In vivo evolution of *env* in SHIV-AD8-infected rhesus macaque after AAV-eCD4-Ig therapy

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CONFLICTS OF INTEREST

No Conflicts of Interest

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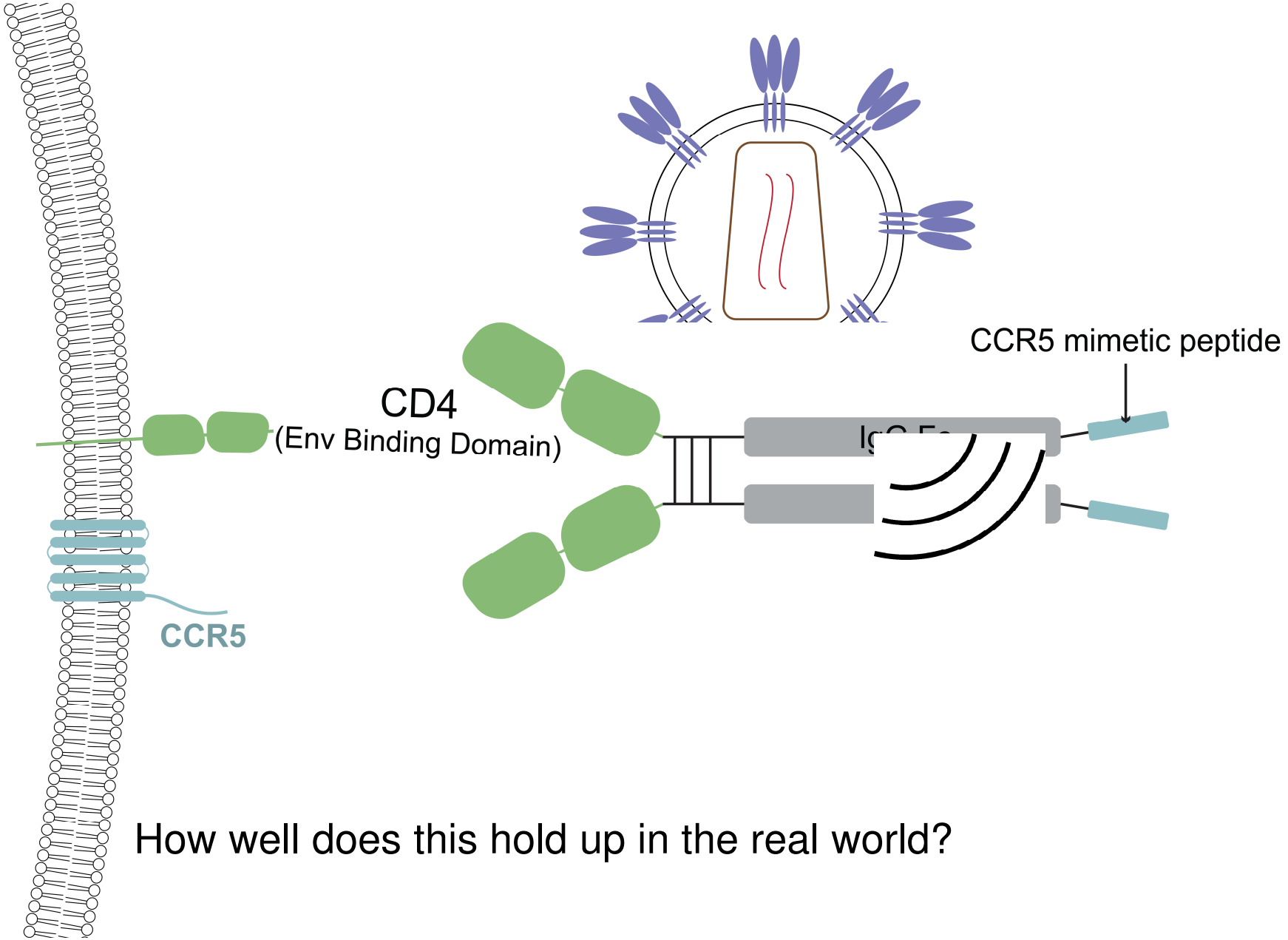
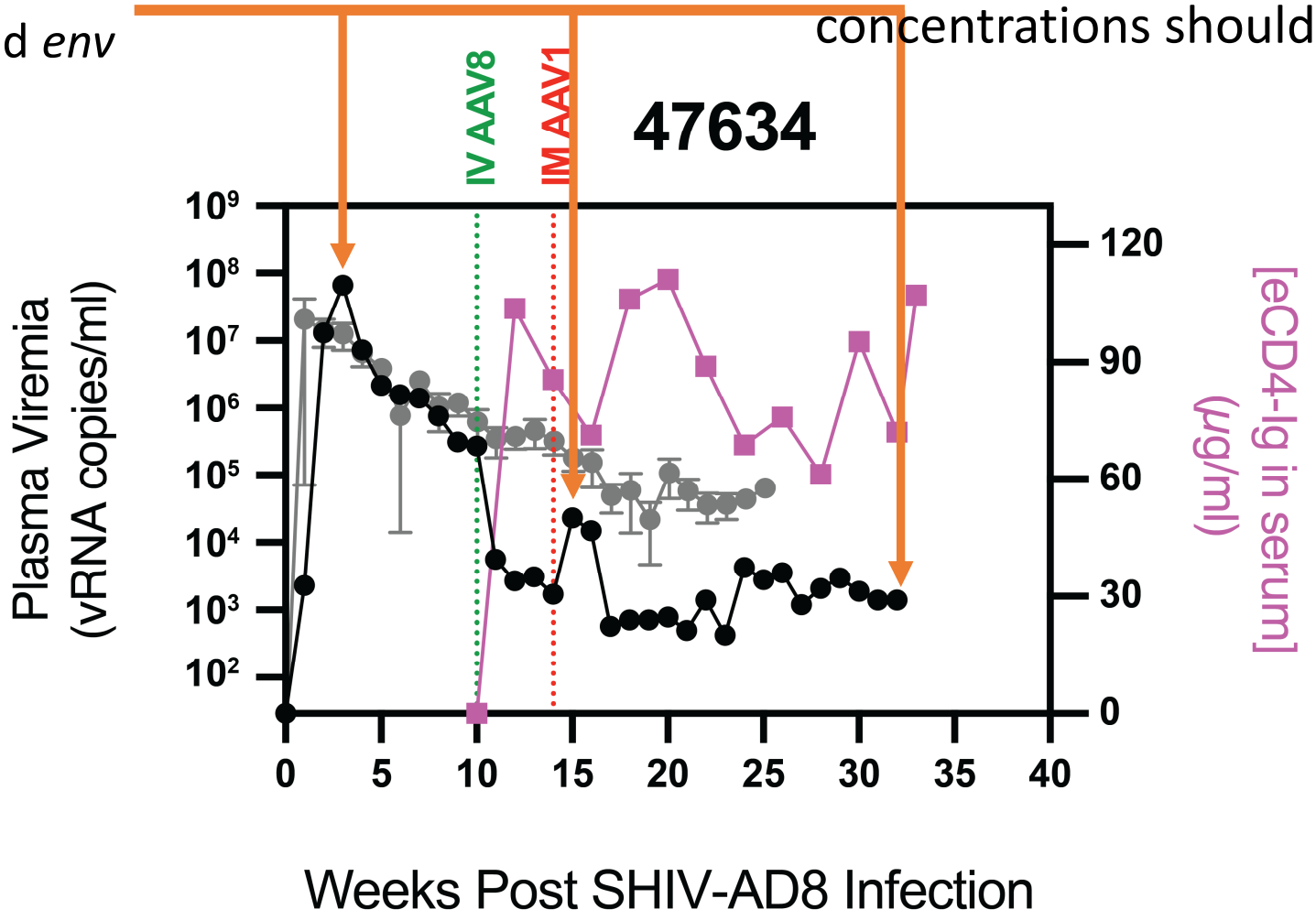


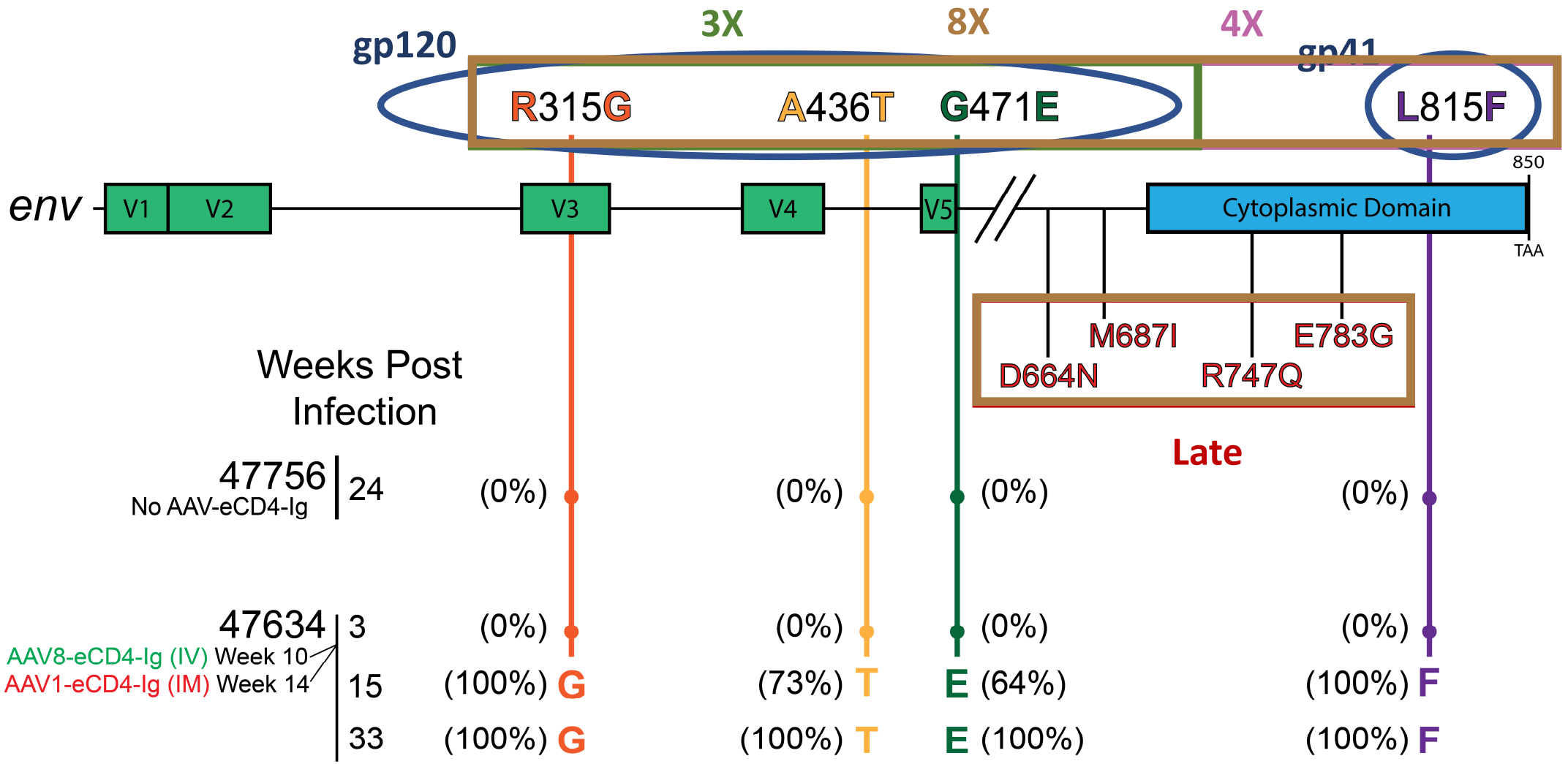
How well does this hold up in the real world?

cloned and sequenced *env*

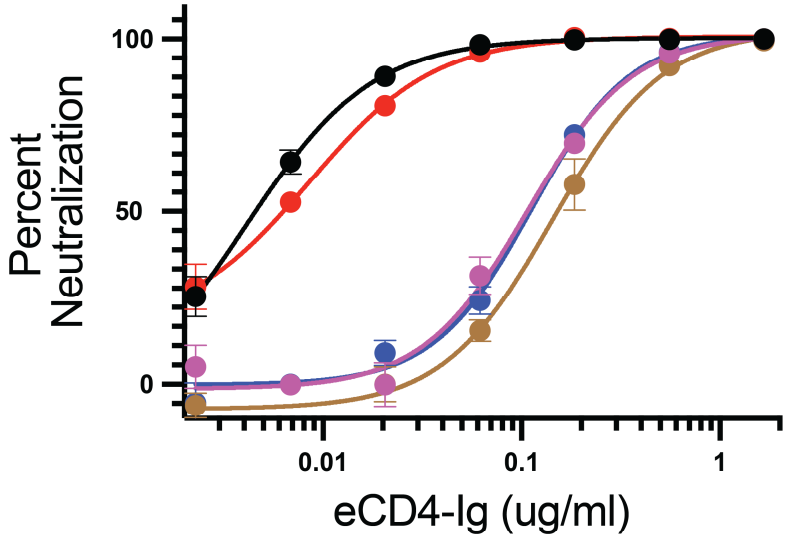
Passive infusion studies tell us these concentrations should suppress!



This virus has probably acquired eCD4-Ig resistance!

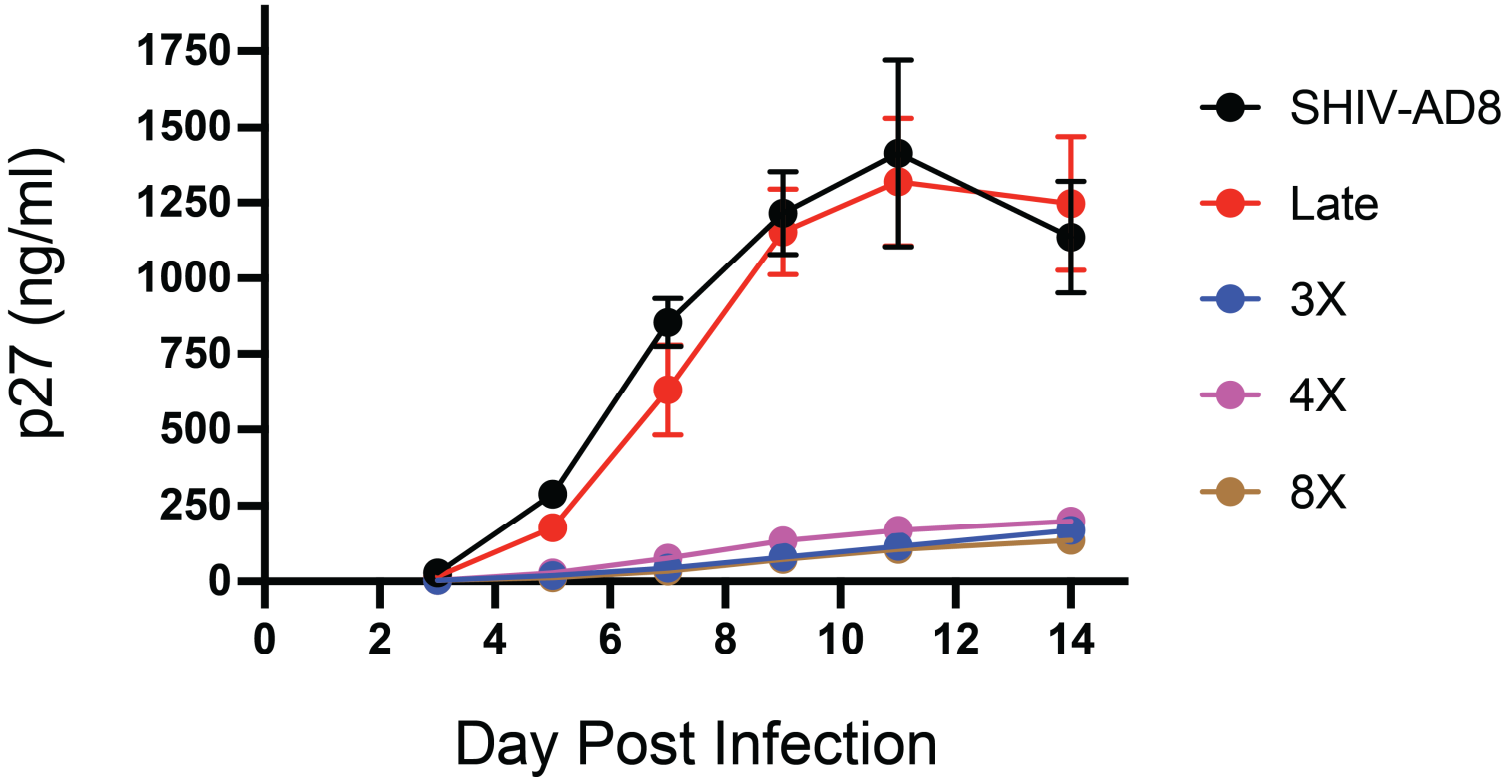

gp120
3X
8X
4X
gp41
R315G
A436T
G471E
L815F
850
env
V1
V2
V3
V4
V5
Cytoplasmic Domain
TAA
M687I
E783G
D664N
R747Q
Late
Weeks Post Infection
47756
No AAV-eCD4-Ig
24
(0%)
(0%)
(0%)
(0%)
47634
AAV8-eCD4-Ig (IV) Week 10
AAV1-eCD4-Ig (IM) Week 14
3
(0%)
(0%)
(0%)
(0%)
15
(100%) G
(73%) T
E (64%)
(100%) F
33
(100%) G
(100%) T
E (100%)
(100%) F

Neutralization in TZM-bl cells

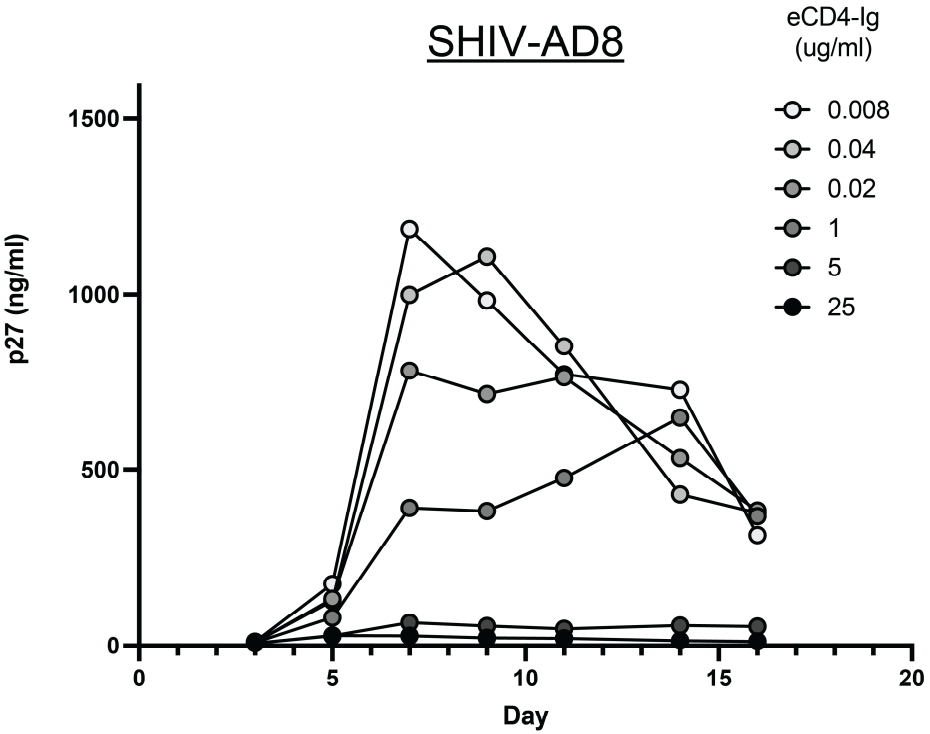


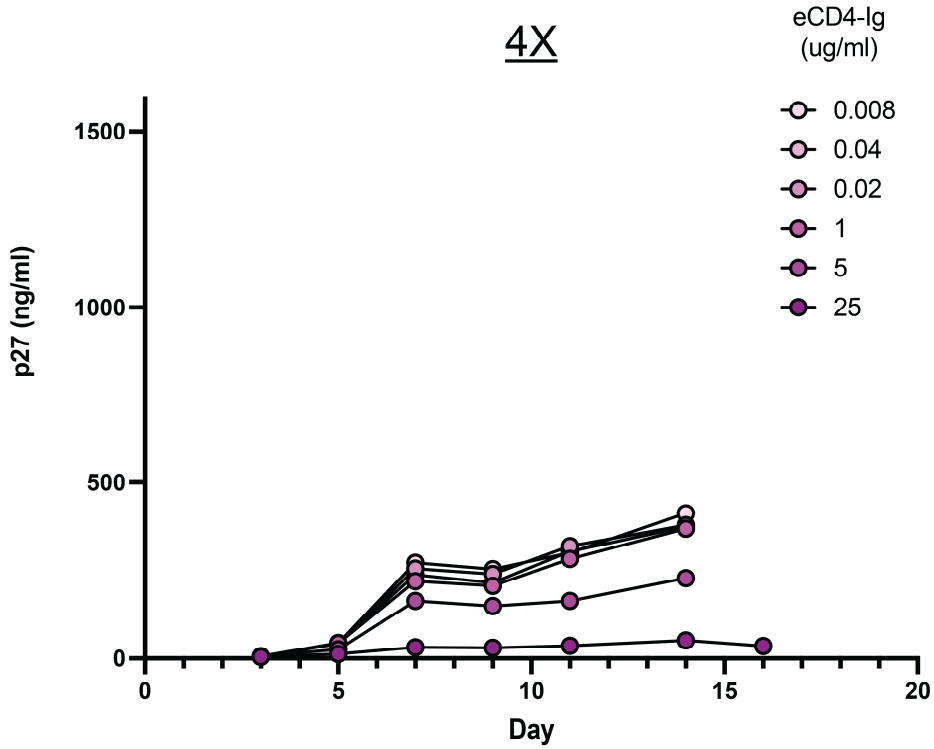
	IC50	Fold Change
SHIV-AD8	0.005	1
Late	0.007	1
3X	0.105	24
4X	0.129	23
8X	0.197	33

Viral replication in rhesus PBMC



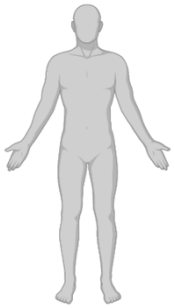
Viral replication in rhesus PBMC w/ eCD4-Ig

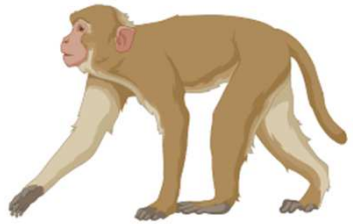




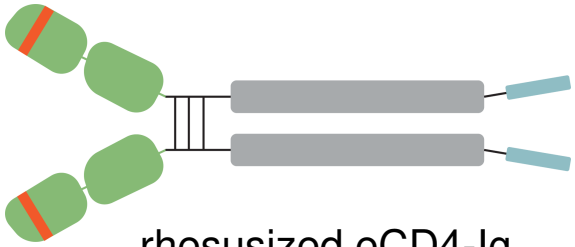
4X is resistant, but has not escaped!

How?





CD4 Residue 39	Asparagine (N)	Isoleucine (I)
Env affinity	↑	↓

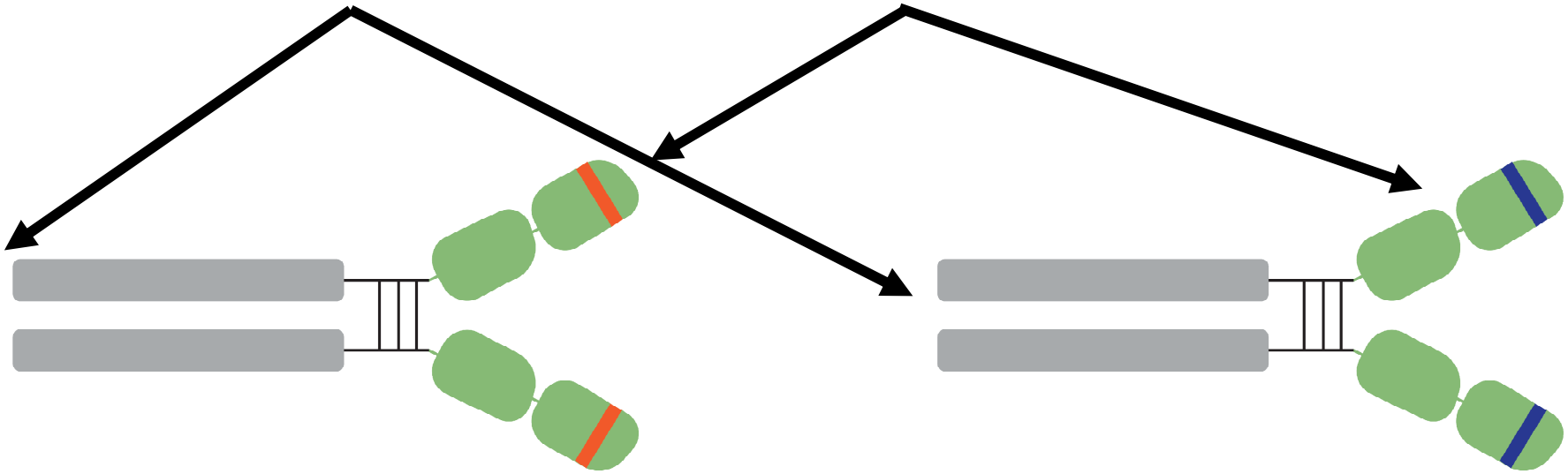


rhesusized eCD4-Ig

rhesus CD4

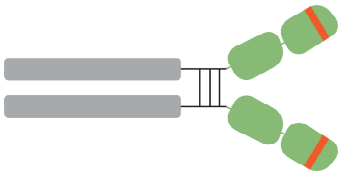
These are is **CD4-Ig**, Not **eCD4-Ig**.
No mimetic peptide!

Soluble proxies for the two types of CD4 domains present in **eCD4-Ig** vs **rhesus cellular CD4**

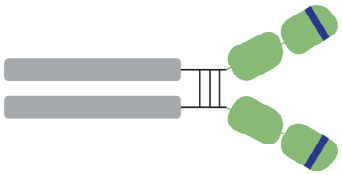


Asparagine
(N)

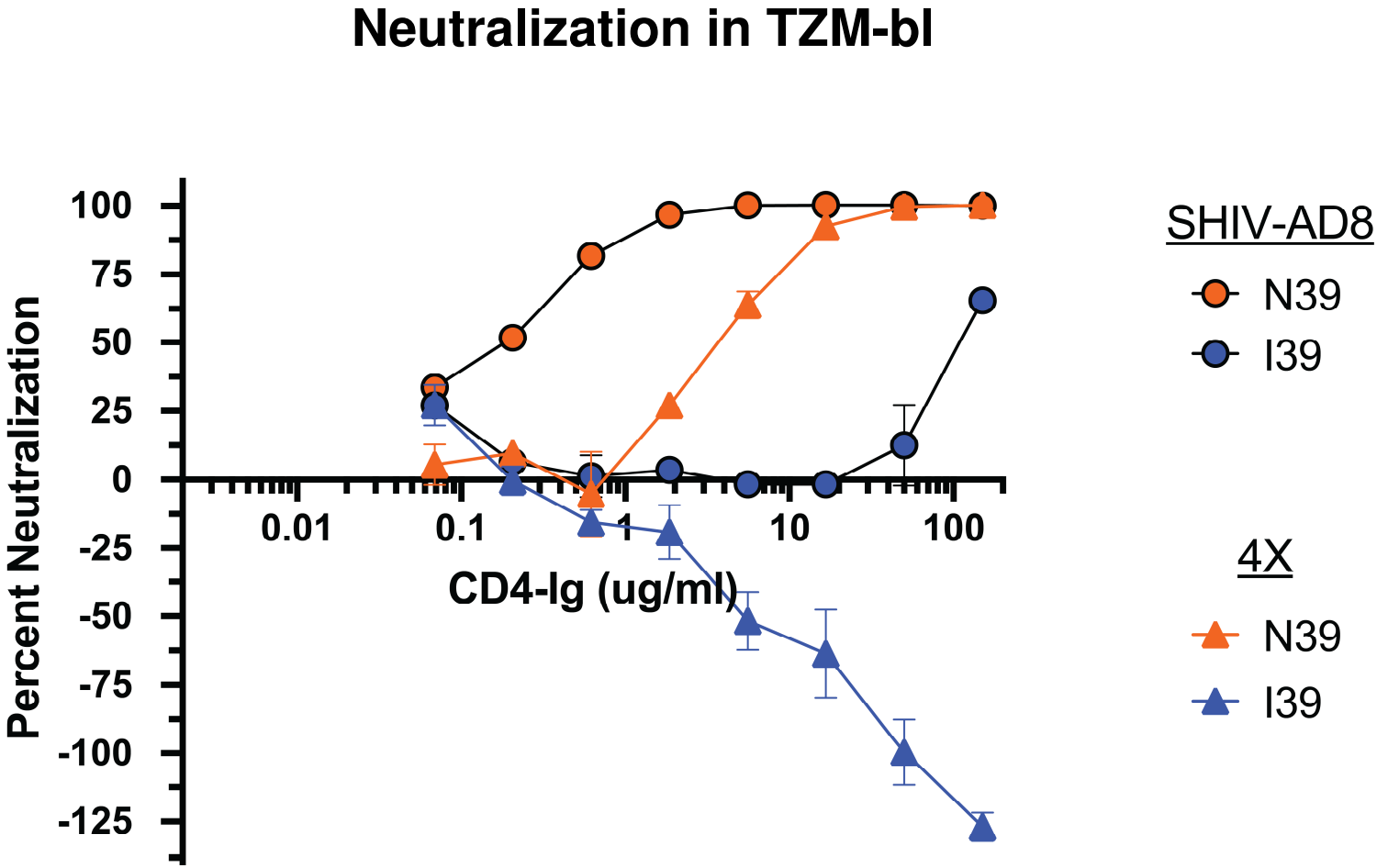
Isoleucine
(I)



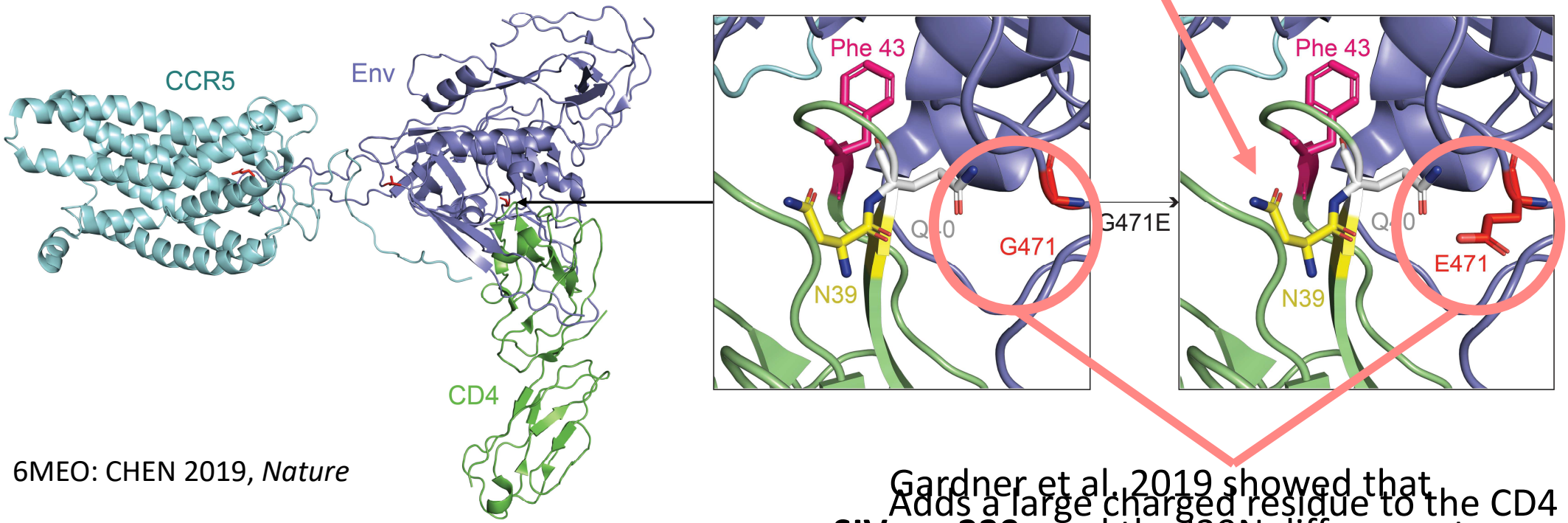
eCD4-Ig
proxy
(N)



Rhesus CD4
proxy
(I)



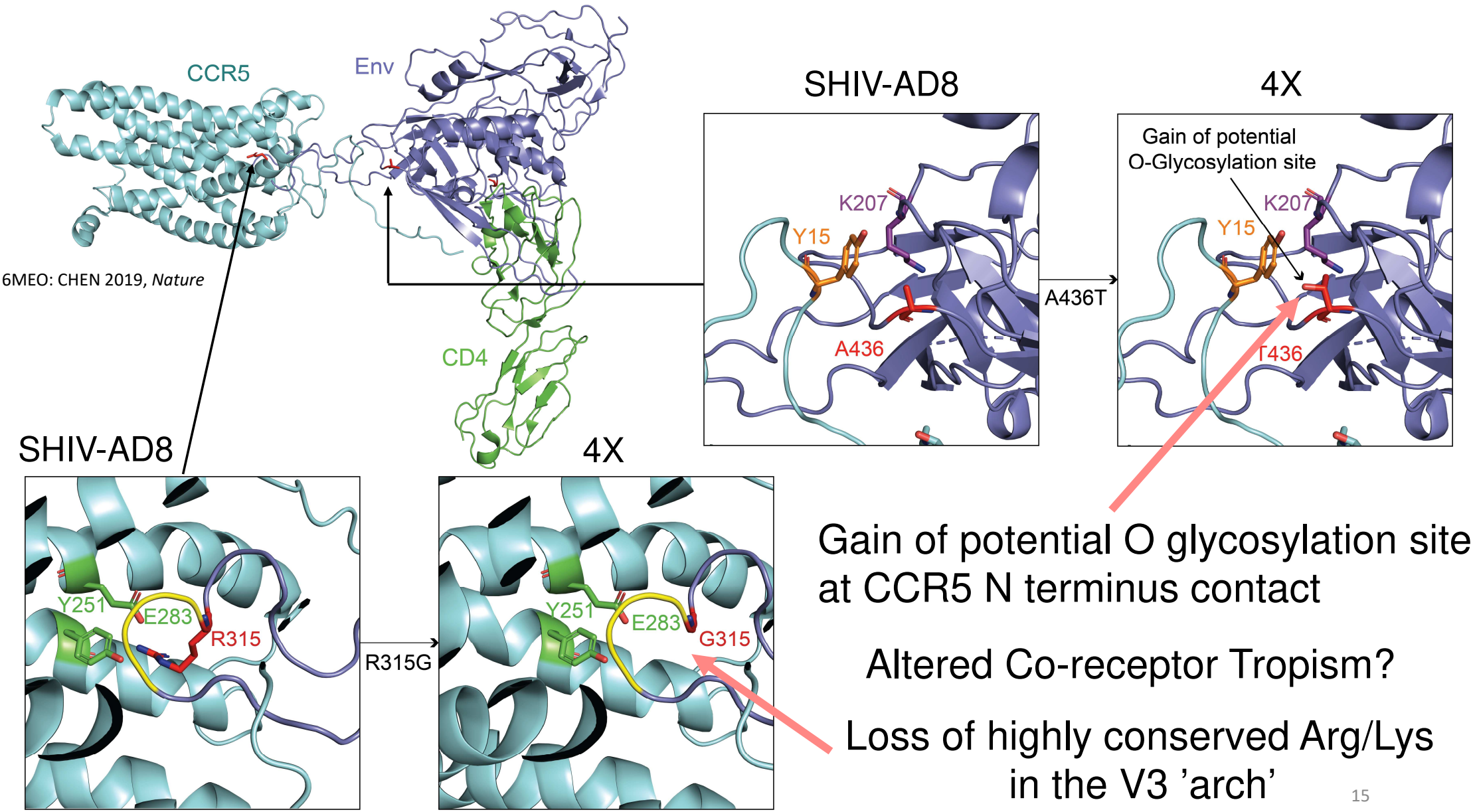
Right next to residue 39!



6MEO: CHEN 2019, *Nature*

Gardner et al. 2019 showed that **SIVmac239** used the I39N difference to develop eCD4-Ig resistance as well!

Adds a large charged residue to the CD4 binding pocket

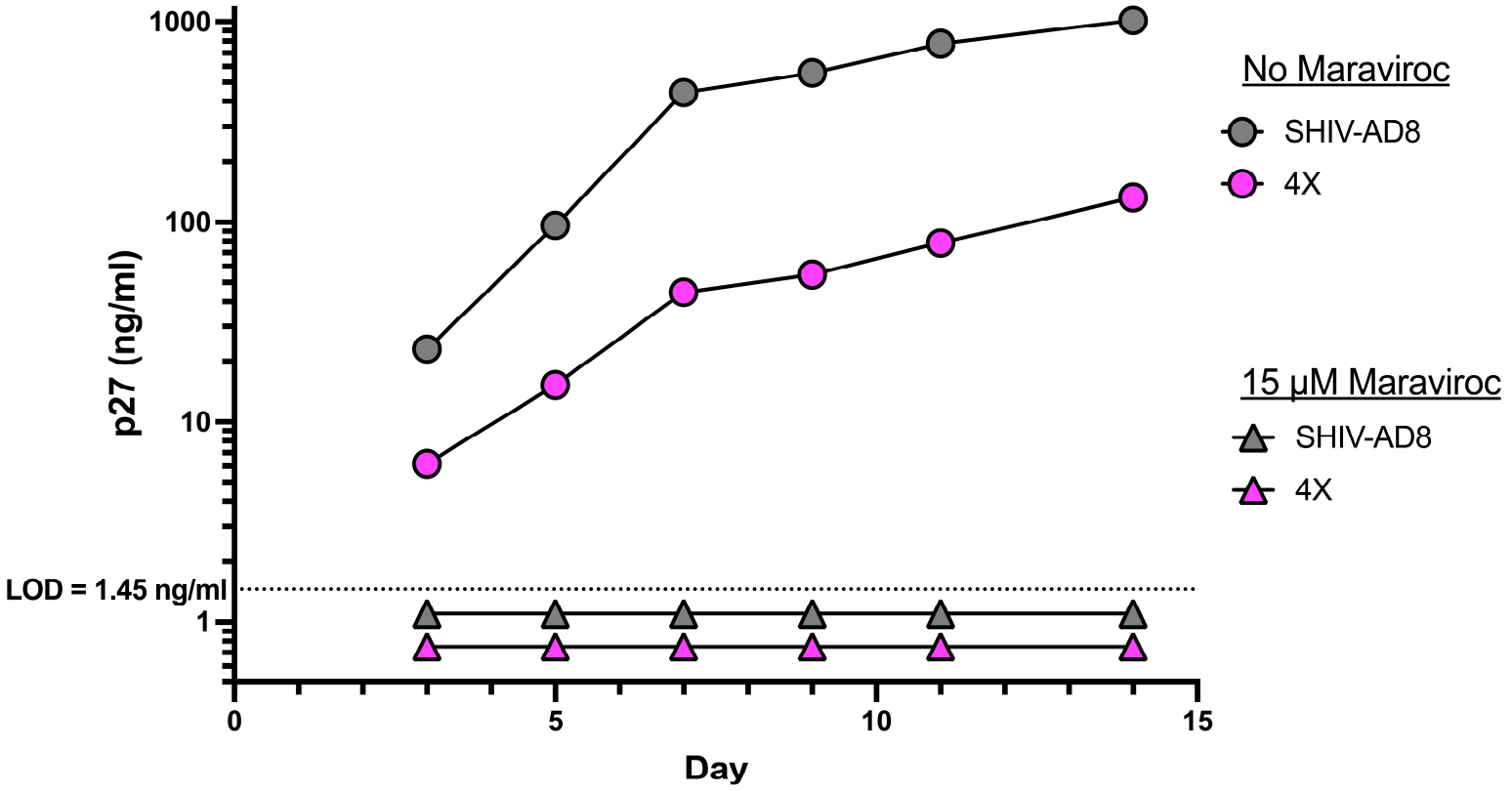


Gain of potential O glycosylation site at CCR5 N terminus contact

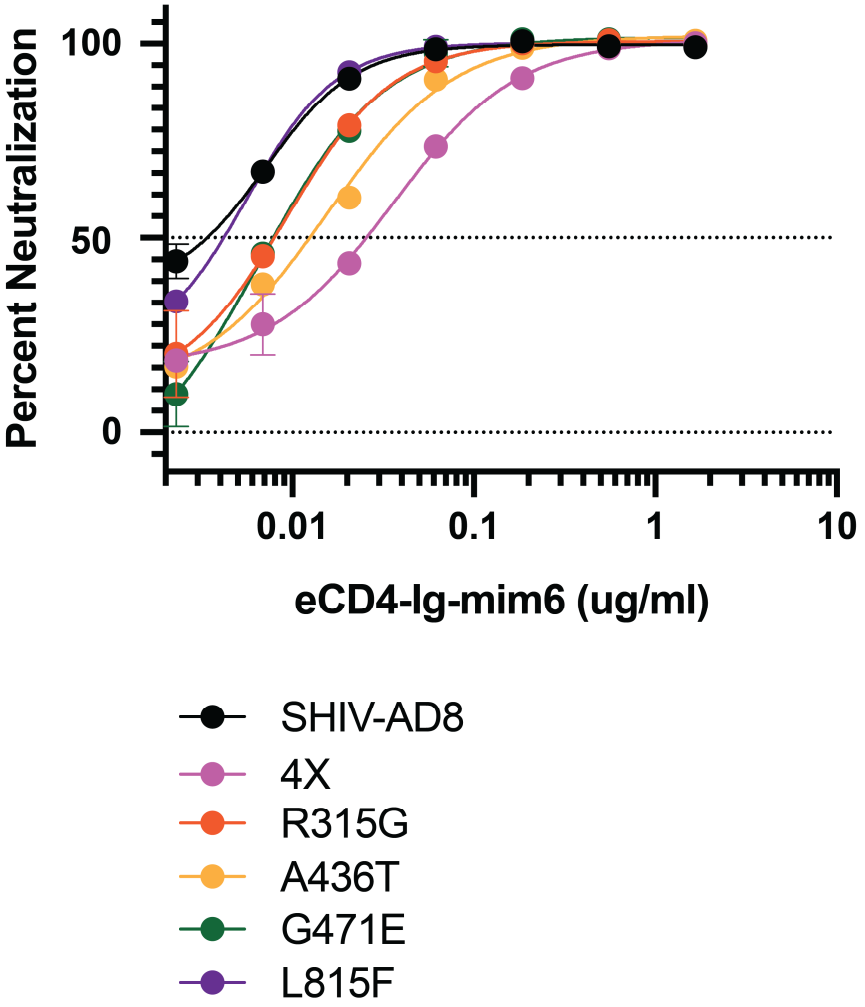
Altered Co-receptor Tropism?

Loss of highly conserved Arg/Lys in the V3 'arch'

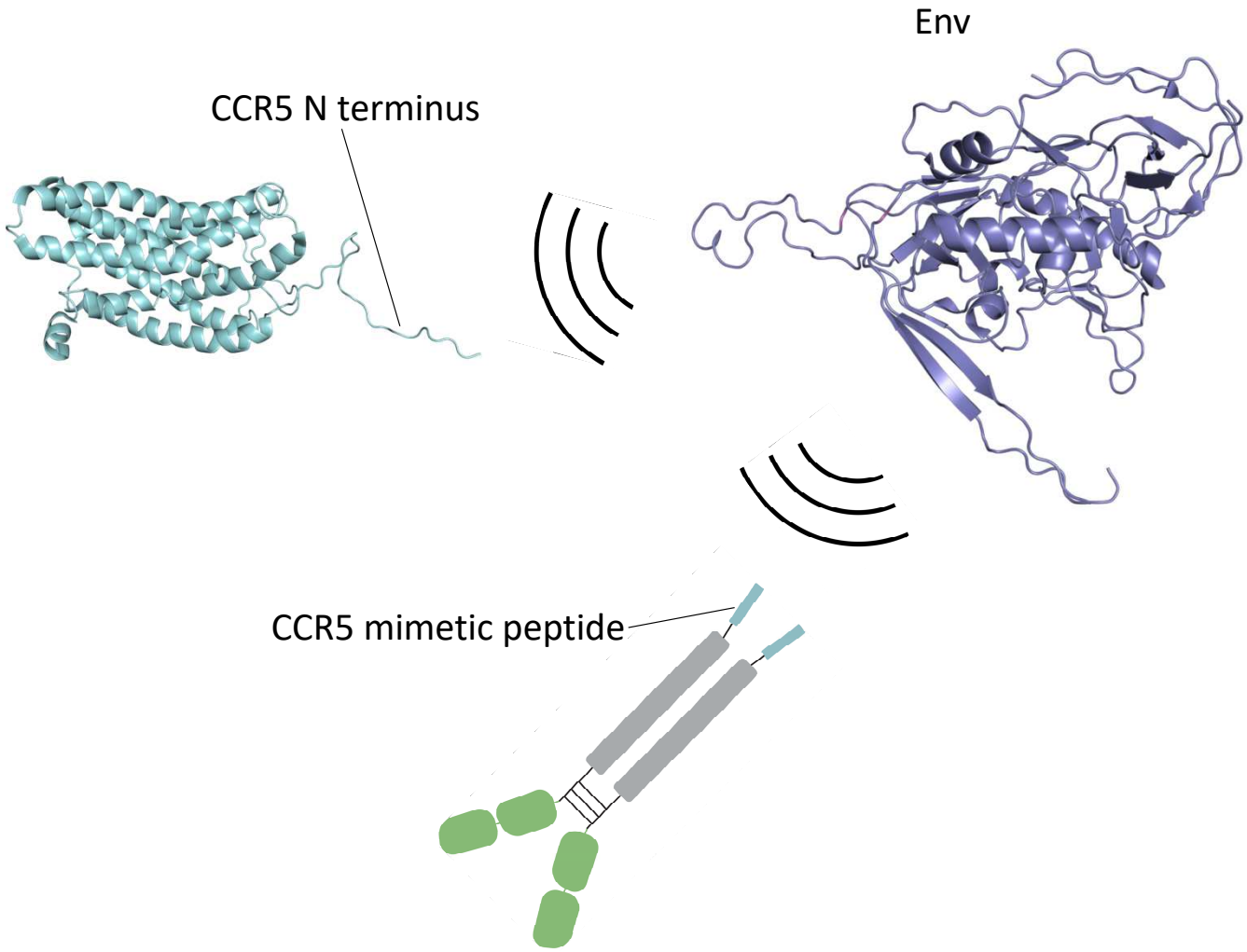
Replication in rhesus PBMC w/ Maraviroc



Singly Cloned 4X Mutations; TZM-bl Assay; Pseudovirus



In the absence of shifted tropism, we **hypothesize** that R315G and A436T help 4X better discriminate between CCR5 and the CCR5 mimetic peptide



env can evolve to exploit differences between eCD4-Ig and the receptors it mimics

BUT! The resistance it can access is limited

eCD4-Ig forces HIV to evolve in a way that limits its own fitness

We could:

Identify inhibitors for co-delivery with eCD4-Ig that block off these narrow resistance pathways

OR

Improve the potency, stability, and/or
AAV expression levels of eCD4-Ig such that even resistant variants are overcome.

I'd Like to Say Thanks to:

The meeting organizers

<u>The Martins Lab</u>

Mauricio Martins

Patricia Hahn

Siddhartha Shandilya

Matt Gardner

<u>California National Primate Research Center</u>

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Jennifer Watanabe

Lincoln Hopkins

Mike Farzan

Emmune Inc.

<u>Viral Loads</u>

Jeff Lifson

<u>AAV Vectors</u>

Guanping Gao

Jun Xie Ran He

Questions?

	bnAb	SHIV-AD8	4X	Fold Change
CD4bs	N6	0.176	0.198	1.1
CD4bs	VRC01	0.386	0.211	1.8
CD4bs	3BNC117	0.054	0.052	1.0
CD4bs V1V2 MPER	N6/PGDM1400/10E8	0.509	0.405	1.3
V3	10-1074	0.067	0.071	1.1
V3	PGT128	0.016	0.017	1.1
CD4bs	VRC-CH31	0.186	0.617	3.3
V3	447-52D	>1.67	>1.67	-
V1V2	PGT145	0.489	0.850	1.7
CD4i	E51	>1.67	>1.67	-
MPER	10E8V4	1.092	1.063	1.0
MPER	4E10	>1.67	>1.67	-

IC50

0 ug/ml 2

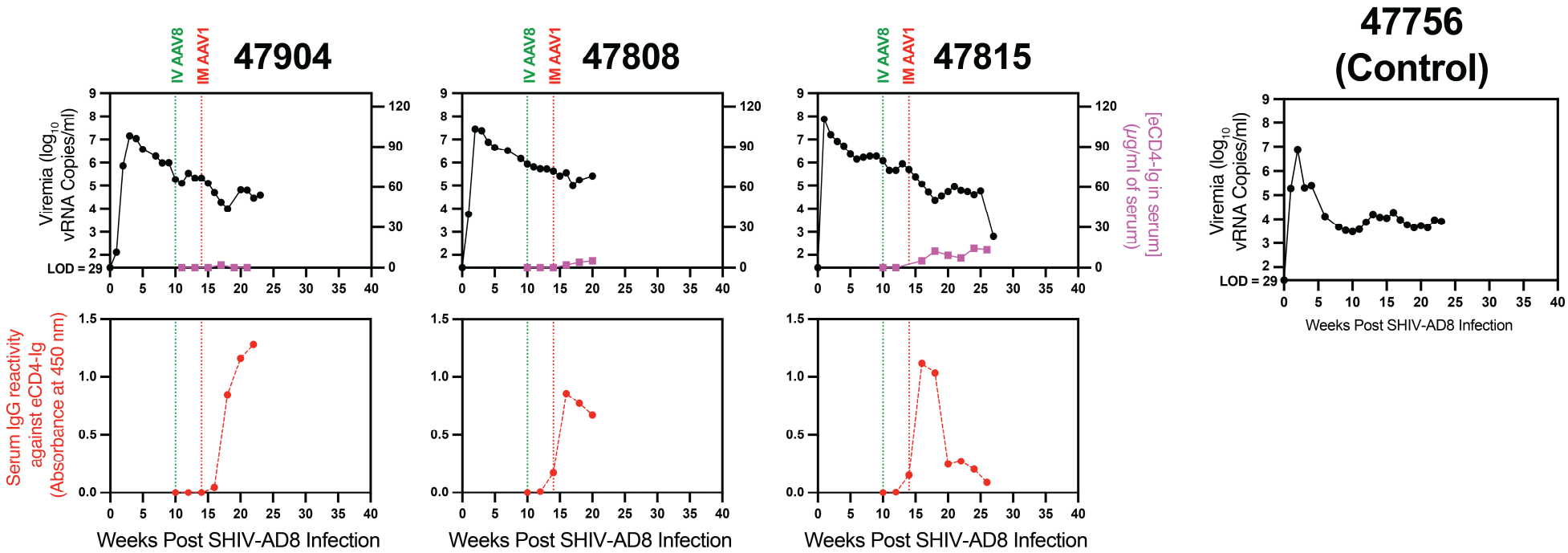
Fold Change (More Sensitive)

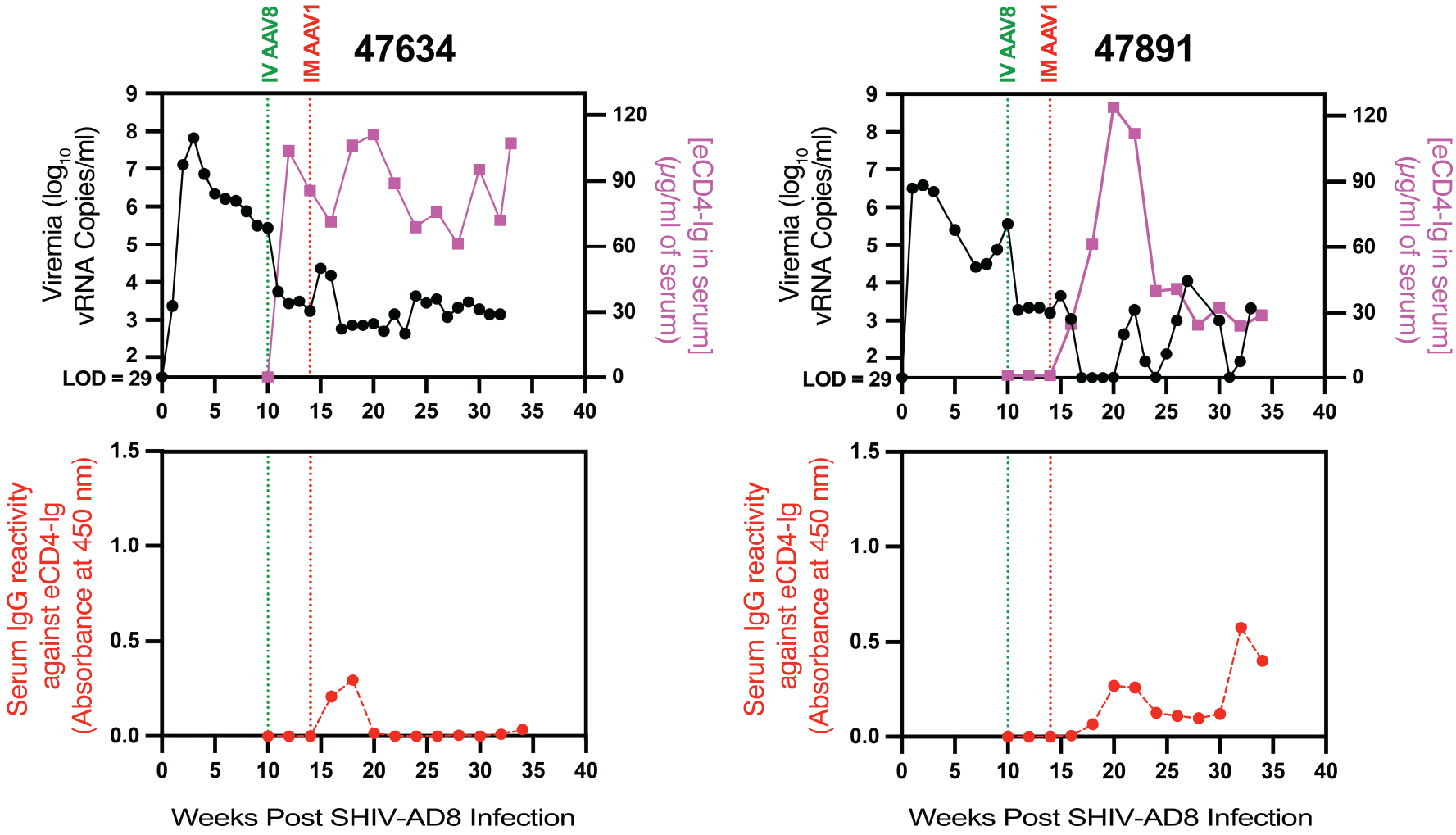
0 3.3

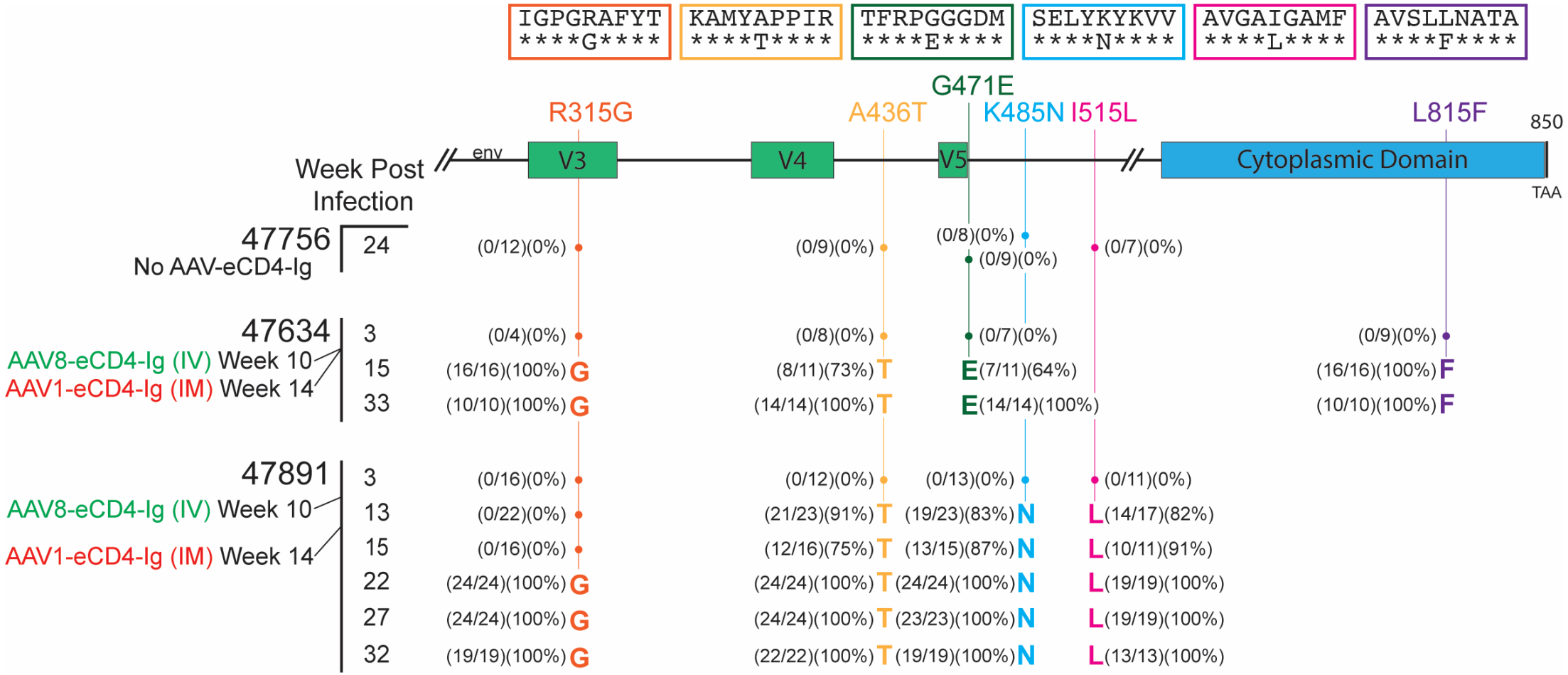
Fold Change (More Resistant)

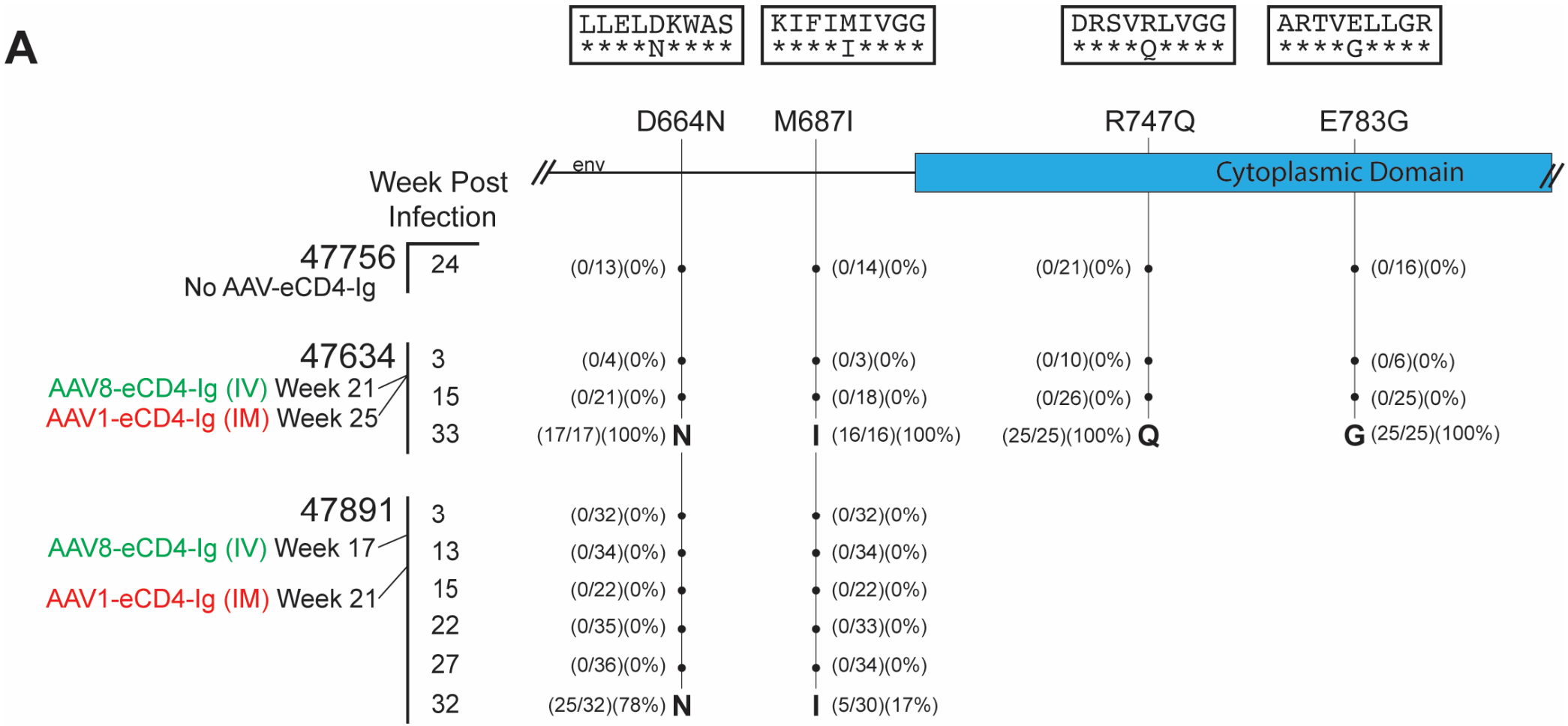
0 3.3

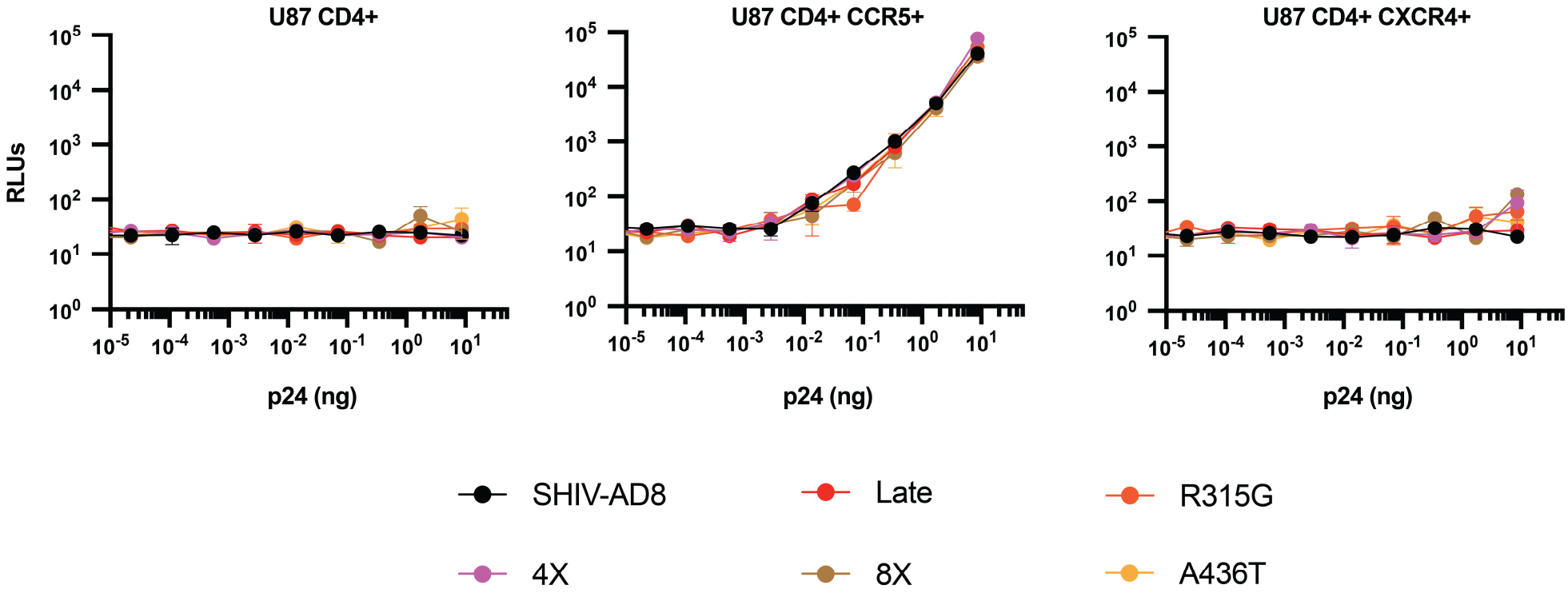
bNAb	Percent Strains Neutralized	Median IC50		Percent Strains Neutralized	Median IC50		Change in Percent Strains Neutralized		Fold Change in Median IC50	Log2 (Fold Change in Median IC50)	Absolute Fold Change	Target Epitope			
VRC07-523-	95%	0.0830		92%	0.0370		-2.70%		0.4458	-1.1656	2.24	CD4BS			
PGT145:	54%	0.3510		78%	0.1582		24.32%		0.4507	-1.1497	2.22	V1V2			
N6:IC50:	95%	0.0692		100%	0.0410		5.41%		0.5925	-0.7550	1.69	CD4BS			
3BNC117:IC	76%	0.2780		70%	0.1775		-5.41%		0.6385	-0.6473	1.57	CD4BS			
VRC07:IC50	84%	0.2630		92%	0.1685		8.11%		0.6407	-0.6423	1.56	CD4BS			
561_02_12:I	78%	0.1065		89%	0.0700		10.81%		0.6573	-0.6054	1.52	CD4BS			*limited Data
561_01_18:I	89%	0.0760		97%	0.0510		8.11%		0.6711	-0.5755	1.49	CD4BS			*limited Data
VRC13:IC50	70%	0.2165		70%	0.1755		0.00%		0.8106	-0.3029	1.23	CD4BS			
PGT128:	51%	0.0309		51%	0.0280		0.00%		0.9074	-0.1401	1.10	V3			
PGT121:	76%	0.0738		62%	0.0770		-13.51%		1.0429	0.0607	1.04	V3			
VRC01: IC50	68%	0.4000		86%	0.4950		18.92%		1.2375	0.3074	1.24	CD4BS			
10-1074V:	62%	0.0230		62%	0.0290		0.00%		1.2609	0.3344	1.26	V3			
10-1074: IC50	59%	0.0467		59%	0.0705		0.00%		1.5092	0.5937	1.51	V3			
10E8:IC50:	97%	0.4110		97%	0.6262		0.00%		1.5236	0.6074	1.52	GP41			
4E10:IC50:	100%	4.4000		95%	7.0700		-5.41%		1.6068	0.6842	1.61	GP41			
2F5:IC50:	11%	16.8500		14%	27.9000		2.70%		1.6558	0.7275	1.66	GP41			*few strains neutralized
DH511.2:IC	97%	1.3048		97%	2.7705		0.00%		2.1234	1.0864	2.12	GP41			
PGDM1400:	59%	0.0180		78%	0.0390		18.92%		2.1667	1.1155	2.17	V1V2			
PG9: IC50	59%	0.0400		68%	0.0911		8.11%		2.2768	1.1870	2.28	V1V2			
VRC26.08:	49%	0.0110		76%	0.0290		27.03%		2.6364	1.3985	2.64	V1V2			
VRC26.25:	57%	0.0003		76%	0.0009		18.92%		2.9500	1.5607	2.95	V1V2			
b12:IC50:	32%	3.2950		24%	15.7000		-8.11%		4.7648	2.2524	4.76	CD4BS			*few strains neutralized
2G12:IC50:	8%	6.5000		0%	50.0000		-8.11%		7.6923	2.9434	7.69	Glycan Shield C2, C3, V4, C4			*few strains neutralized


47904
47808
47815
47756
(Control)
IV AAV8
IM AAV1
Viremia (log10 vRNA Copies/ml)
[eCD4-Ig in serum] (μg/ml of serum)
LOD = 29
Serum IgG reactivity against eCD4-Ig (Absorbance at 450 nm)
Weeks Post SHIV-AD8 Infection

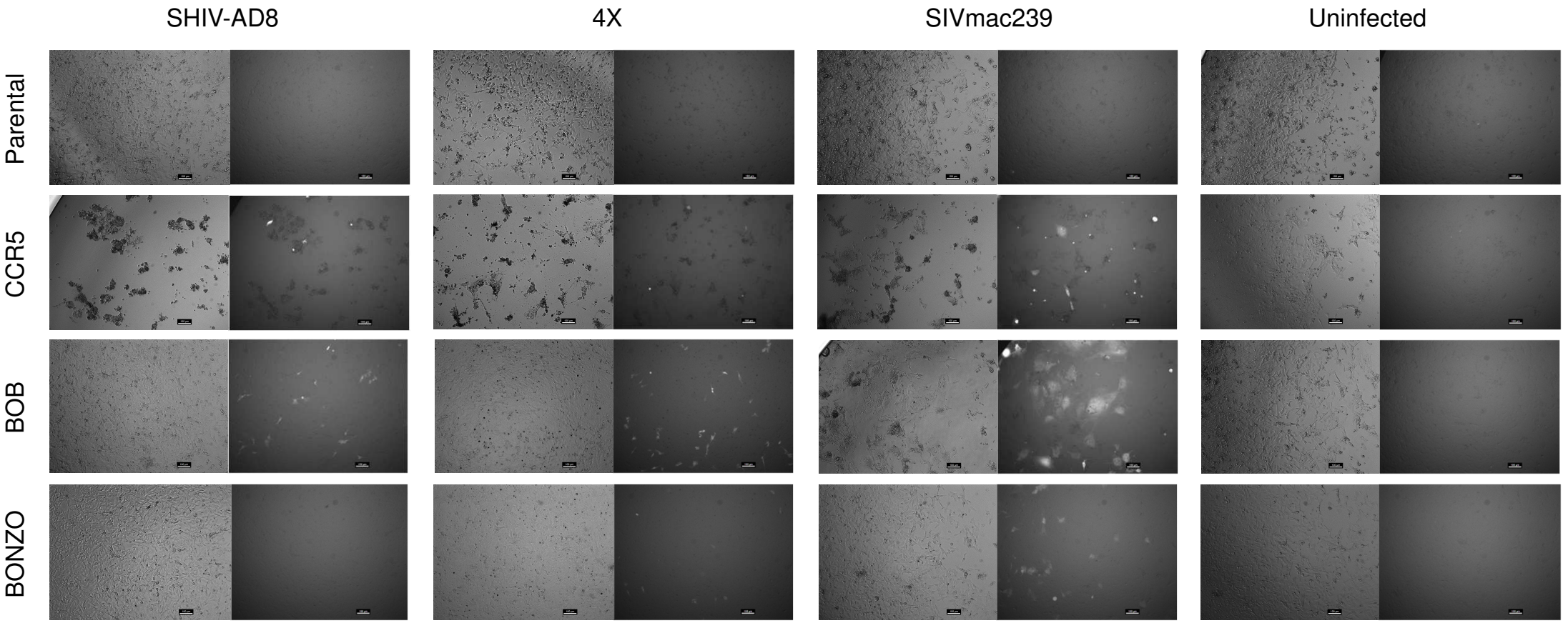

47634
IV AAV8
IM AAV1
Viremia (log10 vRNA Copies/ml)
[eCD4-Ig in serum] (μg/ml of serum)
LOD = 29
Serum IgG reactivity against eCD4-Ig (Absorbance at 450 nm)
Weeks Post SHIV-AD8 Infection
47891
IV AAV8
IM AAV1
Viremia (log10 vRNA Copies/ml)
[eCD4-Ig in serum] (μg/ml of serum)
LOD = 29
Serum IgG reactivity against eCD4-Ig (Absorbance at 450 nm)
Weeks Post SHIV-AD8 Infection


IGPGRAFYT
****G****
KAMYAPPIR
****T****
TFRPGGGDM
****E****
SELYKYKVV
****N****
AVGAIGAMF
****L****
AVSLLNATA
****F****
R315G
G471E
A436T
K485N
I515L
L815F
850
env
V3
V4
V5
Cytoplasmic Domain
TAA
Week Post Infection
47756
No AAV-eCD4-Ig
24
(0/12)(0%)
(0/9)(0%)
(0/8)(0%)
(0/9)(0%)
(0/7)(0%)
47634
AAV8-eCD4-Ig (IV) Week 10
AAV1-eCD4-Ig (IM) Week 14
3
(0/4)(0%)
(0/8)(0%)
(0/7)(0%)
(0/9)(0%)
15
(16/16)(100%) G
(8/11)(73%) T
E(7/11)(64%)
(16/16)(100%) F
33
(10/10)(100%) G
(14/14)(100%) T
E(14/14)(100%)
(10/10)(100%) F
47891
AAV8-eCD4-Ig (IV) Week 10
AAV1-eCD4-Ig (IM) Week 14
3
(0/16)(0%)
(0/12)(0%)
(0/13)(0%)
(0/11)(0%)
13
(0/22)(0%)
(21/23)(91%) T
(19/23)(83%) N
L(14/17)(82%)
15
(0/16)(0%)
(12/16)(75%) T
(13/15)(87%) N
L(10/11)(91%)
22
(24/24)(100%) G
(24/24)(100%) T
(24/24)(100%) N
L(19/19)(100%)
27
(24/24)(100%) G
(24/24)(100%) T
(23/23)(100%) N
L(19/19)(100%)
32
(19/19)(100%) G
(22/22)(100%) T
(19/19)(100%) N
L(13/13)(100%)


A
LLELDKWAS
****N****
KIFIMIVGG
****I****
DRSVRLVGG
****Q****
ARTVELLGR
****G****
D664N
M687I
R747Q
E783G
env
Cytoplasmic Domain
Week Post Infection
47756
No AAV-eCD4-Ig
24
(0/13)(0%)
(0/14)(0%)
(0/21)(0%)
(0/16)(0%)
47634
AAV8-eCD4-Ig (IV) Week 21
AAV1-eCD4-Ig (IM) Week 25
3
15
33
(0/4)(0%)
(0/21)(0%)
(17/17)(100%) N
(0/3)(0%)
(0/18)(0%)
I (16/16)(100%)
(0/10)(0%)
(0/26)(0%)
(25/25)(100%) Q
(0/6)(0%)
(0/25)(0%)
G (25/25)(100%)
47891
AAV8-eCD4-Ig (IV) Week 17
AAV1-eCD4-Ig (IM) Week 21
3
13
15
22
27
32
(0/32)(0%)
(0/34)(0%)
(0/22)(0%)
(0/35)(0%)
(0/36)(0%)
(25/32)(78%) N
(0/32)(0%)
(0/34)(0%)
(0/22)(0%)
(0/33)(0%)
(0/34)(0%)
I (5/30)(17%)


U87 CD4+
U87 CD4+ CCR5+
U87 CD4+ CXCR4+
RLUs
p24 (ng)
SHIV-AD8
Late
R315G
4X
8X
A436T

Day 3 Post Infection – Representative Images.



38 ng/ml p27, spinoculated, +DEAE dextran