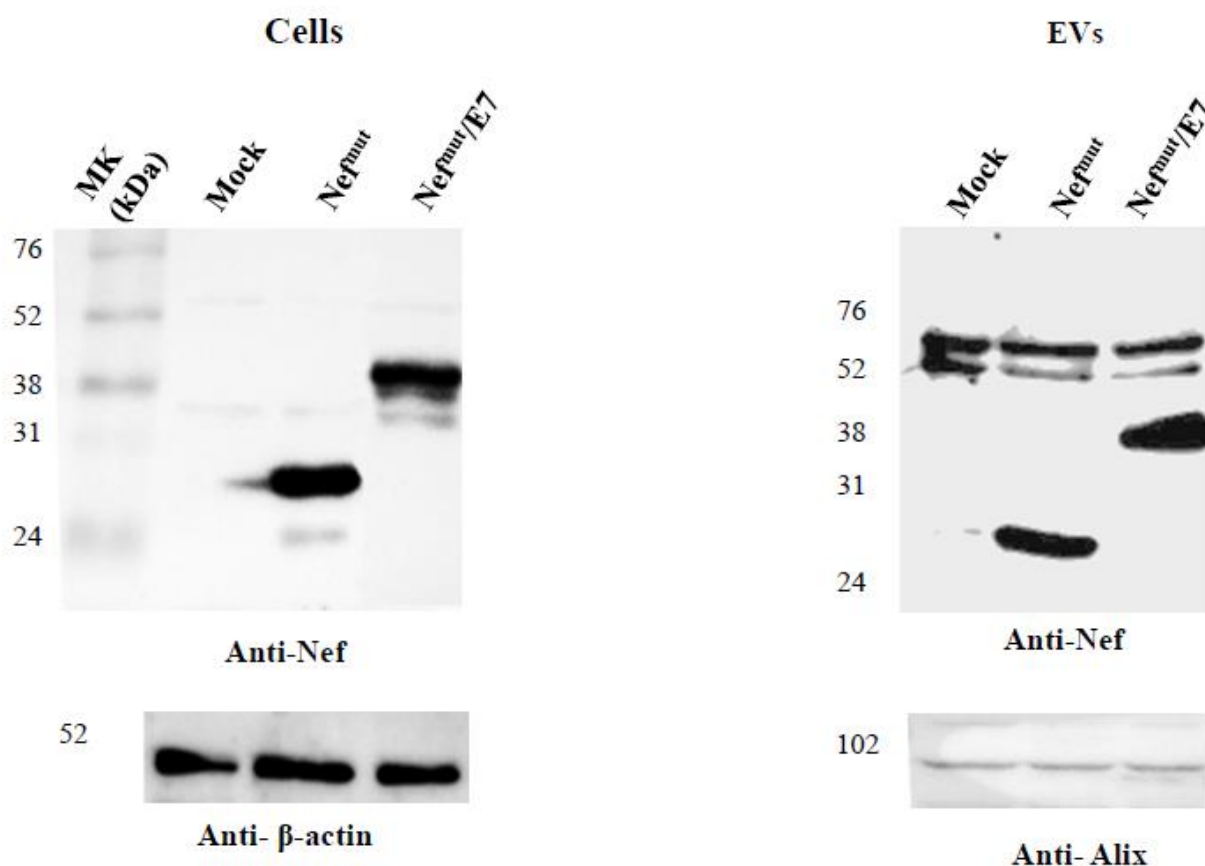
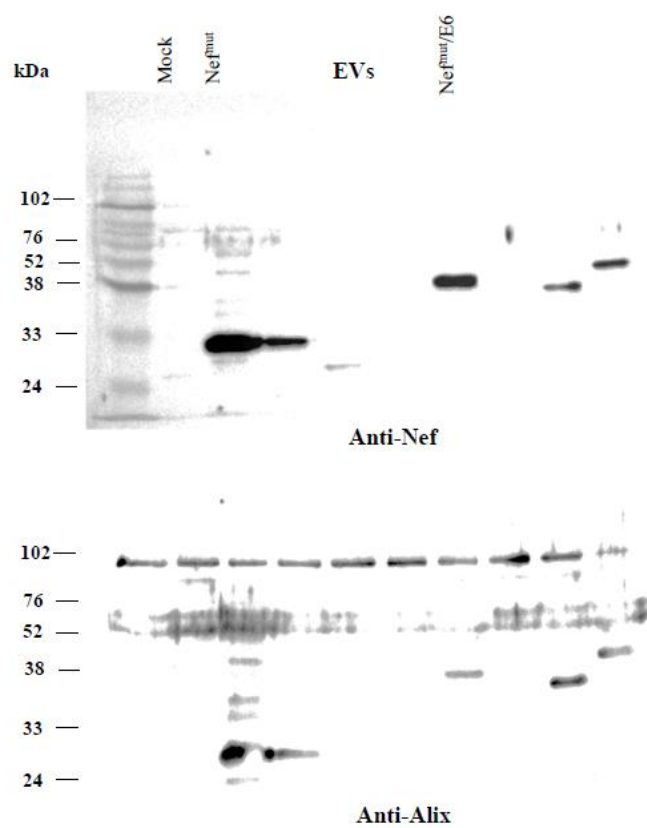
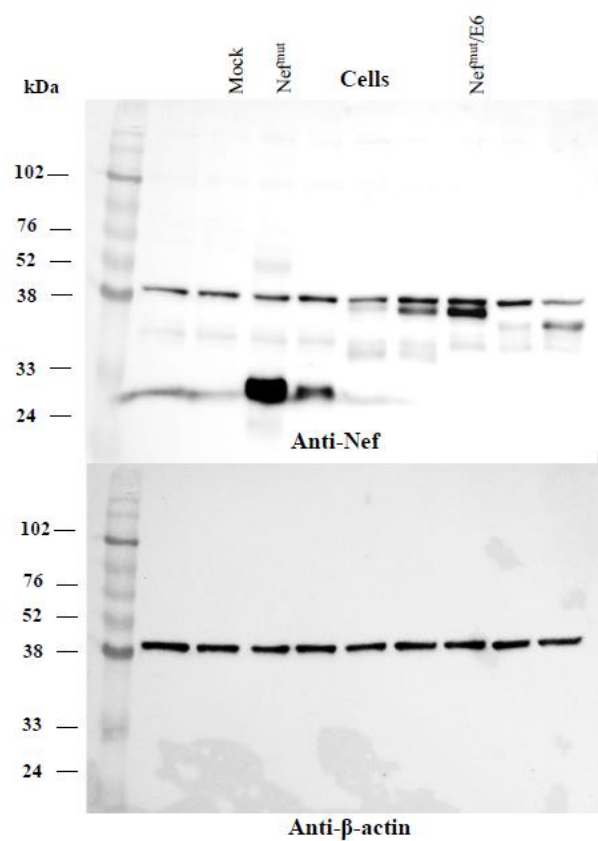


# Supplementary Material: Long-Term Antitumor CD8<sup>+</sup> T Cell Immunity Induced by Endogenously Engineered Extracellular Vesicles

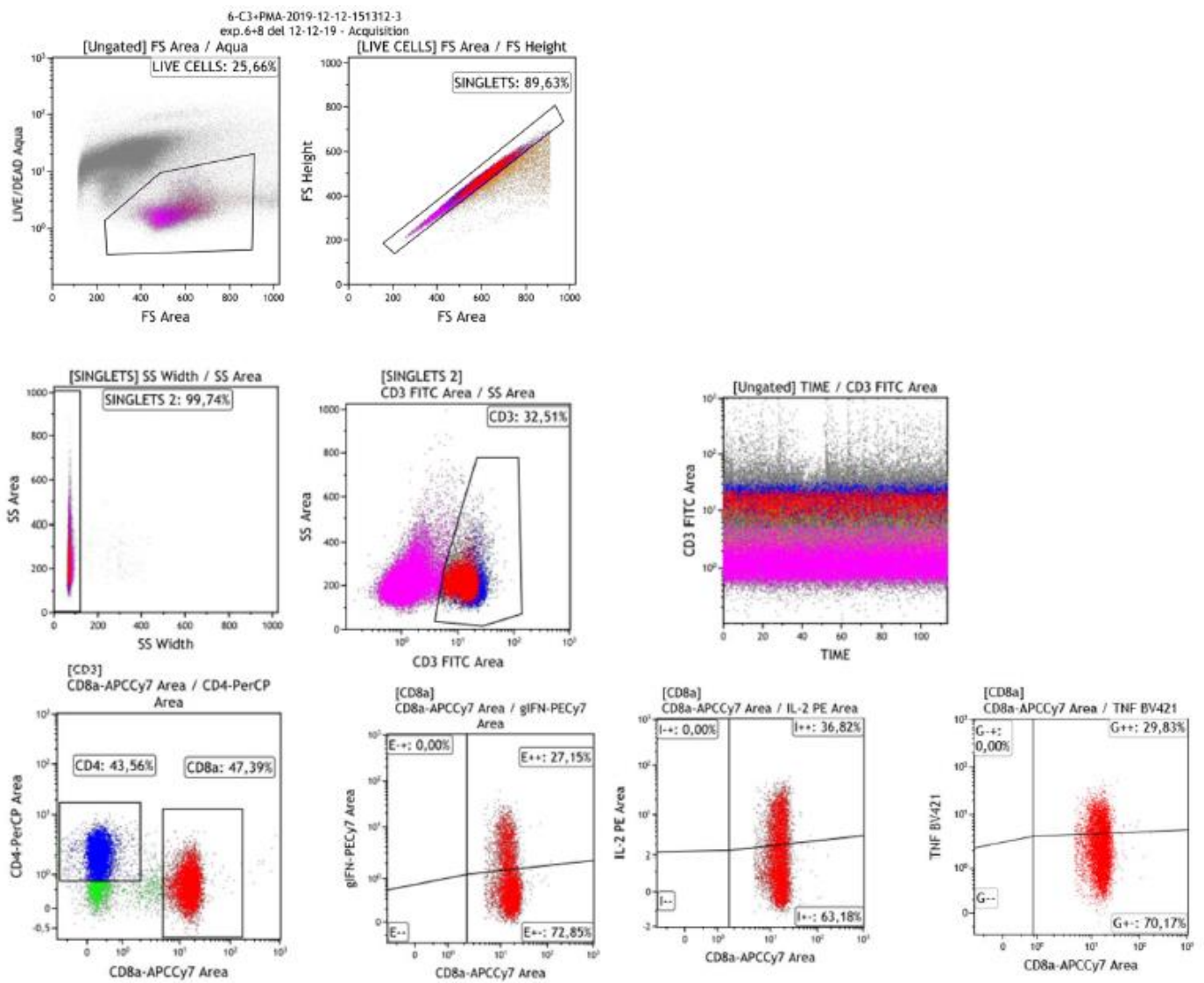
Flavia Ferrantelli, Francesco Manfredi, Chiara Chiozzini, Patrizia Leone, Andrea Giovannelli, Eleonora Olivetta and Maurizio Federico



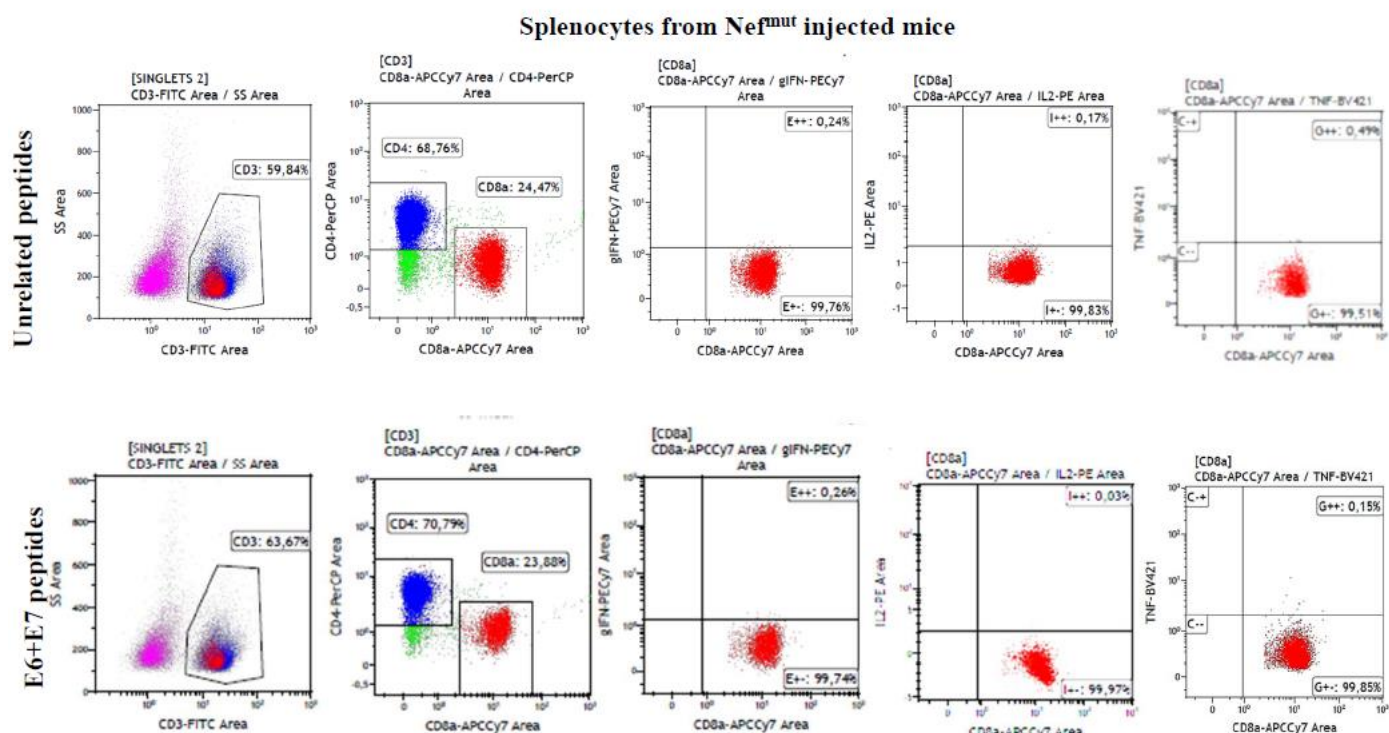
**Figure S1.** Western blot analysis for the expression of Nefmut/E7 in HEK293T cells and respective EVs. Raw data.



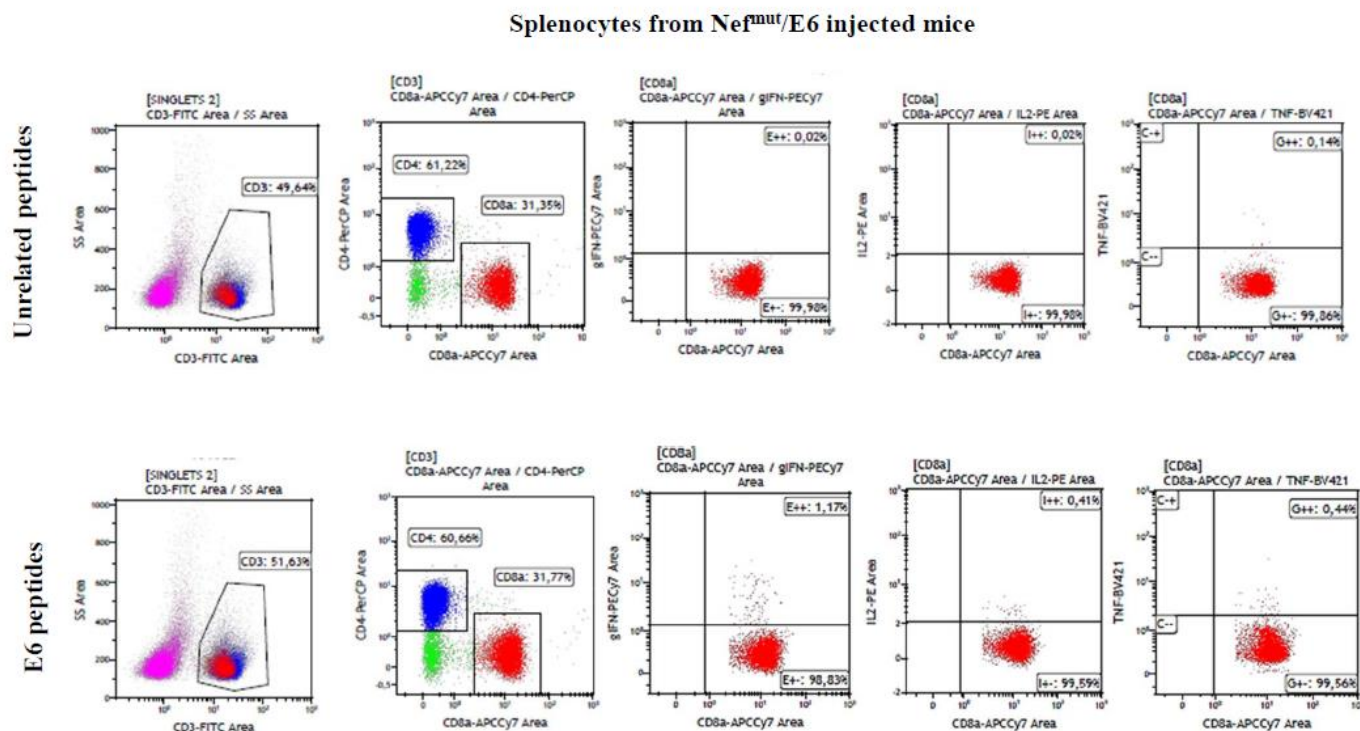
**Figure S2.** Western blot analysis for the expression of Nefmut/E6 in HEK293T cells and respective EVs. Raw data.



**Figure S3.** Gating strategy carried out in flow cytometry analysis of splenocytes from injected mice. shown is the analysis on PMA-treated cells.

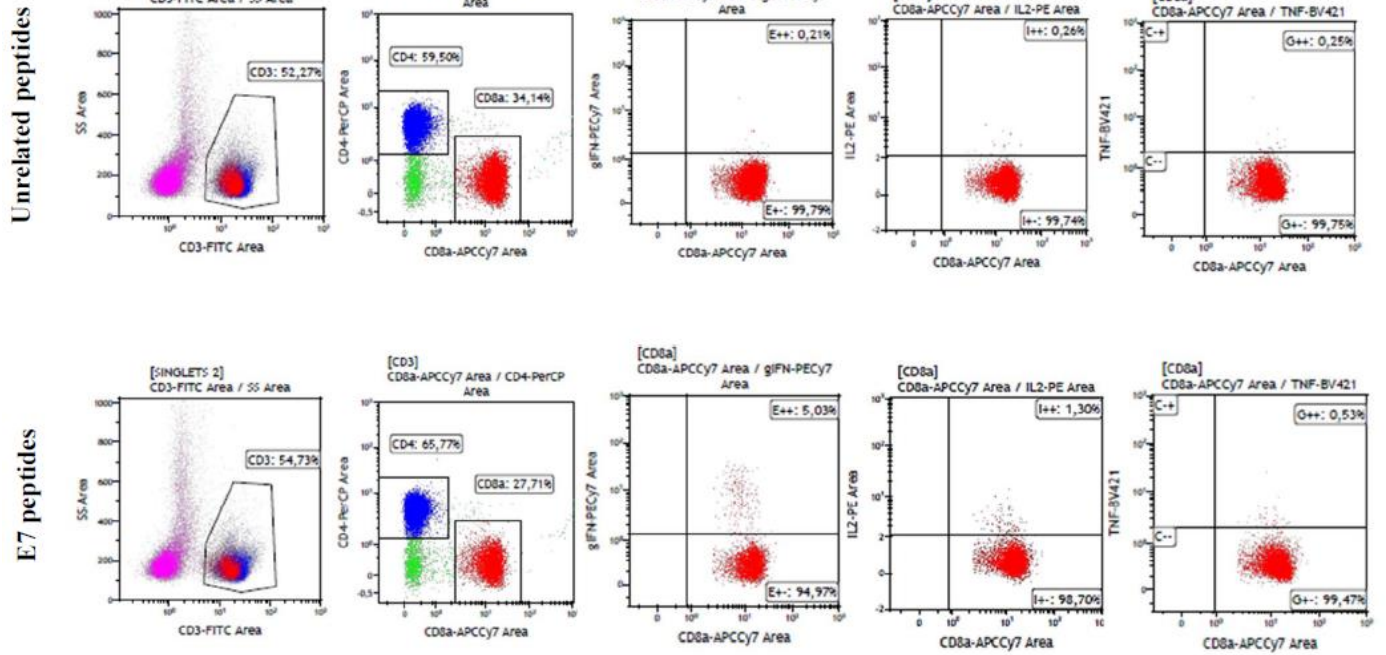


**Figure S4.** Representative flow cytometer plots from the analysis of splenocytes isolated from Nefmut injected mice, and treated with either MHC Class I-matched, unrelated nonamers (upper plots) or HPV16 E6/E7-specific nonamers (lower plots).



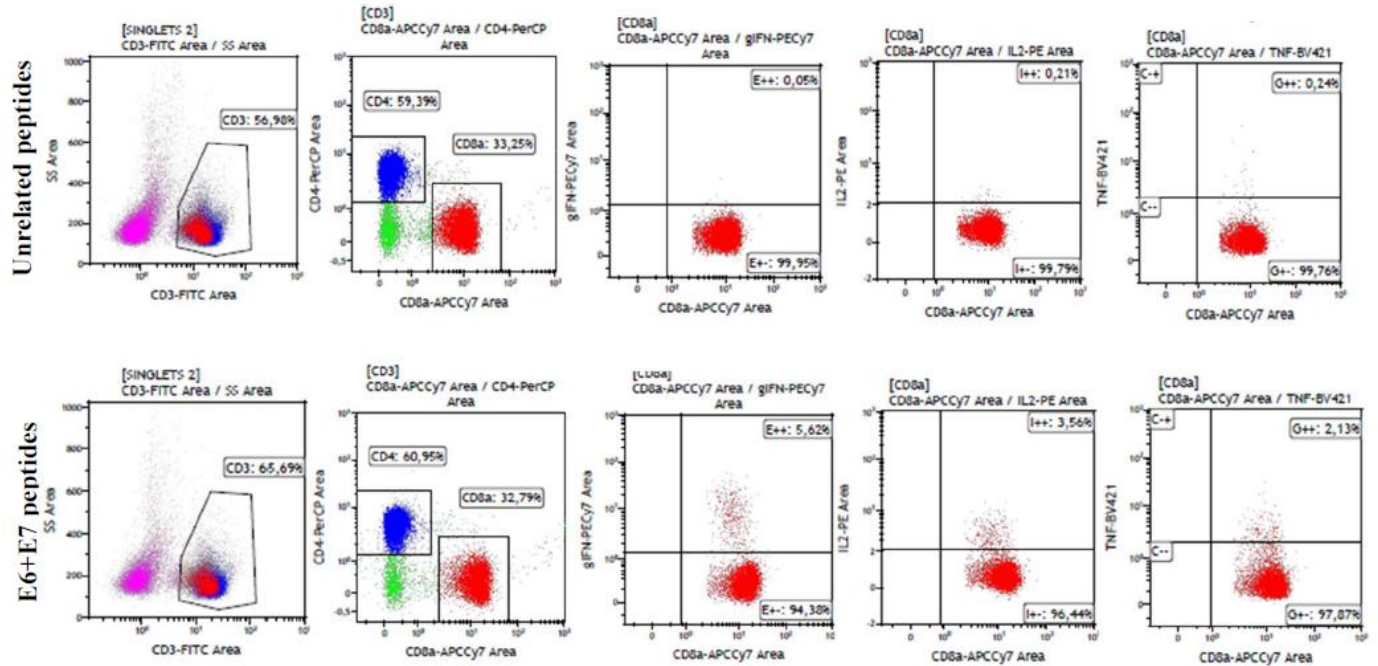
**Figure S5.** Representative flow cytometer plots from the analysis of splenocytes isolated from Nefmut/E6 injected mice, and treated with either MHC Class I-matched, unrelated nonamers (upper plots) or HPV16 E6-specific nonamers (lower plots).

### Splenocytes from Nef<sup>mut</sup>/E7 injected mice

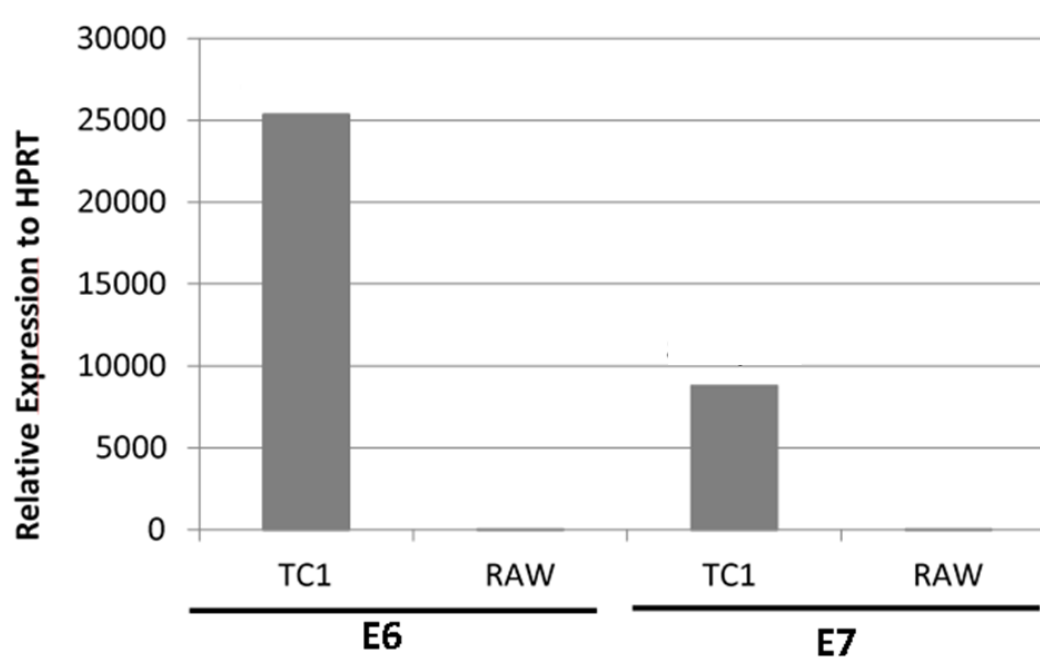


**Figure S6.** Representative flow cytometer plots from the analysis of splenocytes isolated from Nefmut/E7 injected mice, and treated with either MHC Class I-matched, unrelated nonamers (upper plots) or HPV16 E7-specific nonamers (lower plots).

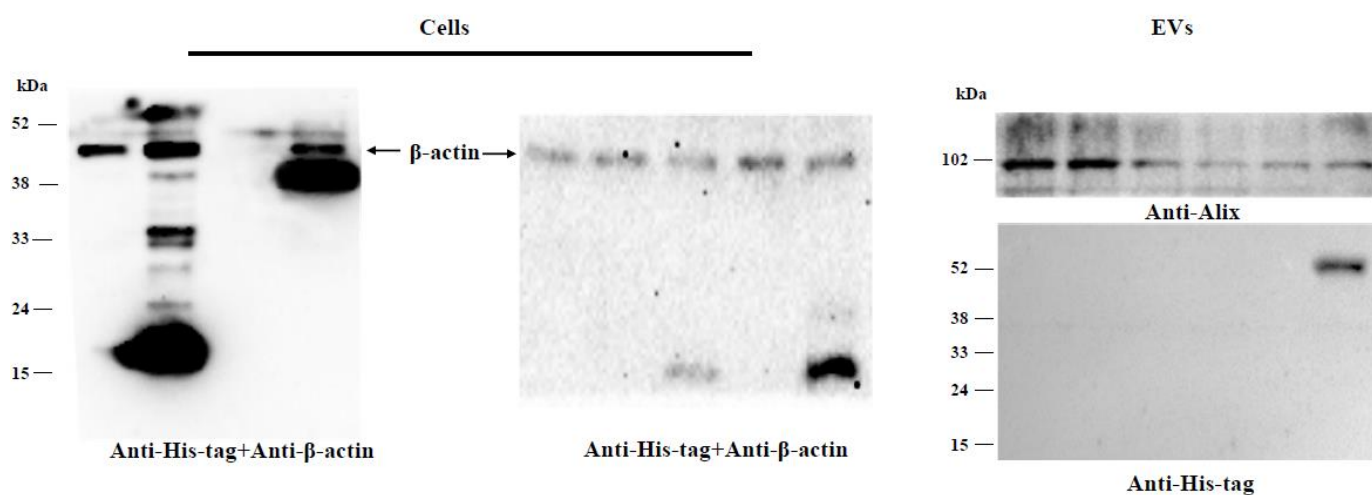
### Splenocytes from Nef<sup>mut</sup>/E6+Nef<sup>mut</sup>/E7 injected mice



**Figure S7.** Representative flow cytometer plots from the analysis of splenocytes isolated from mice injected with Nefmut/E6 and Nefmut/E7 vectors, and treated with either MHC Class I-matched, unrelated nonamers (upper plots) or HPV16 E6+E7-specific nonamers (lower plots).



**Figure S8.** HPV16-E6 and -E7 gene expression in TC-1 cells. HPV16-E6 and -E7 gene expression was quantified by qRT-PCR in TC-1 cells and RAW murine cells as control. Gene expression was normalized by amplifying samples for hypoxanthine guanine phosphoribosyltransferase (HPRT) as house-keeping RNA.



**Figure S9.** Western blot analysis for the expression of HPV16-E6 and -E7 in HEK293T cells and respective EVs. Raw data.