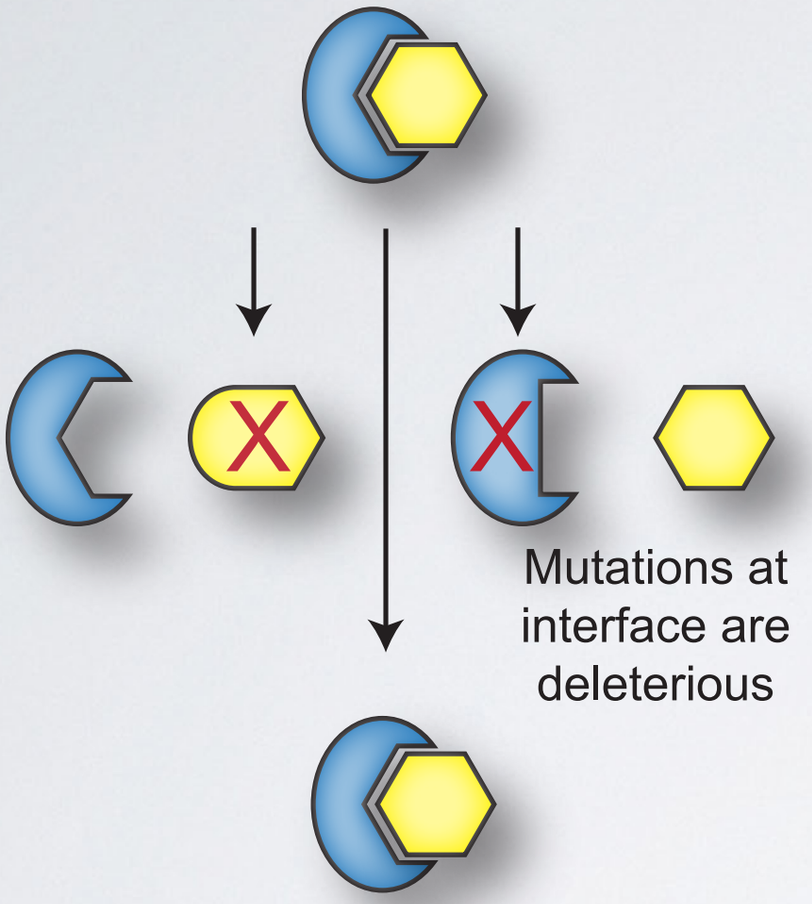
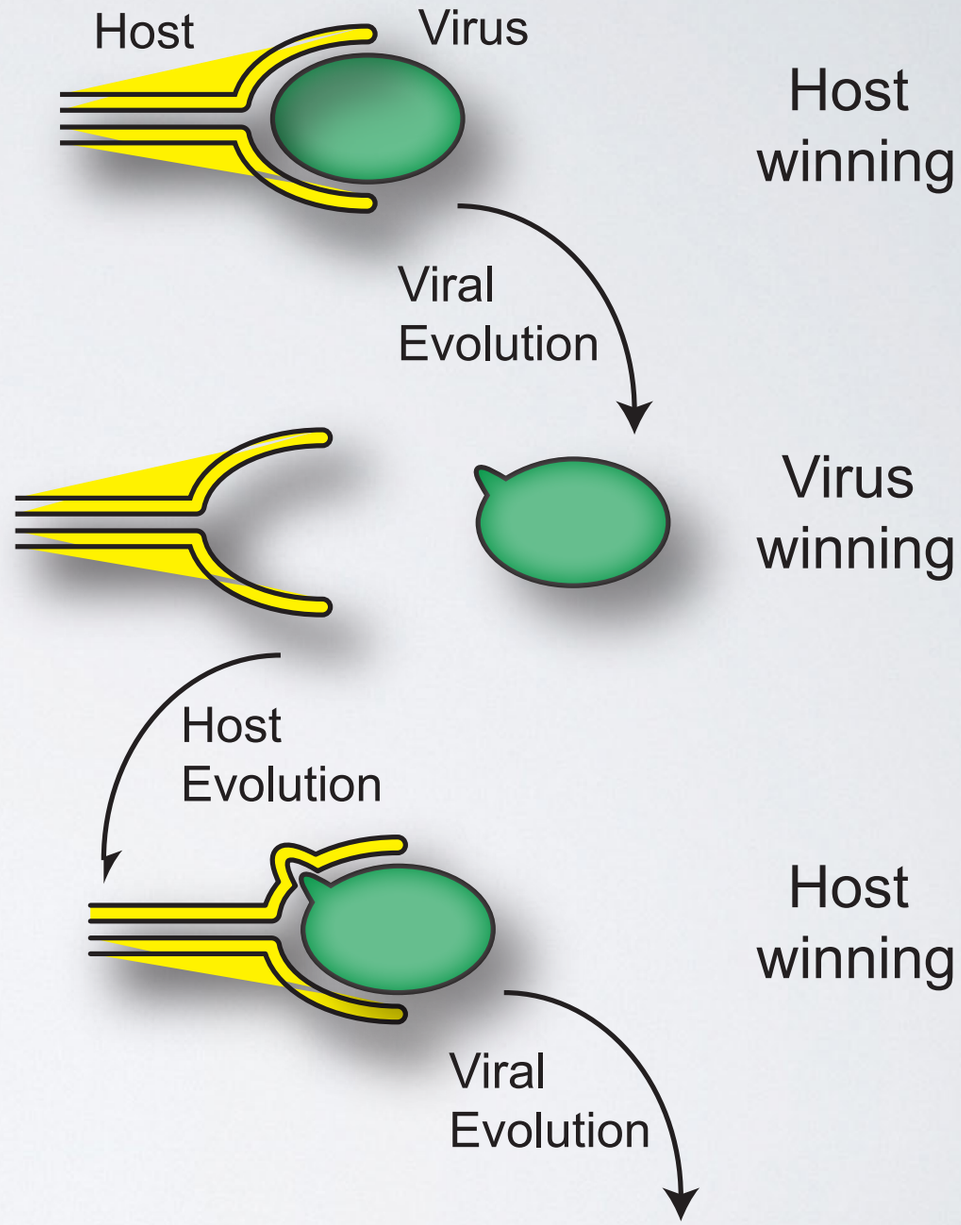
Host-host coevolution

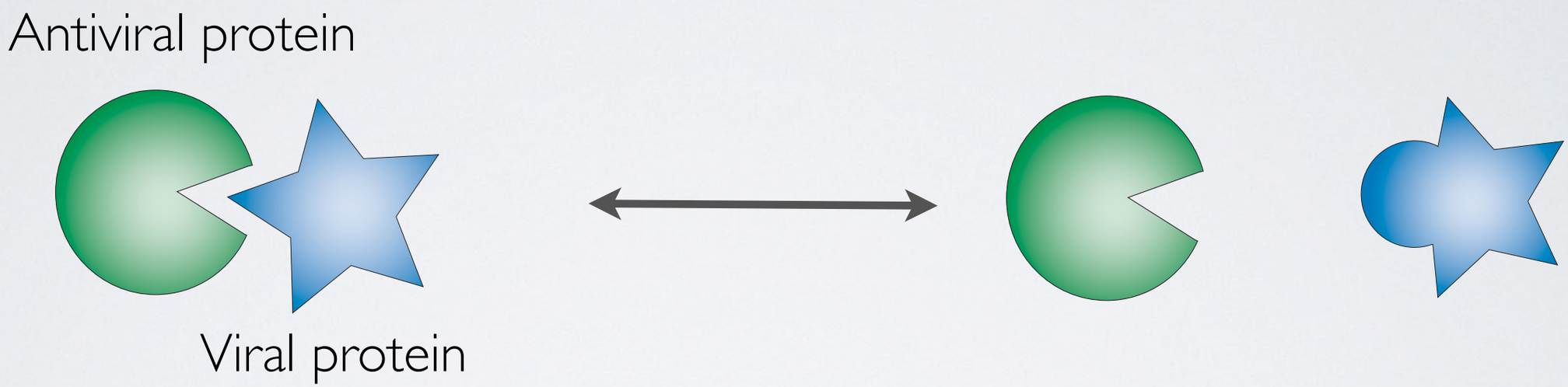


Host-virus arms race



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

"Where" does positive selection occur?

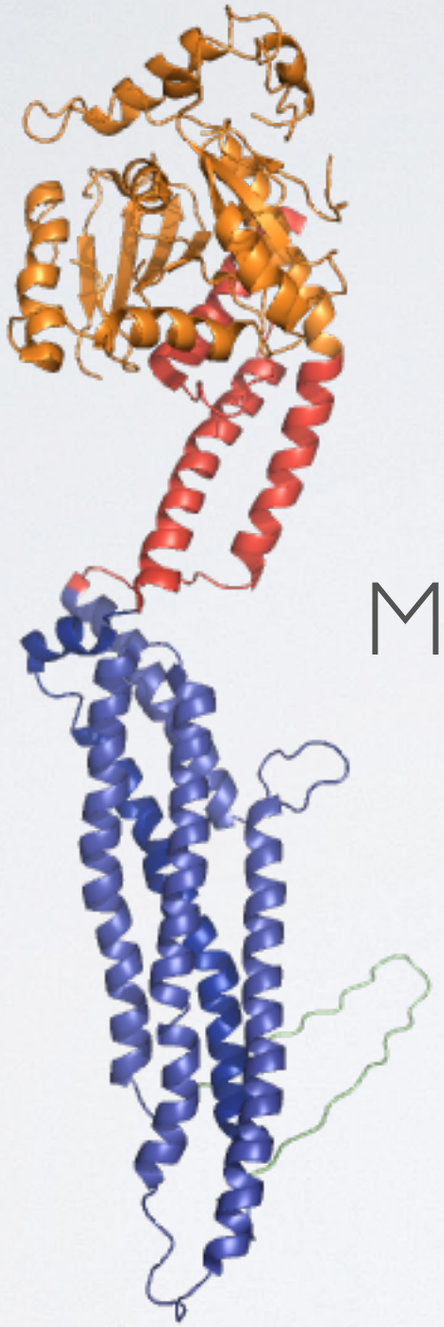


MxA, an IFN-induced Dynamin-like GTPase

GTPase domain

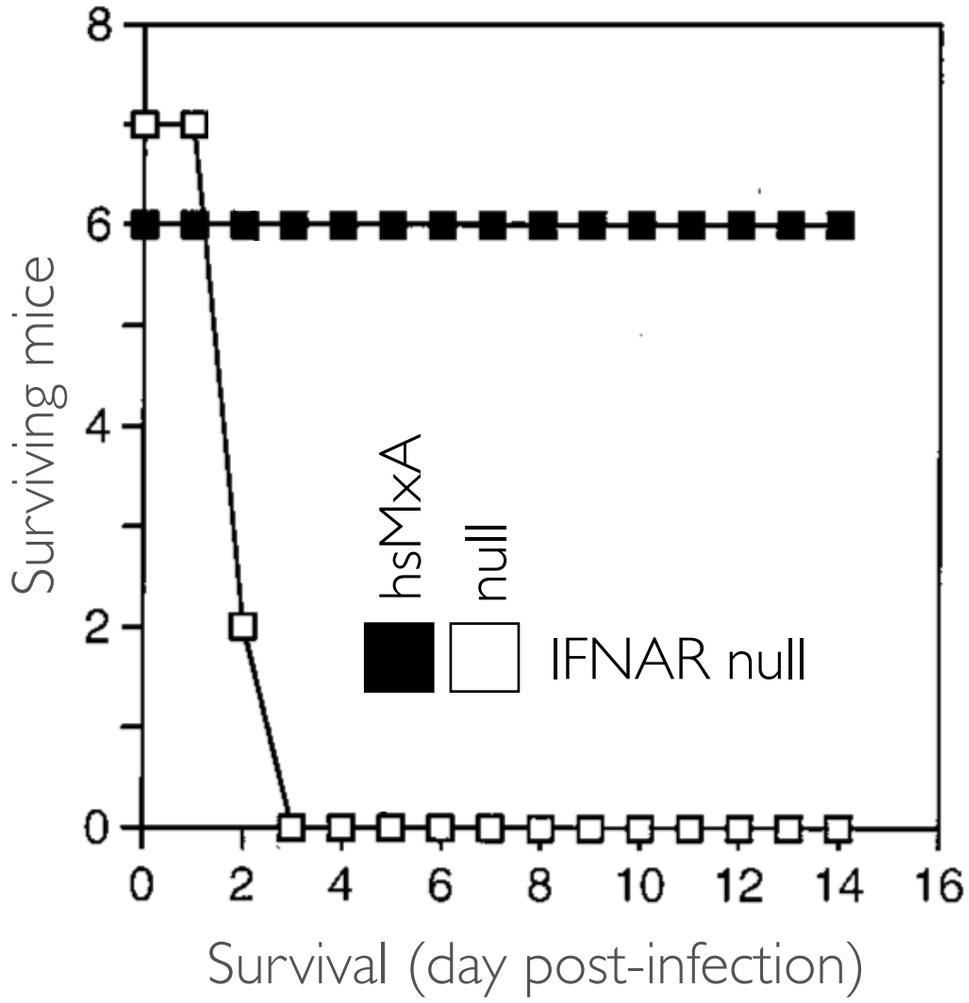
Bundle-signaling element

Stalk domain



MxA

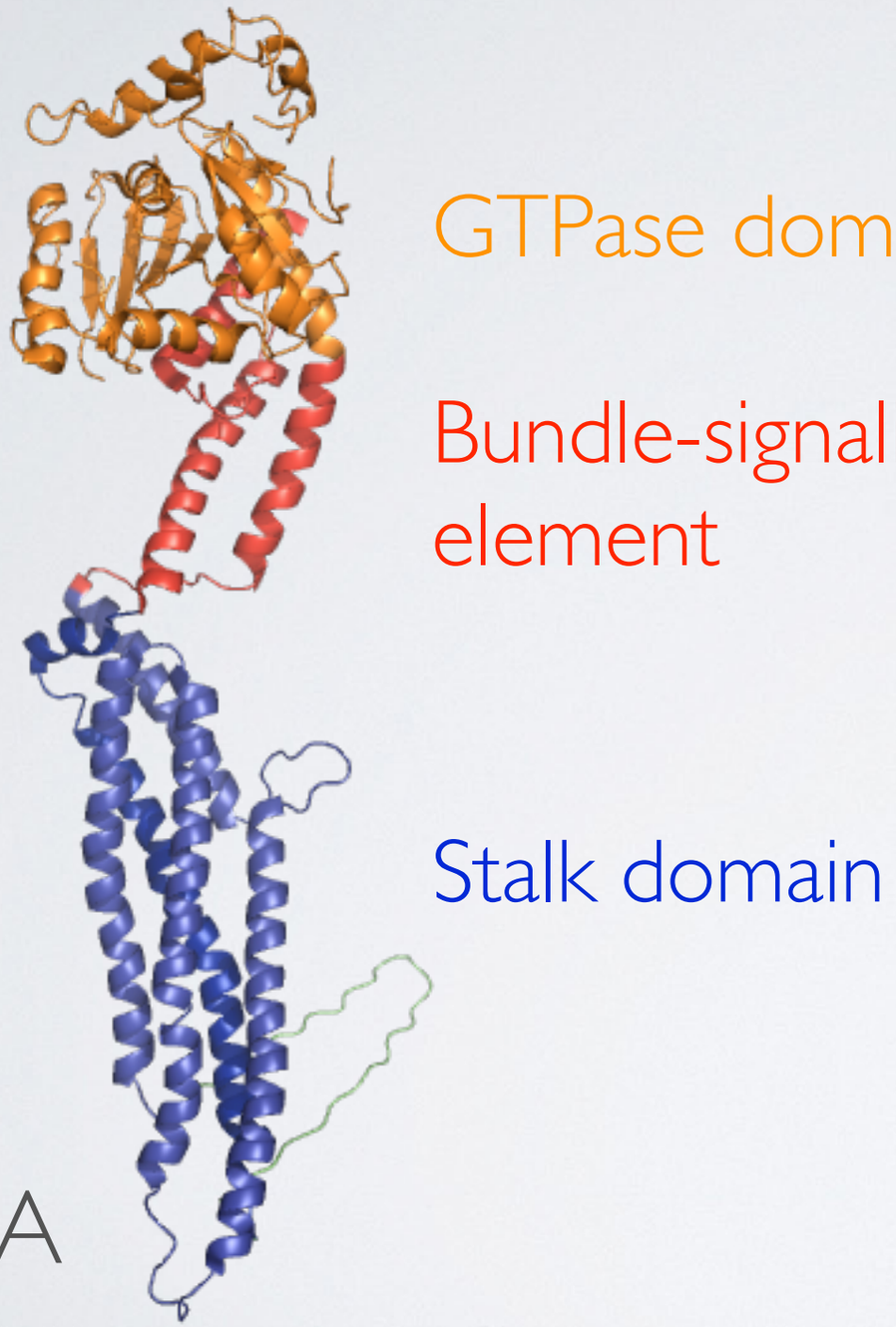
MxA provides robust protection against viral infection



Gao et al. Immunity (2011)

Hefti et al. JVirol (1999)

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

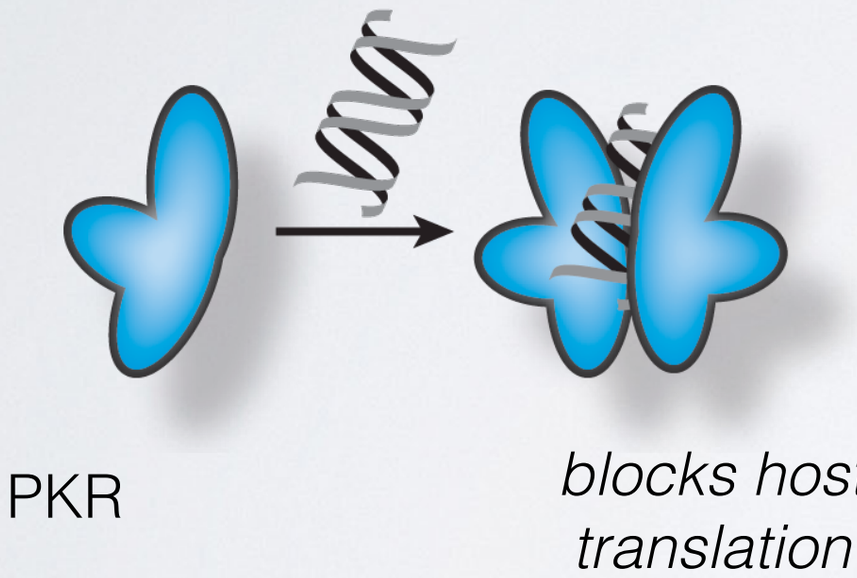
Positive-sense RNA viruses:
Picornaviridae: Coxsackievirus B4
Togaviridae: Semliki Forest virus

DNA viruses:
Asfarviridae: African swine fever virus

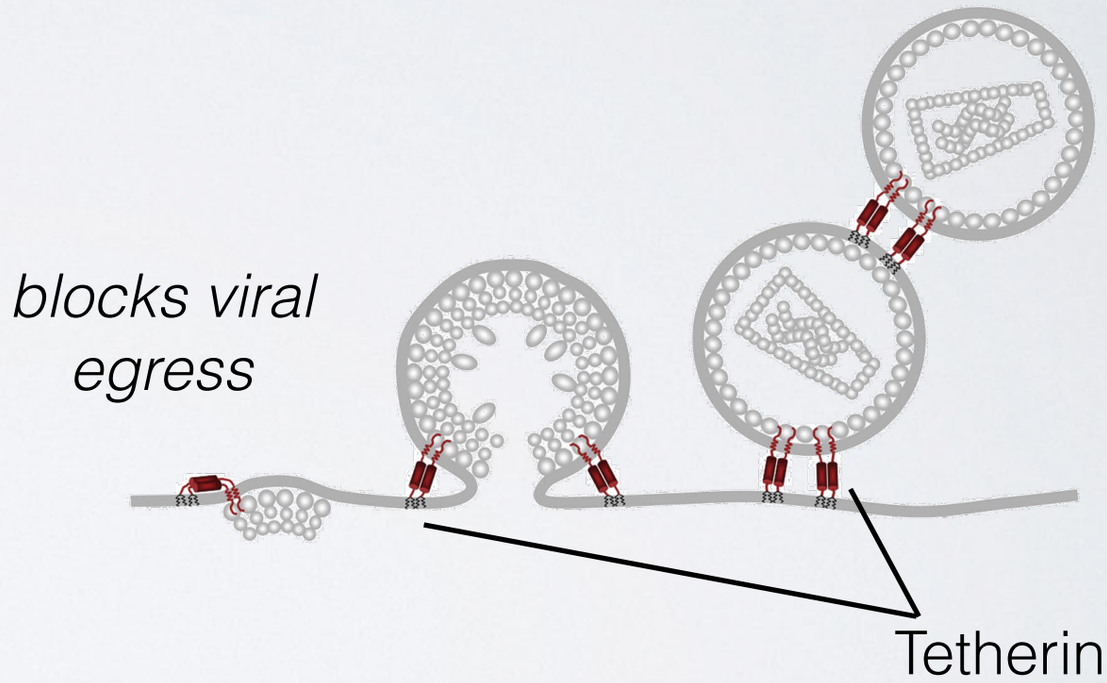
Hepadnaviridae: Hepatitis B virus

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)

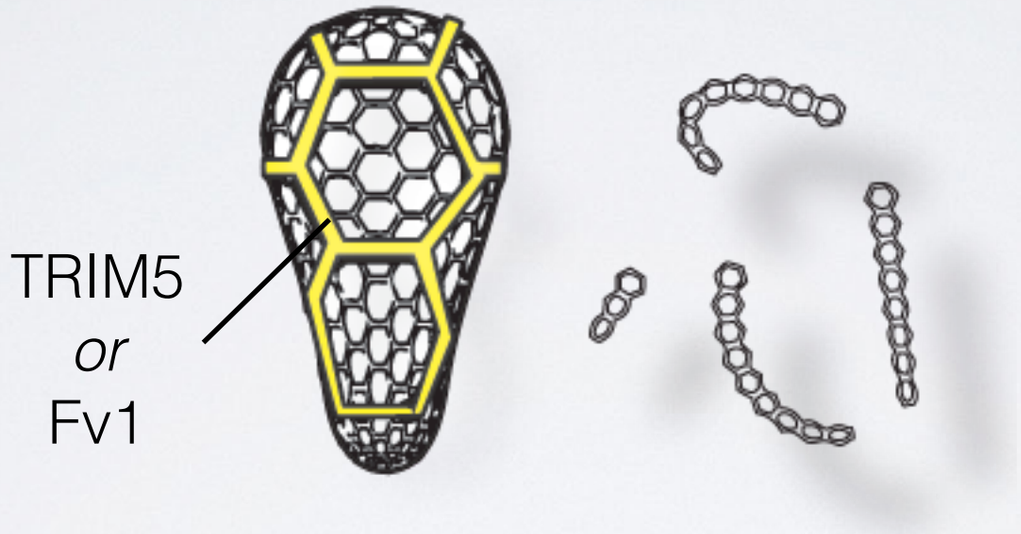


Antivirals such as PKR and Tetherin evolve under positive selection to escape viral antagonism

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)



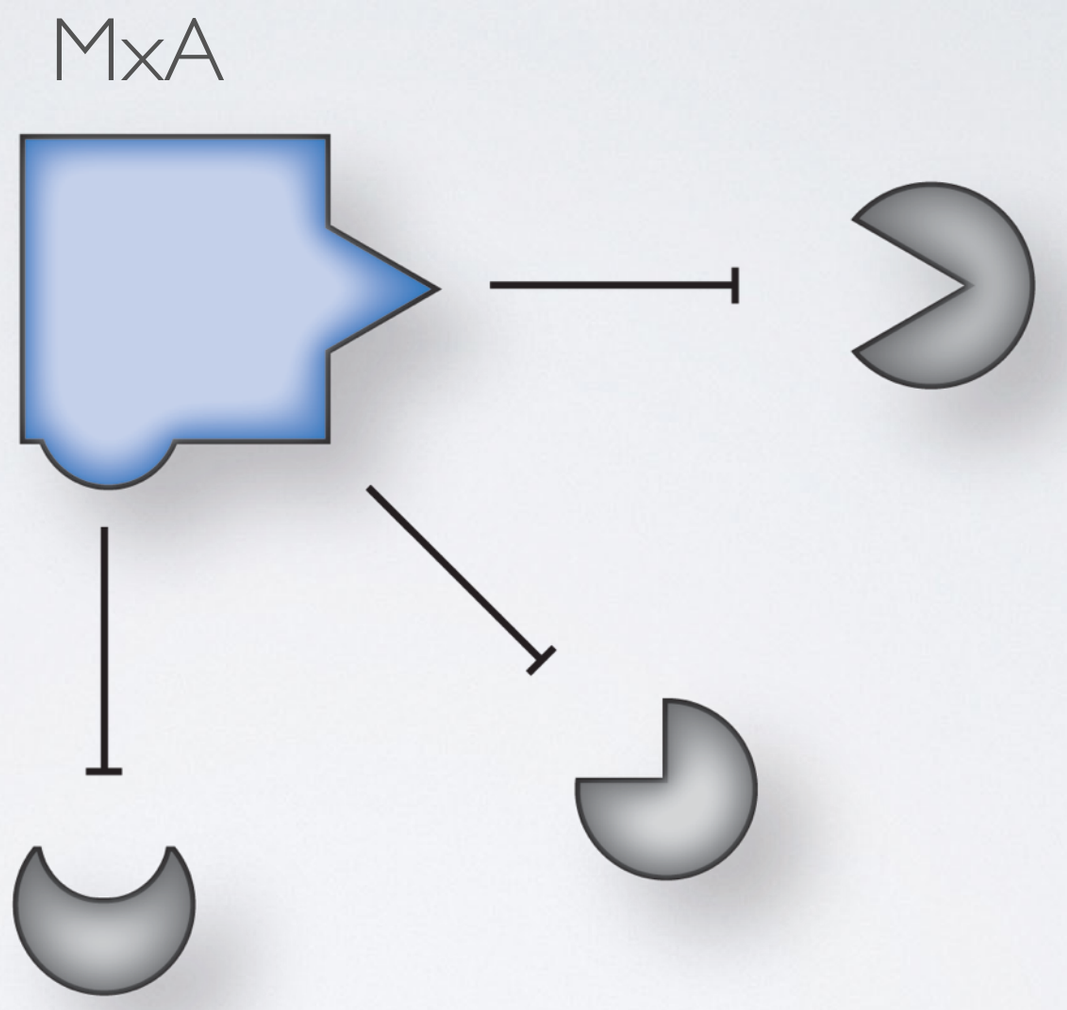
capsid destruction

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

Perez-Caballero et al. Cell (2009),
Mitchell et al. Curr Opin Microbiol (2013)

MxA is an antiviral hybrid

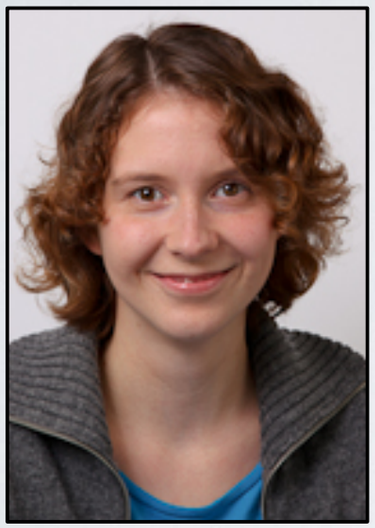
(broadly-acting despite specific recognition of distinct viral targets)

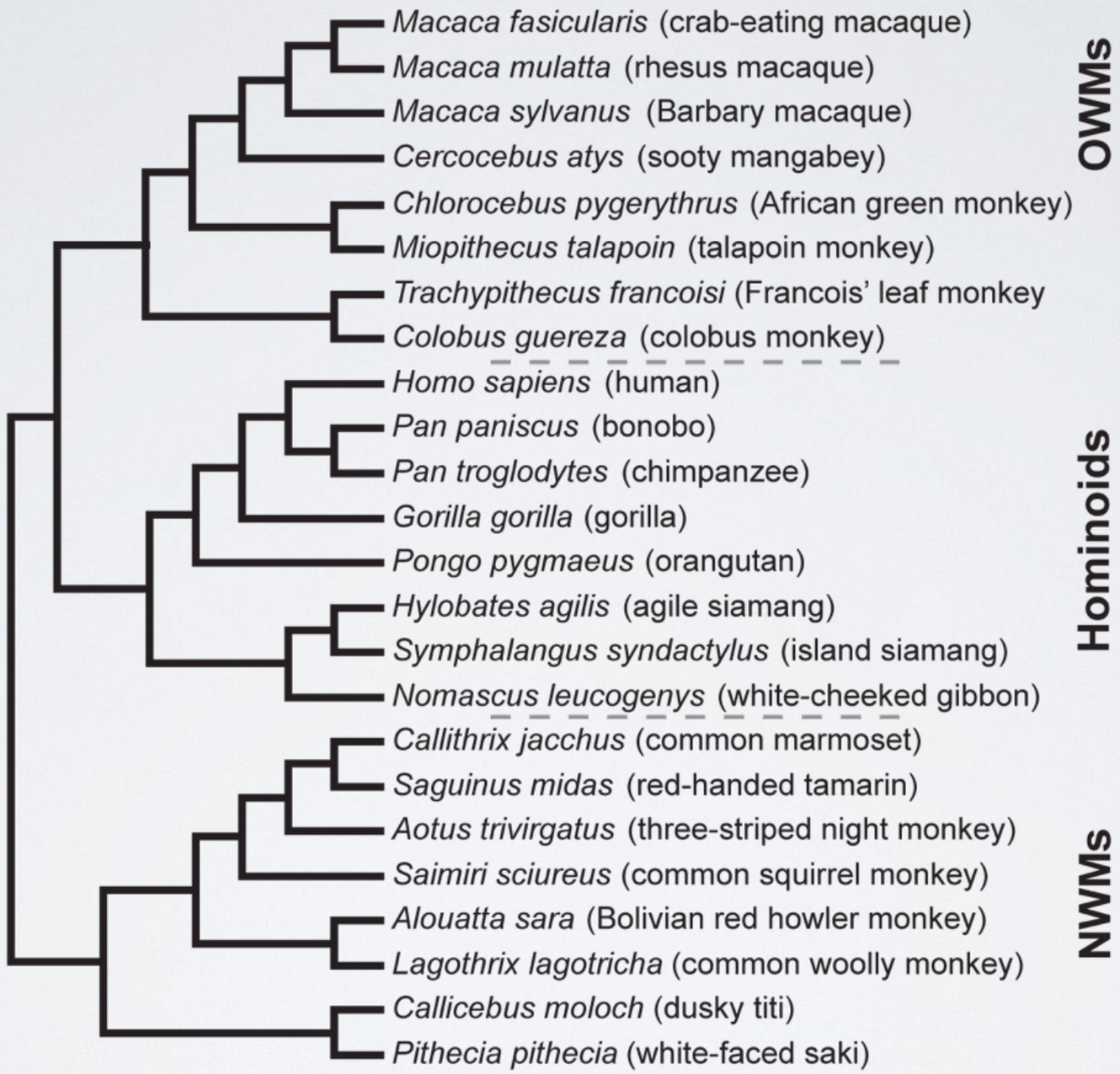


How does MxA maintain antiviral breadth while recognizing multiple, distinct viral targets?

Mitchell et al. Curr Opin Microbiol (2013)

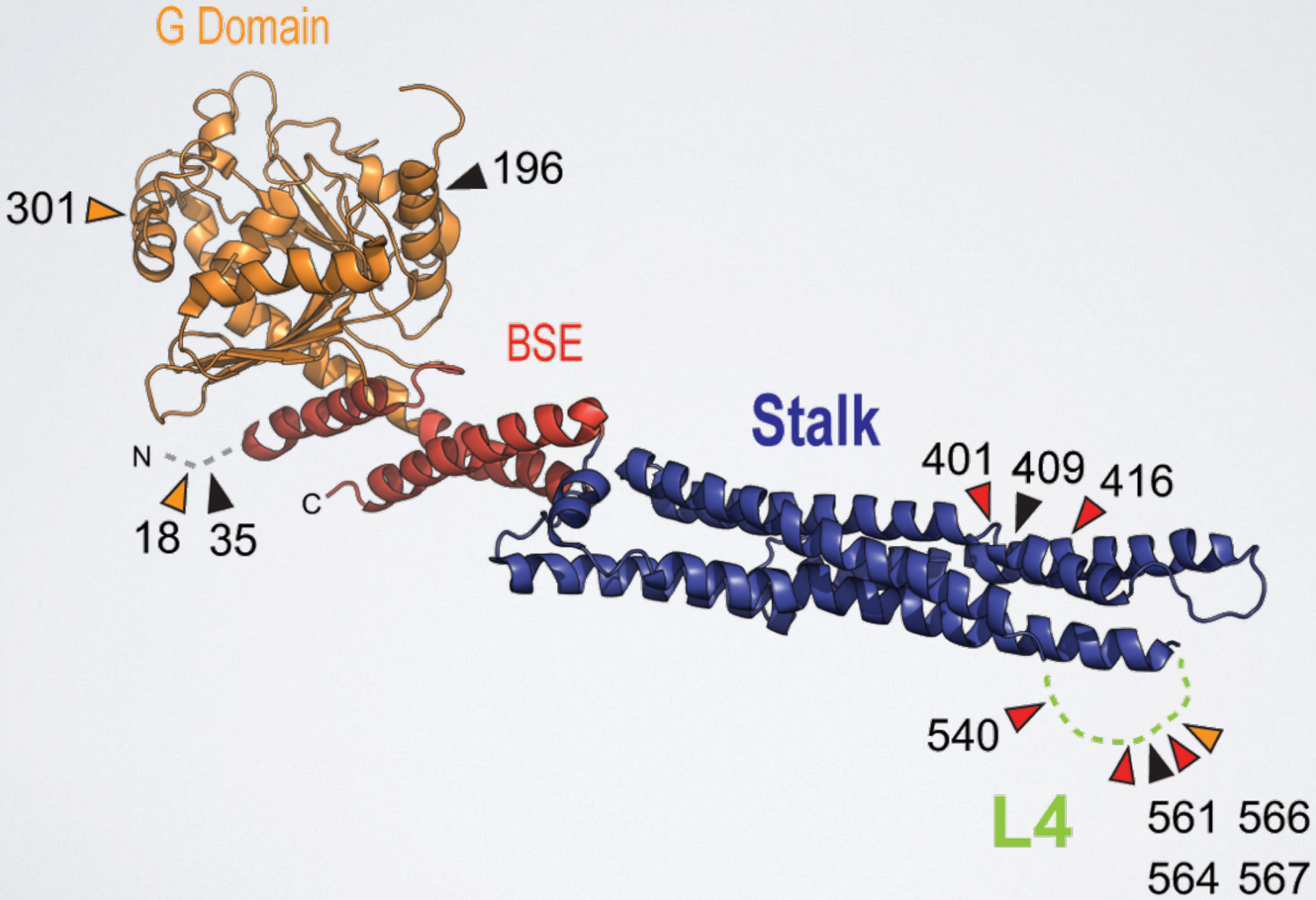
Evolution-guided identification of target recognition in the antiviral large GTPase MxA





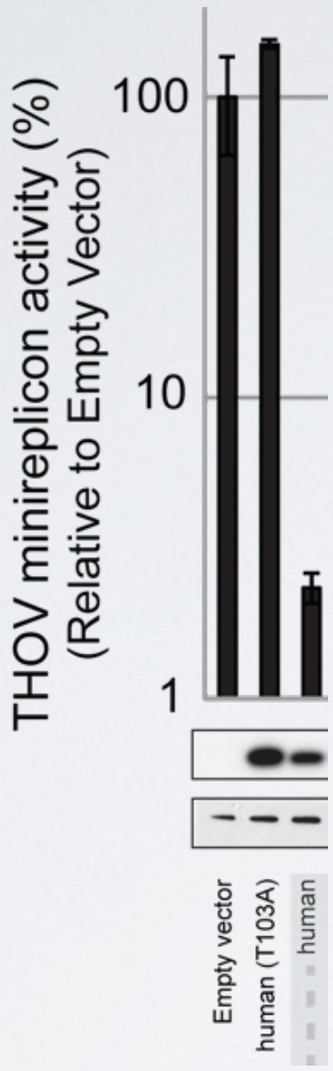
Mitchell, Patzina et al. Cell Host Microbe (2012)

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



Mitchell, Patzina et al. Cell Host Microbe (2012)

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



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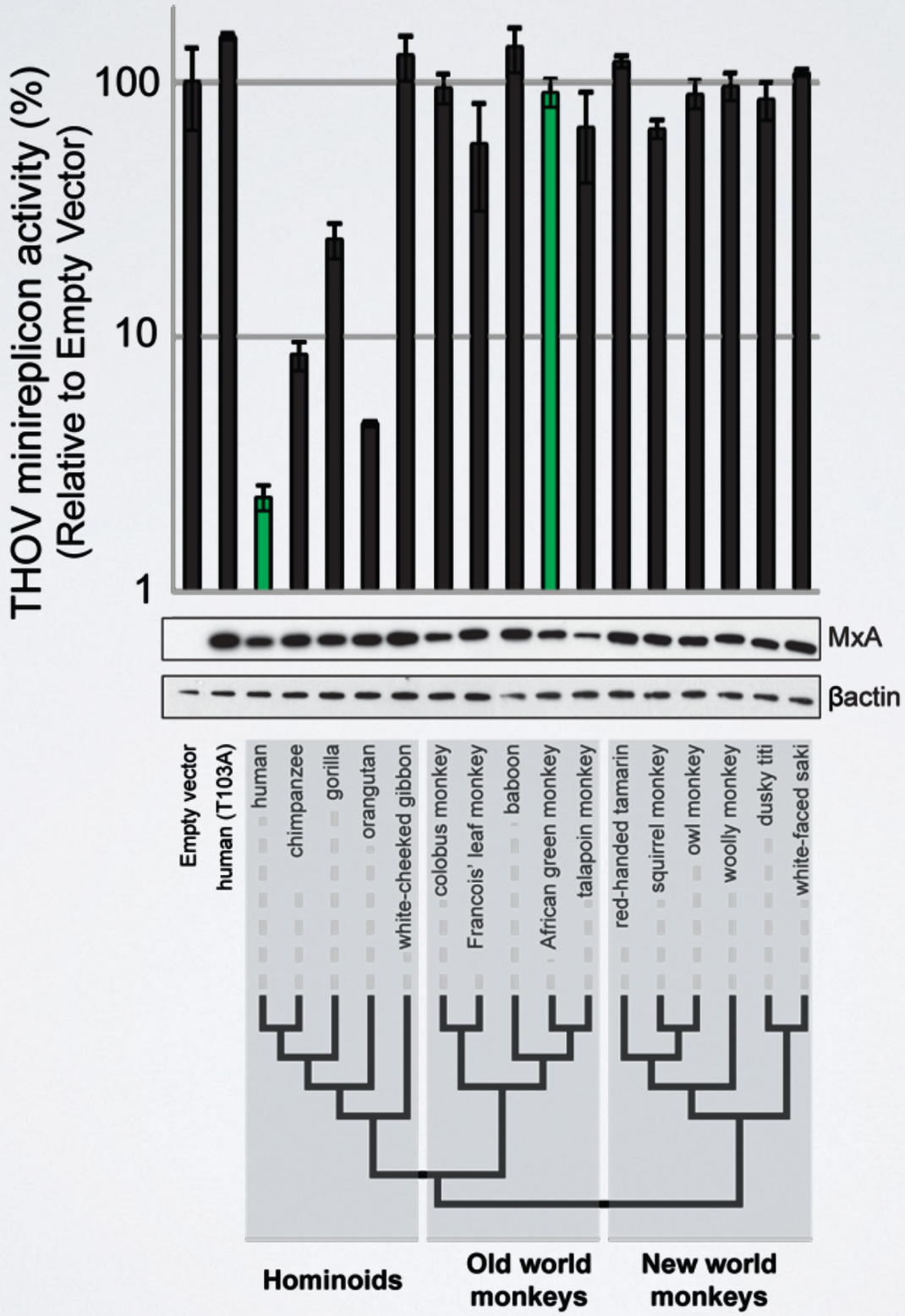

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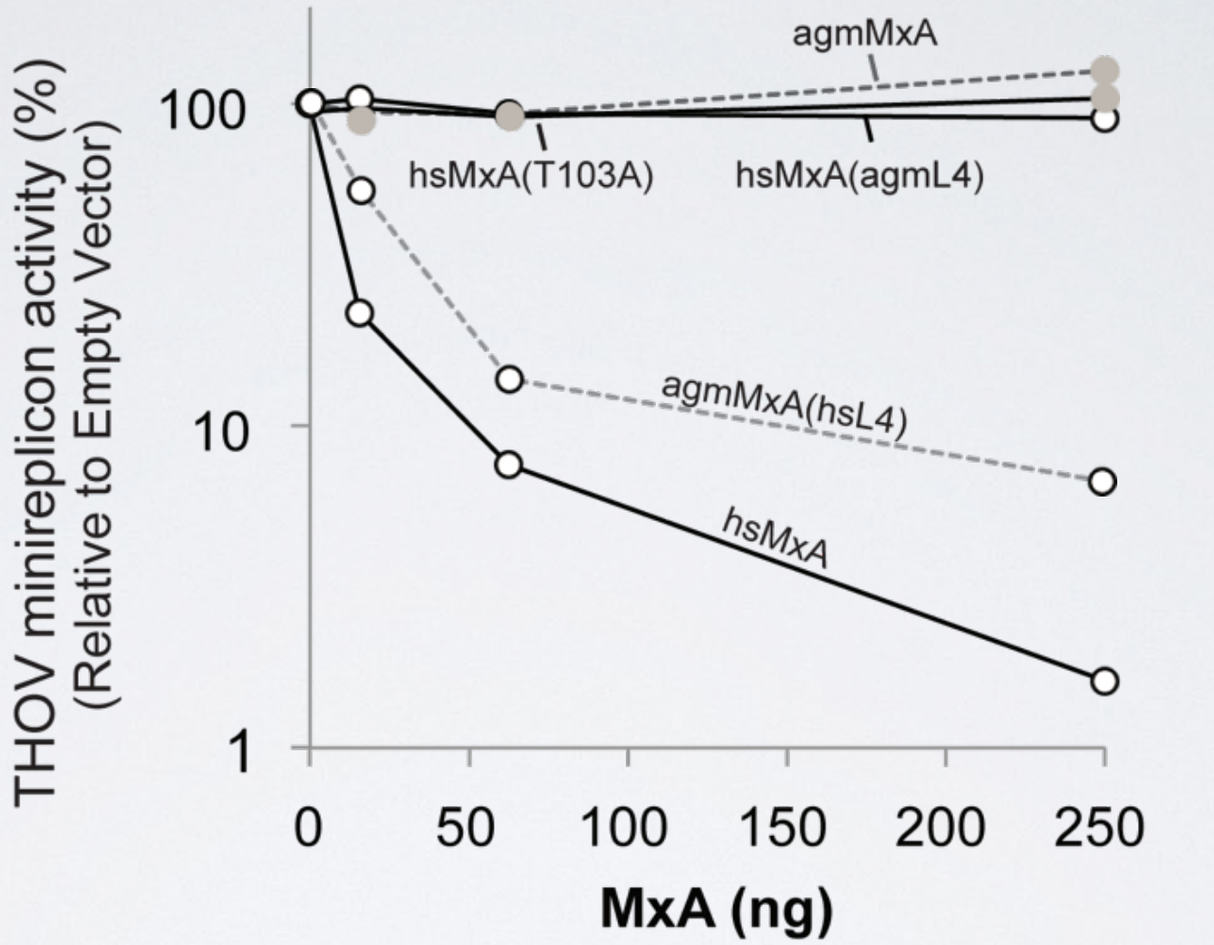
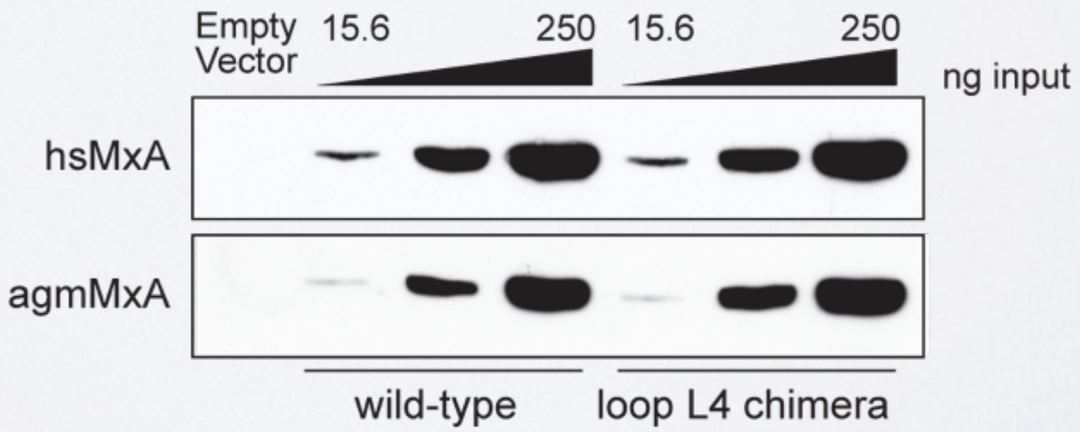
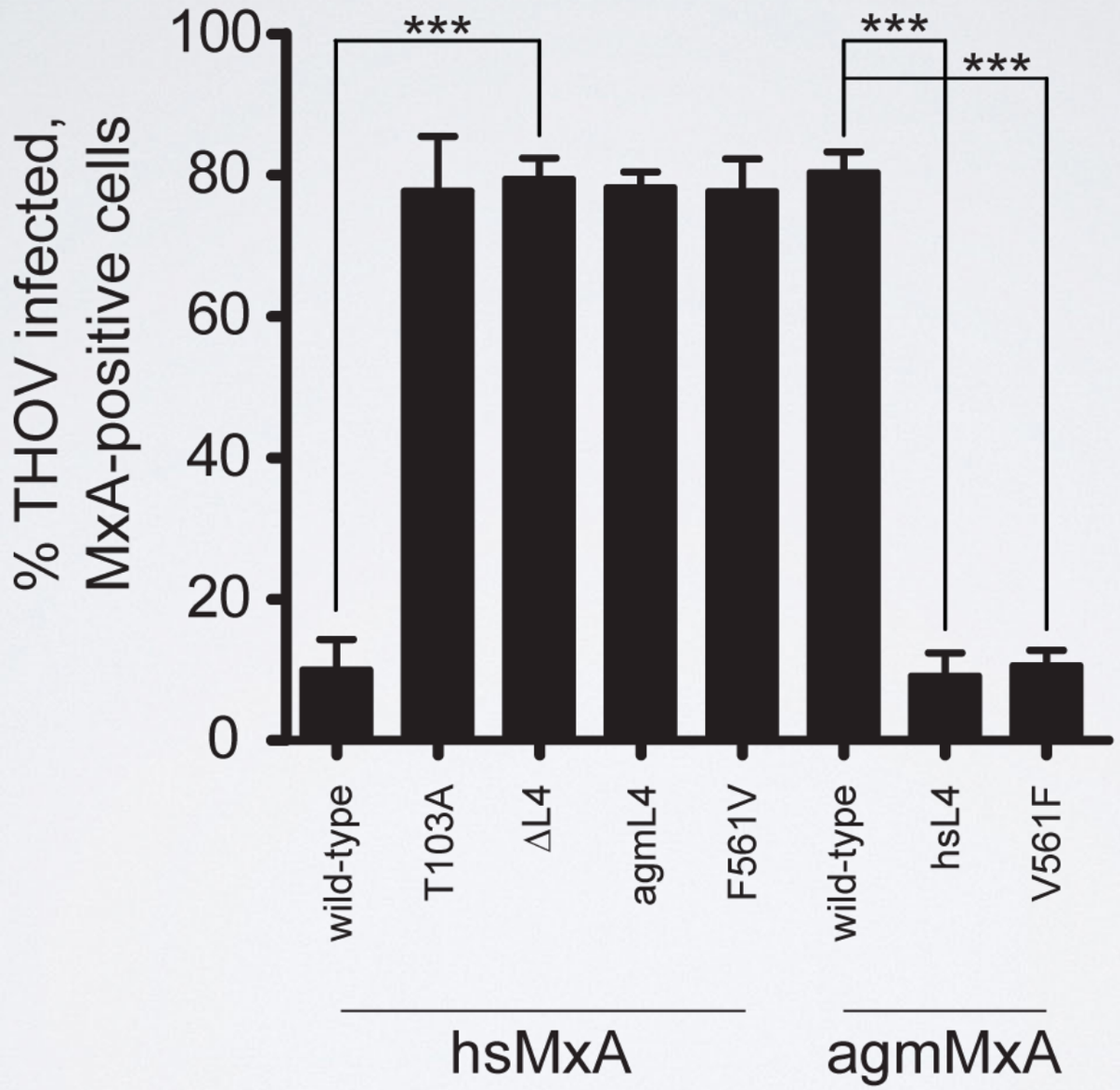
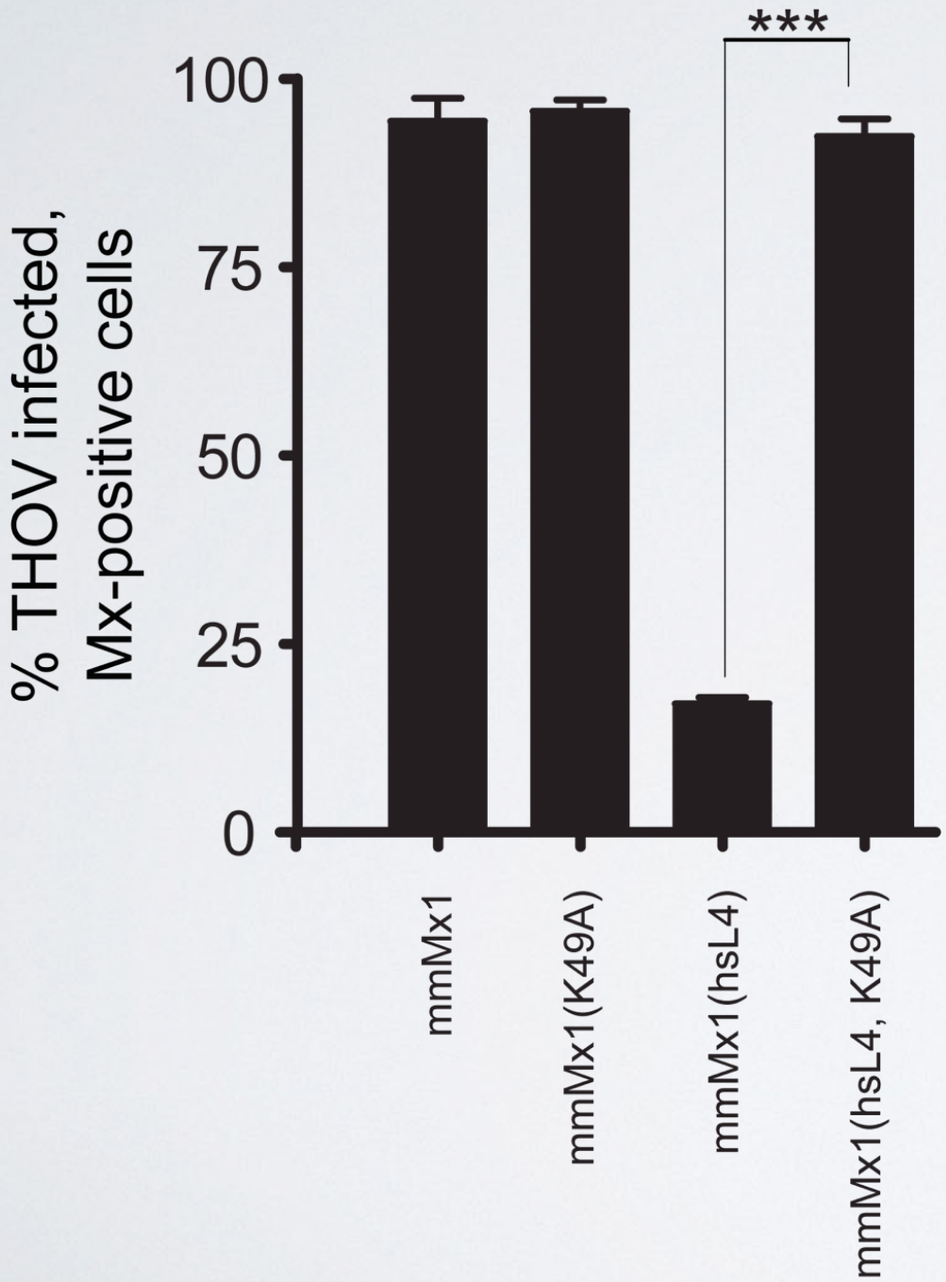


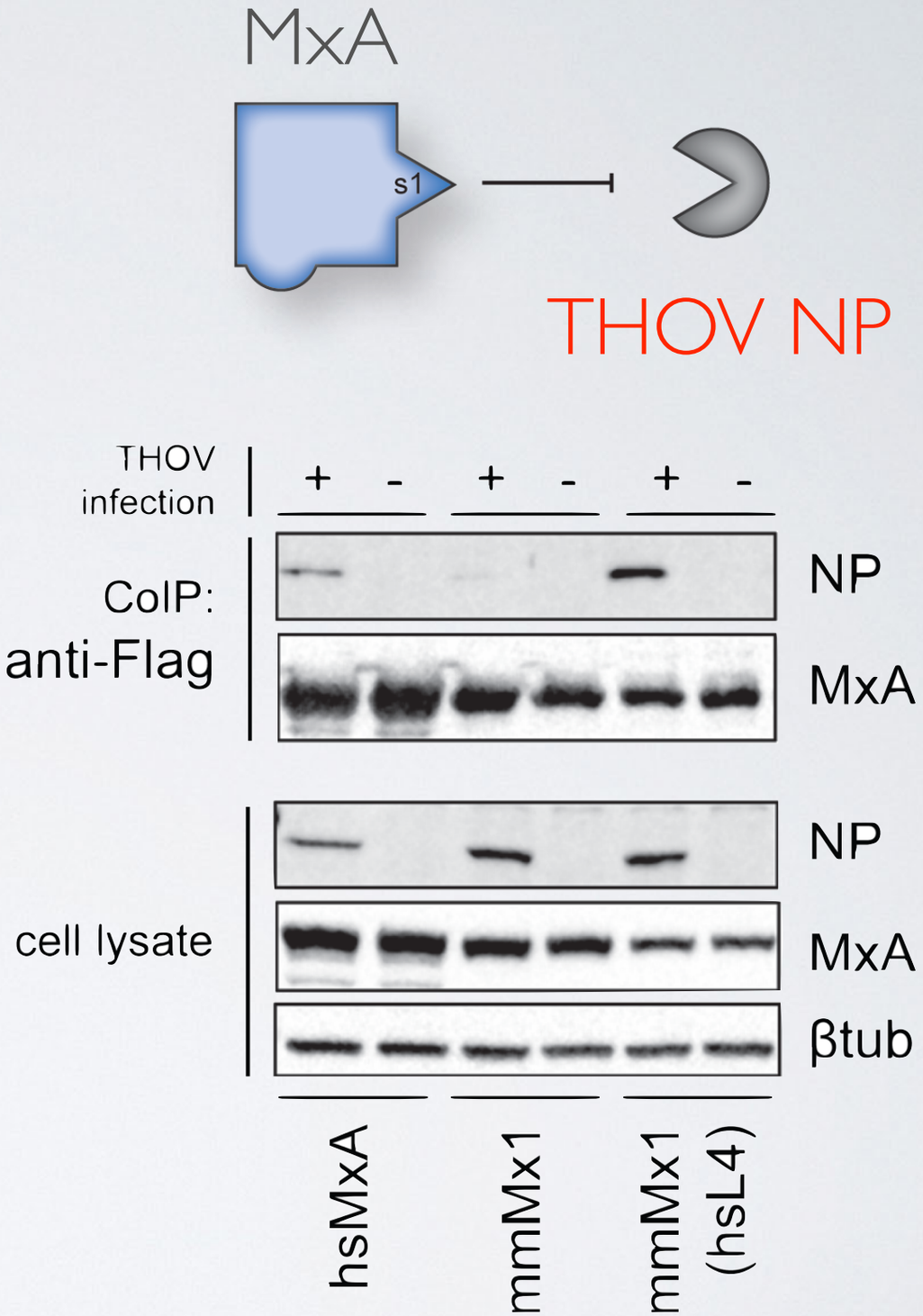


A single residue in L4 confers MxA antiviral specificity for THOV



L4 is a modular unit for MxA target recognition





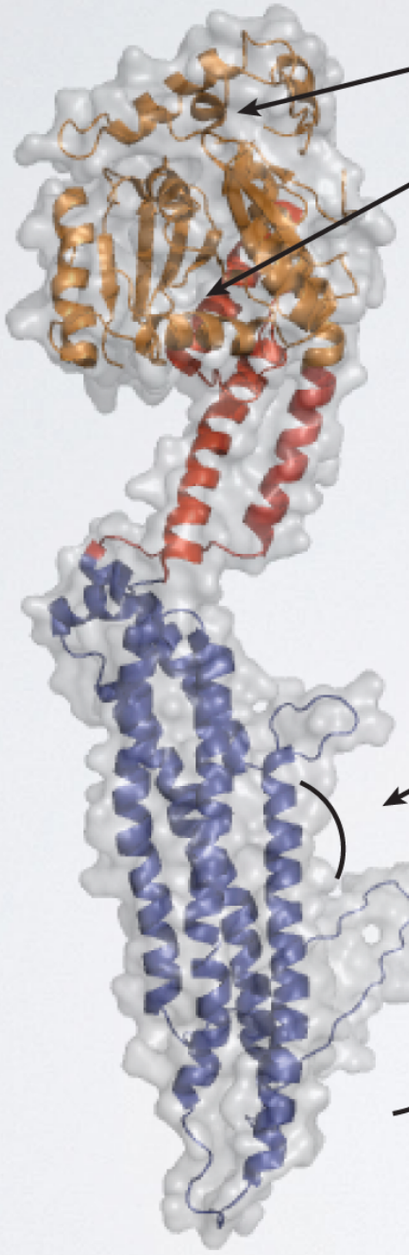
Mitchell, Patzina et al. Cell Host Microbe (2012)

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

G domain

BSE

Stalk

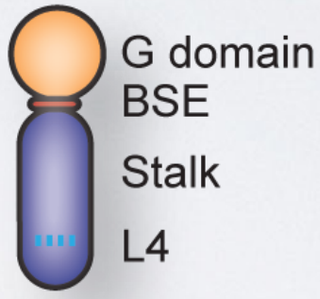


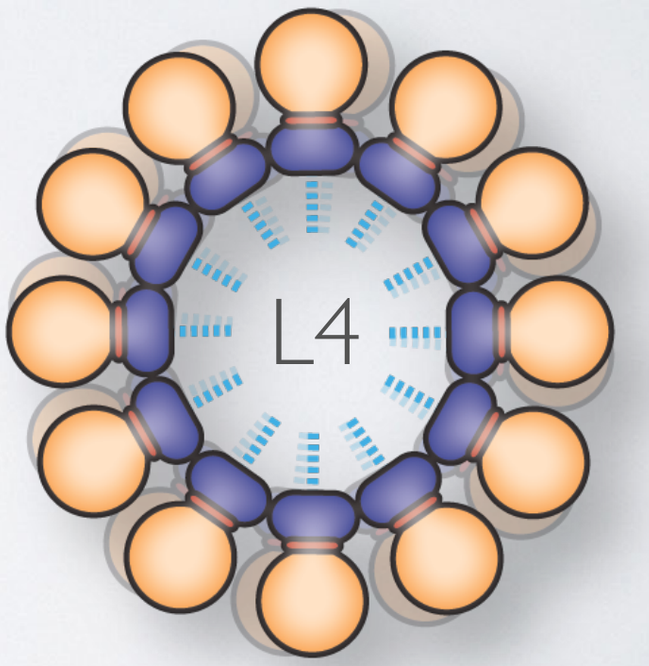
301
196

401
409
416

loop L4
540, 561,
564, 566,
567

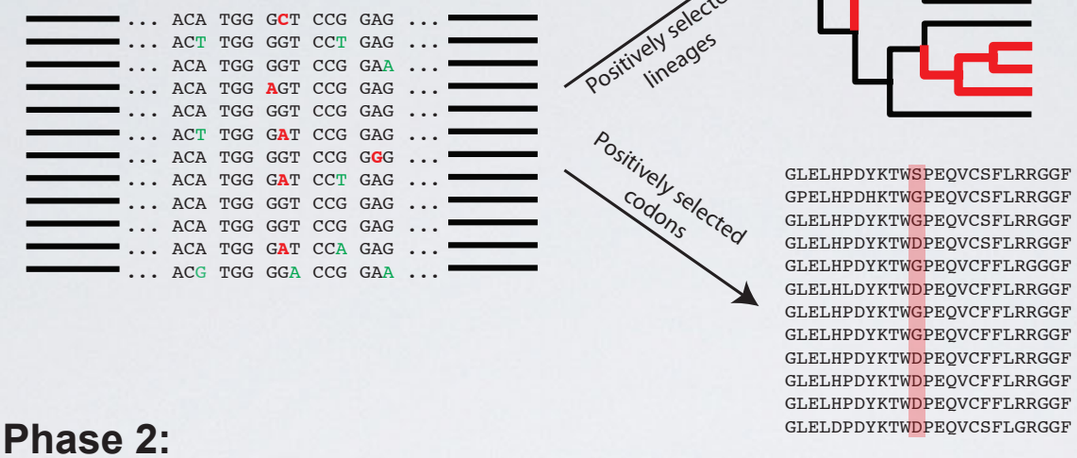
THOV
(*orthomyxoviruses*)





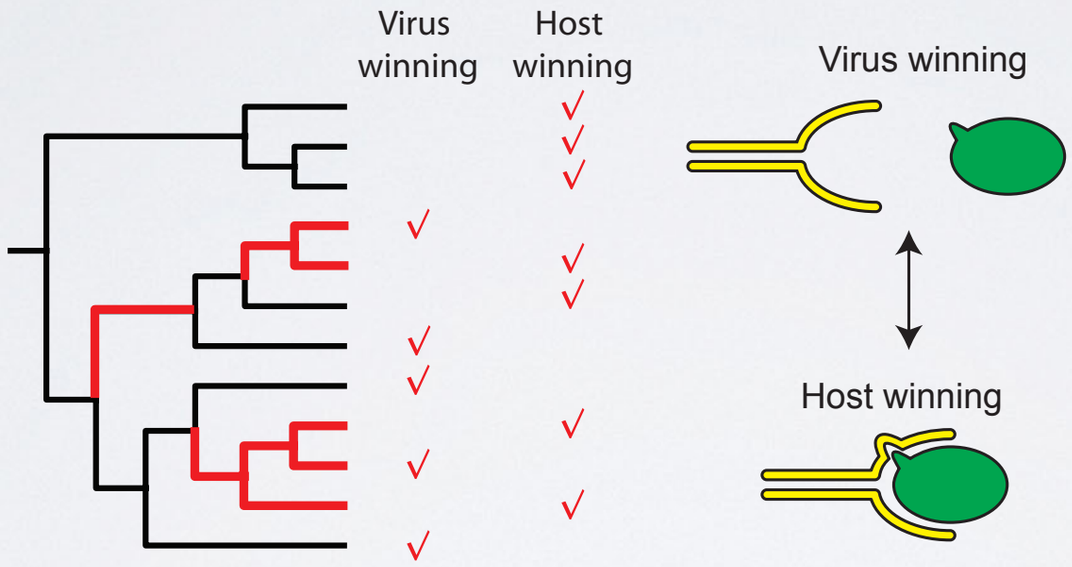
Phase 1:

Analyze sequences for positive selection



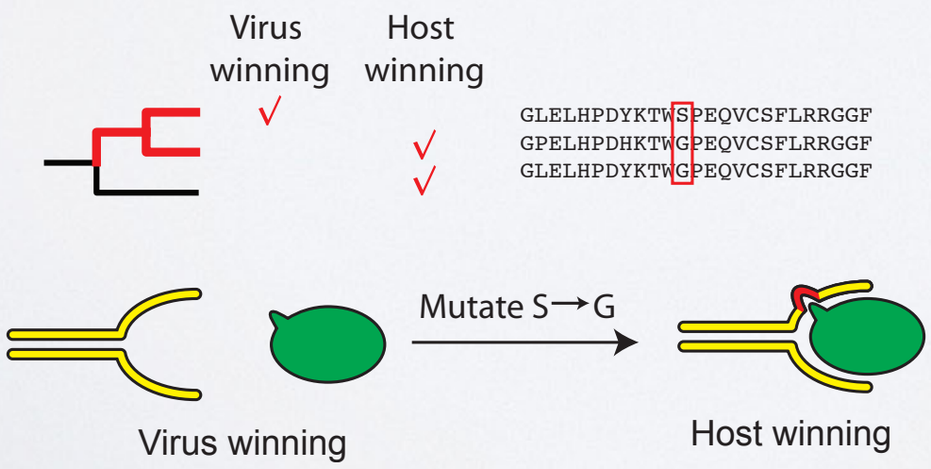
Phase 2:

Determine phenotypic consequences of ortholog variation



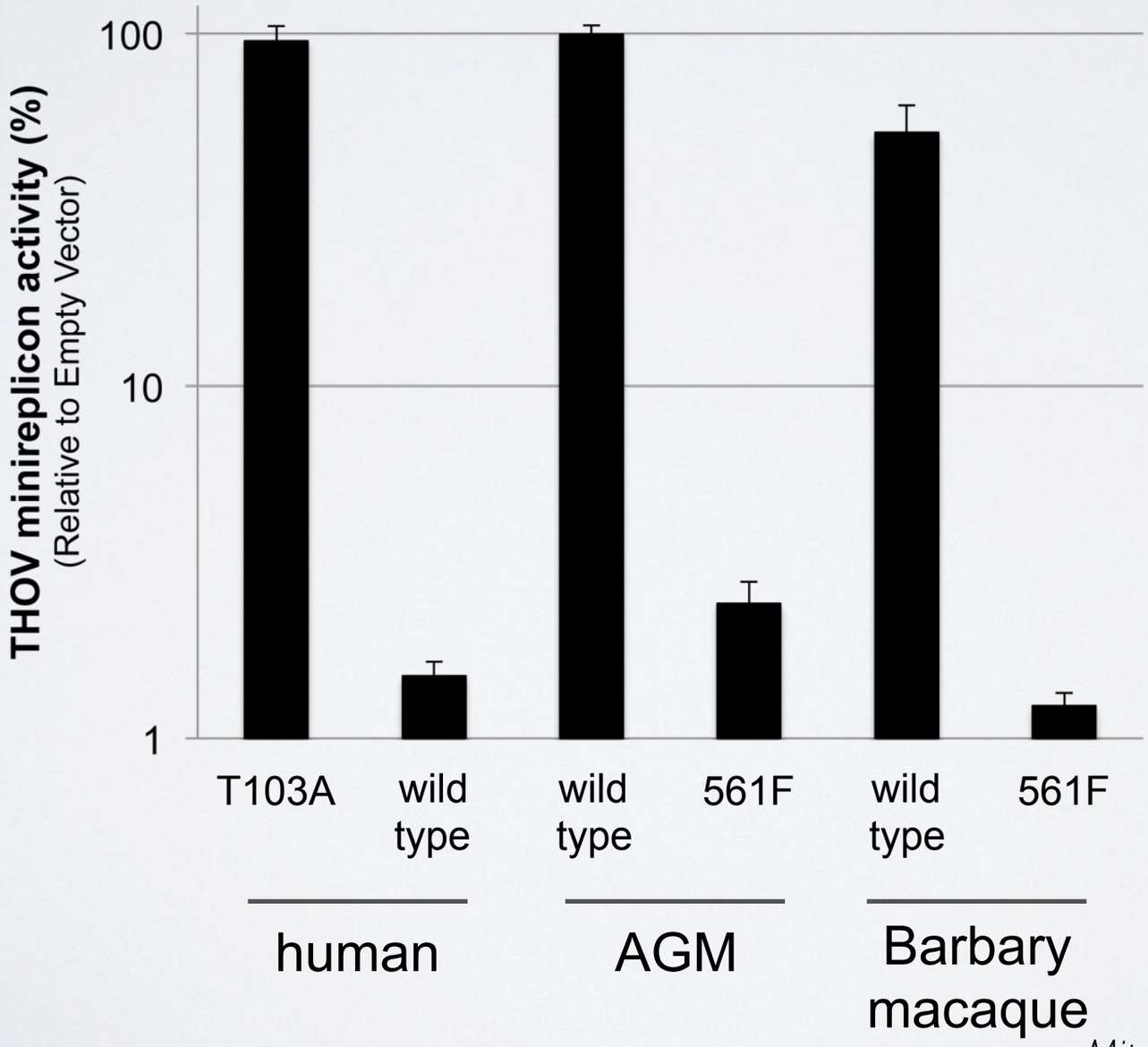
Phase 3:

Map positively selected changes on to host-virus interaction



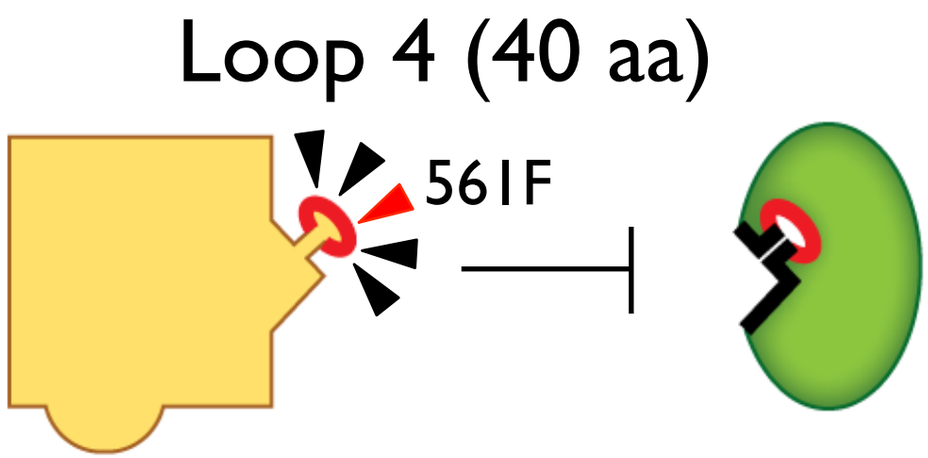
Robustness in MxA antiviral specificity for THOV

```
                    556      561      566      572
human               KKSWDFGAFQSSSATDI
African green monkey KKSWDVGTFQPS-STDI
Barbary macaque     KKSWDVGTFQSS-STDI
```

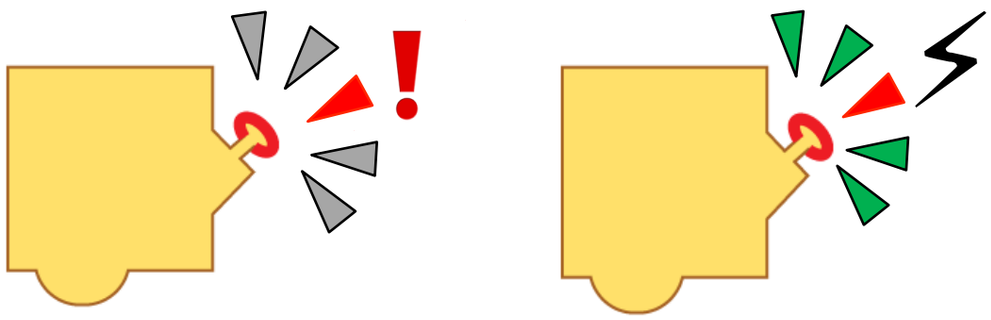


Mitchell, Patzina et al. Cell Host Microbe (2012)

Evolution-guided mutagenesis to understand protein function

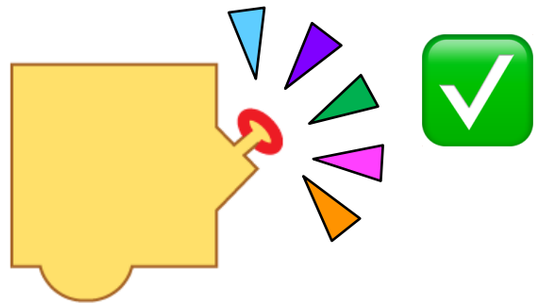


MxA



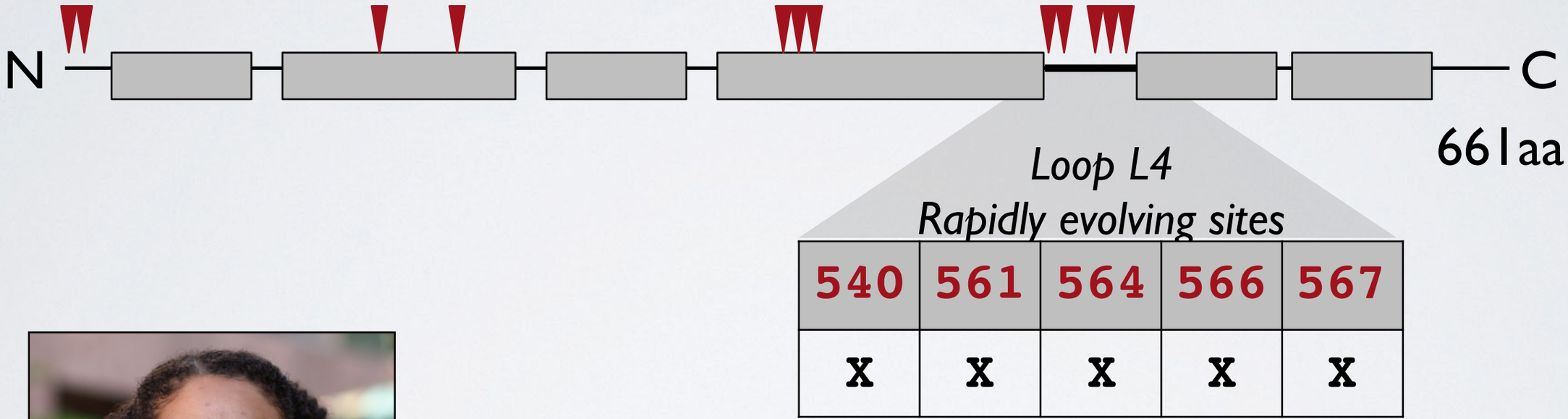
Epistasis?

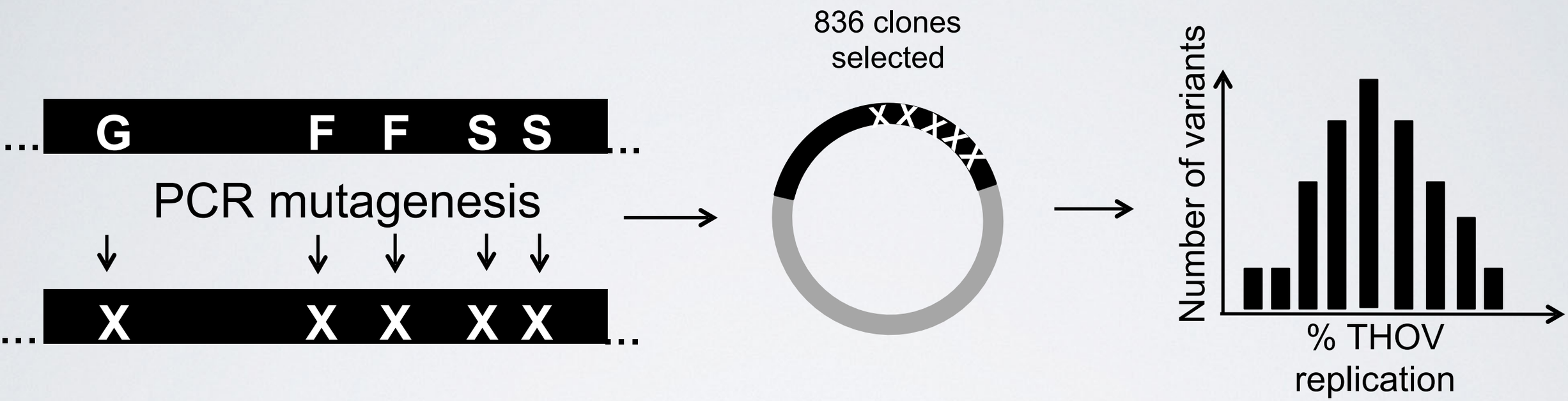
THOV NP



Rules for target recognition?

Combinatorial mutagenesis to understand and expand antiviral specificities





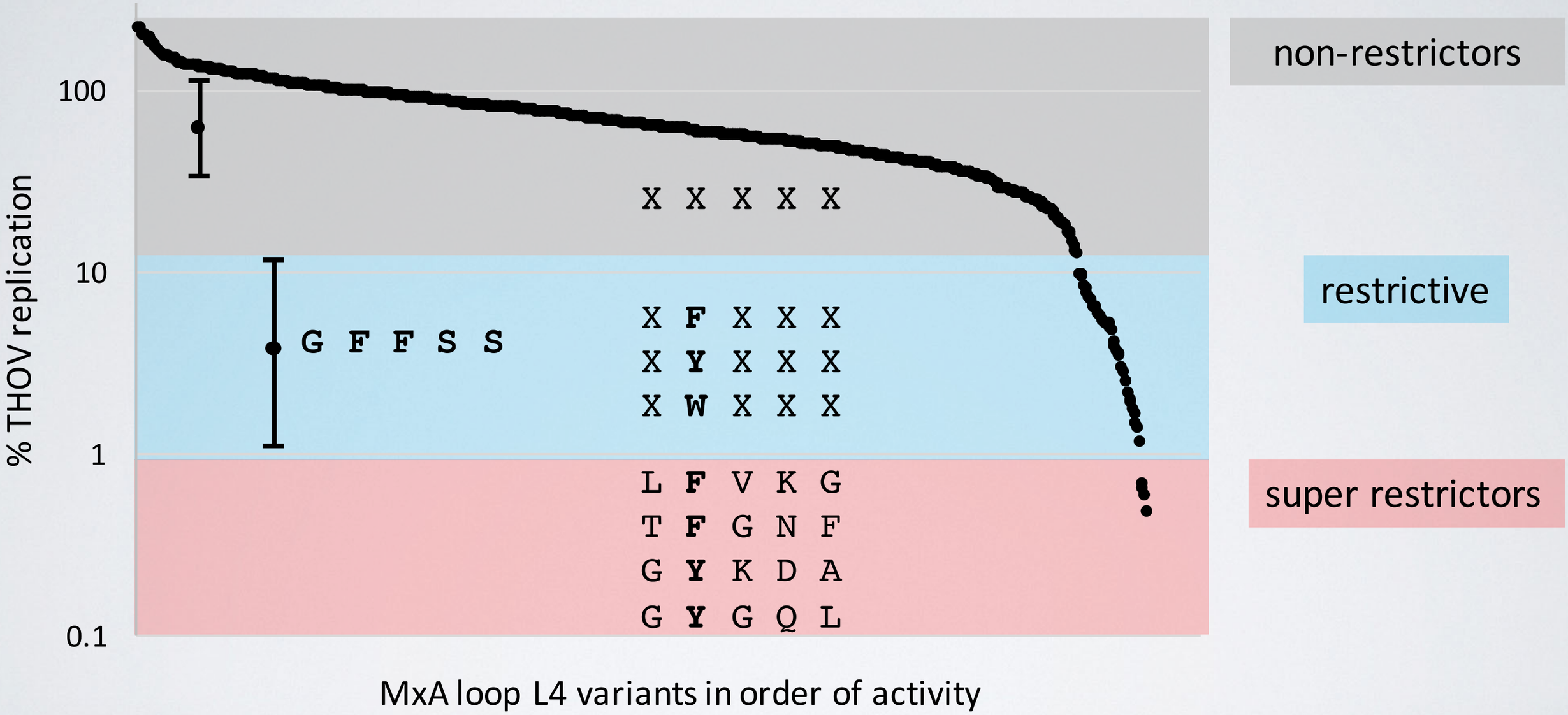
Library design

Select variants

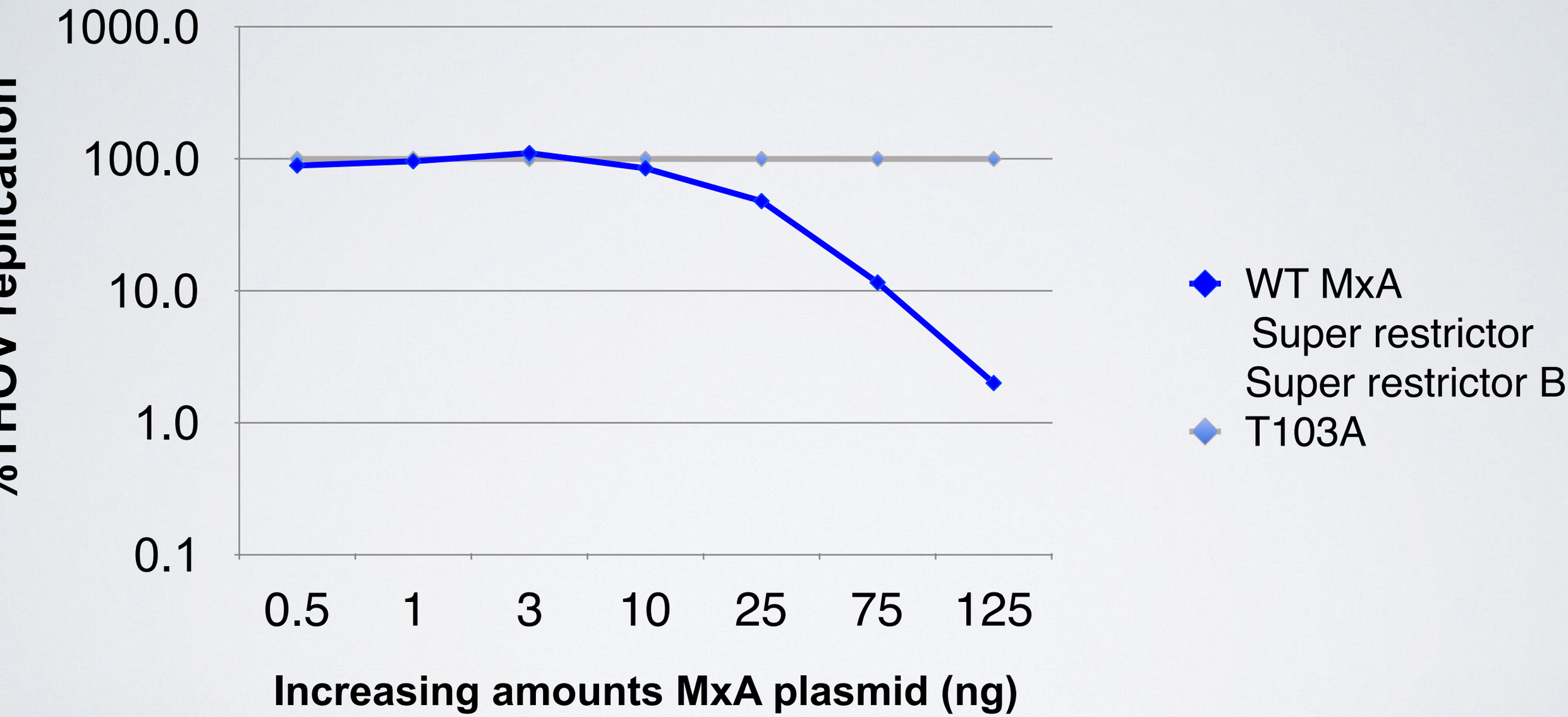
Assess functionality

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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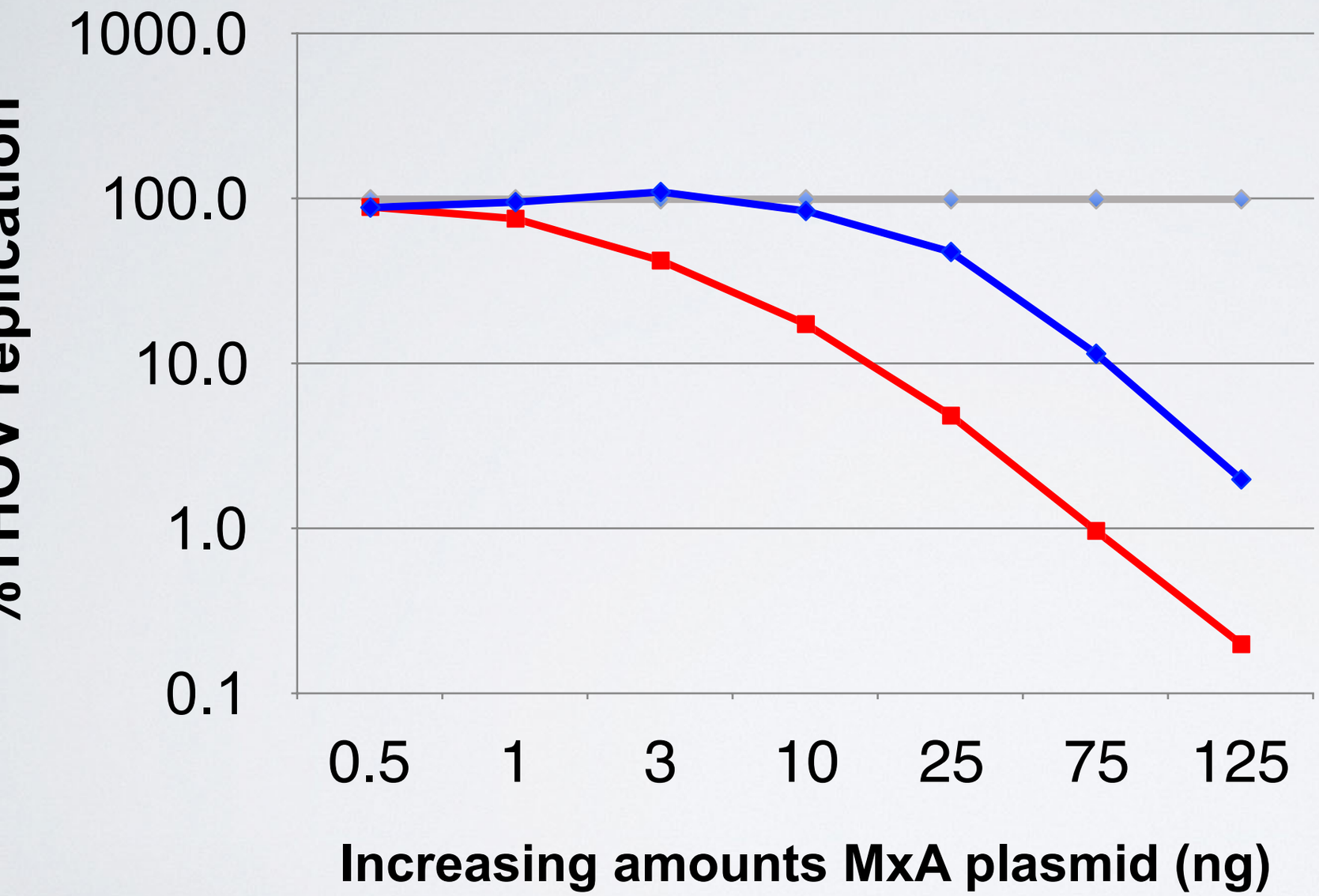
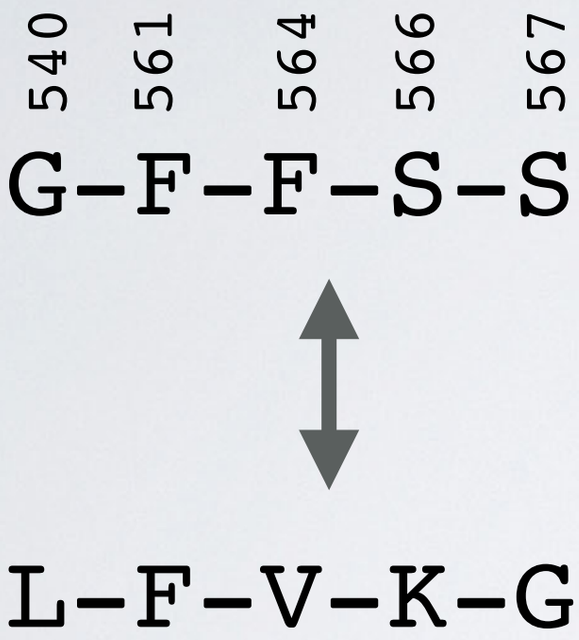


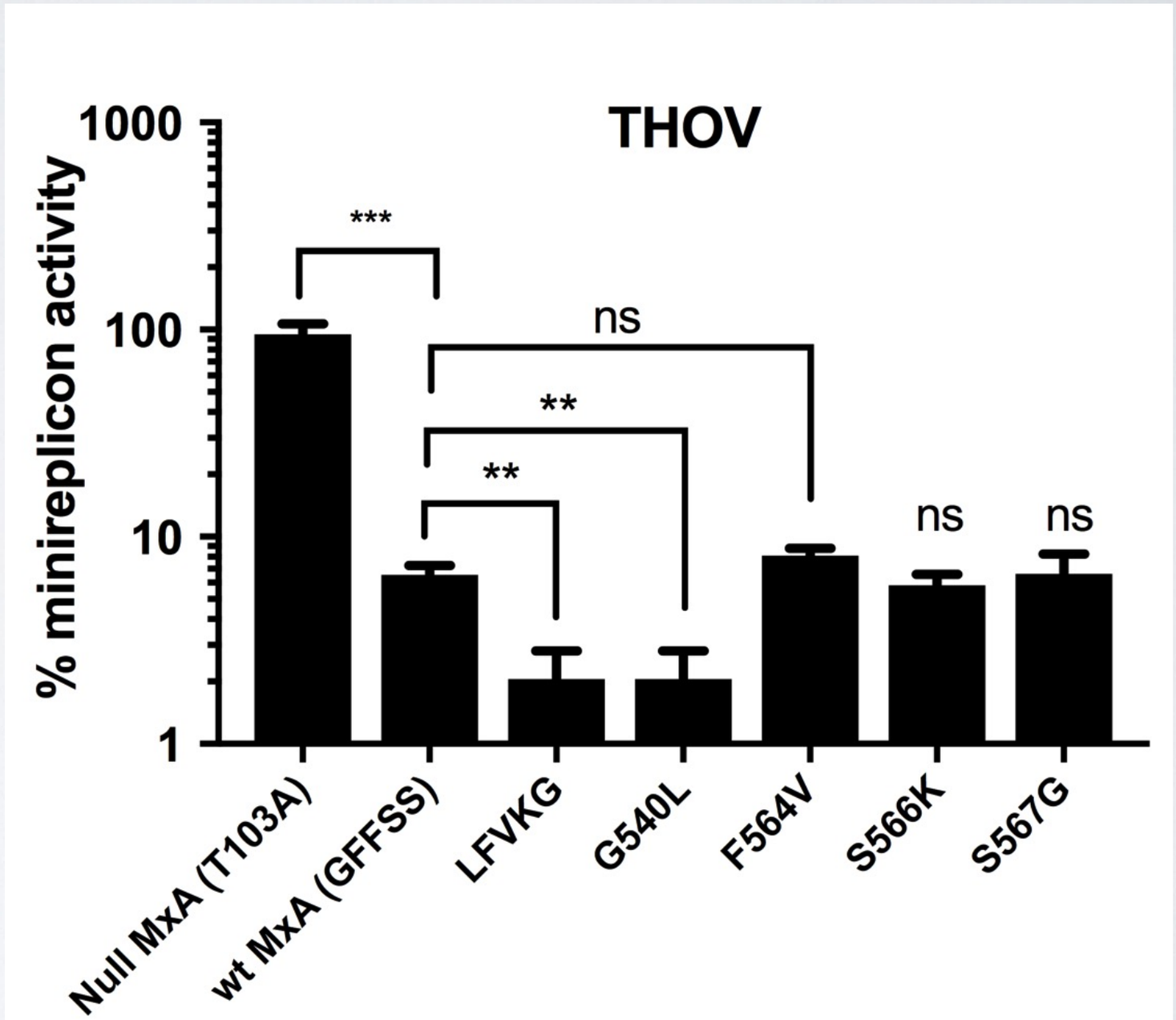
Genetic basis?

Mechanism?

- WT MxA
- Super restrictor
- T103A

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

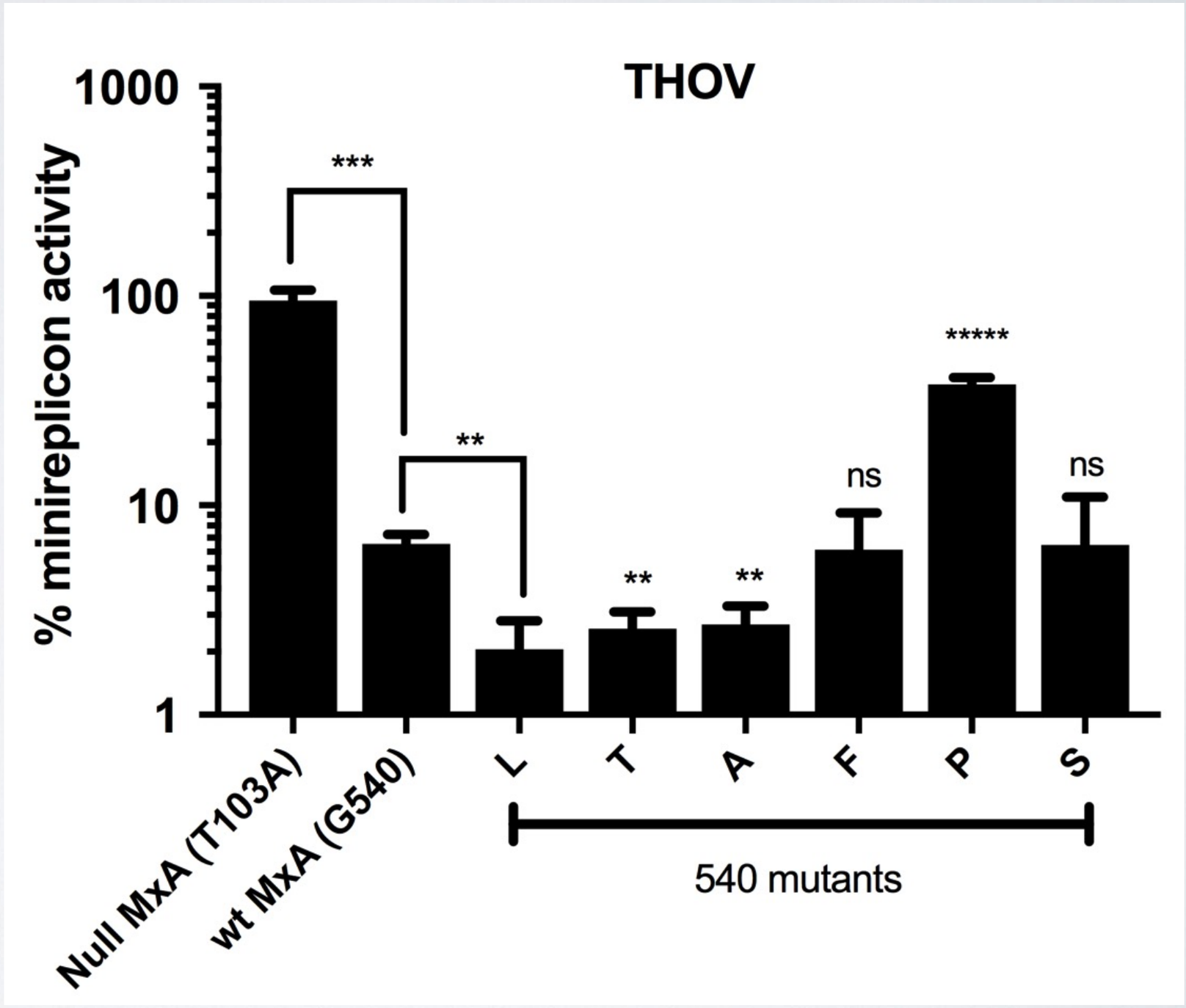




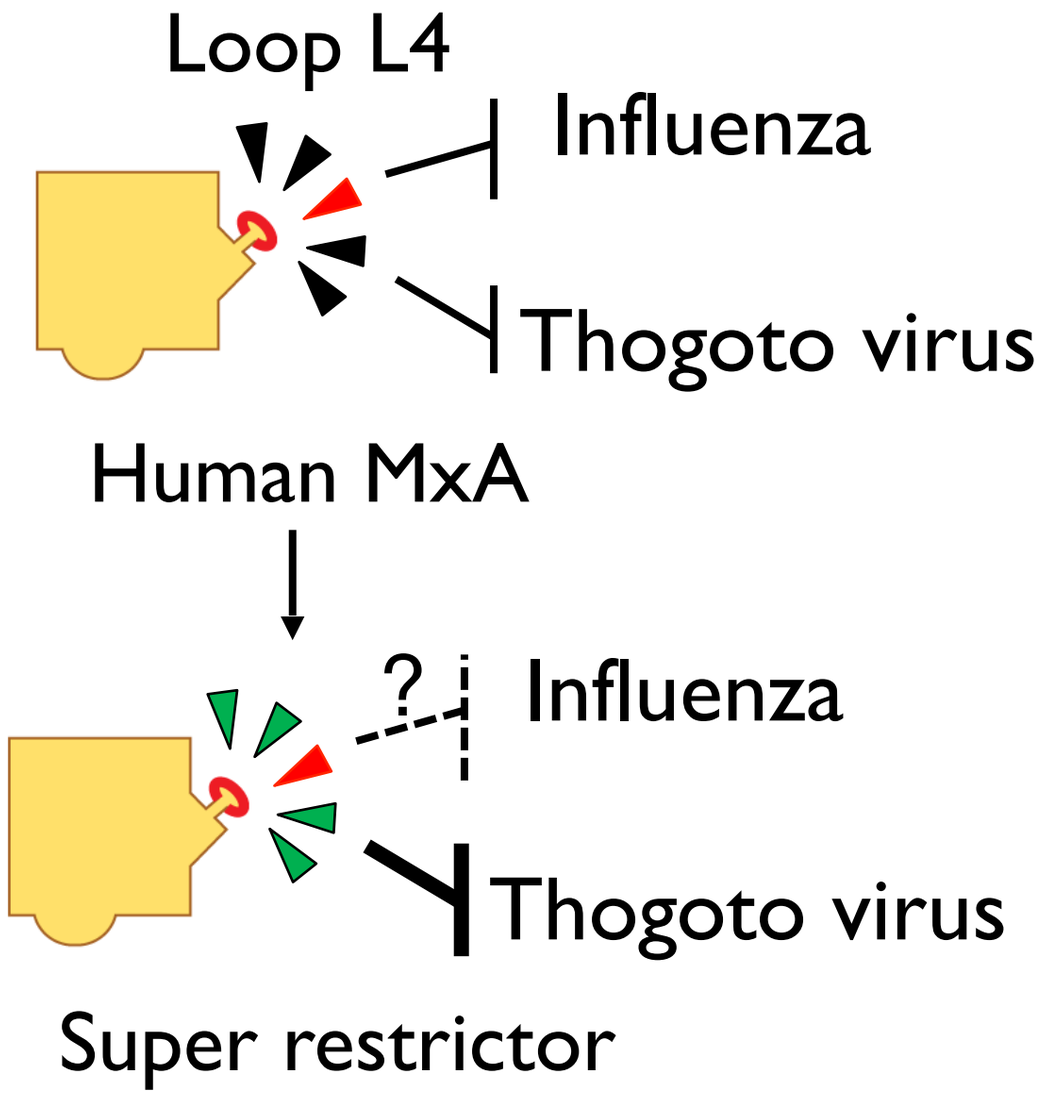
A single amino acid change G540L explains the super-restrictor phenotype

G 540 – F 561 – F 564 – S 566 – S 567

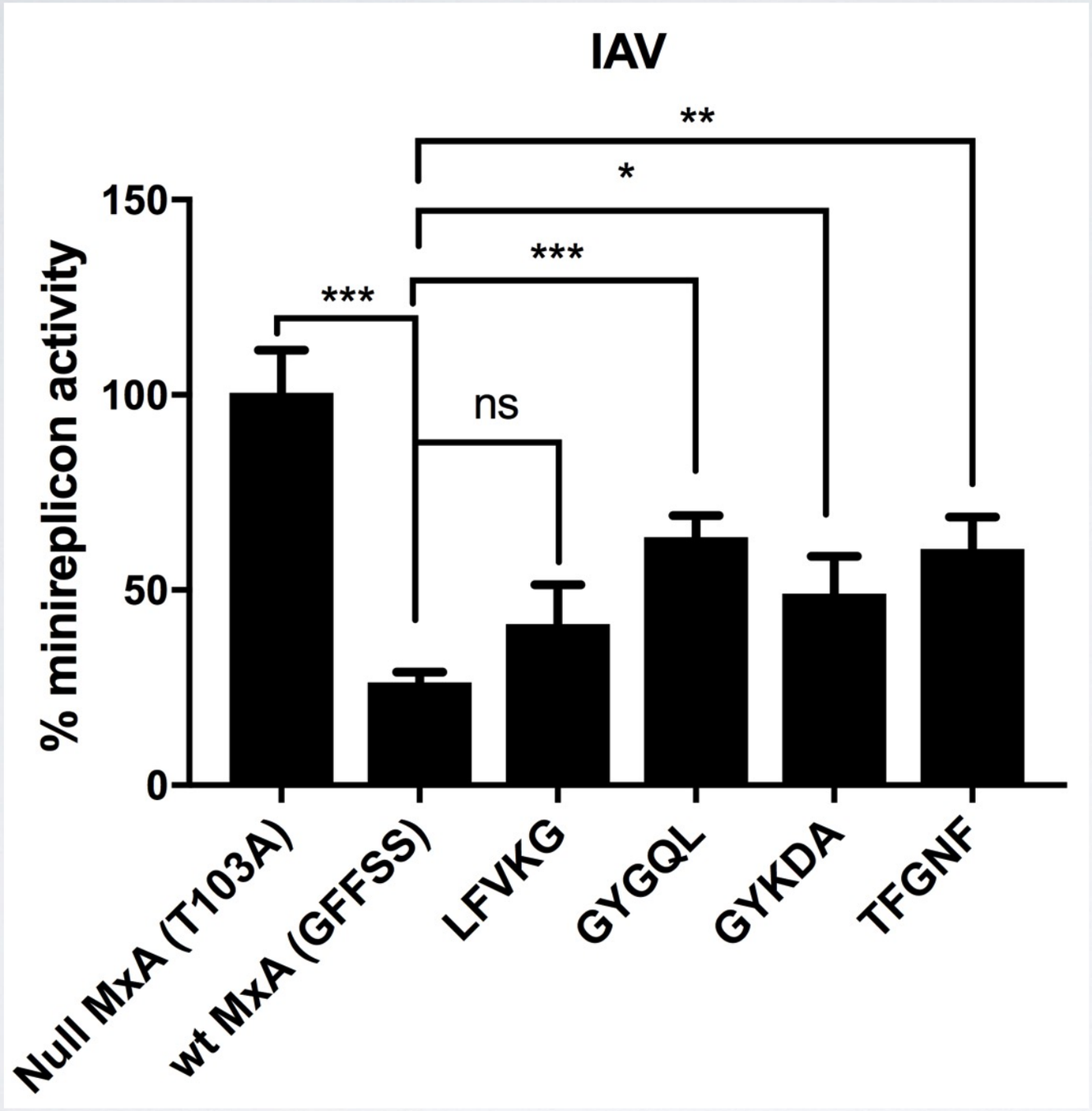
L – F – V – K – G



Does super restriction lead to a specificity tradeoff?

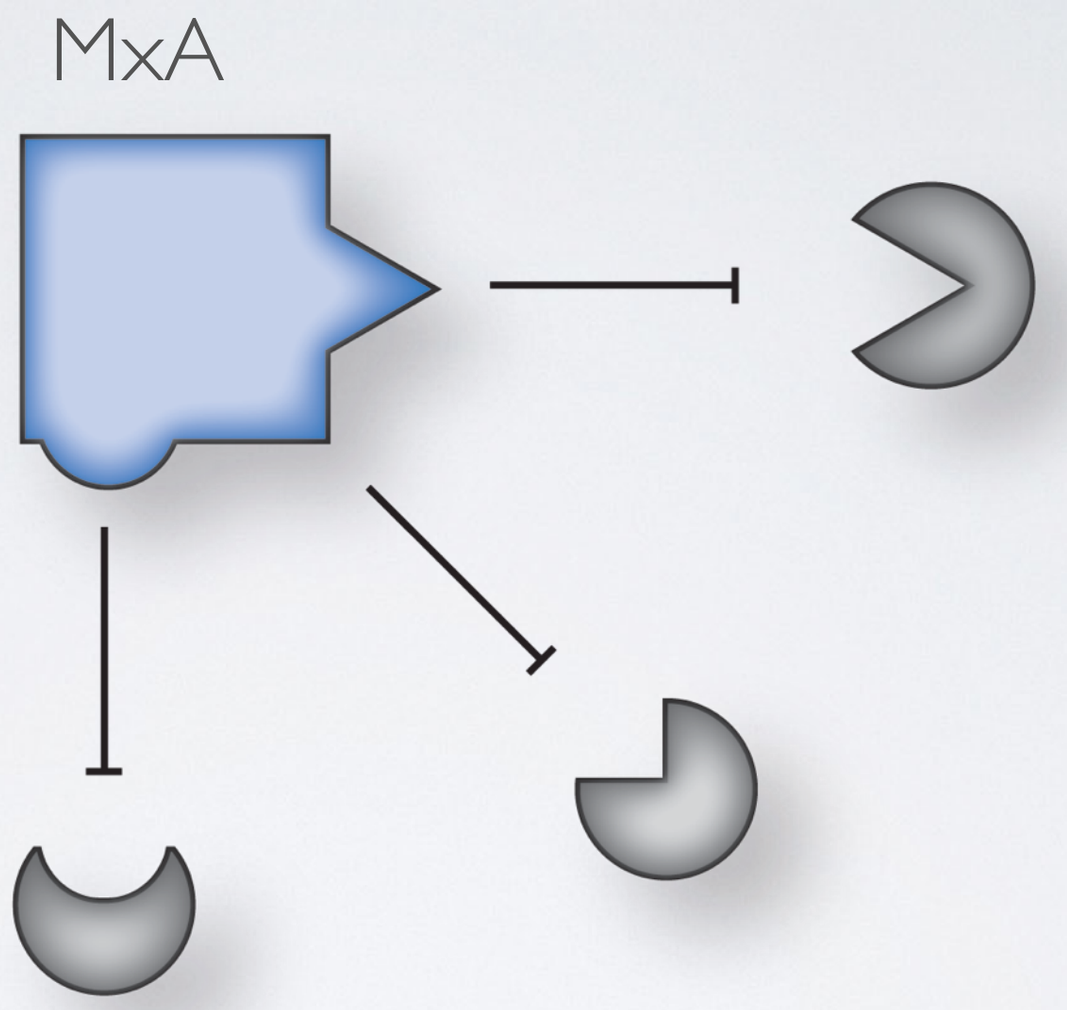


Super-restrictors are virus specific



MxA is an antiviral hybrid

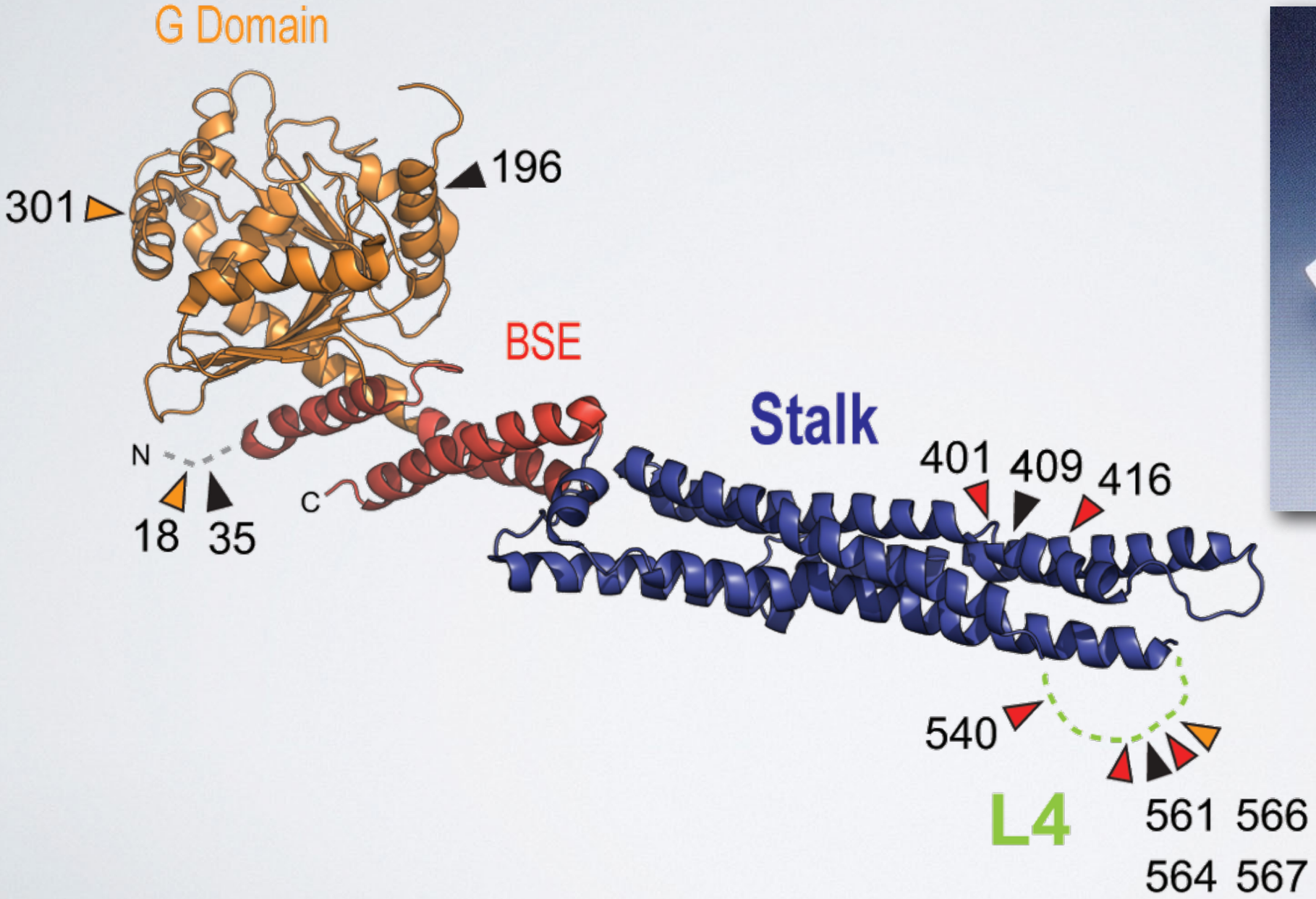
(broadly-acting despite specific recognition of distinct viral targets)



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

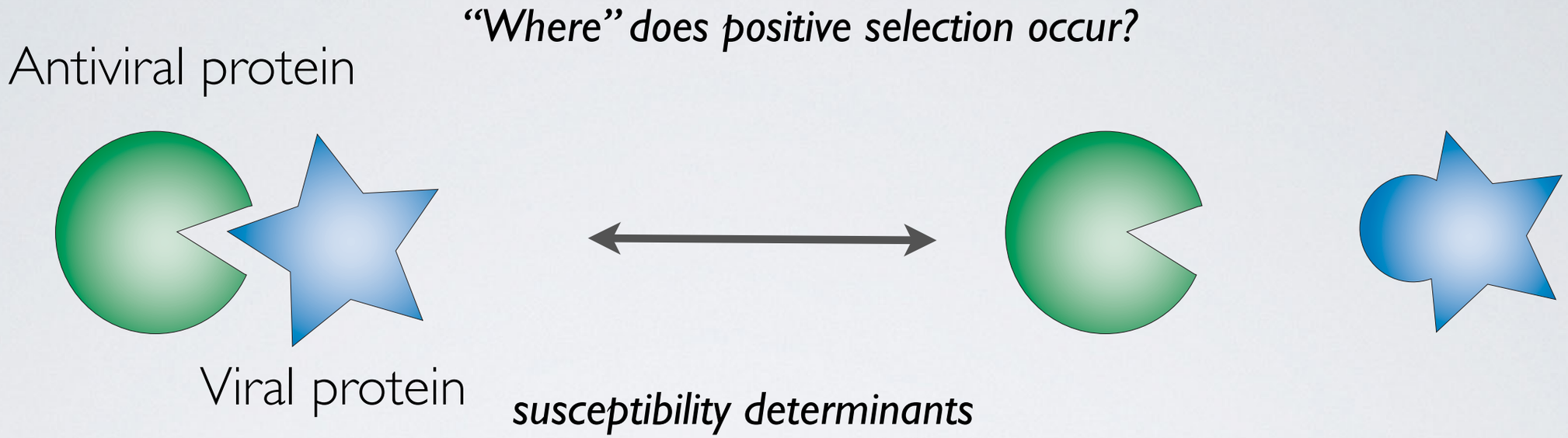
Mitchell et al. Curr Opin Microbiol (2013)


MxA
B
G Domain
B
Stalk
B
1
L4
662


G Domain
301
196
BSE
N
C
18
35
Stalk
401
409
416
540
L4
561
566
564
567

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

"Where" does positive selection occur?



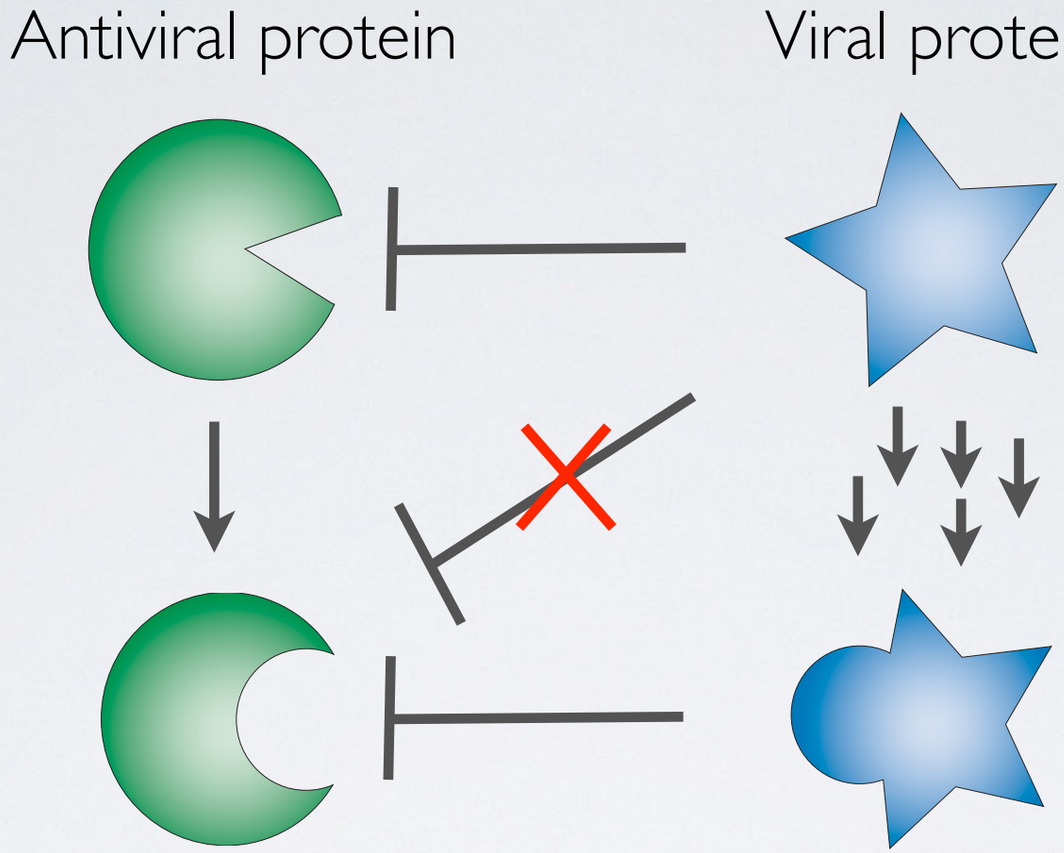
Rules of Engagement: Molecular Insights from Host-Virus Arms Races

Matthew D. Daugherty[1] and Harmit S. Malik[1,2]

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

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

the usual suspects



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