The Growth of Murine Breast Cancer Cells in Dystrophic Mice

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Abstract

The American Cancer Society predicted that 230,480 women would be diagnosed with, and 39,520 women would die from breast cancer (BC) in the United States in 2011. While the incidence of female BC has been decreasing, BC remains the second leading cause of cancer death among women in the United States. Cancer cachexia, the cancer-related loss of muscle, affects up to 25% of BC patients and is associated with poor prognosis and decreased quality of life. Alterations to the dystrophin glycoprotein complex (DGC), a transmembrane, multi-subunit protein complex with structural and signaling roles, have been reported in mammary tumors of BC patients and skeletal muscles of cachectic cancer patients. However, this complex is most frequently studied for its role in Duchenne muscular dystrophy (DMD), a severe, progressive muscle wasting disease. Despite the similar alterations reported in these diseases, it is unclear whether alterations in the DGC in one tissue can impact the progression of disease in another. ***Purpose:*** The purpose of the studies described in this dissertation was to identify differences in body composition, energy expenditure and plasma cytokine content between the C57BL/10ScSn-*Dmd*^{mdx}/J (*mdx*) mouse model of DMD and C57BL/10ScSnJ (BL/10) control mice and to determine whether systemic alteration of the DGC (as observed in the *mdx* mouse) alters the growth of E0771 murine mammary tumors. ***Results:*** There were differences in body composition and plasma cytokine profiles between *mdx* and BL/10 mice. We also found that, relative to controls, the tumor–induced increase in cytokines that promote invasion and

metastasis was not as severe in *mdx* mice. ***Conclusions:*** This study revealed several differences between *mdx* and BL/10 mice and provides support for the suggestion that the *mdx* mouse may not be an accurate model of DMD. In addition, the improved cytokine profile of tumor-bearing *mdx* mice suggests that the acute phase of DMD may be protective against BC invasion and metastasis. Further research should confirm this effect and determine whether alterations in the DGC of the *mdx* mouse are directly or indirectly responsible.

Dedication

This dissertation is dedicated to my parents, who always encouraged us to ask questions, to say "I don't know…but I will find out" and taught us that there is no such thing as "can't"!

"What is it that confers the noblest delight? What is that which swells a man's breast with pride above that which any other experience can bring to him? Discovery! To know that you are walking where none others have walked; that you are beholding what human eye has not seen before; that you are breathing a virgin atmosphere. To give birth to an idea--to discover a great thought--an intellectual nugget, right under the dust of a field that many a brain-plow had gone over before… To be the first--that is the idea. To do something, say something, see something, before anybody else--these are the things that confer a pleasure compared with which other pleasures are tame and commonplace, other ecstasies cheap and trivial…These are the men who have really lived--who have actually comprehended what pleasure is--who have crowded long lifetimes of ecstasy into a single moment."

-Mark Twain, Innocents Abroad (1869)

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List of abbreviations

ADG – average daily gain

ANOVA – analysis of variance

ARMS – amplification-resistant mutation system

ATP – adenosine triphosphate

BC – breast cancer

BL/6 – C57BL/6

BL/10 – C57BL/10ScSnJ

BMI – body mass index

CON_{5-9} – C57BL/10ScSnJ mice that were used experimentally when they were 5 to 9 wk old

$CON+T_{5-9}$ – E0771 tumor bearing C57BL/10ScSnJ mice that were used experimentally when they were 5 to 9 wk old

CON_{6-13} – C57BL/10ScSnJ mice that were used experimentally when they were 6 to 13 wk old

$CON+T_{6-13}$ – E0771 tumor bearing C57BL/10ScSnJ mice that were used experimentally when they were 6 to 13 wk old

CON_{9-13} – C57BL/10ScSnJ mice that were used experimentally when they were 9 to 13 wk old

CS – newborn calf serum

d – day(s)

DGC – dystrophin glycoprotein complex

DMBA – 7,12-dimethylbenz[a]anthracene

DMD – Duchenne muscular dystrophy

DMEM – Dulbecco's Modified Eagle Medium

dpt – days post E0771 tumor cell injection

E0771 – murine mammary adenocarcinoma cell line

EDL – extensor digitorum longus

EGFR – epidermal growth factor receptor

IGF – insulin-like growth factor

IL – interleukin

MDA-MB-231 – human mammary adenocarcinoma cell line

mdx – C57BL/10ScSn-*Dmd*^{mdx}/J; murine model of Duchenne muscular dystrophy

$MDX_{5\text{-}9}$ – C57BL/10ScSN-*Dmd*^{mdx}/J mice that were used experimentally when they were 5 to 9 wk old

$MDX{+}T_{5\text{-}9}$ – E0771 tumor bearing C57BL/10ScSN-*Dmd*^{mdx}/J mice that were used experimentally when they were 5 to 9 wk old

$MDX_{6\text{-}13}$ – C57BL/10ScSN-*Dmd*^{mdx}/J mice that were used experimentally when they were 6 to 13 wk old

MDX+$T_{6\text{-}13}$ – E0771 tumor bearing C57BL/10ScSN-*Dmd*^{mdx}/J mice that were used experimentally when they were 6 to 13 wk old

$MDX_{9\text{-}13}$ – C57BL/10ScSN-*Dmd*^{mdx}/J mice that were used experimentally when they were 9 to 13 wk old

mo – month(s)

MT-W9A – rat mammary tumor

MT-W9B – rat mammary tumor

MT-W9C – rat mammary tumor

P/S – penicillin/streptomycin

REE – resting energy expenditure

RER – respiratory exchange ratio

RH – relative humidity

TEE – total energy expenditure

TNF – tumor necrosis factor

TSA – tumor surface area

UCP – uncoupling protein

wk – week(s)

Chapter 1: Introduction

The American Cancer Society predicted that 230,480 women would be diagnosed with, and 39,520 women would die from BC in the United States in 2011 [1]. While the incidence of female BC decreased by 1.7 – 2.7% per year between 1995 and 2007 (the most recent period for which data are available) [2], BC remains the second leading cause of cancer death among women in the United States [1, 3]. Cancer cachexia, the cancer-related loss of skeletal muscle mass [4], is reported to affect 0.8 to 24.8% of BC patients [5-8]. The occurrence of cachexia is typically associated with a poor prognosis [6, 7] and a decreased quality of life [9] for affected patients.

The dystrophin glycoprotein complex (DGC) is a multi-subunit transmembrane protein complex that acts as a physical and signaling connection between the intracellular and extracellular environments [10]. While the DGC is most frequently studied for its role in muscular dystrophy, alterations to this complex have been reported in BC cells [10, 11] and in the skeletal muscle cells of cachectic tumor-bearing mice and humans [12]. However, it is presently unclear whether alteration in the DGC of one type of tissue can affect progression of disease in another.

Administration of murine BC cells to *mdx* mice provides a unique model in which this relationship can be explored. The *mdx* mouse model of DMD carries a naturally occurring X-linked [13, 14] point mutation resulting in a premature stop codon [15] for the dystrophin gene which prevents expression of the dystrophin protein [16]. In the absence of dystrophin, protein expression of many other components of the DGC is drastically reduced [17, 18]. This model was used in the studies described below to test the ***overarching hypothesis*** that systemic down-regulation of DGC components can effect BC growth and cachexia.

In order to test this hypothesis, we developed the following specific aims:

Specific aim 1: To review the available BC cachexia literature. Presently, there is little information available regarding BC cachexia. The limited information that is available is spread throughout a variety of journals and is frequently reported in articles that are not specific to patients with BC cachexia. The ***purpose*** of the summary described in this aim is to bring the information available in the literature together into a clear and concise summary of the current understanding BC cachexia. This aim will be addressed in Chapter 2 of this dissertation.

Specific aim 2: To determine whether BC growth and cachexia are altered in mdx mice. Alterations to the DGC have been reported in BC, cachexia, DMD, and in the *mdx* mouse, but it is currently unclear whether systemic alteration of the DGC (as observed in the *mdx* mouse) affects BC growth or BC cachexia. Because of the high levels of inflammation present in *mdx* mice [19] and the relationship between chronic inflammation and cancer risk [20], we ***hypothesize*** that BC growth will be enhanced in *mdx* mice. In addition, the results of Acharyya et al. [12] led us to ***hypothesize*** that BC cachexia will be exacerbated in *mdx* mice. In order to address these aims, we injected murine mammary carcinoma cells into *mdx* and BL/10 control mice and compared tumor growth and markers of cachexia (including skeletal muscle function) between genotypes. This aim will be addressed in Chapter 3 of this dissertation.

Specific aim 3: To identify differences in body composition, energy expenditure, and plasma cytokine content between 5- to 13-wk-old BL/10 and mdx mice. Because of the differences in tumor growth observed in our preliminary study (Specific aim 2, Chapter 3), we hope to identify differences between *mdx* and BL/10 mice. We ***hypothesize*** that there will be differences in body mass and composition, energy expenditure, and plasma cytokine profiles between the two genotypes. To test

this hypothesis, we measured body mass and composition, total energy expenditure, and cage activity of 5- to 13-wk-old BL/10 and *mdx* mice. Cytokine profiles were measured in plasma collected from 9- and 13-wk-old BL/10 and *mdx* mice. This aim will be addressed in Chapter 4 of this dissertation.

Specific aim 4: To confirm that the phenotype of the mdx mouse is protective against breast cancer growth. In our pilot study (Specific aim 2, Chapter 3), we observed a protective effect of the *mdx* phenotype on the growth of murine mammary tumors. The purpose of the study described in this aim is to confirm the results of our pilot study and to determine whether this protective effect is dependent on the age (i.e., state of dystrophic myopathy) of the mouse. Based on our pilot study, we ***hypothesize*** that the growth of murine mammary tumors will be blunted in *mdx* mice and that this effect will be more pronounced when the dystrophic phenotype is more severe (i.e., during the acute phase of dystrophic myopathy). In order to test this hypothesis, we measured the growth of murine mammary tumors in *mdx* mice that were in the acute (i.e., 5- to 9-wk-old mice) and chronic (i.e., 9- to 13-wk-old mice) phases of muscular dystrophy and compared it to the growth of mammary tumors in age-matched BL/10 control mice. This aim will be addressed in Chapter 5 of this dissertation.

The results of the studies described in this dissertation (i.e., Specific Aim 3) will enhance the understanding of the *mdx* mouse model of DMD. While the *mdx* mouse is an accurate genetic representation of DMD, the phenotype of this model is not as severe as that of patients with DMD and its appropriateness as a model of DMD has been questioned by scientists. Despite this, the *mdx* mouse remains the most frequently used model of DMD [21]. A better understanding of the phenotypic differences between the *mdx* mouse and patients with DMD will improve the ability of scientists to determine whether use of the *mdx* mouse is appropriate for their study of DMD. The studies described here (i.e, Specific Aims 2 & 4) will also reveal whether the phenotype of the *mdx* mouse can affect the

growth of mammary tumors, and as such, may reveal novel mechanisms for the prevention and treatment of BC.

Chapter 2: Cachexia in breast cancer: a review of the existing literature

Abstract

BC is the second leading cause of cancer mortality among women in the United States. The American Cancer Society predicted that in the United States, as many as 230,480 women would be diagnosed with and 39,520 women would die from BC in 2011. Up to one quarter of those diagnosed with BC can be expected to experience cachexia, a debilitating condition characterized by a loss of lean body mass that is frequently associated with decreased quality of life, poor tolerance and responsiveness to treatment, and decreased survival. Despite the frequency and severity of this condition, BC-specific research of cachexia is limited. Several physiological, metabolic, and immunological changes have been reported in BC cachexia, but an effective treatment for this condition has not been identified. There is some evidence to suggest treatment with megestrol acetate or supplementation with energy modulating vitamins and eicosapentaenoic acid may reduce the severity of BC cachexia, but further research is necessary to confirm that the improvement in body mass associated with these treatments is due to increased functional muscle mass. Improving the understanding of BC cachexia and developing an appropriate treatment could improve the quality of life and survival of patients with advanced BC.

Introduction

The American Cancer Society predicted that 230,480 women would be diagnosed with, and 39,520 women would die from BC in the United States in 2011 [1]. While the incidence of invasive female BC has been decreasing by about 2% per year since 1998 [3], BC remains the second most frequently diagnosed cancer and the second leading cause of cancer death among women in the United States [1, 3]. As many as 24.8% [7] of those diagnosed with BC can be expected to experience a complication known as cachexia.

Cancer cachexia was recently defined as “a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment“ [4]. A diagnosis of cachexia requires a loss of more than 5% of the patient’s body mass in the previous 6 months, a body mass index (BMI) of less than 20 with a 2% loss of body mass, or an appendicular skeletal muscle index consistent with sarcopenia (<7.26 kg/m^2 and <5.45 kg/m^2 for males and females, respectively) with a 2% loss of body mass [4]. The occurrence of cachexia is typically associated with a poor prognosis [6, 7] and a decreased quality of life [9].

Cachexia is reported to affect 0.8 to 24.8% of BC patients [5-8]. This variation in the frequency of cachexia is likely due, in part, to inconsistencies in the definition of cachexia used by physicians and researchers and differences in patient characteristics (e.g., tumor characteristics and stage) across studies. However, it seems clear that cachexia is more prevalent in those with advanced BC (i.e., higher stage; refer to Table 1 and Table 2 for a brief summary of BC staging and treatment) [6, 7, 22, 23], and that patients who develop cachexia have a poorer prognosis than those who do not [6].

Despite the frequency and seriousness of this complication, the mechanisms of cachexia and its treatment remain poorly understood. This is particularly true in the area of BC cachexia. At the present time, articles relating specifically to BC cachexia make up just 2% of articles on cancer cachexia, despite the fact that 27% of those with cancer cachexia are BC patients [7]. In addition, the limited information that is available is spread throughout a variety of journals and is frequently reported in articles that are not specific to patients with BC cachexia. As mentioned in ***Specific aim 1***, the ***purpose*** of this review is to bring the information available in the literature together into a clear and concise summary of the current understanding BC cachexia. Topics that are important to cachexia (e.g., muscle strength, protein metabolism), but have not been studied specifically in BC will be mentioned only briefly.

Table 1: BC staging and treatment (Stages 0-II)

Stage	Description [24]	Treatment options [25, 26]
0	• All cancer cells located within duct • No invasion into tissue surrounding primary tumor • No metastasis to lymph nodes or distant sites	• Lumpectomy followed by radiation • Mastectomy • Adjuvant therapy (e.g., tamoxifen, trastuzumab if tumor is hormone receptor positive)
IA	• Primary tumor ≤ 2 cm in diameter • Invasion into tissue surrounding primary tumor • No metastasis to lymph nodes or distant sites	• Lumpectomy or partial mastectomy followed by radiation • Mastectomy • Adjuvant therapy (e.g., tamoxifen, trastuzumab if tumor is hormone receptor positive)
IB	• Primary tumor ≤ 2 cm in diameter or not found • Invasion into tissue surrounding primary tumor • Metastasis in 1 to 3 axillary lymph nodes (0.2 to 2 mm in diameter) • No metastasis to distant sites	
IIA	• Primary tumor ≤ 2 cm in diameter or not found • Invasion into tissue surrounding primary tumor • Metastasis in 1 to 3 axillary lymph nodes (> 2 mm in diameter) and/or internal mammary lymph nodes • No metastasis to distant sites	• Lumpectomy or partial mastectomy followed by radiation • Mastectomy (followed by radiation if tumor > 5 cm in diameter or lymph nodes are involved) • Adjuvant therapy (e.g., tamoxifen, trastuzumab) if tumor is hormone receptor positive • Chemotherapy (e.g., doxorubicin, cyclophosphamide)
	• Primary tumor 2 to 5 cm in diameter • Invasion into tissue surrounding primary tumor • No metastasis to lymph nodes or distant sites.	
IIB	• Primary tumor 2 to 5 cm in diameter • Invasion into tissue surrounding primary tumor • Metastasis in 1 to 3 axillary lymph nodes and/or internal mammary lymph nodes • No metastasis to distant sites	
	• Primary tumor > 5 cm in diameter • Invasion into tissue surrounding primary tumor, but not chest wall or skin • No metastasis to lymph nodes or distant sites	

Adapted from the "How is breast cancer staged?", "Treatment of stage 0 (non-invasive) breast cancer", and "Treatment of invasive breast cancer, by stage" by the American Cancer Society [24-26]

Table 2: BC staging and treatment (Stages III-IV)

Stage	Description [24]	Treatment options [26]
IIIA	• Primary tumor < 5 cm in diameter or not found • Invasion into tissue surrounding primary tumor • Metastasis in 4 to 9 axillary lymph nodes or internal mammary lymph nodes • No metastasis to distant sites	• Neoadjuvant chemotherapy • Lumpectomy or partial mastectomy followed by radiation • Mastectomy (followed by radiation if tumor > 5 cm in diameter or lymph nodes are involved) • Adjuvant therapy (e.g., tamoxifen, trastuzumab) if tumor is hormone receptor positive • Chemotherapy (e.g., doxorubicin, cyclophosphamide)
	• Primary tumor > 5 cm in diameter • Invasion into tissue surrounding primary tumor, but not chest wall or skin • Metastasis to 1 to 9 axillary lymph nodes or internal mammary lymph nodes • No metastasis to distant sites	
IIIB	• Invasion into tissue surrounding primary tumor, including chest wall or skin • Metastasis to 0 to 9 axillary lymph nodes and internal mammary lymph nodes • No metastasis to distant sites	
	• Inflammatory BC • No metastasis to distant sites	
IIIC	• Primary tumor of any diameter • Invasion into tissue surrounding primary tumor • Metastasis to ≥ 10 axillary lymph nodes, lymph nodes under clavicle, lymph nodes above clavicle, and/or internal mammary lymph nodes • No metastasis to distant sites	
IV	• Primary tumor of any diameter • Invasion into tissue surrounding primary tumor • With or without involvement of local lymph nodes • Metastasis to distant organs or lymph nodes	• Mastectomy (when appropriate) • Adjuvant therapy (e.g., tamoxifen, trastuzumab) if tumor is hormone receptor positive • Chemotherapy (e.g., doxorubicin, cyclophosphamide)
	• Inflammatory BC • Metastasis to distant organs or lymph nodes	

Adapted from the "How is breast cancer staged?" and "Treatment of invasive breast cancer, by stage" by the American Cancer Society [24-26]

Methods

The PubMed database was used to identify BC cachexia-specific articles published from 1809 to present. The primary search for “breast cancer” and “cachexia” resulted in 72 hits, including 51 original research articles and 21 review articles. Secondary searches were performed to identify BC-specific articles that addressed some aspect of cachexia that may have not been identified by our initial search (e.g., “breast cancer” and “muscle”, “breast cancer” and “weight”). The text and reference sections of recovered articles were screened to identify articles that may have been missed by our primary and secondary searches. When areas were identified that were fundamental to understanding cachexia but had limited BC cachexia-specific literature available, additional searches were performed that were not delimited to “breast cancer” (e.g., “cachexia” and “IL-6”).

Results

Systemic changes associated with BC cachexia

The presence of mammary tumors can induce a number of physiological and molecular changes throughout the body. These changes can occur in close proximity to the primary tumor (e.g., changes in mammary tissue macronutrient metabolism), in the circulation (e.g., changes in circulating cytokine content), and in distant tissues (e.g., changes in skeletal muscle protein turnover). Together, these changes, which will be described in detail below, contribute to the loss of body mass typically associated with cachexia.

Physiological changes

Anorexia (i.e., decreased appetite) frequently occurs with cancer cachexia [27] and may contribute to some of the body mass loss observed in patients with BC cachexia. Anorexia can have a number of causes, including nausea, early satiety, depression, chemotherapy and radiation therapy [28, 29], and

appears to be "correlated highly with level of well-being" in terminal cancer patients (*r* not provided, *P*<0.0001) [30]. Decreased gastric emptying and intestinal absorption are also thought to contribute to cancer cachexia. To our knowledge, there is only one report of delayed gastric emptying in a BC patient, but it is unclear whether the patient also had symptoms of cachexia [31]. Similarly, changes in the intestinal absorption of monosaccharides have been observed in BC (n=10, stage IV), but whether these changes correlated to incidence or severity of cachexia in patients with BC was not reported [32]. The fact that nutritional supplementation, including total parenteral nutrition, cannot completely reverse the tissue (especially muscle) wasting observed with cachexia confirms that physiological factors alone are not the only cause of the wasting seen with cancer cachexia [33], but BC-specific research in this area is limited.

Metabolic changes

Increased resting energy expenditure (REE) has been implicated as a potential contributing factor to cachexia in cancer patients. To the authors' knowledge, just one group has compared the REE of BC patients to that of cancer-free controls. On the basis of this one report, the REE of patients (post-surgery but pre-chemotherapy) with invasive (n=17, stage I-III) [34], but not metastatic (n=30, stage IV) [35], BC may be greater than that of healthy controls. Elevations in brown adipose tissue uncoupling protein (UCP) 1 and skeletal muscle UCP2 and UCP3 are thought to contribute to the increased REE observed in a variety of cancer types [27] by dissipating the proton gradient that drives mitochondrial adenosine triphosphate (ATP) synthesis, but this has not been studied in BC patients.

Carbohydrate metabolism

Cancer-induced alterations in macronutrient metabolism are also thought to contribute to the changes observed in cachexia (Figure 1). Researchers hypothesize that the high rate of glycolytic activity typically observed in benign, malignant, and metastatic cancer, including BC [36, 37], is due to the need to meet

the high energy demand of rapidly dividing cancer cells despite intermittent hypoxia [38, 39] and mitochondrial damage [39, 40]. BC-specific data to support this hypothesis are typically reported in rodent models of BC rather than in patients with BC. For example, in cachectic rats with 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors, the activity of glycolytic enzymes (e.g., hexokinase, phosphoglucoisomerase, and aldolase), which are responsible for oxygen independent production of ATP from glucose, are elevated by 57-97% in mammary tumors compared to mammary tissue from BC-free rats [41]. However, the activities of gluconeogenic enzymes (e.g., fructose 1,6-bisphosphatase and glucose 6-phosphatase), which are responsible for production of glucose from non-carbohydrate precursors; citric acid cycle enzymes (e.g., isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase), which produce ATP and reducing equivalents from acetyl CoA; and electron transport chain enzymes (e.g., NADH dehydrogenase and cytochrome *c* oxidase), which play a role in oxygen dependent production of ATP from NADH and $FADH_2$, are decreased by 26-41% in tumors compared to mammary tissue from tumor-free rats [41].

Alterations in mammary and liver carbohydrate metabolism have also been reported in animal models of BC cachexia. The activities of glycolytic, citric acid cycle, and electron transport chain enzymes are 23-46% lower; while the activity of gluconeogenic enzymes is 38-68% greater in mammary tissue of cachectic rats bearing DMBA-induced tumors than in the mammary tissue of tumor-free rats [41, 42]. In the liver, mitochondrial activity of citric acid cycle and electron transport chain enzymes is 33-54% lower in DMBA-induced tumor-bearing cachectic rats compared to tumor-free rats [42].

In addition, alterations in circulating markers of carbohydrate metabolism have been reported in BC cachexia. Decreases in blood riboflavin, niacin, and serum CoQ_{10}, which are necessary for oxidative phosphorylation, have been reported in cachectic rats bearing DMBA-induced mammary tumors [42]. There are no apparent changes in fasting blood glucose in either murine or human models of BC

cachexia [43-45], but there is some evidence of decreased glucose production and glucose tolerance in cachectic cancer patients [including three with stage IV BC] compared to cancer-free controls [46].

Alterations to other markers of carbohydrate metabolism, such as increases in the activity of enzymes responsible for the production of glucose from lactate (i.e., Cori cycle enzymes), have been reported to occur in other types of cancer cachexia [47], but these changes have not been studied in BC cachexia.

Together, these findings suggest that the presence of mammary tumors in rodents is associated with whole-body alterations in carbohydrate metabolism, which could contribute to the cachexia experienced by as many as 25% of patients with BC. Further research is necessary to determine whether alterations in carbohydrate metabolism also occur in patients with BC.

Lipid metabolism

It is presently unclear whether systemic changes in lipid metabolism contribute to BC. The loss of adipose tissue typically observed in patients with cancer cachexia is thought to be due more to increased lipolysis rather than decreased lipogenesis [27]. However, to the authors' knowledge, whole body rates of lipolysis and lipogenesis have not been reported in patients with BC cachexia (Figure 1).

With respect to circulating factors associated with lipid metabolism, plasma lipoprotein lipase, an enzyme responsible for the removal of fatty acids from triglycerides for storage in adipocytes and skeletal muscle cells, is typically decreased in cancer cachexia [48]. However, in studies that included patients with BC cachexia, plasma lipoprotein lipase activity was not decreased (n=7, with and without metastasis [22]; n=7, stage IV [45]) or correlated to weight loss (n=7, with and without metastasis; *r*, *P* not provided) [22].

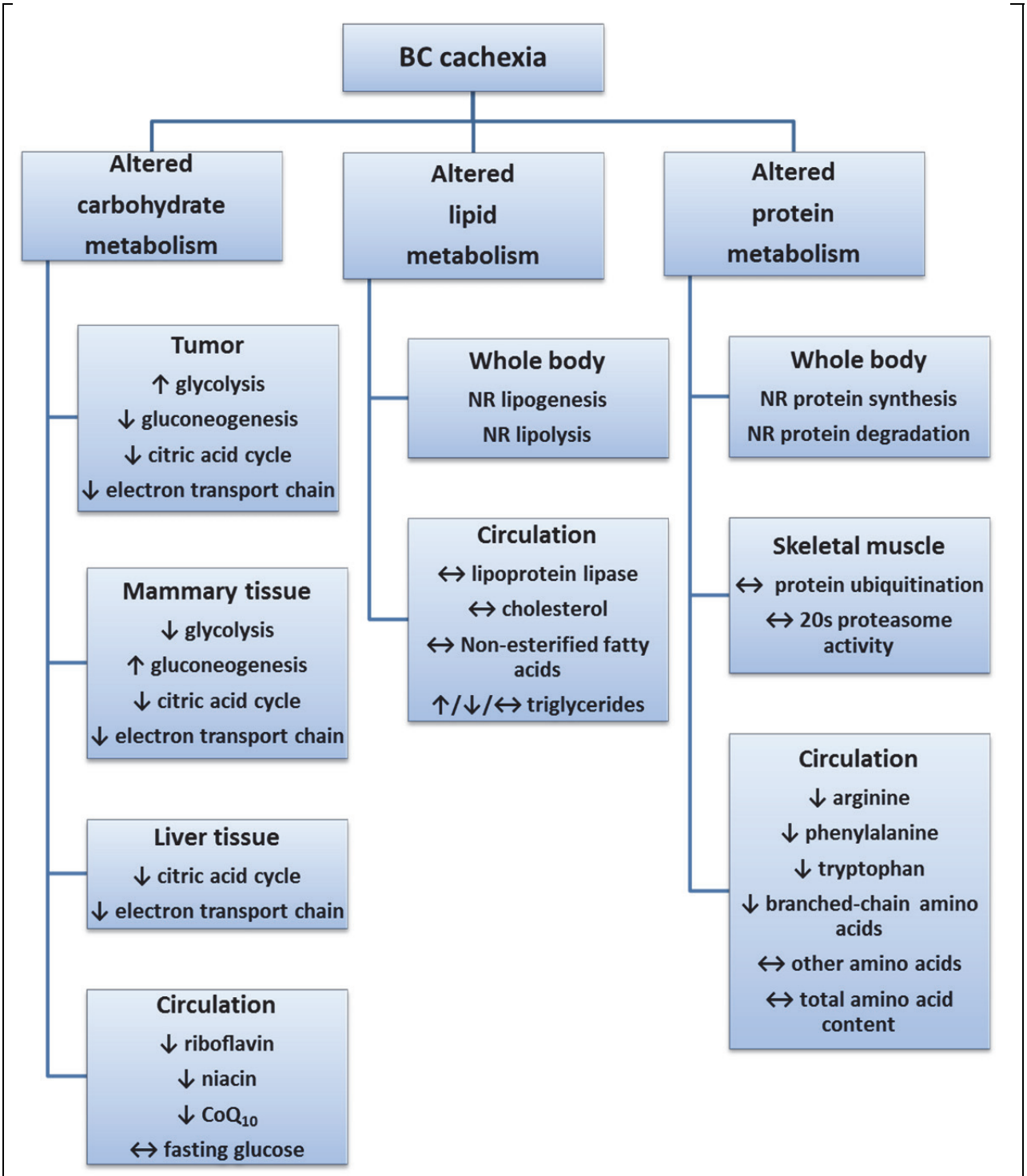

Figure 1: Metabolic changes in BC cachexia

BC cachexia is associated with systemic alterations in macronutrient metabolism. This figure summarizes the changes in markers of carbohydrate, lipid, and protein metabolism that have been reported in the tissues of patients and/or rodents with BC.

↑ increased, ↓ decreased, ↔ not different, NR no information available

Changes in circulating lipids (e.g., triglycerides, cholesterol, and non-esterified fatty acids) have been reported in cachexia associated with a variety of cancer types and are thought to reflect changes in lipid intake and metabolism [49]. However, in most studies that included patients with BC, fasting serum cholesterol was not affected by presence of cancer (n=7, stage IV [45]) or BC cachexia (n=14, with and without metastasis [23]). Non-esterified fatty acid levels also appear to be normal in patients with BC cachexia (n=7, with and without metastasis [22]). Whether circulating triglyceride levels are altered in BC cachexia is unclear. A study by Nomura et al. reported no difference in serum triglyceride content between BC patients, including those with weight loss (n=7, with and without metastasis) and normal controls [22], while a study by Vlassara et al. that included patients with BC reported increased triglyceride content in the plasma of patients with cancer cachexia (n=7, stage IV) [45]. In contrast, when compared to non-cachectic mice with BC, circulating triglycerides are lower in mice with BC cachexia [43]. While research is limited, this information suggests that the lipid metabolism perturbations frequently reported in cancer cachexia are not as apparent in BC cachexia, and as such altered lipid metabolism may not be a major contributor to BC cachexia.

Protein metabolism

Alterations in protein metabolism, via both decreased protein synthesis and increased protein degradation (primarily through the ubiquitin-proteasome pathway) have been implicated as causes of skeletal muscle loss in cancer cachexia [27]. However, there are limited data available to confirm that this is also true in BC cachexia (Figure 1). For example, Kumar et al. reported no difference muscle protein ubiquitination or in the activity of the 20s proteasome between cachectic mice bearing interleukin (IL)-1α over-expressing MCF-7 mammary tumors and non-cachectic mice (bearing empty vector MCF-7 mammary tumors) [43].

There has been one report of alterations in circulating amino acid (AA) levels in patients with BC. Based on that study, it appears that patients with BC (n=22 stage I-III), including those who have decreased body mass, have 17-23% lower post-absorptive venous plasma arginine, phenylalanine, tryptophan, and branched-chain amino acid levels compared to healthy controls, while the content of other amino acids and plasma total amino acid content did not seem to be affected by the presence of BC [8]. Neither plasma arginine nor total amino acid content were correlated to loss of body mass or BMI (*r*, *P* not provided) [8], suggesting that changes in protein metabolism may have occurred prior to measurable changes in body mass [8]. Further research in the area of BC-related changes in protein synthesis and degradation are necessary before a conclusion can be made regarding the role of protein metabolism in BC cachexia.

In summary, the current, albeit limited, information available suggests that the presence of mammary tumors may induce whole-body changes in carbohydrate, lipid, and protein metabolism that may, in part, be responsible for cachexia in BC. Despite its obvious importance, macronutrient metabolism is grossly understudied in BC cachexia and would benefit from further research, particularly in the areas of lipid and protein metabolism. A better understanding of BC-related changes in macronutrient metabolism may reveal novel targets for the treatment and prevention of BC cachexia.

Changes in circulating factors

Cytokines

Cytokines are immune-related signaling molecules often studied for their role in cachexia. It is thought that these molecules are released from tumor cells and cells of the tumor microenvironment [e.g., from tumor associated macrophages, T-cells] and circulate throughout the body, affecting systemic changes. Typically, pro-inflammatory cytokines such as IL-1α, IL-6, lipid mobilizing factor, proteolysis inducing factor, and tumor necrosis factor-α (TNF-α) are thought to promote cachexia [50, 51], while anti-

inflammatory cytokines such as IL-1Ra, IL-4, and IL-10, are thought to protect against cachexia [50, 51]. However, to the authors' knowledge only IL-1α, TNF-α, and IL-6 have been studied for their role in BC cachexia (Figure 2).

IL-1α-secreting mammary tumors (MCF-7IL-1α) induce cachexia in nude mice while vector-treated control tumors (MCF-7) do not [43]. While the tumors in this study had been intentionally modified to secrete IL-1α, there are reports of this cytokine being elevated "naturally" in cachexia. While not studied in BC cachexia specifically, studies suggest that the typical sources of cachexia-inducing elevations in IL-1α are BC cells [52, 53] and cells of the tumor microenvironment [52].

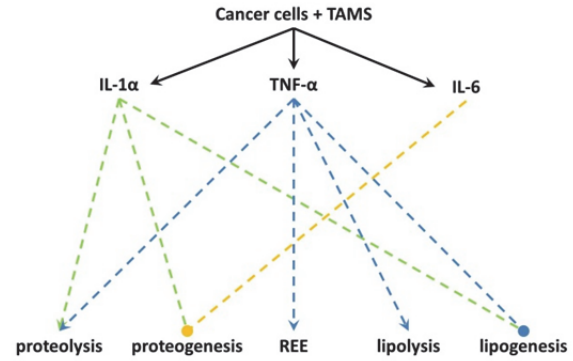

Figure 2: BC cachexia associated cytokines
Cytokines IL-1α, TNF-α, and IL-6 appear to induce cachexia in BC. The mechanisms by which they exert their effects has not been studied in BC specifically, but may be similar to those reported in other types of cachexia. Arrow head: stimulates, Closed circle: inhibits.

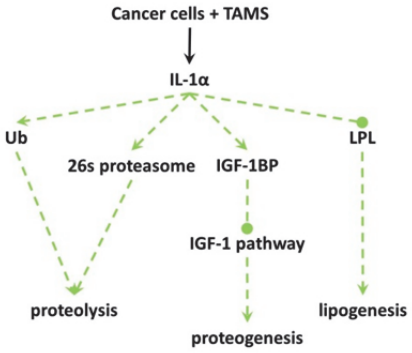

Figure 3: IL-1α mechanism of action
The mechanism of action for IL-1α has not been studied in BC cachexia specifically. However, research in other types of cachexia suggests that it may induce cachexia by promoting the ubiquitin proteasome pathway, inhibiting the IGF-1 pathway, or inhibiting lipoprotein lipase activity. Arrow head: stimulates, Closed circle: inhibits.

When elevated, IL-1α may induce cachexia by increasing protein degradation via the ubiquitin-proteasome pathway [51] and by decreasing protein synthesis via increased hepatic production of insulin-like growth factor (IGF) binding protein, which in turn decreases IGF-1 driven synthesis of new proteins [54] (Figure 3). IL-1α may also induce cachexia by decreasing lipogenesis via lipoprotein lipase inhibition [55]; however, decreased lipoprotein lipase activity may not occur in BC [22] and does not appear to be related to weight loss [22].

In addition, elevated IL-1α may cause anorexia by increasing levels of corticotrophin-releasing hormone, a neurotransmitter thought to decrease food intake, and by decreasing the impact of neuropeptide Y [50], a neurotransmitter that stimulates appetite, thereby exacerbating body mass loss.

IL-6 secreting mammary tumors (i.e., KPL-4 tumors) induce cachexia in nude mice while KPL-1 tumors, which secrete very low levels of IL-6, do not [56]. An inverse relationship between serum IL-6 and cachexia markers (e.g., body mass, performance status) has been reported in other models (both human and mouse) of BC cachexia [43, 57-59]. Researchers believe that IL-6, which is thought to be released from tumor cells and tumor associated macrophages [60], contributes to cachexia by inducing an acute phase response [55]. The mechanism by which the acute phase response results in cachexia has not been confirmed, but it has been suggested that increased production of acute phase proteins (e.g., C-reactive protein) by the liver depletes the pool of AAs available for synthesis of skeletal muscle proteins (Figure 4).

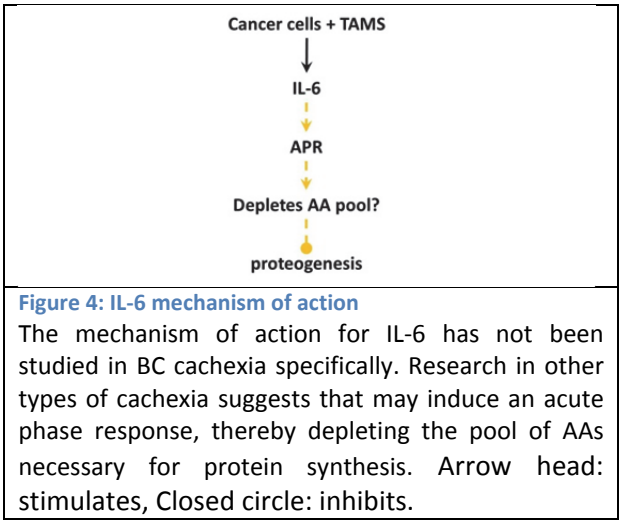

Figure 4: IL-6 mechanism of action
The mechanism of action for IL-6 has not been studied in BC cachexia specifically. Research in other types of cachexia suggests that may induce an acute phase response, thereby depleting the pool of AAs necessary for protein synthesis. Arrow head: stimulates, Closed circle: inhibits.

Elevation of serum TNF-α has been reported in cachectic humans with BC [44], but not in murine models of BC cachexia [43, 56]. After being secreted from tumor cells and/or tumor associated macrophages [60], TNF-α is believed to contribute to cachexia by decreasing both fat and skeletal muscle mass. This cytokine is typically thought to decrease fat mass by inhibiting lipogenesis and increasing lipolysis (Figure 5). With respect to lipogenesis, TNF-α typically acts by inhibiting lipoprotein lipase. However, the activity of this enzyme does not appear to be altered in BC [22], suggesting that TNF-α's pro-lipolytic activity (i.e., stimulation of adipocyte triglyceride lipolysis via a mitogen-activated protein kinase-dependent

pathway [55]) may play a more prominent role in BC cachexia-related loss of fat mass. TNF-α is also thought to affect skeletal muscle mass by uncoupling mitochondrial respiration [51, 55, 60], increasing muscle protein breakdown via the ubiquitin-proteasome pathway [51, 55, 60], inducing apoptosis of skeletal muscle fibers and preventing muscle fiber regeneration [51]. Similar to IL-1, TNF-α may induce cachexia-related anorexia via a corticotrophin-releasing hormone dependent mechanism [51].

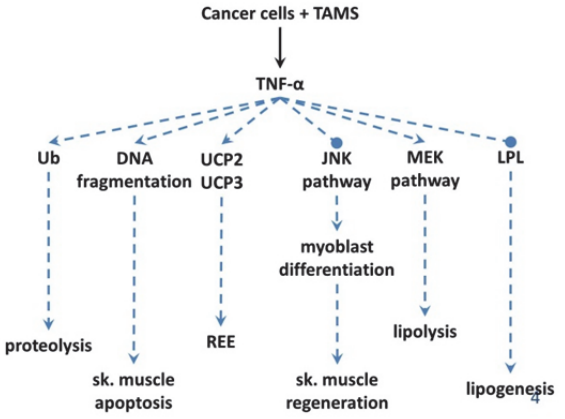

Figure 5: TNF-alpha mechanism of action
TNF-α is thought to induce cachexia via a number of mechanisms. However, these mechanisms have not been studied specifically in BC. Arrow head: stimulates, Closed circle: inhibits.

Serum content of IL-1β [43], IL-5 [43], IL-10 [43], IFN-γ [43, 56], and leukemia inhibitory factor [56], do not seem to be related to cachexia in BC models. Neither the remaining cytokines mentioned at the beginning of this section (i.e., lipid mobilizing factor, proteolysis inducing factor, IL-1Ra, IL-4, IL-10) nor the source and mechanism of IL-1α, IL-6, and TNF-α have been studied in BC specific models of cachexia.

Hormones and other circulating factors

A number of researchers have reported cachexia-related changes in the serum content of hormones and other circulating factors in patients with BC. With respect to metabolism-related factors, fasting plasma glucagon [44], but not insulin [44-46], appears to be increased in cachectic mice and humans with BC. Leptin, a hormone typically thought to signal satiety is increased [61, 62] in patients with BC, but it is unclear whether serum content correlates to cachexia (r=-0.45 to 0.46 or not provided, P=0.03 to 0.07 or not provided) [23, 43, 61, 62]. Serum adiponectin, an adipokine thought to stimulate glucose and lipid oxidation does not appear to be correlated to cachexia (r=0.15, P=0.4) [23]. Ghrelin, an appetite stimulating hormone, appears to be higher in the serum of cachectic patients with a variety of cancer types (including BC) compared to that of non-cachectic cancer patients; there is a modest, positive

correlation between ghrelin and BC-related loss of body mass (r=0.50, P=0.001) [23]. There is limited evidence that serum calcium [43] and serum cortisol [44] are increased while serum albumin and blood hemoglobin are decreased with BC-induced loss of body mass [23]. There is no apparent relationship between white blood cell and lymphocyte content [23], serum growth hormone [44], serum thyroid stimulating hormone [44], and loss of body mass in BC. These studies included, but were not limited to patients with BC [23, 43, 44].

To date, researchers have identified a number of circulating factors (e.g., IL-1α, IL-6, TNF-α, glucagon, leptin, and ghrelin) whose plasma content is altered in BC cachexia. Further research is necessary to determine which of these markers contributes to BC cachexia and to determine the mechanism responsible for the effect.

Physical changes in BC cachexia

Together, the systemic alterations mentioned above, possibly combined with others that have yet to be discovered, result in phenotypic changes that are distressing to both the patient and their family. One such change is a decrease in body mass due to the loss of both lean and fat mass [63, 64]. This decrease in lean body mass seems to result from a disproportionate loss of muscle mass while visceral organ mass is maintained [48, 63, 65]. There are a number of reports of muscle loss in patients with BC cachexia; however, whether this change affects skeletal muscle function is unclear.

Skeletal muscle force output has not been measured in patients with BC cachexia, but has been studied in murine models of cancer cachexia. Absolute isometric force production is decreased in extensor digitorum longus (EDL) muscles isolated from cachectic mice [66, 67]. However, studies reveal mixed results when isometric force output is normalized to muscle mass – in one study, normalized force output from EDL muscles was decreased with cachexia [67] while another indicated that EDL muscles

from tumor-bearing mice may produce more normalized force at submaximal stimulation frequencies (e.g., 40-125 Hz) than those from non-tumor bearing controls [66]. Similarly, mixed results have been reported with respect to the normalized force response to repeated electrical stimuli (i.e., simulated exercise), with one study reporting decreased normalized force output throughout the protocol [67] and another reporting similar normalized force output by EDLs from cachectic and tumor-free control mice [66]. Further research is necessary before conclusions can be made regarding the effect of BC cachexia on skeletal muscle function can be made.

It should be noted that cachexia related skeletal muscle changes have also been reported at the molecular level, but again, BC-specific research in this area is limited. In mice bearing mammary tumors, there was a trend (P=0.0542) for decreased skeletal muscle content of MyoD, a protein that promotes the differentiation of muscle cells. However, in this study, myostatin, which is thought to inhibit muscle cell differentiation, was not expressed in muscles from cachectic or non-cachectic mice [43]. In contrast, more recent literature reports increased skeletal muscle myostatin content in cachexia induced by other types of cancer [68]. Increased NF-κB activation [69] and altered expression of components of the DGC [12], a multi-subunit protein complex that connects the cytoskeleton of muscle cells with the extra-cellular matrix, have also been reported in the cachectic skeletal muscles of humans and mice, but it is unclear whether similar changes occur in BC cachexia.

The aforementioned loss of skeletal muscle mass may, at least partially, be responsible for the weakness, decreased ability to perform typical daily tasks (e.g., self-care) [6, 29], and decreased quality of life [29, 64] typically associated with cachexia in cancer patients (including, but not limited to patients with BC). These symptoms are of utmost concern as they have been associated with decreased tolerance [70] and responsiveness to treatment [6], and decreased survival [6, 29, 30] in cancer patients, highlighting the need for an effective treatment of BC cachexia.

Treatment of BC cachexia

With the exception of tumor reduction or excision (when possible) [70], there are no approved methods for the treatment of BC cachexia [7, 71]. A number of treatments, including nutritional supplementation and treatment with hormonal and anti-mitotic agents, have been tested (Table 3), but researchers have had little success at halting or completely reversing cachexia, an effect that could greatly benefit the quality of life, response to treatment, and even survival [65] of patients with BC.

Nutritional Supplementation

Several studies have explored the use of oral nutritional supplementation to prevent cachexia. For example, mice bearing DMBA-induced mammary tumors that were treated with a combination of energy metabolism modulating vitamins (riboflavin, niacin, and coenzyme Q_{10}: 45, 100, and 40 mg/kg/d, respectively) gained body mass in a similar fashion to those who did not have BC, while untreated tumor bearing mice gained body mass initially, but experienced a rapid loss of body mass towards the end of the 28 d experiment [42]. One potential mechanism for this result is a shift towards aerobic metabolism – treatment with energy modulating vitamins has been shown to restore mammary and liver mitochondrial activity of citric acid cycle and electron transport chain enzymes in cachectic BC-bearing mice [42]. A similar effect is observed when energy modulating vitamins are provided in combination with tamoxifen, an anti-estrogen commonly used for the treatment of estrogen receptor-positive BC [41].

Supplementation with fish oil, which is high in vitamin E and n-3 fatty acids such as eicosapentaenoic acid and docosahexaeonic acid, in an attempt to decrease the inflammation thought to be partially responsible for cancer cachexia [72] has yielded mixed results. In one study, short-term (2 weeks), high-dose (1000 mg/day) treatments with fish oil did not alleviate symptoms of cachexia in cancer patients, including those with BC (n=6, stage IV) [72]. Conversely, BC (MDA-MB-231 human mammary

adenocarcinoma cell line xenograft)-bearing athymic mice treated with 3% fish oil for 2 wk gained more body mass throughout the course of the study than those fed a control diet [73]. In the latter study, however, no tumor-free controls were used, so it is unclear whether the fish oil treatment was preventing body mass loss or simply causing a greater than normal body mass gain [73]. If effective, it is thought that the eicosapentaenoic acid component of this supplement would decrease the content of the pro-inflammatory cytokines (e.g., IL-1, IL-6, TNF-α, and proteolysis inducing factor) [55, 74], thus inhibiting the BC-induced loss of fat and muscle mass thought to be induced by activation of the acute phase response. However, this mechanism has not been explored in BC cachexia specifically.

The use of orally administered proteases as an adjuvant therapy for BC has also been associated with decreased cachexia in patients with BC (n=649; stages I-IV) [5]. However, the mechanisms responsible for this effect, and whether decreased cachexia correlated to decreases in tumor burden were not reported [5]. In addition, the lack of randomization between treatment groups may have affected the results of this study. Further research is necessary before recommendations regarding the use of orally administered proteases as an anti-cachexia treatment can be made.

Oral administration of branched-chain amino acids [75] and the use of enteral and parenteral supplementation (including total parenteral nutrition) [76] have also been studied for their beneficial effects on protein synthesis [77] in cancer cachexia, but have not been studied in patients with BC.

Table 3: Potential BC cachexia treatments

Treatment (route)	Dose	Duration (wk)	Subject (sex)	BC only?	Effect on cancer	Effect on body mass	Effect on other markers?	Indep. effect*	References
5'-dFUrd (PO)	60 mg/kg, 5x/wk	3	nude mice (f)	Y	-	0	NR	N/A	[59]
docetaxel (i.p.)	5-10 mg/kg/wk	3	nude mice (f)	Y	0/-	0	NR	N/A	[59]
docetaxel + 5'dFUrd (i.p.+ PO)	5-10 mg/kg/wk + 60 mg/kg, 5x/wk	3	nude mice (f)	Y	-	0	NR	N/A	[59]
docetaxel + tegafur (i.p.+ PO)	5-10 mg/kg/wk + 100 mg/kg, 5x/wk	3	nude mice (f)	Y	0/-	0	NR	N/A	[59]
eCadherin overexpression (tumor cell transfection)	N/A	4	nude mice (f)	Y	-	+	NR	NR	[78]
energy modulating vitamins (Riboflavin + Niacin + CoQ_{10}) ± tamoxifen (PO)	45+100 + 40 mg/kg/d ± 10 mg/kg/d	4	SD rats (f)	Y	-	+	NR	NR	[41, 42]
Fish oil (eicosapentaenoic acid) (PO)	1,000 mg/d	2	humans (m+f)	N	NR	0	NR, N/Y	N/A	[72]
Hydrazine sulfate (PO)	180 mg/d	4.2	humans (m+f)	N	0/-	0/+	NR, Y	NR	[46]
megestrol acetate (PO)	160-1600 mg/d	≥ 4	humans (m+f)	Y	NR, 0/-	0/+	NR, Y	NR, Y	[28, 79-83]
medroxyprogesterone acetate (IM)	100 mg/kg, 2x/wk	4	nude mice (f)	Y	0	+	NR, Y	NR	[84]
Paclitaxel (i.p.)	9 mg/kg/d	0.7	nude mice (f)	Y	0/-	0	NR	N/A	[85]
rhuMAbHER2 (i.p.)	20 mg/kg initially, then 10 mg/kg/wk	4	nude mice (f)	Y	-	+	Y	NR	[56]
Sagopilone (IV)	10 mg/kg	one dose	nude mice (f)	Y	-	0/+	NR	NR	[85]
Tegafur (PO)	100 mg/kg, 5x/wk	3	nude mice (f)	Y	0/-	0	0	N/A	[59]

Treatments included in this table have been tested in patients or rodents with BC that displayed symptoms of BC cachexia.
*** effect on body mass is independent of treatment's effect on BC, Y yes, - decreased cancer burden, 0 no effect on body mass/tumor burden, NR not reported, N/A not applicable because treatment did not affect body mass,+ increased body mass, N no**

With respect to dietary BC cachexia treatments, supplementation with energy modulating vitamins and fish oil (or eicosapentaenoic acid) appear to be associated with weight gain in rodent models but it is unclear whether they are effective for the treatment of cachexia in patients with BC. Orally administered proteases may also prove beneficial in the treatment of BC cachexia, but randomized controlled trials are necessary before a conclusive decision can be made. Use of branched-chain amino acids and enteral and parenteral supplementation have shown promise in treating patients with cancer cachexia, and may also prove beneficial in preventing cachexia in patients with BC.

Hormonal treatments

Medroxyprogesterone acetate [57, 86] and megestrol acetate [81, 83, 87-90] are hormonal (progestational) anti-BC agents. The use of these drugs is associated with common side effects including increased appetite and body mass gain. They have also been studied as potential treatments for BC cachexia. Treatment with medroxyprogesterone acetate prevented BC (KPL-4 xenograft)-related body mass loss in mice [84], but did not reduce cachexia in cancer patients [91]. However, the latter study included just 2 patients with BC (stage IV) [91]. Research suggests that when medroxyprogesterone acetate treatment does prevent cachexia, it does so by inhibition of the TNF-α-induced release of IL-6 from tumor cells [84], which, as previously described, is thought to decrease cachexia by inhibiting BC-induced body mass loss associated with activation of the acute phase response.

Megestrol acetate appears to be effective in preventing, and even reversing, the loss of body mass and other symptoms [83, 92] of cachexia in cancer patients, including those with advanced BC (n=28, stage IV [83]; n=101, stage IV [93]). Notably, megestrol acetate induced weight gain appears to occur independent of tumor progression (n=28, stage IV [83]; n=40, stage IV [79]; n=161, stage IV [80]) and can be observed in as little as one week [94]. In addition, high (480 mg/day) but not low (160 mg/day) doses of megestrol acetate increased skeletal muscle mass in patients with cancer cachexia, including those

with BC (n=7, stage IV) [28]; however, even with an increase in muscle mass, the ability of patients to perform activities of daily living was not improved [28]. With respect to mechanism of action, research suggests that megestrol acetate increases plasma content of IGF-1 in patients with metastatic BC, which may be responsible for the increase in body mass observed with megestrol acetate treatment [95]. In addition, this drug may induce the differentiation of fibroblasts into adipocytes [87, 96] via a mechanism that is currently unclear, but does not appear to be related to TNF-α inhibition [96].

While information on medroxyprogesterone acetate is limited, there is ample evidence to suggest that megestrol acetate can be used to prevent or treat BC-induced weight loss. Further research is necessary to confirm that the weight gain associated with hormonal treatments is the result of an increase in functional skeletal muscle mass.

Anti-mitotic agents

Anti-mitotic agents are compounds that prevent mitosis by inhibiting proper formation of microtubules. Treatment with such agents (e.g., sagopilone, paclitaxel), has been reported to suppress BC cachexia. For example, treatment of a mouse model of metastatic BC (MDA-MB-231 xenograft) with one dose (10 mg/kg i.v.) of sagopilone, (a synthetic mitotic inhibitor), prior to onset of bone metastasis was associated with a 10.9% greater body mass than treatment with a vehicle after 20 days [85]. In addition, after 23 days of tumor growth in a similar model, post-metastasis treatment with either sagopilone (10 mg/kg i.v.) or paclitaxel (a mitotic inhibitor originally derived from the Pacific Yew tree; currently in use; 9 mg/kg/d i.p. for 4 days) was associated with 6.8 and 9.7% greater body mass, respectively, than those treated with the vehicle [85]. The mechanism by which these anti-mitotic agents affect BC cachexia is unclear, but it is likely that the effect can at least be partially explained by a treatment-induced decrease in tumor burden. [85].

In contrast, treatment with docetaxel (semisynthetic mitotic inhibitor derived from the European Yew tree; currently in use; 10 mg/kg/wk i.p.for 3 wk) did not affect body mass or serum glucose in a mouse model of BC (KPL-4 xenograft) [59] despite decreasing tumor burden (10 mg treatment only). Similarly, treatment of BC with docetaxel in combination with other anti-BC treatments [i.e., 10 mg/kg/wk docetaxel with 5'-deoxy-5-fluorouridine (5'-dFUrd) or Tegafur; 5 mg/kg/wk docetaxel with 5'dfUrd] had no effect on body mass, but was associated with decreased serum IL-6 and an improvement in other markers of cachexia (i.e., serum glucose).

Together, these findings suggest that the effectiveness of anti-mitotic agents against cachexia varies from agent to agent and, at this time, their anti-cachectic effects cannot be isolated from their ability to decrease tumor burden.

Other treatments

There are some reports that treatment of BC patients (among patients with other types of cancers) with hydrazine sulfate, a gluconeogenesis inhibitor, may promote weight gain [97] and improve glucose tolerance [46] by correcting tumor-induced perturbations in carbohydrate metabolism. However, use of this drug is controversial and it is not approved in the United States for treatment of cancer cachexia [98]. A study of E-cadherin secreting mammary tumors suggests that E-cadherin may also protect against BC cachexia; however, upregulation of E-cadherin was also associated with decreased metastasis, which could have contributed to the observed decrease in cachexia [78]. Further research is necessary to determine whether the anti-cachectic effect of E-cadherin is independent of its effects on tumor progression.

A number of other treatments (e.g., anti-TNF-α and anti-IL-6 compounds, anabolic agents, ghrelin mimetics) have shown promising results in cachexia induced by other types of cancer [9], but their

effects on BC-associated cachexia have not been explored. Further research is necessary to determine whether these treatments can be used successfully in BC cachexia and to confirm the efficacy of energy modulating vitamins, eicosapentaenoic acid, orally administered proteases and megestrol acetate at preventing or reversing cachexia related loss of skeletal muscle mass.

Animal models

There are five well-defined models of BC cachexia in the literature (Table 4). In one such model, intracardiac injection of nude mice (female, 4-5 weeks of age) with as few as $1x10^5$ MDA-MB-231 (human metastatic mammary adenocarcinoma) cells induces loss of body mass within 3-4 weeks [78, 85, 99-101], with some exceptions [102]. In addition, injection of $2x10^6$ to $1x10^7$ KPL-4 (human malignant mammary carcinoma) cells into the mammary fat pads of nude or Severe Combined Immunodeficiency mice (female, 4-6 weeks of age) induces cachexia within 3 weeks [56, 59, 84]. Injection of $5x10^5$ IL-1α overexpressing, but not control, MCF-7 cells into the mammary fat pad of nude mice (6-8 weeks of age, estrogen implanted) results in cachexia within 7 weeks [43].

In rats, chemical induction of mammary tumors with one dose of DMBA (25mg/kg, orally) in Sprague-Dawley rats (female, 8 weeks of age) results in a lower body mass four months post-induction compared to tumor-free rats of the same age [41, 42]; however, it is unclear whether this difference is the result of carcinogen toxicity or tumor burden. Subcutaneous implantation of MT-W9B and MT-W9C mammary tumor minces into Wistar/Furth rats (8-9 weeks of age) induces cachexia within two months of treatment, while implantation with MT-W9A tumor mince occasionally resulted in cachexia, but only after several months of tumor growth [103].

While these findings suggest that some models of BC may develop cachexia, it is important to keep in mind that diagnoses of cachexia in these models are typically based on a loss of whole body mass.

Further research is necessary to confirm the loss of muscle mass in the currently available models of cachexia and to confirm that available animal models of BC cachexia mimic the human condition.

Table 4: Summary of potential animal models of BC cachexia

Species	Strain	Sex	Age (wk)	Cell line	Chem. tx	Min. amt	Mode of delivery	Time to mass loss	Refs.
mouse	BALB/*c-nu/nu*	F	4-5	MDA-MB-231	-	$1x10^5$ cells	inject cells into left ventricle	3-4 wk	[78, 99-101]
***mouse**	BALB/*c-nu/nu*	F	3	MDA-MB-231	-	$1x10^5$ cells	inject cells into left ventricle	40-60 d	[102]
mouse	nude	F	4	KPL-4	-	$2x10^6$ cells	inject cells into mammary fat pads	5 wk	[84]
mouse	nude	F	4	KPL-4	-	$5x10^6$ cells	inject cells into mammary fat pad(s)	3 wk	[56]
mouse	nude	F	5	MDA-MB-231	-	$1x10^5$ cells	inject cells into left ventricle	20-23 d	[85]
mouse	nude (w/ estrogen implant)		6-8	MCF-7IL-1α	-	$5x10^5$ cells	inject cells into mammary fat pads	7 wk	[43]
mouse	SCID	F	4	KPL-4	-	$1x10^7$ cells	inject cells into mammary fat pad	3 wk	[56]
rat	Sprague-Dawley	F	8	-	DMBA	25 mg/kg	gastric intubation	3 mo + 28 d	[42]
rat	Sprague-Dawley	F	8	-	DMBA	25 mg	gastric intubation	3 mo + 28 d	[41]
rat	Wistar/Furth	F, M	8-9	MT-W9B	-	0.1 ml	inject tumor mince into mammary fat pad	< 2 mo	[103]
rat	Wistar/Furth	F, M	8-9	MT-W9C	-	0.1 ml	inject tumor mince into mammary fat pad	< 2 mo	[103]
***rat**	Wistar/Furth	F	8-9	MT-W9A	-	0.1 ml	inject tumor mince into mammary fat pad	several months	[103]

* model only induced loss of body mass in some animals

Conclusion

While the ideal treatment for cachexia is tumor excision, a better understanding of the mechanisms driving BC cachexia is important to establish effective treatments for those patients whose tumors cannot be completely excised. Current research suggests that in BC, cachexia related loss of body mass is due, at least partially, to systemic changes in carbohydrate, lipid, and fat metabolism. These changes in metabolism seem to be due to a combination of factors, including high tumor energy demand and the release of cytokines from the tumor and its microenvironment.

A number of treatments (e.g., energy modulating vitamins, eicosapentaenoic acid, orally administered proteases, megestrol acetate) show promise in reducing the severity of BC cachexia. However, the limited availability of data from BC specific, randomized controlled trials prevents researchers and physicians from making well informed recommendations for the treatment of BC cachexia. In addition, it is still necessary to confirm that treatments for cachexia increase functional skeletal muscle mass rather than whole body mass.

This review summarizes the limited scientific information available on BC cachexia and highlights the need for further systemic research in the area. Of particular importance is the identification of the mechanism(s) driving BC cachexia and development of effective therapies for the prevention and reversal of cachexia in patients with BC. Success in this area will improve the quality of life and survival of patients with advanced BC.

In the next chapter, we discuss a study that was designed to determine whether the DGC, a transmembrane protein complex whose structure is altered in BC and in cachexia, plays a role in the progression of BC cachexia. It was our hope that the results of the preliminary study described in Chapter 3 of this dissertation would enhance our understanding of BC cachexia and identify novel targets for the treatment of this devastating disease.

Chapter 3: Preliminary study: Breast cancer growth and cachexia in dystrophic mice

Abstract

Alterations to the DGC, a transmembrane, multi-subunit protein complex with structural and signaling roles, have been reported in BC cells and in cachectic and dystrophic skeletal muscle cells. Despite the similar alterations reported in these diseases, it is unclear whether alterations to the DGC in one tissue can impact the progression of disease in another. ***Purpose:*** The purpose of the studies described in this chapter was to determine whether BC and cachexia are affected by systemic alteration of the DGC. ***Methods:*** Murine BC cells (E0771) were injected into *mdx* and BL/10 mice. Tumor growth and indicators of cachexia (e.g., body mass and skeletal muscle function) were measured. ***Results:*** The presence of E0771 cells did not induce cachexia in either genotype. Tumors were less likely to form and grew more slowly in *mdx* mice than in age-matched BL/10 controls. ***Conclusion:*** The dystrophic phenotype of the *mdx* mouse may be protective against BC. Further research is necessary to confirm this effect.

Introduction

Alterations to the DGC have been reported in BC, cachexia, and muscular dystrophy. A 2005 study by Acharyya et al. reported that colon cancer-related cachexia is exacerbated in *mdx* mice [12], but it is unclear whether this is also the case in BC. The purpose of our preliminary study was to determine whether systemic downregulation of the DGC can affect BC cachexia and/or the growth of mammary tumors.

To address this, we developed the following ***research questions***: (1) Are dystrophin, utrophin, EGFR, and/or HER2/neu expressed in E0771 cells/tumors? (2) What effect does the E0771 murine mammary adenocarcinoma cell line have on mammary gland and uterine mass? (3) Is BC cachexia exacerbated in *mdx* mice (i.e., in the absence of dystrophin and with systemic downregulation of components of the DGC)?, and (4) Is E0771 tumor growth altered in *mdx* mice?

Because high levels of inflammation present in *mdx* mice [19] and the relationship between chronic inflammation and cancer risk [20], we ***hypothesized*** that BC growth would be enhanced in *mdx* mice. In addition, the results of a study described by Acharyya et al. [12] led us to ***hypothesize*** that the presence of BC in *mdx* mice would be associated with lower skeletal muscle function compared to either factor alone and that tumor growth would not be affected by genotype.

Materials and methods

Study design

Skeletal muscle function and tumor growth were compared among the following groups:

1. ***MN6***: C57BL/10ScSn-*Dmd*^{mdx}/J (*mdx)* mice. The *mdx* mouse strain was selected for this study because it carries a naturally occurring X-linked [13, 14] point mutation resulting in a premature stop codon [15] for the dystrophin gene which, in turn, prevents expression of the dystrophin protein [16]. In the absence of dystrophin, protein expression of many other components of the DGC is drastically reduced [17, 18]. Use of this model allowed us to explore the effect that systemic down-regulation of the DGC may have on BC growth
2. ***MY6***: Tumor bearing *mdx* mice. E0771 cells were injected when mice were 9 wk old and tumors were allowed to grow for up to 6 wk.

3. ***10N6***: C57BL/10ScSn/J (BL/10) mice. This strain was selected because it is the parent strain for the *mdx* mouse.
4. ***10Y6***: Tumor-bearing BL/10 mice. As in MY6, E0771 cells were injected when mice were 9 wk old and tumors were allowed to grow for up to 6 wk.

Reagents

Dulbecco's Modified Eagle Medium (DMEM), newborn calf serum (CS) penicillin/streptomycin (P/S), and Trypsin-EDTA were purchased from Mediatech, Inc. (Herndon, VA). All other chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO) or Thermo Fisher Scientific, Inc. (Suwanee, GA).

Maintenance of E0771 murine mammary adenocarcinoma cells

E0771 cells (originated from a C57/BL6 mouse) were a generous gift from Enrico Mihich at the Roswell Park Cancer Institute (Buffalo, NY). This cell line was selected for the studies described in this chapter because it is an aggressive, murine breast cancer cell line that can produce tumors in BL/10 and *mdx* mice without the use of immune suppressive therapy (data not shown). E0771 cells were maintained in 100 mm tissue culture-treated plastic plates containing DMEM supplemented with 5% CS and 1 % P/S (100 U/ml). Plates were kept at 37°C in a humidified atmosphere of 5% CO_2.

Animal husbandry

BL/10 and *mdx* mice were obtained from Dr. Grange's Breeding Colony at Virginia Tech (Blacksburg, VA) and allowed to mature to 9 wk of age. This age was selected because it is early in the "chronic phase of myopathy" previously described in *mdx* mice [104].

Body mass was measured weekly using a standard electronic scale (accuracy 0.1g). Equation 1 was used to determine tumor-free body mass after euthanasia.

Equation 1

$$Tumor\ free\ body\ mass\ = body\ mass\ before\ euthanasia - mass\ of\ harvested\ tumors$$

Food intake was monitored throughout the study. For each measurement, a known mass of food was placed in each cage. After 24 hours, the amount of food remaining in each cage was determined using a standard electronic scale (accuracy 0.1 g) and the difference was divided by the number of mice in the cage.

Throughout the study, mice were group-housed under a 12-hr light/dark cycle with *ad libitum* access to water and standard rodent chow. Genotype was confirmed by a modified [105] *mdx*-amplification-resistant mutation system (ARMS) assay [106]. Mouse health and well-being and the environmental parameters of the facility were monitored daily by animal care staff. All procedures were approved by Virginia Tech's Institutional Animal Care and Use Committee (Protocol: 06-182-HNFE).

E0771 cell injection and tumor growth

On the day of cell injection (i.e., when mice reached 9 wk of age), E0771 cells were suspended in Matrigel (BD Biosciences: Bedford, MA) (5000 cells/40µl of Matrigel). Mice in tumor-bearing groups received 4 subcutaneous injections, one in each quadrant of the back, of the E0771 cell-Matrigel mix. The use of 5,000 cells per injection site was selected because previous work in our lab suggested that this number would be sufficient to produce tumors of a size and/or quality that necessitated euthanasia within approximately 24 d of cell injection (data not shown).

Tumors were allowed to grow until tumor size and/or quality necessitated euthanasia (no longer than 6 wk). Tumor surface area (TSA) was determined weekly using the formula shown in Equation 2 [107]. When the endpoint was reached, tumor-bearing mice and their tumor-free controls were anesthetized by i.p. injection of 0.2 ml of a 1:10 mixture of xylazine:ketamine (~2:20 mg/kg). When a surgical plane of anesthesia was reached, EDL muscles and blood were collected for further analysis (described below).

Following exsanguination, tumors, mammary glands and uteri were harvested and tissue mass was determined using an electronic analytical scale (accuracy 0.0001g).

Equation 2

$$Tumor\ surface\ area = (tumor\ length \div 2) \times (tumor\ width \div 2) \times \pi$$

In vitro skeletal muscle function

EDL muscles were surgically excised from anesthetized mice and secured via 4-0 suture to a dual-mode servomotor (Aurora Scientific: Aurora, Ontario) as previously described [108, 109]. Muscles were allowed to equilibrate in an oxygenated (95% O_2-5% CO_2) physiological salt solution [108] bath for 10 minutes prior to data collection.

Following the equilibration period, baseline function was determined by subjecting isolated EDLs to three twitches and two tetani, each separated by 1 minute. After a 5 minute rest, muscles were subjected to force frequency, fatigue, and recovery protocols.

During the force frequency protocol, isolated EDLs were stimulated for 800 ms at 1, 30, 50, 80, 100 and 150 Hz. Each stimulation was separated by 1 minute. After a 5 minute rest, muscles were subjected to an additional twitch and tetanus (separated by 1 minute) and rested for an additional 5 minutes before beginning the fatigue protocol.

During the fatigue protocol, EDLs were stimulated at 60 Hz for 1 second every 5 seconds for 5 minutes (total of 60 stimulations) then allowed to rest for 5 minutes. Force recovery after the completion of the fatigue protocol was determined by stimulating the muscle at 150 Hz for 800 ms every 5 minutes for 30 minutes.

For all protocols, stress output (g/mm^2) was calculated as the force output (g) for a given stimulation normalized to the estimated cross sectional area (mm^2) of the muscle. Cross sectional area of each muscle was determined by the Equation 3 [110].

Equation 3

$$Muscle\ cross\ sectional\ area\ = muscle\ mass\ in\ g\ \div (1.056 \frac{g}{mm^3} \times muscle\ length\ in\ mm)$$

Plasma collection and analysis

Blood was collected from anesthetized mice via cardiac puncture. Blood was placed in 1.5 ml microcentrifuge tubes containing 10 µl of 0.1 M sodium citrate buffer and separated by centrifugation at 500 RCF for 20 min at 4°C). The plasma portion was removed and stored at -80°C.

Plasma creatine kinase activity was measured using a Creatine Kinase (Two Part) Reagent Set (Pointe Scientific, Inc.: Canton, MI) as directed by the manufacturer.

Plasma was pooled from mice in each treatment group and cytokine levels were measured by Quansys' Multiplex Testing Service using a Q-Plex™ Mouse Cytokine/Chemokine Array (Quansys Biosicences: Logan, UT). This array was selected because it allows for affordable quantification of a wide range of cytokines/chemokines with a small volume of plasma. A 2-fold difference between treatment groups was considered significant.

Tumor cytokine analysis

One randomly selected tumor from each mouse was flash frozen in liquid nitrogen immediately post-harvest and stored at -80°C. At a later date, tumors were homogenized in ice-cold RIPA buffer. Tumor homogenates were pooled from mice in each treatment group and cytokine levels were measured using the aforementioned Q-Plex™ Mouse Cytokine/Chemokine Array.

Protein analysis

E0771 cells and randomly selected E0771 tumors were lysed in ice-cold RIPA buffer. Total protein content was determined by BCA Protein Assay Kit (Thermo Fisher Scientific, Inc.: Rockford, IL). The presence of dystrophin and utrophin (a dystrophin homologue) in E0771 cells was determined by western blot. The presence of epidermal growth factor receptor (EGFR) and HER2/neu in E0771 tumors was also determined by western blot (primary antibodies: sc-31157, sc-284, respectively; Santa Cruz Biotechnology, Inc.: Santa Cruz, CA).

Statistical analyses

Student's *t*-tests were used to determine differences in protein expression, study length, and tumor mass between genotypes. 2x2 ANOVAs (genotype x tumor presence) were used to determine differences in tissue mass, food intake, EDL morphology, final, initial and change in body mass, and EDL twitch properties. Mixed-model ANOVAs with repeated measures (group x time) were used to determine differences in weekly body mass, contractile properties, and TSA among treatment groups. A two-tailed Fisher's Exact test was used to determine differences in the tumor formation success rate between genotypes. ANOVAs were used to determine differences in creatine kinase activity and the effect of plasma on cell growth. Where appropriate, Student's *t*-tests and/or Tukey's HSD were used for post-hoc analyses. Reported values are mean ± standard error. For all analyses, $\alpha = 0.05$.

Results

Question 1: Is dystrophin, utrophin, EGFR, and/or HER2/neu expressed in E0771 cells/tumors?

Neither dystrophin (not shown) nor utrophin (Figure 6) were detectable in E0771 cells. Both EGFR and HER2/neu were present in E0771 tumors (Figure 7; not measured in E0771 cells).

Positive control	**MDA-MB-231**	**MCF-10A**	**E0771**	**SCp2**
Mouse gastrocnemius	Human BC cells	Human mammary epithelial cells	Murine BC cells	Murine mammary epithelial cells

Figure 6: Utrophin content

Utrophin expression in human and murine mammary epithelial cell and human and murine mammary adenocarcinoma lysates. Utrophin was expressed in human, but not murine, cell lines. Expression was depressed in the human mammary adenocarcinoma cell line compared to the non-tumorigenic control.

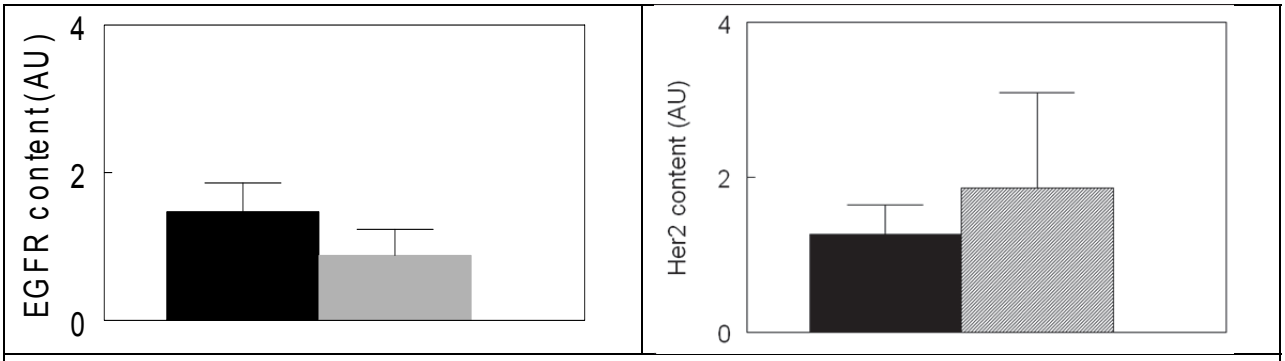

Figure 7: EGFR content

Tumor EGFR and Her2 content was not affected by genotype. (n=4-7 tumors/group).
Black solid: BL/10, Grey solid: *mdx*.

Question 2: What effect does the E0771 murine mammary adenocarcinoma cell line have on mammary gland and uterine mass?

The presence of E0771 tumors did not affect mammary gland mass in BL/10 or *mdx* mice, but was associated with increased uterine mass independent of genotype (Table 5).

Table 5: Tissues masses

	10N6	10Y6	MN6	MY6	Effects
Mammary gland (mg)	123.0±9.3	131.2±19.5	137.6±10.8	119.0±28.1	*P*=0.8567
Uterus (mg)	97.8±15.3	45.9±4.6	97.6±12.7	72.0±2.3	Tumor, *P*=0.0052 (-T > +T)
Heart (mg)	102.4±3.3	92.0±3.7	119.6±3.2†	129.1±7.3*†	Interaction, P=0.0481
Soleus (2) (mg)	13.2±0.4	14.2±0.8	24.0±0.8	23.5±1.7	Genotype, P<0.0001 (mdx > BL/10)
*** different from 10N6, † different from 10Y6. (n = 4-11 mice/group)**					

Question 3: Is BC cachexia exacerbated in *mdx* mice?

E0771 cells did not induce cachexia in BL/10 or *mdx* mice. After 6 wk of E0771 tumor growth, there was no difference in food intake (Table 6), heart mass, soleus mass (Table 5), EDL mass (Table 7), or tumor-free body mass (Table 8 and Figure 8) between tumor-free (i.e., 10N6 and MN6) and tumor-bearing (i.e., 10Y6 and MY6) mice. In addition, the twitch properties (Table 9), stress-frequency profiles (Figure 9A), and fatigue profiles (Figure 9B) of EDL muscles were not affected by the presence of BC in either BL/10 or *mdx* mice. During recovery from fatigue, EDL stress output was greater in MN6 compared to MY6 at 15-20 minutes post-fatigue, but this difference was lost by 25 minutes post-fatigue (Figure 9C). The presence of BC had no effect on the force recovery of BL/10 EDLs after a fatiguing stimulus (Figure 9C).

Table 6: Food intake

	10N6	10Y6	MN6	MY6	Effects
24 hr food intake (3 wk) (g)	3.3±0.2	3.3±0.1	4.2±0.2	4.4±0.2	Genotype, P<0.0001 (mdx > BL/10)
24 hr food intake (6 wk) (g)	3.3±0.4	3.5±0.4	3.3±0.3	5.5±1.2	P=0.1782
(n=3-10 mice/group)					

Table 7: EDL morphology

	10N6	10Y6	MN6	MY6	Effects
Length (mm)	11.2±0.3	10.9±0.2	13.4±0.2	13.2±0.3	Genotype, P<0.0001 (mdx > BL/10)
Mass (mg)	9.4±0.3	8.1±0.3	13.5±0.3	13.2±0.8	Genotype, P<0.0001 (mdx > BL/10)
Cross-sectional area (mm2)	0.78±0.02	0.70±0.02	0.95±0.02*†	1.01±0.04*†	Interaction, P=0.0059
*** different from 10N6, † different from 10Y6. (n = 12-20 muscles)**					

Table 8: Body mass					
	10N6	10Y6	MN6	MY6	Effects
Initial body mass (g)	18.5±0.6	18.8±0.6	24.0±0.6	24.8±0.4	Genotype, P<0.0001 (mdx > BL10)
Final body mass (g)	20.7±0.5	21.9±0.6	27.9±0.5	31.2±0.9	Genotype, P<0.0001 (mdx > BL10) Tumor, P=0.0014 (+T > -T)
Final tumor-free body mass (g)	20.7±0.5	19.7±0.6	27.9±0.5*†	30.3±1.0*†	Interaction, P=0.0189
Change in TF body mass (g)	+2.2±0.1	+1.0±0.4	+3.9±0.3†	+5.6±0.9*†	Interaction, P=0.0129 (Figure 8B)
*** different from 10N6, † different from 10Y6. (n=8-11 mice/group)**					

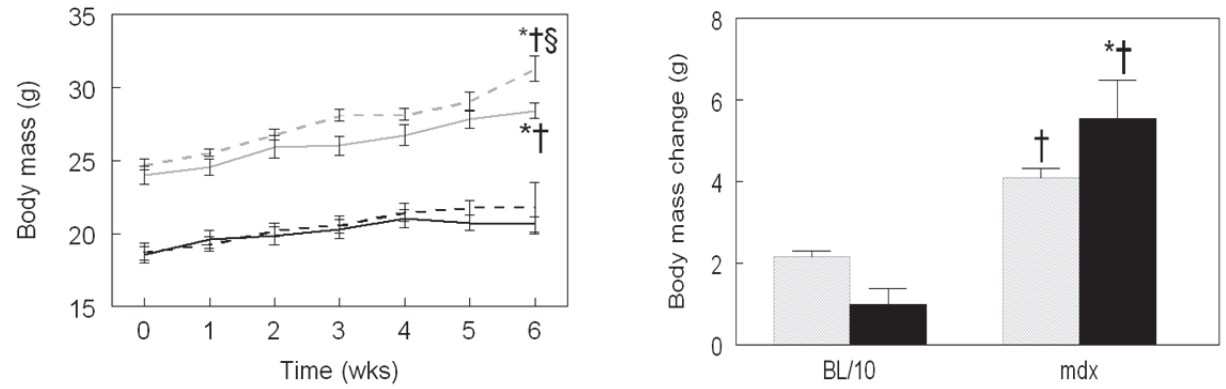

Figure 8: Body mass

(A) The body mass of *mdx* mice was greater than that of BL/10 mice at all time points. At the 6 wk time point, the body mass of MY6 was greater than that of MN6. Black solid: 10N6, Black striped: 10Y6, Grey solid: MN6, Grey striped: MY6. (n=2-11 mice/group) **(B)** The presence of BC was associated with a greater body mass gain in *mdx* mice. Change in body mass was not affected by presence of BC in BL/10 mice. Black solid: with BC, Grey solid: without tumor. (n=8-11 mice/group). * different from 10N6, † different from 10Y6, § different from MN6.

Table 9: EDL twitch properties					
	10N6	10Y6	MN6	MY6	Effects
Time to peak tension (ms)	13.3±0.5	12.9±0.5	12.4±0.4	12.2±0.5	
Twitch force (g)	5.0±0.2	4.3±0.5	4.2±0.1	4.6±0.2	
Twitch stress (g/mm2)	6.7±0.4	5.7±0.6	4.2±0.2	4.7±0.4	Genotype, P=0.0002 (BL/10 > mdx)
Half-relaxation time (ms)	15.9±1.1	13.8±1.3	12.8±0.8	11.9±1.3	
*** different from 10N6, † different from 10Y6. (n = 12-20 muscles)**					

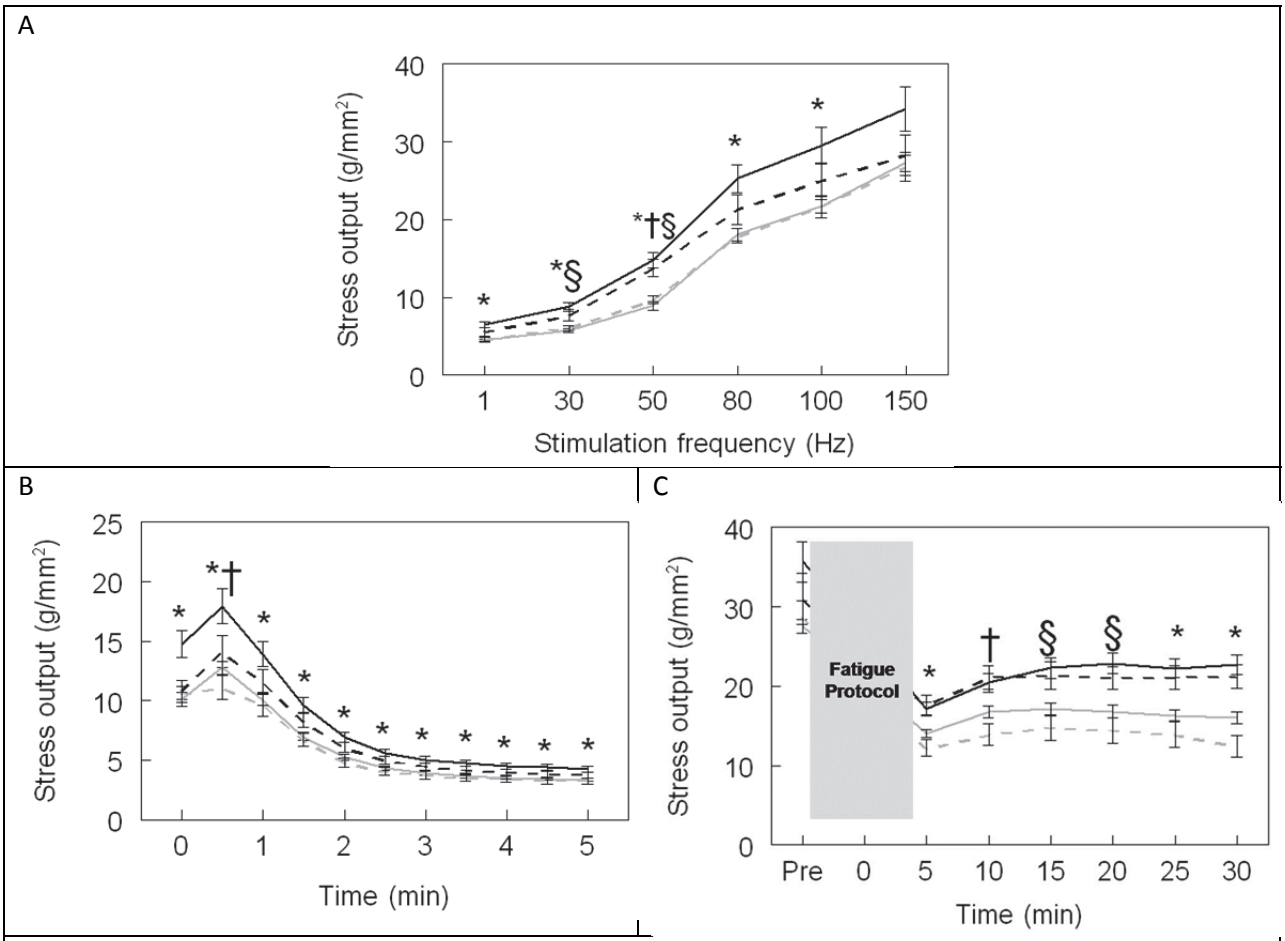

Figure 9: EDL contractile properties

(A) EDL stress frequency curve. An interaction between treatment group and stimulation frequency was observed. At stimulation frequencies of 1-100 Hz, MN6 and MY6 EDLs produced less stress than those from 10N6. There was no difference among groups at 150 Hz.* MN6 and MY6 different from 10N6, † MN6 different from 10Y6, § MY6 different from 10Y6. **(B) EDL fatigue profile.** An interaction between treatment group and time was observed. * MN6 and MY6 different from 10N6, † 10Y6 different from 10N6. **(C) EDL recovery profile.** An interaction between treatment group and time was observed. * MN6 and MY6 different from 10N6 and 10Y6, † MN6 and MY6 different from 10Y6 and MY6 different from 10N6, § MN6 different from 10N6 and MY6 different from 10N6 and 10Y6. (n=11-18 muscles/group) Black solid: 10N6, Black striped: 10Y6, Grey solid: MN6, Grey striped: MY6.

Question 4: Is tumor growth altered in *mdx* mice?

Interestingly, *mdx* mice seem to be somewhat protected from BC. At most time points, fewer injection sites on *mdx* mice developed tumors than those on BL/10 mice (Figure 10A). In addition, excessive tumor burden and/or tumor ulceration necessitated earlier euthanasia of tumor-bearing BL/10 mice compared to tumor-bearing *mdx* mice (Figure 10B).

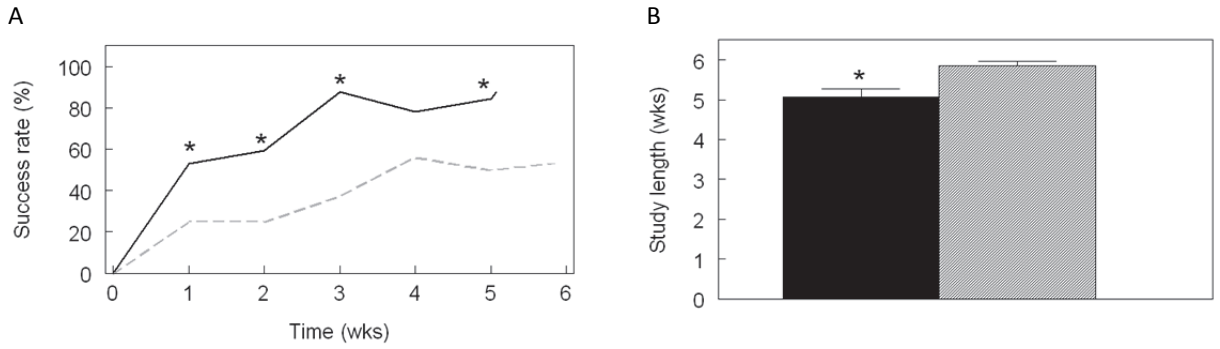

Figure 10: Tumor formation success rate and time to euthanasia

(A) The development of tumors was less frequent at injection sites on mdx mice than at injection sites on BL/10 mice at most time points. (n=8-36 tumors/genotype). (B) Because of excessive tumor burden and/or tumor ulceration, tumor bearing BL/10 mice needed to be euthanized earlier than tumor bearing mdx mice. (n=8 mice/genotype.

Black: BL/10, Grey: *mdx*, * different from mdx.

Tumor size was also affected by genotype. TSA was smaller in *mdx* mice at most time points (Figure 11A), but this effect was lost when injection sites that failed to form tumors were removed from the calculations (Figure 11B). There was no difference in tumor mass per animal between genotypes (Figure 11C), but when tumor mass was corrected for body mass, there was a trend for tumor mass to be smaller in *mdx* mice (Figure 11D).

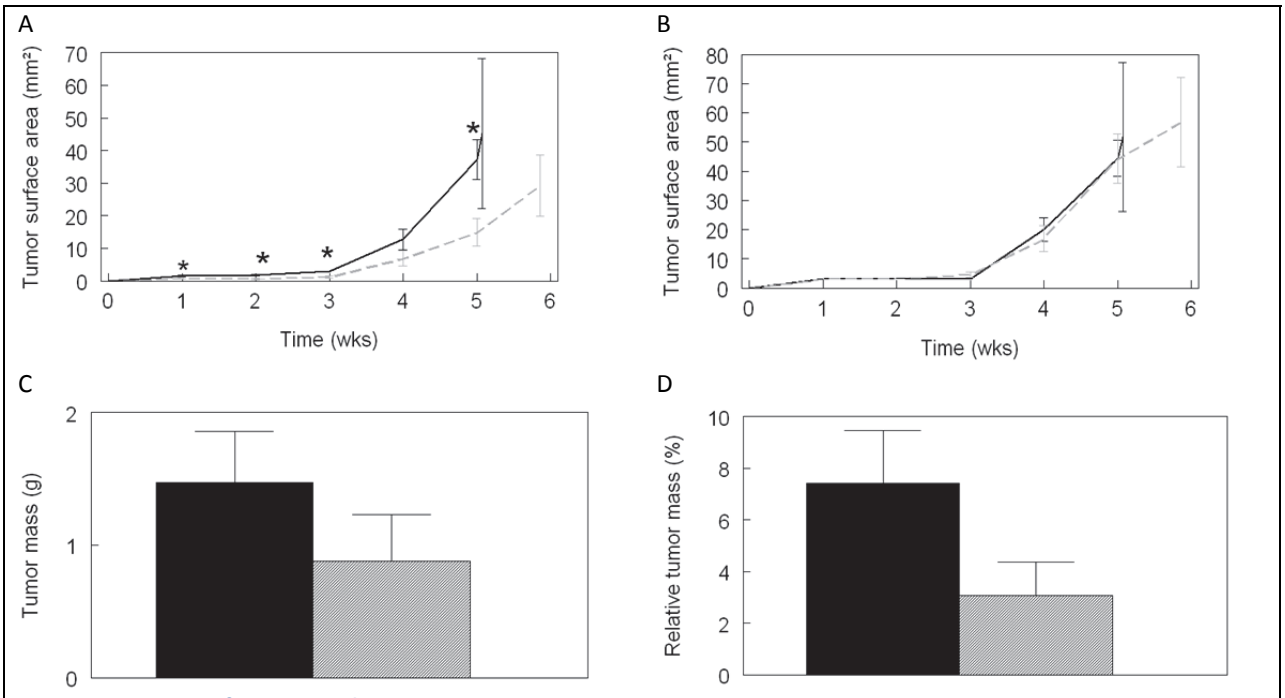

Figure 11: Tumor surface area and mass

(A) An interaction between time and genotype on TSA was observed. Post-hoc analyses suggest that TSA is greater in BL/10 mice compared to mdx mice at weeks 1, 2, 3 and 5. (n=8-36 tumors/genotype). (B) When injection sites that failed to form tumors were removed from calculation, there was no difference in average TSA between genotypes. (n=7-36 tumors/genotype). (C) After 6 weeks of tumor growth, there was no difference in tumor mass between BL/10 and mdx mice. (n=7-9 mice/group). (D) When expressed as a per cent of body mass, there was a trend for tumor mass to be less in mdx compared to BL/10 mice. (n=7-9 mice/group).
Black solid: BL/10, Grey Solid: *mdx* * different from BL/10.

At the molecular level, there was no difference in the amount of IFN-γ, IL-1β, IL-5, IL-6, IL-12p70, IL-17, TNF-α, CCL2, or CCL5 in tumors from C57BL/6J mice (BL/6; syngenic with E0771 cell line) and *mdx* mice after 3 wk of tumor growth (Figure 12A) or in the amount of IFN-γ, IL-1β, IL-6, CCL2, or CCL5 in tumors from BL/10 and *mdx* mice after 6 wk of tumor growth (Figure 12B). Content of IFN-γ, IL-6, CCL2, and CCL5 was greater in tumors collected from *mdx* mice after 3 wk of growth compared to those collected from *mdx* mice after 6 wk of growth (Figure 12C).

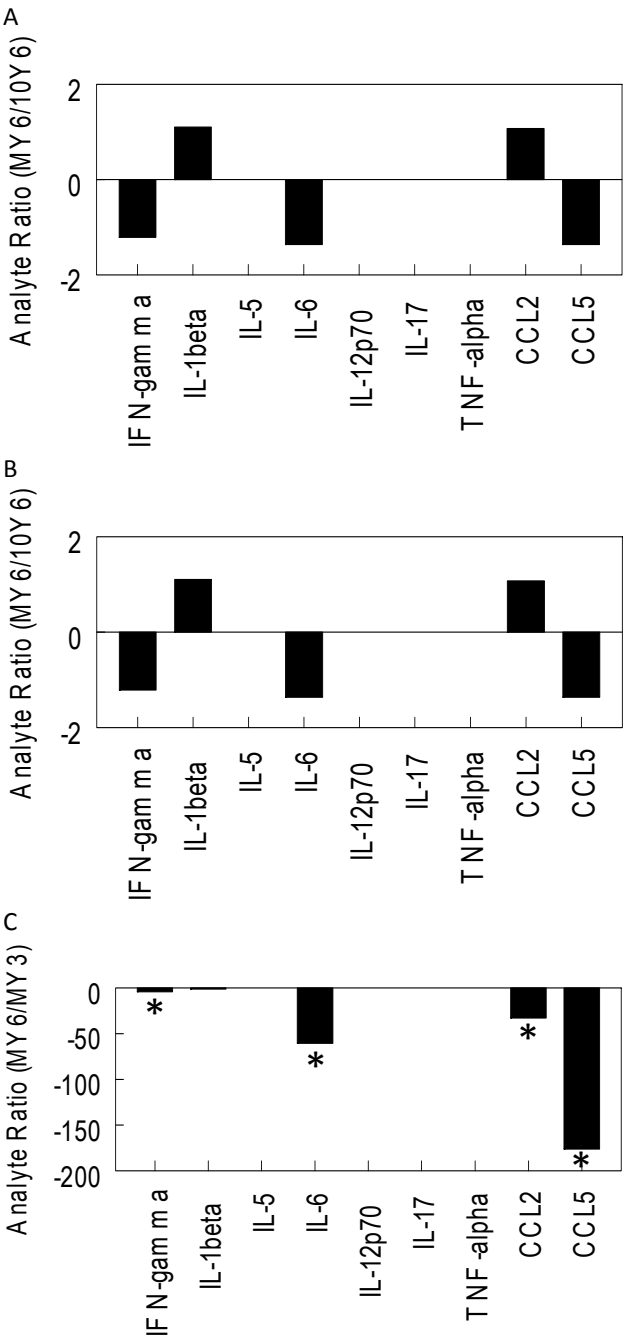

Figure 12: Cytokine profiles

Ratios of select cytokines in pooled (n=5 mice/group) tumor homogenates. (A) There was no difference in cytokine plasma content between MY3 and 6Y3. (B) There was no difference in cytokine plasma content between MY6 and 10Y6. (C) Plasma content of IFN-γ, IL-6, CCL2, and CCL5 decreased over time in tumor-bearing mdx mice. Plasma content of IL-1β did not change over time. * greater than 2-fold difference.

To test the hypothesis that circulating factors may be responsible for the blunting of tumor growth observed in *mdx* mice, we treated E0771 cells with plasma collected from mice in each treatment group. In this study, we found that that treatment with plasma pooled from *mdx* mice did not affect E0771 cell proliferation compared to plasma pooled from BL/10 mice (Figure 13).

We then analyzed the plasma of all treatment groups for a number of factors in an attempt to identify differences in the plasma of BL/10 and *mdx* mice. As reported by others [13], at the 3-wk time point, creatine kinase activity was greater in the plasma of *mdx* mice compared to that of BL/6 mice (BL/6 data for 6-wk time point not available). Plasma creatine kinase activity was not affected by the presence of E0771 tumors in BL/6 or *mdx* mice (Figure 14). Plasma IL-6 and CCL2 content were also elevated in *mdx* mice compared to BL/6 controls (Figure 15B; BL/10 data not available). The presence of E0771

tumors was associated with elevated plasma IL-6, IL-12p70, and CCL2 in both BL/6 and *mdx* mice (Figure 15A, C). Tumor presence was associated with decreased plasma IL-5 and increased CCL5 in BL/6 mice only (Figure 15A).

At the 6-wk time point, IL-6 content was greater in the plasma of *mdx* mice compared to that of BL/10 mice (Figure 16B). The presence of tumors was associated with increased plasma IL-6 and CCL2 content in both BL/10 and *mdx* mice (Figure 16A, C), and increased IFN-γ and CCL5 in *mdx* mice only (Figure 16C).

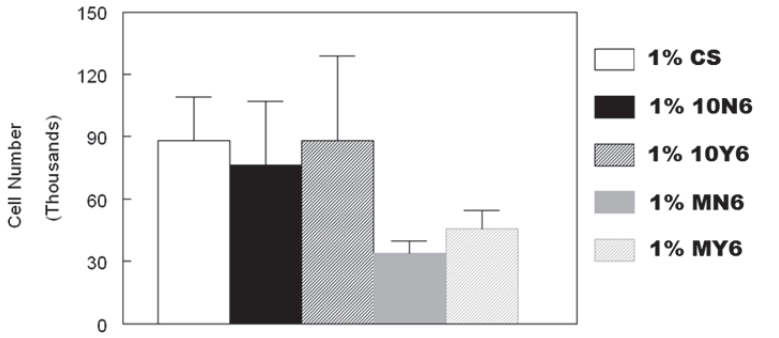

Figure 13: Effect of plasma on cell growth
E0771 cells were treated with pooled plasma (1% in DMEM) from each treatment group. After 48 hrs of treatment, there was no difference in cell number among groups. (n=5 replicates; P=0.4337)

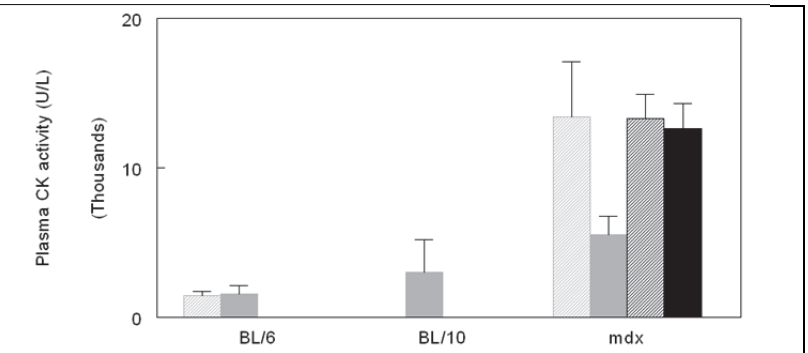

Figure 14: Plasma creatine kinase activity
At the 3 wk time point, plasma creatine kinase was greater in mdx mice than in BL/6 mice. Plasma creatine kinase activity was not affected by the presence of BC in any genotype or at any time point. (n=0-8 mice/genotype)
Striped: w/o BC, Solid: w/ BC, Grey: 3 wk Black: 6 wk

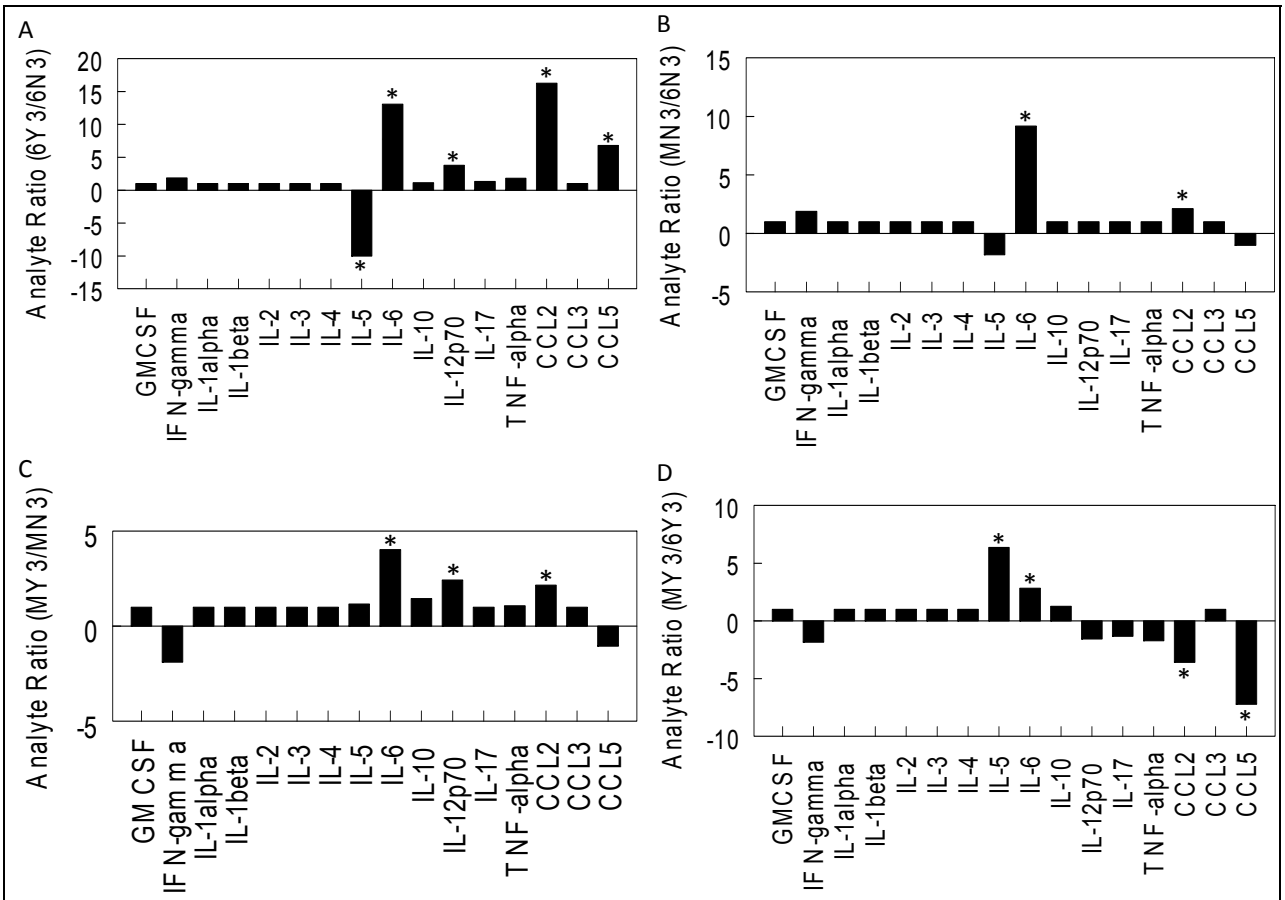

Figure 15: Cytokine profiles

Ratios of select cytokines in pooled (n=5 mice/group) plasma samples. (A) IL-5 content was greater, and IL-6, IL-12p70, CCL2, and CCL5 content was lower in 6Y3 plasma compared to 6N3 plasma. (B) Plasma IL-6 and CCL2 content was greater in MN3 plasma compared to 6N3. (C) Plasma IL-6, IL-12p70, and CCL2 content was greater in MY3 compared to MN3. (D) IL-5 and IL-6 content was greater while CCL2 and CCL5 content was lower in MY3 plasma compared to 6Y3 plasma. * greater than 2-fold difference.

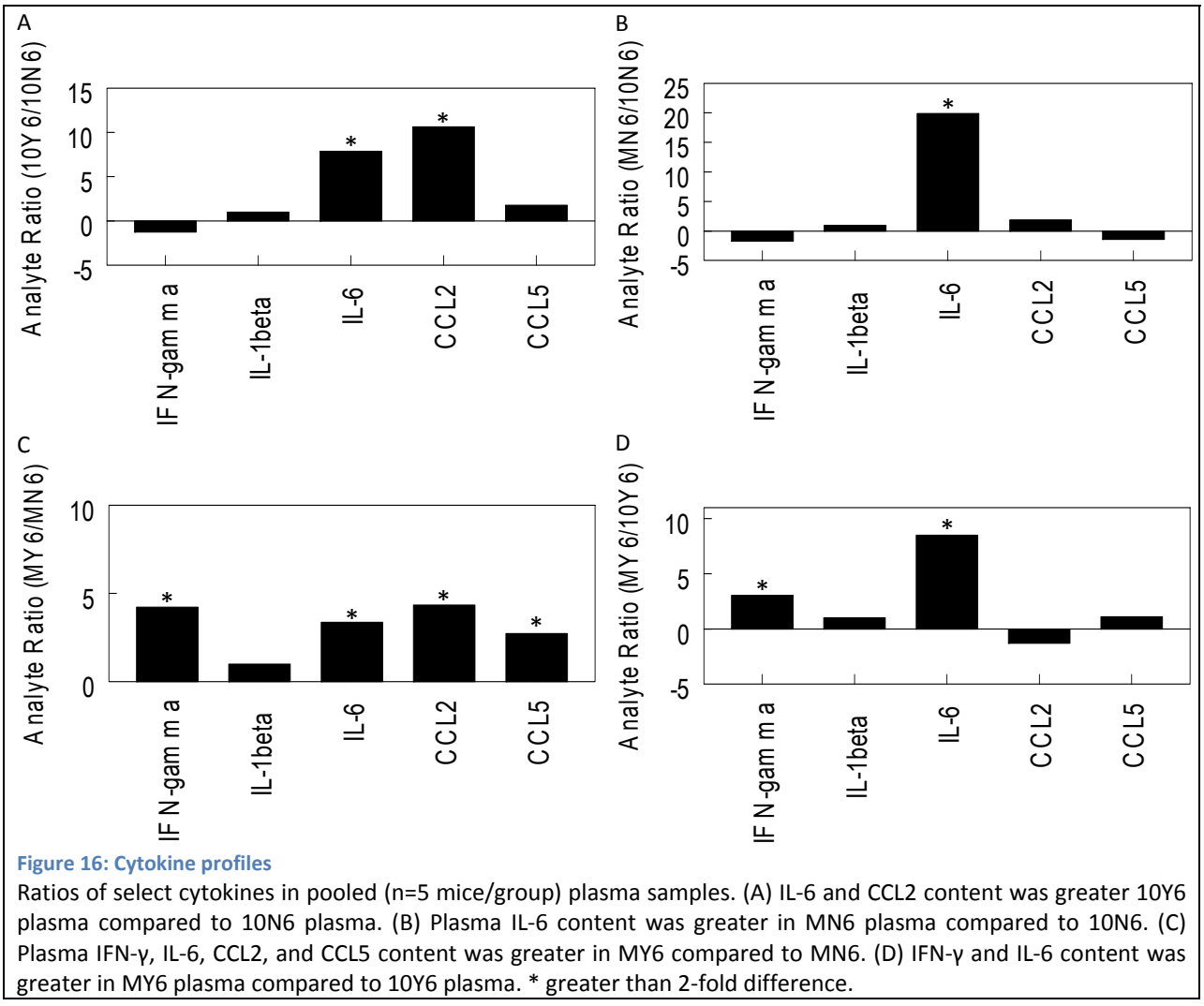

Figure 16: Cytokine profiles

Ratios of select cytokines in pooled (n=5 mice/group) plasma samples. (A) IL-6 and CCL2 content was greater 10Y6 plasma compared to 10N6 plasma. (B) Plasma IL-6 content was greater in MN6 plasma compared to 10N6. (C) Plasma IFN-γ, IL-6, CCL2, and CCL5 content was greater in MY6 compared to MN6. (D) IFN-γ and IL-6 content was greater in MY6 plasma compared to 10Y6 plasma. * greater than 2-fold difference.

Discussion

We hypothesized that BC growth and cachexia would be enhanced with systemic alteration of the DGC (i.e., in *mdx* mice). To determine whether BC growth is affected by alteration of the DGC, we compared the growth of E0771 murine mammary tumors in *mdx* and BL/10 control mice. To determine whether cachexia is affected by alteration of the DGC, we compared the body mass and skeletal muscle function of tumor-bearing *mdx* and BL/10 mice.

In this study, we found that the presence of the E0771 murine mammary tumor has no lasting effect markers of cachexia (e.g., body mass, skeletal muscle function). Therefore, we were unable to confirm our hypothesis that the dystrophic phenotype of the *mdx* mouse exacerbates cachexia.

In contrast to our second hypothesis, we found that the phenotype of the *mdx* mouse may prevent the establishment and growth of BC. However, large within-group variation prevented us from making a definitive conclusion on whether there were differences between groups. This variation could be due to several factors, including genetic differences between the E0771 cell line and the BL/10 and *mdx* host mice and the length of the research period. With respect to the latter factor, BC cells were injected into mice between 9/7/2007 and 3/3/2009 - this occurred for several reasons, including, but not limited to:

1. limited availability of mice of all genotypes at any given time (rather than purchasing a large group of mice at once, mice were used as they were produced by the breeding colony).
2. difficulty in breeding the BL/10 mouse strain (the vendor considers this strain of mice to be fair breeders [111]).
3. an error in cage labeling that resulted in breeder pairs of *mdx*-α7B1 integrin heterozygotes and their offspring to be labeled and used as the BL/10 strain for a period of approximately 8 months.

Whether the phenotype of the *mdx* mouse is protective against BC will be explored further in Chapter 5 of this dissertation. In the next chapter (i.e., Chapter 4), we will further our understanding of the *mdx* mouse by comparing the body composition, energy expenditure, and plasma cytokine profile of 5- to 13-wk-old *mdx* mice to that of age-matched BL/10 control mice.

Chapter 4: Comparison of body composition, energy expenditure, and plasma cytokine content between *mdx* and BL/10 mice

Abstract

DMD is a severe progressive muscle wasting disease that occurs in 1-3 out of every 10,000 male births. The *mdx* mouse is the most commonly used model of this disease. However, the phenotype of the *mdx* mouse is not as severe as that of patients with DMD and thus the usefulness of this model may be limited in some applications. ***Purpose***: The purpose of this study was to compare the body composition, energy expenditure, and cytokine profile of two cohorts (age 5 to 9 wk and 6 to 13 wk) of *mdx* mice to age-matched control mice. ***Results***: In contrast to the decreased muscle mass typically reported in patients with DMD, *mdx* mice in this study had a greater % lean mass and a lower % fat mass. In addition, total energy expenditure (TEE) was not different between *mdx* mice and control mice at the ages tested. Differences in plasma cytokine content were observed in *mdx* mice in both cohorts. ***Conclusion***: Our findings provide support for the theory that the immune system plays a large role in the progression of DMD and suggest that careful consideration of the objective of a study is necessary when determining the appropriateness of the *mdx* mouse as a model of DMD.

Introduction

DMD is an X-linked progressive muscle wasting disease that occurs in 1-3 out of every 10,000 male births [112-114]. Patients with DMD typically develop skeletal muscle weakness and are diagnosed at 2-7 years of age [115-118], are wheelchair bound at 7-13 years of age [115-119], and experience cardiac and/or respiratory complications [115, 116, 118-120] leading to death by 17-30 years of age [115, 117, 118, 121]. While available treatments (e.g., corticosteroids) do prolong the life of patients with DMD, none are able to prevent or reverse the progression of this disease [122].

DMD is caused by inherited or spontaneous genetic mutations to the Xp21.2 locus [123], which codes for dystrophin, a cytoskeletal protein that connects γ-actin to the DGC in the sarcolemma [122]. These mutations prevent the expression of dystrophin [16], resulting in increased sarcolemmal permeability [118, 122], immune cell infiltration [118], and repeated cycles of muscle degeneration and regeneration [118]. These changes are accompanied by whole body alterations in body composition (e.g., increased % fat mass and decreased % lean mass) [124, 125]. Treatment of DMD is typically aimed at preventing or reversing the loss of skeletal muscle mass; however, there is little information available regarding the body composition of the *mdx* mouse [126], a model of DMD frequently used when developing new treatments.

Adequate nutritional management is important for maintaining the health of patients with DMD. Two factors that should be considered when making dietary recommendations are TEE and respiratory exchange ratio (RER). TEE can be used to determine caloric need and is the sum of four components: basal metabolic rate, the thermic effect of food, non-exercise thermogenesis, and exercise thermogenesis [127]. RER is defined as the ratio of volume of carbon dioxide produced to the volume of oxygen consumed and can be used to determine substrate utilization [128]. Knowledge of these values specific to patients with DMD can be used by dietitians to make appropriate recommendations for calorie, carbohydrate, and fat intake. These factors are poorly understood in patients with DMD. [125]

The immune system plays a prominent role in the initiation and progression of the dystrophic phenotype both in patients with DMD and in the *mdx* mouse model of muscular dystrophy. The infiltration of immune cells (e.g., macrophages, eosinophils) occurs prior to disease onset [13, 19] and continues throughout the course of the disease [13, 19]. Cytokines are small proteins that act as intracellular mediators of immunity by affecting the activation and chemotaxis of immune cells [129]. A number of cytokine elevations have been reported in skeletal muscles of patients with DMD and in *mdx*

mice (see Evans et al., 2009 for an extensive review) [19] but information regarding the content of cytokines in the systemic circulation (e.g., plasma) is limited. A pilot study performed in our lab (Chapter 3), revealed plasma cytokine differences between 12- to 15-wk-old *mdx* mice and age-matched controls (i.e., during the chronic phase of the dystrophic phenotype). Whether these differences are present during the acute phase of the dystrophic phenotype remains unclear and is the basis for the study described in this chapter.

Enhanced knowledge of the circulating cytokine profile in patients with DMD and an understanding of how this profile reflects skeletal muscle damage could allow researchers and physicians to establish non-invasive markers of skeletal muscle damage and immune cell infiltration. These markers could, in turn, be used to determine the effectiveness of pharmaceuticals, dietary compounds, and physical therapies as treatments for DMD.

In this chapter we will address these deficiencies in the current understanding of the *mdx* mouse model of DMD. We will report alterations in body composition and energy expenditure, as well as identify shifts in the plasma cytokine profile of *mdx* mice during the acute and chronic phases of muscular dystrophy. Based on a review of the literature and the results of our preliminary study, we ***hypothesize*** that there will be phenotypic differences between *mdx* mice and age-matched BL/10 control mice and that these differences will vary with age (i.e., the severity of the dystrophic phenotype). In addition, we ***hypothesize*** that pattern of these differences will be similar to, but not as severe as those observed between patients with DMD and unaffected controls.

Methods

Animal husbandry

Four-wk-old female C57BL/10ScSn-Dmd^{mdx}/J mice (*mdx*; Stock number: 001801; homozygous for Dmd^{mdx}) and C57BL/10ScSn/J control mice (BL/10; Stock number: 000476) were purchased from The Jackson Laboratory (Bar Harbor, ME). Female *mdx* mice were used in this study to allow the authors to use one set of mice to address the hypothesis described in this chapter, as well as those described in the next (i.e., to reduce the number of mice used for this dissertation). Female *mdx* mice are homozygous for the *mdx* mutation of gene for dystrophin [130] and exhibit a similar pathology to male *mdx* mice [13, 131]. Due to limited availability, mice were purchased in two sets (i.e., $SET_{5\text{-}9}$ and $SET_{6\text{-}13}$; Table 10). To avoid the effect of variation between sets, the data for each set were analyzed separately unless otherwise indicated.

Table 10: Treatment groups

			Mouse age (wk)	
Group	n	Genotype	Initial	End
$SET_{5\text{-}9}$				
$CON_{5\text{-}9}$	9	BL/10	5	9
$MDX_{5\text{-}9}$	10	*mdx*	5	9
$SET_{6\text{-}13}$				
$CON_{6\text{-}13}$	7	BL/10	6	13
$MDX_{6\text{-}13}$	7	*mdx*	6	13

Upon receipt, mice were allowed to acclimate to our facility for 1 to 2 wk. Experimental procedures were performed on $SET_{5\text{-}9}$ when mice were between 5 and 9 wk old (Figure 17). This age range was selected because it falls within the "acute phase of myopathy" previously described in *mdx* mice [104]. Experimental procedures were performed on $SET_{6\text{-}13}$ when mice were between 6 and 13 wk old. This age range was selected because it allowed for comparison between sets while extending in to the "chronic phase of myopathy" previously described in *mdx* mice [104].

Throughout the study, mice were pair-housed under a 12-hr light/dark cycle with *ad libitum* access to water and standard rodent chow. Genotype was confirmed by a modified [105] *mdx*-ARMS assay [106]. Mouse health and well-being and the environmental parameters [i.e., temperature (accuracy 1°C) and

relative humidity (accuracy 1%)] of the facility were monitored daily by animal care staff. All procedures were approved by Virginia Tech's Institutional Animal Care and Use Committee (Protocol: 07-142-HNFE).

Food intake

Food intake was measured at 6, 7, and 8 wk of age for mice in SET_{5-9} and at 11 and 13 wk of age for mice in SET_{6-13}. For each measurement, a known mass of food was placed in each cage. After 24 hours, the mass of the food remaining in each cage was determined using a standard electronic scale (accuracy 0.1 g) and the difference was divided by the number of mice in the cage.

Morphological characteristics

Body mass and composition were measured weekly beginning at 5 wk of age for mice in SET_{5-9} and at 6 wk of age for mice in SET_{6-13}. Body mass was measured using a standard electronic scale (accuracy 0.1g). Body composition (i.e., fat mass, lean mass, and fluid mass) was measured using a Minispec LF-90 TD-NMF (Bruker Optics: Billerica, MA) as recommended by the manufacturer.

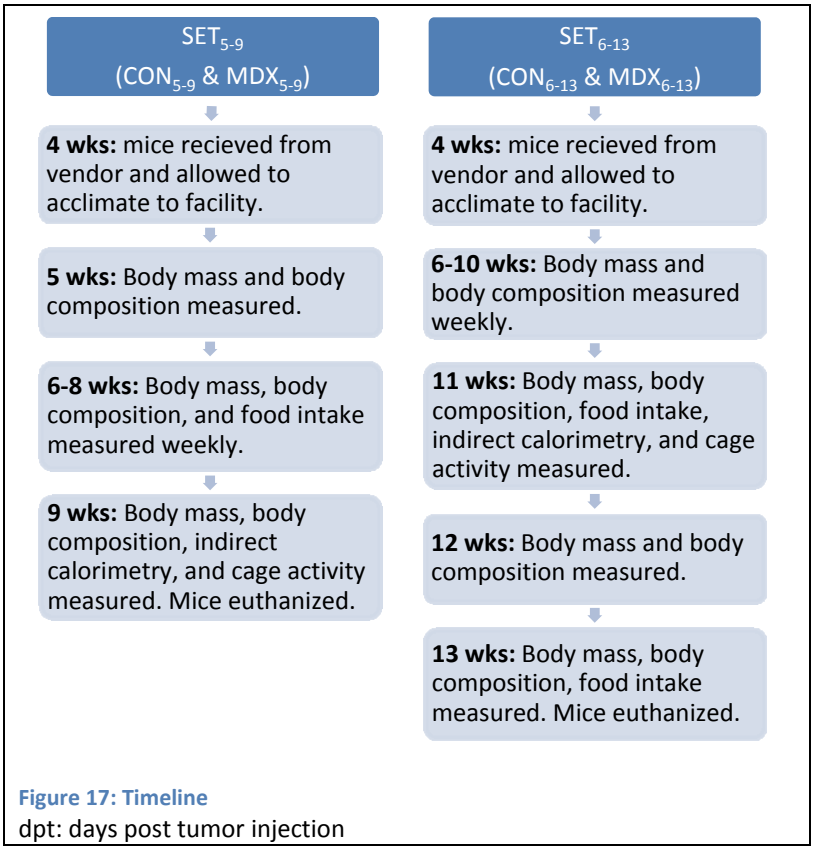

Figure 17: Timeline
dpt: days post tumor injection

Indirect calorimetry and cage activity

Ventilated gas exchange (i.e., O_2 and CO_2) and spontaneous cage activity were determined in 5 randomly selected mice per treatment group. O_2 consumption and CO_2 production were measured on single-housed mice by open circuit calorimetry and used to determine RER (Equation 4) and TEE (Equation 5). An infrared light-beam frame was used to determine spontaneous cage activity in the horizontal (i.e., X and Y) and vertical (i.e., Z) directions. Activity was determined by counting the number of novel interruptions of infrared light beams (i.e., beam breaks) [132]. Measurements were made at 9 wk of age in $SET_{5\text{-}9}$ and at 11 wk of age in $SET_{6\text{-}13}$ by the Virginia Tech Integrated Life Sciences Building Phenotyping Core (Blacksburg, VA) using a PhenoMaster/LabMaster System (TSE Systems: Chesterfield, MO).

Equation 4

$$RER = volume\ of\ CO_2\ produced \div volume\ of\ O_2\ consumed$$

Equation 5

$$TEE = \left(\left(volume\ of\ O_2\ consumed \times \left(3.815 + \left(1.232 \times RER\right)\right)\right) \times 4.1868\right) \div lean\ body\ mass$$

Tissue collection

At 9 wk of age, mice in $SET_{5\text{-}9}$ were anesthetized by inhaled isoflurane and exsanguinated via cardiac puncture. Blood was collected and placed in 1.5 ml microcentrifuge tubes containing 10 µl of 0.1 M sodium citrate buffer and separated by centrifugation at 500 RCF for 20 min at 4°C. The plasma portion was removed and stored at -80°C.

At 13 wk of age, mice in $SET_{6\text{-}13}$ were anesthetized by inhaled CO_2 and exsanguinated via cardiac puncture. Blood was collected and processed as described above.

Immune profile

Cytokine levels were measured in plasma pooled from mice in each treatment group by Quansys' Multiplex Testing Service using a Q-Plex™ Mouse Cytokine/Chemokine Array (21-plex). This array was selected because it provides highly sensitive quantification (i.e., pg/ml) of 21 cytokines from a small volume of plasma (i.e., 30 µl). A 2-fold difference between treatment groups was considered significant.

Statistical analyses

Student's *t*-tests were used to determine differences between sets (i.e., $SET_{5\text{-}9}$ and $SET_{6\text{-}13}$) for temperature and RH. Mixed-model ANOVAs with repeated measures (genotype x age) were used to determine differences in food intake, body mass, and body composition between $CON_{5\text{-}9}$ and $MDX_{5\text{-}9}$ and between $CON_{6\text{-}13}$ and $MDX_{6\text{-}13}$. A 2x2 ANOVA (genotype x set) was used to determine differences in ADG (average daily mass gain). 2x2 ANOVAs (genotype x phase) were also used to determine differences in TEE, RER, and cage activity. Where appropriate, Student's *t*-tests and/or Tukey's HSD were used for post-hoc analyses. Reported values are mean ± standard error. For all analyses, $\alpha = 0.05$.

Table 11: Environmental parameters

	$SET_{5\text{-}9}$	$SET_{6\text{-}13}$
Temperature (°C)		
AM	21±0	21±0
PM	21±0	21±0
AVG	21±0	21±0
Range	21-22	19-21
Relative Humidity (%)		
AM	42±1	30±0
PM	43±1	30±1
AVG	42±1	30±0
Range	34-51	23-41

Acceptable temperature range is 18-26°C. Acceptable RH range is 30-70%.Values are mean±SEM.

Results

Environmental parameters

There were no differences in the morning, afternoon, and daily average temperatures during the period in which the mice in $SET_{5\text{-}9}$ and $SET_{6\text{-}13}$ were housed in our facility (Table 11). The morning, afternoon, and daily average RH values during the period in which the mice in $SET_{6\text{-}13}$ were housed in our facility were lower compared to the period during which the mice in $SET_{5\text{-}9}$ were housed ($P<0.0001$ for all measurements; Table 11).

Food intake

The 24-hr food intake of SET_{5-9} was not affected by genotype (P=0.8995; not shown), age (P=0.0635), or an interaction of the two (P=0.8589). In SET_{6-13}, 24-hr food intake was affected by an interaction of genotype and age (P=0.0003). Post-hoc analyses revealed that the 24-hr food intake of MDX_{6-13} was greater than that of CON_{6-13} at 13 (5.18±0.26 g v. 3.93±0.03 g; P=0.0171), but not 11 wk of age (P=0.1393).

Morphological characteristics

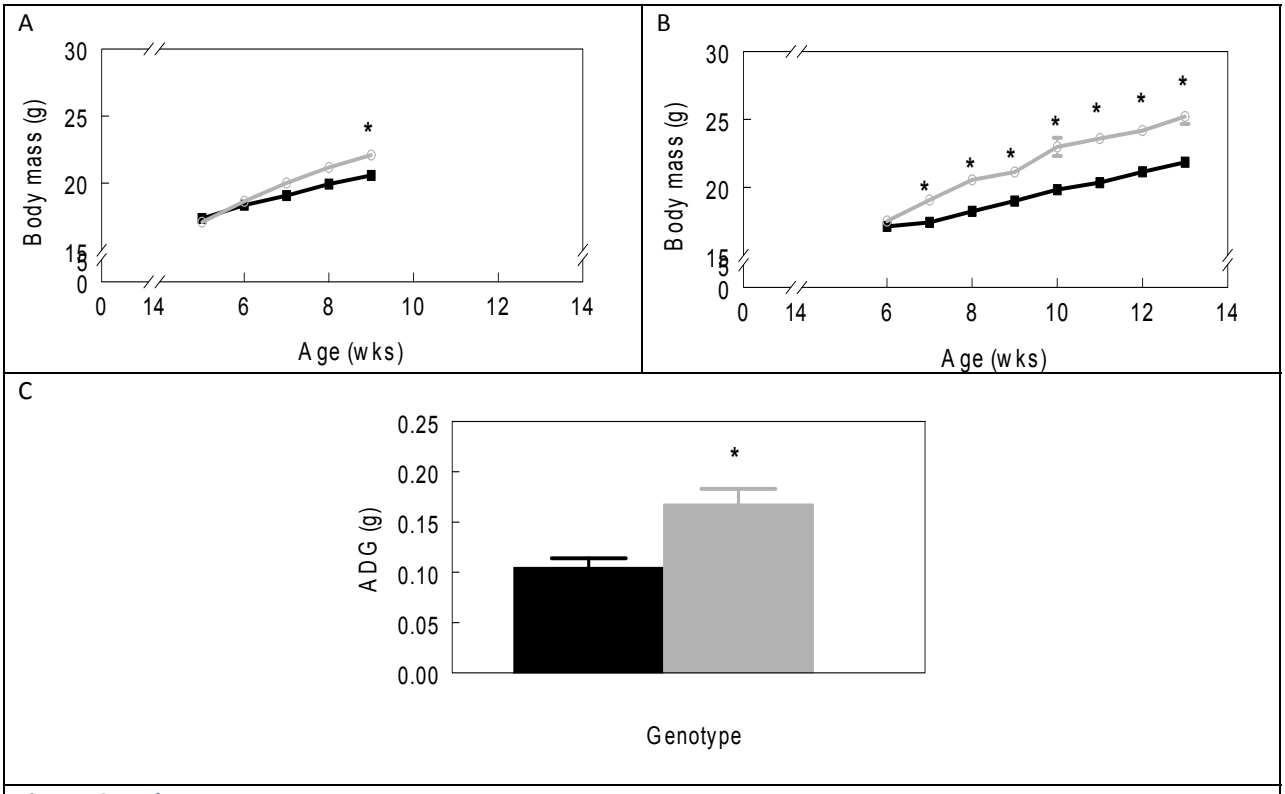

Figure 18: Body mass
(A) The body mass of MDX_{5-9} was greater than CON_{5-9} at 9 wk of age. (B) The body mass of MDX_{6-13} was greater than CON_{6-13} at 7 to 13 wk of age. (C) The ADG from 6 to 9 wk of age was greater in *mdx* mice than in BL/10 mice. Black solid: CON, gray solid: MDX, *genotype difference (P<0.05).

An interaction effect (genotype x age) on body mass was observed in SET_{5-9} (P=0.0044; Figure 18A) and SET_{6-13} (P=0.0025; Figure 18B). Post-hoc analyses revealed that in SET_{5-9}, the body mass of MDX_{5-9} was greater than that of CON_{5-9} at 9 wk only (P=0.0295; Figure 18A). Post-hoc analyses revealed that in SET_{6-}

$_{13}$, the body mass of MDX_{6-13} was greater than that of CON_{6-13} when mice were between 7 and 13 wk old (P<0.05; Figure 18B).

The ADG from 6 to 9 wk of age was greater in *mdx* mice than in BL/10 mice (P=0.0010), independent of SET. ADG was not affected by SET (P=0.9734) or an interaction of genotype and SET (P=0.2701).

A genotype effect in which the % lean mass of MDX_{5-9} was greater than that of CON_{5-9} was observed (P=0.0021; Figure 19A). An interaction of genotype and age on % lean body mass was observed in SET_{6-13} (P=0.0072; Figure 19B). Post-hoc analyses revealed that % lean mass of MDX_{6-13} was greater than that of CON_{6-13} when mice were 6 to 10 and 12 to 13 wk old (P<0.05; Figure 19B).

A genotype effect in which the % fat mass of MDX_{5-9} was less than that of CON_{5-9} was observed for SET_{5-9} (P=0.0160; Figure 19C). An age effect was also observed (P=0.0241) in SET_{5-9}, but post-hoc analysis did not reveal any differences. An interaction effect (genotype x age) on % fat mass was observed in SET_{6-13} (P=0.0498). Post-hoc analyses revealed that % fat mass was less in MDX_{6-13} compared to CON_{6-13} at all time points (P<0.05; Figure 19D).

A genotype effect in which the % fluid mass of *mdx* mice was greater than that of BL/10 mice was observed for SET_{5-9} (P<0.0001; Figure 19E) and SET_{6-13} (P<0.0001; Figure 19F).

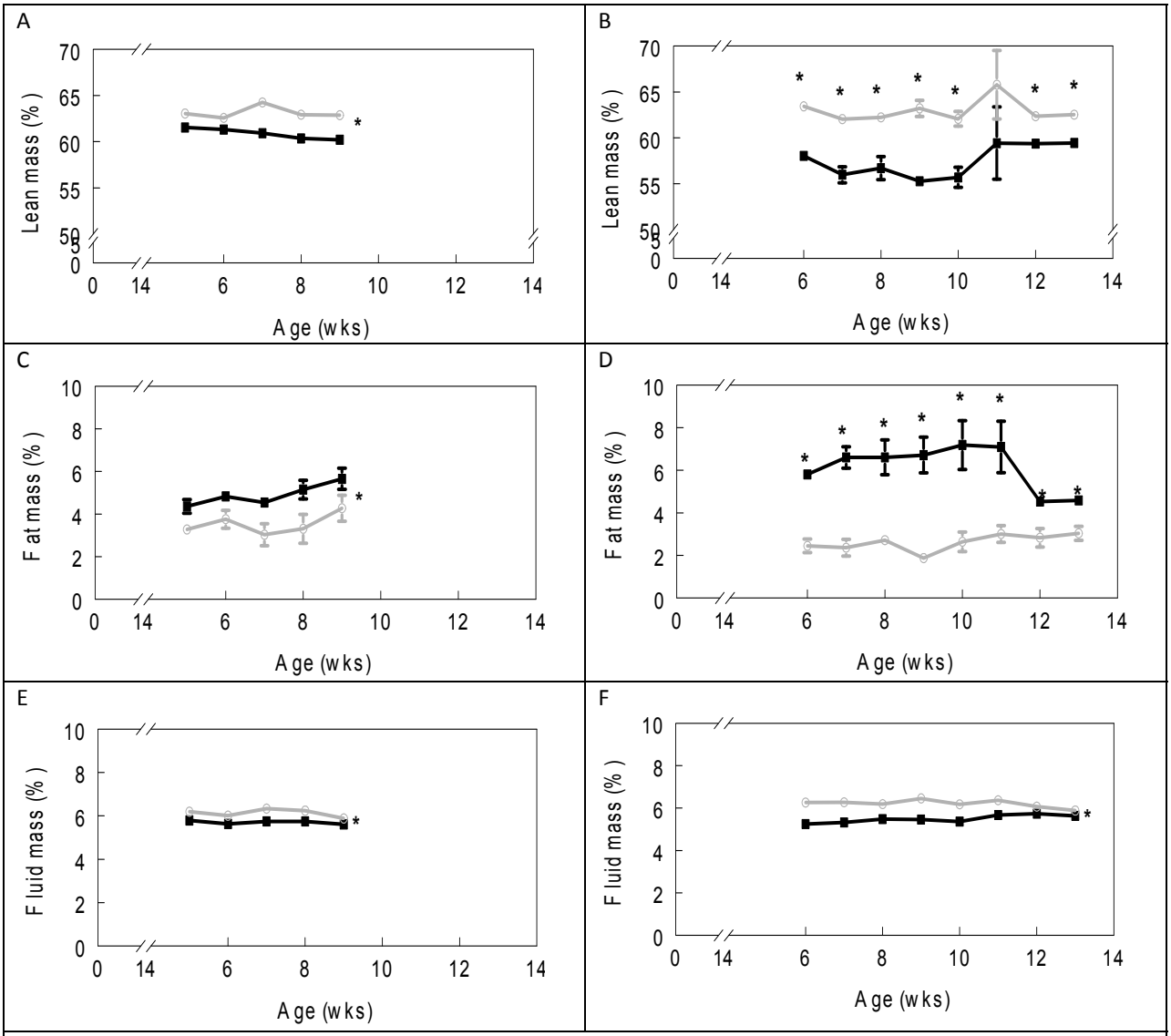

Figure 19: Body composition

(A) A genotype effect (*mdx*>BL/10) on % lean mass was observed for $SET_{5\text{-}9}$. (B) % lean mass was greater in $MDX_{6\text{-}13}$ than in $CON_{6\text{-}13}$ at 6, 7, 8, 9, 10, 12, and 13 wk, but not at 11 wk. (C) Genotype (*mdx*<BL/10) and age effects on % fat mass were observed in $SET_{5\text{-}9}$. (D) % fat mass was lower in $MDX_{6\text{-}13}$ than in $CON_{6\text{-}13}$ at all time points. (E) A genotype effect (*mdx*>BL/10) on % fluid mass was observed for $SET_{5\text{-}9}$. (F) A genotype effect (*mdx*>BL/10) on % fluid mass was observed for $SET_{6\text{-}13}$.

Black solid line: BL/10, gray solid line: *mdx*, *genotype difference (P<0.05).

Indirect calorimetry

Independent of genotype, TEE and RER were greater during the dark phase than during the light phase at 9 (P<0.0001; not shown) and 11 wk of age (P<0.0001 and P=0012, respectively). TEE and RER were not affected by genotype or by an interaction of genotype and phase in either SET (P>0.05).

Horizontal cage activity

In 9-wk-old mice, cage activity in the horizontal plane was greater during the dark phase than during the light phase (P=0.0048; Figure 20A). Horizontal cage activity in this set was not affected by genotype or by an interaction of genotype and phase (P>0.05). The horizontal cage activity of 11-wk-old mice was not affected by genotype, phase, or an interaction of the two (P>0.05).

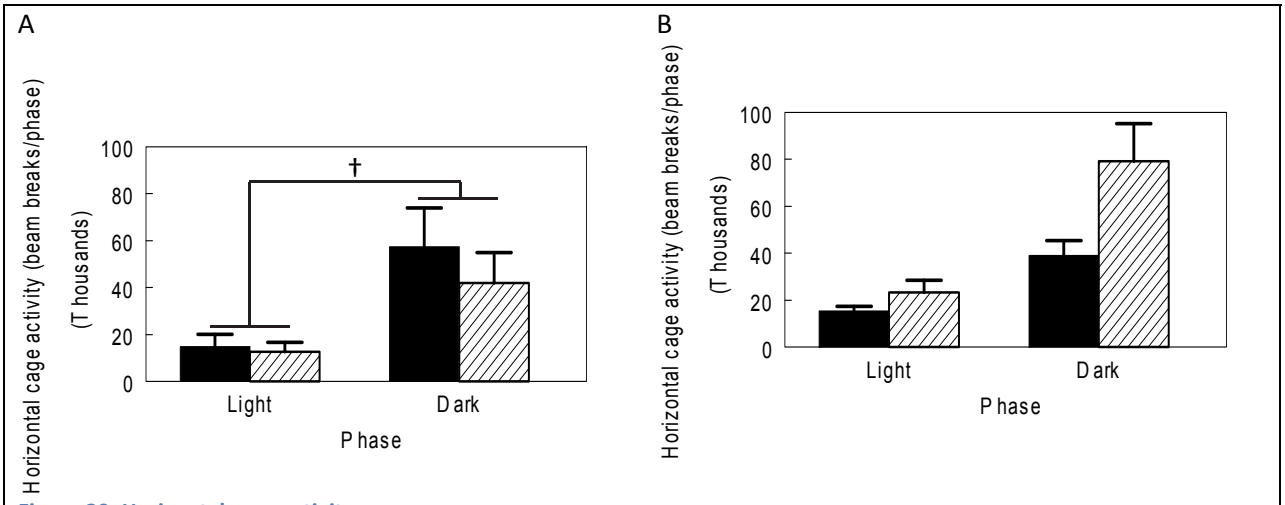

Figure 20: Horizontal cage activity
(A) As expected, cage activity in the horizontal plane was greater during the dark phase than during the light phase in 9-wk-old mice (P=0.0048). (B) An interaction effect (genotype x phase) on horizontal cage activity was observed in 11-wk-old mice (P=0.0426), but post-hoc analyses did not reveal differences among groups.
Black solid: CON, Black striped: MDX, † phase effect

Vertical cage activity

In 9-wk-old mice, cage activity in the vertical plane (Figure 21A) was affected by genotype (P=0.0310) and phase (P=0.0002), but there was no interaction (P=0.1790). Vertical cage activity was decreased in 9-wk-old *mdx* mice relative to age-matched BL/10 mice and increased during the dark phase relative to

the light phase. An interaction of genotype and phase was observed on cage activity in the vertical plane in 11-wk-old mice (P<0.0001; Figure 21B). Post hoc analyses revealed that vertical cage activity of 11-wk-old *mdx* mice was greater than that of 11-wk-old BL/10 mice during both the light phase (P=0.0451) and the dark phase (P=0.0451).

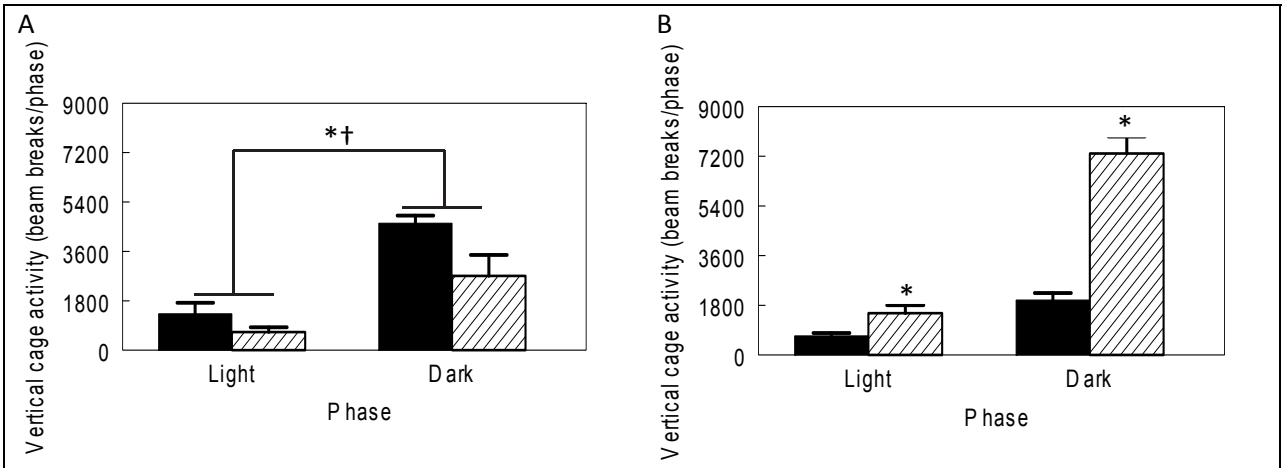

Figure 21: Vertical cage activity
(A) Independent of phase, cage activity in the vertical plane was lower in 9-wk-old *mdx* mice than in BL/10 mice. (B) An interaction effect (genotype x phase) on vertical cage activity was observed in 11-wk-old mice. Post hoc analyses revealed that cage activity in the vertical plane was greater in *mdx* mice during both phases
Black solid: CON, Black striped: MDX, **mdx* different from age-matched controls, † phase effect (P<0.05).

Immune profile

GM-CSF: Plasma GM-CSF was at least 4.0-fold lower in 9-wk-old *mdx* mice than in 9-wk-old BL/10 mice (Table 12). Plasma GM-CSF was below the detection limit for 13-wk-old mice of both genotypes. Plasma GM-CSF was at least 4.0-fold lower in 13-wk-old BL/10 mice relative to 9-wk-old BL/10 mice, but was below the detection limit for *mdx* mice of both ages.

IL-6: Plasma IL-6 content was at least 3.8-fold greater in 9-wk-old mdx mice and at least 5.5-fold greater in 13-wk-old *mdx* mice than in age-matched controls (Table 12). Plasma content of IL-6 was below the detection limit for BL/10 mice at both time points. There was no difference in plasma IL-6 between 9-wk-old and 13-wk-old mdx mice.

IL-12p70: Plasma IL-12p70 was at least 5.8-fold lower in 9-wk-old *mdx* mice than in age-matched controls (Table 12), but was below the detection limit for 9-wk-old mice of both genotypes. Plasma IL-12p70 was at least 5.8-fold lower in 13-wk-old BL/10 mice than in their younger counterparts and was below the detection limit in *mdx* mice of both ages.

CXCL1: Plasma CXCL1 was 4.9-fold greater in 9-wk-old *mdx* mice and 3.7-fold greater in 13-wk-old *mdx* mice than in age-matched controls (Table 12). Age had no effect on plasma CXCL1 content in either genotype.

CCL1: Genotype did not affect the plasma CCL1 content of 9- or 13-wk-old mice (Table 12). However, plasma CCL1 content was 2.8-fold greater in 13-wk-old BL/10 mice and 3.0-fold greater in 13-wk-old *mdx* mice than in their younger counterparts.

CCL2: Plasma CCL2 was 2.8-fold greater in 9-wk-old *mdx* mice and 2.5-fold greater in 13-wk-old *mdx* mice than in age-matched BL/10 mice (Table 12). Age had no effect on plasma CCL2 content in either genotype.

Table 12: Plasma cytokine/chemokine concentrations

	9-wk-old		**13-wk-old**	
	BL/10 (n=9)	***mdx*** (n=9)	**BL/10** (n=7)	***mdx*** (n=6)
	Plasma analyte concentration (pg/ml)			
Cytokines: Colony stimulating factor family				
GM-CSF	6.1	< 1.5	< 1.5	< 1.5
Cytokines: Interferon family				
IFNγ	< 0.1	< 0.1	< 0.1	< 0.1
Cytokines: Interleukin family				
IL-1α	4.8	4.7	3.2	5.4
IL-1β	< 8.8	< 8.8	< 8.8	< 8.8
IL-2	< 0.9	< 0.9	< 0.9	< 0.9
IL-3	< 1.0	< 1.0	< 1.0	< 1.0
IL-4	< 2.2	< 2.2	< 2.2	< 2.2
IL-5	6.2	5.8	4.1	4.6
IL-6	< 2.0	7.6	< 2.0	11.1
IL-10	< 0.5	< 0.5	< 0.5	< 0.5
IL-12p70	22.5	< 3.9	< 3.9	< 3.9
IL-17	< 0.0	< 0.0	< 0.0	< 0.0
Cytokines: TNF family				
TNFα	< 0.7	< 0.7	< 0.7	< 0.7
Chemokines: CXCL family				
CXCL1	35.4	171.7	54.9	202.1
Chemokines: CCL family				
CCL1	16.9	16.6	47.3	49.4
CCL2	21.4	59.4	40.0	100.5
CCL3	< 2.5	< 2.5	< 2.5	< 2.5
CCL5	19.1	14.8	16.0	21.8
CCL11	2736.5	3103.1	4084.6	4518.7
CCL17	281.4	261.1	443.3	621.4
CCL22	163.8	255.6	136.2	220.4

CCL17: Genotype had no effect on plasma CCL17 at either age (Table 12). While age did not affect the plasma CCL17 content of BL/10 mice, plasma CCL17 was 2.4-fold greater in 13-wk-old mice compared to their younger counterparts.

Additional cytokines: Plasma content of IL-1α, IL-5, CCL5, CCL11, and CCL22 was not affected by genotype or age (Table 12). Plasma content of IL-1β, IL-2, IL-3, IL-4, IL-10, IL-17, IFN-γ, TNF-α, and CCL3 was below the detection limit for all groups (Table 12).

Discussion

DMD is the most common [133, 134] and most severe form of muscular dystrophy. Those affected typically experience progressive skeletal muscle weakness [115-118] leading to death by 30 years of age [115, 117, 118, 121]. DMD is caused by mutation of the dystrophin gene that prevents expression of the dystrophin protein [123].

Similar to DMD, the *mdx* mouse carries a naturally occurring X-linked [13, 14] mutation that prevents expression of the dystrophin protein [16]. While this mouse is widely accepted as a genetic model of DMD [135, 136] and remains the most commonly used model of this disease [118, 135, 137], the phenotype of the *mdx* mouse is not as severe as that of patients with DMD [118, 135]. In the study described in this chapter, we identified differences in the body composition, energy expenditure, and plasma cytokine profile of *mdx* mice. We hypothesized that the body composition, energy expenditure, and plasma cytokine profile of *mdx* mice would be different from that of age-matched BL/10 controls. These differences and their implications will be discussed below.

Body composition

DMD is characterized by a progressive loss of skeletal muscle with a concomitant increase of fat and fibrous tissue within the muscle [138-140]. Treatment of DMD is typically aimed at maintaining or increasing muscle mass in affected patients.

The *mdx* mouse is frequently used when developing pharmaceutical and dietary interventions that could be used for patients with DMD. Despite the fact that the end-goal of many of these interventions is the

maintenance of lean body mass, there is little information available regarding body composition of the *mdx* mouse [126]. We attempted to address this issue by measuring the body mass and body composition of female 5- to 13-wk-old *mdx* and BL/10 mice. This age range was selected because it encompasses ages at which cycles of degeneration and regeneration are the most severe in *mdx* mice [118].

Similar to previous findings in both male and female *mdx* mice [126, 141-143] and in young boys with DMD (<13 yr) [125, 144, 145], we report that the body mass of female 7- to 13-wk-old *mdx* mice is greater and increases more rapidly (i.e., greater ADG) than that of age-matched controls (e.g., BL/10). With respect to body composition, patients with DMD typically have lower lean mass [124, 125], higher fat mass [124, 125], and lower body water [146, 147]. In contrast, our findings suggest that increased body mass observed in *mdx* mice is due to an increase in lean mass and fluid mass. *Mdx* mice also appear to have a lower fat mass than age-matched controls. These findings are in conflict with those of Rothwell and Stock [126], who reported an elevation in the % fat mass of female *mdx* mice. However, the mice used in the Rothwell study were considerably older (4 to 5 mo old) than the mice used in our study, and the genotype of the control mice was unclear. The results of our study do agree with recent reports that male and female *mdx* mice have a lower abdominal fat to body mass ratio [139, 148] and a greater hind limb muscle mass [141, 143, 149]. With respect to alterations in total body water, the higher % fluid mass observed here conflicts with the previous reports of lower whole body water in patients with DMD [146, 147], but does correspond to the greater intramuscular albumin in male and female *mdx* mice reported by Dupont-Versteegden [150] and the higher intramuscular water content of patients with DMD reported by Marden [140].

With one exception [126], the results of the body composition analysis performed in this study seem to correspond to previous reports of body composition differences between *mdx* and BL/10 control mice.

However, the pattern of body composition alterations observed in in the *mdx* mouse model of DMD appear to be in conflict with what is typically reported in patients with DMD, suggesting that use of *mdx* mice (male or female) may not be appropriate for researching the effects of treatments on the body composition of DMD patients.

Energy expenditure

Adequate nutritional support is important for maintaining the health and quality of life of patients with DMD. TEE and RER should be taken into account when determining the dietary requirements of a patient with DMD. The TEE – and resulting dietary need of a patient is determined by a number of factors, including: basal/resting metabolic rate and activity level. Previous literature suggests that the TEE of male and female *mdx* mice is suppressed at 4 to 6 wk of age [131] but is similar to the TEE of BL/10 mice at 6 to 12 months of age [131, 151]. The results of the study reported here suggest the return of TEE to BL/10 levels may occur at as early as 9 wk of age in female *mdx* mice, which approximately corresponds to the end of the acute phase of muscle degeneration and regeneration previously described in *mdx* mice [13, 65, 104, 131]. While the TEE of patients with DMD has not been reported, several groups have measured REE, which makes up a large portion of TEE, in patients with DMD with mixed results [152-158].

Physical activity is another factor contributing to TEE. Typically, the cage activity of 2- to 11-wk-old male and female *mdx* mice is less than [131, 159, 160] or equal to [160] that of BL/10 mice while no difference in cage activity is observed in older mice (6- to 12-months old) [131, 160]. Similar to these earlier studies, we report suppressed vertical plane cage activity and no difference in horizontal cage activity in 9-wk-old female *mdx* mice. However, vertical cage activity was greater in 11-wk-old female *mdx* mice relative to age-matched controls. The mechanism responsible for the latter effect is unclear but may be related to the lower relative humidity during the period in which this group of mice was

housed. At this time, it is unclear whether the decreased cage activity typically reported in *mdx* mice is a result of the dystrophic phenotype [131, 159], contributes to the dystrophic phenotype [131, 159], or whether both scenarios are true.

With respect to RER, a measurement that can be used to estimate whole-body substrate utilization (i.e., use of carbohydrates v. fat for ATP production), the results of this study are in agreement with previous reports that whole-body RER of is not affected by muscular dystrophy [151, 161].

With some exceptions, the findings of this study and those of earlier publications suggest that differences in energy expenditure and physical activity between *mdx* and BL/10 mice are closely related to age and/or the severity of disease. During the acute periods of skeletal muscle degeneration and regeneration (i.e., in young mice), TEE and physical activity seem to be suppressed in *mdx* mice. In contrast, when the dystrophic phenotype is less severe (i.e., in older mice) the TEE and physical activity of *mdx* mice are not different from that of age-matched controls. This suggests that use of *mdx* mice should be limited to periods of intense degeneration and regeneration (i.e., the acute phase of the dystrophic phenotype) if energy expenditure or physical activity is a research objective.

Immune profile

Inflammation plays an important role in the progression of DMD [19]. Immune cells such as macrophages and neutrophils can be observed in the skeletal muscle of male and female *mdx* mice [13, 19, 162] and patients with DMD, prior to disease onset, suggesting that the muscle wasting observed in this disease may be initiated, at least partially, by the immune system [19]. This elevation of immune cells within skeletal muscle can be observed throughout the progression of DMD. Cytokine elevations have also been reported in the skeletal muscles of *mdx* mice [163-165] and patients with DMD [166]. Despite the importance of cytokines in the inflammatory response, little is known about the profile of

circulating cytokines in *mdx* mice or patients with DMD. A better understanding of plasma cytokines and how changes in this profile correspond to skeletal muscle damage and immune cell infiltration could be used to develop non-invasive markers for skeletal muscle damage and optimize the treatment of DMD.

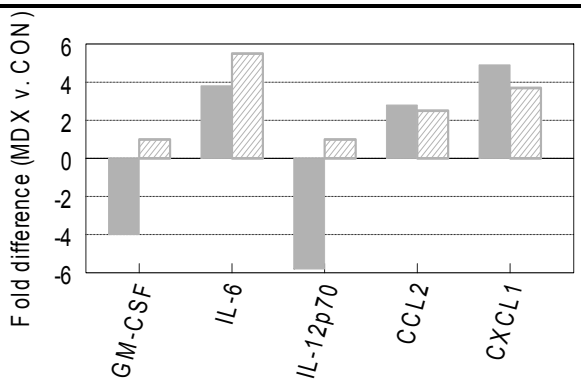

Figure 22: Relative cytokine/chemokine content
Plasma IL-6, CXCL1, and CCL2 were greater in $MDX_{5\text{-}9}$ and $MDX_{6\text{-}13}$ than in respective controls. Plasma GM-CSF and IL-12p70 were lower in $MDX_{5\text{-}9}$ compared to $CON_{5\text{-}9}$. Gray solid bar: Plasma analyte content in $MDX_{5\text{-}9}$ relative to $CON_{5\text{-}9}$, Gray striped bar: Plasma analyte content in $MDX_{6\text{-}13}$ relative to $CON_{6\text{-}13}$

In this study, we screened plasma collected from 9- and 13-wk-old *mdx* mice for a broad array of cytokines to identify those whose content in plasma was altered relative to the plasma of age-matched controls. One cytokine identified in this screen was IL-6, a member of the hematopoietin superfamily. This cytokine can be secreted from a variety of cells, including macrophages (Table 13). When released, it stimulates the activation of lymphocytes (i.e., T cells and B cells). The 4- to 6-fold elevation reported here (Figure 22) corresponds to a previously reported elevation in serum IL-6 in 6-month-old *mdx* mice [167]. Plasma IL-6 elevations have been reported in patients with DMD [168, 169].

Plasma CCL2 content was altered in *mdx* mice in this study. CCL2 is a proinflammatory chemokine that can be released from damaged tissue (e.g., skeletal muscle; Table 13) to attract immune cells [170] such as monocytes to the site of injury, where they become macrophages. The 2- to 3- fold increase we observed in the plasma of *mdx* mice conflicts with the 10-fold increase in serum CCL2 previously reported in 2-month old mice [170], suggesting that content of this cytokine may be variable or transient in *mdx* mice. To the author's knowledge circulating levels of this chemokine have not been reported in patients with DMD, but elevations in plasma content have been reported humans with

exercise-induced skeletal muscle damage [171, 172], which implies that CCL2 may have a role in skeletal muscle repair in patients with DMD as well.

Table 13: Description of cytokines altered in the plasma of *mdx* mice

Cytokine	Alternate name	Source	Target	Reference
IL-6	--	Adipocytes Endothelial cells Macrophages T cells	B cells T cells	[129, 173-175]
IL-12p70	--	Dendritic cells Macrophages	NK cells T cells	[129, 175]
CCL2	MCP-1	Endothelial cells Adipocytes Macrophages Muscle	Basophils Dendritic cells Monocytes NK cells T cells	[19, 129, 170, 173-175]
GM-CSF	--	Endothelial cells Macrophages T cells	Dendritic cells Basophils Eosinophils Monocytes Neutrophils	[129, 174, 175]
CXCL1	KC GROα IL-8	Adipocytes Endothelial cells Macrophages	Fibroblast Neutrophil	[129, 173-175]

Plasma CXCL1 was also elevated in *mdx* mice at both time points. This chemokine is a chemoattractant for neutrophils (Table 13). To the authors' knowledge, neither plasma nor skeletal muscle expression of CXCL1 has been reported in *mdx* mice or patients with DMD, respectively. However, over-expression of CXCL1 has been reported in skeletal muscle with ischemia-reperfusion injury [176, 177], suggesting that this chemokine may have a regular role in the repair of skeletal muscle.

Interestingly, our screen also revealed a transient decrease in the plasma content of IL-12p70 and GM-CSF in *mdx* mice. The observed decrease in IL-12p70 may contribute to the increase in fibrous tissue frequently reported in the muscles of *mdx* mice and patients with DMD [19] as this cytokine is thought to protect against fibrosis [178]. The mechanism responsible for the decreased plasma content of these cytokines is currently unclear.

The elevations in plasma IL-6, CCL2, and CXCL1 observed in this study confirm that immune system alterations in *mdx* mice are maintained beyond the most intense period of skeletal muscle degeneration and regeneration [13, 19, 150] and provides support for continued use of the *mdx* mouse for immune-related DMD research.

The results of the study described here support our hypothesis that the body composition, energy expenditure, and immune profile of *mdx* mice are different from that of BL/10 mice and that these differences vary with age. In addition, we reported instances in which the phenotype of the *mdx* mouse did (i.e., cytokine profile) and did not (i.e., % lean mass and % fat mass) match the symptoms of patients with DMD. Overall, these findings suggest that careful consideration of the objective of a study is necessary when determining the appropriateness of the *mdx* mouse as a model of DMD.

In the next chapter, we will explore whether the phenotypic difference between *mdx* and BL/10 mice affects the growth of mammary tumors.

Acknowledgements

The authors acknowledge Ryan McMillian, Matthew Hulver, Madlyn Frisard and the Mouse Phenotyping Core (Virginia Tech: Blacksburg, VA) for determining total energy expenditure, respiratory exchange ratio, and cage activity.

Chapter 5: Breast cancer growth is not altered in dystrophic mice

Abstract

BC is the second leading cause of cancer death among women in the United States. Changes to the DGC, a protein complex most frequently studied for its role in muscular dystrophy, have been reported in BC. Despite this surprising similarity between BC and muscular dystrophy, the relationship between these two conditions has not been explored. ***Purpose***: The purpose of the study described in this chapter was to determine whether systemic alterations to the DGC affect BC growth. ***Methods***: The growth of mammary tumors in *mdx* mice was compared to growth in BL/10 mice. ***Results***: We found that while the growth of BC was not affected by the *mdx* phenotype, tumor-induced alterations in the plasma cytokine profile were less severe in young *mdx* mice suggesting that these mice may be somewhat protected from BC invasion and metastasis. Future research should determine whether *mdx* mice with BC are less likely to develop metastases than age-matched BL/10 controls.

Introduction

The American Cancer Society predicted that 230,480 women would be diagnosed with, and 39,520 women would die from BC in the United States in 2011 [1]. While the incidence of female BC has been decreasing by approximately 2% per year since 1998 [3], BC remains the second leading cause of cancer death among women in the United States [1, 3]. The DGC is a multi-subunit protein complex that acts as a physical and signaling connection between the intra- and extra-cellular environments [10]. Alterations to this complex are most frequently associated with DMD. However, changes to the DGC have also been reported in BC cell lines [11, 179-183] and tumors [11, 184, 185] and appear to be more pronounced with severe disease [11, 183, 184]. Despite the similarity in alterations to the DGC reported in DMD and in BC, the relationship between these two conditions has not been explored.

The *mdx* mouse carries a naturally occurring X-linked [13, 14] point mutation resulting in a premature stop codon [15] for the dystrophin gene. This mutation prevents expression of the dystrophin protein [16], which results in altered expression of many other components of the DGC [17, 18]. While the *mdx* mouse phenotype is not as severe as that seen in patients with DMD, they do exhibit characteristic skeletal muscle degeneration, regeneration, and weakness. The dystrophic pathology of the *mdx* mouse begins with an acute phase of skeletal muscle degeneration and regeneration that begins as early as 2 wk of age and continues to approximately 8 wk of age [19, 118]. This phase is followed by a chronic phase of pathology characterized by less severe cycles of degeneration and regeneration that continue throughout the lifespan of the mouse [19, 118]. In the previous chapter of this dissertation (i.e., Chapter 4), we identified a number of whole-body differences between *mdx* mice and their controls that were present during the acute and chronic phases of this disease.

Injection of E0771 murine mammary tumor cells into the *mdx* mouse provided us with a novel model to explore the effect of systemic alterations in the DGC on BC growth. The ***specific aim*** of this study was to determine whether the systemic alterations of the DGC found in the *mdx* mouse can impact the growth of mammary tumors, and if so, whether the effect is age-dependent. Based on the results of our preliminary study, we ***hypothesize*** that the growth of E0771 mammary tumors will be slower in *mdx* mice compared to BL/10 mice and that this effect will be more pronounced when the dystrophic phenotype is more severe.

The results of this study could enhance the understanding of the role of the DGC in BC growth and reveal novel mechanisms for the treatment of BC.

Materials and methods

Reagents

DMEM, CS, P/S, and Trypsin-EDTA were purchased from Mediatech, Inc. (Herndon, VA). All other chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO) or Thermo Fisher Scientific, Inc. (Suwanee, GA).

Table 14: Treatment groups

Group	n	Genotype	Treatment	Age (wk)	
				Initial	Final
Set$_{5-9}$					
CON$_{5-9}$	7	BL/10	---	5	9
CON+T$_{5-9}$	7	BL/10	4x5000 E0771 cells	5	9
MDX$_{5-9}$	7	*mdx*	---	5	9
MDX+T$_{5-9}$	7	*mdx*	4x5000 E0771 cells	5	9
SET$_{9-13}$					
CON$_{9-13}$	9	BL/10	---	9	13
CON+T$_{9-13}$	10	BL/10	4x5000 E0771 cells	9	13
MDX$_{9-13}$	10	*mdx*	---	9	13
MDX+T$_{9-13}$	10	*mdx*	4x5000 E0771 cells	9	13

Maintenance of E0771 murine mammary adenocarcinoma cells

E0771 murine mammary adenocarcinoma cells were a generous gift from Enrico Mihich at the Roswell Park Cancer Institute (Buffalo, NY). E0771 cells were maintained in 100 mm tissue culture-treated plastic plates containing DMEM supplemented with 5% CS and 1 % P/S. Plates were kept at 37°C in a humidified atmosphere of 5% CO_2.

Animal husbandry

Four-wk-old female *mdx* mice (Stock number: 001801) and BL/10 control mice (Stock number: 000476) were purchased from The Jackson Laboratory (Bar Harbor, ME). Due to limited availability, mice were purchased in two sets (SET_{5-9} and SET_{6-13}). To avoid the effect of variation among sets, the data for each set were analyzed separately unless otherwise indicated.

Upon receipt, mice in SET_{5-9} were allowed to mature to 5 wk of age (Figure 23). This age was selected because it is considered the youngest age of sexual maturity [186] and falls within the "acute phase of myopathy" previously described in *mdx* mice [104]. When the mice in this set reached 5 wk of age, they were randomized to four treatment groups: CON_{5-9}, $CON+T_{5-9}$, MDX_{5-9}, and $MDX+T_{5-9}$ (Table 14), and those mice which were assigned to tumor-bearing groups received E0771 cell injections (described below).

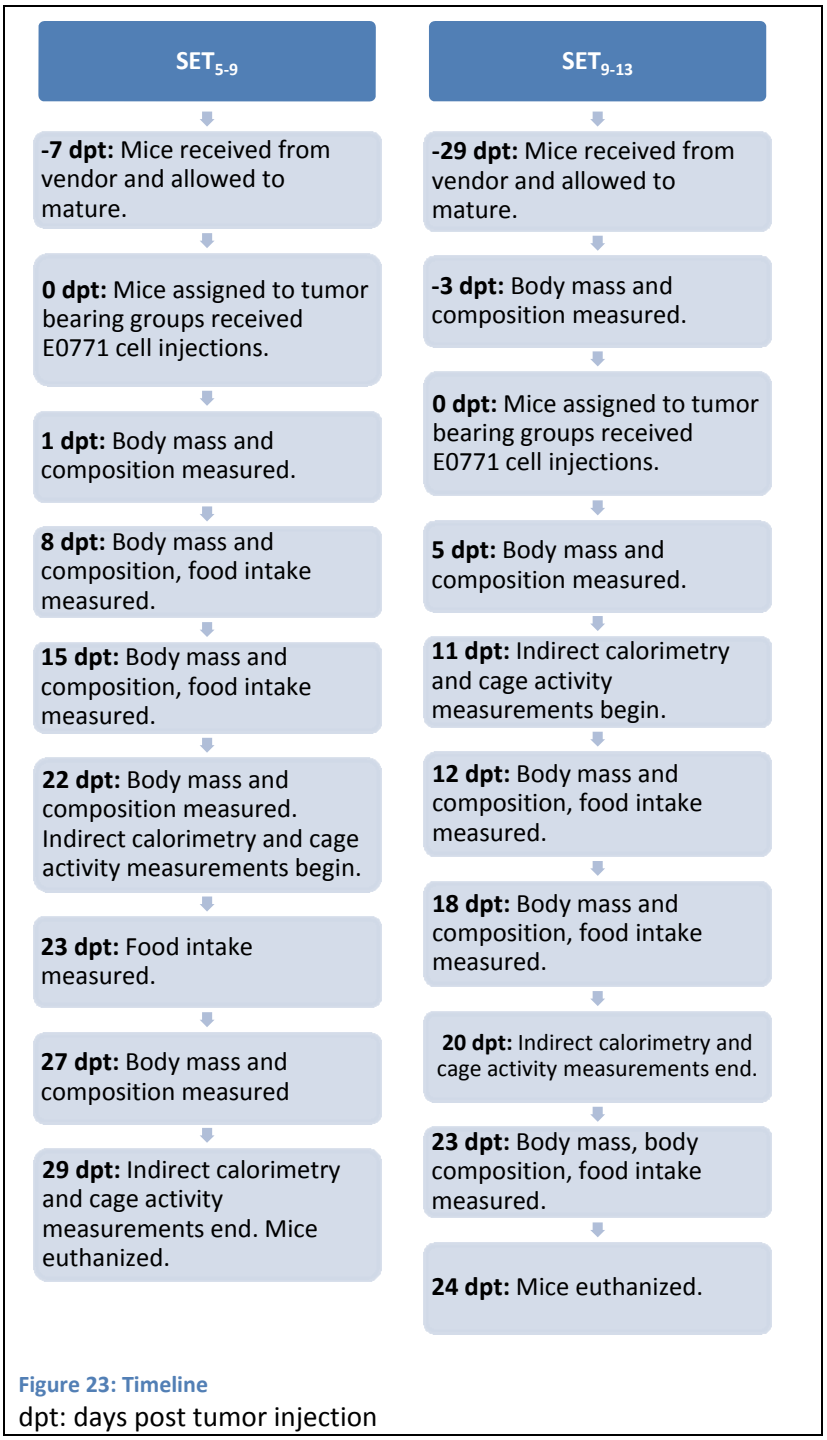

Figure 23: Timeline
dpt: days post tumor injection

Upon receipt, mice in SET_{9-13} were allowed to mature to 9 wk of age (Figure 23). Preliminary work in our lab suggests that an anti-tumor effect can be observed in *mdx* mice of this age. At 9 wk of age, mice were randomized to four treatment groups: CON_{9-13}, $CON+T_{9-13}$, MDX_{9-13}, $MDX+T_{9-13}$ (Table 14), and those mice which were assigned to tumor-bearing groups received E0771 cell injections (described below).

Throughout the study, mice were pair-housed under a 12-hr light/dark cycle with *ad libitum* access to water and standard rodent chow. Genotype was confirmed by a modified [105] *mdx*-ARMS assay [106]. Mouse health and well-being and the environmental parameters [i.e., temperature (accuracy 1°C) and RH (accuracy 1%)] of the facility were monitored daily by animal care staff. All procedures were approved by Virginia Tech's Institutional Animal Care and Use Committee (Protocol: 07-142-HNFE).

E0771 cell injection and tumor growth

On the day of cell injection [i.e., 0 days post tumor cell injection (dpt)], E0771 cells ($SET_{5\text{-}9}$: Passage # 7, $SET_{9\text{-}13}$: Passage # 9) were suspended in Matrigel (BD Biosciences: Bedford, MA) (5000 cells/40µl of Matrigel). Mice in tumor-bearing groups received one 40 µl subcutaneous injection of the cell/Matrigel mixture in each quadrant of the back (i.e., 4 injections total). Tumors were allowed to grow until tumor size and/or quality necessitated euthanasia. TSA was determined weekly using Equation 2 [107].

Food intake

Food intake was measured at 8, 15, and 23 dpt for mice in $SET_{5\text{-}9}$ and at 12 and 23 dpt for mice in $SET_{6\text{-}13}$. For each measurement, a known amount of food was placed in each cage. After 24 hours, the amount of food remaining in each cage was weighed using a standard electronic scale (accuracy 0.1 g) and the difference was divided by the number of mice in the cage.

Morphological characteristics

Body mass and composition were measured weekly beginning at 1 dpt for mice in $SET_{5\text{-}9}$ and at -3 dpt for mice in $SET_{9\text{-}13}$ (Figure 23). Body mass was measured using a standard electronic scale (accuracy 0.1g). Body composition (i.e., fat mass, lean mass, and fluid mass) was measured using a Minispec LF-90 TD-NMF (Bruker Optics: Billerica, MA) as recommended by the manufacturer. Equation 1 was used to determine tumor-free body mass after euthanasia.

Indirect calorimetry and cage activity

Ventilated gas exchange (i.e., O_2 and CO_2) and spontaneous cage activity were determined in 5 randomly selected mice per treatment group. O_2 consumption and CO_2 production were measured on single-housed mice by open circuit calorimetry and used to determine RER (Equation 4) and TEE (Equation 5). An infrared light-beam frame was used to determine spontaneous cage activity in the horizontal (i.e., X and Y) and vertical (i.e., Z) directions. Activity was determined by counting the number of novel interruptions of the infrared light beams (i.e., beam breaks) [132]. Measurements were made at 22-29 dpt for SET_{5-9} and at 11-20 dpt for SET_{9-13} by the Virginia Tech Integrated Life Sciences Building Phenotyping Core (Blacksburg, VA) using a PhenoMaster/LabMaster System (TSE Systems: Chesterfield, MO).

Tissue collection

At 29 dpt, mice in SET_{5-9} were anesthetized by inhaled isoflourane and blood was collected via cardiac puncture. Blood was placed in 1.5 ml microcentrifuge tubes containing 10 µl of 0.1 M sodium citrate buffer and separated by centrifugation at 500 RCF for 20 min at 4°C. The plasma portion was removed and stored at -80°C. Tumors were harvested, weighed individually and stored for further analysis.

At 24 dpt, mice in SET_{6-13} were anesthetized by inhaled CO_2 and blood and tumors were collected and processed as described above.

Tumor substrate oxidation

Immediately after euthanasia, substrate oxidation was determined in one randomly selected tumor from each tumor-bearing mouse in $SET_{9\text{-}13}$. Rates of glucose and fatty acid oxidation were measured using the method described by Frisard et al. [187].

Immune profile

Cytokine levels were measured in plasma pooled from mice in each treatment group by Quansys' Multiplex Testing Service using a Q-Plex™ Mouse Cytokine/Chemokine Array (21-plex). This array was selected because it provides highly sensitive quantification (i.e., pg/ml) of 21 cytokines from a small volume of plasma (i.e., 30 µl). A 2-fold difference between treatment groups was considered significant.

Statistical analyses

Student's *t*-tests were used to determine differences between sets for temperature and RH. 2-way ANOVAs (genotype x tumor presence) were used to determine differences in food intake, body mass, body composition, TEE, RER, and cage activity. Where appropriate, Student's *t*-tests and/or Tukey's HSD were used for post-hoc analyses. Reported values are mean± standard error. For all analyses, $\alpha = 0.05$.

Pearson's χ^2 tests were used to assess differences in tumor formation success rate between genotypes and among tumor locations. Mixed-model ANOVAs with repeated measures (genotype x time) were used to assess differences in TSA between genotypes. Two-way ANOVAs (genotype x tumor location) were used to determine differences in tumor mass between genotypes. Where appropriate, Student's *t*-tests and/or Tukey's HSD were used for post-hoc analyses. A Student's *t*-test was used to assess differences in tumor substrate oxidation between $CON+T_{9\text{-}13}$ and $MDX+T_{9\text{-}13}$. For all analyses, $\alpha = 0.05$.

Results

Environmental parameters

Table 15: Environmental parameters

	SET_{5-9}	SET_{9-13}
Temperature (°C)		
AM	21±0	21±0
PM	21±0	21±0
AVG	21±0	21±0
Range	21-22	19-21
Relative Humidity (%)		
AM	42±1	30±0
PM	43±1	30±1
AVG	42±1	30±0
Range	34-51	23-41

Acceptable temperature range is 18-26°C. Acceptable RH range is 30-70%. Values are mean±SEM.

There were no differences in the morning, afternoon, and daily average temperatures during the period in which the mice in SET_{5-9} and SET_{9-13} were housed in our facility (Table 15). The morning, afternoon, and daily average RH values during the period in which the mice in SET_{9-13} were housed in our facility were lower compared to the period during which the mice in SET_{5-9} were housed ($P<0.0001$ for all measurements; Table 15).

24-hr food intake

The 24-hr food intake of SET_{5-9} was not affected by genotype, tumor presence, or an interaction of the two at any time point ($P>0.05$).

In SET_{9-13}, 24-hr food intake 12 dpt was greater in *mdx* mice than in BL/10 mice (4.2±0.2 g/d v. 3.4±0.1 g/d; $P=0.0015$), but was not affected by tumor presence ($P>0.05$). 24-hr food intake was not affected by genotype or tumor presence at 23 dpt ($P>0.05$). 24-hr food intake was not affected by an interaction of genotype and tumor presence at any time point ($P>0.05$).

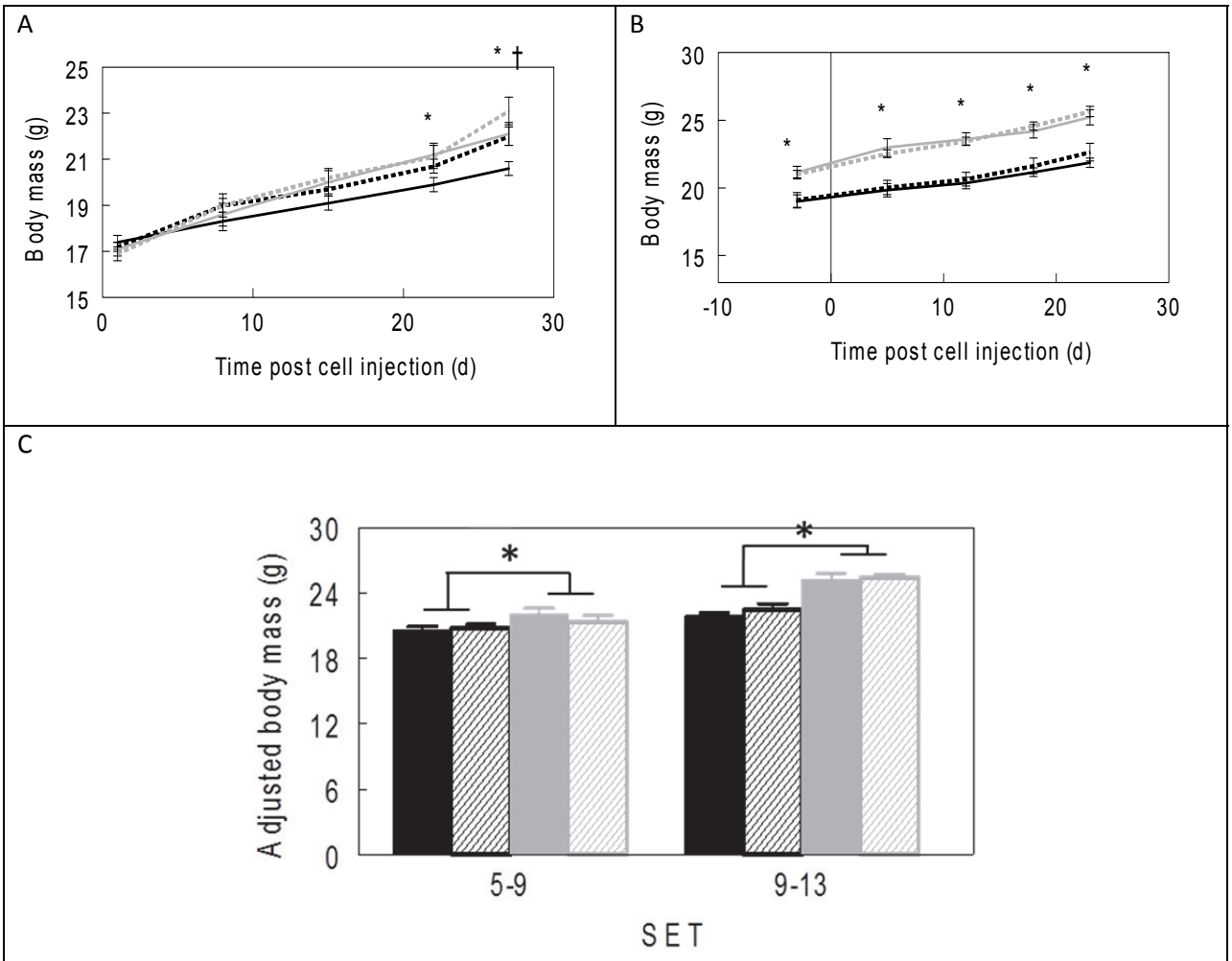

Figure 24: Body mass

(A) In SET_{5-9}, tumor presence was associated with an increased body mass at 27 dpt only. (B) Tumor presence did not affect body mass at any time point in SET_{6-13}. (C) When adjusted for tumor mass, the effect of tumor presence on body mass at 27 dpt in SET_{5-9} was lost.

Values are mean ± SEM; α=0.05. Black: BL/10. Grey: *mdx*. Solid: tumor-free. Dashed/striped: tumor-bearing. * genotype effect. † tumor effect.

Body mass

In $SET_{5-9,}$ body mass was not affected by genotype or tumor presence at 1 to 15 dpt (*P*>0.05;Figure 24A). However, the body mass of *mdx* mice was greater than that of BL/10 mice at 22 (*P*=0.0413) and 27 dpt (*P*=0.0019). The body mass of tumor-bearing mice was greater than that of tumor-free mice at 27 dpt (*P*=0.0131), but this effect was lost when the body mass of tumor-bearing mice was corrected for tumor

mass (P>0.05; Figure 24C). The body mass of SET_{5-9} was not affected by an interaction of genotype and tumor presence at any time point (P>0.05).

For SET_{9-13}, the body mass of *mdx* mice was greater than that of BL/10 mice at all time points (P<0.05; Figure 24B). Neither tumor presence nor an interaction of genotype and tumor presence affected body mass at any time point for SET_{9-13} (P>0.05).

Lean mass

For SET_{5-9}, the % lean mass of *mdx* mice was greater than that of BL/10 mice between 8 and 27 dpt (P<0.05; Figure 25A), but not at 1 dpt (P>0.05). In addition, the % lean mass of tumor bearing mice was less than that of tumor-free mice at 15 dpt (P=0.0114). However, % lean mass of SET_{5-9} was not affected by tumor presence at any other time point or by an interaction of genotype and tumor presence at any time point (P>0.05).

For SET_{9-13}, an interaction of genotype and tumor presence on % lean mass was observed at -3 (P=0.0097; Figure 25B) and 5 dpt (P=0.0246). Post-hoc analyses revealed that for both time points, the % lean mass of MDX_{9-13} and $MDX+T_{9-13}$ was greater than that of CON_{9-13} and $CON+T_{9-13}$. The % lean mass of *mdx* mice was greater than that of BL/10 mice at 18 (P<0.0001) and 23 dpt (P<0.0001), but not at 12 dpt (P=0.0909). The presence of BC had no effect on SET_{9-13} % lean mass at any time point (P>0.05).

Fat mass

For SET_{5-9}, % fat mass was greater in BL/10 mice than in *mdx* mice at all time points (P<0.05; Figure 25C). The % fat mass of tumor-bearing mice was greater than that of tumor-free mice at 1 dpt (P=0.0082), but not at any other time point (P>0.05). % fat mass of SET_{5-9} was not affected by an interaction of genotype and tumor presence at any time point (P>0.05).

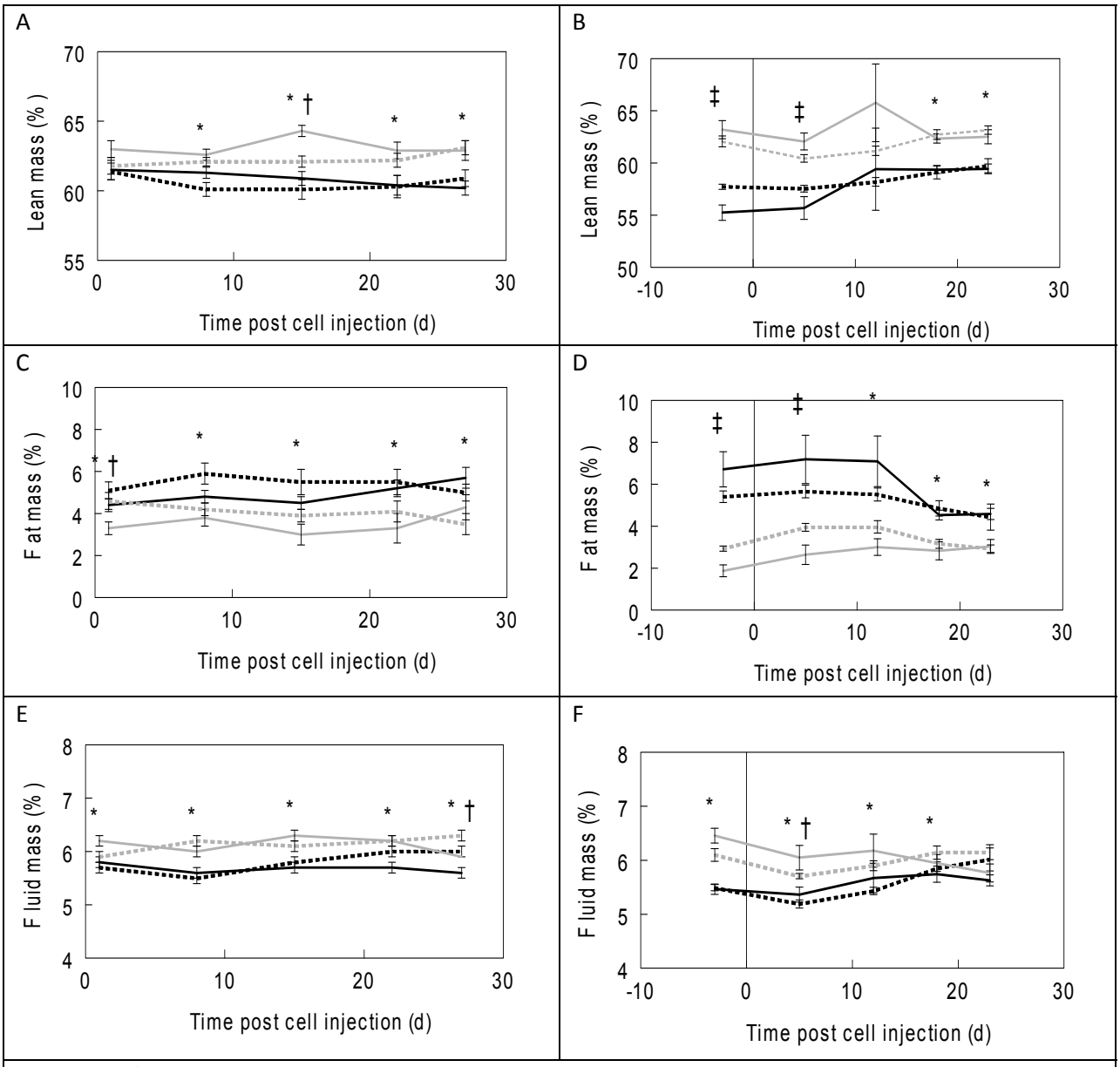

Figure 25: Body composition

(A) In SET_{5-9}, % lean mass was affected by tumor presence at 15 dpt only. (B)Tumor presence had no effect on % lean mass at any time point in SET_{6-13}. (C) In SET_{5-9}, % fat mass was greater in tumor bearing mice at 1 dpt only. (D) In SET_{9-13}, % fat mass was not affected by tumor presence at any time point. (E) In SET_{5-9}, tumor presence was associated with a greater % fluid mass at 27 dpt only. (F)In SET_{9-13}, tumor presence was associated with a greater % fluid mass at 5 dpt only.

Values are mean ± SEM; α=0.05. Black: BL/10. Grey: *mdx*. Solid: tumor-free. Dashed/striped: tumor-bearing. * genotype effect. † tumor effect. ‡ Interaction effect (genotype x tumor).

In SET_{9-13}, an interaction effect (genotype x tumor presence) on % fat mass was observed at -3 (P=0.0183; Figure 25D) and 5 dpt (*P*=0.0416). Post-hoc analyses revealed that at -3 dpt, % fat mass of

$CON_{9\text{-}13}$ and $CON+T_{9\text{-}13}$ was greater than that of $MDX_{9\text{-}13}$ and $MDX+T_{9\text{-}13}$. At 5 dpt, % fat mass of $CON_{9\text{-}13}$ was greater than that of $MDX_{9\text{-}13}$ and $MDX+T_{9\text{-}13}$, and % fat mass of $CON+T_{9\text{-}13}$ was greater than that of $MDX_{9\text{-}13}$. In addition, the % fat mass of BL/10 mice was greater than that of *mdx* mice at 12 to 23 dpt ($P<0.05$). The % fat mass of $SET_{9\text{-}13}$ was not affected by tumor presence at any time point ($P>0.05$).

Fluid mass

For $SET_{5\text{-}9}$, the % fluid mass of *mdx* mice was greater than that of BL/10 mice at all time points ($P<0.05$; Figure 25E). The % fluid mass of tumor-bearing mice was greater than that of tumor-free mice at 27 dpt only ($P=0.0005$). For $SET_{5\text{-}9}$, % fluid mass was not affected by an interaction of genotype and tumor presence at any time point ($P>0.05$).

For $SET_{9\text{-}13}$, the % fluid mass of *mdx* mice was greater than that of BL/10 mice at -3 to 18 dpt ($P<0.05$; Figure 25F), but not at 23 dpt ($P>0.05$). The % fluid mass of tumor-bearing mice was less than that of tumor-free mice at 5 dpt only ($P=0.0250$). The % fluid mass of $SET_{9\text{-}13}$ was not affected by an interaction of genotype and tumor presence at any time point ($P>0.05$).

Indirect calorimetry

The TEE of mice in $SET_{5\text{-}9}$ was not affected by genotype, tumor presence, or an interaction of the two during the light phase or the dark phase ($P>0.05$; not shown). The RER of $SET_{5\text{-}9}$ was greater in tumor-free mice than in tumor-bearing mice during the dark phase (0.96±0.01 v. 0.93±0.01; $P=0.0038$), but was not affected by tumor presence during the light phase ($P>0.05$). The RER of $SET_{5\text{-}9}$ was not affected by genotype or an interaction of genotype and tumor presence during either phase ($P>0.05$).

In $SET_{9\text{-}13}$, the TEE of BL/10 mice was greater than that of *mdx* mice during the light phase (159.42±4.33 kJ/kg/hr v. 134.51±3.11 kJ/kg/hr; $P=0.0004$; not shown), but not during the dark phase ($P>0.05$). The TEE of mice in $SET_{9\text{-}13}$ was not affected by tumor presence or an interaction of genotype and tumor presence

during either the light or the dark phase (P>0.05). The RER of mice in SET_{9-13} was not affected by genotype, tumor presence, or an interaction of the two during either phase (P>0.05).

Cage activity

For SET_{5-9}, cage activity in the horizontal and vertical planes was not affected by genotype, tumor presence, or an interaction of the two during either the light phase or the dark phase (P>0.05; not shown)

Table 16: SET_{9-13} cage activity

phase		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Horizontal cage activity by phase (beam breaks)					
Light (n=5)	Mean	15,391	22,643	23,346	17,902
	SEM	1,926	3,241	5,093	1,771
	Sig?	--	--	--	--
Dark (n=5)	Mean	39,047	44,018	79,158	53,087
	SEM	6,293	10,666	16,046	8,013
	Sig?	--	--	--	--
Vertical cage activity by phase (beam breaks)					
Light (n=5)	Mean	698	1,073	1,520	986
	SEM	103	172	292	112
	Sig?	A	AB	B	AB
Dark (n=5)	Mean	1,991	2,828	7,298	3,025
	SEM	255	810	611	162
	Sig?	A	A	B	A

Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)

For SET_{9-13}, horizontal cage activity was greater in *mdx* mice than in BL/10 mice during the dark phase (P=0.0384; Table 16), but not during the light phase (P>0.05). The horizontal cage activity of SET_{9-13} was not affected by tumor presence or an interaction of genotype and tumor presence during either phase (P>0.05). Vertical cage activity was affected by an interaction of genotype and tumor presence during both the light (P=0.0262) and dark phases (P=0.0002). Post-hoc analyses revealed that vertical cage activity of MDX_{9-13} was greater than that of CON_{9-13} during the light phase and that vertical cage activity of MDX_{9-13} was greater than that of CON_{9-13}, $CON+T_{9-13}$, and $MDX+T_{9-13}$.

Tumor characteristics

Genotype did not affect tumor incidence, surface area, or mass in either set of mice (i.e., SET_{5-9}, SET_{9-13}). In addition, there was no difference in tumor glucose oxidation or tumor palmitate oxidation between genotypes in SET_{9-13}.

Plasma cytokine content

GM-CSF: Plasma GM-CSF content was 2.1-fold greater in CON+T_{5-9} compared to CON_{5-9} (Table 17) and at least 7.4-fold greater in MDX+T_{5-9} compared to MDX_{5-9}. Plasma GM-CSF was below the detection limit in SET_{9-13} BL/10 and *mdx* mice.

IL-1α: In SET_{9-13} BL/10 mice, plasma IL-1α was 7.5-fold greater in CON+T_{9-13} compared to CON_{9-13} (Table 17). Tumor presence did not affect plasma content of IL-1α in SET_{5-9} BL/10 or *mdx* mice or in SET_{9-13} *mdx* mice

IL-5: Plasma IL-5 content was 2.0-fold lower in CON+T_{5-9} compared to CON_{5-9} (Table 17). Tumor presence did not affect plasma content of IL-5 in SET_{5-9} *mdx* mice *or in* SET_{9-13} BL/10 or *mdx* mice.

IL-6: Plasma content of IL-6 was at least 4.1-fold greater in CON+T_{5-9} compared to CON_{5-9} (Table 17). Plasma IL-6 content was 2.6-fold greater in MDX+T_{5-9} compared to MDX_{5-9}. Plasma IL-6 content was more than 6.7-fold greater in CON+T_{9-13} compared to CON_{9-13}. Plasma content of IL-6 was not affected by tumor presence in SET_{9-13} *mdx* mice.

IL-12p70: Tumor presence did not affect plasma content of IL-12p70 in SET_{5-9} BL/10 mice (Table 17). Plasma content of IL-12p70 was below the detection limit in SET_{5-9} *mdx* mice, SET_{9-13} BL/10 mice, and SET_{9-13} *mdx* mice.

IL-17: Plasma content of IL-17 was at least 30.5-fold greater in CON+T_{5-9} compared to CON_{5-9} (Table 17). Plasma content of IL-17 in was below the detection limit in SET_{5-9} *mdx* mice and SET_{9-13} BL/10 and *mdx* mice.

Table 17: Plasma cytokine content

	CON_{5-9} (n=9)	$CON+T_{5-9}$ (n=10)	MDX_{5-9} (n=10)	$MDX+T_{5-9}$ (n=10)	CON_{9-13} (n=9)	$CON+T_{9-13}$ (n=10)	MDX_{9-13} (n=10)	$MDX+T_{9-13}$ (n=10)
Plasma analyte	**Concentration (pg/ml)**							
Cytokines: Colony stimulating factor family								
GM-CSF	6.1	13.0	< 1.5	11.1	< 1.5	< 1.5	< 1.5	< 1.5
Cytokines: Interferon family								
IFNγ	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cytokines: Interleukin family								
IL-1α	4.8	4.6	4.7	3.2	3.2	24.1	5.4	6.6
IL-1β	< 8.8	< 8.8	< 8.8	< 8.8	< 8.8	< 8.8	< 8.8	< 8.8
IL-2	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9
IL-3	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
IL-4	< 2.2	< 2.2	< 2.2	< 2.2	< 2.2	< 2.2	< 2.2	< 2.2
IL-5	6.2	3.1	5.8	3.9	4.1	6.2	4.6	6.4
IL-6	< 2.0	8.2	7.6	19.5	< 2.0	13.4	11.1	9.4
IL-10	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
IL-12p70	22.5	12.4	< 3.9	< 3.9	< 3.9	< 3.9	< 3.9	< 3.9
IL-17	< 0.0	3.1	< 0.0	< 0.0	< 0.0	< 0.0	< 0.0	< 0.0
Cytokines: TNF family								
TNFα	< 0.7	3.2	< 0.7	3.9	< 0.7	5.3	< 0.7	2.9
Chemokines: CXCL family								
CXCL1	35.4	317.1	171.7	294.0	54.9	93.1	202.1	127.8
Chemokines: CCL family								
CCL1	16.9	33.1	16.6	44.3	47.3	56.4	49.4	44.7
CCL2	21.4	639.3	59.4	593.9	40.0	120.0	100.5	127.6
CCL3	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5
CCL5	19.1	50.2	14.8	39.2	16.0	37.3	21.8	26.3
CCL11	2736.5	2639.4	3103.1	2127.2	4084.6	7176.7	4518.7	5616.2
CCL17	281.4	660.7	261.1	1449.3	443.3	636.3	621.4	555.8
CCL22	163.8	323.5	255.6	322.1	136.2	357.6	220.4	291.4

TNFα: Plasma content of TNFα was at least 4.5-fold greater in $CON+T_{5-9}$ compared to CON_{5-9} (Table 17). Plasma TNFα content was at least 5.5-fold greater in $MDX+T_{5-9}$ compared to MDX_{5-9}. Plasma TNFα was more than 7.5-fold greater in $CON+T_{9-13}$ compared to CON_{9-13} and 4.1-fold greater in $MDX+T_{9-13}$ compared to MDX_{9-13}.

CXCL1: Plasma content of CXCL1 was 9.0-fold greater in $CON+T_{5-9}$ compared to CON_{5-9} (Table 17). Plasma CXCL1 was not affected by tumor presence in SET_{5-9} *mdx* mice or SET_{9-13} BL/10 and *mdx* mice.

CCL1: Plasma content of CCL1 was 2.0-fold greater in CON+$T_{5\text{-}9}$ compared to $CON_{5\text{-}9}$ (Table 17) and 2.7-fold greater in MDX+$T_{5\text{-}9}$ compared to $MDX_{5\text{-}9}$. Plasma CCL1 was not affected by tumor presence in either genotype of $SET_{9\text{-}13}$.

CCL2: Plasma content of CCL2 was 29.9-fold greater in CON+$T_{5\text{-}9}$ compared to $CON_{5\text{-}9}$ (Table 17). Plasma content of CCL2 was 10.0-fold greater in MDX+$T_{5\text{-}9}$ compared to $MDX_{5\text{-}9}$. Plasma CCL2 was 3.0-fold greater in CON+$T_{9\text{-}13}$ compared to $CON_{9\text{-}13}$. CCL2 was not affected by tumor presence in $SET_{9\text{-}13}$ *mdx* mice.

CCL5: Plasma CCL5 content was 2.6-fold greater in CON+$T_{5\text{-}9}$ compared to $CON_{5\text{-}9}$ (Table 17), 2.6-fold greater in MDX+$T_{5\text{-}9}$ compared to $MDX_{5\text{-}9}$, and 2.3-fold greater in CON+$T_{9\text{-}13}$ compared to $CON_{9\text{-}13}$. Plasma content of CCL5 was not affected by tumor presence in $SET_{9\text{-}13}$ *mdx* mice.

CCL11: Plasma content of CCL11 was not affected by tumor presence in either genotype at any time point (Table 17).

CCL17: Plasma CCL17 was 2.3-fold greater in CON+$T_{5\text{-}9}$ compared to $CON_{5\text{-}9}$ (Table 17) and 5.6-fold greater in MDX+$T_{5\text{-}9}$ compared to $MDX_{5\text{-}9}$. Plasma content of CCL17 was not affected by tumor presence in $SET_{9\text{-}13}$ BL/10 or *mdx* mice.

CCL22: Plasma CCL22 content was 2.0-fold greater in CON+$T_{5\text{-}9}$ compared to $CON_{5\text{-}9}$ (Table 17) and 2.6-fold greater in CON+$T_{9\text{-}13}$ compared to $CON_{9\text{-}13}$. Tumor presence did not affect plasma CCL22 in *mdx* mice of either set.

Additional cytokines: Plasma content of IL-1β, IL-2, IL-3, IL-4, IL-10, IFNγ, and CCL3 was below the detection limit in all genotypes and sets (Table 17).

Table 18: Description of cytokines whose content was altered in the plasma of E0771 tumor-bearing mice

Cytokine	Alternate names	Known sources	Known targets	Effect on BC	References
Cytokines: Colony stimulating factor family					
GM-CSF	--	Endothelial cells Macrophages T cells	Basophils Dendritic cells Eosinophils Monocytes Neutrophils	Anti-angiogenesis Anti-growth Anti-metastasis	[129, 174, 175, 188-190]
Cytokines: Interleukin family					
IL-1α	--	Epithelial cells Macrophages/Monocytes Tumor cells T cells	Fibroblasts Macrophages T cells Phagocytes	Pro-invasion Pro-metastasis	[129, 189, 191-193]
IL-5	--	Mast cells T cells	B cells Eosinophils		[129, 188, 194]
IL-6	--	Adipocytes Endothelial cells Macrophages T cells Tumor cells	B cells T cells	Pro-growth	[129, 173-175, 191, 195, 196]
IL-17	IL-17A mCTLA-8	T cells Neutrophils NK cells	Epithelial cells Endothelial cells Fibroblasts	Pro-metastasis	[129, 197-199]
Cytokines: TNF family					
TNFα	--	Macrophages NK cells T cells Tumor cells	Endothelial cells	Pro-angiogenesis Pro-growth Pro-invasion Pro-metastasis	[129, 189, 191, 200, 201]

Discussion

Alterations to the DGC have been reported in BC [11, 179-185] and in the *mdx* mouse model of DMD. The results of a pilot study performed in our lab led us to hypothesize that the dystrophic phenotype of the *mdx* mouse could protect against mammary tumor growth (Chapter 3). In the study described in this chapter, we measured the growth of E0771 BC tumors and tumor-induced changes in the plasma cytokine profile in *mdx* mice during the acute and chronic phases of muscular dystrophy. While the dystrophic phenotype did not affect the growth of mammary tumors, we did observe a number of differences in the profile of circulating cytokines between tumor-bearing *mdx* and BL/10 mice.

As reported by others [195, 200][201-205], the presence of E0771 mammary tumors was associated with increased plasma content of a number of tumor promoting cytokines (Figure 26; Table 18 and Table 19). Plasma content of IL-1α, IL-6, IL-17, CCL2, TNFα, CCL5, CXCL1, CCL22, and CCL17 were elevated in at least one treatment group in this study. The presently available body of literature suggests that these cytokines support the growth and progression of BC by promoting tumor cell proliferation [195, 200], invasion [201], and metastasis [201-205] and by enhancing tumor angiogenesis [201, 203].

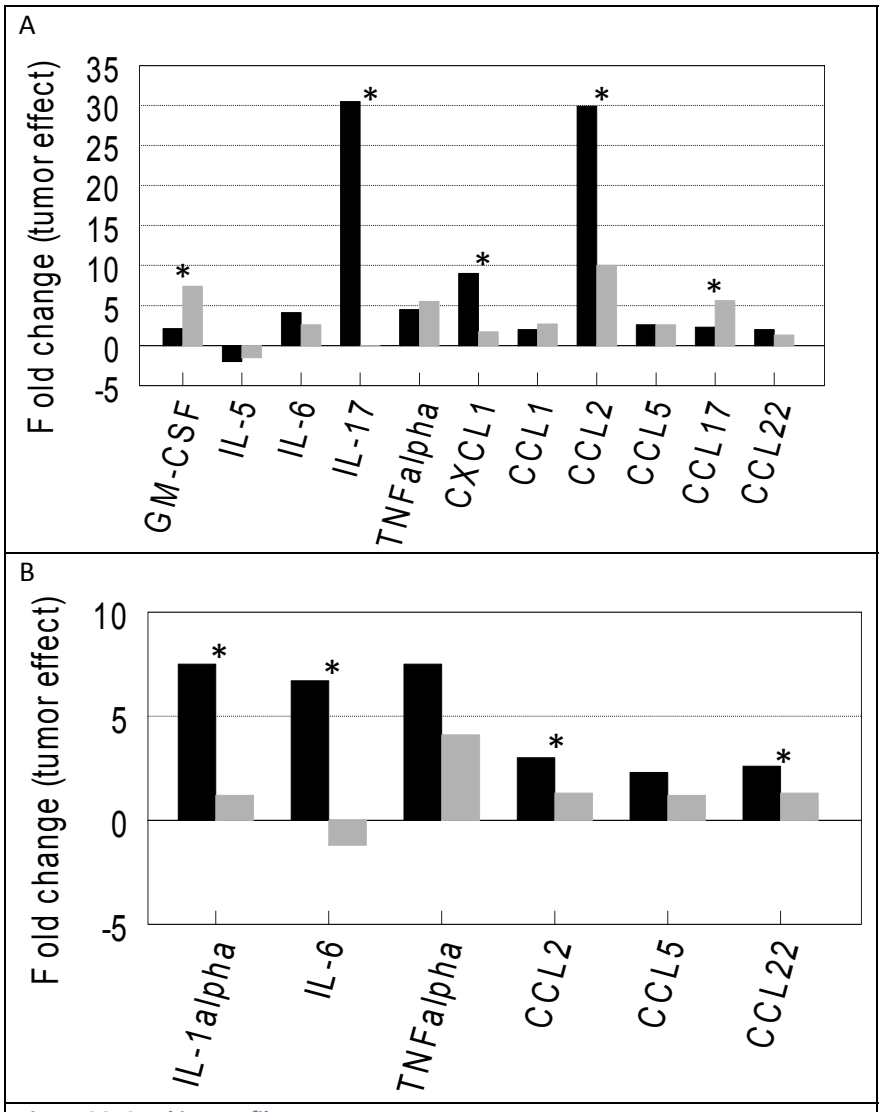

Figure 26: Cytokine profile
(A) In the younger set of mice, the presence of BC in BL/10 mice had a greater effect on plasma content of IL-17, CCL2, and CXCL1 than the presence of BC in *mdx* mice. The presence of BC had a greater effect on plasma GM-CSF and CCL17 in *mdx* mice than the presence of BC in BL/10 mice. (B) In the older set of mice, the presence of BC in BL/10 mice had a greater effect on plasma content of IL-1α, IL-6, CCL2, and CCL22 than the presence of BC in *mdx* mice. Black solid: Analyte content of CON+T relative to CON. Grey solid: Analyte content of MDX+T relative to MDX. * greater than two fold difference between black bar and grey bar.

When comparing the magnitude of tumor-induced changes across genotype and age, it became apparent that both factors affected tumor-related perturbations to the cytokine milieu (Figure 26). With

respect to genotype, the presence of E0771 tumors affected the content of more cytokines in BL/10 mice than in *mdx* mice (6 to 11 cytokines affected v. 1 to 7 cytokines affected). In addition, the tumor-induced changes in plasma cytokine content seemed to be more "tumor-promoting" in BL/10 mice compared to *mdx* mice. For example, the BC-induced increase in some of the cytokines (Table 18 and Table 19) thought to promote tumor invasion and metastasis by stimulating expression of matrix metalloproteinases (i.e., IL-17), promoting angiogenesis, and/or recruiting immune cells to the site of the tumor (i.e., CXCL1, CCL2) [199, 202, 206] was at least 2 times greater in BL/10 mice than in *mdx* mice. In addition, the tumor-induced increase in the anti-tumor cytokine GM-CSF was greater in *mdx* mice than in BL/10 mice. These differences suggest that while the *mdx* phenotype did not affect BC size, it may have a protective effect against BC invasion and metastasis. Further research is necessary to confirm whether *mdx* mice are in fact protected from the invasion and metastasis of BC, and if so, how this protective effect occurs despite the chronic elevations in tumor-promoting cytokines (i.e., IL-6, CXCL1, CCL2) found in *mdx* mice (Chapter 4).

With respect to age, a greater number of cytokines were affected by tumor presence in younger mice (i.e. 5 to 9 wk old, when *mdx* mice were in the "acute phase of myopathy") than in older mice (i.e., 9 to 13 wk old; 7 to 11 cytokines affected v. 1-6 cytokines affected). In addition, tumor-induced changes in plasma cytokines were more pronounced in younger, rather than older mice. These findings suggest that tumor-associated changes in plasma cytokine profiles may be more affected the host than the presence of the tumor itself.

Table 19: Description of chemokines whose content was altered in the plasma of E0771 tumor-bearing mice

Cytokine	Alternate names	Known sources	Known targets	Effect on BC	References
Chemokines: CXCL family					
CXCL1	KC GROα IL-8	Adipocytes Endothelial cells Macrophages	Fibroblasts Neutrophils	Pro-metastasis	[129, 173-175, 197, 206]
Chemokines: CCL family					
CCL1	TCA-3 I-309	T cells	Monocytes Neutrophils T cells		[129, 207]
CCL2	MCP-1	Endothelial cells Adipocytes Macrophages/Monocytes Muscle Tumor cells	Basophils Dendritic cells Monocytes NK cells T cells	Pro-angiogenesis Pro-growth Pro-metastasis	[19, 129, 170, 173-175, 196, 202, 203, 208-211]
CCL3	MIP-1α LD78	Macrophages/Monocytes T cells	Astrocytes Basophils B cells Dendritic cells Eosinophils Fibroblast Monocytes/Macrophages Neutrophils NK cells Osteoclast T cells		[129, 189, 197, 208]
CCL5	RANTES	Monocytes Tumor cells	Basophils Dendritic cells Eosinophils Monocytes/macrophages NK cells T cells	Pro-invasion Pro-metastasis	[129, 204, 208, 212-214]
CCL17	TARC	Macrophages	Dendritic cells Macrophages T cells Thymocytes	Pro-metastasis	[129, 215-218]
CCL22	MDC	Basophils Dendritic cells Eosinophils Neutrophils Monocytes/Macrophages Mast cells Tumor cells	Dendritic cells Endothelial cells Monocytes NK cells T cells Thymocytes	Pro-metastasis	[129, 205, 217, 219, 220]

Together, these differences suggest that tumor-related changes in the circulating cytokine profile of tumor-bearing mice may be driven more by the response of the host to the tumor than by the tumor itself. Identification of the cause(s) of these differences may be useful for the development of novel BC treatments.

Interestingly, despite large (2.6- to > 7.5-fold) tumor-induced increases in plasma content of several cachexia promoting cytokines (i.e., IL-1α, IL-6, TNFα), we found no evidence that tumors derived from this cell line had anything more than a transient effect on food intake, body mass and composition, energy expenditure, or cage activity in *mdx* or BL/10 mice of either age. These results allowed us to arrive at two conclusions: (1) elevations in pro-cachexia cytokines alone are not sufficient to induce cachexia and (2) despite their aggressiveness, E0771 murine mammary adenocarcinoma tumors do not induce cancer-related cachexia or require more than 3 to 4 wk to induce cachexia. Therefore, the murine models of BC described in this article would not be appropriate for the study of BC cachexia.

While we did not observe the hypothesized decrease in the rate of tumor growth in *mdx* mice, the results of our study did reveal that tumor-induced changes in plasma cytokines are less severe in tumor-bearing *mdx* mice than in controls. This finding suggests that the *mdx* phenotype may be somewhat protective against BC invasion and metastasis; however, further research is necessary to confirm this. We also reported several novel findings regarding the E0771 cell line, the most notable of which is that despite inducing elevations in a number of pro-cachectic cytokines, E0771 tumors did not have a lasting effect on body composition, energy expenditure or activity levels, suggesting that elevations in IL-1α, IL-6, TNFα, may not be sufficient to induce BC cachexia.

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Chapter 6: Conclusions

The overarching hypothesis of this dissertation was that systemic down-regulation of DGC components can effect BC growth and cachexia. Three conclusions can be drawn from the experiments used to test this hypothesis. First, there are differences in the body composition and plasma cytokine profiles between *mdx* and BL/10 mice. Some of these differences conflict with the differences typically reported in patients with DMD, suggesting that the appropriateness of the *mdx* mouse as a model of DMD varies with the endpoint of interest. Second, from the absence of a tumor effect on body mass and composition and skeletal muscle function, we can conclude that despite its aggressiveness, the E0771 cell line does not induce cachexia. Finally, tumor–induced increases in pro-invasion and metastasis cytokines were not as severe in *mdx* mouse plasma. This was most noticeable in young *mdx* mice, suggesting that the acute phase of DMD may be protective against BC invasion and metastasis. Further research is necessary to confirm this effect and to determine the mechanism responsible.

Chapter 7: Summary

Alterations to the DGC have been reported in the mammary tumors of patients with BC and in the skeletal muscles of patients with DMD. Despite this similarity, the relationship between these two diseases has not been explored. The studies described in this dissertation were designed to address this gap in the literature.

In Chapter 2, we summarized the body of literature currently available on BC related cachexia. While the availability of BC-specific cachexia articles is relatively limited, there is evidence to suggest that in BC, cachexia related loss of body mass may be due to systemic changes in carbohydrate, lipid, and fat metabolism. These changes in metabolism seem to be due to a combination of factors, including high tumor energy demand and the release of cytokines from the tumor and its microenvironment. Treatments such as megestrol acetate, energy modulating vitamins and eicosapentaenoic acid may prove useful in reducing the severity of BC cachexia. Because of the high incidence of BC among women in the United States and the devastating effects of cachexia on affected patients, further study of cachexia in BC is imperative.

In Chapter 3, we described a pilot study that was designed to measure BC growth and cachexia in *mdx* and BL/10 control mice. The presence of E0771 murine mammary tumors has no lasting effect on markers of cachexia (e.g., body mass, muscle mass, skeletal muscle function) in either genotype. However, E0771 cells injected into *mdx* mice were less likely to form tumors than those injected into BL/10 control mice. When tumors did develop, those in *mdx* mice grew more slowly than those in BL/10 mice. The latter two findings suggest systemic alteration of the DGC, as in the *mdx* mouse model of DMD, may blunt the establishment and growth of BC and prompted the studies described in Chapters 4 and 5 of this dissertation.

In Chapter 4, the body composition, energy expenditure and plasma cytokine profile of *mdx* mice were compared to that of BL/10 mice. We observed differences in the body composition of 5- to 13-wk-old female *mdx* mice and age-matched controls that did not mimic differences typically reported in patients with DMD. In contrast, the altered plasma cytokine profile we observed in *mdx* mice was quite similar to that reported in patients with DMD. These findings provide support for the notion that the *mdx* mouse may not be the most accurate phenotypic model of DMD and that its suitability for use in DMD research may depend on the study objective. Future research should correlate changes in the plasma cytokine profile to progression of skeletal muscle damage. Advances in this area could provide researchers and physicians with a non-invasive method to determine the extent of skeletal muscle damage and the effectiveness of treatments in patients with DMD.

Chapter 5 of this dissertation described a study designed to assess the effect of the *mdx* phenotype on BC growth. In contrast to the results of our pilot study (Chapter 3), BC growth was not slower in *mdx* mice. However, the BC-induced alteration in plasma cytokines was more favorable in *mdx* mice, suggesting that these mice may be somewhat protected from BC invasion and metastasis. Future studies should confirm whether this is the case and, if so, determine the mechanism responsible for this effect.

In conclusion, we believe that a more focused effort is necessary to determine effective treatment for BC cachexia. In addition, we identified a number of differences between *mdx* and BL/10 mice and provided evidence to suggest that the appropriateness of the *mdx* mouse as a model of DMD varies based on the study objective. Finally, our results suggest that BC invasion and metastasis may be blunted in *mdx* mice. Further research in this area could reveal a novel target for the treatment of BC.

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Appendices

Appendix A: Annotated list of figures

Figure 1: Metabolic changes in BC cachexia

BC cachexia is associated with systemic alterations in macronutrient metabolism. This figure summarizes the changes in markers of carbohydrate, lipid, and protein metabolism that have been reported in the tissues of patients and/or rodents with BC.

↑ increased, ↓ decreased, ↔ not different, NR no information available

Figure 2: BC cachexia associated cytokines

Cytokines IL-1α, TNF-α, and IL-6 appear to induce cachexia in BC. The mechanisms by which they exert their effects has not been studied in BC specifically, but may be similar to those reported in other types of cachexia. Arrow head: stimulates, Closed circle: inhibits.

Figure 3: IL-1α mechanism of action

The mechanism of action for IL-1α has not been studied in BC cachexia specifically. However, research in other types of cachexia suggests that it may induce cachexia by promoting the ubiquitin proteasome pathway, inhibiting the IGF-1 pathway, or inhibiting lipoprotein lipase activity. Arrow head: stimulates, Closed circle: inhibits.

Figure 4: IL-6 mechanism of action

The mechanism of action for IL-6 has not been studied in BC cachexia specifically. Research in other types of cachexia suggests that may induce an acute phase response, thereby depleting the pool of AAs necessary for protein synthesis. Arrow head: stimulates, Closed circle: inhibits.

Figure 5: TNF-alpha mechanism of action

TNF-α is thought to induce cachexia via a number of mechanisms. However, these mechanisms have not been studied specifically in BC. Arrow head: stimulates, Closed circle: inhibits.

Figure 6: Utrophin content

Utrophin expression in human and murine mammary epithelial cell and human and murine mammary adenocarcinoma lysates. Utrophin was expressed in human, but not murine, cell lines. Expression was depressed in the human mammary adenocarcinoma cell line compared to the non-tumorigenic control.

Figure 7: EGFR content

Tumor EGFR and Her2 content was not affected by genotype. (n=4-7 tumors/group).

Black solid: BL/10, Grey solid: mdx.

Figure 8: Body mass

The body mass of mdx mice was greater than that of BL/10 mice at all time points. At the 6 wk time point, the body mass of MY6 was greater than that of MN6. Black solid: 10N6, Black striped: 10Y6, Grey solid: MN6, Grey striped: MY6. (n=2-11 mice/group) (B) The presence of BC was associated with a greater body mass gain in mdx mice. Change in body mass was not affected by presence of BC in BL/10 mice. Black solid: with BC, Grey solid: without tumor. (n=8-11 mice/group). * different from 10N6, † different from 10Y6, § different from MN6.

Figure 9: EDL contractile properties

(A) EDL stress frequency curve. An interaction between treatment group and stimulation frequency was observed. At stimulation frequencies of 1-100 Hz, MN6 and MY6 EDLs produced less stress than those from 10N6. There was no difference among groups at 150 Hz.* MN6 and MY6 different from 10N6, † MN6 different from 10Y6, § MY6 different from 10Y6. (B) EDL fatigue profile. An interaction between treatment group and time was observed. * MN6 and MY6 different from 10N6, † 10Y6 different from 10N6. (C) EDL recovery profile. An interaction between treatment group and time was observed. * MN6 and MY6 different from 10N6 and 10Y6, † MN6 and MY6 different from 10Y6 and MY6 different from 10N6, § MN6 different from 10N6 and MY6 different from 10N6 and 10Y6.

(n=11-18 muscles/group) Black solid: 10N6, Black striped: 10Y6, Grey solid: MN6, Grey striped: MY6.

Figure 10: Tumor formation success rate and time to euthanasia

(A) The development of tumors was less frequent at injection sites on mdx mice than at injection sites on BL/10 mice at most time points. (n=8-36 tumors/genotype). (B) Because of excessive tumor burden and/or tumor ulceration, tumor bearing BL/10 mice needed to be euthanized earlier than tumor bearing mdx mice. (n=8 mice/genotype.

Black: BL/10, Grey: mdx, * different from mdx.

Figure 11: Tumor surface area and mass

(A) An interaction between time and genotype on TSA was observed. Post-hoc analyses suggest that TSA is greater in BL/10 mice compared to mdx mice at weeks 1, 2, 3 and 5. (n=8-36 tumors/genotype). (B) When injection sites that failed to form tumors were removed from calculation, there was no difference in average TSA between genotypes. (n=7-36 tumors/genotype). (C) After 6 weeks of tumor growth,

there was no difference in tumor mass between BL/10 and mdx mice. (n=7-9 mice/group). (D) When expressed as a per cent of body mass, there was a trend for tumor mass to be less in mdx compared to BL/10 mice. (n=7-9 mice/group).

Black solid: BL/10, Grey Solid: mdx * different from BL/10.

Figure 12: Cytokine profiles

Ratios of select cytokines in pooled (n=5 mice/group) tumor homogenates. (A) There was no difference in cytokine plasma content between MY3 and 6Y3. (B) There was no difference in cytokine plasma content between MY6 and 10Y6. (C) Plasma content of IFN-γ, IL-6, CCL2, and CCL5 decreased over time in tumor-bearing mdx mice. Plasma content of IL-1β did not change over time. * greater than 2-fold difference.

Figure 13: Effect of plasma on cell growth

E0771 cells were treated with pooled plasma (1% in DMEM) from each treatment group. After 48 hrs of treatment, there was no difference in cell number among groups. (n=5 replicates; P=0.4337)

Figure 14: Plasma creatine kinase activity

At the 3 wk time point, plasma creatine kinase was greater in mdx mice than in BL/6 mice. Plasma creatine kinase activity was not affected by the presence of BC in any genotype or at any time point. (n=0-8 mice/genotype)

Striped: w/o BC, Solid: w/ BC, Grey: 3 wk Black: 6 wk

Figure 15: Cytokine profiles

Ratios of select cytokines in pooled (n=5 mice/group) plasma samples. (A) IL-5 content was greater, and IL-6, IL-12p70, CCL2, and CCL5 content was lower in 6Y3 plasma compared to 6N3 plasma. (B) Plasma IL-6 and CCL2 content was greater in MN3 plasma compared to 6N3. (C) Plasma IL-6, IL-12p70, and CCL2 content was greater in MY3 compared to MN3. (D) IL-5 and IL-6 content was greater while CCL2 and CCL5 content was lower in MY3 plasma compared to 6Y3 plasma. * greater than 2-fold difference.

Figure 16: Cytokine profiles

Ratios of select cytokines in pooled (n=5 mice/group) plasma samples. (A) IL-6 and CCL2 content was greater 10Y6 plasma compared to 10N6 plasma. (B) Plasma IL-6 content was greater in MN6 plasma compared to 10N6. (C) Plasma IFN-γ, IL-6, CCL2, and CCL5 content was greater in MY6 compared to MN6. (D) IFN-γ and IL-6 content was greater in MY6 plasma compared to 10Y6 plasma. * greater than 2-fold difference.

Figure 17: Timeline

dpt: days post tumor injection

Figure 18: Body mass

(A) The body mass of MDX5-9 was greater than CON5-9 at 9 wk of age. (B) The body mass of MDX6-13 was greater than CON6-13 at 7 to 13 wk of age. (C) The ADG from 6 to 9 wk of age was greater in mdx mice than in BL/10 mice.

Black solid: CON, gray solid: MDX, *genotype difference ($P<0.05$).

Figure 19: Body composition

(A) A genotype effect (mdx>BL/10) on % lean mass was observed for SET5-9. (B) % lean mass was greater in MDX6-13 than in CON6-13 at 6, 7, 8, 9, 10, 12, and 13 wk, but not at 11 wk. (C) Genotype (mdx<BL/10) and age effects on % fat mass were observed in SET5-9. (D) % fat mass was lower in MDX6-13 than in CON6-13 at all time points. (E) A genotype effect (mdx>BL/10) on % fluid mass was observed for SET5-9. (F) A genotype effect (mdx>BL/10) on % fluid mass was observed for SET6-13.

Black solid line: BL/10, gray solid line: mdx, *genotype difference (P<0.05).

Figure 20: Horizontal cage activity

(A) As expected, cage activity in the horizontal plane was greater during the dark phase than during the light phase in 9-wk-old mice (P=0.0048). (B) An interaction effect (genotype x phase) on horizontal cage activity was observed in 11-wk-old mice (P=0.0426), but post-hoc analyses did not reveal differences among groups.

Black solid: CON, Black striped: MDX, † phase effect

Figure 21: Vertical cage activity

(A) Independent of phase, cage activity in the vertical plane was lower in 9-wk-old mdx mice than in BL/10 mice. (B) An interaction effect (genotype x phase) on vertical cage activity was observed in 11-wk-old mice. Post hoc analyses revealed that cage activity in the vertical plane was greater in mdx mice during both phases

Black solid: CON, Black striped: MDX, *mdx different from age-matched controls, † phase effect (P<0.05).

Figure 22: Relative cytokine/chemokine content

Plasma IL-6, CXCL1, and CCL2 were greater in MDX5-9 and MDX6-13 than in respective controls. Plasma GM-CSF and IL-12p70 were lower in MDX5-9 compared to CON5-9. Gray solid bar: Plasma analyte content in MDX5-9 relative to CON5-9, Gray striped bar: Plasma analyte content in MDX6-13 relative to CON6-13

Figure 23: Timeline

dpt: days post tumor injection

Figure 24: Body mass

(A) In SET5-9, tumor presence was associated with an increased body mass at 27 dpt only. (B) Tumor presence did not affect body mass at any time point in SET6-13. (C) When adjusted for tumor mass, the effect of tumor presence on body mass at 27 dpt in SET5-9 was lost.

Values are mean ± SEM; α=0.05. Black: BL/10. Grey: mdx. Solid: tumor-free. Dashed/striped: tumor-bearing. * genotype effect. † tumor effect.

Figure 25: Body composition

(A) In SET5-9, % lean mass was affected by tumor presence at 15 dpt only. (B)Tumor presence had no effect on % lean mass at any time point in SET6-13. (C) In SET5-9, % fat mass was greater in tumor bearing mice at 1 dpt only. (D) In SET9-13, % fat mass was not affected by tumor presence at any time point. (E) In SET5-9, tumor presence was associated with a greater % fluid mass at 27 dpt only. (F)In SET9-13, tumor presence was associated with a greater % fluid mass at 5 dpt only.

Values are mean ± SEM; α=0.05. Black: BL/10. Grey: mdx. Solid: tumor-free. Dashed/striped: tumor-bearing. * genotype effect. † tumor effect. ‡ Interaction effect (genotype x tumor).

Figure 26: Cytokine profile

(A) In the younger set of mice, the presence of BC in BL/10 mice had a greater effect on plasma content of IL-17, CCL2, and CXCL1 than the presence of BC in mdx mice. The presence of BC had a greater effect on plasma GM-CSF and CCL17 in mdx mice than the presence of BC in BL/10 mice. (B) In the older set of mice, the presence of BC in BL/10 mice had a greater effect on plasma content of IL-1α, IL-6, CCL2, and CCL22 than the presence of BC in mdx mice.

Black solid: Analyte content of CON+T relative to CON. Grey solid: Analyte content of MDX+T relative to MDX. * greater than two fold difference between black bar and grey bar.

Appendix B: Chapter 4 raw data and statistics

Environmental conditions: Temperature

Raw data

Day	AM Temp (°C)	PM Temp (°C)	Comments
SET_{5-9}			
-7	21	22	SET_{5-9} arrived at facility
-6	21	21	
-5	21		
-4	21		
-3	21	22	
-2	21	21	
-1	21	21	
0	21	21	SET_{5-9} E0771 cell injection
1	21	21	
2	21		
3	21		
4	21	21	
5	21	21	
6	21	22	
7	21	21	
8	21	21	
9	21		
10	21		
11	21		
12	21	21	
13	21	21	
14	21	22	
15	21	21	
16	21		
17	21		
18	21	21	
19	21	21	
20	21	22	
21	21	21	
22	21	21	
23	21		
24	21		
25	22	21	
26	21	21	
27	22	21	
28	21	21	SET_{5-9} euthanized
29	21	21	SET_{5-9} euthanized
SET_{6-13}			
-29	21	21	SET_{6-13} arrived at facility
-28	21	21	
-27	20		
-26	21		

Day	AM Temp (°C)	PM Temp (°C)	Comments
-25	21	21	
-24	21	21	
-23	21	21	
-22	21	21	
-21	21		
-20	21		
-19	20		
-18	21	21	
-17	21	21	
-16	21	21	
-15	21	21	
-14	21	21	
-13	20		
-12	20		
-11	20	21	
-10	19	21	
-9	21	21	
-8	21		
-7	21	21	
-6	21		
-5	21		
-4	21	21	
-3	21	21	
-2	21	21	
-1	21	21	
0	21	21	$SET_{6\text{-}13}$ E0771 cell injection
1	21		
2	21		
3	21	21	
4	21	21	
5	21	21	
6	21	21	
7	21		
8	21		
9	21		
10	21	21	
11	21	21	
12	21	21	
13	21	21	
14	21		
15	21		
16	21		
17	21	21	
18	21	21	
19	21	21	
20	21	21	
21	21	21	
22	21		

Day	AM Temp (°C)	PM Temp (°C)	Comments
23	20		
24	21	21	$SET_{6\text{-}13}$ euthanized

Descriptive statistics

	n	Mean	SEM
AM Temp			
$SET_{5\text{-}9}$	37	21°C	0.042°C
$SET_{6\text{-}13}$	54	21°C	0.062°C
PM Temp			
$SET_{5\text{-}9}$	26	21°C	0.088°C
$SET_{6\text{-}13}$	34	21°C	0°C
Avg. Temp			
$SET_{5\text{-}9}$	37	21°C	0.036°C
$SET_{6\text{-}13}$	54	21°C	0.062°C

Inferential statistics

Student's t-test	P>|t|
AM Temp.	0.0034
PM Temp.	0.0221
Avg. Temp	0.0003

Environmental conditions: Relative humidity

Raw data

Day	AM Humidity (%)	PM Humidity (%)	Comments
SET_{5-9}			
-7	46	49	SET_{5-9} arrived at facility
-6	49	47	
-5	47		
-4	49		
-3	47	43	
-2	49	45	
-1	42	45	
0	46	45	SET_{5-9} E0771 cell injection
1	42	44	
2	47		
3	46		
4	41	45	
5	42	43	
6	42	41	
7	42	44	
8	45	44	
9	34		
10	42		
11	38		
12	42	43	
13	44	48	
14	38	32	
15	37	33	
16	37		
17	41		
18	43	38	
19	39	36	
20	40	41	
21	41	41	
22	43	42	
23	37		
24	38		
25	37	44	
26	38	46	
27	44	51	
28	43	46	SET_{5-9} euthanized
29	50	43	SET_{5-9} euthanized
SET_{6-13}			
-29	29	34	SET_{6-13} arrived at facility
-28	32	30	
-27	33		
-26	31		
-25	33	32	

Day	AM Humidity (%)	PM Humidity (%)	Comments
-24	39	34	
-23	32	30	
-22	32	31	
-21	32		
-20	27		
-19	28		
-18	27	27	
-17	35	41	
-16	28	27	
-15	27	26	
-14	28	26	
-13	27		
-12	27		
-11	27	25	
-10	28	24	
-9	25	26	
-8	26		
-7	28	33	
-6	32		
-5	33		
-4	26	25	
-3	23	23	
-2	24	24	
-1	24	33	
0	33	33	$SET_{6\text{-}13}$ E0771 cell injection
1	33		
2	32		
3	29	30	
4	33	33	
5	34	32	
6	32	32	
7	32		
8	33		
9	32		
10	27	27	
11	27	28	
12	30	28	
13	29	33	
14	30		
15	37		
16	33		
17	28	29	
18	32	30	
19	32	32	
20	28	32	
21	32	30	
22	32		
23	24		

Day	AM Humidity (%)	PM Humidity (%)	Comments
24	25	26	SET_{6-13} euthanized

Descriptive statistics

	n	Mean	SEM
AM Humidity			
SET_{5-9}	37	42%	0.67%
SET_{6-13}	54	30%	0.47%
PM Humidity			
SET_{5-9}	26	43%	0.87%
SET_{6-13}	34	30%	0.66%
Avg. Humidity			
SET_{5-9}	37	42%	0.66%
SET_{6-13}	54	30%	0.46%

Inferential statistics

Student's t-test	P>|t|
AM Humidity	<0.0001
PM Humidity	<0.0001
Avg. Humidity	<0.0001

Food intake: SET_{5-9}

Raw data

		24-hr food intake (g)		
Cage	Group	6 wk	7 wk	8 wk
1	CON_{5-9}	3.0	3.5	4.1
8	CON_{5-9}	3.4	3.4	4.0
10	CON_{5-9}	2.7	4.0	
12	MDX_{5-9}	2.5	3.4	3.3
13	MDX_{5-9}	2.9	3.7	4.0
14	MDX_{5-9}	3.6	4.0	3.8
15	MDX_{5-9}	3.3	3.6	3.6
20	CON_{5-9}	3.6	3.8	3.0
23	CON_{5-9}	2.8	4.2	3.2

Descriptive statistics

	CON_{5-9} (n=14)	**MDX_{5-9} (n=12)**
24-hr food intake (g)		
Mean	3.46	3.46
SEM	0.13	0.13
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
24-hr food intake (g)		
6 wk (n=9)	Mean	3.07
	SEM	0.14
	Sig?	--
7 wk (n=9)	Mean	3.71
	SEM	0.09
	Sig?	--
8 wk (n=8)	Mean	3.62
	SEM	0.15
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		CON_{5-9} (n=4-5)	MDX_{5-9} (n=4)
24-hr food intake (g)			
6 wk	Mean	3.09	3.05
	SEM	0.17	0.25
	Sig?	--	--
7 wk	Mean	3.75	3.65
	SEM	0.15	0.12
	Sig?	--	--
8 wk	Mean	3.56	3.68
	SEM	0.27	0.15
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)					
Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	0.00	0.02	1	6	0.8995
Age	2.01	5.03	2	5	0.0635
Interaction	0.06	0.16	2	5	0.8589

Food intake: $SET_{6\text{-}13}$

Raw data

		24-hr food intake (g)	
Cage	Group	11 wk	13 wk
1	$MDX_{6\text{-}13}$	4.1	4.7
2	$CON_{6\text{-}13}$	3.5	3.9
4	$MDX_{6\text{-}13}$	5.0	5.9
5	$CON_{6\text{-}13}$	3.8	3.9
7	$MDX_{6\text{-}13}$	4.0	5.2
11	$MDX_{6\text{-}13}$		4.9
12	$CON_{6\text{-}13}$		4.0
16	$CON_{6\text{-}13}$	3.6	3.9

Descriptive statistics

	$CON_{6\text{-}13}$ (n=7)	**$MDX_{6\text{-}13}$ (n=7)**
24-hr food intake (g)		
Mean	3.80	4.83
SEM	0.07	0.25
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
24-hr food intake (g)		
11 wk (n=6)	Mean	4.00
	SEM	0.22
	Sig?	A
13 wk (n=8)	Mean	4.55
	SEM	0.27
	Sig?	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		CON_{6-13} (n=3-4)	MDX_{6-13} (n=3-4)
24-hr food intake (g)			
11 Wk	Mean	3.63	4.37
	SEM	0.09	0.32
	Sig?	--	--
13 Wk	Mean	3.93	5.18
	SEM	0.03	0.26
	Sig?	A	B

Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)

Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	2.61	10.44	1	4	0.0319
Age	9.01	36.03	1	4	0.0039
Interaction	2.65	10.62	1	4	0.0311

Post-hoc analyses (interaction, CON_{6-13} v. MDX_{6-13})

Student's t-test	P>|t|
11 Wk	0.1393
13 Wk	0.0171

Body mass: SET_{5-9}

Raw data

		Body mass (g)				
ID	Group	5 Wk	6 Wk	7 Wk	8 Wk	9 Wk
10-101	CON_{5-9}	18.5	20.0	20.7	20.9	22.2
10-102	CON_{5-9}	18.1	19.5	19.8	20.3	21.1
10-115	CON_{5-9}	16.9	17.5	18.0	19.2	19.8
10-116	CON_{5-9}	17.6	19.0	19.5	20.7	20.5
10-119	CON_{5-9}	17.9	18.3	18.7	20.0	20.3
10-122	MDX_{5-9}	16.5	17.0	18.2	19.2	19.8
10-123	MDX_{5-9}	17.5	19.5	20.4	21.6	23.0
10-124	MDX_{5-9}	15.4	18.0	18.5	19.3	20.1
10-125	MDX_{5-9}	16.6	17.1	19.1	21.4	23.0
10-126	MDX_{5-9}	19.2	21.8	23.3	24.0	24.8
10-127	MDX_{5-9}	15.9	16.8	18.9	19.9	21.0
10-128	MDX_{5-9}	17.6	19.4	20.1	21.3	21.7
10-129	MDX_{5-9}	17.1	18.9	20.5	21.9	22.6
10-130	MDX_{5-9}	18.1	19.3	21.1	21.9	22.9
10-131	MDX_{5-9}	17.0				
10-138	CON_{5-9}	17.2	18.2	19.1	19.7	20.0
10-139	CON_{5-9}	17.4	18.6	19.4	20.5	21.9
10-144	CON_{5-9}	16.0	16.4	17.1	18.0	19.3
10-145	CON_{5-9}	16.8	17.6	19.3	20.0	20.1

Descriptive statistics

	CON_{5-9} (n=45)	**MDX_{5-9} (n=46)**
Body mass (g)		
Mean	19.1	19.7
SEM	0.2	0.3
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Body mass (g)		
5 Wk (n=19)	Mean	17.2
	SEM	0.2
	Sig?	A
6 Wk (n=18)	Mean	18.5
	SEM	0.3
	Sig?	B
7 Wk (n=18)	Mean	19.5
	SEM	0.3
	Sig?	BC
8 Wk (n=18)	Mean	20.5
	SEM	0.3
	Sig?	CD
9 Wk (n=18)	Mean	21.3
	SEM	0.4
	Sig?	D
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		**CON_{5-9} (n=9)**	**MDX_{5-9} (n=9-10)**
Body mass (g)			
5 Wk	Mean	17.4	17.1
	SEM	0.3	0.3
	Sig?	--	--
6 Wk	Mean	18.3	18.6
	SEM	0.4	0.5
	Sig?	--	--
7 Wk	Mean	19.1	20.0
	SEM	0.3	0.5
	Sig?	--	--
8 Wk	Mean	19.9	21.2
	SEM	0.3	0.5
	Sig?	--	--
9 Wk	Mean	20.6	22.1
	SEM	0.3	0.5
	Sig?	A	B
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)					
Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	0.11	1.77	1	16	0.2023
Age	39.17	127.31	4	13	<0.0001
Interaction	1.98	6.43	4	13	0.0044

Post-hoc analyses (interaction, CON_{5-9} v. MDX_{5-9})	
Student's t-test	P>|t|
5 Wk	0.5113
6 Wk	0.6508
7 Wk	0.1573
8 Wk	0.0532
9 Wk	0.0295

Body mass: SET_{6-13}

Raw data

		Body mass (g)							
ID	Group	6 Wk	7 Wk	8 Wk	9 Wk	10 Wk	11 Wk	12 Wk	13 Wk
10-201	MDX_{6-13}	16.1	18.1	19.5	19.7	21.0	22.1	22.8	23.3
10-202	CON_{6-13}	17.3	17.5	18.4	19.1	20.9	20.6	22.0	22.3
10-203	CON_{6-13}	16.9	16.8	17.8	18.4	19.6	20.0	21.4	22.4
10-206	MDX_{6-13}	18.9	20.6	22.4	23.2	25.1	24.5	25.7	27.4
10-207	MDX_{6-13}	17.7	18.8	19.4	20.2	22.0	22.6	24.0	25.0
10-208	CON_{6-13}	17.4	17.6	18.2	18.9	18.5	20.6	21.8	22.9
10-210	MDX_{6-13}	17.2	18.8	19.9	20.7	22.1	23.1	22.8	24.5
10-211	MDX_{6-13}	17.9	19.1	20.4	21.3	23.0	24.3	24.8	25.8
10-217	MDX_{6-13}	16.4	17.7	20.2	20.6				
10-218	MDX_{6-13}	18.5	20.4	22.1	22.2	24.7	25.0	25.0	25.3
10-219	CON_{6-13}	18.5	18.9	19.2	20.1	20.9	21.5	21.2	21.4
10-220	CON_{6-13}	17.1	17.0	17.9	18.0	18.8	19.4	20.5	21.5
10-227	CON_{6-13}	17.5	18.1	19.4	21.1	21.6	21.8	21.6	22.3
10-228	CON_{6-13}	15.2	16.1	16.8	17.4	18.5	18.6	19.6	20.2

Descriptive statistics

	CON_{6-13} (n=56)	**MDX_{6-13} (n=52)**
Body mass (g)		
Mean	19.4	21.6
SEM	0.3	0.4
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Body mass (g)		
6 Wk (n=14)	Mean	17.3
	SEM	0.3
	Sig?	A
7 Wk (n=14)	Mean	18.3
	SEM	0.3
	Sig?	AB
8 Wk (n=14)	Mean	19.4
	SEM	0.4
	Sig?	BC
9 Wk (n=14)	Mean	20.1
	SEM	0.4
	Sig?	BCD
10 Wk (n=13)	Mean	21.3
	SEM	0.6
	Sig?	CDE
11 Wk (n=13)	Mean	21.9
	SEM	0.6
	Sig?	DEF
12 Wk (n=13)	Mean	22.6
	SEM	0.5
	Sig?	EF
13 Wk (n=13)	Mean	23.4
	SEM	0.6
	Sig?	F
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		CON_{6-13} (n=7)	MDX_{6-13} (n=6-7)
Body mass (g)			
6 Wk	Mean	17.1	17.5
	SEM	0.4	0.4
	Sig?	--	--
7 Wk	Mean	17.4	19.1
	SEM	0.3	0.4
	Sig?	A	B
8 Wk	Mean	18.2	20.6
	SEM	0.3	0.5
	Sig?	A	B
9 Wk	Mean	19.0	21.1
	SEM	0.5	0.5
	Sig?	A	B
10 Wk	Mean	19.8	23.0
	SEM	0.5	0.7
	Sig?	A	B
11 Wk	Mean	20.4	23.6
	SEM	0.4	0.5
	Sig?	A	B
12 Wk	Mean	21.2	24.2
	SEM	0.3	0.5
	Sig?	A	B
13 Wk	Mean	21.9	25.2
	SEM	0.3	0.6
	Sig?	A	B

Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)

Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	1.671	18.38	1	11	0.0013
Age	249.99	178.57	7	5	<0.0001
Interaction	26.80	19.14	7	5	0.0025

Post-hoc analyses (interaction, CON_{6-13} v. MDX_{6-13})	
Student's t-test	P>|t|
6 Wk	0.4742
7 Wk	0.0101
8 Wk	0.0018
9 Wk	0.0074
10 Wk	0.0036
11 Wk	0.0004
12 Wk	0.0006
13 Wk	0.0008

Average daily gain (6 Wk – 9Wk)

Raw data

ID	Group	ADG (g)
SET_{5-9}		
10-101	CON_{5-9}	0.122
10-102	CON_{5-9}	0.080
10-115	CON_{5-9}	0.115
10-116	CON_{5-9}	0.075
10-119	CON_{5-9}	0.100
10-122	MDX_{5-9}	0.156
10-123	MDX_{5-9}	0.175
10-124	MDX_{5-9}	0.117
10-125	MDX_{5-9}	0.295
10-126	MDX_{5-9}	0.150
10-127	MDX_{5-9}	0.210
10-128	MDX_{5-9}	0.115
10-129	MDX_{5-9}	0.185
10-130	MDX_{5-9}	0.200
10-131	MDX_{5-9}	0.000
10-138	CON_{5-9}	0.090
10-139	CON_{5-9}	0.165
10-144	CON_{5-9}	0.145
10-145	CON_{5-9}	0.139
SET_{6-13}		
10-201	MDX_{6-13}	0.180
10-202	CON_{6-13}	0.090
10-203	CON_{6-13}	0.075
10-206	MDX_{6-13}	0.215
10-207	MDX_{6-13}	0.125
10-208	CON_{6-13}	0.075
10-210	MDX_{6-13}	0.175
10-211	MDX_{6-13}	0.170
10-217	MDX_{6-13}	0.210
10-218	MDX_{6-13}	0.185
10-219	CON_{6-13}	0.080
10-220	CON_{6-13}	0.045
10-227	CON_{6-13}	0.180
10-228	CON_{6-13}	0.110

Descriptive statistics

	CON (n=16)	MDX (n=17)
	Average daily gain (g)	
Mean	0.105	0.168
SEM	0.009	0.015
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

	SET_{5-9} (n=19)	(n=14)
	Average daily gain (g)	
Mean	0.139	0.137
SEM	0.014	0.015
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

		CON (n=7-9)	MDX (n=7-10)
Average daily gain (g)			
SET_{5-9}	Mean	0.115	0.160
	SEM	0.010	0.024
	Sig?	--	--
SET_{6-13}	Mean	0.094	0.180
	SEM	0.016	0.011
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

2-way ANOVA (genotype x set)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.04	0.01	4.54	0.0100
Error	29	0.08	0.00		
Total	32	0.11			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.04	13.26	0.0010	
Set	1	0.00	0.00	0.9734	
Interaction	1	0.00	1.26	0.2701	

Post-hoc analyses (genotype, CON v. MDX)	
Student's t-test	P>|t|
	0.0013

Percent lean mass: SET_{5-9}

Raw data

		Lean mass (% of body mass)				
ID	Group	5 wk	6 wk	7 wk	8 wk	9 wk
10-101	CON_{5-9}	58.7	61.2	59.6	62.4	60.9
10-102	CON_{5-9}	65.7	62.0	61.6	63.0	62.7
10-115	CON_{5-9}	61.8	62.0	62.7	60.9	60.7
10-116	CON_{5-9}	60.4	59.6	61.7	56.4	58.5
10-119	CON_{5-9}	60.2	59.6	62.3	61.8	62.5
10-122	MDX_{5-9}	60.1	60.4	61.7	59.5	57.6
10-123	MDX_{5-9}	62.4	63.6	65.0	65.8	64.3
10-124	MDX_{5-9}	61.8	64.4	63.7	61.9	62.9
10-125	MDX_{5-9}	62.0	62.0	64.7	62.3	64.0
10-126	MDX_{5-9}	66.7	63.2	65.8	64.7	65.0
10-127	MDX_{5-9}	62.2	61.6	63.1	64.1	62.4
10-128	MDX_{5-9}	65.8	63.0	64.4	61.3	62.3
10-129	MDX_{5-9}	62.4	63.7	64.4	62.8	63.9
10-130	MDX_{5-9}	62.7	61.6	65.7	64.0	63.6
10-131	MDX_{5-9}	64.6				
10-138	CON_{5-9}	63.8	61.9	62.2	59.7	59.9
10-139	CON_{5-9}	62.4	62.5	61.3	61.0	58.3
10-144	CON_{5-9}	60.6	62.1	59.8	60.4	58.9
10-145	CON_{5-9}	60.8	61.1	57.8	57.8	59.6

Descriptive statistics

	CON_{5-9} (n=45)	**MDX_{5-9} (n=46)**
Lean mass (% of body mass)		
Mean	60.9	63.1
SEM	0.3	0.3
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Lean mass (% of body mass)		
5 Wk (n=19)	Mean	62.3
	SEM	0.5
	Sig?	--
6 Wk (n=18)	Mean	61.9
	SEM	0.3
	Sig?	--
7Wk (n=18)	Mean	62.6
	SEM	0.5
	Sig?	--
8 Wk (n=18)	Mean	61.6
	SEM	0.6
	Sig?	--
9 Wk (n=18)	Mean	61.5
	SEM	0.5
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		**CON_{5-9} (n=9)**	**MDX_{5-9} (n=9-10)**
Lean mass (% of body mass)			
5 Wk	Mean	61.5	63.1
	SEM	0.7	0.6
	Sig?	--	--
6 Wk	Mean	61.3	62.6
	SEM	0.4	0.4
	Sig?	--	--
7 Wk	Mean	60.9	64.3
	SEM	0.5	0.4
	Sig?	--	--
8 Wk	Mean	60.4	62.9
	SEM	0.7	0.6
	Sig?	--	--
9 Wk	Mean	60.2	62.9
	SEM	0.5	0.7
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)

Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	0.84	13.39	1	16	0.0021
Age	0.53	1.74	4	13	0.2016
Interaction	0.56	1.80	4	14	0.1881

Percent lean mass: $SET_{6\text{-}13}$

Raw data

		Lean mass (% of body mass)							
ID	Group	6 wk	7 wk	8 wk	9 wk	10 wk	11 wk	12 wk	13 wk
10-201	$MDX_{6\text{-}13}$	64.8	60.8	60.4	63.4	60.4	84.2	62.6	60.4
10-202	$CON_{6\text{-}13}$	58.1	57.2	58.5	56.8	57.9	82.1	58.2	59.6
10-203	$CON_{6\text{-}13}$	56.8	56.9	57.1	57.4	57.6	58.0	59.9	57.7
10-206	$MDX_{6\text{-}13}$	64.6	62.8	63.2	62.5	64.2	63.5	62.6	64.5
10-207	$MDX_{6\text{-}13}$	63.9	61.3	61.4	61.3	62.2	61.7	63.0	62.8
10-208	$CON_{6\text{-}13}$	58.6	55.0	54.2	53.1	56.9	58.6	58.6	58.3
10-210	$MDX_{6\text{-}13}$	61.7	63.5	62.7	67.4	62.0	61.5	63.7	62.1
10-211	$MDX_{6\text{-}13}$	62.8	62.2	62.3	60.2	59.4	60.2	60.7	61.1
10-217	$MDX_{6\text{-}13}$	63.0	62.4	63.5	63.5				
10-218	$MDX_{6\text{-}13}$	63.3	61.4	62.2	64.3	64.4	63.7	61.7	64.3
10-219	$CON_{6\text{-}13}$	56.2	52.1	51.7	52.7	50.3	51.7	58.4	60.8
10-220	$CON_{6\text{-}13}$	59.6	57.3	60.6	57.2	58.0	58.1	59.5	59.5
10-227	$CON_{6\text{-}13}$	59.1	59.0	60.3	55.5	56.0	56.0	60.4	59.3
10-228	$CON_{6\text{-}13}$	58.0	54.5	54.8	54.3	53.0	51.6	60.6	61.1

Descriptive statistics

	$CON_{6\text{-}13}$ (n=56)	**$MDX_{6\text{-}13}$ (n=52)**
Lean mass (% of body mass)		
Mean	57.5	62.9
SEM	0.6	.5
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Lean mass (% of body mass)		
6 Wk (n=14)	Mean	60.7
	SEM	0.8
	Sig?	--
7 Wk (n=14)	Mean	59.0
	SEM	1.0
	Sig?	--
8 Wk (n=14)	Mean	59.5
	SEM	1.0
	Sig?	--
9 Wk (n=14)	Mean	59.2
	SEM	1.2
	Sig?	--
10 Wk (n=13)	Mean	58.6
	SEM	1.2
	Sig?	--
11 Wk (n=13)	Mean	62.4
	SEM	2.8
	Sig?	--
12 Wk (n=13)	Mean	60.7
	SEM	0.5
	Sig?	--
13 Wk (n=13)	Mean	60.9
	SEM	0.6
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		CON_{6-13} (n=7)	MDX_{6-13} (n=6-7)
Lean mass (% of body mass)			
6 Wk	Mean	58.0	63.4
	SEM	0.5	0.4
	Sig?	A	B
7 Wk	Mean	56.0	62.0
	SEM	0.9	0.4
	Sig?	A	B
8 Wk	Mean	56.7	62.2
	SEM	1.3	0.4
	Sig?	A	B
9 Wk	Mean	55.3	63.2
	SEM	0.7	0.9
	Sig?	A	B
10 Wk	Mean	55.7	62.1
	SEM	1.1	0.8
	Sig?	A	B
11 Wk	Mean	59.4	65.8
	SEM	3.9	3.7
	Sig?	--	--
12 Wk	Mean	59.4	62.4
	SEM	0.4	0.4
	Sig?	A	B
13 Wk	Mean	59.4	62.5
	SEM	0.5	0.7
	Sig?	A	B
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)					
Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	2.70	29.70	1	11	0.0002
Age	5.30	3.79	7	5	0.0809
Interaction	16.96	12.12	7	5	0.0072

Post-hoc analyses (interaction, CON_{6-13} v. MDX_{6-13})	
Student's t-test	P>|t|
6 Wk	<0.0001
7 Wk	0.0002
8 Wk	0.0039
9 Wk	<0.0001
10 Wk	0.0008
11 Wk	0.2656
12 Wk	0.0003
13 Wk	0.0043

Percent fat mass: SET_{5-9}

Raw data

		Fat mass (% of body mass)				
ID	Group	5 wk	6 Wk	7 wk	8 wk	9 wk
10-101	CON_{5-9}	6.1	3.8	5.5	4.7	5.3
10-102	CON_{5-9}	3.6	4.1	3.7	2.8	3.9
10-115	CON_{5-9}	3.8	4.0	4.3	5.2	5.8
10-116	CON_{5-9}	4.1	5.5	4.7	7.5	7.6
10-119	CON_{5-9}	5.4	6.2	4.2	4.1	3.0
10-122	MDX_{5-9}	4.6	6.2	6.5	7.3	8.6
10-123	MDX_{5-9}	3.2	1.9	1.9	0.6	3.4
10-124	MDX_{5-9}	4.1	3.5	4.0	3.8	4.1
10-125	MDX_{5-9}	3.3	3.7	2.0	4.2	3.2
10-126	MDX_{5-9}	1.7	3.1	2.3	1.6	3.2
10-127	MDX_{5-9}	4.3	5.3	3.0	2.4	5.5
10-128	MDX_{5-9}	3.0	3.5	3.8	4.8	4.5
10-129	MDX_{5-9}	3.5	3.0	2.5	3.6	2.6
10-130	MDX_{5-9}	3.0	3.9	1.5	1.8	3.5
10-131	MDX_{5-9}	2.3				
10-138	CON_{5-9}	3.1	4.7	3.5	4.8	5.7
10-139	CON_{5-9}	4.1	5.6	4.1	5.6	7.1
10-144	CON_{5-9}	5.2	4.6	5.5	5.6	6.2
10-145	CON_{5-9}	4.0	5.2	5.6	6.2	6.6

Descriptive statistics

	CON_{5-9} (n=45)	**MDX_{5-9} (n=46)**
Fat mass (% of body mass)		
Mean	4.9	3.5
SEM	0.2	0.2
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Fat mass (% of body mass)		
5 Wk (n=19)	Mean	3.8
	SEM	0.2
	Sig?	--
6 Wk (n=18)	Mean	4.3
	SEM	0.3
	Sig?	--
7Wk (n=18)	Mean	3.8
	SEM	0.3
	Sig?	--
8 Wk (n=18)	Mean	4.2
	SEM	0.5
	Sig?	--
9 Wk (n=18)	Mean	5.0
	SEM	0.4
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		**CON_{5-9} (n=9)**	**MDX_{5-9} (n=9-10)**
Fat mass (% of body mass)			
5 Wk	Mean	4.4	3.3
	SEM	0.3	0.3
	Sig?	--	--
6 Wk	Mean	4.8	3.8
	SEM	0.3	0.4
	Sig?	--	--
7 Wk	Mean	4.5	3.0
	SEM	0.3	0.5
	Sig?	--	--
8 Wk	Mean	5.2	3.3
	SEM	0.4	0.7
	Sig?	--	--
9 Wk	Mean	5.7	4.3
	SEM	0.5	0.6
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)

Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	0.45	7.25	1	16	0.0160
Age	1.24	4.04	4	13	0.0241
Interaction	0.12	0.40	4	13	0.8084

Percent fat mass: SET_{6-13}

Raw data

		Fat mass (% of body mass)							
ID	Group	6 wk	7 wk	8 wk	9 wk	10 wk	11 wk	12 wk	13 wk
10-201	MDX_{6-13}	2.1	3.5	3.8	1.5	3.6	3.8	4.0	4.0
10-202	CON_{6-13}	5.2	6.0	5.3	5.6	4.8	4.3	5.2	5.2
10-203	CON_{6-13}	6.3	6.2	5.9	5.3	4.8	4.7	4.4	5.4
10-206	MDX_{6-13}	1.5	2.1	2.3	2.0	1.8	2.3	2.4	2.3
10-207	MDX_{6-13}	2.3	1.6	2.7	1.6	3.2	2.8	2.1	2.9
10-208	CON_{6-13}	6.8	8.2	8.0	8.7	5.4	5.6	4.5	5.2
10-210	MDX_{6-13}	3.1	1.1	2.7	1.1	1.9	3.3	2.0	2.8
10-211	MDX_{6-13}	4.0	4.1	3.6	3.4	4.1	4.3	4.4	4.1
10-217	MDX_{6-13}	1.8	2.2	1.6	2.2				
10-218	MDX_{6-13}	2.4	2.1	2.5	1.4	1.4	1.7	2.3	2.3
10-219	CON_{6-13}	6.5	8.5	10.2	10.4	12.2	12.0	5.2	4.0
10-220	CON_{6-13}	4.7	4.9	4.1	4.7	4.9	4.6	4.3	3.8
10-227	CON_{6-13}	5.7	5.6	4.9	4.8	8.2	7.7	4.9	4.8
10-228	CON_{6-13}	5.6	7.0	8.0	7.7	10.2	10.9	3.4	3.8

Descriptive statistics

	CON_{6-13} (n=56)	**MDX_{6-13} (n=52)**
Fat mass (% of body mass)		
Mean	6.1	2.6
SEM	0.3	0.1
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Fat mass (% of body mass)		
6 Wk (n=14)	Mean	4.1
	SEM	0.5
	Sig?	--
7 Wk (n=14)	Mean	4.5
	SEM	0.7
	Sig?	--
8 Wk (n=14)	Mean	4.7
	SEM	0.7
	Sig?	--
9 Wk (n=14)	Mean	4.3
	SEM	0.8
	Sig?	--
10 Wk (n=13)	Mean	5.1
	SEM	0.9
	Sig?	--
11 Wk (n=13)	Mean	5.2
	SEM	0.9
	Sig?	--
12 Wk (n=13)	Mean	3.8
	SEM	0.3
	Sig?	--
13 Wk (n=13)	Mean	3.9
	SEM	0.3
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		CON_{6-13} (n=7)	MDX_{6-13} (n=6-7)
Fat mass (% of body mass)			
6 Wk	Mean	5.8	2.5
	SEM	0.3	0.3
	Sig?	A	B
7 Wk	Mean	6.6	2.4
	SEM	0.5	0.4
	Sig?	A	B
8 Wk	Mean	6.6	2.7
	SEM	0.8	0.3
	Sig?	A	B
9 Wk	Mean	6.7	1.9
	SEM	0.8	0.3
	Sig?	A	B
10 Wk	Mean	7.2	2.6
	SEM	1.2	0.5
	Sig?	A	B
11 Wk	Mean	7.1	3.0
	SEM	1.2	0.4
	Sig?	A	B
12 Wk	Mean	4.5	2.8
	SEM	0.2	0.4
	Sig?	A	B
13 Wk	Mean	4.6	3.0
	SEM	0.3	0.3
	Sig?	A	B
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)					
Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	2.69	29.58	1	11	0.0002
Age	2.67	1.91	7	5	0.2470
Interaction	6.84	4.88	7	5	0.0498

Post-hoc analyses (interaction, CON_{6-13} v. MDX_{6-13})	
Student's t-test	P>|t|
6 Wk	<0.0001
7 Wk	<0.0001
8 Wk	0.0025
9 Wk	0.0008
10 Wk	0.0065
11 Wk	0.0139
12 Wk	0.0087
13 Wk	0.0046

Percent fluid mass: SET_{5-9}

Raw data

		Fluid mass (% of body mass)				
ID	Group	5 wk	6 wk	7 wk	8 wk	9 wk
10-101	CON_{5-9}	5.6	6.1	6.0	6.0	6.1
10-102	CON_{5-9}	5.7	5.5	5.5	5.6	5.0
10-115	CON_{5-9}	6.1	5.7	5.6	5.8	5.9
10-116	CON_{5-9}	5.3	5.2	5.8	5.1	5.5
10-119	CON_{5-9}	6.3	5.4	5.7	6.0	5.8
10-122	MDX_{5-9}	6.0	5.8	6.1	6.0	5.7
10-123	MDX_{5-9}	6.1	6.6	6.3	7.0	5.8
10-124	MDX_{5-9}	5.8	6.2	6.0	5.9	5.9
10-125	MDX_{5-9}	6.0	5.6	6.3	5.8	6.0
10-126	MDX_{5-9}	6.8	6.0	6.5	6.6	6.1
10-127	MDX_{5-9}	6.0	6.1	6.6	6.6	5.8
10-128	MDX_{5-9}	6.4	5.9	6.3	6.0	5.8
10-129	MDX_{5-9}	6.0	6.1	6.3	6.3	6.1
10-130	MDX_{5-9}	6.4	6.1	6.8	6.2	5.9
10-131	MDX_{5-9}	6.65				
10-138	CON_{5-9}	6.1	5.6	6.4	5.7	5.1
10-139	CON_{5-9}	5.8	5.5	5.9	6.0	5.5
10-144	CON_{5-9}	5.8	6.3	5.5	5.7	5.6
10-145	CON_{5-9}	5.7	5.5	5.5	6.0	6.1

Descriptive statistics

	CON_{5-9} (n=45)	**MDX_{5-9} (n=46)**
Fluid mass (% of body mass)		
Mean	5.7	6.1
SEM	0.1	0.1
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Fluid mass (% of body mass)		
5 Wk (n=19)	Mean	6.0
	SEM	0.1
	Sig?	--
6 Wk (n=18)	Mean	5.8
	SEM	0.1
	Sig?	--
7Wk (n=18)	Mean	6.0
	SEM	0.1
	Sig?	--
8 Wk (n=18)	Mean	6.0
	SEM	0.1
	Sig?	--
9 Wk (n=18)	Mean	5.7
	SEM	0.1
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		**CON_{5-9} (n=9)**	**MDX_{5-9} (n=9-10)**
Fluid mass (% of body mass)			
5 Wk	Mean	5.8	6.2
	SEM	0.1	0.1
	Sig?	--	--
6 Wk	Mean	5.6	6.0
	SEM	0.1	0.1
	Sig?	--	--
7 Wk	Mean	5.7	6.3
	SEM	0.1	0.1
	Sig?	--	--
8 Wk	Mean	5.7	6.2
	SEM	0.1	0.1
	Sig?	--	--
9 Wk	Mean	5.6	5.9
	SEM	0.1	0.0
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)

Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	1.61	25.75	1	16	<0.0001
Age	0.82	2.67	4	13	0.0795
Interaction	0.27	0.86	4	13	0.5112

Percent fluid mass: $SET_{6\text{-}13}$

Raw data

		Fluid mass (% of body mass)							
ID	Group	6 wk	7 wk	8 wk	9 wk	10 wk	11 wk	12 wk	13 wk
10-201	$MDX_{6\text{-}13}$	6.5	6.05	6.1	6.6	5.8	7.6	5.7	5.5
10-202	$CON_{6\text{-}13}$	5.3	5.3	5.4	5.3	6.1	7.2	5.8	5.3
10-203	$CON_{6\text{-}13}$	5.4	5.3	5.5	5.5	5.4	5.2	5.7	5.6
10-206	$MDX_{6\text{-}13}$	6.8	6.15	6.3	6.4	6.7	6.0	6.0	6.2
10-207	$MDX_{6\text{-}13}$	6.3	6.6	5.6	6.5	5.5	6.2	6.6	6.4
10-208	$CON_{6\text{-}13}$	5.5	5.6	5.3	5.3	5.0	5.0	5.9	5.7
10-210	$MDX_{6\text{-}13}$	6.2	6.6	5.9	7.2	6.4	5.8	6.1	5.7
10-211	$MDX_{6\text{-}13}$	5.85	6.0	6.0	6.0	5.9	5.9	6.0	5.6
10-217	$MDX_{6\text{-}13}$	6.2	6.2	7.0	6.2				
10-218	$MDX_{6\text{-}13}$	6.2	6.4	6.5	6.4	6.9	6.9	6.1	6.0
10-219	$CON_{6\text{-}13}$	5.4	5.6	5.4	5.8	5.4	6.0	5.8	6.1
10-220	$CON_{6\text{-}13}$	5.2	5.3	5.7	5.3	5.2	5.5	5.0	5.5
10-227	$CON_{6\text{-}13}$	5.1	5.2	6.0	5.9	5.4	5.5	5.7	5.5
10-228	$CON_{6\text{-}13}$	4.9	5.1	5.2	5.3	5.2	5.4	6.4	5.9

Descriptive statistics

	$CON_{6\text{-}13}$ (n=56)	**$MDX_{6\text{-}13}$ (n=52)**
Fluid mass (% of body mass)		
Mean	5.5	6.2
SEM	0.1	0.1
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Age		
Fluid mass (% of body mass)		
6 Wk (n=14)	Mean	5.7
	SEM	0.2
	Sig?	--
7 Wk (n=14)	Mean	5.8
	SEM	0.1
	Sig?	--
8 Wk (n=14)	Mean	5.8
	SEM	0.1
	Sig?	--
9 Wk (n=14)	Mean	6.0
	SEM	0.2
	Sig?	--
10 Wk (n=13)	Mean	5.7
	SEM	0.2
	Sig?	--
11 Wk (n=13)	Mean	6.0
	SEM	0.2
	Sig?	--
12 Wk (n=13)	Mean	5.9
	SEM	0.1
	Sig?	--
13 Wk (n=13)	Mean	5.8
	SEM	0.1
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Age		CON_{6-13} (n=7)	MDX_{6-13} (n=6-7)
Fluid mass (% of body mass)			
6 Wk	Mean	5.3	6.3
	SEM	0.1	0.1
	Sig?	--	--
7 Wk	Mean	5.3	6.3
	SEM	0.1	0.1
	Sig?	--	--
8 Wk	Mean	5.5	6.2
	SEM	0.1	0.2
	Sig?	--	--
9 Wk	Mean	5.5	6.5
	SEM	0.1	0.1
	Sig?	--	--
10 Wk	Mean	5.4	6.2
	SEM	0.1	0.2
	Sig?	--	--
11 Wk	Mean	5.7	6.4
	SEM	0.3	0.3
	Sig?	--	--
12 Wk	Mean	5.7	6.1
	SEM	0.2	0.1
	Sig?	--	--
13 Wk	Mean	5.6	5.9
	SEM	0.1	0.1
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x age)					
Source	Value	Exact F	Num DF	Den DF	*P*>F
Genotype	5.90	64.93	1	11	<0.0001
Age	0.90	0.64	7	5	0.7136
Interaction	3.14	2.24	7	5	0.1954

Total energy expenditure (by phase): SET_{5-9}

Raw data

		TEE by phase (kJ/kg/hr)	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-101	CON_{5-9}	133.37	154.28
10-102	CON_{5-9}	140.52	154.97
10-115	CON_{5-9}	134.42	163.40
10-119	CON_{5-9}	129.69	169.03
10-122	MDX_{5-9}	147.48	170.43
10-124	MDX_{5-9}	140.73	166.66
10-125	MDX_{5-9}	156.25	168.06
10-129	MDX_{5-9}	132.35	156.24
10-130	MDX_{5-9}	134.60	164.26
10-145	CON_{5-9}	146.17	194.26

Descriptive statistics

	CON_{5-9} (n=10)	**MDX_{5-9} (n=10)**
TEE by phase (kJ/kg/hr)		
Mean	152.01	153.71
SEM	6.27	4.48
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		
TEE by phase (kJ/kg/hr)		
Light (0700-1459) (n=10)	Mean	139.56
	SEM	2.64
	Sig?	A
Dark (1500-0659) (n=10)	Mean	166.16
	SEM	3.65
	Sig?	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		CON_{5-9} (n=5)	MDX_{5-9} (n=5)
TEE by phase (kJ/kg/hr)			
Light (0700-1459)	Mean	136.83	142.28
	SEM	2.91	4.37
	Sig?	--	--
Dark (1500-0659)	Mean	167.19	165.13
	SEM	7.30	2.44
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.01	0.09	1	8	0.7718
Phase	7.71	61.7	1	8	<0.0001
Interaction	0.15	1.23	1	8	0.3003

Total energy expenditure (by phase): $SET_{6\text{-}13}$

Raw data

		TEE by phase (kJ/kg/hr)	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-206	$MDX_{6\text{-}13}$	137.70	170.11
10-207	$MDX_{6\text{-}13}$	139.24	204.26
10-208	$CON_{6\text{-}13}$	154.18	164.21
10-210	$MDX_{6\text{-}13}$	140.57	177.44
10-211	$MDX_{6\text{-}13}$	134.67	165.94
10-218	$MDX_{6\text{-}13}$	123.61	168.37
10-219	$CON_{6\text{-}13}$	166.73	181.92
10-220	$CON_{6\text{-}13}$	155.55	190.02
10-227	$CON_{6\text{-}13}$	138.50	171.44
10-228	$CON_{6\text{-}13}$	173.86	202.62

Descriptive statistics

	$CON_{6\text{-}13}$ (n=10)	**$MDX_{6\text{-}13}$ (n=10)**
TEE by phase (kJ/kg/hr)		
Mean	169.90	156.19
SEM	5.89	7.89
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		
TEE by phase (kJ/kg/hr)		
Light (0700-1459) (n=10)	Mean	146.46
	SEM	4.94
	Sig?	A
Dark (1500-0659) (n=10)	Mean	179.63
	SEM	4.67
	Sig?	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		CON_{6-13} (n=5)	MDX_{6-13} (n=5)
TEE by phase (kJ/kg/hr)			
Light (0700-1459)	Mean	157.76	135.16
	SEM	6.04	3.05
	Sig?	--	--
Dark (1500-0659)	Mean	182.04	177.23
	SEM	6.77	7.03
	Sig?	--	--
Within each time point, levels not connected by the same letter are different Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.43	3.43	1	8	0.1011
Phase	8.77	70.13	1	8	<0.0001
Interaction	0.63	5.04	1	8	0.0550

Respiratory exchange ratio (by phase): SET_{5-9}

Raw data

		RER by phase	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-101	CON_{5-9}	0.92	0.96
10-102	CON_{5-9}	0.90	0.93
10-115	CON_{5-9}	0.89	0.98
10-119	CON_{5-9}	0.93	0.99
10-122	MDX_{5-9}	0.89	0.96
10-124	MDX_{5-9}	0.87	0.96
10-125	MDX_{5-9}	0.85	0.98
10-129	MDX_{5-9}	0.91	0.97
10-130	MDX_{5-9}	0.87	0.96
10-145	CON_{5-9}	0.87	0.95

Descriptive statistics

	CON_{5-9} (n=10)	**MDX_{5-9} (n=10)**
RER by phase		
Mean	0.93	0.92
SEM	0.01	0.02
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		
RER by phase		
Light (0700-1459) (n=10)	Mean	0.89
	SEM	0.01
	Sig?	A
Dark (1500-0659) (n=10)	Mean	0.96
	SEM	0.01
	Sig?	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		CON_{5-9} (n=5)	MDX_{5-9} (n=5)
RER by phase			
Light (0700-1459)	Mean	0.90	0.88
	SEM	0.01	0.01
	Sig?	--	--
Dark (1500-0659)	Mean	0.96	0.97
	SEM	0.01	0.00
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.08	0.65	1	8	0.4431
Phase	9.78	78.20	1	8	<0.001
Interaction	0.38	3.02	1	8	0.1205

Respiratory exchange ratio (by phase): $SET_{6\text{-}13}$

Raw data

		RER by phase	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-206	$MDX_{6\text{-}13}$	0.87	0.94
10-207	$MDX_{6\text{-}13}$	0.88	0.94
10-208	$CON_{6\text{-}13}$	0.90	0.95
10-210	$MDX_{6\text{-}13}$	0.88	0.95
10-211	$MDX_{6\text{-}13}$	0.84	0.92
10-218	$MDX_{6\text{-}13}$	0.90	0.96
10-219	$CON_{6\text{-}13}$	0.87	0.86
10-220	$CON_{6\text{-}13}$	0.92	0.98
10-227	$CON_{6\text{-}13}$	0.88	0.98
10-228	$CON_{6\text{-}13}$	0.85	0.85

Descriptive statistics

	$CON_{6\text{-}13}$ (n=10)	**$MDX_{6\text{-}13}$ (n=10)**
RER by phase		
Mean	0.91	0.91
SEM	0.02	0.01
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		
RER by phase		
Light (0700-1459) (n=10)	Mean	0.88
	SEM	0.01
	Sig?	A
Dark (1500-0659) (n=10)	Mean	0.93
	SEM	0.01
	Sig?	B
Among time points, levels not connected by the same letter are different Student's t-test, α=0.05)		

Phase		CON_{6-13} (n=5)	MDX_{6-13} (n=5)
RER by phase			
Light (0700-1459)	Mean	0.89	0.87
	SEM	0.01	0.01
	Sig?	--	--
Dark (1500-0659)	Mean	0.92	0.94
	SEM	0.03	0.01
	Sig?	--	--

Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.00	0.01	1	8	0.9356
Phase	3.02	24.16	1	8	0.0012
Interaction	0.25	2.01	1	8	0.1939

Horizontal cage activity (by phase): SET_{5-9}

Raw data

		Horizontal activity (beam breaks)	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-101	CON_{5-9}	4,182	2,324
10-102	CON_{5-9}	33,941	56,261
10-115	CON_{5-9}	15,575	52,947
10-119	CON_{5-9}	6,976	70,982
10-122	MDX_{5-9}	7,281	21,035
10-124	MDX_{5-9}	22,619	78,611
10-125	MDX_{5-9}	3,702	8,112
10-129	MDX_{5-9}	8,060	39,252
10-130	MDX_{5-9}	21,914	62,728
10-145	CON_{5-9}	13,569	104,344

Descriptive statistics

	CON_{5-9} (n=10)	**MDX_{5-9} (n=10)**
Horizontal activity (beam breaks)		
Mean	36,110	27,331
SEM	10,804	8,039
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase			
Horizontal activity (beam breaks)			
Light (0700-1459) (n=10)		Mean	13,781
		SEM	3,107
		Sig?	A
Dark (1500-0659) (n=10)		Mean	49,659
		SEM	10,221
		Sig?	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Phase		CON_{5-9} (n=5)	MDX_{5-9} (n=5)
Horizontal activity (beam breaks)			
Light (0700-1459)	Mean	14,848	12,715
	SEM	5,208	3,970
	Sig?	--	--
Dark (1500-0659)	Mean	57,371	41,947
	SEM	16,497	12,972
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.06	0.50	1	8	0.5012
Phase	1.86	14.92	1	8	0.0048
Interaction	0.06	0.51	1	8	0.4947

Horizontal cage activity (by phase): $SET_{6\text{-}13}$

Raw data

		Horizontal activity (beam breaks)	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-206	$MDX_{6\text{-}13}$	36,721	112,554
10-207	$MDX_{6\text{-}13}$	9,520	47,683
10-208	$CON_{6\text{-}13}$	15,234	27,286
10-210	$MDX_{6\text{-}13}$	33,425	114,113
10-211	$MDX_{6\text{-}13}$	19,146	36,825
10-218	$MDX_{6\text{-}13}$	17,916	84,615
10-219	$CON_{6\text{-}13}$	16,476	29,462
10-220	$CON_{6\text{-}13}$	17,569	38,886
10-227	$CON_{6\text{-}13}$	8,208	36,946
10-228	$CON_{6\text{-}13}$	19,467	62,656

Descriptive statistics

	$CON_{6\text{-}13}$ (n=10)	**$MDX_{6\text{-}13}$ (n=10)**
Horizontal activity (beam breaks)		
Mean	27,219	51,251
SEM	5017	12,227
Sig?	--	--
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		
Horizontal activity (beam breaks)		
Light (0700-1459) (n=10)	Mean	19,368
	SEM	2,889
	Sig?	A
Dark (1500-0659) (n=10)	Mean	59,102
	SEM	10,522
	Sig?	B
Among time points, levels not connected by the same letter are different Student's t-test, α=0.05)		

Phase		CON_{6-13} (n=5)	MDX_{6-13} (n=5)
Horizontal activity (beam breaks)			
Light (0700-1459)	Mean	15,391	23,346
	SEM	1,926	5,093
	Sig?	--	--
Dark (1500-0659)	Mean	39,047	79,158
	SEM	6,293	16,046
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.61	4.86	1	8	0.0585
Phase	4.43	35.43	1	8	0.0003
Interaction	0.73	5.80	1	8	0.0426

Post-hoc analysis (interaction, CON_{6-13} v. MDX_{6-13})

Student's t-test	P>|t|
Light (0700-1459)	0.2025
Dark (1500-0659)	0.0654

Vertical cage activity (by phase): SET_{5-9}

Raw data

		Vertical activity (beam breaks)	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-101	CON_{5-9}	1,746	4,215
10-102	CON_{5-9}	2,608	4,402
10-115	CON_{5-9}	1,307	4,714
10-119	CON_{5-9}	468	4,141
10-122	MDX_{5-9}	701	1,632
10-124	MDX_{5-9}	863	4,716
10-125	MDX_{5-9}	53	429
10-129	MDX_{5-9}	630	2,977
10-130	MDX_{5-9}	1,061	3,801
10-145	CON_{5-9}	505	5,675

Descriptive statistics

	CON_{5-9} (n=10)	**MDX_{5-9} (n=10)**
Vertical activity (beam breaks)		
Mean	2,978	1,686
SEM	597	502
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase			
Vertical activity (beam breaks)			
Light (0700-1459) (n=10)	Mean	994	
	SEM	234	
	Sig?	A	
Dark (1500-0659) (n=10)	Mean	3,670	
	SEM	499	
	Sig?	B	
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Phase		CON_{5-9} (n=5)	MDX_{5-9} (n=5)
Vertical activity (beam breaks)			
Light (0700-1459)	Mean	1,327	662
	SEM	402	169
	Sig?	--	--
Dark (1500-0659)	Mean	4,629	2,711
	SEM	280	763
	Sig?	--	--
Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.85	6.83	1	8	0.0310
Phase	4.95	39.58	1	8	0.0002
Interaction	0.27	2.17	1	8	0.1790

Vertical cage activity (by phase): $SET_{6\text{-}13}$

Raw data

		Vertical activity (beam breaks)	
ID	Genotype	Light (0700-1459)	Dark (1500-0659)
10-206	$MDX_{6\text{-}13}$	2,354	8,412
10-207	$MDX_{6\text{-}13}$	795	5,842
10-208	$CON_{6\text{-}13}$	739	1,543
10-210	$MDX_{6\text{-}13}$	2,043	7,801
10-211	$MDX_{6\text{-}13}$	1,261	5,833
10-218	$MDX_{6\text{-}13}$	1,148	8,601
10-219	$CON_{6\text{-}13}$	922	1,649
10-220	$CON_{6\text{-}13}$	864	2,535
10-227	$CON_{6\text{-}13}$	338	1,542
10-228	$CON_{6\text{-}13}$	627	2,687

Descriptive statistics

	$CON_{6\text{-}13}$ (n=10)	**$MDX_{6\text{-}13}$ (n=10)**
Vertical activity (beam breaks)		
Mean	1,345	4,409
SEM	252	1,014
Sig?	A	B
Levels not connected by the same letter are different (Student's t-test, α=0.05)		

Phase		
Vertical activity (beam breaks)		
Light (0700-1459) (n=10)	Mean	1,109
	SEM	200
	Sig?	A
Dark (1500-0659) (n=10)	Mean	4,645
	SEM	938
	Sig?	B
Among time points, levels not connected by the same letter are different Student's t-test, α=0.05)		

Phase		CON_{6-13} (n=5)	MDX_{6-13} (n=5)
Vertical activity (beam breaks)			
Light (0700-1459)	Mean	698	1,520
	SEM	103	292
	Sig?	A	B
Dark (1500-0659)	Mean	1,991	7,298
	SEM	255	611
	Sig?	A	B

Within each time point, levels not connected by the same letter are different (Student's t-test, α=0.05)

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x phase)

Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	6.19	49.52	1	8	0.0001
Phase	20.25	162.02	1	8	<0.0001
Interaction	8.15	65.17	1	8	<0.0001

Post-hoc analysis (interaction, CON_{6-13} v. MDX_{6-13})	
Student's t-test	P>|t|
Light (0700-1459)	0.0451
Dark (1500-0659)	0.0004

Cytokine/chemokine: SET_{5-9}

Raw data

	CON_{5-9} (n=9)	**MDX_{5-9} (n=9)**	**Fold difference**
	Plasma analyte concentration (pg/ml)		MDX_{5-9} relative to CON_{5-9}
IL-1α	4.8	4.7	1.0 fold ↓
IL-1β	< 8.8	< 8.8	--
IL-2	< 0.9	< 0.9	--
IL-3	< 1.0	< 1.0	--
IL-4	< 2.2	< 2.2	--
IL-5	6.2	5.8	1.1 fold ↓
IL-6	< 2.0	7.6	> 3.8 fold ↑
IL-10	< 0.5	< 0.5	--
IL-12p70	22.5	< 3.9	> 5.8 fold ↓
IL-17	< 0.0	< 0.0	--
CCL2	21.4	59.4	2.8 fold ↑
IFNγ	< 0.1	< 0.1	--
TNFα	< 0.7	< 0.7	--
CCL3	< 2.5	< 2.5	--
GM-CSF	6.1	< 1.5	> 4.0 fold ↓
CCL5	19.1	14.8	1.3 fold ↓
CCL11	2736.5	3103.1	1.1 fold ↑
CXCL1	35.4	171.7	4.9 fold ↑
CCL22	163.8	255.6	1.6 fold ↑
CCL17	281.4	261.1	1.1 fold ↓
CCL1	16.9	16.6	1.0 fold ↓

Cytokine/chemokine: SET_{6-13}

Raw data

	CON_{6-13} (n=7)	MDX_{6-13} (n=6)	Fold difference		
	Analyte concentration (pg/ml)		MDX_{6-13} relative to CON_{6-13}	CON_{6-13} relative to CON_{5-9}	MDX_{6-13} relative to MDX_{5-9}
IL-1α	3.2	5.4	1.7 fold ↑	1.5 fold ↓	1.1 fold ↑
IL-1β	< 8.8	< 8.8	--	--	--
IL-2	< 0.9	< 0.9	--	--	--
IL-3	< 1.0	< 1.0	--	--	--
IL-4	< 2.2	< 2.2	--	--	--
IL-5	4.1	4.6	1.1 fold ↑	1.5 fold ↓	1.3 fold ↓
IL-6	< 2.0	11.1	> 5.5 fold ↑	--	1.5 fold ↑
IL-10	< 0.5	< 0.5	--	--	--
IL-12p70	< 3.9	< 3.9	--	> 5.8 fold ↓	--
IL-17	< 0.0	< 0.0	--	--	--
CCL2	40.0	100.5	2.5 fold ↑	1.9 fold ↑	1.7 fold ↑
IFNγ	< 0.1	< 0.1	--	--	--
TNFα	< 0.7	< 0.7	--	--	--
CCL3	< 2.5	< 2.5	--	--	--
GM-CSF	< 1.5	< 1.5	--	> 4.0 fold ↓	--
CCL5	16.0	21.8	1.4 fold ↑	1.2 fold ↓	1.5 fold ↑
CCL11	4084.6	4518.7	1.1 fold ↑	1.5 fold ↑	1.5 fold ↑
CXCL1	54.9	202.1	3.7 fold ↑	1.6 fold ↑	1.2 fold ↑
CCL22	136.2	220.4	1.6 fold ↑	1.2 fold ↓	1.2 fold ↓
CCL17	443.3	621.4	1.4 fold ↑	1.6 fold ↑	2.4 fold ↑
CCL1	47.3	49.4	1.0 fold ↑	2.8 fold ↑	3.0 fold ↑

Appendix C: Chapter 5 raw data and statistics

Environmental conditions: Temperature

Raw data

Day	AM Temp (°C)	PM Temp (°C)	Comments
SET_{5-9}			
-7	21	22	SET_{5-9} arrived at facility
-6	21	21	
-5	21		
-4	21		
-3	21	22	
-2	21	21	
-1	21	21	
0	21	21	SET_{5-9} E0771 cell injection
1	21	21	
2	21		
3	21		
4	21	21	
5	21	21	
6	21	22	
7	21	21	
8	21	21	
9	21		
10	21		
11	21		
12	21	21	
13	21	21	
14	21	22	
15	21	21	
16	21		
17	21		
18	21	21	
19	21	21	
20	21	22	
21	21	21	
22	21	21	
23	21		
24	21		
25	22	21	
26	21	21	
27	22	21	
28	21	21	SET_{5-9} euthanized
29	21	21	SET_{5-9} euthanized
SET_{9-13}			
-29	21	21	SET_{9-13} arrived at facility
-28	21	21	
-27	20		
-26	21		
-25	21	21	

Day	AM Temp (°C)	PM Temp (°C)	Comments
-24	21	21	
-23	21	21	
-22	21	21	
-21	21		
-20	21		
-19	20		
-18	21	21	
-17	21	21	
-16	21	21	
-15	21	21	
-14	21	21	
-13	20		
-12	20		
-11	20	21	
-10	19	21	
-9	21	21	
-8	21		
-7	21	21	
-6	21		
-5	21		
-4	21	21	
-3	21	21	
-2	21	21	
-1	21	21	
0	21	21	$SET_{9\text{-}13}$ E0771 cell injection
1	21		
2	21		
3	21	21	
4	21	21	
5	21	21	
6	21	21	
7	21		
8	21		
9	21		
10	21	21	
11	21	21	
12	21	21	
13	21	21	
14	21		
15	21		
16	21		
17	21	21	
18	21	21	
19	21	21	
20	21	21	
21	21	21	
22	21		
23	20		
24	21	21	$SET_{9\text{-}13}$ euthanized

Descriptive statistics

	n	Mean	SEM
AM Temp			
SET5-9	37	21°C	0.042°C
SET9-13	54	21°C	0.062°C
PM Temp			
SET5-9	26	21°C	0.088°C
SET9-13	34	21°C	0°C
Avg. Temp			
SET5-9	37	21°C	0.036°C
SET9-13	54	21°C	0.062°C

Inferential statistics

Student's t-test	P>|t|
AM Temp.	0.0034
PM Temp.	0.0221
Avg. Temp	0.0003

Environmental conditions: Relative humidity

Raw data

Day	AM Humidity (%)	PM Humidity (%)	Comments
SET_{5-9}			
-7	46	49	SET_{5-9} arrived at facility
-6	49	47	
-5	47		
-4	49		
-3	47	43	
-2	49	45	
-1	42	45	
0	46	45	SET_{5-9} E0771 cell injection
1	42	44	
2	47		
3	46		
4	41	45	
5	42	43	
6	42	41	
7	42	44	
8	45	44	
9	34		
10	42		
11	38		
12	42	43	
13	44	48	
14	38	32	
15	37	33	
16	37		
17	41		
18	43	38	
19	39	36	
20	40	41	
21	41	41	
22	43	42	
23	37		
24	38		
25	37	44	
26	38	46	
27	44	51	
28	43	46	SET_{5-9} euthanized
29	50	43	SET_{5-9} euthanized
SET_{9-13}			
-29	29	34	SET_{9-13} arrived at facility
-28	32	30	
-27	33		
-26	31		
-25	33	32	

Day	AM Humidity (%)	PM Humidity (%)	Comments
-24	39	34	
-23	32	30	
-22	32	31	
-21	32		
-20	27		
-19	28		
-18	27	27	
-17	35	41	
-16	28	27	
-15	27	26	
-14	28	26	
-13	27		
-12	27		
-11	27	25	
-10	28	24	
-9	25	26	
-8	26		
-7	28	33	
-6	32		
-5	33		
-4	26	25	
-3	23	23	
-2	24	24	
-1	24	33	
0	33	33	$SET_{9\text{-}13}$ E0771 cell injection
1	33		
2	32		
3	29	30	
4	33	33	
5	34	32	
6	32	32	
7	32		
8	33		
9	32		
10	27	27	
11	27	28	
12	30	28	
13	29	33	
14	30		
15	37		
16	33		
17	28	29	
18	32	30	
19	32	32	
20	28	32	
21	32	30	
22	32		
23	24		

Day	AM Humidity (%)	PM Humidity (%)	Comments
24	25	26	SET_{9-13} euthanized

Descriptive statistics

	n	Mean	SEM
AM Humidity			
SET_{5-9}	7	42%	0.67%
SET_{9-13}	54	30%	0.47%
PM Humidity			
SET_{5-9}	26	43%	0.87%
SET_{9-13}	34	30%	0.66%
Avg. Humidity			
SET_{5-9}	37	42%	0.66%
SET_{9-13}	54	30%	0.46%

Inferential statistics

Student's t-test	P>|t|
AM Humidity	<0.0001
PM Humidity	<0.0001
Avg. Humidity	<0.0001

Food intake: SET_{5-9}

Raw data

		24-hr food intake (g)		
Cage	Group	8 dpt	15 dpt	23 dpt
1	CON_{5-9}	3.0	3.5	4.1
2	$MDX+T_{5-9}$	2.4	3.3	3.5
3	$MDX+T_{5-9}$	2.9	3.7	4.9
4	$CON+T_{5-9}$	2.7	3.5	3.1
5	$CON+T_{5-9}$	3.7	3.5	4.3
8	CON_{5-9}	3.4	3.4	4.0
9	$CON+T_{5-9}$	3.7	3.7	3.2
10	CON_{5-9}	2.7	4.0	
11	$MDX+T_{5-9}$	3.2	3.7	
12	MDX_{5-9}	2.5	3.4	3.3
13	MDX_{5-9}	2.9	3.7	4.0
14	MDX_{5-9}	3.6	4.0	3.8
15	MDX_{5-9}	3.3	3.6	3.6
16	MDX_{5-9}		4.3	4.2
17	$CON+T_{5-9}$	3.5	4.0	3.6
19	$CON+T_{5-9}$	3.5	4.3	3.9
20	CON_{5-9}	3.6	3.8	3
21	$MDX+T_{5-9}$	3.1	4.3	3.3
23	CON_{5-9}	2.8	4.2	3.2
24	$MDX+T_{5-9}$	4.2	3.6	3.6

Descriptive statistics

dpt		**CON_{5-9}**	**$CON+T_{5-9}$**	**MDX_{5-9}**	**$MDX+T_{5-9}$**
24-hr food intake (g)					
8 (n=4-5)	Mean	3.1	3.4	3.0	3.1
	SEM	0.2	0.2	0.3	0.3
	Sig?	--	--	--	--
10 (n=5)	Mean	3.8	3.8	3.8	3.7
	SEM	0.1	0.2	0.2	0.2
	Sig?	--	--	--	--
23 (n=4-5)	Mean	3.6	3.6	3.8	3.8
	SEM	0.3	0.2	0.2	0.4
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
24-hr food intake (g)			
8 (n=9-10)	Mean	3.3	3.1
	SEM	0.1	0.2
	Sig?	--	--
10 (n=10)	Mean	3.8	3.7
	SEM	0.1	0.1
	Sig?	--	--
23 (n=9)	Mean	3.6	3.8
	SEM	0.2	0.2
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{5-9}+MDX_{5-9}	CON+T_{5-9}+ MDX+T_{5-9}
24-hr food intake (g)			
8 (n=9-10)	Mean	3.1	3.3
	SEM	0.1	0.2
	Sig?	--	--
10 (n=10)	Mean	3.8	3.7
	SEM	0.1	0.1
	Sig?	--	--
23 (n=9)	Mean	3.7	3.7
	SEM	0.1	0.2
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

8 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.38	0.13	0.52	0.6728
Error	15	3.64	0.24		
Total	18	4.02			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.11	0.47	0.5051	
Tumor	1	0.20	0.82	0.3807	
Interaction	1	0.06	0.26	0.6197	

10 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.02	0.01	0.06	0.9788
Error	16	1.92	0.12		
Total	19	1.94			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.00	0.02	0.8988	
Tumor	1	0.01	0.07	0.7994	
Interaction	1	0.01	0.10	0.7509	

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.22	0.07	0.26	0.8561
Error	14	4.00	0.29		
Total	17	4.22			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.22	0.76	0.3974	
Tumor	1	0.01	0.03	0.8730	
Interaction	1	0.11	0.00	0.9884	

Food intake: $SET_{9\text{-}13}$

Raw data

		24-hr food intake (g)	
Cage	Group	12 dpt	23 dpt
1	$MDX_{9\text{-}13}$	4.1	4.7
2	$CON_{9\text{-}13}$	3.5	3.9
3	$CON+T_{9\text{-}13}$	3.2	
4	$MDX_{9\text{-}13}$	5.0	
5	$CON_{9\text{-}13}$	3.8	
6	$MDX+T_{9\text{-}13}$	3.9	
7	$MDX_{9\text{-}13}$	4.0	
8	$CON+T_{9\text{-}13}$		
9	$MDX+T_{9\text{-}13}$	4.2	0.0
10	$CON+T_{9\text{-}13}$	3.3	3.1
11	$MDX_{9\text{-}13}$		
12	$CON_{9\text{-}13}$		
13	$MDX+T_{9\text{-}13}$	4.6	
14	$MDX+T_{9\text{-}13}$	3.9	4.7
15	$CON+T_{9\text{-}13}$	3.0	
16	$CON_{9\text{-}13}$	3.6	
10-201	$MDX_{9\text{-}13}$		
10-202	$CON_{9\text{-}13}$		
10-203	$CON_{9\text{-}13}$		
10-204	$CON+T_{9\text{-}13}$		4.4
10-205	$CON+T_{9\text{-}13}$		3.6
10-206	$MDX_{9\text{-}13}$		5.8

		24-hr food intake (g)
10-207	$MDX_{9\text{-}13}$	6.0
10-208	$CON_{9\text{-}13}$	3.9
10-209	$MDX+T_{9\text{-}13}$	4.1
10-210	$MDX_{9\text{-}13}$	5.7
10-211	$MDX_{9\text{-}13}$	4.7
10-212	$CON+T_{9\text{-}13}$	4.3
10-213	$MDX+T_{9\text{-}13}$	
10-214	$MDX+T_{9\text{-}13}$	4.2
10-215	$CON+T_{9\text{-}13}$	
10-216	$CON+T_{9\text{-}13}$	
10-217	$MDX_{9\text{-}13}$	
10-218	$MDX_{9\text{-}13}$	4.9
10-219	$CON_{9\text{-}13}$	3.8
10-220	$CON_{9\text{-}13}$	4.2
10-221	$MDX+T_{9\text{-}13}$	5.7
10-222	$MDX+T_{9\text{-}13}$	5.4
10-223	$MDX+T_{9\text{-}13}$	4.3
10-224	$MDX+T_{9\text{-}13}$	
10-225	$CON+T_{9\text{-}13}$	3.7
10-226	$CON+T_{9\text{-}13}$	3.9
10-227	$CON_{9\text{-}13}$	4.2
10-228	$CON_{9\text{-}13}$	3.6

Descriptive statistics

dpt		$CON_{9\text{-}13}$	$CON+T_{9\text{-}13}$	$MDX_{9\text{-}13}$	$MDX+T_{9\text{-}13}$
24-hr food intake (g)					
12 (n=3-4)	Mean	3.6	3.2	4.4	4.1
	SEM	0.1	0.1	0.3	0.2
	Sig?	--	--	--	--
23 (n=6-7)	Mean	3.9	3.8	5.3	4.1
	SEM	0.1	0.2	0.2	0.7
	Sig?	--	--	--	--

Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)

dpt		CON_{9-13}+ $CON+T_{9-13}$	MDX_{9-13}+ $MDX+T_{9-13}$
24-hr food intake (g)			
12 (n=6-7)	Mean	3.4	4.2
	SEM	0.1	0.2
	Sig?	A	B
23 (n=12-13)	Mean	3.9	4.6
	SEM	0.1	0.4
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
24-hr food intake (g)			
12 (n=6-7)	Mean	4.0	3.7
	SEM	0.2	0.2
	Sig?	--	--
23 (n=12-13)	Mean	4.6	4.0
	SEM	0.2	0.4
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

12 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	2.74	0.91	7.68	0.0075
Error	9	1.07	0.12		
Total	12	3.81			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	2.40	20.19	0.0015	
Tumor	1	0.36	2.99	0.1181	
Interaction	1	0.06	0.48	0.5069	

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	8.50	2.83	2.42	0.0948
Error	21	24.60	1.17		
Total	24	33.10			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	3.93	3.36	0.0810	
Tumor	1	2.74	2.34	0.1414	
Interaction	1	2.09	1.79	0.1958	

Body mass: SET_{5-9}

Raw data

		Body mass (g)				
ID	Group	1 dpt	8 dpt	15 dpt	22 dpt	27 dpt
10-101	CON_{5-9}	18.5	20.0	20.7	20.9	22.2
10-102	CON_{5-9}	18.1	19.5	19.8	20.3	21.1
10-103	$MDX+T_{5-9}$	15.9	17.3	18.3	20.1	20.5
10-104	$MDX+T_{5-9}$	16.6	18.7	20.4	22.0	24.3
10-105	$MDX+T_{5-9}$	17.5	19.2	20.5	20.9	23.0
10-106	$MDX+T_{5-9}$	18.0	20.5	21.4	22.4	23.8
10-107	$CON+T_{5-9}$	17.5	20.1	19.6	20.5	21.6
10-108	$CON+T_{5-9}$	17.6	18.2	19.2	19.7	21.5
10-109	$CON+T_{5-9}$	17.5	20.0	20.5	21.6	23.1
10-110	$CON+T_{5-9}$	16.0	18.5	20.2	20.6	21.4
10-115	CON_{5-9}	16.9	17.5	18.0	19.2	19.8
10-116	CON_{5-9}	17.6	19.0	19.5	20.7	20.5
10-117	$CON+T_{5-9}$	17.0	17.9	18.1	18.9	20.3
10-118	$CON+T_{5-9}$	16.5	18.8	19.2	20.1	21.9
10-119	CON_{5-9}	17.9	18.3	18.7	20.0	20.3
10-120	$MDX+T_{5-9}$	17.3	21.9	22.0	19.1	
10-121	$MDX+T_{5-9}$	15.0	17.6	19.7	21.2	22.9
10-122	MDX_{5-9}	16.5	17.0	18.2	19.2	19.8
10-123	MDX_{5-9}	17.5	19.5	20.4	21.6	23.0
10-124	MDX_{5-9}	15.4	18.0	18.5	19.3	20.1
10-125	MDX_{5-9}	16.6	17.1	19.1	21.4	23.0
10-126	MDX_{5-9}	19.2	21.8	23.3	24.0	24.8
10-127	MDX_{5-9}	15.9	16.8	18.9	19.9	21.0
10-128	MDX_{5-9}	17.6	19.4	20.1	21.3	21.7
10-129	MDX_{5-9}	17.1	18.9	20.5	21.9	22.6
10-130	MDX_{5-9}	18.1	19.3	21.1	21.9	22.9
10-131	MDX_{5-9}	17.0				
10-132	$CON+T_{5-9}$	18.6	20.6	21.7	21.7	23.8
10-133	$CON+T_{5-9}$	17.0	18.9	19.7	21.2	23.9
10-136	$CON+T_{5-9}$	17.0	18.3	19.1	20.4	22.7
10-137	$CON+T_{5-9}$	17.2	18.6	20.1	21.8	20.1
10-138	CON_{5-9}	17.2	18.2	19.1	19.7	20.0
10-139	CON_{5-9}	17.4	18.6	19.4	20.5	21.9
10-140	$MDX+T_{5-9}$	18.1	20.0	21.1	22.1	25.4
10-141	$MDX+T_{5-9}$	16.9	20.4	21.9	24.1	25.1
10-144	CON_{5-9}	16.0	16.4	17.1	18.0	19.3
10-145	CON_{5-9}	16.8	17.6	19.3	20.0	20.1
10-146	$MDX+T_{5-9}$	17.3	17.2	18.2	19.6	20.8
10-147	$MDX+T_{5-9}$	16.3	17.4	18.9	19.9	22.3

Descriptive statistics

dpt		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Body mass (g)					
1 (n=9-10)	Mean	17.4	17.2	17.1	16.9
	SEM	0.3	0.2	0.3	0.3
	Sig?	--	--	--	--
8 (n=9-10)	Mean	18.3	19.0	18.6	19.0
	SEM	0.4	0.3	0.5	0.5
	Sig?	--	--	--	--
15 (n=9-10)	Mean	19.1	19.7	20.0	20.2
	SEM	0.3	0.3	0.5	0.4
	Sig?	--	--	--	--
22 (n=9-10)	Mean	19.9	20.7	21.2	21.1
	SEM	0.3	0.3	0.5	0.5
	Sig?	--	--	--	--
27 (n=9-10)	Mean	20.6	22.0	22.1	23.1
	SEM	0.3	0.4	0.5	0.6
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
Body mass (g)			
1 (n=19-20)	Mean	17.3	17.0
	SEM	0.2	0.2
	Sig?	--	--
8 (n=19)	Mean	18.7	18.8
	SEM	0.2	0.4
	Sig?	--	--
15 (n=19)	Mean	19.4	20.1
	SEM	0.2	0.3
	Sig?	--	--
22 (n=19)	Mean	20.3	21.2
	SEM	0.2	0.3
	Sig?	A	B
27 (n=18-19)	Mean	21.3	22.6
	SEM	0.3	0.4
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{5-9}+MDX_{5-9}	CON+T_{5-9}+ MDX+T_{5-9}
Body mass (g)			
1 (n=19-20)	Mean	17.2	17.0
	SEM	0.2	0.2
	Sig?	--	--
8 (n=18-20)	Mean	18.5	19.0
	SEM	0.3	0.3
	Sig?	--	--
15 (n=18-20)	Mean	19.5	20.0
	SEM	0.3	0.3
	Sig?	--	--
22 (n=18-20)	Mean	20.5	20.9
	SEM	0.3	0.3
	Sig?	--	--
27 (n=18-19)	Mean	21.3	22.5
	SEM	0.4	0.4
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

1 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1.18	0.39	0.49	0.6914
Error	35	28.10	0.80		
Total	38	29.28			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.84	1.05	0.3133	
Tumor	1	0.37	0.46	0.5041	
Interaction	1	0.00	0.00	0.9831	

8 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	2.88	0.96	0.5240	0.6687
Error	34	62.27	1.83		
Total	37	65.15			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.26	0.14	0.7098	
Tumor	1	2.47	1.35	0.2537	
Interaction	1	0.17	0.09	0.7607	

15 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	7.19	2.40	1.48	0.2385
Error	34	55.24	1.62		
Total	37	62.43			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	4.94	3.04	0.0902	
Tumor	1	1.93	1.19	0.2837	
Interaction	1	0.47	0.29	0.5950	

22 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	9.33	3.11	1.96	0.1379
Error	34	53.84	1.58		
Total	37	63.18			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	7.12	4.50	0.0413	
Tumor	1	1.16	0.74	0.3972	
Interaction	1	1.35	0.85	0.3627	

27 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	29.58	9.86	4.80	0.0070
Error	33	67.77	2.05		
Total	36	97.35			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	15.77	7.68	0.0091	
Tumor	1	14.13	6.88	0.0131	
Interaction	1	0.43	0.21	0.6515	

Body mass: $SET_{9\text{-}13}$

Raw data

		Body mass (g)				
ID	Group	-3 dpt	5 dpt	12 dpt	18 dpt	23 dpt
10-201	$MDX_{9\text{-}13}$	19.7	21.0	22.1	22.8	23.3
10-202	$CON_{9\text{-}13}$	19.1	20.9	20.6	22.0	22.3
10-203	$CON_{9\text{-}13}$	18.4	19.6	20.0	21.4	22.4
10-204	$CON+T_{9\text{-}13}$	17.7	18.3	19.3	20.4	20.8
10-205	$CON+T_{9\text{-}13}$	17.3	19.2	19.7	20.8	22.2
10-206	$MDX_{9\text{-}13}$	23.2	25.1	24.5	25.7	27.4
10-207	$MDX_{9\text{-}13}$	20.2	22.0	22.6	24.0	25.0
10-208	$CON_{9\text{-}13}$	18.9	18.5	20.6	21.8	22.9
10-209	$MDX+T_{9\text{-}13}$	21.2	22.6	23.6	24.1	25.5
10-210	$MDX_{9\text{-}13}$	20.7	22.1	23.1	22.8	24.5
10-211	$MDX_{9\text{-}13}$	21.3	23.0	24.3	24.8	25.8
10-212	$CON+T_{9\text{-}13}$	20.4	21.2	21.4	23.4	24.6
10-213	$MDX+T_{9\text{-}13}$	20.7	21.8	23.0	24.3	25.6
10-214	$MDX+T_{9\text{-}13}$	20.8	22.1	23.1	25.0	25.7
10-215	$CON+T_{9\text{-}13}$	21.1	22.2	23.1	24.1	25.0
10-216	$CON+T_{9\text{-}13}$	19.0	19.2	19.7	20.1	20.7
10-217	$MDX_{9\text{-}13}$	20.6				
10-218	$MDX_{9\text{-}13}$	22.2	24.7	25.0	25.0	25.3
10-219	$CON_{9\text{-}13}$	20.1	20.9	21.5	21.2	21.4
10-220	$CON_{9\text{-}13}$	18.0	18.8	19.4	20.5	21.5
10-221	$MDX+T_{9\text{-}13}$	21.6	23.1	24.3	24.7	25.3
10-222	$MDX+T_{9\text{-}13}$	22.3	24.0	24.7	26.1	27.7
10-223	$MDX+T_{9\text{-}13}$	20.4	22.1	22.7	23.7	24.4
10-224	$MDX+T_{9\text{-}13}$	20.1	22.0	22.8	24.1	25.3
10-225	$CON+T_{9\text{-}13}$	20.0	20.9	21.3	21.4	21.9
10-226	$CON+T_{9\text{-}13}$	18.2	19.2	20.0	21.2	23.3
10-227	$CON_{9\text{-}13}$	21.1	21.6	21.8	21.6	22.3
10-228	$CON_{9\text{-}13}$	17.4	18.5	18.6	19.6	20.2

Descriptive statistics

dpt		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Body mass (g)					
-3 (n=7)	Mean	19.0	19.1	21.1	21.0
	SEM	0.5	0.5	0.5	0.3
	Sig?	--	--	--	--
5 (n=6-7)	Mean	19.8	20.0	23.0	22.5
	SEM	0.5	0.5	0.7	0.3
	Sig?	--	--	--	--
12 (n=6-7)	Mean	20.4	20.6	23.6	23.5
	SEM	0.4	0.5	0.5	0.3
	Sig?	--	--	--	--
18 (n=6-7)	Mean	21.2	21.6	24.2	24.6
	SEM	0.3	0.6	0.5	0.3
	Sig?	--	--	--	--
23 (n=6-7)	Mean	21.9	22.6	25.2	25.6
	SEM	0.3	0.6	0.6	0.4
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{9-13}+ CON+T_{9-13}	MDX_{9-13}+ MDX+T_{9-13}
Body mass (g)			
-3 (n=14)	Mean	19.1	21.1
	SEM	0.3	0.3
	Sig?	A	B
5 (n=13-14)	Mean	19.9	22.7
	SEM	0.3	0.3
	Sig?	A	B
12 (n=13-14)	Mean	20.5	23.5
	SEM	0.3	0.3
	Sig?	A	B
18 (n=13-14)	Mean	21.4	24.4
	SEM	0.3	0.3
	Sig?	A	B
23 (n=13-14)	Mean	22.3	25.4
	SEM	0.4	0.3
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
Body mass (g)			
-3 (n=14)	Mean	20.1	20.1
	SEM	0.4	0.4
	Sig?	--	--
5 (n=13-14)	Mean	21.3	21.3
	SEM	0.6	0.5
	Sig?	--	--
12 (n=13-14)	Mean	21.9	22.1
	SEM	0.6	0.5
	Sig?	--	--
18 (n=13-14)	Mean	22.6	23.1
	SEM	0.5	0.5
	Sig?	--	--
23 (n=13-14)	Mean	23.4	24.1
	SEM	0.6	0.6
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

-3 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	28.68	9.56	6.69	0.0019
Error	24	34.30	1.43		
Total	27	62.99			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	28.60	20.01	0.0002	
Tumor	1	0.00	0.00	0.9875	
Interaction	1	0.08	0.06	0.8146	

5 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	54.03	18.01	10.69	0.0001
Error	23	38.75	1.68		
Total	26	92.78			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	53.72	31.88	<0.0001	
Tumor	1	0.11	0.06	0.8015	
Interaction	1	0.72	0.43	0.5197	

12 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	61.96	20.65	16.35	<0.0001
Error	23	29.05	1.26		
Total	26	91.01			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	61.64	48.80	<0.0001	
Tumor	1	0.03	0.03	0.8706	
Interaction	1	0.24	0.24	0.6258	

18 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	2	61.91	20.63	16.21	<0.0001
Error	23	29.27	1.27		
Total	26	91.18			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	59.86	47.03	<0.0001	
Tumor	1	1.24	0.98	0.3337	
Interaction	1	0.01	0.01	0.9246	

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	71.61	23.87	14.45	<0.0001
Error	23	37.98	1.65		
Total	26	109.59			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	67.95	41.15	<0.0001	
Tumor	1	2.47	1.49	0.2339	
Interaction	1	0.22	0.13	0.7202	

Tumor free body mass: SET_{5-9}

Raw data

		Tumor free body mass (g)
ID	Group	29 dpt
10-101	CON_{5-9}	22.2
10-102	CON_{5-9}	21.1
10-103	$MDX+T_{5-9}$	20.3
10-104	$MDX+T_{5-9}$	21.6
10-105	$MDX+T_{5-9}$	22.5
10-106	$MDX+T_{5-9}$	22.7
10-107	$CON+T_{5-9}$	21.2
10-108	$CON+T_{5-9}$	20.7
10-109	$CON+T_{5-9}$	21.3
10-110	$CON+T_{5-9}$	21.0
10-115	CON_{5-9}	19.8
10-116	CON_{5-9}	20.5
10-117	$CON+T_{5-9}$	19.5
10-118	$CON+T_{5-9}$	21.3
10-119	CON_{5-9}	20.3
10-120	$MDX+T_{5-9}$	18.0
10-121	$MDX+T_{5-9}$	19.9
10-122	MDX_{5-9}	19.8
10-123	MDX_{5-9}	23.0
10-124	MDX_{5-9}	20.1
10-125	MDX_{5-9}	23.0
10-126	MDX_{5-9}	24.8
10-127	MDX_{5-9}	21.0
10-128	MDX_{5-9}	21.7
10-129	MDX_{5-9}	22.6
10-130	MDX_{5-9}	22.9
10-131	MDX_{5-9}	
10-132	$CON+T_{5-9}$	21.0
10-133	$CON+T_{5-9}$	22.0
10-136	$CON+T_{5-9}$	21.8
10-137	$CON+T_{5-9}$	18.6
10-138	CON_{5-9}	20.0
10-139	CON_{5-9}	21.9
10-140	$MDX+T_{5-9}$	23.8
10-141	$MDX+T_{5-9}$	23.9
10-144	CON_{5-9}	19.3
10-145	CON_{5-9}	20.1
10-146	$MDX+T_{5-9}$	19.4
10-147	$MDX+T_{5-9}$	21.1

Descriptive statistics

dpt		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Tumor free body mass (g)					
28 (n=9-10)	Mean	20.6	20.8	22.1	21.3
	SEM	0.3	0.3	0.5	0.6
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{5-9}+ $CON+T_{5-9}$	MDX_{5-9}+ $MDX+T_{5-9}$
Tumor free body mass (g)			
28 (n=19)	Mean	20.7	21.7
	SEM	0.2	0.4
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{5-9}+MDX_{5-9}	$CON+T_{5-9}$+ $MDX+T_{5-9}$
Tumor free body mass (g)			
28 (n=18-20)	Mean	21.3	21.1
	SEM	0.4	0.3
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

28 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	12.30	4.10	1.94	0.1418
Error	34	71.87	2.11		
Total	37	84.17			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	9.70	4.59	0.0395	
Tumor	1	0.62	0.29	0.5931	
Interaction	1	2.47	1.17	0.2874	

Tumor free body mass: $SET_{9\text{-}13}$

Raw data

		Tumor free body mass (g)
ID	Group	23 dpt
10-201	$MDX_{9\text{-}13}$	23.3
10-202	$CON_{9\text{-}13}$	22.3
10-203	$CON_{9\text{-}13}$	22.4
10-204	$CON+T_{9\text{-}13}$	20.8
10-205	$CON+T_{9\text{-}13}$	21.8
10-206	$MDX_{9\text{-}13}$	27.4
10-207	$MDX_{9\text{-}13}$	25.0
10-208	$CON_{9\text{-}13}$	22.9
10-209	$MDX+T_{9\text{-}13}$	25.4
10-210	$MDX_{9\text{-}13}$	24.5
10-211	$MDX_{9\text{-}13}$	25.8
10-212	$CON+T_{9\text{-}13}$	24.2
10-213	$MDX+T_{9\text{-}13}$	25.6
10-214	$MDX+T_{9\text{-}13}$	25.5
10-215	$CON+T_{9\text{-}13}$	24.9
10-216	$CON+T_{9\text{-}13}$	20.6
10-217	$MDX_{9\text{-}13}$	
10-218	$MDX_{9\text{-}13}$	25.3
10-219	$CON_{9\text{-}13}$	21.4
10-220	$CON_{9\text{-}13}$	21.5
10-221	$MDX+T_{9\text{-}13}$	25.3
10-222	$MDX+T_{9\text{-}13}$	26.7
10-223	$MDX+T_{9\text{-}13}$	24.4
10-224	$MDX+T_{9\text{-}13}$	24.7
10-225	$CON+T_{9\text{-}13}$	21.7
10-226	$CON+T_{9\text{-}13}$	23.0
10-227	$CON_{9\text{-}13}$	22.3
10-228	$CON_{9\text{-}13}$	20.2

Descriptive statistics

dpt		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Tumor free body mass (g)					
23 (n=6-7)	Mean	21.9	22.4	25.2	25.4
	SEM	0.3	0.6	0.6	0.3
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{9-13}+ $CON+T_{9-13}$	MDX_{9-13}+ $MDX+T_{9-13}$
Tumor free body mass (g)			
23 (n=13-14)	Mean	22.1	25.3
	SEM	0.3	0.3
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
Tumor free body mass (g)			
23 (n=13-14)	Mean	23.4	23.9
	SEM	0.6	0.5
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	68.31	22.77	15.50	<0.0001
Error	23	33.79	1.47		
Total	26	102.10			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	66.71	45.40	<0.0001	
Tumor	1	0.83	0.57	0.4597	
Interaction	1	0.29	0.20	0.6593	

Percent lean mass: SET_{5-9}

Raw data

		Lean mass (%)				
ID	Group	1 dpt	8 dpt	15 dpt	22 dpt	27 dpt
10-101	CON_{5-9}	58.7	61.2	59.6	62.4	60.9
10-102	CON_{5-9}	65.2	62.0	61.6	63.0	62.7
10-103	$MDX+T_{5-9}$	60.9	60.5	62.2	60.5	61.5
10-104	$MDX+T_{5-9}$	61.5	62.5	61.5	60.5	63.0
10-105	$MDX+T_{5-9}$	63.0	63.5	61.2	64.3	61.8
10-106	$MDX+T_{5-9}$	63.9	61.3	62.1	61.5	63.9
10-107	$CON+T_{5-9}$	62.4	60.2	60.6	59.7	60.8
10-108	$CON+T_{5-9}$	60.4	59.7	60.6	59.3	61.3
10-109	$CON+T_{5-9}$	60.4	60.2	60.7	59.3	60.9
10-110	$CON+T_{5-9}$	60.2	58.0	57.6	57.0	60.4
10-115	CON_{5-9}	61.8	62.0	62.7	60.9	60.7
10-116	CON_{5-9}	60.4	59.6	61.2	56.4	58.5
10-117	$CON+T_{5-9}$	63.6	60.7	63.2	63.4	63.2
10-118	$CON+T_{5-9}$	61.8	60.7	61.4	63.6	62.7
10-119	CON_{5-9}	60.2	59.6	62.3	61.8	62.5
10-120	$MDX+T_{5-9}$	61.4	63.2	64.2	61.6	
10-121	$MDX+T_{5-9}$	61.5	62.7	64.5	65.0	65.6
10-122	MDX_{5-9}	60.1	60.4	61.7	59.5	57.6
10-123	MDX_{5-9}	62.4	63.6	65.0	65.8	64.3
10-124	MDX_{5-9}	61.8	64.4	63.7	61.9	62.9
10-125	MDX_{5-9}	62.0	62.0	64.7	62.3	64.0
10-126	MDX_{5-9}	66.7	63.2	65.8	64.7	65.0
10-127	MDX_{5-9}	62.2	61.6	63.1	64.1	62.4
10-128	MDX_{5-9}	65.8	63.0	64.4	61.3	62.3
10-129	MDX_{5-9}	62.4	63.7	64.4	62.8	63.9
10-130	MDX_{5-9}	62.7	61.6	65.7	64.0	63.6
10-131	MDX_{5-9}	64.6				
10-132	$CON+T_{5-9}$	57.9	56.4	55.7	56.7	57.4
10-133	$CON+T_{5-9}$	61.8	62.2	62.8	62.3	61.5
10-136	$CON+T_{5-9}$	61.1	61.0	59.6	60.6	58.8
10-137	$CON+T_{5-9}$	64.5	61.7	59.4	61.2	62.2
10-138	CON_{5-9}	63.8	61.9	62.2	59.7	59.9
10-139	CON_{5-9}	62.4	62.5	61.3	61.0	58.3
10-140	$MDX+T_{5-9}$	60.7	61.1	62.5	61.4	63.2
10-141	$MDX+T_{5-9}$	64.7	62.4	60.9	60.2	63.1
10-144	CON_{5-9}	60.6	62.1	59.8	60.4	58.9
10-145	CON_{5-9}	60.8	61.1	57.8	57.8	59.6
10-146	$MDX+T_{5-9}$	58.5	61.1	60.9	63.1	61.6
10-147	$MDX+T_{5-9}$	62.4	63.4	61.6	63.7	64.4

Descriptive statistics

dpt		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Lean mass (%)					
1 (n=9-10)	Mean	61.5	61.4	63.0	61.8
	SEM	0.7	0.6	0.6	0.6
	Sig?	--	--	--	--
8 (n=9-10)	Mean	61.3	60.1	62.6	62.1
	SEM	0.4	0.5	0.4	0.3
	Sig?	--	--	--	--
15 (n=9-10)	Mean	60.9	60.1	64.3	62.1
	SEM	0.5	0.7	0.4	0.4
	Sig?	--	--	--	--
22 (n=9-10)	Mean	60.4	60.3	62.9	62.2
	SEM	0.7	0.8	0.6	0.5
	Sig?	--	--	--	--
27 (n=9-10)	Mean	60.2	60.9	62.9	63.1
	SEM	0.5	0.6	0.7	0.5
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
Lean mass (%)			
1 (n=19-20)	Mean	61.5	62.4
	SEM	0.4	0.4
	Sig?	--	--
8 (n=19)	Mean	60.6	62.3
	SEM	0.4	0.3
	Sig?	A	B
15 (n=19)	Mean	60.5	63.1
	SEM	0.5	0.4
	Sig?	A	B
22 (n=19)	Mean	60.3	62.5
	SEM	0.5	0.4
	Sig?	A	B
27 (n=18-19)	Mean	60.6	63.0
	SEM	0.4	0.4
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{5-9}+MDX_{5-9}	$CON+T_{5-9}$+ $MDX+T_{5-9}$
Lean mass (%)			
1 (n=19-20)	Mean	62.3	61.6
	SEM	0.5	0.4
	Sig?	--	--
8 (n=18-20)	Mean	61.9	61.1
	SEM	0.3	0.4
	Sig?	--	--
15 (n=18-20)	Mean	62.6	61.1
	SEM	0.5	0.5
	Sig?	A	B
22 (n=18-20)	Mean	61.6	61.2
	SEM	0.6	0.5
	Sig?	--	--
27 (n=18-19)	Mean	61.5	61.9
	SEM	0.5	0.4
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

1 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	16.92	5.64	1.55	0.2183
Error	35	127.13	3.63		
Total	38	144.05			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	9.47	2.61	0.1154	
Tumor	1	4.50	1.24	0.2732	
Interaction	1	2.66	0.73	0.3977	

8 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	35.81	11.94	6.73	0.0011
Error	34	60.26	1.77		
Total	37	96.07			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	26.69	15.06	0.0005	
Tumor	1	6.75	3.81	0.0593	
Interaction	1	1.61	0.91	0.3479	

15 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	89.84	29.95	10.76	<0.0001
Error	34	94.67	2.78		
Total	37	184.51			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	67.24	24.15	<0.0001	
Tumor	1	19.94	7.16	0.0114	
Interaction	1	4.24	1.52	0.2255	

22 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	48.31	16.10	3.79	0.0190
Error	34	144.50	4.25		
Total	37	192.81			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	46.18	10.87	0.0023	
Tumor	1	1.62	0.38	0.5413	
Interaction	1	1.15	0.27	0.6070	

27 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	56.43	18.81	6.09	0.0020
Error	33	101.96	3.09		
Total	36	158.38			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	54.64	17.68	0.0002	
Tumor	1	2.01	0.650	0.4254	
Interaction	1	0.46	0.15	0.7030	

Percent lean mass: $SET_{9\text{-}13}$

Raw data

		Lean mass (%)				
ID	Group	-3 dpt	5 dpt	12 dpt	18 dpt	23 dpt
10-201	$MDX_{9\text{-}13}$	63.4	60.4	84.2	62.6	60.4
10-202	$CON_{9\text{-}13}$	56.8	57.9	82.1	58.2	59.6
10-203	$CON_{9\text{-}13}$	57.4	57.6	58.0	59.9	57.7
10-204	$CON+T_{9\text{-}13}$	57.9	58.7	58.8	58.7	62.2
10-205	$CON+T_{9\text{-}13}$	58.7	57.9	59.9	58.6	59.9
10-206	$MDX_{9\text{-}13}$	62.5	64.2	63.5	62.6	64.5
10-207	$MDX_{9\text{-}13}$	61.3	62.2	61.7	63.0	62.8
10-208	$CON_{9\text{-}13}$	53.1	56.9	58.6	58.6	58.3
10-209	$MDX+T_{9\text{-}13}$	61.9	61.2	61.2	61.9	63.0
10-210	$MDX_{9\text{-}13}$	67.4	62.0	61.5	63.7	62.1
10-211	$MDX_{9\text{-}13}$	60.2	59.4	60.2	60.7	61.1
10-212	$CON+T_{9\text{-}13}$	58.2	57.6	59.0	60.0	60.0
10-213	$MDX+T_{9\text{-}13}$	65.0	60.3	60.0	62.4	63.3
10-214	$MDX+T_{9\text{-}13}$	61.0	59.8	62.0	63.4	62.5
10-215	$CON+T_{9\text{-}13}$	56.8	56.4	57.0	57.2	58.0
10-216	$CON+T_{9\text{-}13}$	57.9	58.5	58.2	59.0	57.2
10-217	$MDX_{9\text{-}13}$	63.5				
10-218	$MDX_{9\text{-}13}$	64.3	64.4	63.7	61.7	64.3
10-219	$CON_{9\text{-}13}$	52.7	50.3	51.7	58.4	60.8
10-220	$CON_{9\text{-}13}$	57.2	58.0	58.1	59.5	59.5
10-221	$MDX+T_{9\text{-}13}$	62.2	59.4	59.8	62.2	62.1
10-222	$MDX+T_{9\text{-}13}$	62.1	60.9	63.2	65.3	64.5
10-223	$MDX+T_{9\text{-}13}$	60.9	62.1	61.3	61.8	62.1
10-224	$MDX+T_{9\text{-}13}$	61.6	59.6	60.9	62.3	64.7
10-225	$CON+T_{9\text{-}13}$	57.2	56.9	57.0	58.1	59.7
10-226	$CON+T_{9\text{-}13}$	57.6	57.0	57.7	62.5	61.5
10-227	$CON_{9\text{-}13}$	55.5	56.0	56.0	60.4	59.3
10-228	$CON_{9\text{-}13}$	54.3	53.2	51.6	60.6	61.1

Descriptive statistics

dpt		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Lean mass (%)					
-3 (n=7)	Mean	55.3	57.7	63.2	62.1
	SEM	0.7	0.2	0.9	0.5
	Sig?	A	A	B	B
5 (n=6-7)	Mean	55.7	57.5	62.1	60.5
	SEM	1.1	0.3	0.8	0.4
	Sig?	A	A	B	B
12 (n=6-7)	Mean	59.4	58.2	65.8	61.2
	SEM	3.9	0.4	3.7	0.4
	Sig?	--	--	--	--
18 (n=6-7)	Mean	59.4	59.1	62.4	62.7
	SEM	0.4	0.6	0.4	0.5
	Sig?	--	--	--	--
23 (n=6-7)	Mean	59.4	59.8	62.5	63.2
	SEM	0.5	0.7	0.7	0.4
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{9-13}+ CON+T_{9-13}	MDX_{9-13}+ MDX+T_{9-13}
Lean mass (%)			
-3 (n=14)	Mean	56.5	62.6
	SEM	0.5	0.5
	Sig?	A	B
5 (n=13-14)	Mean	56.6	61.2
	SEM	0.6	0.5
	Sig?	A	B
12 (n=13-14)	Mean	58.8	63.3
	SEM	1.9	1.8
	Sig?	--	--
18 (n=13-14)	Mean	59.2	62.6
	SEM	0.4	0.3
	Sig?	A	B
23 (n=13-14)	Mean	59.6	62.9
	SEM	0.4	0.4
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
Lean mass (%)			
-3 (n=14)	Mean	59.2	59.9
	SEM	1.2	0.7
	Sig?	--	--
5 (n=13-14)	Mean	58.6	59.0
	SEM	1.1	0.5
	Sig?	--	--
12 (n=13-14)	Mean	62.4	59.7
	SEM	2.8	0.5
	Sig?	--	--
18 (n=13-14)	Mean	60.7	60.9
	SEM	0.5	0.6
	Sig?	--	--
23 (n=13-14)	Mean	60.9	61.5
	SEM	0.6	0.6
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

-3 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	289.98	96.66	33.63	<0.0001
Error	24	68.98	2.87		
Total	27	358.95			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	264.14	91.91	<0.0001	
Tumor	1	3.16	1.10	0.3051	
Interaction	1	22.68	7.89	0.0097	

5 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	161.70	53.90	15.45	<0.0001
Error	23	80.24	3.49		
Total	26	241.95			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	144.67	41.47	<0.0001	
Tumor	1	0.10	0.03	0.8644	
Interaction	1	20.18	5.78	0.0246	

12 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	209.76	69.92	1.48	0.2454
Error	23	1084.30	47.14		
Total	26	1294.06			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	146.76	3.11	0.0909	
Tumor	1	56.86	1.21	0.2835	
Interaction	1	19.14	0.41	0.5303	

18 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	74.90	24.97	15.24	<0.0001
Error	23	37.69	1.64		
Total	26	112.59			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	73.34	44.76	<0.0001	
Tumor	1	0.04	0.02	0.8806	
Interaction	1	0.65	0.40	0.5354	

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	73.45	24.48	11.68	<0.0001
Error	23	48.21	2.10		
Total	26	121.67			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	70.59	33.68	<0.0001	
Tumor	1	1.46	0.70	0.4126	
Interaction	1	0.17	0.08	0.7785	

Percent fat mass: SET_{5-9}

Raw data

		Fat mass (%)				
ID	Group	1 dpt	8 dpt	15 dpt	22 dpt	27 dpt
10-101	CON_{5-9}	6.10	3.75	5.45	4.70	5.25
10-102	CON_{5-9}	3.60	4.05	3.65	2.80	3.85
10-103	$MDX+T_{5-9}$	5.50	5.45	5.00	6.10	5.45
10-104	$MDX+T_{5-9}$	4.10	3.80	4.45	5.45	1.90
10-105	$MDX+T_{5-9}$	5.25	3.65	4.40	2.95	4.85
10-106	$MDX+T_{5-9}$	2.60	4.05	4.00	4.85	1.90
10-107	$CON+T_{5-9}$	4.80	5.40	5.30	6.70	4.75
10-108	$CON+T_{5-9}$	6.25	6.35	5.40	5.95	5.05
10-109	$CON+T_{5-9}$	5.00	5.65	5.65	6.25	5.25
10-110	$CON+T_{5-9}$	6.75	7.75	6.90	8.20	4.85
10-115	CON_{5-9}	3.80	4.00	4.30	5.15	5.75
10-116	CON_{5-9}	4.05	5.50	4.65	7.50	7.55
10-117	$CON+T_{5-9}$	3.25	5.25	3.55	3.25	4.20
10-118	$CON+T_{5-9}$	4.35	5.45	5.40	3.05	4.65
10-119	CON_{5-9}	5.35	6.15	4.15	4.10	2.95
10-120	$MDX+T_{5-9}$	3.73	4.05	2.20	2.40	
10-121	$MDX+T_{5-9}$	5.15	2.80	2.25	2.20	2.05
10-122	MDX_{5-9}	4.55	6.15	6.50	7.25	8.60
10-123	MDX_{5-9}	3.15	1.85	1.85	0.55	3.40
10-124	MDX_{5-9}	4.05	3.50	3.95	3.75	4.10
10-125	MDX_{5-9}	3.30	3.70	1.95	4.20	3.20
10-126	MDX_{5-9}	1.70	3.05	2.30	1.55	3.20
10-127	MDX_{5-9}	4.30	5.25	2.95	2.40	5.45
10-128	MDX_{5-9}	3.00	3.45	3.80	4.80	4.50
10-129	MDX_{5-9}	3.45	3.00	2.50	3.55	2.60
10-130	MDX_{5-9}	3.00	3.90	1.50	1.75	3.45
10-131	MDX_{5-9}	2.30				
10-132	$CON+T_{5-9}$	7.70	9.30	10.20	8.65	7.65
10-133	$CON+T_{5-9}$	3.85	3.70	3.25	3.55	4.40
10-136	$CON+T_{5-9}$	5.25	5.50	4.70	5.05	6.40
10-137	$CON+T_{5-9}$	3.80	4.15	4.20	4.35	2.45
10-138	CON_{5-9}	3.10	4.65	3.50	4.75	5.70
10-139	CON_{5-9}	4.10	5.60	4.10	5.60	7.10
10-140	$MDX+T_{5-9}$	5.60	4.25	4.10	3.90	3.60
10-141	$MDX+T_{5-9}$	3.45	5.10	5.00	6.65	4.35
10-144	CON_{5-9}	5.20	4.60	5.50	5.60	6.15
10-145	CON_{5-9}	3.97	5.20	5.60	6.15	6.60
10-146	$MDX+T_{5-9}$	6.50	5.15	4.00	3.75	4.75
10-147	$MDX+T_{5-9}$	4.00	3.35	3.90	2.25	2.90

Descriptive statistics

dpt		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Fat mass (%)					
1 (n=9-10)	Mean	4.36	5.10	3.28	4.59
	SEM	0.32	0.45	0.28	0.38
	Sig?	--	--	--	--
8 (n=9-10)	Mean	4.83	5.85	3.76	4.17
	SEM	0.28	0.52	0.42	0.27
	Sig?	--	--	--	--
15 (n=9-10)	Mean	4.54	5.46	3.03	3.93
	SEM	0.27	0.63	0.52	0.31
	Sig?	--	--	--	--
22 (n=9-10)	Mean	5.15	5.50	3.31	4.05
	SEM	0.44	0.63	0.67	0.52
	Sig?	--	--	--	--
27 (n=9-10)	Mean	5.66	4.97	4.28	3.53
	SEM	0.49	0.43	0.61	0.46
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

Dpt		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
Fat mass (%)			
1 (n=19-20)	Mean	4.75	3.93
	SEM	0.29	0.27
	Sig?	A	B
8 (n=19)	Mean	5.37	3.97
	SEM	0.32	0.24
	Sig?	A	B
15 (n=19)	Mean	5.02	3.51
	SEM	0.36	0.30
	Sig?	A	B
22 (n=19)	Mean	5.33	3.70
	SEM	0.38	0.42
	Sig?	A	B
27 (n=18-19)	Mean	5.29	3.90
	SEM	0.33	0.38
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Dpt		CON_{5-9}+MDX_{5-9}	$CON+T_{5-9}$+ $MDX+T_{5-9}$
Fat mass (%)			
1 (n=19-20)	Mean	3.79	4.84
	SEM	0.24	0.29
	Sig?	A	B
8 (n=18-20)	Mean	4.30	5.01
	SEM	0.28	0.34
	Sig?	--	--
15 (n=18-20)	Mean	3.79	4.69
	SEM	0.34	0.38
	Sig?	--	--
22 (n=18-20)	Mean	4.23	4.78
	SEM	0.45	0.43
	Sig?	--	--
27 (n=18-19)	Mean	4.97	4.28
	SEM	0.42	0.35
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

1 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	17.63	5.88	4.53	0.0087
Error	35	45.41	1.30		
Total	38	63.04			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	6.19	4.77	0.0358	
Tumor	1	10.18	7.84	0.0082	
Interaction	1	0.79	0.61	0.4393	

8 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	24.15	8.05	5.59	0.0031
Error	34	48.93	1.44		
Total	37	73.08			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	18.01	12.51	0.0012	
Tumor	1	4.78	3.32	0.0772	
Interaction	1	0.89	0.62	0.4373	

15 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	29.64	9.88	4.92	0.0061
Error	34	68.31	2.01		
Total	37	97.94			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	21.83	10.87	0.0023	
Tumor	1	7.74	3.85	0.0580	
Interaction	1	0.00	0.00	0.9881	

22 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	28.54	9.51	3.03	0.0426
Error	34	106.76	3.14		
Total	37	135.30			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	25.62	8.16	0.0073	
Tumor	1	2.81	0.89	0.3510	
Interaction	1	0.36	0.11	0.7376	

27 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	22.63	7.54	3.25	0.0340
Error	33	76.54	2.32		
Total	36	99.17			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	18.29	7.89	0.0083	
Tumor	1	4.79	2.06	0.1602	
Interaction	1	0.01	0.00	0.9531	

Percent fat mass: $SET_{9\text{-}13}$

Raw data

		Fat mass (%)				
ID	Group	-3 dpt	5 dpt	12 dpt	18 dpt	23 dpt
10-201	$MDX_{9\text{-}13}$	1.50	3.60	3.80	4.00	3.95
10-202	$CON_{9\text{-}13}$	5.60	4.75	4.25	5.20	5.20
10-203	$CON_{9\text{-}13}$	5.30	4.75	4.70	4.40	5.35
10-204	$CON+T_{9\text{-}13}$	5.90	6.10	5.30	5.20	2.95
10-205	$CON+T_{9\text{-}13}$	4.25	5.20	4.30	4.55	3.75
10-206	$MDX_{9\text{-}13}$	2.00	1.75	2.25	2.35	2.25
10-207	$MDX_{9\text{-}13}$	1.60	3.20	2.80	2.10	2.90
10-208	$CON_{9\text{-}13}$	8.70	5.35	5.55	4.50	5.20
10-209	$MDX+T_{9\text{-}13}$	3.30	3.75	4.20	3.30	2.85
10-210	$MDX_{9\text{-}13}$	1.10	1.90	3.30	1.95	2.80
10-211	$MDX_{9\text{-}13}$	3.35	4.05	4.25	4.35	4.10
10-212	$CON+T_{9\text{-}13}$	4.75	4.75	5.05	4.10	3.85
10-213	$MDX+T_{9\text{-}13}$	2.40	4.00	4.60	3.90	3.55
10-214	$MDX+T_{9\text{-}13}$	2.95	3.10	3.10	3.15	3.40
10-215	$CON+T_{9\text{-}13}$	6.30	6.50	6.50	5.75	7.45
10-216	$CON+T_{9\text{-}13}$	5.95	4.55	5.30	5.65	5.90
10-217	$MDX_{9\text{-}13}$	2.20				
10-218	$MDX_{9\text{-}13}$	1.40	1.35	1.65	2.25	2.25
10-219	$CON_{9\text{-}13}$	10.35	12.15	12.00	5.15	4.00
10-220	$CON_{9\text{-}13}$	4.65	4.90	4.60	4.25	3.75
10-221	$MDX+T_{9\text{-}13}$	2.70	4.70	5.30	3.55	2.95
10-222	$MDX+T_{9\text{-}13}$	2.80	4.05	3.75	2.05	2.05
10-223	$MDX+T_{9\text{-}13}$	3.15	3.85	3.20	3.10	2.85
10-224	$MDX+T_{9\text{-}13}$	3.25	4.20	3.65	3.10	2.85
10-225	$CON+T_{9\text{-}13}$	5.10	6.10	6.65	5.55	4.10
10-226	$CON+T_{9\text{-}13}$	5.60	6.40	5.50	3.15	3.05
10-227	$CON_{9\text{-}13}$	4.75	8.20	7.70	4.85	4.80
10-228	$CON_{9\text{-}13}$	7.65	10.20	10.85	3.40	3.80

Descriptive statistics

dpt		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Fat mass (%)					
-3 (n=7)	Mean	6.71	5.41	1.88	2.94
	SEM	0.84	0.28	0.28	0.12
	Sig?	A	A	B	B
5 (n=6-7)	Mean	7.19	5.66	2.64	3.95
	SEM	1.15	0.31	0.46	0.18
	Sig?	A	AB	C	BC
12 (n=6-7)	Mean	7.09	5.51	3.01	3.97
	SEM	1.20	0.31	0.40	0.30
	Sig?	--	--	--	--
18 (n=6-7)	Mean	4.54	4.85	2.83	3.16
	SEM	0.23	0.37	0.43	0.22
	Sig?	--	--	--	--
23 (n=6-7)	Mean	4.59	4.44	3.04	2.93
	SEM	0.27	0.62	0.33	0.18
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

Dpt		CON_{9-13}+ CON+T_{9-13}	MDX_{9-13}+ MDX+T_{9-13}
Fat mass (%)			
-3 (n=14)	Mean	6.06	2.41
	SEM	0.46	0.21
	Sig?	A	B
5 (n=13-14)	Mean	6.42	3.35
	SEM	0.61	0.29
	Sig?	A	B
12 (n=13-14)	Mean	6.30	3.53
	SEM	0.64	0.27
	Sig?	A	B
18 (n=13-14)	Mean	4.69	3.01
	SEM	0.21	0.22
	Sig?	A	B
23 (n=13-14)	Mean	4.51	2.98
	SEM	0.33	0.63
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Dpt		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
Fat mass (%)			
-3 (n=14)	Mean	4.30	4.17
	SEM	0.79	0.37
	Sig?	--	--
5 (n=13-14)	Mean	5.09	4.80
	SEM	0.91	0.29
	Sig?	--	--
12 (n=13-14)	Mean	5.21	4.74
	SEM	0.88	0.30
	Sig?	--	--
18 (n=13-14)	Mean	3.75	4.01
	SEM	0.33	0.31
	Sig?	--	--
23 (n=13-14)	Mean	3.87	3.68
	SEM	0.30	0.38
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

-3 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	103.33	34.44	22.58	<0.0001
Error	24	36.61	1.53		
Total	27	139.95			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	93.44	61.25	<0.0001	
Tumor	1	0.11	0.07	0.7912	
Interaction	1	9.78	6.41	0.0183	

5 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	77.46	25.82	8.89	0.0004
Error	23	66.77	2.90		
Total	26	144.23			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	65.65	22.61	<0.0001	
Tumor	1	0.08	0.03	0.8684	
Interaction	1	13.52	4.66	0.0416	

12 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	63.69	21.23	6.65	0.0021
Error	23	73.45	3.19		
Total	26	137.14			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	53.20	16.66	0.0005	
Tumor	1	0.64	0.20	0.6595	
Interaction	1	10.85	3.40	0.0782	

18 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	19.75	6.58	9.82	0.0002
Error	23	15.43	0.67		
Total	26	35.18			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	19.29	28.75	<0.0001	
Tumor	1	0.70	1.04	0.3178	
Interaction	1	0.00	0.00	0.9792	

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	15.90	5.30	5.08	0.0076
Error	23	24.00	1.04		
Total	26	39.90			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	15.64	14.99	0.0008	
Tumor	1	0.12	0.11	0.7416	
Interaction	1	0.00	0.00	0.9631	

Percent fluid mass: SET_{5-9}

Raw data

		Fluid mass (%)				
ID	Group	1 dpt	8 dpt	15 dpt	22 dpt	27 dpt
10-101	CON_{5-9}	5.55	6.10	6.00	6.00	6.05
10-102	CON_{5-9}	5.70	5.45	5.50	5.60	5.00
10-103	$MDX+T_{5-9}$	5.85	6.05	5.95	6.15	5.90
10-104	$MDX+T_{5-9}$	5.85	5.85	5.65	5.75	6.65
10-105	$MDX+T_{5-9}$	6.00	6.80	6.25	6.30	6.00
10-106	$MDX+T_{5-9}$	6.70	6.25	6.15	5.75	6.60
10-107	$CON+T_{5-9}$	6.35	6.40	5.50	5.50	5.50
10-108	$CON+T_{5-9}$	5.45	5.25	5.50	5.80	6.00
10-109	$CON+T_{5-9}$	5.35	5.10	5.70	5.85	6.70
10-110	$CON+T_{5-9}$	5.40	5.65	6.35	5.75	5.70
10-115	CON_{5-9}	6.10	5.65	5.60	5.75	5.90
10-116	CON_{5-9}	5.30	5.20	5.80	5.10	5.50
10-117	$CON+T_{5-9}$	5.95	5.15	5.70	5.95	5.60
10-118	$CON+T_{5-9}$	5.35	5.40	6.25	6.60	6.25
10-119	CON_{5-9}	6.25	5.35	5.70	6.00	5.75
10-120	$MDX+T_{5-9}$	5.67	6.70	6.50	6.35	
10-121	$MDX+T_{5-9}$	5.55	6.10	6.00	6.00	6.05
10-122	MDX_{5-9}	5.70	5.45	5.50	5.60	5.00
10-123	MDX_{5-9}	5.85	6.05	5.95	6.15	5.90
10-124	MDX_{5-9}	5.85	5.85	5.65	5.75	6.65
10-125	MDX_{5-9}	6.00	6.80	6.25	6.30	6.00
10-126	MDX_{5-9}	6.70	6.25	6.15	5.75	6.60
10-127	MDX_{5-9}	6.35	6.40	5.50	5.50	5.50
10-128	MDX_{5-9}	5.45	5.25	5.50	5.80	6.00
10-129	MDX_{5-9}	5.35	5.10	5.70	5.85	6.70
10-130	MDX_{5-9}	5.40	5.65	6.35	5.75	5.70
10-131	MDX_{5-9}	6.10	5.65	5.60	5.75	5.90
10-132	$CON+T_{5-9}$	5.30	5.20	5.80	5.10	5.50
10-133	$CON+T_{5-9}$	5.95	5.15	5.70	5.95	5.60
10-136	$CON+T_{5-9}$	5.35	5.40	6.25	6.60	6.25
10-137	$CON+T_{5-9}$	6.25	5.35	5.70	6.00	5.75
10-138	CON_{5-9}	5.67	6.70	6.50	6.35	
10-139	CON_{5-9}	5.55	6.10	6.00	6.00	6.05
10-140	$MDX+T_{5-9}$	5.70	5.45	5.50	5.60	5.00
10-141	$MDX+T_{5-9}$	5.85	6.05	5.95	6.15	5.90
10-144	CON_{5-9}	5.85	5.85	5.65	5.75	6.65
10-145	CON_{5-9}	6.00	6.80	6.25	6.30	6.00
10-146	$MDX+T_{5-9}$	6.70	6.25	6.15	5.75	6.60
10-147	$MDX+T_{5-9}$	6.35	6.40	5.50	5.50	5.50

Descriptive statistics

dpt		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Fluid mass (%)					
1 (n=9-10)	Mean	5.80	5.72	6.20	5.94
	SEM	0.10	0.11	0.11	0.11
	Sig?	--	--	--	--
8 (n=9-10)	Mean	5.63	5.55	6.01	6.23
	SEM	0.12	0.13	0.09	0.10
	Sig?	--	--	--	--
15 (n=9-10)	Mean	5.74	5.81	6.34	6.13
	SEM	0.10	0.11	0.08	0.08
	Sig?	--	--	--	--
22 (n=9-10)	Mean	5.74	6.01	6.24	6.21
	SEM	0.10	0.11	0.13	0.10
	Sig?	--	--	--	--
27 (n=9-10)	Mean	5.61	6.03	5.88	6.32
	SEM	0.13	0.14	0.04	0.09
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
Fluid mass (%)			
1 (n=19-20)	Mean	5.76	6.07
	SEM	0.07	0.08
	Sig?	A	B
8 (n=19)	Mean	5.58	6.13
	SEM	0.09	0.07
	Sig?	A	B
15 (n=19)	Mean	5.78	6.23
	SEM	0.07	0.06
	Sig?	A	B
22 (n=19)	Mean	5.88	6.22
	SEM	0.08	0.08
	Sig?	A	B
27 (n=18-19)	Mean	5.83	6.10
	SEM	0.11	0.07
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{5-9}+MDX_{5-9}	CON+T_{5-9}+ MDX+T_{5-9}
Fluid mass (%)			
1 (n=19-20)	Mean	6.01	5.83
	SEM	0.09	0.08
	Sig?	--	--
8 (n=18-20)	Mean	5.82	5.89
	SEM	0.09	0.11
	Sig?	--	--
15 (n=18-20)	Mean	6.04	5.97
	SEM	0.09	0.08
	Sig?	--	--
22 (n=18-20)	Mean	5.99	6.11
	SEM	0.10	0.07
	Sig?	--	--
27 (n=18-19)	Mean	5.74	6.17
	SEM	0.07	0.09
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

1 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1.32	0.44	3.90	0.0167
Error	35	3.96	0.11		
Total	38	5.28			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.94	8.27	0.0068	
Tumor	1	0.28	2.48	0.1243	
Interaction	1	0.09	0.75	0.3917	

8 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	3.05	1.02	8.80	0.0002
Error	34	3.92	0.12		
Total	37	6.98			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	2.70	23.40	<0.0001	
Tumor	1	0.04	0.38	0.5418	
Interaction	1	0.22	1.87	0.1809	

15 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	2.14	0.71	8.67	0.0002
Error	34	2.80	0.08		
Total	37	4.93			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	1.96	23.82	<0.0001	
Tumor	1	0.05	0.63	0.4316	
Interaction	1	0.18	2.25	0.1430	

22 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1.44	0.48	4.36	0.0106
Error	34	3.74	0.11		
Total	37	5.17			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	1.14	10.41	0.0028	
Tumor	1	0.12	1.10	0.3012	
Interaction	1	0.22	2.01	0.1659	

27 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	2.41	0.80	7.12	0.0008
Error	33	3.72	0.11		
Total	36	6.13			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.76	6.77	0.0138	
Tumor	1	1.70	15.09	0.0005	
Interaction	1	0.00	0.01	0.9304	

Percent fluid mass: $SET_{9\text{-}13}$

Raw data

		Fluid mass (%)				
ID	Group	-3 dpt	5 dpt	12 dpt	18 dpt	23 dpt
10-201	$MDX_{9\text{-}13}$	6.60	5.75	7.55	5.70	5.50
10-202	$CON_{9\text{-}13}$	5.30	6.10	7.15	5.75	5.30
10-203	$CON_{9\text{-}13}$	5.45	5.40	5.20	5.70	5.55
10-204	$CON+T_{9\text{-}13}$	5.65	5.10	5.35	5.95	6.25
10-205	$CON+T_{9\text{-}13}$	5.45	5.50	5.55	5.80	5.80
10-206	$MDX_{9\text{-}13}$	6.35	6.65	5.95	6.00	6.20
10-207	$MDX_{9\text{-}13}$	6.50	5.50	6.15	6.60	6.35
10-208	$CON_{9\text{-}13}$	5.30	4.95	5.00	5.90	5.65
10-209	$MDX+T_{9\text{-}13}$	5.75	5.70	5.70	6.15	5.90
10-210	$MDX_{9\text{-}13}$	7.15	6.40	5.75	6.05	5.70
10-211	$MDX_{9\text{-}13}$	6.00	5.85	5.90	6.00	5.60
10-212	$CON+T_{9\text{-}13}$	5.65	5.25	5.15	5.60	5.95
10-213	$MDX+T_{9\text{-}13}$	6.35	5.60	5.50	6.15	6.20
10-214	$MDX+T_{9\text{-}13}$	6.30	5.85	5.95	6.25	5.95
10-215	$CON+T_{9\text{-}13}$	5.20	5.05	5.60	6.20	5.60
10-216	$CON+T_{9\text{-}13}$	5.50	5.30	5.05	5.25	5.05
10-217	$MDX_{9\text{-}13}$	6.20				
10-218	$MDX_{9\text{-}13}$	6.40	6.90	6.90	6.05	5.95
10-219	$CON_{9\text{-}13}$	5.75	5.40	6.00	5.80	6.10
10-220	$CON_{9\text{-}13}$	5.30	5.15	5.50	5.00	5.50
10-221	$MDX+T_{9\text{-}13}$	6.50	5.90	5.85	6.30	6.15
10-222	$MDX+T_{9\text{-}13}$	6.15	5.65	6.20	6.60	6.85
10-223	$MDX+T_{9\text{-}13}$	5.70	5.70	6.15	5.55	5.70
10-224	$MDX+T_{9\text{-}13}$	5.95	5.55	5.95	6.00	6.30
10-225	$CON+T_{9\text{-}13}$	5.50	5.00	5.40	5.45	5.55
10-226	$CON+T_{9\text{-}13}$	5.50	5.25	5.55	6.10	6.95
10-227	$CON_{9\text{-}13}$	5.90	5.40	5.50	5.70	5.45
10-228	$CON_{9\text{-}13}$	5.25	5.15	5.35	6.35	5.85

Descriptive statistics

dpt		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Fluid mass (%)					
-3 (n=7)	Mean	5.46	5.49	6.46	6.10
	SEM	0.10	0.06	0.14	0.12
	Sig?	--	--	--	--
5 (n=6-7)	Mean	5.36	5.21	6.18	5.71
	SEM	0.14	0.06	0.23	0.05
	Sig?	--	--	--	--
12 (n=6-7)	Mean	5.67	5.38	6.37	5.90
	SEM	0.27	0.08	0.29	0.09
	Sig?	--	--	--	--
18 (n=6-7)	Mean	5.74	5.76	6.07	6.14
	SEM	0.15	0.13	0.12	0.12
	Sig?	--	--	--	--
23 (n=6-7)	Mean	5.63	5.88	5.88	6.15
	SEM	0.10	0.23	0.14	0.14
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

dpt		CON_{9-13}+ CON+T_{9-13}	MDX_{9-13}+ MDX+T_{9-13}
Fluid mass (%)			
-3 (n=14)	Mean	5.48	6.28
	SEM	0.05	0.10
	Sig?	A	B
5 (n=13-14)	Mean	5.29	5.92
	SEM	0.08	0.12
	Sig?	A	B
12 (n=13-14)	Mean	5.53	6.12
	SEM	0.14	0.15
	Sig?	A	B
18 (n=13-14)	Mean	5.75	6.11
	SEM	0.10	0.08
	Sig?	A	B
23 (n=13-14)	Mean	5.75	6.03
	SEM	0.12	0.10
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

dpt		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
Fluid mass (%)			
-3 (n=14)	Mean	5.96	5.80
	SEM	0.16	0.10
	Sig?	--	--
5 (n=13-14)	Mean	5.74	5.46
	SEM	0.17	0.08
	Sig?	A	B
12 (n=13-14)	Mean	5.99	5.64
	SEM	0.21	0.09
	Sig?	--	--
18 (n=13-14)	Mean	5.89	5.95
	SEM	0.10	0.10
	Sig?	--	--
23 (n=13-14)	Mean	5.75	6.01
	SEM	0.09	0.13
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

-3 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	4.93	1.64	20.78	<0.0001
Error	24	1.90	0.08		
Total	27	6.83			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	4.48	56.65	<0.0001	
Tumor	1	0.19	2.39	0.1353	
Interaction	1	0.26	3.29	0.0821	

5 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	3.53	1.18	10.31	0.0002
Error	23	2.63	0.11		
Total	26	6.16			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	2.89	25.27	<0.0001	
Tumor	1	0.66	5.75	0.0250	
Interaction	1	0.16	1.42	0.2455	

12 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	3.35	1.12	4.11	0.0180
Error	23	6.26	0.27		
Total	26	9.62			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	2.49	9.13	0.0061	
Tumor	1	0.97	3.56	0.0719	
Interaction	1	0.05	0.19	0.6699	

18 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.87	0.29	2.43	0.0907
Error	23	2.73	0.12		
Total	26	3.59			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.83	6.99	0.0145	
Tumor	1	0.02	0.14	0.7166	
Interaction	1	0.01	0.04	0.8385	

23 dpt

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.95	0.32	1.82	0.1713
Error	23	4.01	0.17		
Total	26	4.96			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.47	2.67	0.1159	
Tumor	1	0.45	2.57	0.1223	
Interaction	1	0.00	0.00	0.9592	

Total energy expenditure (by phase): SET_{5-9}

Raw data

		TEE by phase (kJ/kg/hr)	
ID	Group	Light	Dark
10-101	CON_{5-9}	133.37	154.28
10-102	CON_{5-9}	140.52	154.97
10-104	$MDX+T_{5-9}$	137.80	156.57
10-106	$MDX+T_{5-9}$	138.69	193.91
10-107	$CON+T_{5-9}$	141.87	173.40
10-109	$CON+T_{5-9}$	130.53	146.13
10-110	$CON+T_{5-9}$	157.34	171.87
10-115	CON_{5-9}	134.42	163.40
10-119	CON_{5-9}	129.69	169.03
10-121	$MDX+T_{5-9}$	112.98	146.49
10-122	MDX_{5-9}	147.48	170.43
10-124	MDX_{5-9}	140.73	166.66
10-125	MDX_{5-9}	156.25	179.88
10-129	MDX_{5-9}	132.35	156.24
10-130	MDX_{5-9}	134.60	164.26
10-132	$CON+T_{5-9}$	144.26	178.85
10-137	$CON+T_{5-9}$	143.80	165.63
10-141	$MDX+T_{5-9}$	132.62	168.93
10-145	CON_{5-9}	146.17	194.26

Descriptive statistics

phase		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
TEE by phase (kJ/kg/hr)					
Light (n=4-5)	Mean	136.83	143.56	142.28	130.52
	SEM	2.91	4.26	4.37	6.00
	Sig?	--	--	--	--
Dark (n=4-5)	Mean	167.19	167.18	167.50	166.48
	SEM	7.30	5.67	3.87	10.23
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

phase		CON_{5-9}+ $CON+T_{5-9}$	MDX_{5-9}+ $MDX+T_{5-9}$
TEE by phase (kJ/kg/hr)			
Light (n=9-10)	Mean	140.20	137.05
	SEM	2.68	3.95
	Sig?	--	--
Dark (n=9-10)	Mean	167.18	167.04
	SEM	4.36	4.65
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{5-9}+MDX_{5-9}	$CON+T_{5-9}$+ $MDX+T_{5-9}$
TEE by phase (kJ/kg/hr)			
Light (n=9-10)	Mean	139.56	137.76
	SEM	2.64	4.04
	Sig?	--	--
Dark (n=9-10)	Mean	167.34	166.86
	SEM	3.90	5.14
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	467.21	155.74	1.73	0.2028
Error	15	1346.62	89.77		
Total	18	1813.83			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	67.82	0.76	0.3985	
Tumor	1	29.82	0.33	0.5729	
Interaction	1	402.03	4.48	0.0515	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	2.41	0.80	0.00	0.9997
Error	15	3264.73	217.65		
Total	18	3267.14			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.18	0.00	0.9774	
Tumor	1	1.26	0.01	0.9405	
Interaction	1	1.20	0.01	0.9419	

Total energy expenditure (by phase): $SET_{9\text{-}13}$

Raw data

		TEE by phase (kJ/kg/hr)	
ID	Group	Light	Dark
10-204	$CON+T_{9\text{-}13}$	147.12	181.86
10-205	$CON+T_{9\text{-}13}$	165.29	175.23
10-206	$MDX_{9\text{-}13}$	137.70	170.11
10-207	$MDX_{9\text{-}13}$	139.24	204.26
10-208	$CON_{9\text{-}13}$	154.18	164.21
10-209	$MDX+T_{9\text{-}13}$	122.73	143.35
10-210	$MDX_{9\text{-}13}$	140.57	177.44
10-211	$MDX_{9\text{-}13}$	134.67	165.94
10-212	$CON+T_{9\text{-}13}$	185.76	197.21
10-214	$MDX+T_{9\text{-}13}$	135.83	154.58
10-218	$MDX_{9\text{-}13}$	123.61	168.37
10-219	$CON_{9\text{-}13}$	166.73	181.92
10-220	$CON_{9\text{-}13}$	155.55	190.02
10-221	$MDX+T_{9\text{-}13}$	155.34	197.86
10-222	$MDX+T_{9\text{-}13}$	130.20	160.37
10-223	$MDX+T_{9\text{-}13}$	125.18	161.61
10-225	$CON+T_{9\text{-}13}$	152.89	171.87
10-226	$CON+T_{9\text{-}13}$	154.28	183.74
10-227	$CON_{9\text{-}13}$	138.50	171.44
10-228	$CON_{9\text{-}13}$	173.86	202.62

Descriptive statistics

phase		**$CON_{9\text{-}13}$**	**$CON+T_{9\text{-}13}$**	**$MDX_{9\text{-}13}$**	**$MDX+T_{9\text{-}13}$**
TEE by phase (kJ/kg/hr)					
Light (n=5)	Mean	157.76	161.07	135.16	133.86
	SEM	6.04	6.84	3.05	5.82
	Sig?	--	--	--	--
Dark (n=5)	Mean	182.04	181.98	177.22	163.55
	SEM	6.77	4.38	7.03	9.16
	Sig?	--	--	--	--

Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)

phase		CON_{9-13}+ $CON+T_{9-13}$	MDX_{9-13}+ $MDX+T_{9-13}$
TEE by phase (kJ/kg/hr)			
Light (n=10)	Mean	159.42	134.51
	SEM	4.33	3.11
	Sig?	A	B
Dark (n=10)	Mean	182.01	170.39
	SEM	3.80	5.90
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{9-13}+MDX_{9-13}	$CON+T_{9-13}$+ $MDX+T_{9-13}$
TEE by phase (kJ/kg/hr)			
Light (n=10)	Mean	146.46	147.46
	SEM	4.94	6.20
	Sig?	--	--
Dark (n=10)	Mean	179.63	172.77
	SEM	4.67	5.69
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	3133.90	1044.63	6.61	0.0041
Error	16	2527.56	157.97		
Total	19	5661.46			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	3102.36	19.64	0.0004	
Tumor	1	5.00	0.03	0.8610	
Interaction	1	26.54	0.17	0.6873	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1142.98	380.99	1.54	0.2435
Error	16	3966.99	247.94		
Total	19	5109.96			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	675.63	2.72	0.1183	
Tumor	1	235.63	0.95	0.3441	
Interaction	1	231.72	0.93	0.3481	

Respiratory exchange ratio (by phase): SET_{5-9}

Raw data

		RER by phase	
ID	Group	Light	Dark
10-101	CON_{5-9}	0.92	0.96
10-102	CON_{5-9}	0.90	0.93
10-104	$MDX+T_{5-9}$	0.88	0.96
10-106	$MDX+T_{5-9}$	0.88	0.91
10-107	$CON+T_{5-9}$	0.88	0.94
10-109	$CON+T_{5-9}$	0.81	0.88
10-110	$CON+T_{5-9}$	0.89	0.95
10-115	CON_{5-9}	0.89	0.98
10-119	CON_{5-9}	0.93	0.99
10-121	$MDX+T_{5-9}$	0.89	0.94
10-122	MDX_{5-9}	0.89	0.96
10-124	MDX_{5-9}	0.87	0.96
10-125	MDX_{5-9}	0.85	0.98
10-129	MDX_{5-9}	0.91	0.97
10-130	MDX_{5-9}	0.87	0.96
10-132	$CON+T_{5-9}$	0.84	0.93
10-137	$CON+T_{5-9}$	0.90	0.93
10-141	$MDX+T_{5-9}$	0.81	0.92
10-145	CON_{5-9}	0.87	0.95

Descriptive statistics

phase		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
RER by phase					
Light (n=4-5)	Mean	0.90	0.86	0.88	0.86
	SEM	0.01	0.02	0.01	0.02
	Sig?	--	--	--	--
Dark (n=4-5)	Mean	0.96	0.93	0.97	0.93
	SEM	0.01	0.01	0.00	0.01
	Sig?	--	--	--	--

Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)

phase		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
RER by phase			
Light (n=9-10)	Mean	0.88	0.87
	SEM	0.01	0.01
	Sig?	--	--
Dark (n=9-10)	Mean	0.94	0.95
	SEM	0.01	0.01
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{5-9}+MDX_{5-9}	CON+T_{5-9}+ MDX+T_{5-9}
RER by phase			
Light (n=9-10)	Mean	0.89	0.86
	SEM	0.01	0.01
	Sig?	--	--
Dark (n=9-10)	Mean	0.96	0.93
	SEM	0.01	0.01
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.00	0.00	1.47	0.2628
Error	15	0.01	0.00		
Total	18	0.02			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.00	0.63	0.4405	
Tumor	1	0.00	2.92	0.1079	
Interaction	1	0.00	0.74	0.4029	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.01	0.00	4.16	0.0249
Error	15	0.01	0.00		
Total	18	0.01			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.00	0.43	0.5234	
Tumor	1	0.01	11.72	0.0038	
Interaction	1	0.00	0.00	0.9939	

Respiratory exchange ratio (by phase): $SET_{9\text{-}13}$

Raw data

		RER by phase	
ID	Group	Light	Dark
10-204	$CON+T_{9\text{-}13}$	0.82	0.88
10-205	$CON+T_{9\text{-}13}$	0.87	0.91
10-206	$MDX_{9\text{-}13}$	0.87	0.94
10-207	$MDX_{9\text{-}13}$	0.88	0.94
10-208	$CON_{9\text{-}13}$	0.90	0.95
10-209	$MDX+T_{9\text{-}13}$	0.88	0.94
10-210	$MDX_{9\text{-}13}$	0.88	0.95
10-211	$MDX_{9\text{-}13}$	0.84	0.92
10-212	$CON+T_{9\text{-}13}$	0.95	0.96
10-214	$MDX+T_{9\text{-}13}$	0.91	0.94
10-218	$MDX_{9\text{-}13}$	0.90	0.96
10-219	$CON_{9\text{-}13}$	0.87	0.86
10-220	$CON_{9\text{-}13}$	0.92	0.98
10-221	$MDX+T_{9\text{-}13}$	0.86	0.96
10-222	$MDX+T_{9\text{-}13}$	0.87	0.93
10-223	$MDX+T_{9\text{-}13}$	0.88	0.93
10-225	$CON+T_{9\text{-}13}$	0.88	0.93
10-226	$CON+T_{9\text{-}13}$	0.88	0.93
10-227	$CON_{9\text{-}13}$	0.88	0.98
10-228	$CON_{9\text{-}13}$	0.85	0.85

Descriptive statistics

phase		**$CON_{9\text{-}13}$**	**$CON+T_{9\text{-}13}$**	**$MDX_{9\text{-}13}$**	**$MDX+T_{9\text{-}13}$**
RER by phase					
Light (n=5)	Mean	0.89	0.88	0.87	0.88
	SEM	0.01	0.02	0.01	0.01
	Sig?	--	--	--	--
Dark (n=5)	Mean	0.92	0.92	0.94	0.94
	SEM	0.03	0.01	0.01	0.01
	Sig?	--	--	--	--

Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)

phase		CON_{9-13}+ CON+T_{9-13}	MDX_{9-13}+ MDX+T_{9-13}
RER by phase			
Light (n=10)	Mean	0.88	0.88
	SEM	0.01	0.01
	Sig?	--	--
Dark (n=10)	Mean	0.92	0.94
	SEM	0.01	0.00
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{9-13}+MDX_{9-13}	CON+T_{9-13}+ MDX+T_{9-13}
RER by phase			
Light (n=10)	Mean	0.88	0.88
	SEM	0.01	0.01
	Sig?	--	--
Dark (n=10)	Mean	0.93	0.93
	SEM	0.01	0.01
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.00	0.00	0.15	0.9269
Error	16	0.02	0.00		
Total	19	0.02			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.00	0.29	0.5984	
Tumor	1	0.00	0.00	0.9481	
Interaction	1	0.00	0.16	0.6921	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	0.00	0.00	0.35	0.7900
Error	16	0.02	0.00		
Total	19	0.02			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	0.00	1.04	0.3228	
Tumor	1	0.00	0.01	0.9319	
Interaction	1	0.00	0.00	0.9840	

Horizontal cage activity (by phase): SET_{5-9}

Raw data

		Horizontal cage activity by phase (beam breaks)	
ID	Group	Light	Dark
10-101	CON_{5-9}	4,182	2,324
10-102	CON_{5-9}	33,941	56,261
10-104	$MDX+T_{5-9}$	6,246	19,185
10-106	$MDX+T_{5-9}$	15,109	55,800
10-107	$CON+T_{5-9}$	13,799	54,182
10-109	$CON+T_{5-9}$	23,157	30,336
10-110	$CON+T_{5-9}$	8,813	17,282
10-115	CON_{5-9}	15,575	52,947
10-119	CON_{5-9}	6,976	70,982
10-121	$MDX+T_{5-9}$	4,027	5,848
10-122	MDX_{5-9}	7,281	21,035
10-124	MDX_{5-9}	22,619	78,611
10-125	MDX_{5-9}	3,702	8,112
10-129	MDX_{5-9}	8,060	39,252
10-130	MDX_{5-9}	21,914	62,728
10-132	$CON+T_{5-9}$	7,855	28,154
10-137	$CON+T_{5-9}$	12,985	42,737
10-141	$MDX+T_{5-9}$	8,945	54,889
10-145	CON_{5-9}	13,569	104,344

Descriptive statistics

phase		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Horizontal cage activity by phase (beam breaks)					
Light (n=4-5)	Mean	14,849	13,322	12,715	8,582
	SEM	5,208	2,714	3,970	2,397
	Sig?	--	--	--	--
Dark (n=4-5)	Mean	57,372	34,538	41,948	33,931
	SEM	16,497	6,361	12,972	12,661
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

phase		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
Horizontal cage activity by phase (beam breaks)			
Light (n=9-10)	Mean	14,085	10,878
	SEM	2,780	2,421
	Sig?	--	--
Dark (n=9-10)	Mean	45,955	38,384
	SEM	9,162	8,686
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{5-9}+MDX_{5-9}	CON+T_{5-9}+ MDX+T_{5-9}
Horizontal cage activity by phase (beam breaks)			
Light (n=9-10)	Mean	13,782	11,215
	SEM	3,107	1,923
	Sig?	--	--
Dark (n=9-10)	Mean	49,660	34,268
	SEM	10,221	6,162
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	92,515,777	30,838,592	0.43	0.7340
Error	15	1,073,807,236	71,587,149		
Total	18	1,166,323,013			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	55,581,547	0.78	0.3921	
Tumor	1	37,692,271	0.53	0.4792	
Interaction	1	7,993,676	0.11	0.7429	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1,717,718,196	572,572,732	0.74	0.5423
Error	15	11,541,061,444	769,404,096		
Total	18	13,258,779,640			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	302,371,065	0.39	0.5402	
Tumor	1	1,119,709,824	1.46	0.2464	
Interaction	1	258,262,054	0.34	0.5709	

Horizontal cage activity (by phase): SET_{9-13}

Raw data

		Horizontal cage activity by phase (beam breaks)	
ID	Group	Light	Dark
10-204	$CON+T_{9-13}$	20,711	78,724
10-205	$CON+T_{9-13}$	34,327	47,587
10-206	MDX_{9-13}	36,721	112,554
10-207	MDX_{9-13}	9,520	47,683
10-208	CON_{9-13}	15,234	27,286
10-209	$MDX+T_{9-13}$	17,727	46,070
10-210	MDX_{9-13}	33,425	114,113
10-211	MDX_{9-13}	19,146	36,825
10-212	$CON+T_{9-13}$	15,837	12,557
10-214	$MDX+T_{9-13}$	19,901	49,033
10-218	MDX_{9-13}	17,916	84,615
10-219	CON_{9-13}	16,476	29,462
10-220	CON_{9-13}	17,569	38,886
10-221	$MDX+T_{9-13}$	21,321	84,645
10-222	$MDX+T_{9-13}$	11,202	40,319
10-223	$MDX+T_{9-13}$	19,359	45,366
10-225	$CON+T_{9-13}$	24,266	45,357
10-226	$CON+T_{9-13}$	18,076	35,865
10-227	CON_{9-13}	8,208	36,946
10-228	CON_{9-13}	19,467	62,656

Descriptive statistics

phase		**CON_{9-13}**	**$CON+T_{9-13}$**	**MDX_{9-13}**	**$MDX+T_{9-13}$**
Horizontal cage activity by phase (beam breaks)					
Light (n=5)	Mean	15,391	22,643	23,346	17,902
	SEM	1,926	3,241	5,093	1,771
	Sig?	--	--	--	--
Dark (n=5)	Mean	39,047	44,018	79,158	53,087
	SEM	6,293	10,666	16,046	8,013
	Sig?	--	--	--	--

Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)

phase		CON_{9-13}+ CON+T_{5-9}	MDX_{9-13}+ MDX+T_{9-13}
Horizontal cage activity by phase (beam breaks)			
Light (n=10)	Mean	19,017	20,624
	SEM	2,149	2,699
	Sig?	--	--
Dark (n=10)	Mean	41,533	66,122
	SEM	5,896	9,506
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{9-13}+MDX_{9-13}	CON+T_{9-13}+ MDX+T_{9-13}
Horizontal cage activity by phase (beam breaks)			
Light (n=10)	Mean	19,368	20,273
	SEM	2,889	1,912
	Sig?	--	--
Dark (n=10)	Mean	59,103	48,552
	SEM	10,522	6,468
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	218,489,894	72,829,965	1.35	0.2947
Error	16	865,705,633	54,106,602		
Total	19	1,084,195,527			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	12,907,424	0.24	0.6319	
Tumor	1	4,090,601	0.08	0.7869	
Interaction	1	201,491,868	3.72	0.0716	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	4,784,333,607	1,594,777,869	2.69	0.0815
Error	16	9,501,219,834	593,826,240		
Total	19	14,285,553,441			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	3,023,266,730	5.09	0.0384	
Tumor	1	556,544,150	0.94	0.3474	
Interaction	1	1,204,522,726	2.03	0.1736	

Vertical cage activity (by phase): SET_{5-9}

Raw data

		Vertical cage activity by phase (beam breaks)	
ID	Group	Light	Dark
10-101	CON_{5-9}	1,746	4,215
10-102	CON_{5-9}	2,608	4,402
10-104	$MDX+T_{5-9}$	147	1,141
10-106	$MDX+T_{5-9}$	1,765	7,399
10-107	$CON+T_{5-9}$	1,676	4,173
10-109	$CON+T_{5-9}$	538	710
10-110	$CON+T_{5-9}$	761	1,838
10-115	CON_{5-9}	1,307	4,714
10-119	CON_{5-9}	468	4,141
10-121	$MDX+T_{5-9}$	1,316	6,206
10-122	MDX_{5-9}	701	1,632
10-124	MDX_{5-9}	863	4,716
10-125	MDX_{5-9}	53	429
10-129	MDX_{5-9}	630	2,977
10-130	MDX_{5-9}	1,061	3,801
10-132	$CON+T_{5-9}$	110	932
10-137	$CON+T_{5-9}$	1,187	3,755
10-141	$MDX+T_{5-9}$	443	4,023
10-145	CON_{5-9}	505	5,675

Descriptive statistics

phase		CON_{5-9}	$CON+T_{5-9}$	MDX_{5-9}	$MDX+T_{5-9}$
Vertical cage activity by phase (beam breaks)					
Light (n=4-5)	Mean	1,327	854	662	918
	SEM	402	269	169	376
	Sig?	--	--	--	--
Dark (n=4-5)	Mean	4,629	2,282	2,711	4,692
	SEM	280	715	763	1,375
	Sig?	--	--	--	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

phase		CON_{5-9}+ CON+T_{5-9}	MDX_{5-9}+ MDX+T_{5-9}
Vertical cage activity by phase (beam breaks)			
Light (n=9-10)	Mean	1,091	775
	SEM	2,41	183
	Sig?	--	--
Dark (n=9-10)	Mean	3,456	3,592
	SEM	533	773
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{5-9}+MDX_{5-9}	CON+T_{5-9}+ MDX+T_{5-9}
Vertical cage activity by phase (beam breaks)			
Light (n=9-10)	Mean	994	883
	SEM	234	209
	Sig?	--	--
Dark (n=9-10)	Mean	3,670	3,353
	SEM	499	798
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1,174,188	391,396	0.84	0.4905
Error	15	6,948,006	463,200		
Total	18	8,122,194			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	426,145	0.92	0.3527	
Tumor	1	55,017	0.12	0.7352	
Interaction	1	624,453	1.35	0.2638	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	22,591,100	7,530,367	2.45	0.1038
Error	15	46,126,691	3,075,113		
Total	18	68,717,791			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	285,071	0.09	0.7650	
Tumor	1	158,069	0.05	0.8237	
Interaction	1	22,047,852	7.17	0.0172	

Vertical cage activity (by phase): SET_{9-13}

Raw data

		Vertical cage activity by phase (beam breaks)	
ID	Group	Light	Dark
10-204	$CON+T_{9-13}$	956	5,554
10-205	$CON+T_{9-13}$	1,741	2,989
10-206	MDX_{9-13}	2,354	8,412
10-207	MDX_{9-13}	795	5,842
10-208	CON_{9-13}	739	1,543
10-209	$MDX+T_{9-13}$	1,031	2,630
10-210	MDX_{9-13}	2,043	7,801
10-211	MDX_{9-13}	1,261	5,833
10-212	$CON+T_{9-13}$	773	569
10-214	$MDX+T_{9-13}$	1,333	3,014
10-218	MDX_{9-13}	1,148	8,601
10-219	CON_{9-13}	922	1,649
10-220	CON_{9-13}	864	2,535
10-221	$MDX+T_{9-13}$	1,030	3,575
10-222	$MDX+T_{9-13}$	644	3,121
10-223	$MDX+T_{9-13}$	891	2,783
10-225	$CON+T_{9-13}$	871	2,949
10-226	$CON+T_{9-13}$	1,026	2,080
10-227	CON_{9-13}	338	1,542
10-228	CON_{9-13}	627	2,687

Descriptive statistics

phase		CON_{9-13}	$CON+T_{9-13}$	MDX_{9-13}	$MDX+T_{9-13}$
Vertical cage activity by phase (beam breaks)					
Light (n=5)	Mean	698	1,073	1,520	986
	SEM	103	172	292	112
	Sig?	A	AB	B	AB
Dark (n=5)	Mean	1,991	2,828	7,298	3,025
	SEM	255	810	611	162
	Sig?	A	A	B	A
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)					

phase		CON_{9-13}+ CON+T_{9-13}	MDX_{9-13}+ MDX+T_{9-13}
Vertical cage activity by phase (beam breaks)			
Light (n=10)	Mean	886	1,253
	SEM	113	172
	Sig?	--	--
Dark (n=10)	Mean	2,410	5,161
	SEM	424	772
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

phase		CON_{9-13}+MDX_{9-13}	CON+T_{9-13}+ MDX+T_{9-13}
Vertical cage activity by phase (beam breaks)			
Light (n=10)	Mean	1,109	1,030
	SEM	200	98
	Sig?	--	--
Dark (n=10)	Mean	4,645	2,926
	SEM	938	391
	Sig?	A	B
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

Inferential statistics

Light phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	1,740,818	580,273	3.37	0.0448
Error	16	2,757,513	172,345		
Total	19	4,498,331			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	674,546	3.91	0.0654	
Tumor	1	31,601	0.18	0.6742	
Interaction	1	1,034,670	6.00	0.0262	

Dark phase

2-way ANOVA (genotype x tumor presence)					
ANOVA					
Source	DF	SS	MS	F	*P*>F
Model	3	85,255,779	28,418,593	20.28	<.0001
Error	16	22,418,244	1,401,140		
Total	19	107,674,023			
Effects Tests					
Source	DF	SS	F	*P*>F	
Genotype	1	37,853,761	27.02	<0.0001	
Tumor	1	14,759,338	10.53	0.0051	
Interaction	1	3,2642,680	23.30	0.0002	

Tumor incidence: $Set_{5\text{-}9}$

Raw data

Mouse ID	Tumor	Tx Group	Tumor Present?					
			Day 0	Day 7	Day 14	Day 21	Day 27	Day 29
10-103	T1 (UL)	MDX+$T_{5\text{-}9}$	NO	NO	YES	NO	NO	YES
10-103	T2 (UR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	NO	YES	YES
10-103	T3 (LR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-103	T4 (UL)	MDX+$T_{5\text{-}9}$	NO	NO	YES	YES	NO	YES
10-104	T1 (UL)	MDX+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-104	T2 (UR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-104	T3 (LR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-104	T4 (UL)	MDX+$T_{5\text{-}9}$	NO	NO	YES	YES	NO	YES
10-105	T1 (UL)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	NO	YES
10-105	T2 (UR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-105	T3 (LR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-105	T4 (UL)	MDX+$T_{5\text{-}9}$	NO	NO	YES	NO	YES	YES
10-106	T1 (UL)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-106	T2 (UR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-106	T3 (LR)	MDX+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-106	T4 (UL)	MDX+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-107	T1 (UL)	CON+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-107	T2 (UR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-107	T3 (LR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-107	T4 (UL)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-108	T1 (UL)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-108	T2 (UR)	CON+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-108	T3 (LR)	CON+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-108	T4 (UL)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-109	T1 (UL)	CON+$T_{5\text{-}9}$	NO	NO	YES	NO	NO	YES
10-109	T2 (UR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	NO	YES
10-109	T3 (LR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	NO	YES
10-109	T4 (UL)	CON+$T_{5\text{-}9}$	NO	NO	NO	YES	NO	YES
10-110	T1 (UL)	CON+$T_{5\text{-}9}$	NO	YES	YES	YES	NO	YES
10-110	T2 (UR)	CON+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-110	T3 (LR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-110	T4 (UL)	CON+$T_{5\text{-}9}$	NO	NO	NO	NO	NO	YES
10-117	T1 (UL)	CON+$T_{5\text{-}9}$	NO	YES	YES	YES	YES	YES
10-117	T2 (UR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES
10-117	T3 (LR)	CON+$T_{5\text{-}9}$	NO	NO	YES	YES	YES	YES

Mouse	Tumor	Tx Group	Tumor Present?					
10-117	T4 (UL)	CON+T_{5-9}	NO	NO	NO	NO	YES	YES
10-118	T1 (UL)	CON+T_{5-9}	NO	YES	NO	YES	YES	YES
10-118	T2 (UR)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-118	T3 (LR)	CON+T_{5-9}	NO	NO	NO	YES	YES	YES
10-118	T4 (UL)	CON+T_{5-9}	NO	NO	NO	YES	NO	YES
10-120	T1 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-120	T2 (UR)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-120	T3 (LR)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-120	T4 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-121	T1 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-121	T2 (UR)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-121	T3 (LR)	MDX+T_{5-9}	NO	NO	NO	YES	NO	YES
10-121	T4 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-132	T1 (UL)	CON+T_{5-9}	NO	NO	NO	YES	NO	YES
10-132	T2 (UR)	CON+T_{5-9}	NO	YES	YES	YES	NO	YES
10-132	T3 (LR)	CON+T_{5-9}	NO	YES	YES	YES	NO	YES
10-132	T4 (UL)	CON+T_{5-9}	NO	NO	NO	YES	NO	YES
10-133	T1 (UL)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-133	T2 (UR)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-133	T3 (LR)	CON+T_{5-9}	NO	NO	NO	YES	YES	YES
10-133	T4 (UL)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-136	T1 (UL)	CON+T_{5-9}	NO	YES	YES	YES	YES	YES
10-136	T2 (UR)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-136	T3 (LR)	CON+T_{5-9}	NO	YES	YES	YES	YES	YES
10-136	T4 (UL)	CON+T_{5-9}	NO	NO	YES	YES	NO	YES
10-137	T1 (UL)	CON+T_{5-9}	NO	NO	NO	NO	NO	YES
10-137	T2 (UR)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-137	T3 (LR)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-137	T4 (UL)	CON+T_{5-9}	NO	NO	YES	YES	YES	YES
10-140	T1 (UL)	MDX+T_{5-9}	NO	NO	NO	YES	YES	YES
10-140	T2 (UR)	MDX+T_{5-9}	NO	YES	YES	YES	NO	YES
10-140	T3 (LR)	MDX+T_{5-9}	NO	NO	YES	YES	YES	YES
10-140	T4 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	YES	YES
10-141	T1 (UL)	MDX+T_{5-9}	NO	NO	NO	NO	YES	YES
10-141	T2 (UR)	MDX+T_{5-9}	NO	NO	YES	YES	YES	YES
10-141	T3 (LR)	MDX+T_{5-9}	NO	NO	NO	YES	YES	YES
10-141	T4 (UL)	MDX+T_{5-9}	NO	NO	NO	YES	YES	YES
10-146	T1 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES
10-146	T2 (UR)	MDX+T_{5-9}	NO	NO	YES	YES	NO	YES

Mouse	Tumor	Tx Group	Tumor Present?					
10-146	T3 (LR)	MDX+T_{5-9}	NO	NO	NO	YES	NO	YES
10-146	T4 (UL)	MDX+T_{5-9}	NO	NO	YES	NO	NO	YES
10-147	T1 (UL)	MDX+T_{5-9}	NO	NO	YES	YES	YES	YES
10-147	T2 (UR)	MDX+T_{5-9}	NO	YES	YES	YES	YES	YES
10-147	T3 (LR)	MDX+T_{5-9}	NO	YES	YES	YES	YES	YES
10-147	T4 (UL)	MDX+T_{5-9}	NO	YES	YES	YES	YES	YES

Descriptive statistics

Group	***n***	***Tumor Present?***					
		Day 0	*Day 7*	*Day 14*	*Day 21*	*Day 27*	*Day 29*
CON+T_{5-9}	*40*	*0.0%*	*27.5%*	*75.0%*	*90.0%*	*67.5%*	*100.0%*
MDX+T_{5-9}	*40*	*0.0%*	*35.0%*	*85.0%*	*87.5%*	*57.5%*	*100.0%*

Tumor location	***n***	***Tumor Present?***					
		Day 0	*Day 7*	*Day 14*	*Day 21*	*Day 27*	*Day 29*
T1 (UL)	*20*	*0.0%*	*35.0%*	*75.0%*	*80.0%*	*55.0%*	*100.0%*
T2 (UR)	*20*	*0.0%*	*45.0%*	*100.0%*	*95.0%*	*70.0%*	*100.0%*
T3 (LR)	*20*	*0.0%*	*40.0%*	*75.0%*	*100.0%*	*75.0%*	*100.0%*
T4 (UL)	*20*	*0.0%*	*5.0%*	*70.0%*	*80.0%*	*50.0%*	*100.0%*

Inferential statistics

Comparison of treatment group (Pearson's χ^2 test)						
	Day 0	*Day 7*	*Day 14*	*Day 21*	*Day 27*	*Day 29*
P>χ^2	*--*	*0.4693*	*0.2636*	*0.7235*	*0.3556*	*--*

Comparison of tumor location (Pearson's χ^2 test)						
	Day 0	*Day 7*	*Day 14*	*Day 21*	*Day 27*	*Day 29*
P>χ^2	*--*	*0.0291*	*0.0760*	*0.9430*	*0.3047*	*--*

Tumor incidence: $Set_{9\text{-}13}$

Raw data

Mouse ID	Tumor	Tx Group	Tumor Present?					
			Day 0	Day 7	Day 14	Day 21	Day 23	Day 24
10-204	T1 (UL)	CON+$T_{9\text{-}13}$	NO	NO	NO	NO	NO	YES
10-204	T2 (UR)	CON+$T_{9\text{-}13}$	NO	NO	NO	YES	YES	YES
10-204	T3 (LR)	CON+$T_{9\text{-}13}$	NO	NO	YES	NO	NO	YES
10-204	T4 (UL)	CON+$T_{9\text{-}13}$	NO	NO	NO	NO	YES	YES
10-205	T1 (UL)	CON+$T_{9\text{-}13}$	NO	YES	NO	YES	YES	YES
10-205	T2 (UR)	CON+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-205	T3 (LR)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-205	T4 (UL)	CON+$T_{9\text{-}13}$	NO	NO	NO	YES	YES	YES
10-209	T1 (UL)	MDX+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-209	T2 (UR)	MDX+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-209	T3 (LR)	MDX+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-209	T4 (UL)	MDX+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-212	T1 (UL)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-212	T2 (UR)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-212	T3 (LR)	CON+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-212	T4 (UL)	CON+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-213	T1 (UL)	MDX+$T_{9\text{-}13}$	NO	YES	NO	YES	NO	YES
10-213	T2 (UR)	MDX+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-213	T3 (LR)	MDX+$T_{9\text{-}13}$	NO	YES	YES	YES	NO	YES
10-213	T4 (UL)	MDX+$T_{9\text{-}13}$	NO	NO	NO	NO	NO	YES
10-214	T1 (UL)	MDX+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-214	T2 (UR)	MDX+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-214	T3 (LR)	MDX+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-214	T4 (UL)	MDX+$T_{9\text{-}13}$	NO	YES	NO	YES	YES	YES
10-215	T1 (UL)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-215	T2 (UR)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-215	T3 (LR)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-215	T4 (UL)	CON+$T_{9\text{-}13}$	NO	YES	NO	YES	YES	YES
10-216	T1 (UL)	CON+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES
10-216	T2 (UR)	CON+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-216	T3 (LR)	CON+$T_{9\text{-}13}$	NO	NO	YES	YES	YES	YES
10-216	T4 (UL)	CON+$T_{9\text{-}13}$	NO	NO	YES	NO	YES	YES
10-221	T1 (UL)	MDX+$T_{9\text{-}13}$	NO	YES	NO	NO	NO	YES
10-221	T2 (UR)	MDX+$T_{9\text{-}13}$	NO	YES	YES	YES	YES	YES

Mouse	Tumor	Tx Group	Tumor Present?					
10-221	T3 (LR)	MDX+T_{9-13}	NO	YES	NO	YES	YES	YES
10-221	T4 (UL)	MDX+T_{9-13}	NO	YES	YES	NO	NO	YES
10-222	T1 (UL)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-222	T2 (UR)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-222	T3 (LR)	MDX+T_{9-13}	NO	NO	YES	YES	YES	YES
10-222	T4 (UL)	MDX+T_{9-13}	NO	NO	YES	YES	YES	YES
10-223	T1 (UL)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-223	T2 (UR)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-223	T3 (LR)	MDX+T_{9-13}	NO	NO	NO	NO	YES	YES
10-223	T4 (UL)	MDX+T_{9-13}	NO	NO	NO	YES	YES	YES
10-224	T1 (UL)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-224	T2 (UR)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-224	T3 (LR)	MDX+T_{9-13}	NO	YES	YES	YES	YES	YES
10-224	T4 (UL)	MDX+T_{9-13}	NO	NO	YES	YES	YES	YES
10-225	T1 (UL)	CON+T_{9-13}	NO	YES	YES	YES	YES	YES
10-225	T2 (UR)	CON+T_{9-13}	NO	YES	YES	YES	YES	YES
10-225	T3 (LR)	CON+T_{9-13}	NO	NO	YES	YES	YES	YES
10-225	T4 (UL)	CON+T_{9-13}	NO	NO	NO	YES	YES	YES
10-226	T1 (UL)	CON+T_{9-13}	NO	YES	YES	YES	YES	YES
10-226	T2 (UR)	CON+T_{9-13}	NO	NO	YES	YES	YES	YES
10-226	T3 (LR)	CON+T_{9-13}	NO	NO	YES	YES	YES	YES
10-226	T4 (UL)	CON+T_{9-13}	NO	NO	NO	YES	YES	YES

Descriptive statistics

Group	n	Tumor Present?					
		Day 0	Day 7	Day 14	Day 21	Day 23	Day 24
CON+T_{9-13}	40	0.0%	42.9%	71.4%	85.7%	92.9%	100.0%
MDX+T_{9-13}	40	0.0%	57.1%	75.0%	85.7%	82.1%	100.0%

Tumor location	n	Tumor Present?					
		Day 0	Day 7	Day 14	Day 21	Day 23	Day 24
T1 (UL)	20	0.0%	85.7%	71.4%	85.7%	78.6%	100.0%
T2 (UR)	20	0.0%	57.1&	92.9%	100.0%	100.0%	100.0%
T3 (LR)	20	0.0%	35.7%	85.7%	85.7%	85.7%	100.0%
T4 (UL)	20	0.0%	21.4%	42.9	71.4%	85.7%	100.0%

Inferential statistics

Comparison of treatment group (Pearson's χ^2 test)						
	Day 0	Day 7	Day 14	Day 21	Day 23	Day 24
P>χ2	--	0.2850	0.7628	1.0000	0.2254	--

Comparison of tumor location (Pearson's χ^2 test)						
	Day 0	Day 7	Day 14	Day 21	Day 23	Day 24
P>χ2	--	0.0043	0.0150	0.1979	0.3762	--

Tumor surface area: SET_{5-9}

Raw data

Mouse ID	Tumor	Tx Group	Tumor surface area (mm^2)				
			Day 0	Day 7	Day 14	Day 21	Day 27
10-103	T1 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	0.0	0.0
10-103	T2 (UR)	MDX+T_{5-9}	0.0	3.5	3.5	0.0	3.5
10-103	T3 (LR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-103	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	0.0
10-104	T1 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	25.6	88.8
10-104	T2 (UR)	MDX+T_{5-9}	0.0	3.5	3.5	9.7	50.2
10-104	T3 (LR)	MDX+T_{5-9}	0.0	3.5	3.5	7.0	40.7
10-104	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	0.0
10-105	T1 (UL)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	0.0
10-105	T2 (UR)	MDX+T_{5-9}	0.0	3.5	3.5	7.7	40.1
10-105	T3 (LR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-105	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	0.0	3.5
10-106	T1 (UL)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-106	T2 (UR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	76.4
10-106	T3 (LR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	52.9
10-106	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-107	T1 (UL)	CON+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-107	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	18.6
10-107	T3 (LR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-107	T4 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-108	T1 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-108	T2 (UR)	CON+T_{5-9}	0.0	3.5	3.5	15.9	80.6
10-108	T3 (LR)	CON+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-108	T4 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-109	T1 (UL)	CON+T_{5-9}	0.0	0.0	3.5	0.0	
10-109	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	27.2	
10-109	T3 (LR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	
10-109	T4 (UL)	CON+T_{5-9}	0.0	0.0	0.0	3.5	
10-110	T1 (UL)	CON+T_{5-9}	0.0	3.5	3.5	3.5	0.0
10-110	T2 (UR)	CON+T_{5-9}	0.0	3.5	3.5	3.5	56.7
10-110	T3 (LR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-110	T4 (UL)	CON+T_{5-9}	0.0	0.0	0.0	0.0	0.0
10-117	T1 (UL)	CON+T_{5-9}	0.0	3.5	3.5	3.5	41.8
10-117	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	32.6

Mouse ID	Tumor	Tx Group	Tumor surface area (mm^2)				
10-117	T3 (LR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-117	T4 (UL)	CON+T_{5-9}	0.0	0.0	0.0	0.0	3.5
10-118	T1 (UL)	CON+T_{5-9}	0.0	3.5	0.0	3.5	3.5
10-118	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	40.6
10-118	T3 (LR)	CON+T_{5-9}	0.0	0.0	0.0	3.5	3.5
10-118	T4 (UL)	CON+T_{5-9}	0.0	0.0	0.0	3.5	0.0
10-120	T1 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-120	T2 (UR)	MDX+T_{5-9}	0.0	0.0	14.9	52.9	
10-120	T3 (LR)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-120	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-121	T1 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-121	T2 (UR)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-121	T3 (LR)	MDX+T_{5-9}	0.0	0.0	0.0	3.5	
10-121	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-132	T1 (UL)	CON+T_{5-9}	0.0	0.0	0.0	3.5	
10-132	T2 (UR)	CON+T_{5-9}	0.0	3.5	3.5	23.7	
10-132	T3 (LR)	CON+T_{5-9}	0.0	3.5	3.5	3.5	
10-132	T4 (UL)	CON+T_{5-9}	0.0	0.0	0.0	3.5	
10-133	T1 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	32.1
10-133	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	45.2	89.3
10-133	T3 (LR)	CON+T_{5-9}	0.0	0.0	0.0	3.5	3.5
10-133	T4 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-136	T1 (UL)	CON+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-136	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	71.2
10-136	T3 (LR)	CON+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-136	T4 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	0.0
10-137	T1 (UL)	CON+T_{5-9}	0.0	0.0	0.0	0.0	0.0
10-137	T2 (UR)	CON+T_{5-9}	0.0	0.0	3.5	66.7	196.2
10-137	T3 (LR)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-137	T4 (UL)	CON+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-140	T1 (UL)	MDX+T_{5-9}	0.0	0.0	0.0	3.5	115.0
10-140	T2 (UR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	0.0
10-140	T3 (LR)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-140	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	3.5
10-141	T1 (UL)	MDX+T_{5-9}	0.0	0.0	0.0	0.0	37.8
10-141	T2 (UR)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	87.5
10-141	T3 (LR)	MDX+T_{5-9}	0.0	0.0	0.0	3.5	3.5
10-141	T4 (UL)	MDX+T_{5-9}	0.0	0.0	0.0	3.5	3.5

Mouse ID	Tumor	Tx Group	Tumor surface area (mm^2)				
10-146	T1 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	67.5	
10-146	T2 (UR)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	
10-146	T3 (LR)	MDX+T_{5-9}	0.0	0.0	0.0	3.5	
10-146	T4 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	0.0	
10-147	T1 (UL)	MDX+T_{5-9}	0.0	0.0	3.5	3.5	22.4
10-147	T2 (UR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	65.6
10-147	T3 (LR)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	3.5
10-147	T4 (UL)	MDX+T_{5-9}	0.0	3.5	3.5	3.5	57.9

Descriptive statistics

Day		CON+T_{5-9}	MDX+T_{5-9}
Tumor surface area (mm^2)			
0 (n=40)	Mean	0.0	0.0
	SEM	0.0	0.0
	Sig?	--	--
7 (n=40)	Mean	1.0	1.2
	SEM	0.3	0.3
	Sig?	--	--
14 (n=40)	Mean	2.6	3.3
	SEM	0.2	0.4
	Sig?	--	--
21 (n=40)	Mean	7.2	6.8
	SEM	2.0	2.1
	Sig?	--	--
27 (n=28-32)	Mean	22.5	27.6
	SEM	7.2	6.5
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

	CON+T_{5-9} (n=192)	MDX+T_{5-9} (n=188)
Tumor surface area (mm2)		
Mean	6.0	6.5
SEM	1.4	1.2
Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Day		
Tumor surface area (mm^2)		
0 (n=80)	Mean	0.0
	SEM	0.0
	Sig?	A
7 (n=80)	Mean	1.1
	SEM	0.2
	Sig?	AB
14 (n=80)	Mean	2.9
	SEM	0.2
	Sig?	AB
21 (n=80)	Mean	7.0
	SEM	1.4
	Sig?	B
27 (n=60)	Mean	24.9
	SEM	4.8
	Sig?	C
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Inferential statistics

Mixed model ANOVA with repeated measures (group x time)

Source	Value	Exact F	Num DF	Den DF	P>F
Group	0.00	0.09	1	58	0.7611
Time	4.80	66.02	4	55	<0.0001
Interaction	0.12	1.64	4	55	0.1778

Tumor surface area: $SET_{9\text{-}13}$

Raw data

Mouse ID	Tumor	Tx Group	Tumor surface area (mm^2)				
			Day 0	Day 7	Day 14	Day 21	Day 23
10-204	T1 (UL)	CON+$T_{9\text{-}13}$	0.0	0.0	0.0	0.0	0.0
10-204	T2 (UR)	CON+$T_{9\text{-}13}$	0.0	0.0	0.0	3.5	3.5
10-204	T3 (LR)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	0.0	0.0
10-204	T4 (UL)	CON+$T_{9\text{-}13}$	0.0	0.0	0.0	0.0	3.5
10-205	T1 (UL)	CON+$T_{9\text{-}13}$	0.0	3.5	0.0	3.5	3.5
10-205	T2 (UR)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-205	T3 (LR)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-205	T4 (UL)	CON+$T_{9\text{-}13}$	0.0	0.0	0.0	3.5	3.5
10-209	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-209	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-209	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-209	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-212	T1 (UL)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-212	T2 (UR)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	55.7
10-212	T3 (LR)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-212	T4 (UL)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-213	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0	3.5	0.0	3.5	0.0
10-213	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-213	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	0.0
10-213	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0	0.0	0.0	0.0	0.0
10-214	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-214	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-214	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-214	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0	3.5	0.0	3.5	3.5
10-215	T1 (UL)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-215	T2 (UR)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-215	T3 (LR)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-215	T4 (UL)	CON+$T_{9\text{-}13}$	0.0	3.5	0.0	3.5	3.5
10-216	T1 (UL)	CON+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5
10-216	T2 (UR)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-216	T3 (LR)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	3.5	3.5
10-216	T4 (UL)	CON+$T_{9\text{-}13}$	0.0	0.0	3.5	0.0	3.5
10-221	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0	3.5	0.0	0.0	0.0
10-221	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0	3.5	3.5	3.5	3.5

Mouse ID	Tumor	Tx Group	Tumor surface area (mm^2)				
10-221	T3 (LR)	MDX+T_{9-13}	0.0	3.5	0.0	3.5	3.5
10-221	T4 (UL)	MDX+T_{9-13}	0.0	3.5	3.5	0.0	0.0
10-222	T1 (UL)	MDX+T_{9-13}	0.0	3.5	3.5	3.5	3.5
10-222	T2 (UR)	MDX+T_{9-13}	0.0	3.5	3.5	56.0	76.0
10-222	T3 (LR)	MDX+T_{9-13}	0.0	0.0	3.5	3.5	3.5
10-222	T4 (UL)	MDX+T_{9-13}	0.0	0.0	3.5	3.5	3.5
10-223	T1 (UL)	MDX+T_{9-13}	0.0	3.5	3.5	3.5	3.5
10-223	T2 (UR)	MDX+T_{9-13}	0.0	3.5	3.5	3.5	3.5
10-223	T3 (LR)	MDX+T_{9-13}	0.0	0.0	0.0	0.0	3.5
10-223	T4 (UL)	MDX+T_{9-13}	0.0	0.0	0.0	3.5	3.5
10-224	T1 (UL)	MDX+T_{9-13}	0.0	3.5	3.5	38.0	47.3
10-224	T2 (UR)	MDX+T_{9-13}	0.0	3.5	3.5	3.5	19.4
10-224	T3 (LR)	MDX+T_{9-13}	0.0	3.5	3.5	3.5	23.5
10-224	T4 (UL)	MDX+T_{9-13}	0.0	0.0	3.5	3.5	3.5
10-225	T1 (UL)	CON+T_{9-13}	0.0	3.5	3.5	3.5	3.5
10-225	T2 (UR)	CON+T_{9-13}	0.0	3.5	3.5	3.5	3.5
10-225	T3 (LR)	CON+T_{9-13}	0.0	0.0	3.5	3.5	3.5
10-225	T4 (UL)	CON+T_{9-13}	0.0	0.0	0.0	3.5	3.5
10-226	T1 (UL)	CON+T_{9-13}	0.0	3.5	3.5	33.7	46.7
10-226	T2 (UR)	CON+T_{9-13}	0.0	0.0	3.5	3.5	3.5
10-226	T3 (LR)	CON+T_{9-13}	0.0	0.0	3.5	3.5	3.5
10-226	T4 (UL)	CON+T_{9-13}	0.0	0.0	0.0	3.5	3.5

Descriptive statistics

Day		CON+T_{9-13}	MDX+T_{9-13}
Tumor surface area (mm^2)			
0 (n=28)	Mean	0.0	0.0
	SEM	0.0	0.0
	Sig?	--	--
7 (n=28)	Mean	1.5	2.0
	SEM	0.3	0.3
	Sig?	--	--
14 (n=28)	Mean	2.5	2.6
	SEM	0.3	0.3
	Sig?	--	--
21 (n=28)	Mean	4.1	6.1
	SEM	1.1	2.2
	Sig?	--	--
23 (n=28)	Mean	6.7	8.3
	SEM	2.4	3.1
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

	CON+T_{9-13} (n=140)	MDX+T_{9-13} (n=140)
Tumor surface area (mm^2)		
Mean	2.9	3.8
SEM	0.6	0.8
Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Day		
Tumor surface area (mm^2)		
0 (n=56)	Mean	0.0
	SEM	0.0
	Sig?	A
7 (n=56)	Mean	1.8
	SEM	0.2
	Sig?	AB
14 (n=56)	Mean	2.6
	SEM	0.2
	Sig?	AB
21 (n=56)	Mean	5.1
	SEM	1.3
	Sig?	BC
23 (n=56)	Mean	7.5
	SEM	1.3
	Sig?	C
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Inferential statistics

Mixed model ANOVA with repeated measures (genotype x time)					
Source	Value	Exact F	Num DF	Den DF	P>F
Genotype	0.01	0.45	1	54	0.5064
Time	3.47	44.18	4	51	<0.0001
Interaction	0.04	0.45	4	51	0.7694

Tumor mass: SET_{5-9}

Raw data

Mouse ID	Tumor	Tx Group	Tumor mass (g)
10-103	T1 (UL)	MDX+T_{5-9}	0.0070
10-103	T2 (UR)	MDX+T_{5-9}	0.0074
10-103	T3 (LR)	MDX+T_{5-9}	0.1520
10-103	T4 (UL)	MDX+T_{5-9}	0.0092
10-104	T1 (UL)	MDX+T_{5-9}	1.5234
10-104	T2 (UR)	MDX+T_{5-9}	0.8081
10-104	T3 (LR)	MDX+T_{5-9}	0.3548
10-104	T4 (UL)	MDX+T_{5-9}	0.0294
10-105	T1 (UL)	MDX+T_{5-9}	0.0076
10-105	T2 (UR)	MDX+T_{5-9}	0.3404
10-105	T3 (LR)	MDX+T_{5-9}	0.0542
10-105	T4 (UL)	MDX+T_{5-9}	0.0506
10-106	T1 (UL)	MDX+T_{5-9}	0.1558
10-106	T2 (UR)	MDX+T_{5-9}	0.5434
10-106	T3 (LR)	MDX+T_{5-9}	0.2800
10-106	T4 (UL)	MDX+T_{5-9}	0.0981
10-107	T1 (UL)	CON+T_{5-9}	0.1963
10-107	T2 (UR)	CON+T_{5-9}	0.1917
10-107	T3 (LR)	CON+T_{5-9}	0.0086
10-107	T4 (UL)	CON+T_{5-9}	0.0455
10-108	T1 (UL)	CON+T_{5-9}	0.0373
10-108	T2 (UR)	CON+T_{5-9}	0.5769
10-108	T3 (LR)	CON+T_{5-9}	0.0640
10-108	T4 (UL)	CON+T_{5-9}	0.0738
10-109	T1 (UL)	CON+T_{5-9}	0.0850
10-109	T2 (UR)	CON+T_{5-9}	1.4604
10-109	T3 (LR)	CON+T_{5-9}	0.0754
10-109	T4 (UL)	CON+T_{5-9}	0.1651
10-110	T1 (UL)	CON+T_{5-9}	0.0085
10-110	T2 (UR)	CON+T_{5-9}	0.3791
10-110	T3 (LR)	CON+T_{5-9}	0.0027
10-110	T4 (UL)	CON+T_{5-9}	0.0070
10-117	T1 (UL)	CON+T_{5-9}	0.3180
10-117	T2 (UR)	CON+T_{5-9}	0.4029
10-117	T3 (LR)	CON+T_{5-9}	0.0167
10-117	T4 (UL)	CON+T_{5-9}	0.0633
10-118	T1 (UL)	CON+T_{5-9}	0.1096
10-118	T2 (UR)	CON+T_{5-9}	0.4381
10-118	T3 (LR)	CON+T_{5-9}	0.0761
10-118	T4 (UL)	CON+T_{5-9}	0.0073
10-120	T1 (UL)	MDX+T_{5-9}	0.1244
10-120	T2 (UR)	MDX+T_{5-9}	0.7170
10-120	T3 (LR)	MDX+T_{5-9}	0.0609
10-120	T4 (UL)	MDX+T_{5-9}	0.1836
10-121	T1 (UL)	MDX+T_{5-9}	0.5600
10-121	T2 (UR)	MDX+T_{5-9}	1.7674
10-121	T3 (LR)	MDX+T_{5-9}	0.4902
10-121	T4 (UL)	MDX+T_{5-9}	0.1411
10-132	T1 (UL)	CON+T_{5-9}	0.8376
10-132	T2 (UR)	CON+T_{5-9}	1.2475
10-132	T3 (LR)	CON+T_{5-9}	0.0425
10-132	T4 (UL)	CON+T_{5-9}	0.7070
10-133	T1 (UL)	CON+T_{5-9}	0.2887
10-133	T2 (UR)	CON+T_{5-9}	1.4285
10-133	T3 (LR)	CON+T_{5-9}	0.1038
10-133	T4 (UL)	CON+T_{5-9}	0.0974
10-136	T1 (UL)	CON+T_{5-9}	0.0376
10-136	T2 (UR)	CON+T_{5-9}	0.6455
10-136	T3 (LR)	CON+T_{5-9}	0.1634
10-136	T4 (UL)	CON+T_{5-9}	0.0127
10-137	T1 (UL)	CON+T_{5-9}	0.0302
10-137	T2 (UR)	CON+T_{5-9}	1.3562
10-137	T3 (LR)	CON+T_{5-9}	0.0506
10-137	T4 (UL)	CON+T_{5-9}	0.1068
10-140	T1 (UL)	MDX+T_{5-9}	1.1661
10-140	T2 (UR)	MDX+T_{5-9}	0.2281
10-140	T3 (LR)	MDX+T_{5-9}	0.0578
10-140	T4 (UL)	MDX+T_{5-9}	0.1146

Mouse ID	Tumor	Tx Group	Tumor mass (g)
10-141	T1 (UL)	MDX+T_{5-9}	0.0400
10-141	T2 (UR)	MDX+T_{5-9}	1.0558
10-141	T3 (LR)	MDX+T_{5-9}	0.0981
10-141	T4 (UL)	MDX+T_{5-9}	0.0126
10-146	T1 (UL)	MDX+T_{5-9}	0.7933
10-146	T2 (UR)	MDX+T_{5-9}	0.4769
10-146	T3 (LR)	MDX+T_{5-9}	0.0663
10-146	T4 (UL)	MDX+T_{5-9}	0.0493
10-147	T1 (UL)	MDX+T_{5-9}	0.2467
10-147	T2 (UR)	MDX+T_{5-9}	0.4975
10-147	T3 (LR)	MDX+T_{5-9}	0.1003
10-147	T4 (UL)	MDX+T_{5-9}	0.3838

Descriptive statistics

Day		CON+T_{5-9}	MDX+T_{5-9}
Tumor mass (g)			
T1 (n=10)	Mean	0.1949	0.4624
	SEM	0.0794	0.1696
	Sig?	--	--
T2 (n=10)	Mean	0.8127	0.6442
	SEM	0.1581	0.1561
	Sig?	--	--
T3 (n=10)	Mean	0.0604	0.1715
	SEM	0.0154	0.0481
	Sig?	--	--
T4 (n=10)	Mean	0.1286	0.1072
	SEM	0.0662	0.0357
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

	CON+T_{5-9} (n=40)	MDX+T_{5-9} (n=40)
Tumor mass (g)		
Mean	0.2991	0.3463
SEM	0.0662	0.0670
Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Day		
Tumor mass (g)		
T1 (n=20)	Mean	0.3287
	SEM	0.0962
	Sig?	A
T2 (n=20)	Mean	0.7284
	SEM	0.1098
	Sig?	B
T3 (n=20)	Mean	0.1159
	SEM	0.0277
	Sig?	A
T4 (n=20)	Mean	0.1179
	SEM	0.367
	Sig?	A
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Inferential statistics

2-way ANOVA (genotype x tumor position)					
Source	DF	SS	MS	F ratio	P>F
Model	7	5.55	0.79	6.85	<0.0001
Error	72	8.34	0.12		
Total	79	13.89			
Effects tests					
	DF	SS	F ratio	P>F	
Genotype	1	0.04	0.38	0.5370	
Position	3	4.99	14.36	<0.0001	
Interaction	3	0.52	1.50	0.2232	

Tumor mass: $SET_{9\text{-}13}$

Raw data

Mouse ID	Tumor	Tx Group	Tumor mass (g)
10-204	T1 (UL)	CON+$T_{9\text{-}13}$	0.0020
10-204	T2 (UR)	CON+$T_{9\text{-}13}$	0.0074
10-204	T3 (LR)	CON+$T_{9\text{-}13}$	0.0096
10-204	T4 (UL)	CON+$T_{9\text{-}13}$	0.0106
10-205	T1 (UL)	CON+$T_{9\text{-}13}$	0.0271
10-205	T2 (UR)	CON+$T_{9\text{-}13}$	0.2823
10-205	T3 (LR)	CON+$T_{9\text{-}13}$	0.1096
10-205	T4 (UL)	CON+$T_{9\text{-}13}$	0.0049
10-209	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0190
10-209	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0206
10-209	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0890
10-209	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0089
10-212	T1 (UL)	CON+$T_{9\text{-}13}$	0.0160
10-212	T2 (UR)	CON+$T_{9\text{-}13}$	0.2947
10-212	T3 (LR)	CON+$T_{9\text{-}13}$	0.0384
10-212	T4 (UL)	CON+$T_{9\text{-}13}$	0.0788
10-213	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0062
10-213	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0080
10-213	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0024
10-213	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0029
10-214	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0376
10-214	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0901
10-214	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0209
10-214	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0599
10-215	T1 (UL)	CON+$T_{9\text{-}13}$	0.0094
10-215	T2 (UR)	CON+$T_{9\text{-}13}$	0.0443
10-215	T3 (LR)	CON+$T_{9\text{-}13}$	0.0617
10-215	T4 (UL)	CON+$T_{9\text{-}13}$	0.0068
10-216	T1 (UL)	CON+$T_{9\text{-}13}$	0.0062
10-216	T2 (UR)	CON+$T_{9\text{-}13}$	0.0311
10-216	T3 (LR)	CON+$T_{9\text{-}13}$	0.0116
10-216	T4 (UL)	CON+$T_{9\text{-}13}$	0.0032
10-221	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0046
10-221	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0090
10-221	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0071
10-221	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0034
10-222	T1 (UL)	MDX+$T_{9\text{-}13}$	0.5237
10-222	T2 (UR)	MDX+$T_{9\text{-}13}$	0.2176
10-222	T3 (LR)	MDX+$T_{9\text{-}13}$	0.1134
10-222	T4 (UL)	MDX+$T_{9\text{-}13}$	0.1044
10-223	T1 (UL)	MDX+$T_{9\text{-}13}$	0.0038
10-223	T2 (UR)	MDX+$T_{9\text{-}13}$	0.0036
10-223	T3 (LR)	MDX+$T_{9\text{-}13}$	0.0035
10-223	T4 (UL)	MDX+$T_{9\text{-}13}$	0.0047
10-224	T1 (UL)	MDX+$T_{9\text{-}13}$	0.2731
10-224	T2 (UR)	MDX+$T_{9\text{-}13}$	0.1064
10-224	T3 (LR)	MDX+$T_{9\text{-}13}$	0.1094
10-224	T4 (UL)	MDX+$T_{9\text{-}13}$	0.1308
10-225	T1 (UL)	CON+$T_{9\text{-}13}$	0.0951
10-225	T2 (UR)	CON+$T_{9\text{-}13}$	0.0220
10-225	T3 (LR)	CON+$T_{9\text{-}13}$	0.0167
10-225	T4 (UL)	CON+$T_{9\text{-}13}$	0.0562
10-226	T1 (UL)	CON+$T_{9\text{-}13}$	0.2175
10-226	T2 (UR)	CON+$T_{9\text{-}13}$	0.1089
10-226	T3 (LR)	CON+$T_{9\text{-}13}$	0.0024
10-226	T4 (UL)	CON+$T_{9\text{-}13}$	0.0022

Descriptive statistics

Day		CON+T_{9-13}	MDX+T_{9-13}
Tumor mass (g)			
T1 (n=7)	Mean	0.0533	0.1240
	SEM	0.0299	0.0761
	Sig?	--	--
T2 (n=7)	Mean	0.1130	0.0650
	SEM	0.0469	0.0300
	Sig?	--	--
T3 (n=7)	Mean	0.0357	0.0494
	SEM	0.0145	0.0196
	Sig?	--	--
T4 (n=7)	Mean	0.0232	0.0450
	SEM	0.0117	0.0204
	Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)			

	CON+T_{9-13} (n=28)	MDX+T_{9-13} (n=28)
Tumor mass (g)		
Mean	0.0563	0.0709
SEM	0.0153	0.0213
Sig?	--	--
Among time points, levels not connected by the same letter are different (Student's t-test, α=0.05)		

Day		
Tumor mass (g)		
T1 (n=14)	Mean	0.0887
	SEM	0.0404
	Sig?	--
T2 (n=14)	Mean	0.0890
	SEM	0.0276
	Sig?	--
T3 (n=14)	Mean	0.0426
	SEM	0.0119
	Sig?	--
T4 (n=14)	Mean	0.0341
	SEM	0.0117
	Sig?	--
Among time points, levels not connected by the same letter are different (Tukey HSD, α=0.05)		

Inferential statistics

2-way ANOVA (genotype x tumor position)					
Source	DF	SS	MS	F ratio	P>F
Model	7	0.06	0.001	0.96	0.4737
Error	48	0.46	0.001		
Total	55	0.52			
Effects tests					
	DF	SS	F ratio	P>F	
Genotype	1	0.00	0.31	0.5805	
Position	3	0.04	1.26	0.2984	
Interaction	3	0.02	0.87	0.4652	

Tumor substrate oxidation: Glucose, Set_{9-13}

Raw data

ID	Group	Glucose oxidation (nmol/mg protein/hr)
10-205	CON+T_{9-13}	7.06
10-209	MDX+T_{9-13}	3.35
10-212	CON+T_{9-13}	2.46
10-214	MDX+T_{9-13}	3.85
10-215	CON+T_{9-13}	3.86
10-222	MDX+T_{9-13}	17.03
10-224	MDX+T_{9-13}	9.07
10-225	CON+T_{9-13}	10.92
10-226	CON+T_{9-13}	9.50

Descriptive statistics

	n	Glucose oxidation (nmol/mg protein/hr)	
		Mean	SEM
CON+T_{9-13}	5	6.76	1.61
MDX+T_{9-13}	4	8.33	3.18

Inferential statistics

Student's t-test	
P>|t|	0.6806

Tumor substrate oxidation: Palmitate, Set_{9-13}

Raw data

ID	Group	Palmitate oxidation (nmol/mg protein/hr)
10-205	CON+T_{9-13}	4.67
10-209	MDX+T_{9-13}	3.55
10-212	CON+T_{9-13}	4.05
10-214	MDX+T_{9-13}	3.38
10-215	CON+T_{9-13}	3.18
10-222	MDX+T_{9-13}	3.72
10-224	MDX+T_{9-13}	4.21
10-225	CON+T_{9-13}	7.95
10-226	CON+T_{9-13}	5.46

Descriptive statistics

	n	Palmitate oxidation (nmol/mg protein/hr)	
		Mean	SEM
CON+T_{9-13}	5	5.062	0.81298
MDX+T_{9-13}	4	3.715	0.179

Inferential statistics

Student's t-test	
P>|t|	0.1747

Cytokine/chemokine: SET_{5-9}

Raw data

Plasma analyte	Concentration (pg/ml)		Fold difference	Concentration (pg/ml)		Fold difference
	CON_{5-9} (n=9)	$CON+T_{5-9}$ (n=10)	$CON+T_{5-9}$ relative to CON_{5-9}	MDX_{5-9} (n=10)	$MDX+T_{5-9}$ (n=10)	$MDX+T_{5-9}$ relative to MDX_{5-9}
Cytokines: Colony-stimulating factor family						
GM-CSF	6.1	13.0	2.1 fold ↑	< 1.5	11.1	>7.4 fold ↑
Cytokines: Interferon family						
IFNγ	< 0.1	< 0.1	--	< 0.1	< 0.1	--
Cytokines: Interleukin family						
IL-1α	4.8	4.6	1.1 fold ↓	4.7	3.2	1.5 fold ↓
IL-1β	< 8.8	< 8.8	--	< 8.8	< 8.8	--
IL-2	< 0.9	< 0.9	--	< 0.9	< 0.9	--
IL-3	< 1.0	< 1.0	--	< 1.0	< 1.0	--
IL-4	< 2.2	< 2.2	--	< 2.2	< 2.2	--
IL-5	6.2	3.1	2.0 fold ↓	5.8	3.9	1.5 fold ↓
IL-6	< 2.0	8.2	>4.1 fold ↑	7.6	19.5	2.6 fold ↑
IL-10	< 0.5	< 0.5	--	< 0.5	< 0.5	--
IL-12p70	22.5	12.4	1.8 fold ↓	< 3.9	< 3.9	--
IL-17	< 0.0	3.1	>30.5 fold ↑	< 0.0	< 0.0	--
Cytokines: TNF family						
TNFα	< 0.7	3.2	>4.5 fold ↑	< 0.7	3.9	>5.5 fold ↑
Chemokines: CXCL family						
CXCL1	35.4	317.1	9.0 fold ↑	171.7	294.0	1.7 fold ↑
Chemokines: CCL family						
CCL1	16.9	33.1	2.0 fold ↑	16.6	44.3	2.7 fold ↑
CCL2	21.4	639.3	29.9 fold ↑	59.4	593.9	10.0 fold ↑
CCL3	< 2.5	< 2.5	--	< 2.5	< 2.5	--
CCL5	19.1	50.2	2.6 fold ↑	14.8	39.2	2.6 fold ↑
CCL11	2736.5	2639.4	--	3103.1	2127.2	1.5 fold ↓
CCL17	281.4	660.7	2.3 fold ↑	261.1	1449.3	5.6 fold ↑
CCL22	163.8	323.5	2.0 fold ↑	255.6	322.1	1.3 fold ↑

Cytokine/chemokine: SET_{9-13}

Raw data

Plasma analyte	Concentration (pg/ml)		Fold difference	Concentration (pg/ml)		Fold difference
	CON_{9-13} (n=9)	$CON+T_{9-13}$ (n=10)	$CON+T_{9-13}$ relative to CON_{9-13}	MDX_{9-13} (n=10)	$MDX+T_{9-13}$ (n=10)	$MDX+T_{9-13}$ relative to MDX_{9-13}
Cytokines: Colony-stimulating factor family						
GM-CSF	< 1.5	< 1.5	--	< 1.5	< 1.5	--
Cytokines: Interferon family						
IFNγ	< 0.1	< 0.1	--	< 0.1	< 0.1	--
Cytokines: Interleukin family						
IL-1α	3.2	24.1	7.5 fold ↑	5.4	6.6	1.2 fold ↑
IL-1β	< 8.8	< 8.8	--	< 8.8	< 8.8	--
IL-2	< 0.9	< 0.9	--	< 0.9	< 0.9	--
IL-3	< 1.0	< 1.0	--	< 1.0	< 1.0	--
IL-4	< 2.2	< 2.2	--	< 2.2	< 2.2	--
IL-5	4.1	6.2	1.5 fold ↑	4.6	6.4	1.4 fold ↑
IL-6	< 2.0	13.4	>6.7 fold ↑	11.1	9.4	1.2 fold ↓
IL-10	< 0.5	< 0.5	--	< 0.5	< 0.5	--
IL-12p70	< 3.9	< 3.9	--	< 3.9	< 3.9	--
IL-17	< 0.0	< 0.0	--	< 0.0	< 0.0	--
Cytokines: TNF family						
TNFα	< 0.7	5.3	>7.5 fold ↑	< 0.7	2.9	4.1 fold ↑
Chemokines: CXCL family						
CXCL1	54.9	93.1	1.7 fold ↑	202.1	127.8	1.6 fold ↓
Chemokines: CCL family						
CCL1	47.3	56.4	1.2 fold ↑	49.4	44.7	1.1 fold ↓
CCL2	40.0	120.0	3.0 fold ↑	100.5	127.6	1.3 fold ↑
CCL3	< 2.5	< 2.5	--	< 2.5	< 2.5	--
CCL5	16.0	37.3	2.3 fold ↑	21.8	26.3	1.2 fold ↑
CCL11	4084.6	7176.7	1.8 fold ↑	4518.7	5616.2	1.2 fold ↑
CCL17	443.3	636.3	1.4 fold ↑	621.4	555.8	1.1 fold ↓
CCL22	136.2	357.6	2.6 fold ↑	220.4	291.4	1.3 fold ↑