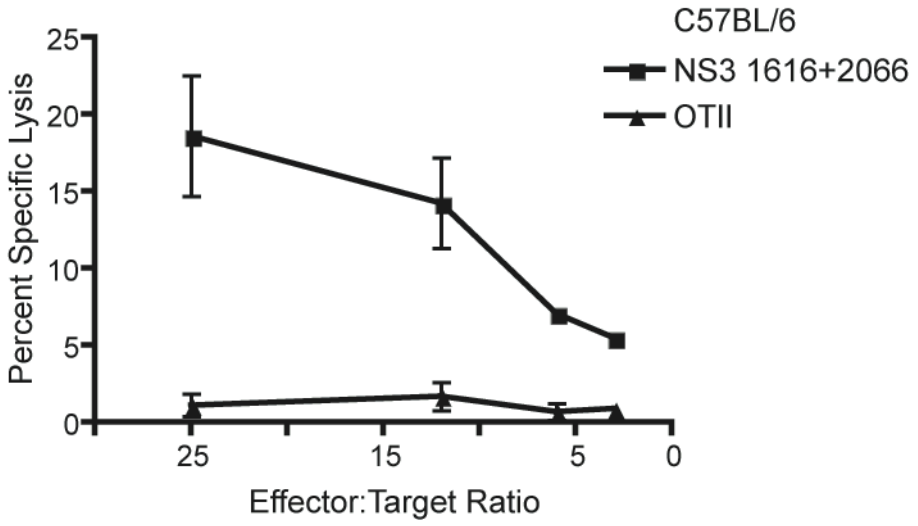
Supplemental Data

Supplemental Table 2

Day Post Infection	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
# of mice	N=4	N=3	N=4	N=5	N=2
Anti-CD3	1.16	1.14	0.52	1.33	0.91
E646-660	ND	0.32	ND	0.29	0.16
E641-655	0.51	0.33	0.10	0.41	0.27
NS3 1616-1630	0.32	0.50	0.15	0.30	0.14
1617-1627	ND	0.11	ND	ND	0.08
1618-1628	ND	0.10	ND	0.17	0.08
1619-1629	ND	0.15	ND	0.28	0.37
1620-1630	ND	0.16	ND	ND	ND
NS3 2066-2080	0.53	0.41	0.41	0.49	0.43
2068-2078	ND	0.25	ND	0.24	ND
2070-2080	ND	0.20	0.01	0.34	0.08

Supplemental Table 2. List of truncated CD4 T cell peptides. This table lists the truncated CD4 T cell peptides used to determine the optimal epitope. The optimal epitope is shown in bold. Responses were determined by ICCS for IFNγ production by splenocytes on day 7 post infection, as described in methods, and are represented as in Table 1. ND-not determined

Supplemental Figure 1



Supplemental Figure 1. Direct *ex vivo* cytotoxicity of peptide pulsed targets by purified CD4 T cells. **A**. Purified CD4 T cells were immediately used in a 6-hour ^{51}Cr release assay with peptide-pulsed target cells (IC21). IC21 cells were peptide pulsed (10-6M) with either a combination of NS3 1616+ NS32066 or OTII control peptide. Results represent the average of 4 mice, and are representative of two independent experiments.

Supplemental methods

$CD4^+$ T cells were isolated from spleens of B6 mice 7 days post-infection with WNV, by negative selection using anti-CD8, B220, NK1.1-coated beads (Miltenyi Biotec). $CD4^+$ T cells were isolated at 80-95% purity. Direct ex vivo CTL activity was determined using radioactively labeled peptide-coated IC21 cells as targets. 1x10^5 IC21 cells (1x10^4 cells per well) were pulsed with ^{51}CR and peptide overnight in a 96 well plate. Cells were washed 3 times with warm media (5% FBS + RPMI) and purified CD4 T cells were serially diluted, then placed into the 96 well plate at indicated effector to target ratio for six hours. After six hours 30µl of supernatant was removed and added to a lumaplate (Packard Co). Radioactivity was measured using TopCount Packard δ/γ radioactivity reader (Packard Co). Percent specific lysis was calculated as [(E - S)/(M - S)] times 100, where E equals the counts per minute released from targets incubated with lymphocytes, S equals the counts per minute released from target cells incubated with no lymphocytes and M equals the counts per minute released from cells after lysis with 1% Nonidet P40 (USB).