Cell

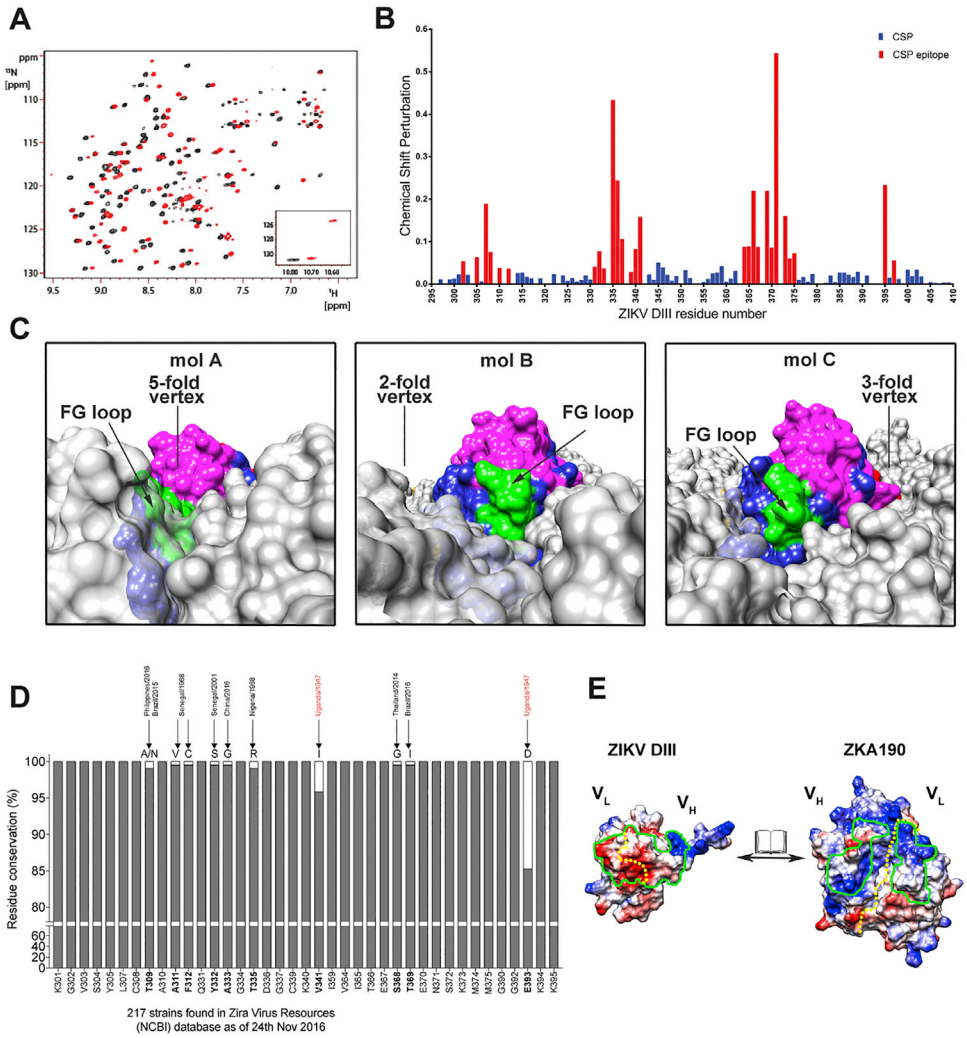
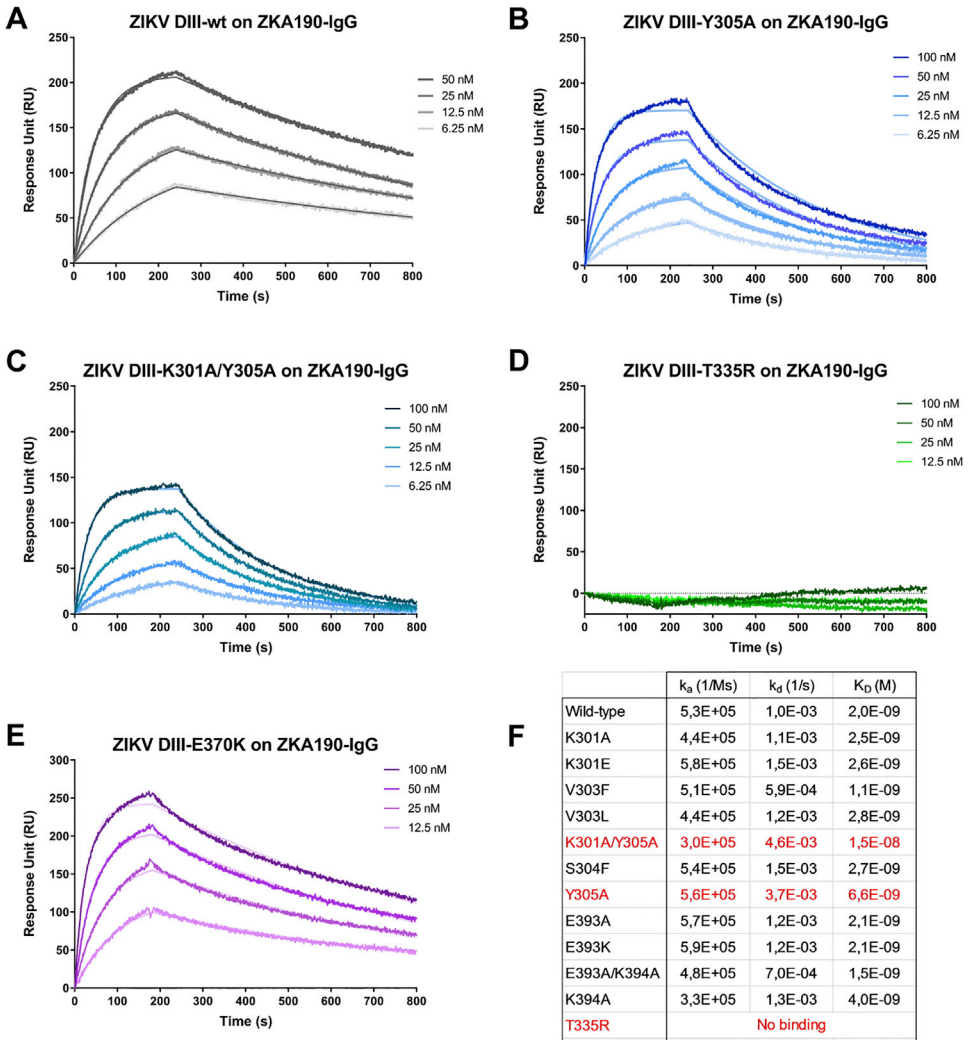


Figure S1. Identification of ZKA190 Epitope and Analysis of its Conservation in ZIKV Strains, Related to Figure 2

(A) Overlay of [15N,1H]-HSQC spectra of 15N-labeled ZIKV DIII in the absence (black) or presence (red) of unlabelled ZKA190 Fab. Differences identify DIII residues affected by mAb binding. (B) NMR epitope mapping of ZKA190 Fab in complex with ZIKV DIII. The chemical shift perturbation (CSP, y axis) is plotted against the DIII residue number. Residues affected by antibody binding are in red. (C) Residues in the FG loop identified by NMR epitope mapping are partially hidden in E protein mol A but largely exposed in mols B and C. DIII of E protein is colored in blue. Residues identified by NMR epitope mapping are colored in magenta, except those in the FG loop, which are colored in green. Adjacent E proteins are shown as gray surface. (D) Level of amino acid residue conservation in ZKA190 epitope as calculated by the analysis of sequences from 217 ZIKV strains found in ZIKV Resources (NCBI) databases as of November 24th 2016. (E) Open-book representation showing charge complementarity between the epitope and paratope of the docking result. Boundaries of the epitope and paratope are circled in green. The borders between heavy and light chains of Fab and its corresponding footprint on DIII are shown as yellow dashed lines.



F

	k_a (1/Ms)	k_d (1/s)	K_D (M)
Wild-type	5,3E+05	1,0E-03	2,0E-09
K301A	4,4E+05	1,1E-03	2,5E-09
K301E	5,8E+05	1,5E-03	2,6E-09
V303F	5,1E+05	5,9E-04	1,1E-09
V303L	4,4E+05	1,2E-03	2,8E-09
K301A/Y305A	3,0E+05	4,6E-03	1,5E-08
S304F	5,4E+05	1,5E-03	2,7E-09
Y305A	5,6E+05	3,7E-03	6,6E-09
E393A	5,7E+05	1,2E-03	2,1E-09
E393K	5,9E+05	1,2E-03	2,1E-09
E393A/K394A	4,8E+05	7,0E-04	1,5E-09
K394A	3,3E+05	1,3E-03	4,0E-09
T335R	No binding		
E370K	5,6E+05	1,3E-03	2,5E-09

Figure S2. Binding of WT or Mutated DIII to ZKA190 IgG, Related to Figure 2

(A–E) SPR data and binding kinetics are shown. (F) DIII mutants that affect (red highlights) or do not affect binding are shown as indicated in the figure.

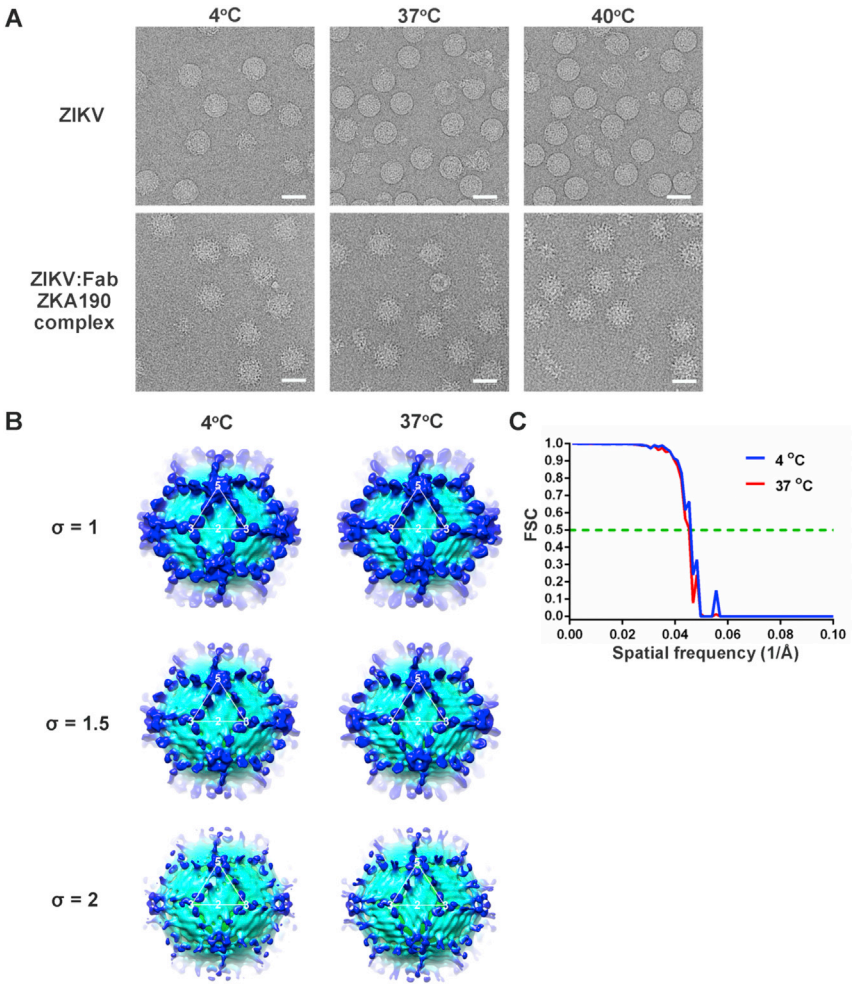


Figure S3. CryoEM Reconstruction of ZIKV:Fab ZKA190 Complex, Related to Figure 2

(A) ZKA190 Fab binds to ZIKV at 4°C, 37°C and 40°C. The uncomplexed ZIKV particles mostly have smooth surfaces at all temperatures. In contrast, in the ZKA190-complexed samples, most particles appear spiky, indicating Fab binding. The scale bar represents 50 nm. (B) The cryoEM maps of ZIKV:ZKA190 Fab complex at 4°C and 37°C, displayed at multiple contour sigma levels. The Fab densities at all positions are similarly reduced at higher contour levels, suggesting equal occupancies. The densities of virus surface and Fab molecules are colored in cyan and blue, respectively. The white triangle highlights one asymmetric unit. Vertices are indicated. (C) Fourier shell correlation (FSC) of the ZIKV:ZKA190 Fab complexes at 4°C and 37°C were plotted against spatial frequency.

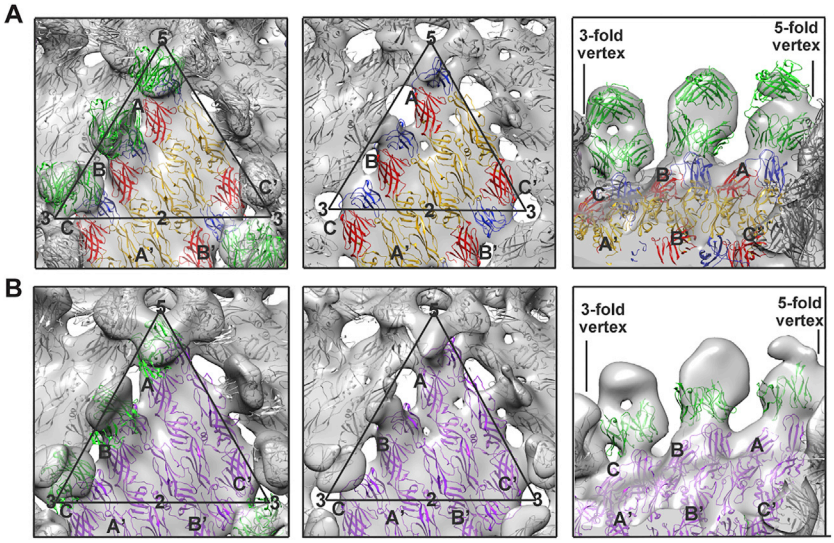


Figure S4. Interpretation of the cryoEM Map and Validation of the Docking Model in the cryoEM Density Map, Related to Figure 2

(A) Interpretation of the cryoEM ZIKV:ZKA190 map by fitting the crystal structure of E protein dimers (PDB 5JHM) and homology model of the Fab into the map. Left, overall view of the fit. Middle, density map displayed at a higher contour level showing the E protein raft is well fitted. Right, density map displayed at a lower contour level showing that the Fab density above molecule C has the best shape corresponding to Fab, compared to those above molecules A and B. The map was first interpreted by displaying the map at high contour so that the shape of the E protein dimers could be easily observed for fitting of the E protein raft, then by using lower contours, Fab ZKA190 was fitted into the density above E protein molecule C. The Fab-E protein (molecule C) complex was subsequently superimposed onto molecules A and B. The positions of the Fab above E protein molecules A and B were slightly adjusted to optimize the fit. Fab molecules are shown as green ribbons. The three individual E proteins in one asymmetric unit are labeled as A, B and C while those in the neighboring asymmetric unit within a raft, A', B' and C'. The cryoEM map is shown as a transparent gray surface. The black triangle represents one asymmetric unit. (B) The docking model of the DIII complexed with the variable region of the Fab (Fv) fits well into the cryoEM density map. The docking model was first superimposed onto E protein dimers (A-C' and B-B') by using their DIIIs. Each E protein dimer:Fv ZKA190 complex was then fitted into the cryoEM density as a rigid body. Left, the overall fit. Middle, E proteins raft shown with the density map displayed at a high contour level. Right, Fv molecules fit well to the density map. E proteins within one raft are colored in purple whereas Fv molecules are colored in green.

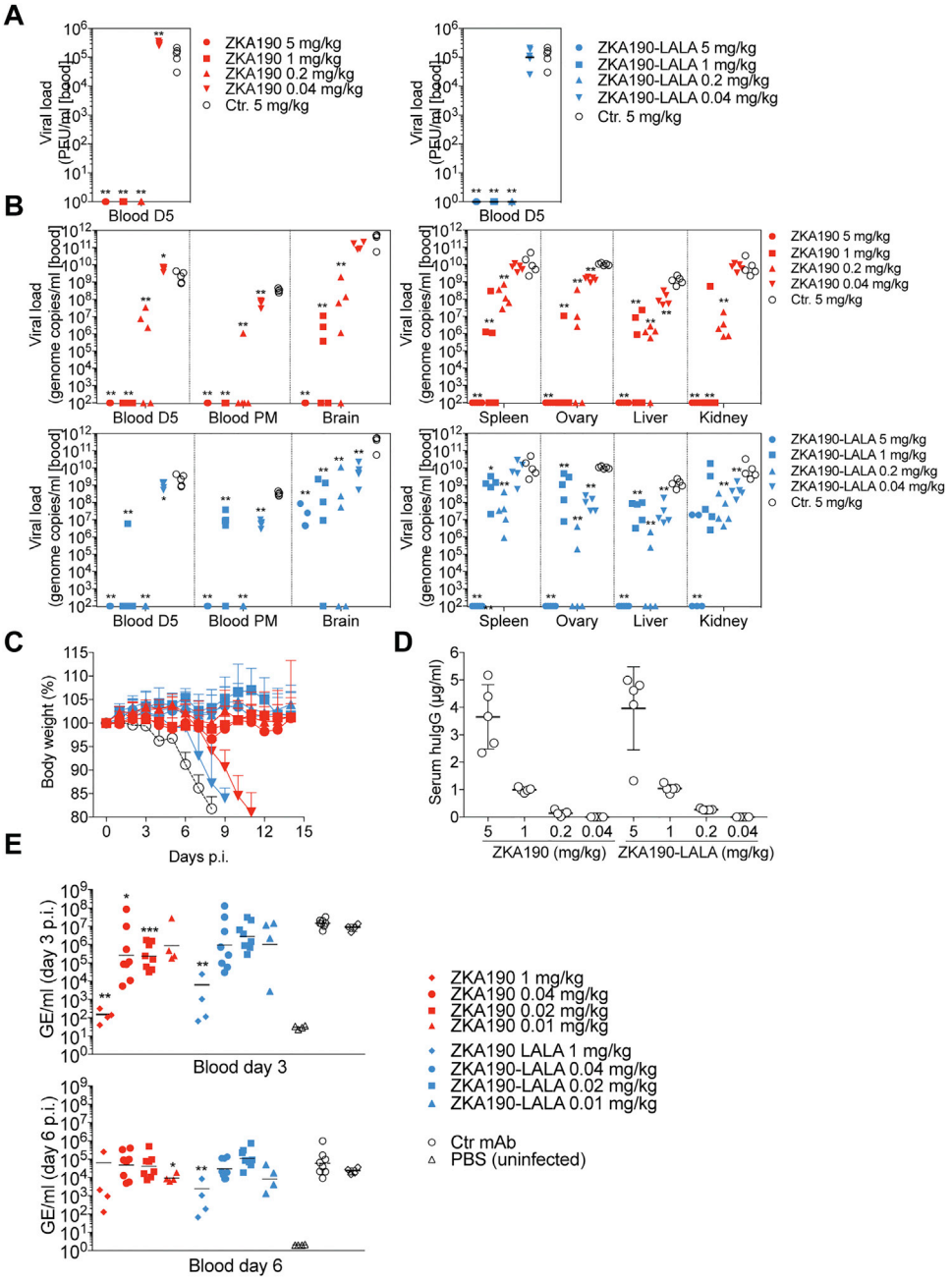
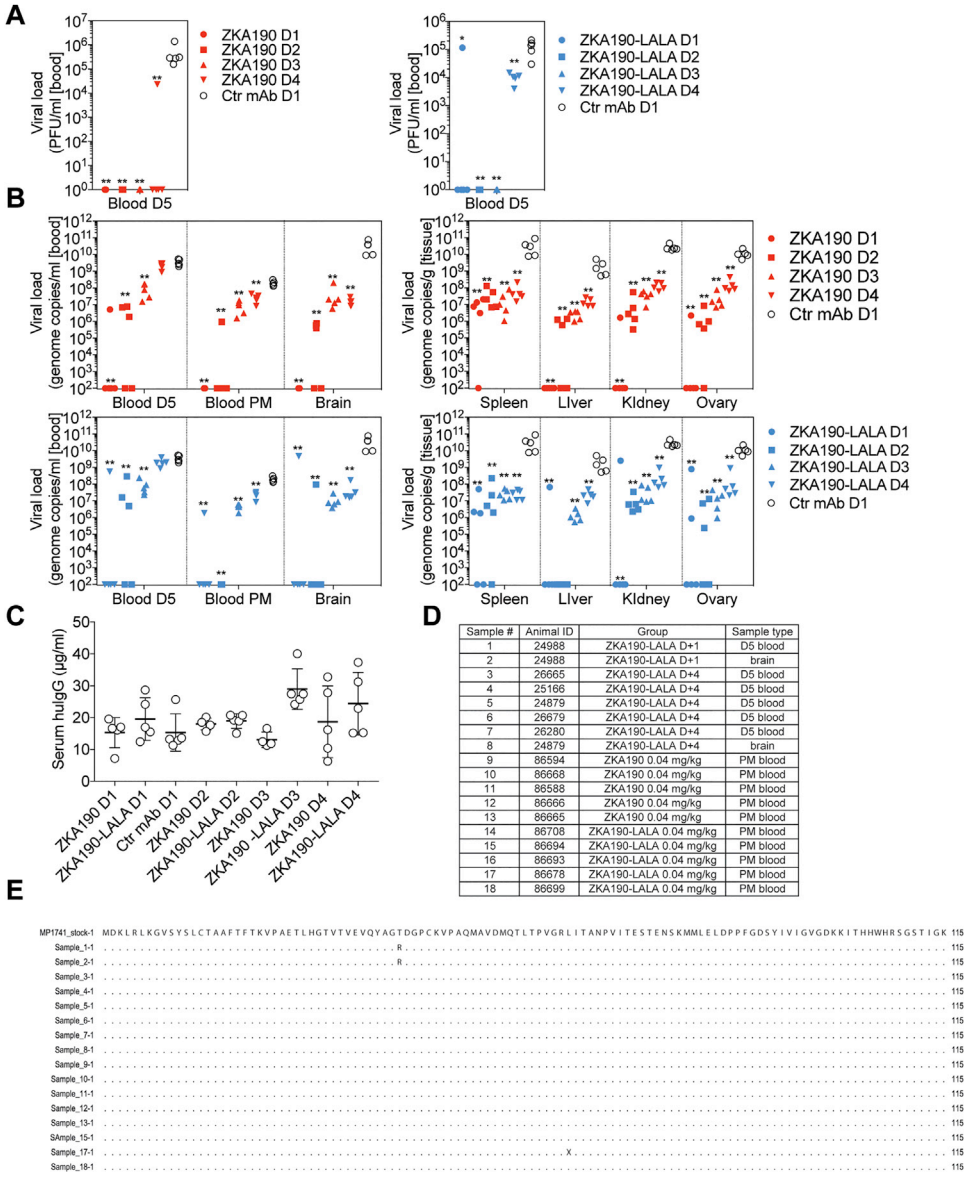


Figure S5. Prophylactic Efficacy of the Anti-ZIKV DIII-specific mAb ZKA190 against ZIKV Strains MP1741 and Nica 2-16, Related to Figure 4

(A–D) Results related to Figure 4A and 4B. (A) Shown is the viremia measured as PFU/mL on day 5 in blood of all animals. (B) Viral load was measured as genomic copies/mL by qPCR on day 5 in blood of all animals and in blood and indicated tissues when animals were culled at the end of the study or when the humane end-points were met. (C) Mice were monitored over a 14-day period for body weight loss. (D) Serum concentrations of human IgG in day 5 blood samples. (E) Results related to Figure 4E–4G. Shown is the viremia measured as genomic copies/mL by qRT-PCR on days 3 and 6 in blood of all animals. Significance was determined compared to control antibody treatment by nonparametric unpaired Mann-Whitney U test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Data in (C) and (D) are represented as mean ± SD for each determination.



Sample #	Animal ID	Group	Sample type
1	24988	ZKA190-LALA D+1	D5 blood
2	24988	ZKA190-LALA D+1	brain
3	26665	ZKA190-LALA D+4	D5 blood
4	25166	ZKA190-LALA D+4	D5 blood
5	24879	ZKA190-LALA D+4	D5 blood
6	26679	ZKA190-LALA D+4	D5 blood
7	26280	ZKA190-LALA D+4	D5 blood
8	24879	ZKA190-LALA D+4	brain
9	86594	ZKA190 0.04 mg/kg	PM blood
10	86668	ZKA190 0.04 mg/kg	PM blood
11	86588	ZKA190 0.04 mg/kg	PM blood
12	86666	ZKA190 0.04 mg/kg	PM blood
13	86665	ZKA190 0.04 mg/kg	PM blood
14	86708	ZKA190-LALA 0.04 mg/kg	PM blood
15	86694	ZKA190-LALA 0.04 mg/kg	PM blood
16	86693	ZKA190-LALA 0.04 mg/kg	PM blood
17	86678	ZKA190-LALA 0.04 mg/kg	PM blood
18	86699	ZKA190-LALA 0.04 mg/kg	PM blood

Figure S6. Therapeutic Efficacy of the Anti-ZIKV DIII-specific mAb ZKA190, Related to Figure 4

(A) Viral load was measured as PFUs on day 5 in blood of all animals. (B) Viral load was measured as genomic copies by qPCR on day 5 in blood of all animals and in blood and indicated tissues when animals were culled at the end of the study or when the humane end-points were met. Significance was determined compared to control antibody treatment by nonparametric unpaired Mann-Whitney U test. *$p < 0.05$; **$p < 0.01$. (C) Serum levels of human IgG in day 5 blood samples. (D) Sequencing of the DIII region from ZIKV ex vivo isolates from ZKA190-treated animals. Full description of sequenced samples. (E) Amino acid sequence alignment of DIII from the 18 selected samples (derived from 16 animals) as compared to the parental sequence of the inoculum virus (MP1751 strain). Data in (C) are represented as mean ± SD for each determination.