

1 **Potential Role of Heterologous Flavivirus Immunity in Preventing Urban Transmission of Yellow Fever**
2 **Virus**

4 Supplementary Files

6 **Supplementary Table 1: FRNT₅₀ measured on Day 0 before YFV challenge.**

NHP ID	Sex	Primary exposure	FRNT ₅₀ (1/n)					
			YFV	DENV1	DENV-2	DENV3	DENV4	ZIKV
NV 289	M	Naïve	<20	<20	<20	<20	<20	<20
NV 259	F	Naïve	<20	<20	<20	<20	<20	<20
UG 171	F	Naïve	<20	<20	<20	<20	<20	<20
UG 253A	M	DENV-2 P81407	<20	<20	<20	<20	<20	#
BC 407	F	DENV-2 P81407	<20	<20	<20	<20	<20	#
FR 423A	M	DENV-2 P81407	<20	<20	2560	<20	<20	#
SB 393	M	DENV-2 P81407	<20	<20	320	<20	<20	#
CP 60	F	DENV-2 P81407	<20	<20	40	<20	<20	#
SB 395	F	DENV-2 P81407	<20	<20	640	<20	<20	#
BC 116	M	DENV-2 P81407	<20	<20	80	<20	<20	#
FR 469	M	DENV-2 P81407	<20	<20	640	<20	<20	#
BC 167	F	DENV-2 P81407	<20	<20	1280	<20	<20	#
FR 840	F	DENV-2 P81407	<20	<20	640	<20	<20	#
BC940	M	DENV-2 NGC	<20	<20	640	<20	<20	#
MB1433	M	DENV-2 NGC	<20	<20	320	<20	<20	#
BC838	F	DENV-2 NGC	<20	<20	640	<20	<20	#
FR 1565	M	ZIKV PRVABC59	<20		#			<20
BC 1173	F	ZIKV PRVABC59	<20		#			320
BC 1169	M	ZIKV PRVABC59	<20		#			160
EC 529	F	ZIKV PRVABC59	<20		#			2560
EC 944	M	ZIKV DakAr 41525	<20		#			640
FR 1221	F	ZIKV DakAr 41525	<20		#			640
UG 2626	F	ZIKV DakAr 41525	<20		#			640
# DENV-2 and ZIKV-exposed samples were not evaluated against each other as it is beyond the scope of this manuscript								

8 **Supplementary Table 2: Log₁₀-transformed YFV viremia on days 1-5 post-infection.**

Primary exposure	FRNT ₅₀ against DENV-2 (1/n)	NHP ID	YFV Viremia (Log ₁₀ FFU/mL) Days Post-Infection				
			1	2	3	4	5
PBS	<LOD	NV 289	<1.70	3.22	5.56	6.23	3.51
	<LOD	NV 259	<1.70	<1.70	2.98	4.19	<1.70
	<LOD	UG 171	<1.70	3.33	6.06	7.08	6.53
DENV-2 P81407	<LOD	UG 253A	<1.70	2.92	6.02	5.04	<1.70
	<LOD	BC 407	<1.70	3.15	5.39	5.32	<1.70
	2560	FR 423A	<1.70	<1.70	<1.70	<1.70	<1.70
	320	SB 393	<1.70	<1.70	4.89	4.68	<1.70
	40	CP 60	<1.70	3.32	5.02	6.28	<1.70
	640	SB 395	<1.70	<1.70	<1.70	<1.70	<1.70
	80	BC 116	<1.70	<1.70	4.95	<1.70	<1.70
	640	FR 469	<1.70	<1.70	3.30	4.08	<1.70
	1280	BC 167	<1.70	<1.70	<1.70	<1.70	<1.70
	640	FR 840	<1.70	<1.70	<1.70	<1.70	<1.70
DENV-2 NGC	640	BC940	†	1.70	3.54	3.93	<1.70
	320	MB1433	†	1.70	3.70	4.93	<1.70
	640	BC838	†	2.98	5.35	5.68	<1.70
Limit of detection (LOD) is 50 FFU/mL (1.7 log ₁₀ FFU/mL)							

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11 **Supplementary Table 3: Log₁₀-transformed YFV viremia on days 1-5 post-infection**

Primary exposure	FRNT ₅₀ against ZIKV (1/n)	NHP ID	YFV Viremia (Log ₁₀ FFU/mL) Days Post-Infection				
			1	2	3	4	5
PBS	<LOD	NV 289	<1.70	3.22	5.56	6.23	3.51
	<LOD	NV 259	<1.70	<1.70	2.98	4.19	<1.70
	<LOD	UG 171	<1.70	3.33	6.06	7.08	6.53
ZIKV PRVABC59	<LOD	FR1565	†	1.81	5.83	7.30	7.52
	320	BC1173	†	3.51	4.98	4.29	<1.70
	160	BC1169	†	<1.70	3.10	4.00	<1.70
	2560	EC529	†	1.70	3.65	2.68	<1.70
ZIKV DakAr 41525	640	EC944	†	<1.70	<1.70	2.10	<1.70
	640	FR1221	†	1.88	4.15	4.24	<1.70
	640	UGZ626	†	<1.70	3.54	2.30	<1.70
† indicates the samples that were lost due to inconsistent storage temperatures							

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Supplementary Table 4: Mosquito infection rate at peak YFV viremia timepoint

Prior flavivirus immunity	NHP ID	Days Post- Infection	YFV Viremia (log ₁₀ FFU/ml)	Total Infected Bodies (x)	Total Engorged (y)	Infection Rate = x/y *100)	Dissemination Rate = Total Infected Legs/x *100
None	NV 289	3	5.56	10	30	33.33	0 (0)
		4	6.23	14	22	63.63	9 (64.3)
	NV 259	3	2.98	0	59	0	†
		4	4.19	2	50	4	0 (0)
	UG 171	3	6.06	29	54	53.7	6 (20.7)
		4	7.08	49	63	77.77	4 (8.2)
DENV-2- seronegative	UG253A	3	6.02		NA*		
		4	5.04		NA		
	BC 407	3	5.39	21	71	29.5	1 (4.8)
		4	5.32	18	69	24.8	2 (11.11)
DENV-2 P8 1407	FR 423A	3	1.40		NA		
		4	1.40		NA		
	SB 393	3	4.89		NA		
		4	4.68		NA		
	CP 60	3	5.02		NA		
		4	6.28		NA		
	SB 395	3	1.40		NA		
		4	1.40		NA		
	BC 116	3	4.95	1	31	3.2	0 (0)
		4	1.40	0	54	0	†
	FR 469	3	3.30	0	75	0	†
		4	4.08	0	73	0	†
	BC 167	3	1.40	0	53	0	†
		4	1.40	0	73	0	†
	FR 840	3	1.40	0	50	0	†
		4	1.40	0	59	0	†
DENV-2 NGC	BC 940	3	3.54	0	73	0	†
		4	3.93	3	66	4.5	1 (33.33)
	MB 1433	3	3.70	1	52	1.9	0 (0)
		4	4.93	6	55	10.9	0 (0)
	BC 838	3	5.35	1	27	3.7	0 (0)
		4	5.68	3	46	6.5	0 (0)
ZIKV PR- seronegative	FR 1565	3	5.83	7	44	9	2 (28.5)
		4	7.30	18	30	60	4 (22.2)
ZIKV PRVABC59	BC 1173	3	4.98	1	27	3.7	0 (0)
		4	4.29	0	36	0	†
	BC 1169	3	3.10	0	56	0	†
		4	4.00	0	59	0	†
	EC 529	3	3.65	0	67	0	†
		4	2.68	0	41	0	†
ZIKV DakAr 41525	EC 944	3	1.40	0	59	0	†
		4	2.10	0	69	0	†
	FR 1221	3	4.15	0	27	0	†
		4	4.24	0	17	0	†
	UG 2626	3	3.54	0	57	0	†
		4	2.30	0	49	0	†

*NA refers to groups of mosquitoes that did not survive for 14 days post-feeding due to technical issues with the incubator.

Dissemination rate (DR) refers to the number of positive mosquito legs/ total number of mosquitoes infected. Legs from mosquitoes that were positive for infection were utilized to measure dissemination. † refers to the samples that were not assayed due to negative infection in bodies

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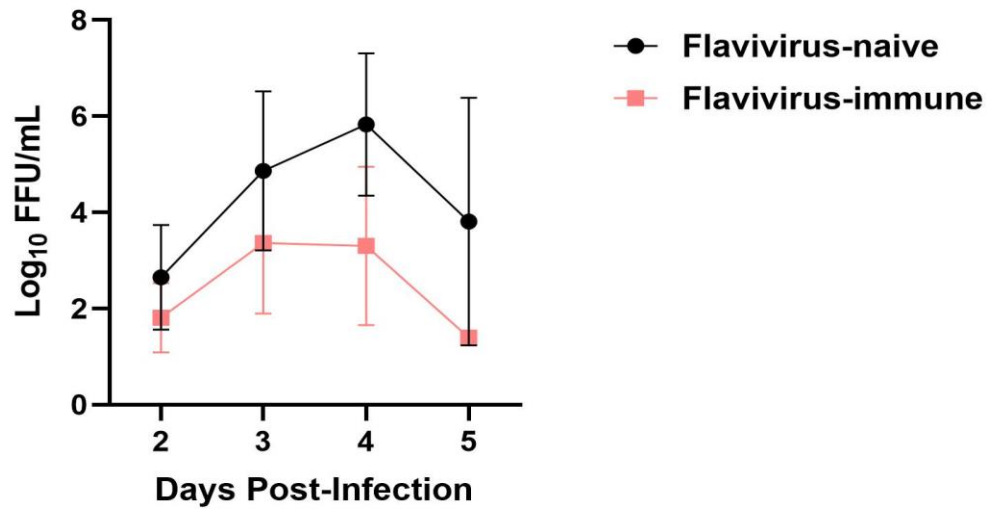
17 **Supplementary Table 5: Infection rate of mosquitoes analyzed by two-tailed Fisher’s Exact Test**

Timepoint	Group 1	Group 2	Significance?	p-value
3 DPI	Flavivirus-naïve	DENV-2 NGC-immune	Yes	<0.0001
	Flavivirus-naïve	DENV-2 P8-immune	Yes	<0.0001
	Flavivirus-naïve	DENV-2-seronegative	No	0.2678
4 DPI	Flavivirus-naïve	DENV-2 NGC-immune	Yes	<0.0001
	Flavivirus-naïve	DENV-2 P8-immune	Yes	<0.0001
	Flavivirus-naïve	DENV-2-seronegative	Yes	0.0026
3 DPI	Flavivirus-naïve	ZIKV PR-immune	Yes	<0.0001
	Flavivirus-naïve	ZIKV DakAr-immune	Yes	<0.0001
	Flavivirus-naïve	ZIKV-seronegative	No	0.0132
4 DPI	Flavivirus-naïve	ZIKV PR-immune	Yes	<0.0001
	Flavivirus-naïve	ZIKV DakAr-immune	Yes	<0.0001
	Flavivirus-naïve	ZIKV-seronegative	No	0.3132

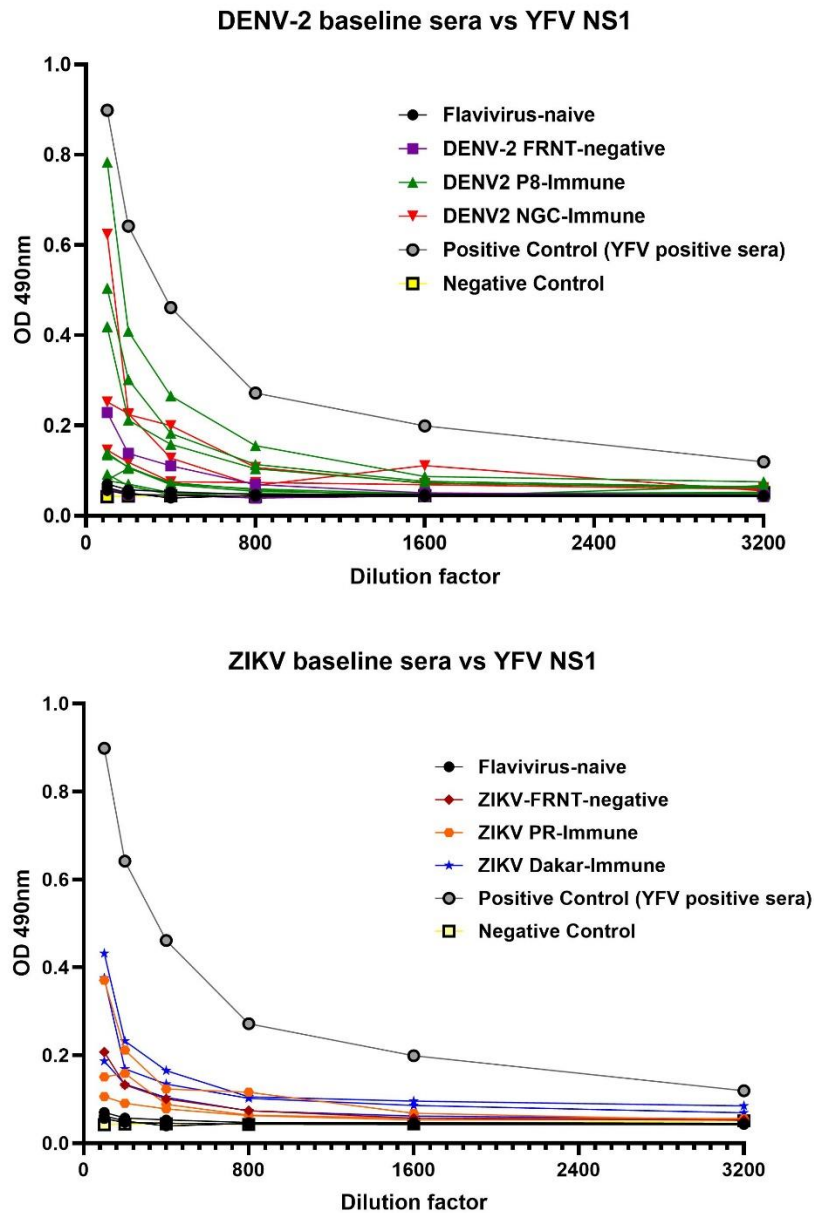
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Combined YFV Viremia

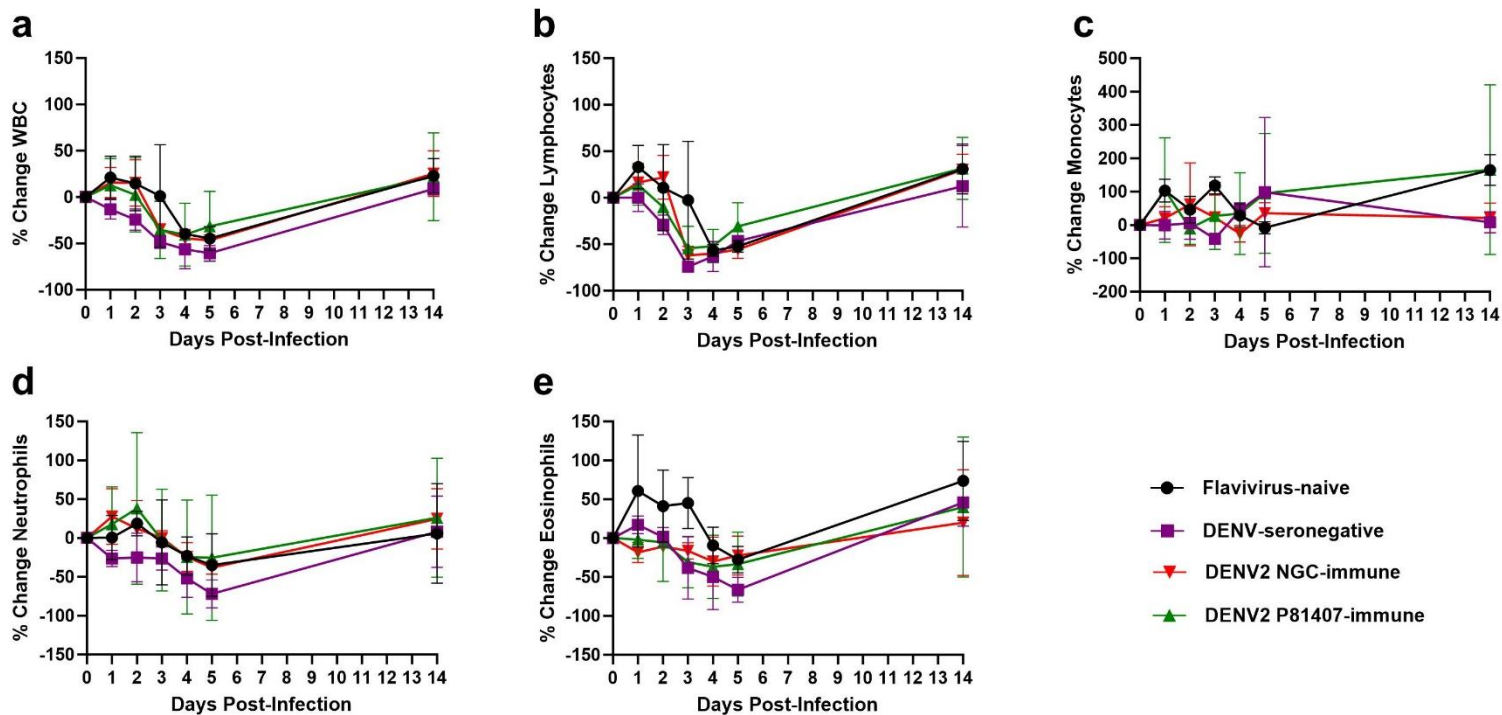


Supplementary Figure 1: Combined YFV viremia levels in DENV-2 (n=11) and ZIKV-immune (n=6) animals compared to flavivirus-naïve animals (n=3) at 2,3,4, and 4 DPI. The two groups were analyzed by two-way ANOVA repeated measures and no significant difference was found ($p>0.05$).



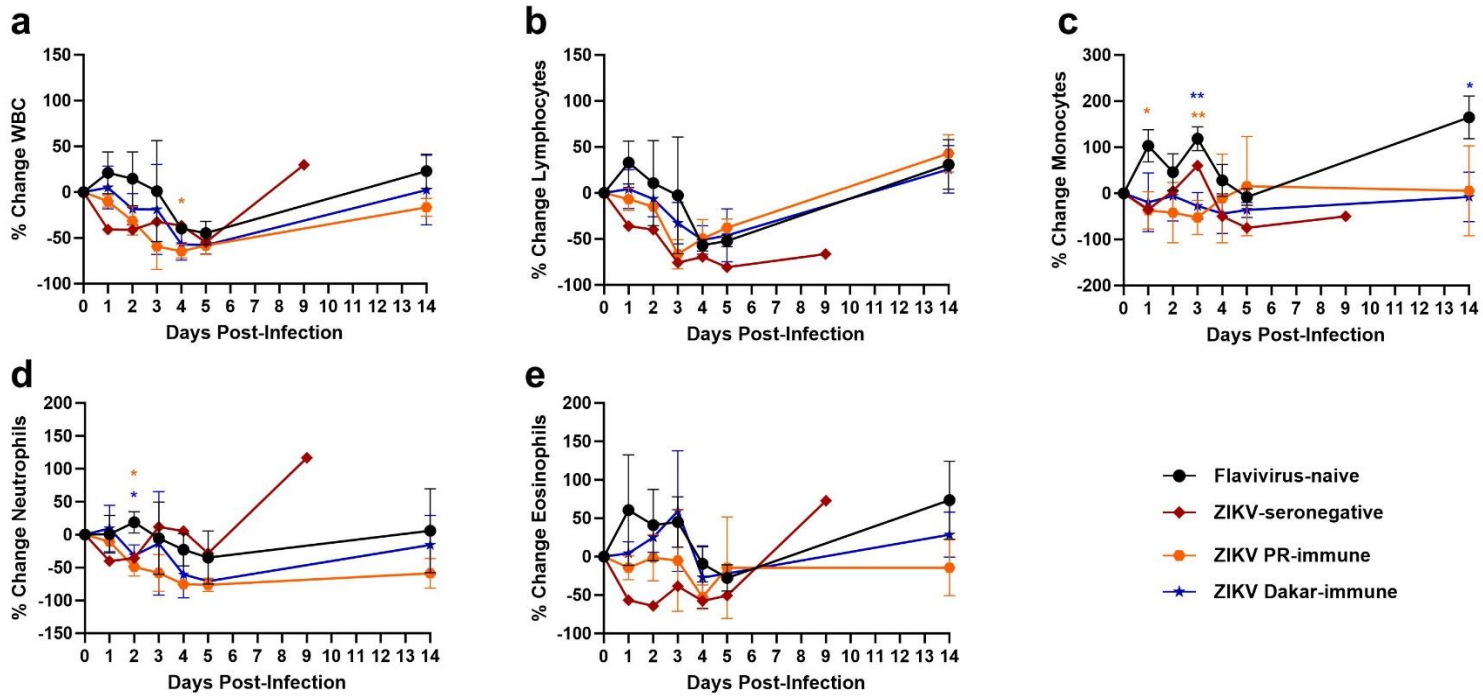
Supplementary Figure 2: Binding antibodies against YFV NS1

Baseline sera collected from all animals on Day 0, prior to YFV challenge was utilized to measure binding antibodies by ELISA against YFV NS1 recombinant protein. Positive control is serum from YFV-infected animal collected at 14 DPI, and negative control is blank (no sample).



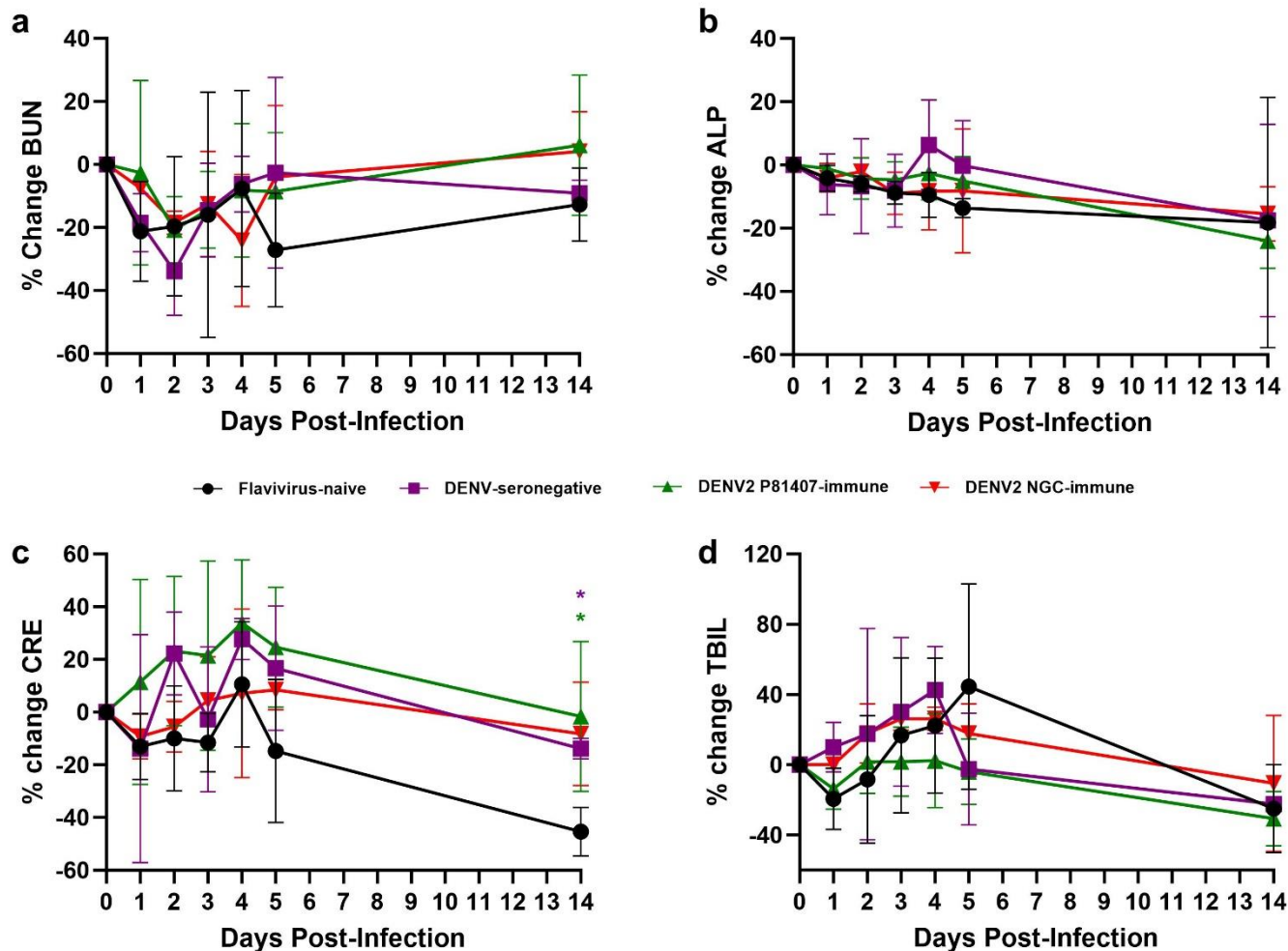
Supplementary Figure 3: Complete blood analyses of flavivirus-naïve and DENV-2 exposed animals

Percentage change in WBCs (a), lymphocytes (b), monocytes (c), neutrophils (d), and eosinophils (e) on days 1-5 and 14 compared with baseline levels in flavivirus-naïve (n=3), DENV-2 NGC (n=3), DENV-2 P8 (n=8), and DENV seronegative (n=2) NHPs. Each group was compared with the other by two-way ANOVA repeated measures. No significant difference was observed in any of the groups.



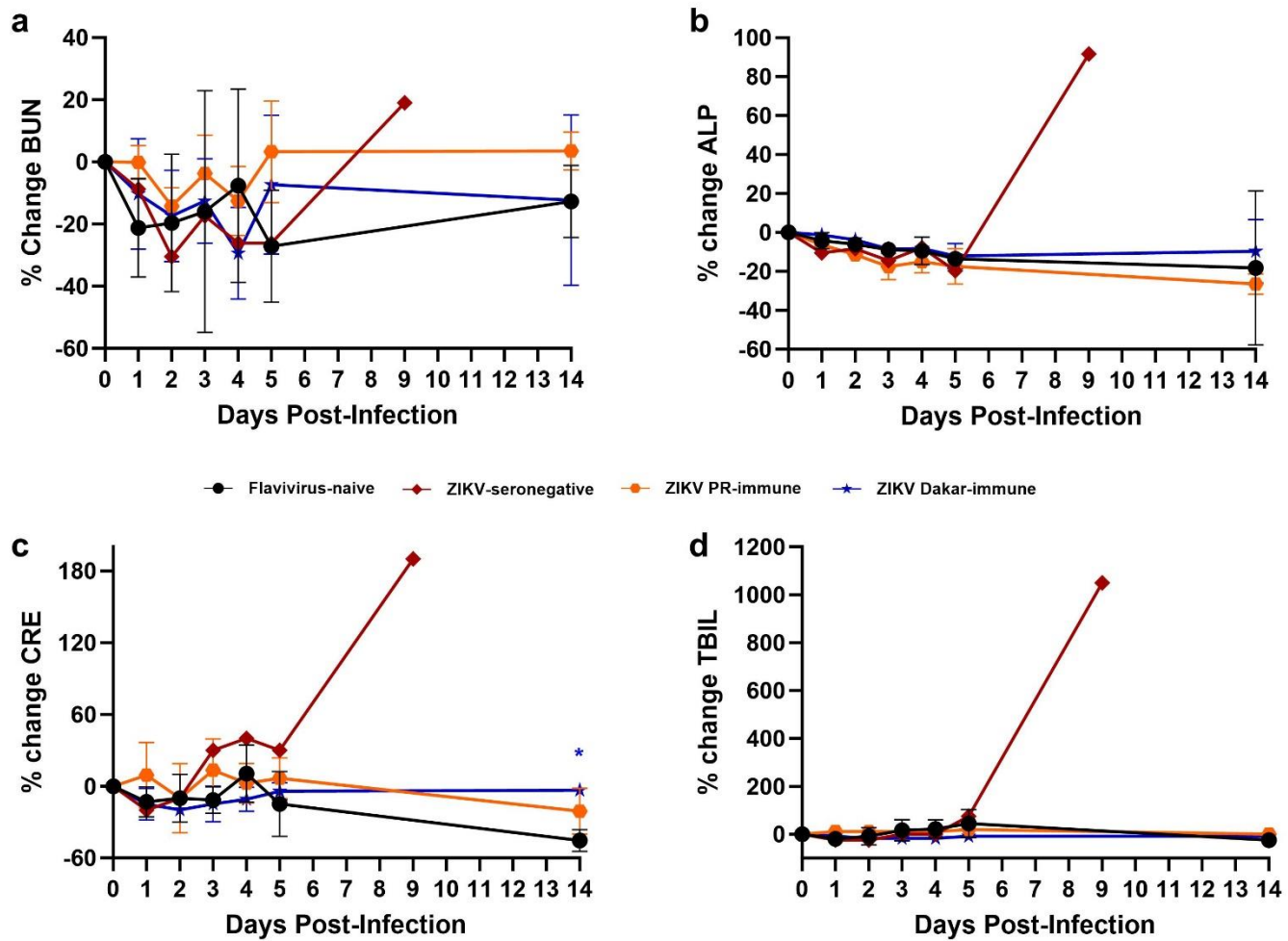
Supplementary Figure 4: Complete blood analyses of flavivirus-naïve and ZIKV-exposed animals

Percentage change in WBCs (a), lymphocytes (b), monocytes (c), neutrophils (d), and eosinophils (e) on 1-5 and 14 DPI compared with baseline levels in flavivirus-naïve (n=3), ZIKV PR (n=3), ZIKV DakAr (n=3) and ZIKV-seronegative (n=1) NHPs. Each group was compared with the other by two-way ANOVA repeated measures. Flavivirus-naïve animals had a significant increase in monocyte level at 1 and 3 DPI compared to ZIKV PR group ($p=0.0237$ and $p=0.0097$ respectively), and at 3 and 14 DPI compared to ZIKV DakAr group ($p=0.0066$ and $p=0.0303$ respectively). Since the ZIKV-seronegative group has only one animal, this group was excluded from analyses. Statistical significance (*) color is attributed to the significant difference between that group compared to the flavivirus-naïve group. * $p<0.05$, ** $p<0.01$.



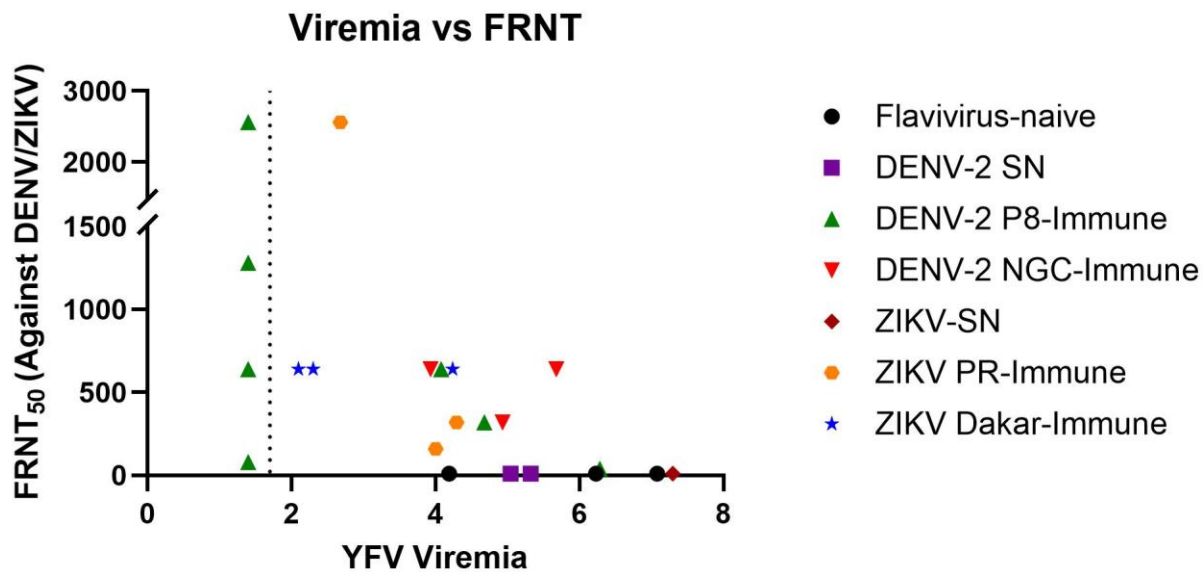
Supplementary Figure 5: Serum chemistries of flavivirus-naïve and DENV-2 exposed animals

Percentage change in blood urea nitrogen (BUN) (a), alanine phosphatase (ALP) (b), creatine (CRE) (c), total bilirubin (TBIL) (d) on 1-5 and 14 DPI compared with baseline levels in flavivirus-naïve and DENV-2-exposed NHPs. Each group was compared with the flavivirus-naïve group by two-way ANOVA repeated measures. Statistical significance (*) color is attributed to the significant difference between that group compared to the flavivirus-naïve group. * $p < 0.05$.



Supplementary Figure 6: Serum chemistries of flavivirus-naïve and ZIKV-exposed animals

Percentage change in blood urea nitrogen (BUN) (a), alanine phosphatase (ALP) (b), creatine (CRE) (c), total bilirubin (TBIL) (d) on 1-5 and 14 DPI compared with baseline levels in flavivirus-naïve and ZIKV-exposed NHPs. Each group was compared with the flavivirus-naïve group by two-way ANOVA repeated measures. Since the ZIKV-seronegative group has only one animal, this group was excluded from analyses. Statistical significance (*) color is attributed to the significant difference between that group compared to the flavivirus-naïve group. * $p < 0.05$.



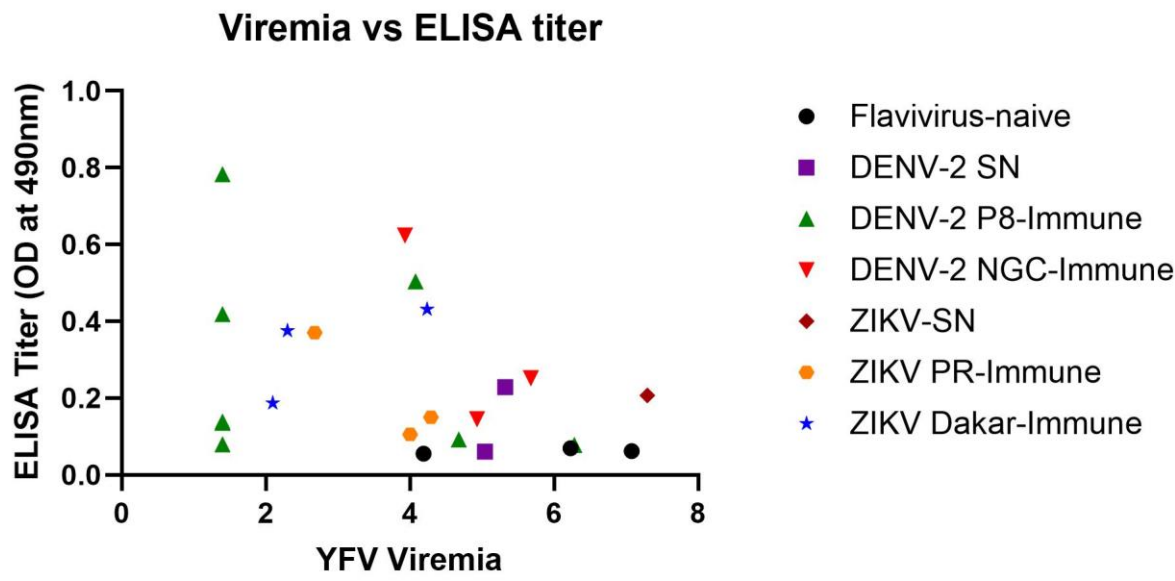
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75 **Supplementary Figure 7: Correlation between YFV Viremia and neutralizing antibody titer**

76 Peak YFV viremia (log₁₀ FFU/mL) levels analyzed against homologous FRNT₅₀ values. A Pearson correlation
77 coefficient was computed to assess the relationship between YFV viremia and neutralizing antibody titer
78 (FRNT₅₀).

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82 **Supplementary Figure 8: Correlation between YFV Viremia and NS1 antibodies**
83 Peak YFV viremia (log₁₀ FFU/mL) levels analyzed against homologous anti-NS1 ELISA titers. A Pearson
84 correlation coefficient was computed to assess the relationship between YFV viremia and anti-NS1 ELISA titer.
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