TABLE S1. Primer sequences

Plasmid constructed	Primer name	Sequence 5′ to 3′ [a]
pDPV388	442	CGCCCGCGGGTTGATGGTTTAGCTGGAGC
	443	CGCGCGGCCG*C*ACTTGTGGCATTACACGGCC
pDPV392	444	GCCCGCGCGGCTCGTAACATAGTTCCACCTCCGCTC
	445	AGCCGCGCGGGCTATGATTAAACTTTAATGGTAAAGT
pDPV416	288	CGCGCGGCCGCCTCGTCTTCTGGAACGACAC
	443	
pDPV421	494	CGCAGATCT*TTA***ATGATGATGATGATGATG**TTTATTTTTTTGTGCACTTGTAAAGGTAGG
	498	CGCAGATCTGCCACCGCGGTGGAGCTC
pDPV422	495	CGCAGATCT*TTA***ATGATGATGATGATGATG**ATTCATAACCCAAATTGGATATCCGC
	498	
pDPV423	496	CGCAGATCT*TTA***ATGATGATGATGATGATG**TTTTTGATGTGACGCTAAATATTCTTTTGC
	498	
pDPV424	497	CGCAGATCT*TTA***ATGATGATGATGATGATG**ATCATAAATTTTTTCAACAGCCTGC
	498	
pDPV426	511	*CCACTTTGTACAAGAAAG*TTGCATTGCTTTAATCATAAATTTTTTCAACAGCC
	512	***GTGGAGAACCTGTACTTCCAGGGT***TATAAAGAGCATCAAGAGAAGAATGC
pDPV432	538	CGAACAGTTCCATCAGCAAAACTAGCGGCAGCAGAAGCAA
	539	TTGCTTCTGCTGCCGCTAGTTTTGCTGATGGAACTGTTCG
	554	CGTGATCCTGCCGACAATACAATTATTGACGGACTAAAGACAGTAG
	555	GCTAGATGATCCGCTGCTTTTTCATTTGGCTTATTTGACTTATTCG
pDPV433	534	AAGTGGAAAAGGTGCAAAAGAATCTTTCGCGAGTGTGAAAATTAAAGATGAAGCAACG
	535	CGTTGCTTCATCTTTAATTTTCACACTCGCGAAAGATTCTTTTGCACCTTTTCCACTT
	554, 555	
pDPV434	536	CTCACAATATGATAATGTTGGAGTATTTACAGCTGTAGTAAATGTGAATGGCGTACGAATTTA
	537	TAAATTCGTACGCCATTCACATTTACTACAGCTGTAAATACTCCAACATTATCATATTGTGAG
	554, 555	
pDPV435	540	AATGATCTGCATAATGAAGTACTTTGCGCAGAATTTGTAGGTACGTTAGGGAAAGAT
	541	ATCTTTCCCTAACGTACCTACAAATTCTGCGCAAAGTACTTCATTATGCAGATCATT
	554, 555	
pDPV436	542	CAAATCTTCATTAATGCAAATAGCGCTGCAGAAGAAAAAGTGAAGAAAATGC
	543	GCATTTTCTTCACTTTTTCTTCTGCAGCGCTATTTGCATTAATGAAGATTTG
	554, 555	
pDPV448	595	GGGTACTGGGGCTATAAAGAGGCACAAAAAAATAAAAAAGGTGGATTTGAAGCAGAAGG
	596	CCTTTTTTATTTTTTTGTGCCTCTTTATAGCCCCAGTACCCTGTGCCGAC
	603	GTCTTCTTTACATAAAAGCGAGCCTTTTACAAAACATAACC
	604	GCTTTACGTTCTTCCATAACTTTCACATCTGGATTG
pDPV449	597	CGCTTGCGATGAATTCACGTGCACAAAAAAATAAAAAAGGTGGATTTGAAGCAGAAGG
	598	CCTTTTTTATTTTTTTGTGCACGTGAATTCATCGCAAGCGTTGTACC
	603, 604	
pDPV450	599	CCGTGCAGCTATTCGTGATGCACAAAAAAATAAAAAAGGTGGATTTGAAGCAGAAGG
	600	CCTTTTTTATTTTTTTGTGCATCACGAATAGCTGCACGGTAACTAAAATCACCG
	603, 604	
pDPV451	601	GTGCAACATCTTGTTTTGAAAGCACAAAAAAATAAAAAAGGTGGATTTGAAGCAGAAGG
	602	CCTTTTTTATTTTTTTGTGCTTTCAAAACAAGATGTTGCACTTTTCTTAGTTCATCTTG
	603, 604	

[a] Restriction sites are underlined, TEV cleavage site regions of pDEST-HisMBP-T are italicized and in bold, *att*R2.1 regions of pDEST-HisMBP-T are italicized and underlined, added stop codons are italicized, and His_6 encoding codons are in bold.

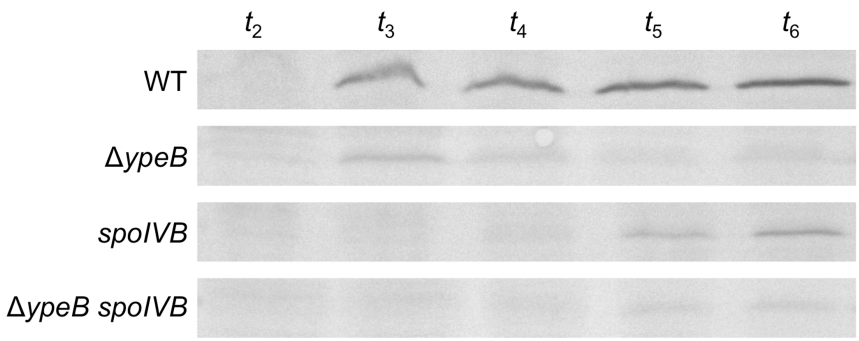

FIG. S1. Disruption of *spoIVB* does not stabilize SleB during Δ*ypeB* spore formation. An approximately 500-bp internal fragment of *B. anthracis spoIVB* (BAS4077) was PCR amplified from the chromosome and inserted into pRP1028 (1) using restriction enzymes BamHI and KpnI. The resulting plasmid, pDPV429, was moved into wild-type and Δ*ypeB* strains of *B. anthracis* by conjugation, as done in the initial stages of the markerless gene replacement procedure (2). Subsequent plasmid integration and disruption of the chromosomal copy of *spoIVB* was achieved by shifting the temperature to 42°C and was verified by PCR. Wild-type (WT), Δ*ypeB*, *spoIVB*, and Δ*ypeB spoIVB* strains were sporulated in modified G broth at 37°C, and samples were collected from 2-6 hours (t_2-t_6) after the initiation of sporulation. Immunoblots were probed with anti-SleB antibodies, and a colorimetric substrate was used for detection.

In the wild-type strain, SleB was strongly detected from 3 hours (t_3) onward after the initiation of sporulation, while only miniscule levels were detected during early *sleB* expression from a Δ*ypeB* strain. In the *spoIVB* mutant, low levels of SleB were observed at t_5 and t_6. This reduction in SleB expression compared with the wild-type can likely be attributed to the sporulation defect in this strain. In a Δ*ypeB spoIVB* mutant, SleB was not restored, even to the low levels seen during sporulation of the *spoIVB* mutant, thus, SpoIVB cannot be solely responsible for the degradation of SleB during spore formation in the absence of YpeB.

References

1. **Bishop-Lilly KA, Plaut RD, Chen PE, Akmal A, Willner KM, Butani A, Dorsey S, Mokashi V, Mateczun AJ, Chapman C, George M, Luu T, Read TD, Calendar R, Stibitz S, Sozhamannan S.** 2012. Whole genome sequencing of phage resistant *Bacillus anthracis* mutants reveals an essential role for cell surface anchoring protein CsaB in phage AP50c adsorption. Virol J **9:**246.
2. **Janes BK, Stibitz S.** 2006. Routine markerless gene replacement in *Bacillus anthracis*. Infect Immun **74:**1949-1953.