

Table S1. Properties of avian and mammalian MXRA8 receptor activity for alphaviruses, Related to Figure 1

Virus	Infection enhanced by avian MXRA8	Infection enhanced by mammalian MXRA8	Infection inhibited by chicken MXRA8-Fc	Infection inhibited by knockout of chicken MXRA8	Cell line used
SINV	✓	X	✓	✓	3T3, CEFs, 293T, HeLa, Jurkat
WEEV	✓	X	✓	-	3T3, CEFs
WHAV	✓	X	✓	-	3T3, CEFs
BABV	✓	X	✓	-	3T3, CEFs
OCKV	✓	X	✓	✓	3T3, CEFs,
AURA	X	X	X	X	3T3, CEFs, 293T, HeLa
CHIKV 181/25	X	✓	-	-	3T3, CEFs, 293T, HeLa, Jurkat
CHIKV 37997	X	✓	-	-	293T
SINV-EEEV	X	X	-	X	3T3, K562
SINV-VEEV	X	X	-	-	3T3, Jurkat

✓, yes; X, no; -, not tested.

Table S2. Cryo-EM model building and refinement statistics, Related to Figures 3 and 7

Asymmetric Unit Model		WEEV-VLP alone (EMDB-27272 PDB: 8DAQ)	WEEV-VLP + duck Mxra8 (EMDB-27271 PDB: 8DAN)	CHIKV-VLP + Du-D1-Mo-D2 (EMDB-28644 PDB: 8EWF)	WEEV-VLP + Du-D1-Mo-D2 (EMDB-40711 PDB: 8SQN)
# of chains		8	12	9	9
# of residues		3412	4472	3697	3677
# of carbohydrates		8	12	9	9
Resolution (Å)		4.35	4.74	3.96	3.89
MolProbity Score		1.98	1.84	2.25	1.91
All-atom clash score		13.52	10.23	21.25	12.07
Rotamer outliers (%)		0.00	0.00	0.00	0.00
C β outliers		0.00	0.00	0.00	0.00
Ramachandran Plot Values	Favored (%)	95.05	95.50	93.42	95.52
	Allowed (%)	4.95	4.50	6.58	4.48
	Outlier (%)	0.00	0.00	0.00	0.00
R.M.S. Deviations	Bond lengths (Å)	0.003	0.002	0.004	0.003
	Bond angles (°)	0.554	0.517	0.679	0.598
Correlation Coefficient		0.75	0.68	0.71	0.72

Table S3. List of contact residues of duck MXRA8 (bound to WEEV-VLP), Du-D1-Mo-D2 (bound to WEEV-VLP), mouse MXRA8 (bound to CHIKV-VLP), or Du-D1-Mo-D2 MXRA8 (bound to CHIKV-VLP), Related to Figures 3 and 7

E1-E2 heterodimer	MXRA8 domain	Duck Mxra8 (+ WEEV) residues PDB: 8DAN	Du-D1-Mo-D2 (+ WEEV) residues	Mouse Mxra8 (+ CHIKV) residues PDB: 6NK6	Du-D1-Mo-D2 (+ CHIKV) residues PDB: 8EWF
Wrap	Domain-I	N114(1), T117(8), D118(20), R190(12), T195(2)	T117(1), R190(8), R197(4)	Q83(1), R84(16), R86(2), Y91(1), A93(1), G94(1), E95(7), R97(14), H139(3), H141(2), H141(1), D146(4)	M86(5), R88(2), Y93(4), A95(1), G96(2), D97(17), R99(1), H141(2), Y148(12)
	Domain-II	None	None	P56(3), R57(8), V59(2), T61(1), D63(14), R64(5), L65(5), H66(5), N247(1), A250(4), R251(9),	P56(5), V59(1), D63(7), R64(9), L65(27), N66(1), A252(2), R253(15), D255(3)
intra	Domain-I	Y81(5), A95(1), R99(7), S103(6), Y104(16), Q106(4), T117(1), A129(2),	A95(9), G96(2), S103(5), Y104(18), Q106(2), R108(1), T117(2), D127(2),	H115(2), D194(5), H196(21), E198(7)	E196(1)
	Domain-II	R64(30), L65(3), E201(3)	D63(1), R64(4), L65(9), N66(11), E201(4)	Q62(1), P236(2), D240(1), E260(1)	E201(3), S229(7), F239(11)
inter	Domain-I	Y81(2), G82(13), D83(7), N84(2)	D83(6)	None	None

	Domain-II	None	None	L210(3), P211(5), G212(5), S214(1), F237(2), R263(12), A264(4), E266(3)	None
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Close-contacts between atomic coordinates are defined as the heavy atoms within 0.39 nm.

Number of contacts per residue are indicated within parentheses.

Table S4. List of contact residues of WEEV-VLP bound to duck MXRA8 (PDB: 8DAN) or Du-D1-Mo-D2 MXRA8 (PDB: 8SQN) and CHIKV-VLP bound to mouse MXRA8 (PDB: 6NK6) or Du-D1-Mo-D2 (PDB: 8EWF), Related to Figures 3 and 7

E1-E2 heterodimer	E1-E2 domain	WEEV residues (duck MXRA8)	WEEV residues (Du-D1-Mo-D2)	CHIKV residues (mouse MXRA8)	CHIKV residues (Du-D1-Mo-D2)
Wrap	E1 Domain-II	Y85(27), S98(3), K223(1), V226(10), K227(2)	Y85(8), S225(1), K227(4)	Y85(8), F87(5), W89(1), D97(2), A98(1), A226(1)	Y85(2), F87(3), D97(16)
	E2 Domain-A	None	None	H18(1), E24(1), H26(6), S27(14), C28(4), H29(3), S72(1), T74(7), R119(12), K120(2), I121(2)	C22(2), G23(1), E24(7), H26(33), C28(1), D71(3), R119(15), K120(6), I121(1)
	E2 Domain-B	None	None	R178(2), T179(5), M181(1), T182(3), T191(5), V192(1), N193(5), T213(1), D214(7), I222(1), D223(8)	T179(2), T182(6), Q183(4), Q184(2), N193(5), D214(2), D223(5)
intra	E1 Domain-I	V34(1), S35(4), R134(1), T141(1), R143(16), D145(5), R157(5)	V34(4), S35(1), K130(2), R134(3), R143(12), D145(6)	K132(1), T143(1), Y147(2)	S130(1), K132(2), A145(6)
	E1 Domain-II	E262(5), E264(1), R267(1)	K69(4), A70(2)	None	None
	E2 Domain-A	D7(3)	None	W64(7)	W64(1)
	E2 β -linker	L152(1), L161(3),	L152(2), K154(8),	V157(2), Q158(15),	V157(2), S159(1),

		E163(16), T164(4), S165(2), H259(7), R263(1), V268(1)	D159(1), L161(7), S165(1), R263(6), L264(1), T265(6), P266(3), T267(4), V268(3)	S159(11), T265(1)	V264(2), T265(5)
inter	E1 Domain-II	K69(8), A70(16)	K69(4), A70(2)	K71(2), S72(5), L73(2), E209(1), K211(14), D212(5)	None
	E2 β - linker	None	None	A164(4), E165(1), E166(1)	None

Close-contacts between atomic coordinates are defined as the heavy atoms within 0.39 nm.

Number of contacts per residue are indicated within parentheses.

Table S5. Properties of chimeric protein Du-D1-Mo-D2 and Mo-D1-Du-D2 MXRA8 receptor activity for alphaviruses, Related to Figure 6.

Virus	Infection enhanced by cell surface expression of Du-D1-Mo-D2 MXRA8	Infection enhanced by cell surface expression of Mo-D1-Du-D2 MXRA8	Binding of Du-D1-Mo-D2 MXRA8-Fc to surface of infected cells	Inhibition of infection by Du-D1-Mo-D2 MXRA8-Fc	<i>In vivo</i> protection by Du-D1-Mo-D2 N66R MXRA8-Fc
SINV	✓	X	✓	✓	-
SINV-WEEV	✓	X	✓	✓	✓
SINV-VEEV	-	-	X	-	-
SINV-CHIKV	✓	X	✓	✓	✓
MAYV	✓	X	✓	-	-

✓, yes; X, no; -, not tested.