Supplemental Figure 1. Development and in vitro comparison of BAFF-R-CD19(t) and CD19-BAFF-R(t) dual targeting CAR T cell constructs. A. Schematics depict the arrangement of BAFF-R and CD19 targeting scFvs designed for each dual-targeting CAR construct. B. FACS analysis plots show CAR positive T cells following the EGFR enrichment. C. Calculated specific lysis are plotted from CTL assays performed against Nalm-6 ALL tumor lines (including wild-type, CD19-/-, and BAFF-R-/- variants) for BAFF-R-CD19(t) and CD19-BAFF-R(t) dual-targeting CAR constructs. Mock T cells were used as allogeneic control. Experiments was conducted in triplicate and analyzed by a Student’s t-test; **P<0.01, ***P<0.001, ****P<0.0001, NS, not significant.
Supplemental Figure 2. Generation of single and dual CAR T cells. Naïve and central memory T cells (Tn/mem) cells were enriched, activated, transduced, and expanded for 13 days. FACS analysis plots depict the T cell identity (Figure A) based on CD3 and transduction efficiency (Figure B) based on EGFR expression for CD19-BAFF-R(l) dual-CAR, CD19 single CAR, and BAFF-R single CAR T cells that were transduced at MOI=2. Mock T cells were used as negative control. CD19-BAFF-R(l) dual-CAR that passed quality control was then utilized for in vitro functional degranulation and cytokine release assays and in vivo survival models as seen in Figure 4.
Supplemental Figure 3. CD3 depletion from patient blood samples. CD3+ cells were depleted from patient samples and CD3 was assessed by flow cytometry pre- and post-T cell depletion. Representative data from 7 samples are presented.