



Supplementary Materials for

A mucosal vaccine against *Chlamydia trachomatis* generates two waves of protective memory T cells

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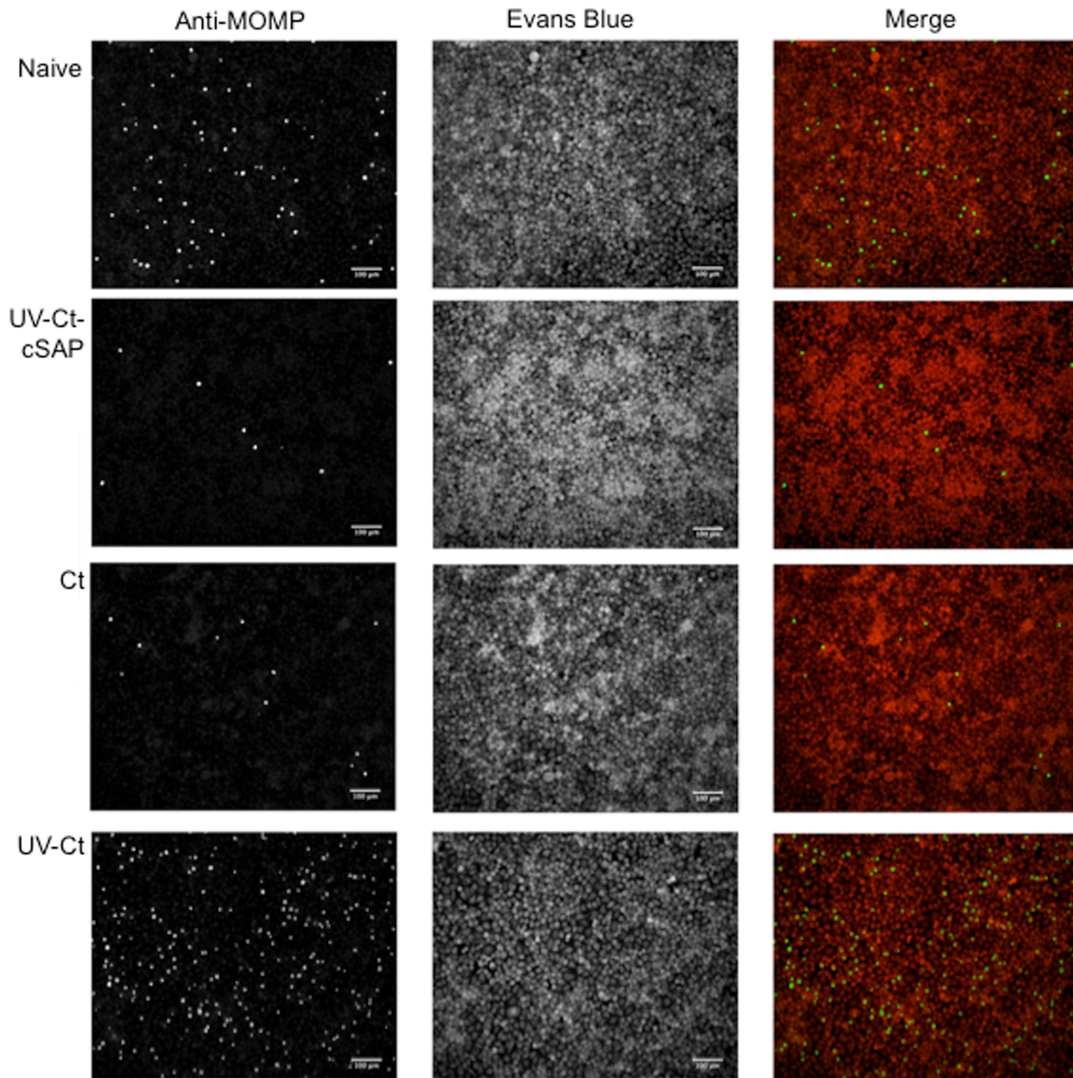
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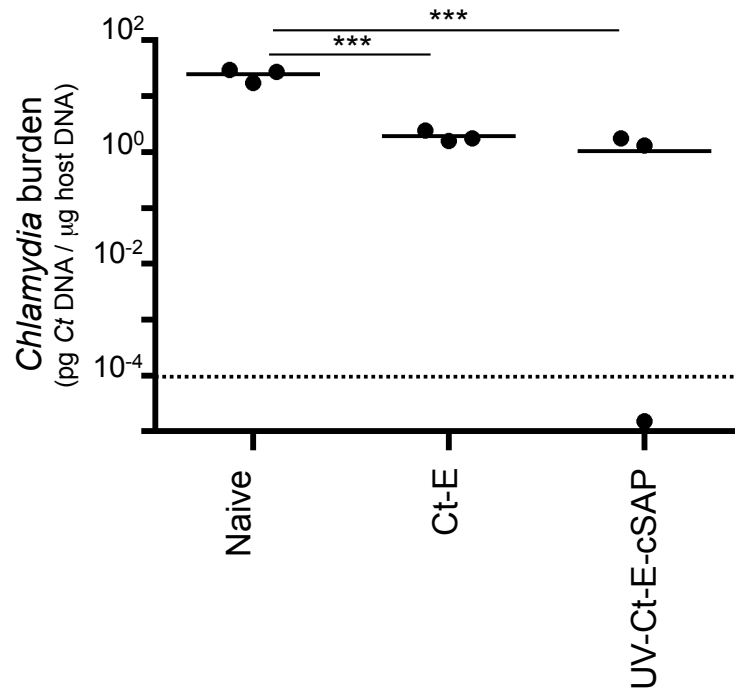
Supplementary Materials:

Figures S1 – S10



Supplementary Figure 1. Assessment of uterine *Ct* burden in immunized mice.

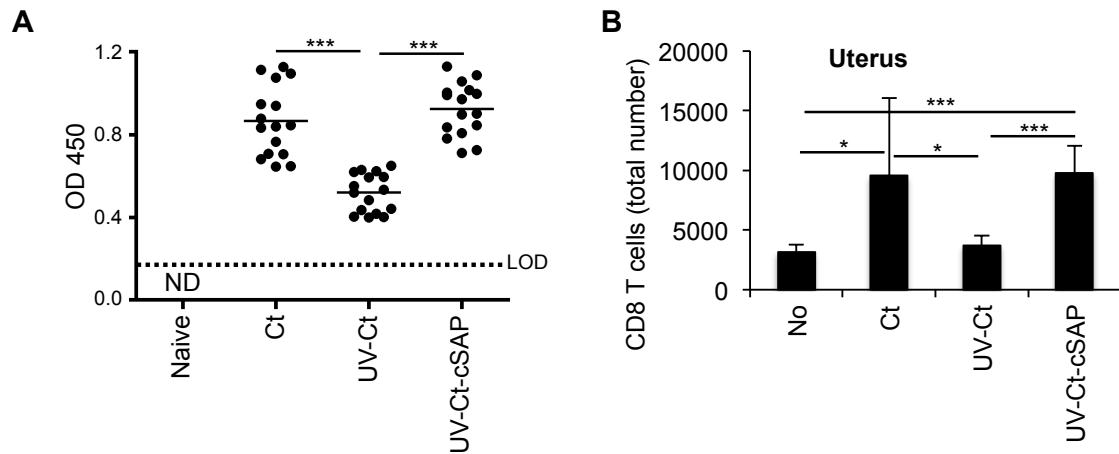
Representative micrographs of McCoy cells stained with Evans blue (red) incubated with titrated tissue homogenates of uteri harvested on day 5 after *Ct* challenge to detect inclusion forming units (IFUs) of *Ct*. IFUs were identified by staining with anti-MOMP mAb (green). Original magnification 40X. Quantitative data are provided in **Fig. 2C**.



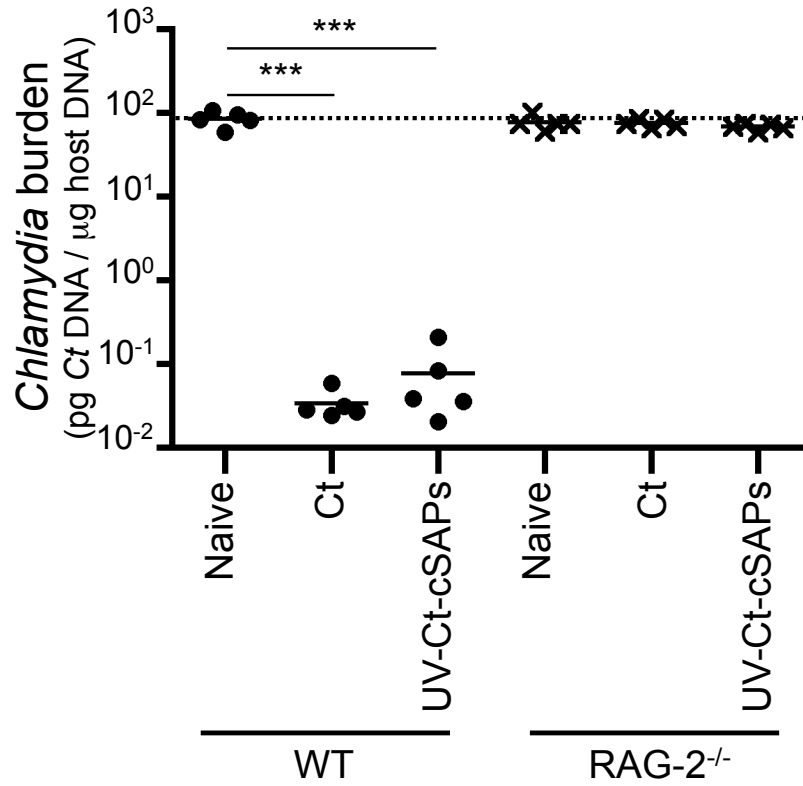
Supplementary Figure 2. Protection against Chlamydia serovar E. *Ct* burden

following i.u. challenge with live *Ct-E* 4 weeks after immunization with *Ct-E* or UV-*Ct-*

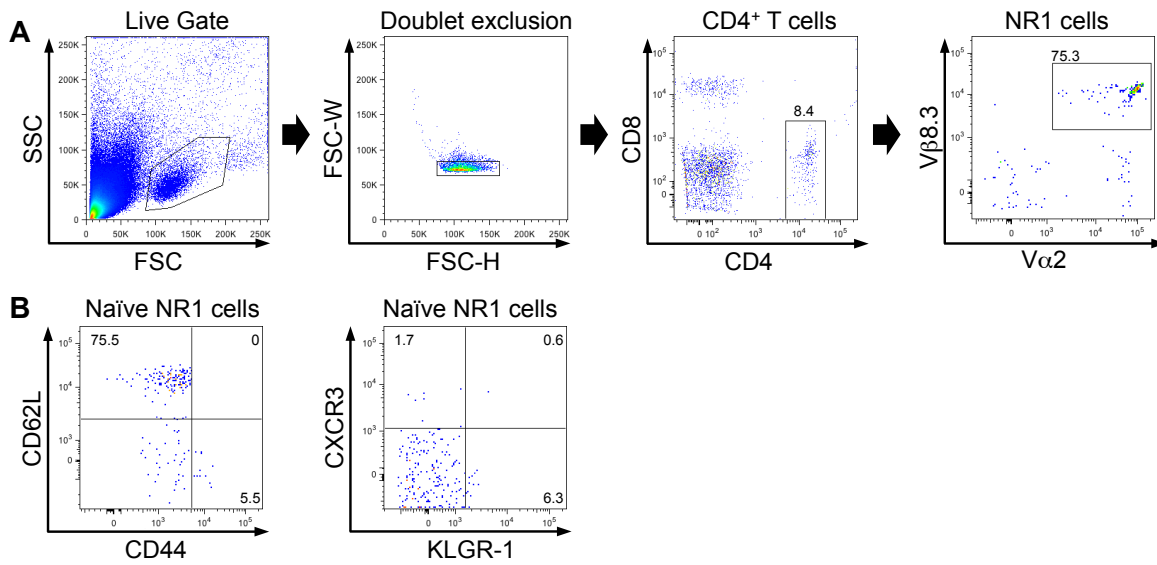
E-cSAP. n = 3 mice/group; broken line indicates limit of detection; *** $P < 0.001$.



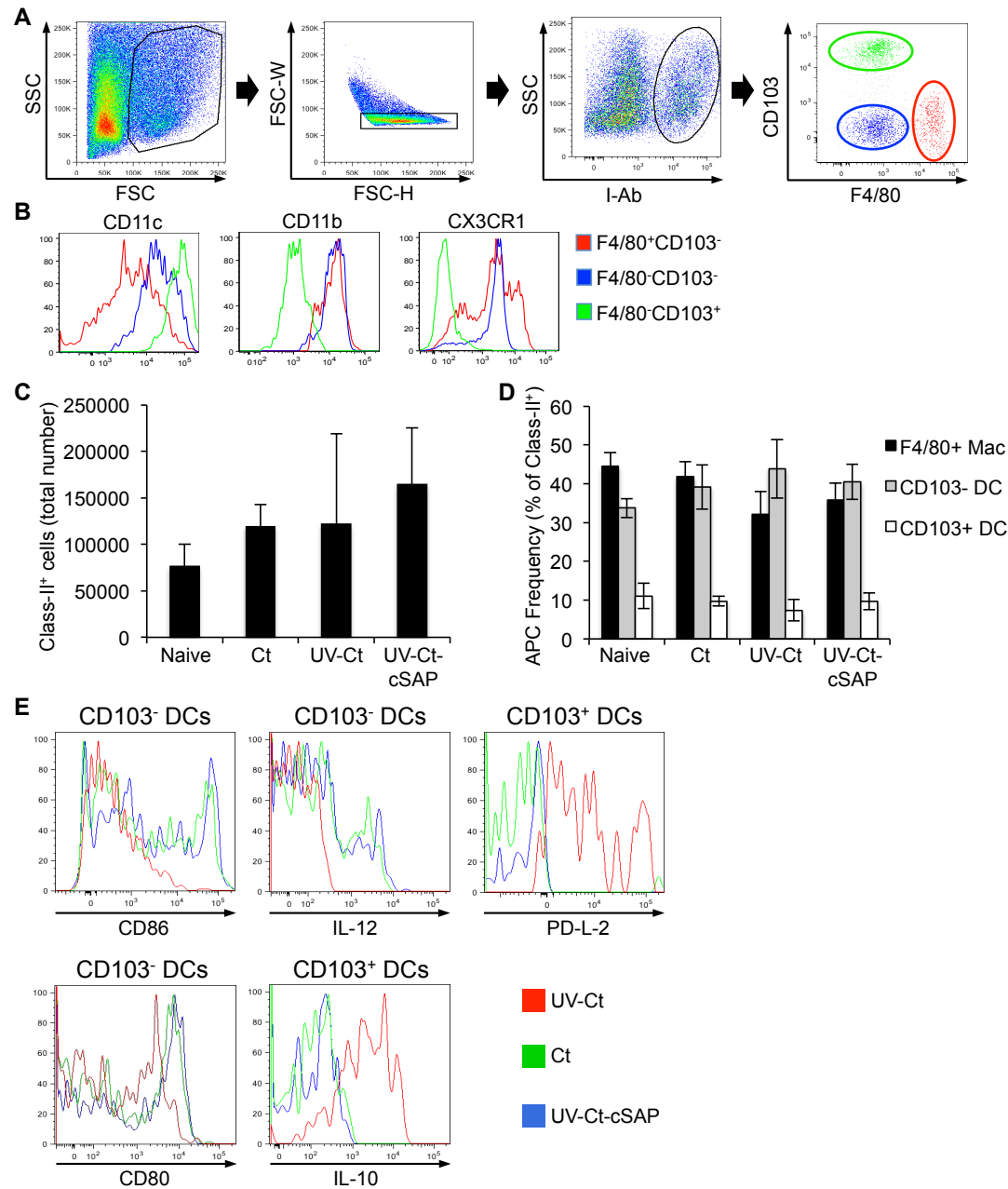
Supplementary Figure 3. *Ct*-specific antibodies and uterine CD8 T cells after UV-*Ct*-cSAP immunization. (A) Anti-*Ct* IgG titers in serum were determined by enzyme-linked immunosorbent assay 4 weeks after immunization. Symbols represent optical densities (OD) of sera from individual mice. Results were pooled from two independent experiments. *** $P < 0.001$. ND, not detected; LOD, limit of detection. (B) Frequency of CD8 T cells in the uterus 4 days after immunization ($n = 4-6$ mice/group; * $P < 0.05$; *** $P < 0.001$). Error bars show mean \pm SEM.



Supplementary Figure 4. Immunized RAG-2^{-/-} mice do not develop immunity or tolerance against *Ct* challenge. *Ct* burden following i.u. challenge with live *Ct* 4 weeks after immunization of RAG-2^{-/-} mice (n= 5 mice/group; *** $P<0.001$).

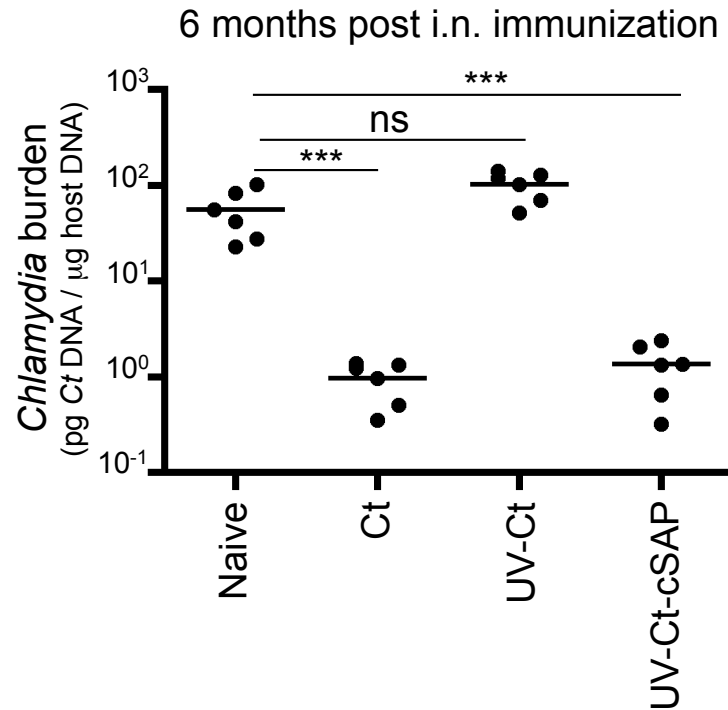


Supplementary Figure 5. Phenotype of NR1 T_N cells. (A) Gating strategy to identify TCR transgenic (i.e. CD4⁺Vβ8.3⁺Vα2⁺) T cells among splenic and LN mononuclear cells from NR1 mice. (B) Representative dot plots of CD4⁺Vβ8.3⁺Vα2⁺ NR1 cells stained for differentiation and activation markers indicate a predominant CD62L⁺ CD44^{low} CXCR3⁻ KLGR1⁻ phenotype characteristic of T_N.



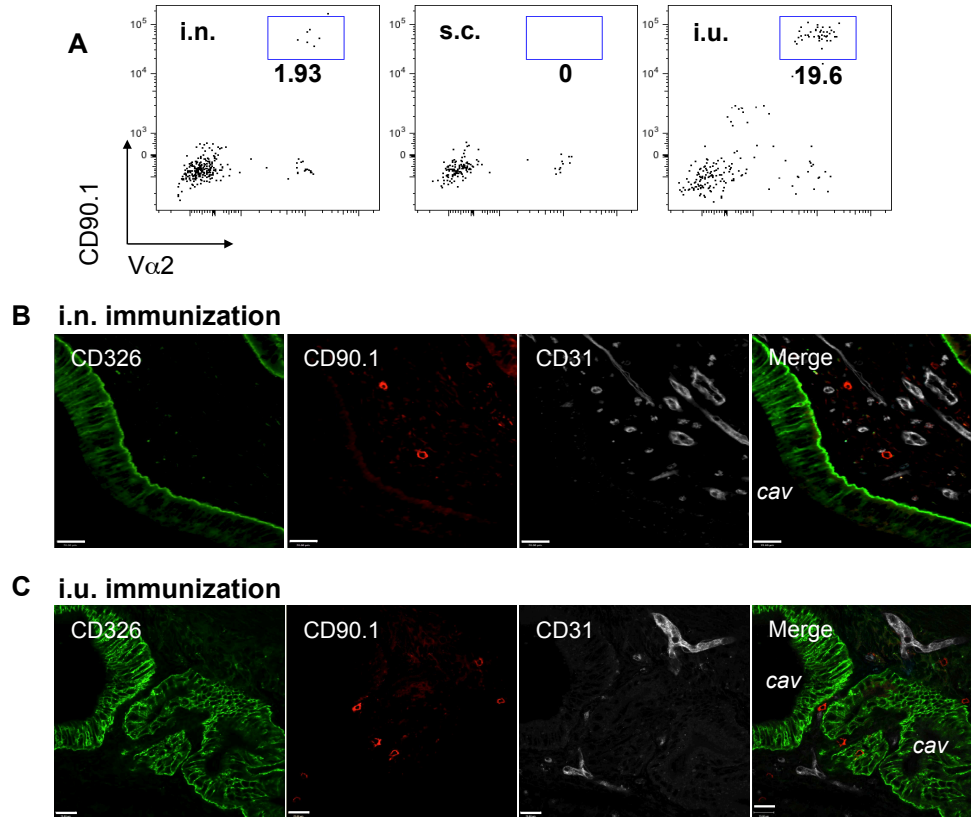
Supplementary Figure 6. Composition of Ag-presenting cells in uteri of naïve and immunized mice. (A) Gating strategy for MHC class II⁺ uterine APC subsets. (B) Representative histogram plots of CD11c, CD11b and CX3CR1 expression on CD45⁺ MHC-II⁺ uterine APC subsets in naïve mice. (C) Total number of uterine APCs and (D) relative distribution of APC subsets 18 hours after immunization, the timepoint at which APC subsets were harvested for experiments in **Fig. 5B** and **D-F**. (n=5 mice/group). (E) Representative

histogram plots of costimulatory molecule and cytokine expression on CD103⁺ and CD103⁻ uterine DC subsets following immunization with *Ct* (green), UV-*Ct* (red) or UV-*Ct*-cSAP (blue). Error bars show mean \pm SEM.

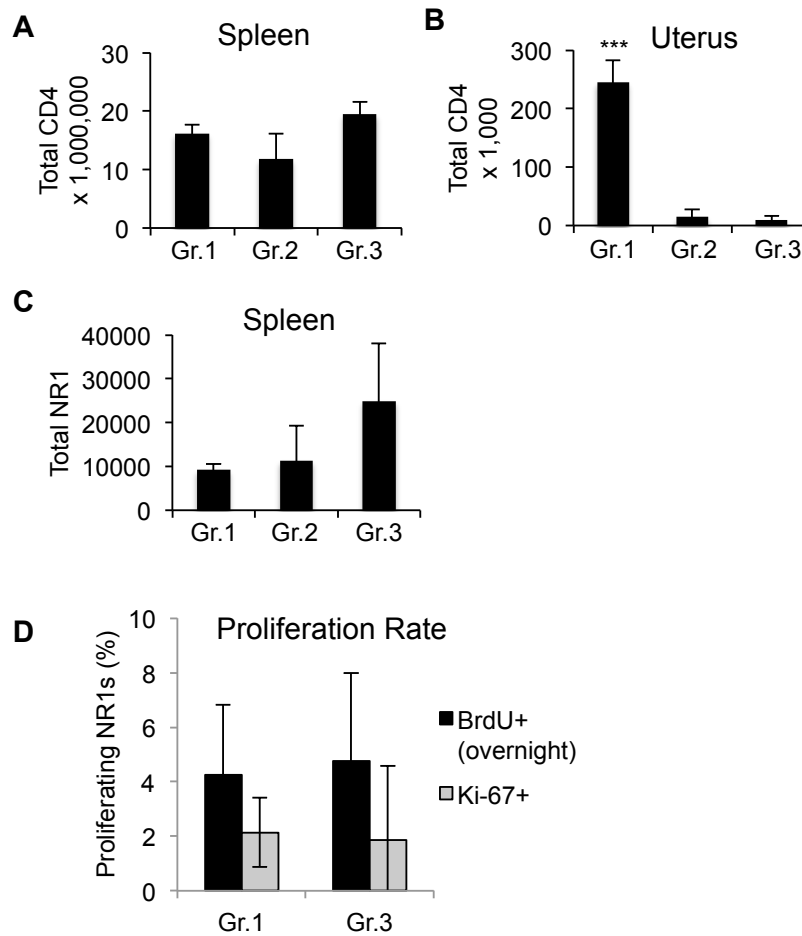


Supplementary Figure 7. Long-term protection of mice after i.n. UV-*Ct*-cSAP

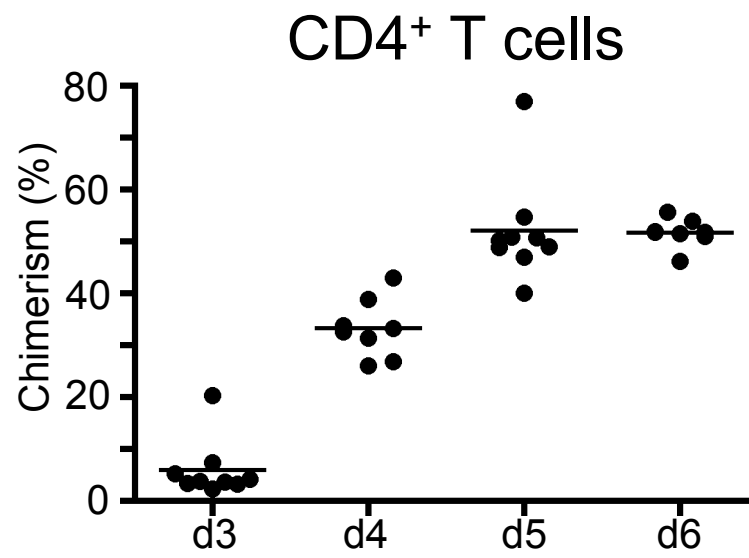
immunization. *Ct* burden was determined by qPCR following i.u. *Ct* challenge 6 months after s.c. or i.n. immunization as shown in **Fig. 1A** (n = 5-6 mice/group; *** $P < 0.001$). ns, non significant.



Supplementary Figure 8. Accumulation of *Ct*-specific T cells in blood and uterus after s.c., i.n. or i.u. immunization with UV-*Ct*-cSAP. (A) Representative FACS plots of uterus resident T cells 30 days after immunization. **(B)** Representative confocal micrographs of uterine mucosa stained with anti-CD326 (epithelial cells, green), anti-CD90.1 (transgenic marker for NR1 cells, red) and anti-CD31 (endothelial cells, grey) 30 days after intranasal or **(C)** intrauterine immunization. *cav*, uterine cavity. Scale bar: 70 μ m. Original magnification 400X.



Supplementary Figure 9. Effect of anti- $\alpha 4$ MAb treatment on bulk CD4 T cells and NR1 cells in spleen and uterus. (A-C) T cell numbers were quantified by flow cytometry in single-cell suspensions of tissues from experimental groups 1, 2 and 3 described in **Fig. 6E** ($n = 5$ mice per group, *** $P < 0.001$). Total number of CD4⁺ T cells in (A) spleen and (B) uterus. (C) Total number of splenic NR1 cells. (D) The proliferation rate of uterine NR1 T_{RM} cells in groups 1 and 3 was determined after *Ct* challenge using FACS to assess BrdU uptake or Ki-67 staining to detect actively dividing cells ($n=4$ mice/group). Error bars show mean \pm SEM.



Supplementary Figure 10. Timecourse of blood chimerism of circulating CD4⁺ T cells in parabiotic mice. Chimerism was determined by flow cytometry during days 3-6 after parabiosis surgery.