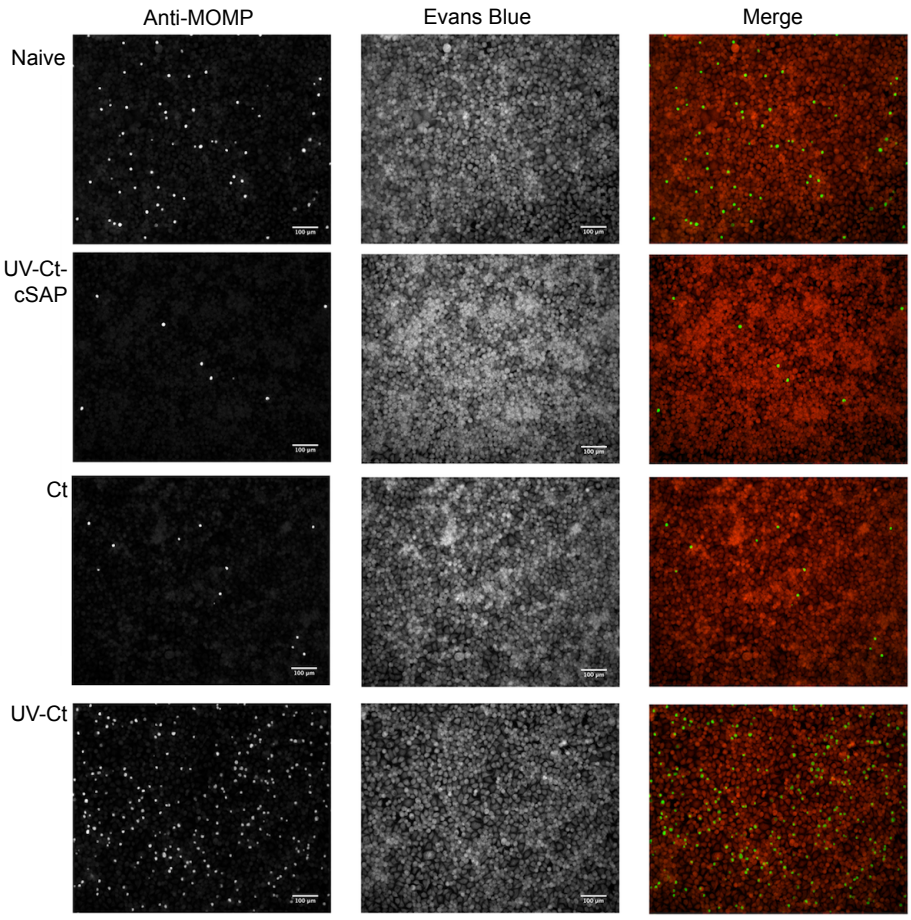
Supplementary Materials:

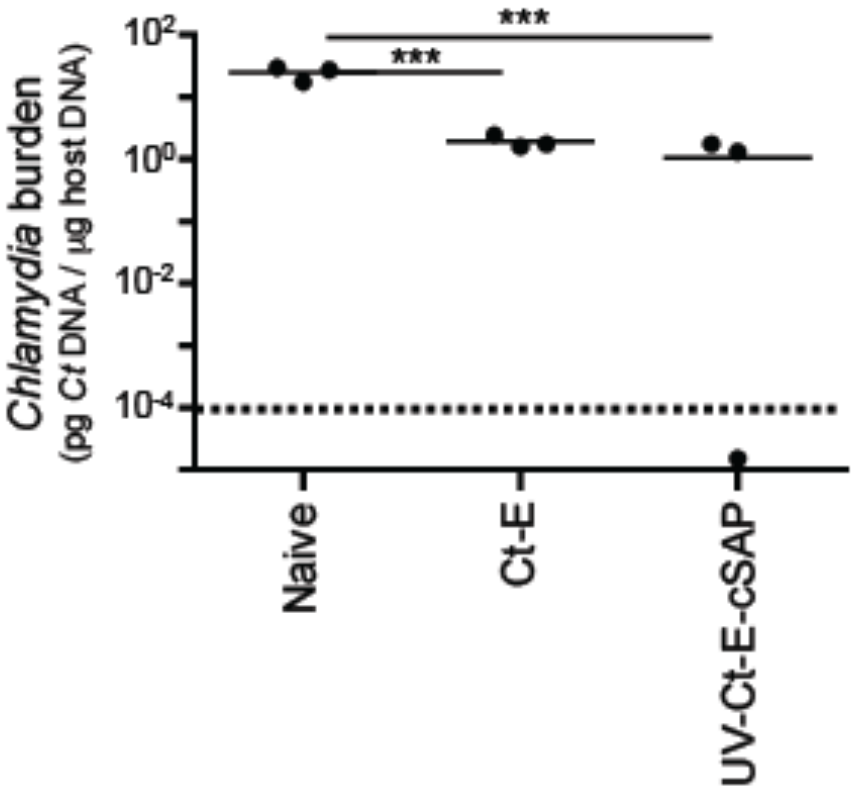
Figures S1 – S10

Supplementary Figure 1

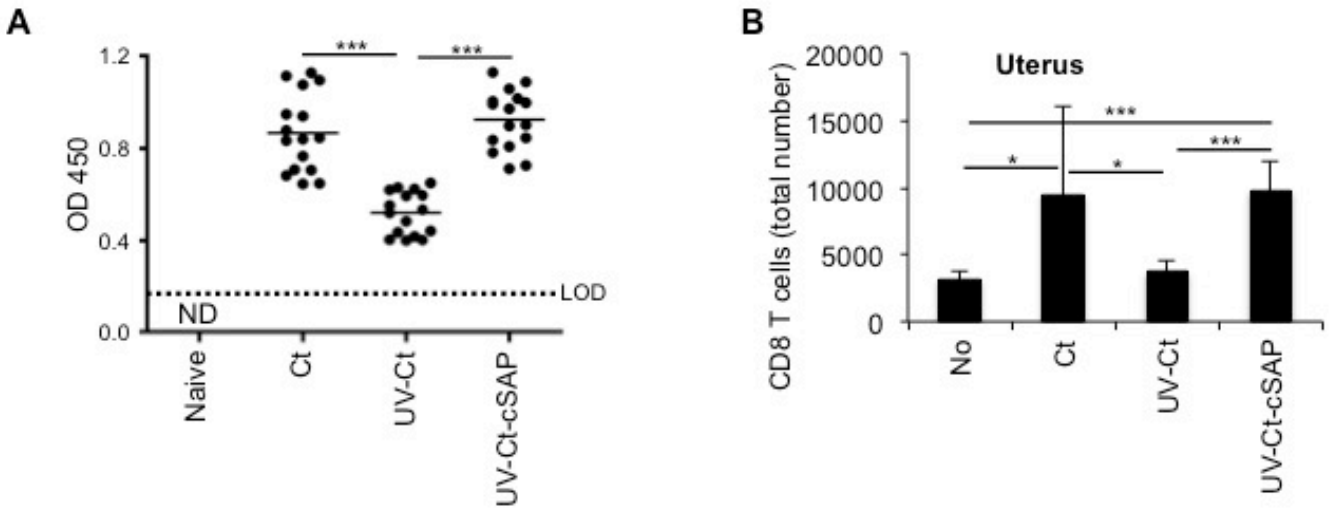


Supplementary Figure 1. Assessment of uterine *Ct* burden in immunized mice.

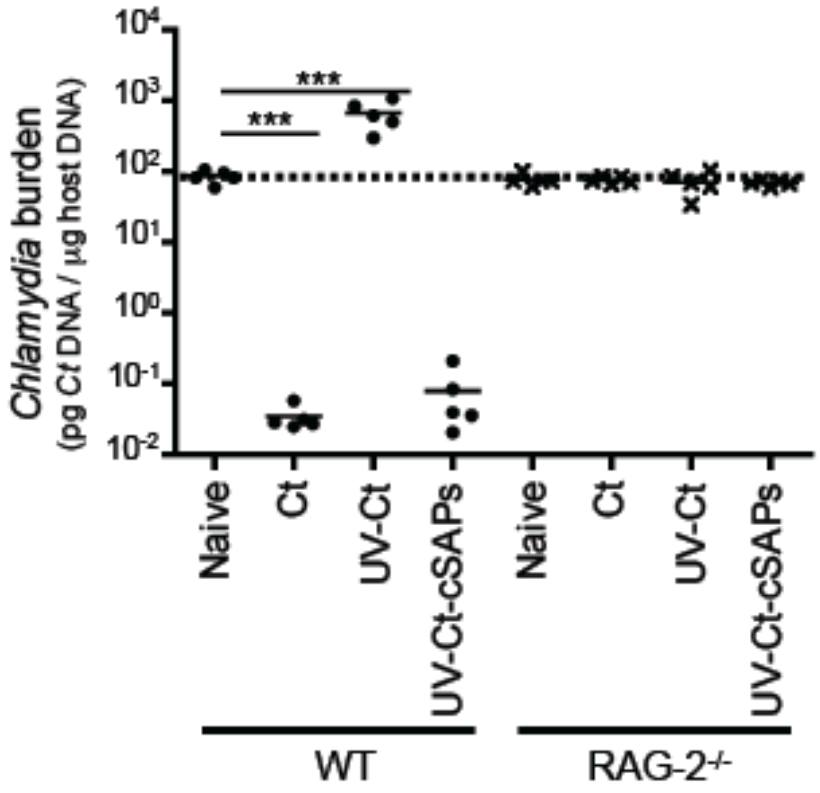
Representative micrographs of McCoy cells stained with Evans blue (red) incubated with titrated tissue homogenates of uteri harvested on day 5 after *Ct* challenge to detect inclusion forming units (IFUs) of *Ct*. IFUs were identified by staining with anti-MOMP mAb (green). Original magnification 40X.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



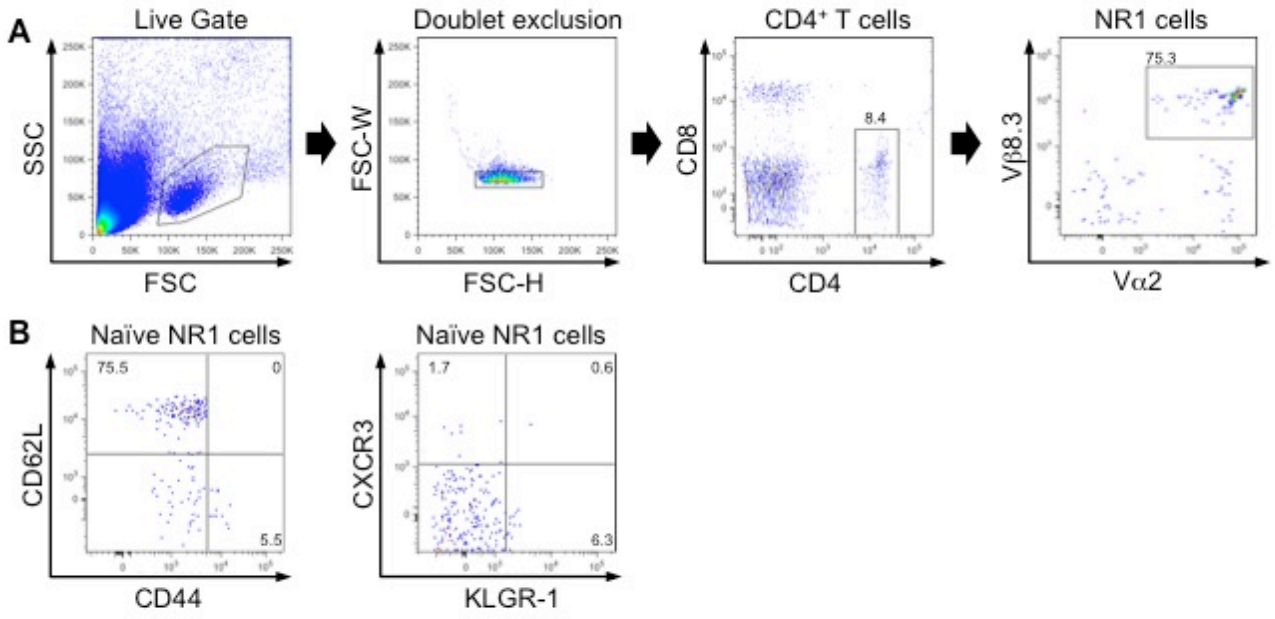
Supplementary Figure 2. Protection against Chlamydia serovar E. *Ct* burden following i.u. challenge with live *Ct-E* 4 weeks after immunization with *Ct*-E or UV-*Ct*-E-cSAP. n = 3 mice/group; broken line indicates limit of detection; *** $P<0.001$.



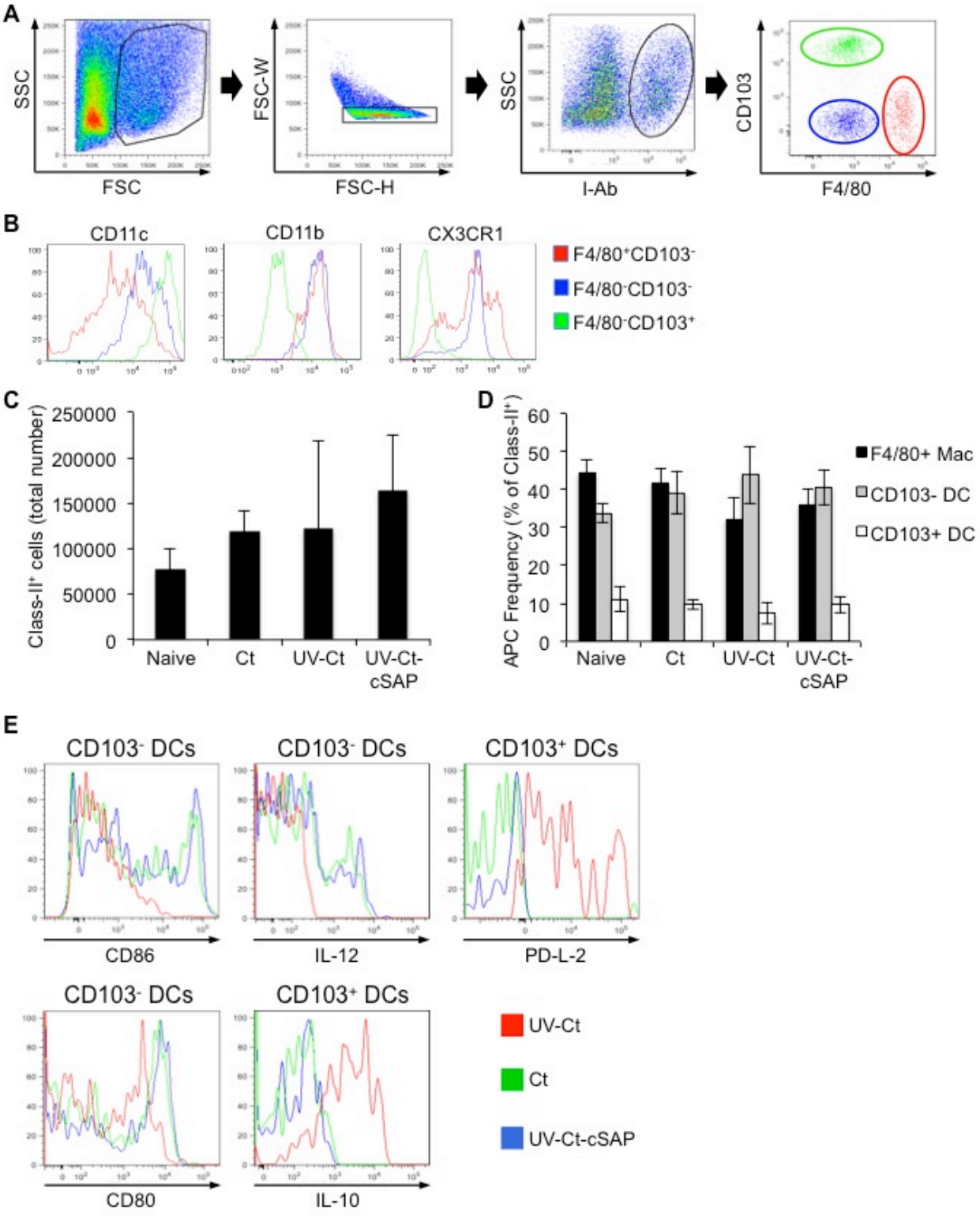
Supplementary Figure 3. *Ct*-specific antibodies and uterine CD8 T cells after UV-*Ct*-cSAP immunization. (**A**) Anti-*Ct* IgG titers in serum were determined by enzyme-linked immunosorbent assay 4 weeks after immunization. Symbols represent optical densities (OD) of sera from individual mice. Results 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 ± 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).

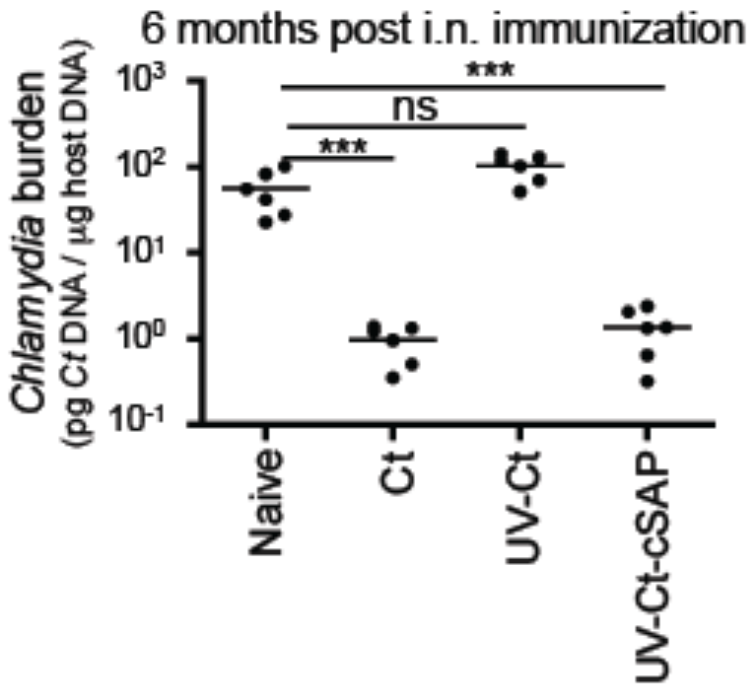


Supplementary Figure 5. Phenotype of NR1 T_N cells. (**A**) Gating strategy to identify TCR transgenic (i.e. $CD4^+V\beta8.3^+V\alpha2^+$) T cells among splenic and LN mononuclear cells from NR1 mice. (**B**) Representative dot plots of $CD4^+V\beta8.3^+V\alpha2^+$ NR1 cells stained for differentiation and activation markers indicate a predominant $CD62L^+$ $CD44^{low}$ $CXCR3^-$ $KLRG1^-$ phenotype characteristic of T_N.

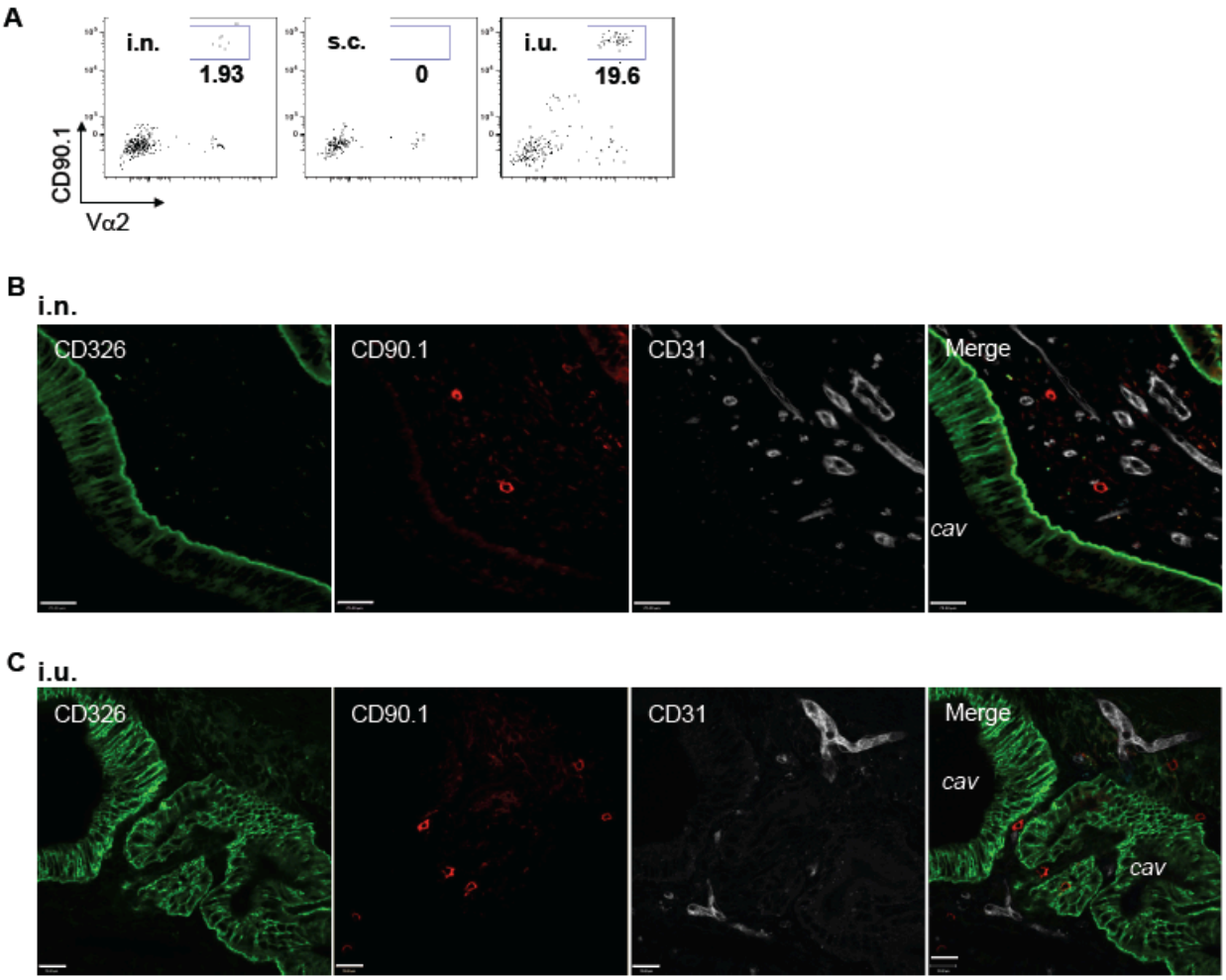


Supplementary Figure 6. Composition of Ag-presenting cells in uteri of naïve and immunized mice. (**A**) Gating strategy for MHC class II$^+$ uterine APC subsets. (**B**)

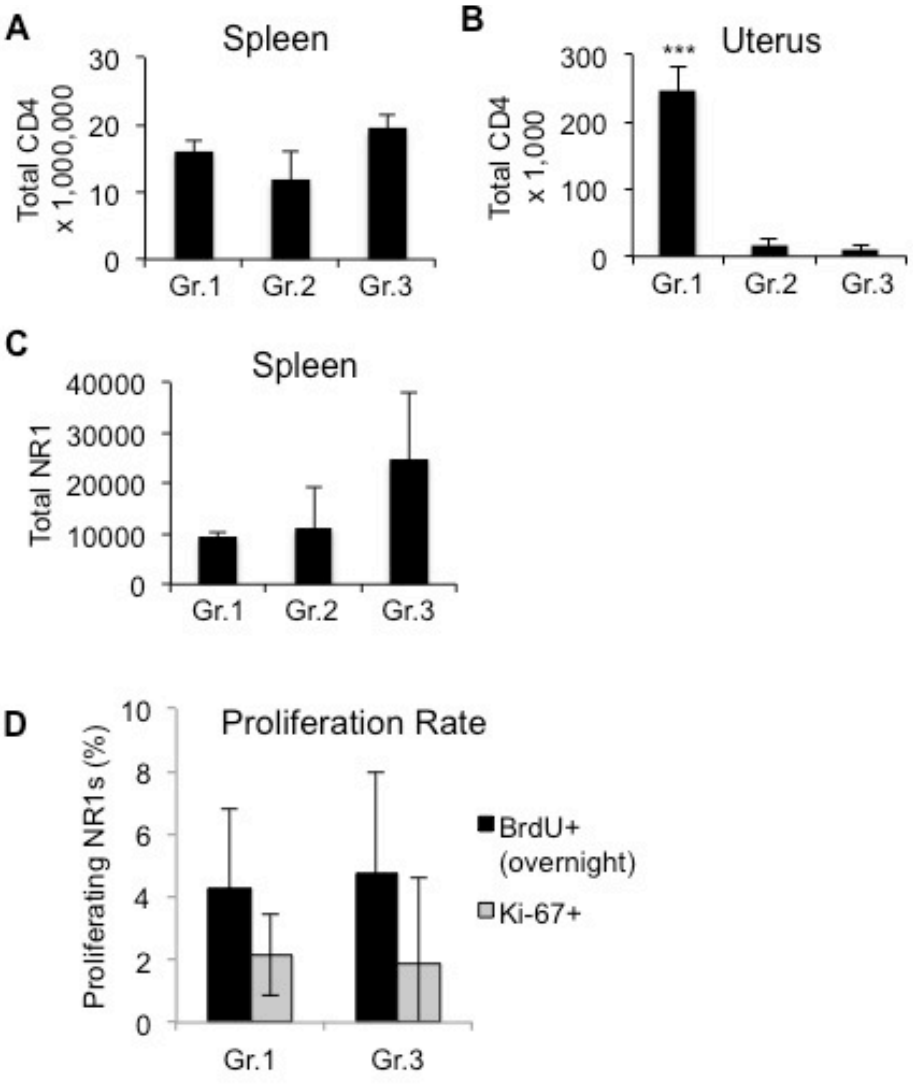
Representative histogram plots of CD11c, CD11b and CX3CR1 expression on $CD45^+$ $MHC\text{-}II^+$ uterine APC subsets in naïve mice. (**C**) Total number of uterine APCs and (**D**) relative distribution of APC subsets 18 hours after immunization, the timepoint at which APC subsets were harvested for 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 ± SEM.



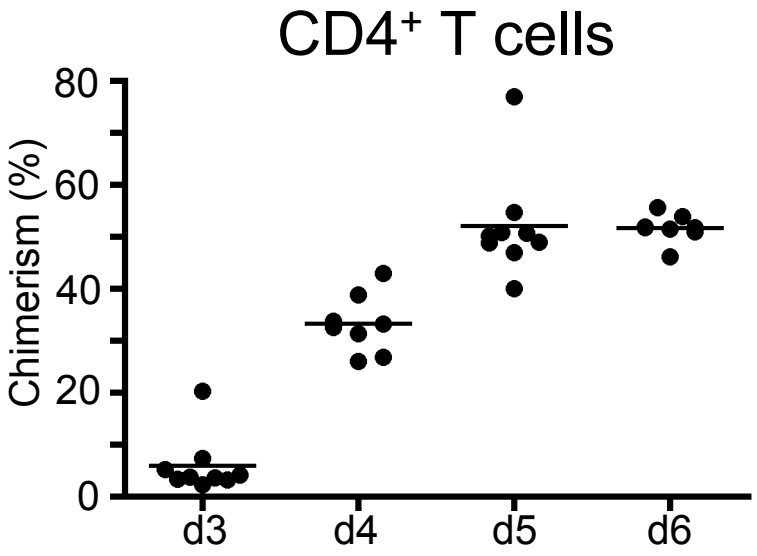
Supplementary Figure 7. Long-term protection of mice after i.n. UV-*Ct*–cSAP immunization. *Ct* burden was determined by qPCR following i.u. *Ct* challenge 6 months after s.c. or i.n. immunization as shown in **Fig. 1A** (n = 5-6 mice/group; ***$P<0.001$). ns, non significant.



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 μm. Original magnification 400X.



Supplementary Figure 9. Effect of anti-α4 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 ± 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.