

Coupled monoubiquitylation of the co-E3 ligase DCNL1 by Ariadne-RBR E3 ubiquitin ligases promotes cullin-RING ligase complex remodeling

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Running title: *DCNL1 monoubiquitylation by Ariadne E3 ligases*

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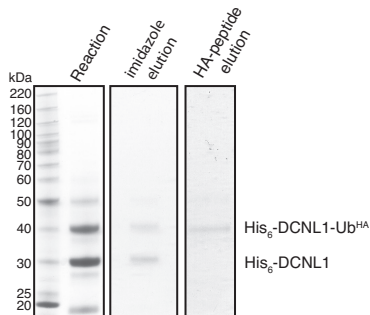
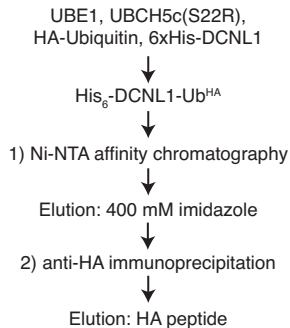
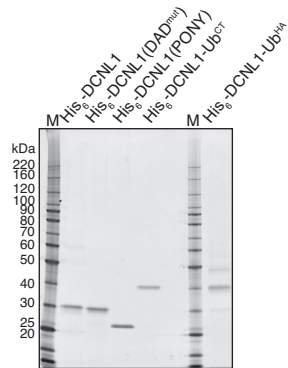
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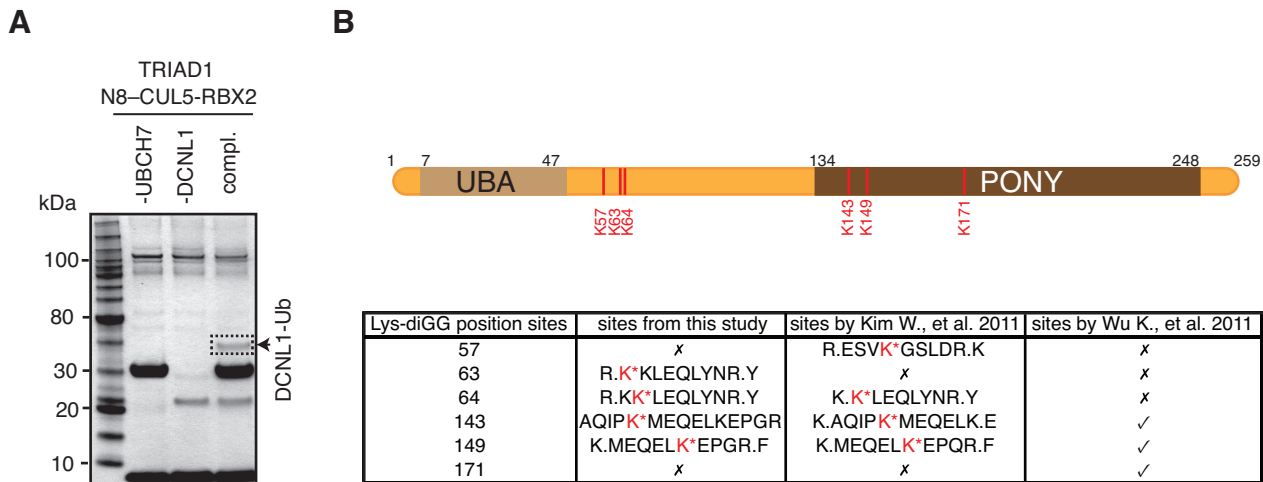
## SUPPORTING INFORMATION

**Figure S1**

**Figure S2**

**A****B**

**Figure S1: *In vitro* monoubiquitylation and purification of DCNL1.** (A) Flow chart summarizing the sequential purification steps of *in vitro* monoubiquitylated DCNL1 as described in Experimental procedures (left), and Coomassie stained SDS PAGE showing reaction products of the monoubiquitylation reaction, fraction after imidazole and HA-peptide elution (right). (B) Coomassie stained SDS-PAGE showing recombinant purified DCNL1 variants.



**Figure S2 Identification of ubiquitinated lysin residues on DCNL1 (A)** Reconstitution of DCNL1 monoubiquitylation with purified recombinant TRIAD1, neddylated CUL5–RBX2 (N8-CUL5–RBX2), and UBCH7. Products of complete and drop-out (-) reactions were separated on SDS-PAGE and detected by coomassie stain. Boxed band indicating mono-ubiquitylated DCNL1 was excised and subjected to MS analysis. **(B)** Table and cartoon summarizing identified Lys-diGG sites including published lysine sites.