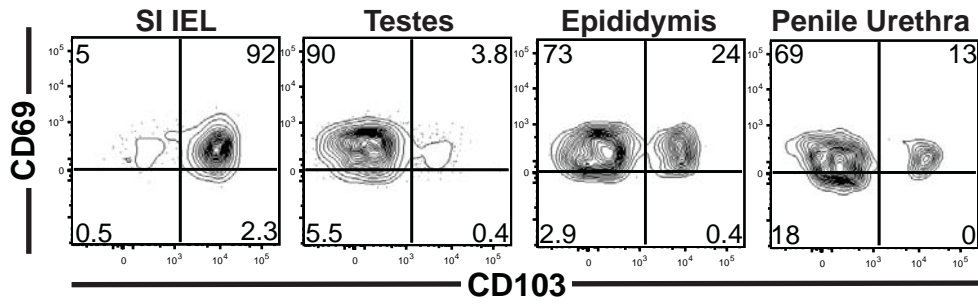
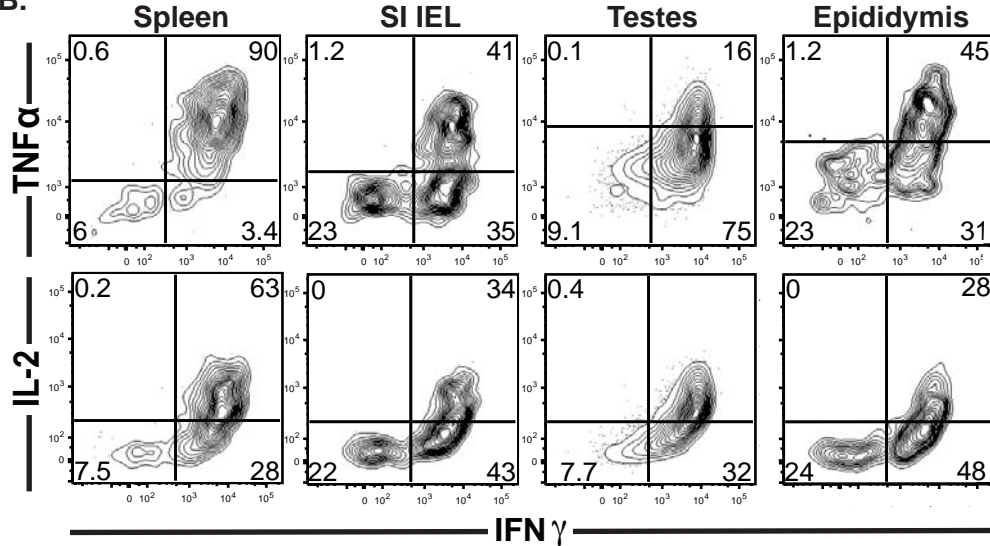


Supplemental Figure 1: Memory CD8 T cells from testes and epididymis are functional

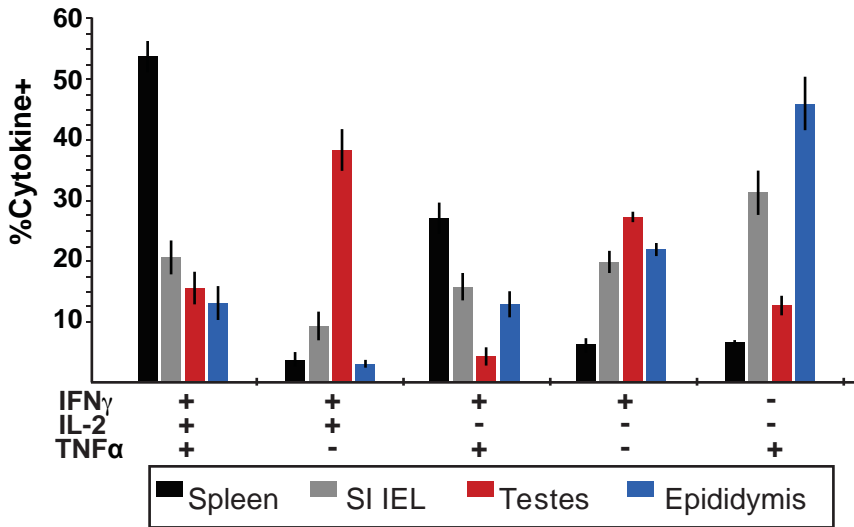
A.



B.

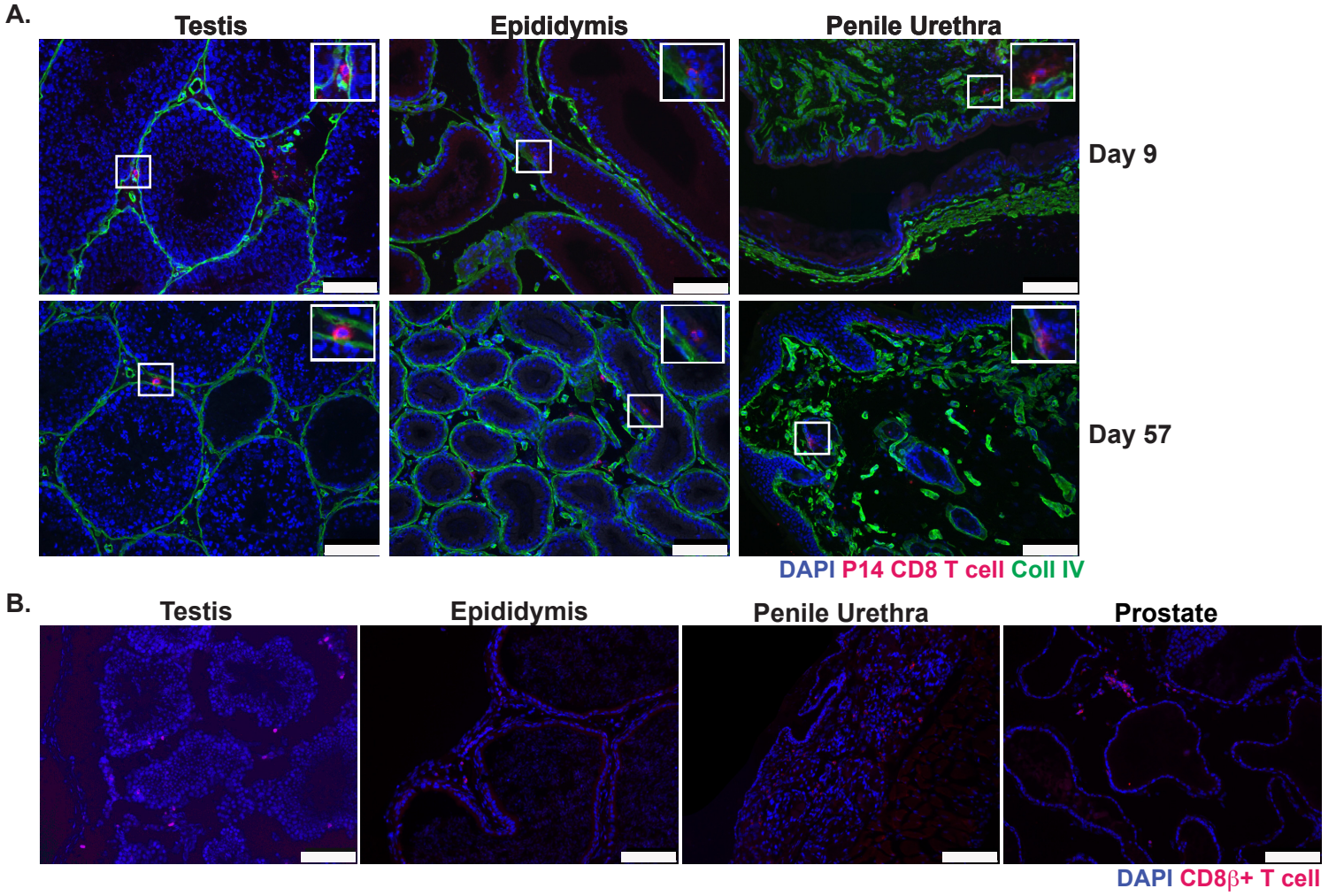


C.



Supplemental Figure 1: P14 CD8 T cells from D120-160 memory immune chimeras (A) stained directly ex vivo for CD103 and CD69. Representative flow plots. (B-C) P14 CD8 T cells from D120-160 memory immune chimeras were stimulated in vitro for 4hrs with gp33 peptide (cognate) or no peptide controls then analyzed by flow cytometry for cytokine production (blood contaminants excluded by intravascular antibody staining), (B) representative flow plots (C) percent P14 from each tissue producing indicated cytokine combination. n=12 from 3 experiments. Graph shows mean and SEM.

Supplemental Figure 2: Effector and memory CD8 T cells are located in the MGT after local lung infection.



Supplemental Figure 2: Effector and memory CD8 T cells are located in the MGT after local lung infection. (A) Naïve P14 CD8 T cells were transferred to C57BL/6 mice prior to intranasal flu-gp33 infection. Representative images of P14 identified in testes, epididymis and penile urethra 9 and 57 days after infection. Thy1.1+ P14 (red), DAPI (blue), Collagen IV (green). n=6-8 from 2 experiments. (B) Pet store mice were bred and litter success was determined before analyzing CD8β + T cell population in testes, epididymis, penile urethra and prostate, CD8β (red), DAPI (blue), n=4 separate breeders

Supplemental Video 1 (linked to Figure 2D): $3-4 \times 10^6$ LCMV D6 effector P14-gfp were i.p. transferred to infection-matched recipients. The MGT was harvested 3h after injection and P14-gfp attachment was visualized by 2-photon microscopy. Video shows attached P14-gfp cells (green) and an embedded P14-gfp CD8 T cell (green) under the collagen capsule layer. A white circle identifies the T cell that is embedded in the tissue. Second harmonic generation (SHG) is shown in blue.

Supplemental Video 2 (linked to Figure 2E): 4×10^6 D6 effector P14-gfp were incubated in vitro with infection-matched tissue for 1-3h before being visualized by 2-photon microscopy. The video shows P14-gfp CD8 T cells (green) under the collagen capsule layer and embedded in the tissue. White circles identify T cells that are embedded. SHG is shown in blue.

Supplemental Video 3 (linked to Figure 3D): Memory P14-gfp CD8 T cells were co-cultured for 2-3h with MGT freshly isolated from mice infected 6 days prior with LCMV. Live imaging was captured by 2-photon microscopy. The first video shows P14-gfp CD8 T cells (green) attached and rolling along the tissue surface. The second video shows P14-gfp CD8 T cells (green) that are embedded. Embedded T cells are identified by white circles. SHG/hoescht are shown in blue.