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Supplemental Information

Tumor Antigen and Receptor Densities Regulate Efficacy of a Chimeric Antigen Receptor Targeting Anaplastic Lymphoma Kinase

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Supplementary Figures

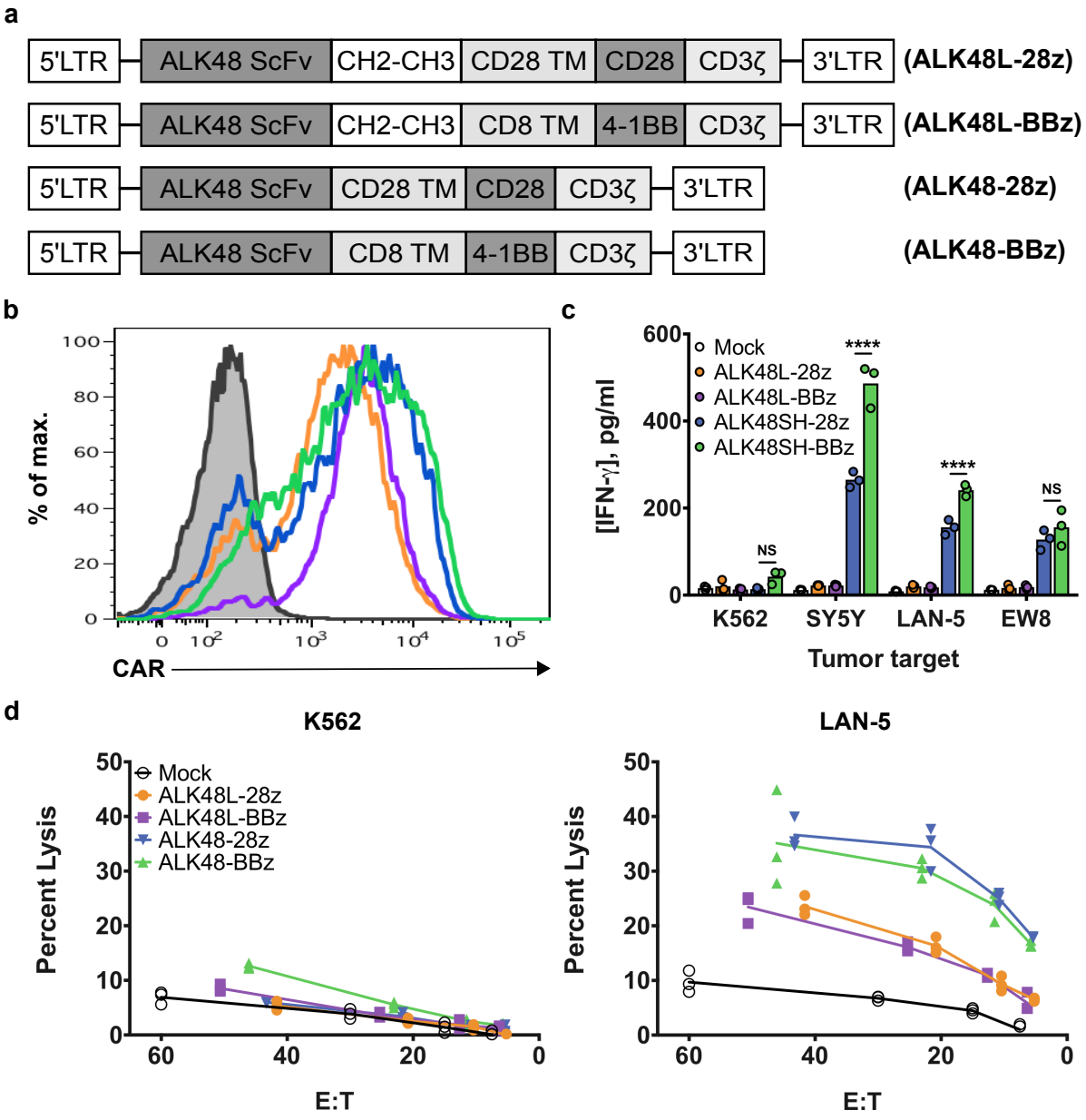

Figure S1. Characterization of ALK48 CAR T cells.

The scFv sequence of the ALK48 antibody was used to construct several CARs targeting ALK. The resulting vectors also contained either a CD28 transmembrane/co-stimulatory motif or a CD8a transmembrane/4-1BB costimulatory motif, and a CD3-zeta

signaling domain. “Long” CAR T cells were created by adding the CH2-CH3 sequence of human IgG1 between the scFv and transmembrane domains (**a**). After retroviral transduction, ALK48 CARs expressed on the surface of the human T cells (**b**). Production of IFN-gamma by ALK48 CAR T cells was evaluated after 24-hour co-incubation with ALK^+ tumor cell lines. Differences in cytokine production between CARs was determined by a two-way ANOVA followed by a Tukey’s multiple comparisons test (**c**). Cytolytic activity of ALK48 CAR T cells was assessed in a standard 6-hour ^{51}Cr release assay against ALK^- (K562) and ALK^+ (LAN-5) tumor lines (**d**). Cytokine release experiments were repeated with three different PBMC donors and cytolytic activity experiments were repeated with two different PBMC donors.

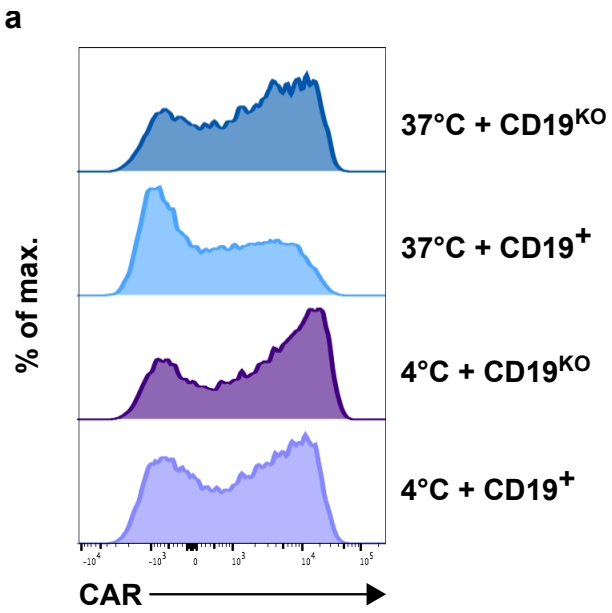

Figure S2. CARs do not down-modulate at 4°C.

CD19.4-1BB.ζ-BFP CAR T cells were co-incubated with $CD19^{+}$ or $CD19^{KO}$ NALM-6-GL tumor cells at 37°C or 4°C for 1 hour. Surface CAR was quantified by flow cytometry. Data is representative of two experiments with different donors (**a**).

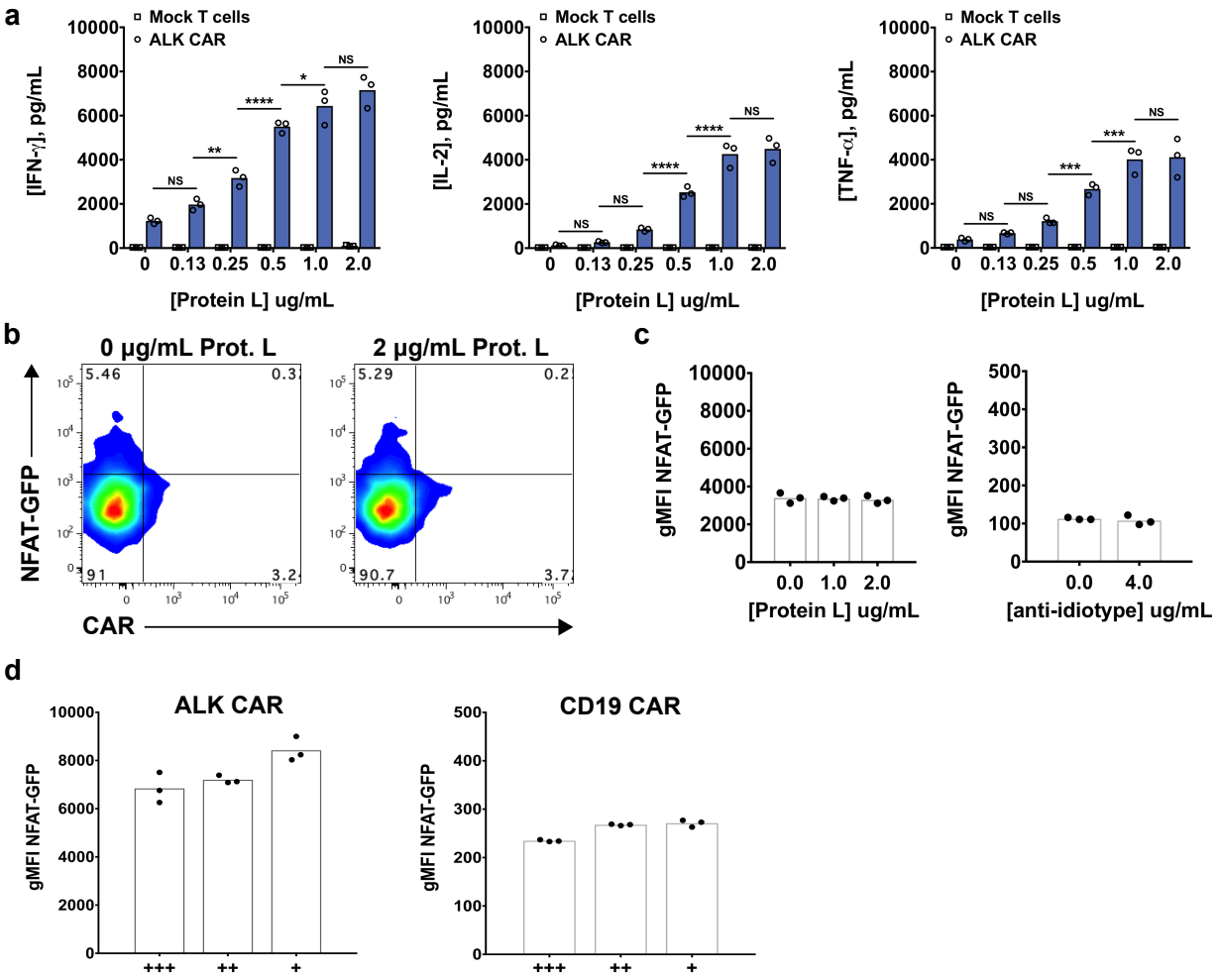

Figure S3. Evaluating CAR T cell activity with an NFAT-GFP reporter.

ALK CAR T cells were added to 96-well plates coated with a range of concentrations of biotinylated Protein L and cytokine release assayed. Differences between adjacent levels of protein L stimulation and CAR groups were determined by a two-way ANOVA followed by a Tukey's multiple comparisons test, and are representative of two experiments with different PBMC donors (**a**). Representative plots and quantification of the geometric mean fluorescence of NFAT-GFP for Mock/NFAT-GFP reporter T cells after stimulation on Protein L or anti-(CD19 scFv)-idiotype antibody coated plates are shown (**b**) and quantified (**c**). High (+++), medium

(++), or low (+) CAR expressing (ALK48.4-1BB.ζ or CD19.4-1BB.ζ-BFP)/NFAT-GFP reporter T cells were stimulated for 24 hours with anti-CD3/CD28 beads and evaluated by flow cytometry for NFAT-GFP expression (**d**). NFAT-GFP reporter data is representative of the three experiments each with the ALK CAR and CD19 CAR as described in Figure 5.