**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 | MGADTSVGIVGLLLT |
| | 2 | TSVGIVGLLLTTAMA |
| | 3 | IVGLLLTTAMAAEVT |
| | 4 | LLTTAMAAEVTRRGS |
| | 5 | AMAAEVTRRGSAYYM |
| | 6 | EVTRRGSAYYMYLDR |
| | 7 | RGSAYYMYLDRNDAG |
| | 8 | YYMYLDRNDAGEAIS |
| Pool 2 | 9 | LDRNDAGEAISFPTT |
| | 10 | DAGEAISFPTTLGMN |
| | 11 | AISFPTTLGMNKCYI |
| | 12 | PTTLGMNKCYIQIMD |
| | 13 | GMNKCYIQIMDLGHT |
| | 14 | CYIQIMDLGHTCDAT |
| | 15 | IMDLGHTCDATMSYE |
| | 16 | GHTCDATMSYECPML |
| Pool 3 | 17 | DATMSYECPMLDEGV |
| | 18 | SYECPMLDEGVEPDD |
| | 19 | PMLDEGVEPDDVDCW |
| | 20 | EGVEPDDVDCWCNTT |
| | 21 | PDDVDCWCNTTSTWV |
| | 22 | DCWCNTTSTWVVYGT |
| | 23 | NTTSTWVVYGTCHHK |
| | 24 | TWVVYGTCHHKKGEA |
| Pool 4 | 25 | YGTCHHKKGEARRSR |
| | 26 | HHKKGEARRSRRAVT |
| | 27 | GEARRSRRAVTLPSH |
| | 28 | RSRRAVTLPSHSTRK |
| | 29 | AVTLPSHSTRKLQTR |
| | 30 | PSHSTRKLQTRSQTW |
| | 31 | TRKLQTRSQTWLESR |
| | 32 | QTRSQTWLESREYTK |
| Pool 5 | 33 | QTWLESREYTKHLIR |
| | 34 | ESREYTKHLIRVENW |
| | 35 | YTKHLIRVENWIFRN |

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|---|---|---|
| | 36 | LIRVENWIFRNPGFA |
| | 37 | ENWIFRNPGFALAAA |
| | 38 | FRNPGFALAAAAIAW |
| | 39 | GFALAAAAIAWLLGS |
| | 40 | AAAAIAWLLGSSTSQ |
| Pool 6 | 41 | IAWLLGSSTSQKVIY |
| | 42 | LGSSTSQKVIYLVMI |
| | 43 | TSQKVIYLVMILLIA |
| | 44 | VIYLVMILLIAPAYS |
| | 45 | VMILLIAPAYSIRCI |
| | 46 | LIAPAYSIRCIGVSN |
| | 47 | AYSIRCIGVSNRDFV |
| | 48 | RCIGVSNRDFVEGMS |
| Pool 7 | 49 | VSNRDFVEGMSGGTW |
| | 50 | DFVEGMSGGTWVDVV |
| | 51 | GMSGGTWVDVVLEHG |
| | 52 | GTWVDVVLEHGGCVT |
| | 53 | DVVLEHGGCVTVMAQ |
| | 54 | EHGGCVTVMAQDKPT |
| | 55 | CVTVMAQDKPTVDIE |
| | 56 | MAQDKPTVDIELVTT |
| Pool 8 | 57 | KPTVDIELVTTTVSN |
| | 58 | DIELVTTTVSNMAEV |
| | 59 | VTTTVSNMAEVRSYC |
| | 60 | VSNMAEVRSYCYEAS |
| | 61 | AEVRSYCYEASISDM |
| | 62 | SYCYEASISDMASDS |
| | 63 | EASISDMASDSRCPT |
| | 64 | SDMASDSRCPTQGEA |
| Pool 9 | 65 | SDSRCPTQGEAYLDK |
| | 66 | CPTQGEAYLDKQSDT |
| | 67 | GEAYLDKQSDTQYVC |
| | 68 | LDKQSDTQYVCKRTL |
| | 69 | SDTQYVCKRTLVDRG |
| | 70 | YVCKRTLVDRGWGNG |
| | 71 | RTLVDRGWGNGCGLF |
| | 72 | DRGWGNGCGLFGKGS |
| Pool 10 | 73 | GNGCGLFGKGSLVTC |
| | 74 | GLFGKGSLVTCAKFA |
| | 75 | KGSLVTCAKFACSKK |
| | 76 | VTCAKFACSKKMTGK |
| | 77 | KFACSKKMTGKSIQP |
| | 78 | SKKMTGKSIQPENLE |
| | 79 | TGKSIQPENLEYRIM |
| | 80 | IQPENLEYRIMLSVH |
| Pool 11 | 81 | NLEYRIMLSVHGSQH |
| | 82 | RIMLSVHGSQHSGMI |
| | 83 | SVHGSQHSGMIVNDT |
| | 84 | SQHSGMIVNDTGHET |
| | 85 | GMIVNDTGHETDENR |
| | 86 | NDTGHETDENRAKVE |
| | 87 | HETDENRAKVEITPN |
| | 88 | ENRAKVEITPNSPRA |
| Pool 12 | 89 | KVEITPNSPRAEATL |
| | 90 | TPNSPRAEATLGGFG |
| | 91 | PRAEATLGGFGSLGL |

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|---|---|---|
| | 92 | ATLGGFGSLGLDCEP |
| | 93 | GFGSLGLDCEPRTGL |
| | 94 | LGLDCEPRTGLDFSD |
| Pool 13 | 95 | CEPRTGLDFSDLYYL |
| | 96 | TGLDFSDLYYLTMNN |
| | 97 | FSDLYYLTMNNKHWL |
| | 98 | YYLTMNNKHWLVHKE |
| | 99 | MNNKHWLVHKEWFHD |
| | 100 | HWLVHKEWFHDIPLP |
| | 101 | HKEWFHDIPLPWHAG |
| | 102 | FHDIPLPWHAGADTG |
| Pool 14 | 103 | PLPWHAGADTGTPHW |
| | 104 | HAGADTGTPHWNNKE |
| | 105 | DTGTPHWNNKEALVE |
| | 106 | PHWNNKEALVEFKDA |
| | 107 | NKEALVEFKDAHAKR |
| | 108 | LVEFKDAHAKRQTVV |
| | 109 | KDAHAKRQTVVVLGS |
| | 110 | AKRQTVVVLGSQEGA |
| Pool 15 | 111 | TVVVLGSQEGAVHTA |
| | 112 | LGSQEGAVHTALAGA |
| | 113 | EGAVHTALAGALEAE |
| | 114 | HTALAGALEAEMDGA |
| | 115 | AGALEAEMDGAKGRL |
| | 116 | EAEMDGAKGRLSSGH |
| | 117 | DGAKGRLSSGHLKCR |
| | 118 | GRLSSGHLKCRLKMD |
| Pool 16 | 119 | SGHLKCRLKMDKLRL |
| | 120 | KCRLKMDKLRLKGVS |
| | 121 | KMDKLRLKGVSYSLC |
| | 122 | LRLKGVSYSLCTAAF |
| | 123 | GVSYSLCTAAFTFTK |
| | 124 | SLCTAAFTFTKIPAE |
| | 125 | AAFTFTKIPAETLHG |
| | 126 | FTKIPAETLHGTVTV |
| Pool 17 | 127 | PAETLHGTVTVEVQY |
| | 128 | LHGTVTVEVQYAGTD |
| | 129 | VTVEVQYAGTDGPCK |
| | 130 | VQYAGTDGPCKVPAQ |
| | 131 | GTDGPCKVPAQMAVD |
| | 132 | PCKVPAQMAVDMQTL |
| | 133 | PAQMAVDMQTLTPVG |
| | 134 | AVDMQTLTPVGRLIT |
| Pool 18 | 135 | QTLTPVGRLITANPV |
| | 136 | PVGRLITANPVITES |
| | 137 | LITANPVITESTENS |
| | 138 | NPVITESTENSKMML |
| | 139 | TESTENSKMMLELDP |
| | 140 | ENSKMMLELDPPFGD |
| | 141 | MMLELDPPFGDSYIV |
| | 142 | LDPPFGDSYIVIGVG |
| Pool 19 | 143 | FGDSYIVIGVGEKKI |
| | 144 | YIVIGVGEKKITHHW |
| | 145 | GVGEKKITHHWHRSG |
| | 146 | KKITHHWHRSGSTIG |
| | 147 | HHWHRSGSTIGKAFE |

148 RSGSTIGKAFEATVR
149 TIGKAFEATVRGAKR
150 AFEATVRGAKRMAVL

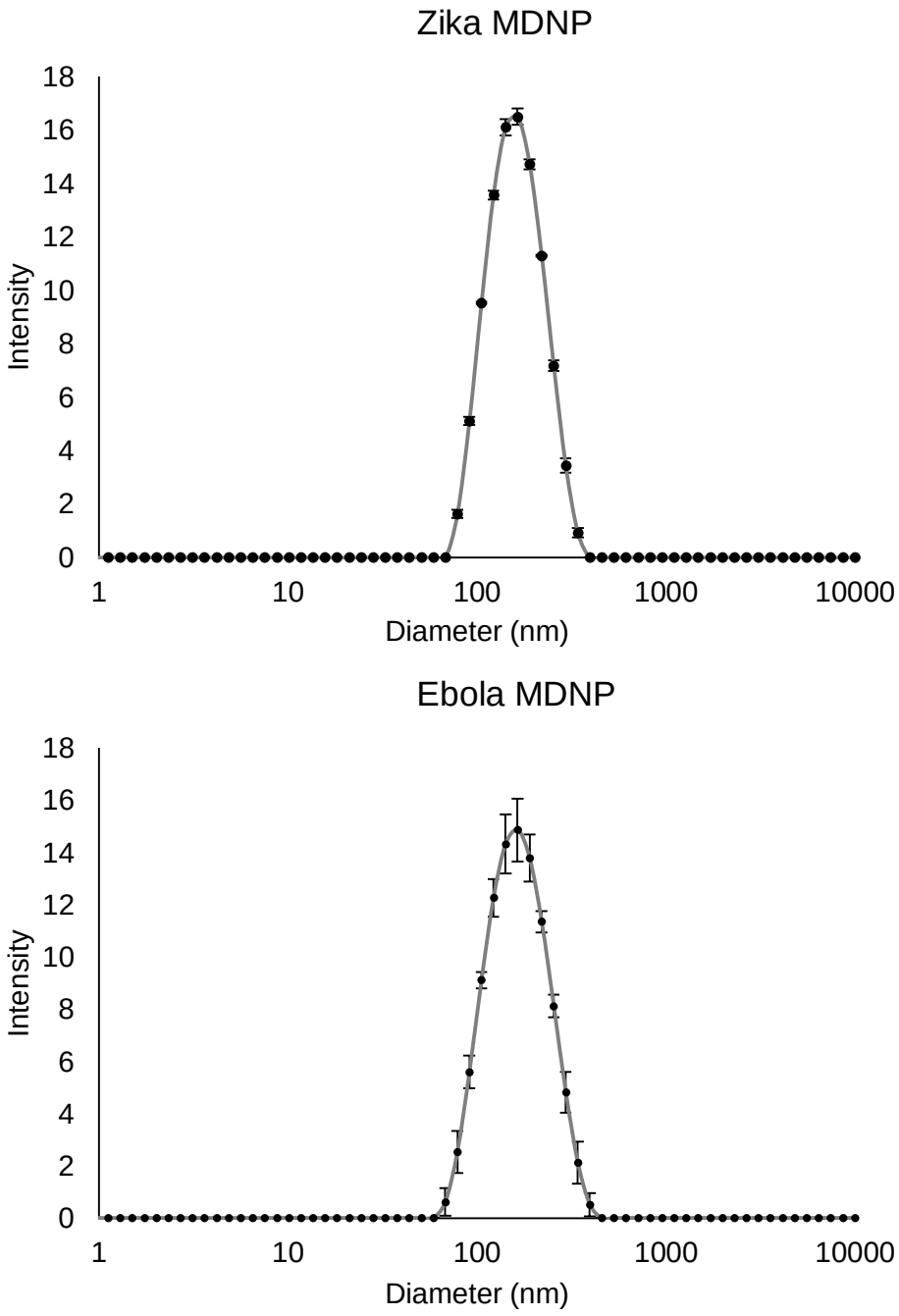


**Figure S1.** Modified dendrimer nanoparticle (MDNP) size distribution. MDNP vaccines suspended in PBS were characterized by dynamic light scattering and the diameters by intensity are shown. N = 3 and error bars represent SD.

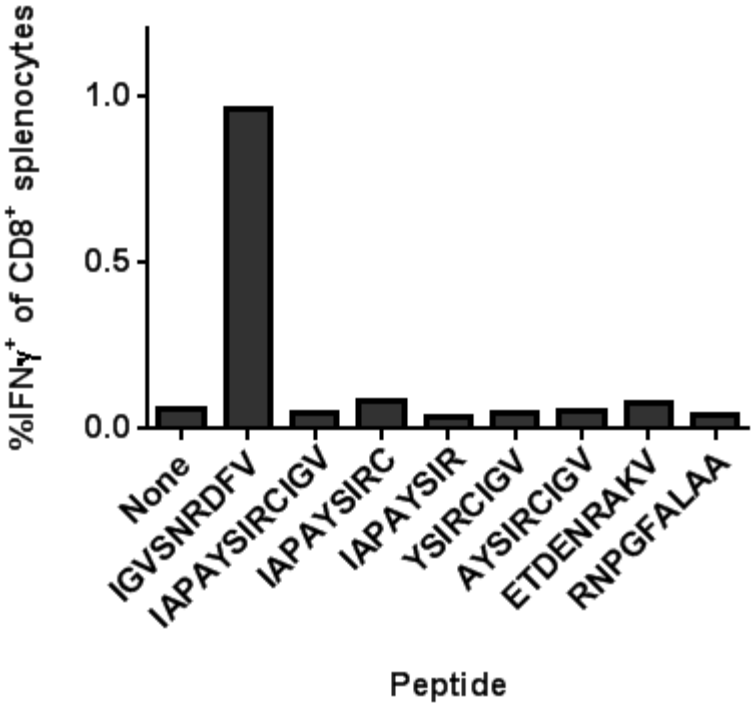


**Figure S2. Screening of additional ZIKV E protein-derived peptides for activation of CD8+ T cells from a single ZIKV-immunized mouse**. Splenocytes from a mouse vaccinated with RNA nanoparticle vaccine against the ZIKV E gene were stimulated *ex vivo* with 0.2 µg/ml of the indicated peptide. After 7 hrs. of culture, intracellular cytokine staining for IFNγ was performed.

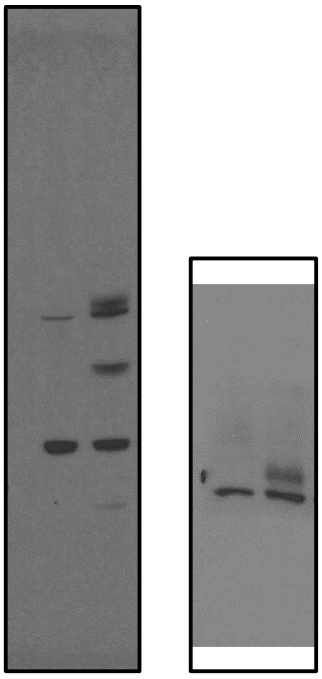

**Figure S3. Raw image data for immunoblots for figures 1b (left) and 1c (right).** Original, unaltered scans of films developed after chemiluminescence reactions of the indicated blots, with entire membrane lengths shown.

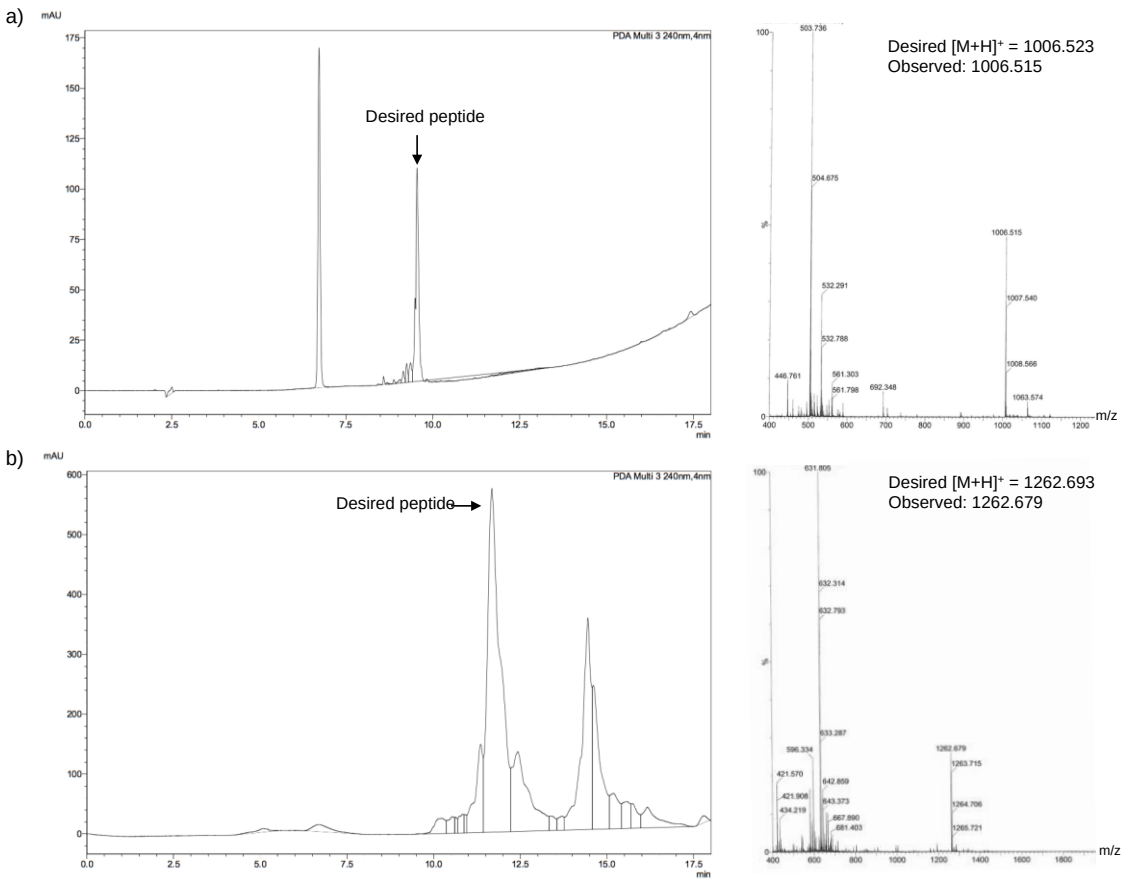


**Figure S4. Representative HPLC and LC-MS characterization of ZIKV-related peptides synthesized to validate the T cell epitopes IGVSNRDFV and IPAYSIRCIGV**. (a) HPLC trace and mass of desired peak corresponding to IGVSNRDFV. (b) HPLC trace and mass of desired peak corresponding to IPAYSIRCIGV.