## Supplementary Material

# Essentiality of a non-RING element in priming donor ubiquitin for catalysis by a monomeric E3

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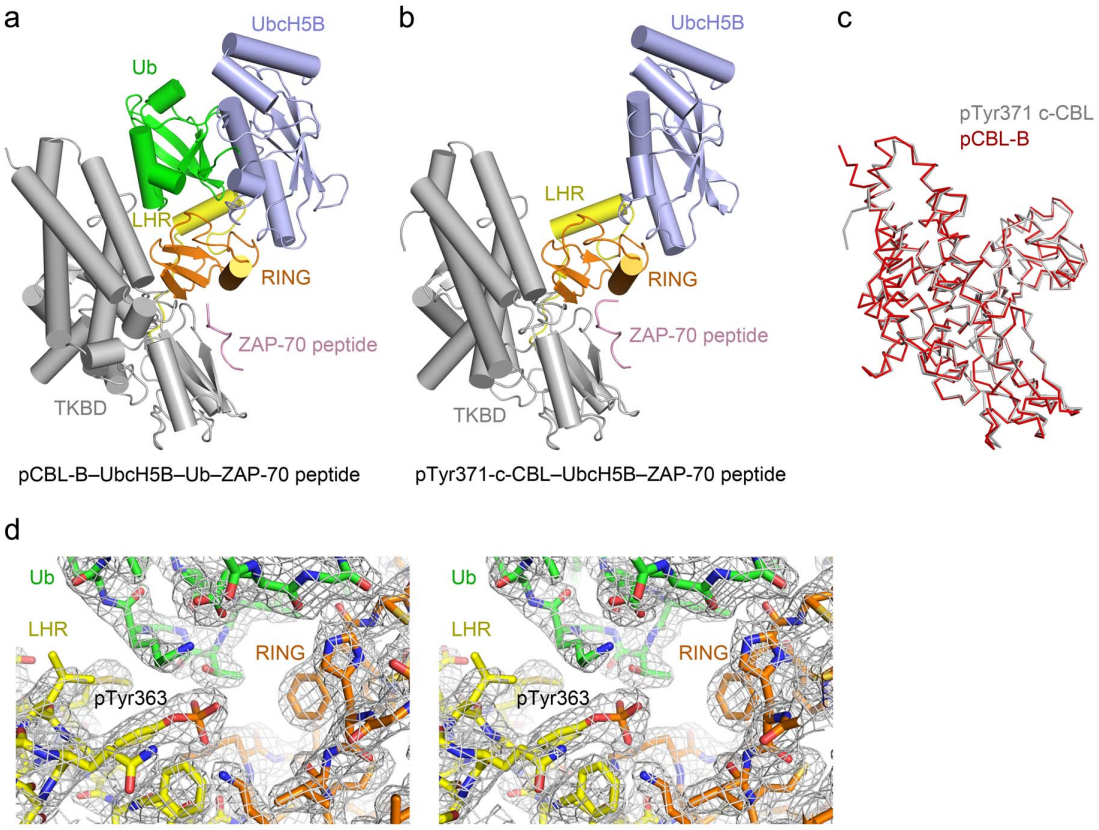
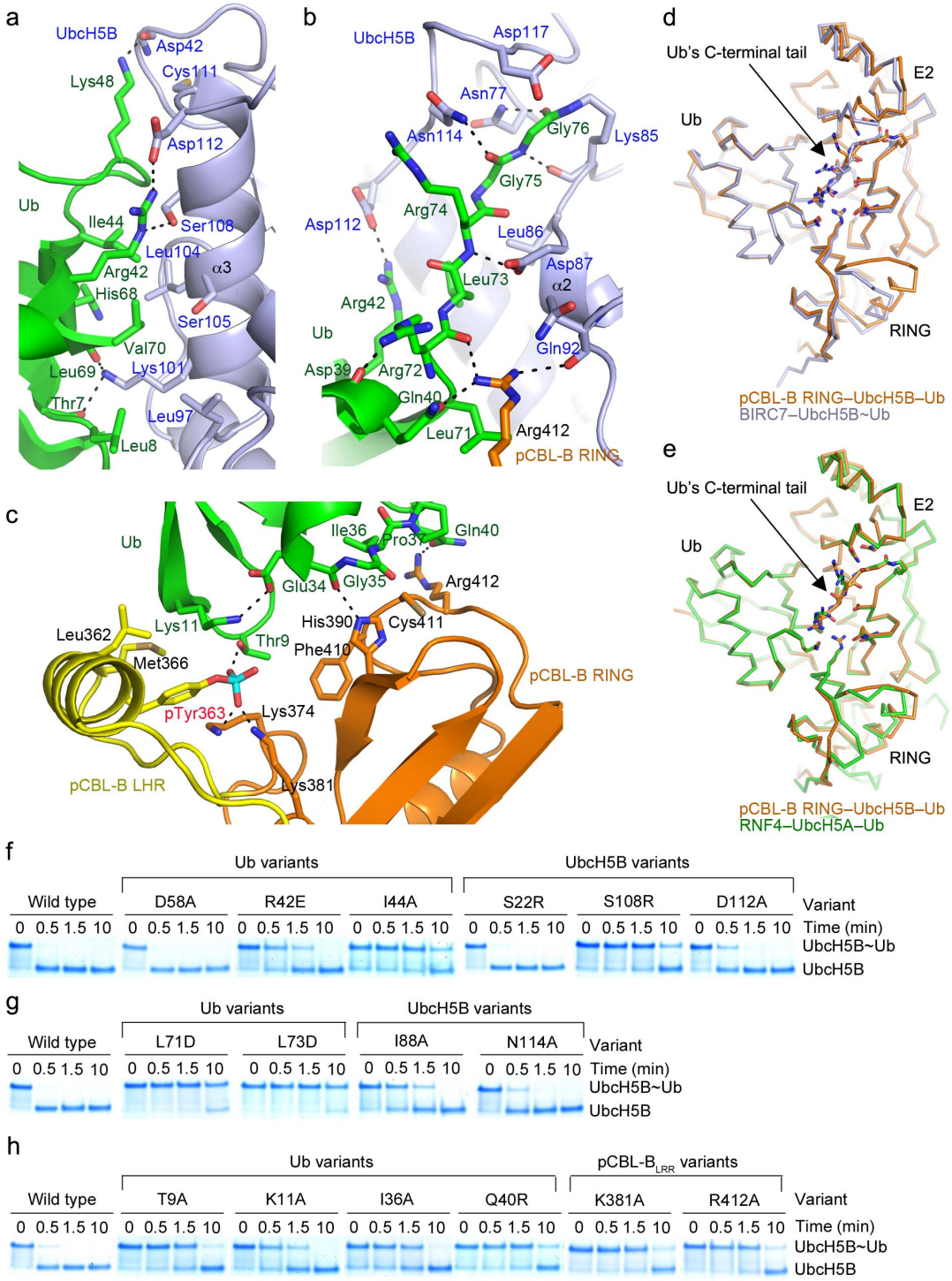
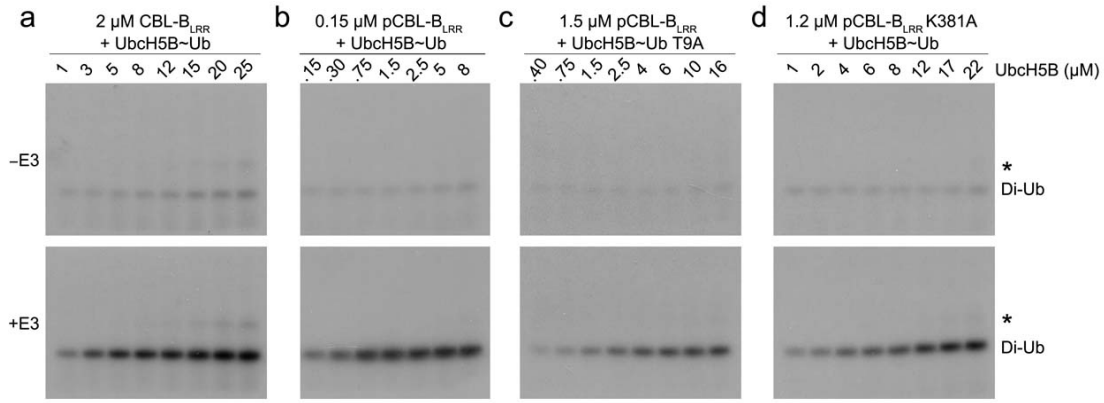



**Supplementary Figure 1.** The structure of pCBL-B–UbcH5B–Ub–ZAP-70 peptide complex is nearly identical to pTyr371-c-CBL–UbcH5B–ZAP-70 peptide complex. (**a**) Structure of pCBL-B–UbcH5B–Ub–ZAP-70 peptide complex. (**b**) Structure of pTyr371-c-CBL–UbcH5B–ZAP-70 peptide complex (PDB 4A4C[1]). Coloring for **a** and **b** is as described in **Figure 1**. (**c**) Superposition of pCBL-B (red) and pTyr371-c-CBL (gray) (r.m.s. deviation of 0.65 Å for all Cα atoms). (**d**) Stereo view of pCBL-B–Ub interface in Chains E–H. Residues are shown as sticks and pTyr363 is labeled. Coloring is as described in **Figure 1**, with N atoms blue, O atoms red and S atoms yellow. 2Fo – Fc electron density (light gray) contoured at 1.0σ is shown.

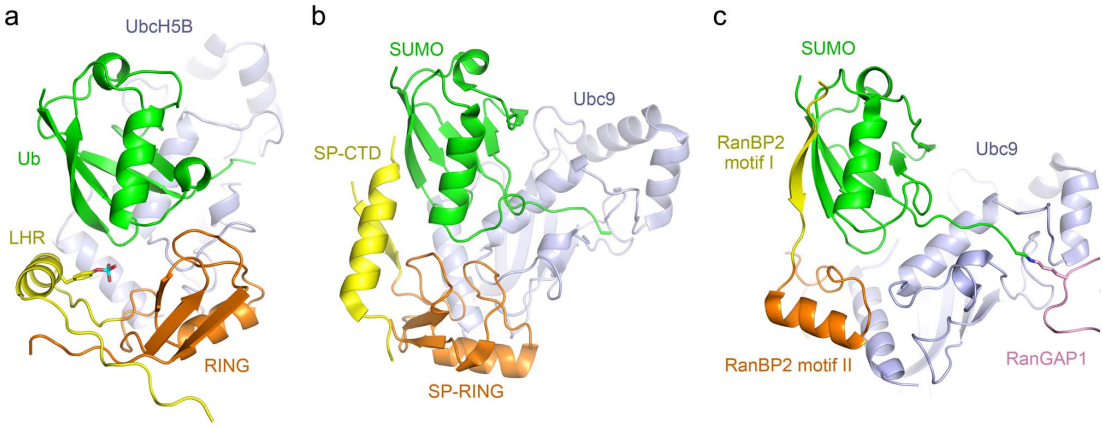



**Supplementary Figure 2.** Effects of pCBL-B$_{LRR}$, UbcH5B and Ub mutations on Ub transfer. (**a**) Close-up of interactions between Ub's Ile44 surface and UbcH5B's α3. (**b**) Close-up of interactions between Ub's tail and UbcH5B's α2. (**c**) Close-up of interactions between Ub's Ile36 surface and pCBL-B's LHR and RING domain. Coloring for **a–c** is as described in **Figure 1**. (**d,e**) Superposition of the RING–E2–Ub portion of pCBL-B complex (orange) onto the BIRC7–UbcH5B~Ub[2] (**d**, light blue)

and RNF4–UbcH5A–$Ub^3$ (**e**, green) complexes. The side chain of Ub's C-terminal tail residues and the corresponding contacting E2 and E3 residues are displayed as sticks, revealing nearly identical Ub interactions. (**f–h**) Non-reduced SDS-PAGE of pulse-chase lysine discharge reactions showing the disappearance of UbcH5B~Ub with pCBL-$B_{LRR}$, UbcH5B and Ub variants over time. Selected mutations on residues shown in **a**, **b**, and **c** were tested in **f**, **g**, and **h** respectively. Ub D58A, which opposes the Ile36 and Ile44 surfaces, was included as a control mutant. UbcH5B S22R, incorporated into our crystallization variant, displays similar activity to wild type.




**Supplementary Figure 3.** Representative reduced autoradiograms of di-Ub kinetic assays. (**a–d)** Reactions were performed in the absence (upper panel) or in the presence of E3 (lower panel). Reactions performed in the absence of E3 were used for background subtraction during quantification (see Methods). CBL-$B_{LRR}$ and Ub variants are specified at the top of each panel. Final UbcH5B and E3 concentrations are indicated. Asterisks show non-reducible E2–Ub bands.




**Supplementary Figure 4.** Comparison of E3–E2–Ub and E3–E2–SUMO complexes. (**a**) A portion of pCBL-B–UbcH5B–Ub–ZAP-70 peptide structure lacking the TKBD. Coloring is as described in **Figure 1**. (**b**) Model of a portion of Siz1 RING E3 (PDB 3I2D[4]) bound to Ubc9 and SUMO generated by aligning Siz1 RING domain to pCBL-B RING domain in **a** and by superposing Ubc9 with SUMO from PDB 1Z5S[5] onto UbcH5B in **a**. (**c**) Structure of RanBP2–Ubc9–SUMO–RanGAP1 complex (PDB 1Z5S) displayed by aligning SUMO to Ub in **a**. The E2-binding domains are colored in orange, E2 in light blue, Ub and SUMO in green and the additional Ub- and SUMO-interacting component in yellow.

**Supplemental References**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3. Plechanovova, A., Jaffray, E.G., Tatham, M.H., Naismith, J.H. & Hay, R.T. Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis. *Nature* **489**, 115-20 (2012).
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