Table S1. Longitudinal analysis of neutralizing antibody titers in selected cohort participants.

985

Year		2004	2005	2006	2007	2008	2009	2010	2011
NT50	DENV1	<10	<10	16	26	61	52	25	304
	DENV2	<10	<10	2150	1347	947	1061	466	1586
	DENV3	<10	<10	32	41	58	35	37	887
	DENV4	<10	<10	<10	<10	<10	<10	<10	<10
Infecting serotype				DENV2					DENV3
Infection outcome				Inapparent					Symptomatic

1791

Year		2004	2005	2006	2007	2008	2009	2010	2011
NT50	DENV1	<10	<10	30	22	47	<10	47	373
	DENV2	<10	353	500	304	334	286	243	475
	DENV3	<10	74	10	33	97	57	36	849
	DENV4	<10	30	18	32	39	65	31	551
Infecting serotype			DENV2						DENV3
Infection outcome			Inapparent						Symptomatic

3243

Year		2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
NT50	DENV1						<10	65	75	166	814
	DENV2						<10	11	26	56	71
	DENV3						<10	762	838	1890	6258
	DENV4						<10	43	23	49	523
Infecting serotype								DENV3			DENV1
Infection outcome								Symptomatic			Inapparent

Table S1. Longitudinal analysis of neutralizing antibody titers in selected cohort participants. Related to Figure 1
Annual samples collected from each of the three participants in this study were used for neutralization assays. A ≥4-fold increase in DENV-specific neutralizing antibody titers (NT_{50}) in paired annual samples indicates a DENV infection during the study year, as highlighted for DENV1 (orange), DENV2 (yellow) and DENV3 (green). All three donors experienced one symptomatic DENV3 infection, which was also confirmed by RT-PCR/virus isolation.

Supplementary Table 2 – capture ELISA data and Neut data from all 3 labs for 15 hmAbs

					ELISA-VUMC (ng/ml)				NEUT-WUSTL (ng/ml)				NEUT-UCB (ng/ml)				UNC-CH (ng/ml)			
mAb Clone	Donor ID	Sample Harvest date	IgG sub-class	light chain	DV1 Thailand /16007/ 1964	DV2 Thailand /16681/ 1964	DV3 Philippines/16562/1964	DV4 Indonesia/1036/1976	DV1 Nauru/ West Pac/1974	DV2 Thailand /S16803 /1974	DV3 Thailand /CH53489/1973	DV4 Columbia/TVP-376/1982	DV1 1265-4	DV2 N172-06	DV3 N2845-09	DV4 N703-99	DV1ic West Pac 74	DV2ic 16803	DV3ic SriLanka 89 Geno III	DV4ic SriLanka 92
66	1037	2013	IgG1	κ	NB	NB	63.2	NB	NN	NN	NN	NN	NN	NN	146.7	NN	NN	NN	66.2	NN
115	1037	2013	IgG1	λ	NB	NB	106.9	NB	NN	NN	5.5	NN	NN	NN	87.0	NN	NN	NN	5.5	NN
144	1037	2013	IgG1	κ	NB	NB	109.1	NB	329	NN	22.0	NN	2951	NN	39.4	NN	NN	NN	18.9	NN
236	985	2011	IgG1	λ	NB	NB	390.0	NB	NN	NN	9.3	NN	NN	NN	15.0	NN	NN	NN	390.8	NN
286	1791	2011	IgG1	κ	NB	NB	286.0	NB	NN	NN	4.4	NN	NN	NN	18.0	NN	NN	NN	26.3	NN
290	1791	2011	IgG1	κ	NB	NB	225.0	NB	NN	NN	79.0	N/A	NN	NN	74.0	NN	NN	NN	4.4	NN
297	1791	2011	IgG1	λ	NB	NB	142.0	NB	NN	NN	5.3	NN	NN	NN	21.0	NN	NN	NN	18.2	NN
298	1791	2011	IgG1	κ	NB	NB	124.0	NB	NN	NN	8.0	N/A	NN	NN	100.0	NN	NN	NN	22.7	NN
354	1791	2011	IgG1	κ	NB	NB	91.0	NB	NN	NN	8.1	N/A	NN	NN	100.0	NN	NN	NN	57.6	NN
404	985	2011	IgG1	κ	NB	NB	37.3	NB	NN	NN	5.0	NN	NN	NN	33.0	NN	NN	NN	7.9	NN
406	985	2011	IgG1	λ	NB	NB	136.0	NB	NN	NN	64.0	NN	NN	NN	224.0	NN	NN	NN	68.2	NN
415	985	2011	IgG1	κ	NB	NB	26.2	NB	NN	NN	731.0	NN	N/A	N/A	N/A	N/A	NN	NN	57.6	NN
419	985	2011	IgG1	κ	NB	NB	44.4	NB	N/A	N/A	N/A	N/A	NN	NN	2.8	NN	NN	NN	1.8	NN
437	985	2011	IgG1	κ	NB	NB	25.8	NB	NN	NN	9.0	NN	NN	NN	207.0	NN	NN	NN	3.9	NN
443	985	2011	IgG3	λ	NB	NB	15.1	NB	NN	NN	11.0	NN	NN	NN	57.0	NN	NN	NN	4.2	NN

NB = Non Binding
NN = Non Neutralizing
N/A = Not Attempted

Table S2. Capture ELISA and FRNT data. Related to Figure 1 and S1.

Live-virus capture ELISA EC_{50} values were determined at Vanderbilt University Medical Center, Vero-81 cell FRNT EC_{50} values for WHO prototype strains for DENV 1-4 were performed at Washington University in St. Louis, U937 neutralization EC_{50} values were determined at U.C. Berkeley, and Vero-81 cell FRNT EC_{50} value were determined at University of North Carolina-CH.

Supplementary Figure 1 – DENV3 hmAbs are TS by Capture ELISA

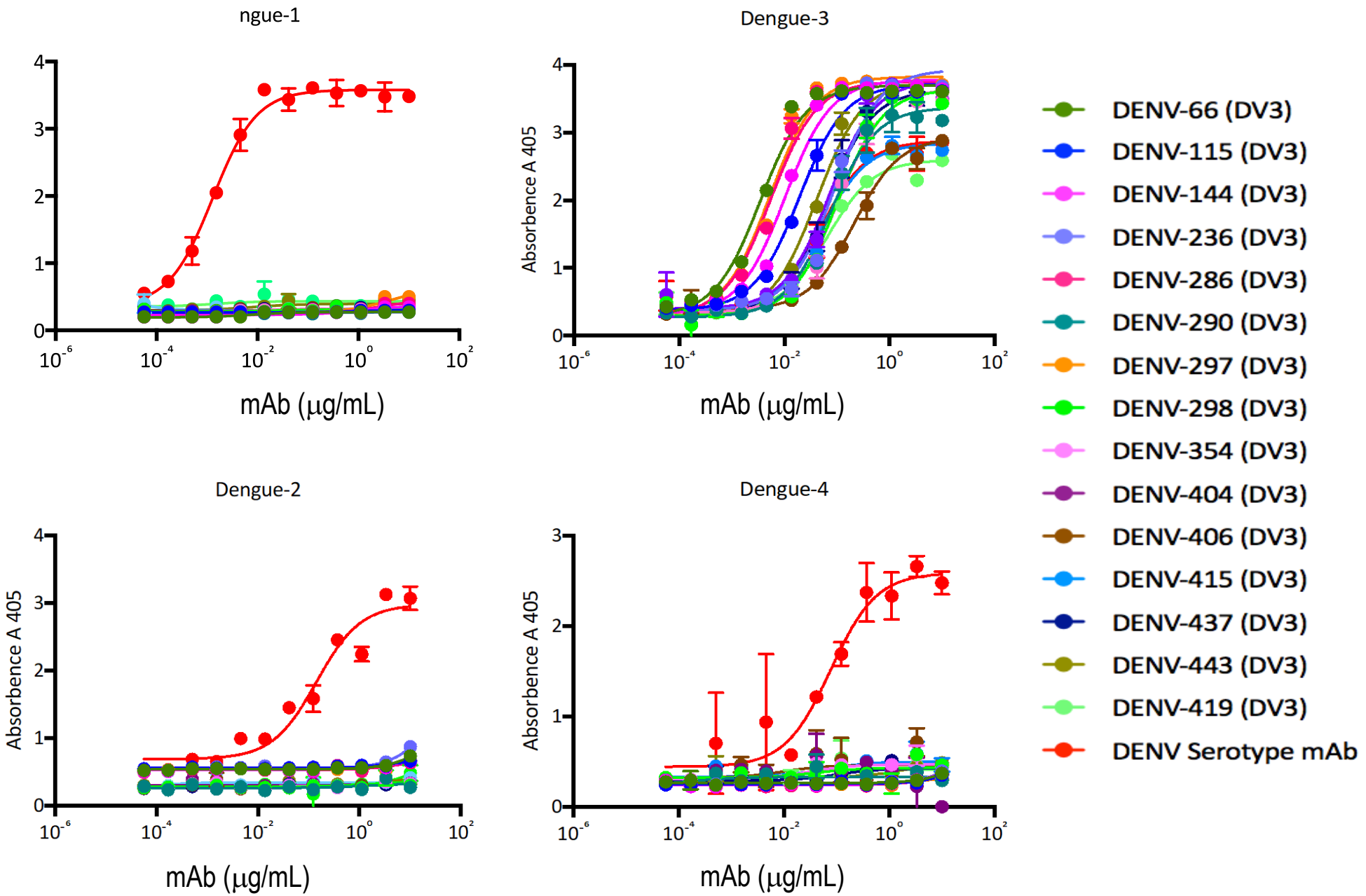


Figure S1. DENV3 hmAbs are type-specific by capture ELISA. Related to Figure 1 and Supplemental Table 1.

Binding curves of individual hmAbs to 4G2 captured DENV1, DENV2, DENV3 or DEMV4 are shown. DENV Serotype mAb are DENV1-specific hmAb 1F4, DENV2-specific hmAb 2D22, DENV3-specific hmAb 5J7 and DENV4-specific hmAb D4-126 and were used as positive controls.

Supplemental Figure 2 – Foci on Vero-81 cells

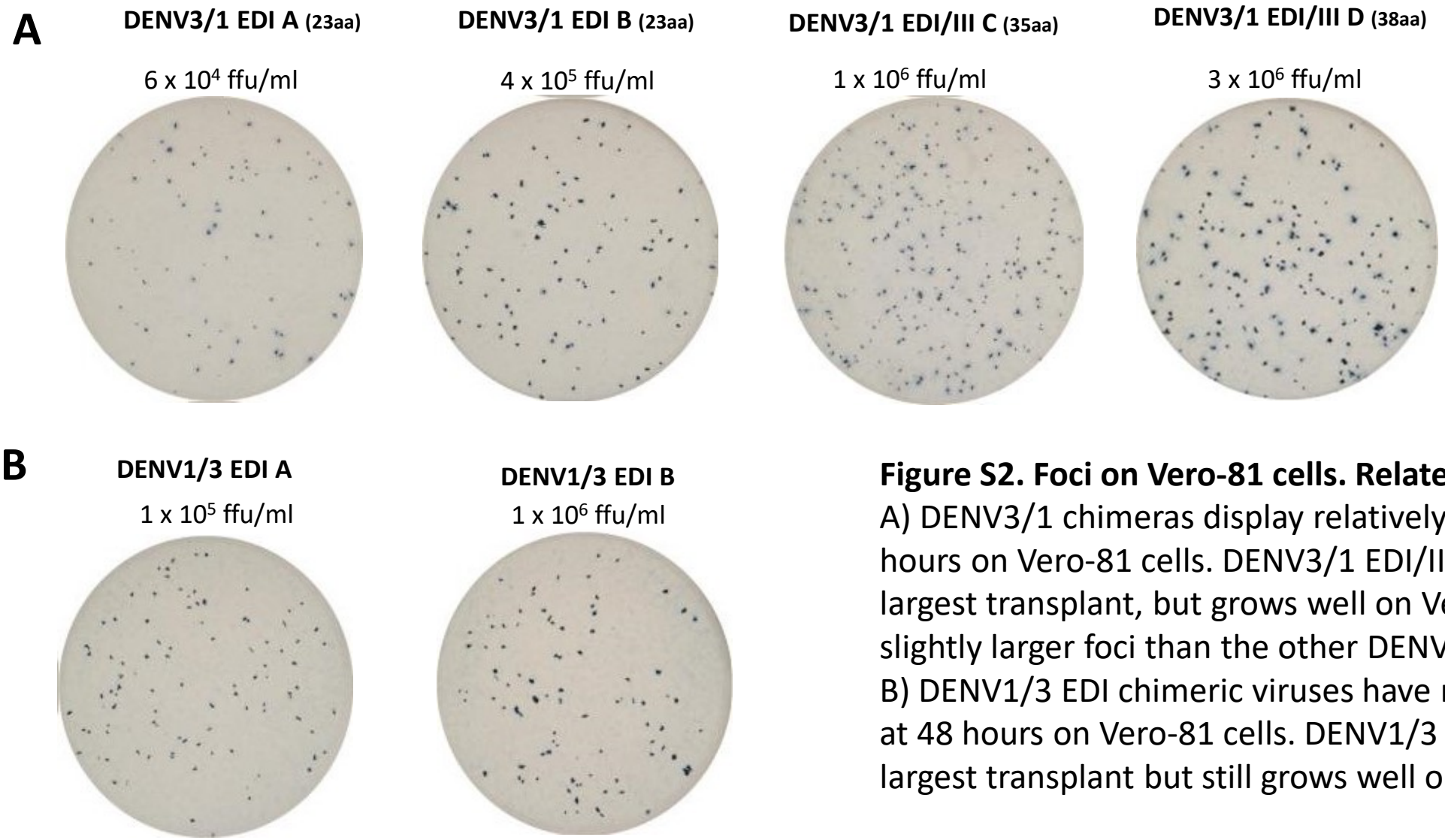


Figure S2. Foci on Vero-81 cells. Related to Figures 3 and 4. A) DENV3/1 chimeras display relatively similar foci at 48 hours on Vero-81 cells. DENV3/1 EDI/III D contains the largest transplant, but grows well on Vero cells and displays slightly larger foci than the other DENV3/1 chimeric viruses. B) DENV1/3 EDI chimeric viruses have relatively similar foci at 48 hours on Vero-81 cells. DENV1/3 EDI B contains the largest transplant but still grows well on Vero-81 cells.

Supplemental Figure 3 - **Neutralization curves for DENV1/3 chimeric viruses.**

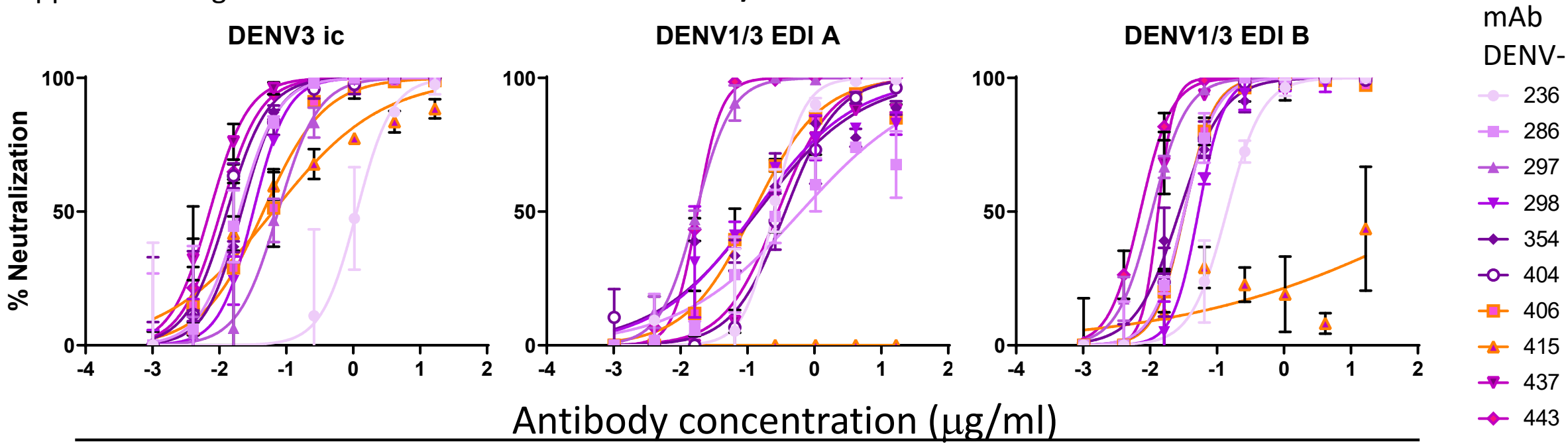
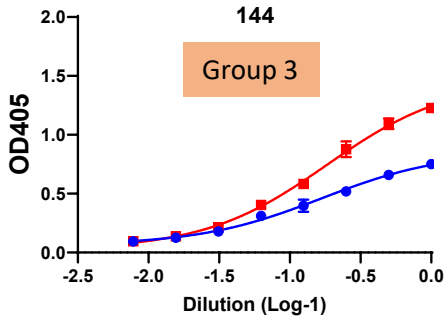
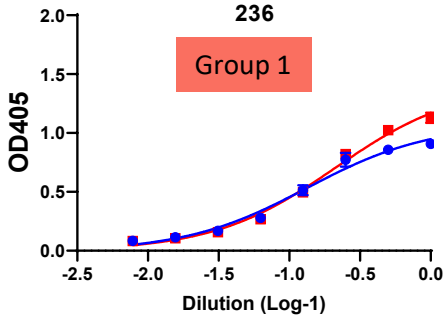


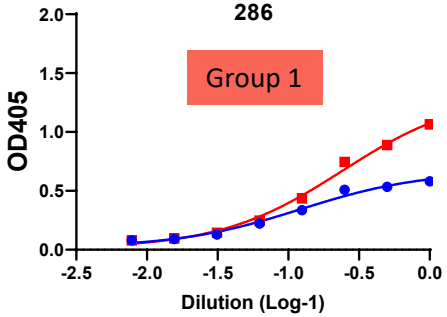
Figure S3. Neutralization curves for DENV1/3 chimeric viruses. Related to Figure 4.

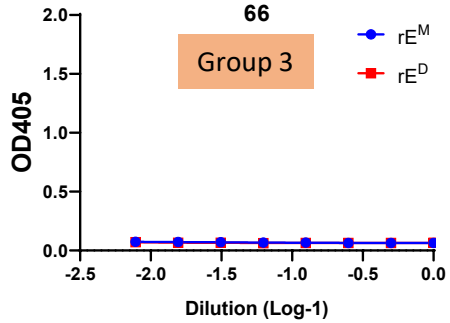
Vero-81 FRNT neutralization curves for parental DENV3 ic and chimeric DENV1/3 EDI-A and DENV1/3 EDI B are shown. Neutralization curves for DENV1/3 EDI B are very similar to neutralization curves for the parental DENV3, indicating a similar interaction of the hmAbs with DENV1/3 EDI B, whereas neutralization curves for DENV1/3 EDI A show reduced slopes. It is interesting that sequence analysis of hmAb variable region genes found that the group 1 EDI antibodies fall into three categories of binding interfaces defined by LOF, GOF and genotype-exchange studies using neutralization assays. Sequence analysis revealed that the group 1b hmAb DENV-236 and -297 have similar heavy chains, even though they originated in two separate individuals. Moreover, these mAbs are also the only two DENV3 hmAbs that neutralized the chimeric DENV1/3 EDI recombinant viruses significantly better than they did the parental DENV3 viruses (**Figure 3**). These mAbs are also the only two hmAbs in the panel that bound equally well to monomeric or dimeric recE and showed an inability to neutralize DENV3 G-IV (**Figure 4C**). These shared characteristics may be a result of their similar heavy chains.

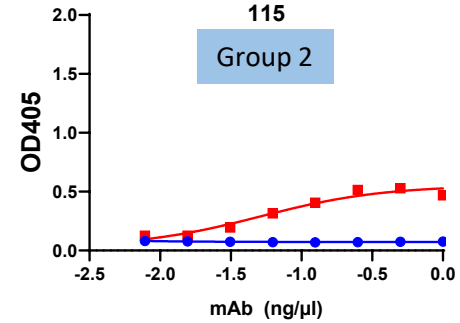
Supplemental Fig 4 - **Monomer/dimer DENV3 E glycoprotein ELISA.**

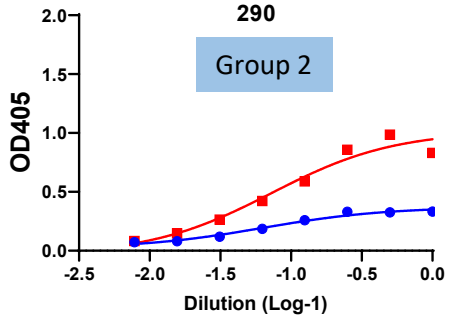


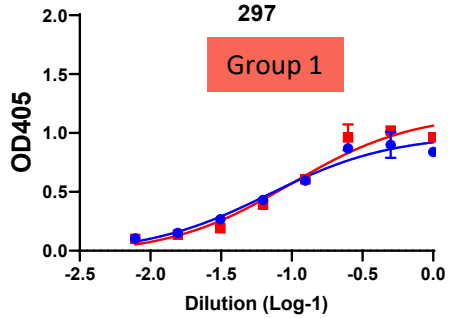


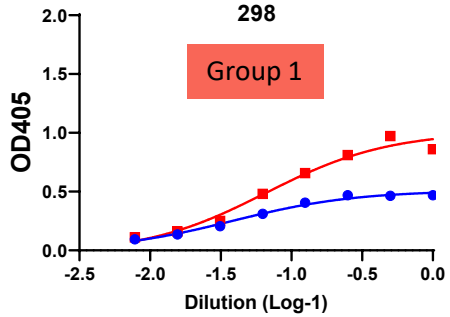


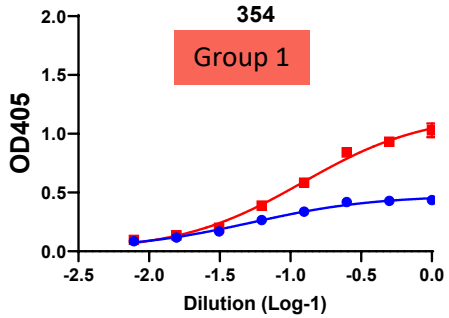


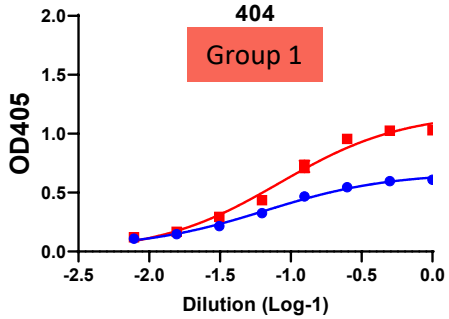


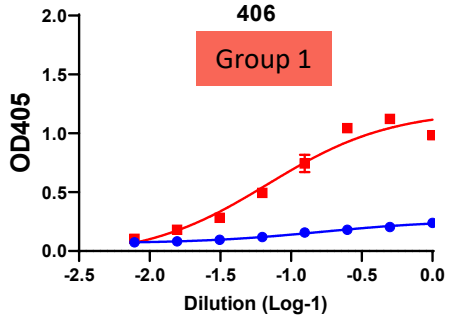


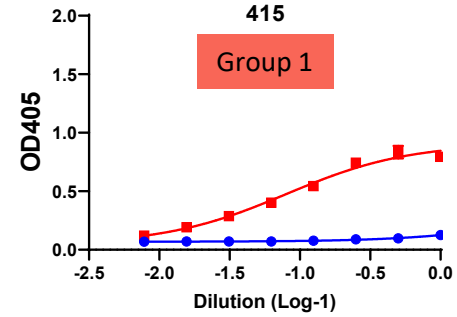


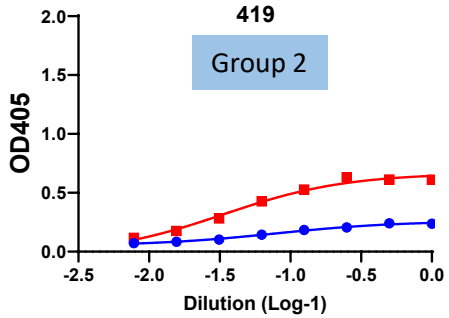


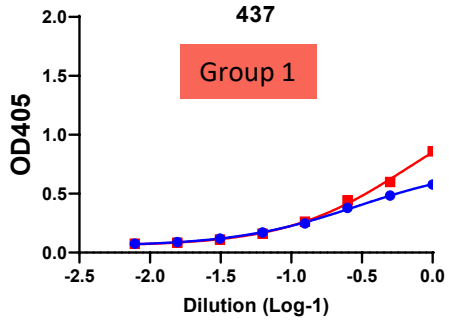


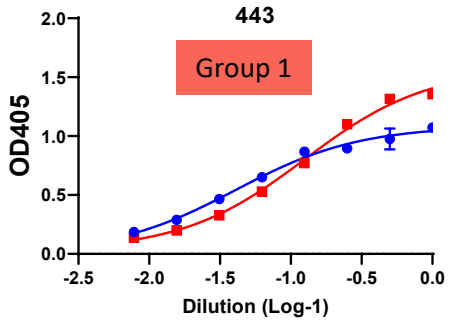


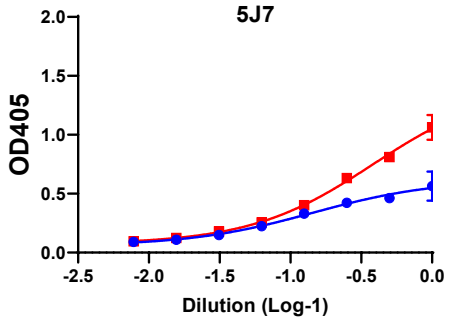












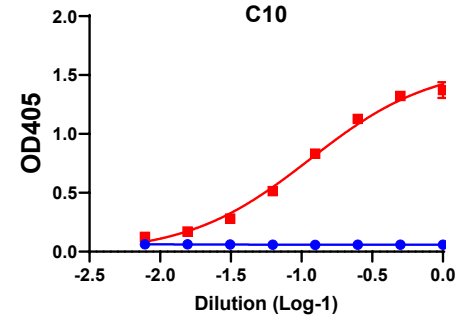
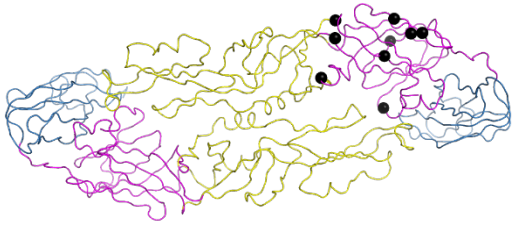


Figure S4. Monomer/dimer DENV3 E glycoprotein ELISA. Related to Virus, rE and rEDIII ELISA in Star Methods Details

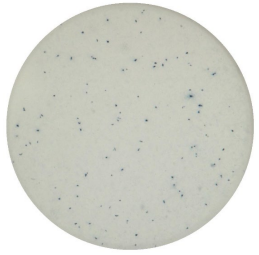
Binding curves for individual hmAbs to ELISA plates coated with rE monomers or stabilized rE dimers. A starting concentration of 2 ng/µL of hmAb was diluted 2-fold (x-axis). Bound hmAbs were detected using anti-human-IgG-alkaline phosphatase and absorbance measurement at 405 nm. In comparison to the other group 1 antibodies, hmAbs DENV-415 and -406 did not bind detectably to recE monomers but did show weak binding to recE dimers, suggesting that they recognize a quaternary epitope. Group 1a hmAbs DENV-286, -298, -354 and -404 showed weak binding to recE monomer but higher binding to recE dimers, suggesting their epitope is presented more completely by recE dimers. Group 1b hmAbs DENV-236 and -297 showed no preference for recE dimers over monomers, implying that they may recognize functional epitopes in the EDI of a single protomer. Among the remaining members of group 1, hmAb DENV-443 showed a slight preference for binding to recE dimers at higher concentrations of antibody, while hmAb DENV-437 showed a low level of binding to dimer or monomeric recE without preference, suggesting that these mAbs may recognize unique epitope configurations. In contrast, all of the group 2 hmAbs (*e.g.*, DENV-115, -290 and -419) showed a clear preference for binding to recE dimers, suggesting that they recognize quaternary epitopes. Group 3 hmAb DENV-66 did not neutralize a genotype II DENV3 virus, and consequently, did not bind recE monomers or dimers based on a genotype II E protein sequence, while the other group 3 hmAb DENV-144 preferred dimer recE and showed a similar pattern of binding as hmAb 5J7, which is known to bind a quaternary epitope. A recombinant form of the CR EDE1 epitope-specific hmAb C10 was used as a positive control, because its epitope is displayed on recE dimers and not monomers, as can be clearly seen in the binding pattern of rEDE1 C10.

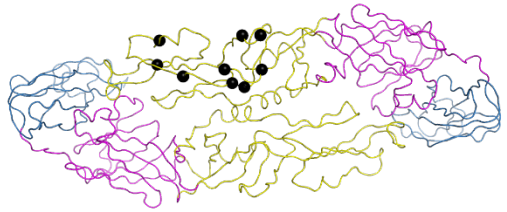
Supplemental Fig. 5

A **Gain-of-Function –** DENV3 Puerto Rico backbone with Domain 1, 2 or 3 changed to Sri Lanka

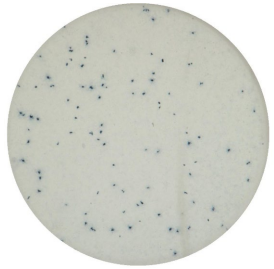


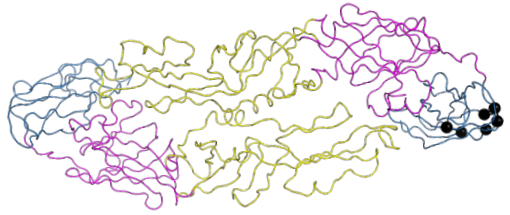
DENV3 G-IV with G-III EDI



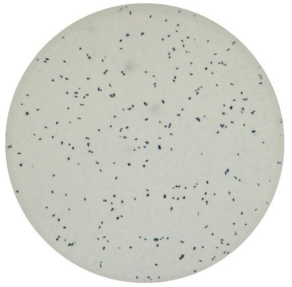


DENV3 G-IV with G-III EDII



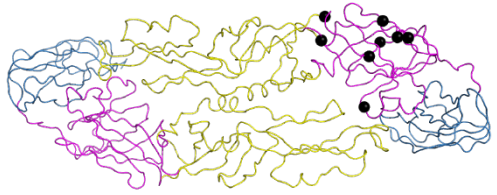


DENV3 G-IV with G-III EDII

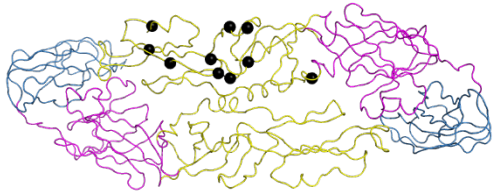


DENV3	Genotype	6	22	50	62	63	68	81	113	120	124	132	139	169	171	172	224	226	270	301	302	322	380	383	386
Puerto Rico 1977	IV	V	E	V	G	A	T	T	S	H	L	H	I	A	V	T	A	V	I	S	G	I	T	K	R
DENV3 G-IV w G-III EDI	IV/III	I	D	A	G	A	T	T	S	H	L	Y	V	T	A	I	A	V	N	S	G	I	T	K	R
DENV3 G-IV w G-III EDII	IV/III	V	E	V	E	G	I	V	L	Q	P	H	I	A	V	T	T	T	I	S	G	I	T	K	R
DENV3 G-IV w G-III EDIII	IV/III	V	E	V	G	A	T	T	S	H	L	H	I	A	V	T	A	V	I	T	N	V	I	N	K
Sri Lanka 1989	III	I	D	A	E	G	I	V	L	Q	P	Y	V	T	A	I	T	T	N	T	N	V	I	N	K
domain			I		II									I				II				III			

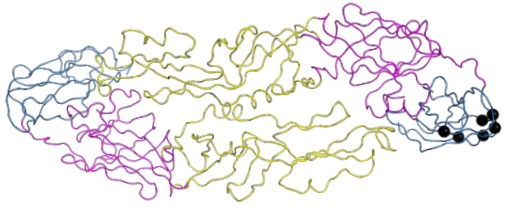
B **Loss-of-Function –** DENV3 Sri Lanka backbone with Domain 1, 2 or 3 changed to Puerto Rico



DENV3 G-III with G-IV EDI



DENV3 G-III with G-IV EDII



DENV3 G-III with G-IV EDIII

DENV3	Genotype	6	22	50	62	63	68	81	113	120	124	132	139	169	171	172	224	226	270	301	302	322	380	383	386
Sri Lanka 1989	III	I	D	A	E	G	I	V	L	Q	P	Y	V	T	A	I	T	T	N	T	N	V	I	N	K
DENV3 G-III w G-IV EDI	III/IV	V	E	V	E	G	I	V	L	Q	P	H	I	A	V	T	T	T	N	T	N	V	I	N	K
DENV3 G-III w G-IV EDII	III/IV	I	D	A	G	A	T	T	S	H	L	Y	V	T	A	I	A	V	I	T	N	V	I	N	K
DENV3 G-III w G-IV EDIII	III/IV	I	D	A	E	G	I	V	L	Q	P	Y	V	T	A	I	T	T	N	S	G	I	T	K	R
Puerto Rico 1977	IV	V	E	V	G	A	T	T	S	H	L	H	I	A	V	T	A	V	I	S	G	I	T	K	R
domain			I		II									I				II				III			

Figure S5. Gain-of-function and loss-of-function genotype domain-swap chimeras. Related to Figures 5 and 6.

A) Gain-of-Function DENV3 genotype chimeric viruses. DENV3 G-IV backbone with EDI, EDII or EDII changed to G-III. PyMOL software representations of gain-of-function viruses. Transferred residues from a DENV3 Sri Lanka strain are designated by black spheres on a DENV3 Puerto Rico strain backbone. Foci at 48 hours on Vero-81 cells are shown. Amino acid alignment of changes residues are shown. Puerto Rico DENV3 residues are shown in purple and Sri Lanka DENV3 residues are shown in blue. Domain map of residues is shown in gray.

B) Loss-of-Function DENV3 genotype chimeric viruses. DENV3 G-III backbone with EDI, EDII or EDII changed to G-IV. PyMOL software representations of loss-of-function viruses. Transferred residues from Puerto Rico DENV3 are designated by black spheres on a Sri Lanka DENV3 backbone. Amino acid alignment of changes residues are shown. Puerto Rico DENV3 residues are shown in purple and Sri Lanka DENV3 residues are shown in blue. Domain map of residues is shown in gray.