A *tps1Δ* persister-like state in *Saccharomyces cerevisiae* is regulated by *MKT1*

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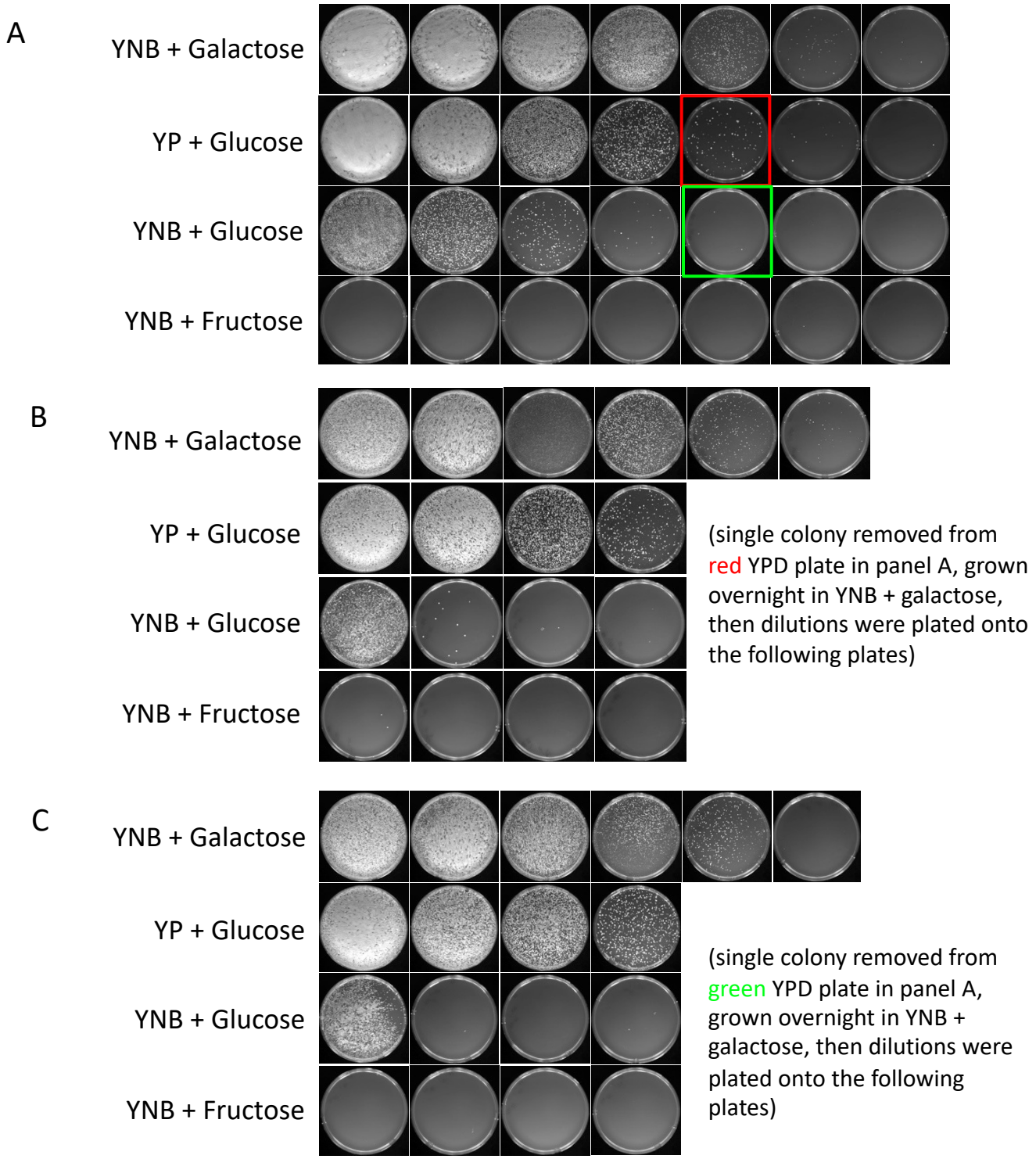
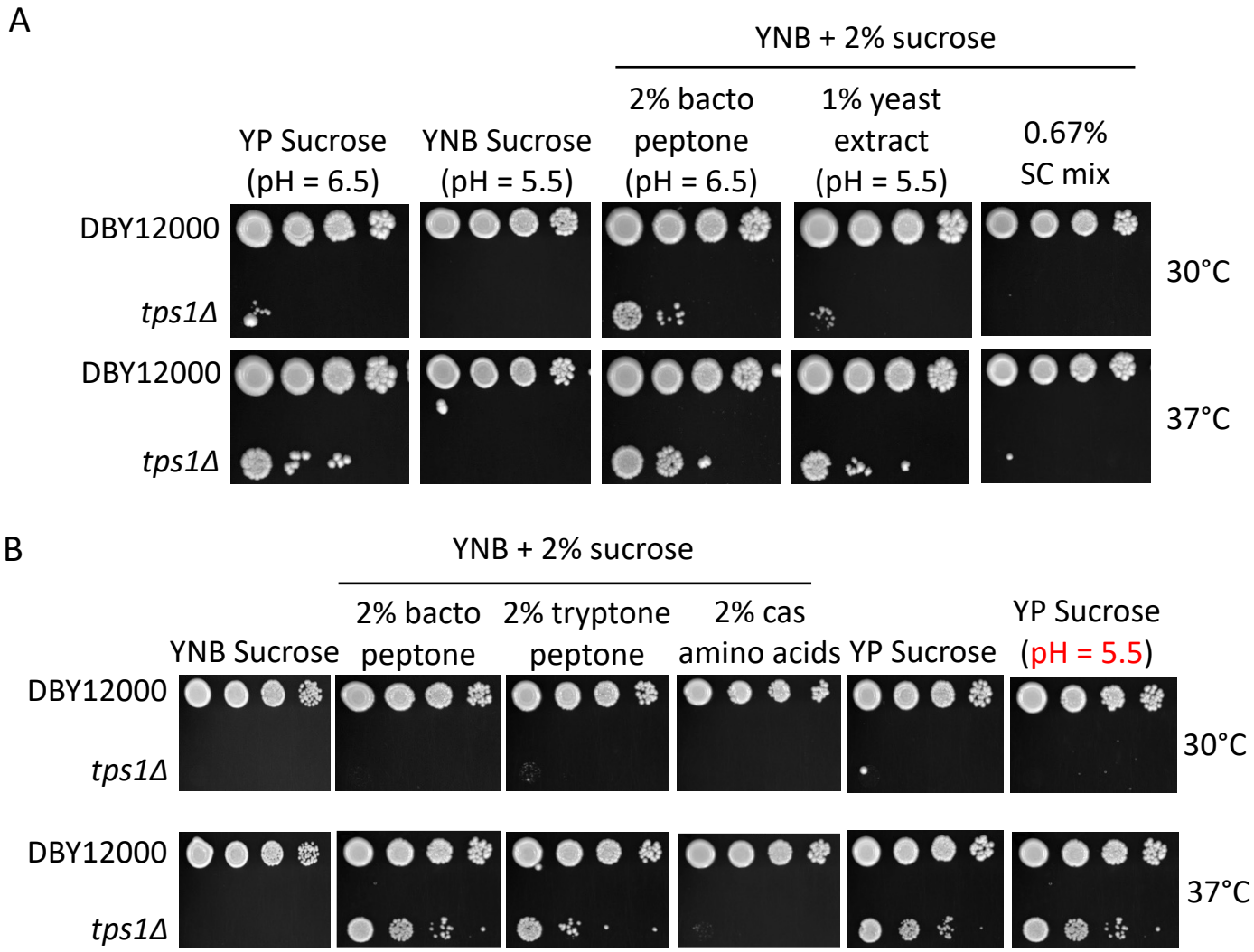
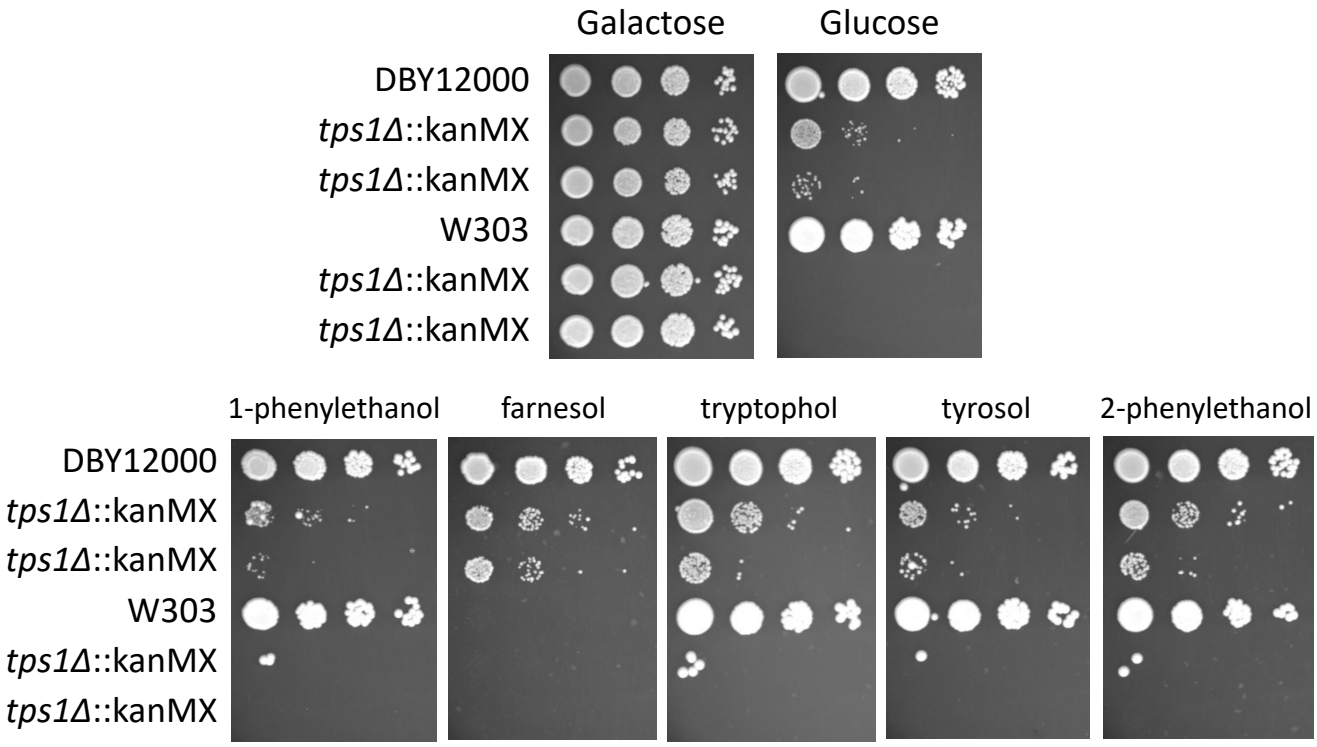
Supplemental Figures/Tables



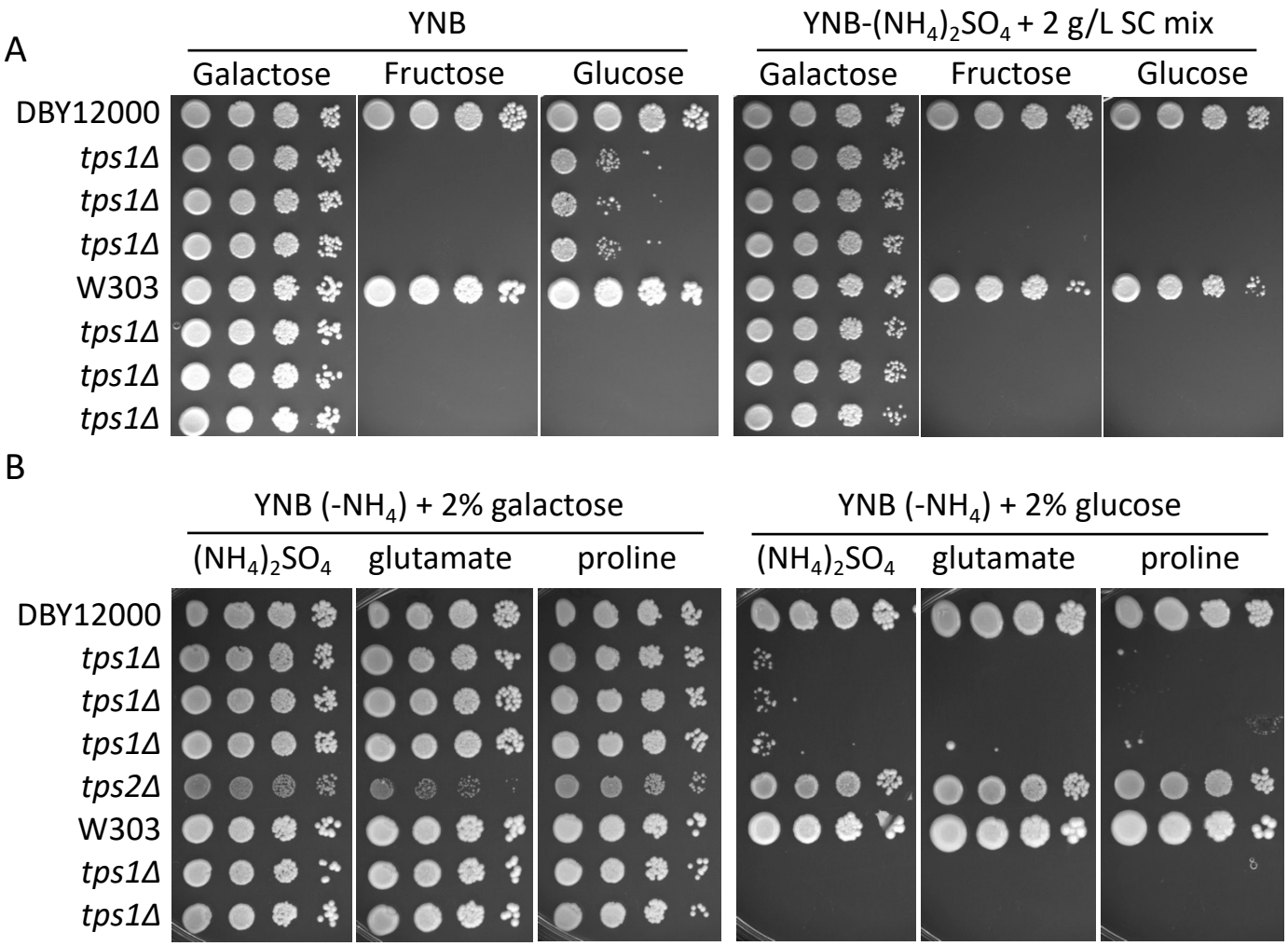
Supplemental Figure 1. The *tps1Δ* persister-like state is non-genetic and is enhanced by rich media. **A**. *tps1Δ* cells were grown overnight in YNB + 2% galactose, then 1:10 serial dilutions were spread onto the indicated media. Plates were incubated for 2-3 days at 30°C before photographing. **B**. A single colony from the plate indicated by red outline in panel A was re-grown in YNB + 2% galactose, then treated as described for panel A. **C**. A single colony from the plate indicated by green outline in panel A was re-grown in YNB + 2% galactose, then treated as described for panel A. Strain used in this figure is DBY12383.



Supplemental Figure 2. Multiple types of peptone influence the *tps1Δ* persister-like state, and this is independent of media pH. **A**. Wild type (DBY12000) and *tps1Δ* cells were grown overnight in YNB + 2% galactose, then 1:10 serial dilutions were spread onto the indicated media (initial dilution OD_{600} = 1.0). Plates were incubated for 3 days at the indicated temperatures before photographing. The pH of each type of plate was measured with a pH strip and listed in parentheses. **B**. Similar experiment as shown in panel A, though performed on a different day with a number of different media conditions as indicated. The media pH indicated in red for YP Sucrose was adjusted to 5.5 using HCl (typical pH for minimal media without amino acid supplementation). Strains used in this figure: DBY12000, DBY12383.

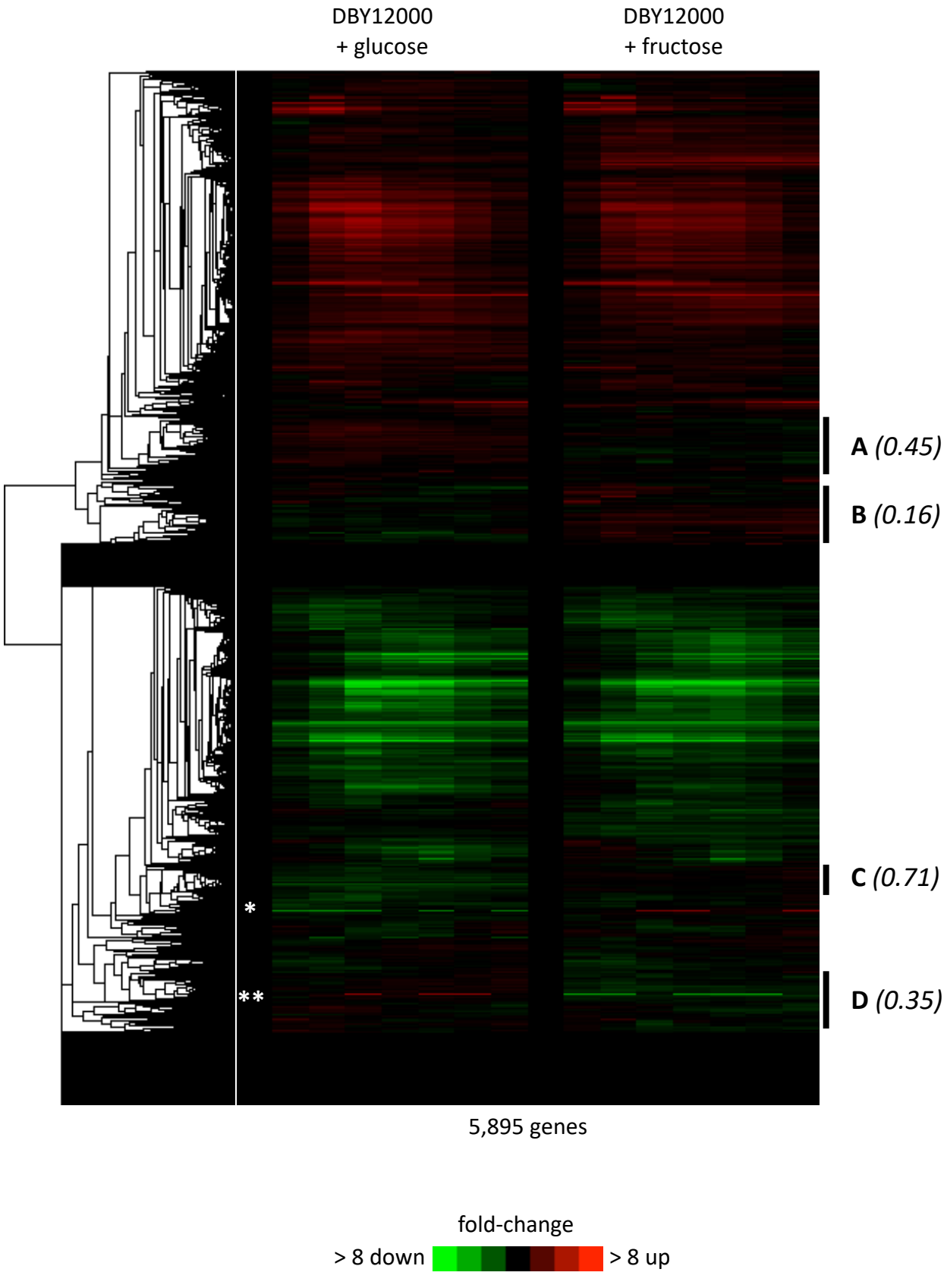


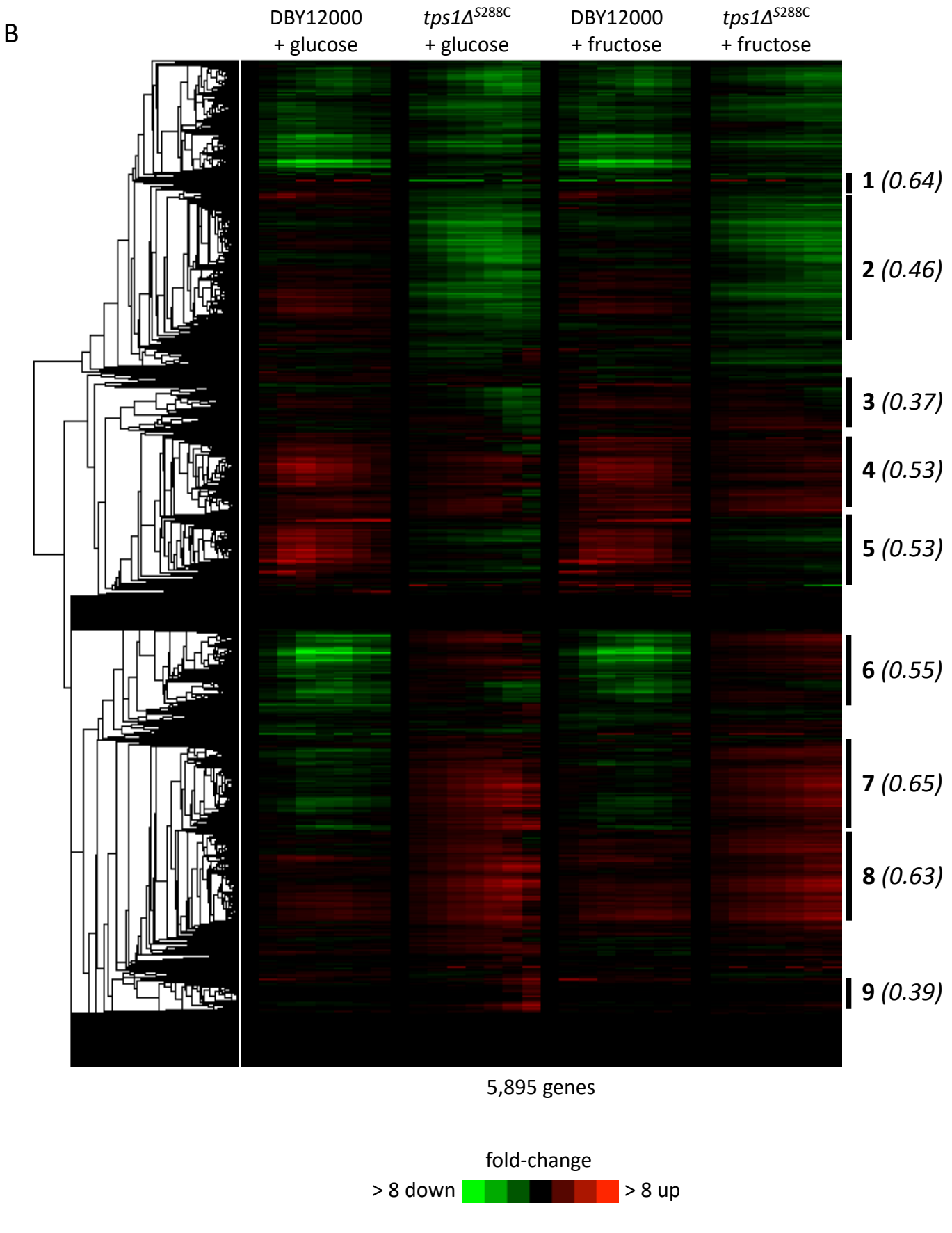
Supplemental Figure 3. Yeast quorum sensing molecules do not influence the *tps1Δ* persister-like state. The indicated strains were grown overnight in YNB + 2% galactose, then 1:10 serial dilutions were prepared (initial dilution OD_{600} = 1.0). Strains were spotted onto the indicated media. Top row: comparing two carbon sources as indicated (both present at 2%). Bottom row: all YNB + 2% glucose plates containing the indicated quorum sensing molecules at 500 µM. Two biological replicates of *tps1Δ* were included for each strain background. Notably, the W303 wild type strain appears unable to grow in the presence of 500 µM farnesol. Strains used in this figure: DBY12000, DBY12383, DBY15117, DBY15121.



Supplemental Figure 4. The *tps1Δ* persister-like state is dependent on ammonia as a nitrogen source. A. The indicated strains were grown overnight in YNB + 2% galactose, then 1:10 serial dilutions were prepared (initial dilution OD_{600} = 1.0). Strains were spotted onto the indicated media containing carbon sources at 2%. Three biological replicates of *tps1Δ* were included for each strain background. **B**. The indicated strains were grown overnight in YNB + 2% galactose, then 1:10 serial dilutions were prepared (initial dilution OD_{600} = 1.0). Strains were spotted onto the indicated media containing various nitrogen sources. Usable nitrogen was present at 76 mM (the amount in typical minimal medium). 2-3 biological replicates of *tps1Δ* were included for each strain background. All plates were incubated for 3 days at 30°C before photographing. Strains used in this figure: DBY12000, DBY12383, DBY15117, DBY15121, DBY12118.

A

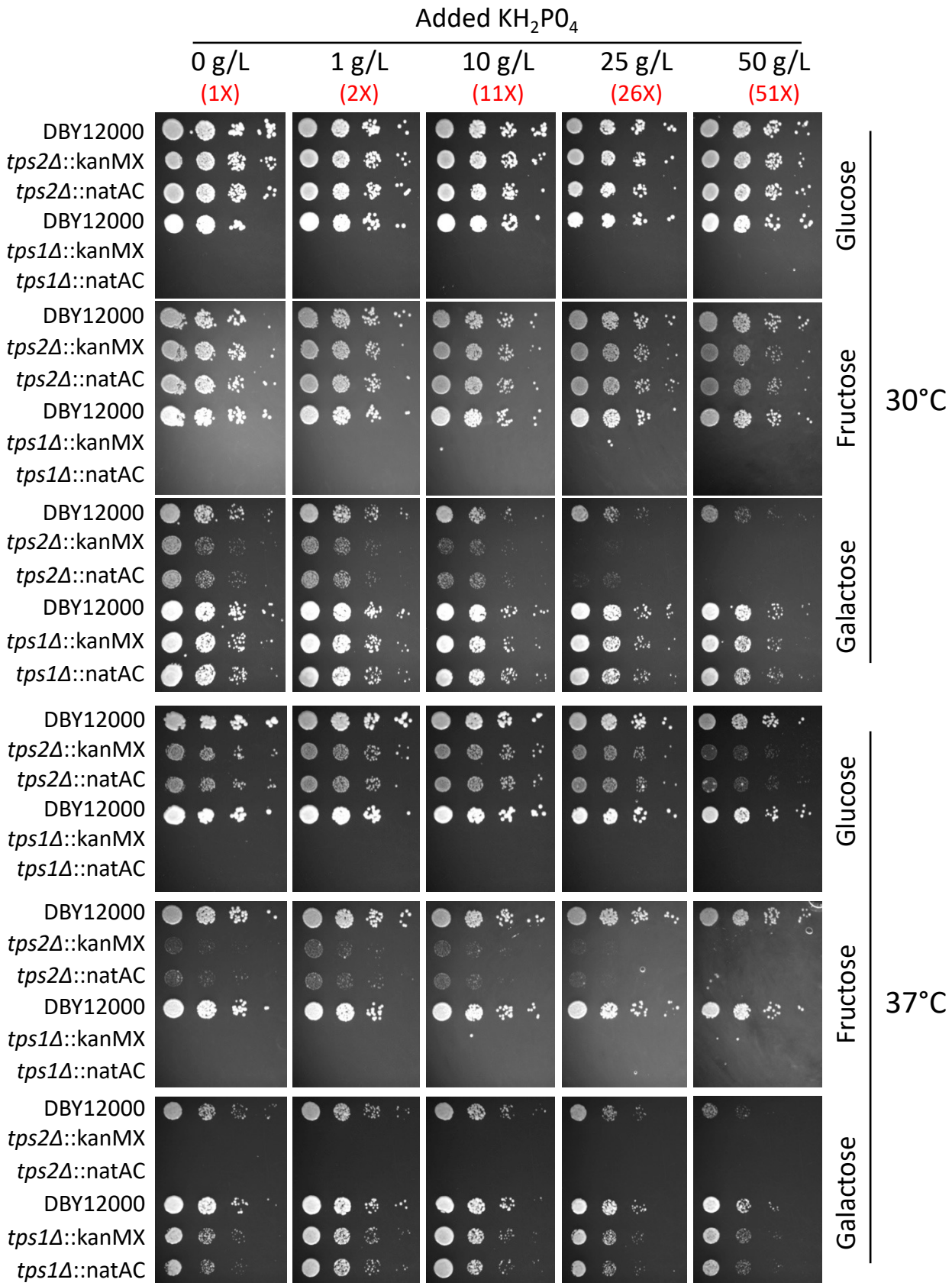

DBY12000
+ glucose
DBY12000
+ fructose
A (0.45)
B (0.16)
C (0.71)
D (0.35)
*
**
5,895 genes
fold-change
> 8 down
> 8 up


B
DBY12000
+ glucose
tps1Δ^S288C
+ glucose
DBY12000
+ fructose
tps1Δ^S288C
+ fructose
1 (0.64)
2 (0.46)
3 (0.37)
4 (0.53)
5 (0.53)
6 (0.55)
7 (0.65)
8 (0.63)
9 (0.39)
5,895 genes
fold-change
> 8 down
> 8 up

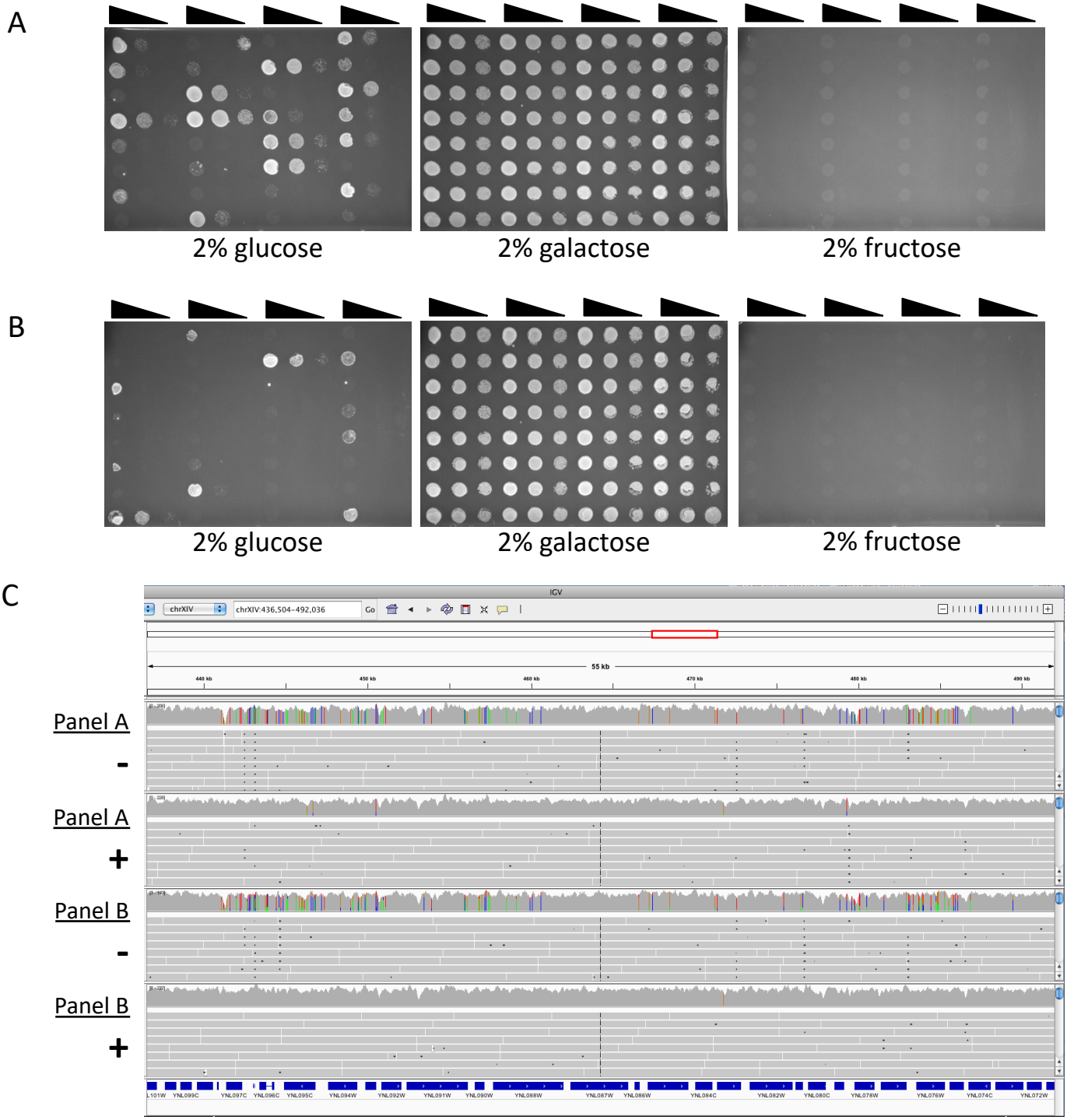
C

Cluster	Significantly enriched GO terms
A	chromatin (M), transcription factor complex (M), RNAPII holoenzyme (M), nucleus (C), chromosome (C), DNA binding (F), transcription factor activity (F), transcription from RNAPII promoter (P), mitotic cell cycle (P), chromatin organization (P), DNA repair (P), organelle fission (P)
B	endoplasmic reticulum (C), vacuoule (C), cytoplasmic vesicle (C), lipid metabolism (P), Golgi vesicle tranport (P)
C	endomembrane system (C), vacuole (C), endoplasmic reticulum (C), transmembrane transporter activity (F), lipid metabolism (P), transmembrane transport (P), protein glycosylation (P), Golgi vesicle transport (P)
D	chromatin (M), transcription factor complex (M), chromosome (C), cytoplasmic vesicle (C), DNA binding (F), transcription factor activity (F), transcription from RNAPII (P), mitotic cell cycle (P), chromatin organization (P)
1	transcription from RNAPII (P), signaling (P), response to chemical (P)
2	DNA binding (F), kinase activity (F), enzyme regulator activity (F), transcription from RNAPII (P), mitotic cell cycle (P), chromatin organization (P), organelle fission (P)
3	membrane (C), endoplasmic reticulum (C), ion transport (P), lipid metabolism (P), transmembrane transport (P), cell wall (P), transferase activity (F), hydrolase activity (F), glycosyl transferase activity (F)
4	ribonucleoprotein complex (M), ribosome (M), nucleus (C), nucleolus (C), structural constituent of ribosome (F), rRNA processing (P), cytoplasmic translation (P), ribosomal biogenesis (P)
5	ribonucleoprotein complex (M), ribosome (M), nucleus (C), nucleolus (C), ATPase activity (F), mRNA binding (F), helixase activity (F), rRNA processing (P), ribosomal biogenesis (P)
6	mitochondrial ribosome (M), mitochondrion (C), oxidoreductase activity (F), transmembrane transport activity (F), mitochondrion organization (P), generation of precursor metabolites and energy (P), protein complex biogenesis (P), nucleobase metabolism (P)
7	proteasome (M), oxidoreductase activity (F), protein complex biogenesis (P), proteolysis (P), lipid metabolism (P), cytoskeletal organization (P)
8	cytoplasmic ribosome (M), ribosome (C), structural constituent of ribosome (F), cytoplasmic translation (P), cellular amino acid metabolism (P)
9	spindle pole body (M), centromere (M), microtubule (M), plamsa membrane (C), chromosome (C), cytoplasmic vesicle (C), ATPase activity (F), organelle fission (P), cell cycle (P), cellular response to DNA damage (P), chromosome segregation (P)

Supplemental Figure 5. The gene expression response of wild type and *tps1Δ* cells to glucose and fructose exhibits strain- and condition-dependent effects. Strains were grown to early log phase in YNB + 2% galactose before addition of glucose or fructose to 2% as indicated. Cells were sampled at 0, 2.5, 5, 10, 15, 30, 60, and 120 minutes for RNA preparation. RNA-seq libraries and sequencing were performed as described in Materials and Methods. Data was analyzed as described in Materials and Methods. **A** - Heat-map showing wild type only. Indicated genes are **YDR524W-C* (gene of unknown function) and **RPL41B (ribosomal 60S subunit L41B). **B** - Heat-map showing wild type and *tps1Δ*. In both A and B, individual clusters are indicated to the right of the heat-map, along with the Pearson correlation for each cluster shown parenthetically. **C** - Significantly enriched GO terms from highlighted clusters in panels A and B. GO terms were identified by searching using the SGD (www.yeastgenome.org) GO Slim Mapper tool, and examining Process (P), Function (F), Component (C), and Macromolecular Complex (M) data sets. Listed are terms manually curated as highly significant. Strains used in this figure: DBY12000, DBY12383.



Supplemental Figure 6. Neither *tps1Δ* nor *tps2Δ* growth defects are restored by addition of exogenous phosphate to growth media. Strains were grown overnight in minimal media (glucose-containing for *tps2Δ*, galactose-containing for *tps1Δ*), then 1:10 serial dilutions were prepared (initial dilution OD_{600} = 1.0) and spotted onto the indicated media and incubated at indicated temperatures for 3 days before photographing. Strains used in this figure: DBY12000, DBY12134, DBY12383, DBY12118, DBY12813.



Supplemental Figure 7. Segregation of *tps1Δ* persister-like activity to a region on chromosome XIV. Segregants from DBY12689 (**A**) and DBY12688 (**B**) were spotted onto the indicated minimal media and grown 3 days at 30°C (3 x 10-fold serial dilutions; initial dilution OD_{600} = 1.0). **C**. Strains with *tps1Δ* persister-like activity (**+**) and strains without (**-**) were pooled from both sets of segregants and sequenced. Shown is a IGV browsing window zoomed into chromosome XIV. Because the reference for sequence alignment was an S288C derivative, all W303-specific mutations appear as colored vertical bars. Note that strains that fail to exhibit *tps1Δ* persister-like activity (-) all contain the W303 genetic material for this region, while the opposite is true for those segregants that do exhibit *tps1Δ* persister-like activity (**+**).

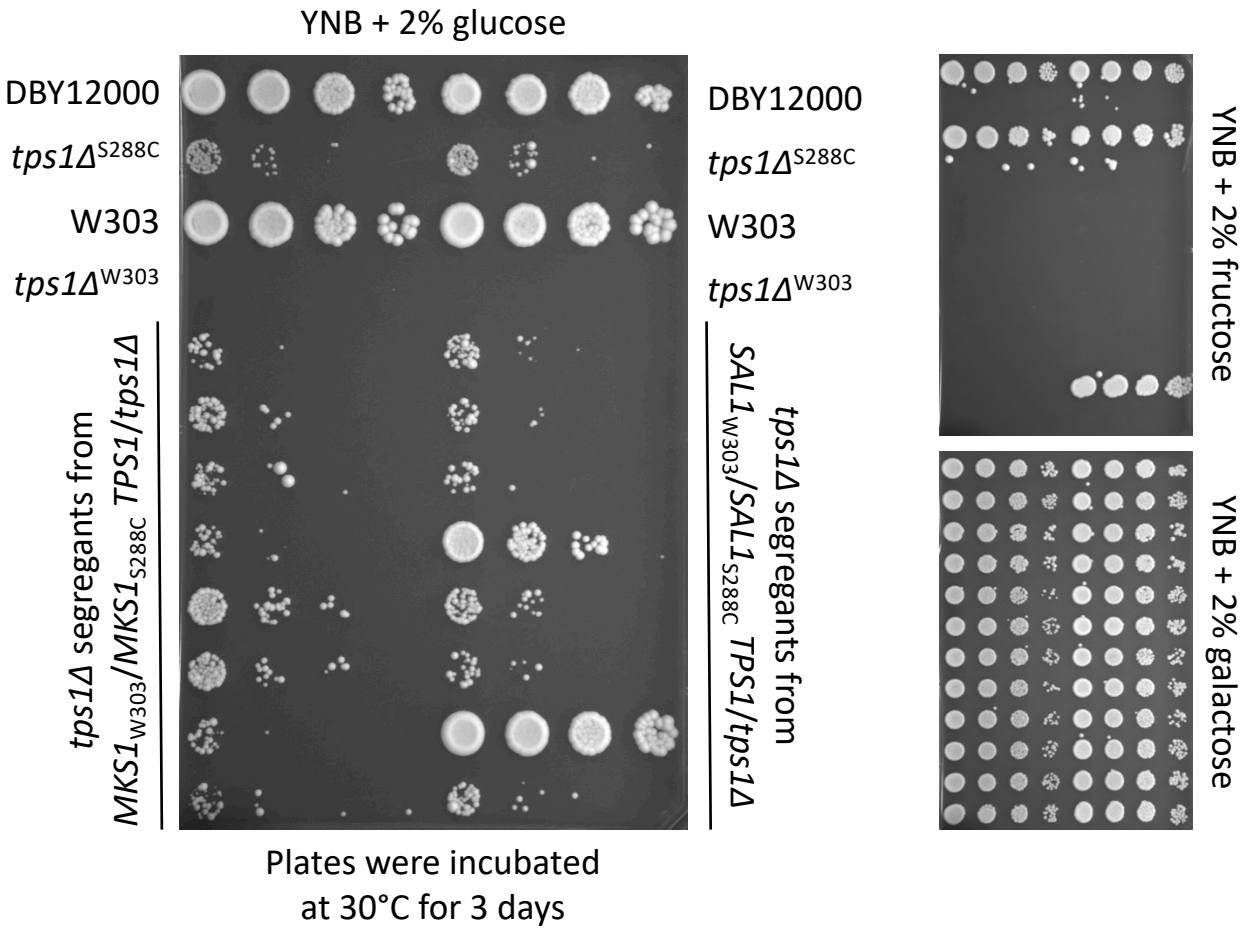
Supplemental Figure 8. Potential causative mutations on chromosome XIV between 440,000 and 490,000 kb (within coding regions)

Gene	Function	Changes[a]
PHO23	histone deacetylase complex component	F->L
tRNA-Leu	encodes leucine tRNA	see notes[b]
YNL095C	putative protein of unknown function	F->L, V->F, T->I, V->A, D->E
APP1	component of endocytic pathway	A->T
YNL092W	putative SAM-dependent methyltransferase	A->E
NST1	protein of unknown function	E->D, R->G
RHO2	involved in cell polarity and microtubule assembly	F->C
SNN1	subunit of endosomal maturation complex	I->M
MKT1	forms complex with Pbp1 in glucose-deprivation stress granules; allelic variation affects mitochondrial genome stability, drug resistance, and more	D->G, K->R
SAL1	ADP/ATP transporter active in mitochondria during fermentation	V-M, G->W, G->A, frameshift[c]
SWS2	putative mitochondrial ribosomal small subunit	F->L
TPM1	major isoform of tropomyosin; binds/stabilizes actin cables and filaments	H->Q
NIS1	protein localized to the bud neck during G2/M	Y->M, L->W, E->D, S->R
APJ1	hsp40 with a role in SUMO-mediated protein degradation	D->N
MKS1	pleiotropic negative transcriptional regulator; involved in Ras/cAMP signaling and nitrogen regulation	H->Y, P->A, D->E, P->T, E->G
IMP4	component of the SSU processome	S->G

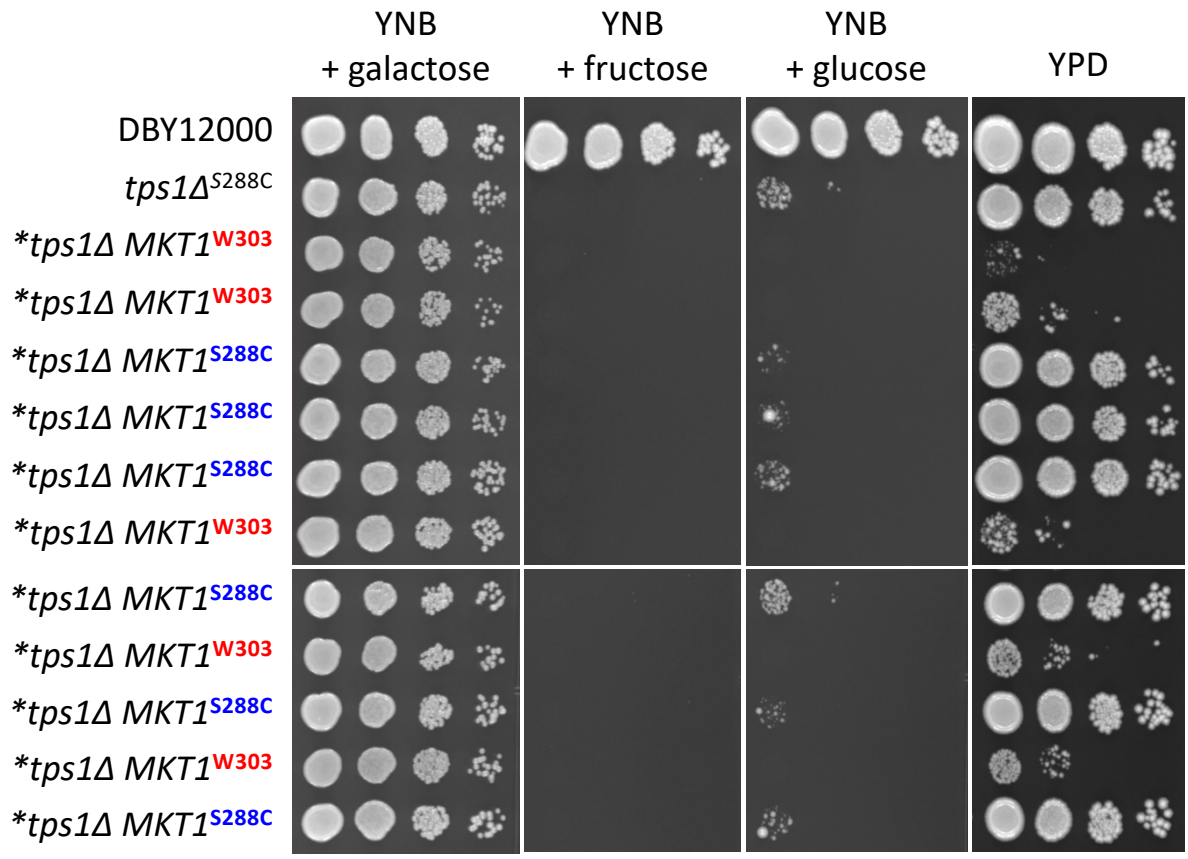
[a]amino acid changes in S288C-background compared to W303 background (S288C->W303)

[b]single base change present in S288C compared to W303

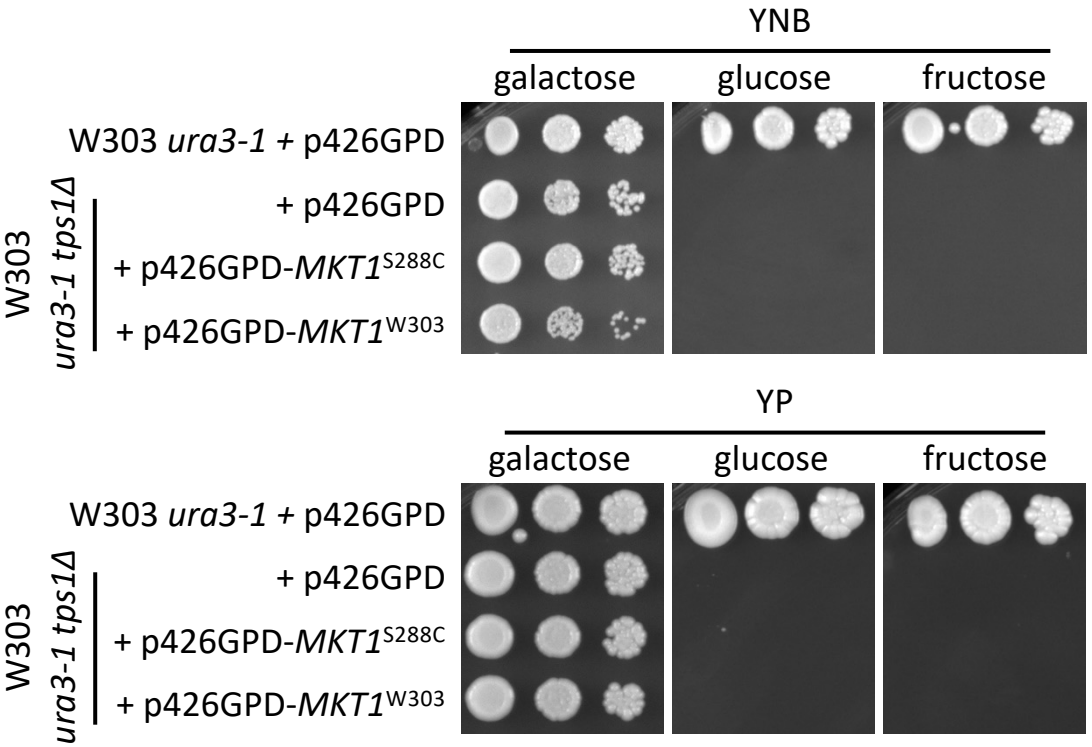
[c]frameshift mutation truncates S288C *SAL1*



Supplemental Figure 9. Mutations in *MKS1* or *SAL1* in S288C compared to W303 are not responsible for *tps1Δ* persister-like activity. The indicated strains, including *URA3*$^{+}$ segregants from DBY12796 (*MKS1* test) and DBY12795 (*SAL1* test) were grown overnight in YNB + 2% galactose. Next, 1:10 serial dilutions were prepared (initial dilution OD_{600} = 1.0) and strains were spotted onto the indicated media, then incubated for 3 days at 30°C.



Supplemental Figure 10. *MKT1* regulates the *tps1Δ* persister-like state . *URA3*$^{+}$ *tps1Δ* segregants from DBY12821 (along with the indicated wild type and *tps1Δ* controls) were grown overnight in YNB + 2% galactose liquid before 10-fold serial dilutions were prepared and spotted onto the indicated media. The initial dilution had an OD_{600} of 1.0. Listed carbon sources were present at 2%. Plates were incubated at 30°Cfor 3 days. Cells from each strain shown were grown overnight in YP + 2% Galactose for genomic DNA preparation, *MKT1* PCR amplification, and sequencing. The identified allele present is indicated for each strain: red for the W303 allele, or blue for the S288C allele.



Supplemental Figure 11. Overexpression of *MKT1*^{S288C} in W303 tps1 does not induce persister-like state, suggesting the *MKT1*^{S288C} allele is recessive. Indicated strains were grown overnight in YNB + 2% galactose liquid before 10-fold serial dilutions were prepared and spotted onto the indicated media. The initial dilution had an OD_{600} of 1.0. Listed carbon sources were present at 2%. Plates were incubated at 30°C for 3 days. Strains used in this figure: DBY15039, PGY185.