

Supplemental Figure Legends

Fig. S1

Intramuscular application of high doses of MVA leads to altered T cell differentiation. Groups of mice (n=4) were vaccinated intramuscularly with 10^8 IU or 10^9 IU MVA OVA. On day 8 post prime, splenocytes were analyzed for intracellular IFN γ against VACV- B8R $_{20}$ or OVA $_{257}$ and is shown as relative and absolute number (A). IFN γ positive cells were further analyzed for multifunctionality (TNF α and IL-2 production). B shows two representative plots of the distribution of multifunctional subpopulations after stimulation with VACV- B8R $_{20}$ and relative amount of polyfunctional (IFN γ^+ , TNF α^+ and IL-2+) T cells. Multimer positive cells were further analyzed for CD62L and CD127 subpopulations. c shows two representative plots of the distribution of memory T cell subpopulations of VACV- B8R $_{20}$ and the relative distribution of memory T cell subpopulations specific for OVA $_{257}$ or VACV- B8R $_{20}$. (■ 10^8 IU, □ 10^9 IU). Analysis of one representative of two independent experiments.

Fig. S2

Mucosally applied lyophilized MVA OVA induces mucosal immunity. Groups of mice (n=4) were vaccinated intranasally with lyophilized MVA OVA (10^9 IU) or intramuscularly (10^8 IU). On day 300 post prime fecal pellets were collected and analyzed for VV-specific IgA by ELISA. (*= p<0,05)

Fig. S3

Comparable impairment to overcome vectors-specific immunity for mucosally or i.m. applied MVA. Groups of naive or previously immunized mice (n=4) (MVA wt 10^8 IU i.p.) were vaccinated intranasally with lyophilized MVA OVA (10^9 IU) or intramuscularly (10^8 IU). 8 days later, splenocytes were analyzed for intracellular IFN γ production upon stimulation with OVA $_{257}$ peptide. Absolute numbers of IFN γ^+ SIINFEKL specific T-cells in spleen are shown. One representative of two independent experiments.