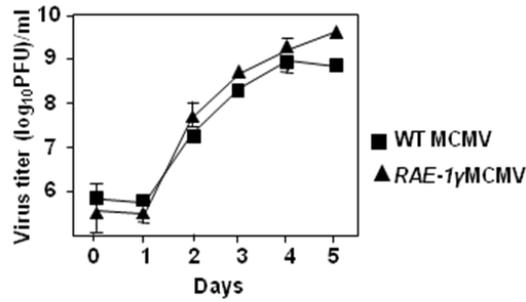
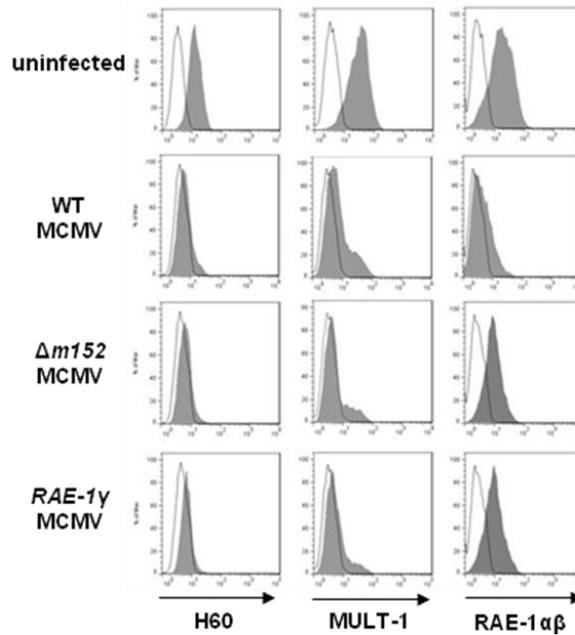


A



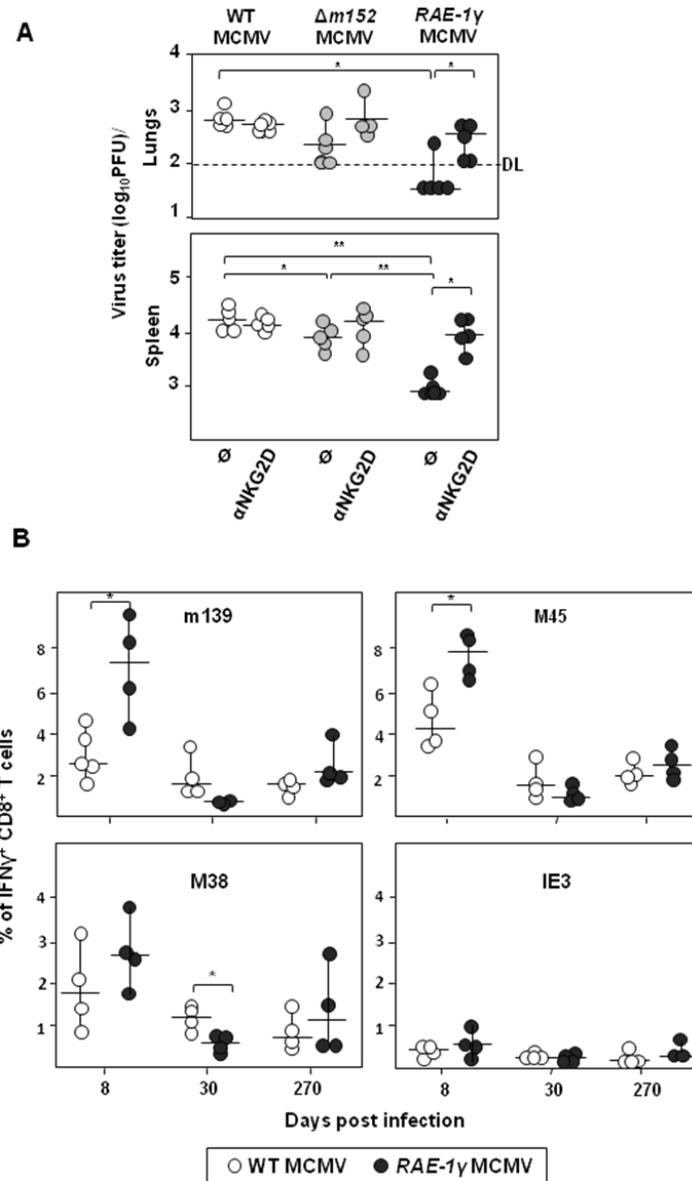
B



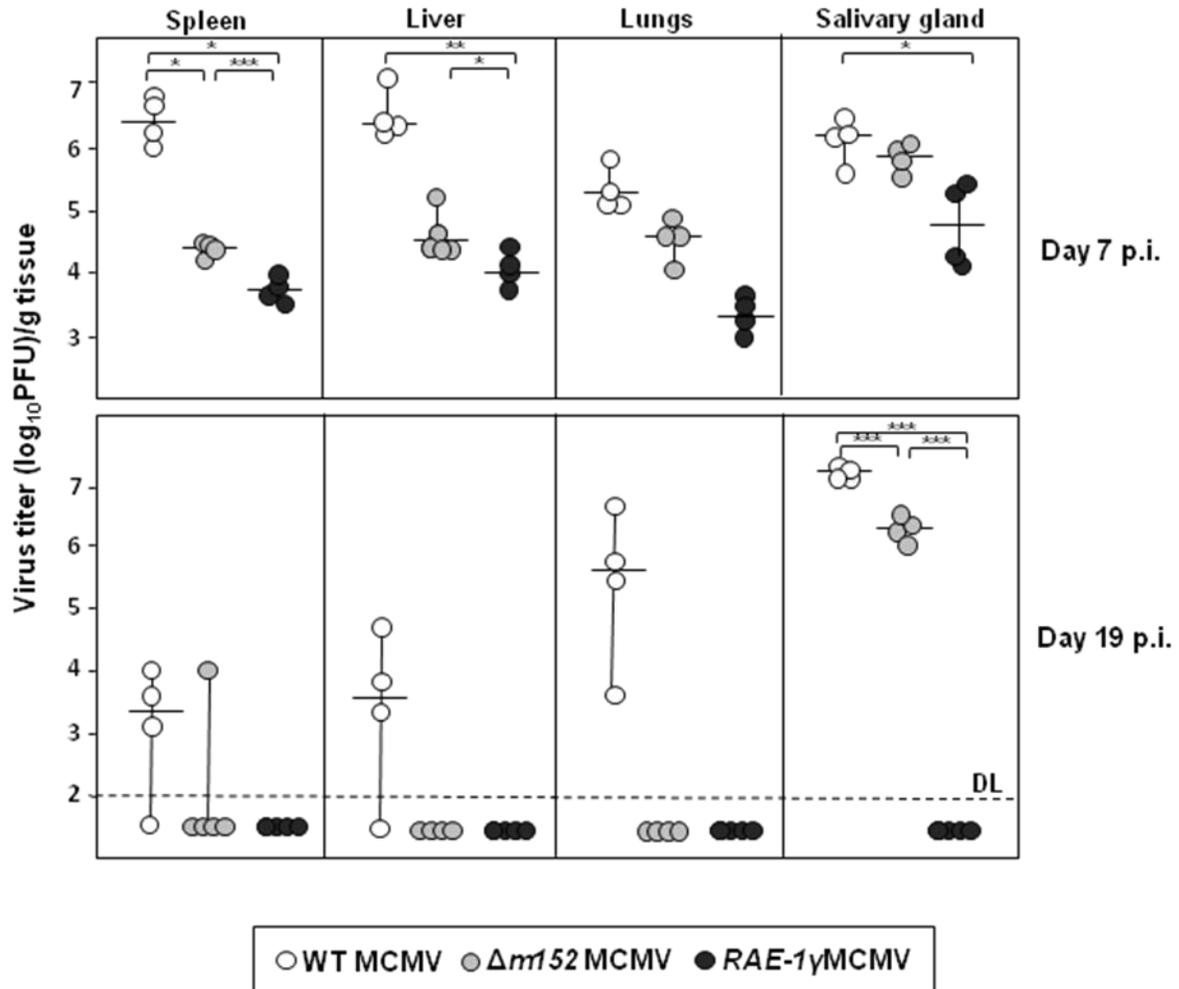
Supplementary Figure 1.

(A) Insertion of RAE-1 γ in place of $m152$ MCMV gene does not affect virus replication in vitro. MEF was infected with RAE-1 γ MCMV or WT MCMV at 0.1 PFU per cell. Supernatants were harvested at indicated time p.i. and virus titers were determined by plaque assay. Means \pm standard errors for the representative of 3 experiments are shown

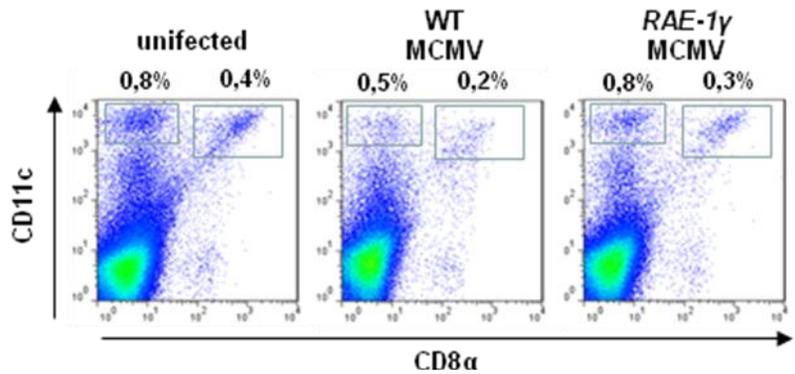
(B) Compared to $\Delta m152$ MCMV infection, the insertion of RAE-1 γ in place of $m152$ MCMV gene does not change the expression of other NKG2D ligands on infected cells. NIH 3T3 (left panel), SVEC 4-10 (middle panel) and B12 cells (right panel) were infected with indicated viruses at 3 PFU per cell and 12 h later analyzed for the surface expression of NKG2D ligands by staining with the biotinylated anti-H60 Ab, anti-MULT-1 Ab or anti-RAE-1 $\alpha\beta$ Ab, followed by PE-conjugated goat anti-rat IgG or PE-labeled streptavidin. Cells incubated with the secondary Ab in the absence of the primary Ab were used as negative control (thin line). Each histogram represents 10,000 gated propidium iodide-negative cells



Supplementary Figure 2. *RAE-1γ*MCMV is attenuated in *Ly49H*⁺ C57BL/6 mice. (A) Untreated C57BL/6 mice or C57BL/6 mice injected with blocking anti-NKG2D Ab were i.v. injected with 5×10^5 PFU of the indicated viruses. Viral titers were determined in spleen 3 d p.i. by plaque assay. (B) C57BL/6 mice were f.p. injected with 2×10^5 PFU of *RAE-1γ*MCMV or WT MCMV. Splenocytes were isolated at different time p.i., stimulated with the indicated peptides and stained for IFN- γ production. The percentage of IFN- γ ⁺CD8⁺ T cells for individual mice (circles) and median values (horizontal bars) are shown.



Supplementary Figure 3. *RAE-1γ*MCMV in newborn mice is attenuated compared to MCMV lacking the m152 gene only. Neonatal BALB/c mice were i.p. injected with 500 PFU of *RAE-1γ*MCMV, WT MCMV or Δ m152 MCMV 6 h post partum and viral titers were determined by plaque assay at the indicated time p.i. Individual mice (circles) and median values (horizontal bars) are shown. DL, detection limit.



Supplementary Figure 4. *RAE-1 γ MCMV* does not affect the frequency of cDCs in spleen of infected mice. Splenocytes were isolated from naïve BALB/c mice or BALB/c mice i.v. injected with 2×10^5 PFU of *RAE-1 γ MCMV* or WT MCMV 3 d p.i. and analyzed for the frequency of CD11b cDCs (CD11c^{hi}CD8 α ⁻) and CD8 α cDCs (CD11c^{hi}CD8 α ⁺) within the NKp46⁻ TCR β ⁻ population. Numbers in dot plots represent the percentage of CD11b cDCs and CD8 α cDCs within the total splenocyte population for a representative animal from a group of three mice.