

Supplemental Information

A Combination of Two Human Monoclonal

Antibodies Prevents Zika Virus

Escape Mutations in Non-human Primates

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Mouse ID	Treatment	day 4	day 5	day 7
PO32-1	10-1074		wt	
PO32-B1	10-1074		wt	
PO32-B2	10-1074		wt	
PO32-B3	10-1074		wt	
PO38-A	10-1074	wt		wt
PO38-B	10-1074	wt		wt
PO38-C	10-1074	wt		wt
PO32-6	Z004		wt	
PO32-7	Z004		wt	
PO32-8	Z004		wt	
PO38-D	Z004	wt		
PO38-E	Z004	wt		
PO38-F	Z004	wt		
PO38-G	Z004	K394R		
PO38-H	Z004	wt		

Figure S1. ZIKV mutations in *Ifnar1*^{-/-} mice. Related to Figure 1.

Summary of the analysis of ZIKV EDIII sequences from infected mouse blood at the indicated time points. Empty cells represent sequences not determined, yellow cells are samples from symptomatic mice, wt is wild type EDIII sequence. The only identified mutation (K394R, in red) was in a mouse pre-treated with Z004.

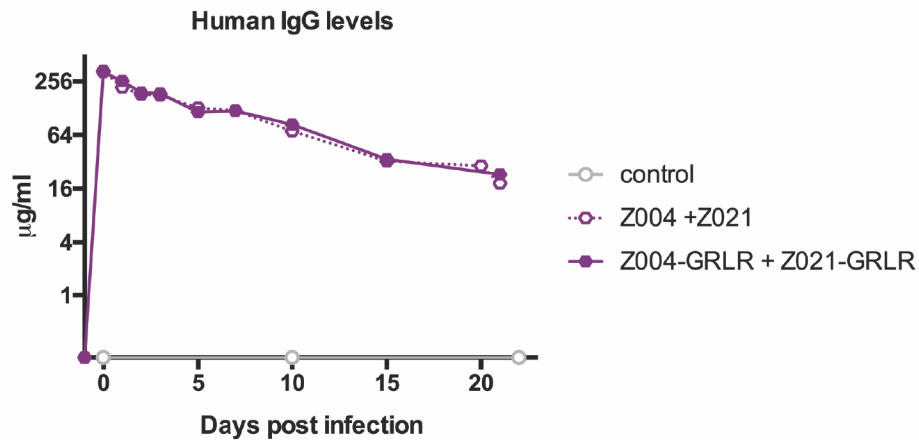
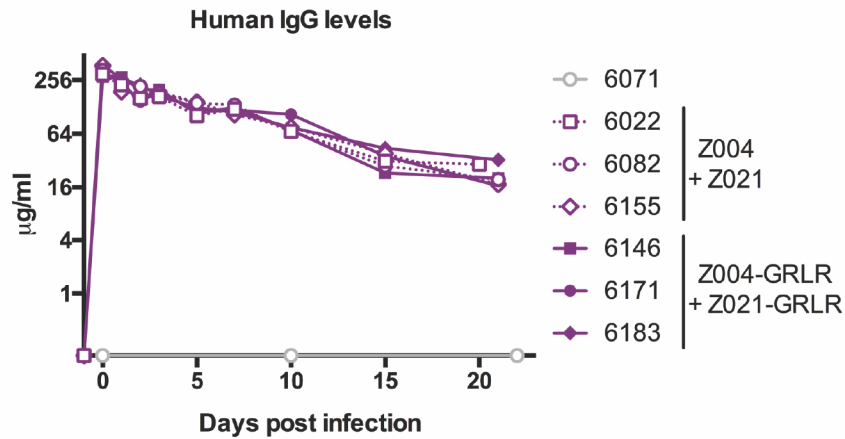


Figure S2. Human IgG levels in macaque plasma over time. Related to Figure 4.

The levels of human IgG antibodies were determined by ELISA. The left panel displays human IgGs in individual macaques, the right panel shows the mean for each group. Macaques were administered 15mg/kg of each of the antibodies on day -1. The mean peak antibody levels on the day of infection (day 0) were 334 $\mu\text{g/ml}$ in the Z004 + Z021 group and 326 $\mu\text{g/ml}$ in the Z004-GRLR + Z021-GRLR group. The antibody levels on day 15 were 32 $\mu\text{g/ml}$ in the Z004 + Z021 group and 34 $\mu\text{g/ml}$ in the Z004-GRLR + Z021-GRLR group, resulting in plasma half-lives of 4.4 and 4.6 days, respectively.

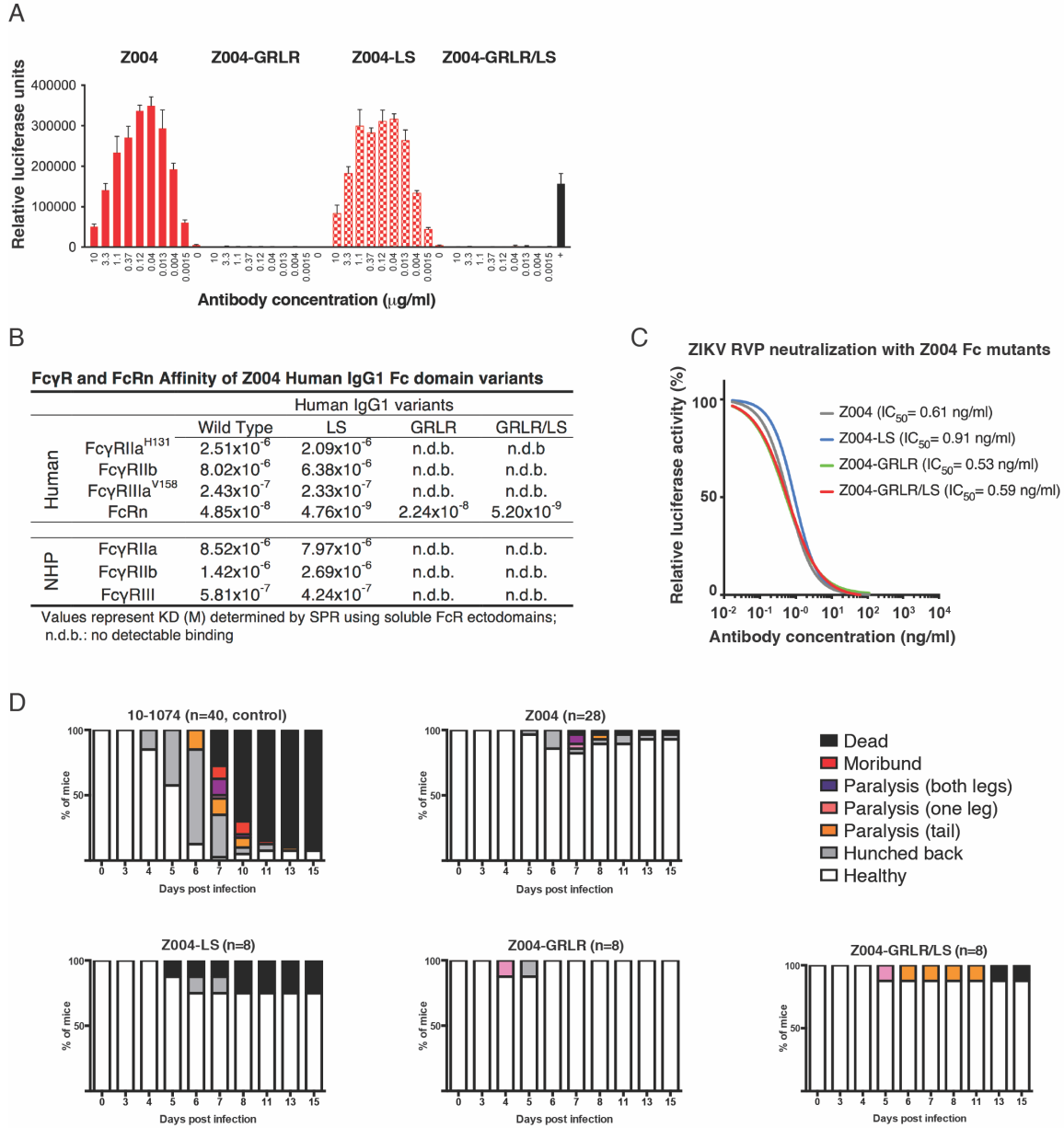


Figure S3. Z004 containing mutations in the antibody Fc portion that prevent Fcγ receptor binding (GRLR)(Horton et al., 2008; Lu et al., 2016) and extend the half-life (LS)(Ko et al., 2014; Zalevsky et al., 2010) remains effective against ZIKV. Related to Figure 4.

(A) Z004-mediated ADE of Fcγ receptor bearing K-562 cells is abrogated by GRLR and GRLR/LS substitutions. Data are represented as mean ± SD of triplicates. The positive control (+) is wild type Z004 antibody (10 ng/ml).

(B) Surface plasmon resonance (SPR) binding profile of Z004 bearing the LS and GRLR mutations alone or in combination.

(C-D) Z004 antibodies bearing the LS and GRLR substitutions alone or in combination remain effective against ZIKV RVPs *in vitro* (C) or ZIKV *in vivo* following prophylactic administration (D).

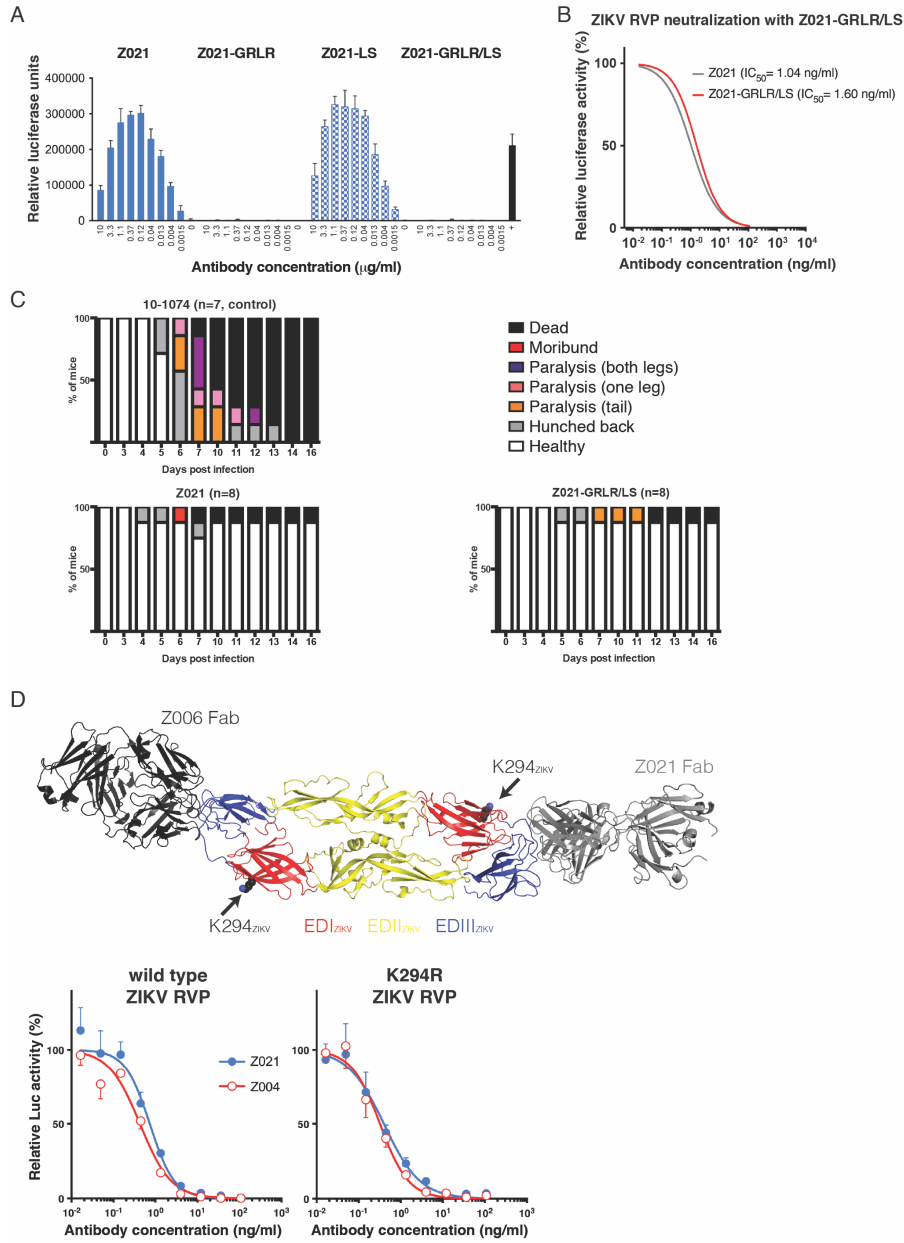


Figure S4. Z021 with substitutions that prevent Fc γ receptor binding (GRLR) and extend the half-life (LS) remains effective against ZIKV. Related to Figure 4.

(A) Z021-mediated ADE is abrogated by GRLR and GRLR/LS substitutions. Data are represented as mean \pm SD of triplicates. The positive control (+) is wild type Z004 antibody (10 ng/ml).

(B-C) Z021 antibodies bearing the LS and GRLR substitutions remain effective against ZIKV RVPs *in vitro* (B) or ZIKV *in vivo* following prophylactic administration (C).

(D) K294R arising in macaque 6171 is not a virus escape mutation. On top is the location of the K294 residue on the structure of the sE dimer of ZIKV (PDB ID: 5JHM). The structures of the Z004-related antibody Z006 and of Z021 in complex with the EDIII of ZIKV are structurally aligned to the sE dimer to show the location of K294 relative to the binding sites of the antibodies. The ZIKV EDIII from the Z006-EDIII and Z021-EDIII structures are omitted for clarity. At the bottom, Z004 and Z021 similarly neutralize wild type (left) and K294R mutant (right) ZIKV RVPs. Data are represented as mean \pm SD of triplicates and a representative of two experiments is plotted. Values are relative to no antibody control.

Table S1- Structural statistics for Z021 bound to ZIKV EDIII and DENV1 EDIII. Related to Figure 3.

Statistics for the highest-resolution shell are shown in parentheses.

	Z021-ZIKV EDIII	Z021-DENV1 EDIII
Data Collection		
Resolution Range (Å)	75.25 - 2.48 (2.57 - 2.48)	50.51 - 2.07 (2.13 - 2.07)
Space group	P2 ₁ 2 ₁ 2 ₁	C 2 2 2 ₁
Cell dimensions		
a, b, c (Å)	73.89, 105.62, 107.24	60.54, 91.60, 187.14
α, β, γ (°)	90, 90, 90	90, 90, 90
Total reflections	111776 (12819)	185501 (14638)
Unique reflections	30524 (3427)	31907 (2463)
Multiplicity	3.7 (3.7)	5.8 (5.9)
Completeness (%)	99.7 (99.9)	99.7 (99.9)
Mean I/σ(I)	7.8 (2.2)	15.0 (2.3)
Wilson B-factor (Å ²)	44.52	50.34
R _{merge}	0.091 (0.486)	0.046 (0.702)
R _{pim}	0.077 (0.418)	0.030 (0.461)
CC _{1/2}	0.993 (0.696)	0.997 (0.724)
Refinement		
R _{work} /R _{free}	18.2% / 22.8%	18.4% / 23.2%
Number of atoms	4163	4191
Protein atoms	4095	4065
Solvent atoms	68	126
Protein residues	537	532
RMS (bonds) (Å)	0.008	0.006
RMS (angles) (°)	1.22	1.12
Clashscore	2.97	3.85
Average B-factor (Å ²)	48.71	67.96
Number of TLS groups	5	5

Table S2- Contact residues between Z021 and ZIKV EDIII and Z021 and DENV1 EDIII. Related to Figure 3.

Contacts are defined as residues in which any atom is within 4 Å of an atom from a residue on the interacting partner using AntibodyDatabase (West et al., 2013). The table is organized by antibody residue, listing all antigen residues contacted by each antibody residue. Asterisk (*) indicates that experimental density for side chain is not present and therefore side chain position cannot be accurately modeled. It is unlikely that these residues are actually involved in binding.

Chain	Z021 Fab	ZIKV EDIII	Closest distance (Å)	Z021 Fab	DENV1 EDIII	Closest distance (Å)
HC	T31	G392	3.92	T31	G383	3.93
	Y32	T309	3.70	Y32	E384*, K385*	2.68
	Y33	S306, L307	2.90	Y33	V300, M301, T303	2.82
	Y52	L307, T309, V391	3.13	Y52	M301, A382	3.47
	D56	Y305	3.46	D56	Y299	3.78
	H58	S304, Y305, S306	3.67	H58	S298, Y299	3.07
	P97	T309	3.49	P97	T303	3.39
	G98	T309, A310, G334, T335, D336	2.63	G98	T303, G304, Y326, G328, T329, D330	2.69
	G99	T335, D336	3.38	G99	T329, D330	3.47
	R100	T335, D336, G337, S368	2.92	R100	T329, D330, A331	2.97
LC	S30	S368	3.46			
	Y32	T335, S368, T369, E370	3.10	Y32	T329, K361, E362	2.51
	D50	T335	2.95	D50	T329	2.79
	K53	E370	3.02			
	R91	S306, D336, G337, P338	2.85	R91	V300, D330, A331, P332	2.89
	N93	P338	3.38	N93	S298, P332	3.34
	W94	V303, Y305, P338	3.80			
	L95A	S306	3.64	L95A	S298, V300	3.43

Table S3. List of primers used in this study. Related to STAR Methods.

Primer ID	Primer sequence	Comments
Mutagenesis of Iγ1 expression vector to produce GRLR and LS Fc mutant antibodies		
DFRp1455	TGAACTCCTGAGGGGACCGTCAGTC	For G236R in GRLR mutant
DFRp1456	GGTGCTGGGCACGGTGGG	For G236R in GRLR mutant
DFRp1457	AACAAAGCCCCGCCAGCCCCC	For L328R in GRLR mutant
DFRp1458	GGAGACCTTGCACTTGTACTCCTTG	For L328R in GRLR mutant
DFRp1459	ATGCTCCGTGCTGCATGAGGC	For M428L in LS mutant
DFRp1460	GAGAAGACGTTCCCCTGC	For M428L in LS mutant
DFRp1461	GCTCTGCACAGCCACTACAGC	For N434S in LS mutant
DFRp1462	CTCATGCAGCACGGAGCATG	For N434S in LS mutant
Mutagenesis of ZIKV EDIII expression vector to produce mutant EDIII proteins		
E393A/K394A	TTGTCATAGGAGTCGGGGCGGGCGAAGATCACCCACCACTG	For E393A/K394A mutant
E393D	CATAGGAGTCGGGGGACAAGAAGATCACCCAC	For E393D mutant
K394R	GTCATAGGAGTCGGGGGAGCGTAAGATCACCCACCACTGG	For K394R mutant
Mutagenesis of pZIKV/HPF/CprM*E* to produce mutant ZIKV RVPs		
RU-O-24379	ACTTGGTCATGATACTGCTGATTGCCCGGCATACAGCATCAGGTGCATAGGAGT	Forward outer
RU-O-24380	TTCGAACCGCGGGCTGGGTCTATTAAGCAGAGACAGCTGTGGATAAGAAGATC	Reverse outer
RU-O-25002	AGGAGTCGGGGCGGGCGAAGATCACCCAC	Forward E393A/K394A
RU-O-25003	GTGGGTGATCTTCGCCGCCCGACTCCT	Reverse E393A/K394A
DFRp1500	AAATGTCGCCTGAGAATGGATAAACTTAGATTG	Forward K294R
DFRp1501	TCTAAGTTTATCCATTCTCAGGCGACATTTCAA	Reverse K294R
Primers to amplify and sequence the ZIKV EDIII region		
1618p	ACAAGGAGTGGTTCCATGACA	First set (macaque)
2204n	TTTTCCGATGGTGCTGCCAC	First set (macaque)
1970p	GTATGCAGGGACAGATGGACC	Second set (macaque)
2537n	ACCGCATCTCGTTTCCTTCTT	Second set (macaque)
DFRp1284	GGATGATCGTTAATGACACAG	First PCR (mouse)
DFRp1469	ACCATCTTCCCAGGCTTG	First PCR (mouse)
DFRp1283	GGATCCTGATTTGAAAGCTGC	Sequencing primer (mouse)
DFRp1472	TTCCACGACATTCCATTACC	Nested PCR (mouse)
DFRp1470	ATCTACGGGGGAGTCAGGATG	Nested PCR (mouse)