Supplementary Information for

An RNA nanoparticle vaccine against Zika virus elicits antibody and CD8+ T cell responses in a mouse model

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Table S1. Peptide pools tested in initial screen for CD8-stimulating epitopes.

| Pool # | Peptide # | Sequence          |
|--------|-----------|-------------------|
| Pool 1 | 1         | MGADTVGIVGLLLT    |
| Pool 2 | 9         | LDRDAGEAISFPTT    |
| Pool 3 | 17        | DATMSYECPLMDEGV   |
| Pool 4 | 25        | YGTCHHKGKEARRSR   |
| Pool 5 | 33        | QTWLESREYKHLIR    |
| Pool 1 | 2         | TSVGIVGLLLTTAMA   |
| Pool 2 | 10        | DAGEAISFPTTLMGN   |
| Pool 3 | 18        | SYECPMLDEGVEPDD   |
| Pool 4 | 26        | HHKKEARRSRARRAVT  |
| Pool 5 | 34        | ESREYTKHLIRVENW   |
| Pool 1 | 3         | IVGLLTTAMAAEVT    |
| Pool 2 | 11        | AISFPTTLMNKNCYI   |
| Pool 3 | 19        | PMLDEGVEPDDVCW    |
| Pool 4 | 27        | GARRSRRAVTLPSH    |
| Pool 5 | 35        | YTKHLIRVENWIFRN   |
| Pool 1 | 4         | LLTTAMAAEVT       |
| Pool 2 | 12        | PTTLGMNKCYIQIMD   |
| Pool 3 | 20        | EVDVDCWNCNTTSTWV  |
| Pool 4 | 28        | RSRAVTLPSSHSTRK   |
| Pool 5 | 33        | QTWLESREYKHLIR    |
| Pool 1 | 5         | AMAAEVTRRGSAYYM   |
| Pool 2 | 13        | GNMKCYIQIMDLGHT   |
| Pool 3 | 21        | GHTCDATMSYECPLM   |
| Pool 4 | 29        | A/VTLPSHSTRKLQTR  |
| Pool 5 | 34        | ESREYTKHLIRVENW   |
| Pool 1 | 6         | EVTRRGSAYYMAYLDR  |
| Pool 2 | 14        | CYIQIMDLGHTCDAT   |
| Pool 3 | 22        | NTTSWVYGTCHHK     |
| Pool 4 | 30        | PSRARRATLPSHSTRK  |
| Pool 5 | 35        | YTKHLIRVENWIFRN   |
| Pool 1 | 7         | RGSAYYMYLDRNDAG   |
| Pool 2 | 15        | IMDLGHTCDATMSYE   |
| Pool 3 | 23        | TWVYGTCHHKKGAE    |
| Pool 6 | 36 | LIRVENWIFRNPGFA |
| Pool 6 | 37 | ENWIFRNPGFALAAA |
| Pool 6 | 38 | FRNPGFALAAAAIAW |
| Pool 6 | 39 | GFALAAAAIAWLLGS |
| Pool 6 | 40 | AAAIAWLLGSSTSQ |

| Pool 6 | 41 | IAWLLGSSTSQKVIY |
| Pool 6 | 42 | LGSTSQKVIYLVMI |
| Pool 6 | 43 | TSQKVIYLVMLLIA |
| Pool 6 | 44 | VIYLVMLIIAPAYS |
| Pool 6 | 45 | VMILLIAPAYSIRCI |
| Pool 6 | 46 | LIAPAYSIRCIGVSN |
| Pool 6 | 47 | AISIRCIGVSNRDFV |
| Pool 6 | 48 | RCIGVSNRDFVEGMS |

| Pool 7 | 49 | VSNRDVFEGMSGGTW |
| Pool 7 | 50 | DFVEMGSSTGWDDV |
| Pool 7 | 51 | GMSGTWVDVLEHG |
| Pool 7 | 52 | GTWVDVLEHGCVT |
| Pool 7 | 53 | DVWLDHGCCTVMAQ |
| Pool 7 | 54 | EHGCCTVMAQDKPT |
| Pool 7 | 55 | CVTMAQDKPTVDIE |
| Pool 7 | 56 | MAQDKPTVDIELVT |

| Pool 7 | 57 | KPTVDIELVTTTVSN |
| Pool 7 | 58 | DIELVTTVSNMAEV |
| Pool 7 | 59 | VTTVSNAEVRSYIC |
| Pool 7 | 60 | VSNMAEVRSCYEAS |
| Pool 7 | 61 | AEVRSYEASISDM |
| Pool 7 | 62 | SYEASISDMASDS |
| Pool 7 | 63 | EASISDMASDSRCPT |
| Pool 7 | 64 | DMASDSRCPTQGAE |

| Pool 8 | 65 | SDSRCPTQGEAYLDK |
| Pool 8 | 66 | CPTQGEAYLDKQSDT |
| Pool 8 | 67 | GEAYLDKQSDTQYVC |
| Pool 8 | 68 | LDQSDTQYVCCKRTL |
| Pool 8 | 69 | SDTQYVCKRTLVDGR |
| Pool 8 | 70 | YVCKRTLVDGRGWNG |
| Pool 8 | 71 | RTLVDRGWNGCGLGF |
| Pool 8 | 72 | DRGWNGCGLFGKGS |

| Pool 9 | 73 | GNGCGLFGKSGLVC |
| Pool 9 | 74 | GLFGKSGLVTACKFA |
| Pool 9 | 75 | KGSLVTCAKFACS KK |
| Pool 9 | 76 | VTCAKFACS KMTGK |
| Pool 9 | 77 | KFACS SKMTGKSIQP |
| Pool 9 | 78 | SKMTGKSIQPENLE |
| Pool 9 | 79 | TGKSIQPENLEYRIM |
| Pool 9 | 80 | IQOPENLEYRIMLSVH |

| Pool 10 | 81 | NLEYRIMLSVHGSQH |
| Pool 10 | 82 | RIMLSVHGSQSOMI |
| Pool 10 | 83 | SVHGSQHSMIVNDT |
| Pool 10 | 84 | SQHSMIVNDTGHE T |
| Pool 10 | 85 | GMIVNDTGHTDENR |
| Pool 10 | 86 | NDTGHTDENRAKVE |
| Pool 10 | 87 | HETDENRAKVEITPN |
| Pool 10 | 88 | ENRAKVEITPNSPRA |

| Pool 11 | 89 | KVEITPNSPRAEATL |
| Pool 11 | 90 | TPNSPRAEATLGGFG |
| Pool 11 | 91 | PRAEATLGGFGSLGL |
| Pool 13 |
|---|
| 92 | ATLGGFGSLGLDCEP |
| 93 | GFGSLGLDCEPRTGL |
| 94 | LGLDCEPRTGGLDSD |

| Pool 14 |
|---|
| 95 | CEPRTGLDFSGLLYYL |
| 96 | TGLDFSGLLYYLMTNN |
| 97 | FSDLYYLTMNKHWNL |
| 98 | YLYTLMNKHHLVHLK |
| 99 | MNKHHLVHKEWFHD |
| 100 | HLVHKEWFHIDILPL |
| 101 | HKEWFHIDILPWHAG |
| 102 | FHDILPWHAGADTG |

| Pool 15 |
|---|
| 103 | PLPWHAGADTGTPHW |
| 104 | HAGADTGTPWHNNKE |
| 105 | DTGTPWHNNKEALVE |
| 106 | PHWNKEALVEFKDA |
| 107 | NKEALVEFKDAHAKR |
| 108 | LVEFKDAHAKRQTVV |
| 109 | KDAHAKRQTVVVLGS |
| 110 | AKRQTVVVLGSQEGA |

| Pool 16 |
|---|
| 111 | TVVVLGSQEGAVHTA |
| 112 | LGSQEGAVHTALAGA |
| 113 | EAGAVHTALAGALEAE |
| 114 | HTALAGALEAEEMDGA |
| 115 | AGALEAEEMDGAKGRL |
| 116 | EAEMDAGKRLSSGH |
| 117 | DGAKRGLSSGHDLKCR |
| 118 | GRLSSGHLKCRKLMD |

| Pool 17 |
|---|
| 119 | SGHLKCRKLKDMLRL |
| 120 | KCRLKMDKLRLKGVLS |
| 121 | KMDKRLRGVSYSILC |
| 122 | LRLKGVSYSILCTAAT |
| 123 | GVSYSLCTAATFVT |
| 124 | SLCTAATFFKIPAE |
| 125 | AAFTFKIPELATLHG |
| 126 | FTKIPAEATLHGTVT |

| Pool 18 |
|---|
| 127 | PAETLHGTGVTVQY |
| 128 | LHGTGVTVQYAGTD |
| 129 | VTVEVQYAGTDGCK |
| 130 | QYAGTDGCKVFAQ |
| 131 | GTDGCPVKPAQMAD |
| 132 | PCKVPAQMADVMQTL |
| 133 | PAQAMAVDQMQLTPVG |
| 134 | AVDMQTLTPVGRSL |

| Pool 19 |
|---|
| 135 | QTLTPVGRSLTANPV |
| 136 | PVGRSLTANPVITES |
| 137 | LITANPVITESTENS |
| 138 | NPVITESTENSKML |
| 139 | TESTENSKMMELEDP |
| 140 | ENSKMMLELDPPFGD |
| 141 | MLELDPPFGDSYIV |
| 142 | LDPPFGDSYIVIGVG |

| Pool 19 |
|---|
| 143 | FGDSYIVIGVGEKIK |
| 144 | YIVIVGVEKKITHHW |
| 145 | GVGEKKITHWHHRSIG |
| 146 | KITHWHHRSYSTIG |
| 147 | HWHRSGSTIGKAFE |
148 RSGSTIGKAFEATVR
149 TIGKAFEATVRGAKR
150 AFEATVRGAKRMAVL
Figure S1. Modified dendrimer nanoparticle (MDNP) size distribution. MDNP vaccines suspended in PBS were characterized by dynamic light scattering and the diameters by intensity are shown. N = 3 and error bars represent SD.
Figure S2. Screening of additional ZIKV E protein-derived peptides for activation of CD8+ T cells from a single ZIKV-immunized mouse. Splenocytes from a mouse vaccinated with RNA nanoparticle vaccine against the ZIKV E gene were stimulated ex vivo with 0.2 μg/ml of the indicated peptide. After 7 hrs. of culture, intracellular cytokine staining for IFNγ was performed.
Figure S3. Raw image data for immunoblots for figures 1b (left) and 1c (right). Original, unaltered scans of films developed after chemiluminescence reactions of the indicated blots, with entire membrane lengths shown.
Figure S4. Representative HPLC and LC-MS characterization of ZIKV-related peptides synthesized to validate the T cell epitopes IGVSNRDFV and IPAYSIRCIGV. (a) HPLC trace and mass of desired peak corresponding to IGVSNRDFV. (b) HPLC trace and mass of desired peak corresponding to IPAYSIRCIGV.