Supplemental material

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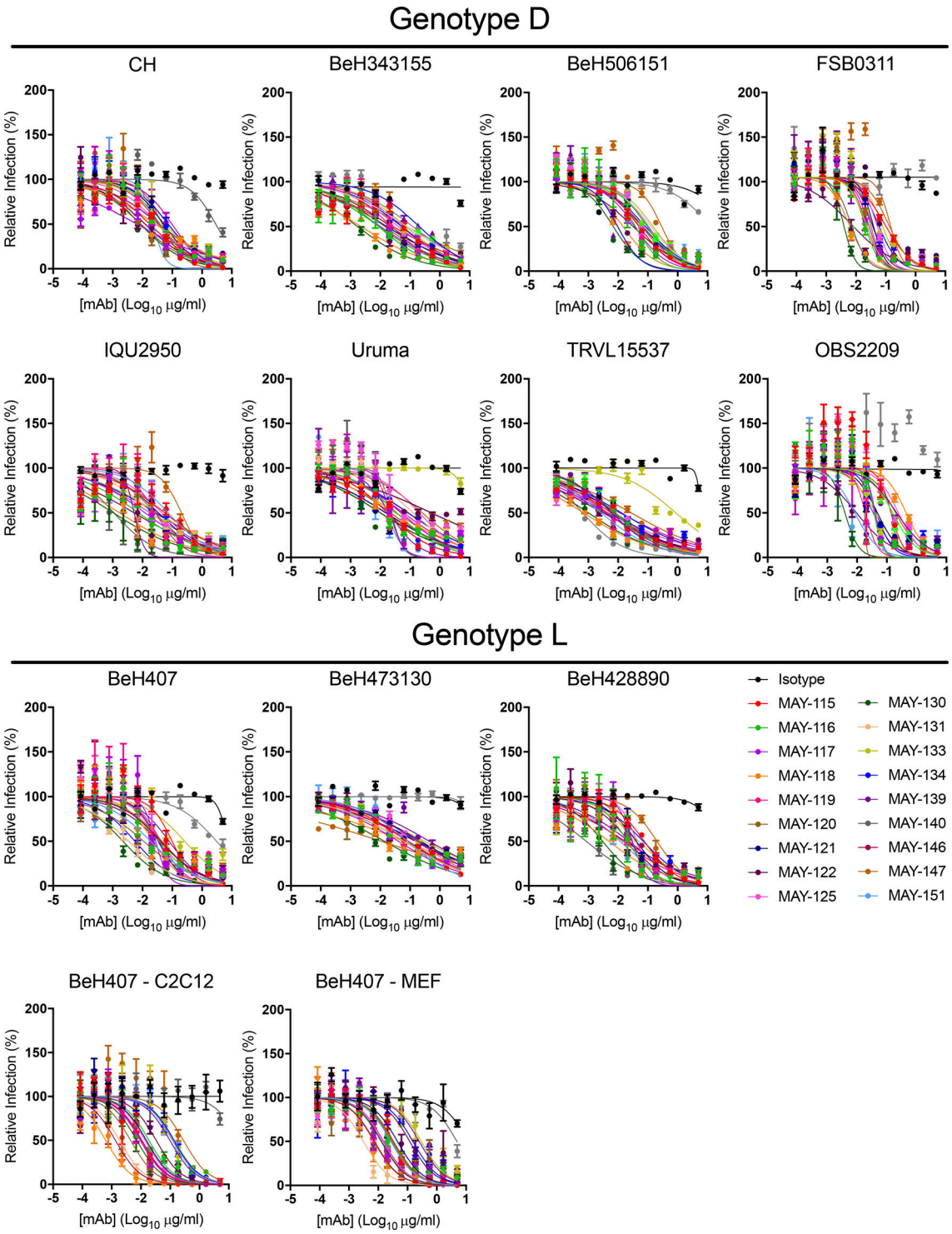
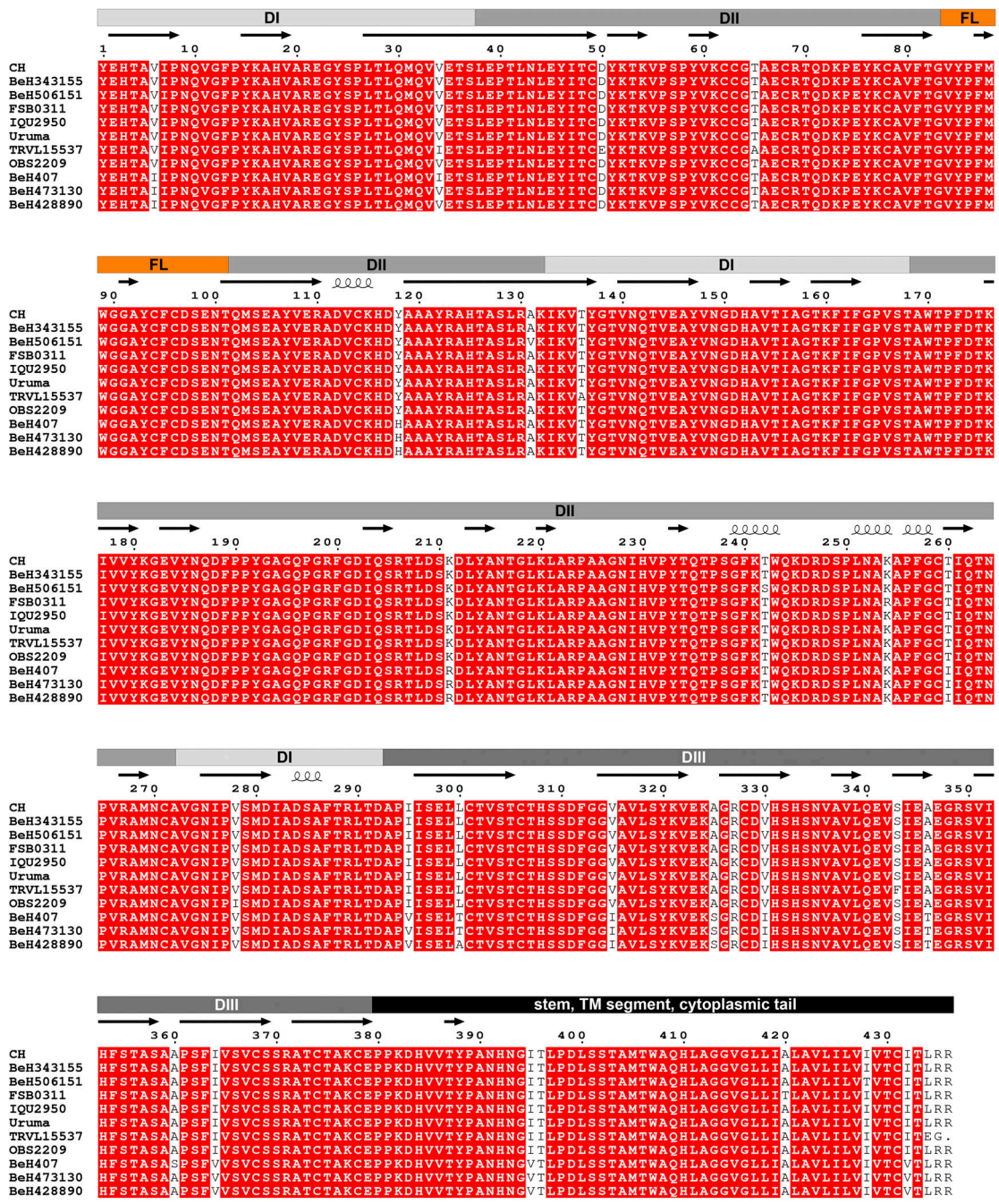


Figure S1. **Anti-MAYV mAbs neutralization of different MAYV strains.** Related to Fig. 1. Serial dilutions of mAbs were incubated with 10^2 FFU of the indicated MAYV strain before inoculation of Vero cells, unless otherwise indicated. mAb neutralization of MAYV-BeH407 also was tested in C2C12 myoblasts and MEFs. Cells were overlaid with methylcellulose and incubated for 18 h. Viral foci were stained, counted, and plotted relative to a no-antibody control. Data are representative of two experiments performed in triplicate. Error bars represent SD within one experiment.

A

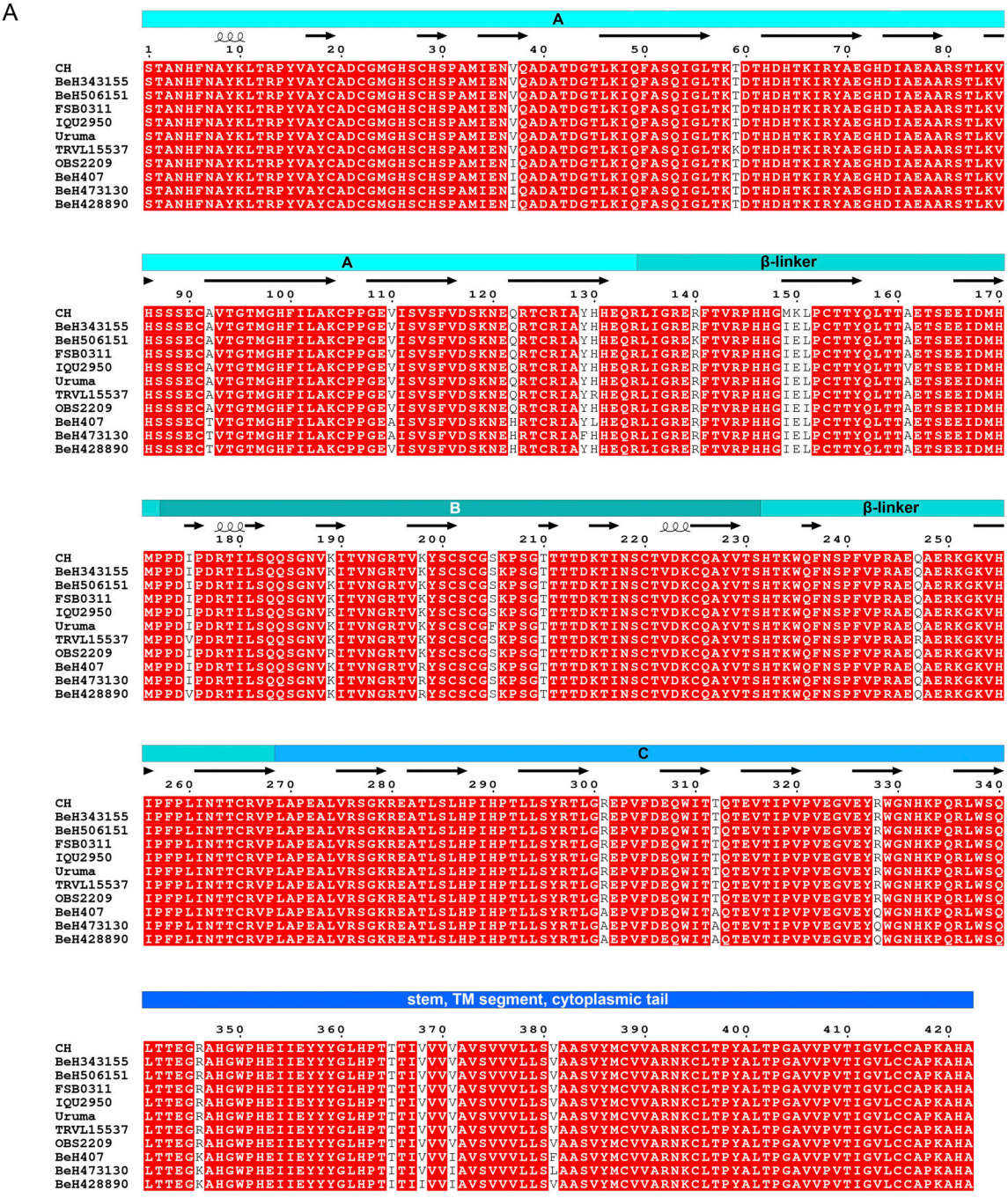


B

	Consensus	CH	BeH473130	BeH506151	IQU2950	BeH407	TRVL15537	Uruma	OBS2209	FSB0311	BeH428890	BeH343155
Consensus		100	97	99.3	99.8	96.6	97.7	100	99.8	99.5	97	100
CH	100		97	99.3	99.8	96.6	97.7	100	99.8	99.5	97	100
BeH473130	97	97		96.3	96.8	99.5	94.7	97	96.8	96.6	99.8	97
BeH506151	99.3	99.3	96.3		99.1	95.9	97	99.3	99.1	98.9	96.3	99.3
IQU2950	99.8	99.8	96.8	99.1		96.3	97.5	99.8	99.5	99.3	96.8	99.8
BeH407	96.6	96.6	99.5	95.9	96.3		94.7	96.6	96.3	96.1	99.3	96.6
TRVL15537	97.7	97.7	94.7	97	97.5	94.7		97.7	97.5	97.3	94.7	97.7
Uruma	100	100	97	99.3	99.8	96.6	97.7		99.8	99.5	97	100
OBS2209	99.8	99.8	96.8	99.1	99.5	96.3	97.5	99.8		99.3	96.8	99.8
FSB0311	99.5	99.5	96.6	98.9	99.3	96.1	97.3	99.5	99.3		96.6	99.5
BeH428890	97	97	99.8	96.3	96.8	99.3	94.7	97	96.8	96.6		97
BeH343155	100	100	97	99.3	99.8	96.6	97.7	100	99.8	99.5	97	

Figure S2. **Amino acid alignment of MAYV E1 proteins.** Related to Figs. 1 and 4 and Table 1. **(A)** MAYV strains were subjected to next-generation sequencing, and the consensus E1 amino acid sequences were compiled and aligned using Geneious v10.2.2 software. Red indicates sequence agreement among strains, whereas residues that vary among strains are highlighted white. Components of domains I (DI), II (DII), and III (DIII), the FL (orange), and the stem, transmembrane, and cytoplasmic tail (black) are indicated above the sequences. Predicted β-sheet (arrows) and α-helical (spirals) secondary structure are indicated above the sequences. **(B)** The amino acid similarity was compiled in a matrix with each strain. TM, transmembrane.

A



B

	Consensus	CH	BeH473130	BeH506151	IQU2950	BeH407	TRVL15537	Uruma	OBS2209	FSB0311	BeH428890	BeH343155
Consensus		99.5	96.7	99.8	99.8	97.2	98.8	98.9	98.8	99.8	97.2	100
CH	99.5		96.2	99.3	99.3	96.7	98.3	98.4	98.4	99.3	96.7	99.5
BeH473130	96.7	96.2		96.5	96.5	98.8	95.5	95.6	96	96.5	98.6	96.7
BeH506151	99.8	99.3	96.5		99.5	96.9	98.6	98.6	98.6	99.5	96.9	99.8
IQU2950	99.8	99.3	96.5	99.5		96.9	98.6	98.6	98.6	99.5	96.9	99.8
BeH407	97.2	96.7	98.8	96.9	96.9		96.2	96	96.5	96.9	98.6	97.2
TRVL15537	98.8	98.3	95.5	98.6	98.6	96.2		97.7	97.7	98.6	96.4	98.8
Uruma	98.9	98.4	95.6	98.6	98.6	96	97.7		98.1	98.9	96	98.9
OBS2209	98.8	98.4	96	98.6	98.6	96.5	97.7	98.1		98.8	96.5	98.8
FSB0311	99.8	99.3	96.5	99.5	99.5	96.9	98.6	98.9	98.8		96.9	99.8
BeH428890	97.2	96.7	98.6	96.9	96.9	98.6	96.4	96	96.5	96.9		97.2
BeH343155	100	99.5	96.7	99.8	99.8	97.2	98.8	98.9	98.8	99.8	97.2	

Figure S3. **Amino acid alignment of MAYV E2 proteins.** Related to Figs. 1 and 4 and Table 1. **(A)** MAYV strains were subjected to next-generation sequencing and consensus E2 amino acid sequences were compiled and aligned using Geneious v10.2.2 software. Sequence agreement among strains is indicated by red color whereas residues that vary among strains are highlighted in white. Components of domains A, B, C, the B-linker, and the stem, transmembrane, and cytoplasmic tail (dark blue) are indicated above the sequences. Predicted β-sheet (arrows) and α-helical (spirals) secondary structure are indicated above the sequences. **(B)** The amino acid similarity was compiled in a matrix with each strain. TM, transmembrane.

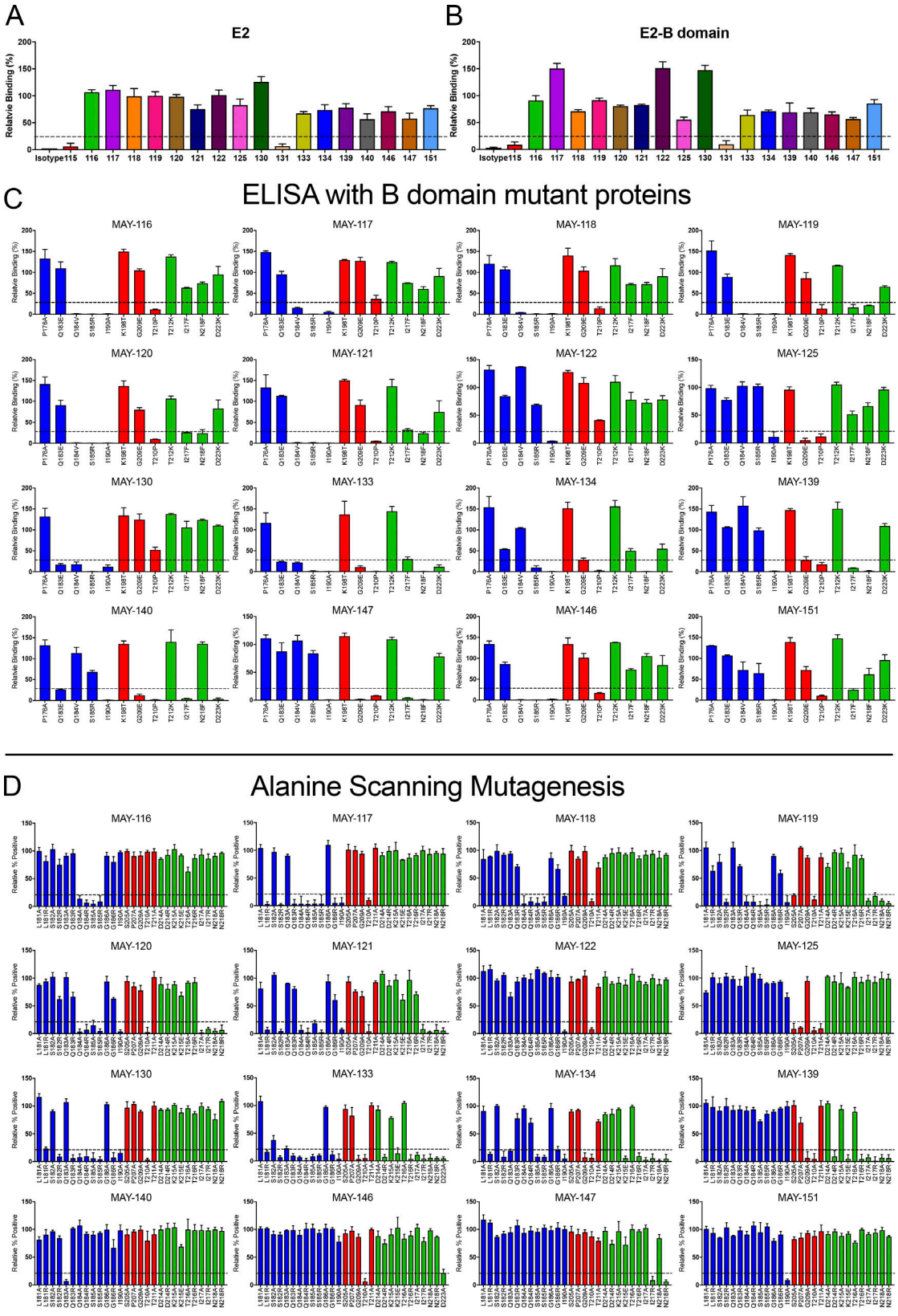


Figure S4. **Binding and mapping of mAbs to recombinant MAYV E2-B domain**. Related to Fig. 4. **(A and B)** Binding of mAbs to E2 protein. ELISA plates were coated with 100 ng/well of recombinant MAYV E2 (A) or the E2 B domain (B) before incubation with 500 ng/ml of anti-MAYV mAbs. Antibody binding to recombinant proteins was detected by an anti-mouse secondary antibody. Binding of the mAbs to the proteins is shown relative to an oligoclonal mixture of MAYV mAbs. Data are the mean and SD of two experiments, each with three replicates. **(C and D)** Mapping of anti-E2 mAbs to MAYV B domain. E2 B domain–specific mAbs were mapped by ELISA (C) and flow cytometry (D) as described in Fig. 4.

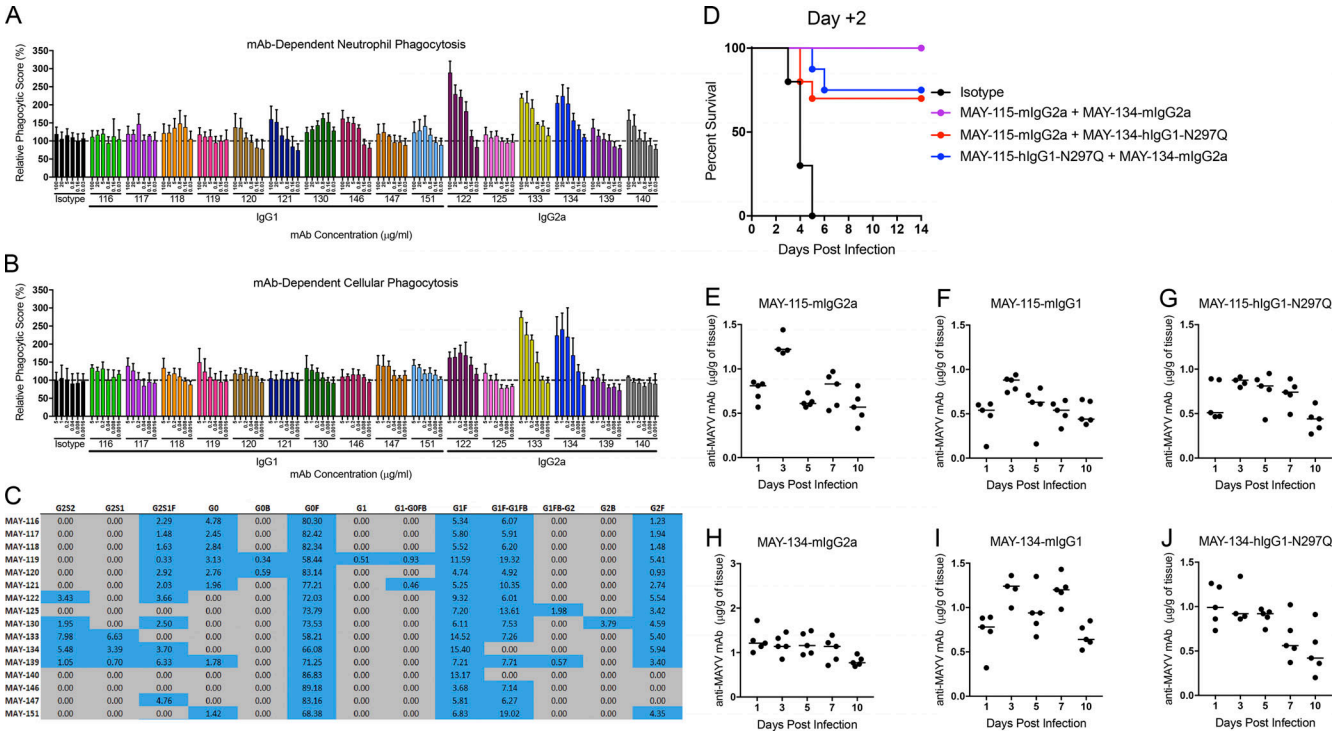


Figure S5. **Effector function and glycan profiles of anti-MAYV mAbs.** Related to Fig. 7. **(A and B)** Anti-MAYV E2 mAbs were evaluated for neutrophil- (A) and monocyte-dependent (B) phagocytosis of MAYV E2 protein-conjugated beads relative to a no-antibody–treated control; data are the average of two experiments performed in duplicate. Error bars represent SD of each replicate. **(C)** The glycan profiles of the mAbs were detected by capillary electrophoresis of isolated Fc regions. The relative abundance of the indicated glycans is shown with glycans present shaded in blue. **(D)** Efficacy of combination therapy with isotype switched mAbs. 100 µg each of a combination of MAY-115 and MAY-134 of the indicated IgG subclass was given to anti-Ifnar1 mAn treated mice beginning at 2 dpi, and survival was monitored. Data are from two experiments (n = 10). **(E–J)** The clearance rate of isotype-switched mAbs in vivo. Antibody clearance in vivo. WT C57BL/6J mice were inoculated with 200 µg/mouse of MAY-115 (E–G) or MAY-134 (H–J) of mouse IgG2a (E and H), human IgG1 (F and I), or human IgG1-N297Q (G and J). At the indicated time points, mice were sacrificed and perfused with PBS. The feet were harvested, homogenized, and analyzed for levels of anti-MAYV mAbs by ELISA.

Table S1. **MAYV strains**

Strain	GenBank accession number	Genotype	Date isolated	Source of isolate	Country of origin
CH	DQ487410	D	1997	Human	Peru
BeH343155	MK573244	D	1978	Human	Brazil
BeH506151	MK573241	D	1991	Human	Brazil
FSB0311	MK573245	D	2002	Human	Bolivia
IQU2950	MK573243	D	2000	Human	Peru
Uruma	MK573246	D	1955	Human	Bolivia
TRVL15537	MK573240	D	1957	Mosquito	Trinidad
OBS2209	MK573242	D	1995	Human	Peru
BeH407	MK573238	L	1955	Human	Brazil
BeH473130	KY618133	L	1988	Human	Brazil
BeH428890	MK573239	L	1984	Human	Brazil

MAYV strains used in this study are listed along with GenBank accession numbers, genotype, isolation date, host source, and country of origin. All strains were provided by the World Reference Center for Emerging Viruses and Arboviruses and described in Powers et al. (2006).

Table S2. **Kinetic and equilibrium binding to E2 protein by anti-MAYV mAbs as measured by BLI**

MAYV mAb	k_a (10^5 M^{-1} s^{-1})	k_d (10^{-4} s^{-1})	$t_{1/2}$ (s)	$K_{D,\ kinetic}$ (nM)	$K_{D,\ equilibrium}$ (nM)
115	n.b.	n.b.	n.b.	n.b.	n.b.
116	1.72 ± 0.29	17.7 ± 4.1	405 ± 90	10.8 ± 4.5	19.9 ± 2.1
117	2.84 ± 0.72	8.49 ± 1.58	834 ± 141	3.25 ± 1.56	10.6 ± 2.5
118	3.07 ± 0.67	22.8 ± 3.6	309 ± 48	7.91 ± 3.37	13.4 ± 4.0
119	2.45 ± 0.73	16.8 ± 2.0	417 ± 47	7.36 ± 2.98	14.3 ± 4.0
120	1.99 ± 0.23	16.0 ± 1.3	436 ± 35	8.05 ± 0.44	17.7 ± 1.0
121	1.38 ± 0.25	49.9 ± 7.0	141 ± 20	37.1 ± 8.9	46.6 ± 12.7
122	5.85 ± 0.20	8.52 ± 1.72	839 ± 191	1.45 ± 0.25	3.96 ± 0.78
125	1.01 ± 0.22	46.2 ± 2.7	150 ± 9	47.5 ± 12.1	58.3 ± 18.8
130	13.8 ± 2.4	10.3 ± 0.3	710 ± 187	0.78 ± 0.32	2.63 ± 0.93
131	n.b.	n.b.	n.b.	n.b.	n.b.
133	0.20 ± 0.02	49.7 ± 3.1	140 ± 9.0	257 ± 42	243 ± 64
134	0.28 ± 0.04	21.5 ± 7.4	346 ± 104	81.0 ± 37.3	151.5 ± 69.4
139	1.37 ± 0.20	11.4 ± 5.7	695 ± 269	8.90 ± 5.9	22.1 ± 11.0
140	0.23 ± 0.04	17.7 ± 1.3	393 ± 28	76.8 ± 7.1	170.2 ± 29.4
146	2.45 ± 0.42	20.0 ± 0.3	347 ± 5	8.33 ± 1.34	17.8 ± 4.9
147	0.73 ± 0.04	12.4 ± 1.8	567 ± 81	17.2 ± 3.5	38.0 ± 8.3
151	1.81 ± 0.29	38.1 ± 7.1	186 ± 35	21.6 ± 6.2	28.8 ± 7.1

Binding of anti-MAYV mAbs to recombinant MAYV E2 protein was measured by BLI. Values are mean ± SD of three experiments. The rates of association (k_a), disassociation (k_d), the $t_{1/2}$ of association, and the kinetic ($K_{D,\ kinetic}$) and equilibrium ($K_{D,\ equilibrium}$) association constants are shown with SDs. $K_{D,\ kinetic} = k_d/k_a$; $t_{1/2} = \ln(2)/k_d$. n.b., nonbinding; these mAbs recognize epitopes in E1 protein.

Reference

Powers, A.M., P.V. Aguilar, L.J. Chandler, A.C. Brault, T.A. Meakins, D. Watts, K.L. Russell, J. Olson, P.F. Vasconcelos, A.T. Da Rosa, et al. 2006. Genetic relationships among Mayaro and Una viruses suggest distinct patterns of transmission. *Am. J. Trop. Med. Hyg.* 75:461–469. https://doi.org/10.4269/ajtmh.2006.75.461