Supplementary Information

Efficient secretory expression of leghemoglobin in *Saccharomyces cerevisiae*

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Supplementary Figures 1-3

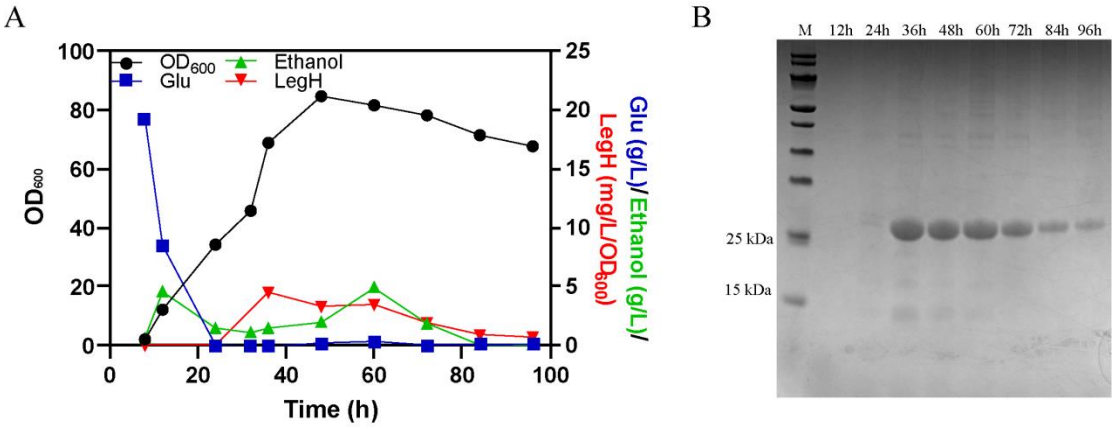

Figure S1 The DO-STAT strategy used for fed-batch fermentation at 5-L fermenter level (S4 strain)

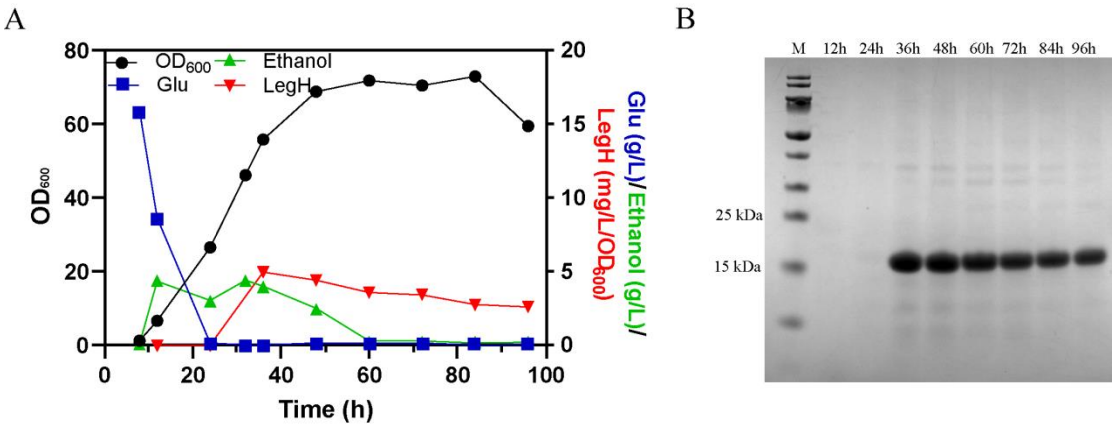

Figure S2 The pH-STAT strategy used for fed-batch fermentation at 5-L fermenter level (S4 strain)

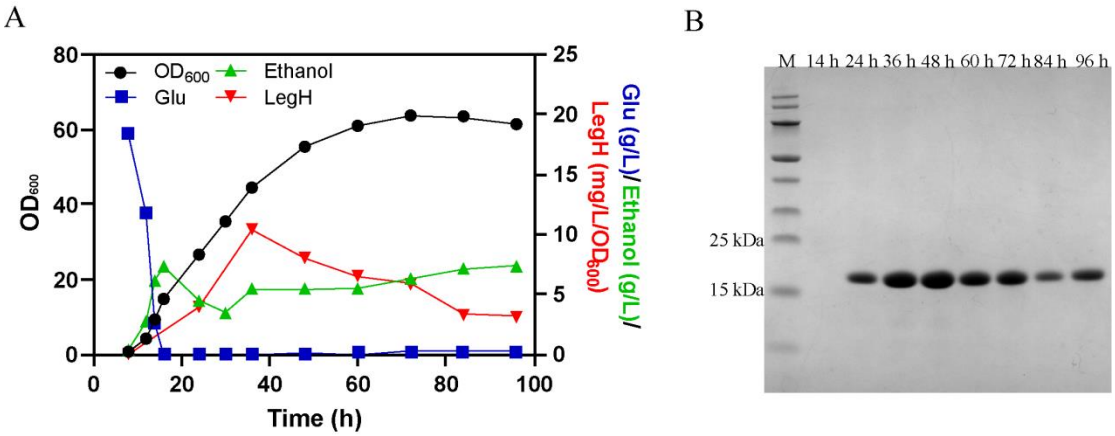

Figure S3 The exponential feeding strategy used for fed-batch fermentation at 5-L

fermenter level (S4 strain)

Supplementary Tables 1-4

Table S1 Strains and plasmids used in this study

Strains and plasmids	Description	Source
Strains		
DH5α	*E. coli* str. K-12 F$^-$ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *hsdR17*($_{rK^-mK^+}$) λ$^-$	Lab stock
CEN.PK2-1C	MATα, ura3-52, trp1-289, leu2-3,112, his3Δ1, MAL2-8C, SUC2	Lab stock
GAL1-LegH (S4)	CEN.PK2-1C harboring plasmid pESC-P_{GAL1}-LegH	Lab stock
CRM_{GAL1}-$CORE_{TEF1}$-LegH	CEN.PK2-1C harboring plasmid pESC-CRM_{GAL1}-$CORE_{TEF1}$-LegH	This study
CRM_{GAL1}-$CORE_{TDH3}$-LegH	CEN.PK2-1C harboring plasmid pESC-CRM_{GAL1}-$CORE_{TDH3}$-LegH	This study
CRM_{GAL1}-$CORE_{GAL7}$-LegH	CEN.PK2-1C harboring plasmid pESC-CRM_{GAL1}-$CORE_{GAL7}$-LegH	This study
GAL1-UAS×2/×3/×4-LegH	GAL1 harboring with pESC-$P_{GAL1\text{-}UAS\times2/\times3/\times4}$-LegH	This study
Δ*GAL80*	CEN.PK2-1C derived strain, Δ*GAL80*	This study
Δ*GAL80*-Δ*MCA1*	CEN.PK2-1C derived strain, Δ*GAL80* Δ*MCA1*	This study
Δ*GAL80*-Δ*PEP4*	CEN.PK2-1C derived strain, Δ*GAL80* Δ*PEP4*	This study
Δ*GAL80*-Δ*VPS10*	CEN.PK2-1C derived strain, Δ*GAL80* Δ*VPS10*	This study
Δ*GAL80*-Δ*PEP4*-Δ*VPS10*	CEN.PK2-1C derived strain, Δ*GAL80* Δ*PEP4*Δ*VPS10*	This study

Strains and plasmids	Description	Source
GAL1-LegH-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-LegH	This study
MFα-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα-LegH	This study
α(Δ27-29)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ27-29}$-LegH	This study
α(Δ30-43)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ30-43}$-LegH	This study
α(Δ44-70)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ44-70}$-LegH	This study
α(Δ55-70)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ55-70}$-LegH	This study
α(Δ57-60)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ57-60}$-LegH	This study
α(Δ57-70)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ57-70}$-LegH	This study
α(Δ79-83)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{Δ79-83}$-LegH	This study
α(1×57-60)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα$_{(57-60)×1}$-LegH	This study
Ost1(Δ27-29)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ27-29}$-LegH	This study
Ost1(Δ30-43)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ30-43}$-LegH	This study
Ost1(Δ44-70)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ44-70}$-LegH	This study
Ost1(Δ55-70)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ55-70}$-LegH	This study

Strains and plasmids	Description	Source
Ost1(Δ57-60)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ57-60}$-LegH	This study
Ost1(Δ57-70)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ57-70}$-LegH	This study
Ost1(Δ79-83)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{Δ79-83}$-LegH	This study
Ost1(1×57-60)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-OFα$_{(57-60)×1}$-LegH	This study
MFα^{V38A}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{V38A}-LegH	This study
MFα^{G40D}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{G40D}-LegH	This study
MFα^{V50A}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{V50A}-LegH	This study
MFα^{L63S}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{L63S}-LegH	This study
MFα^{L64S}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{L64S}-LegH	This study
MFα^{F65S}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{F65S}-LegH	This study
MFα^{I66T}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MFα^{I66T}-LegH	This study
PIR3-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-PIR3-LegH	This study
NCW2-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-NCW2-LegH	This study
PSG1-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-PSG1-LegH	This study

Strains and plasmids	Description	Source
MID2-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-MID2-LegH	This study
GAS5-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-GAS5-LegH	This study
FET3-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-FET3-LegH	This study
CSI2-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-CSI2-LegH	This study
FLO10-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-FLO10-LegH	This study
PHO5-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-PHO5-LegH	This study
PIR1-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-PIR1-LegH	This study
SWP1-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-SWP1-LegH	This study
FLO10α^{L63S}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-FFα^{L63S}-LegH	This study
FLO10(Δ30-43)-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-FF$\alpha_{\Delta 30\text{-}43}$-LegH	This study
FLO10(Δ30-43)α^{L63S}-Δ*GAL80*	Δ*GAL80* harboring plasmid pESC-P_{GAL1}-FF$\alpha_{\Delta 30\text{-}43}{}^{L63S}$-LegH	This study
α-Δ*GAL80*-Δ*PEP4*	Δ*GAL80*-Δ*PEP4* harboring plasmid pESC-P_{GAL1}-MFα-LegH	This study
α-Δ*GAL80*-Δ*VPS10*	Δ*GAL80*-Δ*VPS10* harboring plasmid pESC-P_{GAL1}-MFα-LegH	This study
α-Δ*GAL80*-Δ*MCA1*	Δ*GAL80*-Δ*MCA1* harboring plasmid pESC-P_{GAL1}-MFα-LegH	This study

Strains and plasmids	Description	Source
α-Δ*GAL80*-Δ*PEP4*-Δ*VPS10*	Δ*GAL80*-Δ*PEP4*-Δ*VPS10* harboring plasmid pESC-P_{GAL1}-MFα-LegH	This study
FLO10α^{L63S}-Δ*GAL80*-Δ*PEP4*	Δ*GAL80*-Δ*PEP4* harboring plasmid pESC-P_{GAL1}-FFα^{L63S}-LegH	This study
Plasmids		
pESC	Empty plasmid with bidirectional gene expression cassettes (P_{GAL1}-T_{CYC1} and P_{GAL10}-T_{ADH1}, 2u)	Lab stock
pESC-P_{GAL1}-LegH	pESC inserted with soy leghemoglobin under P_{GAL1}	Lab stock
pESC-CRM_{GAL1}-$CORE_{TEF1}$-LegH	pESC inserted with soy leghemoglobin under CRM_{GAL1}-P_{TEF1}	This study
pESC-CRM_{GAL1}-$CORE_{TDH3}$-LegH	pESC inserted with soy leghemoglobin under CRM_{GAL1}-P_{TDH3}	This study
pESC-CRM_{GAL1}-$CORE_{GAL7}$-LegH	pESC inserted with soy leghemoglobin under CRM_{GAL1}-P_{GAL7}	This study
pESC-$P_{GAL1\text{-}UAS\times2}$-LegH	pESC-P_{GAL1}-LegH with two UAS_{GAL1}	This study
pESC-$P_{GAL1\text{-}UAS\times3}$-LegH	pESC-P_{GAL1}-LegH with three UAS_{GAL1}	This study
pESC-$P_{GAL1\text{-}UAS\times4}$-LegH	pESC-P_{GAL1}-LegH with four UAS_{GAL1}	This study
pESC-P_{GAL1}-MFα-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα leader	Lab stock
pESC-P_{GAL1}-MF$\alpha_{\Delta27\text{-}29}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MF$\alpha_{\Delta27\text{-}29}$ mutant leader	This study
pESC-P_{GAL1}-MF$\alpha_{\Delta30\text{-}43}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MF$\alpha_{\Delta30\text{-}43}$ mutant leader	This study
pESC-P_{GAL1}-MF$\alpha_{\Delta43\text{-}70}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MF$\alpha_{\Delta43\text{-}70}$ mutant leader	This study

Strains and plasmids	Description	Source
pESC-P_{GAL1}-MFα$_{\Delta 55\text{-}70}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα$_{\Delta 55\text{-}70}$ mutant leader	This study
pESC-P_{GAL1}-MFα$_{\Delta 57\text{-}70}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα$_{\Delta 57\text{-}70}$ mutant leader	This study
pESC-P_{GAL1}-MFα$_{\Delta 57\text{-}60}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα$_{\Delta 57\text{-}60}$ mutant leader	This study
pESC-P_{GAL1}-MFα$_{\Delta 79\text{-}83}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα$_{\Delta 79\text{-}83}$ mutant leader	This study
pESC-P_{GAL1}-MFα$_{(57\text{-}60)\times 1}$-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα$_{(57\text{-}60)\times 1}$ mutant leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 27\text{-}29}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 27\text{-}29}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 30\text{-}43}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 30\text{-}43}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 43\text{-}70}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 43\text{-}70}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 55\text{-}70}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 55\text{-}70}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 57\text{-}70}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 57\text{-}70}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 57\text{-}60}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 57\text{-}60}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{\Delta 79\text{-}83}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{\Delta 79\text{-}83}$ hybrid leader	This study
pESC-P_{GAL1}-OFα$_{(57\text{-}60)\times 1}$-LegH	pESC-P_{GAL1}-LegH with Ost1-pro-MFα$_{(57\text{-}60)\times 1}$ hybrid leader	This study
pESC-P_{GAL1}-MFα^{V38A}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{V38A} mutant leader	This study

Strains and plasmids	Description	Source
pESC-P_{GAL1}-MFα^{G40D}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{G40D} mutant leader	This study
pESC-P_{GAL1}-MFα^{V50A}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{V50A} mutant leader	This study
pESC-P_{GAL1}-MFα^{L63S}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{L63S} mutant leader	This study
pESC-P_{GAL1}-MFα^{L64S}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{L64S} mutant leader	This study
pESC-P_{GAL1}-MFα^{F65S}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{F65S} mutant leader	This study
pESC-P_{GAL1}-MFα^{I66T}-LegH	pESC-P_{GAL1}-LegH with pre-pro-MFα^{I66T} mutant leader	This study
pESC-P_{GAL1}-CSI2-LegH	pESC-P_{GAL1}-LegH with CSI2-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-FET3-LegH	pESC-P_{GAL1}-LegH with FET3-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-FLO10-LegH	pESC-P_{GAL1}-LegH with FLO10-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-GAS5-LegH	pESC-P_{GAL1}-LegH with GAS5-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-MID2-LegH	pESC-P_{GAL1}-LegH with MID2-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-NCW2-LegH	pESC-P_{GAL1}-LegH with NCW2-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-PHO5-LegH	pESC-P_{GAL1}-LegH with PHO5-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-PIR1-LegH	pESC-P_{GAL1}-LegH with PIR1-pro-MFα hybrid leader	This study

Strains and plasmids	Description	Source
pESC-P_{GAL1}-PIR3-LegH	pESC-P_{GAL1}-LegH with PIR3-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-PSG1-LegH	pESC-P_{GAL1}-LegH with PSG1-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-SWP1-LegH	pESC-P_{GAL1}-LegH with SWP1-pro-MFα hybrid leader	This study
pESC-P_{GAL1}-FFα^{L63S}-LegH	pESC-P_{GAL1}-LegH with FLO10-pro-MFα^{L63S} hybrid leader	This study
pESC-P_{GAL1}-FFα$_{\Delta30\text{-}43}$-LegH	pESC-P_{GAL1}-LegH with FLO10-pro-MFα$_{\Delta30\text{-}43}$ hybrid leader	This study
pESC-P_{GAL1}-FFα$_{\Delta30\text{-}43}{}^{L63S}$-LegH	pESC-P_{GAL1}-LegH with FLO10-pro-MFα$_{\Delta30\text{-}43}{}^{L63S}$ hybrid leader	This study

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35 **Table S2 Primers used in this study**

Primer	Sequence (5'-3')
CRM-TEF1-F	AAGCATAGCAATCTAATCTAAGTTTGGATCCATGGGTGCTTTTACTGAAAAAC
CRM-TEF1-R	CGAAGAAAAAGAATAGATCAAAAATCATCGCTTCGCT
URM-TEF1-F	GATTTTTGATCTATTCTTTTTCTTCGTCGAAAAAGGCAAT
URM-TEF1-R	TAAAAGCACCCATGGATCCAAACTTAGATTAG
CRM-TDH3-F	AACACACATAAACAAACAAAGGATCCATGGGTGCTTTTACTGAAAAAC
CRM-TDH3-R	ATAGAAGGTGTAATAGATCAAAAATCATCGCTTCGCT
URM-TDH3-F	GATTTTTGATCTATTACACCTTCTATTACCTTCTGCT
URM-TDH3-R	TTCAGTAAAAGCACCCATGGATCCTTTGTTTGTTTATGT
CRM-GAL7-F	CAGTTGAATATTCCCTCAAAAGGATCCATGGGTGCTTTTACTGAAAAACAAG
CRM-GAL7-R	CGACCTGCTTTTATATAGATCAAAAATCATCGCTTCGCT
URM-GAL7-F	GATCTATATAAAAGCAGGTCGGAAAT
URM-GAL7-R	CACCCATGGATCCTTTTGAGGGAATATTC
UAS-1-F	CCGCCCTTTAGTGAGGGTTGAATTCAGTACGGATTAGAAGCCGCCGAGCGGGTGACAG
UAS-1-R	CTCGGCGGCTTCTAATCCGCTCGGAGCAGTGCGGCGCGAGGCACATCTGCGTTTC
GAL10-F	GCTTGTTCGATAGAAGACAGTAGCTTCATTTTCAAAAATTCTTAC
GAL10-R	GCGGCTTCTAATCCGTCAATATAGCAATGAGCAGTTAAGCGTATTACT
GAL4p-F	AGTGAGGGTTGAATTCAGTATTACTCTTTTTTTGGGTTTGGT
GAL4p-GAL10-R	GTAAGAATTTTTGAAAATGAAGCTACTGTCTTCTATCGAACAAGCATGCG

Primer	Sequence (5’-3’)
GAL10-RPCR-F	GCTTAACTGCTCATTGCTATATTGACGGATTAGAAGCCGCCG
GAL4-RPCR-R	CCAAAAAAAGAGTAATACTGAATTCAACCCTCACTAAAGGGCGGCCGCACT
TEF1-F	CGATAGAAGACAGTAGCTTCATTAGTTCTAGAAAACTTAGATTAGATTGC
TEF1-R	GGCGGCTTCTAATCCGATAGCTTCAAAATGTTTCTACTCCTTTTTTACTC
GAL4p-F	AGTGAGGGTTGAATTCAGTATTACTCTTTTTTTGGGTTTGGT
GAL4p-TEF1-R	CTAAGTTTTCTAGAACTAATGAAGCTACTGTCTTCTATCG
TEF1-RPCR-F	GGAGTAGAAACATTTTGAAGCTATCGGATTAGAAGCCGCCGAGC
GAL4-RPCR-R	CCAAAAAAAGAGTAATACTGAATTCAACCCTCACTAAAGGGCGGCCGCACT
2729-F	TTGTGCCGTTGTTGTAGTGTTGACTGGAGC
2729-R	ACTACAACAACGGCACAAATTCCGGCTGAAGCT
3043-F	CCCCTTCTAATTCATCTTCTGTTGTAGTGTTGACT
3043-R	AGAAGATGAATTAGAAGGGGATTTCGATGTTGCT
4370-F	AATGCTGGCATCTAAGTAACCGATGACAGCTTCAG
4370-R	TACTTAGATGCCAGCATTGCTGCTAAAG
5570-F	AATGCTGGCTGGCAAAACAGCAACATCGAAAT
5570-R	GTTTTGCCAGCCAGCATTGCTGCTAAAG
5770-F	AATGCTGGCGGAAAATGGCAAAACAGCAACAT
5770-R	CCATTTTCCGCCAGCATTGCTGCTAAAG
5760-F	ATAACCCGTTGGAAAATGGCAAAACAGCAACAT
5760-R	CATTTTCCAACGGGTTATTGTTTATAAATACTACT

Primer	Sequence (5’-3’)
7983-F	GCCTCTCTTTTTTCTTCTTTAGCAGCAATGCT
7983-R	CTAAAGAAGAAAAAAGAGAGGCTGAAGCTCAATTAAC
(57-60) ×1-F	AATAACCCGTTATTTGTGCTGTTATTTGTGCTGTTGGAAAA TGGCAAAACAGCAACAT
(57-60) ×1-R	AGCACAAATAACGGGTTATTATTGTTTAT
Ost1-F-2	TGTTTTTTCAACGTGTCTTCTGCTGCTCCAGTCAACACTA CAACAGAAG
Ost1-R-1	GTTGTAGTGTTGACTGGAGCAGCAGAAGACACGTTGAAA AAAC
1-F	CCCGAAAAGTGCCACCTGAACGAAGCATCTGTGCTTCATT TTGT
2-R	TTCGTTCAGGTGGCACTTTTCGGGGAAATGT
α-V38A-F	TTCCGGCTGAAGCTGCTATCGGTTAC
α-V38A-R	GTAACCGATAGCAGCTTCAGCCGGAATTTGTGCC
α-G40D-F	GAAGCTGTCATCGATTACTCAGATTTAG
α-G40D-R	CTAAATCTGAGTAATCGATGACAGCTTCAGCCGGAATTTG TG
α-V50A-F	GATTTCGATGCTGCTGTTTTGCCATTTTC
α-V50A-R	GAAAATGGCAAAACAGCAGCATCGAAATCCCCTTCTAAAT C
α-L63S-F	CACAAATAACGGGTCTTTGTTTATAAATAC
α-L63S-R	GTATTTATAAACAAAGACCCGTTATTTGTGCTGTTGGAAAA TG
α-L64S-F	CAGCACAAATAACGGGTTATCTTTTATAAATAC
α-L64S-R	GTATTTATAAAAGATAACCCGTTATTTGTGCTGTTGGAAAA TG
α-F65S-F	CACAAATAACGGGTTATTGTCTATAAATACTAC
α-F65S-R	GTAGTATTTATAGACAATAACCCGTTATTTGTGCTGTTGG

Primer	Sequence (5’-3’)
α-I66T-F	GTTATTGTTTACTAATACTACTATTGCCAGC
α-I66T-R	GCTGGCAATAGTAGTATTAGTAAACAATAACCCGTTATTTG TGCTG
PIR3-F	ATTAGTCGTCTCCGCTTTAGCTGCTACATCTTTAGCTGCTC CAGTCAACACTACAACAG
PIR3-R	TAAAGCGGAGACGACTAATGGCTTTTTATATTGCATGGATC CGTTTTTTCTCCTTGACG
NCW2-F	TATTATTTACCACCTTAATTACTCTAGCCGCTGCTGCTCCA GTCAACACTACAACAG
NCW2-R	TTAAGGTGGTAAATAATATGGAACAGGCCTTCATGGATCC GTTTTTTCTCCTTGACG
PSG1-F	CTTTTCTTTGGCATCGCTTTATCAACATGTTCATGGTGCTC CAGTCAACACTACAACAG
PSG1-R	AGCGATGCCAAAGAAAAGAAGATAAGTATACTATCGTGAA ATCTCATGGATCCGTTTTTTCTCCTTGACG
MID2-F	TTACTTTTAATACTGTCATGCATATCGACGATACGCGCAGC TCCAGTCAACACTACAACAG
MID2-R	GCATGACAGTATTAAAAGTAATAAGCGGAAACTATTCTTG GTTGTGAAAGACAACATGGATCCGTTTTTTCTCCTTGACG
GAS5-F	ACAAGTGCCTTCGTTTTAAGTGCTGGTTTGGCTCAGGCTG CTCCAGTCAACACTACAACAG
GAS5-R	TAAAACGAAGGCACTTGTAAGAGAACGTAGTAACATGGA TCCGTTTTTTCTCCTTGACG
FET3-F	AGCCGTTTTGCTTTTCTCGATGCTCTCGCTAGCACAAGCG GCTCCAGTCAACACTACAACAG
FET3-R	GAAAAGCAAAACGGCTATAGAGAGCAAAGCGTTAGTCAT GGATCCGTTTTTTCTCCTTGACG
CSI2-F	GATACTACTACTTCATTTGTTTGCTCTACAAGAATTTCAAC

Primer	Sequence (5’-3’)
CSI2-R	TTGTCTCCGCTGCTCCAGTCAACACTACAACAG CAAATGAAGTAGTAGTATCACTTTCCAAATGGAGATTTCT GGCAGTCTCATGGATCCGTTTTTTCTCCTTGACG
FLO10-F	TTTGACCGGCCTATTTTTGCTATCTGTAGCTAATGTTGCTCT AGGTGCTCCAGTCAACACTACAACAG
FLO10-R	CAAAAATAGGCCGGTCAAAAATATATATCGAGCAGCCACA GGCATGGATCCGTTTTTTCTCCTTGACG
PHO5-F	TGTTTATTCAATTTTAGCCGCTTCTTTGGCCAATGCAGCTC CAGTCAACACTACAACAG
PHO5-R	AGCGGCTAAAATTGAATAAACAACAGATTTAAACATGGAT CCGTTTTTTCTCCTTGACG
PIR1-F	ATTAGTTGCCTCCGCCTTAGTTGCTACATCTTTAGCTGCTC CAGTCAACACTACAACAG
PIR1-R	AAGGCGGAGGCAACTAATGATTTTTTGTATTGCATGGATC CGTTTTTTCTCCTTGACG
SWP1-F	TTCAAAACACTTGCGGCCTTGGTGTCGTGCATATCGTTCG TCCTCGCTGCTCCAGTCAACACTACAACAGAAGAT
SWP1-R	ATGCACGACACCAAGGCCGCAAGTGTTTTGAAGAATTGC ATGGATCCGTTTTTTCTCCTTGACGTTAAAGTAT

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37 **Table S3 Elements of the promoters used in this study**

Elements of the promoters	Sequence (5'-3')
CRM_{GAL1}	AGTACGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCG AAGGAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTC GCGTTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTC CGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGA AGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCA AATGAACGAATCAAATTAACAACCATAGGATGATAATGCGA TTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAG CGATGATTTTTGATCTA
$CORE_{TEF1}$	TTCTTTTTCTTCGTCGAAAAAGGCAATAAAAATTTTTATCA CGTTTCTTTTTCTTGAAAATTTTTTTTTGATTTTTTTCTCTT TCGATGACCTCCCATTGATATTTAAGTTAATAAACGGTCTTC AATTTCTCAAGTTTCAGTTTCATTTTTCTTGTTCTATTACAA CTTTTTTTACTTCTTGCTCATTAGAAAGAAAGCATAGCAAT CTAATCTAAGTTT
$CORE_{TDH3}$	TTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAA GCTGAAAAAAAAGGTTGAAACCAGTTCCCTGAAATTATTC CCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGAT TGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAATTCTACT TTTATAGTTAGTCTTTTTTTTAGTTTTAAAACACCAAGAACT TAGTTTCGAATAAACACACATAAACAAACAAA
$CORE_{GAL7}$	TATAAAAGCAGGTCGGAAATATTTATGGGCATTATTATGCAG AGCATCAACATGATAAAAAAAAAACAGTTGAATATTCCCTC AAAA
UAS_{GAL1}	CGGATTAGAAGCCGCCGAGCGGGTGACAGCCCTCCGAAG GAAGACTCTCCTCCGTGCGTCCTCGTCTTCACCGGTCGCG TTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCG

38 **Table S4 Hybrid signal peptides used in this study**

Signal peptides	Sequence (5'-3')
MFα	ATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGC AGCATCCTCCGCATTAGCTGCTCCAGTCAACACTACA ACAGAAGATGAAACGGCACAAATTCCGGCTGAAGC TGTCATCGGTTACTCAGATTTAGAAGGGGATTTCGAT GTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACG GGTTATTGTTTATAAATACTACTATTGCCAGCATTGCT GCTAAAGAAGAAGGGGTATCTCTCGAGAAAAGAGA GGCTGAAGCT
pro-MFα	GCTCCAGTCAACACTACAACAGAAGATGAAACGGC ACAAATTCCGGCTGAAGCTGTCATCGGTTACTCAGAT TTAGAAGGGGATTTCGATGTTGCTGTTTTGCCATTTT CCAACAGCACAAATAACGGGTTATTGTTTATAAATAC TACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGT ATCTCTCGAGAAAAGAGAGGCTGAAGCT
pre-Ost1	AGGCAGGTTTGGTTCTCTTGGATTGTGGGATTGTTCC TATGTTTTTTCAACGTGTCTTCTGCT
pre-PIR3	ATGCAATATAAAAAGCCATTAGTCGTCTCCGCTTTAG CTGCTACATCTTTAGCT
pre-NCW2	ATGAAGGCCTGTTCCATATTATTTACCACCTTAATTAC TCTAGCCGCTGCT
pre-PSG1	ATGAGATTTCACGATAGTATACTTATCTTCTTTTCTTT GGCATCGCTTTATCAACATGTTCATGGT
pre-MID2	ATGTTGTCTTTCACAACCAAGAATAGTTTCCGCTTAT TACTTTTAATACTGTCATGCATATCGACGATACGCGCA
pre-GAS5	ATGTTACTACGTTCTCTTACAAGTGCCTTCGTTTTAA GTGCTGGTTTGGCTCAGGCT
pre-FET3	ATGACTAACGCTTTGCTCTCTATAGCCGTTTTGCTTTT

Signal peptides	Sequence (5'-3')
pre-CSI2	CTCGATGCTCTCGCTAGCACAAGCG ATGAGACTGCCAGAAATCTCCATTTGGAAAGTGATA CTACTACTTCATTTGTTTGCTCTACAAGAATTTCAACT TGTCTCCGCT
pre-FLO10	ATGCCTGTGGCTGCTCGATATATATTTTTGACCGGCCT ATTTTTGCTATCTGTAGCTAATGTTGCTCTAGGT
pre-PHO5	ATGTTTAAATCTGTTGTTTATTCAATTTTAGCCGCTTC TTTGGCCAATGCA
pre-PIR1	ATGCAATACAAAAAATCATTAGTTGCCTCCGCCTTAG TTGCTACATCTTTAGCT
pre-SWP1	ATGCAATTCTTCAAAACACTTGCGGCCTTGGTGTCGT GCATATCGTTCGTCCTCGCT

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