SUPPLEMENTARY DATA

FANCI-FANCD2 stabilizes the RAD51-DNA complex by binding RAD51 and protects the 5′-DNA end

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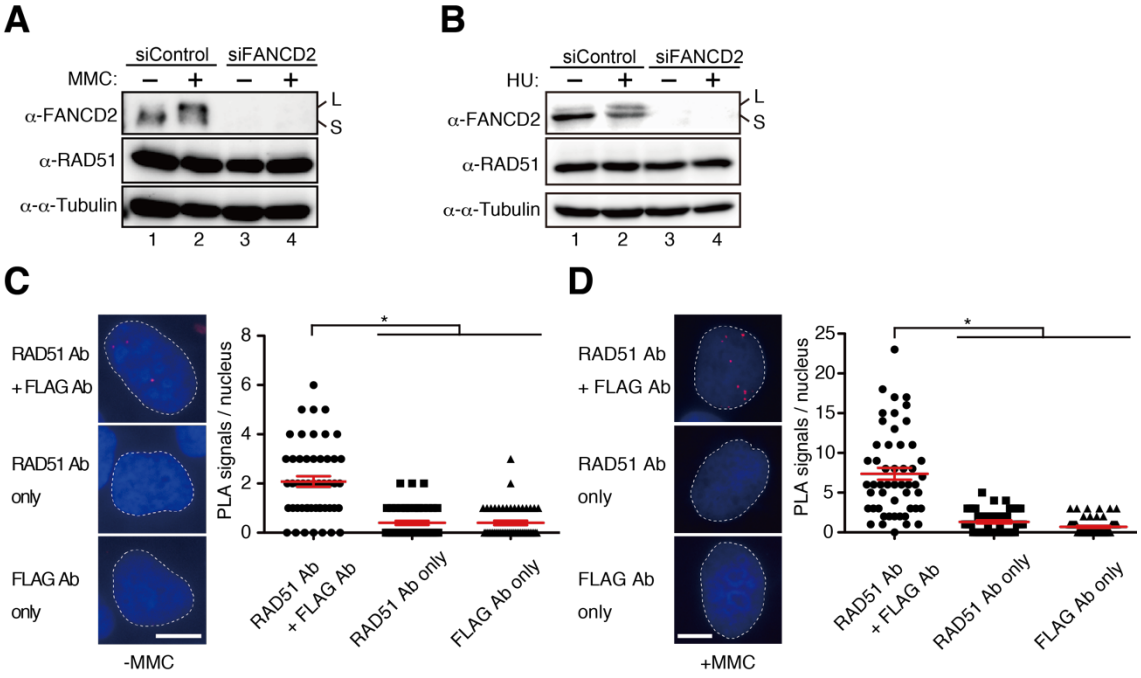
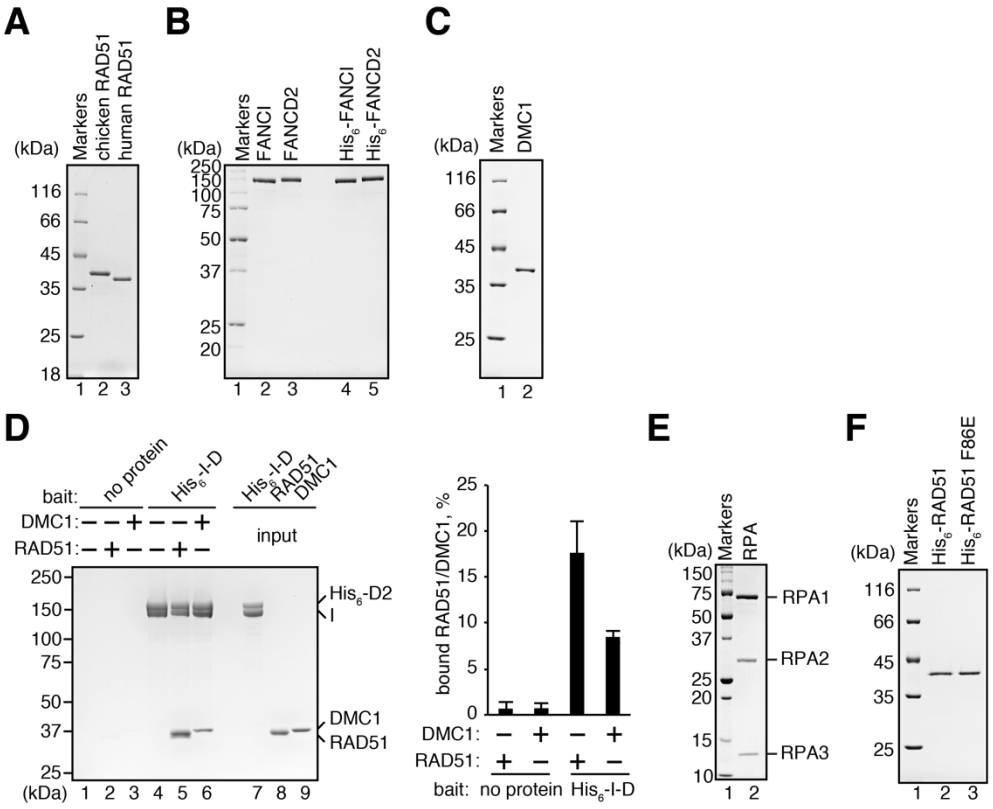
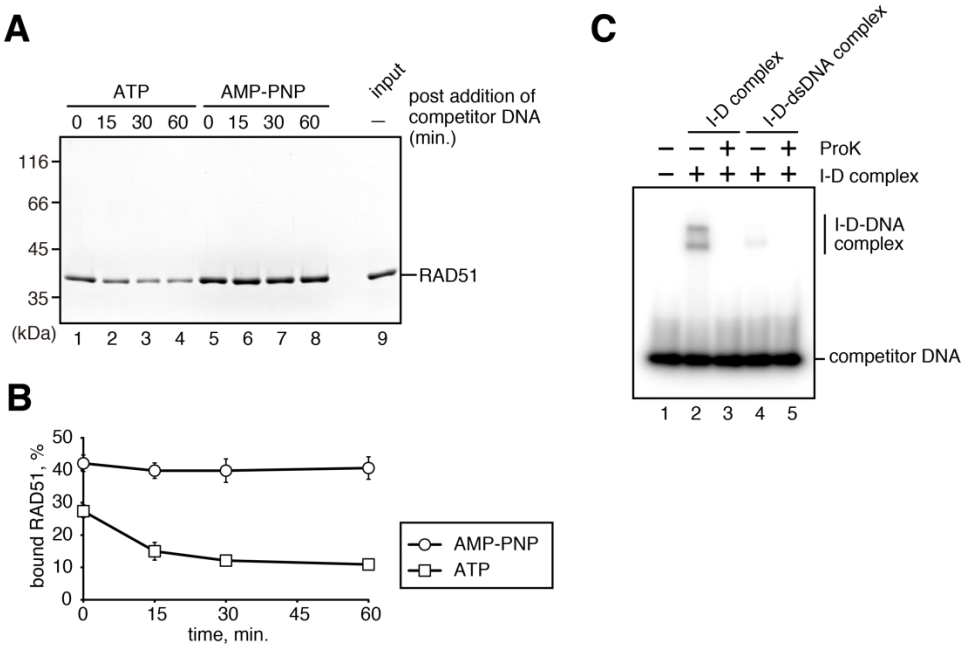
Supplementary Figures and Legends



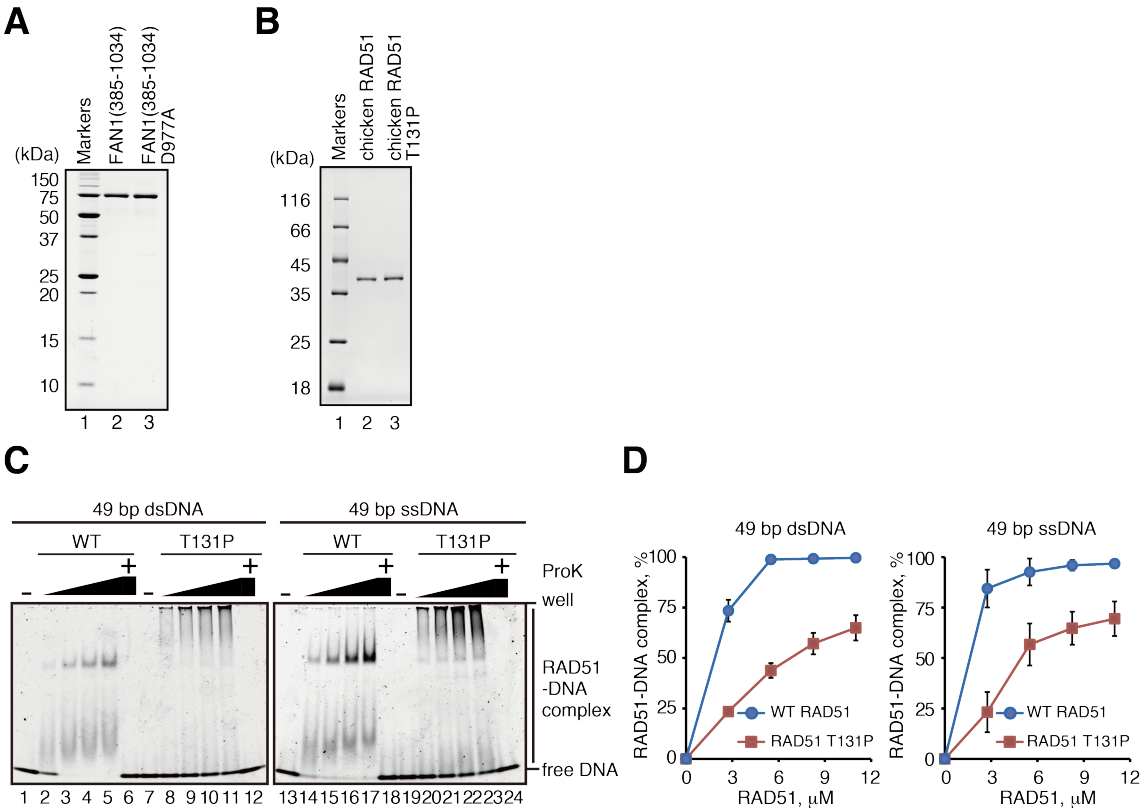
Supplementary Figure S1. Validation of FANCD2 knock-down experiments and PLA. (**A** and **B**) FANCD2 expression levels in 1BR3.hTERT fibroblasts and U2OS cells, in the presence of control siRNA (siControl) or FANCD2 siRNA (siFANCD2). After siRNA transfection, total cellular proteins were prepared from untreated or MMC-treated (100 ng/mL, 24 h) fibroblasts (A) and untreated or HU-treated (4 mM, 5 h) U2OS cells (B). Anti-FANCD2 (top), anti-RAD51 (middle), and anti-α-tubulin (bottom) antibodies were used to detect the corresponding proteins. (**C** and **D**) Validation of the *in situ* PLA. In the top panel, a representative PLA image is shown for untreated (C) or MMC-treated (D) U2OS cells expressing FLAG-tagged FANCD2, with a combination of anti-FLAG and anti-RAD51 antibodies. In the middle and bottom panels, representative PLA images are shown for the omission of the anti-FLAG or anti-RAD51 antibody, respectively. The numbers of PLA signals are represented as dot plots. Statistical differences were determined by the Student's t test; *P<0.0001. Bar: 10 μm.



Supplementary Figure S2. Protein purification and pull-down assay. (**A**-**C**) The purified proteins were analyzed by 12% SDS-PAGE with Coomassie Brilliant Blue staining. (A) Purified human and chicken RAD51. (B) Purified chicken FANCI, FANCD2, His_6-FANCI, and His_6-FANCD2. (C) Purified human DMC1. (**D**) Pull-down assay with the His_6-tagged I-D complex. The RAD51 and DMC1 bound to the His_6-tagged I-D complex were copelleted with the Ni-NTA beads, and the proteins were analyzed by 5-20% SDS-PAGE with Coomassie Brilliant Blue staining. The amounts of RAD51 and DMC1 in the bound fractions were quantitated, and the mean percentages of three independent experiments are indicated with the standard deviations. (**E**) Purified human RPA was analyzed by 15% SDS-PAGE with Coomassie Brilliant Blue staining. (**F**) Purified chicken His_6-RAD51 and His_6-RAD51 F86E mutant were analyzed by 12% SDS-PAGE with Coomassie Brilliant Blue staining.



Supplementary Figure S3. RAD51 transfer assay. (**A**) Time course experiments of the RAD51 transfer assay with ssDNA beads. RAD51 transfer reactions were performed in the presence of ATP (lanes 1-4) or AMP-PNP (lanes 5-8). The reaction times were 0 min (lanes 1 and 5), 15 min (lanes 2 and 6), 30 min (lanes 3 and 7), and 60 min (lanes 4 and 8). The RAD51 retained on the ssDNA beads was analyzed by 10% SDS-PAGE with Coomassie Brilliant Blue staining. (**B**) Graphical representation of the experiments shown in panel A. The amounts of RAD51 retained on the ssDNA beads were quantitated, and the mean percentages of three independent experiments are plotted with standard deviations. (**C**) DNA binding assay with the I-D complex and the I-D-dsDNA complex. The ^{32}P-labeled ssDNA, with the same sequence as the competitor DNA used in the RAD51 transfer assay, was incubated with the I-D complex (0.8 μM, lanes 2 and 3) or the I-D-dsDNA complex (0.8 μM, lanes 4 and 5), and the samples were analyzed by 6% PAGE. Lanes 3 and 5 indicate control experiments, in which the samples were deproteinized before electrophoresis.



Supplementary Figure S4. Purification of FAN1 and RAD51 T131P. (**A**) Purified chicken FAN1 nuclease domain (amino acids 385-1034). The purified FAN1 nuclease domain and the FAN1 nuclease domain D977A mutant, in which the Asp977 residue located in the nuclease active center was replaced by Ala, were analyzed by 15% SDS-PAGE with Coomassie Brilliant Blue staining. (**B**) Purified chicken RAD51 T131P. Purified RAD51 and RAD51 T131P were analyzed by 15% SDS-PAGE with Coomassie Brilliant Blue staining. (**C**) DNA binding assay of RAD51 T131P. The 49-mer dsDNA or 49-mer ssDNA was incubated with increasing amounts (0, 3, 6, 9, and 12 μM) of wild-type (WT) RAD51 or RAD51 T131P, and the samples were analyzed by PAGE with SYBR Gold staining. Lanes 6, 12, 18, and 24 indicate control experiments with 12 μM protein, in which the samples were deproteinized before electrophoresis. (**D**) Graphic representation of the experiments shown in panel C. The intensity of the free DNA band was quantitated, and the amounts of DNA bound to the proteins were estimated. The mean percentages of three independent experiments are plotted against the protein concentration, with standard deviations.