Supplemental Figure Legends

supplemental Figure 1. The FANCD2 CUE domain adopts a triple-alpha helical organization characteristic of ubiquitin-binding domains. (A) The crystal structure of the murine Fancd2-Fanci (ID) complex was recently solved by Joo and colleagues.[31] Here we have oriented the ID structure from the PDB file (#3S4W) to demonstrate the triple-helical secondary structure of the Fancd2 CUE domain, depicted in red. (B) The Fancd2 CUE domain is in close proximity to K522 (highlighted in yellow) of Fanci (equivalent residue to K523 of human FANCI), the residue that undergoes monoubiquitination. Fancd2 and Fanci are shown in blue and green, respectively.

supplemental Figure 2. FANCD2 noncovalently interacts with ubiquitin. (A) The integrated densities of bound protein bands from Figure 2B were quantified using ImageJ software and plotted. (B) The integrated densities of input and Ub-sepharose-bound protein bands from Figure 2D were quantified using ImageJ software and the ratios of Ub-bound protein to input were calculated and plotted.

supplemental Figure 3. **Mutation of the CUE domain impairs FANCD2 protein stability.** The integrated densities of protein bands from Figure 3A were quantified using ImageJ software and plotted. While the integrated band densities for a single experiment are shown, these experiments were repeated multiple times with very similar findings.

supplemental Figure 4. Deletion of the CUE domain destabilizes FANCD2. Whole-cell lysates from COS-7 cells transiently expressing wild type FANCD2-H6/V5 (D2-wt), FANCD2-

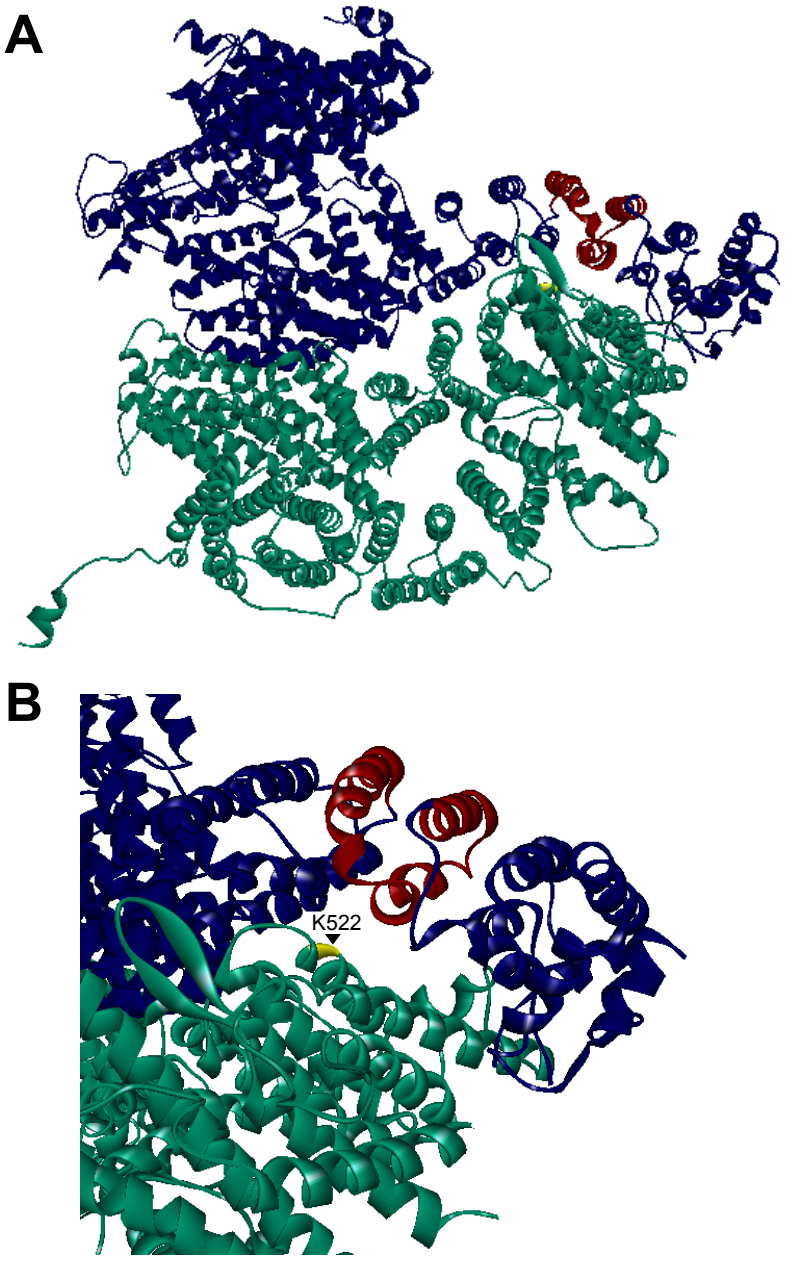
H6/V5 K561R (D2-K-R), FANCD2-H6/V5 with a 100 amino acid (aa) amino-terminal deletion (D2-ΔN-100), and FANCD2-H6/V5 with a 254 aa amino-terminal deletion (D2-ΔN-254) encompassing the entire CUE domain, were generated and proteins were resolved and immunoblotted with antibodies against V5 and tubulin.

supplemental Figure 5. The CUE domain is required for FANCD2 and FANCI nuclear foci formation. FA-D2 cells reconstituted with wild type or mutant FANCD2 were untreated or treated with 250 nM MMC for 12 hours and then fixed and immunostained with antibodies against FANCD2 (A) or FANCI (B). The percentage of nuclei with greater than 5 foci were scored and plotted in the indicated histograms.

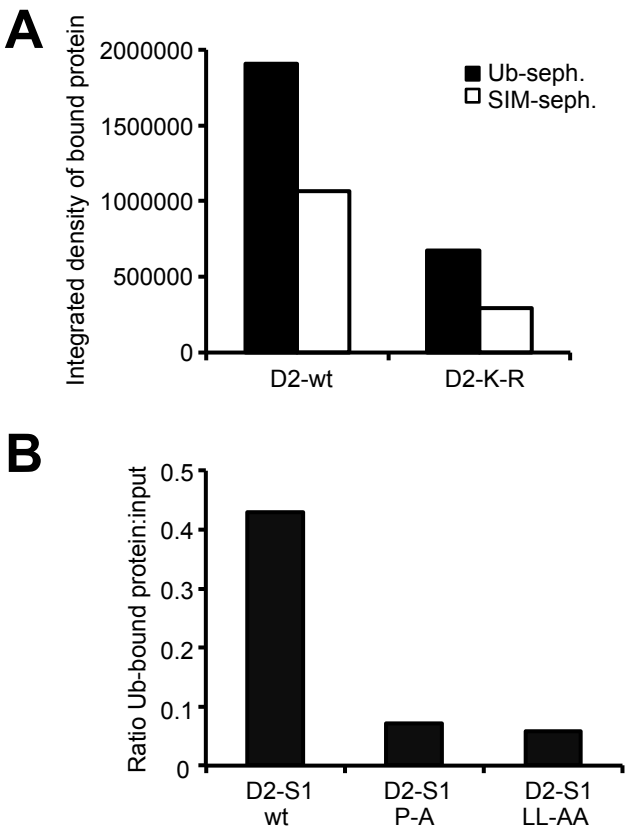
supplemental Figure 6. FA-D2 cells stably expressing the FANCD2 CUE mutants show an intermediate accumulation in G2/M following mitomycin C exposure. FA-D2 cells stably expressing wild type or mutant FANCD2 were untreated (NT) or treated with 10 nM mitomycin C (+MMC) for 48 h, fixed, stained with propidium iodide and analyzed by flow cytometry. This experiment was performed twice with similar findings.

supplemental Figure 7. Schematic model of the role of the FANCD2 CUE domain in the stabilization of the FANCD2-FANCI heterodimer. (A) We propose that FANCD2-FANCI heterodimerization is mediated in part through a noncovalent interaction between the FANCD2 CUE domain and monoubiquitin covalently attached to K523 on FANCI. We also speculate the existence of a reciprocal noncovalent interaction between a UBD on FANCI and monoubiquitin covalently attached to K561 on FANCD2. (B) Upon disruption of the FANCD2 CUE domain,

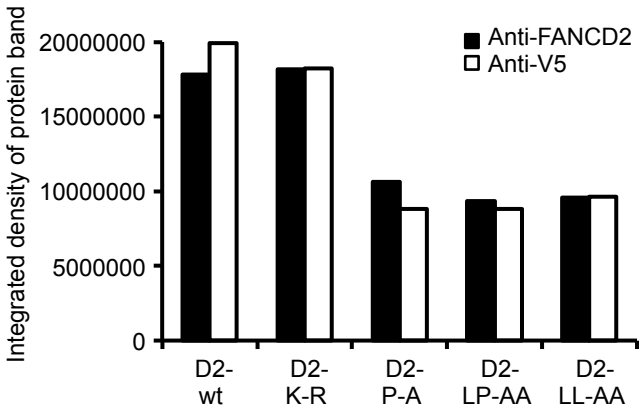
efficient heterodimerization of FANCD2 and FANCI no longer occurs potentially rendering FANCI susceptible to premature deubiquitination by the USP1/UAF1 complex and/or FANCD2 susceptible to polyubiquitination and subsequent degradation by the proteasome.



supplemental Figure 1



supplemental Figure 2



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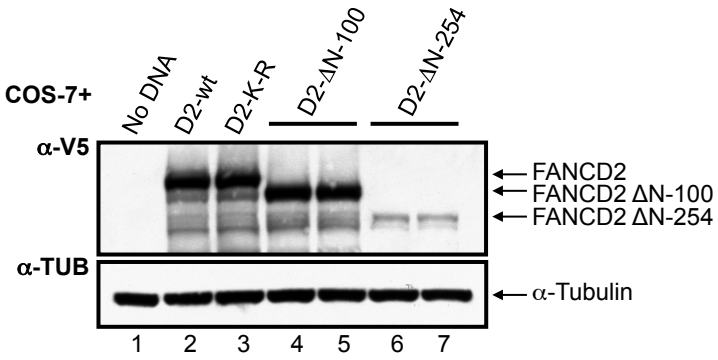

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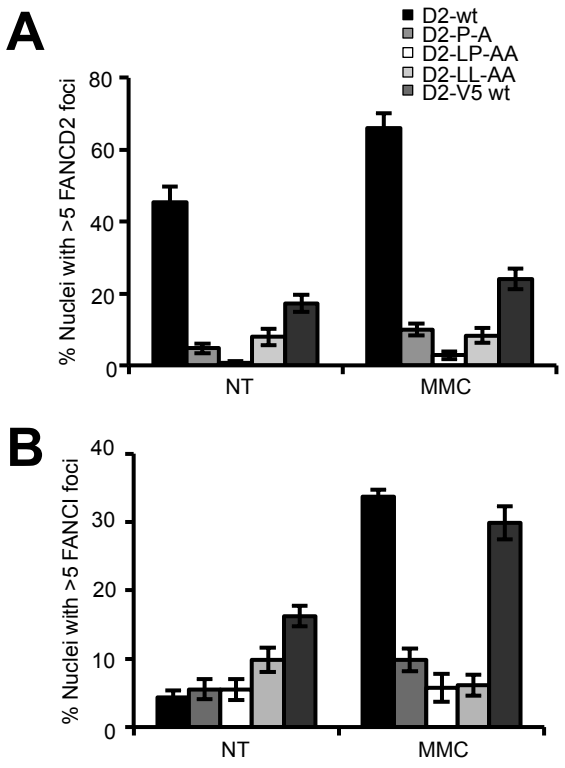

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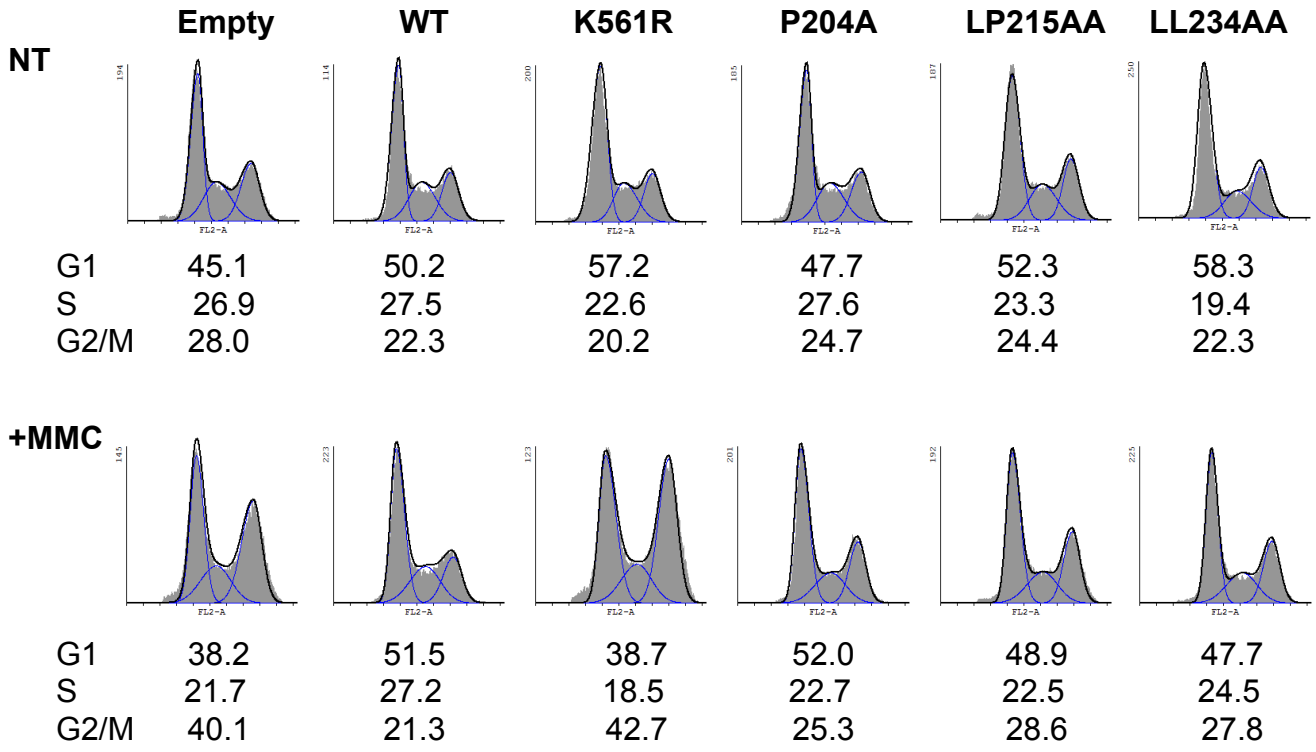

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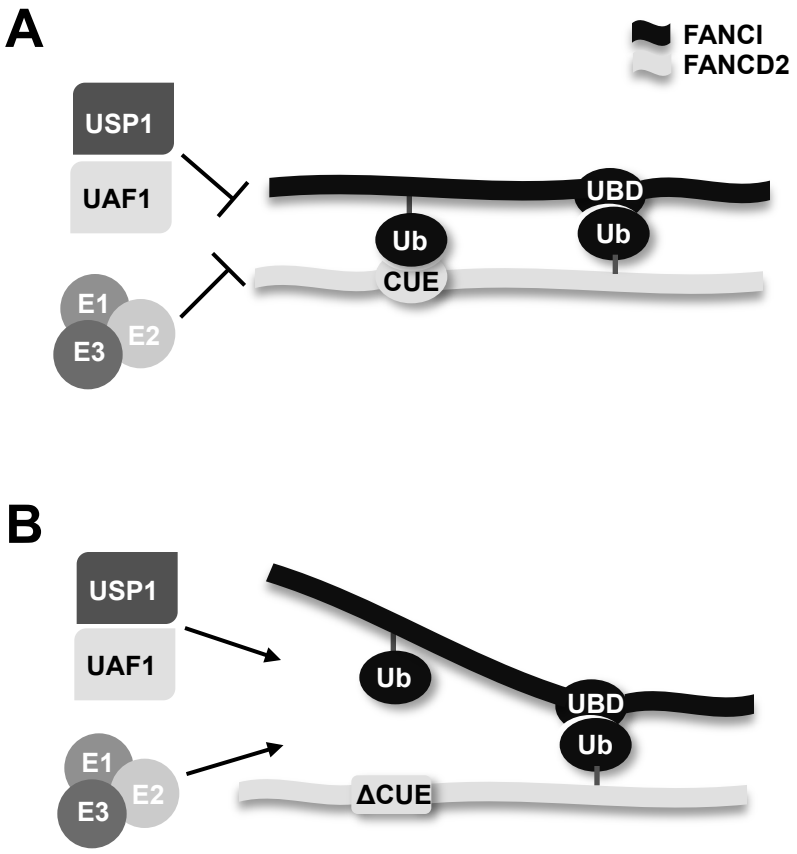

supplemental Figure 7