

## Supplementary Information

### **Molecular basis of 5-hydroxytryptophan synthesis in *Saccharomyces cerevisiae***

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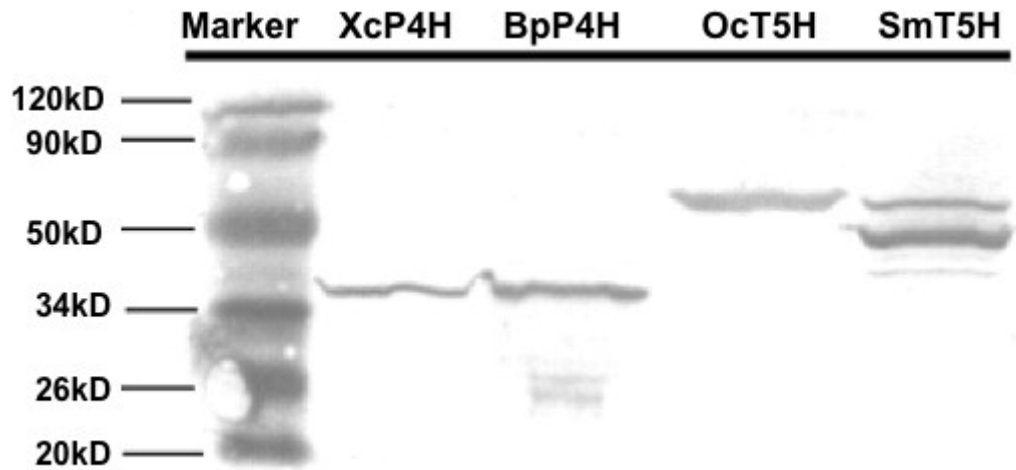
**Table S1** The plasmids used in this study.

Name	Description
H1	pRS415-tef1p-XcP4He-tef1t-tpi1p-dfr1-tpi1t-pdc1p-phhb-pdc1t
H2	pRS415-tef1p-XcP4He-tef1t-tpi1p-folm-tpi1t-pdc1p-phhb-pdc1t
H3	pRS415-tef1p-XcP4He-tef1t
H4	pRS416-tpi1p-dfr1-tpi1t-pdc1p-phhb-pdc1t
H5	pRS416-tpi1p-folm-tpi1t-pdc1p-phhb-pdc1t
H6	pRS415-tef1p-BpP4H-tef1t
H7	pRS415-tef1p-McP4H-tef1t
H8	pRS415-tef1p-OcT5H-tef1t
H9	pRS415-tef1p-SmT5H-tef1t
H10	pRS415-tef1p-OcP4H-tef1t
H11	pRS415-tef1p-HsT3H-tef1t
H12	pRS416-tpi1p-gch-tpi1t-fba1p-pts-fba1t-gpdp-spr-gpdt-gpm1p-dhpr-gpm1t-pdc1p-phhb-pdc1t

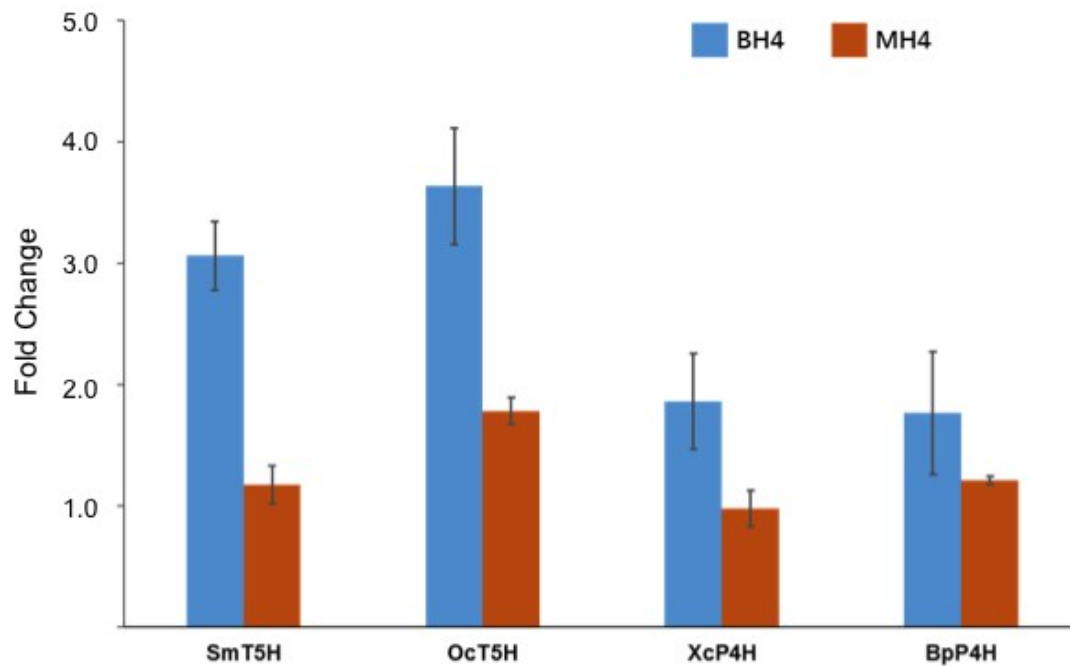
**Table S2** The primers used in this study.

Name	Sequence (5'-3')
pRS-TEF1p-F	CAGTGAGCGCGTAATACGACTCACTATAGGGCGAATTGATAGCTTCAAAATGTTTCTA
TS-TEF1p-R	CATTAAAGCTCAATGAGCCATTTTGTAAATAAAACCTAGATTAGATTGC
TEF1p-TS-F	CAATCTAATCTAAGTTTAAATTACAAAATGGCTCAATTGAGCTTAAATGC
TEF1t-TS-R	CTAGAAAAGTCTTATCAATCTCCTCATACTTGAATCGGGTCTATGTATAC
TS-TEF1t-F	ACATAGACCCGATTCAAGTATGAGGAGATTGATAAGACTTTTCTAGTTGC
pRS-TEF1t-R	AAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCGATAGCGCCGATCAAAGTAT
pRS-TPI1p-F	CAGTGAGCGCGTAATACGACTCACTATAGGGCGAATTGTATATCTAGGAACCCATCAG
GGPPS-TPI1p-R	AAGTAGTTGTCGAAATATGACATTTTATGTTTATGTATGTGTTTTTGTAA
TPI1p-GGPPS-F	TTAAATCTATAACTACAAAAACACATACATAAACTAAAAATGTCATATTCGACAACATA
TPI1t-GGPPS-R	AGATAATATTTTATATAATTATATTAATCCTACTTTCTTCTTAATTGTGAAT
GGPPS-TPI1t-F	ATTCACAATTAGAAGAAGAAAGTAGGATTAATATAATTATATAAAAAATATTATCT
pRS-TPI1t-R	AAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTATATAACAGTTGAAATTTG
PDC1p-TPI1t-R	GAACATATGCTCACCCAGTCGCATGTATATAACAGTTGAAATTTGGATAA
TPI1t-PDC1p-F	TTATCCAAATTTCAACTGTTATATACATGCGACTGGGTGAGCATATGTTC
tHMGR-PDC1p-R	CTTCAGTTTTACCAATTGGTCCATTTTGATTGATTGACTGTGTTATTT
PDC1p-tHMGR-F	AAATAACACAGTCAAATCAATCAAAATGGACCAATTGGTGAAAACCTGAAG
PDC1t-tHMGR-R	TAACTAATAATTAGAGATTAATCGCTTAGGATTAATGCAGGTGACGG
tHMGR-PDC1t-F	CCGTCACCTGCATTAATCCTAAGCGATTTAATCTCTAATTATTAGTTAA
pRS-PDC1t-R	AAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCGCAGTTTGAATTGAGTAA
ENO2p-PDC1t-R	CTTCTATACCCGAGCGTCGACACGGCAGTTTTGAATTGAGTAACCATT
PDC1t-ENO2p-F	AATGGTTACTCAATTCAAAACTGCCGTGTCGACGCTGCGGGTATAGAAAAG
ERG20-ENO2p-R	TCCTAATTTCTTTTTCTGAAGCCATTATTATTGTATGTTATAGTATTAGTTGCTT
ENO2p-ERG20-F	AAGCAACTAATACTATAACATACAATAAATAGGCTTCAGAAAAAGAAATTAGGA
ENO2t-ERG20-R	GACTAATAATTCTTAGTTAAAAGCACTCTATTTGCTTCTCTGTAAACTTTG

ERG20-ENO2t-F	CAAAGTTTACAAGAGAAGCAAATAGAGTGCTTTTAACTAAGAATTATTAGTC
pRS-ENO2t-R	AAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCAGGTATCATCTCCATCTCCC
TEF1p-GDH1-F	AAAGAAAGCATAGCAATCTAATCTAAGTTTAAATTACAAAATGTCAGAGCCAGAATTCA
TEF1t-GDH1-R	ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTAAATACATCACCTTGGT
GDH1-K74V-F	CCGCCAAGGGTCCATACGTTGGTGGTCTACGTTTCCATCC
GDH1-K74V-R	GGATGAAACGTAGACCACCAACGTATGGACCCTTGGCGG
GDH1-T177S-F	CTCCTGGGAAGGTGTCTTATCTGGTAAGGGTTTGAAGTGG
GDH1-T177S-R	CCAGTTCAAACCCTTACCAGATAAGACACCTTCCCAGGAG
TEF1p-GDH2-F	AAAGAAAGCATAGCAATCTAATCTAAGTTTAAATTACAAAATGGATCAGACATATTCTCT
TEF1t-GDH2-R	ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCTTAAATCACACCCTGCGCCA
GDH2-K92V-F	CTGCCATCGGCCCGTACGTTGGCGGTATGCGCTTCCATCC
GDH2-K92V-R	GGATGGAAGCGCATACCGCCAACGTACGGGCCGATGGCAG
GDH2-T195S-F	CAATACCGCCTGCGTCTTCTCTGGTAAGGGCCTTTCATTT
GDH2-T195S-R	AAATGAAAGGCCCTTACCAGAGAAGACGCAGGCGGTATTG
TEF1p-R	TTTGTAATTAACCTTAGATTAGATTGC
TEF1p-XcP4H-F	AAAGAAAGCATAGCAATCTAATCTAAGTTTAAATTACAAAATGAATACTGCCCCAGAAG
TEF1t-XcP4H-R	ATATAAAAGATATGCAACTAGAAAAGTCTTATCAATCTCCCTACACATCACCGTCTCTAG
TEF1t-F	GGAGATTGATAAGACTTTTCTAGTTGC
TPI1p-TEF1t-R	ACGGGTAATCTTCCACCAACCTGATGGGTTTCTAGATATAGATAGCGCCGATCAAAGTAT
TEF1t-TPI1p-F	ATTCGATATTGTCGTAACAAATACTTTGATCGGCGCTATCTATATCTAGGAACCCATCAG
TPI1p-R	TTTTAGTTTATGTATGTGTTTTTTGTA
TPI1p-DFR1-F	TAAATCTATAACTACAAAAACACATACATAAACTAAAAATGGCTGGAGGAAAGATTCC
TPI1t-DFR1-R	AAAGAAAAGAAAGATAATATTTTTATATAATTATATTAATCTCATTACGATTGTATAGAG
TPI1t-F	GATTAATATAATTATATAAAAAATATTATCT
TPI1p-FolM-F	TAAATCTATAACTACAAAAACACATACATAAACTAAAAATGGGTAAAACCCAGCCCTT
TPI1t-FolM-R	AAAGAAAAGAAAGATAATATTTTTATATAATTATATTAATCTTAACGCAGATGACGACCGC
PDC1p-R	TTTGATTGATTGACTGTGTTATTT
PDC1p-PCD-F	TCATAACCTCACGAAAATAACACAGTCAAATCAATCAAATGACCGCACTACCCAAGC
PDC1t-PCD-R	TGCTTATAAACTTTAACTAATAATTAGAGATTAATCGCTCATTTGCGCCCTCGGCGG
PDC1t-F	GCGATTTAATCTCTAATTATTAGTTAA



**Figure S1.** Western blot of P4Hs and T5Hs. Two P4Hs, XcP4H (34.0 kD) and BpP4H (33.9 kD), and two T5Hs, OcT5H (58.4 kD) and SmT5H (51.9 kD) were His-tagged and expressed in pRS415 plasmid, followed by yeast protein extraction and western blotting using the previously established protocols<sup>1,2</sup>. The tagged protein was detected by DAB.



**Figure S2.** *In vitro* enzyme activities of P4Hs and T5Hs. Two P4Hs, XcP4H and BpP4H, and two T5Hs, OcT5H and SmT5H were expressed in pRS415 plasmid, followed by culturing the recombinant yeast strains in the same condition as described in the article and measuring the enzyme activities using a previously established protocol<sup>3</sup>. All of the enzyme activities were normalized to that of XcP4H using MH4 as the cofactor. All of the enzymes demonstrated higher activities when using BH4 as the cofactor. The activities of OcT5H and SmT5H were 50%~75% higher than that of XcP4H and BpP4H when using BH4 as the cofactor.

#### References:

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- 2 Sheng, J., Huang, L., Zhu, X., Cai, J. & Xu, Z. Reconstitution of the peptidoglycan cytoplasmic precursor biosynthetic pathway in cell-free system and rapid screening of antisense oligonucleotides for Mur enzymes. *Applied microbiology and biotechnology* **98**, 1785-1794, doi:10.1007/s00253-013-5467-8 (2014).
- 3 Moran, G. R. & Fitzpatrick, P. F. A continuous fluorescence assay for tryptophan hydroxylase. *Anal Biochem* **266**, 148-152, doi:10.1006/abio.1998.2956 (1999).