SI Appendix

SI Appendix includes six supplementary figures, six supplementary figure legends, three tables and supplementary references.

Supplementary Figure 1

Growth curves, dissociation and recruitment kinetics in WT, Shp1 knock out and Shp1-AID strains. A-C) Densitometric analysis of Western blot band intensities of immunoprecipitations in Fig 1A. For quantifications in A-C the signal intensities for each Shp1 variant at time point 0 were set to 1. A) Skp1 signals were normalized to ^{12xMyc}Met30 to quantify Met30 dissociation from the core ligase. B) Shp1^{3xHA} signals were normalized to ^{12xMyc}Met30 to determine recruitment of Shp1 to SCF^{Met30}. Cdc48^{RGS6H} signals were normalized to ^{12xMyc}Met30 to resolve recruitment into SCF^{Met30}, t-test: p*< 0.1, p**<0.05, p***< 0.01. D) Densitometry of Cdc48 ^{RGS6H} /^{12xMyc}Met30 ratios, WT at time point 0 was set to 1. E) *shp1*Δ mutants show a significant growth defect at 30°C. WT, and Shp1 K.O. strains were grown at 30°C in YEPD medium and samples were taken at indicated time points. Optical density was measured at 600nm. F) Shp1-AID gets sufficiently down-regulated upon auxin exposure. Strains expressing endogenous ^{12xMyc}Met30, Shp1$^{3xHA-AID}$ and the F-Box protein ^{2xFLAG}OsTir under the constitutive ADH promoter were cultured at 30°C in YEPD. Samples were taken at indicated time-points after the addition of 500µM auxin (IAA). Protein levels were analyzed by Western blotting. The amido black stain of the membrane is shown as a loading control. G) Depicted strains do not show a significant growth defect at 30°C in the absence or presence of auxin. Strains were grown at 30°C in YEPD medium or YEPD containing 500 µM IAA and samples were taken at indicated time points. Optical density was measured at 600nm. H) Densitometric analysis of Western blot band intensities of immunoprecipitations in Fig 1D. Skp1 signals were normalized to ^{12xMyc}Met30 to determine Met30 dissociation from the core ligase during cadmium stress.

Supplementary Figure 2

A) Amino acid sequence of Shp1 - Specific domains/motifs are labeled and color-coded. B) Schematic of the most important Shp1 variants used throughout this study. C) Spotting assay - Serial dilutions of depicted strains were made and spotted onto YEPD plates. Plates were incubated for two days at 30°C. D) Shp1 WT, ΔCIM1 &2 and K.O. strains were grown at 30°C in YEPD medium and samples were taken at indicated time points. Optical density was measured at 600nm. E) Cdc48 binding is

significantly decreased in ΔCIM1 and ΔCIM2. $SHP1^{3xHA}$ variants were immunoprecipitated and co-purifications of $Cdc48^{RGS6H}$ were analyzed by Western blotting. F) Met30 protein stability is unaffected in *shp1*Δ deletion mutants. Depicted strains were grown at 30°C in YEPD medium, cycloheximide (100 µg/ml) was added, and samples were collected at indicated time points. Protein stability was analyzed by immunoblotting followed by densitometric analysis. Asterisk next to Met30 levels of K.O. (*shp1*Δ) indicates longer exposure of the Western blot panel to show similar starting amounts of Met30 in CHX time course. G) *MET30* RNA levels are decreased in *shp1*Δ cells. Depicted cultured were grown at 30°C in YEPD medium. RNA was extracted and expression of *MET30* was analyzed by RT-qPCR and normalized to 18S rRNA levels.

Supplementary Figure 3

A) Quantification of spot assays shown in Figure 3A. The intensities of yeast spots were determined using ImageJ. The intensity of the lowest dilution of WT was set to 1. The intensities of *shp1 variant* spots were normalized to WT (n=3), data are represented as mean ±SD. B&C) The expression of Met4-dependent genes in response to methionine starvation. Depicted yeast strains were grown at 30°C in YEDP medium to OD_{600} of 0.6. For Methionine starvation, cultures were washed with water and shifted to minimal medium without methionine for 30 minutes and then harvested. For heavy metal stress induction, cultures were treated with 100µM $CdCl_2$ and samples were harvested after 40 min exposure. RNA was extracted and expression of Met4 target genes *MET25* and *GSH1* was analyzed by RT-qPCR and normalized to 18S rRNA levels (n=2). Set I shown in A, set II shown in B.

Supplementary Figure 4

A-D) Quantification data of ΔUBX_{Ct} was integrated in the graphs shown in Suppl. Fig 1 A-D. For quantifications in A-C the signal intensity of each Shp1 variant at time point 0 was set to 1. A) co-immunoprecipitated Skp1 signals were normalized to immunoprecipitated ^{12xMyc}Met30 to quantify Met30 dissociation from the core ligase. B) $Shp1^{3xHA}$ signals were normalized to ^{12xMyc}Met30. Then the ratio of co-immunoprecipitated $Shp1^{+Cd}/Shp^{w/o\ Cd}$ was determined to analyze recruitment of Shp1 to SCF^{Met30}. $Cdc48^{RGS6H}$ signals were normalized to ^{12xMyc}Met30 and the ratio of co-immunoprecipitated $Cdc48^{+Cd}/Cdc48^{w/o\ Cd}$ was analyzed to resolve recruitment of Cdc48 to SCF^{Met30}, t-test: p*< 0.1, p**<0.05, p***< 0.01. D) Densitometry of co-immunoprecipitated $Cdc48^{RGS6H}$/ immunoprecipitated ^{12xMyc}Met30 ratio. WT at time point 0 was set to 1. E) Shp1ΔCIM2 is recruited to SCF^{Met30} during cadmium stress.

WT and *shp1ΔCIM2* mutants were cultured at 30°C in YEPD medium and treated with 100 µM $CdCl_2$ and samples were harvested after 20 min of exposure. ^{12xMyc}Met30 was immunoprecipitated and co-precipitated proteins were analyzed by Western blotting. F) Full panel of immunoprecipitations that was partially shown in Fig 4C. A strain expressing endogenous ^{12xMyc}Met30, Shp1$^{3xHA-AID}$ and the F-Box protein ^{2xFLAG}OsTir under the constitutive ADH promoter was cultured at 30°C in YEPD medium in the absence and presence of auxin for the indicated time to gradually down-regulate endogenous Shp1-AID levels. Cells were exposed to 100µM $CdCl_2$ and samples were harvested after 20 min. ^{12xMyc}Met30 was immunoprecipitated and co-purified Skp1 was analyzed by Western blotting. G) Densitometric analysis of Western blot band intensities of immunoprecipitations in Suppl. Fig 4F. Shp1^{3xHA} signals were normalized to ^{12xMyc}Met30. Then the ratio of co-immunoprecipitated Shp1^{+Cd}/Shp$^{w/o\ Cd}$ was determined to follow Shp1 recruitment. H) Densitometry of Shp1^{3xHA}/^{12xMyc}Met30 ratio. Quantifications of Western blots shown in Fig. 4B and 4C.

Supplementary Figure 5

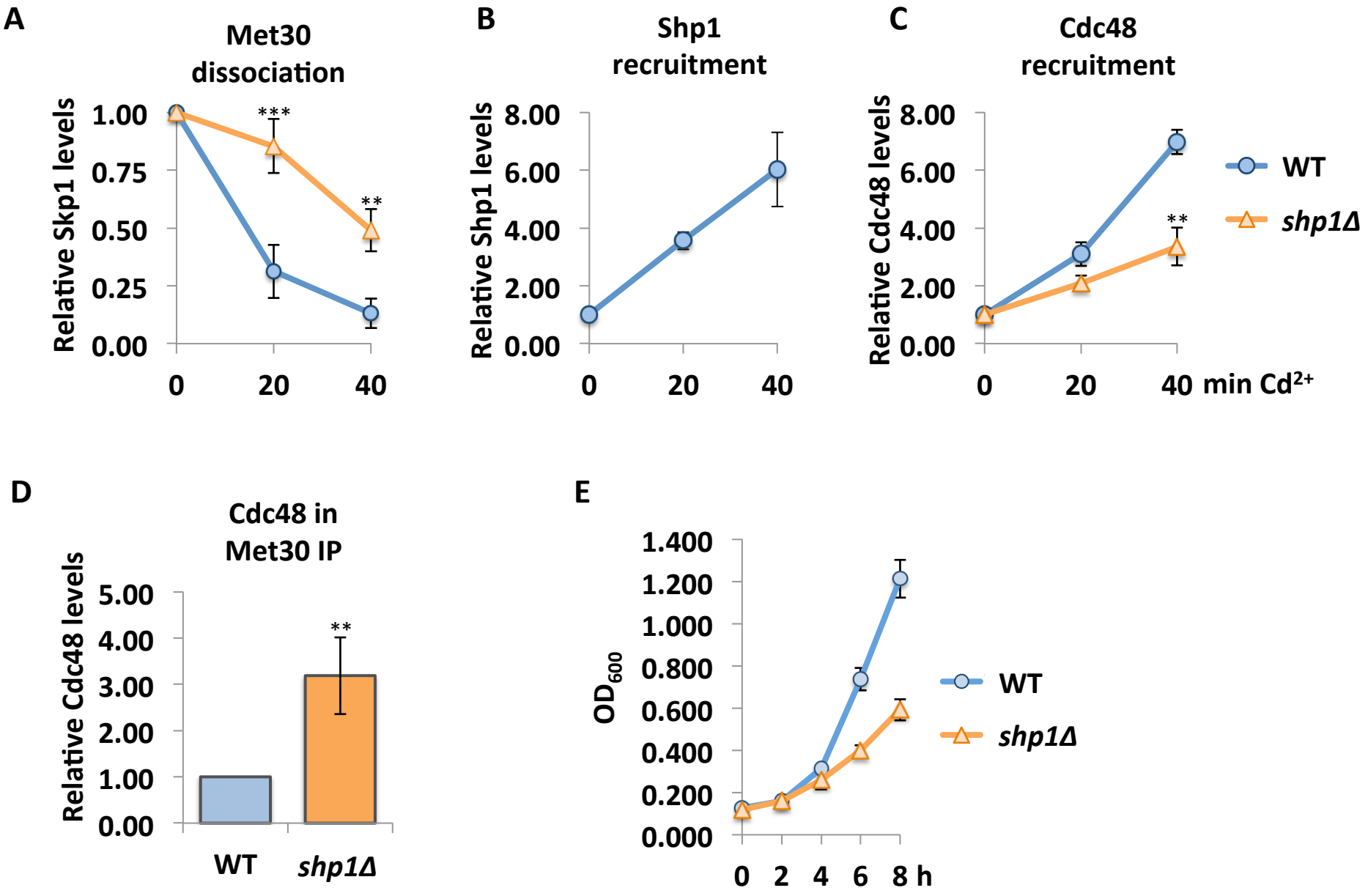
A) Quantification of spot assays shown in Figure 5A. The Intensities of yeast spots were determined using ImageJ. The intensity of the lowest dilution of WT was set to 1. The intensities of *shp1-Δsep* spots were normalized to WT (n=3), data are represented as mean ±SD. B) *Shp1-Δsep* mutants do not show a significant growth defect at 30°C. Wild-type and *shp1-*ΔSEP cells were grown at 30°C in YEPD medium and samples were taken at indicated time points to measure optic density at 600nm. C) The expression of Met4-dependent genes in response to methionine starvation is not altered in *shp1-Δsep* mutants. Depicted yeast strains were grown at 30°C in YEDP medium to OD_{600} of 0.6. For Methionine starvation, cultures were washed with water and shifted to minimal medium without methionine for 30 minutes and then harvested. RNA was extracted and expression of Met4 target genes *MET25* and *GSH1* was analyzed by RT-qPCR and normalized to 18S rRNA levels (n=3), data are represented as mean ±SD. D-F) Densitometric analysis of Western blot band intensities of immunoprecipitations in Fig 5A. For quantifications in D-F the signal intensities for each Shp1 variant at time point 0 were set to 1. D) Skp1 signals were normalized to ^{12xMyc}Met30 to quantify Met30 dissociation from the core ligase. E) Shp1^{3xHA} signals were normalized to ^{12xMyc}Met30 to determine recruitment of Shp1 to SCF^{Met30}. F) Cdc48^{RGS6H} signals were normalized to ^{12xMyc}Met30 to resolve recruitment into SCF^{Met30}, t-test: p*< 0.1, p**<0.05, p***< 0.01. G) Cdc48 binding is marginally decreased in *shp1-Δsep* mutants. SHP1^{3xHA} WT and ΔSEP were

immunoprecipitated and co-precipitation of $Cdc48^{RGS6H}$ was analyzed by Western blotting.

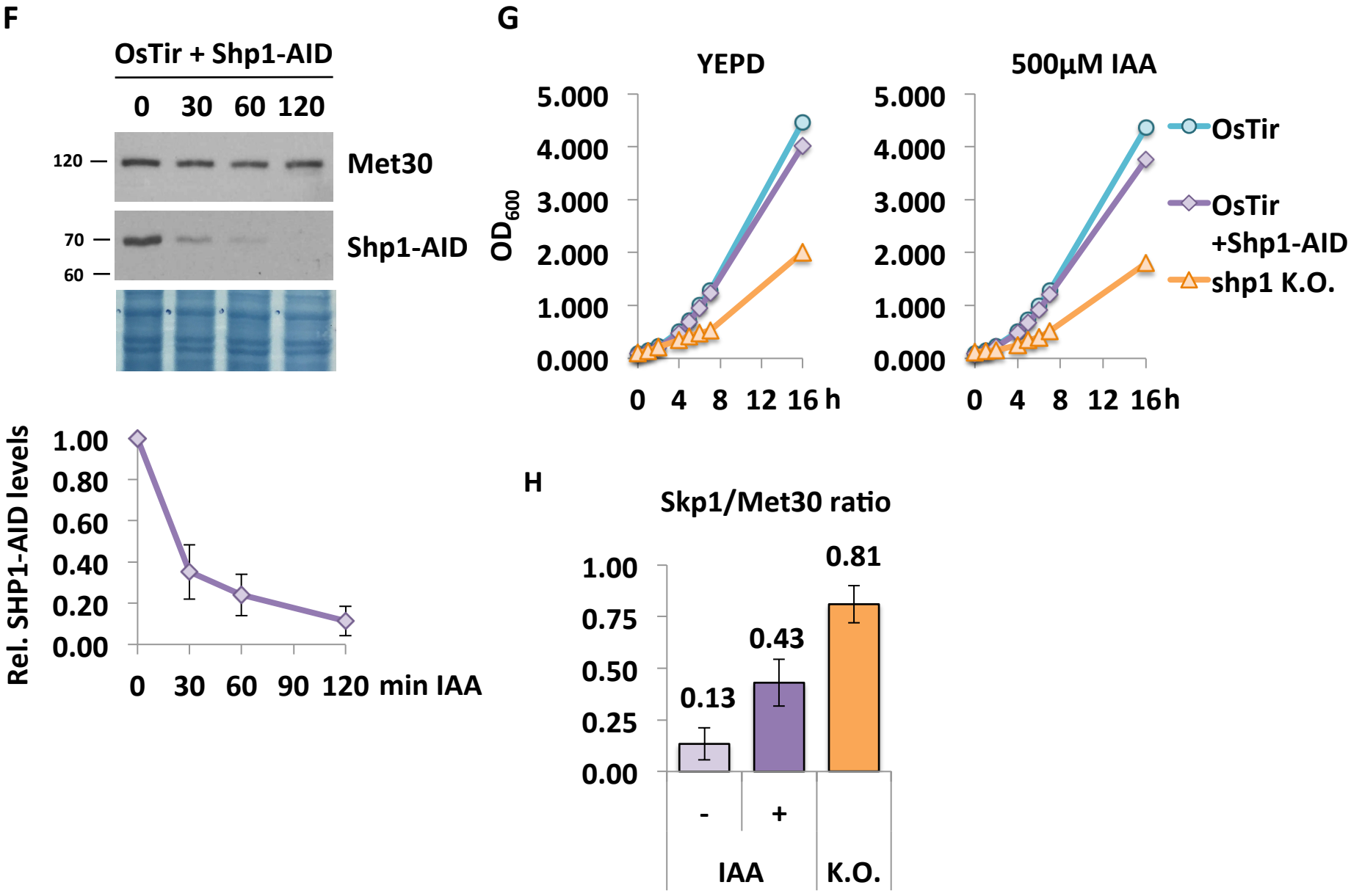
Supplementary Figure 6

A&B) Cdc48 co-factors Ufd1 and Npl4 are recruited to SCF^{Met30} during heavy metal stress. Strains expressing endogenous ^{12xMyc}Met30, Skp1, and $Ufd1^{3xHA}$ or $Npl4^{3xHA}$ respectively were cultured at 30°C in YEPD medium, treated with 100 µM $CdCl_2$, and samples were harvested at indicated time points. ^{12xMyc}Met30 was immunoprecipitated and co-precipitated proteins were analyzed by Western blotting. C) Cdc48 cofactors Npl4 and Ufd1 are involved in 'Skp1-free' Met30 degradation. Wild type, *npl4-1*, and *ufd1-2* temperature sensitive strains expressing ^{12myc}Met30ΔFbox were cultured at permissive temperature (25°C) and then shifted to non-permissive temperature (37°C) for 1.5 h. Cycloheximide (100 µg/ml) was added and samples were collected at the time intervals indicated. Met30ΔFbox protein stability was analyzed by immunoblotting with anti-myc antibodies. Tubulin was used as a loading control.

Suppl. Figure 1



Suppl. Figure 1



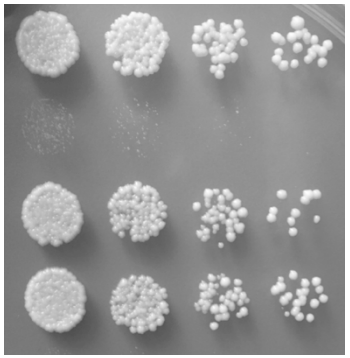
Suppl. Figure 2

A

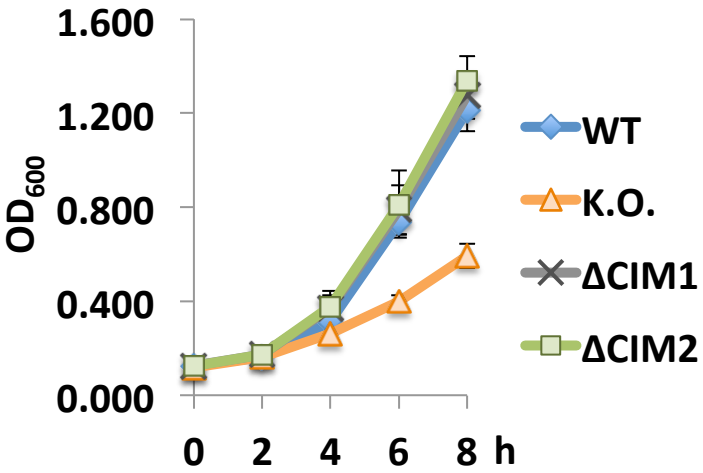
MAEIP**DETIQQFMALTNVSHNIAVQYLSEFGDLNEALNSYYASQTD**DQKDRREEAHWNRQQEKAL **UBA**
KQEAFSTNSSNKAINTEHVGGLCPKPGSSQGSNEYLKRKGSTSPEPTKGSSRSGSGNNSRFMSFSDMV
RGQADDDDEDQPRNTFAGGETSGLEVTDPSDPNSLLKDLLEKARRGGQMGAENGFRDDEDHEMGA
NRFTGRGFRLGSTIDAADEVVEDNTSQSQRRPEKVTREI**TFWKEGFQVADGPLYRYDDPANSFYLSEL** **SEP**
NQGRAPLKLLDVQFGQEVEVNVYKKLDESYKAPTRK***LGGFSGQGQRL***GSPIPGESSPAEVPKNETPA **BS1/SHP motif → CIM1**
AQEQPMPDNEPK**QGDTSIQIRYANGKREVLHCNSTDTVKFLYEHVTSNANTDPSRNFTLNYAFPIKP** **UBX** **FPI → CIM2**
ISNDETTLKDADLLNSVVVQRWA* (423 aa; 47kD) **Additional Cdc48 binding sites**

B

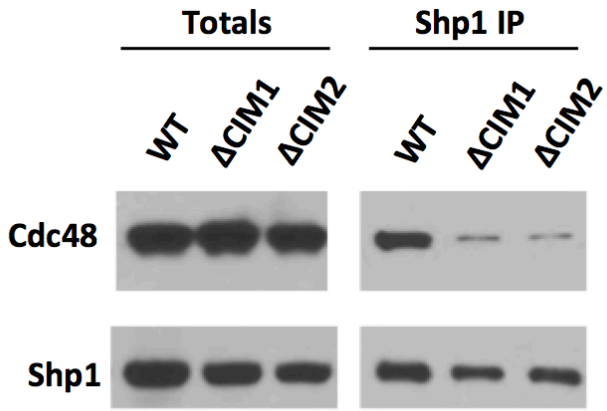
C



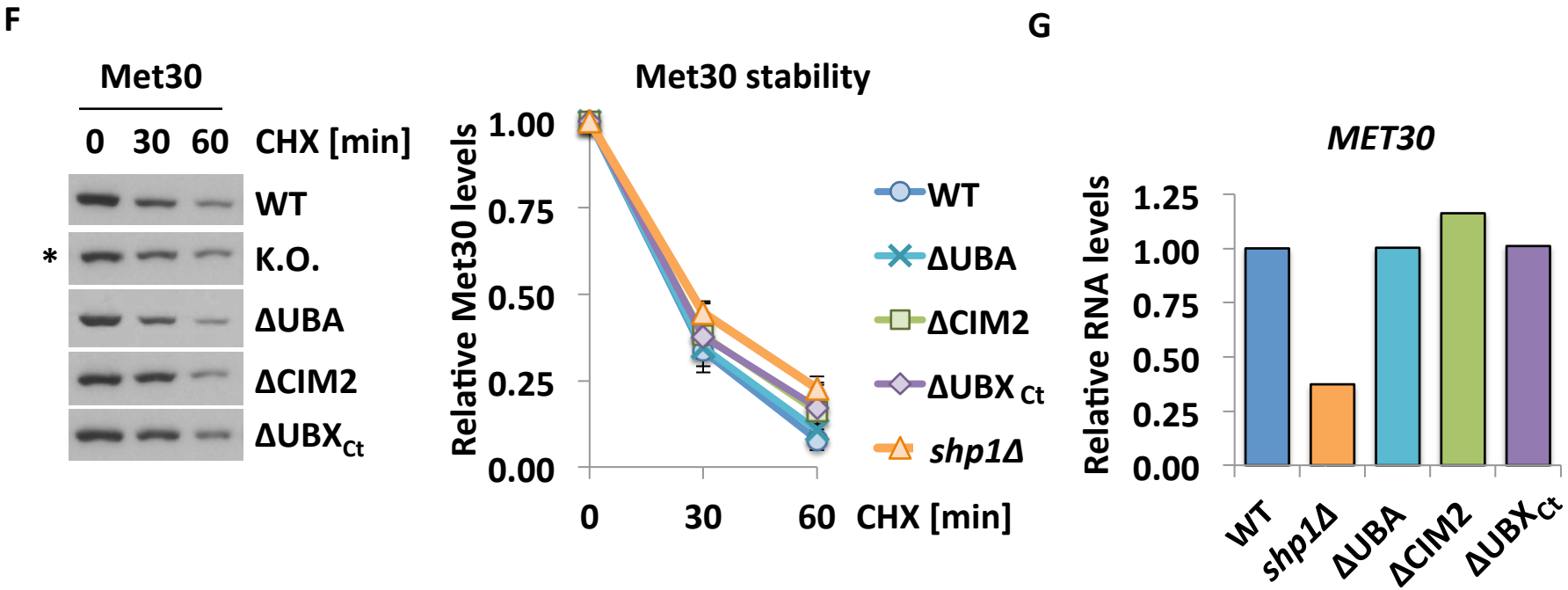
D



E

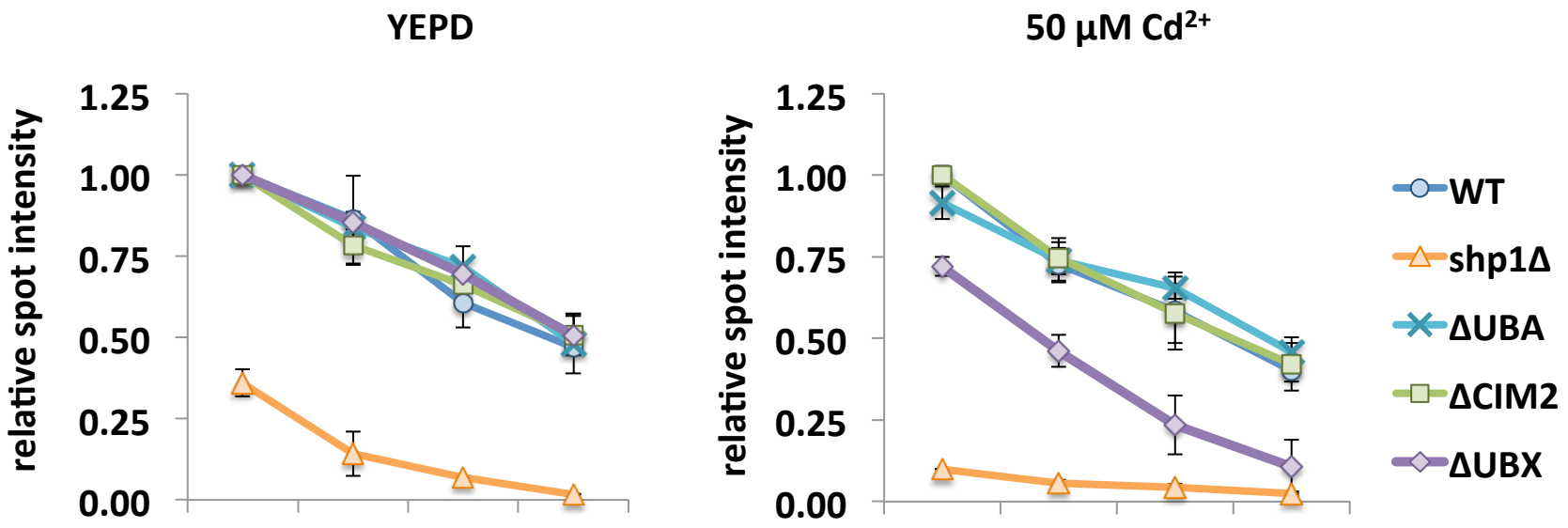


Suppl. Figure 2

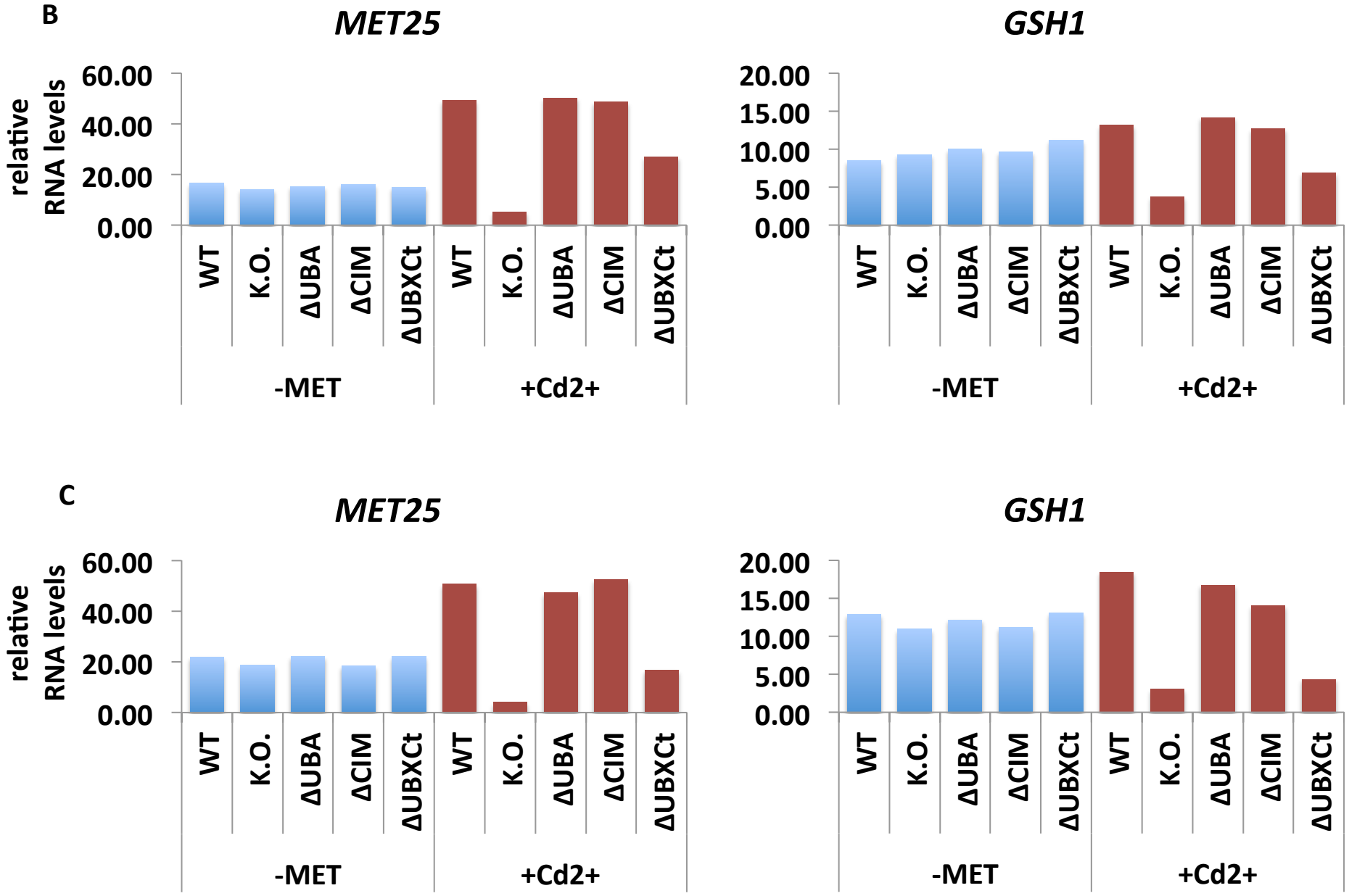


Suppl. Figure 3

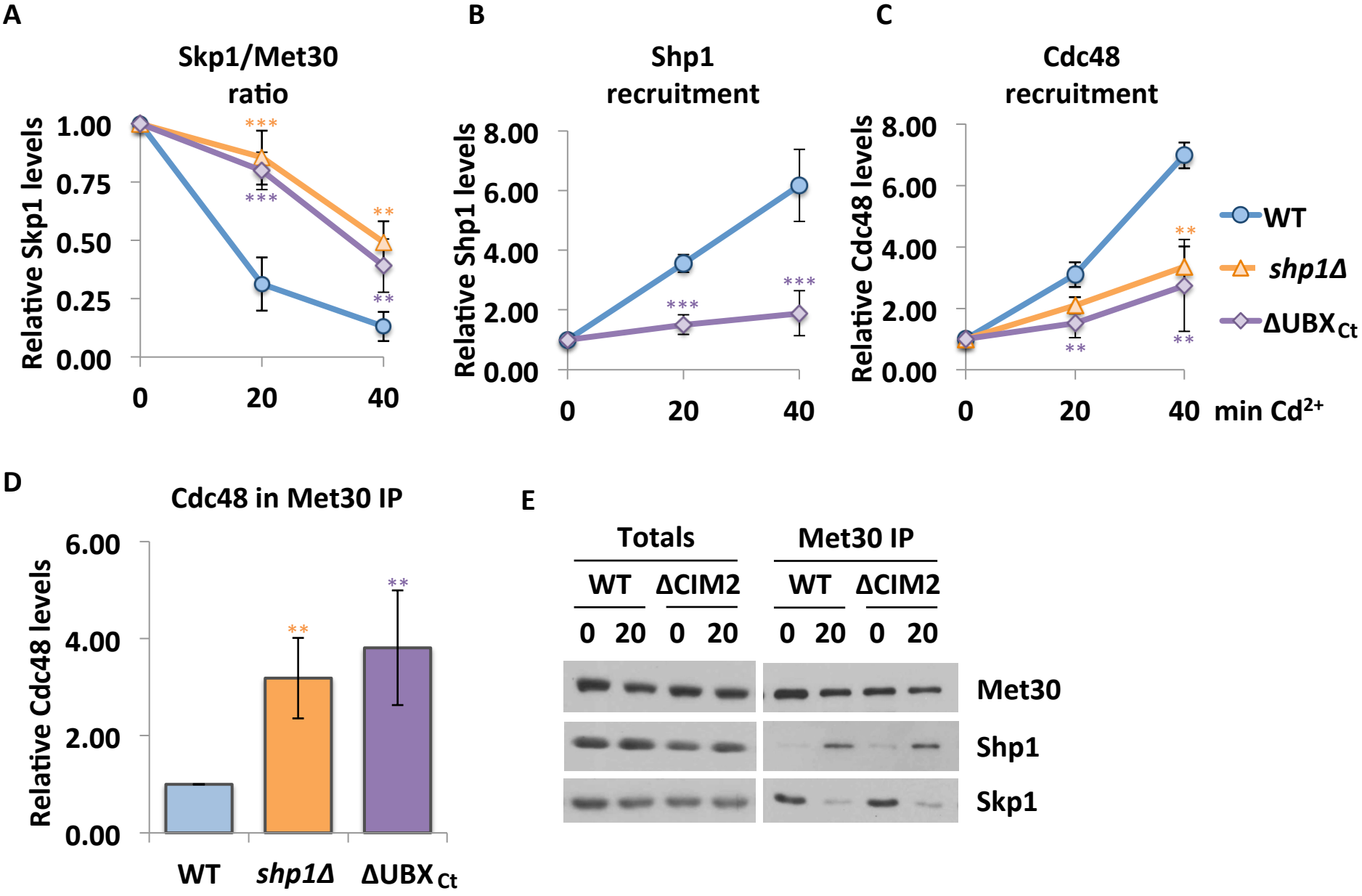
A



Suppl. Figure 3

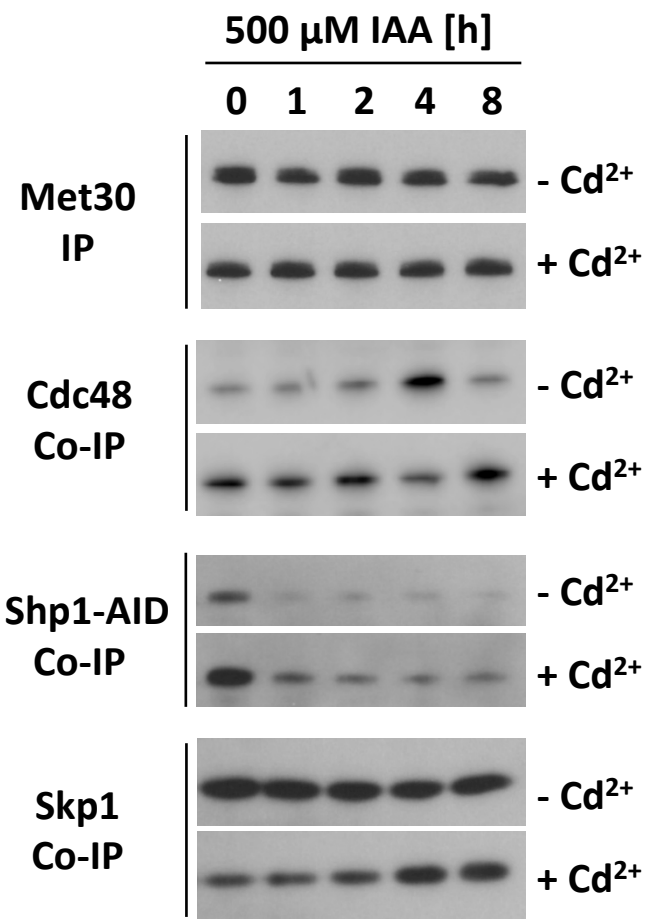


Suppl. Figure 4



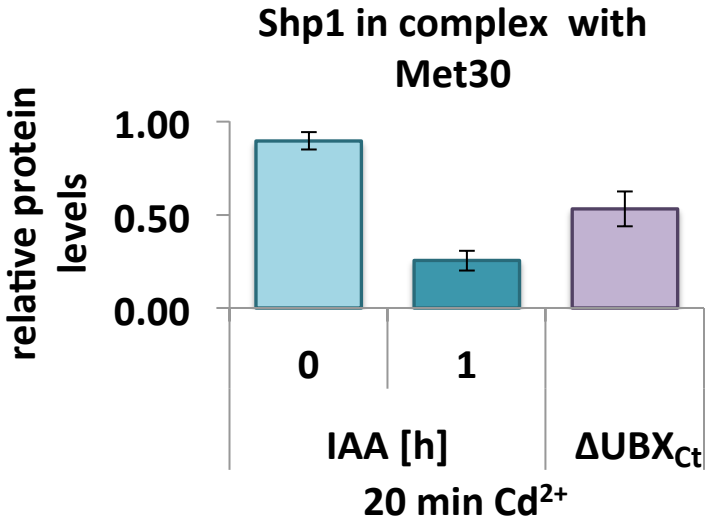
Suppl. Figure 4

F



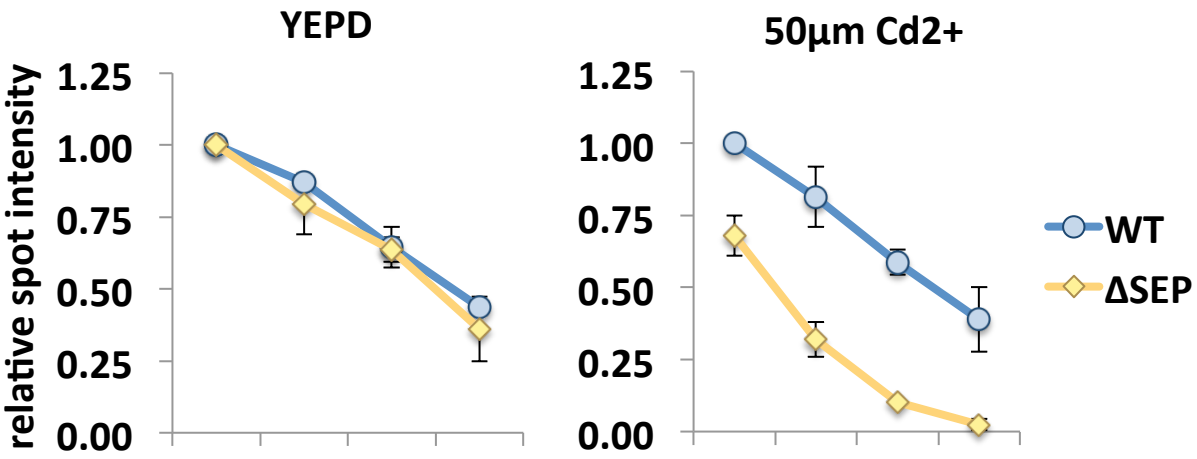
G

H

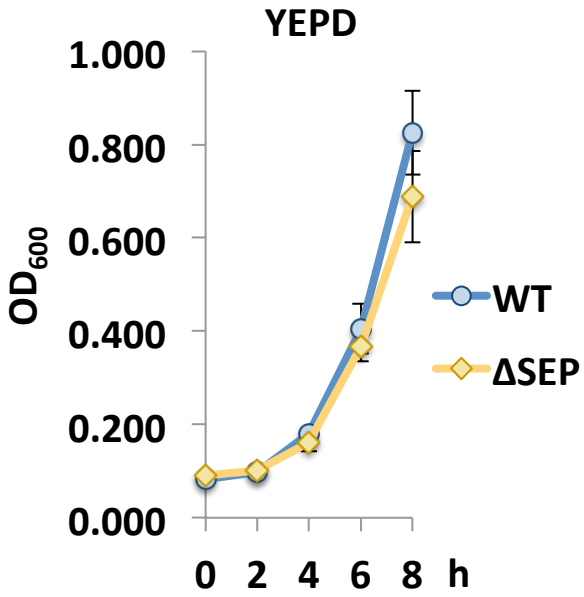


Suppl. Figure 5

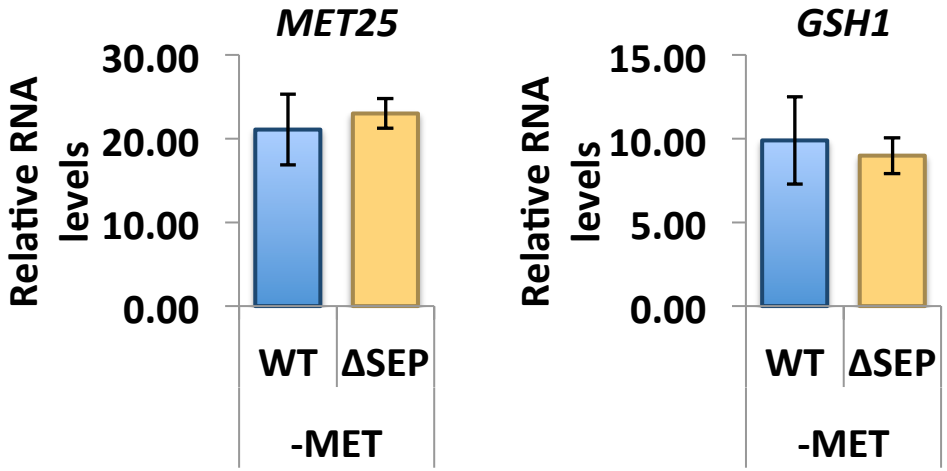
A



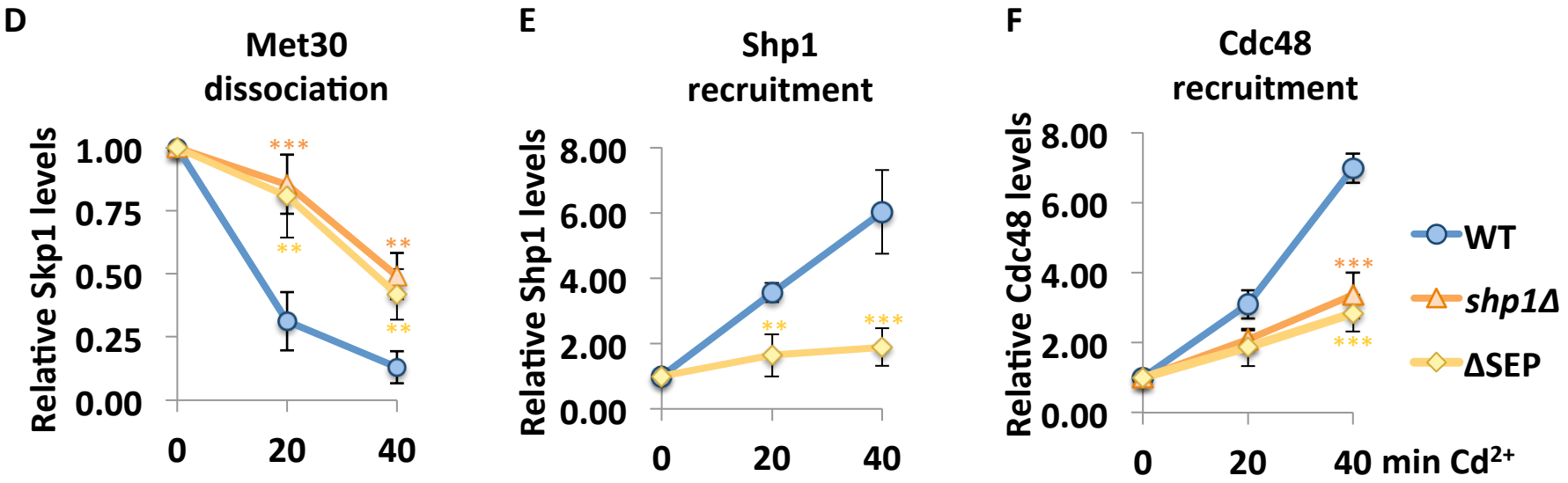
B

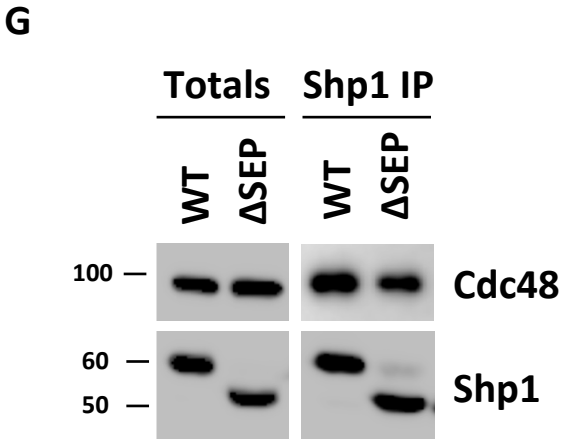


C



Suppl. Figure 5





Suppl. Figure 6

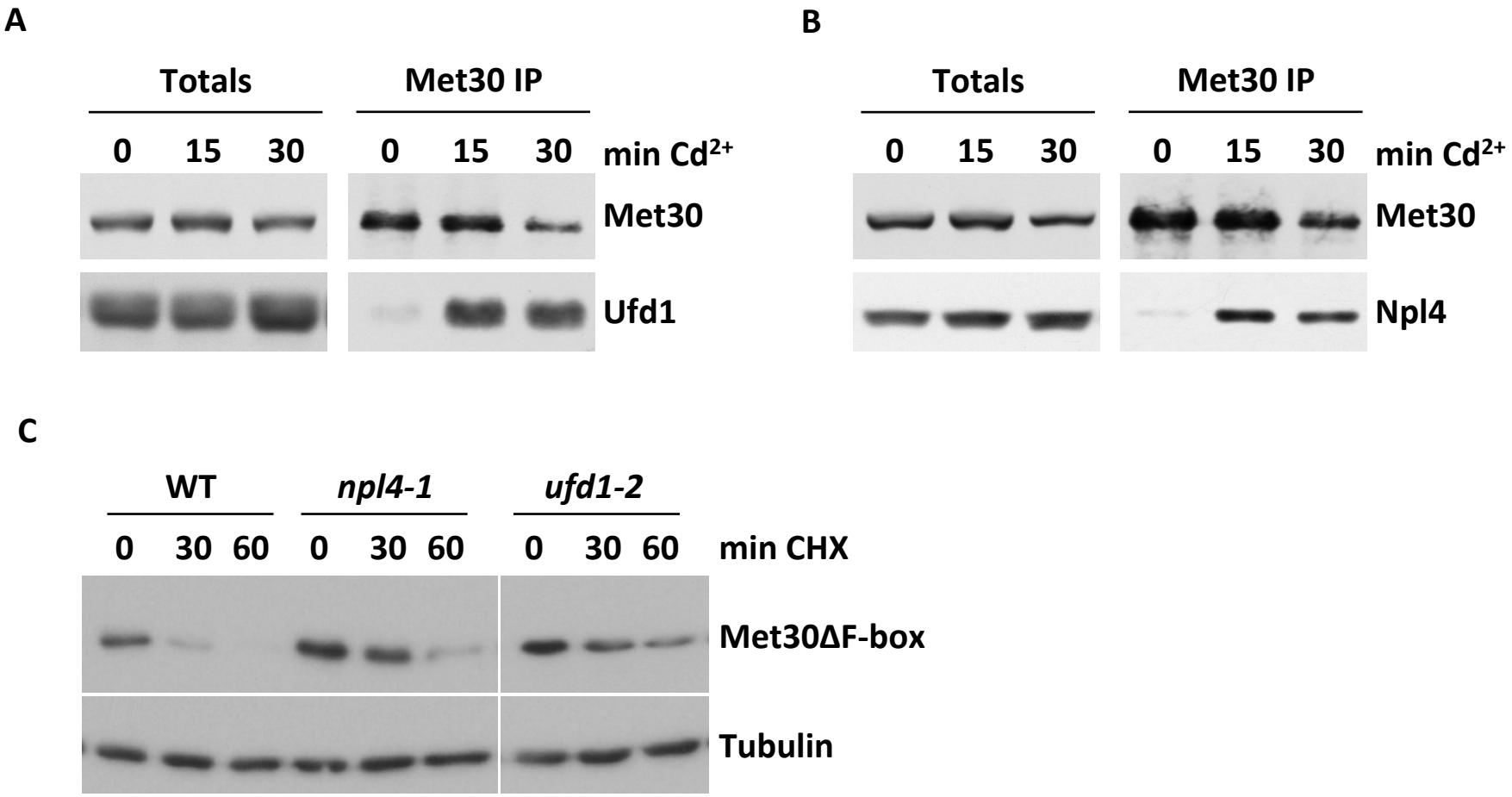


Table S1 - Yeast strains

Strain	relevant genotype	Source
15Daub	a bar1Δ ura3Δns, ade1 his2 leu2-3112 trp1-1	Reed et al., 1985; (1)
PY236	*pep4::URA3*	Kaiser et al., 2000; (2)
PY1073	*12mycMET30::ZEO pep4::URA3*	Yen et al.; 2012; (3)
PY1630	*CDC48RGS6xHis::KAN 12MycMET30::ZEO pep4::URA*	Yen et al.; 2012; (3)
PY1729	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp1::HYG pep4::URA*	Yen et al.; 2012; (3)
PY2230	*2xFLAGOsTir::URA Shp13xHA_AID::TRP YCpLEUprmet30_9xMYCMET30*	This Study
PY2231	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp13xHA::TRP pep4::URA*	This Study
PY2232	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp13xHA ∆UBA pep4::URA*	This Study
PY2234	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp13xHA ∆CIM1::TRP pep4::URA*	This Study
PY2235	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp13xHA ∆CIM2::TRP pep4::URA*	This Study
PY2236	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp13xHA ∆UBX::TRP pep4::URA*	This Study
PY2237	*CDC48RGS6xHis::KAN 12MycMET30::ZEO Shp13xHA ∆SEP::TRP pep4::URA*	This Study

Table S2 – Plasmids

Plasmid	relevant genotype	Source
pP491	*pFa6a3xHA::TRP*	Longtine et al., 1998 ;(4)
pP699	*YCpLEUpmet30_9xMYCMET30*	Flick et al., 2006; (5)
p1716	*pADH_OsTir9xMYC::URA*	Ulrich et al,; 2013; (6)
pP1216	*pADH_OsTir2xFLAG::URA*	This Study
p1736	pKAN-pCUP1-9xMYC-AID	Ulrich et al,; 2013; (6)
pP1217	pFa6a3xHA_AID::TRP	This Study
pML107	pSNR52sgRNA casette CAS9-gWY001 casette LEU2	Laughery et al.; 2015;(7)
pML107gShp1∆ UBA	pSNR52sgRNA shp1 -13 casette CAS9-gWY001 casette LEU2	This Study
pML107gShp1∆ CIM1	pSNR52sgRNA shp1 907 casette CAS9-gWY001 casette LEU2	This Study
pML107gShp1∆ CIM2	pSNR52sgRNA shp1 1184 casette CAS9-gWY001 casette LEU2	This Study
pML107gShp1∆ SEP	pSNR52sgRNA shp1 785 casette CAS9-gWY001 casette LEU3	This Study

Table S3 – Primers (Cloning and 90mer repair fragments for CRISPR)

No	Primer	Sequence	used for
1	OsTir-2xFLAG BamHI F	AAAggatccGACTACAAAGACGATGA TGACAAAGACTACAAAGACGATGA TGACAAATAAagatctcggccgccacc	pMK_2xFLAGOsTir
2	OsTir-2xFLAG BamHI R	TTTggatccagcaccTAGGATTTTAACA AAATTTGGTGCATCATCCC	
3	AID in pFa6a3xHA::TRP F	TACGCTGCTCAGTGCCCTAAAGAT CCAGCCAAACCTCC	pFa6a3xHA_AID::TRP
4	AID in pFa6a3xHA::TRP R	TAGAAGTGGCGCGCCTCAACCAGC TCCCAAGTCCTTAGATT	
5	shp1 -13 gRNA F	GAAAGATAAATGATCAATTAACTCA TTATTTAGGTAGTTTTAGAGCTAGA AATAGCAAGTTAAAA	pML107gShp1ΔUBA
6	shp1 -13 gRNA F	TTTTAACTTGCTATTTCTAGCTCTA AAACTACCTAAATAATGAGTTAATT GATCATTTATCTTTC	
9	shp1 907 gRNA F	GAAAGATAAATGATCACTGGGCGG TTTTTCAGGCCAGTTTTAGAGCTAG AAATAGCAAGTTAAAA	pML107gShp1ΔCIM1
10	shp1 907 gRNA R	TTTTAACTTGCTATTTCTAGCTCTA AAACTGGCCTGAAAAACCGCCCAG TGATCATTTATCTTTC	
11	shp1 1184 gRNA F	GAAAGATAAATGATCAGCTTATTGG TTTGATAGGAAGTTTTAGAGCTAGA AATAGCAAGTTAAAA	pML107gShp1ΔCIM2
12	shp1 1184 gRNA R	TTTTAACTTGCTATTTCTAGCTCTA AAACTTCCTATCAAACCAATAAGCT GATCATTTATCTTTC	
13	PK1126 SHP1 Cterm tagging 5´	CGCTGATCTG CTGAACTCCG TTGTCGTGCA AAGATGGGCA CGGATCCCCG GGTTAATTAA	SHP1-3xHA
14	PK1127 SHP1 Cterm tagging 3´	GTTGAAGTCT TTTCCCGTTT CTGTTTTTGT ATATTTATGC GAATTCGAGC TCGTTTAAAC	
15	PK1098 SHP1 KO 5'	AGAAACGTCG GTAGCACAAC AATTAACTCA TTATTTAGGT CGGATCCCCG GGTTAATTAA	K.O. repair
16	PK1099 SHP1 KO 3'	GTTGAAGTCT TTTCCCGTTT CTGTTTTTGT ATATTTATGC GAATTCGAGC TCGTTTAAAC	
17	Shp1ΔUBA F	TATATAAGAAACGTCGGTAGCACA ACAATTAACTCATTATTTATGAGAG AGGAAGCACATTGGAACAGACAGC AGGAGAAGGCCCTCAAG	UBA repair
18	Shp1ΔUBA R	CTTGAGGGCCTTCTCCTGCTGTCT GTTCCAATGTGCTTCCTCTCTCATA AATAATGAGTTAATTGTTGTGCTAC CGACGTTTCTTATATA	

21	Shp1ΔCIM1 F	tataaaaaattagatgagtcttataaagctccgacg agaaaaGCGGCCGCTggatctcctatcccg ggtgaatcgtcacctgcggaggttcca	CIM1 repair
22	Shp1ΔCIM1 R	tggaacctccgcaggtgacgattcacccgggata ggagatccAGCGGCCGCttttctcgtcggag ctttataagactcatctaatttttata	
23	Shp1ΔCIM2 F	gaacactgacccatcgaggaatttcaccttgaatta tgcTGGtGctGGTaaaccaataagcaacgat gagacaacattgaaggacgctg	CIM2 repair
24	Shp1ΔCIM2 R	cagcgtccttcaatgttgtctcatcgttgcttattggttt ACCagCaCCAgcataattcaaggtgaaattcc tcgatgggtcagtgttc	
25	ShpΔUBX F	tgacccatcgaggaatttcaccttgaattatgcTttt cctatcaaaCCAATAtAGCAACGATGA GACAACattgaaggacgctga	UBX repair
26	ShpΔUBX R	tcagcgtccttcaatGTTGTCTCATCGTTG CTaTATTGGtttgataggaaaAgcataattca aggtgaaattcctcgatgggtca	
27	shp1 785 gRNA F	GAAAGATAAATGATCAATTTGAGC GAGTTAAATCAAGTTTTAGAGCTAG AAATAGCAAGTTAAAA	pML107gShp1ΔSEP
28	shp1 785 gRNA R	TTTTAACTTGCTATTTCTAGCTCTA AAACTTGATTTAACTCGCTCAAATT GATCATTTATCTTTC	
29	Shp1ΔSEP F	TCACAATCACAACGTAGACCAGAA AAAGTCACAAGAGAAATTGCGGCC GCTCCGACGAGAAAACTGGGCGG TTTTTCAGGCCAGGGCCAAAGA	SEP repair
30	Shp1ΔSEP R	TCTTTGGCCCTGGCCTGAAAAACC GCCCAGTTTTCTCGTCGGAGCGGC CGCAATTTCTCTTGTGACTTTTTCT GGTCTACGTTGTGATTGTGA	

Supplementary References

1. S. I. Reed, J. A. Hadwiger, A. T. Lorincz, Protein kinase activity associated with the product of the yeast cell division cycle gene CDC28. *Proc. Natl. Acad. Sci. U. S. A.* (1985) https:/doi.org/10.1073/pnas.82.12.4055.

2. P. Kaiser, K. Flick, C. Wittenberg, S. I. Reed, Regulation of Transcription by Ubiquitination without Proteolysis. *Cell* **102**, 303–314 (2000).

3. J. L. L. Yen, *et al.*, Signal-Induced Disassembly of the SCF Ubiquitin Ligase Complex by Cdc48/p97. *Mol Cell* **48** (2012).

4. M. S. Longtine, *et al.*, Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast* (1998) https:/doi.org/10.1002/(SICI)1097-0061(199807)14:10<953::AID-YEA293>3.0.CO;2-U.

5. K. Flick, S. Raasi, H. Zhang, J. L. Yen, P. Kaiser, A ubiquitin-interacting motif protects polyubiquitinated Met4 from degradation by the 26S proteasome. *Nat. Cell Biol.* (2006) https:/doi.org/10.1038/ncb1402.

6. M. Morawska, H. D. Ulrich, An expanded tool kit for the auxin-inducible degr on system in budding yeast. **4**, 1–4.

7. M. F. Laughery, *et al.*, New vectors for simple and streamlined CRISPR-Cas9 genome editing in Saccharomyces cerevisiae. *Yeast* (2015) https:/doi.org/10.1002/yea.3098.