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# MONOCLONAL ANTIBODIES AGAINST ZIKA VIRUS AND PURIFIED ZIKA E PROTEIN

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To generate a panel of antibodies against ZIKV, we serially infected Irf3/ mice 30 days apart with ZIKV MR-766 (Uganda, 1947) and ZIKV H/PF/2013 (French Polynesia, 2013). Irf3/ mice were used instead of wild-type (WT) mice, because ZIKV strains are deficient in evading type I interferon-mediated immunity. 3 days before myeloma-splenocyte fusion, mice were boosted intravenously with ZIKV H/PF/2013 or recombinant DIII (amino acids 299 to 407 of the ZIKV E protein). After screening 2,000 hybridomas, we isolated six mAbs that recognized ZIKV E protein by ELISA

A cDNA encoding the full-length prM and ectodomain of E of ZIKV (strain H/PF/2013, residues 123-696) was placed in the mammalian expression vector pFM1.2 downstream of a human IL-2 signal sequence peptide (MPLLLLLPLLWAGAL) and terminated with a hexahistidine affinity tag. The protein was expressed by transient transfection of Expi293F cells using HYPE-5 reagent in serum-free Expi293 medium. Cell supernatants were harvested 72 h after transfection. The soluble E protein was recovered by capture on nickel agarose beads and purified by passage over S200 Superdex.

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