

Molecular arms races between primate and viral genomes

Harmit S. Malik
Division of Basic Sciences
Fred Hutchinson Cancer Research Center

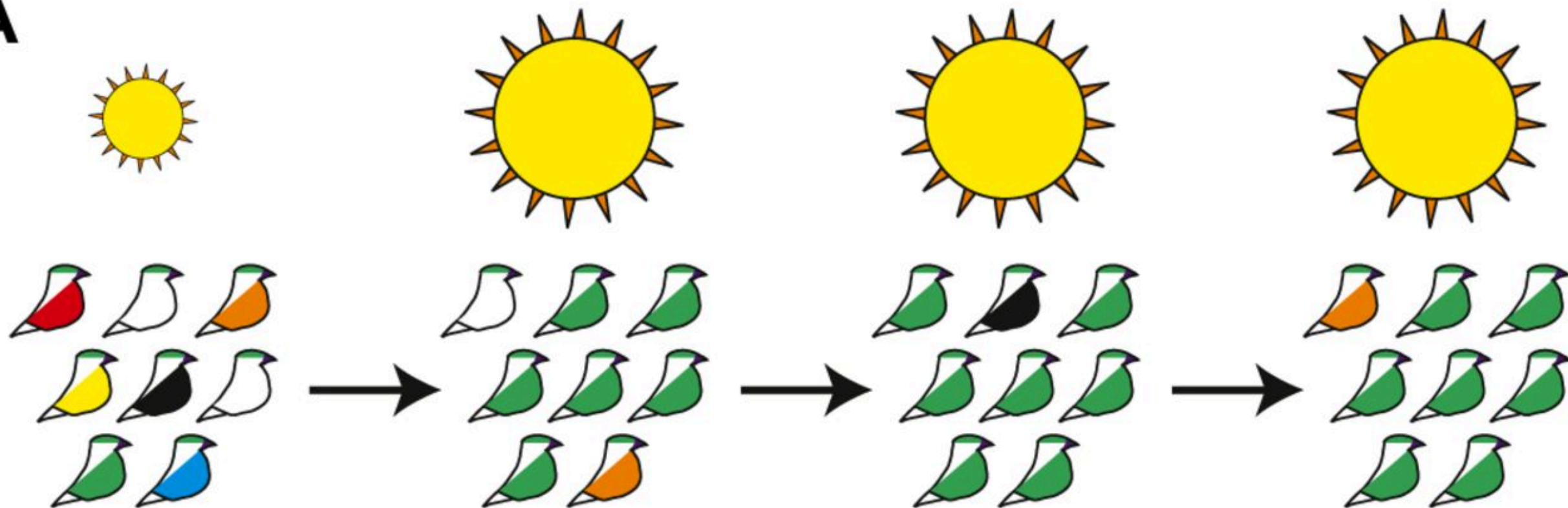
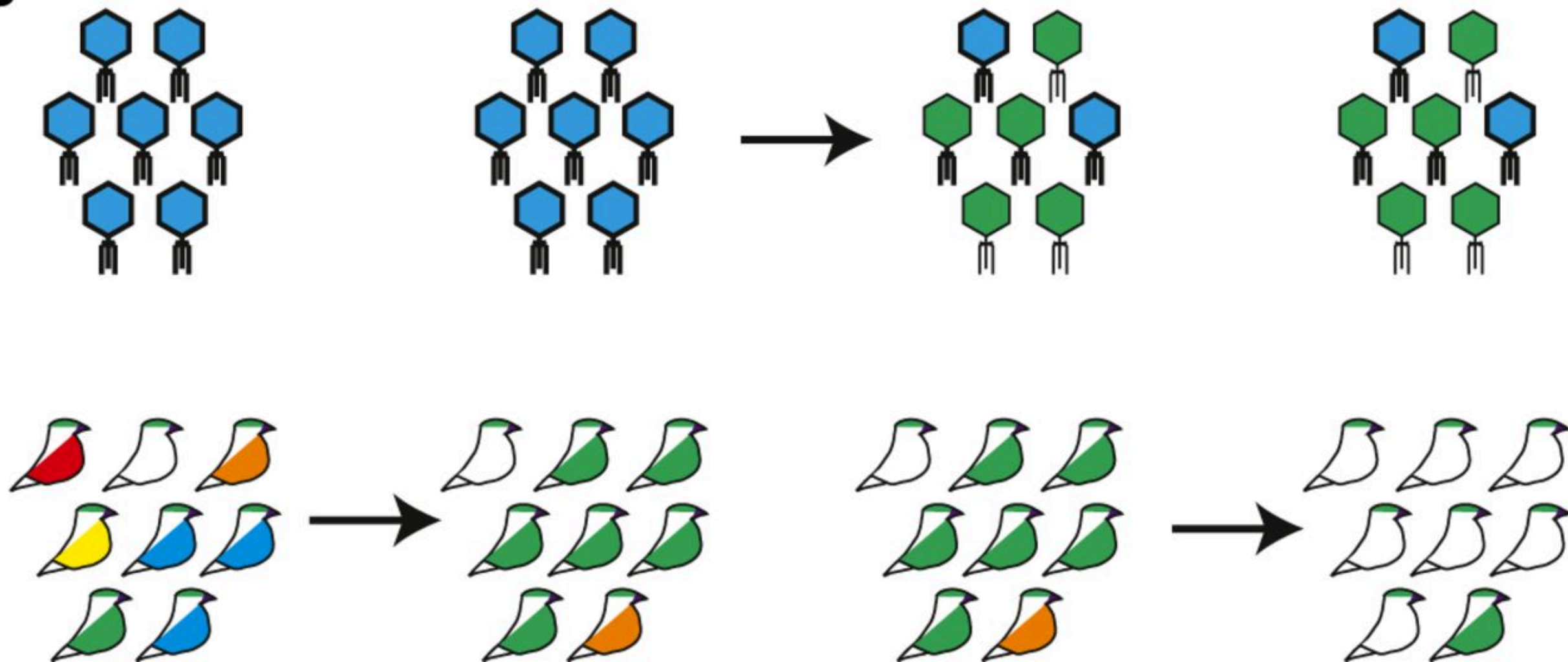


The Red Queen: “It takes all the running you can do, to keep in the same place.”

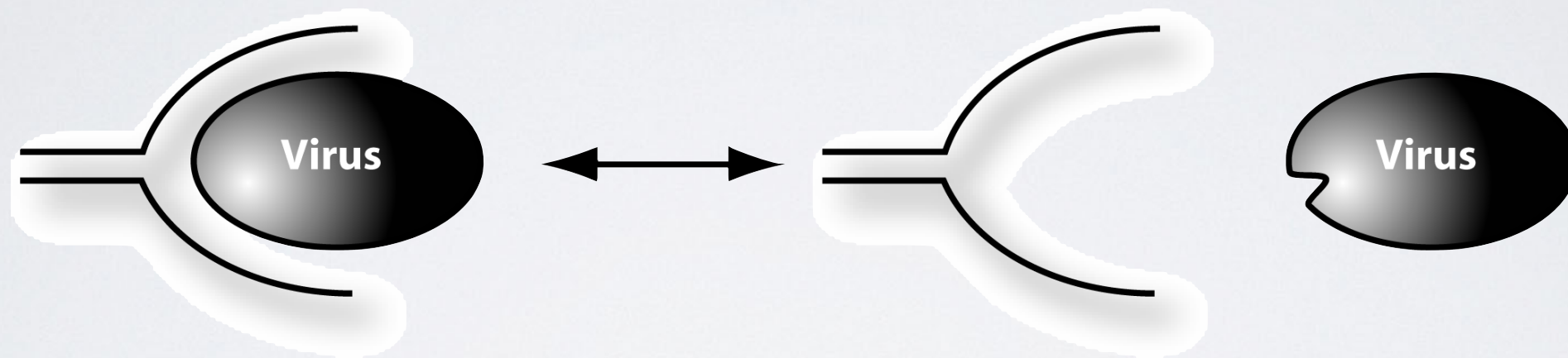
--Lewis Carroll, “Through the Looking Glass”

The Red Queen Hypothesis: “For an evolutionary system, continuing development is needed just in order to maintain its fitness relative to the systems it is co-evolving with.”

--Leigh Van Valen (1973)

A**B**

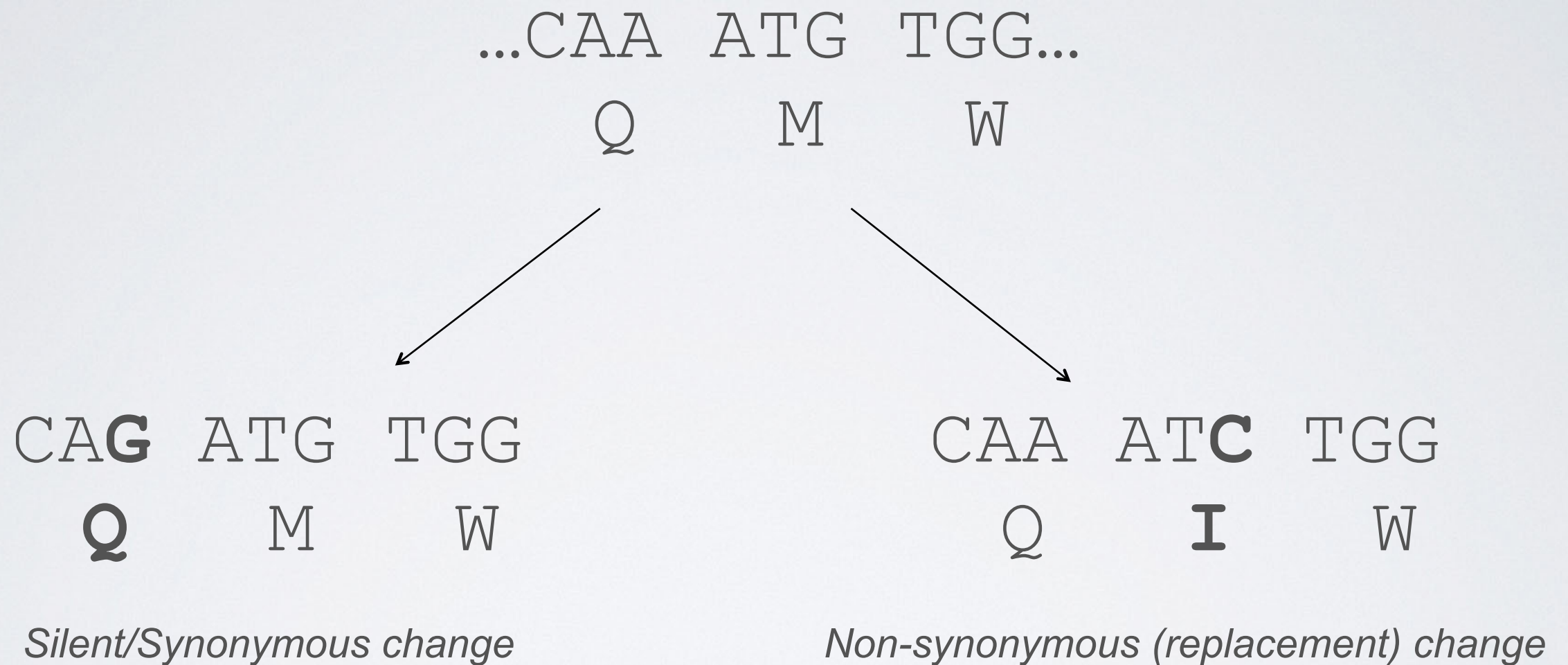
...the usual suspects



One party is always 'losing'
There is always an evolutionary advantage to be gained by innovation

Quantifying 'innovation' in protein-coding genes

(a brief primer)



Quantifying 'innovation' in protein-coding genes

(a brief primer)

Diversifying selection

Positive selection (adaptive evolution)

... CAT GCG CGT ATG ATA ACT ... (..HARMIT..)
... CCT GCG CTT ATG ATG ACT ... (..pAlMmT..)
... CAT GTG CGA ATG ATA ACT ... (..HvRMIT..)
... CCT GCG AGT AGT GTA ACT ... (..pAssaT..)

$\frac{\text{Actual number of synonymous changes}}{\text{Possible number of synonymous changes}}$

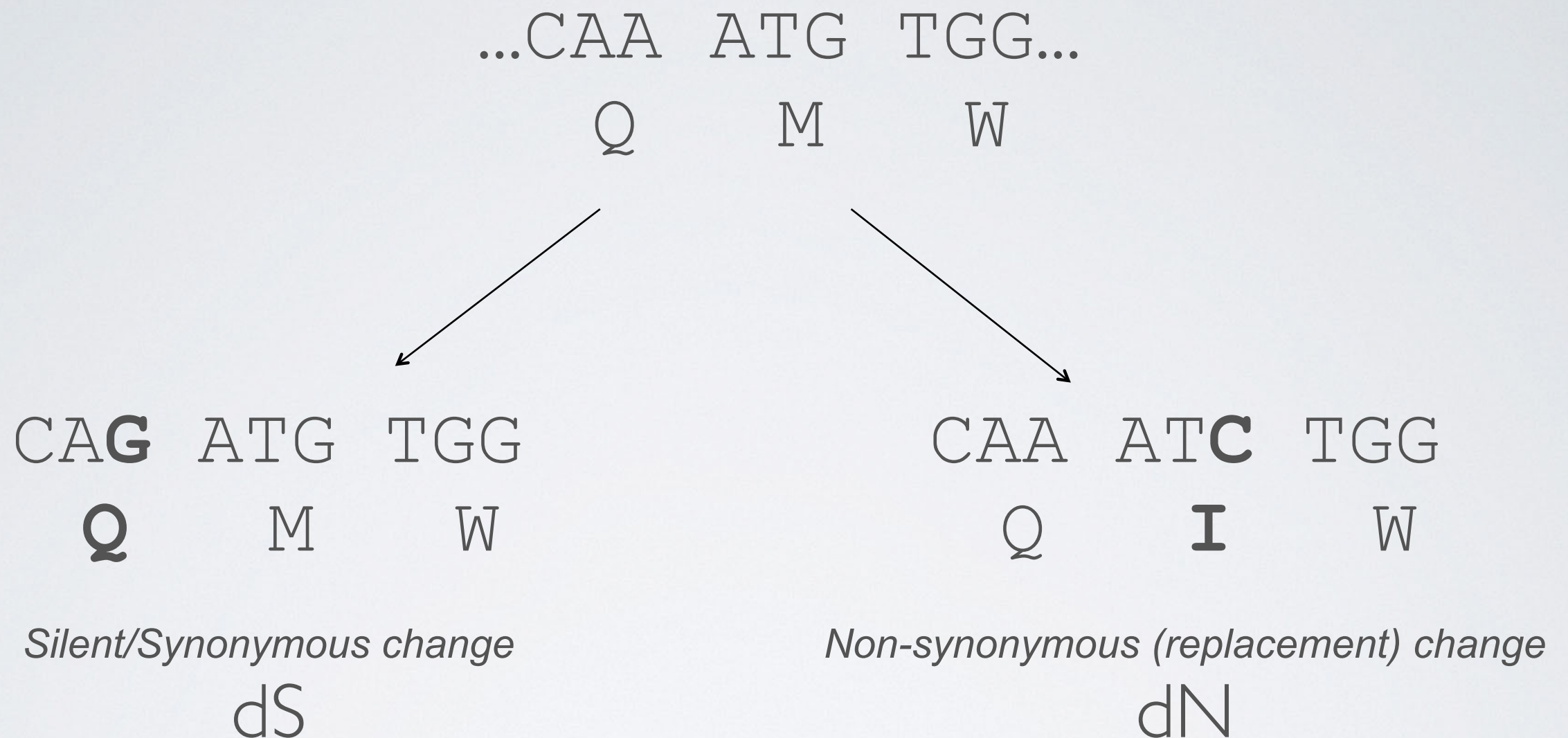
<

$\frac{\text{Actual number of replacement changes}}{\text{Possible number of replacement changes}}$

$$K_S < K_A \text{ or } R$$

Quantifying 'innovation' in protein-coding genes

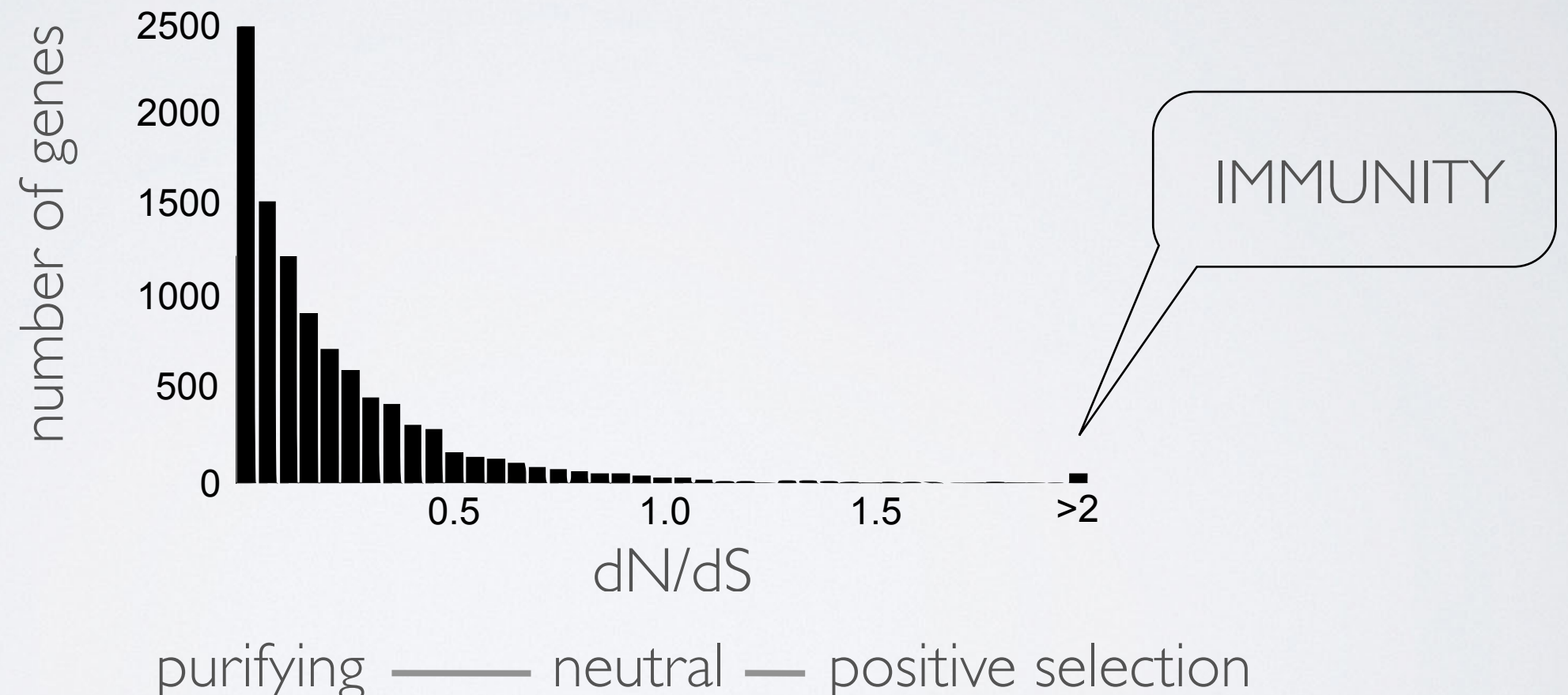
(a brief primer)



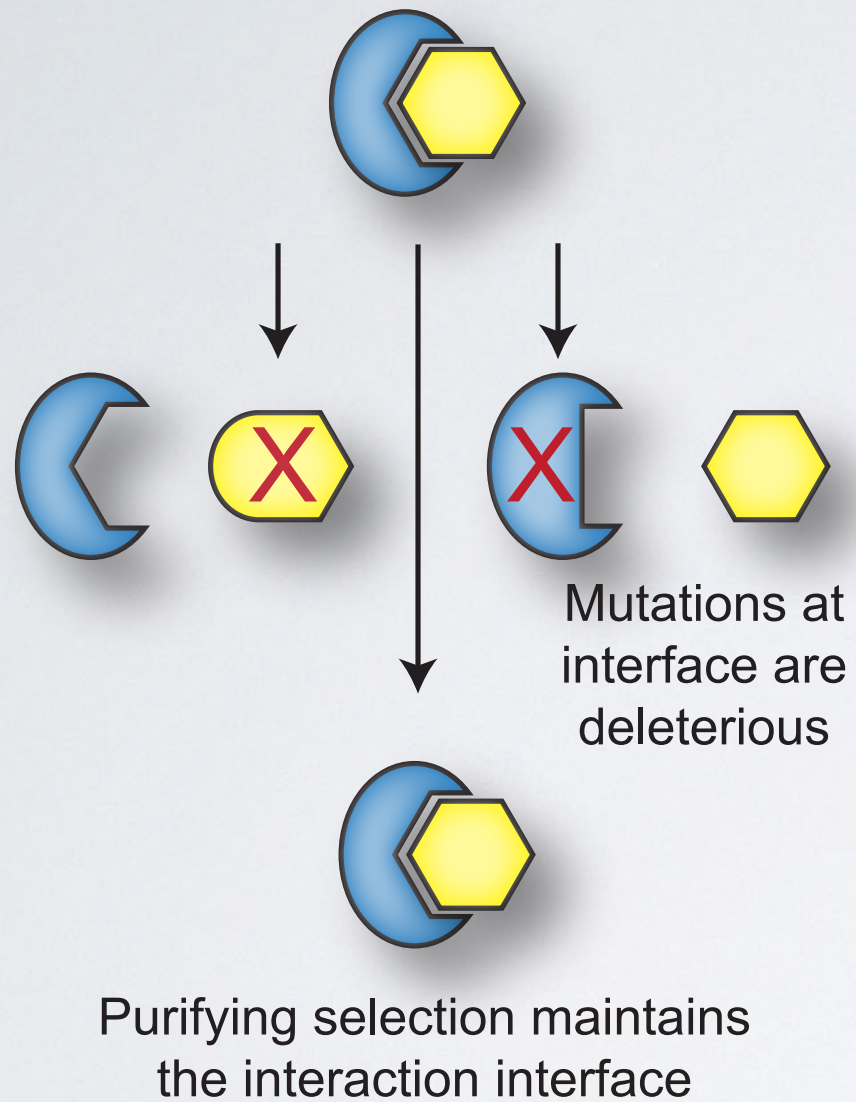
$dN/dS < 1$: purifying selection, high conservation

$dN/dS > 1$: positive selection, constant innovation

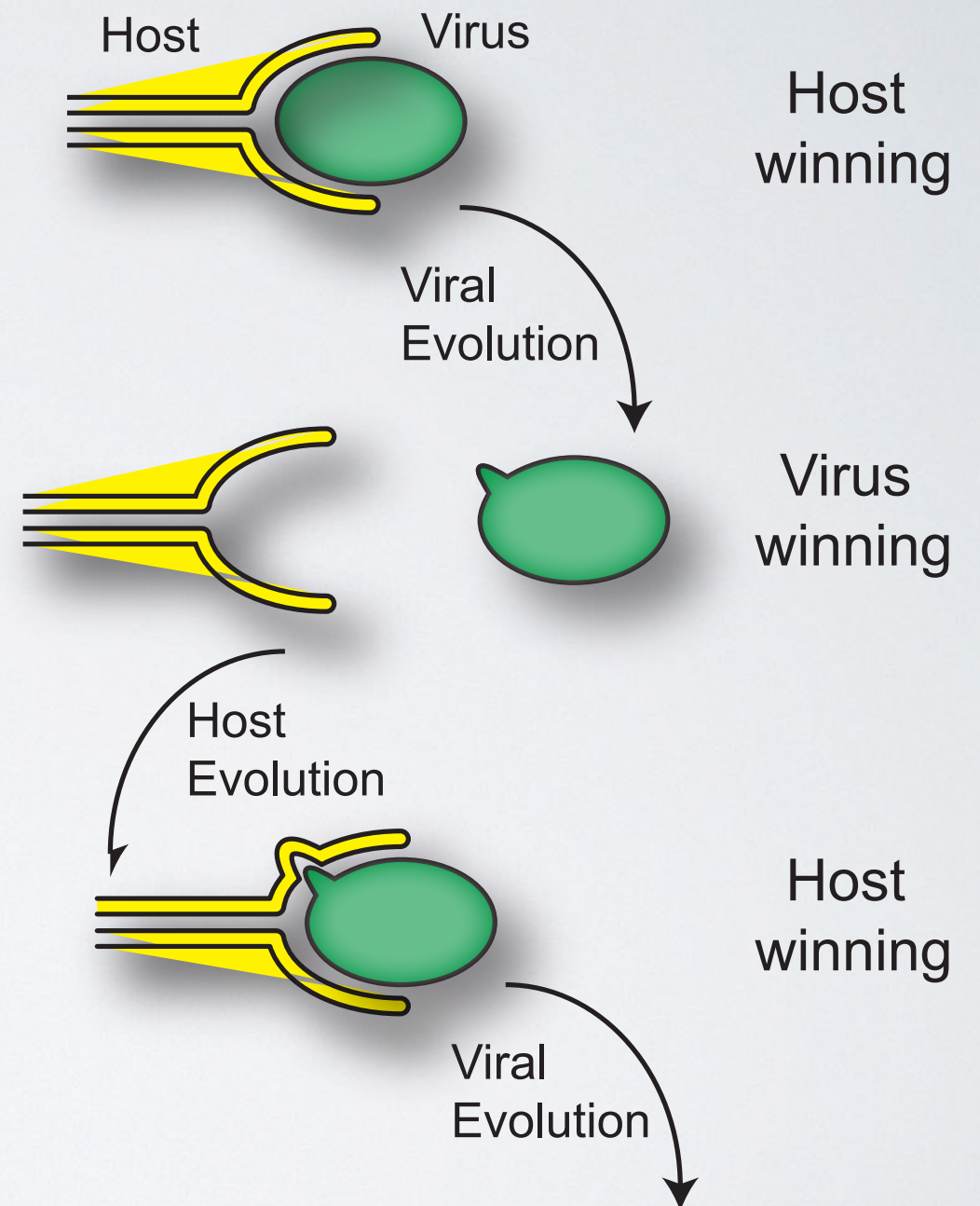
GENOMIC VIEW OF SELECTION



Host-host coevolution

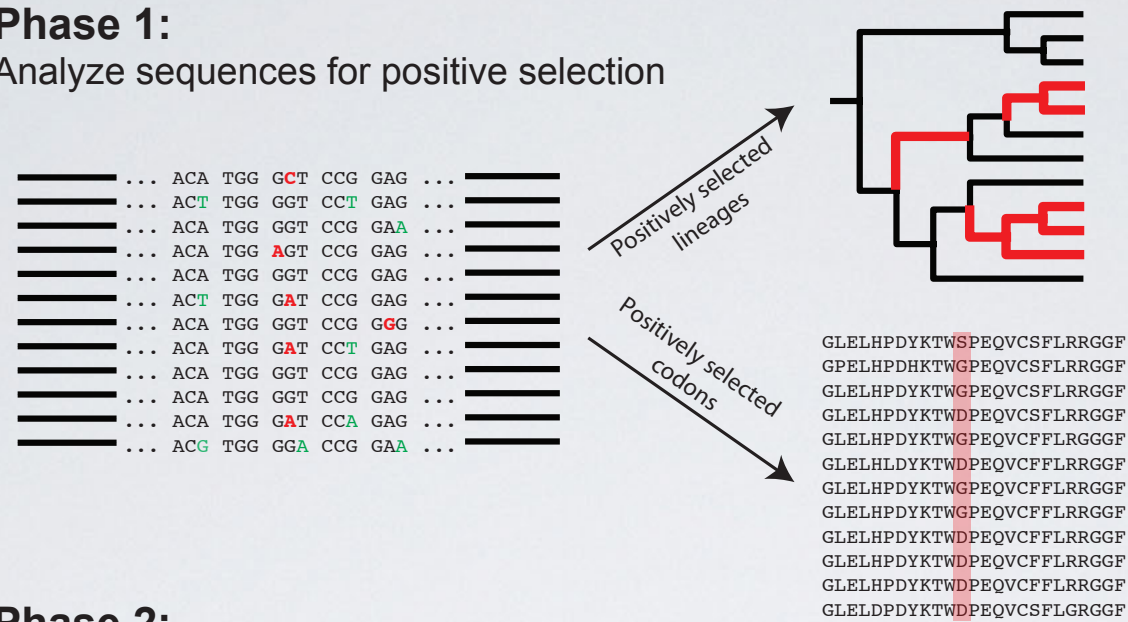


Host-virus arms race



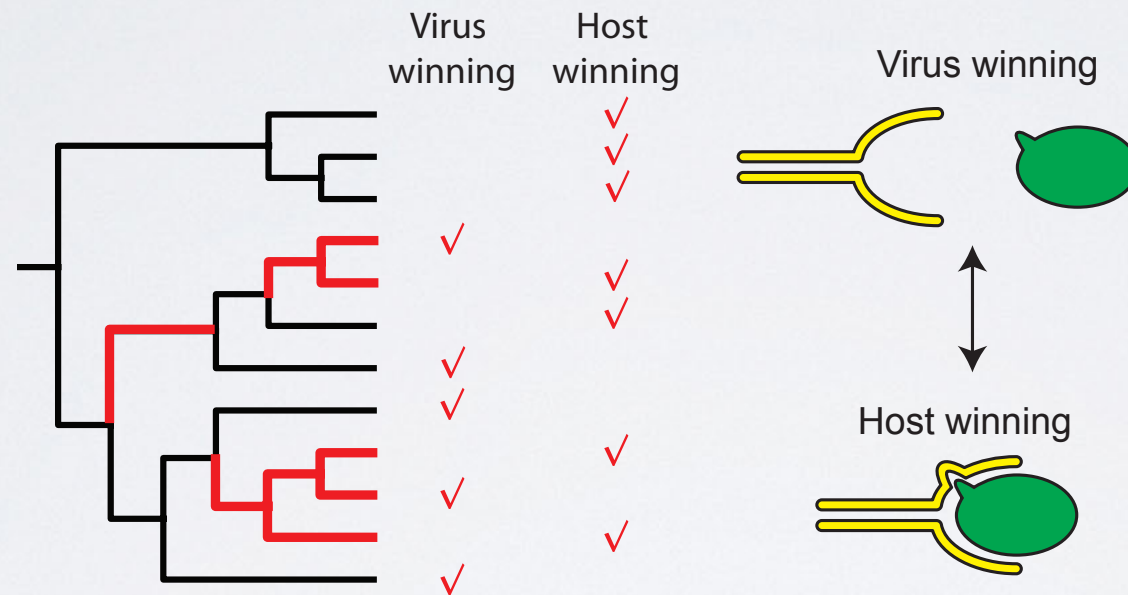
Analyze sequences for positive selection

Analyze sequences for positive selection



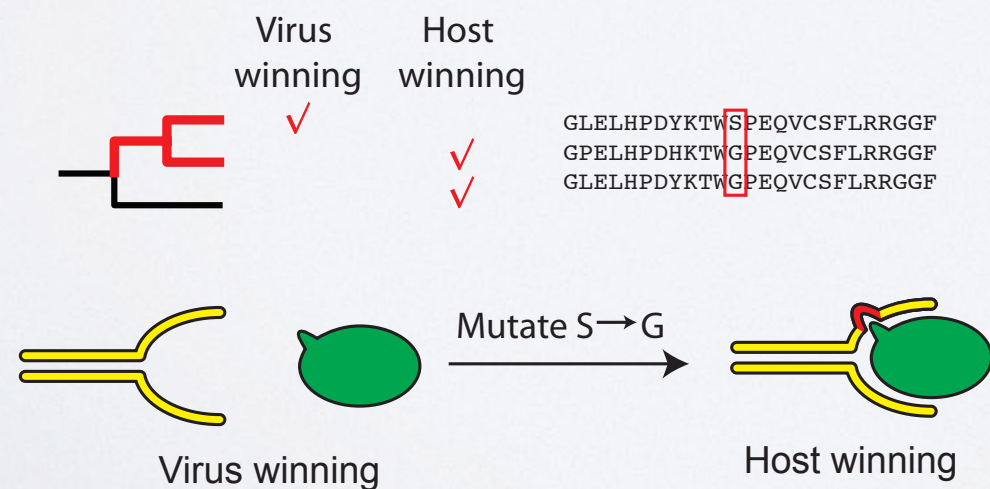
Phase 2:

Determine phenotypic consequences of ortholog variation



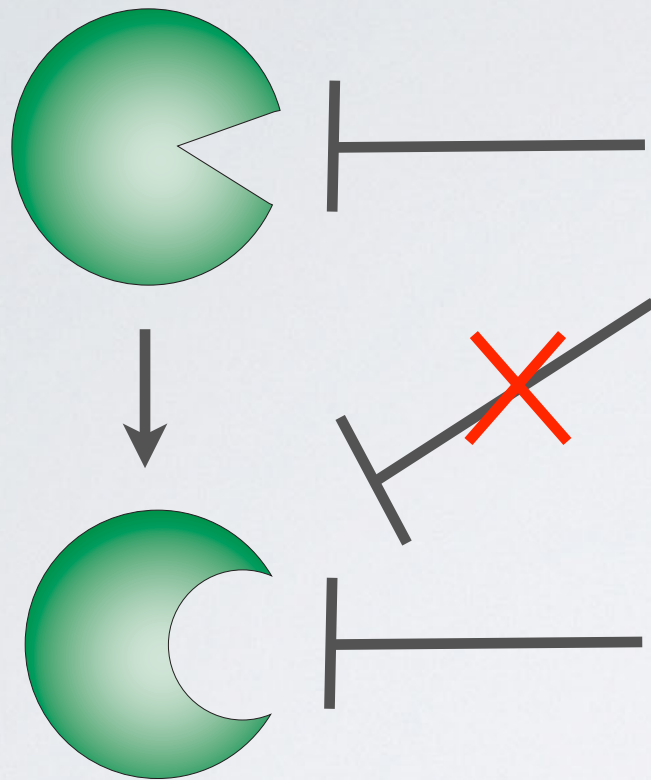
Phase 3:

Map positively selected changes on to host-virus interaction

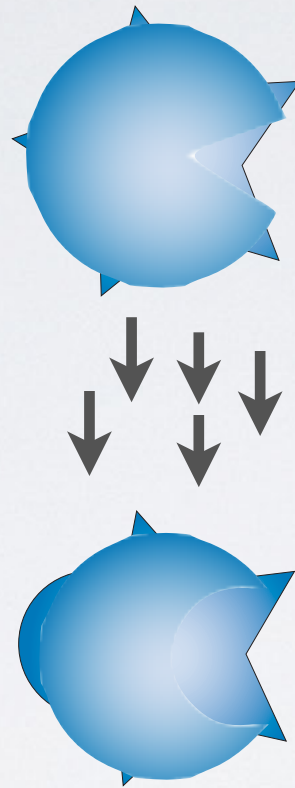


HOST-PATHOGEN EVOLUTION

Antiviral protein



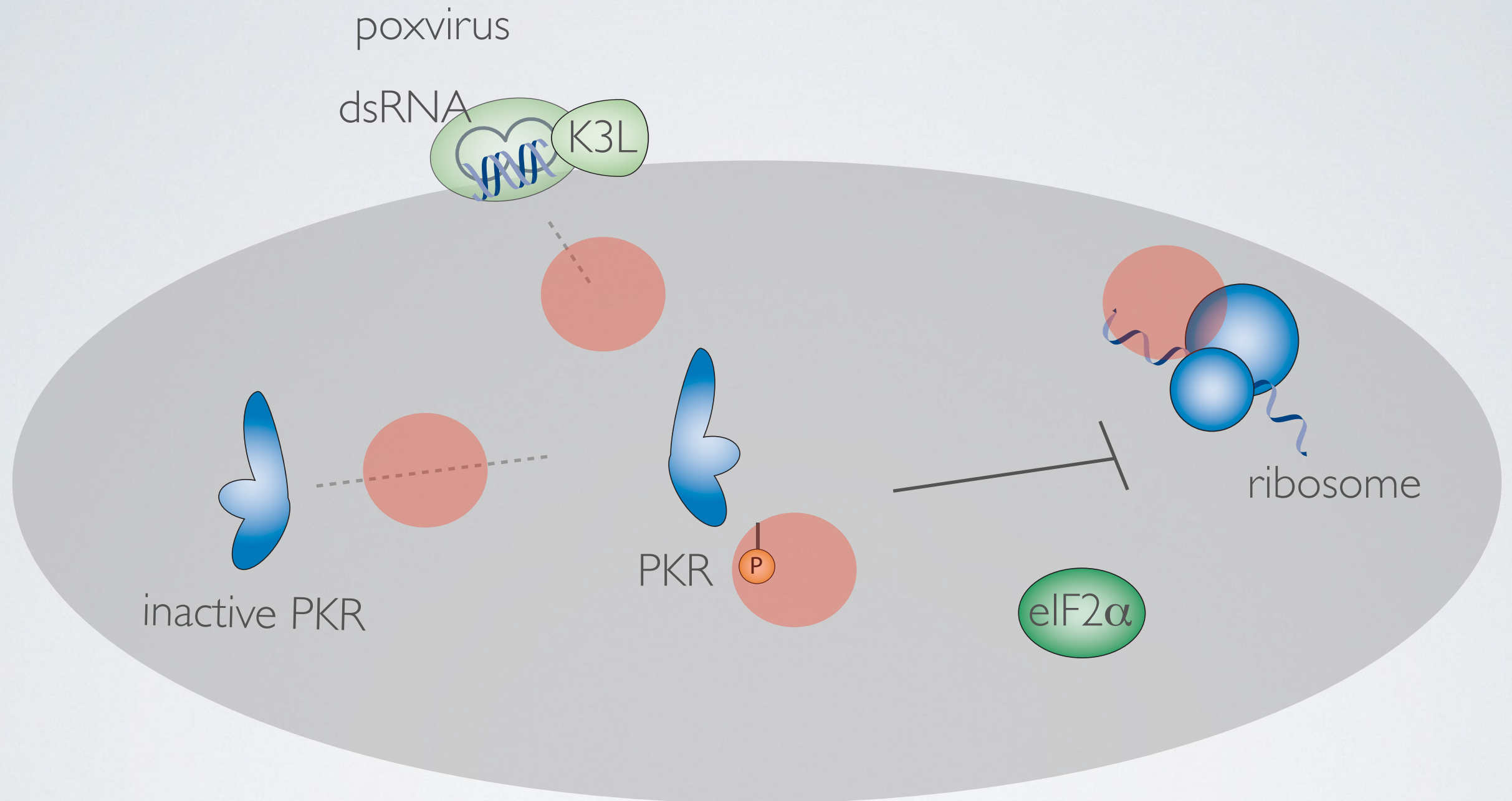
Viral protein



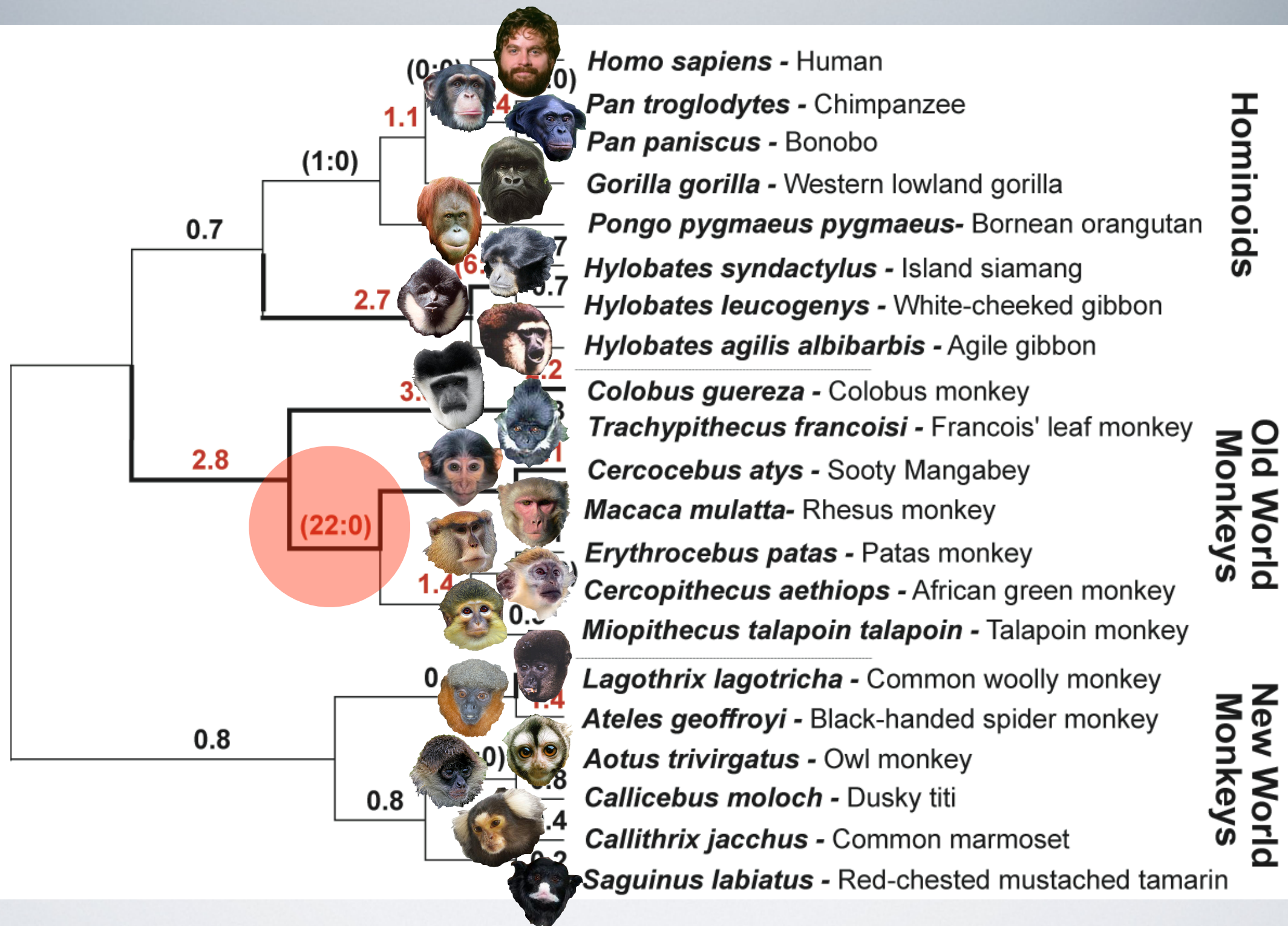
Nels Elde, former postdoc
Now Associate Professor, University of Utah

- the challenge of pathogen mimicry

PROTEIN KINASE R (PKR)



SELECTION ON PRIMATE PKR



Elde, et al., Nature 2009

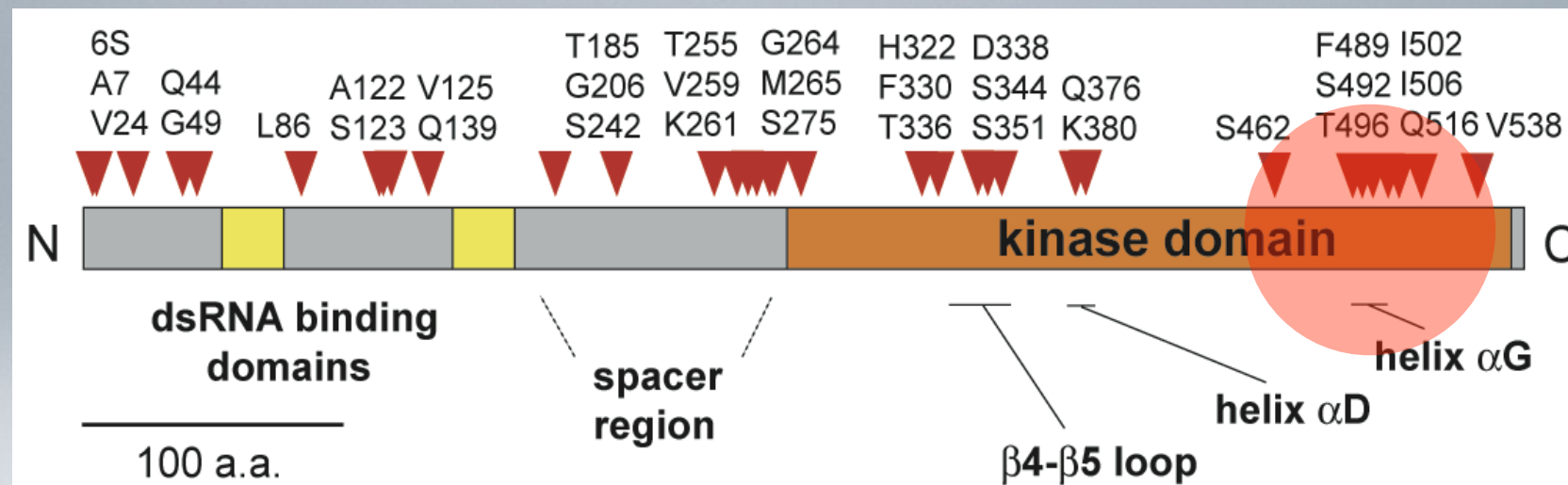
POSITIVE SELECTION ON PKR

Us11 (herpes virus)
NS1A (influenza)
E3L (poxvirus)

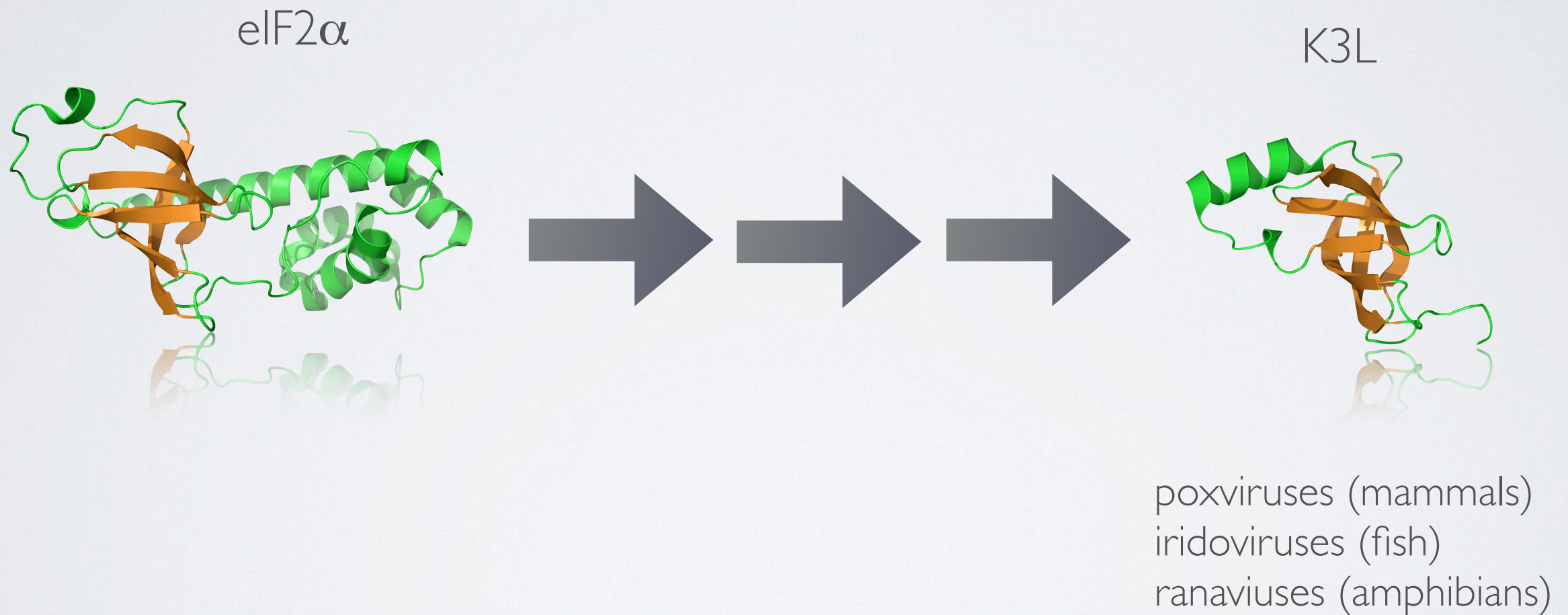
NS5A (HCV)

K3L & E3L (poxvirus)
RelF2H (ranavirus)

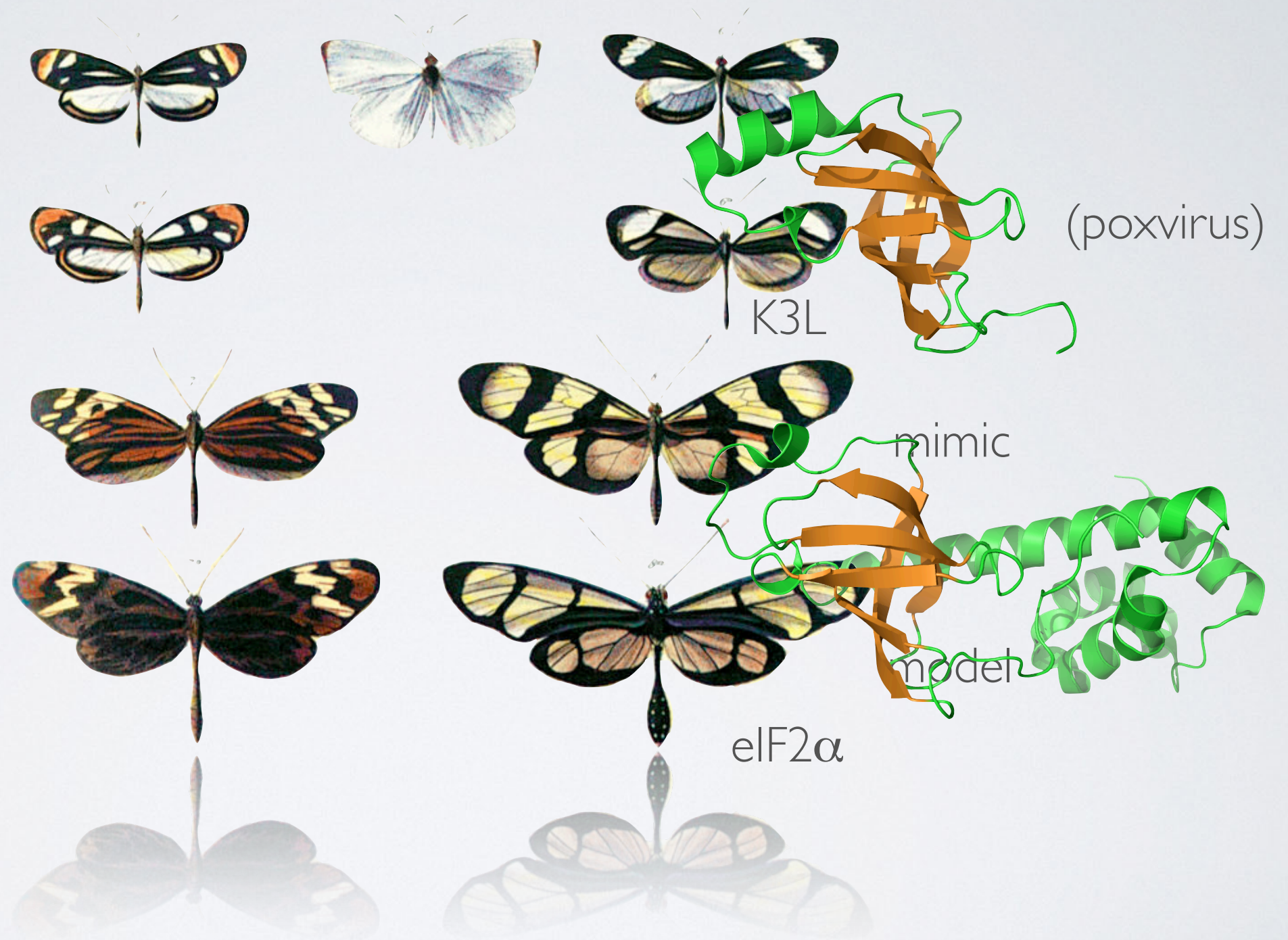
sites under
positive selection:



THE ORIGINS OF MIMICRY



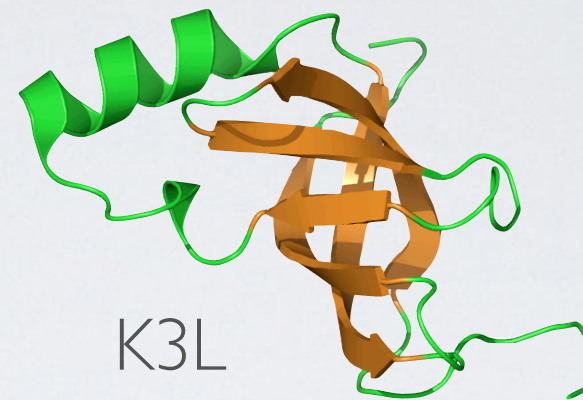
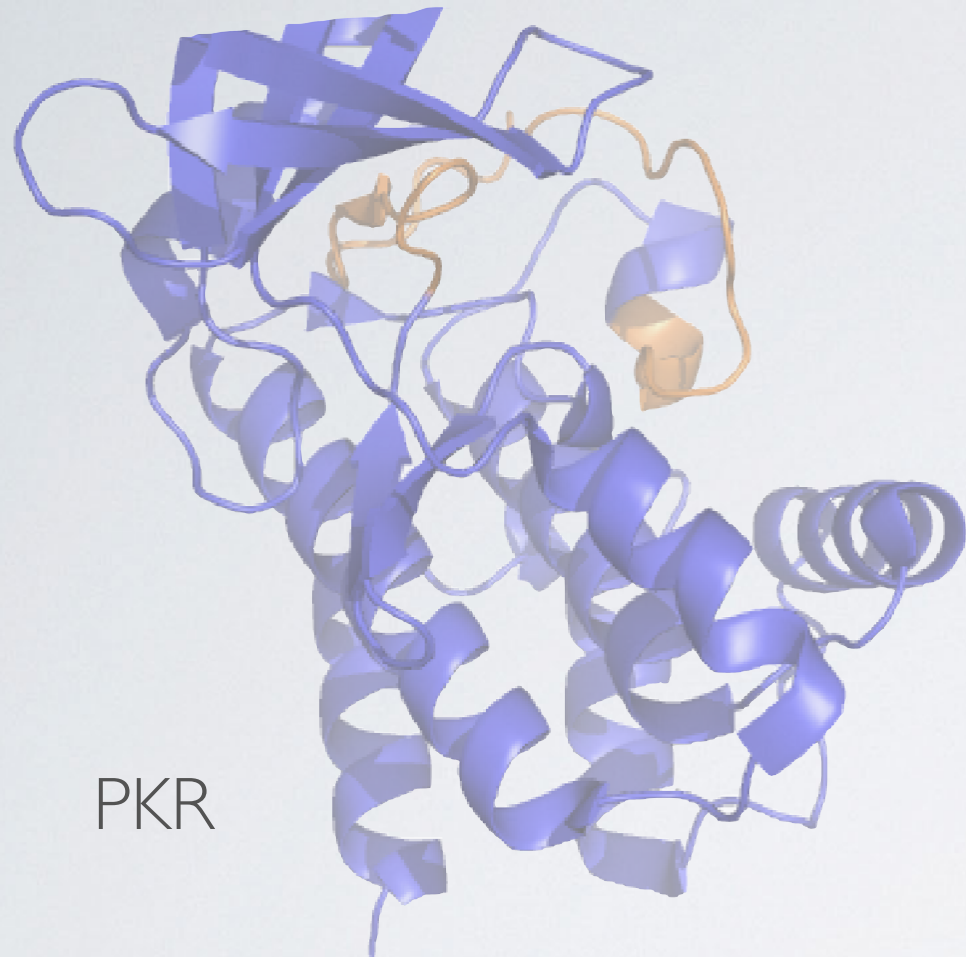
MIMICRY AND CONFLICT



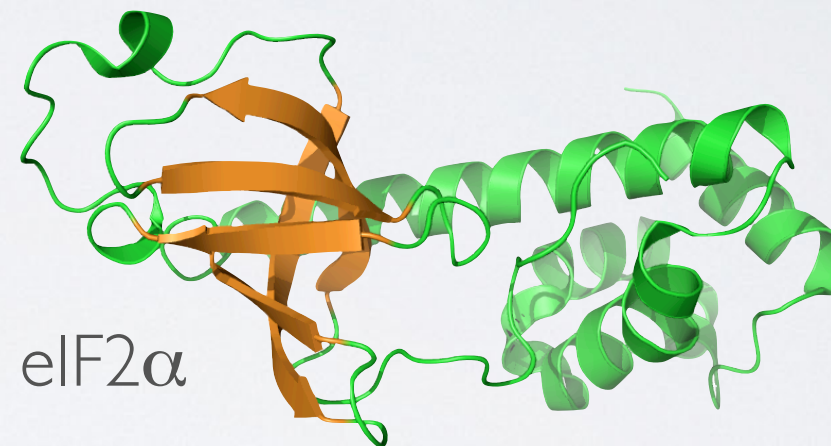
Henry Walter Bates, 1862

Dar, et al., Mol. Cell 2002

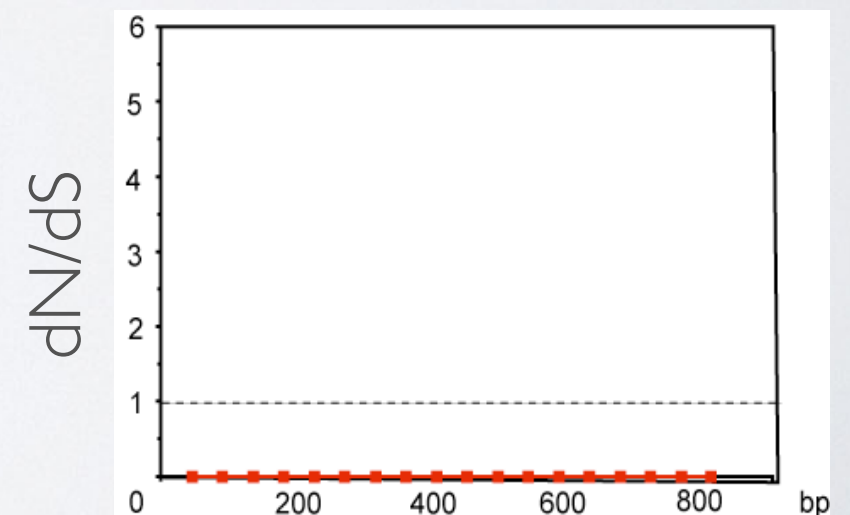
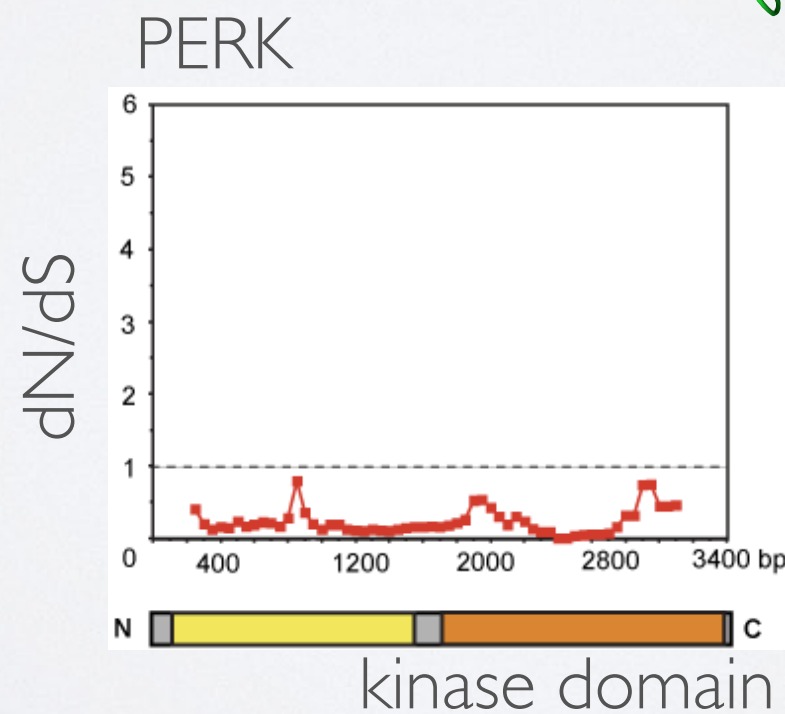
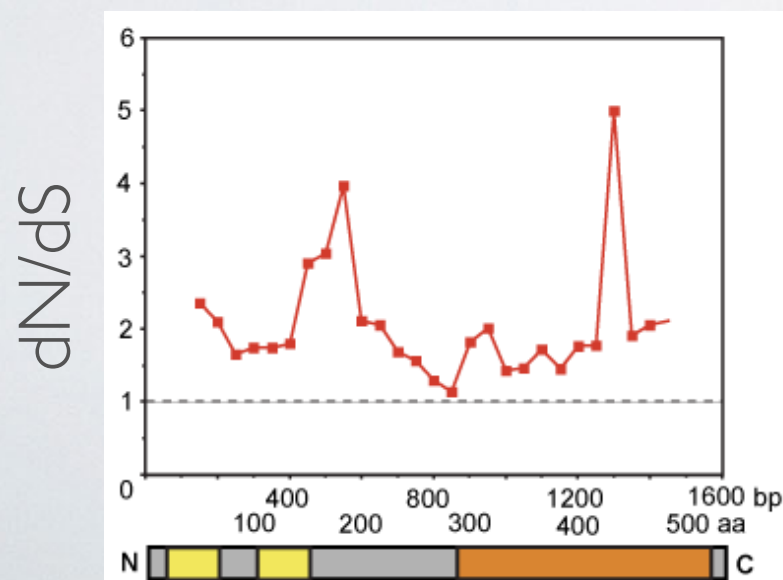
THE CHALLENGE OF MIMICRY



avoid this interaction

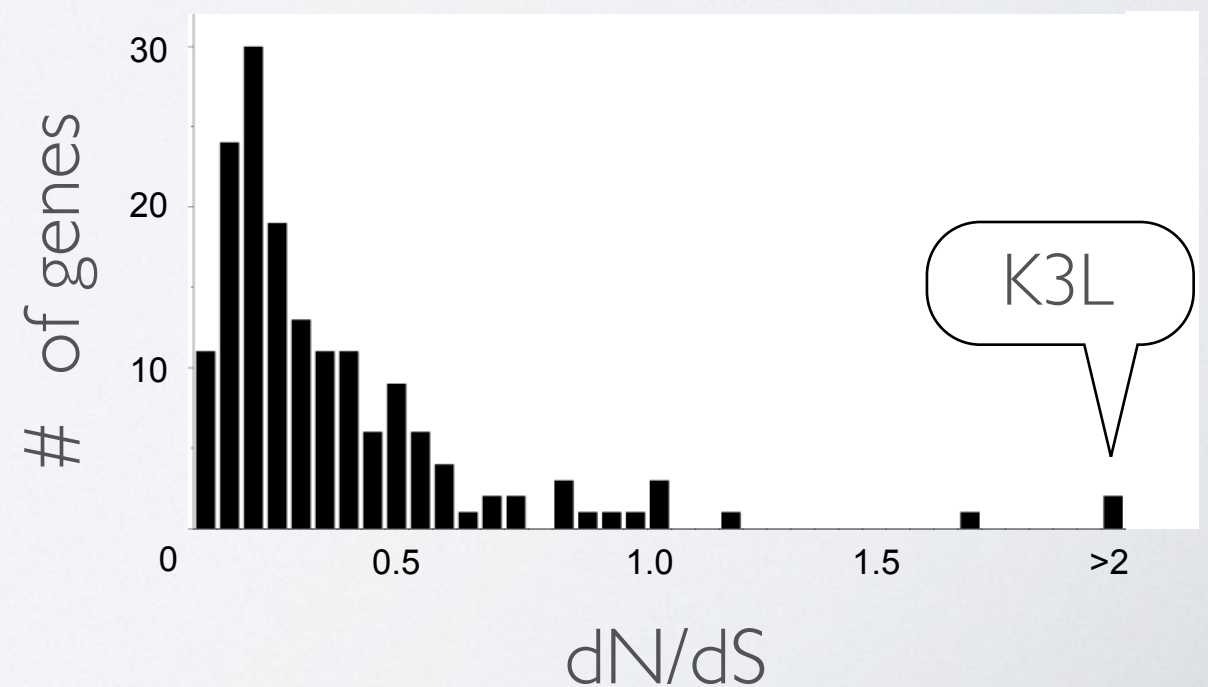
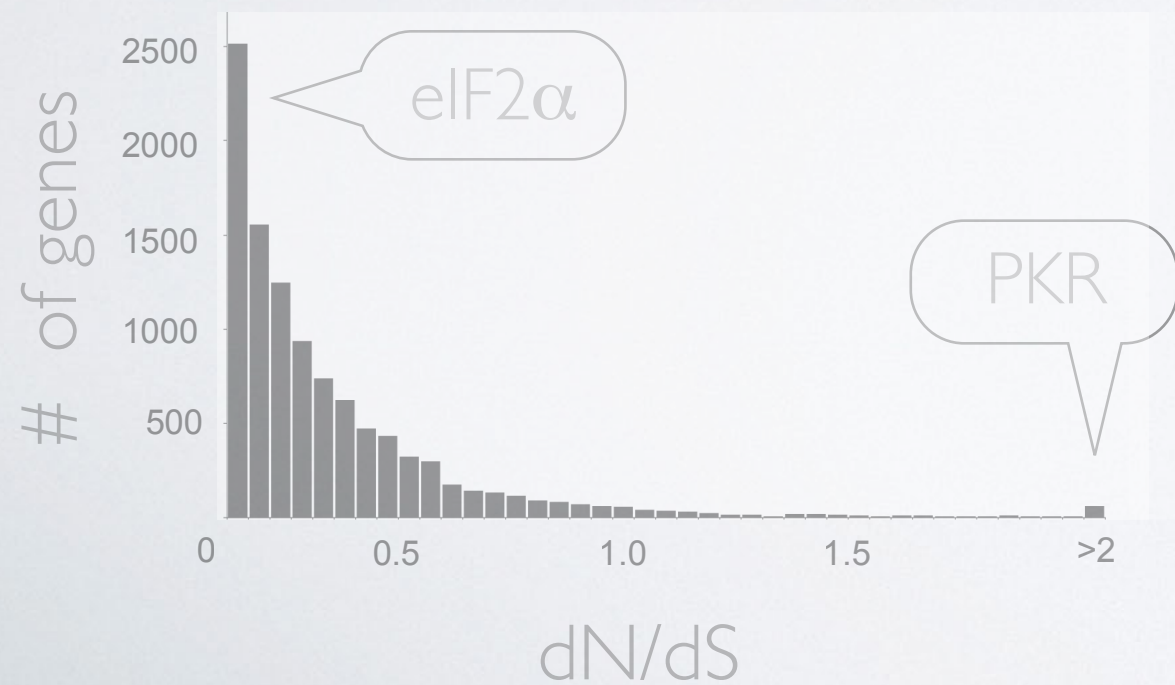
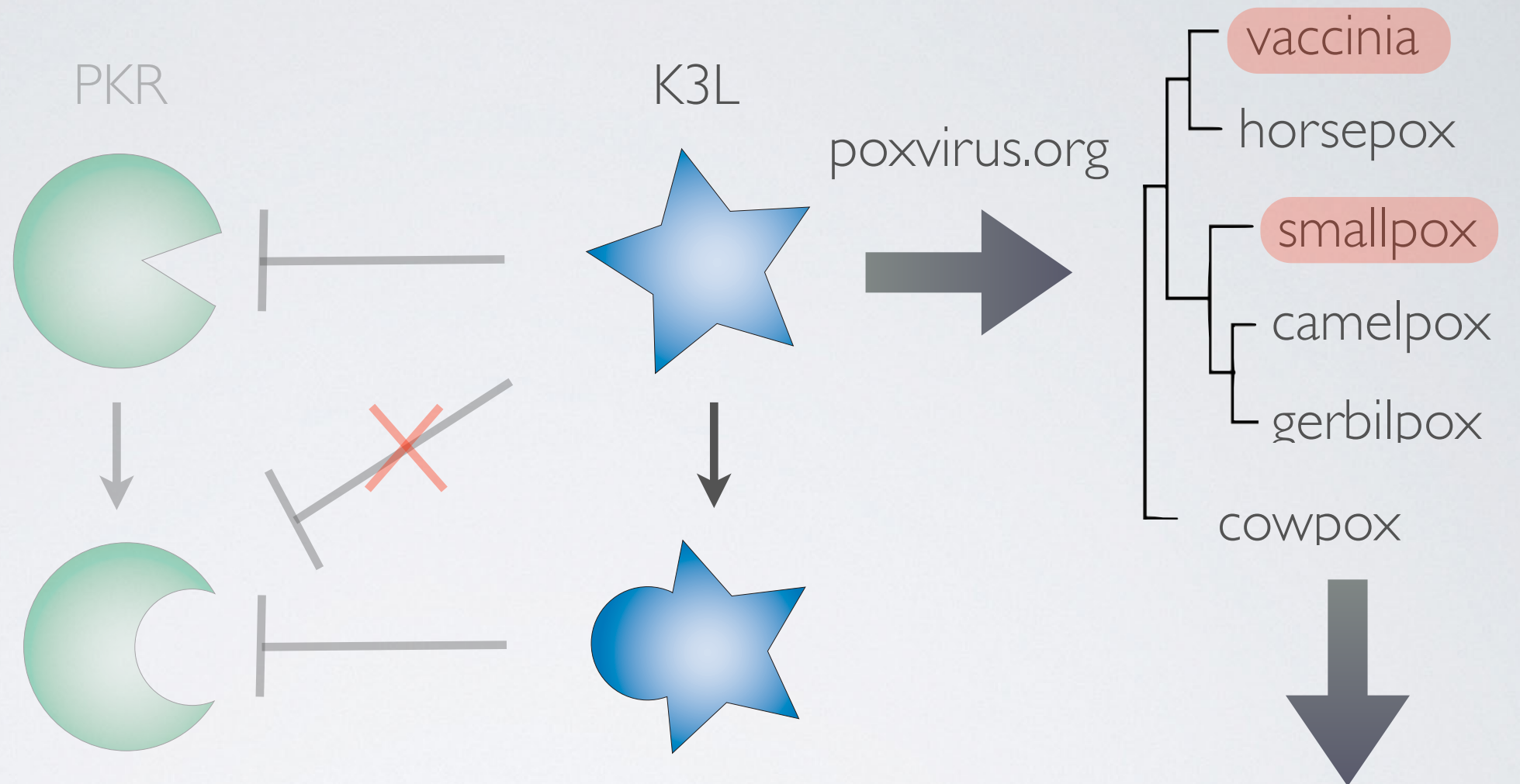


maintain
this interaction

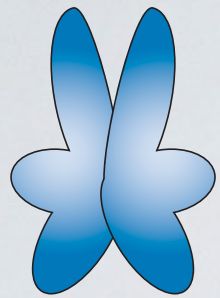


Dar, et al., Cell 2005

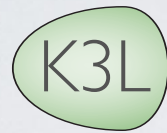
THE EVOLUTION OF MIMICRY



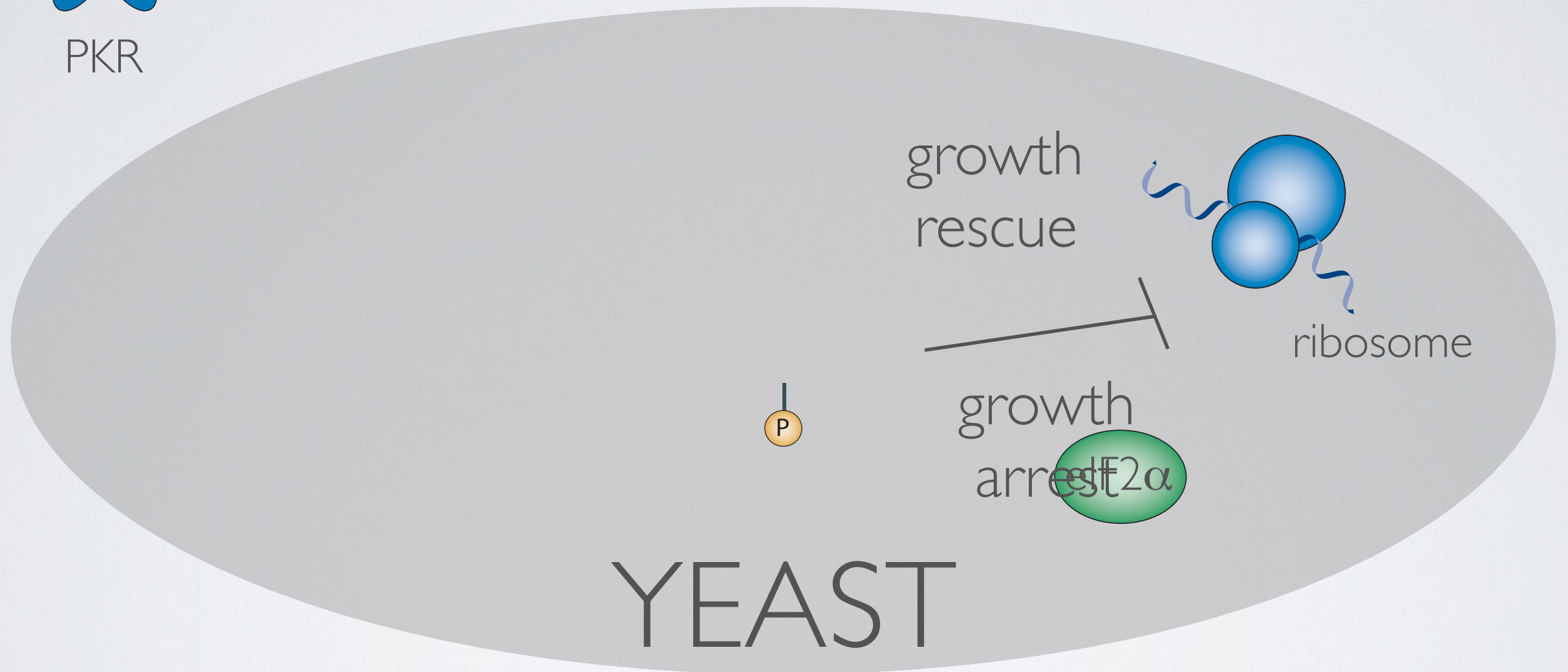
YEAST ASSAY FOR PKR ACTIVITY



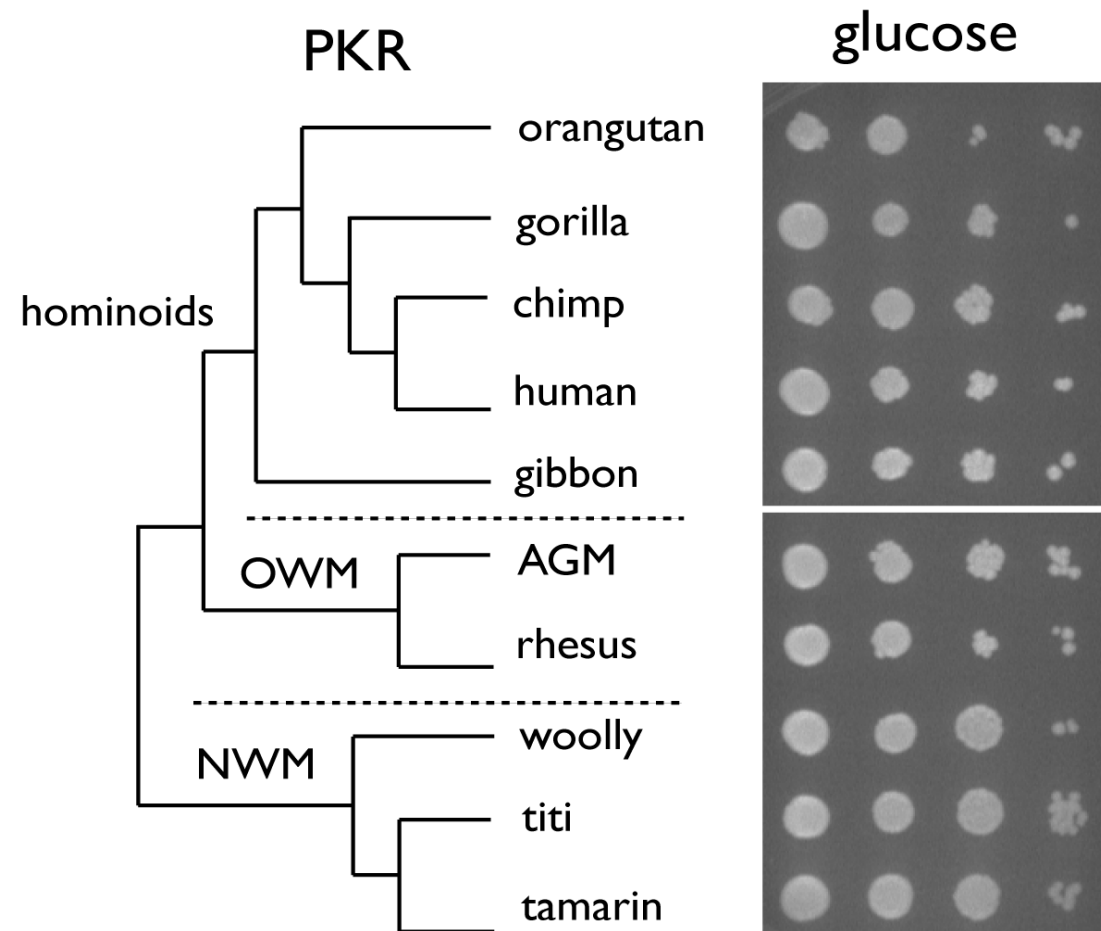
PKR



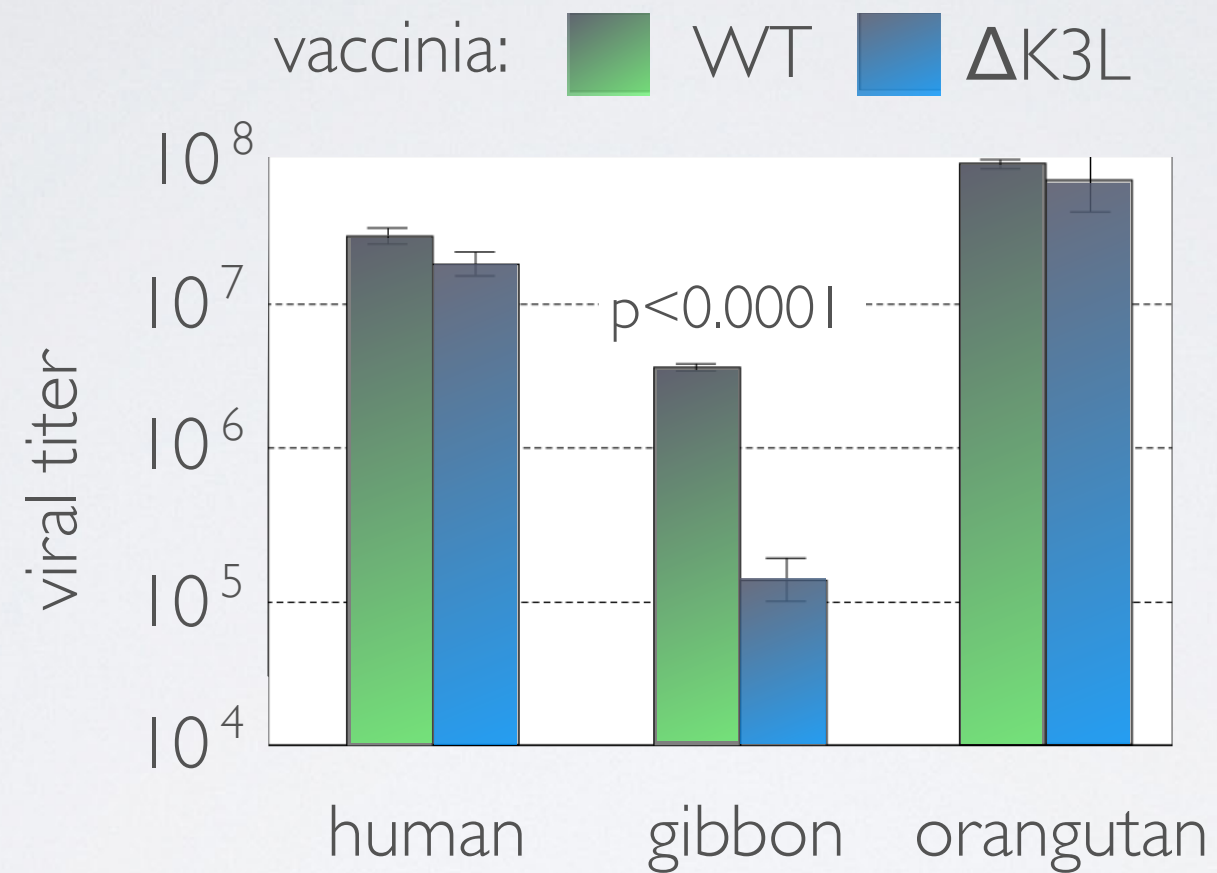
K3L



YEAST ASSAY FOR PKR ACTIVITY

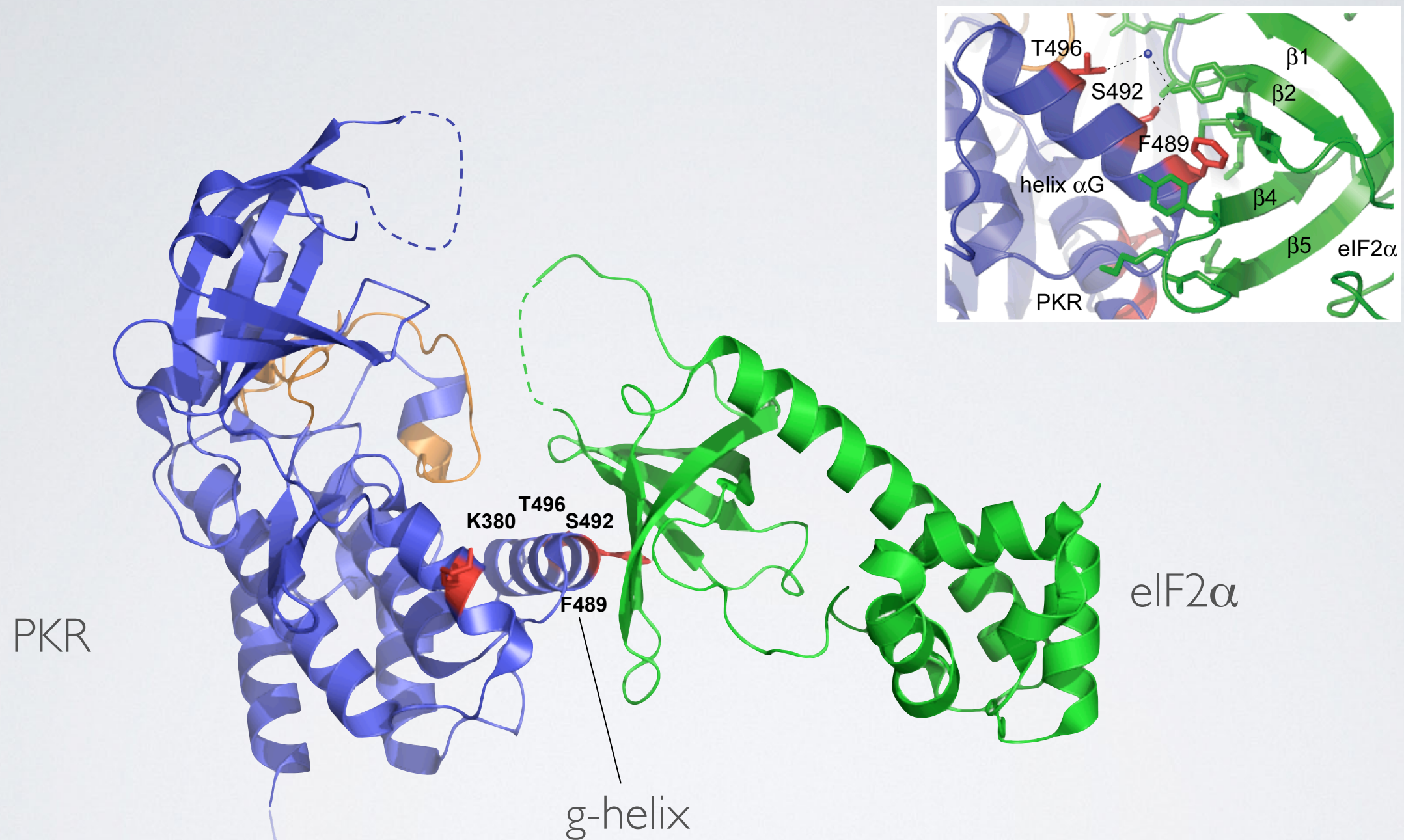


K3L & GIBBON CELL INFECTION

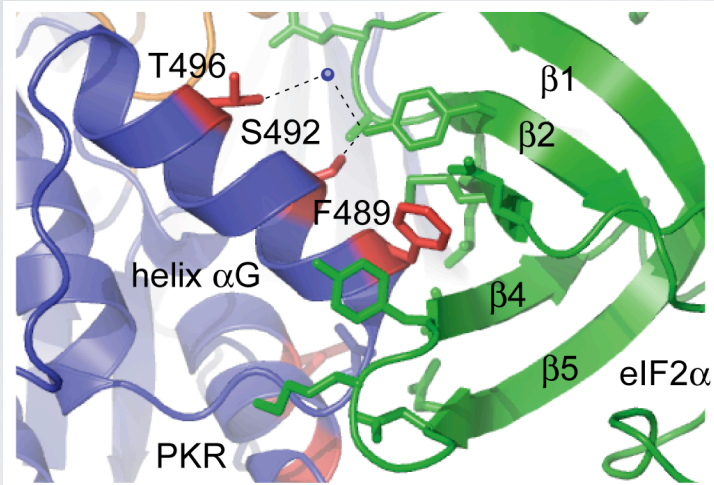


Adam Geballe

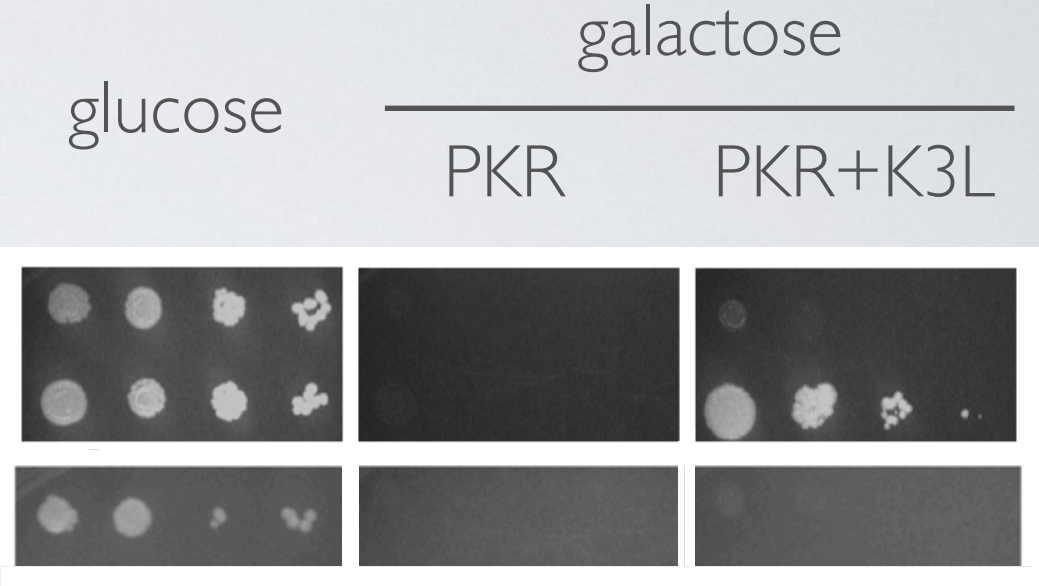
SELECTION AT THE INTERFACE



G-HELIX RESISTS K3L



human
gibbon
orangutan



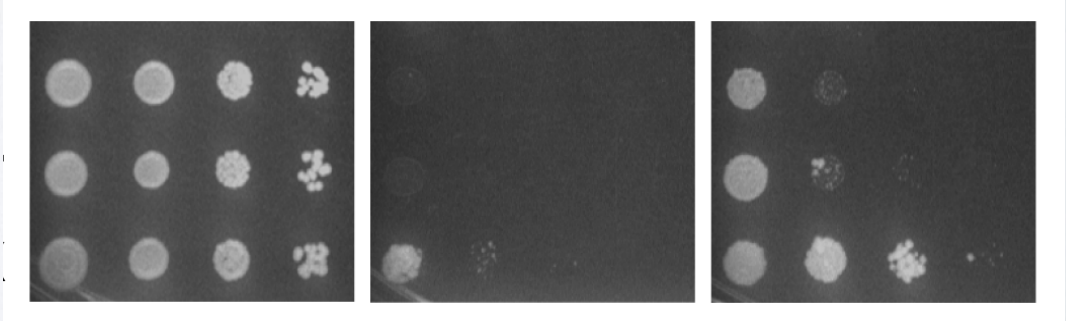
helix αG

489 492 496

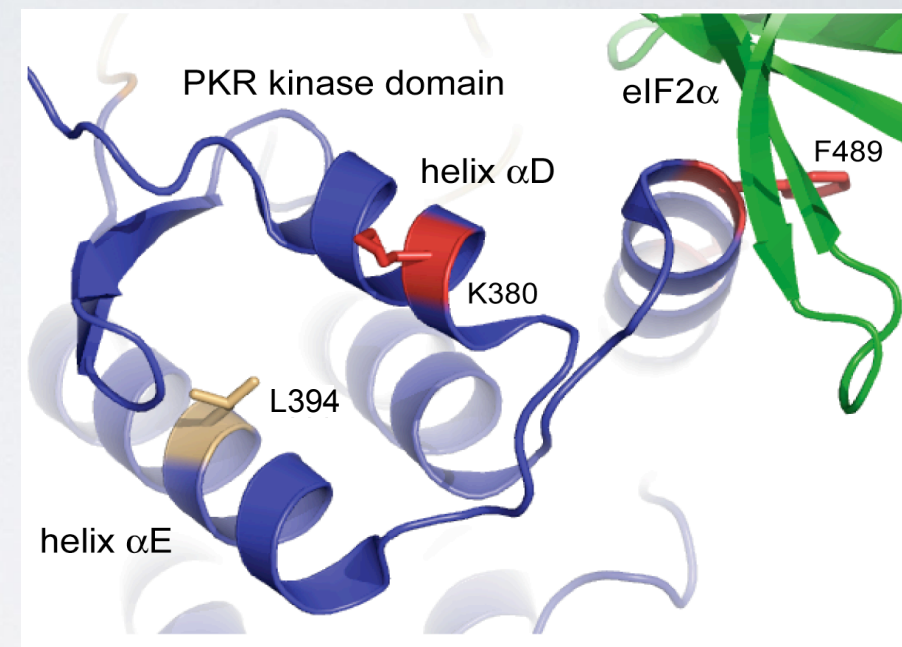
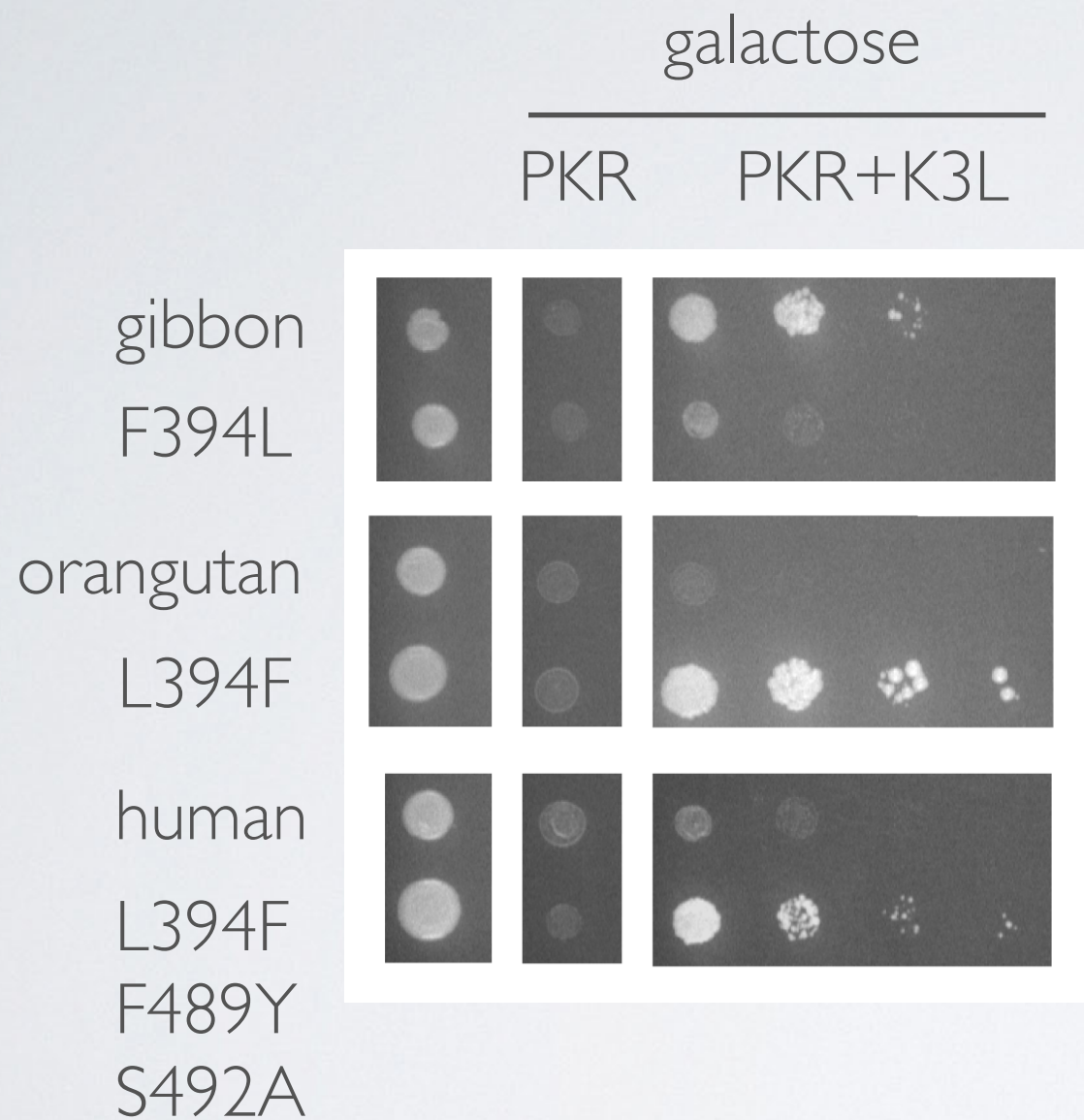
human	DTAFETSKFFTDLR
gibbon	DTAYETAKFFTDLR
orangutan	DTASETAKFFKDLR
rhesus	DTASETLQLFRDLR
owl	DTALETSKFFGDLR

gibbon

FST
YST
SAK

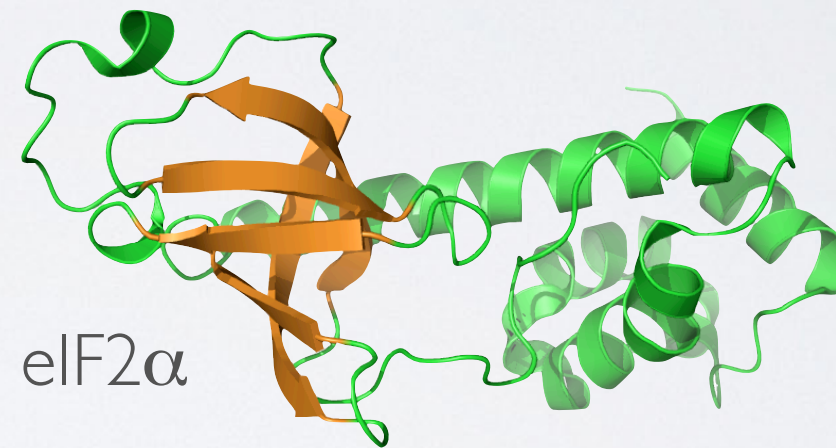
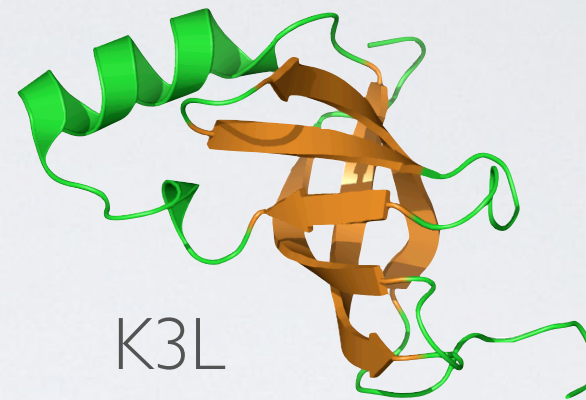
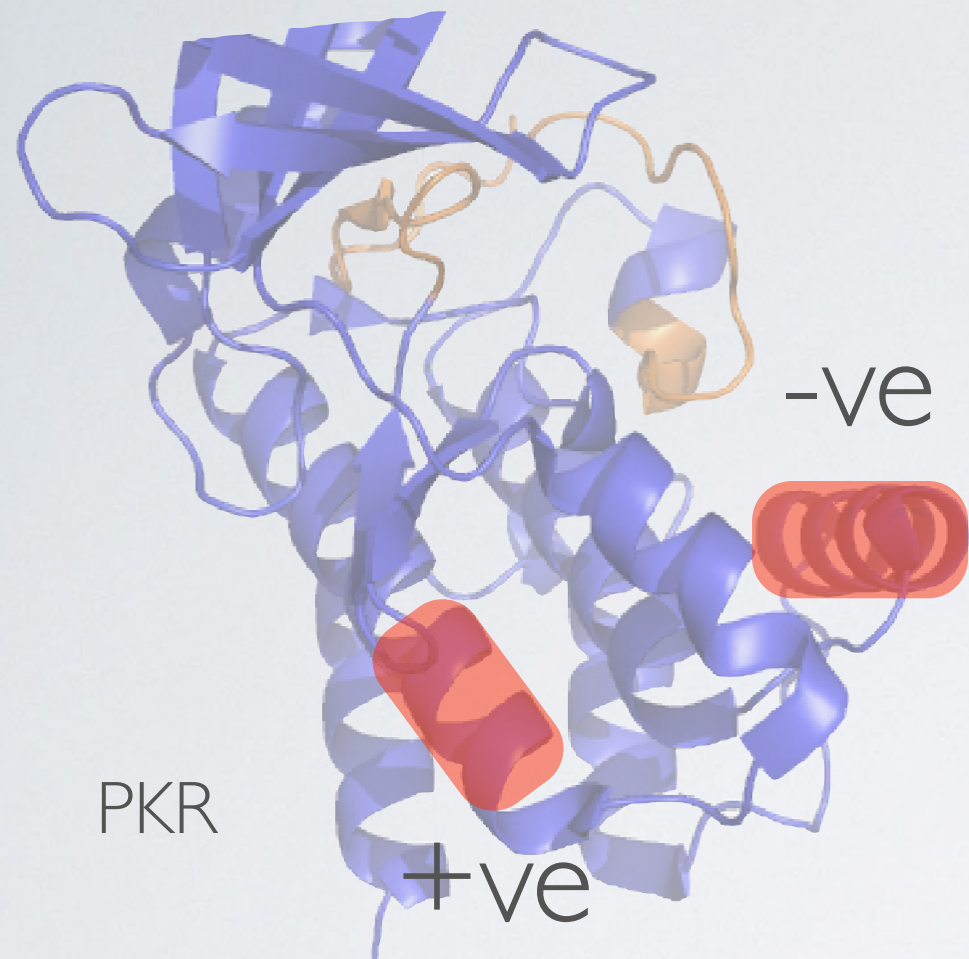


LEU394 OVERRIDES K3L

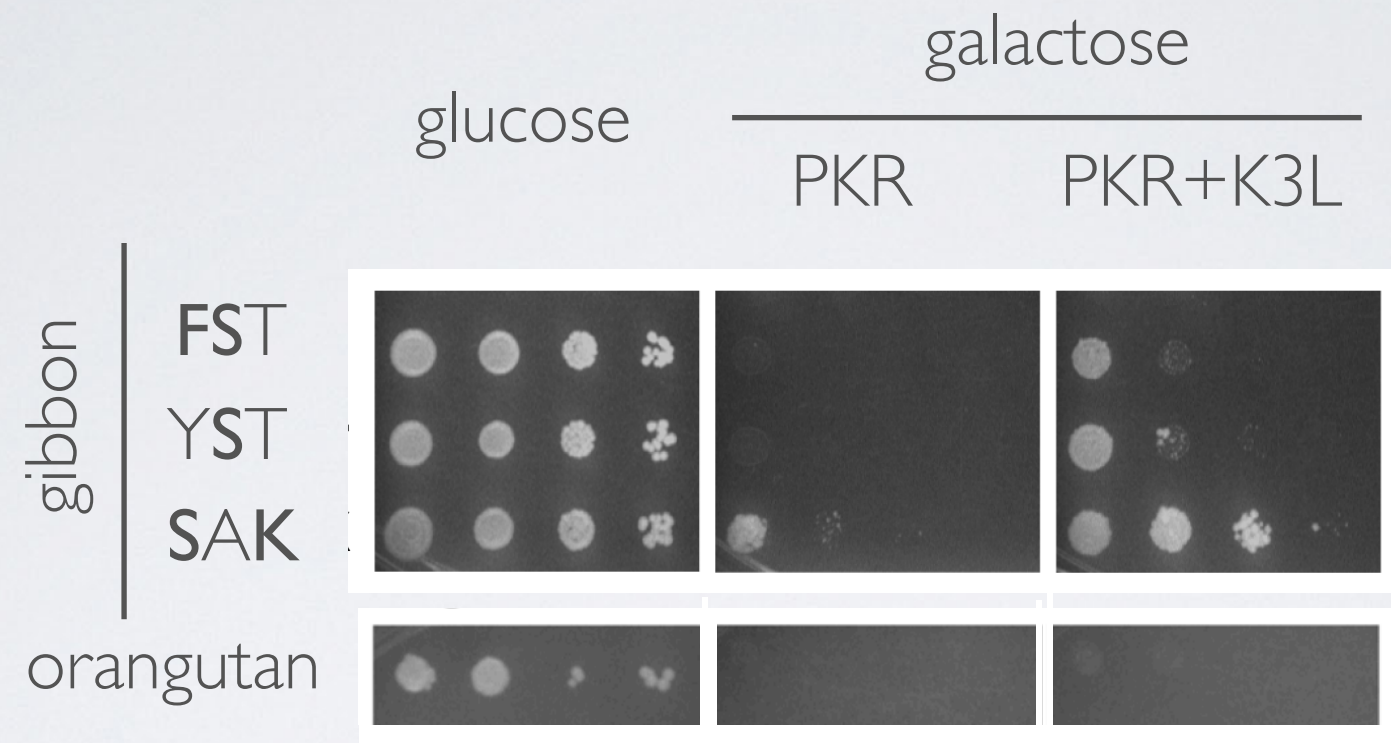


	394	
human	LELF ³⁹⁴ EQ	primates
orangutan	LELF ³⁹⁴ EQ	
gibbon	LE ^F FFEQ	
rhesus	LELF ³⁹⁴ EQ	
owl	FE ^F FFEQ	
mouse	LDLYEQ	other mammals
rabbit	LE ^F FWQ	
dog	LELF ³⁹⁴ EQ	
pig	LE ^F FFEQ	

OVERCOMING MIMICRY



- evolution on multiple surfaces
- flexibility for substrate recognition



Intragenic “restoration” of function

A COST TO FLEXIBILITY

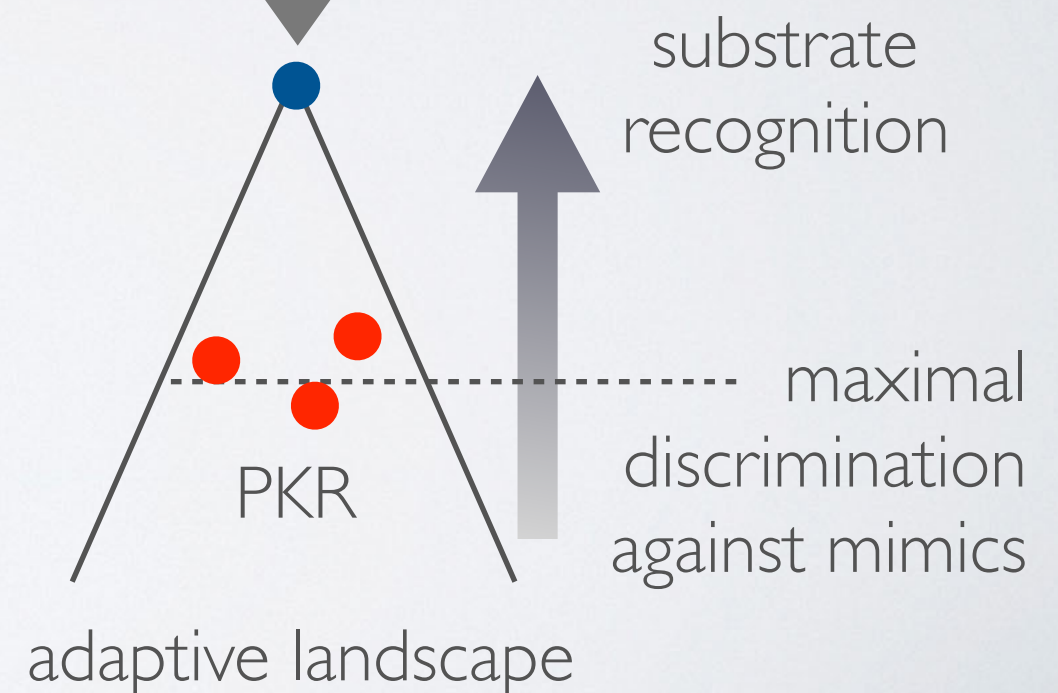
	αG helix																		
	489	492	496		530	533	537		1024	1027	1031		944	947	951				
human	TAFETSKFF	TDLRD			TE	MERA	EVLTGLRT		TQ	MERV	RTLTDVRN		TAS	ERIF	VLNQLRD				
rhesus	TASE	TLQLFR	DLRG		TE	MERA	EVLTGLRT		TQ	MERV	RTLTDVRN		TAS	ERIF	VLNQLRD				
mouse	TESE	KIKFF	ESLRK		TE	MERA	TVLTGVRT		TQ	MERV	RI	LT	DVRN		TAS	ERIF	VLNQLRD		
cow	TSLE	TQKFF	DDLNR		TE	MERA	HVLTGLRT		TQ	MERV	RI	LT	DVRD		TAS	ERIF	VLNQLRD		
dog	TVSE	TLKIF	KE	LRA	TE	MERV	VHILTGLRS		TQ	MERV	RV	LT	DVRN		TAS	ERIF	VLNQLRD		
	*	*	*	**	*****	:	***:	*	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****

PKR

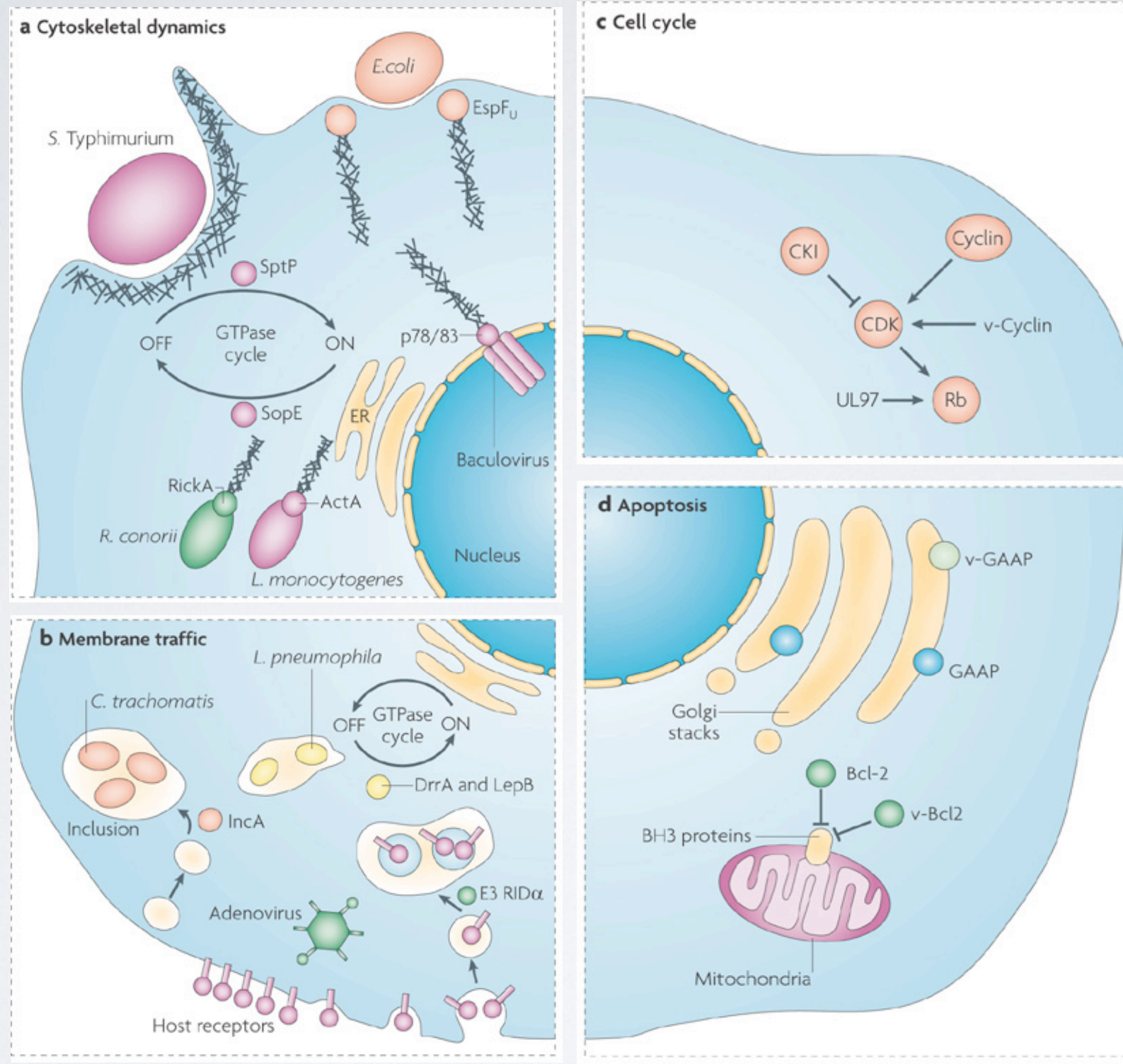
HRI

PERK

GCN2

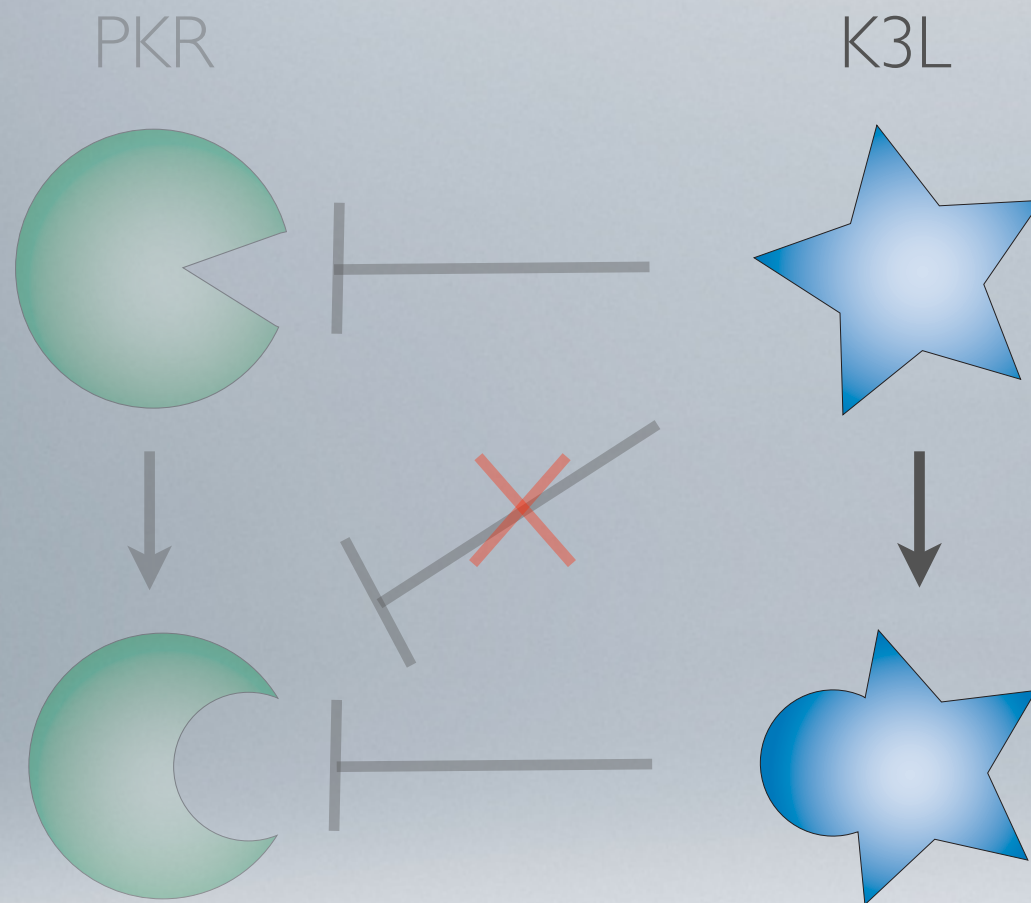


MIMICRY AGAINST THE CELL



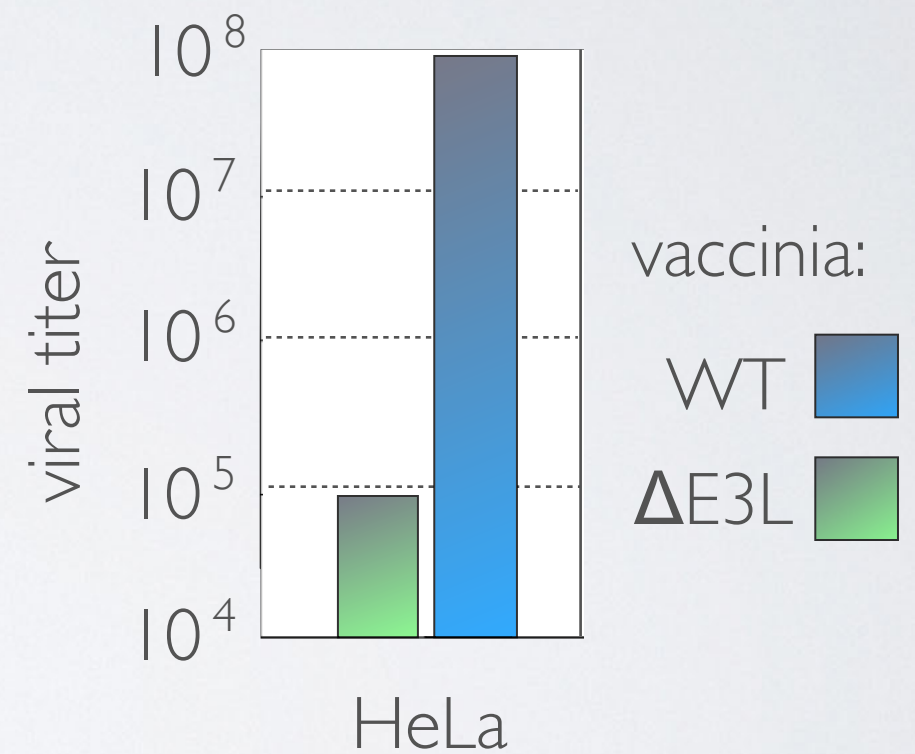
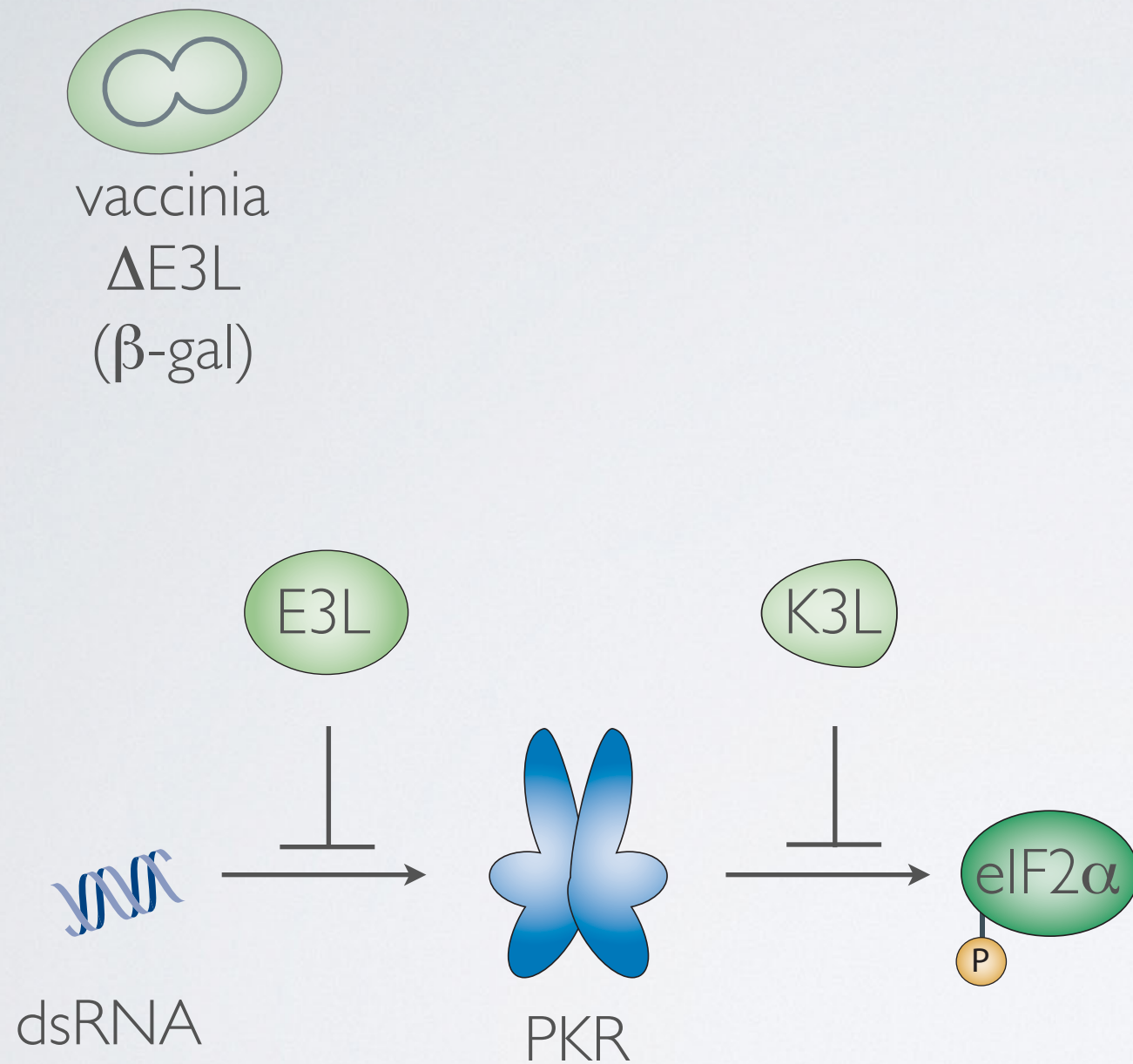
Evolutionary “winners” may in fact be losers...

PKR VERSUS K3L



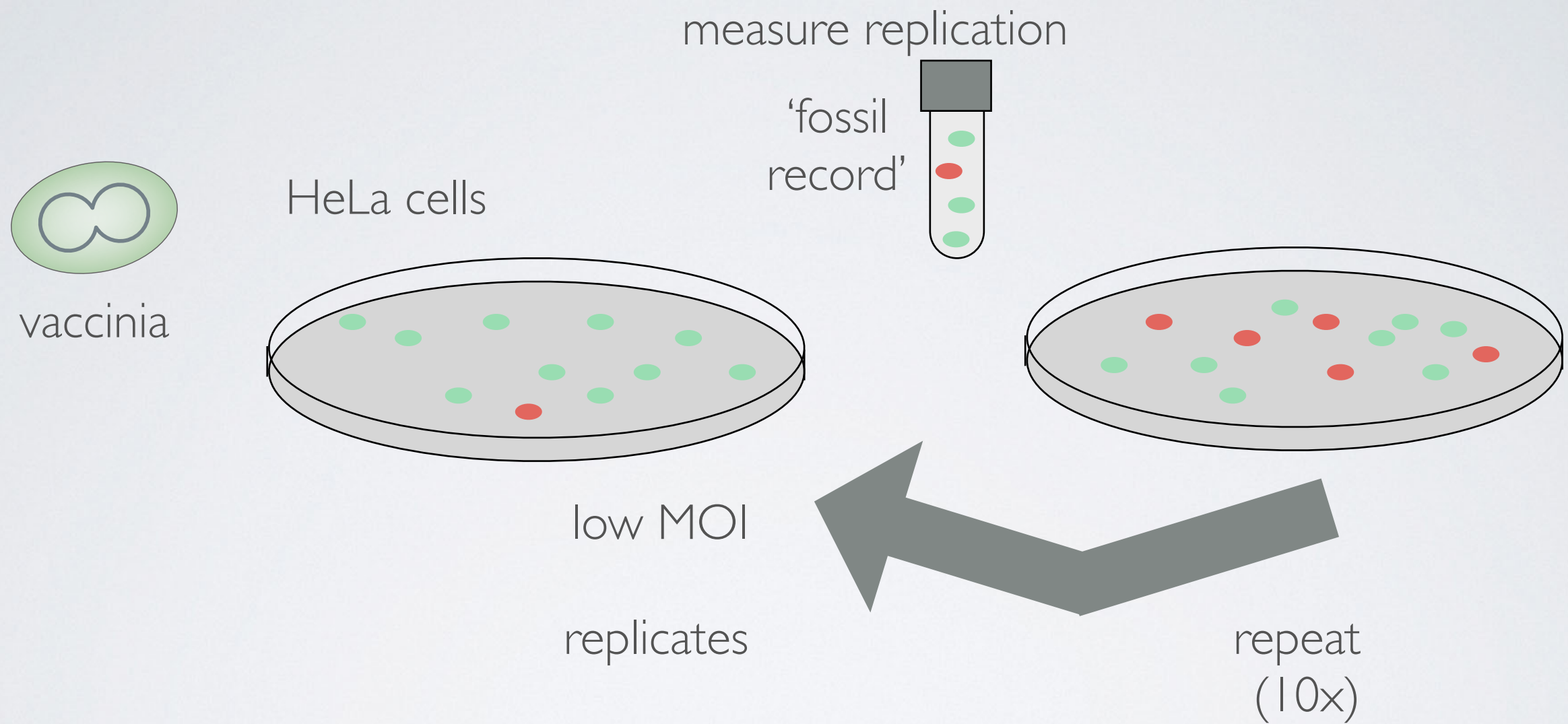
Nels Elde, former postdoc
Now Associate Professor, University of Utah

EXPERIMENTAL EVOLUTION

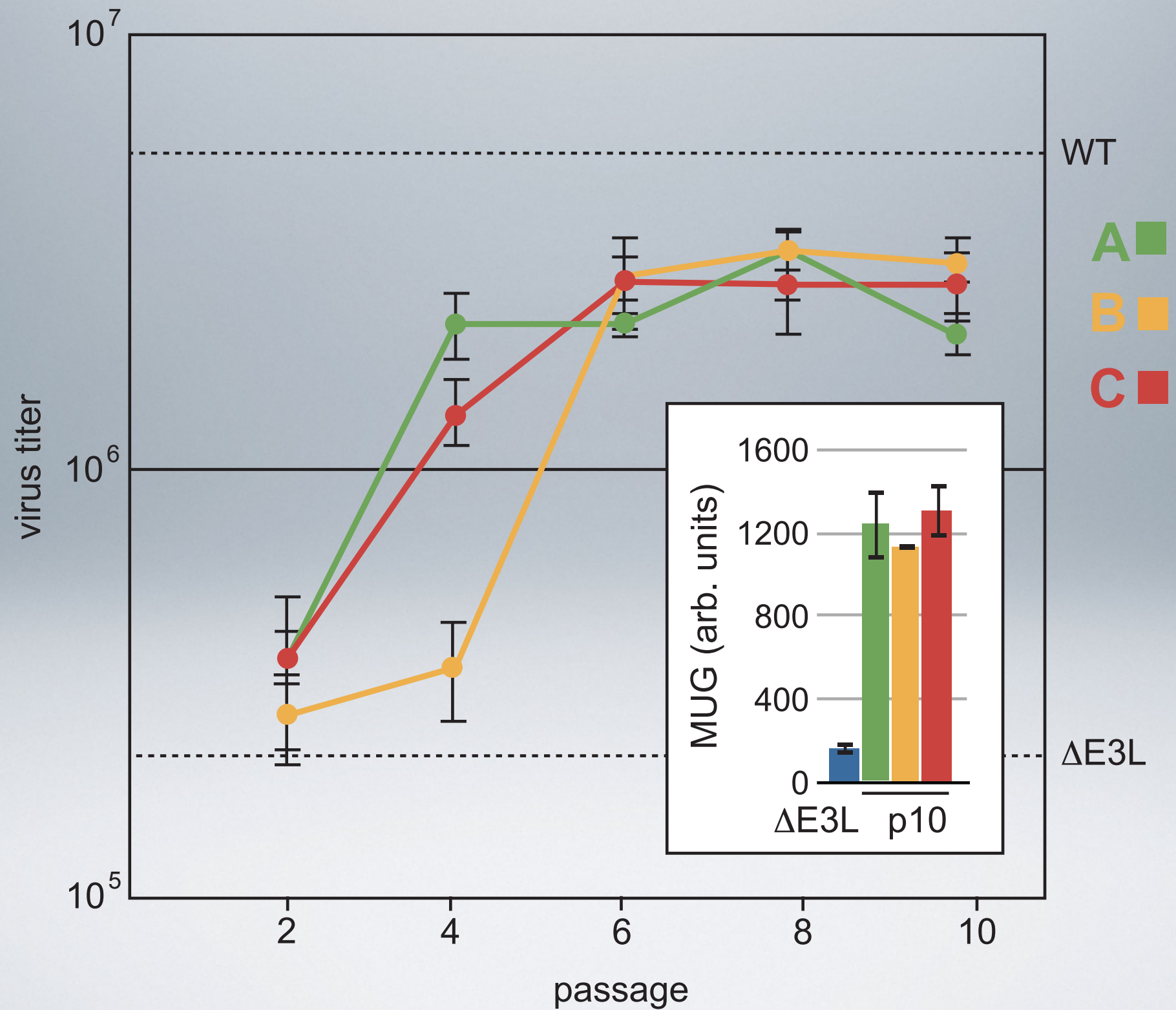


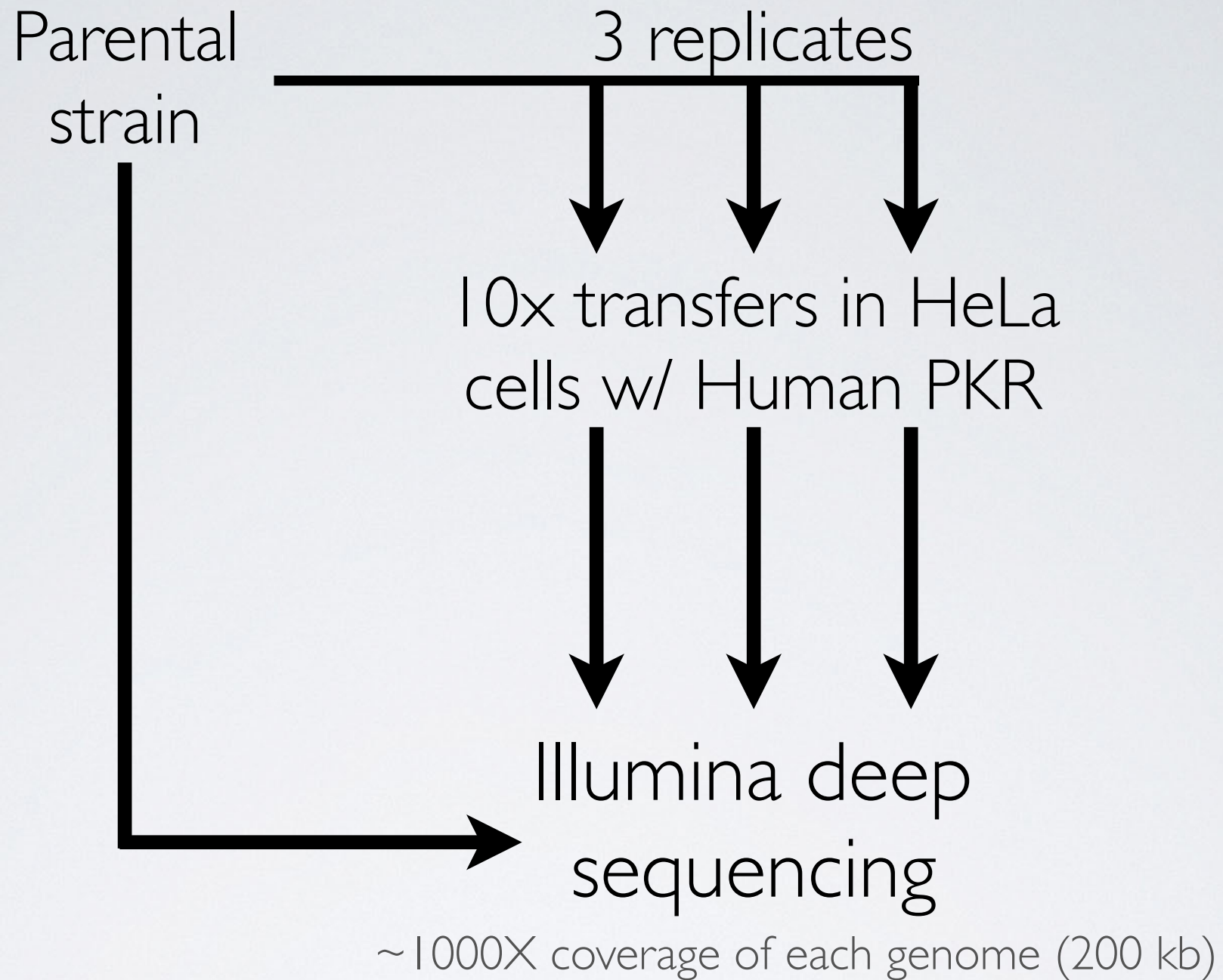
adapted from
Langland and Jacobs, Virology, 2002

EXPERIMENTAL EVOLUTION

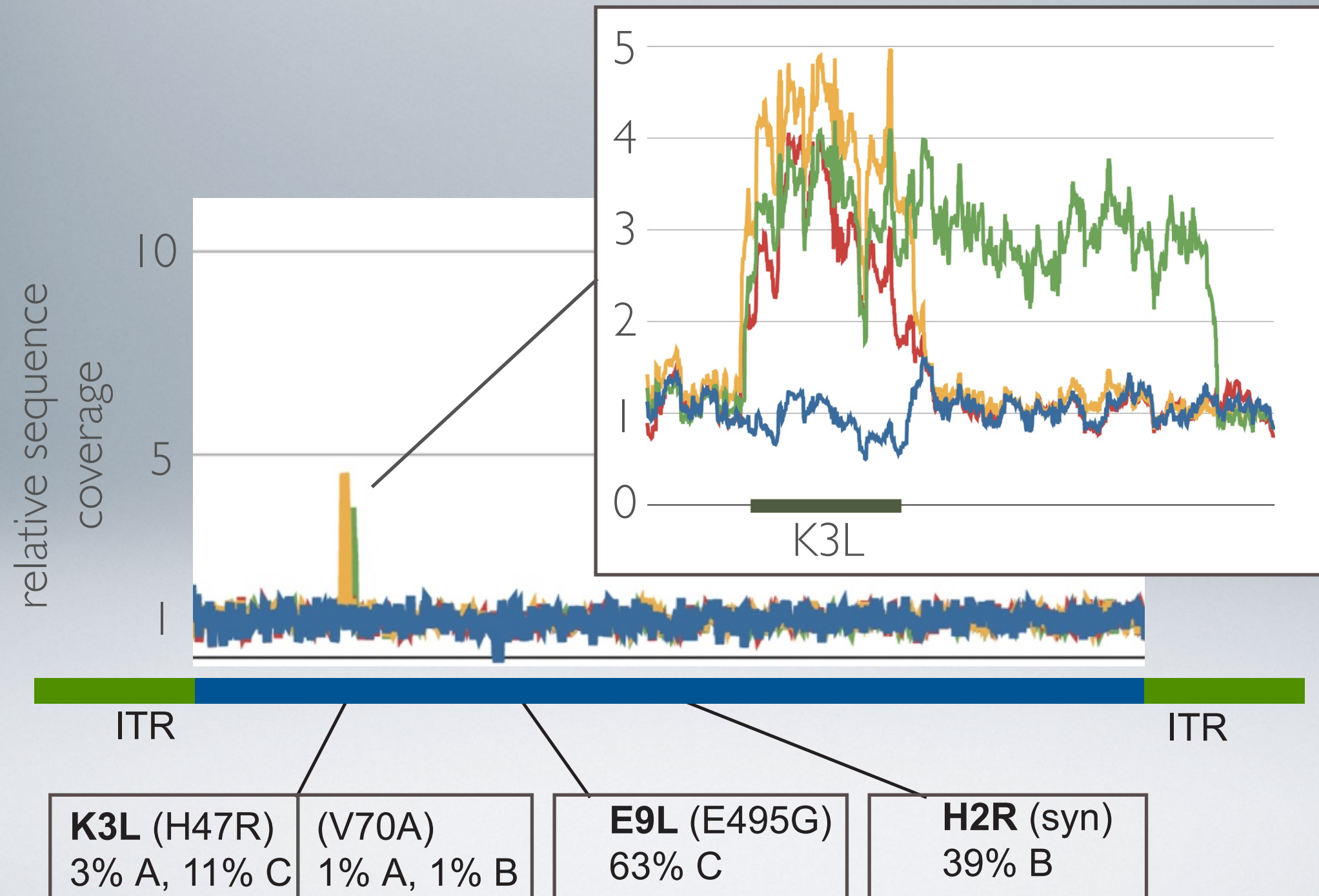


EVOLUTION OF VACCINIA

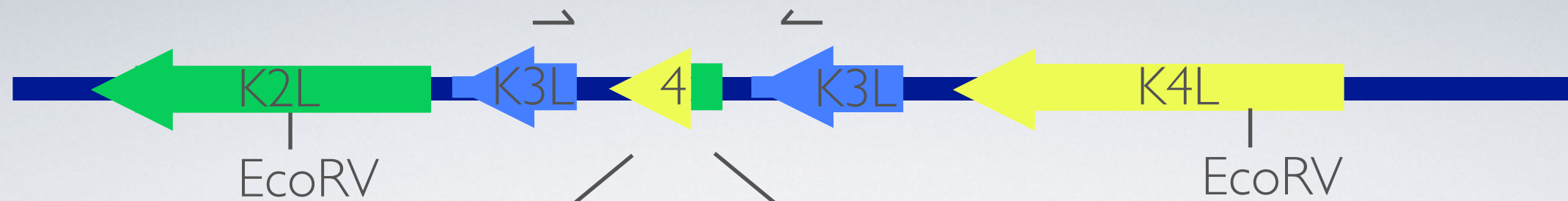
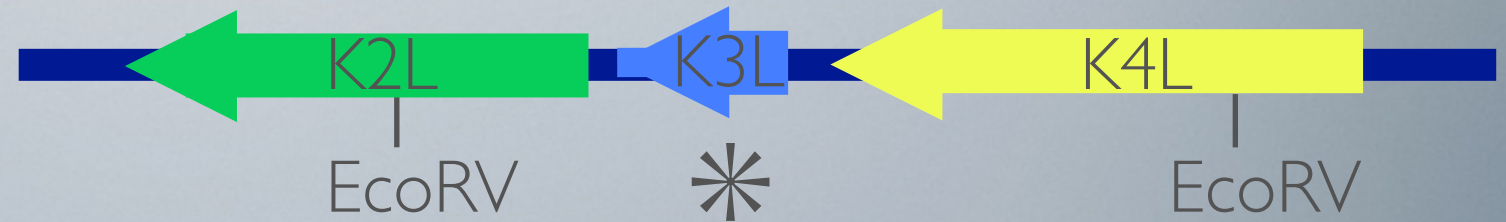
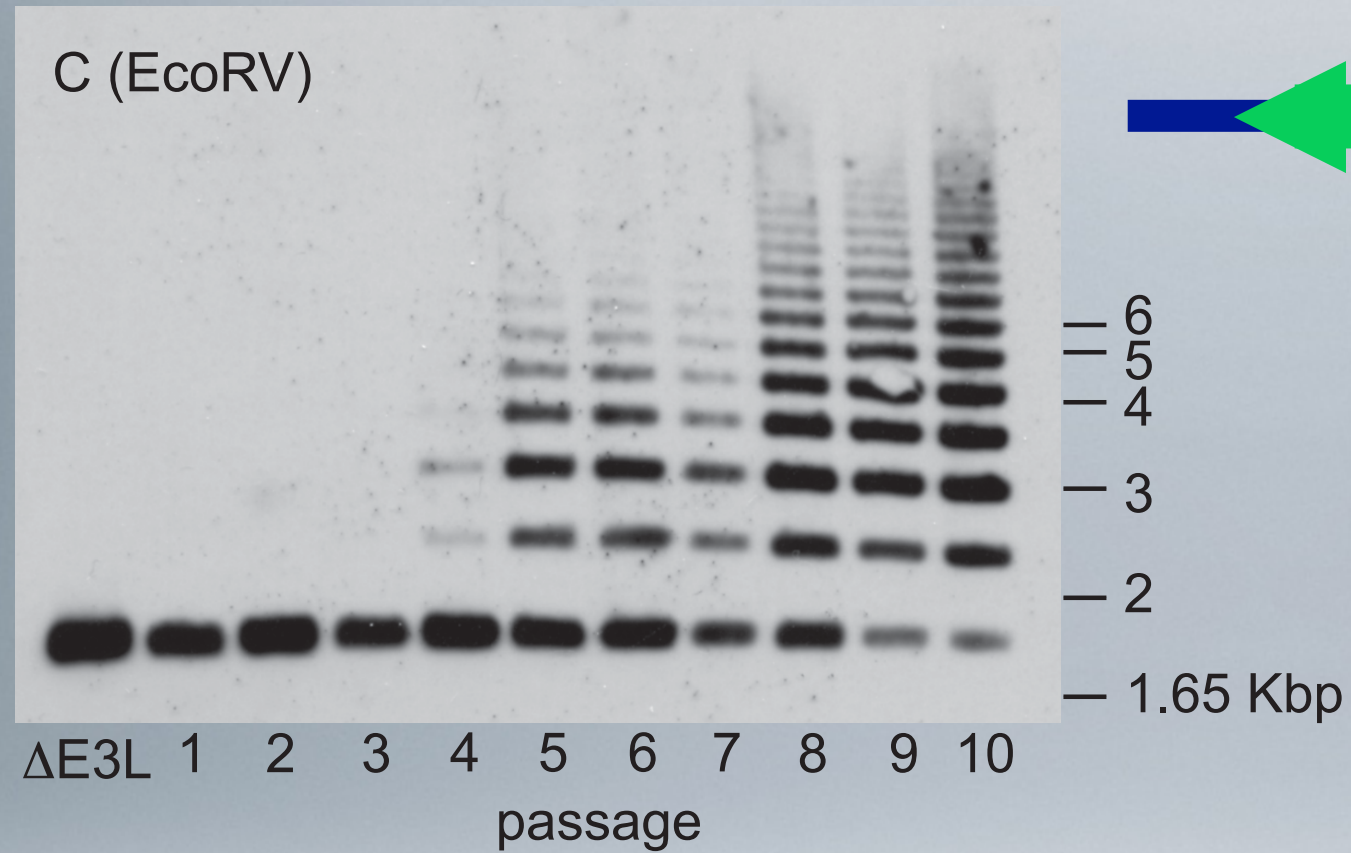




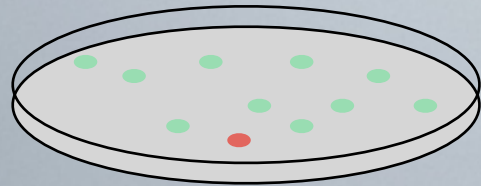
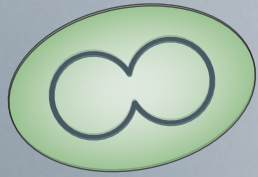
EVOLUTION OF VACCINIA



EVOLUTION OF CNV

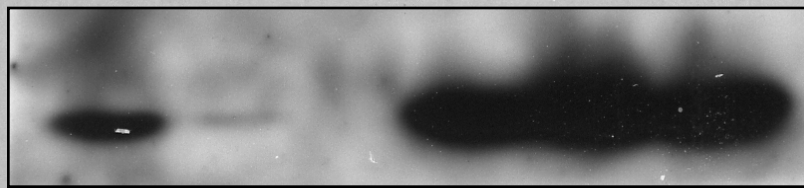


ADAPTIVE K3L DUPLICATIONS

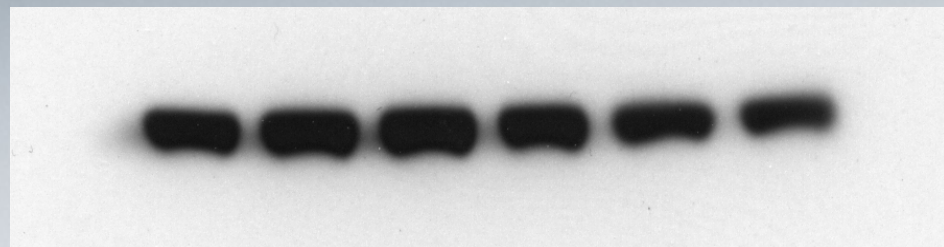


K3L
siRNA

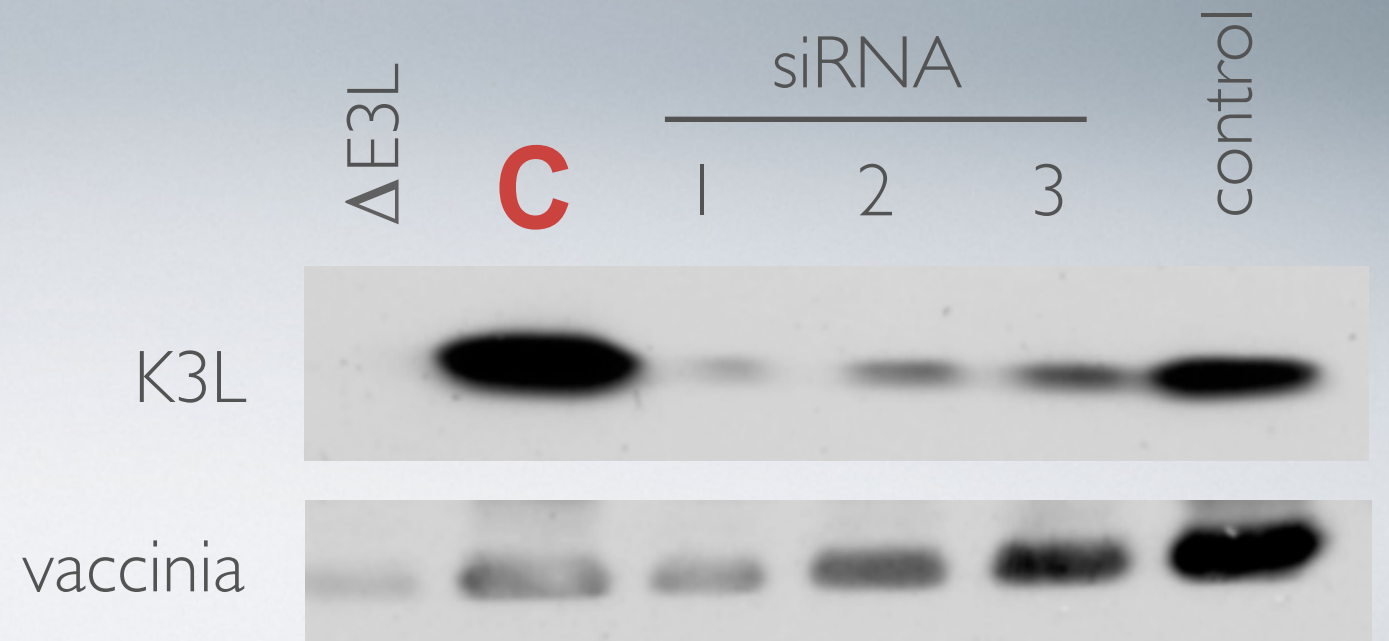
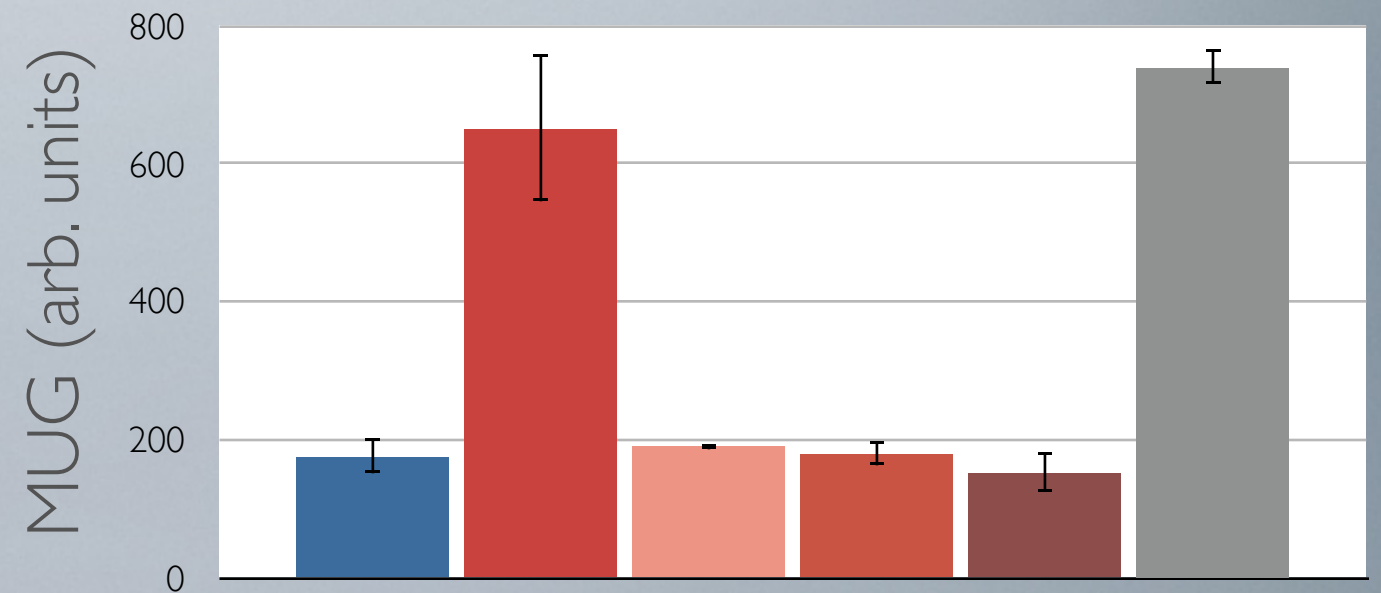
WT Δ E3L mock passage 10
A **B** **C**



K3L



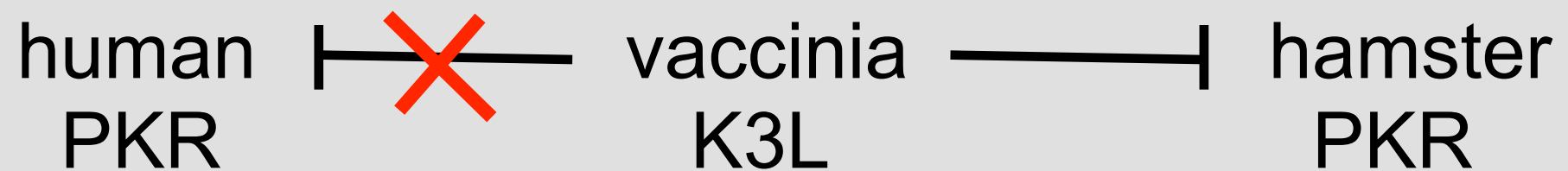
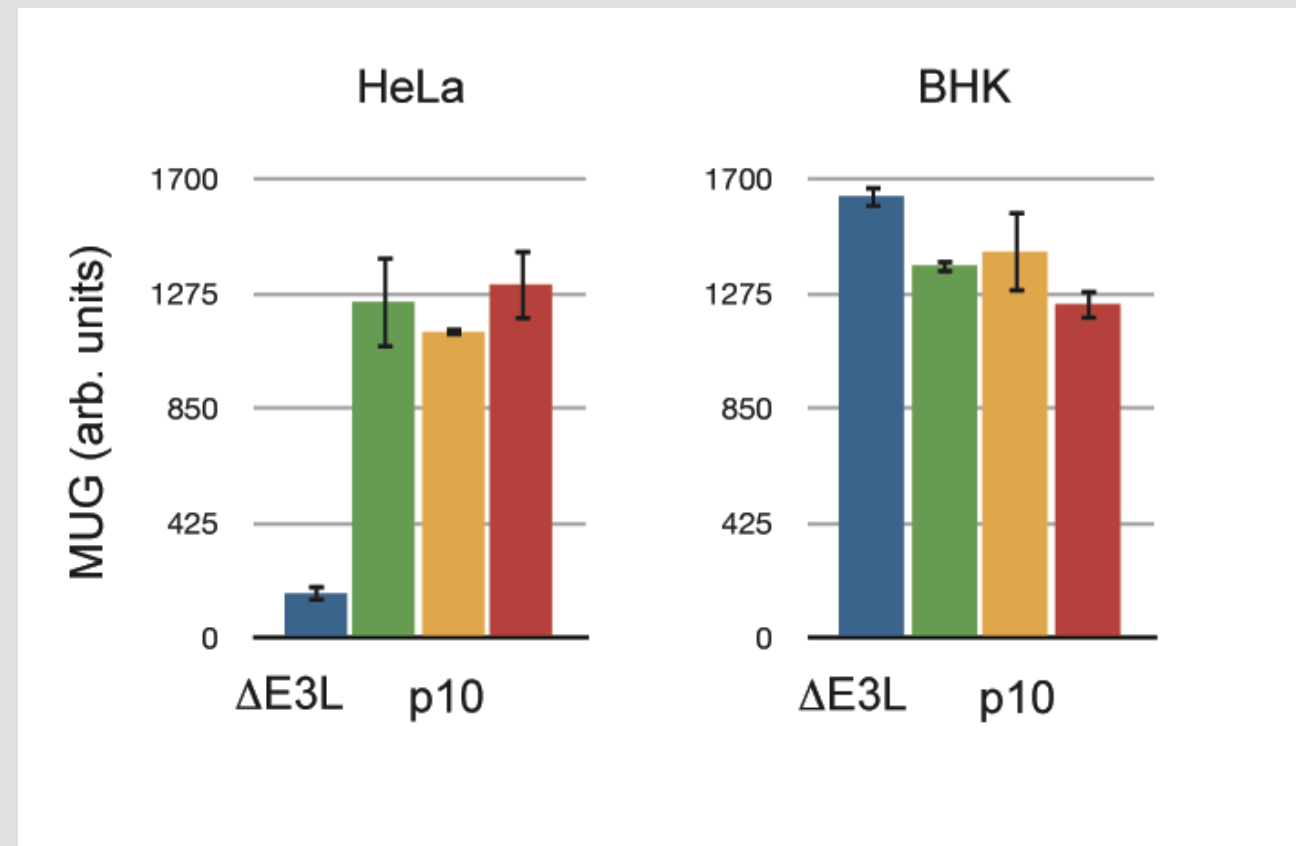
actin

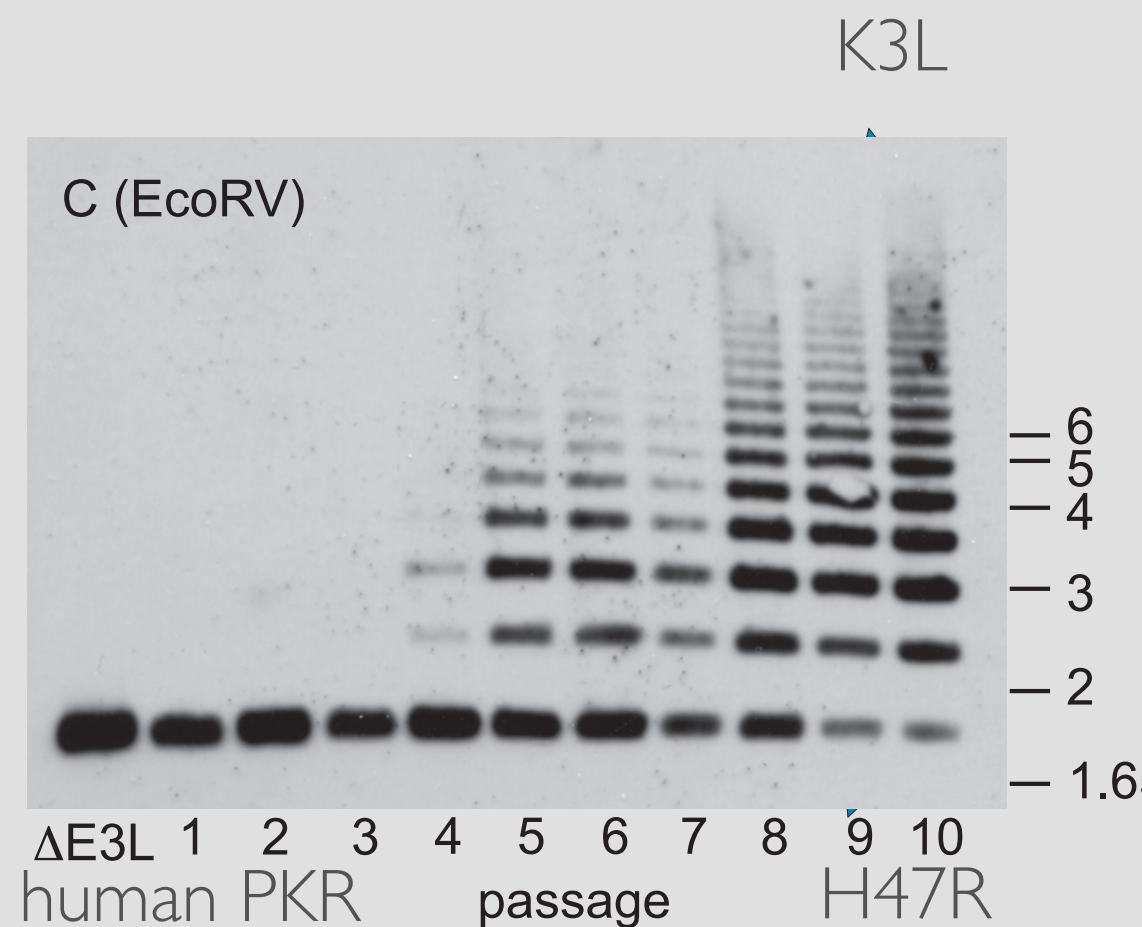
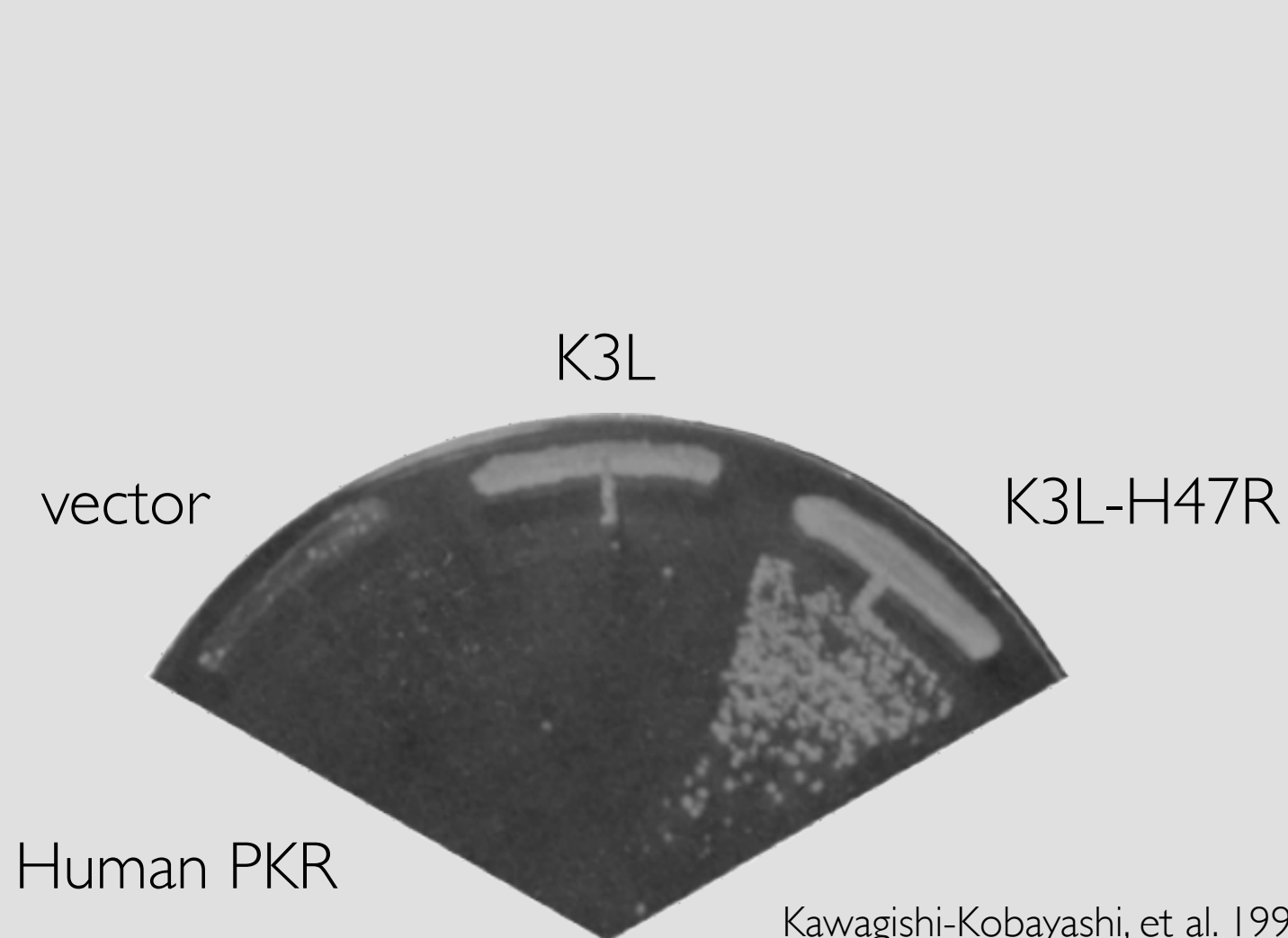
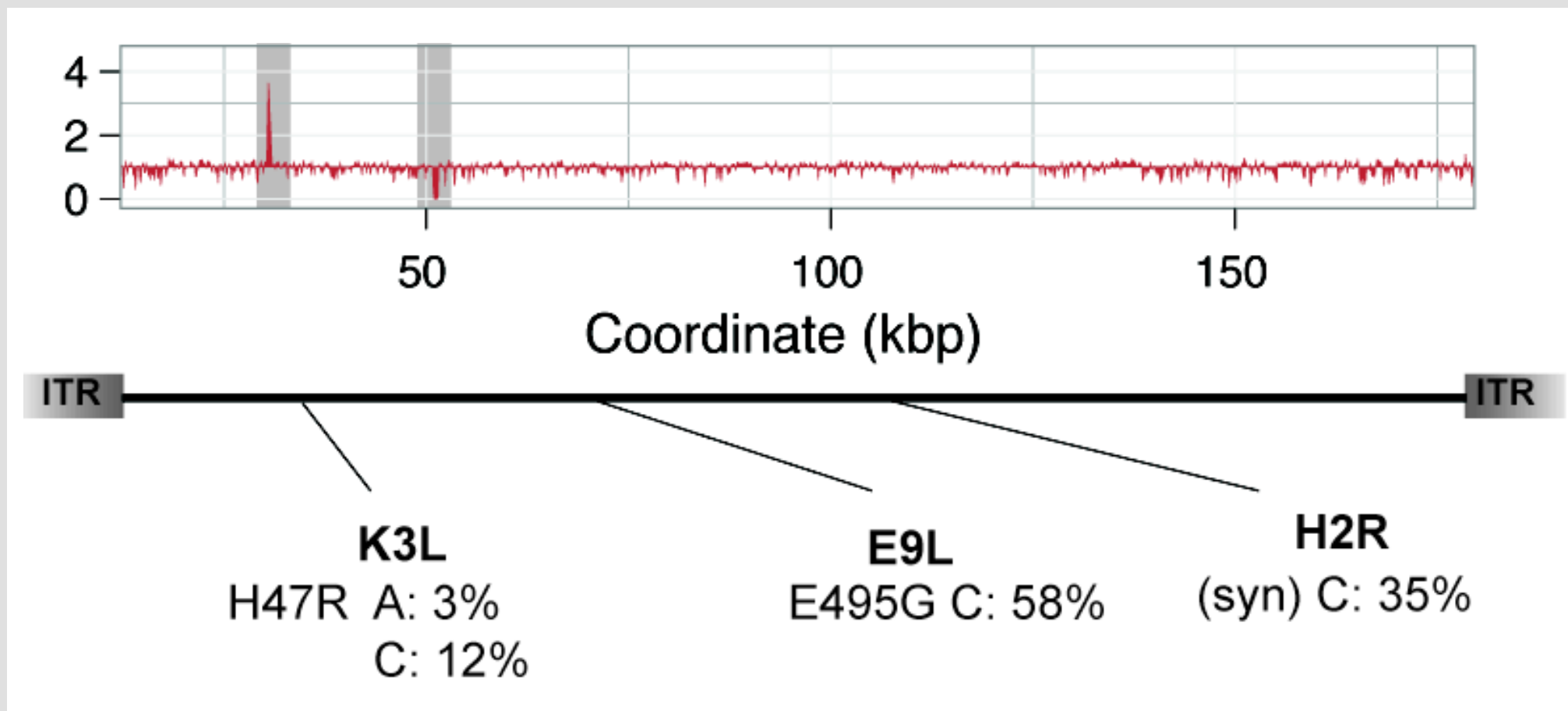


K3L

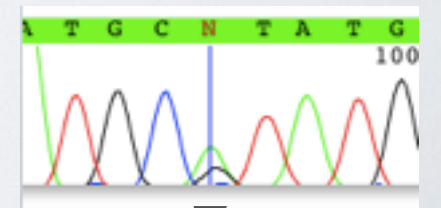
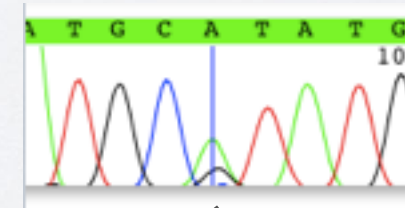
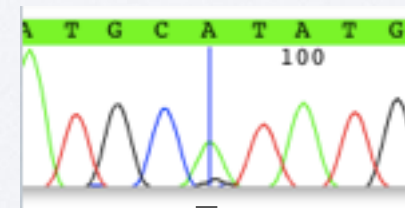
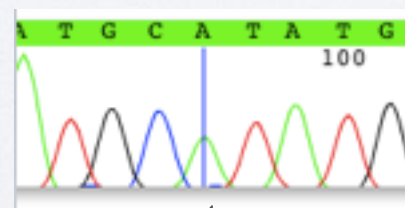
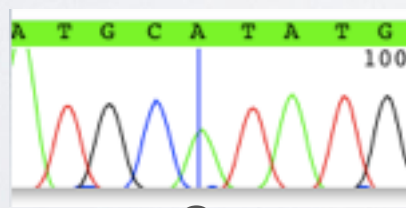
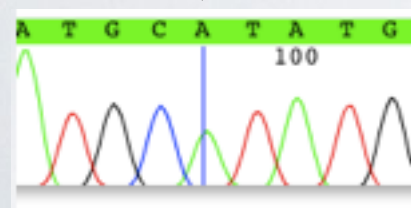
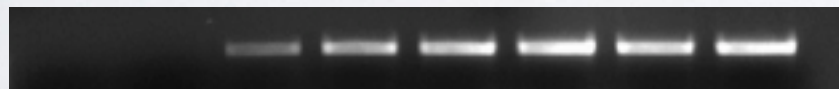
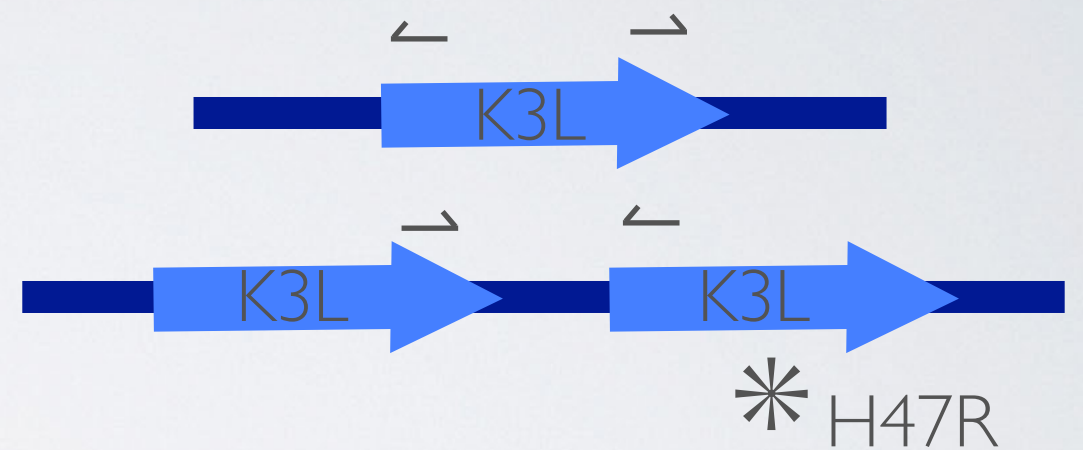
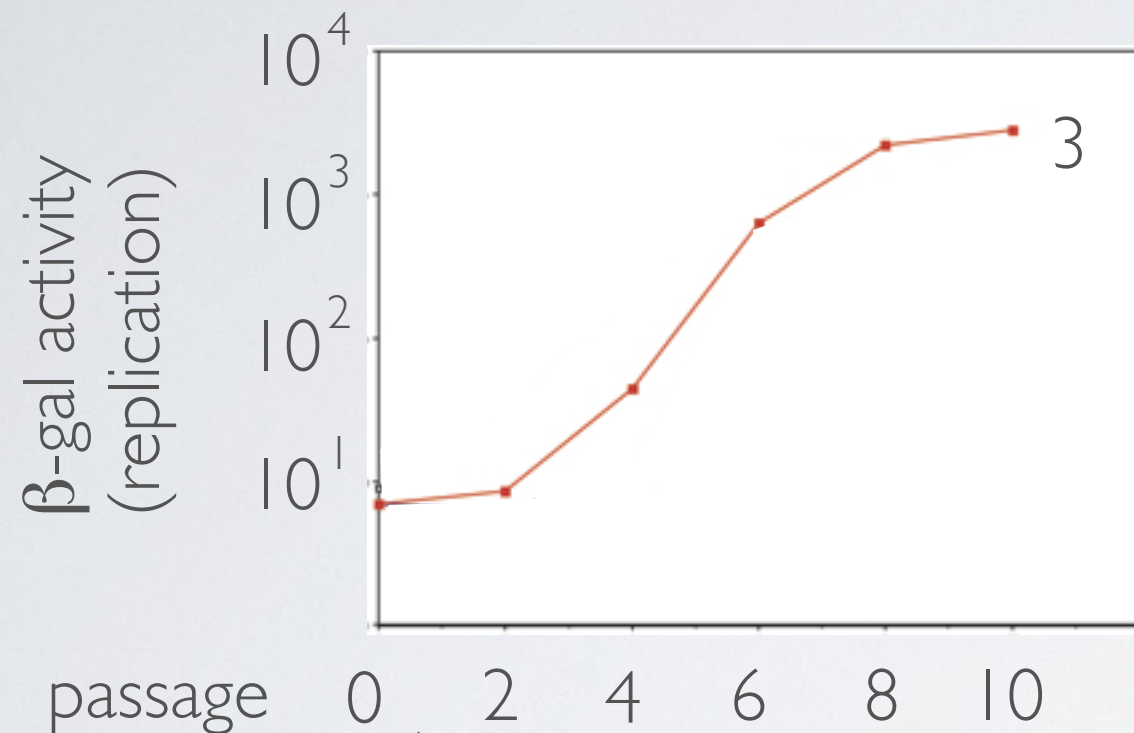
vaccinia

A trade-off associated with K3L gene expansion

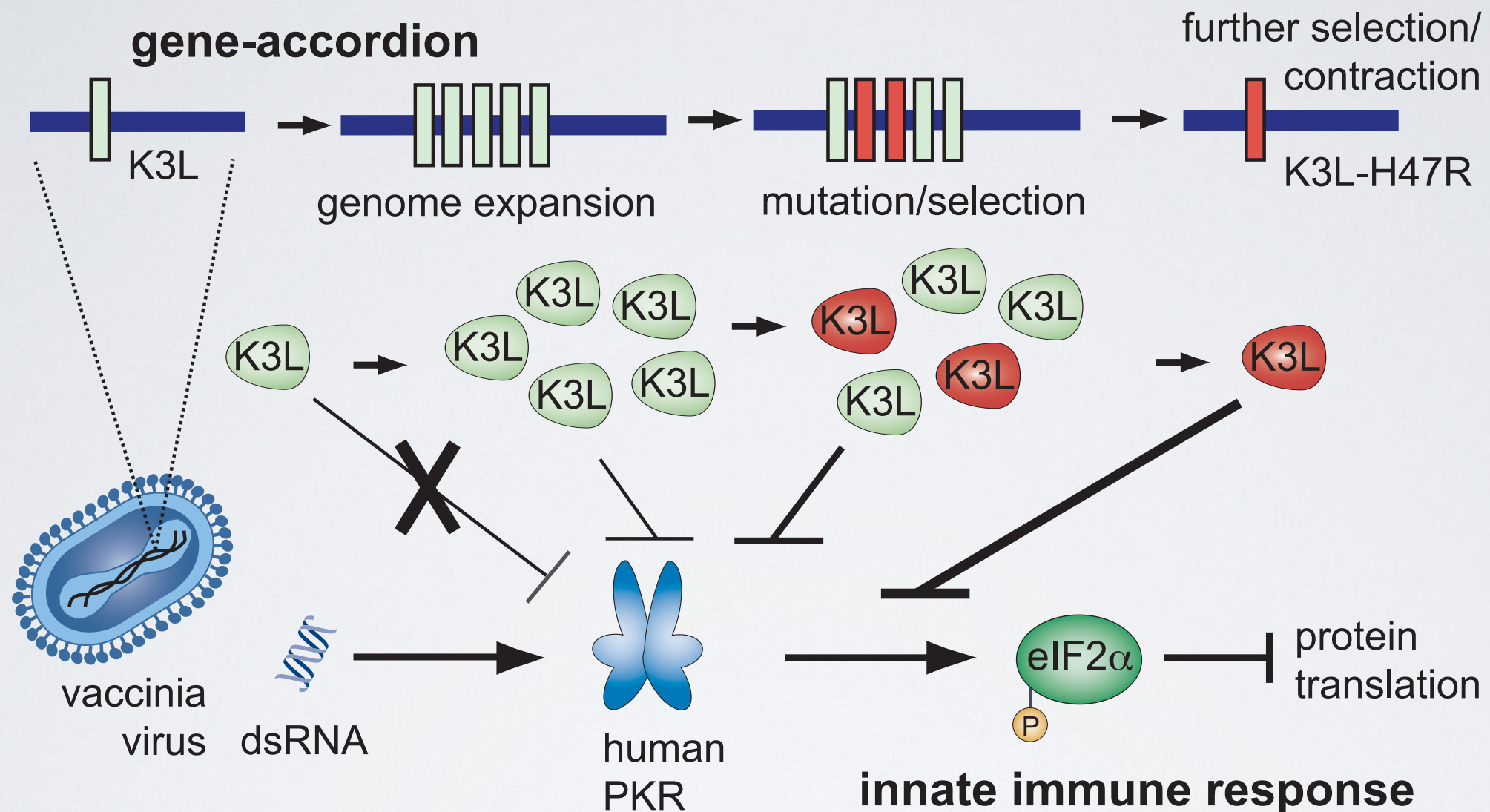




ADAPTATION VIA DUPLICATION



“How” does positive selection occur?

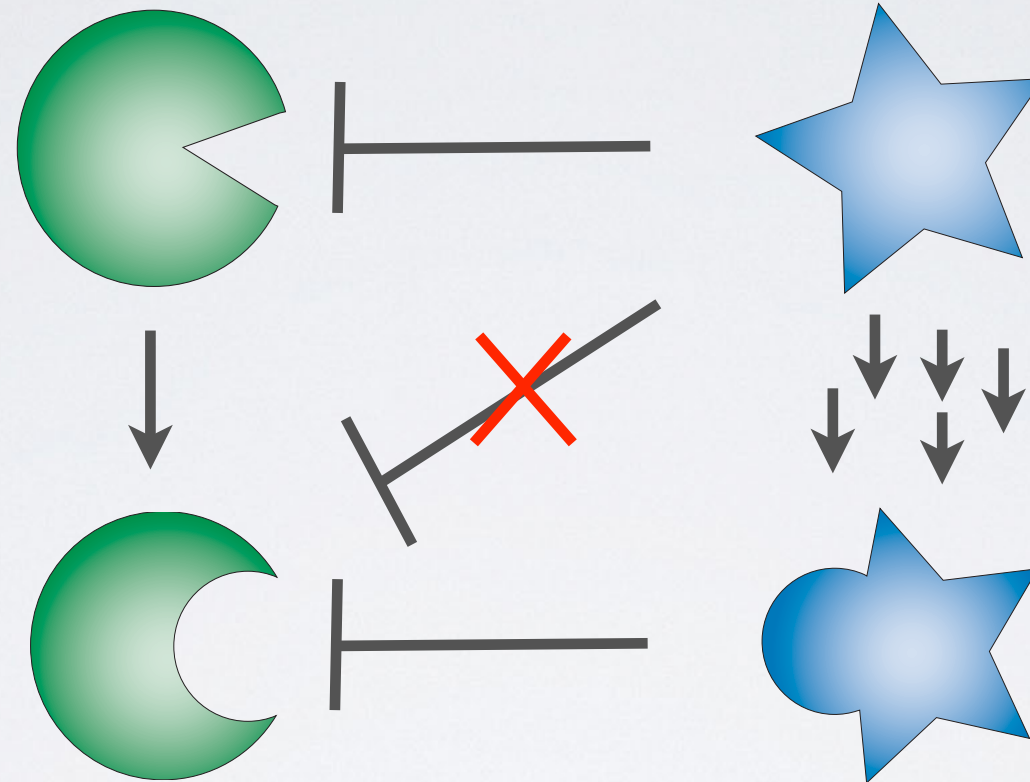


Experimental evolution of vaccinia virus reveals that positive selection proceeds through “hidden” intermediates

the usual suspects

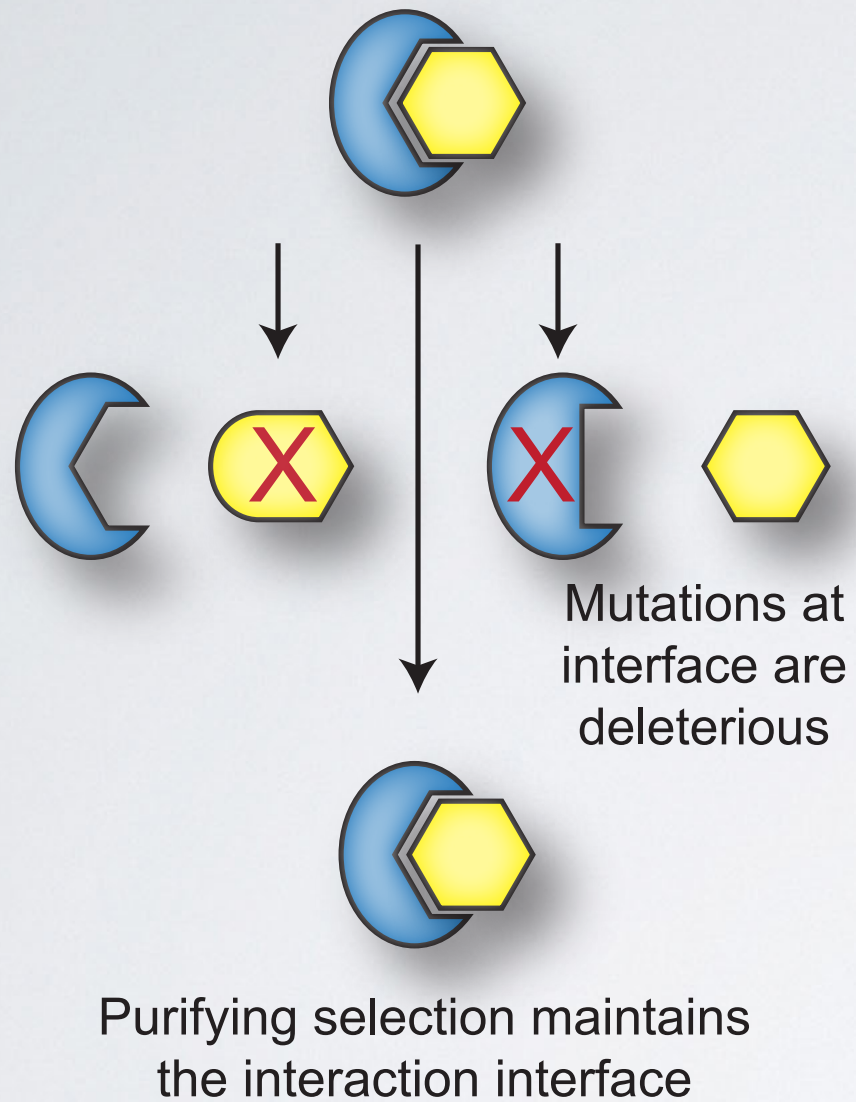
Antiviral protein

Viral protein

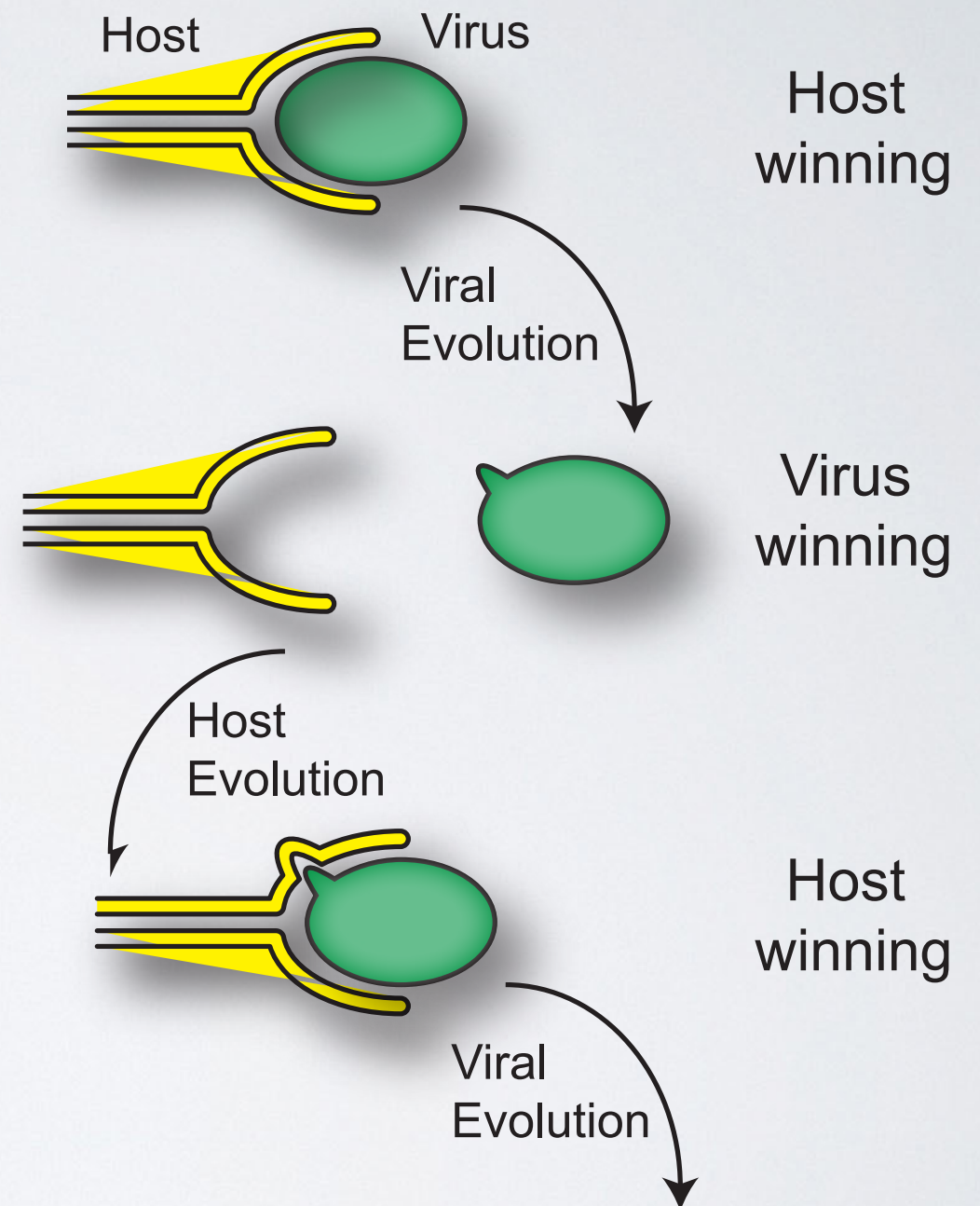


Conflict → 'Rapid evolution'

Host-host coevolution



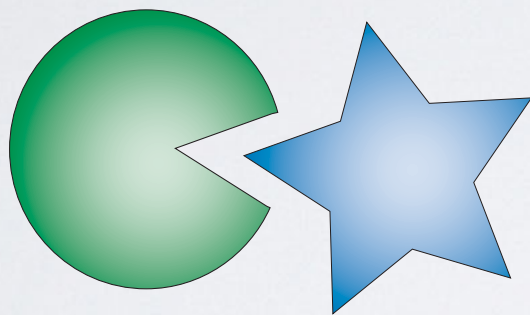
Host-virus arms race



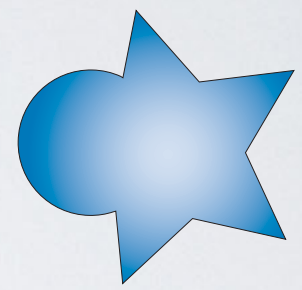
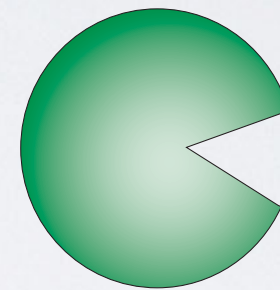
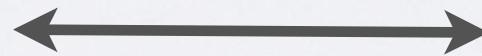
Positive selection identifies 'specificity' domains
(host-virus interaction surfaces)

“Where” does positive selection occur?

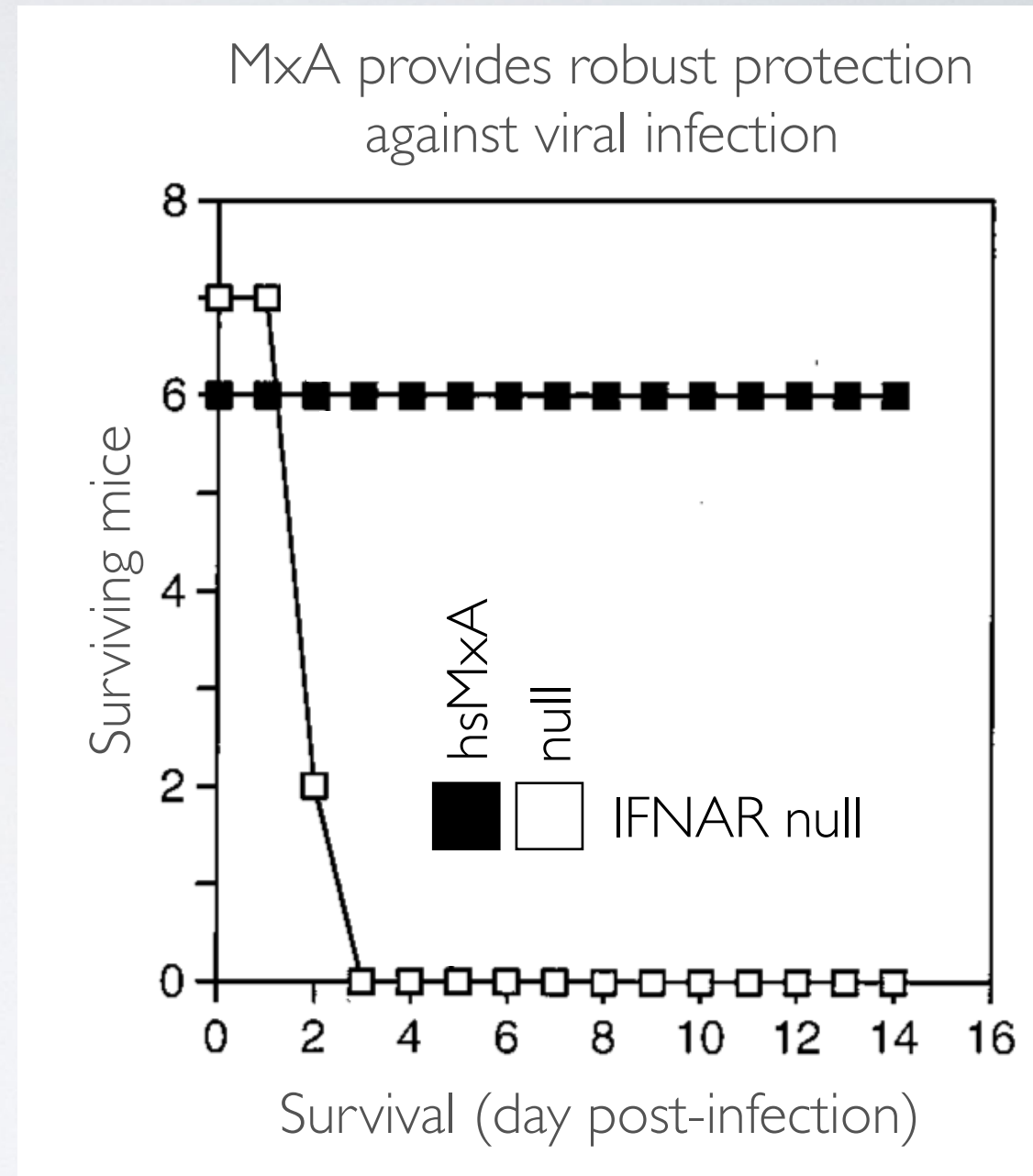
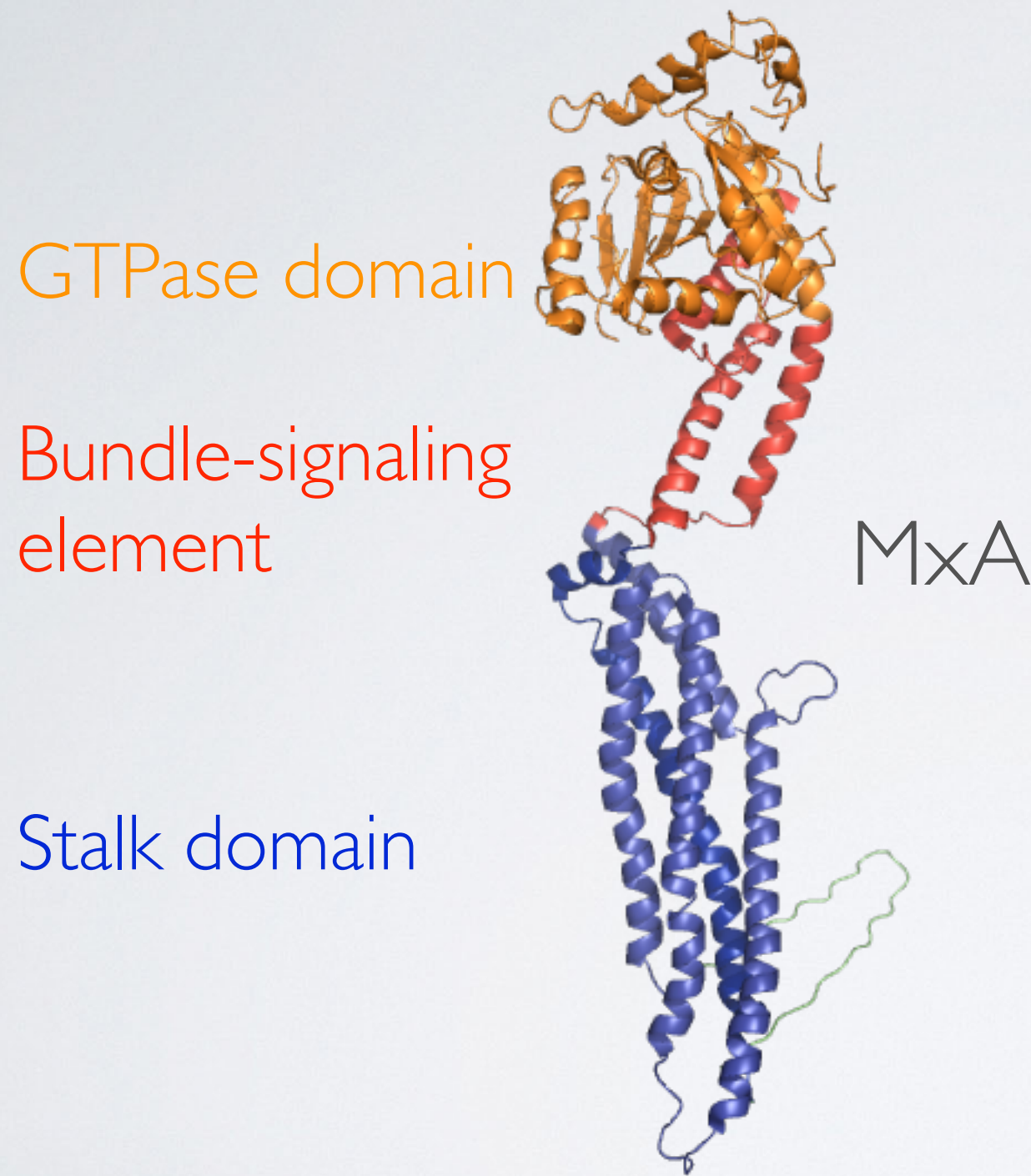
Antiviral protein



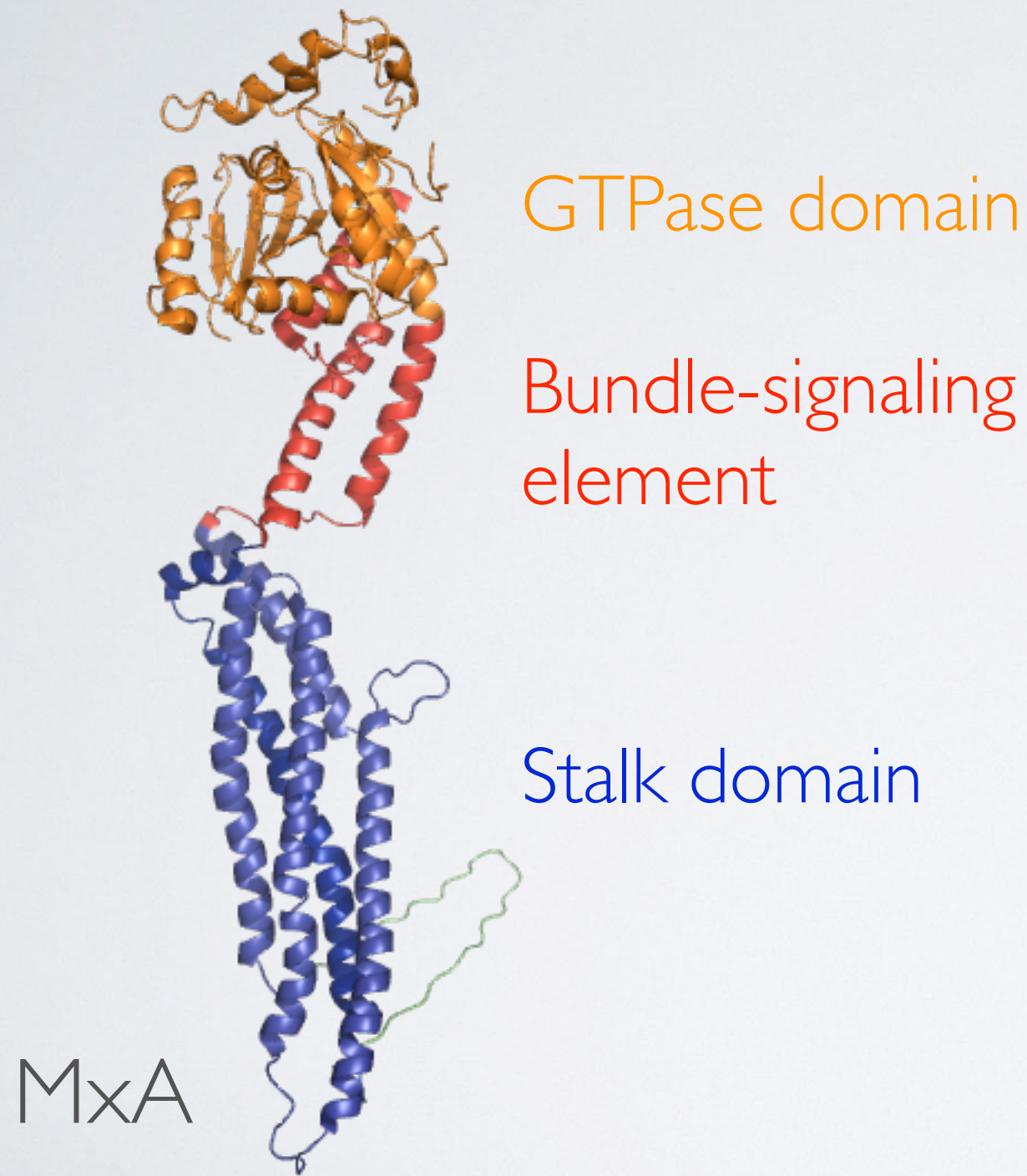
Viral protein



MxA, an IFN-induced Dynamin-like GTPase



MxA broadly inhibits the replication of multiple virus families



MxA-sensitive viruses

Negative-sense RNA viruses:

Orthomyxoviridae: Influenza A virus, Thogoto virus

Paramyxoviridae: Measles virus,

Bunyaviridae: Rift valley fever virus

Rhabdoviridae: Vesicular stomatitis virus

Positive-sense RNA viruses:

Picornaviridae: Coxsackievirus B4

Togaviridae: Semliki Forest virus

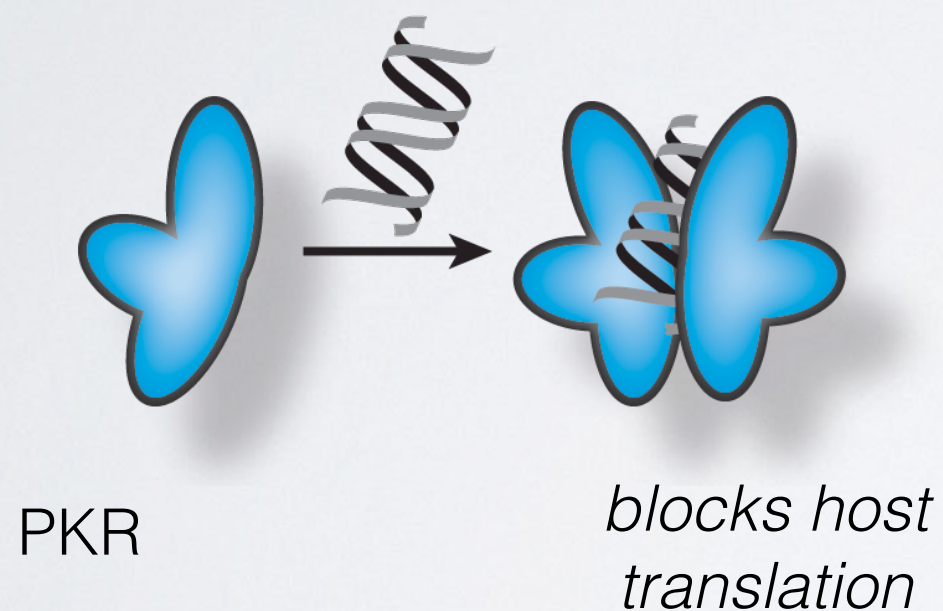
DNA viruses:

Asfarviridae: African swine fever virus

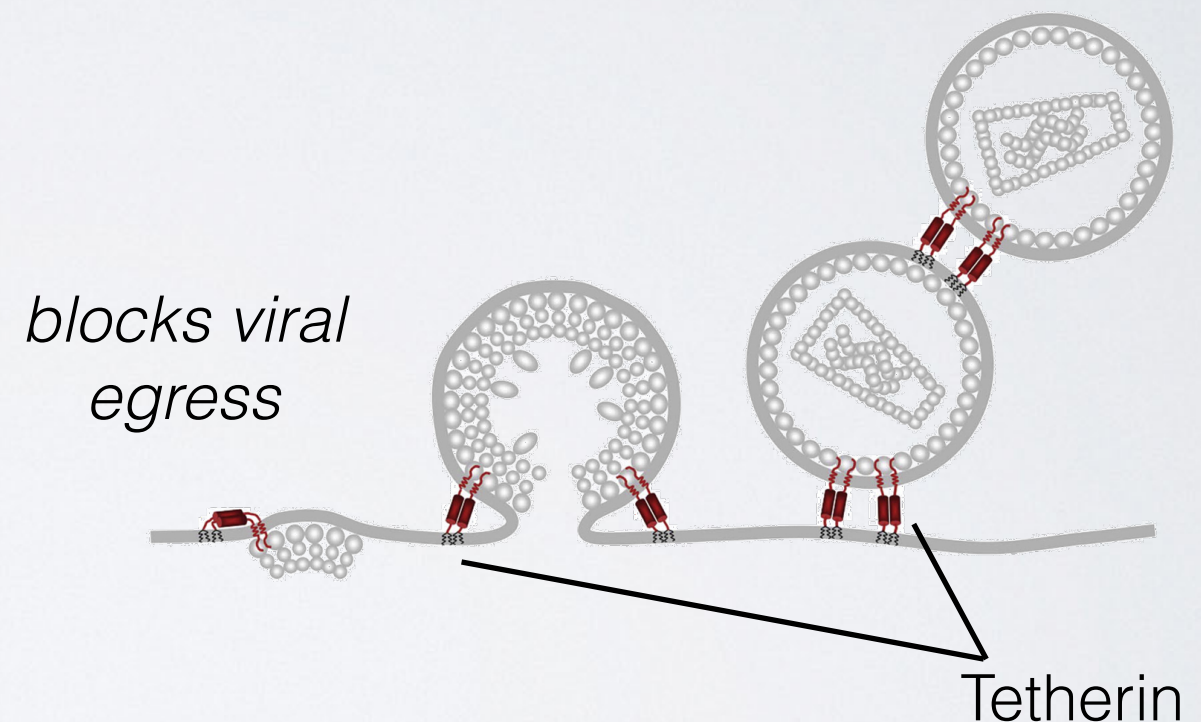
Hepadnaviridae: Hepatitis B virus

Breadth versus **specificity** in antiviral effector proteins

Activates on recognizing a generic feature of viral replication
(e.g. PKR and dsRNA)



Targets via a host cofactor commonly used by viruses
(e.g. Tetherin and lipid membranes)



Antivirals such as PKR and Tetherin evolve
under positive selection to escape viral antagonism

*Perez-Caballero et al. Cell (2009),
Aiken & Joyce. Nature (2011),
Mitchell et al. Curr Opin Microbiol (2013)*

Breadth versus **specificity** in antiviral effector proteins

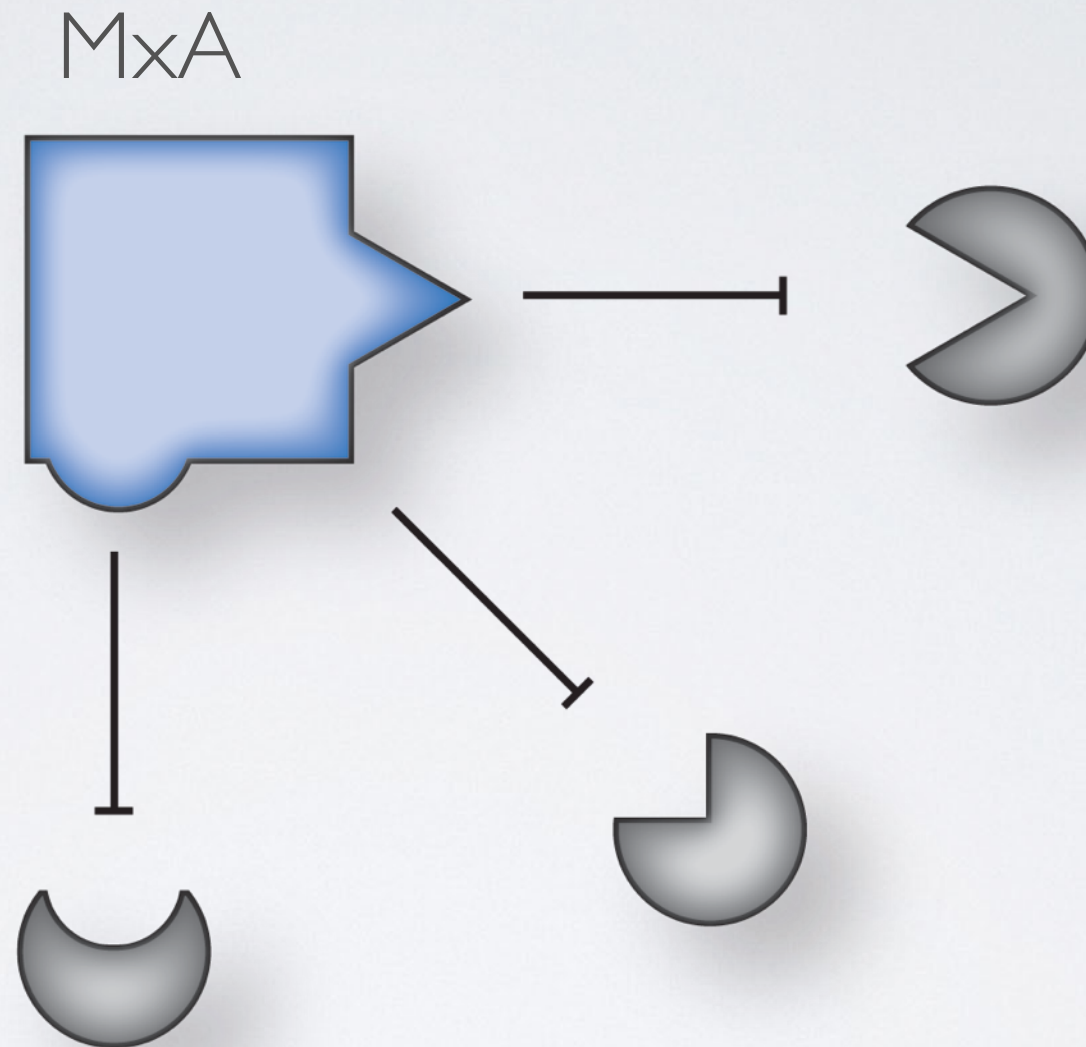
Specific target recognition,
narrowly-acting
(e.g. Trim5 and Fv1; retroviral capsid
lattice)



Antivirals such as TRIM5 and Fv1 evolve under
positive selection to re-establish viral recognition

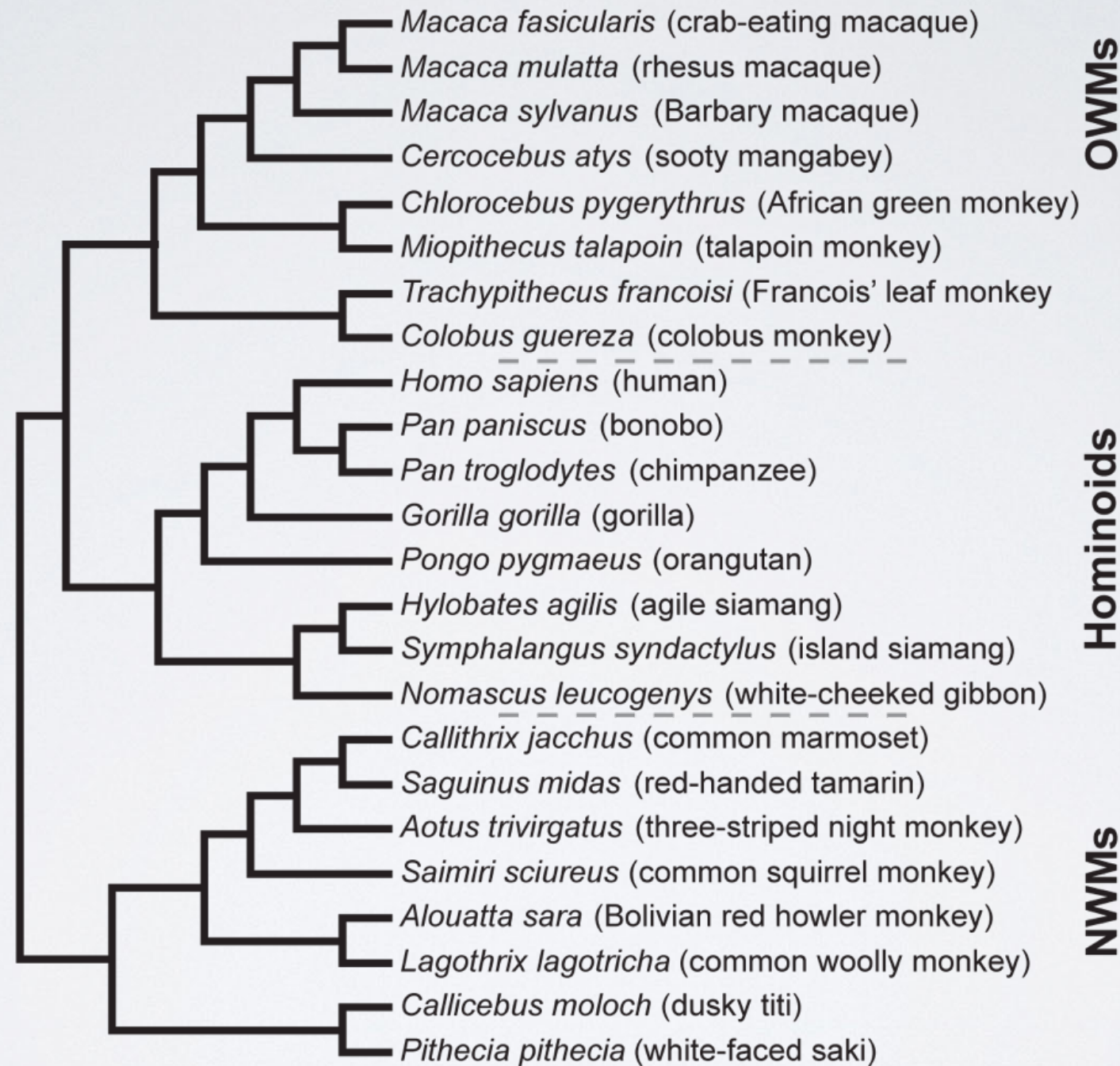
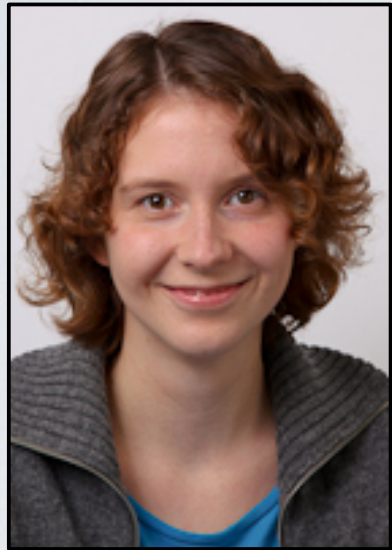
MxA is an antiviral hybrid

(broadly-acting despite specific recognition of distinct viral targets)

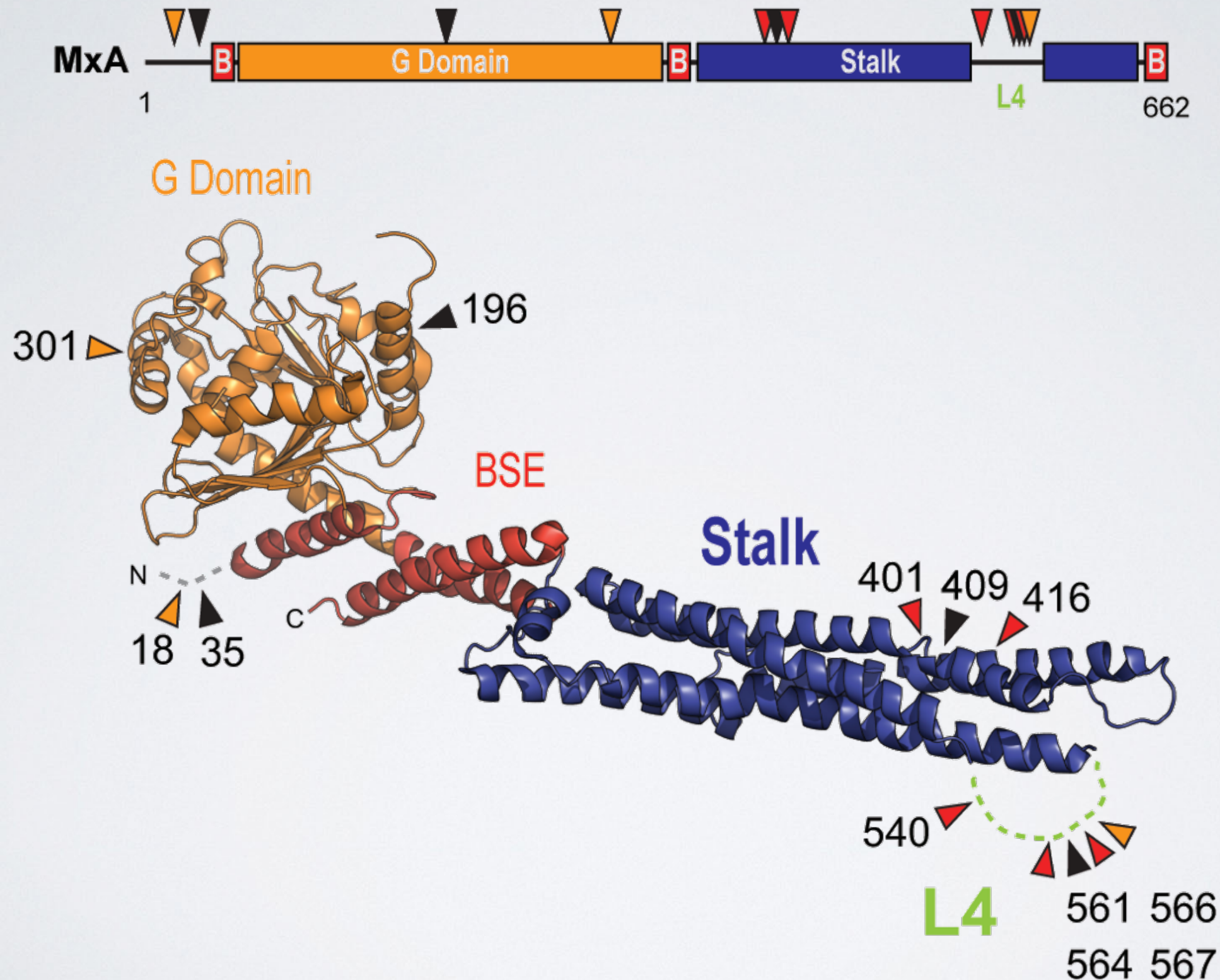


How does MxA maintain antiviral breadth while recognizing multiple, distinct viral targets?

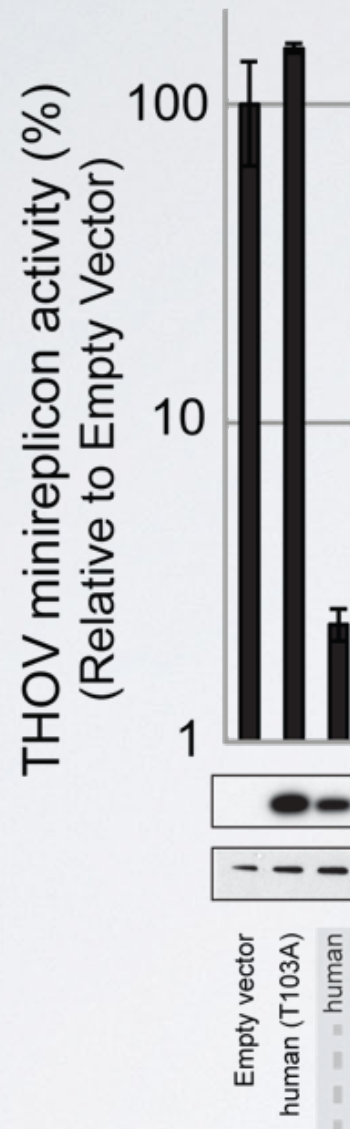
Evolution-guided identification of target recognition in the antiviral large GTPase MxA



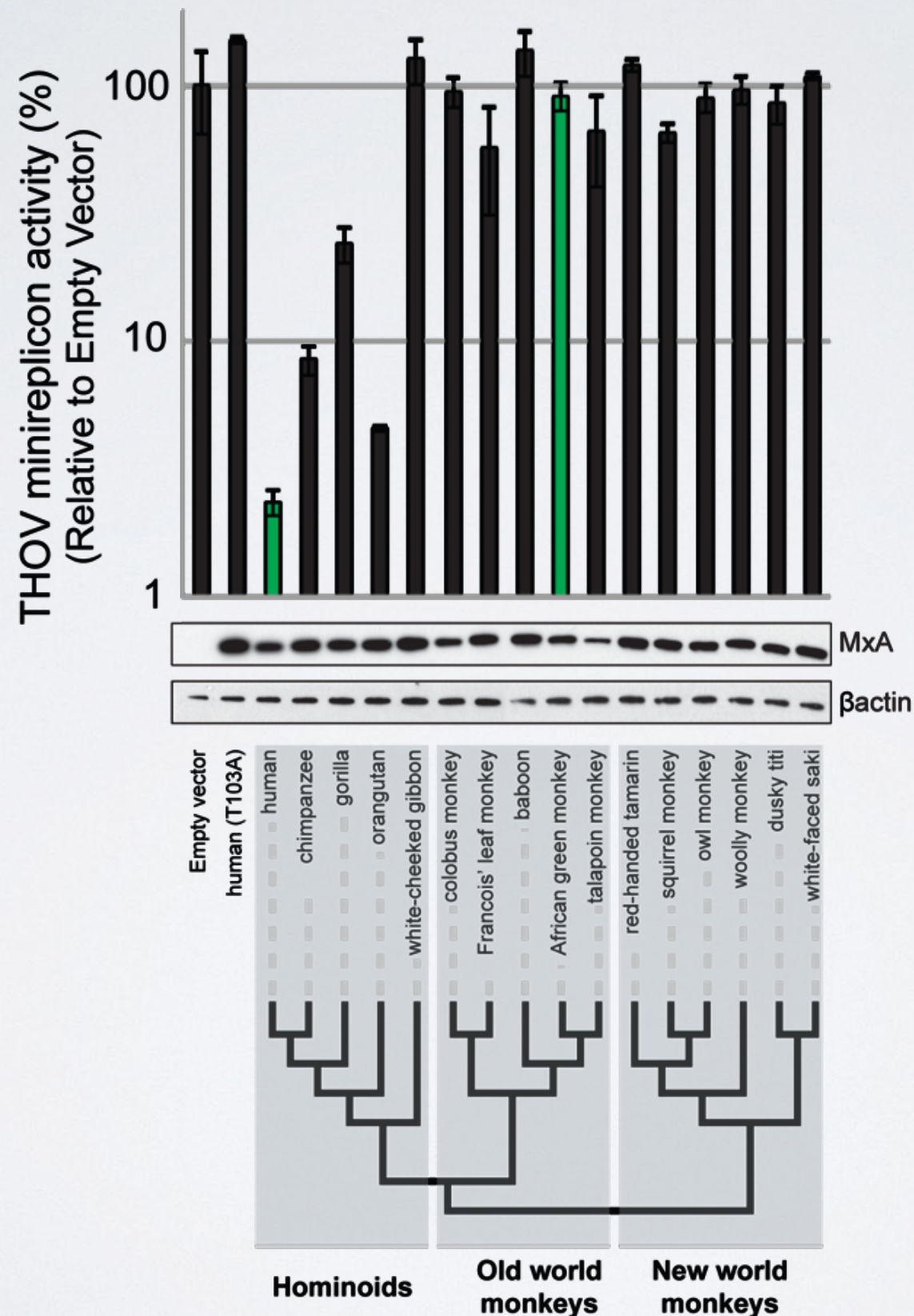
Evolution-guided identification of target recognition in the antiviral large GTPase MxA



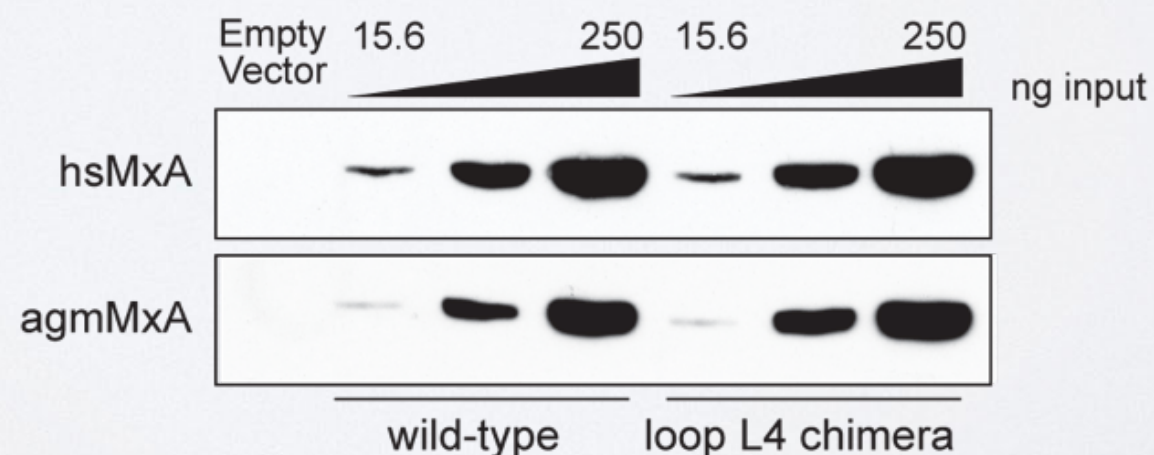
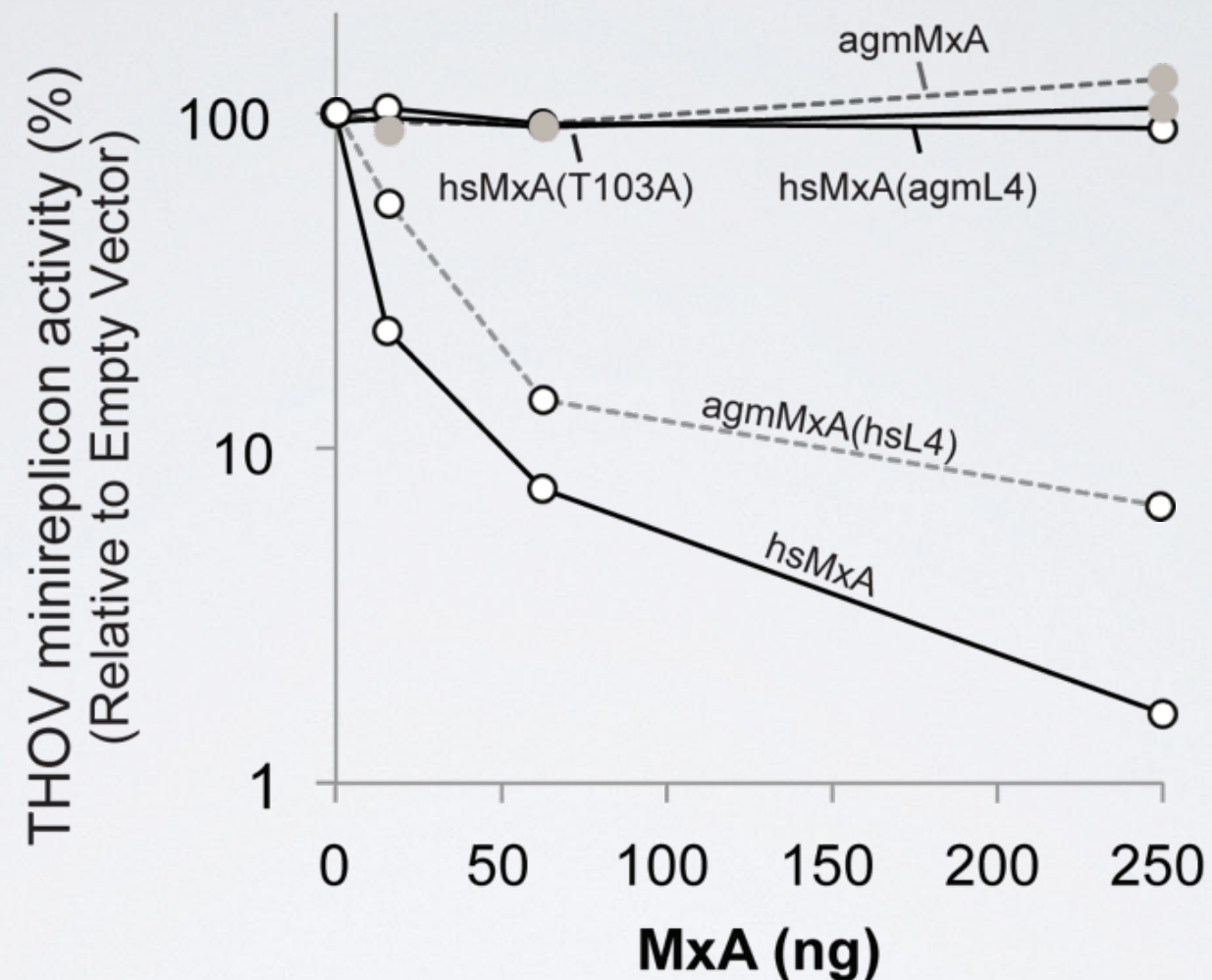
Primate MxA orthologs vary in their ability to restrict a distant influenza relative, Thogoto Virus (THOV)



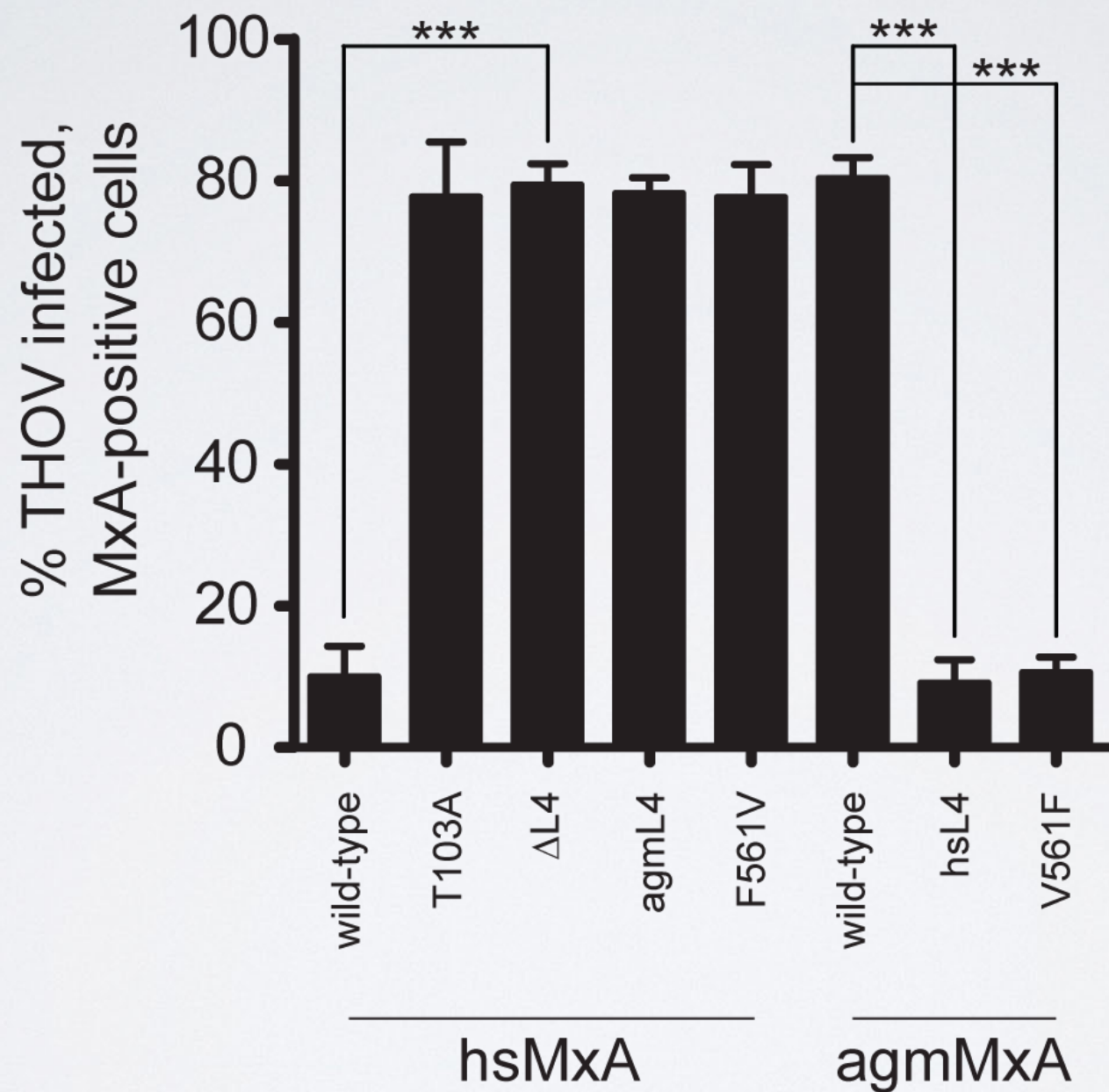
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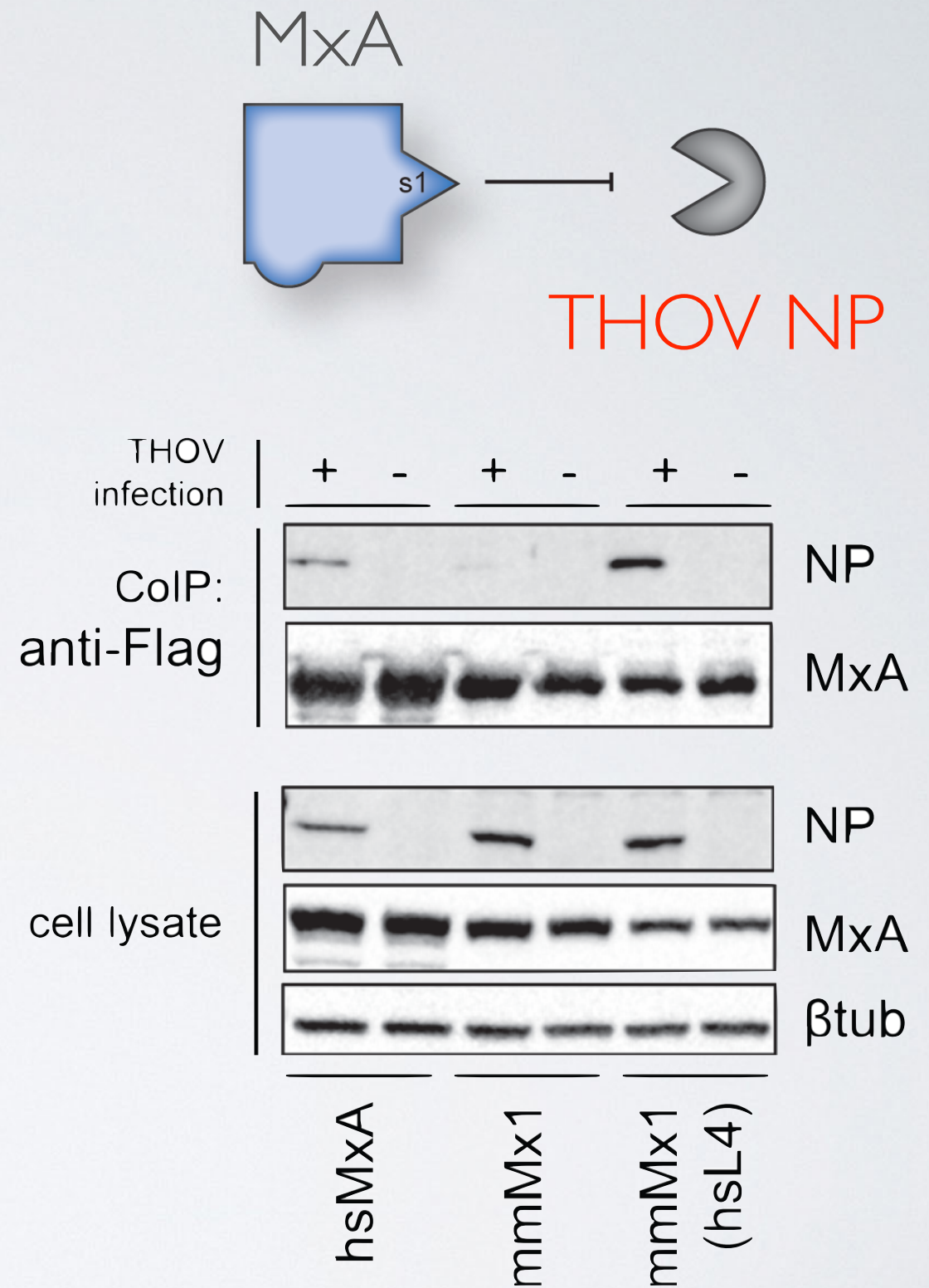
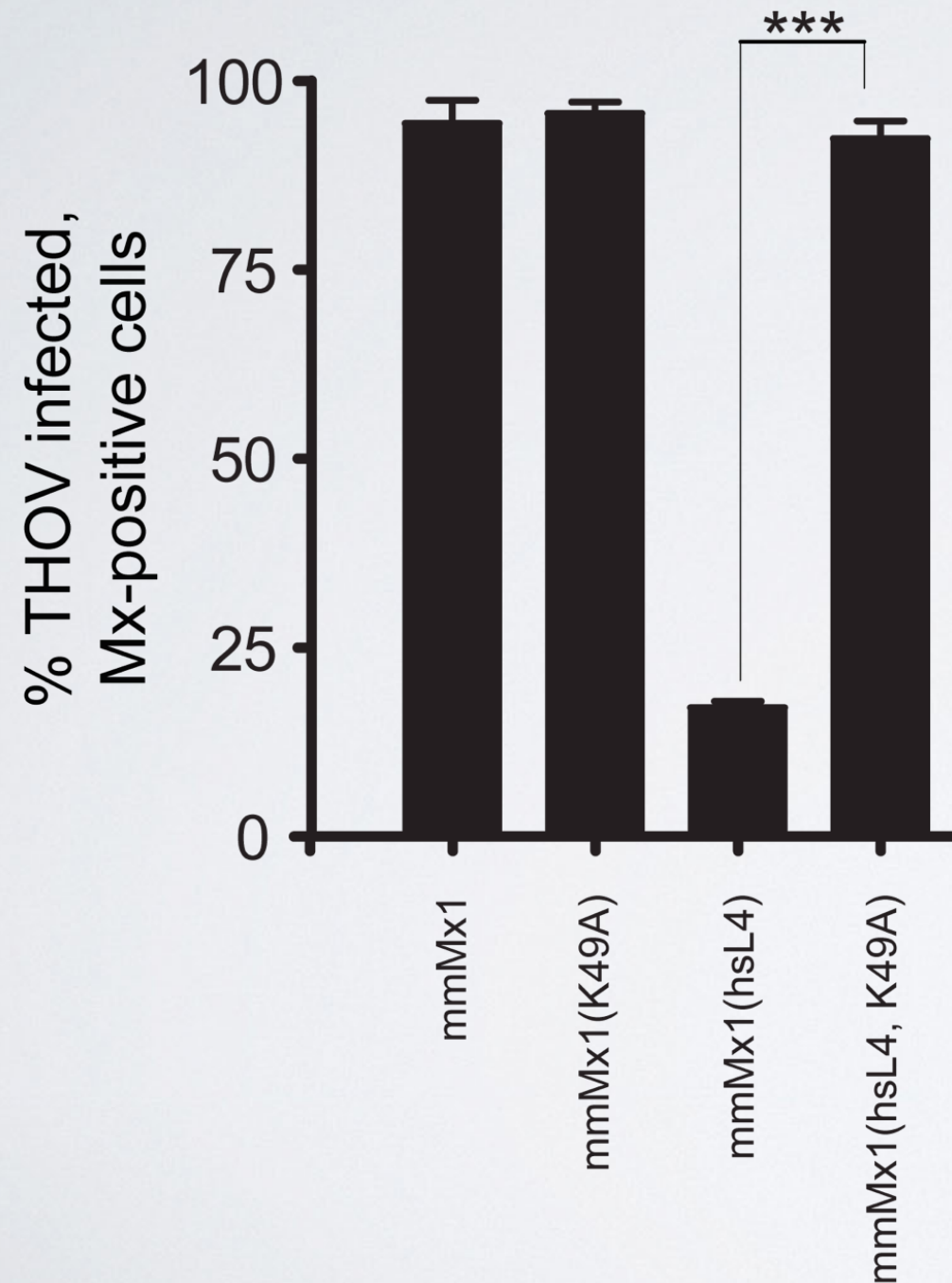
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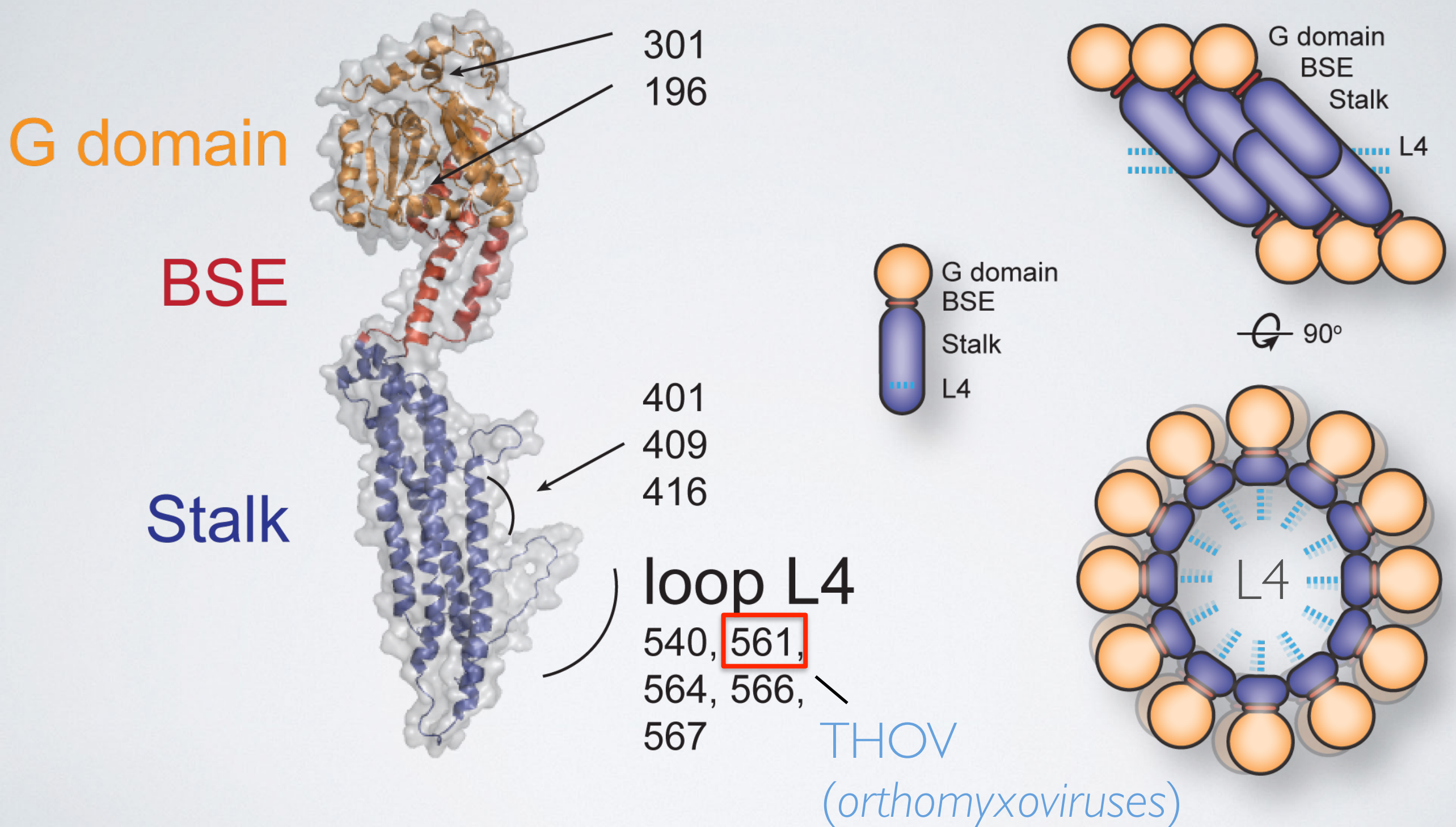
A single residue in L4 confers MxA antiviral specificity for THOV



L4 is a modular unit for MxA target recognition

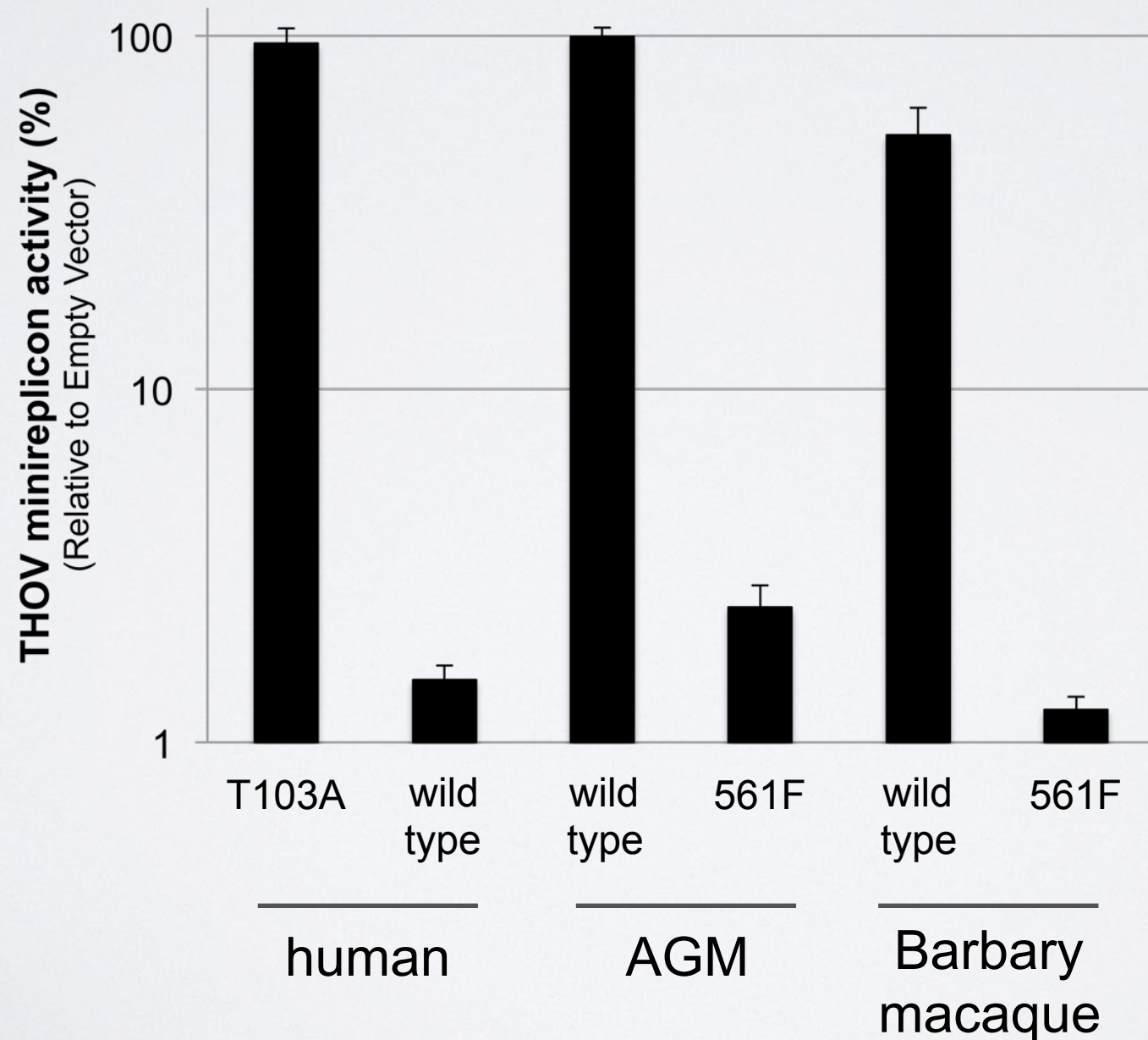


L4 positioning may explain large phenotypes of single amino acid changes in MxA

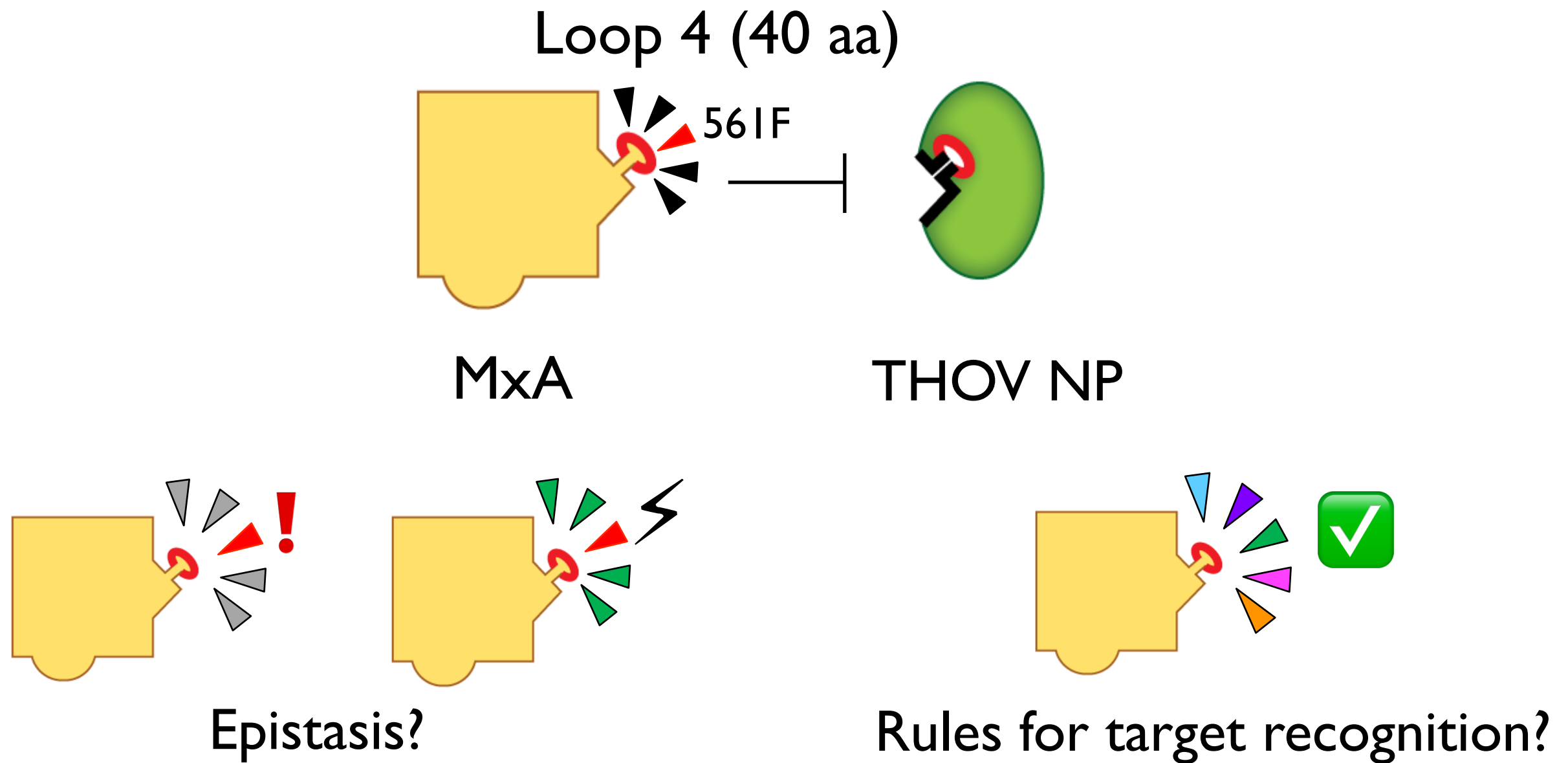


Robustness in MxA antiviral specificity for THOV

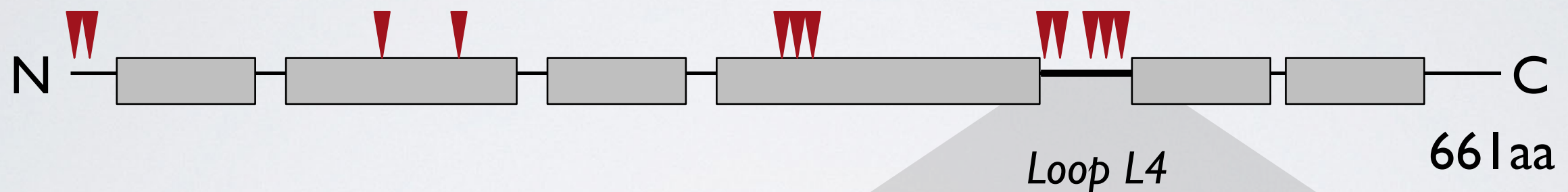
	556	561	566	572									
human	KKSWD	F	G	A	F	Q	S	S	A	T	D	I	
African green monkey	KKSWD	V	G	T	F	Q	P	S	-	S	T	D	I
Barbary macaque	KKSWD	V	G	T	F	Q	S	S	-	S	T	D	I



Evolution-guided mutagenesis to understand protein function



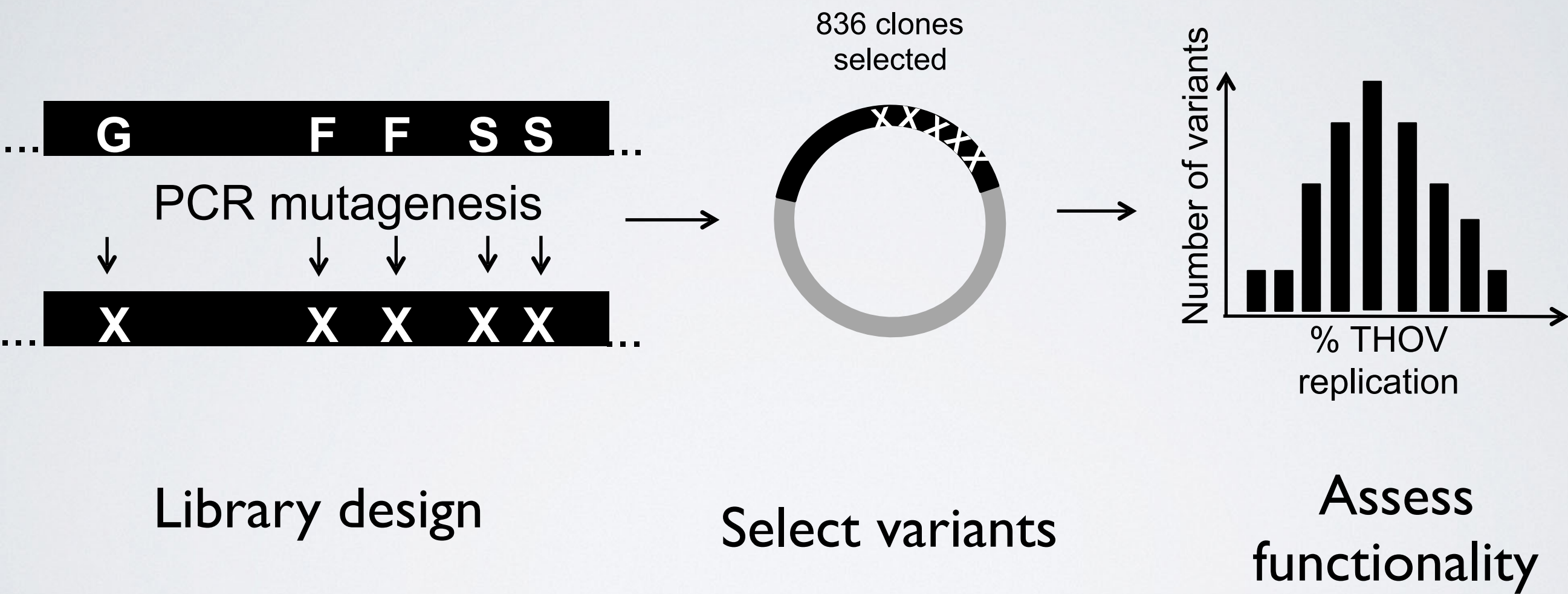
Combinatorial mutagenesis to understand and expand antiviral specificities



Rapidly evolving sites

540	561	564	566	567
X	X	X	X	X

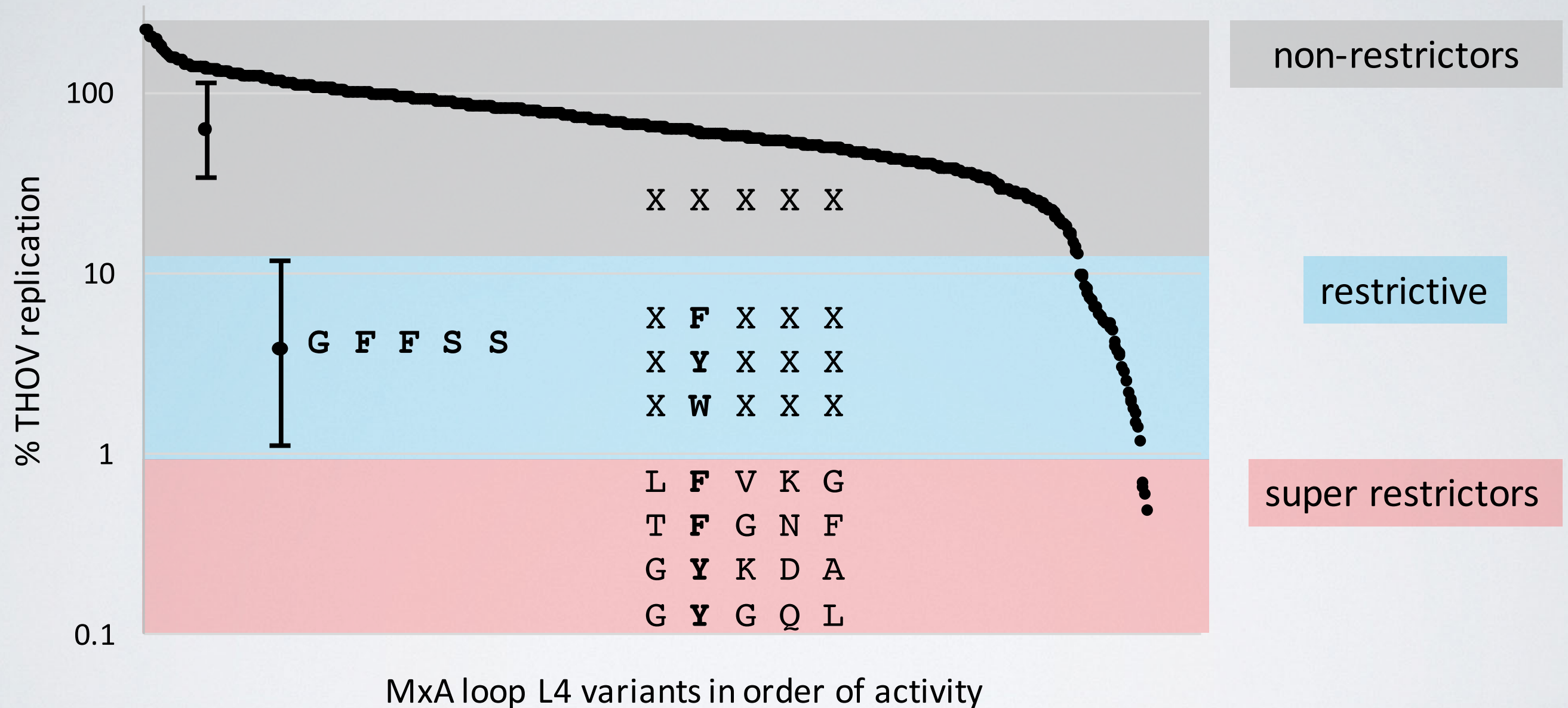




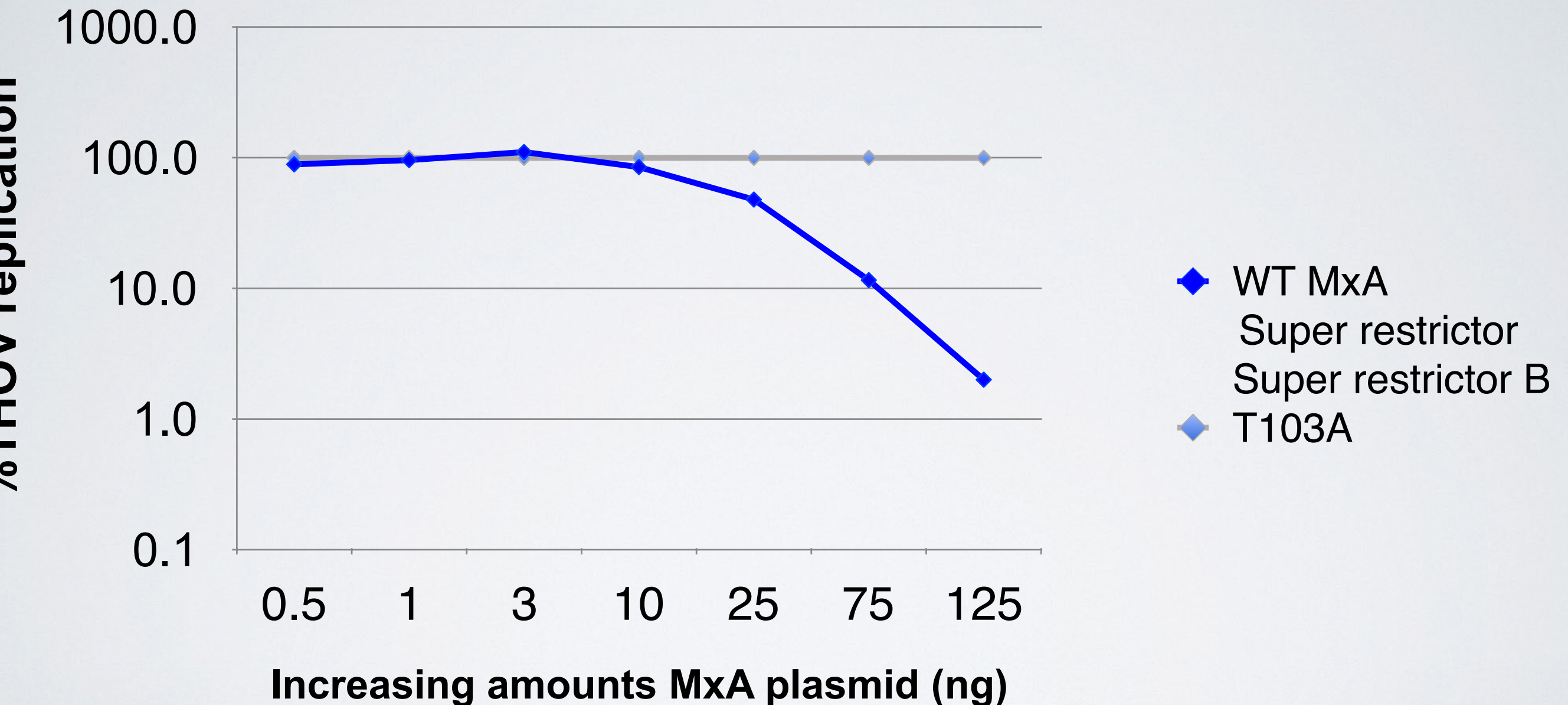
Functional distribution of MxA loop L4 variants



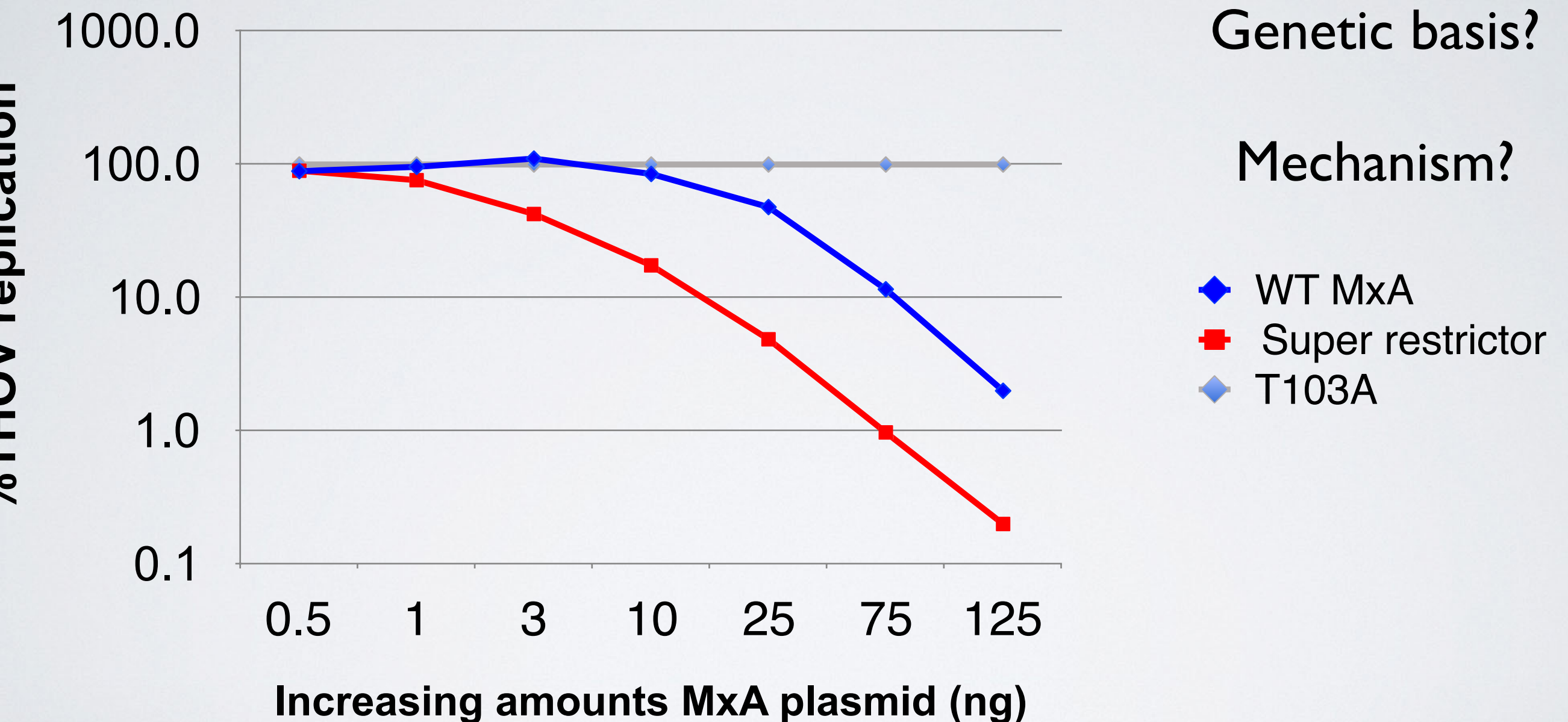
Functional distribution of MxA loop L4 variants



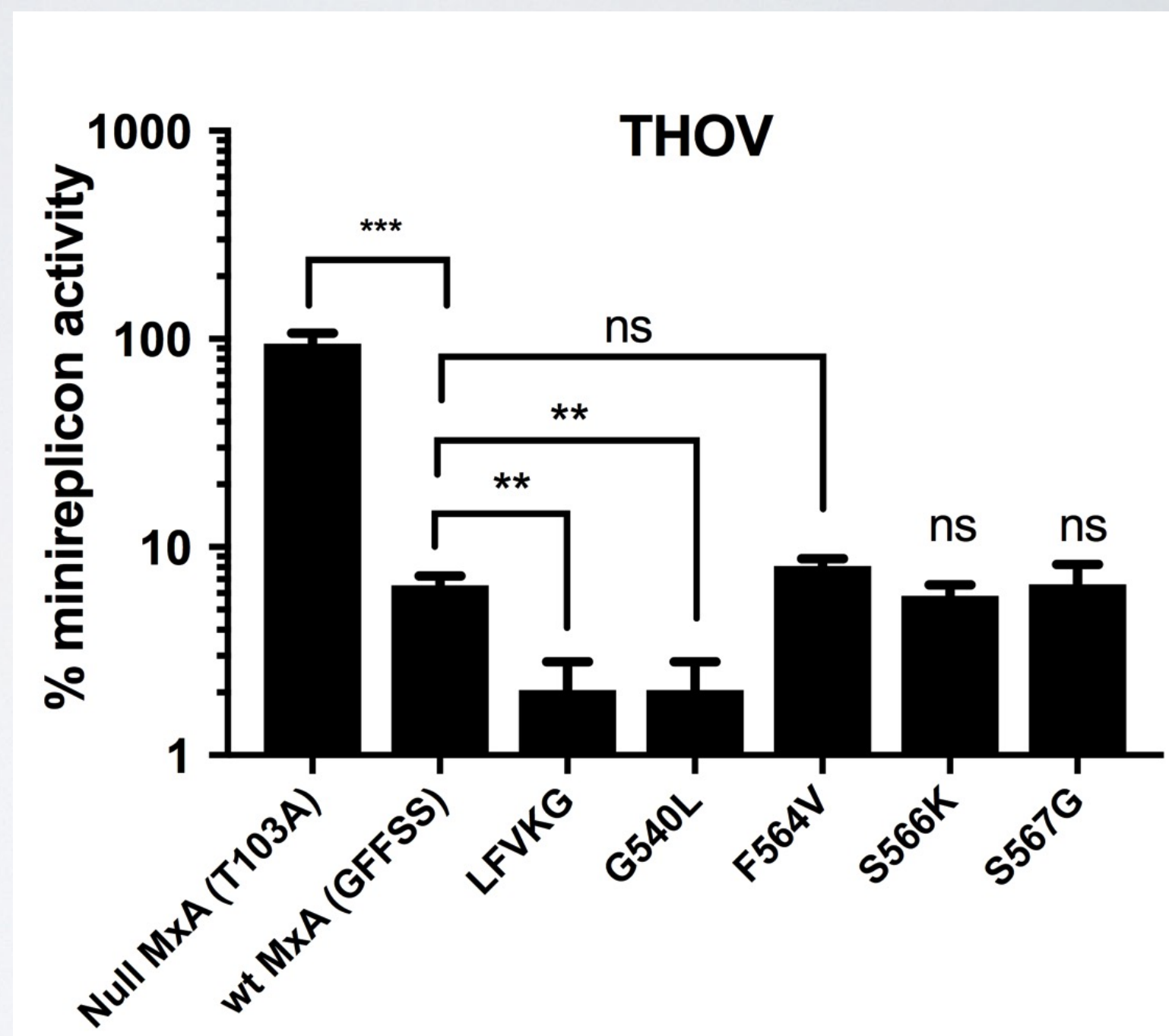
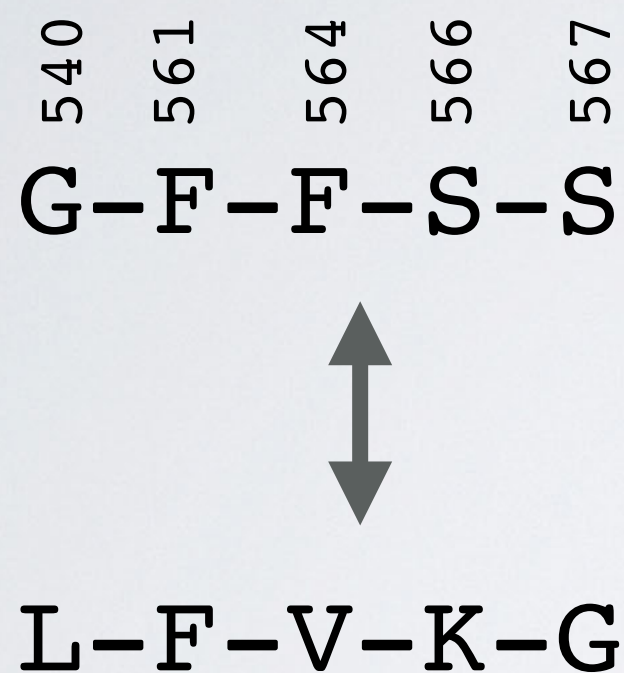
Some MxA loop L4 variants have increased antiviral activity against Thogoto virus



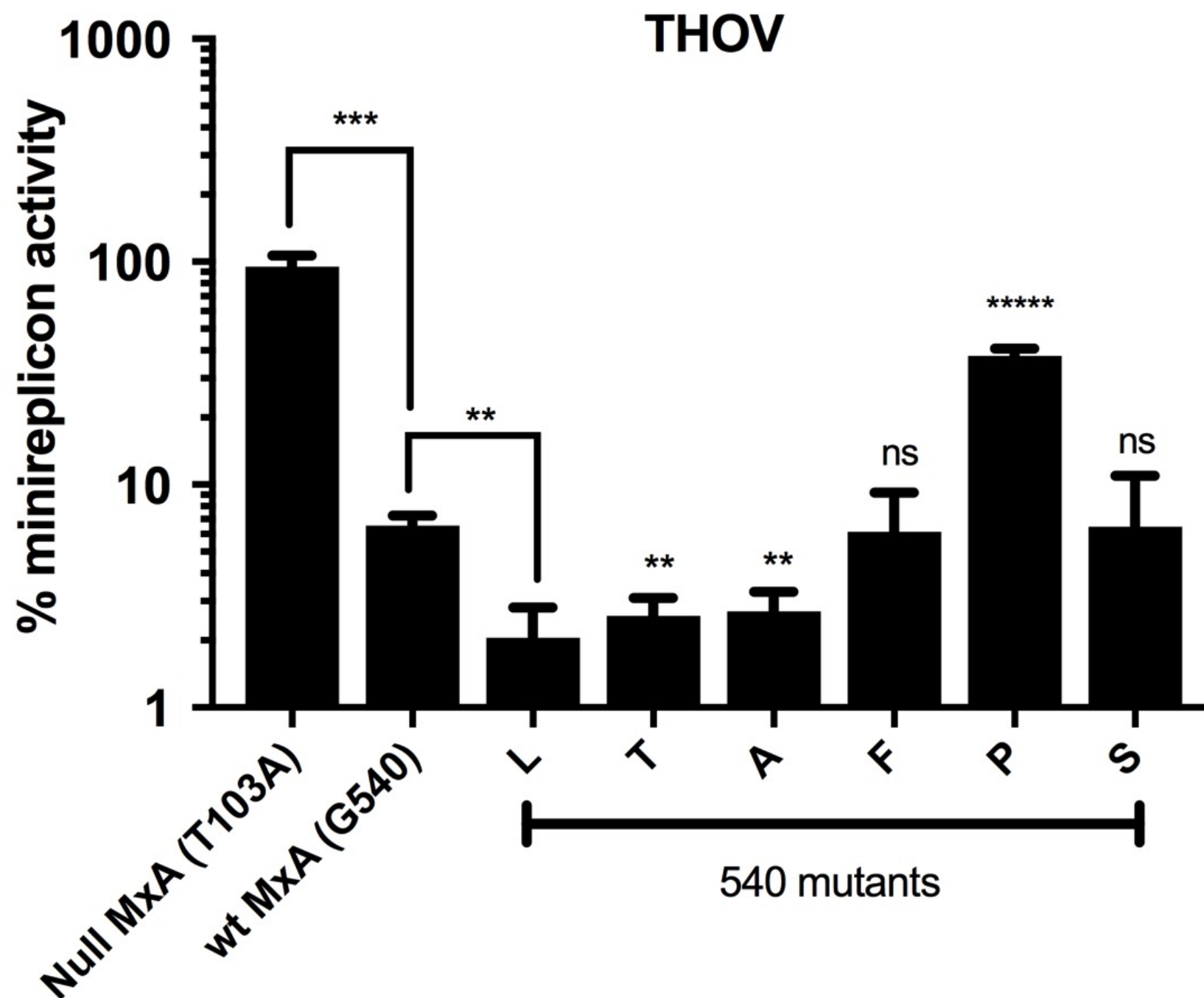
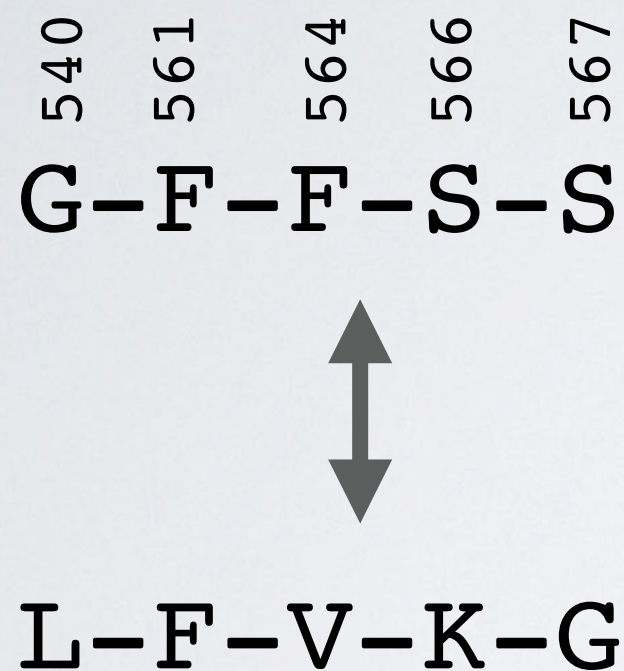
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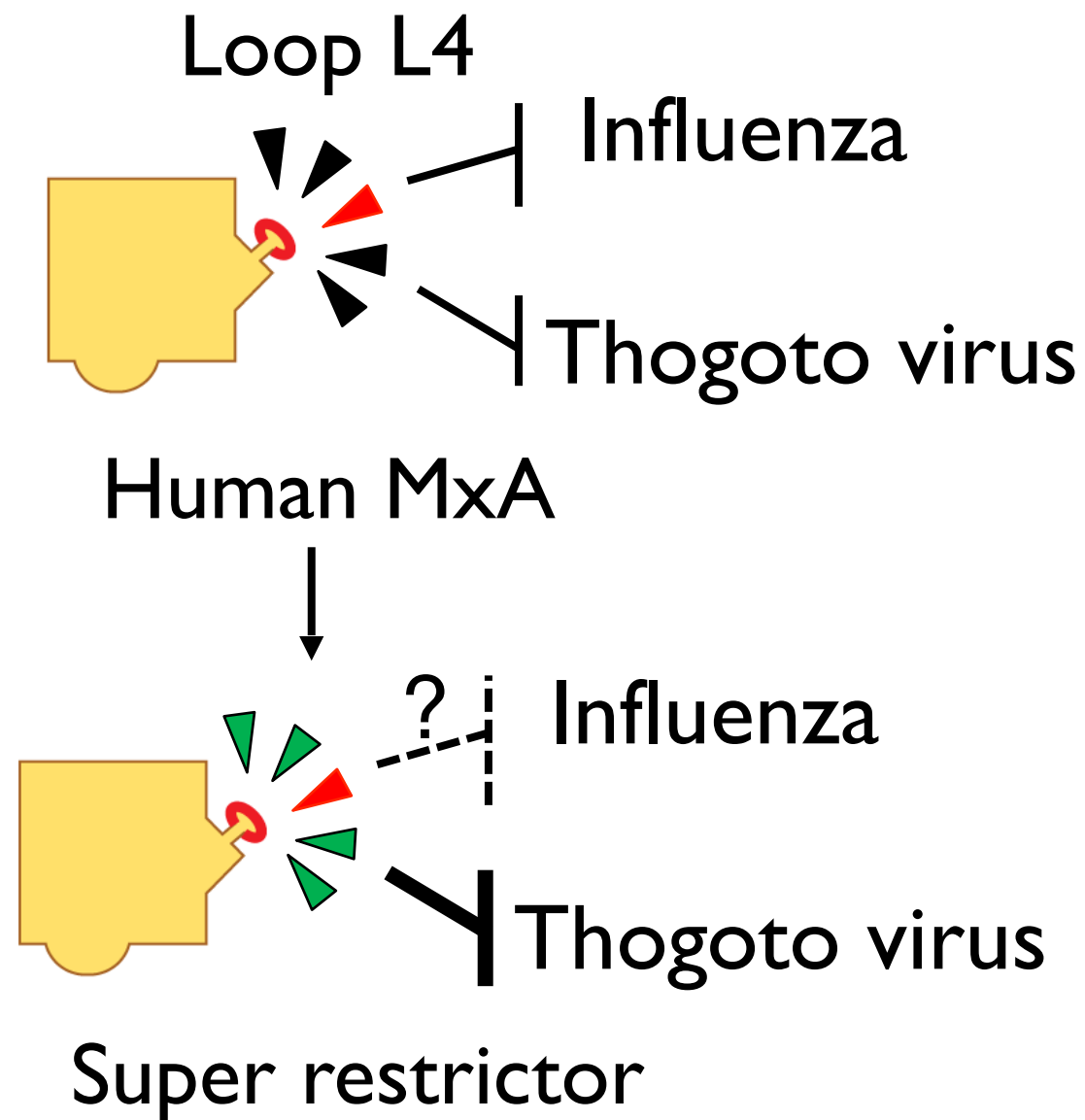
A single amino acid change G540L explains the super-restrictor phenotype



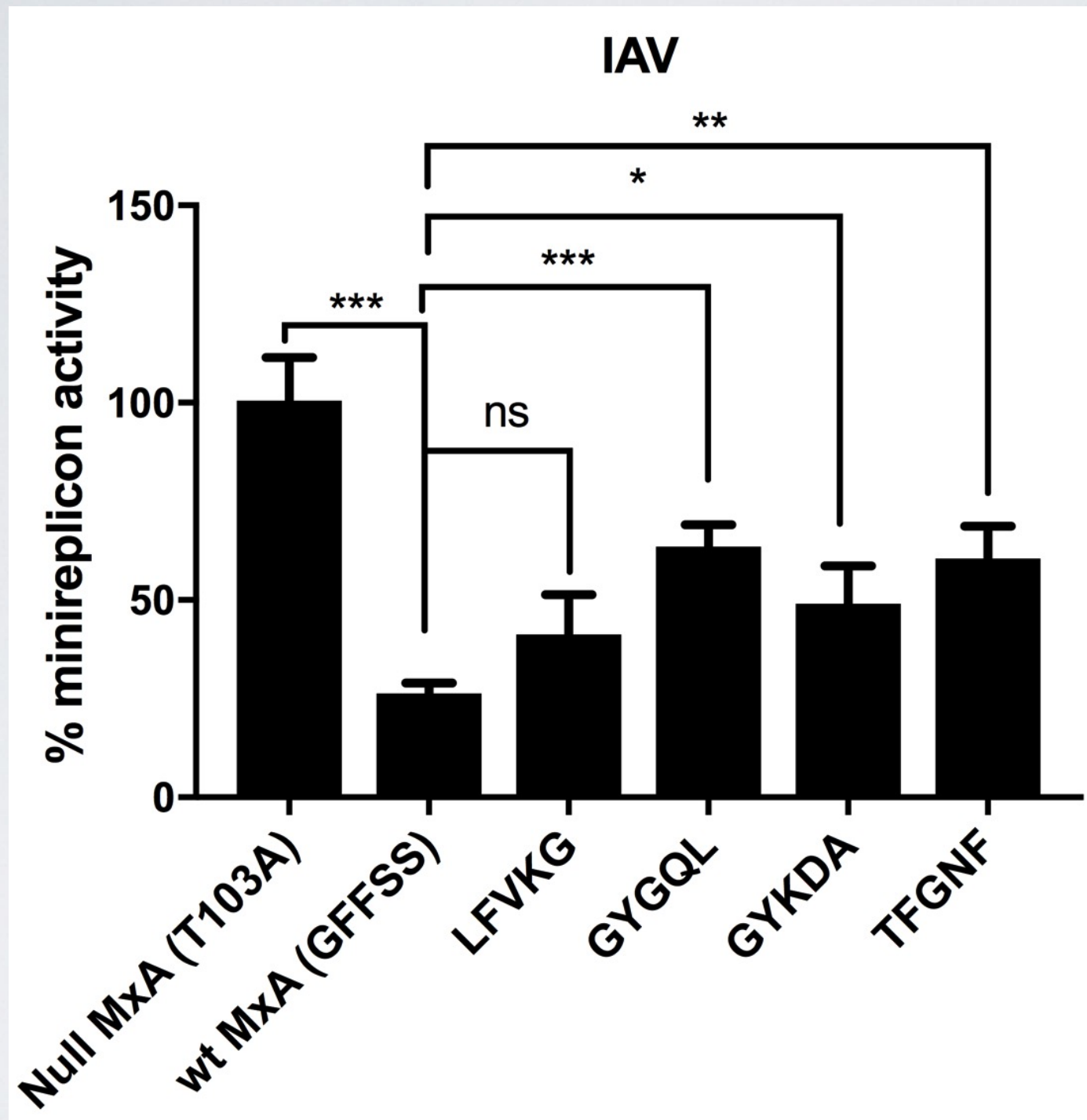
A single amino acid change G540L explains the super-restrictor phenotype



Does super restriction lead to a specificity tradeoff?

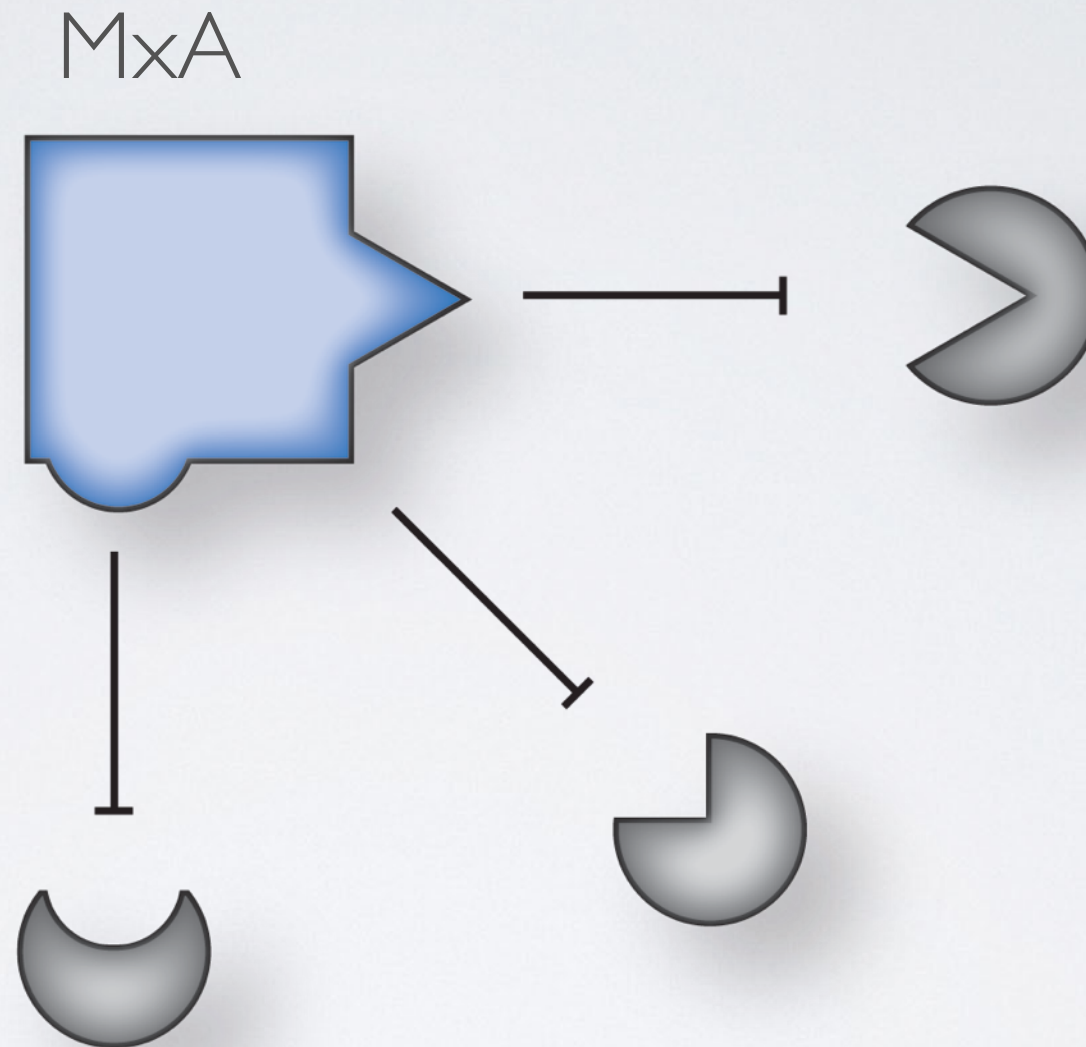


Super-restrictors are virus specific

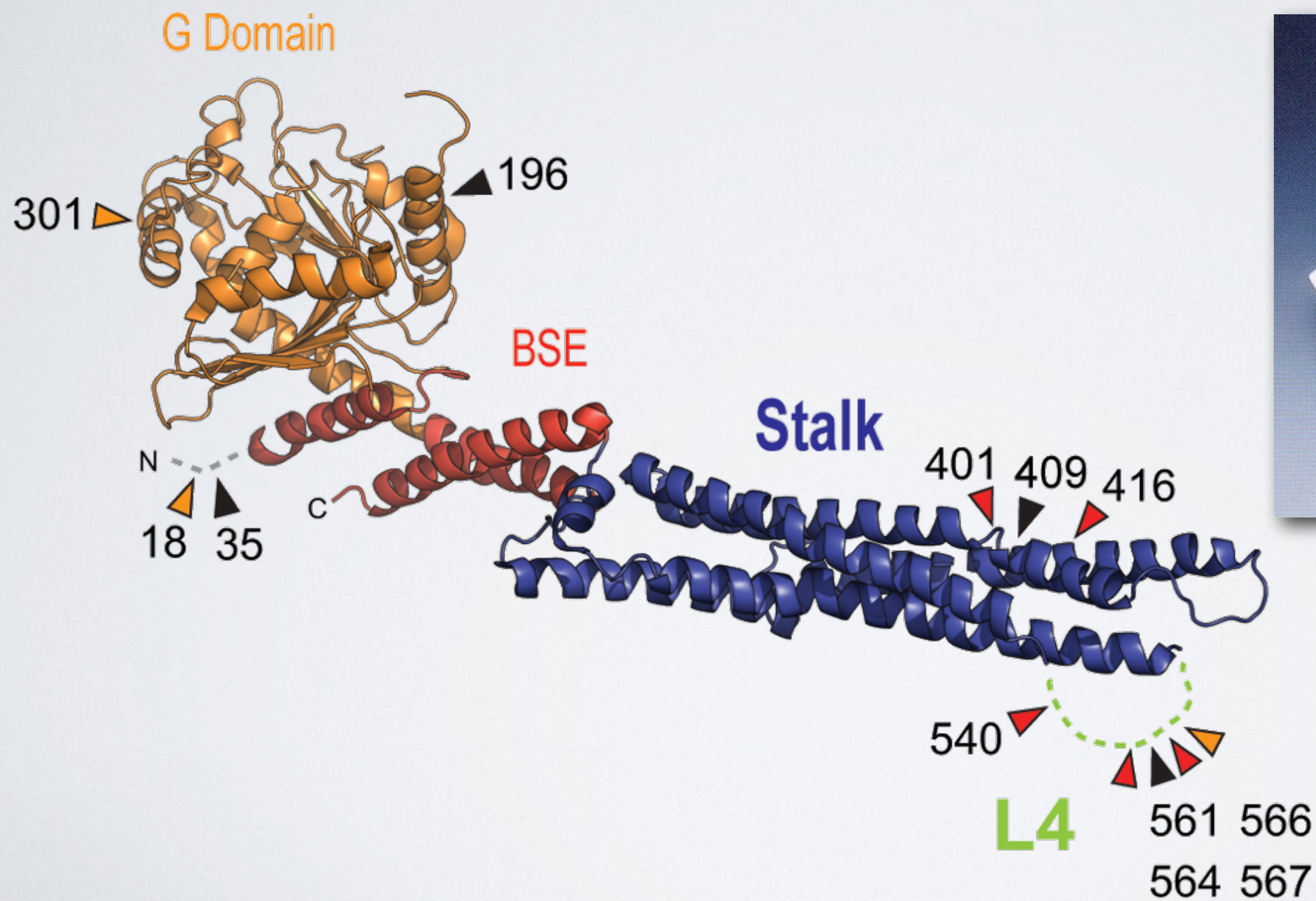


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Rules of Engagement: Molecular Insights from Host-Virus Arms Races

Matthew D. Daugherty¹ and Harmit S. Malik^{1,2}

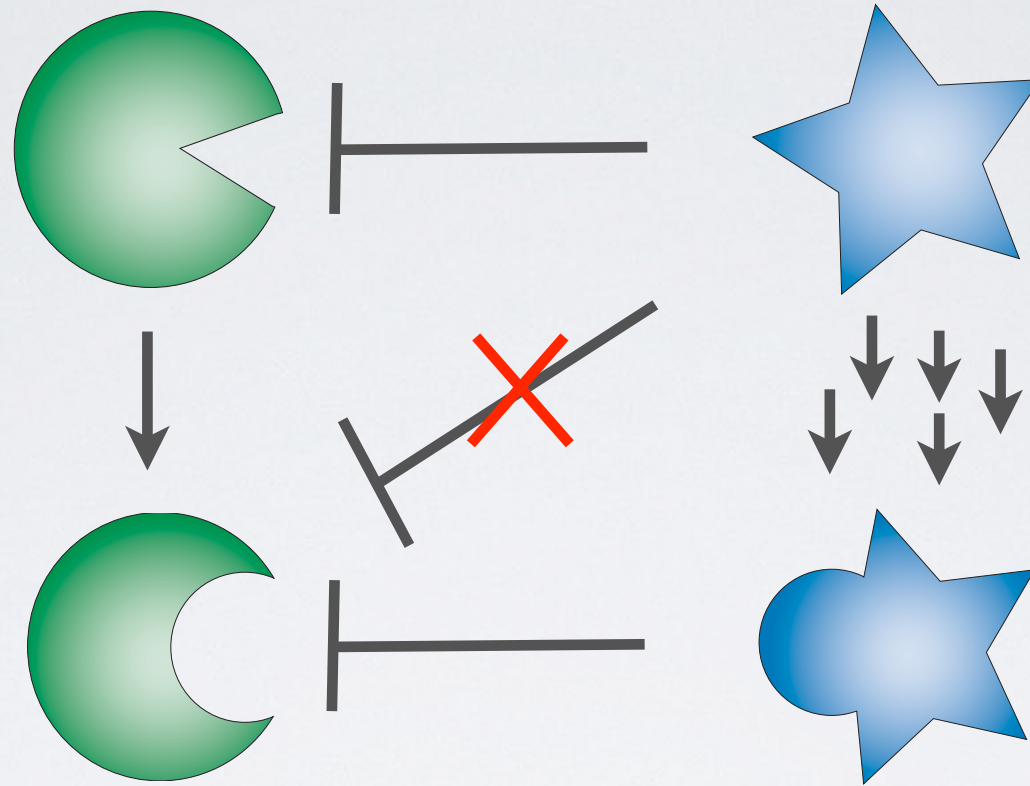
¹Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

²Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109; email: hsmalik@fhcrc.org

the usual suspects

Antiviral protein

Viral protein



*When, where, **how**?*