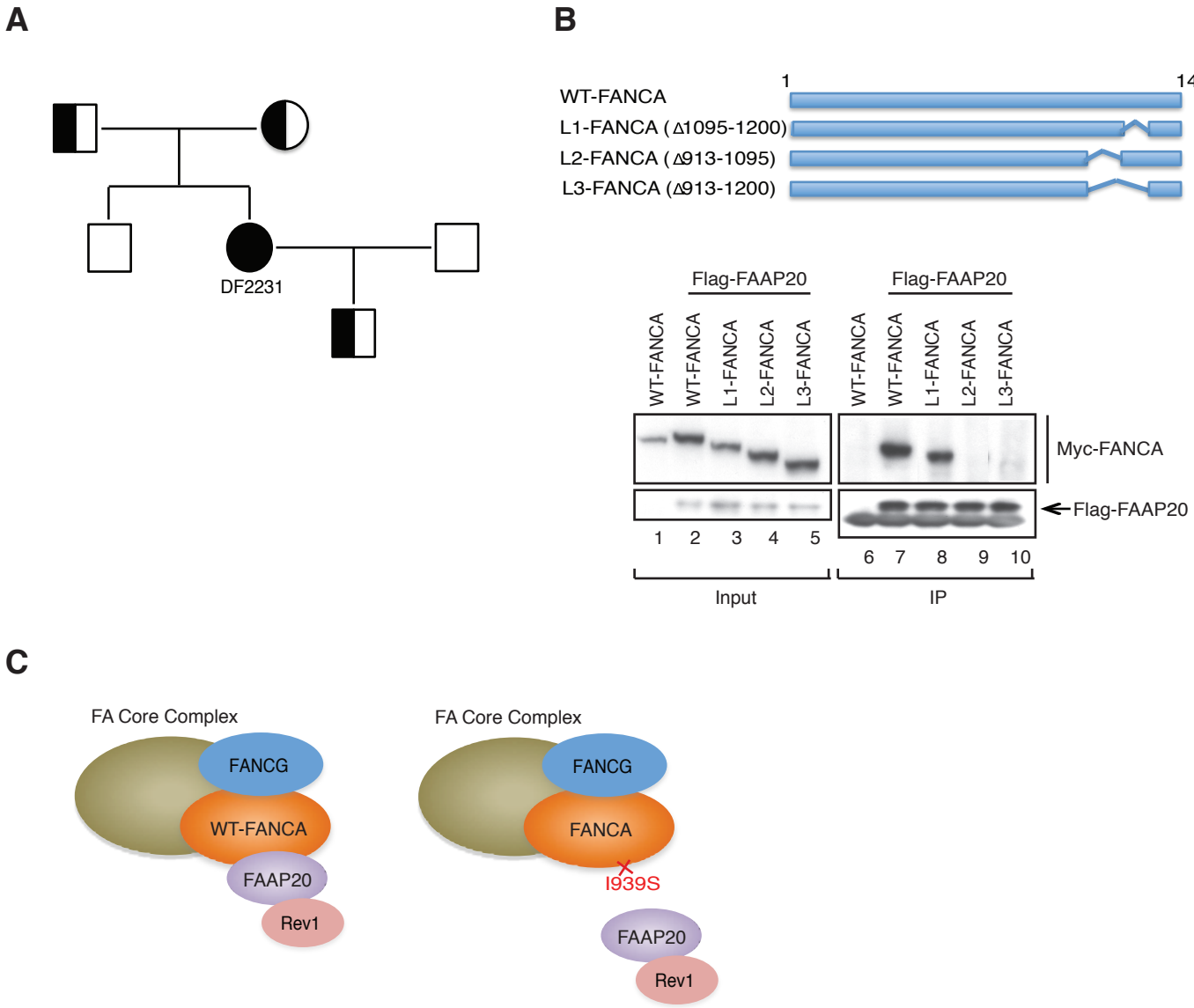

Figure S1 FANCA amino acid 913 to 1095 is critical for FAAP20 intera
(A) Family tree of FANCA mutation status of the DF2231 patient. (B) (Top) Schematic representation of FANCA mutants. (Bottom) Immunoblot of anti-Flag IP immune complexes isolated from cell lysates from 293T cells, transfected with the Myc-FANCA or Flag-FAAP20 cDNAs, as indicated. (C) Cartoon representation of the FA core complex formation in the presence of (Left) FANCA-WT or (Right) FANCA-I939S.

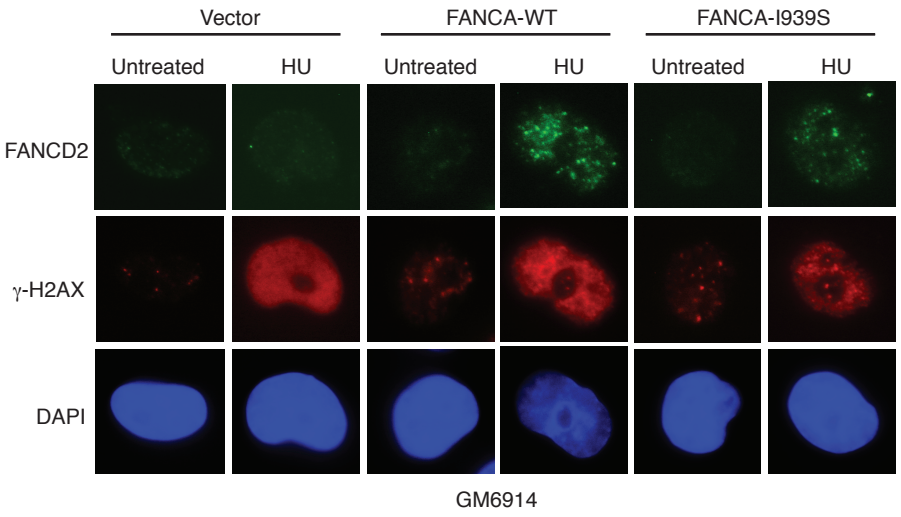

Figure S2 FANCA-I939S is efficient in promoting FANCD2 foci formation
Immunostaining of FANCD2 and γH2AX in GM6914 cells complemented with vector, FANCA-WT, or FANCA-I939S treated with HU for 24 h. Original magnification, x60 for foci. Three independent experiments were performed.

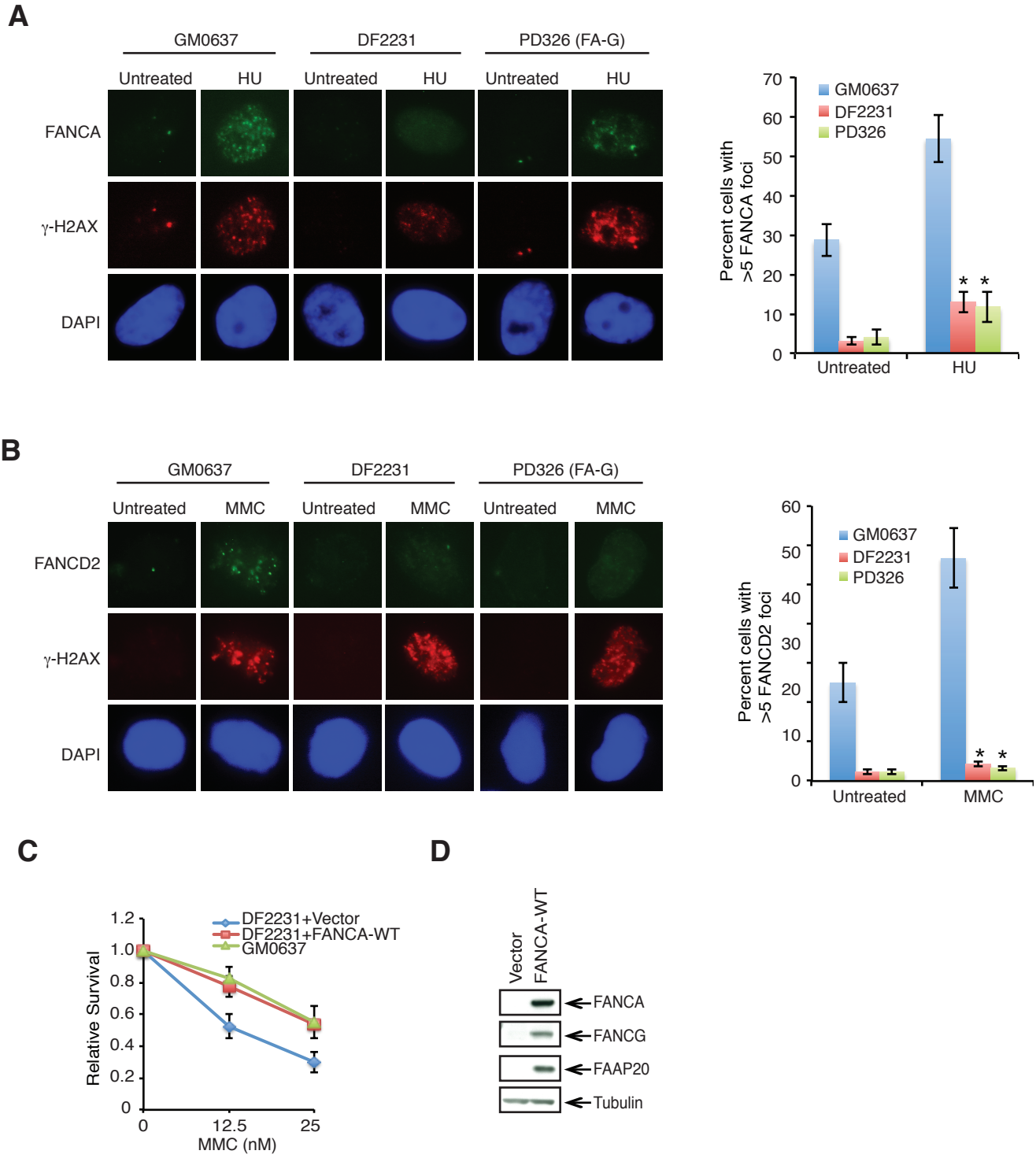

Figure S3 The Full-length mutant FANCA protein in DF2231 cells is sumoylated and fails to form FANCA and FANCD2 foci

(A) Immunostaining of FANCA and (B) FANCD2 in WT (GM0637), DF2231, and FA-G (PD326) cells treated with HU (1 mM) or MMC (50 ng ml-1). Original magnification, x60 for foci. Data shown are mean ± SEM from three independent experiments. * $p<0.05$ compared with HU or MMC treated GM0637 cells. 2 tailed t test (assuming unequal variance). (C) Relative survival of GM0637 and DF2231 cells, complemented with vector or FANCA-WT, treated with increasing doses of MMC and plated for 5 days. Three independent experiments were performed. (D) Immunoblot of analysis of cell lysates from the complemented DF2231 cells.

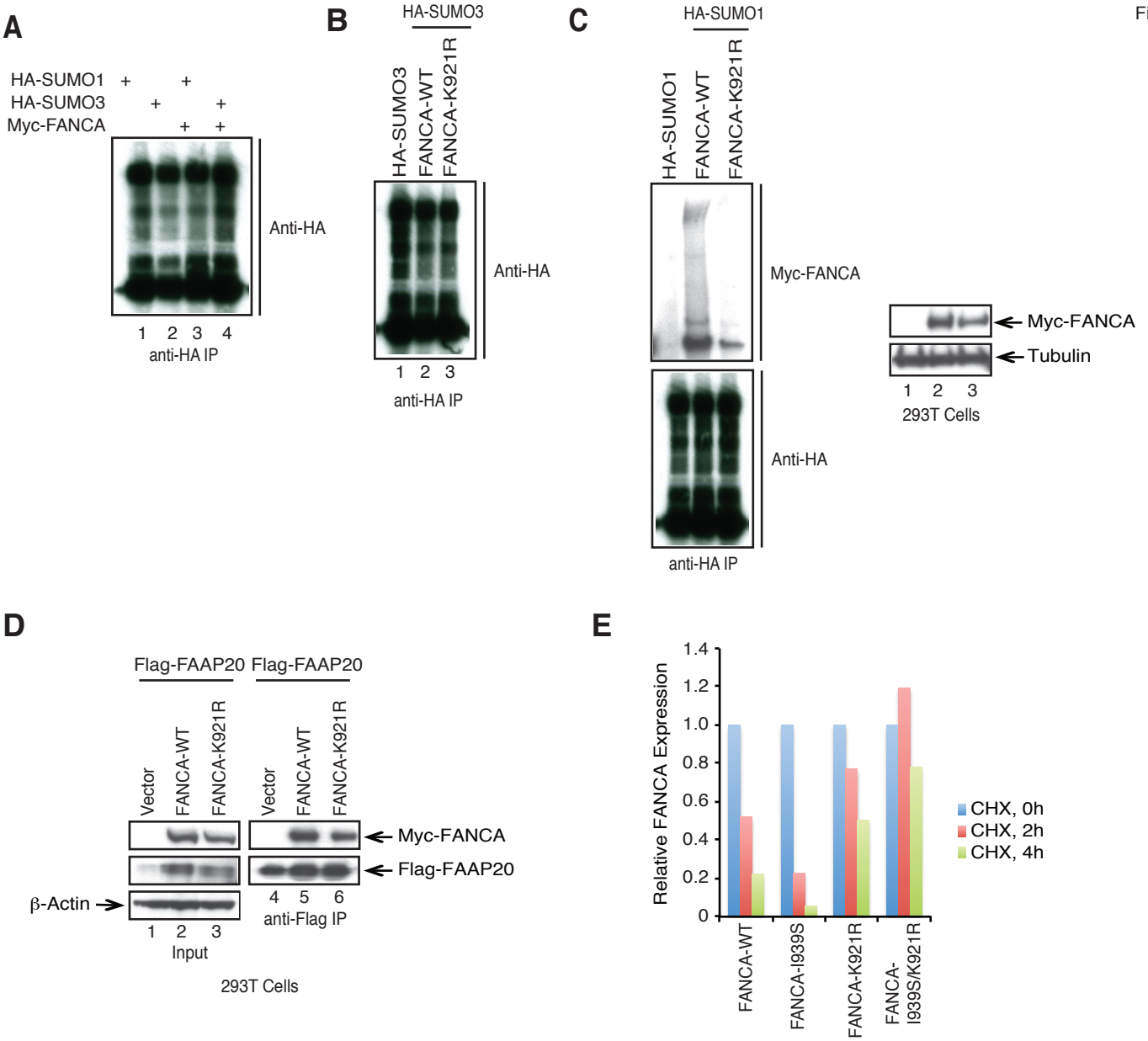

Figure S4 FANCA SUMO conjugation mutants have normal FAAP20 interaction
(A) 293T cells were transfected with HA-tagged SUMO or co-transfected with HA-tagged SUMO with Myc-tagged FANCA-WT. SUMO expression was determined under denaturing conditions by anti-HA immunoprecipitiation followed by anti-HA immunoblot. (B) 293T cells were transfected with HA-tagged SUMO3 or Myc-tagged FANCA-WT or K921R with HA-SUMO3 as indicated. SUMO expression was determined under denaturing conditions by anti-HA immunoprecipitiation followed by anti-HA immunoblot. (C) 293T cells were transfected with HA-tagged SUMO1 or Myc-tagged FANCA-WT or K921R with HA-SUMO1 as indicated. FANCA sumoylation and SUMO expression was determined under denaturing conditions by anti-HA immunoprecipitiation followed by anti-HA immunoblot. (D) Immunoblots of anti-Flag immunoprecipitation from 293T cells stably expressing Flag-FAAP20 and transfected FANCA-WT or FANCA-K921R (E) Bar graph showing relative levels of FANCA expression, corresponding to Figure 4F from the main text.

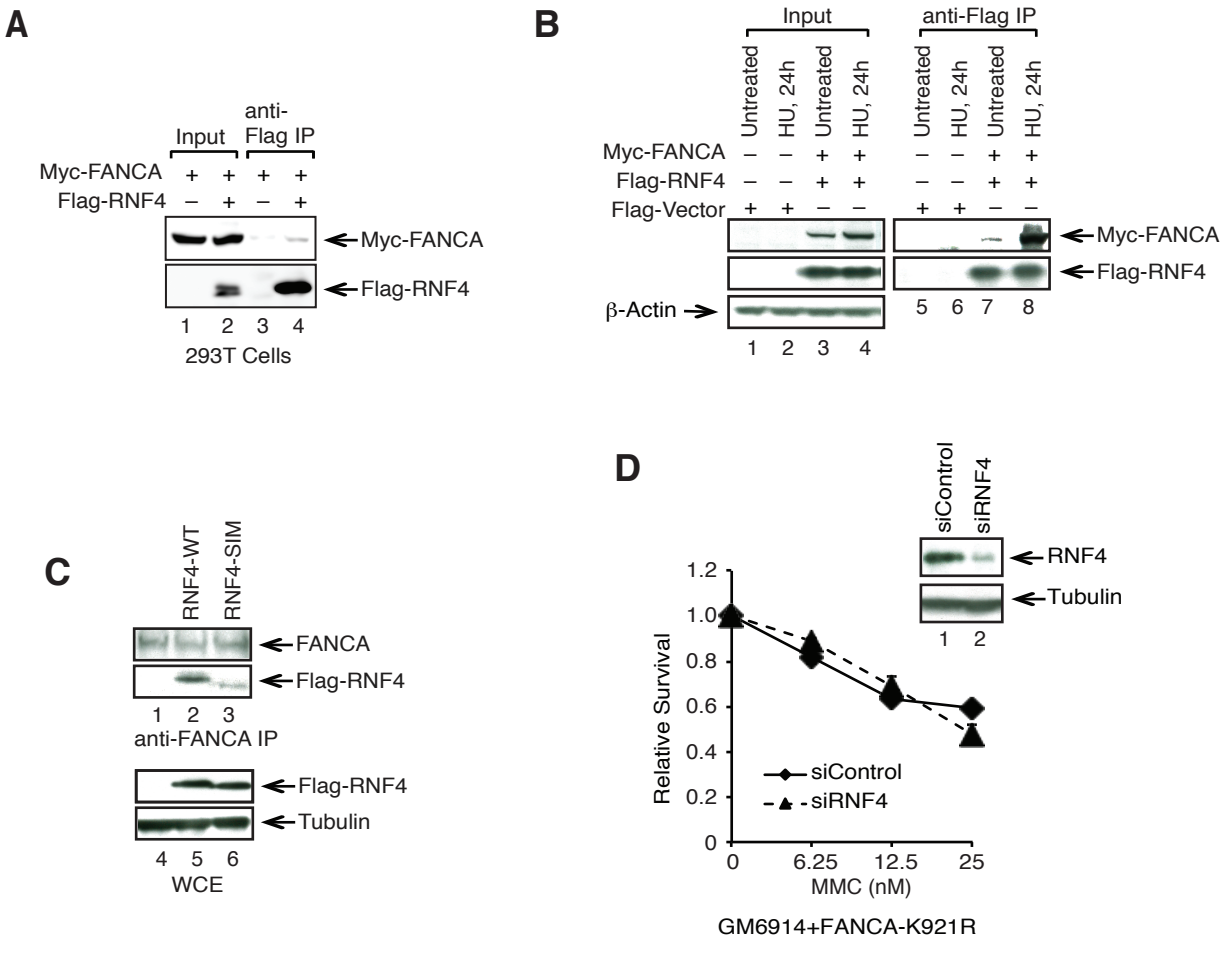

Figure S5 FANCA-WT interacts with RNF4 and the interaction is DNA damage dependent
(A) Immunoblot of anti-Flag immunoprecipiates from 293T cells transfected with Myc-FANCA or co-transfected with Myc-FANCA and Flag-RNF4. (B) Immunoblot of anti-Flag immunoprecipiates from 293T cells transfected with empty Flag-tagged vector or co-transfected with Myc-FANCA and Flag-RNF4. Transfected cells were treated with 1mM HU for 24h. (C) Upon FAAP20 depletion, mutation of the SIM domains of RNF4 reduces the coimmunoprecipitation of RNF4 and FANCA. (D) Survival of GM6914 expressing FANCA-K921R, after transfection with siRNA to RNF4. Three independent experiments were performed.

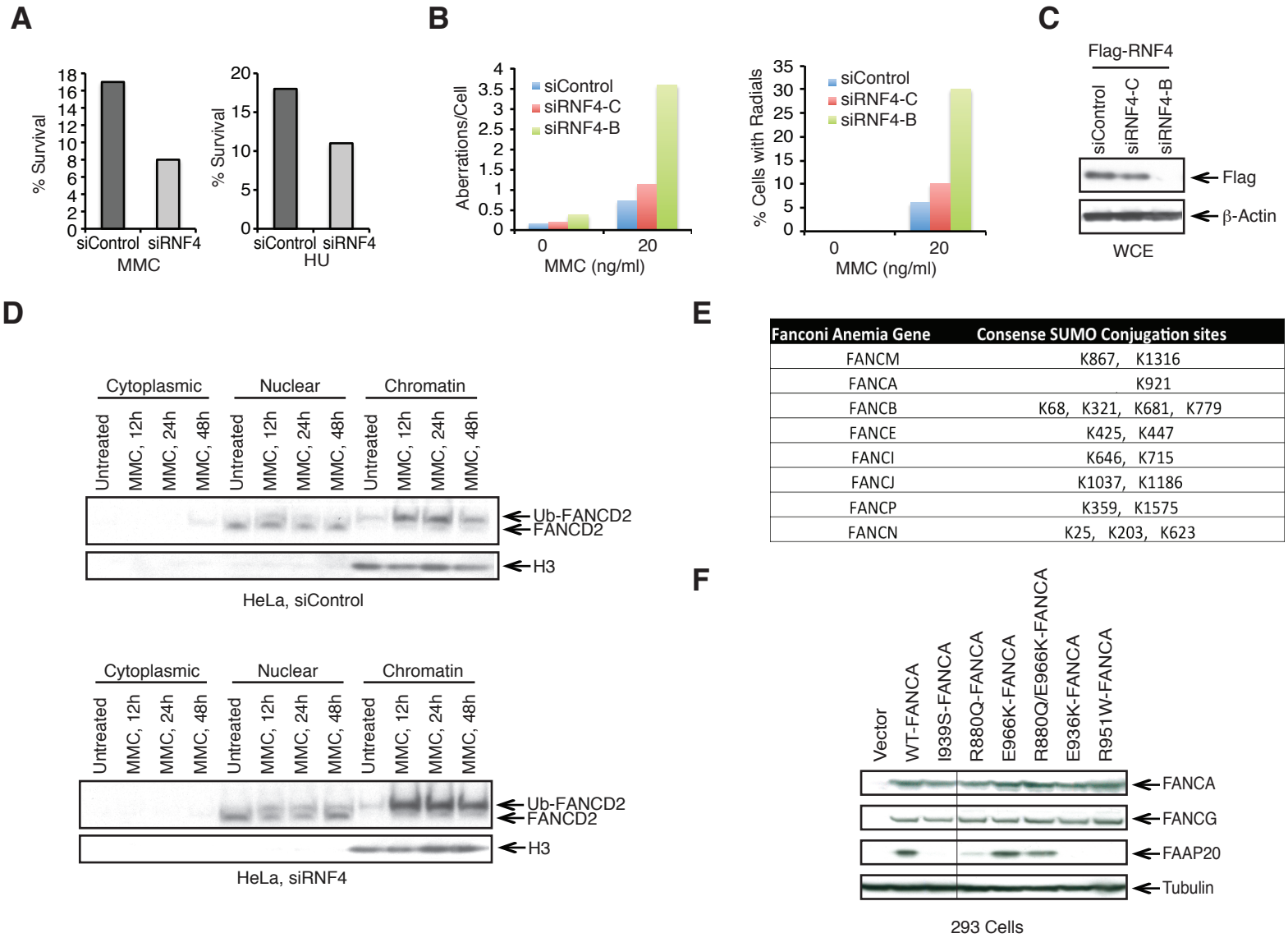

Figure S6 RNF4 suppression results in increased DNA damage sensitivity and chromosome aberrations and other SUMO consensus conjugation sites on Fanconi Anemia proteins

(A) Colony survival assay of U2OS cells transfected with siRNAs oligos against control or RNF4 exposed to increasing doses of MMC or HU and plated for 8 days. (B) Quantification of chromosomal aberrations and radial chromosomes of U2OS cells transfected with siRNAs against control or RNF4 (A or B) exposed 20 ng ml-1 MMC. (C) Immunoblot of RNF4 derived from cell lysates from A. (D) HeLa cells were exposed to MMC and siRNAs, as indicated. Cells were fractionated, and fractionated cellular proteins were immunoblotted with an antibody to FANCD2 or Histone 3. (E) A table listing SUMO consense sites on multiple FANC proteins. (F) Immunoblots of 293T cells stably expressing Flag-FAAP20 and transfected with the indicated FANCA variants. The thin black line indicates that noncontiguous lanes from the same blot are shown.