

Supplementary Materials for
**TET2 regulates early and late transitions in exhausted CD8⁺ T cell
differentiation and limits CAR T cell function**

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Sci. Adv. **10**, eadp9371 (2024)
DOI: 10.1126/sciadv.adp9371

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Figs. S1 to S6
Tables S1, S2, S6 and S7
Legends for tables S3 to S5

Other Supplementary Material for this manuscript includes the following:

Tables S3 to S5

Supplemental Figure 1

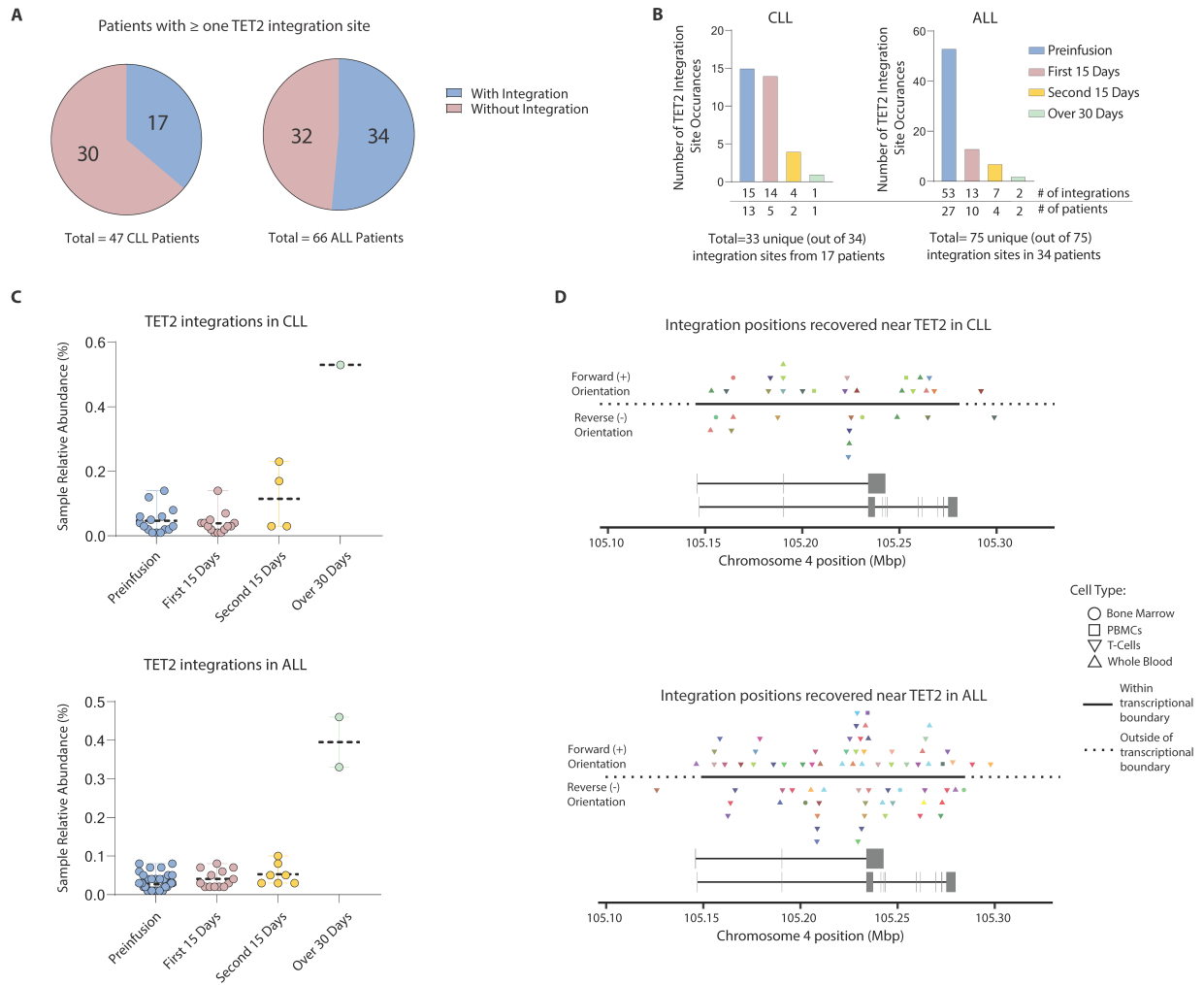


Fig. S1. The TET2 locus is a frequent site of lentivirus integration in CAR T-cell treated patients.

(A) Number of chronic lymphocytic leukemia (CLL) and acute lymphocytic leukemia (ALL) patients with or without \geq one observed CAR lentiviral integration into *TET2*. (B) Distribution of the number of *TET2* integration sites at specified binned timepoints. For CLL patient p04409-09, the same integration was detected within the first 15 days and again in the second 15 days. (C) Scatter plots showing the relative abundance of each *TET2* patient integration at specified timepoints for CLL (top) and ALL (bottom) patient cohorts. Each dot represents one integration site. Dotted line represents mean, error bars represent SEM. (D) Location of each integration clone within the *TET2* transcriptional boundary for CLL (top) and ALL (bottom) patient cohorts. Color coded for patient; symbol shape represents cell type profiled.

Supplemental Figure 2

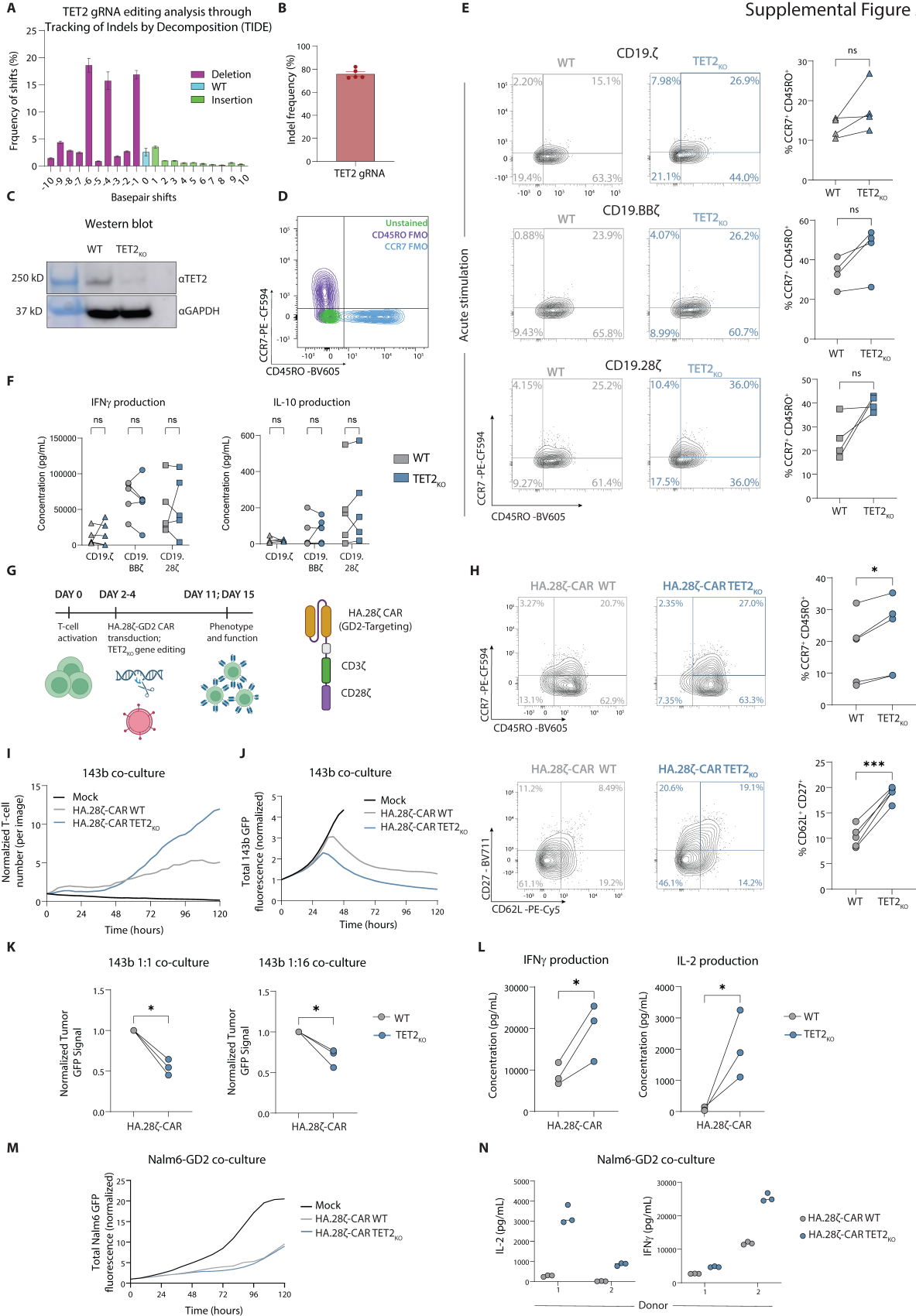


Fig. S2. Validation of TET2KO and function of TET2-disrupted CAR T-cell during acute stimulation and in a tonic CAR signaling model.

(A-B) Frequency of base pair shifts after CRISPR knockout by Tracking of Indels by Decomposition (TIDE) (A) and average indel frequency (B), $n = 5$. (C) Western blot against TET2 in WT and *TET2_{KO}* CAR T-cells. (D) Unstained, fluorescent minus one (FMO) and full stain overlays for CD45RO and CCR7 from figure 1B. (E) Example plots and data of CD19.CD3 ζ , CD19.BB ζ , and CD19.CD28 ζ CAR T-cells \pm *TET2_{KO}* distribution of CCR7⁺ CD45RO⁺ central memory-associated markers in CD8⁺ populations after 1 stimulation (acute stimulation), $n = 4$. (F) IFN γ and IL-10 production from supernatant 24 hours after 5th stimulation, $n = 5$. (G) GD2-targeting HA.28 ζ CAR T-cell manufacturing and *TET2* editing schematic. (H) Example plots and data of CD8⁺ CCR7⁺CD45RO⁺ (**top**) and CD27⁺CD62L⁺ (**bottom**) HA.28 ζ CAR T-cell subsets \pm *TET2_{KO}* at day 11, $n = 5$. (I) HA.28 ζ CAR T-cell \pm *TET2_{KO}* growth in 143b-GL osteosarcoma tumor cell co-culture. (J) Tumor GFP florescence after HA.28 ζ CAR T-cell \pm *TET2_{KO}* co-culture with 143b-GL cells, run in triplicate. (K) Normalized tumor GFP signal from 1:1 and 1:16 E:T co-culture with 143b-GL cells, $n = 3$. (L) IFN γ and IL-2 production after HA.28 ζ CAR T-cell \pm *TET2_{KO}* co-culture with 143b-GL cells, $n = 3$. (M) Tumor GFP florescence after HA.28 ζ CAR T-cell \pm *TET2_{KO}* co-culture against Nalm6-GD2⁺ cells, run in triplicate. (N) Cytokine production from HA.28 ζ CAR T \pm *TET2_{KO}* against Nalm6-GD2⁺ cells, $n = 2$, run in triplicate. Data shown as mean \pm SEM (A-B) or individual values (E, F, H, K, L) from independent donors or as mean of technical replicates (I, J, M). ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by paired t-test. Schematic (G) created with BioRender.com.

Supplemental Figure 3

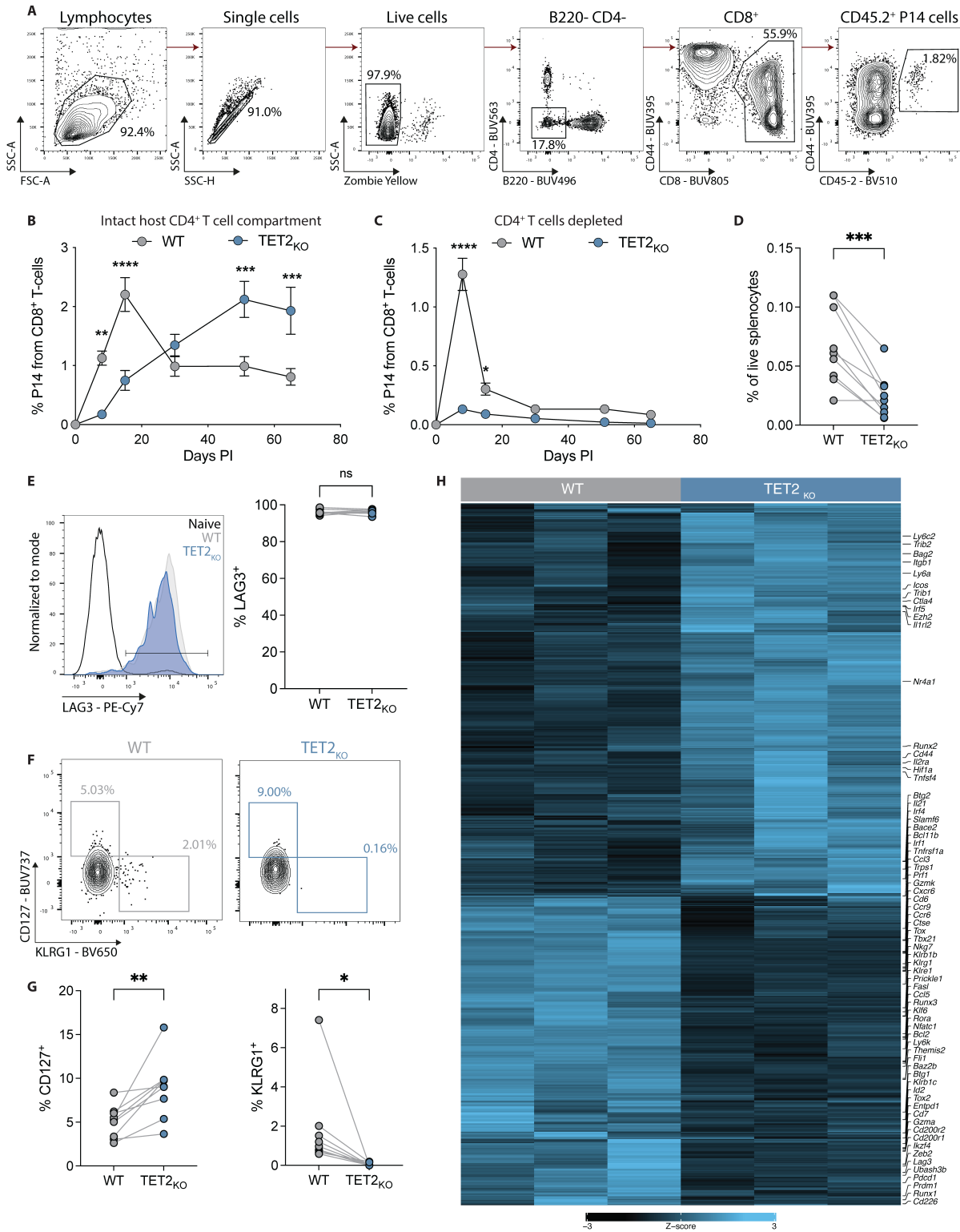


Fig. S3. TET2 regulates differentiation of exhausted CD8+ T-cells.

(A) Gating strategy for identification of co-transferred WT and *TET2_{KO}* P14 cells. (B) Frequency of WT and *TET2_{KO}* P14 cells in blood during chronic LCMV infection without CD4⁺ T-cell depletion. n = 15, mean ± SEM shown. Representative of 2 experiments. (C) Frequency of WT and *TET2_{KO}* P14 cells in blood during chronic LCMV infection. Mice were treated at day -1 and day +1 with CD4-depleting antibody GK1.5, n = 8-10, mean ± SEM shown. Representative of at least 4 experiments. (D) Frequency of WT and *TET2_{KO}* P14 cells in spleen. (E) Example plot and data showing LAG3 expression on WT compared to *TET2_{KO}* P14 cells. (F-G) Example plots (F) and data (G) comparing KLRG1⁺ T_{EFF} and CD127⁺ T_{MEM} frequencies within WT and *TET2_{KO}* P14 cells. (H) Heatmap comparing genes differentially expressed between WT and *TET2_{KO}* P14 cells at day 15 p.i. with LCMV clone 13. (B, C) * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001 by mixed-effects model with Šídák's multiple comparisons test. (D, E, F) n = 9, spleen at day 30 p.i. with LCMV clone 13. Data for individual mice shown; representative of at least 4 experiments. ns p > 0.05; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001 by paired t-test.

Supplemental Figure 4

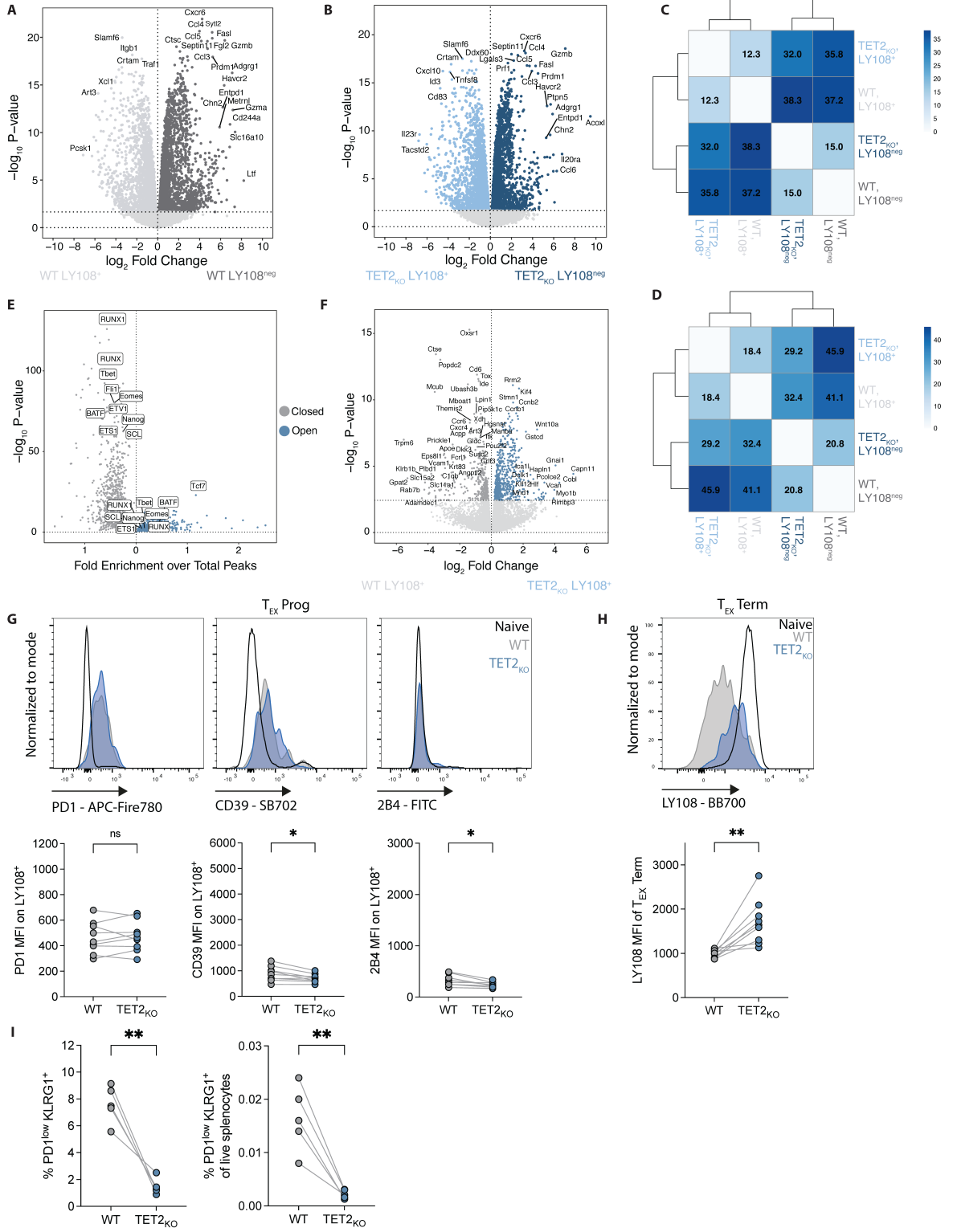


Fig. S4. TET2 loss alters terminal TEX differentiation.

(A-B) Volcano plot highlighting DEGs between LY108⁺ and LY108^{neg} WT P14 cells (A) or LY108⁺ and LY108^{neg} *TET2*_{KO} P14 cells (B). Dotted line at log₁₀ P-value 0.05. (C-D) Distance analysis for RNA-seq (C) and ATAC-seq (D) data comparing T_{EX} subsets between WT and *TET2*_{KO} P14 cells. (E) Volcano plot showing changes in transcription factor accessibility in WT LY108^{neg} cells versus *TET2*_{KO} LY108^{neg} cells. x-axis represents fold enrichment over total peaks; y-axis represents -log₁₀ P-value. (F) Volcano plot highlighting DEG in WT compared to *TET2*_{KO} LY108⁺ T_{EX} cells. (G) Example plots and data comparing expression of PD1, CD39 and 2B4 on *TET2*_{KO} LY108⁺ T_{EX} cells to WT LY108⁺ T_{EX} cells. (H) Example plots and data comparing expression of LY108 on *TET2*_{KO} to WT terminally exhausted T_{EX}. (I) Proportion and absolute frequency of PD1^{low} KLRG1⁺ T_{EFF}-like WT and *TET2*_{KO} P14 cells in spleen at day 6 p.i. with LCMV clone 13, n = 5. (G-H) n = 9, spleen at day 30 p.i. with LCMV clone 13. (G, H, I) Data for individual mice shown; representative of 2-3 independent experiments. * p < 0.05; ** p < 0.01 by paired t-test.

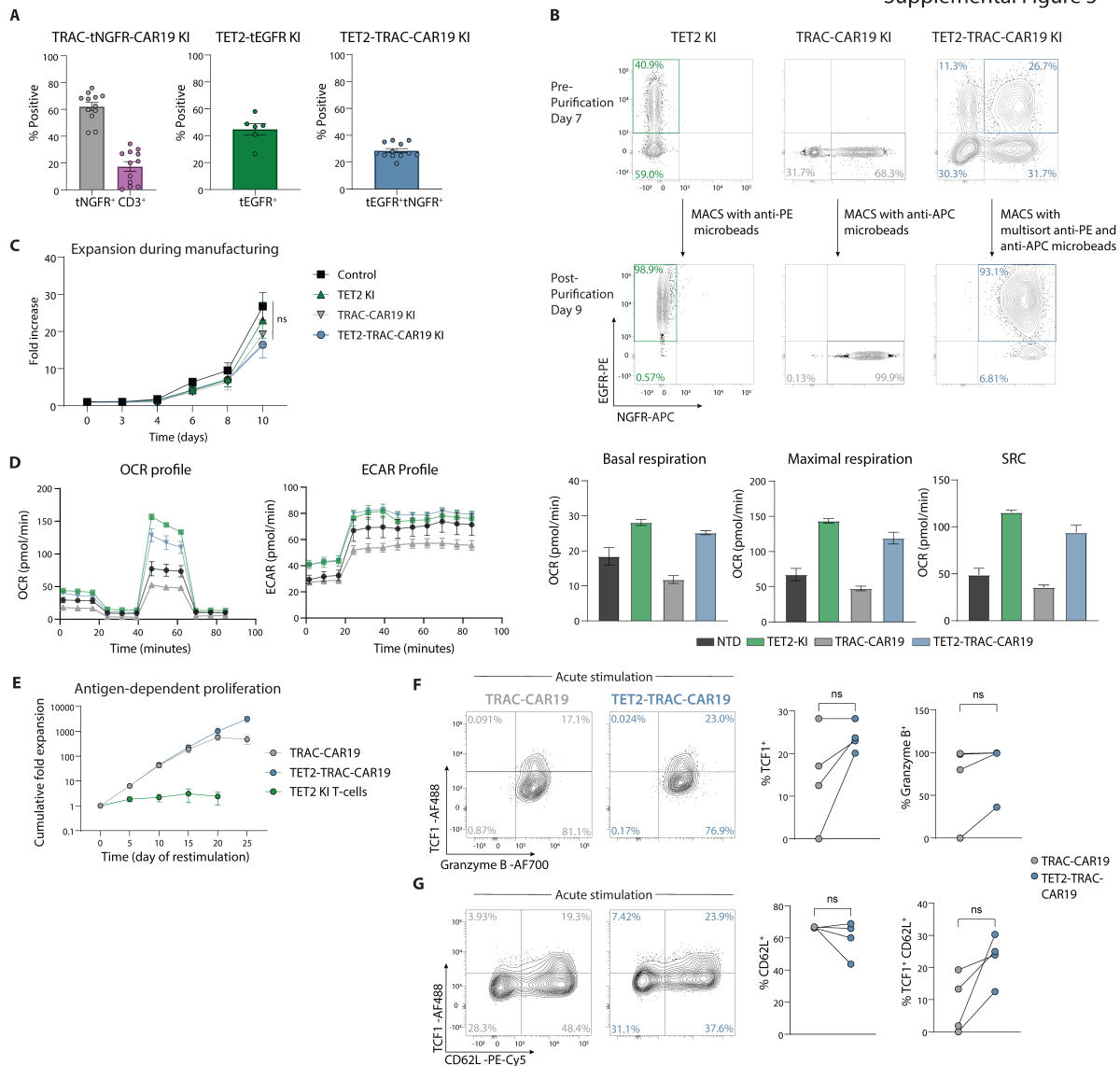


Fig. S5. Engineering of TET2-TRAC-CAR19 dual knock-in T-cells.

(A) Summary of TRAC-tNGFR knock-in (KI), CD3 knock-out (KO), TET2-tEGFR KI and TET2-TRAC-CAR19 KI editing efficiencies at day 7 during expansion, $n = 6 - 12$. (B) Example plots showing TET2-KI, TRAC-CAR19-KI and TET2-TRAC-CAR19 KI cells pre- (day 7) and post-purification (day 9). (C) T-cell expansion during manufacturing and CRISPR-AAV editing; mean \pm SEM shown, $n = 4$, ns $p > 0.05$ by one-way ANOVA test with a post hoc Tukey's multiple comparison test. (D) OCR and ECAR, basal respiration, maximal respiration and SRC profiles of NTD, TET2-KI, TRAC-CAR19 and TET2-TRAC-CAR19 T-cells at the end of manufacturing, $n = 1$, run in triplicate. (E) Antigen-dependent proliferation of TET2-TRAC-CAR T-cells compared to TET2-KI T-cells, $n = 5$. (F) Example plots and data of TCF1 and granzyme B expression on $CD8^+$ CAR T-cells after 1 stimulation (acute stimulation), $n = 4$. (G) Example plots and data of $CD8^+$ TCF1 and CD62L expression on $CD8^+$ CAR T-cells after 1 stimulation (acute stimulation), $n = 4$. Data shown as mean \pm SEM (C, E) or individual values (A, F, G) from independent donors or as mean \pm SEM of technical replicates (D). (F, G) ns $p > 0.05$ by paired t-test.

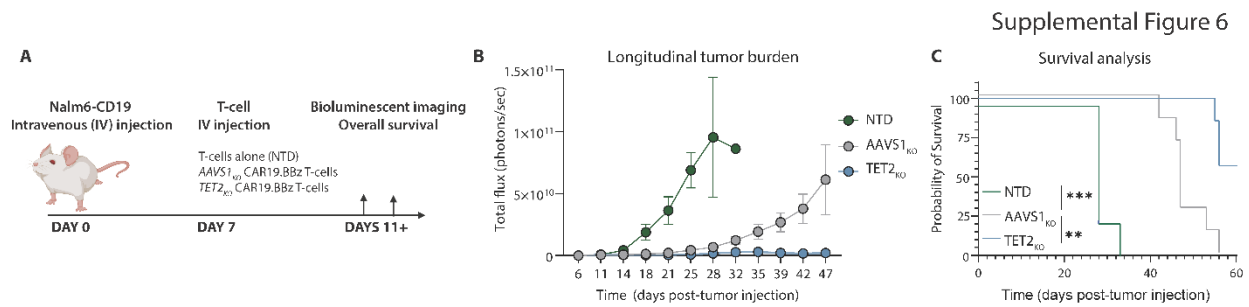


Fig. S6. In Vivo tumor response of *TET2*_{KO} CAR T-cells.

(A) Overview of *in vivo* experimental design, n = 4-7 mice per experimental group; data representative of one experiment. (B) Longitudinal analysis of AAVS1_{KO} and TET2_{KO} CAR T-cell group tumor burden compared to mice receiving non-transduced (NTD) T-cells. Data shown as mean ± SEM. (C) Overall group survival, ** p < 0.01; *** p < 0.001 by Mantel-Cox log-rank test for survival analysis. Schematic (A) created with BioRender.com.

Patient	Cell Type	Time Point	Genomic Position	Relative Abundance
p03712-12	T-cells	Preinfusion	chr4-105264619	0.05
p03712-16	T-cells	Preinfusion	chr4-105225145	0.12
p03712-29	T-cells	Preinfusion	chr4+105291992	0.03
p03712-31	T-cells	Preinfusion	chr4+105183562	0.01
p03712-40	T-cells	Preinfusion	chr4-105163547	0.01
p03712-40	T-cells	Preinfusion	chr4+105182603	0.01
p03712-43	T-cells	Preinfusion	chr4+105190119	0.02
p03712-48	T-cells	Preinfusion	chr4+105223086	0.02
p03712-55	T-cells	Preinfusion	chr4+105221969	0.04
p03712-57	T-cells	Preinfusion	chr4-105187373	0.06
p03712-60	T-cells	Preinfusion	chr4-105223721	0.06
p03712-60	T-cells	Preinfusion	chr4+105265365	0.03
p04409-06	T-cells	Preinfusion	chr4-105224248	0.02
p04409-07	T-cells	Preinfusion	chr4+105267927	0.08
p04409-09	PBMC	Preinfusion	chr4+105253503	0.14
p03712-03	T-cells	First 15 Days	chr4+105160930	0.01
p03712-03	T-cells	First 15 Days	chr4+105200164	0.01
p03712-03	T-cells	First 15 Days	chr4-105298816	0.01
p03712-18	Whole Blood	First 15 Days	chr4-105248829	0.04
p03712-18	Whole Blood	First 15 Days	chr4+105153241	0.07
p03712-18	Whole Blood	First 15 Days	chr4+105251281	0.04
p03712-18	Whole Blood	First 15 Days	chr4+105260695	0.04
p03712-18	Whole Blood	First 15 Days	chr4-105224201	0.03
p03712-29	Whole Blood	First 15 Days	chr4+105228115	0.14
p04409-09*	T-cells	First 15 Days	chr4+105190185	0.02
p04409-09	T-cells	First 15 Days	chr4+105257004	0.02
p04409-22	Whole Blood	First 15 Days	chr4-105152755	0.03
p04409-22	Whole Blood	First 15 Days	chr4-105164454	0.03
p04409-22	Whole Blood	First 15 Days	chr4+105263836	0.05
p04409-09	PBMC	Second 15 Days	chr4+105206047	0.23
p04409-09*	Whole Blood	Second 15 Days	chr4+105190185	0.03
p04409-09	Bone Marrow	Second 15 Days	chr4-105230899	0.03
p04409-22	Bone Marrow	Second 15 Days	chr4+105164450	0.17
p03712-47	Bone Marrow	>30 Days	chr4-105155581	0.53

Table S1. Recovered Integrations within 50KB of the *TET2* locus in CLL patient cohort (NCT01029366).

Patient	Cell Type	Time Point	Genomic Position	Relative Abundance
p959-101	T-cells	Preinfusion	chr4-105122207	0.02
p959-103	T-cells	Preinfusion	chr4-105268362	0.04
p959-103	T-cells	Preinfusion	chr4+105197328	0.08
p959-104	T-cells	Preinfusion	chr4-105205781	0.05
p959-104	T-cells	Preinfusion	chr4+105165499	0.05
p959-107	T-cells	Preinfusion	chr4-105229630	0.01
p959-107	T-cells	Preinfusion	chr4+105151872	0.01
p959-108	T-cells	Preinfusion	chr4-105243661	0.06
p959-111	T-cells	Preinfusion	chr4+105219760	0.02
p959-112	T-cells	Preinfusion	chr4-105226123	0.02
p959-112	T-cells	Preinfusion	chr4+105151786	0.02
p959-112	T-cells	Preinfusion	chr4+105236100	0.04
p959-113	T-cells	Preinfusion	chr4-105158827	0.01
p959-113	T-cells	Preinfusion	chr4+105221551	0.01
p959-113	T-cells	Preinfusion	chr4+105271906	0.01
p959-115	T-cells	Preinfusion	chr4-105229150	0.02
p959-117	T-cells	Preinfusion	chr4+105173039	0.03
p959-118	T-cells	Preinfusion	chr4-105162564	0.01
p959-118	T-cells	Preinfusion	chr4-105204835	0.01
p959-118	T-cells	Preinfusion	chr4-105227631	0.01
p959-118	T-cells	Preinfusion	chr4-105241238	0.01
p959-118	T-cells	Preinfusion	chr4+105182278	0.01
p959-118	T-cells	Preinfusion	chr4+105224901	0.01
p959-121	T-cells	Preinfusion	chr4-105204911	0.04
p959-123	T-cells	Preinfusion	chr4-105239813	0.01
p959-123	T-cells	Preinfusion	chr4+105171995	0.04
p959-123	T-cells	Preinfusion	chr4+105224760	0.03
p959-123	T-cells	Preinfusion	chr4+105243003	0.01
p959-125	T-cells	Preinfusion	chr4-105231290	0.02
p959-131	T-cells	Preinfusion	chr4-105225782	0.03
p959-131	T-cells	Preinfusion	chr4+105187755	0.03
p959-132	T-cells	Preinfusion	chr4+105250821	0.07
p959-132	T-cells	Preinfusion	chr4+105260601	0.07
p959-133	T-cells	Preinfusion	chr4+105256735	0.02
p959-133	T-cells	Preinfusion	chr4-105260182	0.02
p959-136	T-cells	Preinfusion	chr4-105186871	0.03
p959-136	T-cells	Preinfusion	chr4+105157216	0.03
p959-136	T-cells	Preinfusion	chr4+105203882	0.03
p959-141	T-cells	Preinfusion	chr4+105228898	0.03
p959-141	T-cells	Preinfusion	chr4+105294015	0.03
p959-144	T-cells	Preinfusion	chr4+105175287	0.02
p959-144	T-cells	Preinfusion	chr4+105202132	0.02
p959-146	T-cells	Preinfusion	chr4+105154691	0.02
p959-157	T-cells	Preinfusion	chr4+105243230	0.03
p959-158	T-cells	Preinfusion	chr4-105160134	0.01
p959-158	T-cells	Preinfusion	chr4+105227059	0.01
p959-169	T-cells	Preinfusion	chr4+105225389	0.04
p959-172	T-cells	Preinfusion	chr4+105273807	0.05
p959-174	T-cells	Preinfusion	chr4-105204656	0.08
p959-158	T-cells	Preinfusion	chr4-105191937	0.01
p959-158	T-cells	Preinfusion	chr4-105257743	0.01

p959-158	T-cells	Preinfusion	chr4-105271578	0.01
p959-158	T-cells	Preinfusion	chr4+105284604	0.01
p959-101	Whole Blood	First 15 Days	chr4+105206388	0.05
p959-105	Whole Blood	First 15 Days	chr4+105231121	0.06
p959-113	PBMC	First 15 Days	chr4+105230717	0.02
p959-121	Whole Blood	First 15 Days	chr4+105223180	0.08
p959-125	Whole Blood	First 15 Days	chr4+105229646	0.02
p959-125	Whole Blood	First 15 Days	chr4+105258957	0.02
p959-139	Whole Blood	First 15 Days	chr4-105275946	0.07
p959-139	Whole Blood	First 15 Days	chr4+105142570	0.03
p959-140	Whole Blood	First 15 Days	chr4-105269087	0.03
p959-141	Whole Blood	First 15 Days	chr4-105201535	0.07
p959-146	Whole Blood	First 15 Days	chr4-105185661	0.04
p959-160	Whole Blood	First 15 Days	chr4+105217644	0.02
p959-160	Whole Blood	First 15 Days	chr4+105262568	0.02
p04409-29	PBMC	Second 15 Days	chr4+105269578	0.08
p959-143	Whole Blood	Second 15 Days	chr4-105259704	0.05
p959-153	Bone Marrow	Second 15 Days	chr4-105280290	0.10
p959-160	Whole Blood	Second 15 Days	chr4-105208249	0.03
p959-160	Whole Blood	Second 15 Days	chr4+105227846	0.03
p959-160	Whole Blood	Second 15 Days	chr4+105262098	0.03
p959-160	Bone Marrow	Second 15 Days	chr4-105247460	0.05
p959-100	Bone Marrow	>30 Days	chr4-105198703	0.33
p959-160	Whole Blood	>30 Days	chr4-105238384	0.46

Table S2. Recovered Integrations within 50KB of the *TET2* locus in ALL patient cohort.

Table S3 (separate file)

RNA-sequencing data comparing WT and *TET2_{KO}* P14 CD8⁺ T cells at d15 post infection with LCMV clone13.

Table S4 (separate file)

ATAC-sequencing data comparing WT and *TET2_{KO}* P14 CD8⁺ T cells at d15 post infection with LCMV clone13.

Table S5 (separate file)

RNA-sequencing data comparing WT and *TET2_{KO}* CAR T-cells at d20 of chronic restimulation.

Target	Clone	Fluorochrome	Source	Cat #	RRID
LIVE-DEAD Aqua	NA	NA	Invitrogen	L34957	NA
EGFR	AY13	PE	BioLegend	352904	AB_10896794
CD271 (NGFR)	ME20.4	APC	BioLegend	345108	AB_10645515
CD3	SK7	APC-H7	BD Biosciences	560176	AB_1645475
CD4	OKT4	BV785	BioLegend	317442	AB_2563242
CD8	RPA-T8	BV650	BD Biosciences	563822	AB_2744462
CCR7	150503	PE-CF594	BD Biosciences	562381	AB_11153301
CD45RO	UCHL1	BV605	BD Biosciences	562791	AB_2744411
CD62L	DREG-56	PE-Cy5	BioLegend	304808	AB_314468
CD27	L128	BV711	BD Biosciences	563167	AB_2738042
PD1	EH12.2H7	BV421	BioLegend	329920	AB_10960742
LAG3	7H2C65	PE-Cy7	BioLegend	369208	AB_2629835
TIM3	7D3	PE	BD Biosciences	565570	AB_2716866
KLRG1	SA231A2	APC-Fire750	BioLegend	367718	AB_2687392
CD56	NCAM16.2	BV711	BD Biosciences	563169	AB_2738043
TCF1 (intracellular)	C63D9	Alexa Fluor 488	Cell Signaling Technology	64445	AB_2797627
Granzyme B (intracellular)	GB11	Alexa Fluor 700	BD Biosciences	560213	AB_1645453
F(ab') ₂ fragment specific	NA	Biotin-SP	Jackson ImmunoResearch	115-065-072	AB_2338565
Biotin	NA	Streptavidin-PE	BioLegend	405203	NA
Human IgG	1268C	PE	R&D Systems	F0157	NA

Table S6. Antibodies for CAR T-cell studies.

Target	Clone	Fluorochrome	Source	Cat #	RRID
LiveDead	NA	AquaVivid	ThermoFisher	L34957	NA
LiveDead	NA	Zombie Yellow	BioLegend	423104	NA
LiveDead	NA	Zombie NIR	BioLegend	423106	NA
B220	RA3-6B2	APC-eF780	ThermoFisher	47-0452-82	AB_1518810
B220	RA3-6B2	BUV496	BD Biosciences	612950	AB_2870227
CD4	RM4-5	APC-eF780	ThermoFisher	47-0042-82	AB_1272183
CD4	GK1.5	BUV563	BD Biosciences	612923	AB_2870208
CD8a	53-6.7	BUV805	BD Biosciences	612898	AB_2870186
CD8a	53-6.7	BV650	BD Biosciences	563234	AB_2738084
CD8a	53-6.7	APC	Invitrogen	17-0081-83	AB_469336
CD39	24DMS1	SB702	Invitrogen	67-0391-82	AB_2717143
CD44	IM7	BUV396	BD Biosciences	740215	AB_2739963
CD44	IM7	BV711	BioLegend	103057	AB_2564214
CD44	IM7	BV785	BioLegend	103059	AB_2571953
CD44	IM7	APC	BD Biosciences	559250	AB_398661
CD45.1	A20	Pacific Blue	BioLegend	110722	AB_492866
CD45.1	A20	PE-Cy5	ThermoFisher	15-0453-81	AB_468758
CD45.1	A20	AF700	BioLegend	110724	AB_493733
CD45.2	104	BV570	BioLegend	109833	AB_10900987
CD45.2	104	BV785	BioLegend	109839	AB_2562604
CD45.2	104	AF700	BioLegend	109822	AB_493731
CD62L	MEL-14	BV605	BD Biosciences	563252	AB_2738098
CD62L	MEL-14	PE-CF594	BD Biosciences	562404	AB_11154046
CD69	H1.2F3	BV480	BD Biosciences	746813	AB_2744067
CD127	SB/199	BUV737	BD Biosciences	612841	AB_2870163
CD127	SB/199	PE-CF594	BD Biosciences	562419	AB_11153131
CD244 (2B4)	eBio244F4	FITC	eBioscience	11-2441-85	AB_657877
CD244 (2B4)	2B4	FITC	BD Biosciences	553305	AB_394769
CX3CR1	SA011F11	BV605	BioLegend	149027	AB_2565937
CX3CR1	SA011F11	BV650	BioLegend	149033	AB_2565999
CXCR3	CXCR3-173	Biotin	BioLegend	126503	AB_1027658
GFP	Polyclonal	AF488	ThermoFisher	A-21311	AB_221477
gp33	NA	PE	In house	NA	NA
Granzyme B	GB11	BV421	BD Biosciences	563389	AB_2738175
IFN γ	XMG1.2	PerCP-Cy5.5	BioLegend	505822	AB_961361
KI67	B56	A700	BD Biosciences	561277	AB_10611571
KLRG1	2F1	FITC	eBioscience	11-5893-80	AB_1311268
KLRG1	2F1	PerCP-eF710	Invitrogen	46-5893-82	AB_10670282
KLRG1	2F1	BV650	BD Biosciences	740553	AB_2740254
LAG3	C9B7W	PE-Cy7	BioLegend	125226	AB_2715764
LAG3	C9B7W	AF647	BioRad	MCA2386A647T	AB_2133342
LY108	13G3	BV421	BD Biosciences	740090	AB_2739850
LY108	13G3	BB700	BD Biosciences	742272	AB_2871448
PD-1	RMPI-30	PE-Cy7	BioLegend	109110	AB_572017
PD-1	29F.1A12	APC-Fire780	BioLegend	135239	AB_2629767
TCF-1	S33-966	PE	BD Biosciences	564217	AB_2687845
TIGIT	1G9	BV421	BD Biosciences	565270	AB_2688007
TIGIT	1G9	PE-Dazzle594	BioLegend	142110	AB_2566573
TIM3	RMT3-23	BV605	BioLegend	119721	AB_2616907
TIM3	RMT3-23	BV785	BioLegend	119725	AB_2716066
TNF-alpha	MP6-XT22	Pacific Blue	BioLegend	506318	AB_893639
TOX	REA473	APC	Miltenyi	130-118-335	AB_2751485

Table S7. Antibodies for LCMV studies.