Supplemental Figure 1



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(I) 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(I) 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



Supplmental 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.