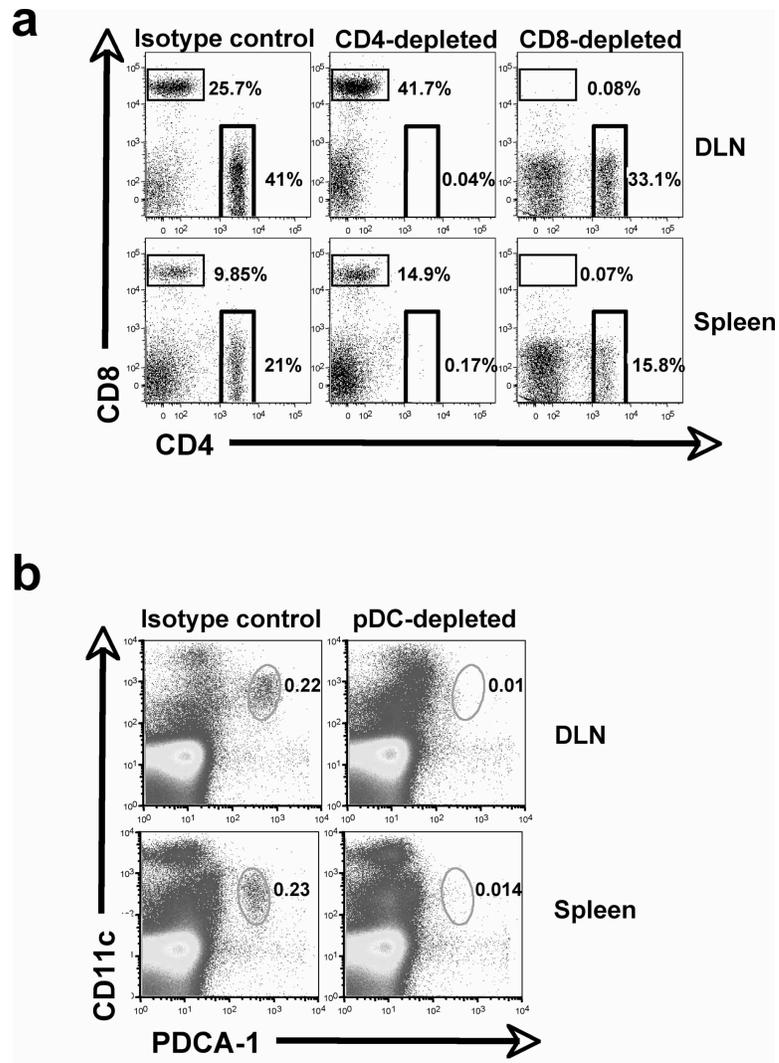


**Supplemental Figure 1.** Expression of TLR9 by primary mouse vaginal keratinocytes. Mouse vaginal epithelial cells were isolated and cultured using a modified method of human vaginal epithelial cell culture (1) adapted for mouse cells. Immediately after collecting the vaginal tract, the tissue was placed in sterile DMEM supplemented with penicillin and streptomycin. Vaginal tissues were carefully dissected into ~0.5 mm blocks, and several blocks were placed in a tissue culture plate pretreated with collagen and fibronectin, and were allowed to adhere for 30 min before being covered with Williams complete medium supplemented with fetal bovine serum (10%), insulin (5 µg/ml), transferrin (5 µg/ml), selenium (6.7 ng/ml), epidermal growth factor (5 ng/ml), penicillin and streptomycin. Plates were incubated at 37 °C in an atmosphere of 5% CO<sub>2</sub>. Once cells reached confluence (~2 weeks), the epithelial cell clusters were collected by trypsinization and plated on 24 well plates. These cells were stimulated with zymosan (100 µg/ml), Poly I:C (50 µg/ml), LPS (10 µg/ml), R848 (10 µg/ml), CpG (5 µg/ml), HSV-2 (10<sup>6</sup>pfu/ml) or media alone for 24h, and RNA was isolated for real time RT-PCR. cDNAs were normalized to HPRT and relative values were plotted on y-axis.

1. Gilbert, R.O., Elia, G., Beach, D.H., Klaessig, S., and Singh, B.N. 2000. Cytopathogenic effect of *Trichomonas vaginalis* on human vaginal epithelial cells cultured in vitro. *Infect Immun* 68:4200-4206.



**Supplemental Figure 2.** Efficiency of depletion of cellular subsets by in vivo antibody treatments. **(a)** CD4 and CD8 T cells were depleted at -3, -1 and 1, 3, 5d post infection by i.p injection of 400  $\mu$ g anti-CD4 (GK1.5; rat IgG2b) and anti-CD8 (53-6.72; rat IgG2a) Abs. Mice injected with rat IgG (500  $\mu$ g) at the same time were used as a control. Total cells from the spleen and vagina draining lymph nodes were isolated and CD4 and CD8 expressing cells were enumerated by flow cytometry. **(b)** pDCs were depleted at -1 and 1d by i.p. injection of 500  $\mu$ g of anti-mPDCA-1. Mice injected with the same amount of rat IgG was used as controls. Total cells from the spleen and draining lymph nodes were isolated and PDCA-1 expressing pDCs were analyzed by flow cytometry.