Supplemental figure legends

Figure S1. Transient expression of a reporter gene and induction of neutralizing antibodies against the capsid.

(A) Depoprovera-treated BALB/c mice (n=5 / group) were inoculated Ivag with $1x10^7$ IU HPV16 or HPV45 PsV expressing luciferase (16Luc and 45Luc) or sham-treated. *In vivo* luciferase expression was measured daily after Ivag instillation and is expressed as mean luminescence in photons / second. (B) One month after ivag instillation of HPV16-luciferase PsV (L1 content of 1μg) blood sample were assessed for the presence of neutralizing antibodies against HPV16. Data are representative of 3 experiments.

Figure S2. Homologous prime/boost with 16MM2 Pseudovirus does not enhance $CD8^+$ T cell response in the genital tract.

Depoprovera-treated mice (n=5 / group) were immunized with $5x10^7$ IU HPV16MM2 and one month later were immunized with $5x10^7$ IU HPV16MM2 or HPV45MM2. Two weeks after the second immunization, cervicovaginal cell suspensions were analyzed by flow cytometry for the presence of K^dM2_{82}-$tetramer^+CD8^+$ T shown as mean percentage + SD (*$P < 0.05$; Kruskal-Wallis/Dunn's test).

Figure S3. Cytokine production by $CD8^+$ T cells after Ivag prime/boost immunization with HPV16MM2/HPV45MM2 as described in Fig. 5A.

Two weeks after the last immunization, the production of IFN-γ, TNF-α and IL-2 by $CD8^+$ T cells was measured by intracellular cytokine staining after a 5hr *in vitro* peptide

stimulation of cervico-vaginal, spleen and ILN cell suspensions. White and back bars represent mean + SD (n=5 / group) of the percentage of cytokine production by $CD8^+$ T cells stimulated with $M2_{(82-90)}$ peptide after subtraction of cytokine production of unstimulated $CD8^+$ T cells from HPV-MM2 or HPV-CTRL immunized groups, respectively. Data are representative of 3 experiments.

Figure S4. Effect of FTY720 treatment on the phenotype of primary and secondary $tetramer^+CD8^+$ T cells in the lymph node and cervico-vaginal mucosa.

Depoprovera-treated BALB/c mice were immunized Ivag as described in Fig. 10. Representative flow cytometry plots of CD62L and CD127 expression of M2-$tetramer^+CD8^+$ T lymphocytes in cervico-vaginal and ILN cell suspensions collected 2 weeks after priming or boost. Data are representative of 3 experiments.

Figure S5. Effect of CD4-depleting antibody i.p. injection on $CD4^+$ T cells, conventional DC and plasmacytoid DC in the cervico-vaginal mucosa.

Depoprovera-treated mice (n=5 / group) were injected i.p. at day-3 and -1 with 100µg of a rat anti-CD4 antibody (clone: GK1.5). At day 0 the presence in the cervico-vaginal mucosa of $CD4^+$ T cells ($CD8^-CD4^+$), conventional DC ($CD3^-CD19^-NKp46^-IA/IE^+CD11c^+$) and plasmacytoid DC ($CD3^-CD19^-NKp46^-IA/IE^+PDCA1^+$) was assessed by flow cytometry. (A) Representative dot plots of CD4 (clone RM4-4) versus CD8 expression and IA/IE versus PDCA1 expression on $CD3^-CD19^-NKp46^-$ cells. (B) Absolute number of cells per cervico-vaginal mucosa, each symbol correspond to individual and horizontal bar to mean ± SD (*$P<0.05$, Mann-Whitney U-test).

Figure S6. CD4 depletion does not alter Ivag gene delivery and abrogates IgG response against L1.

Depoprovera-treated BALB/c mice (n=5 / group) were inoculated Ivag with $5x10^7$ IU HPV16 PsV expressing luciferase (equivalent to 5µg L1 protein) or sham-treated. *In vivo* luciferase expression was measured on day 2 after Ivag instillation and is expressed as mean luminescence in photon / second + SD (A). One month after Ivag instillation, anti-L1 IgG content in serum was determined by Elisa and is expressed as geometric mean end point titer ± 95% CI (B). (C) Analysis of CD62L and CD127 expression by M2-tetramer$^+$CD8$^+$ T cells in the ILN after a single HPV PsV Ivag immunization. Representative flow cytometry plot are shown. Percentage of M2-tetramer$^+$CD8$^+$ T cells is indicated in each quadrant. Data are representative of 3 experiments (*$P<0.05$, Mann-Whitney U-test).

Figure S7. HPV Ivag prime/boost immunization does not reduce viral titers in the cervicovaginal mucosa after vaginal challenge with a recombinant vaccinia virus expressing an irrelevant epitope.

Depo-Provera-treated mice were prime/boost immunized Ivag with $5x10^7$ IU HPV-MM2, with HPV-Luc or were sham-treated. One month after the last immunization mice were challenged Ivag with either $1x10^7$ pfu of a control recombinant vaccinia virus expressing the irrelevant epitope $NP_{(147-155)}$ (A) or $M2_{(82-90)}$-vaccinia virus (B). Five days after challenge, vaginal tissue was collected and recombinant vaccinia virus titers were determined by plaque assays. Results are expressed as pfu per vagina for individual

mice (Symbols) and geometric mean (horizontal bar) ± 95% CI (**P < 0.01; Kruskal-Wallis/Dunn's test).

Supplemental methods

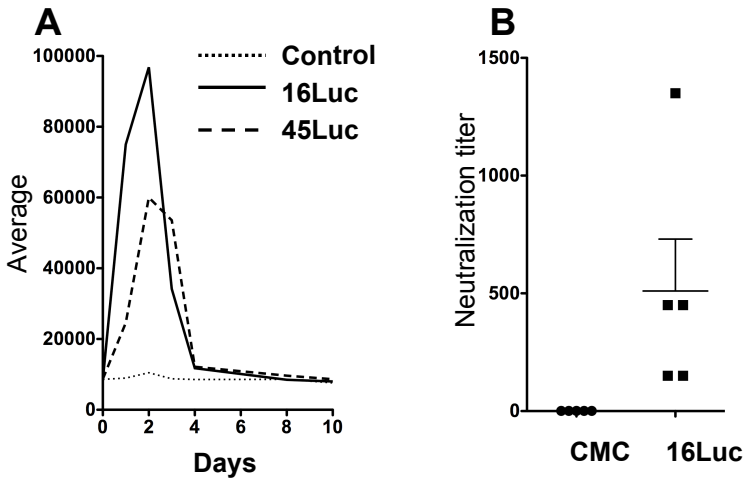
In vitro neutralization. The in vitro neutralization of HPV pseudovirions has been described previously (1). Briefly, serial dilutions of serum sample from individual mice were incubated with pseudovirus expressing secreted alkaline phosphatase (SEAP). The pseudovirus/sera mixtures were then used to infect 293TT cells and supernatants were analyzed for SEAP activity 3 days later. The neutralization titer was defined as the reciprocal of the highest dilution of serum yielding a 50% reduction in SEAP activity.

VLP ELISA. Serum samples from individual mice were assayed for antibody against HPV 16L1 VLP by ELISA using HRP-conjugated affinity purified goat antibodies to mouse IgG or IgA (Southern Biotechnology Associates) as detection reagents. Titers were defined as the reciprocal of the highest sample dilution giving a signal at least equal to 3-fold that of background. Results were expressed as geometric mean antibody titer (GMT) and 95% CI.

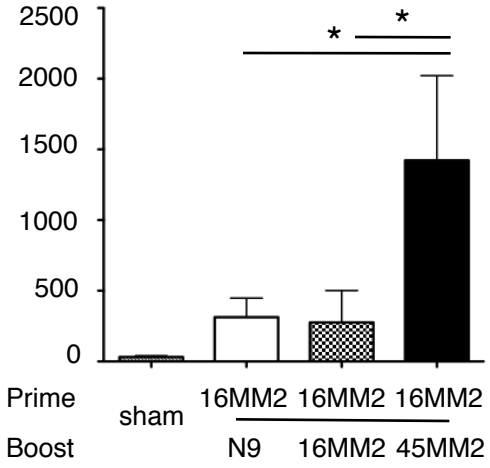
Supplemental References

1. Pastrana, D.V., Buck, C.B., Pang, Y.Y., Thompson, C.D., Castle, P.E., FitzGerald, P.C., Kruger Kjaer, S., Lowy, D.R., and Schiller, J.T. 2004. Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. *Virology* 321:205-216.

Supplemental Figure 1



Supplemental Figure 2



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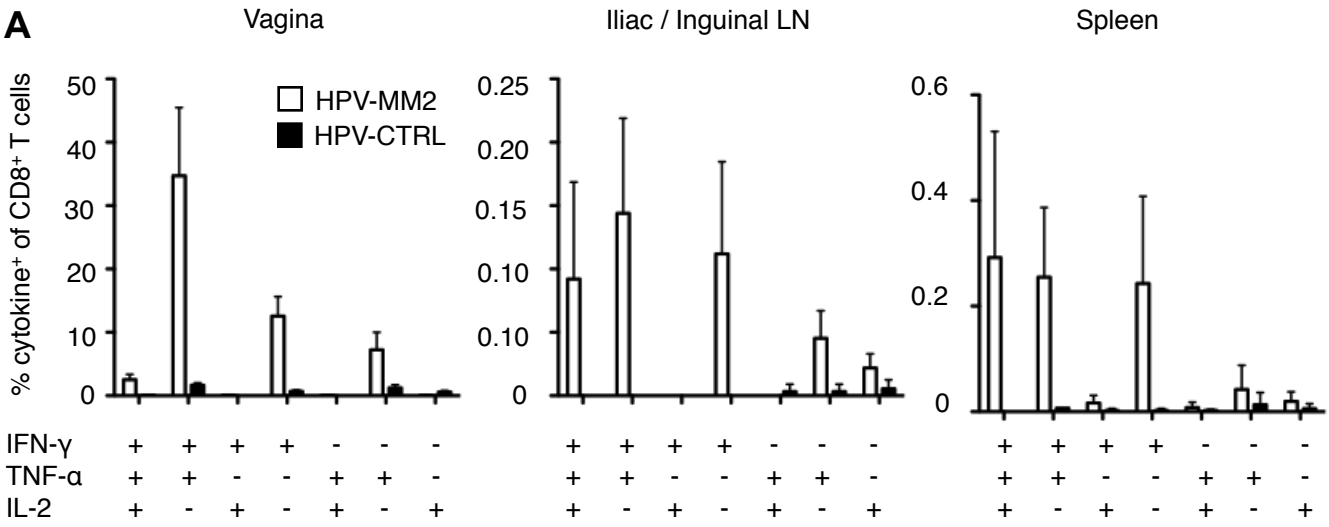

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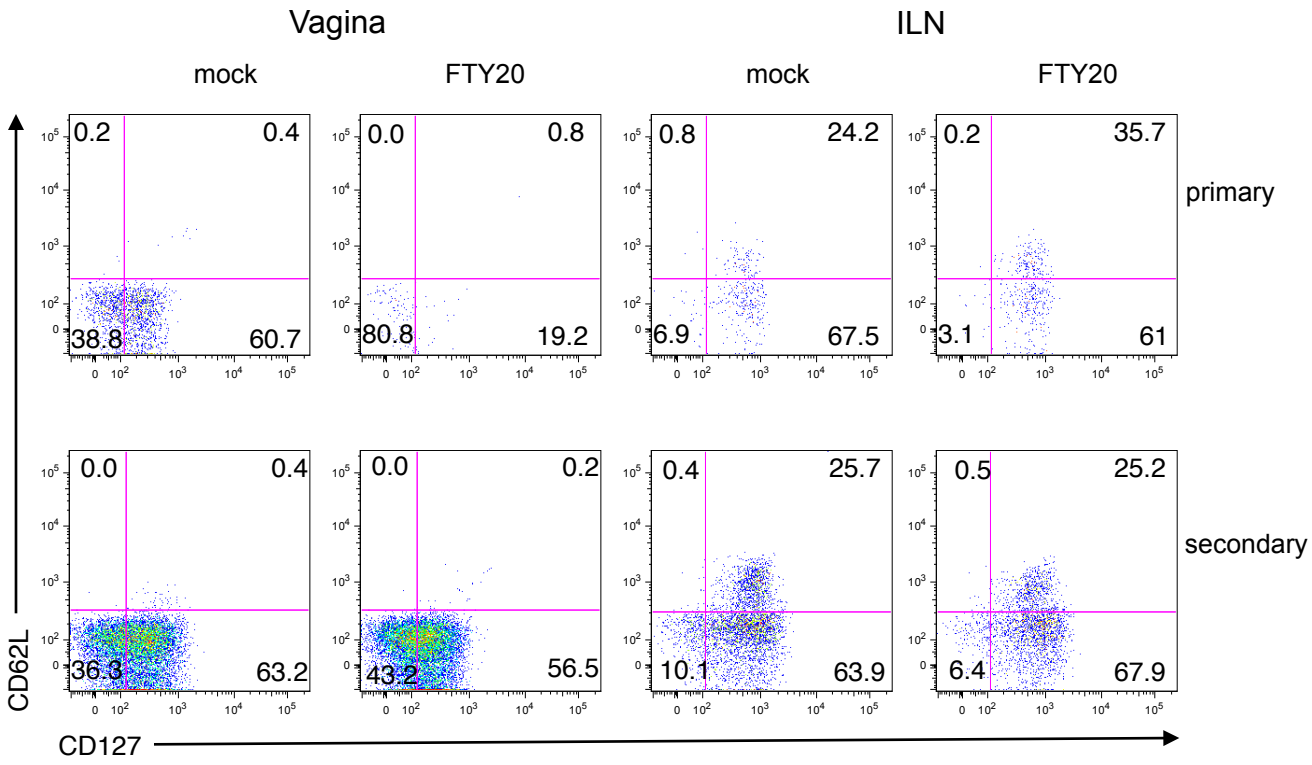

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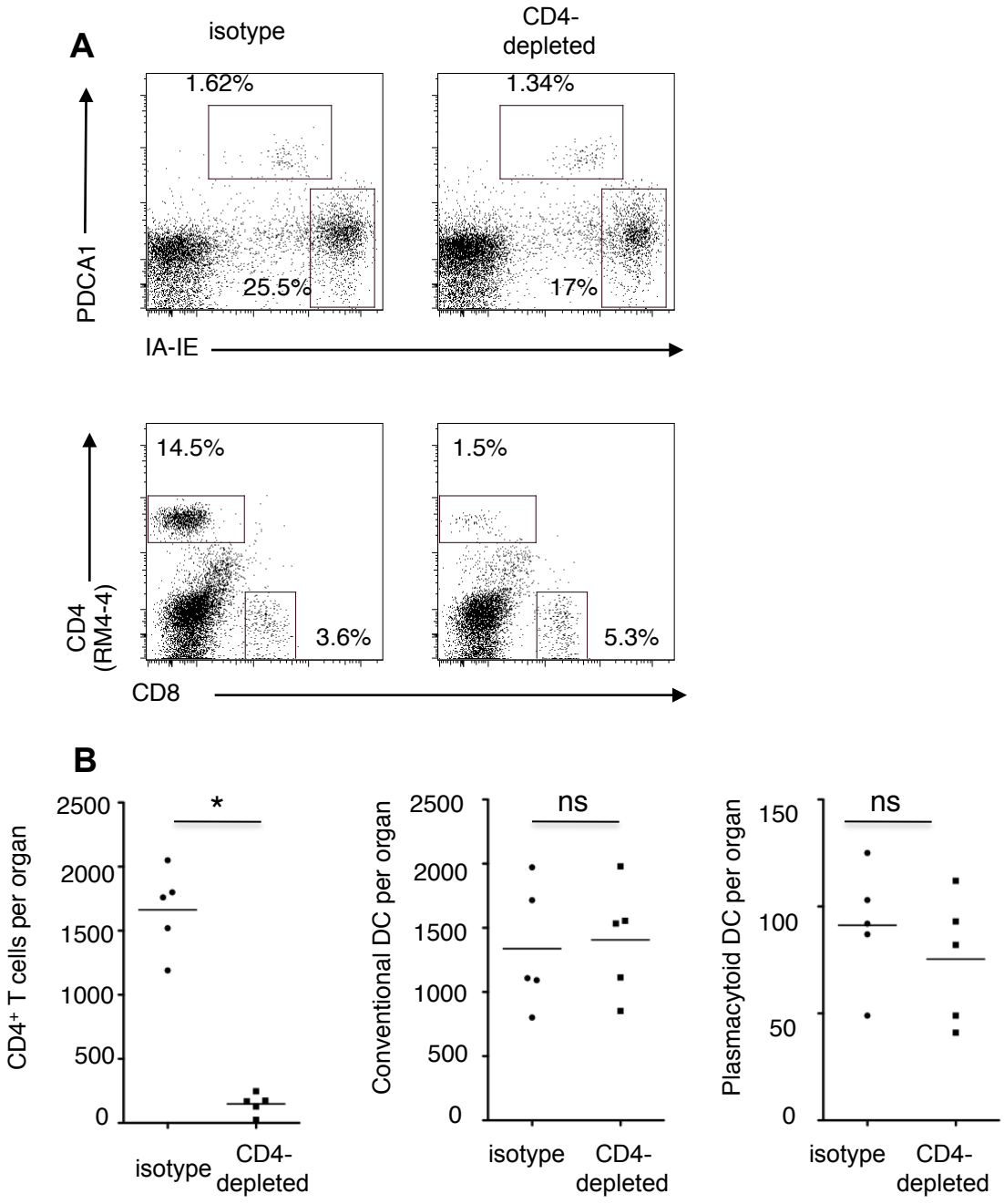
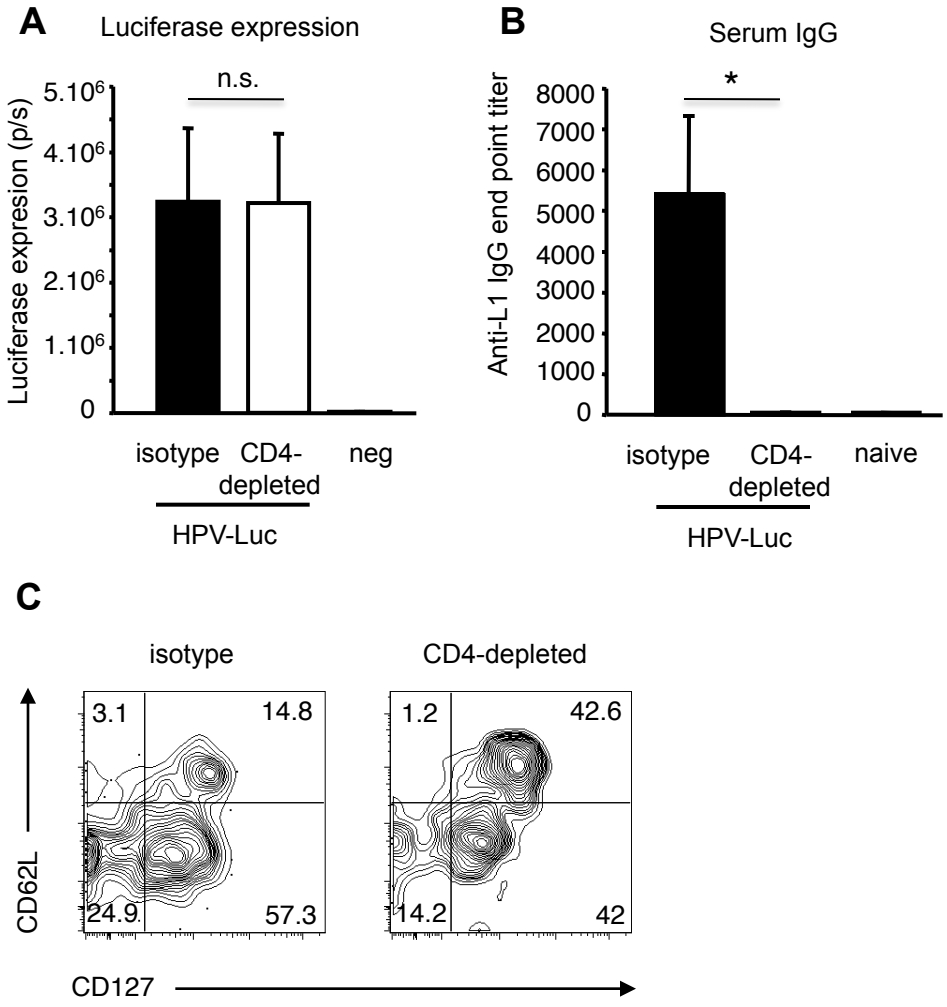


Supplemental Figure 6



Supplemental Figure 7