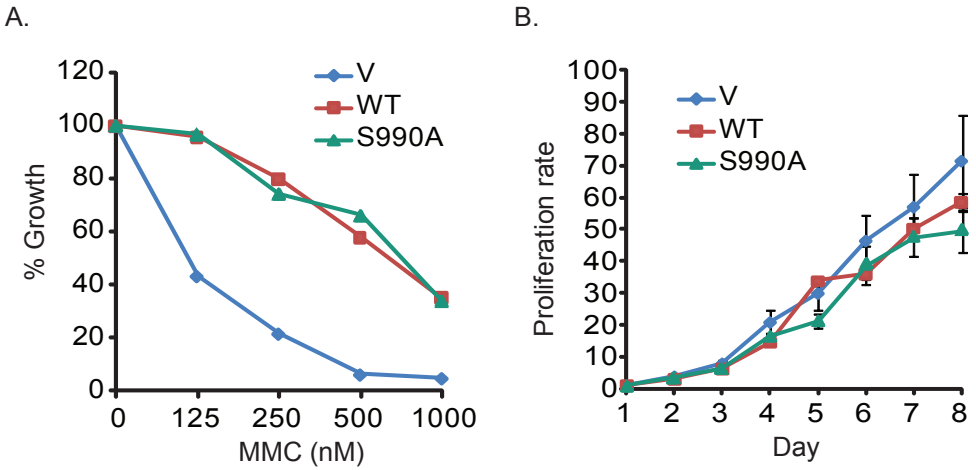
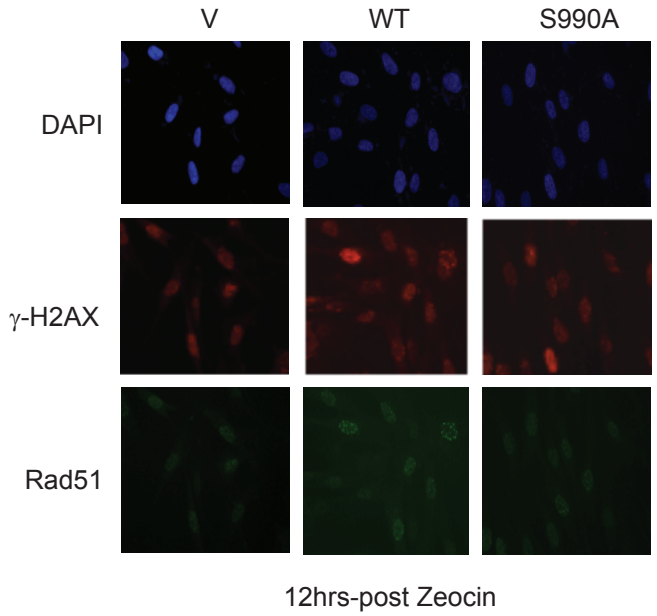
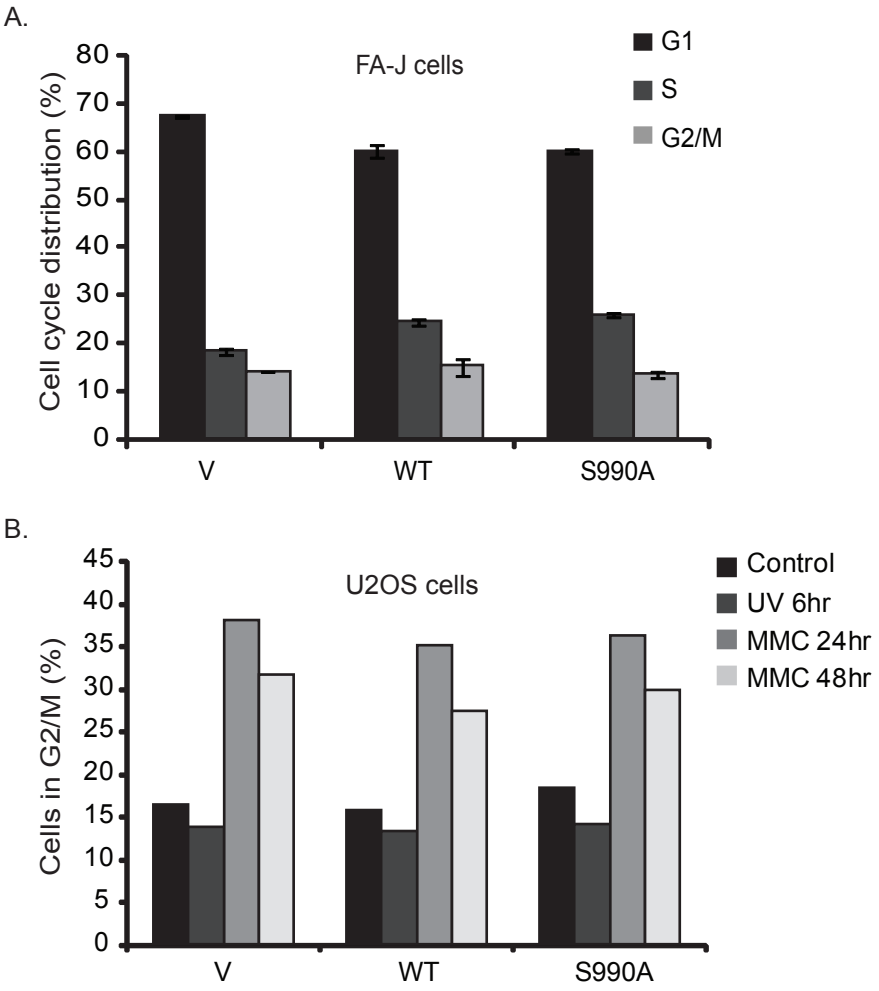


Supplemental Figure 1. FANCJ^{S990A}, similar to FANCJ^{WT} restores normal ICL response in FA-J cells. (A) The FA-J cells expressing vector, FANCJ^{WT}, or FANCJ^{S990A} were plated at low density, treated with the indicated doses of MMC and allowed to grow for 5-8 days. The cells were then collected and counted to analyze percent growth. Data is from one representative experiment. (B) The FA-J cell lines were plated at low density. Cells were collected and counted everyday for 8 days to determine proliferation rate.

Supplemental Figure 2

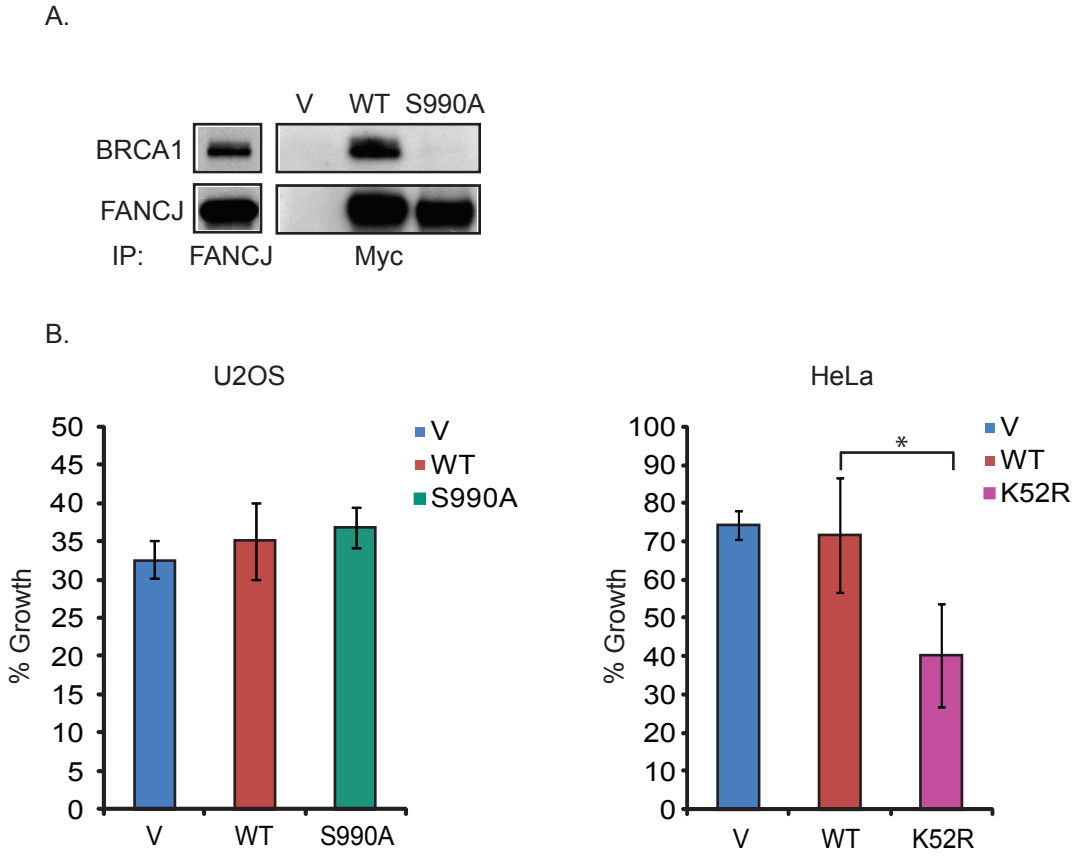


Supplemental Figure 2. FANCJ^{S990A} reduces Rad51 foci following DSBs in FA-J cells. FA-J cells stably expressing vector, FANCJ^{WT}, or FANCJ^{S990A} were treated with 12.5μg/ml of zeocin and immunoflourescence was performed with γ-H2AX and RAD51 Abs.



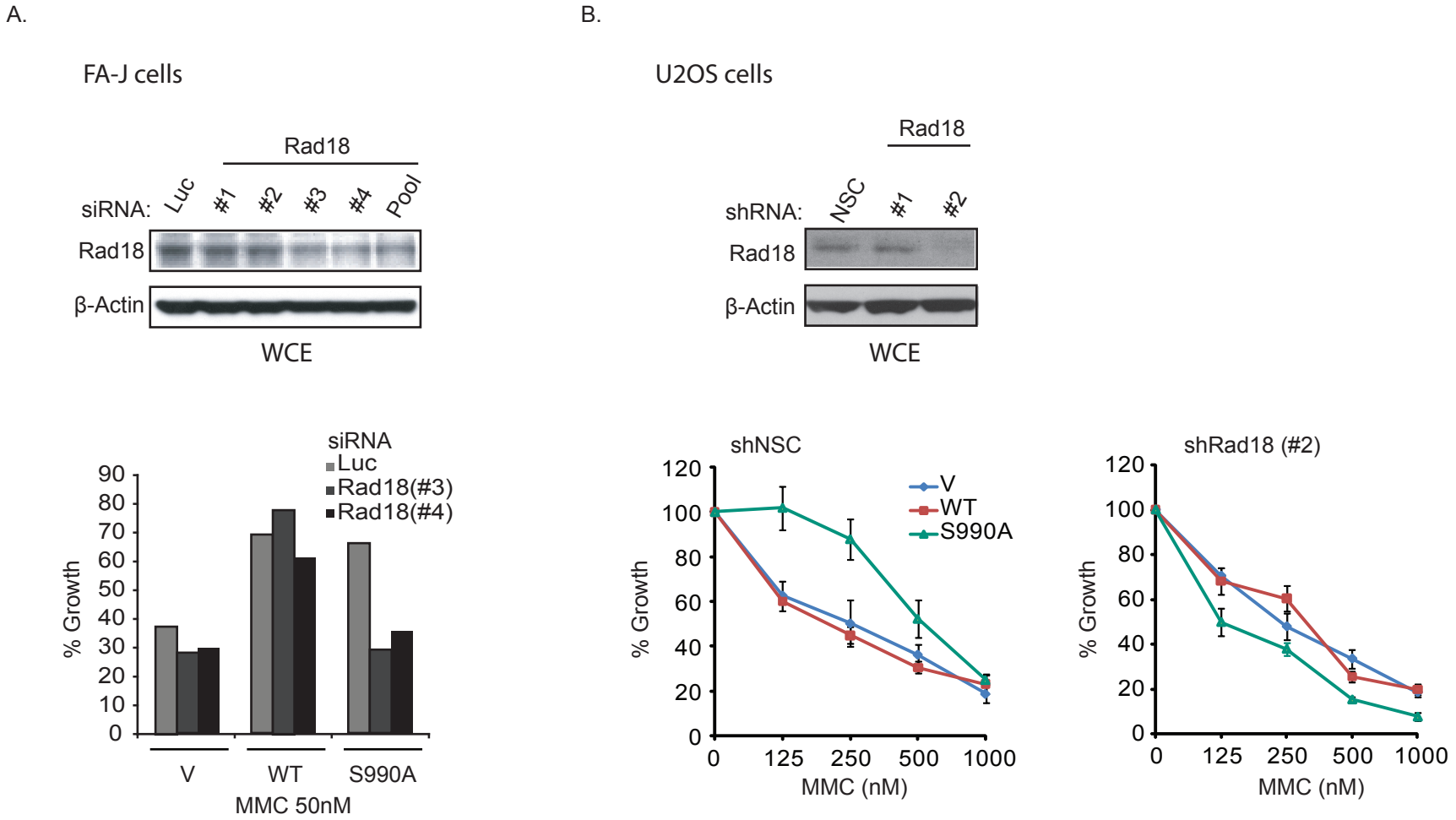
Supplemental Figure 3. FANCJ^{S990A}, as compared to FANCJ^{WT}-complemented FA-J cells, show similar cell cycle distributions in response to DNA damage. (A) The FA-J cells expressing vector, FANCJ^{WT}, or FANCJ^{S990A} were treated with 12.5μg/ml zeocin, 12h later cells were collected and analyzed by FACS to determine the cell cycle distribution. Data represent mean ± SD for 3 independent experiments. (B) U2OS cells stably expressing vector, FANCJ^{S990A}, or FANCJ^{WT} were treated with UV or MMC and collected at the indicated time points and analyzed by FACS to determine the percentage of cells in G2/M. Data represent one experiment performed in triplicate.

Supplemental Figure 4



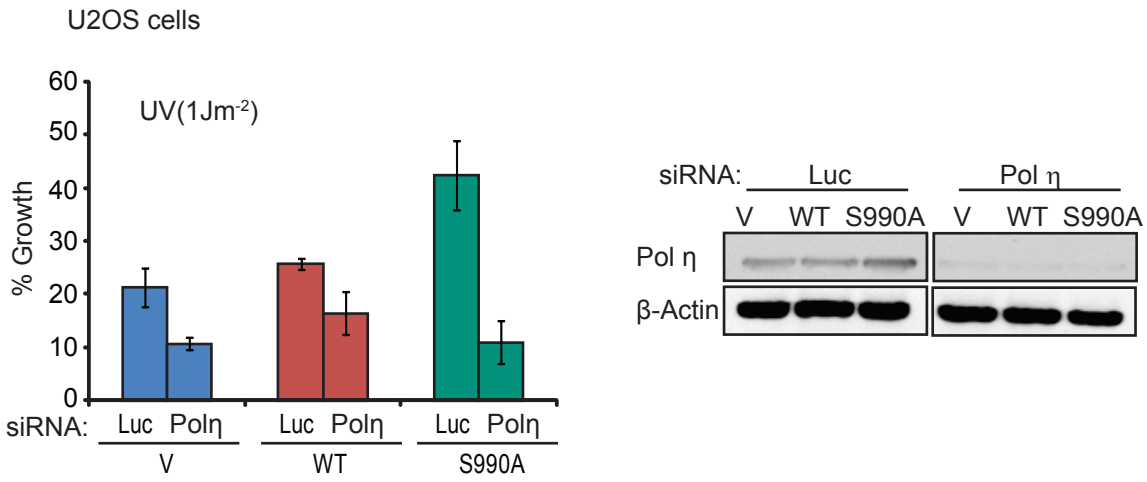
Supplemental Figure 4. Exogenous and endogenous FANCJ bind to similar levels of BRCA1, and unlike of FANCJ^{S990A}, FANCJ^{K52R}- over-expression resulted in MMC sensitivity. (A) MCF7 cells un-transfected or transfected with vector, FANCJ^{WT}, or FANCJ^{S990A} were immunoprecipitated with anti-FANCJ or Myc Ab respectively, and blotted with the indicated Abs. (B) U2OS or HeLa cells stably expressing vector, FANCJ^{WT}, FANCJ^{S990A} or FANCJ^{K52R} were treated with 500nM or 10nM MMC, respectively. Cells were grown for 5-8 days, collected, and counted to analyze percent growth. Data represent mean percent ± SD of growth from 3 independent experiments.

Supplemental Figure 5



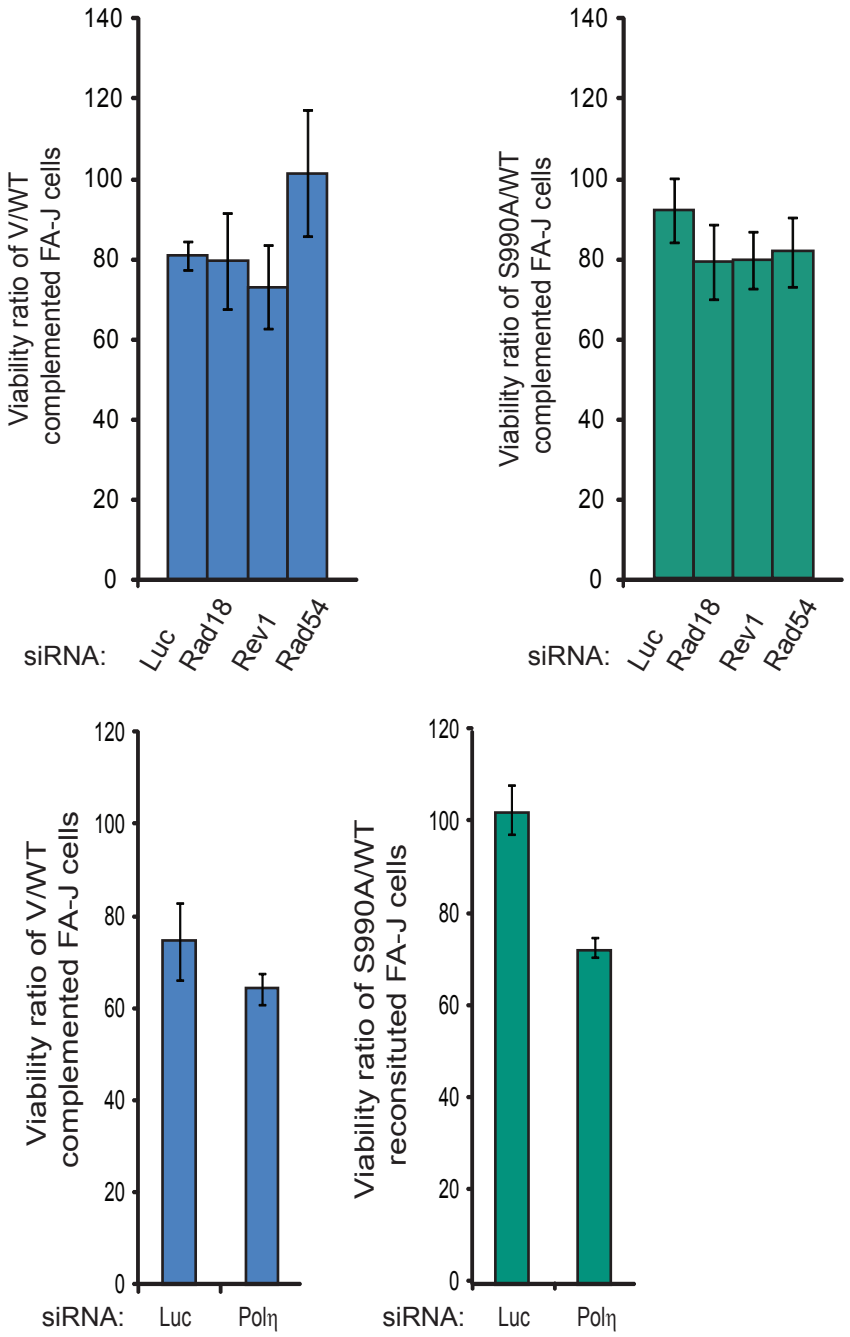
Supplemental Figure 5. FANCJ^{S990A} promotes MMC resistance in a Rad18 dependent manner (A) FA-J cells stably expressing vector, FANCJ^{WT}, or FANCJ^{S990A} were transfected with siRNA to Luc, Rad18 #1, #2, #3, #4, or pool (1+2+3+4). Cells were then collected and immunoblotted with the indicated Abs. Similar to the pool, siRNAs #3 and #4 depleted Rad18, whereas, siRNAs #1 and #2 did not demonstrate signficant depletion of Rad18. Stable FA-J cells expressing Luc, Rad18 siRNAs #3, or #4 were seeded and treated with MMC. (B) U2OS cells were infected with pGIPZ shRNA to non-silencing control (NSC), Rad18 #1, or Rad18 #2. Cells were then collected and immunoblotted with the indicated Abs. Rad18 #2 shRNA showed significant depletion compared to control. Thus, cells stably expressing shRNA to Rad18 #2 or control were transfected with vector, FANCJ^{WT}, or FANCJ^{S990A}. Cells were seeded, treated with the indicated dose of MMC, 6 days later cells were collected, and counted to analyze percent growth. Representive experiments are shown with ± SD in B.

Supplemental Figure 6



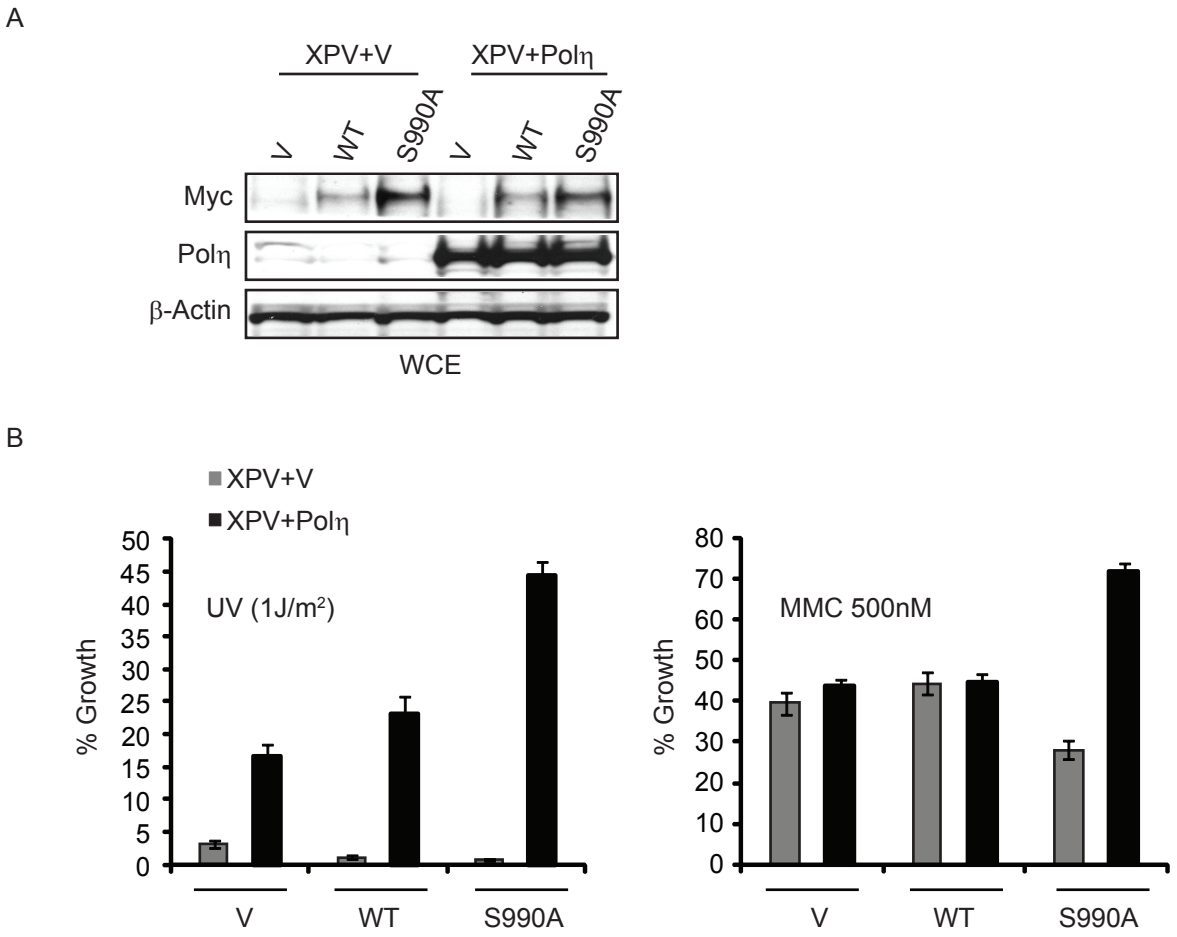
Supplemental Figure 6. FANCJ^{S990A} promotes UV resistance in polη-dependent manner. U2OS cells transiently expressing vector, FANCJ^{WT}, or FANCJ^{S990A} were transfected with siRNA to either luc or polη and incubated for 24hrs. Cells were collected and lysed for immunoblot with the indicated Abs, or plated at low density, treated with UV, and allowed to grow for 5 days. The cells were then collected and counted to analyze percent growth. Data represent mean percent ± SD of growth from 3 independent experiments.

Supplemental Figure 7



Supplemental Figure 5. Growth of the FA-J cell lines treated with siRNA. FA-J cells stably expressing vector, FANCJ^{WT}, or FANCJ^{S990A} were transfected with siRNA to luc, Rad18, Rev1, Rad54 or Polη incubated for 48h. Cells were allowed to grow for 5-8 days, then collected and counted to analyze percent growth. Viability was calculated as shown in Y-axis.

Supplemental Figure 8



Supplemental Figure 8. FANCJ^{S990A} promotes UV and MMC resistance in a polη-dependent manner. (A) XPV cells complemented with vector or Polη were transiently transfected with vector, FANCJ^{WT}, or FANCJ^{S990A}. Cells were either collected and immunobloted with the indicated Abs or (B) treated with the indicated dose of UV or MMC and allowed to grow for 6 days. Cells were then collected and counted to analyze percent growth. Data represent mean percent ± SD of growth from 3 independent experiments.