

Expanded View Figures

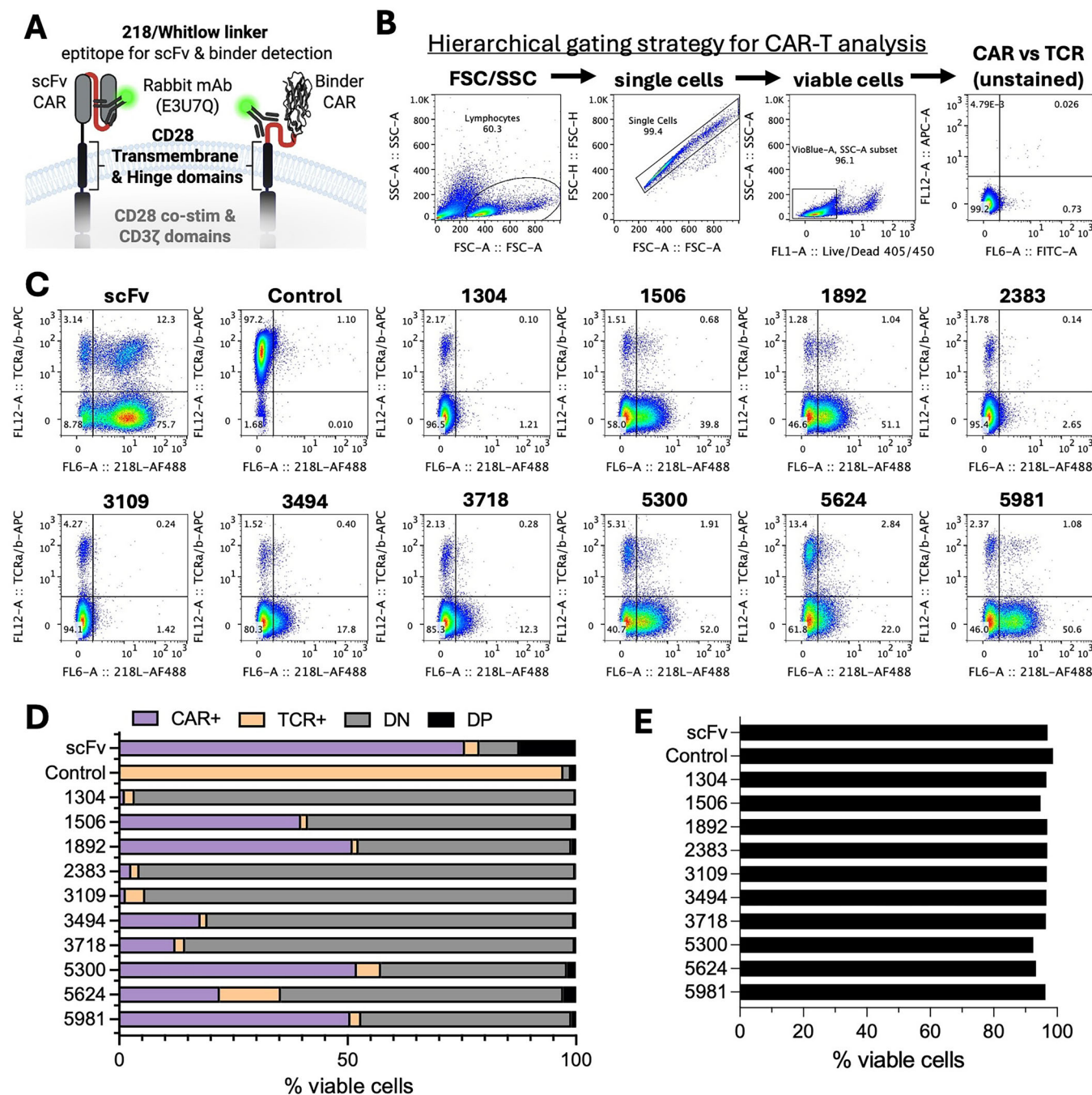


Figure EV1. CAR and TCR expression determined by flow cytometry.

(A) 218/Whitlow linker present in the scFv was designed into the binder construct as an antibody epitope. (B) Hierarchical gating strategy for viable CAR-T enumeration, as shown in (C) scatter plots depicting CAR and TCR surface expression for controls and binder variant CAR-T cells. (D) graphical representation of flow cytometry data showing T cell populations expressing CAR, TCR, neither, or both. (E) Viability assessment of controls and binder variant CAR-T cells. Source data are available online for this figure.

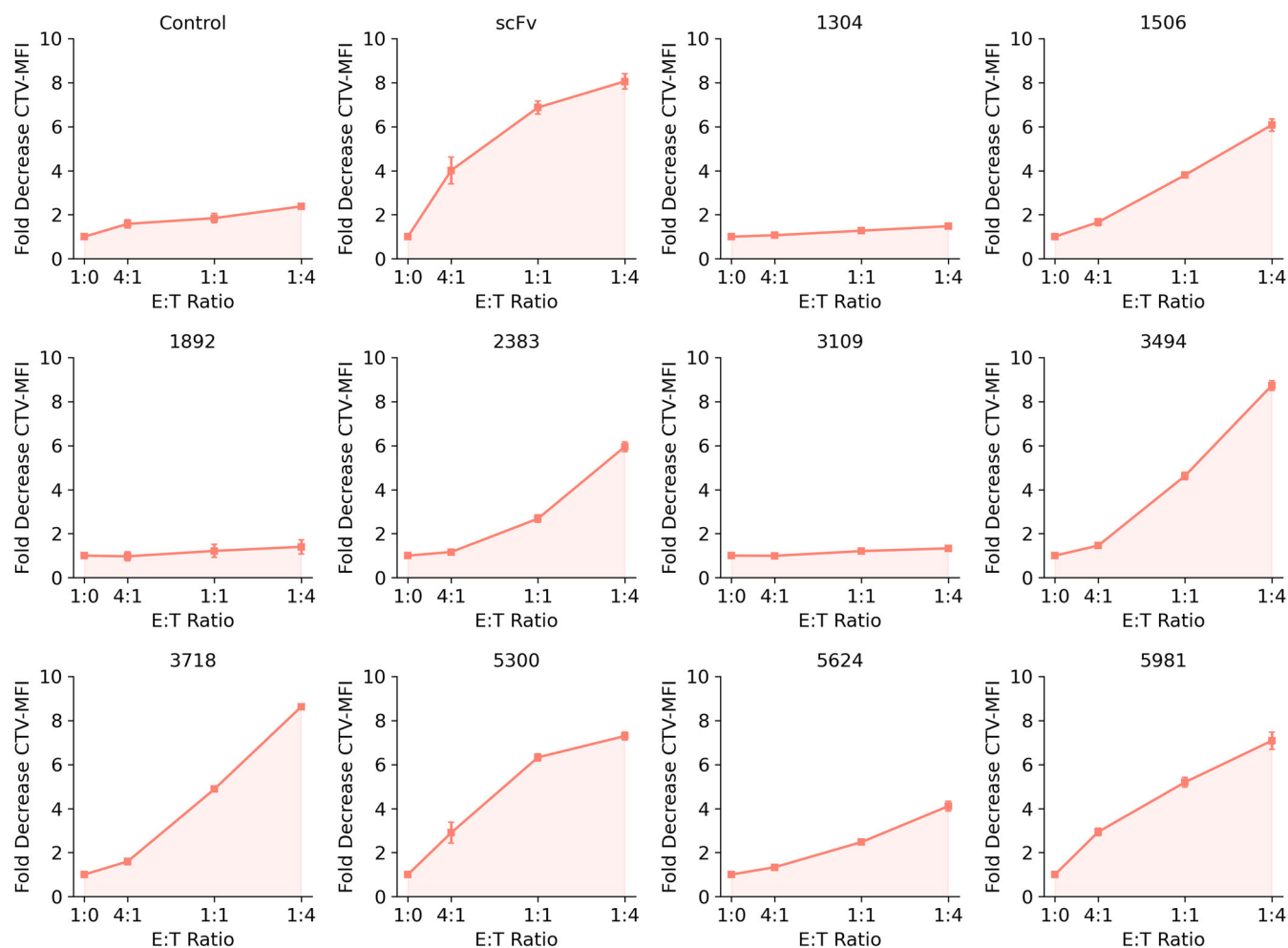


Figure EV2. Proliferation via a decrease in CTV-MFI.

Proliferation was measured over 5 days following stimulation with Raji (CD20+) targets across multiple E:T ratios by decrease in median fluorescence intensity (MFI) of the cell trace violet (CTV) signal. Data represent three technical replicates from one donor; bars indicate standard error centered on the mean. Source data are available online for this figure.

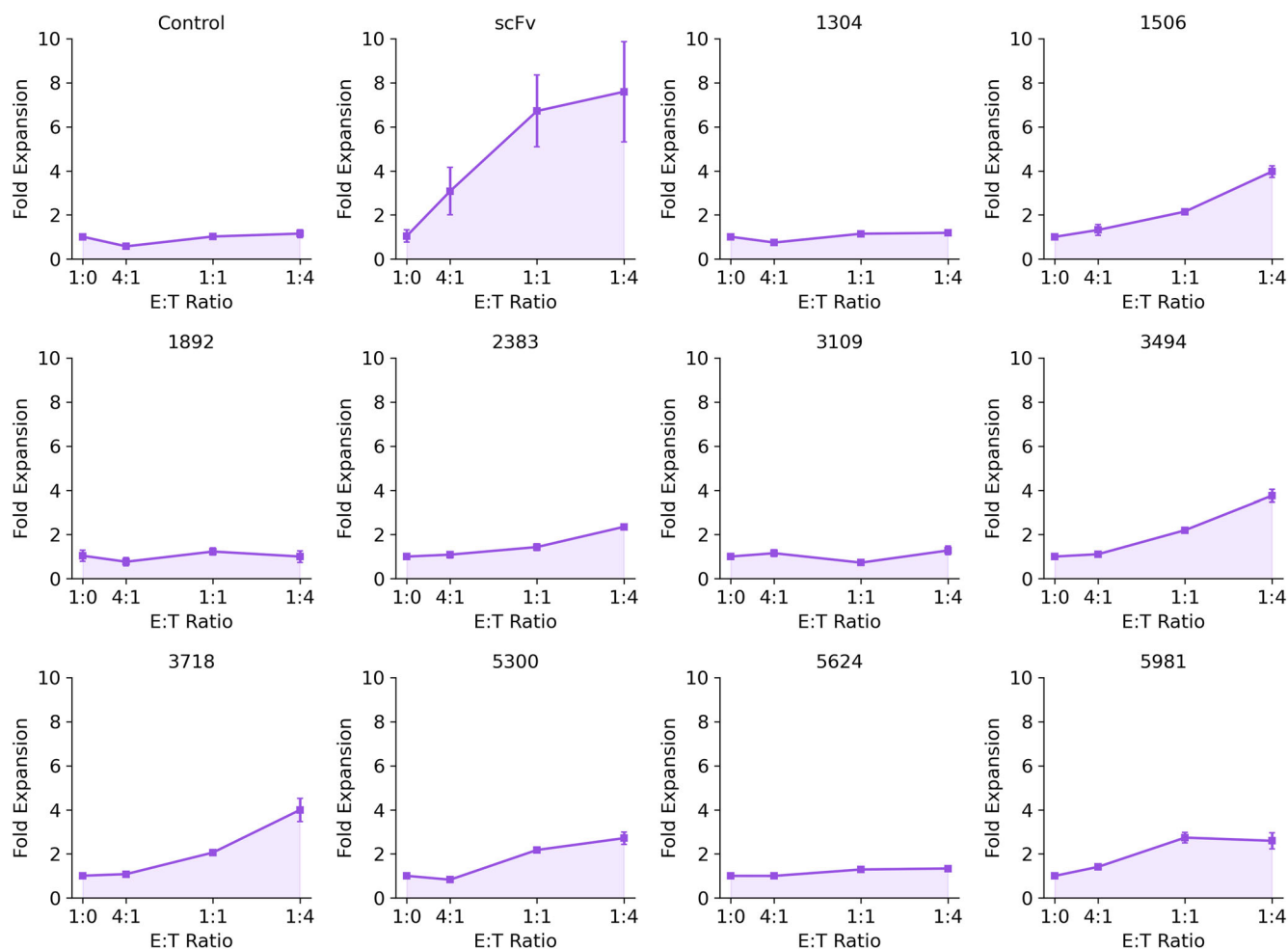


Figure EV3. Fold expansion by cell counts.

Total CAR-T expansion was measured over 5 days following stimulation with Raji (CD20+) targets across multiple E:T ratios by total viable CAR-T yield using volumetric flow cytometry. Data represent three technical replicates from one donor; bars indicate standard error centered on the mean. Source data are available online for this figure.

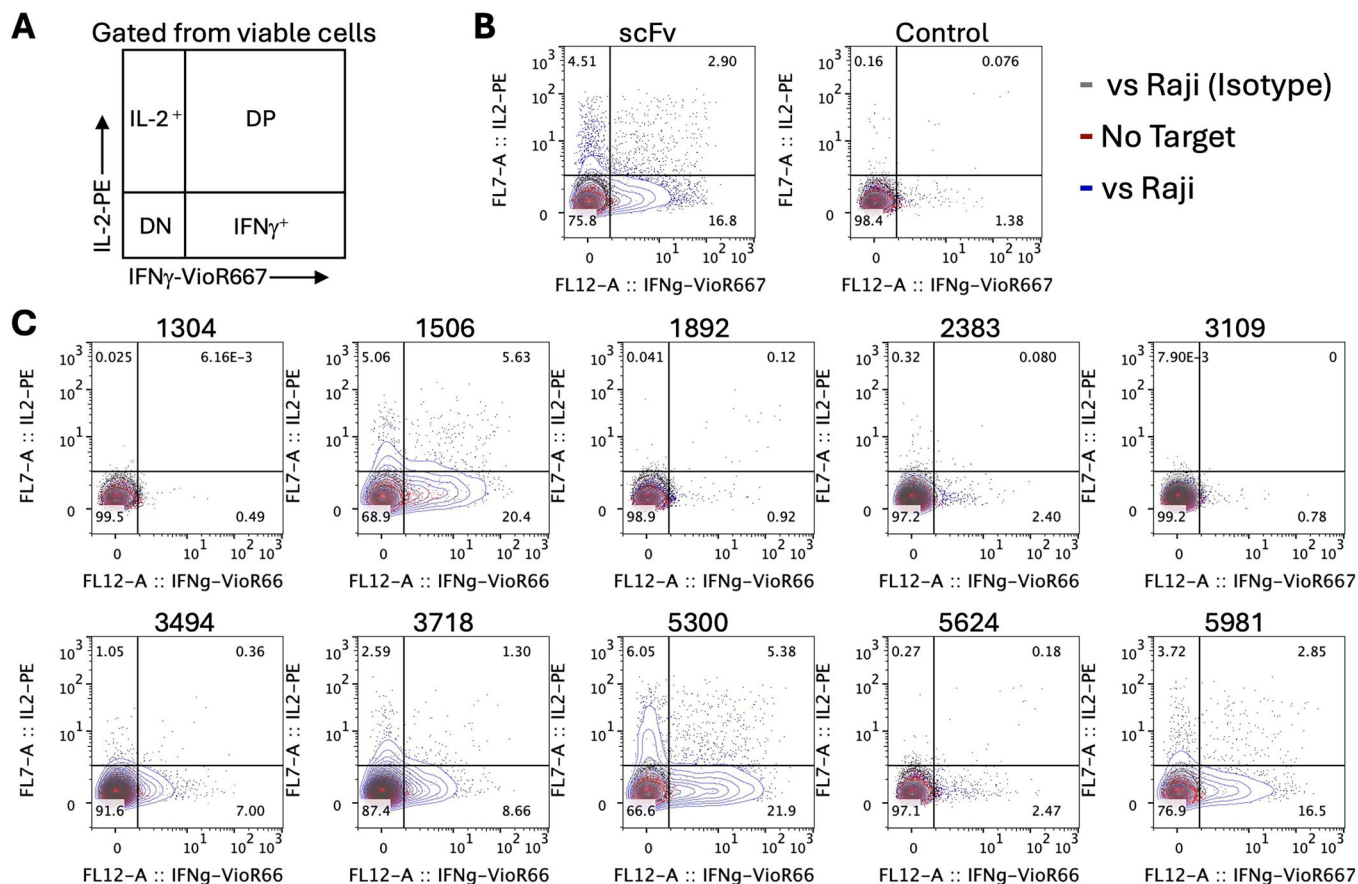


Figure EV4. CAR-T cell cytokine generation induced by CD20⁺ Raji target cells.

(A) Gating strategy illustrating cytokine-generating populations as analyzed by flow cytometry. (B) CD20 scFv CAR and Control (unedited, TCR + CAR-neg T cells), were stained with fluorochrome-conjugated antibodies after incubating alone (red) or in co-culture with Raji target cells. Specifically, anti-IFN γ and anti-IL2 antibodies, or the requisite isotype antibody controls, were used to analyze both controls and (C) individual CD20 binders as CAR-T cells. Source data are available online for this figure.

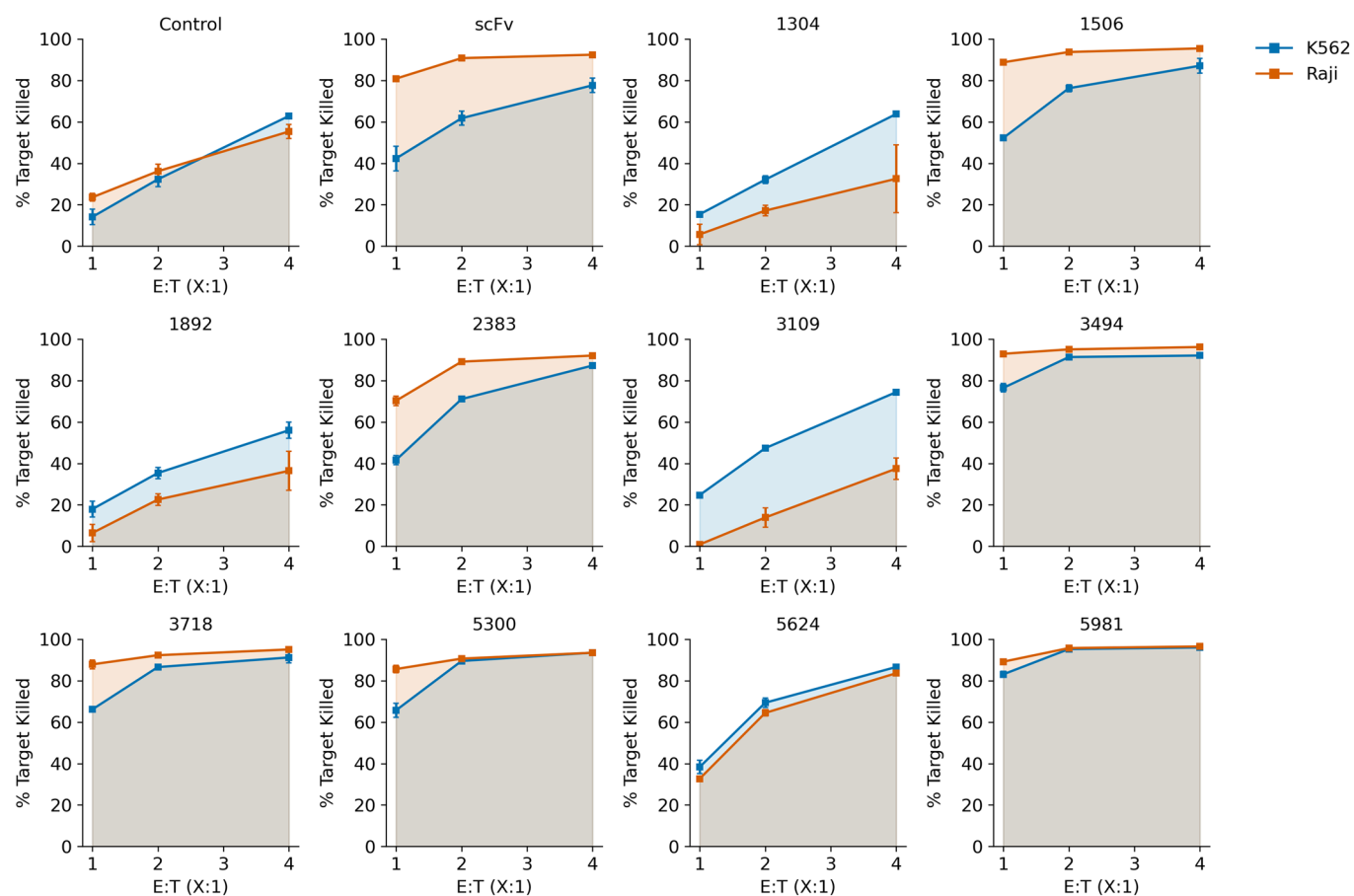


Figure EV5. Raji-specific cytotoxicity vs K562 control.

GFP assay readouts across multiple E:T ratios for Raji (CD20+) and K562 (CD20-) targets, overlaid for direct comparison. Data represents three technical replicates from one donor, bars indicate standard error centered on the mean. Source data are available online for this figure.