

**Supplementary information**

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**Mitochondrial complexome reveals quality-control pathways of protein import**

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In the format provided by the authors and unedited

## **Supplementary Information Guide**

### **Mitochondrial complexome reveals quality control pathways of protein import**

Uwe Schulte et al.

**Supplementary Figure 1. Uncropped versions of gels/blots of this study.**

**Supplementary Table 1. MS data related to identification/quantification of mitochondrial proteins (separate Excel file).** Sheet 1: MitCOM proteins (total of 906 proteins); sheet 2: mitochondrial proteins with single-peptide detection (total of 49 proteins).

**Supplementary Table 2. Data underlying abundance-mass profiles of MitCOM proteins (separate Excel file).**

**Supplementary Table 3. MS data related to identification/quantification of non-mitochondrial proteins (total of 985 proteins) (separate Excel file).**

**Supplementary Table 4. Yeast strains used in this study.**

**Supplementary Table 5. Plasmids used in this study.**

**Supplementary Table 6. Antibodies used in this study.**

**Supplementary Figure 1. Uncropped versions of gels/blots of this study.**

Figure 3b

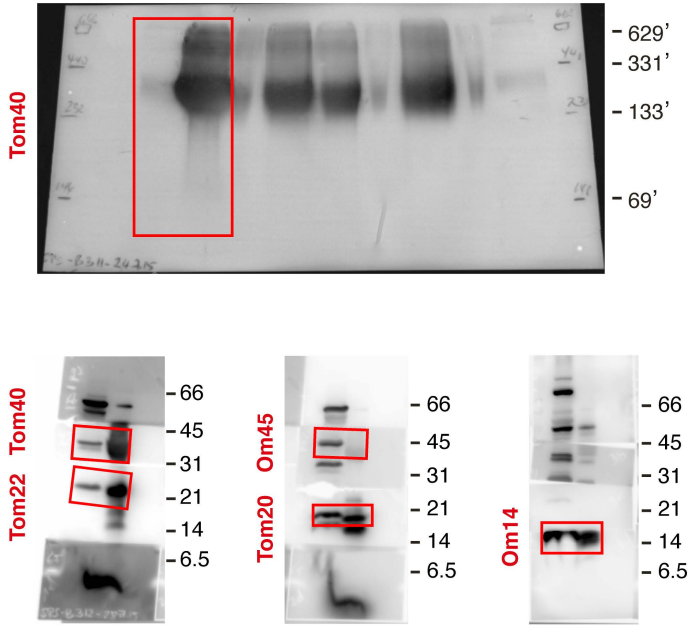


Figure 3e

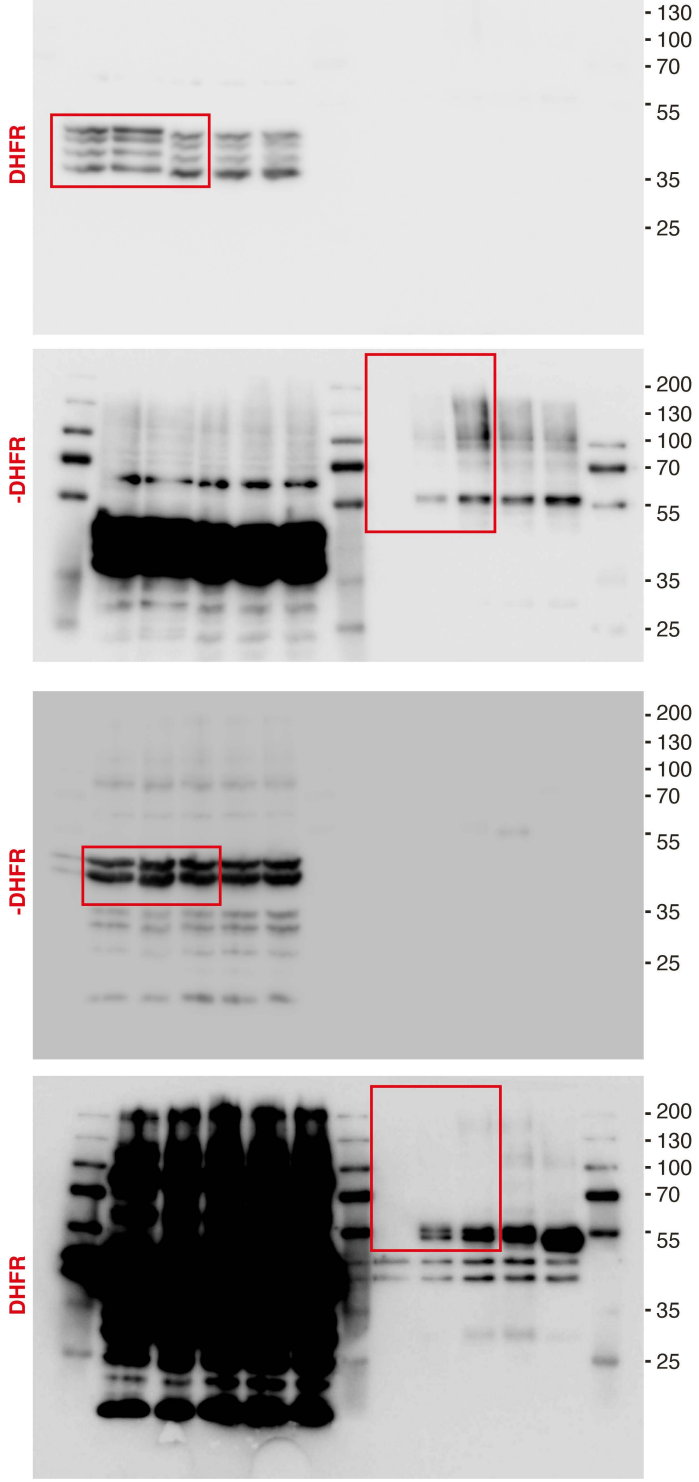


Figure 3d

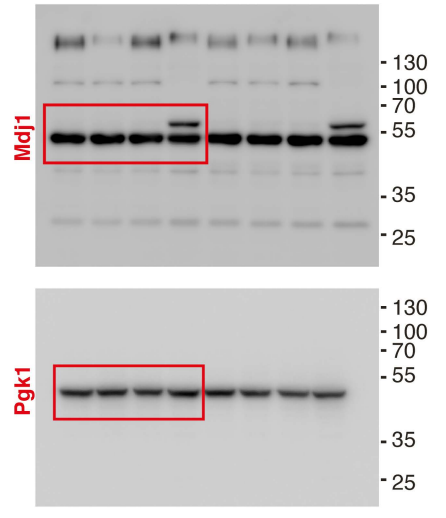


Figure 4b left panel

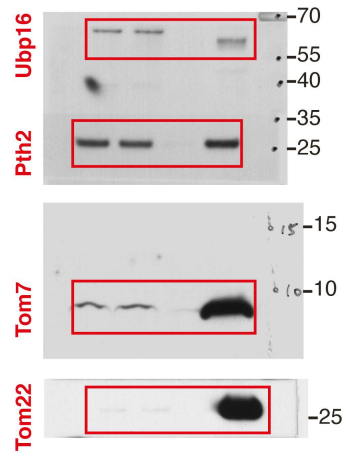


Figure 4b right panel

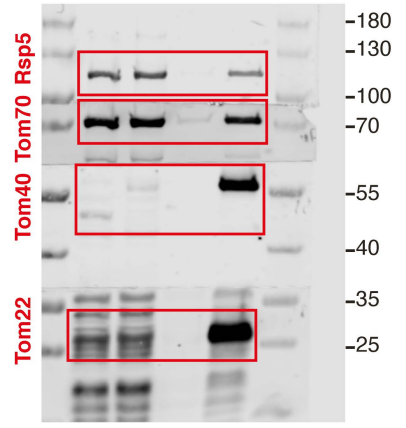


Figure 4c

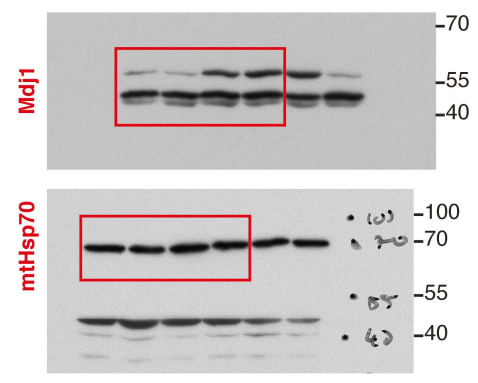
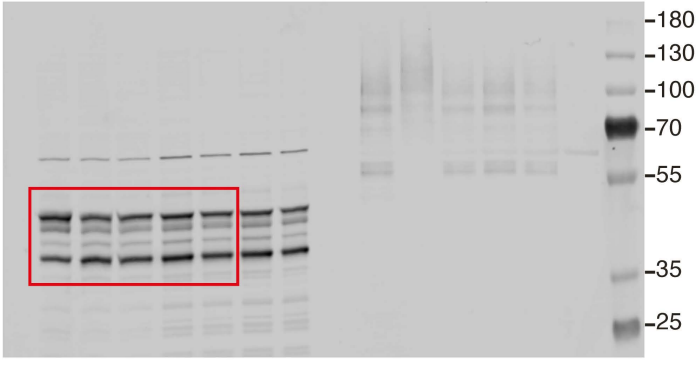
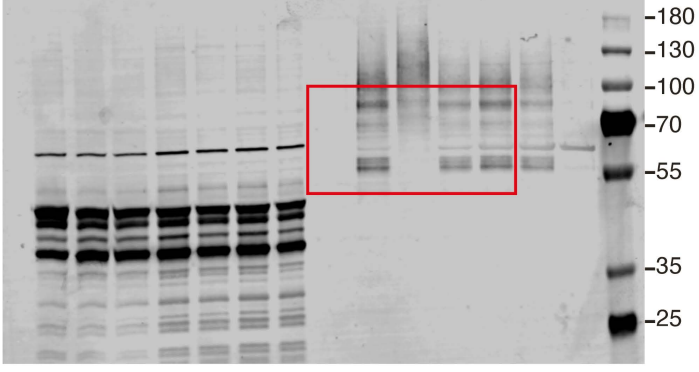


Figure 4d

DHFR



DHFR



Mdj1

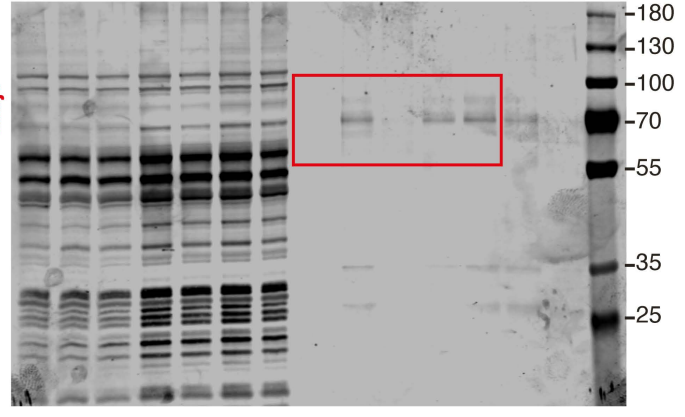


Figure 4e

Mdj1

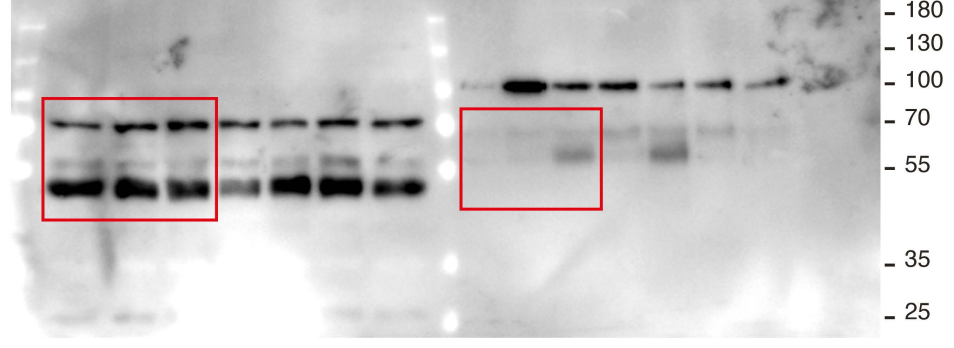
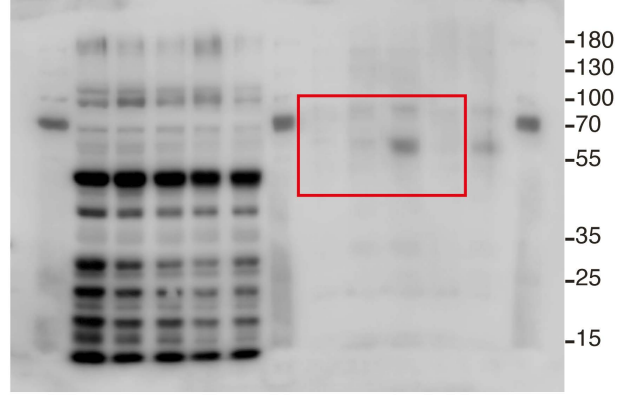


Figure 4f

Mdj1



Mdj1



ubiquitin

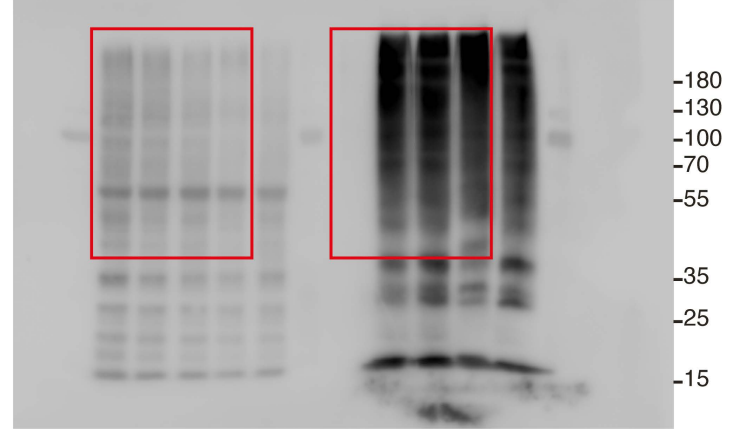


Figure 4g

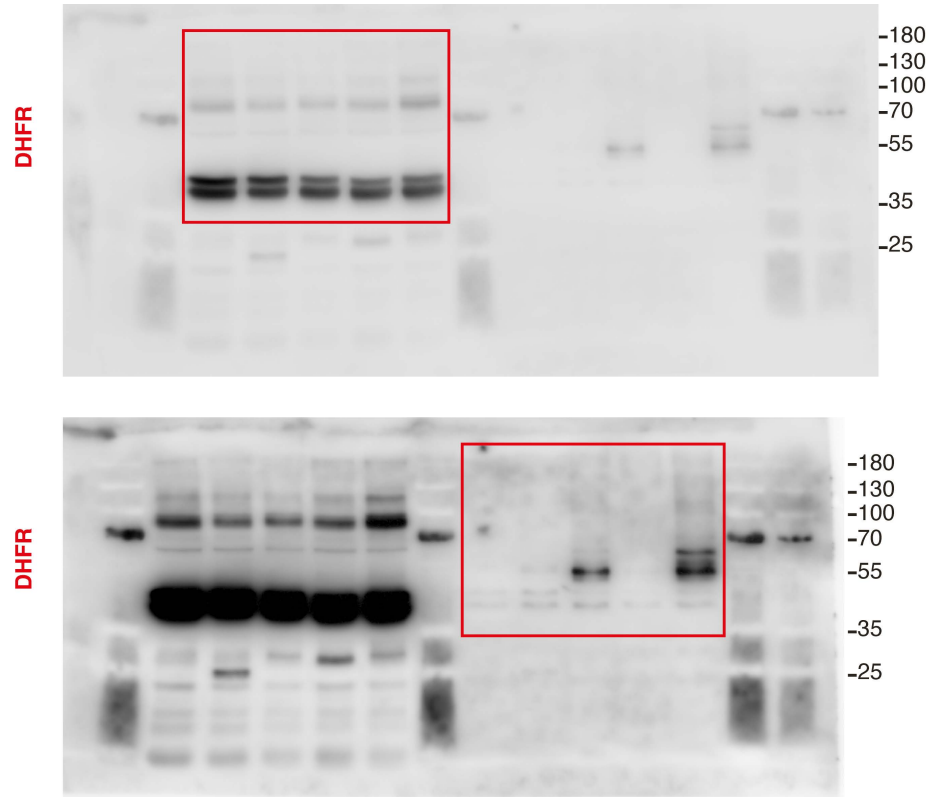


Figure 5c

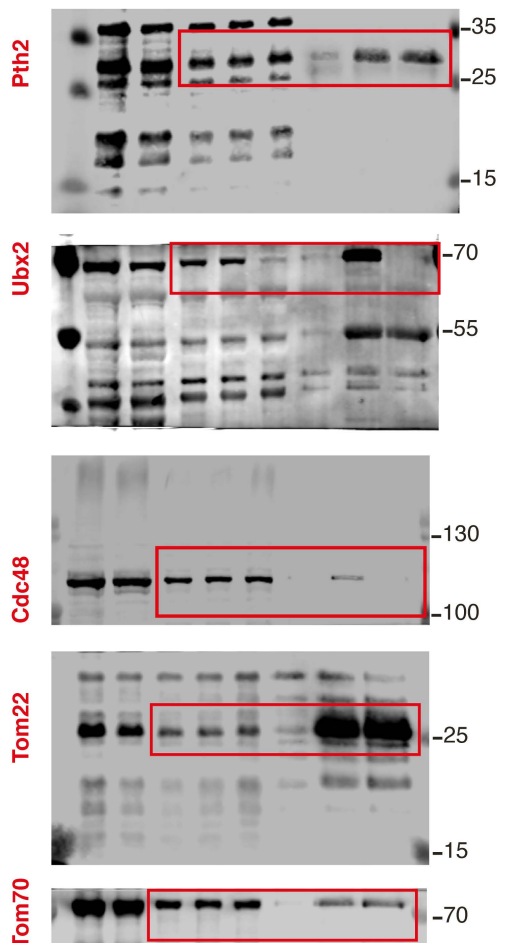


Figure 5g

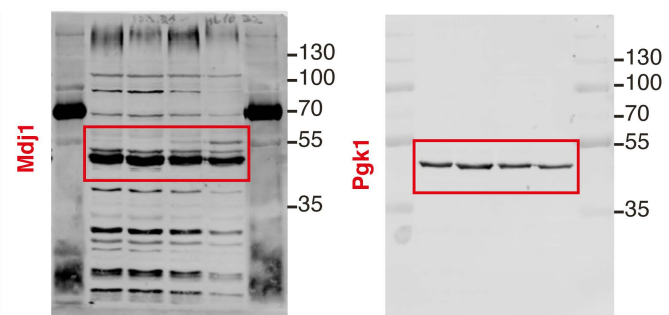


Figure 5a

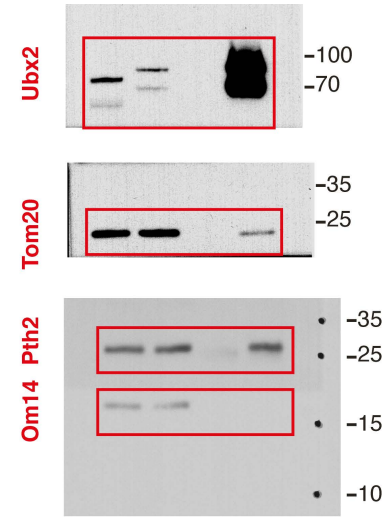


Figure 5b

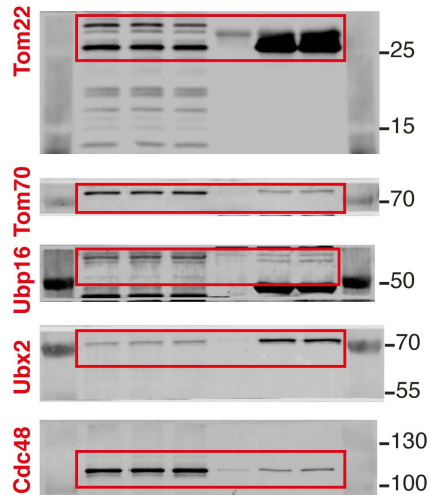
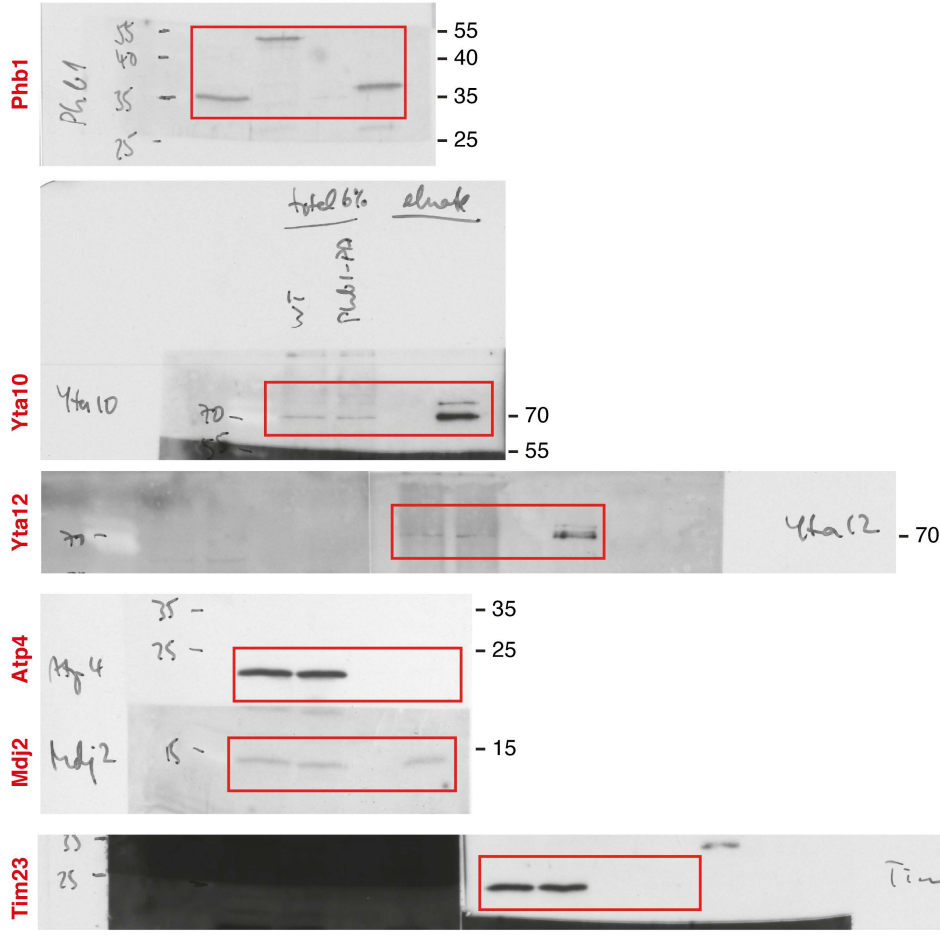


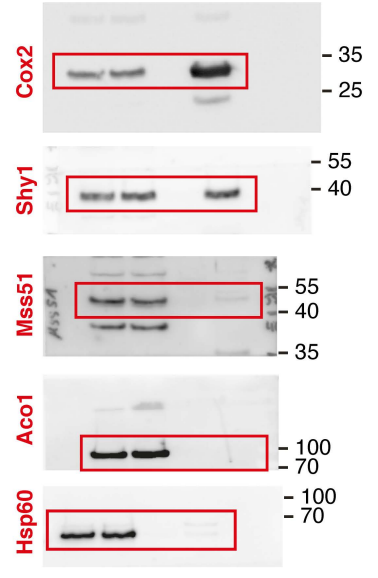
Figure 5e



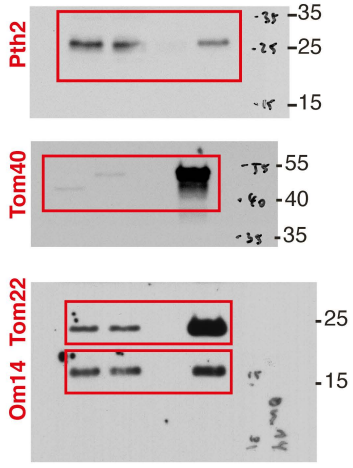
Extended Data Figure 8f



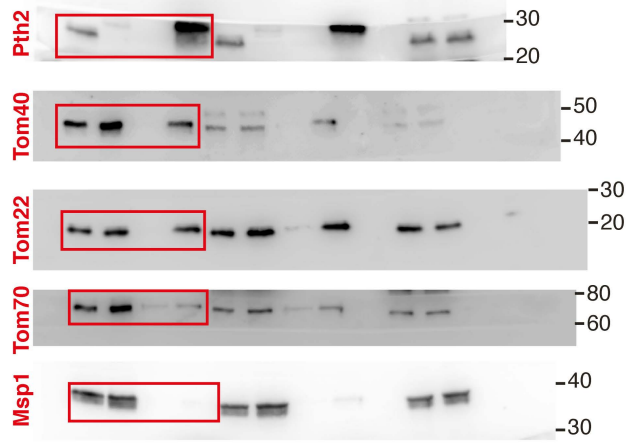
Extended Data Figure 8h



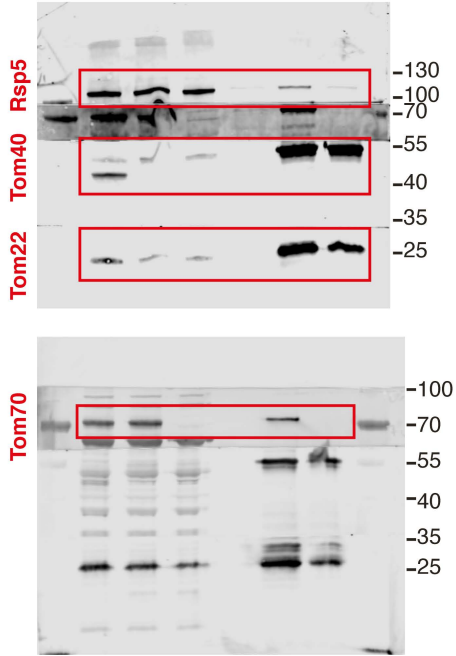
Extended Data Figure 9b



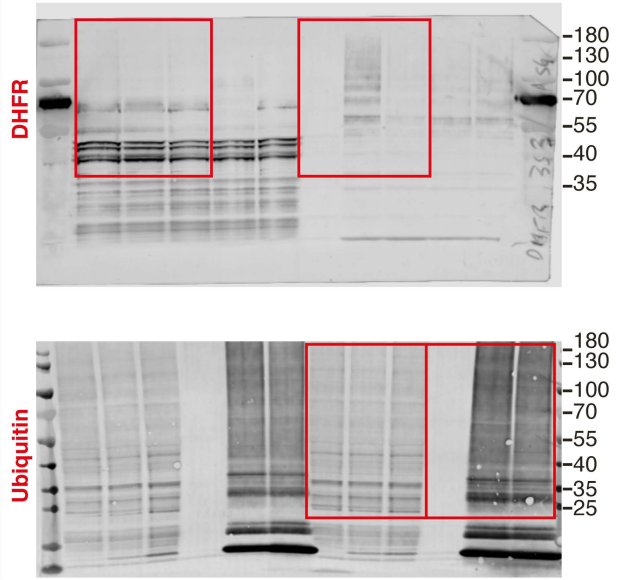
Extended Data Figure 9c



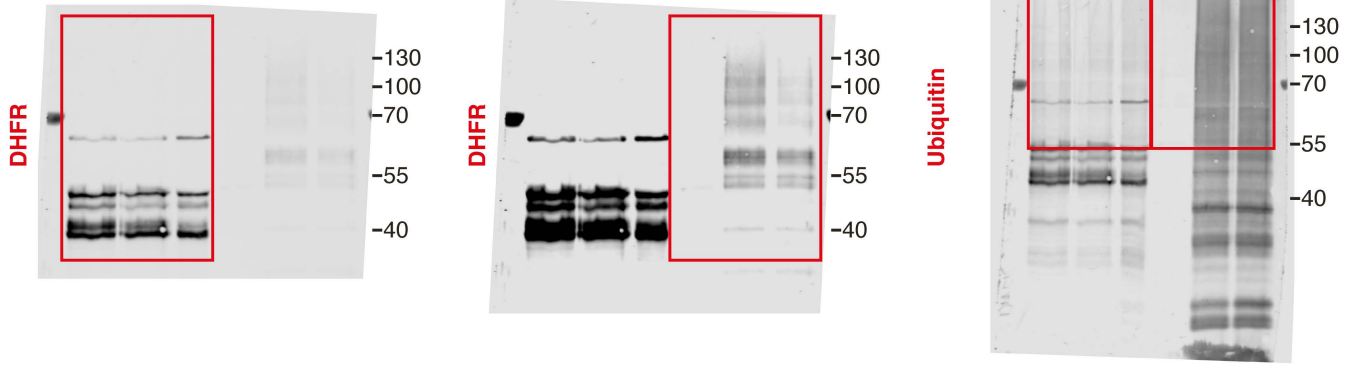
Extended Data Figure 9f



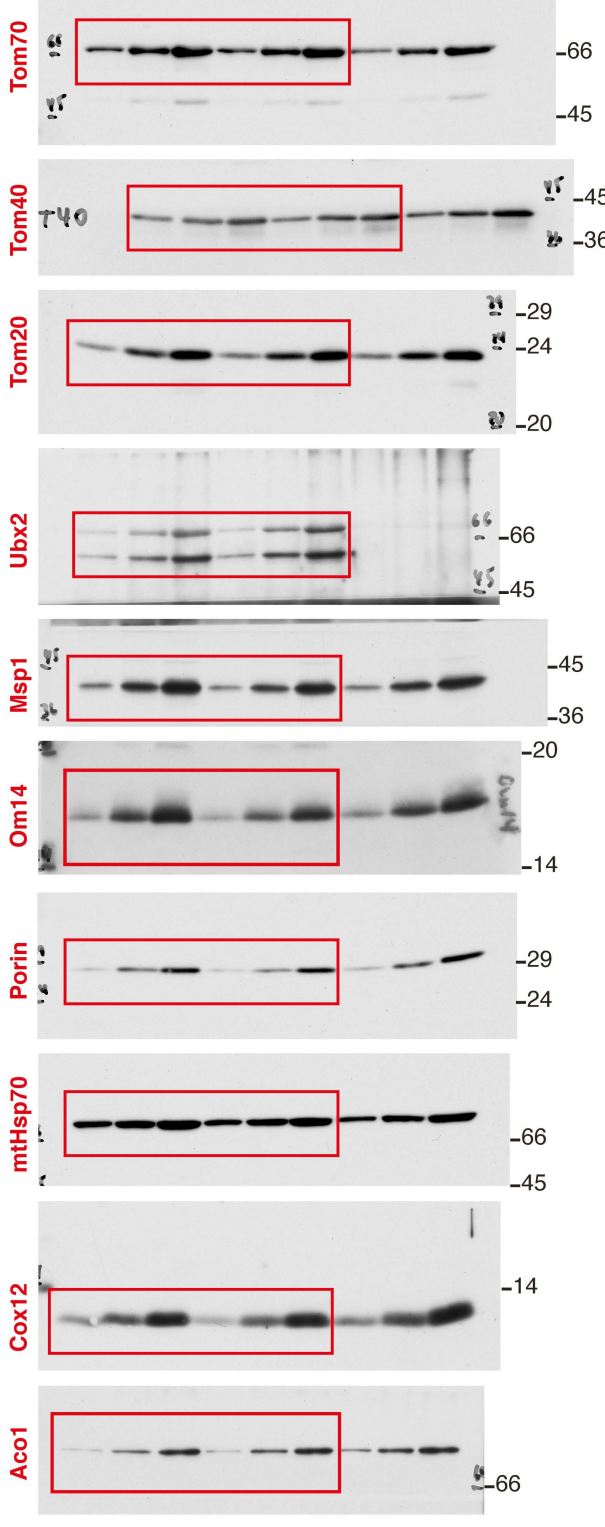
Extended Data Figure 9g



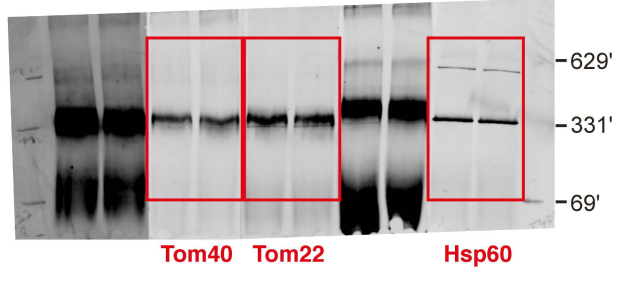
Extended Data Figure 9h



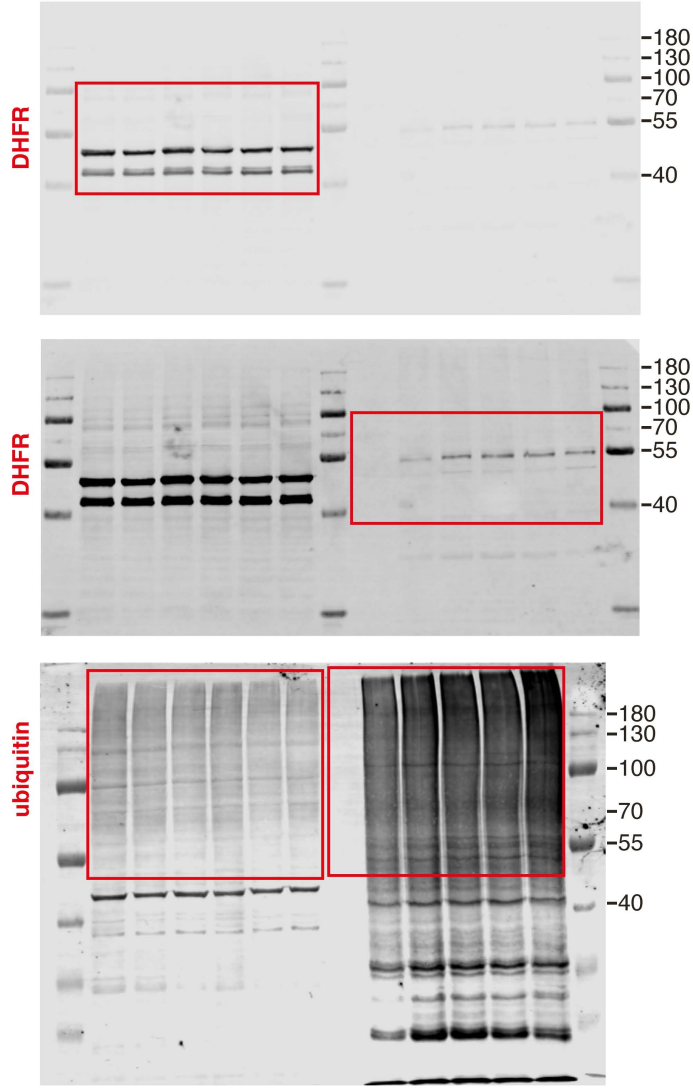
Extended Data Figure 10a



Extended Data Figure 10b

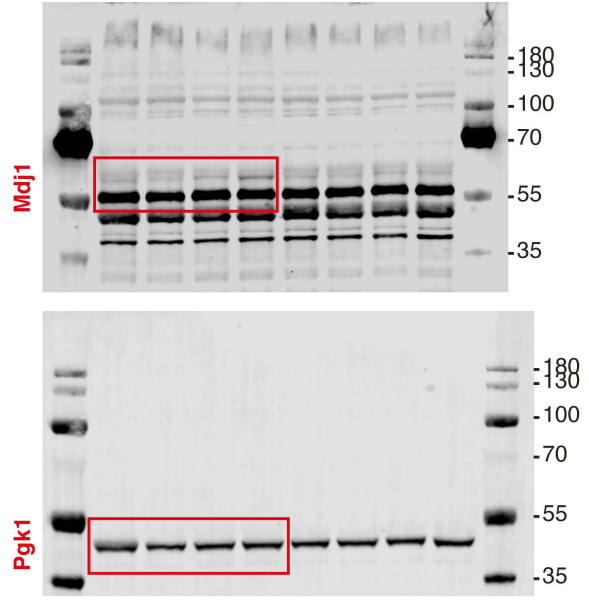


Extended Data Figure 10c

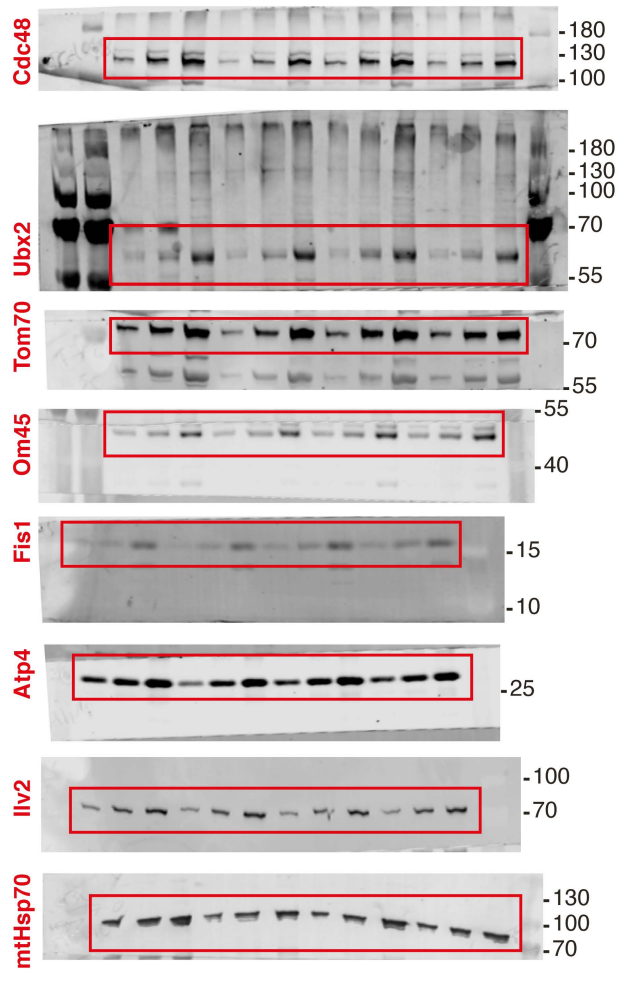




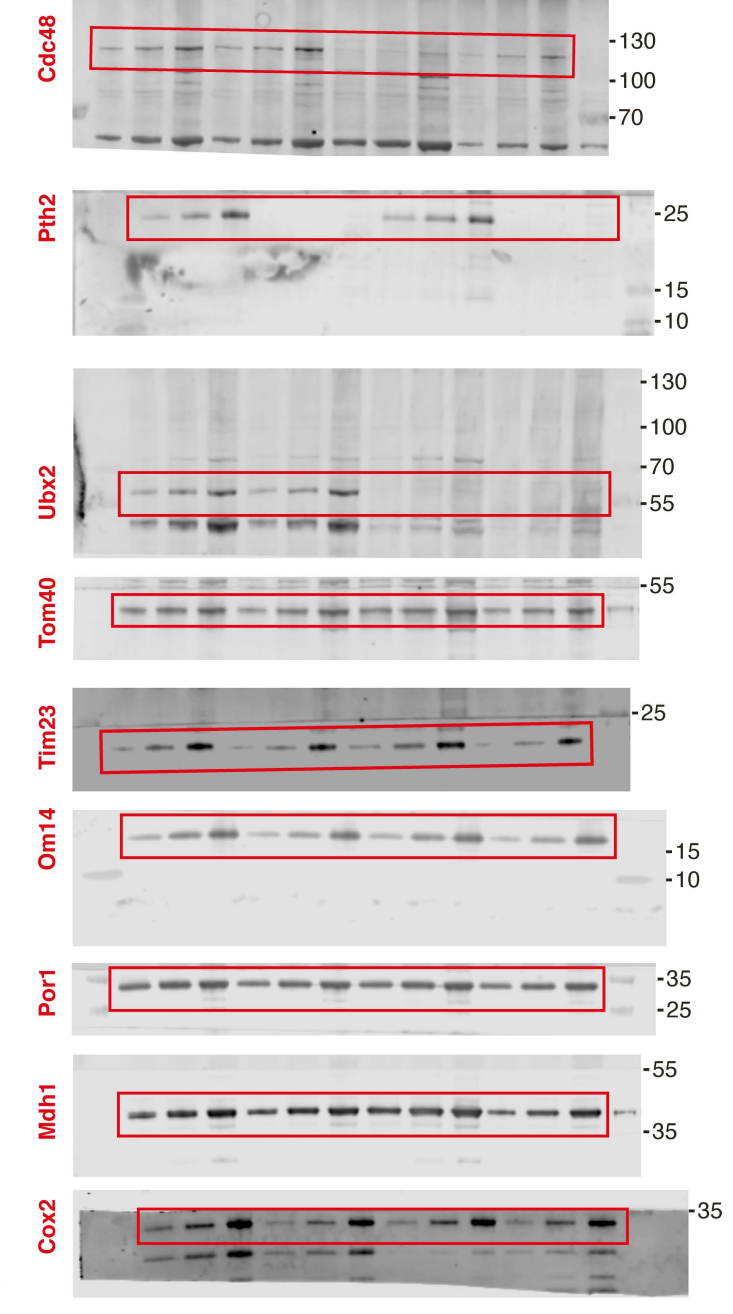
Extended Figure 10e



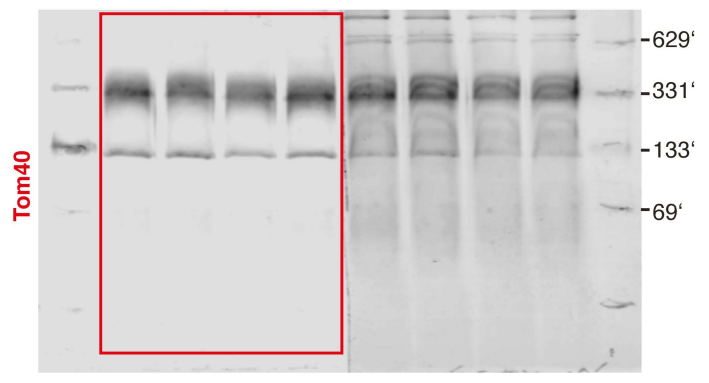
Extended Figure 10g



Extended Figure 10f



Extended Figure 10h



**Supplementary Table 4. Yeast strains used in this study.**

Genotype	Source	Identifier
YPH499 (WT) <i>MATa</i> , <i>ura3-52</i> , <i>lys2-801_amber</i> , <i>ade2-101_ochre</i> , <i>trp1-Δ63</i> , <i>his3-Δ200</i> , <i>leu2Δ1</i>	Ref. 43	TB1
YPH499 <i>cox4::COX4-10His::HIS3MX6</i>	Ref. 42	NP3297
YPH499 <i>Phb1<sup>ProtA</sup></i>	this study	NP4063
YPH499 <i>Tom22<sup>His</sup> Tom40<sup>Strep</sup></i>	this study	NP4420
YPH499 <i>pth2::PTH2-10His::HIS3MX6</i>	this study	TB3
YPH499 <i>tom20::TOM20-10HIS-HIS3MX6</i>	Ref. 21	NP3718
BY4741 (WT) <i>MATa</i> , <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	EUROSCARF	TB2
BY4741 <i>tom40::TOM40-3HA-HIS3MX6</i>	Ref. 17	TB4
BY4741 <i>pth2::kanMX4</i> , <i>tom40::TOM40-3HA-HIS3MX6</i>	this study	TB5
BY4741 <i>ubx2::kanMX4</i> , <i>tom40::TOM40-3HA-HIS3MX6</i>	Ref. 17	TB6
BY4741 <i>ubx2::UBX2-3HA::HIS3MX6</i>	Ref. 17	TB45
BY4741 <i>pth2::KanMX4</i>	EUROSCARF	TB8
BY4741 <i>pam17::hphNT1</i>	Ref. 17	TB9
BY4741 <i>pth2::KanMX4 pam17Δ::hphNT1</i>	this study	TB7
BY4741 <i>ubx2::KanMX4</i>	EUROSCARF	TB22
BY4741 <i>ubx2::KanMX4 pth2::HIS3MX6</i>	this study	TB27
BY4741 <i>ubp16::KanMX4</i>	EUROSCARF	TB21
BY4741 <i>ubp16::KanMX4</i> , <i>pth2::HIS3MX6</i>	this study	TB36
BY4741 <i>pth2::kanMX4 vms1::HIS3MX6</i>	this study	TB35
BY4741 <i>dsk2::KanMX4</i>	this study	TB102
BY4741 <i>dsk2::KanMX4</i> , <i>pam17::hphNT1</i>	this study	TB100
BY4741 <i>rsp5::RSP5-1-KanMX4</i>	EUROSCARF	TB20
BY4741 <i>mdm30::KanMX4</i>	EUROSCARF	TB101
BY4741 <i>mfb1::KanMX4</i>	EUROSCARF	TB103
BY4741 <i>rsp4::RSP5-1-KanMX4</i> <i>ubp16::HIS3MX6</i>	this study	TB39
BY4741 <i>rsp5::RSP5-1-KanMX4</i> <i>pam17::hphNT1</i>	this study	TB12
BY4741 <i>ubp16::KaMX4 pam17::hphNT1</i>	this study	TB13
BY4741 <i>pre9::kanMX4 pam17::hphNT1</i>	Ref. 17	TB11
BY4741 <i>mdm30::KanMX4 pam17::hphNT1</i>	this study	TB72
BY4741 <i>mfb1::KanMX4 pam17::hphNT1</i>	this study	TB73
BY4741 <i>hrd1::KanMX4 pam17::hphNT1</i>	this study	TB71
BY4741 <i>doa10::KanMX4 pam17::hphNT1</i>	this study	TB70
BY4741 <i>vms1::KanMX4</i>	EUROSCARF	TB23
BY4741 <i>pth2::KanMX4 pam17Δ::hphNT1</i> + pRS416	this study	TB166
BY4741 <i>pth2::KanMX4 pam17Δ::hphNT1</i> + pRS416 pPTH2 Pth2	this study	TB167
BY4741 <i>pth2::KanMX4 pam17Δ::hphNT1</i> + pRS416 pPTH2 Pth2 D174A	this study	TB168
BY4741 <i>pth2::KanMX4 pam17Δ::hphNT1</i> + pRS416 pPTH2 Pth2 ΔTM (Δ aa12-32)	this study	TB169
BY4741 <i>pth2::kanMX4 vms1::HIS3MX6</i> + pRS416	this study	TB171
BY4741 <i>pth2::kanMX4 vms1::HIS3MX6</i> + pRS416 pPTH2 Pth2	this study	TB172
BY4741 <i>pth2::kanMX4 vms1::HIS3MX6</i> + pRS416 pPTH2 Pth2 D174A	this study	TB173
BY4741 <i>pth2::kanMX4 vms1::HIS3MX6</i> + pRS416 pPTH2 Pth2 ΔTM (Δ aa12-32)	this study	TB174

BY4741 <i>tom70::hphNT1</i>	this study	TB98
BY4741 <i>tom70::hphNT1, tom40::TOM40-3HA-HIS3MX6</i>	this study	TB113

**Supplementary Table 5. Plasmids used in this study.**

<b>Plasmid</b>	<b>Source</b>	<b>Identifier</b>
pDP-CYB2(1-84)-DHFR-HB(81-181) (referred to as <i>b</i> <sub>2</sub> -DHFR)	Ref. 99	pTB7
pDP-CYB2(1-84) $\Delta$ (47-65)-DHFR-HB(81-181) (referred to as <i>b</i> <sub>2</sub> $\Delta$ -DHFR)	Ref. 99	pTB8
pDP-CYB2(1-84) $\Delta$ (47-65)-DHFR(L251G, P252G)-HB(81-181) (referred to as <i>b</i> <sub>2</sub> $\Delta$ -DHFR <sup>GGxY</sup> )	this study	pTB128
pADH His-Ubiquitin	M. Glickman, Ref. 41	pTB3
pFA6a His3MX6	Ref. 46	pTB2
pFA6a hphNT1	Ref. 44	pTB6
pFA6a KanMX6	Ref. 46	pTB1
pFA6a 3HA-His3MX6	Ref. 45	pTB12
pRS416	Ref. 43	pTB94
pRS416 pPTH2 Pth2	this study	pTB91
pRS416 pPTH2 Pth2 D174A	this study	pTB92
pRS416 pPTH2 Pth2 $\Delta$ TM ( $\Delta$ aa12-32)	this study	pTB93

**Supplementary Table 6. Antibodies used in this study.**

<b>Antibody</b>	<b>Source</b>	<b>Identifier</b>
Rabbit polyclonal anti-Aco1	Ref. 42	GR 947
Rabbit polyclonal anti-Atp4	Ref. 69	GR 1970
Rabbit polyclonal anti-Cdc48	Ref. 17	GR 5015
Rabbit polyclonal anti-Cox2	Ref. 17	GR 1948
Rabbit polyclonal anti-Cox12	Ref. 42	GR1937
Rabbit polyclonal anti-Cox14	Ref. 88	GR 1544
Rabbit polyclonal anti-Fis1	Ref. 21	GR 310
Rabbit polyclonal anti-Hsp60	Ref. 42	170
Rabbit polyclonal anti-Ilv2	Ref. 100	GR1010
Rabbit polyclonal anti-Mdh1	Ref. 101	GR1088
Rabbit polyclonal anti-Mdj1	Ref. 17	121
Rabbit polyclonal anti-Mdj2	Ref. 42	GR 1842
Rabbit polyclonal anti-mtHsp70	Ref. 42	GR 119
Rabbit polyclonal anti-Msp1	Ref. 17	GR 1468
Rabbit polyclonal anti-Mss51	Ref. 42	GR 1952
Rabbit polyclonal anti-Om14	Ref. 17	GR 3041
Rabbit polyclonal anti-Om45	Ref. 17	GR 1311
Rabbit polyclonal anti-Phb1	Ref. 102	298-11
Rabbit polyclonal anti-Por1	Ref. 21	GR 3621
Rabbit polyclonal anti-Pth2	Ref. 21	GR 797
Rabbit polyclonal anti-Rsp5	this study	GR 5064
Rabbit polyclonal anti-Shy1	Ref. 42	GR 1094
Rabbit polyclonal anti-Tim23	Ref. 17	GR 133
Rabbit polyclonal anti-Tom7	Ref. 80	GR 230
Rabbit polyclonal anti-Tom20	Ref. 69	GR 3225
Rabbit polyclonal anti-Tom22	Ref. 69	GR 3227
Rabbit polyclonal anti-Tom40	Ref. 69	168
Rabbit polyclonal anti-Tom70	Ref. 69	GR 657
Rabbit polyclonal anti-Ubp16	Ref. 21	GR 5040
Rabbit polyclonal anti-Ubx2	Ref. 17	GR1484
Rabbit polyclonal anti-Yta10	Ref. 6	GR1550-3
Rabbit polyclonal anti-Yta12	this study	GR1437-3
Rabbit polyclonal anti-Pgk1	Ref. 17	GR 753
mouse-monoclonal anti-DHFR	Santa Cruz	sc-377091
mouse-monoclonal anti-Pgk1	Invitrogen	459250
mouse-monoclonal anti-ubiquitin	Santa Cruz	sc-8017