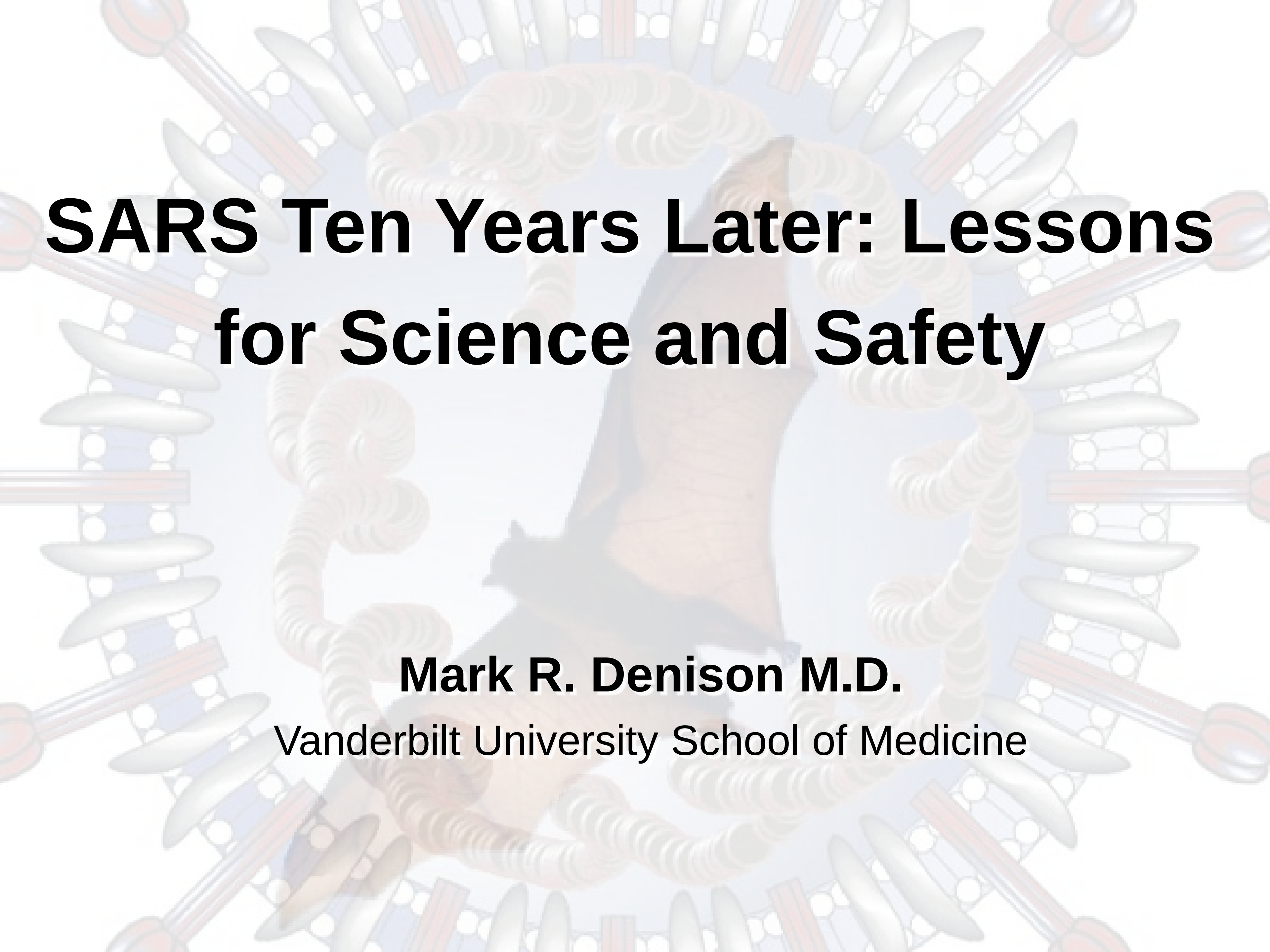
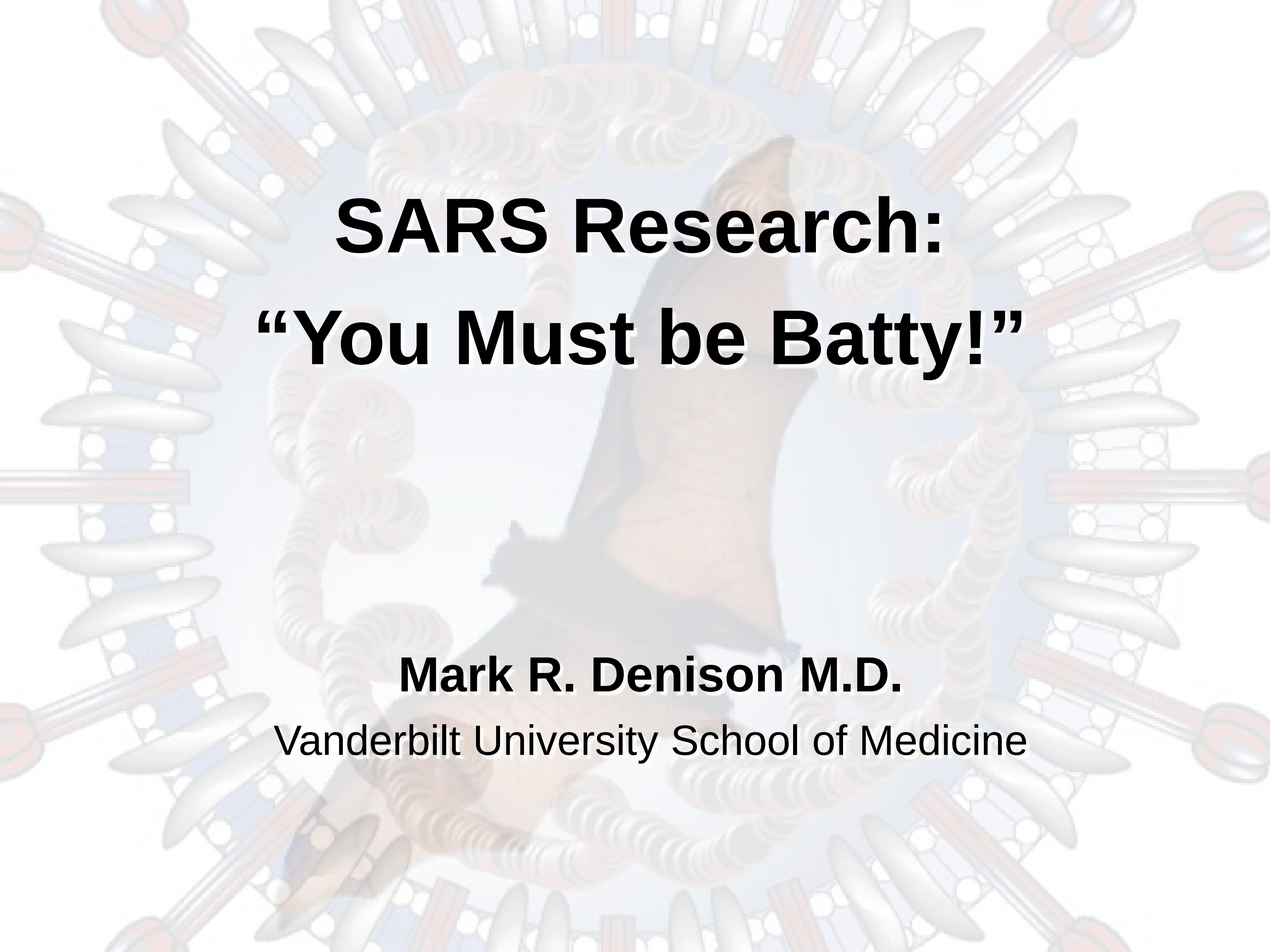


SARS Ten Years Later: Lessons for Science and Safety

Mark R. Denison M.D.
Vanderbilt University School of Medicine



SARS Research:
“You Must be Batty!”

Mark R. Denison M.D.

Vanderbilt University School of Medicine

Acknowledgements

Vanderbilt

Michelle Becker
Xiaotao Lu
Lance Eckerle
Clint Smith

UNC

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Rachel Graham
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Boyd Yount

NIH AI50083, AI59943

SERCEB (South-East Regional Center of Excellence in Emerging Infections and Biodefense)

NIH Microbial Genome Sequencing Project HHSN272200900007C

Zoonotic Viruses and Human Disease

- Zoonotic emergence and potential pandemic viruses are increasing
- Mechanisms of trans-species virus movement and adaptation are unknown
- Delays in response to natural or intentional emergence can be devastating
- New approaches are needed for rapid recovery and study of identified or predicted zoonotic precursor viruses

Research Goals

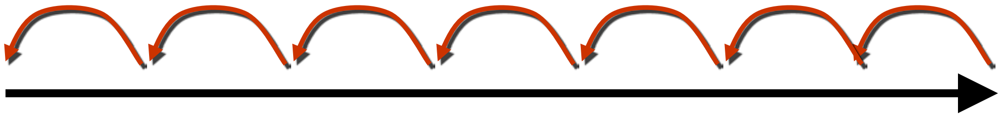
- To define mechanisms of trans-species movement of zoonotic viruses to humans
- To develop broadly applicable approaches to attenuate and treat CoVs and other families of viruses.

Jumping species – a high jump?

Or Hurdles?


Transmission
Stability
Shedding
Adaptation
Evasion
Replication
Transmission

Studying the Trans-Species Movement of Zoonotic Viruses

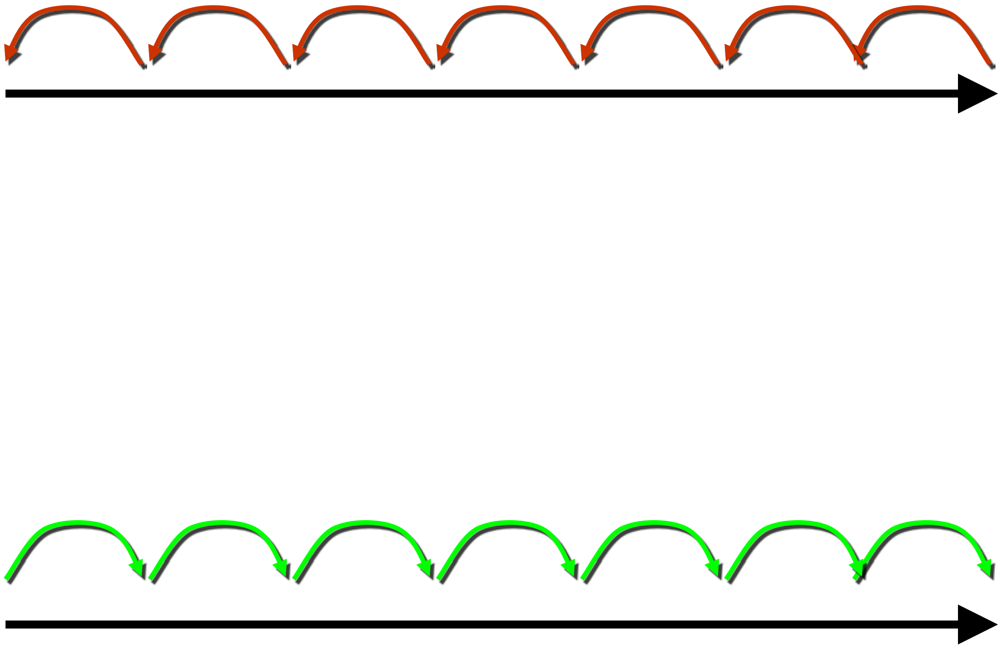


Retrospective, Bioinformatic

Putative Zoonotic Source Virus **(non-cultivatable**)

Emerged Human Virus

Studying the Trans-Species Movement of Zoonotic Viruses



Retrospective, Bioinformatic

Putative Zoonotic Source Virus **(non-cultivatable**)

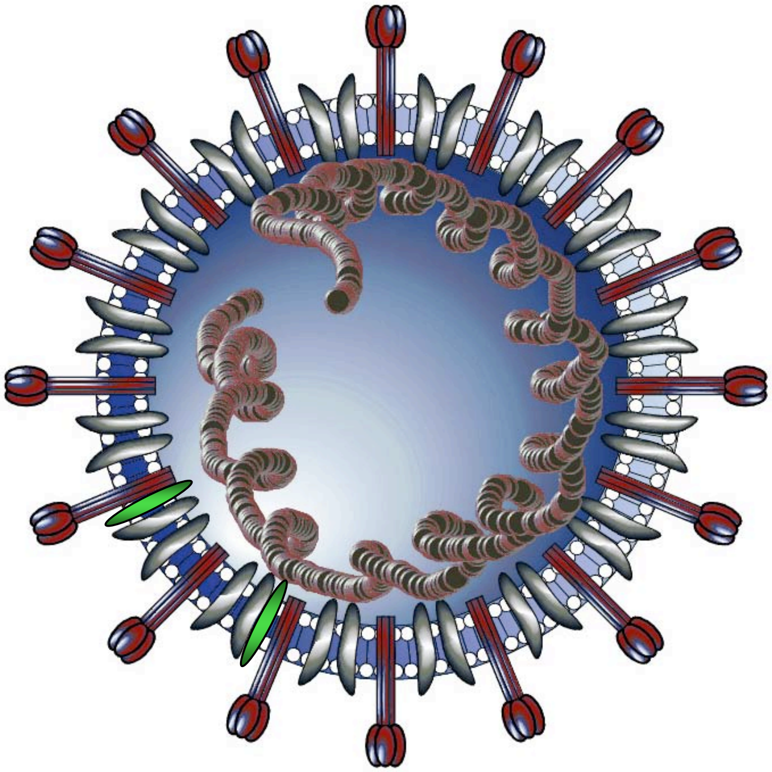
Emerged Human Virus

Putative Precursor - **(synthetic-viable)**

Forward, Biological

Relationship to Emerged Human Virus

Coronaviruses



- Broad diversity across mammalian and avian species
- Demonstrate trans-species capacity in lab and in nature
- Source of multiple human viruses including SARS-CoV
- Likely zoonotic origin (Bats)
- Evidence for frequent new human and zoonotic CoVs

Coronavirus Diseases

Virus	Host	Disease
MHV	mice	hepatitis, encephalitis
TGEV	pigs	gastroenteritis, pneumonia
BCoV	cattle	gastroenteritis, pneumonia
CCoV	dogs	gastroenteritis
FIPV	cats	peritonitis, enteritis
AJ-CoV	cheetah	peritonitis
IBV	chickens	tracheitis, renal
SW-1	beluga whale	hepatitis
BAT-CoV	bats	asymptomatic?
SARS-CoV	Human	SARS
NL63	Human	bronchiolitis, pneumonia
HKU-1	Human	bronchiolitis, pneumonia
HCoV-OC43	Human	colds, pneumonia,
HCoV-229E	Human	colds, pneumonia,

What was (is) SARS?

- Severe Acute Respiratory Syndrome
- A new human coronavirus (SARS-CoV)
- Demonstrated potential for pandemic disease
- November 2002 through July 2003.
- > 8500 Cases, > 774 deaths, 32 countries
- Confirmed coronavirus trans-species movement and severe human disease

Where did SARS-CoV come from?

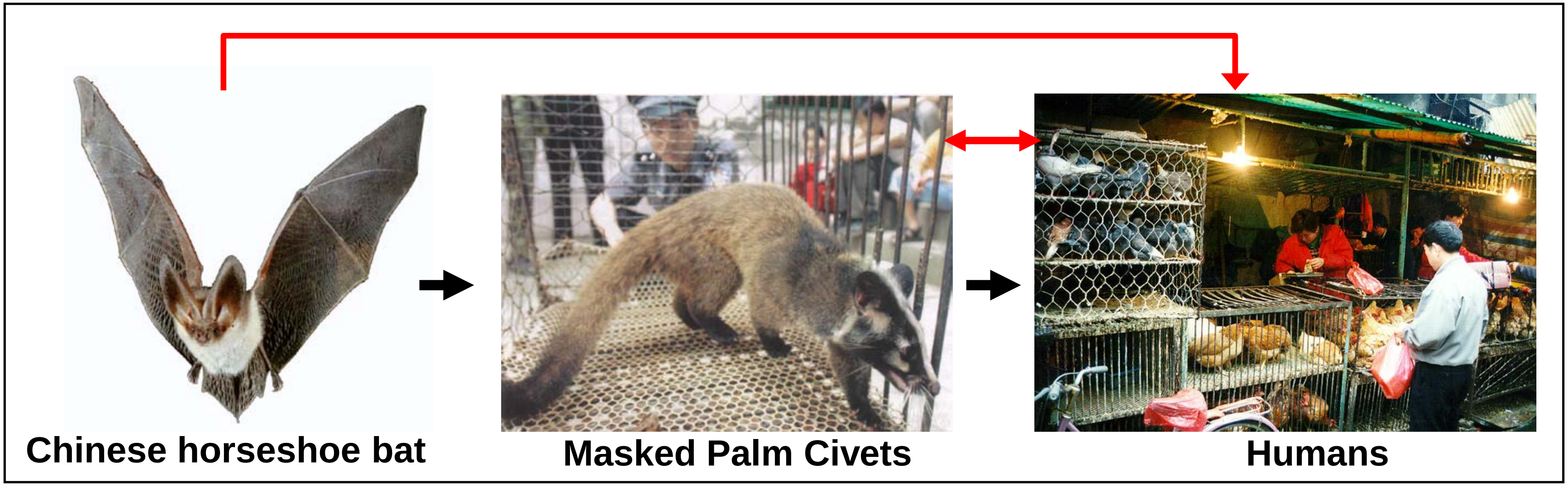
- Direct transmission from animal reservoir?
- Mutations in animal or human virus?
- Recombination between different coronaviruses?

Bats and SARS-like coronaviruses

Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Lau et al., PNAS 2005

Bats Are Natural Reservoirs of SARS-Like Coronaviruses. Li et al., Science 2005

SARS Coronavirus Spillover



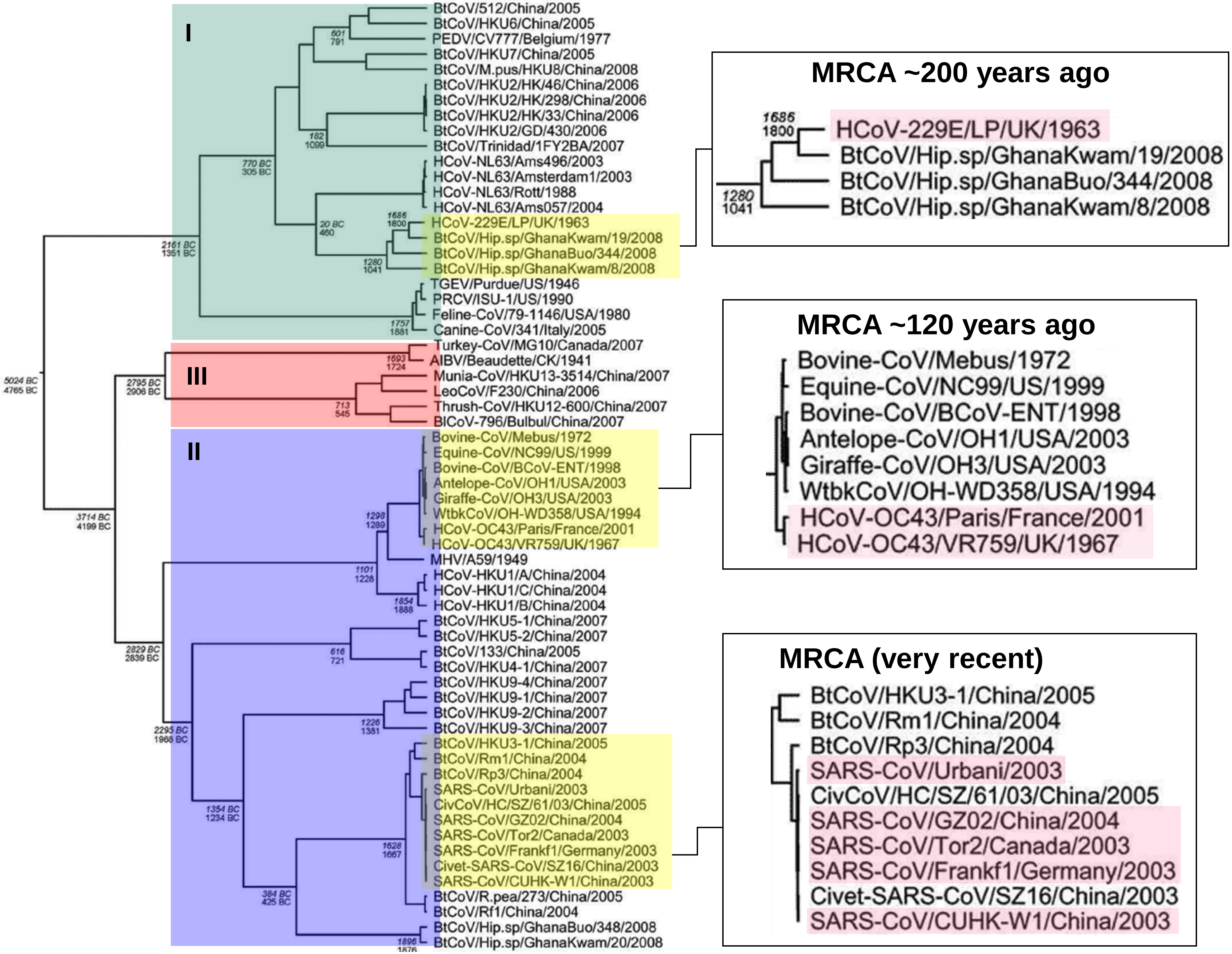
Chinese horseshoe bat

Masked Palm Civets

Humans

Bats and SARS-CoV

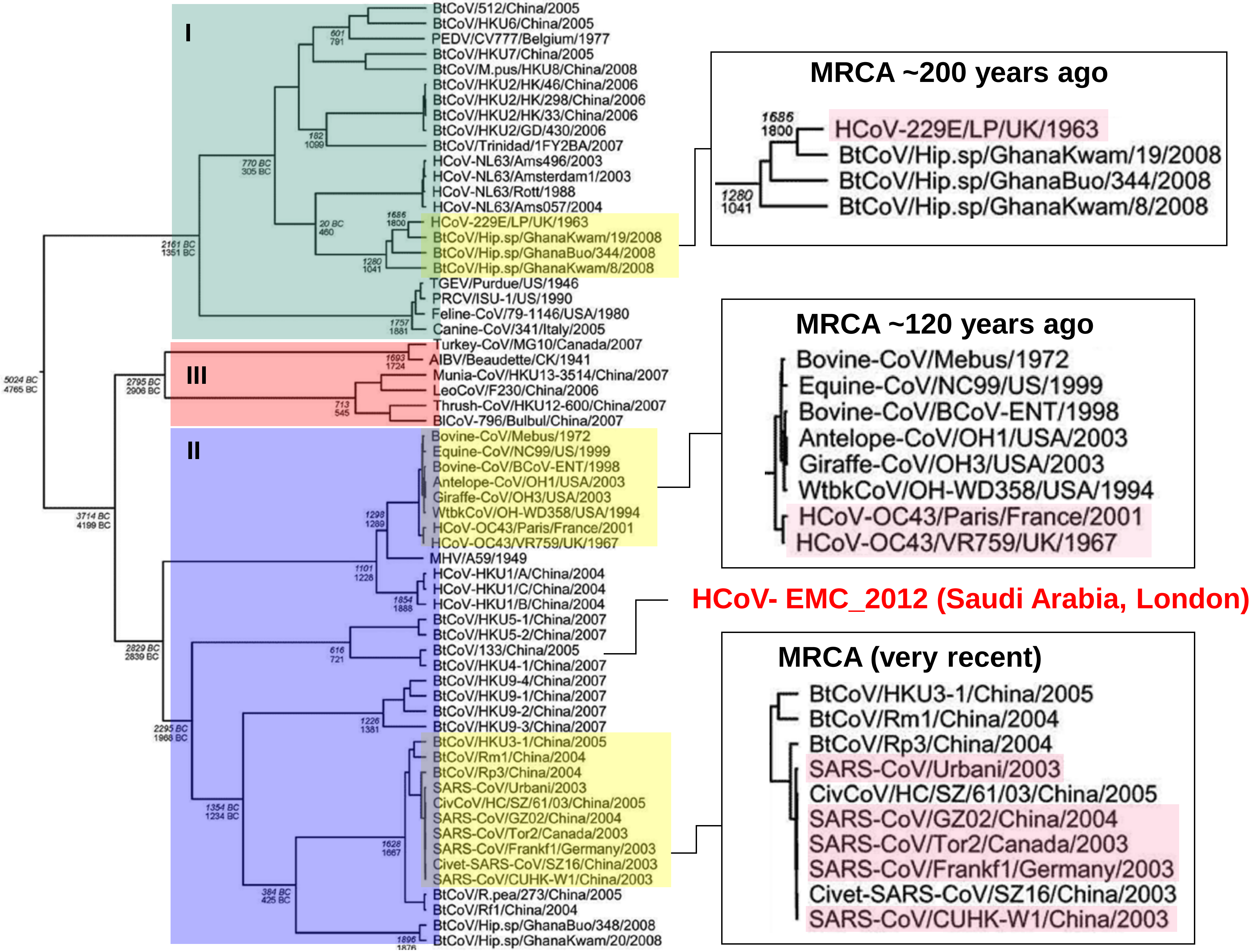
- SARS-CoV is most closely related to beta Bat-CoV, but the precise SARS-CoV precursor has not been found.
- Bats have no apparent disease from CoVs
- Bat-CoVs only recently been isolated in culture
- Mechanisms of host-species switching and adaptation are not known



MRCA ~200 years ago

MRCA ~120 years ago

MRCA (very recent)



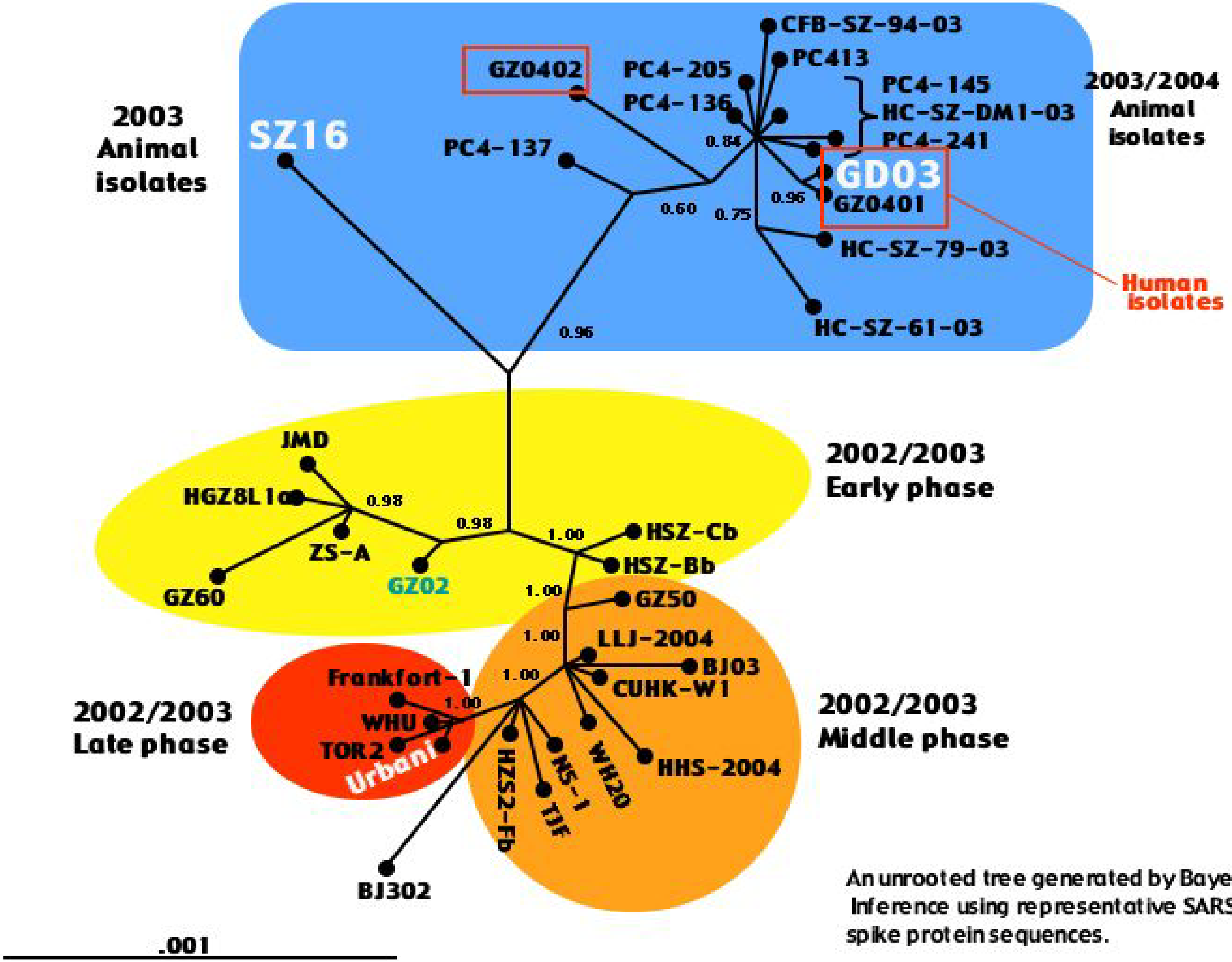
MRCA ~200 years ago

MRCA ~120 years ago

HCoV- EMC_2012 (Saudi Arabia, London)

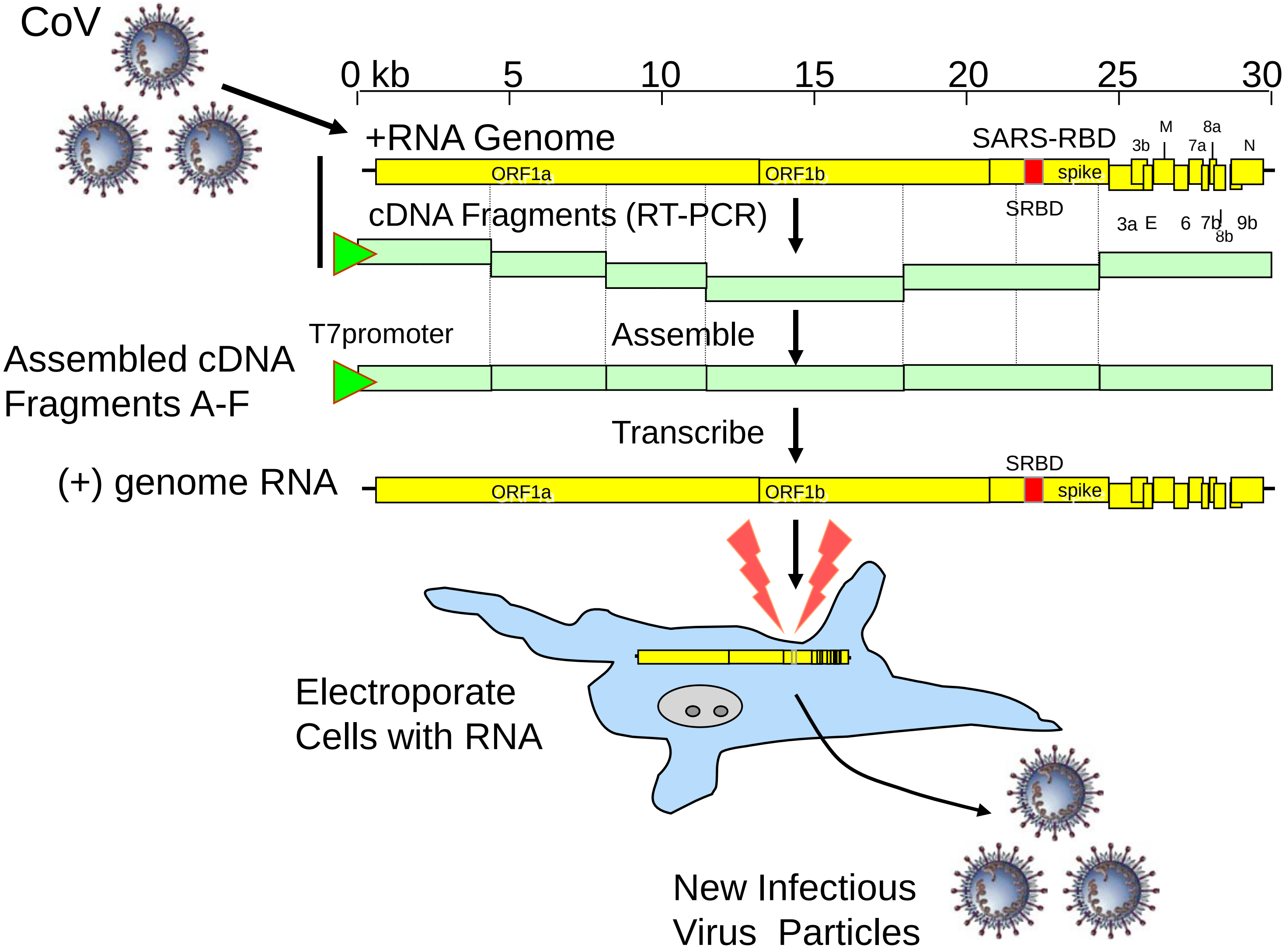
MRCA (very recent)

SARS is not SARS is not SARS



An unrooted tree generated by Bayesian Inference using representative SARS spike protein sequences.

Reverse Genetics: Cloning and Recovery of Recombinant Coronavirus



SARS-CoV

+RNA Genome

cDNA Fragments (RT-PCR)

T7promoter

Assemble

Assembled cDNA Fragments A-F

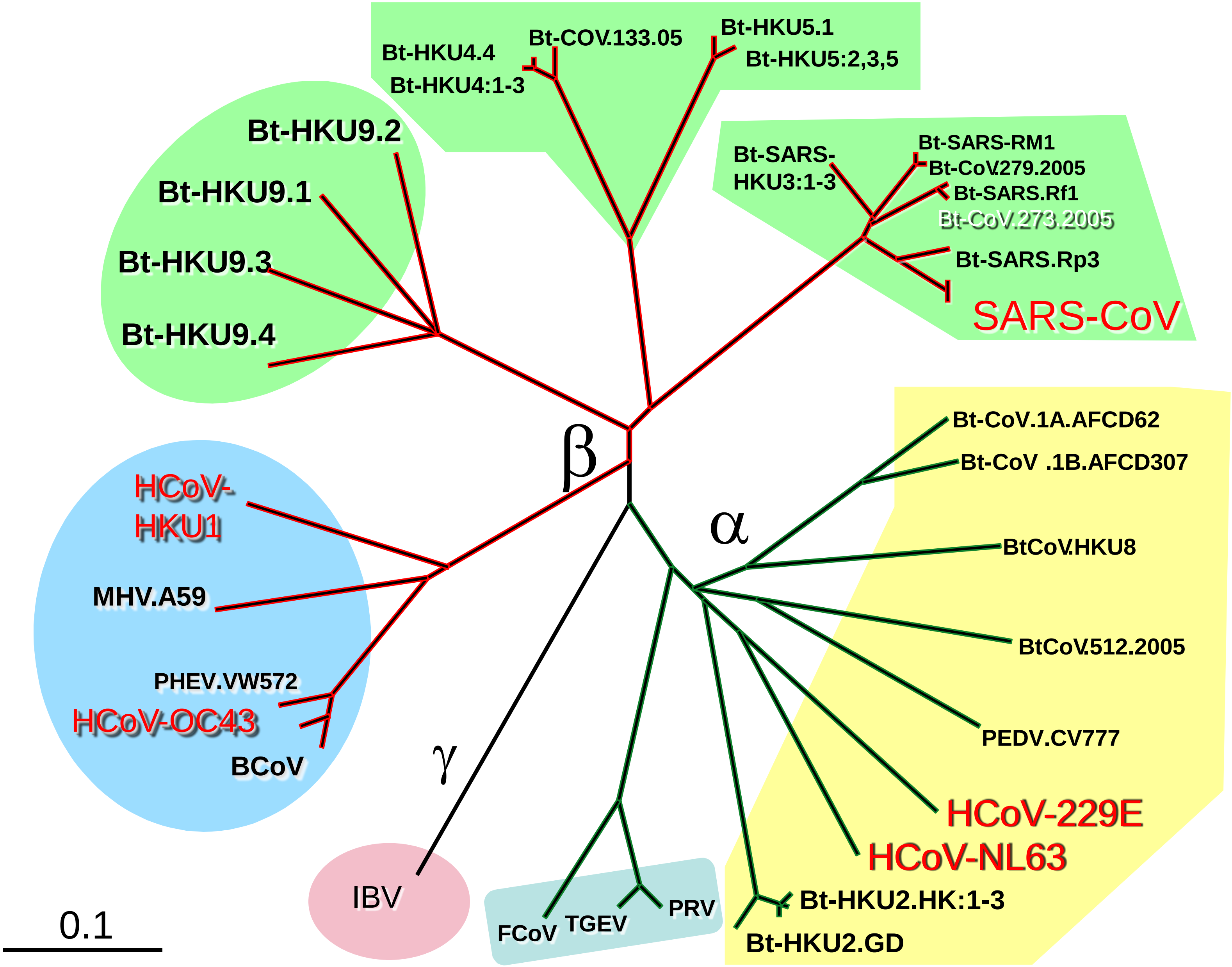
Transcribe

(+) genome RNA

Electroporate Cells with RNA

New Infectious Virus Particles

SRBD - 🟥
SARS Receptor
Binding Domain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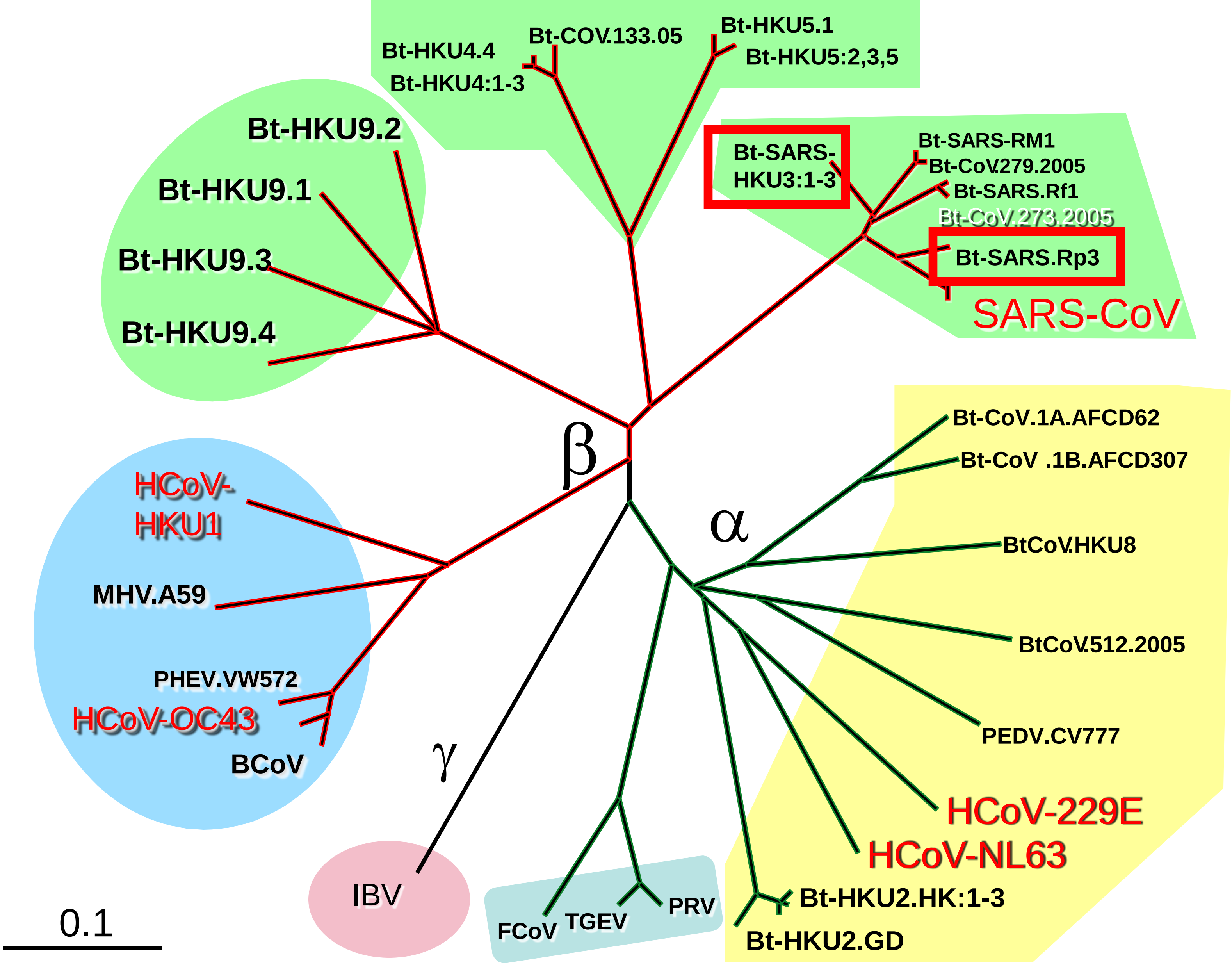
Bt-COV.133.05
Bt-HKU4:1-3
Bt-HKU5.1
Bt-HKU5:2,3,5
Bt-HKU9.2
Bt-HKU9.1
Bt-HKU9.3
Bt-HKU9.4
Bt-SARS-HKU3:1-3
Bt-SARS-RM1
Bt-CoV.279.2005
Bt-SARS.Rf1
Bt-CoV.273.2005
Bt-SARS.Rp3
SARS-CoV
β
Bt-CoV.1A.AFCD62
Bt-CoV .1B.AFCD307
α
BtCoV.HKU8
BtCoV.512.2005
PEDV.CV777
HCoV-229E
HCoV-NL63
Bt-HKU2.HK:1-3
Bt-HKU2.GD
HCoV-HKU1
MHV.A59
PHEV.VW572
HCoV-OC43
BCoV
γ
IBV
FCoV
TGEV
PRV
0.1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Bt-COV.133.05
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IBV
FCoV
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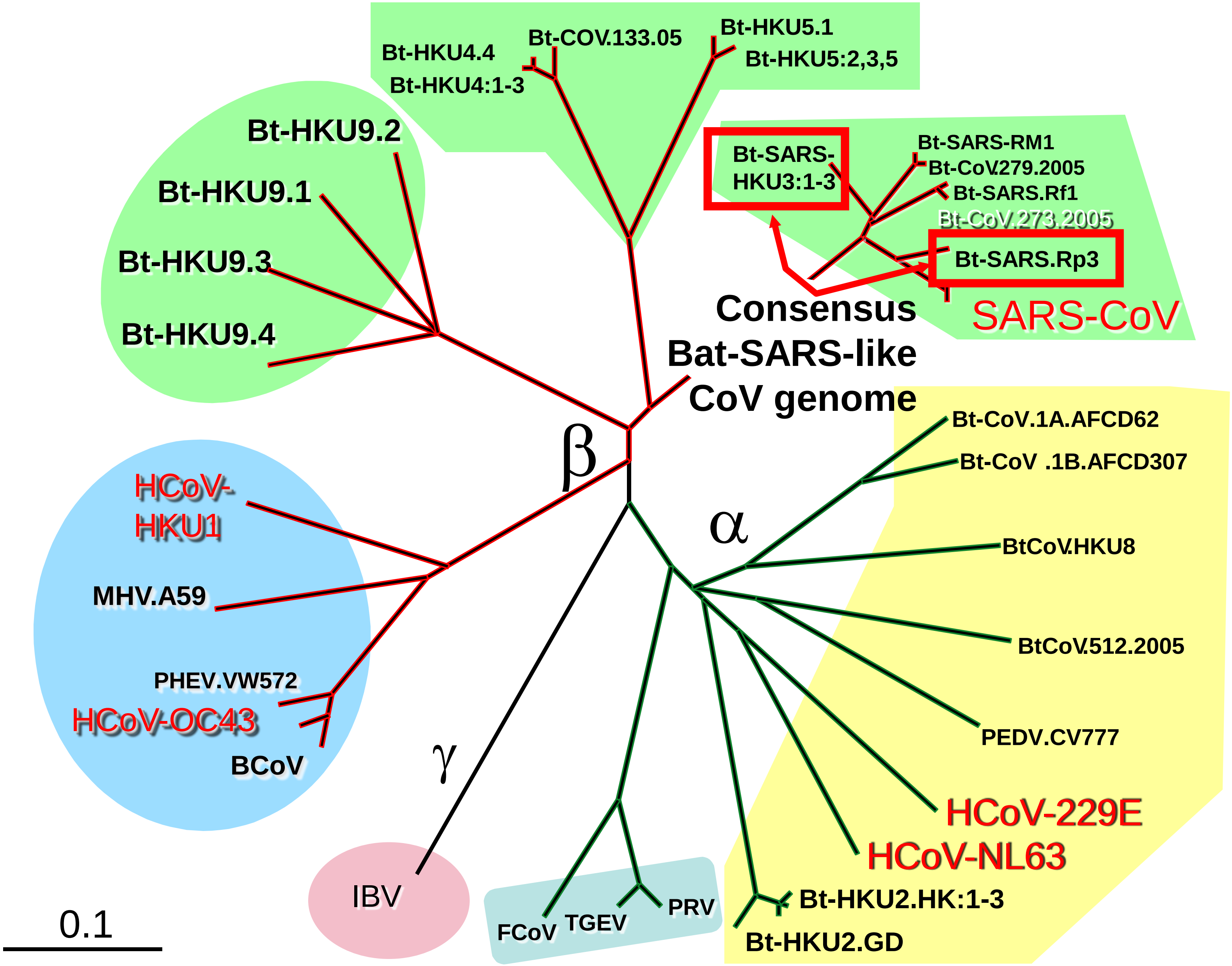
PRV
0.1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Bt-HKU5:2,3,5
Bt-HKU9.2
Bt-HKU9.1
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Bt-SARS-HKU3:1-3
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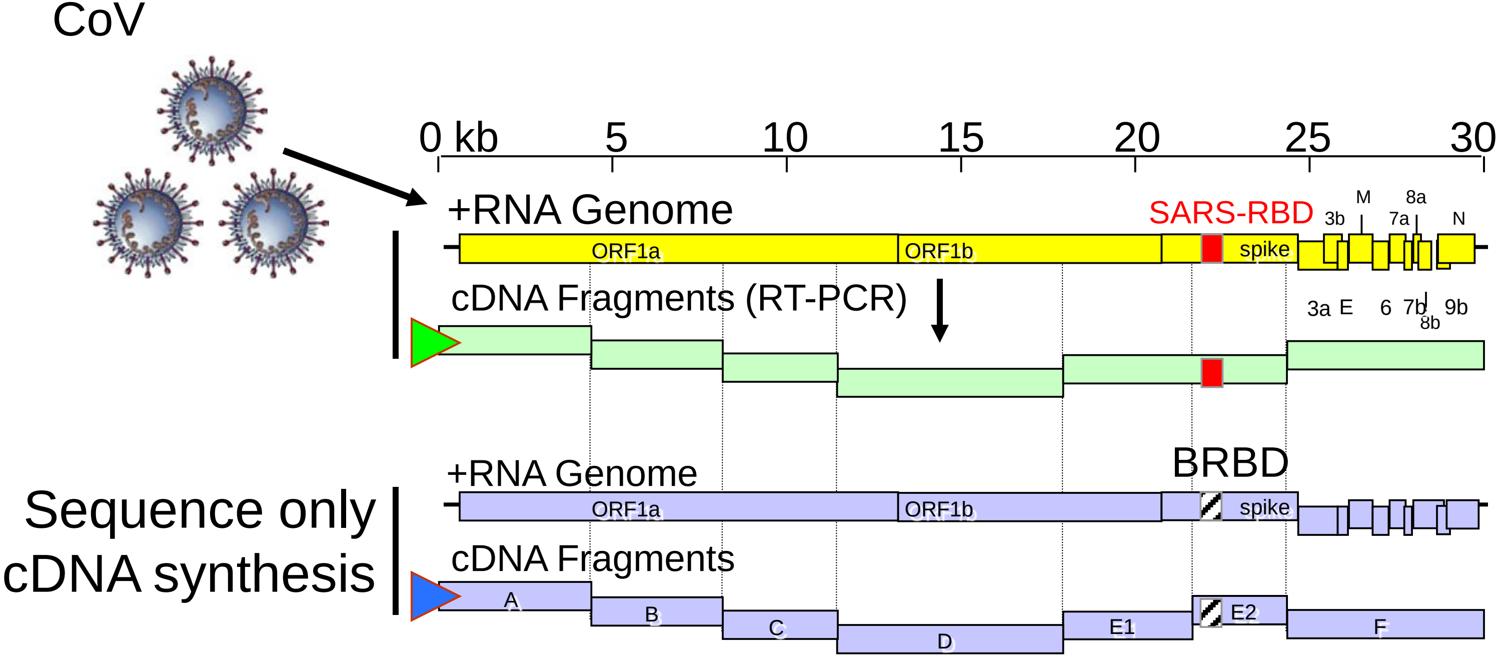
Bt-CoV.279.2005
Bt-SARS.Rf1
Bt-CoV.273.2005
Bt-SARS.Rp3
Consensus Bat-SARS-like CoV genome
SARS-CoV
β
Bt-CoV.1A.AFCD62
Bt-CoV .1B.AFCD307
α
BtCoV.HKU8
BtCoV.512.2005
PEDV.CV777
HCoV-229E
HCoV-NL63
Bt-HKU2.HK:1-3
Bt-HKU2.GD
HCoV-HKU1
MHV.A59
PHEV.VW572
HCoV-OC43
BCoV
γ
IBV
FCoV
TGEV
PRV
0.1

Synthesis of Bat-CoV genome



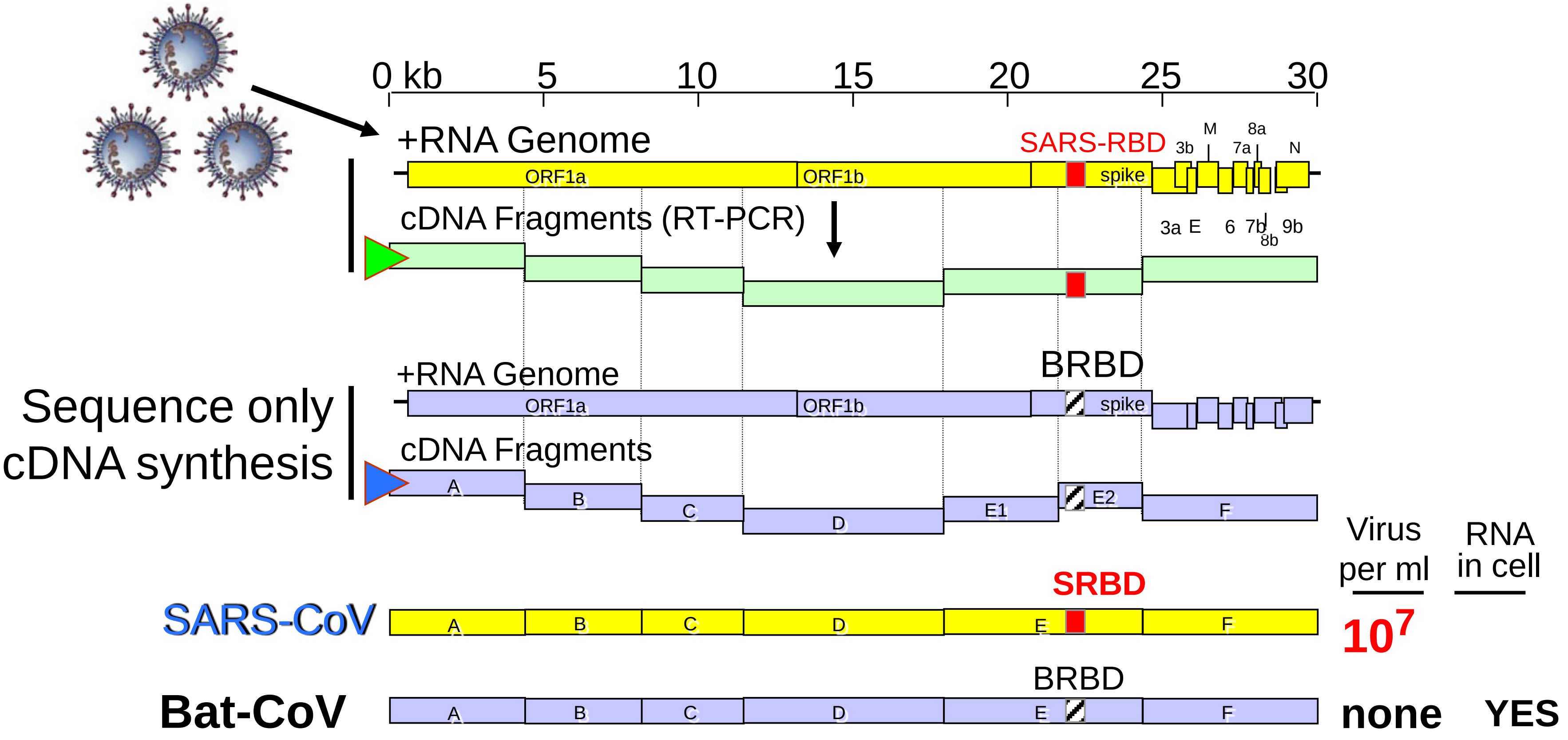
Sequence only
cDNA synthesis

SRBD - 🟥
SARS Receptor
Binding Domain

BRBD - ▨
Bat Receptor
Binding Domain

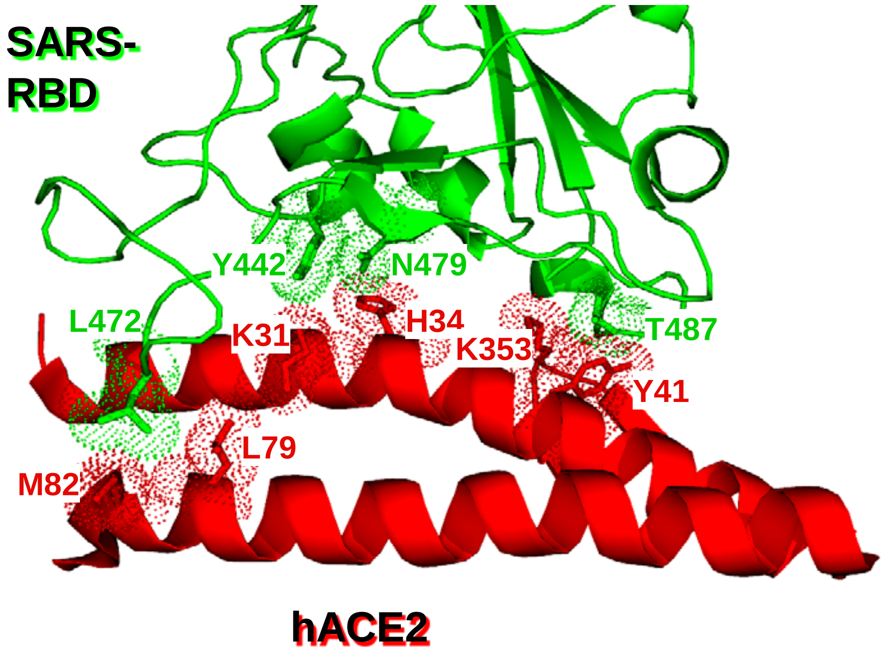
Becker et al. PNAS 2008 vol. 105: 19944-19949

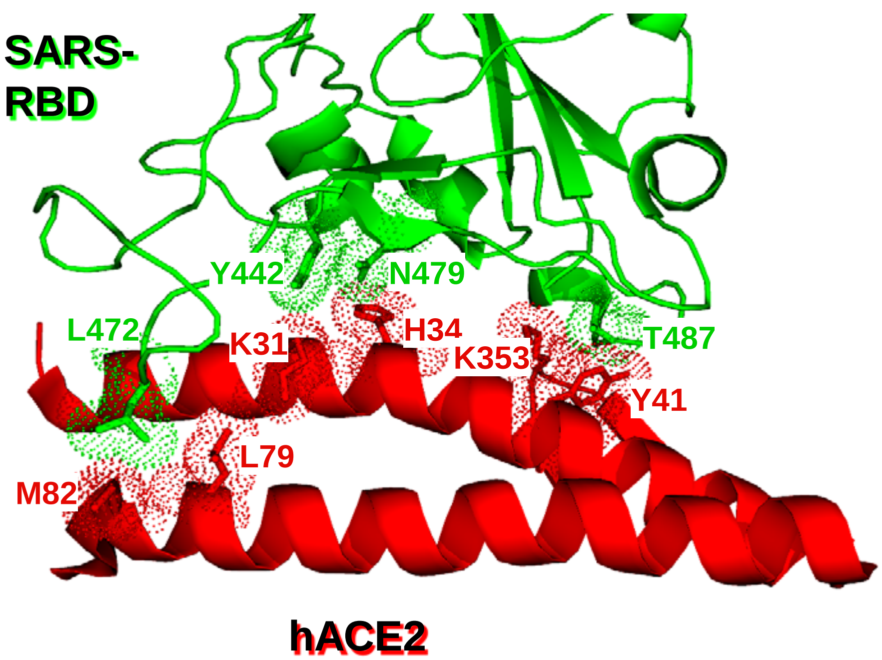
Synthesis of Bat-CoV genome

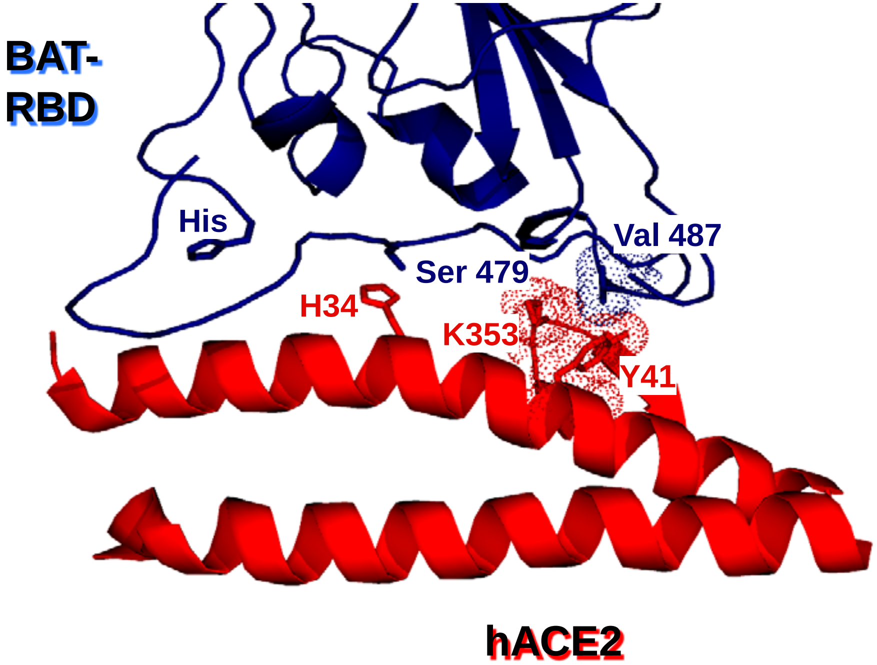


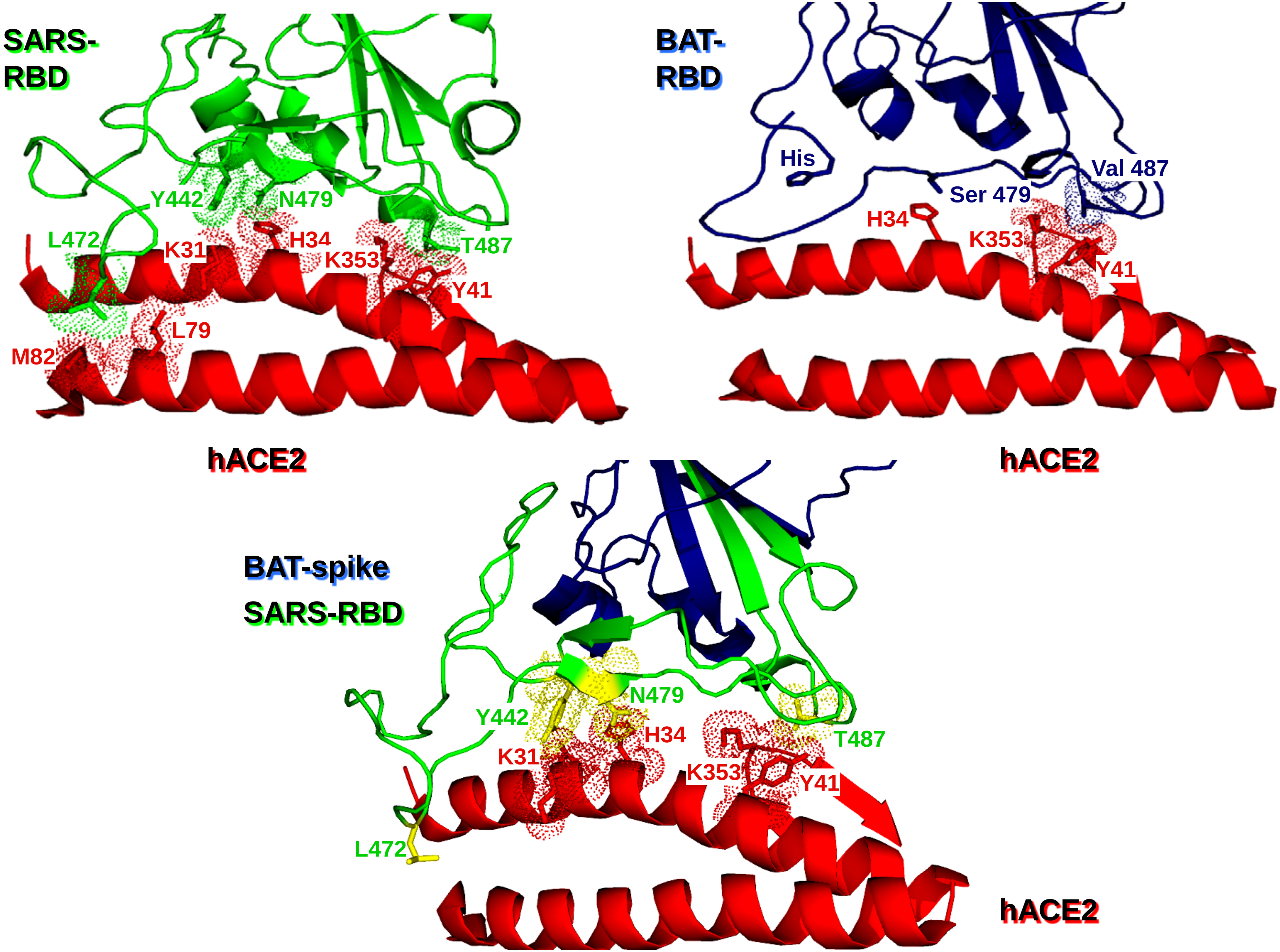
Synthetic Bat-CoV genome replicates but does not cause productive infection

Becker et al., PNAS 2008 vol. 105: 19944-19949

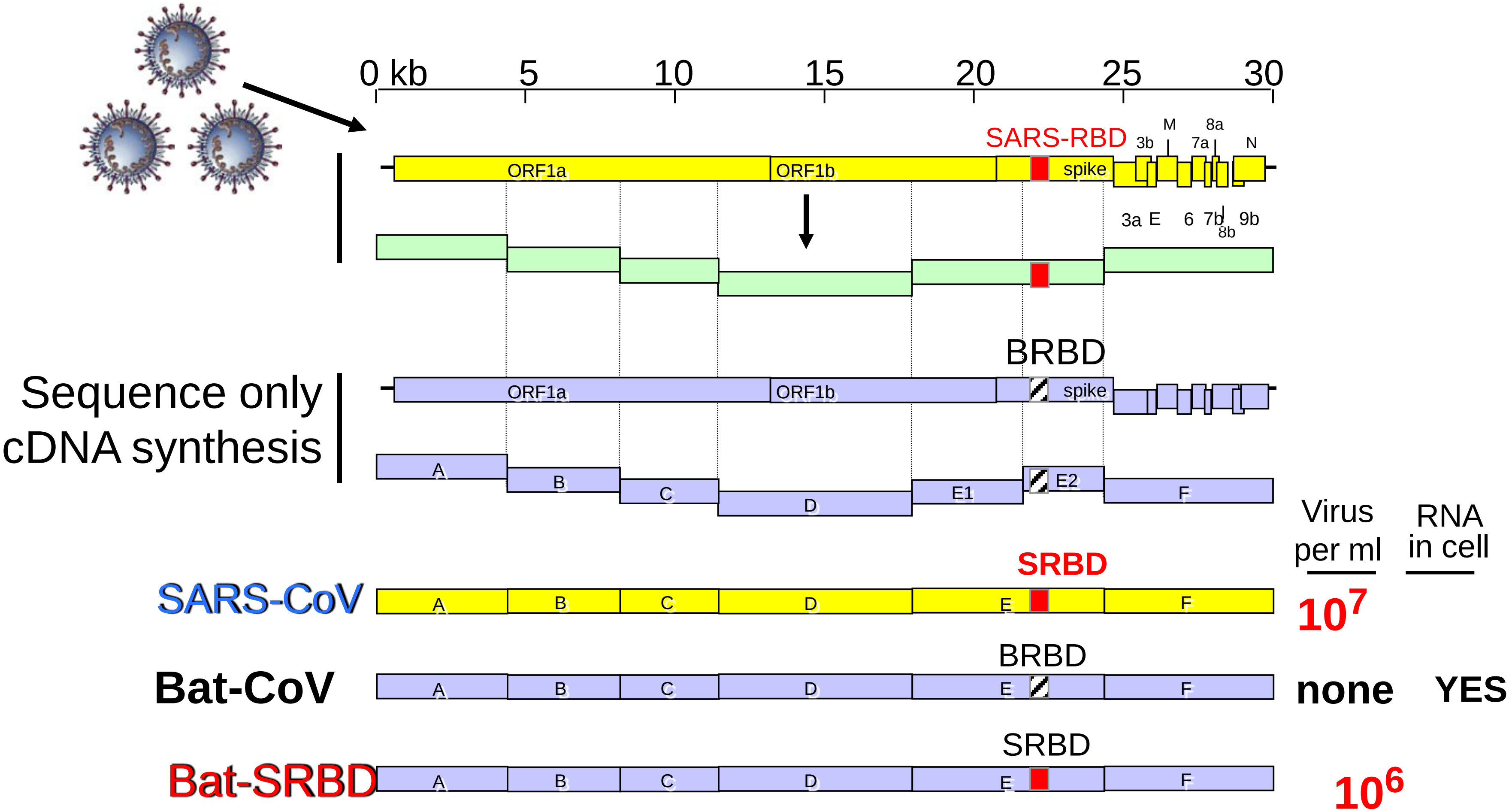

SARS-
RBD
Y442
N479
L472
K31
H34
K353
T487
Y41
L79
M82
hACE2


SARS-
RBD
Y442
N479
L472
K31
H34
K353
T487
Y41
L79
M82
hACE2


BAT-
RBD
His
Val 487
Ser 479
H34
K353
Y41
hACE2


SARS-RBD
Y442
N479
L472
K31
H34
K353
T487
Y41
L79
M82
hACE2
BAT-RBD
His
Val 487
Ser 479
H34
K353
Y41
hACE2
BAT-spike
SARS-RBD
N479
Y442
H34
T487
K31
K353
Y41
L472
hACE2

Synthesis of Bat-CoV genome

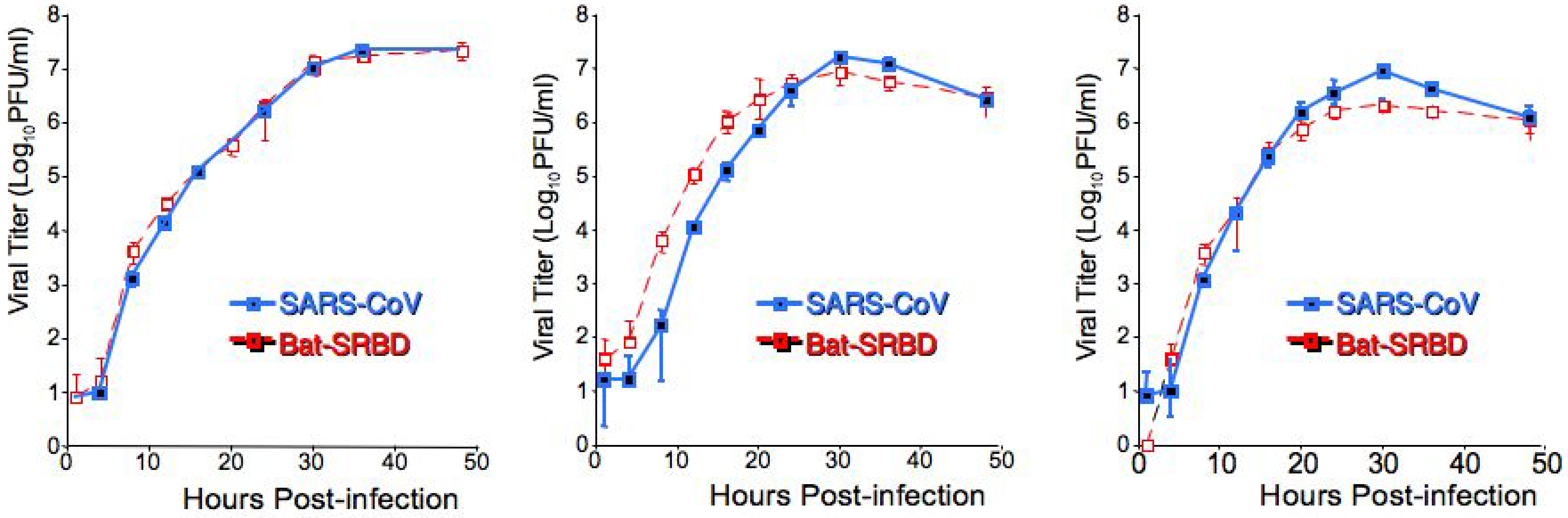


Sequence only
cDNA synthesis

	Virus per ml	RNA in cell
SARS-CoV	10^7	
Bat-CoV	**none**	**YES**
Bat-SRBD	10^6	

SARS-RBD is sufficient for Bat-CoV productive infection in Vero cells

ACE-2 is sufficient for infection of murine cells, but not mice.



Vero-E6
(primate)

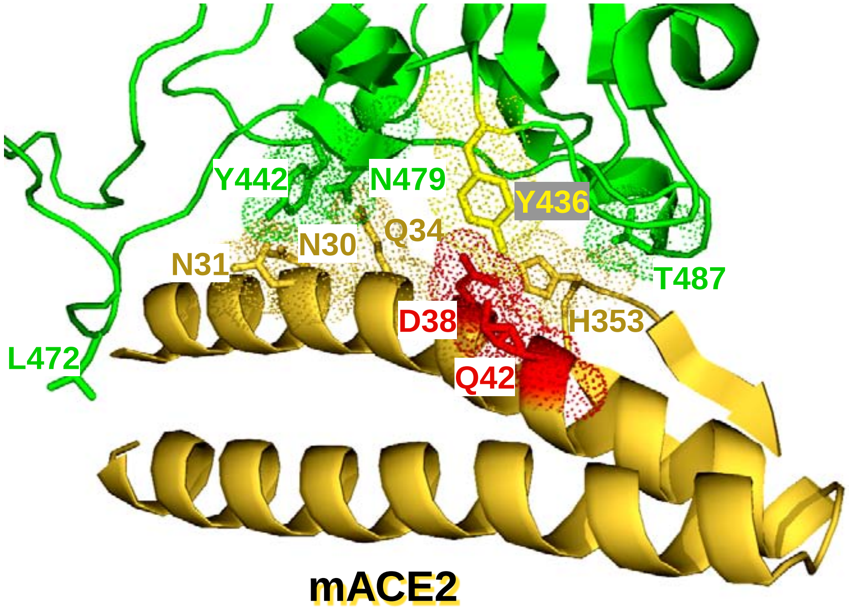
mouse DBT cells
+ civet ACE2

mouse DBT cells
+ human ACE2

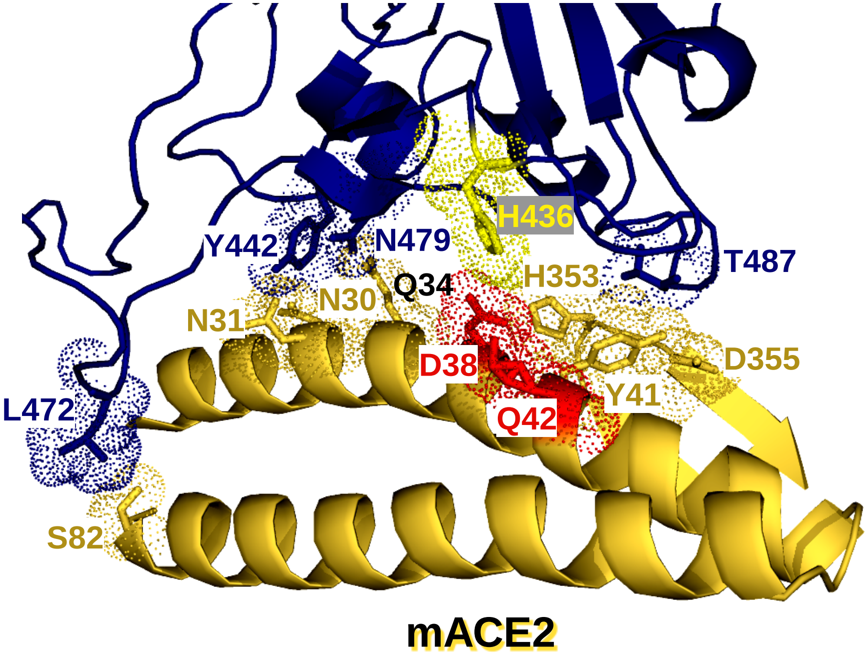
Becker et al., PNAS 2008

Mouse-adapted spike Y436H – predicts better binding to mACE2

SARS-RBD

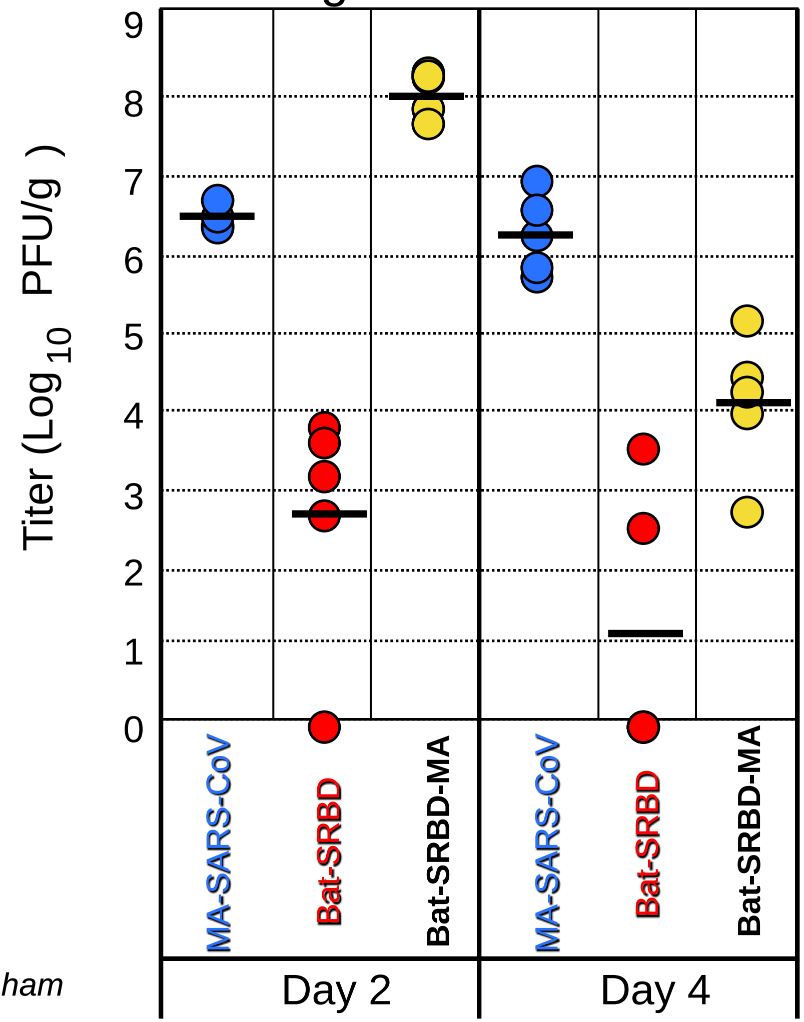


MA-SRBD (Y436H)

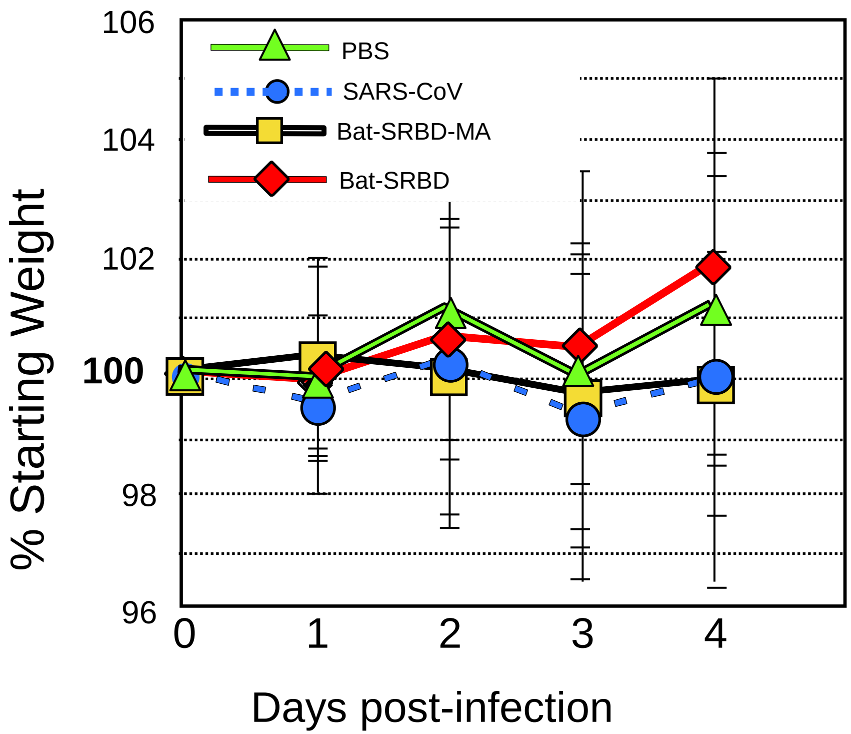


Bat-SRBD-MA replicates in aged BALB/c mouse lungs but does not cause Illness

Lung virus titers

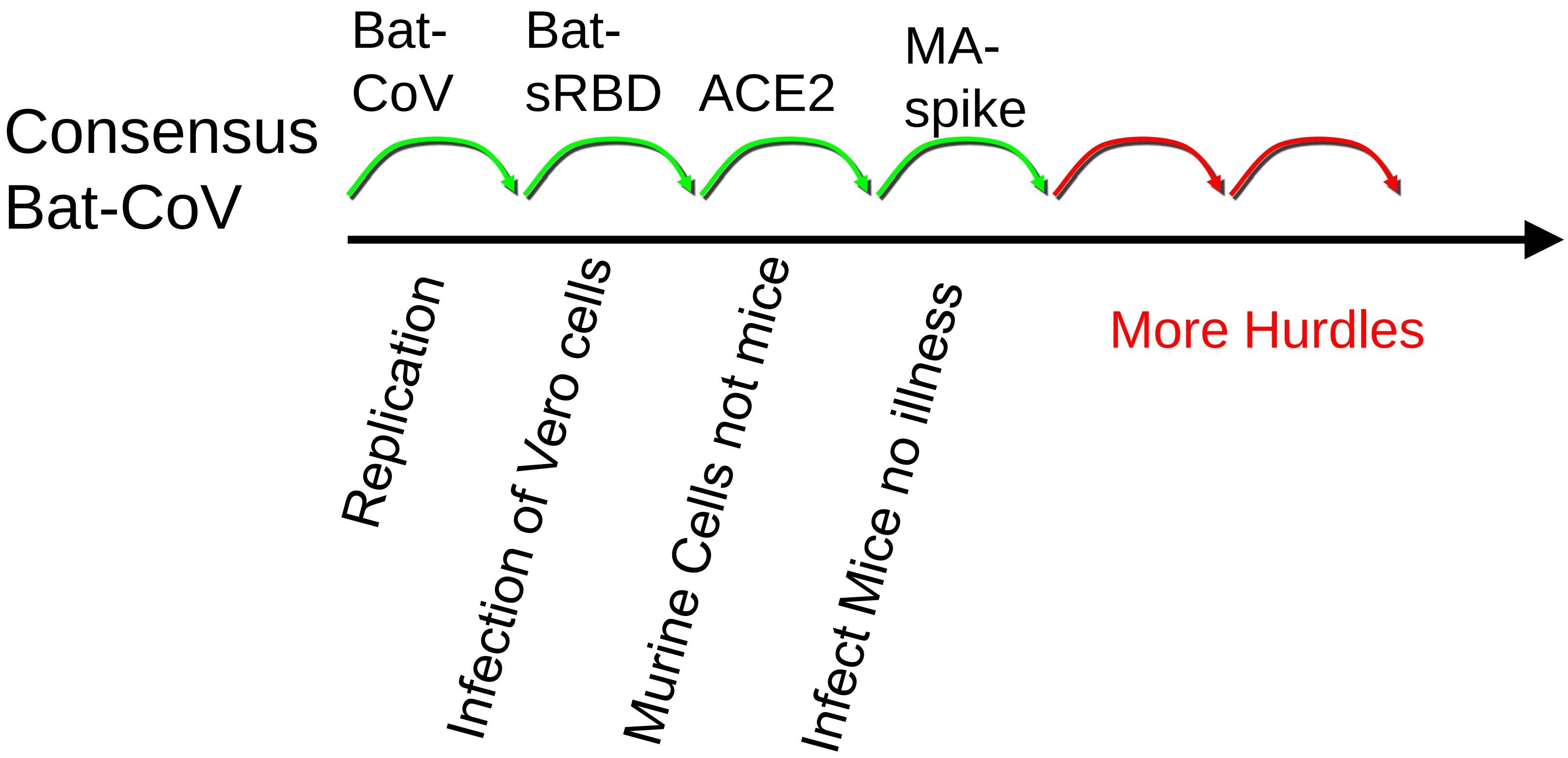


Weight loss



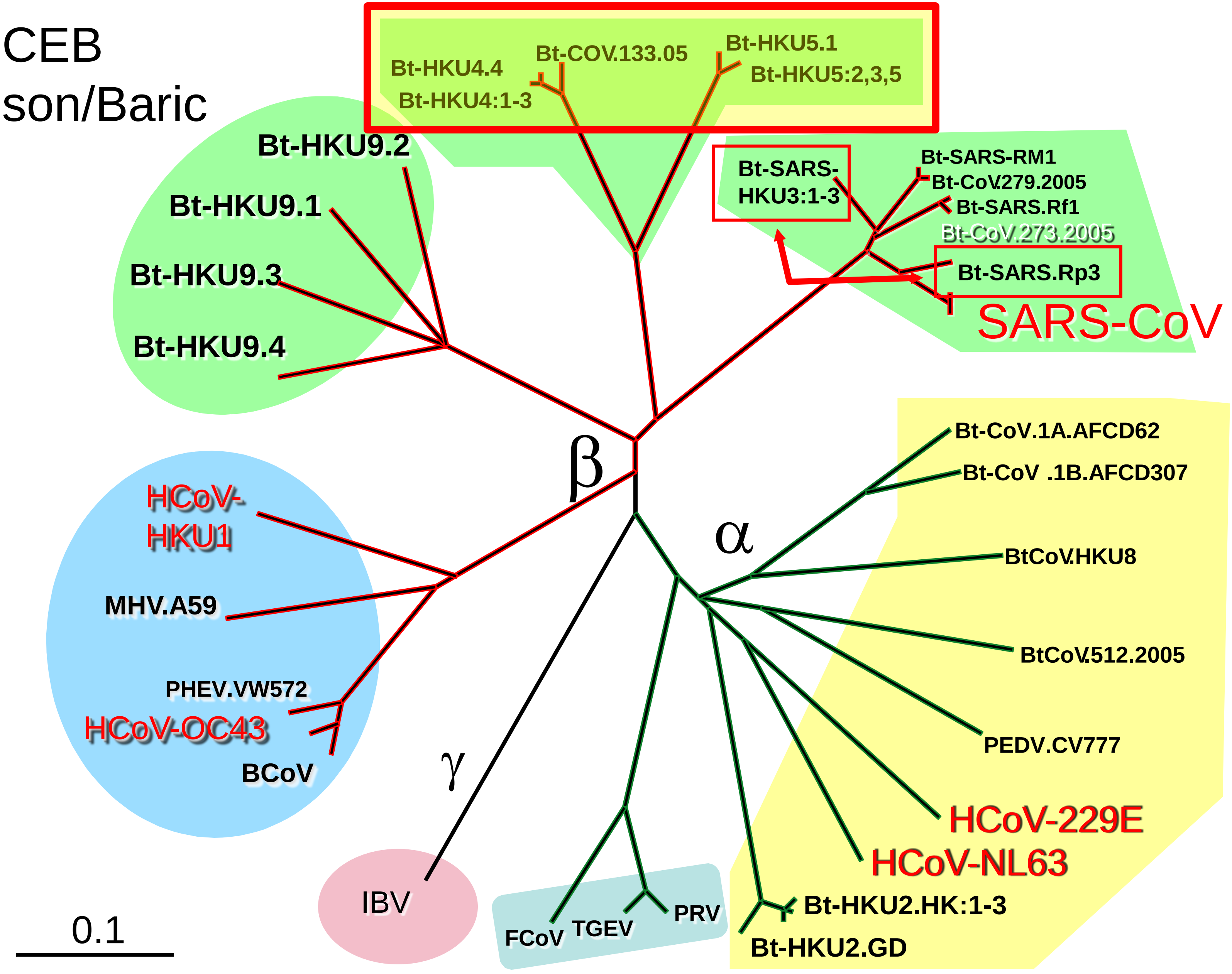
Graham

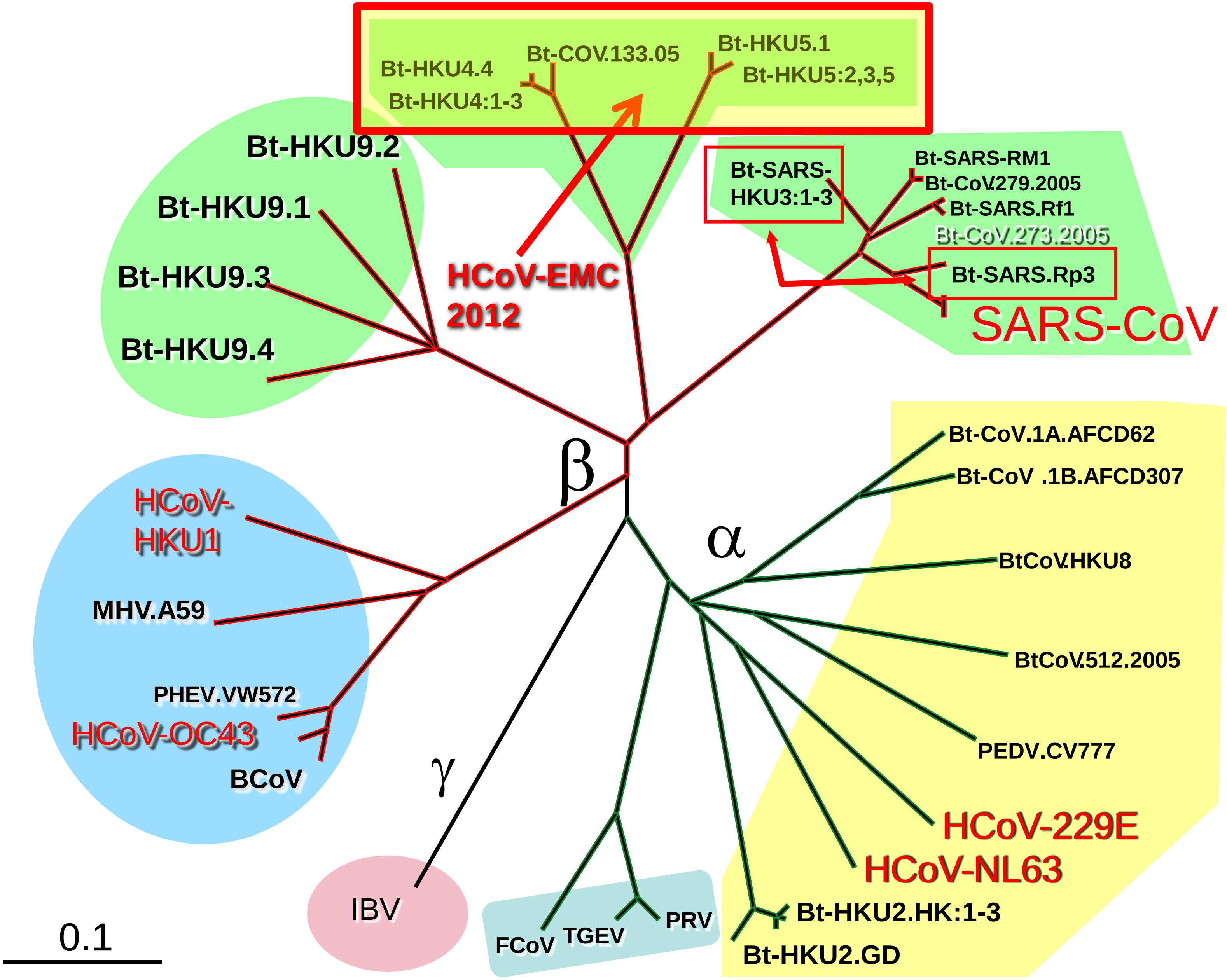
Studying the Trans-Species Movement of Bat Coronaviruses



Consensus Bat-CoV

SERCEB
Denison/Baric

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Bt-HKU9.2
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PRV
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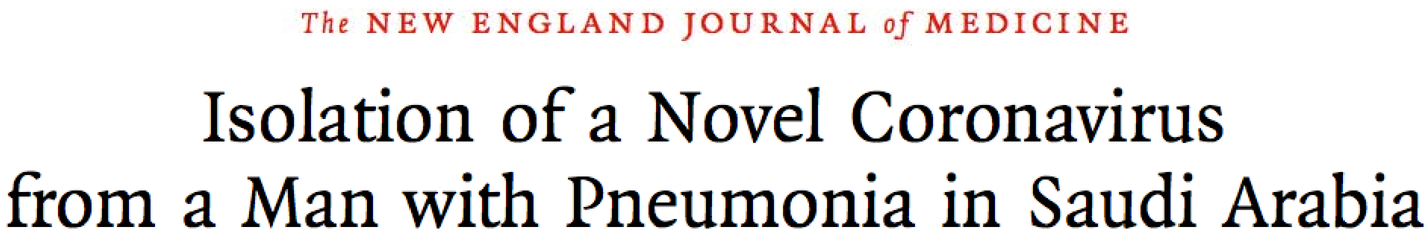


The NEW ENGLAND JOURNAL of MEDICINE

Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia

Ali Moh Zaki, Sander van Boheemen, Theo M. Bestebroer,
Albert D.M.E. Osterhaus, and Ron A.M. Fouchier

- A previously unknown coronavirus from the sputum of a 60-y/o man in Saudi Arabia
- Acute pneumonia and renal failure with a fatal outcome
- HCoV-EMC replicated in cell culture, with CPE and syncytia.
- Novel β coronavirus – closest relatives Bt-CoV HKU4 and HKU5.
- The clinical picture was remarkably similar to SARS in 2003

October 17, 2012, at NEJM.org.N Engl J Med 2012. DOI: 10.1056/NEJMoa1211721

SARS: Still Relevant After all These Years

Mark R. Denison M.D.
Vanderbilt University School of Medicine

Challenges Ahead

- SARS-CoV on Select Agent List
  - How will this impact discovery, collaborations, new investigators?
  - What is *real* cost to investigators?
  - How will we be able to respond to new human CoVs that are “not circulating in humans”? (like EMC-2012)

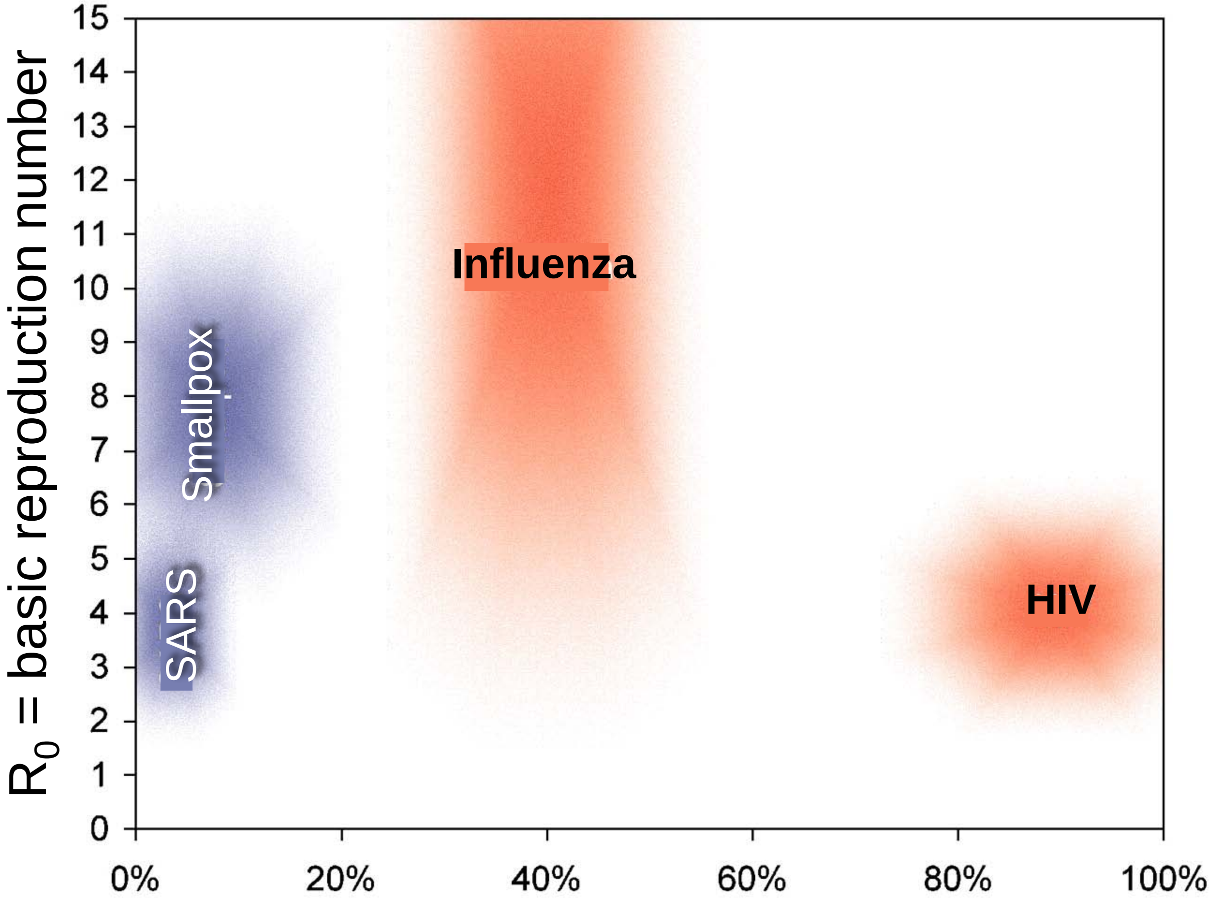
Advances at risk?

- Why did public health interventions succeed?

Why did SARS-CoV allow itself to be controlled?

- Coordinated public health measures – Why did they work?
- Why don’t they work with Influenza? With HIV?

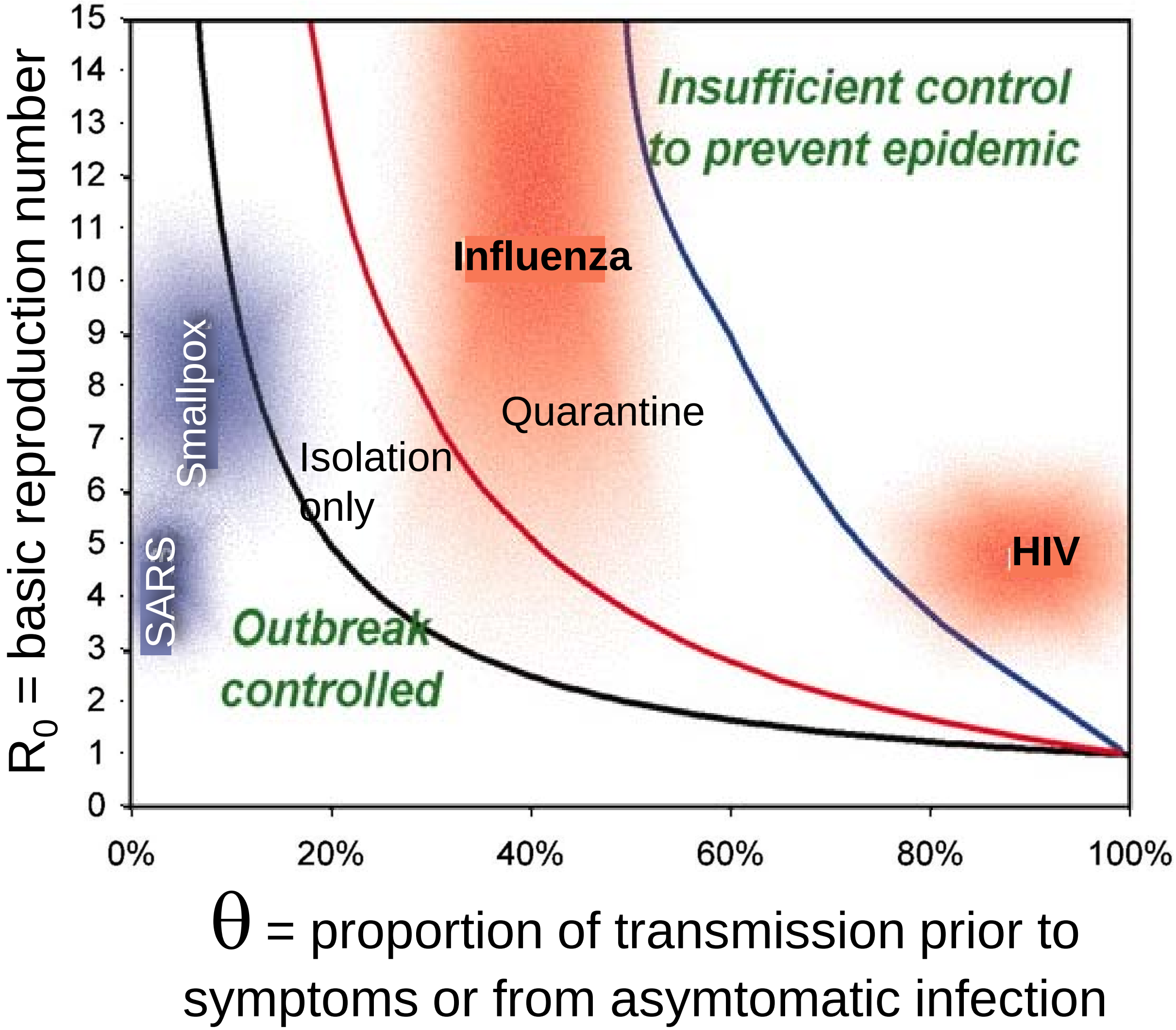
Principles of Epidemic Control



θ = proportion of transmission prior to symptoms or from asymtomatic infection

Fraser C et al. PNAS 2004;101:6146-6151

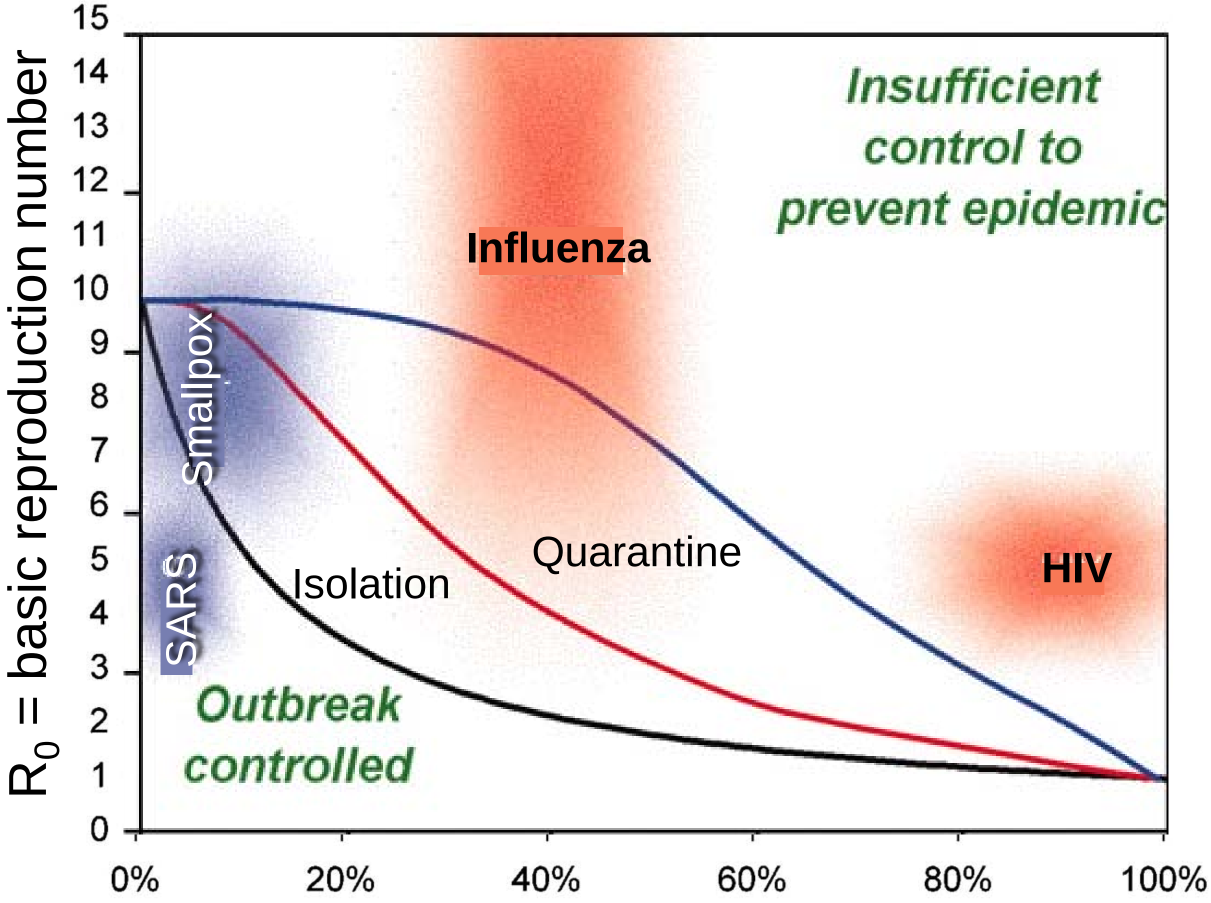
Isolation of 100% of symptomatic individuals



θ = proportion of transmission prior to symptoms or from asymtomatic infection

Fraser C et al. PNAS 2004;101:6146-6151

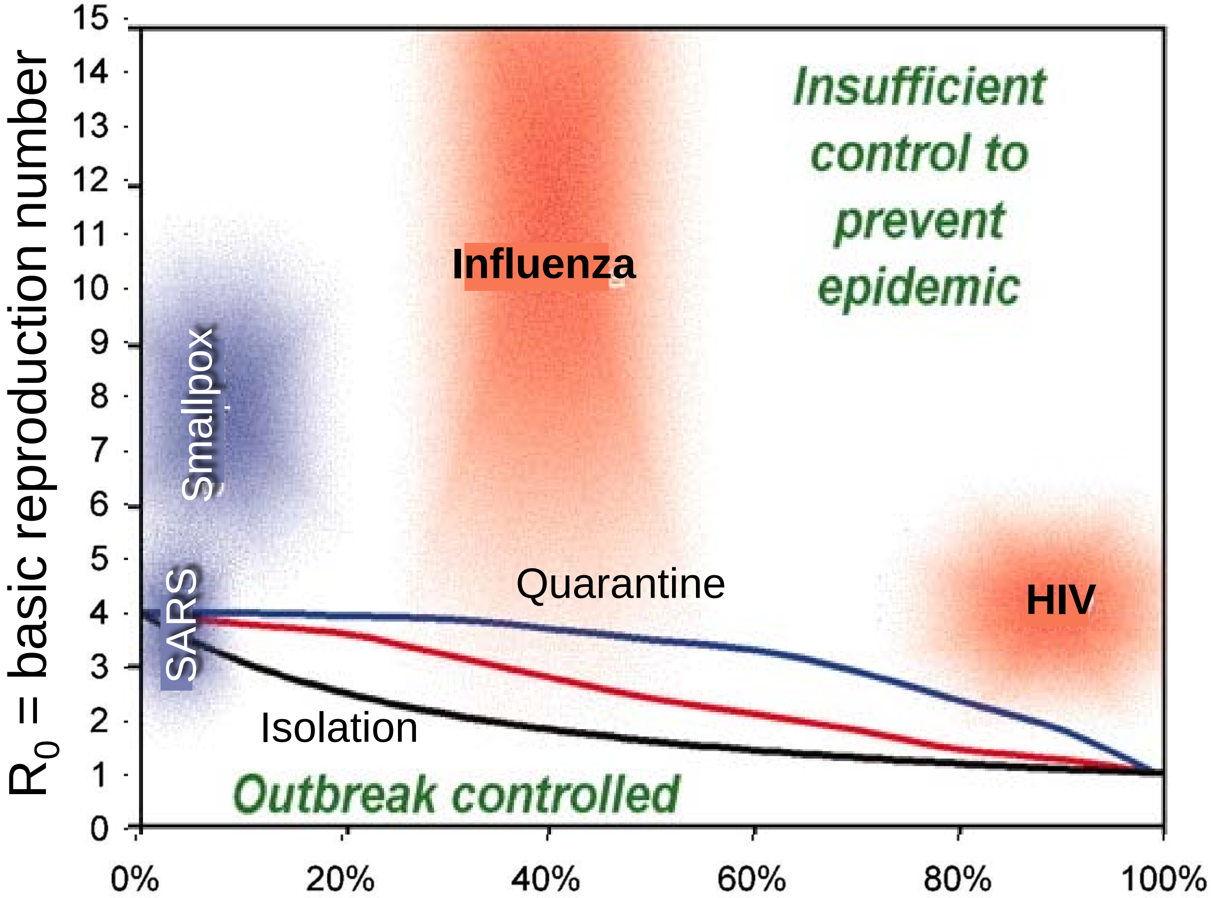
Isolation of 90% of symptomatic individuals



θ = proportion of transmission prior to symptoms or from asymtomatic infection

Fraser C et al. PNAS 2004;101:6146-6151

Isolation of 75% of symptomatic individuals



θ = proportion of transmission prior to symptoms or from asymtomatic infection

Fraser C et al. PNAS 2004;101:6146-6151

Why did SARS-CoV allow itself to be controlled by interventions?

- **Toronto 2003 –** biphasic epidemic: Epidemic - control – relaxed isolation – recurrent epidemic – control and elimination
- **China 2004** - lab-associated infections –

"WHO commends the Chinese authorities for taking swift action to contain the latest outbreak once it was recognized and reported, by way of extensive contact tracing and the quarantine and medical observation of such individuals. Once again, it has been demonstrated that SARS is a containable disease." (WHO health alert: http://www.who.int/csr/don/2004_05_18a/en/index.html)

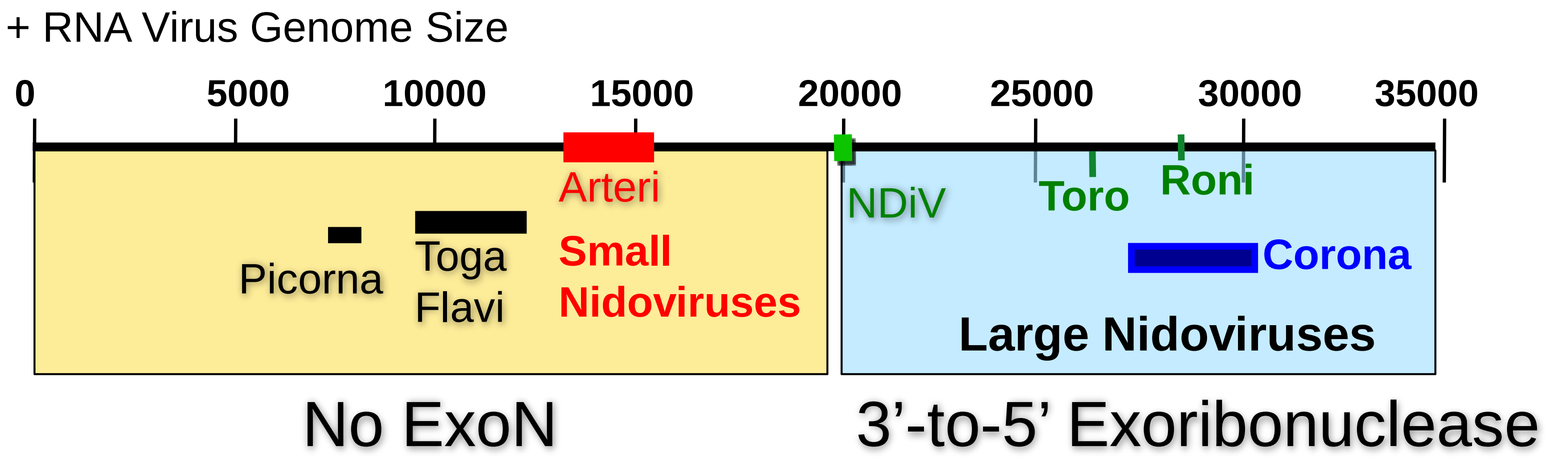
Why did SARS-CoV allow itself to be controlled by interventions?

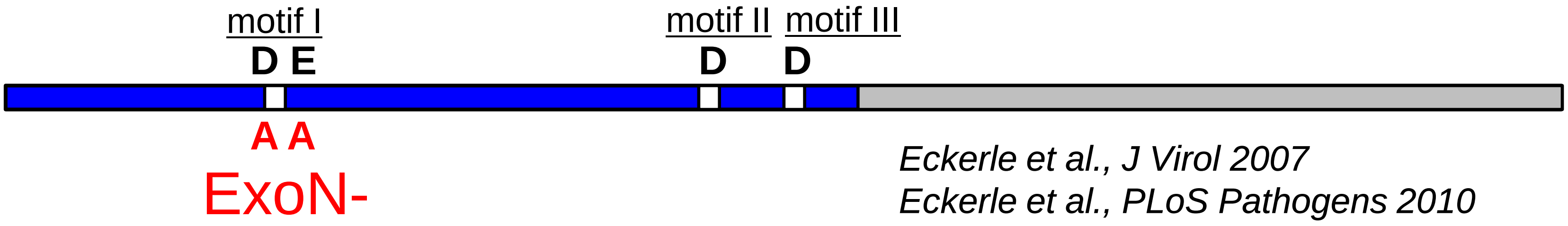
- Coordinated Public Health Measures – why did they work – who gets credit?
- Why don’t they work with Influenza? With HIV?
- SARS-Achilles Heel – low R_0 low θ – controllable by isolation only
- SARS may be uniquely sensitive to public health interventions – Other CoVs?
-

Advances at risk?

- *Busting Myths: Increased mutation rate is dangerous and leads to more virulent virus*

CoVs Encode a 3’-to-5’ Exo-ribo-nuclease

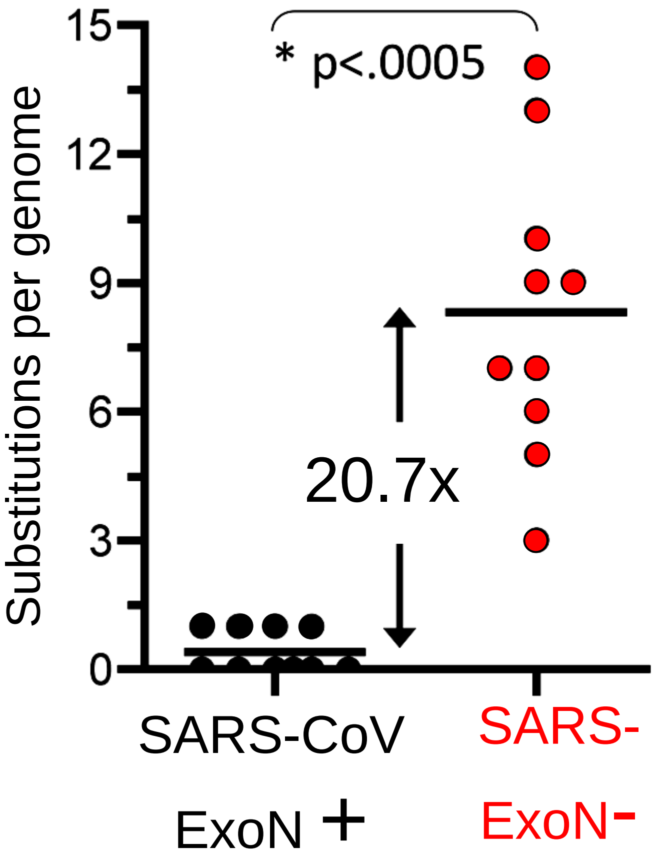




Eckerle et al., J Virol 2007
Eckerle et al., PLoS Pathogens 2010

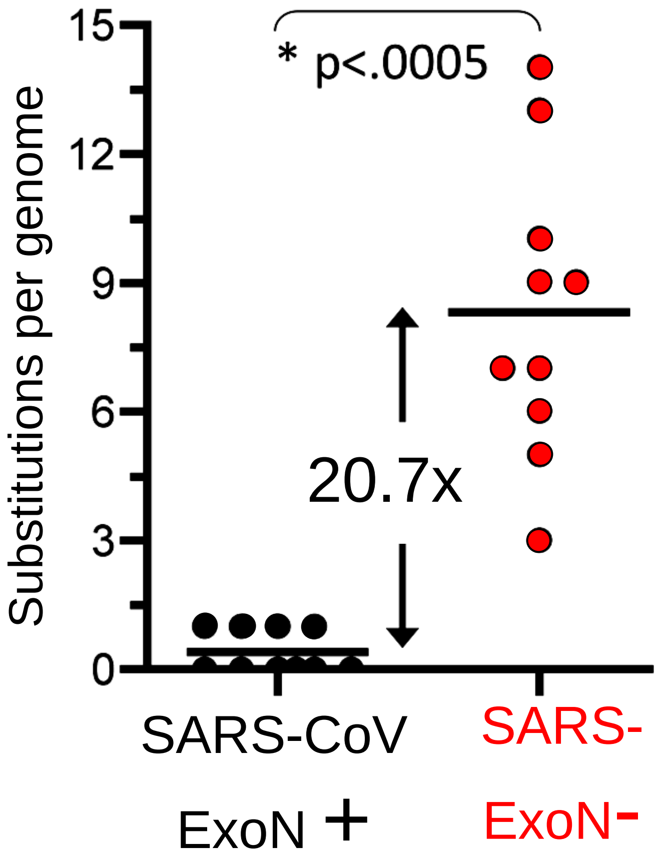
What is the effect of ExoN inactivation on replication fidelity, replication and pathogenesis?

ExoN- mutants have 20-fold increase in mutation frequency *(mutator phenotype)*



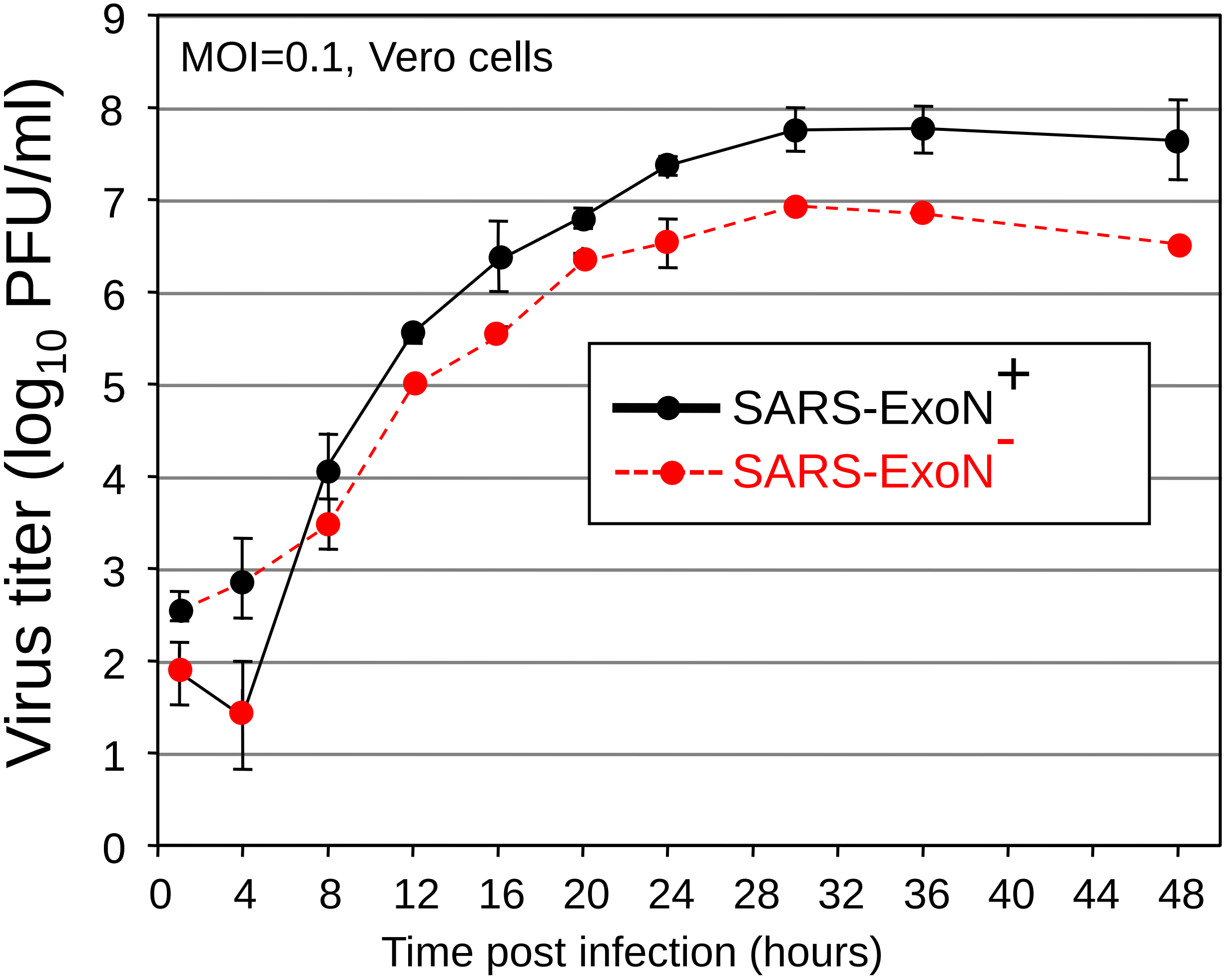
Eckerle et al., J Virol 2007
Eckerle et al., PLoS Pathogens 2010
Michelle Becker

Wildtype CoVs have a 20-fold lower mutation rate than other RNA viruses!



Eckerle et al., J Virol 2007
Eckerle et al., PLoS Pathogens 2010
Michelle Becker

SARS-CoV (ExoN$^+$) and SARS-ExoN$^-$ mutants have similar replication



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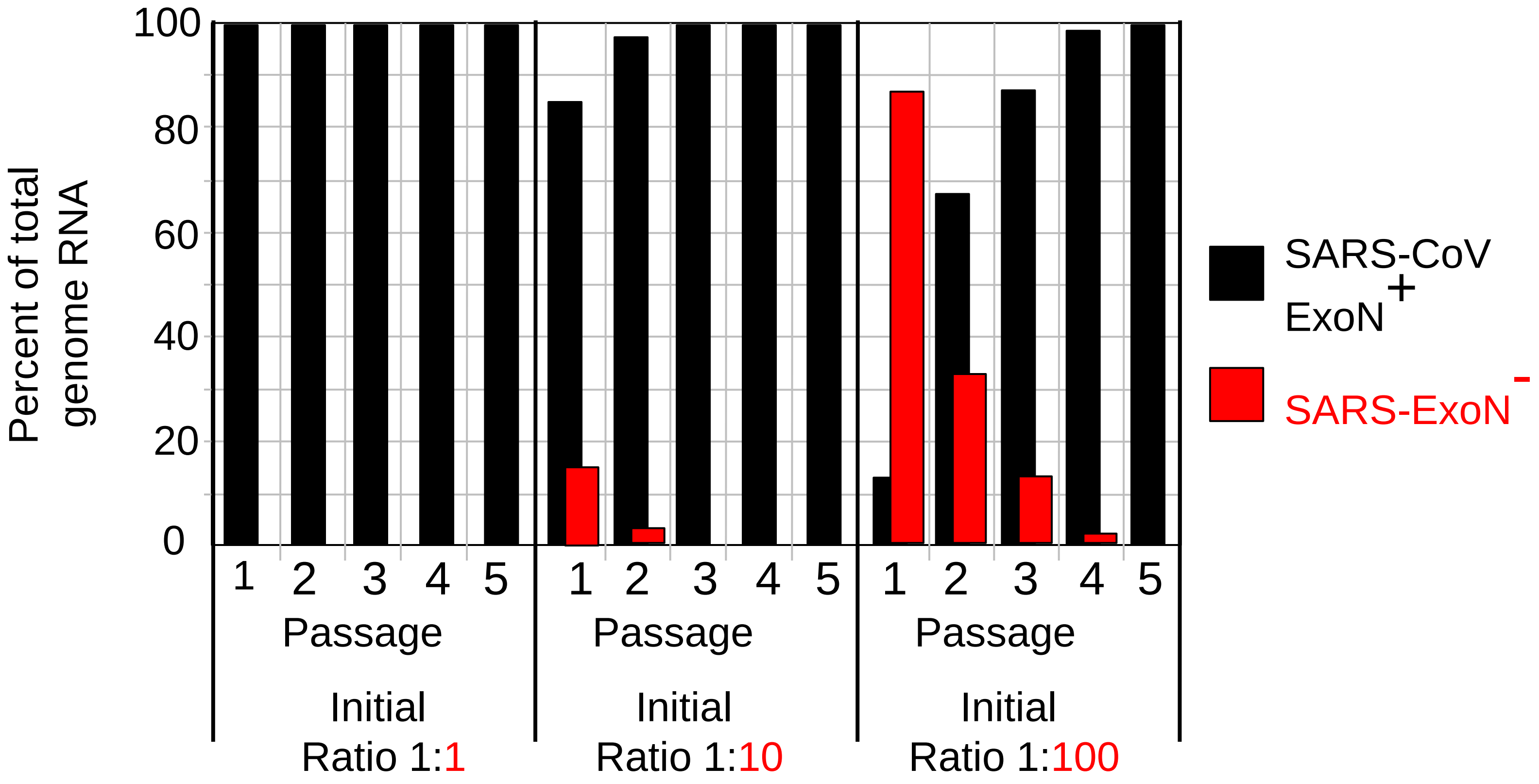
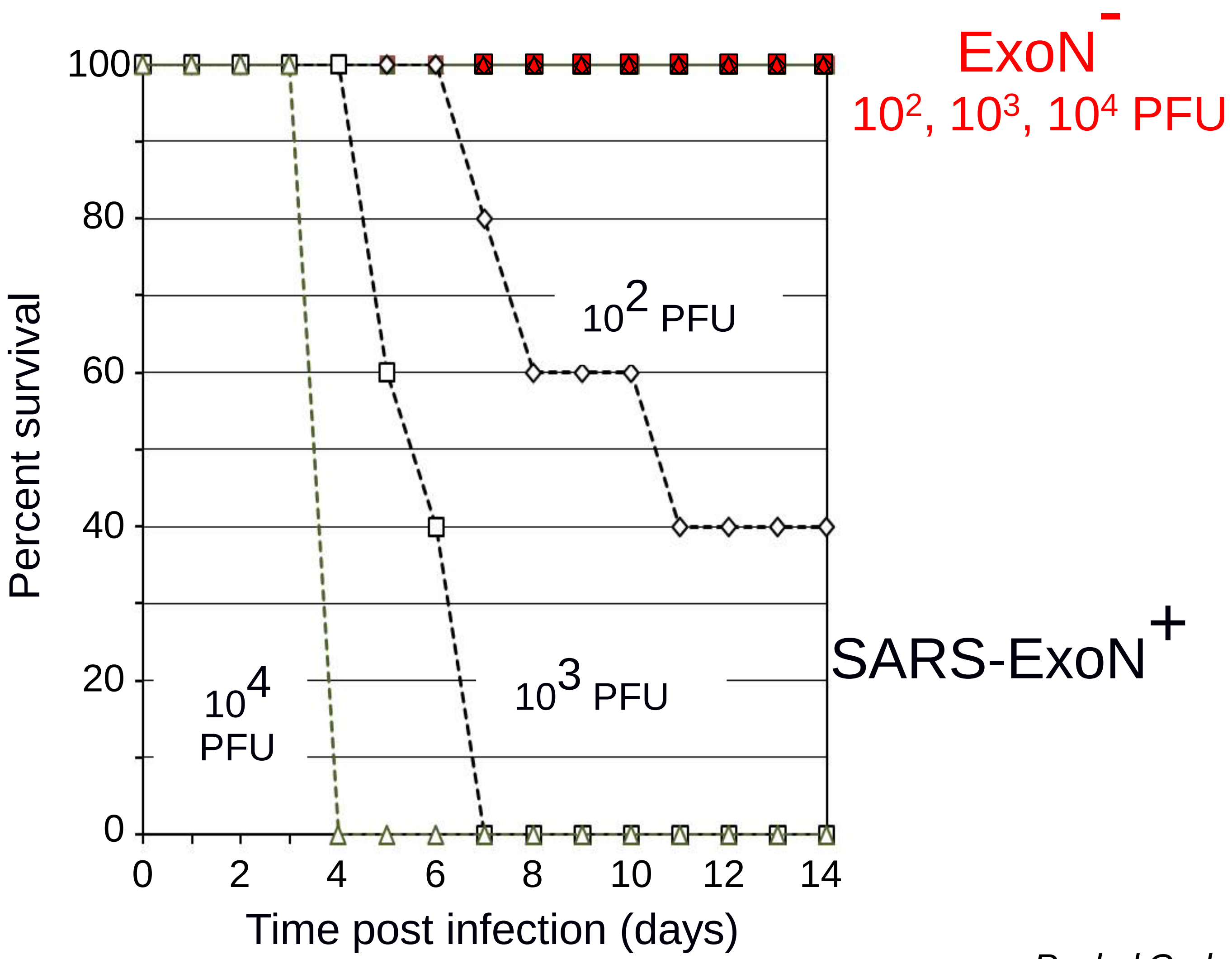
SARS-ExoN$^-$ is less fit than SARS-ExoN$^+$



Rachel Graham

SARS ExoN$^-$ is attenuated in an aged BALB/c mouse model of lethal SARS



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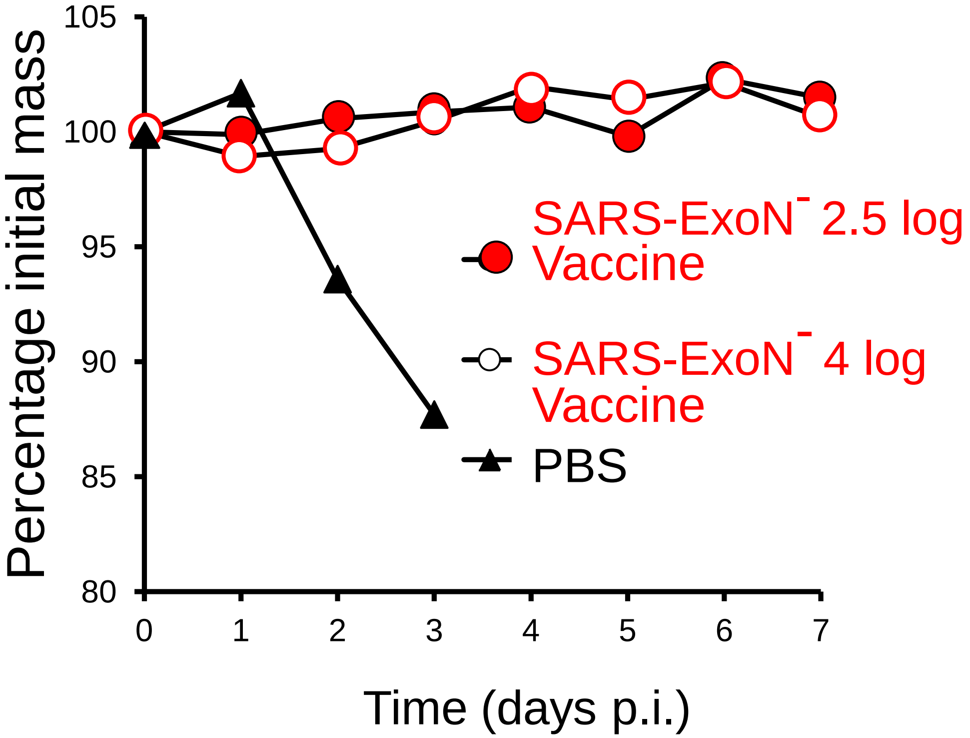
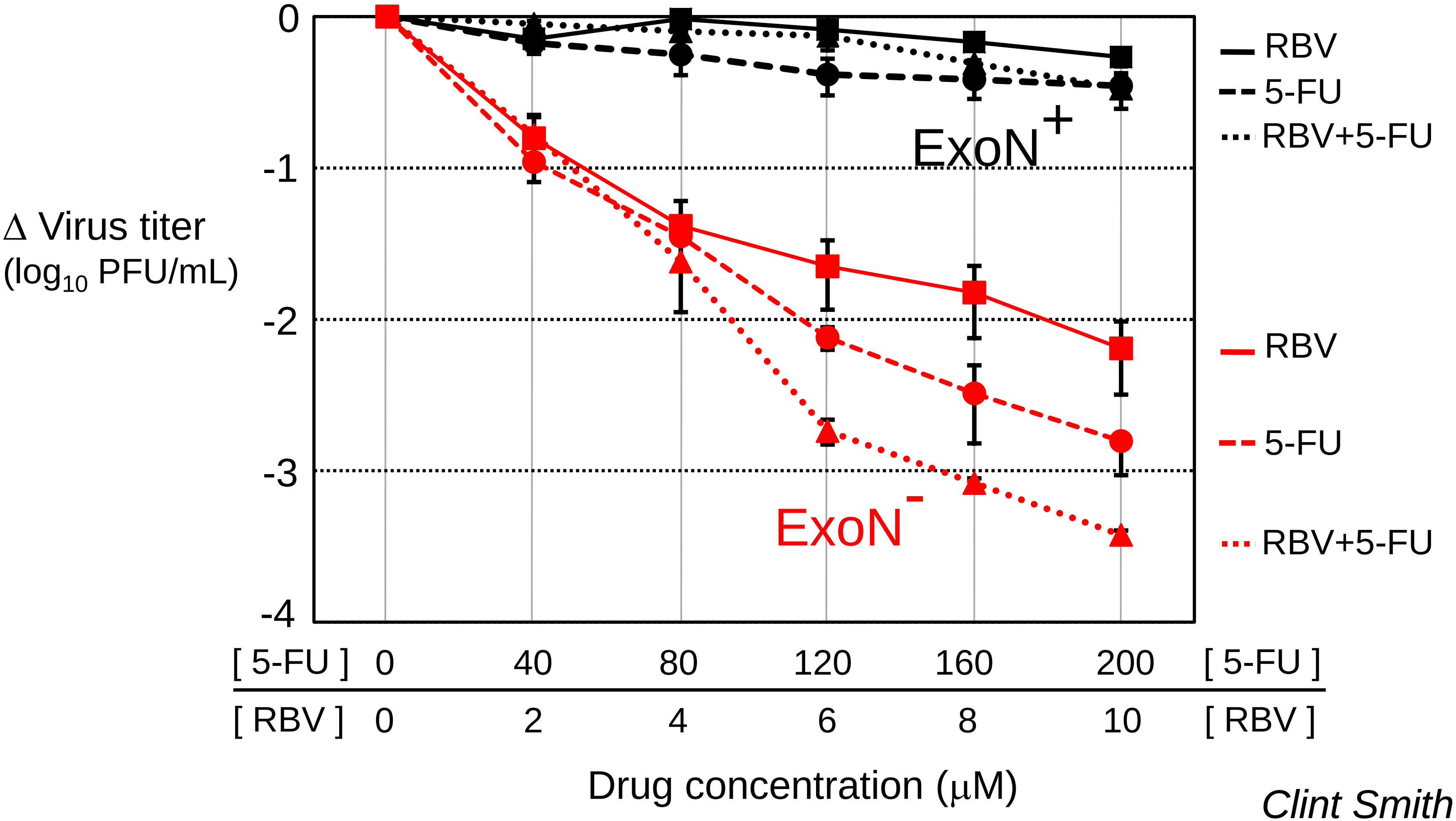
ExoN- mutant protects mice from lethal SARS-CoV challenge



Rachel Graham

ExoN^{-} mutant is sensitive to inhibition by Ribavirin and nucleoside analog RNA mutagens



Clint Smith

Summary

- The ExoN^- mutant genotype and mutator phenotype is stable in vitro and in animal infection.
- ExoN- mutants are attenuated – and protect from lethal SARS-CoV challenge.
- ExoN- mutants have not reverted to virulence.
- ExoN- mutants are profoundly sensitive to RNA mutagens such as Ribavirin

State of the Ideas –before

- RNA viruses do not proofread
- Increased mutation rate = increased virulence and transmission
- Increased mutation rate enhances fitness
- Mutator phenotype decreases safety of working with pathogen

State of the Ideas –before

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Myth

BUSTED

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New State of the Ideas

- Proofreading in SARS-CoV and other CoVs
- Increasing mutation rate impairs virus replication, attenuates, blocks virus ability to restore virulence, and protects.
- Potential attenuation of any known or emerging coronavirus by the same exact mutations.
- Increased safety of ExoN^- attenuated vaccines-sensitivity to RNA mutagens.

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