Figure S1

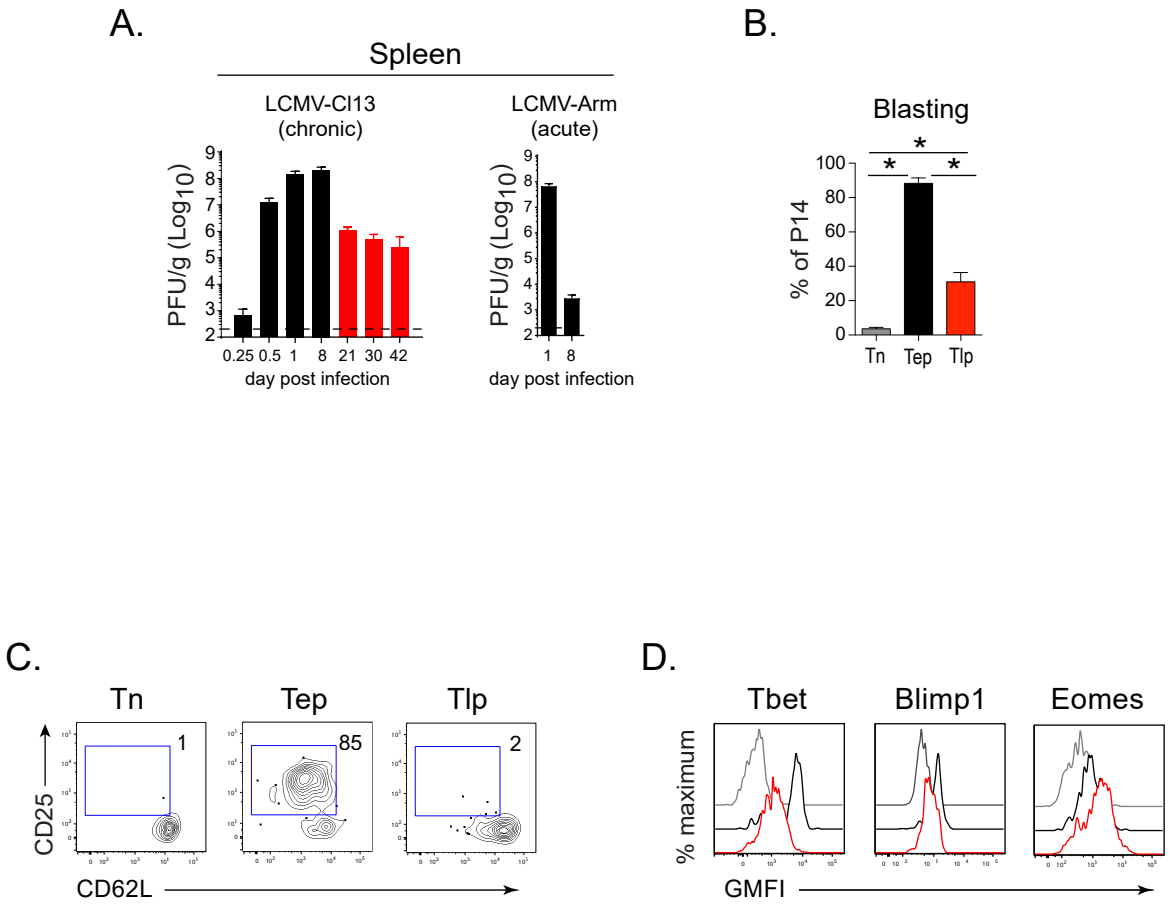

A.
Spleen
LCMV-Cl13
(chronic)
LCMV-Arm
(acute)
PFU/g (Log10)
0.25 0.5 1 8 21 30 42
day post infection
1 8
day post infection
B.
Blasting
% of P14
Tn Tep Tlp
C.
Tn
Tep
Tlp
1
85
2
CD25
CD62L
D.
Tbet
Blimp1
Eomes
% maximum
GMFI

Figure S1. Splenic viral titers during LCMV infection and differentiation profile of virus-specific CD8$^+$ T cells primed in established persistent infection 60 hours after priming. Related to Figure 1

(A) Splenic viral titers during LCMV-Cl13 infection (left) and LCMV-Arm infection (right). Infectious viral titers were determined by plaque assay.

(B) Proportion of blasting Tn, Tep or Tlp P14 cells 60hrs after priming.

(C) Proportion of CD25^{hi} CD62L^{lo} P14 cells 60hrs after priming.

(D) GMFI of Tbet, Blimp1 and Eomes in Tn (gray), Tep (black) and Tlp (red) virus-specific P14 cell.

Data are representative of 3 or more independent experiments with 3-5 mice per group. Error bars indicate standard deviation (SD). Significance was determined by one-way ANOVA, * $p<0.05$.

Figure S2

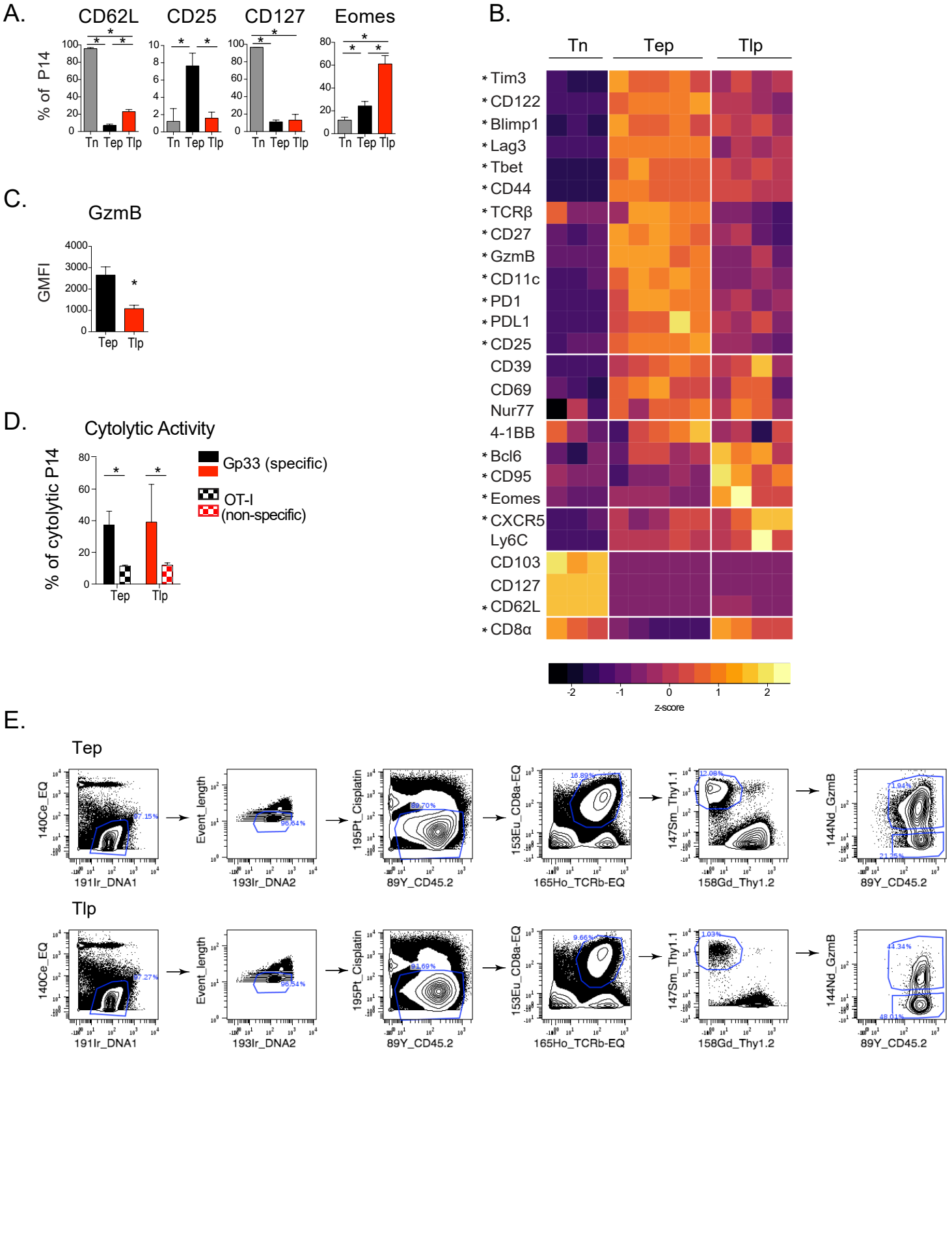

A.
CD62L
CD25
CD127
Eomes
% of P14
Tn Tep Tlp
B.
Tn
Tep
Tlp
* Tim3
* CD122
* Blimp1
* Lag3
* Tbet
* CD44
* TCRβ
* CD27
* GzmB
* CD11c
* PD1
* PDL1
* CD25
CD39
CD69
Nur77
4-1BB
* Bcl6
* CD95
* Eomes
* CXCR5
Ly6C
CD103
CD127
* CD62L
* CD8α
z-score
C.
GzmB
GMFI
Tep Tlp
D.
Cytolytic Activity
% of cytolytic P14
Gp33 (specific)
OT-I
(non-specific)
Tep Tlp
E.
Tep
Tlp
140Ce_EQ
191Ir_DNA1
Event_length
193Ir_DNA2
195Pt_Cisplatin
89Y_CD45.2
153Eu_CD8a-EQ
165Ho_TCRb-EQ
147Sm_Thy1.1
158Gd_Thy1.2
144Nd_GzmB

Figure S2. Virus-specific CD8$^+$ T cells primed in an established persistent infection undergo an alternative pathway of transcriptional and effector differentiation 8 days after priming. Related to Figure 2

(A) Proportion of CD62L, CD25, CD127 and Eomes positive Tn, Tep and Tlp P14 T cells 8 days after priming.

(B) Heat map depicts MSI or GMFI of indicated extracellular markers and transcription factors derived from CyTOF and flow cytometry. Rows are scaled by z-score. *,represents $p<0.05$ between Tep and Tlp cells. Significance was calculated by t-test.

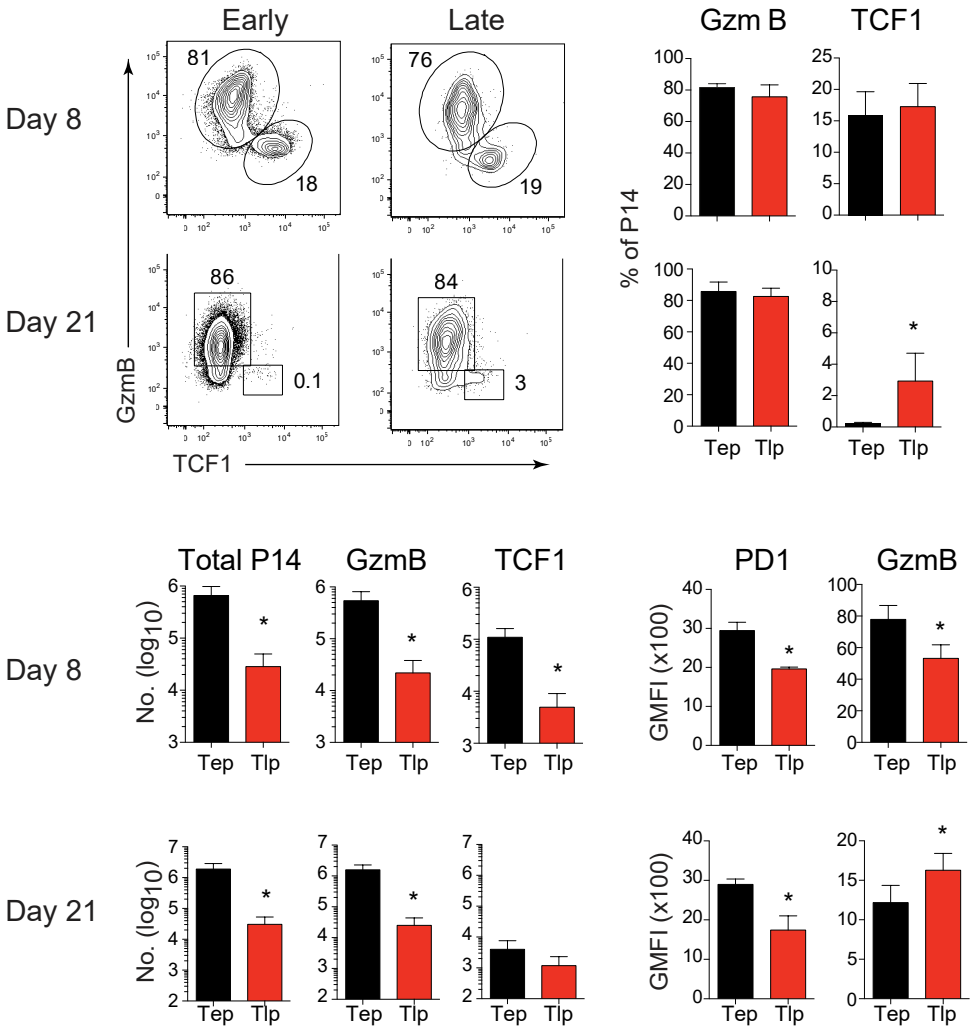
(C) Bar graph depicts the GMFI of GzmB$^+$ virus-specific P14 T cells.

(**D**) Percentage of cytolytic Tep or Tlp P14 cells to targets labeled with LCMV-GP$_{33-41}$ (solid) or non-specific OT-I peptide (patterned).

(**E**) Back gating of GzmB$^+$ and GzmB$^-$ Tep and Tlp populations by mass cytometry.

Data are representative of 3 or more independent experiments with 3-5 mice per group. Error bars indicate SD. Significance was determined by t-test or one-way ANOVA (Figure A). *, $p<0.05$.

A.


Early
Late
Gzm B
TCF1
Day 8
Day 21
81
18
76
19
86
0.1
84
3
GzmB
TCF1
% of P14
Tep
Tlp
Total P14
GzmB
TCF1
PD1
GzmB
No. (log10)
GMFI (x100)

B.

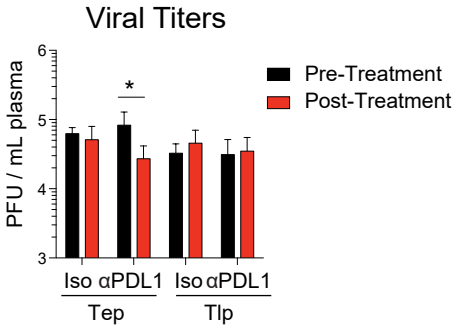

Viral Titers
Pre-Treatment
Post-Treatment
PFU / mL plasma
Iso
αPDL1
Tep
Tlp

Figure S3. Characterization of liver-infiltrating Tep and Tlp, and viral titers pre- and post-anti-PDL1 blockade. Related to Figure 3

(**A**) $CD8^+$ P14 Tep and Tlp cell responses were analyzed in the liver 8 days and 21 days following transfer. Flow plots and bar graphs show both proportion and number of $GzmB^+$ and $TCF1^+$ P14 Tep or Tlp cell subsets. Bar graphs also show GMFI of PD1 and GzmB in P14 T cells.

(B) Mice were $CD4^+$ depleted prior to LCMV infection to maintain high viral titers. Graph shows plasma viral titers pre- (day 20 after priming) and post- (day 35 after priming) isotype or anti-PDL1 antibody treatment.

Data are representative of 2 independent experiments with 5 mice per group. Error bars indicate SD. Significance was determined by t-test. *, $p<0.05$.

Figure S4

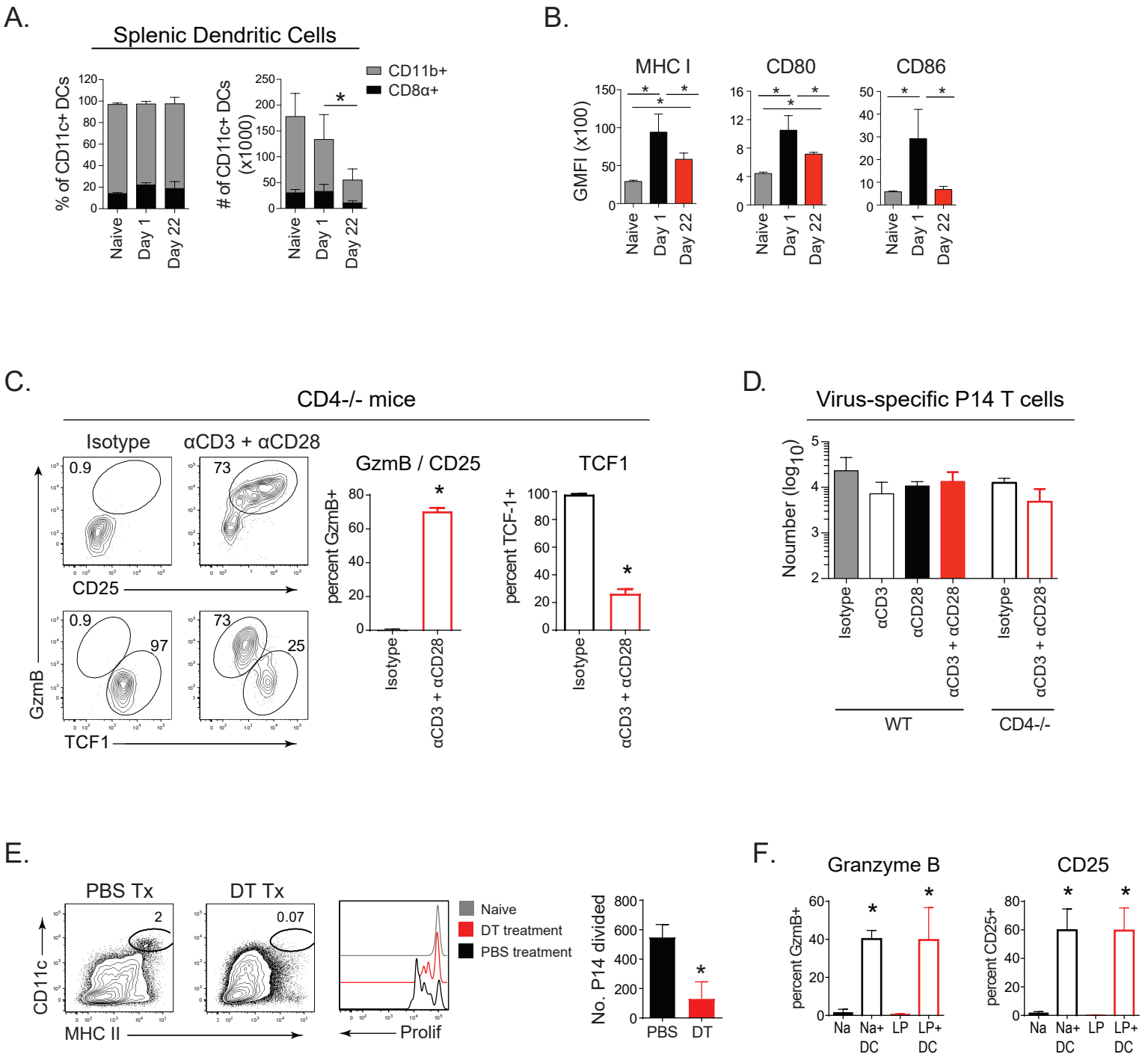

A.
Splenic Dendritic Cells
% of CD11c+ DCs
# of CD11c+ DCs (x1000)
CD11b+
CD8α+
Naive
Day 1
Day 22
B.
MHC I
CD80
CD86
GMFI (x100)
C.
CD4-/- mice
Isotype
αCD3 + αCD28
CD25
GzmB
TCF1
GzmB / CD25
percent GzmB+
TCF1
percent TCF-1+
D.
Virus-specific P14 T cells
Number (log10)
αCD3
αCD28
WT
CD4-/-
E.
PBS Tx
DT Tx
CD11c
MHC II
Prolif
Naive
DT treatment
PBS treatment
No. P14 divided
PBS
DT
F.
Granzyme B
CD25
percent GzmB+
percent CD25+
Na
Na+ DC
LP
LP+ DC

Figure S4. Role of dendritic cells and T cell stimulatory signal strength in Tlp cell differentiation. Related to Figure 4

(A) Proportion and number of $CD8\alpha^+$ and $CD11b^+$ DCs in naïve mice (day 0) and at 1 day and 22 days after LCMV-Cl13 infection (i.e., 1 day after early and late priming).

(B) GMFI of MHC I, CD80 and CD86 on macrophages ($CD45^+$, $CD3^-$, $NK1.1^-$, $B220^-$, $CD11c^-$, $Ly6G^-$, $CD11b^+$) in naïve mice (day 0, gray) and at 1 day (black) and 22 days (red) after LCMV-Cl13 infection.

(C) $CD8^+$ P14 T cells were transferred into $CD4^{-/-}$ mice that had been infected 21 days earlier with LCMV-Cl13. Four hours after P14 T cell transfer, mice were treated i.p. with anti-CD3 and anti-CD28 or isotype control. Flow plots and bar graphs depict the frequency of $GzmB^+$ $CD25^+$ effector and $TCF\text{-}1^+$ memory-like P14 cells 60 hours after transfer.

(D) Bar graphs represent the number of Tlp P14 cells in WT and $CD4^{-/-}$ mice 60 hours after priming and treatment with isotype, anti-CD3 and/or anti-CD28 agonist antibodies.

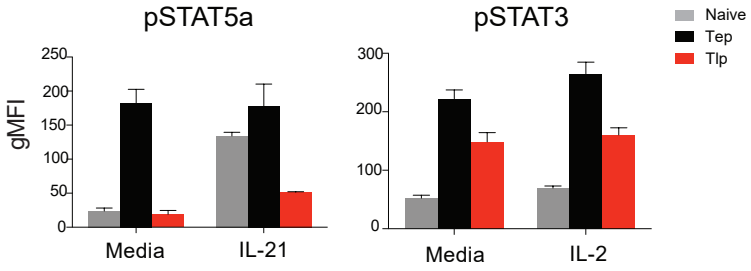
(E) DT or control PBS was injected into LCMV-Cl13-infected CD11c-DTR mice to deplete $CD11c^+$ DCs prior to late priming of P14 T cells (on D20 after LCMV-Cl13 infection). Flow plots depict DC depletion with DT treatment at time of sacrifice (40 hours after P14 priming). Histogram depicts division of late primed P14 T cells with DT treatment (red) or with control PBS treatment (black). Bar graph show the number of divided P14 T cells.

(F) $CD8^+$ P14 T cells were transferred into naïve mice (Na) or into mice infected 21 days before with the LCMV-Cl13(V35A) variant (Tlp = LP). At the time of priming, a group of naïve (Na + DC) and LCMV-Cl13(V35A) infected mice (LP + DC) received $GP_{33\text{-}41}$ peptide loaded bmDC (open bars). Bar graphs represent the percent of GzmB and CD25 expressing P14s 60 hours after transfer.

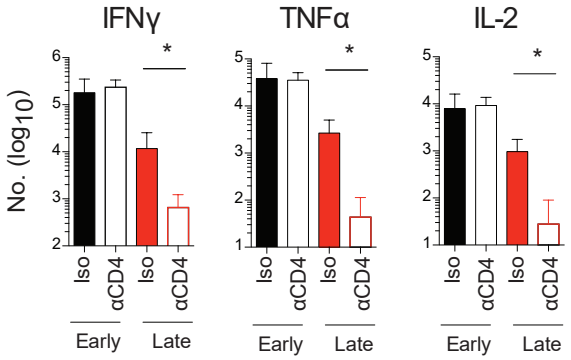
Data are representative of 2 independent experiments with 4-5 mice per group. Error bars indicate SD. Significance was determined by t-test. *, $p<0.05$

Figure S5

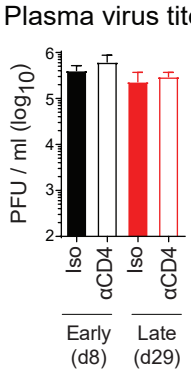
A.



B.



C.



D.

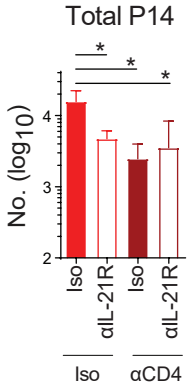


Figure S5. $CD4^+$ T cell help and IL-21 mediated effects on Tep and Tlp cells. Related to Figure 6

(A) Bar graphs depict the GMFI of phosphoSTAT5a and phoshoSTAT3 in P14 Tn, Tep or Tlp cells 60 hours after priming in vivo and then cultured without stimulation (media) or following IL-2 or IL-21 treatment for 30 minutes.

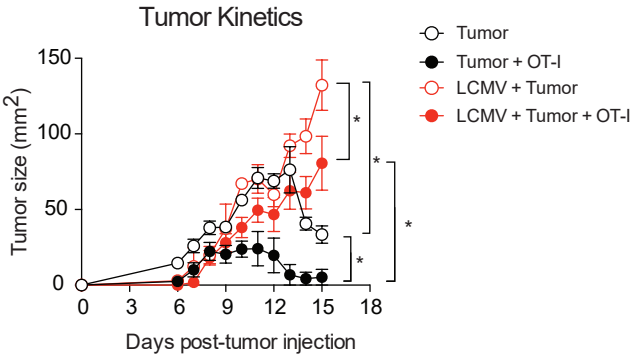
(**B-C**) Mice were either $CD4^+$ depleted (open bars) or isotype control treated (shaded bars) prior to LCMV-Cl13 infection. $CD8^+$ P14 T cells were transferred into naïve mice that were immediately infected with LCMV-Cl13 (black) or into mice that had been infected with LCMV-Cl13 21 days before (red). **(B)** Bar graphs depict the number of IFNγ, TNFα and IL-2 producing P14 T cells and **(C)** the plasma virus titers at day 8 (early prime) and day 29 (late prime) in isotype treated mice (i.e., $CD4^+$ non-depleted) or $CD4^+$ depleted mice.

(D) Mice were either $CD4^+$ depleted or isotype control treated prior to LCMV-Cl13 infection. $CD8^+$ P14 T cells were transferred into mice that had been infected with LCMV-Cl13 21 days before. Mice were either treated with isotype control (shaded bars) or anti-IL-21R blocking antibody (open bars) starting at day 20 through sacrifice at day 29. Bar graph depicts the number of total P14 T cells at day 8 post priming (day 29 of LCMV-Cl13 infection).

Data are representative of 2 independent experiments with 4-5 mice per group. Error bars indicate SD. Significance was determined by t-test *, $p<0.05$.

Figure S6

A.


Tumor Kinetics
Tumor
Tumor + OT-I
LCMV + Tumor
LCMV + Tumor + OT-I
Tumor size (mm2)
150
100
50
0
0 3 6 9 12 15 18
Days post-tumor injection
*
*
*
*

B.

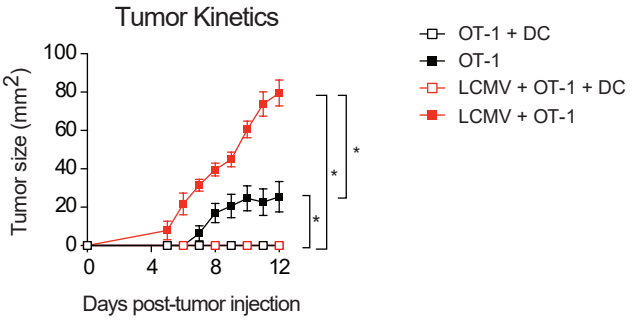

Tumor Kinetics
OT-1 + DC
OT-1
LCMV + OT-1 + DC
LCMV + OT-1
Tumor size (mm2)
100
80
60
40
20
0
0 4 8 12
Days post-tumor injection
*
*
*

Figure S6. Tumor kinetics in chronically infected mice, effect of OT-I cells and dendritic cells. Related to Figure 7

(A) Naïve (black) or mice infected with LCMV-Cl13 for 21 days (red) either received OT-I cells (shaded circles) or no cells (open circles). One day later EG7 tumors were injected into all mice. Graph depicts tumor growth kinetics on the indicated day after tumor injection.

(B) OT-I cells were injected into naïve mice (black) or mice that had been infected for 21 days with LCMV-Cl13 (red). One day later EG7 tumors were injected into all mice either alone (closed squares) or in combination with OVA-peptide pulsed DCs (open squares). Graph depicts tumor growth kinetics on the indicated day after tumor injection.

Data are representative of 2 independent experiments with 4-5 mice per group. Error bars indicate SD. Statistical differences between groups was measured by two-way ANOVA * $p<0.05$.

Antibody Specificity	Metal Tag	Clone	Manufacturer	Catalog number
CD45	89Y	30-F11	Fluidigm (d)	3089005B
Ly6c	115Ln	HK14	BioLegend	128002
CD44	141Pr	IM7	BioLegend	103002
CXCR5	142Nd	145502	BioLegend	L138D7
GzmB	144 Nd	GB11	Thermofischer	MA1-80734
Eomes	146 Nd	Dan11mag	eBioscience	14-4875-80
Thy1.1	147Sm	HIS51	eBioscience	14-0900-85
CD11b	148Nd	M1/70	Fluidigm (d)	3148003B
CD69	149Sm	H1.273	BioLegend	104502
Ly6G	150Nd	1A8	BioLegend	127602
CD25	151Eu	3C7	Fluidigm (d)	3151007B
BCL6	152Sm	K112.91	BD Bioscence	561520
CD8a	153Eu	53-6.7	Fluidigm (d)	3153012B
CD103	155Gd	M290	BD Bioscience	553699
PDL1	156Gd	M1H5	eBioscience	14-5982-85
Thy1.2	158Gd	53-21	Thermofischer	14-0902-82
CD39	159Tb	24DMS1	eBioscience	14-0391-82
CD4	160Gd	rm4-5	BioLegend	100506
Tbet	161Dy	4B10	Fluidigm (d)	3161014B
TCRβ	165Ho	109202	BioLegend	H57-597
B220	166Er	RA3-6B2	Thermofischer	14-0452-85
SLAM	169Tm	TC15-12F12.2	BioLegend	115933
Nk1.1	170Er	PK136	Fluidigm (d)	3170002B
CD95	172Yb	15A7	eBioscience	14-0951-85
CD11c	174Yb	N418	BioLegend	117302
PD1	175Lu	RMP-30	BIoLegend	109104
MHC-II	209Bi	M5/114.15.2	Fluidigm (d)	3209006B

Table S1. Antibody panel for mass cytometry. Related to Figure 1 and Figure 2

Antibodies that were purchased directly conjugated are labeled with (d). Other antibodies were conjugated with Fluidigm's Maxpar conjugation kit according to manufacturer's instructions.

Pathway in Paper	Pathway Name	Database	Accession number
Myc targets	HALLMARK_MYC_TARGETS_V1	MSIGDB_C2	HALLMARK_MYC_TARGETS_V1
Ribosome Biogenesis	RIBOSOME BIOGENESIS	GOBP	GO_0042254
Translation	TRANSLATION	REACTOME	R-HSA-72766.3
MTORC1 signaling	HALLMARK_MTORC1_SIGNALING	MSIGDB_C2	HALLMARK_MTORC1_SIGNALING
DNA replication	DNA REPLICATION	REACTOME DATABASE ID RELEASE 61	69306
Glycolysis	GLYCOLYSIS	REACTOME DATABASE ID RELEASE 61	70171
Integrin family interactions	INTEGRIN FAMILY CELL SURFACE INTERACTIONS	PID NCI-NATURE	INTEGRIN FAMILY CELL SURFACE INTERACTIONS
Regulation of cell motility	REGULATION OF CELL MOTILITY	GOBP	GO_2000145
Signaling by focal adhesion kinase	SIGNALING EVENTS MEDIATED BY FOCAL ADHESION KINASE	PID NCI-NATURE	SIGNALING EVENTS MEDIATED BY FOCAL ADHESION KINASE
Actin cytoskeleton organization	REGULATION OF ACTIN CYTOSKELETON ORGANIZATION	GOBP	GO_0032956
Cell-cell adhesion	Cell-Cell adhesion	GOBP	GO_0098609

Table S2. Selected pathways from Enrichment Map Gene Set. Related to Figure 5B

Pathway Name in Paper	Standard Name
Effector vs memory cells CD8 T cells	GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_DN GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_UP
Acute vs chronic LCMV CD8 T cells	GSE30962_ACUTE_VS_CHRONIC_LCMV_PRIMARY_INF_CD8_TCELL_DN GSE30962_ACUTE_VS_CHRONIC_LCMV_PRIMARY_INF_CD8_TCELL_UP
IL-2 treated CD8 T cell	GSE39110_UNTREATED_VS_IL2_TREATED_CD8_TCELL_DAY3_POST_IMMUNIZATION_DN
IL-21 treated CD8 T cell	GSE19198_CTRL_VS_IL21_TREATED_TCELL_24H_DN

Table S3. Selected pathways from immunesigDB. Related to Figure 5C and 5E