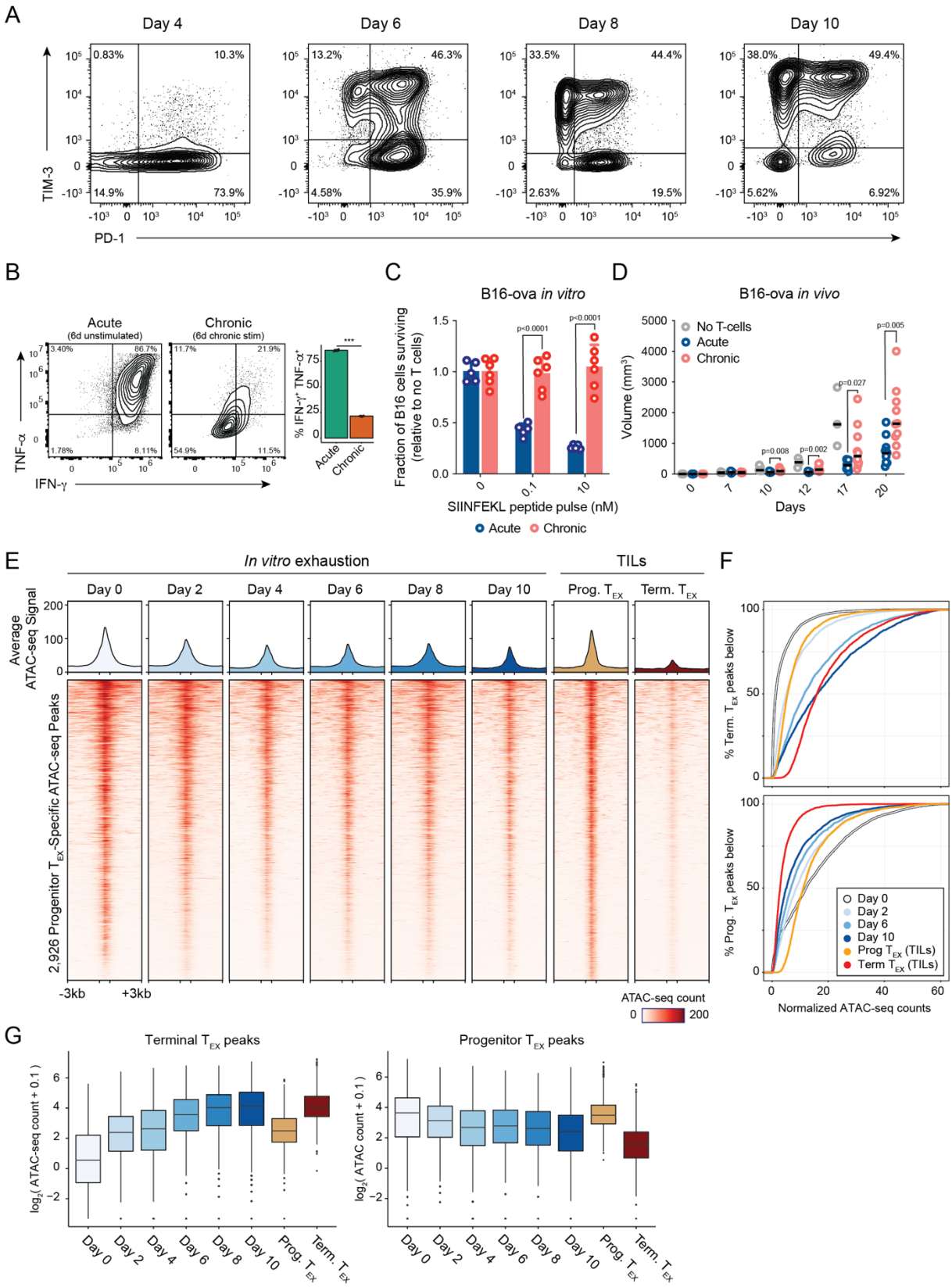

Figure S1. Additional characterization of *in vitro* assay, related to Figure 1. (A) Surface phenotype of chronically stimulated T cells throughout the *in vitro* exhaustion assay. **(B)** Effector cytokine production of

acutely (left) and chronically (right) stimulated T cells after 6 days of chronic stimulation (day 8 after isolation), n=3. Cells were restimulated with PMA and ionomycin 8 days after initial stimulation. **(C)** Survival of B16 cells after co-culture with acutely or chronically stimulated OT-1 T cells, n=3 or n=4 as indicated. Tumor cells were pulsed with cognate peptide (SIINFEKL). **(D)** B16-ovalbumin tumor growth *in vivo* after adoptive transplant of acutely or chronically stimulated T cells, n=10 except for "No T-cells" (n=3). **(E)** Heatmap showing ATAC-seq coverage of each peak in the "Progenitor T_{EX} peak set" for each time point in the *in vitro* exhaustion assay. Reference data from TILs is also included. **(F)** Empirical cumulative distribution of peak accessibility for peaks in the Term. T_{EX} peak set (top) and Prog. T_{EX} peak set (bottom) for the indicated time points *in vitro*. Reference profiles from TILs are included as indicated. **(G)** Box plots for the indicated peak sets in the *in vitro* exhaustion assay and reference TIL samples, n=3,537 Terminal T_{EX} peaks or n=2,926 Progenitor T_{EX} peaks. Each dot represents one peak. Box plots show 25^{th}, 50^{th} (median), and 75^{th} percentiles with outliers shown as dots. For **(E-G)**, one representative replicate is shown for each sample.

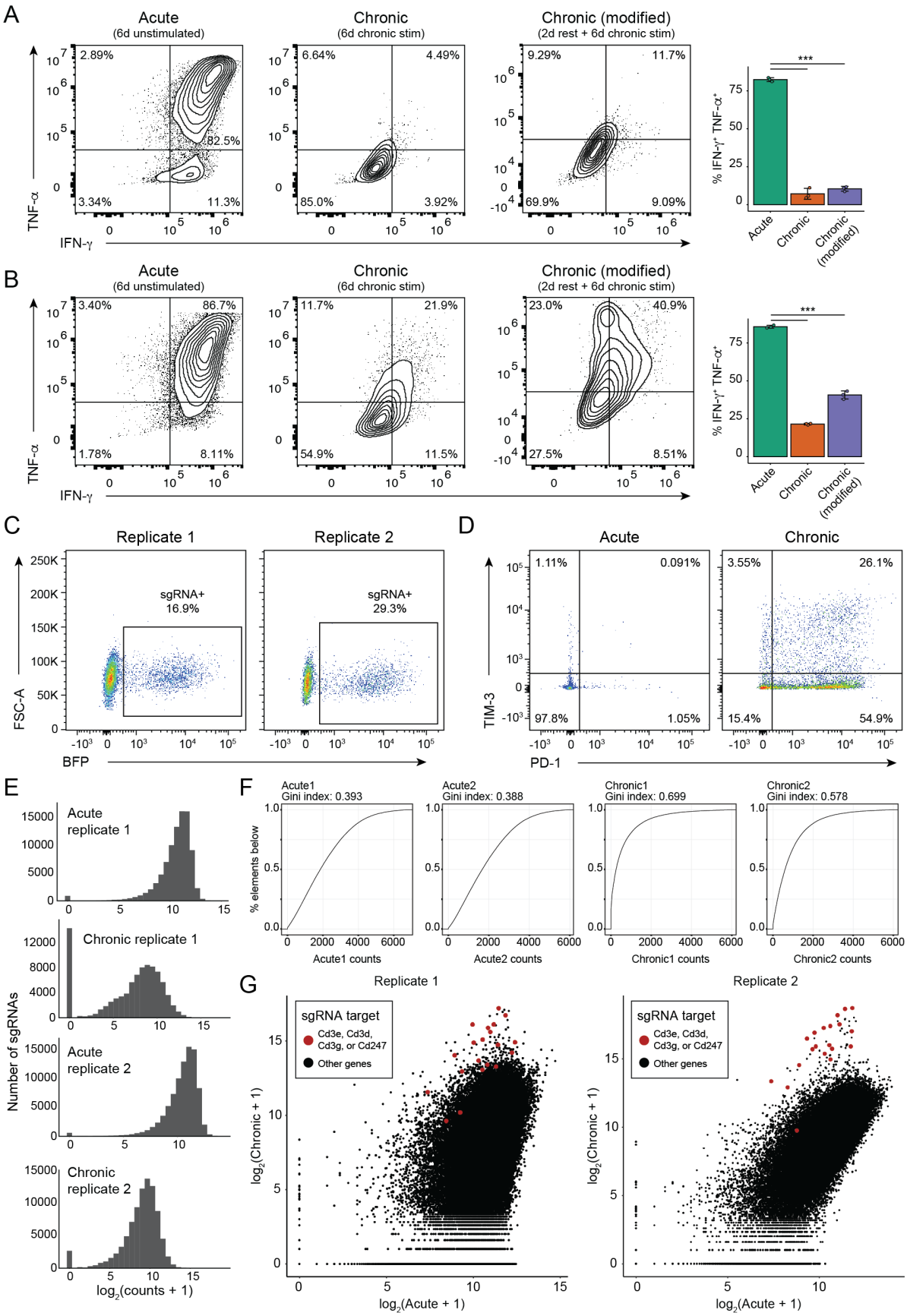
A
Acute
(6d unstimulated)
Chronic
(6d chronic stim)
Chronic (modified)
(2d rest + 6d chronic stim)
2.89%
82.5%
3.34%
11.3%
6.64%
4.49%
85.0%
3.92%
9.29%
11.7%
69.9%
9.09%
TNF-α
IFN-γ
% IFN-γ+ TNF-α+
***
Acute
Chronic
Chronic (modified)
B
Acute
(6d unstimulated)
Chronic
(6d chronic stim)
Chronic (modified)
(2d rest + 6d chronic stim)
3.40%
86.7%
1.78%
8.11%
11.7%
21.9%
54.9%
11.5%
23.0%
40.9%
27.5%
8.51%
TNF-α
IFN-γ
% IFN-γ+ TNF-α+
***
Acute
Chronic
Chronic (modified)
C
Replicate 1
Replicate 2
sgRNA+
16.9%
sgRNA+
29.3%
FSC-A
BFP
D
Acute
Chronic
1.11%
0.091%
97.8%
1.05%
3.55%
26.1%
15.4%
54.9%
TIM-3
PD-1
E
Acute
replicate 1
Chronic replicate 1
Acute
replicate 2
Chronic
replicate 2
Number of sgRNAs
log2(counts + 1)
F
Acute1
Gini index: 0.393
Acute2
Gini index: 0.388
Chronic1
Gini index: 0.699
Chronic2
Gini index: 0.578
% elements below
Acute1 counts
Acute2 counts
Chronic1 counts
Chronic2 counts
G
Replicate 1
Replicate 2
sgRNA target
Cd3e, Cd3d,
Cd3g, or Cd247
Other genes
log2(Chronic + 1)
log2(Acute + 1)

Figure S2. Validation of assay modifications and quality control data for *in vitro* genome wide screen, related to Figure 2. (A-B) Comparison of cytokine production after acute stimulation, chronic stimulation (6 days of anti-CD3 stimulation), or the modified chronic stimulation protocol (6 days of anti-CD3 stimulation after a 48-hour rest). **(A)** Cytokine production after anti-CD3 re-stimulation, n=3. **(B)** Cytokine production after PMA re-stimulation, n=3. **(C)** Expression of BFP on day 2 of the screen. **(D)** Surface phenotype of cells before gDNA extraction. **(E)** sgRNA representation of each sample, n=2000 sgRNAs. **(F)** Gini index and empirical cumulative distribution function shown for each sample in the genome-wide screen. **(G)** sgRNA count correlations (Acute vs Chronic) for each replicate. CD3 subunits are shown in red, all other sgRNAs in black.

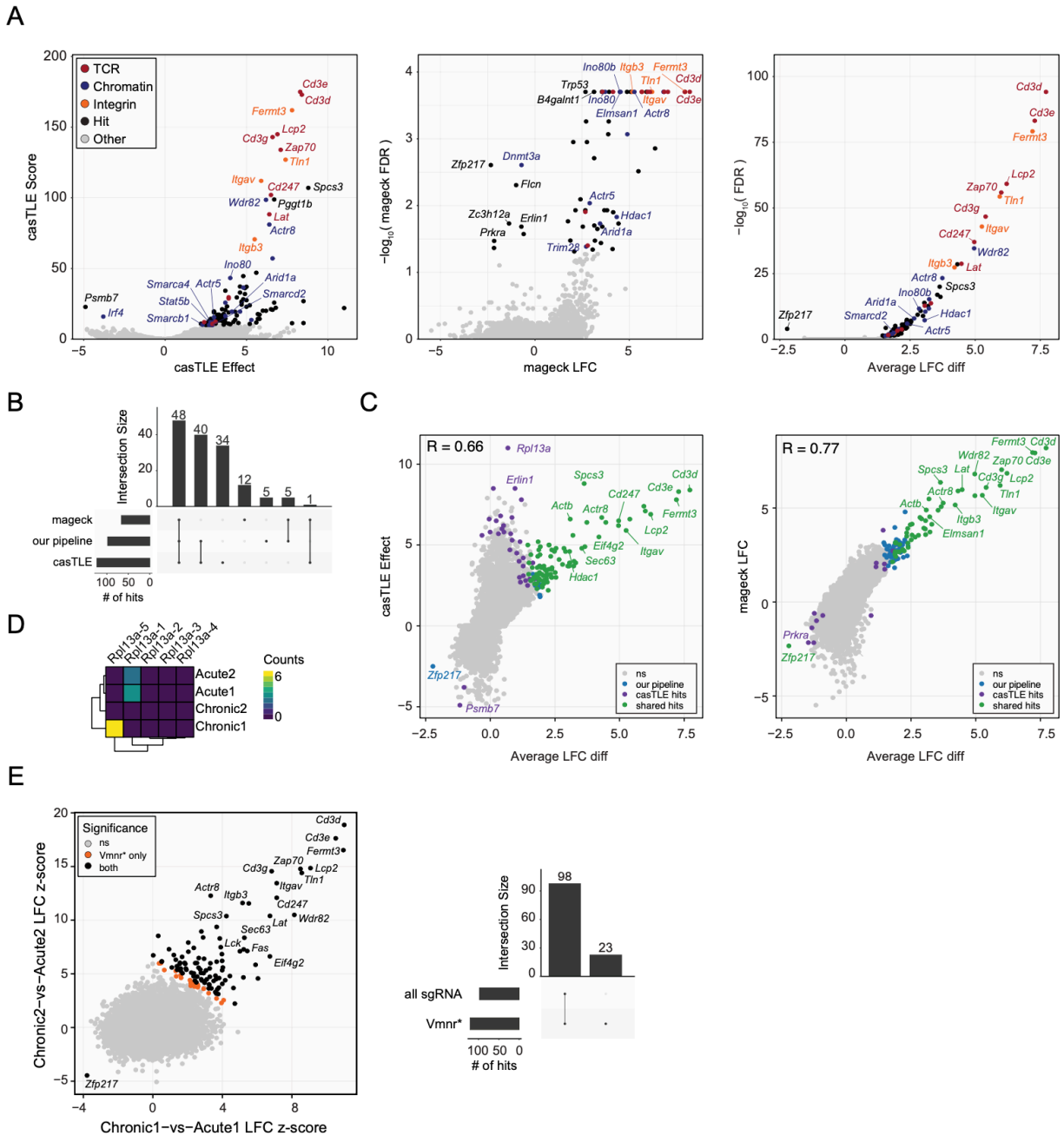

Figure S3. Comparison of CRISPR analysis strategies, related to Figure 2. (A) Volcano plots of genome wide CRISPR screen results using casTLE (left), MAGeCK (center), and our pipeline (right). **(B)** Comparison of hit lists for each of the three pipelines. **(C)** Comparison of LFC difference computed by our pipeline to the casTLE Effect (left) and MAGeCK LFC (right). **(D)** Counts table shown for *Rpl13a*. **(E)** Genome wide screen results when z-scores are computed relative to all sgRNAs or a set of olfactory receptors (Vmnr* genes).

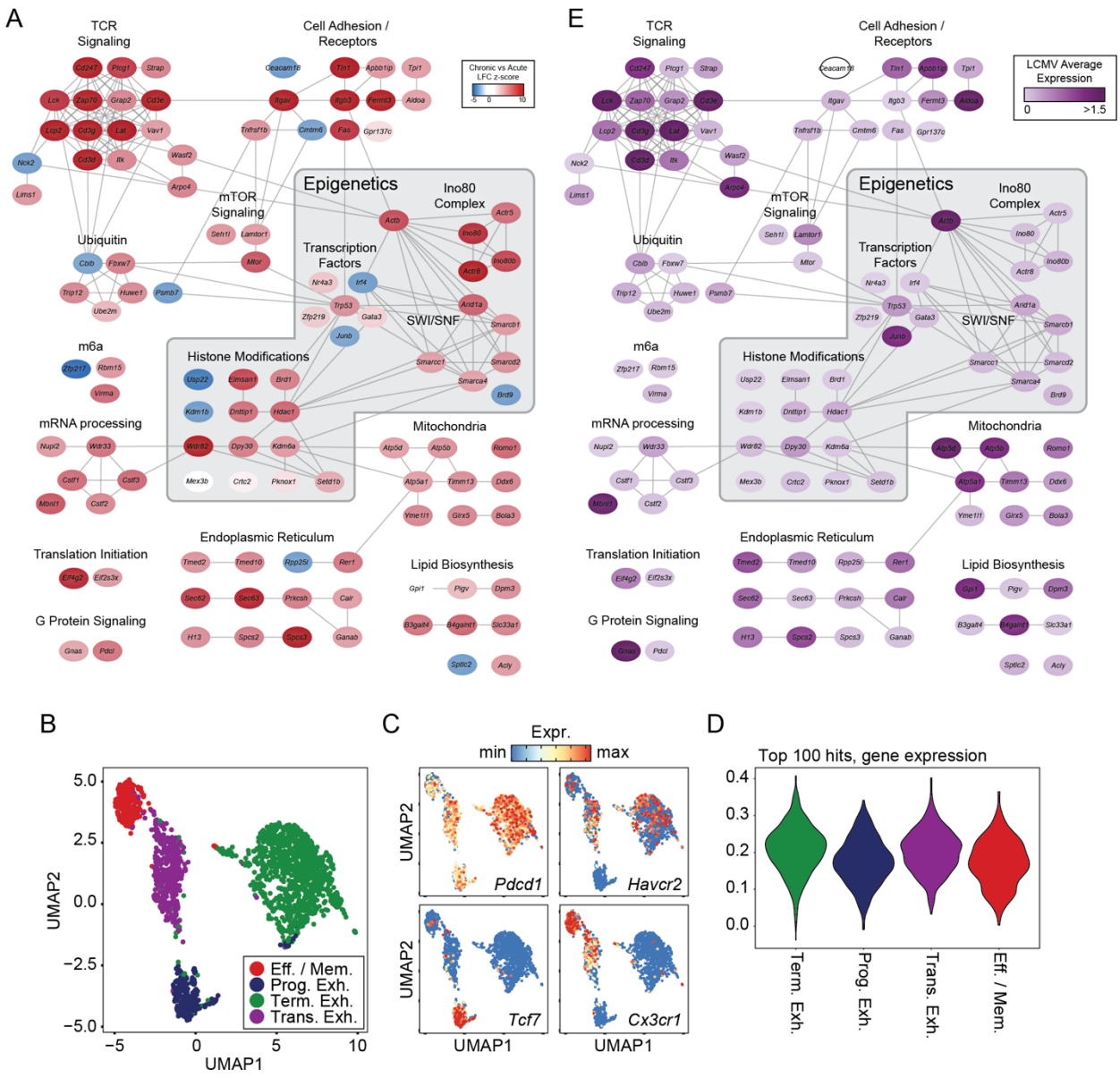

Figure S4. Cytoscape network representation of top hits and LCMV clone 13 expression analysis, related to Figure 2. (A) Top positive and negative hits from the genome-wide screen are shown. Each protein is represented by a node in the cytoscape network, colored by its z-score in the genome-wide screen. Nodes are connected if there is a high confidence protein-protein interaction in the string-db database (Szklarczyk et al., 2019). **(B)** Cell types identified in previously published scRNA-seq data (Raju et al., 2021). **(C)** Expression of *Pdcd1*, *Havcr2*, *Tcf7*, and *Cx3cr1* in single cells. **(D)** Expression of the gene module containing the top 100 *in vitro* hits across clusters. **(E)** Cytoscape network of top hits colored by average expression across all single cells.

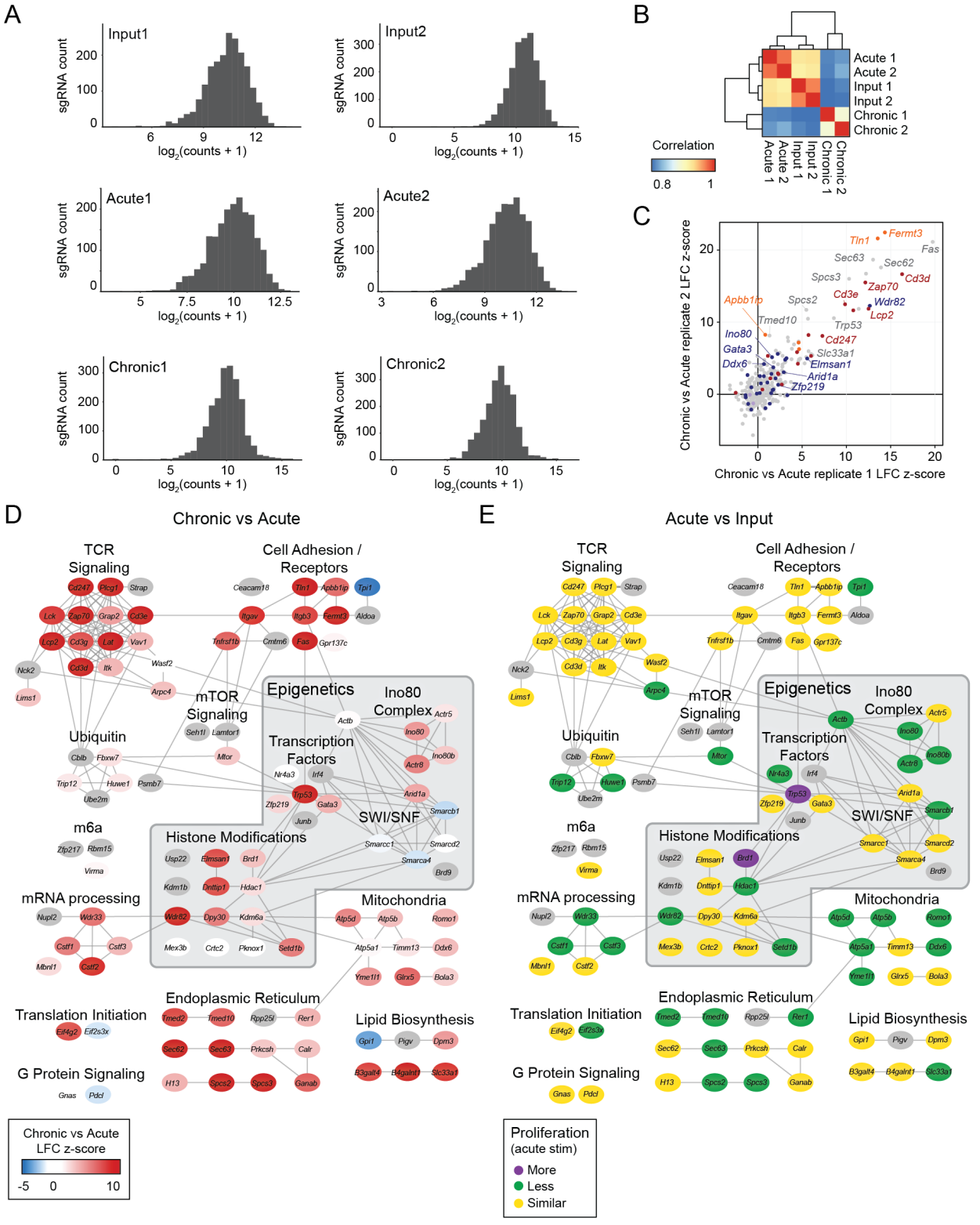

Figure S5. Additional data for targeted *in vitro* screening, related to Figure 2. (A) sgRNA representation of each sample in the *in vitro* mini-pool screen. **(B)** Correlation of the sgRNA counts of each sample in the mini-pool screen. **(C)** Correlation of the Chronic vs Acute replicate z-scores, n=2. **(D)**

Cytoscape interaction network with genes colored by their z-score in the Chronic vs Acute mini-pool screen. **(E)** Cytoscape interaction network with genes colored by their fitness categorization in acute stimulation.

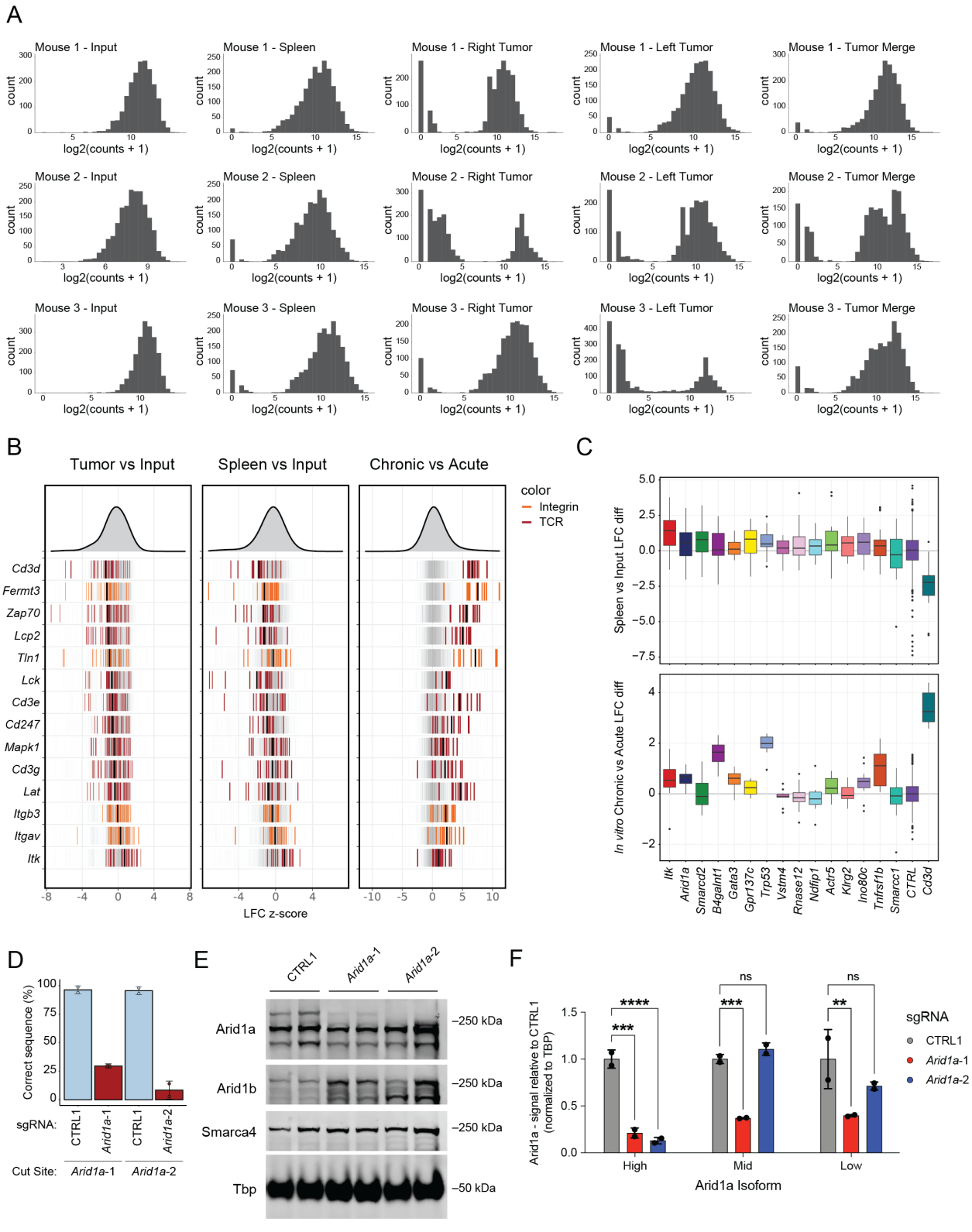

Figure S6. Additional data for targeted *in vivo* screening and validation of *Arid1a*-targeting sgRNAs, related to Figures 3 and 4. (A) sgRNA pool coverage for each sample in the *in vivo* mini-pool screen. **(B)** sgRNA z-scores in MC-38 tumors (n=36 sgRNA-replicates), MC-38 spleens (n=18 sgRNA-replicates), and

in vitro mini-pool Chronic vs Acute (n=12 sgRNA-replicates) for selected genes in the “TCR signaling” and “Integrin signaling” categories. **(C)** Boxplot of spleen vs input (n=18 except for CTRL (n=600)) and acute vs chronic (n=12 except for CTRL (n=400)) log fold change for each sgRNA targeting the indicated gene, with the mean control log fold change subtracted. Box plots show 25th, 50th (median), and 75th percentiles with outliers shown as dots. **(D)** Sanger sequencing (TIDE) analysis of editing efficiency of *Arid1a* sgRNAs, n=2 replicates per sgRNA. Error bars denote mean ± SD. **(E)** Western blot analysis of protein knockdown for Arid1a sgRNAs, as well as Arid1b and Smarca4 expression. **(F)** Quantification of protein knockdown for each identified isoform of *Arid1a* (panel C three bands), n=2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

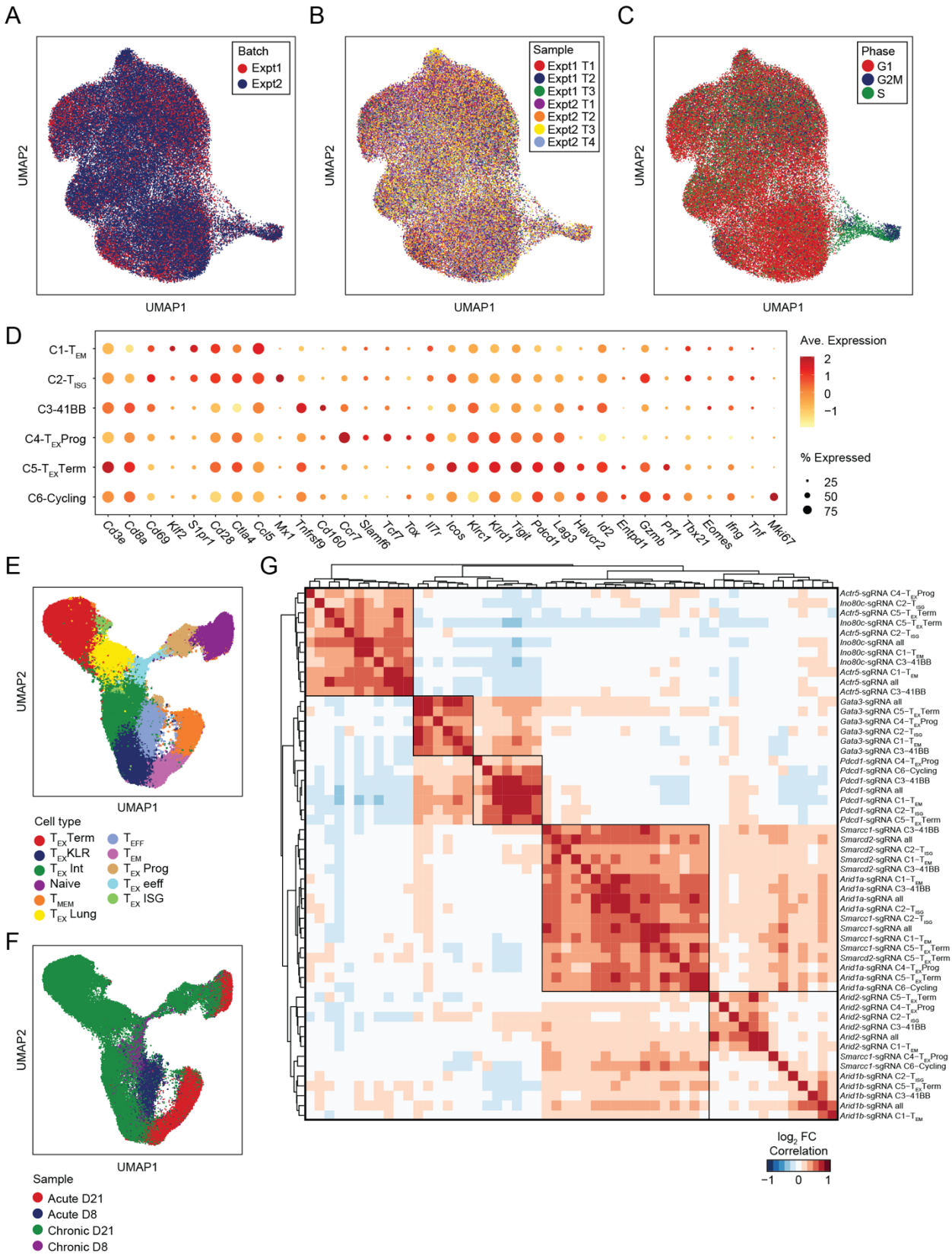
A
B
C
Batch
Expt1
Expt2
Sample
Expt1 T1
Expt1 T2
Expt1 T3
Expt2 T1
Expt2 T2
Expt2 T3
Expt2 T4
Phase
G1
G2M
S
UMAP1
UMAP2
D
C1-T_EM
C2-T_ISG
C3-41BB
C4-T_EXProg
C5-T_EXTerm
C6-Cycling
Cd3e
Cd8a
Cd69
Klf2
S1pr1
Cd28
Ctla4
Ccl5
Mx1
Tnfrsf9
Cd160
Ccr7
Slamf6
Tcf7
Tox
Il7r
Icos
Klrc1
Klrd1
Tigit
Pdcd1
Lag3
Havcr2
Id2
Entpd1
Gzmb
Prf1
Tbx21
Eomes
Ifng
Tnf
Mki67
Ave. Expression
2
1
0
−1
% Expressed
25
50
75
E
Cell type
T_EXTerm
T_EXKLR
T_EX Int
Naive
T_MEM
T_EX Lung
T_EFF
T_EM
T_EX Prog
T_EX eeff
T_EX ISG
F
Sample
Acute D21
Acute D8
Chronic D21
Chronic D8
G
log2 FC
Correlation
−1
0
1

Figure S7. Additional data on the *in vivo* Perturb-seq experiment, related to Figure 6. (A) scRNA-seq profiles of TILs colored by each independent experiment (n=2 independent experiments). **(B)** scRNA-seq profiles of TILs colored by each sample (n=7 replicates). **(C)** scRNA-seq profiles of TILs colored by predicted phase of the cell cycle. **(D)** Additional marker genes shown for each cluster. **(E)** Expanded reference LCMV dataset with single cell profiles colored by LCMV cluster. Data from (Daniel et al., 2021). **(F)** Expanded LCMV dataset with single cell profiles colored by LCMV infection (Acute corresponds to Armstrong infection while Chronic corresponds to Clone 13) and time point (Day 8 or Day 21 post infection). **(G)** Heatmap of the correlation of gene expression differences subsetted on each cluster. The indicated gene knockdown was compared to CTRL1 cells within each cluster. Comparisons with <150 cells in the comparison groups are excluded due to lack of statistical power.

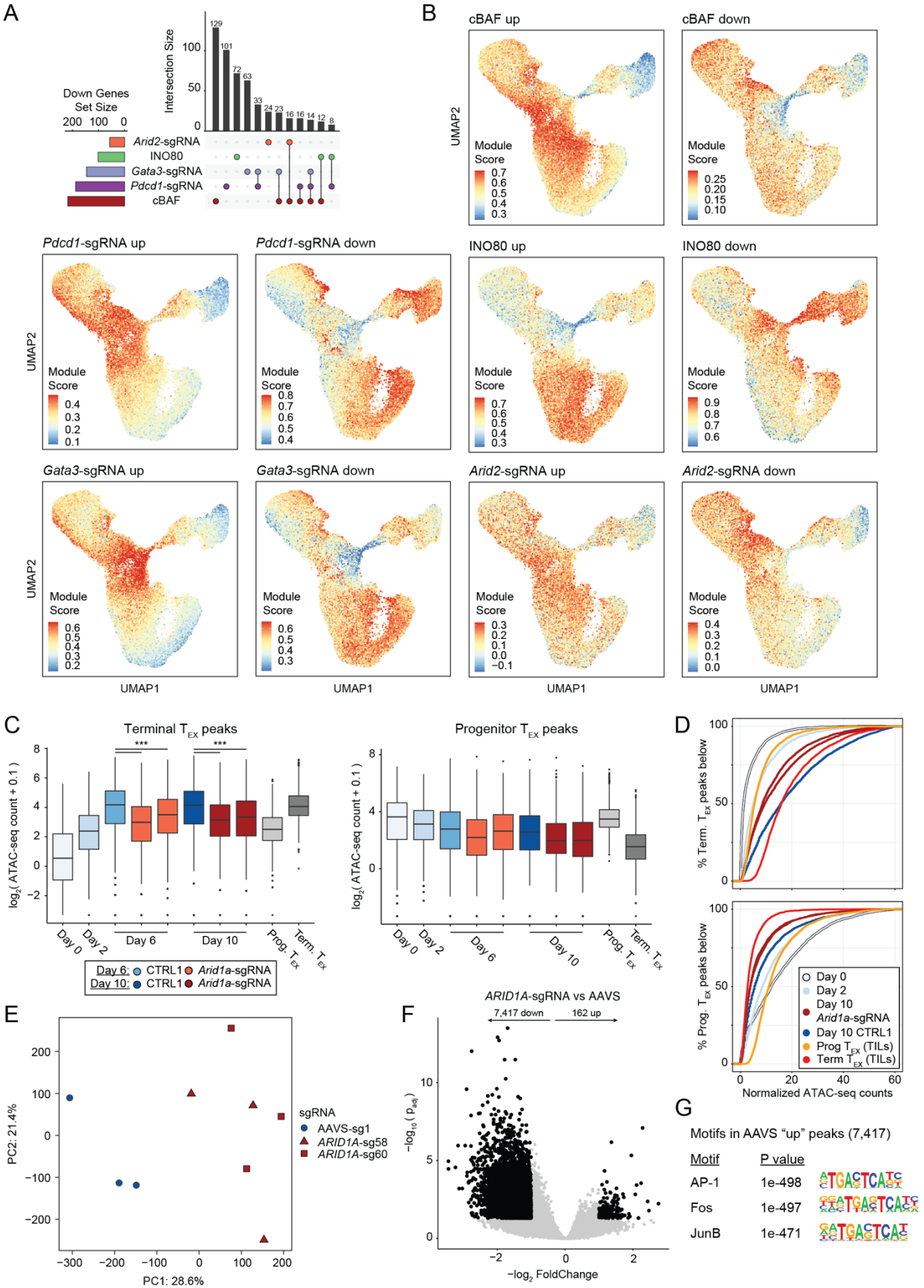
A
Down Genes Set Size
200 100 0
Intersection Size
129
101
72
63
33
24
23
16
16
14
12
8
100
50
0
Arid2-sgRNA
INO80
Gata3-sgRNA
Pdcd1-sgRNA
cBAF
B
cBAF up
cBAF down
Pdcd1-sgRNA up
Pdcd1-sgRNA down
INO80 up
INO80 down
Gata3-sgRNA up
Gata3-sgRNA down
Arid2-sgRNA up
Arid2-sgRNA down
UMAP1
UMAP2
Module Score
C
Terminal T_EX peaks
Progenitor T_EX peaks
log2(ATAC-seq count + 0.1)
***
Day 0
Day 2
Day 6
Day 10
Prog. T ex
Term. T ex
Day 6: CTRL1 Arid1a-sgRNA
Day 10: CTRL1 Arid1a-sgRNA
D
% Term. T_EX peaks below
% Prog. T_EX peaks below
Day 0
Day 2
Day 10
Arid1a-sgRNA
Day 10 CTRL1
Prog T_EX (TILs)
Term T_EX (TILs)
Normalized ATAC-seq counts
E
PC2: 21.4%
PC1: 28.6%
sgRNA
AAVS-sg1
ARID1A-sg58
ARID1A-sg60
F
ARID1A-sgRNA vs AAVS
7,417 down
162 up
−log10 (padj)
−log2 FoldChange
G
Motifs in AAVS "up" peaks (7,417)
Motif
P value
AP-1
1e-498
Fos
1e-497
JunB
1e-471

Figure S8. Additional data on up- and downregulated gene sets and additional ATAC-seq data, related to Figures 7 and 8. (A) Comparison of gene sets downregulated by perturbation of cBAF subunits, INO80 subunits, or *Pdcd1*-sgRNA, *Gata3*-sgRNA, or *Arid2*-sgRNA. **(B)** Module scores of the indicated gene sets computed for each cell in the expanded LCMV reference dataset. **(C)** Box plots for the indicated peak sets in the *in vitro* exhaustion assay and reference TIL samples. Each dot represents one peak, n=3,537 Terminal T_{EX} peaks or n=2,926 Progenitor T_{EX} peaks**.** Box plots show 25th, 50th (median), and 75th percentiles with outliers shown as dots. Significance determined by Wilcoxon test, *** $p < 0.001$. **(D)** Empirical cumulative distribution of peak accessibility for peaks in the Term. T_{EX} peak set (top) and Prog. T_{EX} peak set (bottom) for the indicated samples *in vitro*. Reference profiles from TILs are included as indicated. **(E)** Principal component analysis of ATAC-seq data of primary human T cells chronically stimulated for six days, n=3 per sgRNA. **(F)** Differential peaks between *ARID1A*-sgRNA and AAVS primary human T cells. **(G)** HOMER analysis of TF motifs enriched in AAVS 'up' peaks. Selected highly ranked motifs are shown. Results in **(E-G)** are merged from three different human donors in two independent experiments with two different *ARID1A* targeting sgRNAs per donor.