Supplementary Figure 1. Time-dependent radioresistance in glioma CSCs. Human patient-derived (A) IN528, (B) T3565, and (C) T4121 glioma CSCs and matched non-CSC cells and control U87 human glioma cells were irradiated with 5 Gy X ray or subjected to sham operation without irradiation. Cells were trypsinized and cultured as single-cell suspension at day 7 to avoid overgrown neurospheres. Cell viability was determined after different time, and expressed as a percentage of non-irradiated cells that were harvested at the same time point after irradiation (mean ± SEM, n = 3).
Supplementary Figure 2. Delayed H2AX phosphorylation in glioma CSCs. T3565, T3961 and T4121 glioma CSCs and matched non-CSC cells were irradiated with 5 Gy X ray. Cell lysates were collected at different time points post-irradiation, and subjected to immunoblot analysis with anti-phospho-H2AX-Ser139 and anti-GAPDH antibodies. Representative blots are shown.
Supplementary Figure 3. Clustering analysis of RNA-seq data for DNA damage and repair genes in glioma CSCs.
RNA was isolated from IN528, T4121, and T3961 glioma CSCs and matched non-CSC cells, and subjected to transcriptome analysis by RNA deep sequencing. FPKM values were calculated for the DNA repair genes (available at http://sciencepark.mdanderson.org/labs/wood/dna_repair_genes.html; Wood et al, Science 2001; Friedberg et al, DNA repair and mutagenesis, 2nd Ed, 2006; Lange et al, Nat Rev Cancer 2011), clustered, and expressed in a heat map.
Supplementary Figure 4. DNA-PK (PRKDC) expression is remarkably higher in glioma CSCs, and moderately increased in normal brains and GBM tumor cells, compared with ATM and ATR expression. (A) RNA was isolated from IN528, T4121, and T3961 glioma CSCs and matched non-CSC cells, and subjected to transcriptome analysis by RNA deep sequencing. FPKM values of ATM, ATR, and DNA-PK (PRKDC) were calculated, and expressed as individual dots and means ± SEMs. (B) mRNA expression of ATM, ATR, and DNA-PK (PRKDC) in normal brain and GBM tumors was analyzed in TCGA RNA-seq database (available at http://gliovis.bioinfo.cnio.es). n = 4 for normal brains, and n = 156 for GBM patients.
Supplementary Figure 5. DNA-PK knockdown sensitizes glioma CSCs to radiation at different time. IN528 and T3565 CSCs were transduced with lentivirus that express shRNAs targeting control GFP, DNA-PK, or ATM, followed by puromycin selection for stable expression cell lines. Cells were subjected to viability analysis at different time points after 2 or 5 Gy X-ray radiation (mean ± SEM, n = 3).
Supplementary Figure 6. Radiation-induced ROS generation in CSCs and non-CSCs. IN528 (A) CSCs and (B) matched non-CSCs were irradiated with 5 Gy X-ray. Different time after radiation, intracellular total ROS levels were measured by flow cytometry-based analysis at different time points post-irradiation (mean ± SEM, n = 3).
**Supplementary Figure 7.** Effects of DNA-PK inhibition on radiation-induced aneuploidy in non-CSCs. (A) IN528 and (B) T3565 non-CSCs were pretreated with 1 μM NU7441 (DNA-PK inhibitor) or KU60019 (ATM inhibitor), and irradiated by 5 Gy X-ray. Different time after irradiation, cells were analyzed by flow cytometry for aneuploidy (mean ± SEM, n = 3).