Supplementary information appendix

Supplementary Materials and Methods

Plasmid construction

To make plasmids pPKB1 and pPKB2, the *AUP1* reading frame full length and an alternative insert lacking 34 amino acids from the N-terminal of the reading frame, respectively, were amplified using a PCR reaction. The primers P1 (5'-ATACTAACTAGTTACAACATGCGGCTGGGGAATCT ATG-3') and P2 (5'-ATACTAATCGATTTAGTTTAATTTTGTTTCGTTAGATTG-3) contain *ClaI* and *SpeI* linkers (SI appendix table III). The PCR product was digested then with *ClaI* and *SpeI* and ligated into a *ClaI* and *SpeI* digested pCU416. To construct plasmid PKB32, the Mdh1-GFP open reading frame (ORF) was amplified by HAY840 yeast genomic DNA using a PCR reaction and oligonucleotides primers P7 Forward: 5'-ATACTAACTAGTTACAACA TGTTGTCAAGAGTAGCTAAAC-3' and P8 Reverse: 5'-ATACTAATCGATGACCTCATACTATACCTG-3' containing *ClaI* and *SpeI* restriction sites (SI appendix table IV). The PCR product was digested with *ClaI-SpeI* and ligated into a *ClaI* and *SpeI* digested pCU416, generating a fusion protein under the control of the *CUP1* promoter. Plasmid PKB65 was generated by cloning the Mdh1-GFP reading frame, together with 800 bases of 5' and 500 bases of 3' sequences was amplified using a PCR and primers P5 Forward: 5'-ATACTAGGATCCC AAAAGATCGACGCAATG-3' and P6 Reverse: 5'-ATACTAGAGCTCGACCTCATACTATACCTG-3' (table 4) containing *BamHI* and *SacI* linkers. The PCR product was digested with *BamHI-SacI* and ligated into a *BamHI-SacI* digested pRS415. All the plasmids were verified by sequencing (Hylabs, Rehovot).

Site directed mutagenesis

Site directed mutagenesis protocol was carried out according to the Stratagene QuikChange system. *Pfu* DNA Polymerase (Fermentas) was used with a thermocycling protocol followed by removal of the parental strands with *DpnI* digestion. Thermocycling conditions were as follows: Initial denaturation at 95°C for 2 min, then (steps 2-4), 30 sec at 95 °C, 60 sec at 55°C, and 12 min at 68 °C. Steps 2-4 repeated for 18 cycles. PCR products were treated with DpnI (fermentas) for 1 hr at 37°C, and precipitated using with 70% ethanol. DNA pellets were then dried and resuspended with 20 µl TE buffer pH 8. All mutagenesis products were transformed into *E. coli* DH5α, and mutations were verified by sequencing. The

oligonucleotide primers used to create site-directed MDH1-GFP variants and combined variants are listed in SI appendix Table III.

Culture conditions

Yeast were grown in synthetic dextrose medium (0.67% yeast nitrogen base w/o amino acids (Difco), 2% glucose, auxotrophic requirements and vitamins as required) or in SL medium (0.67% yeast nitrogen base w/o amino acids (Difco), 2% lactate pH 6, 0.1% glucose, auxotrophic requirements and vitamins as required). All culture growth and manipulation were at 26 ^{0}C. Yeast transformation was according to Gietz and Woods (1).

For nitrogen starvation experiments, cells were grown to mid-log phase (OD_{600} of 0.4-0.6) in synthetic dextrose medium (SD), washed with distilled water, and resuspended in nitrogen starvation medium (0.17 YNB-N (Difco), 2% glucose) for the times indicated in individual experiments. Overexpression studies with *CUP1* promoter-based vectors were carried out by supplementing the medium with 5 μM $CuSO_4$, for both control (empty vector) and overexpressing cells.

Calculation of statistical significance of differences in % free GFP signals from immunoblots

Data (% free GFP) were log-transformed before analysis in order to stabilize variances. A repeated measures ANOVA was performed to compare mutants and proteins simultaneously. Thereafter, mutants were compared for each protein and proteins were compared for each mutant by the Tukey HSD test ($p<0.05$). ANOVA analysis was carried out using JMP 12 software.

Generation of selectivity profile comparisons and heat maps

To compare between different genotypes, % free GFP values which were recorded from immunoblots of Mdh1-GFP, Aco1-GFP, Aco2-GFP, Qcr2-GFP and Idp1-GFP were normalized, such that each value of % free GFP was divided by the average value of all % free GFP measured for the same protein over all genotypes. This centered the data distribution such that all proteins contributed equally to the correlation calculation. Normalized data points of 3 biological replicates were averaged. We then calculated a Pearson correlation value (r (correlation coefficient) and p – values (significance of correlation) between all phenotype

pairs (vector of normalized averaged % free GFP per protein) using the rcorr function in R (Hmisc package). A heat map was generated using the heatmap.2 function in R (gplots package). The heat map cells contains the r correlation measured and colored from negative correlation in red to positive correlation in yellow. The dendogram was generated by calculating the Euclidean distance measure between correlation vectors of each genotype using the complete linkage method.

Cell fractionation. Cells (20 OD_{600} units) were collected by centrifugation at 3,500 xg, 5 min, 4°C. The cells were spheroplasted in medium containing 1 M sorbitol and 0.6 mg/ml zymolyase (0.67 % yeast nitrogen base, 2 % glucose, auxotrophic requirements and vitamins as required, 1 M sorbitol, 40 mM HEPES pH 7, 0.6 mg/ml zymolase) for 30 min at 37°C. Spheroplasts were collected by centrifugation at 200xg g for 5 min. The spheroplasts were resuspended on ice in lysis buffer (0.2 M sorbitol, 50 mM potassium acetate, 2 mM EDTA, 40 mM HEPES pH 7, plus protease inhibitors), transferred to a pre-cooled dounce homogenizer and dounced 15 times with a tight fitting pestle. The lysate was then transferred to eppendorf tubes and centrifuged at 300 xg, 4°C, 5 min. 1 ml of cleared supernatant was saved as total extract fraction (T). The rest was transferred to clean eppendorf tubes and centrifuged at 13.000x g, 10 min, 4°C. 1 ml of the supernatant was labeled as S13 (cytosolic proteins). The pellet was labeled as P13. The S13 and total extract fractions (1 ml each) were precipitated with 10% cold TCA by adding 500 µl of 30% TCA, while the P13 fraction was first resuspended in 1 ml lysis buffer and then precipitated with 10 % TCA. For immunoblot analysis, 0.5 OD_{600} equivalents were loaded per lane on SDS-PAGE gels.

Preparation of whole-cell extracts for western blot analysis. Cells (10 OD_{600} units) were treated with 10% cold trichloroacetic acid (TCA) and washed three times with cold acetone. The dry cell pellet was then resuspended in 100 µl cracking buffer (50 mM Tris pH 6.8, 6 M urea, 1 mM EDTA, 1% SDS) and vortexed in Tomy MT-360 microtube mixer at maximum speed, with an equal volume of acid-washed glass beads (425-600 µm diameter), for a total of 30 min. Lysates were clarified by centrifugation at 17,000 xg for 5 min and total protein was quantified in the supernatant using the BCA protein assay (Thermo Scientific, Rockford, IL). SDS loading buffer (final concentrations of 100 mM Tris pH 6.8, 20% glycerol, 2% SDS, 500 mM β-mercaptoethanol) was added to the lysate and the samples

were warmed to 60°C for 5 min prior to loading on gels. ImageJ software was used for band quantification.

Immunoblotting. SDS-10% polyacrylamide gels were transferred to nitrocellulose membrane by using either a wet or semidry blotting transfer system. Membranes were blocked for 1 h with TPBS (137 mM NaCl, 2.7 mM KCL, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1 % Tween and 5 % milk powder (BD Difco Skim Milk), followed by incubation with anti-GFP rabbit (1:5000) for 1.5 h, then washed 4x 2 min with TPBS, incubated for 1.5 h with HRP conjugated secondary antibody (1:10000). The membranes were then washed 4x with TPBS, incubated with SuperSignal Chemiluminescence substrate (Thermo Scientific), and exposed to a Molecular Imager ChemiDoc™ XRS imaging system.

Supplementary figure legends

Supplementary Figure 1. **All the mitochondrial reporters used in this study generate free GFP in an Atg32- dependent fashion**. Cells expressing Qcr2-GFP, Idp1-GFP, Aco1-GFP and Aco2-GFP were grown, collected and analyzed as described in the legend to Figure 1B.

Supplementary Figure 2. **A sample blot comparing free GFP release from Aco1-GFP in WT (HAY75), *aup1Δ* (HAY809), *ptc5Δ* (PKY37), *pkp1Δ* (PKY61), *pkp2Δ* (PKY70) and *pkp1Δ pkp2Δ* (PKY629) *cells***. Experimental details are as described in the legend to Figure 1.

Supplementary Figure 3. **A sample blot comparing free GFP release from Aco2-GFP in WT, *aup1Δ*, *ptc5Δ*, *pkp1Δ*, *pkp2Δ*, and *pkp1Δ pkp2Δ cells***. Experimental details are as described in the legend to Figure1.

Supplementary Figure 4. **A sample blot comparing free GFP release from Qcr2-GFP in WT, *aup1Δ*, *ptc5Δ*, *pkp1Δ*, *pkp2Δ*, and *pkp1Δ pkp2Δ cells***. Experimental details are as described in the legend to Figure 1.

Supplementary Figure 5. **A sample blot comparing free GFP release from Idp1-GFP in WT, *aup1Δ*, *ptc5Δ*, *pkp1Δ*, *pkp2Δ*, and *pkp1Δ pkp2Δ cells***. Experimental details are as described in the legend to Figure 1.

Supplementary Figure 6. **A sample blot comparing free GFP release from Mdh1-GFP in WT, *aup1Δ*, *ptc5Δ*, *pkp1Δ*, *pkp2Δ*, and *pkp1Δ pkp2Δ cells***. Experimental details are as described in the legend to Figure 1.

Supplementary Figure 7. **A sample blot comparing free GFP release from mtDHFR-GFP in WT, *aup1Δ*, *ptc5Δ*, *pkp1Δ*, *pkp2Δ*, and *pkp1Δ pkp2Δ cells***. Experimental details are as described in the legend to Figure1.

Supplementary Figure 8. **The general differential centrifugation fractionation properties of the T199A mutant in Mdh1-GFP are identical to those of the wild-type**. Cells expressing WT Mdh1-GFP (PKY366) and the T199A (PKY368) variant were grown, lysed and fractionated into total lysate, 13,000 xg pellet and 13,000 xg supernatant as described in "Materials and Methods". GAPDH was used as a cytosolic marker.

Supplementary Figure 9. **Free GFP release patterns from different Mdh1-GFP variants expressed in WT yeast cells**. Cells (PKY365) were treated and sampled as described in the legends to Figures 4C and 4D.

Supplementary Figure 10. **Expression of Mdh1^{T199A}-GFP does not affect mitophagy of Qcr2-RFP.** Cells (PKY535) co-expressing Qcr2-RFP and Mdh1-GFP, Mdh1^{T199A}-GFP or Mdh1^{T59A} were treated and sampled as described in the legends to Figures 7A and 7B. **(A)** Samples were immunoblotted and probed with anti-GFP antibodies. **(B)** The same samples were probed with anti-RFP antibodies. WT cells not expressing RFP were also tested in the α-RFP blot as a specificity control. **(C)** Quantification and reproducibility of normalized data from 4 independent biological repetitions. Mdh1-GFP data were normalized to WT Mdh1-

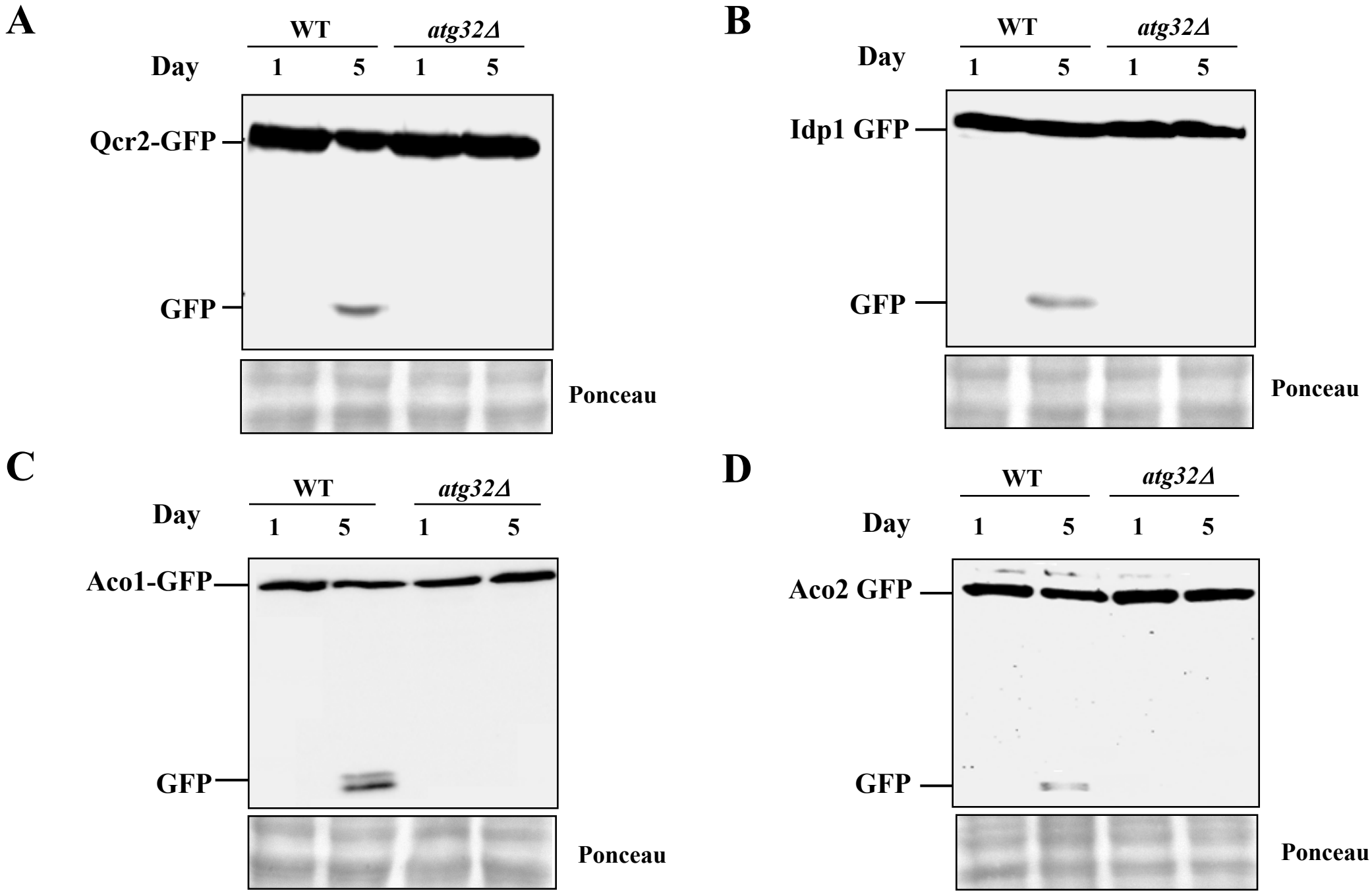
GFP and Qcr2-RFP data were normalized to the Qcr2-RFP data of samples from cells expressing WT Mdh1-GFP.

Supplementary Figure 11. **Alanine point mutants do not affect Mdh1 function and cellular physiology**. Cells (*mdh1Δ*, strain PKY365) harboring empty vector or plasmids encoding MDH1 and the various MDH1 point mutants were sown at OD 0.08 on synthetic lactate medium, and optical density at 600 nm was recorded over 72 hours.

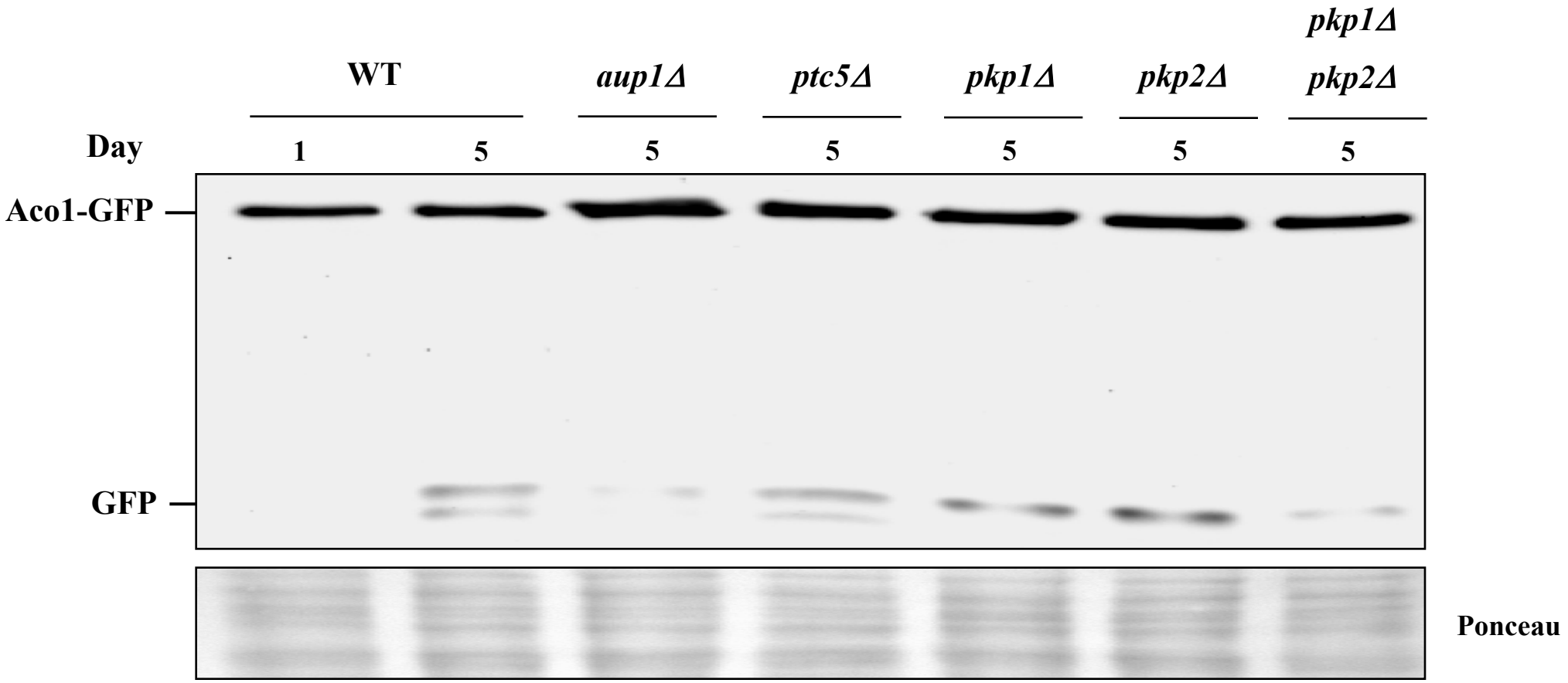
Supplementary Figure 12. **Comparison of endogenous expression levels versus *CUP1* promoter-driven overexpression for Idp1-GFP and Mdh1-GFP**. Cells expressing endogenous Idp1-GFP and Mdh1-GFP (PKY15 and PKY17 (WT) ; PKY186 and 133 (*aup1Δ*)) were grown in complete minimal medium and 30 μg protein samples were analyzed by immunoblotting. Cells overexpressing Mdh1-GFP and Idp1-GFP under the *CUP1* promoter from plasmids (PKY67 and PKY 302 (WT); PKY146 and PKY303 (*aup1Δ*)) were grown on minimal medium lacking uracil and supplemented with 5 μM $CuSO_4$. 30 μg protein samples were analyzed by immunoblotting with anti-GFP antibody.

Supplementary Figure 13. **Survival curve in synthetic lactate medium for *pkp1Δ pkp2Δ* (PKY672) cells compared with WT (PKY17) cells**. Cells were seeded in fresh medium at an OD_{600} of 0.08 and viability was assayed by sampling the cells daily and plating them onto SD complete medium. Viable colonies were counted and normalized to day 1. Data shown is an average of three independent biological repetitions; bars denote standard error.

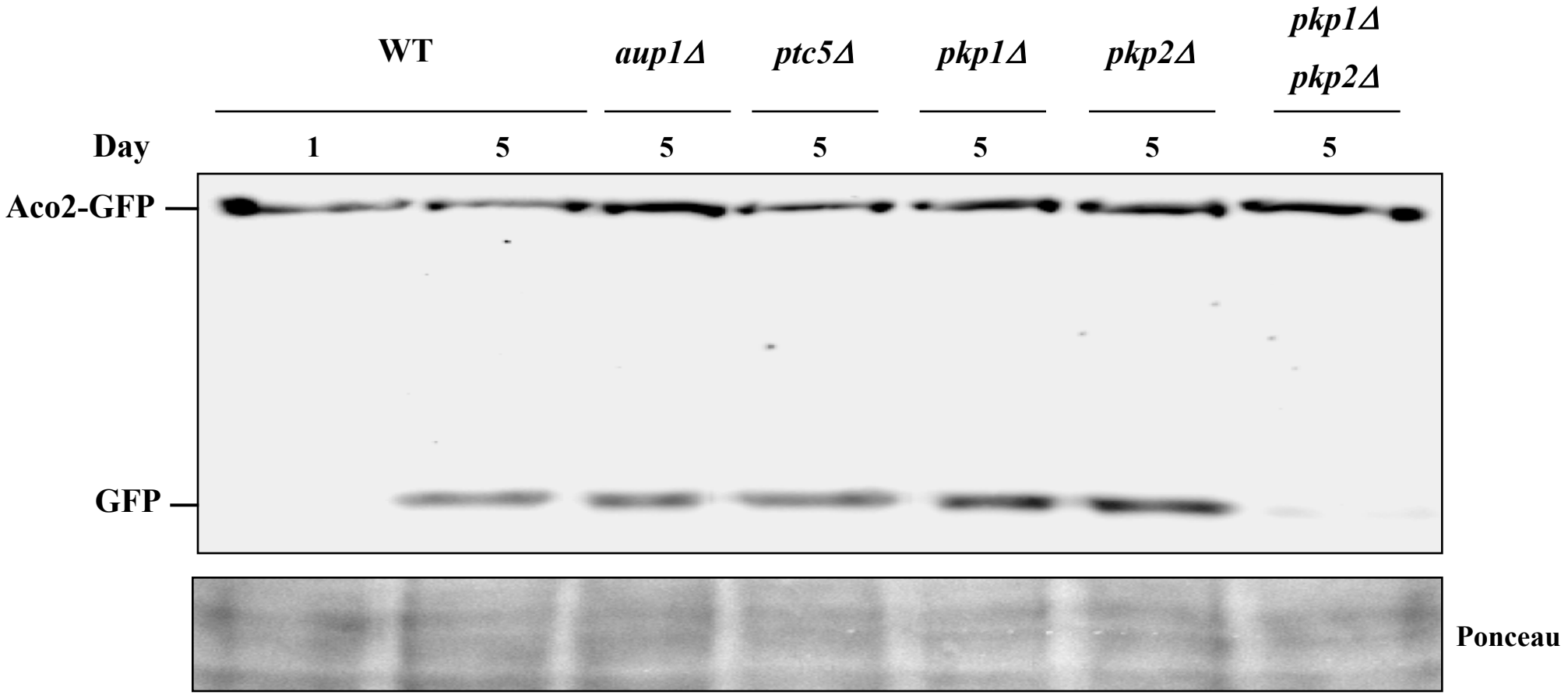
Supplementary Figure 14. **Segregation of Mdh1-RFP from Qcr2-GFP in WT (PKY1025) and *aup1Δ* (PKY1054) cells**. Cells co-expressing integrated Mdh1-RFP and Qcr2-GFP were imaged on days 1 and 2 of the incubation (to avoid interference from vacuolar signal, top panels) and the overlap between the red and green channels was quantified as described in 'Materials and Methods'. Graph (bottom) shows quantification of the overlap between the two channels using the JACoP script in ImageJ (bars denote SE, N=3 biological repeats). Scale bar =1 μm.



Supplementary Figure 1



Supplementary Figure 2



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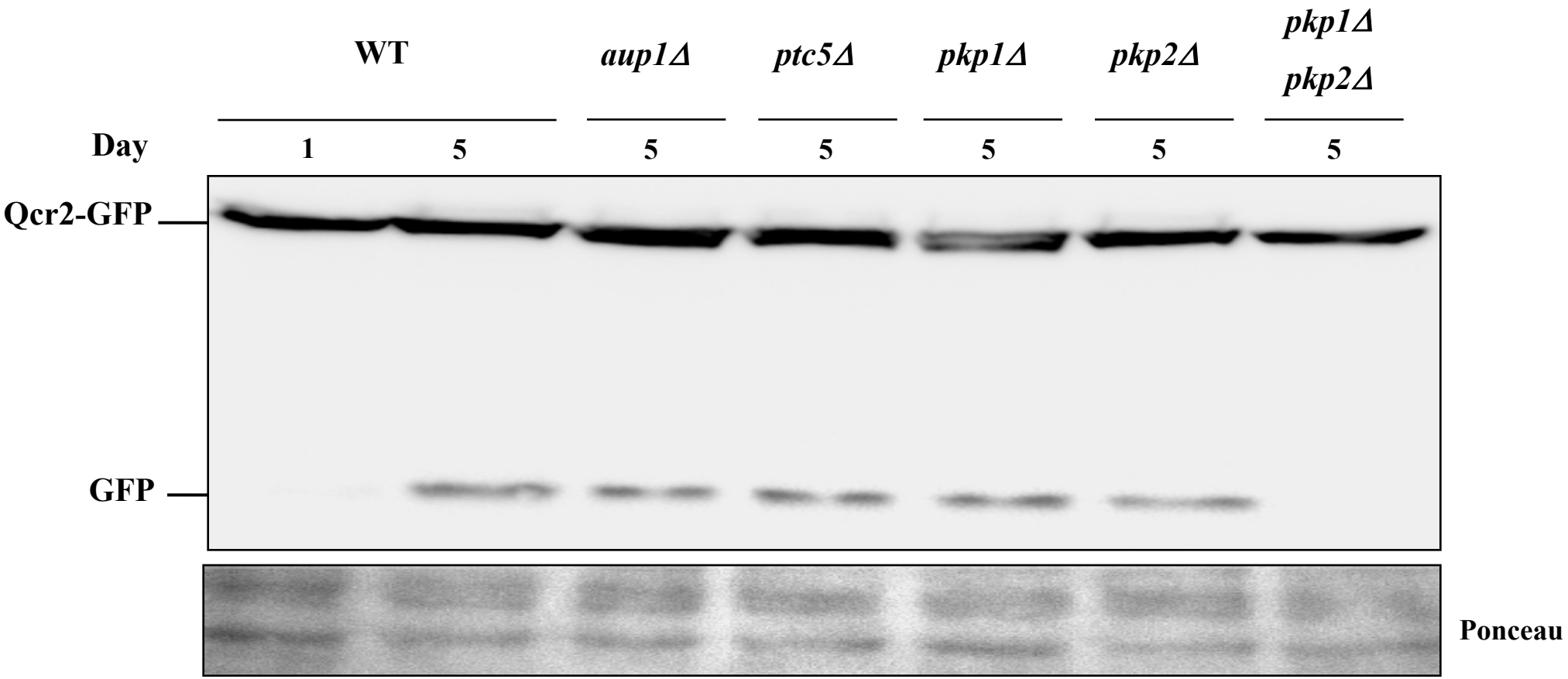

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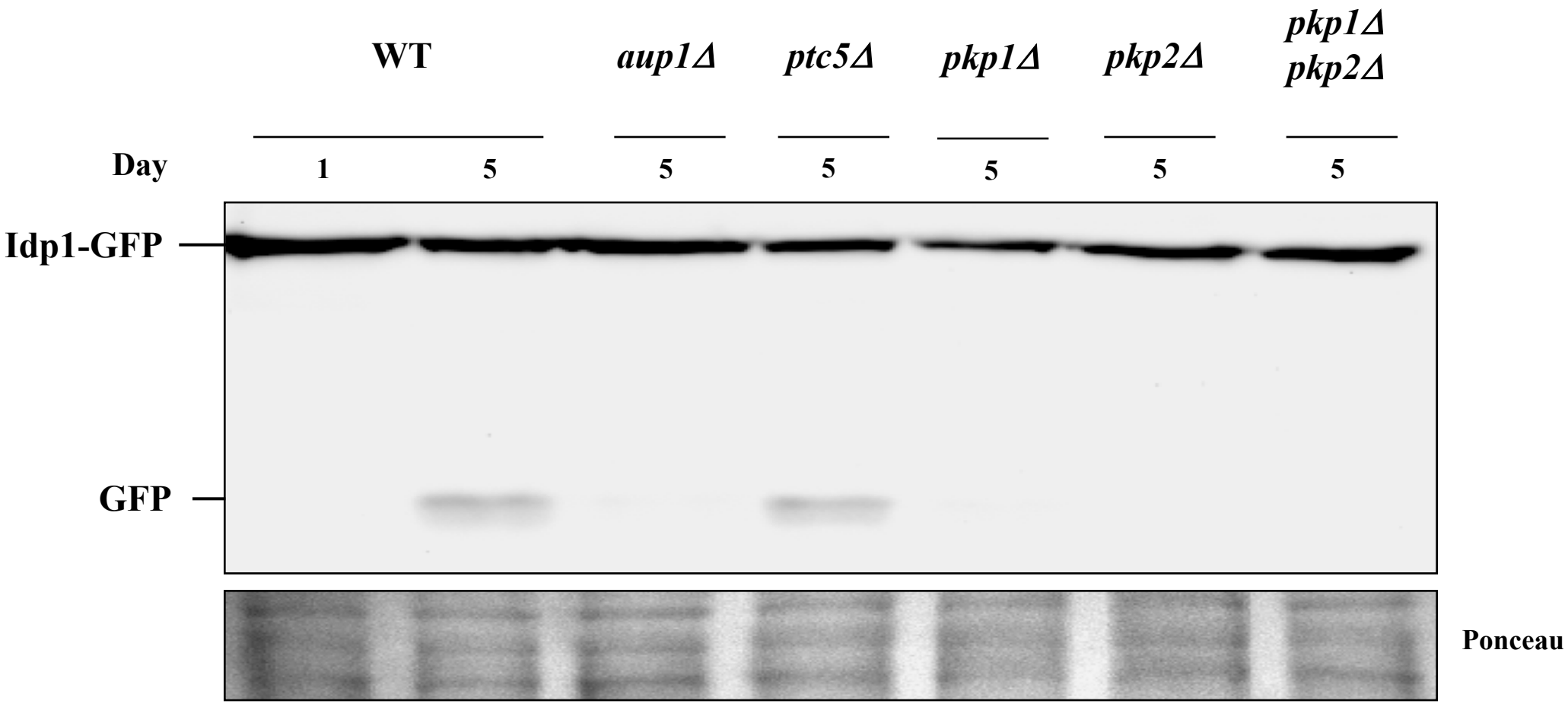

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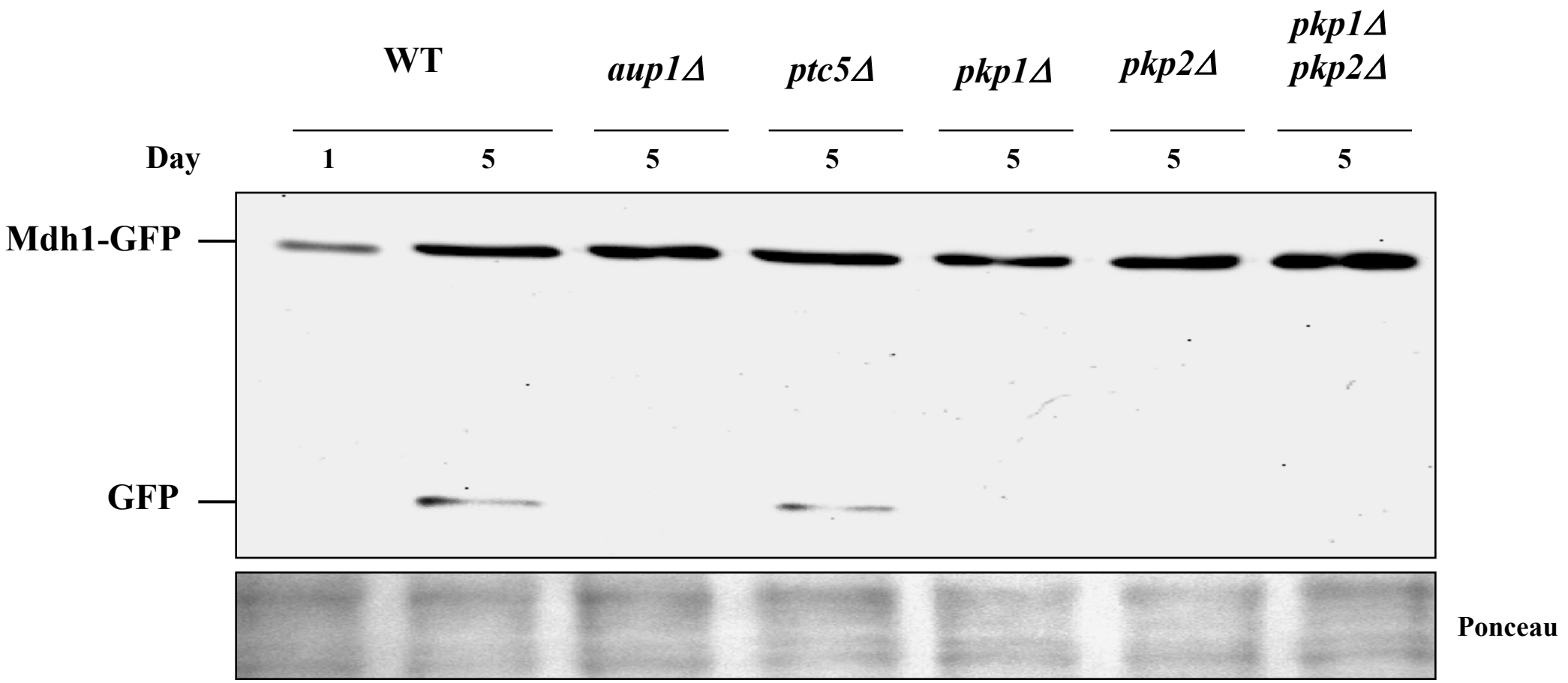

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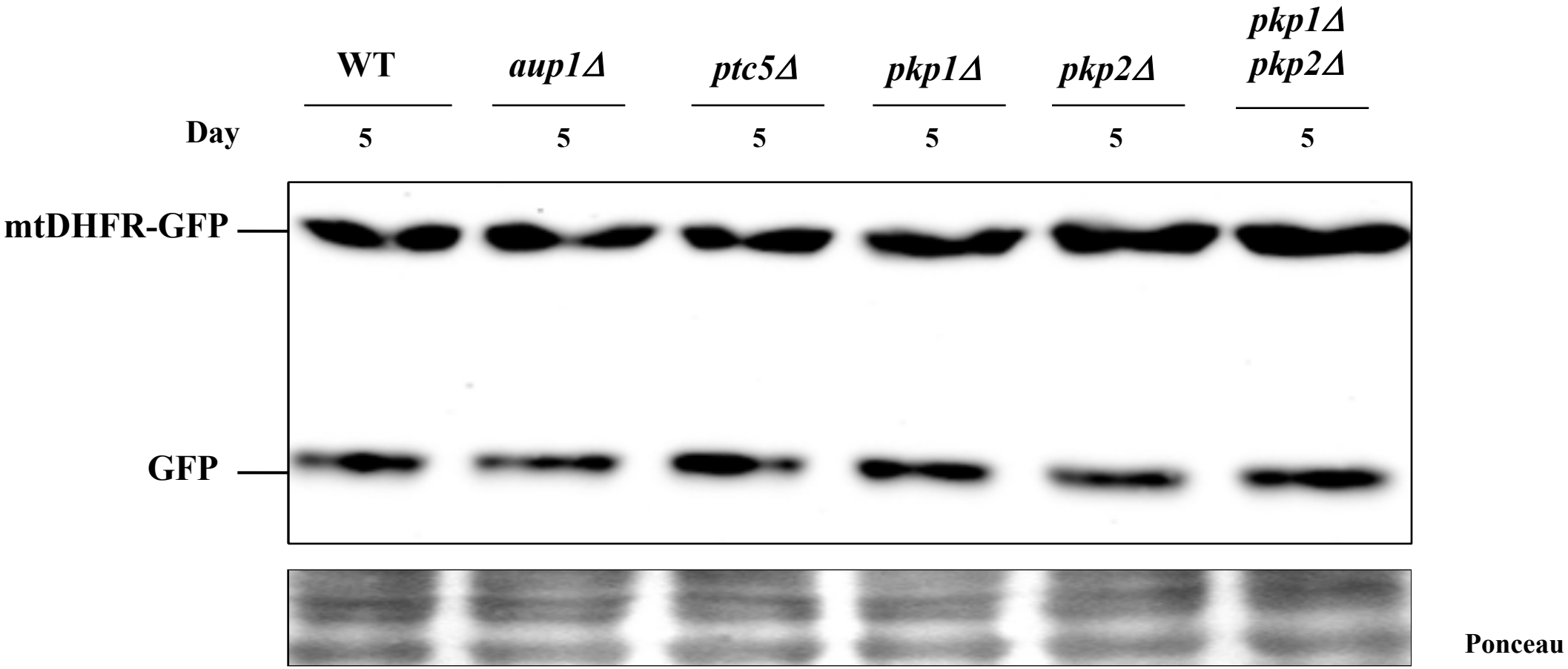

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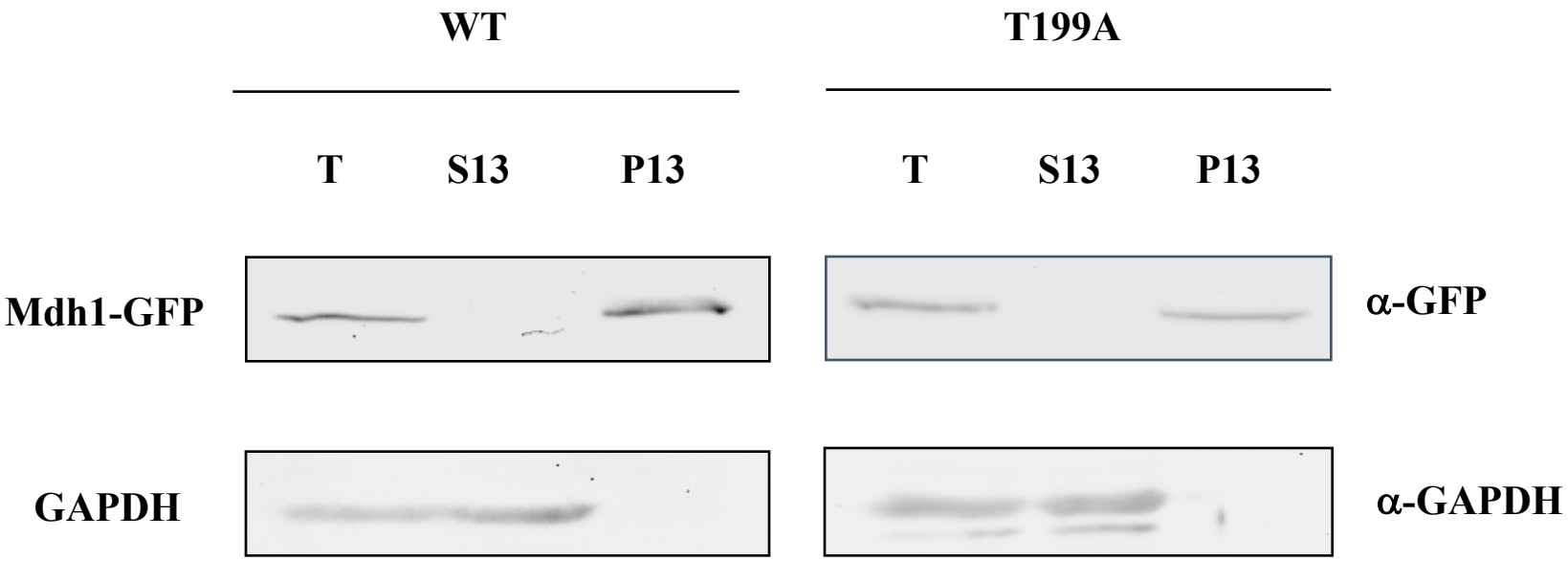

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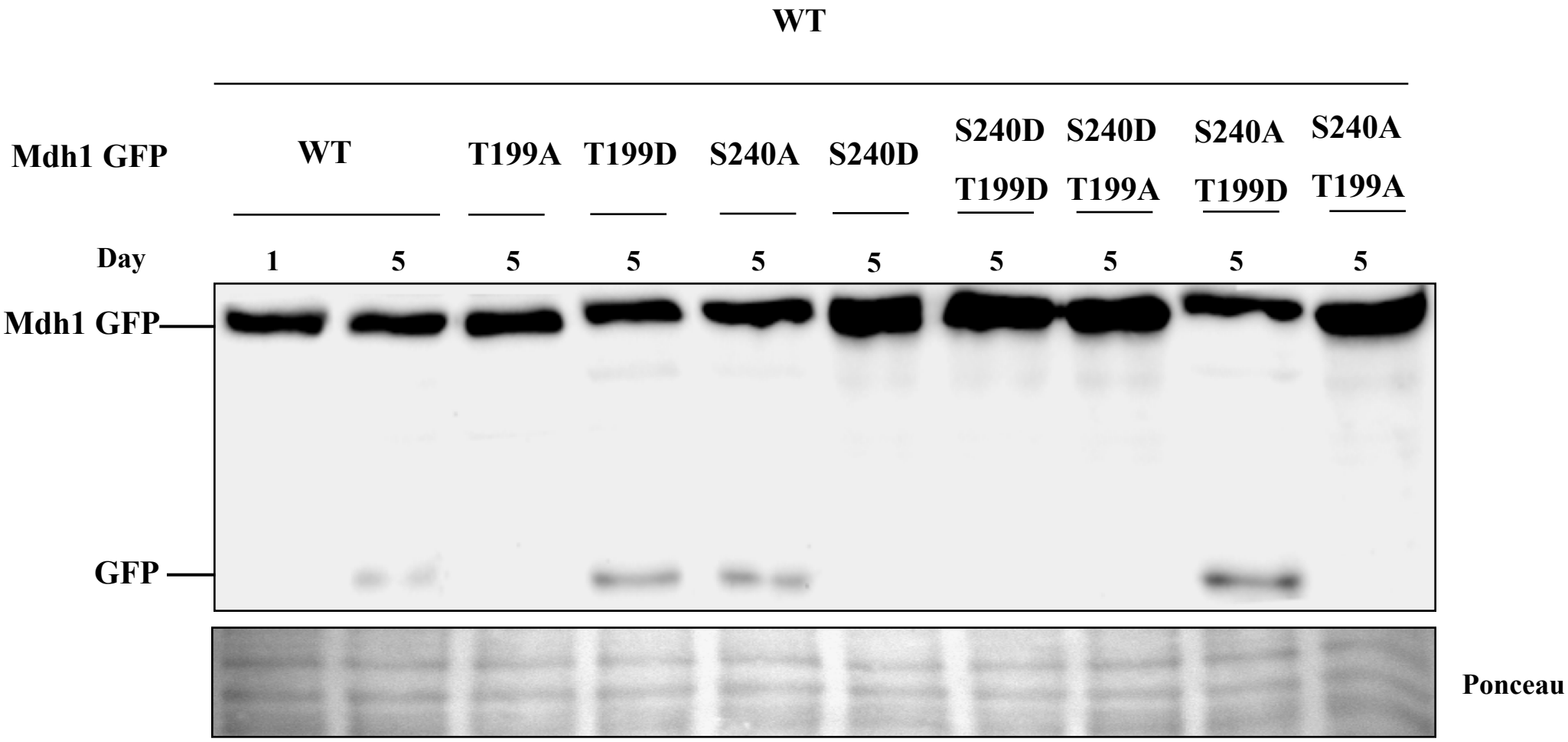
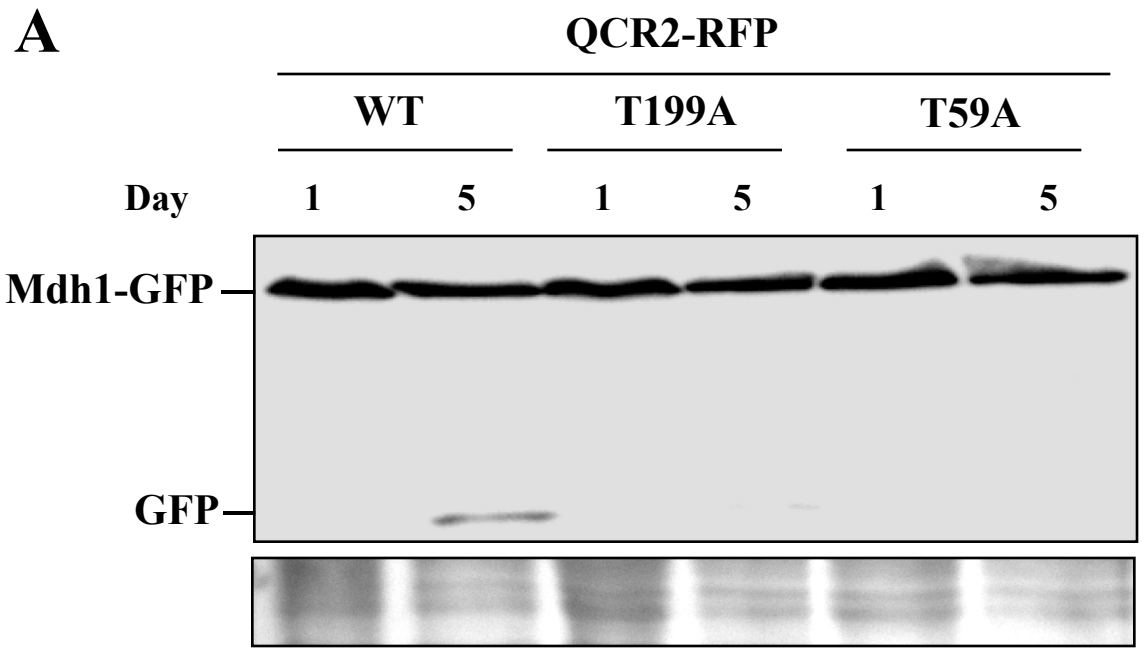
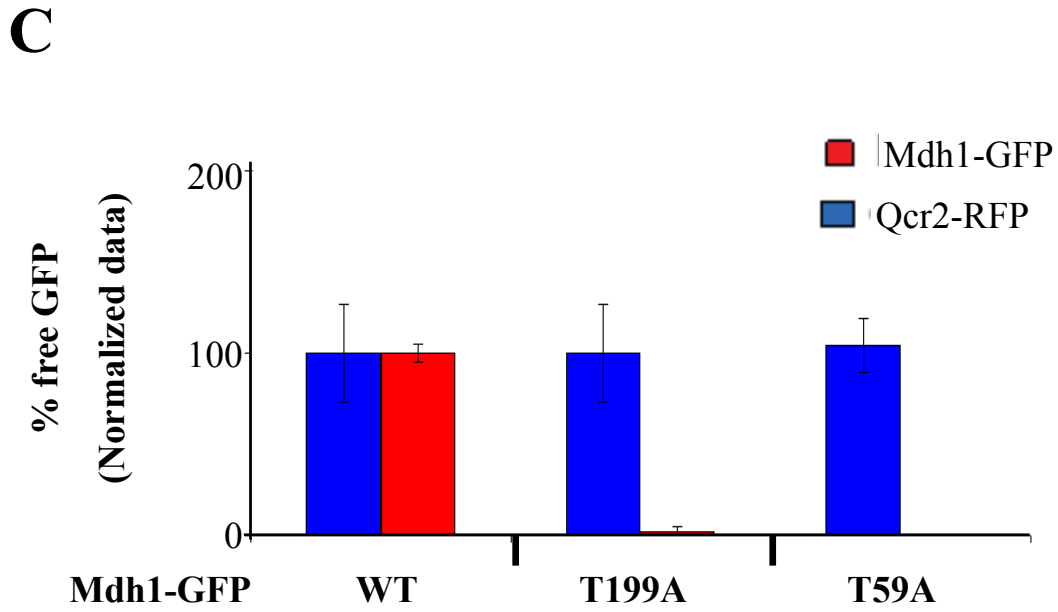
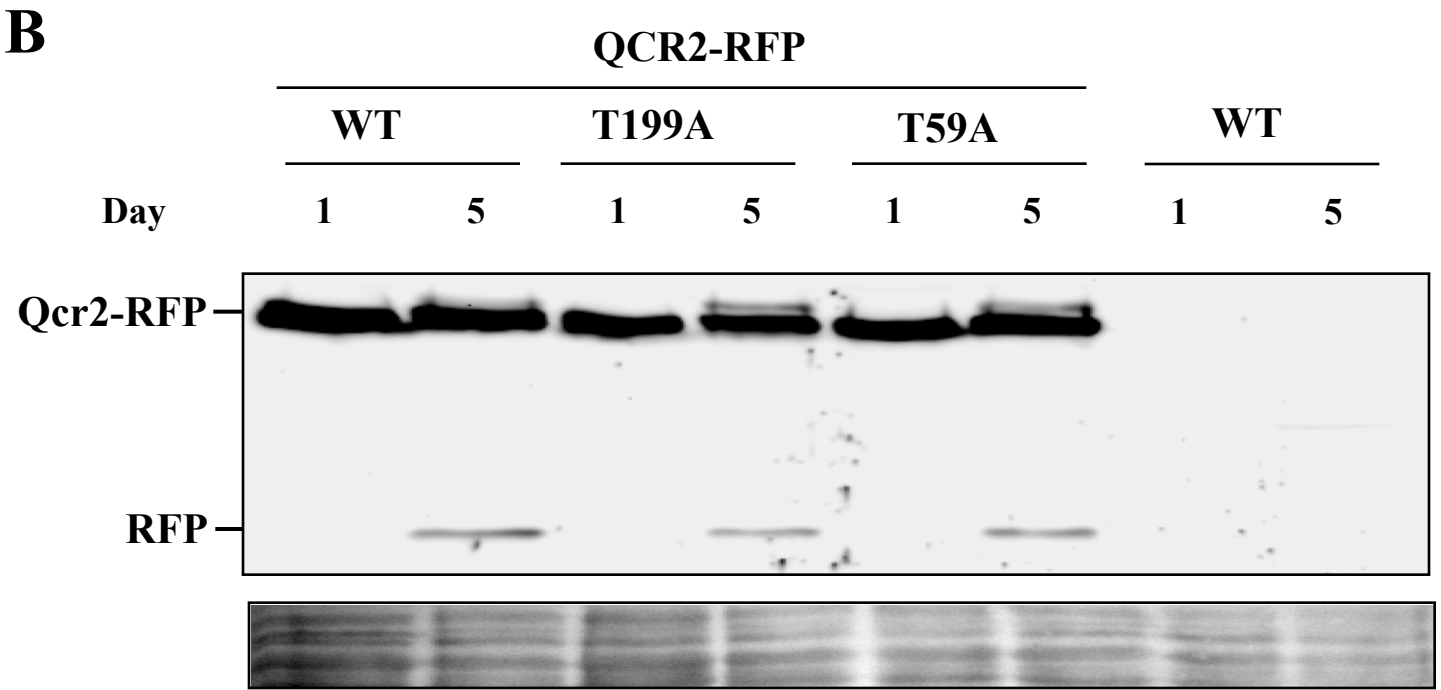


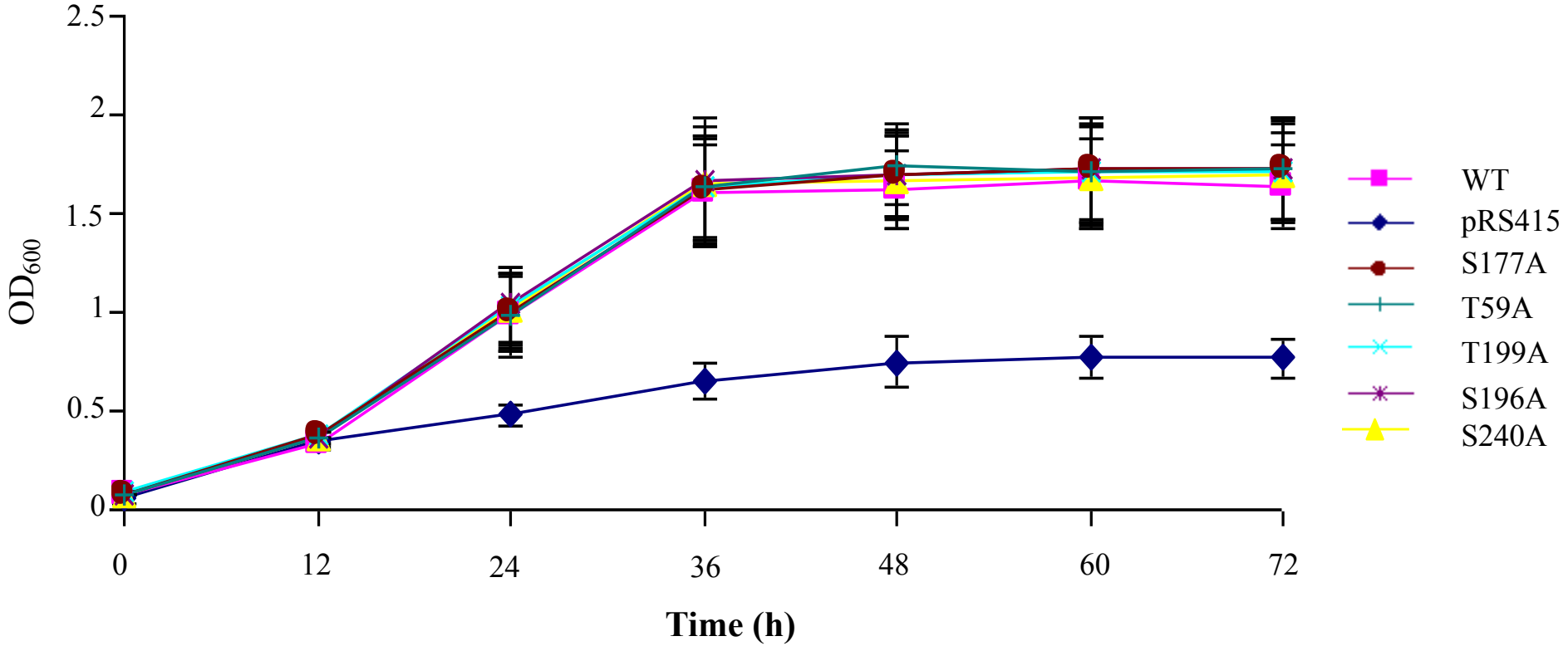
Supplementary Figure 9







Supplementary Figure 10



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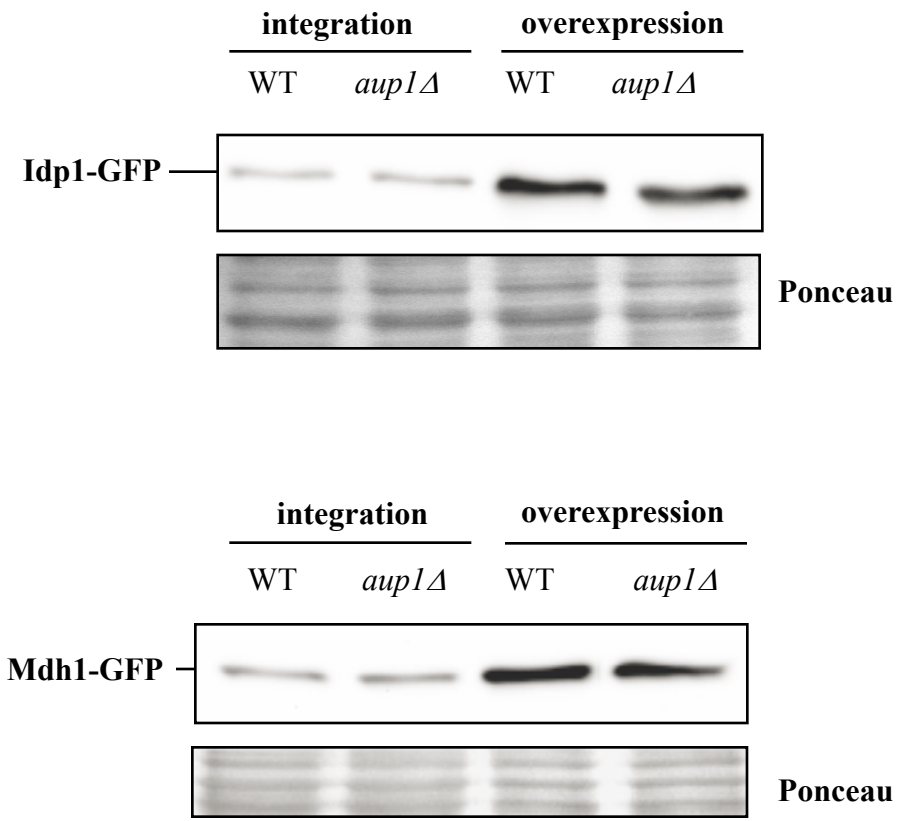

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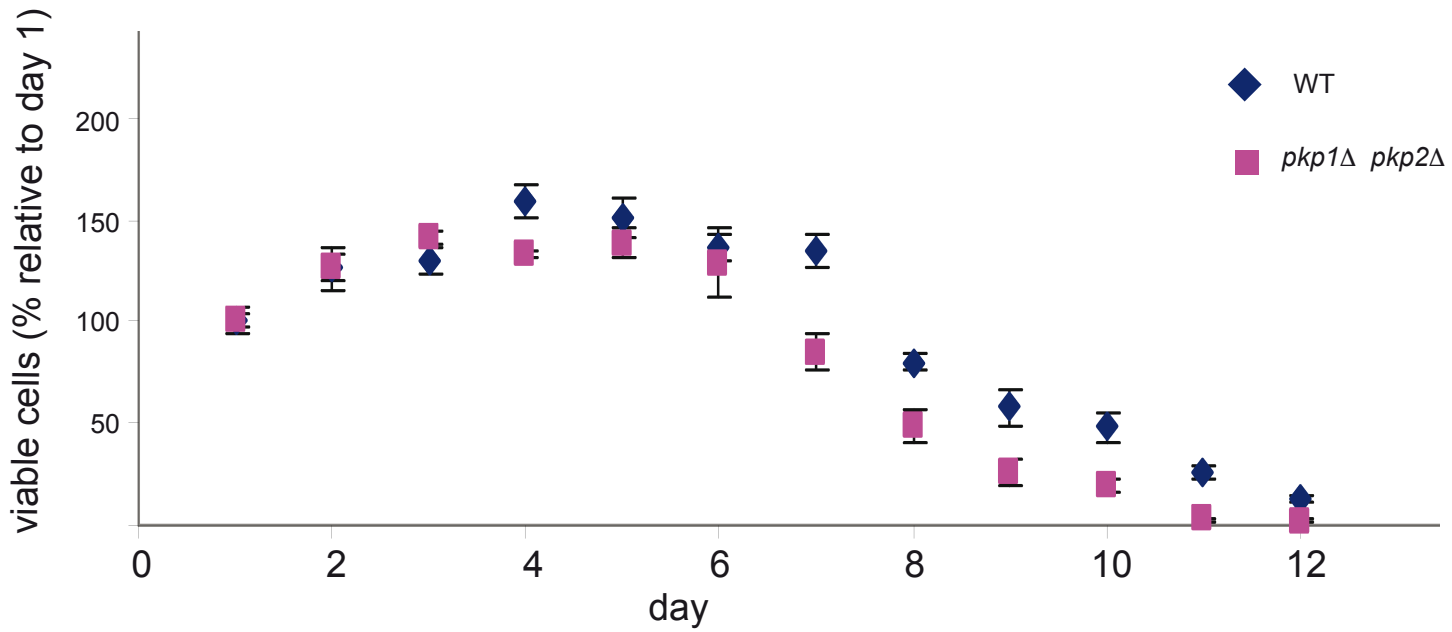
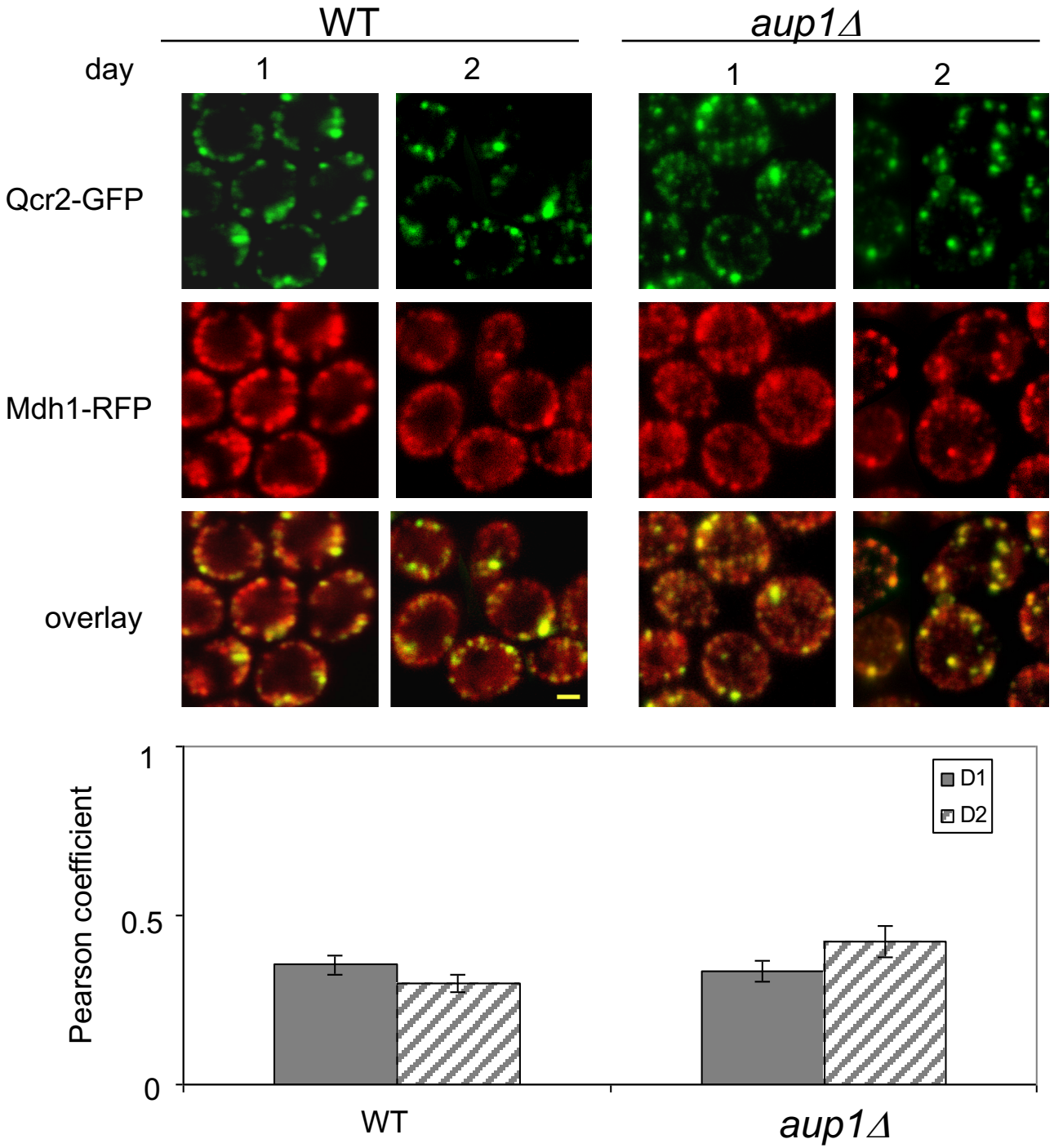


Supplementary Figure 13



Supplementary Figure 14

Supplementary Table I. Statistical significance of the data from Figure 1G. Pairwise comparisons of % free GFP values (between different genotypes over the same protein, and between different proteins over the same genotype) were analyzed using the Tukey HSD test. In each row, means without a common lowercase letter differ significantly over proteins. In each column, means without a common uppercase letter differ significantly over mutants. Values not connected by a common letter are significantly different (Tukey HSD, $p<0.05$).

	Aco2-GFP	Qcr2-GFP	Aco1-GFP	Mdh1-GFP	Idp1-GFP
WT	**52.53** ^{ABa}	**2.13** ^{ABc}	**21.33** ^{ABb}	**14.33** ^{Ab}	**12.66** ^{Ab}
aup1Δ	**34.50** ^{Ba}	**2.50** ^{ABb}	**5.66** ^{Dab}	**2.00** ^{Bb}	**1.33** ^{BCb}
ptc5Δ	**40.06** ^{ABa}	**4.30** ^{Ac}	**33.00** ^{Aa}	**13.33** ^{Ab}	**14.00** ^{Ab}
pkp1Δ	**44.80** ^{ABa}	**1.72** ^{ABcd}	**14.33** ^{BCb}	**0.33** ^{Bd}	**3.00** ^{Bc}
pkp2Δ	**59.13** ^{Aa}	**1.79** ^{ABc}	**10.00** ^{CDb}	**0.00** ^{Bd}	**0.33** ^{Cd}
pkp1Δ pkp2Δ	**0.33** ^{Cb}	**0.00** ^{Bb}	**11.33** ^{BCa}	**0.33** ^{Bb}	**0.33** ^{Cb}

Supplementary Table II. Yeast Strains used in this study

Strain	Genotype	Reference/source
***Saccharomyces cerevisiae* strains**		
HAY75 Wild type, progeny of SEY 6211 and SEY6210	*MATα leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9*	(Abeliovich et al. *Mol. Biol. Cell*, 2003)
HAY809	HAY75, *aup1Δ::HIS5 S.p.*	(Tal et al., *JBC*, 2007)
HAY1239	HAY75, *atg32Δ::G418*^{R}	(Abeliovich et al., *Nat Commun,* 2013)
TVY1	HAY75*, pep4Δ::LEU2*	(Gerhardt et al., *J. Biol. Chem,* 1998)
PKY9	HAY75, ACO2-GFP::*G418*^{R}	This study
PKY11	HAY75, ACO1-GFP::*G418*^{R}	This study
PKY15	HAY75, IDP1-GFP::*G418*^{R}	This study
PKY16	HAY75, QCR2-GFP::*G418*^{R}	This study
PKY17	HAY75, MDH1-GFP::*G418*^{R}	This study
PKY133	HAY809, Mdh1-GFP::G418^{R}	This study
PKY37	HAY75*, ptc5Δ::NAT*^{R}	This study
PKY38	PKY37, QCR2-GFP::*G418*^{R}	This study
PKY39	PKY37, MDH1-GFP::*G418*^{R}	This study
PKY51	PKY37, IDP1-GFP::*G418*^{R}	This study
PKY61	HAY75*, pkp1Δ::HIS5 S.p.*	This study
PKY69	PKY61, MDH1-GFP::*G418*^{R}	This study
PKY70	HAY75, *pkp2Δ, HIS5 S.p.*	This study
PKY71	PKY37, ACO1-GFP::*G418*^{R}	This study
PKY75	PKY61, ACO1-GFP::*G418*^{R}	This study
PKY76	PKY61, ACO2-GFP::*G418*^{R}	This study
PKY87	PKY61, QCR2-GFP::*G418*^{R}	This study
PKY94	PKY37, ACO2-GFP::*G418*^{R}	This study
PKY114	PKY70, ACO1-GFP::*G418*^{R}	This study
PKY115	PKY70, ACO2-GFP::*G418*^{R}	This study
PKY116	PKY70, QCR2-GFP::*G418*^{R}	This study
PKY117	PKY70, MDH1-GFP::*G418*^{R}	This study
PKY122	PKY61, IDP1-GFP::*G418*^{R}	This study
PKY129	PKY70, IDP1-GFP::*G418*^{R}	This study
PKY136	HAY809, QCR2-GFP::*G418*^{R}	This study
PKY151	HAY809, ACO1-GFP::*G418*^{R}	This study
PKY152	HAY809, ACO2-GFP::*G418*^{R}	This study
PKY186	HAY809, IDP1-GFP::*G418*^{R}	This study
PKY334	HAY1239, MDH1-GFP:: *HIS5 S.p*	This study
PKY365	HAY75, *mdh1Δ::HIS5 S.p.*	This study
PKY392	TVY1, MDH1 GFP::*G418*^{R}	This study
PKY395	TVY1, *aup1Δ::HIS5 S.p.*	This study
PKY415	PKY395, Mdh1-GFP::*G418*^{R}	This study
PKY456	PKY365, *aup1Δ::HIS5 S.p.*	This study
PKY488	PKY365, *pkp2Δ* (DP)	This study
PKY520	TVY1, *arg4Δ:: G418*^{R}	This study
PKY629	PKY70, *pkp1Δ (DP)*	This study
PKY661	PKY395, *arg4Δ:: G418*^{R}	This study
PKY629	PKY70, *pkp1Δ (DP)*	This study
PKY670	PKY629, QCR2-GFP::*G418*^{R}	This study
PKY672	PKY629, MDH1-GFP::*G418*^{R}	This study
PKY673	PKY629, ACO1-GFP::*G418*^{R}	This study

PKY681	PKY629, IDP1-GFP::*G418*^{R}	This study
PKY682	PKY629, ACO2-GFP::*G418*^{R}	This study
PKY734	TVY1, *aup1Δ:: NAT*^{R}	This study
PKY826	TVY1, Cit1-HA:: *HIS5 S.p.*	This study
PKY827	TVY1, Mic60-HA:: *HIS5 S.p.*	This study
PKY824	PKY734, Cit1-HA:: *HIS5 S.p.*	This study
PKY825	PKY734, Mic60-HA:: *HIS5 S.p.*	This study
HAY339	*atg1Δ :: LEU2*	This study
PKY915	TVY1, *pkp2Δ::HIS5 S.p.*	This study
PKY535	PKY365, QCR2-RFP::*G418*^{R}	This study
PKY1025	HAY75, Mdh1-RFP:: *G418*^{R}, Qcr2-GFP:: *HIS5 S.p.*	This study
PKY1054	HAY75, *aup1Δ:: NAT*^{R} , Mdh1-RFP:: *G418*^{R}, Qcr2-GFP:: *HIS5 S.p.*	This study
PKY1032	TVY1, Idh1-HA:: *HIS5 S.p.*	This study
PKY1040	PKY734, Idh1-HA:: *HIS5 S.p.*	This study

Supplementary Table III. Plasmids used in this study

Plasmid	Description	Backbone vector	RE sites in backbone vector/insert	Yeast Markers	Insert	Cloning Primers	Reference/ source
pCU416	-	-	*URA3*	-	-	-	(Labbe S et al.., 1999)
pRS415	-	-	*LEU2*	-	-	-	(Sikorsky R et al., 1989)
pDJB11	pCU416-mtRFP	pCU416	HindIII, XhoI	*URA3*	mtRFP	-	(Journo et al., 2009)
pDJB12	pCU414-mtRFP	pCU414	HindIII, XhoI	*TRP1*	mtRFP	-	This study
pPKB1	pCU416-AUP1	pCU416	ClaI, SpeI	*URA3*	AUP1	P1,P2	This study
pPKB2	pCU416-*34Δ*AUP1	pCU416	ClaI, SpeI	*URA3*	34ΔAUP1	P3,P4	This study
pPKB24	pRS415-MDH1^{T199A}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{T199A}-GFP	P5,P6	This study
pPKB25	pRS415-MDH1^{S177A}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{S177A}-GFP	P5,P6	This study
pPKB26	pRS415-MDH1^{S196A}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{S196A}-GFP	P5,P6	This study
pPKB27	pRS415-MDH1^{S240A}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{S240A}-GFP	P5,P6	This study
pPKB28	pRS415-MDH1^{T59A}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{T59A}-GFP	P5,P6	This study
pPKB37	pRS415-MDH1^{S196D}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{S196D}-GFP	P5,P6	This study
pPKB40	pRS415-MDH1^{T199D}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{T199D}-GFP	P5,P6	This study
pPKB45	pRS415-MDH1$^{T199A-s240A-S196A-}$	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-	P5,P6	This study

	$^{S177A-T59A}$_GFP				MDH1$^{T199A-s240A-S196A-S177A-T59A}$_GFP		
pDJB11	pCU416-IDP1-GFP	pCU416	-	*URA3*	IDP1-GFP	-	(Journo et al, 2009)
pPKB65	pRS415-MDH1-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1-GFP	P5,P6	This study
pPKB73	pRS415-MDH1^{T59D}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{T59D}-GFP	P5,P6	This study
pPKB74	pRS415-MDH1^{S240D}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{S240D}-GFP	P5,P6	This study
pPKB75	pRS415-MDH1^{S177D}-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1^{S177D}-GFP	P5,P6	This study
pPKB76	pRS415-MDH1$^{T199D-s240D}$-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1$^{T199D-s240D}$-GFP	P5,P6	This study
pPKB77	pRS415-MDH1$^{T199D-s240A}$-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1$^{T199D-s240A}$-GFP	P5,P6	This study
pPKB78	pRS415-MDH1$^{T199A-s240D}$-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1$^{T199A-s240D}$-GFP	P5,P6	This study
pPKB79	pRS415-MDH1$^{T199A-s240A}$-GFP	pRS415	BamHI, SacI	*LEU2*	[MDH1p]-MDH1$^{T199A-s240A}$-GFP	P5,P6	This study
pPKB82	pCU416-MDH1-GFP	pCU416	ClaI, SpeI	*URA3*	[MDH1p]-MDH1-GFP	P7,P8	This study
pPKB83	pCU416-*17Δ*MDH1-GFP	pCU416	ClaI, SpeI	*URA3*	17ΔMDH1-GFP	P9,P10	This study
pPKB84	pCU416-*17Δ*MDH1 ^{T199A} -GFP	pCU416	ClaI, SpeI	*URA3*	17ΔMDH1 ^{T199A} -GFP	P9,P10	This study
pPKB88	pRS414-MDH1-GFP	pRS414	BamHI, SacI	*TRP1*	[MDH1p]-MDH1-GFP	P5,P6	This study
pPKB89	pCU416-PKP1	pCU416	ClaI, SpeI	*URA3*	PKP1	P11,P12	This study

pPKB90	pCU416-PKP2	pCU416	ClaI, SpeI	*URA3*	PKP2	P13,P14	This study
pKB33	pYX142 mt-GFP	pYX142		*LEU2*	mtGFP	-	Westermann B., et al., 2000
pKB115	pYX142 mt-DHFR-GFP	pYX142	BamHI, BglII	*LEU2*	Mt-DHFR-GFP	P15, P16	This study

Supplementary Table IV. Oligonucleotides used in this study

Primers	Purpose	Primers
P1	Amplification of AUP1 for the construction of pKPB1	Forwrard:5' ATACTAACTAGTTACAACATGCGGCTGGGGAATCTATG 3'
P2		Reverse: 5 'ATACTAATCGATTTAGTTTAATTTTGTTTCGTTAGATTG3'
P3	Amplification of aup1Δ34 for the construction of pKPB2	Forward:5'ATACTAACTAGTTACAACATGAGTAGGATCAACCGGTTAG3'
P4		Reverse: 5 'ATACTAATCGATTTAGTTTAATTTTGTTTCGTTAGATTG3'
P5	Amplification of MDH1-GFP for the construction of pPKB65 , under native promoter of MDH1.	Forward: 5 'ATACTAGGATCCCAAAAGATCGACGCAATG3'
P6		Reverse: 5'ATACTAGAGCTCGACCTCATACTATACCTG3'
P7	Amplification of MDH1-GFP for the construction of pKPB82	Forward: 5'ATACTAACTAGTTACAACATGTTGTCAAGAGTAGCTAAAC3'
P8		Reverse: 5'ATACTAATCGATGACCTCATACTATACCTG3'
P9	Amplification of MDH1Δ17 for the construction of pPKB83&pPKB84	Forward: 5'ATACTAACTAGTTACAACATGTATAAAGTGACTGTTTTG
P10		Reverse: 5' ATACTAATCGATGACCTCATACTATACCTG
P11	Amplification of PKP1 for the construction of pKPB89	Forward: 5' ATACTAACTAGTTACAACATGTGGAAGATTATGCGTTC
P12		Reverse: 5' ATACTAATCGATTTACTTTTTGGAGAGTAGTG
P13	Amplification of PKP2 for the construction of pPKB90	Forward: 5' ATACTAGGATCCTACAACATGTCTAAGTATCAAATTAA
P14		Reverse: 5' ATACTAATCGATTCACACTTTATCTAATTGTAG
P15	Amplification of DHFR for the construction of pKB115	Forward: 5' ATACTAGGATCCTACAACATGATCAGTCTGATTGCGG
P16		Reverse: 5' ATACTAAGATCTCCGCCGCTCCAGAATCTC
D1	Amplification of natMX4 cassette for the deletion of *ptc5Δ*	Forward: 5' TTTCAACAGAAGAAGTGCTTTTACTTCTCT CAATCTCTCC3'
D2		Reverse: 5'ATCCTCTGGTATATACCTACCTCAGCATAAGTTTATATC
D3	Amplification of *His5 S.p.* cassette for the deletion of *pkp1Δ*	Forward:5'ACCAACTCTTTGTATTTGAAAAGCTTGCAAACGTTGCTATCCCG GGCTGCAGGAATTC3'
D4		Reverse:5'TAAGTACAGCTCGTCATCGTCTGGCTTTAAAAGATTGATCTCGA CGGTATCGATAAGC3'
D5		Forward:5'ATCGTGAGAGATTGGTCAATGGATAAATAGTTCTGTAATT CCCGGGCTGCAGGAATTC 3'

D6	Amplification of *His5 S.p.* cassette for the deletion of *pkp2Δ*	Reverse: 5'GGATAACTCATTGGTTCTAGCGACGTGTGCATGTACGGTT TCGACGGTATCGATAAGC 3'
D7	Amplification of *His5 S.p.* cassette for the deletion of *mdh1Δ*	Forward:5'AAGAAAAAAAACAAAAGGAAAAGGAAGGATACCATATACACC CGGGCTGCAGGAATTC3'
D8		Reverse:5'TTCGGATTTGAACAAGGGAGAGTCCACGAAGGAAGGCTCGTCG ACGGTATCGATAAGC3'
D9	Amplification of $G418^R$ cassette for the deletion of *arg4Δ*	Forward:5'GCTCAAAAGCAGGTAACTATATATAACAAGACTAAGGCGGTG GTCTTGCGCAACACG
D10		Reverse:5'CTAATTTAATTGGGATTTCAAATTATCCAATTGCTTCAATGAAT TCGAGCTCGTTTAAAC
T1	Amplification of *His5 S.p.* cassette for the tagging of Mic60-HA	Forward:5'GCGCCTGGTAGAGATTCTTGACTGTGAAATAAGGACGTTGTAC CCATACGATGTTCCTG
T2		Reverse:5'ATCGGAAATGACGAGTAACAGCTAACTTACATTTTAGGTTCGA CGGTATCGATAAGC
T3	Amplification of *His5 S.p.* cassette for the tagging of Cit1-HA	Forward:5'AAAATACAAGGAGTTGGTAAAGAAAATCGAAAGTAAGAACTA CCCATACGATGTTCCTG
T4		Reverse:5'GTAGGCATAGGGGACTCAAAGCGTAAAAATCATGAAGTCATCG ACGGTATCGATAAGC
M19	Site Directed Mutagenesis of S240A on Mdh1-GFP	Forward:5'GCAAAGAATGGTGCTGGCGCTGCTACGTTGTCAATGGC3'
M20		Reverse:5' GCCATTGACAACGTAGCAGCGCCAGCACCATTCTTTGC 3'
M1	Site Directed Mutagenesis of T199A on Mdh1-GFP	Forward: 5'CGGTGGACATTCTGGTATCGCGATCATCCCATTGATTTCG3'
M2		Reverse:5'CGAAATCAATGGGATGATCGCGATACCAGAATGTCCACCG3'
M3	Site Directed Mutagenesis of S196A on Mdh1-GFP	Forward: 5'CGGTGGACATGCTGGTATTGCCATCATCCCATTGATTTCG3'
M4		Reverse: 5'CGAAATCAATGGGATGATGGCAATACCAGCATGTCCACCG3'
M5	Site Directed Mutagenesis of S177A on Mdh1-GFP	Forward: 5'GAGCCGCCAGATTCATCGCAGAAGTCGAGAACAC3'
M6		Reverse: 5'GTGTTCTCGACTTCTGCGATGAATCTGGCGGCTC3'
M7	Site Directed Mutagenesis of T59A on Mdh1-GFP	Forward: 5'GGGCGCAAAAGGTGTTGCCGCCGATTTGTCTCATATTC3'
M8		Reverse: 5'GAATATGAGACAAATCGGCGGCAACACCTTTTGCGCCC3'
M9	Site Directed Mutagenesis of S240D on Mdh1-GFP	Forward: 5'CAAAGAATGGTGCTGGCGATGCTACGTTGTCAATGGC3'
M10		Reverse: 5'GCCATTGACAACGTAGCATCGCCAGCACCATTCTTTG3'
M11	Site Directed Mutagenesis of T199D on Mdh1-GFP	Forward: 5'GGTGGACATTCTGGTATTGATATCATCCCATTGATTTCGC3'
M12		Reverse: 5'GCGAAATCAATGGGATGATATCAATACCAGAATGTCCACC3'
M13		Forward: 5'CGTCATCGGTGGACATGATGGTATTACCATCATC3'

M14	Site Directed Mutagenesis of S196D on Mdh1-GFP	Reverse: 5'GATGATGGTAATACCATCATGTCCACCGATGACG3'
M15	Site Directed Mutagenesis of S177D on Mdh1-GFP	Forward: 5'GAGCCGCCAGATTCATCGATGAAGTCGAGAACACCG3'
M16		Reverse: 5'CGGTGTTCTCGACTTCATCGATGAATCTGGCGGCTC3'
M17	Site Directed Mutagenesis of T59D on Mdh1-GFP	Forward: 5'GCGCAAAAGGTGTTGCCGATGATTTGTCTCATATTCCAAC3'
M18		Reverse: 5'GTTGGAATATGAGACAAATCATCGGCAACACCTTTTGCGC3'

SI reference list

1. Gietz RD, Woods RA (2002) Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. *Methods Enzymol* 350:87–96.