Supplemental Figure 1. Siglec-6 expression in primary cells and cell lines

A) *SIGLEC6* mRNA qPCR analysis in primary $CD34^+$HSC cells, B and T-cells and the indicated cell lines. Each dot represents an independent data point. **B)** Flow cytometry analysis comparing Siglec-6 levels in the indicated cell lines. **C**) Table representing the percentage of Siglec-6^+ B-cells in different healthy subjects. **D**) Representative flow cytometry analysis of Siglec-6 levels in primary and relapsed CLL ($CD5^+CD19^+$) and T-cells ($CD3^+$) from a donor used as reference.

Supplemental Figure 2. CAR-T cell proliferation in response to plate bound Siglec-6.

Quantitation of CAR-T cell proliferation by CPD dilution after three days culture in multiwell plates coated with recombinant Siglec-6-Fc, showing the percentage of cells in each cycle. T-test analysis indicated no significant differences between JML1-CAR-short and JML1-CAR. Data corresponds to four independent experiments, n=4,.

Supplemental Figure 3. Cytotoxic activity of JML1-CAR and JML1-short-CAR T-cells against Siglec-6^{hi} U937 cells.

In vitro cytotoxic analysis after incubation of Siglec-6^- internal negative cells CCRF-CEM (CPD^+) and, Siglec-6^{hi} U937 cells ($CFSE^+$) with the indicated CAR-T cells for 4-6 hours at the indicated effector to target cell ratios. **B)** Quantitation of cytotoxicity at the indicated effector to target cell ratios in two independent experiments. Cytotoxic activity is derived as described in the Methods section. T-test of JML1-CAR vs JML1-short-CAR is shown, $p<0.001$ (***), $p<0.01$ (**) or $p<0.05$ (*), n=4.

Supplemental Figure 4. Identification of the Siglec-6 domain that binds JML1.

ELISA showing the binding of JML1 Fab to the indicated Siglec-6-ectodomains. Bottom: Cartoon representations of the Siglec-6 ectodomain constructs used in the ELISA and the expression cassette map. V = Ig-like V-type domain (membrane-distal), C = Ig-like C2-type domains I (middle) and II (membrane proximal). Data are representative of two independent experiments and were analyzed using GraphPad Prism software. Statistics were calculated using an unpaired Welch's t test (** P≤ 0.01)

Supplemental Figure 5. Siglec-6 levels in primary CLL

A) Surface Siglec-6 levels in primary CLL samples shown in Figure 5E, comparing Siglec-6 levels in CLL and T-cells from the same patient. **B)** quantitation of the percentage of Siglec-6+ remaining CLL cells after treatment with the indicated CARs as in Figure 5G. Lines represent a single CLL sample treated with either CAR. T-test p=0.0385, n=6.

Supplemental Figure 6. JML1-CAR-short and JML1-CAR have antitumor activity in a xenograft mouse model of CLL

A) Surface expression of Siglec-6 analyzed by flow cytometry in the indicated cell lines. **B)** Image of *in vivo* luminescence at different times after injection of the indicated cell lines. **C)** Quantitative analysis of luminescence observed in (B) at the indicated times. **D)** Image of *in vivo* luminescence at different times after injection of MEC1-002 cells and the indicated CAR-T cells. **E)** Quantitative analysis of luminescence observed in (D) at the indicated times. Average radiance corresponds to [p/s/cm²/sr]. T-test of JML1-CAR against No CAR n=5. p<0.05 (*). Experiments of groups of 5 mice shown were repeated three times.

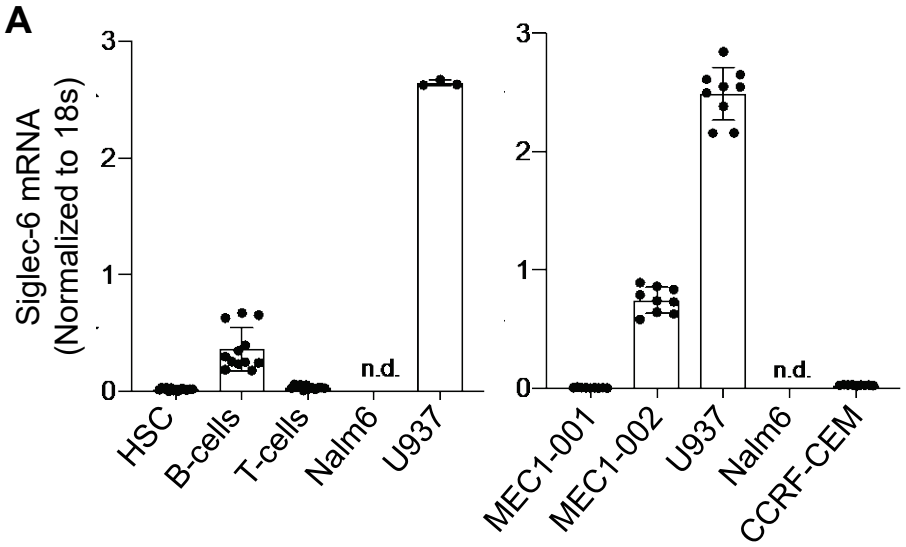
Supplemental Method:

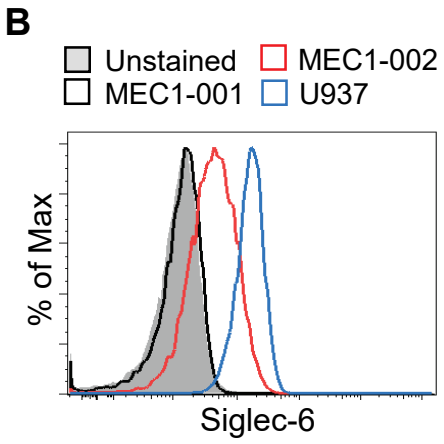
Siglec-6 epitope mapping

Each domain mapping Siglec-6 construct was expressed as a human IgG-Fc domain fusion protein by transfecting 293 Freestyle cells (ThermoFisher Scientific) with pCEP4 plasmids

containing human IgG1 Fc (99-329) fused at its C-terminus to Siglec-6 residues 27-128, 27-145, 27-235, or 27-347. Cell culture supernatants were sterile-filtered and purified via affinity chromatography using a HiTrap Protein A HP column (GE Healthcare). Proteins were 90% pure as determined by SDS PAGE.

For the domain mapping ELISA, 50 ng of each Siglec-6 construct was coated directly into individual polystyrene wells of a 96-well plate and subsequently incubated with the human Fab JML-1 or an isotype control at 1.5 ng/µL. Binding was detected with peroxidase-conjugated goat anti-human IgG, F(ab')2 -specific secondary antibody (Jackson ImmunoResearch) and BioFX ABTS one-component HRP substrate. Signal was quantified at 405 nm using SpectraMax M5 instrument with SoftMax Pro software (Molecular Devices).

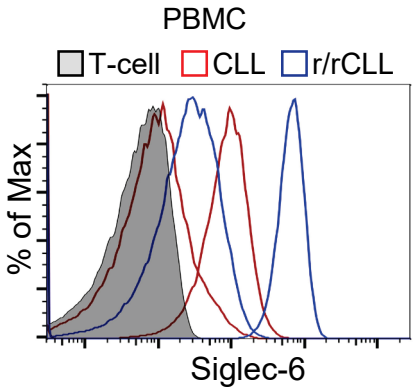




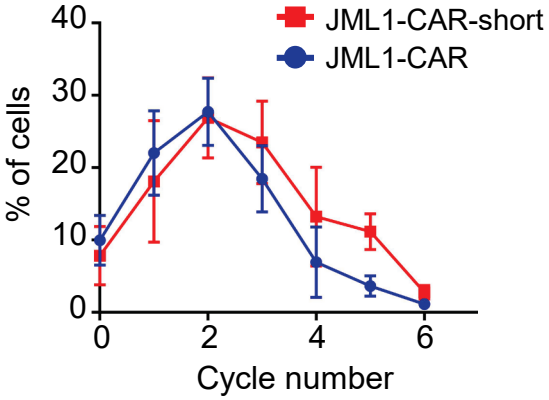
C

% Siglec-6^{+}B-cells

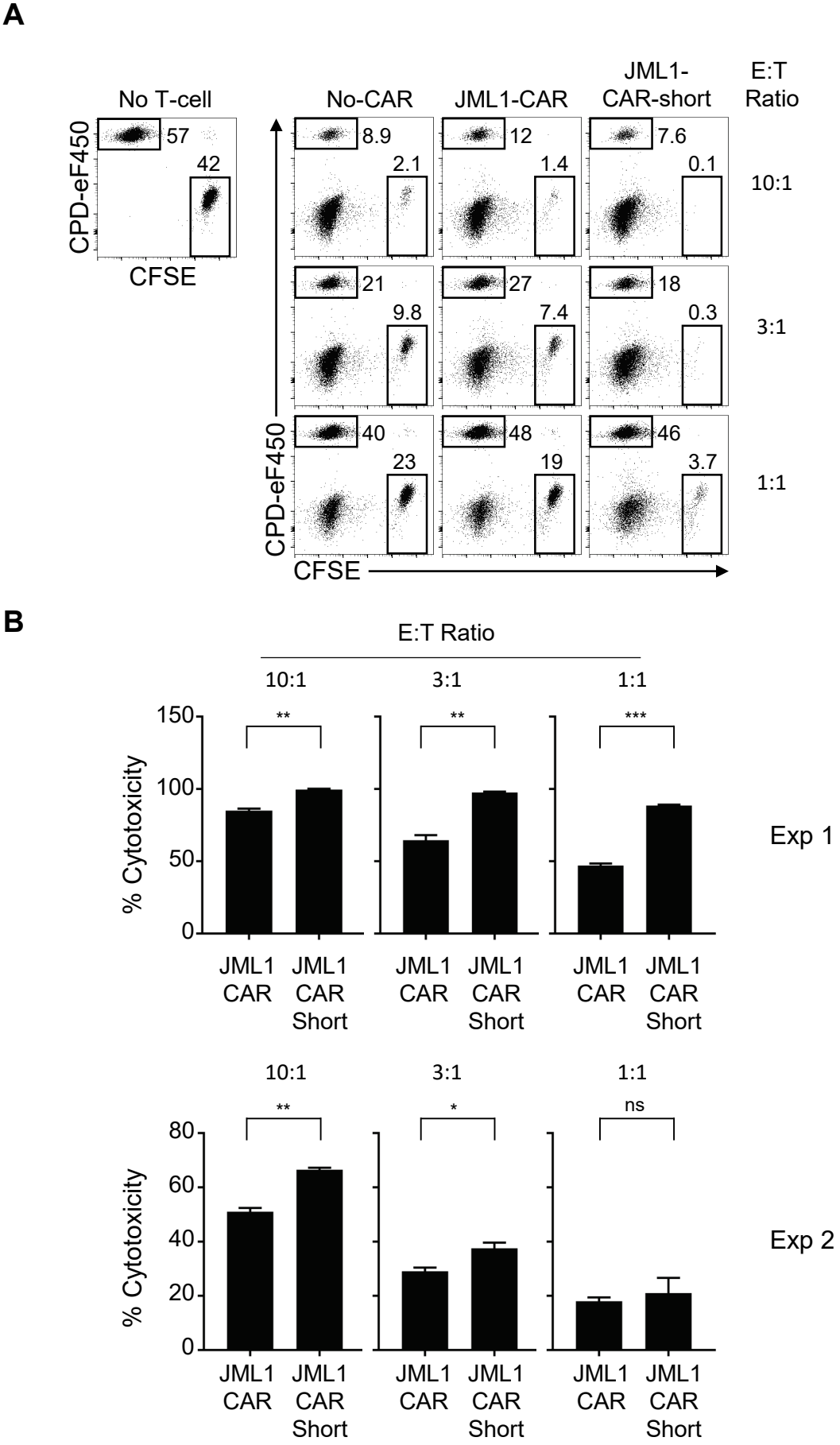
PBMC1	57
PBMC2	37
PBMC3	25
PBMC4	35.6
PBMC5	43.1
PBMC6	31.8
PBMC7	33
PBMC8	13
Mean	34.4
SD	12.8



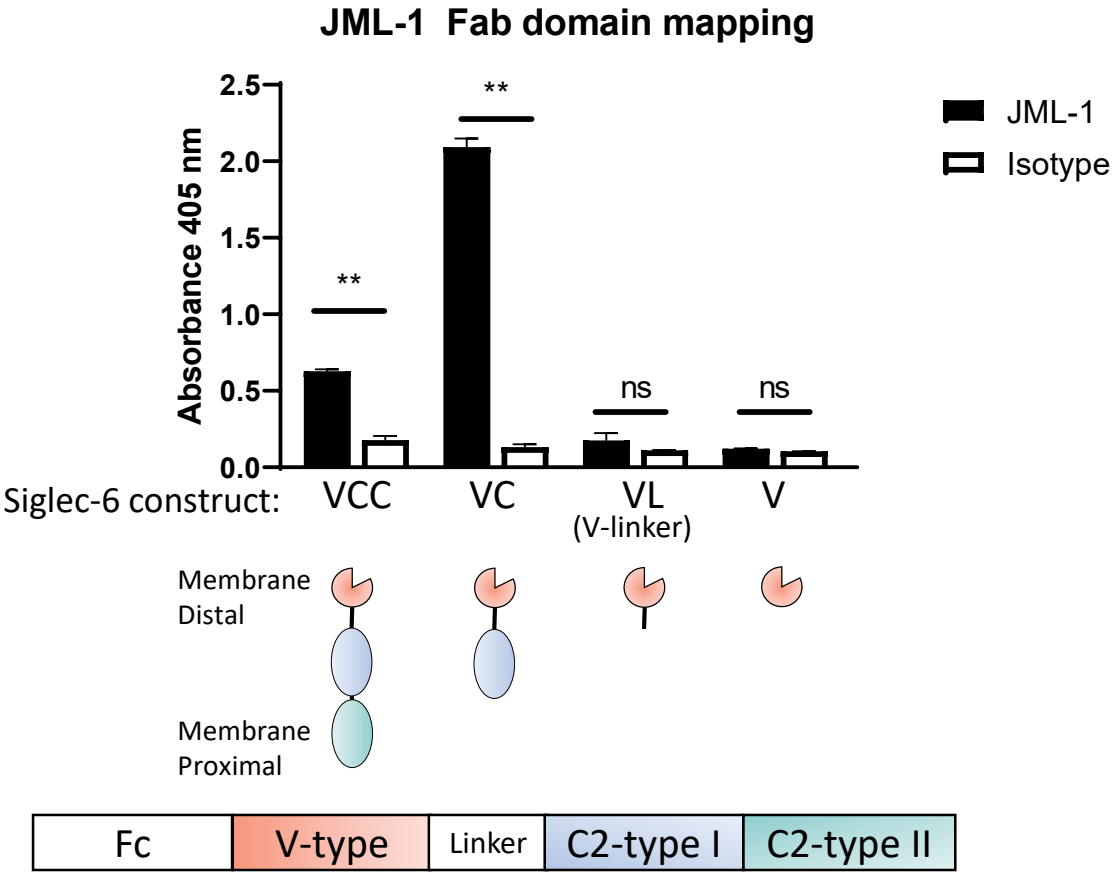
Supplemental Figure 1



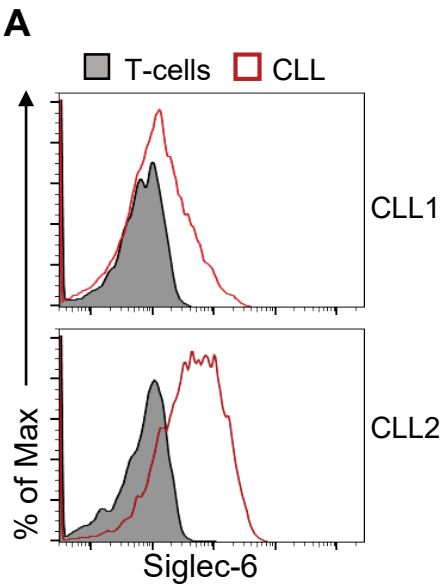
Supplemental Figure 2

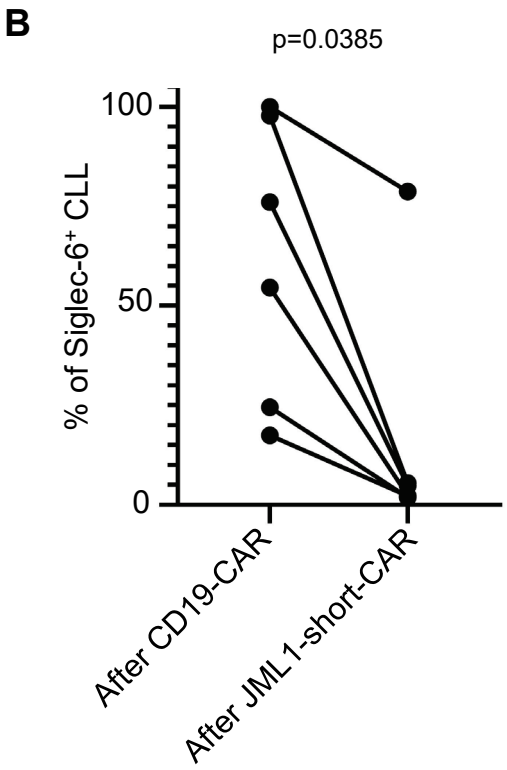


Supplemental Figure 3

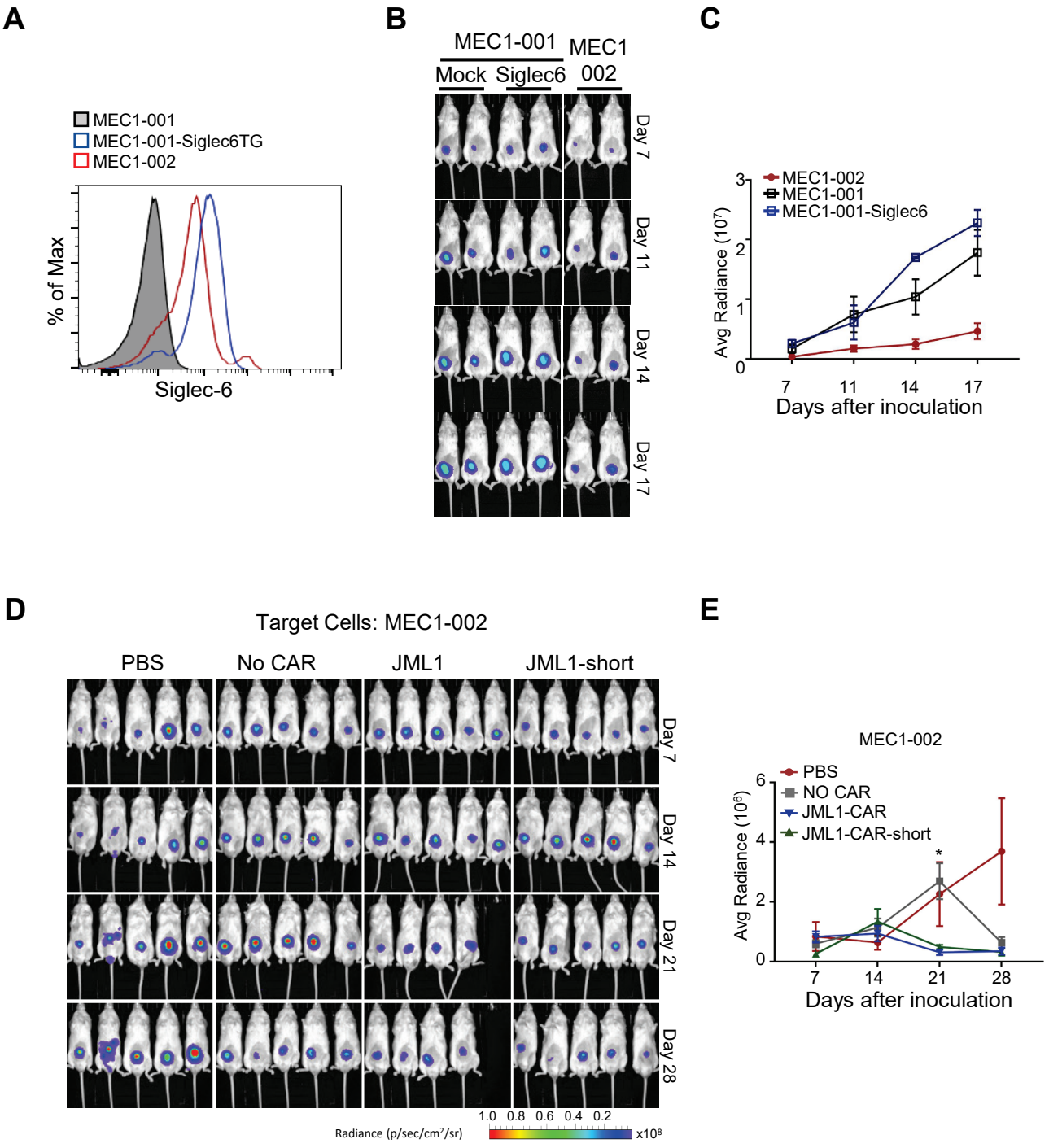


Supplemental Figure 4





Supplemental Figure 5



Supplemental Figure 6