SUPPLEMENTAL FIGURE LEGEND

Fig. S1. DNA-damage-induced phosphorylation of FANCM is ATR dependent. **(*A*)** Time-dependent up-regulation of FANCM phosphorylation (pFANCM) and FANCD2 monoubiquitination (FANCD2-UB). HeLa cells were treated with 100 ng/mL MMC for the indicated times were analyzed by immunoblotting using anti-FANCM and anti-FANCD2. **(*B*)** Inhibition of HU-induced FANCM hyperphosphorylation in ATR knockdown cells. Defective phosphorylation correlated with reduced FANCD2 monoubiquitination in HEK293 cells. Cells treated with 1.5 mM HU for 16 h two days after being transfected with either siControl or siATR#1 were analyzed by immunoblotting with anti-ATR, anti-FANCM, anti-FANCD2, and anti-Actin. **(*C*)** Immunoblot showing that suppression of ATR using a second siRNA inhibits HU-induced hyperphosphorylation of FANCM. HEK293 cells were transfected with either a control siRNA (siControl) or a second siRNA targeting ATR (siATR#2). Two days post-transfection, the cells were treated with 1.5 mM HU for 16 h and were analyzed by immunoblotting. The decrease in FANCM phosphorylation correlates with the decrease in FANCD2 monoubiquitination.

Fig. S2. Peptides released from FANCM protein were analyzed for putative phosphorylation sites by mass spectrometry (MS). Yellow shading denotes the peptides covered by MS analysis. PIKK kinases (ATR, ATM, and DNA-PK) consensus site, S/TQ is shaded in green and the site identified in this study as being phosphorylated is shaded in red. 85% of the FANCM protein was represented by the peptides released by three different tryptic digestions.

Fig. S3. Immunoblot showing that antibody raised against phosphorylated S1045 specifically recognizes the ectopically expressed wildtype, but not S1045A mutant, form of FANCM. HeLa cells were transfected with a FLAG-tagged form of either the FANCMWT or FANCMS1045A mutant protein. Two days post transfection, the cells were treated with either 1.5 mM HU or 100 ng/mL MMC for 16 h. Cells were lysed and immunoprecipitated using anti-M2 agarose and immunoblotted with pFANCM (S1045A) antibody.

Fig. S4. UV mediated DNA-damage-induced phosphorylation of FANCM at S1045. HeLa cells were irradiated with 20 J/m_2 UV, and in 0 and 6 h, cell extracts were subjected to immunoprecipitation

with anti-FANCM antibody. Phosphorylation of of endogenous FANCM was detected by immunoblotting with an antibody that detects specifically FANCM phosphorylated at S1045 - pFANCM (S1045).

Fig. S5. Phosphorylation at S1045 is not required for FANCM binding to the FA core complex but for FANCD2 monoubiquitination. (A) Immunoblot showing the presence of FA core proteins in complexes immunoprecipitated (IP) with wildtype (WT) and S1045A mutant forms of FANCM. U2OS cells stably expressing the WT or S1045A mutant form of FANCM were immunoprecipitated with anti-FLAG M2 agarose and immunoblotted with the indicated antibodies. **(B)** Immunoblot showing FANCD2 monoubiquitination status in cells described in main figure 3A, after treatment with 100 ng/mL MMC for 16 h and immunoblotting with anti-FANCD2. Numbers below the FANCD2 immunoblot represent percentage of total FANCD2 that is monoubiquitinated, as measured by densitometry.

Fig. S6. MMC-induced chromatin association of the FA-core complex is impaired in S1045A-expressing cells. Immunoblot shows amount of FANCA associated with chromatin. Endogenous FANCM was stably knocked down in HEK293 cells and wildtype (WT) or S1045A mutant FANCM was ectopically expressed. Cells were treated with 100 ng/mL MMC for 16 h and chromatin fractionation was carried out as described in Methods. Chromatin fractions were immunoblotted using antibodies against FANCA (component of the FA-core complex) and H2A (control for chromatin loading). Numbers below the immunoblot represent fold increase of FANCA in chromatin after MMC treatment compared to levels in untreated samples.

Fig. S7. Recruitment of FANCM to ICL site is ATR dependent. The recruitment of endogenous FANCM to the Psoralen-ICLs were studied by immunofluorescence in Control and ATR knockdown HeLa cells. γ-H2AX was used as positive control for ICL sites. Representative images are shown in panel **A**, and quantitation is shown in **B**. Error bars in the graph indicate the ±SD, n=3. After the laser treatment (to induce ICLs), cells were incubated at 37°C for 10min and fixed immediately in freshly prepared 4% formaldehyde in PBS for 10 min at room temperature. For immunofluorescence staining, cells were incubated at 37ºC for 1 hr with anti-γH2AX (Upstate, Millipore) and anti-FANCM antibodies.

Fig. S8. S1045A mutant-expressing cells do not show any gross change in cell-cycle distribution. Cells-cycle analysis of HeLa cells in which endogenous FANCM was knocked down using an shRNA against its 3'UTR (shFANCM-UTR), and a wildtype (WT), S1045A or K117R mutant form of the protein was ectopically expressed.

Fig. S9. Expression of FLAG tagged FANCMK117R in the context of knockdown of the endogenous protein. U2OS cells stably transduced with shRNA targeting the 3'UTR of FANCM (shFANCM-UTR) were further transduced with retroviral vector alone (Vector), or with retrovirus carrying $FANCM_{K117R}$. U2OS cells stably expressing a non-targeting shRNA (shControl) served as control.