SUPPLEMENTAL MATERIAL

Brien et al., http://www.jem.org/cgi/content/full/jem.20090222/DC1

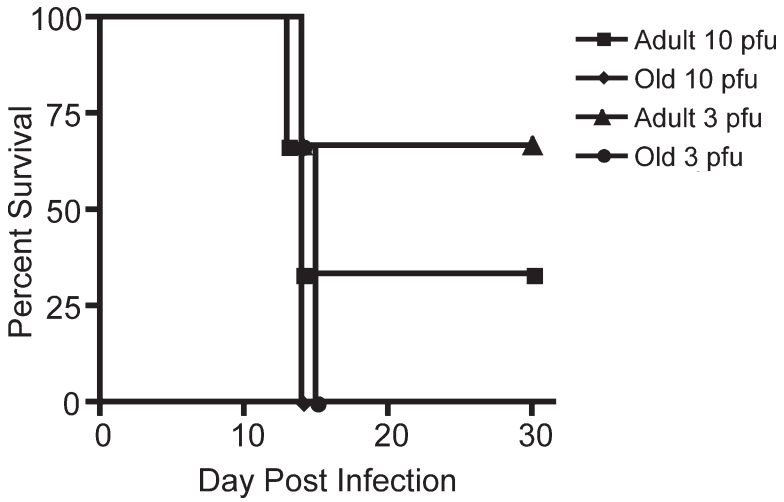

Figure S1. Survival of adult and old BALB/c mice infected with WNV. Adult and old BALB/c mice were infected with strain NY99 i.p. at 10 or 3 PFU/animal ($n = 3$). At both doses, adult mice survived at a higher frequency then their old counterparts. The experiment was repeated three times with comparable results.

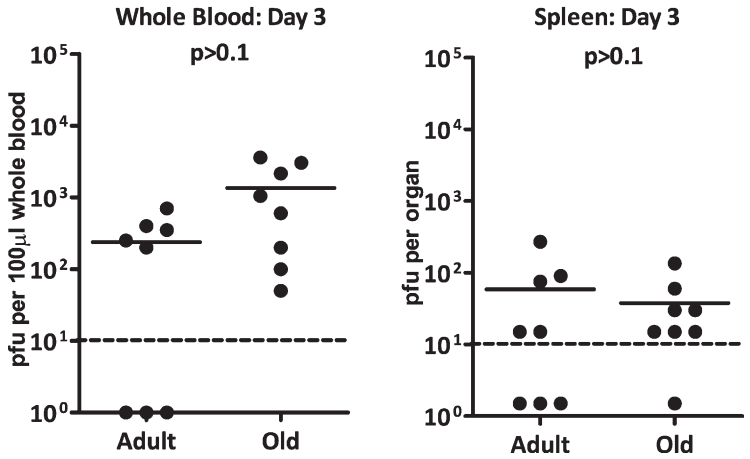

Figure S2. Viral titer in the organs of adult and old mice at indicated days after administration of a WNV dose lethal to old but not adult mice. Adult and old mice ($n = 8$) were given 1,200 PFU WNV 385–99 s.c. and sacrificed at the indicated days after infection, and viral titer in blood and spleen was determined by plaque assay and shown to be statistically equivalent ($P > 0.1$ and $P > 0.7$, respectively). Results are representative of two experiments and illustrate that systemic WNV titers do not differ between adult and old mice early after infection and do not correlate to the disease outcome. The dashed line represents the limit of detection for the assay. Horizontal bars indicate mean values.

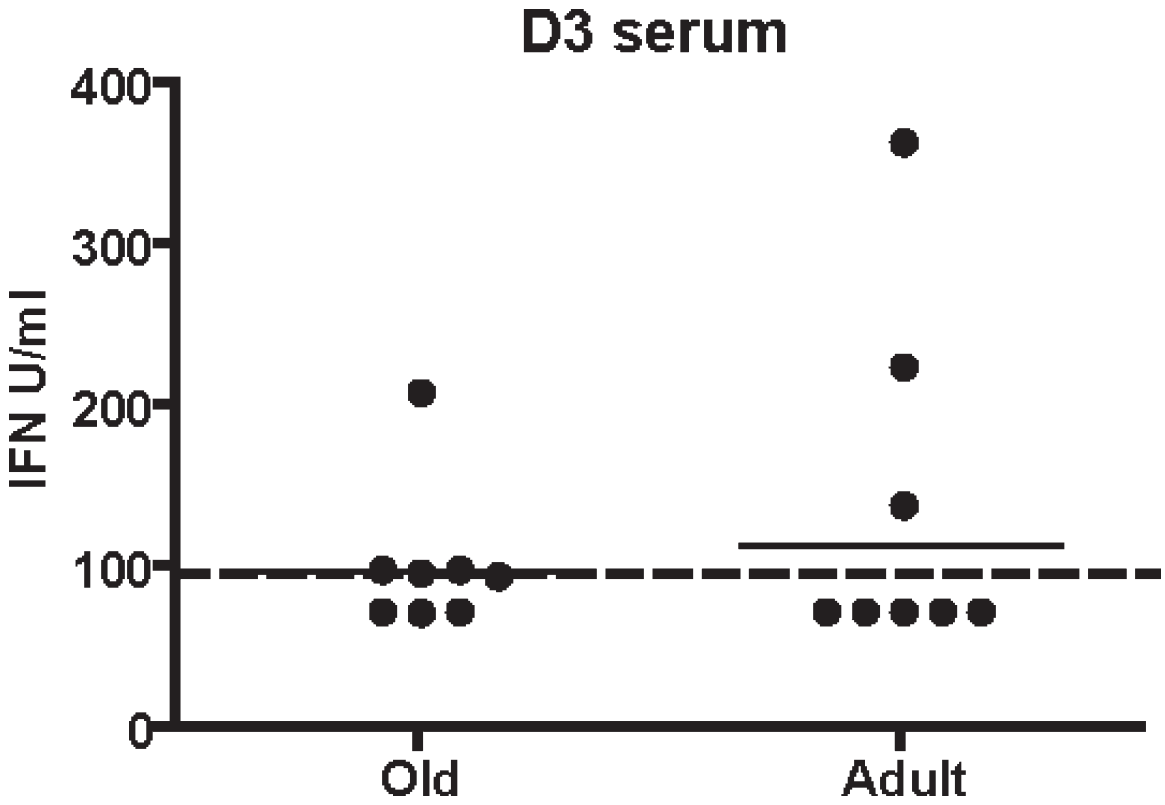

Figure S3. IFN-I activity in the serum of old and adult mice. Old and adult mice were infected s.c. with WNV at a dose that caused decreased survival of old mice compared with adult animals. These mice were bleed 3 d after infection, blood was allowed to clot and then spun down, and serum was kept at −80. A VSV-based bioassay for mouse IFN-I was completed using this serum. Sensitivity of the assay in our hands does not allow us to make categorical claims; however, within its limits, it does not appear that there are major defects in IFN-I production in old animals. Results are representative of two independent experiments. Horizontal bars indicate mean values. The dashed line indicates limit of detection.

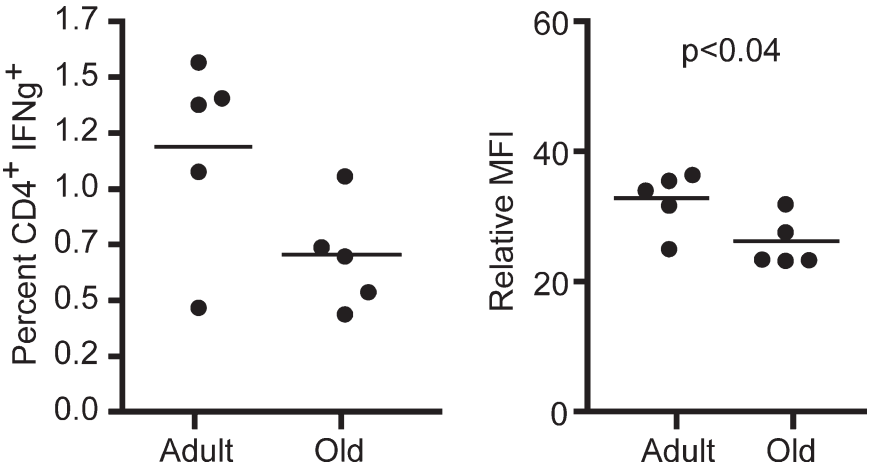

Figure S4. Response of adult and old CD4 T cells to class II MHC-restricted WNV peptide epitopes. B6 mice were infected with 600 PFU/mouse WNV 31 s.c. and responses analyzed on day 7 using immunodominant $NS3_{2066}$ and ENV_{641} peptides for brief in vitro stimulation. In this experiment, old mice showed a trend toward mobilizing fewer antigen-specific $CD4^+$ T cells than adult mice ($P < 0.09$) and their CD4 T cells produced significantly less IFN-γ per cell than CD4 T cells from adult mice ($P < 0.04$). In other experiments, these and other measures of the response were found to be reduced in old mice, sometimes significantly and sometimes not, depending on the size of the experimental groups. However, old animals always showed a trend toward lower responses compared with adults (in 12/12 experiments). Horizontal bars indicate mean values.

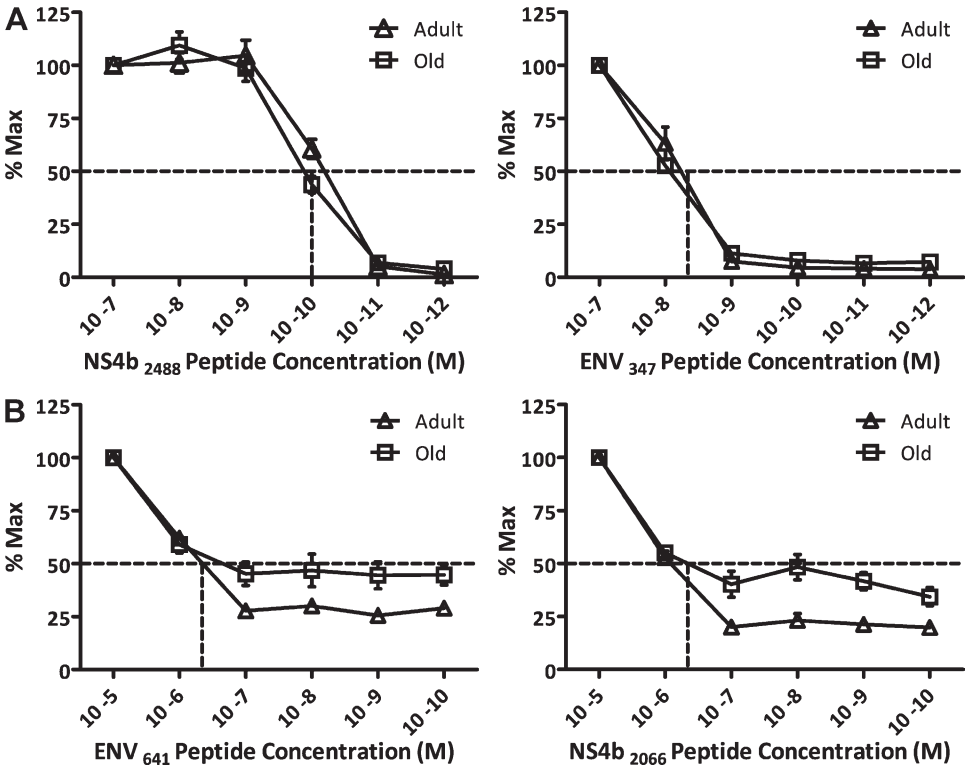

Figure S5. Peptide sensitivity of 8-d-old and adult CD8 and CD4 T cells is largely superimposable. 8 d after WNV 385–99 infection s.c. (dose that produces increased mortality in old animals), splenic CD8 and CD4 T cells were isolated and tested for the ability to produce IFN-γ upon brief (6 h) stimulation with decreasing doses of indicated CD8 (A) or CD4 (B) peptides. Results were normalized to 100% values, taken as those obtained at the highest peptide concentrations. CD8 cells showed no difference in peptide sensitivity. CD4 cells also showed largely superimposable values in the linear phase and their 50% stimulation points were identical; however, there was slightly higher response by the adult cells at lower peptide concentrations. Results depict 12 animals/group from two independent experiments. The dashed lines indicate 50% of the maximum response. Error bars represent SEM.

Table S1. Old mice exhibit increased WNV mortality regardless of route of injection or viral isolate

Mouse group	Virus strain	Virus dose (route)	Live/total	MST	Fisher exact test p-value	Chi-squared test p-value
				days		
Intraperitoneal infection						
Adult	NY99	20 pfu (i.p.)	0/5	14.5	ND	ND
Old	NY99	20 pfu (i.p.)	0/5	13.5	ND	ND
Adult	NY99	10 pfu (i.p.)	0/3	14	ND	ND
Old	NY99	10 pfu (i.p.)	0/3	13	ND	ND
Adult	NY99	6 pfu (i.p.)	9/20	18.5	ND	ND
Old	NY99	6 pfu (i.p.)	4/11	14.5	ND	ND
Adult	NY99	3 pfu (i.p.)	19/20	U	ND	ND
Old	NY99	3 pfu (i.p.)	5/21	14.5	ND	ND
Adult	NY99	1 pfu (i.p.)	20/21	U	ND	ND
Old	NY99	1 pfu (i.p.)	5/21	15	ND	ND
Adult	NY99	0.5 pfu (i.p.)	21/22	U	ND	ND
Old	NY99	0.5 pfu (i.p.)	13/20	15	ND	ND
Subcutaneous infection						
Adult	NY99	80 pfu (s.c.)	10/10	U	0.0006	0.00039
Old	NY99	80 pfu (s.c.)	1/7	12	0.0006	0.00039
Adult	NY99	40 pfu (s.c.)	8/10	U	0.023	0.0176
Old	NY99	40 pfu (s.c.)	2/9	12	0.023	0.0176
Adult	NY99	20 pfu (s.c.)	10/10	U	0.0031	0.004
Old	NY99	20 pfu (s.c.)	3/9	12	0.0031	0.004

B6 mice of indicated ages (adult, 4–6 mo; old, 19–20 mo) were infected as described above and in Materials and methods. Survival was scored over 45 d. i.p. results are pooled from three independent experiments, whereas s.c. results come from two independent experiments. Statistical significance is denoted for the s.c. groups along with the tool used for calculation. U, undefined.

[AQ2]