

Table S1, related to Figure 1. Human E1-specific mAb VLP and recombinant protein binding in presence of the nonionic detergent Tween®20.

Binding Group	EEEV mAb	EC ₅₀ (ng/mL; [Tween®20])						
		Virus-like particles (VLPs)			Protein			
		EEEV	VEEV	WEEV	EEEV E1	CHIKV E1	MAYV E1	EEEV E3E2/E2
EEEV-specific	EEEV-76	30	>	>	7	>	>	>
	EEEV-104	8	>	>	36	>	>	>
	EEEV-109	9	>	>	2	>	>	>
	EEEV-126	11	>	>	13	>	>	>
	EEEV-127	9	>	>	7	>	>	>
	EEEV-312	1,895	>	>	13	>	>	>
	EEEV-320	22	>	>	17	>	>	>
	EEEV-377	11	>	>	9	>	>	>
	EEEV-379	85	>	>	5	>	>	>
	EEEV-398	24	>	>	9	>	>	>
	EEEV-400	11	>	>	8	>	>	>
EEEV-VEEV	EEEV-157	11	1,731	>	3	>	>	>
New World	EEEV-179	11	20	16	34	>	>	>
Broadly-reactive	EEEV-307	6	8	>	14	33	33	>
	EEEV-354	7	5	>	21	14	41	>
Pan-alphavirus	EEEV-138	8	5	9	15	61	16	>
	EEEV-342	16	8	35	35	24	40	>
	EEEV-346	3	2	3	14	12	14	>
	EEEV-368	5	5	6	19	12	29	>
	EEEV-387	9	11	10	10	40	50	>

> symbol indicates > 5 µg/mL

Table S2, related to Figures 2, 3, S2, S3, and S4. Human E1-specific mAb epitope mapping.

Binding Group	EEEV mAb	Epitope	Critical Binding Residues ¹ or Potential Interacting Peptides ²
EEEV-specific	EEEV-76	Domain II (central β sheet, ij loop)	T218, L220
	EEEV-104	Domain II (central β sheet, gh loop)	Y93, Y193
	EEEV-109	Domain III	289-300, 348-353
	EEEV-126	Domain II (cd loop)	Q79
	EEEV-127	Domain II (ij loop)	L220
	EEEV-312	Domain II (cd loop, central β sheet, ij loop)	V80, F81, Y85, F87, W89, G90, G91, F95, N100, T218, L220
	EEEV-320	Domain II (central β sheet)	G182
	EEEV-377	Domain II (ij loop)	L220
	EEEV-379	Unknown	ND
	EEEV-398	Domain I	G165, S168, S169, W171
	EEEV-400	Domain II central β sheet, gh loop	K197
EEEV-VEEV	EEEV-157	Domain III	353-366
New World	EEEV-179	Domain I (DI), Domain II (DII), Fusion loop, Domain III (DIII), DI/DII hinge region, DI/DIII linker	Y93, A286, N361; 37-45, 80-92, 107-113, 141-146, 147-162, 191-201, 202-214, 221-228, 229-254, 262-271, 288-300
Broadly-reactive	EEEV-307	Fusion loop	W89, G91, F95
	EEEV-354		W89, Y93, F95
Pan-alphavirus	EEEV-138		F95, N100
	EEEV-342		W89, F95, N100
	EEEV-346		W89, F95, N100
	EEEV-368		G91, F95
	EEEV-387		F95

¹Critical binding residues were determined through cell-surface-display antigen binding, EEEV and CHIKV E1 alanine-scanning mutagenesis library analyses.

²Potential interacting peptides were determined by comparison of relative deuterium uptake between unbound and bound states of the EEEV E1 glycoprotein through HDX-MS.

ND = not determined

Table S3, related to Figure 5. Human E1-specific mAb temperature-independent binding.

EEEV mAb	Temperature-dependent binding EC ₅₀ (ng/mL; [1× PBS])																	
	Virus-like particles (VLPs)									Protein								
	EEEV			VEEV			WEEV			EEEV E1			CHIKV E1			MAYV E1		
	22°C	37°C	42°C	22°C	37°C	42°C	22°C	37°C	42°C	22°C	37°C	42°C	22°C	37°C	42°C	22°C	37°C	42°C
76	40	55	123	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
104	105	102	115	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
109	16	15	21	>	>	>	>	>	>	198	188	189	>	>	>	>	>	>
126	43	41	62	>	>	>	>	>	>	690	1,020	395	>	>	>	>	>	>
127	35	42	58	>	>	>	>	>	>	374	294	252	>	>	>	>	>	>
312	>	>	>	>	>	>	>	>	>	94	44	65	>	>	>	>	>	>
320	180	143	135	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
377	50	41	53	>	>	>	>	>	>	409	4,220	630	>	>	>	>	>	>
379	739	663	791	>	>	>	>	>	>	49	33	50	>	>	>	>	>	>
398	152	135	163	>	>	>	>	>	>	1,096	289	262	>	>	>	>	>	>
400	44	35	55	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
157	174	192	174	>	>	>	>	>	>	222	230	214	>	>	>	>	>	>
179	49	55	40	519	530	412	395	360	698	2,686	1,100	1,651	>	>	>	>	>	>
307	85	86	68	192	346	210	>	>	>	927	328	262	>	>	>	357	943	397
354	94	107	84	142	86	122	>	>	>	410	235	348	405	1,342	765	356	342	226
138	23	25	26	53	39	35	112	99	127	194	249	292	401	419	646	836	688	724
342	53	66	52	64	96	51	340	371	1,063	567	309	324	285	2,708	1,300	371	3,175	629
346	18	19	15	20	25	13	30	34	32	122	182	131	148	272	172	113	186	168
368	83	58	81	338	110	69	293	155	253	317	265	237	1,020	1,607	346	355	247	409
387	118	69	130	461	250	158	163	102	168	232	626	351	2,017	4,931	4,933	2,440	1,044	3,597

> symbol indicates > 5 µg/mL

Table S4, related to Figure 4. Human E1-specific mAb acidic pH-dependent or pH-independent binding.

EEEV mAb	Binding EC ₅₀ (ng/mL; [in 1× PBS]) for indicated antigen and pH (5.4 or 7.4)											
	Virus-like particles (VLPs)						Protein					
	EEEV		VEEV		WEEV		EEEV E1		CHIKV E1		MAYV E1	
	5.4	7.4	5.4	7.4	5.4	7.4	5.4	7.4	5.4	7.4	5.4	7.4
EEEV-76	292	790	>	>	>	>	>	>	>	>	>	>
EEEV-104	154	300	>	>	>	>	>	>	>	>	>	>
EEEV-109	726	1,183	>	>	>	>	579	675	>	>	>	>
EEEV-126	174	330	>	>	>	>	>	>	>	>	>	>
EEEV-127	114	297	>	>	>	>	>	>	>	>	>	>
EEEV-312	172	>	>	>	>	>	318	285	>	>	>	>
EEEV-320	164	499	>	>	>	>	>	>	>	>	>	>
EEEV-377	197	509	>	>	>	>	>	>	>	>	>	>
EEEV-379	>	>	>	>	>	>	615	396	>	>	>	>
EEEV-398	1,970	1,078	>	>	>	>	>	>	>	>	>	>
EEEV-400	198	246	>	>	>	>	>	>	>	>	>	>
EEEV-157	1586	1118	>	>	>	>	945	748	>	>	>	>
EEEV-179	108	202	>	>	1,790	1,760	>	>	>	>	610	>
EEEV-307	147	299	342	3,001	4,363	4,009	862	1,315	>	1,647	>	1,211
EEEV-354	245	562	412	3,053	>	>	692	978	4,696	2,889	4,028	2,020
EEEV-138	90	221	143	277	137	308	696	711	556	337	2,014	1,020
EEEV-342	154	578	267	664	394	2,912	3,451	2,998	2,750	>	2,795	965
EEEV-346	12	31	21	43	20	59	64	131	177	88	534	365
EEEV-368	282.7	979.2	503.8	>	587.3	3,400	1,883	722.4	>	>	2,134	1,350
EEEV-387	237	626	599	2,941	195	769	2,958	>	>	>	>	3,536

> symbol indicates > 5 µg/mL

Table S5, related to Figures 5 and S6. Human E1-specific mAb neutralization activity.

Binding group	EEEV mAb	IC ₅₀ (ng/mL [37°C; 42°C])					Egress inhibition (yes or no)
		SINV/EEEV	SINV/VEEV	SINV/WEEV	CHIKV	MAYV	SINV/EEEV
EEEV-specific	EEEV-76	>	NA				no
	EEEV-104	>					yes
	EEEV-109	>					yes
	EEEV-126	>					yes
	EEEV-127	>					no
	EEEV-312	>					no
	EEEV-320	>					no
	EEEV-377	>					no
	EEEV-379	>					no
	EEEV-398	>					no
EEEV-400	>	no					
EEEV-VEEV	EEEV-157	>	>	>	>	>	no
New World	EEEV-179	>	>	>	>	>	yes
Broadly-reactive	EEEV-307	>	>	>	>	>	no
	EEEV-354	>	3,505; 5,042	>	>	>	no
Pan-alphavirus	EEEV-138	>	>	>	>	>	no
	EEEV-342	>	3,558; 3,732	>	>	>	no
	EEEV-346	>	2,044; 3,207	>	>	>	no
	EEEV-368	>	>	>	>	>	no
	EEEV-387	>	2,112; >	>	>	>	no

> symbol indicates > 5 µg/mL

NA = not applicable