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SUPPLEMENTARY MATERIALS

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VACCINES

Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys

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Zika virus (ZIKV) is responsible for a major ongoing epidemic in the Americas and has been causally associated with fetal microcephaly. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority. Here we demonstrate that three different vaccine platforms protect against ZIKV challenge in rhesus monkeys. A purified inactivated virus vaccine induced ZIKV-specific neutralizing antibodies and completely protected monkeys against ZIKV strains from both Brazil and Puerto Rico. Purified immunoglobulin from vaccinated monkeys also conferred passive protection in adoptive transfer studies. A plasmid DNA vaccine and a single-shot recombinant rhesus adenovirus serotype 52 vector vaccine, both expressing ZIKV premembrane and envelope, also elicited neutralizing antibodies and completely protected monkeys against ZIKV challenge. These data support the rapid clinical development of ZIKV vaccines for humans.

The explosive and unprecedented ZIKV outbreak in the Americas (1, 2) prompted the World Health Organization to declare this epidemic a public health emergency of international concern. ZIKV has been causally associated with fetal microcephaly, intrauterine growth retardation, and other congenital malformations in both humans (3–6) and mice (7–9), and it has also been linked with neurologic disorders such as Guillain-Barre syndrome (10). Several reports have shown that ZIKV can infect placental and fetal tissues, leading to prolonged viremia in pregnant women (11) and nonhuman primates (12). ZIKV also appears to target cortical neural progenitor cells (7–9, 13, 14), which likely contributes to neuropathology.

We recently reported the protective efficacy of two vaccines against ZIKV challenges in mice: a purified inactivated virus (PIV) vaccine from ZIKV strain PRVABC59 and a DNA vaccine expressing an optimized premembrane and envelope (prM-Env) immunogen from ZIKV strain BeH815744 (15). These studies used ZIKV challenge strains from Brazil (ZIKV-BR; Brazil/ZK/2015) (9) and Puerto Rico (ZIKV-PR; PRVABC59). ZIKV replication in

mice was dependent on the mouse strain (15) and may be less extensive than in nonhuman primates (12). We therefore evaluated the immunogenicity and protective efficacy of inactivated virus, DNA-based, and vector-based vaccines against ZIKV challenge in rhesus monkeys.

ZIKV PIV vaccine study

We first immunized 16 rhesus monkeys by the subcutaneous route with 5 μ g of ZIKV PIV vaccine plus alum ($n = 8$) or sham vaccine (alum only) ($n = 8$) at weeks 0 and 4 (fig. S1). All PIV-vaccinated animals developed ZIKV Env-specific binding antibodies, as measured by enzyme-linked immunosorbent assays (ELISAs), and ZIKV-specific neutralizing antibodies, as measured by micro-neutralization (MN50) assays, at week 2 after initial immunization. Median log antibody titers at week 2 were 1.87 (Fig. 1A) and 2.27 (Fig. 1B) in ELISAs and MN50 assays, respectively. After the week 4 boost immunization, median log antibody titers increased substantially to 3.54 (Fig. 1A) and 3.66 (Fig. 1B), respectively, at week 6. In contrast, sham control monkeys did not develop detectable ZIKV-specific antibody responses (fig. S2).

Binding antibody titers correlated with neutralizing antibody titers in the PIV-vaccinated animals ($P < 0.0001$, coefficient of correlation $R = 0.88$, Spearman rank correlation test; fig. S3). Only minimal antibody-dependent cellular phagocytosis responses were observed. The majority of PIV-vaccinated monkeys (Fig. 1, C and D), but not sham control animals (fig. S4), also developed modest cellular immune responses, primarily to Env, as measured by interferon (IFN)- γ enzyme-linked immunospot (ELISPOT) assays.

To assess the protective efficacy of the PIV vaccine against ZIKV challenge, we infected PIV-immunized and sham control monkeys by the subcutaneous route with 10^6 viral particles [vp; 10^3 plaque-forming units (PFU)] of ZIKV-BR or ZIKV-PR ($n = 4$ per group) (15). Viral loads after ZIKV challenge were quantitated by reverse transcription polymerase chain reaction (15), and viral infectivity was confirmed by growth in Vero cells. ZIKV-specific MN50 titers increased after challenge, particularly in the sham controls (fig. S5). Sham control monkeys exhibited 6 to 7 days of detectable viremia, with median peak viral loads of 5.82 log copies/ml (range, 5.21 to 6.29 log copies/ml; $n = 8$) on day 3 to 5 after challenge (Fig. 2A). Virus was also detected in the majority of sham control animals in urine and cerebrospinal fluid (CSF) on day 3, as well as in colorectal secretions and cervicovaginal secretions on day 7 (Fig. 2, B to E). In contrast, PIV-vaccinated monkeys showed complete protection against ZIKV challenge, as evidenced by no detectable virus (<100 copies/ml) in the blood, urine, CSF, colorectal secretions, or cervicovaginal secretions in any animal after challenge ($n = 8$; $P = 0.0002$, Fisher's exact test comparing PIV-vaccinated animals with sham controls). We were unable to assess ZIKV in semen in the male animals in this study because of inadequate sample volumes. No major differences in plasma viral loads were observed between the sham controls that received ZIKV-BR versus those that received ZIKV-PR (fig. S6).

Adoptive transfer studies

We next explored the mechanism of the observed protection through adoptive transfer studies.

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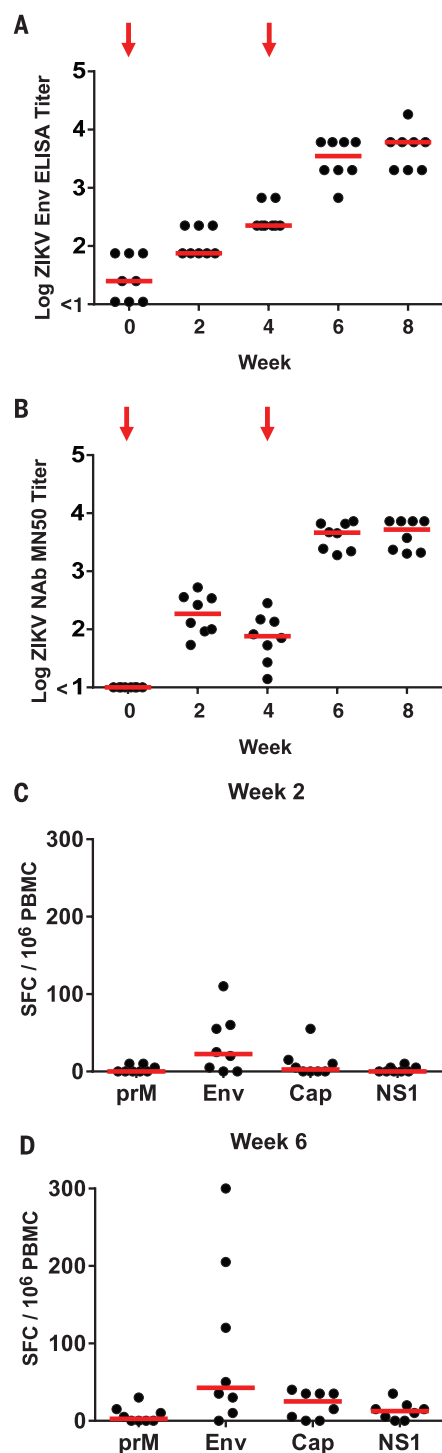


Fig. 1. Immunogenicity of the ZIKV PIV vaccine. (A) ZIKV Env-specific ELISA titers and (B) ZIKV-specific MN50 titers after immunization of rhesus monkeys by the subcutaneous route with 5 µg of PIV vaccine at weeks 0 and 4 (red arrows). The maximum measurable log MN50 titer in this assay was 3.86. NAb, neutralizing antibody. (C) Cellular immune responses measured by IFN-γ ELISPOT assays for prM, Env, Cap (capsid), and NS1 (nonstructural protein 1) at week 2 and (D) week 6. Red bars reflect medians. SFC, spot-forming cells. PBMC, peripheral blood mononuclear cells.

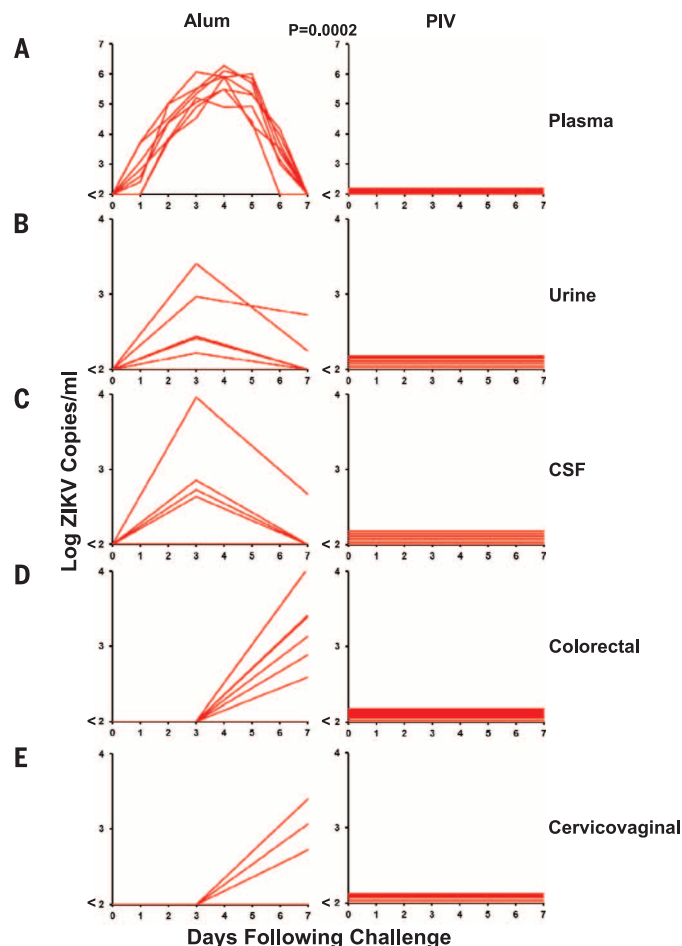


Fig. 2. Protective efficacy of the ZIKV PIV vaccine. PIV-vaccinated and sham control (alum-vaccinated) rhesus monkeys ($n = 8$ per group) were challenged by the subcutaneous route with 10^6 vp (10^3 PFU) of ZIKV-BR or ZIKV-PR. Each group contained six female and two male animals. Viral loads are shown for (A) plasma, (B) urine, (C) CSF, (D) colorectal secretions, and (E) cervicovaginal secretions. Viral loads were determined on days 0, 1, 2, 3, 4, 5, 6, and 7 for the plasma samples (A) and on days 0, 3, and 7 for the other samples [(B) to (E)]. Data are shown for all eight animals, except in the case of cervicovaginal secretions (E), where data for the six females are shown. The P value was determined by Fisher's exact test.

Using protein G affinity chromatography, we purified immunoglobulin G (IgG) from plasma from PIV-vaccinated monkeys at week 8. Vaccine-elicited, ZIKV-specific IgG was then infused into four groups of naïve Balb/c mice ($n = 5$ per group) as fivefold serial dilutions of the purified IgG preparation, which had a log ELISA titer of 3.30 and a log MN50 titer of 3.30. After infusion, these groups of recipient mice (designated I, II, III, and IV) had median log ELISA titers of 2.83, 2.35, 1.40, and <1.00 (Fig. 3A) and median log MN50 titers of 2.93, 1.77, 1.14, and <1.00 (Fig. 3B), respectively. Mice were then challenged by the intravenous route with 10^5 vp (10^2 PFU) of ZIKV-BR, as we have previously described (15). The higher two doses of purified IgG provided complete protection after ZIKV challenge, whereas the lower two doses of purified IgG resulted in reduced viremia compared with sham-infused control mice (Fig. 3, C to E).

Vaccine-elicited, ZIKV-specific IgG was also infused into two groups of naïve rhesus monkeys ($n = 2$ per group). After infusion, these groups of recipient monkeys (designated I and II) had respective median log MN50 titers of 2.11 and 1.22 (Fig. 4A). Monkeys were then challenged with 10^6 vp (10^3 PFU) of ZIKV-BR. In the animals that received the higher IgG dose, one animal was completely protected and the other showed a blip of viremia on days 3 to 5 (Fig. 4B). No en-

hancement of viral replication was observed at subtherapeutic IgG concentrations. Taken together, these data demonstrate that purified IgG from PIV-vaccinated rhesus monkeys provided passive protection against ZIKV challenge after adoptive transfer to both rodents and primates.

ZIKV DNA and adenovirus vaccine study

To evaluate the immunogenicity and protective efficacy of DNA- and vector-based ZIKV vaccines, we immunized 12 rhesus monkeys with a plasmid DNA vaccine (15) or a rhesus adenovirus serotype 52 (RhAd52) vector-based vaccine (16) (fig. S1). Monkeys were immunized by the intramuscular route with 5 mg of DNA vaccine expressing prM-Env at weeks 0 and 4 ($n = 4$), with 10^{11} vp of RhAd52 vector expressing prM-Env as a single immunization at week 0 ($n = 4$), or with sham vaccine at weeks 0 and 4 ($n = 4$). The DNA-prM-Env vaccine induced ZIKV-specific neutralizing antibody titers in all animals after the week 4 boost immunization, although only minimal MN50 titers were detected after the initial priming immunization (Fig. 5A). In contrast, the RhAd52-prM-Env vaccine induced ZIKV-specific neutralizing antibody responses in all animals at week 2 after the initial priming immunization (Fig. 5A). Moreover, the RhAd52-prM-Env vaccine induced a substantial breadth of antibody responses against linear ZIKV Env epitopes, as measured by peptide

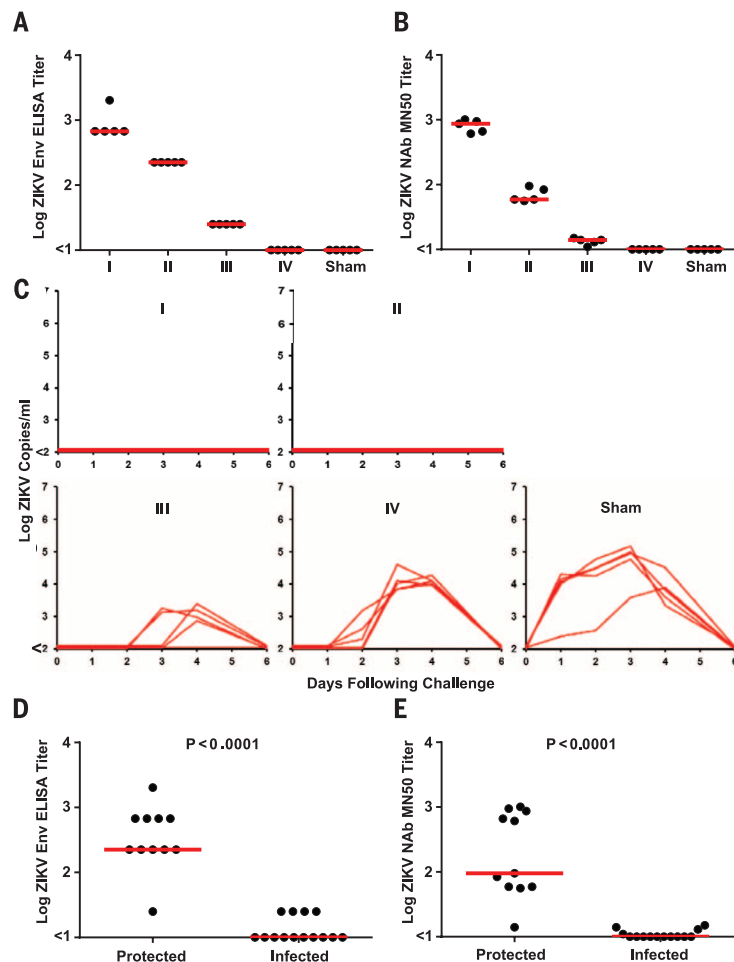


Fig. 3. Adoptive transfer studies in mice. (A) ZIKV Env-specific ELISA titers and (B) ZIKV-specific MN50 titers in serum from recipient Balb/c mice ($n = 5$ per group), measured 1 hour after adoptive transfer of fivefold serial dilutions of IgG purified from PIV-vaccinated rhesus monkeys (groups I, II, III, and IV) or sham controls. (C) Plasma viral loads in mice after challenge with 10^5 vp (10^2 PFU) of ZIKV-BR. (D and E) Immune correlates of protection and infection. Red bars reflect medians. P values were determined by t tests.

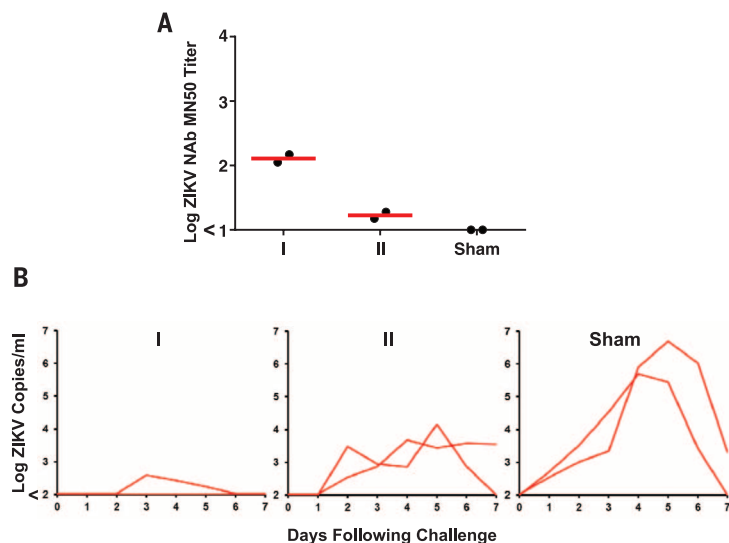


Fig. 4. Adoptive transfer studies in rhesus monkeys. (A) ZIKV-specific MN50 titers in serum from recipient rhesus monkeys ($n = 2$ per group), measured 1 hour after adoptive transfer of fivefold dilutions of IgG purified from PIV-vaccinated rhesus monkeys (groups I and II) or sham controls. (B) Plasma viral loads in rhesus monkeys after challenge with 10^6 vp (10^3 PFU) of ZIKV-BR. Red bars reflect medians.

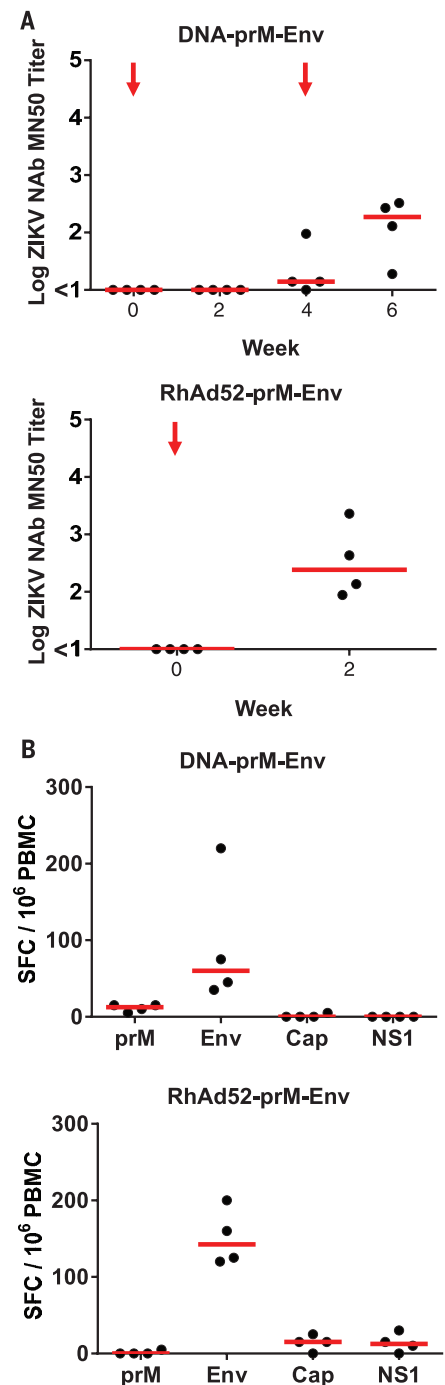


Fig. 5. Immunogenicity of the ZIKV DNA-prM-Env and RhAd52-prM-Env vaccines. (A) ZIKV-specific MN50 titers measured after immunization of rhesus monkeys by the intramuscular route with 5 mg of DNA-prM-Env vaccine at weeks 0 and 4 (red arrows) or with 10^{11} vp RhAd52-prM-Env as a single immunization at week 0. (B) Cellular immune responses measured by IFN- γ ELISPOT assays for prM, Env, Cap, and NS1 at week 6 for the DNA-prM-Env vaccine or at week 4 for the RhAd52-prM-Env vaccine. Red bars reflect medians.

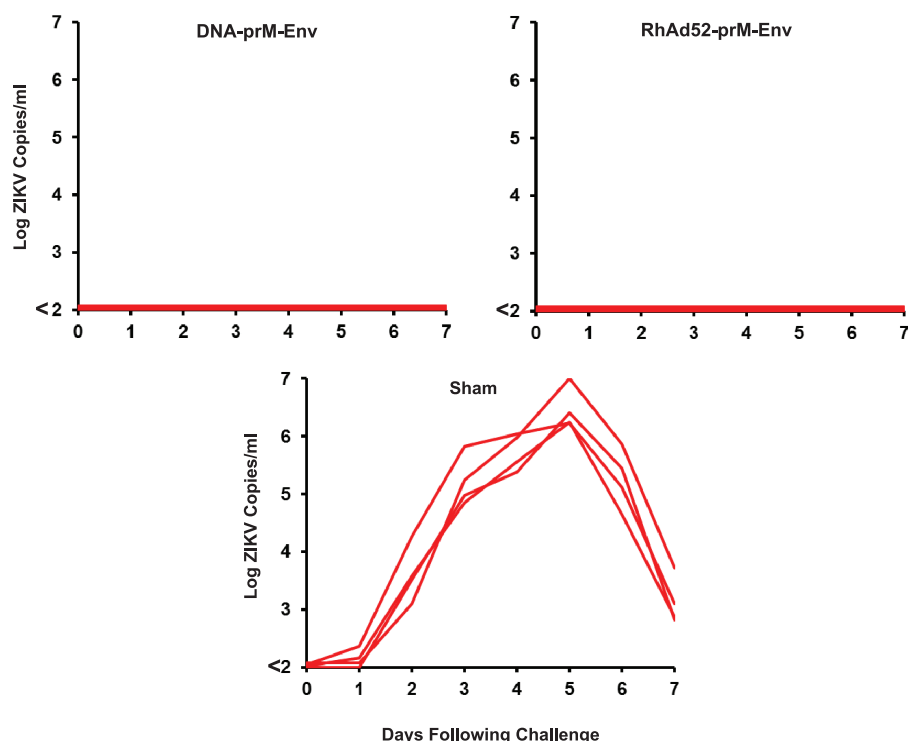


Fig. 6. Protective efficacy of the ZIKV DNA-prM-Env and RhAd52-prM-Env vaccines. Rhesus monkeys vaccinated with the DNA-prM-Env vaccine, the RhAd52-prM-Env vaccine, or sham vaccine ($n = 4$ per group) were challenged by the subcutaneous route with 10^6 vp (10^3 PFU) of ZIKV-BR. Plasma viral loads are shown.

microarray assays, compared with the other vaccines tested (17) (fig. S7). The DNA-prM-Env vaccine also induced detectable Env-specific IFN- γ ELISPOT responses after the week 4 boost immunization, and the RhAd52-prM-Env vaccine induced Env-specific cellular immune responses after the initial week 0 priming immunization (Fig. 5B). Monkeys were challenged 4 weeks after the final vaccination. Both the DNA- and the RhAd52-prM-Env vaccines provided complete protection against subcutaneous challenge with 10^6 vp (10^3 PFU) of ZIKV-BR, as measured by plasma viral loads (Fig. 6).

Discussion

We demonstrate that three different vaccine platforms provide complete protection against ZIKV challenge in rhesus monkeys. No specific clinical safety adverse effects related to the vaccines were observed. We recently reported the protective efficacy of the PIV vaccine and the DNA-prM-Env vaccine in mice (15). The present data confirm and extend these prior studies by demonstrating robust protection against ZIKV challenge in nonhuman primates and specifically using the vaccination doses, routes, and schedules that are typically evaluated in clinical trials. Although the PIV vaccine and the DNA-prM-Env vaccine appeared comparably immunogenic in mice (15), the former proved more potent in rhesus monkeys under the conditions tested (Figs. 1 and 5). To generalize these observations to a vector-based vaccine, we also evaluated the RhAd52-prM-Env vaccine, which proved highly immunogenic and afforded complete protection after a single

immunization in monkeys (Fig. 5). Rhesus adenovirus vectors have the potential advantage of minimal baseline vector-specific neutralizing antibodies in human populations (16).

The adoptive transfer studies demonstrate that vaccine-elicited antibodies are sufficient for protection against ZIKV challenge. Moreover, passive protection in mice and rhesus monkeys was observed at relatively low antibody titers (Figs. 3 and 4). Such antibody titers are likely achievable in humans with these vaccine platforms, thus raising optimism for the development of a ZIKV vaccine for humans. Future preclinical and clinical studies will need to address the potential impact of cross-reactive antibodies against dengue virus and other flaviviruses. Secondary infection with a heterologous dengue serotype can be clinically more severe than initial infection, which may or may not reflect antibody-dependent enhancement (18, 19). Cross-reactive antibodies for ZIKV and dengue virus have also been described (20, 21), and dengue-specific antibodies have been reported to increase ZIKV replication in vitro (22). The relevance and implications of these findings for ZIKV vaccine development remain to be determined.

The consistent and robust antibody-based correlates of vaccine protection against ZIKV challenge in both rodents and primates suggest the generalizability of these findings. Similar correlates of protection, and specifically neutralizing antibody titers >10 , have been reported for other flavivirus vaccines in humans (23–25). Together, these data suggest a path forward for clinical development of ZIKV vaccines in humans. PIV

vaccines have been evaluated previously in clinical trials for other flaviviruses, including dengue virus, tick-borne encephalitis virus, and Japanese encephalitis virus (26–30). Phase 1 clinical trials with the ZIKV PIV vaccine, as well as other candidate ZIKV vaccines, are expected to begin later this year.

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SUPPLEMENTARY MATERIALS

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Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys

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Editor's Summary

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Supplementary Materials for

Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys

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Materials and Methods

Animals, vaccines, and challenges. 34 outbred, Indian-origin male and female rhesus monkeys (*Macaca mulatta*) were housed at Bioqual, Rockville, MD. In the first vaccine study, monkeys were immunized by the s.c. route with 5 μ g ZIKV purified inactivated virus (PIV) vaccine derived from the PRVABC59 isolate (15) with alum (Alhydrogel; Brenntag Biosector, Denmark) or alum alone at weeks 0 and 4 (N=8/group). In the second vaccine study, monkeys were immunized by the i.m. route with 5 mg DNA vaccines expressing prM-Env (amino acids 216–794 of the polyprotein derived from the BeH815744 isolate and optimized for high expression) (15) at weeks 0 and 4, a single immunization of 10^{11} VP RhAd52 (16) expressing prM-Env at week 0, or sham controls (N=4/group). Rhesus monkeys were challenged four weeks after the final immunization by the s.c route with 10^6 viral particles (VP) [10^3 plaque-forming units (PFU)] ZIKV-BR (Brazil ZKV2015) or ZIKV-PR (PRVABC59) (15). For adoptive transfer studies, Balb/c mice were infused i.v. with IgG purified from PIV vaccinated monkeys at week 8 and were challenged by the i.v. route with 10^5 VP (10^2 PFU) ZIKV-BR. Rhesus monkeys were infused i.v. with IgG purified from PIV vaccinated monkeys at week 8 and were challenged by the s.c. route with 10^6 VP (10^3 PFU) ZIKV-BR. Animals were randomly allocated to groups. Immunologic and virologic assays were performed blinded. All animal studies were approved by the appropriate Institutional Animal Care and Use Committee (IACUC).

RT-PCR. RT-PCR assays were utilized to monitor viral loads, essentially as previously described (15). RNA was extracted from plasma or other samples with a

QIAcube HT (Qiagen, Germany). The wildtype ZIKV BeH815744 Cap gene was utilized as a standard. RNA was purified (Zymo Research, CA, USA), and RNA quality and concentration was assessed by the BIDMC Molecular Core Facility. Log dilutions of the RNA standard were reverse transcribed and included with each RT-PCR assay. Viral loads were calculated as virus particles (VP) per ml and were confirmed by PFU assays. Assay sensitivity was 100 copies/ml.

PFU assay. Vero WHO cells were seeded in a MW6 plate to reach confluency at day 3. Cells were infected with log dilutions of ZIKV for 1 h and overlaid with agar. Cells were stained after 6 days of infection by neutral red staining. Plaques were counted, and titers were calculated by multiplying the number of plaques by the dilution and divided by the infection volume.

ELISA. Monkey ZIKV Env ELISA kits (Alpha Diagnostic International, TX, USA) were used to determine endpoint binding antibody titers using a modified protocol. 96-well plates coated with ZIKV Env protein were first equilibrated at room temperature with 300 µl of kit working wash buffer for 5 min. 6 µl of monkey serum was added to the top row, and 3-fold serial dilutions were tested in the remaining rows. Samples were incubated at room temperature for 1 h, and plates washed 4 times. 100 µl of anti-human IgG HRP-conjugate working solution was then added to each well and incubated for 30 min at room temperature. Plates were washed 5 times, developed for 15 min at room temperature with 100 µl of TMB substrate, and stopped by the addition of 100 µl of stop solution. Plates were analyzed at 450nm/550nm on a VersaMax microplate reader using Softmax Pro 6.0 software (Molecular Devices, CA, USA). ELISA endpoint titers were

defined as the highest reciprocal serum dilution that yielded an absorbance >2-fold over background values. Log₁₀ endpoint titers are reported.

Neutralization assay. A high-throughput ZIKV microneutralization (MN) assay was utilized for measuring ZIKV-specific neutralizing antibodies, essentially as previously described (15). Briefly, serum samples were serially diluted three-fold in 96-well micro-plates, and 100 µl of ZIKV-PR containing 100 PFU were added to 100 µl of each serum dilution and incubated at 35°C for 2 h. Supernatants were then transferred to microtiter plates containing confluent Vero cell monolayers (World Health Organization, NICSC-011038011038). After incubation for 4 d, cells were fixed with absolute ethanol:methanol for 1 h at –20°C and washed three times with PBS. The pan-flavivirus monoclonal antibody 6B6-C1 conjugated to HRP (6B6-C1 was a gift from JT Roehrig, CDC) was then added to each well, incubated at 35°C for 2 h, and washed with PBS. Plates were washed, developed with 3,3',5,5'-tetramethylbenzidine (TMB) for 50 min at room temperature, stopped with 1:25 phosphoric acid, and absorbance was read at 450 nm. For a valid assay, the average absorbance at 450 nm of three non-infected control wells had to be ≤ 0.5, and virus-only control wells had to be ≥ 0.9. Normalized absorbance values were calculated, the MN50 titer was determined by a log mid-point linear regression model. The MN50 titer was calculated as the reciprocal of the serum dilution that neutralized ≥ 50% of ZIKV, and seropositivity was defined as a titer ≥ 10, with the maximum measurable titer 7,290. Log₁₀ MN50 titers are reported.

Antibody peptide microarrays. IgG binding to linear peptides spanning ZIKV Env was measured with peptide microarrays (JPT Peptide Technologies, Berlin, Germany), essentially as previously described (17). Briefly, microarrays consisted of 3

identical subarrays containing 153 overlapping 15 amino acid ZIKV Env peptides, which covered 98.2% of available ZIKV Env sequences. Serum was incubated with the microarrays and Alexa Fluor 647-conjugated anti-human IgG. The readout and image processing was performed with Genepix 4300A scanner/software. Mean fluorescent intensity (MFI) equaled the mean of triplicate peptides and was corrected by subtracting values from matched peptides on control microarrays incubated with secondary antibody alone. The threshold for positivity was >5x noise distribution of the sample size.

ELISPOT. ZIKV-specific cellular immune responses were assessed by interferon- γ (IFN- γ) ELISPOT assays using pools of overlapping 15-amino-acid peptides covering the prM, Env, Cap, and NS1 proteins (JPT, Berlin, Germany), essentially as we previously described (15). 96-well multiscreen plates (Millipore, MA, USA) were coated overnight with 100 μ l/well of 10 μ g/ml anti-human IFN- γ (BD Biosciences, CA, USA) in endotoxin-free Dulbecco's PBS (D-PBS). The plates were then washed three times with D-PBS containing 0.25% Tween 20 (D-PBS-Tween), blocked for 2 h with D-PBS containing 5% FBS at 37°C, washed three times with D-PBS-Tween, rinsed with RPMI 1640 containing 10% FBS to remove the Tween 20, and incubated with 2 μ g/ml of each peptide and 2×10^5 monkey PBMC in triplicate in 100 μ l reaction mixture volumes. Following an 18 h incubation at 37°C, the plates were washed nine times with PBS-Tween and once with distilled water. The plates were then incubated with 2 μ g/ml biotinylated anti-human IFN- γ (BD Biosciences, CA, USA) for 2 h at room temperature, washed six times with PBS-Tween, and incubated for 2 h with a 1:500 dilution of streptavidin-alkaline phosphatase (Southern Biotechnology Associates, AL, USA). Following five washes with PBS-Tween and one with PBS, the plates were developed

with nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl-phosphate chromogen (Pierce, IL, USA), stopped by washing with tap water, air dried, and read using an ELISPOT reader (Cellular Technology Ltd., OH, USA). The numbers of spot-forming cells (SFC) per 10^6 cells were calculated. The medium background levels were typically <15 SFC per 10^6 cells.

IgG purification and adoptive transfer. Polyclonal IgG was purified from plasma from PIV vaccinated monkeys at week 8 using protein G purification kits and pooled (Thermo Fisher Scientific, MA, USA). The purified IgG preparation had a log ELISA titer of 3.30 and a log MN50 titer of 3.30. Purified IgG was infused into groups of naïve recipient Balb/c mice or rhesus monkeys by 5-fold serial dilutions prior to ZIKV-BR challenge. Mice received 200, 40, 8, 1.5, or 0 μ l of the IgG preparation. Monkeys received 10, 2, or 0 ml of the IgG preparation.

Statistical analyses. Analysis of virologic and immunologic data was performed using GraphPad Prism v6.03 (GraphPad Software, CA, USA). Comparisons of groups was performed using t-tests and Wilcoxon rank-sum tests. Correlations were assessed by Spearman rank-correlation tests.

Author Contributions

P.A., R.A.L., N.L.M., S.J.T., and D.H.B. designed the studies. P.A., M.B., M.K., Z.L., D.N., O.N., R.N., and N.B.M. produced the DNA and Ad vaccines and conducted the virologic assays. R.A.D., K.M., R.G.J., K.H.E., N.L.M., and S.J.T. produced the PIV vaccines and conducted the virus neutralization assays. J.P.S.P. and P.M.A.Z. developed the challenge virus. J.M., B.F., and M.G.L. led the clinical care of the rhesus monkeys. P.A., R.A.L., C.A.B., E.T.M., E.N.B., A.A., A.L.B., C.C., A.C., P.B.G., D.J., J.J., B.C.L., S.M., K.M., M.S., G.H.N., K.E.S., and G.A. conducted the monkey and mouse studies and performed the immunologic assays. D.H.B. wrote the paper with all co-authors.

Supplementary Figure Legends

Figure S1. Vaccine schedules. Immunization and challenge schedules for the ZIKV purified inactivated virus (PIV) vaccine, plasmid DNA vaccine, and rhesus adenovirus serotype 52 (RhAd52) vaccine studies. Red arrows indicate vaccinations, and black arrows indicate ZIKV challenges. The numbers reflect study weeks.

Figure S2. MN50 titers in the sham controls in the ZIKV PIV vaccine study. ZIKV-specific microneutralization (MN50) titers following immunization of rhesus monkeys with sham (alum only) at weeks 0 and 4 (red arrows). Red bars reflect medians.

Figure S3. Correlation of binding and neutralizing antibody titers in the ZIKV PIV vaccine study. Correlations of binding ELISA titers and microneutralization (MN50) titers at weeks 2 and 6 combined from the ZIKV PIV vaccine study. P-value reflects a Spearman rank-correlation test.

Figure S4. IFN- γ ELISPOT assays in the sham controls in the ZIKV PIV vaccine study. Cellular immune responses by IFN- γ ELISPOT assays to prM, Env, Cap, and NS1 at week 2 and week 6 following immunization of rhesus monkeys with sham (alum only) at weeks 0 and 4. Red bars reflect medians.

Figure S5. MN50 titers following ZIKV challenge in the ZIKV PIV vaccine study. ZIKV-specific microneutralization (MN50) titers following ZIKV-BR challenge in

rhesus monkeys that received the ZIKV PIV vaccine or sham (alum only). The maximum measurable log MN50 titer in this assay was 3.86. Red bars reflect medians.

Figure S6. Viral loads in the ZIKV PIV vaccine study. Plasma viral loads in PIV vaccinated monkeys and sham controls following challenge with ZIKV-BR or ZIKV-PR (N=4/group).

Figure S7. ZIKV Env peptide microarrays. Serum samples from week 6 from rhesus monkeys immunized with ZIKV PIV, DNA-prM-Env, and RhAd52-prM-Env vaccines were assayed for linear antibody reactivity using ZIKV Env peptide microarrays. Colors indicate individual monkeys.

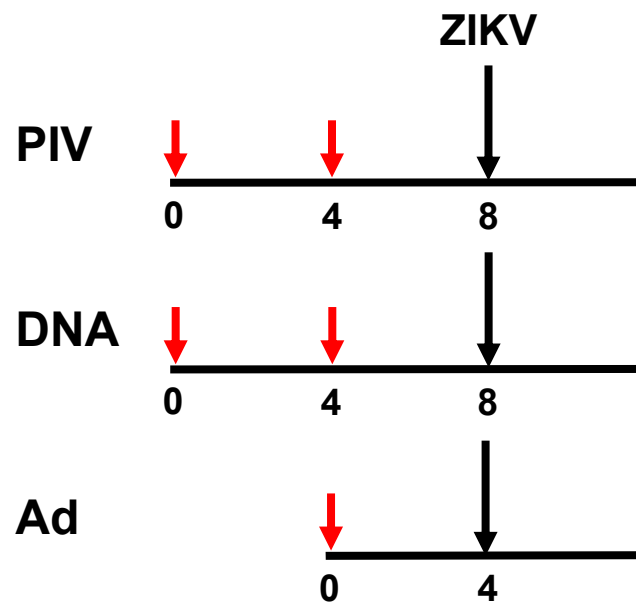


Figure S1

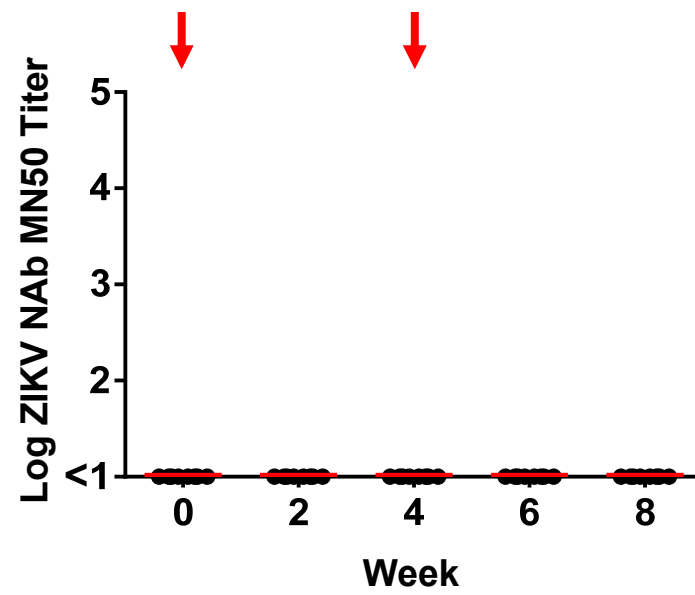


Figure S2

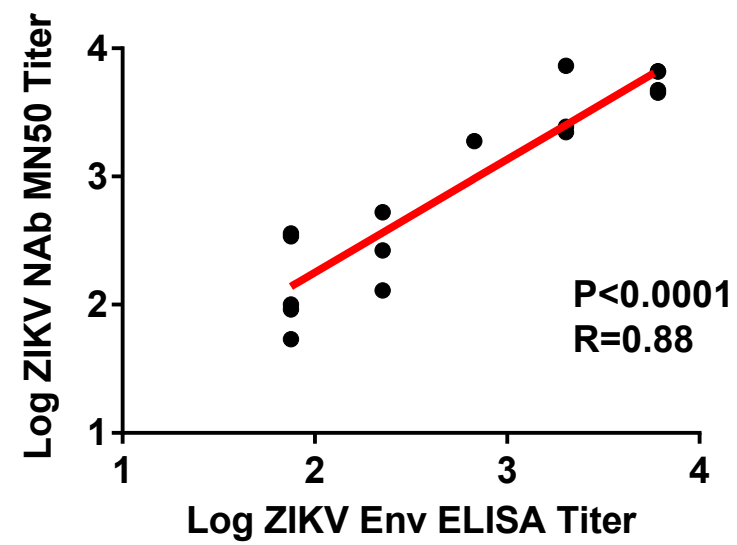


Figure S3

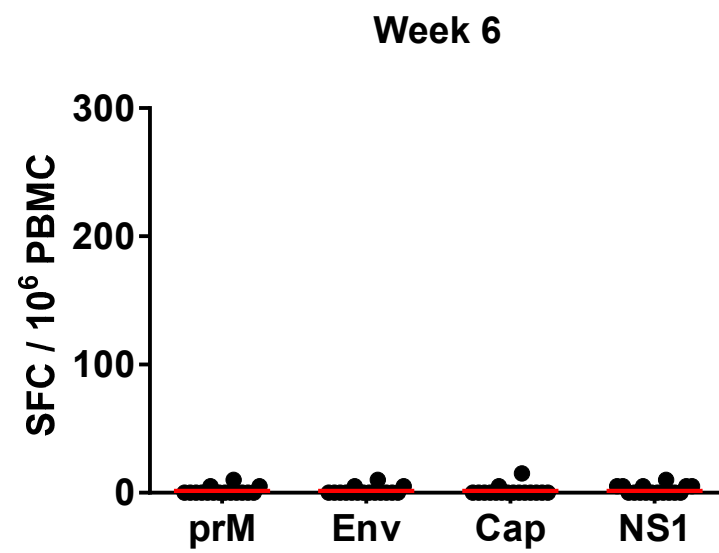
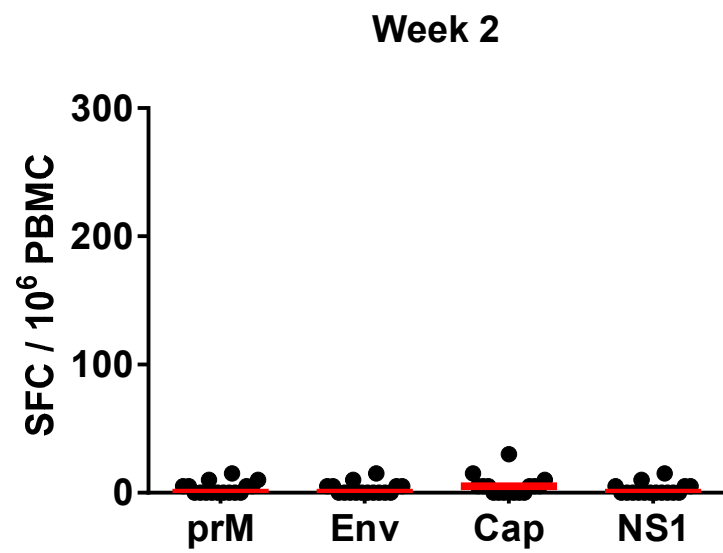
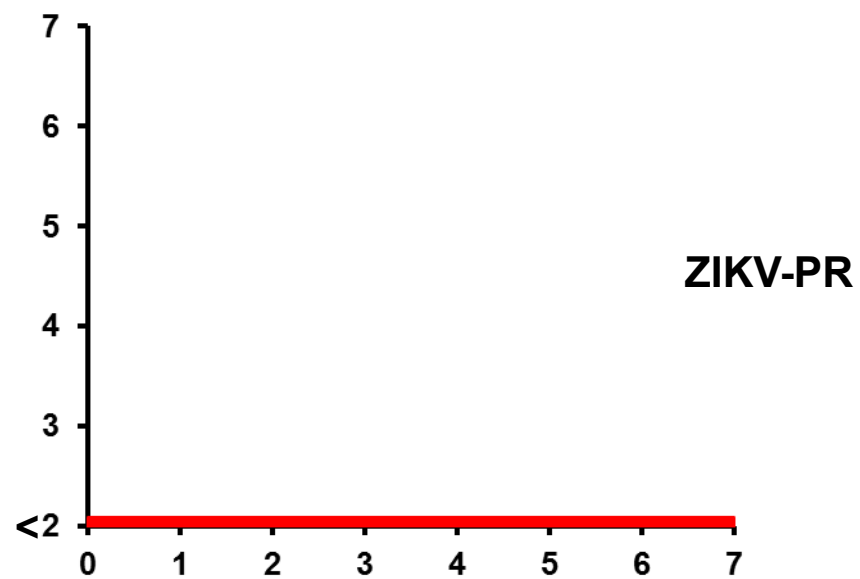
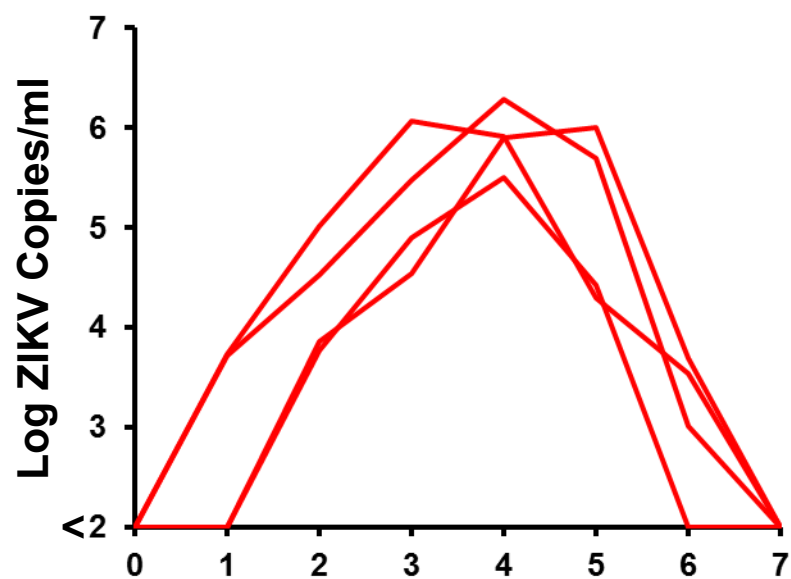
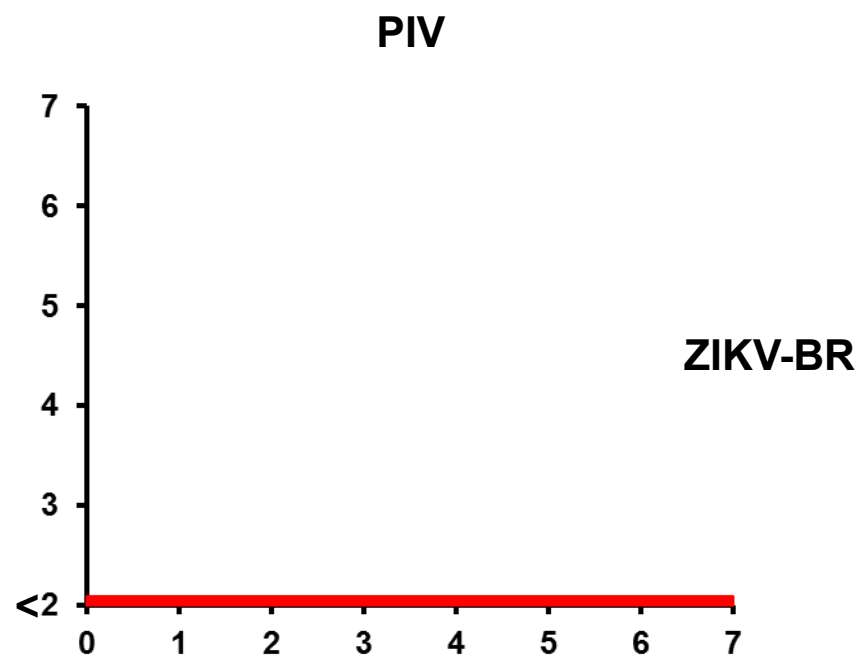
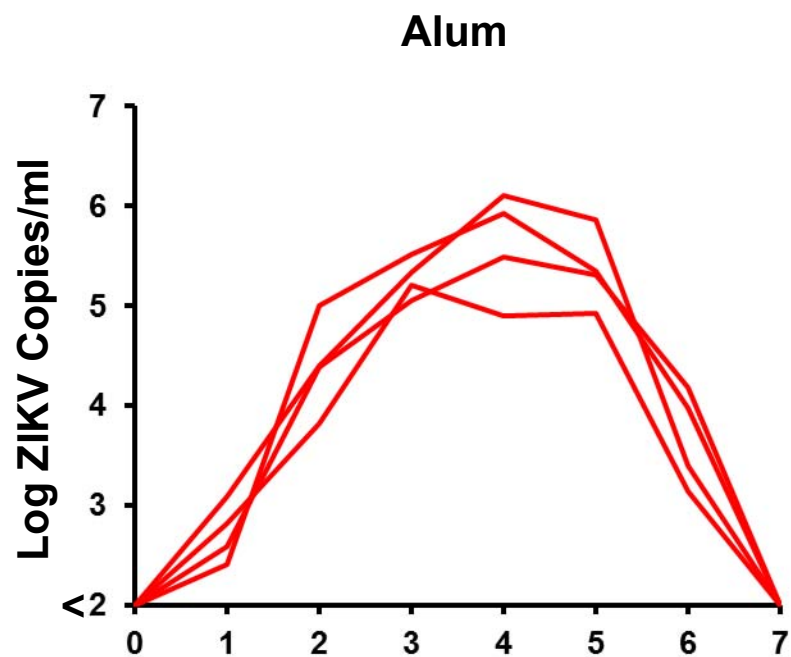


Figure S4



Days Following Challenge

Figure S6

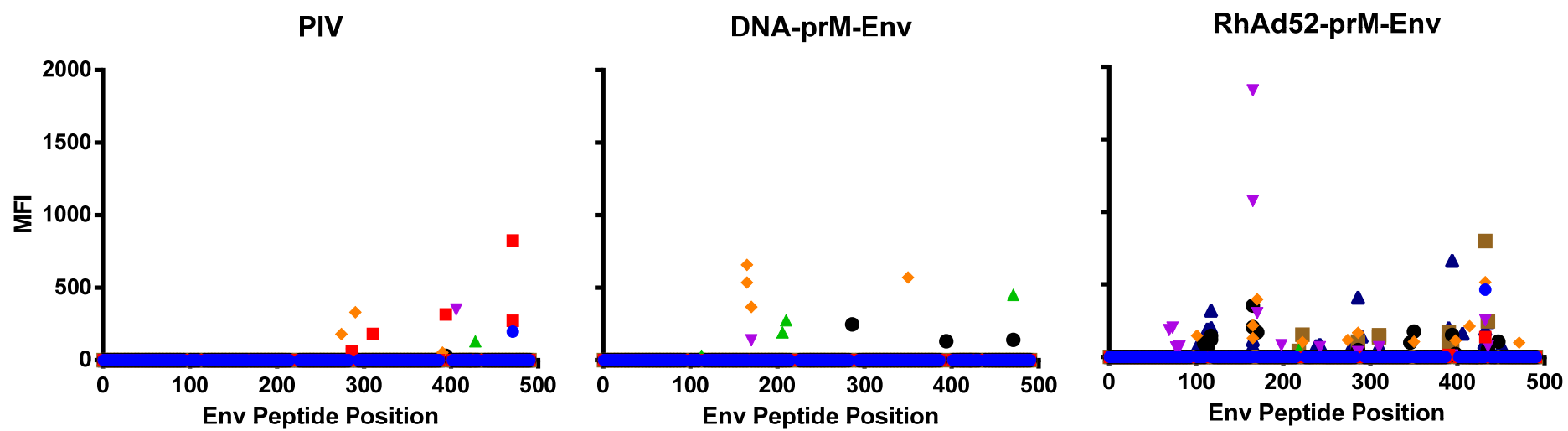


Figure S7

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