# **Supplementary Figure S1.**



Figure S1. Cell cycle distribution of human prostate and non-small cell lung carcinoma cells during the time course of fractionated radiotherapy (3 fractions of 2 Gy). Unirradiated cells (0 Gy) were used as control. Cells were incubated with BrdU for 10 min at indicated time points. Arrows indicate time of irradiation (2 Gy). Results show mean  $\pm$  STDEV (n = 3, \* P < 0.05, \*\* P < 0.01, Student's t-test).

### **Supplementary Figure S2.**



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Figure S2. (A) Clonogenic survival and (B) SD/MF ratios of 4 wildtype MEF cell lines. (C) Colony formation assay of WT#1, WT#2, and 53bp1<sup>-/-</sup> MEF irradiated with 6 Gy single dose or 3 times 2 Gy fractionated radiation with indicated time intervals between fractions. Unirradiated cells were used as control.

# Supplementary Figure S3.



Figure S3. Cell cycle distribution of wildtype (WT) MEF during the course of fractionated radiotherapy (3 fractions of 2 Gy) with a 2 h or 24 h time interval between fractions.

## **Supplementary Figure S4.**



Figure S4. (A) Survival of wildtype (WT) MEF and  $53bp1^{-/-}$  MEF after single dose radiation (SD). (B) Cell cycle distribution of WT, *Atm-/-*, and 53bp1-/- MEF irradiated with a 24 h time interval between fractions. At indicated time points, cells were incubated with BrdU for 10 min. Staining was performed with anti-BrdU antibody and PI. Unirradiated cells (0 Gy) were used as control. Arrows indicate time of irradiation. Results show mean  $\pm$  STDEV (n = 3, \* P < 0.05, \*\* P < 0.01, Student's t-test).

## **Supplementary Figure S5.**



Figure S5. Cell cycle distribution of unirradiated (0 Gy) and irradiated (3 x 2 Gy) *Rif1fl/fl* and ) *Rif1fl/fl+Cre* at indicated time points during the time course of fractionated radiotherapy (3 fractions of 2 Gy). Cells were incubated with BrdU prior to fixation and staining with Anti-BrdU antibodies. Results show mean  $\pm$  STDEV (n = 3, \*\* P < 0.01, Student's t-test).

### **Supplementary Figure S6.**



Figure S6. (A) Cell cycle distribution of unirradiated (0 Gy) and irradiated (3 x 2 Gy)  $53bp1^{-/-}$  and  $Brca1\Delta 11$  and (B)  $53bp1^{-/-}Brca1\Delta 11$  cells at indicated time points. Cells were incubated with BrdU prior to fixation and staining with Anti-BrdU antibodies. (C) Class switch recombination (CSR) in wildtype (WT),  $53bp1^{-/-}$ ,  $53bp1^{-/-}/Brca1\Delta 11$ ,  $Rnf168^{-/-}$ , and $Rnf168^{-/-}/Brca1\Delta 11$  splenic B cells. WT MEF were used as control. CSR efficiency in WT B cells was set to 100%.

# Supplementary Figure S7.



Figure S7. (A) Western blot showing p53 expression and serine-15 phosphorylation as well as p21 expression in wild type (WT) MEF at 1 h or 24 h after a single 6 Gy dose of IR.  $\beta$ -actin served as loading control. (B) Fold change of *Cdkn1a* mRNA expression in WT cells at 24 h after indicated radiation treatment analyzed using qRT-PCR.