

# **Tumoricidal, Temozolomide- and Radiation-Sensitizing Effects of K<sub>Ca</sub>3.1 K<sup>+</sup> Channel Targeting In Vitro Are Dependent on Glioma Cell Line and Stem Cell Fraction**

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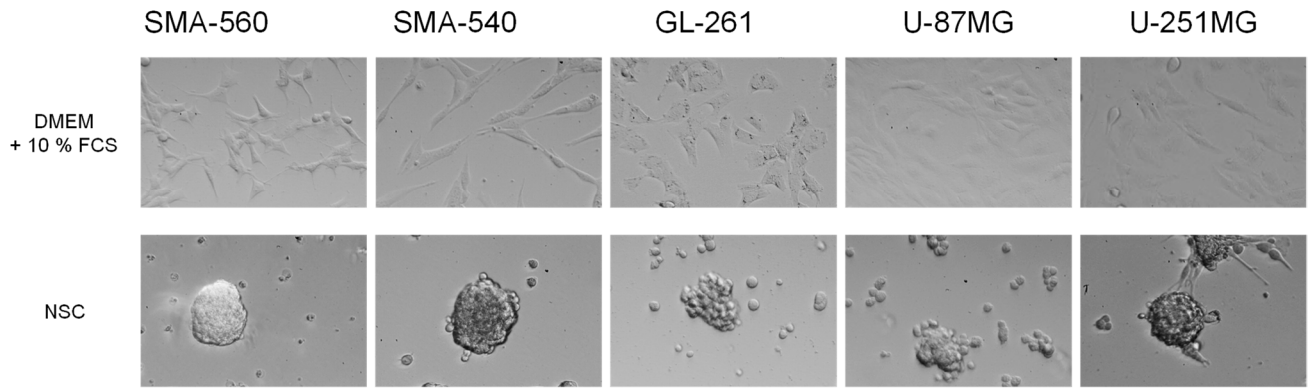
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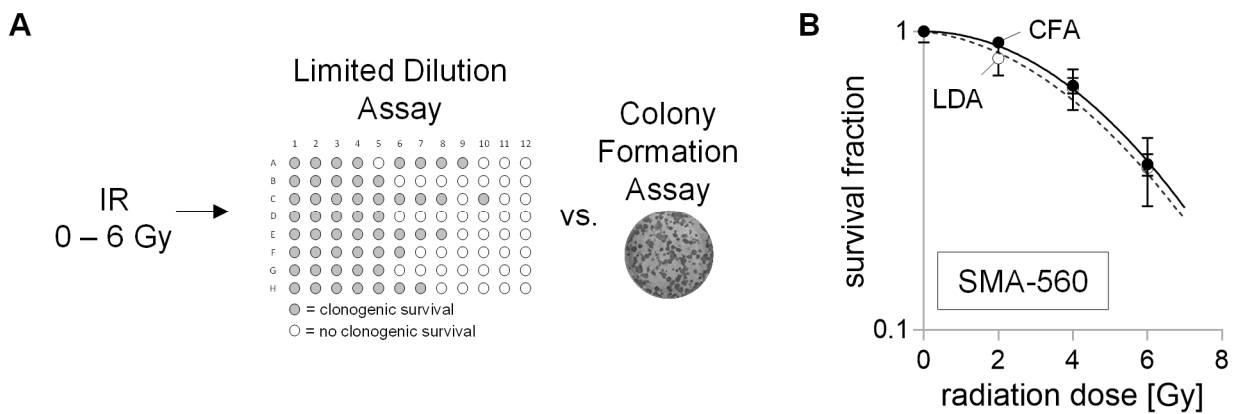
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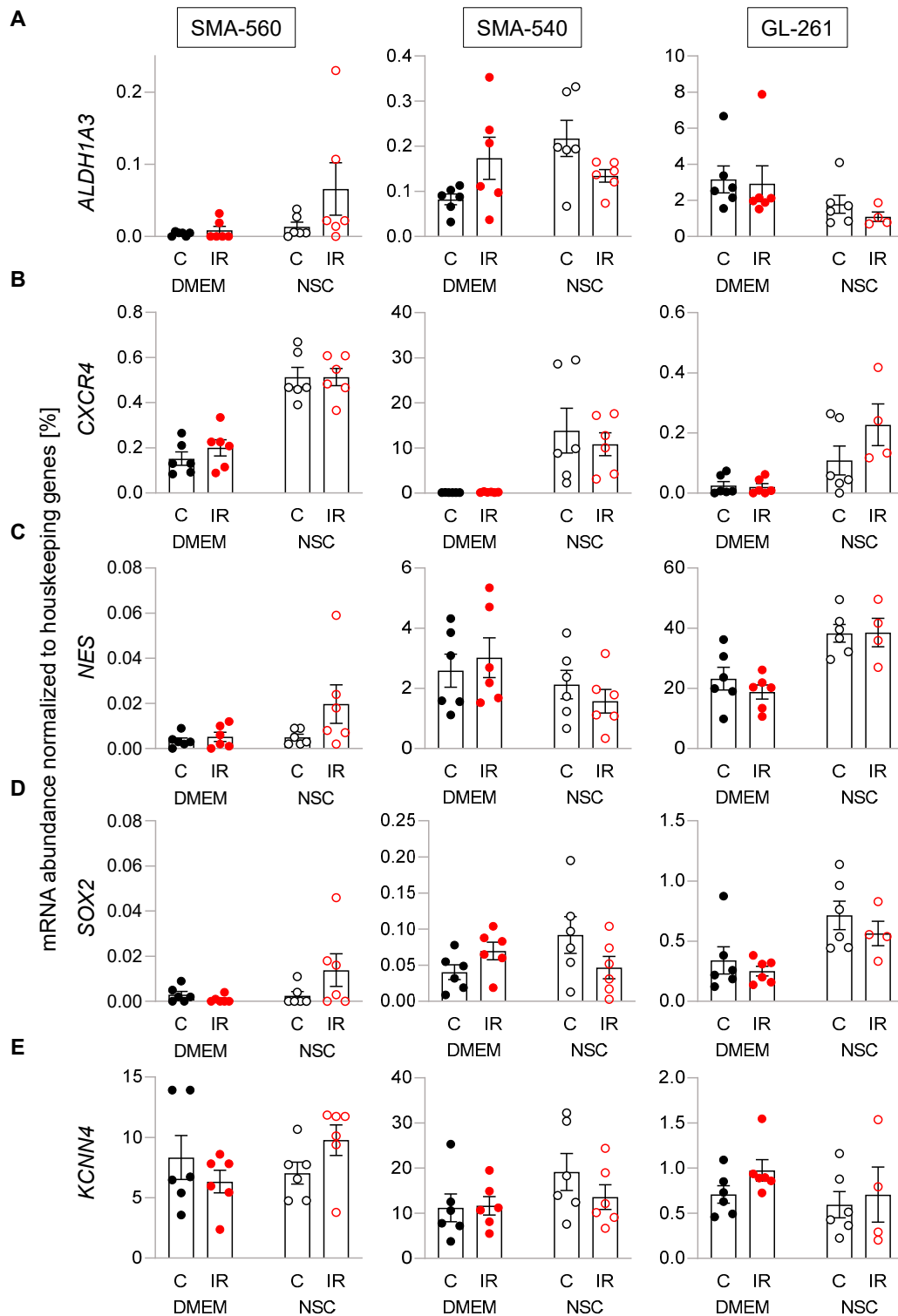
## Supplementary Figures



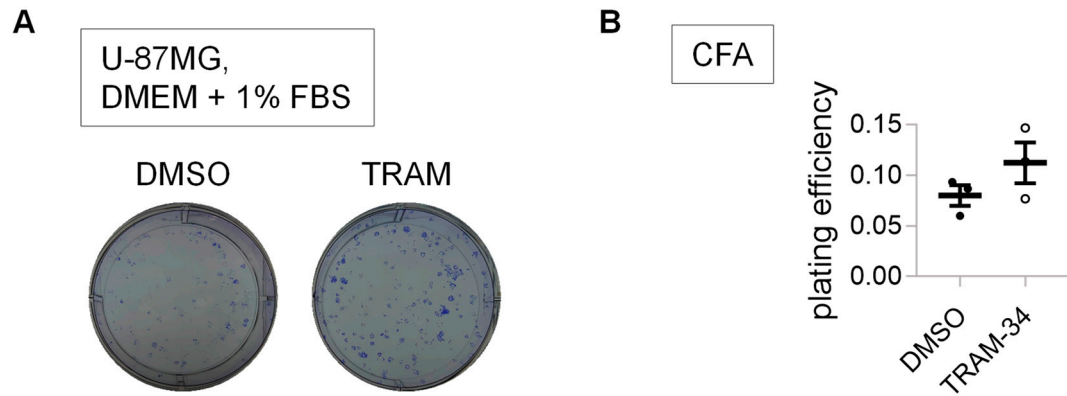
**Supplementary Figure S1.** Representative images of growth phenotypes in “bulk” cell-“differentiating” DMEM/10% FBS (top) and glioma stem cell-enriching NSC (bottom) medium. Note the adherent and spheroid-like phenotype of DMEM- and NSC-grown cells, respectively.



**Supplementary Figure S2.** Survival fractions of irradiated (0-6 Gy) SMA-560 cells grown in DMEM + 10% FBS determined by limited dilution assay (LDA; B, open circles) or colony formation assay (CFA; B, closed circles) are not different. Depicted is in (A, left) a schematic drawing of a 96-well plate where SMA-560 cell suspensions were sequentially 1:2-diluted (well 1 to 12 from left to right). Seeded cell number-dependent clonogenic growth after 7 d incubation is indicated by grey color. In (A, right) a micrograph of fixed and stained SMA-560 colonies typically obtained in colony formation assay is shown. Data in (B) are mean values ( $\pm$  standard error) of three experimental units and three to four observational units each. Survival fractions were fitted according to the linear quadratic model and given as follows: CFA, solid line; LDA, dashed line.



**Supplementary Figure S3.** Irradiation does not affect mRNA abundance of (A) *ALDH1A3*, (B) *CXCR4*, (C) *Nestin*, (D) *SOX2* or (E) *KCNN4* in SMA-560 (left), SMA-540 (middle) or GL-261 (right) cells systematically irrespective of culture condition. Cells were irradiated with 4 Gy and incubated for another 24 hours before cell pellets were collected and subsequently analyzed via RT qPCR. Bar graphs indicate mean values ( $\pm$  standard errors) of two to three experimental units and two observational units each. C = control (0 Gy), IR = irradiation with 4 Gy.



**Supplementary Figure S4.** TRAM-34 does not exhibit tumoricidal effects on U-87MG cells when incubated in DMEM + 1% FBS. A-B. Representative images of colony formation (A) and plating efficiencies (B) after treatment with vehicle (DMSO, closed black circles) or TRAM-34 (5  $\mu$ M, open circles). Data are individual values and mean  $\pm$  standard error of one experimental unit with three observational units each.