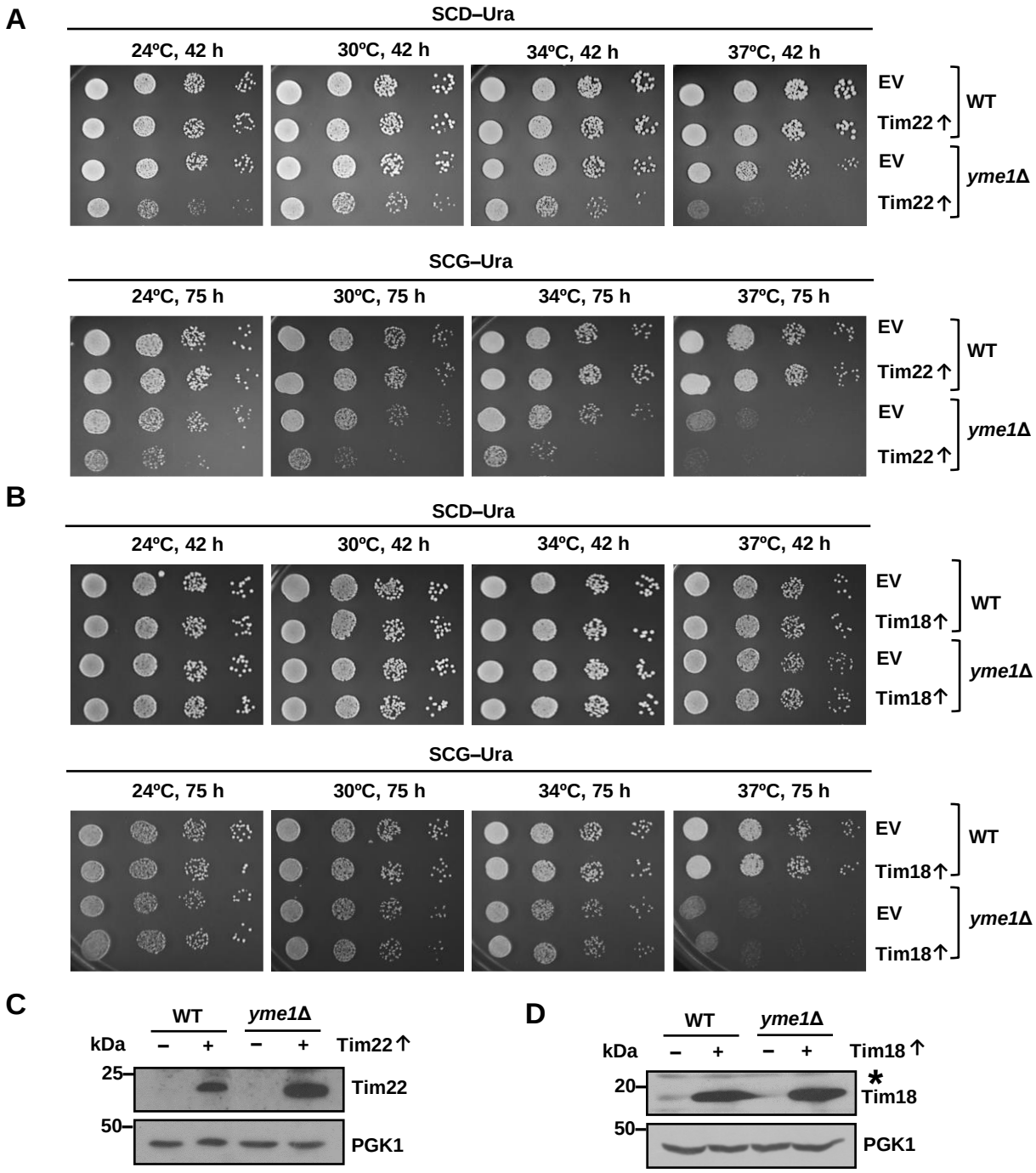


Fig. S1. The overexpression of Tim22 aggravates the growth defects of the *yme1*Δ strain. (A,B) Growth phenotype analysis upon overexpression. WT and *yme1*Δ strains overexpressing either Tim22 or Tim18 under the control of centromeric plasmid pRS416$_{TEF}$ were serially diluted and spotted on the indicated media. (C,D) Examination of steady-state levels of overexpressed proteins. The overexpression of Tim22 and Tim18 were examined by immunoblotting in the whole-cell extracts of the indicated strains. The asterisk indicates the presence of a non-specific band. Data in A–D are representative of *n*=3 experiments.

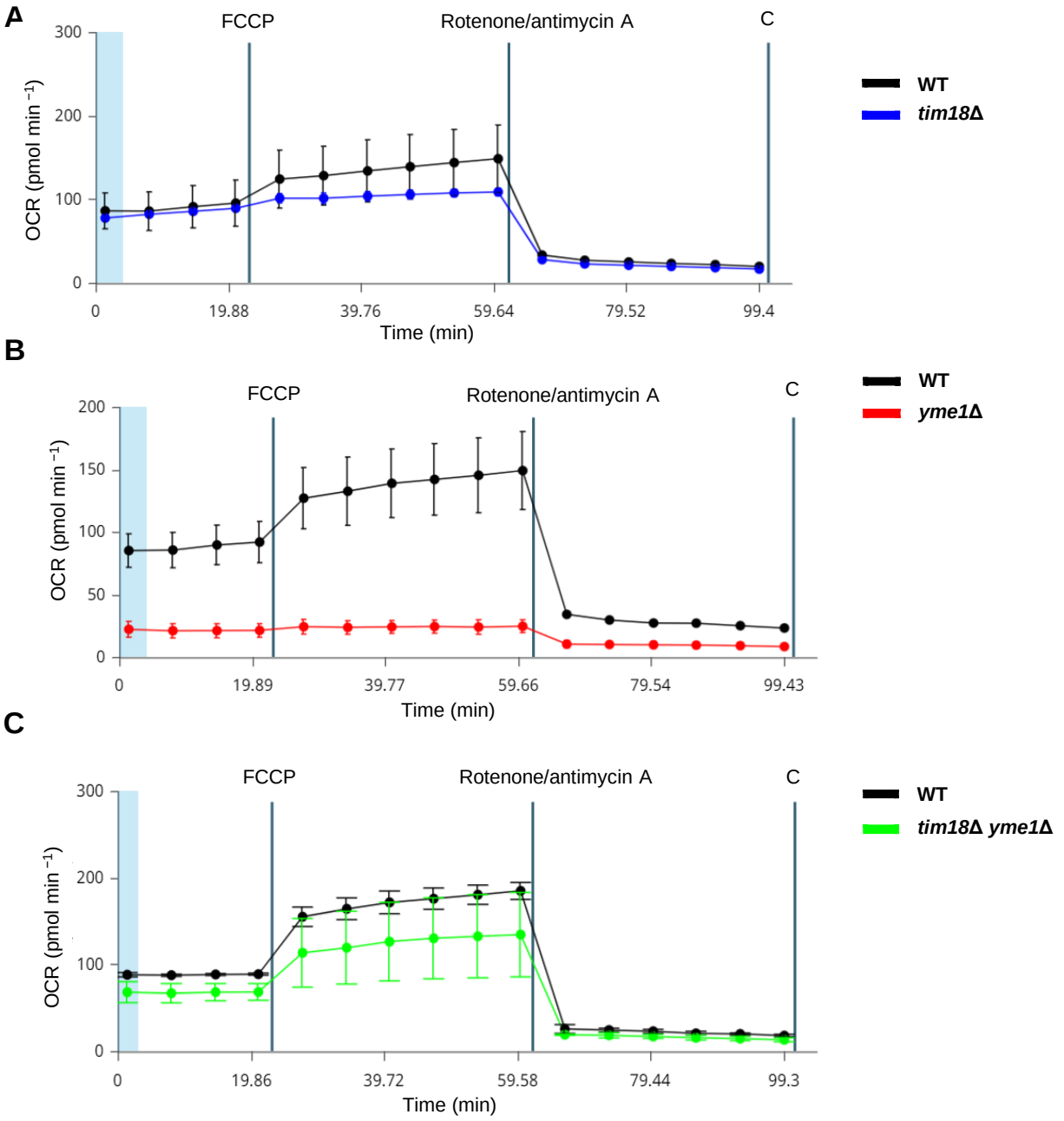


Fig. S2. The deletion of Tim18 rescues the OCR of cells lacking Yme1. (A-C) Measurement of OCRs. The respiratory capacity of WT and the indicated deletion strains were determined with a Seahorse XF HS mini analyzer. FCCP and rotenone/antimycin A were sequentially added to analyze mitochondrial respiratory efficiency. Each graph represents an individual experiment showing the pattern of OCRs for WT and the deletion strains.

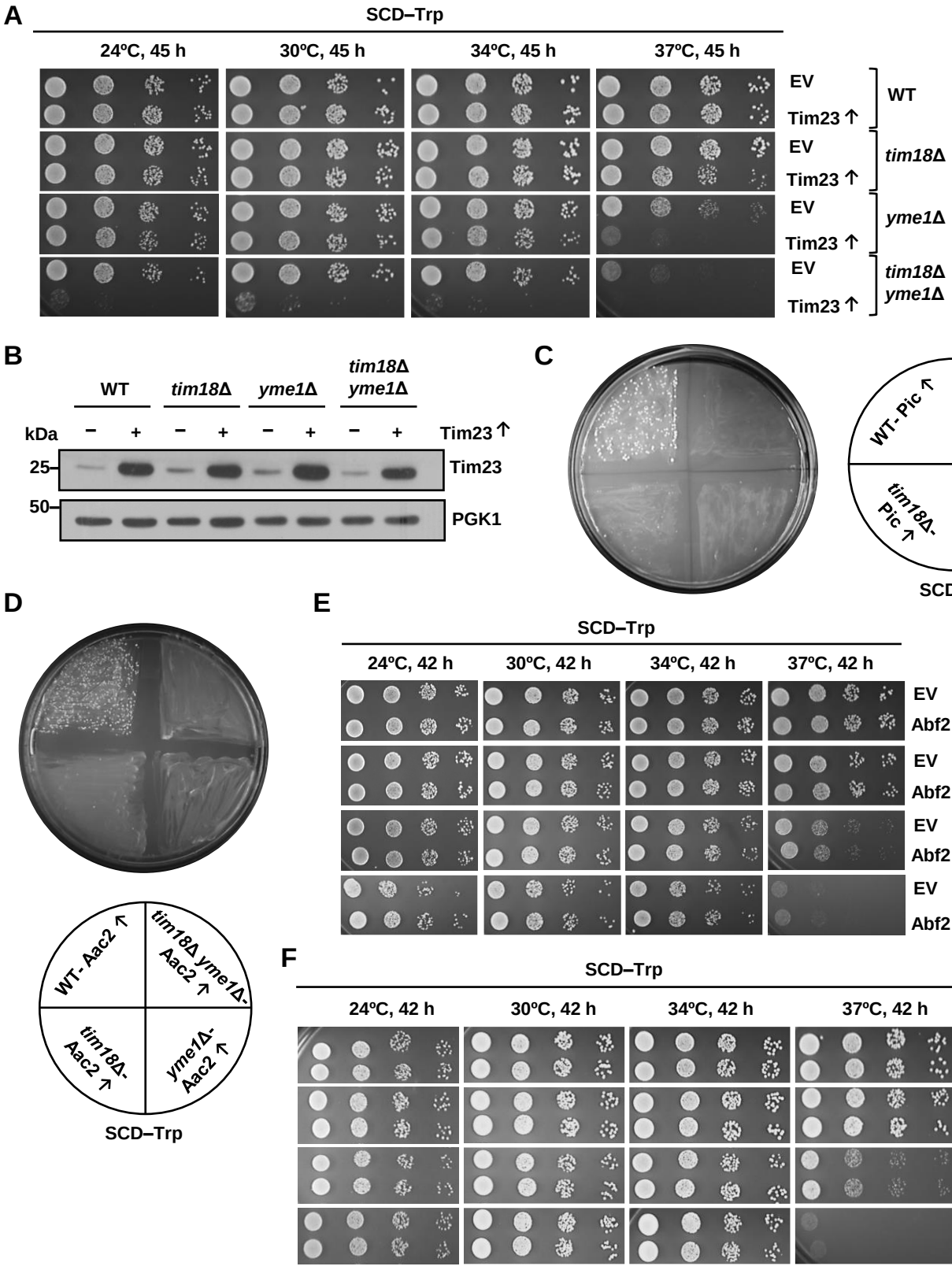


Fig. S3. The overexpression of the TIM22 pathway substrates escalates the growth defects of the *yme1*Δ cells. (A) Growth phenotype analysis upon overexpression of Tim23. Ten-fold serially diluted WT and deletion strains overexpressing Tim23 under the control of centromeric plasmid $pRS414_{TEF}$ were spotted on the SCD–Trp medium and incubated at different temperatures. (B) Estimation of the Tim23 overexpression. The overexpression of Tim23 was examined in the whole-cell extracts of the indicated strains by immunoblotting. (C,D) Assessment of Pic and Aac2 overexpression on the growth of WT and deletion strains. The ORFs of Pic and Aac2 were cloned in the $pRS414_{TEF}$ plasmid and transformed in WT and deletion strains, followed by plating on selection medium (SCD–Trp). (E,F) Examination of growth phenotype upon overexpression of Abf2 and Tom6. WT and deletion strains encompassing either EV, Abf2, or Tom6 overexpressing plasmids were grown to the mid-log phase, serially diluted, and spotted on the specified medium. The images shown are representative of *n*=3 biological replicates.

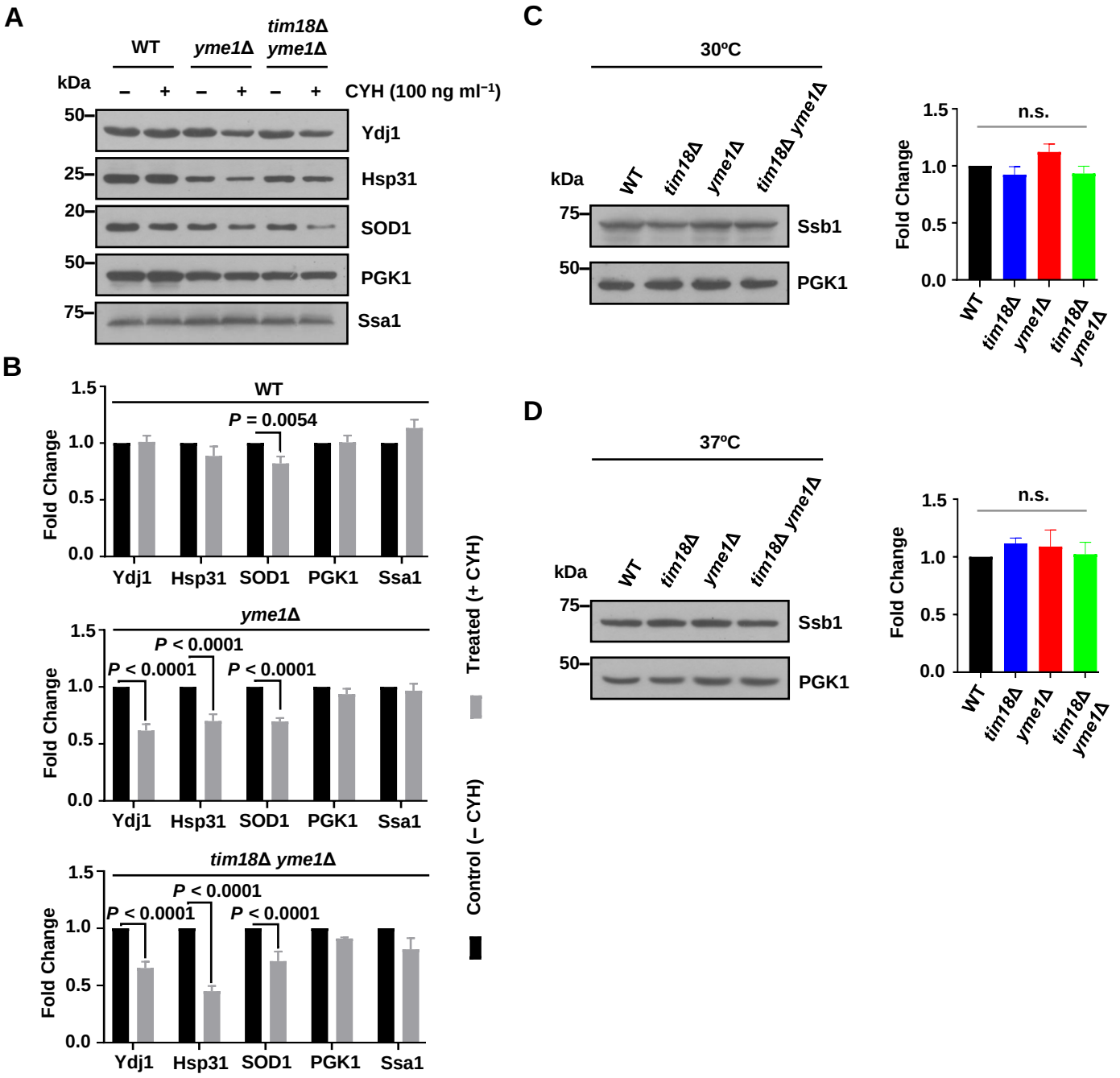


Fig. S4. Partial suppression of cytosolic protein synthesis does not rescue the growth defects of *yme1*Δ cells. (A,B) Assessment of the cytosolic protein inhibition upon CYH treatment. WT, *yme1*Δ, and *tim18*Δ*yme1*Δ strains were treated with CYH (100 ng ml^{-1}) for 180 min, followed by immunoblot analysis of different cytosolic proteins in whole-cell extracts. (C,D) Estimation of Ssb1 steady-state protein levels. The expression of Ssb1 in the whole-cell extracts of WT and the indicated deletion strains was measured by immunoblotting at permissive and elevated temperatures. Data represent mean±s.e.m. of n=3 biological replicates. One-way ANOVA with Tukey's multiple comparisons test was used for determining statistical significance. n.s., not significant.

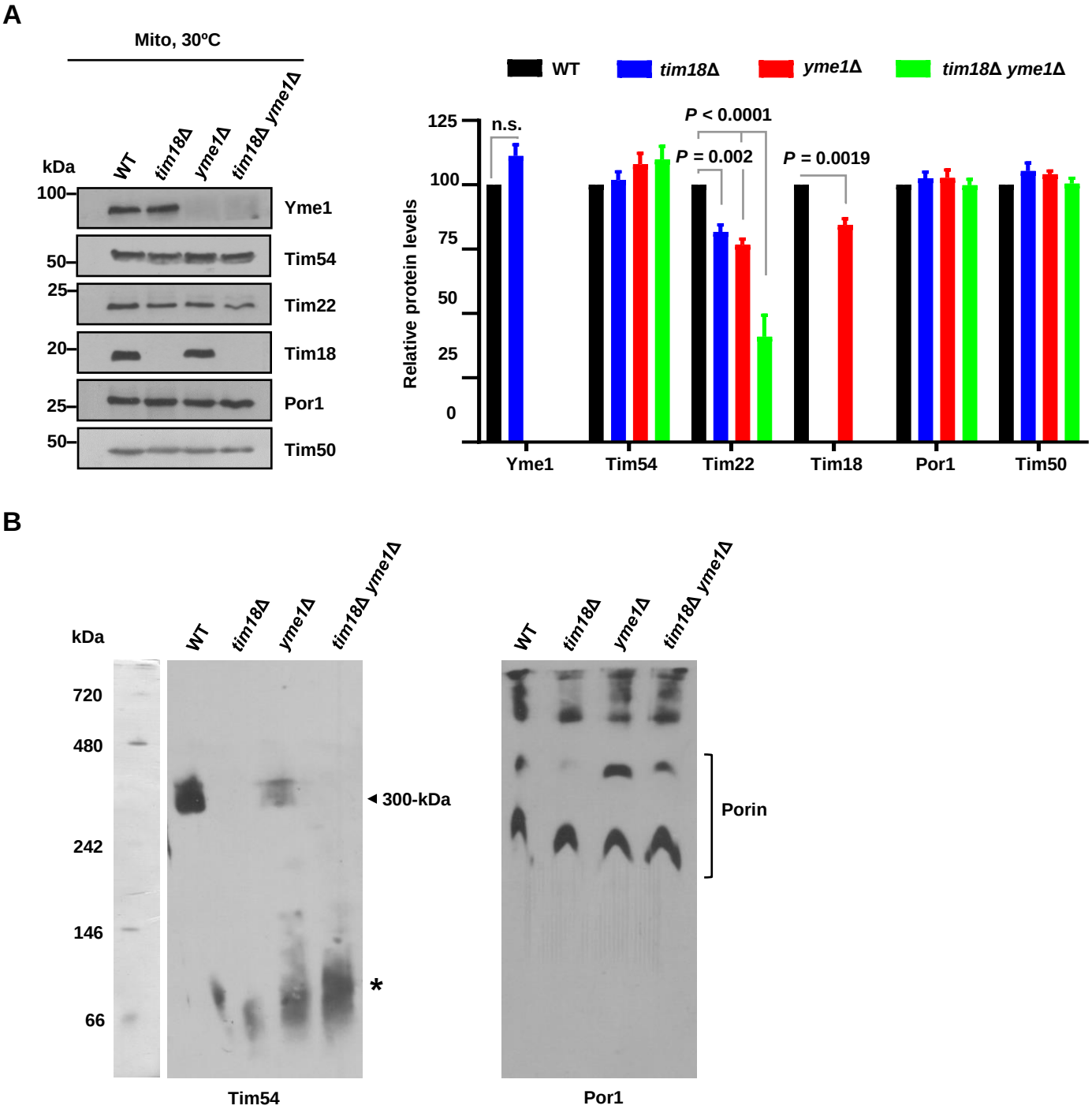


Fig. S5. The loss of Yme1 affects the steady-state levels of the TIM22 complex components. (A) Measurement of the protein levels of the carrier translocase machinery. 100 μg of mitochondria isolated from indicated strains grown in YPG medium at 30°C were assessed by immunoblotting. Protein amounts were quantified using ImageJ software and were plotted as percentages by setting the intensities of WT mitochondria as 100%. Data specify mean±s.e.m. of *n*=3 biological replicates. Two-way ANOVA with Tukey's multiple-comparisons test was used for calculating statistical significance. n.s., not significant. (B) Analysis of the stability of the carrier translocase machinery using BN-PAGE. Mitochondria isolated from WT, *tim18*Δ, *yme1*Δ, *and tim18*Δ *yme1*Δ cells grown at 37°C in YPG for 24 h were solubilized in digitonin buffer, and proteins were examined by BN-PAGE followed by immunoblotting with the indicated antibodies. Arrowhead represents the TIM22 complex (~300-kDa), and the asterisk indicates possible intermediate subcomplexes. The data shown are representative of *n*=3 biological replicates.

Fig. S6. Blot transparency

Fig. 1

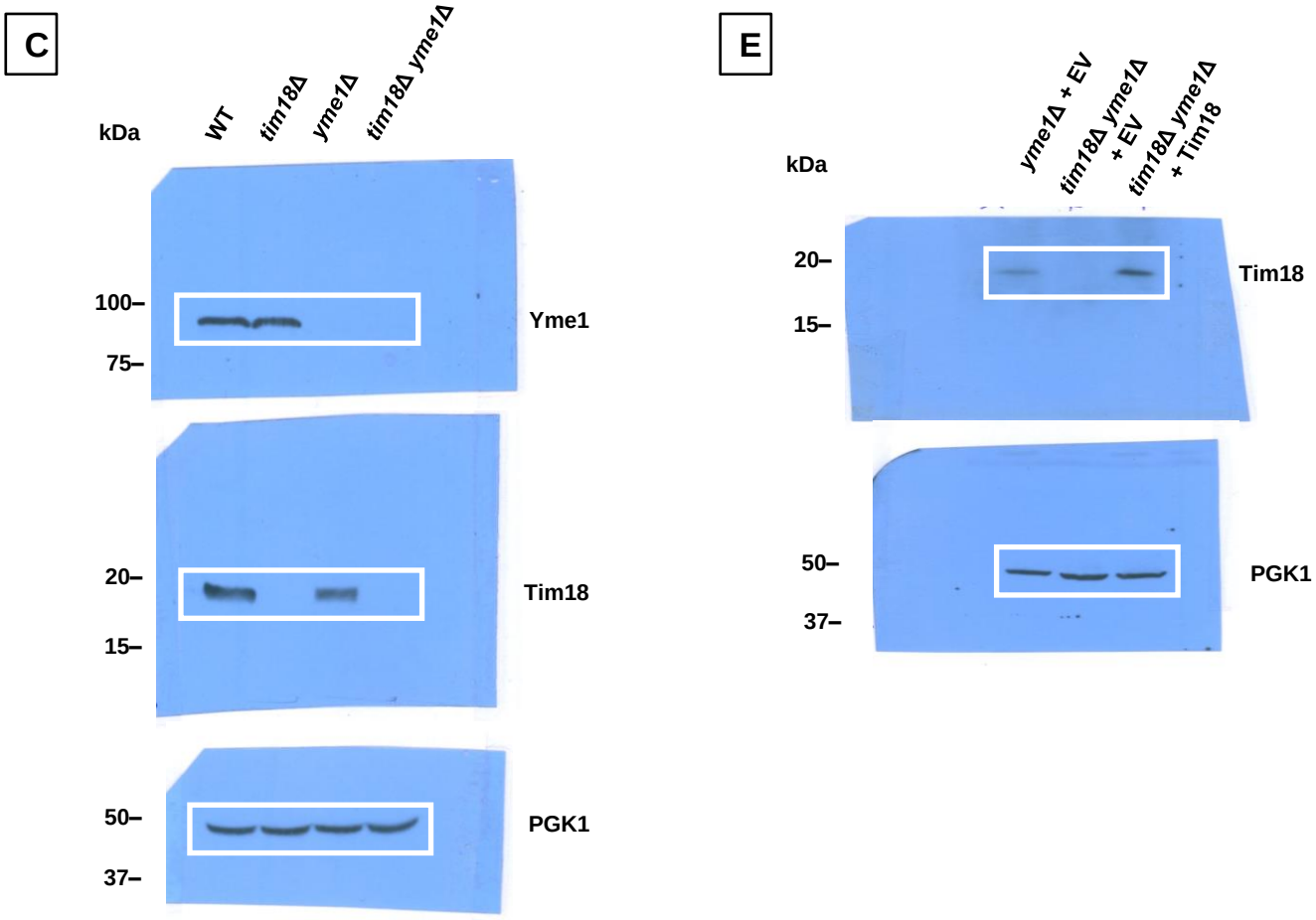


Fig. 3

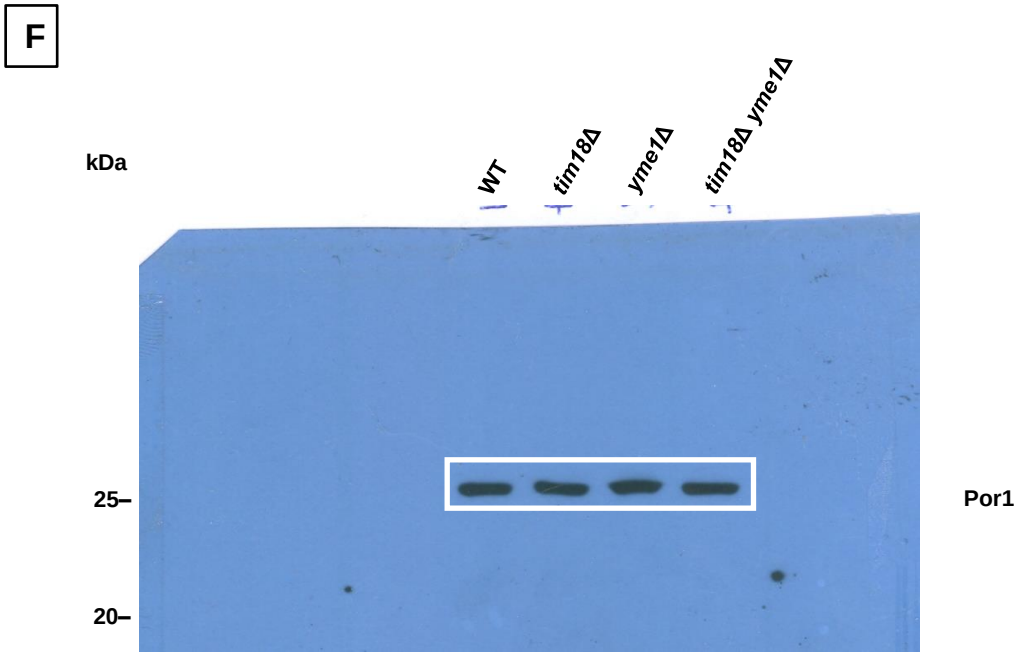


Fig. 4

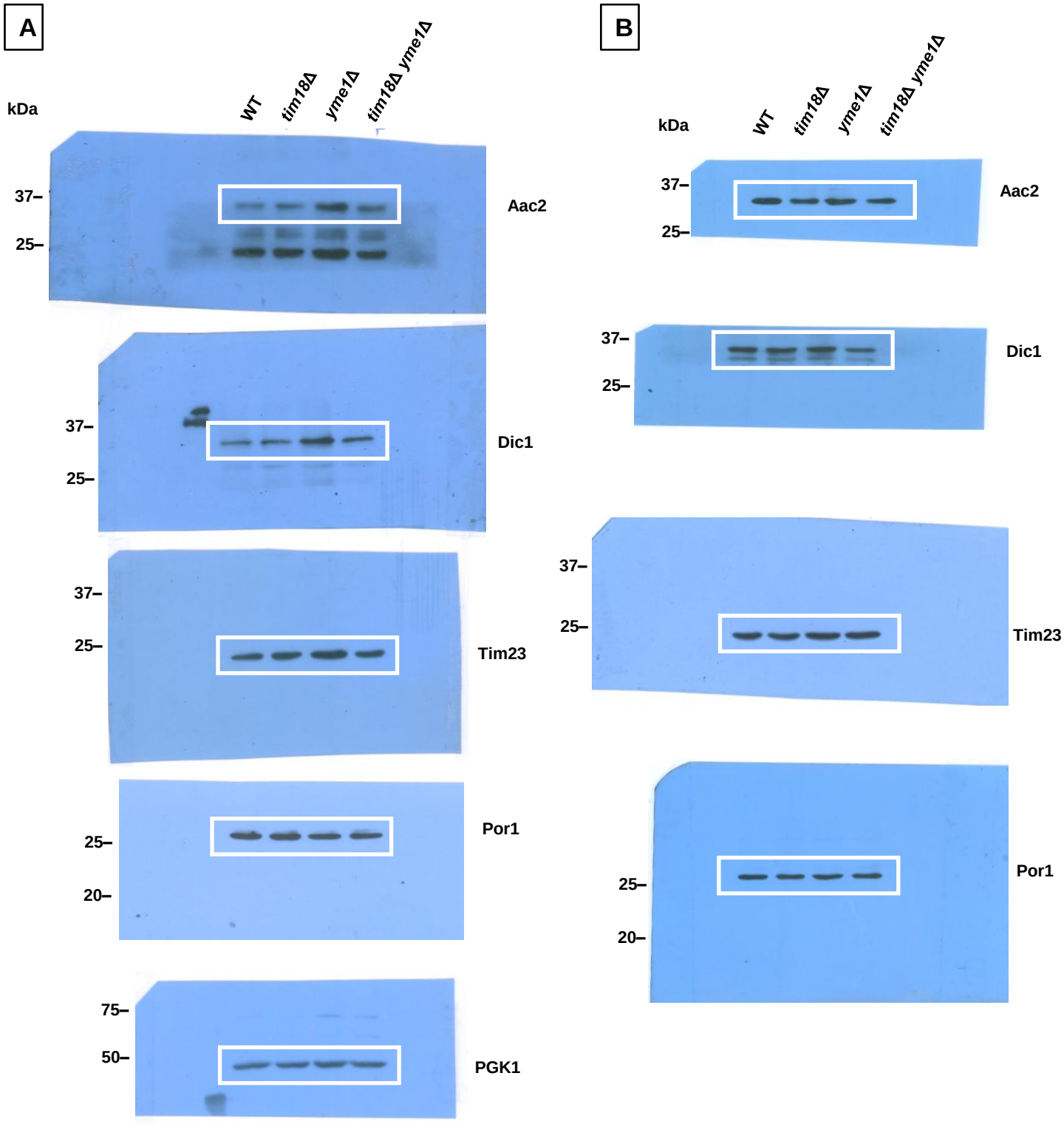


Fig. 5

B

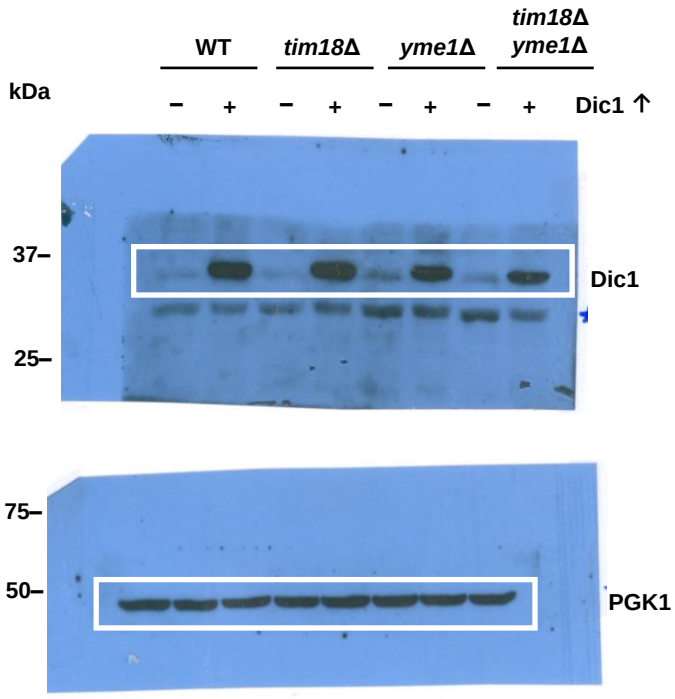


Fig. 6

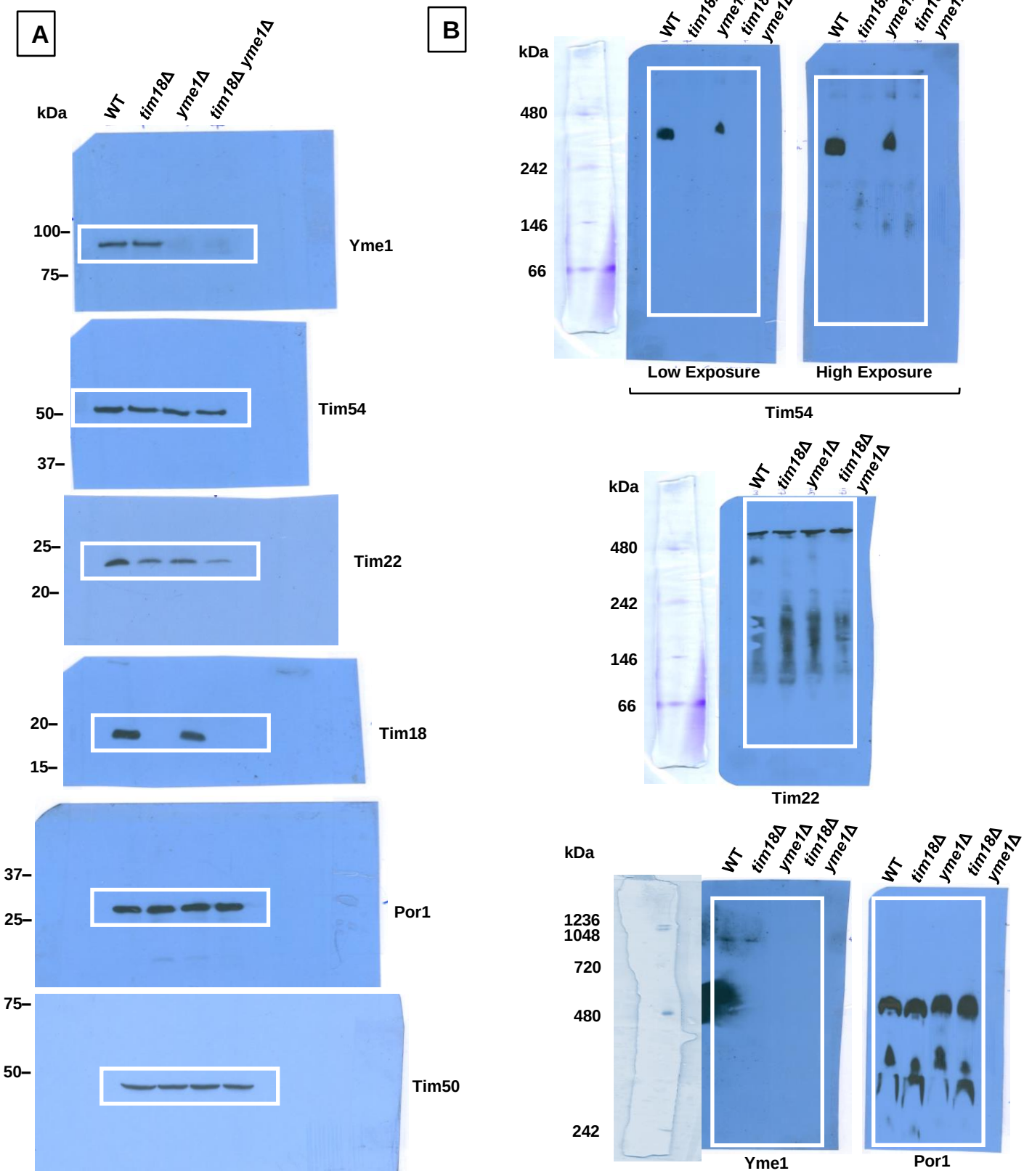


Fig. 6

C

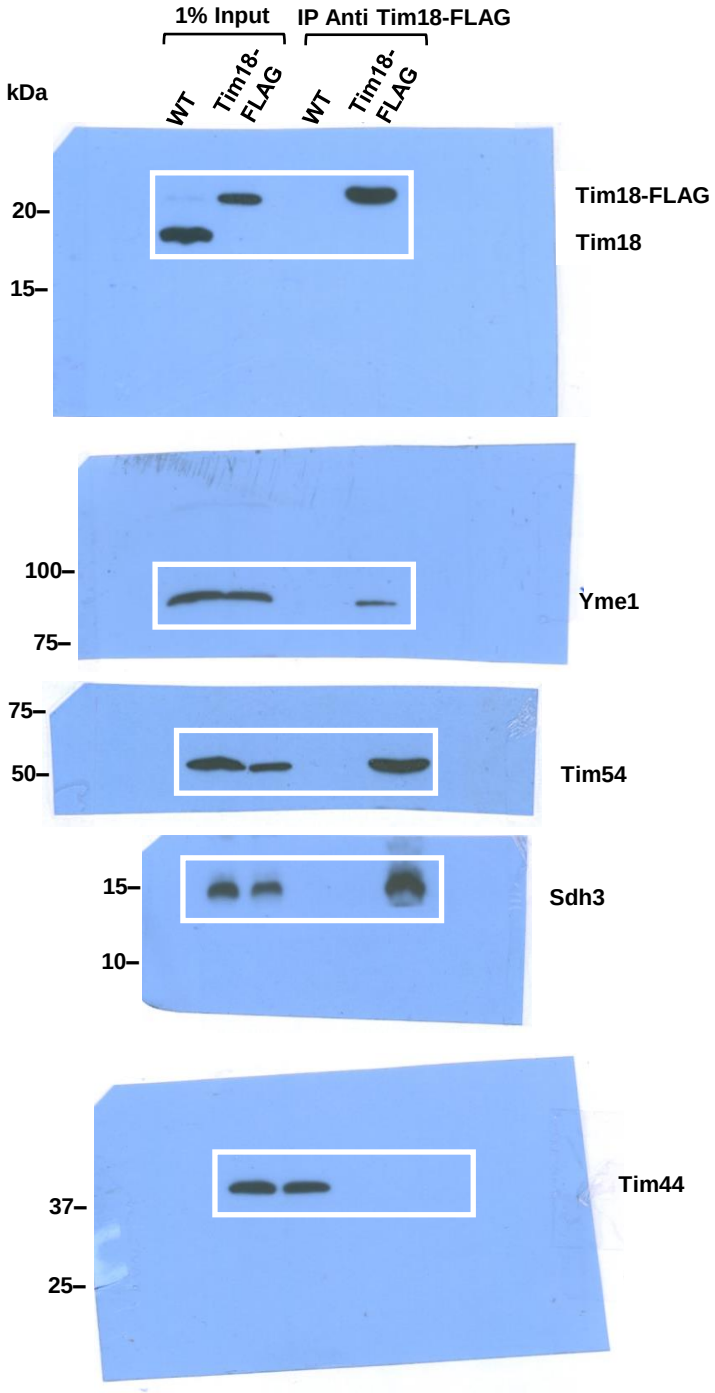


Fig. 6

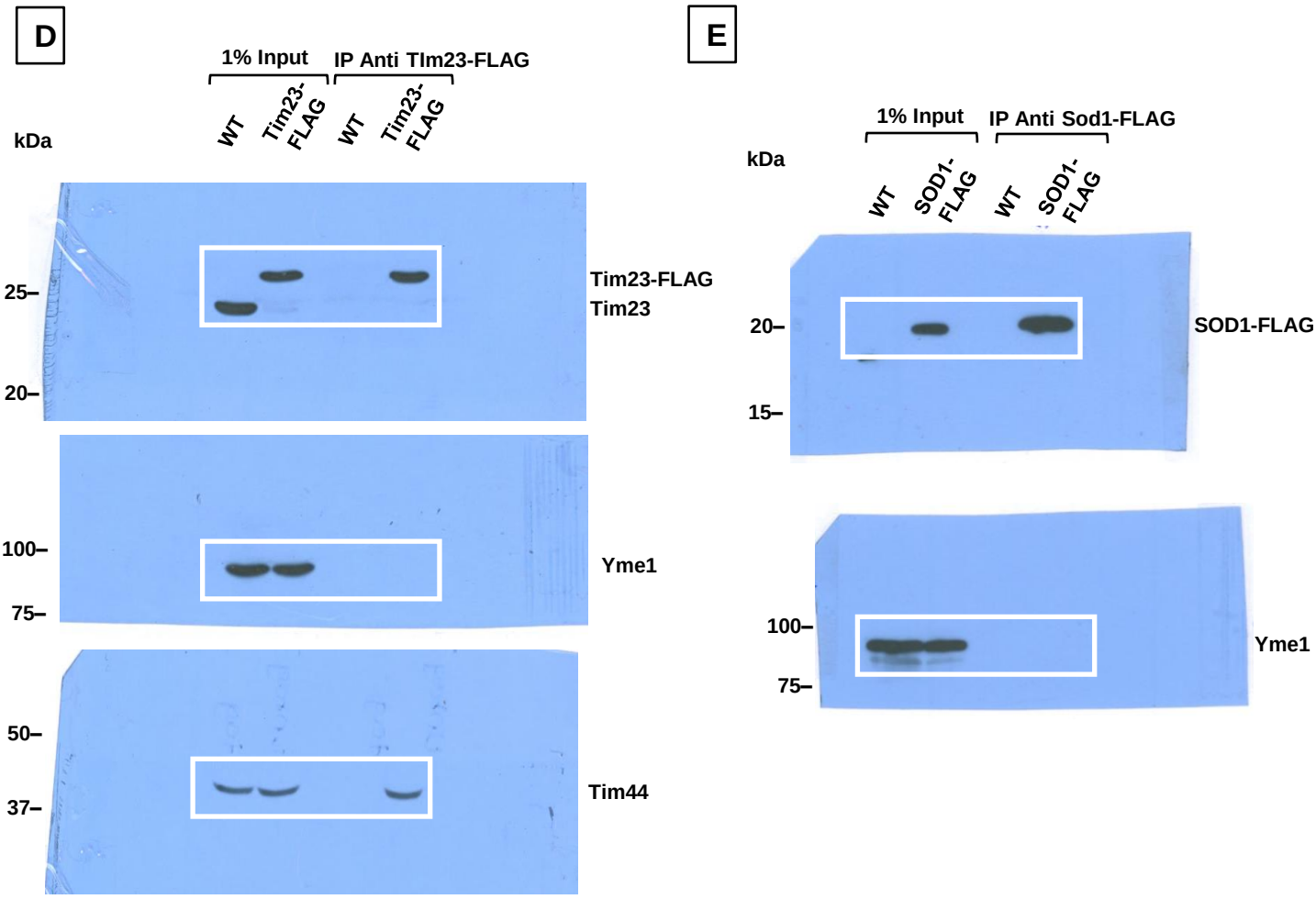
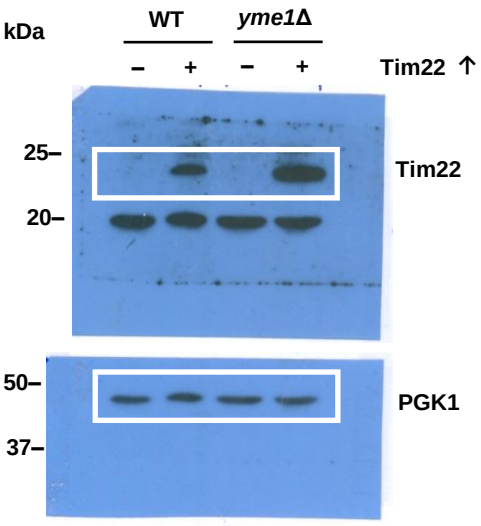


Fig. S1

C



D

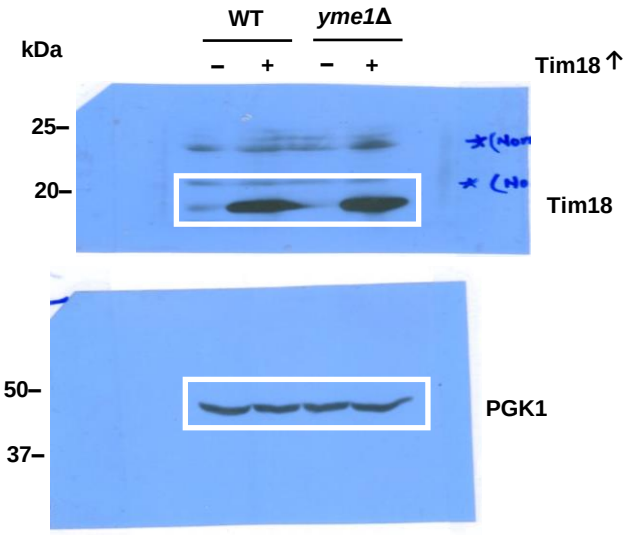


Fig. S3

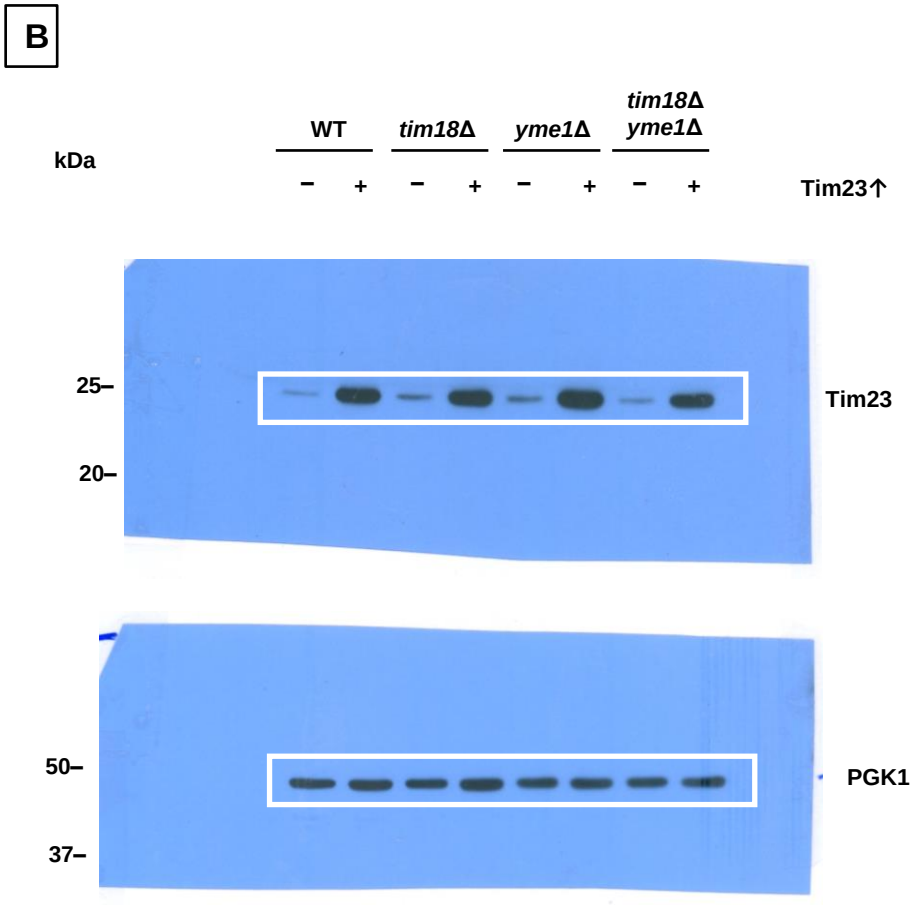


Fig. S4

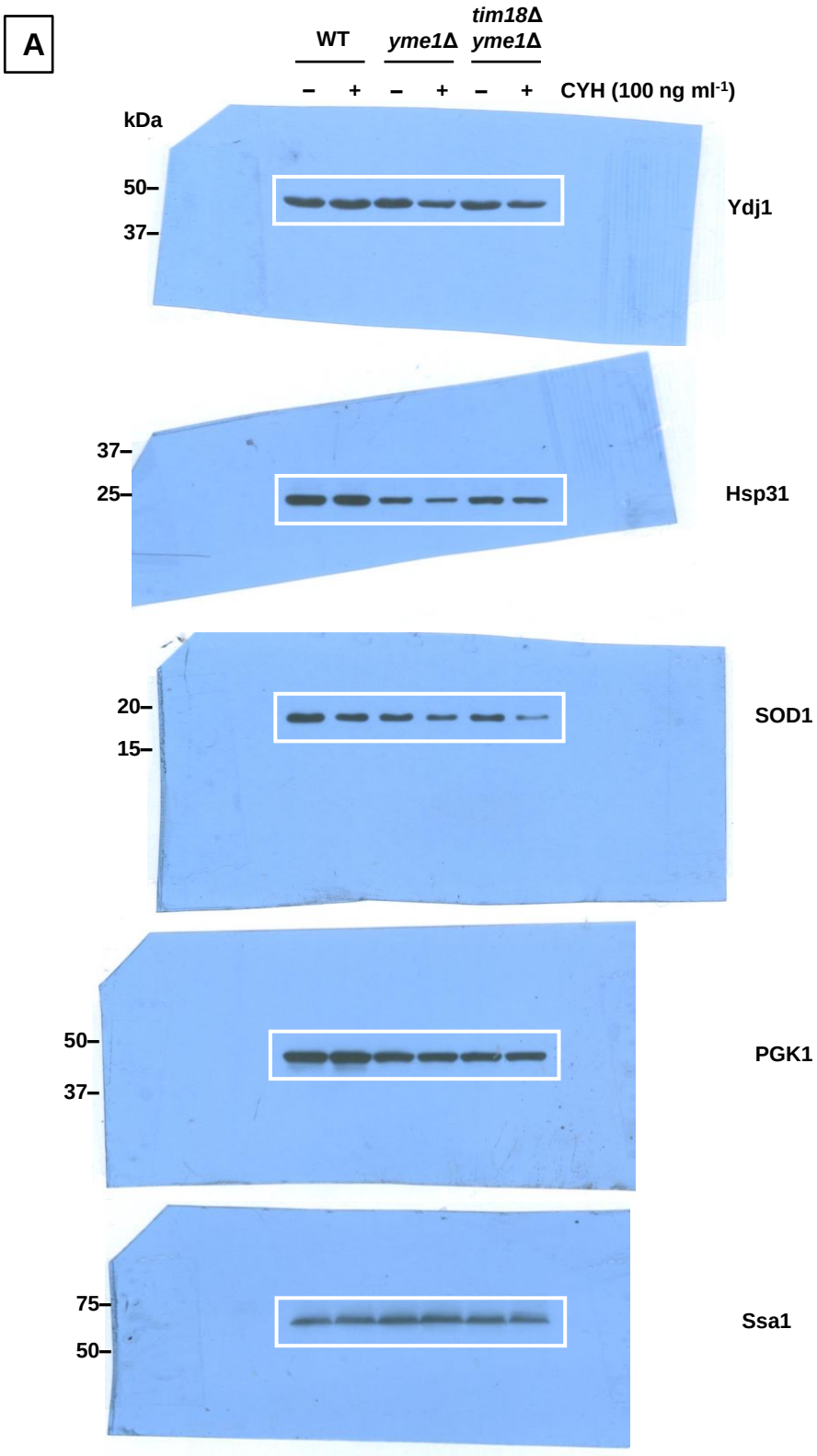


Fig. S4

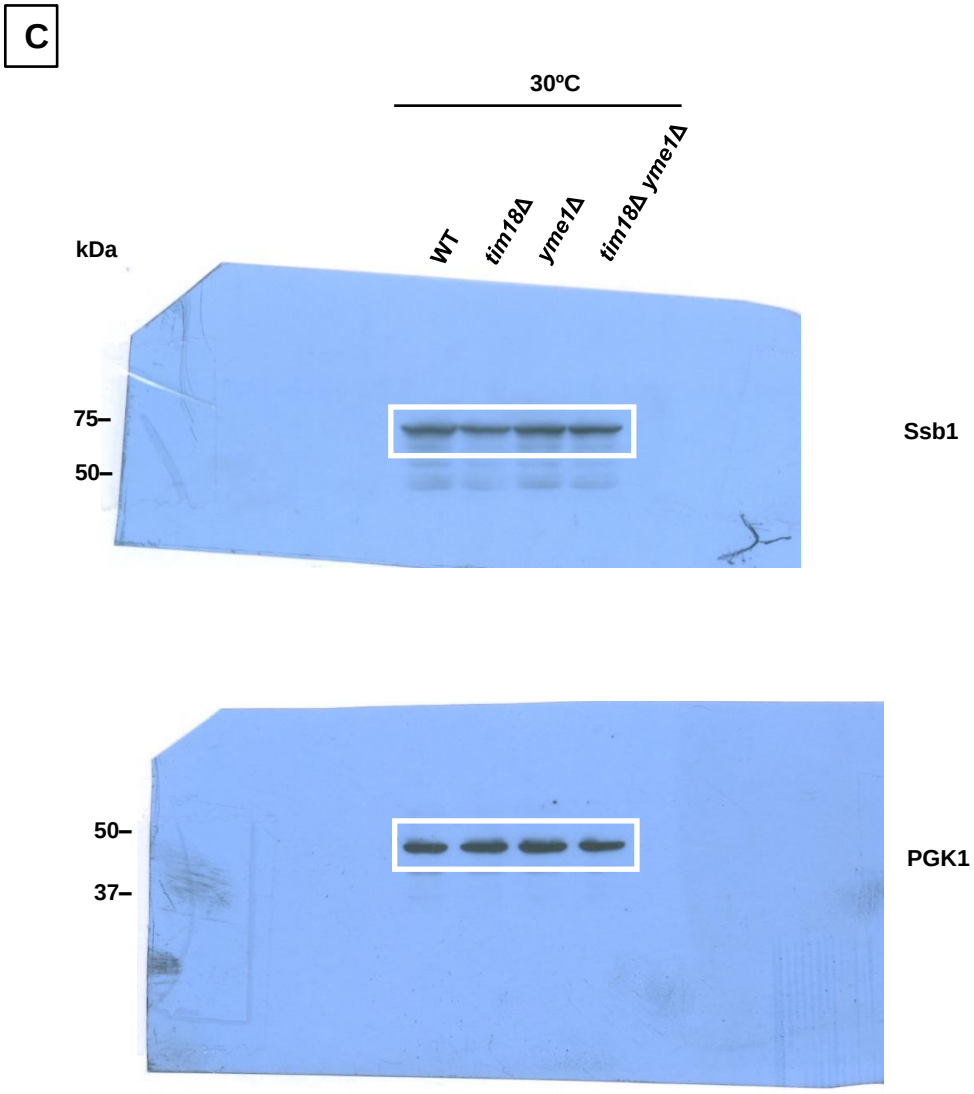


Fig. S4

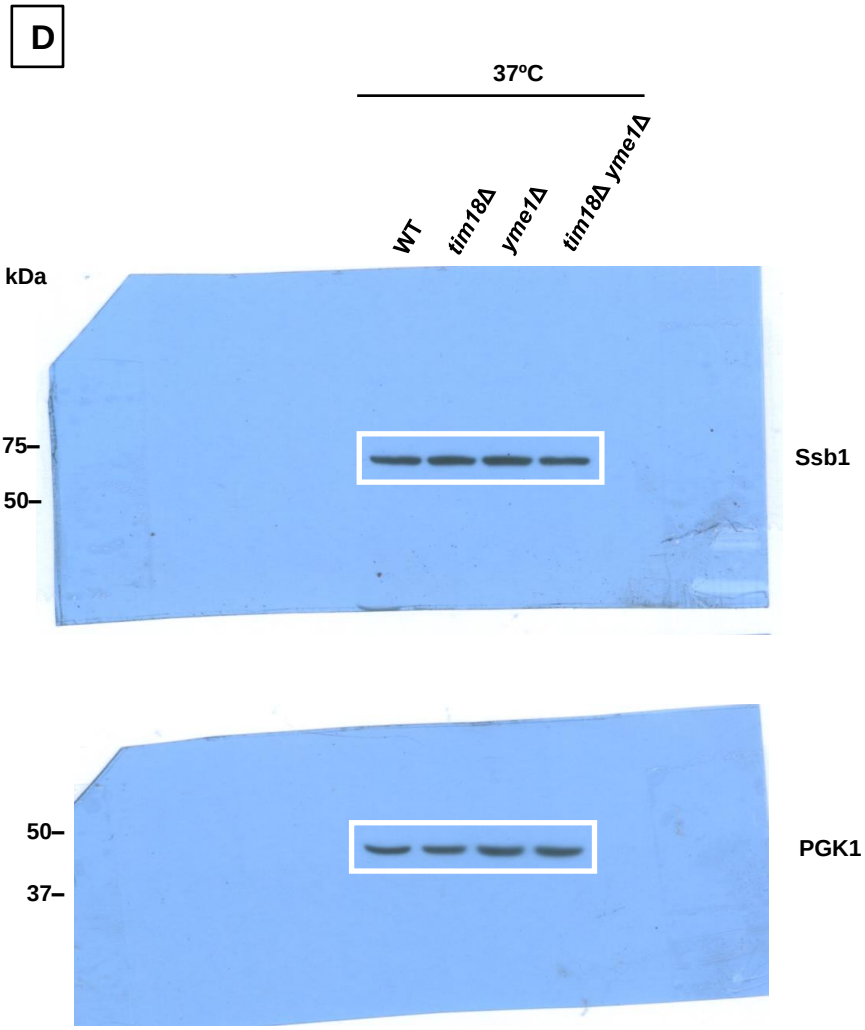


Fig. S5

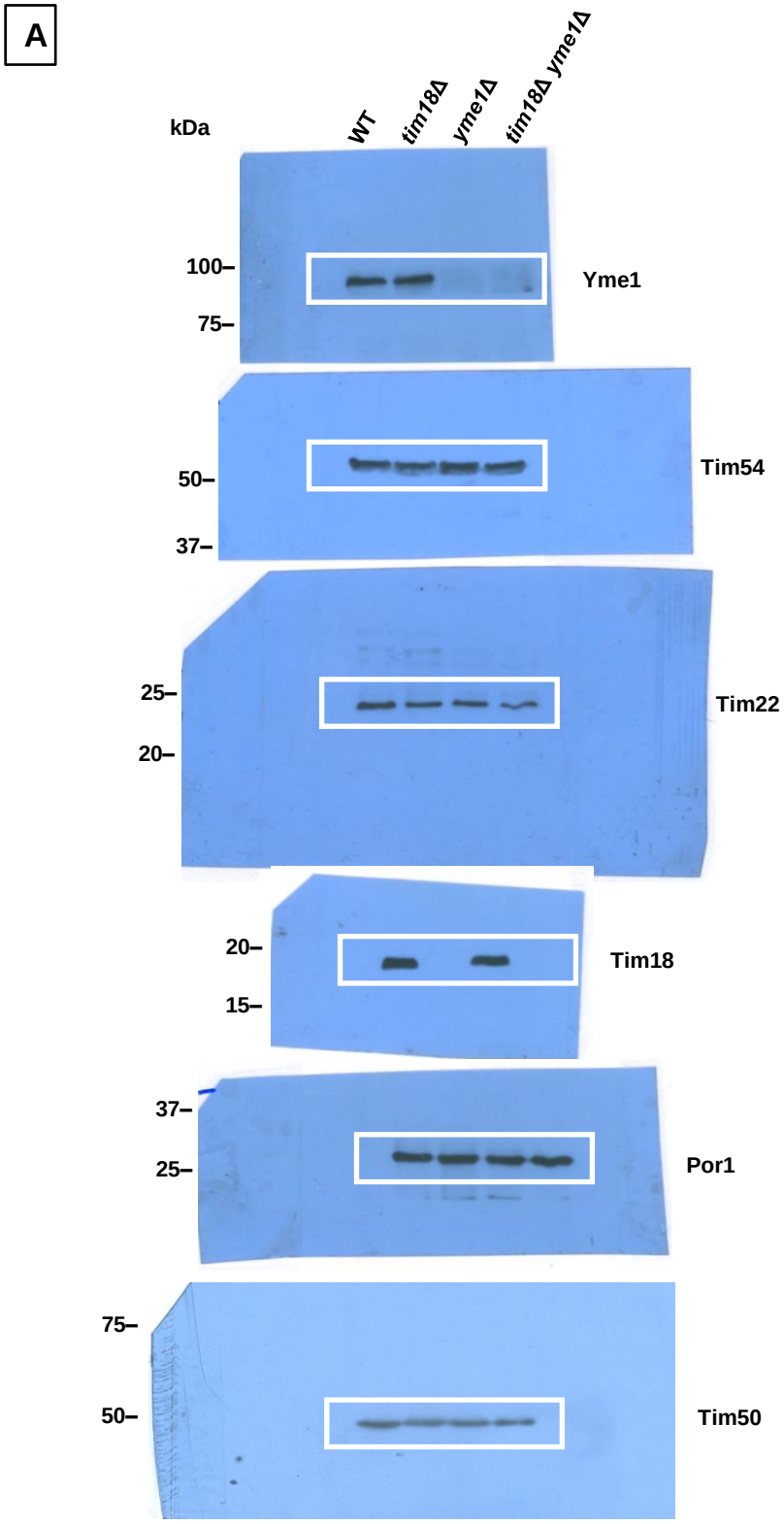


Fig. S5

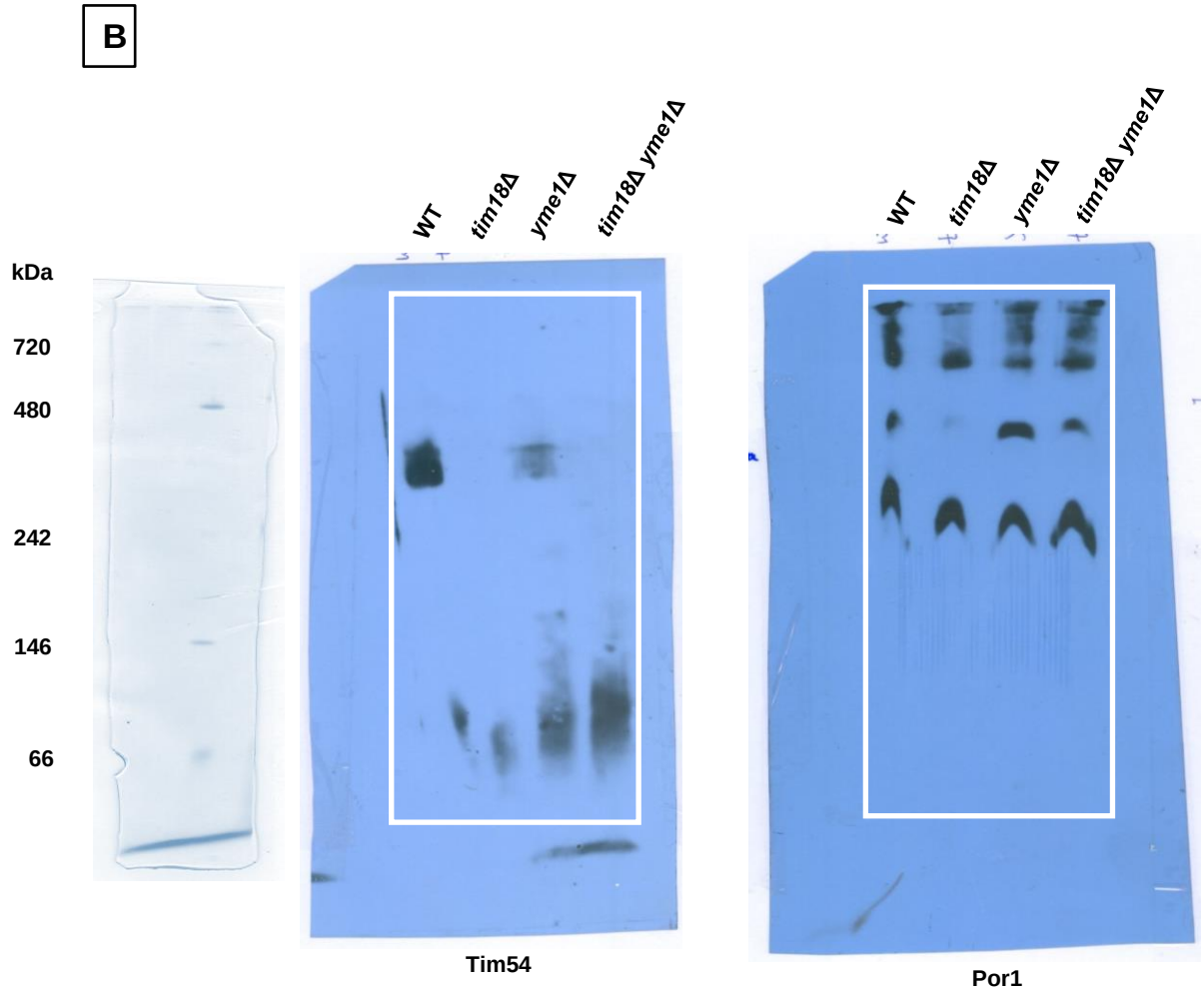


Table S1. Yeast strains used in this study

Name	Genotype	Source
WT W303	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 GAL2 met2-1 lys2-2*	Hines et al., 2011
tim18Δ	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 GAL2 met2-1 lys2-2 tim18Δ::KanMX4*	This study
yme1Δ	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 GAL2 met2-1 lys2-2 yme1Δ::HphNT1*	This study
tim18Δ yme1Δ	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 GAL2 met2-1 lys2-2 tim18Δ::KanMX4 yme1Δ::HphNT1*	This study
K127A *yme1Δ*	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 tim22Δ::CgHIS3* [pRS314-$tim22_{K127A}$] *yme1Δ::HphNT1*	This study
WT Tim22-316	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 tim22 Δ::CgHIS3,* [pRS316-*Tim22*]	Okamoto et al., 2014
Tim18FLAG/WT Tim22-316	*MATa ade2-1 his3-11, 15 ura3-1 leu2-3, 112 trp1-1 can1-100 tim22 Δ::CgHIS3, TIM18-FLAG::KanMX6* [pRS316-*Tim22*]	Okamoto et al., 2014
PTY44	*MATa lys2 ura3-52 leu2-3,112 trp1-Δ1 [rho$^+$ TRP1]*	Thorsness et al., 1993
PTY44/*tim18Δ*	*MATa lys2 ura3-52 leu2-3,112 trp1-Δ1 tim18Δ::KanMX4 [rho$^+$ TRP1]*	This study
PTY52	*MATa lys2 ura3-52 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 [rho$^+$ TRP1]*	Thorsness et al., 1993
PTY52/*tim18Δ*	*MATa lys2 ura3-52 leu2-3,112 trp1-Δ1 yme1-Δ1::URA3 tim18Δ::KanMX4 [rho$^+$ TRP1]*	This study
PJ53/Tim23-FLAG	*trp1-1/trp1-1, ura3-1/ura3-1, leu2-3,112/leu2-3/112, his3-11,15/his3-11,15 ade2-1/ade2-1, can1-100/can1-100 GAL2$^+$/GAL2$^+$, met2-Δ1/met2-Δ1, lys2-Δ2/lys2-Δ2, TIM23-FLAG::KanMX4*	This study
PJ53/SOD1-FLAG	*trp1-1/trp1-1, ura3-1/ura3-1, leu2-3,112/leu2-3/112, his3-11,15/his3-11,15 ade2-1/ade2-1, can1-100/can1-100 GAL2$^+$/GAL2$^+$, met2-Δ1/met2-Δ1, lys2-Δ2/lys2-Δ2, sod1Δ::HphNT1* [pRS416$_{GPD}$-*SOD1-FLAG*]	This study

Table S2. Plasmid constructs and primers used in this study

	Primers used for deletion of *TIM18* and *YME1*gene	
Construct Name	**Orientation (5'-3')**	**Source**
*tim18*Δ Fwd	CGGTGATGCGAGGTGCAACAACTGAGTAATTTAATACCTTTGGCGTACGCTGCAGGTCGAC	This study
*tim18*Δ Rev	GAAATCTTAGAAATGCAAAAAAAAAGAAAAAGTATGGGTGAGTCAATCGATGAATTCGAGCTCG	This study
*yme1*Δ Fwd	GCAATTTTTAATTATAATACATTGTGGATAGAACGAAAACAGAGACGTGCGTACGCTGCAGGTCGAC	This study
*yme1*Δ Fwd	CGGTCTTGAGGTAGGTTCCTTCATACGTTTAACTTCTTAGAATAAAATCAGGATGGCGGCGTTAGTATCG	This study

	Primers used for plasmid construction		
Construct Name	**Orientation (5'-3')**	**Restriction Site**	**Source**
pRS316-Tim18 Fwd	GCGAGCGGCCGCAAAGTTTAGGTCATCACTTCCC	NotI	This study
pRS316-Tim18 Rev	ACGCGTCGACATAATAAAAAAGGCACTTTAGA	SalI	This study
$pRS416_{TEF}$-Tim22 Fwd	GCGGGATCCATGGACTACAAAGACGATGAC	BamHI	This study
$pRS416_{TEF}$-Tim22 Rev	ACGCGTCGACTCATTCTTTAAAATCGTTTTG	SalI	This study
$pRS416_{TEF}$-Tim18 Fwd	GCGTCTAGAATGCTATTGTTTCCTGGCTTGAAG	XbaI	This study
$pRS416_{TEF}$-Tim18 Rev	ACGCGTCGACTTAACGGGTGTTTTGCAACC	SalI	This study
$pRS414_{TEF}$-AAC2 Fwd	CGCGGATCCATGTCTTCCAACGCCCAAG	BamHI	This study
$pRS414_{TEF}$-AAC2 Rev	ACGCGTCGACTTATTTGAACTTCTTACC	SalI	This study

pRS414$_{TEF}$-PIC Fwd	CTAGACTAGTATGTCTGTGTCTGCTGCTCC	SpeI	This study
pRS414$_{TEF}$-PIC Rev	CCGCTCGAGCTAATGACCACCACCACC	XhoI	This study
pRS414$_{TEF}$-DIC1 Fwd	CGCGGATCCATGTCAACCAACGCAAAAGAG	BamHI	This study
pRS414$_{TEF}$-DIC1 Rev	ACGCGTCGACCTACTTGTCTTCCTTTGGC	SalI	This study
pRS414$_{TEF}$-Tim23 Fwd	CGGACTAGTATGTCGTGGCTTTTTGGAG	SpeI	This study
pRS414$_{TEF}$-Tim23 Rev	CCGCTCGAGTCATTTTTCAAGTAGTCTTTTC	XhoI	This study
pRS414$_{TEF}$-Tom6 Fwd	CGCGGATCCATGGACGGTATGTTTG	BamHI	This study
pRS414$_{TEF}$-Tom6 Rev	ACGCGTCGACTTATAATTGTGGGGCC	SalI	This study
pRS414$_{TEF}$-Abf2 Fwd	CGCGGATCCATGAACAGTTACAGCCTATTA	BamHI	This study
pRS414$_{TEF}$-Abf2 Rev	CCGCTCGAGCTAGTTGAGAGGGTAGCGAGC	XhoI	This study
pRS416$_{GPD}$ SOD1 Fwd	CGCGGATCCATGGTTCAAGCAGTCGCAGTG	BamHI	This study
pRS416$_{GPD}$ SOD1 Rev	TGCGGTCGACTTACTTGTCGTCATCGTCTTTGTAG TCGTTGGTTAGACCAATGACACC	SalI	This study

*** Restriction sites are underlined**