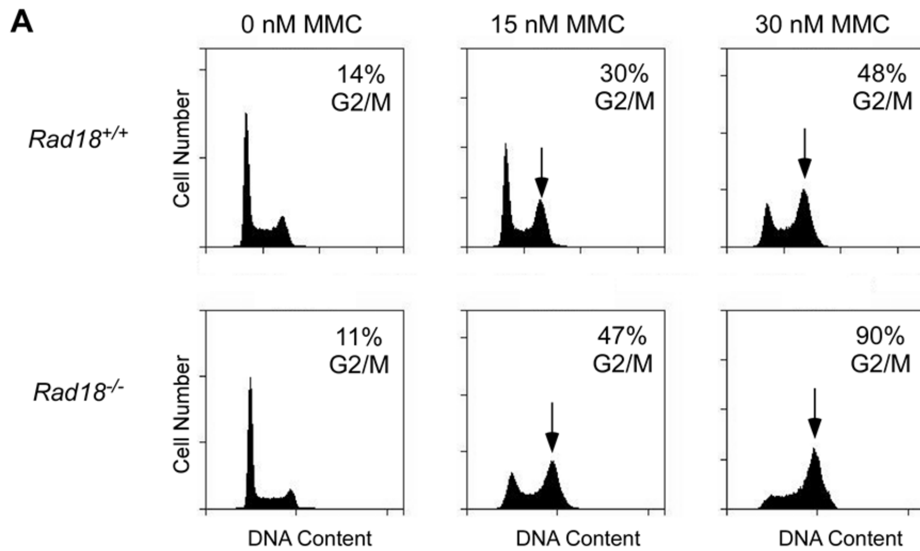
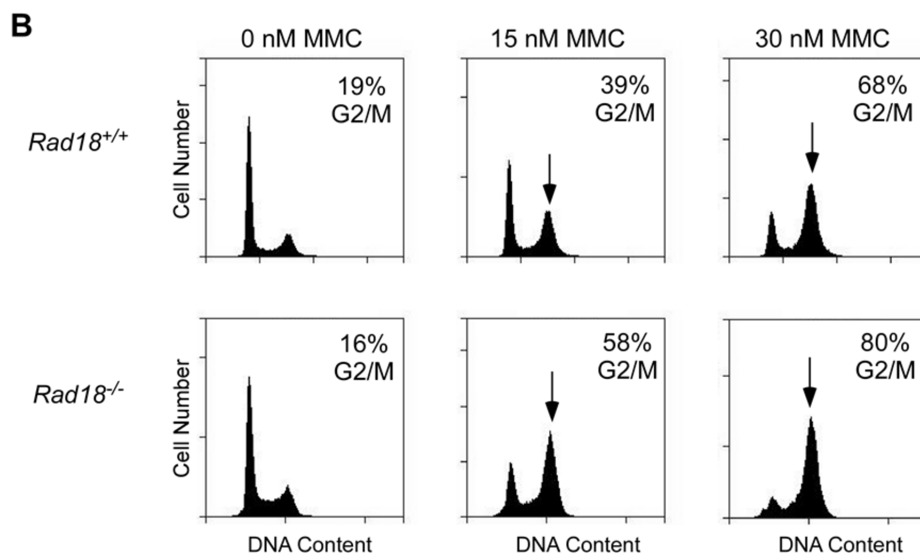
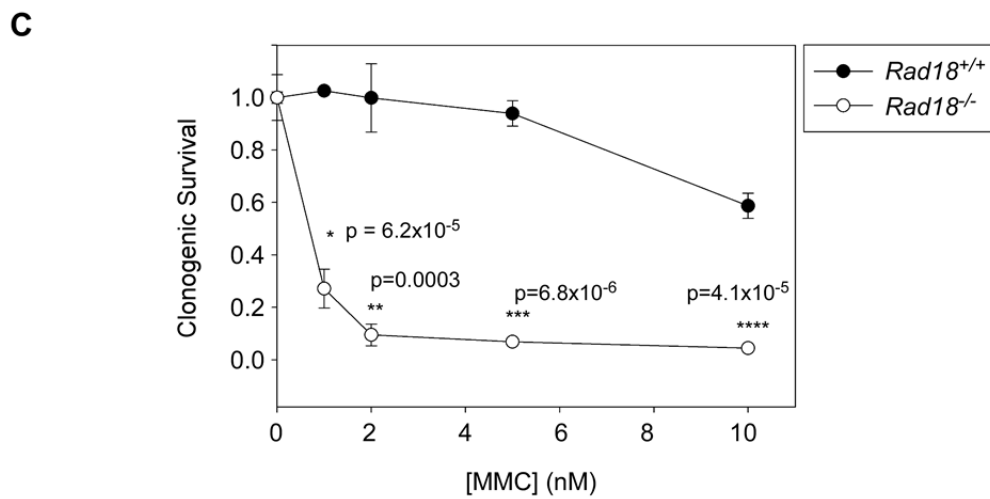




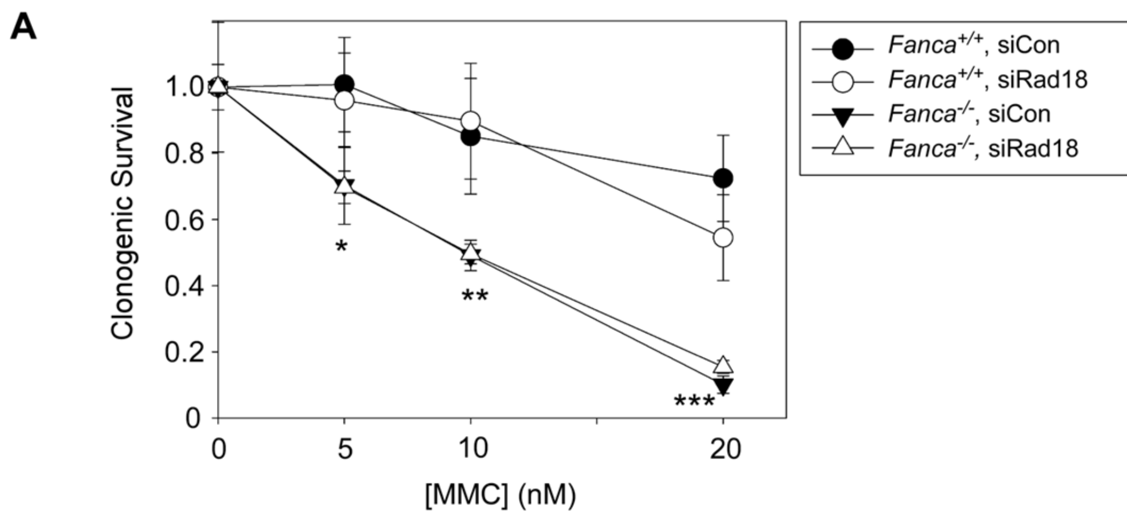


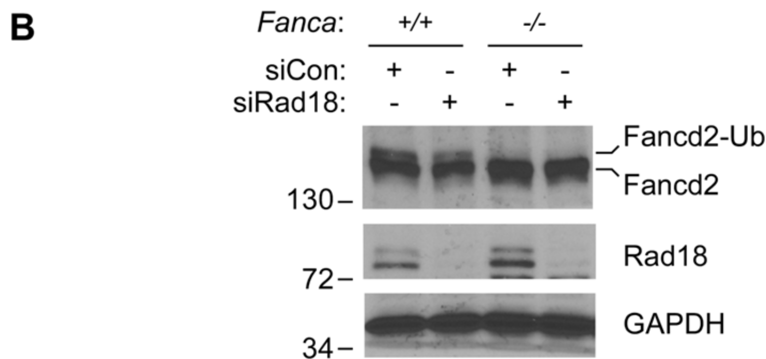
Supplementary Figure 1 Aberrant MMC-Induced G2 arrest and MMC-sensitivity *Rad18$^{-/-}$* MEF

(A) Replicate cultures of log-phase isogenic MEF derived from *Rad18*$^{+/+}$ and *Rad18*$^{-/-}$ littermates were incubated for 48 hr with MMC (0, 15nM or 30nM). Nuclei from the resulting cells were stained with propidium iodide and DNA contents of all samples were analyzed by flow cytometry. All MEF data shown in **Fig. 1** were obtained using these isogenic MEF.

(B) In an experiment identical to that described in panel **(A)** above, but using isogenic *Rad18*$^{+/+}$ and *Rad18*$^{-/-}$ MEF derived from a different pregnant female, cells were incubated for 48 hr with MMC (0, 15nM or 30nM) or were left untreated for controls. Nuclei from the resulting cells were stained with propidium iodide and DNA contents of all samples were analyzed by flow cytometry.

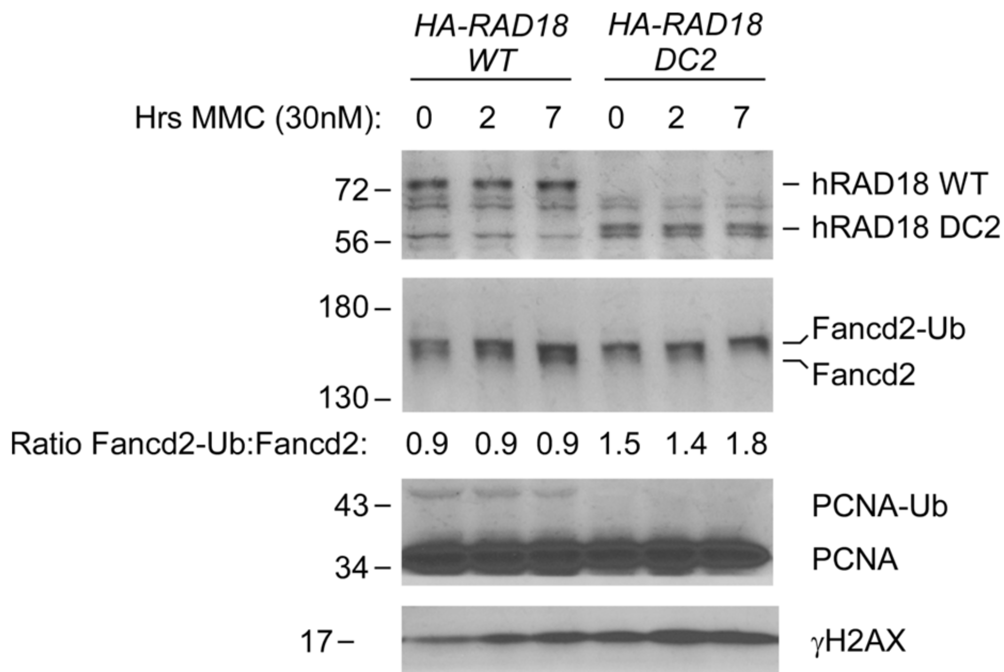
(C) Replicate cultures of the isogenic *Rad18*$^{+/+}$ and *Rad18*$^{-/-}$ MEF described in panel B above were incubated with various doses of MMC for 2 days and subsequently analyzed for clonogenic survival. On the survival curves, each data point represents the mean of three replicate determinations, and error bars represent the range. For each dose of MMC, we performed unpaired Student's t-test between between groups. For cells that received 1, 2, 5, or 10 nM MMC, the p values are indicated on the plot and demonstrate highly significant differences between MMC-tolerance of *Rad18*$^{+/+}$ and *Rad18*$^{-/-}$ cells.





Supplementary Figure 2 MMC-sensitivity of *Fanca*$^{-/-}$ MEF.

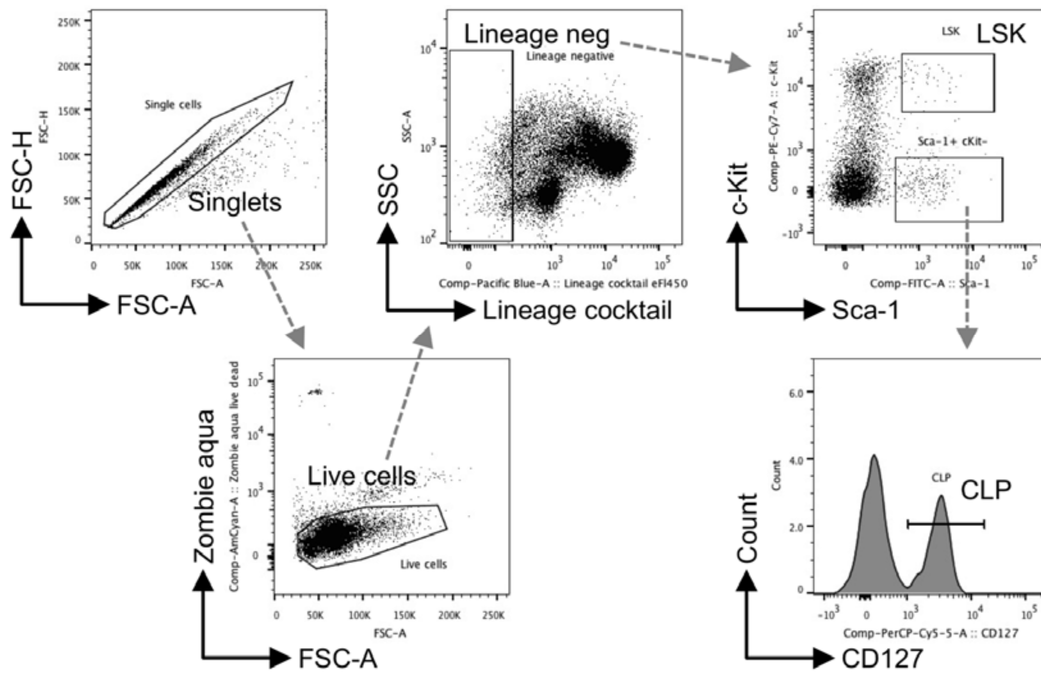
Primary cultures of *Fanca*$^{+/+}$ and *Fanca*$^{-/-}$ MEF were transfected with control non-targeting siRNA or with siRNA against Rad18. siRNA-transfected cultures were re-plated and incubated with various doses of MMC for 2 days and analyzed for clonogenic survival **(A).** On the survival curves, each data point represents the mean of three replicate determinations, and error bars represent the range. For each dose of MMC, we performed ANOVA between groups followed by Tukey's multiple comparison of means test. Results of the Tukey test indicate significant difference in MMC-sensitivity between *Fanca*$^{+/+}$ and *Fanca*$^{-/-}$ cells at every MMC concentration (all p values were <0.003, as indicated by the asterisks on the graph) but no difference in sensitivity between siCon and siRad18 groups. Some cultures of siRNA transfected cells were harvested 48 hr after re-plating to obtain protein extracts for SDS-PAGE and immunoblotting with the indicated antibodies **(B).**



Supplementary Figure 3 Immunoblot analysis of PCNA and Fancd2 from *HA-RAD18 WT* and *HA-RAD18 DC2* MEF.

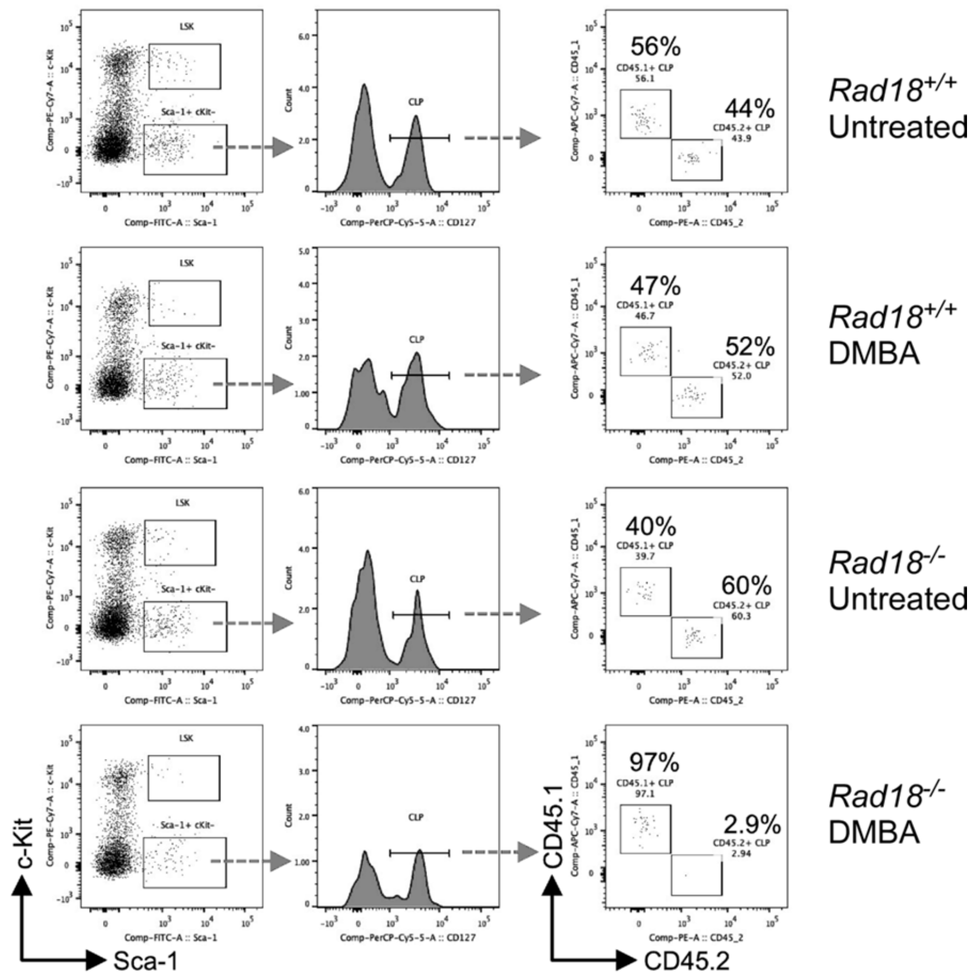
Exponentially-growing cultures of HA-RAD18 WT and HA-RAD18 DC2 MEF were treated with 30 nM MMC for 2 or 7 hr. Protein extracts from MMC-treated (and control untreated) cells were harvested and analyzed by immunoblotting with the indicated antibodies. MMC induced a DNA damage response in as evidenced by increased levels of γH2AX in the drug-treated cultures relative to untreated controls. The relatively low level of MMC used in this experiment did not trigger an increase in PCNA ubiquitination above basal levels in the HA-RAD18 WT cells. As expected from our previous work (Durando et al., 2013), PCNA-mono-ubiquitination was attenuated in HA-RAD18 DC2 cells relative to HA-RAD18 WT MEF. Basal levels of γH2AX were higher in the HA-RAD18 DC2 cells relative to HA-RAD18 WT MEF, most likely because of increases in replication stalling and spontaneous DNA damage due to dampened TLS. Fancd2 mono-ubiquitination was increased in the HA-RAD18 DC2 cells relative to HA-RAD18 WT MEF (as evidenced by increased ratios of modified:unmodified Fancd2). Taken together, these results show that Fancd2 ubiquitination is not compromised in cells

expressing HA-RAD18 DC2. On the contrary, the decreased PCNA mono-ubiquitination in HA-RAD18 DC2 cells is associated with compensatory increases in γH2AX and FA pathway activation.



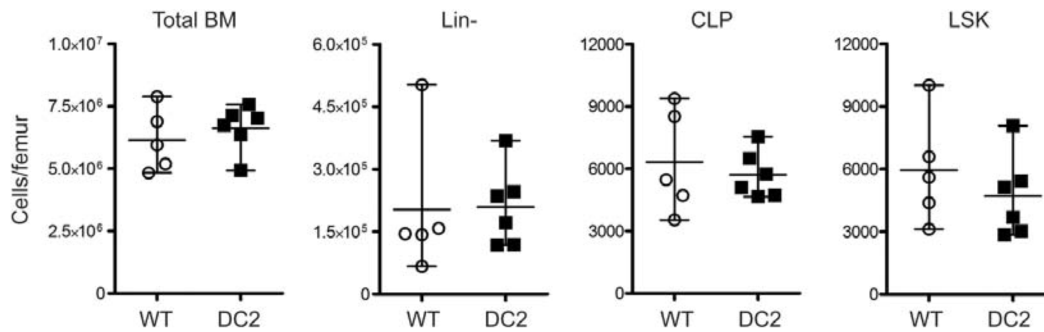
Supplementary Figure 4

Gating strategy for identification of hematopoietic progenitors progenitor gating strategy (no congenic markers).



Supplementary Figure 5

CD45.1 and CD45.2 gating for the mixed BM transfers, beginning with lineage negative cells in the left panels, c-kit/Sca-1 gating in the middle, and then CLP as the example population show (with frequencies).



Supplementary Figure 6 Effect of DMBA treatment on hematopoietic progenitors in *HA-RAD18 WT* and *HA-RAD18 DC2* mice.

HA-RAD18 WT and *HA-RAD18 DC2* mice were administered 6 doses of DMBA as described in the legend to Fig. 4. Bone marrow cells from the DMBA-treated mice were stained with antibodies against appropriate cell surface markers and assessed for various hematopoietic cell subsets using flow cytometry. Statistical analysis was performed using an unpaired, two-tailed Student's t-test. There was no significant difference in the DMBA-sensitivity of LSK cells (or other hematopoietic subsets, not shown) between *HA-RAD18 WT* and *HA-RAD18 DC2* mice.