**Supplementary Figure Legends**

**Supplementary Figure 1. Local and systemic immune responses produced by different vaccination routes.** C57BL/6 mice (5 per group) were vaccinated with pNGVL4a-Sig/E7(detox)/HSP70 (50μg per mouse) twice with 7 day interval intramuscularly or cervicovaginally, followed by TA-HPV boost intramuscularly or cervicovaginally 7 days after the second DNA vaccination. 7 days after the last immunization, mice were sacrificed and splenocytes and cervicovaginal cells were isolated and analyzed by flow cytometry. **A**. Representative flow cytometry analysis and **B**, Bar graph showing the number of E7-specific CD8+ T cells in splenocytes. **C**, Representative flow cytometry and **D**, Bar graph showing the number of E7-specific CD8+ T cells in the cervicovaginal cells. Values are shown as mean ± SD, \*p<0.05, \*\*p<0.01, ns, not significant.

**Supplementary Figure 2.** **Characterization of MAdCAM-1 expression in cervicovaginal tissue after vaccination.** C57BL/6 mice (5 per group) were vaccinated with pNGVL4a-Sig/E7(detox)/HSP70 (50μg per mouse) prime followed six days later by TA-HPV (1x107 per mouse) boost either within cervicovaginal tissues or intramuscularly. One week after the last immunization, cervicovaginal tissuse were isolated and immunohistochemistry were performed to detect MAdCAM-1 expression. Arrows identified MAdCAM-1 expression in corresponding vessels (original magnification ×200). **A**. Immunohistochemistry (IHC) staining of MAdCAM-1 (**a.** Isotype staining control, **b**. Naïve, **c**. IM, **d**. ICV). **B.** Quantitation of MAdCAM-1 positive cells/200 × high power field (HPF).

**Supplementary Figure 3.** **Migration of CSFE-labeled α4β7 expressing E7-specific CD8+ T cells into spleen and vagina of WT or Itgb7-/- mice.** WT and Itgb7-/- mice were vaccinated within cervicovagional tissues with pNGVL4a-Sig/E7(detox)/HSP70 (50μg per mouse) prime followed 7 days later by TA-HPV (1x107 per mouse) boost. One week after the last immunization, single cells from spleen were prepared. The cells were stained with anti-mouse CD8a-FITC, HPV16 E7aa49-57 loaded H-2Db tetramer-PE and anti-mouse α4β7-APC. 7-AAD was used to exclude dead cells. The cells were acquired with FACSCalibur flow cytometer and analyzed with CellQuest Pro software. (**A**) The figure representative flow cytometry images of α4β7 expression by HPV16 E7-specific CD8+ T cells by splenocytes. 7 days after the last immunization, lymphocytes were isolated from spleen of WT or Itgb7-/- mice and labeled with CFSE. 4 ×107 splenocytes were then adoptively transferred into WT mice through retrooribital injections. One day after cell transfer, lymphocytes in spleen and cervicovagianal tissues were isolated and stained with Abs against CD8, which were analyzed by flow cytometry. The percentage of positive cells was used to calculate labeled cells recovered. Representative flow cytometry analysis of splenocytes (**B**) and cervicovaginal cells (**D**). Summary of flow cytometry analysis of CFSE+ CD8+ T cells in splenocytes (**C**) and in cervicovaginal tissues (**E**). Values are shown as mean ± SD, \*p<0.05, \*\*p<0.01, ns, not significant.

**Supplementary Figure 4.** **Comparison of HPV16 E7-specific CD8+ T cells induced by HPV16 E7 long peptide after either intramuscular or intracervicovaginal vaccination.** Female C57BL/6 mice (5 per group) were vaccinated with 10μg/mouse of HPV16 E7aa43-62 peptide mixed with 1μg/mouse of CpG1826 within cervicovaginal tissues or intramuscularly. The mice were boosted with the same regimen 7 days later. One week after the last immunization, single cells from spleen and cervicovaginal tissue were prepared. The cells were stained with anti-mouse CD8a-FITC, HPV16 E7aa49-57 loaded H-2Db tetramer-PE. 7-AAD was used to exclude dead cells. The cells were acquired with FACSCalibur flow cytometer and analyzed with CellQuest Pro software. **A.** Representative flow cytometry image of HPV16 E7-specific CD8+ T cells in spleen. **B.** Summary of HPV16 E7-specific CD8+ T cells in spleen. **C.** Representative flow cytometry image of HPV16 E7-specific CD8+ T cells in cervicovaginal tissue. **D.** Summary of HPV16 E7-specific CD8+ T cells in cervicovaginal tissue.

**Supplementary Figure 5.** **Comparison of anti-tumor effect generated by either pNGVL4a-Sig/E7(detox)/HSP70 intracervicovaginal vaccination followed by intracervicovaginal TA-HPV boost or intracervicovaginal TA-HPV vaccination.** Female C57BL/6 mice (5 per group) were injected with 2 × 104 of TC-1/luciferase intracervically on day 0. The mice were then vaccinated with either 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 or PBS within cervicovaginal tissues on day 2. The mice were boosted with the same regimen on day 5. pNGVL4a-Sig/E7(detox)/HSP70 and one group of PBS vaccinated mice were further vaccinated with 1 × 107 pfu of TA-HPV within cervicovaginal tissue on day 8. The expression of luciferase in the vagina was monitored by luminescence. **A**. Luminescence images of representative mice challenged with luciferase-expressing TC-1 tumor and treated according to the various treatment regimens. **B**. Bar graph showing luminescence intensity, values are shown as mean ± SD, \*\*p<0.01, \*\*\*p<0.001, ns, not significant.

**Supplementary Figure 6.** **Analysis of HPV16 E7-specific CD8+ T cells after intracervicovaginal vaccination with or without CD4+ T cell depletion.** Female C57BL/6 mice (5 per group) were injected with 100 μg/mouse of anti-mouse CD4 antibody (clone GK1.5) or PBS as described in 7A. The mice were then vaccinated with 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 within cervicovaginal tissues. After 7 days, the mice were boosted with 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 and further boosted with 1x107/mouse of TA-HPV 7 days later within cervicovaginal tissues. One week after the last immunization, single cells from spleen, draining lymph nodes (DLN) and cervicovaginal tissues were prepared. The cells were stained with anti-mouse CD8a-FITC and HPV16 E7aa49-57 loaded H-2Db tetramer-PE. 7-AAD was used to exclude dead cells. The cells were acquired with FACSCalibur flow cytometer and analyzed with CellQuest Pro software. **A.** Schema of the experiment. Summary of the frequency of HPV16 E7-specific CD8+ T cells in spleen (**B**), DLN (**C**) and cervicovaginal tissue (**D).**

**Supplementary Figure 7.** **Detection of CD103 and s1p1 expression by HPV16 E7-specific CD8+ T cells after intracervicovaginal vaccination.** FemaleC57BL/6 mice (5 per group) were vaccinated with 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 within cervicovaginal tissues. After 7 days, the mice were boosted with 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 and further boosted with 1x107/mouse of TA-HPV 7 days later within cervicovaginal tissues. One week after the last immunization, single cells from spleen, draining lymph nodes (DLN) and cervicovaginal tissues were prepared. The cells were stained with anti-mouse CD8a-FITC, CD103-PerCP-Cy5.5 and HPV16 E7aa49-57 loaded H-2Db tetramer-APC. For intracellular staining of S1P1, the surface stained cells were permeabilized and fixed using the Cytofix/Cytoperm kit according to the manufacturer’s instructions (BD Pharmingen, San Diego, CA) followed by the addition of PE-conjugated anti-mouse s1p1 antibody. The cells were acquired with FACSCalibur flow cytometer and analyzed with CellQuest Pro software. **A.** Representative flow cytometry image of CD103 expression by HPV16 E7-specific CD8+ T cells. **B.** Summary of CD103 expression by HPV16 E7-specific CD8+ T cells. **C.** Representative flow cytometry image of s1p1 expression by either CD103+ or CD103- HPV16 E7-specific CD8+ T cells. **D.** Summary of s1p1 expression by either CD103+ or CD103- HPV16 E7-specific CD8+ T cells.

**Supplementary Figure 8.** **Comparison of HPV16 E7-specific CD8+ T cells induced by pNGVL4a-Sig/E7(detox)/HSP70 intracervicovaginal vaccination followed by either intracervicovaginal or subcutaneous TA-HPV boost.** Female C57BL/6 mice (5 per group) were vaccinated with 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 within cervicovaginal tissues. After 7 days, the mice were boosted with 50μg/mouse of pNGVL4a-Sig/E7(detox)/HSP70 and further boosted with 1x107/mouse of TA-HPV 7 days later either within cervicovaginal tissues or subcutaneously. One week after the last immunization, single cells from spleen and cervicovaginal tissues were prepared. The cells were stained with anti-mouse CD8a-FITC and HPV16 E7aa49-57 loaded H-2Db tetramer-PE. 7-AAD was used to exclude dead cells. The cells were acquired with FACSCalibur flow cytometer and analyzed with CellQuest Pro software. **A.** Representative flow cytometry image of HPV16 E7-specific CD8+ T cells in spleen. **B.** Summary of HPV16 E7-specific CD8+ T cells in spleen. **C.** Representative flow cytometry image of HPV16 E7-specific CD8+ T cells in cervicovaginal tissue. **D.** Summary of HPV16 E7-specific CD8+ T cells in cervicovaginal tissue.