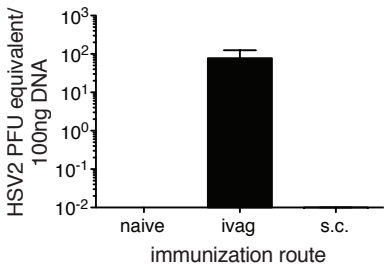
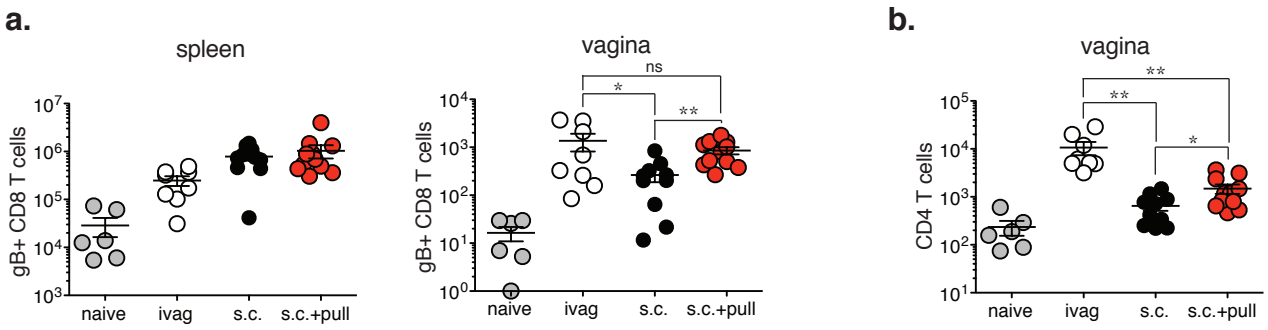
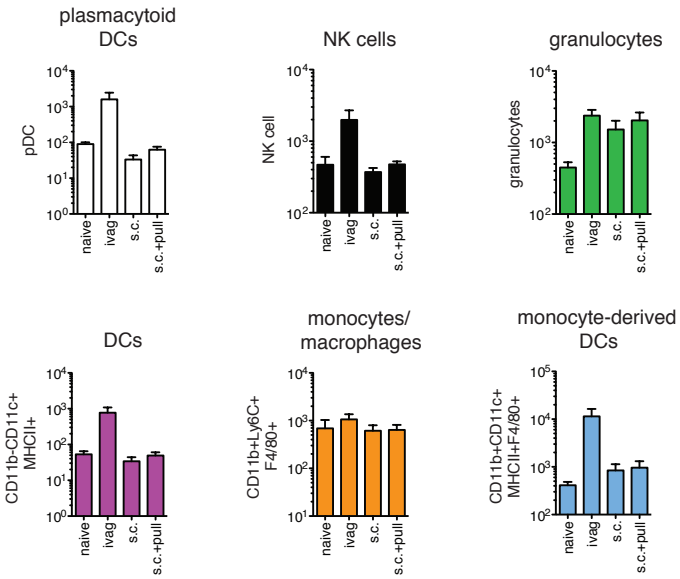


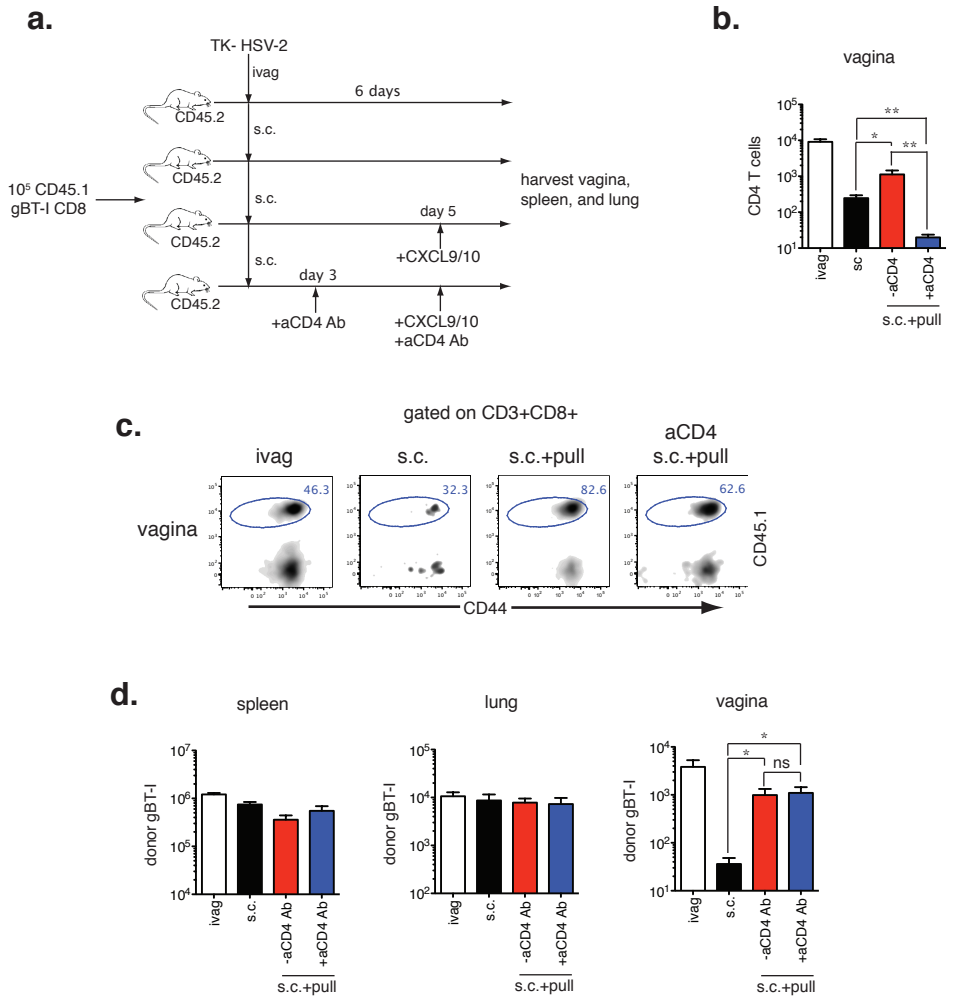
Supplementary Figure 1. **HSV-2 antigen is not present in vagina after subcutaneous immunization.** Mice were immunized either ivag or s.c. with TK- HSV-2. On day 5 p.i., vaginal tissue from immunized and naive mice were harvested and HSV-2 antigen was measured by qPCR. Data represent two independent experiments. Error bar is SEM.



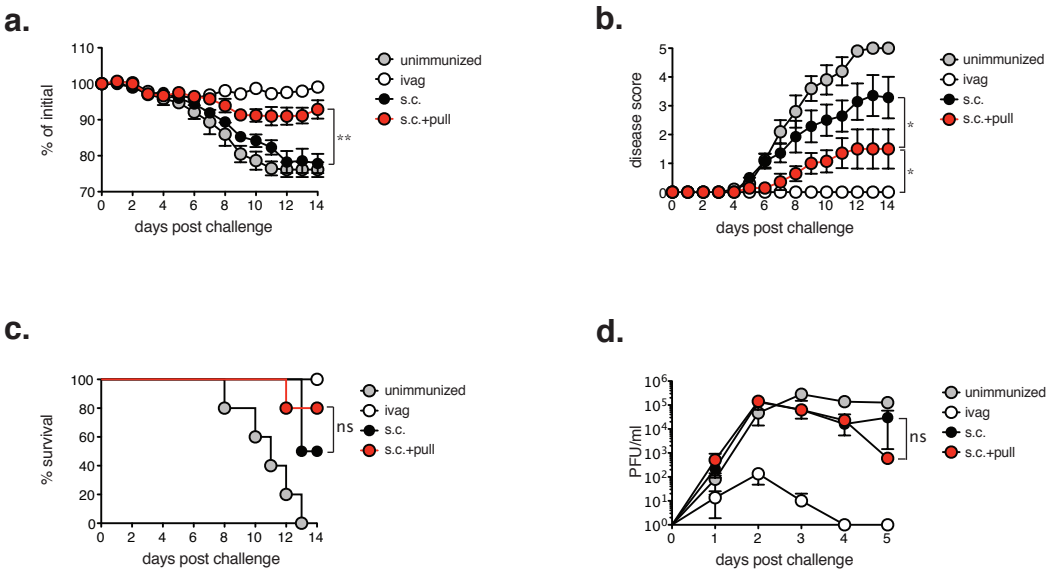
Supplementary Figure 2. Endogenous HSV-specific CD8 T cells are recruited to the vagina by prime and pull. Depo-treated naive mice were immunized ivag or s.c. with TK- HSV-2. Five days p.i., s.c. immunized mice were treated ivag with CXCL9 and CXCL10 (s.c.+pull) or PBS (s.c.). One day later, endogenous HSV-specific T cells were ennumerated in the indicated tissues. a, gB-specific CD8 T cells in the vagina and spleen were identified by MHCI tetramer. b, CD44+ CD4 T cell numbers in the vagina. Statistical significance was measured by unpaired Student's t-test. *$p<0.05$. **$p<0.01$ and ns = not significant. n=6-11 per group. Data are pooled from three independent experiments. Error bars show SEM.



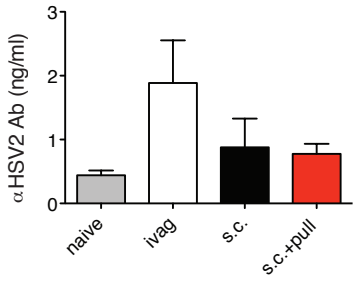
Supplementary Figure 3. Inflammatory innate cells are not recruited by prime and pull. Five days post ivag or s.c. immunization with TK- HSV-2, mice were treated with the chemokine pull. Recruitment of different cell populations were analyzed one day post-pull in the vagina. Naive mice were unimmunized. Difference between s.c. and s.c.+pull is not significant for any cell population by unpaired Student's t-test. n=3 (naive), n=9 (ivag, s.c., s.c.+pull). Data are pooled from three independent experiments. Error bars show SEM.



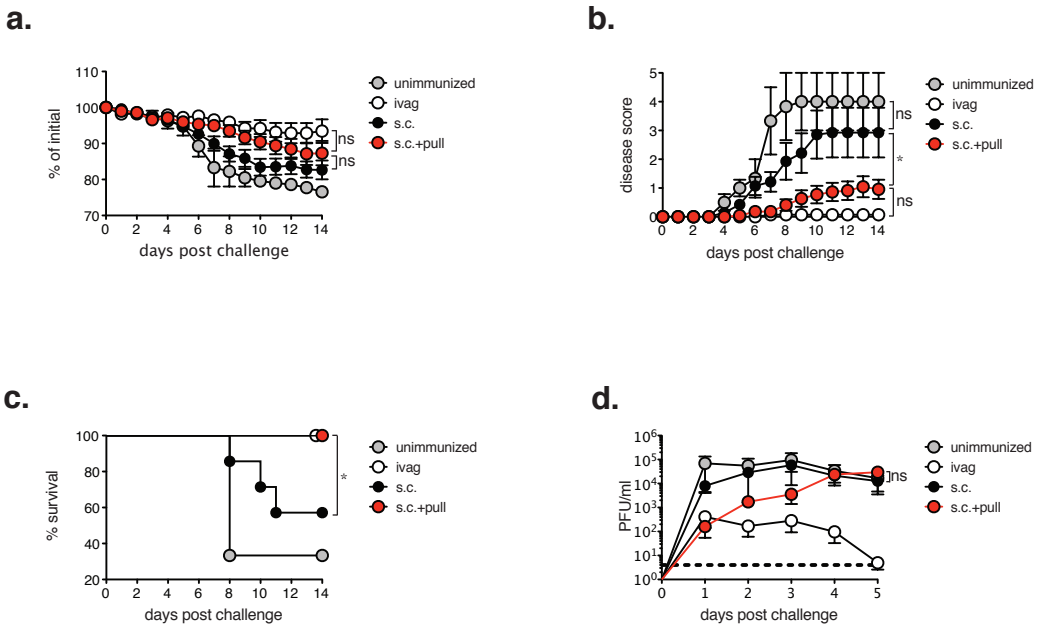
Supplementary Figure 4. CD8 T cell recruitment during prime and pull does not require CD4 T cell help. a, Experimental schematic. Mice were immunized ivag or s.c. with TK- HSV-2. At day 3 and day 5 p.i., s.c. immunized mice were injected intraperitoneally with aCD4 antibody (Ab). At day 5 p.i., chemokine pull was applied vag. T cell numbers were determined one day post-pull. b, CD4 T cell numbers iwere determined in the vaginas of depleting antibody-treated and untreated mice. c, Frequency of donor gBT-I CD8 T cells was measured in the vagina one day post-pull. Plots are gated on total CD8 T cells. Numbers in plots indicate percent of total CD8 T cells that are gBT-I. d, Number of donor gBT-I CD8 T cells was measured in the indicated tissues. n=6 per group. Statistical significance was measured by unpaired Student's t-test. *p<0.05, **p<0.01, ns = not significant. Data are pooled from 2 independent experiments. Error bars show SEM.



Supplementary Figure 5. Prime and pull provides protection against lethal WT HSV-2 challenge in the absence of TCR Tg CD8 T cells. Depo-treated mice were immunized either ivag or s.c. with TK- HSV-2. At 5 days p.i., s.c. immunized mice were treated with either the chemokine pull or PBS. Four weeks post-pull, mice were challenge ivag with a lethal dose of WT HSV-2. Weight loss (a), disease scores (b), survival (c) and viral titers (d) were monitored for two weeks post challenge. *p<0.05, **p<0.01, and ns = not significant. Statistical significance was measured by two-way ANOVA (a, b, and d) or log-rank (Mantel-Cox) test (c). n=5 (unimmunized, ivag, s.c.+pull), n=6 (s.c.). Data are pooled from two independent experiments. Error bars show SEM.



Supplementary Figure 6. HSV-specific antibodies in the vagina are not affected by chemokine pull. Vaginal washes from unimmunized, ivag immunized, s.c. immunized and prime and pull mice were collected at 3-4 weeks post-pull. HSV-specific antibodies were measured by ELISA. Data are pooled from two independent experiments. Error bars show SEM.



Supplementary Figure 7. Prime and pull mice are protected 12 weeks post-pull from WT HSV-2 challenge. 10^5 gBT-I CD8 T cells were adoptively transferred to Depo-treated recipients and mice were immunized ivag or s.c. with TK- HSV-2 or left unimmunized. Five days p.i., s.c. immunized mice were treated intravaginally with PBS (s.c.) or 3ug each CXCL9 and CXCL10 (s.c.+pull). All groups were Depo-treated again 1-2 weeks post-pull. At 10-12 weeks post-pull, mice were challenged ivag with WT HSV-2 and monitored for weight loss (a), disease score (b) and survival (c). Viral titers were measured from vaginal washes harvested during the first 5 days of challenge (d). Statistical significance was measured by two-way ANOVA (a, b, and d) or log-rank (Mantel-Cox) test (d). *$p<0.05$, ns = not significant. n=3 (unimmunized), n=7 (ivag, s.c.), n=11 (s.c.+pull). Data are pooled from three independent experiments. Error bars show SEM.

Supplementary Text

List of *p*-values for main figures

Figure 1. c, *p = 0.0225 (ivag vs. s.c. + pull) and **p = 0.0019 (s.c. vs. s.c. + pull). **D,** **p = 0.0037 (ivag vs. s.c. + pull), **p = 0.0082 (s.c. vs s.c. + pull).

Figure 2. (b) In the vagina, *p = 0.0171 (naïve vs. d5), *p = 0.0399 (d5 vs. d15) and *p = 0.0376 (d5 vs. d28). In the spleen, ***p<0.0001 (naïve vs d5), **p=0.0016 (d5 vs d15) and **p=0.0025 (d5 vs d28). For frequency, **p = 0.0023 (naïve vs. d5) and ***p < 0.0001 (d5 vs. d15, d5 vs. d28). **c,** *p = 0.0207 (naïve vs. d5), *p = 0.0438 (d5 vs. d28). **d,** ***p < 0.0001 (naïve vs. d5), **p = 0.0033 (d5 vs. d15) and ***p = 0.0009 (d5 vs. d28).

Figure 3. a, *p = 0.0231 (ivag vs. s.c. + pull) and ***p = 0.0006 (s.c. vs. s.c. + pull). **c**, **p = 0.0053 (ivag vs. s.c. + pull). **d**, **p = 0.0081 (ivag vs. s.c.), *p = 0.0382 (s.c. vs. s.c. + pull), **e**,*p = 0.0490 (ivag vs. s.c. + pull).

Figure 4. a, ***p = 0.0006 (s.c. vs. s.c. + pull), *p = 0.0425 (ivag vs. s.c. + pull). **b,** ***p < 0.0001 (s.c. vs. s.c. + pull), **p = 0.0063 (ivag vs. s.c. + pull). **c,** **p = 0.002 (s.c. vs s.c.+pull). **d**, ***p = 0.0002 (unimmunized vs. s.c. + pull), ns = not significant (s.c. vs. s.c.+pull). **e**, ***p = 0.0007 (unimmunized vs s.c.), **p = 0.0017 (unimmunized vs. s.c. + pull), and *p = 0.0328 (s.c. vs. s.c. + pull).