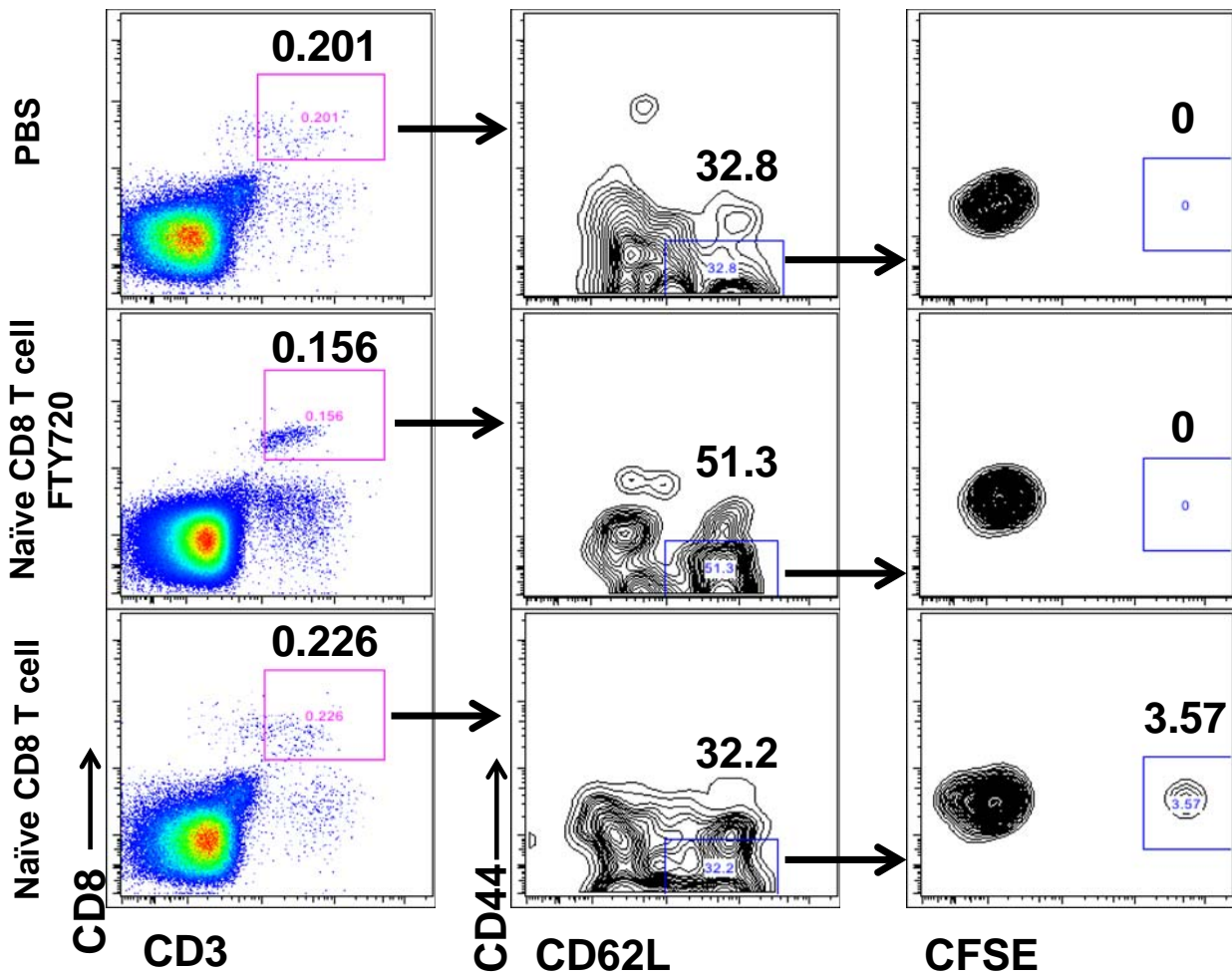


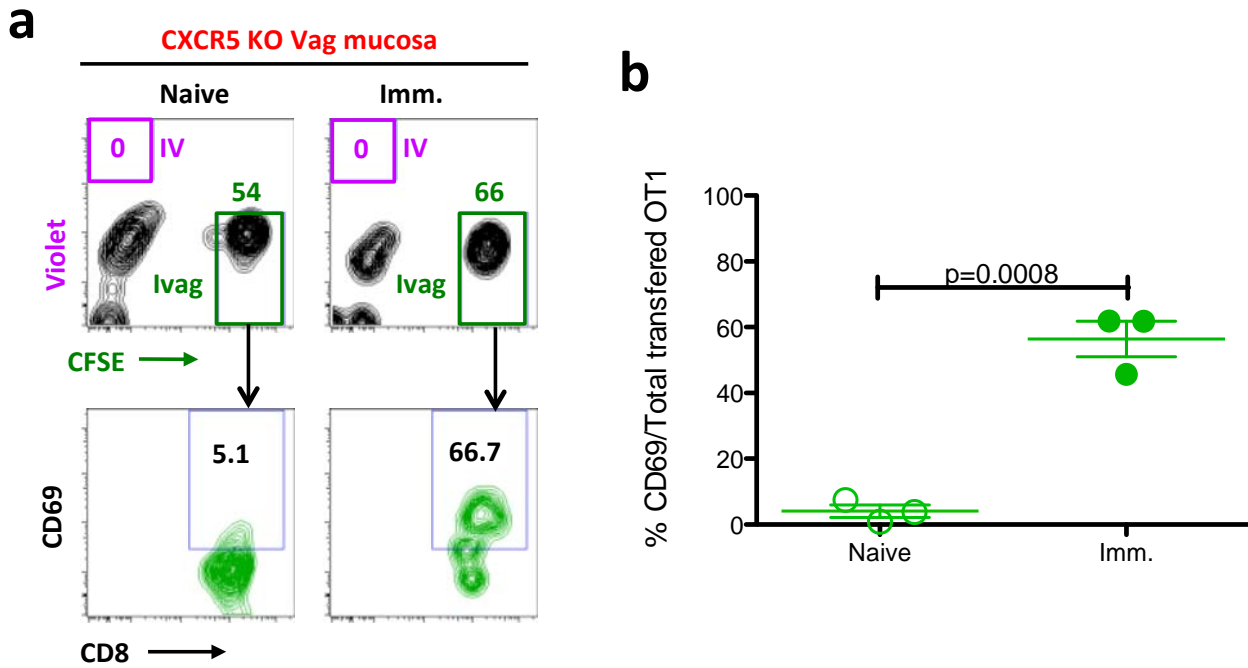
Supplementary figure 1. Naïve CD8⁺ T cells present in vaginal mucosal parenchyma.

Naïve *BALB/c* or *C57BL/6* mice received either PBS or 25 ng of APC-labeled anti-Thy1.2 antibody through IV injection. One minute after the injection, all the mice were euthanized. The cells from both digested vagina and retro orbital venous plexus were collected. **(a)** Gating strategy. **(b)** The median percentage (median \pm interquartile range) of Thy1.2 negative naïve CD8⁺ T cells per total naïve CD8⁺ T cells summarized from the pool of 4 independently repeated experiments (n=13). The p value was generated using a Mann-Whitney test.



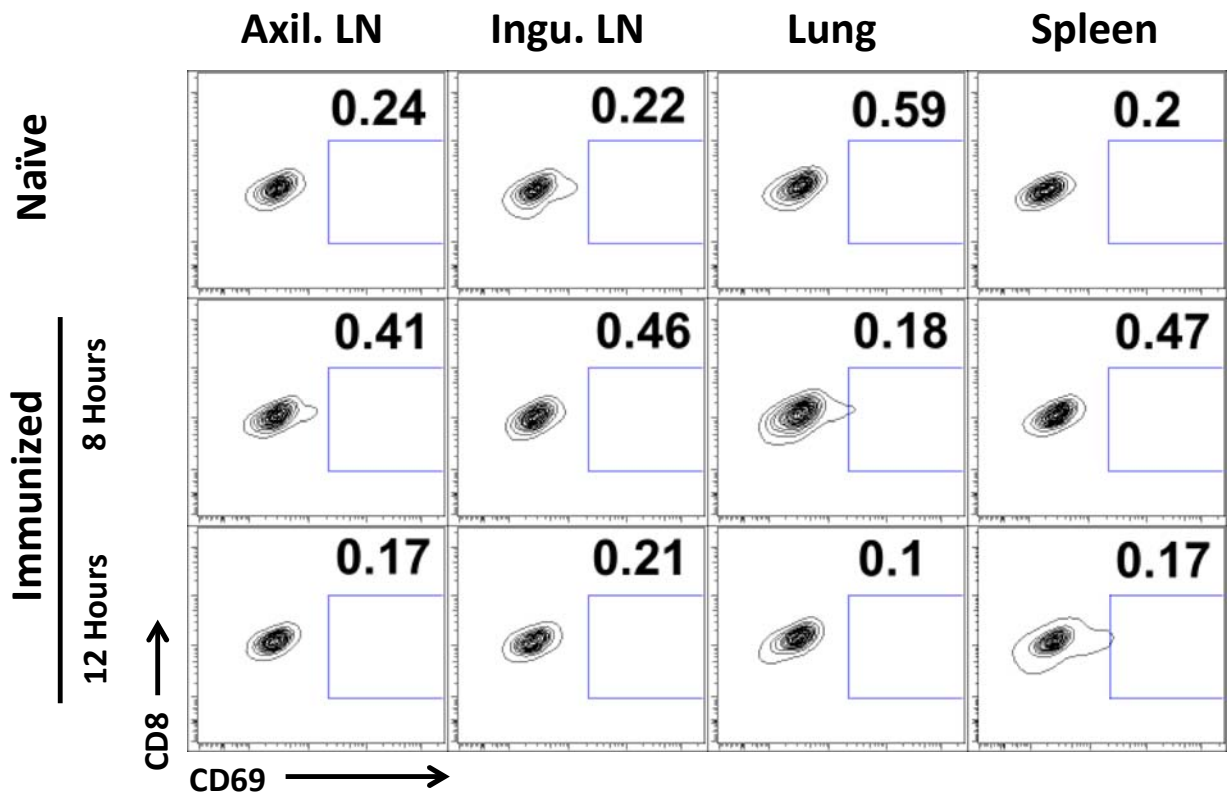
Supplementary figure 2. The FTY720-induced blockage of vaginal infiltration of adoptively transferred naive CD8⁺ T cells.

Representative figures from 2 independently repeated experiments (n=2) show the percentage of adoptively transferred naive CD8⁺ T cells in the vaginal tissues of the mice receiving naive CD8⁺ T cells, naive CD8⁺ T cells plus FTY720 or PBS. The flow cytometry data were collected using the pool of 5 digested vaginal tissues.



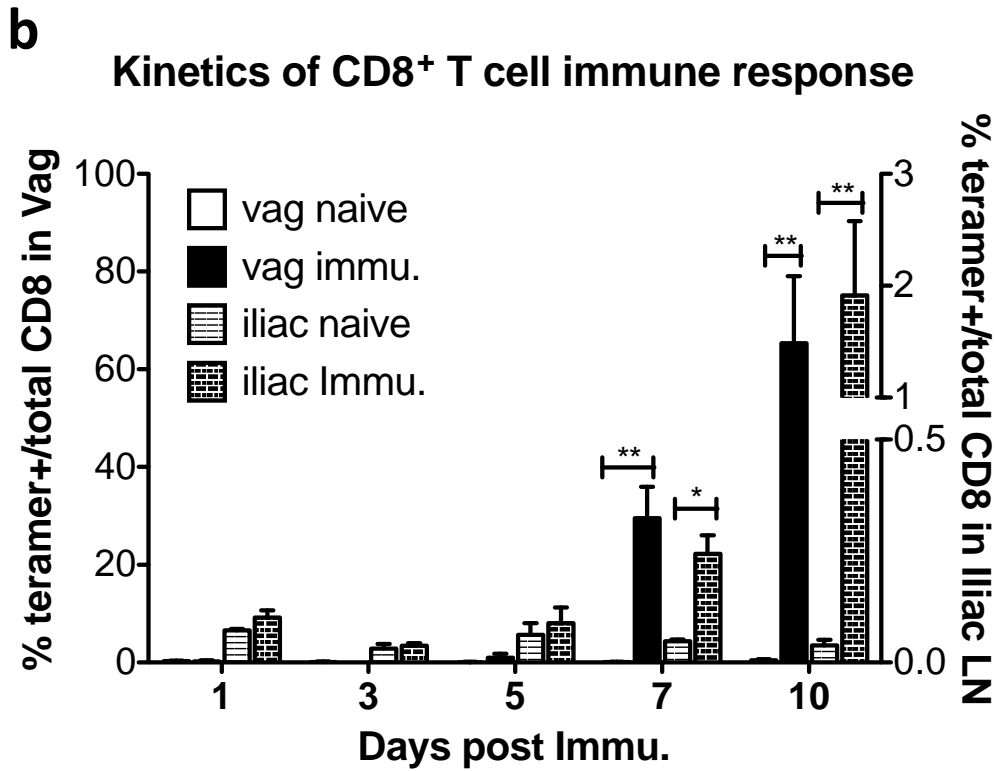
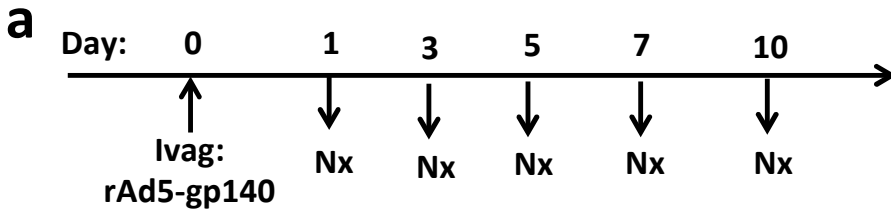
Supplementary figure 3. The CD69 expression of Ivag vs IV transferred naïve OT-I CD8⁺ T cells in LN-deficient CXCR5 KO mice at 24 hours PI

(a) The total and CD69-expressing transferred naïve OT-I CD8⁺ T cells in the vaginal mucosa. (b) The percentage of CD69 expression in each group. This is representative of 2 independently repeated experiments (n=3) with the same results. The p value was generated using a Student's *t* test.



Supplementary figure 4. The CD69 expression of CD8⁺ T cells in different tissue compartments of naïve and immunized RAG-I KO, OT-I transgenic mice that were sacrificed at 8 hours or 12 hours PI.

This is representative of 2 independently repeated experiments (n=3) with the same results.



Supplementary figure 5. The kinetics of CD8⁺ T cell immune induction after Ivag immunization.

This is representative of 2 independently repeated experiments (n=5 at each time point) with the same results. (a) The experimental design. (b) The percentage of Ag-specific CD8⁺ T cells in vaginal mucosa and iliac LN over time (*, p<0.05, **, p<0.01). The p values were generated using Student's *t* test.