Supplementary Information for

A Combination of Two Human Monoclonal Antibodies Limits Fetal Damage by Zika Virus in Macaques

Koen K.A. Van Rompay*, Lark L. Coffey, Tania Kapoor, Anna Gazumyan, Rebekah I. Keesler, Andrea Jurado, Avery Peace, Marianna Agudelo, Jennifer Watanabe, Jodie Usachenko, Anil Singapuri, Ramya Immareddy, Amir Ardeshir, Jackson B. Stuart, Stylianos Bournazos, Jeffrey V. Ravetch, Paul J. Balderes, Ivo C. Lorenz, Shannon R. Esswein, Jennifer Keeffe, Pamela J. Bjorkman, Qiao Wang, Charles M. Rice, Margaret R. MacDonald, Michel C. Nussenzweig*, Davide F. Robbiani*

Koen K.A. Van Rompay, kkvanrompay@ucdavis.edu; Michel C. Nussenzweig, nussen@rockefeller.edu; Davide F. Robbiani, drobbiani@rockefeller.edu

This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.2000414117

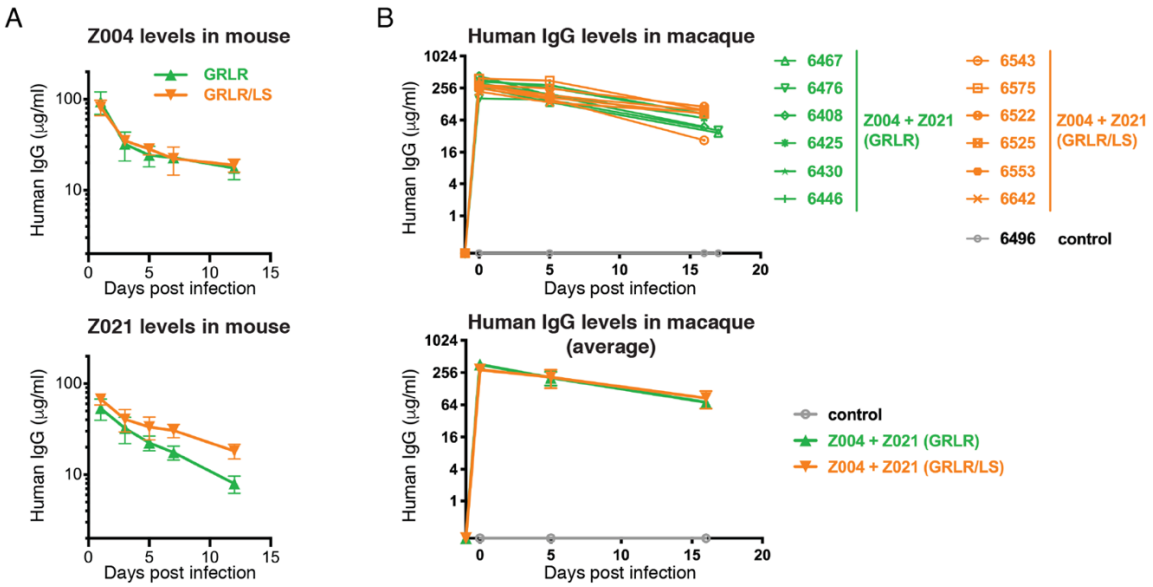

Fig. S1. Levels of Z004 and Z021 human antibodies in mice and macaques. (A) Amount of human IgG antibodies in serum over time upon injection of 100µg of either Z004 or Z021 into FcγR/FcRn humanized mice. Four mice per group; shown is the mean ± SD. **(B)** Levels of human IgG antibodies in macaque plasma. The top panel displays human IgGs in individual macaques; the bottom panel shows the mean for each group. Macaques were administered 15 mg/kg of each of the antibodies on day -1. The mean peak antibody levels on the day of infection (day 0) were 364 µg/ml in the $Z004^{GRLR}$ + $Z021^{GRLR}$ group and 289 µg/ml in the $Z004^{GRLR/LS}$ + $Z021^{GRLR/LS}$ group. The antibody levels on day 16 were 71 µg/ml in the $Z004^{GRLR}$ + $Z021^{GRLR}$ group and 85 µg/ml in the $Z004^{GRLR/LS}$ + $Z021^{GRLR/LS}$ group, resulting in plasma half-lives of 6.8 and 9 days, respectively. Samples from macaques 6467 and 6476 (both treated with GRLR antibodies) were excluded from the calculation of the half-life since they were sampled on a different day. Control is plasma from a ZIKV infected, untreated animal.

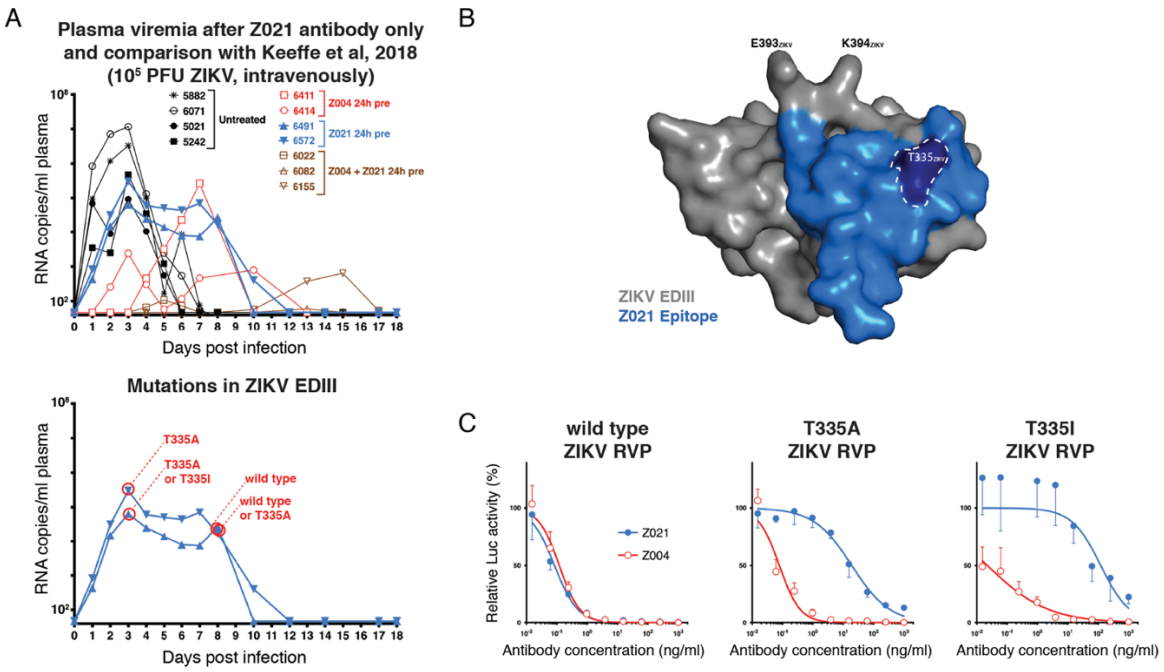

Fig. S2. Administration of Z021 alone induces the emergence of resistant ZIKV in macaque. (A) Macaques were administered the Z021 monoclonal antibody 24 hours before intravenous inoculation with 10^5 PFU of Brazilian ZIKV. The top graph shows the measurement of plasma viremia over time upon Z021 treatment (blue), alongside the values previously reported for Z004 alone, Z004 + Z021, and untreated controls, for which the same virus, dose and route of administration were used (1). The bottom graph shows the time points analyzed that revealed T335 mutations in the virus EDIII region. **(B)** The epitope of ZIKV EDIII recognized by the Z021 antibody is shown in blue (PDB: 6DFI). The T335 residue is highlighted, as well as residues E393 and K394 that when mutated can confer resistance to Z004 (1). **(C)** Z004 similarly neutralizes RVPs corresponding to wild type ZIKV sequence or ZIKV mutated at the T335 residue, while Z021 is less potent against viruses containing T335A or T335I. Data are represented as mean ± SD of triplicates and are relative to isotype control.

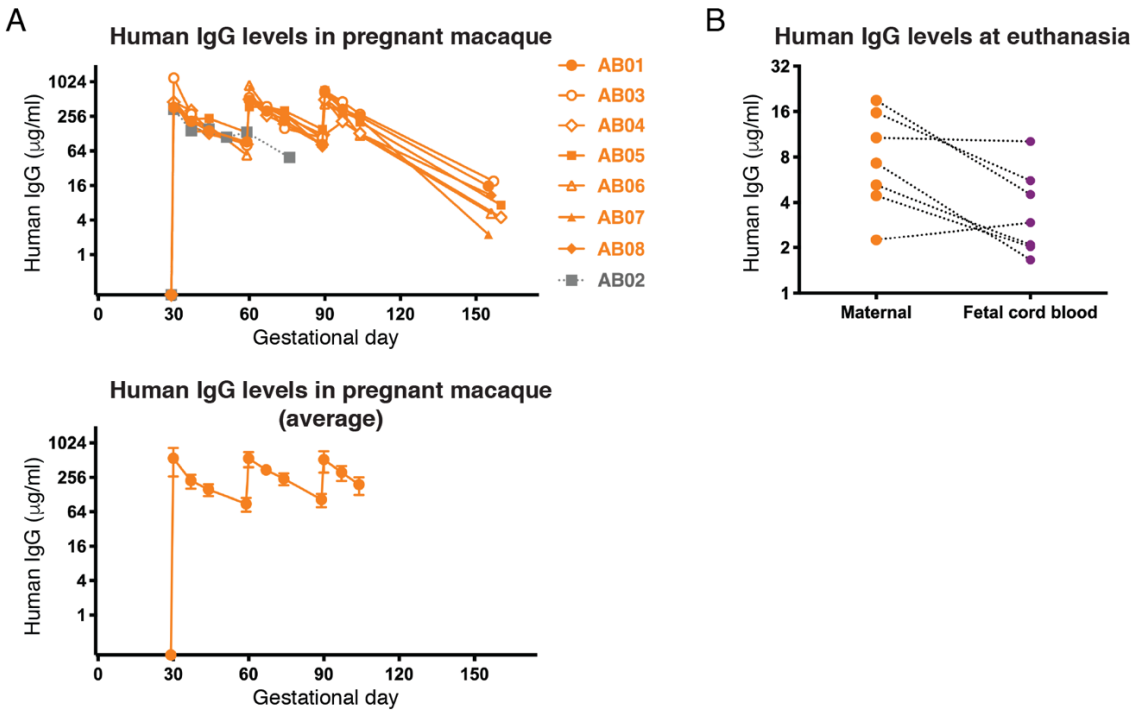

Fig. S3. Plasma antibody levels of Z004$^{GRLR/LS}$ + Z021$^{GRLR/LS}$ in dams and fetuses. **(A)** Levels of human IgG antibodies in plasma of pregnant macaques. The top panel displays human IgGs in individual macaques; the bottom panel shows the mean with standard deviation. Macaques were administered 15mg/kg of each of the antibodies 24 hours before each of the virus challenges, which occurred on GD30, GD60 and GD90. The mean peak antibody levels were 526 µg/ml (GD30), 547 µg/ml (GD60) and 522 µg/ml (GD90); and the calculated half-lives were 11.7 days (GD30-GD59), 12 days (GD60-GD89) and 9.6 days (GD90-GD104). In grey in the top panel are the values for the pregnancy that was prematurely terminated due to fetal loss, which did not receive a 2nd dose of antibodies, and which was excluded from the calculations for the average (bottom panel) and for the half-life values. **(B)** Human IgG levels in the dam and respective fetus (cord blood) at the time of euthanasia.

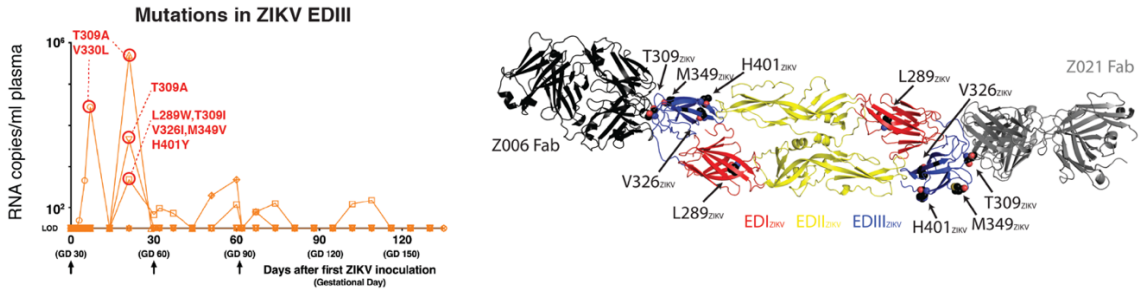

Fig. S4. Mutations in Zika virus emerge in plasma from antibody-treated pregnant macaques. On the left is a graphic summary of the identified mutations. Most of the mutations had already emerged in the antibody treated nonpregnant macaques (see Fig. 3). One exception was a virus detected in one animal with mutations resulting in 5 amino acid substitutions (L289W, T309I, V326I, M349V, H401Y); the effect of these mutations on antibody sensitivity could not be evaluated because we were unable to produce RVPs corresponding to these changes. We were unable to amplify the EDIII from most samples with RNA copies/ml below 10^3. On the right, the mutations are mapped on the structure of the sE dimer of ZIKV (PDB ID: 5JHM). The structures of the Z004-related antibody Z006 (PDB ID:5VIG) and of Z021 (PDB ID:6DFI) in complex with the EDIII of ZIKV are structurally aligned to the sE dimer to show the location of the mutated residues relative to the binding sites of the antibodies. Except for T309, all mutations are outside of the epitopes of both antibodies. The ZIKV EDIII from the Z006-EDIII and Z021-EDIII structures are omitted for clarity.

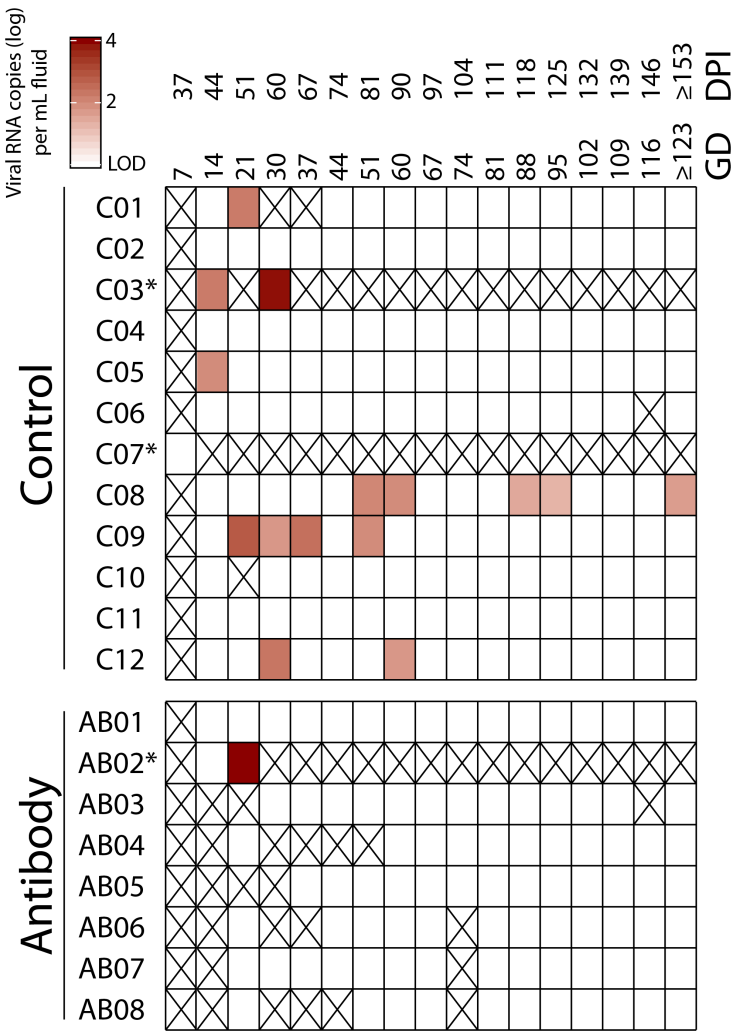

Fig. S5. Detection of ZIKV RNA in amniotic fluid. Amniotic fluid was collected regularly by amniocentesis and tested for ZIKV RNA by qRT-PCR. Results are indicated as $\log_{10}$ ZIKV RNA copies/ml amniotic fluid, the mean of triplicate qRT-PCR replicates. Blank cells indicate samples that tested negative, i.e., below the limit of detection (~1.5 to 2.3 $\log_{10}$ ZIKV RNA copies/ml amniotic fluid, depending on volume). The intensity of red highlights the quantity of viral RNA detected. Cross-out indicates samples not tested, because either not collected (GD 37), not able to collect despite amniocentesis attempt, or early pregnancy termination; * indicates the 2 control animals and one antibody-treated animal with early fetal death. The ≥ GD 153 time point is the time of euthanasia. DPI: days post-infection; GD: gestational day.

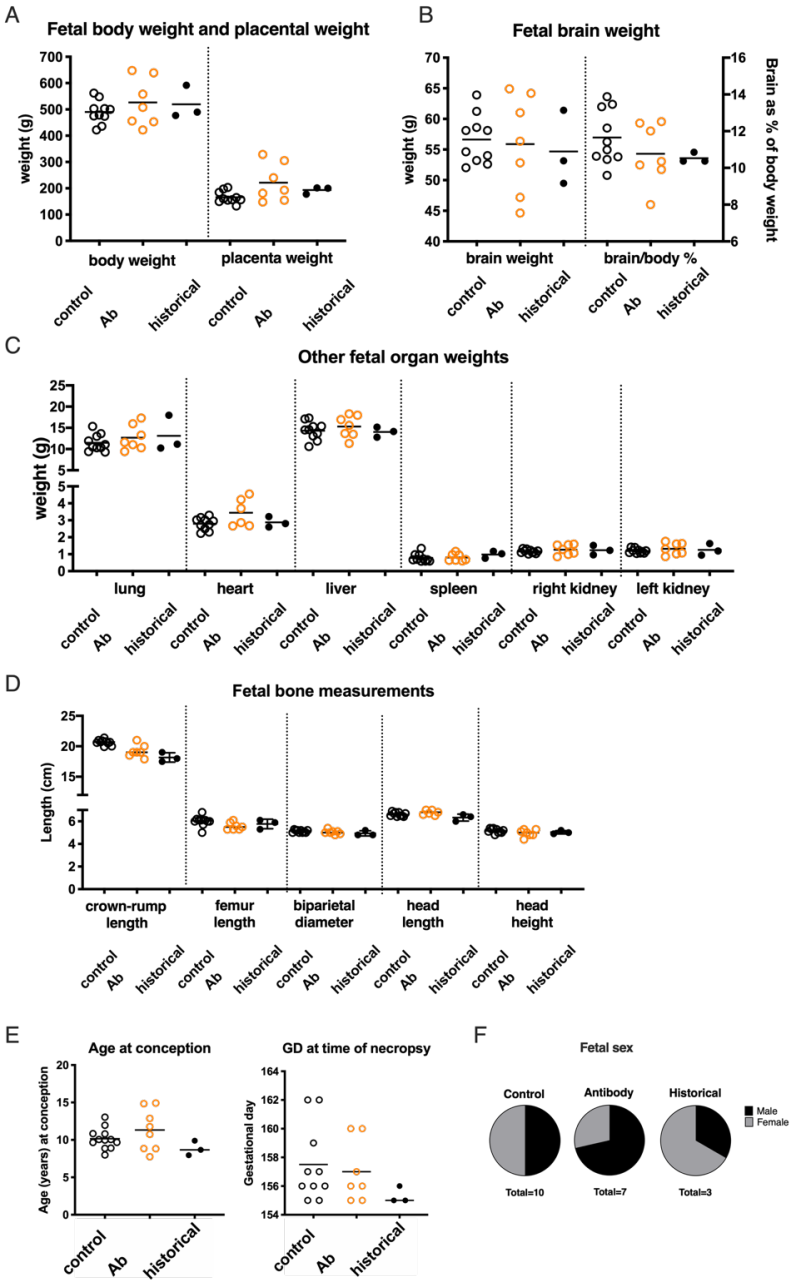

Fig. S6. Fetal measurements at euthanasia from ZIKV infected macaques and from non-infected historical controls. All measurements were collected on fetuses delivered via hysterotomy and immediately euthanized. The graphs include only fetuses that made it to the experimental endpoint (GD155-162) and exclude fetuses with early death. The historical controls are uninfected fetuses that also underwent intensive sampling as described earlier (2). **(A)** Fetal body and placental weights. **(B)** Fetal brain weights, including brain weight expressed as a percentage of total body weight. **(C)** Fetal organ weights. **(D)** Fetal bone measurements. **(E)** Age at conception and gestational day (GD) at time of euthanasia. **(F)** Sex of the fetuses.

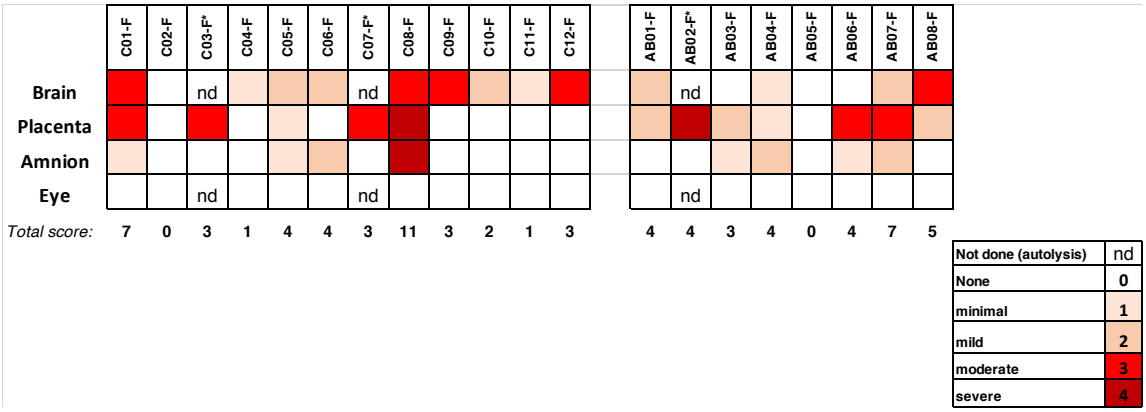

Fig. S7. Fetal pathology scores. Fetal brain (13-20 sections per animal), placenta (1-2 sections per animal), amnion (1-2 sections per animal) and eye (2 sections per animal) were evaluated blindly by a pathologist and were assigned a score of 0 to 4 based on severity (Tables S2, S3 and (3)). Asterisks indicate animals with early fetal death, for which some tissues could not be evaluated due to autolysis.

Table S1. Histology of tissues from antibody treated dams.

Animal ID	Spleen	Mesenteric lymph node	Obturator lymph node	Inguinal lymph node	Uterus
AB01	Moderate lymphoid hyperplasia	Mild lymphoid hyperplasia	Moderate hemorrhage in sinuses	Moderate lymphoid hyperplasia	Small numbers of perivascular lymphocytes in endometrium and myometrium (WNL)
AB02	Mild lymphoid hyperplasia	Mild lymphoid hyperplasia	N/A	N/A	Small numbers of perivascular lymphocytes in the myometrium (WNL)
AB03	Mild lymphoid hyperplasia	Severe lymphoid hyperplasia	WNL	Mild lymphoid hyperplasia	Small numbers of perivascular lymphocytes in endometrium and myometrium (WNL)
AB04	Mild lymphoid hyperplasia	Mild lymphoid hyperplasia	Moderate macrophages in sinuses with hemosiderin	Moderate hemorrhage in sinuses; mild lymphoid hyperplasia	Thick decidua with hemosiderin-laden macrophages; mild to moderate numbers perivascular lymphocytes in endometrium and myometrium
AB05	Moderate lymphoid hyperplasia	Mild lymphoid hyperplasia	Moderate hemorrhage in sinuses	Moderate hemorrhage in sinuses	Small numbers of perivascular lymphocytes in endometrium (WNL)
AB06	Moderate lymphoid hyperplasia	Mild lymphoid hyperplasia	Moderate macrophages in sinuses	Mild lymphoid hyperplasia	Small numbers of perivascular lymphocytes in endometrium (WNL)
AB07	Mild lymphoid hyperplasia	Moderate lymphoid hyperplasia	WNL	Mild lymphoid hyperplasia	Small numbers of perivascular lymphocytes in endometrium and myometrium (WNL)
AB08	Mild lymphoid hyperplasia	WNL	WNL	WNL	Small numbers of perivascular lymphocytes in endometrium and myometrium (WNL)

See in Van Rompay et al (ref. 3) for control pregnant macaques C01-C12; N/A is 'not available' and WNL is 'within normal limits'

Table S2. Histology of fetal tissues from antibody treatment group.

Fetus ID	Central nervous system	Eye	Placenta	Amnion	Umbilical cord	Mesenteric lymph node	Lung	Liver	Kidney	Ileum
AB01-F	19 sections: B5, B6, B7: segmental attenuation/loss of ependyma, mild, with gliosis	WNL	Basal plate and decidua have mild multifocal neutrophilic vasculitis and few interstitial neutrophils	WNL	WNL	WNL	Multifocal mild congestion and hemorrhage; rare squames	Mild EMH; mild glycogen; no GB	WNL	WNL
AB02-F	Severe autolysis	Severe autolysis	Placental separation; hemorrhage, fibrin and necrosis within decidua	WNL	N/A	N/A	Severe autolysis	Severe autolysis	N/A	N/A
AB03-F	17 sections: B2: single focus mineral near ventricle	WNL	Basal plate and villous parenchyma multifocal areas of necrosis with neutrophils (slide 3-1, not the best sxn)	Very small numbers of neutrophils within the stroma	WNL	WNL	Rare squames	Rare EMH; moderate glycogen; no GB	WNL	WNL
AB04-F	17 sections: B2, B3: rare individual cells in ventricular lumen; B4, B5: segmental attenuation/loss of ependyma, mild, with gliosis; cystic choroid plexus	WNL	Increased syncytial knots	Cystic change to epithelium; hemorrhage and hemosiderin-laden macrophages in stroma; thick decidua	WNL	WNL	Mild squames	Moderate EMH; moderate glycogen	WNL	WNL
AB05-F	17 sections: B5: single focus mineral near ventricle	WNL	WNL	WNL	WNL	WNL	Rare squames	Mild EMH; moderate glycogen	WNL	WNL
AB06-F	19 sections: B6: LGN less layering	WNL	Multifocal necrosis/loss of trophoblasts with neutrophils along fetal plate; large mid-disc infarct extending from the basal plate into the villous parenchyma with necrosis/mineralization of the basal plate and decidua; increased syncytial knots	Single area of moderate numbers of neutrophils within decidua	WNL	WNL	Mild squames	Rare EMH; moderate glycogen	WNL	WNL
AB07-F	20 sections: Parietal lobe (10): multifocal mineralization near ventricle and adjacent neuronal rests; B2,B3: single rest mineralization	WNL	Severe decidual thickening with hemorrhage, edema and necrosis; multifocal ischemic necrosis of placenta full thickness (chronic and acute); mild lymphocytes and moderate neutrophils in decidua	Severe decidual thickening with multifocal small areas of hemorrhage and fibrin; mild lymphocytes	WNL	N/A	Moderate hemorrhage within alveoli	Mild EMH; moderate glycogen	WNL	Moderate hemorrhage within lumen; cytoplasmic protein droplets in enterocytes
AB08-F	19 sections: Parietal lobe (10), lateral ventricle (15), B3, B4, B5: single to few focus/foci periventricular mineralization; Lat ventricle (15), B4: mild loss of ependyma with gliosis; B6, B7: moderate ependymal loss	WNL	Fetal plate multifocal trophoblast necrosis with fibrin and neutrophils, multifocal very mild neutrophils in fetal vessels; decidua multifocal necrosis with neutrophils	WNL	WNL	WNL	Moderate squames	Rare EMH; mild glycogen	WNL	WNL

See in Van Rompay et al (ref. 3) for control pregnant macaques C01-C12; N/A is 'not available', WNL is 'within normal limits' and EMH is extra-medullary hematopoiesis

Table S3. Fetal pathology scoring.

Score	Placenta/fetal membranes	Fetal neuropathology
0	none - no lesions observed	none - no lesions observed
1	minimal - focal, small lesion in section, not observed in control animals but significance questionable, could be background lesion	minimal - focal, small lesion in one brain section, not observed in control animals but significance questionable, could be background lesion
2	mild – mild, multifocal lesions in section, single layer	mild – mild ependymal loss in multiple brain sections +/- very few mineral foci
3	moderate – mild to moderate lesions extensively throughout section, multiple layers	moderate – mild to moderate ependymal loss in multiple sections, mineralization +/- cuffing
4	severe – widespread lesions throughout section, multiple layers	severe – widespread lesions in multiple brain sections

Table S4. Macaques used in the study.

Animal ID	Treatment	Date of birth	Sex	Total prior pregnancies	Delivery of live infants	Age at necropsy (in years)
LOW DOSE SUBCUTANEOUS ZIKV CHALLENGE (NONPREGNANT)						
6467	Z004+Z021 GRLR	5/2/15	M	n.a.	n.a.	2.8
6476	Z004+Z021 GRLR	5/11/15	M	n.a.	n.a.	2.8
6408	Z004+Z021 GRLR	3/7/15	M	n.a.	n.a.	3.3
6425	Z004+Z021 GRLR	3/30/15	M	n.a.	n.a.	3.2
6430	Z004+Z021 GRLR	4/3/15	M	n.a.	n.a.	3.2
6446	Z004+Z021 GRLR	4/17/15	M	n.a.	n.a.	3.2
6543	Z004+Z021 GRLR/LS	4/21/16	F	n.a.	n.a.	2.8
6575	Z004+Z021 GRLR/LS	7/2/16	F	n.a.	n.a.	2.6
6522	Z004+Z021 GRLR/LS	3/19/16	F	n.a.	n.a.	2.9
6525	Z004+Z021 GRLR/LS	4/1/16	F	n.a.	n.a.	2.8
6553	Z004+Z021 GRLR/LS	5/6/16	F	n.a.	n.a.	2.7
6642	Z004+Z021 GRLR/LS	4/27/17	M	n.a.	n.a.	1.8
6496	untreated control	6/11/15	M	n.a.	n.a.	3.0
6315	untreated control	4/7/14	M	n.a.	n.a.	4.0
6500	untreated control	6/18/15	M	n.a.	n.a.	2.8
6501	untreated control	6/21/15	M	n.a.	n.a.	3.0
LOW DOSE SUBCUTANEOUS ZIKV CHALLENGE (PREGNANT)						
AB01	Z004+Z021 GRLR/LS	3/19/04	F	10	9	15.3
AB02	Z004+Z021 GRLR/LS	2/21/04	F	8	7	15.1
AB03	Z004+Z021 GRLR/LS	4/28/08	F	6	5	11.1
AB04	Z004+Z021 GRLR/LS	3/25/10	F	4	2	9.3
AB05	Z004+Z021 GRLR/LS	3/15/06	F	6	3	13.3
AB06	Z004+Z021 GRLR/LS	4/21/07	F	4	3	12.1
AB07	Z004+Z021 GRLR/LS	4/3/10	F	3	0 (1 abortion, 2 experimental harvests)	9.3
AB08	Z004+Z021 GRLR/LS	5/1/11	F	2	2	8.2

See in Van Rompay et al. (ref. 3) for control pregnant macaques C01-C12; n.a. is 'not applicable'.

Table S5. List of primers used in this study.

Primer ID	Primer sequence	Comments
Generation of pZIKV/HPF/CprM*PRVABC59E*		
RU-O-24379	ACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGCAT CAGGTGCATAGGAGT	Forward with BspHI
RU-O-24380	TTCGAACCGCGGCTGGGTCCTATTAAGCAGAGACAGCTG TGGATAAGAAGATC	Reverse with SacII
Mutagenesis of pZIKV/HPF/CprM*PRVABC59E* to produce mutant ZIKV RVPs		
DFRp1477	ACTTGGTCATGATACTGCTGATTG	Forward outer with BspHI
DFRp1584	AACCGCGGCTGGGTCCTATTAAGCAGA	Reverse outer with SacII
DFRp1505	GACAGTCACAGTGGAGTTACAGTACGCAGGGAC	Forward V330L
DFRp1506	GTCCCTGCGTACTGTAACTCCACTGTGACTGTC	Reverse V330L
DFRp1503	GTGTCATACTCCTTGTGTGCTGCAGCGTTCACA	Forward T309A
DFRp1504	TGTGAACGCTGCAGCACACAAGGAGTATGACAC	Reverse T309A
DFRp1557	GTGTCATACTCCTTGTGTATTGCAGCGTTCACA	Forward T309I
DFRp1558	TGTGAACGCTGCAATACACAAGGAGTATGACAC	Reverse T309I
Mutagenesis of pZIKV/HPF/CprM*E* to produce mutant ZIKV RVPs		
DFRp1477	ACTTGGTCATGATACTGCTGATTG	Forward outer with BspHI
DFRp1481	CGAACCGCGGGCCCTCTAGATCAA	Reverse outer with SacII
DFRp1546	GCAGGGGCAGATGGACCTTGC	Forward T335A
DFRp1547	GCAAGGTCCATCTGCCCCTGC	Reverse T335A
DFRp1548	GCAGGGATAGATGGACCTTGC	Forward T335I
DFRp1549	GCAAGGTCCATCTATCCCTGC	Reverse T335I

SI References

1. Keeffe JR, *et al.* (2018) A Combination of Two Human Monoclonal Antibodies Prevents Zika Virus Escape Mutations in Non-human Primates. *Cell reports* 25(6):1385-1394.e1387.
2. Coffey LL, *et al.* (2018) Intraamniotic Zika virus inoculation of pregnant rhesus macaques produces fetal neurologic disease. *Nature communications* 9(1):2414.
3. Van Rompay KKA, *et al.* (2019) DNA vaccination before conception protects Zika virus-exposed pregnant macaques against prolonged viremia and improves fetal outcomes. *Science translational medicine* 11(523).