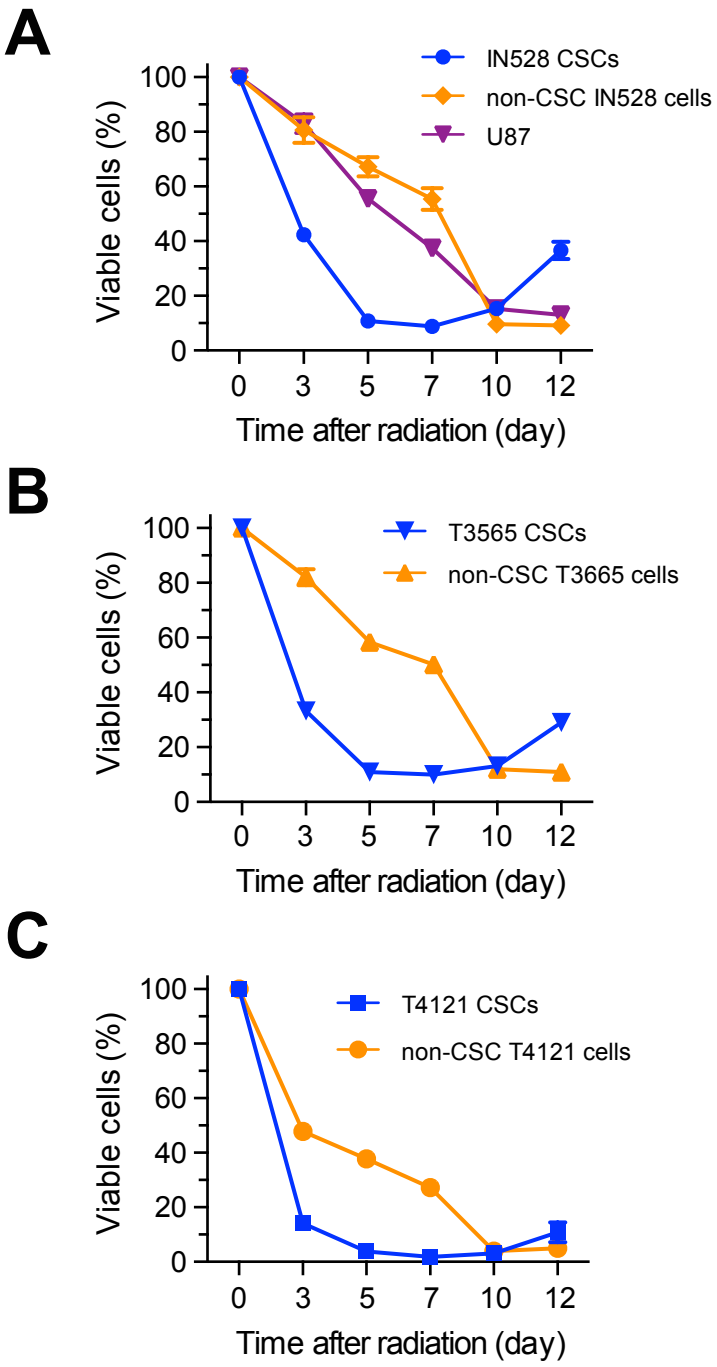
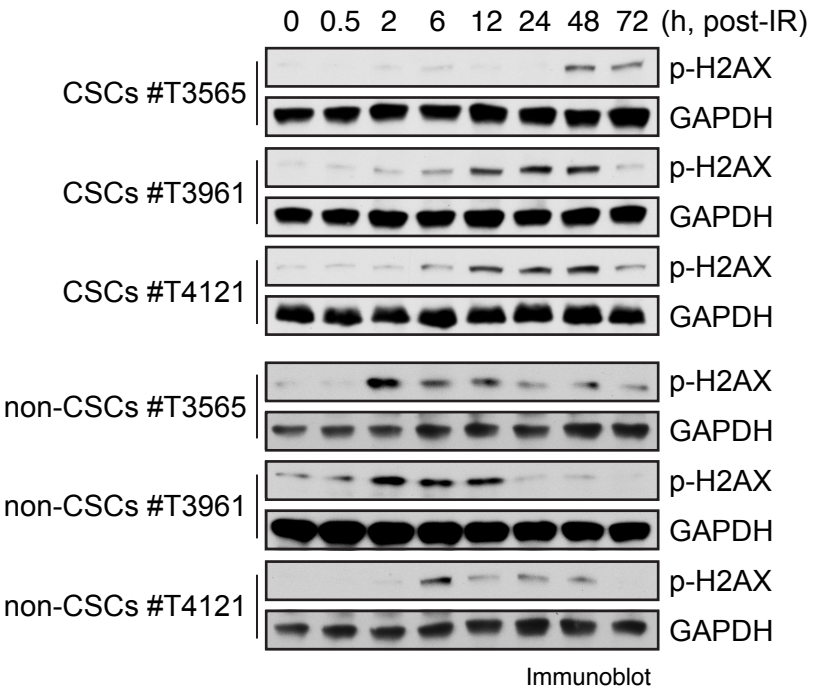
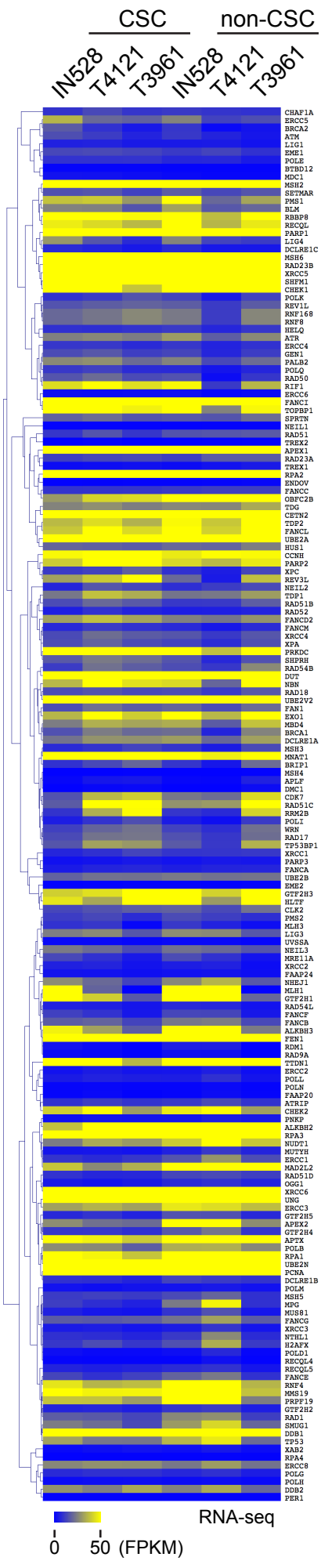


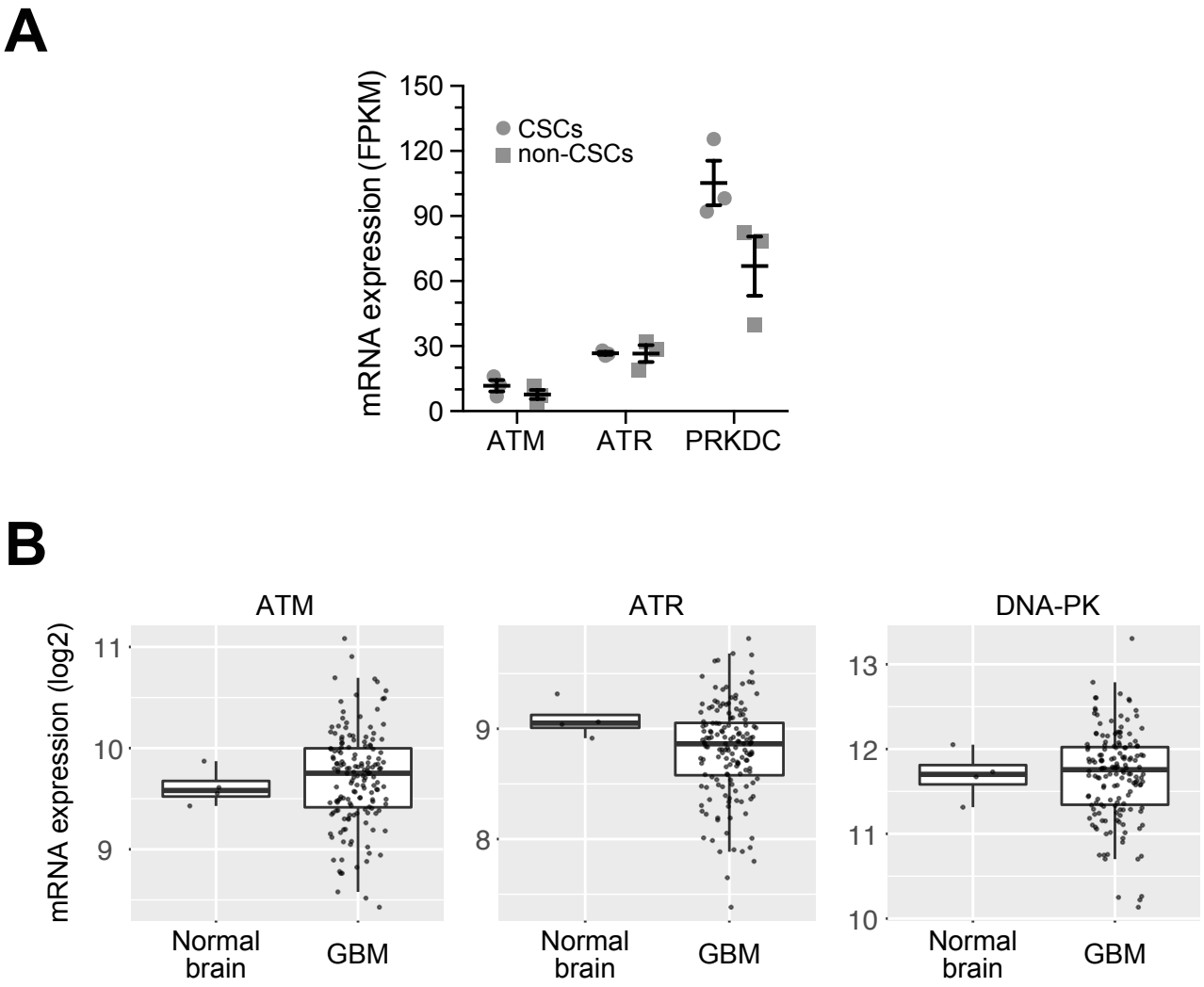
Supplementary Figure 1. Time-dependent radioresistance in glioma CSCs.
Human patient-derived (**A**) IN528, (**B**) T3565, and (**C**) T4121 glioma CSCs and matched non-CSC cells and control U87 human glioma cells were irradiated with 5 Gy X ray or subjected to sham operation without irradiation. Cells 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).



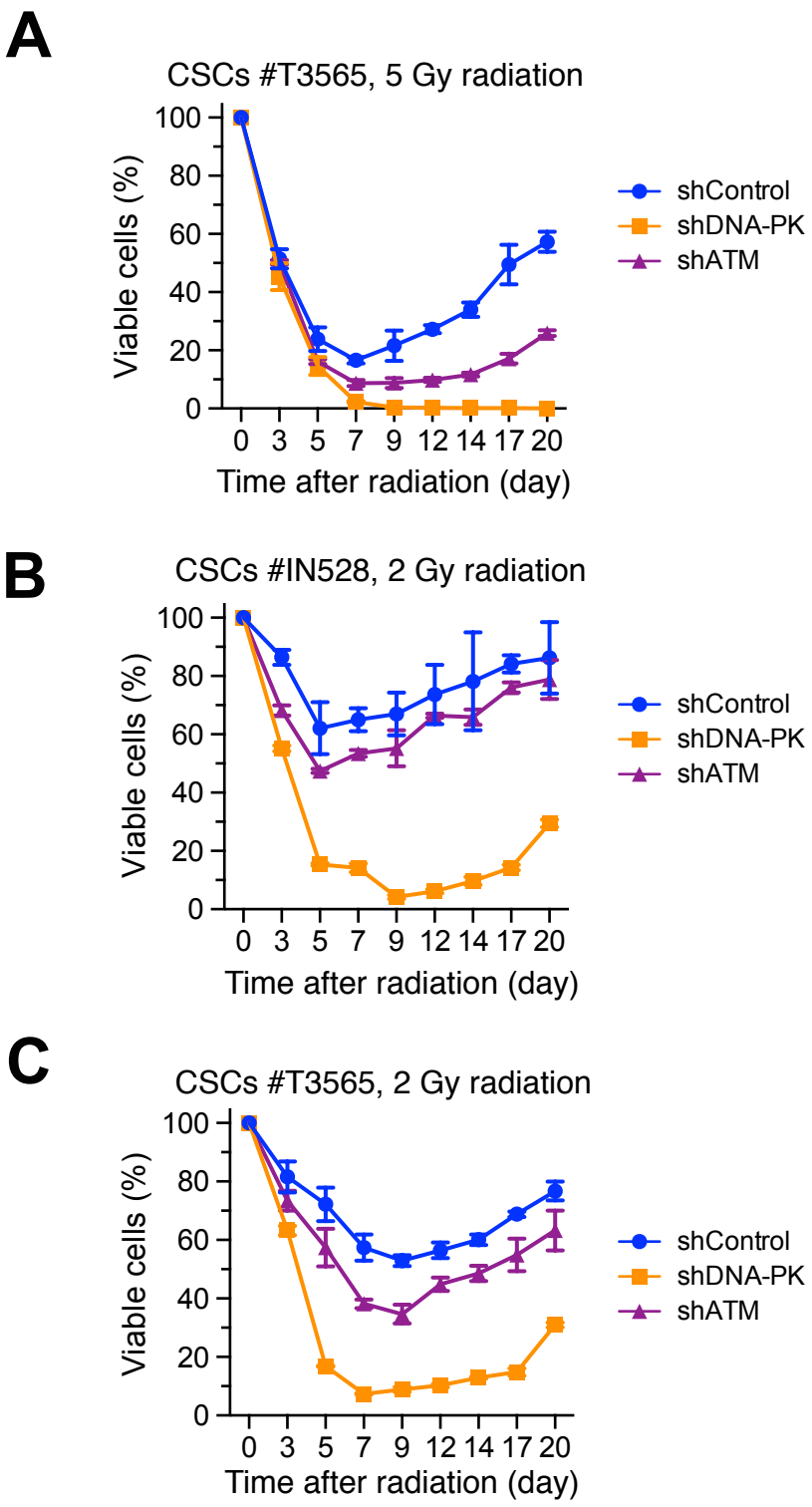
Supplementary Figure 2. Delayed H2AX phosphorylation in glioma CSCs.
T3565, T3961 and T4121 glioma CSCs and matched non-CSC cells were irradiated with 5 Gy X ray. Cell lysates were collected at different time points post-irradiation, and subjected to immunoblot analysis with anti-phospho-H2AX-Ser^{139} and anti-GADPH antibodies. Representative blots are shown.



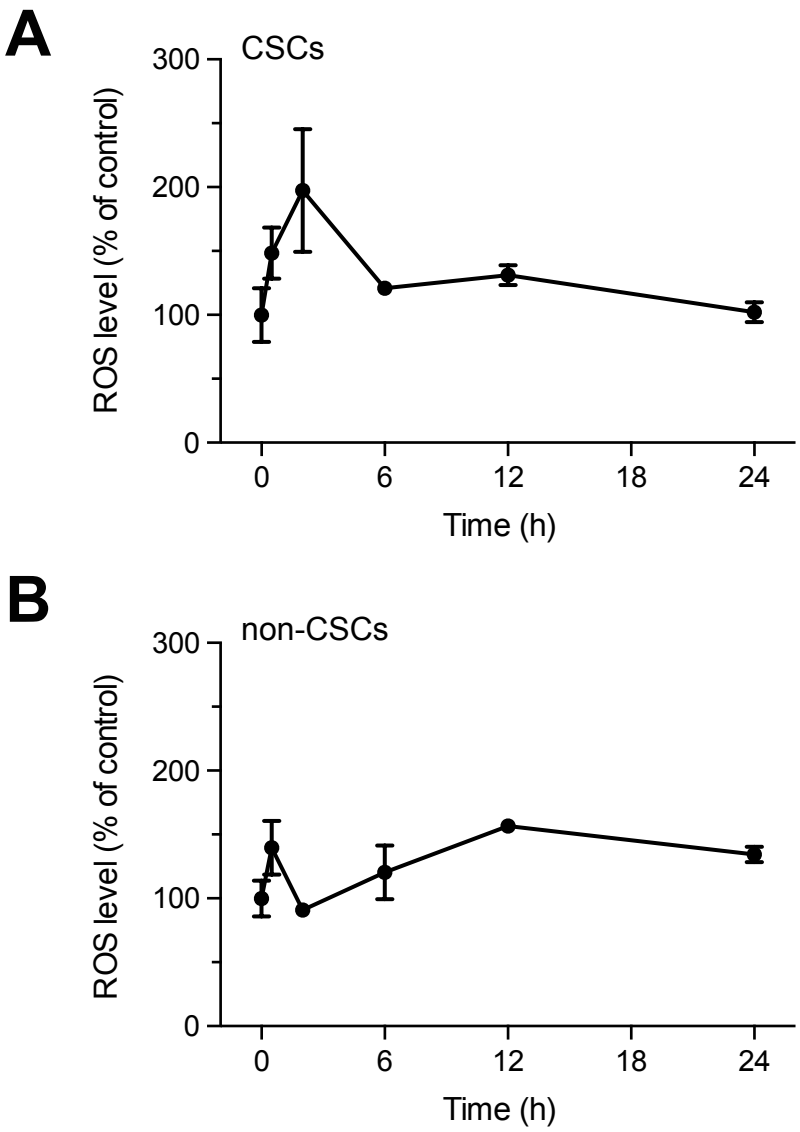
Supplementary Figure 3. Clustering analysis of RNA-seq data for DNA damage and repair genes in glioma CSCs.
RNA was isolated from IN528, T4121, and T3961 glioma CSCs and matched non-CSC cells, and subjected to transcriptome analysis by RNA deep sequencing. FPKM values were calculated for the DNA repair 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.



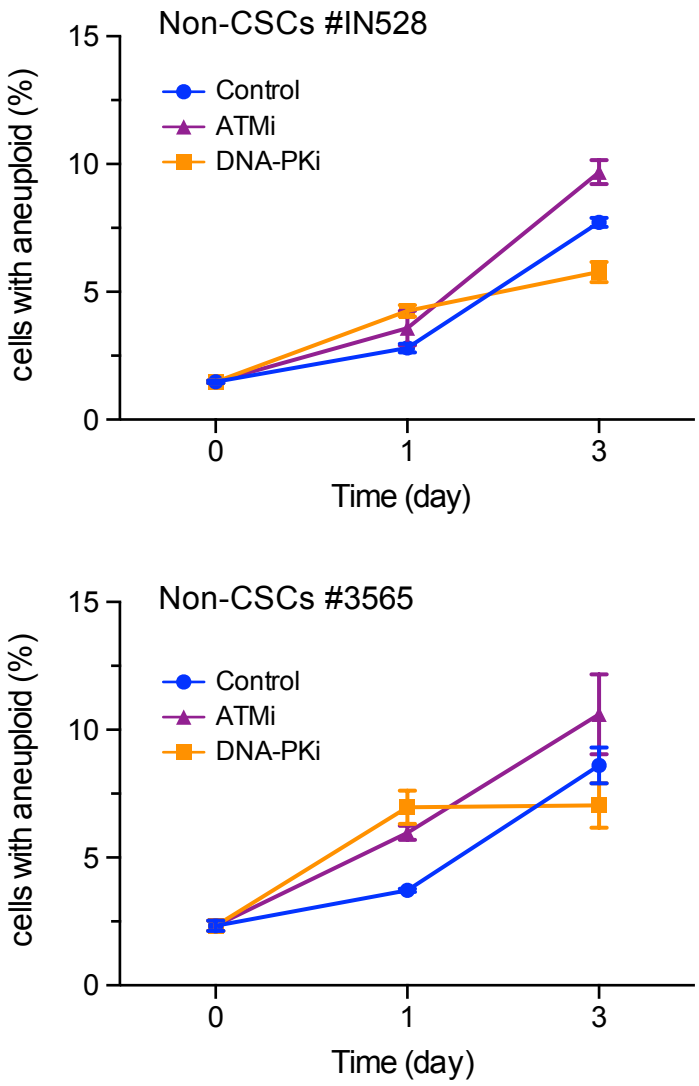
Supplementary Figure 4. DNA-PK (PRKDC) expression is remarkably higher in glioma CSCs, and moderately increased in normal brains and GBM tumor cells, compared with ATM and ATR expression.
(**A**) RNA was isolated from IN528, T4121, and T3961 glioma CSCs and matched non-CSC cells, and subjected to transcriptome 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 anueploidy 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).