Figure S1. FMRP-Mediated γH2AX Induction and BRCA1 Phosphorylation, Related to Figures 1, 3, and 7

(A and B) Induction of γH2AX foci formation is impaired in FMRP KO (B) as compared to wild-type MEFs (A) in response to aphidicolin (APH) treatment.

(C) Left graph, wild-type but not FMRP KO and Dot1L mutant MEFs exhibited a 3-fold (cells with < 10 foci) and 4-fold (cells with > 10 foci) increase in γH2AX foci in response to APH.

(D) Western blot showing the levels of expression of exogenous Flag-HA-FMRP (lane 2) and FMRP Agenet domain mutants (lanes 3 and 4) in FMRP KO MEFs.

(E) HeLa cells were subjected to scrambled or two independent FMRP shRNAs (FMRP shRNA1 and FMRP shRNA2). Cells were then treated with DMSO as a control or APH for 24 hr, followed by western blotting for BRCA1 phospho-Ser1423. In contrast to control RNAi cells (3.5-fold induction in Ser1423 phosphorylation), FMRP RNAi cells showed no phosphorylation of BRCA1 at Ser1473 in response to APH treatment (compare lane 2 to 4 and 6).

(1) Unlike in HeLa cells, in 293 cells FMRP RNAi-mediated dampening of phospho-Ser1423 could be partially attributed to the increased levels of phospho-Ser1423 even in the absence of APH treatment, suggesting that the FMRP effect on BRCA1 phosphorylation might be context dependent. Blots are representative of three experiments.