Figure S1. Competition-binding of human mAbs against DENV2 antigen

2nd / 1st	1B22	1C6	1H10	1L13	2H12	2K2	2M2	4E9	4F8	4G21	5E15	5G22	3D18
1B22	X	+	+	+	+	+	+	+	+	+	+	+	O
1C6	+	X	+	+	+	+	+	+	+	+	+	+	O
1H10	+	+	X	+	+	+	+	+	+	+	+	+	O
1L13	+	+	+	X	+	+	+	+	+	+	+	+	O
2H12	+	+	+	+	X	+	+	+	+	+	+	+	O
2K2	+	+	+	+	+	X	+	+	+	+	+	+	O
2M2	+	+	+	+	+	+	X	+	+	+	+	+	O
4E9	+	+	+	+	+	+	+	X	+	+	+	+	O
4F8	+	+	+	+	+	+	+	+	X	+	+	+	O
4G21	+	+	+	+	+	+	+	+	+	X	+	+	O
5E15	+	+	+	+	+	+	+	+	+	+	X	+	O
5G22	+	+	+	+	+	+	+	+	+	+	+	X	O
3D18	O	O	O	O	O	O	O	O	O	O	O	O	X

The symbol "**+**" indicates that the antibody pairing competed for the same site on the antigen, defined as >75% reduction of binding of the 2nd antibody compared to un-competed binding; the symbol "**O**" indicates that the antibody pairing did not compete for the same site on the antigen. Each of the antibodies successfully competed for binding with self (grey X). As a control reagent we used the human mAb 3D18 that recognizes the DENV E protein fusion loop.

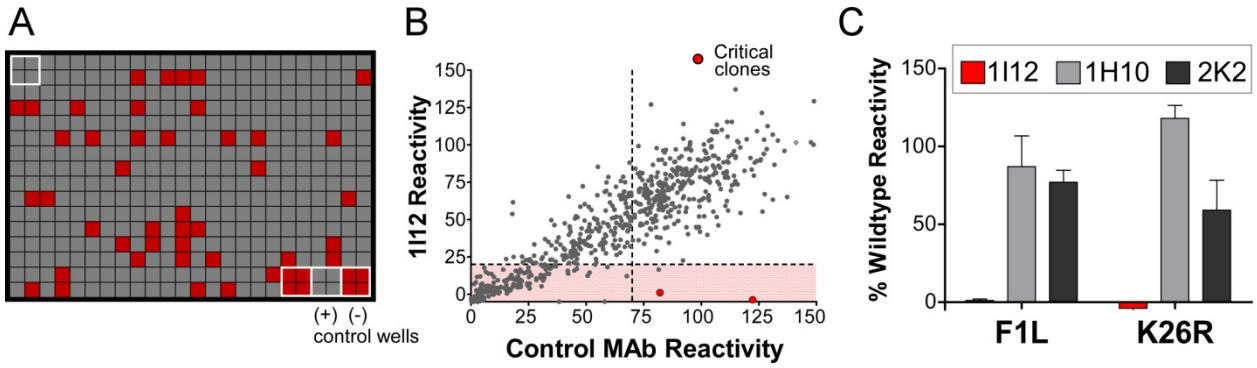

Figure S2. Identifying critical residues for prM MAb binding. (A) The shotgun mutagenesis mutation library for DENV3 prM/E is arrayed in 384-well plates, with each well of each mutation array plate containing one mutant with a defined substitution. MAb reactivity results for a representative 384-well plate are illustrated. Eight positive (wild-type prM/E) and eight negative (mock-transfected) control wells were included on each plate. (B) Human HEK293T cells expressing the DENV3 prM/E envelope mutation library were tested for immunoreactivity with MAb 1I12, which was measured using an Intellicyt high-throughput flow cytometer. Using algorithms described elsewhere (Davidson and Doranz 2014; U.S. patent application 61/938,894), clones with reactivity of <20% relative to that of wild-type DENV2 prM/E yet >70% reactivity for a control MAb were initially identified to be critical for 1I12 binding. (C) Mutation of two individual residues reduced MAb 1I12 binding (red bars) but did not greatly affect the binding of other conformation-dependent MAbs (gray bars). Bars represent the mean and range of at least two replicate data points.

Table S1. Critical residues identified by shotgun mutagenesis epitope mapping			
MAb	**Mutation Array**	**Epitope Location**	**Critical Residues [numbering on PDB ID #3C6E (prM) or PDB ID# 1UZG (E protein)**
1B22	**DENV3**	prM	L3
1C6	**DENV3**	prM	F1, K26
1E16	**DENV3**	prM	F1, E18, K26
1E23	**DENV3**	prM	L3, S5, E9, K26
1G6	**DENV3**	prM/ E protein Fusion Loop/Domain II	S5, G102
1G10	**DENV3**	prM	E18, K26
1H7.2	**DENV3**	prM	K26
	DENV4	prM	F1, K26
1H10	**DENV3**	prM	L3, S5
1I12	**DENV3**	prM	F1, K26
1K20	**DENV3**	prM	K26
1L13	**DENV3**	prM	F1, L3, E18, K26
1O6	**DENV3**	prM	F1, E18, K26
2B17	**DENV3**	prM	F1, L3, K26
2G3	**DENV3**	prM	F1, L3, S5, E9, K26
2H12	**DENV3**	prM	K26
2H21	**DENV3**	prM	K26
	DENV4	prM	F1, E18, L24, K26
2J9	**DENV3**	prM	F1, L3, K26
2K2	**DENV3**	prM	L3
	DENV4	prM	L3
2M2	**DENV3**	(Western blot indicates binding to prM and Env)	No critical residues identified
	DENV4	(Western blot indicates binding to prM and Env)	No critical residues identified
4E9	**DENV3**	prM	No critical residues identified
4F8	**DENV3**	prM	L3, S5
4G21	**DENV3**	prM	S5, E18
5E15	**DENV3**	prM	L3
5G22	**DENV3**	prM	L3, S5, E9, K26
5M22	**DENV4**	prM	K26

MAb	Mutations								
	F1L	L3R	S5R	E9D	E18D	K26R	W101 C	G102R	H242R
1B22	51 (9)	32 (7)	40 (14)	57 (10)	95 (18)	94 (17)	69 (15)	84 (13)	128 (37)
1C6	27 (1)	28 (5)	40 (3)	60 (6)	43 (2)	7 (1)	79 (1)	61 (7)	ND
1E16	18 (1)	62 (3)	29 (3)	44 (1)	22 (5)	3 (1)	97 (5)	77 (8)	99 (4)
1E23	59(3)	1 (1)	1 (1)	26 (5)	99 (8)	0 (1)	77 (0)	52 (5)	64 (3)
1G6	61(24)	44 (10)	20 (9)	55 (9)	96 (22)	72 (13)	20 (2)	10 (12)	3 (2)
1G10	26(1)	66 (22)	33 (3)	39 (4)	23 (2)	5 (2)	120 (7)	83 (3)	75 (11)
1H7.2	72(1)	33 (0)	62 (1)	84 (1)	98 (12)	24 (5)	88 (8)	71 (3)	98 (2)
1H10	87(15)	1 (5)	25 (4)	65 (16)	84 (14)	118 (8)	93 (32)	71 (16)	110 (3)
1I12	1 (0)	62 (2)	48 (3)	55 (8)	74 (4)	-4 (1)	64 (5)	ND	ND
1K20	59 (1)	62 (3)	58 (1)	50 (0)	83 (15)	15 (1)	64 (9)	69 (10)	87 (4)
1L13	10 (1)	14 (2)	57 (3)	66 (19)	15 (3)	8 (0)	69 (8)	ND	ND
1O6	1 (1)	58 (7)	51 (3)	50 (3)	6 (1)	10 (1)	89 (3)	74 (11)	102 (14)
2B17	0 (1)	10 (1)	46 (1)	42 (5)	84 (6)	2 (1)	68 (12)	62 (3)	80 (1)
2G3	-2 (2)	-1 (2)	25 (1)	27 (9)	59 (15)	-3 (2)	69 (0)	59 (3)	63 (4)
2H12	29 (2)	31 (2)	41 (6)	39 (5)	42 (0)	26 (2)	92 (5)	81 (2)	102 (9)
2H21	59 (3)	83 (0)	65 (8)	51 (12)	112 (8)	5 (2)	83 (1)	81 (14)	97 (2)
2J9	1 (0)	16 (0)	42 (14)	47 (10)	95 (17)	0 (0)	82 (9)	77 (1)	103 (7)
2K2	77 (8)	1 (1)	51 (5)	61 (3)	108 (9)	59 (19)	63 (6)	86 (9)	103 (19)
2M2	85 (2)	74 (4)	64 (5)	86 (3)	119 (3)	89 (2)	41 (6)	77 (4)	102 (3)
4E9	53 13)	65 (21)	54 (10)	64 (28)	135 (16)	50 (11)	39 (5)	73 (13)	109 (32)
4F8	59 (3)	4 (0)	24 (6)	49 (9)	86 (15)	99 (3)	104 (12)	81 (7)	89 (13)
4G21	36 (5)	48 (4)	21 (5)	36 (7)	0 (1)	76 (0)	84 (2)	71 (4)	97 (12)
5E15	38(6)	3 (0)	38 (12)	52 (13)	122 (11)	86 (14)	96 (6)	73 (2)	101 (17)
5G22	30(1)	0 (0)	1 (0)	28 (1)	81 (7)	2 (2)	58 (6)	55 (0)	74 (1)
5M22	48(7)	47 (6)	49 (8)	55 (0)	69 (11)	66 (6)	50 (5)	62 (5)	88 (6)

Table S2. Summary data for DENV3 residues critical for MAb binding. Reactivities for mAbs with DENV3 prM/E mutants are expressed as percent of their binding to wild-type prM/E, with ranges (half of the maximum minus minimum values) in parentheses. At least two replicate values were obtained for each experiment. ND, not determined