

UBA6 specificity for ubiquitin E2 conjugating enzymes reveals a priority mechanism of BIRC6

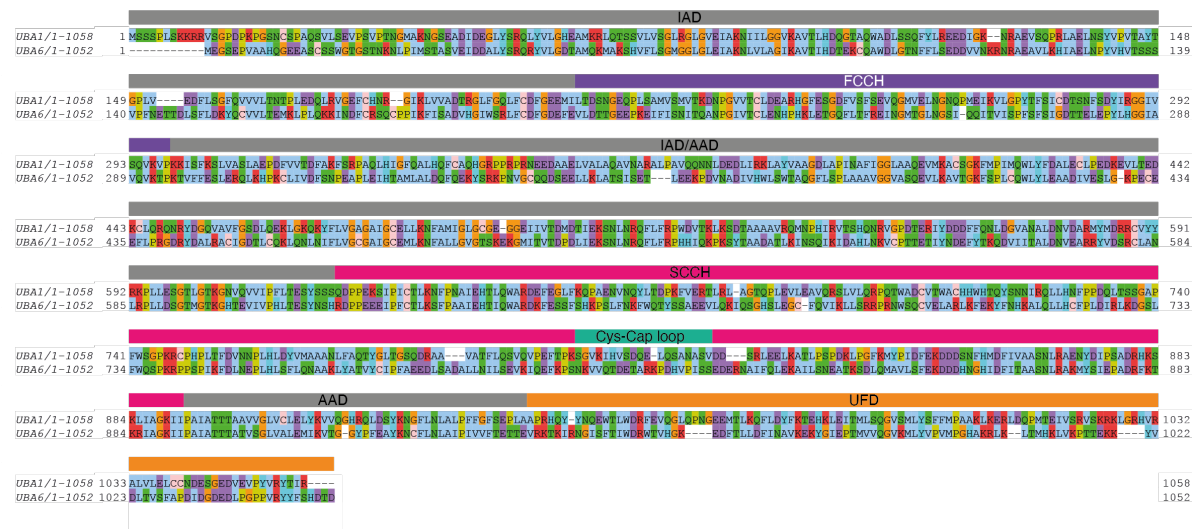
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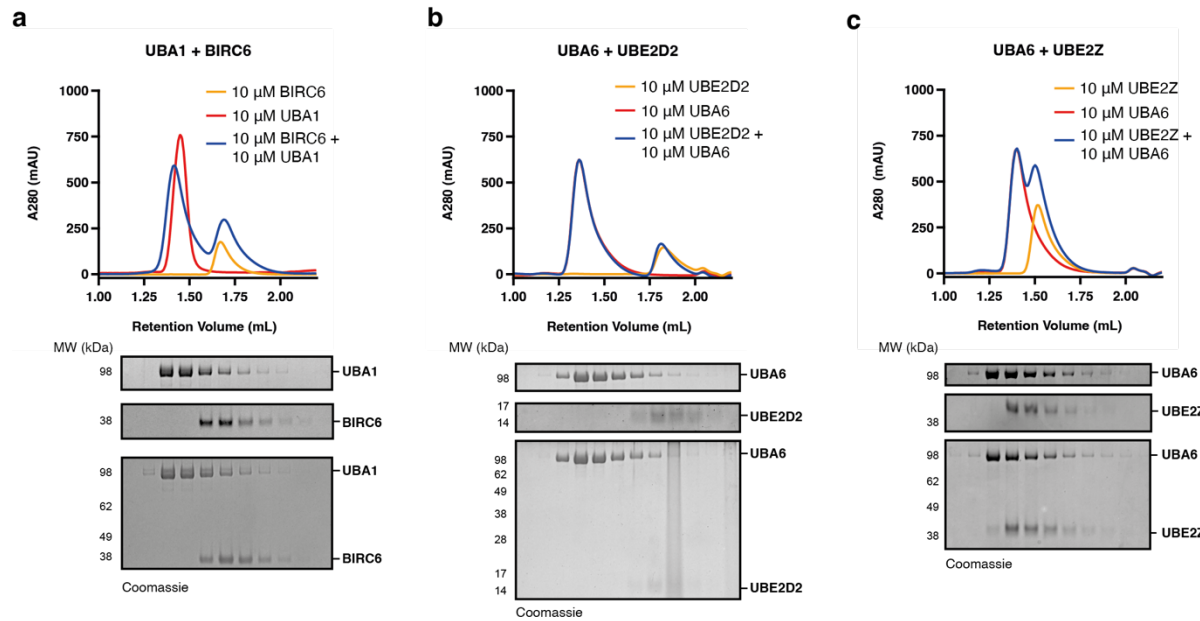
Supplementary Figure 1

a



Supplementary Figure 1
The canonical E1 architecture is conserved between UBA1 and UBA6
a, Sequence alignment between the human E1 enzymes for ubiquitin (UBA1, UBA6). Residues are coloured based on their properties defined by the Clustal colouring scheme.

Supplementary Figure 2



Supplementary Figure 2

Analytical Size Exclusion Chromatography (SEC) of E1-E2 combinations.

a, Analytical SEC of BIRC6^{UBC} with UBA1. SDS-PAGE gel analysis of fractions between Retention Volume of 1.25 – 2.0 ml.

b-c, Analytical SEC of the UBA6-active E2s UBE2D2 (**b**) and UBE2Z (**c**) with UBA6. SDS-PAGE gel analysis of fractions between Retention Volume of 1.25 – 2.0 ml.

Supplementary Figure 3

a

E2	N-terminal extension	C-terminal extension	Class
UBE2A	3	3	I
UBE2B	3	3	I
UBE2C	29	4	II
UBE2D1	-	-	I
UBE2D2	-	-	I
UBE2D3	-	-	I
UBE2D4	-	-	I
UBE2E1	46	-	II
UBE2E2	54	-	II
UBE2E3	60	-	II
UBE2G1	4	5	I
UBE2G2	3	2	I
UBE2H	5	32	III
UBE2J1	9	173	III
UBE2J2	11	112	III
UBE2K	2	46	III
UBE2L3	1	5	I
UBE2N	1	3	I
UBE2O	952	180	IV
UBE2Q1	250	N/A*	II
UBE2Q2	203	N/A*	II
UBE2R1	7	63	III
UBE2R2	7	65	III
UBE2S	10	65	III
UBE2T	1	45	III
UBE2U	3	172	III
UBE2W	2	N/A*	I
UBE2Z	98	101	IV
BIRC6	4572	117	IV

Extensions measured as residues beyond the core UBC domain, as found in the UBE2D subfamily. A limit of 15 residues was chosen for consistency with earlier E2 classifications.
Class I: No extension
Class II: N-terminal extension (> 15 residues)
Class III: C-terminal extension (> 15 residues)
Class IV: N- and C- terminal extensions (> 15 residues)
* UBE2Q1/2 and UBE2W have a different C-terminal fold to the canonical UBC domain

Supplementary Figure 3

Human E2 ubiquitin-conjugating enzymes are classed into four groups determined by domain architecture

a, Table summarising N- and C-terminal extensions present across human E2 enzymes.

Supplementary Figure 4

a

UBA6 WT
Unpaired t-Test significance
(% UBE2D2~Ub with competitor vs no competitor)

[Competitor] (μM)					
Competitor	0.75	1.5	3.0	4.5	6.0
BIRC6 ^{UBC}	ns	ns	ns	0.024	0.005
BIRC6 ^{UBC} C4666A	ns	ns	0.023	0.010	0.007
BIRC6 ^{UBC} A4575D	ns	ns	ns	ns	ns
BIRC6 ^{UBC} A4575D, C4666A	ns	ns	ns	ns	ns
UBE2Z	ns	ns	ns	ns	ns
UBE2Z CA	ns	ns	ns	ns	ns

b

Unpaired t-Test significance
(% E2~Ub with UBA6 WT vs ΔCys-Cap Loop)

E2	1 min	5 min	30 min
BIRC6 ^{UBC} WT	0.0004	0.002	0.029
BIRC6 ^{UBC} A4575D	ns	ns	ns
UBE2Z	0.016	ns	ns
UBE2D2	ns	ns	ns

c

UBA6 ΔCys-Cap Loop
Unpaired t-Test significance
(% UBE2D2~Ub with competitor vs no competitor)

[Competitor] (μM)					
Competitor	0.75	1.5	3.0	4.5	6.0
BIRC6 ^{UBC}	0.021	0.015	0.019	0.010	0.014
BIRC6 ^{UBC} C4666A	0.033	0.020	0.012	0.011	0.009
BIRC6 ^{UBC} A4575D	ns	ns	ns	ns	ns
BIRC6 ^{UBC} A4575D, C4666A	ns	ns	ns	ns	ns
UBE2Z	ns	ns	ns	ns	ns
UBE2Z CA	ns	ns	ns	ns	ns

Supplementary Figure 4

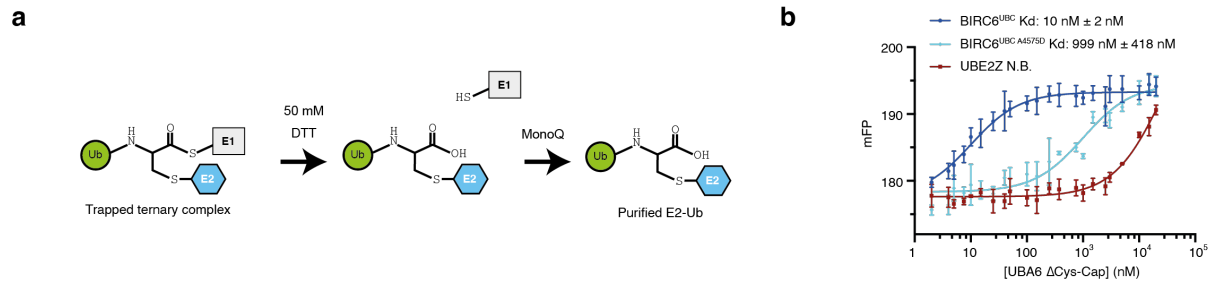
Statistical analysis of transthiolation assays.

a, Table of unpaired t-test significance values when comparing mean uninhibited UBE2D2 transthiolation to the activity measured with competitor E2s at increasing concentrations across three independent technical replicates (related to **Fig. 5a, b**).

b, Table of unpaired t-test significance values when comparing the proportion of loaded E2 formed by UBA6 WT and UBA6 ΔCys-Cap loop at each time point across three independent technical replicates (related to **Fig. 6b, c**).

c, Table of unpaired t-test significance values when comparing mean uninhibited UBE2D2 transthiolation the activity measured with competitor E2s at increasing concentrations across three independent technical replicates (related to **Fig. 6e, f**).

Supplementary Figure 5



Supplementary Figure 5

Affinity measurements of E2s towards UBA6 ΔCys-Cap loop.

a, Following generation of the trapped E1-UbDha-E2 ternary complex as described in **Extended Figure 5d**), the E1~UbDha thioester bond was collapsed by addition of DTT and the liberated E2-UbDha was purified by anion exchange chromatography (Mono Q).

b, Fluorescence polarisation measurements of UBA6 ΔCys-Cap loop binding to BIRC6^{UBC}, BIRC6^{UBC} A4575D and UBE2Z. Binding was detected for BIRC6^{UBC} (Kd: 10 ± 2 nM) and BIRC6^{UBC} A4575D (Kd: 999 ± 418 nM). Although no Kd could be fitted to UBE2Z, the FP response indicates a stronger interaction than seen towards UBA6 WT (**Fig. 2g**). Data shown is mean ± SD from experiments each performed in technical triplicate.

Supplementary Data Table 1

Summary of vectors and protein purification steps for recombinant proteins used in this study.

Protein	Construct length	Vector	N-terminal tag	Expression system	Ion exchange column	Gel filtration column
Ubiquitin	1-76	Mod. pOPINB	None	<i>E. coli</i>	SP	Superdex 75
MC-Ubiquitin	1-78	Mod. pOPINB	None	<i>E. coli</i>	SP	Superdex 75
Ubiquitin G76C	1-76	Mod. pOPINB	None	<i>E. coli</i>	SP	Superdex 75
UBA1	1-1058	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA1-6UFD	1-1053	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA1-6SCCH	1-1065	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA6	1-1052	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA6-1UFD	1-1057	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA6-1SCCH	1-1045	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA6 ΔCys-	1-1037	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
Cap loop						
UBA6 C625S	1-1052	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBA6-UFD	940-1052	pOPINS	His ₆ -SUMO	<i>E. coli</i>	Resource S	Superdex 75
dUBA1	184-1191	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	Resource Q	Superdex 200
UBE2A	1-152	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2B	1-152	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2C	1-179	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2D1	1-147	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2D2	1-147	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2D3	1-147	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2D4	1-147	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2E1	1-193	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource S	Superdex 75
UBE2E2	1-201	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2E3	1-207	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2G1	1-170	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2G2	1-165	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2H	1-183	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2J1	1-285	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2J2	1-230	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2K	1-200	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2L3	1-154	pOPINB	His ₆ -GST-3C	<i>E. coli</i>	Resource S	Superdex 75
UBE2N	1-152	pGEX6P1	GST-3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2O	1-1292	pGB	C-terminal His ₆	<i>Sf9</i>	Resource Q	Superdex 200
UBE2Q1	1-422	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2Q1 ΔN	246-422	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2Q1 ΔC	1-399	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2Q1 ΔNC	246-399	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2Q2	1-375	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2Q2 ΔN	197-375	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2Q2 ΔC	1-352	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2Q2 ΔNC	197-352	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2R1	1-236	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2R2	1-238	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2S	1-222	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource S	Superdex 75
UBE2T	1-197	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2U	1-150	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
UBE2W	1-151	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
UBE2Z	1-354	pOPINB	His ₆ -3C	<i>E. coli</i>	Resource Q	Superdex 75
BIRC6 ^{UBC}	4498-4820	pOPINS	His ₆ -SUMO	<i>E. coli</i>	Resource Q	Superdex 75
BIRC6	1-4857	pACEBAC	Twin-Strep-3C	<i>Sf9</i>	MonoQ	Superose 6
dBIRC6	4577-4879	pOPINB	His ₆ -3C	<i>E. coli</i>	N/A	Superdex 75
h/dBIRC6 chimeras	various	pOPINS	His ₆ -SUMO	<i>E. coli</i>	N/A	Superdex 75

Supplementary Data Table 2

Primers used in this study

Primer	Primer sequence
BIRC6-co-4498-S_Fwd	GCGAACAGATCGGTGGTGCTAATCAAGAAAAGAAGCTGGGCG
BIRC6-co-4820-B_Rev	ATGGTCTAGAAAGCTTTAttattaGTCAGGGTCCAAGCCTTCAGG
BIRC6-co-C4666A_Fwd	GACGGCAAAGTCgctCTGTCCATCCTGAACACC
BIRC6-co-C4666A_Rev	GGATGGACAGagcGACTTTGCCGTCGTTGTACAGG
BIRC6-co-A4702E_Fwd	CTCTGATCCTGGTCgaaGAGCCCTACTTCAACGAGCCTGG
BIRC6-co-A4702E_Rev	GTTGAAGTAGGGCTCttcGACCAGGATCAGAGACTGGACAGAC
BIRC6-co-R4576E_Fwd	CTGCTCGTGCTgagCGTTTGGCTCAAGAAGCTGTGaccC
BIRC6-co-R4576E_Rev	CTTGAGCCAAACGctcAGCACGAGCAGCGGAGTTAGCGTC
BIRC6-co-Q4580R_Fwd	ggCGTTTGGCTcgaGAAGCTGTGACCCTGTCCACCAGCC
BIRC6-co-Q4580R_Rev	CACAGCTTctcgAGCCAAACGCCTAGCACGAGCAGCGG
BIRC6-co-E4604R_Fwd	CGTGCGTTGCGACGAGcgaCGTctgGACATTATGAAGGTGCTG
BIRC6-co-E4604R_Rev	CAGCACCTTCATAATGTCcagACGtgcCTCGTCGCAACGCACG
BIRC6-co-L4606R_Fwd	CGTGCGTTGCGACGAGgaaCGTcggGACATTATGAAGGTGCTG
BIRC6-co-L4606R_Rev	CAGCACCTTCATAATGTCccgACGttcCTCGTCGCAACGCACG
BIRC6-co-R4713E_Fwd	CTACTTCAACGAGCCTGGTtacGAGgaaTCCCGTGGTACTCC
BIRC6-co-R4713E_Rev	GGAGTACCACGGGAttcCTCgtaACCAGGCTCGTTGAAGTAG
BIRC6-co-Y4711R_Fwd	CTACTTCAACGAGCCTGGTcgcGAGcgtTCCCGTGGTACTCC
BIRC6-co-Y4711R_Rev	GGAGTACCACGGGAacgCTCgcgACCAGGCTCGTTGAAGTAG
BIRC6-co-N4682S_Fwd	CGAGGAAAAGTGGtccCCTCAGACCTCCAGCTTCctgCAGG
BIRC6-co-N4682S_Rev	GGAGGTCTGAGGggaCCACTTTTCCTCGGGACGACCATGCCAGG
BIRC6-co-L4689S_Fwd	CCTCAGACCTCCAGCTTctcgCAGGTCCTGGTGTCTGTCCAGTCTC
BIRC6-co-L4689S_Rev	GACACCAGGACCTGcgaGAAGCTGGAGGTCTGAGGgttCACTTTTCCTC
BIRC6-co-S4697D_Fwd	CTGGTGTCTGTCCAGgatctgATCCTGGTCGCTGAGCCCTACTTCAACG
BIRC6-co-S4697D_Rev	CAGCGACCAGGATcagatcCTGGACAGACACCAGGACCTGcagGAAGC
BIRC6-co-L4698A_Fwd	CTGGTGTCTGTCCAGtctcgATCCTGGTCGCTGAGCCCTACTTCAACG
BIRC6-co-L4698A_Rev	CAGCGACCAGGATcgcagaCTGGACAGACACCAGGACCTGcagGAAGC
BIRC6-co-V4701T_Fwd	CAGtctctgATCCTGaccGCTGAGCCCTACTTCAACGAGCCTGGTTACG
BIRC6-co-V4701T_Rev	GAAGTAGGGCTCAGCggtCAGGATcagagaCTGGACAGACACCAGG
BIRC6-co-R4654A_Fwd	GTGGTCACTCCGTGgctTTCAACCCCAACCTGTACAACGACGG
BIRC6-co-R4654A_Rev	CAGGTTGGGGTTGAAagcCACGGAGTGACCACCAAGTGGTTTC
BIRC6-co-Y4460A_Fwd	CAACCCCAACCTGgccaacGACGGCAAAGTCTGCCTGTCCATCCTG
BIRC6-co-Y4460A_Rev	GGCAGACTTTGCCGTCgttggcCAGGTTGGGGTTGAAacgCACGGAG
BIRC6-co-N4461E_Fwd	CAACCCCAACCTGtacgagGACGGCAAAGTCTGCCTGTCCATCCTG
BIRC6-co-N4461E_Rev	GGCAGACTTTGCCGTCctcgttaCAGGTTGGGGTTGAAacgCACGGAG
BIRC6-co-A4575D_Fwd	CTCCGCTGCTCGTgatAGGCGTTTGgctCAAGAAGCTGTGACC
BIRC6-co-A4575D_Rev	CTTGagcCAAACGCCTatcACGAGCAGCGGAGTTAGCGTCGTTGG
BIRC6-co-A4579E_Fwd	gctAGGCGTTTGgaaCAAGAAGCTGTGACCCTGTCCACCAGCCTGCC

BIRC6-co-A4579E_Rev	GTCACAGCTTCTTGttcCAAACGCCTagcACGAGCAGCGGAGTTAG
BIRC6-co-A4579L_Fwd	gctAGGCGTTTTGcttCAAGAAGCTGTGACCCTGTCCACCAGCCTGCC
BIRC6-co-A4579L_Rev	GTCACAGCTTCTTGaagCAAACGCCTagcACGAGCAGCGGAGTTAG
GSSG-hBIRC6-co-4555_FWD	GGGCTCTTCCGGGGTGAAC TACCACTACATGTCCC
hBIRC6-4541-co-GSSG_REV	CCCCGGAAGAGCCCTCGAAGGTGTGCGAACTG
BIRC6-co-W4673A_Fwd	CTGAACACCgcgCATGGTCGTCCCGAG
BIRC6-co-W4673A_Rev	GACGACCATGcgGCTGTT CAGGATGGACAG
BIRC6-co-W4673R_Fwd	CTGAACACCcgtCATGGTCGTCCCGAG
BIRC6-co-W4673R_Rev	GACGACCATGacgGGTGTTCAGGATGGACAG
dBIRC6_4577_B_FWD	AAGTTCTGTTTCAGGGCCCGGAGCCCCAGAGCAAATC
dBIRC6_4879-B_REV	ATGGTCTAGAAAGCTTTAttaCAAGTCCTCGAGGCC
dBIRC6-4648_hBIRC6-4590_FWD	TTACACTGTCCACCAGCCTAccttt
dBIRC6-4648_hBIRC6-4590_REV	aggaggatgaggacaaaggTAGGCTGGTGGACAGTGTA
BIRC6-co-E4603T_E4604D_Fwd	CGTGCGTTGCGACaccgatCGTctgGACATTATGAAGGTGCTG
BIRC6-co-E4603T_E4604D_Rev	CAGCACCTTCATAATGTcagACGatcGGTGTGCGCAACGCACG
hBIRC6-4708_dBIRC6-4768_FWD	CTGAGCCCTACTTCAACGAGcccgggcttgaaag
hBIRC6-4708_dBIRC6-4768_REV	acttctttcaaagcccggCTCGTTGAAGTAGGGC
BIRC6-co-4631ffpp_Fwd	CGAGTTGACGTGttctttccgcccGACTACCCTaaccagCCTCCAC
BIRC6-co-4631ffpp_Rev	ttAGGGTAGTCcggcgggaaagaaCACGTGCGAACTCGAAGCAACCG
BIRC6-co-SS4638NQ_Fwd	CCTCAAGACTACCCTaaccagCCTCCACTGGTCAACCTGGAACCC
BIRC6-co-SS4638NQ_Rev	GACCAAGTGGAGGctggttAGGGTAGTCTTGAGGGAAGTACACG
BIRC6-co-G4650R_Fwd	GAAACCACTGGTcgtCACTCCGTGCGTTTCAACCCCAAC
BIRC6-co-G4650R_Rev	CGCACGGAGTGacgACCAGTGGTTTCCAGGTTGACCAG
BIRC6-co-l4669V_Fwd	GTCTGCCTGTCCgtcCTGAACACCTGGCATGGTCGTCCCGAGG
BIRC6-co-l4669V_Rev	GCCAGGTGTT CAGgacGGACAGGCAGACTTTGCCGTGCTTG
hBIRC6-4589_dBIRC6-4649_FWD	CCTGTCCACCAGCCTGccc
hBIRC6-4589_dBIRC6-4649_REV	GAAGTACTAAAGCTGAGGGGcaggctggtggacag
dBIRC6-4767_hBIRC6-4709_FWD	CCGAGCCCTATTTCAATGAAcctggttacgagc
dBIRC6-4767_hBIRC6-4709_REV	GAACGCTCGTAACCAGGttcattgaaatagggctcg
hUBA1-1-B_Fwd	AAGTTCTGTTTCAGGGCCCGATGTCCAGCTCGCCGCTGTG
hUBA1-1058-B_Rev	ATGGTCTAGAAAGCTTTAttaGCGGATGGTGTATCGGACATAG
UBA6-co-1_B_Fwd	AAGTTCTGTTTCAGGGCCCGATGGAAGGTTCCGAGCCTGTG
UBA6-co-1052-B_Rev	ATGGTCTAGAAAGCTTTAttaGTCGGTGTGTCGTGAGAGAAGTAG
UBA6-co-C625A_fwd	GAGGAGATCCCATTCCGCCACACTGAAGAGC
UBA6-co-C625A_rev	GCTCTTCAGTGTGGCGAATGGGATCTCCTC
UBA6-co-C625S_Fwd	GAGATCCCATTCTcaACCCTGAAGTCATTCCCCGCTG
UBA6-co-C625S_Rev	GAATGACTTCAGGGTtgaGAATGGGATCTCTTCTCTGGAGGG
UBA6_940_Cys_B_FWD	AAGTTCTGTTTCAGGGCCCGTGCGAAGTCCGCAAGACTAAGATCCG
UBA6-co-M998R_Fwd	GTACGTGCCCCGTGaggCCCGGTCACGCTAAGCGTC
UBA6-co-M998R_Rev	GCGTGACCGGGcctCACGGGCACGTACAGCATCTTG

UBA6-co-V990Y_Fwd	GGTGGTCCAGGGTtacAAGATGCTGTACGTGCCCCGTG
UBA6-co-V990Y_Rev	CGTACAGCATCTTgtaACCCTGGACCACCATGGTAGG
UBA6-co-M992E_Fwd	CAGGGTgtcAAGgagCTGTACGTGCCCCGTGatgC
UBA6-co-M992E_Rev	GGCACGTACAGctcCTTgacACCCTGGACCACCATG
UBA6_GSlinker_FWD	GGCGGTTCCGGTGGCTCCGGTGGGgacgagcgtaacgct
UBA6_GSlinker_REV	GGAGCCACCCGAACCGCCcttgaactcttgatcttact
UBA6-co-937-UB1-UFD_Fwd	cccatcgtggtgttcaccGAACCCCTTGCCGCACC
UBA6-co-937-UB1-UFD_Rev	GGTGCGGCAAGGGGTTcggtagaacaccacgatggg
UBA6-614_UBA1-622_FWD	CGAGTCCTACAACCTCCCAcaggaccacctgag
UBA6-614_UBA1-622_REV	cttctcaggtgggtcctgGTGGGAGTTGTAGGACTC
UBA1-891_UBA6-892_FWD	CTGATTGCAGGGAAGATCATCcccgcctatc
UBA1-891_UBA6-892_REV	gtggtagcgatagcgggGATGATCTTCCCTGCA
UBA1-621_UBA6-615_FWD	GAGTCGTACAGTTCCAGCagggaccctccagaggaagag
UBA1-621_UBA6-615_REV	cctctgagggtccctGCTGGAACGTGACGACTC
UBA6-891_UBA1-892_FWD	GTATCGCTGGCAAGATCATCccagccattgc
UBA6-891_UBA1-892_REV	cgtggcaatggctggGATGATCTTGCCAGCGATA
dUBA1-isoform1-B-Fwd	AAGTTCTGTTTCAGGGCCCCGatgctgagcggtcagcaattgatggcgg
dUBA1-full-length-B-Rev	ATGGTCTAGAAAGCTTTATTAaggcgagcgtgtaacggacgtagggcacc
UBE2A-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGAGCACACCGGCACGTGC
UBE2A-co-152-B_Rev	ATGGTCTAGAAAGCTTTAttaACAATCACGCCAGCTCTGTTCAAC
UBE2B-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGAGCACACCGGCACGTGC
UBE2B-co-152-B_Rev	ATGGTCTAGAAAGCTTTAttaGCTATCATTCCAGCTCTGTTCAACA
UBE2C-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGGCAAGCCAGAATCGTGATCC
UBE2C-co-179-B_Rev	ATGGTCTAGAAAGCTTTAttaCGGTTCTTGCTGGTAACCTG
UBE2D1_1_B_Fwd	AAGTTCTGTTTCAGGGCCCCGatggcgctgaagaggattcagaaaag
UBE2D1_147_B_Rev	ATGGTCTAGAAAGCTTTAttaCATTGCATATTTCTGAGTCCATTC
UBE2D2-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGGCCCTGAAACGCATTCAAAAG
UBE2D2-co-147-B_Rev	ATGGTCTAGAAAGCTTTAttaCATTGCATATTTCTGGGTCCATTAC
UBE2D3-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGGCACTGAAACGCATTAACAAAGAAC
UBE2D3-co-147-B_Rev	ATGGTCTAGAAAGCTTTAttaCATTGCATATTTCTGGGTCCATTAC
UBE2D4-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGGCCCTGAAACGTATTAGAAAAG
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UBE2E3-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGAGCAGCGATCGTCAGCGT
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UBE2G1-co-1-B_Fwd	AAGTTCTGTTTCAGGGCCCCGATGACCGAACTGCAGAGCGCA
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	TCTG
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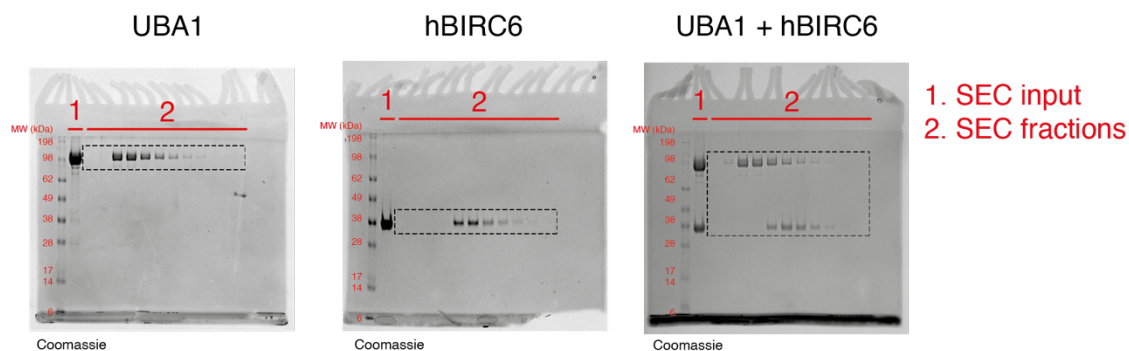
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UBE2Z-co-C188A_Rev	GGTGCCTAAAATGCTCAGGGCAACTTTACCATTGCGG
Ub_G76C-HindIII_Rev	ggtaccAAGCTTttattaacaTCCACGCAAGCGCAAAACAAGGTG
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Source Data for Supplementary Information

Uncropped SDS-PAGE gels for Supplementary Figure 2

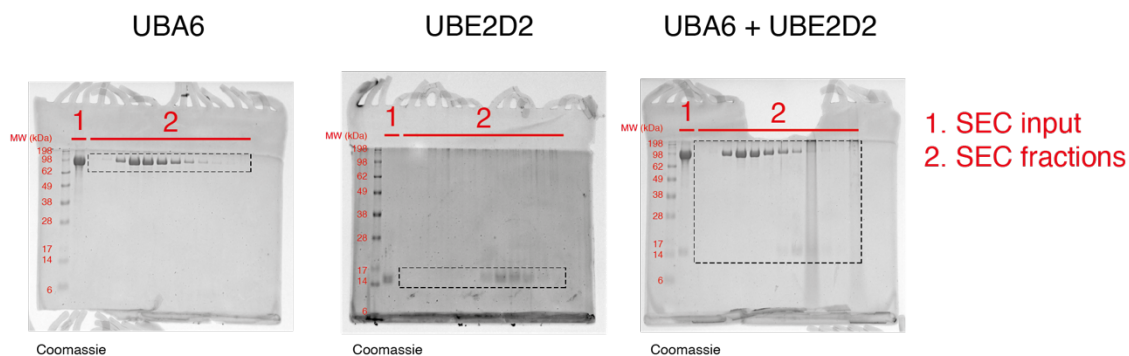
Supplementary Figure 2a

Dashed black box - cropped image in corresponding figure.



Supplementary Figure 2b

Dashed black box - cropped image in corresponding figure.



Supplementary Figure 2c

Dashed black box - cropped image in corresponding figure.

