Supplementary Figures

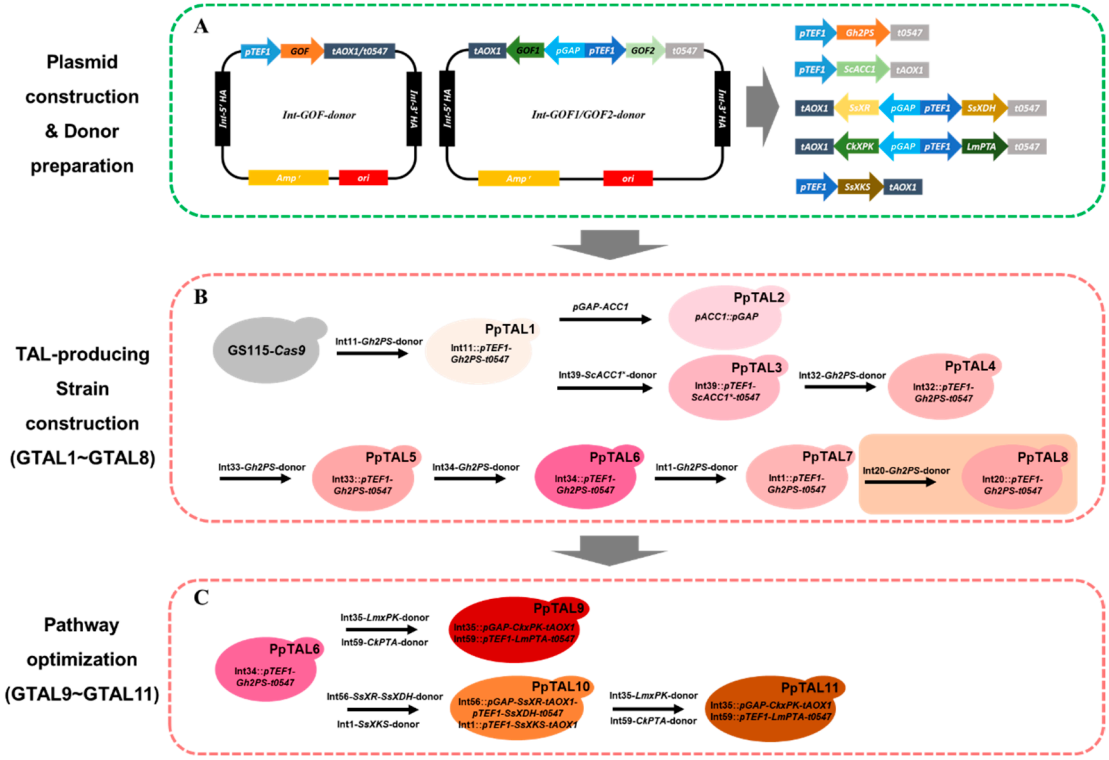

Figure S1. Schematic diagram of plasmid and strain construction in this study. (A) Schematic diagram of gene integration expression cassette and plasmid construction. (B) Schematic diagram of TAL-producing strains construction. (C) Schematic diagram of the pathway optimization strain construction for enhanced acetyl-CoA synthesis and/or xylose utilization.

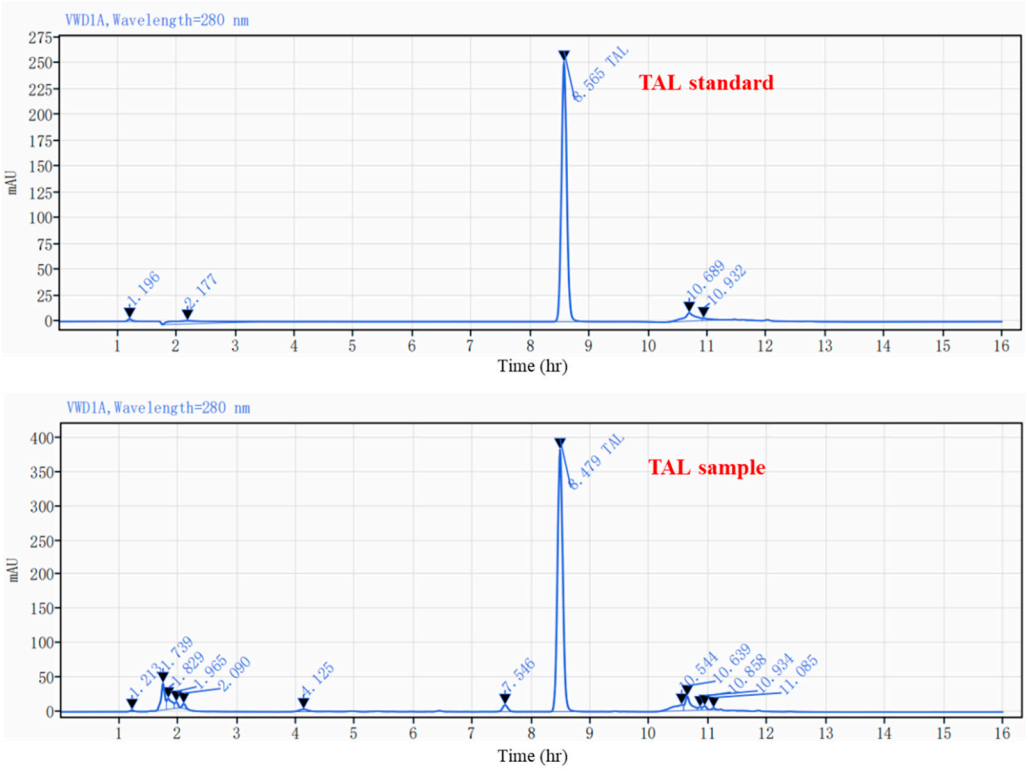

Figure S2. HPLC analysis for the identification and quantification of TAL. The peak corresponding to TAL was observed at ~8.50 min.

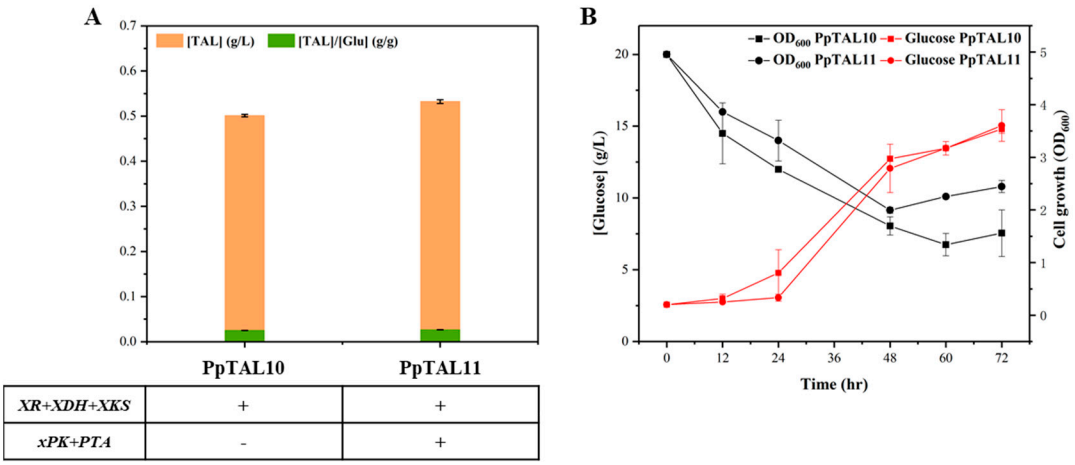

Figure S3. TAL production from glucose. (A) Comparison of TAL titer of the xylose utilization strain with or without the PK pathway in SCD medium. (B) Glucose consumption and cell growth profiles of PpTAL10 and PpTAL11 in SCD medium. The data represent three biological replicates and the error bars represent standard deviations.

Supplementary Tables

Table S1. List of Plasmids Used in This Study.

Name	Genotype	Description
HZP-gRNA-Int1	PARS1, zeocin, *pSER*, Int1	The sgRNA helper plasmids
HZP-gRNA-Int11	PARS1, zeocin, *pSER*, Int11	
HZP-gRNA-Int20	PARS1, zeocin, *pSER*, Int20	
HZP-gRNA-Int32	PARS1, zeocin, *pSER*, Int32	
HZP-gRNA-Int33	PARS1, zeocin, *pSER*, Int33	
HZP-gRNA-Int34	PARS1, zeocin, *pSER*, Int34	
HZP-gRNA-Int35	PARS1, hygromycin B, *pSER*, Int35	
HZP-gRNA-Int39	PARS1, zeocin, *pSER*, Int39	
HZP-gRNA-Int56	PARS1, zeocin, *pSER*, Int56	
HZP-gRNA-Int59	PARS1, zeocin, *pSER*, Int59	
HZP-gRNA-Det*PFK1*	PARS1, zeocin, *pSER*, Det*PFK1*	
HZP-gRNA-Det*PYK1*	PARS1, zeocin, *pSER*, Det*PYK1*	
Int1-donor	Amp, Int1-*pTEF1-Hind*III-*Nde* I -*t0547*	The integration donor helper plasmids containing an upstream and a down-stream homologous arm of ~500 bp as well as the restriction sites for genes or genes cassettes
Int11-donor	Amp, Int11-*pTEF1-Hind*III-*Nde* I -*t0547*	
Int20-donor	Amp, Int20-*pTEF1-Hind*III-*Nde* I -*t0547*	
Int32-donor	Amp, Int32-*pTEF1-Hind*III-*Nde* I -*t0547*	
Int33-donor	Amp, Int33-*pTEF1-Hind*III-*Nde* I -*t0547*	
Int34-donor	Amp, Int34-*pTEF1-Hind*III-*Nde* I -*t0547*	
Int35-donor	Amp, Int35-*pGAP-Ava* I -*Bgl* II -*tAOX1*	
Int39-donor	Amp, Int39-*pTEF1-Hind*III-*Nde* I -*tAOX1*	

Int56-donor	Amp, Int56-*pTEF1-Hind*Ⅲ*-Nde* Ⅰ*-t0547-pGAP-Ava* Ⅰ*-Bgl* Ⅱ*-tAOX1*	
Int1-donor	Amp, Int1-*pTEF1-Hind*Ⅲ*-Nde* Ⅰ*-tAOX1*	
Int59-donor	Amp, Int59-*Not* Ⅰ*-Nde* Ⅰ	
Det*PFK1*-donor	Amp, Det*PFK1*-HA	The deletion donor helper plasmids containing an upstream and a downstream homologous arm of ~500 bp
Det*PYK1*-donor	Amp, Det*PYK1*-HA	
Int1-*Gh2PS*-donor	Amp, Int1-*pTEF1-Gh2PS-t0547*	Genes cloned into the integration donor helper plasmids for site-specific integration
Int11-*Gh2PS*-donor	Amp, Int11-*pTEF1-Gh2PS-t0547*	
Int20-*Gh2PS*-donor	Amp, Int20-*pTEF1-Gh2PS-t0547*	
Int32-*Gh2PS*-donor	Amp, Int32-*pTEF1-Gh2PS-t0547*	
Int33-*Gh2PS*-donor	Amp, Int33-*pTEF1-Gh2PS-t0547*	
Int34-*Gh2PS*-donor	Amp, Int34-*pTEF1-Gh2PS-t0547*	
Int35-*CkxPK*-donor	Amp, Int35-*pGAP-CkxPK-tAOX1*	
Int39-*ScACC1**-donor	Amp, Int39-*pTEF1-ScACC1*-tAOX1*	
Int56-*SsXR-SsXDH*-donor	Amp, Int56-*pTEF1-SsXDH-t0547-pGAP-SsXR-tAOX1*	
Int1-*SsXKS*-donor	Amp, Int1-*pTEF1-SsXKS-tAOX1*	
Int59-*LmPTA*-donor	Amp, Int59-*pAOX1-LmPTA-tAOX1*	

Table S2. List of Primers Used in This Study.

Primers	**Sequences (5′-3′)**
sgRNA-Int1-F[1]	ACGCTATCTGAAGTATTTACTGGG
sgRNA-Int1-R	AAACCCCAGTAAATACTTCAGATA
sgRNA-Int11-F	ACGCTATTAAAAAAGACGATCCCG
sgRNA-Int11-R	AAACCGGGATCGTCTTTTTTAATA
sgRNA-Int20-F	ACGCAGAAGAAAATGCGAAACAGG
sgRNA-Int20-R	AAACCCTGTTTCGCATTTTCTTCT
sgRNA-Int32-F	ACGCGTGACGAAAGAGATGAGGTG
sgRNA-Int32-R	AAACCACCTCATCTCTTTCGTCAC
sgRNA-Int33-F	ACGCCCGTCACTATGAGGACAAAG
sgRNA-Int33-R	AAACCTTTGTCCTCATAGTGACGG
sgRNA-Int34-F	ACGCGATCAGTTCATTGATAGACA
sgRNA-Int34-R	AAACTGTCTATCAATGAACTGATC
sgRNA-Int35-F	ACGCACTGATTTGCCAGAAGAACA
sgRNA-Int35-R	AAACTGTTCTTCTGGCAAATCAGT
sgRNA-Int39-F	ACGCGATTCAGTAGAGTCCTATTG
sgRNA-Int39-R	AAACCAATAGGACTCTACTGAATC
sgRNA-Int56-F	ACGCGAAATGTTGCTCGGTGCGCG
sgRNA-Int56-R	AAACCGCGCACCGAGCAACATTTC
sgRNA-Int59-F	ACGCAATGATGTCAATCCCATACG
sgRNA-Int59-R	AAACCGTATGGGATTGACATCATT
Gh2PS-F	TATTCTCACTACATACATTTTAGTTATTCGCCAACGATGG-GAAGTTACTCTAGCGATG
Gh2PS-R	TTAGTTTAGTCTTAAACTAAGCGAAACTACGTACGTCAATTCCCATTTGC-TACAGCTG

*ScACC1**-F	TTTATTCTCACTACATACATTTTAGTTATTCGCCAACATGAGCGAAGAAA-GCTTATTC
*ScACC1**-R	GGCAAATGGCATTCTGACATCCTCTTGATTAGAATCTAG-TCATTTCAAAGTCTTCAAC
SsXR-F	CAATTGAACAACTATCAAAACACACATGCCTTCTATTAAGTTGAACTCTG
SsXR-R	AATGGCATTCTGACATCCTCTTGAATTAGACGAAGATAGGAATCTTGTCC
SsXDH-F	CATACATTTTAGTTATTCGCCAACAATGACTGCTAACCCTTCCTTG
SsXDH-R	TAAACTAAGCGAAACTACGTACGCATTACTCAGGGCCGTCAATGA
SsXKS-F	ACATACATTTTAGTTATTCGCCAACATGACCACTACCCCATTTGA
SsXKS-R	TGACATCCTCTTGATTAGAATCTAGTTAGTGTTTCAATTCACTTTCCATCT
CkxPK-F	TCAATTGAACAACTATCAAAACACACAATGGCTGATTTCGACTCAAA
CkxPK-R	TTGAAGTGAAAAAAAGAAATGCACGACTTATGCAAATTAGCATCTCAC-TTAATCTTCTGT
LmPTA-F	ACATACATTTTAGTTATTCGCCAACGCATGAAGCTAATGGAAAACATCTTC
LmPTA-R	TAAACTAAGCGAAACTACGTACGCTTATTAGCCTTGCGCTTGC
Int1-*Gh2PS*-F	TACTTCTTTTGCAACGTAAATACTAGTCTAAAAGCTCAAGGAGATTTCAGAGGAG-TTG
Int1-*Gh2PS*-R	GCCTTAAAGGAAATTTGGAACTCCAGTGCCACTTTCGCTAGGGTTGTTCCGTTAG-CAC
Int11-*Gh2PS*-F	ATTAAATTTGGCAATTCCTCTAACATGCCATGGGATCAAGGAGATTTCAGAGGAG-TTG
Int11-*Gh2PS*-R	TGATATGAGAAGCAAACAAGATTTATAGACAGATTAGCTAGGGTTGTTCCGTTAG-CAC
Int20-*Gh2PS*-F	AAGTTTTTTATGAGACGATGATTTCGAAAGGTTCTACAAGGAGATTTCAGAGGAG-TTG
Int20-*Gh2PS*-R	AGCCCTCTTGGAGCTGTGTTATTTACTATTGAGGAAGCTAGGGTTGTTCCGTTAG-CAC
Int33-*Gh2PS*-F	ACCCACATTTTCCTCCTAGCACGTGAATCTTTTAAACAAGGAGATTTCAGAGGAG-TTG
Int33-*Gh2PS*-R	TCTAACTAGTTTGCCGCGACCACCATACAATATTTAGCTAGGGTTGTTCCGTTAG-CAC
Int34-*Gh2PS*-F	GAAGCAAAAATGACTGTAATCCTTATACGGCTGATTCAAGGAGATTTCAGAG-GAGTTG
Int34-*Gh2PS*-R	GTACATTTAATCATGTGTTTTAAAACTGATGATGAAGCTAGGGTTGTTCCGTTAG-CAC
Int35-*CkxPK*-donor	GTCATCTTCCTTGCTTTCGTCTTATTCGATGCTACTAATTGCCTGCTACTCTGGTCCC
Int35-*CkxPK*-donor	ATCTCCACATATCAAGGAATTATCATAACCAGTCTCTTTTCATCTCAC-TTAATCTTCTGTACTCT
Int39-*ScACC1**-F	TGCCCAGCAGGACATTGAAAAAGCAG
Int39-*ScACC1**-R	AGCAGCTGGAGCAGAAGAGGAAG
Int56-*SsXR-SsXDH*-F	GAACTTCCATAGGACTTTCTGCA
Int56-*SsXR-SsXDH*-R	GAGCTCGTGGTATTCAACCCA
Int1-*SsXKS*-F	GTCCATACTTCTTTTGCAACGTAAATACTAGTCTAAAAGCCAA-TAACTGTCGCCTCTTTTATCTG
Int1-*SsXKS*-R	TCAGGCCTTAAAGGAAATTTGGAACTCCAGTGCCACTTTCGCTCTCAC-TTAATCTTCTGTACTC
Int59-*LmPTA*-donor	TCCAATCAGGGGTTGAGTGA
Int59-*LmPTA*-donor	GGTCAAAGACTTAAGACCGC

Note:

[1] IntX refers to the genomic integration site of *P. pastoris*.

Table S3. List of Gene Coding Sequences Used in This Study.

Gene	Sequences (5′-3′)
Gh2PS	ATGGGAAGTTACTCTAGCGATGATGTGGAGGTTATTCGAGAGGCCGG- TAGAGCCCAGGGCTTAGCGACCATACTTGCGATTGGCACCGCTACTCCACCGAATTCTGT AGCGCAAGCCGACTATGCGGATTACTATTTTCGAGTCACTAAATCAGAGCACATGGTG- GAT- TTAAAGGAGAAATTCAAACGAATTTGTGAAAAGACGGCGATCAAGAAACGGTATCTGGC GTTGACGGAGGACTACCTACAAGAGAATCCAAC- GATGTGCGAATTCATGGCCCCTTCTCTAAACGCTCGCCAGGACCTTGTCGTGACTGGCGT ACCAATGCTCGGTAAAGAGGCAGCCGTAAAGGCCATAGACGAATGGGGATTACCCAA- GAG- TAAAATCACCCACCTAATTTTTTGTACAACGGCAGGGGTCGATATGCCGGGGGCAGATTA CCAGCTGGTCAAGCTGCTTGGGTTGAGCCCATCCGTTAAGCGTTACATGTTGTATCAG- CAGGGTTGCGCTGCCGGGGGTACTGTTTTACGCTTGGCCAAAGATCTTGCGGAGAACAA CAAGGGTAGTAGGGTCCTCATCGTGTGCTCCGAAATTACTGCGATACTCTTTCAC- GGACCCAACGAAAATCATCTCGACTCACTTGTCGCTCAAGCCCTGTTCGGTGACGGGGC AGCTGCCCTTATAGTGGGTTCGGGACCGCACCTAGCTGTTGAGCGCCCTATCTTCGA- GATTGTATCAACGGACCAGACAATATTGCCGGATACAGAGAAGGCAATGAAATTACATCT CCGGGAGGGGGGATTAACCTTTCAACTTCATCGGGATGTGCCCCTCATGGTCG- CAAAAAACATCGAAAACGCTGCTGAAAAAGCATTGTCCCCTCTAGGTATTACCGACTGG AATTCTGTGTTTTGGATGGTCCATCCCGGCGGCCGTGCAATACTAGAC- CAAGTAGAAAGGAAACTCAATTTAAAGGAAGACAAGCTTAGGGCGTCGCGTCATGTGCT GTCAGAATATGGGAACCTGATATCGGCGTGTGTACTCTTCATCATCGATGAAGTAC- GGAAAA- GAAGTATGGCTGAAGGAAAGAGCACTACGGGCGAGGGGTTAGACAGCGGCGTTTTGTTT GGATTCGGACCTGGCATGACCGTTGAAACAGTTGTACTAAGGTCGGTTAGAGTGACAG- CAGCTGTAGCAAATGGGAATTGA
*ScACC1**	ATGAGCGAAGAAAGCTTATTCGAGTCTTCTCCACAGAAGATGGAGTAC- GAAATTACAAAC- TACTCAGAAAGACATACAGAACTTCCAGGTCATTTCATTGGCCTCAATACAGTAGATAAA CTAGAGGAGTCCCCGTTAAGGGACTTTGTTAAGAGTCACGGTGGTCACACGGTCATA- TCCAA- GATCCTGATAGCAAATAATGGTATTGCCGCCGTGAAAGAAATTAGATCCGTCAGAAAATG GGCATACGAGACGTTCGGCGATGACAGAAC- CGTCCAATTCGTCGCCATGGCCACCCCAGAA- GATCTGGAGGCCAACGCAGAATATATCCGTATGGCCGATCAATACATTGAAGTGCCAGGT GGTACTAATAATAACAACTACGCTAACGTAGACTTGATCGTAGACATCGCCGAAAGAG- CAGACGTAGACGCCGTATGGGCTGGCTGGGGTCACGCCTCCGAGAATCCACTATTGCCTG AAAAATTGTCCCAGTCTAAGAGGAAAGTCATCTTTATTGGGCCTCCAGGTAAC- GCCATGAGGTCTTTAGGTGATAAAATCTCCTCTACCATTGTCGCTCAAAGTGCTAAAGTCC CATGTATTCCATGGTCTGGTACCGGTGTTGACACCGTTCACGTGGACGAGAAAAC- CGGTCTGGTCTCTGTCGACGATGACATCTATCAAAAGGGTTGTTGTACCTCTCCTGAAGAT GGTTTACAAAAGGCCAAGCGTATTGGTTTTCCTGTCATGAT- TAAGGCATCCGAAGGTGGTGGTGGTAAAGGTATCAGACAAGTTGAACGTGAAGAAGATT TCATCGCTTTATACCACCAGGCAGCCAAC- GAAATTCCAGGCTCCCCCATTTTCATCATGAAGTTGGCCGGTAGAGCGCGTCACTTGGAA GTTCAACTGCTAGCAGATCAGTACGGTACAAATATTTCCTTGTTCGGTAGA- GACTGTTCCGTTCAGAGACGTCATCAAAAAATTATCGAAGAAGCACCAGTTACAATTGCC AAGGCTGAAACATTTCACGAGATGGAAAAGGCTGCCGTCAGACTGGGGAAACTAG- TCGGTTATGTCTCTGCCGGTACCGTGGAGTATCTATATTCTCATGATGATGGAAAATTCTAC TTTTTAGAATTGAACCCAAGATTACAAGTCGAGCATCCAACAAC- GGAAATGGTCTCCGGTGTTAACTTACCTGCAGCTCAATTACAAATCGCTATGGGTATCCCT

	ATGCATAGAATAAGTGACATTAGAACTTTATATGG- TATGAATCCTCATTCTGCCTCAGAAATCGATTTCGAATTCAAAACTCAAGATGCCACCAA GAAACAAAGAAGACCTATTCCAAAGGGTCATTGTACCGCTTGTCGTATCACATCAGAA- GATCCAAACGATGGATTCAAGCCATCGGGTGGTACTTTGCATGAACTAAACTTCCGTTCTT CCTCTAATGTTTGGGGTTACTTCTCCGTGGGTAACAATGGTAATATTCACTCCTTTTCG- GACTCTCAGTTCGGCCATATTTTTGCTTTTGGTGAAAATAGACAAGCTTCCAGGAAACAC ATGGTTGTTGCCCTGAAGGAATTGTCCATTAGGGGTGATTTCAGAACTACTGTGGAA- TACTT- GATCAAACTTTTGGAAACTGAAGATTTCGAGGATAACACTATTACCACCGGTTGGTTGGA CGATTTGATTACTCATAAAATGACCGCTGAAAAGCCTGATCCAACTCTTGCCGTCATTT- GCGGTGCCGCTACAAAGGCTTTCTTAGCATCTGAAGAAGCCCGCCACAAGTATATCGAAT CCTTACAAAAGGGACAAGTTCTATCTAAAGACCTACTGCAAACTATGTTCCCTGTAGAT- TTTATCCATGAGGGTAAAAGATACAAGTTCACCGTAGCTAAATCCGGTAATGACCGTTAC ACATTATTTATCAATGGTTCTAAATGTGATATCAT- ACTGCGTCAACTAGCTGATGGTGGTCTTTTGATTGCCATAGGCGGTAAATCGCATACCATC TATTGGAAAGAAGAAGTTGCTGCTACAAGATTATCCGTTGACTCTATGACTACTTTGTT- GGAAGTTGAAAACGATCCAACCCAGTTGCGTACTCCATCCCCTGGTAAATTGGTTAAATT CTTGGTGGAAAATGGTGAACACATTATCAAGGGCCAACCATATGCAGAAATT- GAAGTTATGAAAATGCAAATGCCTTTGGTTTCTCAAGAAAATGGTATCGTCCAGTTATTAA AGCAACCTGGTTCTACCATTGTTGCAGGTGATATCATGGCTATTATGACTCTTGAC- GATCCATCCAAGGTCAAGCACGCTCTACCATTTGAAGGTATGCTGCCAGATTTTGGTTCTC CAGTTATCGAAGGAACCAAACCTGCCTATAAATTCAAGTCATTAGTGTCTACTTT- GGAAAACATTTTGAAGGGTTATGACAACCAAGTTATTATGAACGCTTCCTTGCAACAATT GATAGAGGTTTTGAGAAATCCAAAACTGCCTTACTCAGAATGGAAAC- TACACATCTCTGCTTTACATTCAAGATTGCCTGCTAAGCTAGATGAACAAATGGAAGAGT TAGTTGCACGTTCTTTGAGACGTGGTGCTGTTTTCCCAGCTAGACAATTAAGTAAATT- GATTGATATGGCCGTGAAGAATCCTGAATACAACCCCGACAAATTGCTGGGCGCCGTCGT GGAACCATTGGCGGATATTGCTCATAAGTACTCTAACGGGTTAGAAGCCCATGAACATTC- TA- TATTTGTCCATTTCTTGGAAGAATATTACGAAGTTGAAAAGTTATTCAATGGTCCAAATGT TCGTGAGGAAAATATCATTCTGAAATTGCGTGATGAAAACCCTAAAGATCTAGA- TAAAGTT- GCGCTAACTGTTTTGTCTCATTCGAAAGTTTCAGCGAAGAATAACCTGATCCTAGCTATCT TGAAACATTATCAACCATTGTGCAAGTTATCTTCTAAAGTTTCTGCCATTTTCTC- TACTCCTCTACAACATATTGTTGAACTAGAATCTAAGGCTACCGCTAAGGTCGCTCTACAA GCAAGAGAAATTTTGATTCAAGGCGCTTTACCTTCGGTCAAGGAAA- GAACTGAACAAATT- GAACATATCTTAAAATCCTCTGTTGTGAAGGTTGCCTATGGCTCATCCAATCCAAAGCGCT CTGAACCAGATTTGAATATCTTGAAGGACTTGATCGATTCTAATTACGTT- GTGTTCGATGTTTTACTTCAATTCCTAACCCATCAAGACCCAGTTGTGACTGCTGCAGCTG CTCAAGTCTATATTCGTCGTGCTTATCGTGCTTACACCATAGGAGATATTAGAGTTCAC- GAAGGTGTCACAGTTCCAATTGTTGAATGGAAATTCCAACTACCTTCAGCTGCGTTCTCC ACCTTTCCAACTGTTAAATCTAAAATGGGTATGAACAGGGCTGTTGCTGTTTCAGATTT- GTCATATGTTGCAAACAGTCAGTCATCTCCGTTAAGAGAAGGTATTTTGATGGCTGTGGAT CATTTAGATGATGTTGATGAAATTTTGTCACAAAGTTTGGAAGTTATTCCTCGTCAC- CAATCTTCTTCTAACGGACCTGCTCCTGATCGTTCTGGTAGCTCCGCATCGTTGAGTAATG TTGCTAATGTTTGTGTTGCTTCTACAGAAGGTTTCGAATCTGAAGAGGAAATTTTGG- TAAGGTTGAGAGAAATTTTGGATTTGAATAAGCAGGAATTAATCAATGCTTCTATCCGTCG TATCACATTTATGTTCGGTTTTAAAGATGGGTCTTATCCAAAGTATTATACTTTTAAC- GGTCCAAATTATAACGAAAATGAAACAATTCGTCACATTGAGCCGGCTTTGGCCTTCCAA CTGGAATTAGGAAGATTGTCCAACTTCAACATTAAACCAATTTTCACTGATAA- TAGAAACATCCATGTCTACGAAGCTGTTAGTAAGACTTCTCCATTGGATAAGAGATTCTTT

	ACAAGAGGTATTATTAGAACGGGTCATATCCGTGATGACATTTCTATTCAAGAA- TATCTGACTTCTGAAGCTAACAGATTGATGAGTGATATATTGGATAATTTAGAAGTCACCG ACACTTCAAATTCTGATTTGAATCATATCTTCATCAACTTCATTGCGGTGTTTGA- TATCTCTCCAGAAGATGTCGAAGCCGCCTTCGGTGGTTTCTTAGAAAGATTTGGTAAGAG ATTGTTGAGATTGCGTGTTTCTTCTGCCGAAATTAGAATCATCATCAAA- GATCCTCAAACAGGTGCCCCAGTACCATTGCGTGCCTTGATCAATAACGTTTCTGGTTATG TTATCAAAACAGAAATGTACACCGAAGTCAAGAACGCAAAAGGTGAATGGG- TATTTAAGTCTTTGGGTAAACCTGGATCCATGCATTTAAGACCTATTGCTACTCCTTACCCT GTTAAGGAATGGTTGCAACCAAAACGTTATAAGGCACACTTGATGGGTACCACAT- ATGTC- TATGACTTCCCAGAATTATTCCGCCAAGCATCGTCATCCCAATGGAAAAATTTCTCTGCAG ATGTTAAGTTAACAGATGATTTCTTTATTTCCAACGAGTTGATTGAAGATGAAAAC- GGCGAATTAACTGAGGTGGAAAGAGAACCTGGTGCCAACGCTATTGGTATGGTTGCCTTT AAGATTACTGTAAAGACTCCTGAATATCCAAGAGGCCGTCAATTTGTTGTTGTTGCTAAC- GA- TATCACATTCAAGATCGGTTCCTTTGGTCCACAAGAAGACGAATTCTTCAATAAGGTTACT GAATATGCTAGAAAGCGTGGTATCCCAAGAATTTACTTGGCTG- CAAACTCAGGTGCCAGAATTGGTATGGCTGAAGAGATTGTTCCACTATTTCAAGTTGCAT GGAATGATGCTGCCAATCCGGACAAGGGCTTCCAA- TACTTATACTTAACAAGTGAAGGTATGGAAACTTTAAAGAAATTTGACAAAGAAAATTCT GTTCTCACTGAACGTACTGTTATAAACGGTGAAGAAAGATTTGTCATCAAGA- CAATTATTGGTTCTGAAGATGGGTTAGGTGTCGAATGTCTACGTGGATCTGGTTTAATTGCT GGTGCAACGTCAAGGGCTTACCACGATATCTTCACTATCACCTTAGTCACTT- GTAGATCCGTCGGTATCGGTGCTTATTTGGTTCGTTTGGGTCAAAGAGCTATTCAGGTCGA AGGCCAGCCAATTATTTTAACTGGTGCTCCTGCAATCAACAAAATGCTGGGTAGA- GAAGTTTATACTTCTAACTTACAATTGGGTGGTACTCAAATCATGTATAACAACGGTGTTT CACATTTGACTGCTGTTGACGATTTAGCTGGTGTAGAGAAGATTGTT- GAATGGATGTCTTATGTTCCAGCCAAGCGTAATATGCCAGTTCCTATCTTGGAAACTAAAG ACACATGGGATAGACCAGTTGATTTCACTCCAACTAATGATGAAACTTACGATGTAA- GATGGATGATTGAAGGTCGTGAGACTGAAAGTGGATTTGAATATGGTTTGTTTGATAAAG GGTCTTTCTTTGAAACTTTGTCAGGATGGGCCAAAGGTGTTGTCGTTGG- TAGAGCCCGTCTT- GGTGGTATTCCACTGGGTGTTATTGGTGTTGAAACAAGAACTGTCGAGAACTTGATTCCT GCTGATCCAGCTAATCCAAATAGTGCTGAAACATTAATTCAAGAACCTGGTCAAGTTTGG- CATCCAAACTCCGCCTTCAAGACTGCTCAAGCTATCAATGACTTTAACAACGGTGAACAA TTGCCAATGATGATTTTGGCCAACTGGAGAGGTTTCTCTGGTGGTCAACGTGA- TATGTTCAACGAAGTCTTGAAGTATGGTTCGTTTATTGTTGACGCATTGGTGGATTACAAA CAACCAATTATTATCTATATCCCACCTACCGGTGAACTAAGAGGTGGTTCATGGGTTGTT- GTCGATCCAACTATCAACGCTGACCAAATGGAAATGTATGCCGACGTCAACGCTAGAGCT GGTGTTTTGGAACCACAAGGTATGGTTGGTATCAAGTTCCGTAGAGAAAAATTGCTG- GACAC- CATGAACAGATTGGATGACAAGTACAGAGAATTGAGATCTCAATTATCCAACAAGAGTTT GGCTCCAGAAGTACATCAGCAAATATCCAAGCAATTAGCTGATCGTGAGAGAGAAC- TATTGCCAATTTACGGACAAATCAGTCTTCAATTTGCTGATTTGCACGATAGGTCTTCACG TATGGTGGCCAAGGGTGTTATTTCTAAGGAACTGGAATGGACCGAGGCAC- GTCGTTTCTTCTTCTGGAGATTGAGAAGAAGATTGAACGAAGAATATTTGATTAAAAGGT TGAGCCATCAGGTAGGCGAAGCATCAAGATTAGAAAAGATCGCAAGAATT- AGATCGTGG- TACCCTGCTTCAGTGGACCATGAAGATGATAGGCAAGTCGCAACATGGATTGAAGAAAA CTACAAAACTTTGGACGATAAACTAAAGGGTTTGAAATTAGAGTCATTCGCTCAA- GACTTAGCTAAAAAGATCAGAAGCGACCATGACAATGCTATTGATGGATTATCTGAAGTT ATCAAGATGTTATCTACCGATGATAAAGAAAAATTGTTGAAGACTTTGAAATGA

CkxPK	ATGGCTGATTTCGACTCAAAGGAATACTTAGAATTGGTTGATAAATGGTGGAGAGCTACT- A- ACTACTTGTCCGCTGGTATGATCTTCTTGAAGTCAAACCCATTATTTTCCGTTACTAACACA CCTATCAAGGCTGAAGATGTTAAAGTCAAGCCAATTGGTCATTGGGGTACTA- TATCTGGTCAAACATTCTTGTATGCTCACGCAAACAGATTGATTAACAAATACGGTTTGAA TATGTTTTACGTTGGTGGTCCAGGTCATGGTGGTCAAGTAATGGTTACAAACGCTTACTT- GGATGGTGCATATACCGAAGACTACCCTGAAATTACTCAAGATATCGAGGGTATGAGTCA TTTGTTTAAAAGATTCTCTTTCCCAGGTGGTATTGGTTCACATATGACTGCTCAAACAC- CTGGTTCATTGCACGAAGGTGGTGAATTGGGTTATTCCTTAAGTCATGCCTTCGGTGCTGT TTTAGATAATCCAGACCAAGTCGCCTTTGCTGTTGTCGGTGACGGTGAA- GCAGAAACAGGTCCTTCTATGGCCTCATGGCACTCCATAAAATTTTTGAATGCCAAGAAC GATGGTGCTGTTTTACCAGTCTT- GGACTTAAATGGTTTCAAAATCTCTAACCCTACAATTTTTAGTAGAATGTCTGATGAAGAA ATAACCAAGTTTTTCGAAGGTTTGGGTTACTCACCAAGATTCATTGAAAAC- GATGACATCCATGATTATGCAACTTACCACCAATTGGCTGCAAACATCTTGGATCAAGCT ATCGAAGACATCCAAGCAATCCAAAATGATGCCAGAGAAAACGGTAAATACCAAGAC- GGTGAAATACCAGCCTGGCCTGTTATTATAGCTAGATTGCCAAAGGGTTGGGGTGGTCCT ACACATGATGCATCCAATAACCCAATCGAAAATAGTTTTAGAGCCCATCAAGTTCCATT- GCCTTTAGAACAACACGATTTGGCAACTTTACCAGAATTCGAAGACTGGATGAACTCTTA CAAGCCTGAAGAATTGTTTAATGCTGATGGTTCATTGAAAGAC- GAATTAAAGGCAATCGCCCCAAAAGGTGACAAGAGAATGTCCGCCAATCCTATTACAAA CGGTGGTGCTGATAGAAGTGACTTGAAGTTACCAAACTGGAGAGAATTCGCAAACGATA- TAAACGATGACACTAGAGGTAAAGAATTCGCTGATTCAAAGAGAAACATGGACATGGCA ACATTGTCTAACTACTTAGGTGCCGTTTCACAATTGAATCCAACCAGATTCAGAT- TTTTCGGTCCTGATGAAACTATGTCCAATAGATTGTGGGGTTTGTTTAATGTCACCCCTAG ACAATGGATGGAAGAAATTAAAGAACCACAAGATCAATTGTTGTCTCCTACTGG- TAGAATCATTGACTCACAATTATCCGAACATCAAGCAGAAGGTTGGTTGGAAGGTTATAC CTTAACTGGTAGAGTCGGTATTTTCGCTAGTTACGAATCATTTTTGAGAGTAGTTGATAC- CATGGTAACTCAACATTTCAAGTGGTTGAGACACGCTTCTGAACAAGCATGGAGAAACG ATTACCCATCCTTGAACTTAATAGCCACAAGTACCGCTTTCCAACAA- GATCATAATGGTTACACACACCAAGACCCAGGCATGTTGACCCATTTGGCAGAAAAGAA ATCTAACTTCATCAGAGAATATTTGCCTGCTGATGGTAACTCTTTGTTAGCTGTACAA- GAAA- GAGCATTTTCAGAAAGACATAAGGTTAATTTGTTGATCGCTTCTAAGCAACCTAGACAAC AATGGTTCACTGTAGAAGAAGCAGAAGTTTTGGCCAACGAAGGTTTAAAAA- TAATCGATTGGGCATCTACTGCCCCATCTTCAGATGTTGACATTACATTTGCTTCAGCAGG TACAGAACCTACCATAGAAACTTTGGCCGCTTTGTGGTTAATCAATCAA- GCATTTCCAGATGTCAAGTTTAGATACGTAAACGTCGTAGAATTGTTGAGATTGCAAAAG AAATCTGAACCTAACATGAACGATGAAAGAGAATTAAGTGCTGAAGAATTCAA- TAAGTACTTCCAAGCAGACACACCAGTTATTTTCGGTTTTCATGCTTACGAAAACTTAATC GAATCATTTTTCTTTGAAAGAAAGTTTACTGGTGACGTCTATGTACACGGTTACAGAGAA- GATGGTGACATTACTACAACCTATGATATGAGAGTTTACTCCCATTTGGACAGATTCCACC AAGCCAAAGAAGCAGCCGAAATCTTGAGTGCTAACGGTAAAATAGATCAAGCTG- CAGCCGACACTTTCATAGCTAAAATGGATGACACATTGGCAAAGCATTTTCAAGTTACCA GAAATGAAGGTAGAGATATCGAAGAATTCACTGATTGGACCTGGTCACCATTAAAATAA- TAA
LmPTA	ATGAAGCTAATGGAAAACATCTTCGGGCTGGCCAAGGCGGACAAAAAAAA- GATCGTGTT- GGCGGAAGGCGAAGAGGAGCGTAACATTAGGGCTTCCGAGGAAATAATCAGAGACGGG ATAGCCGATATAATCCTAGTTGGCTCAGAATCAGTCATTAAGGAAAATGCCGCTAAATTT- GGCGTGAATCTGGCAGGGGTCGAGATAGTCGATCCAGAAACGTCCTCCAAGACTGCCGG CTACGCGAACGCTTTCTATGAGATCCGTAAAAATAAGGGTGTCACACTAGAAAAAGCG-

	GA- TAAAATAGTCCGTGATCCAATTTATTTTGCCACCATGATGGTCAAACTAGGTGATGCTGAT GGGCTAGTGTCTGGAGCCATTCACACAACGGGTGATTTACTAAGGCCAGGCCTG- CAGATCGTTAAGACAGTTCCCGGCGCGAGTGTGGTGTCCTCTGTCTTCTTGATGAGTGTTC CTGACTGCGAGTACGGCGAG- GATGGTTTTCTGTTATTCGCTGACTGTGCTGTTAATGTATGCCCGACCGCAGAGGAATTATC TAGCATAGCAATCACAACCGCCGAGACGGCAAAAAACCTTTGTAA- GATCGAGCCCAGAGTCG- CAATGCTATCTTTCTCTACAATGGGTTCCGCGTCCCATGAACTAGTCGACAAAGTGACTAA GGCCACAAAGTTGGCTAAGGAGGCACGTCCCGACTTGGATATCGACGGTGAATT- GCAATT- AGATGCGTCACTAGTAAAGAAAGTAGCTGACCTAAAGGCTCCCGGCTCCAAGGTGGCAG GTAAGGCTAATGTGTTGATTTTTCCGGATATACAGGCTGGCAACATAGGGTATAAACTAG- TCCAGCGTTTTGCGAAAGCTGAAGCAATCGGACCCATTTGCCAAGGATTTGCCAAGCCTA TAAACGATTTGAGTAGAGGATGTTCTGTCGACGACATTGTAAAGGTCGTGGCGGTCAC- GGCGGTTCAGGCGCAAGCGCAAGGCTAATAA
SsXR	ATGCCTTCTATTAAGTTGAACTCTGGTTACGACATGCCAGCCGTCGGTTTCGGCTGTT- GGAAAGTCGACGTCGACACCTGTTCTGAACAGATCTACCGTGCTATCAAGACCGGTTAC AGATTGTTCGACGGTGCCGAAGATTACGCCAACGAAAAGTTAGTTGGTGCCGGTGTCAA- GAAGGCCATTGACGAAGGTATCGTCAAGCGTGAAGACTTGTTCCTTACCTCCAAGTTGTG GAACAACTACCACCACCCAGACAACGTCGAAAAGGCCTT- GAACAGAACCCTTTCTGACTT- GCAAGTTGACTACGTTGACTTGTTCTTGATCCACTTCCCAGTCACCTTCAAGTTCGTTCCA TTAGAAGAAAAGTACCCACCAGGATTCTACTGTGGTAAGGGTGACAACTTCGACTAC- GAA- GATGTTCCAATTTTAGAGACCTGGAAGGCTCTTGAAAAGTTGGTCAAGGCCGGTAAGATC AGATCTATCGGTGTTTCTAACTTCCCAGGTGCTTTGCTCTTGGACTTGTTGAGAGGTGC- TAC- CATCAAGCCATCTGTCTTGCAAGTTGAACACCACCCATACTTGCAACAACCAAGATTGAT CGAATTCGCTCAATCCCGTGGTATTGCTGTCAC- CGCTTACTCTTCGTTCGGTCCTCAATCTTTCGTTGAATTGAACCAAGGTAGAGCTTTGAAC ACTTCTCCATTGTTCGAGAACGAAACTATCAAGGCTATCGCTGCTAAGCACGG- TAAGTCTCCAGCTCAAGTCTTGTTGAGATGGTCTTCCCAAAGAGGCATTGCCATCATTCC AAAGTCCAACACTGTCCCAAGATTGTTGGAAAACAAGGACGTCAACAGCTTCGACTT- GGAC- GAACAAGATTTCGCTGACATTGCCAAGTTGGACATCAACTTGAGATTCAACGACCCATGG GACTGGGACAAGATTCCTATCTTCGTCTAA
SsXDH	ATGACTGCTAACCCTTCCTTGGTGTTGAACAAGATCGACGACATTTCGTTCGAAACTTAC- GATGCCCCAGAAATCTCTGAACCTACCGATGTCCTCGTCCAGGTCAAGAAAACCGGTATC TGTGGTTCCGACATCCACTTCTACGCCCATGGTAGAATCGGTAACTTCGTTTTGACCAA- GCCAATGGTCTTGGGTCACGAATCCGCCGGTACTGTTGTCCAGGTTGGTAAGGGTGTCAC CTCTCTTAAGGTTGGTGACAACGTCGCTATCGAACCAGGTATTCCATCCAGAT- TCTCCGAC- GAATACAAGAGCGGTCACTACAACTTGTGTCCTCACATGGCCTTCGCCGCTACTCCTAAC TCCAAGGAAGGCGAACCAAACCCACCAGGTAC- CTTATGTAAGTACTTCAAGTCGCCAGAA- GACTTCTTGGTCAAGTTGCCAGACCACGTCAGCTTGGAACTCGGTGCTCTTGTTGAGCCA TTGTCTGTTGGTGTCCACGCCTCCAAGTTGGGTTCCGTTGCTTTCGGCGACTACGTT- GCCGTCTTTGGTGCTGGTCCTGTTGGTCTTTTGGCTGCTGCTGTCGCCAAGACCTTCGGTG CTAAGGGTGTCATCGTCGTTGACATTTTCGACAACAAGTTGAAGATGGCCAAGGACATT- GGTGCTGCTACTCACACCTTCAACTCCAAGACCGGTGGTTCTGAAGAATTGATCAAGGCT TTCGGTGGTAACGTGCCAAACGTCGTTTTGGAATGTACTGGTGCTGAACCTT-

	GTATCAAGTT- GGGTGTTGACGCCATTGCCCCAGGTGGTCGTTTCGTTCAAGTTGGTAACGCTGCTGGTCC AGTCAGCTTCCCAATCACCGTTTTCGCCATGAAGGAATTGACTTT- GTTCGGTTCTTTCAGA- TACGGATTCAACGACTACAAGACTGCTGTTGGAATCTTTGACACTAACTACCAAAACGGT AGAGAAAATGCTCCAATTGACTTTGAACAATTGATCACCCACAGATA- CAAGTTCAAGGACGC- TATTGAAGCCTACGACTTGGTCAGAGCCGGTAAGGGTGCTGTCAAGTGTCTCATTGACGG CCCTGAGTAA
SsXKS	ATGACCACTACCCCATTTGATGCTCCAGATAA- GCTCTTCCTCGGGTTCGATCTTTCGACTCAGCAGTTGAAGATCATCGTCACCGATGAAAA CCTCGCTGCTCTCAAAACCTACAATGTCGAGTTCGA- TAGCATCAACAGCTCTGTCCAGAAGGGTGTCATTGCTATCAACGACGAAATCAGCAAGG GTGCCATTATTTCCCCCGTTTACATGTGGTTGGATGCCCTTGACCATGTTTTTGAAGA- CATGAAGAAGGACGGATTCCCCTTCAACAAGGTTGTTGGTATTTCCGGTTCTTGTCAACA GCACGGTTCGGTATACTGGTCTAGAACGGCCGAGAAGGTCTTGTCCGAATTGGAC- GCTGAATCTTCGTTATCGAGCCAGATGAGATCTGCTTTCACCTTCAAGCACGCTCCAAAC TGGCAGGATCACTCTACCGGTAAAGAGCTTGAAGAGTTCGAAAGAG- TGATTGGTGCTGATGCCTTGGCTGATATCTCTGGTTCCAGAGCCCATTACAGATTCACAGG GCTCCAGATTAGAAAGTTGTCTACCAGATTCAA- GCCCGAAAAGTACAACAGAACTGCTCG- TATCTCTTTAGTTTCGTCATTTGTTGCCAGTGTGTTGCTTGGTAGAATCACCTCCATTGAAG AAGCCGATGCTTGTGGAATGAACTTGTACGATATCGAAAAGCGCGAGTTCAACGAA- GAGCTCTTGGCCATCGCTGCTGGTGTCCACCCTGAGTTGGATGGTGTAGAACAAGACGGT GAAATTTACAGAGCTGGTATCAATGAGTTGAAGAGAAAGTTGGGTCCTGTCAAAC- CTATAACATACGAAAGCGAAGGTGACATTGCCTCTTACTTTGTCACCAGATACGGCTTCA ACCCCGACTGTAAAATCTACTCGTTCACCGGAGACAATTTGGCCACGATTATCTCGTT- GCCTTTGGCTCCAAATGATGCTTTGATCTCATTGGGTACTTCTACTACAGTTTTAATTATCA CCAAGAACTACGCTCCTTCTTCTCAATACCATTTGTTTAAACATCCAACCATGCCTGAC- CAC- TACATGGGCATGATCTGCTACTGTAACGGTTCCTTGGCCAGAGAAAAGGTTAGAGACGA AGTCAACGAAAAGTTCAATGTAGAAGACAAGAAGTCGTGG- GACAAGTTCAATGAAATCTT- GGACAAATCCACAGACTTCAACAACAAGTTGGGTATTTACTTCCCACTTGGCGAAATTGT CCCTAATGCCGCTGCTCAGATCAAGAGATCGGTGTTGAACAGCAAGAACGAAATT- GTAGAC- GTTGAGTTGGGCGACAAGAACTGGCAACCTGAAGATGATGTTTCTTCAATTGTAGAATCA CAGACTTTGTCTTGTAGATTGAGAACTGGTCCAATGTTGAGCAAGAGTGGAGAT- TCTTCTGCTTCCAGCTCTGCCTCACCTCAACCAGAAGGTGATGGTACAGATTTGCACAAG GTCTACCAAGACTTGGTTAAAAAGTTTGGTGACTTGTTCACTGATGGAAAGAAGCAAAC- CTTTGAGTCTTTGACCGCCAGACCTAACCGTTGTTACTACGTCGGTGGTGCTTCCAACAA CGGCAGCATTATCCSCAAGATGGGTTCCATCTTGGCTCCCGTCAACGGAAAC- TACAAGGTT- GACATTCCTAACGCCTGTGCATTGGGTGGTGCTTACAAGGCCAGTTGGAGTTACGAGTGT GAAGCCAAGAAGGAATGGATCGGATACGATCAGTATATCAACAGATTGTTT- GAAGTAAGTGACGAGATGAATCTGTTCGAAGTCAAGGATAAATGGCTCGAATATGCCAA CGGGGTTGGAATGTTGGCCAAGATGGAAAGTGAATTGAAACACTAA

Table S4. Comparison of TAL Titer, Yield, Productivity from different carbon sources.

Strain	Medium	Titer (g/L)	Yield (g/g)	Volumetric productivity (g/L·h)
PpTAL1	YPD	0.95	0.0477	0.0133
PpTAL2	YPD	0.93	0.0467	0.0130
PpTAL3	YPD	1.07	0.0536	0.0149
PpTAL4	YPD	1.51	0.0753	0.0209
PpTAL5	YPD	2.01	0.100	0.0279
PpTAL6	YPD	2.44	0.122	0.0339
PpTAL7	YPD	2.55	0.127	0.0354
PpTAL8	YPD	2.72	0.136	0.0378
PpTAL10	SCD	0.50	0.0251	0.00696
PpTAL11	SCD	0.53	0.0266	0.00739
PpTAL10	SCX	0.54	0.0271	0.00754
PpTAL11	SCX	0.83	0.0413	0.0115
PpTAL8	SCM	0.021	0.00103	0.000285
PpTAL9	SCM	0.057	0.00285	0.000793