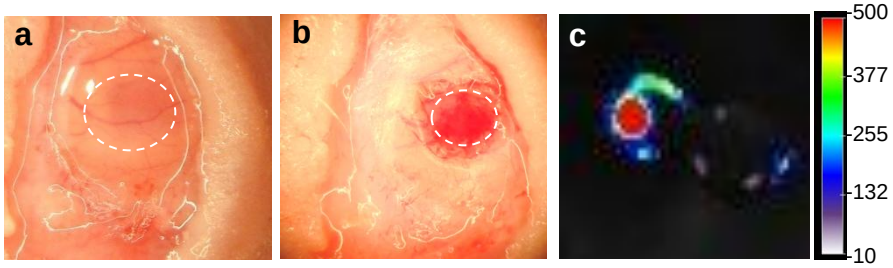
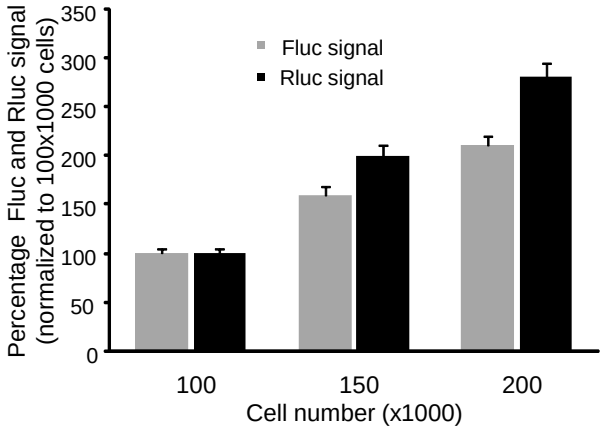
Encapsulated Therapeutic Stem Cells Transplanted in the Tumor Resection Cavity Induce Cell Death in GBMs

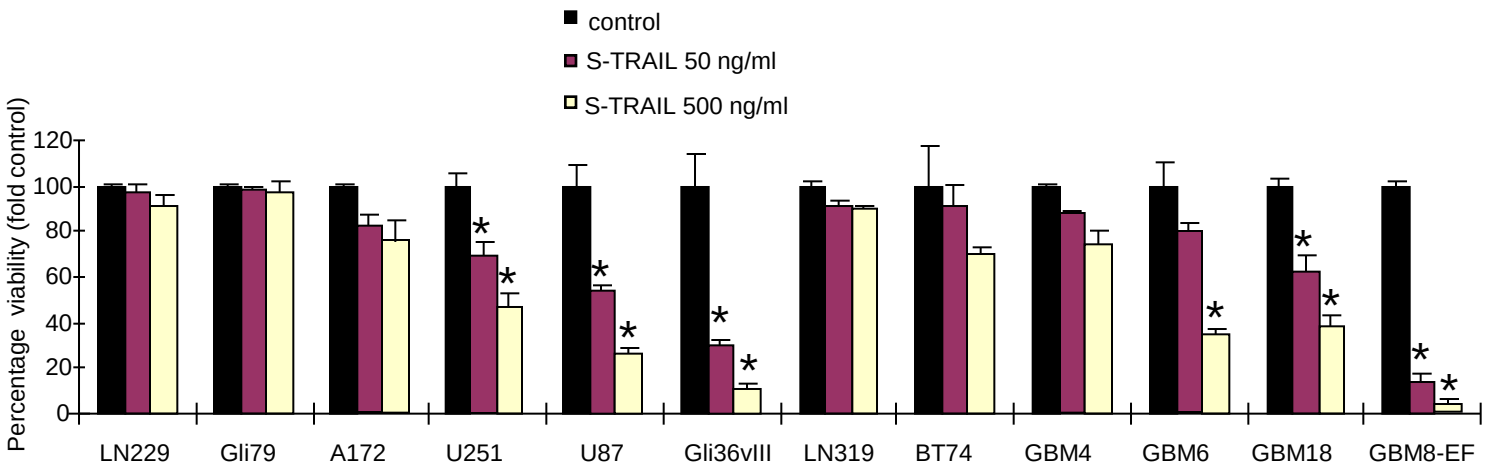
Timo M. Kauer, Jose-Luiz Figueiredo, Shawn Hingtgen and Khalid Shah



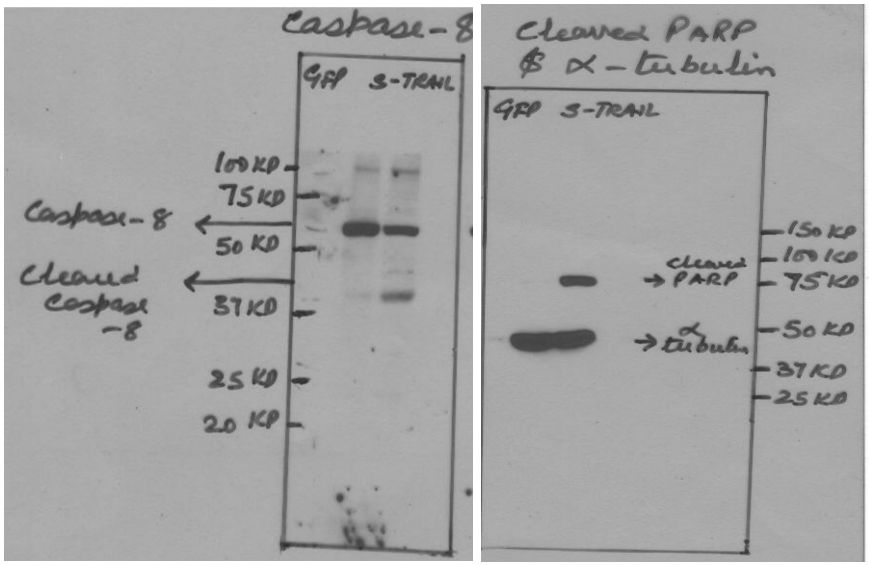
Supplementary Figure 1: **Tumor resection:** U87-Fluc-mCherry tumor cells were implanted in mice with cranial window. Light images of the cranial window with intact U87-Fluc-mCherry tumor (a) and the resected tumor (b). (c) Resected U87-mCherry-Fluc *in vivo* tumor specimen imaged for Fluc bioluminescence. Dotted circle indicates the tumor implantation site (in a) and tumor resection site (in b).



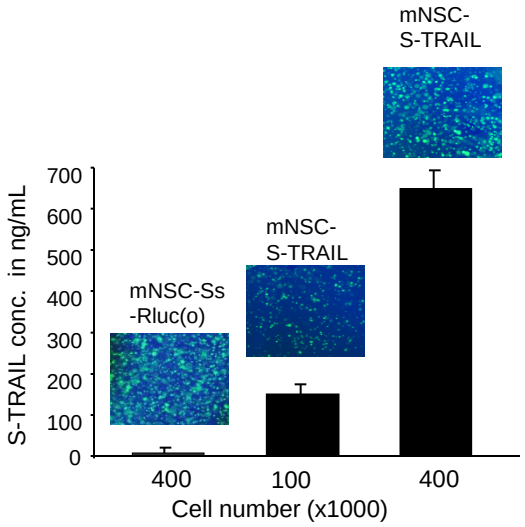
Supplementary Figure 2: ***In vitro*** **characterization of sECM encapsulated mNSC:** mNSC were either transduced with LV-GFP-Fluc or LV-Ss-Rluc(o) and different cell numbers were encapsulated in sECM and imaged for cell viability (Fluc activity) and protein secretion (Rluc(o)) activity. Plot revealing the correlation between cell number and Fluc or Rluc activity is shown.



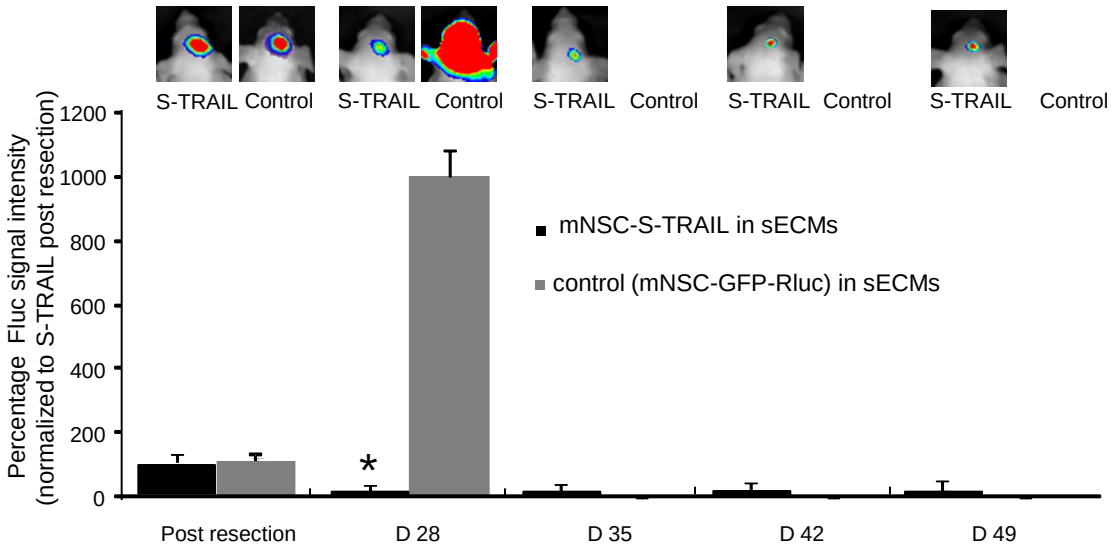
Supplementary Figure 3: **Sensitivity/resistance of GBM cells to TRAIL mediated apoptosis:** Different established GBM (LN229, Gli79, A172, U251, U87, Gli36vIII, LN319) and primary GBM (BT74, GBM4, GBM6, GBM18, and GBM8-EF) lines were incubated with different concentration of S-TRAIL and GBM cell viability was determined 24 h post-incubation. Plot revealing the percentage cell viability is shown. *$p<0.05$ versus control determined by ANOVA; data are mean ±s.e.m.



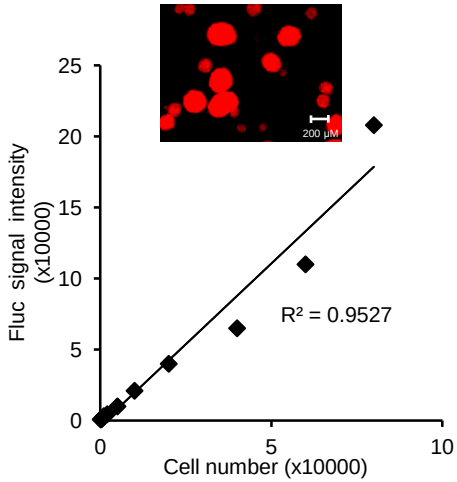
Supplementary Figure 4: **Western blot analysis:** Un-cropped Western blots of the data shown in Fig. 4f



Supplementary Figure 5: **TRAIL secretion of sECM encapsulated mNSC:** mNSC were either transduced with LV-Ss-Rluc(o) or LV-S-TRAIL and different cell numbers were encapsulated in sECM and incubated in growth medium and 24 h later ELISA for S-TRAIL was performed on conditioned medium. Representative photomicrographs of encapsulated mNSC expressing S-TRAIL or Ss-Rluc(o) and plot revealing the correlation between the S-TRAIL concentration in the medium and the number of encapsulated mNSC.



Supplementary Figure 6: **sECM encapsulated mNSC-S-TRAIL reduces tumor volumes over time:** Mice were implanted with U87mCherry-Fluc GBM cells in a cranial window, resected and implanted with sECM encapsulated mNSC-S-TRAIL or mNSC-GFP-Rluc. Mice were followed for changes in tumor volume by serial Fluc bioluminescence imaging. Plot reveals the percentage Fluc signal intensity post tumor resection over 49 days. Representative images are shown. *$p<0.05$ versus controls at day 28; determined by students t test; data are mean ±s.e.m.



Supplementary Figure 7: **Engineered human GBM8 cells for *in vitro* and *in vivo* studies:** Regression analysis indicating linear correlation between primary human GBM8 cells expressing Fluc-mCherry cell number and Fluc activity. Representative photomicrograph of GBM8-mCherry-Fluc cells in culture is shown. Data was derived from the experiments performed in triplicate.