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SUPPLEMENTARY INFORMATION

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Intravital mucosal imaging of CD8$^+$ resident memory T cells shows tissue-autonomous recall responses that amplify secondary memory

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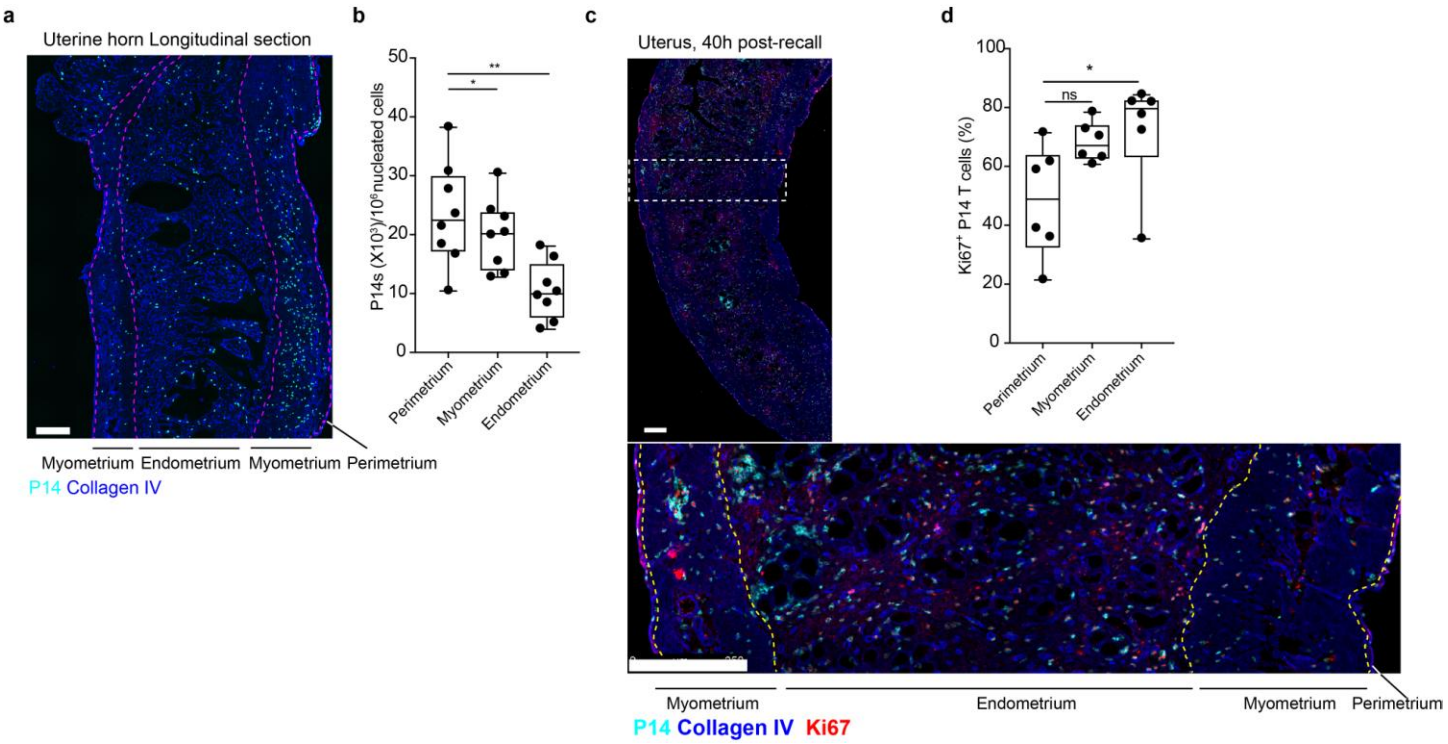
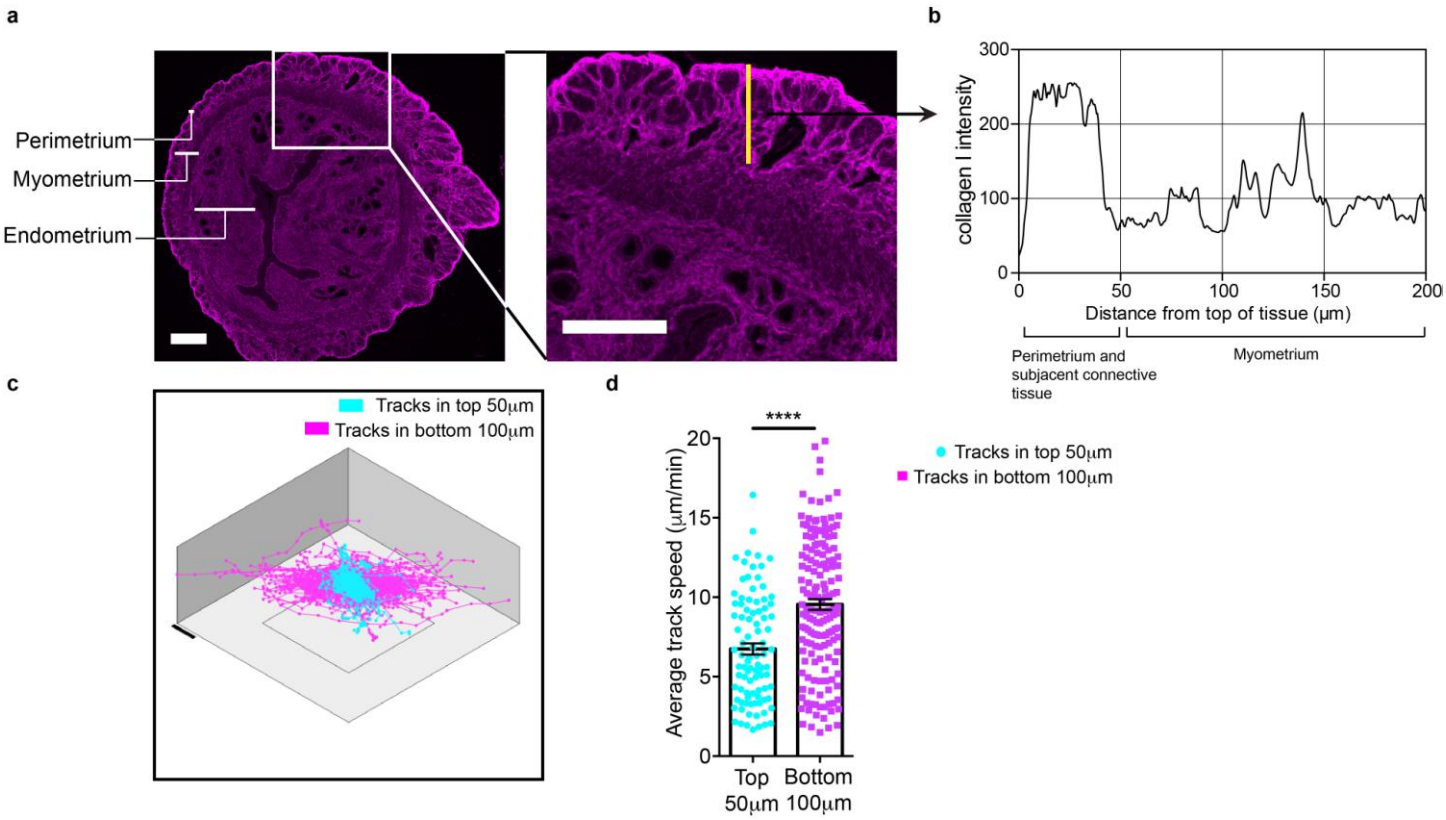


Supplementary Figure 1

LCMV-specific $CD8^+$ T cells are present throughout the uterus and upregulate Ki67 after local reactivation.

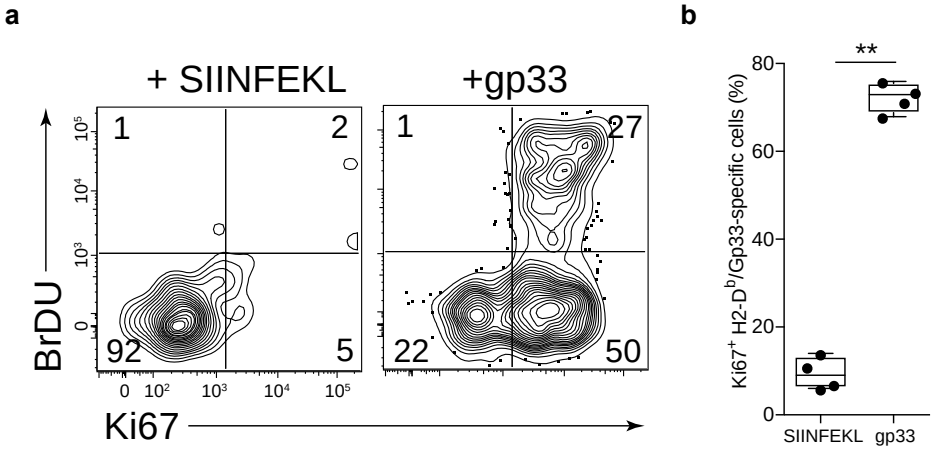
$CD90.1^+$ P14 $CD8^+$ T cells were transferred to C57BL/6J mice one day prior to infection with LCMV Armstrong. **a**) 60 days later, longitudinal sections of the murine uterine horn were stained for collagen-IV (blue) and CD90.1 (cyan), scale bars = 250 µm. **b**). P14 $CD8^+$ T cells were enumerated by QIM in the three indicated layers of the uterus. Data are representative of two independent experiments, with 4 mice/experiment. **c-d**) P14 immune chimeras were t.c. challenged with gp33 peptide and the FRT was harvested 40h later. The uterus was stained for collagen-IV (blue), P14 (cyan) and Ki67 (red), scale bars = 250 µm, and the proportion of P14 $CD8^+$ T cells that were $Ki67^+$ was enumerated. Data are representative of two independent experiments, with 3 mice/experiment. * $p<0.05$, ** $p<0.01$, One way ANOVA (**b**), Kruskal–Wallis ANOVA (**d**). Box plots with individual data points shown. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the minimum and maximum data points.



Supplementary Figure 2

CD8$^+$ T cells in uterine perimetrium migrate slower than those in myometrium.

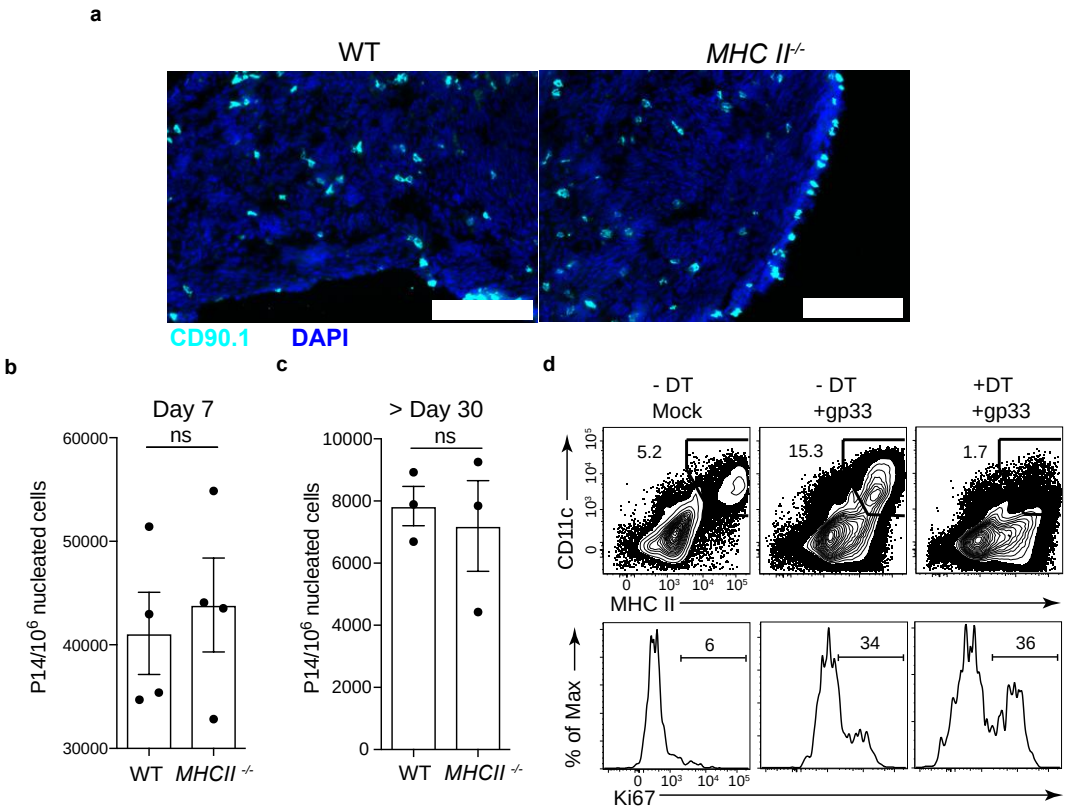
a) Transverse sections of the murine uterine horn were stained for collagen-I (magenta) and the three major layers of the uterine horn are highlighted (scale bars, 200 μm). **b**) Collagen-I staining intensity across the region highlighted by the yellow line (representing 200 μm) indicates that collagen density is higher in the perimetrium and subjacent connective tissue (top 50 μm) than myometrium (bottom 150 μm). **c**&**d**) GFP$^+$ P14 CD8$^+$ T cells were transferred to C57BL/6J mice one day before recipients were infected with LCMV Armstrong. 40 days later, intravital imaging of uterine horn was performed. **c**) A 3D isometric plot depicts superimposed tracks of several GFP$^+$ P14 CD8$^+$ T cells from the top 50 μm (cyan) and bottom 100 μm (magenta) of the uterus after normalizing starting coordinates to the origin. **d**) Average track speeds. **** $p<0.0001$ Mann-Whitney U-test, bars indicate mean ± S.E.M.



Supplementary Figure 3

Proliferation of endogenous LCMV-specific $CD8^+$ T cells within the FRT after local challenge.

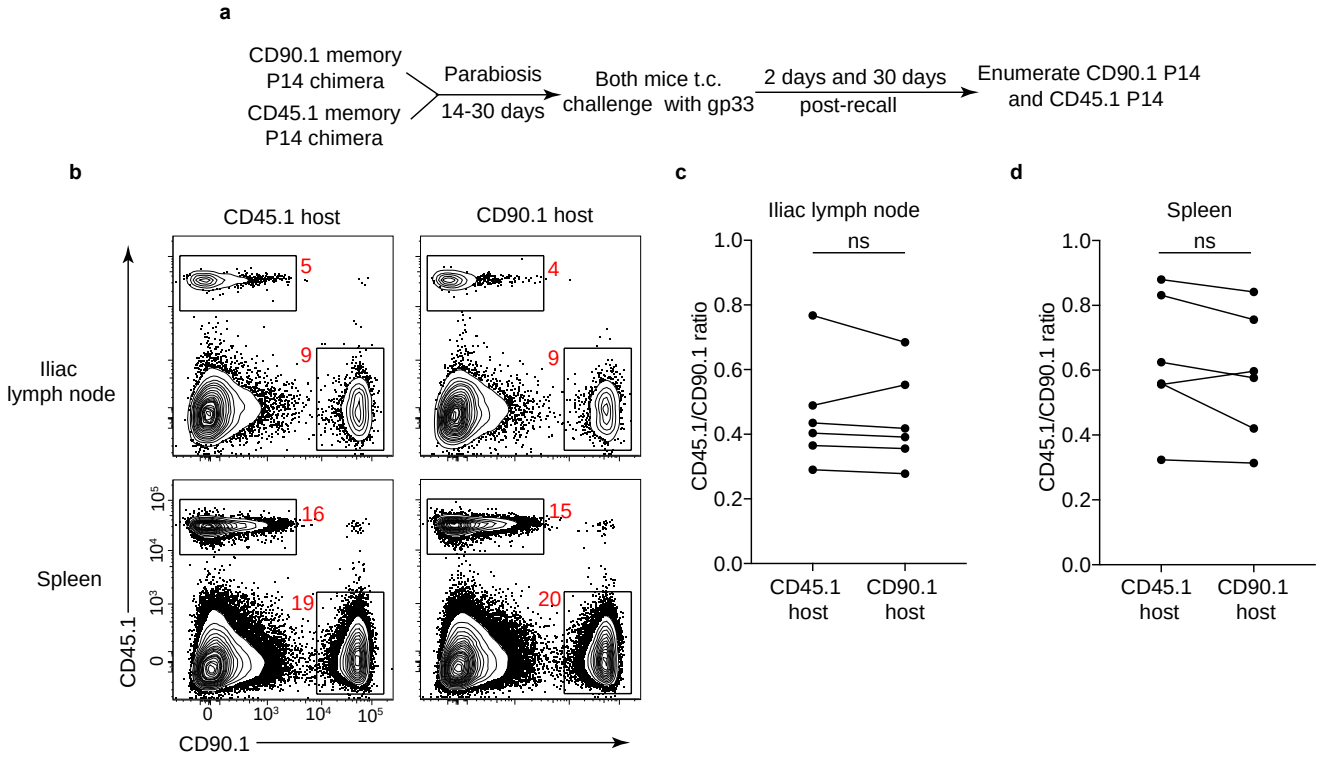
C57BL/6 mice were infected with LCMV Armstrong. >90 days later, rechallenged with gp33 t.c. 46h after peptide challenge, mice were injected i.p. with BrdU. Two hours later, H-$2D^b$/gp33 MHC I $tetramer^+$ $CD8^+$ T cells that were isolated from the FRT were stained with anti-Ki67 and anti-BrdU antibodies to determine that cells were proliferating. SIINFEKL constituted a control peptide that did not reactivate T_{RM} cells. **a**) representative flow cytometry. **b**) Summary of data from one of two similar experiments (n=4 per group per experiment). ** $p<0.01$, Mann-Whitney U-test, box plots with individual data points shown. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the minimum and maximum data points.



Supplementary Figure 4

$CD4^+$ T cell help is dispensable for $CD8^+$ T cell migration and retention in the FRT, and depletion of dendritic cells does not impair in situ proliferation.

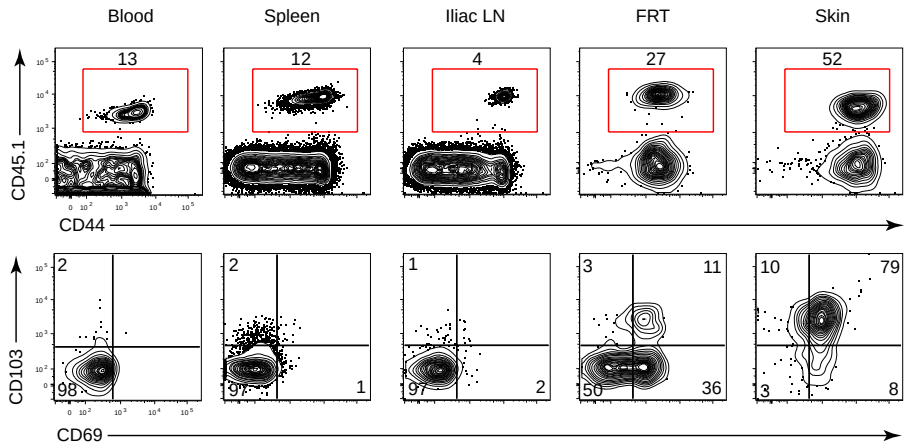
Wild type (WT) or MHC II^- mice received $5X10^4$ P14 $CD8^+$ T cell and were infected with LCMV Armstrong one day later. **a**) Representative immunohistochemistry images showing P14 $CD8^+$ T cells (stained using anti-CD90.1 antibody, cyan) in the uterine horn 30 days post-infection in both strains of mice (scale bars, 250 µm). **b**&**c**) Number of P14 CD8+ T cells enumerated by QIM on indicated days. **d**) P14 immune chimeras were made as described in CD11c-DTR bone chimeric mice as described in **Fig. 4e**. Dendritic cells were identified as $CD11c^+$/MHC II bright $CD45^+$ cells. Flow cytometric plots of $CD45^+$ leukocytes (top row) and $CD90.1^+$ P14 $CD8^+$ T cells (bottom row) isolated from the FRT indicate that the depletion of DCs via diphtheria toxin (DT) did not impair induction of P14 proliferation program 24h after gp33 t.c. Data are representative of two separate experiments with 3 mice/group per experiment. ns= not significant, Mann-Whitney U-test, bars indicate mean ± S.E.M.



Supplementary Figure 5

Equilibration of P14 CD8$^+$ memory T cells in lymph nodes and spleen after parabiosis.

a) Schematic for experiments in **Fig. 6**. Both CD45.1$^+$ P14 and CD90.1$^+$ P14 LCMV immune chimeras were generated in separate C57BL/6 (CD45.2$^+$/CD90.2$^+$) mice. 60 days after LCMV infection, mice underwent parabiosis surgery. 14-30 days later, both mice were challenged t.c. with gp33 peptide. CD45.1$^+$ and CD90.1$^+$ P14 were assessed in the spleens and FRTs of both parabiont pairs at day 2 and day 30 post-recall. **b)** Representative plots, gated on CD8$^+$ lymphocytes, and **c&d)** plots indicating the ratio of CD45.1$^+$ to CD90.1$^+$ P14 memory CD8$^+$ T cells in individual parabiont pairs before gp33 peptide challenge. Data are representative of two separate experiments with at least three parabiont pairs/experiment totaling 12 individual mice in individual groups. Wilcoxson signed rank test. ns-not significant.



Supplementary Figure 6

VSV infection establishes broadly distributed $CD8^+$ T cell memory.

Naïve CD45.1+ OT-I $CD8^+$ T cells were intravenously transferred to C57Bl/6 mice. The following day, recipients were infected with VSV-OVA i.v. 120 days later, lymphocytes were isolated from the indicated tissues and analyzed by flow cytometry. Top row gated on CD8β+ lymphocytes, bottom row gated on CD45.1+ OT-I $CD8^+$ T cells.