

Table S1 Primers used in this study.

Figure S1 Expression of *ScTHI5* in *C. glabrata* confers a growth advantage during thiamine starvation conditions. Transformation of *C. glabrata* wild-type cells with a plasmid containing the *CgPMU3* promoter (1000 bp) driving expression of *ScTHI5* either with or without a stop codon at the end of the *ScTHI5* ORF. Without a stop codon, a YFP fusion protein is formed, and we confirmed that expression was thiamine starvation regulated by flow cytometry (left). Streaking these two plasmid containing strains with a third strain containing an empty vector on SD-his-thiamine and replica plating twice to dilute the residual thiamine demonstrated that the *CgPMU3p-ScTHI5* plasmids conferred a growth advantage in the absence of thiamine (right).

Figure S2 The frequency of abundant sequences increases after selection in thiamine starvation conditions. After selection and next generation sequencing (NGS) of the samples, we calculated the abundance of each 11 bp sequence. We then graphed the frequency of the 20 most abundant sequences in each sample relative to the percent of the total sequences. After selection, the majority of sequences are represented by less than 20 sequences.

Table S2 Raw data from the seven samples that we sequenced. At the bottom of each column, we indicate how many sequencing reads we acquired from the NGS, and the proportion of those sequences that were unique (i.e. only observed as one sequence out of the total). We randomly acquired an additional 2000-5000 sequences to confirm that we had no bias in selecting a relatively small subset of the total NGS run, and noted only slight changes (i.e. sequences may change from the 10th most abundant to the 12th most abundant). There were no sequences identified that appeared in multiple samples below the top 25 sequences with either dataset. We judged that the data did not change based on sampling error.

Table S3 List of sequences observed in wild-type selections 1-3 (Figure S2) that were represented in at least two replicates. While we examined the top 30 sequences in the selection samples, none of the sequences that were less abundant than the top 20 led to a fold enrichment >15x, and given that the fold enrichment still resulted in less than 1% of the final proportion, we believe we have examined all of the relevant sequences.

Figure S3 Alignment of sequences in Table S3 with *C. glabrata* THI promoters and *CgPMU3* promoter. We aligned the sequences highly enriched by selection with the *CgPMU3* 11 bp element, and other *CgTHI* promoter 13 bp elements, asking what nucleotides were likely required for elevated expression. We note that sequences A-D became abundant post selection, and in most cases were not even observed in the preselection sequences (i.e. less than 1/8986), suggesting that the selection enriched for sequences that conferred elevated expression. Sequences A and G have a lot of similarity to *CgPET18* and sequence E matches *CgPMU3* in 8 of 11 nucleotides, as the surrounding nucleotides confer the GA in front of the CGTACA.

Figure S4 Scanning mutagenesis of the 13 bp THI UAS in the *CgPET18* promoter suggests

the xxCCGTxxAxxTG nucleotides are critical for expression. Single mutations were introduced into the full-length *CgPET18* promoter, replacing the native nucleotide with an A (or C if an A was in that position) and measuring fluorescence in thiamine starvation conditions.

Figure S5 Schematic of *S. cerevisiae* promoters and location of mutations and deletions made in this study. Presented is 300 bp of the *ScTHI5* promoter and 200 bp of the *ScTHI20* promoter with both strands shown, as well as the first 15 bp of the ORF. Based on MEME analysis (located under the sequences), we identified regions (boxed within the sequence) that shared similarity to the THI UAS identified in *C. glabrata*. We replaced the entire boxed region in *ScTHI5p* and in *ScTHI20p* with the sequences shown above them, and did not observe a significant decline in expression of the promoter (Figure 7A). Additionally, we replaced the *ScTHI20* region with 13 Ts and did not see a significant decline in expression (data not shown). Realignment of these regions with the THI UAS consensus suggested numerous regions that were similar to the THI UAS (highlighted in yellow). Regions in green indicate putative Thi2 binding sites based on Nosaka, 2006. When we remove regions of the promoter overlapping with the Thi2 binding site (highlighted in grey), we observe a large decline in expression of the promoters (Figure 7B and 7C).

Figure S6 Expression of *S. cerevisiae* THI promoters is dependent on Pdc2, Thi2, and Thi3. While deleting *ScPDC2* and *ScTHI3* reduces almost all expression of the *ScTHI4* promoter, deletion of *THI2* allows for regulation, albeit with lower expression.

Table S1 Primers used in this study.

	Strain/Plasmid	Primer number	Primer Sequence	Notes	
<i>Scpdc2ΔNATMX6</i>	<i>NATMX6</i>	01770	ATGCTTTCCATTACAGCAAGATATAAATTTGTCTAATGGcgatccccgggttaattaa		
		01771	CTAAAAAAGCCCTGTGTACCAGGTAAGTGAAGTTATAGAATTCGAGCTCGTTAAAC		
<i>ScPDC2 -pRS316</i>	<i>ScPDC2</i> ORF	01854	ggtggcgccgctctagaactagtggatccTTTACTACCTCAATCTGTGA		
		01855	taagcttgatatacgaattcctcgagcccgATCGAATCATCATTATATAC		
<i>ScADH1 p-ScPDC1 -pRS315</i>	<i>ScADH1</i> p	0808	ggagctccacgcggtggcgccgctctagaactagtGGTGTACCAGTATAAATAG		
		0809	cagtgaaataattcttcaccttttagacatGGATCTGTATAGAGATAGTTGATTG		
	<i>ScPDC1</i> ORF	02174	GCTATACCAAGCATACAATCAACTATCTCATATACaatgtctgaaattactttggg		
		02175	gacaacaccagtgaaataattcttcaccttttagacatCGGCAACTTCTTGTCTGGTC		
<i>Cg pmu1Δ pmu2Δ pmu3ΔNATMX6</i>	<i>NATMX6</i>	0226	catgcttaacctctaagtatcctacatagactgctctctgCGGATCCCCGGGTAAATAA		
		0229	tgatatagatgatcgaggattttaagatactacgagaattgGAATTCGAGCTCGTTAAAC		
<i>CgPMU3 p-ScTH15 -YFP-pRS313</i>	<i>CgPMU3</i> p (wild-type)	01479	ggtggcgccgctctagaactagtggatccgggctgacgctgttacaac	-1000 bp (5')	
		02177	ACAAAAATGTGATCTTGTCTGTAGACATtgtaacgagagtggatata	at ATG with homology to ScTH15 (3')	
	<i>CgPMU3</i> p (with Ns in UAS)	02207	CTTAAATATGGATGCTTTCTC	-250 bp (3') (use with 1479)	
		02205	gagaagcatccatatttaagaNNNNNNNNNNNatacttgtaaacaattcttctc	-250 bp to put Ns in UAS (5')	
		02206	TATTTGGATTGCGCTCTAATTAAGAGAAGCATCCATATTTAAAG	upstream of -250 bp to put Ns in UAS (5')	
		01478	accagtgaaataattcttcaccttttagacattgtaacgagagtggatata	at ATG with homology to YFP (3')	
		02178	atcaaatatatactccactctcgttacaaATGTCTACAGACAAGATCAC	5' ScTH15 ORF	
	<i>ScTH15</i> ORF	02179	accagtgaaataattcttcaccttttagacatAGCTGGGAAGGCCAATCTCT	3' ScTH15 ORF without stop codon with homology to YFP	
		02180	accagtgaaataattcttcaccttttagacatTTAAGCTGGAAGGCCAATCTCT	3' ScTH15 ORF witht stop codon with homology to YFP	
	<i>CgPMU3 p-YFP-pRS313</i>	-1000 bp	01479	ggtggcgccgctctagaactagtggatccgggctgacgctgttacaac	use with 01478
		-900 bp	01480	ggtggcgccgctctagaactagtggatccactctatagacottccaga	use with 01478
		-800 bp	01481	ggtggcgccgctctagaactagtggatcccttgaattattgttttttgt	use with 01478
		-700 bp	01482	ggtggcgccgctctagaactagtggatccctctccctttttctgtatttgt	use with 01478
		-600 bp	01483	ggtggcgccgctctagaactagtggatccacgttactattatgtgttaac	use with 01478
		-500 bp	01484	ggtggcgccgctctagaactagtggatccctctcgctactatttctgt	use with 01478
		-400 bp	01485	ggtggcgccgctctagaactagtggatccctctctactaggatcaaatagt	use with 01478
		-300 bp	01486	ggtggcgccgctctagaactagtggatcccaagagaagcatcccatttta	use with 01478
-262 bp		01510	ggtggcgccgctctagaactagtggatccagagaagcatcaacgatactct	use with 01478	
-257 bp		02103	ggtggcgccgctctagaactagtggatccCGTACACGATCTGTTAA	use with 01478	
-252 bp		02104	ggtggcgccgctctagaactagtggatccAACGATCTGTTAACAATT	use with 01478	
-247 bp		02105	ggtggcgccgctctagaactagtggatccTACTTGTTAACAATCTTTTC	use with 01478	
-200 bp		01487	ggtggcgccgctctagaactagtggatccggatctactaaagcatctaa	use with 01478	
-100 bp		01488	ggtggcgccgctctagaactagtggatccgttcttagactaaagtattat	use with 01478	
at ATG with homology to YFP (3')		01478	accagtgaaataattcttcaccttttagacattgtaacgagagtggatata		
mutate GACGTACAACG to CGATGACAGCA		01977	gagaagcatccatatttaagaCGATGACAGCAatacttgtaaacaattctcttc	use with 01478	
		01978	gaagaattgtttaacaagatATGCTGTACGTctctaaataggatgctcttc	use with 01479	
mutate GACGTACAACG to AAGGTACAACG		02107	GAGAAGCATCCATATTTAAAGaaagtcaaacgATACTTGTTTAAC	use with 01478	
		02108	GTTAACAGTATcgttgttacttTCTTAAATATGGATGTTCTCTC	use with 01479	
mutate GACGTACAACG to GACAAACAACG		02109	AAGCATCCATATTTAAAGagacaacaacgATACTTGTTTAAACAT	use with 01478	
		02110	ATTGTTAAACAAGTATcgttgtttctTCTTAAATATGGATGCTT	use with 01479	
mutate GACGTACAACG to GACGTACCCCG		02111	ATCCATATTTAAAGagcgtaccocgATACTTGTTTAAACAATCTCT	use with 01478	
		02112	AGAATTTGTTAAACAAGTATcggggtacgtCTTAAATATGGAT	use with 01479	
mutate GACGTACAACG to GACGTACAAA		02113	CCATATTTAAAGagcgtacaaaaTACTTGTTTAAACAATCTCTTC	use with 01478	
		02114	GAAAGAATTGTTAAACAAGTATtttgtacgtCTTAAATATGG	use with 01479	
mutate -257 bp G → T		02262	tatttaagaTAGTACAACGatacttgttaacaattcttctc	use with 01478	
		02264	ttgttaacaagatCGTTGTACGTATctttaaattggatgctctctc	use with 01479	
mutate -256 bp A → T		02265	tatttaagaGTGTCACAACGatacttgttaacaattcttctc	use with 01478	
		02266	ttgttaacaagatCGTTGTACGATctttaaattggatgctctctc	use with 01479	
mutate -255 bp C → T		02267	tatttaagaGATGTACAACGatacttgttaacaattcttctc	use with 01478	
		02268	ttgttaacaagatCGTTGTACATCTctttaaattggatgctctctc	use with 01479	
mutate -254 bp G → T		02269	tatttaagaGACTTTACAACGatacttgttaacaattcttctc	use with 01478	
		02270	ttgttaacaagatCGTTGTAAAGTctctttaaattggatgctctctc	use with 01479	
mutate -253 bp T → A		02271	tatttaagaGAGGAACAACGatacttgttaacaattcttctc	use with 01478	
		02272	ttgttaacaagatCGTTGTCTGCTctttaaattggatgctctctc	use with 01479	
mutate -252 bp A → T		02273	tatttaagaGACGTTCAACGatacttgttaacaattcttctc	use with 01478	
		02274	ttgttaacaagatCGTTGAACGTctttaaattggatgctctctc	use with 01479	
mutate -251 bp C → T		02275	tatttaagaGACGTATACAACGatacttgttaacaattcttctc	use with 01478	
		02276	ttgttaacaagatCGTTATACGTctttaaattggatgctctctc	use with 01479	
mutate -250 bp A → T		02277	tatttaagaGACGTACTACGatacttgttaacaattcttctc	use with 01478	
		02278	ttgttaacaagatCGTAGTACGTctttaaattggatgctctctc	use with 01479	
mutate -249 bp A → T		02280	tatttaagaGACGTACATCTGatacttgttaacaattcttctc	use with 01478	
		02281	ttgttaacaagatCGATGTACGTctttaaattggatgctctctc	use with 01479	
mutate -248 bp C → T		02282	tatttaagaGACGTACAATGatacttgttaacaattcttctc	use with 01478	
		02283	ttgttaacaagatCATTGTACGTCTctttaaattggatgctctctc	use with 01479	
mutate -247 bp G → T		02284	tatttaagaGACGTACAACATatacttgttaacaattcttctc	use with 01478	
		02285	ttgttaacaagatAGTTGTACGTCTctttaaattggatgctctctc	use with 01479	
mutate -257 bp G → C	02360	gaagcatcccatatttaagaCAGGTACAACGatacttgttaacaattcttctc	use with 01478		
	02361	aaagtattgttaacaagatCGTTGTACGTGctttaaattggatgctctctc	use with 01479		
mutate -256 bp A → G	02362	gaagcatcccatatttaagaGGCGGTACAACGatacttgttaacaattcttctc	use with 01478		
	02363	aaagtattgttaacaagatCGTTGTACGCTctttaaattggatgctctctc	use with 01479		
mutate -255 bp C → G	02364	gaagcatcccatatttaagaGAGGTACAACGatacttgttaacaattcttctc	use with 01478		
	02365	aaagtattgttaacaagatCGTTGTACCTctttaaattggatgctctctc	use with 01479		
mutate -254 bp G → C	02366	gaagcatcccatatttaagaGACCTACAACGatacttgttaacaattcttctc	use with 01478		
	02367	aaagtattgttaacaagatCGTTGTAGTCTctttaaattggatgctctctc	use with 01479		
mutate -253 bp T → G	02368	gaagcatcccatatttaagaGACGGACAACGatacttgttaacaattcttctc	use with 01478		
	02369	aaagtattgttaacaagatCGTTGTCCGCTctttaaattggatgctctctc	use with 01479		
mutate -252 bp A → G	02370	gaagcatcccatatttaagaGACGTGCAACGatacttgttaacaattcttctc	use with 01478		
	02371	aaagtattgttaacaagatCGTTGCACGTCTctttaaattggatgctctctc	use with 01479		
mutate -251 bp C → G	02372	gaagcatcccatatttaagaGACGTAGAACGatacttgttaacaattcttctc	use with 01478		
	02373	aaagtattgttaacaagatCGTTGTACGTCTctttaaattggatgctctctc	use with 01479		
mutate -250 bp A → G	02374	gaagcatcccatatttaagaGACGTACGACGatacttgttaacaattcttctc	use with 01478		
	02375	aaagtattgttaacaagatCGTGTACGTCTctttaaattggatgctctctc	use with 01479		
mutate -249 bp A → G	02376	gaagcatcccatatttaagaGACGTACAGCGatacttgttaacaattcttctc	use with 01478		
	02377	aaagtattgttaacaagatCGCTGTACGTCTctttaaattggatgctctctc	use with 01479		
mutate -248 bp C → G	02378	gaagcatcccatatttaagaGACGTACAAGGatacttgttaacaattcttctc	use with 01478		
	02379	aaagtattgttaacaagatCCTGTACGTCTctttaaattggatgctctctc	use with 01479		
mutate -247 bp G → C	02380	gaagcatcccatatttaagaGACGTACAACATatacttgttaacaattcttctc	use with 01478		
	02381	aaagtattgttaacaagatGGTTGTACGTCTctttaaattggatgctctctc	use with 01479		
<i>CgPMU3</i> UAS:: <i>PacI</i>		02455	gagaagcatcccatatttaagaTTAATTAAtacttgttaacaattcttctc	use with 01478	
		02456	gaaagaattgttaacaagatTTAATTAAtcttaaattggatgctctctc	use with 01479	
<i>CgPMU3</i> UAS:: <i>CgPET18</i> UAS		02534	gattggcgtctaaattaagagaagcatccatatttaagaTTGTTCCGTGTAAATGAatac	use with 01478	
		02536	agatgtgtccaaaggaagaattgttaacaagatTCATTTACAGGAAACAatctt	use with 01478	
<i>CgPMU3</i> UAS:: <i>CgTH10</i> UAS		02532	gattggcgtctaaattaagagaagcatccatatttaagaGTTTCCCTCTAAGTGAaata	use with 01478	
		02533	agatgtgtccaaaggaagaattgttaacaagatTTCACTTAGAGGGAACGctct	use with 01478	
<i>CgPMU3</i> p + <i>ScTH12</i> site (forward)		02672	TGGATTGCCGTCTAATTAAAGgaaacccttagagTAAGAGACGTACACGATAC	use with 01478	
		02673	GTATCGTTGTACGTCTTAActctaaaggggttctcCTTAATTAGACGGCAATCCA	use with 01478	
<i>CgPMU3</i> p + <i>ScTH12</i> site (reverse)		02674	TGGATTGCCGTCTAATTAAAGctctaaaggggttctcTAAGAGACGTACACGATAC	use with 01478	
		02675	GTATCGTTGTACGTCTTAAggaacccttagagCTTAATTAGACGGCAATCCA	use with 01479	

CgPET18 p-YFP-pRS313	-1000 bp	01718	ggtggcgccgcgctctagaactagtaggataccAGAGATTACTACAACTGTTTG	use with 01720
	-400 bp	01719	ggtggcgccgcgctctagaactagtaggataccTTTACGCTACACCTTCGAG	use with 01720
	-328 bp	02457	ccgcggtggcgccgcgctctagaactagtaggataccTGGATATCAGATTGTTCCGCT	use with 01720
	-318 bp	02458	ccgcggtggcgccgcgctctagaactagtaggataccGATATCAGATTGTTCCGCTG	use with 01720
	-314 bp	02459	ccgcggtggcgccgcgctctagaactagtaggataccTATCAGATTGTTCCGCTGTA	use with 01720
	-312 bp	02460	ccgcggtggcgccgcgctctagaactagtaggataccTACAGATTGTTCCGCTGTA	use with 01720
	-300 bp	01844	ggtggcgccgcgctctagaactagtaggataccAGTGGATATCAGATTGTTCCG	use with 01720
	-200 bp	01843	ggtggcgccgcgctctagaactagtaggataccTATTTTGAATAAAGATGTT	use with 01720
	-100 bp	01842	ggtggcgccgcgctctagaactagtaggataccGCAATGAGGTATAAATAGGGC	use with 01720
	at ATG with homology to YFP (3') PMU3 UAS	01720	accagtgaataattcttcaaccttagacattTGTTTACAAACATTTGGTTA	
		02599	ACAGCCATACGGCCTTATGgATCAATAATTTTGTAAATAAG	use with 01720
		02600	TTTATTACAAAATATTAGTccATAAGGCCGTATGGCTGTG	use with 01718
	THI UAS: <i>Pacl</i>	02497	GAGCTAATAAAGTGGATATTtaattaaCCGTGTAAATGAATAAGTTATG	use with 01720
		02498	CATAACTTATTCACTTACACGGTtaattaaATATCCACTTTTATTAGCTC	use with 01718
	THI UAS: <i>PMU3</i> UAS	02668	AGTGGATATCAGATTGgaactacaacgAATAAGTTATGCATCTCG	use with 01720
		02669	TGCATAACTTATTcgttgtacgtcCAATCTGATATCCACTTTAT	use with 01718
	mutate -305 bp T -> A	02605	ATAAAGTGGATATCAGATTGatccgtgtaaatgAATAAGTTATGCATCTC	use with 01720
		02606	GATGCATAACTTATTcattttacacggatCAATCTGATATCCACTTTAT	use with 01718
	mutate -304 bp T -> A	02607	ATAAAGTGGATATCAGATTGtaccgtgtaaatgAATAAGTTATGCATCTC	use with 01720
		02608	GATGCATAACTTATTcattttacacggtaCAATCTGATATCCACTTTAT	use with 01718
	mutate -303 bp C -> A	02609	ATAAAGTGGATATCAGATTGttacgtgtaaatgAATAAGTTATGCATCTC	use with 01720
		02610	GATGCATAACTTATTcattttacacgtaaCAATCTGATATCCACTTTAT	use with 01718
	mutate -302 bp C -> A	02611	ATAAAGTGGATATCAGATTGttcagtgtaaatgAATAAGTTATGCATCTC	use with 01720
		02612	GATGCATAACTTATTcattttacactgaaCAATCTGATATCCACTTTAT	use with 01718
	mutate -301 bp G -> A	02613	ATAAAGTGGATATCAGATTGttccatgtaaatgAATAAGTTATGCATCTC	use with 01720
		02614	GATGCATAACTTATTcattttacatggaaCAATCTGATATCCACTTTAT	use with 01718
	mutate -300 bp T -> A	02615	AAAGTGGATATCAGATTGttccagtgtaaatgAATAAGTTATGCATCTC	use with 01720
		02616	GAGATGCATAACTTATTcattttactcggaaCAATCTGATATCCACTTT	use with 01718
	mutate -299 bp G -> A	02617	AAAGTGGATATCAGATTGttccgtgtaaatgAATAAGTTATGCATCTC	use with 01720
		02618	GAGATGCATAACTTATTcattttacacggaaCAATCTGATATCCACTTT	use with 01718
	mutate -298 bp T -> A	02619	AAAGTGGATATCAGATTGttccgtgtaaatgAATAAGTTATGCATCTCG	use with 01720
		02620	CGAGATGCATAACTTATTcattttacacggaaCAATCTGATATCCACTTT	use with 01718
	mutate -297 bp A -> C	02621	AAAGTGGATATCAGATTGttccgtgtcaatgAATAAGTTATGCATCTCG	use with 01720
		02622	CGAGATGCATAACTTATTcattgtacacggaaCAATCTGATATCCACTTT	use with 01718
	mutate -296 bp A -> C	02623	AAAGTGGATATCAGATTGttccgtgtacatgAATAAGTTATGCATCTCG	use with 01720
		02624	CGAGATGCATAACTTATTcattgtacacggaaCAATCTGATATCCACTTT	use with 01718
	mutate -295 bp A -> C	02625	AAAGTGGATATCAGATTGttccgtgtcaatgAATAAGTTATGCATCTCG	use with 01720
		02626	CGAGATGCATAACTTATTcattgtacacggaaCAATCTGATATCCACTTT	use with 01718
	mutate -294 bp T -> A	02627	AAAGTGGATATCAGATTGttccgtgtcaaatgAATAAGTTATGCATCTCG	use with 01720
		02628	CGAGATGCATAACTTATTcattttacacggaaCAATCTGATATCCACTTT	use with 01718
	mutate -293 bp G -> A	02629	AAAGTGGATATCAGATTGttccgtgtcaaatAATAAGTTATGCATCTCG	use with 01720
		02630	CGAGATGCATAACTTATTcattttacacggaaCAATCTGATATCCACTTT	use with 01718
CgTH10 p-YFP-pRS313	-1000 bp	01588	GGTGGCGCGCCTCTAGAAGTGTGGATCC TACTCATCATCTTTACGCA	use with 1587
	-900 bp	01589	GGTGGCGCGCCTCTAGAAGTGTGGATCC CGAAGATCCAGCAGAAAT	use with 1587
	-800 bp	01590	GGTGGCGCGCCTCTAGAAGTGTGGATCC TGAACCGGATTTTCTCTTTT	use with 1587
	-700 bp	01591	GGTGGCGCGCCTCTAGAAGTGTGGATCC GGACATTGAGACTGGTTAAT	use with 1587
	-600 bp	01592	GGTGGCGCGCCTCTAGAAGTGTGGATCC GTGACTCGTGAAAGGATGA	use with 1587
	-580 bp	02316	ACC CGCGTGGCGCGCCTCTAGAAGTGTGGATCCATGGCGAGGTTCCCTCTA	use with 1587
	-575 bp	02404	accgcggtggcgccgcgctctagaactagtaggataccCGAGGTTTCCCTCTAAGTGA	use with 1587
	-570 bp	02405	accgcggtggcgccgcgctctagaactagtaggataccTTTCCCTCTAAGTGAATGCT	use with 1587
	-565 bp	02406	accgcggtggcgccgcgctctagaactagtaggataccCTCTAAGTGAATGCTTTTGG	use with 1587
	-564 bp	02502	ccgcggtggcgccgcgctctagaactagtaggataccTCTAAGTGAATGCTTTTGGAA	use with 1587
	-563 bp	02503	ccgcggtggcgccgcgctctagaactagtaggataccCTAAGTGAATGCTTTTGGAA	use with 1587
	-562 bp	02504	ccgcggtggcgccgcgctctagaactagtaggataccTAAGTGAATGCTTTTGGAA	use with 1587
	-561 bp	02505	ccgcggtggcgccgcgctctagaactagtaggataccAAGTGAATGCTTTTGGAA	use with 1587
	-560 bp	02317	ACC CGCGTGGCGCGCCTCTAGAAGTGTGGATCCAGTGAATGCTTTTGGAAATGC	use with 1587
	-540 bp	02318	ACC CGCGTGGCGCGCCTCTAGAAGTGTGGATCCGGATAAGTGGATGAGACGG	use with 1587
	-520 bp	02319	ACC CGCGTGGCGCGCCTCTAGAAGTGTGGATCCCTGCCCATGTGTGGTGTAA	use with 1587
	-500 bp	01593	GGTGGCGCGCCTCTAGAAGTGTGGATCC CGATTGGCACTACGGCAAGA	use with 1587
	-400 bp	01594	GGTGGCGCGCCTCTAGAAGTGTGGATCC CACACTGGTACAGTAATAAT	use with 1587
	-300 bp	01595	GGTGGCGCGCCTCTAGAAGTGTGGATCC CACCACTGGCGTGCACATA	use with 1587
	-200 bp	01596	GGTGGCGCGCCTCTAGAAGTGTGGATCC TATAAGGAAAAAATTTT	use with 1587
	-100 bp	01597	GGTGGCGCGCCTCTAGAAGTGTGGATCC TAGAGCTTTTGTCTACTTT	use with 1587
	at ATG with homology to YFP (3') THI UAS: <i>Pacl</i>	01587	ACCAGTGAATAATTCTTCACTTTTACAT TGTTGACGGCTGTATGGG	
		02549	CGTGAAGGATGAATGGCGAGGTTtaattaaATGCTTTTGAATGCTGGATAAG	use with 1587
		02550	CTTATCCAGCATTTCCAAAAGCATTTtaattaaAACCTCGCCCATTCATCCTTTCACG	use with 1588
	THI UAS: <i>PMU3</i> UAS	02670	TGAATTGGCGAGGTgaactacaacgAATGCTTTTGAATGCTGGA	use with 1587
		02671	ATTCAAAAGCATTtgctgtacgtcACCTGCCCATTCATCTCTT	use with 1588
	<i>CgTH10</i> p + <i>ScTH2</i> site (forward)	02707	TGAATTGGCGgaaacaccttagagAGGTTtccctctaagtgaATGCTTTTGAATGCTGG	use with 1587
		02708	AAGCATTcaacttagagggaaACCTCctctaagggtttccGCCATTCATCCTTTCACGAG	use with 1588
	<i>CgTH10</i> p + <i>ScTH2</i> site (reverse)	02709	TGAATTGGCGctctaagggtttccGAGGTTtccctctaagtgaATGCTTTTGAATGCTGG	use with 1587
		02710	AAGCATTcaacttagagggaaACCTCGgaaacaccttagagGCCATTCATCCTTTCACGAG	use with 1588
	<i>CgTH10</i> p + <i>ScTH2</i> site (forward) + THI UAS	02711	CTCGTGAAGGATGAATGGCGgaaacaccttagagAGGTAATGCTTTTGAATGCTGG	use with 1587
		02712	CCAAAAGCATTAACCTCctctaagggtttccGCCATTCATCCTTTCACGAGTCAACCAC	use with 1588
CgTH20 p-YFP-pRS313	-1000 bp	01784	ggtggcgccgcgctctagaactagtaggataccCCGATATTCATCAACT	use with 01786
	-500 bp	01785	ggtggcgccgcgctctagaactagtaggataccATTTTTCGCTCTTTTAT	use with 01786
	-400 bp	01846a	ggtggcgccgcgctctagaactagtaggataccAACTGAACGAAGTTCGAACAA	use with 01786
	-300 bp	01847a	ggtggcgccgcgctctagaactagtaggataccCAATATCATCAAAATCAACT	use with 01786
	-295 bp	02499	ccgcggtggcgccgcgctctagaactagtaggataccATCATCAAAATCAACTAAAT	use with 01786
	-290 bp	02500	ccgcggtggcgccgcgctctagaactagtaggataccCAAAATCAACTAAATTTAC	use with 01786
	-285 bp	02501	ccgcggtggcgccgcgctctagaactagtaggataccTCAACTAAATTTCACTCTAA	use with 01786
	-280 bp	02441	ccgcggtggcgccgcgctctagaactagtaggataccTAAATTTCACTCTAATTTGA	use with 01786
	-260 bp	02442	ccgcggtggcgccgcgctctagaactagtaggataccATAATTTTGTGATACCTAT	use with 01786
	-240 bp	02443	ccgcggtggcgccgcgctctagaactagtaggataccGATAAATAATATAAATTCG	use with 01786
	-220 bp	02444	ccgcggtggcgccgcgctctagaactagtaggataccATTGTTCTAAGGGCACTGGT	use with 01786
	-200 bp	01848a	ggtggcgccgcgctctagaactagtaggataccTGCACAACTGGATGGGTGG	use with 01786
	-180 bp	02445	ccgcggtggcgccgcgctctagaactagtaggataccGGATGAGAAGGCCAAGGCTC	use with 01786
	-100 bp	01849a	ggtggcgccgcgctctagaactagtaggataccTCTGTTGTAAATATCTGGTCT	use with 01786
	at ATG with homology to YFP (3') PMU3 UAS	01786	accagtgaataattcttcaaccttagacattTATTGTAAAGTTCCTCGAGCT	
		02603a	CGATCAACTGAACGAAGcTGCCTGCGAAACCTCGCTATTGACGTTTTT	use with 01786
		02604	ATGACGAGGTTTCGCGAGCAgcTTCGTTTCAGTTGATCGTTG	use with 01784
CgTH14 p-YFP-pRS313	-950 bp	01249	ggtggcgccgcgctctagaactagtaggataccctgattatgtcagatgtgaata	use with 01250
	-750 bp	01408	ggtggcgccgcgctctagaactagtaggataccTCAAAAATAACCCCATACAC	use with 01250
	-650 bp	01409	ggtggcgccgcgctctagaactagtaggataccTTAAGAGGTGATCGATAGAG	use with 01250
	-550 bp	01410	ggtggcgccgcgctctagaactagtaggataccTTGACTAGTAAGCCAAAGCCATG	use with 01250
	-450 bp	01411	ggtggcgccgcgctctagaactagtaggataccCTAAGTTTGTGACAAATGACC	use with 01250
	-430 bp	01443	ggtggcgccgcgctctagaactagtaggataccATTAACAAATACAGAAATAC	use with 01250
	-410 bp	01444	ggtggcgccgcgctctagaactagtaggataccACCTTTTTTTTTTCTTTTGTCT	use with 01250
	-390 bp	01445	ggtggcgccgcgctctagaactagtaggataccTGTGGAATCGAATTTAAACATG	use with 01250
	-370 bp	01446	ggtggcgccgcgctctagaactagtaggataccAATGCCCTTGAATTTGCATATT	use with 01250
	-350 bp	01412	ggtggcgccgcgctctagaactagtaggataccTATTTTAAACAAAATATCAAG	use with 01250
	-250 bp	01413	ggtggcgccgcgctctagaactagtaggataccACCACTGTATCTTGAAG	use with 01250
	at ATG with homology to YFP (3')	01250	accagtgaataattcttcaaccttagacatttttatttttagtaattat	

ScTHI5 p-YFP-pRS313	-1000 bp at ATG with homology to YFP (3') Mutate putative ancestral site at -217 bp from caactagcagaag to ggcaatccctgtg	O2398 O2397	accgcggtggcgccgctctagaactagtggaatccGTCCCTGTCGACTTGCTGAA accagtgaaataattcttcaccttagacatAGCTTGGAAGTGTGTTTATT	use with O2397
	Delete -220 bp to -210 bp	O2838 O2839 O2643 O2644	TGAACTATGAAATATACGAATTATTggcaatccctgtgGCTTGAAATATTACTTAT ATAAGTAAATATTTCAGCCacagggaattgccAAATAATTCGTATATTTCATAGTTCA ACTATGAAATATACGAATTaaGAAGGCTTGAAATATTACT AGTAATATTTCAGGCCTTCttAATTCGTATATTTCATAGT	use with O2397 use with O2398 use with O2397 use with O2398
	Delete -210 bp to -200 bp	O2645 O2646	ATACGAATTATTCAACTAGcaAAATATTACTTATTCAGATT AATCTGAATAAGTAATATTtgCTAGTTGAATAATTCGTAT	use with O2397 use with O2398
	Delete -200 bp to -190 bp	O2647 O2648	TTCAACTAGCAGAAGGCTTgtATTCAAGATTATGAAAATGG CCATTTTCATAATCTGAATacAAGCCTTCCTGCTAGTTGAA	use with O2397 use with O2398
	Delete -190 bp to -180 bp	O2649 O2650	AGAAGGCTTGAAATATTACTaTGAAAATGGTGTGTTATA TATAACAGCACCATTTCtataGTAATATTTCAGCCTTCT	use with O2397 use with O2398
	Delete -120 bp to -110 bp	O2651 O2652	CTCTTGGCAGCAGCAAAcaAAACCTCGTATTATCATCT AGATGATAATACGAGGTTTtgTTTGCTGTCTGCGCAAGAG	use with O2397 use with O2398
	Delete -110 bp to -100 bp	O2653 O2654	GACAGCAAAcaAAACATATaaTTATCATCTTCATGAACAA TTGTTTCATGAAGATGATAattATATGTTTTGTTGCTGTC	use with O2397 use with O2398
	Delete -100 bp to -90 bp	O2655 O2656	AAAACATATAAAAACCTCGttCATGAACAATATTCTGTC GACAAGAATATTGTTTCATGaaCGAGGTTTTATATGTTTT	use with O2397 use with O2398
ScTHI20 p-YFP-pRS313	-1000 bp at ATG with homology to YFP (3') Mutate putative ancestral site at -150to-137 from cagctggcagaaa to gtccctttcggg	O2391 O2390	accgcggtggcgccgctctagaactagtggaatccGTGACCAATTAATTCTATAT accagtgaaataattcttcaccttagacatAGTAAATGCGAGACGTTTT	use with O2390
	Delete -170 bp to -160 bp	O2840 O2841 O2635 O2636	GTTAGGAAACCCTTAGAGCTATGCGtcccctttcgggAAATCCTATTATTTAAACC GGTTTAAATAATAGGATTTCcgaaggggacGCATAGCTCTAAGGGTTTCCTAAC ATGATCCCCGATGAAGGTTaaGAGCTATGCCAGCTGGCAG CTGCCAGCTGGCATAGCTCttAACCTTCATCGGGGATCAT	use with O2390 use with O2391 use with O2390 use with O2391
	Delete -160 bp to -150 bp	O2637 O2638	ATGAAGGTTAGGAAACCCTtaAGCTGGCAGAAAAATCCTA TAGGATTTTTTCTGCCAGCtaAGGGTTCCTAACCTTCAT	use with O2390 use with O2391
	Delete -150 bp to -140 bp	O2639 O2640	GAAACCCTTAGAGCTATGcCaAAATCCTATTATTAAAC GTTTAAATAATAGGATTTTTtgGCATAGCTTAAGGGTTTC	use with O2390 use with O2391
	Delete -140 bp to -130 bp	O2641 O2642	GAGCTATGCCAGCTGGCAGatTATTAAACCATCAGACAA TTGTCTGATGGTTTAAATAatCTGCCAGCTGGCATAGCTC	use with O2390 use with O2391
ScTHI4 p-YFP-pRS313	-950 bp at ATG with homology to YFP (3')	O1241 O1242	ggtggcgccgctctagaactagtggaatccAAATGATAATTAAATATACTAG accagtgaaataattcttcaccttagacatTTTGATAGTTAGTTGATTTTT	use with O1242

Figure S1

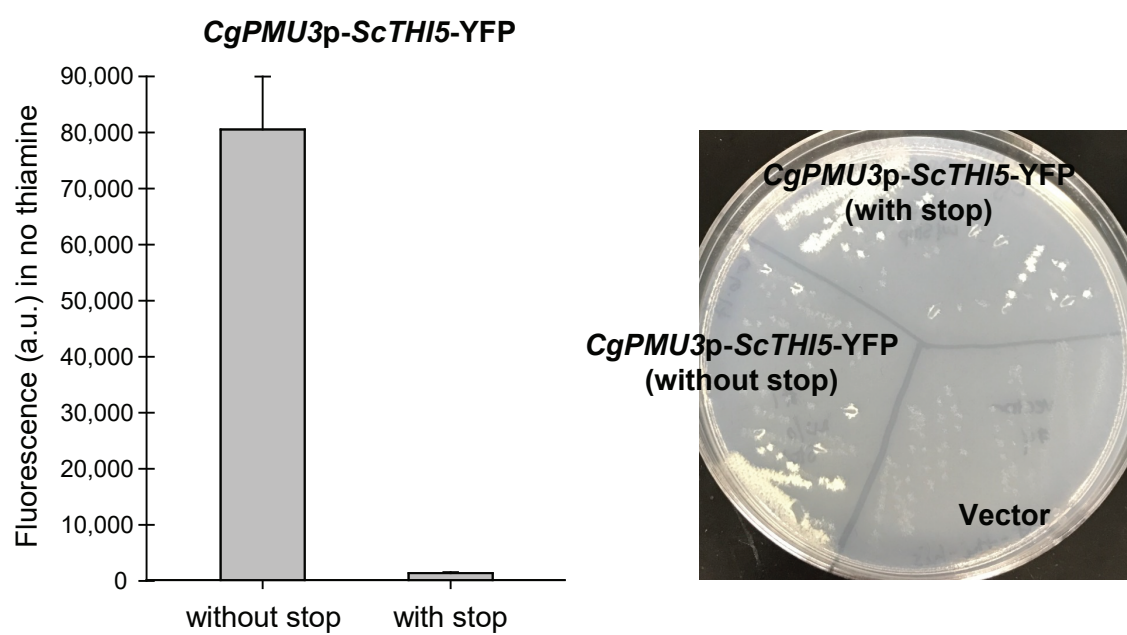


Figure S2

Frequency of top 20 most abundant sequences in each sample

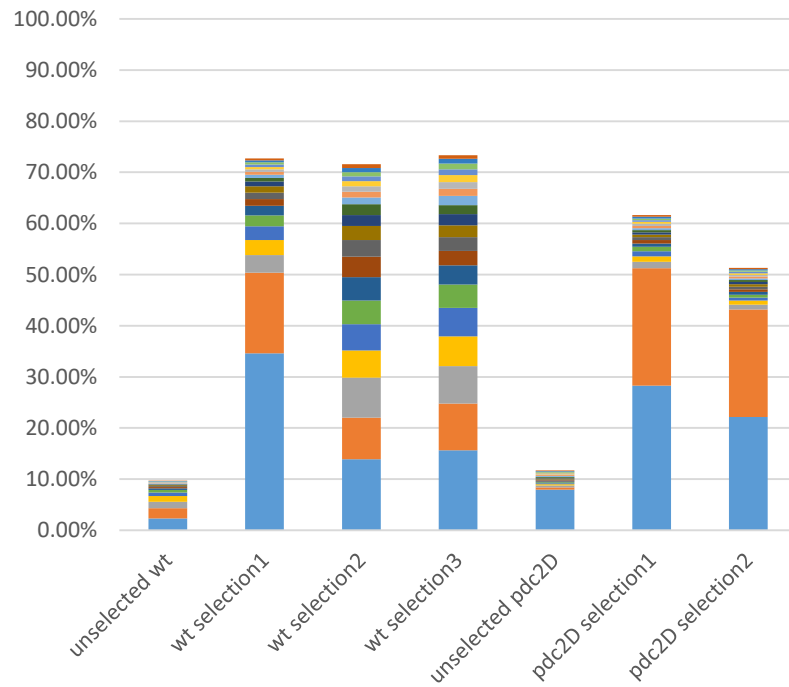


Table S2

Raw data from Next Generation sequencing runs sorted by abundance of sequence													
sample 1 unselected wt		sample 2 selection1 wt		sample 3 selection2 wt		sample 4 selection3 wt		sample 5 unselected pdc2D		sample 6 selection1 pdc2D		sample 7 selection2 pdc2D	
CAGTTACCCGG	2.3%	ATCGTATATTA	34.6%	CAGTTACCCGG	13.9%	CAGTTACCCGG	15.7%	GACGTACAAG	7.9%	CTTCGTATGAA		GTGTAAGTTGA	22.2%
ATCGTATATTA	2.0%	TTTCGTGTAAA	15.8%	TTTCGTGTAAA	8.1%	ATCGTATATTA	9.1%	GTGTAAGTTGA	0.4%	GTGTAAGTTGA	23.0%	CTTCGTATGAA	21.0%
CGTAGTTCAATG	1.3%	TCACGTGGCAT	3.4%	ATCGTATATTA	7.8%	TTTACCCGTGAC	7.3%	CTTCGTATGAA	0.4%	CAGTTACCCGG	1.3%	GACGTACAAG	1.0%
CACACTTTACG	1.1%	GTGTAAGTTGA	2.9%	CGTTCTCCGTG	5.3%	TTTCGTGTAAA	5.8%	TTCCGTATGTT	0.2%	ATCGTATATTA	1.0%	GAGTGCCAATT	0.8%
CATTCCGAAAG	0.7%	ATCGTATATATG	2.7%	TTTACCCGTGAC	5.1%	CACACTTTAAG	5.6%	CTTAGTGCCTG	0.2%	TTTAACCCGTGAC	1.0%	GTGTAAGTTGC	0.6%
CGTACCATGCG	0.5%	ATCGTATATTAAG	2.1%	CACACTTTAAG	4.6%	CGTTCTCCGTG	4.6%	CTGTATGTATG	0.2%	GTGGAAGTTGA	0.9%	CTTCGTATTTGC	0.6%
ATCGTATTTATG	0.3%	CGTACATTAAG	1.9%	CGTAGTTCAATG	4.6%	CGTAGTTCAATG	3.7%	CTCCGTATGAA	0.2%	CTCCGTATGAA	0.7%	CAGTTACCCGG	0.5%
CACTGTGCAAG	0.2%	ATCGTATGCAA	1.3%	CATTCCGAAAG	4.0%	CATTCCGAAAG	2.8%	GCCTGATATGTG	0.2%	CTTCGTATGAG	0.7%	GGGTAAACATT	0.5%
CCCGTACTTACA	0.2%	ATCGTTCAATTA	1.3%	CTTCGTATGAA	3.3%	CACGTGTGACAG	2.7%	AAGCTTCGCAC	0.2%	CTTAGTATGAA	0.5%	CTTCGTATGAC	0.5%
ATCGTATTTCAA	0.2%	TTGCTTATAG	1.2%	CACTGTGCAAG	2.7%	ATCGTATTTCAA	2.4%	TTCTCTGCAAG	0.2%	TTCTCTGCAAG	0.5%	CTTCGTGTGAA	0.5%
CAAAAAGTATTT	0.1%	CCCGTACTACA	0.9%	ATCGTATTTATG	2.2%	CGTACATTAAG	2.2%	GACTGTACGTG	0.2%	GTGCGTATGAA	0.5%	GTGCTTGTGGA	0.5%
CAACTAGTGCAG	0.1%	AAGATCACGTT	0.7%	CGTACCATGCG	2.1%	CTTCGTATGAA	1.8%	TTGTGTGATTT	0.2%	GTGTAAGTTGG	0.4%	TTTCGTGTAAA	0.4%
CGTTAGTATATG	0.1%	ATTCGTATGAA	0.6%	CGTACATTAAGGG	1.3%	CGTCATGGGTG	1.8%	GATCAGCATTTG	0.2%	CGTAGTTCATG	0.4%	TTTCGTGTGAA	0.3%
GCAACTGACTTT	0.1%	ATCGTATTTTAA	0.6%	CGTACATTAAG	1.1%	ATCGTATTTATG	1.4%	CACAACGCGTC	0.2%	CGTTCTCCGTG	0.4%	CACAACGCGTC	0.3%
TACAATGAAT	0.1%	ATCGTAGTTTA	0.5%	CGTCATGGGTG	1.0%	CGTGTAAAGG	1.3%	GAATTACAGGA	0.2%	CATTGTTTACG	0.4%	AGATTCCGATT	0.3%
ATCATATCCATC	0.1%	ATCTTATATTA	0.5%	TCACGTGGCAT	1.0%	TCACATATTAAG	1.3%	TTTTGTGCGCA	0.2%	CTTCGTGTGAA	0.4%	ATCGTATATTA	0.3%
ATCGTTTCATPA	0.1%	AATCGTGTAAA	0.5%	CTCTCTGTAAG	0.9%	GTGTAAGTTGA	1.2%	TTGCTTCCAG	0.2%	TTTCGTGTAAA	0.3%	CTTCGTATGAA	0.3%
CTCAGTTTCGTC	0.1%	CTCGTAAACAA	0.5%	GTGTAAGTTAG	0.9%	GTCTTACCCGG	1.1%	GTTCAGCTGAT	0.2%	CACACTTTTACG	0.3%	GTTCGTATGGA	0.3%
TACTCTGTAGC	0.1%	TCACGTGACCT	0.4%	TTTCGTATTAAG	0.8%	TCACGTGGCAT	1.0%	CTTCGACAGGA	0.1%	CGTCGTATGAA	0.3%	GACCAATATAT	0.3%
CATTGAGTACG	0.1%	GATATCATTTCT	0.3%	CTTCCTTACGAA	0.7%	ATCGTTCAATPA	0.7%	TTCAGTGTCCG	0.1%	GTGTAAGTTGA	0.3%	GTGTAATTTGA	0.3%
6128 out of 8986 unique		483 out of 2117 unique		356 out of 1750 unique		416 out of 2082 unique		4347 out of 8175 unique		764 out of 2398 unique		2677 out of 10382 unique	
Percent unique	68.2%		22.8%		20.3%		20.0%		53.2%		31.9%		25.8%

Table S3

Sequences that were highly enriched in wild-type by selection in no thiamine conditions

		Fold enrichment	Final proportion	Fold enrichment in <i>pdc2Δ</i>	times observed in wt selection
A	TTTCGTGTAAA	888x	9.9%	<3x	3
B	TTTACCCTGAC	561x	6.2%	6.8x	2
C	CGTTCTCCGTG	445x	5.0%	<3x	2
D	TCACGTGGCAT	161x	18.0%	<3x	3
E	CGTACATAAAG	133x	1.5%	<3x	3
F	CGTCATGGGTG	126x	1.4%	<3x	2
G	CGTGTTAAGGG	119x	1.3%	<3x	2
H	TTCGTTATAAG	91x	1.0%	<3x	2

Figure S3

CgPET18		--TTCCGTGTAAAATG--
A		--TTTCGTGTAAA---
G		-----CGTGTTAAGGG
B		TTTACCCTGAC-----
CgPET18		--TTCCGTG-TAAATG--
C		-----CGTTC TCCGTG
F		-----CGTCATGGGTG
CgTHI20		--T TCACTCTAATTT
CgTHI10		--TTCCCTCTAAGTG
CgTHI4		--TGCCCTTGAATTG
H		--TTCGT TATAAG--
CgPMU3	(AGA)	-GACGTACAACG-
E	(AGA)	---CGTACATAAAG-
D	(AGA)	TCACGTGGCAT-

Figure S4

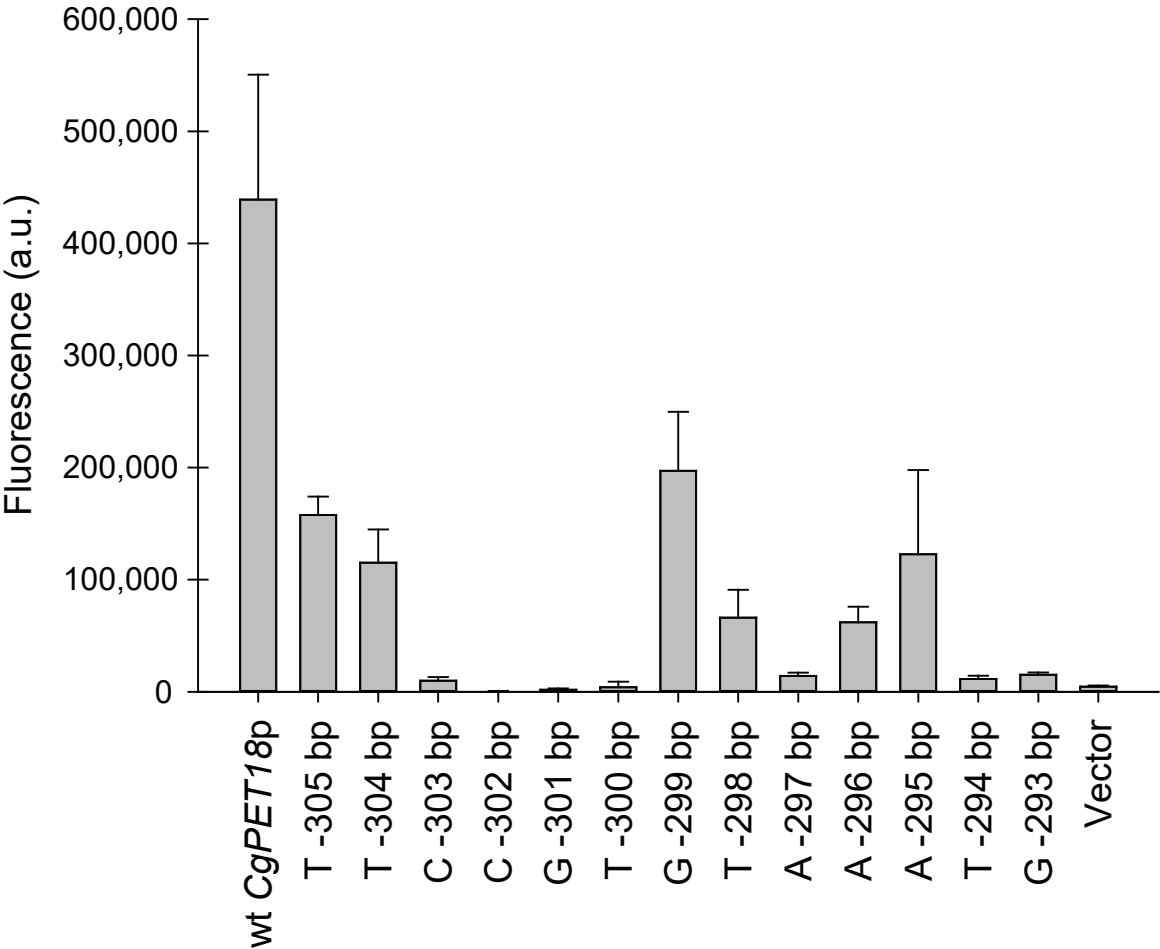
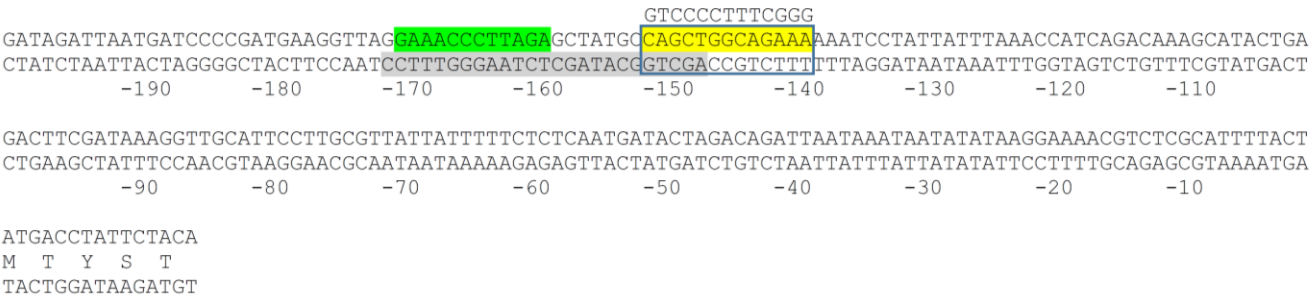


Figure S5

ScTHI5



ScTHI20



-  = candidate THI UAS
-  = candidate Thi2 binding site
-  = Region required for expression in Figure 7B and 7C
-  = site of mutation of THI UAS in Figure 7A

Name 	Strand 	Start 	p-value 	Sites 
2. CgTHI10pr	-	3	1.00e-7	T CACTTAGAGGGGAA AC
5. ScTHI5	+	14	7.52e-6	ACGAATTATT CAACTAGCAGAAAG GCTTGAAATA
6. ScTHI20	+	21	9.95e-6	AGAGCTATGC CAGCTGGCAGAAA AAATCCTATT
1. CgPET18pr	-	3	1.06e-5	T CATTTACACGGAA CA
3. CgTHI4pr	-	3	1.12e-5	G CAATTC AAGGGCA TT
4. CgTHI20pr	-	3	2.34e-5	C AAATTAGAGTGAA AT

Figure S6

