Supporting information for

Comparison of cross-regulation by different OTUB1-E2 complexes

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SUPPLEMENTAL INFORMATION
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Tables S1 – S4

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Antibody	Catalogue Number	Supplier	Species	Dilution in 2% BSA, 0.02% Sodium Azide & PBS buffer
UBE2E1	A-630	Boston Biochem	Rabbit	1:800
UBE2E2	Ab-177485	Abcam	Rabbit	1:5000
UBE2E3	PA-551889	Thermo Fisher Scientific	Rabbit	1:100
UBE2N	4E11	Invitrogen	Mouse	1:1000
UBE2D	A-615	Boston Biochem	Rabbit	1:400
K48 Ub	4289S	Cell Signaling Technology	Rabbit	1:1000
Ub wt	sc-8017	Santa Cruz	Mouse	1:1000

Table S1. Primary Antibodies.

Antibody	Catalogue Number	Supplier	Species	Anti-Species	Dilution in 5% blocking buffer & TBST
HRP-Conjugate IgG	12-348	Millipore	Goat	Rabbit	1:5000
Alex Fluor 594 conjugate	A-21203	ThermoScientific	Donkey	Mouse	1:5000

Table S2. Secondary Antibodies

Proteins Interacting with OTUB1	K_d (μM)	Stoichiometry (N)	ΔH (kcal/mol)	ΔS (cal/mol)
UBE2D1	9.3 ± 1.7	0.8 ± 0.01	-3 ± 0.04	12.7
UBE2D2	3.9 ± 1.3	0.9 ± 0.01	-5 ± 0.12	8.5
UBE2D3	8.1 ± 3.6	1.2 ± 0.03	-3.8 ± 0.11	11.5
UBE2N	8.1 ± 2.1	0.7 ± 0.02	-5.2 ± 0.16	6
UBE2E1	7.3 ± 1.4	0.9 ± 0.01	-1.3 ± 0.03	19.3

Table S3. ITC parameters measured by titrating in OTUB1 into E2 binding partners or K48 diUbiquitin. Experimental data shown in Figure 2.

Proteins in Cell	Proteins in Syringe	K_d (μM)	Stoichiometry (N)	ΔH (kcal/mol)	ΔS (cal/mol)
OTUB1 (C91S)	K48 diUb	84.0 ± 5.0	0.8 ± 0.02	-12.8 ± 0.4	-24.1
OTUB1 (C91S) and UBE2D1	K48 diUb and UBE2D1	12 ± 3.9	1.4 ± 0.02	-10.3 ± 0.4	-12
OTUB1 (C91S) and UBE2D3	K48 diUb and UBE2D3	13.2 ± 3.6	1.1 ± 0.03	-75.3 ± 0.5	-22.2
OTUB1 (C91S) and UBE2N	K48 diUb and UBE2N	22.3 ± 3.5	2.4 ± 0.03	-2.2 ± 0.04	13.9

Table S4. ITC parameters measured by titrating in OTUB1 into E2 binding partners or K48 diUbiquitin. Experimental data shown in Figure 2.

Figure S1

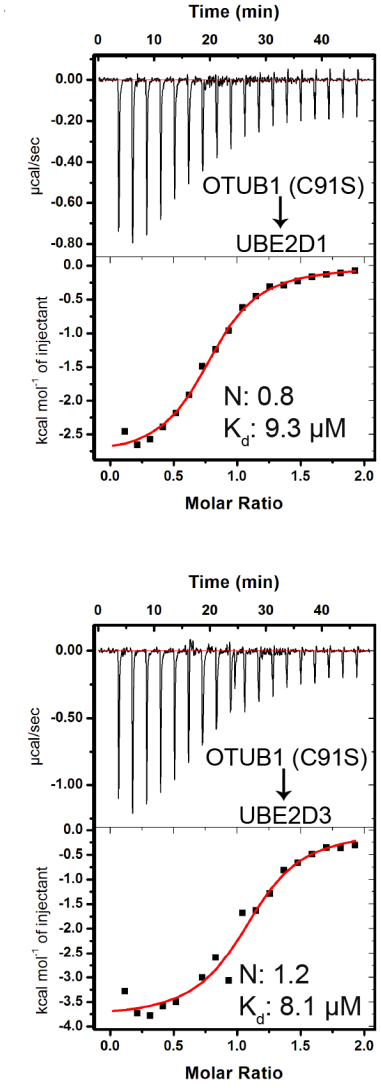

Figure S1. OTUB1 catalytic mutant binds E2s with the same affinity as wild type OTUB1. ITC measurement of the affinity of OTUB1-C91S for UBE2D1 (top) and UBE2D3 (bottom).

Figure S2

A **B**

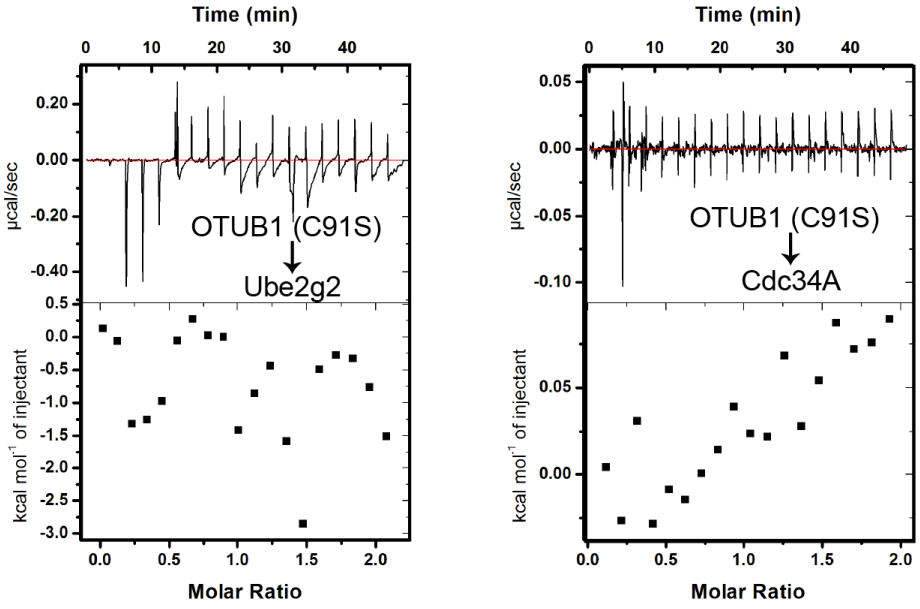

Figure S2. OTUB1 does not bind to CDC34A or UBE2G2. ITC measurements in which OTUB1 (1.5 mM) is titrated into a cell containing 150 µM of either Cdc34A or Ube2g2.

Figure S3

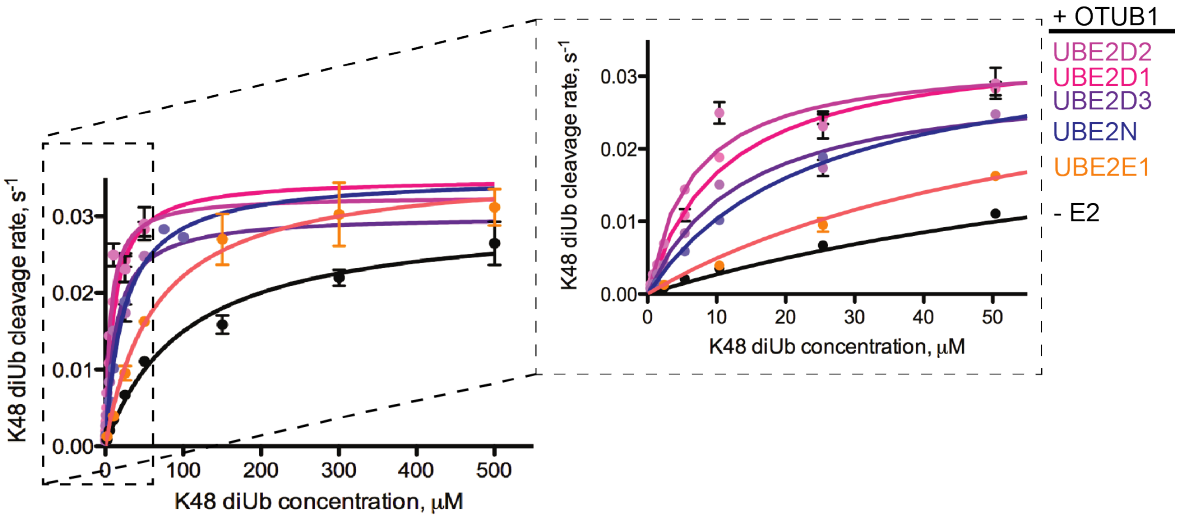

Figure S3. Full kinetic titration showing effect of E2 enzymes on OTUB1 DUB activity Full range titration of K48 diUb (0.4 — 500 μM) to track cleavage by OTUB1 (50 nM) in the absence and presence of UBE2D1, UBE2D2, UBE2D3, UBE2N, and UBE2E1 (10 μM). Inset indicates region of plot that is shown in Figure 4a.

Figure S4

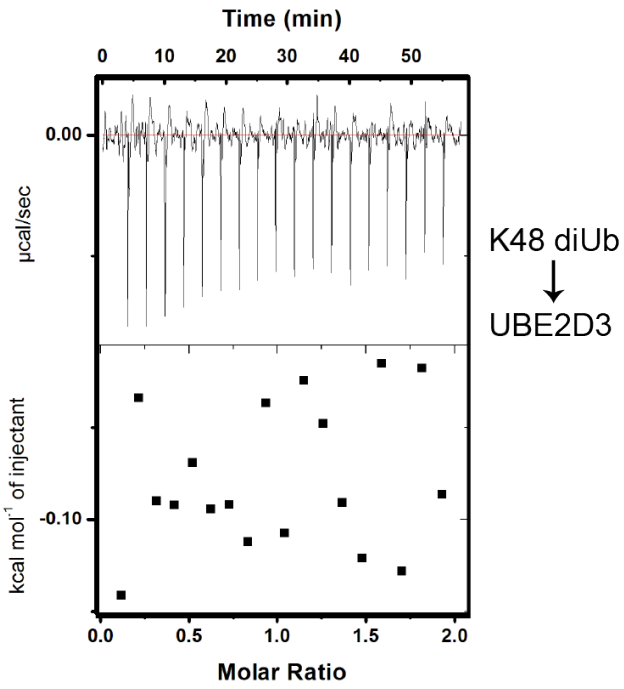

Figure S4. UBE2E3 does not interact detectably with K48 dibuquitin. ITC measurements of Binding of UBE2D3 to K48 diubiquitin.

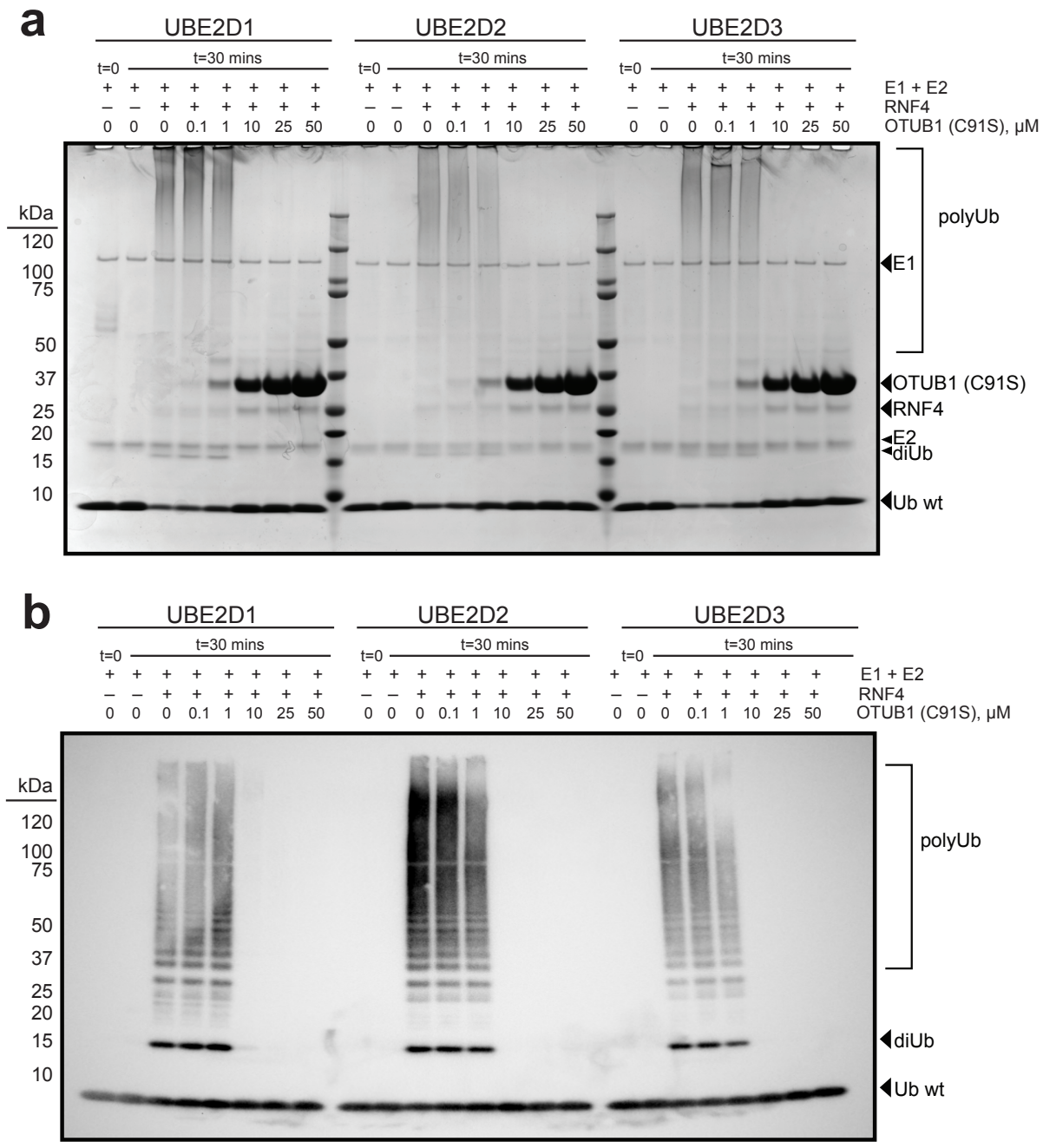

Figure S5. OTUB1 facilitated inhibition of UBE2D. End point reactions quenched at t= 0 and 30 mins with increasing concentrations of OTUB1 (C91S) (0-50 μM). Each reaction contained 150 nM E1, 2 μM E2, 50 μM Ub wt, and where applicable, 2 μM E3. **(a)** SDS-PAGE gel stained with Coomassie showing UBE2D(1-3) inhibition by OTUB1 **(b)** UBE2D inhibition by OTUB1 transferred to membrane and blotted against Ub wt

Figure S6

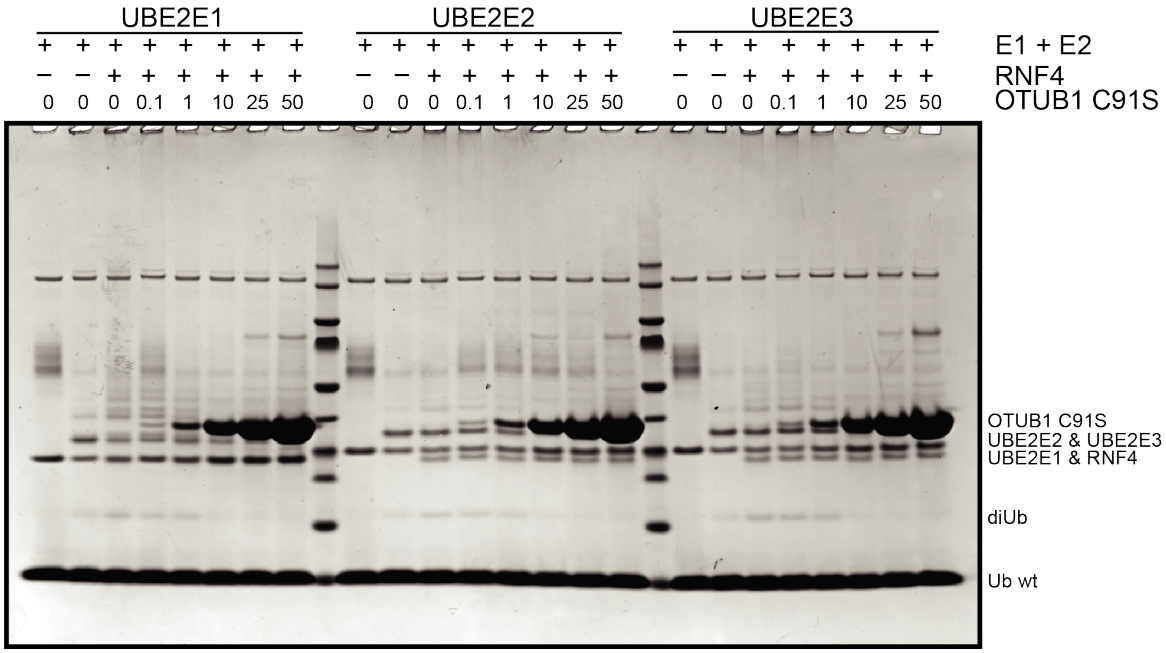

Figure S6. Coomassie stained gel of OTUB1 facilitated inhibition of UBE2E proteins. End point reactions quenched at t= 0 and 30 mins with increasing concentrations of OTUB1 (C91S) (0-50 μM). Each reaction contained 150 nM E1, 2 μM E2, 50 μM Ub, and where applicable, 2 μM E3.