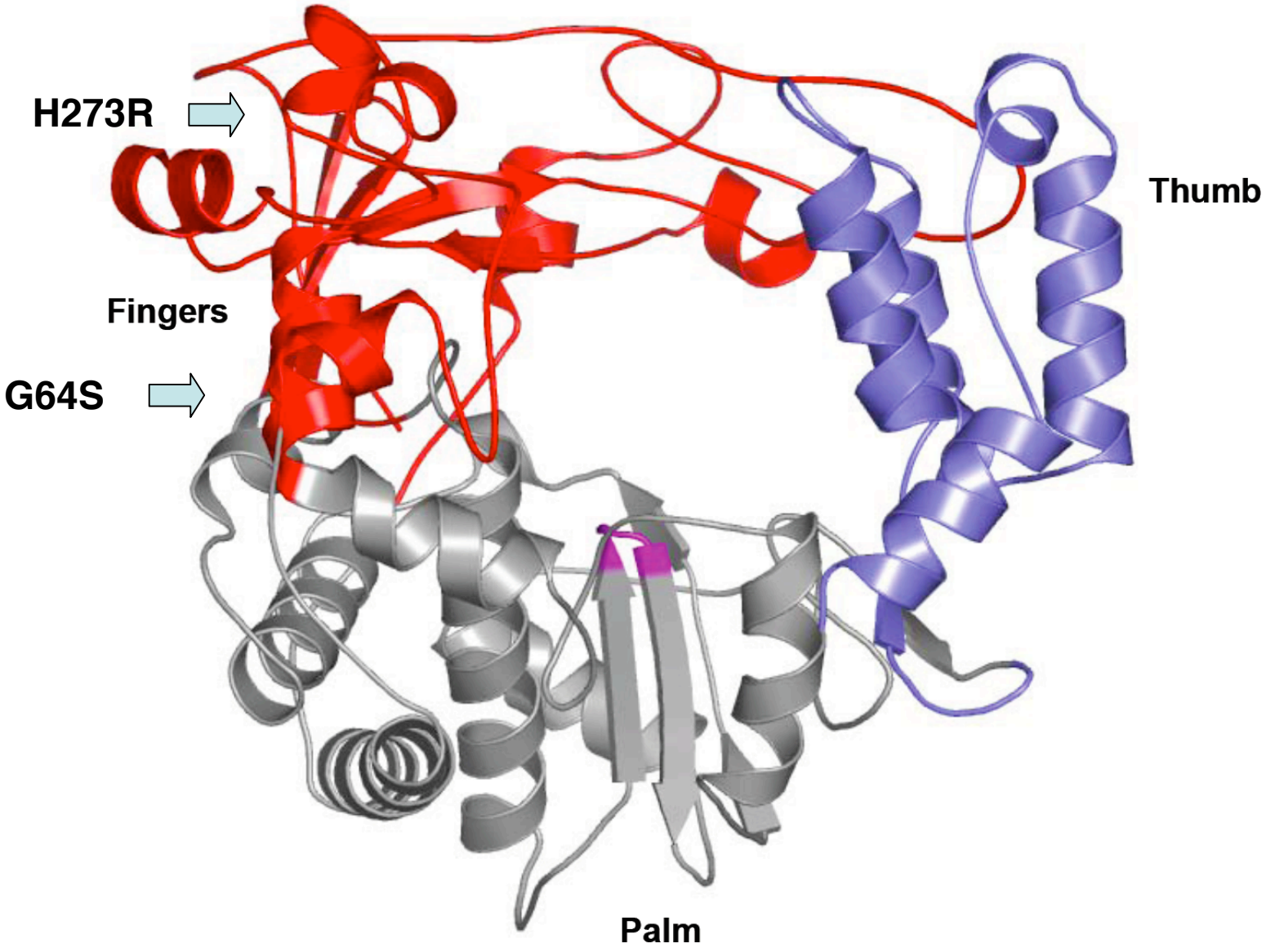
Replication fidelity based vaccines: a novel approach to Polio vaccine design

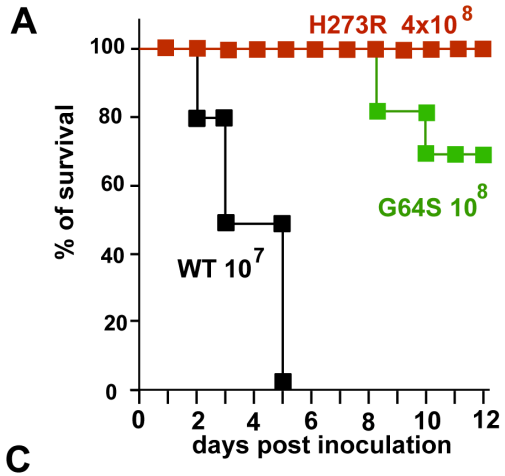
Mutations that decrease or increase mutation rate



Replication fidelity mutants

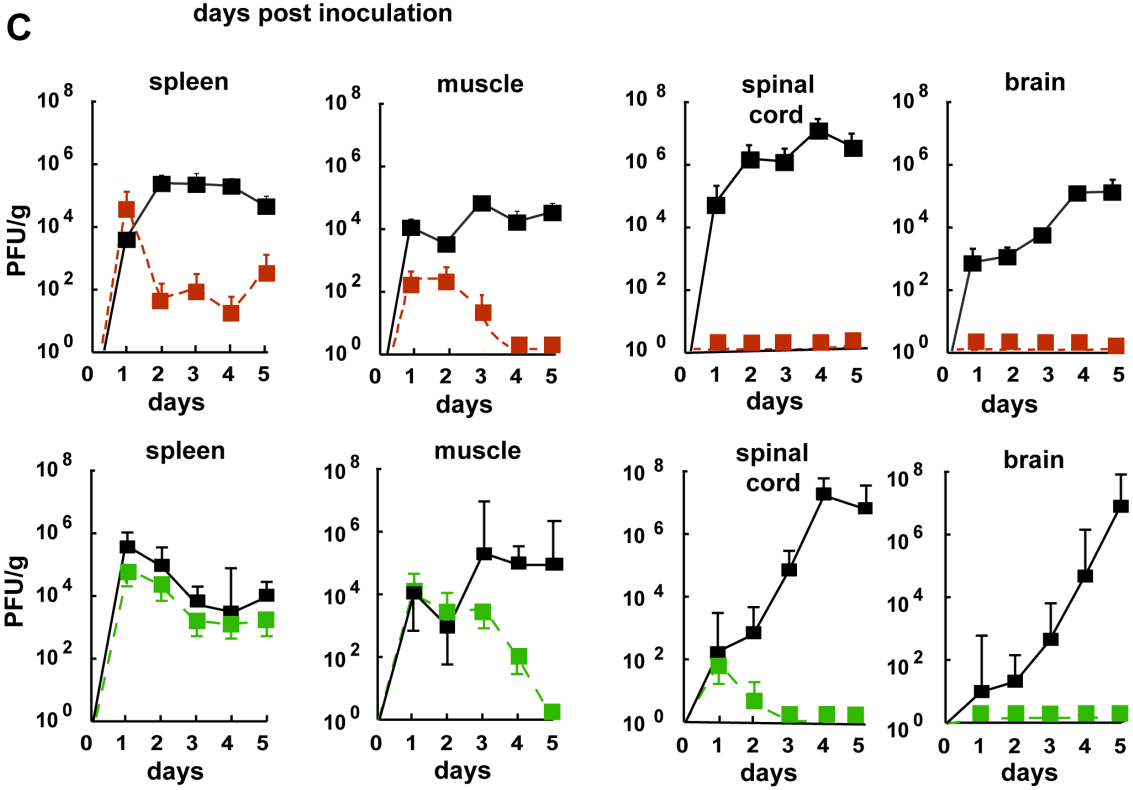
Virus	Total number of mutations	Mutation per genome
wildtype	22/86,700	1.89
Ser64	4/86,700	0.34
H273R	65/161,330	3.00

Both low and high-fidelity mutants are attenuated

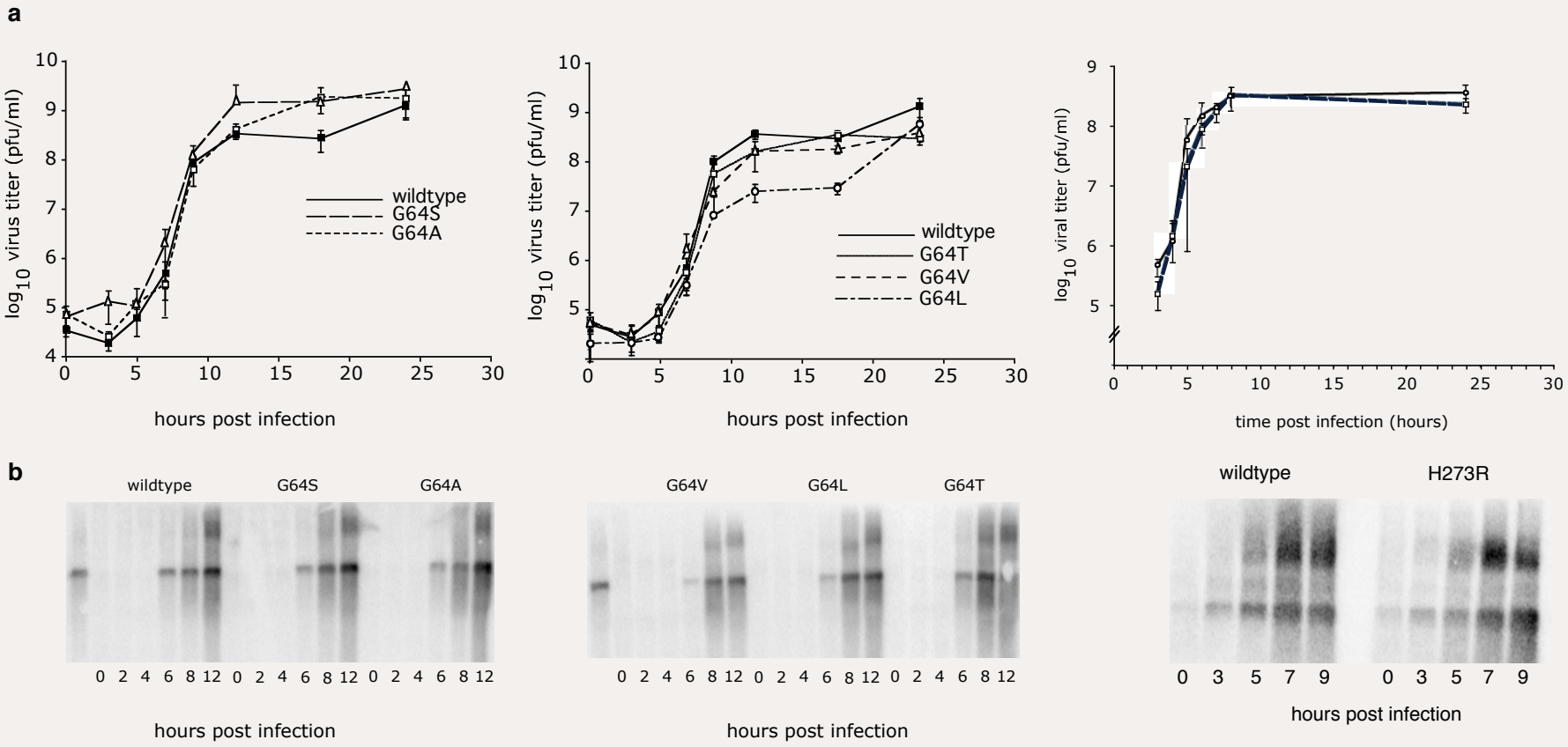


B

Virus	LD_{50} (PFU)
WT	1.2×10^6
H273R	$> 4 \times 10^8$
G64S	3.9×10^8



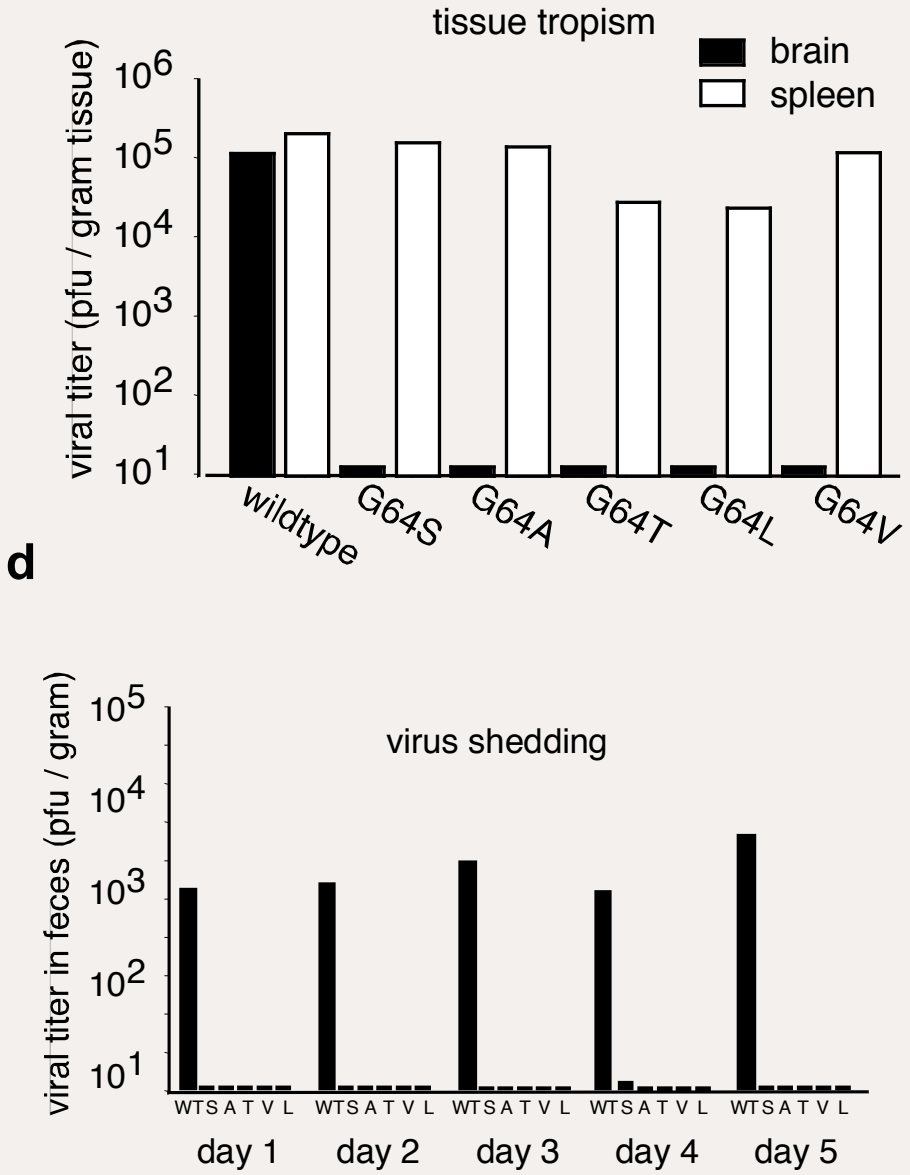
Fidelity and replication



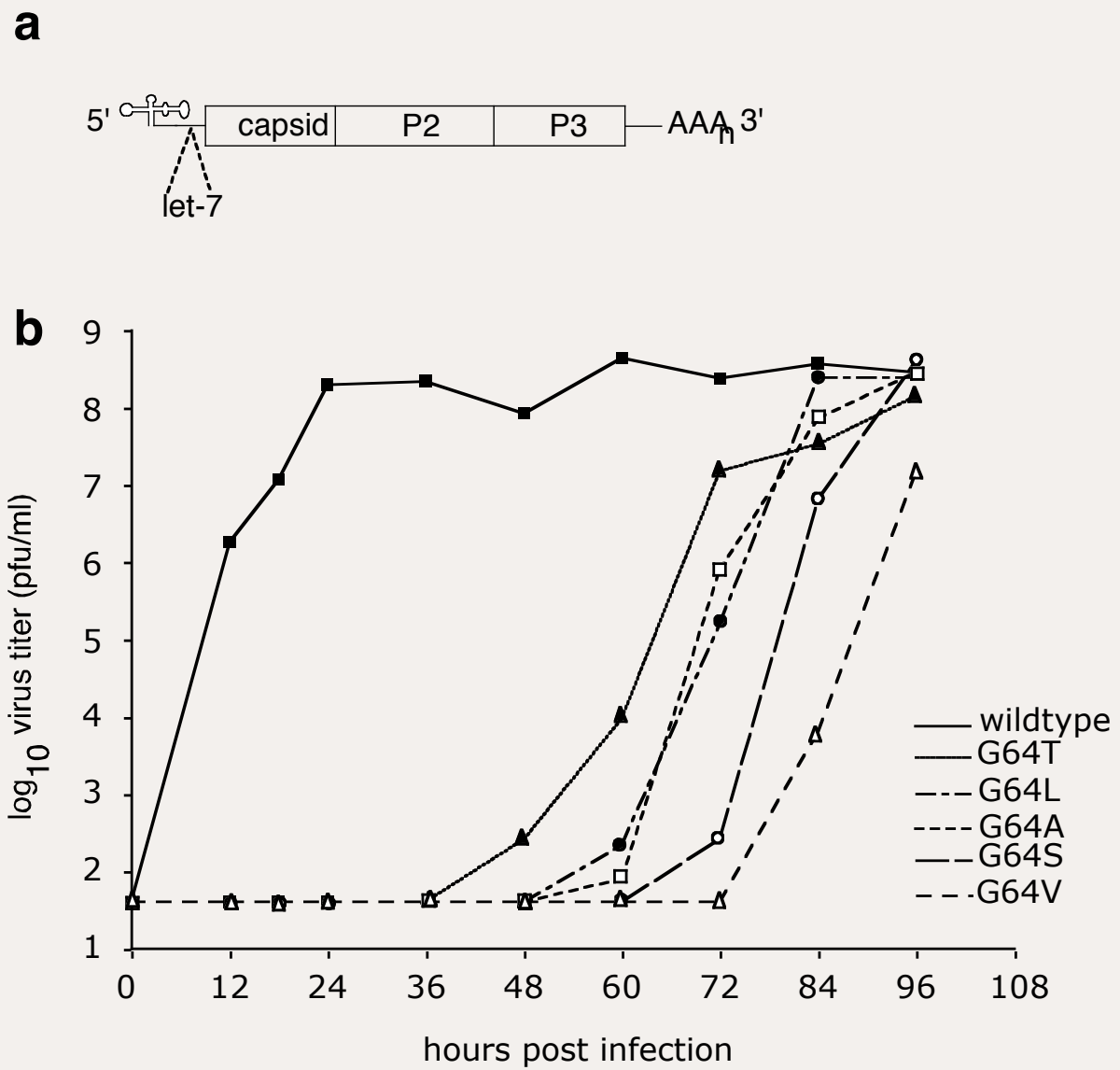
High fidelity vaccines : attenuated & reduced shedding

Virus	LD50(pfu)
Ser64	3.9×10^8
Ala64	4.9×10^7
Thr64	$>1.8 \times 10^8$
Val64	$>1.0 \times 10^8$
Leu64	$>2.3 \times 10^8$
Wildtype	1.2×10^6
Sabin	$> 1.0 \times 10^8$

40-300X less virulent



Lower risk of reversion and mutation to higher fitness

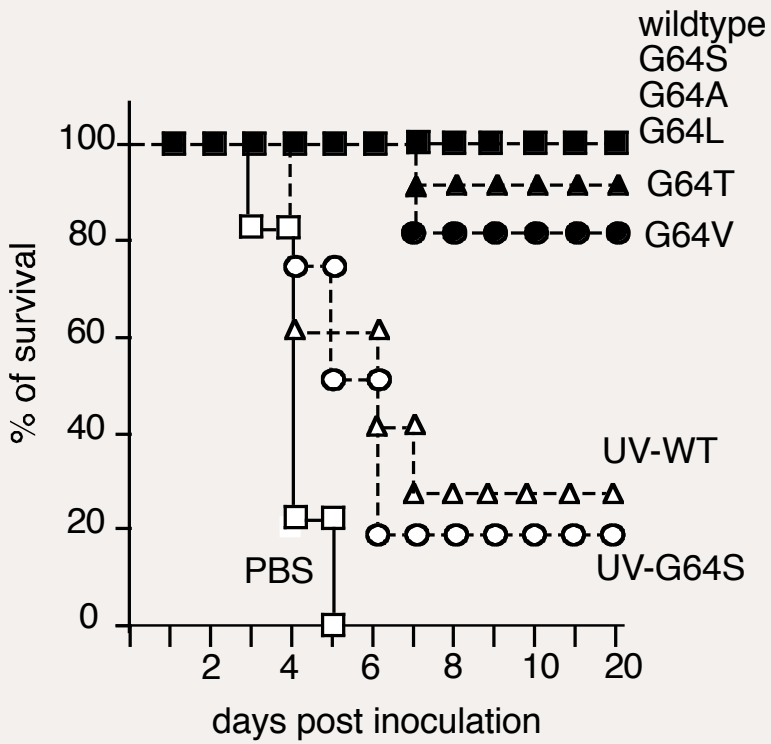


A single dose confers long lasting protection

a

virus		neutralization (% of inhibition)
wildtype	10^6	95 ± 7.8
G64S	10^7	99 ± 1.7
G65A	10^7	99 ± 1.1
G64T	10^7	43 ± 6.2
G64V	10^7	54 ± 19.5
G64L	10^7	58 ± 23.4
PBS		2 ± 7.8
UV-WT	10^7	14 ± 7.8

b



A novel approach to poliovirus vaccines

Novel OPV?

full immune response
single dose, long lasting immunity - YES

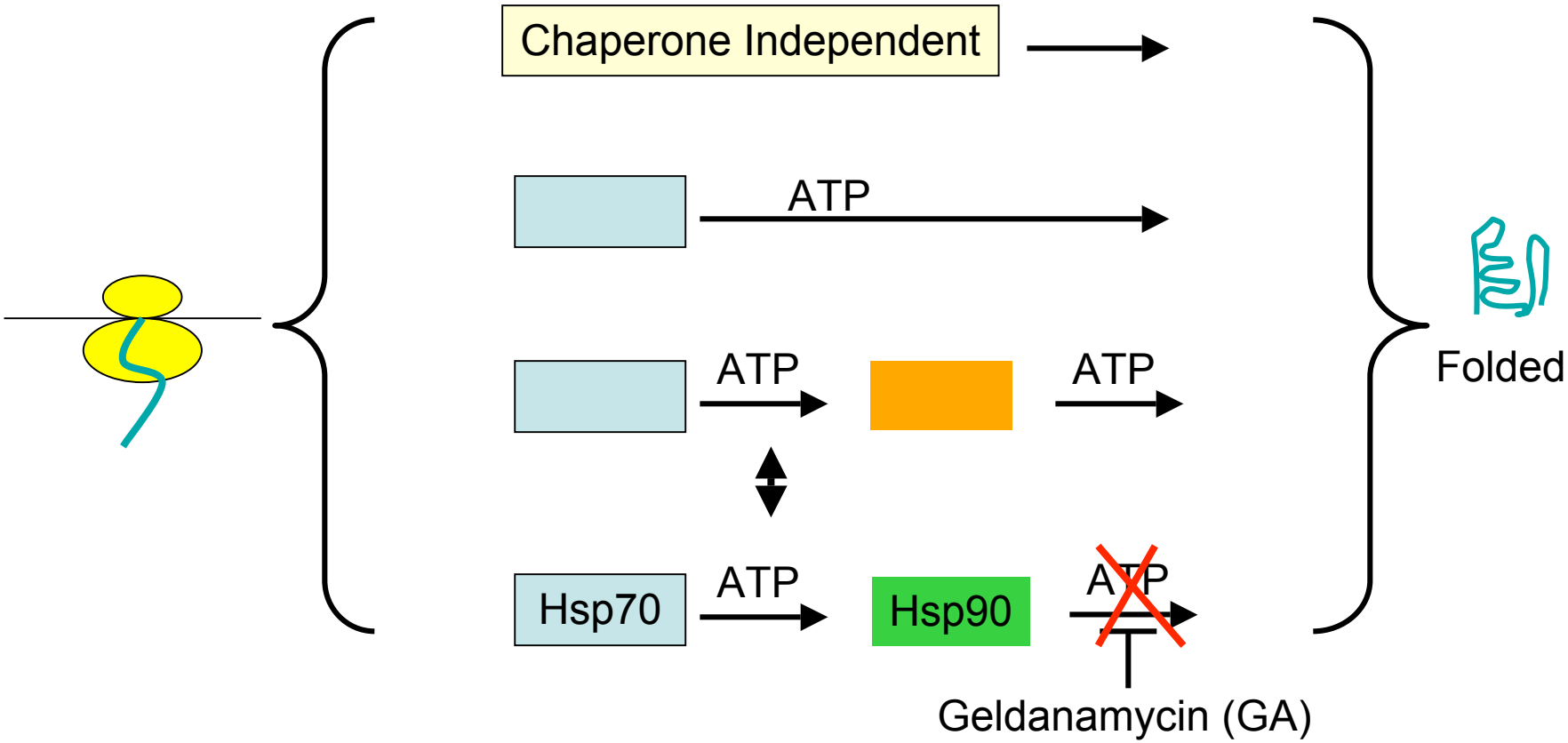
1. attenuation - YES
2. mutation/reversion - REDUCED
3. shedding/dissemination - REDUCED

Novel IPV production?

1) antigenic determinants intact
2) high replication rates in culture
3) safe

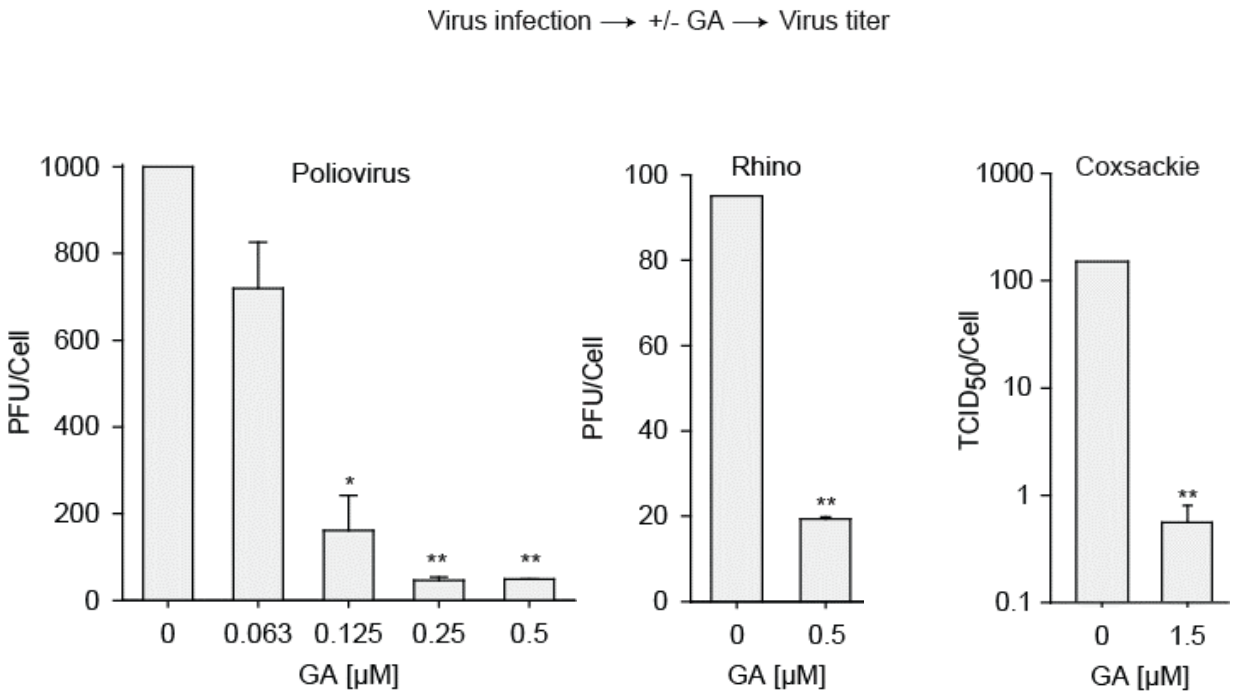
Inhibitors of molecular chaperons as antivirals

Multiple Cytosolic Folding Pathways



Can a protein evolve to use alternate folding pathways?

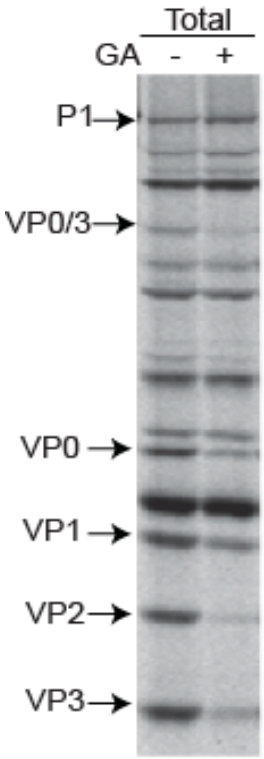
GA Inhibits Several Picornavirus Replication



Hsp90 is required for picornavirus replication

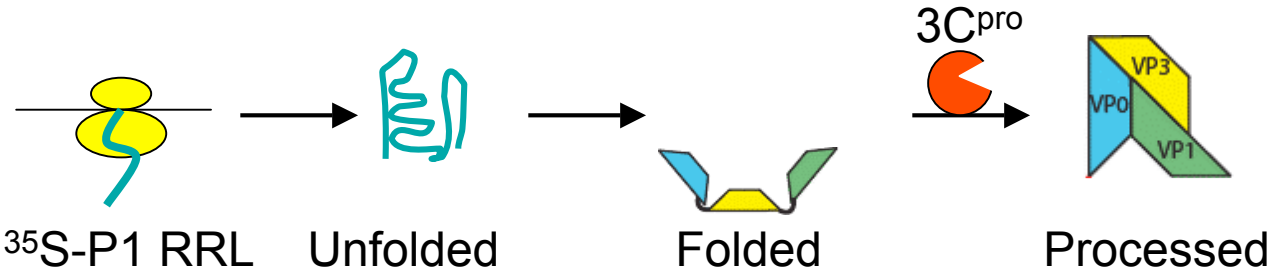
GA Selectively Inhibits P1 Processing

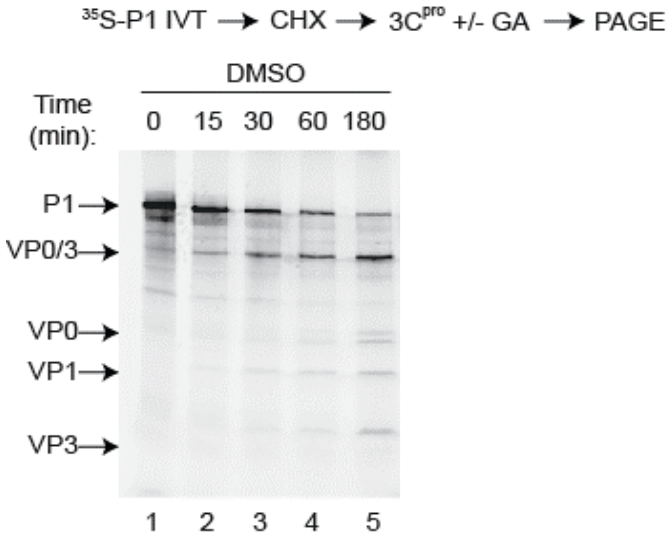
Poliovirus infect → +/- GA → ^{35}S-Met/Cys



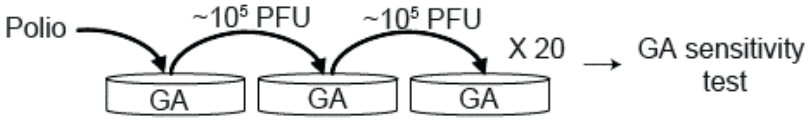
- GA does not inhibit P2 or P3 processing
- P1 is the only viral protein bound by Hsp90

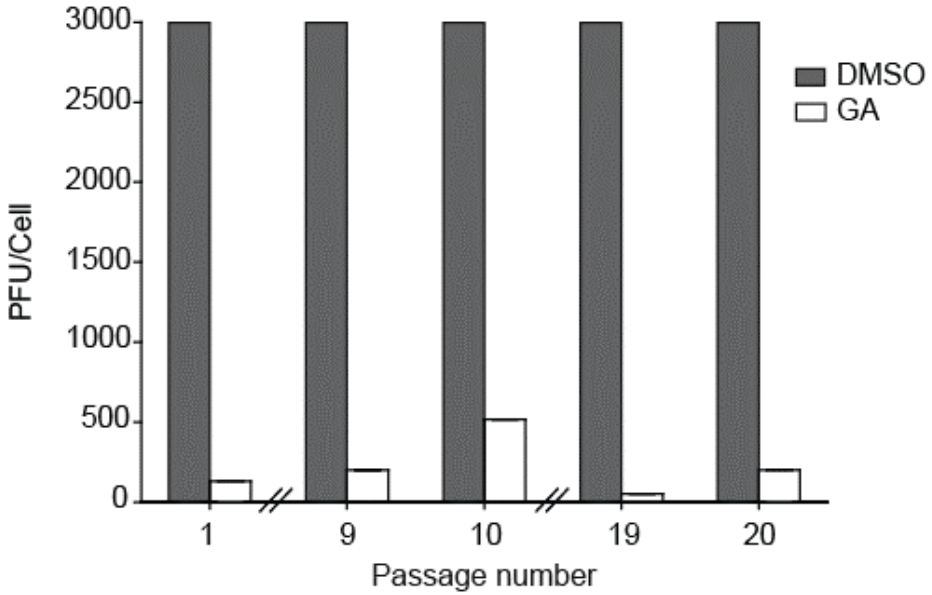
Hsp90 Is Directly Involved in P1 Processing



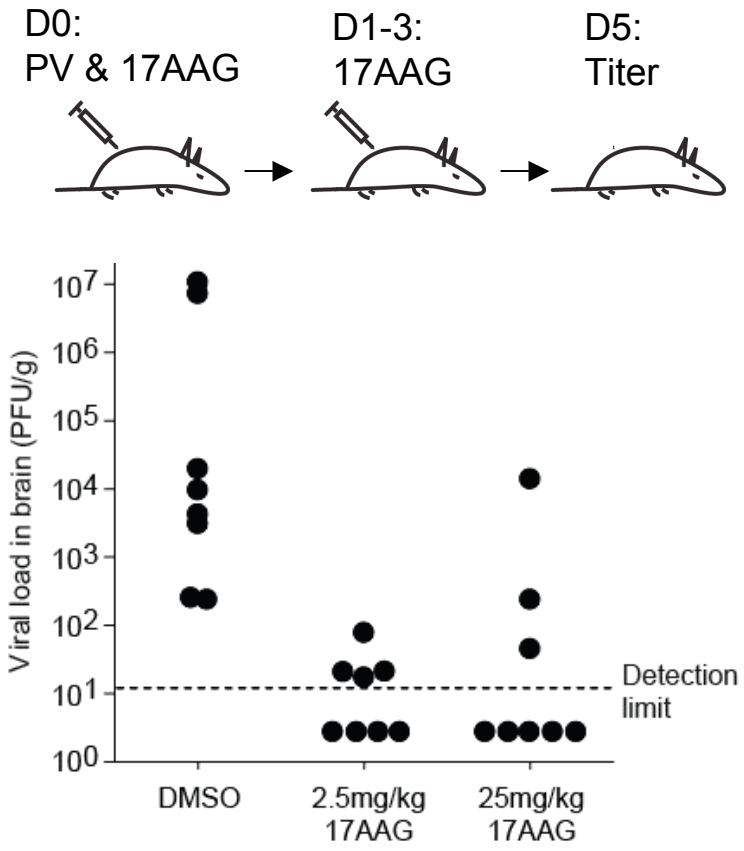


Hsp90 Requirement Cannot be Bypassed





17-AAG Reduces Polio Replication In Vivo



N=8/grp; p<.005

Conclusions

Implication for Hsp90 inhibitors as antivirals:

- Inhibit replication of picornaviruses & **several others *in vitro***
- Inhibit poliovirus replication in infected animals
- Does not elicit drug resistance
- Safe for human use (phase II clinical trials)

Acknowledgements

UCSF - Andino Lab

Marco Vignuzzi

Emily Wendt
Cecily Burrill

Penn State University - Cameron Lab

Craig Cameron

J.J. Arnold
Victoria Korneeva

NIH-NIAID

Stanford University - Frydman Lab

Judith Frydman

Ron Geller