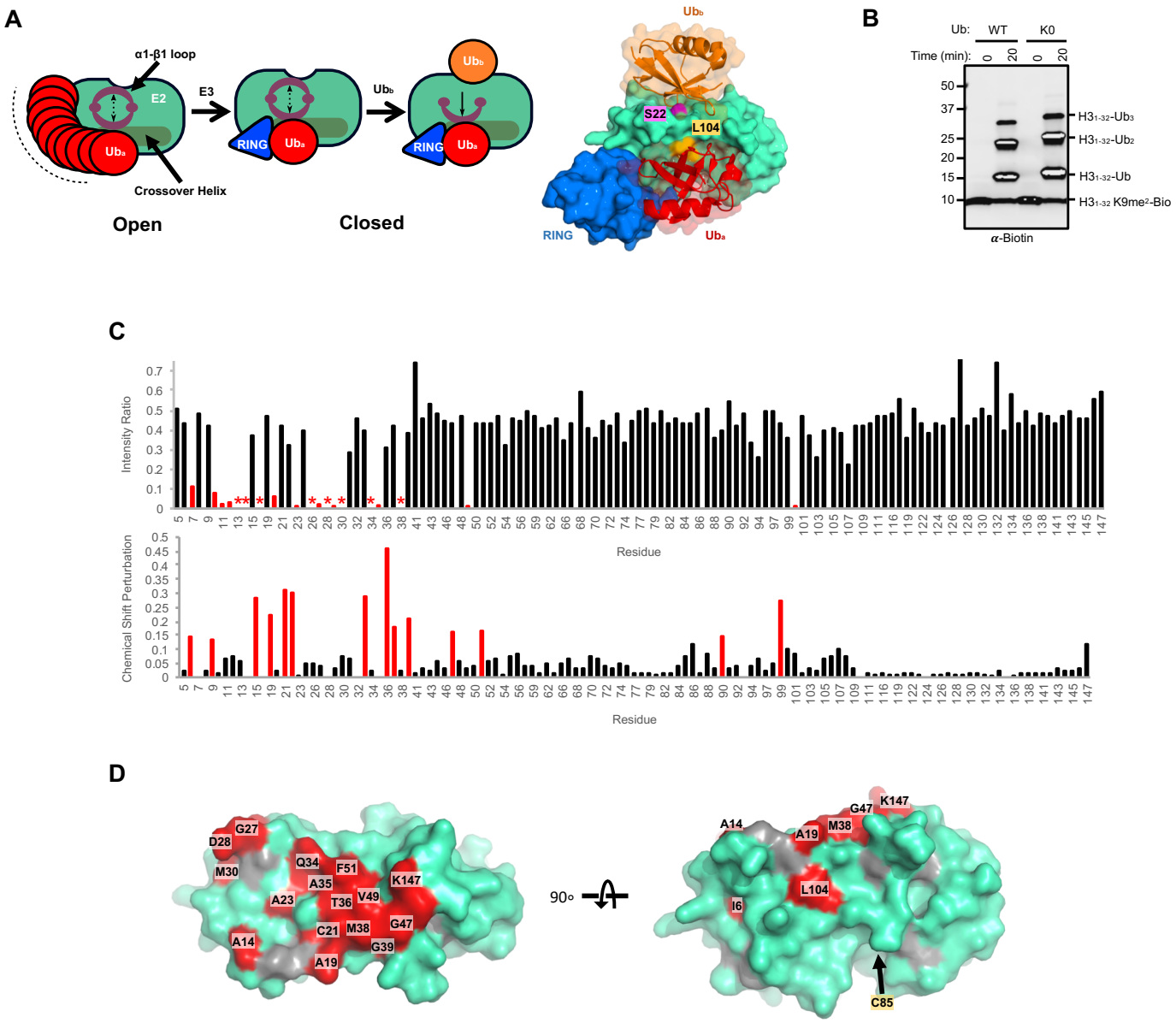
Supplemental Information

A bifunctional role for the UHRF1 UBL domain in the control of hemi-methylated DNA-dependent histone ubiquitylation

Paul A. DaRosa, Joseph S. Harrison, Alex Zelter, Trisha N. Davis, Peter Brzovic, Brian Kuhlman, Rachel E Klevit



Supplemental Figure S1 | Related to Figures 1 and 2. The UHRF1-UBL binds to the backside of Ube2D E2s

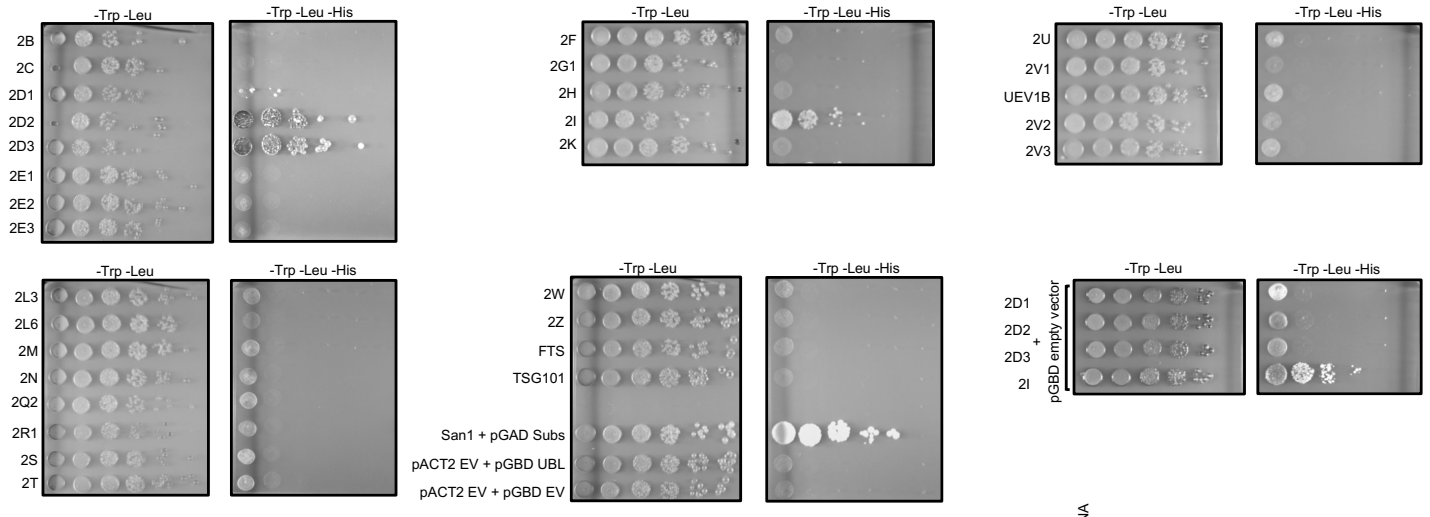
(A) Cartoon of the E2~Ub activation mechanism by RING E3 ubiquitin ligases. In the absence of a RING, the conjugated ubiquitin is flexible relative to the E2 (*left*). RING domain binding promotes a "closed" conformation, where the thioester-linked ubiquitin (Ub_a) interacts with a face on the E2 to facilitate aminolysis (*left center*). Binding of Ub at a noncovalent site (Ub_b; *orange*) further promotes the closed conformation, purportedly by inducing the α1-β1 loop of Ube2D into a conformation that interacts with Ub_a (*right center*). Structure of the RNF38-Ube2D2-Ub complex (PDB 3V4L) showing the locations of the noncovalent and thioester-linked

ubiquitin in the closed conformation (*right*). The location of residues at the center of the E2/Ub_b and E2/Ub_a interfaces are shown for orientation (S22 and L104 respectively).
(B) Substrate (H3-peptide, $H3_{1-32}K9me^2$-biotin) ubiquitylation assay with either WT ubiquitin or K0 ubiquitin (mutations: K6R, K11R, K27M, K29R, K33R, K48R, K63R) in the presence of he5mc DNA.
(C) ^1H-^{15}N HSQC intensity ratios (bound/free intensity; *top*) and chemical shift perturbations (CSPs; *bottom*) upon addition of 450 μM UHRF1-UBL to 150 μM ^{15}N-Ube2D3. Red bars and/or red asterisks indicate residues corresponding to $^1H^{15}N$-HSQC peaks with CSPs and peak intensity reductions greater than the average shift/intensity loss by one standard deviation. These residues are plotted on the surface in Figure 2C.
(D) Surface representation of the binding site of ubiquitin (125 μM) on ^{15}N-Ube2D3 (250 μM) analyzed as in panel C and Figure 2C from previously published data (Brzovic and Klevit, 2006).

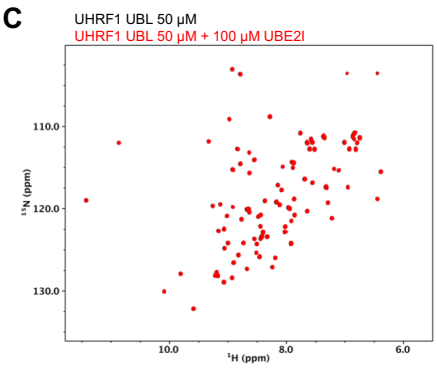
A

AD: E2 (pACT2)	Ube2B	Ube2C	Ube2D1	Ube2D2	Ube2D3	Ube2E1	Ube2E2	Ube2E3	Ube2F	Ube2G1	Ube2H	Ube2I	Ube2K	Ube2L3	Ube2L6	Ube2M	Ube2N	Ube2Q2	Ube2R1	Ube2S	Ube2T	Ube2U	Ube2V1	Ube2V2	UbesV1 Isoform 1	Ube2V2	Ube2V3	Ube2W	Ube2Z	AKTIP	TSG101	Empty Vector
BD: UHRF1 UBL [pGBD(120)]	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
pGBD(120) Empty Vector	ND	ND	–	–	–	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–

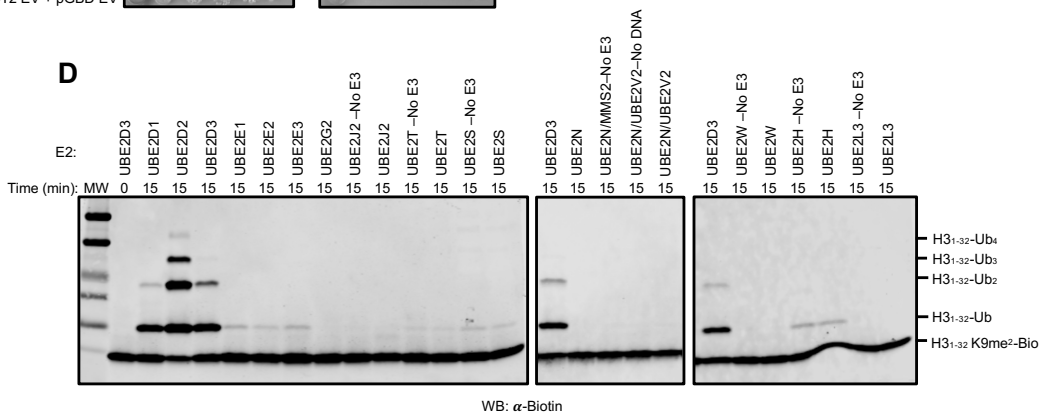
B



C



D



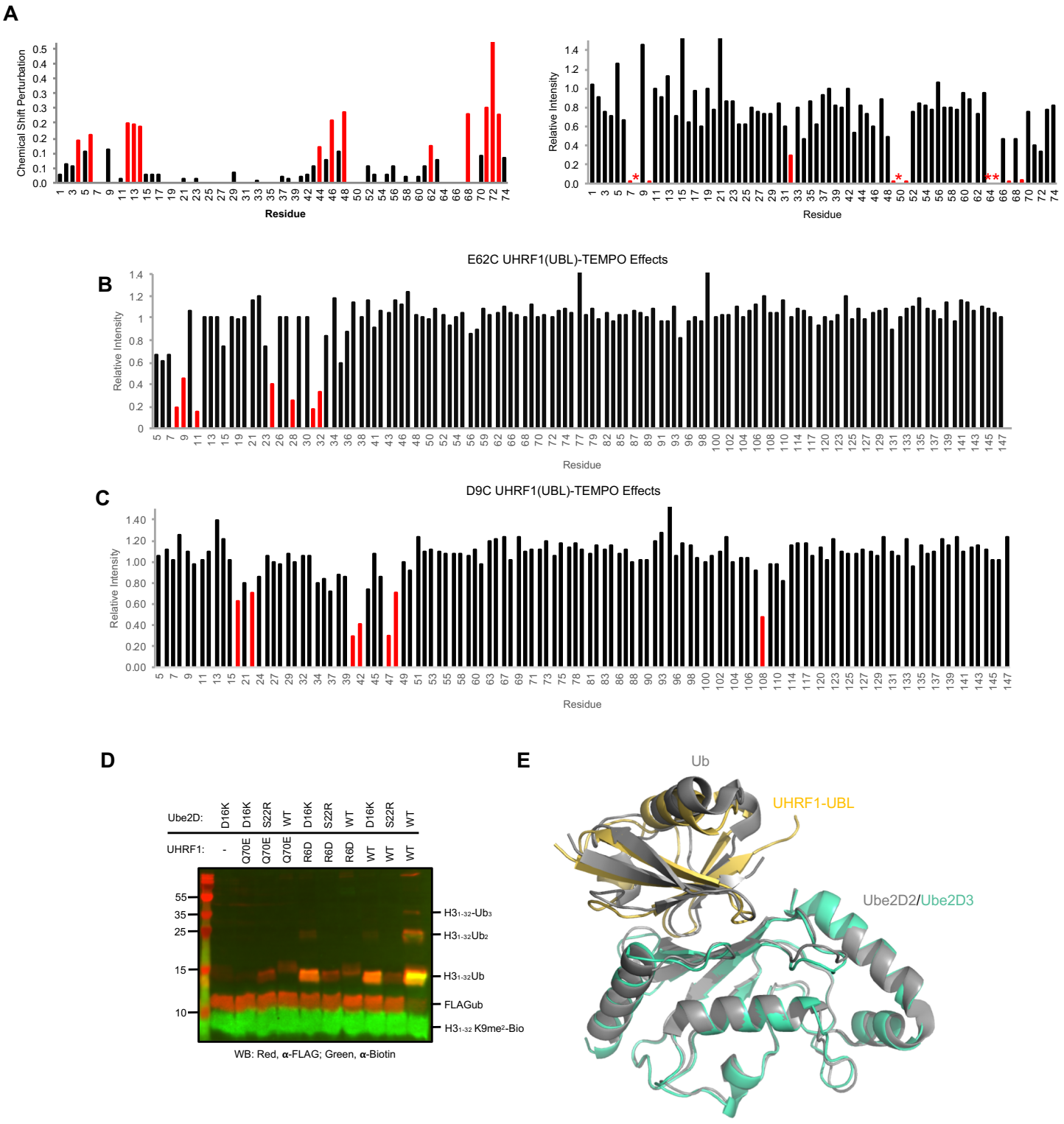
Supplemental Figure S2 | Related to Figure 2. Ube2Ds support UHRF1 histone ubiquitylation

(A) Summary of directed yeast two-hybrid (Y2H) of UHRF1-UBL (BD; bait in pGBD-C1 a.k.a. pBGD(120) vector) against 31 different E2s (AD; prey in pACT2 vector).

(B) Y2H growth with indicated transfections summarized in (A). San1 + pGAD Sub indicates a positive control (Rosenbaum et al., 2011).

(C) $^{1}H^{15}N$-HSQC of 50 μM ^{15}N-UHRF1-UBL in the absence (black) or presence (red) or 100 μM Ube2I. Ube2I showed a positive interaction in the Y2H with UHRF1-UBL and the empty pGBD(120) vector, but no interaction was seen by NMR with Ube2I.

(D) UHRF1 substrate ($H3_{1-32}K9me^{2}$-biotin; $H3_{1-32}$) ubiquitylation with the indicated E2 enzymes. Only Ube2D family members show E3-dependent ubiquitylation of $H3_{1-32}K9me^{2}$. Western blots (WB) are for the C-terminal biotin tag on $H3_{1-32}K9me^{2}$.



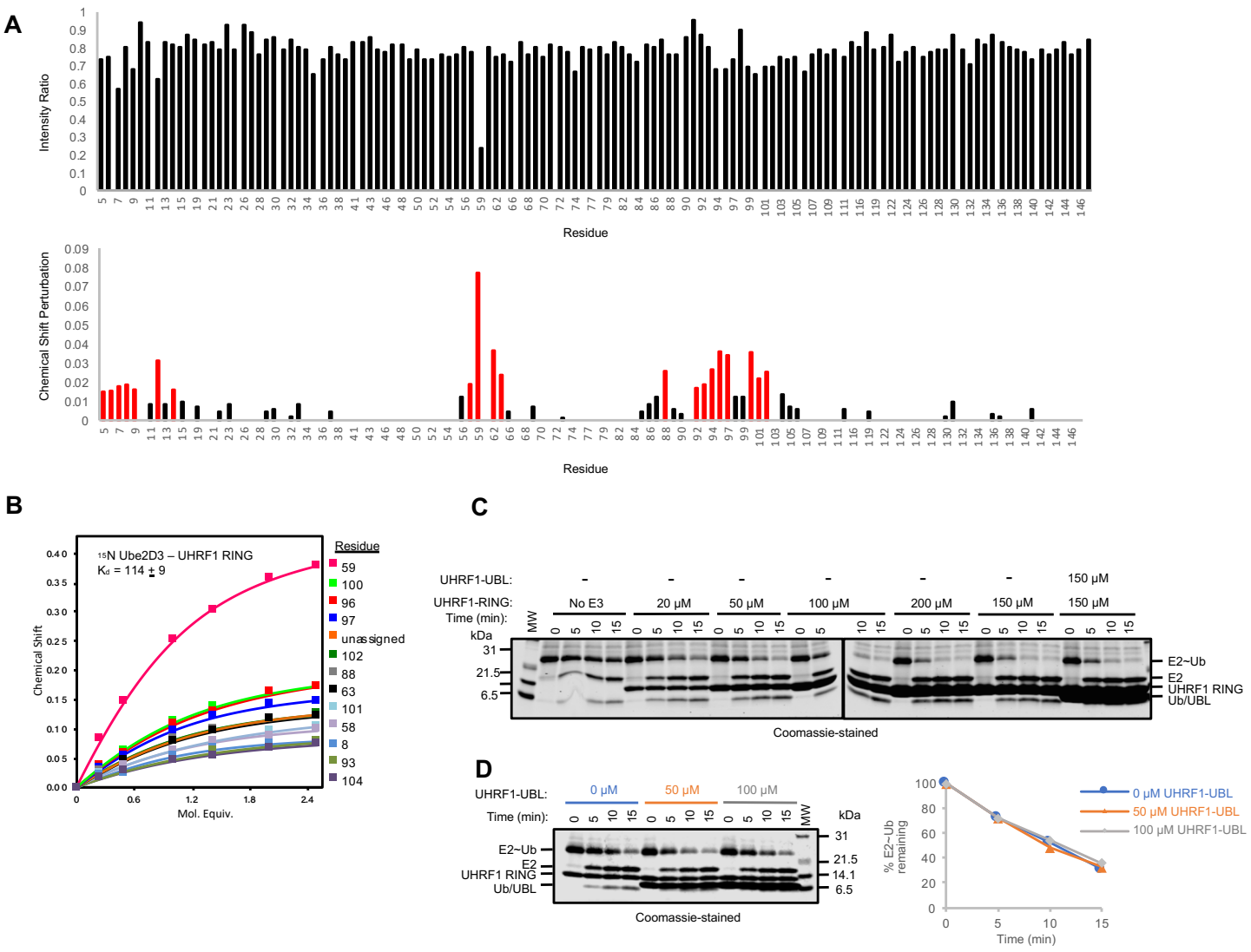
Supplemental Figures S3 | Related to Figure 3. The UHRF1-UBL binds to the backside of Ube2D using a similar surface as Ub

(A) CSPs (*left*) and intensity ratios (*right*) upon addition of 225 μM Ube2D3 to 150 μM ^{15}N-UHRF1-UBL. The binding residues colored in (A) are plotted on the surface of the UBL structure in Figure 3A (*right*).

(**B and C**) Effects of the paramagnetic spin label TEMPO conjugated at positions E62 (*B*) and D9 (*C*) of the UHRF1-UBL on ^{15}N-Ube2D. Red bars indicated the top 5% of peaks that experience spin label effects in the ^{1}H^{15}N-HSQC (highlighted on the surface of the E2 shown in Figure 3B).

(**D**) Substrate **(**$H3_{1-32}K9me^{2}$-biotin; $H3_{1-32}$) ubiquitylation reactions between pairs of Ube2D1 and UHRF1 mutants designed to test aspects of the preliminary models of UHRF1-UBL/Ube2D. This is the same gel as shown in Figure 3C, but with additional lanes showing Q70E-UHRF1, which was omitted for simplicity.

(**E**) Structural alignment of the UHRF1-UBL/Ube2D3 model (with the UBL in gold and Ube2D3 in green) with the backside ubiquitin interaction seen in PDB 3V4L (gray) using the E2 enzymes to align the structures. While there are differences in the interactions, the relative orientation of the proteins is strikingly similar.



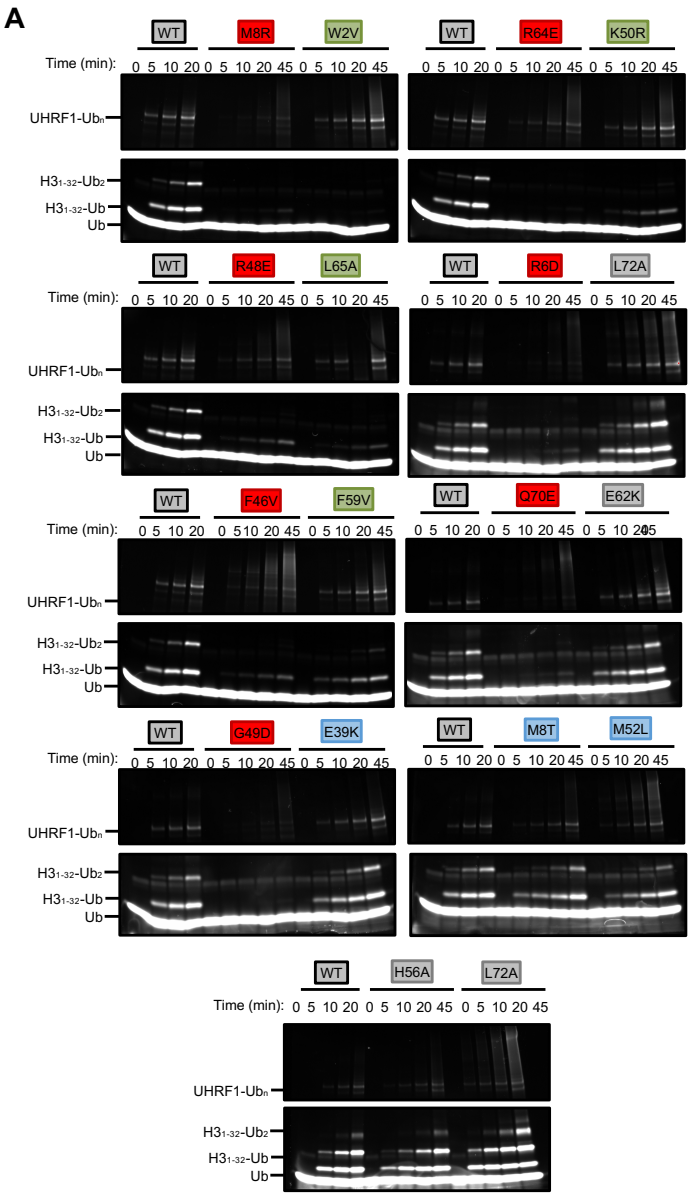
Supplemental Figures S4 | Related to Figure 4. The UHRF1-RING binding and activity is not affected by the UHRF1-UBL

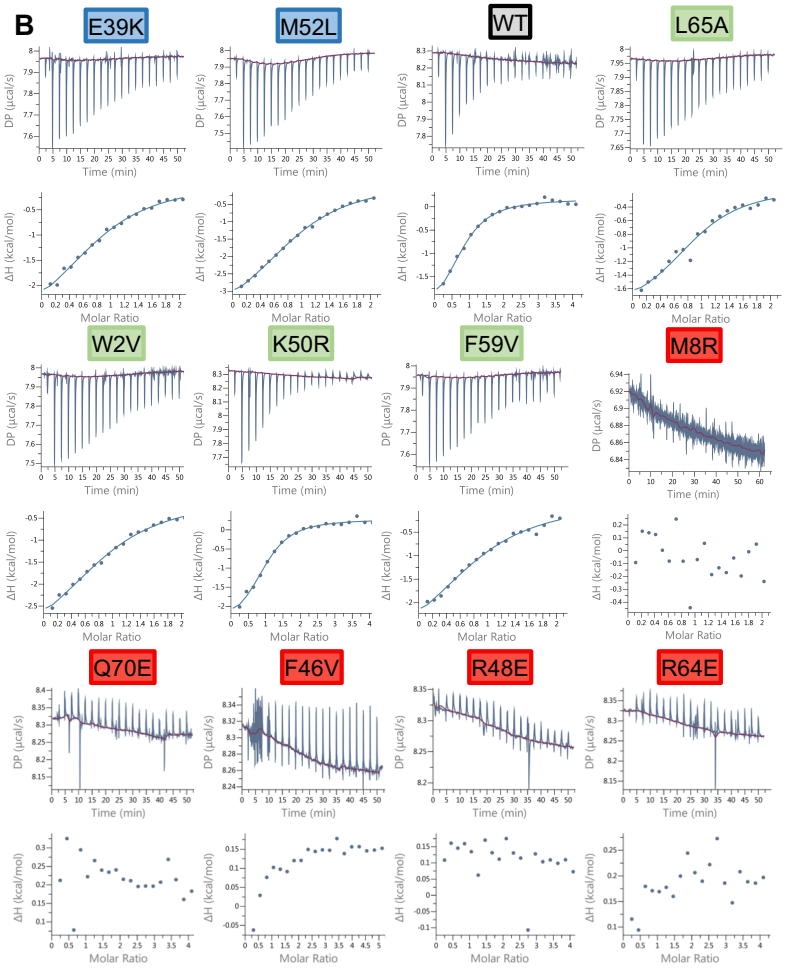
(A) Peak intensity ratios (*top*) and chemical shift perturbations (CSPs; *bottom*) of Ube2D induced by UHRF1-RING binding: 50 μM UHRF1-RING domain was added to 200 μM ^{15}N Ube2D3(S22R/C85S). Red bars indicate residues corresponding to ^{1}H^{15}N-HSQC peaks with CSPs greater than the average shift by one standard deviation. These residues are plotted on the Ube2D3 surface in Figure 4A.

(B) Binding curves generated from ^{1}H^{15}N-HSQC peak chemical shift perturbations of 200 μM ^{15}N-Ube2D3(S22R/C85S) as a function of UHRF1-RING concentration. The K_d value was determined from a 1-site, group fitting of the curves shown.

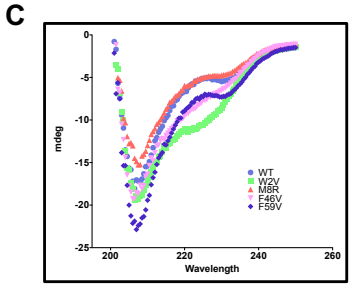
(**C**) Aminolysis of wild type Ube2D3~Ub (thioester linked) in the presence of increasing UHRF1-RING domain and 40 mM lysine and, where indicated, 150 μM UHRF1-UBL. The presence of the UBL has no detectible enhancement of UHRF1-RING mediated aminolysis of the E2~Ub conjugate.

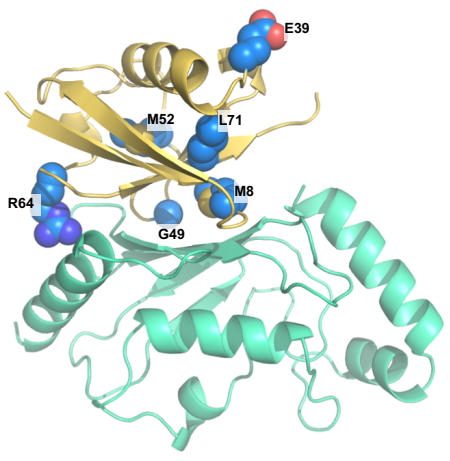
(**D**) Aminolysis assay as in (C) with 20 μM UHRF1-RING and increasing UHRF1-UBL as indicated. Densitometry (*right)* shows clearly that the UHRF1-UBL does not enhance RING-mediated aminolysis of Ube2D3~Ub.





UBL	N	Kd (µM)	Kd Error (µM)	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
WT	0.80	29.0	6.9	-2.67	-6.19	-3.52
W2V	0.95	41.7	14.7	-3.62	-5.98	-2.36
K50R	0.96	20.4	4.4	-2.85	-6.4	-3.55
F59V	0.93	47.2	26.5	-3.43	-5.9	-2.47
L65A	0.93	22.1	13.3	-1.87	-6.35	-4.48
E39K	0.89	40.1	15.6	-3.11	-6	-2.89
M52L	0.95	34.5	5.8	-4.24	-6.09	-1.85
Q70E	NA	ND	NA	NA	NA	NA
F46V	NA	ND	NA	NA	NA	NA
R48E	NA	ND	NA	NA	NA	NA
R64E	NA	ND	NA	NA	NA	NA
M8R	NA	ND	NA	NA	NA	NA





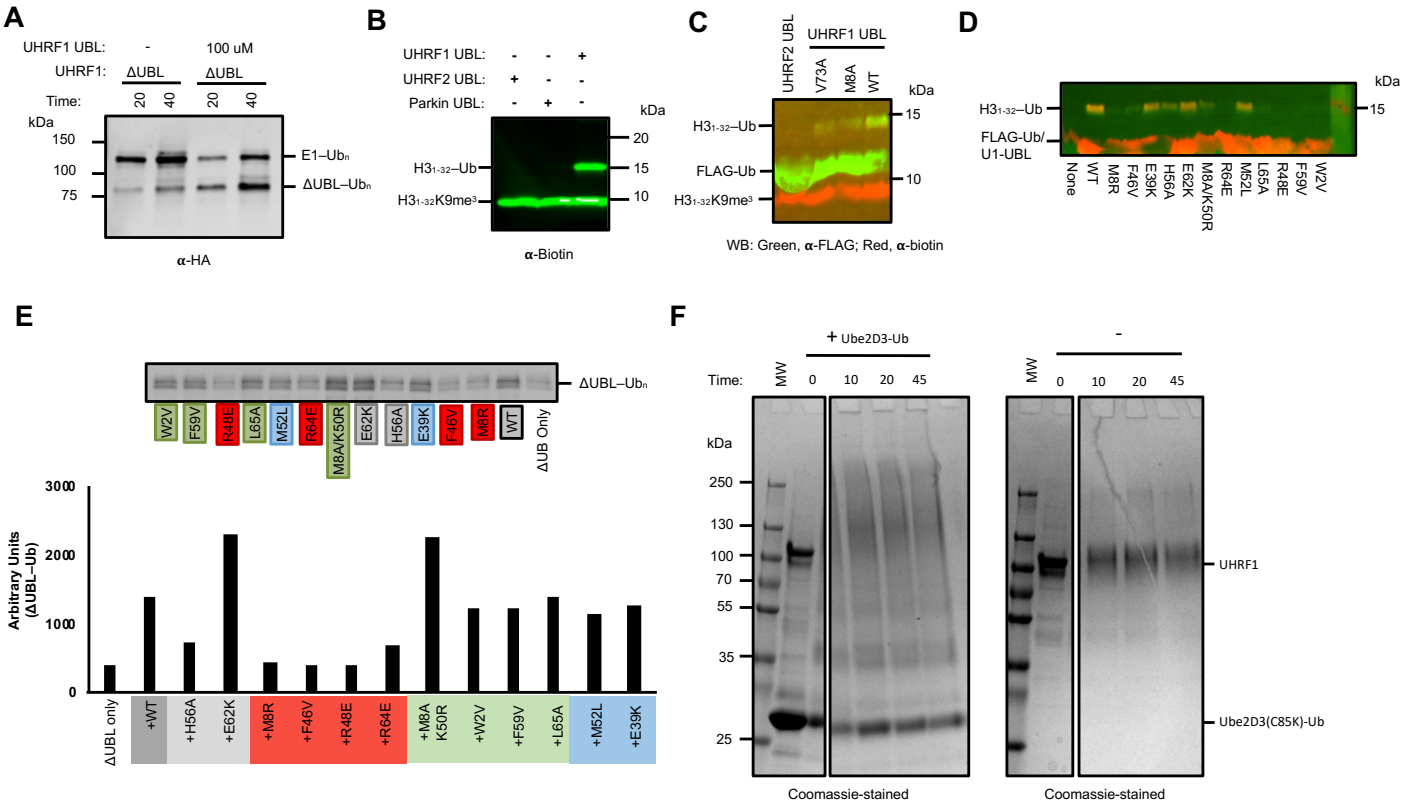
Supplemental Figure S5 | Related to Figure 5. Mutant UHRF1 ubiquitylation and UBL binding properties

(A) Full-length UHRF1 H3-peptide ($H3_{1-32}K9me^2$-biotin; $H3_{1-32}$) time course ubiquitylation reactions with the indicated mutants. These assays show representative gels of biological duplicates used to quantify rates from densitometry shown in Figure 5B.

B) ITC isotherms of binding between UBL variants and Ube2D1 (*top)* and a table of thermodynamic parameters derived from the titrations (*bottom*). Binding curves were fit to a single-site binding model. Mutant names are colored as in Figure 5A and B.

(**C**) Circular dichroism of the indicated UBL mutants at 20 μM concentration. The peak at 207 nm is indicative of a folded β-sheet-containing protein.

(D) The location of the UBL mutations identified in human cancers found in COSMIC are shown in spheres in the model of the UBL/E2 interaction (see **Supplemental File 2**).



Supplemental Figure S6 | Related to Figure 5. A surface of the UHRF1-UBL, not involved in E2 binding, is essential for histone ubiquitylation.

(A) ΔUBL-UHRF1 auto-ubiquitylation (ΔUBL-Ub) rescue experiments with the indicated UBLs at 100 μM concentration.

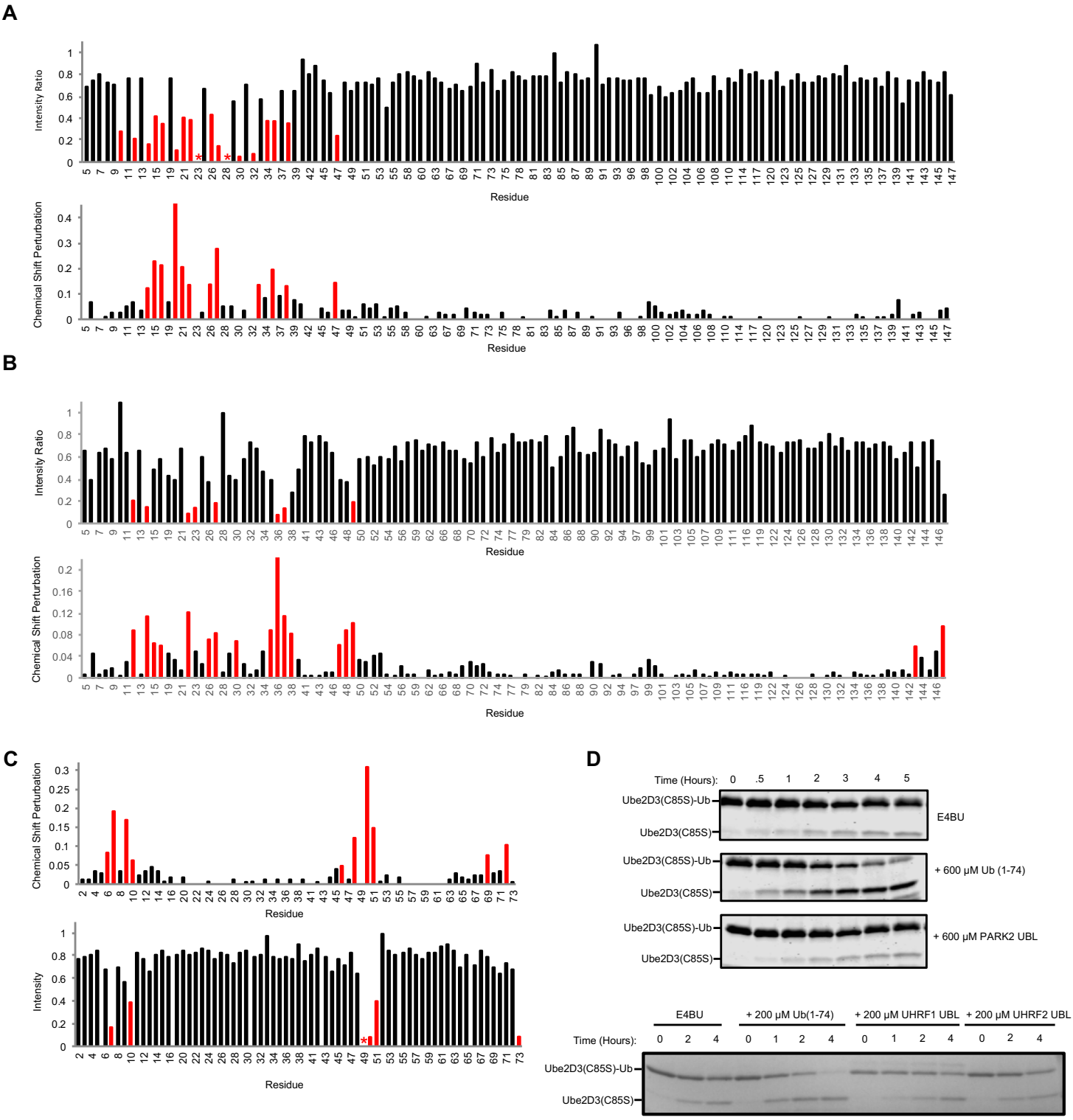
(**B**) ΔUBL-UHRF1 substrate ($H3_{1-32}K9me^2$-biotin; $H3_{1-32}$) ubiquitylation rescue experiments with 100 μM of the indicated UBLs and Ube2D3.

(**C**) ΔUBL-UHRF1 substrate ($H3_{1-32}$) ubiquitylation rescue experiments with the indicated UBLs and Ube2D1. In this experiment the UHRF2 UBL is present at 500 μM, well above the measured K_d of binding to Ube2D (see Figure 6).

(**D**) ΔUBL -UHRF1 substrate ($H3_{1-32}K9me^2$-biotin; $H3_{1-32}$) ubiquitylation rescue experiments with the indicated UHRF1-UBL mutants added *in trans* with Ube2D1. This blot and a replicate with most of the UBL variants tested are quantified in Figure 5F.

(**E**) ΔUBL-UHRF1 auto-ubiquitylation rescue experiments with the indicated UBL variants at 100 μM (*top*). Densitometry quantification of the ΔUBL auto-ubiquitylation in the above blot (*bottom*). The mutations are colored as in Figure 5A.

(**F**) UHRF1 crosslinking with BS3 (lysine-lysine crosslinker) in the presence of he5mc DNA, a $H3_{1-32}K9m^2$ peptide and, where indicated, a stable isopeptide-linked Ube2D-Ub conjugate (Ube2D3(C85K)-Ub) at the indicated time (min) (see Methods).


A
Intensity Ratio
Residue
Chemical Shift Perturbation
Residue
B
Intensity Ratio
Residue
Chemical Shift Perturbation
Residue
C
Chemical Shift Perturbation
Residue
Intensity
Residue
D
Time (Hours): 0 .5 1 2 3 4 5
Ube2D3(C85S)-Ub
Ube2D3(C85S)
E4BU
Ube2D3(C85S)-Ub
Ube2D3(C85S)
+ 600 μM Ub (1-74)
Ube2D3(C85S)-Ub
Ube2D3(C85S)
+ 600 μM PARK2 UBL
E4BU
+ 200 μM Ub(1-74)
+ 200 μM UHRF1 UBL
+ 200 μM UHRF2 UBL
Time (Hours): 0 2 4 0 1 2 4 0 1 2 4 0 2 4
Ube2D3(C85S)-Ub
Ube2D3(C85S)

Supplemental Figure S7 | Related to Figure 6. UHRF2 and PARKIN UBLs also bind to Ube2D.

(**A**) $^{1}H^{15}N$-HSQC Ube2D3(C85S) intensity ratios (*top*) and CSPs (*bottom*) upon addition of 150 μM UHRF2 UBL to 150 μM ^{15}N Ube2D3(C85S).

(**B**) $^{1}H^{15}N$-HSQC Ube2D3(C85S) intensity ratios (*top*) and CSPs (*bottom*) upon addition of 150 μM PARKIN UBL to 150 μM ^{15}N Ube2D3(C85S).

(C) $^{1}H^{15}N$-HSQC UHRF2 UBL CSPs (*top*) and intensity ratios (*bottom*) upon addition of 75 μM Ube2D3(C85S) to 150 μM ^{15}N-UHRF2 UBL. Red bars and/or red asterisks indicate residues with CSPs/intensity loss greater than 1 standard deviation from the mean. These residues are plotted on the surface representations of Ube2D and UHRF2 UBL in Figure 6. Assignments for the PARKIN UBL were not obtained.

(**D**) E2-Ub hydrolysis assay showing hydrolysis of Ube2D3(C85S)-O-Ub (oxyester-linked) in the presence of the E4B U-box domain (E4BU; residues 1092–1173) and 600 μM Ub(1-74) (residues 1-74 of Ub), 600 μM Parkin UBL (*top*), or 200 μM Ub(1-74), 200 μM UHRF1-UBL, or 200 μM UHRF2 UBL (*bottom*). While Ub(1-74) promotes the hydrolysis of Ube2D3(C85S)-O-Ub, no additional hydrolysis is observed for UHRF1-UBL, UHRF2 UBL, and the Parkin UBL.