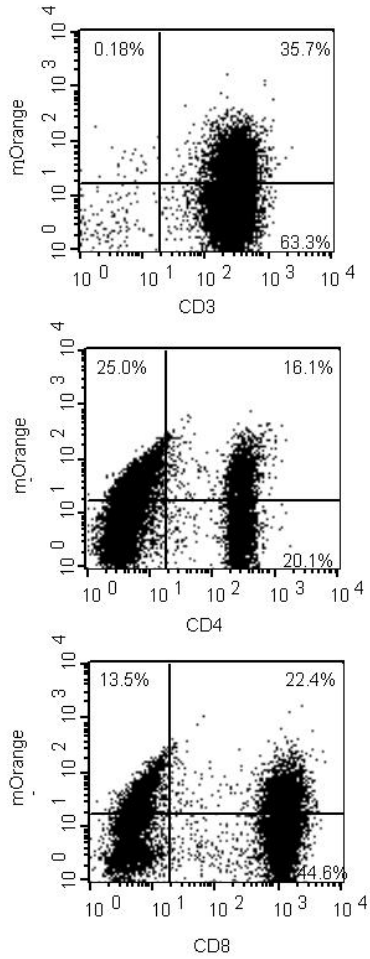
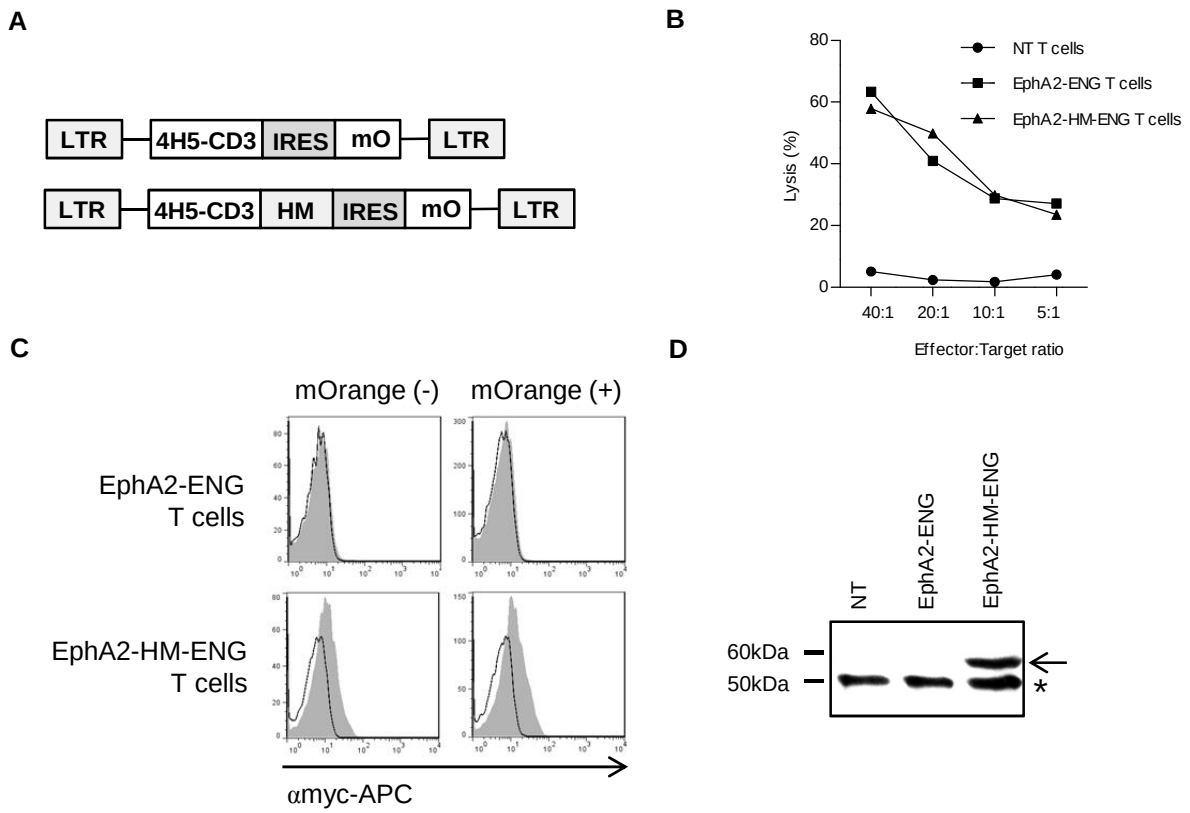


Supplementary Figure 1: Transduction of CD4- and CD8-positive T cells. CD3/CD28-activated T cells were transduced with RD114-pseudotyped retroviral particles encoding EphA2-ENG and mOrange. Five to 7 days post transduction mOrange expression was determined by FACS analysis. mOrange expression was detected in CD3-, CD4-, and CD8-positive T cells.

Supplementary Figure 1

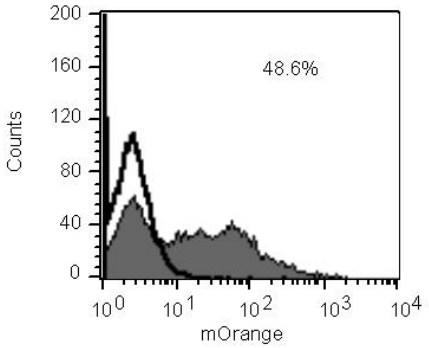


Supplementary Figure 2: Generation of T cells expressing EphA2-ENG with a 6xHis-Myc tag. (**A**) Scheme of retroviral vectors encoding EphA2-ENG and EphA2-ENG with a c-terminal 6xHis-Myc tag (EphA2-HM). EphA2-HM was generated by inserting a 6xHisMyc-tag before the stop codon. EphA2-ENG and EphA2-HM ENG T cells were generated by retroviral transduction and post transduction 73% (EphA2-ENG) or 57% (EphA2-HM ENG) T cells were positive for mOrange by FACS analysis (data not shown). (**B**) Cytotoxicity assay using NT, EphA2-ENG and EphA2-HM T cells as effectors and EphA2-positive U373 cells as target cells. There was no significant difference between EphA2- and EphA2-HM ENG T cells, indicating that the tag does not interfere with the function of the engager molecule. (**C**) FACS analysis of EphA2- and EphA2-HM ENG T cells gated on mOrange positive and mOrange negative cells (Open curve: isotype, Filled curve: αmyc-APC (Abcam, Cambridge, MA). EphA2-ENG T cells showed no cell surface staining. EphA2-ENG-HM T cells were stained with αmyc-APC. Transduced (mOrange+) and non-tranduced T cells (mOrange-) were positive, indicating that transduced T cells secrete engager molecules that bind to non-transduced T cells. (**D**) Media from NT, EphA2-ENG or EphA2-HM ENG T cells were incubated with His Mag Sepharose excel (GE Healthcare). Beads were washed and the bound fraction was eluted according to the manufacturer's instruction. The eluted fraction was separated by SDS-PAGE, and blotted with αMyc (Abcam) followed by a HRP-conjugated goat mouse IgG antibody (Santa Cruz Biotechnology); *: unspecific band.

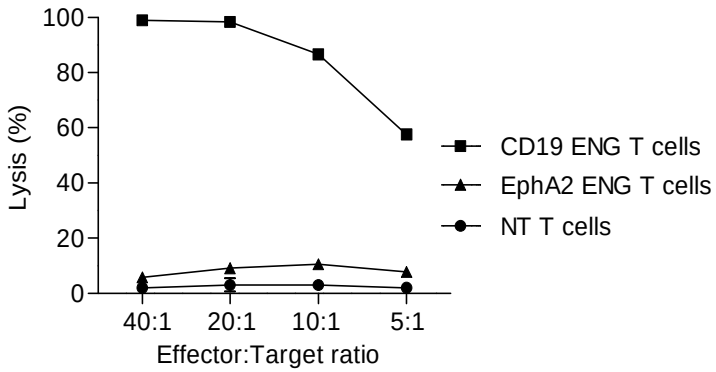
Supplementary Figure 2

A

B

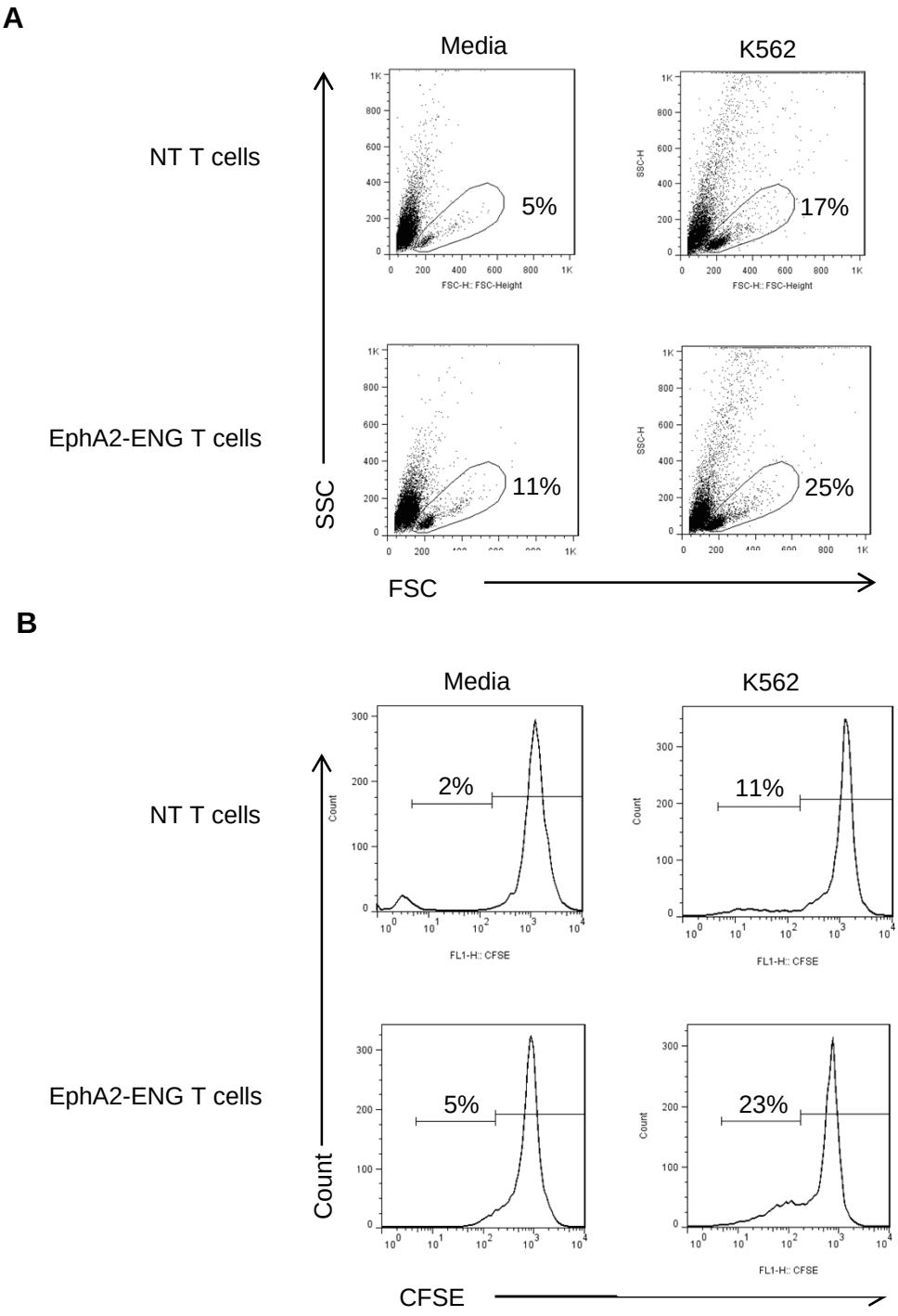


C



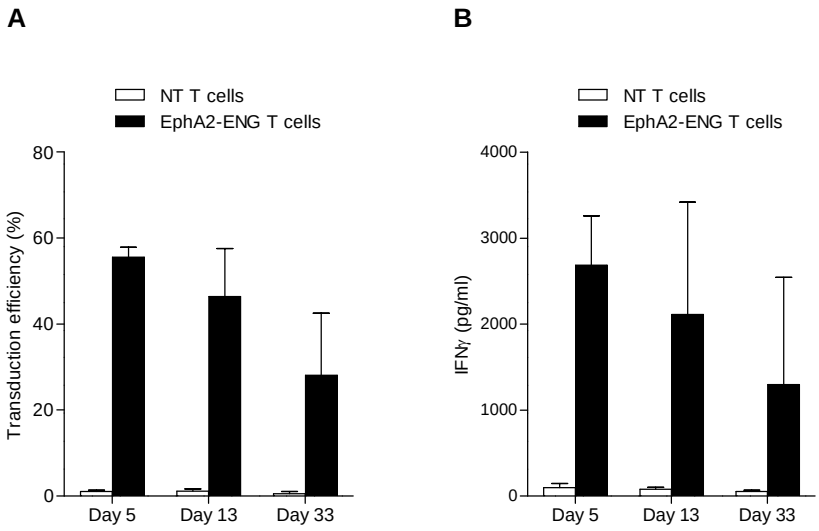
Supplementary Figure 3: Generation of CD19-ENG T cells. (**A**) Scheme of retroviral vector. (**B**) FACS analysis for mOrange of transduced and NT T cells. (**C**) Cytotoxicity assay using NT, CD19-ENG, and EphA2-ENG T cells as effectors and CD19-positive BV173 cells as targets.

Supplementary Figure 3



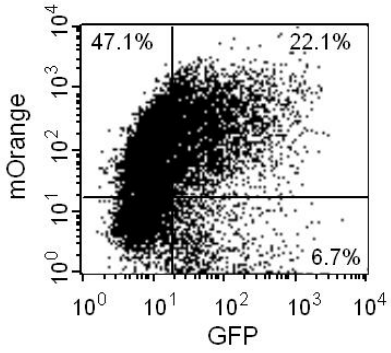
Supplementary Figure 4: Minimal proliferation of EphA2-ENG T cells in the absence of antigen. To evaluate if EphA2-ENG T cells proliferate in the absence of antigen, EphA2-ENG T cells and non-transduced (NT) T cells were labeled with 1.5 μmol/L carboxyfluorescein diacetate succinimidyl ester (CFSE). Subsequently cells were cultured in the absence or presence of irradiated K562 at a T-cell to K562 ratio of 10:1. After 7 days, cells were analyzed by FACS analysis. (**A**) Forward (FSC) and side scatter (SSC). (**B**) FACS analysis for CFSE. On day 7, only 25% of EphA2-ENG T cells were alive and of the viable cells only 23% had proliferated in the presence of K562, indicating that EphA2-ENG T cells do not significantly proliferate in the absence of antigen.

Supplementary Figure 4



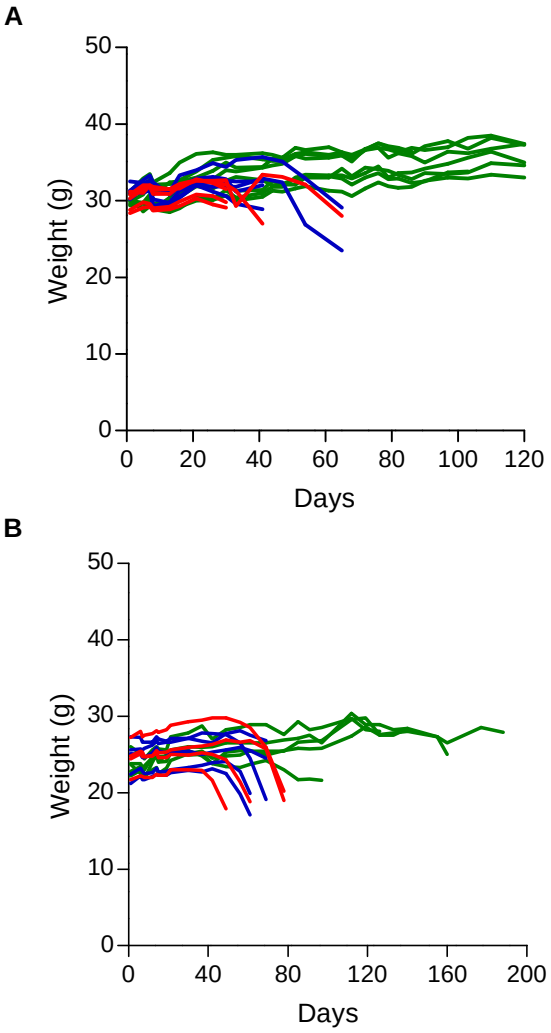
Supplementary Figure 5: Functional stability of EphA2-ENG T cells after transduction. Five, 13 and 33 days after transduction, EphA2-ENG T cells expanded with IL2 were harvested, (**A**) analyzed by FACS for mOrange and cultured for 48 hours. (**B**) Media from these EphA2-ENG T cells was cocultured with U373 cells and non-transduced (NT) T cells for 24 hours. IFNγ secretion was detected by ELISA assay (n=3).

Supplementary Figure 5



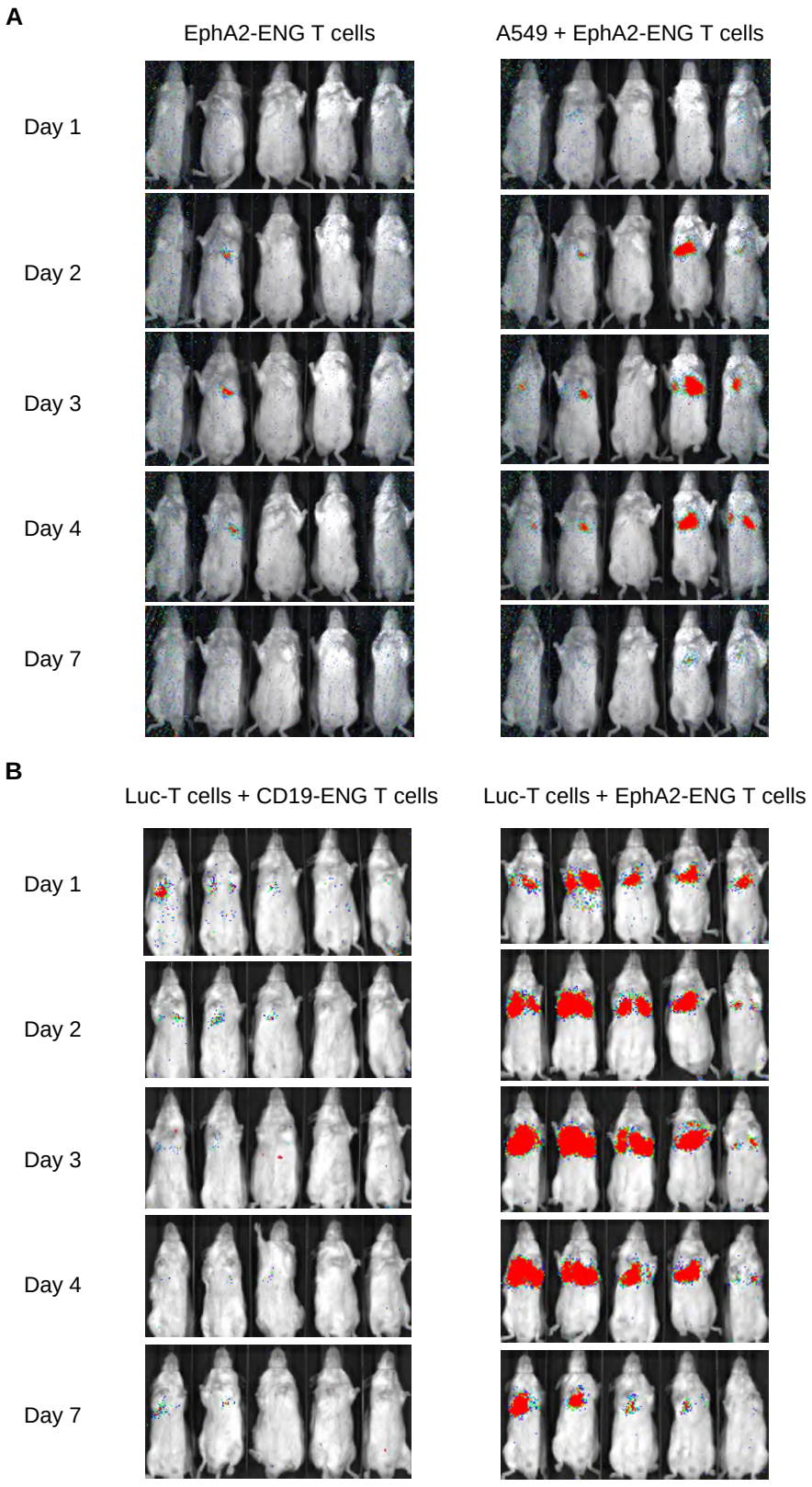
Supplementary Figure 6: FACS analysis of GFP.ffLuc and Engager expressing T cells. CD3/CD28-activated T cells were transduced with RD114-pseudotyped retroviral particles encoding EphA2-ENG and mOrange, and eGFP.ffLuc. FACS analysis for mOrange and GFP.

Supplementary Figure 6



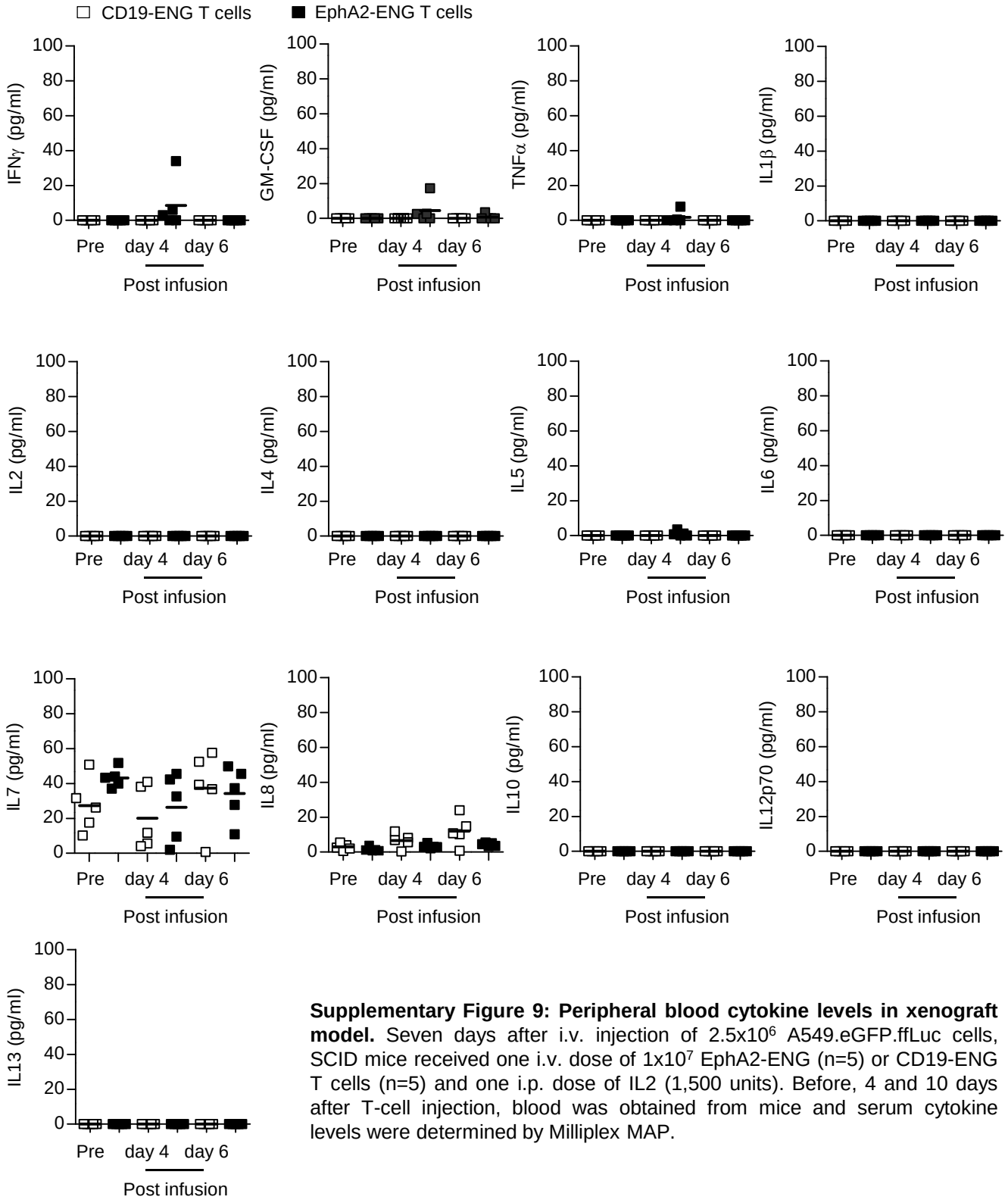
Supplementary Figure 7: Weight change of mice treated with ENG T cells. The experiment is described in the text and the corresponding antitumor activity is shown in Figure 7. (**A**) U373 glioma SCID xenograft model. (**B**) A549 lung tumor SCID xenograft model.

Supplementary Figure 7



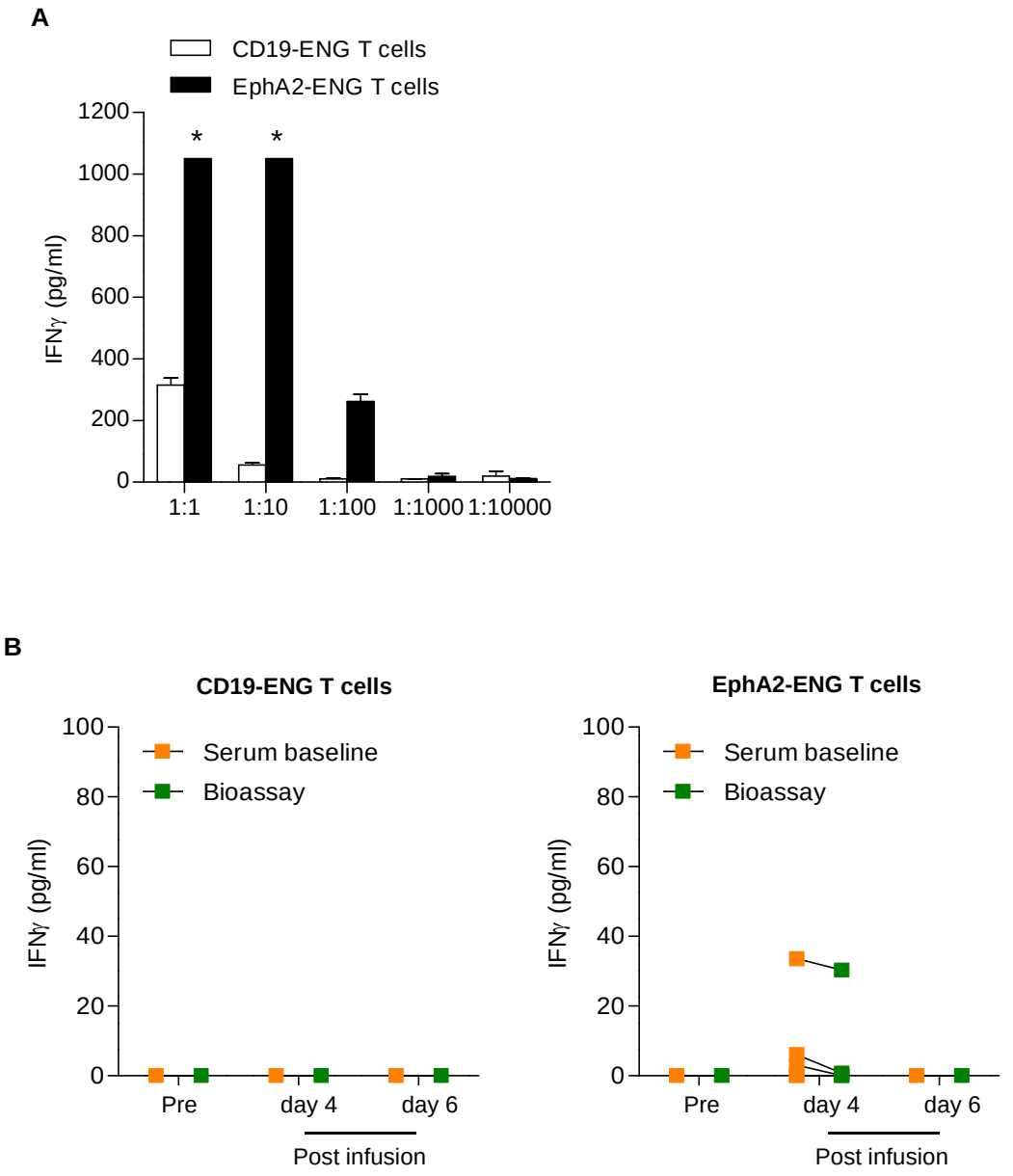
Supplementary Figure 8: T cells localize to lung tumors post injection. The experiment is described in the text and the quantitative data is shown in Figure 6. (**A**) A549 tumor- or non-tumor-bearing mice received an i.v. injection of an admixture of eGFP.ffLuc-expressing EphA2-ENG and NT T cells. (**B**) A549 tumor-bearing mice received an i.v. injection of an admixture of EphA2-ENG and eGFP.ffLuc-expressing NT cells or CD19-ENG and eGFP.ffLuc-expressing NT cells.

Supplementary Figure 8



Supplementary Figure 9: Peripheral blood cytokine levels in xenograft model. Seven days after i.v. injection of $2.5x10^6$ A549.eGFP.ffLuc cells, SCID mice received one i.v. dose of $1x10^7$ EphA2-ENG (n=5) or CD19-ENG T cells (n=5) and one i.p. dose of IL2 (1,500 units). Before, 4 and 10 days after T-cell injection, blood was obtained from mice and serum cytokine levels were determined by Milliplex MAP.

Supplementary Figure 9



Supplementary Figure 10: Bioassay of media or serum. (**A**) 50 μl media from EphA2-ENG or CD19-ENG T cells was diluted and cocultured with U373 (EpA2+) cells and non-transduced (NT) T cells in a total volume 200 μl of for 24 hours in a 96 well format at and effector to target (E:T) ratio of 10:1. IFNγ secretion was detected by ELISA assay (n=2). * > 1050 pg/ml. (**B**) The same set up was used to detect engager molecules in mice. Seven days after i.v. injection of $2.5x10^6$ A549.eGFP.ffLuc cells, mice received one i.v. dose of $1x10^7$ EphA2-ENG (n=5) or CD19-ENG T cells (n=5) and one i.p. dose of IL2 (1,500 units). Before, 4 and 10 days after T-cell injection blood was obtained from mice. Serum was tested for the presence of engager molecules using the same assay as desrcibed above. IFNγ levels was determined in the serum before (Serum baseline; orange squares) and after 24h of coculture ((Bioassay; green squares).

Supplementary Figure 10