

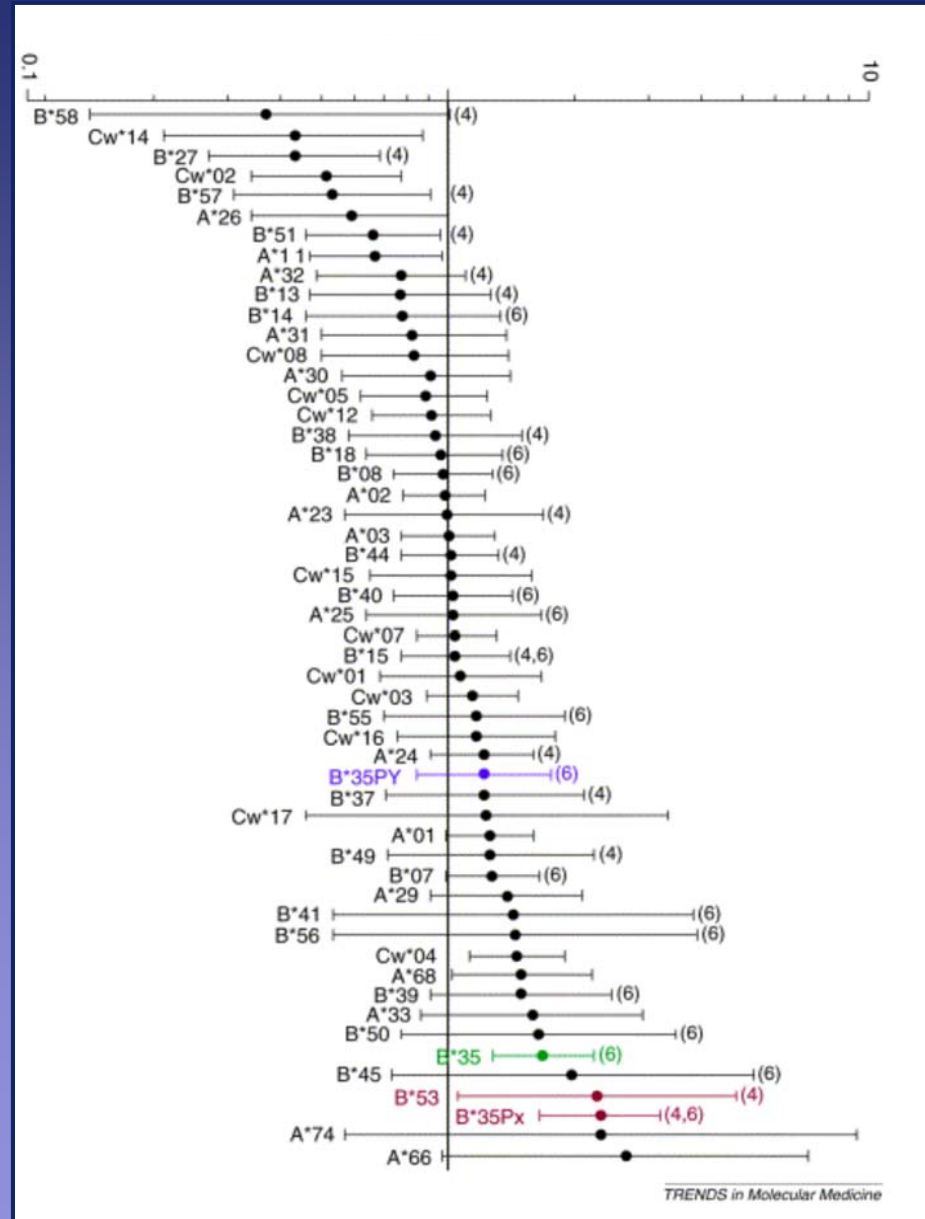


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Sheraton Seattle Hotel & Towers

**CTL escape mutations in HIV capsid
decrease viral fitness
by altering cyclophilin A dependence
revealing regions of Gag
susceptible to immune pressure**

**Arne Schneidewind, Mark Brockman, Todd Allen
Partners AIDS Research Center
Massachusetts General Hospital, Boston**

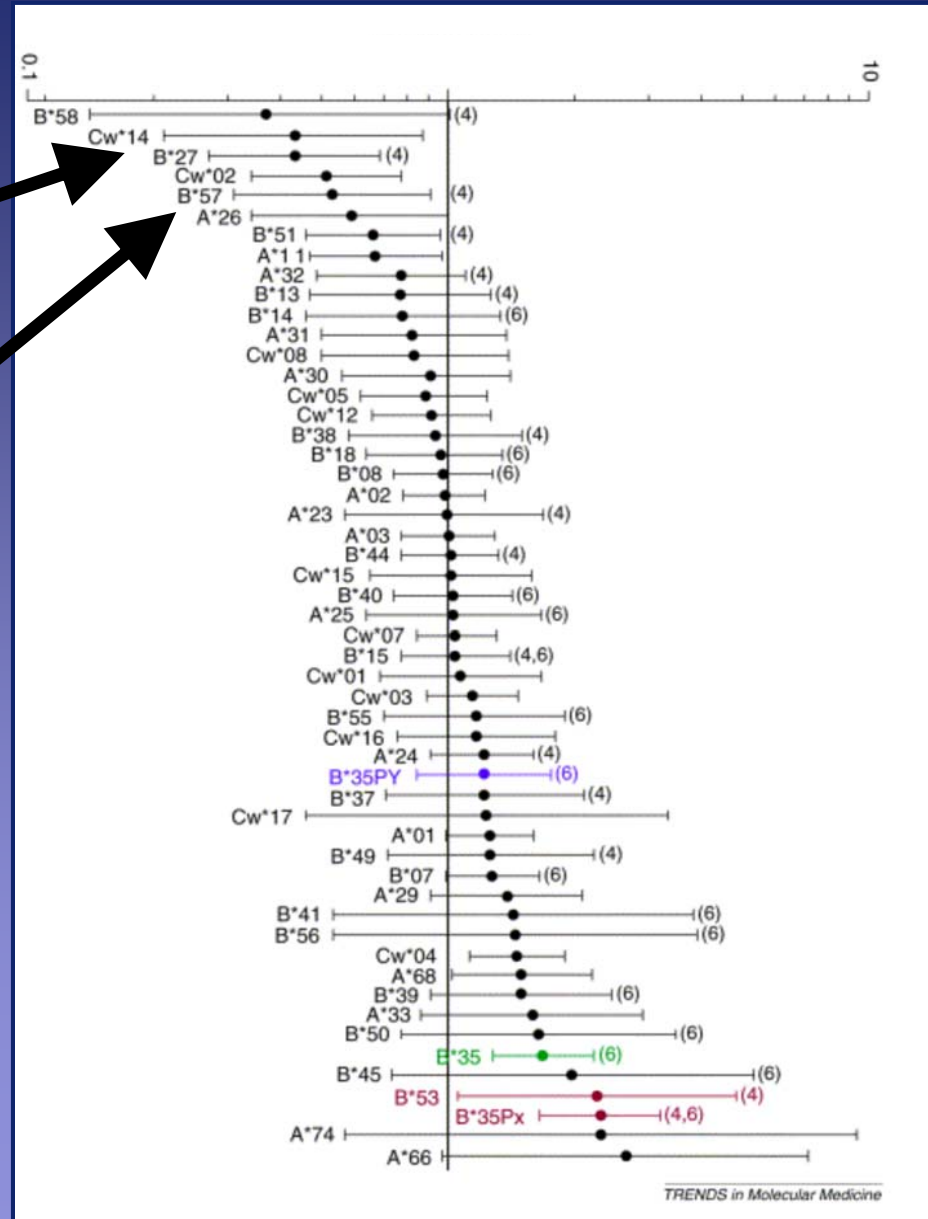
Relative hazard for AIDS defining event



Relative hazard for AIDS defining event

HLA-B27

HLA-B57

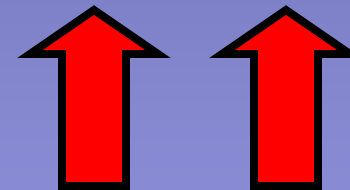


CTL escape in B57-TW10

	220	230	240	250	
NL4-3	D R L H P V <u>H A G P I A</u> P G Q M R E P R G S D I A G T		T S T L Q E Q I G W	M T H N P P I P	
Cons Gag-B				N	
Pt14	Q	V	L	N	A
Pt35	Q	V		N	
Pt101	M Q	V	I	N	Q S
Pt102				N	
Pt105				N	N
Pt106	v Q	V	L	N	V A
Pt107				N	A N
Pt110	Q	V	I	N	A N
Pt114			I	T N	A N
Pt119	Q	V	I	N	A
Pt123	Q	V	I	N	A
Pt203		V		N	N

CTL escape in B57-TW10

	220			230			240			250																																	
NL4-3	D	R	L	<u>V</u>	<u>H</u>	<u>A</u>	<u>G</u>	<u>P</u>	<u>I</u>	A	P	G	Q	M	R	E	P	R	G	S	D	I	A	G	T	T	S	T	L	Q	E	Q	I	G	W	M	T	H	N	P	P	I	P
Cons Gag-B																							N																		
Pt14																							N		A															
Pt35																							N															
Pt101	M		Q	V		I			N	Q		S																													
Pt102																							N															
Pt105																							N			N															
Pt106	v	Q		V		L			N	V		A																													
Pt107																							N		A	N															
Pt110	Q		V		I			N	A		N																														
Pt114																							T	N		A	N														
Pt119	Q		V		I			N	A																																
Pt123	Q		V		I			N	A																																
Pt203																							N			N															

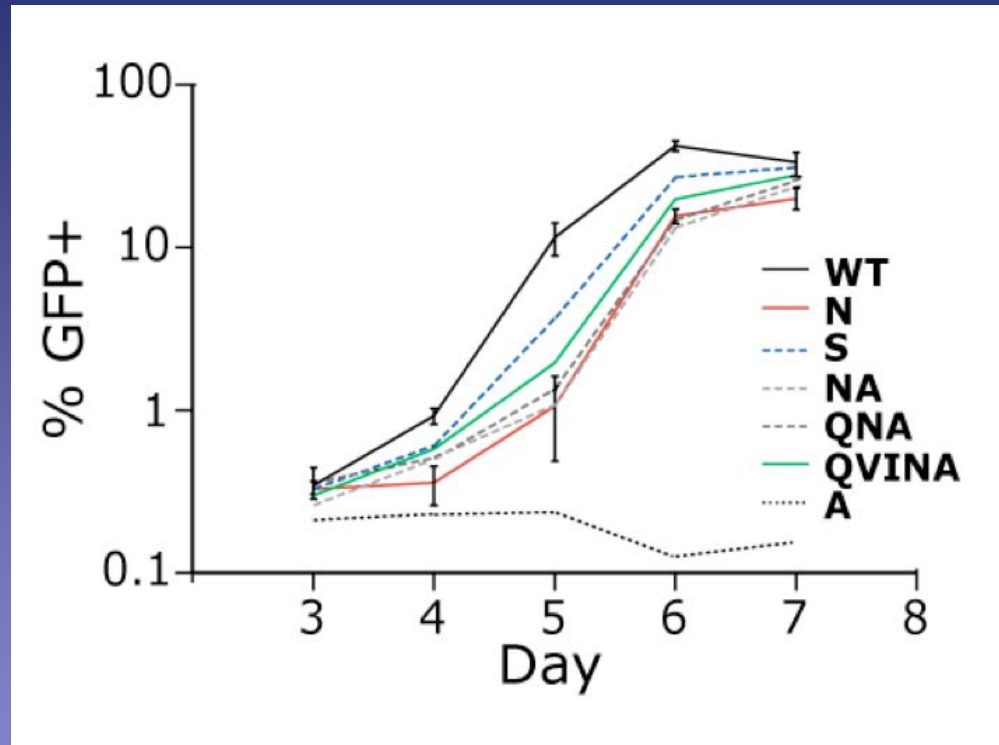


T₂₄₂N **G₂₄₈A**

CTL escape in B57-TW10

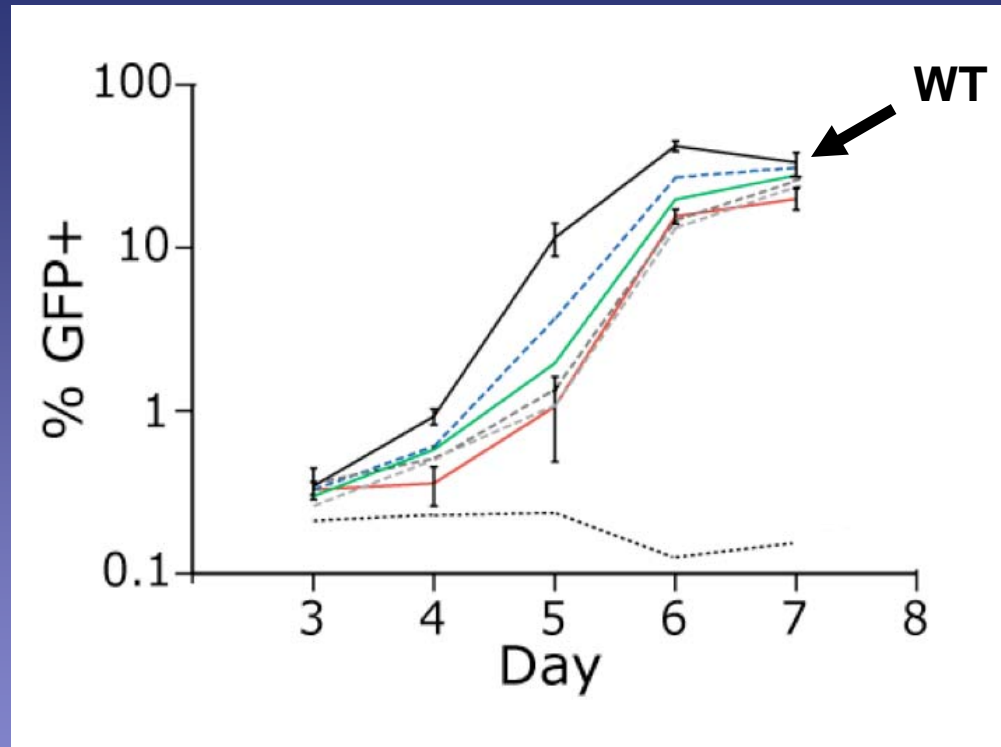
	220	230	240	250
NL4-3	D R L H P V H A G P I A P G Q M R E P R G S D I A G T	T S T	L Q E Q I G W	M T H N P P I P
Cons Gag-B				N
Pt14	Q	V	L	N
Pt35	Q	V		N
Pt101	M Q	V	I	N
Pt102				N
Pt105				N
Pt106	v Q	V	L	N
Pt107				N
Pt110	Q	V	I	N
Pt114			I	N
Pt119	Q	V	I	N
Pt123	Q	V	I	N
Pt203		V		N
WT (NL4-3)				N
N				N
S				S
A				A
W				W
NA				N
QNA	Q			N
QVINA	Q	V	I	N

Fitness cost of CTL escape in B57-TW10



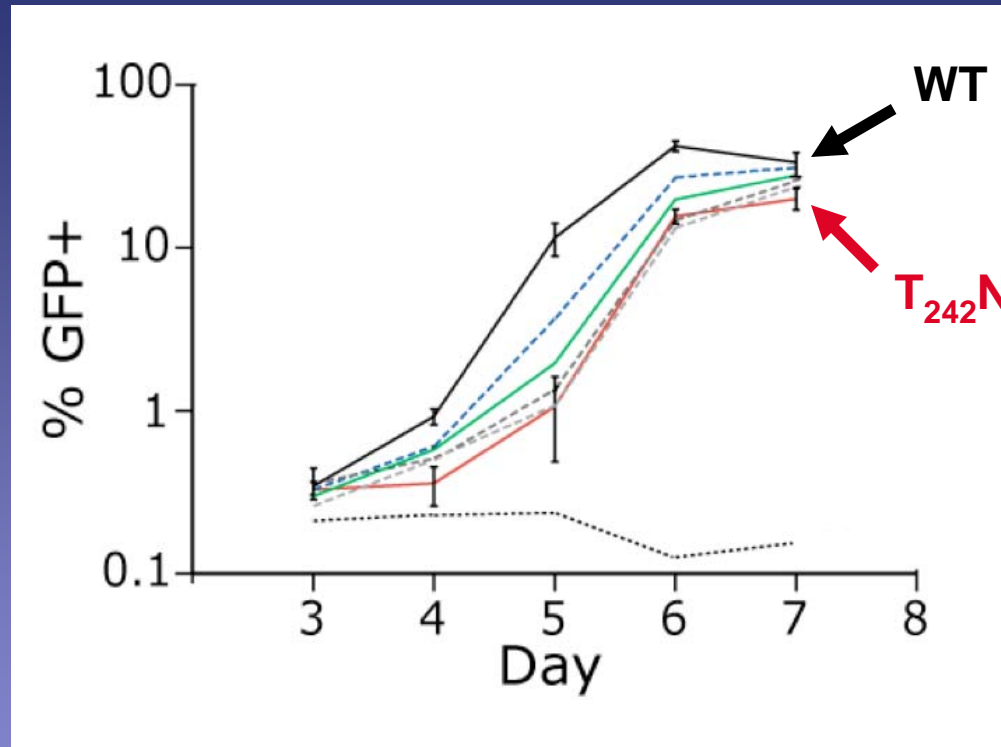
	220	230	240	250
NL4-3	DRLHPVHAGPIAPGQMR	REPRGSDIAGTTSTLQEQIGW	MTHNPP	IP
Cons Gag-B				N
WT (NL4-3)				
N				N
S				S
A				A
W				W
NA				N
QNA		Q		N
QVINA		Q	V	I

Fitness cost of CTL escape in B57-TW10



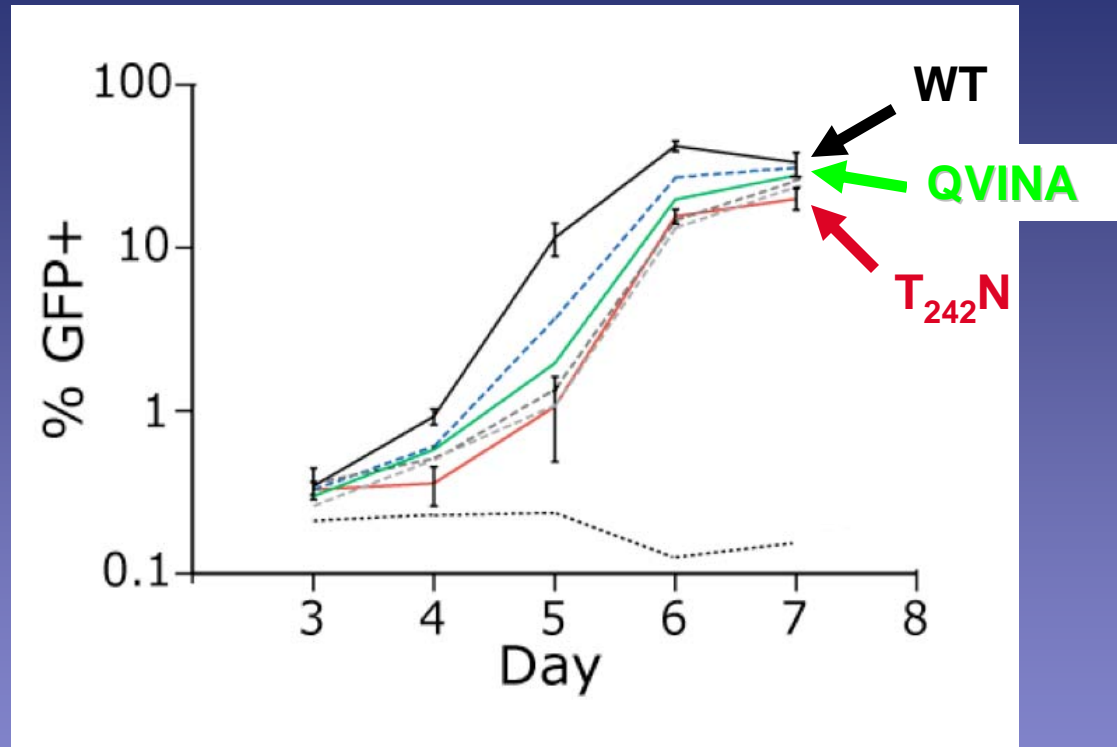
	220	230	240	250
NL4-3	DRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTHNPPIP			
Cons Gag-B				N
WT (NL4-3)				
N				N
S				S
A				A
W				W
NA				N
QNA		Q		N
QVINA		Q	V	I

Fitness cost of CTL escape in B57-TW10



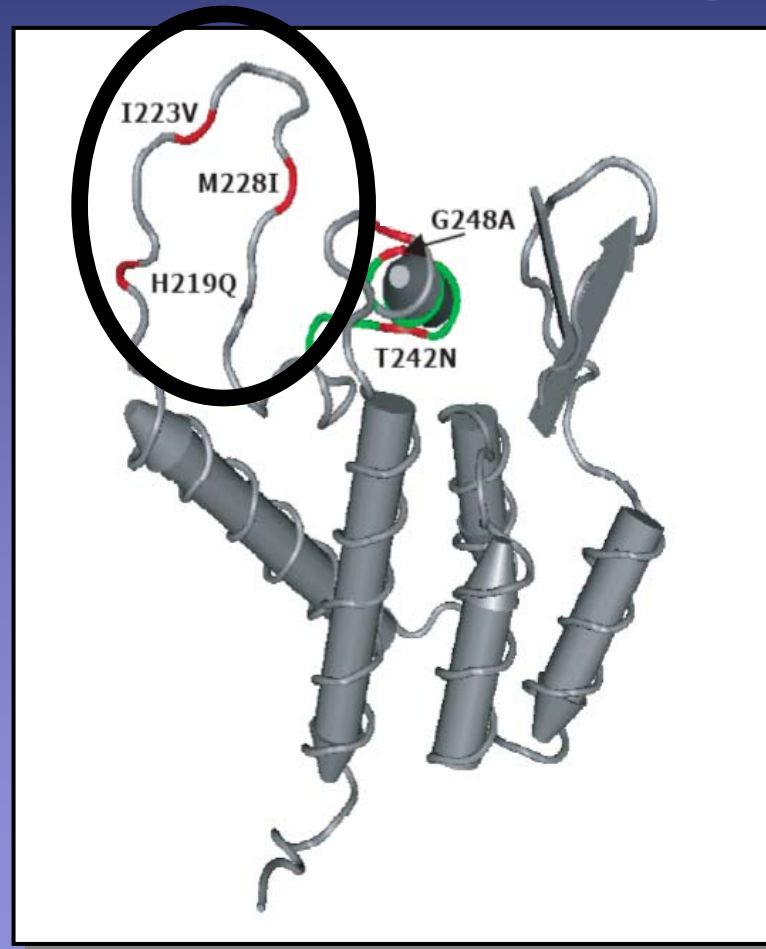
	220	230	240	250
NL4-3	DRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTHNPPIP			
Cons Gag-B				N
WT (NL4-3)				
N				N
S				S
A				A
W				W
NA				N
QNA		Q		N
QVINA		Q	V	I

Fitness cost of CTL escape in B57-TW10

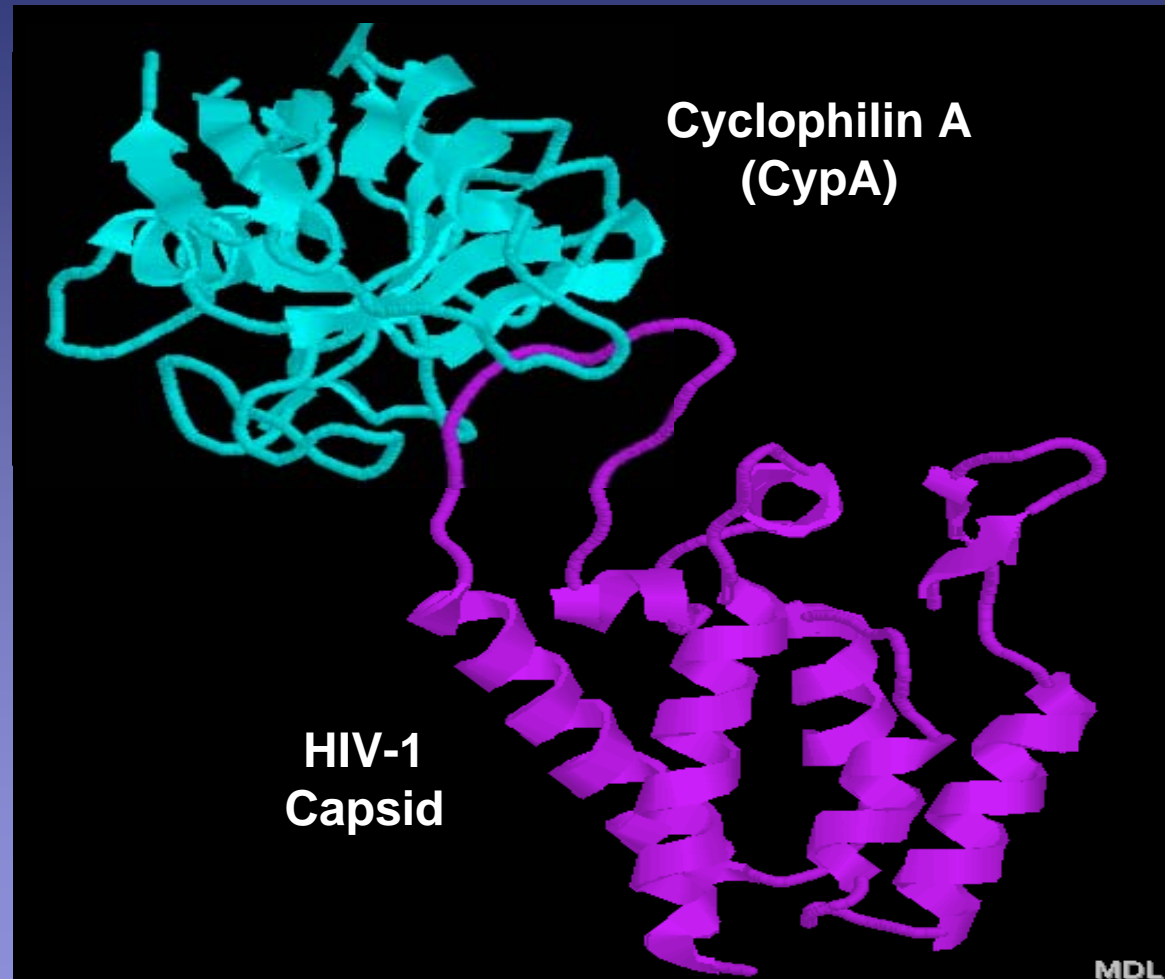


	220	230	240	250
NL4-3	DRLHPVHAGPIAPGQMR	REPRGSDIAGTTSTLQEQ	IGWMTHNPP	IP
Cons Gag-B				N
WT (NL4-3)				
N				N
S				S
A				A
W				W
NA				N
QNA		Q		N
QVINA	Q	V	I	N

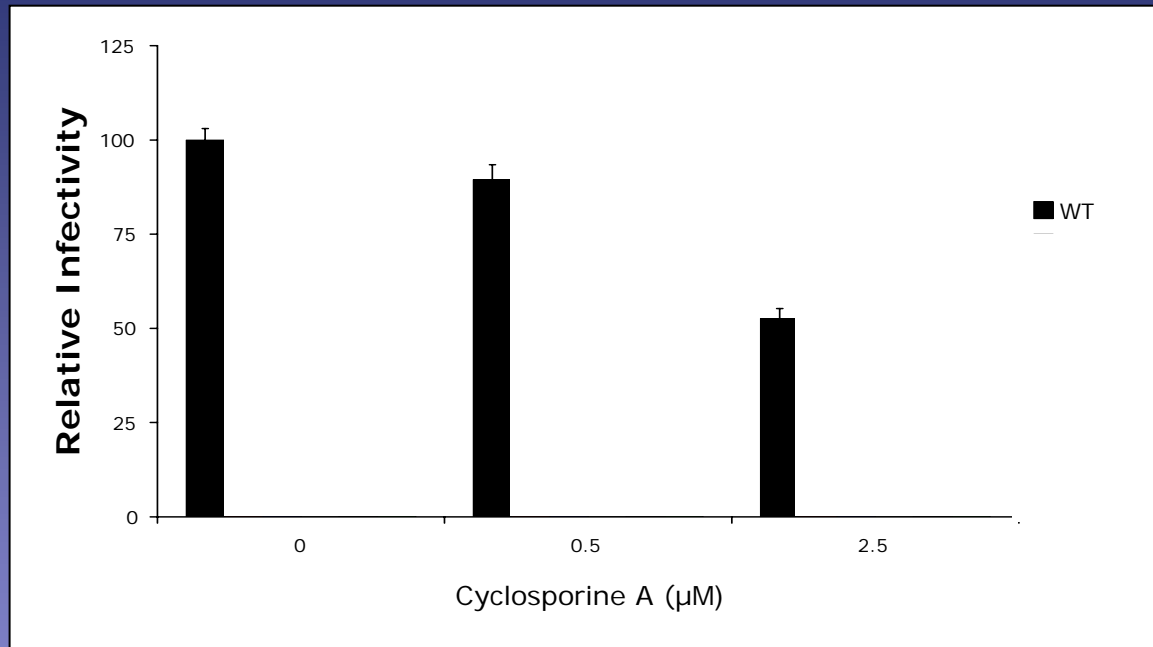
Position of B57-TW10 Proximity to the cyclophilin A binding loop



Interaction of capsid and cyclophilin A



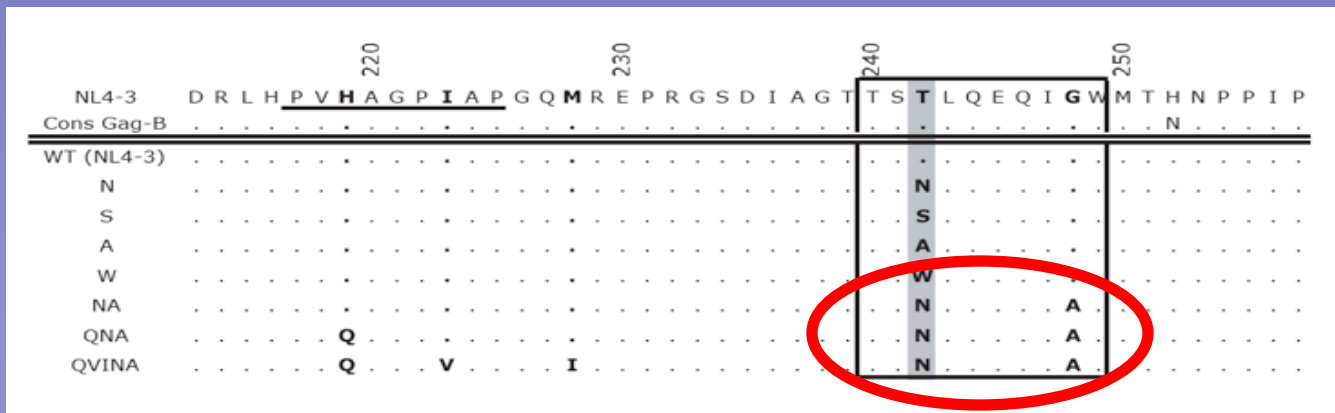
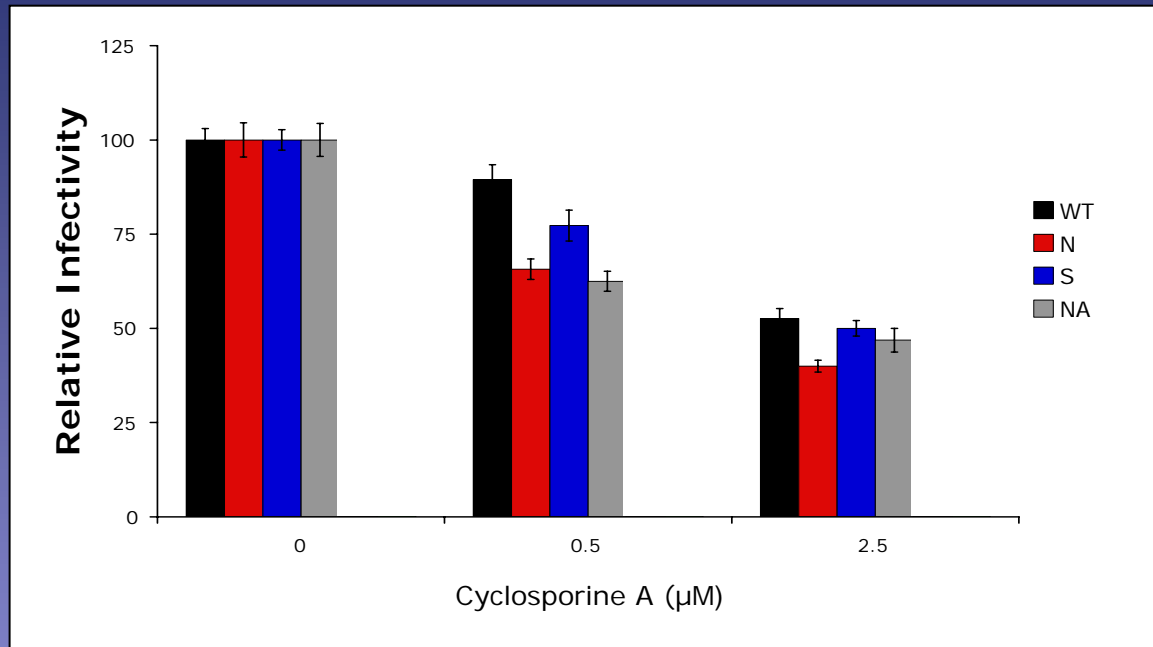
Cyclophilin A is required for normal infectivity



	220	230	240	250
NL4-3	DRLHPVHAGPIAPGQMREPRGSDIAGT	TSTLQEQIGW	MTHNPP	IP
Cons Gag-BN			
WT (NL4-3)			
N			
S			
A			
W			
NA			
QNA	Q			A
QVINA	Q	V	I	A

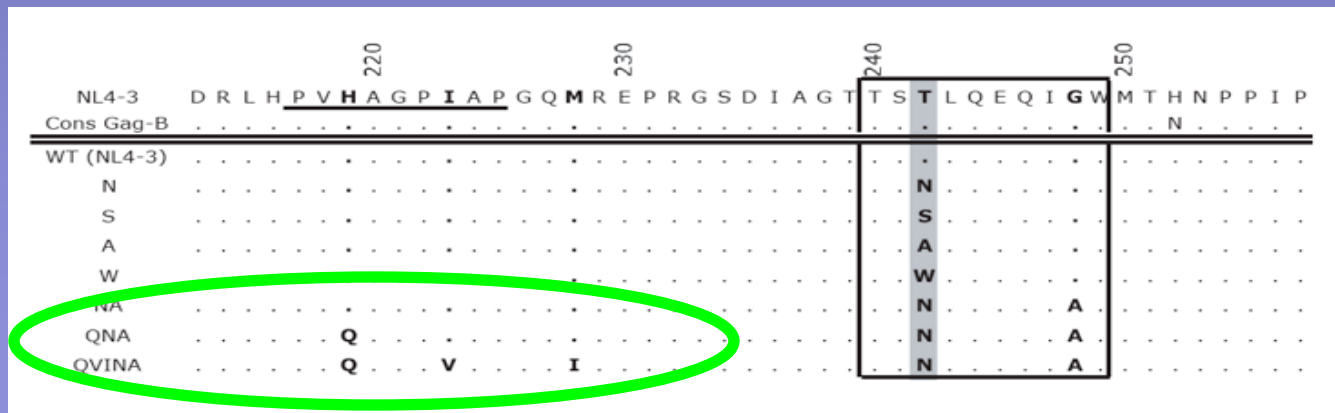
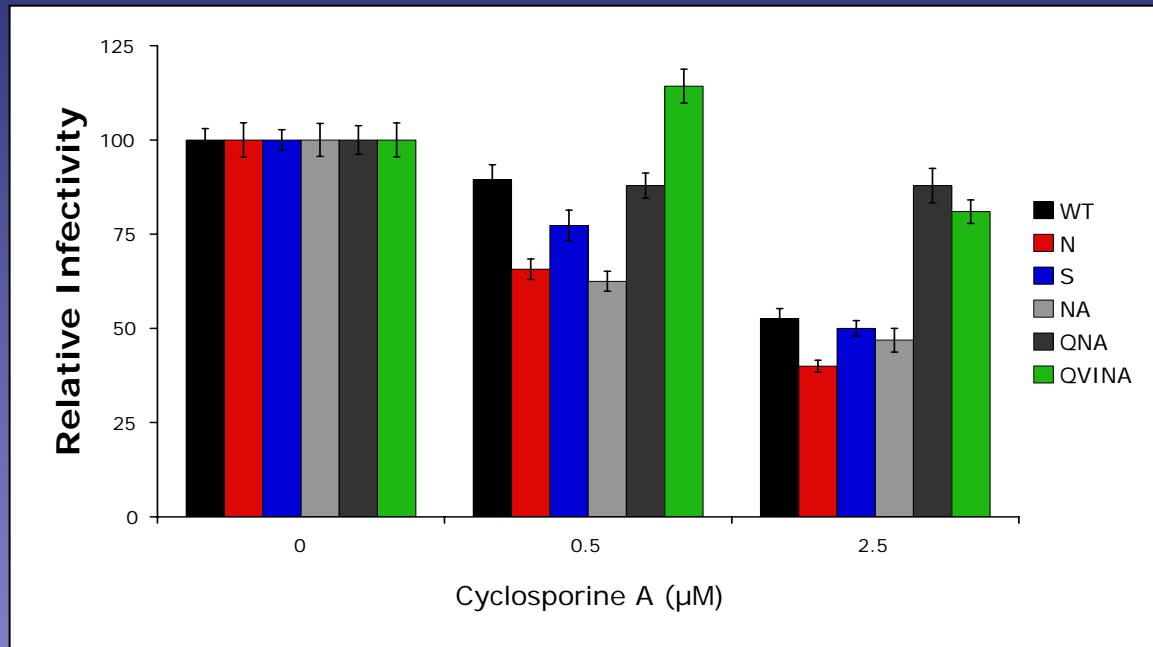
Cyclophilin A is required for normal infectivity

T₂₄₂N increases sensitivity to CsA

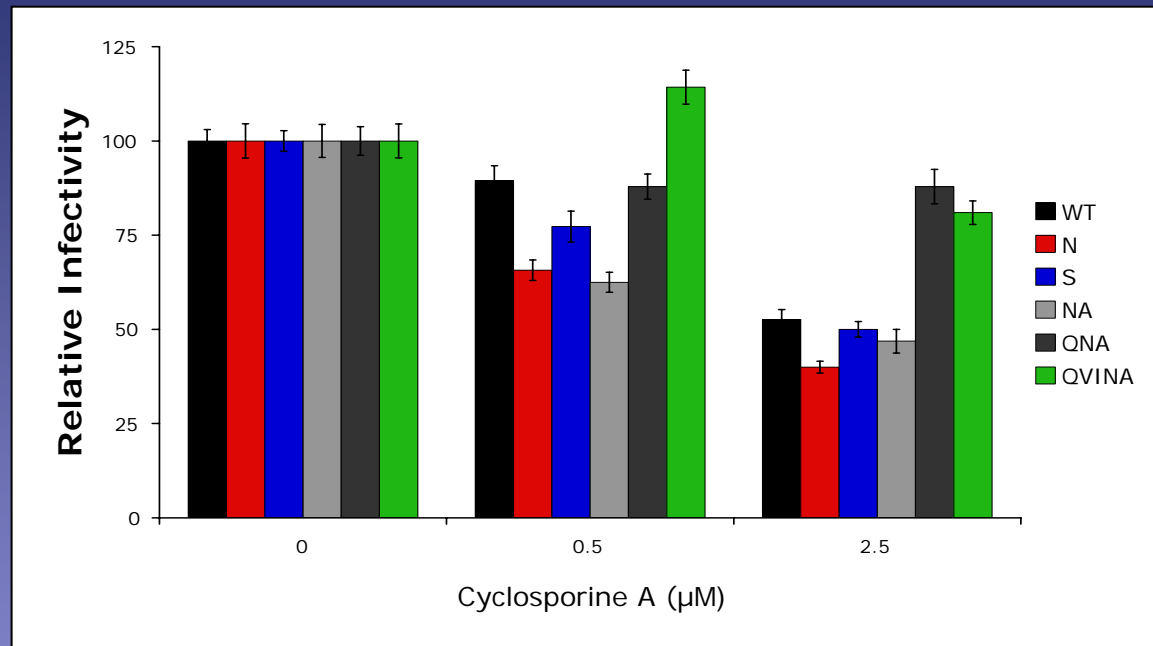


Cyclophilin A is required for normal infectivity

QVI-mutations render HIV CypA-independent



Conclusions TW10



- CTL escape in TW 10 reduces viral replication and increases dependence on CypA
- Compensatory mutations restore viral fitness and render HIV CypA-independent

CTL escape in B27-KK10

	170	180	190	200	210	220	230	240	250	260	270									
	VIPMF	SALSEG	ATPQDL	NTMLNT	VGGHQA	AMQMLK	ETINEE	AAEWDR	LHPVHAG	PIAPGQ	MREPRG	SDIAGT	TSTLQEQ	IGWMTN	NNPPI	PVGEI	YKRW	IILGL	NKIV	MY
L8146
F758	T.....	S.S.....	K.....
M124	V.....	A.....
AC160	M.....	M.....
AC88	V.....	M.....
11504	M.....
F7148	I.....	MQ.....	I.....	A.....	K.....	M.....
17630	A.....	D.....	d.....	H.....	K.....	M.....
18030	A.....	K.....	M.....
M101	A.....	V.....	v.....	K.....	M.....
SW	A.....	V.....	H.....	K.....	M.....
025	A.....	I.....	a.....	s.a.....	K.....	M.....
CR0339X	A.....	I.....	H.....	K.....	M.....	K.....
PRLS24	A.....	K.....	M.....
L8129	A.....	V.....	K.....	M.....
007 96	A.....	H.....	K.....	M.....
777 96	A.....	K.....	M.....
CR0312W	T.....	H.....	V.....	K.....	M.....
CR0206U	V.....	NP.....	K.....	I.....
L8118	T.....	S.....	V.....	T.....
L8159	V.....	I.....	T.....	S.....	V.....	T.....

	170	175	255	260	265	270	275
pNL4-3	V I P M F	S A L S E G	// P P I P	V G E I Y	K R W I I L G L N K	I V R M Y	
L268M	//	M.....
R264K	//	K.....
S173A	A.....	//

CTL escape in B27-KK10: L₂₆₈M and R₂₆₄K inside the epitope

	170	180	190	200	210	220	230	240	250	260	270											
	VIPMF	SALSEG	ATPQDL	NTMLNT	VG	GHQAAMQ	MLKETINE	EEAAEW	DR	LHPV	HAGPIAP	GQMPRE	PRGSDI	AGTTST	LQEQIG	WMTN	PP	IP	PEIYK	YILGLN	KIVRM	
L8146
F758	T.....	S.S.....
M124	V.....	A.....
AC160	M.....
AC88	V.....
11504
F7148	I.....
17630	A.....
18030	A.....
M101	A.....	V.....	v.....
SW	A.....	V.....
025	A.....	I.....
CR0339X	A.....	I.....
PRLS24	A.....
L8129	A.....	V.....
007 96	A.....
777 96	A.....
CR0312W	T.....	H.....
CR0206U	V.....
L8118	T.....
L8159

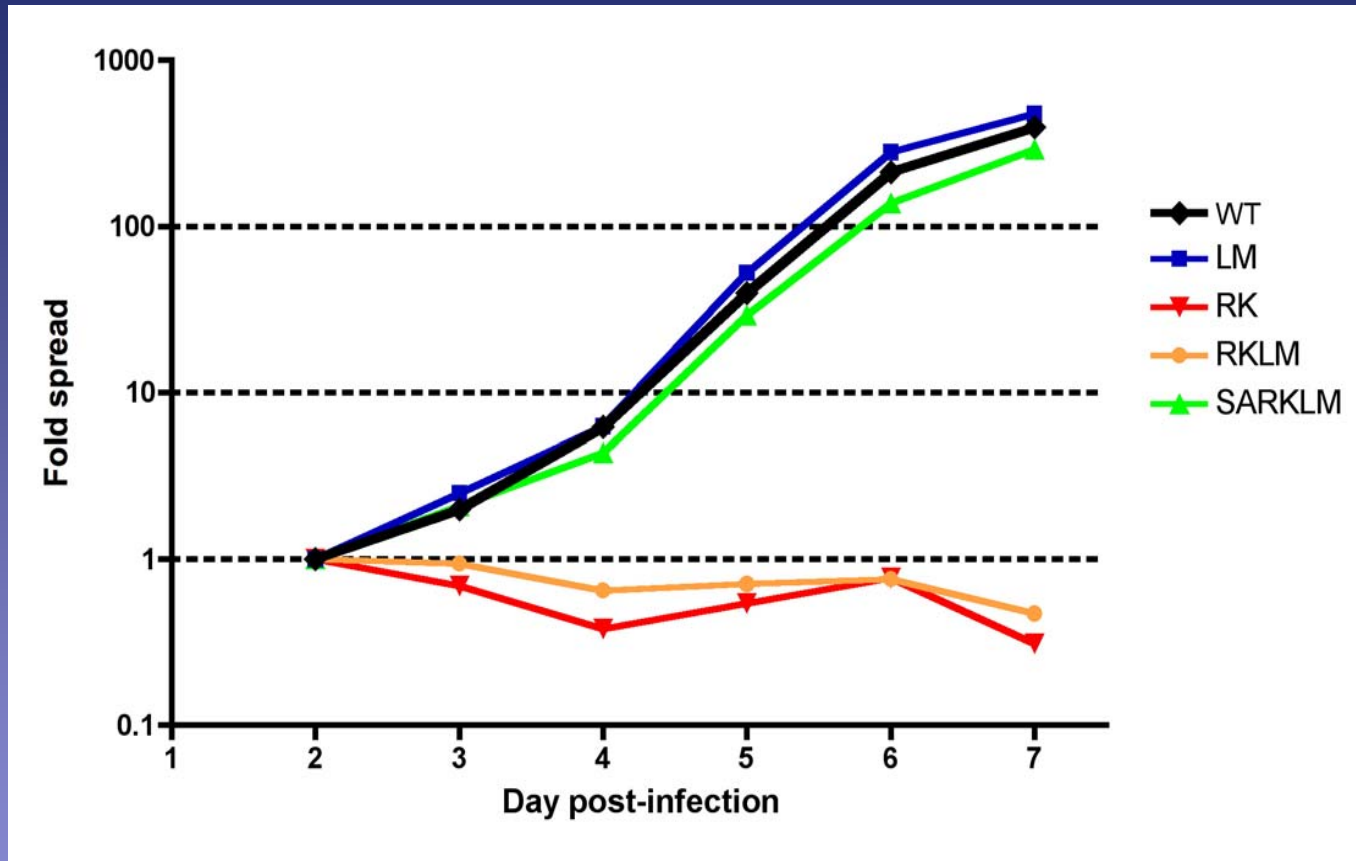
	170	175	255	260	265	270	275
pNL4-3	V I P M F	S A L S E G	// P P I P V G E I Y		K R W I I L G L N K	I V R M Y	
L268M	//
R264K	//
S173A	A	//

CTL escape in B27-KK10: S₁₇₃A upstream of the epitope

	170	180	190	200	210	220	230	240	250	260	270	
	PMFS	ALSEGAT	PD	LNTML	NTVGGHQAAMQ	MLKETINEEAAEW	DR	LHPVHAGPIAP	GQ	MPRGSDIAGT	STLQEQIGW	M
L8146												
F758		T								S	S	K
M124				V			A					
AC160						M						M
AC88							V					M
11504												M
F7148				I		MQ		I		A		K
17630	A							D		d	H	K
18030	A											K
M101	A					V	v					K
SW	A					V				H		K
025	A			I			a			s	a	K
CR0339X	A			I						H		K
PRLS24	A											K
L8129	A						V					K
007 96	A									H		K
777 96	A											K
CR0312W	T	H					V					K
CR0206U	V						NP					K
L8118	T									S	V	T
L8159						V	I			T	S	V

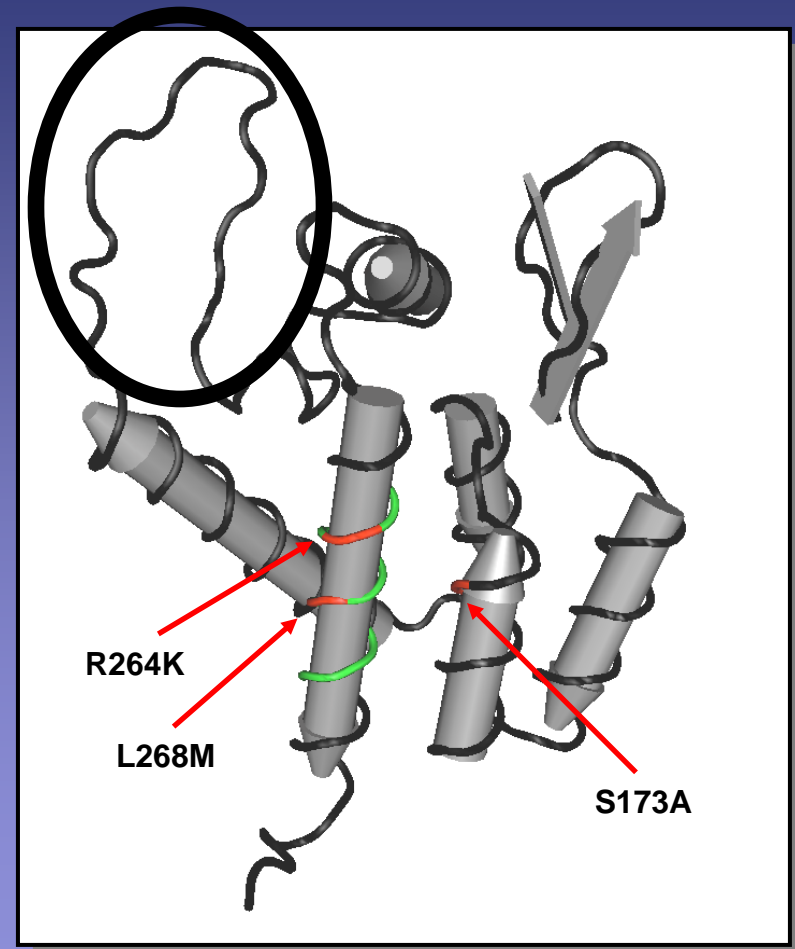
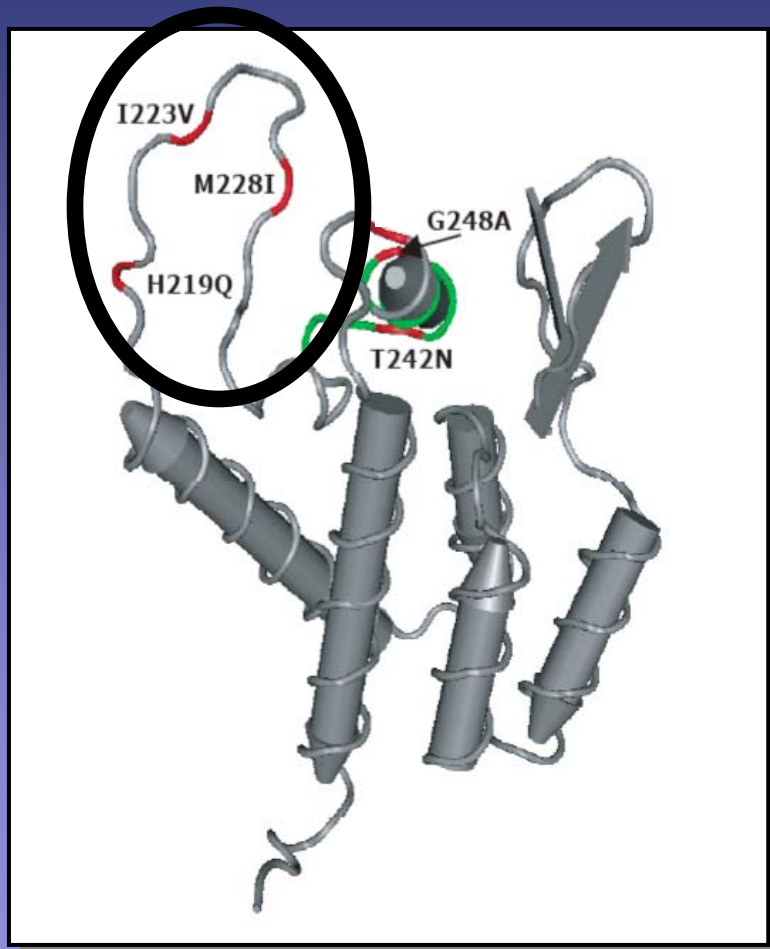
	170	175	255	260	265	270	275
pNL4-3	V I P M F S	A L S E G	//	P P I P V G E I Y	K R W I I L G L N K	I V R M Y	
L268M			//			M	
R264K			//		K		
S173A		A	//				

Fitness cost of CTL escape in B27-KK10



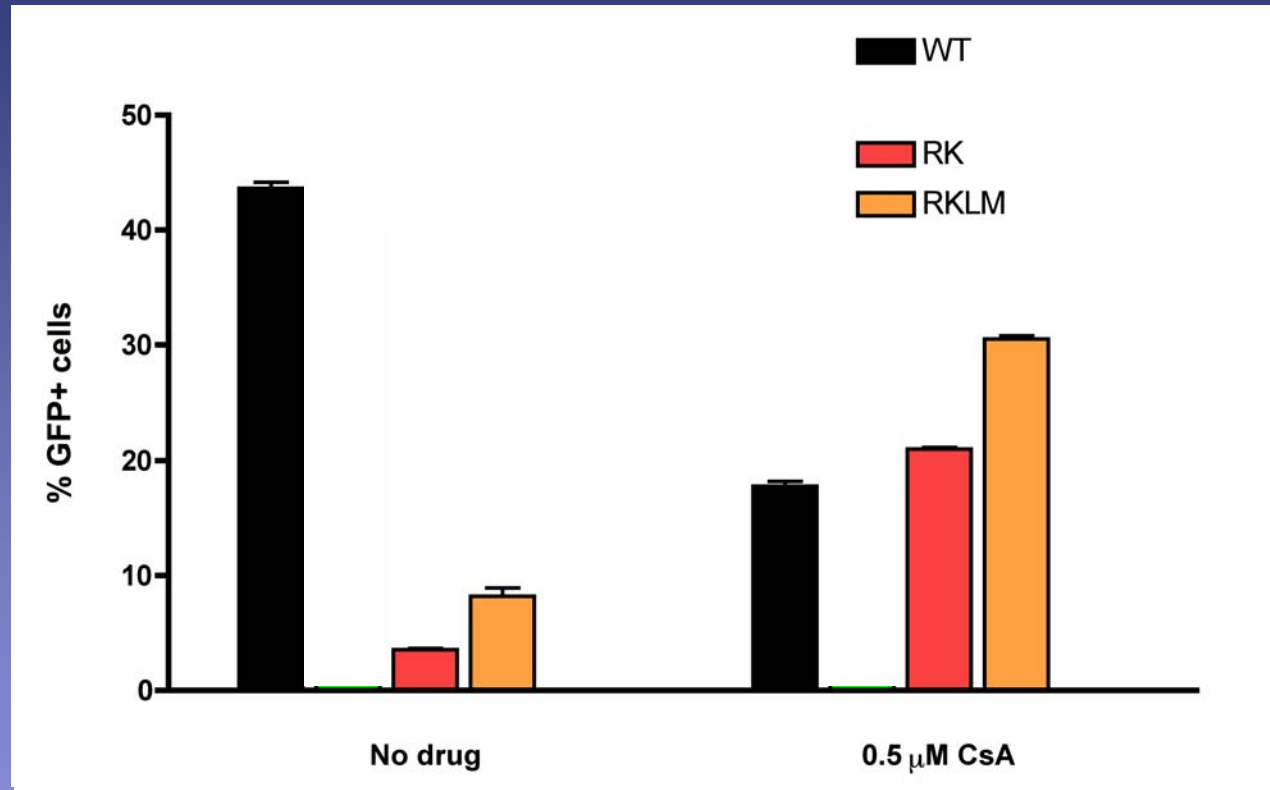
	170	175	255	260	265	270	275
pNL4-3	V I P M F	S A L S E G	// P P I P V G E I Y		K R W I I L G L N K	I V R M Y	
L268M	.	.	//	.	.	M	.
R264K	.	.	//	.	K	.	.
S173A	.	A	//

Position of B57-TW10 and B27-KK10 Influence on the CypA phenotype?



Cyclophilin A is required for normal infectivity

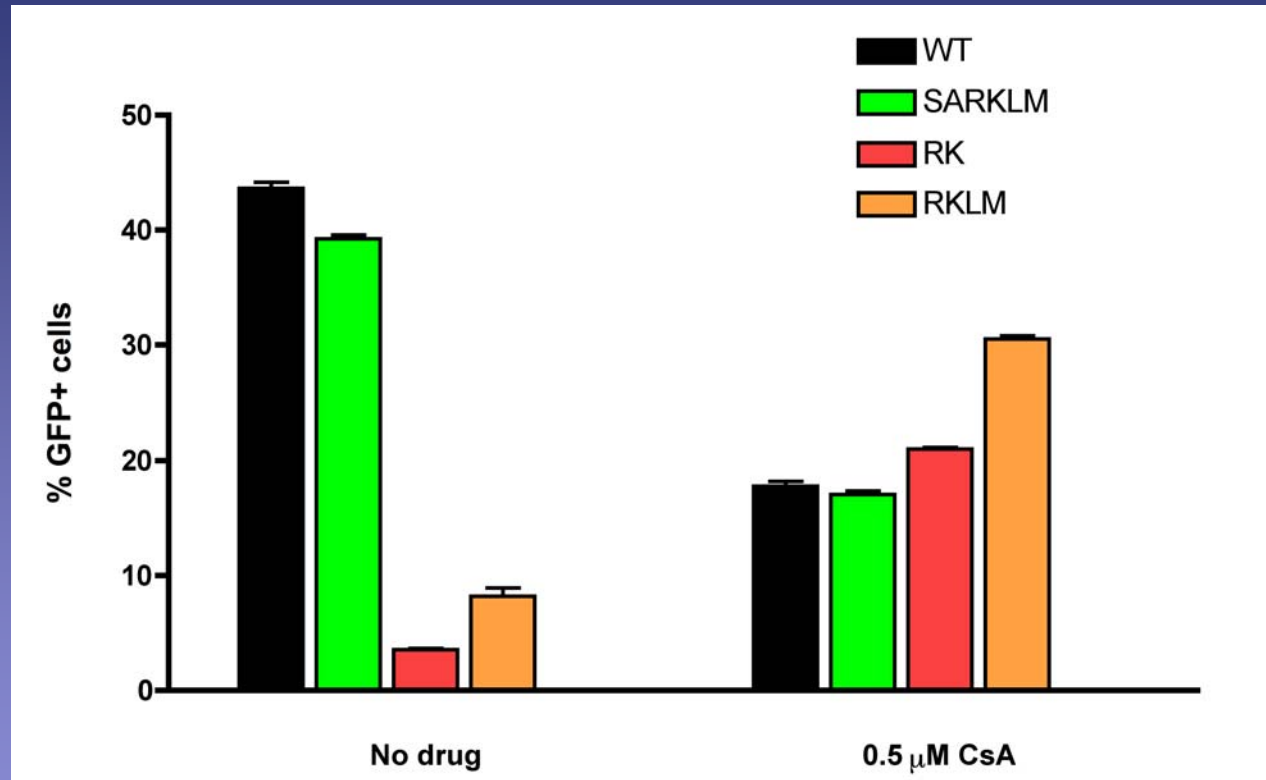
R₂₆₄K renders HIV Cyclosporine A-dependent



	170	175	255	260	265	270	275
pNL4-3	V I P M F	S A L S E G	// P P I P V G E I Y	K R W I I L G L N K	I V R M Y		
L268M	.	.	//	.	.	M	.
R264K	.	.	//	.	K	.	.
S173A	.	A	//

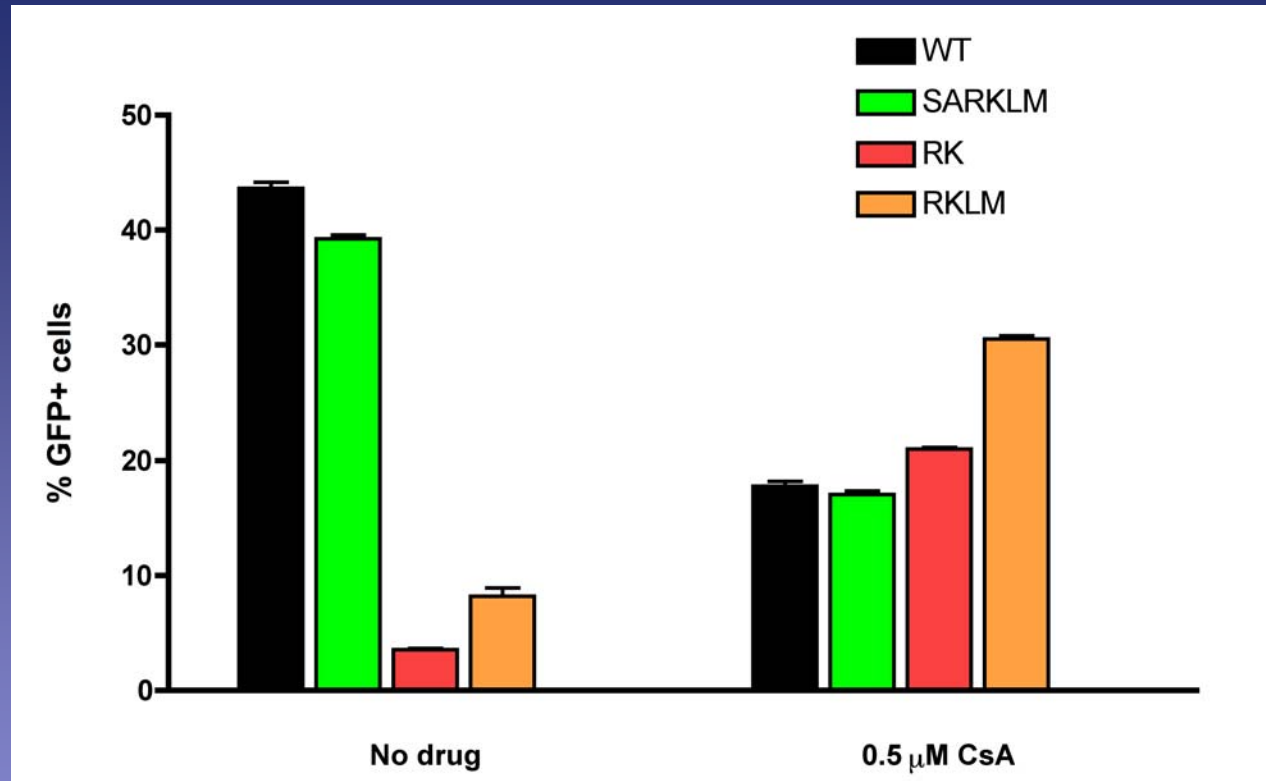
Cyclophilin A is required for normal infectivity

S₁₇₃A restores normal CypA phenotype



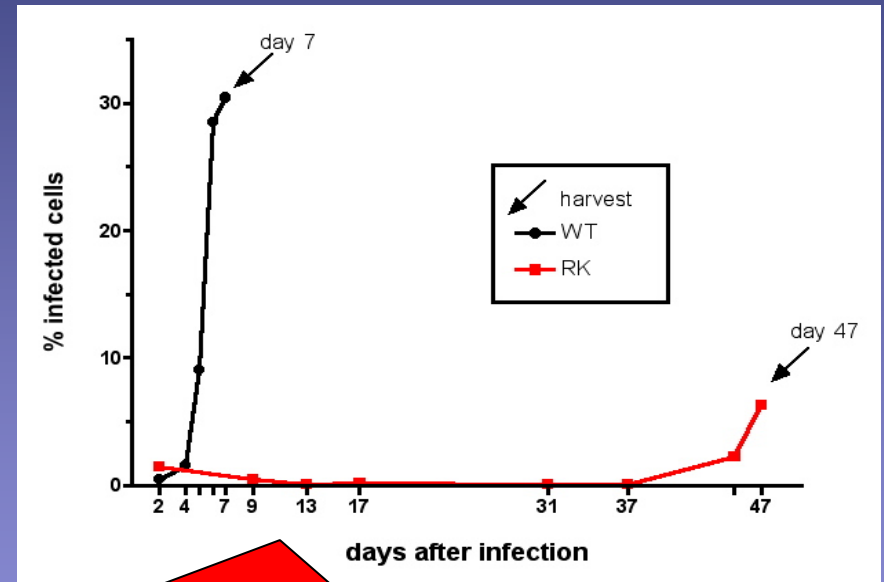
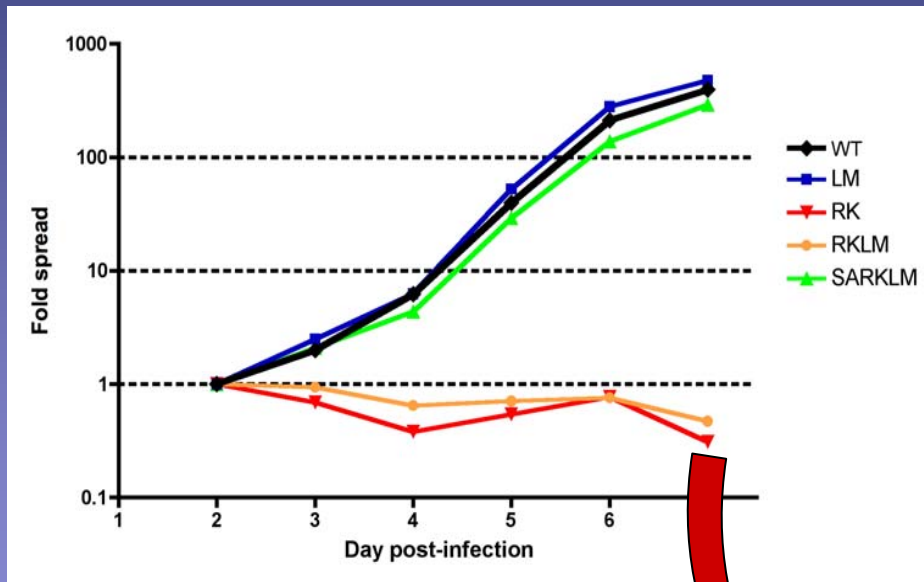
	170	175	255	260	265	270	275
pNL4-3	V I P M F	S A L S E G	// P P I P V G E I Y	K R W I I L G L N K	I V R M Y		
L268M	.	.	//	.	.	M	.
R264K	.	.	//	.	K	.	.
S173A	.	A	//

Summary KK10



- CTL escape in KK 10 dramatically reduces viral replication and renders HIV CsA-dependent
- Compensatory mutation restores viral fitness and CypA-dependent phenotype

Alternative compensation for $R_{264}K$



Summary

- Two highly protective HLA alleles (B57 and B27) immunodominantly present epitopes from adjacent conserved regions of the HIV-1 capsid protein
- Escape in both epitopes is associated with a reduction in viral fitness and efficient replication requires one or more compensatory mutations
- Defects of TW10 and KK10 escape variants are modulated by cellular cyclophilin A action
 - * Compensation in B57-TW10 escape renders HIV cyclophilin A-independent
 - * Escape in B27-KK10 renders HIV cyclosporine A-dependent
- Functional constraints on capsid limit the generation of escape mutations and their effectiveness
- The combination of a strong immune pressure and a significant fitness cost imposed by CTL escape might explain the protective effect of certain HLA-haplotypes

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Sandra Müller

Thomas Harrer

Andrew McMichael

Bruce Walker

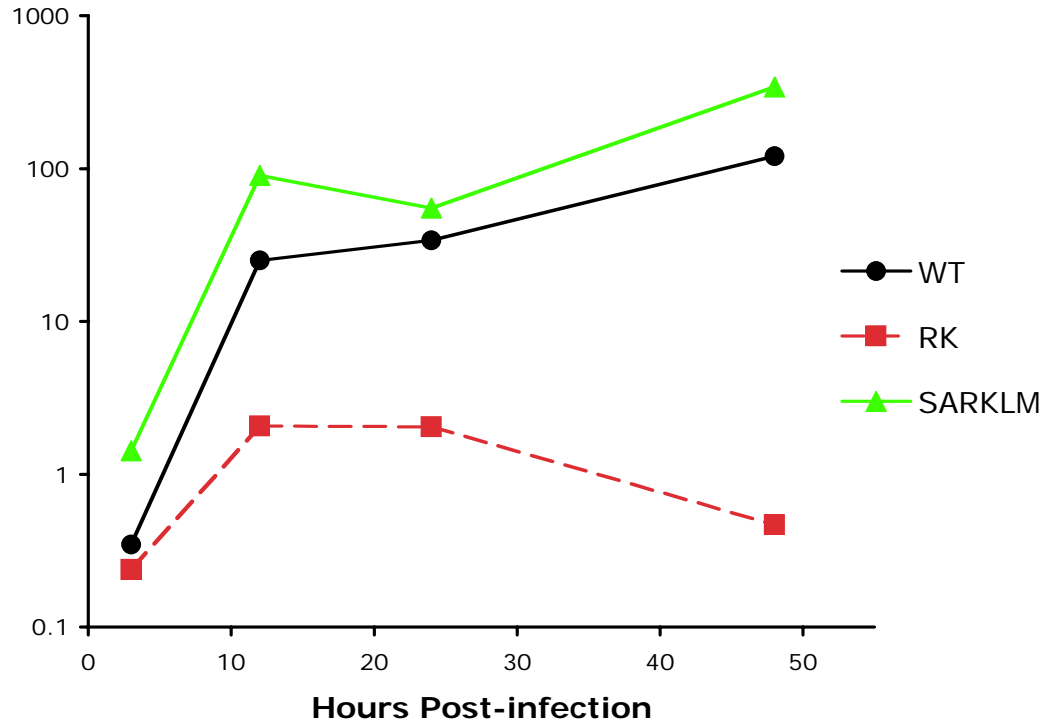
Todd Allen

All blood donors

Funding: R01 AI054178; R21 AI067078; AIEDRP

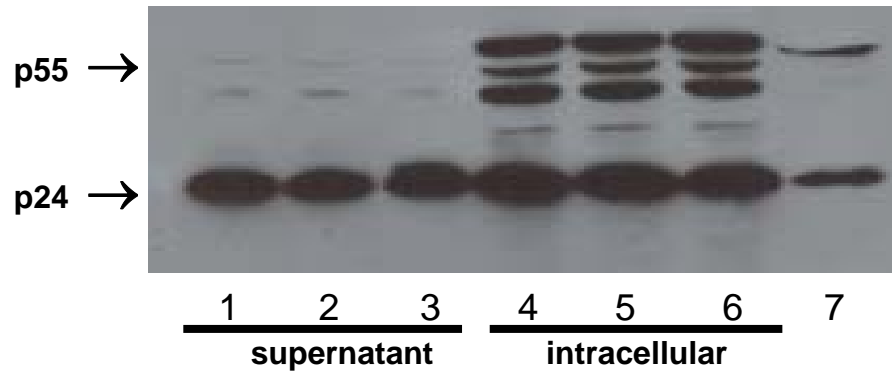
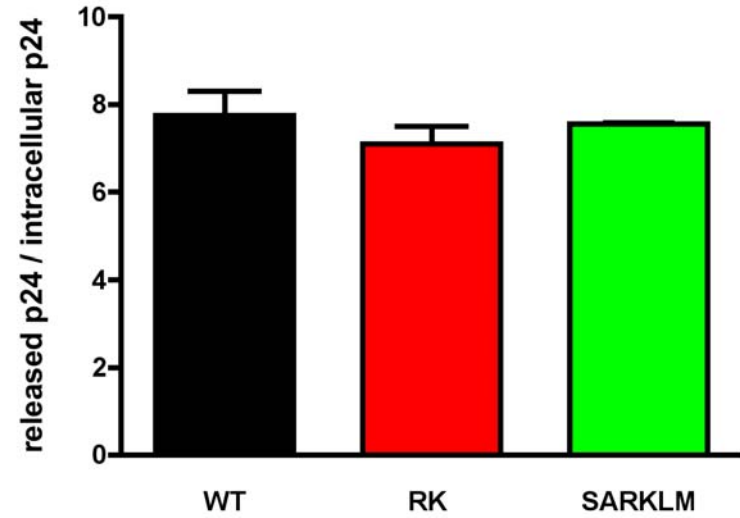
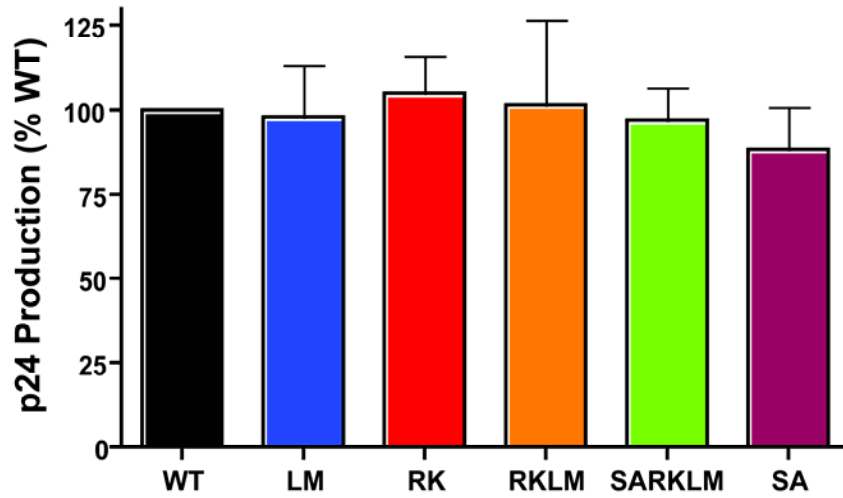


Rescue slide

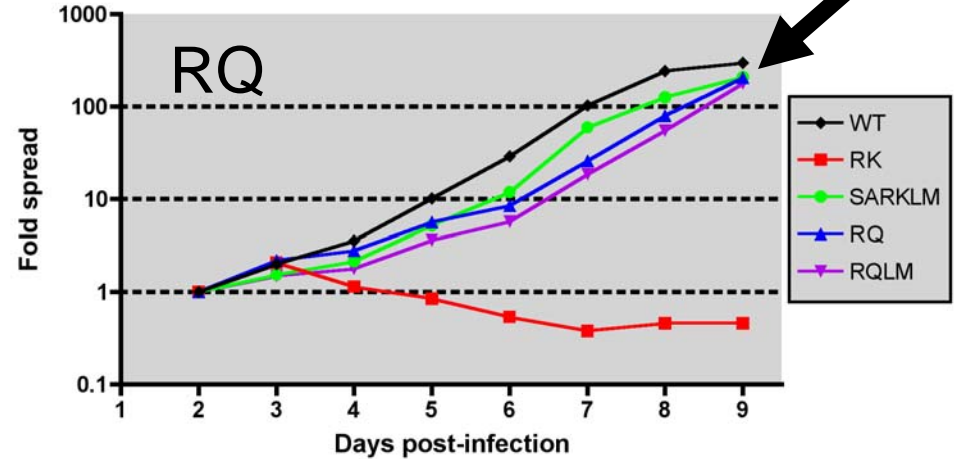
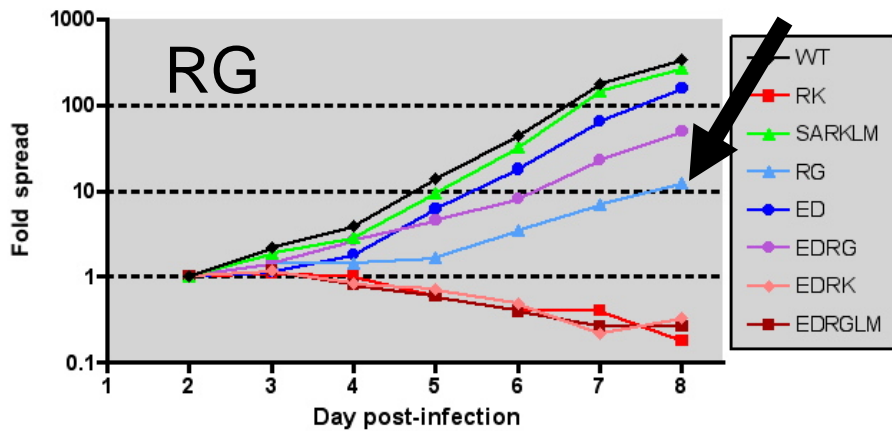
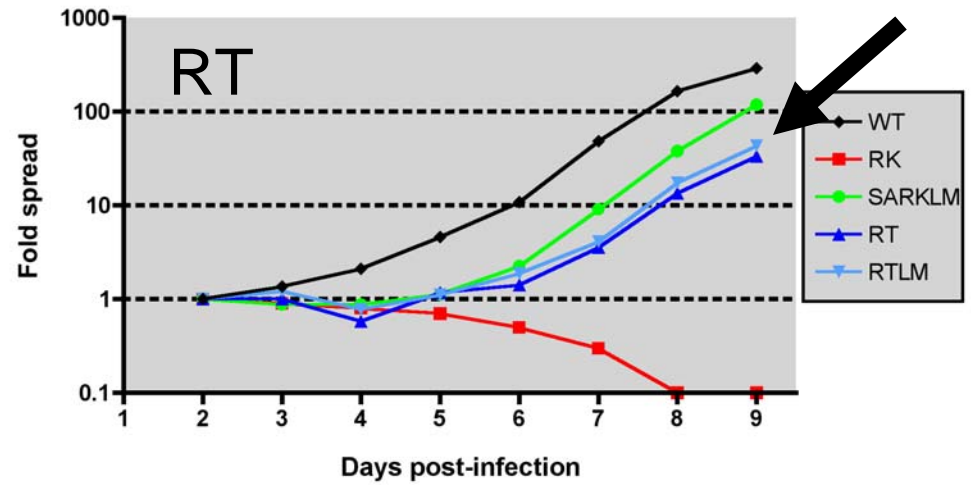
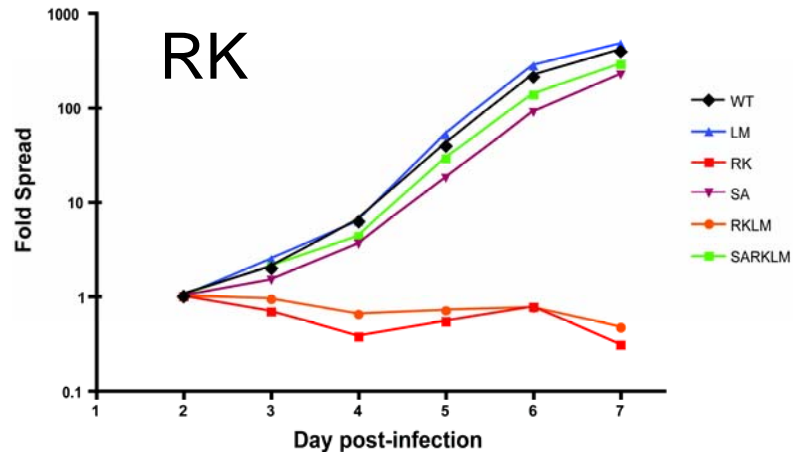


	170	175	255	260	265	270	275																													
pNL4-3	V	I	P	M	F	S	A	L	S	E	G	//	P	P	I	P	V	G	E	I	Y	K	R	W	I	I	L	G	L	N	K	I	V	R	M	Y
L268M	//	M
R264K	//	K
S173A	A	//

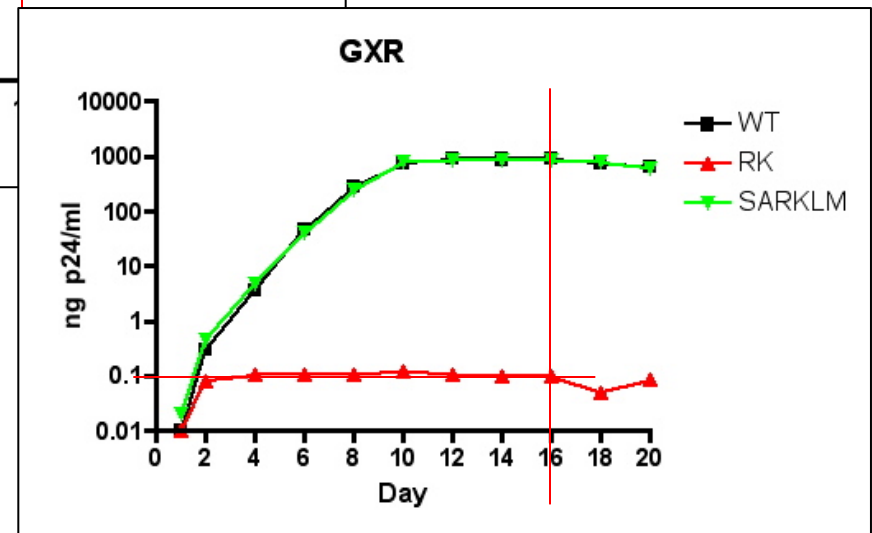
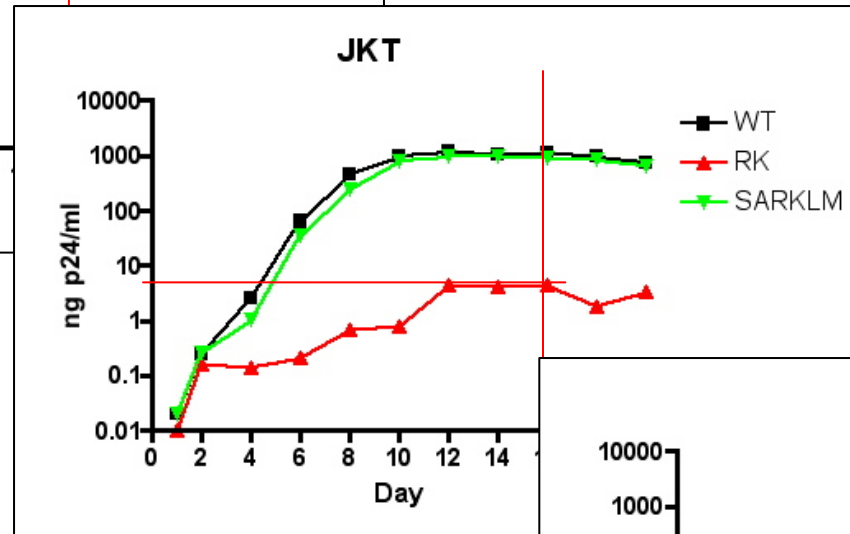
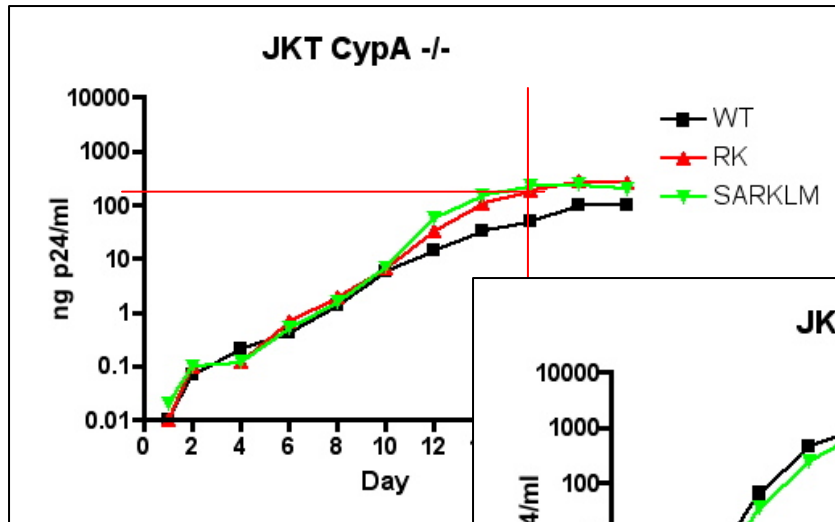
Rescue slide



Rescue slide



Rescue slide



Rescue slide

