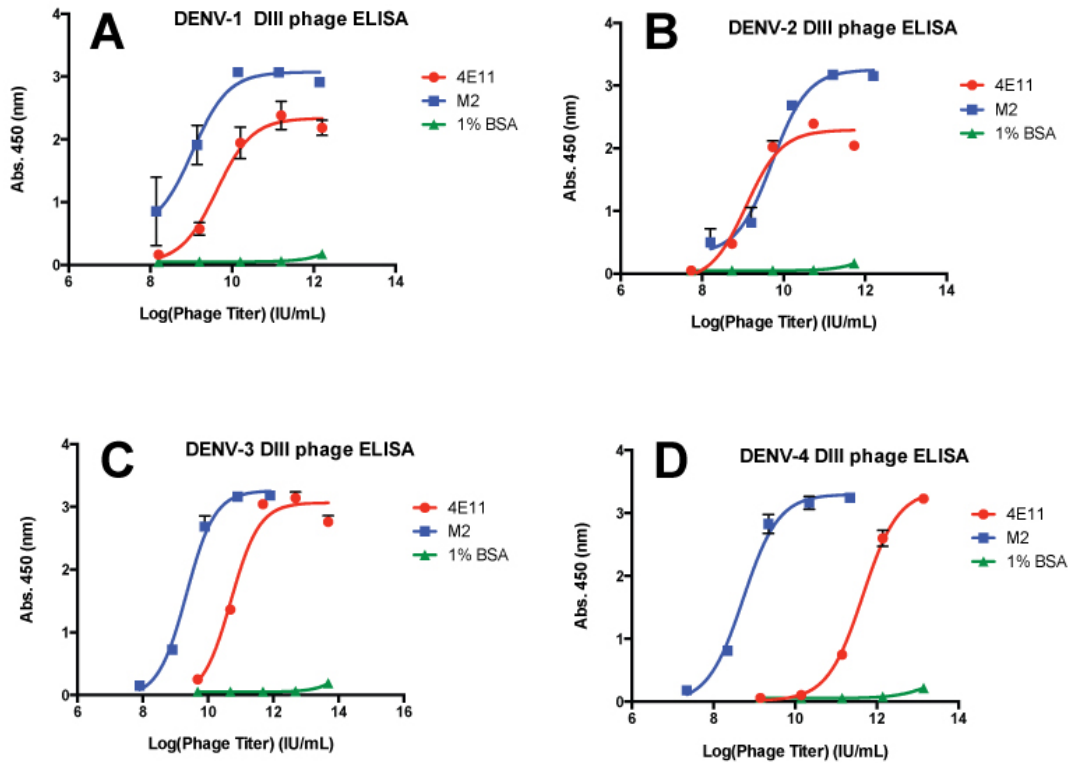


**Supporting Information for “Comprehensive Mapping of Functional Epitopes on Dengue Virus Glycoprotein E DIII for Binding to Broadly Neutralizing Antibodies 4E11 and 4E5A by Phage Display”**

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**Figure S1 Validation of Phage-Expressed DIII.** Phage ELISA for DENV-1 (A), DENV-2 (B), DENV-3 (C), and DENV-4 (D) against anti-FLAG antibody M2, 4E11, and 1% BSA.

**Table S1 – Percent Identity and Similarity of DENV Glycoprotein E DIII Domains.<sup>A</sup>**

	<b>DENV-1</b>	<b>DENV-2</b>	<b>DENV-3</b>	<b>DENV-4</b>
<b>DENV-1</b>	--	65/79	70/87	55/71
<b>DENV-2</b>	65/79	--	60/80	62/77
<b>DENV-3</b>	70/87	60/80	--	51/68
<b>DENV-4</b>	55/71	62/77	51/68	--

<sup>A</sup> For each pairwise alignment, the % identity/% similarity are shown.

**Primers used for Library synthesis (listed 5' -> 3'):**

**DENV-1:**

Library 1:

GTGATGTGCACCGGKCCCTTCRMASYTGMARMAGMAGYTGCCGAAACCCAGCAT,  
CCGATTGTGACCGACRMAGMAAAASCAGTGRMCATCGAAACCGAACCG,

Library 2:

GGTACAGTGCTGGTGS MAGTGRMATATGMAGGCACCGATGCCSCATGCAAAAATCCCGTTT,  
ATCGTGGGCGCCGGCGMARMAGCCSYTRMASYTKCKSGTTCAAGAAAGGCAGC;

**DENV-2:**

Library 1:

AGCATGTGCACCGGCRMATTTMARYTGYTRMAGMARYTGCCGAAACCCAGCAT,  
AACCCGATTGTGACCGMARMAGMTAGCSCAGTGRMCATTGAAGCCGAACCG,

Library 2:

GGCACCATTGTGATCSSTGTGSMATATGMAGGCGATGGTAGCSCATGCAAAAATCCCGTTC,  
ATCATTGGCGTGGAGSCAGSTCAGSYTRMASYTRMCKSGTTCAAGAAGGGCAGC;

**DENV-3:**

Library 1:

GCCATGTGCCTGAACRCTTTTCGYTSYTRMARMAGMAGYTAGCGAAACCCAGCAT,  
CCGGTGGTGACCAAGRMAGMAGAGSCAGTGRMCATCGAAGCCGAACCG,

Library 2:

GGCACAATCCTGATCRMAGTGGMATACRMAGGCGAGGATGCCSCATGCAAAAATCCCGTTT,  
GTGATCGGCATCGGCGMTRMAGCCSYTRMARYTRMCKSGTACAAAAAAGGTAGC;

**DENV-4:**

Library 1:

ACCATGTGCAGCGGCRMATTKCCRYTGMTRMAGMARYGGCCGAAACCCAGCAT,  
CCGTTCGCCGAAAACRCTRMCAGCGYTACRCMCATCGAACTGGAACCG,

Library 2:

GGCACCACCGTGGTGRMAGTGRMATAACGMAGGCGCAGGTGCCSCATGCAAAGTGCCGATC,  
GTGATTGGCGTGGGCGMTKCCGCCSYTRCTSYTSMKSGTTCCGCAAAGGCAGC.

**Table S2 – Number of ELISA-Positive Sequences Used for Analysis**

<b>Library</b>	<b>4E11 # Sequences</b>	<b>M2 # Sequences</b>
<b>4E11:</b>		
DENV-1 Library #1	125	151
DENV-1 Library #2	121	130
DENV-2 Library #1	95	144
DENV-2 Library #2	160	63
DENV-3 Library #1	169	156
DENV-3 Library #2	114	157
DENV-4 Library #1	157	157
<b>4E5A:</b>		
DENV-1 Library #1	48	151
DENV-1 Library #2	51	130
DENV-4 Library #1	103	157
DENV-4 Library #2	75	167

“Library 1” positions: 305, 307, 308, 309, 310, 311, 312, 360 (DENV-2 only), 361, 362, 364, 366

“Library 2” positions: 323, 325, 327, 332, 384, 385, 387, 388, 389, 390, 391

**Table S3 – Frequency of Observed Residues from Combinatorial Alanine Scanning against 4E11 for DENV-1**

Res.	4E11 Selection (Function)				M2 Selection (Display)			
	WT	Ala	m2	m3	WT	Ala	m2	m3
S305	72	69	-	-	100	71	-	-
K307	73	16	12 (E)	39 (T)	81	21	26 (E)	43 (T)
L308	78	17	6 (P)	38 (V)	93	26	9 (P)	43 (V)
E309	84	56	-	-	99	71	-	-
K310	119	5	4 (E)	13 (T)	79	25	48 (E)	19 (T)
E311	84	55	-	-	126	45	-	-
V312	101	38	-	-	106	65	-	-
Q323	57	20	24 (E)	21 (P)	42	28	41 (E)	23 (P)
K325	54	14	18 (E)	36 (T)	55	21	36 (E)	22 (T)
E327	85	37	-	-	99	33	-	-
P332	121	3	-	-	112	22	-	-
K361	62	28	28 (E)	22 (T)	83	15	33 (E)	31 (T)
E362	83	56	-	-	104	57	-	-
P364	61	79	-	-	80	81	-	-
N366	40	42	35 (D)	23 (T)	62	33	27 (D)	40 (T)
E384	93	39	-	-	114	49	-	-
K385	50	17	29 (E)	37 (T)	47	21	50 (E)	43 (T)
L387	88	5	3 (P)	37 (V)	74	28	20 (P)	40 (V)
K388	62	24	17 (E)	30 (T)	44	34	46 (E)	39 (T)
L389	62	7	1 (P)	62 (V)	48	24	21 (P)	70 (V)
S390	80	52	-	-	87	76	-	-
W391	63	12	28 (G)	29 (S)	43	26	58 (G)	35 (S)

**Table S4 – Frequency of Observed Residues from Combinatorial Alanine Scanning against 4E11 for DENV-2**

Res.	4E11 Selection (Function)				M2 Selection (Display)			
	WT	Ala	m2	m3	WT	Ala	m2	m3
S305	57	10	14 (E)	14 (T)	53	23	37 (E)	32 (T)
K307	70	2	2 (E)	21 (T)	48	22	31 (E)	44 (T)
I308	60	4	5 (T)	26 (V)	61	13	15 (T)	56 (V)
V309	60	35	-	-	103	42	-	-
K310	75	7	4 (E)	9 (T)	52	27	38 (E)	28 (T)
E311	66	29	-	-	97	48	-	-
I312	60	3	2 (T)	30 (V)	48	26	19 (T)	52 (V)
R323	14	22	42 (G)	82 (P)	15	16	12 (G)	21 (P)
Q325	85	45	30 (E)	0 (P)	22	13	18 (E)	11 (P)
E327	137	23	-	-	38	26	-	-
P332	157	3	-	-	28	31	-	-
E360	72	34	-	-	87	59	-	-
K361	41	28	15 (E)	22 (T)	31	33	52 (E)	32 (T)
D362	69	37	-	-	93	54	-	-
P364	56	50	-	-	63	84	-	-
N366	34	15	41 (D)	16 (T)	37	15	68 (D)	26 (T)
P384	140	20	-	-	24	40	-	-
G385	94	66	-	-	45	19	-	-
L387	94	8	0 (P)	58 (V)	14	16	14 (P)	20 (V)
K388	38	40	53 (E)	29 (T)	19	19	11 (E)	15 (T)
L389	81	6	0 (P)	73 (V)	14	14	11 (P)	24 (V)
N390	75	34	7 (D)	44 (T)	19	13	17 (D)	14 (T)
W391	154	2	1 (G)	3 (S)	13	11	24 (G)	13 (S)

**Table S5 – Frequency of Observed Residues from Combinatorial Alanine Scanning against 4E11 for DENV-3**

Res.	4E11 Selection (Function)				M2 Selection (Display)			
	WT	Ala	m2	m3	WT	Ala	m2	m3
T305	77	93	-	-	68	88	-	-
V307	137	33	-	-	115	42	-	-
L308	158	5	0 (P)	7 (V)	95	16	8 (P)	38 (V)
K309	30	76	22 (E)	42 (T)	51	40	26 (E)	40 (T)
K310	103	16	20 (E)	31 (T)	47	17	73 (E)	20 (T)
E311	128	42	-	-	97	60	-	-
V312	160	10	-	-	132	25	-	-
K323	17	2	67 (E)	28 (T)	44	14	75 (E)	25 (T)
E325	109	5	-	-	140	18	-	-
K327	105	2	0 (E)	7 (T)	55	22	53 (E)	28 (T)
P332	114	0	-	-	84	74	-	-
K361	65	46	28 (E)	31 (T)	48	39	55 (E)	15 (T)
E362	113	57	-	-	111	47	-	-
P364	83	87	-	-	64	94	-	-
N366	30	27	43 (D)	69 (T)	40	23	44 (D)	51 (T)
D384	62	52	-	-	99	59	-	-
K385	69	12	19 (E)	14 (T)	55	18	49 (E)	49 (T)
L387	110	0	0 (P)	4 (V)	50	33	26 (P)	49 (V)
K388	98	3	0 (E)	13 (T)	38	35	40 (E)	43 (T)
I389	93	0	0 (T)	21 (V)	58	32	20 (T)	47 (V)
N390	63	38	9 (D)	4 (T)	39	34	47 (D)	38 (T)
W391	95	6	5 (G)	6 (S)	39	34	55 (G)	30 (S)

**Table S6 – Frequency of Observed Residues from Combinatorial Alanine Scanning against 4E11 for DENV-4**

Res.	4E11 Selection (Function)				M2 Selection (Display)			
	WT	Ala	m2	m3	WT	Ala	m2	m3
K305	109	23	11 (E)	15 (T)	75	36	35 (E)	12 (T)
S307	156	2	-	-	138	20	-	-
I308	121	0	0 (T)	37 (V)	89	5	12 (T)	52 (V)
D309	2	155	-	-	58	100	-	-
K310	157	1	0 (E)	0 (T)	38	8	87 (E)	25 (T)
E311	132	26	-	-	119	39	-	-
M312	92	9	0 (T)	57 (V)	85	17	16 (T)	40 (V)
K323			ND <sup>A</sup>				ND <sup>A</sup>	
K325			ND <sup>A</sup>				ND <sup>A</sup>	
E327			ND <sup>A</sup>				ND <sup>A</sup>	
P332			ND <sup>A</sup>				ND <sup>A</sup>	
T361	124	34	-	-	110	48	-	-
N362	54	15	66 (D)	23 (T)	62	18	47 (D)	31 (T)
V364	136	21	-	-	128	29	-	-
N366	22	20	87 (D)	29 (T)	28	47	55 (D)	27 (T)
D384			ND <sup>A</sup>				ND <sup>A</sup>	
S385			ND <sup>A</sup>				ND <sup>A</sup>	
L387			ND <sup>A</sup>				ND <sup>A</sup>	
T388			ND <sup>A</sup>				ND <sup>A</sup>	
L389			ND <sup>A</sup>				ND <sup>A</sup>	
H390			ND <sup>A</sup>				ND <sup>A</sup>	
W391			ND <sup>A</sup>				ND <sup>A</sup>	

<sup>A</sup> ND = not determined due to inefficient library screening

**Table S7 – Frequency of Observed Residues from Combinatorial Alanine Scanning against 4E5A for DENV-1**

Res.	4E5A Selection (Function)				M2 Selection (Display)			
	WT	Ala	m2	m3	WT	Ala	m2	m3
S305	23	25	-	-	100	71	-	-
K307	30	5	4 (E)	9 (T)	81	21	26 (E)	43 (T)
L308	35	1	1 (P)	11 (V)	93	26	9 (P)	43 (V)
E309	24	24	-	-	99	71	-	-
K310	44	1	1 (E)	2 (T)	79	25	48 (E)	19 (T)
E311	32	16	-	-	126	45	-	-
V312	32	16	-	-	106	65	-	-
Q323	22	10	7 (E)	12 (P)	42	28	41 (E)	23 (P)
K325	22	4	13 (E)	12 (T)	55	21	36 (E)	22 (T)
E327	38	13	-	-	99	33	-	-
P332	42	9	-	-	112	22	-	-
K361	22	4	14 (E)	8 (T)	83	15	33 (E)	31 (T)
E362	27	21	-	-	104	57	-	-
P364	19	29	-	-	80	81	-	-
N366	15	19	6 (D)	8 (T)	62	33	27 (D)	40 (T)
E384	37	14	-	-	114	49	-	-
K385	20	7	7 (E)	17 (T)	47	21	50 (E)	43 (T)
L387	35	4	2 (P)	10 (V)	74	28	20 (P)	40 (V)
K388	30	7	9 (E)	5 (T)	44	34	46 (E)	39 (T)
L389	21	3	3 (P)	24 (V)	48	24	21 (P)	70 (V)
S390	30	21	-	-	87	76	-	-
W391	22	4	14 (G)	10 (S)	43	26	58 (G)	35 (S)

**Table S8 – Frequency of Observed Residues from Combinatorial Alanine Scanning against 4E5A for DENV-4**

Res.	4E5A Selection (Function)				M2 Selection (Display)			
	WT	Ala	m2	m3	WT	Ala	m2	m3
K305	90	7	4 (E)	2 (T)	75	36	35 (E)	12 (T)
S307	98	5	-	-	138	20	-	-
I308	78	0	2 (T)	23 (V)	89	5	12 (T)	52 (V)
D309	5	98	-	-	58	100	-	-
K310	100	1	0 (E)	2 (T)	38	8	87 (E)	25 (T)
E311	97	6	-	-	119	39	-	-
M312	75	4	1 (T)	22 (V)	85	17	16 (T)	40 (V)
K323	64	11	0 (E)	0 (T)	57	18	71 (E)	23 (T)
K325	61	1	3 (E)	10 (T)	63	16	55 (E)	35 (T)
E327	45	30	-	-	116	53	-	-
P332	75	0	-	-	85	84	-	-
T361	87	16	-	-	110	48	-	-
N362	47	8	37 (D)	10 (T)	62	18	47 (D)	31 (T)
V364	96	7	-	-	128	29	-	-
N366	14	17	46 (D)	26 (T)	28	47	55 (D)	27 (T)
D384	54	12	-	-	127	42	-	-
S385	58	17	-	-	120	48	-	-
L387	50	3	0 (P)	22 (V)	42	43	12 (P)	72 (V)
T388	53	22	-	-	100	69	-	-
L389	52	0	0 (P)	23 (V)	62	23	30 (P)	52 (V)
H390	42	2	1 (D)	30 (P)	34	19	46 (D)	68 (P)
W391	55	4	1 (G)	15 (S)	45	19	50 (G)	53 (S)