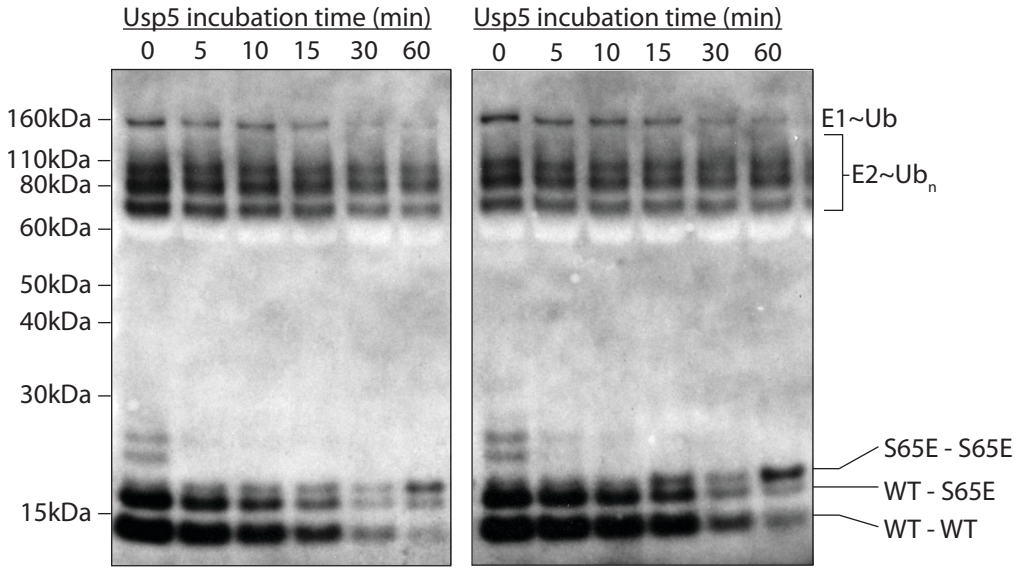
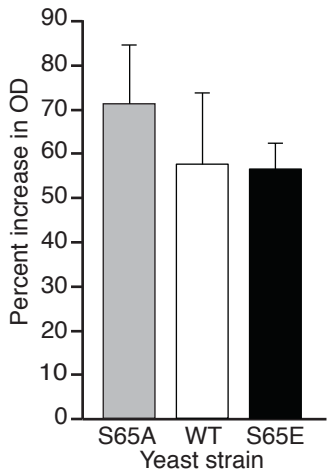
Appendix Table of Contents

Figure S1



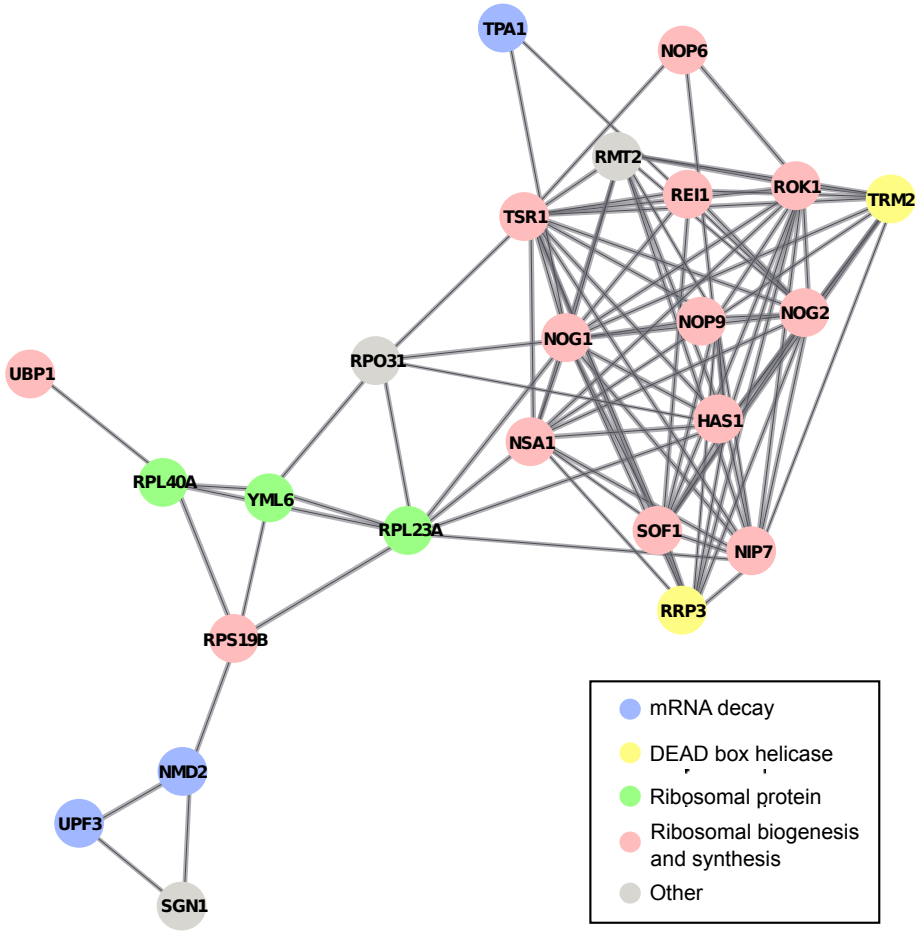
Timecourse of di-ubiquitin disassembly with the DUB Usp5.
Anti-ubiquitin immunoblotting of in vitro generated mixed chains comprised of both WT and His-tagged S65E ubiquitin. Chains were subjected to disassembly with Usp5, and the presence of the dimers containing specific linkage type connections was measured over time. Two independent replicate experiments are shown.

Figure S2



Measurement of yeast growth rates.
The percent increase in OD600 over a 2 h timespan for yeast strains expressing S65A, WT, or S65E ubiquitin (for each strain n = 4).

Figure S3



Proteins with significantly repressed turnover in S65E ubiquitin mutants. Protein-protein interaction network of proteins with the largest protein turnover delay in S65E ubiquitin mutant cells relative to WT or S65A ubiquitin cells.

Appendix Table S1. Yeast strains used in this study.

Strains	Genotype	Ubiquitin expressed in 2μ plasmids	Source or reference	Experiments
LSY207	MATa lys2–810 leu2–3,-112 ura3–52 his3-Δ200 trp1–1[am] ubi1-Δ1::TRP1 ubi2-Δ2::ura3 ubi3-Δub-2 ubi4-Δ2::LEU2 pdr5::KanMX [pUB221]	P_{CUP1}-6His-myc-ubiquitin	1, 2	Fig. 4B
DS147	MATa lys2–810 leu2–3,-112 ura3–52 his3-Δ200 trp1–1[am] ubi1-Δ1::TRP1 ubi2-Δ2::ura3 ubi3-Δub-2 ubi4-Δ2::LEU2 pdr5::KanMX [pUB100][	P_{GPD}-ubiquitin (WT)	This study	Fig. 4A, Fig. 5, Fig. 7
DS181	MATa lys2–810 leu2–3,-112 ura3–52 his3-Δ200 trp1–1[am] ubi1-Δ1::TRP1 ubi2-Δ2::ura3 ubi3-Δub-2 ubi4-Δ2::LEU2 pdr5::KanMX [pUB100][	P_{GPD}-ubiquitin (S65A)	This study	Fig. 4A, Fig. 5, Fig. 7
DS182	MATa lys2–810 leu2–3,-112 ura3–52 his3-Δ200 trp1–1[am] ubi1-Δ1::TRP1 ubi2-Δ2::ura3 ubi3-Δub-2 ubi4-Δ2::LEU2 pdr5::KanMX [pUB100][	P_{GPD}-ubiquitin (S65E)	This study	Fig. 4A, Fig. 5, Fig. 7
DS208	BY4742: MATα his3Δ, leu2Δ, ura3Δ, lys2Δ	P_{CUP1}-6His-myc-ubiquitin	1	Fig. 6
DS209	BY4742: MATα his3Δ, leu2Δ, ura3Δ, lys2Δ	P_{CUP1}-6His-myc-ubiquitin S65A mutant	This study	Fig. 6
DS210	BY4742: MATα his3Δ, leu2Δ, ura3Δ, lys2Δ	P_{CUP1}-6His-myc-ubiquitin S65E mutant	This study	Fig. 6

1. Spence J, Gali RR, Dittmar G, Sherman F, Karin M, Finley D (2000) Cell 102:67-76
2. Swaney DL, Beltrao P, Starita L, Guo A, Rush J, Fields S, Krogan NJ, Villén J (2013) Nature Methods 10(7) 676-682

Appendix Table S2. The effect of H_2O_2 stress on ubiquitin signaling. Log_2(H2O2/control) quantifying the response to 5mM H_2O_2 exposure for global ubiquitin protein, S65 and S57 phosphorylation, and ubiquitin chain abundances. To account for any errors in mixing of cells, all log_2 ratios were normalized such that the median of the lysate protein quantification distribution was centered at $log_2 = 0$.

	Hydrogen peroxide exposure time (min)			
Analyte measured	10	30	90	240
Total ubiquitin protein	-0.01	0.07	0.41	0.72
Ubiquitin S65 Phosphorylation	-0.28	1.16	2.75	3.85
Ubiquitin S57 Phosphorylation	-0.27	-0.03	0.64	1.73
K6 ubiquitin chains	0.86	0.87	1.17	1.27
K11 ubiquitin chains	1.45	2.20	2.97	3.77
K27 ubiquitin chains	-0.22	-0.68	-0.80	-0.92
K29 ubiquitin chains	1.45	1.49	1.87	2.41
K33 ubiquitin chains	0.99	0.96	1.57	2.23
K48 ubiquitin chains	2.32	2.54	3.09	3.25
K63 ubiquitin chains	2.22	2.82	3.35	3.76

Appendix Table S3. Enrichment of gene ontology terms in S65E cells. Ubiquitylation sites from the top 10% most increasing sites in S65E cells vs WT cells were analyzed for gene ontology enrichment as compared to all ubiquitylation sites identified. Analysis was performed using the STRING website.

Gene ontology ID	Term	Type of process	NumberOfGenes	p-value	p-value_bonferroni corrected
GO:0002181	cytoplasmic translation	biological process	26	5.53E-20	2.22E-16
GO:0006412	translation	biological process	30	2.54E-14	1.02E-10
GO:0044267	cellular protein metabolic process	biological process	54	3.49E-12	1.40E-08
GO:0019538	protein metabolic process	biological process	52	9.86E-11	3.95E-07
GO:0034645	cellular macromolecule biosynthetic process	biological process	37	5.91E-06	2.37E-02
GO:0009059	macromolecule biosynthetic process	biological process	37	7.47E-06	2.99E-02
GO:0044260	cellular macromolecule metabolic process	biological process	62	4.67E-05	1.87E-01
GO:0042254	ribosome biogenesis	biological process	18	9.36E-05	3.75E-01
GO:0006407	rRNA export from nucleus	biological process	4	1.39E-04	5.58E-01
GO:0051029	rRNA transport	biological process	4	1.39E-04	5.58E-01
GO:0003735	structural constituent of ribosome	molecular function	21	1.64E-11	3.51E-08
GO:0005198	structural molecule activity	molecular function	21	1.47E-06	3.15E-03
GO:0044445	cytosolic part	cellular component	33	2.17E-22	1.78E-19
GO:0022626	cytosolic ribosome	cellular component	26	5.73E-20	4.70E-17
GO:0005829	cytosol	cellular component	42	8.39E-20	6.88E-17
GO:0022625	cytosolic large ribosomal subunit	cellular component	13	2.79E-10	2.29E-07
GO:0044391	ribosomal subunit	cellular component	20	5.71E-10	4.68E-07
GO:0022627	cytosolic small ribosomal subunit	cellular component	11	7.22E-10	5.92E-07
GO:0030686	90S preribosome	cellular component	10	1.22E-05	9.99E-03
GO:0015934	large ribosomal subunit	cellular component	11	1.43E-05	1.17E-02
GO:0015935	small ribosomal subunit	cellular component	9	1.56E-05	1.28E-02
GO:0022624	proteasome accessory complex	cellular component	5	9.54E-05	7.82E-02
GO:0005838	proteasome regulatory particle	cellular component	5	9.54E-05	7.82E-02
GO:0031597	cytosolic proteasome complex	cellular component	5	2.22E-04	1.82E-01
GO:0034515	proteasome storage granule	cellular component	5	2.22E-04	1.82E-01
GO:0030684	preribosome	cellular component	10	7.92E-04	6.49E-01
sce03010	Ribosome	KEGG pathway	47	5.53E-32	5.03E-30
sce03050	Proteasome	KEGG pathway	8	2.15E-04	1.96E-02

No enriched Interpro or PFAM domains were identified for these proteins.