Supplemental Materials and Methods

TCGA Analysis/ PECAN expression analysis

Analysis of *SNAI2* expression across the TCGA database confirmed sarcoma tumors highly express *SNAI2* compared to other cancer types. Analysis of *SNAI2* expression in a different cohort of approximately 2000 pediatric cancers from the St. Jude-PeCan portal confirmed that *SNAI2* is highly expressed in RMS tumors and especially the ERMS sub-type compared to other pediatric cancers with osteosarcoma tumors expressing higher *SNAI2* (Supplemental Figure 1D).

SNAI1 CRISPR KO Cell Generation

Cloning sgRNAs is previously described by Addgene lentiCRISPR v2 (https://www.addgene.org/52961/). After plating ~1.5x106 293T cells in 100mm plate, 9 μg sgRNA SNAI1 lenti-CRISPR (see Supplemental Table 5)(54), 5 μg, lentiviral 2nd Gen packaging plasmid (psPAX2) and 1 μg envelope plasmid (pMD2G), DMEM basic media without serum to 1 ml total, and 40-45 μl TansIt-LT1 transfection reagent (~3:1 ratio Transit-LT1/DNA) was added in a 1.5 mL tube, mixed, and incubated for 15-20 minutes at room temperature. Fresh DMEM 10% FBS media with no antibiotic was given 24h after initial plating, and 1 mL of prepared DNA complexes was added to different regions of the plate. After 48h, media was collected and filtered using a 0.45 μm filter. Media was aspirated from target cells, and 3 mL of fresh media without antibiotics with 16-20 μg/mL of protamine and 2-3 mL infecting media was added. Target cells were left to incubate for 48h at 37 ^{o}C. After 48h, virus-containing media was replaced with fresh media containing puromycin (12 μg /mL) and was incubated for an additional 72h. Cells were then characterized by PCR and western blot to confirm SNAI1 knockout.

Senescence Cell Histochemical Staining

RMS cells were seeded into 6 well plates (0.1 – 0.5 x 10^6 cells/well) and radiated after 24h. Cells were fixed after 120h with 4% paraformaldehyde. Cells were then stained using the Senescence Cells Histochemical Staining Kit (Sigma Aldrich) according to the provided protocol. Cells were allowed to incubate with the stain at 37^{o}C without

CO_2 until cells were stained blue (2 hours to overnight). Percentage of cells positive for β-galactosidase was assessed using ImageJ. Significance was determined using Student's t test.

Immunofluorescence Staining

Immunofluorescence staining was performed similar to in Ignatius et. al., 2017 (27). Cells were plated at 4,000 cells/well (no IR) and 10,000 cells/well (receiving IR), grown in 10% FBS DMEM or RPMI growth media, fixed at 72 hpIR (0 or 15 Gy) in 4% paraformaldehyde/PBS, permeabilized in 0.5% Triton X-100/PBS, and incubated with rabbit anti-MEF2C (CST; Catalog No. 5030) and anti-myosin heavy chain (DSHB) in 1% BSA/PBS. Secondary antibody detection was performed with Alexa Flour 488 goat anti-mouse and Alexa Fluor 594 goat anti-rabbit (Invitrogen). Cells were counterstained with DAPI (1:10,000) and imaged. Images were processed in ImageJ and Adobe Photoshop.

Supplemental References

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