

Figure S1. Ly49G2+ NK cells licensed by MHC I D^k class I molecules in R7 congenic mice. Freshly prepared splenocytes from R7 and R2 congenic mice were incubated with immobilized anti-CD16/CD32 in the presence of Brefeldin A for four hours and stained for DX5, CD3, 4D11, and intracellular IFN- γ . Relative IFN- γ productivity was plotted as a ratio of the percentage of IFN- γ + cells among the Ly49G2+ NK cells to the percentage of IFN- γ + cells among the Ly49G2- NK cells as described (Xie et al., 2010). Data are representative of three experiments with 2-8 animals per genotype. (*p<0.05 by Student's T-test)

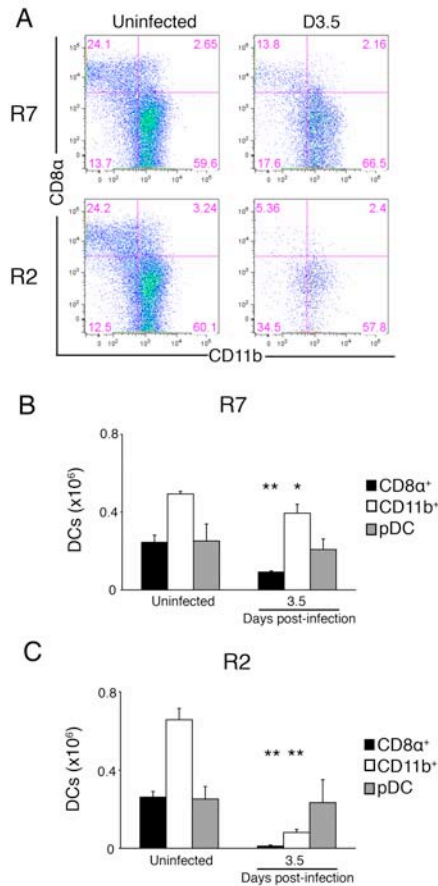


Figure S2. MHC I D^k supports splenic dendritic cells following MCMV infection.

(A) Representative dot plots showing the proportion of CD8α⁺ and CD11b⁺ cDC subsets in uninfected and infected R7 and R2 spleens. The dot plots were gated on CD3⁻, CD19⁻, CD11c^{hi}, MHC II⁺ cells. (B) The graph represents the mean ± SEM for live gated cDC and pDC (CD3⁻, CD19⁻, MHC II⁺, CD11c^{mid}, PDCA-1⁺) subsets in R7 spleen. (C) The graph represents the mean ± SEM for live gated DC subsets in non-Tg spleen. Shown is one representative experiment of two, with 3-4 mice per strain per time point.

Both CD8α⁺ and CD11b⁺ cDC subsets were significantly higher on d 3.5 in R7 than R2 mice (p<0.001), whereas cDC subsets were comparable in uninfected animals. (* p<0.05, **p<0.005 compared to respective subset in uninfected mice by Student's T-test).

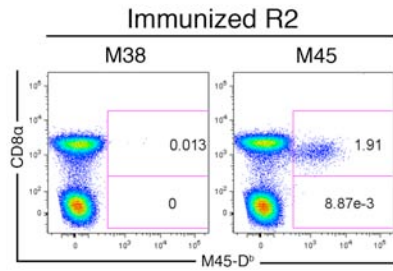


Figure S3. M45-HGIRNASFI-D^b-restricted CD8 T cell responsiveness in immunized R2 congenic mice. Ex vivo splenocytes isolated 6 d after immunization with the indicated peptides plus anti-CD40 and poly I:C and stained for CD19, CD3, CD8 and virus-specific T cell receptors using M45-D^b tetramers prior to flow cytometric analysis. Shown is one representative experiment of two, with 3-4 mice per strain per treatment group.

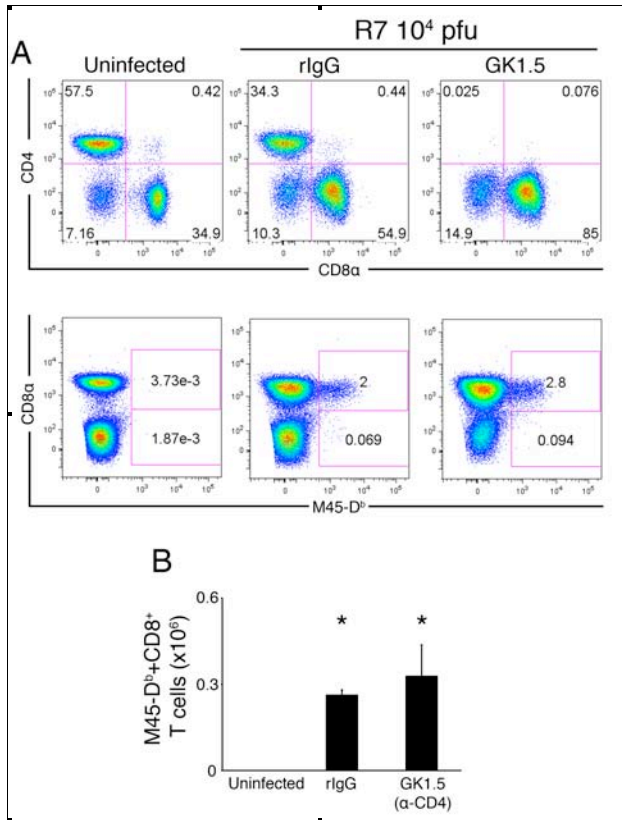


Figure S4. MHC I D^k-dependent NK cell enhancement of MCMV-specific CD8 T cell responsiveness is CD4 T cell-independent. (A) Representative dot plots showing the proportion of CD4 T cells and M45-D^b-specific CD8 T cells in spleens of uninfected R7, infected R7 treated with control rat IgG, infected R7 depleted of CD4⁺ T cells (GK1.5). The dot plots were gated on CD19⁻, CD3⁺ cells. (B) The graph represents the mean ± SEM for live gated M45-D^b-specific CD8 T cells in uninfected and infected R7 animals given the indicated treatments. The data are representative of two experiments with 4 mice per treatment group. (* p<0.05 by Student's T-test compared to uninfected).

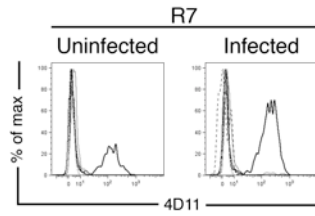


Figure S5. Ly49G2 is not expressed on cDCs, CD8 T cells, or MCMV-specific CD8 T cells. Ex vivo splenocytes from MCMV-infected (10^4 PFU; d 6) R7 were stained and analyzed for Ly49G2 (mAb 4D11) on NK cells (solid black line), CD11c^{hi} cDCs (solid gray line), CD8 T cells (black dotted line), and M45-D^b tetramer positive CD8 T cells (black long dash line) with 4D11 FMO as negative control (dashed gray line).