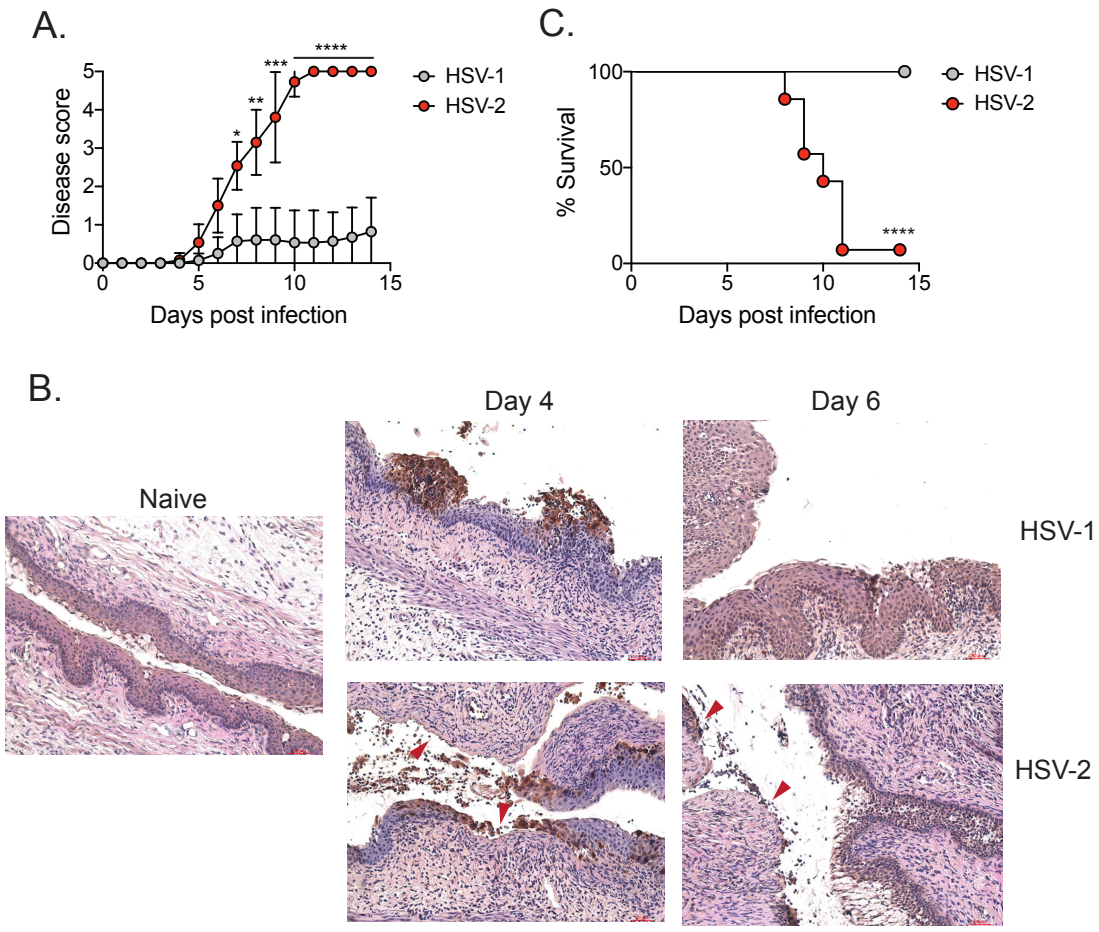
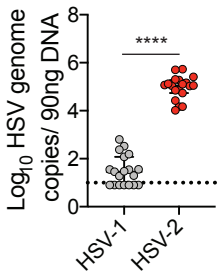
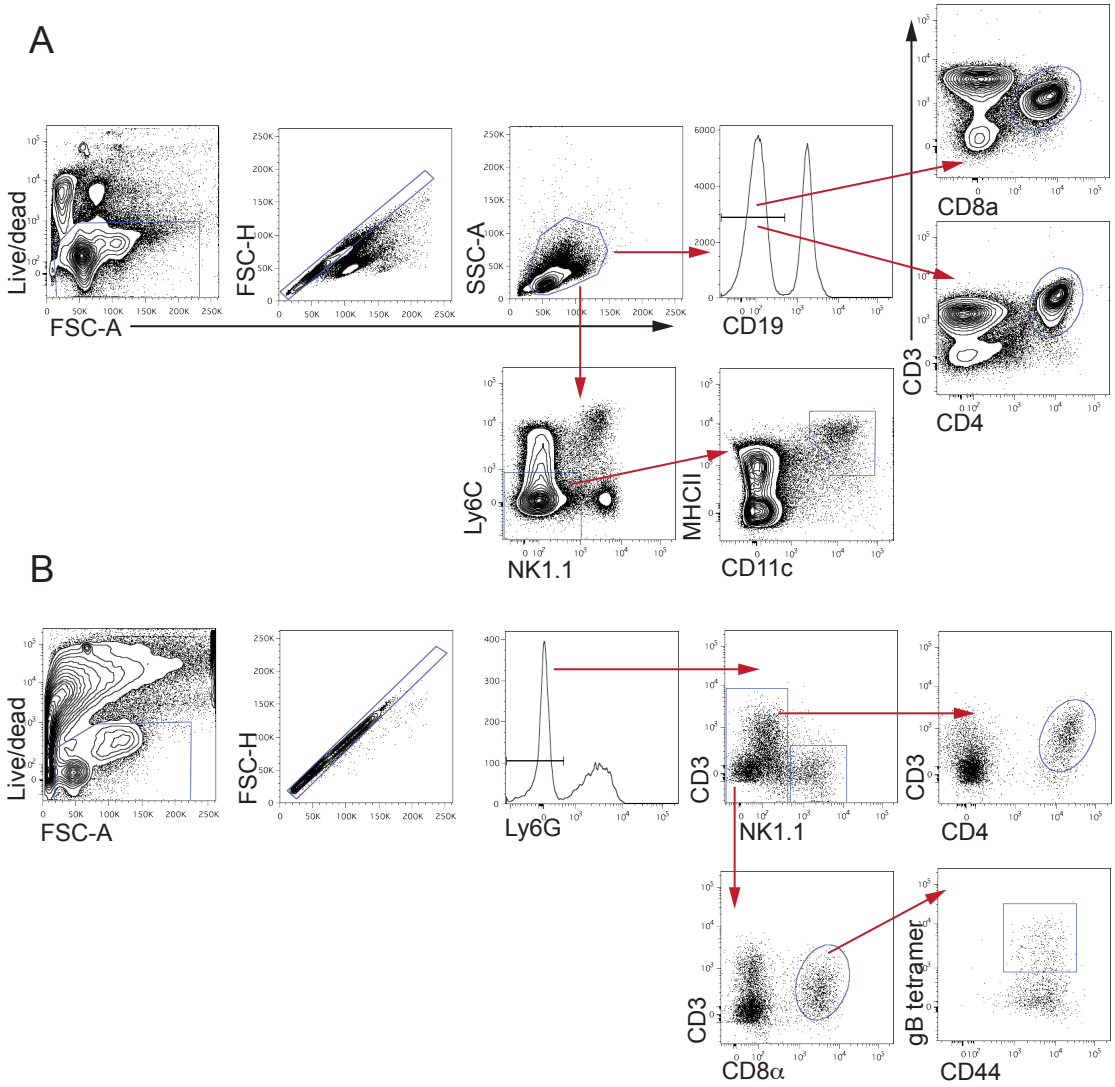


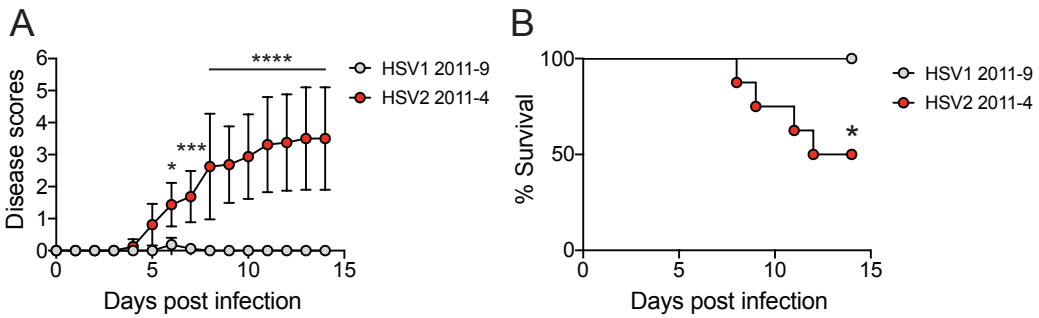
Supplemental Figure 1. Vaginal infection with HSV-1 and HSV-2 lead to different disease outcomes. C57BL/6 female mice were treated with Depo-Provera and inoculated with 10^4 PFU HSV-1 McKrae or HSV-2 186 syn+. Disease score (A) and survival (C) were monitored for 2 weeks (n=14 HSV-1, n=14 HSV-2). B) Representative images of vaginal tissue sections were probed with antibodies against HSV antigens in naive mice or after infection on the indicated days. Red arrows show areas of epithelial denuding. Experiment was performed once. Error bars in (A) show S.D. Statistical significance was measured by two-way ANOVA with Bonferroni multiple comparisons test (A) or log-rank test (B). *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.



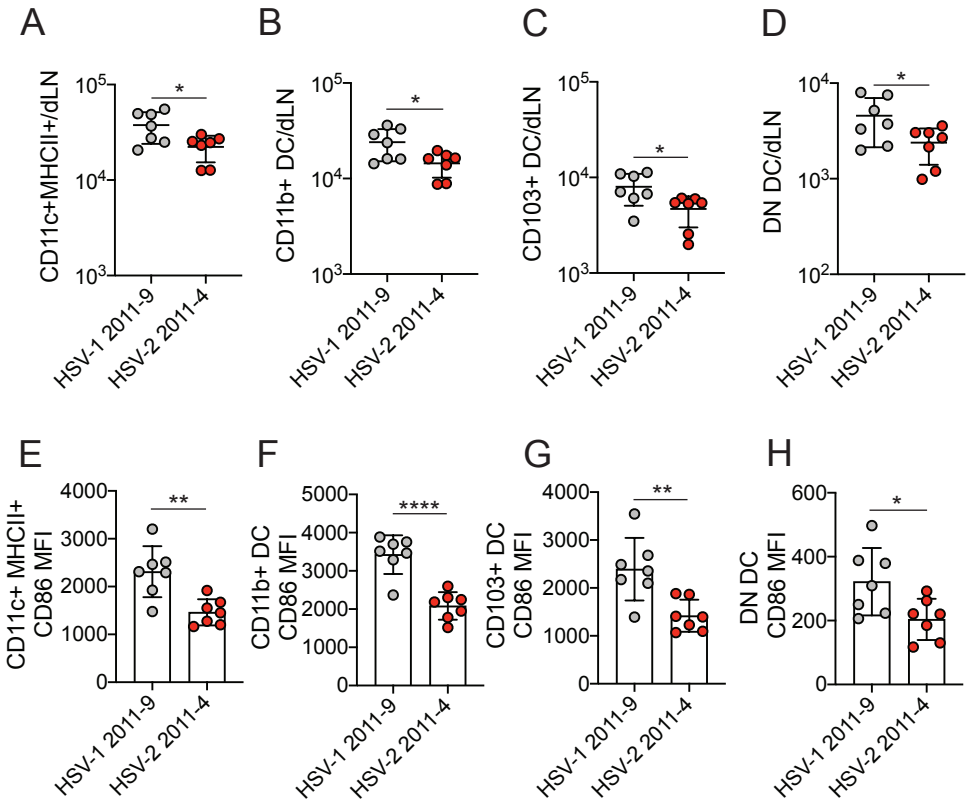
Supplemental Figure 2. Less viral DNA is present in the ganglia after HSV-1 infection compared to HSV-2. C57BL/6 female mice were treated with Depo-Provera and inoculated with 10^4 PFU HSV-1 McKrae (n=19) or HSV-2 186 syn+ (n=19). At 6 d.p.i., sacral lumbar ganglia were harvested and DNA was extracted. Viral DNA was measured by quantitative PCR (qPCR). Dotted line in graph shows limit of detection for our assay. Horizontal bars show mean, error bars show 95% CI. Data are pooled from 3 independent experiments. Statistical signifcance was measured by Mann-Whitney test on log-transformed data. ****p<0.001.



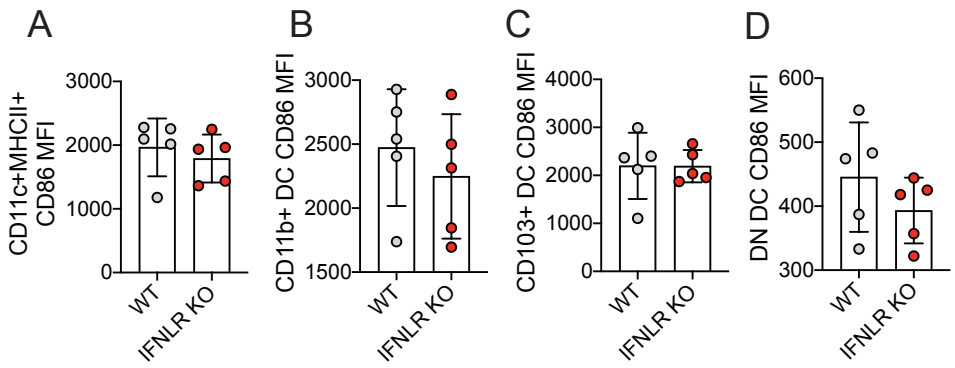
Supplemental Figure 3. Gating strategy for flow cytometric analysis. A) Gating strategy for identifying T cells and DCs in the dLN. B) Gating strategy for identifying T cells and tetramer+ populations in the vagina.



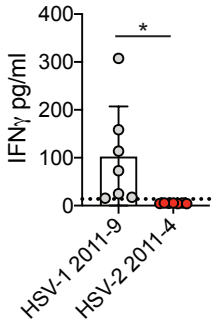
Supplemental Figure 4. A low passage clinical HSV-1 isolate induces less disease compared to a low passage clinical HSV-2 isolate. C57BL/6 females were treated with Depo-Provera and inoculated with 10^4 PFU HSV-1 2011-9 (n=8) or HSV-2 2011-4 (n=8). Disease scores (A) and survival (B) were monitored over the course of two weeks. Error bars show S.D.. Data are pooled from 2 independent experiments. Statistical significance was measured by two-way ANOVA with Bonferroni's multiple comparisons test (A) or by log-rank test (B). *p<0.05, ***p<0.005, ****p<0.001.



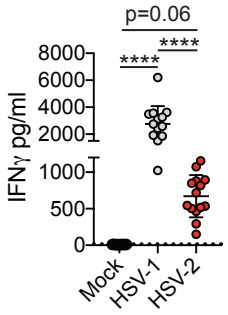
Supplemental Figure 5. A low passage clinical HSV-1 isolate induces more rapid DC maturation compared to a low passage clincal HSV-2 isolate. C57BL/6 females were treated with Depo-Provera and inoculated with 10^4 PFU HSV-1 2011-9 (n=7) or HSV-2 2011-4 (n=7). At 2 d.p.i., dLNs were harvested and DCs were analyzed. Total DCs (A), CD11b+ DC (B), CD103+ DC (C) and DN DC (D) were counted. Level of CD86 expression on total DCs (E), CD11b+ DC (F), CD103+ DC (G) and DN DC (H) was measured by flow cytometry. Horizontal bars show mean, error bars show S.D.. Data are pooled from 2 independent experiments. Statistical significance was measured by two-tailed Student's t-test. *p<0.05, **p<0.01, ****p<0.001.



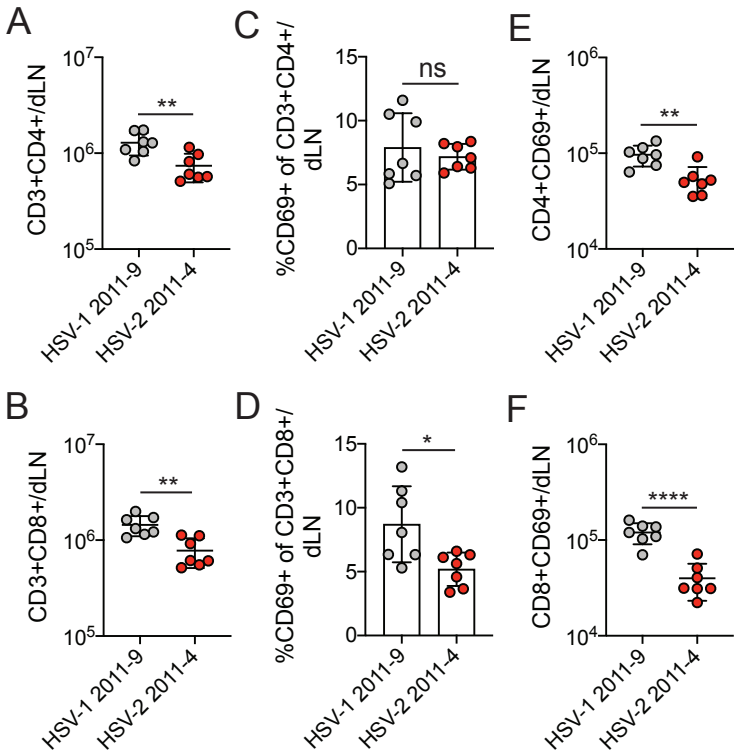
Supplemental Figure 6. IFNλ has minimal impact on DC maturation after HSV-1 vaginal infection. IFNλ receptor-deficient females (n=5) or wildtype controls (n=5) were Depo-treated and infected vaginally with 10^4 PFU HSV-1 McKrae. At 2 d.p.i., dLN were harvested and CD86 expression was assessed on total CD11c+ MHCII+ cells (A), CD11b+ DC (B), CD103+ DC (C) and DN DC (D). Vertical bars show S.D.



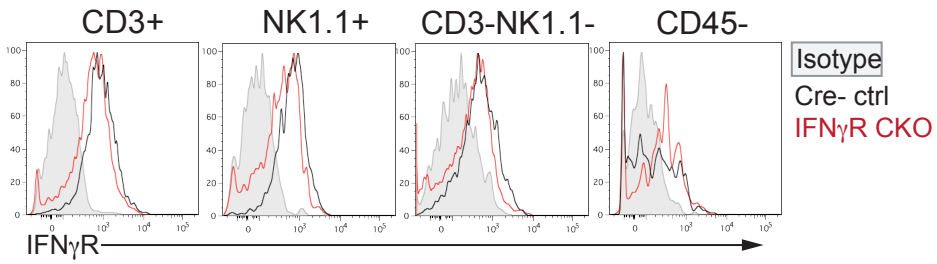
Supplemental Figure 7. Difference in IFNγ production at 1 day post-infection is maintained after inoculation with clinical isolates. Mice were infected with 10^5 PFU HSV-1 2011-9 (n=7) or 10^5 PFU HSV-2 2011-4 (n=7). IFNγ was measured in vaginal washes collected at 1 d.p.i.. Data are pooled from 2 independent experiments. Statistical significance was measured by two-tailed Student's t-test, *p<0.05.



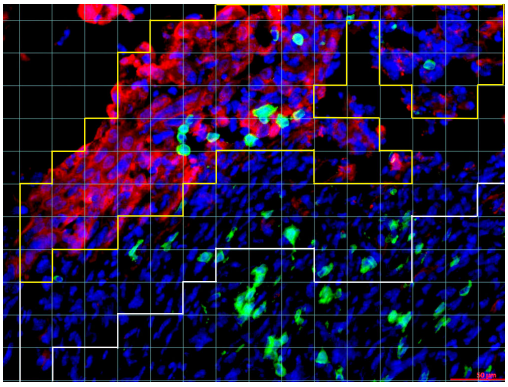
Supplemental Figure 8. Robust IFNγ production in vagina can be detected in the vagina 2 days after HSV-2 infection. Mice were infected as described in Figure 1. IFNγ was measured by ELISA in vaginal washes collected on 2 d.p.i.. Data are pooled from 3 independent experiments. Statistical significance was measured by one-way ANOVA with Tukey's multiple comparisons test. ****p<0.001.



Supplemental Figure 9. Infection with a low passage clinical isolate of HSV-1 leads to more rapid T cell activation compared to infection with a low passage clinical isolate of HSV-2. Mice were infected as described in Supplemental Figure 3 and dLNs were harvested at 2 d.p.i.. Total number of CD4+ (A) and CD8+ (B) T cells were counted. The frequency of CD4+ (C) and CD8+ (D) T cells expressing CD69 was measured by flow cytometry. E. Total number of CD4+ T cells expressing CD69. F. Total number of CD8+ T cells expressing CD69. Horizontal bars show mean, error bars show S.D.. Data are pooled from 2 independent experiments (HSV-1 2011-9: n=7, HSV-2 2011-4: n=7). Statistical significance was measured by two-tailed Student's t-test. *p<0.05, **p<0.01, ****p<0.001, ns = not significant.



Supplemental Figure 10. IFNγR expression in the vagina is similar between mice lacking IFNγR in the peripheral nervous system and littermate controls. IFNγR cell surface expression was measured by flow cytometry six days after HSV-1 infection. Open histograms show IFNγR expression levels on the indicated cell populations for the CKO or ctrl genotypes. Shaded histogram show isotype control staining.



HSV NK cell DAPI

Supplemental Figure 11. Example of NK cell quantification method from immunofluorescent images. A grid of 400 px^2 squares were overlaid on top of the images in Image J. Region within the yellow lines were considered “infected”, and the region between the white and yellow lines was considered “proximal”. The number of squares in each region and the number of NK cells within each region was manually counted using the cell counter function in Image J. Total number of NK cells was divided by total number of squares within each area to obtain the number of NK cells per 400 px^2.

Antibody target/Reagent	Fluorophores used
CD3	Pacific Blue, APC
CD4	PE, PE/Dazzle 594
CD8a	Brilliant Violet 605, PECy7, APC
CD8b.2	FITC
CD69	PeCy7
CD44	Alexa Fluor 700
CD45.1	Pacific Blue
HSV tetramer	APC
CD119 (IFNgR)	PE
Armenian hamster IgG isotype	PE
IFNg	PerCPCy5.5
granzyme B	APC
Tbet	PE/Dazzle 594
CD11c	PECy7
CD11b	APC
I-A/I-E	Alexa Fluor 700
CD103	PE
CD86	Pacific Blue
NK1.1	PerCPCy5.5, FITC
NKp46	PE/Dazzle 594
CD19	FITC, PE
Ly6G	PerCPCy5.5, PE
Ly6C	Brilliant Violet 605
Live/Dead Aqua	AmCyan

Supplemental Table 1. List of antibodies and fluorophores used.