**Supplementary Figure S1. *BRCA1* mutation confirmation and *RAD51* gene expression in SUM149 cells.**

(A) BRCA1 status of the four TNBC cell lines used in the present study was confirmed by Western blot. (B) SUM149 cells were sorted for ALDH negative and positive cells, then radiated with a single dose of 4 Gy. RNA was collected 12 hours after radiation to determine changes in DNA repair proteins. The genes shown had the highest and lowest fold change.

**Supplementary Figure S2. RAD51 foci is associated with CSC population of *BRCA1*-mutant TNBC cells and effect of long-term PARP inhibition on RAD51.**

(A) RAD51 foci scoring in sorted ALDEFLUOR-positive and –negative cells HCC1937 and MDA-MB-231 cells. HCC1937 and MDA-MB-231 cells were sorted based on aldehyde dehydrogenase activity. Sorted ALDEFLUOR-positive and –negative cells were seeded onto 96 well cell culture plates and radiated at 4Gy single dose the following morning; cells were then fixed at 0 and 12 post radiation and stained for RAD51. Nuclei with ≥ 5 foci were counted as RAD51 positive cells. (B) TNBC cells were treated with/without PARPi (1µM), cells were lysed after 7 days for Western blot. Mean±SEM from ≥ 3 biological repeats. HCC1937 0h vs 12h p=0.086

**Supplementary Figure S3: Knockdown of *RAD51* prevents foci formation in SUM149 and SUM159 cells.**

*RAD51* KD was induced by 72 hours of doxycycline treatment, and then cells were seeded onto chamber slides overnight and radiated at 4Gy single dose the following day to induce DNA double strand breaks, the cells were then fixed, permeabilized and stained with γH2X and RAD51, followed by Alexa-488 and Alexa-549 secondary antibodies. DAPI was applied in the mounting medium to stain nucleus.

**Supplementary Figure S4. The effect of *RAD51* KD on TNBC viable cell count, CSCs, and cell cycle.**

SUM149 and SUM159 cells were plated, and *RAD51* KD was induced for 7 days prior to ALDEFLUOR assay. (A) Cell number, (B) ALDEFLUOR percentage and (C) absolute CSC number (cell number times ALDEFLUOR-positive) after 3 days of *RAD51* KD in SUM149 and SUM159. Cell cycle was assessed by propidium iodide staining in (D) SUM149 and (E) SUM159 cells after 3 days of *RAD51* KD. Mean±SEM from 3 biological repeats. \*p<0.05, \*\*p<0.01.

**Supplementary Figure S5. *RAD51* KD sensitizes TNBC CSCs to PARPi.**

(A) MDAMB231 and (B) HCC1937 cells were plated and *RAD51* KD was induced for 3 days prior to olaparib treatment at 0, 10, 100 nM. Aldehyde dehydrogenase activity was assessed after 7 days treatment and absolute cancer stem cell number was calculated by multiplying percentage of ADLEFLUOR-positive cells and total cell number for (C) MDAMB231 and (D) HCC1937 cells. Mean±SEM from 3 biological repeats.

**Supplementary Figure S6. Efficiency of *RAD51* shRNA knockdown with additional shRNA vectors.**

SUM159 and SUM149 cells were infected with two additional pTripZ-RAD51-shRNA vectors (1, 4). Cells were lysed after three days of ± doxycycline induction, 50ug lysate from each sample was used for Western blot and β-actin was used as loading control. Quantification was performed using ImageJ, the intensity of the RAD51 band was normalized against β-actin.

**Supplementary Figure S7. Knocking down *RAD51* with two additional shRNA vectors also sensitizes CSCs to PARPi.**

Infected SUM149 and SUM159 cells were incubated with or without doxycycline for 3 days, and then the cells were treated with PARPi (0, 100nM) for 5 days prior to ALDEFLUOR assay. The absolute CSC number was calculated as ALDEFLUOR+ve (%) times total cell number. Mean±SEM from 3 biological repeats. \*p<0.05, \*\*\*p<0.001.

**Supplementary Figure S8. “Physiologic” *RAD51* knockdown decreases cancer stem cells in SUM149 cells.**

SUM149 *RAD51* KD cells were induced with low concentrations of doxycycline for 3 days to achieve downregulation of RAD51 (A) RNA expression and (B) protein levels. (C) SUM149 cells were plated and 2-fold *RAD51* KD was induced for 3 days prior to olaparib treatment at 10μM. Aldehyde dehydrogenase activity was assessed after 3 days treatment and absolute cancer stem cell number was calculated by multiplying percentage of ADLEFLUOR-positive cells and total cell number. Data is presented as Mean±SEM from ≥3 technical repeats; adjusted P value presented: \*p,0.05; \*\*p<0.005; \*\*\*\*p<0.0001.