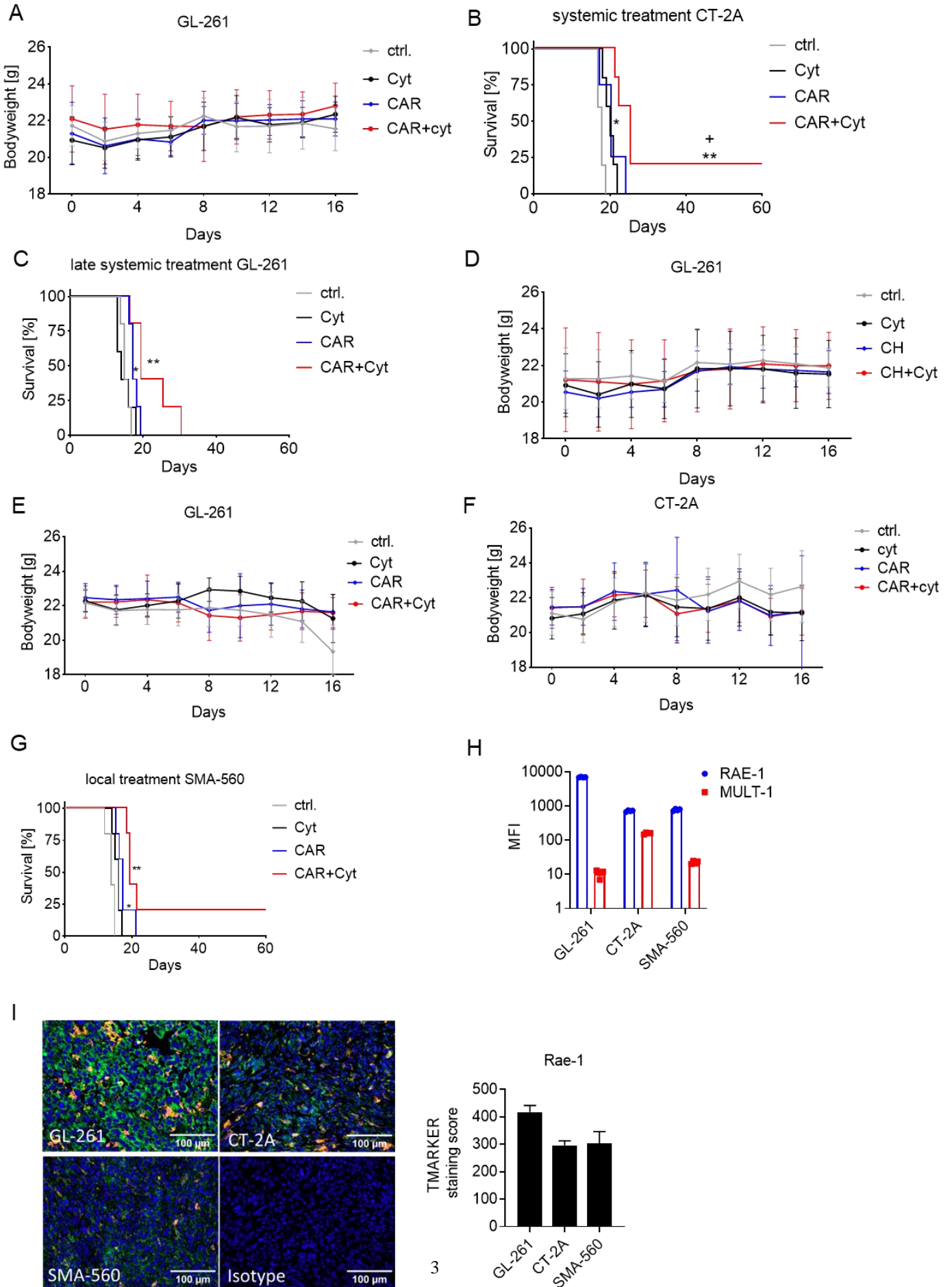


Supplementary Figure 2



47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71

**Supplementary Figure 2. Bodyweight measurements, therapeutic activity of modified T cells and target antigen expression**

**A.** Murine T cells were mock-electroporated (ctrl.) or transfected with mRNAs encoding mIL12 and mIFN $\alpha$ 2 (Cyt), the NKG2D CAR (CAR), or all three proteins (CAR + Cyt). Subsequently, 5x10<sup>6</sup> cells were i.v. injected at days 4, 7, 10 and 13 after brain inoculation of GL-261 cells. Bodyweight was assessed every other day. **B.** Same setup as in A but CT-2A glioma cells were used as a model and survival was monitored. Kaplan Meier curve is shown. **C.** Same setup as in A but treatment was administered at days 10, 13, and 16 after tumor implantation and survival was monitored. **D.** Same setup as in A, but the NKG2D CAR was retrovirally transduced (CH). **E** and **F.** GL-261(C) or CT-2A (D) glioma-bearing mice were treated intratumorally at days 7 and 12 after tumor implantation with the different modified T cells as indicated in A. Mean  $\pm$  SD are shown. **G.** Same setup as in E but SMA-560 glioma-bearing mice were treated and survival was monitored. **H.** RAE-1 and MULT-1 were detected on GL-261, CT-2A and SMA-560 glioma cells by flow cytometry *in vitro*. Mean Fluorescence Intensity (MFI) is shown. **I.** *Ex vivo* glioma-bearing mouse brains comprising GL-261, CT-2A and SMA-560 cells were sectioned and stained for RAE-1 (green), CD45 (red) and DAPI (blue). Representative images are shown and quantification of RAE-1 staining intensity is shown on the right. Scale bar = 100  $\mu$ M.