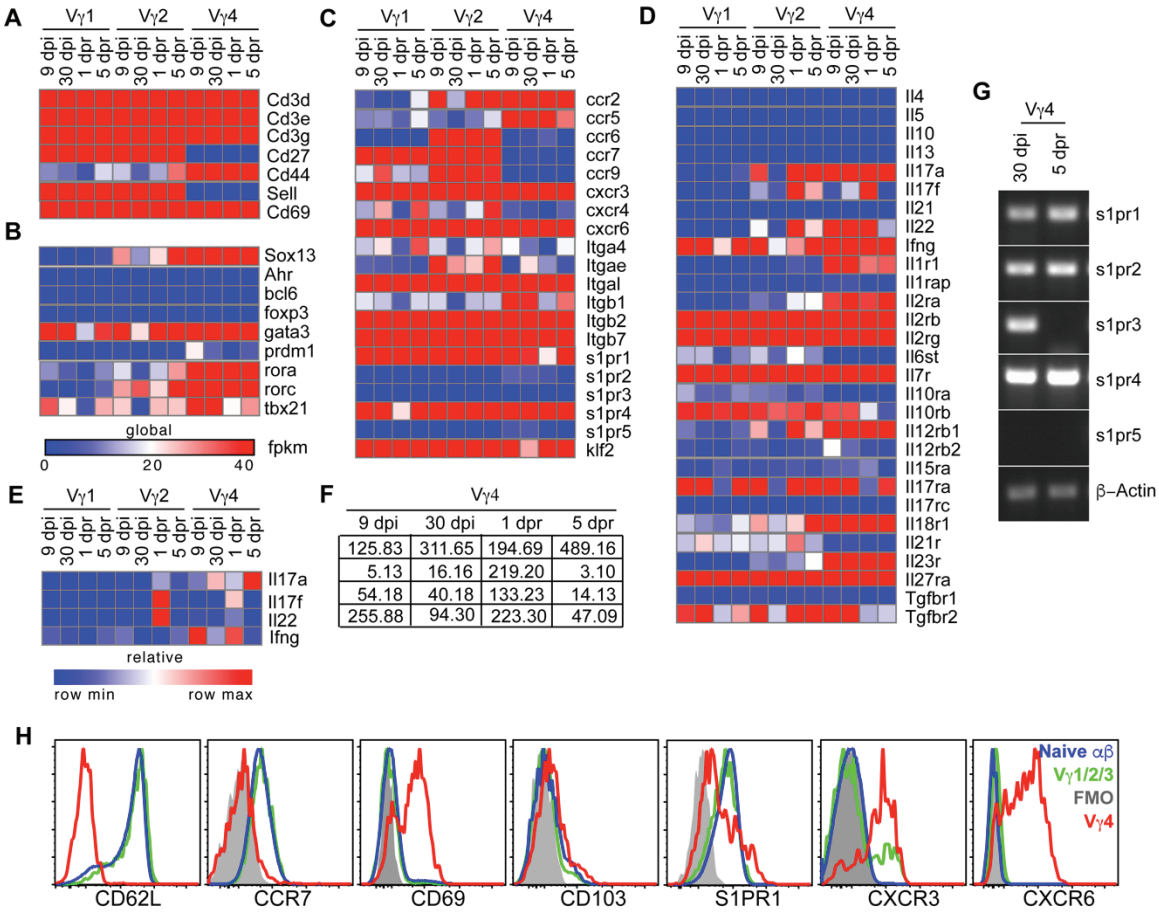

Fig. S1: RNA-seq profile of *Lm*-elicited Vγ4+ γδ T cells. Heat maps generated using fpkm values from RNA-seq analysis of sorted Vγ1.1+ (Vγ1.1+ CD27-CD44lo GL3+ TCRβ- CD3+ CD45+ live singlets) or Vγ2+ (Vγ2+ CD27-CD44lo GL3+ TCRβ- CD3+ CD45+ live singlets) or *Lm*-elicited Vγ4+ γδ T cells (Vγ1.1-Vγ2-Vγ3- CD27- CD44hi GL3+ TCRβ- CD3+CD45+ live singlets) at 9, 30 dpi and 1, 5 dpr following oral *Lm* infection. Heat maps were generated using global analysis from a range of 0 to 40 fpkm. **A)** T cell surface molecules, **B)** Transcription factors, **C)** Molecules involved in lymph node migration and **D)** Cytokine/Cytokine receptors expression. **E)** Heat map and **F)** table generated using fpkm values from RNA-seq analysis of **E)** Vγ1.1+ or Vγ2+ or **E and F)** *Lm*-elicited Vγ4+ γδ T cells displayed as relative expression of gene for IL-17A,

IL-17F, IL-22 and IFNγ. **G)** 2% Agarose gel showing band from RT-PCR for S1PR1, S1PR2, S1PR3, S1PR4, S1PR5 and βActin on RNA isolated from sorted *Lm*-elicited Vγ4+ γδ T cells at Memory (30 dpi) or Recall (5 dpr). **H)** CD62L, CCR7, CD69, CD103, S1PR1, CXCR3 and CXCR6 expression on *Lm*-elicited Vγ4+ γδ T cells from MLN at >30 dpi (*Lm*-immune). Corresponding FMO showing *Lm*-elicited Vγ4+ γδ T cells, naïve αβ T cells (CD27+CD44lo TCRβ+CD3+CD45+) and rest of γδ T cells (Vγ1.1/Vγ2/Vγ3) in the MLN (Vγ1.1/Vγ2/Vγ3 CD27+CD44lo GL3+ TCRβ- CD3+CD45+). Plots are representative of 3 independent experiments, n=3-5 mice

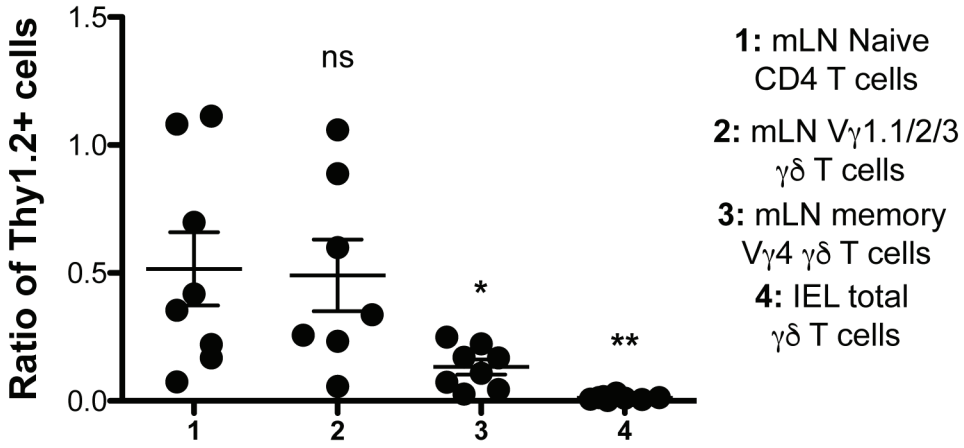

Fig. S2: Ratio of absolute numbers of Thy1.2+ cells found between Thy1-mismatched *Lm*-immune host after parabiosis. Data are expressed as Mean ± SEM analyzed by One-Way ANOVA. *p<0.05 and **p<0.001 calculated using Tukey's Multicomparison Test. Data are from 1 experiment. Parabionts were sacrificed after 9 days. Each dot represents one mouse. All comparisons were made against naïve CD4 αβ T cell ratios.

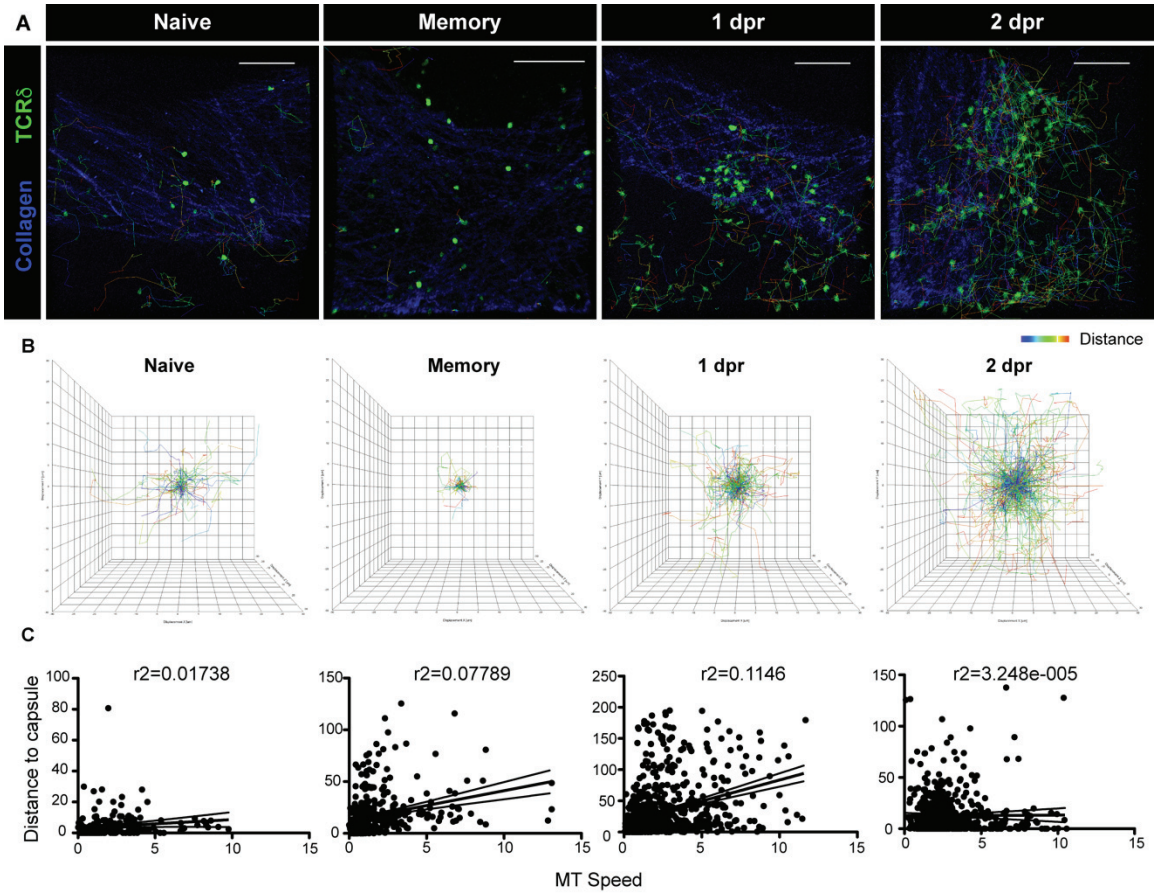

Fig. S3: Dynamics of memory and recalled γδ Trm in the MLN. A) Maximum intensity projection images from time-lapse movies showing γδ T cells in the interfollicular area of MLN (imaging depth ~ 100-200 μm under the LN capsule) at 0, 30 dpi and 1 and 2 dpr following oral *Lm* infection. Scale bars represent 100 μm. Cell tracks are color coded to display track length. Data from Movies S1, S2, S3 and S4 representing 1 mouse out of 3 from 3-5 independent experiments. **B)** Superimposed tracks of γδ T cells in IFA normalized to their starting coordinates. Data from Movies S1, S2, S3 and S4. **C)** Scatter plots displaying Mean Track Speed (MT Speed) vs Distance to the MLN capsule for each cell track from all the movies collected in each time point: Naïve (n=3), Memory (n=4), 1 dpr (n=4) and 2 dpr (n=5). R square values were calculated using linear regression analysis displaying a 95% confidence band on both sides of the best-fit line.

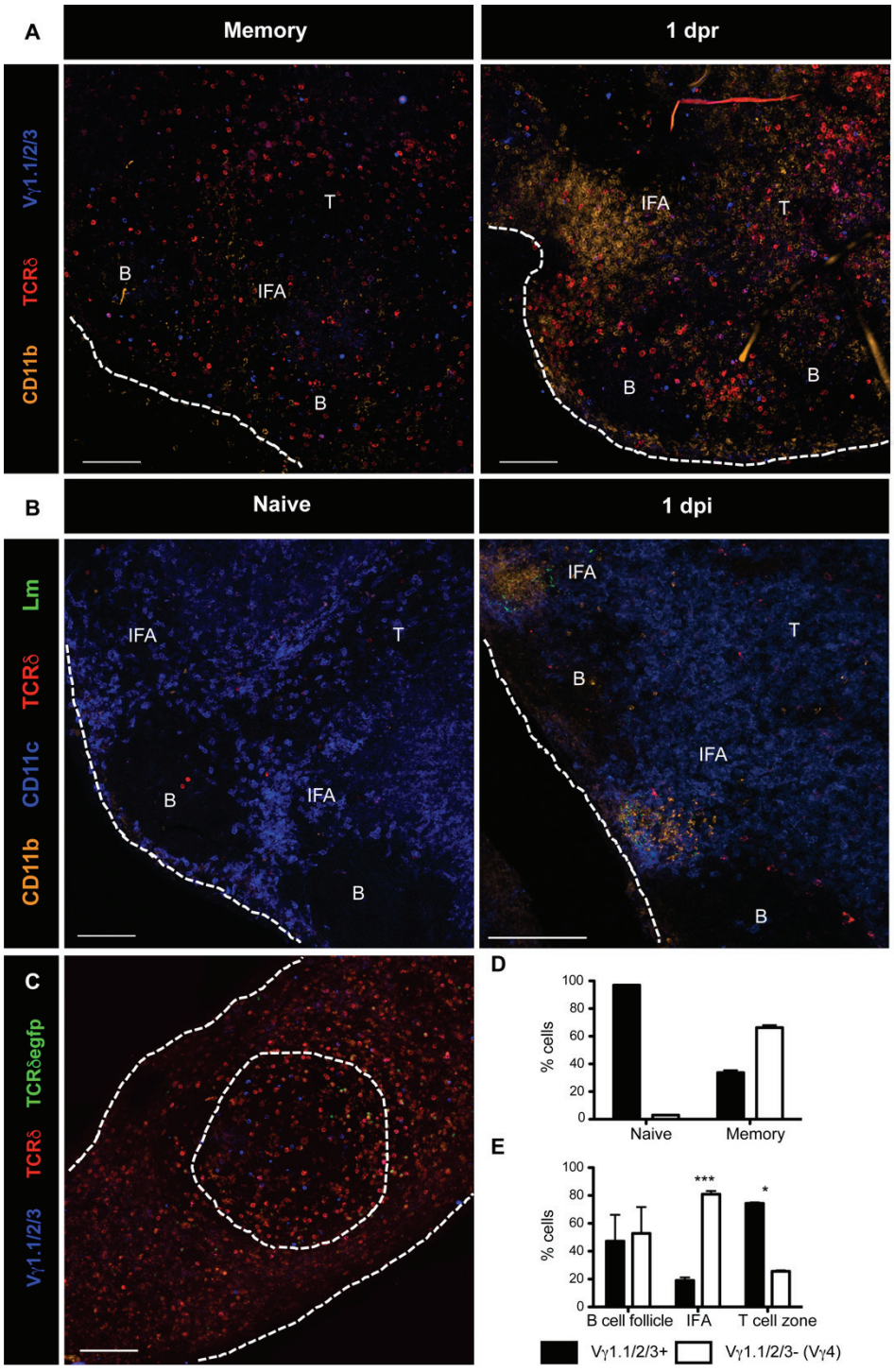

Fig. S4: A) Majority of the memory Vγ4+ γδ T cells in *Lm*-immune mice are located in interfollicular areas of the MLN. Thick sections (~400 µm) from *Lm*-immune (Memory) (left panel) or 1 dpr mice (right panel) were immunostained with anti-

Vγ1.1/Vγ2/Vγ3 antibodies and the indicated proteins. Scale bars 100 μm. **B) Myeloid clusters that form after primary oral *Lm* infection contain low numbers of γδ T cells.** Sections of MLN from naïve mice (left panel) or mice infected with *Lm* for 1 day (dpi) (right panel) were immunostained for *Lm* and the indicated proteins**.** Data are representative of 2 independent experiments, n=3. Scale bars 100 μm. . B: B cell zone. T: T cell zone. IFA: Interfollicular area. M: Medulla. **C) Most of the γδ T cells (gfp+) at memory phase imaged by multi-photon microscopy are Vγ4+ γδ T cells, and are located in the IFA.** Thick section (~400 μm) from *Lm*-immune (Memory) was immunostained with anti-Vγ1.1/Vγ2/Vγ3 antibodies and the indicated proteins. Scale bar 100 μm **D)** Percentage of Vγ1/2/3+ vs Vγ1/2/3- (Vγ4+) cells found at Naïve (n=1) or Memory (n=6) using spots by Imaris. **E)** Location of Vγ1/2/3+ vs Vγ4+ cells found at memory (n=4) using spots by Imaris. ***$p<0.001$ and *$p<0.05$ calculated by Two-Way ANOVA and Bonferroni post-test analysis.

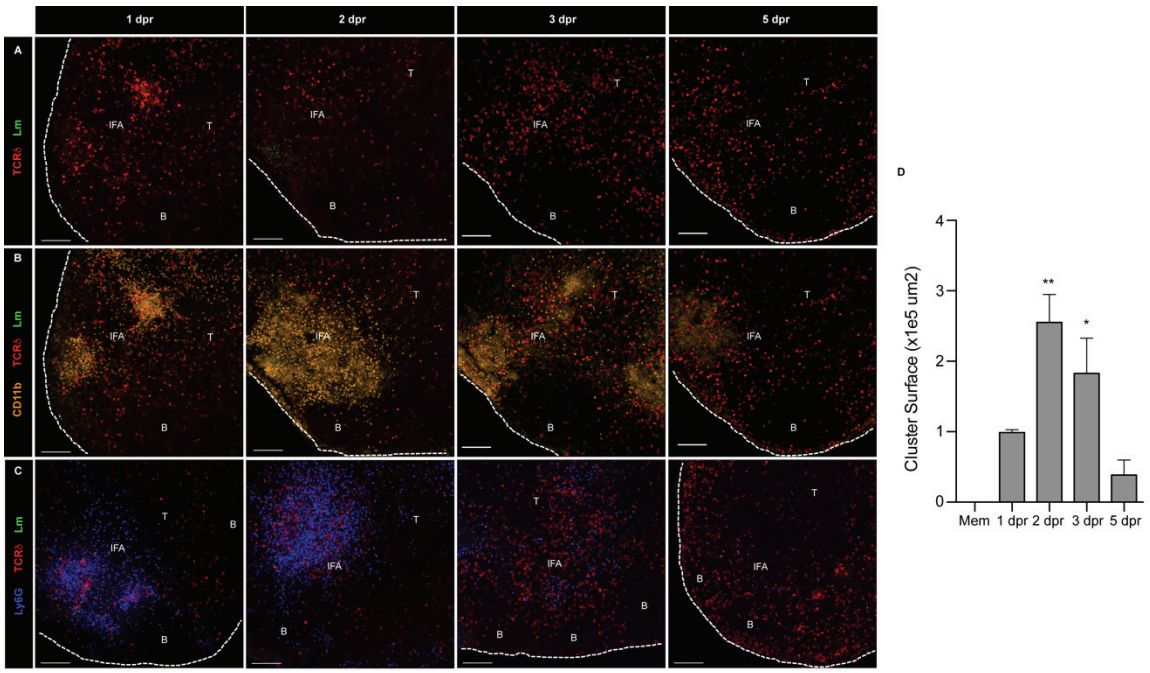

Fig. S5: Localization of neutrophils and *Lm*-elicited γδ T cells in the MLN during oral *Lm* recall. A-C) Sections of MLN from *Lm*-immune at 1, 2, 3 and 5 dpr were immunostained for *Lm* and the indicated proteins. Data are representative of 2 independent experiments, n=3 mice/group. Scale bars 100 μm. D) quantification of the cluster formation was done by using the Imaris software to render a defined surface for gd/CD11b clusters using the fluorescent staining for gd cells and CD11b+ cells. (n=3 for each time point). **p<0.01 and *p<0.05 calculated by One-Way ANOVA analysis using Tukey Multicomparison test.

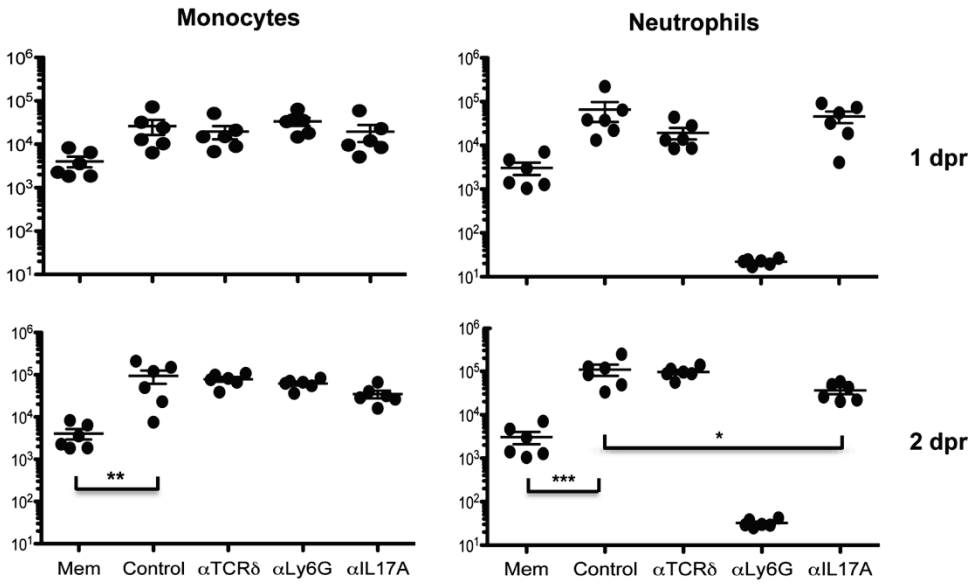

Fig. S6: Neutrophil numbers in the MLN are reduced at 2 dpr upon IL-17A blockade. Absolute numbers of monocytes (Ly6ChiCD11bhi CD3-CD19-CD45+) and neutrophils (Ly6GhiCD11bhi CD3-CD19-CD45+) detected in MLN of *Lm*-immune mice at 1 or 2 dpr. Data represent 2 independent experiments, n=4 mice/group. Data are expressed by Mean ± SEM by One-Way ANOVA. *p<0.05, **p<0.01, ***p<0.001.

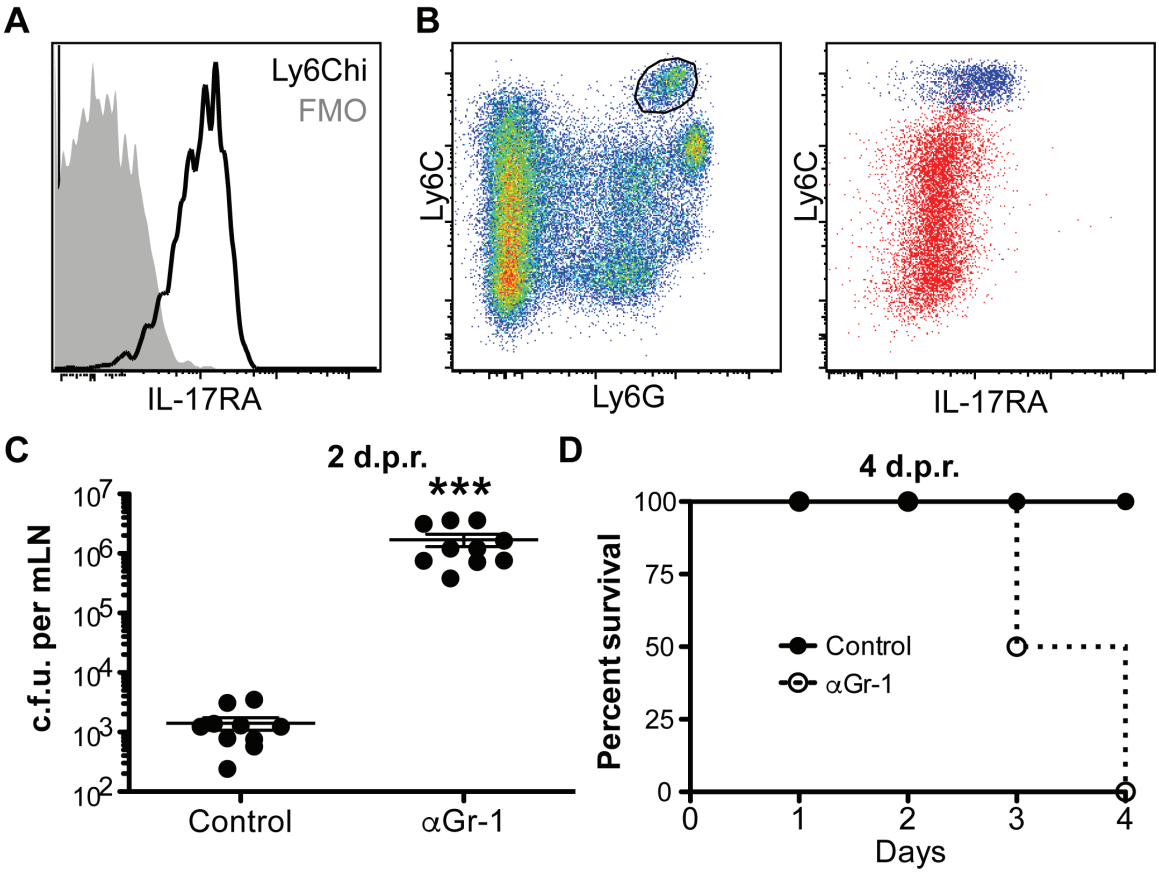

Fig. S7: IL-17RA+ myeloid cells are critical for bacterial clearance. A) IL-17R expression on Ly6C+CD11b+ cells from MLN at 1 dpr. **B)** Ly6C vs. Ly6G (Ly6C+ CD11b+ gate), Ly6C vs. IL-17RA (Ly6C+CD11b+ gate in blue, whole MLN red) on CD3-B220- cells and IL-17R expression on Ly6C+CD11b+ cells from MLN at 1 dpr. **C and D**) c.f.u. from MLN at 2 (**B**) or 4 (**C**) dpr after treatment with LTF-2 (IgG2b control) or RB6-8C5 (rat IgG2b αGr-1) antibody as described in Material and Methods section. Data are expressed as Mean ± SEM and analyzed by student *t* test. *+*=p<0.001. Data shown are representative of two independent experiments, n= 7 to 10 (2 dpr) or 5-10 (4 dpr) mice per group.

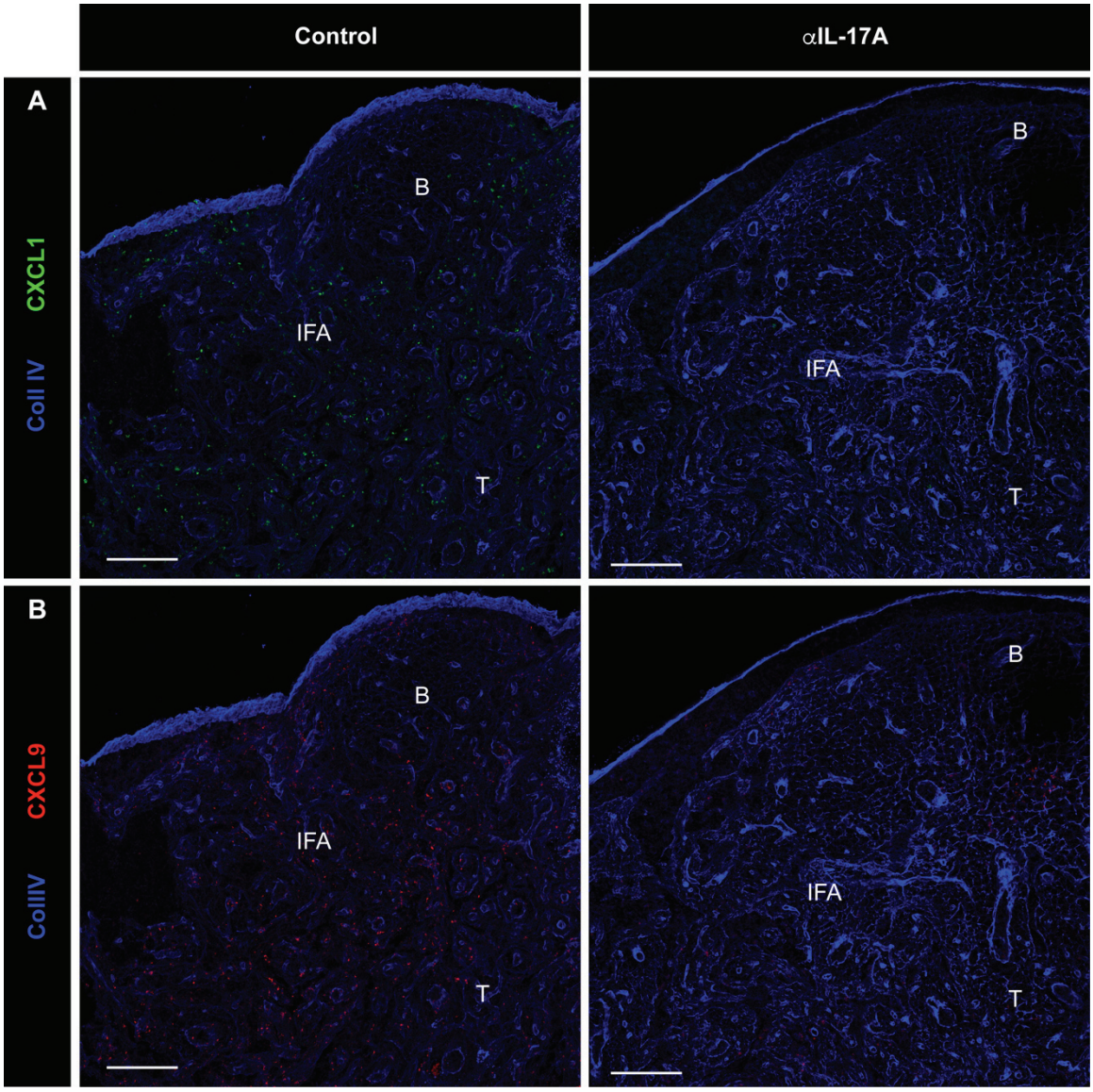

Fig. S8: IL-17A regulates CXCL1 and CXCL9 production in the MLN during recall and CXCR3 enables the local redistribution and migration of γδ Trm cells near the sites of *Lm* replication within the MLN. Sections of MLN at 1 dpr treated with either HRPN (Control) or 17F3 (αIL-17A) antibodies and stained for CXCL1 (**A and B**), or CXCL9 (**C and D**) and Collagen IV. B: B cell zone. T: T cell zone. IFA: Interfollicular area. Scale bars 100 μm.

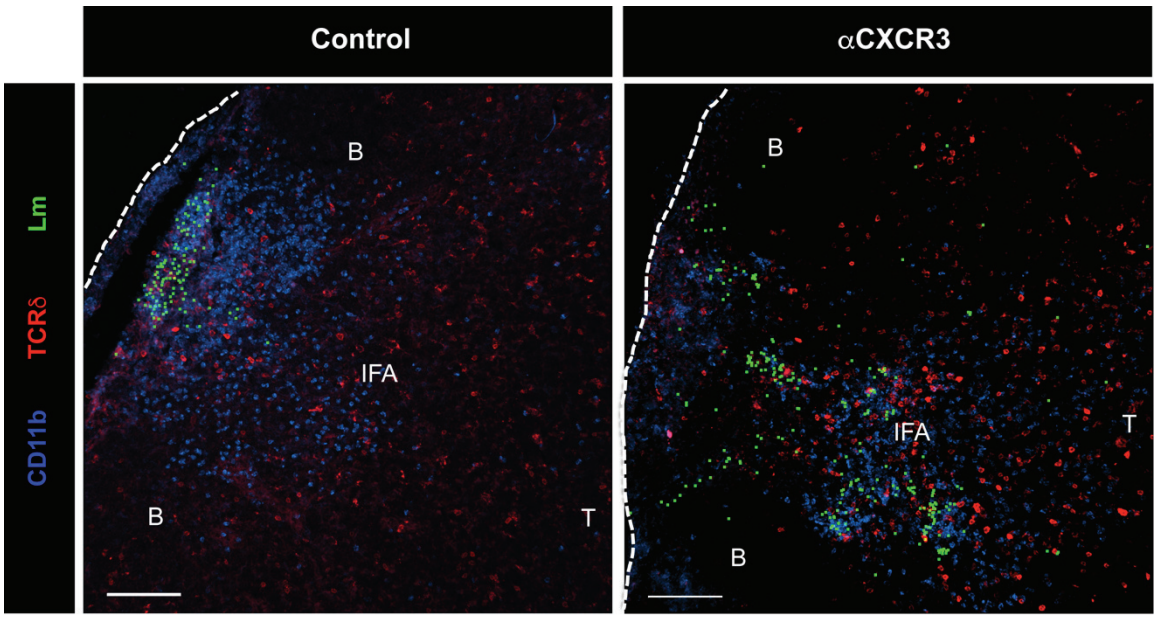

Fig S9: Sections of MLN from mice at 1 dpr that were treated with Armenian hamster IgGs or CXCR3-173 (αCXCR3) antibodies were immunostained for *Lm* (*Lm* rendered using Spot function of Imaris), and the indicated proteins. B: B cell zone. T: T cell zone. IFA: Interfollicular area. Data are representative of 2 independent experiments, n=3/group. Scale bars 100 μm.

Supplementary Movies

Movie S1, related to Figure 2. Movement of γδ T cells in naïve mice (0 dpi) show a normal random motility pattern. Mesenteric lymph nodes from naive mice were imaged using two-photon microscopy. γδ T cells (green) and collagen fibers (blue). Maximum intensity projection time series images were acquired by two-photon microscopy. The display rate is 10 frames/second. The step size when a single frame was collected was 4μm. Stills of this video are shown in Figure 2A.

Movie S2, related to Figure 2. γδ T cells in memory mice (>30 dpi) exhibited a limited motility pattern. Mesenteric lymph nodes from memory mice were imaged using two-photon microscopy. γδ T cells (green) and collagen fibers (blue). Maximum intensity projection time series images were acquired by two-photon microscopy. The display rate is 10 frames/second. The step size when a single frame was collected was 4μm. Stills of this video are shown in Figure 2A.

Movie S3, related to Figure 2. Reactivated γδ T cells in *Lm*-immune mice upon oral *Lm* recall (1 dpr) are highly motile. Mesenteric lymph nodes from memory mice that were re-infected (1 dpr) with LM were imaged using two-photon microscopy. γδ T cells (green) and collagen fibers (blue). Maximum intensity projection time series images were acquired by two-photon microscopy. The display rate is 10 frames/second. The step size when a single frame was collected was 4μm. Stills of this video are shown in Figure 2A.

Movie S4, related to Figure 2. Movement of γδ T cells in *Lm*-immune mice upon oral *Lm* recall (2 dpr) is rapid. Mesenteric lymph nodes from memory mice that were re-infected (2 dpr) with LM were imaged using two-photon microscopy γδ T cells (green) and collagen fibers (blue). Maximum intensity projection time series images were acquired by two-photon microscopy. The display rate is 10 frames/second. The step size when a single frame was collected was 4μm. Stills of this video are shown in Figure 2A.

Supplemental Methods

Mice

Female Balb/cJ mice were purchased from The Jackson Laboratory (Bar Harbor) and were maintained in specific pathogen-free conditions. Previously described C57/BL6 TCRδ-H2B-eGFP mice (1) obtained from Dr. I. Prinz (Hannover Medical School) were backcrossed 8 times into Balb/c background using speed congenics (Dartmouse, Dartmouth College, NH). F1 mice used in two-photon microscopy were generated mating fully backcrossed Balb/c TCRδ-H2B-eGFP mice with Balb/c TCR αβ knock-out (ko) mice, stock #004364 (The Jackson Laboratories, Bar Harbor, ME). Eight to ten week old age-matched mice were used for experiments. All animal experiments were performed in accordance to the University of Connecticut Health Center Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

Bacteria and Infections

Lm strain EGDe with recombinant InlA (2) was used for most infections. Mice were infected and recalled as described before (3). Bacterial burden was quantitated as described before (3).

Single cell preparation and Flow Cytometry

Single-cell suspensions were prepared from MLNs, pLNs (consisting of the axillary, brachial, and inguinal lymph nodes), IELs, and the LP as described before (3). Live cells were enumerated from single-cell suspensions with the use of a Vi-CELL Cell Viability Analyzer (Beckman Coulter). Staining of single cell suspensions were made at 4 °C for 40 min in the dark with various combinations of directly fluorochrome-conjugated antibodies purchased from Biolegend (San Diego, CA) or eBiosciences, Inc (San Diego, CA) as described before (3). After staining, cells were fixed for 20 min with 2% paraformaldehyde. S1PR1 staining was performed as described before (4). In vivo cytokine detection was performed by treating mice with brefeldin-A (250μg/500 ul PBS/mouse i.v.). MLNs were harvested and digested with collagenase IV in presence of GolgiPlug (BD Biosciences, San Jose, CA) before proceeding to intracellular staining. For the identification of IL-17A and IFNγ, cells were permeabilized and fixed with Cytofix/Cytoperm buffer (BD) for 20 min at 4 C and then stained with an antibody specific as described before. CCR7 detection was performed by staining single cell suspension of the MLN with CCL19-human Fc followed by using Alexa Fluor 488 goat anti-human Fc (Life Technologies, Grand Island, NY) as secondary detection antibody.

RNA sequencing and RT-PCR

Cell suspensions obtained as described before from MLNs at 9, 30 dpi and 1, 5 dpr were counted using Vi-CELL Cell Viability Analyzer (Beckman Coulter). γδ T cells were enriched by negative magnetic isolation with sheep anti-rat Dynabeads (Thermo Scientific) and rat anti-mouse antibodies to MHC class II (I/A-E), CD4 (GK1.5) and CD8a (2.43). CD27+CD44lo Vγ1.1+ or Vγ2+ γδT cells and CD27– $CD44^{hi}$ Vγ1.1-Vγ2-

Vγ3- γδ T cells were sorted from the enriched population on a FACS Aria II (BD). Total RNA was extracted using RNAeasy Plus Microkit (Qiagen, Valencia, CA) and was sequenced using Illumina HiSeq200/2500 PE100 by Otogenetics Corporation (Norcross, GA). Data was analyzed on DNAnexus platform (DNAnexus, Inc., Mountain View, CA) using TopHat (Known Genes) v1.1.0 and Cuffdiff v1.0.1 apps mapping to mm10 mouse genome. Data Accesion Number at Sequence Read Archive (NCBI): BioProject PRJNA299800 and BioSample SRP065262. Data was plotted using Gene-E software (Broad Institute, Cambridge, MA). RNA from Lm-elicited Vγ4 γδ T cells sorted as described above was extracted using Trizol (Life Technologies, Carlsbad, CA, USA), DNA contamination was removed by DNA-free (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA was made using iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). PCR was performed using GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA) and the following primers: βActin: forward 5'-AGA GGG AAA TCG TGC GTG AC-3' and reverse 5'-CAA TAG TGA TGA CCT GGC CGT-3', S1PR1: forward 5' ATG GTG TCC ACT AGC ATC CC 3' and reverse 5'- CGA TGT TCA ACT TGC CTG TGT AG -3', S1PR2: forward 5'- ATG GGC GGC TTA TAC TCA GAG -3' and reverse 5'- GCG CAG CAC AAG ATG ATG AT -3', S1PR3: forward 5'- ACT CTC CGG GAA CAT TAC GAT -3' and reverse 5'- CAA GAC GAT GAA GCT ACA GGT G -3' , S1PR4: forward 5'- GTC AGG GAC TCG TAC CTT CCA -3' and reverse 5'- GAT GCA GCC ATA CAC ACG G -3' and S1PR5: forward 5'- GCT TTG GTT TGC GCG TGA G -3' and reverse 5'- GGC GTC CTA AGC AGT TCC AG -3'.

Parabiosis

Parabiosis surgery was performed using a modified procedure as described before (5) in accordance with the University of Connecticut Institutional Animal Care Committee. See also Supplemental information. Briefly, pairs of age-matched female Thy1.1+ Balb/c naive or *Lm*-immune mice were co-housed for a week with Thy1.2+ *Lm*-immune (>30 dpi) Balb/c mice before parabiosis procedure. Mice were anaesthetized using a mixture of ketamine hydrochloride at 90-120 mg/kg bodyweight (Ketaject, Clipper Distributing Company L.L.C, St. Joseph, MO) and xylazine at 10 mg/kg bodyweight (TranquiVed, Akorn, Inc., Decatur, IL) injected intraperitoneally (i.p.). Skin in the corresponding flanks of mice was shaved, hair was removed with ethanol and site of incision was cleaned with Betadine solution (Purdue Products L.P., Stamford, CT). A lateral skin incision was made from 1 cm behind the ear to just past the hind limb. Skin was loosened from connective tissue along the incision and the fascia of the pair was sutured with catgut (Ethicon 4-0 chromic gut sutures, Henry Schein, Denver, PA) in three matching points: scapulae, below the inguinal lymph node and close to the thigh. The corresponding skin edges were approximated symmetrically and joined with a 9-mm stainless steel wound clips (AUTOCLIP, Clay Adams, Parsippany, NJ). Mice were given 60 μg Buprenex (Reckitt Benckiser Hull, England) and 1 ml of warm sterile tepid saline solution and place under heat lamp until they resume movement. Mice were injected with Buprenex for the next two days. Mice were joined for at least 9 days.

In Vivo Antibody Treatments

Mice were given i.p. injections using the following amount of antibodies: 200 μg of HRPN (rat IgG1 control, BioXcell), 17F3 (αIL-17A, mouse IgG1, BioXcell), anti-Cδ TCR (GL4) or 250 μg of Armenian Hamster IgG (control, BioXcell) or CXCR3-173

(αCXCR3, Armenian hamster IgG, BioXcell) or 500 μg of LTF-2 (rat IgG2b control, BioXcell) or RB6-8C5 (αGr-1, rat IgG2b, BioXcell). anti-Cδ TCR was injected on days -3, -1 and +1 and everyday until the last day of recall. HRPN, 17F3, Armenian Hamster IgG and CXCR3-173 were injected on days -1, 0 and everyday until the last day of recall. HRPN, 1A8, LTF-2 and RB6-8C5 were injected on days -1, 0 and everyday until the last day of recall.

Confocal Microscopy

MLNs were fixed overnight in paraformaldehyde-lysine-periodate solution (PLP) as described before (4). Briefly, MLN were fixed in PLP solution (0.05 M phosphate buffer, 0.1 M L-lysine (pH 7.4, Sigma-Aldrich, St. Louis, MO), 2 mg/ml $NaIO_4$ (Sigma-Aldrich) and 4% (v/v) paraformaldehyde (Electron Microscopy Sciences, ProSciTech)), washed 4 times with PBS for 15 minutes and dehydrated in sucrose overnight at 4°C (30% in phosphate buffer). Tissues were snap frozen in OCT compound (Tissue-Tek, Sakura Finetek) using isopentane (Sigma-Aldrich) and liquid nytrogen before storage at -80°C. 30-μm frozen sections were cut using a cryostat (Leica CM1850) and air-dried before storage at -80°C. Sections were incubated with 0.3 M Glycine (Sigma-Aldrich) for 30 min, blocked for 45 min with 5 % normal goat serum (NGS, Gibco), 5% of fetal bovine serum (FBS, Gibco), Fc block anti-CD16/32, 0.05% Tween 20 (Bio-Rad) in PBS and mounted on Shandon Coverplate disposable immunostaining chambers (Thermo Scientific) in Sequenza Slide Rack (Thermo Scientific). Sections were stained for primary antibodies (Rabbit anti-LLO (Dako)) for 1.5 hr at RT, washed 3 times with PBS

for 5 min and stained for secondary or fluorophore-conjugated antibodies for 1 hr. at RT (alexa fluor 488 conjugated goat anti-rabbit (Life Technologies), FITC-conjugated CXCL1, PE or alexa fluor 546 conjugated anti-TCRδ (GL3), APC-conjugated CD11c, APC-conjugated Vγ1.1 or Vγ2 or Vγ3, alexa fluor 647 Ly6G alexa fluor 647 αIL-17A or alexa fluor 660-conjugated CXCL9, Pacific blue or eFluor 450 conjugated CD11b and BD Horizon V500 conjugated B220) or DAPI. Stained sections were mounted using Prolong Gold Antifade (Life Technologies). Images were acquired using an LSM 780 (Carl Zeiss) and images were processed using Imaris (Bitplane, CT, US). Easy 3D setup after Thresholding Background Substraction and adjustment of minimum and maximum with gamma 1.0. Masking was used before Spot detection to digitally quantite images.

Two Photon Microscopy

MLNs were attached to a small plastic coverslip using a thin film of Vetbond (3M, St Paul, MN) that was then placed at the base of the imaging chamber and continously superfused with warm (35 to 37°C) DMEM medium bubbled with 95% O_2 and 5 % CO_2. A MaiTai Ti:sapphire laser (Newport/Spectra-Physics) was tuned to 960 nm for excitation of green fluorescent protein (gfp) and second harmonic. Stacks of 25-30 optical sections (512 x 512 pixels) of 4 μm each were acquired in 25-30 seconds for a period of 60-90 minutes below the MLN capsule close to interfollicular areas on an Ultima IV multiphoton microscope (Prairie Technologies) to provide imaging volumes of ~100μm in depth. Emitted Light and second harmonic signals were captured by 3 band-pass filters, 460/50 (second Harmonic), 525/50 (GFP) and collected with non-descanned detectors. Imaging data was analyzed using Imaris 3D software (Bitplane, Inc.) using

drift correction and plotting using MotilityLab suite (Washington University, St. Louis, MO) or Prism 5 (Graph Pad).

Statistical Analysis

A Mann-Whitney, One-Way or Two-Way ANOVA or Linear Regression Analysis was performed using Prism 5 (Graph Pad).

References:

1. Prinz I, *et al.* (2006) Visualization of the earliest steps of gammadelta T cell development in the adult thymus. *Nature immunology* 7(9):995-1003.
2. Wollert T, *et al.* (2007) Extending the host range of Listeria monocytogenes by rational protein design. *Cell* 129(5):891-902.
3. Sheridan BS, *et al.* (2013) gammadelta T cells exhibit multifunctional and protective memory in intestinal tissues. *Immunity* 39(1):184-195.
4. Benechet AP, *et al.* (2016) T cell-intrinsic S1PR1 regulates endogenous effector T-cell egress dynamics from lymph nodes during infection. *Proceedings of the National Academy of Sciences of the United States of America* 113(8):2182-2187.
5. Klonowski KD, *et al.* (2004) Dynamics of blood-borne CD8 memory T cell migration in vivo. *Immunity* 20(5):551-562.