

### Supplementary Figure 1.

**A.** The percentage of G2 (CENPF<sup>+</sup>) cells was determined after knockdown of SETDB1, SUV39 or HP1. The ratio of CENP<sup>+</sup>: DAPI cells was assessed in a minimum of 200 cells.

**B and C.** Following siRNA mediated knockdown of control (CTR), SETDB1, SUV39, HP1, 1BR3 hTERT cells were irradiated with 3 Gy, RPA (C) and RAD51 (D) foci number were enumerated at the indicated times.

### Supplementary Figure 2.

**A.** Following transfection of U2OS DR-GFP cells with siRNAs targeting control (CTR), SETDB1, SUV39, HP1 or BRCA1, cells were re-transfected with pCBASceI and stained with propidium iodide. The percentage of G1, S and G2/M phase cells was assessed by FACS analysis.

**B.** The *I-SceI* plasmid (pCBASceI) includes an HA-Tag. After transfection of U2OS DR-GFP cells with pCBASceI, *I-SceI* expression level were determined by staining with HA antibody.

**C.** HR efficiency was also determined after SETDB1 knockdown using each siRNA oligonucleotide. Results represent the mean  $\pm$  S.E.M of three experiments. Asterisks indicate a statistically significant difference between siCTR and siSETDB1#A or siSETDB1#B ( $P < 0.05$ ; t-test).

**D.** Knockdown efficiency of two distinct siRNA SETDB1 oligonucleotides, singly or combined, was assessed by western blotting.

**E and F.** Following transfection of siRNAs targeting control (CTR), SETDB1, SUV39 or HP1, H1299 dA3-1 cells, which harbour a construct that allows NHEJ to be monitored, were re-transfected with pCBASceI (3,30). The percentage of GFP<sup>+</sup> cells was measured by FACS 48 h after transfection with *I-SceI*. GFP<sup>+</sup> cells were normalised to the percentage of EGFP positive cells and the level of the S/G2 population (**E**).

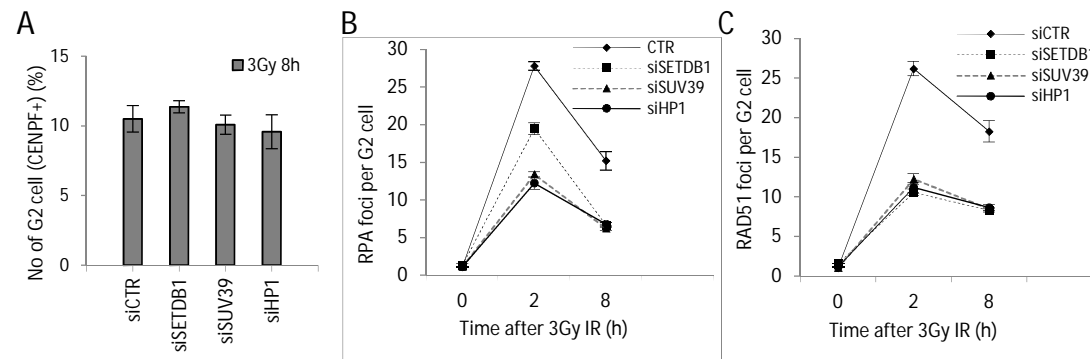
The level of HA-tagged *I-SceI* was assessed by staining with HA antibody following knock down (**F**).

**Supplementary Figure 3.**

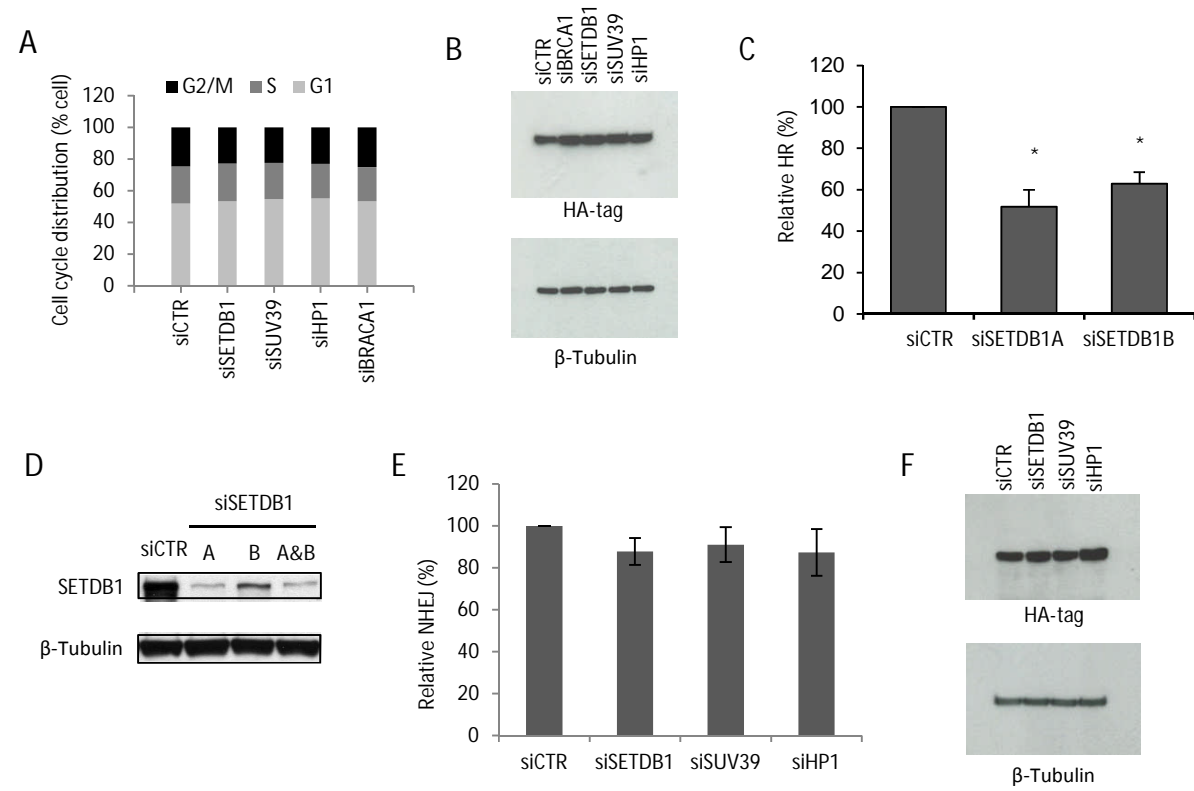
**A.** 1BR3 hTERT cells were transfected with siRNAs targeting control (CTR) or EXO1/BLM. The knockdown efficiency was verified by immunoblotting.

**B and C.** 1BR3 hTERT cells were transfected with siRNAs targeting control (CTR) or SETDB1, 48 h later cells were irradiated with 3 Gy and co-stained with  $\gamma$ H2AX/ 53BP1 or  $\gamma$ H2AX/BRCA1 antibody at the indicated times post IR. The percentage of overlapped  $\gamma$ H2AX/ 53BP1 foci (**A**) and  $\gamma$ H2AX/BRCA1 foci (**B**) was quantified in deconvolved Delta Vision 2D images by the softWoRx® suite software. The Pearson Coefficient of Correlation monitors how closely two intensities overlap on a pixel-by-pixel basis (full overlap is 1.0).

Supplementary figure 1



Supplementary figure 2



### Supplementary figure 3

