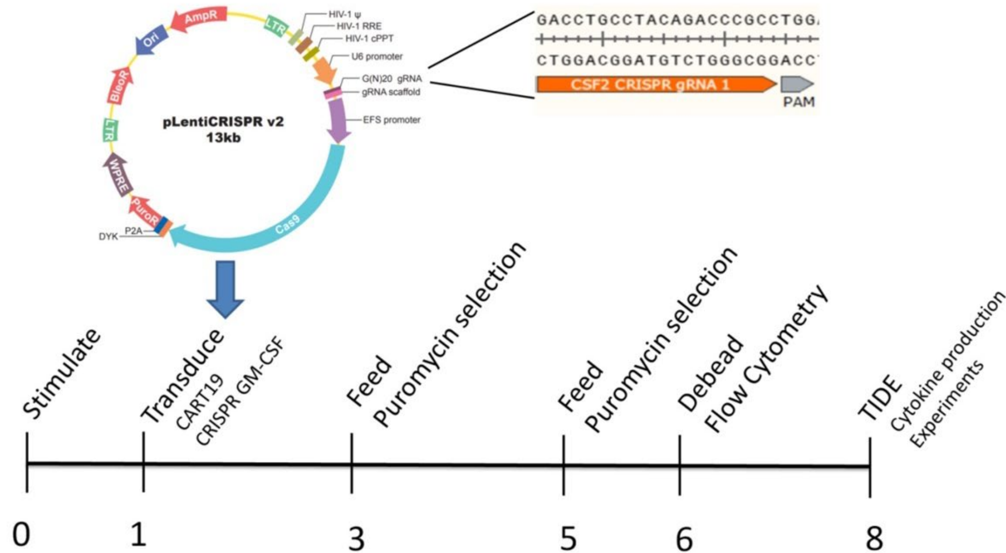


Fig. S1. Generation of GM-CSF^{KO} CART19 cells via lentiviral transduction of T cells. Schema of experimental design in which GM-CSF^{KO} CART19 cells were generated via transduction of a lentiviral vector carrying Cas9 and gRNA under the control of a U6 promoter.

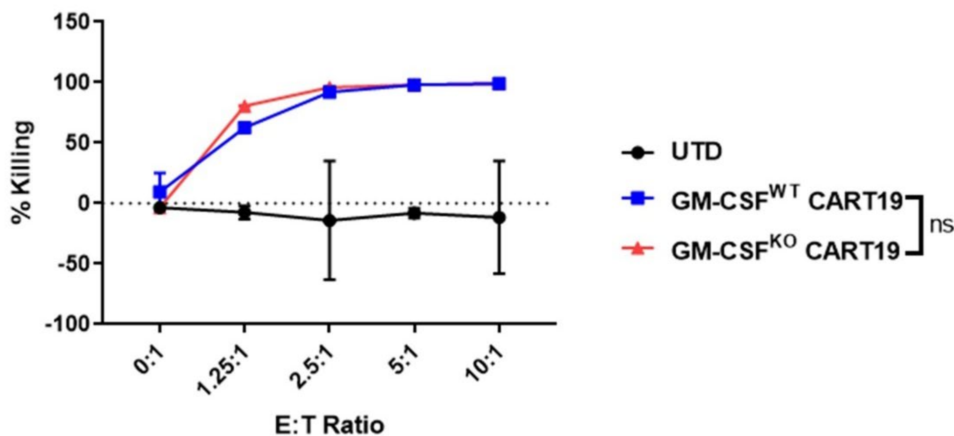
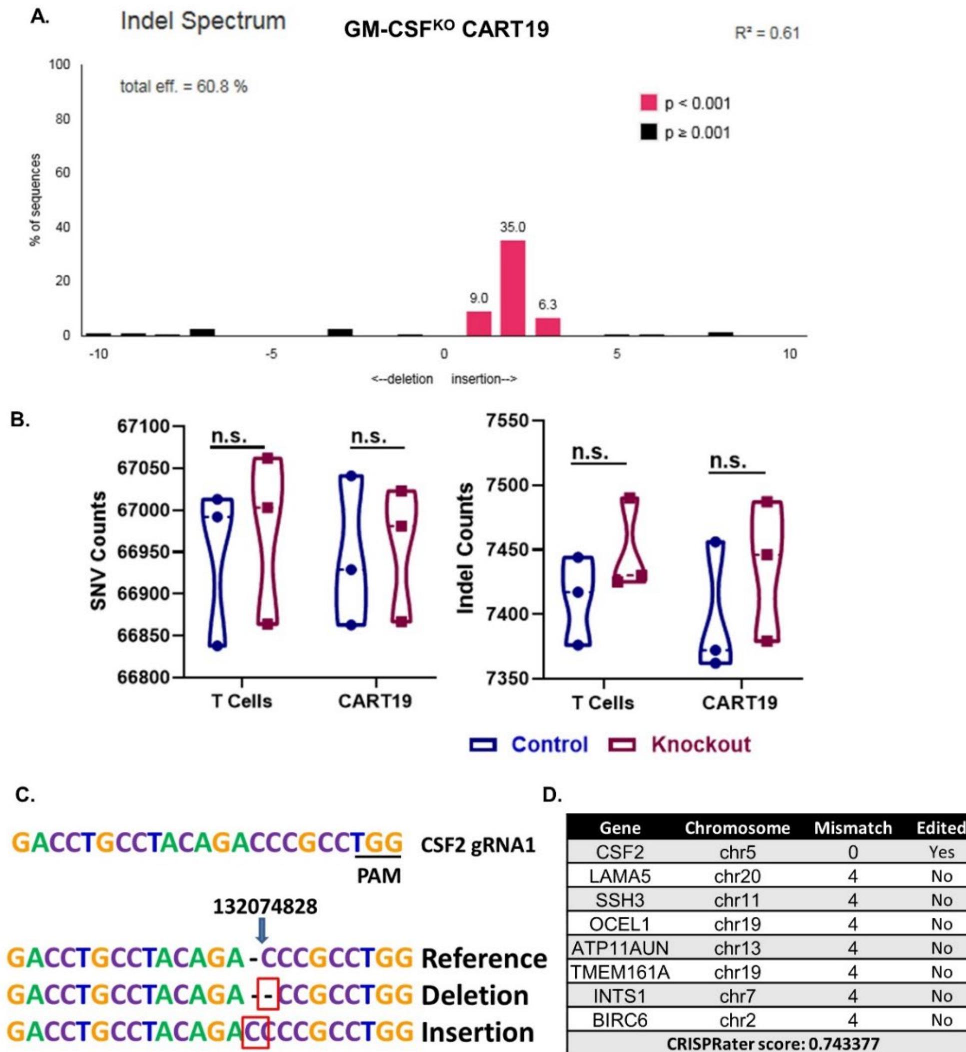


Fig. S2. GM-CSF^{KO} CART19 or GM-CSF^{WT} CART19 cells do not show a difference in killing. Untransduced T cells, GM-CSF^{KO} CART19, and GM-CSF^{WT} CART19 were co-cultured with CD19^{+} Nalm6 at different effector:target ratios (two-way ANOVA; ns = not significant).



Gene	Chromosome	Mismatch	Edited
CSF2	chr5	0	Yes
LAMA5	chr20	4	No
SSH3	chr11	4	No
OCEL1	chr19	4	No
ATP11AUN	chr13	4	No
TMEM161A	chr19	4	No
INTS1	chr7	4	No
BIRC6	chr2	4	No
CRISPRater score: 0.743377			

Fig. S3. CRISPR/Cas9 editing of GM-CSF in CART cells is precise and specific. (A) Representative TIDE sequence to verify allelic modification frequency in GM-CSF^{KO} CART19. DNA was isolated from GM-CSF^{KO} CART19 cells followed by PCR and Sanger sequencing of the resulting PCR product. Sequencing results were run through TIDE software in order to calculate efficacy of knockout based on number of insertions and deletions on the targeted gene region. **(B) There is not a significant difference in number of single nucleotide variants (SNVs) or insertions and deletions (indels) between GM-CSF^{KO} conditions and controls.** Analysis of single nucleotide polymorphisms by whole exome sequencing on three biological replicates (Wilcoxon signed rank test; n.s. = not significant; 3 biological replicates). **(C) CSF2 gene-specific editing is precise.** Insertion or deletion of cytosine at base pair 132074828 is the only SNV or indel identified in chromosome 5 (CSF2, exon 3) on three biological replicates. **(D) Potential off targets predicted by available tools are not edited.** CCTop predicted targets in exonic regions. Only edit found in our dataset is CSF2 (CRISPRater score: 0.743377).

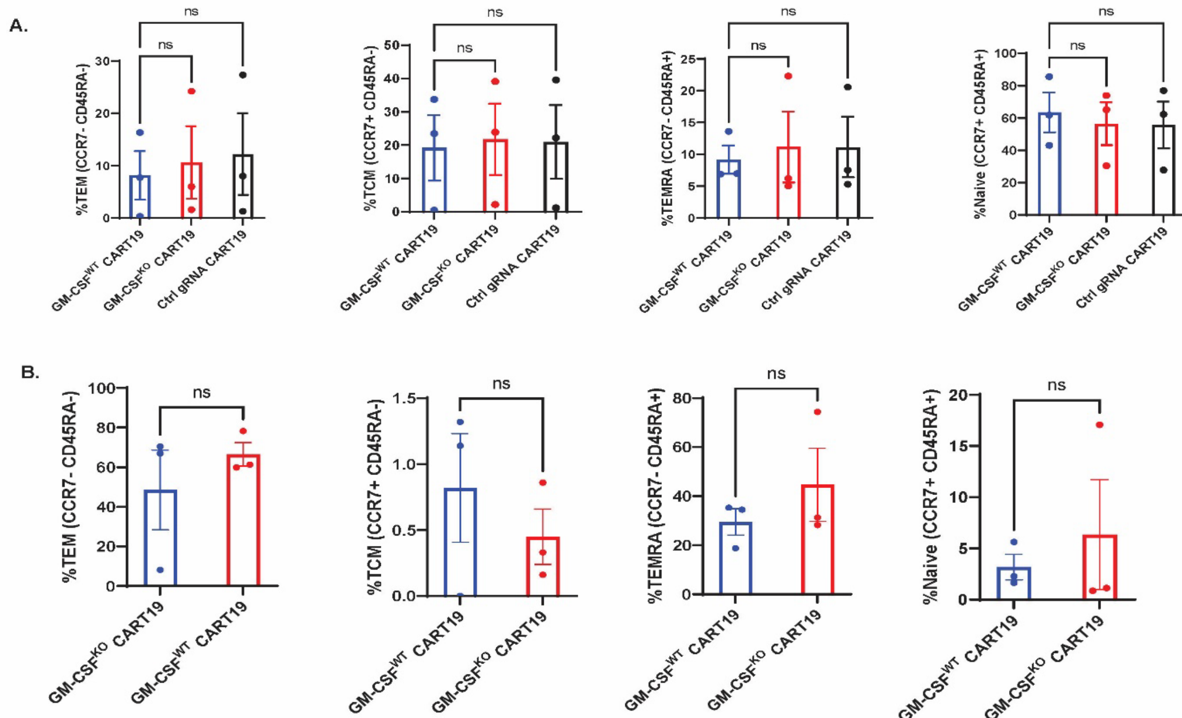


Fig. S4. Memory phenotype of GM-CSF^{KO} CART19 is not altered at early timepoints *in vitro* or later timepoints *in vivo*. (A) Memory phenotype is not altered by GM-CSF disruption *in vitro*. CCR7 and CD45RA is measured using flow cytometry on GM-CSF^{WT} CART19, GM-CSF^{KO} CART19, and non-targeting control gRNA CART19 at the end of manufacturing (one-way ANOVA; ns = not significant). **(B) Memory phenotype is not altered by GM-CSF disruption *in vivo*.** CCR7 and CD45RA is measured using flow cytometry on the harvested splenic cells from mice treated with GM-CSF^{WT} CART19 or GM-CSF^{KO} CART19 (t-test; ns = not significant).

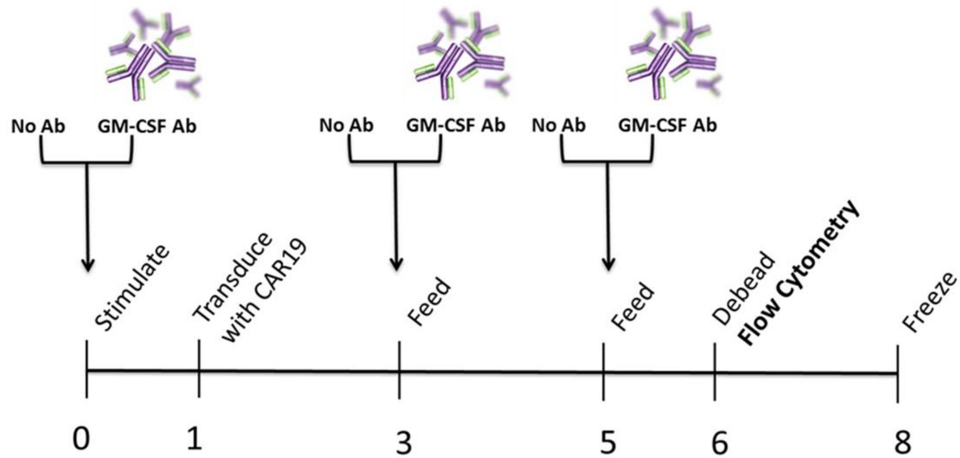


Fig. S5. Generation of CART19 cells in the presence or absence of GM-CSF blocking antibody. Schema of CART19 cells production in the presence of GM-CSF blocking antibody (administered on days 0, 3 and 5 of CART19 production).

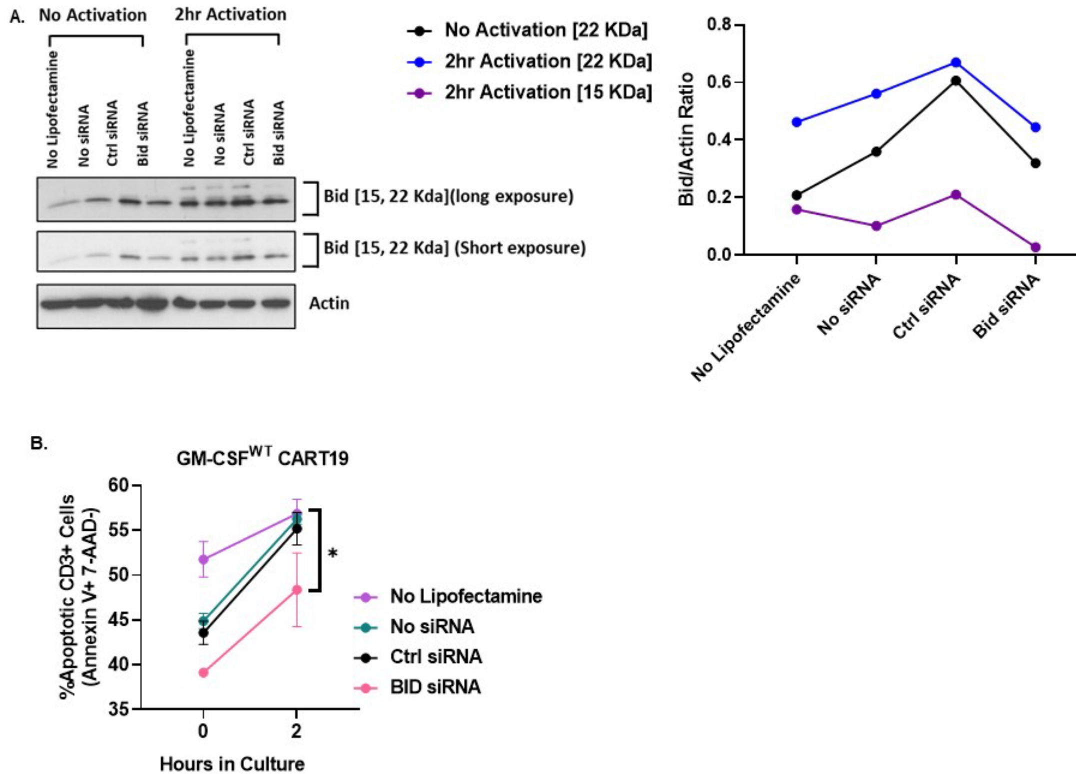


Fig. S6. Depletion of Bid via siRNA results in reduced apoptosis on CART19 cells. (A) Confirmation of Bid knockdown with siRNA. Western blot was performed on the following CART19 conditions: No lipofectamine, No siRNA, Ctrl siRNA, and Bid siRNA. These CART19 cells were measured at baseline and after they were activated with $CD19^+$ antigen (irradiated Nalm6) for 2 hours prior to harvesting for western blot. Western blot is depicted (left) as well as the band quantification (right). **(B) Bid blockade with siRNA on CART19 cells results in reduced apoptosis.** CART19 stimulated by CD3/CD28 beads were cultured in the presence of an Opti-MEM and lipofectamine master mix that had either BID siRNA, control siRNA, or no siRNA. After 24 hours, CART19 cells were de-beaded and co-cultured with irradiated $CD19^+$ Nalm6 cells. Apoptosis was measured via flow cytometric staining of Annexin V, 7-AAD, and CD3 at 0hr and 2hr (two-way ANOVA; * $p<0.05$;1 biological replicate, 2 technical replicates).