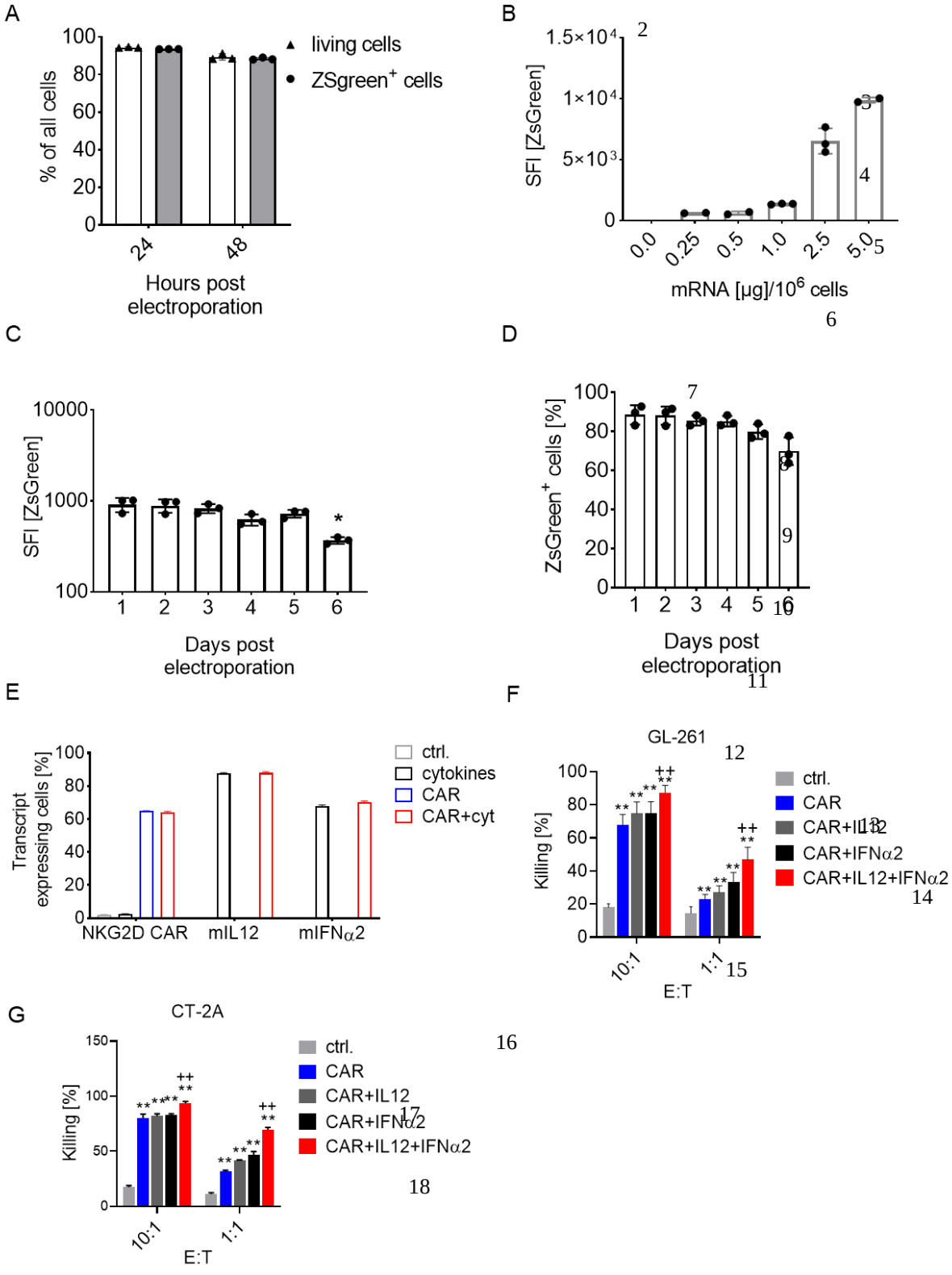
Supplementary Figure 1



Supplementary Figure 1. Electroporation of murine T cells for mRNA transfection and cytolytic activity of multifunctional CAR T cells. A. Murine T cells were electroporated with 2.5 µg mRNA encoding for ZSgreen at a voltage of 1600 mV applied in 3 pulses of 10 ms pulse width. The percentage of living cells and ZSgreen positive cells of all cells was determined by flow cytometry 24 h or 48 h after electroporation. **B**. Electroporation setup as in A, but increasing concentrations of mRNA were transfected and fluorescence intensity was detected 24 h later by flow cytometry. **C** and **D**. Electroporation as in A, but fluorescence intensity or percentage of fluorescent cells was determined for up to 6 days after electroporation by flow cytometry. **E.** Murine T cells were mock-electroporated with the parameter indicated in A or with mRNAs encoding either for the NKG2D CAR, or mIL12 and mIFNα2 or all three proteins. Transgene expression at 24 h was determined by flow cytometry. Data are presented as mean ± SD (* $p < 0.05$; compared to day 1 after electroporation). **F** and **G**. Murine T cells were mock electroporated (ctrl.), or with mRNAs encoding for either the NKG2D CAR (CAR), or the NKG2D CAR and mIL12 or mIFNα2 (CAR + IL12 and CAR + IFNα2), or all three mRNAs (CAR + IL12 + IFNa2). Subsequently, they were used as effector cells in co-culture with GL-261 (F) or CT-2A (G) glioma cells at the indicated effector:target ratios.