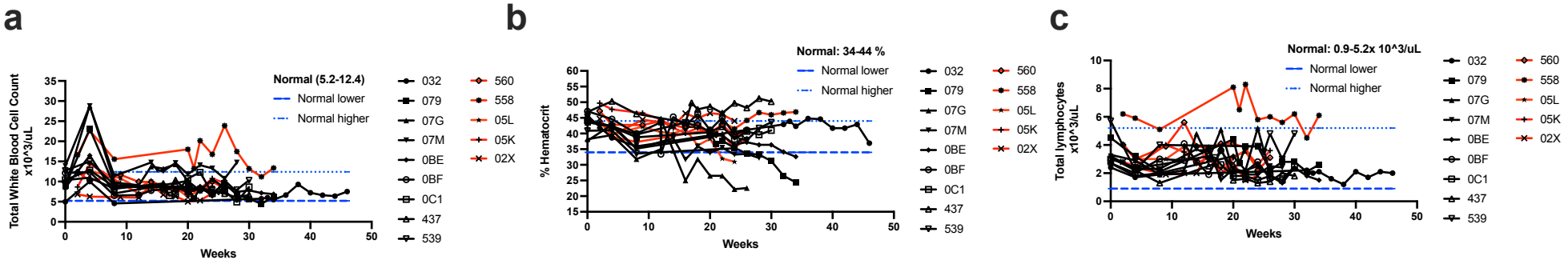
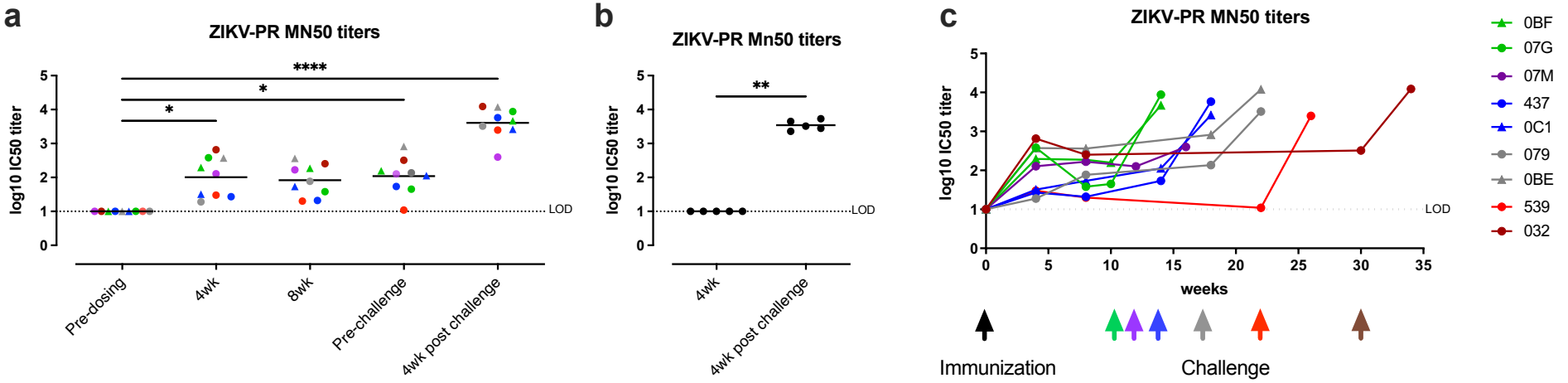
Suppl. Table 1: Timing between vaccination and challenge

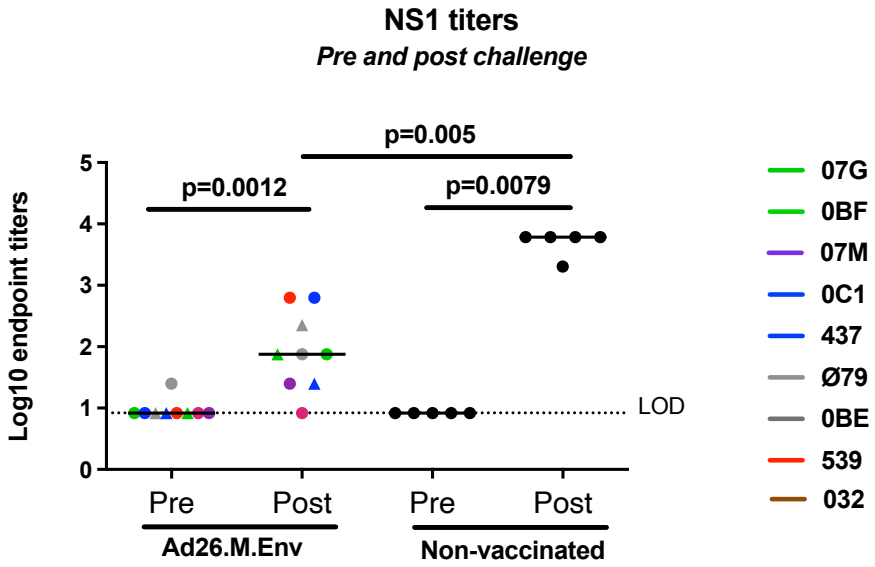
Sub-Study #	Animal #	Vaccination Date	Challenge Date	Time difference b/w vaccination & challenge
17-20.1	560	N/A	12/27/17	N/A
	05L	N/A		
	05K	N/A		
	02X	N/A		
17-20.2	07G	10/30/17	1/10/18	2mo. 11ds
	0BF	10/30/17		
17-20.3	07M	10/30/17	1/24/18	2mo. 25ds
17-20.4	0C1	10/30/17	2/7/18	3mo. 8ds
	437	10/30/17		
17-20.5	079	10/30/17	3/7/18	4mo. 5ds
	0BE	10/30/17		
	558	N/A		
17-20.6	539	10/30/17	4/4/18	5mo. 5ds
17-20.7	032	10/30/17	5/30/18	7mo. 0ds



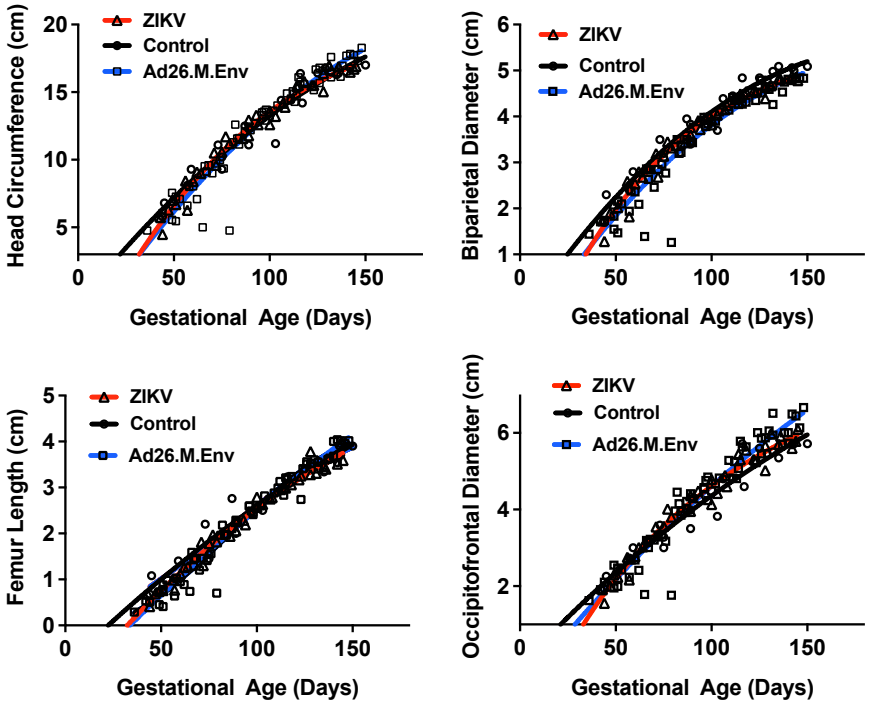
Suppl. Fig. 1. Clinicopathological monitoring following vaccination in breeding female macaques. Blood was collected for complete blood cell count (CBC) during pregnancy in vaccinated, challenged (black) and non-vaccinated, challenged (red) macaques. Total white blood cell count **(a)**, % hematocrit **(b)**, and total lymphocyte count **(c)** are shown for the 42-week gestational period. Normal reference values for rhesus macaques are indicated by the blue dashed lines.



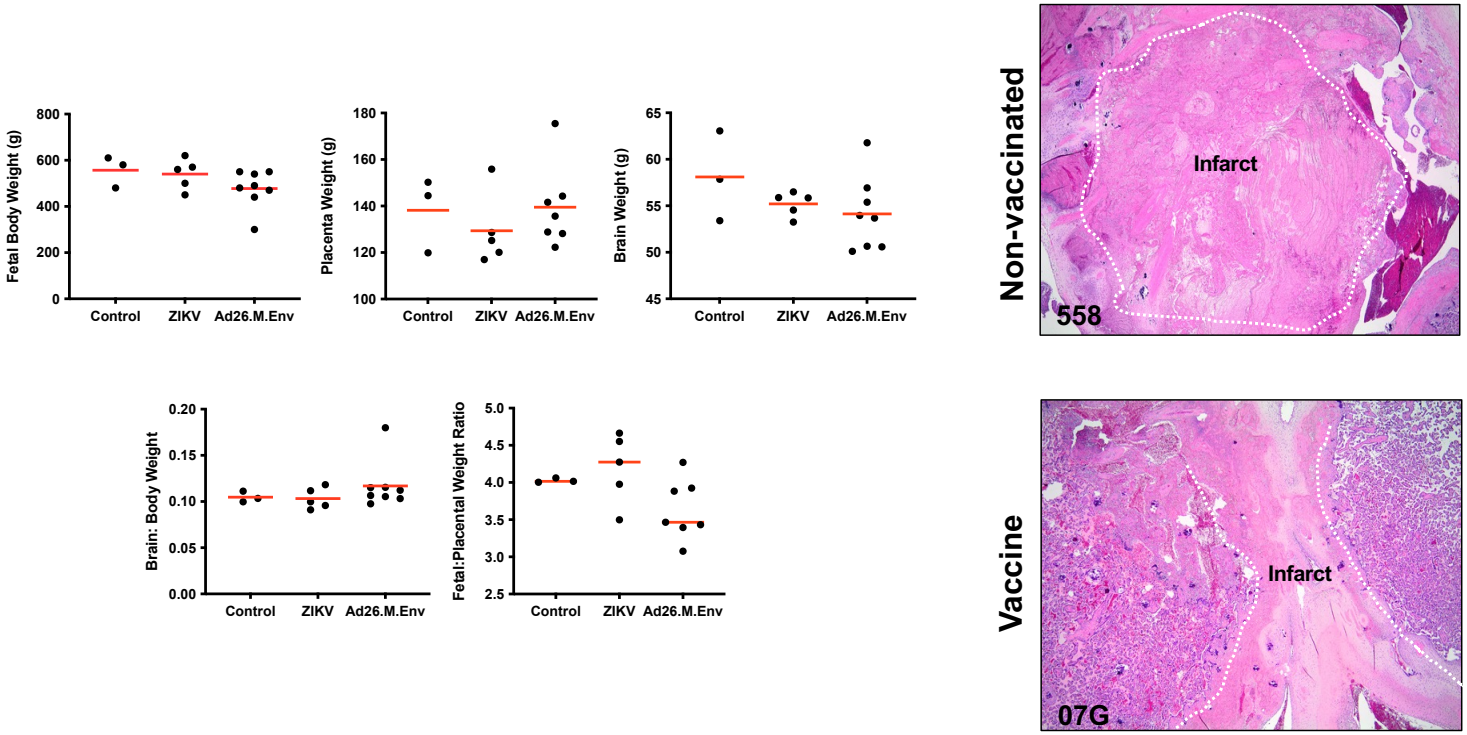
Suppl. Figure 2. Kinetics of ZIKV neutralizing antibody titers by MN50 in sera of dams pre- and post-challenge. ZIKV-PR neutralizing antibody responses in sera of **(a,c)** dams (n= 9) immunized with 10^{11} vp Ad26.M.Env and **(b)** non-vaccinated dams determined by MN50. Neutralizing antibody titers are reported as the log10 of the inverse of the serum dilution that reduce the number of input virus by 50% (IC50). The dashed line shows the lower limit of detection (LOD) defined as the log10 of one dilution below the start dilution of the samples (1 log10 for MN50). Individual animals of group 1 (Ad26.M.Env immunized) are color coded to represent number of weeks between immunization and challenge; green (10wk); purple (12 wk); blue (14 wk); gray (18 wk); red (22 wk); brown (30 wk). Colored connective lines represent the neutralization response in time. Black arrow indicates time of vaccination; colored arrows indicate time of challenge matched to inter-immunization and challenge interval color in figure legend. **(a)** One way ANOVA, followed by Friedman test for multiple comparisons **(b)** Mann-Whitney test; * p=/< 0.05; ** p=/< 0.01; *** p=< 0.001.



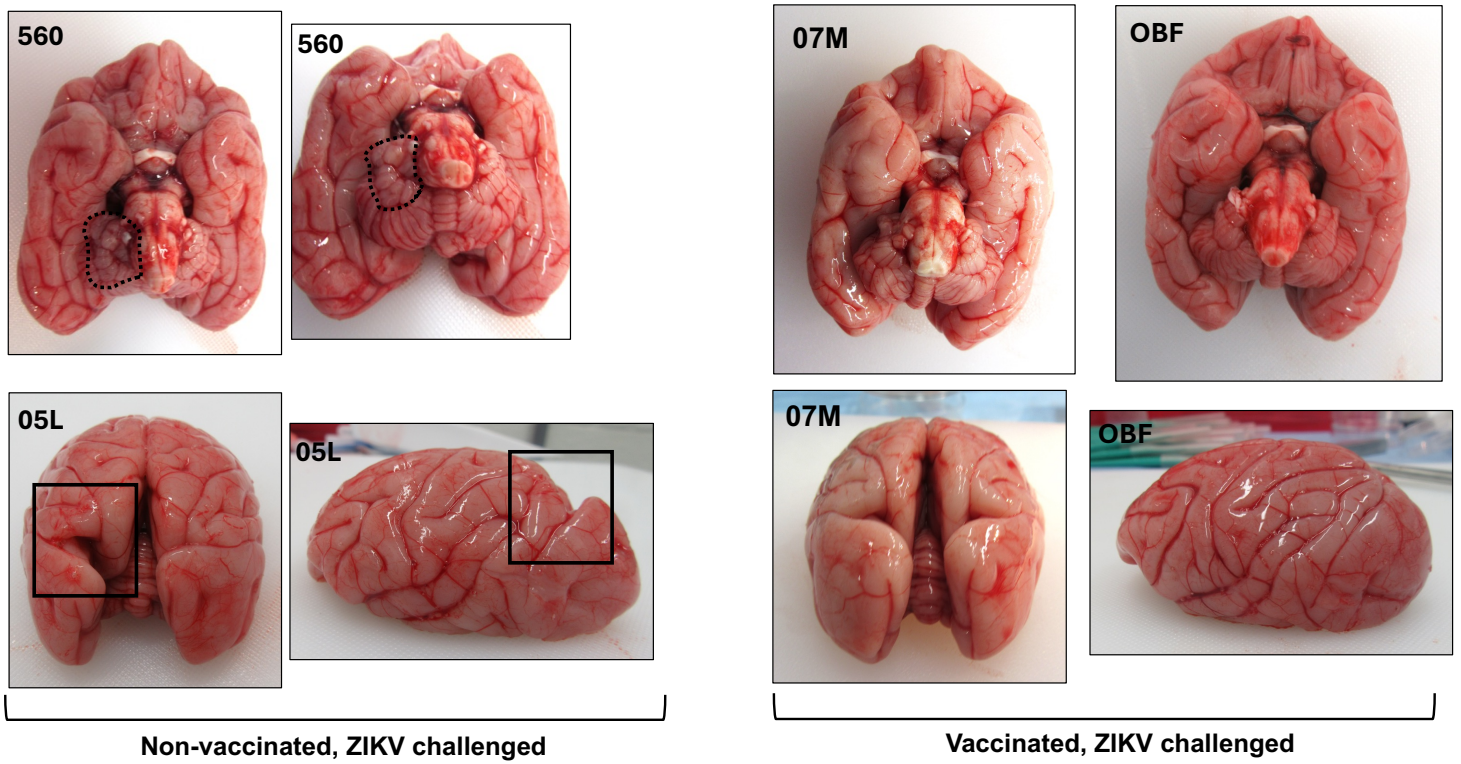
Suppl. Fig. 3. NS1-specific antibody titers in sera of dams pre- and post-ZIKV challenge. NS1-specific antibody titers in serum isolated pre-challenge and 4 weeks post-challenge from dams immunized with 10^{11} vp Ad26.M.Env (n=9) or non-vaccinated control animals (n=5), determined by using a commercially available ELISA kit. NS1-specific antibody titers are reported as the log10 of the inverse of the first dilution above 2x the background value of naïve sera. The mean response per group is indicated with a horizontal line. The dotted line represents the background level, defined as one dilution step below the start dilution of the samples (0.92 log10). Individual animals of group 1 (Ad26.M.Env immunized) are color coded to represent number of weeks between immunization and challenge; green (10wk); purple (12 wk); blue (14 wk); gray (18 wk); red (22 wk); brown (30 wk). Mann-Whitney test.



Suppl. Fig. 4. Perinatal ultrasonography of fetal macaques during gestation. Fetal development was monitored perinatally every 2 weeks via ultrasound by measuring (**a**) head circumference (**b**) biparietal diameter (**c**) femur length and (**d)** occipitofrontal diameter. Each symbol represents a fetal measurement at the indicated gestational age in days. All fetuses had at least 2 measurements for each parameter, but not every fetus had all measurements at each assessment. Red (non-vaccinated, ZIKV challenged); Blue (Ad26.M.Env vaccinated, ZIKV challenged); Black (non-vaccinated, non-challenged).



Suppl. Fig. 5. Placental and brain weights to fetal weight and placental pathology. Fetal, placental, and brain weights of unfixed specimens at time of necropsy for controls (n=3); vaccinated and challenged (n=9); non-vaccinated, challenged (n=5) dams and fetuses where available. Symbols are representative of individual weights or matched ratios. Fetal brain to body weight ratio and fetal weight to placental weight ratios across groups. For some organs, weights were not captured prior to fixation and therefore individual weights and ratios are not included. All comparisons were non-statistically significant (n.s.) by one-way ANOVA followed by Kruskal-Wallis test. Representative placental pathology from non-vaccinated and Ad.26.M.Env vaccinated dams showing material infarction in placentas at term in both groups.



Suppl. Fig. 6. Gross brain abnormalities in non-vaccinated, ZIKV infected fetal macaques. Images of non-fixed fetal brain specimens at time of necropsy from fetuses (560) and (05L) born to ZIKV challenged, non-vaccinated dams and fetuses (07M and OBF) born to dams Ad26.M.Env vaccinated prior to ZIKV challenge. Fetus 560 has a cerebellar malformation (dashed black line); Fetus 05L has asymmetry of the left parietal lobe (black box).