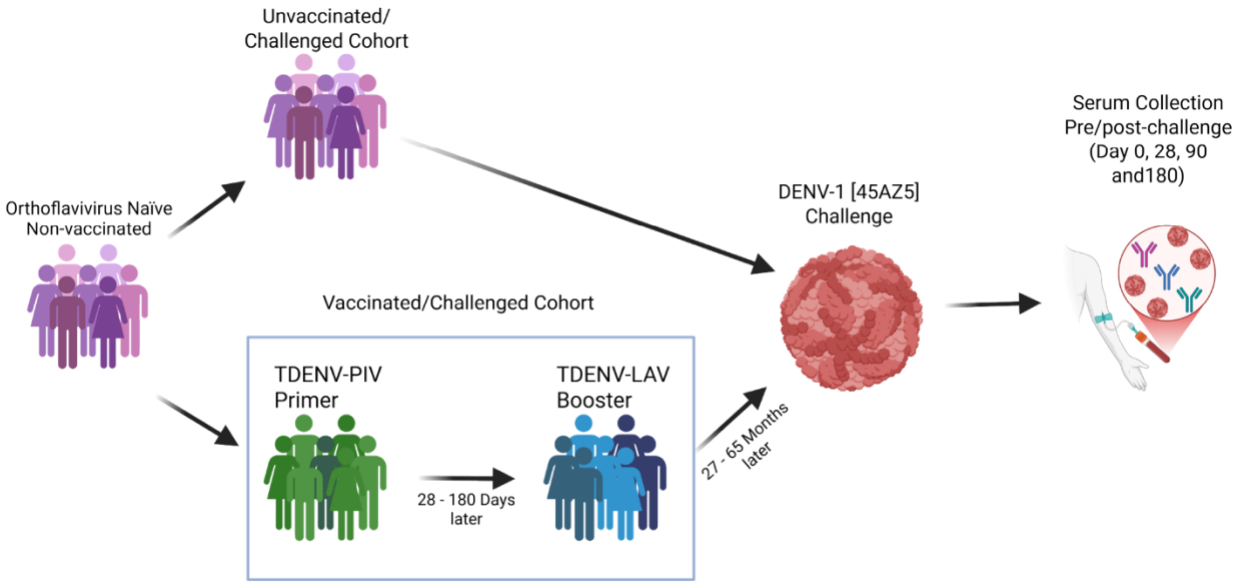
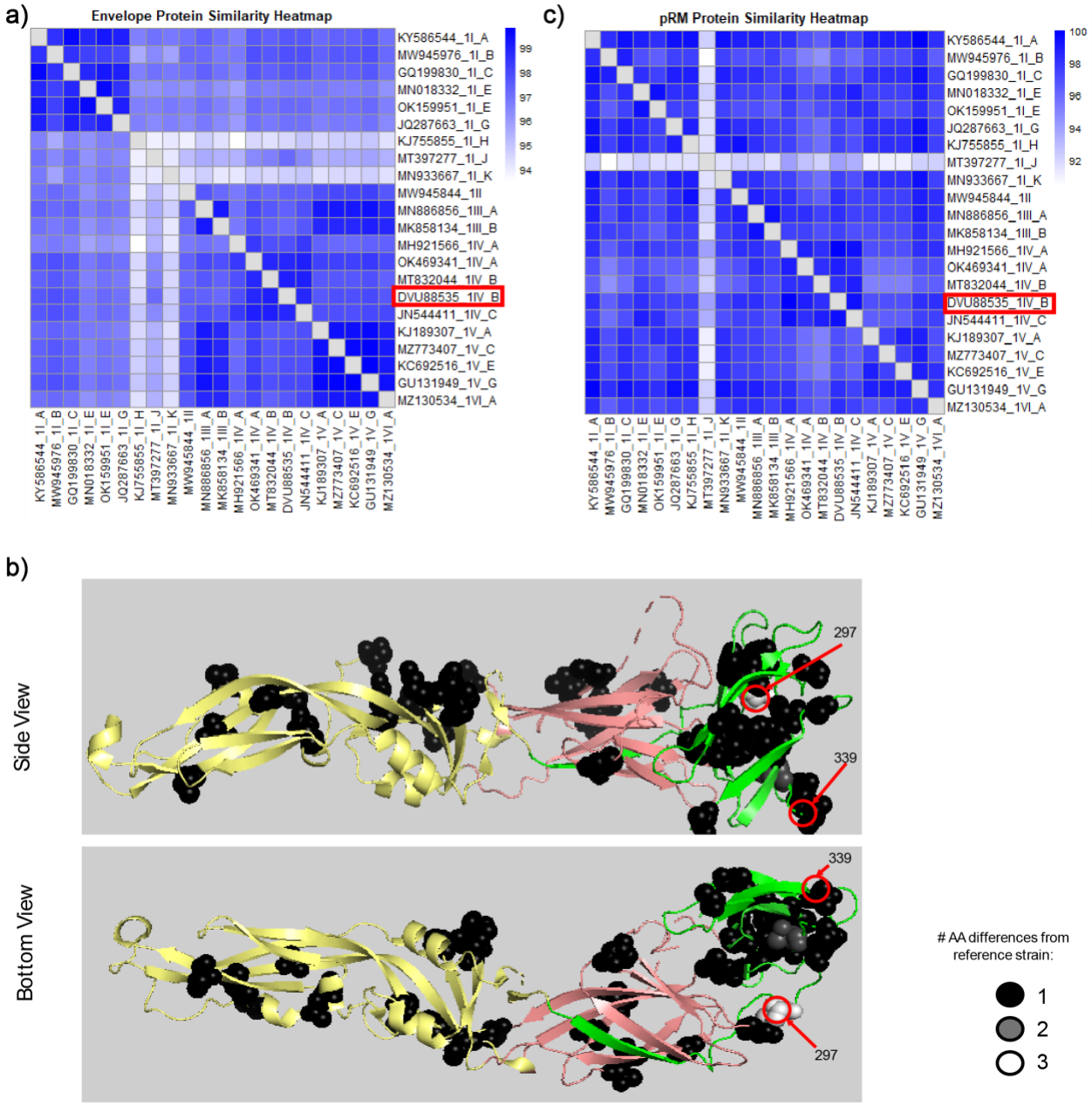
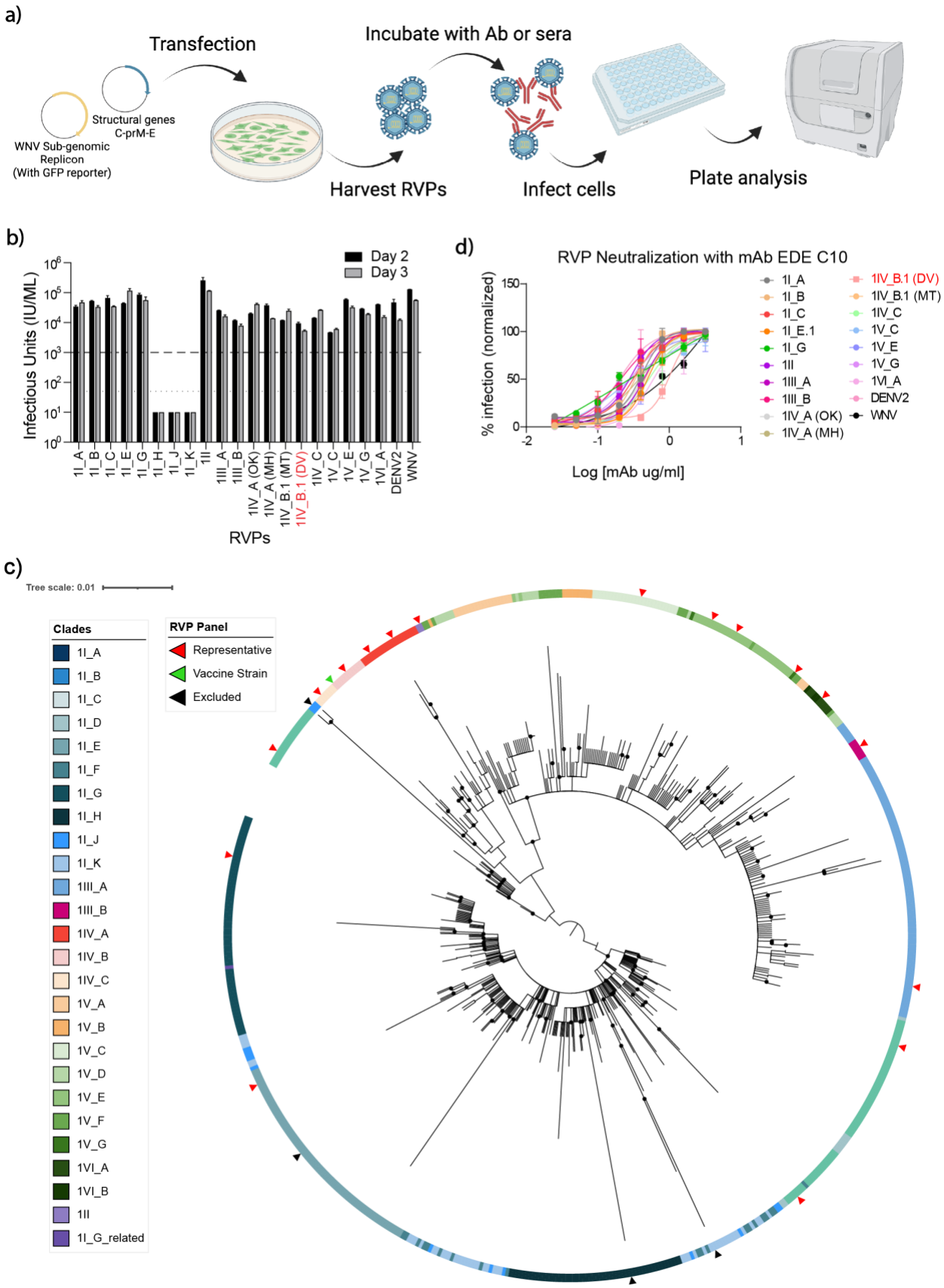
Supplemental Figures



Supplemental Figure 1. Workflow of Cohort Selection and Dengue Virus-1 Challenge after Tetravalent Prime-Boost Vaccination. These were the steps during the TDENV-PIV study to separate vaccinated and unvaccinated cohorts and challenge them with the experimental DENV-1 45AZ5 strain. Serum from each cohort was collected pre-challenge (day 0) and post-challenge (day 28, 90, and 180).

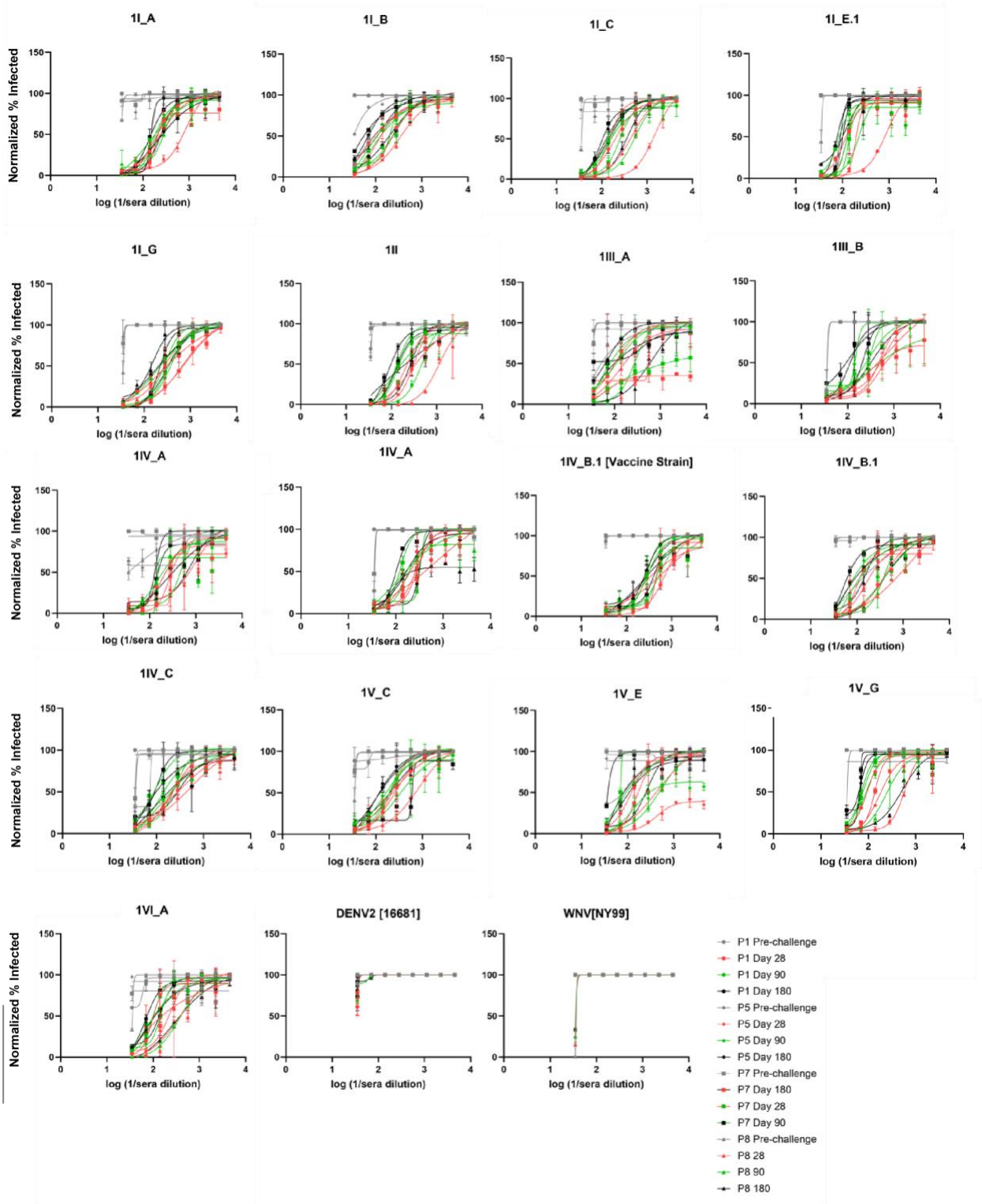


Supplemental Figure 2. Genetic similarity matrix of the prM and envelope protein among DENV-1 representatives. (A) The genetic similarity matrix of the envelope protein from our panel. The vaccine strain is highlighted with a red rectangle. The similarity matrix was calculated using the pairwise/multiple sequence alignment algorithm in Geneious Prime. **(B)** Amino acid variation on the envelope protein. The envelope protein (PDB: 7A3O) of the reference strain was isolated in PyMOL. The spheres represent the number of amino acid differences from the reference strain. The black, grey, and white spheres represent 1-, 2-, and 3-amino acid differences, respectively. Note that the structure used to display the amino acid residue differences utilizes the WestPac-74 cryo-EM structure and may not reflect all of the conformational changes from each variant. **(C)** The genetic similarity matrix of the envelope proteins from our panel.

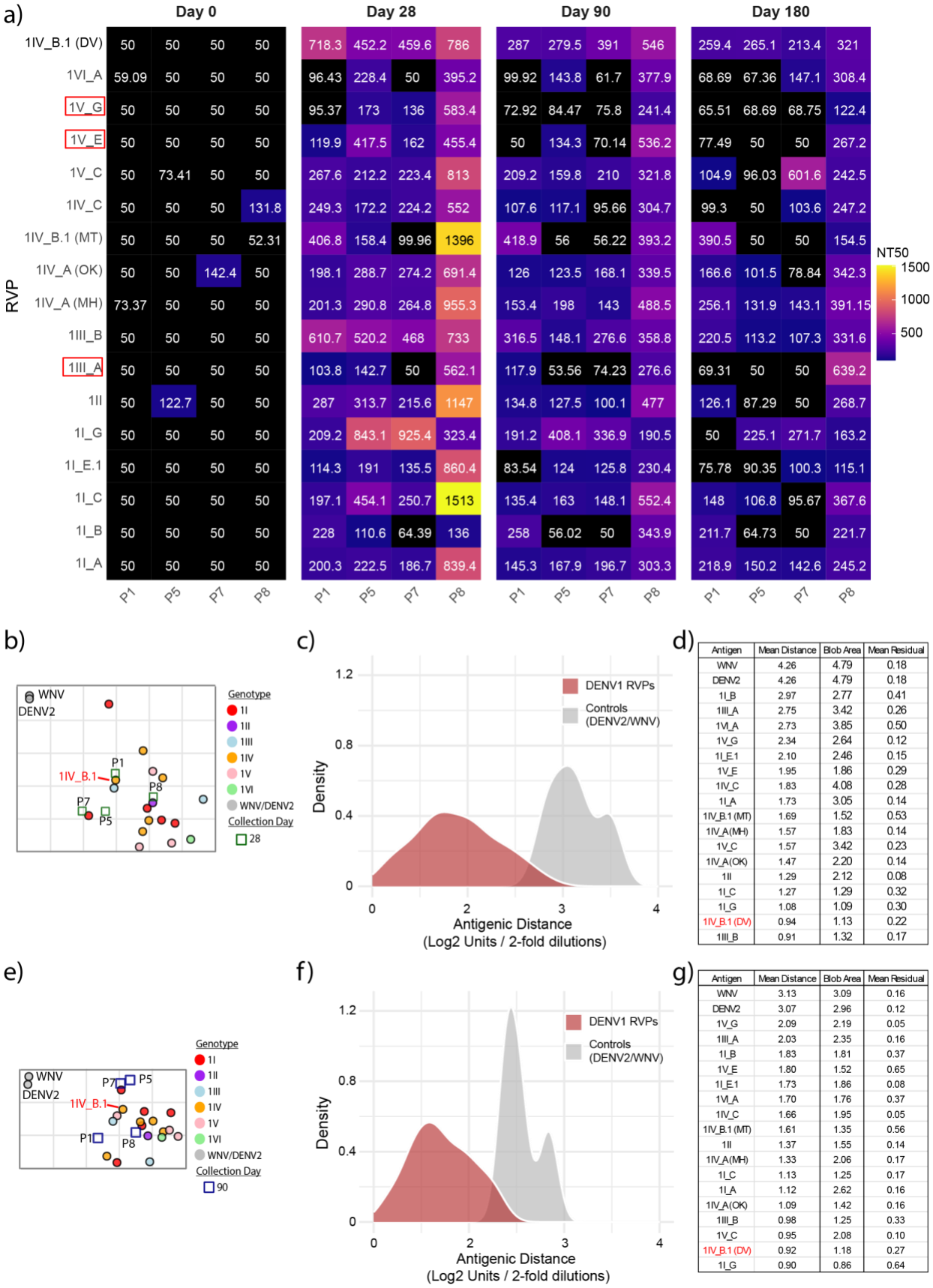


Supplemental Figure 3. DENV-1 major lineages show variable expression in Vero cells and

neutralization profiles using mAb. (A) The reporter virus particle generation and neutralization workflow. These are the steps used to generate representative reporter virus particles and test them against a panel of serum samples. **(B)** RVPs were incubated with Vero E6 Progen cells and imaged at 48 and 72 h. The black and red dotted lines represent the titers used in the neutralization assay and the background of the assay, respectively. **(C)** Maximum-likelihood tree of 624 DENV1 envelope sequences. Twenty representative strains were selected by PARNAS (excluded and downstream representatives highlighted in black and red, respectively) to represent the global diversity across DENV-1 major lineages, as shown in the legend. The TDENV reference strain used to generate the TDENV-PIV/LAV vaccine is highlighted as a neon-green square, and its corresponding major lineage is highlighted as a red rectangle, as shown in the legend. The black circles on each node indicate an ultrafast bootstrap value of at least 90. **(D)** The neutralization profile of individual RVPs tested against mAb EDE C10. Each strain is color-coded as shown in the legend. The vaccine derivative strain is highlighted in red.

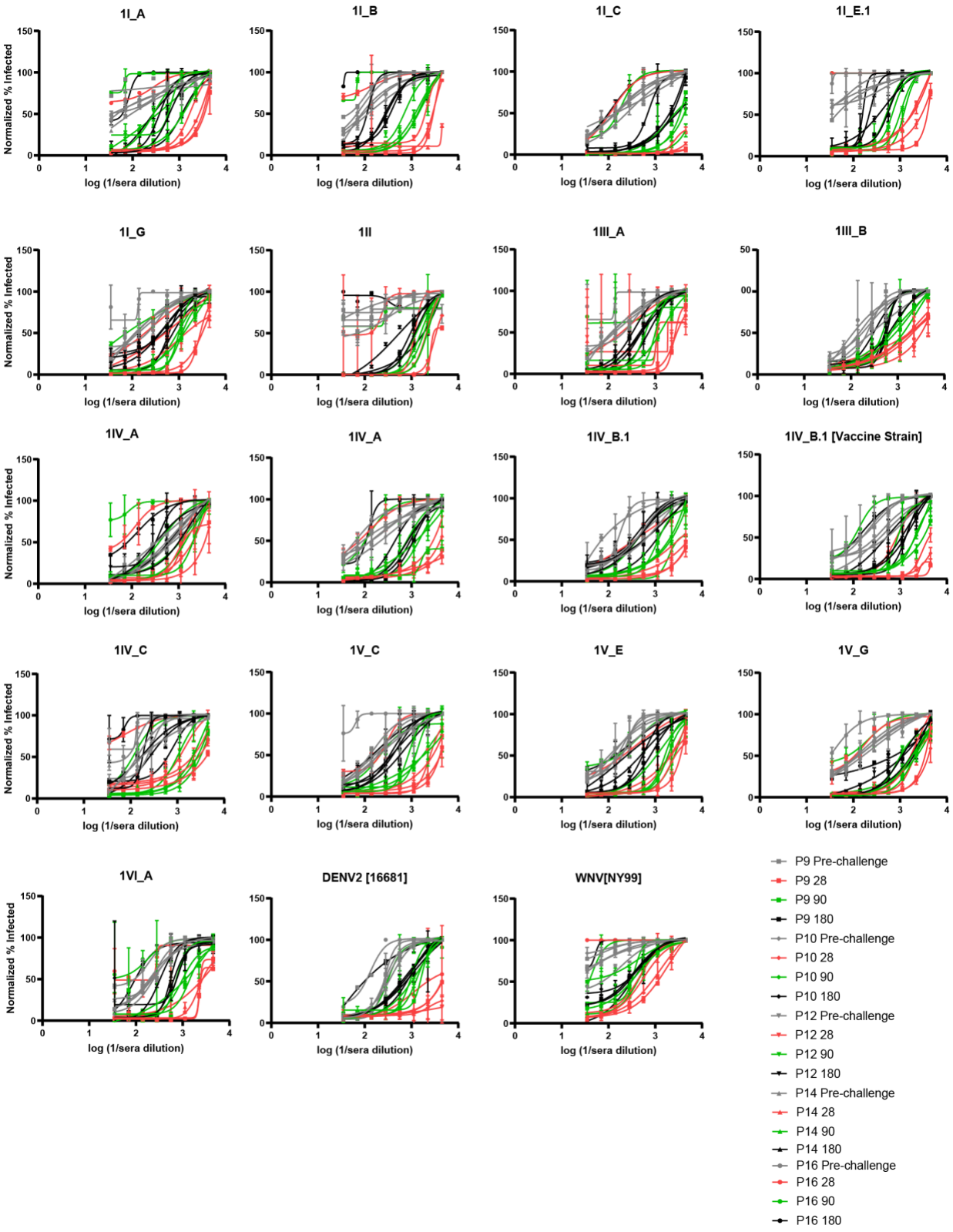


Supplemental Figure 4. The neutralization profiles of individual RVPs tested against serum from unvaccinated, U.S. dengue-naive individuals challenged with DENV-1 (strain 45AZ5).

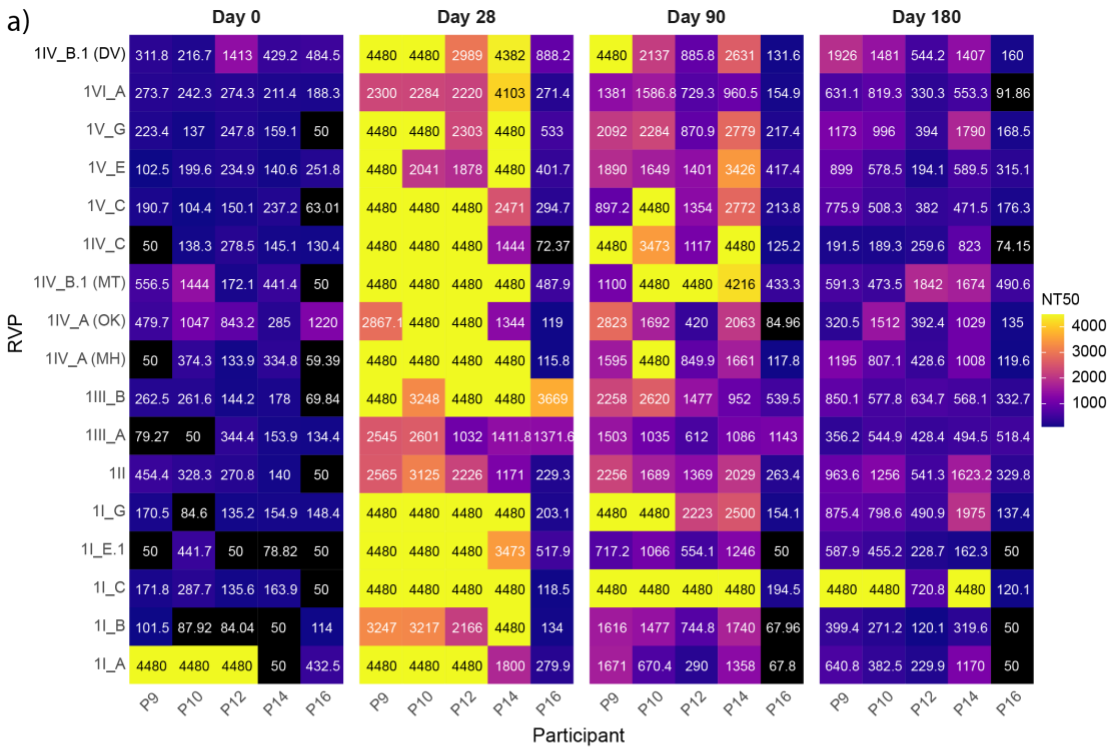


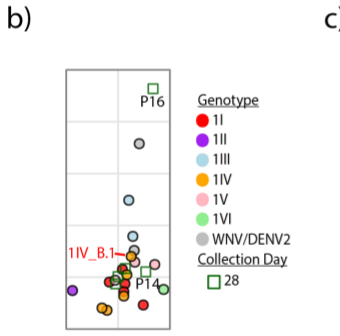
Supplemental Figure 5. Antigenic cartography profiles of DENV-1 major lineages against

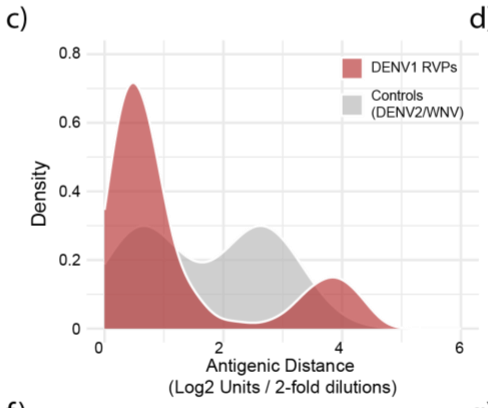
serum from unvaccinated, U.S. dengue-naive individuals challenged with DENV-1 (strain 45AZ5). (A) Heatmap showing neutralization titers (NT_{50}) for each participant in the unvaccinated cohort (n = 4) tested against a panel of major DENV-1 lineages (n = 17). Each tile represents the NT50 value for a given serum–lineage pair. The lower limit of detection was set at 50, and the maximum antibody dilution tested was 1:4480. **(B-D)** Antigenic profiles were created for the unvaccinated challenge cohort at day 28. **(B)** Antigenic map showing the relative positions of all strains based on neutralization distances. **(C)** Density distribution of antigenic distances across all lineages. Dashed vertical lines indicate the 2 AU and 3 AU thresholds, corresponding to 4-fold and 8-fold reductions in neutralization, respectively. Red distributions represent all DENV-1 strains in this study, and grey distributions represent antigenic controls (DENV-2 [strain: 16681] and WNV [strain: NY99]). **(D)** Summary table of each strain's antigenic profile, including the mean antigenic distance across all sera, blob area (reflecting the positional uncertainty of each antigen), and mean residual (indicating the agreement between predicted distances and observed neutralization titers). **(E-G)** Antigenic profiles were created for the unvaccinated challenge cohort at day 90, structured identically to panels **B–D**.



Supplemental Figure 6. The neutralization profiles of individual RVPs tested against serum from vaccinated, U.S. dengue-naive individuals challenged with DENV-1 (strain 45AZ5).

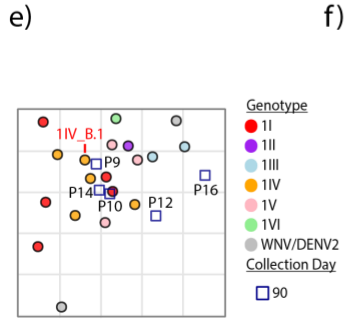


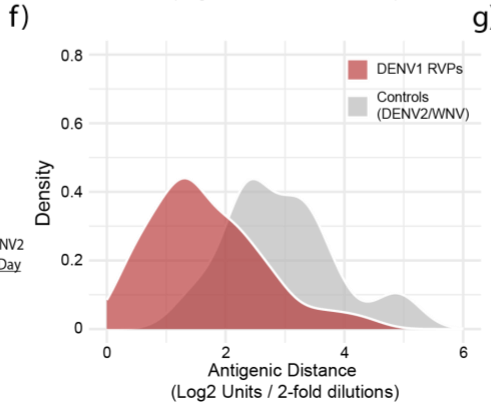




d)

Antigen	Mean Distance	Blob Area	Mean Residual
WNV	2.27	1.09	0.84
1II	1.70	1.94	0.29
1III_A	1.61	1.49	0.49
1IV_A(OK)	1.51	1.03	0.60
1IV_C	1.50	0.88	0.85
1VI_A	1.41	1.39	0.16
1I_B	1.40	1.21	0.50
1III_B	1.21	0.61	1.06
1V_E	1.15	1.19	0.34
1IV_A(MH)	1.11	0.66	0.58
1I_C	1.10	0.66	0.57
DENV2	1.07	0.77	0.61
1I_A	1.06	0.85	0.30
1IV_B.1 (MT)	1.00	0.71	0.41
1I_G	0.99	0.65	0.36
1V_G	0.97	0.85	0.32
1V_C	0.97	0.79	0.17
1I_E.1	0.90	0.71	0.21
1IV_B.1 (DV)	0.90	0.62	0.26



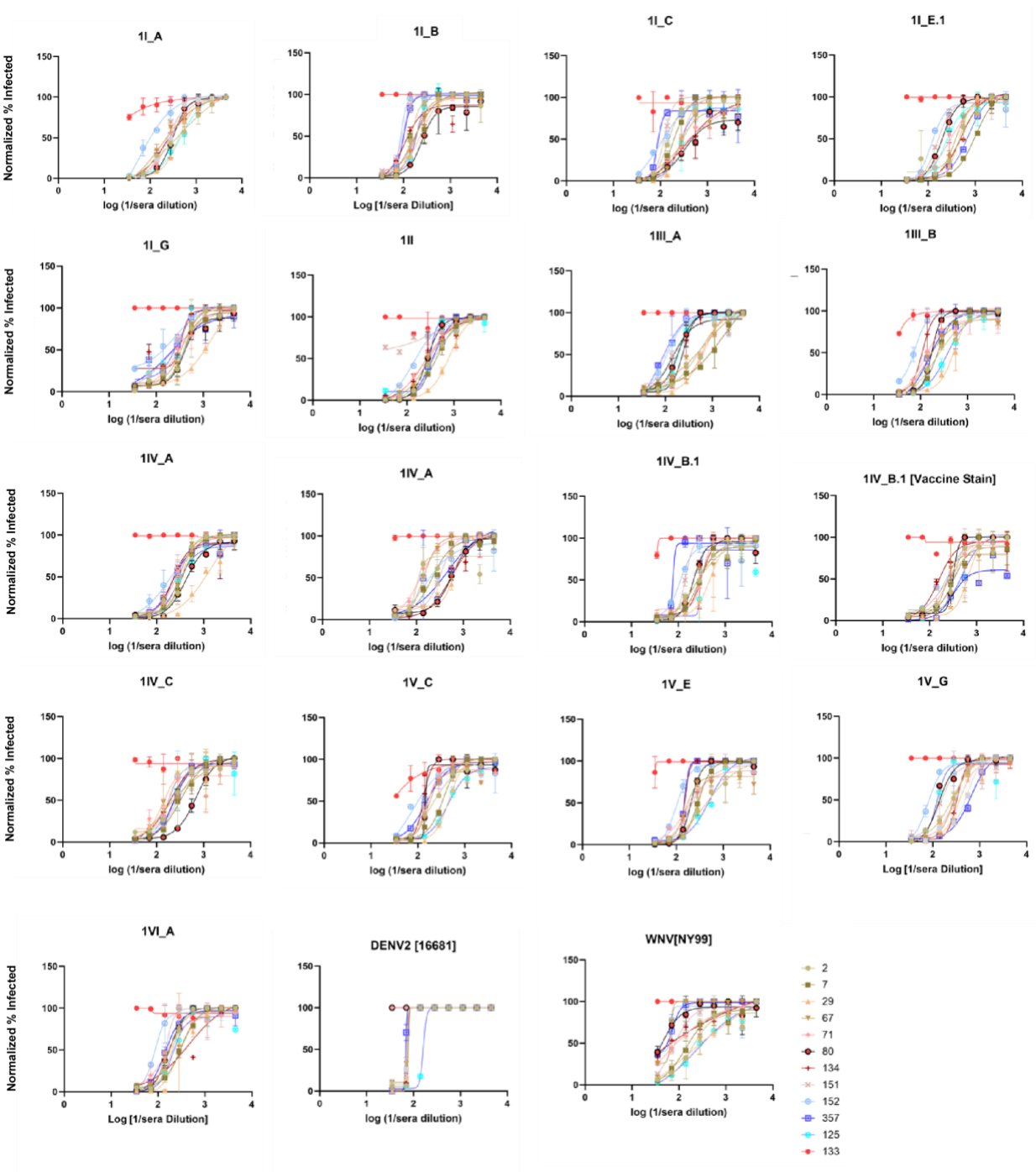


g)

Antigen	Mean Distance	Blob Area	Mean Residual
WNV	3.67	3.41	0.25
1I_E.1	2.82	2.54	0.12
1I_A	2.78	2.59	0.29
1I_B	2.22	2.59	0.07
DENV2	2.20	1.42	0.62
1VI_A	2.16	1.71	0.28
1IV_A(OK)	2.07	1.75	0.28
1III_A	1.87	1.20	0.57
1IV_A(MH)	1.70	1.68	0.43
1V_G	1.47	3.11	0.30
1IV_B.1 (MT)	1.46	1.21	0.14
1II	1.45	1.34	0.13
1III_B	1.42	1.21	0.43
1V_C	1.38	1.21	0.49
1V_E	1.28	1.29	0.38
1IV_C	1.19	0.86	0.31
1IV_B.1 (DV)	1.08	0.77	0.64
1I_G	1.03	1.27	0.46
1I_C	0.94	0.64	0.53

Supplemental Figure 7. Antigenic cartography profiles of DENV-1 major lineages against

serum from vaccinated, U.S. dengue-naive individuals challenged with DENV-1 (strain 45AZ5). (A) Heatmap showing neutralization titers (NT_{50}) for each participant in the vaccinated cohort (n = 5) tested against a panel of major DENV-1 lineages (n = 17). Each tile represents the NT50 value for a given serum–lineage pair. The lower limit of detection was set at 50, and the maximum antibody dilution tested was 1:4480. **(B-D)** Antigenic profiles were created for the vaccinated challenge cohort at day 28. **(B)** Antigenic map showing the relative positions of all strains based on neutralization distances. **(C)** Density distribution of antigenic distances for all lineages with a given genetic distance. Dashed vertical lines indicate the 2-AU and 3-AU thresholds, corresponding to 4-fold and 8-fold reductions in neutralization, respectively. Red distributions represent all DENV-1 strains in this study, and grey distributions represent antigenic controls (DENV-2 [strain: 16681] and WNV [strain: NY99]). **(D)** Summary table of each strain's antigenic profile, including the mean antigenic distance across all sera, blob area (reflecting the positional uncertainty of each antigen), and mean residual (indicating the agreement between predicted distances and observed neutralization titers). **(E-G)** Antigenic profiles were created for the vaccinated challenge cohort at day 90, structured identically to panels **B–D**.



Supplemental Figure 8. The neutralization profiles of individual RVPs tested against serum from naturally infected individuals.