**SUPPLEMENTARY MATERIALS**

**Title: A Zika Vaccine Targeting NS1 Protein Protects Immunocompetent Adult Mice in a Lethal Challenge Model**

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**SUPPLEMENTARY TEXT**

**Utility of NS1 immunization**. The idea of a flavivirus vaccine based on the NS1 protein is not new; but there has not been an incentive, at least until now, to further develop such a vaccine beyond the preclinical stages, since the standard approaches (e.g. PIV or LAV) using prME immunogens have been quite adequate in generating effective vaccines against TBE, JEV, yellow fever and DENV(*1*). Only recently, due to the ADE concern (ZIKV and DENV have the highest sequence identity of all flaviviruses(*2*), efforts are being directed toward alternative vaccines (e.g. using a stabilized E protein without the ADE epitopes, E protein-derived domain III, or an NS1 approach) against both ZIKV and DENV (personal communications). For development of an alternative NS1 based-DENV vaccine, however, it may be necessary to delete or mutate the NS1 a.a. residues 305-311 (GKLITEW), which has 75% identity with human plasminogen (residue 590-597) (GTLISPEW), to avoid potential induction of plasminogen cross-reactive antibodies that has been suggested, through molecular mimicry, to contribute to pathogenesis of dengue hemorrhagic fever and dengue shock syndrome(*3*).  Vaccination of inbred C3H/HeN mice with a modified DENV2 NS1 protein (deletion of the C-terminal a.a. 271-352 of DEN2 or replacement with the corresponding JEV NS1 a.a, reduced its pathology(*4*).  Nevertheless, immunization with a native NS1, fully protected IFN receptor-deficient mice from lethal challenge with DENV-2 without induction of any vascular leakage(*5*).  In contrast to cross reactivity of E specific antibodies among flaviviruses, especially between DENV serotypes and now between DENV and ZIKV, a concern for ADE(*6, 7*), no cross reactivity was found among antibodies directed to NS1 proteins in humans infected with ZIKA and DENVs(*8*). Moreover, the molecular mimicry between NS1 epitopes and human plasminogen, seems to be limited to DENVs and no NS1-induced pathology has been reported for any other flaviviruses so far, eliminating any safety concerns over using native NS1 as an effective alternative immunogen for ZIKV immunization.

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