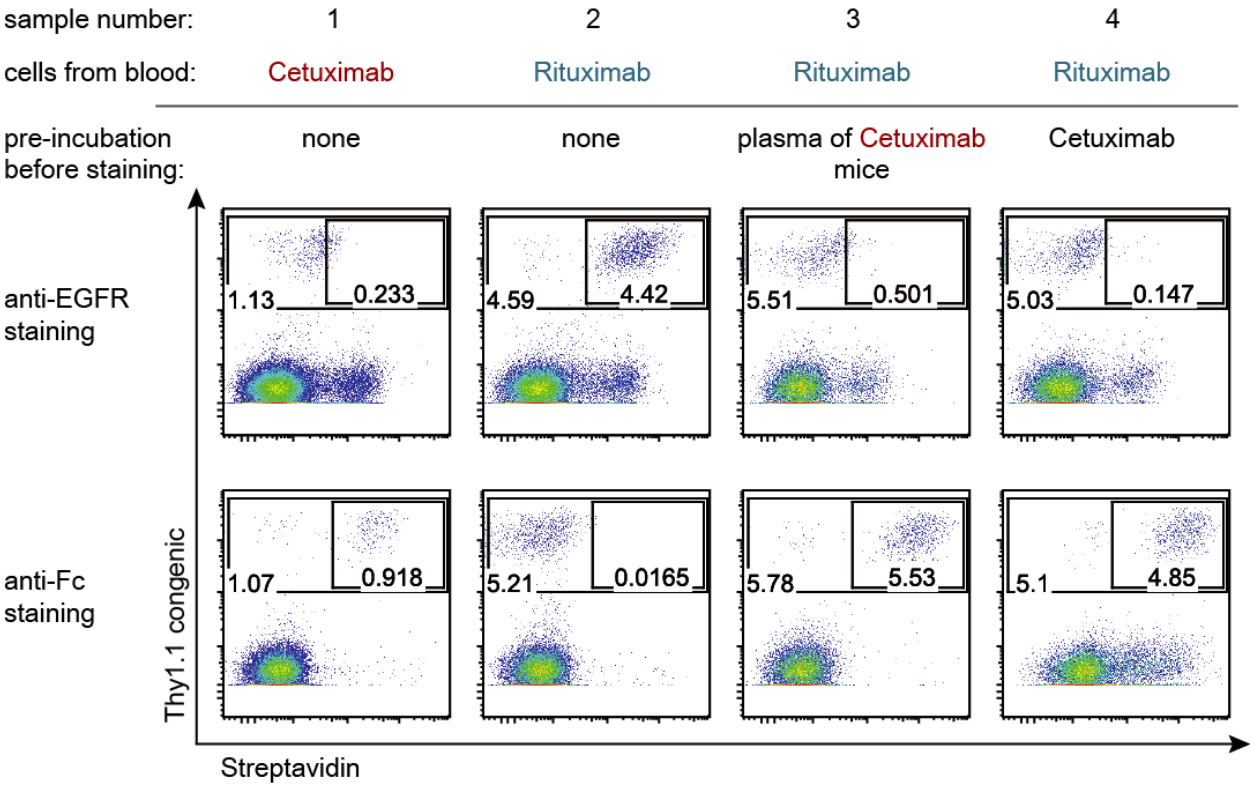
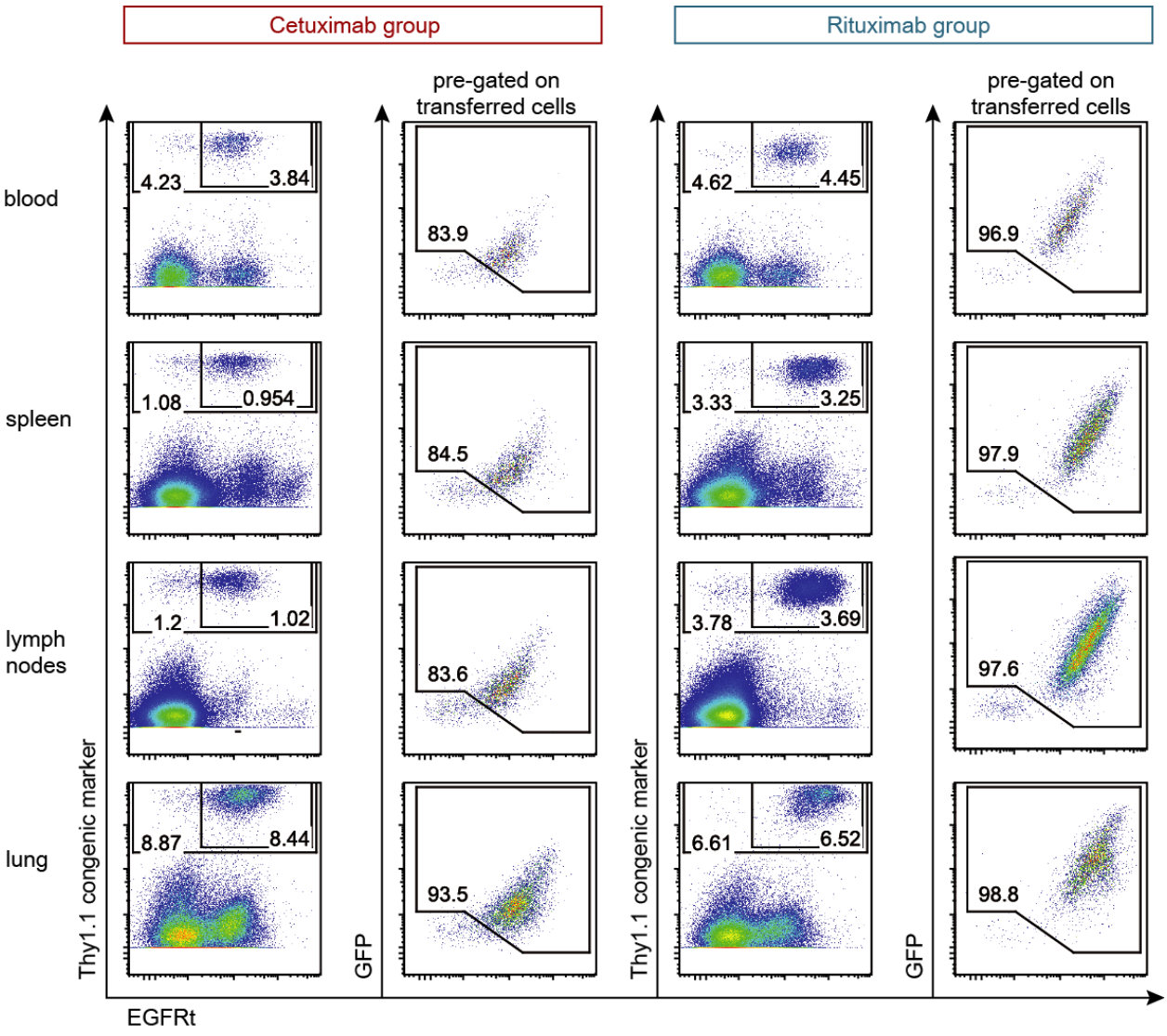
Supplemental Materials

Supplemental Figure 1.



Supplemental Figure 1. Cell-bound Cetuximab reduces EGFR staining intensity. Blood cells were obtained from Cetuximab- (sample 1) or Rituximab-treated mice (sample 2-4) and either not pre-incubated (sample 1, 2) or pre-incubated with 1:2 diluted plasma from Cetuximab-treated mice (sample 3) or 1:2 diluted Cetuximab (sample 4) for 30 minutes at 37°C. Accessible EGFR binding sites were detected with biotinylated Cetuximab and streptavidin-PE (upper row). Fc parts of cell-bound Cetuximab antibodies were detected with a biotinylated Fc-specific Fab reagent and streptavidin-PE (lower row). This experiment was performed repeatedly throughout 5 depletion studies on blood samples from several timepoints.

Supplemental Figure 2.

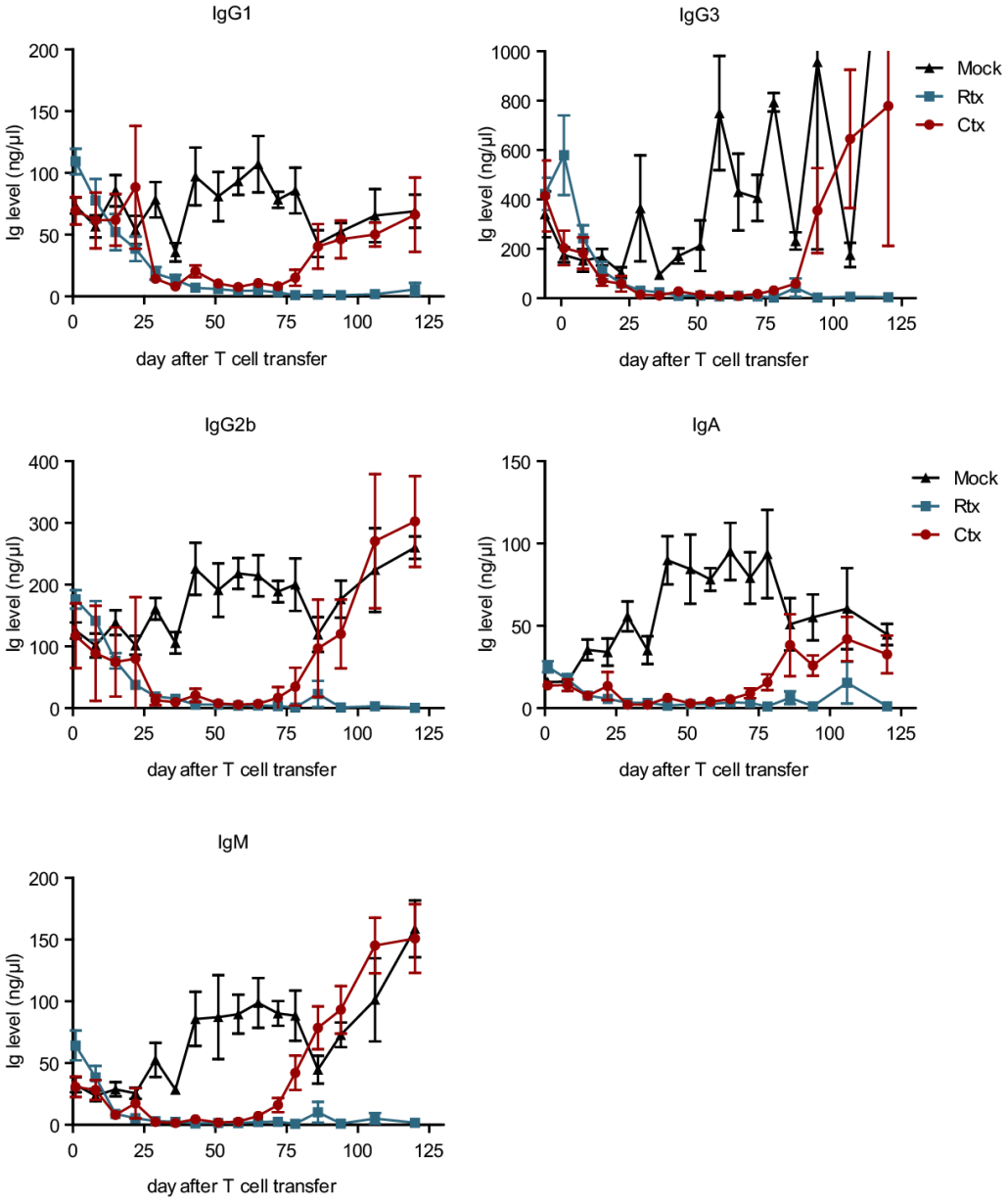


Supplemental Figure 2. Cetuximab mediates partial depletion of EGFRt$^+$ T cells in solid organs. Mice were infused with 250,000 GFP/EGFRt$^+$ Thy1.1$^+$ OT-I cells, vaccinated with MVA-OVA and treated with Cetuximab or Rituximab. On the final day of the experiment (day 238), mice were sacrificed and blood, spleen, lymph nodes and lung were obtained. Samples were stained with Thy1.1 and EGFR-specific mAbs. A. Representative stainings of the different organs from the Cetuximab as well as the Rituximab group are shown. T cells characterized from different solid organs from 3 different depletion studies. For the GFP vs. EGFRt plots, cells were pre-gated on Thy1.1. Frequencies of cells within the respective gates are indicated.

Supplemental Figure 3.

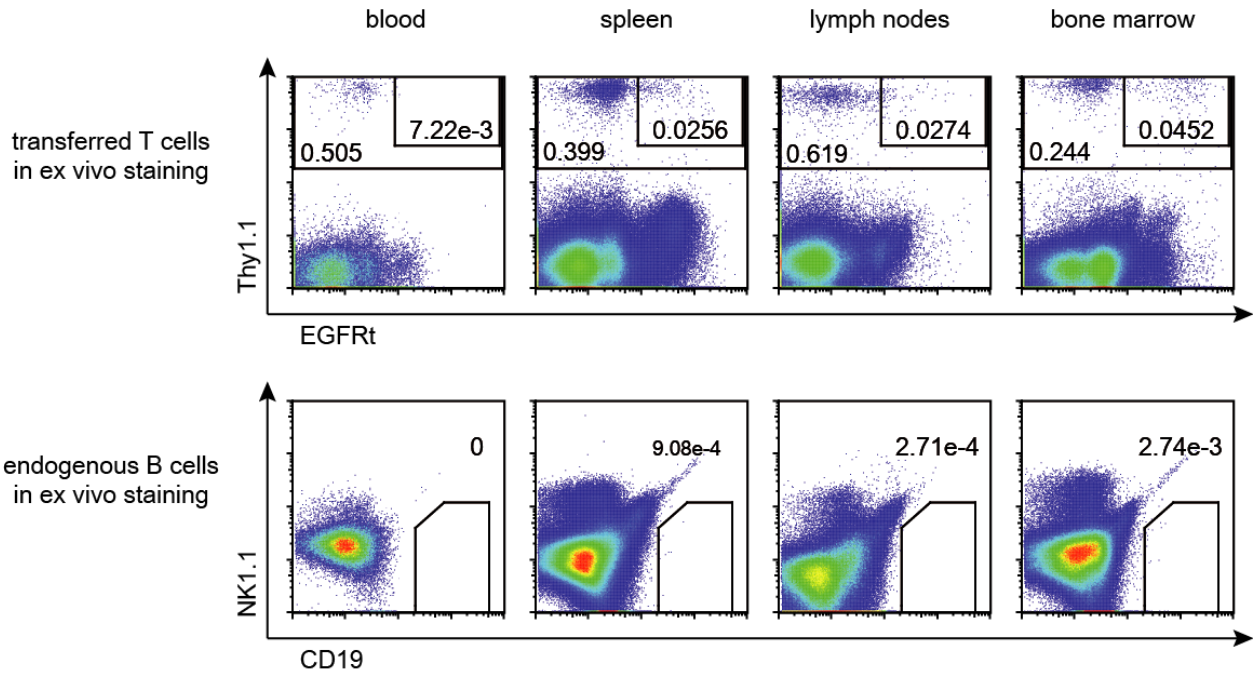
Supplemental Figure 3. (**A**) The m1928E construct encodes for a mouse CD19 CAR and EGFRt. The m1928E CAR/EGFRt construct encompasses a mouse CD19-specific single chain variable fragment (scFv), a spacer derived from CD8 extracellular domain (EC), CD8 transmembrane (TM) domain, CD28 costimulatory domain, CD3ζ signaling domain, a porcine teschovirus-1 peptide (P2A) and the EGFRt surface marker. The gene construct is flanked by long-term repeats (LTR). (**B**) The m19BBE construct encodes for a mouse CD19-specific single chain variable fragment (scFv), a spacer derived from CD8 extracellular domain (EC), CD8 transmembrane (TM) domain, 4-1BB costimulatory domain, CD3ζ signaling domain, a porcine teschovirus-1 peptide (P2A) and the EGFRt surface marker.

Supplemental Figure 4.



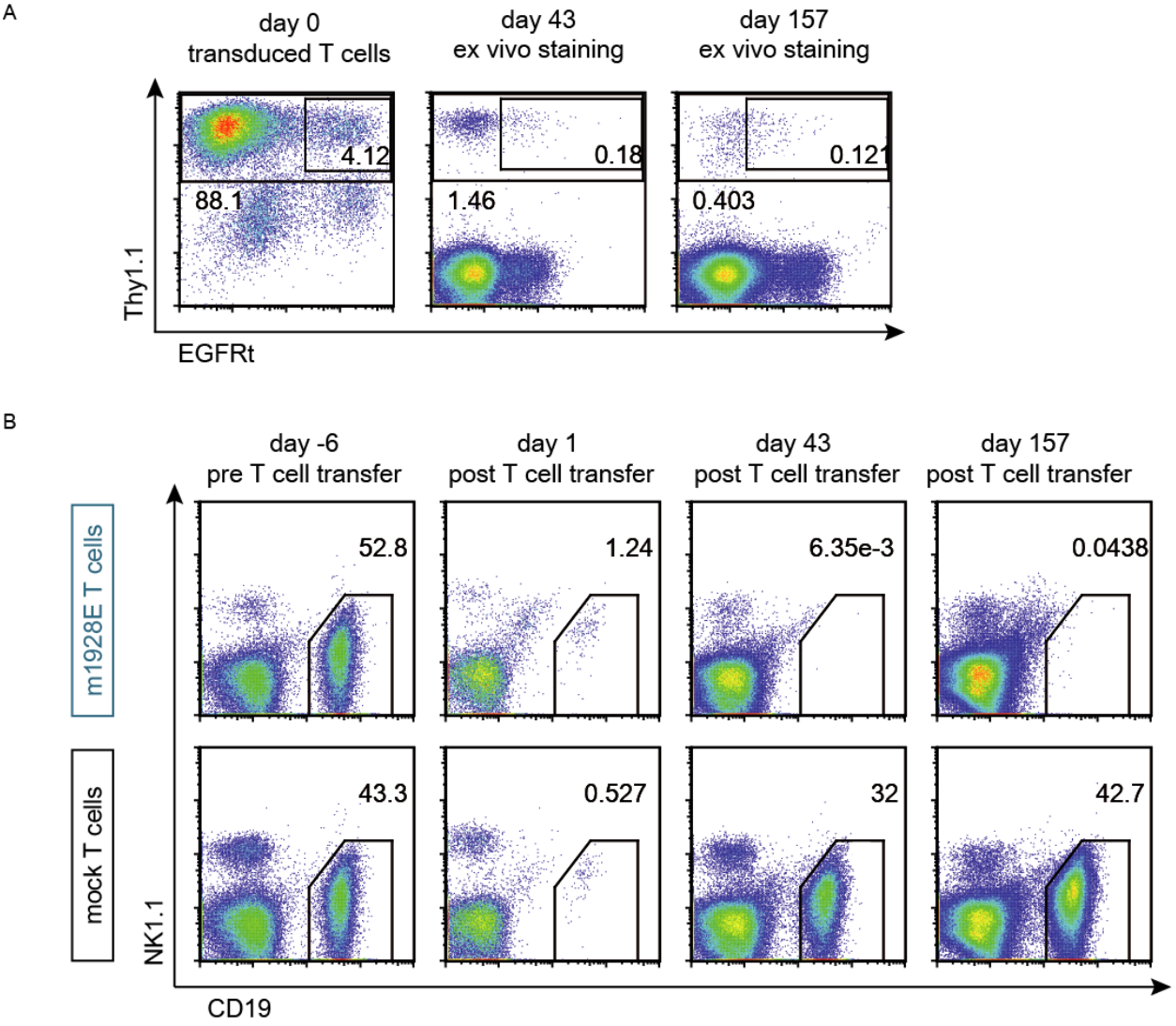
Supplemental Figure 4. Adoptive transfer of mCD19 CAR-T cells is associated with hypogammaglobulinemia. Wildtype C57BL/6 mice received 1.2x10^6 m1928E or mock-transduced Thy1.1$^+$ cells on day 0 and were infused with either Cetuximab (Ctx, red) or Rituximab (Rtx, blue) on day 50 and 55. Blood plasma was collected over the course of the experiment and analyzed by ELISA. Levels of the immunoglobulin (Ig) isotypes IgG1, IgG3, IgG2b, IgA and IgM over time are shown for each of the mouse groups. Means +/- SEM are shown in each graph; n=6 per group.

Supplemental Figure 5.



Supplemental Figure 5. m1928E$^+$ T cells persist long-term at low levels. Blood, spleen, lymph nodes and bone marrow were obtained on day 240 (after CAR T cell infusion) from a mouse that had been infused with 1.2x10^6 m1928E-transduced Thy1.1$^+$ cells on day 0 and treated with Rituximab twice on day 50 and 55. Transferred EGFRt$^+$ cells were detected with Thy1.1 and EGFR-specific mAbs (upper row). Numbers indicate frequencies of gated cells among living lymphocytes. Samples were stained with CD19 and NK1.1-specific antibodies and gates were set on endogenous B cells among Thy1.1$^-$ living lymphocytes (lower row). Numbers indicate B cell frequencies.

Supplemental Figure 6.



Supplemental Figure 6. Surviving m1928E^{+} T cells mediate sustained B cell depletion. (**A**) 8x10^{6} m1928E-transduced, unsorted cells were transferred into irradiated wildtype C57BL/6 mice. Transduced T cells were stained with Thy1.1 and EGFR-specific mAbs at the day of T cell transfer (day 0) and similar *ex vivo* stainings were performed on blood samples from day 43 or day 157 post T cell infusion. (**B**) To examine long-term CD19 CAR-mediated T cell functionality, blood samples were obtained on day -6 before and on day 1, 43 and 157 after T cell transfer and stained with CD19 and NK1.1-specific mAbs. Numbers indicate endogenous B cell frequencies among Thy1.1^{-} living lymphocytes. Samples from representative mice that received m1928E-transduced or mock T cells are shown in the upper and lower row, respectively