TABLE S1, Related to Figures 3, S2, and S3. Summary table of EEEV E2 neutralizing antigenic determinants recognized by human anti-EEEV mAbs.

EEEV mAb	E2 Domain	Critical EEEV E2 alanine residues[a]	Allosteric EEEV E2 alanine residues[b]	Reduction in neutralization potency for SINV/EEEV escape mutant viruses[c]
EEEV-12	A	No reduction	N/A	M68T (L227R)
EEEV-33	A (N-link/A/Arch 1/B/Arch 2)	**9**, 74, **116, 118, 120**	**34**, **163, 166, 172, 240**	(M68T)
EEEV-147	N-link/Arch 1	**9, 10**	**163, 166**	Minimal reduction
EEEV-7	B	**205, 206, 207**, 213	N/A	M68T
EEEV-106	B	**205, 206, 207**, 213	N/A	M68T
EEEV-27	A/B (N-link/A/Arch 1/B/Arch 2)	**9**, 73, **178, 186, 190, 202, 205-207**, 213-**216, 222, 229**	**33-34, 65, 98, 109, 128, 145 163, 165-166, 172, 185, 226-**227, **240**	M68T
EEEV-129	B	**205, 207**, 213	N/A	M68T
EEEV-21	B	**178**, 194, **202, 205, 207**, 213, 215	**185**	(M68T)
EEEV-94	A/B (N-link/A/B)	**9, 202, 205**, 215	**33, 185**	Minimal reduction

EEEV-143	B	**202**	N/A	(M68T)
EEEV-93	A/B (N-link/A/Arch 1/B/Arch 2)	9, 73, **178, 202, 205-206**, 215-**216**	**33-34, 98, 128, 145, 163, 165-166, 172, 185, 226, 240**	M68T, L227R
EEEV-97	B	**178, 190, 200, 202, 207**, 215, **219, 222**	**185**	M68T, G192R, L227R

[a] Surface-exposed critical alanine residues (<25% binding relative to WT) are identified as determined through alanine-scanning mutagenesis library analyses for each mAb. See **Figure 3B** for a heat map representation of average percent binding of human anti-EEEV mAbs to critical residues and **Figure S2** for bar graph representation of human anti-EEEV mAb binding to critical residues.

[b] Critical alanine residues (<25% binding relative to WT) that are not surface exposed, as determined through alanine-scanning mutagenesis library analyses for each mAb are identified. The identified residues may result in a loss of binding phenotype due to allosteric effects on the epitope. N/A = not applicable

[c] Neutralization potency of human anti-EEEV mAbs for the SINV/EEEV escape mutant viruses (M68T, G192R, and L227R). Minimal reduction indicates that neutralization potency of respective human anti-EEEV mAb is similar to neutralization activity against WT SINV/EEEV. Escape mutants with a >10-fold reduction in neutralization potency are indicated for each mAb. Parentheses include escape mutants with > 5-fold reduction in neutralization potency. See **Figure S3** for neutralization curves of human anti-EEEV mAbs to SINV/EEEV escape mutant viruses (M68T, G192R, and L227R).

Bold indicates residues distinct from those previously identified with a loss-of-binding phenotype for the murine anti-EEEV mAbs (Kim et al., 2019).

Table S2, Related to STAR Methods. Parameters used for high-resolution data collection of SINV/EEEV:rEEEV-33 Fab, SINV/EEEV:rEEEV-143 Fab, EEEV VLP, and EEEV VLP:rEEEV-143 Fab.

	Parameters	SINV/EEEV: rEEEV-33 Fab	SINV/EEEV: rEEEV-143 Fab	EEEV VLP	EEEV VLP: rEEEV-143 Fab
Data Deposition	EMDB	22223	22188	22276	22277
	PDB			6XO4	6XOB
Microscope setting	Microscope	Titan-Krios	Glacios	Titan Krios	Titan Krios
	Acceleration voltage (kV)	300	200	300	300
	Detector			Falcon 3EC	Falcon 3EC
	Magnification (x)	18,000	22,000	96,000	75,000
	Pixel size (Å)	1.64	2.0	0.8608	1.11
	Dose Exposure (e⁻/Å2)	50	25	30	30
	Defocus range (µm)	1.0 – 2.5	1.0 – 2.5	0.8-2	1-2.5
Data	# Micrographs			1,745	~4,800
	# particles	~18,000	~10,000	3,935	3,600
	# particle after 2D	~12,900	~7,200	3,569	2,471
	Final particles #	~12,900	~7,200	3,469	1,300
	Symmetry	icosahedral	icosahedral	icosahedral	icosahedral
	Resolution FSC=0.143 (Å)	7.2	8.3	4.2	8.5
Model refinement and validation	Protein residues	-	-	3,992	5,700
	Map CC	-	-	0.79	0.69
	Bond lengths (Å)	-	-	0.006	0.004
	Bond angles	-	-	0.671	0.816
	Ramachandran	-	-		
	Outliers (%)	-	-	0	0
	Allowed (%)	-	-	10.93	21.64
	Favored (%)	-	-	89.07	78.36
	Poor rotamers (%)	-	-	5.41	21.99
	MolProbity score	-	-	2.9	3.98
	Clash score	-	-	18.8	53.27
	CaBLAM score	-	-	6.42	7.28