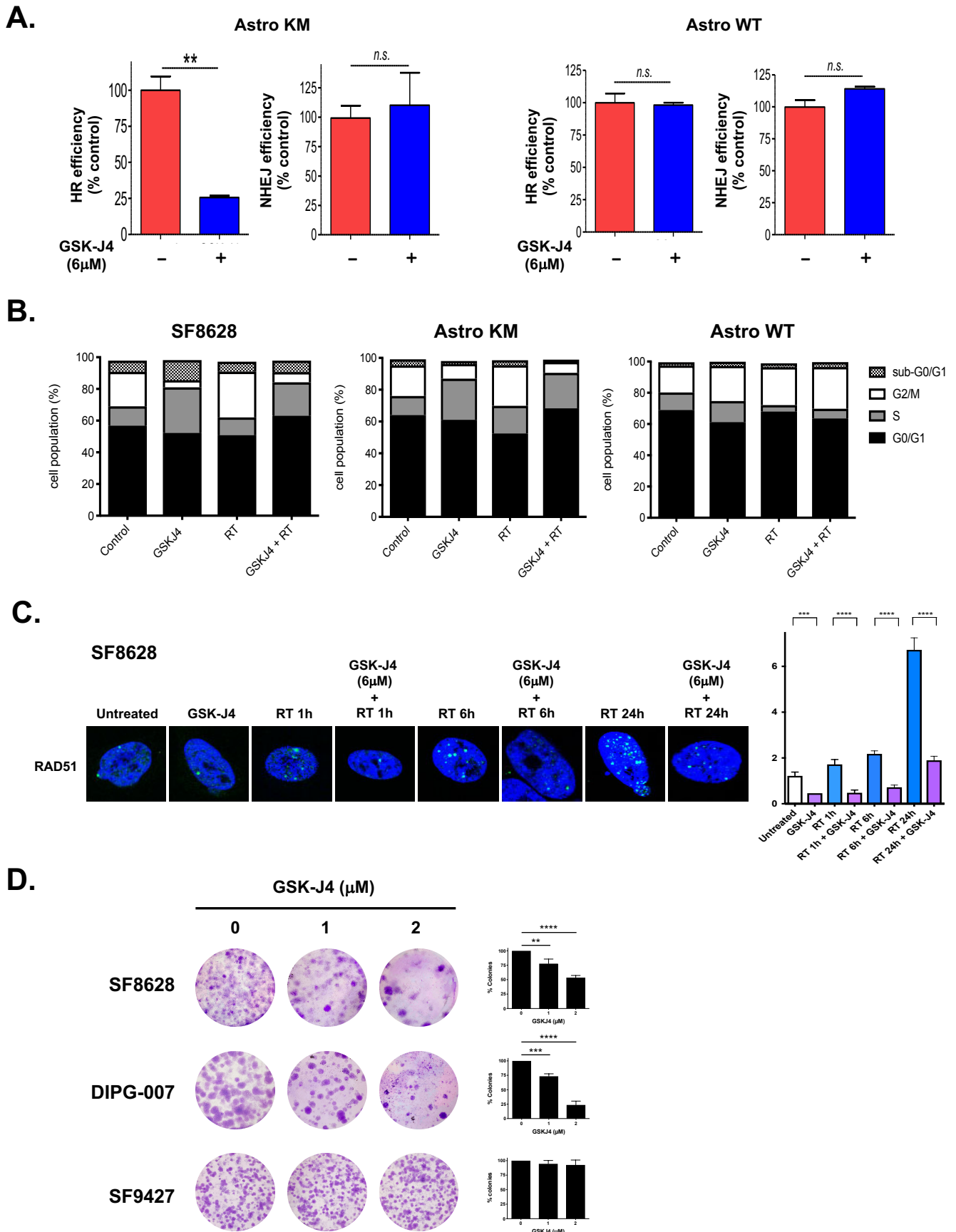


# Supplementary Figure 2



**Supplementary Figure 2. Effects of GSK-J4 on DNA damage repair, cell cycle, and cell proliferation.** **(A)** DNA repair assay showing effect of GSK-J4 (6  $\mu$ M) on homologous recombination (HR) and nonhomologous end-joining (NHEJ) pathways in human astrocytes expressing K27M (Astro KM) and wild-type (Astro WT) *H3F3A* transgenes. Value shown are based on averages from duplicate or triplicate samples and error bars represent SD (mean  $\pm$  SD). Unpaired *t*-test values for comparisons between samples: \*\* indicates  $P = 0.0089$  in Astro KM and n.s. indicates no significance in both Astro KM and WT. **(B)** Effect of GSK-J4 on RAD51 foci formation in irradiated SF8628 K27M DIPG cells. Left: representative images of nuclei from each treatment, showing RAD51 foci. Right: Mean and SD values for RAD51 foci/nucleus are shown, and are based on foci counts from two independent experiments. Unpaired *t*-test values for RAD51 comparisons between treatments: \*\*\*\* indicate  $p < 0.0001$ . \*\*\* indicated  $p = 0.0005$  between untreated vs. GSK-J4 only. **(C)** Effects of mono- and combination treatments on cell cycle distributions. SF8628 or human astrocyte cells were then treated with 6  $\mu$ M GSK-J4, radiation treatment (RT) (6 Gy), or with GSK-J4 and RT. GSK-J4 treatments were initiated 72 hours before RT ( GSK-J4 + RT). Vehicle only (DMSO) was used as a control. At 24 hours after RT, cells were fixed and stained with propidium iodide for determination of cell-cycle distributions by flow cytometric analysis. **(D)** GSKJ4 colony forming effect on cells with or without K27M. GSK-J4 treatment reduced clonal growth of K27M DIPG cells. Value shown are normalized to 100 % of vehicle control (DMSO) based on averages from triplicate samples and error bars represent SD (mean  $\pm$  SD). Unpaired *t*-test values for comparisons between treatments: \*\* indicates  $P = 0.0084$  between control vs. 1.0  $\mu$ M GSK-J4 in SF8628, \*\*\*\* indicates  $P < 0.0001$  between control vs. 2.0  $\mu$ M GSK-J4 in SF8628, \*\*\* indicates  $P = 0.0004$  between control vs. 1.0  $\mu$ M GSK-J4 in DIPG-007, \*\*\*\* indicates  $P < 0.0001$  between control vs. 2.0  $\mu$ M GSK-J4 in DIPG-007.