Supplementary Information (SI) Guide:

SI Figures

SI Fig. 1: Kinetics of *BATF3* and *MYC* expression in WT and *TET2*-edited CAR T cells.

a, Differentially accessible genomic regions between $TET2_{bed}$ Rv-1928z+41BBL and WT Rv-1928z+41BBL. Both samples were isolated from mice at day 90. The red dots are peaks with |fold change| >1.5 and (p_{adj}<0.1). **b,** Differential genomic accessibility in *BATF3* and *MYC* regions ([$TET2_{bed}$/WT Rv-1928z+41BBL]; p_{adj}<0.05). p values in **a,b** are adjusted for multiple comparisons by BKY method. **c,** Schematic of *in vitro* repeated rechallenge assay for BATF3 and MYC FACS analysis. **d,** CAR T cells were cultured with (stimulated) or without (unstimulated) irradiated 3T3-CD19. 24 hours later, BATF3 and MYC intracellular FACS was performed on pooled CAR T cells (pooled from 3 wells) for each condition. **e,** Kinetic analysis of *BATF3* (top) and *MYC* (bottom) expression for Rv-1928z+41BBL CAR T cells. Fraction of positive cells is represented in red font; MFI is represented in green font. **f, g,** Day 15 BATF3 (**f**) and MYC (**g**) population fractions for WT and *TET2*-edited Rv-1928z+41BBL CAR T cells. p values in **f,g** were determined through two-sided χ2 test. Exact p values are available in SI Table 4.

SI Fig. 2: JQ1 and dexamethasone treatment inhibit $TET2_{bed}$ CAR T cell proliferation.

a, *BATF3* gRNA targeting site (left panel). Pre-infusion CAR T cell BATF3 flow cytometry quantification. MFI is indicated. **b-d,** Schematics of the cell proliferation assay (**b**). The cells were either treated with DMSO, JQ1 (500nM) or dexamethasone (dexa, 1μm). DMSO normalized cell counts for JQ1 (**c**) and dexa (**d**). p-values were determined by two-sided

unpaired t-test (**c, d**). **e-g,** Schematics of qPCR study (**e**). CAR T cells were treated with DMSO, JQ1 or dexa at the same dose as cell proliferation assay for 12 hours. Transcripts were normalized to *B2M* for each sample. DMSO normalized *BATF3* and *MYC* levels under JQ1 treatment (**f**) and dexa (**g**). Multiple unpaired unpaired t-tests corrected by BKY method (**f, g**). Data in **c, d, f, g** is presented as mean±SD. (n=4). $p < 0.05$ was considered statistically significant. p values are denoted: $p > 0.05$, not significant, NS; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Exact p values are available in SI Table 4.

SI Fig. 3: Effector loci accessibility in long-term persisting WT and *TET2$_{bed}$* CAR T cells.

a-d, Genome accessibility in WT and *TET2$_{bed}$* CAR T cells for *IL2* (**a**), *GZMB* (**b**), *IFNG* (**c**), and *TNF* (**d**). These genome accessibility tracks are representative of n=3.

SI Fig. 4: Flow Cytometry gating strategy.

a, Gating strategy for Extended Data Fig. 1a, 1b, 2a and 2b (left panel). **b**, Gating strategy for Extended Data Fig. 1a, 1b, 2a, 2b (right panel), 1g, 2e and 3a. **c**, Gating strategy for Extended Data Fig. 1i, 2g and 3c. **d**, Gating strategy for Fig. 5f. **e**, Gating strategy for Extended Data Fig. 8l.

SI Table Titles

SI Table 1: Exome analysis of hyper-proliferative *TET2$_{bed}$* CAR T cells.

1a, Translocation analysis in *TET2$_{bed}$* hyper-proliferative CAR T cells. **1b,** Mutation analysis in *TET2$_{bed}$* hyper-proliferative CAR T cells. **1c,** Copy number analysis in *TET2$_{bed}$* hyper-proliferative CAR T cells.

SI Table 2: Retroviral integration site analysis in hyper-proliferative *TET2$_{bed}$* CAR T cells.
2a, Retrovirus integration site analysis for *TET2$_{bed}$* Rv-1928z+41BBL (17-1). **2b,** Retrovirus integration site analysis for *TET2$_{bed}$* Rv-1928z (2-2).
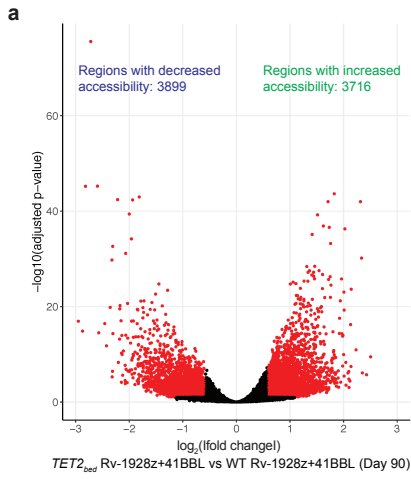
SI Table 3: Replicate information on representative figures.
3a-d, Replicate information for selected panels in Extended Data Fig. 1 (**a**), Extended Data Fig. 2 (**b**), Extended Data Fig. 3 (**c**), and Extended Data Fig. 8 (**d**).
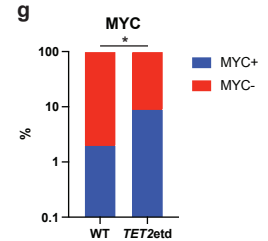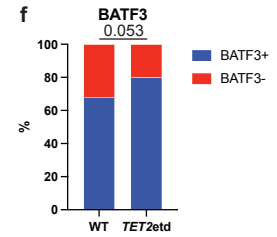
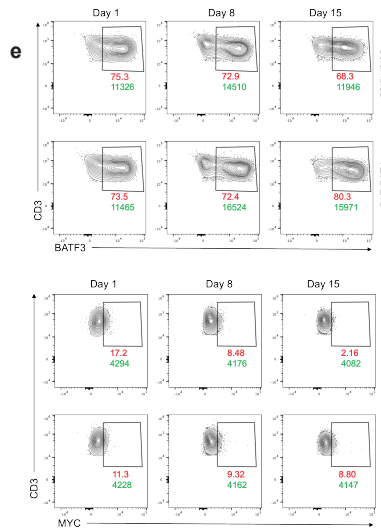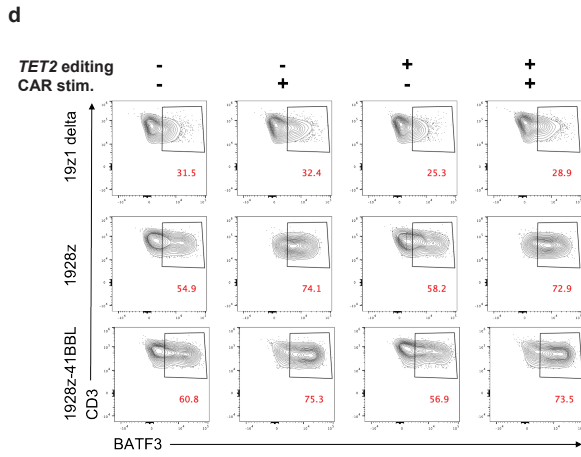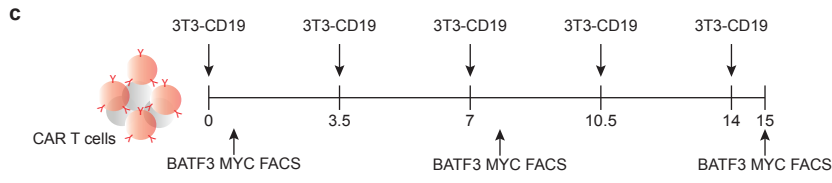**SI Table 4: Exact p values for figures.**
4a-i, Exact p values for selected panels in Fig. 2 (**a**), Fig. 4 (**b**), Fig. 5 (**c**), Extended Data Fig. 1 (**d**), Extended Data Fig. 4 (**e**), Extended Data Fig. 8 (**f**), Extended Data Fig. 10 (**g**), SI Fig. 1 (**h**), SI Fig. 2 (**i**).
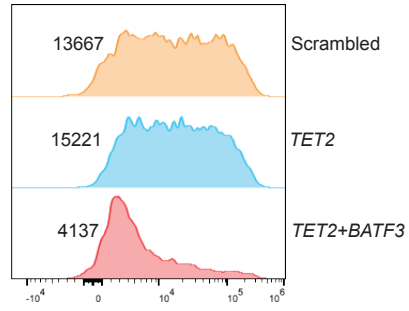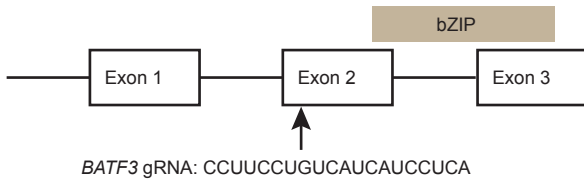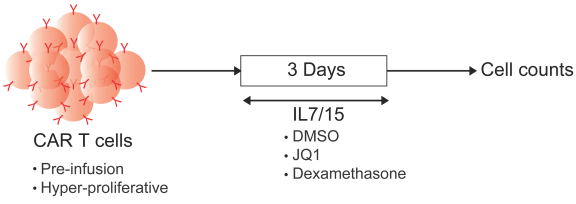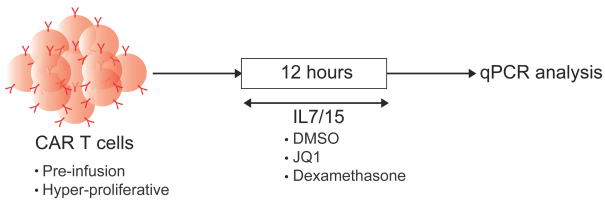
SI Table 5: List of antibodies used in the flow cytometry.

a

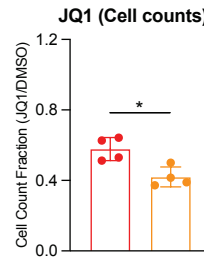Regions with decreased accessibility: 3899

Regions with increased accessibility: 3716

−log10(adjusted p-value)

log$_2$(|fold change|)

$TET2_{bed}$ Rv-1928z+41BBL vs WT Rv-1928z+41BBL (Day 90)

b

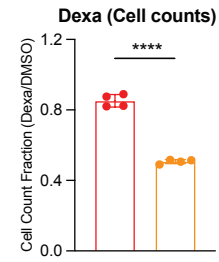| BATF3					
region	baseMean	log2(FoldChange)	lfcSE	padj	distance
Chr1: 212698931-212700763	367.594042	0.779699416	0.118779277	5.15E-09	0
Chr1: 212691505-212692274	76.48321453	1.387572691	0.226312834	6.29E-08	7712
Chr1: 212697149-212697351	16.65639557	1.328313103	0.449082429	0.025786478	2635
MYC					
Chr8: 127733639-127739180	866.4256011	0.654547727	0.108930937	1.24E-07	0
Chr8: 128240088-128240842	127.2572027	0.885809796	0.170965886	8.51E-06	-504655
Chr8: 128298313-128299429	92.70707583	0.855069117	0.201923454	0.000469664	-562880
Chr8: 128070065-128070549	70.30376769	0.76244445	0.218212186	0.005834982	-334632
Chr8: 128540410-128541456	149.6362575	0.592900353	0.171427424	0.006508655	-804977
Chr8: 128076678-128077354	93.32698554	0.679631031	0.205552617	0.010170037	-341245
Chr8: 128078292-128078558	24.79958049	1.154992909	0.365992552	0.015449671	-342859
Chr8: 128657313-128657875	86.82943888	0.630751681	0.201528077	0.016564504	-921880
Chr8: 127963134-127963657	46.0053605	0.808901717	0.260841767	0.017859574	-227701
Chr8: 128338716-128339162	36.71976028	0.885855782	0.295396593	0.02327404	-603283
Chr8: 128668823-128669069	25.28793685	0.984485442	0.363342827	0.046241898	-933390
Chr8: 128184558-128185194	64.50611487	0.605748536	0.225118266	0.048182484	-449125

c

d

e

f

BATF3
0.053

g

MYC

a

Exon 1 — Exon 2 — Exon 3

bZIP

BATF3 gRNA: CCUUCCUGUCAUCAUCCUCA

13667 Scrambled
15221 *TET2*
4137 *TET2+BATF3*

b

CAR T cells
• Pre-infusion
• Hyper-proliferative

→ 3 Days → Cell counts

IL7/15
• DMSO
• JQ1
• Dexamethasone

e

CAR T cells
• Pre-infusion
• Hyper-proliferative

→ 12 hours → qPCR analysis

IL7/15
• DMSO
• JQ1
• Dexamethasone

□ Pre-infusion $TET2_{etd}$ *TRAC*-1928z   □ $TET2_{bed}$ *TRAC*-1928z (Day 75)

c JQ1 (Cell counts)

Cell Count Fraction (JQ1/DMSO)

*

d Dexa (Cell counts)

Cell Count Fraction (Dexa/DMSO)

****

f JQ1 (qPCR)

Norm. transcripts (JQ1/DMSO)

**** ****

BATF3   MYC

g Dexa (qPCR)

Norm. transcripts (Dexa/DMSO)

**** ****

BATF3   MYC

a

15 kb

WT Rv-1928z+41BBL

TET2$_{bed}$ Rv-1928z+41BBL

IL2

b

7.1 kb

WT Rv-1928z+41BBL

TET2$_{bed}$ Rv-1928z+41BBL

GZMB   XR_001750665.1

c

8.9 kb

WT Rv-1928z+41BBL

TET2$_{bed}$ Rv-1928z+41BBL

IFNG

d

6.7 kb

WT Rv-1928z+41BBL

TET2$_{bed}$ Rv-1928z+41BBL

LTA   TNF