

Supplemental Information

Cooperativity Enables Non-neutralizing

Antibodies to Neutralize Ebolavirus

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Supplemental Information:

Table S1: ELISA based competition assay for EBOV GP Δ TM binding, Related to main Figure 1. Pairs of mAbs were tested for their binding to EBOV GP Δ TM to identify cooperative or synergistic combinations. Antibodies with overlapping epitopes naturally competed (i.e. 2G4, 4G7, KZ52) as did self-self pairs, while mAb pairs highlighted in red demonstrated >50% increase in binding and those in orange showed 25-50% increase as a result of pairing. Binding is normalized to control antibody, where < 1 = competition, 1= no change and > 1= enhancement.

	Detection mAb (@EC50)	Competing mAb (saturating)											
		FVM02	FVM04	FVM09	FVM20	h4B8	h17C6	c13C6	KZ52	c6D8	c2G4	c4G7	1H3
Ebola GP Δ TM	FVM02-biotin	0.17	0.83	0.85	0.91	0.95	0.99	0.83	1.00	0.92	1.03	0.89	0.99
	FVM04-biotin	0.92	0.18	0.75	0.82	0.87	0.86	0.23	0.98	0.94	0.95	0.81	0.94
	FVM20-biotin	0.84	0.77	0.26	0.35	1.20	0.95	0.74	0.52	0.84	4.00	3.56	0.83
	m4B8	1.03	0.80	0.94	0.95	0.13	0.32	0.94	1.08	0.99	1.07	1.12	1.10
	m17C6	0.99	0.05	0.92	0.97	0.51	0.60	0.83	1.19	1.04	1.03	1.11	1.12
	m8C4	0.82	0.83	1.99	1.71	0.95	0.91	0.45	0.53	0.97	0.59	0.69	0.82
	m13C6	0.93	0.24	0.89	0.90	0.87	0.91	0.07	0.93	0.98	1.13	1.22	1.13
	m16G8	0.77	0.88	0.87	0.91	0.92	0.92	0.89	1.05	0.94	1.03	1.11	1.14
	m2G4	0.94	0.81	1.41	1.29	0.98	1.02	0.83	0.11	0.94	0.32	0.34	0.86
	m4G7	0.92	0.88	1.08	1.05	1.03	1.06	0.87	0.14	0.96	0.24	0.29	1.07
	KZ52-biotin	0.96	0.92	0.47	0.83	1.02	1.03	0.89	0.29	0.91	0.47	0.38	0.93

Table S2: ELISA based competition assay for SUDV GP Δ TM binding, Related to main Figure 1. A similar panel of mAbs to those in Table S1 were evaluated for binding to SUDV GP Δ TM. Color codes match that described in the Table S1 legend.

	Detection mAb (@EC50)	Competing mAb (saturating)						
		FVM02	FVM04	FVM09	FVM20	h4B8	h17C6	c13C6
Sudan GPΔTM	FVM02-biotin	0.21	0.88	0.91	1.01	1.01	1.04	1.02
	FVM04-biotin	0.94	0.21	0.94	1.03	1.01	1.02	1.03
	FVM20-biotin	0.87	0.66	0.55	0.95	1.15	1.02	1.14
	m8C4	0.92	1.05	1.31	1.12	1.07	1.07	1.25
	m13C6	0.96	0.21	1.55	1.29	1.20	1.15	0.93
	m4B8	1.01	0.58	0.90	0.97	0.13	0.90	1.08
	m17C6	1.00	0.07	0.88	1.03	0.24	0.97	1.12
	m16G8	0.87	0.87	0.84	1.02	0.97	1.07	1.07
	16F6	0.87	0.94	0.63	0.99	1.03	1.03	1.04

Figure S1: Analysis of synergy between mAb pairs in neutralization of EBOV or SUDV, Related to main Figure 3. The combination index (CI) and dose reduction index (DRI) obtained by CompuSyn analysis are shown for the mAb pairs, with the first mAb in the panel titles tested at various concentrations in the presence of a fixed concentration of the second mAb. Fa: Fraction affected (neutralized). Related to main Figure 2. CI=1: additive effect, CI<1: synergy; CI>1: antagonism. DRI>1 indicates a potential fold reduction in effective dose as a result of drug combination.

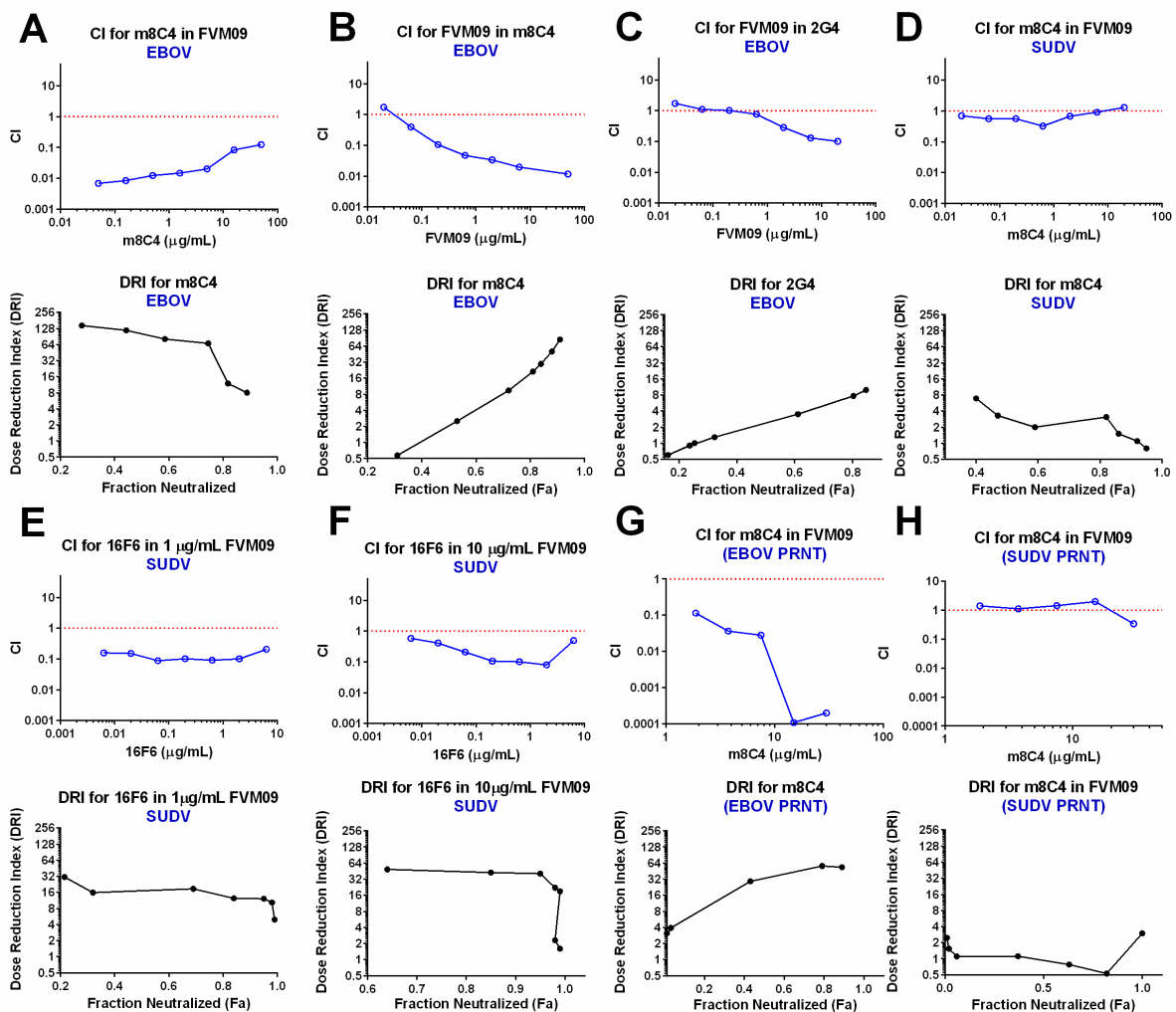


Figure S2: Synergy analysis of FVM09 and FVM02 combination with FVM04, Related to main Figure 3. Synergy analysis of FVM04 paired with FVM09 (A-D) or with FVM02 (D-F) shows no synergy between FVM04 and FVM09 and moderate synergy at lower concentrations between FVM04 and FVM02. For panels A&D, each data point represents the average of three measurements \pm SD.

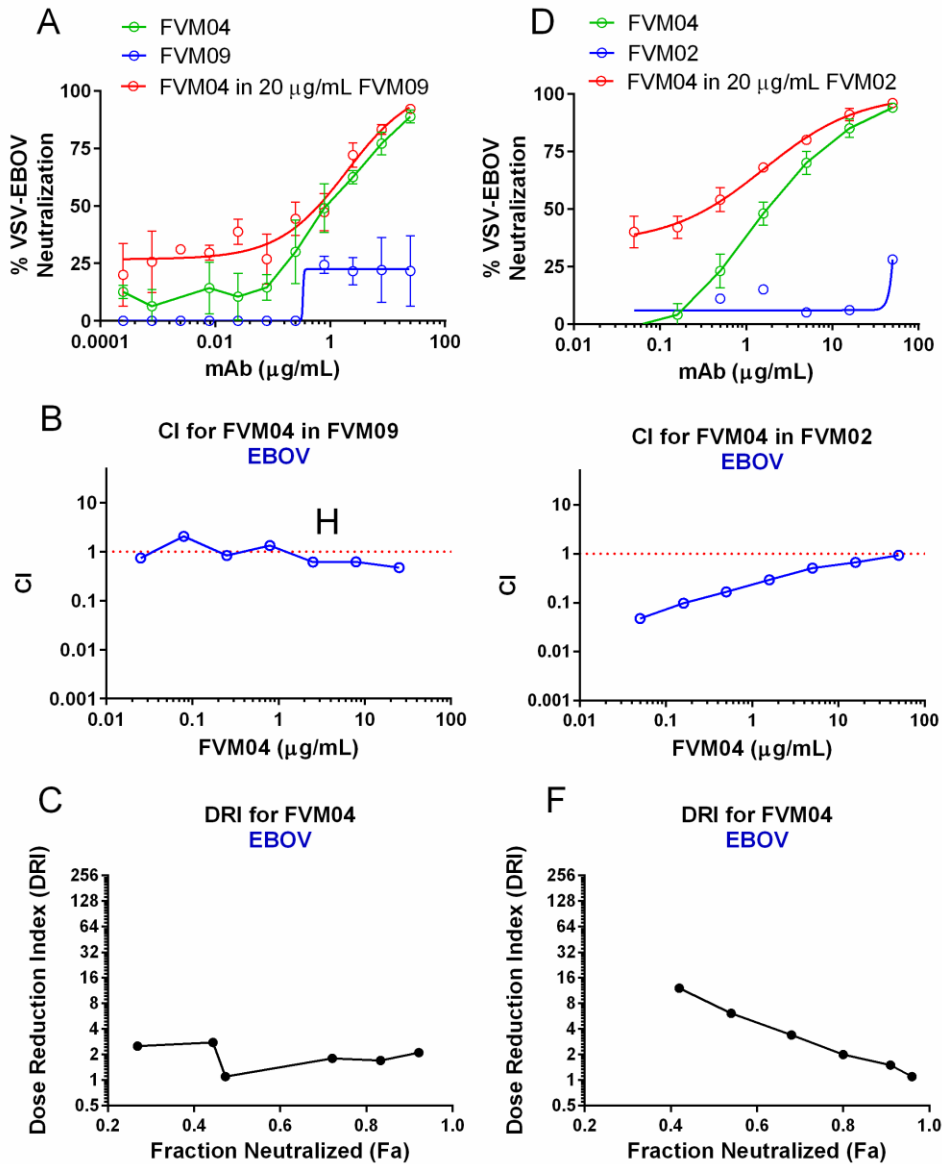


Figure S3: Cooperative neutralization mediated by Fab fragments, Related to main Figure

3. (A) Relative binding of FVM09 full IgG and FVM09 Fab to GP- Δ TM. (B) VSV-EBOV GP-Luc neutralization mediated by Fab in the presence of m8C4 at 13 μ g/ml. (C) Relative binding to GP- Δ TM of m8C4 IgG and m8C4 Fab alone or in the presence of FVM09. (D) The weak neutralizing activity of m8C4 Fab is enhanced in the presence of FVM09 at a fixed concentration of 20 μ g/ml. (E) Cooperativity between FVM09 and m8C4 Fab fragments is seen in neutralizing VSV-SUDV GP-Luc. All data points represents the average of three measurements \pm SD.

