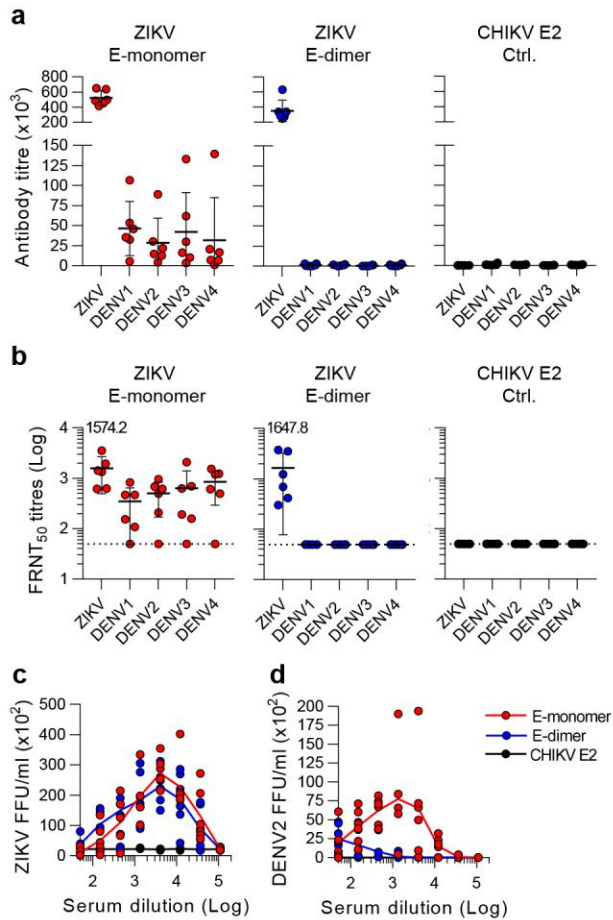


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A protective Zika virus E-dimer-based subunit vaccine engineered to abrogate antibody-dependent enhancement of dengue infection

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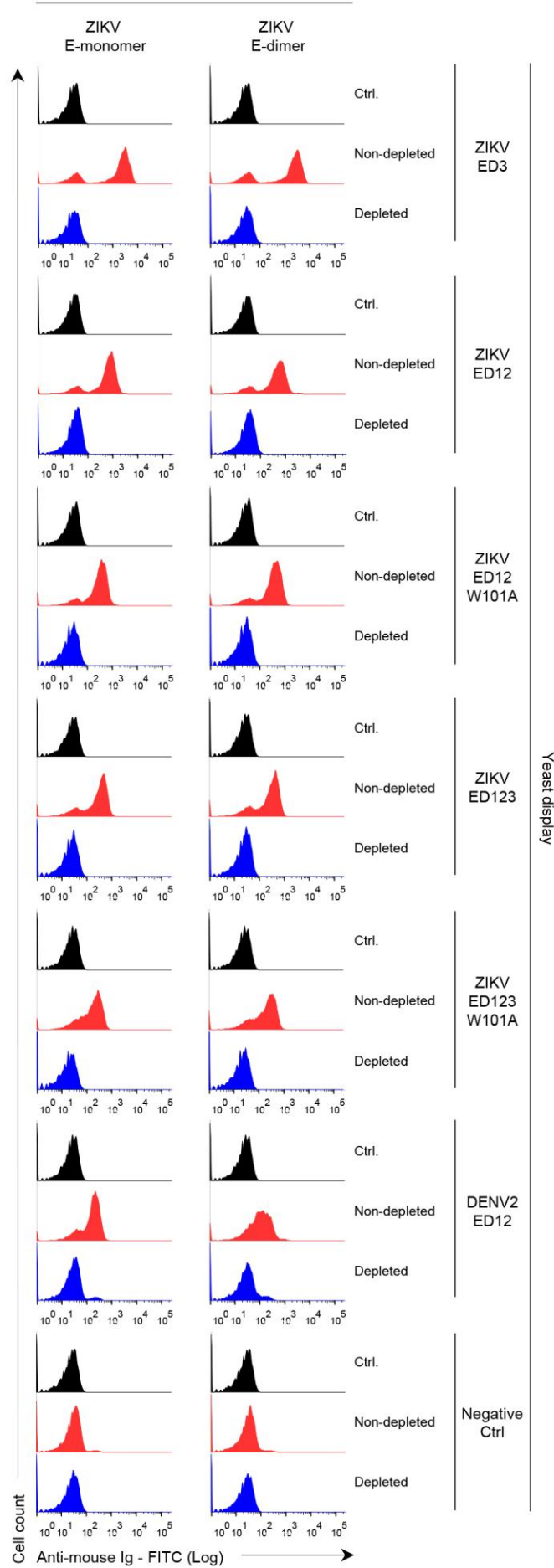


Supplementary Figure 1

Binding, neutralizing and ADE activities of outbred CD1 mouse sera following immunization with ZIKV E-monomer and E-dimer proteins.

Three groups of female CD1 mice ($n=6$ animals per group) were immunized with E-dimer, E-monomer, or CHIKV E2 as before, using Addavax as adjuvant. **(a)** ELISA analysis showing the antibody titres obtained from ZIKV E-monomer (left panel), ZIKV E-dimer (middle panel) and CHIKV E2 (right panel), immunized-mouse serum on captured ZIKV and DENV 1-4 virions (for each virus, $n=6$ individual mouse serum per group). **(b)** FRNT₅₀ titres against ZIKV and DENV 1-4 were calculated for animals immunized with ZIKV E-monomer (left panel), ZIKV E-dimer (middle panel) and CHIKV E2 (right panel) (for each virus, $n=6$ individual mouse serum per group); in **(a-b)** individual measurements, as well as arithmetic mean \pm s.d. values are shown. **(c-d)** Infection enhancement curves (FFU/ml) of U937 cells infected with ZIKV PF13 **(c)** and DENV-2 16681 **(d)** viruses in presence of serially-diluted mouse immune serum ($n=6$ individual mouse serum per group). The data are shown as single data points and arithmetic mean.

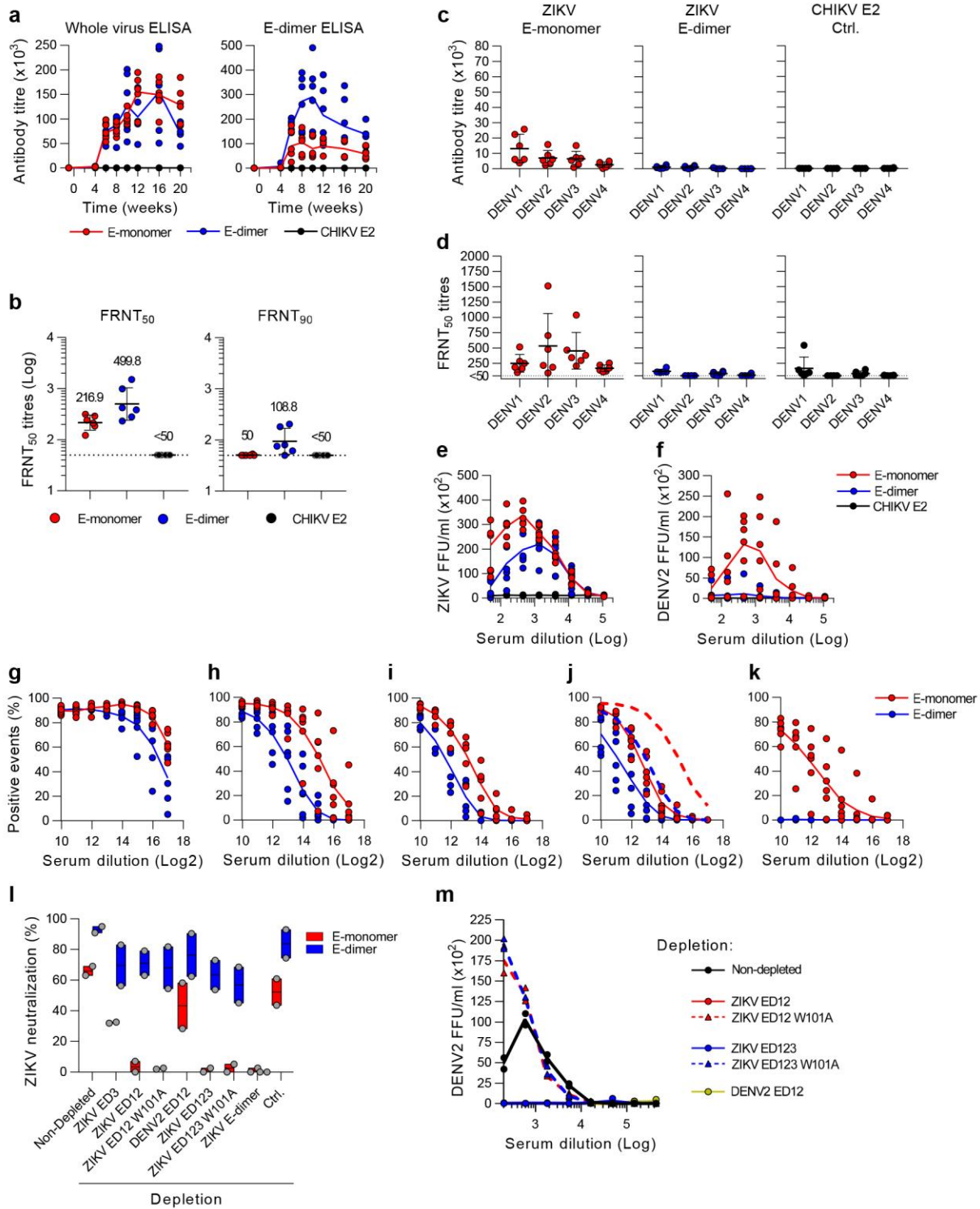
Pooled mouse immune serum



Supplementary Figure 2

Depletion of specific antibody populations from mouse immune sera.

Serum was collected from BALB/c mice 10 weeks post prime, pooled by group and incubated with a series of antigen -displaying yeast to remove specific fractions of antibodies. The depleted sera were tested by flow cytometry for any remaining reactivity and additional rounds of depletion were performed until all the antigen-specific activity was removed. Flow cytometry profiles showing the reactivity of each sera before (red) and after (blue) depletion with each antigen displaying -yeast are shown; histogram plots were used to facilitated comparison and a negative control using mock yeast (black) was added to demonstrate complete depletion of antibody. The data shown is representative of three independent experiments performed for each depletion and sera.

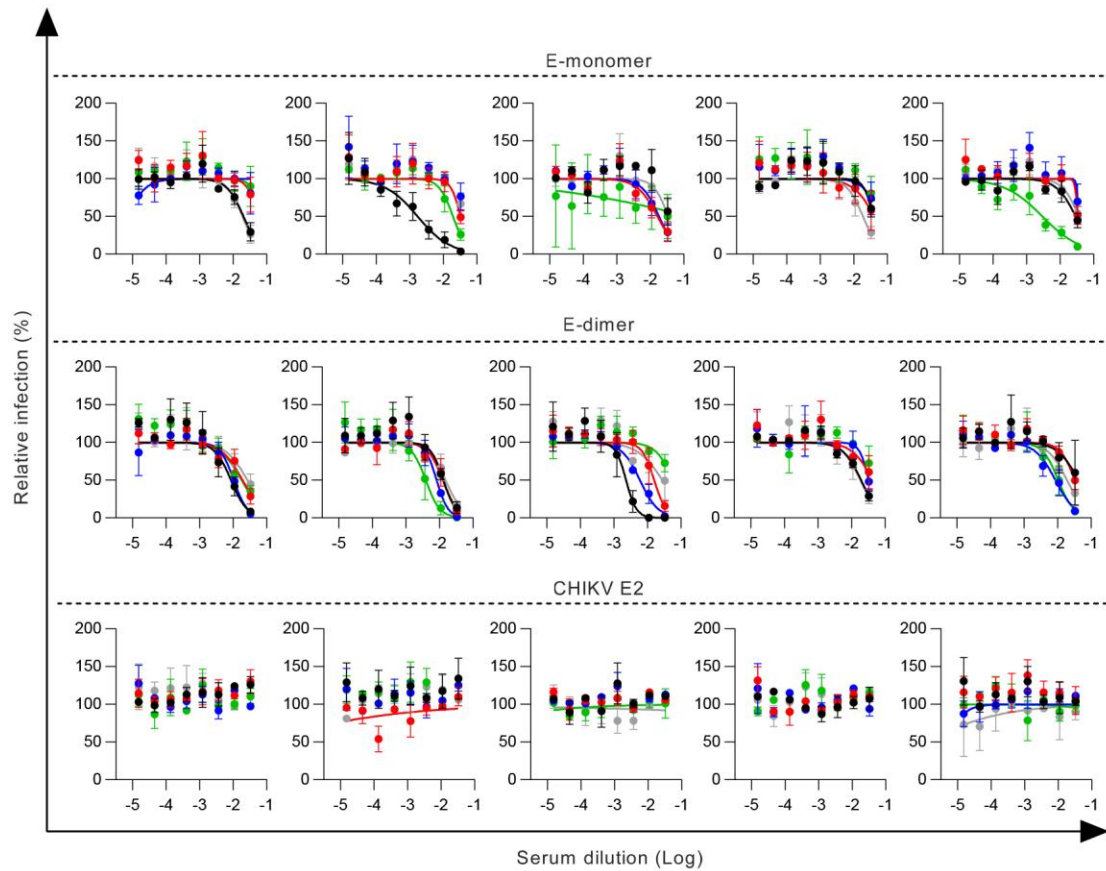


Supplementary Figure 3

Profiles and in-depth characterization of the immune response to ZIKV E-monomers and E-dimers using alum as adjuvant.

Three groups of female BALB/C mice ($n=6$ animals per group) were immunized with E-dimer, E-monomer, or CHIKV E2, Alum was used as adjuvant throughout. **(a)** ELISA analysis showing the antibody titres of mouse immune serum to captured ZIKV PF13 virions (left) and ZIKV E-dimers (right) ($n=6$ individual mouse serum for each group and time point); single data points and arithmetic mean (solid lines) are shown. **(b)** FRNT₅₀ (left) and FRNT₉₀ (right) titres against ZIKV were calculated for individual animals in each group ($n=6$ individual mouse serum for each group). The dashed lines indicate the limit of detection of the assay. Individual measurements, as well as geometric mean \pm s.d. values are noted. **(c)** ELISA analysis showing the antibody titres of ZIKV E-monomer- (left panel), ZIKV E-dimer- (middle panel) and CHIKV E2- (right panel) immune mouse serum to captured DENV 1-4 virions (for each virus, $n=6$ individual mouse serum per group). **(d)** FRNT₅₀ titres against DENV 1-4 were also calculated (for each virus, $n=6$ individual mouse serum per

group); in **(b-d)** individual measurements, as well as arithmetic mean \pm s.d. values are shown. **(e-f)** Infection enhancement curves (FFU/ml) of U937 cells infected with ZIKV PF13 **(e)** and DENV-2 16681 **(f)** viruses in presence of mouse immune serum (for each virus, $n=6$ individual mouse serum per group). The data are shown as single data points and arithmetic mean (solid lines). **(g-k)** Flow cytometry profiles of E-monomer and E-dimer mouse immune serum against yeast displaying ZIKV ED3 **(g)**, ED12 **(h)**, ED123 **(i)** and ED12 carrying the W101A mutation **(j)** as well as DENV-2 ED12 **(k)**; in all cases, $n=6$ individual mouse serum for each group. Data is presented as individual measurements and arithmetic mean (solid lines). In **(j)**, profiles obtained with the wild type ZIKV ED12 were included for comparison (dashed lines). **(l)** Serum was depleted of specific antibodies using antigen-displaying yeasts and analyzed for ZIKV neutralization activity, FRNT₅₀ titres were calculated (for each group, $n=2$ independent depletion experiments using pooled sera). The data is presented as floating bars showing mean (central line), maximum and minimal values together with individual measurements for each group and depletion. **(m)** Infection enhancement curves (FFU/ml) of U937 cells infected with DENV-2 16681 in presence E-monomer immune mouse serum after depletion of specific antibodies as in **l** (for each depletion, $n=2$ independent experiments using depleted-pooled). Data are shown as single data points and arithmetic mean.



Supplementary Figure 4

ZIKV-neutralizing activities of WT C57Bl/6J mice immunized with ZIKV E-monomer and E-dimer proteins.

WT C57Bl/6J were immunized and boosted with ZIKV E-monomer, ZIKV E-dimer or CHIKV E2 proteins using Addavax as adjuvant. Neutralizing potential of the serum was determined by FRNTs and individual mouse neutralization curves are shown. $n=25$ animals per group. Each point represents the mean of two technical replicates with error bars denoting the range, each graph shows the results of 5 individual animals.