

SUPPLEMENTAL MATERIAL

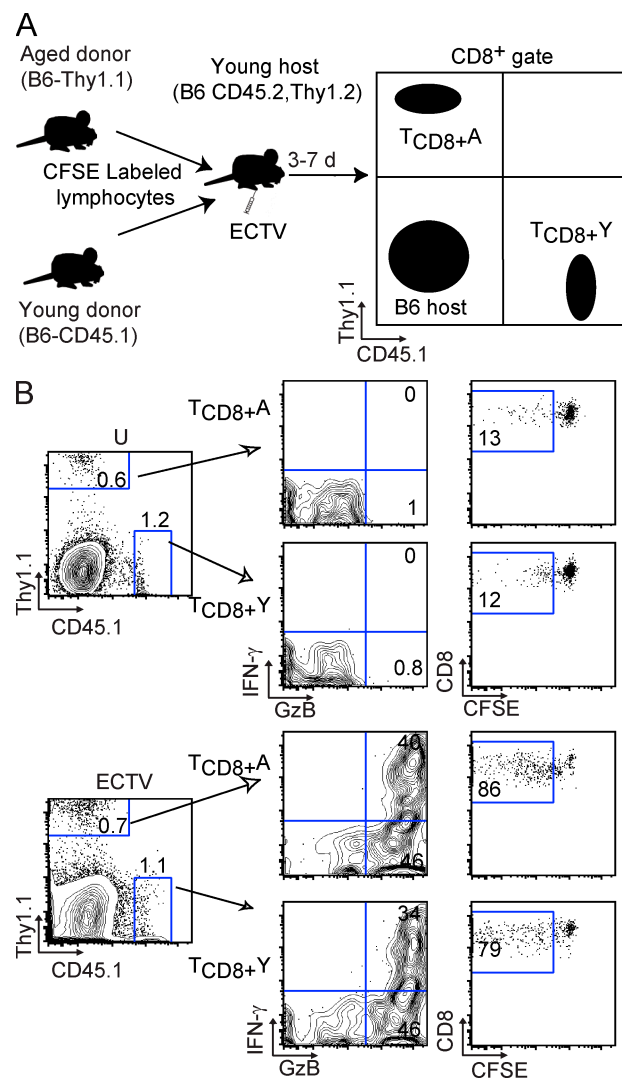
Fang et al., <http://www.jem.org/cgi/content/full/jem.20100282/DC1>

Figure S1. The defective T_{CD8+} cell response of aged mice to ECTV is not T cell intrinsic. A reciprocal experiment to that depicted in Fig. 4 A and B is shown. (A) Experimental design for the adoptive transfer of CFSE-labeled lymphocytes from aged (B6-Thy1.1) and young (B6-CD45.1) donor mice into young B6 (B6 CD45.2-Thy1.2) hosts followed by ECTV infection and flow cytometry analysis gating on CD8⁺ cells. (B) B6 mice, as in A, were euthanized on the indicated dpi. Cells from the D-LN and spleen were restimulated ex vivo for 5 h with infected cells in the presence of brefeldin A and stained for surface CD3, CD8, Thy1.1, and CD45.1, and for intracellular IFN- γ and GzB, followed by flow cytometry. Representative plots correspond to spleens of individual mice 7 dpi. Plots corresponding to an uninfected control mouse are also shown for comparison. Plots were gated on CD8⁺ cells. Data are representative of two independent experiments.

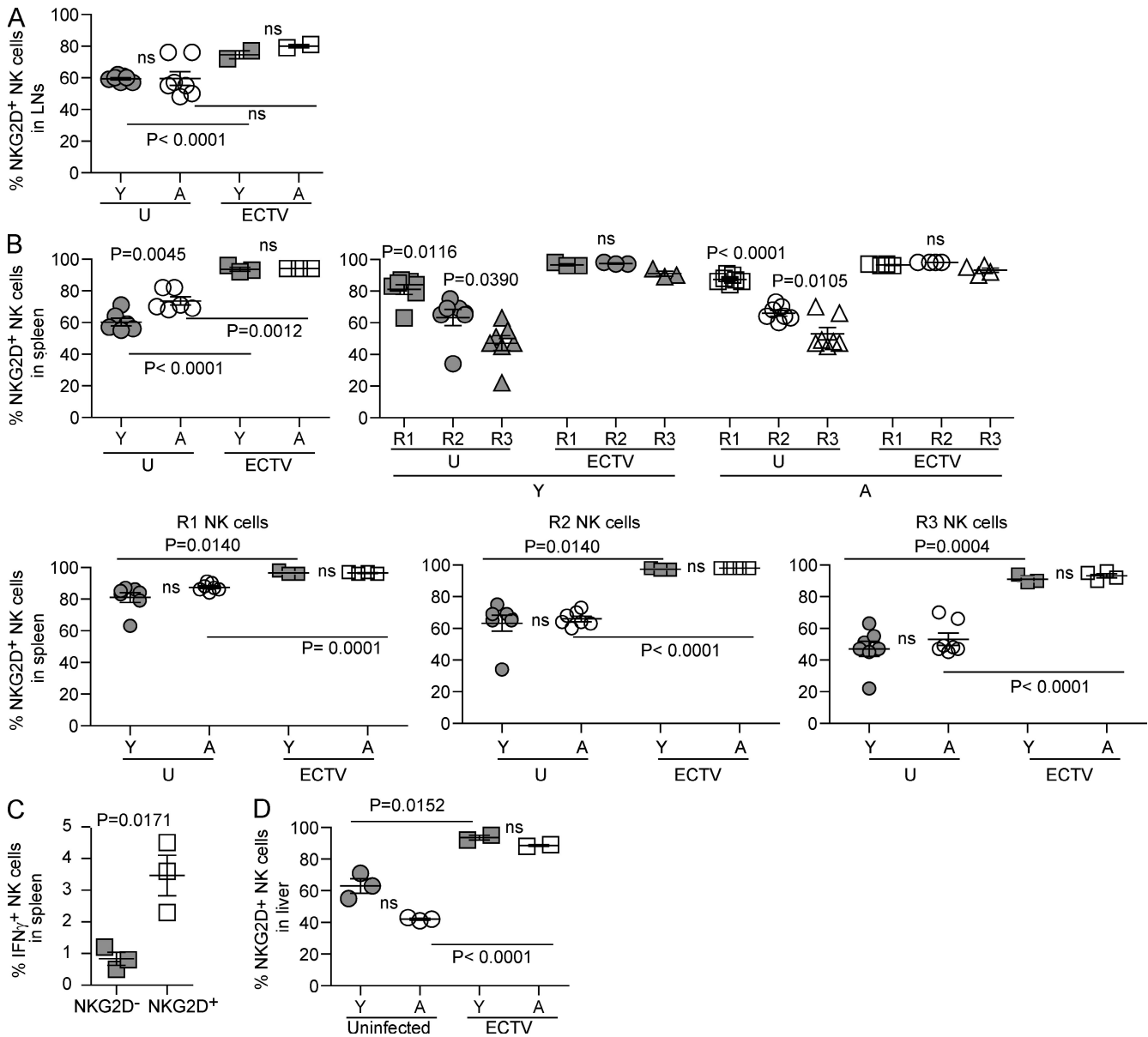


Figure S2. Aged mice are not different from young mice in terms of NKG2D expression under normal conditions or during infection. Young or aged mice were left uninfected or infected with WT ECTV. (A) 2-dpi groups of mice were euthanized, and the D-LNs were made into single cell suspensions and stained for CD3, NK1.1, and NKG2D. The proportion of CD3⁺NK1.1⁺ cells that expressed NKG2D was determined by flow cytometry. Each symbol in the uninfected groups corresponds to individual mice. Each symbol in the infected groups corresponds to pooled D-LNs from two or three mice. Data are representative of two independent experiments. (B) 5-dpi groups of mice were euthanized, and their spleens were made into cell suspensions, stained for surface CD3, NK1.1, CD27, and CD11b, and analyzed by flow cytometry. The same data are analyzed in several ways to allow for the various comparisons. Top left, NKG2D in all NK cells young versus aged in uninfected or ECTV infected. Top right, R1 versus R2 versus R3 in uninfected or ECTV-infected young or aged mice. Bottom, NKG2D expression in R1 (left), R2 (center), or R3 (right) NK cells in young versus aged uninfected or ECTV-infected mice. (C) The NK cells in the young groups in B were analyzed for IFN- γ expression by intracellular staining. (D) As in A, but with lymphocytes recovered from the livers by percoll gradient centrifugation 5 dpi. Data correspond to three to five individual mice from infected mice and six to eight individual naive mice from each group. In all panels, the horizontal bar represents the mean and the vertical bars the SEM.