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Supplemental information

CRISPR screens unveil nutrient-dependent lysosomal and mitochondrial nodes impacting intestinal tissue-resident memory CD8$^+$ T cell formation

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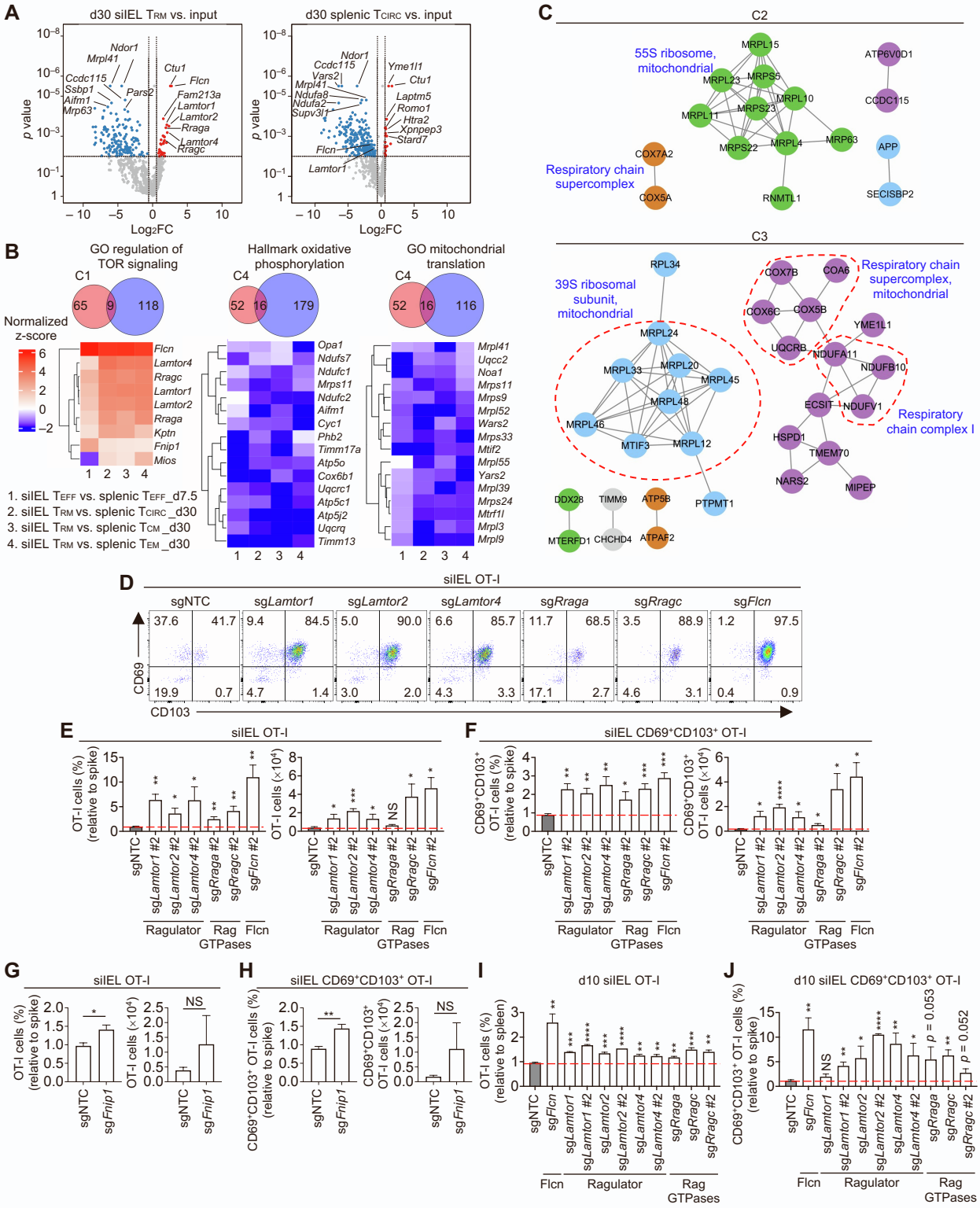

Figure S1. Protein-protein interaction analysis and validation of lysosome-associated genes as negative regulators of CD8⁺ T_{RM} development (related to Figure 1). (A) Volcano plots depicting genes targeted by sgRNAs that were enriched (denoted in red) or depleted (denoted in blue) in day 30 p.i. siIEL T_{RM} versus input cells or day 30 p.i. splenic T_{CIRC} versus input cells from *in vivo* CRISPR screen (as described in Figure 1A). (B) Venn diagrams and heatmaps showing the genes in the GO regulation of TOR signaling signature that overlapped with C1 genes, or genes in the Hallmark oxidative phosphorylation signature and GOBP mitochondrial translation signature that overlapped with C4 genes (C1 and C4 genes identified as described in Figure 1B). (C) PPI networks of C2 and C3 genes (genes identified as described in Figure 1B). The red dotted lines indicate known protein complexes that are labeled by blue colored text. (D) CD103 and CD69 expression on siIEL OT-I cells transduced with the indicated sgRNAs. (E and F) Frequencies (relative to spike) and numbers of indicated sgRNA-transduced total (E) or CD69⁺CD103⁺ (F) OT-I cells in siIEL at day 21 p.i. (from dual-color transfer system) ($n \geq 3$ per group). (G and H) Frequencies (relative to spike) and numbers of indicated sgRNA-transduced total (G) or CD69⁺CD103⁺ (H) OT-I cells in siIEL at day 21 p.i. (from dual-color transfer system) ($n \geq 4$ per group). (I and J) Frequencies (normalized to sgNTC spike first and then to the relative frequency among splenic OT-I cells) of total (I) or CD69⁺CD103⁺ (J) siIEL OT-I cells at day 10 p.i. (from dual-color transfer system) ($n \geq 2$ per group). Two-tailed unpaired Student's t test (E–J). NS, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Data (mean ± SEM) are compiled from two (E, F, I, and J) or represent ≥1 (D, G, and H) experiments.

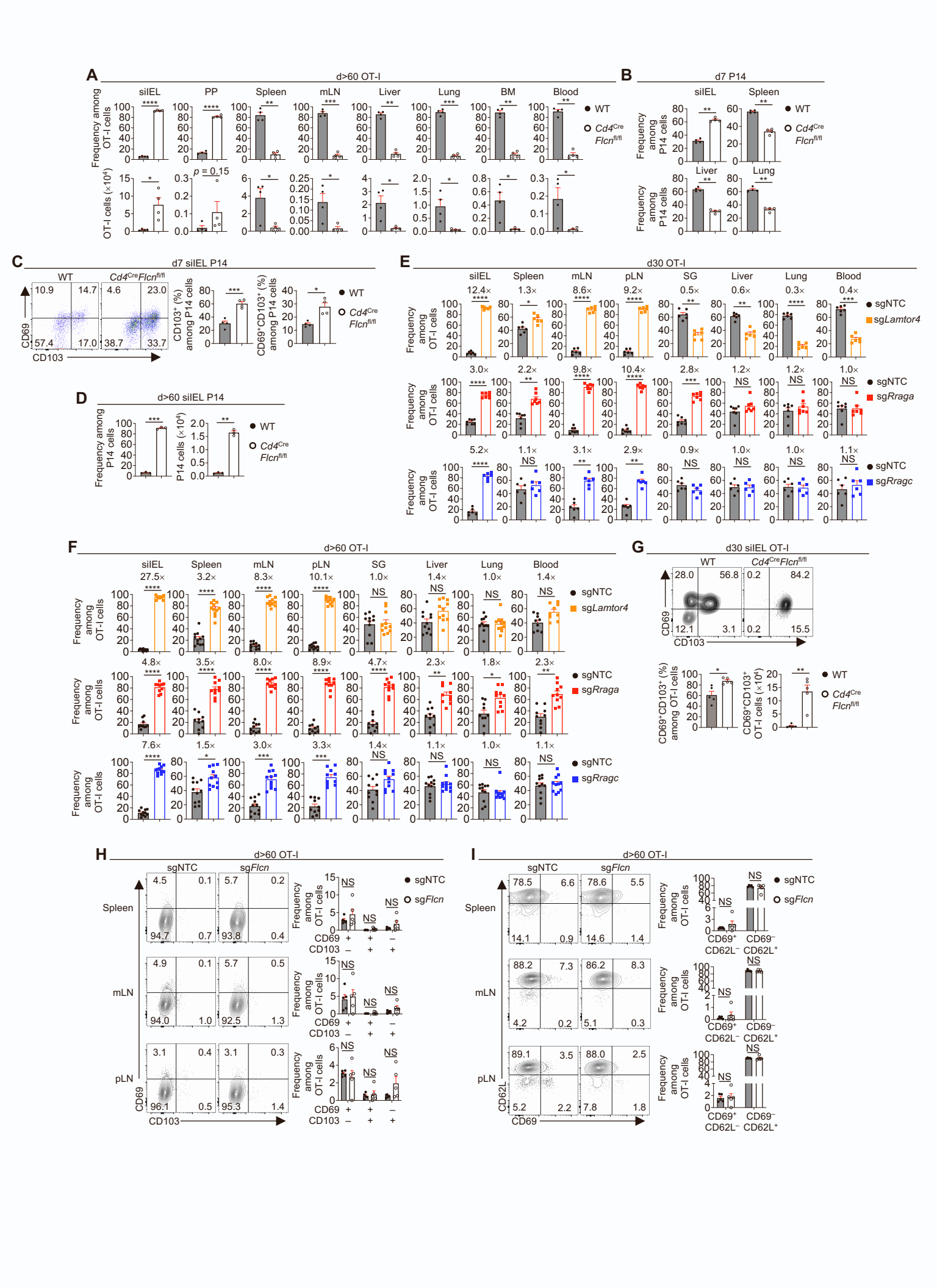
A
d>60 OT-I
siIEL
PP
Spleen
mLN
Liver
Lung
BM
Blood
Frequency among OT-I cells
OT-I cells (×10⁴)
p = 0.15
WT
Cd4Cre Flcnfl/fl
B
d7 P14
siIEL
Spleen
Liver
Lung
Frequency among P14 cells
C
d7 siIEL P14
WT
Cd4Cre Flcnfl/fl
CD69
CD103
10.9
14.7
57.4
17.0
4.6
23.0
38.7
33.7
CD103+ (%) among P14 cells
CD69+CD103+ (%) among P14 cells
D
d>60 siIEL P14
Frequency among P14 cells
P14 cells (×10⁴)
E
d30 OT-I
siIEL
Spleen
mLN
pLN
SG
Liver
Lung
Blood
12.4×
1.3×
8.6×
9.2×
0.5×
0.6×
0.3×
0.4×
3.0×
2.2×
9.8×
10.4×
2.8×
1.2×
1.2×
1.0×
5.2×
1.1×
3.1×
2.9×
0.9×
1.0×
1.0×
1.1×
sgNTC
sgLamtor4
sgRraga
sgRragc
Frequency among OT-I cells
F
d>60 OT-I
siIEL
Spleen
mLN
pLN
SG
Liver
Lung
Blood
27.5×
3.2×
8.3×
10.1×
1.0×
1.4×
1.0×
1.4×
4.8×
3.5×
8.0×
8.9×
4.7×
2.3×
1.8×
2.3×
7.6×
1.5×
3.0×
3.3×
1.4×
1.1×
1.0×
1.1×
sgNTC
sgLamtor4
sgRraga
sgRragc
Frequency among OT-I cells
G
d30 siIEL OT-I
WT
Cd4Cre Flcnfl/fl
CD69
CD103
28.0
56.8
12.1
3.1
0.2
84.2
0.2
15.5
CD69+CD103+ (%) among OT-I cells
CD69+CD103+ (×10⁴) OT-I cells
H
d>60 OT-I
sgNTC
sgFlcn
Spleen
mLN
pLN
CD69
CD103
4.5
0.1
94.7
0.7
5.7
0.2
93.8
0.4
4.9
0.1
94.0
1.0
5.7
0.5
92.5
1.3
3.1
0.4
96.1
0.5
3.1
0.3
95.3
1.4
Frequency among OT-I cells
CD69 + + –
CD103 – + +
I
d>60 OT-I
sgNTC
sgFlcn
Spleen
mLN
pLN
CD62L
CD69
78.5
6.6
14.1
0.9
78.6
5.5
14.6
1.4
88.2
7.3
4.2
0.2
86.2
8.3
5.1
0.3
89.1
3.5
5.2
2.2
88.0
2.5
7.8
1.8
Frequency among OT-I cells
CD69+ CD62L–
CD69– CD62L+

Figure S2. Deletion of Flcn selects for CD8$^+$ T cell tissue residency in the small intestine (related to Figure 2). (A) Naive WT (CD45.1$^+$) and *Cd4*^{Cre}*Flcn*$^{fl/fl}$ (CD45.1$^+$CD45.2$^+$) OT-I cells were co-transferred into WT (CD45.2$^+$) mice at a 1:1 ratio, followed by infection with LM-OVA. Frequencies and numbers of OT-I cells in the indicated tissues at day >60 p.i. In blood, cell numbers are relative to 10^6 peripheral blood mononuclear cells (n = 4 per group). (B–D) Naive WT (CD45.2$^+$Thy1.1$^+$Thy1.2$^+$) and *Cd4*^{Cre}*Flcn*$^{fl/fl}$ (CD45.2$^+$Thy1.2$^+$) P14 cells were co-transferred into WT (CD45.1$^+$) mice at a 1:1 ratio, followed by LCMV-Armstrong infection one day later. Frequencies among P14 cells in indicated tissues at day 7 p.i. (n = 4 per group) (B). Frequencies of CD103$^+$ or CD69$^+$CD103$^+$ siIEL P14 cells at day 7 p.i. (n = 4 per group) (C). Frequencies and numbers of P14 cells at day >60 p.i. (n = 3 per group) (D). (E and F) Frequencies among indicated sgRNA-transduced OT-I cells in indicated tissues at days 30 (E) or >60 (F) p.i. (from dual-color transfer system) ($n \geq 6$ per group). Fold-change relative to sgNTC is indicated. (G) Frequencies and numbers of CD69$^+$CD103$^+$ siIEL OT-I cells at day 30 p.i. (from mice as described in A) (n = 5 per group). (H and I) Frequencies of indicated sgRNA-transduced CD69$^+$CD103$^-$, CD69$^+$CD103$^+$, and CD69$^-$CD103$^+$ OT-I cells (H) or CD69$^+$CD62L$^-$ and CD69$^-$CD62L$^+$ OT-I cells (I) in indicated tissues at day >60 p.i. (from dual-color transfer system) (n = 5 per group). Two-tailed paired Student's t test (A–I). NS, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Data (mean ± SEM) are compiled from three (F) or represent ≥2 (A–E and G–I) experiments.

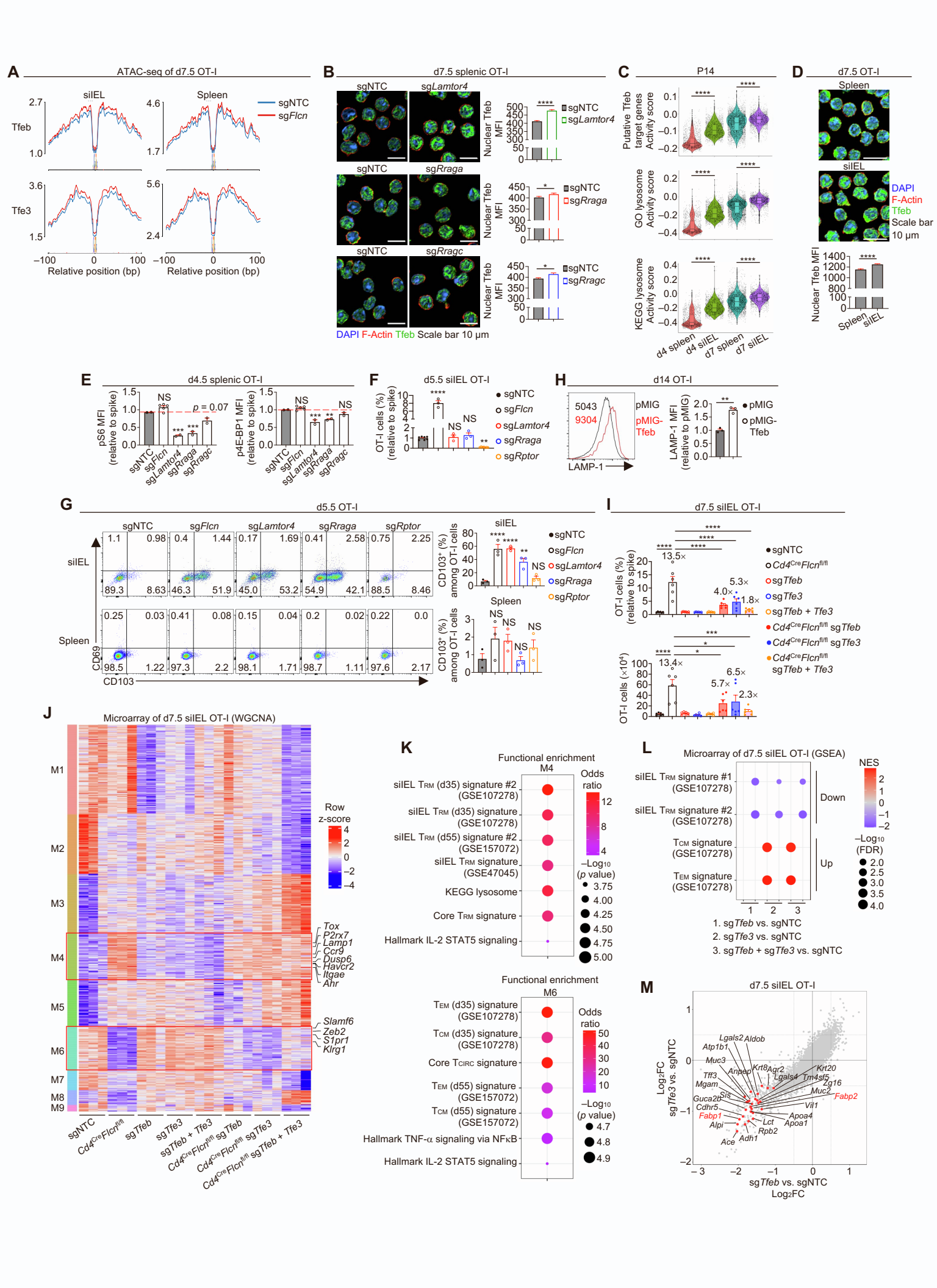
A
ATAC-seq of d7.5 OT-I
siIEL
Spleen
sgNTC
sgFlcn
Tfeb
Tfe3
Relative position (bp)
B
d7.5 splenic OT-I
sgNTC
sgLamtor4
sgRraga
sgRragc
Nuclear Tfeb MFI
DAPI F-Actin Tfeb Scale bar 10 μm
C
P14
Putative Tfeb target genes Activity score
GO lysosome Activity score
KEGG lysosome Activity score
d4 spleen
d4 siIEL
d7 spleen
d7 siIEL
D
d7.5 OT-I
Spleen
siIEL
DAPI
F-Actin
Tfeb
Scale bar 10 μm
Nuclear Tfeb MFI
E
d4.5 splenic OT-I
pS6 MFI (relative to spike)
p4E-BP1 MFI (relative to spike)
p = 0.07
F
d5.5 siIEL OT-I
OT-I cells (%) (relative to spike)
sgRptor
H
d14 OT-I
5043
9304
pMIG
pMIG-Tfeb
LAMP-1
LAMP-1 MFI (relative to pMIG)
G
d5.5 OT-I
CD69
CD103
CD103+ (%) among OT-I cells
I
d7.5 siIEL OT-I
OT-I cells (%) (relative to spike)
OT-I cells (×10⁴)
Cd4CreFlcnfl/fl
sgTfeb
sgTfe3
sgTfeb + Tfe3
Cd4CreFlcnfl/fl sgTfeb
Cd4CreFlcnfl/fl sgTfe3
Cd4CreFlcnfl/fl sgTfeb + Tfe3
J
Microarray of d7.5 siIEL OT-I (WGCNA)
M1
M2
M3
M4
M5
M6
M7
M8
M9
Row z-score
Tox
P2rx7
Lamp1
Ccr9
Dusp6
Havcr2
Itgae
Ahr
Slamf6
Zeb2
S1pr1
Klrg1
K
Functional enrichment
M4
siIEL TRM (d35) signature #2 (GSE107278)
siIEL TRM (d35) signature (GSE107278)
siIEL TRM (d55) signature #2 (GSE157072)
siIEL TRM signature (GSE47045)
KEGG lysosome
Core TRM signature
Hallmark IL-2 STAT5 signaling
Odds ratio
–Log10 (p value)
Functional enrichment
M6
TEM (d35) signature (GSE107278)
TCM (d35) signature (GSE107278)
Core TCIRC signature
TEM (d55) signature (GSE157072)
TCM (d55) signature (GSE157072)
Hallmark TNF-α signaling via NFκB
Hallmark IL-2 STAT5 signaling
L
Microarray of d7.5 siIEL OT-I (GSEA)
siIEL TRM signature #1 (GSE107278)
siIEL TRM signature #2 (GSE107278)
TCM signature (GSE107278)
TEM signature (GSE107278)
Down
Up
NES
–Log10 (FDR)
1. sgTfeb vs. sgNTC
2. sgTfe3 vs. sgNTC
3. sgTfeb + sgTfe3 vs. sgNTC
M
d7.5 siIEL OT-I
Log2FC sgTfe3 vs. sgNTC
sgTfeb vs. sgNTC Log2FC
Lgals2
Aldob
Atp1b1
Muc3
Anxa10
Krt8
Agr2
Krt20
Tff3
Lgals4
Tm4sf5
Zg16
Mgam
Muc2
Fabp2
Gsta
Guca2b
Vil1
Cdhr5
Fabp1
Apoa4
Alpi
Lct
Apoa1
Rpb2
Ace
Adh1

Figure S3. Regulation and function of Tfeb and Tfe3 activities and mTORC1 signaling in T_{RM} formation (related to Figure 3). (A) Footprinting analysis of ATAC-seq data from sgNTC- or sg*Flcn*-transduced OT-I cells from siIEL and spleen at day 7.5 p.i. (as described in Figure 3A). (B) Nuclear Tfeb levels (based on MFI) in indicated sgRNA-transduced OT-I cells from spleen at day 7.5. p.i. (from dual-color transfer system) ($n \geq 131$ cells per group). (C) Violin plots showing the activity scores of Tfeb putative target genes (see STAR Methods) or GO lysosome and KEGG lysosome gene signatures in P14 cells from indicated tissues profiled by scRNA-seq at days 4 and 7 p.i. (GSE131847; see STAR Methods). (D) Nuclear Tfeb levels (based on MFI) in OT-I cells from spleen or siIEL at day 7.5 p.i. ($n \geq 385$ per group cells). (E) Quantification (relative to spike; based on MFI) of phosphorylated S6 and 4E-BP1 (pS6 and p4E-BP1) in indicated sgRNA-transduced OT-I cells from spleen at day 4.5 p.i. (from dual-color transfer system) ($n \geq 2$ per group). (F) Frequency (relative to spike) of indicated sgRNA-transduced OT-I cells in siIEL at day 5.5 p.i. (from dual-color transfer system) ($n \geq 3$ per group). (G) Flow cytometry analysis of CD69 and CD103 expression on OT-I cells transduced with indicated sgRNAs in siIEL or spleen at day 5.5 p.i. (left). Right, frequency of $CD103^+$ OT-I cells transduced with indicated sgRNAs in siIEL or spleen at day 5.5 p.i. ($n = 3$ per group). (H) Intracellular LAMP-1 expression (relative to the average of the pMIG group; based on MFI) in pMIG ($mCherry^+$) or pMIG-Tfeb (GFP^+)-transduced OT-I cells from spleen at day 14 p.i. (from dual-color transfer system) ($n = 3$ per group). (I) WT (from $Flcn^{fl/fl}Cas9^+$ mice) or Flcn-deficient (from $Cd4^{Cre}Flcn^{fl/fl}Cas9^+$ mice) OT-I cells were transduced or co-transduced with indicated sgRNAs ($Ametrine^+$ or GFP^+), and then co-transferred at a 1:1 ratio with OT-I cells transduced with sgNTC (spike; $Ametrine^+$ or GFP^+) followed by LM-OVA infection (from dual-color transfer system). Frequency (relative to spike) and number of indicated sgRNA-transduced OT-I cells in siIEL at day 7.5 p.i. is shown. Fold-change relative to sgNTC is indicated ($n = 6$ per group). (J) Heatmap depicting gene expression patterns in the nine gene modules (M1–M9) identified by transcriptome profiling and WGCNA of indicated sgRNA-transduced OT-I cells (as described in I) in siIEL at day 7.5 p.i. ($n = 3$ per group). (K) Functional enrichment analysis of the M4 and M6 genes (defined in J) using the KEGG and Hallmark signatures, curated T_{RM}, T_{EM}, and T_{CM} signatures (GSE107278, GSE157072, GSE47045; see STAR Methods), and core T_{RM} and T_{CIRC} signatures (see STAR Methods). (L and M) OT-I cells transduced with indicated sgRNAs in siIEL at day 7.5 p.i. were profiled for transcriptome analysis (as described in J). GSEA using curated siIEL T_{RM}, splenic T_{CM} and splenic T_{EM} gene signatures (GSE107278; see STAR Methods) was performed. Bubble plot showing enriched (red) and reduced (blue) gene signatures for the indicated comparisons (L). FC/FC plot of the indicated pairwise comparisons. Shared reduced genes in sg*Tfeb*- versus sgNTC-transduced OT-I cells compared with sg*Tfe3*- versus sgNTC-transduced OT-I cells (based on $\log_2FC < -0.5$) in the curated siIEL T_{RM} gene signatures #1 and #2 (GSE107278; see STAR Methods) were highlighted in red (M). Two-tailed paired Student's t test (B and H), two-tailed unpaired Student's t test (D), one-way ANOVA (E–G and I), Wilcoxon rank sum test (C), or Fisher's exact test (K). NS, not significant; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Data (mean ± SEM) are compiled from two (E, F, and I) or represent ≥2 (B, D, G, and H) experiments.

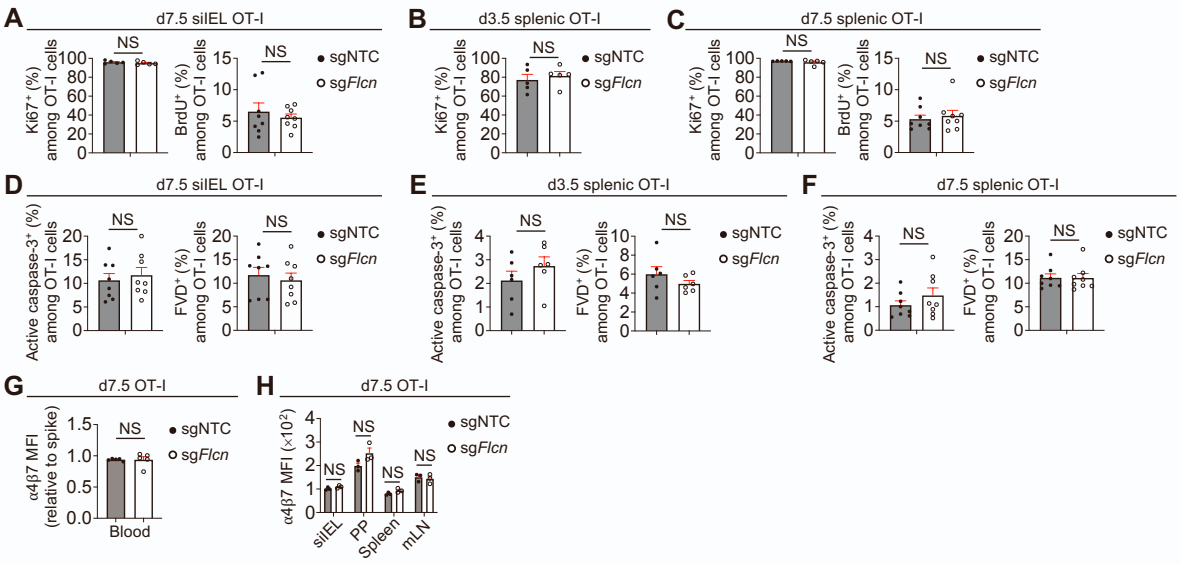

Figure S4. Flcn deficiency does not impair CD8⁺ T cell proliferation or survival (related to Figure 4). (A) Frequencies of Ki67⁺ and bromodeoxyuridine (BrdU)⁺ cells among sgNTC- and sg*Flcn*-transduced OT-I cells in siIEL at day 7.5 p.i. (from dual-color transfer) ($n \geq 5$ per group). (B) Frequency of Ki67⁺ cells among sgNTC- and sg*Flcn*-transduced OT-I cells in spleen at day 3.5 p.i. ($n = 5$ per group). (C) Frequencies of Ki67⁺ and BrdU⁺ cells among sgNTC- and sg*Flcn*-transduced OT-I cells in spleen at day 7.5 p.i. ($n \geq 5$ per group). (D) Frequencies of active caspase-3⁺ and fixable viability dye (FVD)⁺ cells among sgNTC- and sg*Flcn*-transduced OT-I cells in siIEL at day 7.5 p.i. ($n \geq 5$ per group). (E) Frequencies of active caspase-3⁺ and FVD⁺ cells among sgNTC- and sg*Flcn*-transduced OT-I cells in spleen at day 3.5 p.i. ($n \geq 5$ per group). (F) Frequencies of active caspase-3⁺ and FVD⁺ cells among sgNTC- and sg*Flcn*-transduced OT-I cells in spleen at day 7.5 p.i. ($n \geq 5$ per group). (G) $\alpha 4\beta 7$ expression (relative to spike; based on MFI) on sgNTC- and sg*Flcn*-transduced OT-I cells in the blood at day 7.5 p.i. (from dual-color transfer system) ($n = 5$ per group). (H) $\alpha 4\beta 7$ expression (based on MFI) on sgNTC- and sg*Flcn*-transduced OT-I cells from indicated tissues at day 7.5 p.i. ($n = 3$ per group). Two-tailed paired Student's t test (A–F) or two-tailed unpaired Student's t test (G and H). NS, not significant. Data (mean ± SEM) represent ≥2 experiments (A–H).

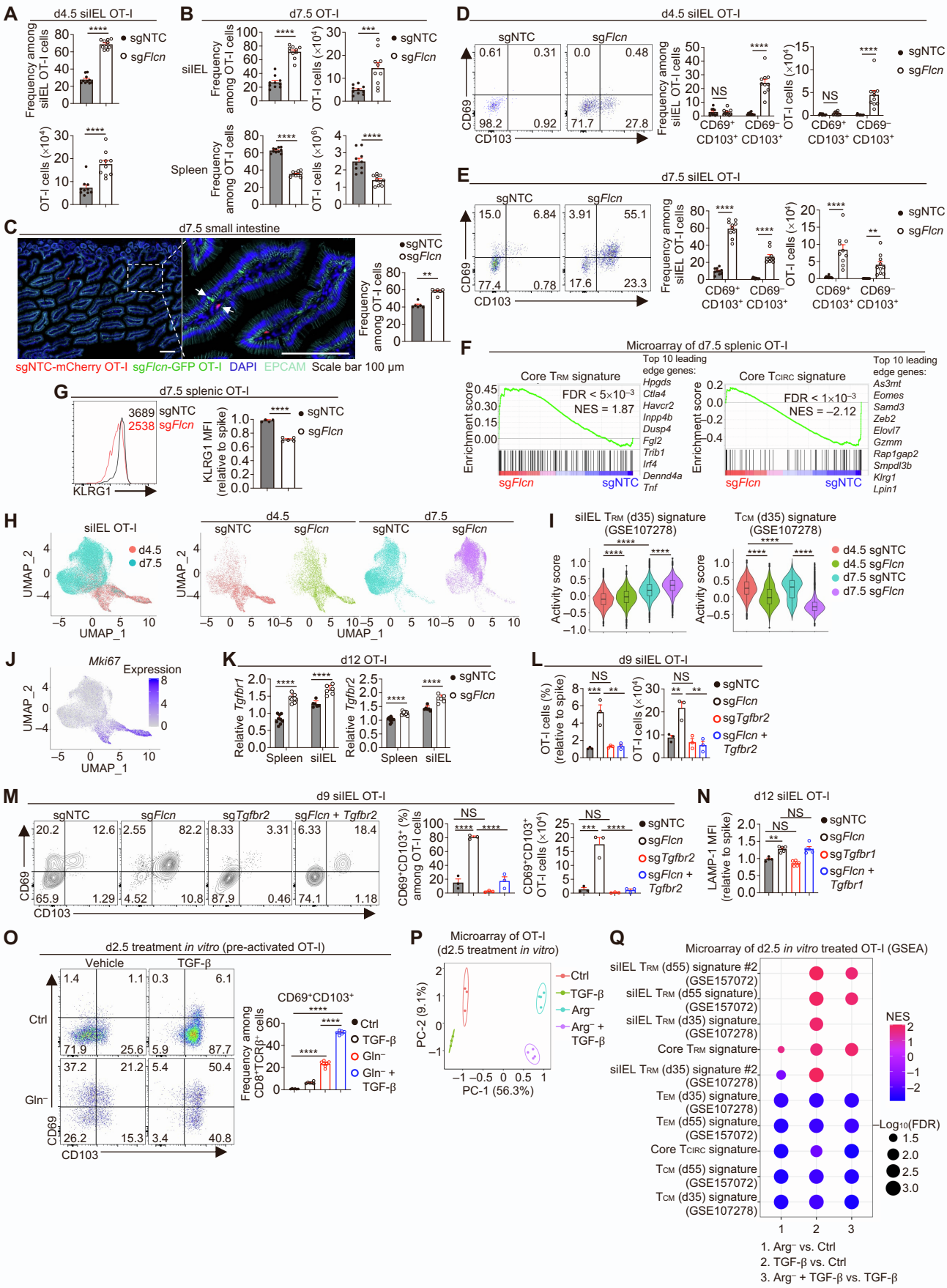
A
d4.5 siIEL OT-I
Frequency among siIEL OT-I cells
OT-I cells (×10⁴)
sgNTC
sgFlcn
B
d7.5 OT-I
siIEL
Spleen
Frequency among OT-I cells
OT-I cells (×10⁴)
OT-I cells (×10⁶)
C
d7.5 small intestine
sgNTC-mCherry OT-I sgFlcn-GFP OT-I DAPI EPCAM Scale bar 100 μm
Frequency among OT-I cells
D
d4.5 siIEL OT-I
CD69
CD103
Frequency among siIEL OT-I cells
OT-I cells (×10⁴)
NS
CD69⁺ CD103⁺
CD69⁻ CD103⁺
E
d7.5 siIEL OT-I
F
Microarray of d7.5 splenic OT-I
Core TRM signature
FDR < 5×10⁻³
NES = 1.87
Top 10 leading edge genes:
Hpgds
Ctla4
Havcr2
Inpp4b
Dusp4
Fgl2
Trib1
Irf4
Dennd4a
Tnf
Core TCIRC signature
FDR < 1×10⁻³
NES = –2.12
Top 10 leading edge genes:
As3mt
Eomes
Samd3
Zeb2
Elovl7
Gzmm
Rap1gap2
Smpdl3b
Klrg1
Lpin1
Enrichment score
G
d7.5 splenic OT-I
KLRG1
KLRG1 MFI (relative to spike)
H
siIEL OT-I
d4.5
d7.5
UMAP_1
UMAP_2
I
siIEL TRM (d35) signature (GSE107278)
TCM (d35) signature (GSE107278)
Activity score
d4.5 sgNTC
d4.5 sgFlcn
d7.5 sgNTC
d7.5 sgFlcn
J
Mki67
Expression
K
d12 OT-I
Relative Tgfbr1
Relative Tgfbr2
Spleen
siIEL
L
d9 siIEL OT-I
OT-I cells (%) (relative to spike)
OT-I cells (×10⁴)
sgTgfbr2
sgFlcn + Tgfbr2
M
d9 siIEL OT-I
sgTgfbr2
sgFlcn + Tgfbr2
CD69⁺CD103⁺ among OT-I cells (%)
CD69⁺CD103⁺ OT-I cells (×10⁴)
N
d12 siIEL OT-I
LAMP-1 MFI (relative to spike)
sgTgfbr1
sgFlcn + Tgfbr1
O
d2.5 treatment in vitro (pre-activated OT-I)
Vehicle
TGF-β
Ctrl
Gln⁻
CD69
CD103
CD69⁺CD103⁺
Frequency among CD8⁺TCRβ⁺ cells
Gln⁻ + TGF-β
P
Microarray of OT-I (d2.5 treatment in vitro)
PC-2 (9.1%)
PC-1 (56.3%)
Arg⁻
Arg⁻ + TGF-β
Q
Microarray of d2.5 in vitro treated OT-I (GSEA)
siIEL TRM (d55) signature #2 (GSE157072)
siIEL TRM (d55 signature) (GSE157072)
siIEL TRM (d35) signature (GSE107278)
Core TRM signature
siIEL TRM (d35) signature #2 (GSE107278)
TEM (d35) signature (GSE107278)
TEM (d55) signature (GSE157072)
Core TCIRC signature
TCM (d55) signature (GSE157072)
TCM (d35) signature (GSE107278)
NES
–Log10(FDR)
1. Arg⁻ vs. Ctrl
2. TGF-β vs. Ctrl
3. Arg⁻ + TGF-β vs. TGF-β

Figure S5. Targeting Flcn promotes early accumulation and programming of siIEL CD8$^+$ T_{RM} cells (related to Figure 5). (A and B) Frequency and number of total OT-I cells transduced with indicated sgRNAs in siIEL at day 4.5 p.i. (A) or in siIEL or spleen at day 7.5 p.i. (B) (n = 10 per group). (C) Confocal images and frequency of sgNTC (mCherry$^+$)- and sg*Flcn* (GFP$^+$)-transduced OT-I cells in the small intestine at day 7.5 p.i. (from dual-color transfer system). White arrows indicate representative sgNTC (red) and sg*Flcn* (green) OT-I cells (n = 3, with two imaging fields quantified per mouse). (D and E) Frequencies and numbers of sgNTC- or sg*Flcn*-transduced CD69$^+$CD103$^+$ and CD69$^-$CD103$^+$ siIEL OT-I cells at day 4.5 (D) or 7.5 (E) p.i. (n = 10 per group). (F) sgNTC (Ametrine$^+$)- and sg*Flcn* (GFP$^+$)-transduced total OT-I cells were sort-purified from the spleen of the same host on day 7.5 p.i. and profiled by microarray assay. GSEA enrichment plots of core T_{RM} and T_{CIRC} gene signatures (see STAR Methods) are shown. (G) KLRG1 expression (relative to spike; based on MFI) on indicated sgRNA-transduced OT-I cells in spleen at day 7.5 p.i. (from dual-color transfer system) (n = 5 per group). (H–J) sgNTC (Ametrine$^+$)- or sg*Flcn* (GFP$^+$)-transduced OT-I cells from siIEL of the same host were sort-purified at days 4.5 and 7.5 p.i. and profiled by scRNA-seq (n = 2 biological replicates per time point, and each replicate was pooled from 2 mice). UMAP plot depicting all siIEL OT-I cells and individual perturbations (H). Violin plots showing activity scores of curated siIEL T_{RM} signature or T_{CM} signature (GSE107278; see STAR Methods) (I). UMAP plot depicting *Mki67* expression (J). (K) Relative *Tgfbr1* and *Tgfbr2* expression in sgNTC- or sg*Flcn*-transduced OT-I cells in spleen or siIEL at day 12 p.i. ($n \geq 6$ samples per group). (L and M) Frequencies (relative to spike) and numbers of indicated sgRNA-transduced total (L) or CD69$^+$CD103$^+$ (M) OT-I cells in siIEL at day 9 p.i. (from dual-color transfer system) (n = 3 per group). (N) Intracellular LAMP-1 expression (relative to spike; based on MFI) in indicated sgRNA-transduced OT-I cells in siIEL at day 12 p.i. (from dual-color transfer system) ($n \geq 4$ per group). (O) Frequency of CD69$^+$CD103$^+$ OT-I cells after naive OT-I cells were cultured *in vitro* with anti-CD3 and anti-CD28 for 48 h, expanded in rhIL-2, rmIL-15, and rmIL-7 for 24 h, and then transferred into Ctrl or Gln$^-$ RPMI medium supplemented with the above cytokines with or without rhTGF-β1 for 2.5 days (n = 6 technical replicates per group). (P and Q) OT-I cells were cultured as indicated in (O), followed by transcriptome profiling. Principal component analysis of the transcriptomes of the indicated groups is shown (P). Bubble plot showing the enriched (red) and reduced (blue) signatures in each of the indicated comparisons, based on GSEA using core T_{RM} and T_{CIRC} signatures (see STAR Methods), and curated T_{RM}, T_{EM}, and T_{CM} signatures (GSE107278 and GSE157072; see STAR Methods) (Q). Two-tailed paired Student's t test (A–C), two-way ANOVA (D, E, and K), two-tailed unpaired Student's t test (G), one-way ANOVA (L–O), or Wilcoxon rank sum test (I). NS, not significant; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Data (mean ± SEM) represent ≥3 (A, B, D, E, G, L, M, and O) or one (C, K, and N) experiments.

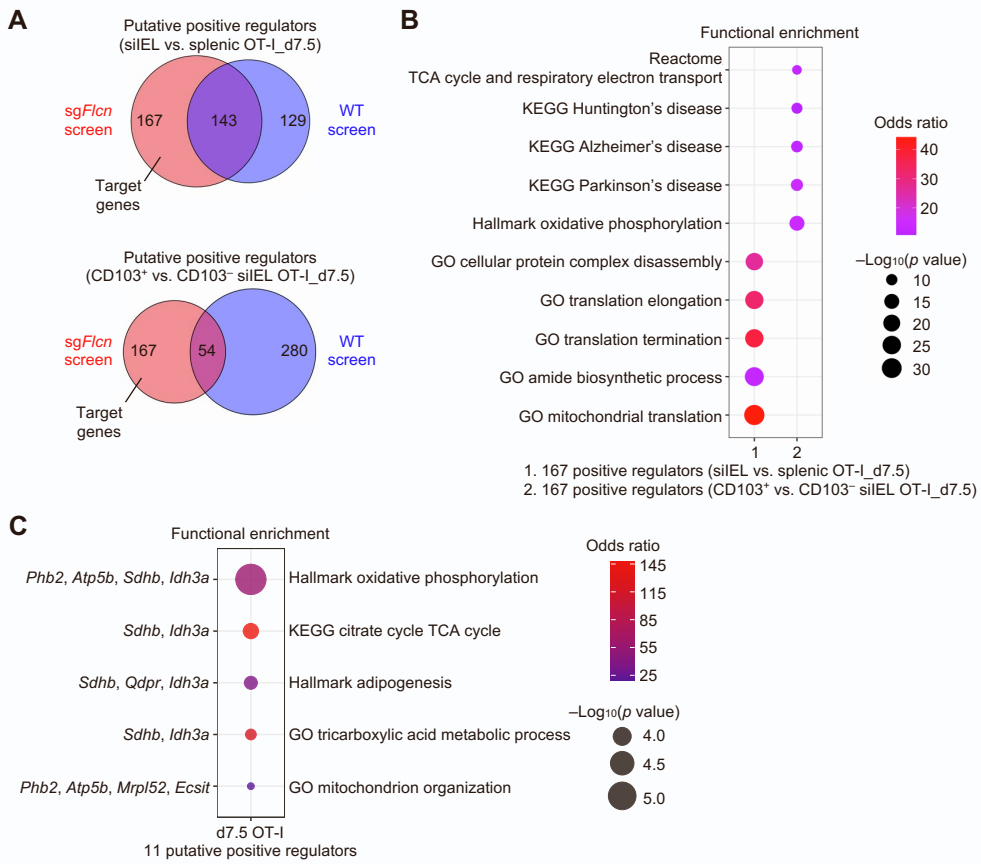

Figure S6. Genetic interaction screen identifies putative positive regulators of Flcn-deficient siIEL OT-I and CD103$^+$ OT-I cell accumulation (related to Figure 6). (A) Venn diagrams depicting the putative positive regulators identified from the *in vivo* CRISPR screen of sg*Flcn*-transduced OT-I cells (described in Figure 6A) and the *in vivo* CRISPR screen of WT OT-I cells (described in Figure 1A). The discrete and overlapping perturbation effects were compared in day 7.5 siIEL OT-I cells (day 7.5 siIEL OT-I cells versus day 7.5 splenic OT-I cells) and day 7.5 siIEL CD103$^+$ OT-I cells (day 7.5 siIEL CD103$^+$ OT-I cells versus day 7.5 siIEL CD103$^-$ OT-I cells) (see STAR Methods). (B) Functional enrichment analysis of the target genes defined in (A) using the Reactome, KEGG, Hallmark, and GO signatures. Bubble plot showing the top 5 enriched pathways in each of the indicated putative positive regulators. (C) Functional enrichment analysis of the 11 genes defined in Figure 6F using the KEGG, Hallmark, and GO signatures. Fisher's exact test (B and C).