# Supporting Information

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### SI Materials and Methods

#### Construction of Plasmids.

***Construction of plasmids pTR785 and pTR791***. Plasmids pTR785 and pTR791 contain the Ubc7 ORF under its own promoter (1,000 bp downstream of the ATG) and a C-terminal 3HA tag. Notably, it is essential to have a large promoter fragment of ∼1,000 bp because a shorter promoter of 500 bp did not induce Ubc7 expression. A PCR fragment containing the Ubc7 promoter, ORF, and 3HA was amplified from strain TRy344 and cloned into pJET1.2/blunt plasmid (Thermo Scientific) to obtain pTR723. The UBC7p-Ubc7-3HA fragment was excised from pTR723 with BglII and was cloned into the BamHI site of pRS303 to obtain pTR785 or to pRS313 to obtain pTR791.

***Construction of plasmids pTR948 and pTR953***. A PCR fragment of the $Ubc7_{KO}$ ORF was amplified from plasmid pTR468 and cloned into pTR791 cut with BlpI by homologous recombination to obtain pTR948. Then a PvuII fragment containing Ubc7p-$Ubc7_{KO}$-3HA from pTR948 was cloned into pRS303 to generate pTR953.

***Construction of plasmid pTR717***. A *met25p-Deg1*-Flag-Vma12-Ura3 fragment was cut from pTR458 with PvuII and ligated into the SmaI site of the YDp-K vector.

***Construction of plasmid pTR941***. A CPY*-HA fragment was cut from pTR414 with SalI and EcoRI and was cloned into pRS314.

***Construction of plasmids pTR1454 and pTR1456***. *UBC7*p-Ubc7-3HA and *UBC7*p-$Ubc7_{R118}$-3HA fragments were excised from pTR785 and pTR1086, respectively, with NcoI/SalI and were cloned into pRS314 plasmid.

***Construction of plasmid pTR1475***. The NcoI/SalI fragment of Hrd1 RING (amino acids 287–531) was cut from pTR1469 and cloned into the pET-42b(+) plasmid.

***Construction of plasmid pTR1544***. The Flag epitope was added to the N terminus of $Ub_{R48}$ (pTR843) by transfer PCR (1).

***Plasmids made by site directed mutagenesis***. pTR977, pTR1077, pTR1078, pTR1086, and pTR1111 were generated using pTR785 as a template. pTR1119, pTR1121, pTR1125, pTR1127, and pTR1129 were generated using pTR953 as a template. pTR1138, pTR1500, pTR1502, pTR1514, pTR1528, and pTR1617 were generated using pTR526 as a template. pTR1530 and pTR1621 were generated using pTR1454 as a template. pTR1611 and pTR1615 were generated using pTR1544 as a template.

#### Construction of Yeast Strains.

***Construction of yeast strains TRy763, TRy1045, TRy1070, TRy1072, TRy1074, and TRy1184***. pTR785 or its related derivatives were linearized with AfeI and integrated into the *HIS3* locus of TRy109 by homologous recombination.

***Construction of yeast strains to construct TRy508, TRy802, TRy832, TRy864, TRy957, TRy960, TRy975, TRy989, TRy1033, TRy1035, TRy1037, TRy1041, TRy1043, and TRy1045.*** pTR717 was linearized with HpaI and integrated into the *LYS2* locus of TRy109 by homologous recombination to obtain yeast strain TRy505. Then pTR785 or its related derivatives were linearized with AfeI and transformed into the *HIS3* locus of TRy505.

***Construction of yeast strain TRy1126***. To knock out the *UBC7* ORF, a PCR fragment containing the KanMX cassette surrounded by 150 bp of *UBC7* flanking regions was integrated into yeast strain YPH499 (2).

***Construction of yeast strains TRy1281 and TRy1282***. Plasmid pRH373 (3) containing UBC7-2HA was linearized with BsgI and integrated into TRy1126 or TRy1148, respectively, at the *TRP1* locus.

***Construction of yeast strain TRy786***. Yeast strain TRy786 was constructed by crossing yeast strains TRy508 and TRy457.


1. Erijman A, Shifman JM, Peleg Y (2014) A single-tube assembly of DNA using the transfer-PCR (TPCR) platform. *Methods Mol Biol* 1116:89–101.
2. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122(1):19–27.
3. Gardner RG, Shearer AG, Hampton RY (2001) In vivo action of the HRD ubiquitin ligase complex: Mechanisms of endoplasmic reticulum quality control and sterol regulation. *Mol Cell Biol* 21(13):4276–4291.


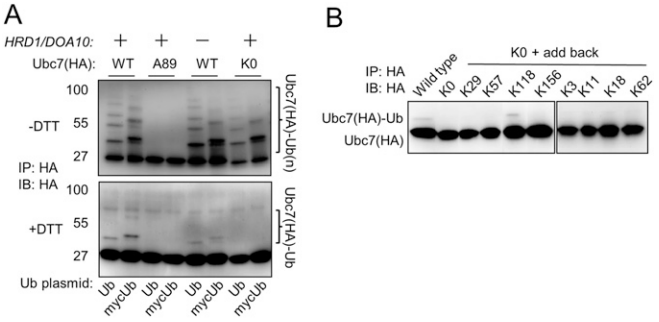


**Fig. S1.** Cue1-bound Ubc7 is auto-ubiquitylated in vivo on both DTT-sensitive and -resistant acceptor sites. Ubc7 was immunoprecipitated using anti-HA polyclonal Abs from yeast cell extracts coexpressing Ubc7-3HA and either $Ub_{A76}$ or N-tagged Myc-$Ub_{A76}$. Ubc7 was eluted from the beads with SDS gel-loading buffer containing 8 M urea either with or without DTT, resolved by SDS/PAGE, and then subjected to immunoblot analysis with anti-HA Abs. (*A*) Different patterns of Ubc7 ubiquitylation in cells expressing the E2 variants Ubc7, $Ubc7_{A89}$, or $Ubc7_{KO}$ in which the two ERAD E3 ligases Doa10 and Hrd1 are either present or deleted. (*B*) Identification of the DTT-resistant monoubiquitylation site. Ubc7 variants expressing a single Lys residue at the indicated position were isolated by immunoprecipitation with anti-HA Abs from the respective yeast strains. The immune complexes were resolved by reducing SDS/PAGE and were subjected to immunoblot analysis with anti-HA Abs.

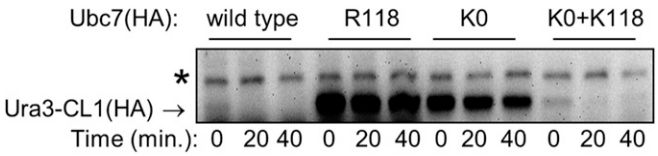


**Fig. S2.** Extended exposure of the immunoblot described in Fig. 1*A* revealed expression of Ura3-*CL1* at time 0 of the CHX-chase experiment that disappeared rapidly in wild-type cells but was substantially stabilized in cells expressing $Ubc7_{R118}$ or $Ubc7_{KO}$. The asterisk indicates a cross-reacting protein that serves as a loading control.

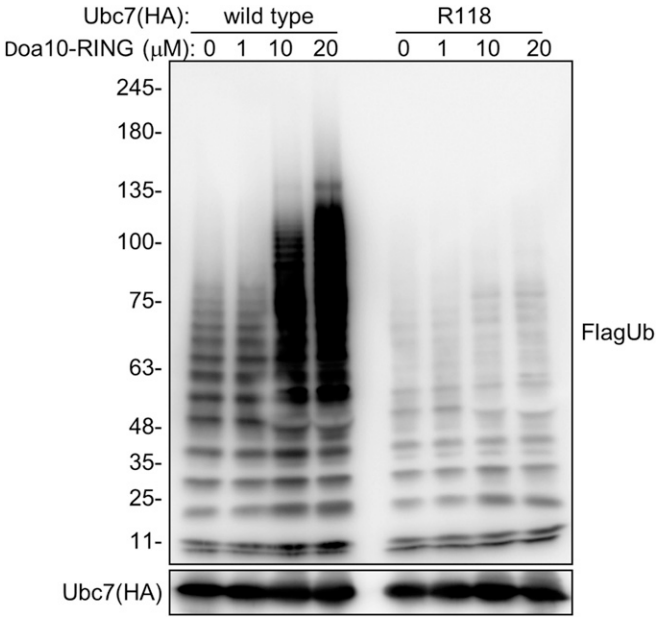


**Fig. S3.** Doa10 RING activates wild-type Ubc7 but not $Ubc7_{R118}$. The in vitro ubiquitylation assay was performed as described in the legend of Fig. 2*D* with increasing concentrations of GST-Doa10 RING. Ub conjugates were separated by SDS/PAGE followed by immunoblot analysis with anti-FLAG (Ub) and with anti-HA (Ubc7) Abs.

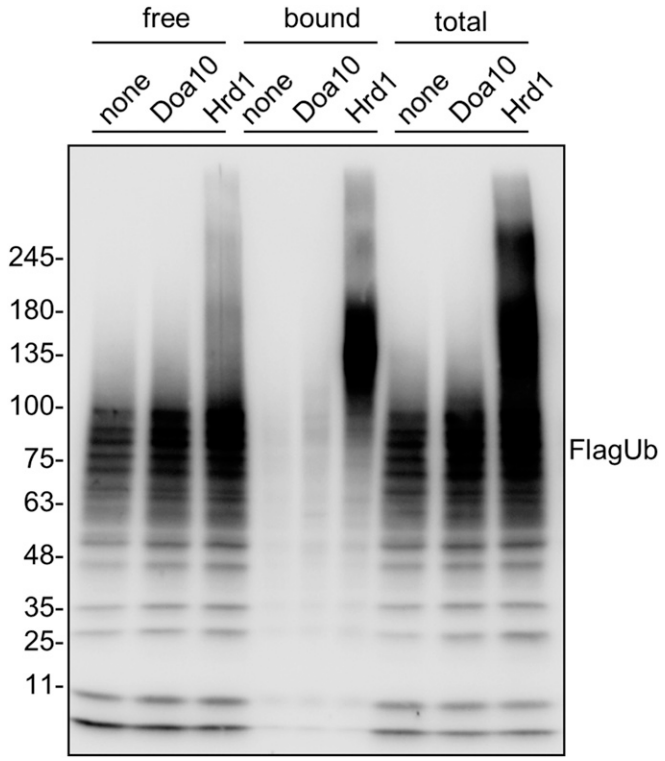


**Fig. S4.** Detection of free polyUb chains after GST pull-down. In vitro ubiquitylation assays were performed as described in the legend to Fig. 2 *D* and *E*. Reactions were terminated with a stop solution containing 4 mM EDTA, 20 mM *N*-ethylmaleimide (NEM); then aliquots were removed for determination of total (free and E3-conjugated) ubiquitylation (Total). GST pull down separated E3-conjugated (GST-bound) and free (not GST-bound) polyUb chains. The GST-bound material was eluted with 50 mM glutathione. The various polyUb samples were resolved by SDS/PAGE and subjected to immunoblot analysis with anti-FLAG Abs.

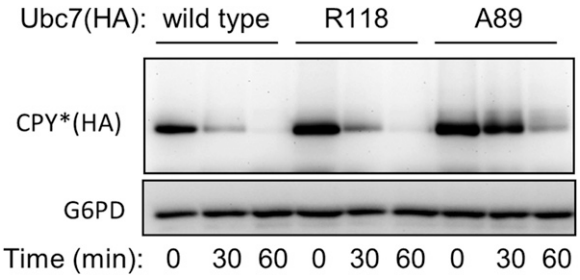


**Fig. S5.** Analysis of CPY* degradation in cells expressing wild-type Ubc7, $Ubc7_{R118}$, or $Ubc7_{A89}$. CHX-chase experiments were performed; then CPY*-HA was visualized by immunoblot analysis with anti-HA Abs. G6PD staining provides a loading control.

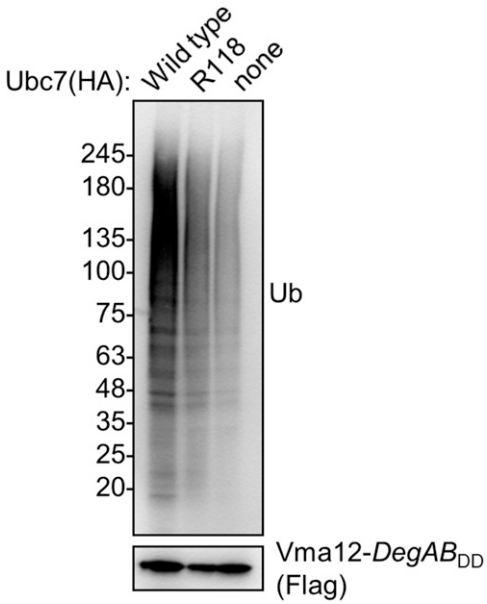


**Fig. S6.** In vivo ubiquitylation of Vma12-*DegAB*$_{DD}$ by Ubc7 and Ubc7$_{R118}$. Vma12-*DegAB*$_{DD}$ was isolated by immunoprecipitation from *ubc7Δ* cells expressing the indicated Ubc7 variants. The immune complexes were subjected to immunoblot analysis with anti-FLAG (VMA12) and anti-Ub Abs.

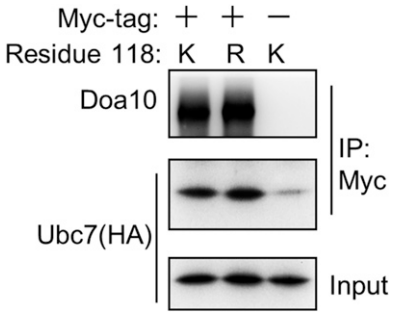


**Fig. S7.** Replacing Lys118 of Ubc7 with Arg did not affect E2–E3 interaction. Cells expressing Myc-tagged Doa10 and either ubc7 or Ubc7$_{R118}$ were lysed and subjected to immunoprecipitation with anti-Myc antibodies. Ubc7 association with Doa10 was determined by analysis of the Doa10 complexes with anti-HA Abs.

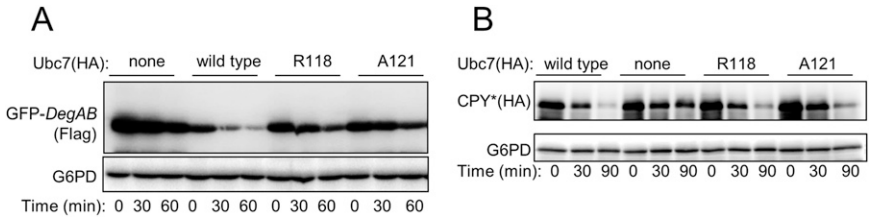


**Fig. S8.** The α2 helix of Ubc7 is essential for the degradation of Doa10 substrates but is dispensable for the Hrd1 substrates. (*A*) CHX-chase analysis of the degradation of GFP-*DegAB* in cells expressing wild-type Ubc7, Ubc7$_{R118}$, or Ubc7$_{A121}$. GFP-*DegAB* was subjected to Western blot analysis and detected by anti-GFP Abs. (*B*) A CHX-chase analysis as described in *A*, except that the substrate used was CPY*-HA. Proteins were visualized with anti-HA Abs. In *A* and *B*, G6PD staining provides a loading control.

**Table S1. Plasmids**

| Plasmid | Relevant markers | Source |
|---|---|---|
| pTR120 | pET-42b(+)-GST-Doa10(1-112) | M. Hochstrasser |
| pTR224 | pOC9-Ura3-*CL1* | (1) |
| pTR414 | pCPY*HA (pDN431) | (2) |
| pTR458 | pRS414-MET25p-*Deg1*-Vma12-URA3 | M. Hochstrasser |
| pTR468 | pCR2.1-Ubc7$_{K0}$-2HA | (3) |
| pTR525 | pTYB2-Ubc7-2HA-ChitinBD | (4) |
| pTR526 | pTYB2-Ubc7-2HA-ChitinBD Cue1$^{\Delta TM}$ | (4) |
| pTR717 | YDp-K-*met25p*-Deg1-Flag-Vma12-Ura3 | This study |
| pTR723 | pJET1.2/blunt -*Ubc7p*-Ubc7-3HA | This study |
| pTR785 | pRS303-*Ubc7p*-Ubc7-3HA | This study |
| pTR791 | pRS313-*Ubc7p*-Ubc7-3HA | This study |
| pTR843 | pET14-Ub$_{R48}$ | A. Ciechanover |
| pTR849 | pRS303-*Ubc7p*-Ubc7$_{R57,R62,R70}$-3HA | This study |
| pTR913 | pRS414-Flag-Vma12-*DegAB* | (5) |
| pTR941 | pRS314-CPY*-HA | This study |
| pTR948 | pRS313-*Ubc7p*-Ubc7$_{K0}$-3HA | This study |
| pTR953 | pRS303-*Ubc7p*-Ubc7$_{K0}$-3HA | This study |
| pTR977 | pRS303-*Ubc7p*-Ubc7$_{A89}$-3HA | This study |
| pTR1077 | pRS303-*Ubc7p*-Ubc7$_{R3,R7,R11}$-3HA | This study |
| pTR1078 | pRS303-*Ubc7p*-Ubc7$_{R156}$,161R-3HA | This study |
| pTR1086 | pRS303-*Ubc7p*-Ubc7$_{R118}$-3HA | This study |
| pTR1111 | pRS303-*Ubc7p*-Ubc7$_{R18,R29}$-3HA | This study |
| pTR1114 | pRS414-Flag-Vma12-*DegAB*$_{D950,D951}$ | (5) |
| pTR1119 | pRS303-*Ubc7p*-Ubc7$_{K0+K3}$-3HA | This study |
| pTR1121 | pRS303-*Ubc7p*-Ubc7$_{K0+K11}$-3HA | This study |
| pTR1123 | pRS303-*Ubc7p*-Ubc7$_{K0+K18}$-3HA | This study |
| pTR1125 | pRS303-*Ubc7p*-Ubc7$_{K0+K57}$-3HA | This study |
| pTR1127 | pRS303-*Ubc7p*-Ubc7$_{K0+K62}$-3HA | This study |
| pTR1129 | pRS303-Ubc7p-Ubc7$_{K0+K118}$-3HA | This study |
| pTR1138 | pTYB2-Ubc7$_{R118}$-2HA-ChitinBD-Cue1$^{\Delta TM}$ | This study |
| pTR1404 | pCAU-KHN | (6) |
| pTR1406 | pCAU-KWW | (7) |
| pTR1454 | pRS314-*Ubc7p*-Ubc7-3HA | This study |
| pTR1456 | pRS314-*Ubc7p*-Ubc7$_{R118}$-3HA | This study |
| pTR1469 | pET30-His-Hrd1(287-531aa) | M. Hochstrasser |
| pTR1475 | pET-42b(+)-GST-Hrd1(287-531aa) | This study |
| pTR1500 | pTYB2-Ubc7$_{Q118}$-2HA-ChitinBD Cue1$^{\Delta TM}$ | This study |
| pTR1502 | pTYB2-Ubc7$_{M118}$-2HA-ChitinBD Cue1$^{\Delta TM}$ | This study |
| pTR1514 | pTYB2-Ubc7$_{A118}$-2HA-ChitinBD Cue1$^{\Delta TM}$ | This study |
| pTR1544 | pET14-FlagUb$_{R48}$ | This study |
| pTR1528 | pTYB2-Ubc7$_{A121}$-2HA-ChitinBD-Cue1$^{\Delta TM}$ | This study |
| pTR1530 | pRS314-*Ubc7p*-Ubc7$_{A89}$-3HA | This study |
| pTR1611 | pET14 -FlagUb$_{A8,R48}$ | This study |
| pTR1615 | pET14-FlagUb$_{A70,R48}$ | This study |
| pTR1617 | pTYB2-Ubc7$_{E118}$-2HA-ChitinBD-Cue1$^{\Delta TM}$ | This study |
| pTR1621 | *pRS314* -*Ubc7p*-Ubc7$_{E118}$-3HA | This study |

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1. Gilon T, Chomsky O, Kulka RG (1998) Degradation signals for ubiquitin system proteolysis in Saccharomyces cerevisiae. *EMBO J* 17(10):2759–2766.
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3. Ravid T, Hochstrasser M (2007) Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. *Nat Cell Biol* 9(4):422–427.
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5. Furth N, et al. (2011) Exposure of bipartite hydrophobic signal triggers nuclear quality control of Ndc10 at the endoplasmic reticulum/nuclear envelope. *Mol Biol Cell* 22(24):4726–4739.
6. Vashist S, et al. (2001) Distinct retrieval and retention mechanisms are required for the quality control of endoplasmic reticulum protein folding. *J Cell Biol* 155(3):355–368.
7. Vashist S, Ng DT (2004) Misfolded proteins are sorted by a sequential checkpoint mechanism of ER quality control. *J Cell Biol* 165(1):41–52.

**Table S2. Yeast strains**

| Yeast | Genotype | Source |
|---|---|---|
| TRy109 | α, his3-Δ200, ura3-5, lys2-801, trp1-1, ubc7Δ::LEU2 | (1) |
| TRy327 | a, ura3-52, trp1-Δ63, his3-Δ200, leu2Δ1, lys2-801, doa10-N1-1319myc13-His3MX6, TRP1:: pRH373-2HA-UBC7, ubc7Δ-KanMX | (2) |
| TRy344 | a, ura3-52, lys2-801:::LYS2-Deg1URA3,trp1-Δ63, his3-Δ200, leu2:::LEU2-Deg1lacZ, UBC7-3HA-His3MX6 | M. Hochstrasser |
| TRy457 | a, his3-Δ200, leu2-3,112, ura3-52, lys2-801, trp1-1, hrd1Δ::LEU2, doa10Δ::HIS3 | This study |
| TRy505 | α, his3-Δ200, leu2-3,112, ura3-5, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS, trp1-1, ubc7Δ::LEU2 | This study |
| TRy508 | α, his3-Δ200::pRS303/Ubc7p-Ubc7-3HA::HIS, leu2-3,112, ura3-5, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS, trp1-1, ubc7Δ::LEU2 | This study |
| TRy556 | α, his3-Δ200::pRS303::HIS, leu2-3,112, ura3-5, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS, trp1-1, ubc7Δ::LEU2 | This study |
| TRy763 | α, his3-Δ200::pRS303/Ubc7p-Ubc7-3HA::HIS, leu2-3,112, ura3-5, lys2-801, trp1-1, ubc7Δ::LEU2 | This study |
| TRy786 | α, his3-Δ200::pRS303/Ubc7p-Ubc7-3HA::HIS, leu2-3,112, ura3-5, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS, trp1-1, ubc7::LEU, doa10Δ::HIS3 | This study |
| TRy802 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy832 | α, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{A89}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU2 | This study |
| TRy864 | α, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{R57,R62,R70}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU2 | This study |
| TRy957 | α, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{R3,R7,R11}$-3HA::HIS, leu2-3,112,ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU2 | This study |
| TRy960 | α, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{R1,R56,R161}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU2 | This study |
| TRy975 | α, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{R118}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU2 | This study |
| TRy989 | α, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{R18,R29}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU2 | This study |
| TRy1033 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0+K3}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy1035 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0+K11}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy1037 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0+K18}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy1041 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0+K57}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy1043 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0+K62}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy1045 | a, his3-Δ200::pRS303/-Ubc7p-Ubc7$_{K0+K118}$-3HA::HIS, leu2-3,112, ura3-52, lys2-801::Met25p-Deg1-Flag-Vma12-Ura3::LYS2, trp1-1,ubc7::LEU | This study |
| TRy1070 | α, his3-Δ200::pRS303/Ubc7p-Ubc7$_{K0+K118}$-3HA::HIS, leu2-3,112, ura3-5, lys2-801, trp1-1, ubc7Δ::LEU2 | This study |
| TRy1072 | α, his3-Δ200::pRS303/Ubc7p-Ubc7$_{K0}$-3HA::HIS, leu2-3,112, ura3-5, lys2-801, trp1-1, ubc7Δ::LEU2 | This study |
| TRy1074 | α, his3-Δ200::pRS303/Ubc7p-Ubc7$_{R118}$-3HA::HIS, leu2-3,112, ura3-5, lys2-801, trp1-1, ubc7Δ::LEU2 | This study |
| TRy1126 | a, ura3-52, trp1-Δ63, his3-Δ200, leu2Δ1, lys2-801, ubc7Δ-KanMX | This study |
| TRy1148 | a, ura3-52, trp1-Δ63, his3-Δ200, leu2Δ1, lys2-801, doa10-N1-1319myc13-His3MX6, ubc7Δ-KanMX | M. Hochstrasser |
| TRy1184 | α, his3-Δ200::pRS303/Ubc7p-Ubc7$_{A89}$-3HA::HIS, leu2-3,112, ura3-5, lys2-801, trp1-1, ubc7Δ::LEU2 | This study |
| TRy1281 | a, ura3-52, trp1-Δ63, his3-Δ200, leu2Δ1, lys2-801, TRP1:: pRH373-2HA-UBC7, ubc7Δ-KanMX | This study |
| TRy1282 | a, ura3-52, trp1-Δ63, his3-Δ200, leu2Δ1, lys2-801, doa10-N1-1319myc13-His3MX6, TRP1:: pRH373-2HA-UBC7$_{R11\&}$ ubc7Δ-KanMX | This study |

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2. Ravid T, Hochstrasser M (2007) Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. *Nat Cell Biol* 9(4):422–427.