fermentation

Supplementary Materials

Supplementary File S1: A detailed description of plasmid and strain construction

Plasmid pRSII426-Gal1/10: The *CPS1* terminator, *Gal1/Gal10* bidirectional promoter, and *HIS5* terminator fragments were amplified from *S. cerevisiae* BCC39850's gDNA using primers CPS1t-hom-F and CPS1t-hom-R, Gal1-10-hom-F and Gal1-10-hom-R, HIS5t-hom-F and HIS5t-hom-R, respectively. The three fragments were placed between the KpnI/SacI site in pRSII426 to form pRSII426-Gal1/10 using homologous recombination in yeast.

Plasmid pRSII426-Gal1/10-AsSesTPS: The *Aquilaria sinensis* sesquiterpene synthase gene (*AsSesTPS*, GenBank accession number: AGV40227) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *AsSesTPS* gene fragment was amplified from the pUC57-AsSesTPS using primers AsSesTPS-F and AsSesTPS-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-AsSesTPS.

Plasmid pRSII426-Gal1/10-AsSesTPS1: The *Aquilaria sinensis* sesquiterpene synthase 1 gene (AsSesTPS1, GenBank accession number: QWB49536) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *AsSesTPS1* gene fragment was amplified from the pUC57-AsSesTPS1 using primers AsSesTPS1-F and AsSesTPS1-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-AsSesTPS1.

Plasmid pRSII426-Gal1/10-AsASS1: The *Aquilaria sinensis* sesquiterpene synthase gene (*AsASS1*, GenBank accession number: AFV99464) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *AsASS1* gene fragment was amplified from the pUC57-AsASS1 using primers AsASS1-F and AsASS1-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-AsASS1.

Plasmid pRSII426-Gal1/10-AcHS1: The *Aquilaria crassna* humulene synthase gene (*AcHS1*, GenBank accession number: AMQ67165) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *AcHS1* gene fragment was amplified from the pUC57-AcHS1 using primers AcHS1-F and AcHS1-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-AcHS1.

Plasmid pRSII426-Gal1/10-AmdGS1: The *Aquilaria microcarpa* delta-guaiene synthase gene (*AmdGS1*, GenBank accession number: AHH25146) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *AmdGS1* gene fragment was amplified from the pUC57-AmdGS1 using primers AmdGS1-F and AmdGS1-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-AmdGS1.

Plasmid pRSII426-Gal1/10-ZzBES2: The *Zingiber zerumbet* beta-eudesmol synthase gene (*ZzBES2*, GenBank accession number: BAG12021) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *ZzBES2* gene fragment was amplified from the pUC57-ZzBES2 using primers ZzBES2-F and ZzBES2-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-ZzBES2.

Plasmid pRSII426-Gal1/10-ClTPS2: The *Clausena lansium* terpene synthase gene (*ClTPS2*, GenBank accession number: ADR71055) codon-optimized for *S. cerevisiae* expression was synthesized by GenScript and was provided as a pUC57 plasmid. The *ClTPS2* gene fragment was amplified from the pUC57-ClTPS2 using primers ClTPS2-F and ClTPS2-R. The purified fragment was ligated to the BamHI/SalI site of pRSII426-Gal1/10 to create pRSII426-Gal1/10-ClTPS2.

Plasmid pRSII426-Gal1/10-FPPS-GSG-AcHS1: The FPP synthase gene (ERG20) was amplified from *S. cerevisiae* TBRC 1590's gDNA using primers ERG20gsg-SpeI-F and E20gsgAcHS1-R. The AcHS1 gene was amplified from pUC57-AcHS1 using primers AcHS1gsg-F and AcHS1gsg-EcoRI-R. These two DNA fragments were assembled together by overlap-extension PCR (OE-

PCR). The resulting cassette was ligated to the *Spe*I/*EcoR*I sites of pRSII426-Gal1/10 to create pRSII426-Gal1/10-FPPS-GSG-AcHS1.

Plasmid pRSII426-Gal1/10-FPPS-GSG-AmdGS1: The FPP synthase gene (ERG20) was amplified from *S. cerevisiae* TBRC 1590's gDNA using primers ERG20gsg-SpeI-F and E20gsgAmdGS1-R. The AmdGS1 gene was amplified from pUC57-AmdGS1 using primers AmdGS1gsg-F and AmdGS1gsg-EcoRI-R. These two DNA fragments were assembled together by overlap-extension PCR (OE-PCR). The resulting cassette was ligated to the *Spe*I/*EcoR*I sites of pRSII426-Gal1/10 to create pRSII426-Gal1/10-FPPS-GSG-AmdGS1.

Plasmid pRSII426-Gal1/10-FPPS-GSG-ZzBES2: The FPP synthase gene (ERG20) was amplified from *S. cerevisiae* TBRC 1590's gDNA using primers ERG20gsg-SpeI-F and E20gsgZzBES2-R. The ZzBES2 gene was amplified from pUC57-ZzBES2 using primers ZzBES2gsg-F and ZzBES2gsg-EcoRI-R. These two DNA fragments were assembled together by overlap-extension PCR (OE-PCR). The resulting cassette was ligated to the *Spe*I/*EcoR*I sites of pRSII426-Gal1/10 to create pRSII426-Gal1/10-FPPS-GSG-ZzBES2.

Plasmid pRSII426-Gal1/10-FPPS-GSG-ClTPS2: The FPP synthase gene (ERG20) was amplified from *S. cerevisiae* TBRC 1590's gDNA using primers ERG20gsg-SpeI-F and E20gsgClTPS2-R. The ClTPS2 gene was amplified from pUC57-ClTPS2 using primers ClTPS2gsg-F and ClTPS2gsg-EcoRI-R. These two DNA fragments were assembled together by overlap-extension PCR (OE-PCR). The resulting cassette was ligated to the *Spe*I/*EcoR*I sites of pRSII426-Gal1/10 to create pRSII426-Gal1/10-FPPS-GSG-ClTPS2.

Plasmid pRPR1-gRNA-ERG9p: To construct pRPR1-gRNA-ERG9p for promoter replacement, the gRNA-ERG9p fragment was amplified from pRPR1-gRNA handle-RPR1t using primers ERG9p-gRNA-F and gRNA-Rev. The 0.13-kb PCR band was gel purified and ligated to the *Hin*dIII/*Xho*I site of pRPR1-gRNA handle-RPR1t to yield pRPR1-gRNA-ERG9p.

Strain construction

Strain BCC39850hlu: The markerless CRISPR/Cas9 system was employed to create the multi-autotrophic strains BCC39850hlu. Donor DNA for each gene deletion was obtained by overlap extension PCR (OE-PCR). For example, to obtain donor DNA for *HIS3* deletion, the 500-bp upstream and 500-bp downstream fragments were amplified from BCC39850's genomic DNA using primers ScHIS3_ups_frag_F and ScHIS3_ups_frag_R, and primers ScHIS3_dws_frag_F and ScHIS3_dws_frag_R, respectively. The two DNA fragments were assembled together to form the donor DNA by OE-PCR. The purified donor DNA was introduced into competent cells along with the corresponding pRPR1-gRNA plasmid and p414-TEF1p-Cas9-CYC1t. Transformants were selected on a yeast minimal medium with appropriate amino acid dropout(s). Colony PCR of transformants was performed to verify gene deletion.

Strain FPPY001_39850: The ERG10-Gal1/10-ERG20-Ura integration cassette was amplified from pRSII416-ERG10-Gal1/10-ERG20-Ura3 using primers YPRCd15-int-F and YPRCd15-int-R. The PCR product was purified and transformed into *S. cerevisiae* BCC39850hlu to generate strain FPPY001_39850. Colony PCR was used to verify the genomic integration: YPRCd15-up-F and loxP-seq-R to verify the 5′ end and ERG20-RT-F and YPRCd15-dw-R to verify the 3′ end. The URA3 selectable marker (for selection on medium lacking uracil) was recycled using the loxP-Cre recombinase system.

Strain FPPY002_39850: The tHMG1-Gal1/10-ERG8-Ura integration cassette was amplified from pRSII416-tHMG1-Gal1/10-ERG8-Ura3 using primers ARS308-int-F and ARS308-int-R. The PCR product was purified and transformed into strain FPPY001_39850 to generate strain FPPY002_39850. Colony PCR was used to verify the genomic integration: ARS308-up-F and Ura3-dw-R to verify the 5′ end and ERG8-RT-F and ARS308-dw-R to verify the 3′ end. The URA3 selectable marker was recycled using the loxP-Cre recombinase system.

Strain FPPY003_39850: The ERG13-Gal1/10-IDI1-Ura integration cassette was amplified from pRSII416-ERG13-Gal1/10-IDI1-Ura3 using primers ARS1021-int-F and ARS1021-int-R. The PCR product was purified and transformed into strain FPPY002_39850 to generate strain FPPY003_39850. Colony PCR was used to verify the genomic integration: ARS1021-up-F and

Ura3-dw-R to verify the 5′ end and IDI1-RT-F and ARS1021-dw-R to verify the 3′ end. The URA3 selectable marker was recycled using the loxP-Cre recombinase system.

Strain FPPY004_39850: The tHMG1-Gal1/10-ERG19-Ura integration cassette was amplified from pRSII416-tHMG1-Gal1/10-ERG19-Ura3 using primers ARS720-int-F and ARS720-int-R. The PCR product was purified and transformed into strain FPPY003_39850 to generate strain FPPY004_39850. Colony PCR was used to verify the genomic integration: ARS720-up-F and Ura3-dw-R to verify the 5′ end and ERG19-RT-F and ARS720-dw-R to verify the 3′ end. The URA3 selectable marker was recycled using the loxP-Cre recombinase system.

Strain FPPY005_39850: The tHMG1-Gal1/10-ERG12-Ura integration cassette was amplified from pRSII416-tHMG1-Gal1/10-ERG12-Ura3 using primers ARS1309-int-F and ARS1309-int-R. The PCR product was purified and transformed into FPPY004_39850 to generate strain FPPY005_39850. Colony PCR was used to verify the genomic integration: ARS1309-up-F and Ura3-dw-R to verify the 5′ end and ERG12-RT-F and ARS1309-dw-R to verify the 3′ end. The URA3 selectable marker was recycled using the loxP-Cre recombinase system.

Strain FPPY005_39850 P_{MET3}-ERG9: The CRISPR/Cas9 system was employed to create strain FPPY005_39850 P_{MET3}-ERG9. Donor DNA was amplified from *S. cerevisiae* BCC39850's gDNA using primers Met3p_F and Met3p_R. The PCR band of the donor DNA was gel purified and then transformed into competent FPPY005_39850 cells along with the pRPR1-gRNA-ERG9p and p414-TEF1-Cas9-CYC1t-KAN plasmids. Transformants were selected on a minimal yeast medium with 100 μg/mL G418 and L-leucine dropouts.

Table S1. Primers used in this study.

Primer Name	**Primer Sequence (5' to 3')**
Plasmid construction primers	
tHMG1t-R	TAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGTTTTTGAGTTTTTTTCGTTG
tHMG1-F	TACCTCTATACTTTAACGTCAAGGAGAAAAAACTATAATGGCAGACCAATTGGTGAAAAC

Gal1-10-F	TATAGTTTTTTCTCCTTGACG
Gal1-10-R	TTATATTGAATTTTCAAAAATTCTTAC
ERG12-F	CAAAAAAAAAGTAAGAATTTTTGAAAATTCAATATAAATGTCATTACCGTTCTTAAC
ERG12t-R	CTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGGATTGAATGGCTATTTAAC
ERG8-F	ATCCAAAAAAAAAAGTAAGAATTTTTGAAAATTCAATATAAATGTCAGAGTTGAGAGCC
ERG8t-R	GGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTTCTAGAAAGTTTATTTATCGTTC
ERG19-F	ATCCAAAAAAAAAAGTAAGAATTTTTGAAAATTCAATATAAATGACCGTTTACACAGCA
ERG19t-R	GCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTCATGTAGGGAGGTCATGATA
ERG13-F	GGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACAGATTTATTGTGTTATAAATATAG
ERG13t-R	ATACTTTAACGTCAAGGAGAAAAAACTATAATGAAACTCTCAACTAAAC
IDI1-F	AAAGTAAGAATTTTTGAAAATTCAATATAAATGACTGCCGACAACAAT
IDI1t-R	GAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTAAGAGAAAAAAAAAATGTGAAC
ERG10-F	GGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGTAATTAGTGGAACTTGTG
ERG10t-R	ATACTTTAACGTCAAGGAGAAAAAACTATAATGTCTCAGAACGTTTAC
ERG20-F	AAAGTAAGAATTTTTGAAAATTCAATATAAATGGCTTCAGAAAAAGAA
ERG20t-R	GAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTTTCTCGTACTACCCGTAA

LoxP-Ura3-F	ATCTCGAGATAACTTCGTATAGCATACATTATACGAAGTTATAATGTGGCTGTGGTTTCA
LoxP-Ura3-R	TATGTCGACATAACTTCGTATAATGTATGCTATACGAAGTTATAATCATTACGACCGAGA
CPS1t hom F	GCGTAATACGACTCACTATAGGGCGAATTGGGTACCTTATCATCATCATTTAAATTTTGA
CPS1t hom R	AATATAAACTAGTAAGCTTGAATTCGCGCAATGATTGAATAGT
Gal-1-10 hom F	ATTGCGCGAATTCAAGCTTACTAGTTTATATTGAATTTTCAAAAATTCTTAC
Gal-1-10 hom R	AATCTATGTCGACTCTAGAGGATCCTATAGTTTTTTCTCCTTGACG
HIS5t hom F	AACTATAGGATCCTCTAGAGTCGACATAGATTAATTTAAACAGTATATG
HIS5t hom R	ATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCAAATTCATCCTCTATCATAGA
AsSesTPS1_F	ATATGGATCCAAAACAATGTCTGCTGCACAAGTT
AsSesTPS1_R	ATATGTCGACTCAAATAGTAATTGGATGAACTAAC
AsSesTPS_F	ATATGGATCCAAAACAATGGCAGAAACTAATAGACC
AsSesTPS_R	ATATGTCGACTTAATCCAATGGTAATTGATGAAC
AsASS1_F	ATATGGATCCAAAACAATGTCATCTGCAAAATTGGG
AsASS1_R	ATATGTCGACTCAAATTTCAATAGCATGTCTC
AcHS1_F	ATATGGATCCAAAACAATGTCACCAGCACAAGCA
AcHS1_R	ATATGTCGACTCAGATTGTAAATGGATGGACC
AmdGS1_F	ATATGGATCCAAAACAATGTCATCTGCTAAATTGGG
AmdGS1_R	ATATGTCGACTCAAATTTCAATAGCATGTCTC
ZzBES2_F	ATATGGATCCAAAACAATGGAAAAACAATCC
ZzBES2_R	ATATGTCGACTTACTTGTTAAAATAGTCACAGG
ClTPS2_F	ATATGGATCCAAAACAATGTCAACTCAACAAGTTTC
ClTPS2_R	ATATGTCGACTTAATCATCCAATTTAACTGGATC
Gal1-10_KpnI_F	ATATATGGTACCAGTACGGATTAGAAGCCG
HIS5t_EcoRI_ R	ATATATGAATTCAAATTCATCCTCTATCATAGAAAC
Ups_rDNA_HA_F	ACCTACCGACCAACTTTC

Ups_rDNA_HA_R	GCTTCTAATCCGTACTAGGACATGCCTTTGATATG
Gal1-10-TPS2-F	ATCAAAGGCATGTCCTAGTACGGATTAGAAGCCG
TPS2-His5t-R	TTGTTGTCTGATTTGTAAATTCATCCTCTATCATAGAAAC
Dws_rDNA_HA_F	GATAGAGGATGAATTTACAAATCAGACAACAAAGG
Dws_rDNA_HA_R	GCGAAACCACAGCCAAG
Primers for constructing GSG-linked and GGGGS-linked enzyme fusion plasmids	
ERG20gsg-SpeI-F	ATATATACTAGTAAAACAATGGCTTCAGAAAAAGAAATTAG
E20gsgClTPS2-R	TGAGTTGACATTCCAGAACCTTTACTTCTCTTGTAAACCTTG
ClTPS2gsg-F	AGAGAAGTAAAGGTTCTGGAATGTCAACTCAACAAGTTTC
ClTPS2gsg-EcoRI-R	ATATATGAATTCTTAATCATCCAATTTAACTGG
E20gsgAcHS1-R	GCTGGTGACATTCCAGAACCTTTACTTCTCTTGTAAACCTTG
AcHS1gsg-F	AGAGAAGTAAAGGTTCTGGAATGTCACCAGCACAAGCA
AcHS1gsg-EcoRI-R	ATATATGAATTCTCAGATTGTAAATGGATGGACC
E20gsgAmdGS1-R	GCAGATGACATTCCAGAACCTTTACTTCTCTTGTAAACCTTG
AmdGS1gsg-F	AGAGAAGTAAAGGTTCTGGAATGTCATCTGCTAAATTGGG
AmdGS1gsg-EcoRI-R	ATATATGAATTCTCAAATTTCAATAGCATGTCTC
E20gsgZzBES2-R	TGTTTTTCCATTCCAGAACCTTTACTTCTCTTGTAAACCTTG
ZzBES2gsg-F	AGAGAAGTAAAGGTTCTGGAATGGAAAAACAATCCTTAACC
ZzBES2gsg-EcoRI-R	ATATATGAATTCTTACTTGTTAAAATAGTCACAGG
E20ggggsClTPS2-R	GTTGACATggacccaccgcctccTTTACTTCTCTTGTAAACCTTG
ClTPS2ggggs-F	GAAGTAAAggaggcggtgggtccATGTCAACTCAACAAGTTTC
E20ggggsAmdGS1-R	GATGACATggacccaccgcctccTTTACTTCTCTTGTAAACCTTG
AmdGS1ggggs-F	GAAGTAAAggaggcggtgggtccATGTCATCTGCTAAATTGGG
E20ggggsAcHS1-R	GGTGACATggacccaccgcctccTTTACTTCTCTTGTAAACCTTG
AcHS1ggggs-F	GAAGTAAAggaggcggtgggtccATGTCACCAGCACAAGCA
E20ggggsZzBES2-R	TTTTCCATggacccaccgcctccTTTACTTCTCTTGTAAACCTTG
ZzBES2ggggs-F	GAAGTAAAggaggcggtgggtccATGGAAAAACAATCCTTAACC
CRISPR gRNA plasmid primers	
ERG9p-gRNA-F	ATAAAGCTTCTTTCCATTTATATAGCCCGGTTTTAGAGCTAGAAATAG CA

HIS3-gRNA-F	ATAAAGCTTCATGCTCTGGCCAAGCATTCGTTTTAGAGCTAGAAATAGCA
URA3-gRNA-F	ATAAAGCTTTGTTAGCGGTTTGAAGCAGGGTTTTAGAGCTAGAAATAGCA
LEU2-gRNA-F	ATAAAGCTTGTTGTCAGAGAATTAGTGGGGTTTTAGAGCTAGAAATAGCA
gRNA-Rev	ATACTCGAGAAAAAAGCACCG
Genome integration primers	
YPRCd15-int-F	AAGAAAGAAAAACTAACACATTAATGTAGTTTTAAAATTTCAGGGTACCGGGCCCATAAC
YPRCd15-int-R	AATTTTTATTCTAGCATATATTTAAGTTTGTTTGCGAAACCCCGGCCGCTCTAGAACTAG
ARS308-int-F	TGAAATTTCAACATTAACTTCGAATTTTTTTCTTTTTATCTAACCCCCCCTCGAGATAAC
ARS308-int-R	GTGGTAGCAATATGTAGCAAAGAAGACAAGTAATCCTTCTAGAAAGTTTATTTATCGTTC
ARS1021-int-F	ACTCAAATTTTCCAGTGTCTCTTAGCAGTTAAACCATTCCTGGGGTACCGGGCCCATAAC
ARS1021-int-R	GAATTTCATCACGTGCGTATTATCTCTTAACTCATAATGCCACGGCCGCTCTAGAACTAG
ARS720-int-F	TGTTACTGTTGATTGTTCGTTTATTTGTATAATTGAGTTTACACCCCCCCTCGAGATAAC
ARS720-int-R	ATAAGTTTGCTTTTTGTCACTCTCTTGGCCCTAATTACCATGTAGGGAGGTCATGATATG
ARS1309-int-F	CATTCTAGTATCAAAGAAACTTACTATGACGCAGTTTAGGATCCCCCCCCTCGAGATAAC
ARS1309-int-R	CTGAATAAACAAGGGGCTTTACGATGGAGTAGTAGACCTGGGATTGAATGGCTATTTAAC
Met3p_F	GTTTTGGGTTTAGTGCCTAAACGAGCAGCGAGAACACGATTTAGTACTAACAGAGACTTT

Met3p_R	GACCGGATGCAATGCCAATTGTAATAGCTTTCCCATGTTAATTATACT TTATTCTTGTTA
ScHIS3_ups_frag_F	TCGACGTGGGCCTTTTTC
ScHIS3_ups_frag_R	CTTTAAATAATCGGTGTCACTACATCTTTGCCTTCGTTTATC
ScHIS3_dws_frag_F	GATAAACGAAGGCAAAGATGTAGTGACACCGATTATTTAAAG
ScHIS3_dws_frag_R	TAACCACCACGACGGTTG
ScURA3_ups_frag_F	CATCATCTCATGGATCTGCAC
ScURA3_ups_frag_R	CATTTACTTATAATACAGTTTTTTACATGATTTATCTTCGTTTCCTG
ScURA3_dws_frag_F	CAGGAAACGAAGATAAATCATGTAAAAAACTGTATTATAAGTAAATG
ScURA3_dws_frag_R	GCGTTTTGTTCTTGGAAAC
ScLEU2_ups_frag_F	TAATTGGTTGTTTGGCCG
ScLEU2_ups_frag_R	CATAAAAAAAGAGAATCTTTTTACATTAGAATGGTATATCCTTG
ScLEU2_dws_frag_F	CAAGGATATACCATTCTAATGTAAAAAGATTCTCTTTTTTTATG
ScLEU2_dws_frag_R	GATTTAGTACTGAAGAGGAGG
Colony PCR primers for strain verification	
YPRCd15-up-F	TCCAAATCACGTCAAGAC
loxP-seq-R	GTCGACCTCGAGATAACT
ERG20-RT-F	CTACAACACTCCAGGCGGTA
YPRCd15-dw-R	GGTTTCGATTGTTGGCAAAGAC
ARS308-up-F	CAAACCAACAGATATAGGC
Ura3-dw-R	CATTACGACCGAGATTCCC
ERG8-RT-F	GTTACCGAACATCGTGGCAA
ARS308-dw-R	GAAGGGTTCGGTTTATATG
ARS1021-up-F	ATAACTGCTTGCAGCGGC
IDI1-RT-F	GACGTCAAATGACGAAAGCG
ARS1021-dw-R	CGTGGATTTATGAATCCGGG
ARS720-up-F	TTGAGCGGTTTGTTACTG
ERG19-RT-F	AAATTGTCTGCGCGACCTAC
ARS720-dw-R	GCGAACACTTGTCATTTG
ARS1309-up-F	TAGTTTGTAGCTGGTGGC
ERG12-RT-F	AGATCTTGTTGCTCGCGTTC

ARS1309-dw-R	CCCGCATATGATCTGGAC
HIS3 upst F	AACACAGTCCTTTCCCGC
HIS3 dwst R	GCCTCGTTCAGAATGACAC
LEU2 upst F	CCGGAACCGGCTTTTCAT
LEU2 dwst R	TCCTCCTTTTTCTCCTTCTTG
URA3 upst F	CGAGCAGAAGGAAGAACG
URA3 dwst R	CATTACGACCGAGATTCCC
MET3p_seq_F	GTGACCAGAAAAGTCACGTG
ERG9_int_R	GCATTTCCGTCGAAACTCCA
Real-time PCR primers to quantify the relative expression levels of mevalonate pathway genes	
TAF10-RT-F	GCGAGAGCTAGGCAGCTATT
TAF10-RT-R	ATCGTTCACCGTCAGAACAA
ERG10-RT-F	CCAGACAAGTTGCTTTGGCT
ERG10-RT-R	CCAGCTACGACAACATCAGC
ERG20-RT-F	CTACAACACTCCAGGCGGTA
ERG20-RT-R	TGCAACAACTCAATGCACCA
tHMG1-RT-F	GACTACGACCGCGTATTTGG
tHMG1-RT-R	ACAACCCTCTGTAGTTGCCA
ERG8-RT-F	GTTACCGAACATCGTGGCAA
ERG8-RT-R	AAGGAGGCCAAAGCTGTAGT
ERG13-RT-F	GCCATTGTAGTTTGCGGTGA
ERG13-RT-R	TCAGGACCGATCCACATAGC
IDI1-RT-F	GACGTCAAATGACGAAAGCG
IDI1-RT-R	AAGACGGAGAATGCACGATG
ERG19-RT-F	AAATTGTCTGCGCGACCTAC
ERG19-RT-R	ACCAGCTGCTGTAGGAAAGT
ERG12-RT-F	AGATCTTGTTGCTCGCGTTC
ERG12-RT-R	CTCGTCATCGGTGCCTTTAC

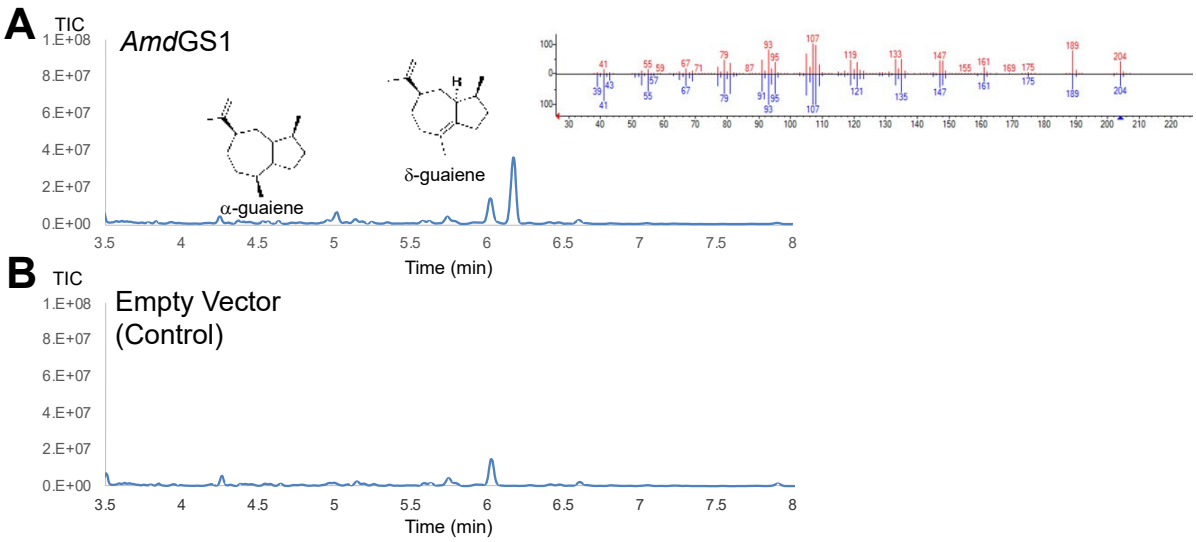

Figure S1. Total ion chromatograms (left panel) and mass spectra (right panel) obtained from GC-MS analysis of dodecane samples from the cultivation of strains FPPY005_39850 harboring pRSII426-Gal1/10-*Amd*GS1 (**A**) and FPPY005_39850 harboring the empty vector pRSII426-Gal1/10 (**B**).

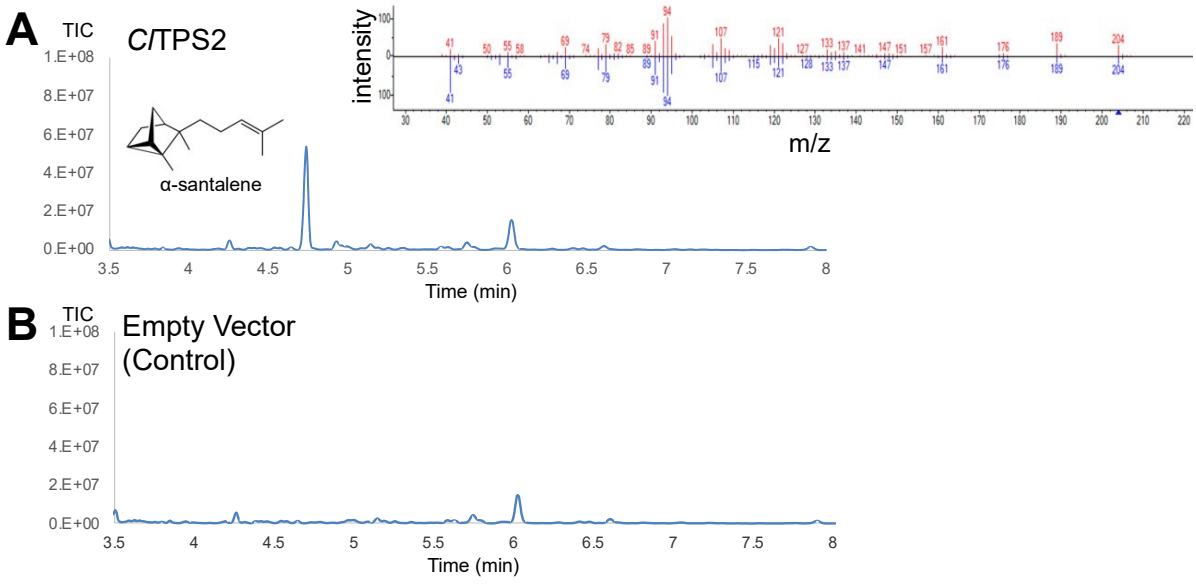

Figure S2. Total ion chromatograms (left panel) and mass spectra (right panel) obtained from GC-MS analysis of dodecane samples from the cultivation of strains FPPY005_39850 harboring

pRSII426-Gal1/10-*Cl*TPS2 (**A**) and FPPY005_39850 harboring the empty vector pRSII426-Gal1/10 (**B**).

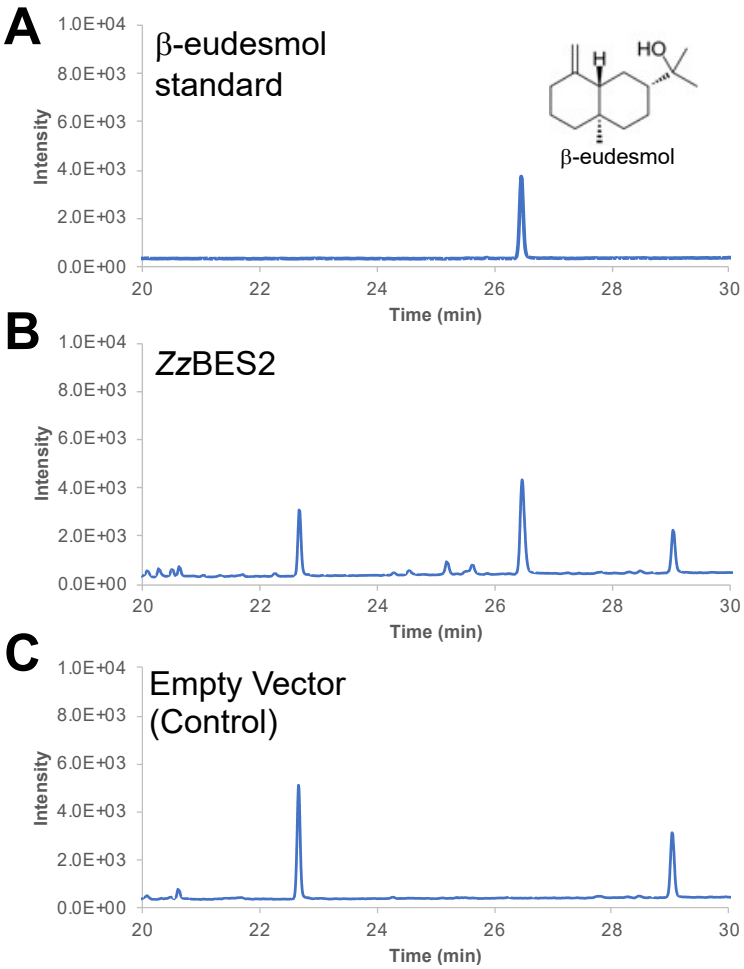

Figure S3. GC-FID analysis of an authentic standard for b-eudesmol (**A**) and dodecane samples from the cultivation of strains FPPY005_39850 harboring pRSII426-Gal1/10-ZzBES2 (**B**) and FPPY005_39850 harboring the empty vector pRSII426-Gal1/10 (**C**).

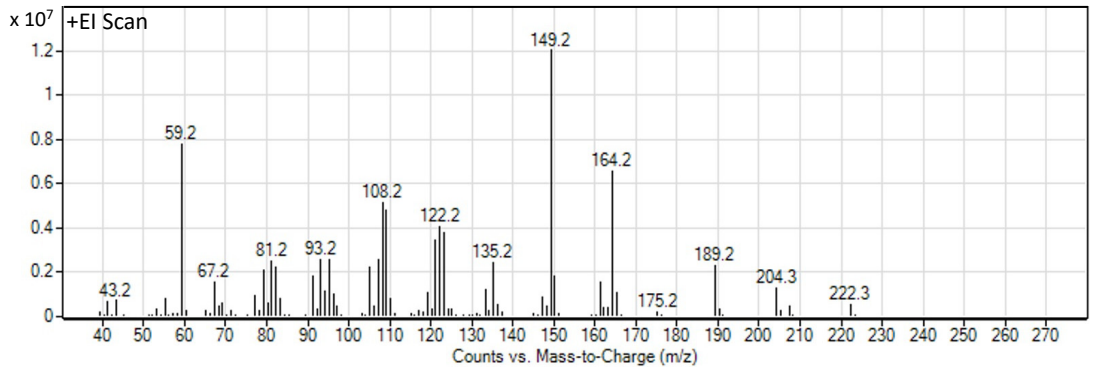

Figure S4. Mass spectra obtained from GC-MS analysis of the dodecane sample containing b-eudesmol from the cultivation of strain FPPY005_39850 harboring pRSII426-Gal1/10-ZzBES2.

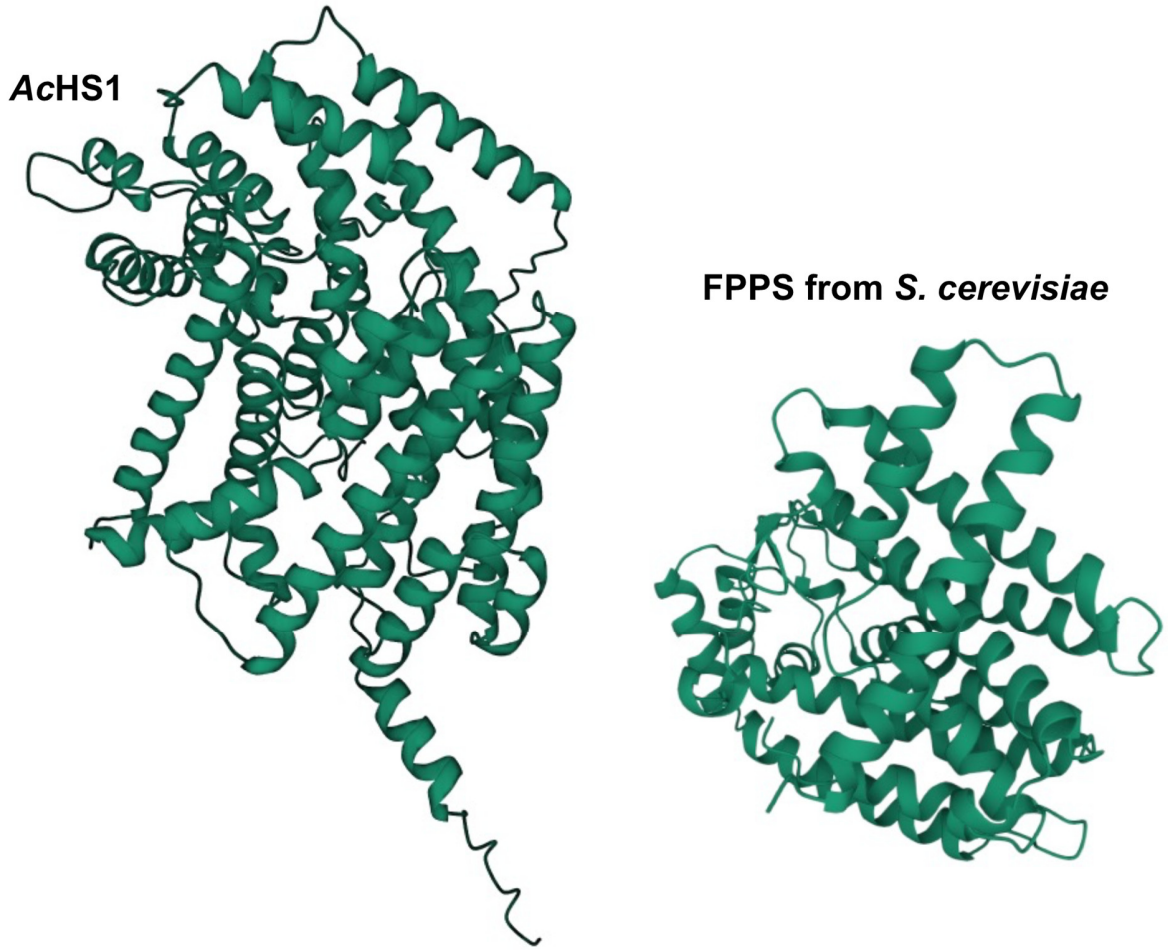

Figure S5. AlphaFold-predicted structures of the *Ac*HS1 and FPPS as standalone enzymes.

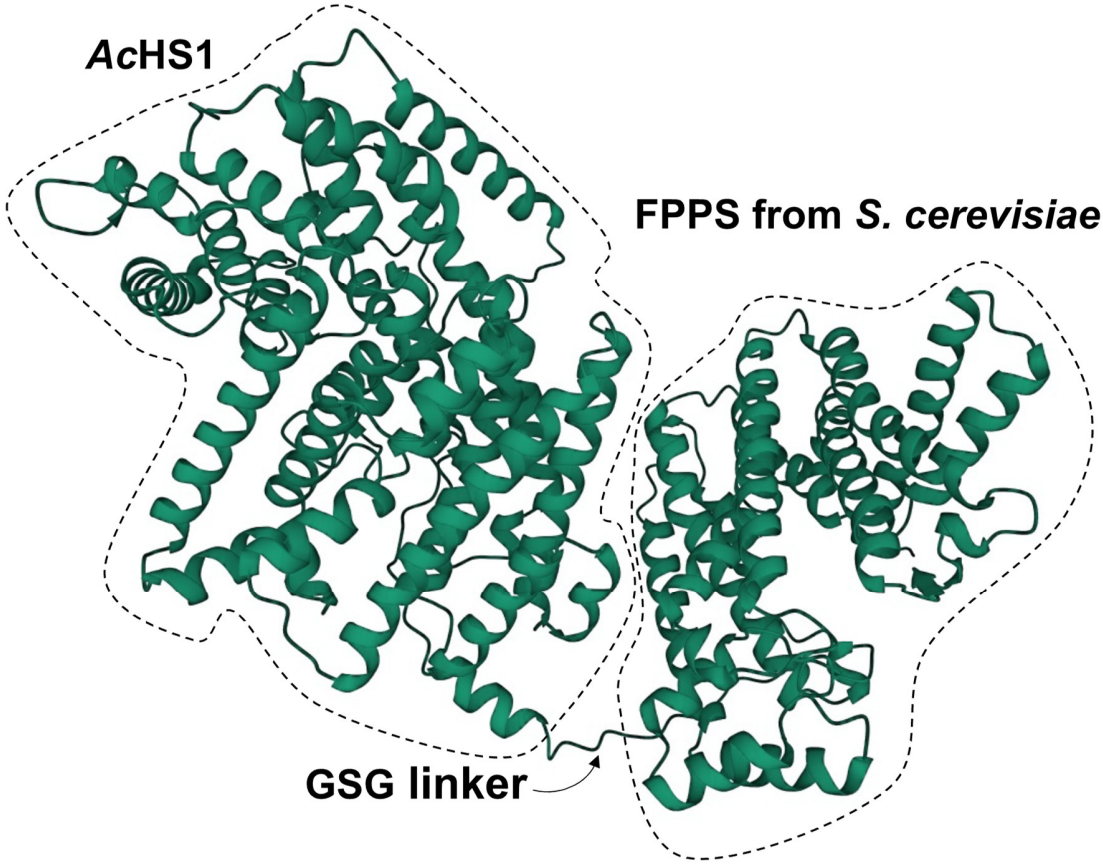

Figure S6. AlphaFold-predicted structure of the FPPS-GSG-*Ac*HS1 enzyme fusion.

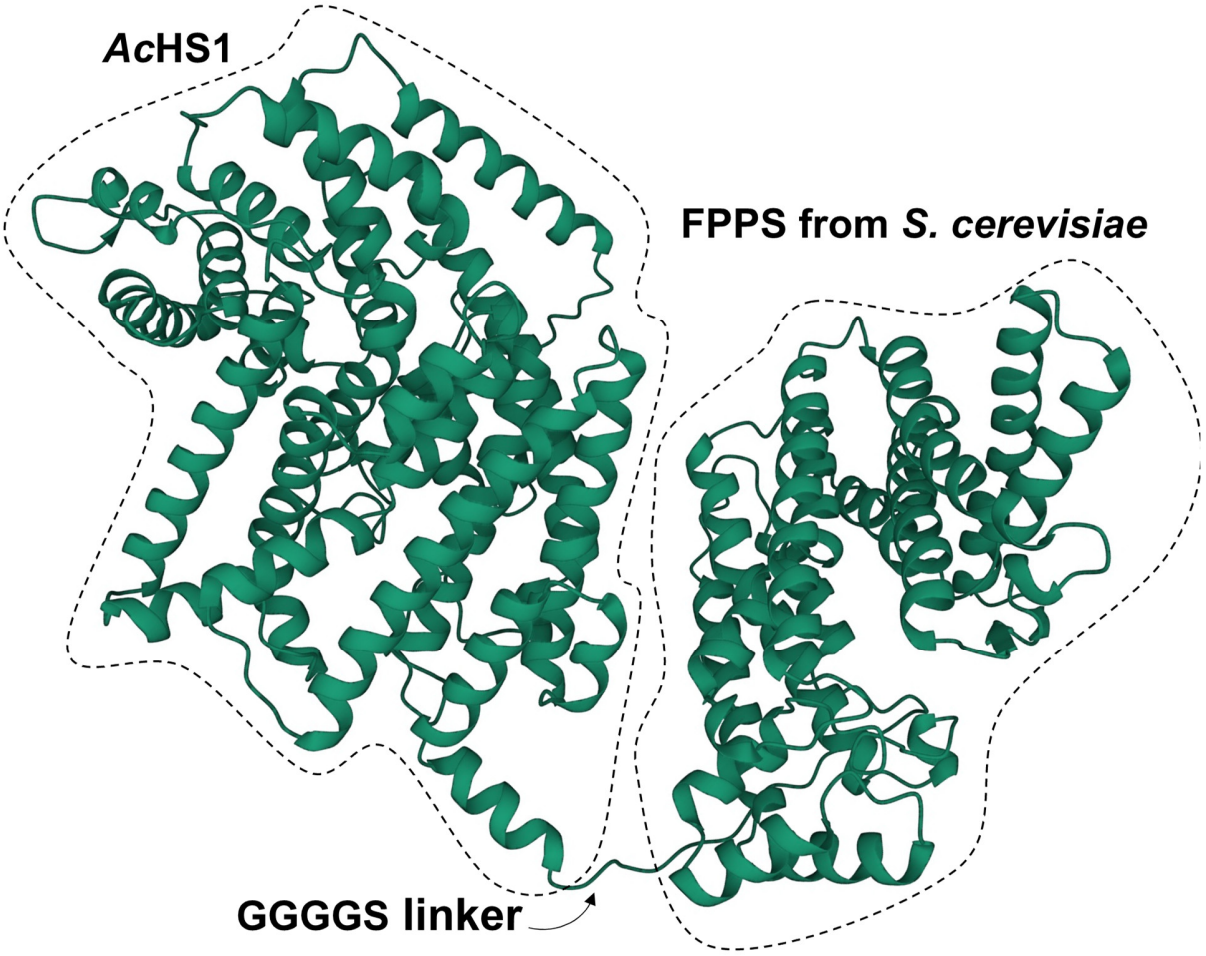

Figure S7. AlphaFold-predicted structure of the FPPS-GGGGS-*Ac*HS1 enzyme fusion.

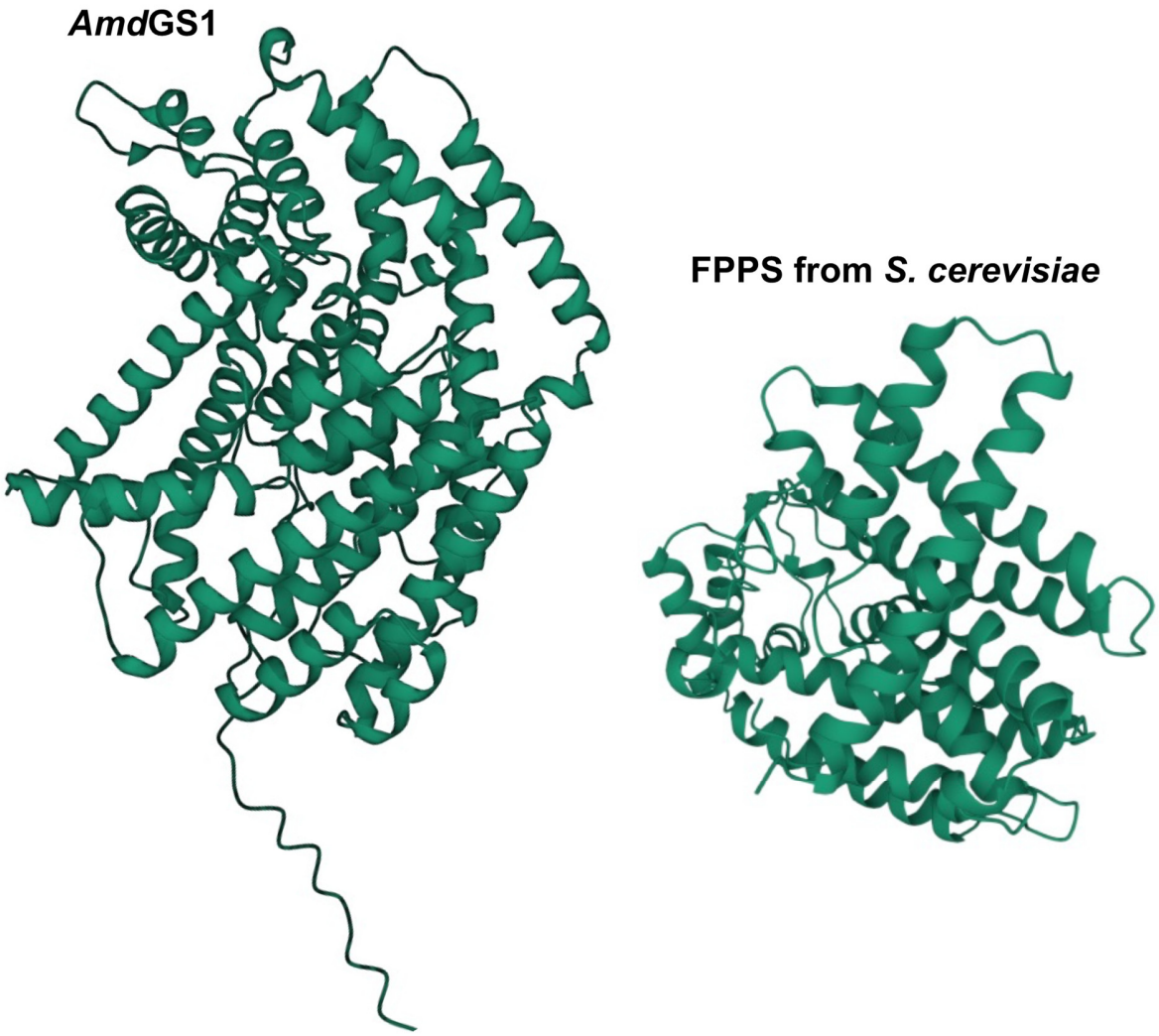

Figure S8. AlphaFold-predicted structures of the *Amd*GS1 and FPPS as standalone enzymes.

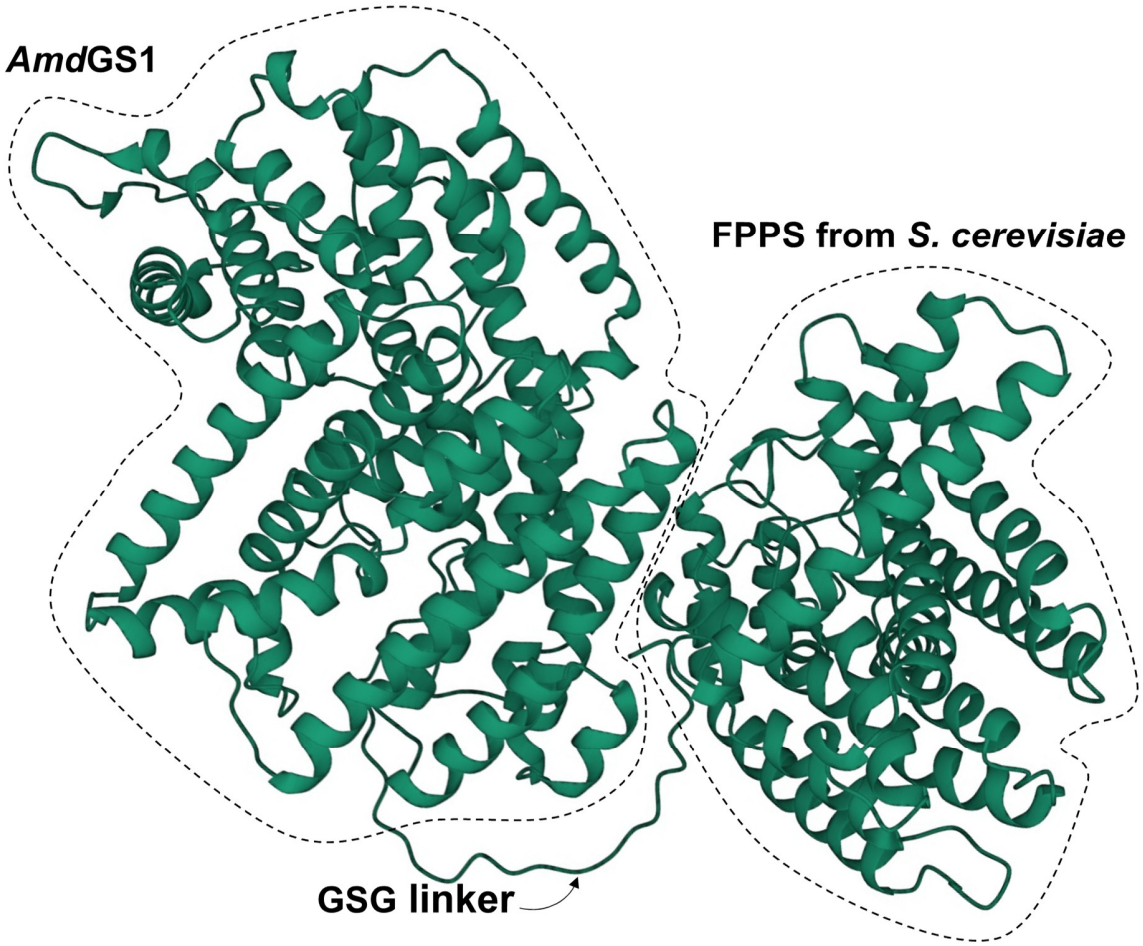

Figure S9. AlphaFold-predicted structure of the FPPS-GSG-*Amd*GS1 enzyme fusion.

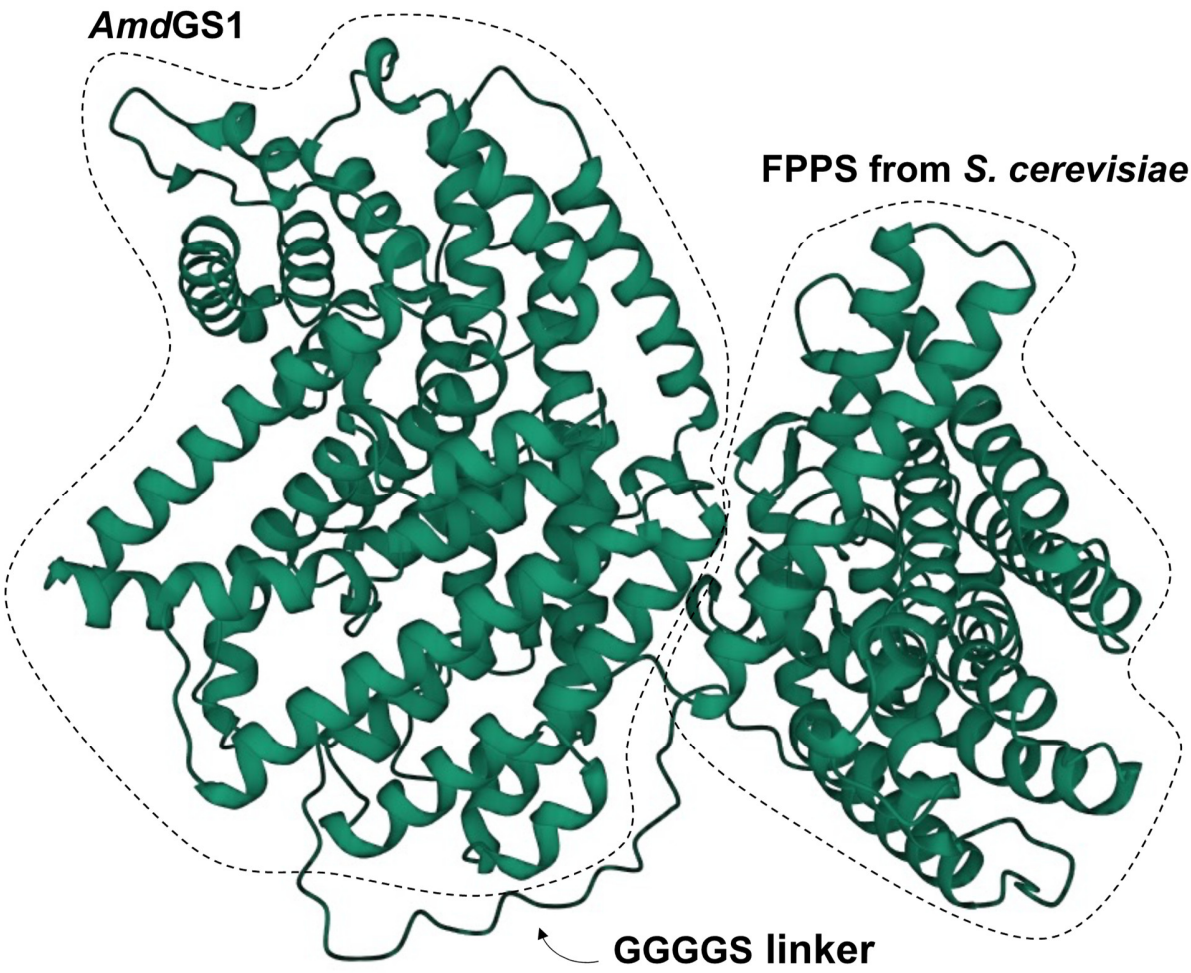

Figure S10. AlphaFold-predicted structure of the FPPS-GGGGS-*Amd*GS1 enzyme fusion.

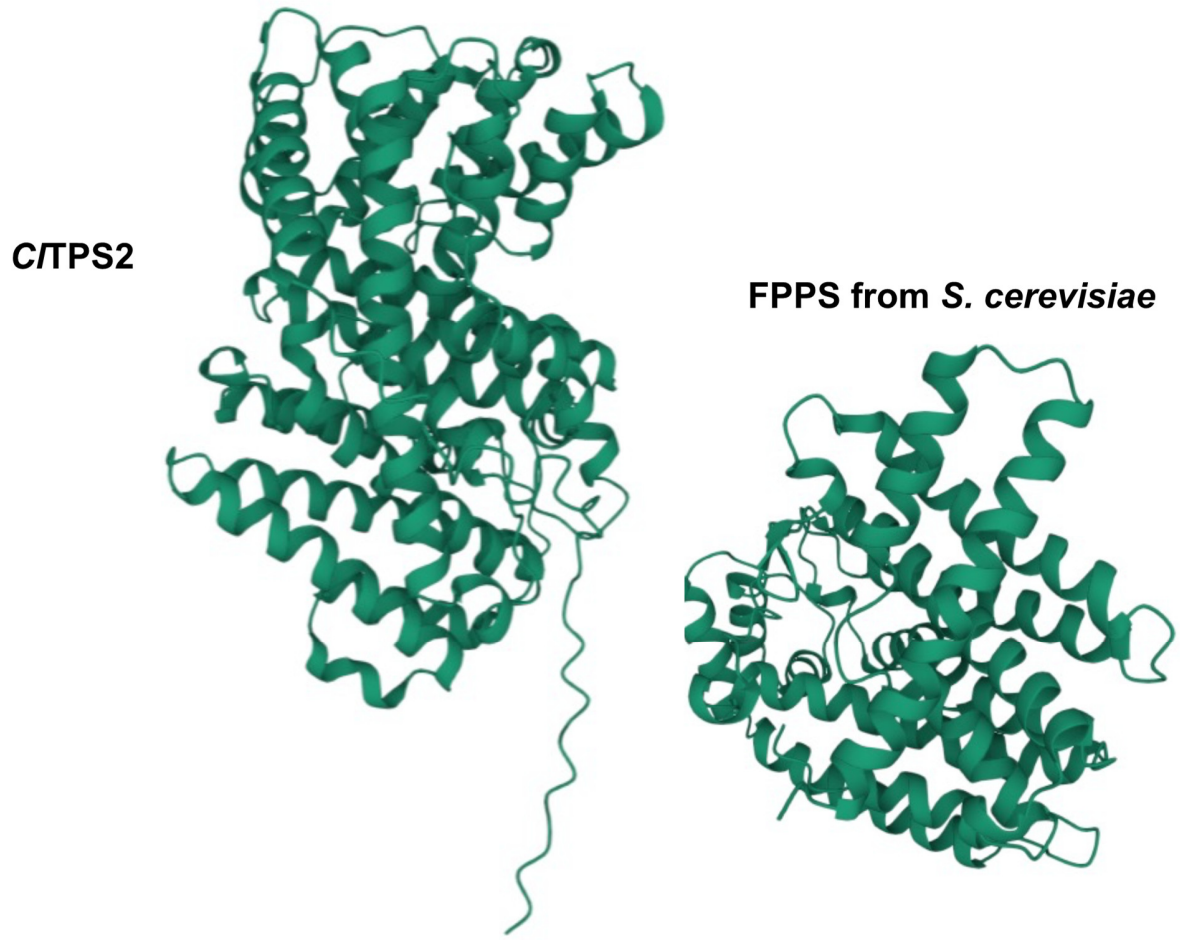

Figure S11. AlphaFold-predicted structures of the *Cl*TPS2 and FPPS as standalone enzymes.

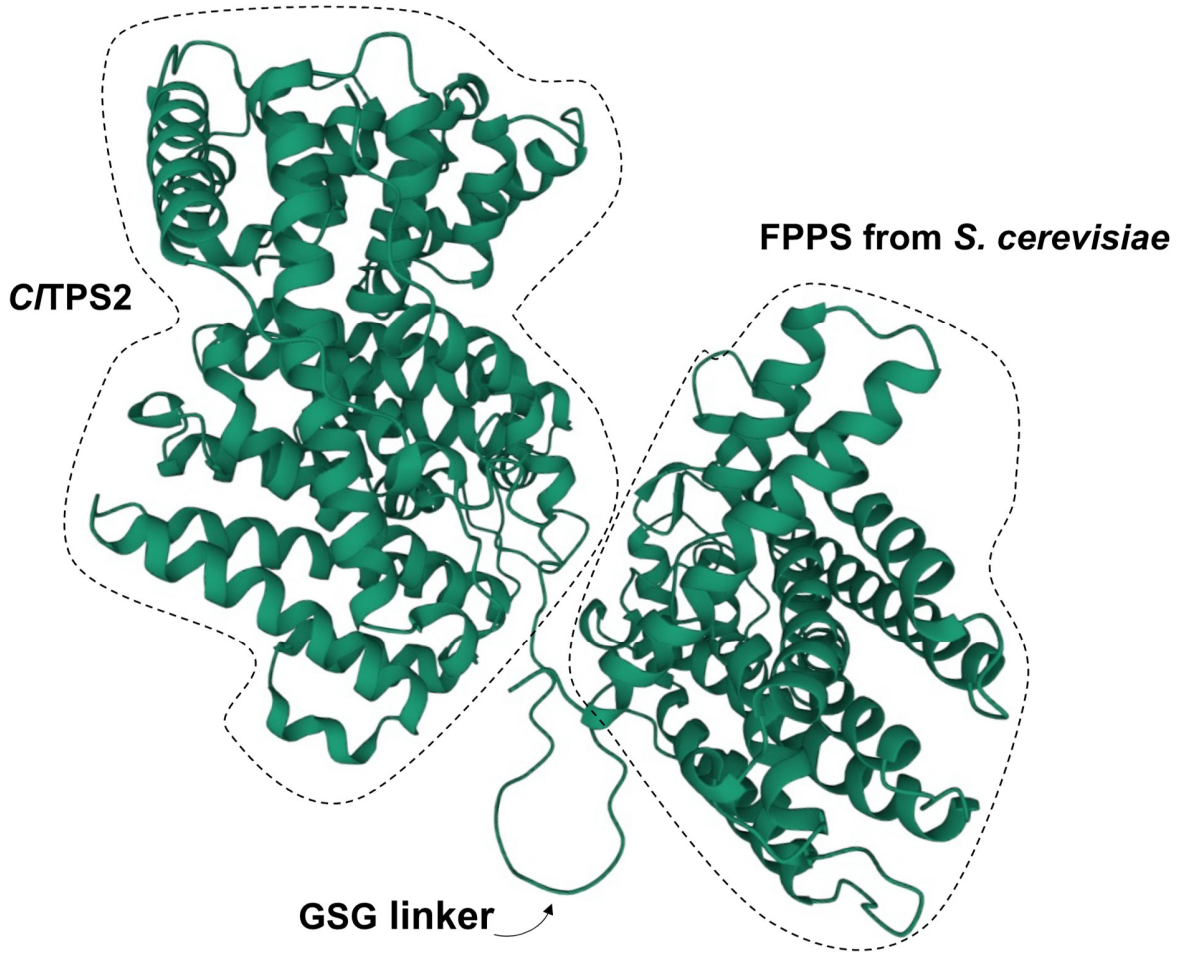

Figure S12. AlphaFold-predicted structure of the FPPS-GSG-*Cl*TPS2 enzyme fusion.

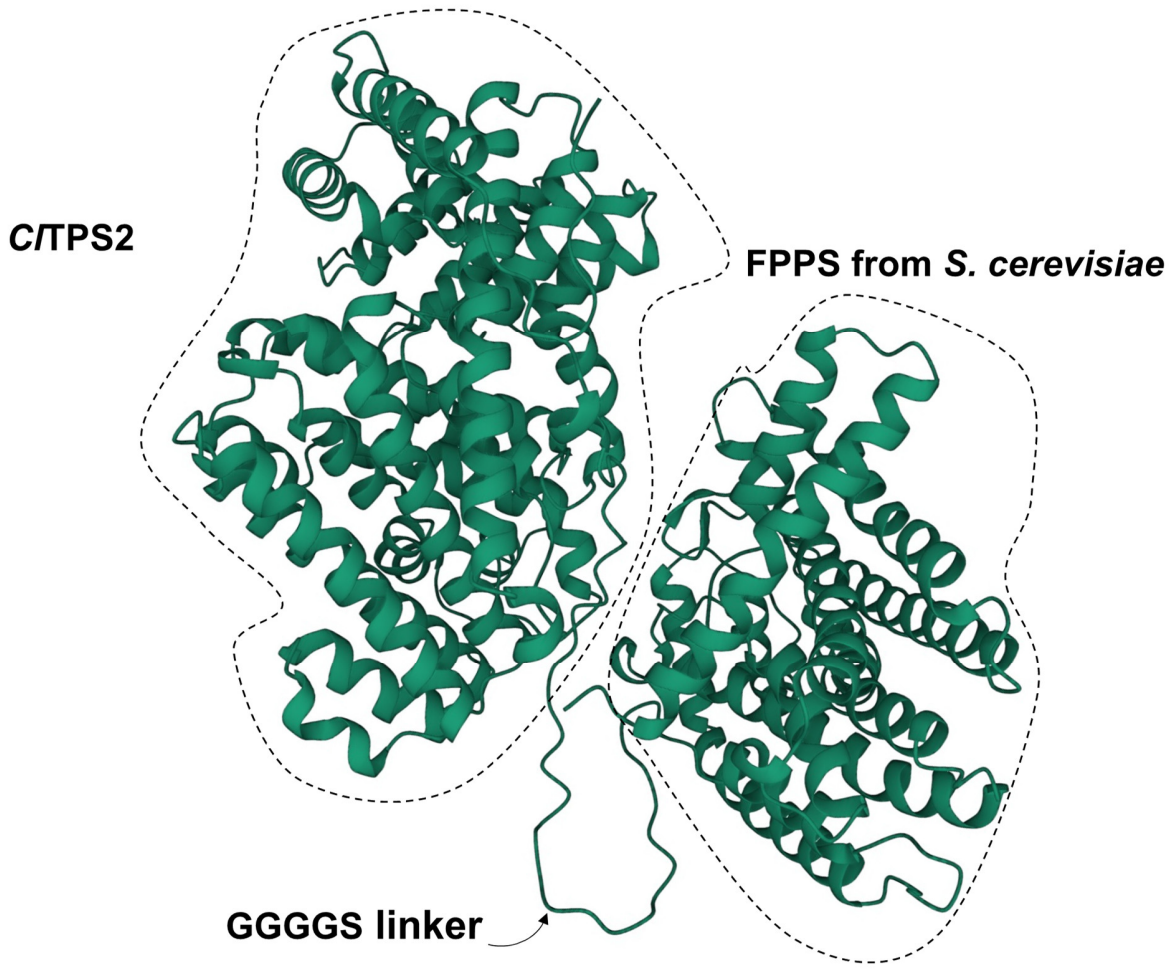

Figure S13. AlphaFold-predicted structure of the FPPS-GGGGS-*Cl*TPS2 enzyme fusion.

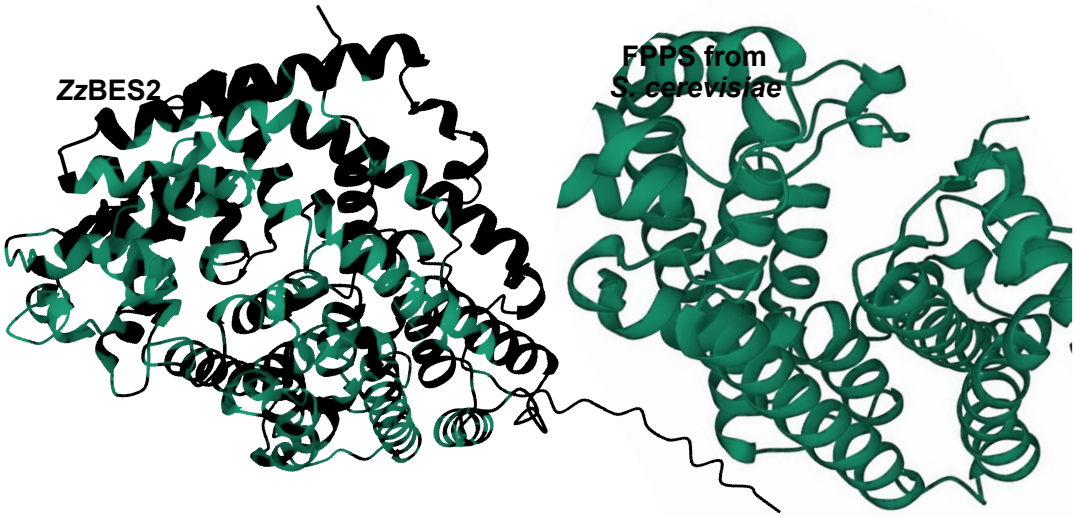

Figure S14. AlphaFold-predicted structures of the *Zz*BES2 and FPPS as standalone enzymes.

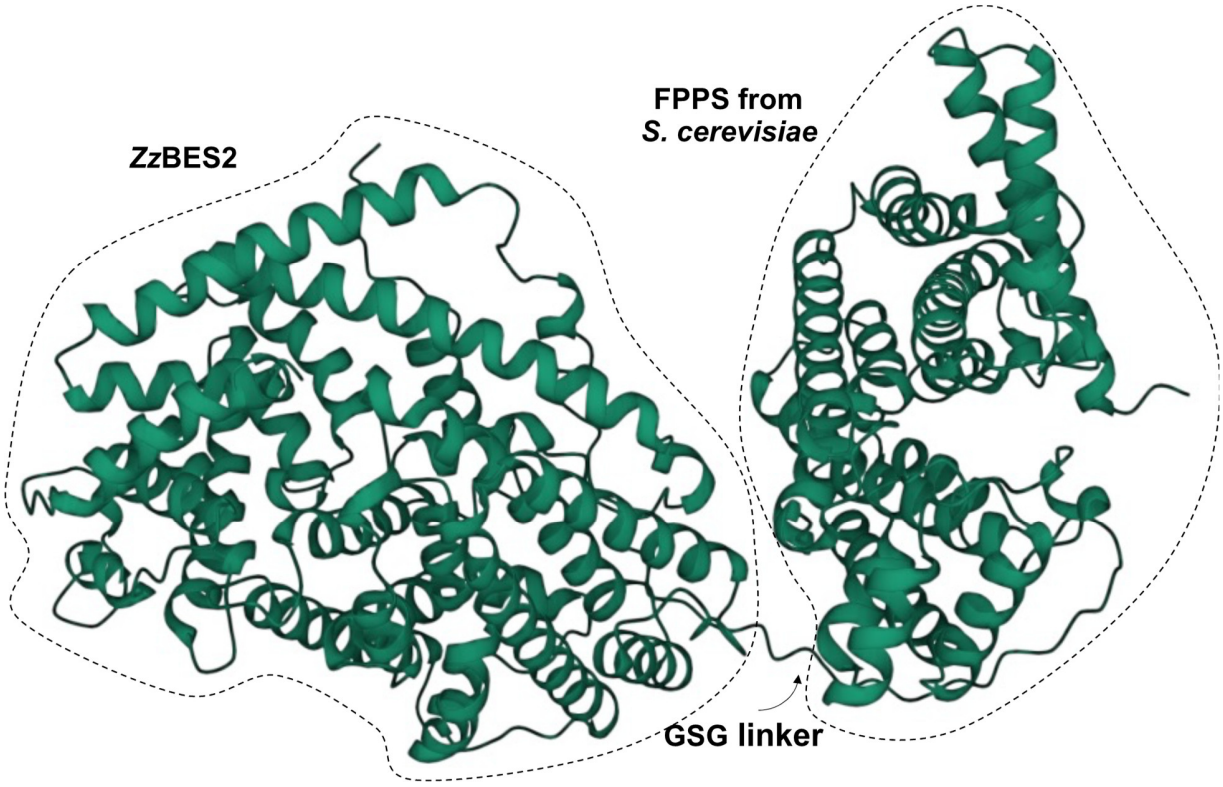

Figure S15. AlphaFold-predicted structure of the FPPS-GSG-ZzBES2 enzyme fusion.

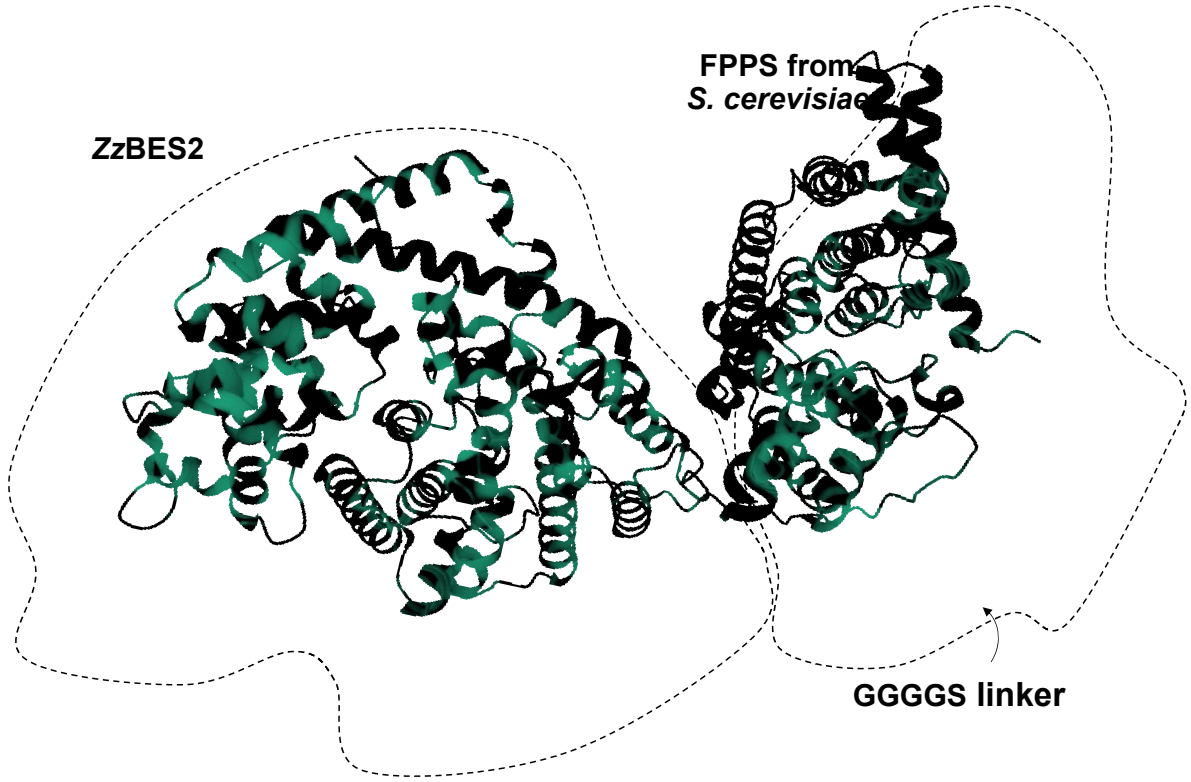

Figure S16. AlphaFold-predicted structure of the FPPS-GGGGS-*Zz*BES2 enzyme fusion.