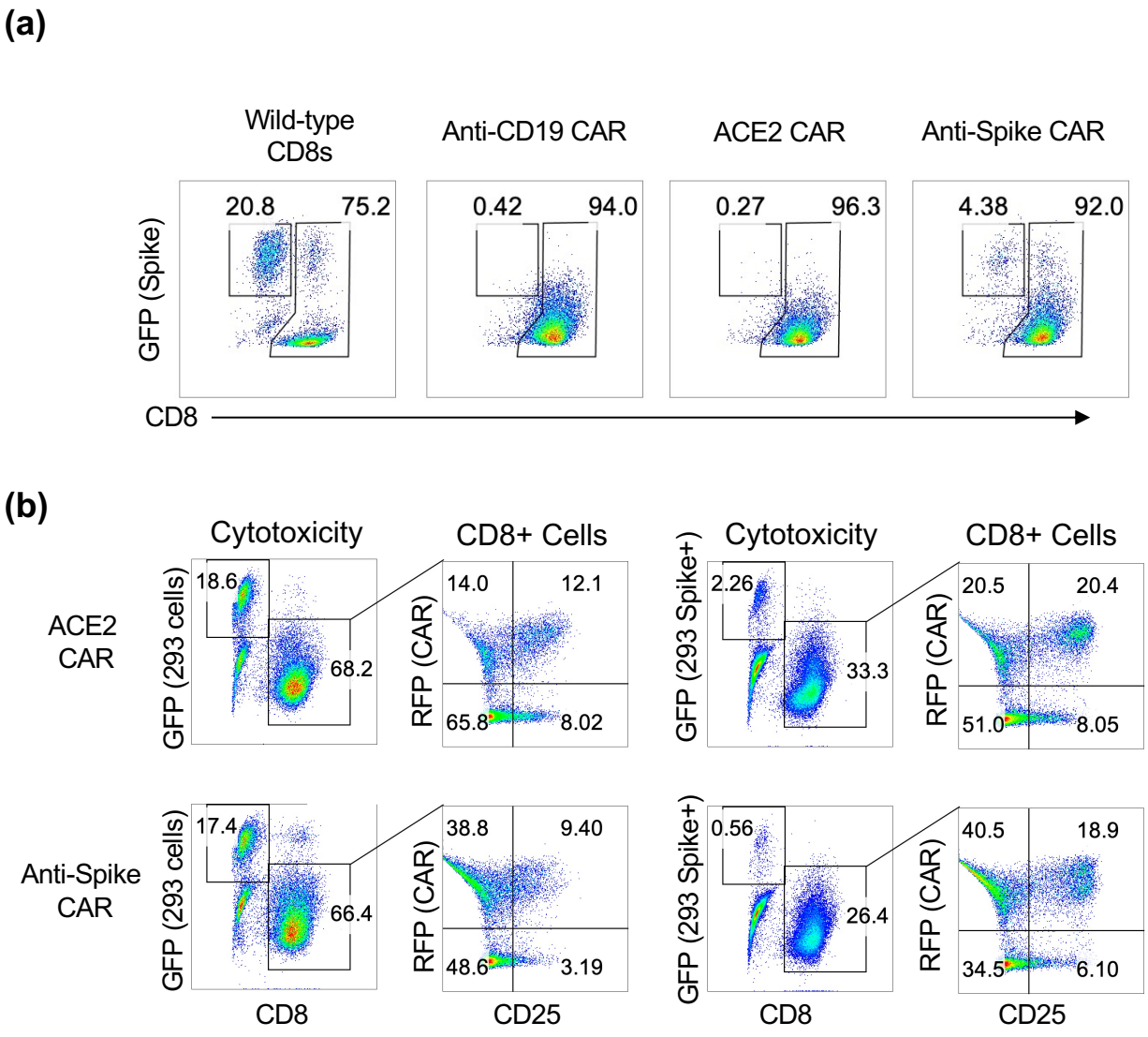
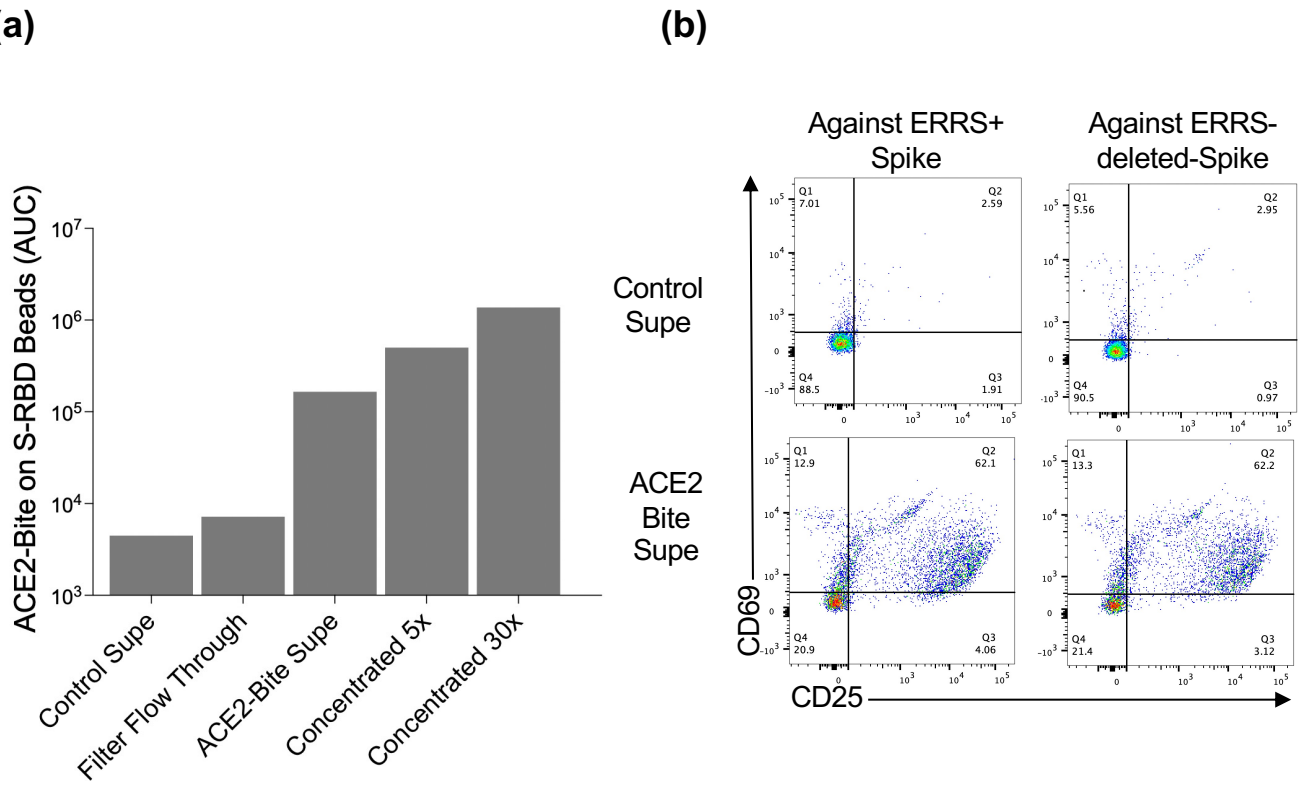
Supplementary figure 1



Supplementary figure 1: Selective cytotoxicity of ACE2 CAR and anti-Spike CAR-T cells for different spike-expressing target cells.

(a) CAR-engineered T cells cytotoxicity assays with Spike-expressing target B cell line (T2 cells) at 8:1 E:T ratio. Wild-type CD8 T cells were used as a negative control and anti-CD19 CAR-expressing CD8 T cells were used as a positive control. **(b)** CAR-engineered T cell cytotoxicity assays with Spike expressing and control 293 target cells. Control 293s were engineered with a GFP-expressing empty vector. Spike-expressing and control 293s were identified by GFP expression. Effector cells were identified by CD8 staining. T cell activation was determined via CD25 staining. CAR-expressing T cells co-expressed RFP with CAR constructs. Panels show representative experiments replicated three times with similar results

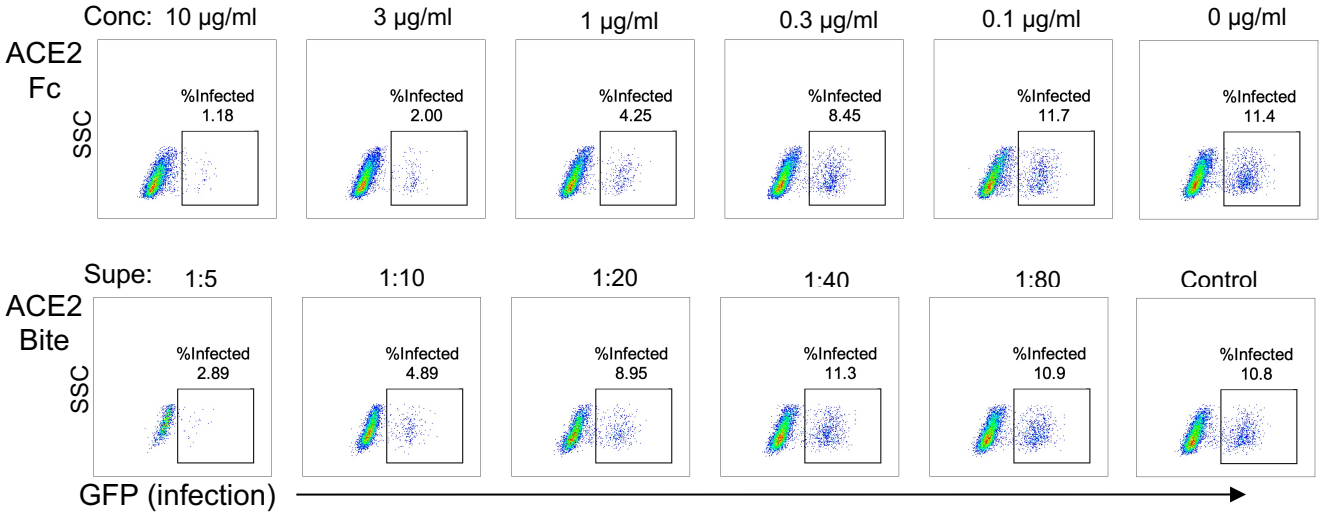
Supplementary figure 2



Supplementary figure 2: ACE2-Bite concentrations determined by a bead-based capture assay and ACE2-Bite activity against wild-type and truncated Spike protein.

(a) Area under the curve (AUC) values of ACE2-Bite molecules in supernatants from different conditions. ACE2-Bite supernatant was concentrated 5-folds and 30-folds. Flow through supernatant from the concentration process (Filter flow through) and wild-type control supernatant were used as controls. Fluorescent beads coated with Spike-Receptor binding domain (S-RBD) were used to capture ACE2-Bite molecules in supernatants titrated from 1:1 to 1:1000 by 10-fold serial dilutions were detected via a recombinant CD3-Fc fusion protein and an anti-Fc antibody. The geometric mean of anti-Fc antibody fluorescence was used to generate the curves which were used to calculate the area under the curve values. **(b)** T cell activation against 293 cells transfected with Spike protein plasmids with and without endoplasmic reticulum retention domain analyzed by CD69 and CD25 expression on T cells.

Supplementary figure 3



Supplementary figure 3: Neutralization efficiency of ACE2-Bite compared to recombinant ACE2-Fc protein

FACS plots show neutralization data of delta variant spike protein pseudotyped virus infection when pre-incubated with different concentrations of ACE2-Fc (top panel) or different dilutions of ACE2-Bite supernatant (bottom panel). The infection levels were determined 3 days later via flow cytometry based on GFP expression.