

Supplementary Information

Proteolysis of adaptor protein Mmr1 during budding is necessary for mitochondrial homeostasis in *Saccharomyces cerevisiae*

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Supplementary Table 1 | E3 ubiquitin ligases co-purified with Mmr1-HA

Proteins annotated as E3 ubiquitin ligase were extracted using data from Supplementary Data 1 and listed. Detected peptides are also listed.

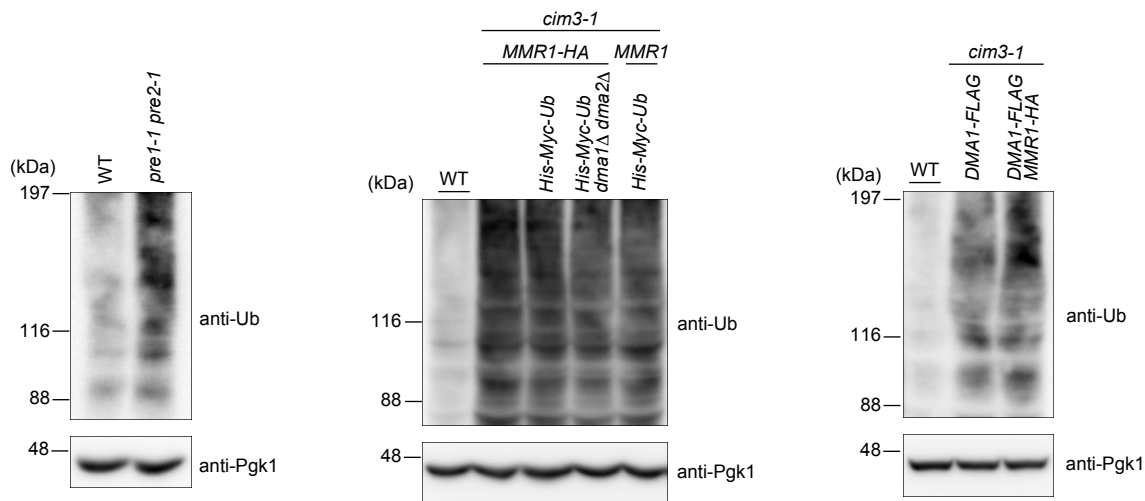
Accession	Description	ORF	Detected peptide
P38823	E3 ubiquitin-protein ligase DMA1 [OS=Saccharomyces cerevisiae S288C]	YHR115C	[K].SSSGTFLNHQR.[L] [R].LTPFIDTSSTSVANQGLFFDPIIR.[T]
P53924	E3 ubiquitin-protein ligase DMA2 [OS=Saccharomyces cerevisiae S288C]	YNL116W	[R].NIVGGADGSTIVNNSQEMYK.[N] [K].SSSGTFLNHQR.[L] [K].LTTGIEEDCSICLCK.[I] [K].AGPGSQLVIGR.[Y] [K].IPEQYHPVVF.[S] [R].DGDILQLGMDFR.[G] [R].LTPFIDSSSTTNQGLFFEPPIR.[K]
P19812	E3 ubiquitin-protein ligase UBR1 [OS=Saccharomyces cerevisiae S288C]	YGR184C	[R].QGVPDNVHIDLLTSR.[I] [K].LSNPELTVFPDSLEDAVDIDK.[I] [K].ILPESSTHSLSPINDVETPTSR.[T] [R].LQHILYFDNR.[Y] [R].NGMSVLHQASYK.[N] [K].TLCSEYLNATECR.[D] [K].ITSQOTIPFYK.[I] [K].SDVFSESIVR.[K] [R].LFPFTEENYR.[T] [K].EIIISLLTCLK.[L] [R].QGVNLQETEK.[E] [K].LFLNAIR.[I] [R].YIDLILADGNQIPLGHHK.[I] [R].SIHNLPHYR.[Y] [K].ICLTGCVK.[V] [K].NNPELGSYSR.[D] [K].ISDFSLR.[S] [K].SYSISFK.[Q]
P12868	E3 ubiquitin-protein ligase pep5 [OS=Saccharomyces cerevisiae S288C]	YMR231W	[K].VLRPIIEGER.[M]
P24814	SCF E3 ubiquitin ligase complex F-box protein GRR1 [OS=Saccharomyces cerevisiae S288C]	YJR090C	[R].LIDLSGCENITDK.[T] [R].ITDASLFQLSK.[L] [R].IQYVDFACCTNLNTR.[T] [R].TLYELADLPK.[L] [R].ALETENMEIR.[N] [K].ITANNNMDELVELLANK.[C] [R].MSSEEVNSLLQVVK.[T] [R].EINSEMCHIVR.[K] [K].FLQSVDTIGIR.[D] [R].QIFCVFSGK.[G] [K].SQLDLFLR.[T] [K].SLYAYCHSK.[L] [K].VVDDMPSLR.[L] [R].VQGFYVPOAR.[N] [K].TFCEDPFSDVDDQDYVVPQGVNR.[E] [R].KFHELNDHIDDFEVNVASLVR.[V] [R].DVSDDVFDLTATYCPR.[V] [R].NFIVHSPMLK.[R]
P39940	E3 ubiquitin-protein ligase RSP5 [OS=Saccharomyces cerevisiae S288C]	YER125W	[R].FGEVTVDLKPDGR.[N]
Q07963	E3 ubiquitin-protein ligase UBR2 [OS=Saccharomyces cerevisiae S288C]	YLR024C	[K].FTSCEPVLIR.[A]
P53119	Probable E3 ubiquitin-protein ligase HUL5 [OS=Saccharomyces cerevisiae S288C]	YGL141W	[K].LSHNFLSSYPNSLGNR.[Q] [K].LLNMTTDEIK.[S] [R].DNVLEDAFNAFNSIGER.[F] [R].ASFLSYSSASK.[L] [K].SLDLTFEIDEPESSAK.[V] [K].FVTSVPQAPLQGFK.[A]

Supplementary Table 2 | Yeast Strains used in this study.

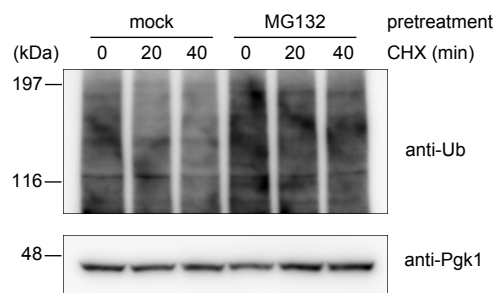
Name	Genotype	Source
W303-1a	<i>MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	Y. Kikuchi
JB291	<i>MATa ura3 his3-11,15 leu2-3,112</i>	1, 2
JB290	<i>JB291, pre1-1 pre2-1</i>	1, 2
BY4741	<i>MATa his3 Δ1 leu2 Δ ura3 Δ met15 Δ</i>	3
YTK4652	<i>W303-1a, pdr5 Δ::HPT</i>	This study
YPH499	<i>MATa his3 Δ200 lys2-801 ade2-101 leu2 Δ1 ura3-52 trp1 Δ63</i>	4
CMY253	<i>MATa ade2-101 ura3-52 his3 Δ200 leu2 Δ1 lys2-801</i>	5
CMY762	<i>CMY253, cim3-1</i>	5
YTK3487	<i>CMY762, MMRI-3HA::LEU2</i>	This study
YTK5423	<i>CMY762, DMA1-5FLAG::HIS3</i>	This study
YTK5425	<i>CMY762, DMA1-5FLAG::HIS3 MMRI-3HA::LEU2</i>	This study
YTK6168	<i>CMY762, His-Myc-Ubiquitin::URA3</i>	This study
YTK5810	<i>CMY762, MMRI-3HA::LEU2 His-Myc-Ubiquitin::URA3</i>	This study
YTK5893	<i>CMY762, MMRI-3HA::LEU2 dma1 Δ::KanMX4 dma2 Δ::HISMx6 His-Myc-ubiquitin::URA3</i>	This study
YTK5414	<i>W303-1a, dma1 Δ::KanMX4</i>	This study
YTK5415	<i>W303-1a, dma2 Δ::HISMx6</i>	This study
YTK5416	<i>W303-1a, dma1 Δ::KanMX4 dma2 Δ::HISMx6</i>	This study
YTK5334	<i>W303-1a, P_{GPD}-Mt-GFP-T_{CYC1}::URA3</i>	This study
YTK5341	<i>W303-1a, P_{GPD}-Mt-GFP-T_{CYC1}::URA3 dma1 Δ::KanMX4 dma2 Δ::HISMx6</i>	This study
YOK5303	<i>W303-1a, P_{GPD}-Mt-mCherry-T_{CYC1}::URA3 MYO2-GFP::LEU2</i>	This study
YOK5304	<i>W303-1a, P_{GPD}-Mt-mCherry-T_{CYC1}::URA3 MYO2-GFP::LEU2 dma1 Δ::KanMX4 dma2 Δ::HISMx6</i>	This study
YOK5182	<i>W303-1a, P_{GPD}-Mt-mCherry-T_{CYC1}::URA3</i>	This study
YOK5185	<i>W303-1a, P_{GPD}-Mt-mCherry-T_{CYC1}::URA3 dma1 Δ::KanMX4 dma2 Δ::HISMx6</i>	This study
YOK5595	<i>W303-1a, P_{GPD}-Mt-mCherry-T_{CYC1}::URA3 MMRI-GFP::ADE2</i>	This study
YOK5596	<i>W303-1a, P_{GPD}-Mt-mCherry-T_{CYC1}::URA3 MMRI-GFP::ADE2 dma1 Δ::KanMX4 dma2 Δ::HISMx6</i>	This study
YTK5879	<i>W303-1a, ste20 Δ::KanMX4</i>	This study
YOK5305	<i>W303-1a, STE20-GFP::LEU2</i>	This study
YOK5407	<i>W303-1a, CLA4-GFP::LEU2</i>	This study
YTK5433	<i>W303-1a, mdm12 Δ::NatNT2</i>	This study
BY25598	<i>W303-1a, P_{ADHI}-OsTIR1-9Myc::URA3</i>	This study
YOK5411	<i>BY25598, CLA4-3HA-AID::KanMX6</i>	This study
YOK5408	<i>BY25598, ste20 Δ::NatNT2</i>	This study
YOK5409	<i>BY25598, ste20 Δ::NatNT2 CLA4-3HA-AID::KanMX6</i>	This study
YOK5434	<i>BY25598, ste20 Δ::NatNT2 CLA4-3HA-AID::KanMX6 P_{GPD}-Mt-GFP-T_{CYC1}::LEU2</i>	This study
YTK5777	<i>W303-1a, mmr1 Δ::KanMX4 MMRI::URA3</i>	This study
YTK5778	<i>W303-1a, mmr1 Δ::KanMX4 MMRI(S414A)::URA3</i>	This study
YTK5984	<i>W303-1a, mmr1 Δ::KanMX4 MMRI(S266A)::URA3</i>	This study
YTK6021	<i>W303-1a, mmr1 Δ::KanMX4 MMRI(S155A)::URA3</i>	This study
YTK6316	<i>W303-1a, mmr1 Δ::KanMX4 MMRI(S76A)::URA3</i>	This study
YOK5455	<i>W303-1a, P_{GPD}-Mt-GFP-T_{CYC1}::LEU2 mmr1 Δ::KanMX4 MMRI::URA3</i>	This study
YOK5456	<i>W303-1a, P_{GPD}-Mt-GFP-T_{CYC1}::LEU2 mmr1 Δ::KanMX4 MMRI(S414A)::URA3</i>	This study
YOK5464	<i>W303-1a, sod1 Δ::KanMX4</i>	This study
YOK5466	<i>W303-1a, sod2 Δ::LEU2</i>	This study

YOK5468	W303-1a, <i>sod1</i> Δ:: <i>KanMX4</i> <i>sod2</i> Δ:: <i>LEU2</i>	This study
YOK5479	W303-1a, <i>dma1</i> Δ:: <i>NatNT2</i> <i>dma2</i> Δ:: <i>HISMX6</i> <i>sod1</i> Δ:: <i>KanMX4</i>	This study
YOK5480	W303-1a, <i>dma1</i> Δ:: <i>NatNT2</i> <i>dma2</i> Δ:: <i>HISMX6</i> <i>sod2</i> Δ:: <i>LEU2</i>	This study
YOK5481	W303-1a, <i>dma1</i> Δ:: <i>NatNT2</i> <i>dma2</i> Δ:: <i>HISMX6</i> <i>sod1</i> Δ:: <i>KanMX4</i> <i>sod2</i> Δ:: <i>LEU2</i>	This study
YOK5482	W303-1a, <i>mmr1</i> Δ:: <i>NatNT2</i> <i>MMR1</i> :: <i>URA3</i>	This study
YOK5483	W303-1a, <i>mmr1</i> Δ:: <i>NatNT2</i> <i>MMR1</i> (<i>S414A</i>):: <i>URA3</i>	This study
YOK5485	W303-1a, <i>mmr1</i> Δ:: <i>NatNT2</i> <i>MMR1</i> :: <i>URA3</i> <i>sod1</i> Δ:: <i>KanMX4</i>	This study
YOK5486	W303-1a, <i>mmr1</i> Δ:: <i>NatNT2</i> <i>MMR1</i> (<i>S414A</i>):: <i>URA3</i> <i>sod1</i> Δ:: <i>KanMX4</i>	This study
YOK5261	BY4741, <i>dma1</i> Δ:: <i>NatNT2</i> <i>dma2</i> Δ:: <i>HIS3</i>	This study
4814	BY4741, <i>ubr1</i> Δ:: <i>KanMX4</i>	6
YOK5581	BY4741, <i>pep5</i> Δ:: <i>KanMX4</i>	This study
6902	BY4741, <i>grr1</i> Δ:: <i>KanMX4</i>	6
1579	BY4741, <i>ubr2</i> Δ:: <i>KanMX4</i>	6
4508	BY4741, <i>hul5</i> Δ:: <i>KanMX4</i>	6
6122	BY4741, <i>ycck3</i> Δ:: <i>KanMX4</i>	6
4015	BY4741, <i>vps41</i> Δ:: <i>KanMX4</i>	6
FAY51R	YPH499, <i>rsp5-1</i>	7

a

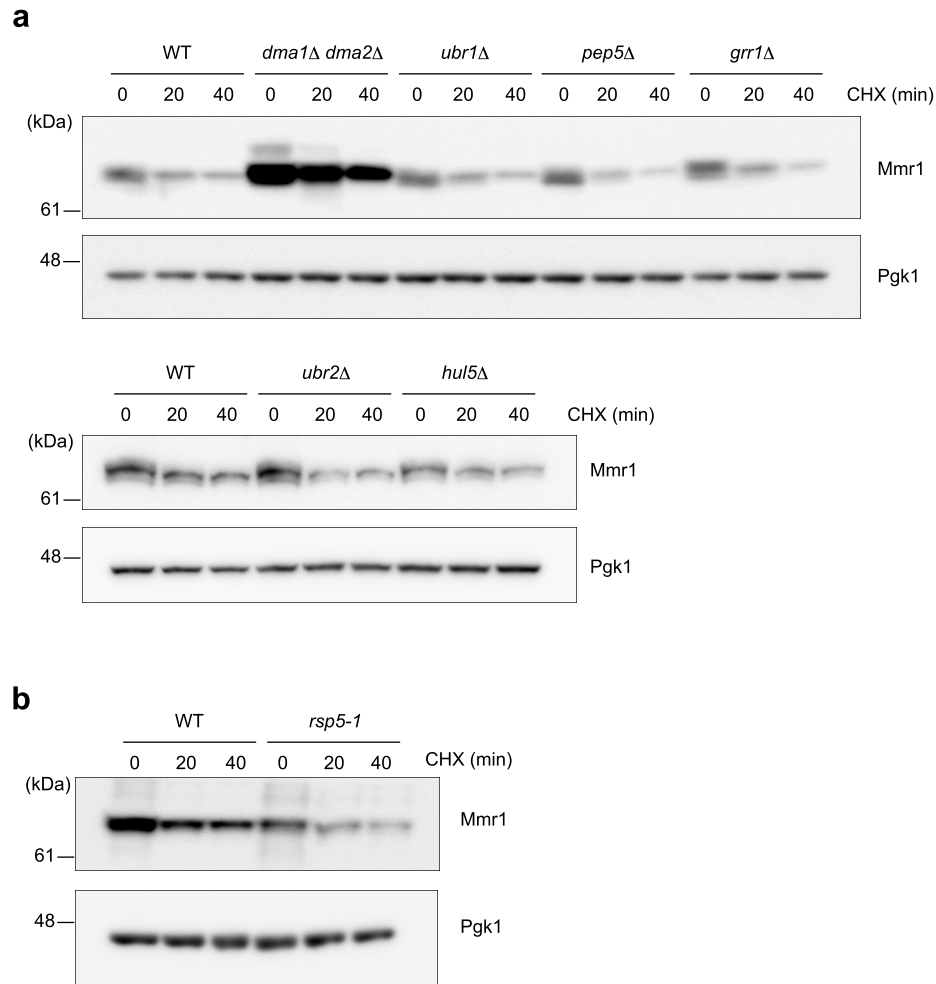


b



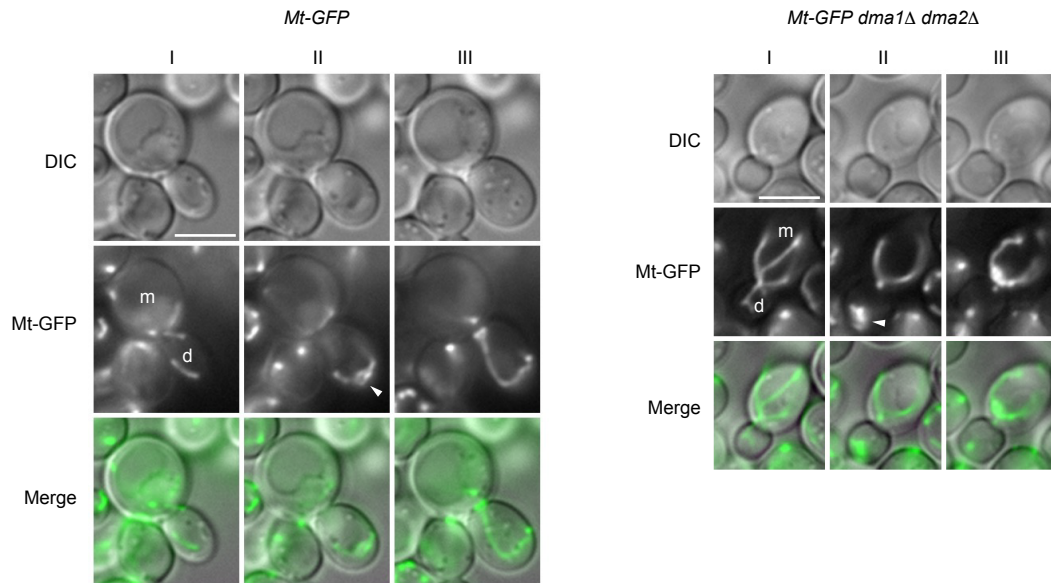
Supplementary Fig. 1 | Ubiquitinated proteins are accumulated in proteasome mutants and cells treated with MG132

a) JB291 (WT), JB290 (*pre1-1 pre2-1*), CMY253 (WT), YTK3487 (*cim3-1 MMR1-HA*), YTK5810 (*cim3-1 MMR1-HA His-Myc-Ub*), YTK5893 (*cim3-1 MMR1-HA His-Myc-Ub dma1Δ dma2Δ*), YTK6168 (*cim3-1 His-Myc-Ub*), YTK5423 (*cim3-1 DMA1-FLAG*), and YTK5425 (*cim3-1 DMA1-FLAG MMR1-HA*) cells were grown to log phase in YPD medium at permissive temperature (25°C), further incubated for 2 h at the restrictive temperature (37°C), and harvested. Their total lysates were subjected to immunoblot analysis with anti-ubiquitin (Ub) or, to demonstrate uniform loading, anti-Pgk1 antibody. b) YTK4652 (*pdr5Δ*) cells were grown to log phase in YPD medium at 30°C, treated with MG132 or mock-treated with dimethyl sulfoxide for 40 min, treated with cycloheximide (CHX), and harvested. Their total lysates were subjected to immunoblot analysis with anti-Ub or anti-Pgk1 antibodies. Similar results were obtained from two independent experiments.



Supplementary Fig. 2 | Dma1 and Dma2 are involved in Mmr1 turnover

a) BY4741 (WT), YOK5261 (*dma1Δ dma2Δ*), 4814 (*ubr1Δ*), YOK5581 (*pep5Δ*), 6902 (*grr1Δ*), 1579 (*ubr2Δ*), and 4508 (*hul5Δ*) cells grown to log phase in YPD medium at 30°C were treated with cycloheximide (CHX) and harvested. Their total lysates were prepared and subjected to immunoblot analysis with anti-Mmr1 or, to demonstrate uniform loading, anti-Pgk1 antibody. b) YPH499 (WT) and FAY51R (*rsp5-1*) cells grown to early log phase in YPD medium at 25°C were further cultured for 2 h at 37°C, and subjected to CHX-chase assay. Mmr1 and Pgk1 were detected via immunoblotting as in a. Similar results were obtained from two independent experiments.



Supplementary Fig. 3 | Mitochondria dynamics during budding

YTK5334 (*Mt-GFP*) and YTK5341 (*Mt-GFP dma1Δ dma2Δ*) cells were grown to log phase in synthetic complete medium and serially observed under a fluorescence microscope using glass-base dishes. Representative images are shown. Cells with mitochondria being transported into the growing bud (I), stacked temporally at the bud-tip (II; arrowhead), and moving dynamically in *Mt-GFP* daughter cells or pulled back to the bud-neck and stacked in *Mt-GFP dma1Δ dma2Δ* cells (III) are shown. For movies of *Mt-GFP* and *Mt-GFP dma1Δ dma2Δ* cells, please see Supplementary Movie 1 and 2, respectively. m and d, mother cell and daughter cell, respectively. DIC, Differential interference contrast. Bar, 5 μ m.

a

Master IDQ6324	Mitochondrial MYO2 receptor-related protein 1 [OS=Saccharomyces cerevisiae S288C]
C Confide Annotated Sequence	Modifications
High [K].KLTSSAVVATSTSK.[T]	
High [K].TTSEGGQNSNPDPINIEK.[N]	
High [K].LDTGPIGNSLLVPTSLSK.[L]	
High [R].FMLEVMKDPISQAQR.[S]	1xOxidation [M2(100)]
High [R].FMLEVMKDPISQAQR.[S]	
High [R].GFQLNLPVQVEK.[K]	
High [K].RGFQLNLPVQVEK.[K]	
High [R].NAGSFQNLNLSPTK.[L]	
High [K].NAEGLLPSYVPVPATPLEDPENHGVK.[K]	
High [K].MLKPEYLSQTTSNLPLLSALLK.[N]	
High [K].NAEGLLPSYVPVPATPLEDPENHGVK.[V]	
High [K].LTTSSAVVATSTSK.[T]	
High [K].DLNLNLPDNNNGFVQYGLKGNNNNNR.[Y]	1xGG [K20(100)]
High [K].DLNLNLPDNNNGFVQYGLK.[G]	
High [R].VVSOGSTQCLSTEVENELPK.[D]	1xCarbamidomethyl [C9]
High [R].VVSOGSTQCLSTEVENELPK.[D]	1xCarbamidomethyl [C9]; 1xPhospho [S6(99.6)]
High [R].SSESVAQVQK.[K]	
High [R].YSFISSTDYEPWCDDGQQHISMQMAMANAEEANSR.[E]	1xCarbamidomethyl [C16]
High [K].QLELEITELK.[L]	
High [K].LVHSMITTNR.[Y]	
High [K].ERNPPSFEAK.[V]	
High [K].VCAEPEILRK.[N]	1xCarbamidomethyl [C2]
High [R].FQFLNNTPK.[M]	
High [K].VCAEPEILR.[K]	1xCarbamidomethyl [C2]
High [K].KTTSEGGQNSNPDPINIEK.[N]	
High [K].LVHSMITTNR.[Y]	1xOxidation [M5(100)]
High [R].FMLEVMK.[D]	1xOxidation [M2(100)]
High [K].DPSQAQR.[S]	
High [R].VSVLDLK.[K]	
High [K].IKQLELEITELK.[L]	1xGG [K2(100)]
High [K].TEGNSAR.[I]	
High [R].FMLEVMK.[D]	
High [R].LARVSVLDLKK.[I]	1xPhospho [S5(100)]
High [K].MLKPEYLSQTTSNLPLLSALLK.[N]	1xOxidation [M1(100)]
High [R].LARVSVLDLK.[K]	1xPhospho [S5(100)]
High [K].IKQLELEITELK.[L]	
High [R].VSVLDLKK.[I]	
High [R].FMLEVMK.[D]	2xOxidation [M2(100); M6(100)]
High [R].VSVLDLK.[K]	1xPhospho [S2(100)]
High [K].KFFNCK.[K]	1xCarbamidomethyl [C5]
High [K].KLTSSAVVATSTSKTEGNSAR.[I]	

b

1 MNSPTMKSEQLTPKLSPMFCLDDQRNAGSFQNLNLSPTKLKLDTGPIGNSLLYPTSLSKLSELSRGGRSKQRRGSDTMRSVSPIRFQFLNNTPKMLKPE 100

101 YLSQTTSNLPLLSALLKNSKTTSEGGQNSNPDPINIEKNIQSIKDKLEQLRSSVESVAQVQKKERNPPSFEAKVCAEPEILRKNAEGLLPSYVPVPATP 200

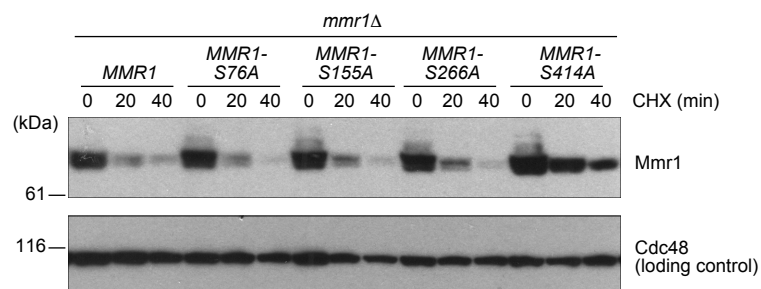
201 LEDPENHGVKVEDKGLRVVSGGSTQCLSTEVENELPKDLNLNLPDNNNGFVQYGLKGNNNNNRYSFISSTSTDYEPWCDDGQQHISMQMAMANAEEAN 300

301 SREKSNLDIKIKQLELEITELKLQNEKLVHSMITTNRYYEERFMLEVMKDPISQAQRSQRDIERKVKQLEKKFFNCKKVLKKLTTSSAVVATSTSKTEGNS 400

401 ARIPCPKTRLARVSVLDLKKIEEQPDSSSGTSSEEDHLTNDTDDANTSEDNLNVAFEEEPSTAISTTASVQSGESKRGFQLNLPVQVEKKEK* 491

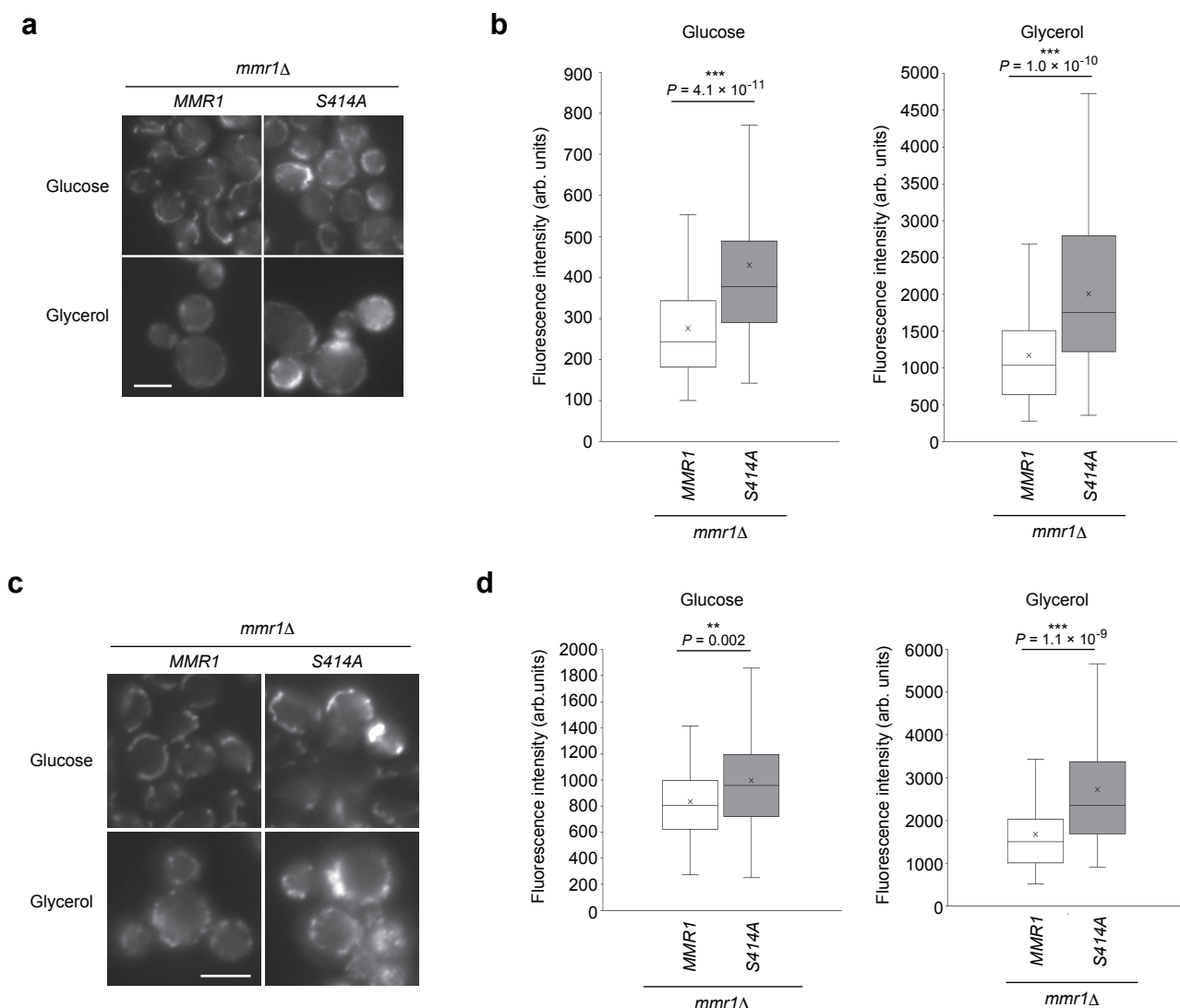
Supplementary Fig. 4 | Phosphorylation sites in Mmr1 detected by MS analysis

a) Mmr1-derived peptides detected by protein MS analysis are listed with detected modifications. Phosphorylation of Mmr1 at S224 and S414 residues was detected with the best site possibility of 99.6% and 100%, respectively. b) Deduced amino acid sequence of Mmr1. S224 and S414 residues are indicated by cyan and red arrowheads, respectively. Motifs that match to the RxS phosphorylation consensus sequence of Ste20 and Cla4 are boxed.



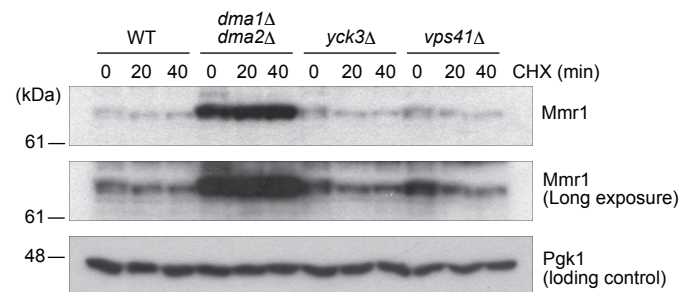
Supplementary Fig. 5 | S414 residue is important for Mmr1 turnover

YTK5777 (*mmr1Δ MMR1*), YTK6316 [*mmr1Δ MMR1(S76A)*], YTK6021 [*mmr1Δ MMR1(S155A)*], YTK5984 [*mmr1Δ MMR1(S266A)*], and YTK5778 [*mmr1Δ MMR1(S414A)*] cells were grown to log phase in YPD medium at 30°C and subjected to cycloheximide (CHX) chase. Their total lysates were prepared and subjected to immunoblot analysis with anti-Mmr1 or, to demonstrate uniform loading, anti-Cdc48 antibody. Similar results were obtained from two independent experiments.



Supplementary Fig. 6 | *MMR1(S414A)* cells have elevated respiratory activity and produce more ROS

a) YTK5675 (*mmr1Δ MMR1*), and YTK5764 [*mmr1Δ MMR1(S414A)*] cells were grown to log phase in synthetic complete medium (either glucose or glycerol was used as the carbon source), loaded with 50 nM TMRM for 1 h, and observed under a fluorescence microscope. Bar, 5 μ m. b) Intensity of TMRM signal. Imaging data from (a) were used for the analysis. c) YTK5675 (*mmr1Δ MMR1*), and YTK5764 [*mmr1Δ MMR1(S414A)*] cells were grown to log phase in synthetic complete medium (either glucose or glycerol was used as the carbon source), loaded with 5 μ M CellROX Green reagent for 1 h, and observed under a fluorescence microscope. Bar, 5 μ m. d) Intensity of CellROX Green reagent signal. Imaging data from (c) were used for the analysis. In (b) and (d), the box covers the region from the 1st quartile to the 3rd quartile. The horizontal line and the cross mark represent the median and mean, respectively. The whiskers at either side of the box extend to 1.5 interquartile ranges from the quartiles. In (b), for cells cultured in glucose-containing medium, 120 and 101 cells for *mmr1Δ MMR1* and *mmr1Δ MMR1(S414A)* cells, respectively, were analyzed, whereas in glycerol-containing medium, 102 and 108 cells for *mmr1Δ MMR1* and *mmr1Δ MMR1(S414A)* cells, respectively, were analyzed. In (d), for cells cultured in glucose-containing medium, 110 cells both for *mmr1Δ MMR1* and *mmr1Δ MMR1(S414A)* cells were analyzed, whereas in glycerol-containing medium, 105 and 102 cells for *mmr1Δ MMR1* and *mmr1Δ MMR1(S414A)* cells, respectively, were analyzed. Significance was tested by Mann-Whitney's *U* test. Similar results were obtained from two independent experiments.



Supplementary Fig. 7 | Yck3 and Vps41 are dispensable for rapid turnover of Mmr1

BY4741 (WT), YOK5261 (*dma1Δ dma2Δ*), 6122 (*yck3Δ*), and 4015 (*vps41Δ*) cells grown to log phase in YPD medium at 30°C were treated with cycloheximide (CHX) and harvested. Their total lysates were prepared and subjected to immunoblot analysis with anti-Mmr1 or, to demonstrate uniform loading, anti-Pgk1 antibody. Similar results were obtained from two independent experiments.

Fig. 1a

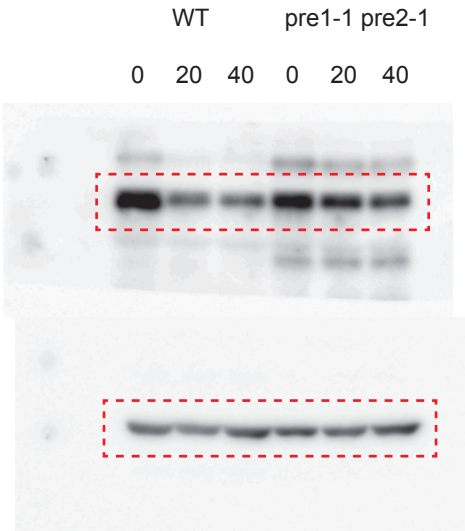


Fig. 1c

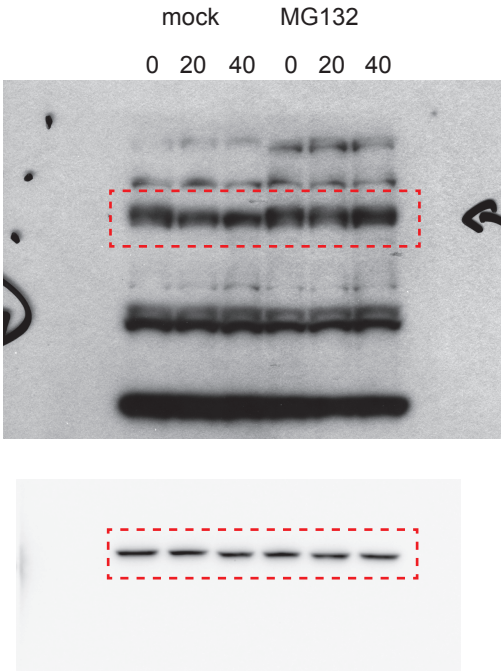


Fig. 2a



Fig. 2d

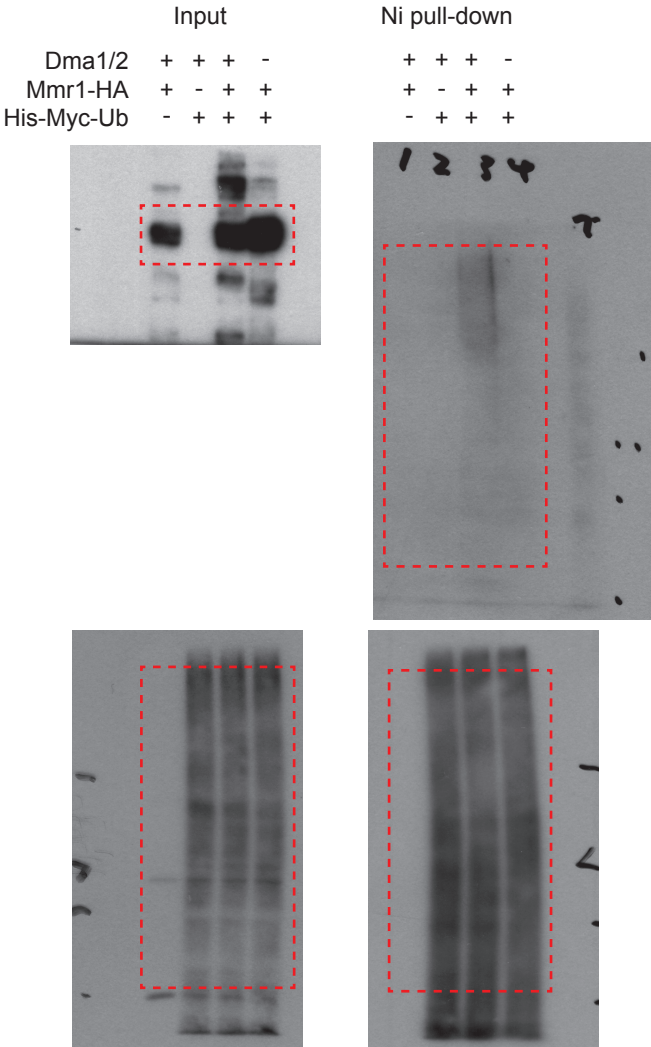


Fig. 2c

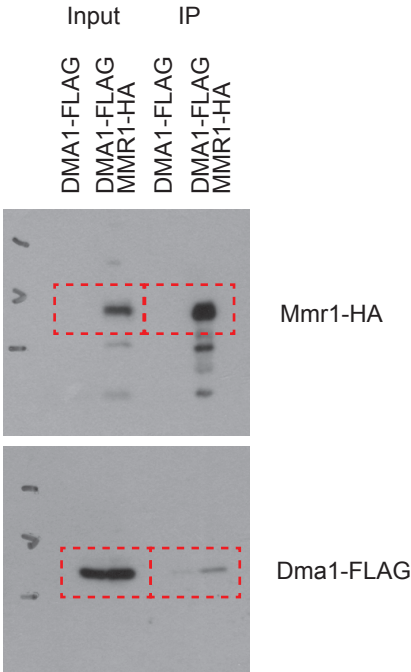


Fig. 5a

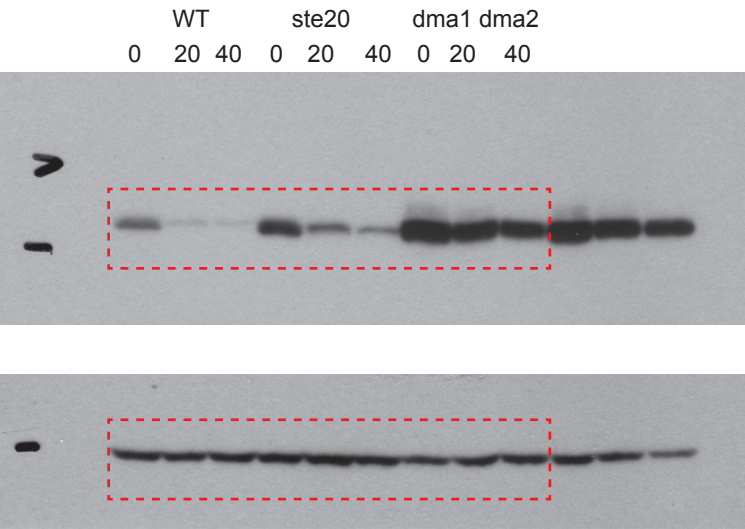


Fig. 5b

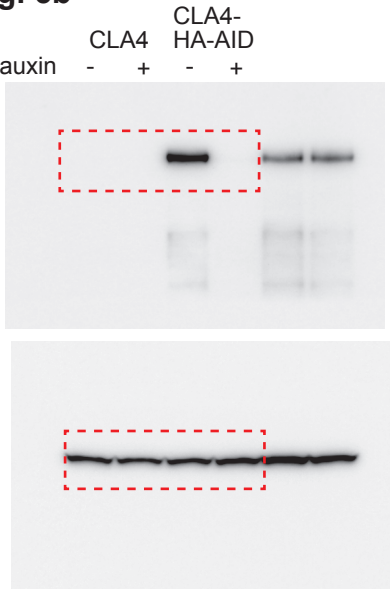


Fig. 5c

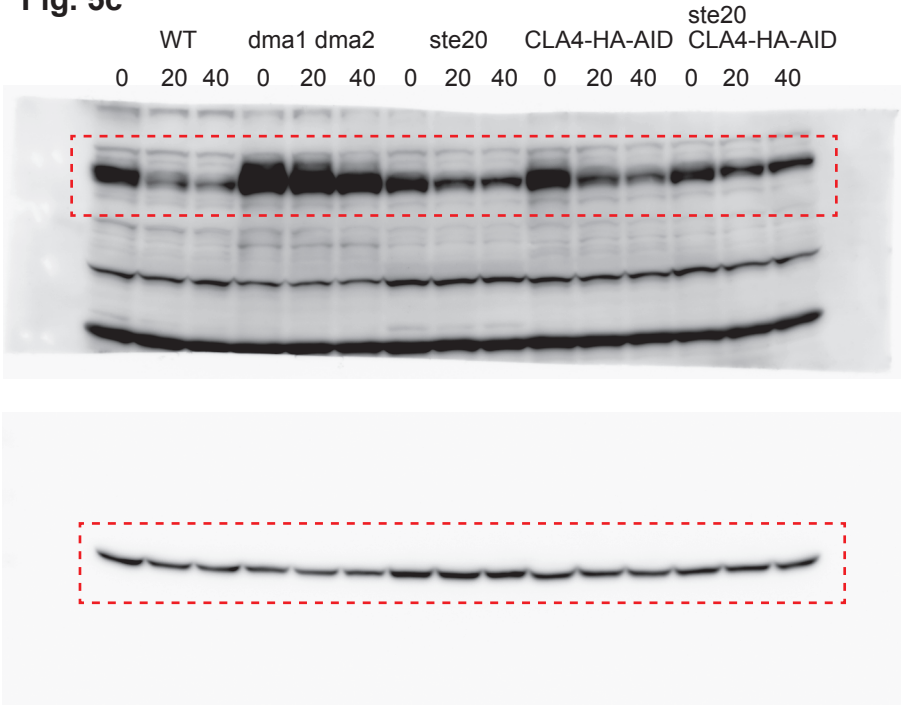


Fig. 5e

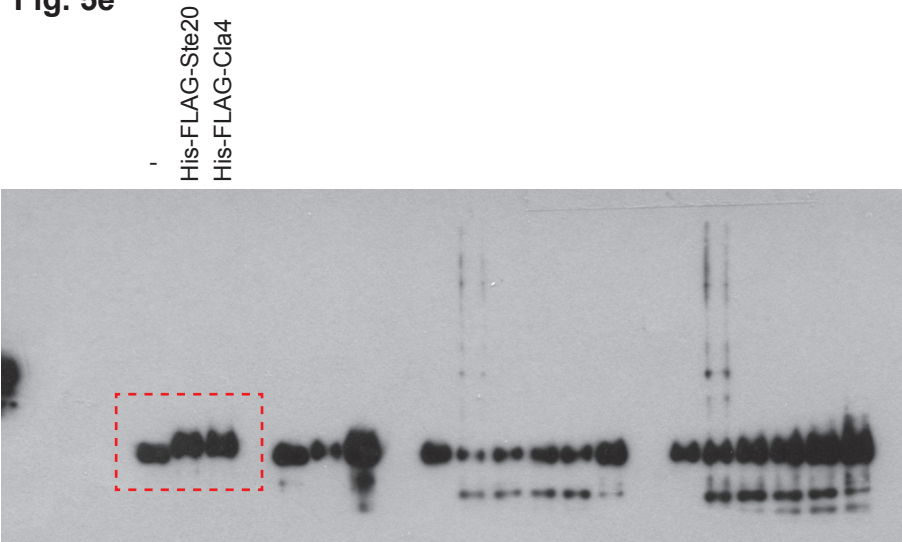


Fig. 5g

Myc-Uba1-His	-	+	-	+	+	+	-	+	-	+	+	+
His-Ubc4	-	+	+	-	+	+	-	+	+	-	+	+
His-FLAG-Dma1	-	+	+	+	-	+	-	+	+	+	-	+
His-Ub	-	+	+	+	+	-	-	+	+	+	+	-
His-HA-Mmr1	+	+	+	+	+	+	+	+	+	+	+	+
His-FLAG-Ste20	+	+	+	+	+	+	-	-	-	-	-	-
His-FLAG-Cla4	-	-	-	-	-	-	+	+	+	+	+	+

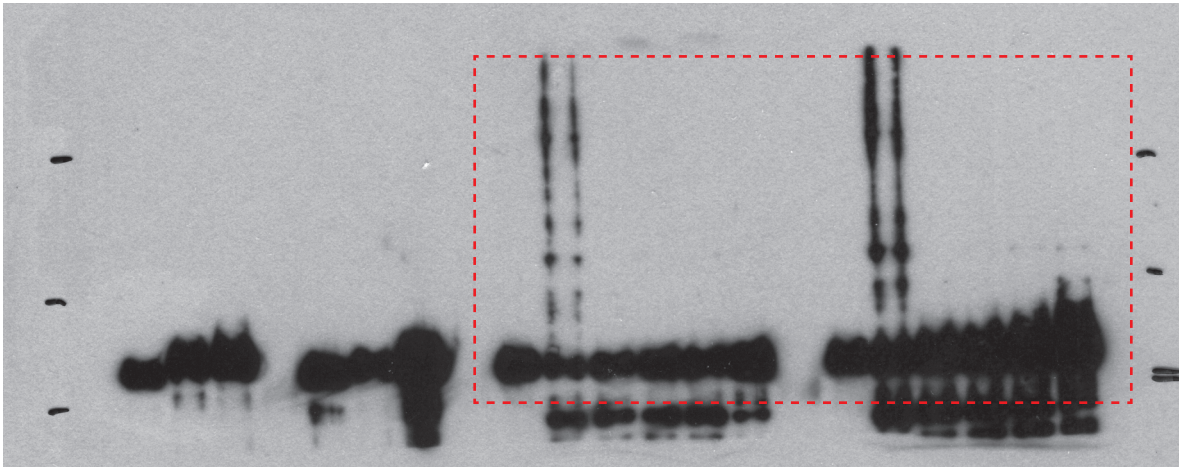


Fig. 5h

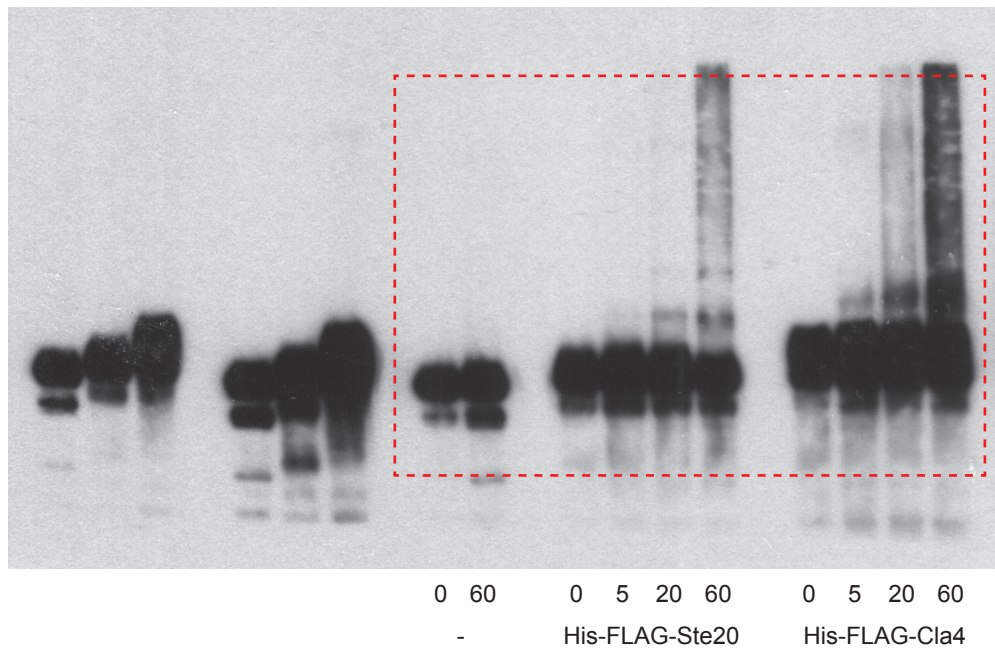


Fig. 6a

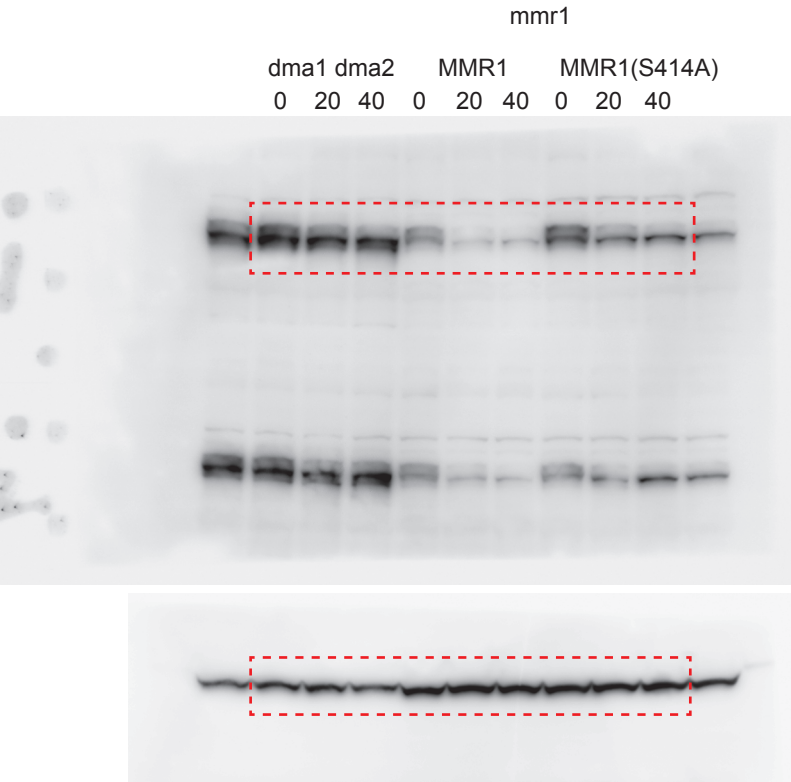
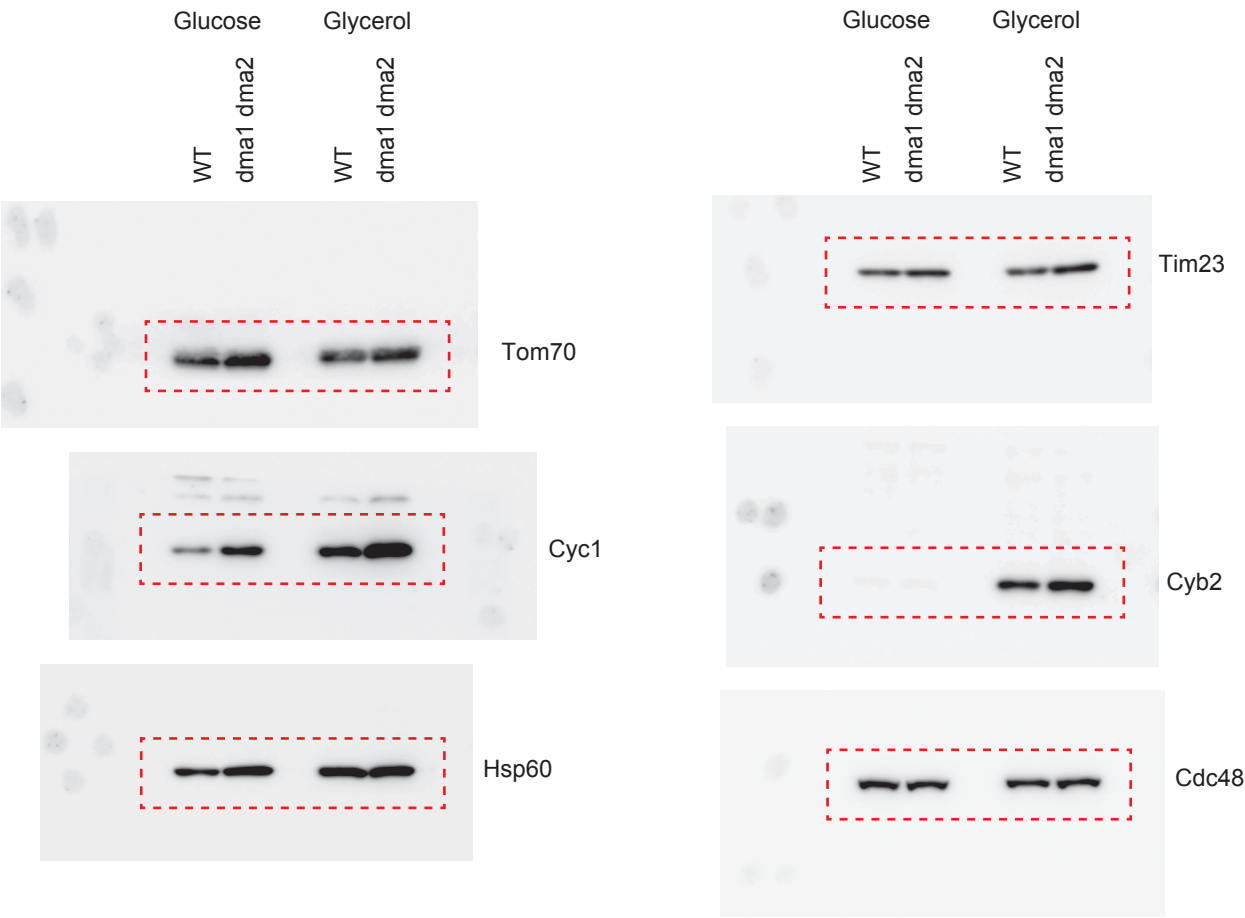
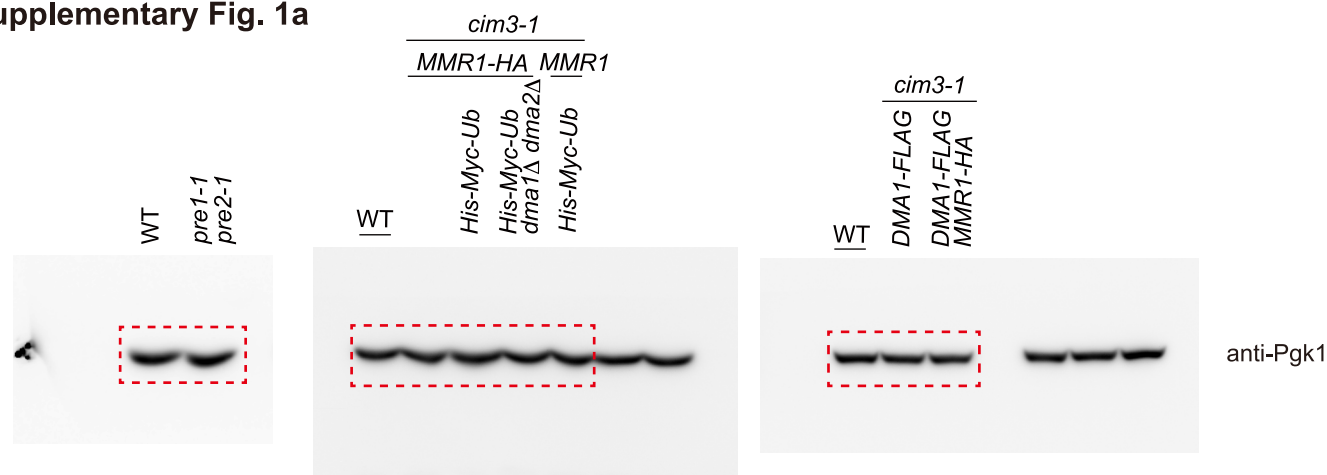


Fig. 7d



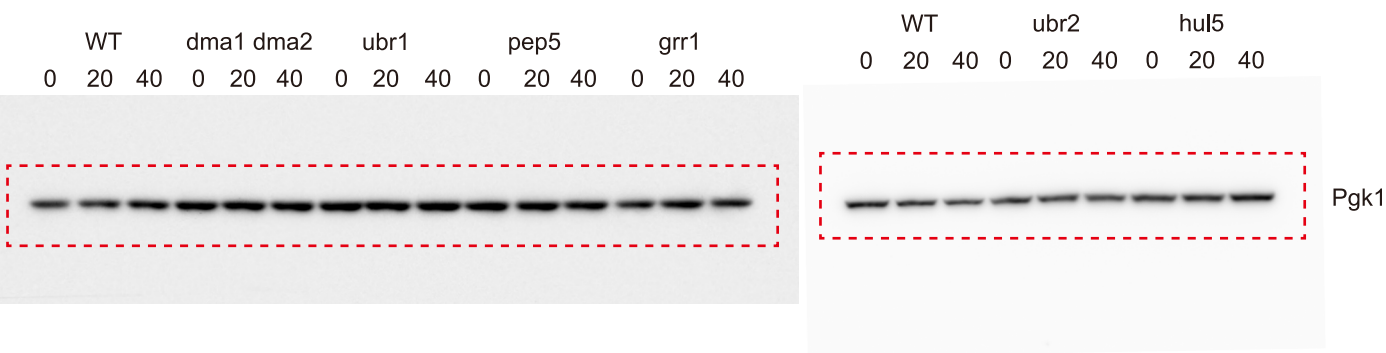
Supplementary Fig. 1a



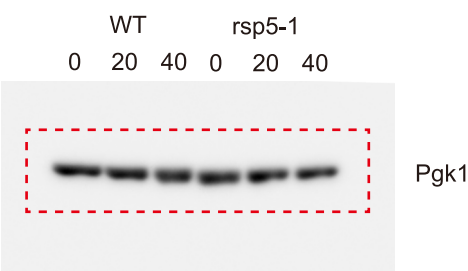
Supplementary Fig. 1b



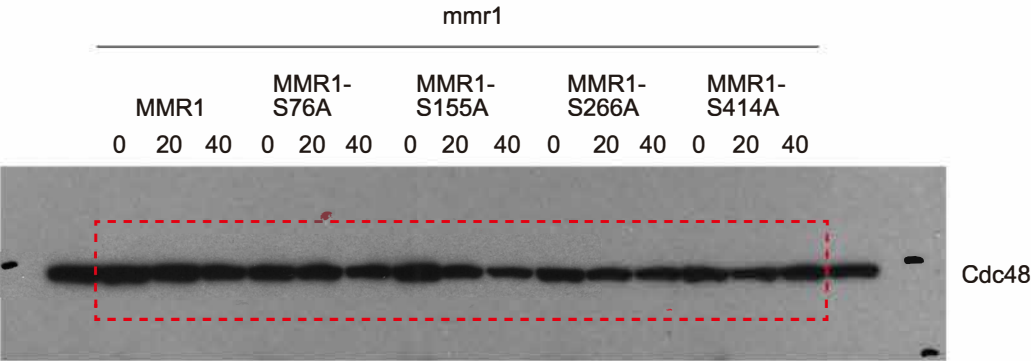
Supplementary Fig. 2a



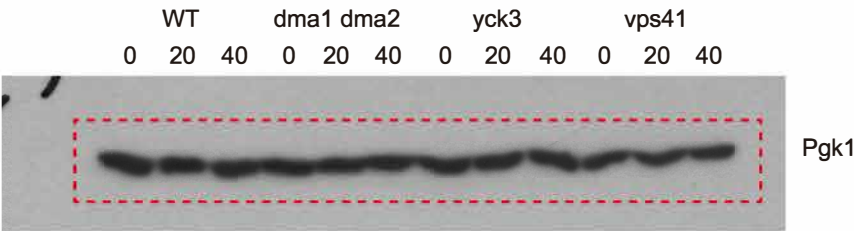
Supplementary Fig. 2b



Supplementary Fig. 5



Supplementary Fig. 7



Supplementary References

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