Table S1. Antigen panel. Antigens used for the immunoassay are listed along with the manufacturer and their expression system. Some flavivirus antigens were produced in-house as previously reported (Merbah et al., Journal of Immunological Methods, 2020). The panel included 23 antigens corresponding to Envelope (E) and non-structural 1 (NS1) from different flaviviruses (ZIKV, DENV1-4, YFV, WNV, JEV and TBEV) and to E1 from the alphavirus Chikungunya. Anti-human IgG was used as a control.

Antigen	Manufacturer†	Catalog no.	Expression system
AntiHuman_IgG	SouthernBiotech	9042–01	Mouse
ZIKV_PRV.WT_E.dom12	Produced in-house	NA	Drosophila S2-insect cells
ZIKV_PRV.WT_E.dom3	Produced in-house	NA	Expi293F-human cells
ZIKV_Sur_NS1	The Native Antigen Company	ZIKVSU-NS1–100	HEK293-human cells
ZIKV_FP13_NS1	R&D Systems	9450-ZK-100	HEK293-human cells
ZIKV_Sur_E	The Native Antigen Company	ZIKVSU-ENV-100	HEK293-human cells
ZIKV_PRV.WT_E	Produced in-house	NA	Drosophila S2-insect cells
ZIKV_PRV.FL4_E	Produced in-house	NA	Drosophila S2-insect cells
ZIKV_ConAf_E	Produced in-house	NA	Drosophila S2-insect cells
JEV_SA14_E	Produced in-house	NA	Drosophila S2-insect cells
JEV_SA14_NS1	The Native Antigen Company	JEV-NS1–100	HEK293-human cells
YFV_17D_E	Produced in-house	NA	Drosophila S2-insect cells
YFV_17D_NS1	The Native Antigen Company	YFV-NS1–100	HEK293-human cells
DENV.4_Dom81_NS1	The Native Antigen Company	DENV4-NS1–100	HEK293-human cells
DENV.4_Dom81_E	The Native Antigen Company	DENV4-ENV-100	HEK293-human cells
DENV.3_PR98_NS1	Cal Bioreagents	A256	Baculovirus-insect cells
DENV.3_PR98_E	Cal Bioreagents	A252	Baculovirus-insect cells
DENV.2_Ind01_NS1	Cal Bioreagents	A255	Baculovirus-insect cells
DENV.2_Ind01_E	Cal Bioreagents	A251	Baculovirus-insect cells
DENV.1_VN07_NS1	Cal Bioreagents	A254	Baculovirus-insect cells
DENV.1_VN07_E	Cal Bioreagents	A250	Baculovirus-insect cells
WNV_NY99_E	The Native Antigen Company	REC31614–100	HEK293-human cells
WNV_NY99_NS1	The Native Antigen Company	WNV-NS1–100	HEK293-human cells
TBEV_Neu_NS1	The Native Antigen Company	TBEV-NS1–100	HEK293-human cells
CHIKV_NA_E1	The Native Antigen Company	CHIKV-E1–100	HEK293-human cells

*CHIKV, chikungunya virus; DENV, dengue virus; JEV, Japanese encephalitis virus; TBEV, tickborne encephalitis virus; YFV, yellow fever virus; WNV, West Nile virus; ZIKV, Zika virus.

Table S2. Demographic information of the study participants

Priming Vaccine		Naïve (n = 25)		JEV (n = 24)		YFV (n = 24)	
Investigational Vaccine		Placebo	ZPIV	Placebo	ZPIV	Placebo	ZPIV
Age at Enrollment	Mean (SD)	33.4 (11.1)	30.3 (8.4)	31.4 (10.2)	30.9 (6.9)	32.5 (4.4)	28.9 (5.9)
Sex	Female	1	10	3	10	2	14
	Male	4	10	2	9	2	6
BMI	Mean (SD)	26.5 (5.0)	26.3 (3.6)	25.8 (4.1)	25.5 (4.0)	25.4 (2.3)	24.8 (2.4)
Ethnicity	Hispanic or Latino	0	3	0	2	0	2
	Not Hispanic or Latino	5	17	5	17	4	18
Race	American Indian or Alaska Native	0	2	0	0	0	0
	Asian	1	0	1	0	0	1
	Black or African American	3	7	1	6	1	5
	Multiple	0	1	0	0	0	2
	White	1	10	3	13	3	12

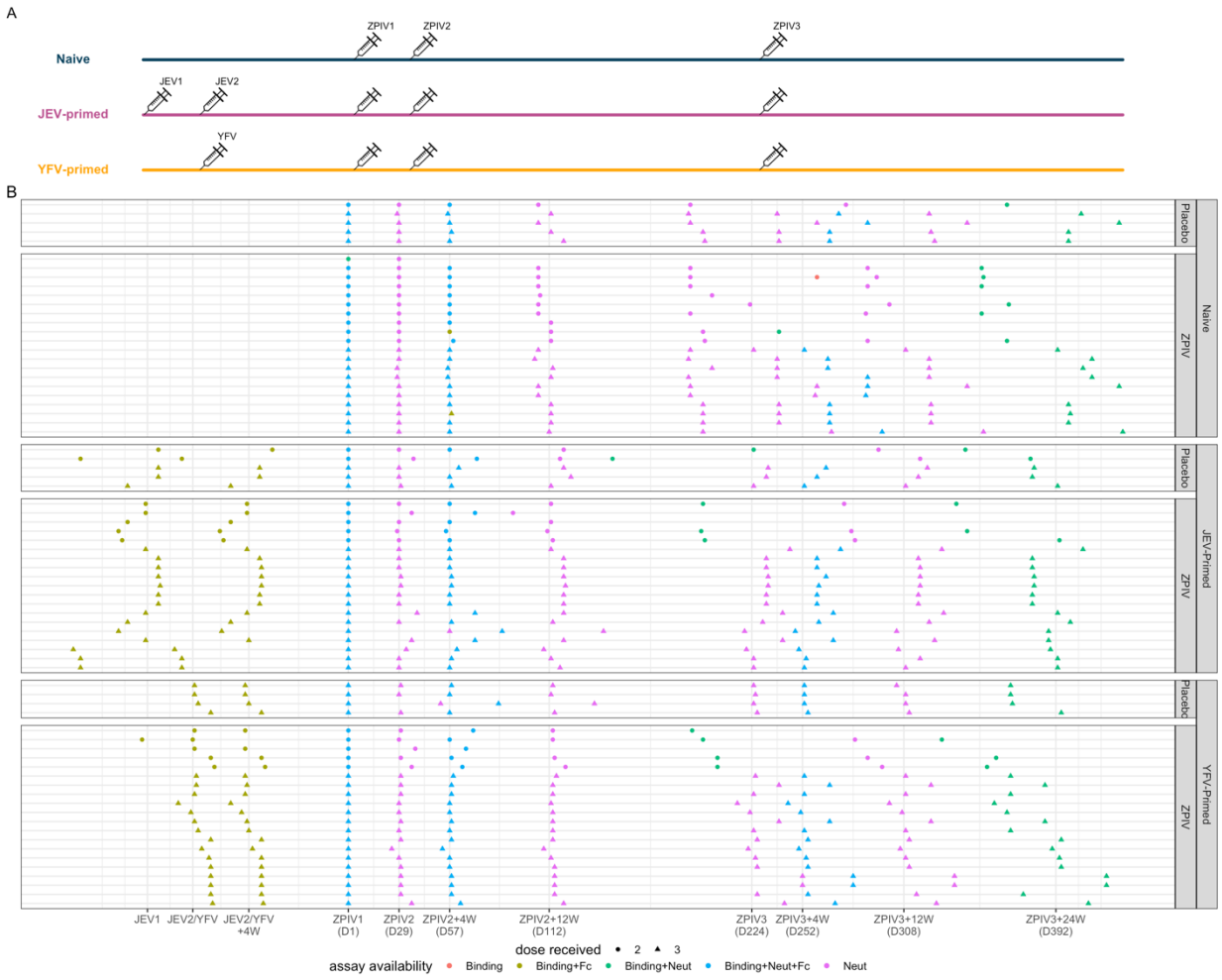

Fig. S1. Timeline of immunizations and sample collection for experimental assays. (A) Immunization schedule for the three groups labeled based on their vaccination status prior to ZPIV vaccination: Naïve, JEV-primed, YFV-primed (B) Description of the experimental data generated at each time point: antibody binding (Binding), Fc effector function (Fc) and neutralization (Neut) assays. JEV1: first dose of JEV vaccination (at enrollment); JEV2: second dose of JEV vaccination; YFV: YVF vaccination (at enrollment); ZPIV1: first dose of ZPIV vaccination (at ZPIV baseline); ZPIV2 and ZPIV3: second and third dose of ZPIV.

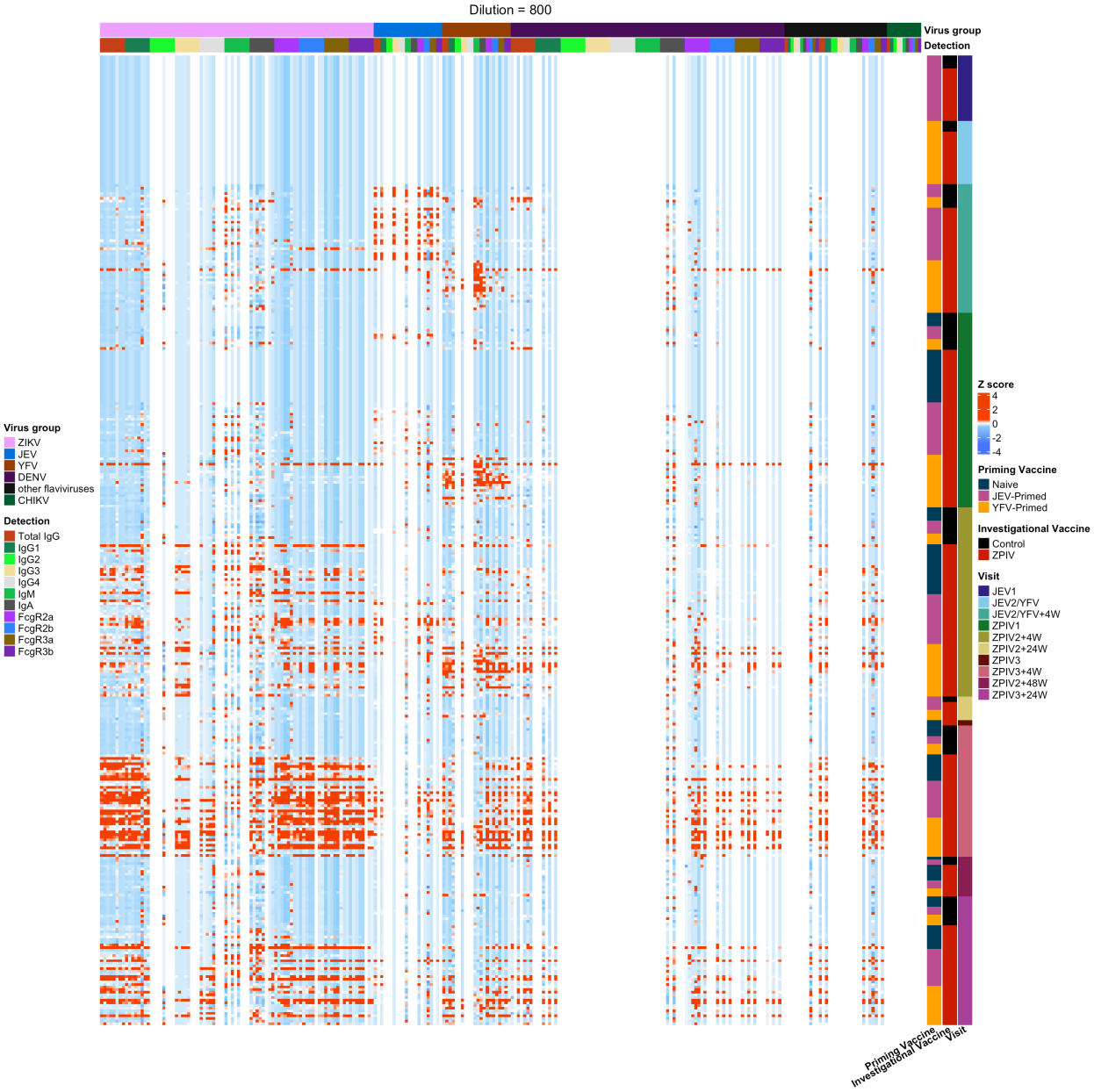

Fig. S2. Binding antibody responses to 23 E and NS1 flavivirus antigens and one alphavirus E antigen. The heatmap shows antibody binding responses presented as z-scores for 371 samples collected longitudinally. Each row represents a sample. Each column corresponds to a specific antigen and detection combination (feature). Features with near zero variance (fewer than three samples with a fold over baseline > 3) were shown as blank columns.

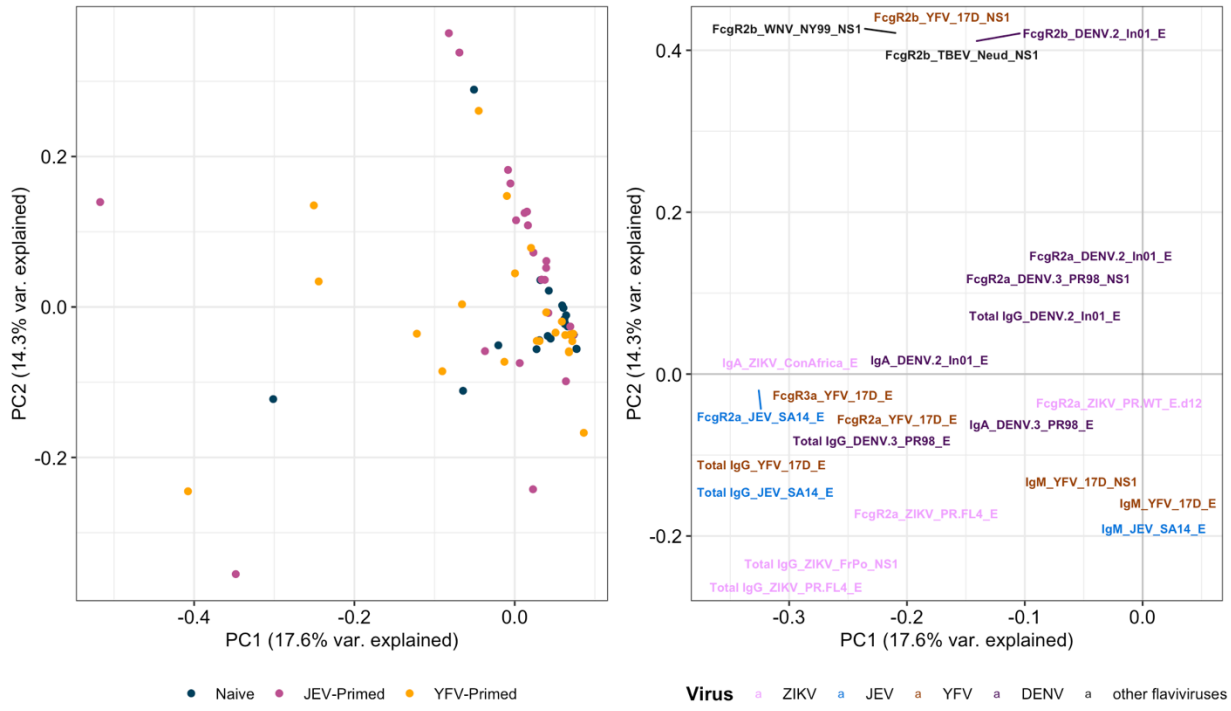

Fig. S3. Flavivirus-specific binding antibody profile at study enrollment. Principal component analysis based on antibody binding responses measured to 23 E and NS1 flavivirus antigens and one alphavirus E antigen. Score (left panel) and loading (right panel) plots of the first two PCs show limited variation of binding features principally driven by a few individuals with no evidence of clustering based on study groups.

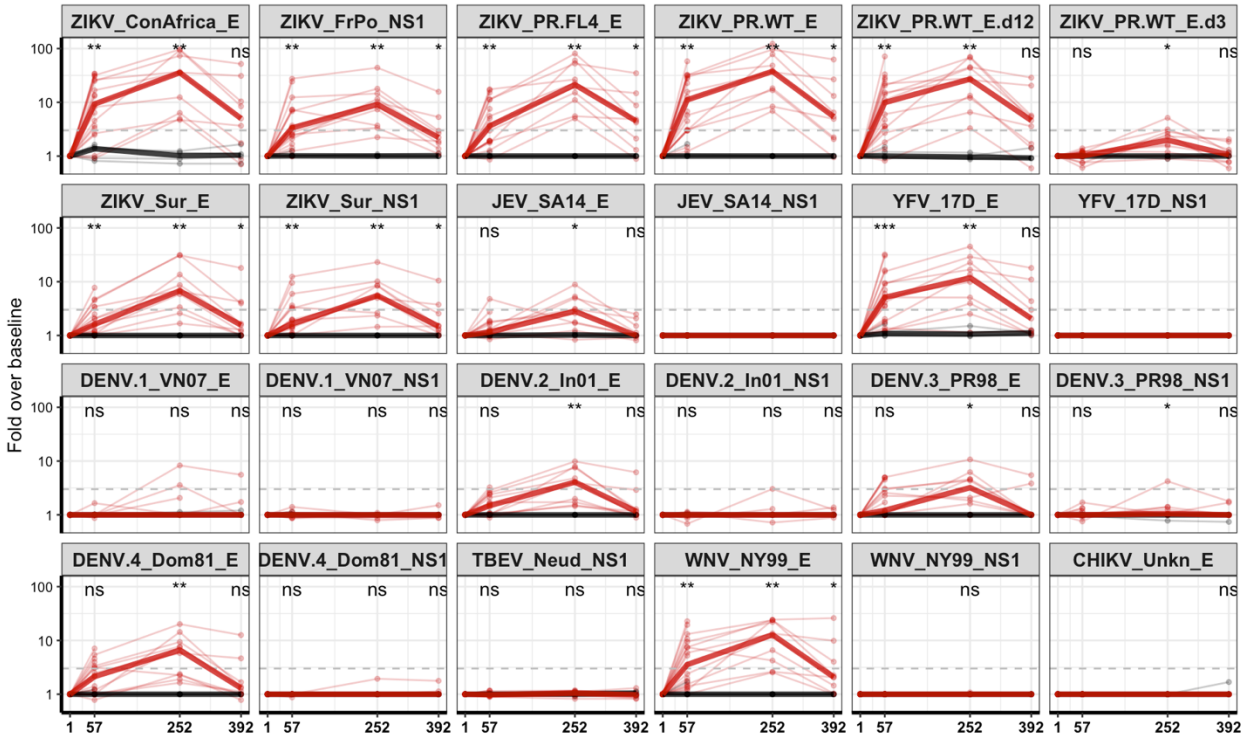

Fig. S4. IgG responses following ZPIV vaccination in the naïve group. Binding antibody responses are shown as fold over baseline at baseline (day 1, first vaccination), four weeks after the second dose (day 57), four weeks after the third dose (day 252) and five months later (day 392). Samples from vaccine and placebo recipients in red and black, respectively. The significance of Wilcoxon rank sum test is shown in each panel. ns: $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

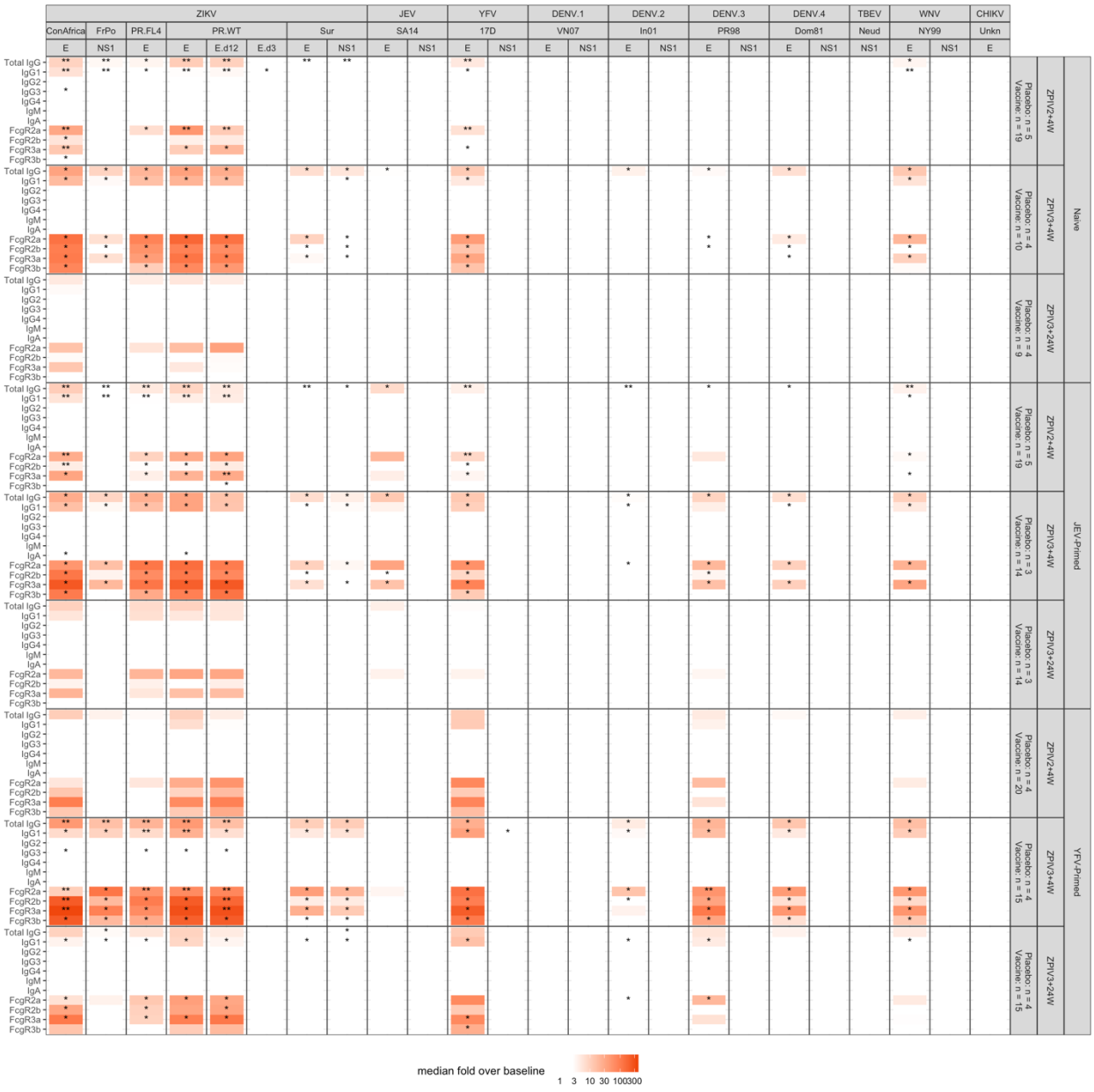

Fig. S5. Median fold over baseline for antibody binding responses in vaccine recipients. Antibody binding responses against 24 antigens and 11 detections are represented for each vaccine group and at each time point. Significant differences of responses between vaccine and placebo recipients using Wilcoxon rank sum test adjusted using the Benjamini–Hochberg procedure are reported with asterisks: *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

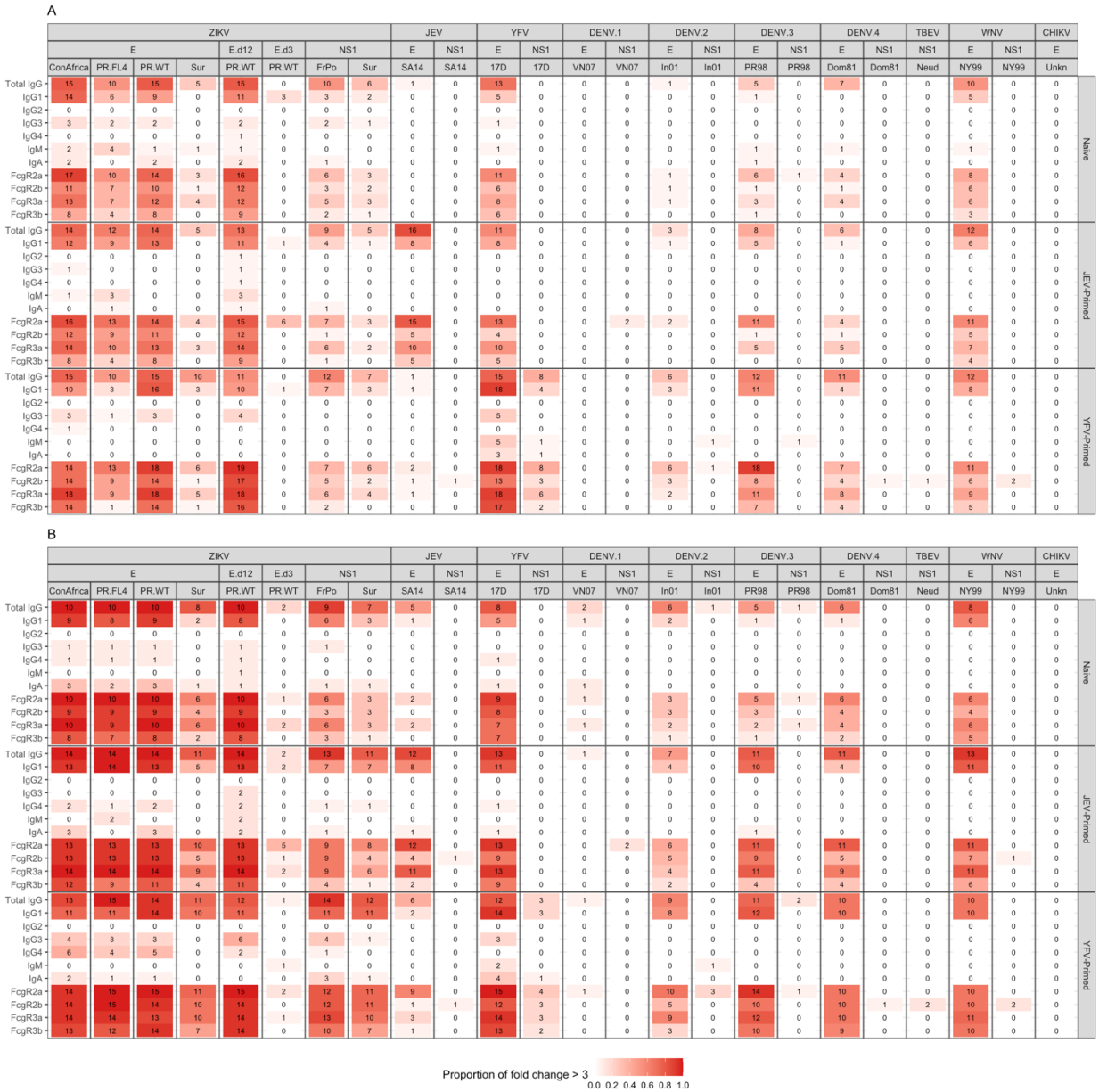

Fig. S6. Proportion of positive samples four weeks after the second (A) and third (B) ZPIV vaccinations. Number of positive samples using a fold over baseline >3 across the 24 antigens and 11 detection reagents tested.

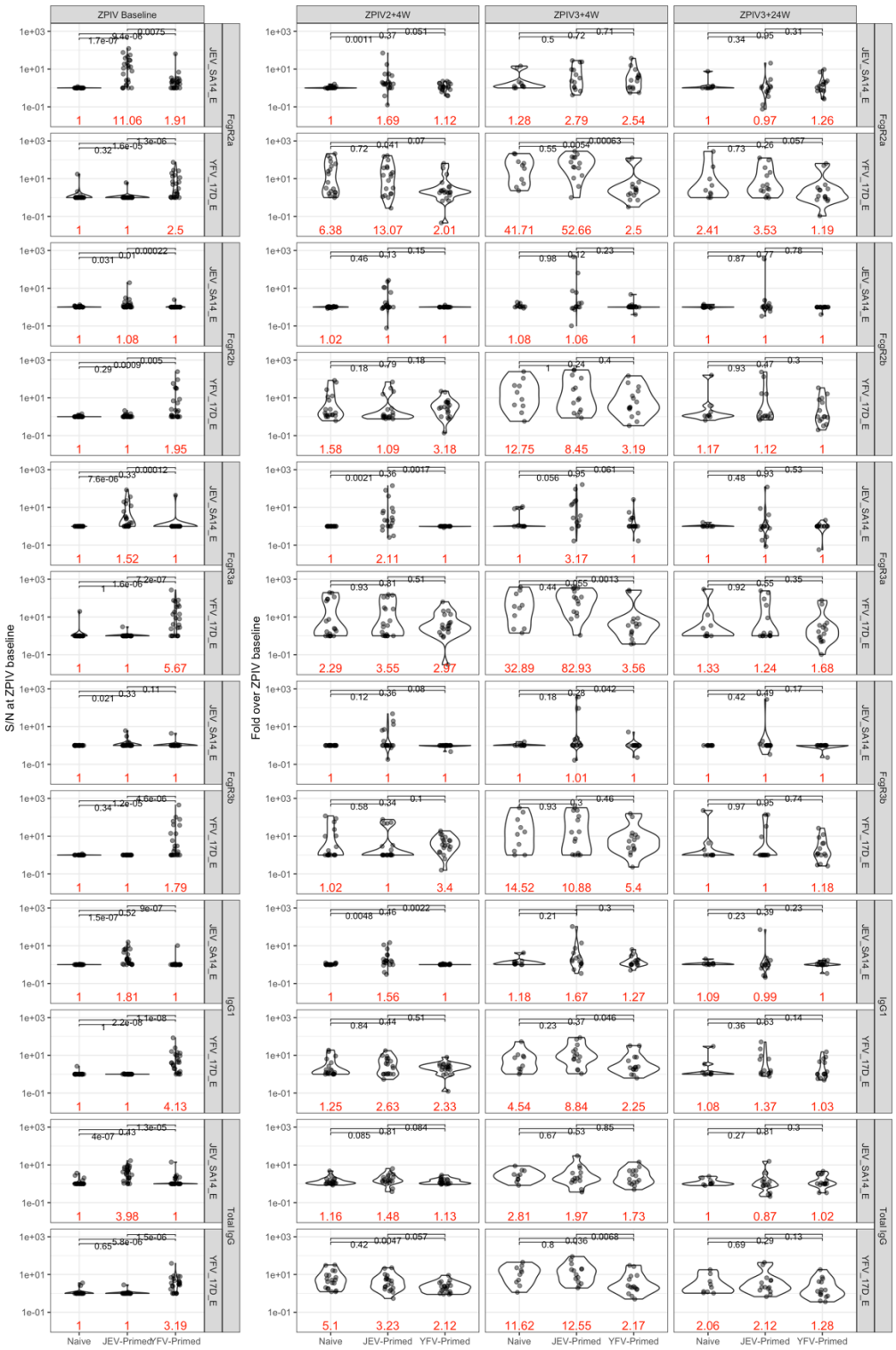

Fig. S7. Antibody responses against JEV and YFV across time points. The left panel shows the signal to noise ratio following the priming vaccination(s), at the time of the first ZPIV dose (ZPIV baseline). The right panel shows the fold increase calculated from the ZPIV baseline at 4 weeks after the second (Day 57) and third (Day 252) doses and at the last visit (ZPIV3+24W, Day 392). Median values of each group were shown in red at the bottom.

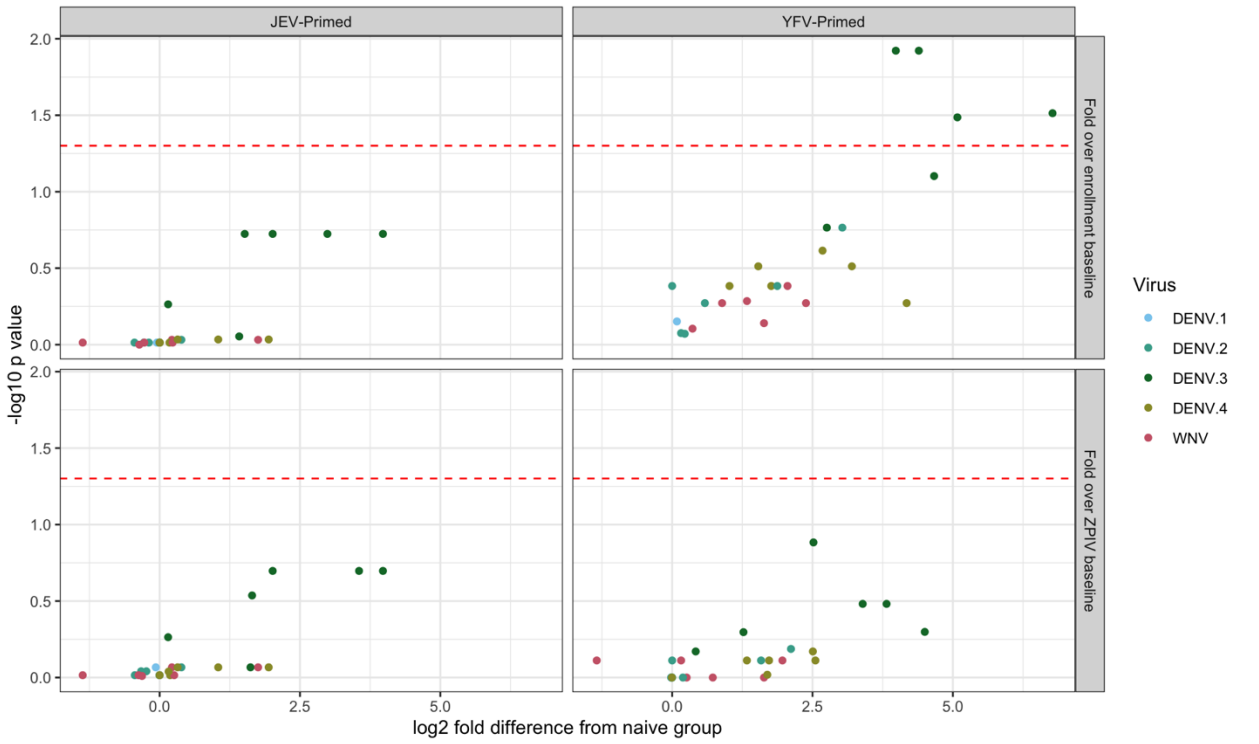

Fig. S8. Higher antibody binding responses against flaviviruses not included in the vaccines in the two primed groups. The fold difference in antibody responses is calculated between each primed groups and the naïve group using median fold over levels at enrollment visit (top) and at ZPIV baseline (bottom). Antibody responses correspond to total IgG, IgG1 and four Fc gamma receptors four weeks after the third ZPIV vaccination against the flaviviruses not part of the vaccines: DENV1-4 and WNV. P values of Wilcoxon rank sum tests are adjusted using the Benjamini–Hochberg procedure and a p value of 0.05 is shown with a red dashed line.

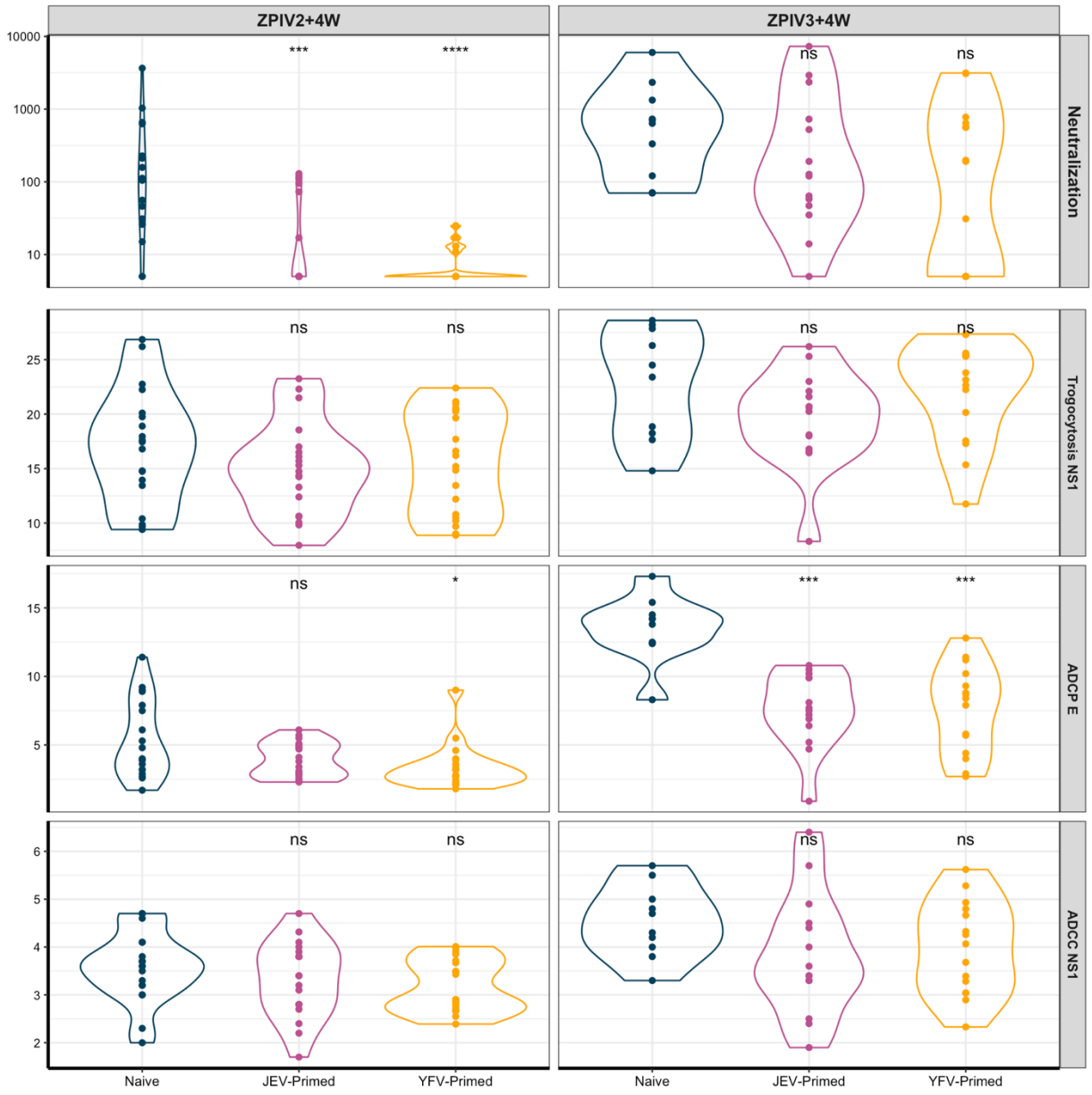

Fig. S9. Neutralizing antibody responses and Fc effector function responses against ZIKV in vaccine recipients four weeks after the second and third ZPIV vaccination. Functional responses are compared between the naïve group and the two primed groups. The significance of Wilcoxon rank sum test is shown in each panel. ns: $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

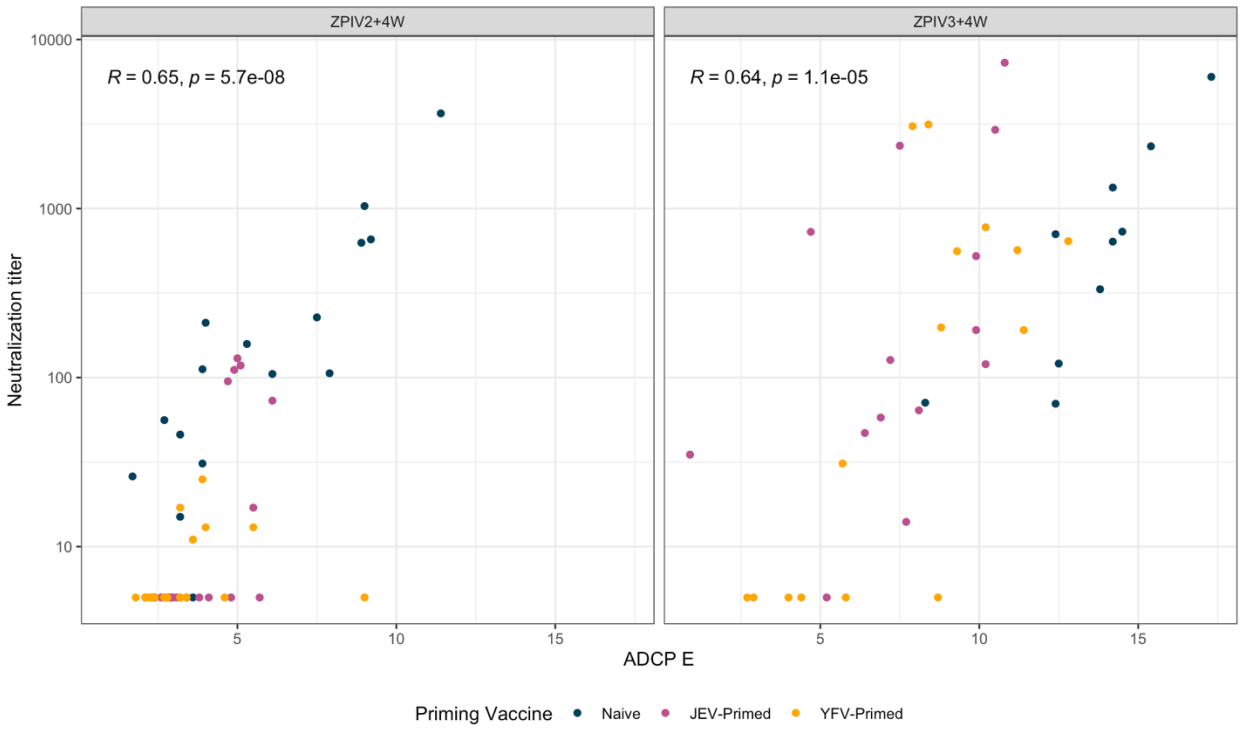

Fig. S10. Relationship between neutralization titer and ADCP against ZIKV four weeks after the second and third dose of ZPIV vaccination. Spearman's correlations were calculated with Rho and p values shown in each panel.

Fig. S11. Relationship between functional and binding antibody responses against ZIKV four weeks after the third ZPIV vaccination. Spearman correlations between four functional responses (neutralization, ADCC, ADCP, trogocytosis) and IgG response and antibody binding to four Fcγ receptors are reported with Rho values and significant relationships shown in red (p values < 0.05 adjusted using the Benjamini–Hochberg procedure).

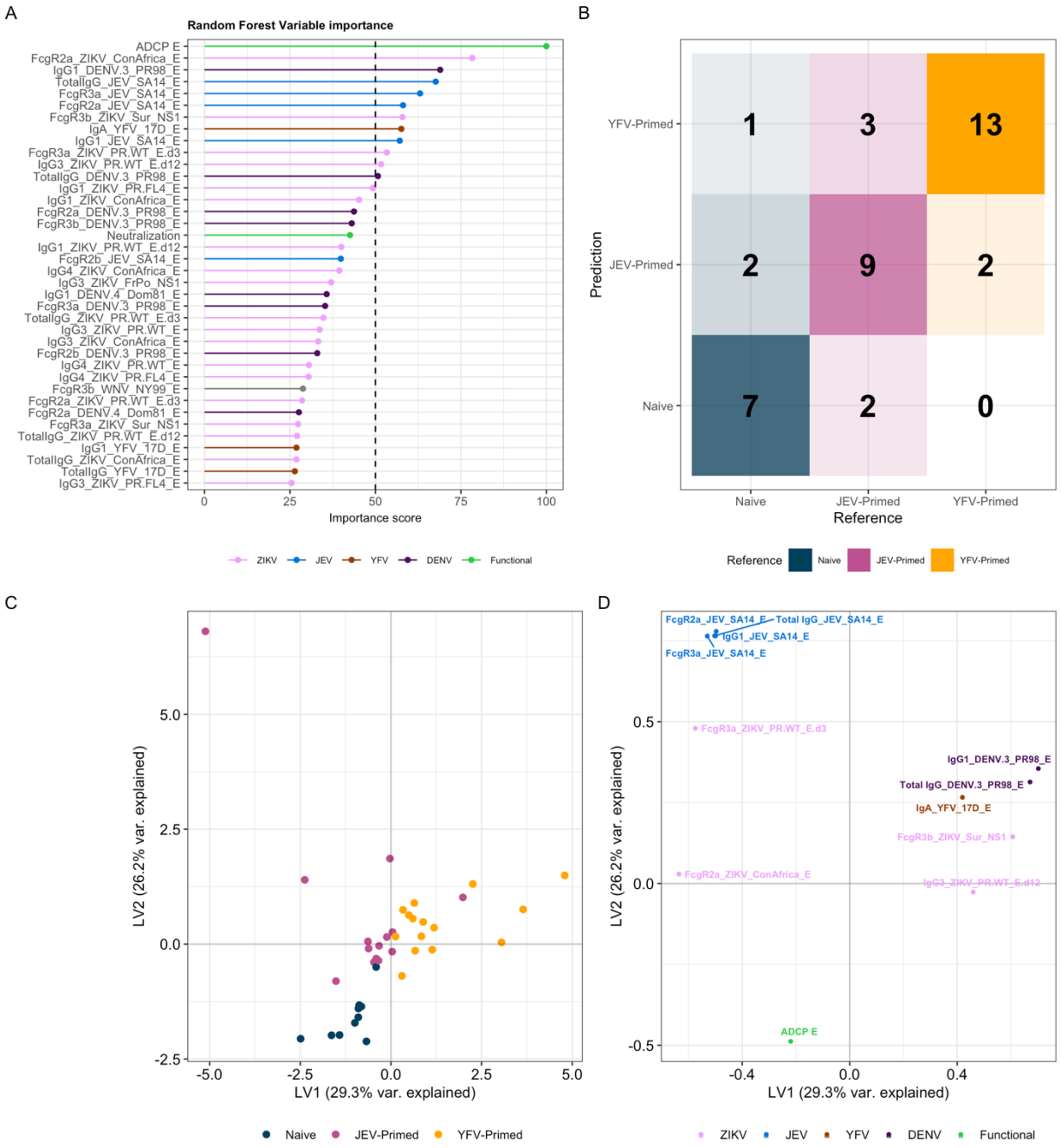

Fig. S12. Immune profiles four weeks after the third ZPIV vaccination (day 252). Random Forest models selected features that are reported with their importance score (A) and used to classify participants in each vaccine group (B). PLSDA models are shown with PLSDA scores (C) and loadings (D).

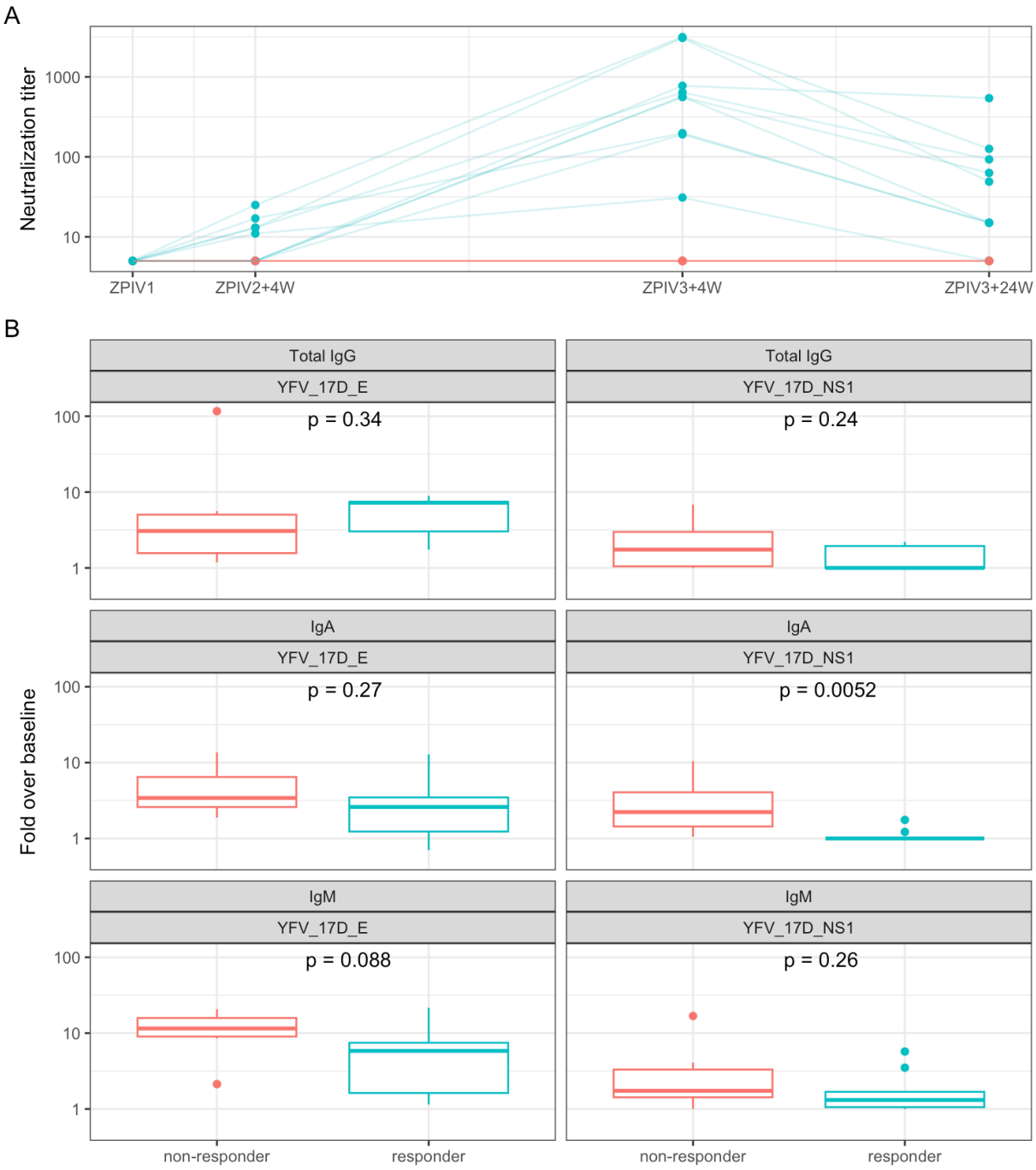

Fig. S13. Higher YFV antibody binding responses after the YFV vaccination in participants who did not mount ZPIV neutralization responses following ZPIV vaccination. (A) Participants who received a priming YFV vaccination were classified as responders (N=9) and non-responders (N=6) based on the ZPIV neutralization titers measured following ZPIV vaccination. (B) IgG, IgA and IgM responses to YFV E and NS1 antigens in responders and non-responders four weeks after the priming YFV vaccination. Neutralization responders and non-responders were compared using Wilcoxon rank sum test and the p value is reported in each panel.

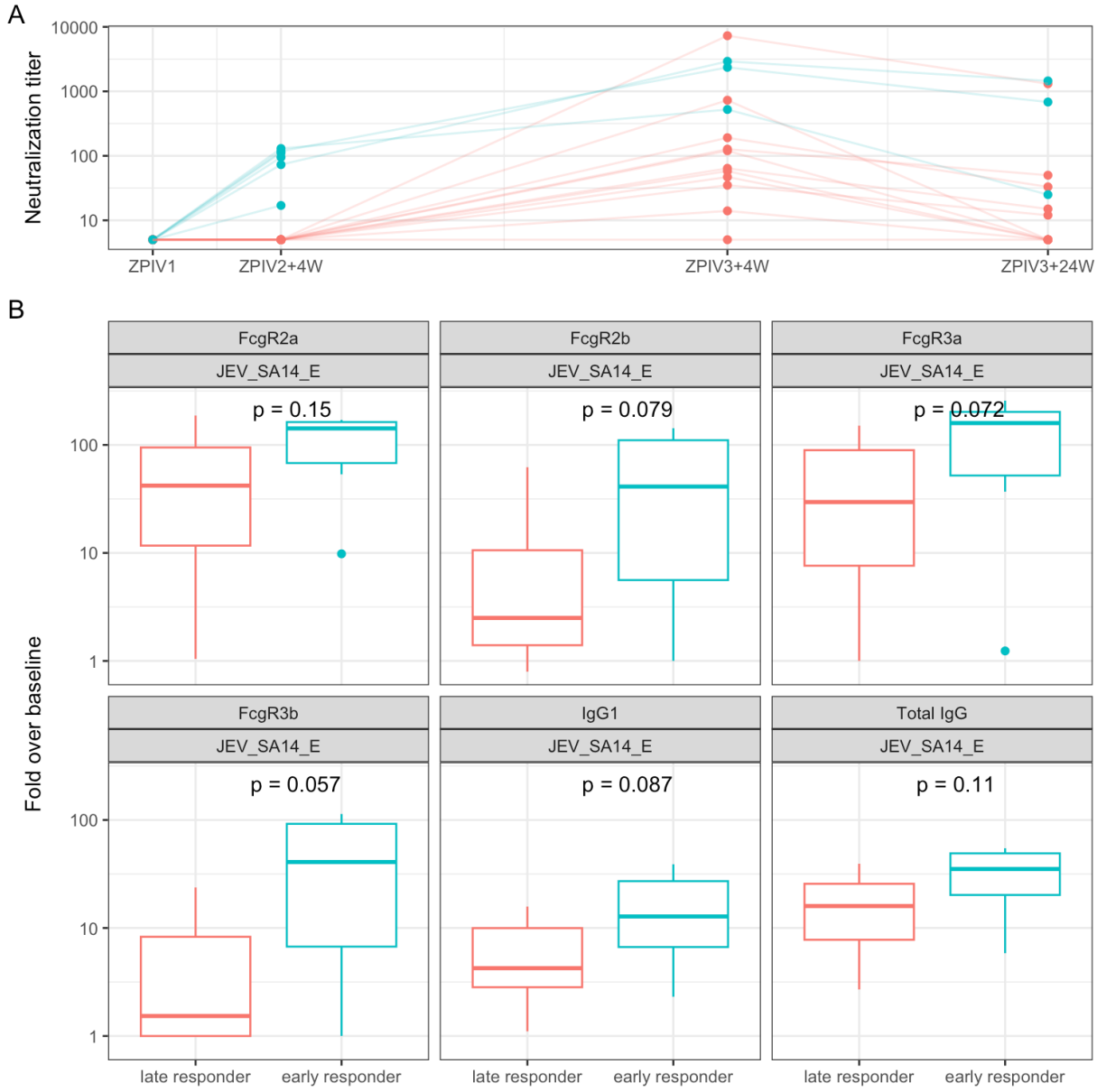

Fig. S14. Higher JEV antibody binding responses after the JEV vaccination in participants with early ZPIV neutralization responses following ZPIV vaccination. (A) Participants who received a priming JEV vaccination were classified as early responders (N=6) and late responders (N=13) based on the ZPIV neutralization titers measured following ZPIV vaccination. (B) IgG and Fcγ receptors responses to JEV E antigens in early and late responders four weeks after the priming JEV vaccination. Early and late responders to ZPIV neutralization were compared using Wilcoxon rank sum test and the p value is reported in each panel.