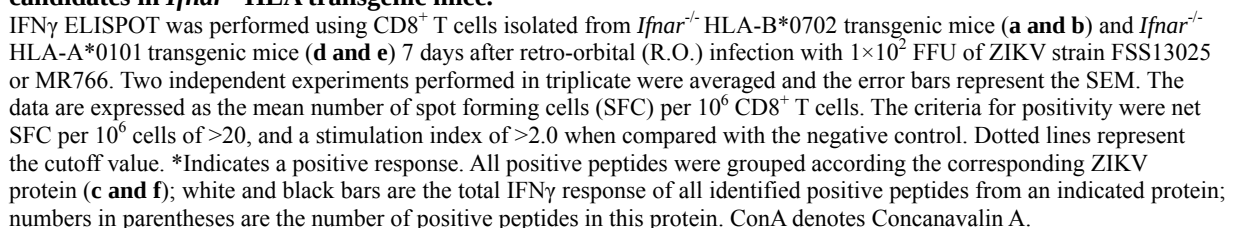
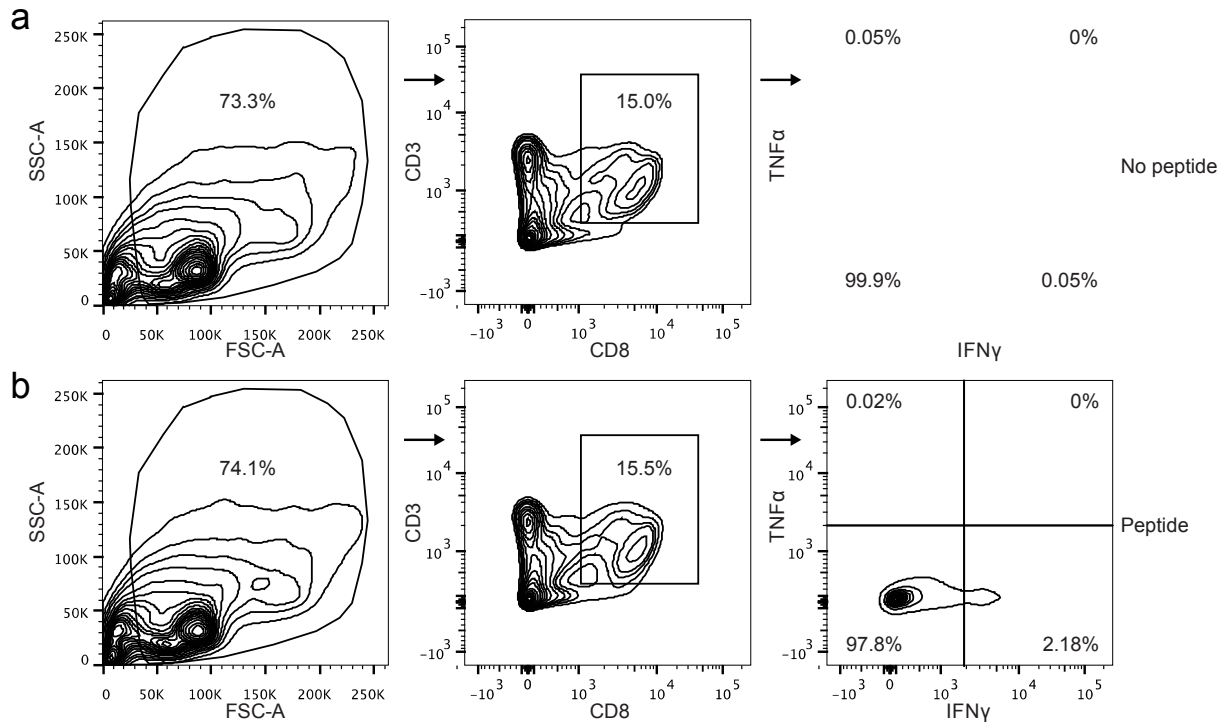


In the format provided by the authors and unedited.

Identification of Zika virus epitopes reveals immunodominant and protective roles for dengue virus cross-reactive CD8⁺ T cells

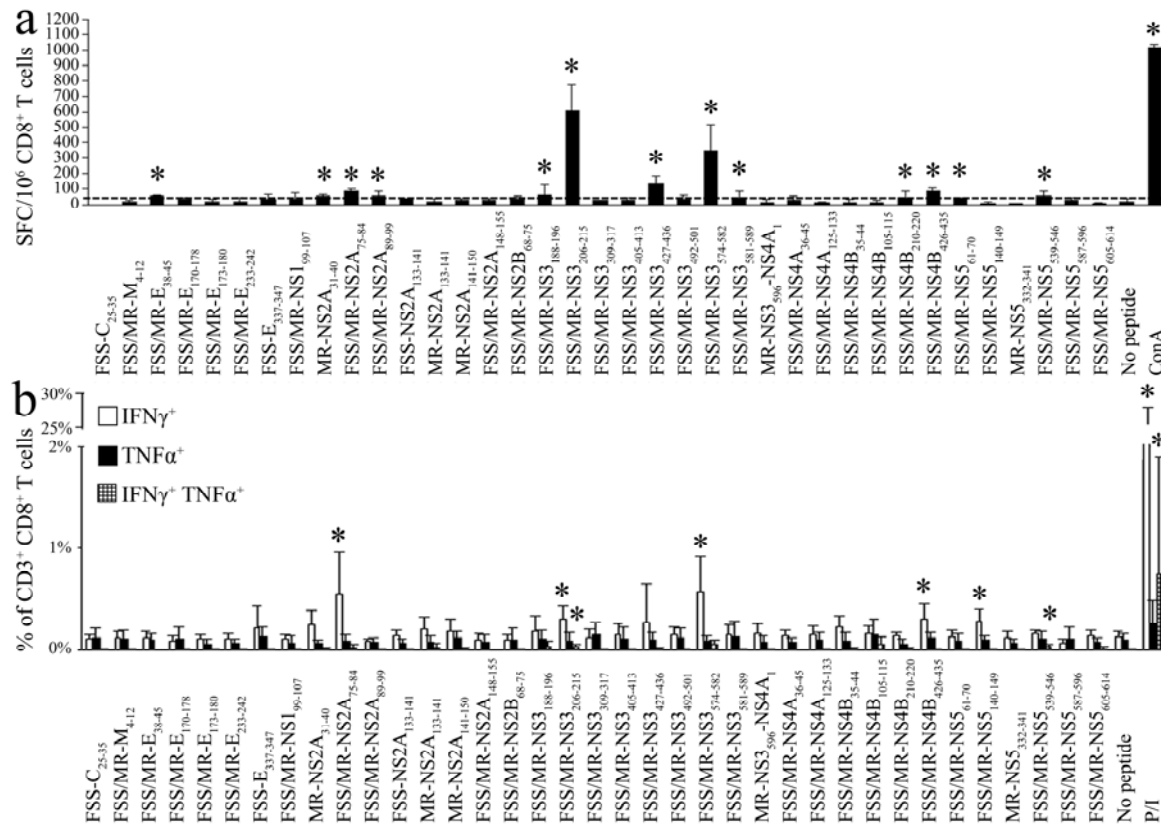
Jinsheng Wen, William Weihao Tang, Nicholas Sheets, Julia Ellison, Alessandro Sette, Kenneth Kim and Sujan Shresta





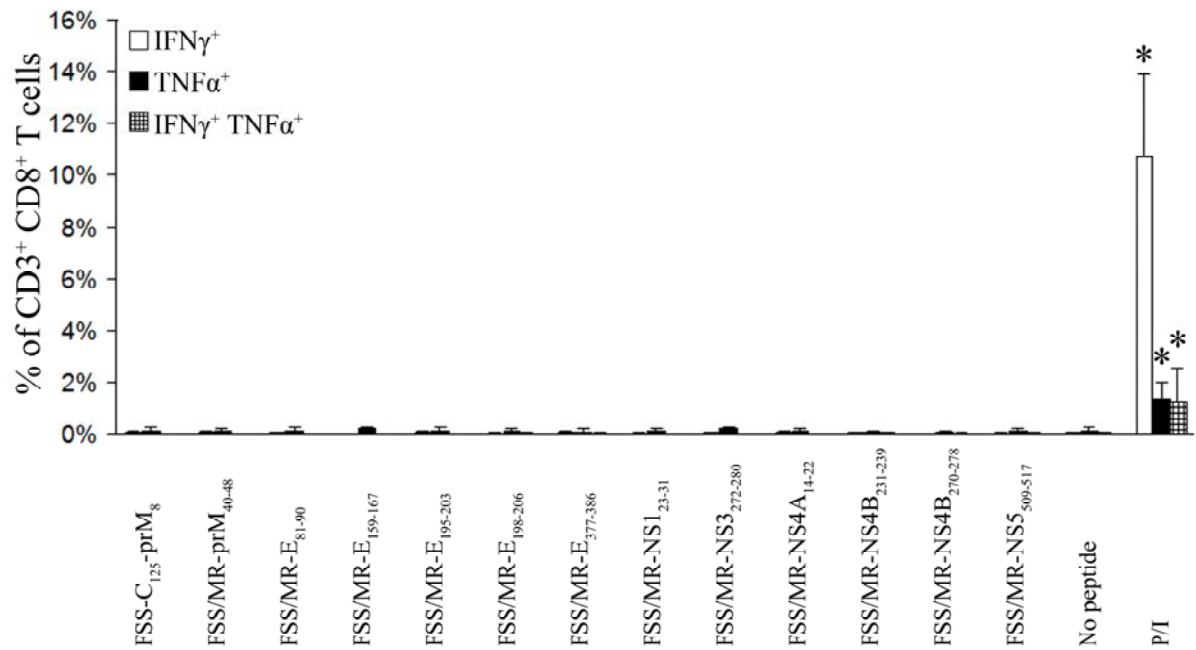
Supplementary Figure 2. Representative ICS data confirming CD8⁺ T cell production of IFN γ upon stimulation with an epitope identified via IFN γ ELISPOT.

Splenocytes isolated from *Ifnar*^{-/-} HLA-B*0702 transgenic mice and *Ifnar*^{-/-} HLA-A*0101 transgenic mice 7 days after R.O. infection with 1×10^2 FFU of ZIKV strain FSS13025 or MR766 were stimulated with each of the 37 positive HLA-B*0702-binding peptides or 13 positive HLA-A*0101-binding peptides identified via IFN γ ELISPOT and then ICS assay was performed. A representative flow cytometric figure shows the CD3⁺ CD8⁺ T cell gate and the percentages of IFN γ ⁺ and/or TNF α ⁺ cells (**a and b**).



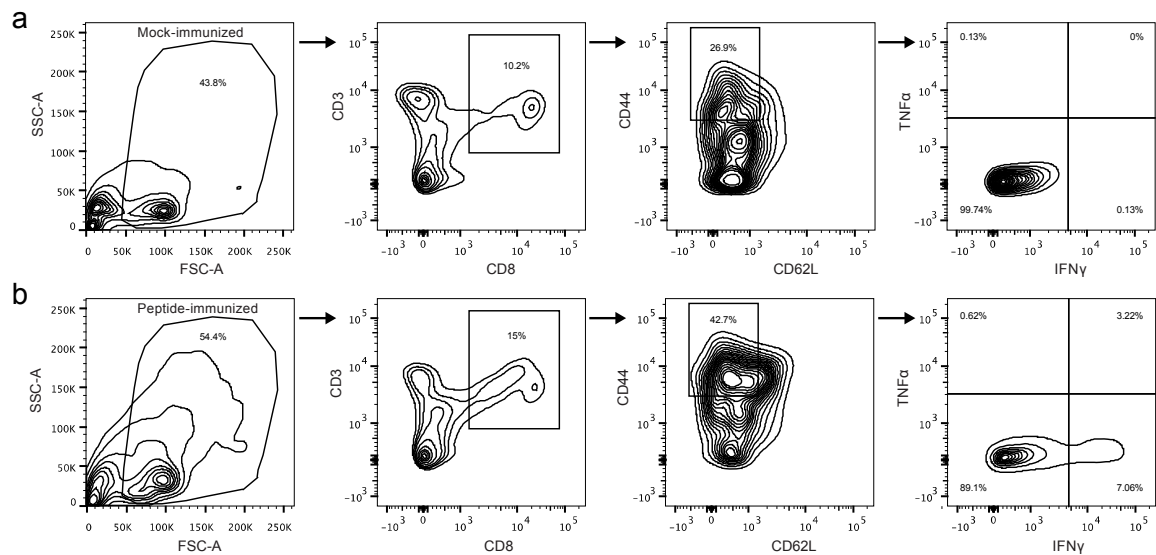
Supplementary Figure 3. Identification of HLA-B*0702-restricted ZIKV epitopes that are cross-reactive with DENV.

Ifnar^{-/-} HLA-B*0702 transgenic mice were infected R.O. with 2×10⁴ FFU of DENV2 strain S221 for 7 days. CD8⁺ T cells isolated from splenocytes were stimulated with each of the 37 ZIKV epitopes identified via IFNγ ELISPOT to perform IFNγ ELISPOT (a) and ICS assay (b). Data represent the average of two independent IFNγ ELISPOT experiments and two independent ICS experiments and are expressed as mean ± SEM. Dotted line corresponds to the cutoff value. *, *P* < 0.05. Two-tailed Mann-Whitney test. See Supplementary Tables 2 and 3 for exact *n* and *P* values.



Supplementary Figure 4. Identification of ZIKV HLA-A*0101-restricted epitopes that are cross-reactive with DENV.

Ifnar^{-/-} HLA-A*0101 transgenic mice were infected R.O. with 2×10^4 FFU of DENV2 strain S221 for 7 days. CD8⁺ T cells isolated from splenocytes were stimulated with each of the 13 ZIKV epitopes identified via IFN γ ELISPOT to perform ICS assay. Data are expressed as mean $\pm$ SEM. See Supplementary Tables 2 and 3 for exact *n* and *P* values.



Supplementary Figure 5. Representative flow cytometric data showing IFN γ ⁺ and/or TNF α ⁺ cells in peptide-immunized mice.

Mock-immunized mice and peptide-immunized mice were challenged R.O. with 1×10^4 FFU of ZIKV FSS13025 for 3 days. Representative flow cytometric data show how CD3⁺ CD8⁺ CD44⁺ CD62L⁻ T cells were gated and the percentages of IFN γ ⁺ and/or TNF α ⁺ cells in mock- (**a**) and peptide-immunized (**b**) mice were determined by ICS.

Supplementary Table 1. ZIKV/DENV cross-reactive peptides and their variants in DENV2.

Peptides ^a	Sequences ^b	Conservation ^c	Reference
FSS/MR766-NS2A ₇₅₋₈₄	RPALLVSFI F	20%	(21)
DENV2-NS2A ₇₄₋₈₃	RPTFAAGLLL		
FSS/MR766-NS3 ₂₀₆₋₂₁₅	APTRVVA AEM	100%	(21)
DENV2-NS3 ₂₀₅₋₂₁₄			
FSS/MR766-NS3 ₅₇₄₋₅₈₂	KPRWMDAR V	78%	(21)
DENV2-NS3 ₅₇₅₋₅₈₃	KPRWLDAR I		
FSS/MR766-NS4B ₄₂₆₋₄₃₅	RPGAF CIKVL	60%	
DENV2-NS4B ₄₂₃₋₄₃₂	NTQ FCIKVL		
FSS/MR-NS5 ₅₃₉₋₅₄₆	VPTGR TTW	88%	
DENV2-NS5 ₅₃₈₋₅₄₅	VPTS RTTW		
FSS/MR766-NS5 ₁₄₀₋₁₄₉	RPRV CTKEEF	70%	(21)
DENV2-NS5 ₁₄₀₋₁₄₉	TPRM CTREEF		
FSS/MR-NS3 ₁₈₈₋₁₉₆	LP EIVRE AI	89%	(21)
DENV2-NS3 ₁₈₇₋₁₉₅	LPAIVRE AI		
MR766-NS2A ₃₁₋₄₀	VVMILGGF SM	40%	
DENV2-NS2A ₃₁₋₄₀	VT LITGN MSF		
FSS/MR-NS2A ₈₉₋₉₉	TPRES M L LAL	30%	
DENV2-NS2A ₈₇₋₉₇	TSKEL M MTT I		
FSS/MR-NS3 ₄₂₇₋₄₃₆	GPMPVTH ASA	90%	
DENV2-NS3 ₄₂₈₋₄₃₇	GPMPVTH SSA		
FSS/MR-NS4B ₂₁₀₋₂₂₀	S PNKYW NSST A	36%	
DENV2-NS4B ₂₀₈₋₂₁₈	N PGR F WN TTI A		
FSS/MR-NS5 ₆₁₋₇₀	APTQ GSASS L	50%	
DENV2-NS5 ₆₁₋₇₀	TKQT GSASSM		
FSS/MR-E ₃₈₋₄₅	KPTVD IEL	75%	
DENV2-E ₃₈₋₄₅	KPTLD FEL		
FSS/MR-NS3 ₅₈₁₋₅₈₉	RVCSD HAAL	56%	
DENV2-NS3 ₅₈₂₋₅₉₀	R I YSD PLAL		

^a ZIKV peptides in **bold** are positive as determined via both IFNγ ELISPOT and ICS assays in DENV2-infected mice.

^b Amino acid residues in **red** are conserved between ZIKV peptide and DENV2 variant.

^c % shared amino acids between ZIKV and DENV2.