

Figure S1. Induction of genes during thiamine starvation of *C. glabrata* wild-type. *C. glabrata* wild-type was grown in thiamine replete and starvation conditions for four hours . qPCR was performed on reverse-transcribed RNA from these samples to determine the fold induction in thiamine starvation relative to replete. These data confirmed induction of the most highly induced genes during thiamine starvation as determined by the RNA-seq data in Figure 1. Fold induction for *CgPMU2* is 25.6 ± 6.22 and for *CgTHI6* is 26.7 ± 7.28 . Error bars represent the standard deviation of the average of fold induction for three independently grown biological replicates. All seven genes are considered induced based on a Student's T test, $P < 0.01$

Species	GeneID	GeneName	Protein length
S. cerevisiae	YBR093C	PHO5	467
S. cerevisiae	YBR092C	PHO3	467
S. cerevisiae	YHR215W	PHO12	467
S. cerevisiae	YAR071W	PHO11	467
S. cerevisiae	YDL024C	DIA3	468
S. mikatae	2.235	NA	467
S. mikatae	2.234	NA	467
S. mikatae	2.233	NA	467
K. lactis	KLLAO019338g	NA	469
K. lactis	KLLAO00286g	NA	476
K. lactis	KLLAOA00176g	NA	476
K. lactis	KLLAO0D00258g	NA	492
K. lactis	KLLAOE24949g	NA	492
S. pombe	SPBP4G.02	pho1	453
S. pombe	SPBC428.03c	pho4	463
S. pombe	SPBC21H.03c	N/A	463

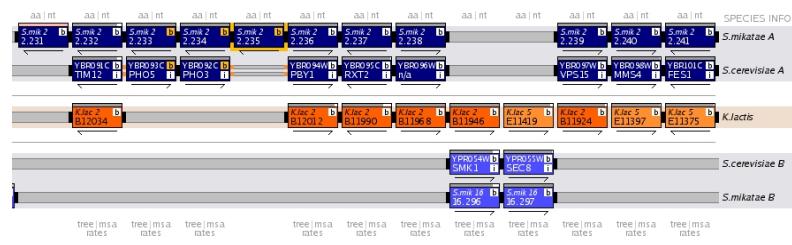


Figure S2. Syntenic relationship between *PHO5* homologs in *S. cerevisiae* and *S. mikatae*. Rows in the figure represent chromosomes and boxes represent genes. Genes are aligned in columns based on inferred syntenic relationship between species, where missing boxes indicate no syntenic homolog detected. *S. cerevisiae* and *S. mikatae* belong to a group of species whose common ancestor has experienced a whole-genome-duplication (WGD). Therefore, each species has two sets of chromosomes (A vs B) that correspond to a single set of ancestral chromosomes. *K. lactis*, as a pre-WGD species, represents the ancestral state. Other features in this figure are explained in detail on the Yeast Gene Order Browser help page (<http://ygb.ucd.ie/>), from which this screenshot was taken on Oct 7, 2017 (Byrne and Wolfe, 2006). This plot shows that the three *PHO5* homologs in *S. mikatae* form a single cluster, suggesting a possible origination through tandem duplication. The syntenic region in *S. cerevisiae* contains just two genes, *ScPHO5* and *ScPHO3*, which are also arranged in tandem. Note that *S. mikatae* likely experienced an additional duplication event as indicated by the yellow box around 2.235. The other three *PHO5* homologs in *S. cerevisiae* are on different chromosomes. We also looked at *K. lactis* and found that none of its five *PHO5* homologs are syntenic with those in *S. cerevisiae* or *S. mikatae*. The distantly related *S. pombe* is not included in YGOB.

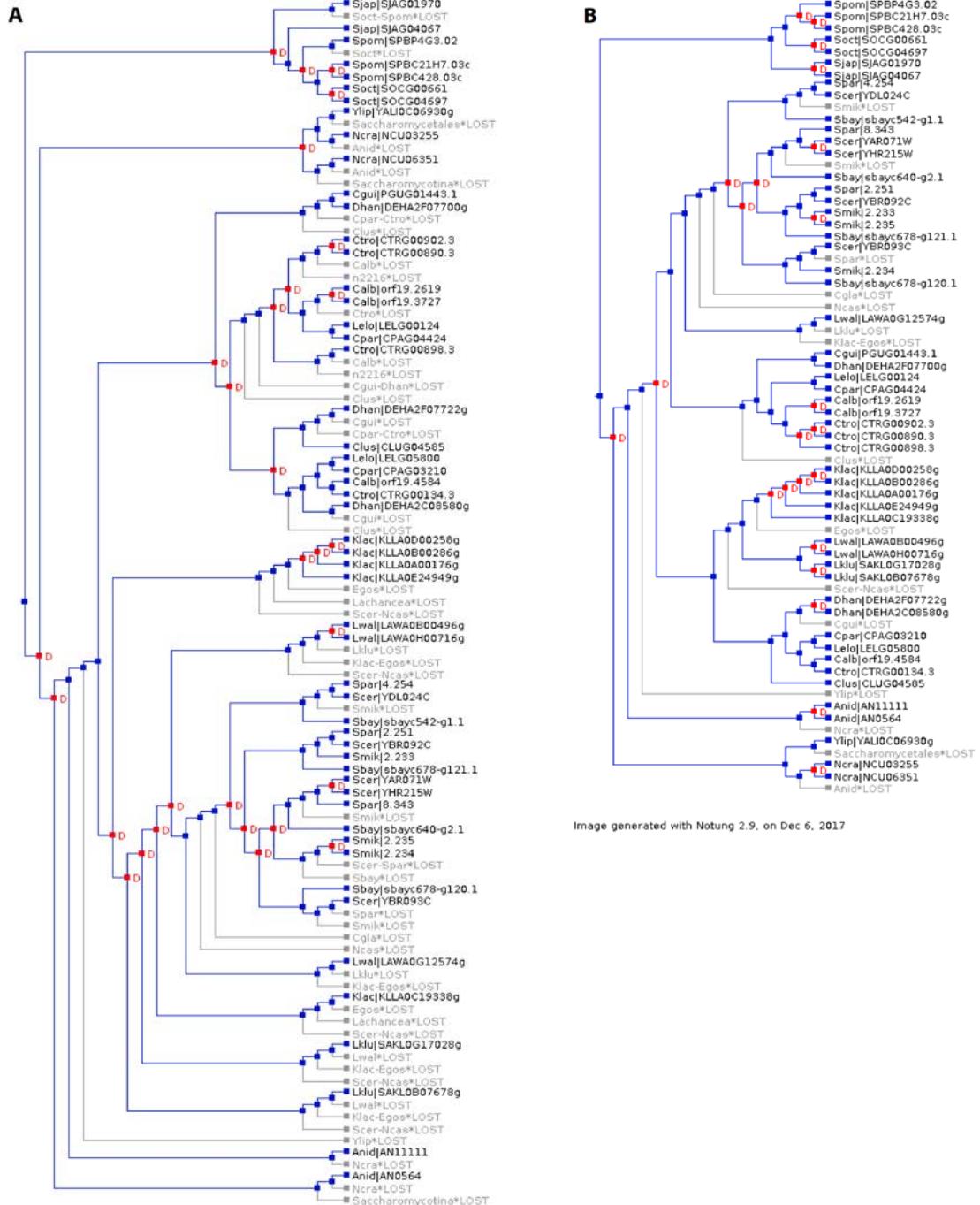


Image generated with Notung 2.9, on Dec 6, 2017

Source of sequences:
 Scer|YBR093C
 Scer|YDL024C
 Scer|YBR092C
 Scer|YHR215W
 Scer|YAR071W
 Smik|2.233
 Smik|2.234
 Spar|2.235
 Spar|4.254
 Spar|8.343

Figure S3. Reconciled gene tree for the PHO5 family showing inferred duplication and loss events. (A) Reconciled tree without rearrangement. Inferred duplication events are marked with "D" in red font. Loss events are shown in gray. Species and gene names are labeled at the leaf nodes, separated by "|". (B) Reconciled and rearranged gene tree. During the rearrangement operation, Notung (v2.9) explores alternative topologies by swapping weakly supported branches with bootstrap values below 90/100 in the reconstructed gene tree. The result is a topology that minimizes the total event score (23/15 duplication/loss events compared to 29/48 before rearrangement).

Sbay|sbayc678-g120.1
Sbay|sbayc542-g1.1
Sbay|sbayc678-g121.1
Sbay|sbayc640-g2.1
Lwal|LAWA0G12574g
Lwal|LAWA0H00716g
Lwal|LAWA0B00496g
Klac|KLLAOCl9338g
Klac|KLLAOB00286g
Klac|KLLAOA00176g
Klac|KLLAOD00258g
Klac|KLLAOE24949g
Lklu|SAKLOG17028g
Lklu|SAKL0B07678g
Clus|CLUG04585
Dhan|DEHA2C08580g
Dhan|DEHA2F07700g
Dhan|DEHA2F07722g
Cgui|PGUG01443.1
Ctro|CTRG00898.3
Ctro|CTRG00134.3
Ctro|CTRG00890.3
Ctro|CTRG00902.3
Calb|orf19.4584
Calb|orf19.3727
Calb|orf19.2619
Cpar|CPAG03210
Cpar|CPAG04424
Lelo|LELG00124
Lelo|LELG05800
Ylip|YALI0C06930g
Anid|AN11111
Anid|AN0564
Ncra|NCU03255
Ncra|NCU06351
Sjap|SJAG01970
Sjap|SJAG04067
Soct|SOCG04697
Soct|SOCG00661
Spom|SPBP4G3.02
Spom|SPBC21H7.03c
Spom|SPBC428.03c

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ATG ATC TTG CAT AGA TTG TTG TTG TTG AAT TTG GAT TGG AAC CCA GAT AAC ACA GCT CAA GAT TTG GAT TTT AGA GCA TTG CCA GGT TAC TTC AAG G < 100
M I L H R L L L L N L D W N P D N T A Q D L D F R A L P G Y F K G
10 20 30 40 50 60 70 80 90

GT TAC ACA GTT CAA GAT ACT CCA GAT ACA AGA ATT AAT GCT ACT GAA TTG GAT TTC TTG GCA TTC GAT GAT AGA TTT TCA AAC TGG CAA CAA TTG TAC CA < 200
Y T V Q D T P D T R I N A T E L D F L A F D D R F S N W Q Q L Y Q
110 120 130 140 150 160 170 180 190

A GCT ATC CCA AAC GAT ACT GAA ACA CAT TCT TAC AAA TTA GTT ATT TTG GCA AGA CAT GGT CAA GGT TAT CAT AAT GCT GCT TAT GAT AGA TAC GGT GAA < 300
A I P N D T E T H S Y K L V I L A R H G Q G Y H N A A Y D R Y G E
210 220 230 240 250 260 270 280 290

AAA GCA TGG TAT GAT TAC TGG TCA AGA TTA GAA GGT GGC CAA TAC GGT ATT TGG TTT GAT GCA GAA TTA ACT CCA TTG GGT AAA AAG CAA GCA TTG GAA G < 400
K A W Y D Y W S R L E G D Q Y G N W F D A E L T P L G K K Q A L E A
310 320 330 340 350 360 370 380 390

CA GGT CAA ACT TAT TTG ACA AAT TTG ACT GAT GGT TTG CAA AGA TTG CCT GAT AAG TTT TTC GTT TCT CCA TTA AGA AGA TGT TTG GAT ACA TGT ATC AG < 500
G Q T Y L T N L T D G L Q R L P D K F F V S P L R R C L D T C I R
410 420 430 440 450 460 470 480 490

A GAA TGG GAA CCA ATC TTC GCT AAG CAT AAG CCA GCA AAT TCA ACT GTT CAT GTT AAA GTT ATT GAA TAC TTA AGA GAA ACA TTG GGT ATC GAT ACT TCT < 600
E W E P I F A K H K P A N S T V H V K V I E Y L R E T L G I D T S
510 520 530 540 550 560 570 580 590

AAC GAA AGA GTT TCT CAT TCA CAA GCA TTG GCA GAA TAC CAA GAT CAT AGA TAC AAC ACA TCA GAT GTT ACT GTT CAT TTC GAT TAT CCT GGT GAC TAC T < 700
N E R V S H S Q A L A E Y Q D H R Y N T S D V T V H F D Y P G D Y S
610 620 630 640 650 660 670 680 690

CT GAA AAA GAT CAA TTA TGG CAA CCA GAT CAT TTG GAA ACA AAA GCT GAA ATG GAT AGA AGA ACT AGA ATT GGT TTG AGA GRA ATG TTT TCT TCA GTT AA < 800
E K D Q L W Q P D H L E T K A E M D R R T R I G L R E M F S S V N
710 720 730 740 750 760 770 780 790

C ACT ACA GAT AAA GTT ATT TCT TTA ACA TGT CAT TCA GAT GTT ATT GAA TCT ATT TTG AGA AAC ATC AAG CAT CCA GCT ATC GAT CAT TTG CAA ACT GGT < 900
T T D K V I S L T C H S D V I E S I L R N I K H P A I D H L Q T G
810 820 830 840 850 860 870 880 890

AAA TTG GTT TAC ACT GTT GTT GAA TTG TCT AAG GCT CCA GCA GAT GAT CAA TCA ATG TTG GTT GTT TCT TAA
K L V Y T V V E L S K A P A D D Q S M L V V S *
910 920 930 940 950 960 970

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Figure S4. Sequence of PMU2/3 ancestor. CgPmu1-3 were aligned using a MUSCLE protein alignment. We identified every case where CgPmu2 and CgPmu3 had the same amino acid and it was different from CgPmu1. This identified 55 amino acids out of the 323 amino acids present in CgPmu1. We then ordered from GENEWIZ (South Plainfield, NJ) a synthetic sequence replacing codons in CgPMU1 with a codon to put the CgPmu2/3 codon in its place.

Table S2. Strains used in this study.

Strain number	Strain	Reference
Yeast strains		
DG5	<i>C. glabrata</i> wild-type (<i>his3-</i>) (BG99)	Cormack and Falkow Genetics 1999
DG141	<i>Cgthi3::NATMX6</i> in DG5	Iosue et al PlosOne 2016
DG176	<i>C. glabrata</i> wild-type (<i>his3-ura3-</i>)	Iosue et al PlosOne 2016
DG2	<i>Cgpho4 ::KANMX6</i> in DG5	Kerwin and Wykoff Genetics 2009
DC3	<i>S. cerevisiae</i> wild-type K699 ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3	Wykoff and O'Shea Genetics 2001
DP1	<i>Schizosaccharomyces pombe</i> wild-type 972 h-	From D. Moazed laboratory
DG29	<i>Cgpmu2::NATMX6</i> in DG5	Orkwis et al Genetics 2010
DG30	<i>Cgpmu3::NATMX6</i> in DG5	Orkwis et al Genetics 2010
DG314	<i>Cgpmu3::CgURA3</i> in DG176	This study
DG320	<i>CgURA3::ScPHO3</i> in DG314	This study
DG318	<i>CgURA3::ScPHO5</i> in DG314	This study
DG327	<i>CgURA3::S. mikataePHO5 g5</i> in DG314	This study
DG328	<i>CgURA3::S. mikataePHO5 g4</i> in DG314	This study
DG332	<i>CgURA3::CgPMU1</i> in DG314	This study
DG317	<i>CgURA3::CgPMU2</i> in DG314	This study
DG345	<i>CgURA3:: Ancestral PMU2/3</i> in DG314	This study
DG319	<i>CgURA3::K. lactis KLLAO_C19338g</i> in DG314	This study
DG373	<i>CgURA3::K. lactis KLLAO_D00258g</i> in DG314	This study
DG326	<i>CgURA3:: S. kluyveriPHO5</i> in DG314	This study
DG331	<i>CgURA3::Y. lipolytica PHO5</i> in DG314	This study
DG329	<i>CgURA3::S. pombePHO1</i> in DG314	This study
DG330	<i>CgURA3::S. pombePHO4</i> in DG314	This study
DG338	<i>ScPHO5 p-ScPHO 3-YFP-pRS313</i> in DG29	This study
DG337	<i>ScPHO5 p-ScPHO5 -YFP-pRS313</i> in DG29	This study
DG335	<i>ScPHO5 p-S. mikatae PHO5 g5 -YFP-pRS313</i> in DG29	This study
DG336	<i>ScPHO5 p-S. mikatae PHO5 g4 -YFP-pRS313</i> in DG29	This study
DG200	<i>ScPHO5 p-CgPMU1 -YFP-pRS313</i> in DG29	Orkwis et al Genetics 2010
DG198	<i>ScPHO5 p-CgPMU 2-YFP-pRS313</i> in DG29	Orkwis et al Genetics 2010
DG199	<i>ScPHO5 p-CgPMU3 -YFP-pRS313</i> in DG29	Orkwis et al Genetics 2010
DG343	<i>ScPHO5 p-Ancestral PMU2/3 -YFP-pRS313</i> in DG29	This study
DG333	<i>ScPHO5 p-K. lactis KLLAO_C19338g -YFP-pRS313</i> in DG29	This study
DG367	<i>ScPHO5 p-K. lactis KLLAO_D00258g -YFP-pRS313</i> in DG29	This study
DG334	<i>ScPHO5 p-S. Kluyveri PHO5 -YFP-pRS313</i> in DG29	This study
DG341	<i>ScPHO5 p-Y. lipolytica PHO5 -YFP-pRS313</i> in DG29	This study
DG339	<i>ScPHO5 p-S. pombe PHO1 -YFP-pRS313</i> in DG29	This study
DG340	<i>ScPHO5 p-S. pombe PHO4 -YFP-pRS313</i> in DG29	This study
DG197	<i>ScPHO5 p-YFP-pRS313</i> in DG29	Orkwis et al Genetics 2010
Bacterial strains		
DB346	3 Kb <i>CgPMU2 p-YFP-pRS313</i>	Kerwin and Wykoff FEMS 2012
DB347	1 Kb <i>CgPMU3 p-YFP-pRS313</i>	This study
DB146	<i>ScPHO5 p-YFP-pRS313</i>	Orkwis et al Genetics 2010
DB147	<i>ScPHO5 p-CgPMU2 -YFP-pRS313</i>	Orkwis et al Genetics 2010
DB148	<i>ScPHO5 p-CgPMU3 -YFP-pRS313</i>	Orkwis et al Genetics 2010

Table S3. Primers used in this study.

<i>ScPHO3</i>	0152	TTGAGGTCAAGTTCAAACCT
	02068	GTAATGGGTGCCCAAGGATGG
	02069	ACGTACCAATTCACTTCGG
<i>ScACT1</i>	0155	TCCTACGAACCTCCAGATGGT
	0156	GGCAGATTCCAAACCCAAAAA
<i>CgPMU1</i>	0191	gtgcacacgtgtatgaacgtg
	0192	cattgggtgatcatctgttaggc
<i>CgPMU2</i>	0664	gtgttgtactgttcatttgattatctcg
	0641	gatacatcgatcacagtgtaaactag
<i>CgPMU3</i>	0195	ctctgcagaaatccaggatc
	0196	ctacgagaattgtatgtatcatc
<i>CgACT1</i>	075	gaccaaaactacttacaactcc
	076	ccactttcgtcgatattctgtctg
<i>SpPHO1</i>	069	gctaatacgcttgc当地atccctc
	070	cttagatgtccaaagatgttcaagag
<i>SpPHO4</i>	02070	ccaggctcgaggctgtcttggg
	02071	gacatgtcgagttaaaatgg
<i>SpACT1</i>	066	ggttttcatgaggctacttacaac
	067	ccgcctctatcatatcttgcctg
<i>CgTHI4</i>	01119	ggacatgaaacaatggccaaagc
	01120	ctcagcgaagtgttcaagat
<i>CgTHI20</i>	01285	CTACATCAGTTCTTCCTTA
	01286	ATTCCTGTCCAAAGGAACTTC
<i>CgTHI10</i>	01287	CGTGGTTGGGTGGTCTATGT
	01288	ATCCAGAGTTGTTGACCGTA
<i>CgPET18</i>	01291	ACCCAGCTCTTATTTCTTAC
	01292	TAGACTCACGTCTATCTAC
<i>CgTHI6</i>	01289	ACATCGGCGTTGGTACATTA
	01290	TGTGGAATTGCAGCTAGATC