PNAS

Supporting Information for
prM-reactive Antibodies Reveal a Role for Partially Mature Virions in Dengue Virus Pathogenesis.

Kimberly A. Dowd[a,1], Devika Sirohi[b,1], Scott D. Speer[a,2], Laura A. VanBlargan[c,3], Rita E. Chen[c], Swati Mukherjee[a,4], Bradley M. Whitener[c,] Jennifer Govero[c,5], Maya Aleshnick[a,6], Bridget Larman[a], Soila Sukupolvi-Petty[c,7], Madhumati Sevvana[b,8], Andrew S. Miller[b], Thomas Klose[b], Aihua Zheng[d,9], Scott Koenig[e], Margaret Kielian[d], Richard J. Kuhn[b,10], Michael S. Diamond[c,f,10], Theodore C. Pierson[a,10]

[a]Viral Pathogenesis Section, Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD 20892
[b]Department of Biological Sciences and Purdue Institute of Inflammation, Immunology, and Infectious Disease, Purdue University, West Lafayette, IN 47907
[c]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110
[d]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, 10461
[e]MacroGenics, Inc. Rockville, MD 20850
[f]Departments of Molecular Microbiology and Pathology & Immunology, Washington University School of Medicine, St. Louis, MO 63110

[10]To whom correspondence may be addressed. Email: piersontc@mail.nih.gov (T.C.P.), diamond@borcim.wustl.edu (M.S.D.), kuhnr@purdue.edu (R.J.K.)

This PDF file includes:

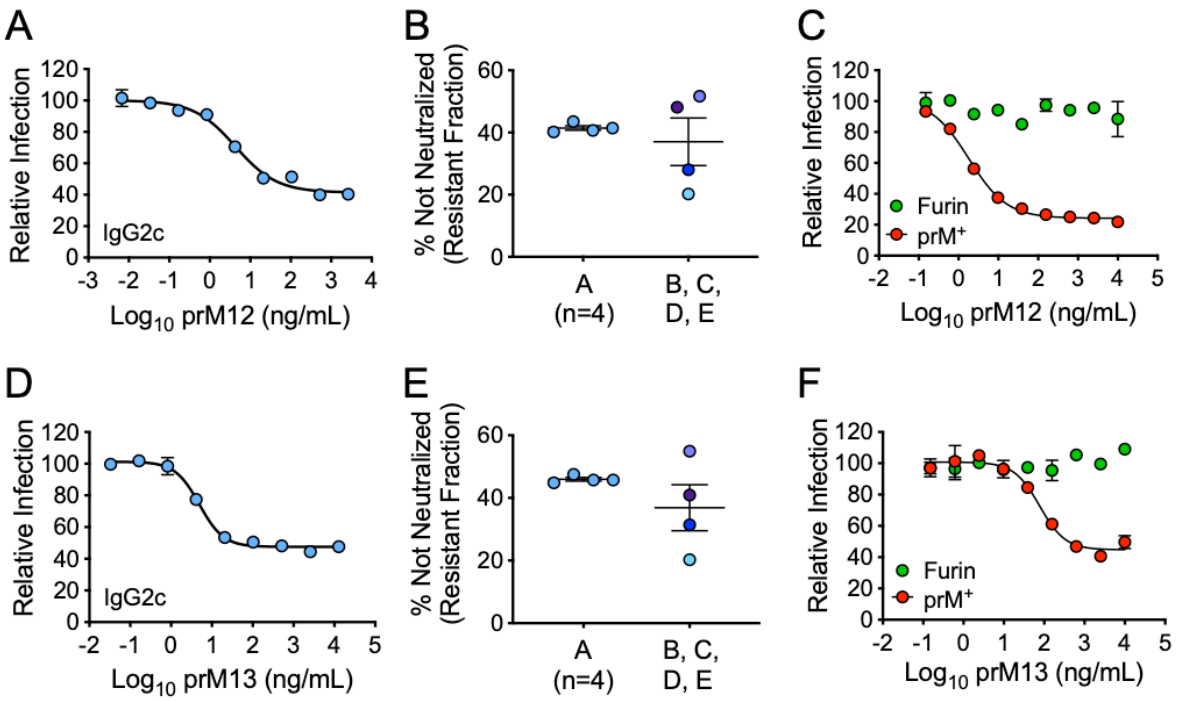

Fig. S1. Neutralization of DENV2 by mAbs prM12 and prM13. GFP-expressing DENV2 reporter virus particles (RVPs) were incubated with serial dilutions of prM12 (**A-C**) or prM13 (**D-F**) for 1 hour at 37°C, followed by infection of Raji-DCSIGNR cells. Infectivity was assessed 48 hours later by flow cytometry. (**A, D**) Representative dose-response curve of standard DENV2 RVPs (stock A) neutralized by mAb prM12 or prM13. Error bars indicate the range of duplicate infections. A resistant fraction of non-neutralized virions is apparent at saturating concentrations of antibody. (**B, E**) The resistant fraction (calculated as the % of virions not neutralized at saturation) is consistent when four independent prM12 or prM13 neutralization assays are performed with aliquots from the same RVP preparation (stock A), but varies when the assay is repeated with four distinct preparations of RVPs collected from independent transfections (stocks B, C, D, E). Horizontal line and error bars indicate the mean and standard error of the mean, respectively. (**C, F**) Representative prM12 or prM13 neutralization curves of DENV2 RVPs with varying levels of uncleaved prM (Furin < prM^+). Error bars indicate the range of duplicate infections. Data are representative of two independent experiments.

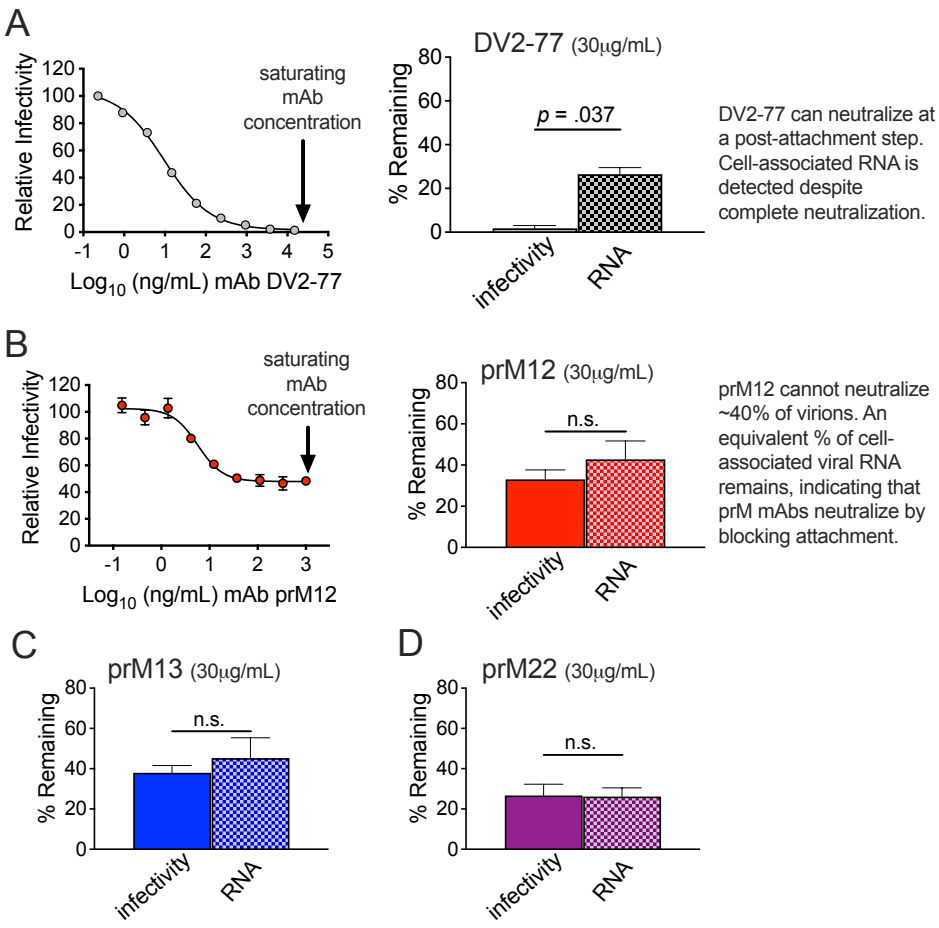

Fig. S2. DENV prM mAbs neutralize by blocking attachment to cells. DENV2 RVPs incubated with a saturating concentration (30 μg/mL) of the indicated mAbs were used to infect Raji-DCSIGNR cells, and the % remaining infectivity (resistant fraction) was assessed by flow cytometry. In parallel, antibody-virus complexes were incubated with Raji-DCSIGNR cells treated with ammonium chloride (to allow for virus entry but not uncoating) and qRT-PCR was used to evaluate the % remaining cell-associated viral RNA as compared to results obtained in the absence of antibody. (**A**) The control E DIII-specific mAb DV2-77 can neutralize the virus at a post-attachment step, resulting in unequal RNA versus infectivity values. A representative dose-response curve is shown in the left panel. On the right, bar graphs indicate the mean and standard error of 3 independent experiments performed with triplicate or quadruplicate technical replicates. (**B**) A representative prM12 dose-response curve is shown in the left panel. On the right (for prM12), and in panels (**C-D**) for prM13 and prM22, bar graphs indicate the mean and standard error of 5 independent experiments performed with triplicate or quadruplicate technical replicates. The entire data set was analyzed by mixed-effects analysis with Bonferroni's multiple comparisons test. n.s.= not significant

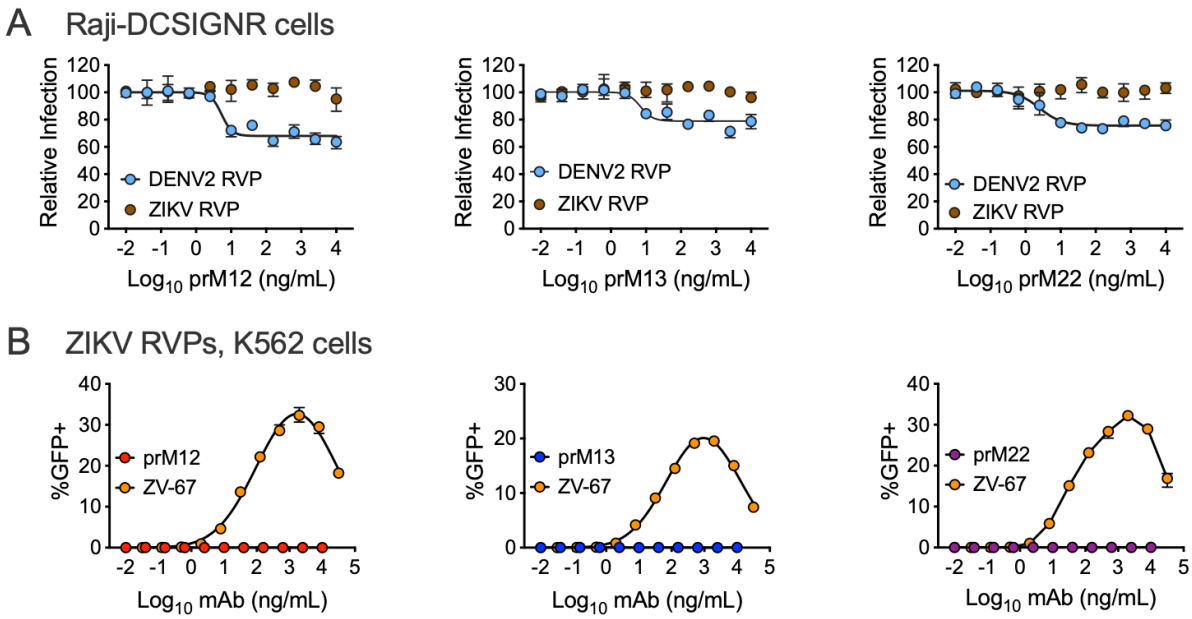

Fig. S3. DENV2 prM mAbs do not cross-react with ZIKV. GFP-expressing reporter virus particles (RVPs) were incubated with serial dilutions of the indicated mAbs for 1 hour at 37°C, followed by infection of Raji-DCSIGNR cells (neutralization; panel A) or K562 cells (ADE; panel B). Infectivity was assessed 48 hours later by flow cytometry. prM mAbs do not cross-react with ZIKV, as indicated by an inability to neutralize (**A**) or enhance (**B**) infection of ZIKV RVPs. ZV-67 is a control mAb specific for the ZIKV E protein. Data are representative of four independent experiments; error bars indicate the range of quadruplicate infections.

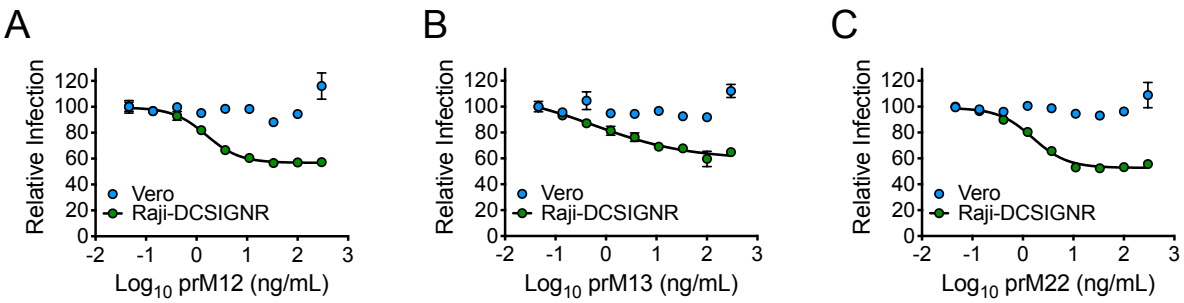

Fig. S4. Cell-type differences in neutralization of DENV2 by prM-specific mAbs. DENV2 RVPs were incubated with serial dilutions of mAb prM12 (**A**), prM13 (**B**), or prM22 (**C**) for 1 hour at 37°C, followed by infection of Raji-DCSIGNR or Vero cell substrates. Infectivity was assessed 48 hours later by flow cytometry. Error bars indicate the range of duplicate infections. Data are representative of two independent experiments.

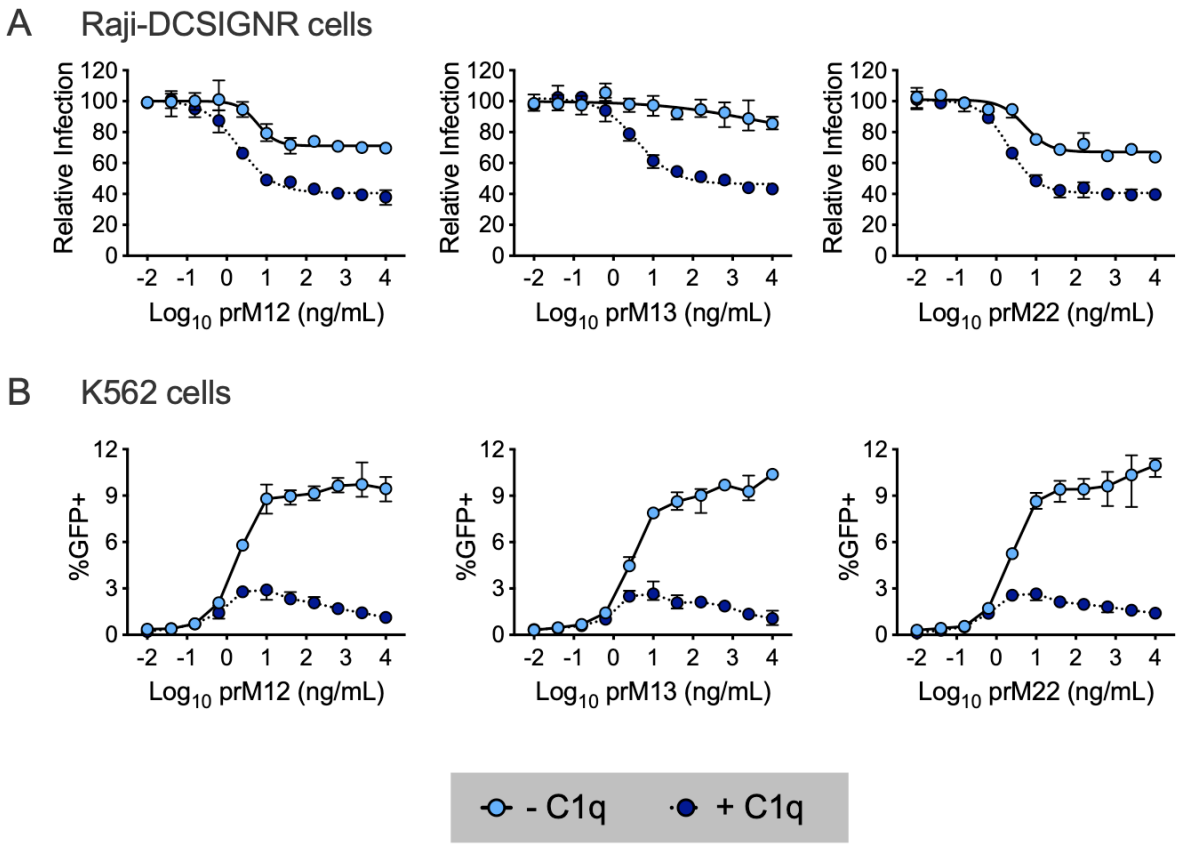

Fig. S5. C1q modulates neutralization and enhancement by prM-specific mAbs. DENV2 RVPs were incubated with serial dilutions of the indicated mAbs for 1 hour at 37°C, followed by infection of Raji-DCSIGNR cells (neutralization; panel A) or K562 cells (ADE; panel B). Infectivity was assessed 48 hours later by flow cytometry. Addition of C1q (50 μg/mL) lowers the stoichiometric threshold for neutralization, resulting in decreases in both the % of virions resistant to neutralization (**A**), and the % of virions susceptible to ADE (**B**). Data are representative of two independent experiments; error bars indicate the range of quadruplicate infections.

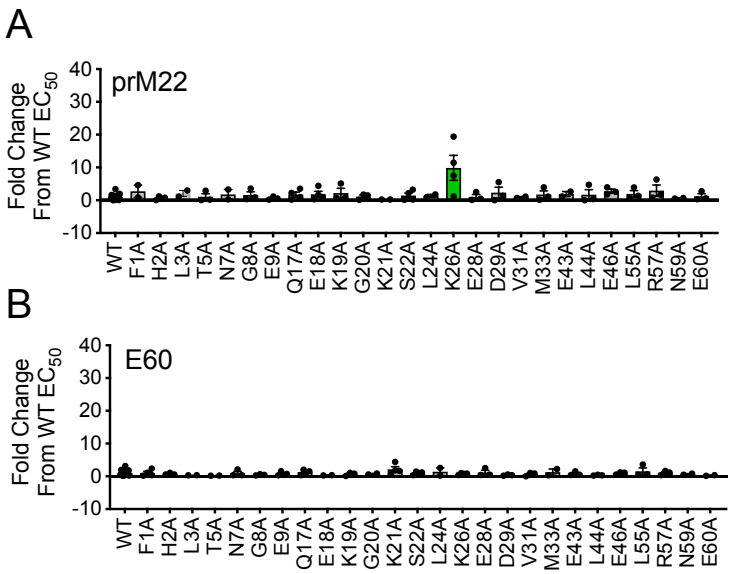

Fig. S6. DENV prM alanine scanning to identify amino acids contacted by prM mAbs. A panel of variant RVPs individually incorporating an alanine at 26 surface-exposed residues on prM was generated. Wild-type (WT) and variant RVPs were incubated with serial dilutions of the indicated mAbs for 1 hour at 37°C, followed by infection of Raji-DCSIGNR cells. Infectivity was assessed 48 hours later by flow cytometry. Shown are EC_{50} values for mAb prM22 (**A**) and the E DII fusion loop-specific mAb E60 (**B**) against the entire panel of 26 variant RVPs incorporating an alanine at the indicated prM residue. Data are expressed as the fold change from the average EC_{50} value against WT RVPs (2-4 independent experiments per mutant; WT EC_{50} value calculated from 20 and 19 independent experiments for mAb prM22 and E60, respectively). The horizontal line and error bars indicate the mean and standard error, respectively. E60 is a maturation state-sensitive E DII-specific antibody, used here as a control to demonstrate that the overall antigenicity and prM content of the variants was similar to WT.

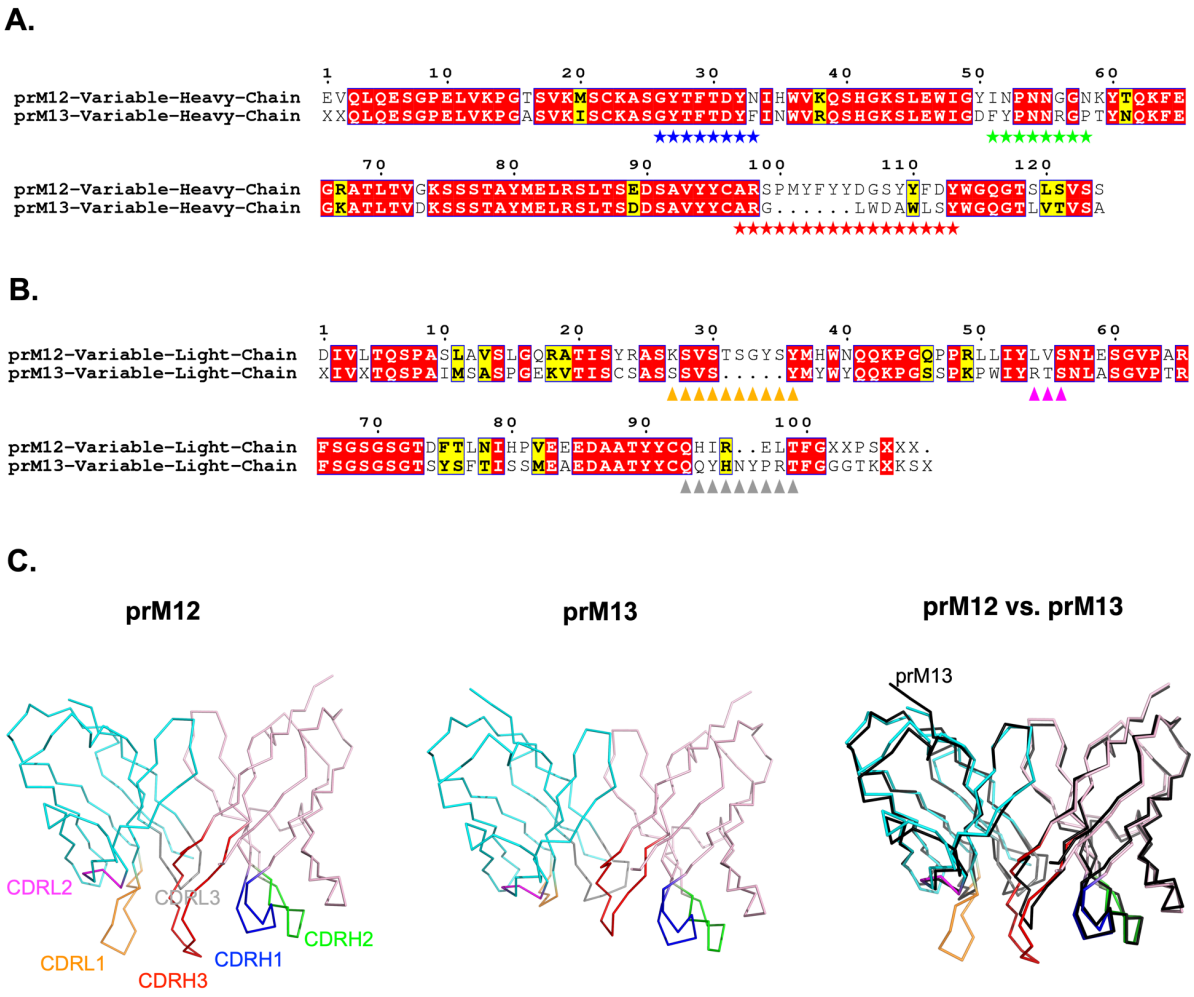

Fig. S7. Comparision of the sequences and modeled structures of the variable domain of prM12 and prM13 Fabs. The sequences for the gamma heavy (**A**) and kappa light (**B**) chains of the variable domains of prM12 and prM13 Fabs were aligned using ESPript 3.0. Red depicts positions with strict amino acid identity while yellow shows conservation of amino acids with similar properties. X represents amino acids that could not be acertained by sequencing. The residues constituting the CDR loops for the heavy chain are emphasized with stars; blue, green and red for CDRH1, CDRH2 and CDRH3, respectively. CDRL1, CDRL2 and CDRL3 residues in the light chain are marked with orange, magenta and gray triangles, respectively. (**C**) The structures of the variable domain of prM12 (left panel) and prM13 (middle panel) were modeled using I-TASSER and illustrated in a backbone trace using PyMOL. The heavy chain is denoted in pink and the light chain in cyan with the CDR loops colored using the scheme described above. The right panel shows the prM13 structure (black) superimposed on the prM12 structure (colored as before). The differences between the two Fab structures are most pronounced in the CDRH3 (red) and CDRL1 (orange) loops, which are substantially longer in prM12.

A

Region		pr		M		
Residue #		28	83	120	135	162
Strain	D2S20	G	T	A	I	A
	16681	E	M	V	M	T

B

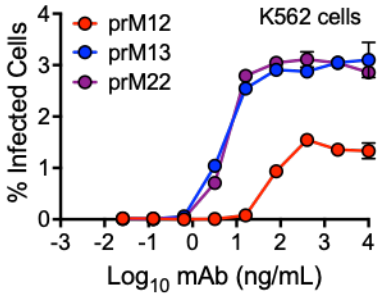

Fig. S8. prM mAbs interact with the mouse adapted DENV2 D2S20 strain. (**A**) Differences in the prM amino acid sequences of strains D2S20 and 16681 are shown, where numbers refer to the amino acid positions in prM. (**B**) The DENV2 D2S20 virus stock used to infect mice in **Figure 6** was incubated with serial dilutions of the indicated mAbs for 1 hour at 37°C, followed by infection of K562 cells. Infectivity was assessed 24 hours later by flow cytometry using an Alexa Fluor 488 conjugated E-protein specific mAb. Error bars indicate the range of duplicate infections.

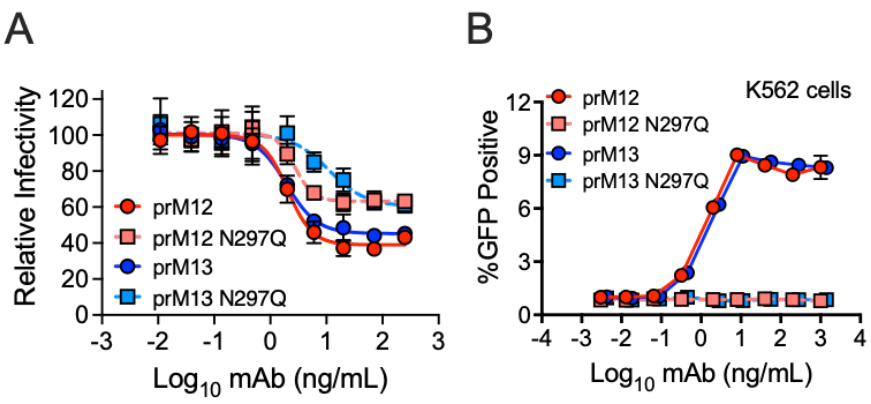

Fig. S9. Characterization of chimeric human Fc prM mAbs. DENV2 16681 RVPs were incubated with serial dilutions of the indicated wild-type or aglycosyl (N297Q) chimeric human Fc mAbs for 1 hour at 37°C, followed by infection of (**A**) Raji-DCSIGNR cells or (**B**) K562 cells. Infectivity was assessed 48 hours later by flow cytometry. Error bars indicate the range of duplicate infections.

Table S1. Binding characteristics of DENV2 prM-reactive mAbs

			Reactivity to heterologous DENV serotypes[a]		
mAb	Isotype		DENV1	DENV3	DENV4
prM12	IgG2c		+	++	+
prM13	IgG2c		++	++	+++
prM22	IgG2c		+++	++	+++

[a]Determined by flow cytometry of infected Vero cells

Table S2. Cryo-EM data acquisition, reconstruction and data availability

	Immature DENV-Fab prM12	Immature DENV-Fab prM13
Magnification (X)	81000	81000
Voltage	300	300
Electron dose ($e^-/Å^2$)	32	32
Defocus range (μm)	-1 to -2.5	-1 to -2.5
Pixel size (Å)	0.865 (Super-resolution)	0.865 (Super-resolution)
Number of micrographs	927	991
Particle included in the final reconstruction	5881	5458
Imposed symmetry	Icosahedral	Icosahedral
Map Resolution (Å) (FSC criterion =0.143)	10.2	9.8
EMDB Accession code	29020	29021
PDB Accession code	8FE3	8FE4

Table S3. Sumf values for soluble prM-E and prM12 Fab fitted into the cryo-EM density of the asymmetric trimeric spike (determined by EMFIT)

		P1	P2	P3
Soluble prM-E		**39.5**	**36.4**	**38.7**
	pr	39.39	37.67	39.12
	E- Domain 1	38.28	34.47	38.25
	E- Domain 2	46.00	45.35	47.36
	E- Domain3	30.29	23.10	24.66
prM12 Fab: Variable Domain		**41.6**	**42.8**	**42.7**
	Heavy Chain	42.97	43.09	43.58
	Light Chain	39.97	42.50	41.58
prM12 Fab: Constant Domain		**32.1**	**35.9**	**32.6**
	Heavy Chain	32.32	34.01	31.44
	Light Chain	31.95	37.57	33.59

Table S4: Sumf values for soluble prM-E and prM13 Fab fitted into the cryo-EM density of the asymmetric trimeric spike (determined by EMFIT)

		P1	P2	P3
Soluble prM-E		**39.4**	**37.0**	**37.6**
	pr	40.97	39.50	39.36
	E- Domain 1	38.59	36.54	37.49
	E- Domain 2	44.63	43.49	44.97
	E- Domain3	30.68	24.63	24.04
prM13 Fab: Variable Domain		**45.5**	**45.0**	**45.5**
	Heavy Chain	45.68	45.27	45.43
	Light Chain	45.33	44.71	45.52
prM13 Fab: Constant Domain		**34.3**	**39.1**	**37.1**
	Heavy Chain	31.10	35.63	36.13
	Light Chain	37.22	42.24	37.96