**Supplementary figure legends**

Fig. S1. Representative flow cytometry plots of IFNγ+, TNFα+, GZB+ and double positive TNFα+IFNγ+ CD8 T cells after in-vitro re-stimulation. Representative flow cytometry analyses of IFNγ+, TNFα+, GZB+ and double positive TNFα+IFNγ+ CD8 T cells. Populations are gated on single cell live CD3+CD8+ lymphocytes. 0.5x106 TC-1 cells were implanted into the flank of the mice at day 0. The vaccine and the control treatments were administered at day 12 when the mean tumor size was ~100 mm3. Mice received subcutaneous injections of PBS (n=8), or 15 μg of E7LP either in a free form (Free E7LP, n=8) or conjugated to NPs (NP-E7LP, n=8); both vaccinated groups also received 20 μg of CpG as adjuvant. Mice were sacrificed 9 days after immunization and spleens were harvested for analyses. Whole splenocytes were re-stimulated in vitro with the HPV16 E7 CD8 peptide RAHYNIVTF and intracellular staining for IFNγ, TNFα and GZB was performed.

Fig. S2. Mean TC-1 tumor volumes. The comparison was stopped when the first mouse in any of the groups reached a veterinary endpoint. Significant differences were present only at the last timepoint (day 22): PBS vs Free E7LP = n.s.; NP-E7LP vs NP-E7LP 2x = n.s.; PBS vs NP-E7LP/NP-E7LP 2x = p<0.0001; Free E7LP vs NP-E7LP/NP-E7LP 2x = p<0.0001; Previous timepoint were all n.s.. Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S3. Survival of subcutaneous TC-1 tumor-bearing mice and TC-1 tumor growth curves. (A) Mice survival. Statistics: PBS vs NP-E7LP p=0.0001, PBS vs NP-E7LP+anti-CD4 p<0.0001, PBS vs NP-E7LP+anti-CD8 p=0.7944, PBS vs NP-E7LP+anti-CD4+anti-CD8 p=0.0684, NP-E7LP vs NP-E7LP+anti-CD4 p=1, NP-E7LP vs NP-E7LP+anti-CD8 p=0.0004, NP-E7LP vs NP-E7LP+anti-CD4+anti-CD8 p=0.0002, NP-E7LP+anti-CD4 vs NP-E7LP+anti-CD8 p=0.0002, NP-E7LP+anti-CD8 vs NP-E7LP+anti-CD4+anti-CD8 p=0.0505, NP-E7LP+anti-CD4 vs NP-E7LP+anti-CD4+anti-CD8 p<0.0001. (B) Individual TC-1 tumor growth curves. (C) Mean TC-1 tumor volumes. The comparison was stopped when the first mouse in any of the groups reached a veterinary endpoint. Significant differences were present only at the last 2 timepoints (day 19 and 22): PBS vs NP-E7LP+anti-CD8 / NP-E7LP+anti-CD8+anti-CD4 = n.s.; NP-E7LP vs NP-E7LP+anti-CD4 = n.s.; NP-E7LP vs PBS / NP-E7LP+anti-CD8 / NP-E7LP+anti-CD8+anti-CD4 = p<0.0001 (for both timepoints); NP-E7LP+anti-CD4 vs PBS / NP-E7LP+anti-CD8 / NP-E7LP+anti-CD8+anti-CD4 = p<0.0001 (for both timepoints); Previous timepoint were all n.s.. 0.5x106 TC-1 cells were implanted into the flank of the mice at day 0. The vaccine and the control treatments were administered when the mean tumor size was ~100 mm3. Mice received subcutaneous injections of PBS (n=8) or 15 μg of E7LP conjugated to NPs (NP-E7LP, n=28) combined with 20 μg of CpG as adjuvant. The NP-E7LP treated mice were then split into 4 different groups that remained untreated (n=7), received 10 mg/kg of anti-CD8 depleting antibody (n=6) every 4 days, or 10 mg/kg of anti-CD4 depleting antibody (n=8) every 4 days, or both (n=7). Mice were monitored to follow tumor growth and survival. Mice that showed CD4 or CD8 T cell depletion less than 95% were excluded from the analyses. Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S4. Tumor growth curves of early-immunized TC-1 tumor-bearing mice. 0.5x106 TC-1 cells were implanted into the flank of the mice at day 0. The vaccine and the control treatments were administered at day 7 when the mean detectable tumor volume was 22 mm3, and 32% of the mice lacked a palpable mass. Mice received subcutaneous injections of PBS (n=5) or 15 μg of E7LP either in a free form (Free E7LP, n=4) or conjugated to NPs (NP-E7LP, n=10); both vaccinated groups also received 20 μg of CpG as adjuvant. (A) Mean tumor volume per group. (B) Individual tumor volumes. Black stars refer to Free E7LP vs NP-E7LP, Green stars refer to Free E7LP vs PBS and blue stars refer to NP-E7LP vs PBS. Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S5. Quantification of intra-tumoral CD8 T cells performed on histological sections of subcutaneous TC-1 tumors. The values are calculated as the percentage of the DAPI+ CD8 T cell nuclei area on the total DAPI+ area. Each mouse is represented by a data point obtained by the mean calculated between every field. 4 samples per group were stained and 2 fields per samples were analyzed using Fiji. Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S6. Flow cytometry analyses of intra-tumoral (A) CD11b+F4/80+ macrophages, (B) CD206+CD11b+F4/80+ “M2 like” macrophages, C) CD11bHighLy6C+Ly6G- monocytes, (D) CD11bHigh Ly6C- Ly6G+ neutrophils, (E) F4/80-CD11b+CD11C+ DCs and (F) F4/80-CD11b-CD11C+ DCs. 0.5x106 TC-1 cells were implanted into a flank of each mouse at day 0. The vaccine and the control treatments were administered when the mean tumor size was ~100 mm3. Mice received subcutaneous injections of PBS (n=8) or 15 μg of E7LP either in a free form (Free E7LP, n=8) or conjugated to NPs (NP-E7LP, n=8); both vaccinated groups also received 20 μg of CpG as adjuvant. Mice were sacrificed 9 days after immunization and tumors were harvested and processed for flow cytometry analyses. All the cells were gated on single, live cells. Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S7. Quantification of intra-tumoral CD8 T cells performed on histological sections. The values are calculated as the percentage of the DAPI+ CD8 T cell nuclei area on the total DAPI+ area. Each mouse is represented by a data point obtained by quantification performed on the whole tissue section. (A) Intravaginal TC-1 tumors: 3 (PBS and NP-E7LP) or 4 (Free E7LP) samples were stained and images of the whole tissue were acquired and analyzed using Fiji. (B) TC-1 lung metastases: 4 (PBS) or 8 (Free E7LP and NP-E7LP) samples were stained and images of the whole tissue were acquired. All the lung metastases identified on each sample were selected and analyzed using Fiji. Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S8. Analyses of relapsing tumors. (A) Tumor growth curve of TC-1 tumor treated with NP-E7LP vaccine, depicting response, stable disease and relapse phase. (B) Gene expression and *E7* sequence analysis comparing untreated TC-1 tumors with TC-1 tumors after relapsing from NP-E7LP treatment. *E7, H2Db, B2M, TAP1* and *PSMB5* gene expression of TC-1 (n=3) and TC-1 relapse (n=6) tumors were determined by real time PCR. The *E7* sequence encoding for the MHC-I restricted CD8 peptide of 8 relapsing TC-1 tumors was sequenced by Sanger sequencing (5 clones per tumor). Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Fig. S9. Quantification of immunological markers evaluated by immuno-histology on tumor tissue sections at different phases of therapeutic response. As the tumor sections were very homogeneous in cell distribution and staining intensity in each separate treatment phase, three representative fields per sample were imaged and subsequently analyzed using Fiji. Each mouse tumor is represented as a data point averaging the values calculated for each field for that tumor. The panels show A) mean CD8 T cells infiltrates per field, calculated as the percentage of the DAPI+/CD8+ area relative to the total DAPI+ nuclear area; and the mean staining intensity per field for: (B) ICOS; (C) F4/80. Treatment groups: PBS n=2; Response n=4; Stable n=4; Relapse n=4.

Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Additional statistics:

CD8: PBS vs Response p=0.0011, PBS vs Stable p=0.0026, PBS vs Relapse p=0.0843.

ICOS: PBS vs Response p=0.0131, PBS vs Stable p=0.6762, PBS vs Relapse p=0.8011.

F4/80: PBS vs Response p=0.0265, PBS vs Stable p=0.0182, PBS vs Relapse p=0.4305.

Fig. S10. Magnified images of macrophages in the tumor microenvironment at different stages of disease progression. Images are derived from the fields shown In Fig. 5. Immuno-fluorescent staining for F4/80, CD11c and MRC1 is shown in 10 μm sections derived from frozen OCT-embedded tumors that were collected at the indicated time-points as in Fig S8A. PBS treated tumors were collected at the endpoint. Scale bars are 50 μm. Single channel images of F4/80, CD11c, MRC1 (top lane and bottom left) and overlays of F4/80+CD11c and F4/80+MRC1 (bottom center and bottom right) are shown.

Fig. S11. Quantification of immunological markers evaluated by immuno-histology on tumor tissue sections at different phases of therapeutic response. As the tumor sections were very homogeneous in cell distribution and staining intensity in each separate treatment phase, three representative fields per sample were imaged and subsequently analyzed using Fiji. Each mouse tumor is represented as a data point averaging the values calculated for each field for that tumor. The panels show the mean staining intensity per field for: (A) CD11c; (B) MRC1; and (C) PDL1. Treatment groups: PBS n=2; Response n=4; Stable n=4; Relapse n=4.

Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Additional statistics:

CD11c: PBS vs Response p=0.0053, PBS vs Stable p=0.0012, PBS vs Relapse p=0.0596.

MRC1: PBS vs Response p=0.0222, PBS vs Stable p=0.0192, PBS vs Relapse p=0.0716.

PDL1: PBS vs Response p=0.0753, PBS vs Stable p=0.1279, PBS vs Relapse p=0.2043.

Fig. S12. Mean TC-1 tumor volumes in mice treated with the agonistic antibody anti-41BB. The comparison was stopped when the first mouse in any of the groups reached a veterinary endpoint. Significant differences were present only at the last timepoint (day 18): Free E7LP vs Free E7LP+anti-41BB = n.s.; NP-E7LP vs NP-E7LP+anti-41BB = p=0.0124; Free E7LP vs NP = n.s.; Free E7LP+anti-41BB vs NP-E7LP+anti-41BB = n.s.; Free E7LP+anti-41BB vs NP-E7LP = n.s.; Previous timepoint were all n.s..

Fig. S13. Analyses of intra-tumoral adaptive immune cell infiltrates upon therapeutic vaccination in combination with the agonistic antibody anti-41BB. (A) Flow cytometry analyses of intra-tumoral E7-specific CD8 T cells using tetramers recognizing the HPV16 E7 CD8 peptide RAHYNIVTF. (B, C) CD44+KLRG1+ terminal effector E7 specific CD8 T cells. (D) Intra-tumoral CD8 T cell to Treg ratio. (E-H) Flow cytometry analyses of 41BB (E), GITR (F), ICOS (G) and OX-40 (H) expression on E7 specific CD8 T cells. (I-K) Flow cytometry analyses of IFNγ (I), TNFα (J) and GZB (K) production by CD8 T cells after in-vitro re-stimulation with the HPV16 E7 CD8 peptide RAHYNIVTF. 0.5x106 TC-1 cells were implanted into the flank of the mice at day 0. Mice were immunized when mean tumor volume was ~170mm3 and sacrificed at day 9. Selected groups also received 3 doses of anti-41BB I.P. every 3 days starting on the day of immunization. Groups: Free E7LP (n=6), NP-E7LP (n=5), Free E7LP+anti-41BB (n=6) and NP-E7LP+anti41BB (n=3).

Statistics: \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p<0.0001; n.s. = not significant.

Additional statistics: (B) NP-E7LP vs Free E7LP: p=0.0095; (C) NP-E7LP vs Free E7LP: p=0.9179; (D) NP-E7LP vs Free E7LP: p=0.008; (E) NP-E7LP vs Free E7LP: p=0.0376; (L) NP-E7LP vs Free E7LP: p=0.0111; (M) NP-E7LP vs Free E7LP: p=0.0155; (N) NP-E7LP vs Free E7LP: p=0.0131.