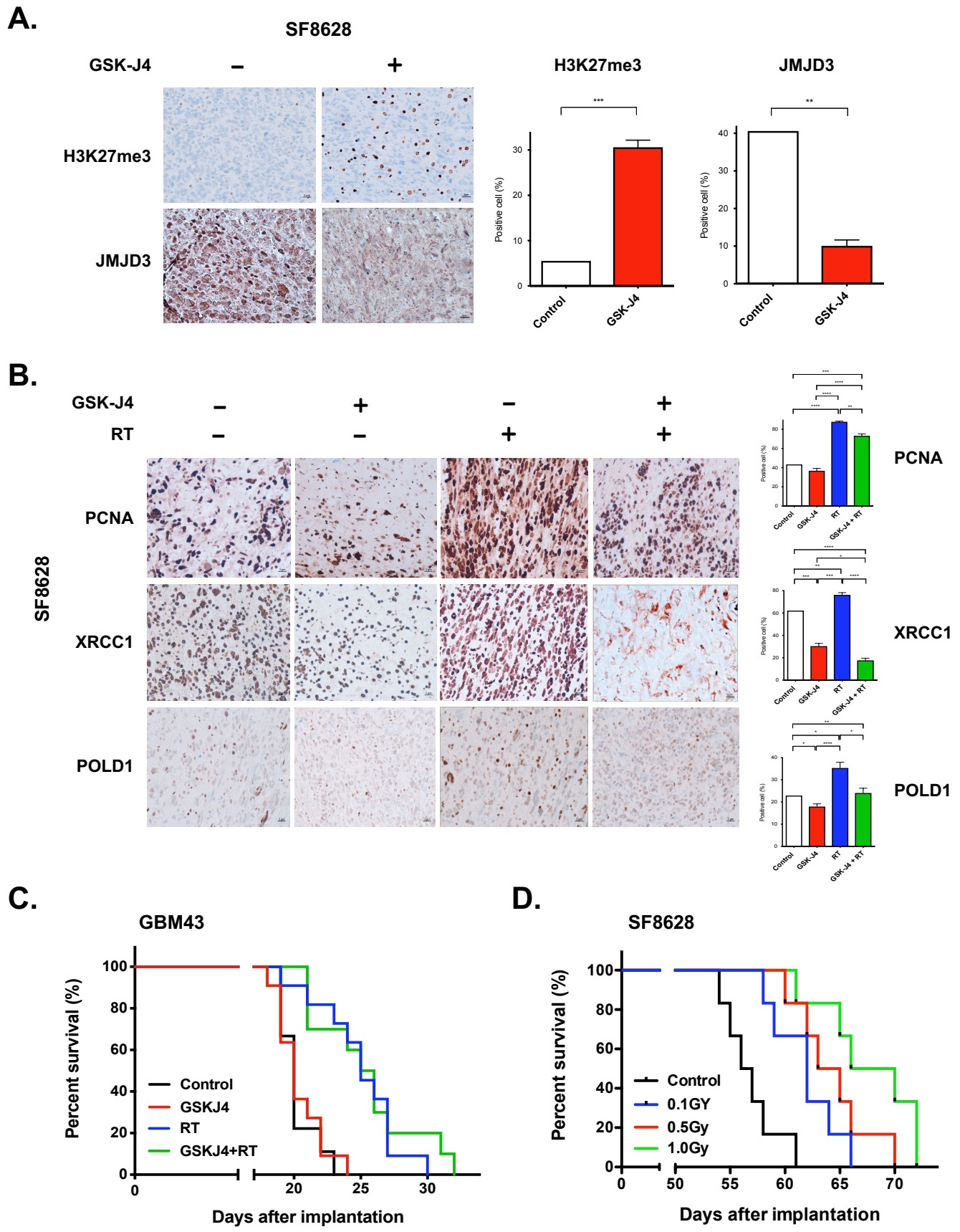


Supplementary Figure S3



Supplementary Figure S3. Effects of GSK-J4 on expression of histone modification enzymes and DNA damage repair proteins, and radiation antitumor activity *in vivo*. **(A)** Effect of GSKJ4 treatment on K27me3 and JMJD3 expression in SF8628 K27M tumor. Left: Image results show GSKJ4 treatment (+) increased tumor cell K27me3 positivity and decrease tumor cells JMJD3 positivity. Right: The graph shows the number of positive cells from vehicle and GSKJ4 treated tumors, with values based on average positive cells counted in three high-powered fields (mean \pm SEM). Unpaired *t*-test values for comparisons between vehicle and GSKJ4 treatment: K27me3, $***P = 0.0002$; JMJD3, $**P = 0.0022$. **(B)** Effect of GSKJ4 treatment on expression of DNA damage repair proteins. Left: Images of representative PCNA, XRCC1, and POLD1 staining for intracranial tumor from mice euthanized at the end of treatment. Right: Mean and SD values representing the average number of positive cells in four high-powered fields in each tumor. Unpaired *t*-test values for comparisons between treatments. : $****P < 0.0001$; PCNA, $***P = 0.0004$ between control vs. GSK-J4 + RT, $***P = 0.0003$ for between GSK-J4 vs. GSK-J4 + RT; XRCC1, $***P = 0.0004$ between control vs. GSK-J4, $**p = 0.0046$ between control vs. RT, $***P = 0.0001$ between GSK-J4 vs. RT, $*P = 0.00149$ between GSK-J4 vs. GSK-J4 + RT; POLD1, $*P = 0.0253$ between control vs. GSK-J4; $*P = 0.0102$ between control vs. RT; $**P = 0.0038$ between control vs. GSK-J4 + RT; $*P = 0.0291$ between RT vs. GSK-J4 + RT. **(C)** Survival plots for GBM43 xenografts. Statistical analysis was performed using a log-rank test with Holm adjustment., $**P = 0.0003$ between control vs. RT, $***P = 0.0002$ between control vs. GSK-J4 + RT, $**P = 0.0005$ between GSK-J4 vs. GSK-J4 + RT. There is no significance different between control vs. GSK-J4 and RT vs. GSK-J4 + RT. **(D)** Analysis of intracranial DIPG radiation dose response. Animals receiving intracranial implantation with SF8628 cells (1.0×10^5) were irradiated with 0.1, 0.5, or 1.0 Gy per day for 3 days a week for 2 weeks. Radiation efficacy was assessed by survival benefit to animals. Statistical analysis was performed using a log-rank test with Holm adjustment., $*P = 0.0061$ between control vs. 0.1 Gy, $**P = 0.0016$ between control vs. 0.5 Gy, $**P = 0.0012$ between control vs. 1.0 Gy.