

Antibody Cocktails

Antibody panel to assess transduced CB cells in xenograft mice:

- anti-mouse CD45.1 PE, cat#553776 BD Biosciences
- anti-human CD45 BUV805, cat#612891 BD Biosciences
- anti-human CD3 BUV395, cat#564001 BD Biosciences
- anti-human CD19 APC-eFluor780, cat#47-0198-42 eBioscience
- anti-human CD33 APC, cat#340474 BD Biosciences
- anti-human CD34 PE-Cy7, cat#348801 BD Biosciences
- anti-human CD41a BV650, cat#740575 BD Biosciences
- anti-human CD42b BUV661, cat#741614 BD Biosciences
- anti-human CD56 BUV737, cat#748609 BD Biosciences
- anti-human CD117 PerCP-Cy5.5, cat#313214 Biolegend

Antibody panel to assess cultured CB cells:

- anti-human CD14 APC-Cy7, cat#557831 BD Biosciences
- anti-human CD15 BUV805, cat#742057 BD Biosciences
- anti-human CD33 BUV395, cat#745709 BD Biosciences
- anti-human CD34 PE-Cy7, cat#348801 BD Biosciences
- anti-human CD41a BV650, cat#740575 BD Biosciences
- anti-human CD42b BUV661, cat#741614 BD Biosciences
- anti-human CD45 APC, cat#17-9459-42 eBioscience
- anti-human CD56 BUV737, cat#748609 BD Biosciences
- anti-human FOLR1 PE, cat#908304 Biolegend

Antibody panel to assess unmodified/ CAR T and leukemia cells in xenograft mice:

- anti-mouse CD45.1 APC/Cy7, cat#110716 Biolegend
- anti-human CD45 BUV805, cat#612891 BD Biosciences
- anti-human CD3 PE-Cy7, cat#563423 BD Biosciences
- anti-human FOLR1 PE, cat#908304 Biolegend

Antibody panel to assess FOLR1 expression in normal HSC, MPP and MLP subsets:

- anti-human CD34 BUV395 cat#563778 BD Biosciences
- anti-human CD38 PE-Cyanine7, cat#25-0389-42 eBioscience
- anti-human CD45 BUV805, cat#612891 BD Biosciences
- anti-human CD45RA APC-eFluor780, cat#47-0458-42 eBioscience
- anti-human CD90 PE-Cy5, cat#555597 BD Bioscience
- anti-human FOLR1 PE, cat#908304 Biolegend

Antibody panel to assess FOLR1 expression in normal CLP, GMP, CMP and MEP subsets:

- anti-human CD10 B605, cat#562978 BD Biosciences
- anti-human CD34 BUV395 cat#563778 BD Biosciences
- anti-human CD38 PE-Cyanine7, cat# 25-0389-42 eBioscience
- anti-human CD45 BUV805, cat# 612891 BD Biosciences
- anti-human CD45RA APC-eFluor780, cat# 47-0458-42 eBioscience
- anti-human CD123 APC cat#658172 BD Biosciences
- anti-human FOLR1 PE, cat#908304 Biolegend

Supplemental Figure 1. C/G-CB cells form tight clusters in mouse bone marrow. (Related to Fig. 1) Histology of femurs taken from primary, secondary and tertiary transplants of C/G-CB cells.

Supplemental Figure 2. Expression of *FOLR1* transcript in C/G-CB cells cultured on ECs.

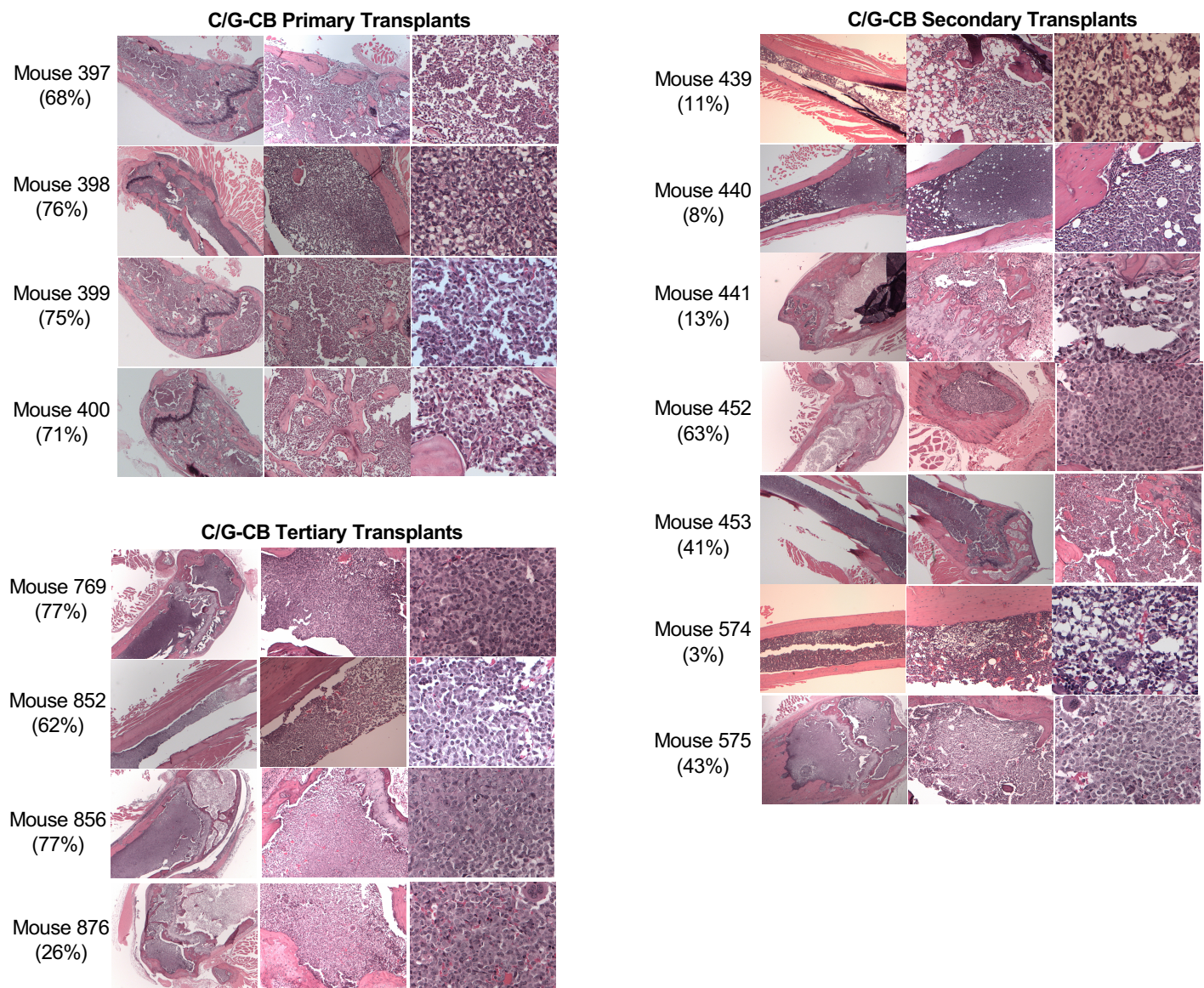
RT-PCR analysis of *FOLR1* expression in engineered CB cells and in fusion positive cell lines M07e and WSU-AML. Expression is normalized as fold-change relative to GFP-CB/EC Wk 3 samples.

Supplemental Figure 3. Expression of C/G transcript in C/G-CB cells. RT-PCR analysis of C/G expression in engineered CB cells and in fusion positive cell lines M07e and WSU-AML.

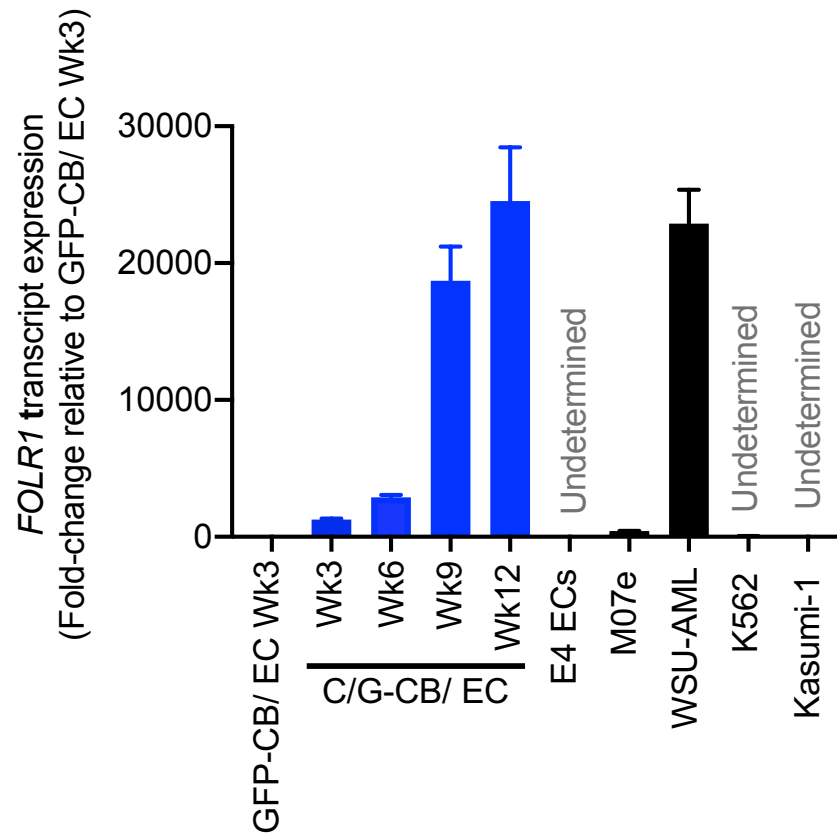
Supplemental Figure 4. FOLR1 CAR constructs and reactivity of short, intermediate, and long FOLR1 CAR T cells.

a. Schematic diagram of second-generation FOLR1 CAR constructs with different IgG4 spacer lengths. SP= GM-CSFR signal peptide; scFv= single-chain variable fragment; TM = transmembrane domain; CD = costimulatory domain; SD = stimulatory domain; tCD19 = transduced marker truncated CD19. **b.** Expression of FOLR1 in C/G-CB, M07e, WSU-AML, Kasumi-1 *FOLR1*⁺ and Kasumi-1 parental cells. Blue= stained with PE-labeled anti-FOLR1. Grey= Isotype control. **c.** Cytolytic activity of CD8 T cells unmodified or transduced with short, intermediate or long FOLR1 CAR construct against C/G-CB, M07e, WSU-AML, Kasumi-1 *FOLR1*⁺ and Kasumi-1 parental cells in a 6-hour assay. Shown is mean percent specific lysis +/- SD from 3 technical replicates at indicated Effector:Target (E:T) ratios. **d.** Concentration of secreted IL-2, IFN- γ , and TNF- α in the supernatant following 24 hour of T cell/AML co-culture at 1:1 E:T ratio. Mean +/- SD from 3 technical replicates is shown. **e.** Representative flow plots showing expression of NFAT, NF- κ B and AP-1 in Jurkat Nur77 reporter transduced with FOLR1 CAR constructs cultured alone (top) or co-incubated with Kasumi-1 *FOLR1*⁺ target cells for 24 hours at 1:1 E:T ratio (bottom). Kasumi-1 *FOLR1*⁺ cells were labeled with Violet Cell Proliferation Dye to differentiate from Jurkat cells. Transduced Jurkat cells were gated based on tCD19 expression. Number in top right corner indicates the percentage of positive cells.

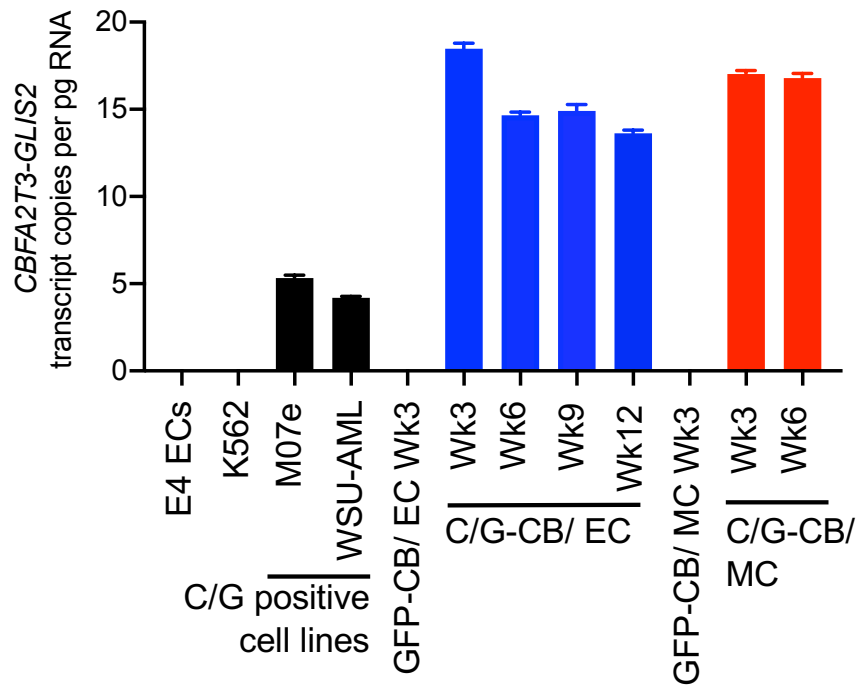
Analysis was performed on day 4 post transduction. **f.** Quantification of percent NFAT⁺, NF- κ B⁺ and AP-1⁺ cells in e.



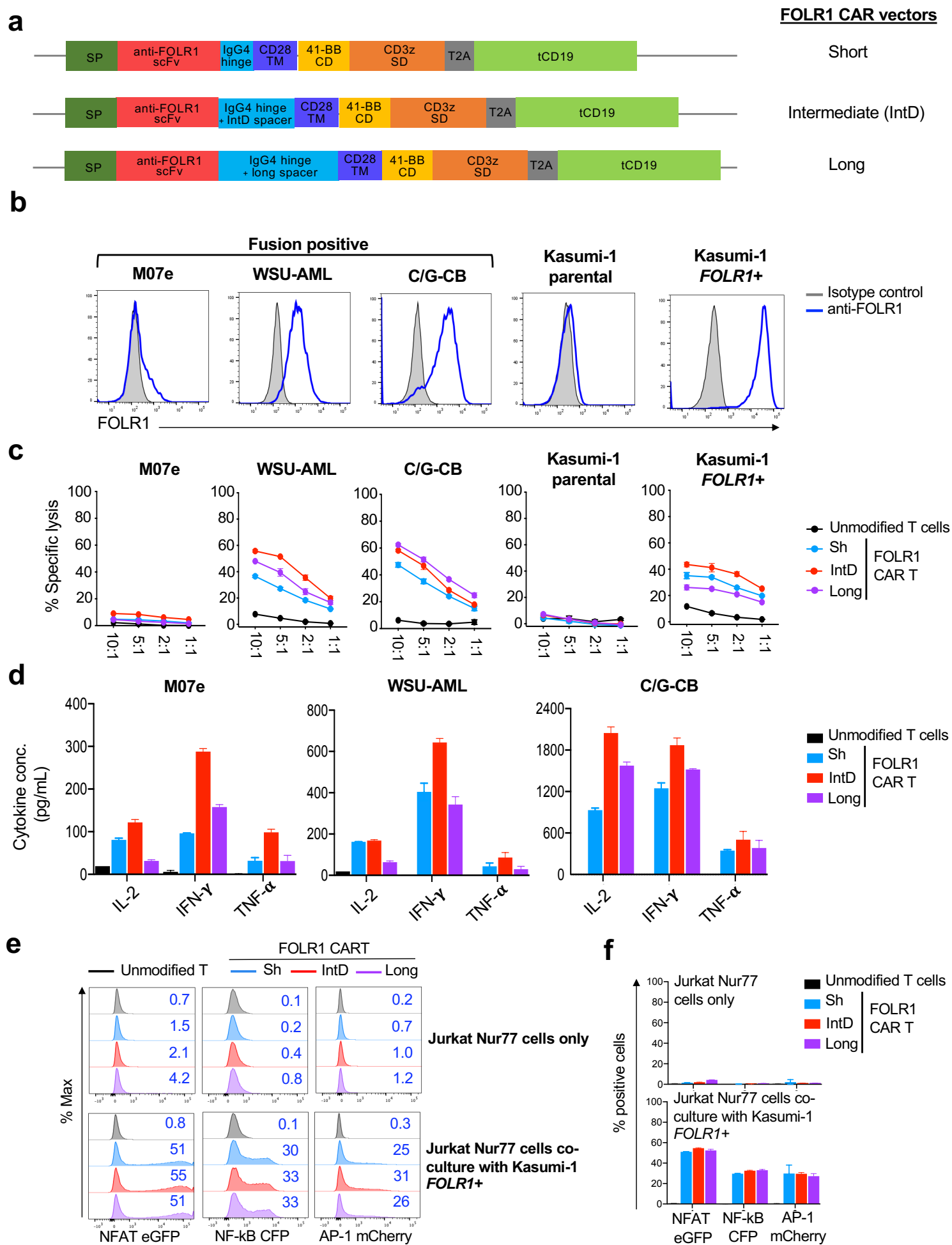
Supplemental Figure 1. C/G-CB cells form tight clusters in mouse bone marrow. (Related to Fig. 1) H&E stains of femurs taken from primary, secondary and tertiary transplants of C/G-CB cells. Magnification: 2.5X(left), 10X (middle), 40X (right).



Supplemental Figure 2. Expression of *FOLR1* transcript in C/G-CB cells cultured on ECs. RT-PCR analysis of *FOLR1* expression in engineered CB cells and in fusion positive cell lines M07e and WSU-AML. Expression is normalized as fold-change relative to GFP-CB/EC Wk 3 samples.



Supplemental Figure 3. Expression of C/G transcript in C/G-CB cells. RT-PCR analysis of C/G expression in engineered CB cells and in fusion positive cell lines M07e and WSU-AML.



Supplemental Figure 3.

Supplemental Figure 4. FOLR1 CAR constructs and reactivity of short, intermediate and long FOLR1 CAR T cells. **a.** Schematic diagram of second-generation FOLR1 CAR constructs with different IgG4 spacer lengths. SP= GM-CSFR signal peptide; scFv= single-chain variable fragment; TM = transmembrane domain; CD = costimulatory domain; SD = stimulatory domain; tCD19 = transduced marker truncated CD19. **b.** Expression of FOLR1 in C/G-CB, M07e, WSU-AML, Kasumi-1 *FOLR1*⁺ and Kasumi-1 parental cells. Blue= stained with PE-labeled anti-FOLR1. Grey= Isotype control. **c.** Cytolytic activity of CD8 T cells unmodified or transduced with short, intermediate or long FOLR1 CAR construct against C/G-CB, M07e, WSU-AML, Kasumi-1 *FOLR1*⁺ and Kasumi-1 parental cells in a 6-hour assay. Shown is mean percent specific lysis +/- SD from 3 technical replicates at indicated Effector:Target (E:T) ratios. **d.** Concentration of secreted IL-2, IFN- γ , and TNF- α in the supernatant following 24 hour of CD8 T cell/AML co-culture at 1:1 E:T ratio. Mean +/- SD from 3 technical replicates is shown. **e.** Representative flow plots showing expression of NFAT, NF-kB and AP-1 in Jurkat Nur77 reporter transduced with FOLR1 CAR constructs cultured alone (top) or co-incubated with Kasumi-1 *FOLR1*⁺ target cells for 24 hours at 1:1 E:T ratio (bottom). Kasumi-1 *FOLR1*⁺ cells were labeled with Violet Cell Proliferation Dye to differentiate from Jurkat cells. Transduced Jurkat cells were gated based on tCD19 expression. Number in top right corner indicates the percentage of positive cells. Analysis was performed on day 4 post transduction. **f.** Quantification of percent NFAT⁺, NF-kB⁺ and AP-1⁺ cells in e.