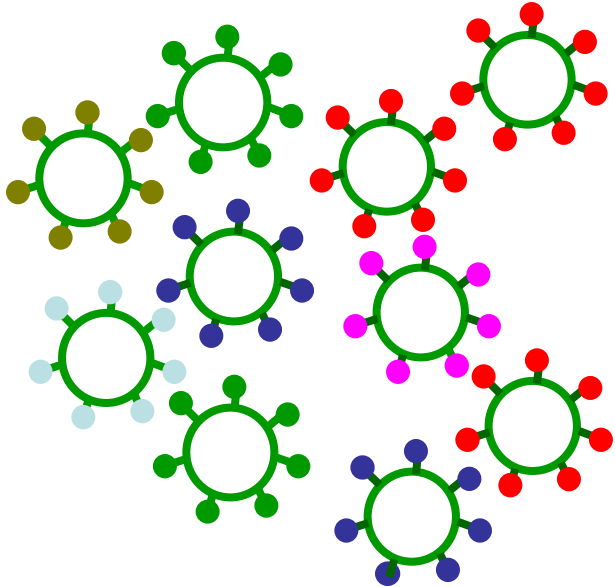

Evaluation of early subtype A, C, and D HIV-1 envelope variants to identify strategies to expose neutralization epitopes

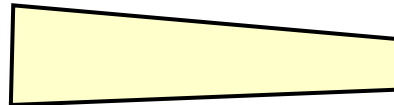
Catherine Blish, Minh-An Nguyen, Dana Panteleeff, Stephanie Rainwater, Zahra Jalalian-Lechak, and Julie Overbaugh

Transmitted, or Early, HIV-1 variants

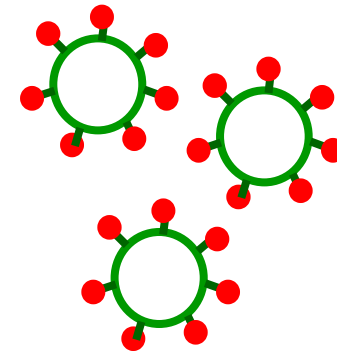
Chronically infected index case



Bottleneck at transmission



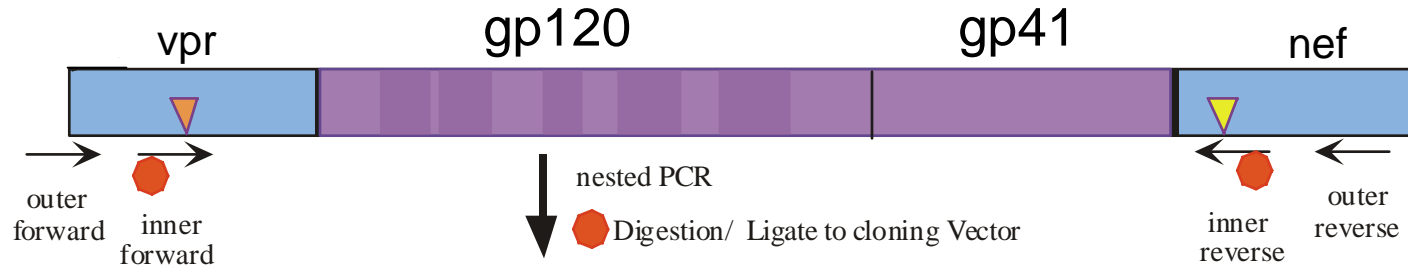
Newly infected person



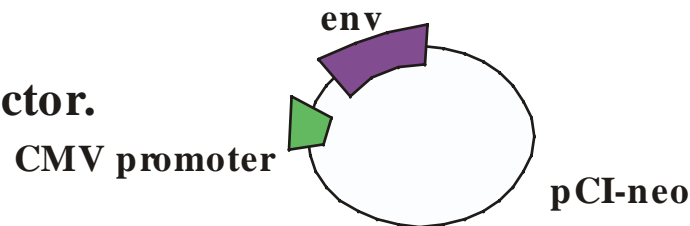
“Early variants”

- These early variants are the most logical targets for a protective vaccine, as they are the strains that must be blocked to prevent new infections.
- Could these early variants contain unique targets for neutralization?

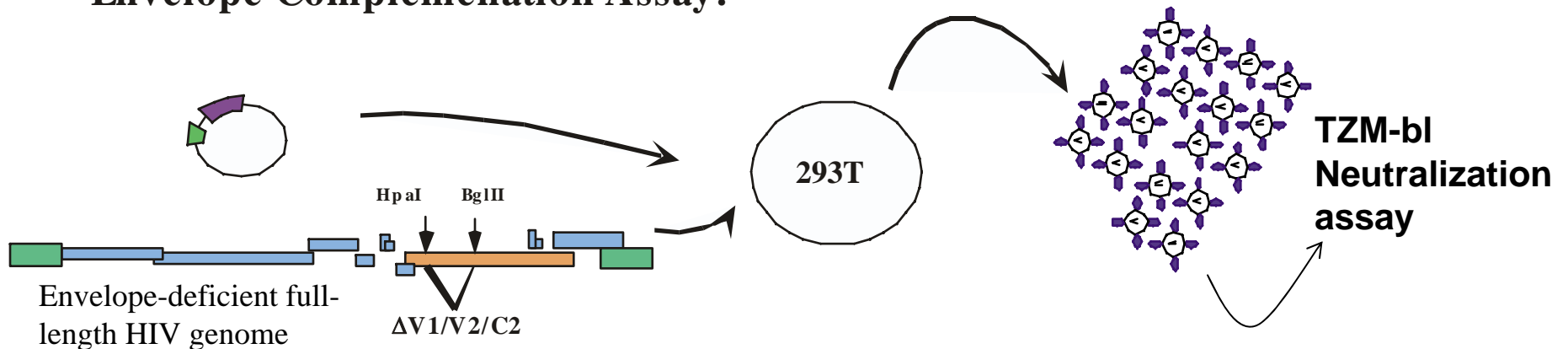
Generation of full-length envelope clones and pseudoviruses from HIV-1 Subtypes A, C, and D in Kenya



Clone into an expression vector.



Envelope Complementation Assay:



Sensitivity of early subtype A, C, D, and A/D recombinant pseudoviruses to plasma pools

Subtype	Viral pseudotype	Kenya 1991	Kenya 98-00	Subtype A	Subtype B	Subtype C	Subtype D
A	Q769b9*		52	63	111	70	78
	Q769d22				62		
	Q769h5						
	Q842d12		116	75	90	52	
	Q842d14	56	194	129	129	78	
	Q842d16*		136	140	82	88	55
	Q259d2.17	59	66	57	110	57	
	Q259d2.26	63	59	74	117	127	59
	Q259.w6*	66	173	72	68	63	64
	Q461c2						
	Q461d1*	283	469	580	334	484	375
	Q461e2						
	Q168a2				71		
	Q168b23*	164	372	441	379	370	218
Q23ENV.17	72	152	122	77	82		

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Increasing
Neutralization
sensitivity ↓

Subtype	Viral pseudotype	Kenya 98-00	Subtype-specific
A/D	QA790.A4	60	
	QA790.C1	150	200
	QA790.C8		
	QA790.E2	75	
C	QC406.F3	88	130
	QB099.C9	97	80
	QB099.A10	77	
	QB099.B1	78	
D	QA465.A1		
	QA465.D1		80
	QA013.H1		
	QA013.M12		
	QC100.D83	50	70
	QD435.A4	68	110
	QD435.E1		55

Sensitivity of early subtype A, C, D, and A/D recombinant pseudoviruses to plasma pools

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	QB099.A10	77	
	QB099.B1	78	
D	QA465.A1		
	QA465.D1		80
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	QA013.M12		
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Q461e2							
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	QB099.C9	97	80
	QB099.A10	77	
	QB099.B1	78	
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	QA465.D1		80
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Increasing
Neutralization
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	QA013.M12		
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There is a broad range of neutralization sensitivities among these early variants to plasma samples

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	QB099.C9	97	80
	QB099.A10	77	
	QB099.B1	78	
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	QA465.D1		80
	QA013.H1		
	QA013.M12		
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	Q842d14	56	194	129	129	78	
	Q842d16*		136	140	82	88	55
	Q259d2.17	59	66	57	110	57	
	Q259d2.26	63	59	74	117	127	59
	Q259.w6*	66	173	72	68	63	64
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	QB099.C9	97	80
	QB099.A10	77	
	QB099.B1	78	
D	QA465.A1		
	QA465.D1		80
	QA013.H1		
	QA013.M12		
	QC100.D83	50	70
	QD435.A4	68	110
	QD435.E1		55

There is a broad range of neutralization sensitivities among these early variants to plasma samples

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	Q769h5						
	Q842d12		116	75	90	52	
	Q842d14	56	194	129	129	78	
	Q842d16*		136	140	82	88	55
	Q259d2.17	59	66	57	110	57	
	Q259d2.26	63	59	74	117	127	59
	Q259.w6*	66	173	72	68	63	64
	Q461c2						
	Q461d1*	283	469	580	334	484	375
	Q461e2						
	Q168a2				71		
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Q23ENV.17	72	152	122	77	82		

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Increasing
Neutralization
sensitivity



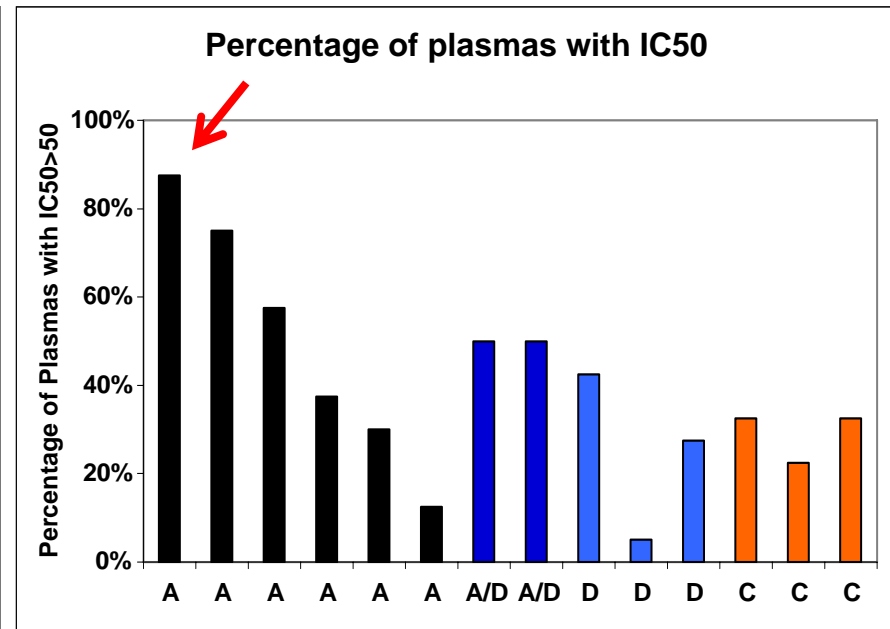
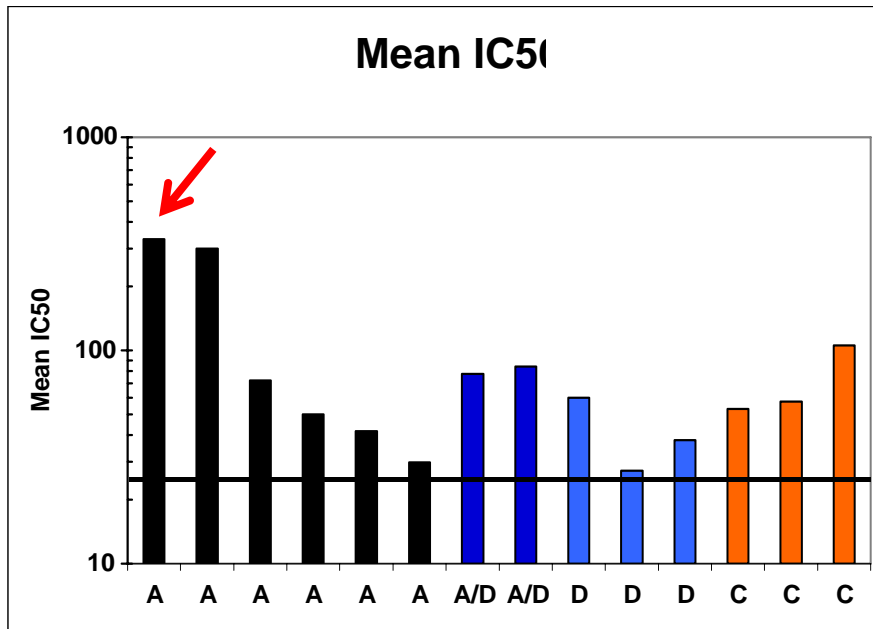
Subtype	Viral pseudotype	Kenya 98-00	Subtype-specific
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	QA790.C1	150	200
	QA790.C8		
	QA790.E2	75	
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	QB099.A10	77	
	QB099.B1	78	
D	QA465.A1		
	QA465.D1		80
	QA013.H1		
	QA013.M12		
	QC100.D83	50	70
	QD435.A4	68	110
	QD435.E1		55

There is a broad range of neutralization sensitivities among these early variants to plasma samples.

Subtype A variants that are susceptible to subtype A plasma are also generally susceptible to plasma samples from subjects infected with other subtypes

Range of neutralization sensitivities of a panel of Kenyan HIV-1 envelope variants

- Developed a panel of Kenyan variants, which can be used to supplement existing virus panels.
- Tested ability of 40 Kenyan plasmas from 1-3 years post-infection to neutralize these viruses.



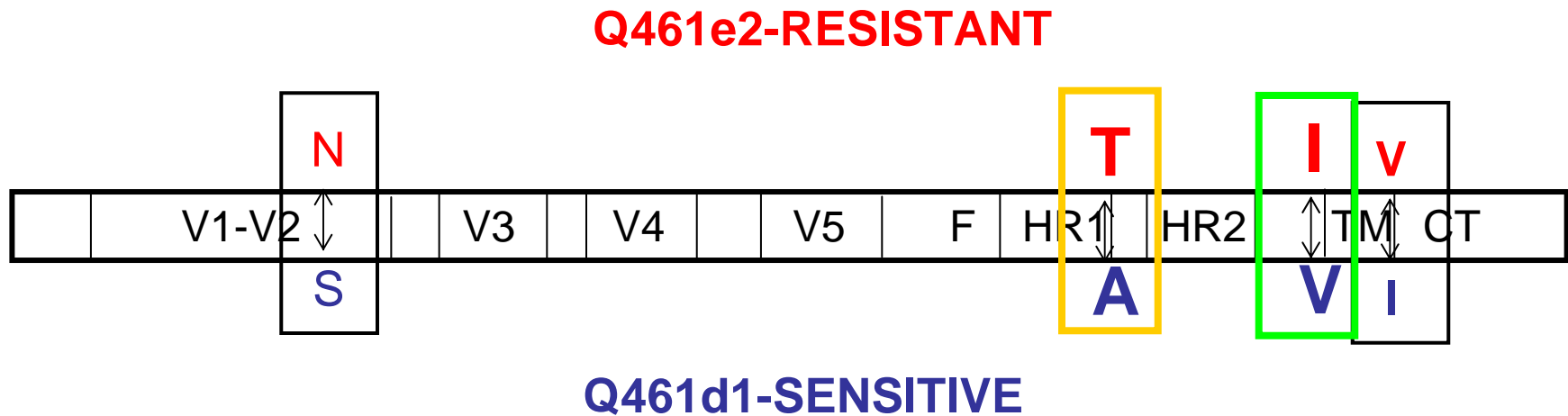
This Kenyan panel represents a range of neutralization sensitivities, and can be used to screen plasma samples for their ability to neutralize circulating variants.

The subtype A variant Q461d1 was remarkably sensitive to neutralization

Viral pseudotype	Plasma						Monoclonal Ab		
	Kenya 1991	Kenya 98-00	Subtype A	Subtype B	Subtype C	Subtype D	4E10	2F5	b12
Q769b9*		52	63	111	70	78	20		
Q769d22				62			18	15	
Q769h5									
Q842d12		116	75	90	52			22.5	
Q842d14	56	194	129	129	78		3.7	0.99	
Q842d16*		136	140	82	88	55		14.6	
Q259d2.17	59	66	57	110	57				
Q259d2.26	63	59	74	117	127	59			
Q259.w6*	66	173	72	68	63	64			
Q461c2							2.8	2.2	
Q461d1*	283	469	580	334	484	375	0.18	0.11	1.0
Q461e2									
Q168a2				71			20.7	9.1	
Q168b23*	164	372	441	379	370	218	0.41	0.01	
Q23ENV.17	72	152	122	77	82			23.3	

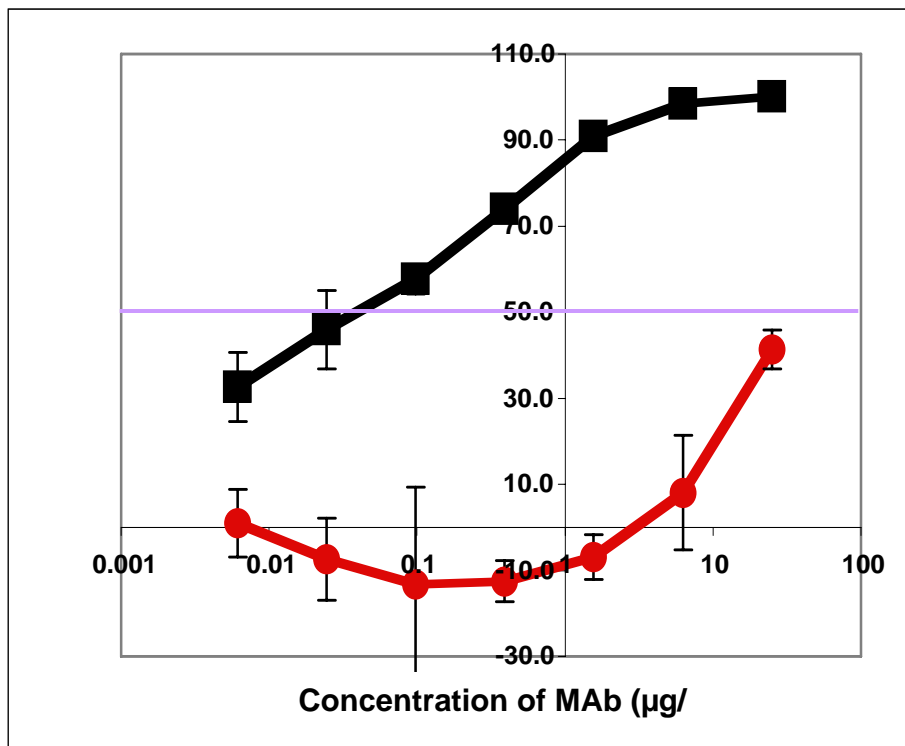
Increasing Neutralization sensitivity ↓

Sequence differences in HIV-1 envelope variants

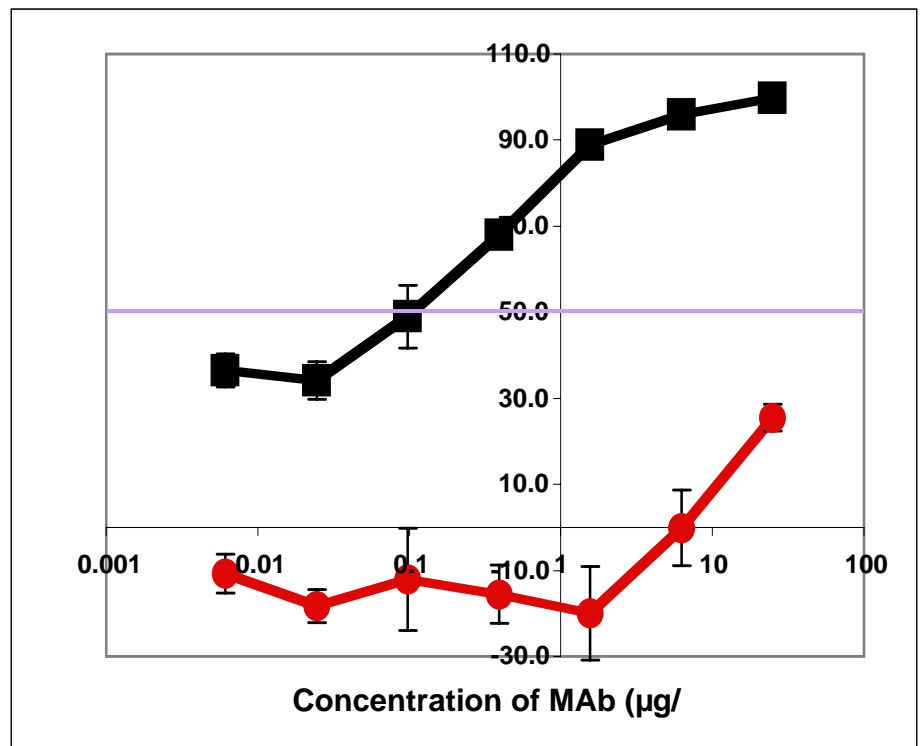


Two substitutions in gp41 alter neutralization by 2F5 and 4E10

2F5



4E10

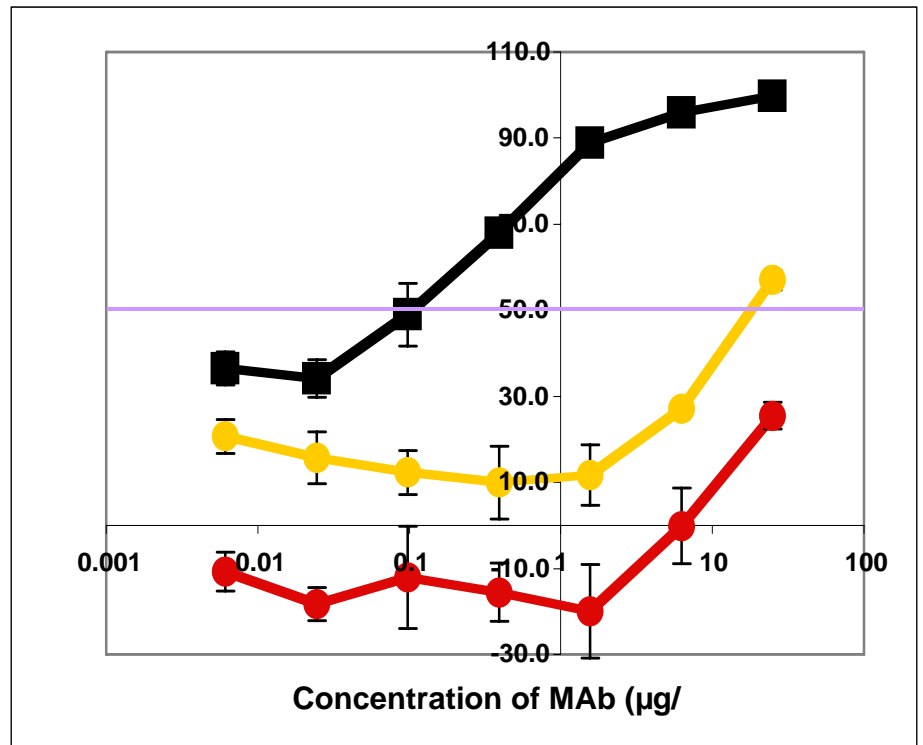
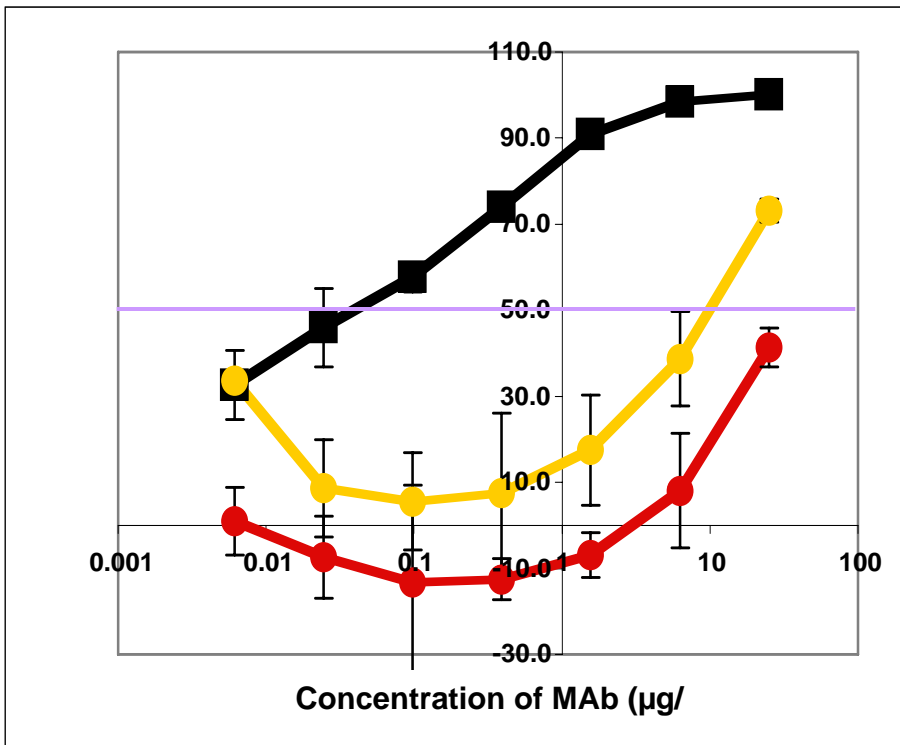


■ Q461d1-SENSITIVE
● Q461e2-RESISTANT

Two substitutions in gp41 alter neutralization by 2F5 and 4E10

2F5

4E10

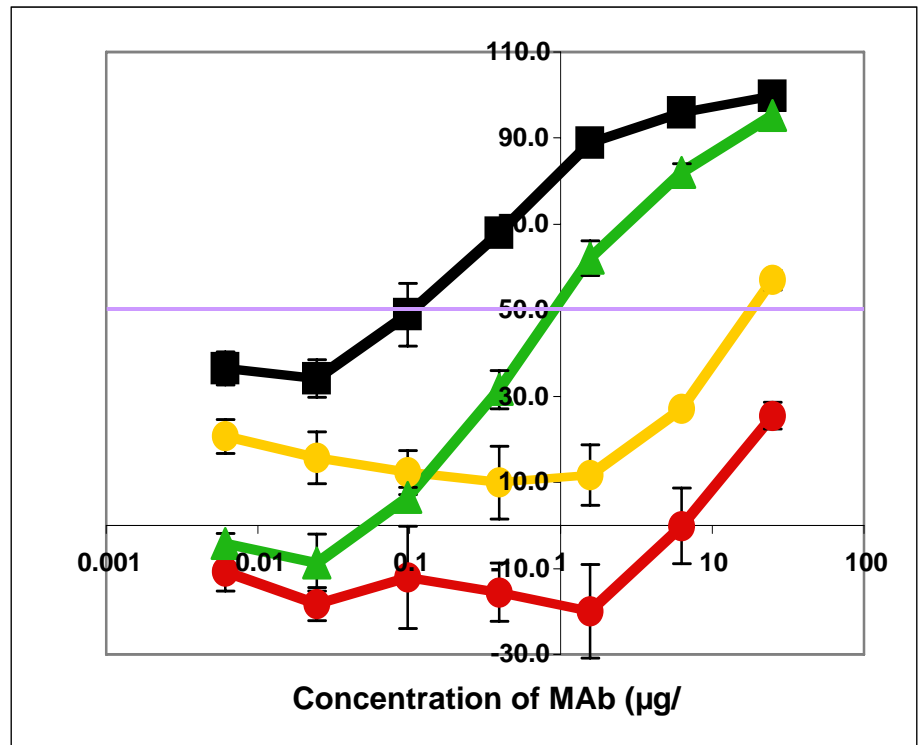
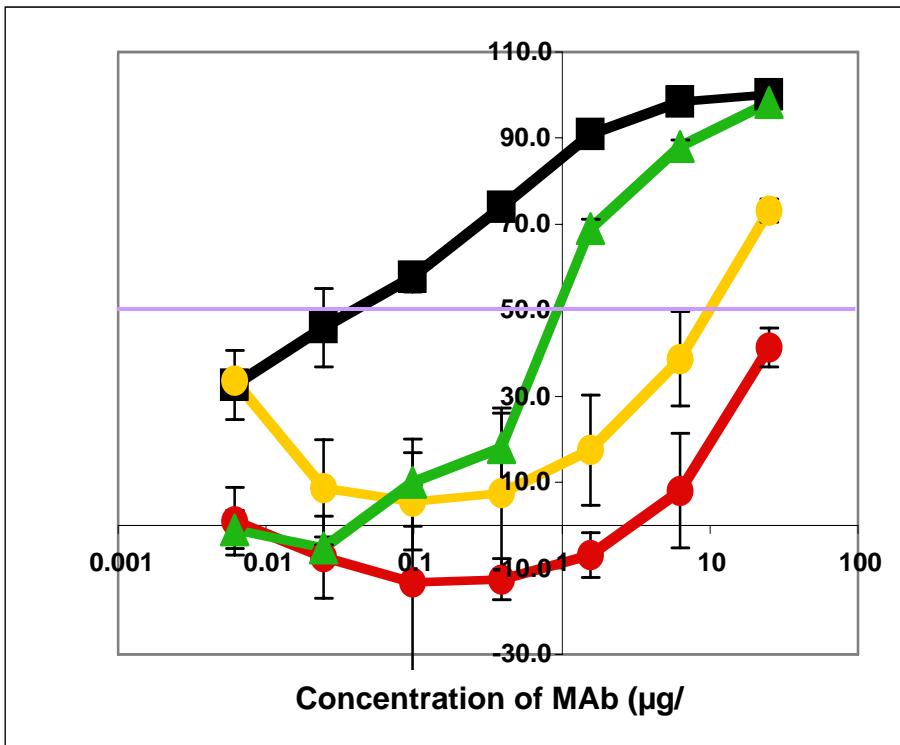


■ Q461d1-SENSITIVE
● Q461e2-RESISTANT
● Q461e2.TA

Two substitutions in gp41 alter neutralization by 2F5 and 4E10

2F5

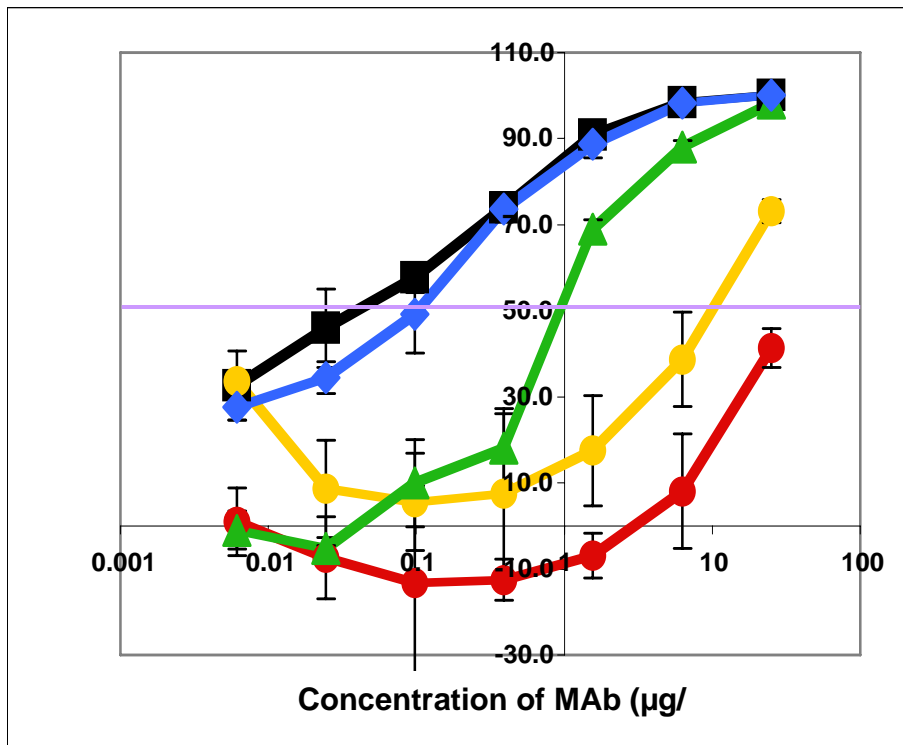
4E10



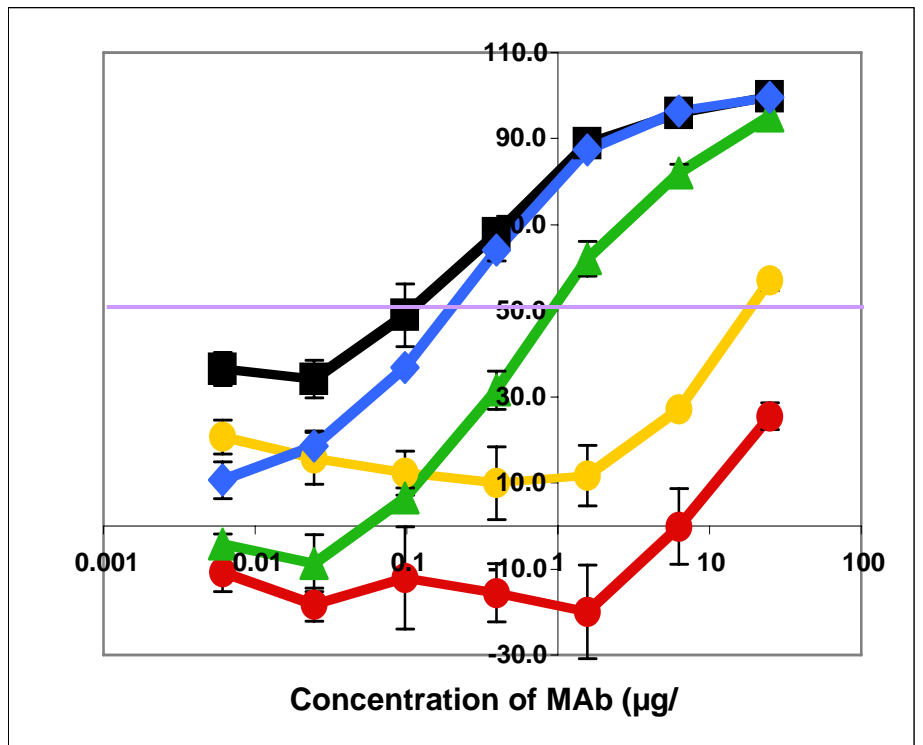
- Q461d1-RESISTANT
- Q461e2-SENSITIVE
- Q461e2.TA
- ▲ Q461e2.IV

Two substitutions in gp41 alter neutralization by 2F5 and 4E10

2F5



4E10

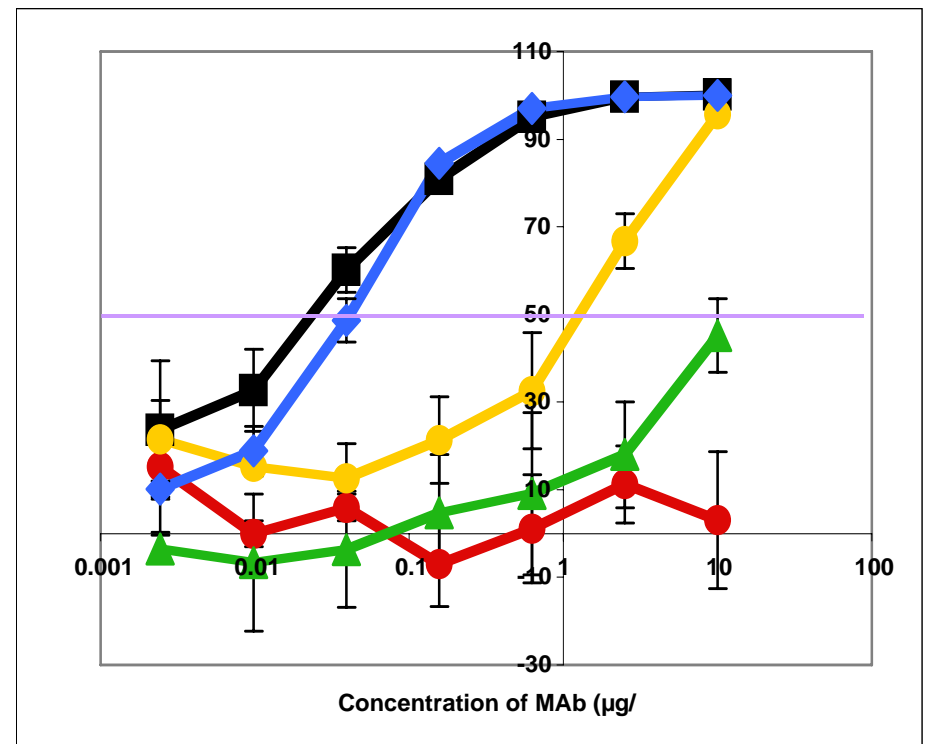
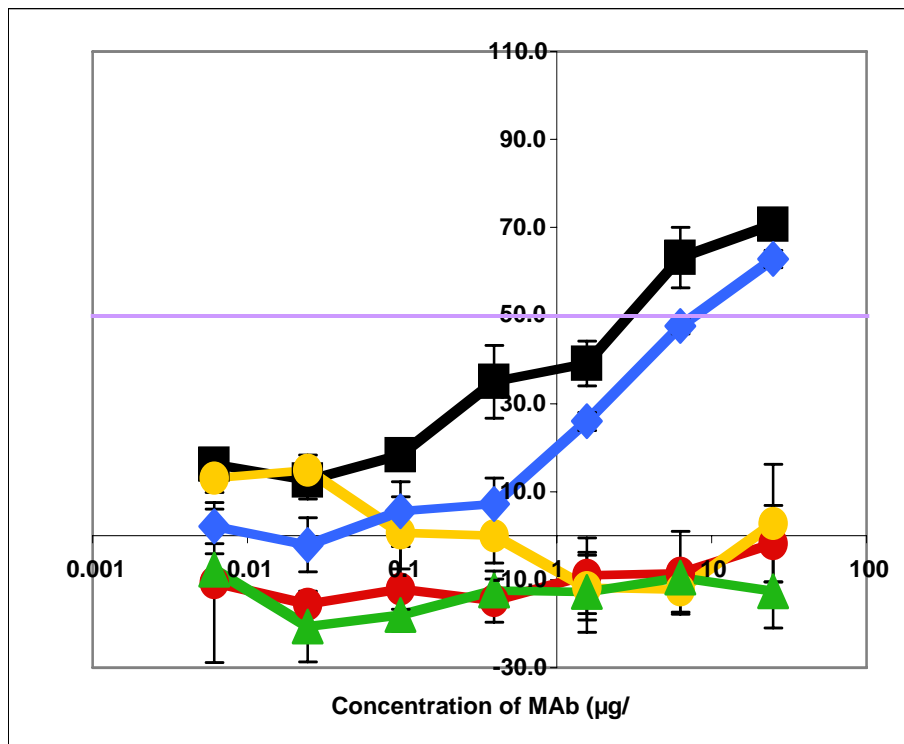


- Q461d1-SENSITIVE
- Q461e2-RESISTANT
- Q461e2.TA
- ▲ Q461e2.IV
- ◆ Q461e2.TAIV

Two substitutions in gp41 alter neutralization by b12 and sCD4

b12

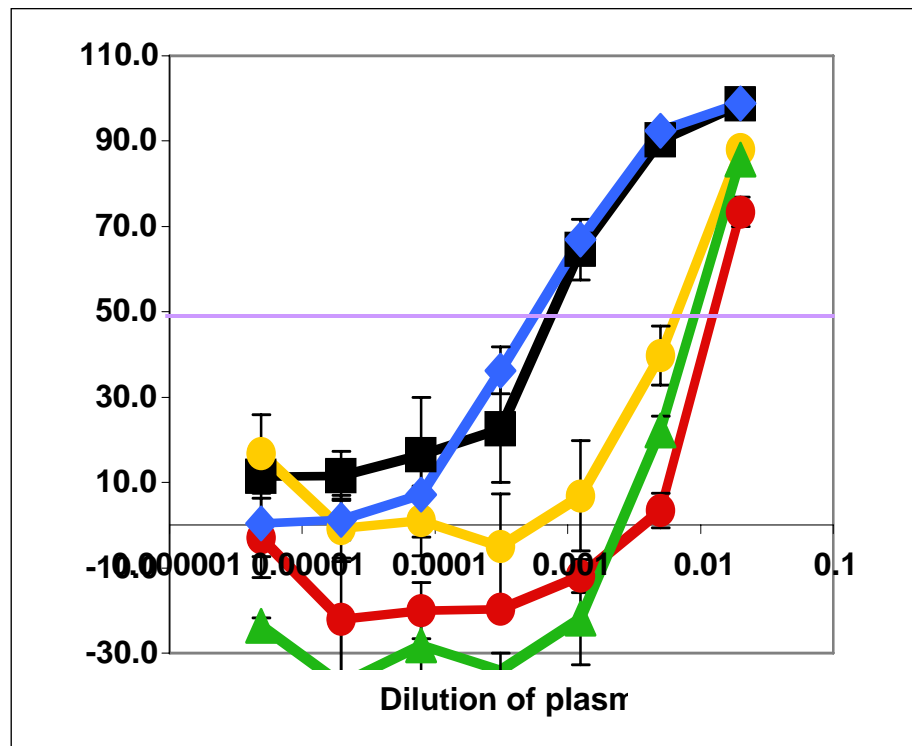
sCD4



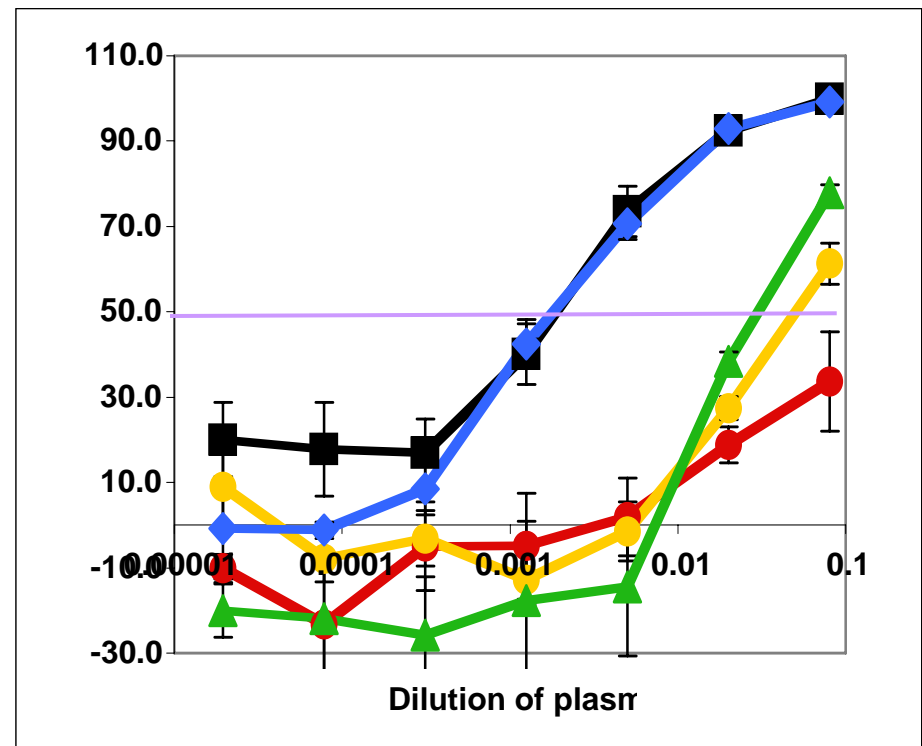
- Q461d1-SENSITIVE
- Q461e2-RESISTANT
- Q461e2.TA
- ▲ Q461e2.IV
- ◆ Q461e2.TAIV

Substitutions in gp41 alter neutralization by autologous and heterologous plasmas

Autologous plasma

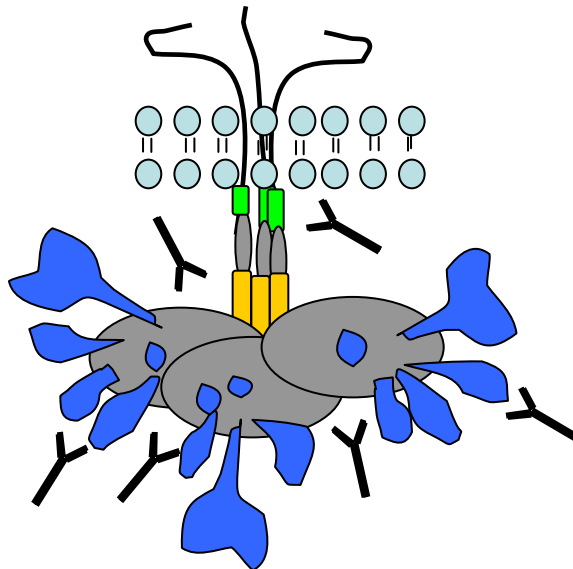


Plasma pool



- Q461d1-SENSITIVE
- Q461e2-RESISTANT
- Q461e2.TA
- ▲ Q461e2.IV
- ◆ Q461e2.TAIV

- The enhanced neutralization sensitivity of these variants does not appear to result from changes in:
 - Envelope infectivity
 - Envelope concentration of the virions
 - Envelope shedding
 - Apparent differences in fusion kinetics
- Thus, these gp41 mutations appear to alter the conformation of the HIV-1 Envelope, allowing neutralizing antibodies access to conserved regions that are normally shielded

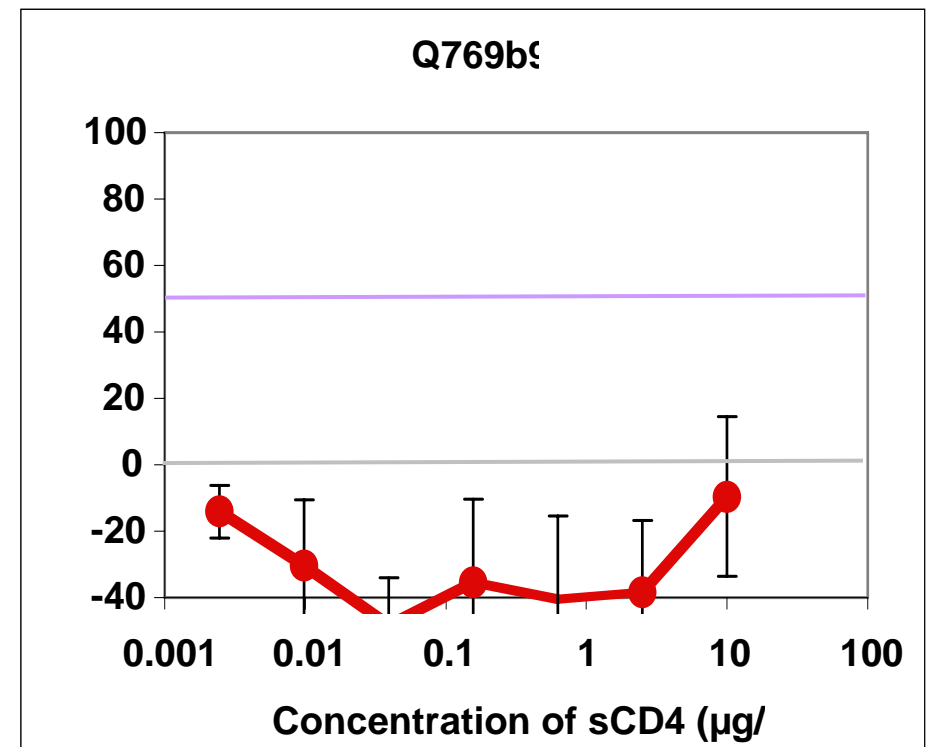
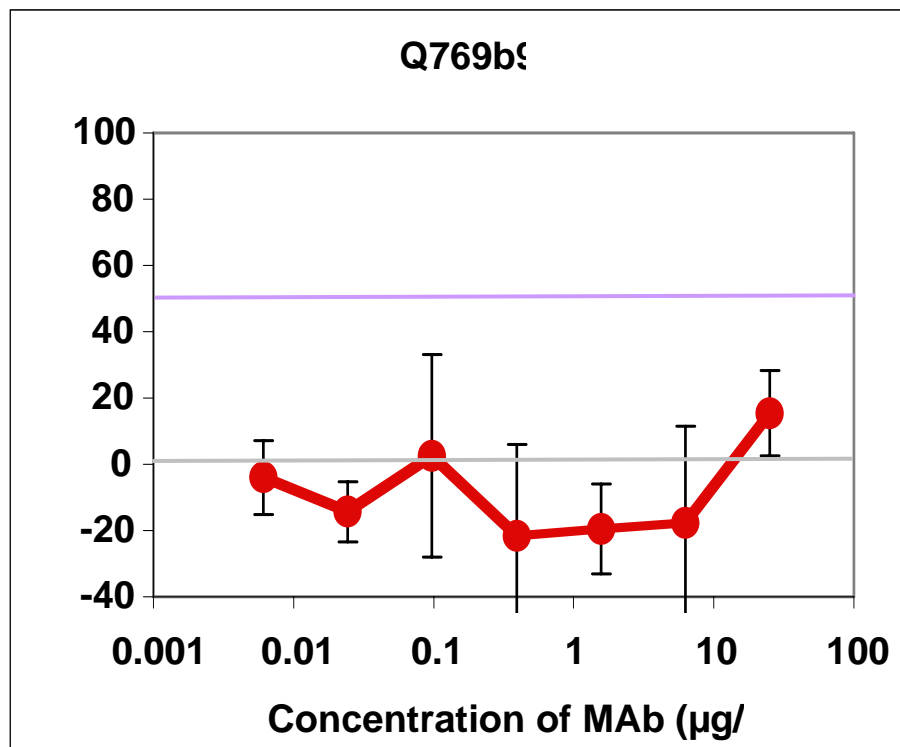


**Will these mutations also expose
neutralization epitopes on other HIV-1
envelope variants?**

gp41 HR1 and MPER mutations expose neutralization epitopes on an unrelated HIV-1 subtype A envelope variant

2F5

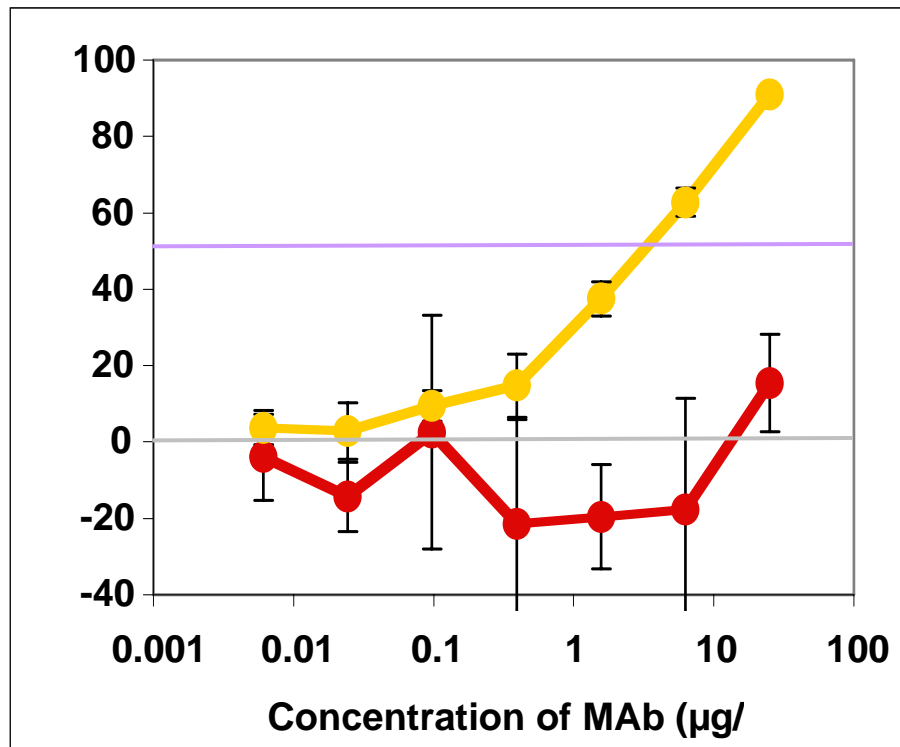
sCD4



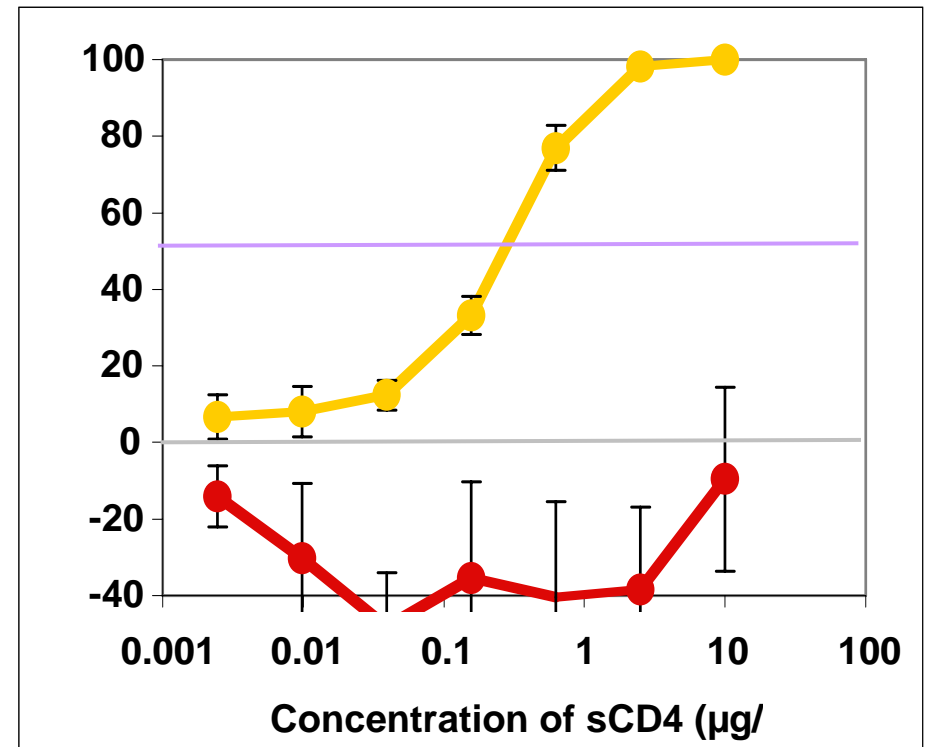
—●— Q769b9

gp41 HR1 and MPER mutations expose neutralization epitopes on an unrelated HIV-1 subtype A envelope variant

2F5



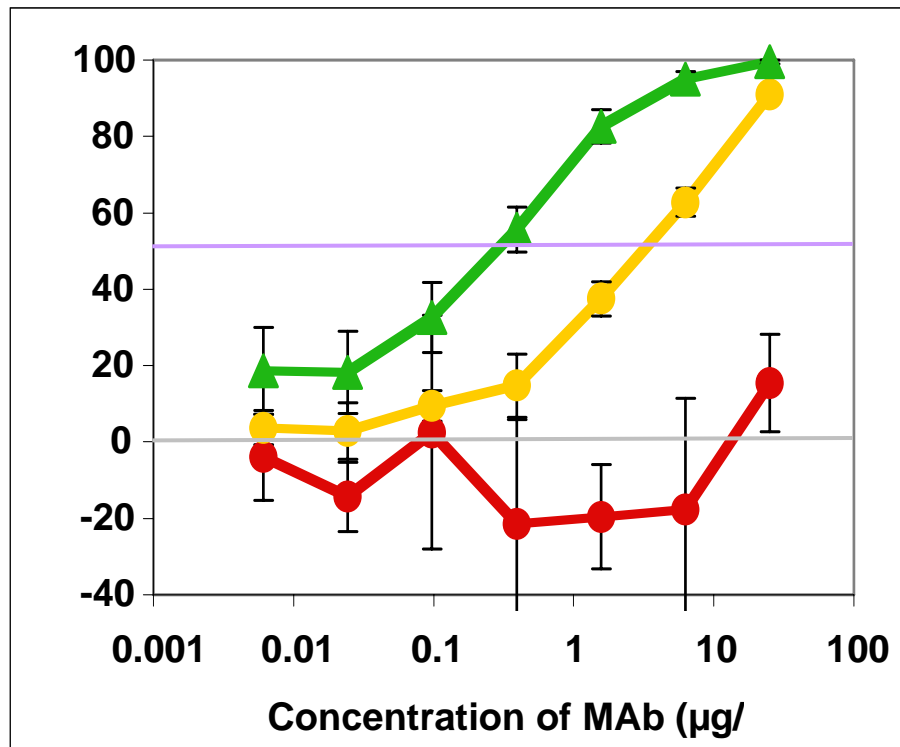
sCD4



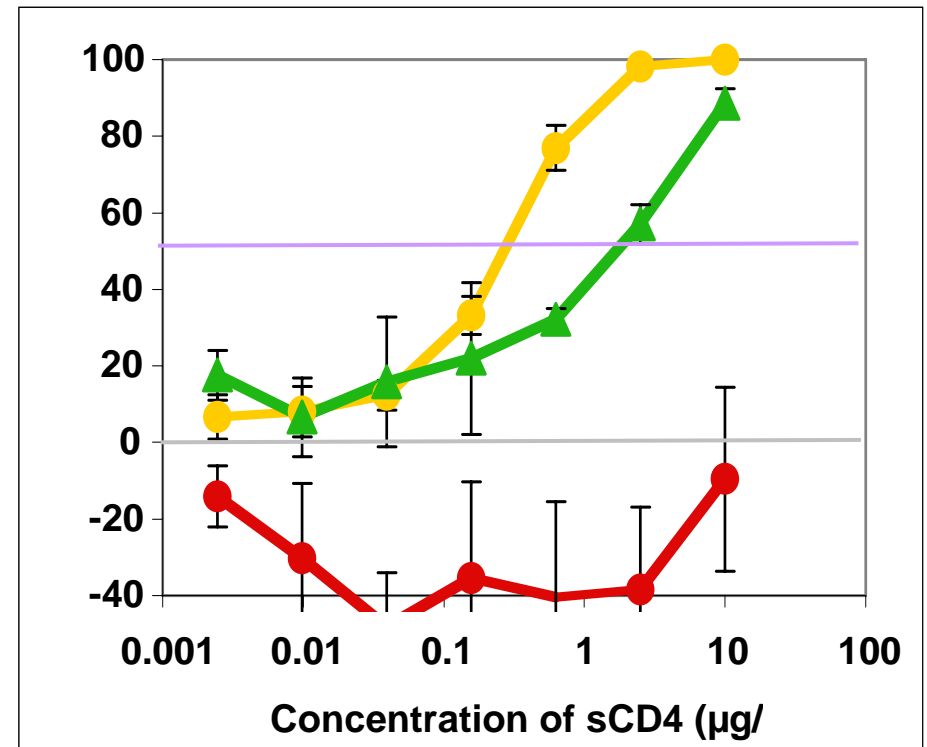
● Q769b9
● Q769b9.TA

gp41 HR1 and MPER mutations expose neutralization epitopes on an unrelated HIV-1 subtype A envelope variant

2F5



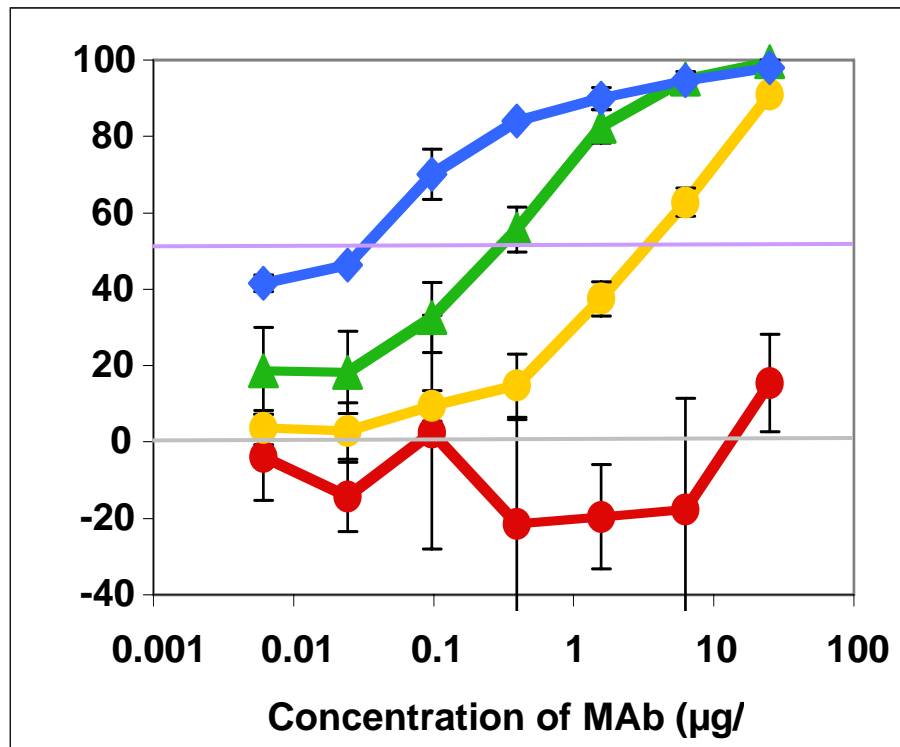
sCD4



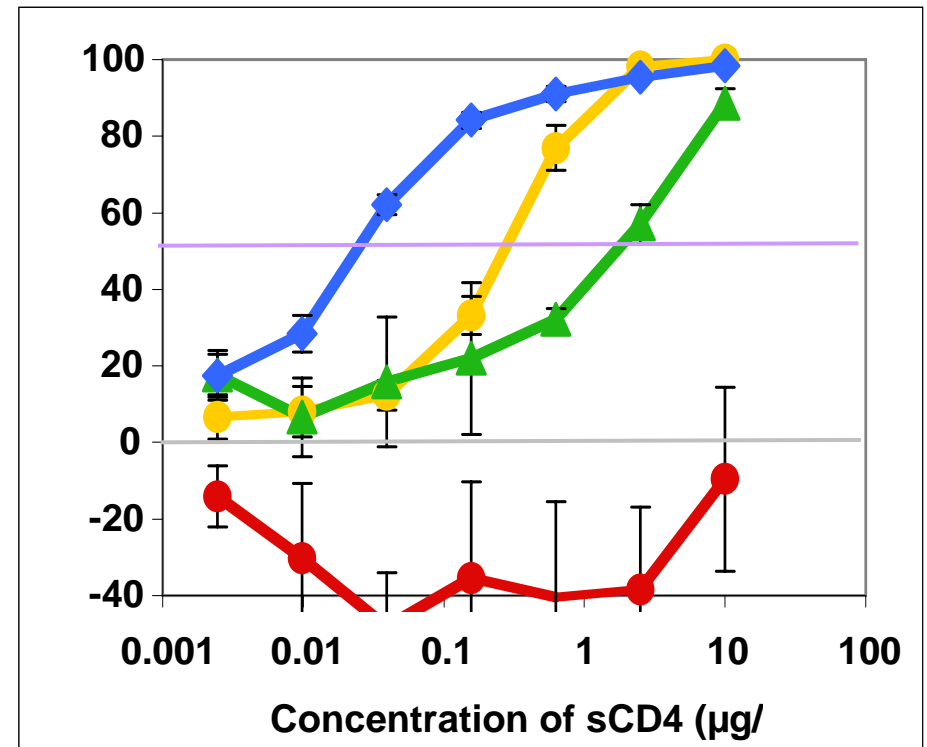
- Q769b9
- Q769b9.TA
- ▲ Q769b9.IV

gp41 HR1 and MPER mutations expose neutralization epitopes on an unrelated HIV-1 subtype A envelope variant

2F5



sCD4

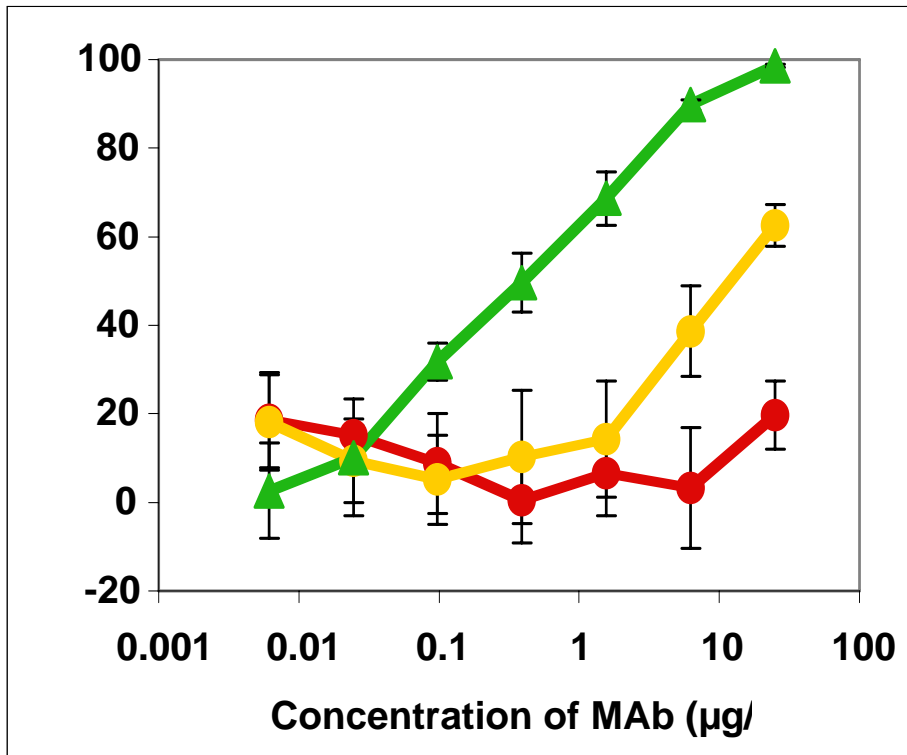


- Q769b9
- Q769b9.TA
- ▲ Q769b9.IV
- ◆ Q769b9.TAIV

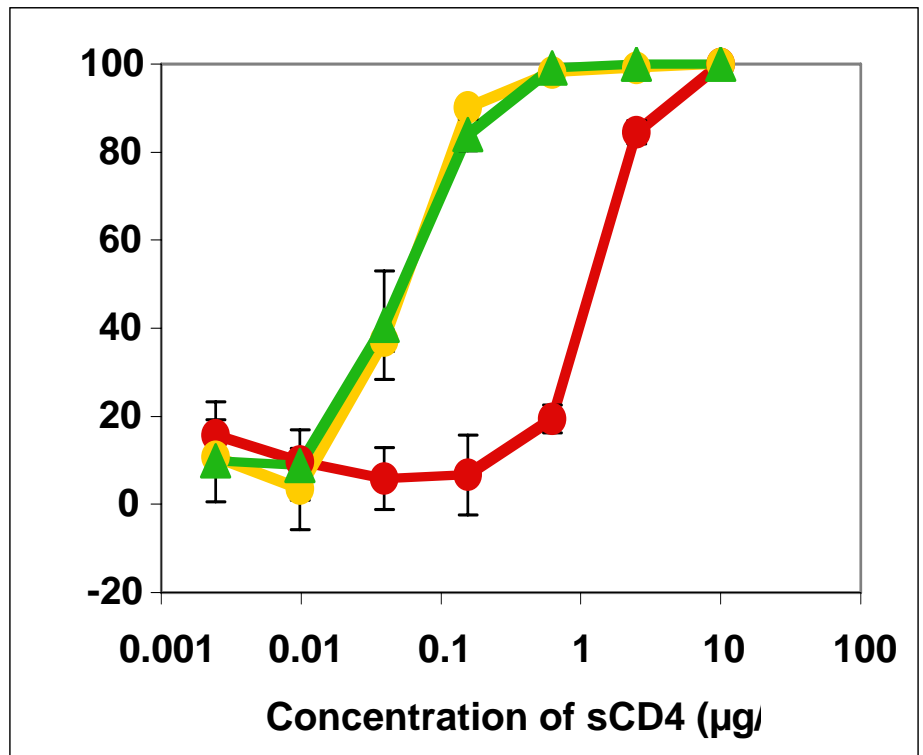
Will these mutations expose neutralization epitopes even on HIV-1 envelope variants of a different subtype?

Substitutions in gp41 HR1 and MPER in subtype B HIV-1 enhance neutralization sensitivity

2F5



sCD4

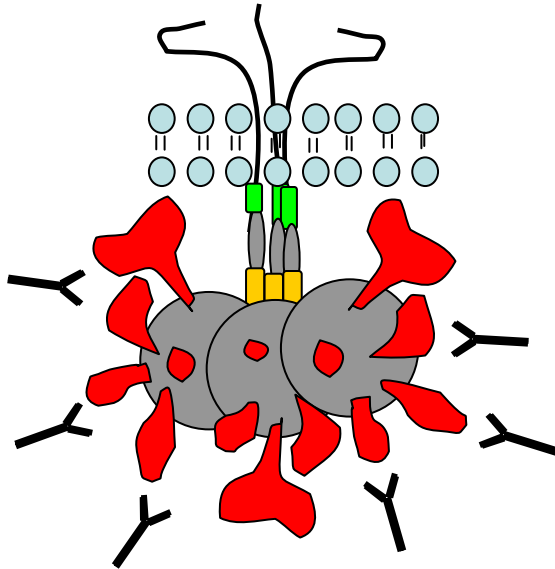


- YU-2
- YU-2.TA
- ▲ YU-2.IV

CONCLUSIONS

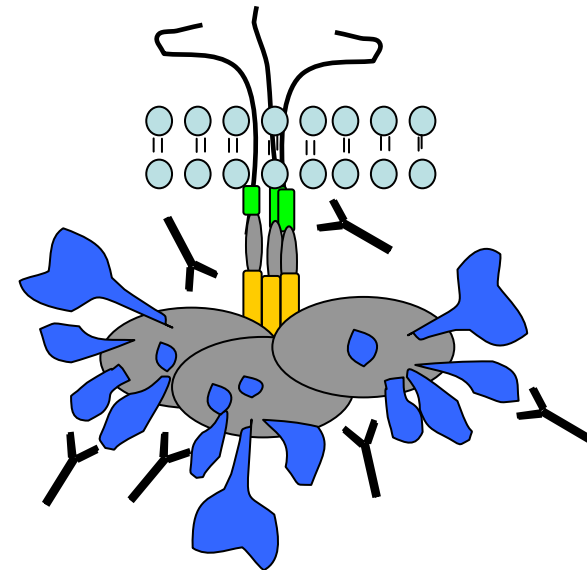
- **Evaluation of early isolates can lead to the generation of virus panels to screen vaccine sera AND also allow identification of features that may eventually help direct vaccine efforts**
- **Two unique mutations in gp41, one in HR1 and one in the MPER, dramatically enhance neutralization of diverse HIV-1 envelope proteins**
 - **This enhancement of neutralization occurs to antibodies that recognize gp120 as well as gp41 and to plasma antibodies**
- **This enhancement of neutralization appears to occur through conformational changes that allow normally shielded epitopes to be exposed to neutralizing antibodies**

Substitutions in gp41 as a model for presentation of conserved epitopes



Resistant viruses:

- “Closed” conformation
- Poor Ab access to conserved regions



Sensitive “mutant” viruses:

- “Open” conformation
- Better Ab access to conserved regions

Structural and immunization studies to test these hypotheses are underway

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Funding:
NIH grant K08 AI068424 to CAB
and AI38518 to JO

