

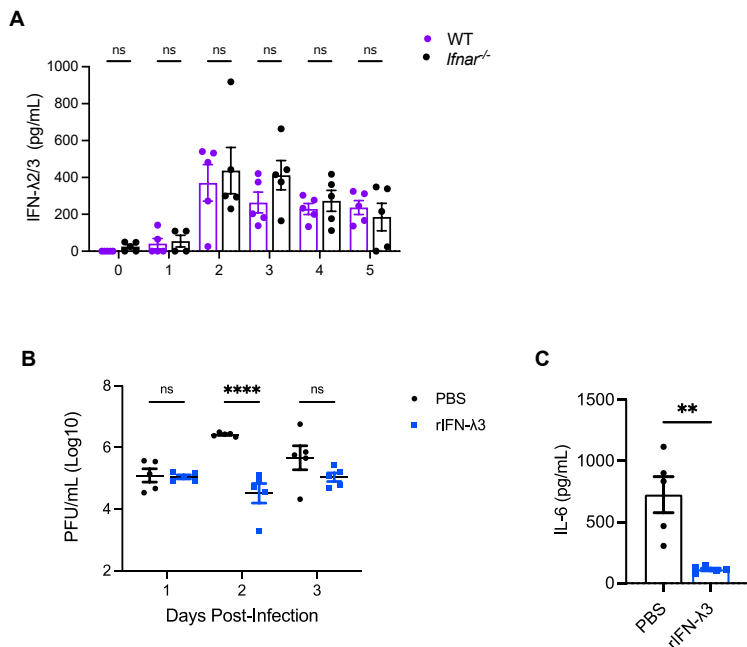


Fig. S1. IFN-λ3 treatment inhibits IL-6-mediated pathology and rescues lymphocyte-derived IFN-γ.

A. WT ($n = 5$) and *Ifnar*^{-/-} ($n = 5$) mice were infected with 10^4 PFU HSV-2 333, and IFN-λ 2/3 levels quantified in the vaginal washes. B-C *Ifnar*^{-/-} mice were infected with 10^4 PFU HSV-2 strain 333 and administered PBS ($n = 5$) or 5 μg IFN-λ3 ($n = 5$) 1 DPI. B. Viral titres in vaginal washes. C. IL-6 levels in vaginal washes 3 DPI. ns, not significant. ** $P < 0.01$, **** $P < 0.0001$. Statistical significance determined by two-way ANOVA with Sidak's multiple comparisons test (A, B) or two-tailed student *t*-test (C).

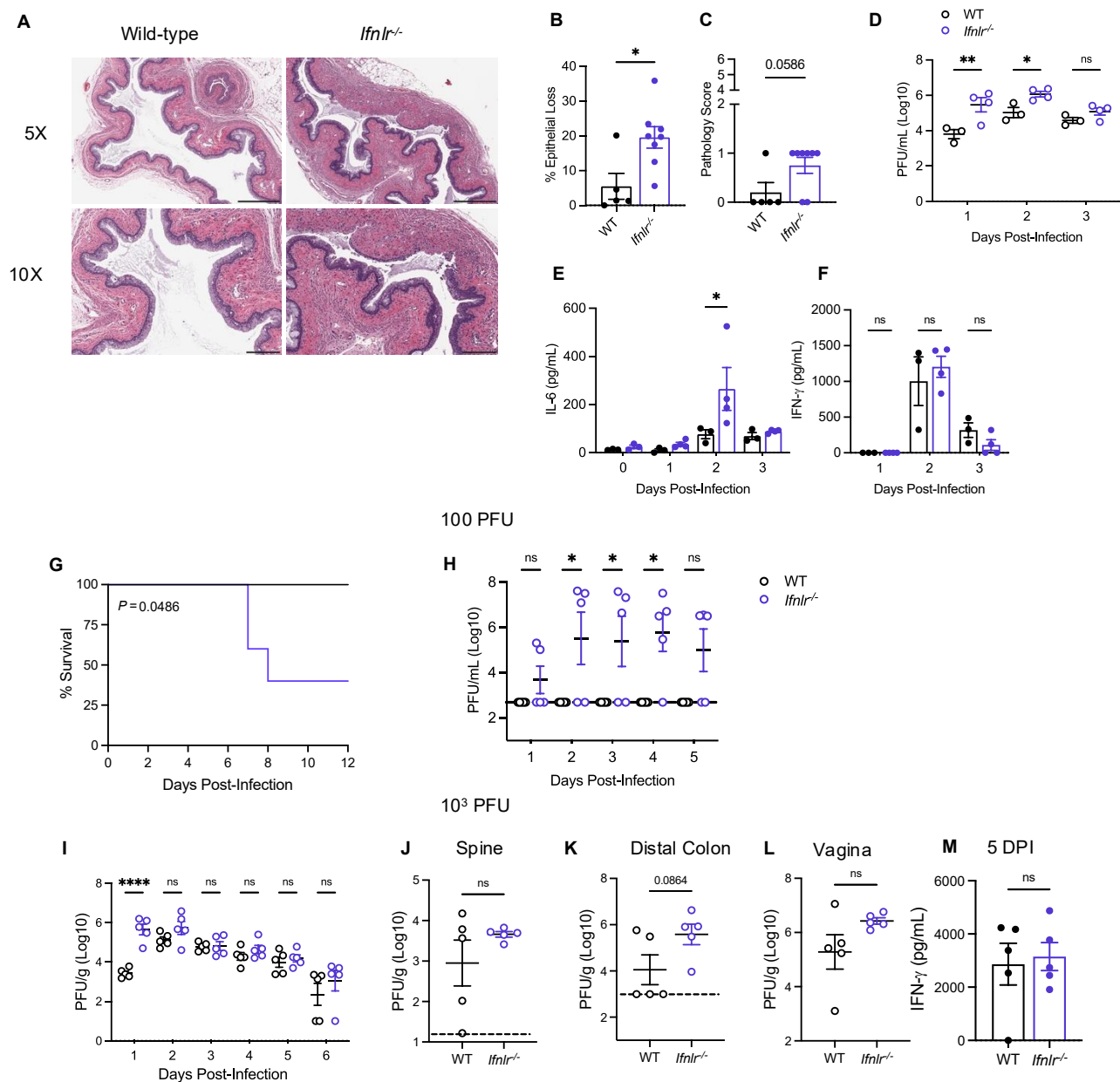


Fig. S2. Loss of *Ifnlr* impairs early antiviral immune responses. A-F. WT and *Ifnlr*^{-/-} mice were infected with 10⁴ PFU HSV-2 333. A. Representative H&E staining. B. % epithelial loss and C. pathology score of WT (*n* = 5) and *Ifnlr*^{-/-} (*n* = 8) mice. D. Viral titres in vaginal washes of WT (*n* = 3) and *Ifnlr*^{-/-} (*n* = 4) mice. E. IL-6 levels in vaginal washes. F. IFN- γ levels in vaginal washes. G-H. WT (*n* = 5) and *Ifnlr*^{-/-} (*n* = 5) mice were infected with 100 PFU HSV-2 strain 333. G. Survival Curve. H. Viral titers in the vaginal washes. I-J. WT (*n* = 5) and *Ifnlr*^{-/-} (*n* = 5) mice were infected with 10³ PFU HSV-2 strain 333. I. Viral titers in vaginal washes. J. Viral titers in spine 6 DPI. K. Viral titers in distal colon 6 DPI. L. Viral titers in whole vaginal tissue 6 DPI. M. IFN- γ levels in vaginal washes 5 DPI. ns, not significant. * *P* < 0.05, ** *P* < 0.01, **** *P* < 0.0001. Statistical significance determined by Kaplan-Meier survival analysis (G) two-way ANOVA with Sidak's multiple comparisons test (D, E, F, H, I) or two-tailed Student's *t*-test (B, C, J, K, L, M).

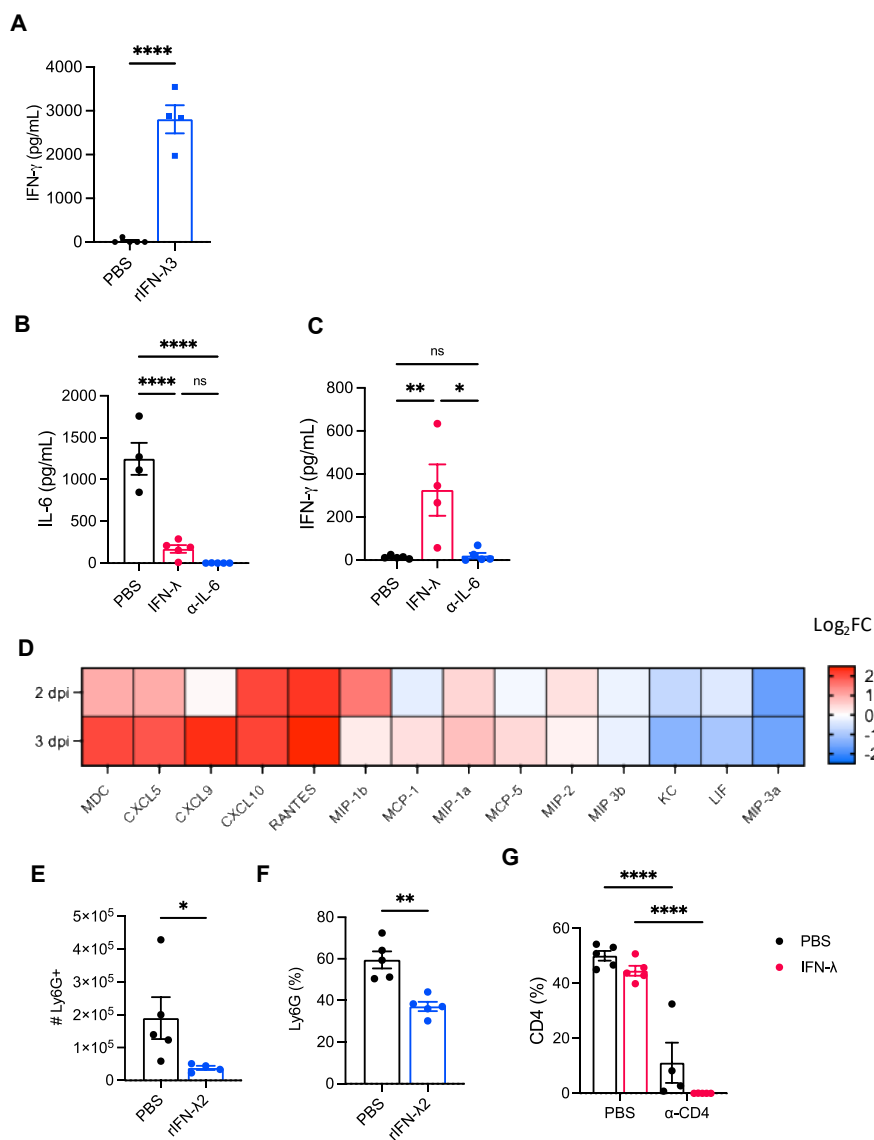


Fig. S3. IFN- λ induces late-stage IFN- γ production independent of increasing T cell recruitment. A. *Ifnar*^{-/-} mice were administered PBS ($n = 5$) or IFN- λ ($n = 4$), and IFN- γ levels were measured 5 DPI in the vaginal washes. B-C, *Ifnar*^{-/-} mice were administered PBS ($n = 4$), IFN- λ ($n = 4$), or α -IL-6 ($n = 5$) during HSV-2 infection B. IL-6 levels in vaginal washes 3 DPI. C. IFN- γ levels in vaginal washes 5 DPI. D-F, *Ifnar*^{-/-} mice were infected with 104 PFU HSV-2 strain 333 and administered PBS ($n = 5$) or IFN- λ ($n = 5$). D. Log₂FC of cytokine production 2 and 3 DPI, as fold change over PBS-treated mice. E. Total number of neutrophils (CD45⁺/Ly6G⁺). F. Proportion of neutrophils to total CD45⁺ cells. G. *Ifnar*^{-/-} mice treated with IFN- λ 1 DPI intravaginally were administered PBS ($n = 5$), α -CD4 ($n = 5$), IFN- λ ($n = 4$), or IFN- λ + α -CD4 ($n = 5$), 1 and 2 days before infection. Proportion of CD4⁺ T cells (CD45⁺/CD3⁺/TCR β ⁺/TCR γ δ ⁻/CD8⁻/CD4⁺) to total CD45⁺ cells in the dLN 5 DPI. ns, not significant. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. Statistical significance determined by two-way ANOVA with Sidak's multiple comparisons test (G), one-way ANOVA with Sidak's multiple comparisons test (B, C), with or two-tailed student t -test (E, F).

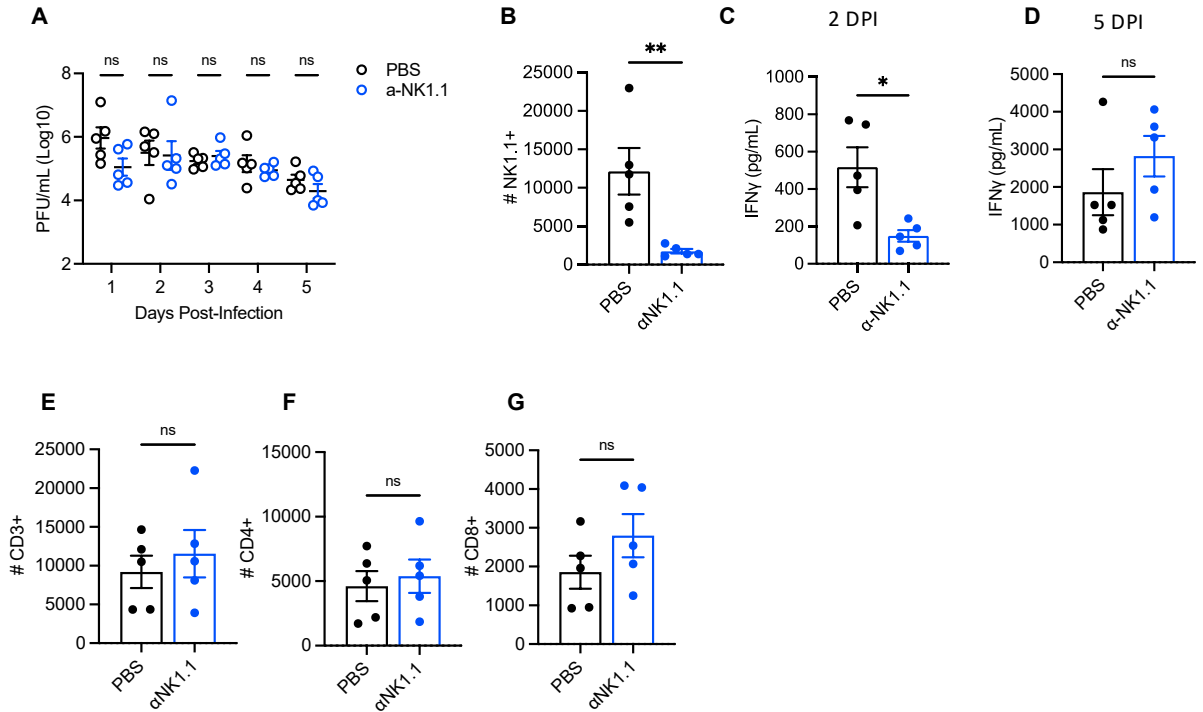


Fig. S4. NK cell depletion does not impact T cell function. A-J. WT mice were infected with 104 PFU HSV-2 strain 333 and administered PBS ($n = 5$) or α -NK1.1 ($n = 5$). Tissues were collected 5 DPI. A. Viral titres. B. Total number of NK cells in the vaginal tissue 5 DPI. C. IFN- γ in the vaginal washes 2 DPI. D. IFN- γ in the vaginal washes 5 DPI. E-H. Total number of CD45 + cells in the vaginal tissue E. Total number of T cells (CD45+/Ly6G-/CD3+) F. Total number of CD4+ T cells (CD45+/Ly6G-/CD3+/TCR β + /CD4+/CD8-) G. Total number of CD8+ T cells (CD45+/Ly6G-/CD3+/TCR β + /CD4-/CD8+). ns, not significant. ** $P < 0.01$. Statistical significance determined by two-way ANOVA with Sidak's multiple comparisons test (A), two-tailed student t -test (B-G).

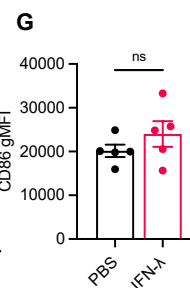
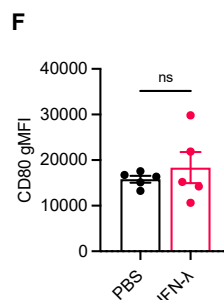
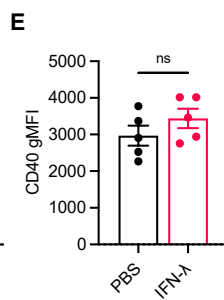
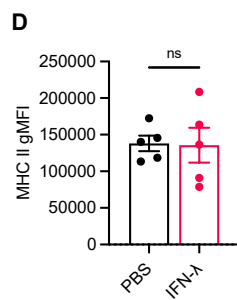
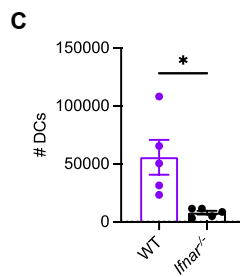
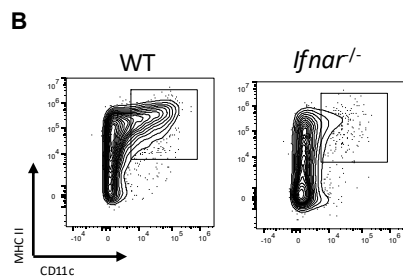
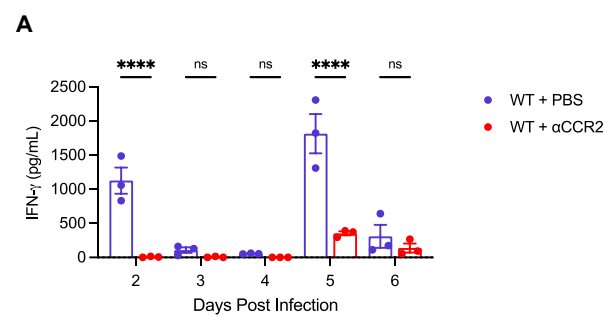


Fig S5. CCR2+ cells are necessary for CD4+ T cell IFN- γ production. A. WT mice were administered PBS ($n = 3$) or α -CCR2 ($n = 3$), and IFN γ levels were measured 2-6 DPI. B WT ($n = 5$) and *Ifnar*^{-/-} mice ($n = 5$) were infected with 104 PFU HSV-2 and vaginal tissue was collected 5 DPI. Representative flow plots for CD11b⁺ DCs. C. Total number of CD11b⁺ DCs (CD45⁺/Ly6G⁻/B220⁻/CD3⁻/CD11b⁺/CD11c⁺/MHC II⁺) in the vaginal tissue. D. *Ifnar*^{-/-} mice ($n = 5$) were infected with 104 PFU HSV-2 and treated with PBS ($n = 5$) or IFN- λ ($n = 5$) 1 DPI intravaginally. Vaginal tissue was collected 5 DPI. E. MHC II gMFI of CD11b⁺ DCs treated mice. F. CD40 II gMFI of CD11b⁺ DCs. G. CD80 gMFI of CD11b⁺ DCs. H. CD80 gMFI of CD11b⁺ DCs. ns, not significant. * $P < 0.05$, **** $P < 0.001$. Statistical significance determined by two-way ANOVA with Sidak's multiple comparisons test (A), two-tailed student t -test (C-G).

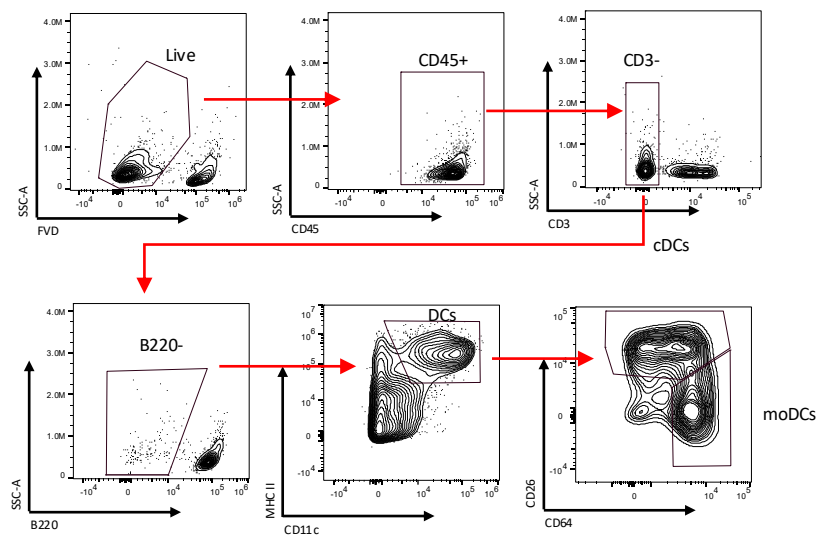
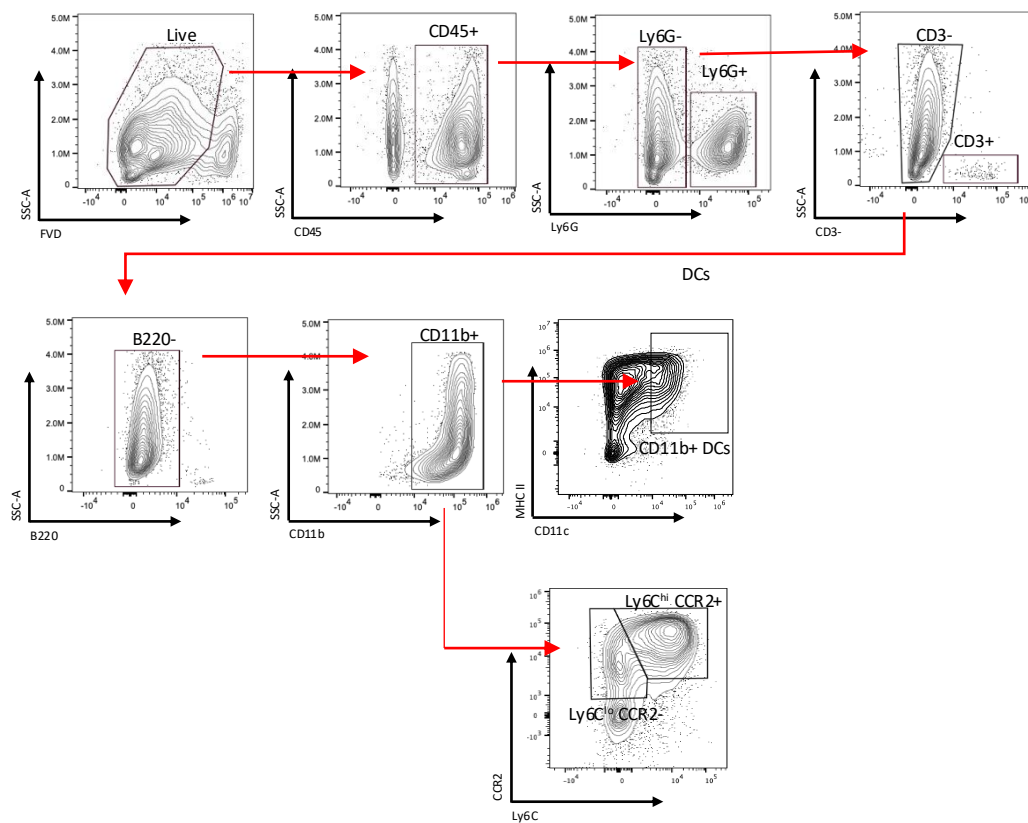
A**B**

Fig S6. Gating Strategy for DCs in the dLN and vaginal tract. A. Gating strategy for DCs in the LN. B. Gating strategy for DCs in the vaginal tract