

Figure S1. Identification of hDNA2 ubiquitination sites using liquid chromatography with tandem mass spectrometry (LC-MS/MS). 3xFlag-hDNA2 was isolated from 293T cells expressing 3xFlag-hDNA2, myc-hTRAF6, and 6His-ubiquitin; resolved by SDS-PAGE; and analyzed using LC-MS/MS. Ubiquitinated peptides were found using MaxQuant software. Mass spectrometry results for fragments of ubiquitinated K29, K194, K275, K303, K377, and K434 residues are shown. Asterisks (*) indicate ubiquitinated lysine residues. b and y ions are indicated in blue and red, respectively.

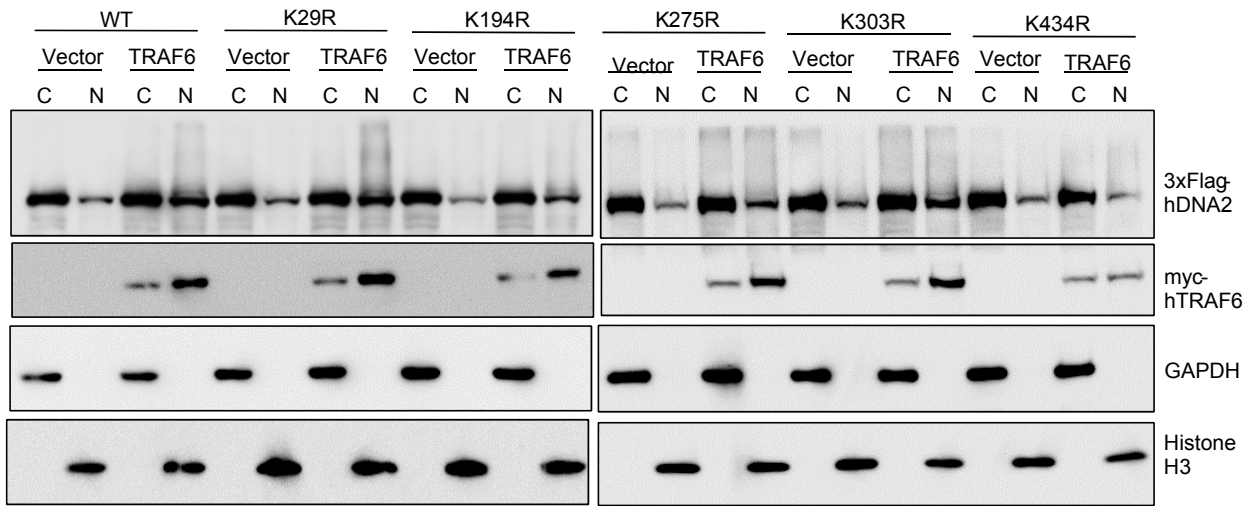


Figure S2. Nuclear localization of WT and hDNA2 mutants. Vectors encoding 3xFlag-tagged WT, K29R, K194R, K275R, K303R, or K434R hDNA2 were co-transfected with empty vectors or vectors encoding myc-hTRAF6 in 293T cells. 3xFlag-hDNA2 proteins in the CE and NE were analyzed by western blot analysis using an anti-Flag antibody. GAPDH and histone H3 were used as markers for the cytoplasmic and nuclear fractions, respectively.

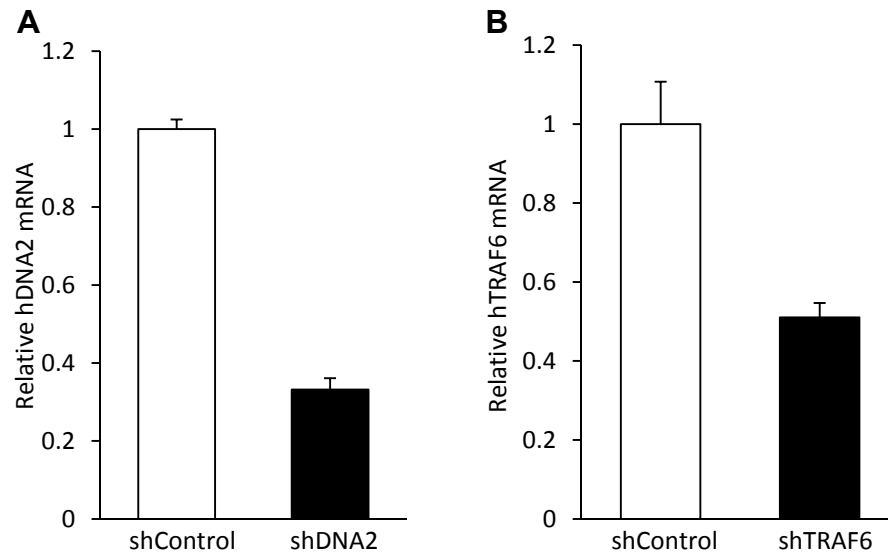


Figure S3. Confirmation of knockdown efficiency using quantitative RT-PCR. Relative levels of **(A)** hDNA2 and **(B)** hTRAF6 mRNA were measured in HeLa cells transfected with a control shRNA plasmid (shControl) or shRNA plasmids against hDNA2 or hTRAF6, respectively. Total RNA was extracted from these cells and quantitative RT-PCR was used to quantify the levels of hDNA2, hTRAF6, and control β -actin mRNA. hDNA2 and hTRAF6 mRNA levels were normalized to β -actin mRNA levels, and the normalized hDNA2 and hTRAF6 mRNA levels in shControl cells was arbitrarily set to 1. The values shown are the means \pm s.e.m. of three independent assays.

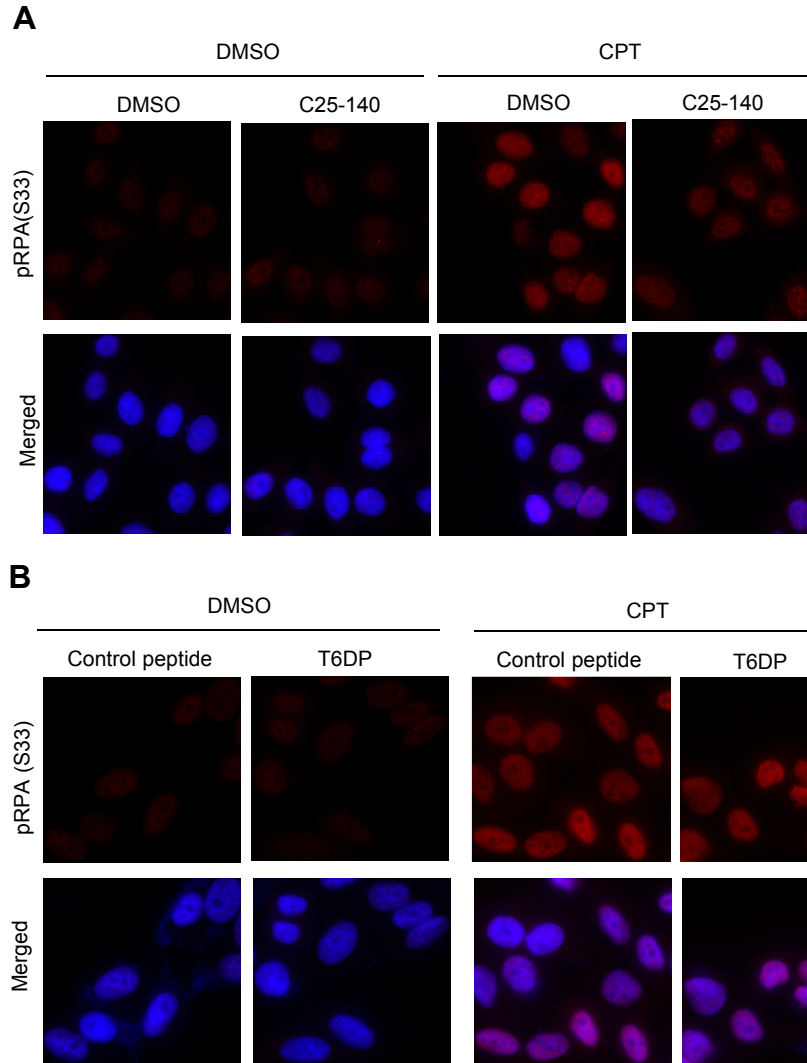


Figure S4. Representative immunofluorescence (IF) staining for pRPA (S33). HeLa cells were pretreated with **(A)** DMSO or C25-140 (30 μ M, 48 h) or **(B)** a control or T6PD peptide (100 μ M, 24 h). The cells were then treated with DMSO or CPT (1 μ M, 4 h). In each case, red: pRPA (S33), blue: DAPI-stained nuclei.

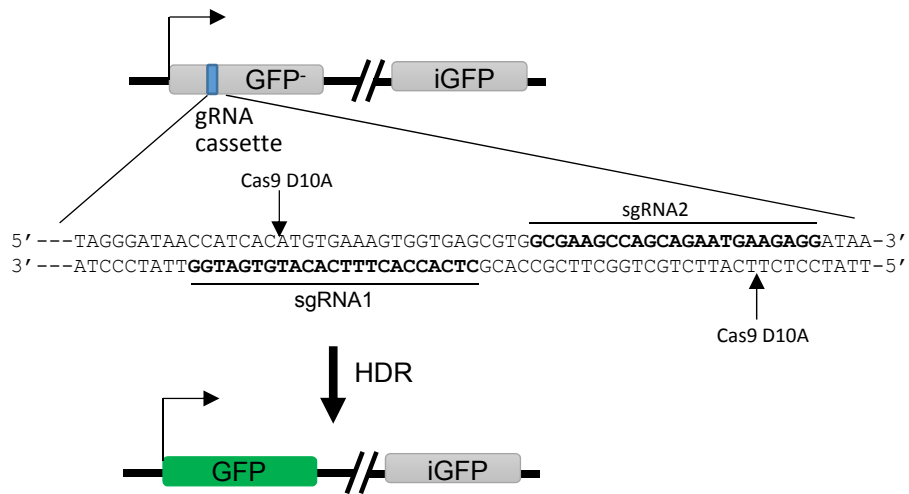


Figure S5. Schematic of the CRISPR/Cas9 D10A double-nicking-based DR-GFP reporter HDR assay. A cassette containing two CRISPR/CAS9 target sites (underlined) was inserted into the GFP gene (GFP⁻). Expression of Cas9 D10A nickase and a pair of single strand guide sequences (sgRNA1 and sgRNA2) results in a DSB. DNA end resection and subsequent recombination with a downstream GFP fragment (iGFP) will produce a functional GFP gene whose product can be detected and quantified using flow cytometry.