Supporting Information

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SI Methods

Tetramers and Peptides. Peptides specific for lymphocytic choriomeningitis virus (LCMV) T-cell epitopes $GP_{33–41}$ (KAVYNFATC), $GP_{34–41}$ (AVYNFATC), $GP_{276–286}$ (SGVENPGGYCL), and $NP_{396–404}$ (FQPQNGQFI) (1) were purchased from 21st Century Biochemicals. Fluorescently labeled MHCI tetramers recognizing D^bGP33 were purchased from Beckman Coulter. D^bGP34, D^bGP276, and D^bNP396 tetramers were obtained from the National Institutes of Health Tetramer Core Facility.

Antibodies and Flow Cytometry. Fluorescently labeled antibodies were purchased from BD Biosciences, eBioscience, and BioLegend. Flow cytometric data were acquired with LSR II, LSR Fortessa, and FACS Canto cytometers (BD Biosciences) by using FACS Diva software. Data were analyzed using by Flowjo software v8.8.6 (Treestar).

Cell Purification and Adoptive Transfers. Dendritic cells used for cell transfer experiments were isolated by magnetic cell separation (MACS) technology with anti-CD11c magnetic microbeads (Miltenyi Biotec) according to manufacturer's instructions, from collagenase-DNase treated spleens of 6- to 10-wk-old uninfected B6.FasKI or B6.CD11c-Cre.FasKI mice. Naïve CD8 T cells used in transfer experiments were obtained as follows. Splenocytes from P14 and OT1 mice were labeled with a mixture of biotinylated antibodies recognizing CD44, CD19, CD4, CD11b, CD11c, and NK1.1. The cells were then washed and incubated with streptavidin microbeads for 15 min at 4 °C, before being loaded onto MACS separation columns. Cells that did not bind to the columns were collected and were typically 99% naïve $CD44^{lo}$ CD8 T cells. P14 cells were then transferred at 1×10^5 cells per mouse for LCMV-Armstrong infection experiments or labeled with 5 μM CFSE (Molecular Probes) for 5 min at room temperature in PBS, washed, mixed 1:1 (8×10^5 cells each) with CFSE-labeled OT1 cells and transferred i.v. into recipient mice. LCMV-activated P14 T cells were isolated by staining splenic cells from LCMV-infected recipient mice with biotinylated anti-CD45.1 antibody, followed by incubation with streptavidin microbeads and purification by MACS selection as described.

Intracellular Cytokine Assay. Single-cell suspensions from spleens and lymph nodes were plated at 2×10^6 cells per well in 96-well plates and stimulated for 5 h at 37 °C with 1 μM viral peptide in the presence of brefeldin A and 0.2 U/mL recombinant IL-2. Stimulated cells were then resuspended in FACS buffer (PBS, 2% FCS, and 0.1% NaN_3) and stained for 25 min at 4 °C with fluorescently tagged antibodies. Subsequently, cells were washed, permeabilized by using BD Cytofix/Cytoperm solution, and stained with monoclonal antibodies for various cytokines according to manufacturer's instructions (BD Biosciences).

T-Cell Activation. BMDCs from B6.CD11c-Cre.FasKI and B6.FasKI mice were grown for 5 d in GM-CSF as described, treated with Mitomycin C (Sigma, 25 μg/mL for 30 min at 37 °C), washed three times, and used as antigen-presenting cells (10^4 cells per well of a U-bottom 96-well plate) with 10^5 MACS-purified T cells (using anti-CD4 or anti-CD8 beads for positive selection) and varying amount of OVA (Sigma) in Click's medium (Irvine Scientific) for 72 h. Eighteen hours before harvesting, 1 μCi of ^{3}HThymidine was added per well to measure T-cell proliferation.

Antinuclear Antibody Staining. Antinuclear antibody staining was done exactly as described (2).

Microarray Analysis. Spleens from LCMV-clone 13–infected B6.FasKI and B6.CD11c-Cre.FasKI mice were collagenase-DNase digested and sorted flow cytometrically to isolate DCs. RNA isolated from three samples (each sample obtained from spleens of three mice) each of FACS-sorted B6.FasKI and B6.CD11c-Cre.FasKI DCs was hybridized to Illumina Mouse WG-6 Expression BeadChip (Illumina) arrays at the Functional Genomics Facility core at the University of Chicago. Probe level data were normalized by using $\log_2$ average signal intensity and summarized to gene expression by using median of probe values. Gene expression data were visualized by using a volcano plot.

In addition to looking for significantly ($P < 0.05$) and differentially regulated genes (>1 log fold), we applied a set of heuristic filters to further identify genes relevant to dendritic cells (DC). First, we restricted our analysis to genes that were also subjected to expression profiling in the ImmGen microarray database (immgen.org). Second, we use a statistical filter to identify genes that were overexpressed, in either all DCs or Plasmacytoid DCs, relative to all other cell types in ImmGen. We assessed overexpression by performing a one-sided two-sample t test, testing against the alternative hypothesis that the mean expression of a gene in DCs is greater than the mean across other cell types. We corrected for multiple hypothesis testing (3) and reported genes $q < 0.05$.

1. van der Most RG, et al. (1998) Identification of Db- and Kb-restricted subdominant cytotoxic T-cell responses in lymphocytic choriomeningitis virus-infected mice. *Virology* 240(1):158–167.
2. Stranges PB, et al. (2007) Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity. *Immunity* 26(5):629–641.
3. Storey J (2002) A direct approach to false discovery rates. *J R Stat Soc Series B Stat Methodol* 64(3):479–498.

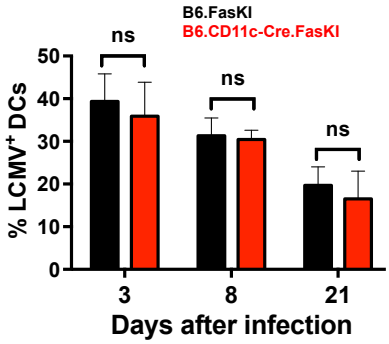

Fig. S1. Percentage of LCMV-clone 13–infected splenic DCs from infected mice of indicated genotypes. Data are compiled from two independent experiments, 3–8 mice per time point. Error bars represent SEM.

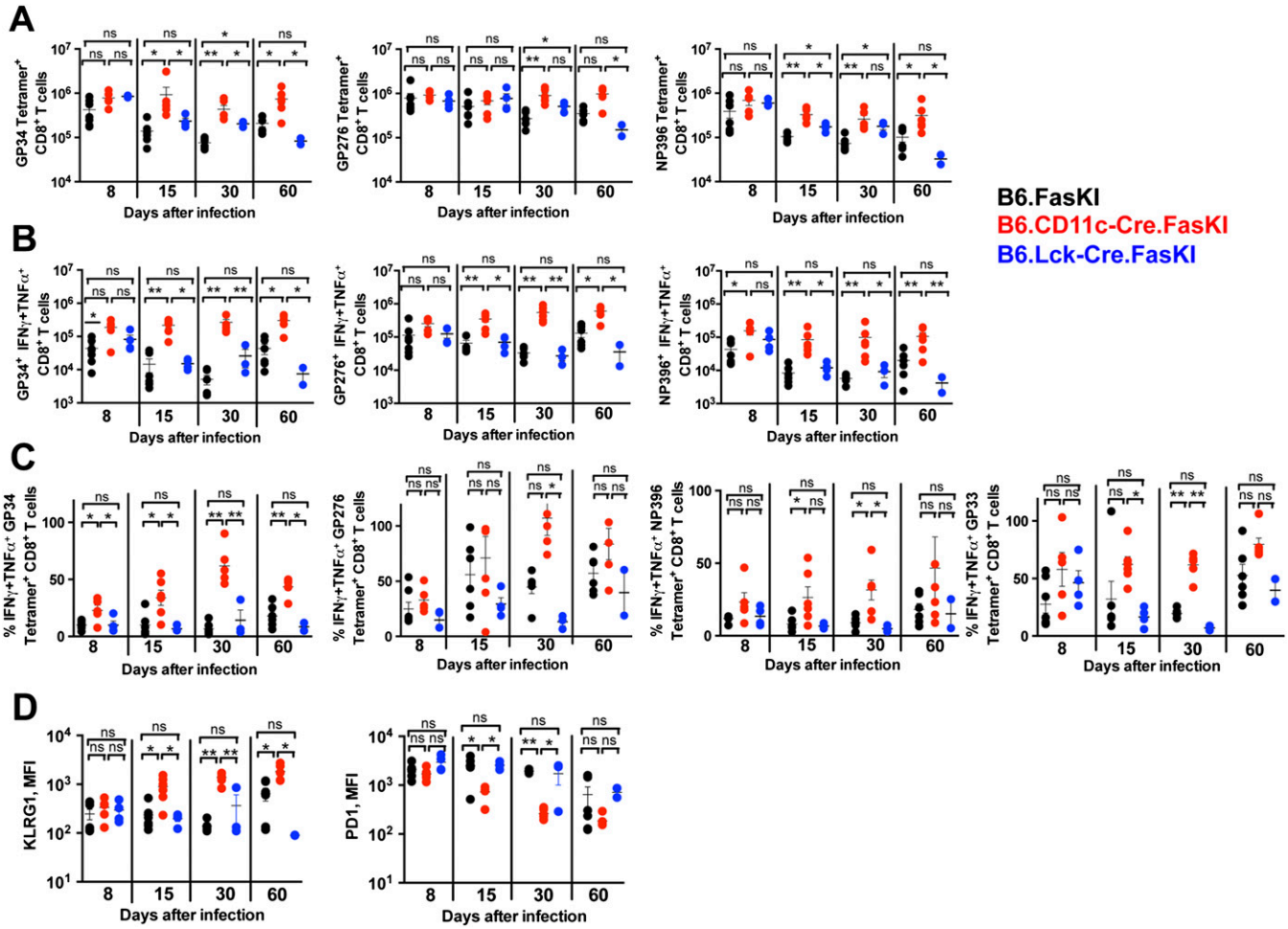

Fig. S2. Analysis of LCMV-specific T cells after infection with LCMV-clone 13. (*A*) LCMV-clone 13–infected mice were analyzed at indicated time points for D^b-GP34, GP276, and NP396 tetramer-positive T-cell numbers. (*B*) IFN-γ and TNF-α double-producing T cells numbers were identified after 5 h in vitro stimulation with indicated LCMV peptides. (*C*) Percent functional T cells were calculated as the frequency of IFN-γ and TNF-α double-producing T cells of the total tetramer-positive cells for each epitope. (*D*) Expression levels of KLRG1 and PD1 were calculated and expressed as median fluorescence intensity (MFI) ± SEM. Data are compiled from two independent experiments with 2–7 mice per time point. Error bars represent SEM. Statistical significance was calculated by ordinary one-way ANOVA (*$P < 0.05$; **$P < 0.005$; ns, not significant).

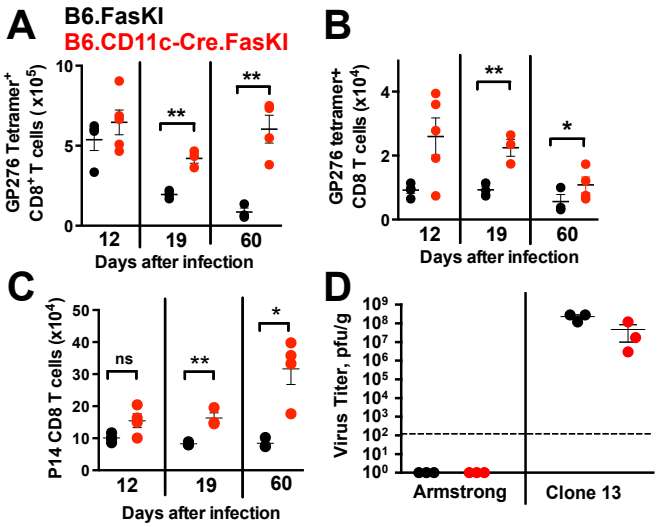

Fig. S3. The fate of T cells specific to LCMV in mice with Fas-sufficient and Fas-deficient DCs. B6.FasKI and B6.CD11c-Cre.FasKI mice infected with LCMV-Armstrong were analyzed at 12, 19, and 60 d after infection, for total numbers of D^b GP276 tetramer-positive T-cell numbers in the spleen (*A*) and lymph nodes (*B*). (*C*) B6.FasKI and B6.CD11c-Cre.FasKI mice were injected with 1×10^5 naïve P14 (GP33-specific) T cells ($CD45.1^+$) 1 d before infection with LCMV-Armstrong and were analyzed for splenic P14 T-cell numbers. Data represent 3–4 mice per time point. (*D*) B6.FasKI and B6.CD11c-Cre.FasKI mice infected with LCMV-Armstrong cleared infection by day 8.

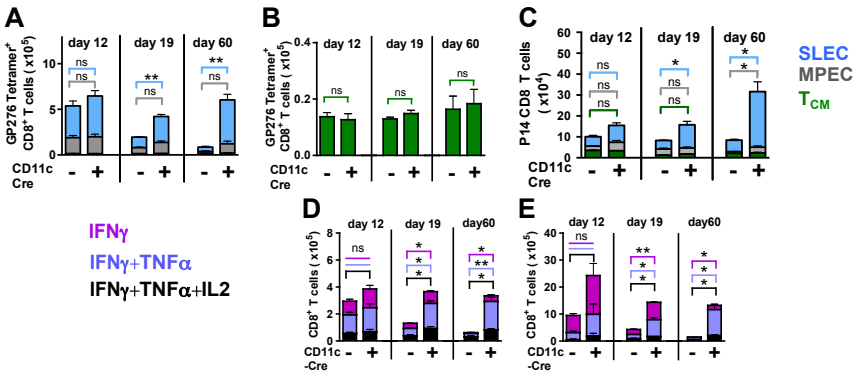

Fig. S4. The fate of effector and memory T cells specific to LCMV in mice with Fas-sufficient and Fas-deficient DCs. (*A* and *B*) Composition of memory T cells: SLECs ($CD44^{hi}$, $KLRG1^{hi}$, $CD127^{lo}$, $CD62L^{lo}$) in blue, MPECs ($CD44^{hi}$, $KLRG1^{lo}$, $CD127^{hi}$, $CD62L^{lo}$) in gray, and T_{CM} ($CD44^{hi}$, $CD127^{hi}$, $KLRG1^{lo}$, $CD62L^{hi}$) in green, in the spleens of Cre^- and Cre^+ mice. (*C*) Same for GP33-reactive P14 cells transferred into LCMV-Armstrong–infected Cre^- and Cre^+ mice. Data represent 3–4 mice per time point. (*D* and *E*) Splenic $CD8^+$ T cells from B6.FasKI and B6.CD11c-Cre.FasKI mice infected with LCMV-Armstrong were analyzed at indicated time points for number of splenic $CD8^+$ T cells producing IFN-γ, IFN-γ + TNF-α, or IFN-γ + TNF-α + IL-2 in response to 5 h in vitro stimulation with GP276 (*D*) or NP396 peptide (*E*). Data are compiled from two independent experiments, 3–5 mice per time point. *P* values comparing groups of mice were obtained by unpaired Student *t* test. Error bars represent SEM (*$P < 0.05$; **$P < 0.005$; ns, not significant).

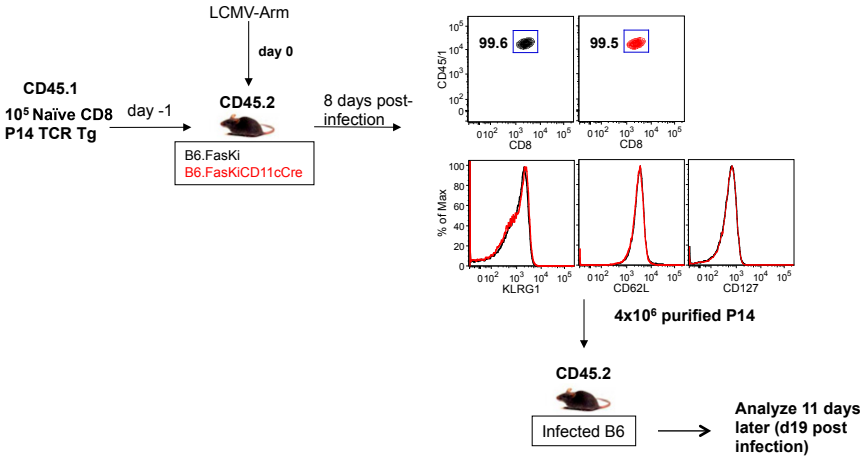

Fig. S5. Scheme for P14 T-cell transfer experiment shown in Fig. 4*F*.

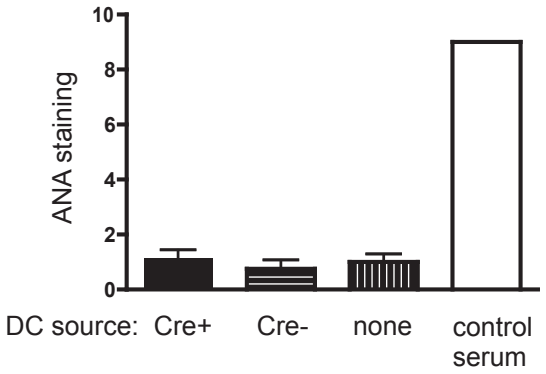

Fig. S6. Staining of permeabilized HEP-2 cells with sera from the recipients of indicated splenic DCs obtained 21 d after DC transfer. There are 3–4 mice per group. Error bars, SEM. Control serum was from B6.lpr mouse. The relative scale was used as published (1).

1. Stranges PB, et al. (2007) Elimination of antigen-presenting cells and autoreactive T cells by Fas contributes to prevention of autoimmunity. *Immunity* 26(5):629–641.

Table S1. Exhaustive list of differentially expressed genes between B6.FasKI and B6.CD11c-Cre.FasKI DCs

Gene	*P* value	Fold change	DCs	pDC
H2-Ob	0.0004	2.2771		Yes
Cd2	0.003	2.4813		
Gimap1	0.0049	2.1285		
Cd37	0.0079	2.0225		
Fcrla	0.008	2.2205	Yes	Yes
Ubd	0.0092	2.53		
H2-M2	0.0094	3.573		Yes
Sct	0.0095	3.9637		Yes
Cd19	0.0117	2.0496		
Gimap7	0.0133	2.284		
Msc	0.0134	3.2174		
2010001M09Rik	0.0139	4.6618	Yes	
Ly6d	0.0143	2.03	Yes	
Jakmip1	0.0145	2.3167		
Blk	0.0146	2.107		
Ighg	0.0166	3.2143		
Cd72	0.0166	2.234		
Faim3	0.0185	2.6733		
Pou2af1	0.0185	2.6401		
Cd79b	0.0236	2.178		
AA467197	0.0251	2.9428		Yes
Igl-V1	0.0252	3.391		Yes
Fah	0.0259	4.0618		
Igh-6	0.0274	3.9011		
Sspn	0.0284	2.4888		
AI324046	0.0308	6.1522		
Prg2	0.0324	2.2093		
Tnfrsf4	0.0374	2.3174		Yes
eGFP	**0.00000**	**31.4**		
Lyz1	0.0127	−2.8155		Yes

Up-regulation of eGFP, which is a part of the CD11c-Cre construct, in the B6.CD11cCre.FasKi DCs serves as a control (highlighted in bold). Genes reported to be expressed in (but not restricted to) DCs or plasmacytoid DCs are shown.