List of Supplemental Materials for Hickey et al. (2020)_*Genetics*

"Protein Quality Control Degron-containing Substrates are Differentially Targeted in the Cytoplasm and Nucleus by Ubiquitin Ligases"

*All supplemental materials except Supplemental Table 5 are within this single PDF.

Supplemental Figures:

Supplemental Figure 1. Supports Figure 1

Supplemental Figure 2. Supports Figure 1

Supplemental Figure 3. Supports Figure 2

Supplemental Figure 4. Supports Figure 9

Supplemental Figure 5. Supports Figure 11

Supplemental Tables:

Supplemental Table 1. Plasmids used in this study

Supplemental Table 2. Yeast strains used in this study

Supplemental Table 3. Targeted Ubiquitin System, version 2.1 (TUS2.1)

Supplemental Table 4. Targeted Ubiquitin System, version 2.0 (TUS2.0)

Supplemental Table 5. Excel file with raw data on human C-terminal 23-mers

Supplemental Figure Legends

Supplemental Figure 1. A screen for ubiquitylation machinery involved in targeting the *Deg2* degron revealed potential roles for Ubr1 and Dia2. (A) An updated targeted ubiquitin system (TUS) yeast gene deletion collection was compiled to identify specific ubiquitylation pathways in *Saccharomyces cerevisiae*. Each well of the 96-well plate contains a haploid yeast strain lacking the coding sequence of a single gene. See Supplemental Table 3 for organization of the strains in the plate. (B) Listed in this table are the TUS2.0 strains that grew better than a control strain (*trp1*Δ) on media lacking uracil in the primary YUMI screen. Growth scores are relative to one another and +++ growth was slower than expected for a strain with maximal Ura3 fusion protein stabilization. (C) Image and key for a single plate from the primary screen. This plate shows better growth of the *dia2*Δ strain than any other strains in column 10 of the TUS2.0.

Supplemental Figure 2. Combining deletion of *SLX8* with deletion of either *UBR1* or *DIA2* leads to robust growth based on the *Deg2*-Ura3-3HA reporter. (A) Schematic for mating-based generation of haploids lacking *SLX8* plus another gene and also carrying the *Deg2* reporter for testing by growth assay. (B) Growth assays for select strains in which each strain lacked the *SLX8* gene in addition to a gene identified in the original screen, as indicated. Genes deleted that are not listed in Supplemental Figure 1 were those whose enhanced growth (compared to the control strain) in the primary screen was questionable. An apparent slightly better growth for any strain in the primary screen could have been due to a relatively heavy streak of the yeast by Christopher Hickey. Relatively slow growth of the *slx8*Δ *doa4*Δ strain compared to either the *slx8*Δ *ubr1*Δ or *slx8*Δ *dia2*Δ strain suggests that combining loss of the Slx5/Slx8 activity with general defects in

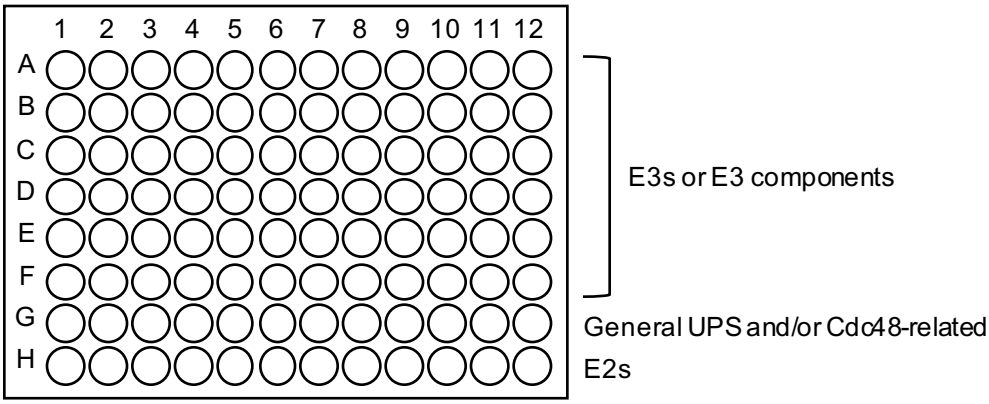
ubiquitin homeostasis (also known to be a phenotype of *doa1*Δ or *ubx1*Δ strains) does not lead to additive effects on the stabilization of the *Deg2* reporter.

Supplemental Figure 3. Additional data on degradation of MATa1 and degrons derived from MATa1. (A) The N-terminal half of MATa1 is not stabilized in cells lacking Doa10. Immunoblot data following cycloheximide-chase in MHY500 (*WT*) or MHY1685 (*doa10*Δ) cells carrying a plasmid expressing the MATa1(1-68)-Ura3-3HA protein. (B) MATa1-Ura3-3HA is targeted by Ubr1 and an E3 or E3s operating with Ubc4/Ubc5. Immunoblot data following cycloheximide-chase in MHY500 (*WT*), MHY9550 (*ubc4*Δ *ubc5*Δ *ubr1*Δ + UBC4), MHY9552 (*ubc4*Δ *ubc5*Δ + ubc4-N78S), or MHY9554 (*ubc4*Δ *ubc5*Δ *ubr1*Δ + ubc4-N78S) cells carrying a plasmid expressing the MATa1-Ura3-3HA protein.

Supplemental Figure 4. Comparing the reporter proteins made in the current study terminating with VVLVVVF with the reporter protein from Maurer et al. also terminating in VVLVVF and called “10-34”. (A) Amino acid sequence shown is that following the matched NLS- and m.nls-reporter proteins in the current study, as described in Figure 4. Growth assays on media lacking either leucine or uracil for the indicated strains expressing the indicated reporter protein. (B) Amino acid sequence shown is that following the Ura3 protein in the Ura3-10-34 degron reporter from Maurer et al. In red are the 3 HA epitopes. Any of the amino acids upstream of VVLVVVF could contribute to overall degron function. Growth assays on media lacking either leucine or uracil for the indicated strains expressing the original Ura3-10-34 protein. Strains used in this figure are BY4741 (WT), MHY7665 (*ubr1*Δ), MHY11286 (*san1*Δ), and MHY6780 (*ltn1*Δ).

Supplemental Figure 5. Additional figures for bioinformatic analysis of the human C-terminal 23-mers from human proteins that were fused to GFP in Koren et al. (A) There is little correlation between theoretical isoelectric point of the 23 amino acid peptides and the protein stability index (PSI) determined for the corresponding GFP-23mer. (B) Displayed are the percentages of peptides that end in the indicated amino acid (single letter code) in three peptide sets: [1] the entire collection of 16,345 peptides, [2] a set of peptides that yielded PSI scores of 1.5 or lower and had relatively low hydrophobicity, and [3] a set of peptides that yielded PSI scores less than 2 and had very low hydrophobicity. A dashed line at 5% represents the theoretical percentage if each of the 20 amino acids were used equally. See Supplemental Table 5 for all data on hydrophobicity.

A.

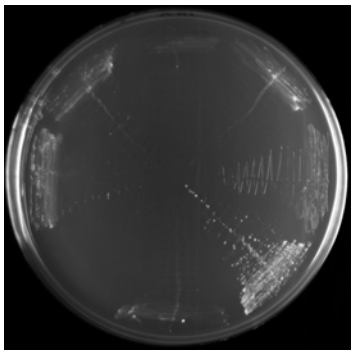


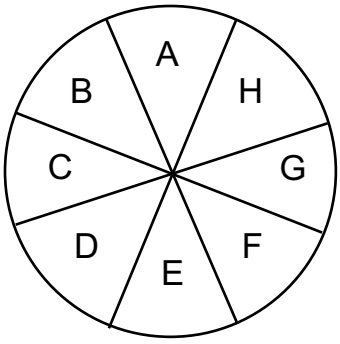
B.

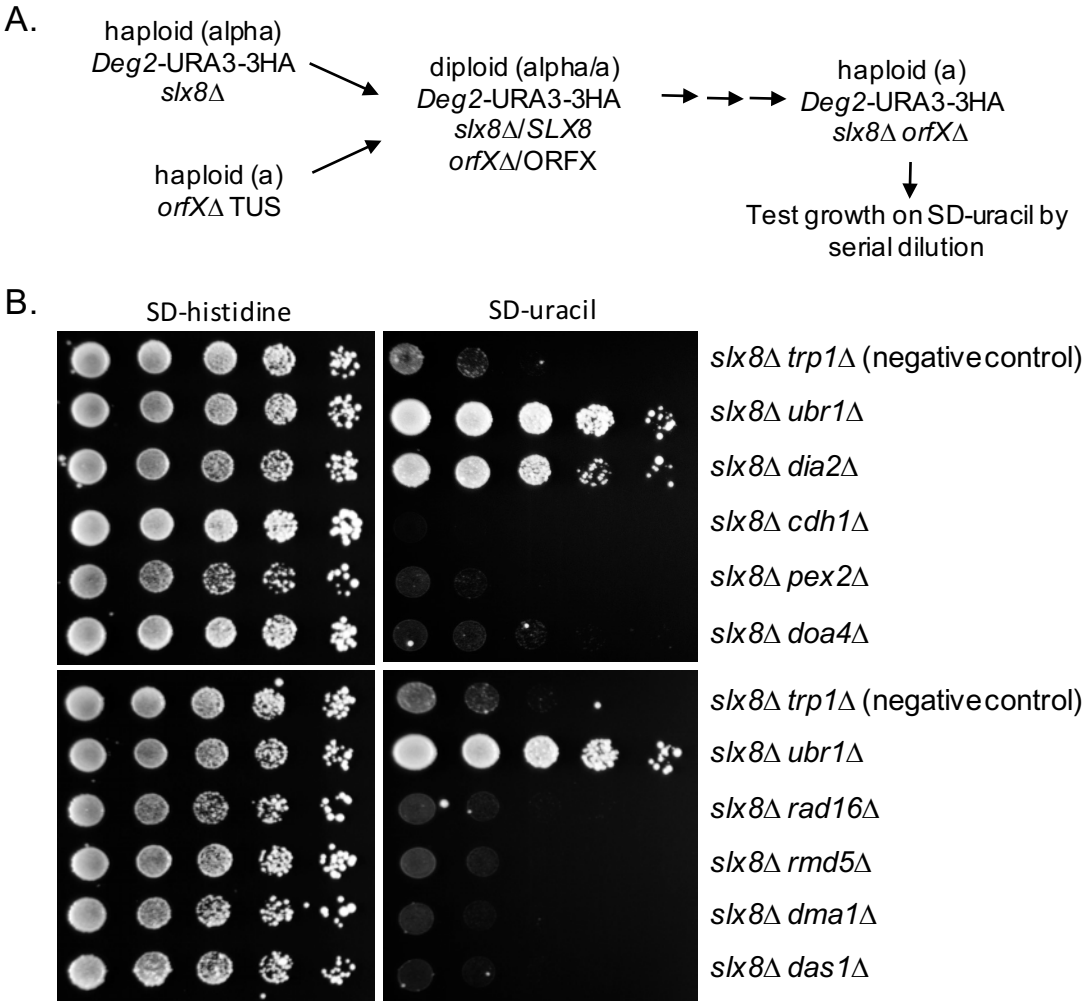
Well	Gene deleted	Growth Score	Comments
A9	*SLX5*	+	Slx5/8 is known E3 for *Deg2* and MATα2
C1	*SLX8*	++	Slx5/8 is known E3 for *Deg2* and MATα2
C6	*UBR1*	++	E3 for the N-end pathway and PQC
F10	*DIA2*	+++	Known to affect transcription generally
G5	*DOA4*	++	DUB; deletion affects UPS generally
G6	*UBX1*	++	Deletion affects UPS generally
H1	*DOA1*	+++	Deletion affects UPS generally
H2	*UBC2*	++	E2; operates with the E3 Ubr1
H3	*UBC4*	++	E2; known E2 for *Deg2* and MATα2

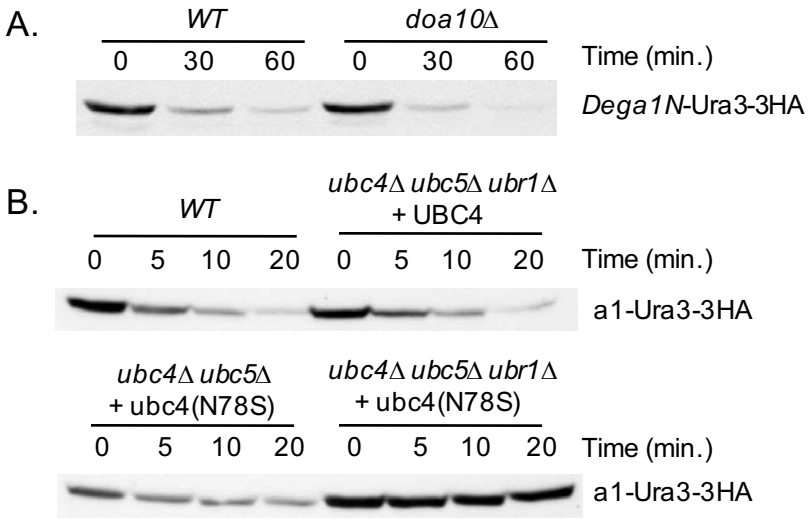
C.

TUS2.0, column 10
A. *bre1*Δ
B. *tom1*Δ
C. *fyv10*Δ
D. No prey strain
E. *asi1*Δ
F. *dia2*Δ
G. *ubx5*Δ
H. *ubc12*Δ

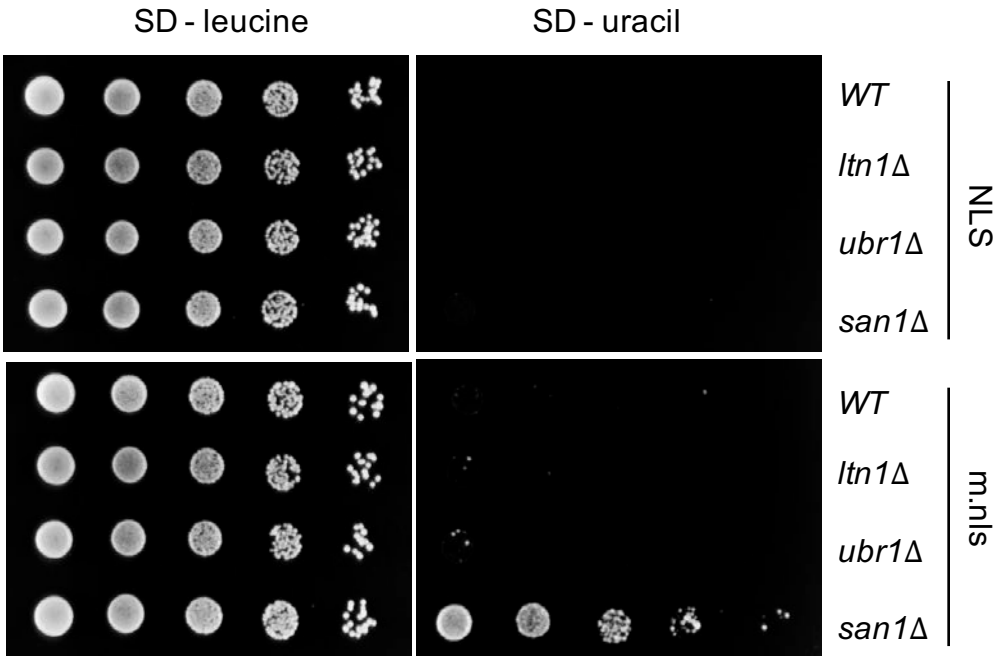




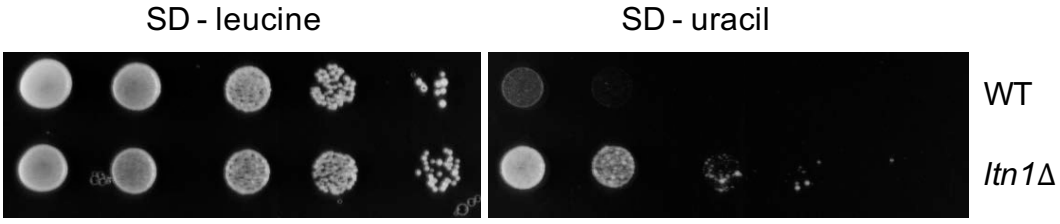

A.
haploid (alpha)
Deg2-URA3-3HA
slx8Δ
haploid (a)
orfXΔ TUS
diploid (alpha/a)
Deg2-URA3-3HA
slx8Δ/SLX8
orfXΔ/ORFX
haploid (a)
Deg2-URA3-3HA
slx8Δ orfXΔ
Test growth on SD-uracil by serial dilution
B.
SD-histidine
SD-uracil
slx8Δ trp1Δ (negative control)
slx8Δ ubr1Δ
slx8Δ dia2Δ
slx8Δ cdh1Δ
slx8Δ pex2Δ
slx8Δ doa4Δ
slx8Δ trp1Δ (negative control)
slx8Δ ubr1Δ
slx8Δ rad16Δ
slx8Δ rmd5Δ
slx8Δ dma1Δ
slx8Δ das1Δ


A.
WT
doa10Δ
0 30 60 0 30 60
Time (min.)
Dega1N-Ura3-3HA
B.
WT
ubc4Δ ubc5Δ ubr1Δ + UBC4
0 5 10 20 0 5 10 20
Time (min.)
a1-Ura3-3HA
ubc4Δ ubc5Δ + ubc4(N78S)
ubc4Δ ubc5Δ ubr1Δ + ubc4(N78S)
0 5 10 20 0 5 10 20
Time (min.)
a1-Ura3-3HA

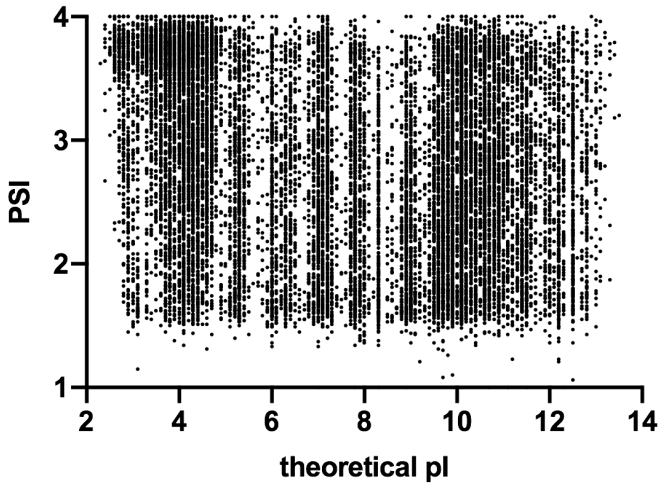
A. YPYDVPDYAGSVVLVVVF



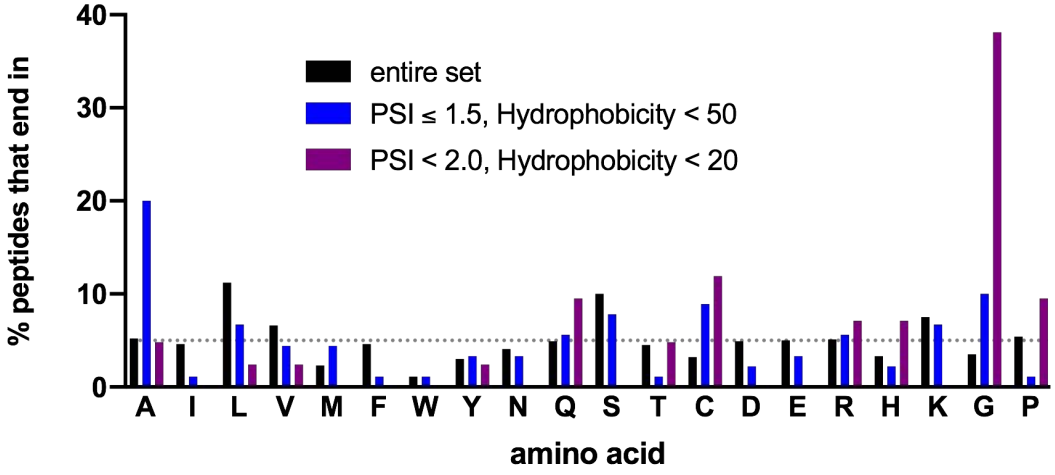
B. QNDLGRIFYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAAQCGPDPVVLVVVF



A.


PSI
theoretical pI
4
3
2
1
2
4
6
8
10
12
14

B.


% peptides that end in
amino acid
entire set
PSI ≤ 1.5, Hydrophobicity < 50
PSI < 2.0, Hydrophobicity < 20
40
30
20
10
0
A I L V M F W Y N Q S T C D E R H K G P

Supplemental Table 1. Plasmids used in this study.

Plasmid	Source
p415MET25promoter-α2(103-189)-URA3-3HA-6His	Hickey et al., 2015
p414MET25promoter-α2(103-189)-URA3-3HA-6His	Hickey et al., 2015
pAG32-MET25promoter-α2(103-189)-URA3-3HA-6His-CYC1TT	This study
p415MET25promoter-a1(cDNA)-URA3-3HA-6His	This study
p415TEF1promoter-a1(cDNA)-URA3-3HA-6His	This study
p415MET25promoter-a1(cDNA; 1-68; Y34Y)-URA3-3HA-6His	This study
p415MET25promoter-a1(cDNA; 69-126)-URA3-3HA-6His	This study
p414MET25promoter-a1(cDNA; 69-126)-URA3-3HA-6His	This study
pRS306-UBC4promoter-UBC4	Stoll et al., 2011
pRS306-UBC4promoter-UBC4(N78S)	Stoll et al., 2011
p415MET25promoter-URA3-HA-MCS	This study
p415MET25promoter-URA3-HA-a1(20-40)	This study
p415MET25promoter-URA3-HA-DegOOF	This study
pRS313-RFP-PUS1	Han et al., 2007
p415MET25promoter-NLS-GFP-URA3-HA-MCS	This study
p415MET25promoter-mutant.nls-GFP-URA3-HA-MCS	This study
p415MET25promoter-NLS-GFP-URA3-HA-STOP	This study
p415MET25promoter-mutant.nls-GFP-URA3-HA-STOP	This study
p415MET25promoter-NLS-GFP-URA3-HA-DegOOF	This study
p415MET25promoter-mutant.nls-GFP-URA3-HA-DegOOF	This study
p415MET25promoter-NLS-GFP-URA3-HA-CL1	This study
p415MET25promoter-mutant.nls-GFP-URA3-HA-CL1	This study
p415MET25promoter-NLS-GFP-URA3-HA-DegF7H	This study
p415MET25promoter-mutant.nls-GFP-URA3-DegF7H	This study
p415MET25promoter-NLS-GFP-URA3-HA-pentaV	This study
p415MET25promoter-mutant.nls-GFP-URA3-pentaV	This study
p415MET25promoter-NLS-GFP-URA3-HA-VVLVVVF	This study
p415MET25promoter-mutant.nls-GFP-URA3-HA-VVLVVVF	This study
pSM2720 = p415MET25promoter-URA3-3HA-“10-34”	Maurer et al., 2016
pcDNA5-FRT/TO-NLS-eGFP-HA-MCS	This study
pcDNA5-FRT/TO-mutant.nls-eGFP-HA-MCS	This study
pcDNA5-FRT/TO-NLS-eGFP-HA-STOP	This study
pcDNA5-FRT/TO-mutant.nls-eGFP-HA-STOP	This study
pcDNA5-FRT/TO-NLS-eGFP-HA-DegF7H	This study
pcDNA5-FRT/TO-mutant.nls-eGFP-HA-DegF7H	This study

Notes: HA = epitope tag; TT = transcriptional terminator; MCS = multiple cloning site; NLS = nuclear localization signal; STOP = stop codon.

Supplemental Table 2. Yeast strains used in this study.

Strain	Genotype	Source
MHY2994	*MATα his3*Δ1 *leu2Δ0, met15Δ0, ura3Δ0 lyp1*Δ *can1*Δ*:MFA1pr-HIS3*	Tong et al., 2001.
MHY9368	*MATα his3*Δ1 *leu2Δ0, met15Δ0, ura3Δ0 lyp1*Δ *can1*Δ*::MFA1pr-HIS3 Deg2-Ura3-3HA::hphMX*	This study
MHY9369	*MATα his3*Δ1 *leu2Δ0, met15Δ0, ura3Δ0 lyp1*Δ *can1*Δ*::MFA1pr-HIS3 slx8*Δ*::natMX Deg2-Ura3-3HA::hphMX*	This study
MHY501	*MATα his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1*	Chen et al., 1993
MHY500	*MATa his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1*	Chen et al., 1993
MHY606	Diploid equivalent of MHY500 and MHY501	Chen et al., 1993
MHY3716	*MATα his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 slx8*Δ*::kanMX4*	Xie et al., 2010
MHY474	*MATα his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1*Δ*::LEU2*	Bartel et al., 1990
MHY9394	*MATα his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1*Δ*::LEU2 slx8*Δ*::kanMX4*	This study
MHY3718	*MATα his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 slx8*Δ*::kanMX4 doa10*Δ*::HIS3*	Xie et al., 2010
MHY9396	*MATα his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 slx8*Δ*::kanMX4 doa10*Δ*::HIS3 ubr1*Δ*::LEU2*	This study
MHY2836	*MATa his3-*Δ*200 leu2-*Δ*1 ura3-52 lys2-801 trp1-*Δ*63 ade2-101*	Ghislain et al., 1993
MHY4464	*MATa his3-*Δ*200 leu2-*Δ*1 ura3-52 lys2-801 trp1-*Δ*63 ade2-101 cim3-1*	Ghislain et al., 1993
MHY9380	*MATa his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1*Δ*::LEU2*	This study
MHY513	*MATa his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubc4*Δ*::HIS3*	Chen et al., 1993
MHY9496	*MATa his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1*Δ*::LEU2 ubc4*Δ*::HIS3*	This study
MHY9384	*MATa his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1*Δ*::LEU2 ubc4*Δ*::HIS3 doa10*Δ*::HIS3*	This study
BY4741	*MAT**a** his3*Δ1 *leu2Δ0, met15Δ0, ura3Δ0*	Brachmann et al., 1998
MHY7665	*MAT**a** his3*Δ1 *leu2Δ0, met15Δ0, ura3Δ0 ubr1*Δ*::kanMX4*	Deletion library
MHY1685	*MATa his3-*Δ*200 leu2-3,112 ura3-52 lys2-801 trp1-1 doa10*Δ*::HIS3*	Swanson et al., 2001

MHY9550	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubc4Δ::HIS3 ubc5Δ::LEU2 ubr1Δ::natMX pRS306-UBC4 (integrated)*	This study
MHY9552	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubc4Δ::HIS3 ubc5Δ::LEU2 pRS306-ubc4-N78S (integrated)*	This study
MHY9554	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubc4Δ::HIS3 ubc5Δ::LEU2 ubr1Δ::natMX pRS306-ubc4-N78S (integrated)*	This study
MHY4995	*MATa ade2-1 his3-11,15 leu2-3,112 ura3-1 trp1-1 can1-100 pRS313-UBA1*	Ghaboosi et al., 2007
MHY4996	*MATa ade2-1 his3-11,15 leu2-3,112 ura3-1 trp1-1 can1-100 pRS313-uba1-204*	Ghaboosi et al., 2007
MHY6197	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 doa10Δ::hphMX4*	This study
MHY6199	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 hrd1Δ::kanMX4*	This study
MHY6194	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 hrd1Δ::kanMX4 doa10Δ::hphMX4*	This study
MHY8940	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 asi1Δ::kanMX6*	This study
MHY8949	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 asi1Δ::hphMX doa10Δ::HIS3*	This study
MHY1631	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 doa10Δ::HIS3*	Swanson et al., 2001
MHY9532	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1Δ::natMX*	This study
MHY3178	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 san1Δ::kanMX*	This study
MHY9564	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 ubr1Δ::natMX doa10Δ::HIS3*	This study
MHY3208	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 san1Δ::kanMX doa10Δ::HIS3*	This study
MHY9563	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 san1Δ::kanMX ubr1Δ::natMX*	This study
MHY9567	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 san1Δ::kanMX ubr1Δ::natMX doa10Δ::HIS3*	This study
MHY6780	*MATa his3Δ1 leu2Δ0, met15Δ0, ura3Δ0 ltn1Δ::kanMX4*	Deletion library

Hickey et al._*Genetics*_Supplemental Table 3
Targeted Ubiquitin System (TUS) library, version 2.1

all strains are mating type a
the background (wild type) strain is called BY4741 = his3Δ1 leu2Δ0 ura3Δ0 met15Δ0

POSITION	ORF	NAME	Motif	Notes
A1	YAL002W	VPS8	RING	
A2	YBL104C	SEA4	RING	
A3	YBR062C	YBR062C	RING	
A4	YBR114W	RAD16	RING	
A5	YBR158W	AMN1	F box	
A6	YBR203W	COS111	F box	
A7	YBR280C	SAF1	F box	
A8	YCR066W	RAD18	RING	
A9	YDL013W	SLX5	RING	
A10	YDL074C	BRE1	RING	
A11	YDL190C	UFD2	U-box	
A12	YDR049W	VMS1	zinc finger, C2H2	peptidyl-RNA hydrolase
B1	YDR103W	STE5	unlikely an E3	mating deficient strain
B2	YDR131C	YDR131C	F box	
B3	YDR143C	SAN1	RING	
B4	YDR219C	MFB1	F box	
B5	YDR255C	RMD5	RING	
B6	YDR265W	PEX10	RING	
B7	YDR266C	HEL2	RING	
B8	YDR306C	PFU1	F box	
B9	YDR313C	PIB1	RING	
B10	YDR457W	TOM1	HECT	
B11	*empty*	*empty*	*empty*	
B12	YER068W	MOT2/NOT4	RING	
C1	YER116C	SLX8	RING	
C2	YGL003C	CDH1	APC/C activator	
C3	YGL131C	SNT2	RING	
C4	YGL141W	HUL5	HECT	
C5	YGR003W	CUL3	CULLIN	
C6	YGR184C	UBR1	RING	
C7	YHL010C	ETP1	RING	
C8	YHR115C	DMA1	RING	
C9	YIL030C	SSM4/DOA10	RING	
C10	YIL097W	FYV10	degenerate RING	strain incorrect?
C11	YJL047C	RTT101	CULLIN	
C12	YJL149W	DAS1	F box	
D1	YJL157C	FAR1	RING	
D2	YJL204C	RCY1	F box	
D3	YJL210W	PEX2	RING	
D4	YJR036C	HUL4	HECT	
D5	YJR052W	RAD7	F box	
D6	YJR090C	GRR1	F box	slow growth; flocculant

D7	YKL010C	UFD4	HECT	
D8	YKL034W	TUL1	RING	
D9	YKR017C	HEL1	RING	
D10	YLR024C	UBR2	RING	made by Hickey
D11	YLR032W	RAD5	RING	
D12	YLR097C	HRT3	F box	
E1	YLR148W	PEP3/VPS18	RING	
E2	YLR224W	UCC1	F box	
E3	YLR247C	IRC20	RING	
E4	YLR352W	LUG1	F box	
E5	YLR368W	MDM30	F box	
E6	YLR427W	MAG2	RING	
E7	YML068W	ITT1	RING	
E8	YML088W	UFO1	F box	slow growth
E9	YMR026C	PEX12	RING	
E10	YMR119W	ASI1	RING	
E11	YMR231W	PEP5/VPS11	RING	
E12	YMR247C	RKR1/LTN1	RING	
F1	YMR258C	ROY1	F box	
F2	YNL008C	ASI3	RING	
F3	YNL023C	FAP1	RING	
F4	YNL116W	DMA2	RING	
F5	YNL230C	ELA1	F box	
F6	YNL311C	SKP2	F box	
F7	YOL013C	HRD1	RING	
F8	YOL054W	PSH1	RING	
F9	YOL138C	RTC1	RING	
F10	YOR080W	DIA2	F box	
F11	YOR191W	ULS1	RING	
F12	YPR093C	ASR1	RING	
G1	YDR069C	DOA4	deubiquitylase	slow growth
G2	YGR135W	PRE9	proteasome subunit α3	slow growth
G3	YDL020C	RPN4	transcription factor	
G4	YKL213C	DOA1	UPS- and Cdc48-related	slow growth
G5	YNL155W	CUZ1	UPS- and Cdc48-related	
G6	YBL058W	SHP1/UBX1	UBX domain	slow growth
G7	YML013W	UBX2	UBX domain	
G8	YDL091C	UBX3	UBX domain	
G9	YMR067C	UBX4	UBX domain	
G10	YDR330W	UBX5	UBX domain	
G11	YJL048C	UBX6	UBX domain	
G12	YBR273C	UBX7	UBX domain	
H1	YDR177W	UBC1	E2	slow growth
H2	YGL058W	UBC2	E2	slow growth
H3	YBR082C	UBC4	E2	
H4	YDR059C	UBC5	E2	
H5	YER100W	UBC6	E2	made by Hickey
H6	YMR022W	UBC7	E2	
H7	YEL012W	UBC8	E2	

H8	YGR133W	UBC10	E2
H9	YOR339C	UBC11	E2
H10	YLR306W	UBC12	E2
H11	YDR092W	UBC13	E2
H12	YOR153W	PDR5	"Wild Type" for UPS

KEY:

Yellow = known or likely E3s, or parts of E3 complexes.

Green = General UPS and/or Cdc48-associated factors

Blue = ubiquitin-conjugating enzymes (E2s)

E2 and E3 genes NOT covered by TUS2.1 (deletion is inviable, unless noted)

ORF	NAME	Motif	Notes
YER125W	RSP5	HECT	
YLL036C	PRP19	U-box	
YGL116W	CDC20	APC/C activator	
YDL008W	APC11	RING	APC/C RING protein
YOL133W	HRT1	RING	RING for SCFs
YDR460W	TFB3	RING	
YLR323C	CWC24	RING	spliceosome
YDR328C	SKP1	SCF Skp1 subunit	
YDL132W	CDC53	Cullin	
YFL009W	CDC4	F box	
YIL046W	MET30	F box	
YMR094W	CTF13	F box	
YPL046C	ELC1	Elongin C	the deletion is viable
YDR054C	UBC3/CDC34	E2	E2 for SCFs

Hickey et al._*Genetics*_Supplemental Table 4
Targeted Ubiquitin System (TUS) library, version 2.0

all strains are mating type a
the background (wild type) strain is called BY4741 = his3Δ1 leu2Δ0 ura3Δ0 met15Δ0

POSITION	ORF	NAME	Motif	Notes
A1	YAL002W	VPS8	RING	
A2	YBL104C	SEA4	RING	
A3	YBR062C	YBR062C	RING	
A4	YBR114W	RAD16	RING	
A5	YBR158W	AMN1	F box	
A6	YBR203W	COS111	F box	
A7	YBR280C	SAF1	F box	
A8	YCR066W	RAD18	RING	
A9	YDL013W	SLX5	RING	
A10	YDL074C	BRE1	RING	
A11	YDL190C	UFD2	U-box	
A12	YDR049W	VMS1	zinc finger, C2H2	peptidyl-RNA hydrolase
B1	YDR103W	STE5	RING-like	mating deficient strain
B2	YDR131C	YDR131C	F box	
B3	YDR143C	SAN1	RING	
B4	YDR219C	MFB1	F box	
B5	YDR255C	RMD5	RING	
B6	YDR265W	PEX10	RING	
B7	YDR266C	HEL2	RING	
B8	YDR306C	PFU1	F box	
B9	YDR313C	PIB1	RING	
B10	YDR457W	TOM1	HECT	
B11	*empty*	*empty*	*empty*	
B12	YER068W	MOT2/NOT4	RING	
C1	YER116C	SLX8	RING	
C2	YGL003C	CDH1	APC/C activator	
C3	YGL131C	SNT2	RING	
C4	YGL141W	HUL5	HECT	
C5	YGR003W	CUL3	Cullin	
C6	YGR184C	UBR1	RING	
C7	YHL010C	ETP1	RING	
C8	YHR115C	DMA1	RING	
C9	YIL030C	SSM4/DOA10	RING	
C10	YIL097W	FYV10	degenerate RING	strain incorrect?
C11	YJL047C	RTT101	Cullin	
C12	YJL149W	DAS1	F box	
D1	YJL157C	FAR1	RING	
D2	YJL204C	RCY1	F box	
D3	YJL210W	PEX2	RING	
D4	YJR036C	HUL4	HECT	
D5	YJR052W	RAD7	F box	
D6	YJR090C	GRR1	F box	slow growth; flocculant

D7	YKL010C	UFD4	HECT	
D8	YKL034W	TUL1	RING	
D9	YKR017C	HEL1	RING	
D10	*empty*	*empty*	*empty*	
D11	YLR032W	RAD5	RING	
D12	YLR097C	HRT3	F box	
E1	YLR148W	PEP3/VPS18	RING	
E2	YLR224W	UCC1	F box	
E3	YLR247C	IRC20	RING	
E4	YLR352W	LUG1	F box	
E5	YLR368W	MDM30	F box	
E6	YLR427W	MAG2	RING	
E7	YML068W	ITT1	RING	
E8	YML088W	UFO1	F box	
E9	YMR026C	PEX12	RING	
E10	YMR119W	ASI1	RING	
E11	YMR231W	PEP5/VPS11	RING	
E12	YMR247C	RKR1/LTN1	RING	
F1	YMR258C	ROY1	F box	
F2	YNL008C	ASI3	RING	
F3	YNL023C	FAP1	RING	
F4	YNL116W	DMA2	RING	
F5	YNL230C	ELA1	F box	
F6	YNL311C	SKP2	F box	
F7	YOL013C	HRD1	RING	
F8	YOL054W	PSH1	RING	
F9	YOL138C	RTC1	RING	
F10	YOR080W	DIA2	F box	
F11	YOR191W	ULS1	RING	
F12	YPR093C	ASR1	RING	
G1	YDR152W	GIR2	RWD domain	
G2	YCR059C	YIH1	RWD domain	
G3	YDR283C	GCN2	RWD domain	
G4	YDR128W	MTC5/SEA3	RWD domain	
G5	YDR069C	DOA4	DUB (general effects)	slow growth
G6	YBL058W	SHP1/UBX1	UBX domain	slow growth
G7	YML013W	UBX2	UBX domain	
G8	YDL091C	UBX3	UBX domain	
G9	YMR067C	UBX4	UBX domain	
G10	YDR330W	UBX5	UBX domain	
G11	YJL048C	UBX6	UBX domain	
G12	YBR273C	UBX7	UBX domain	
H1	YKL213C	DOA1	Ub and Cdc48	
H2	YGL058W	UBC2	E2	
H3	YBR082C	UBC4	E2	
H4	YDR059C	UBC5	E2	
H5	dubious strain - supposed to be ubc6			
H6	YMR022W	UBC7	E2	
H7	YEL012W	UBC8	E2	

H8	YGR133W	UBC10	E2
H9	YOR339C	UBC11	E2
H10	YLR306W	UBC12	E2
H11	YDR092W	UBC13	E2
H12	*empty*	*empty*	*empty*

KEY:

Yellow = known or likely E3s, or parts of E3 complexes.

Pink = RWD domain, a domain with some connection to the UPS

Green = Cdc48-associated factors

Blue = ubiquitin-conjugating enzymes (E2s)

E2 & E3 genes NOT covered by TUS2.0 (gene deletion is inviable, unless noted)

ORF	NAME	Motif	Notes
YER125W	RSP5	HECT	
YLL036C	PRP19	U-box	
YGL116W	CDC20	APC/C activator	
YDL008W	APC11	RING	APC/C
YOL133W	HRT1	RING	RING for SCFs
YDR460W	TFB3	RING	
YLR323C	CWC24	RING	spliceosome
YDR328C	SKP1	SCF Skp1 subunit	
YDL132W	CDC53	Cullin	
YFL009W	CDC4	F BOX	
YIL046W	MET30	F BOX	
YMR094W	CTF13	F BOX	
YPL046C	ELC1	Elongin C	the deletion is viable
YLR024C	UBR2	RING	entry in library bad
YDR177W	UBC1	E2	*(E2 for APC/C)
YDR054C	UBC3	E2	E2 for SCFs
YER100W	UBC6	E2	the deletion is viable

*UBC1 can be deleted in certain strain backgrounds, but was not included in deletion library