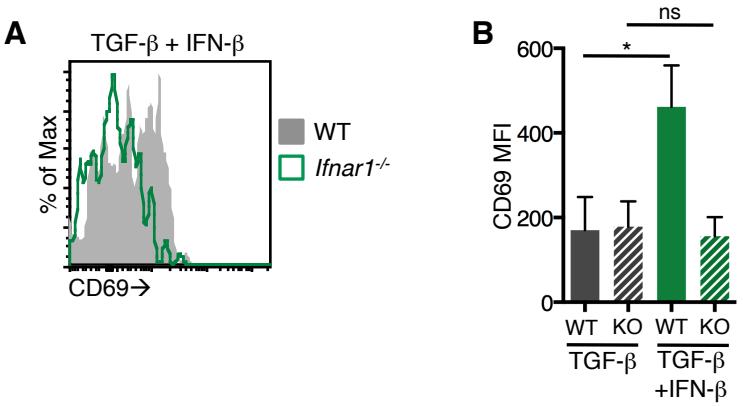

Figure S1, related to Figure 1. Cytokine-mediated CD69 expression is T cell intrinsic. Mice received an equal number of WT and *Ifnar1*$^{-/-}$ OT-I T cells and were infected with Yptb-OVA. Seven days post infection, cells from the MLNs and spleen were cultured with the indicated cytokines for 20 hours and CD69 expression was analyzed. (A) Representative histograms of CD69 expression on WT (shaded histogram) and *Ifnar1*$^{-/-}$ (open histogram) OT-I T cells stimulated with TGF-β and IFN-β. (B) CD69 MFI from triplicate samples from one representative experiment of 2. Error bars represent SD. *$p<0.05$ by unpaired t-test.

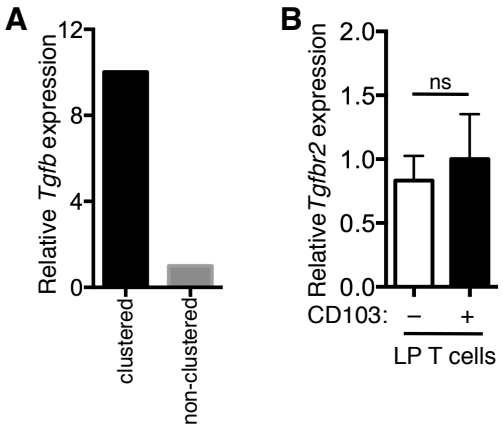

Figure S2, related to Figure 2. *Tgfb* expression is elevated in proinflammatory microenvironments.
(A) Laser capture microdissection was used to isolate areas of the LP with and without $CD8^+$ T cell clustering. RNA was isolated and *Tgfb* levels were determined by qRT-PCR and normalized to *actb* expression. Data shown are from one representative experiment of 2. (B) $CD103^+$ and $CD103^-$ OT-I T cells were sorted from the LP at the indicated timepoints and expression of *Tgfbr2* was determined by qRT-PCR. Values are expressed relative to *actb* expression. Mean and SD represent technical replicates, representative of 2 or more experiments. No significant difference by unpaired t-test.

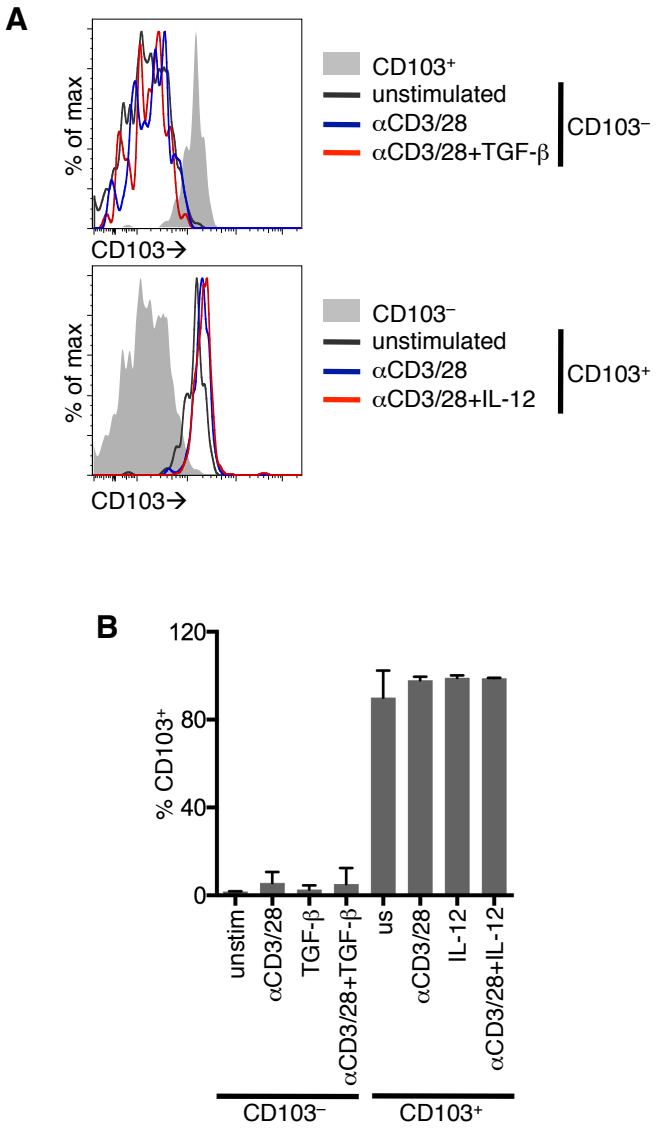

Figure S3, related to Figure 3. The phenotype of Trm subsets is stable *ex vivo.* CD103^{+}CD69^{+} and CD103^{-}CD69^{+} CD8^{+} T cells were sorted from the LP greater than 30 days after Yptb-OVA infection. Sorted cells were left unstimulated (unstim) or stimulated with α-CD3/CD28 beads with or without IL-12 or TGF-β for 24 hours. Representative histograms from a single experiment (A) and the percent of CD103^{+} cells pooled from two experiments (B) are shown.

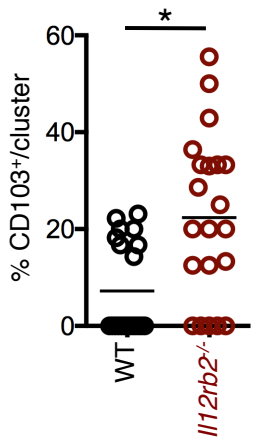

Figure S4, related to Figure 5. *Il12rb2*$^{-/-}$ OT-I T cells aberrantly express CD103 in LP T cell clusters. The percent of CD103^{+} WT or *Il12rb2*$^{-/-}$ OT-I T cells in individual LP T cell clusters was determined at 9 days post infection. *$p<0.005$ by Mann-Whitney test.

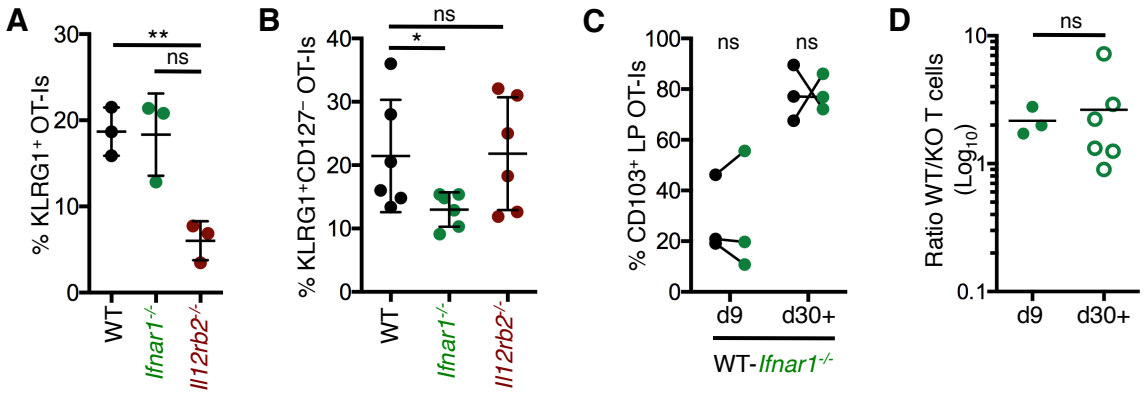

Figure S5, related to Figure 6. Role of cytokine signaling in KLRG1 expression and Trm differentiation during VSV-OVA infection. (A-D) Mice received an equal number of WT, *Ifnar1*$^{-/-}$, and *Il12rb2*$^{-/-}$ OT-I T cells and were infected orally with Yptb-OVA (A) or intraperitoneally with VSV-OVA (B-D). (A) Seven days post Yptb-OVA infection, the percentage of KLRG1^{+} OT-I T cells in the spleen was determined. (B) The percentage of KLRG1^{+}CD127^{-} OT-I T cells in the blood on day 7 after VSV-OVA infection. (C) The percentage of WT and *Ifnar1*$^{-/-}$ OT-I T cells expressing CD103 on days 7 or greater than 30 days post infection. Lines connect data points from individual mice. (D) Ratio of WT to *Ifnar1*$^{-/-}$ OT-I T cells in the LP. Ns=not significant, *$p<0.05$, **$p<0.005$ by paired t-test (A,B,C) or Mann-Whitney test (D).

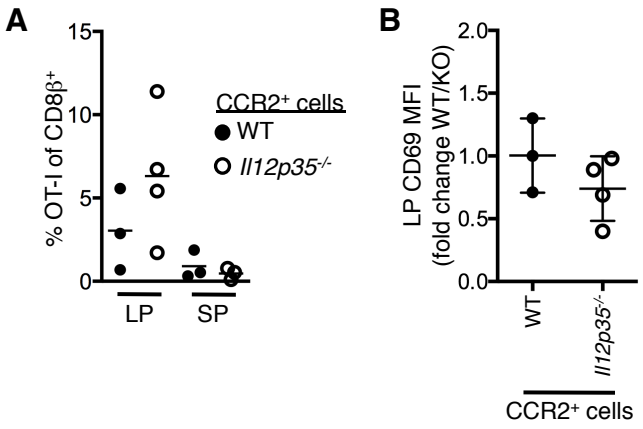

Figure S6, related to Figure 7. Expansion and CD69 expression on OT-I T cells from mice lacking IL-12-producing monocytes. WT:CCR2-DTR or *Il12p35*$^{-/-}$:CCR2-DTR mixed bone marrow chimeric mice received 1x10^4 WT OT-I T cells and were infected with Yptb-OVA. Mice were given daily injections of diphtheria toxin beginning at 5 days post infection. Twelve days after infection, OT-I T cells in the LP and spleen were analyzed. (A) Percentage of OT-I T cells in the CD8b$^+$ cell population. (B) CD69 MFI expressed as the ratio of the CD69 MFI of OT-I T cells from *Il12p35*$^{-/-}$ chimeras/averaged CD69 MFI from WT chimeras from the same experiment.

Table S1, related to Figure 3. qRT-PCR primers.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
Itgae	cctgtgcagcatgtaaaagaatg	caaggatcggcagttcagatac
Il18rap	agactacttcctgagcacaaga	ccttggcaattcgattcaccc
Klrk1	ccttggcaattcgattcaccc	ccttgttgcacaatactggctg
Il18r1	acttttgctgtggagacgttac	ccggcttttctctatcagtgaat
Cd244	ccaccttccaaagcaagtaca	agaaacaacgatgtggggtga
Cd69 (Skon et al., 2013)	tggtcctcatcacgtccttaataa	tccaacttctcgtacaagcctg
Tgfbr2	ccgctgcatatcgtcctgtg	agtggatggatggtcctattaca
Tgfb	tgacgtcactggagttgtacg	ggttcatgtcatggatggtgc
Tbx21	agcaaggacggcgaatgtt	gggtggacatataagcggttc
Actb	ggctgtattcccctccatcg	ccagttggtaacaatgccatg

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Laser Capture Microdissection

Mice were infected with $2x10^8$ Yptb-OVA and 7 days after infecton, intestinal tissues were frozen in OCT media (Sakura). Sections of 7-8μm were placed on polyethylene naphthalate membrane slides (Leica), stained with anti-CD8α antibodies (53-6.7, Biolegend), and dehydrated with ethanol/xylene. Areas containing $CD8^+$ T cell clusters or areas without T cell clustering were cut from the LP using a Leica LMD7000. RNA was isolated using the RNeasy RNA isolation kit (QIAgen) and qRT-PCR was performed using the SYBR one step RT-PCR kit (QIAgen). *Tgfb* expression was normalized to *Actb;* primers used are listed in Table S1.

Stability of CD103 expression *ex vivo*

$CD103^+CD69^+$ and $CD103^-CD69^+$ $CD8\alpha\beta^+$ cells were sorted from the LP to >95% purity using a FACSAriaII (BD Bioscience). Sorted T cells were stimulated with anti-CD3/anti-CD28 Dynabeads (Thermo Fisher Scientific) and/or IL-12 (20ng/ml, Peprotech) and TGF-β (0.5ng/ml, R&D Systems) for 24 hours and CD103 expression was analyzed by flow cytometry.

VSV-OVA infection

For infections, mice received $1x10^4$ naïve OT-I T cells or $5x10^3$ each of WT and cytokine receptor-deficient OT-I T cells i.v. and were then infected i.p with $1x10^7$ PFU of vesicular stomatitis virus expressing ovalbumin (VSV-OVA) (Turner et al., 2008).

SUPPLEMENTAL REFERENCES

Skon, C.N., Lee, J.-Y., Anderson, K.G., Masopust, D., Hogquist, K.A., and Jameson, S.C. (2013). Transcriptional downregulation of S1pr1 is required for the establishment of resident memory CD8+ T cells. Nat. Immunol. *14*, 1285–1293.

Turner, M.J., Jellison, E.R., Lingenheld, E.G., Puddington, L., and Lefrançois, L. (2008). Avidity maturation of memory CD8 T cells is limited by self-antigen expression. J. Exp. Med. *205*, 1859–1868.