Supplementary information

A novel interplay between the Fanconi anemia core complex and ATR-ATRIP kinase during DNA crosslink repair

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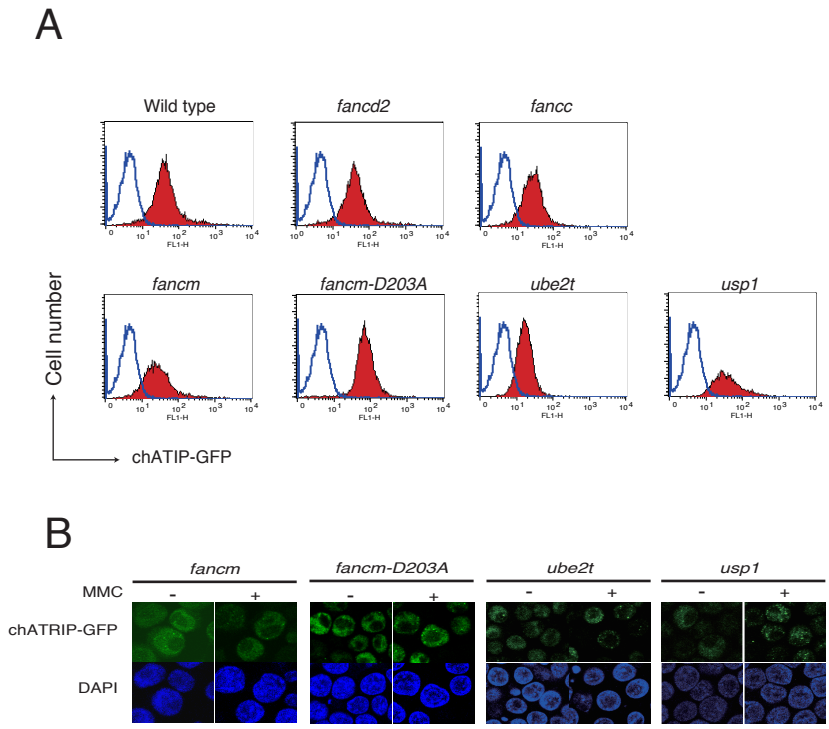
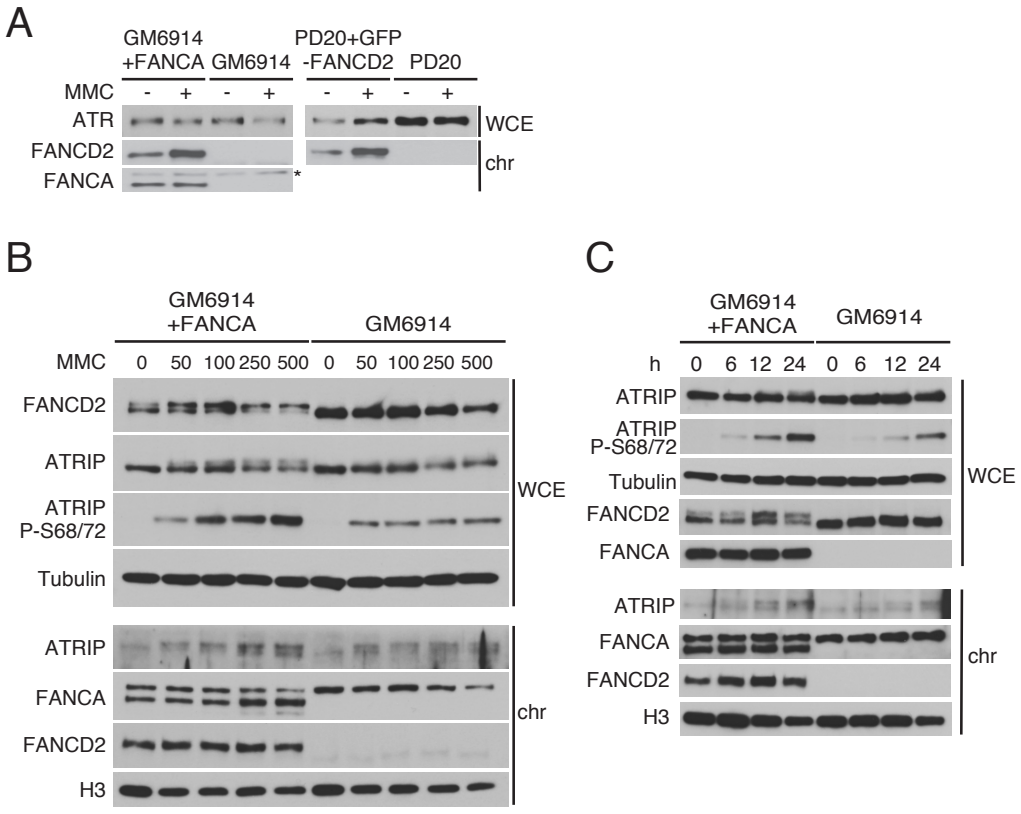
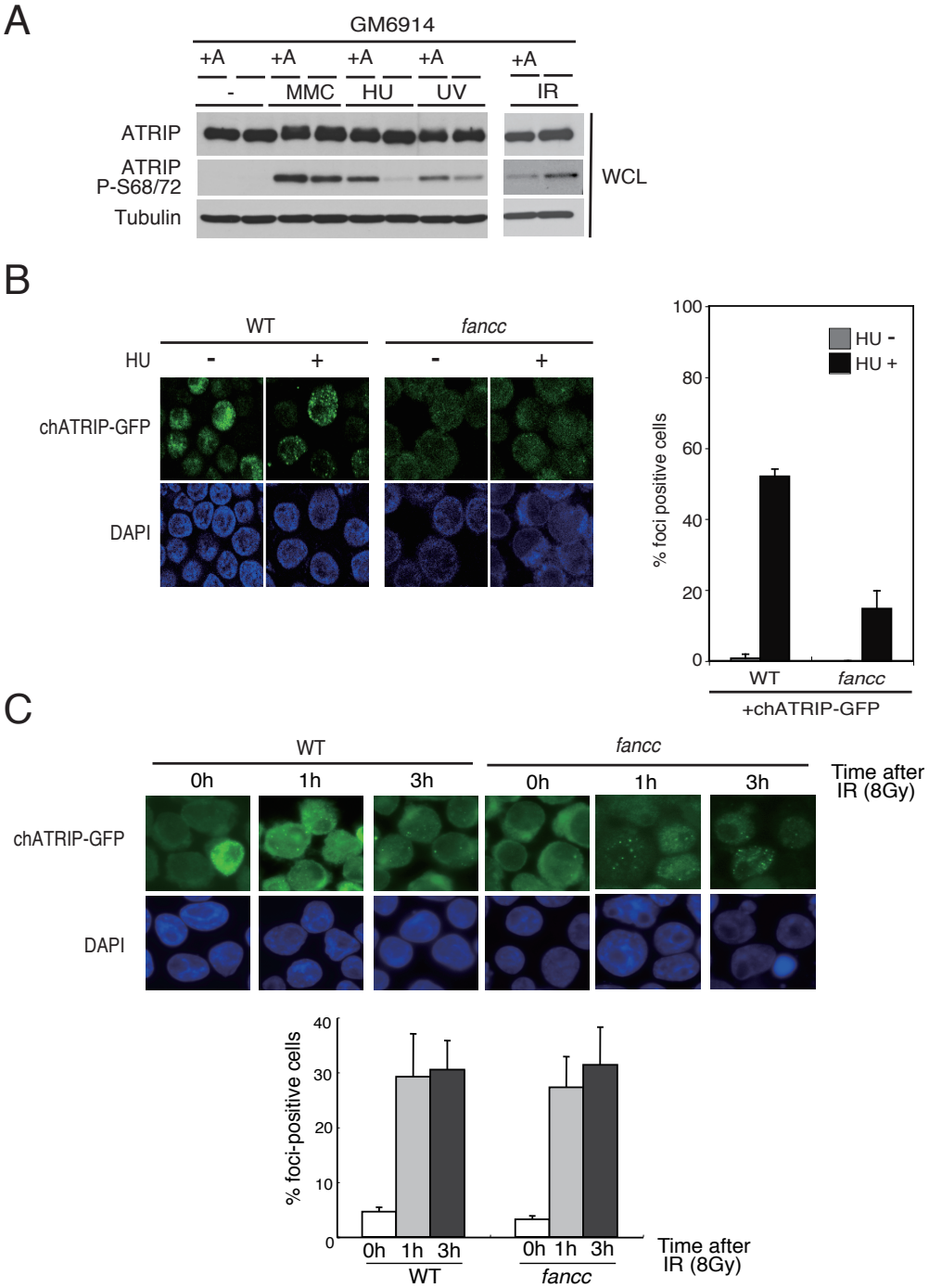


Supplementary Fig. S1. Foci formation of chicken ATRIP-GFP in DT40 mutant cells. (**A**) FACS analysis of the DT40 cell clones. Cells with indicated genotype were stably transfected with chicken ATRIP-GFP expression vector, and clones expressing comparable levels of ATRIP-GFP were identified by FACS analysis. **(B)** Cells were stimulated with MMC (500 ng/ml for 6h), and observed using confocal laser scanning microscopy.

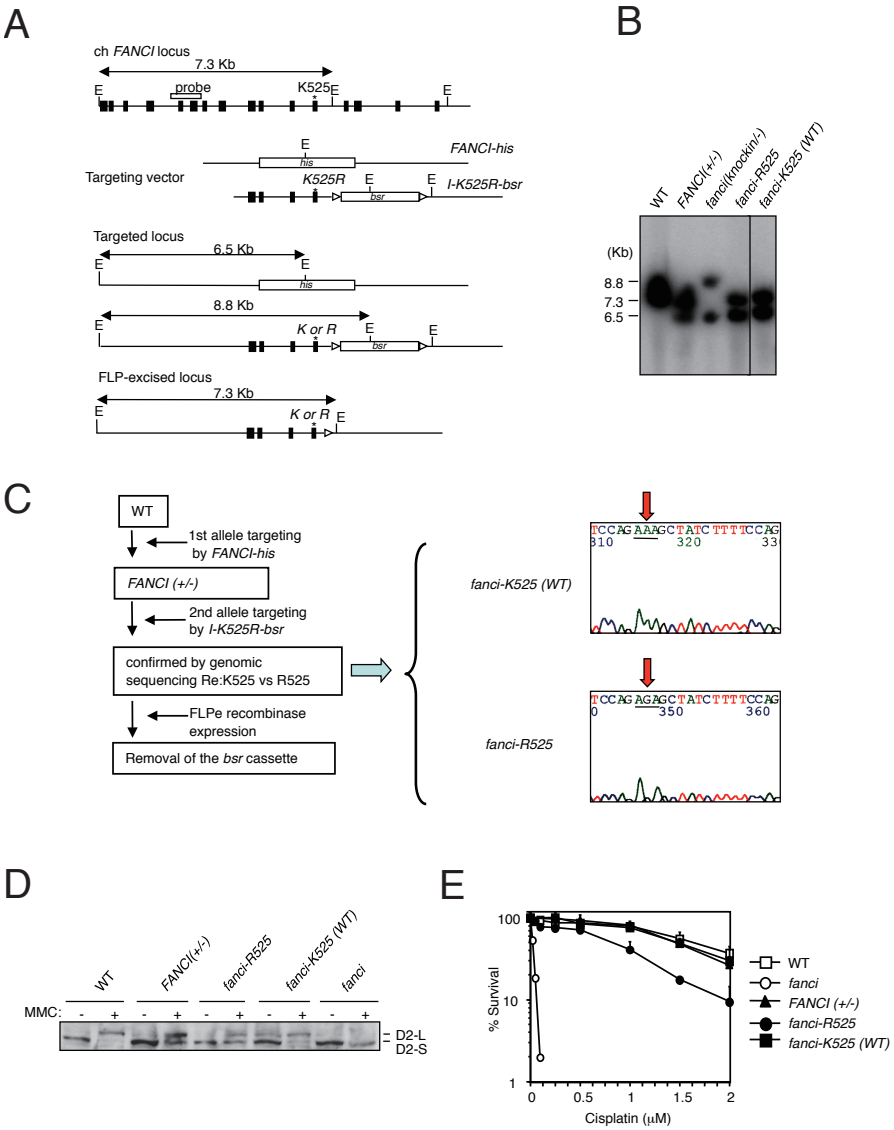


Supplementary Fig. S2. Western blotting analysis of human cells deficient in the FA pathway. (**A**) GM6914 (FA-A) and PD20 (FA-D2) were treated with or without MMC stimulation (100 ng/ml for 24 h). Cells were then fractionated, and analyzed by western blotting. (**B** and **C**) Dose response (**B**) and time course (**C**) experiments in GM6914 (FA-A) and complemented control cells. Cells were treated with increasing concentrations of MMC (ng/ml for 24 h) or treated with MMC (100 ng/ml) for the indicated durations, and analyzed with western blotting.



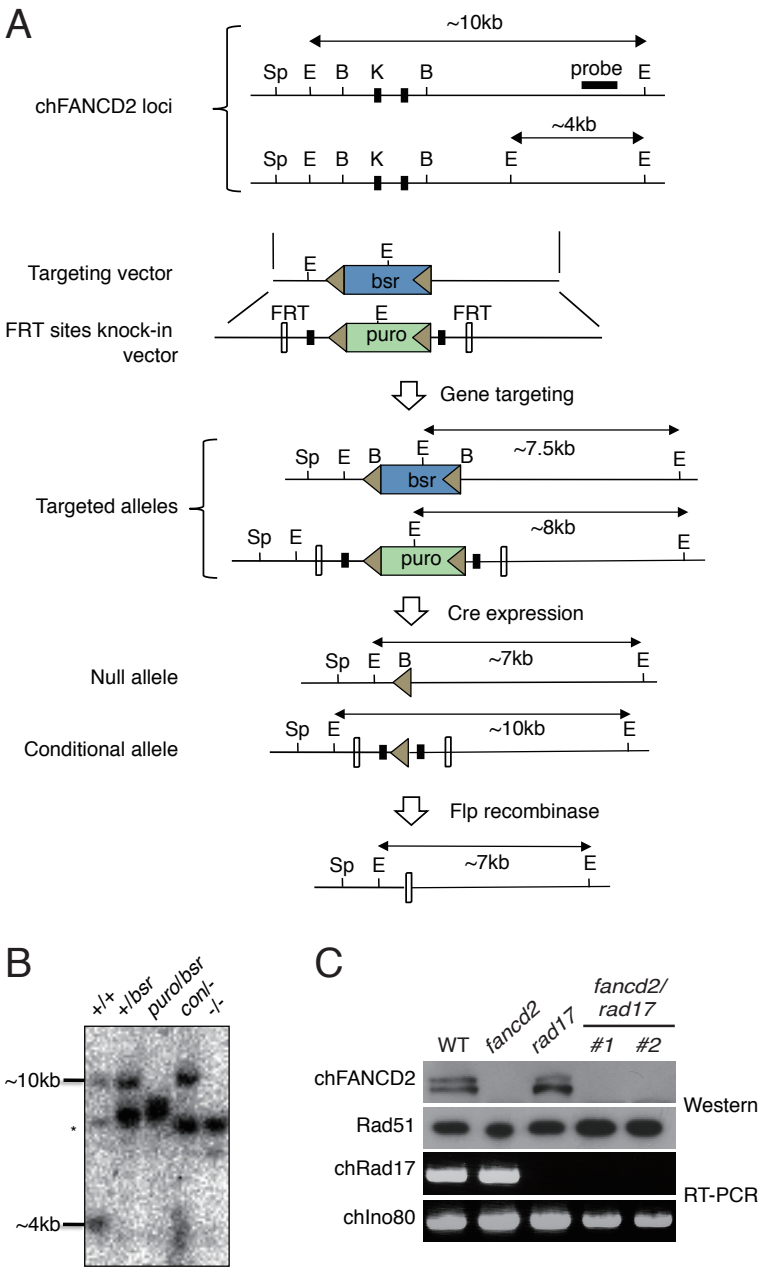
Supplementary Fig. S3. ATRIP phosphorylation or localization in cells lacking the core complex in response to various DNA damages. (**A)** FA-A (GM6914) and complemented control (+A) cells were treated with MMC (100 ng/ml for 24 h),

hydroxyurea (1 mM for 6h), UV (6h after 30 J/m^2), or IR (30 min after 10 Gy) and analyzed with western blotting. (**B**) WT or *fancc* DT40 cells expressing chicken ATRIP-GFP were treated with HU (1 mM for 6h) and ATRIP-GFP foci formation was analyzed using confocal laser scanning microscopy. (**C**) WT or *fancc* DT40 cells expressing chicken ATRIP-GFP were exposed to IR (8 Gy) and ATRIP-GFP foci formation was analyzed at the indicated time points using fluorescent microscopy. The bar graphs represent mean and SD of % GFP-foci positive cells. 50 nuclei were scored, and nuclei containing >4 bright GFP foci were defined as foci-positive.



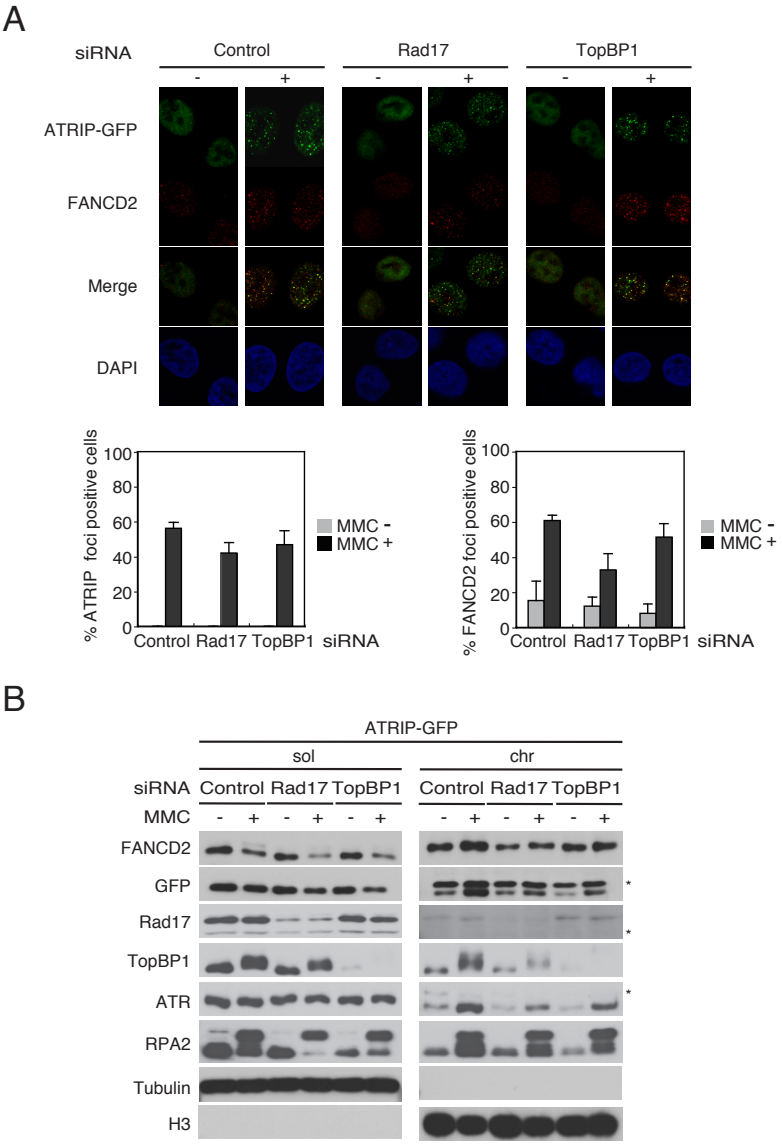
Supplementary Fig. S4. Generation of FancI K525R "knock-in" mutation. (**A**) A schematic of the *FANCI* disruption or "knock-in" vector. (**B**) Southern blot analysis confirmed the genotypes in the process of "knock-in" (right panel). (**C**) Summary of the procedures. Gene targeting with "knock-in" vector *I-K525R-bsr* yielded two types of cells each carrying wild type sequence (K525) or the mutation K525R in the exon encoding the monoubiquitination site K525 as confirmed by genomic PCR followed by

direct sequencing. *fanci-K525 (WT)* cells were technically a heterozygous knock-out, since they are expected to produce normal FancI protein from non-disrupted allele. Finally, the *bsr* cassette flanked by the FRT sequences was excised by expression of FLP recombinase. (**D)** FancD2 monoubiquitination in various mutant clones. (**E)** Colony survival assay of the indicated clones in the presence of cisplatin. *fanci* K525R mutant cells were only very mildly sensitive to cisplatin.



Supplementary Fig. S5. Generation of the conditional *fancd2* cells and *fancd2/rad17* double knockout DT40 cells. (**A**) A schematic of the *FANCD2* disruption or FRT sites "knock-in". The *FANCD2* alleles were sequentially modified by the disruption vector and FRT "knock-in" vector. The *bsr* or *puro* cassette was removed by transient expression of Cre recombinase. (**B**) Southern blot analysis confirmed the genotypes in

the process of “knock-in” and excision of the genomic region by expression of FLP recombination. con/-, cells carrying FANCD2 conditional allele. Asterisk indicates position of a non-specific band. (**C**) Verification of *fancd2*/*rad17* double knockout clones. Cells with indicated genotypes were analyzed with western blotting and RT-PCR analysis.



Supplementary Fig. S6. Analysis of the FA pathway activation in siRad17- or siTopBP1-treated cells. (**A**) A549 cells expressing ATRIP-GFP was transfected with the indicated siRNA oligo, treated or not with MMC (100 mg/ml for 24 h), and fixed. After staining with anti-FANCD2, images were captured by confocal laser scanning microscopy. The bar graphs represent mean and SD of %ATRIP or FANCD2

foci-positive cells among 50 nuclei in three independent experiments. Nuclei with >4 bright foci were scored as positive. (**B**) siRNA-treated cells were fractionated into soluble (sol) and chromatin (chr) fractions and analyzed by western blotting. Asterisks (*) indicate non-specific bands.