EVOLUCIÓN Y PATOLOGÍA DE LOS CORONAVIRUS HUMANOS: DESASRROLLO DE VACUNAS Y ANTIVIRALES

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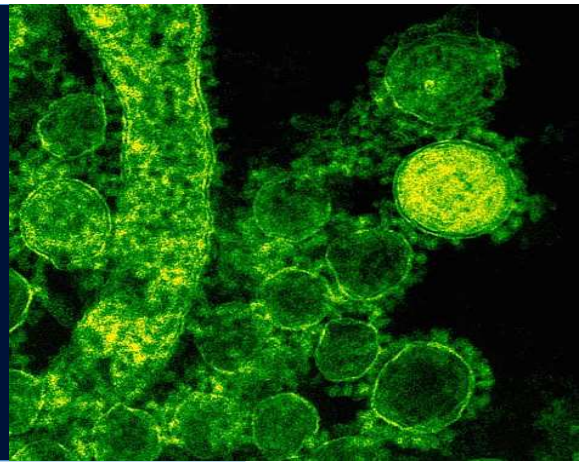
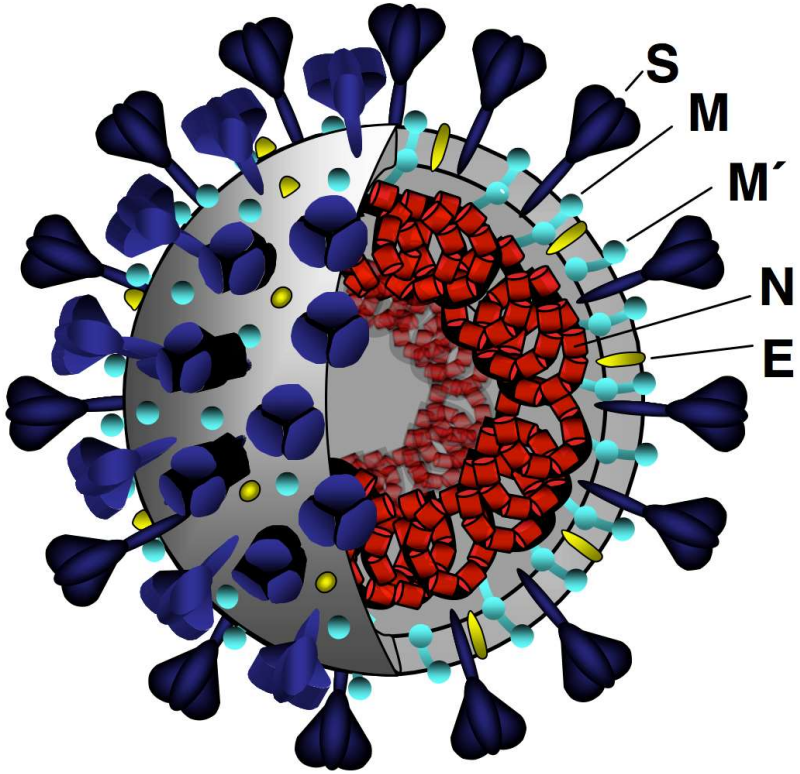
MAYO 12, 2022

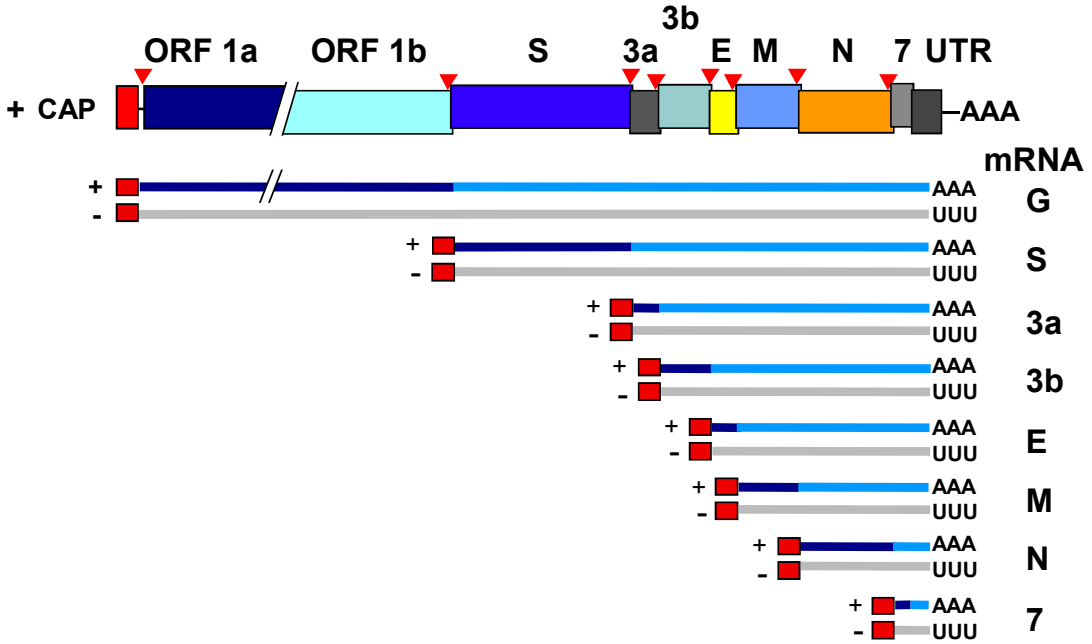
VIRUSES ARE GREAT GENERATORS OF GENETIC VARIABILITY



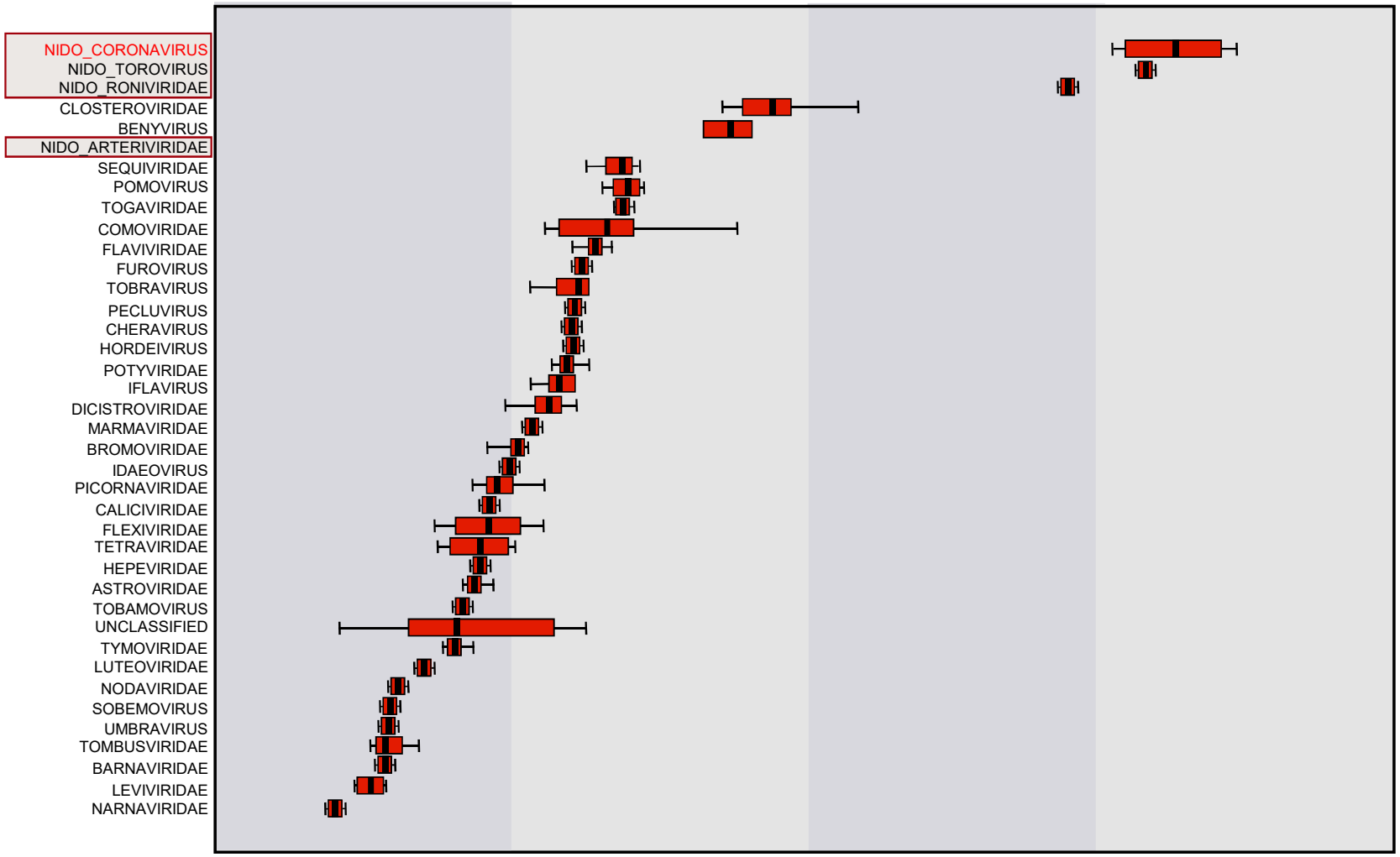
- BY SHUTTLING GENES INTO AND OUT OF THEIR HOST, VIRUSES SEEM TO BE A MAJOR DRIVING FORCE IN THE EVOLUTION OF HIGHER ORGANISMS
- IN HUMANS, VIRUSES ARE CONTINUALLY REINVENTING THEMSELVES THANKS TO A HIGHLY CREATIVE PROCESS NEVER SEEN BEFORE IN NATURE

CORONAVIRUS STRUCTURE AND GENE EXPRESSION

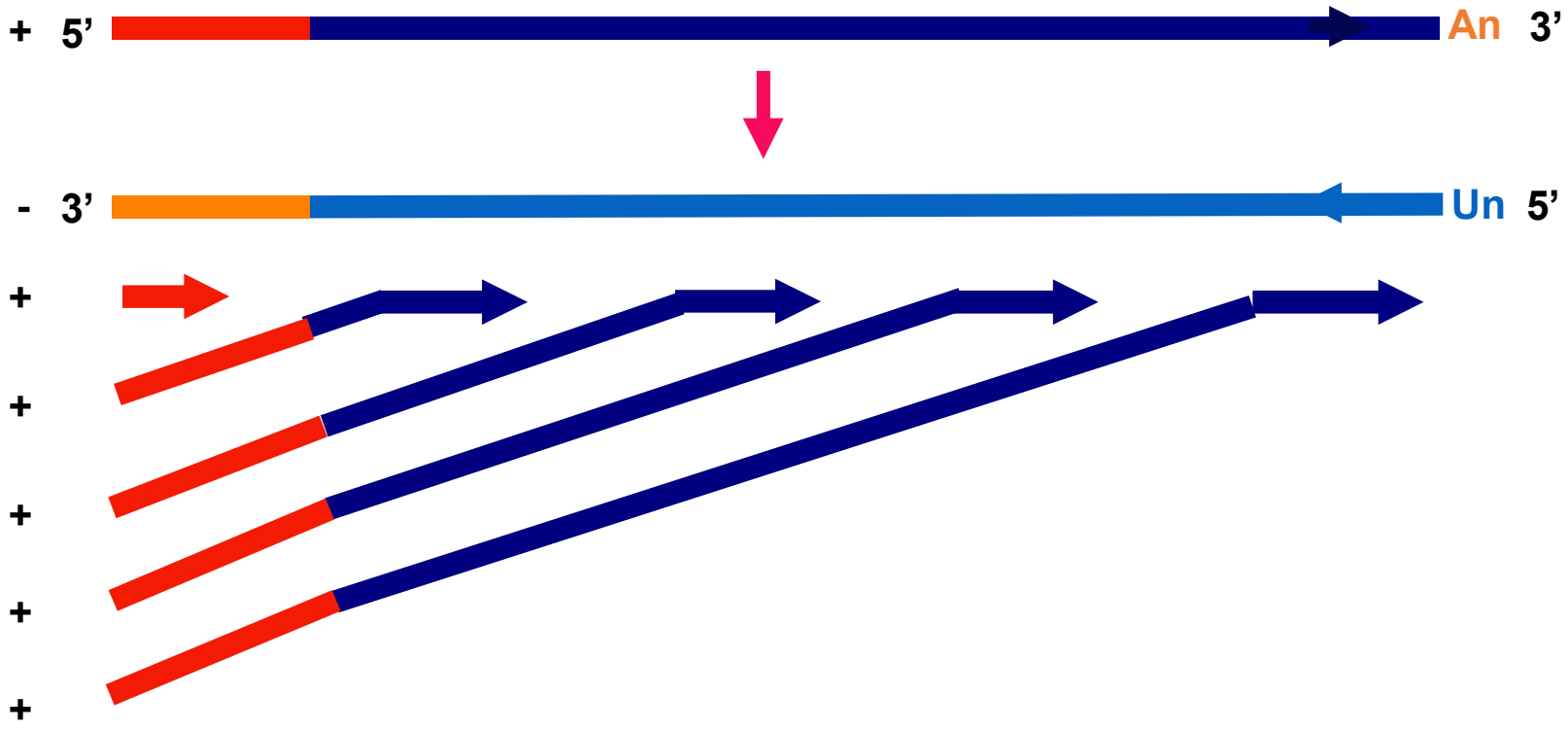




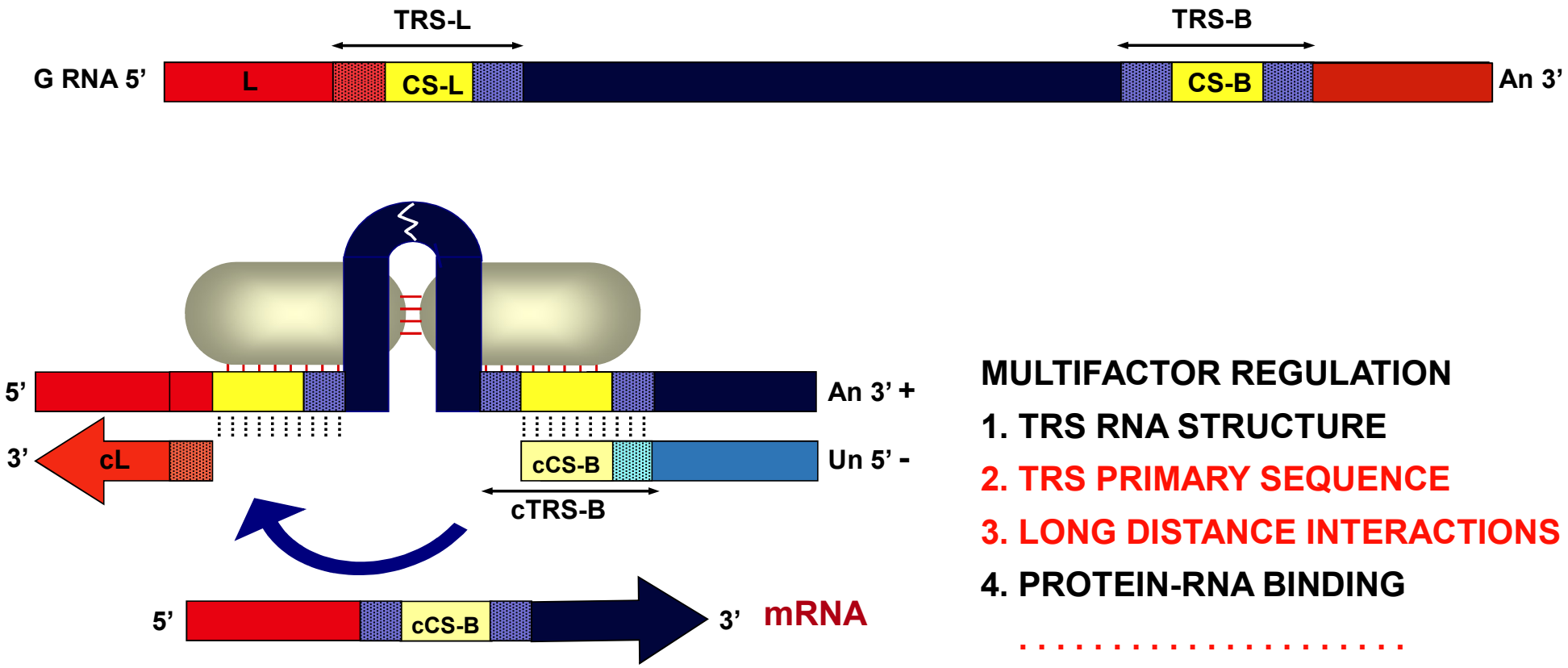
RNA VIRUS GENOME SIZE IN NUCLEOTIDES



CORONAVIRUS RNA REPLICATION



REGULATION OF sgmRNA LEVELS



MULTIFACTOR REGULATION

1. **TRS RNA STRUCTURE**
2. **TRS PRIMARY SEQUENCE**
3. **LONG DISTANCE INTERACTIONS**
4. **PROTEIN-RNA BINDING**

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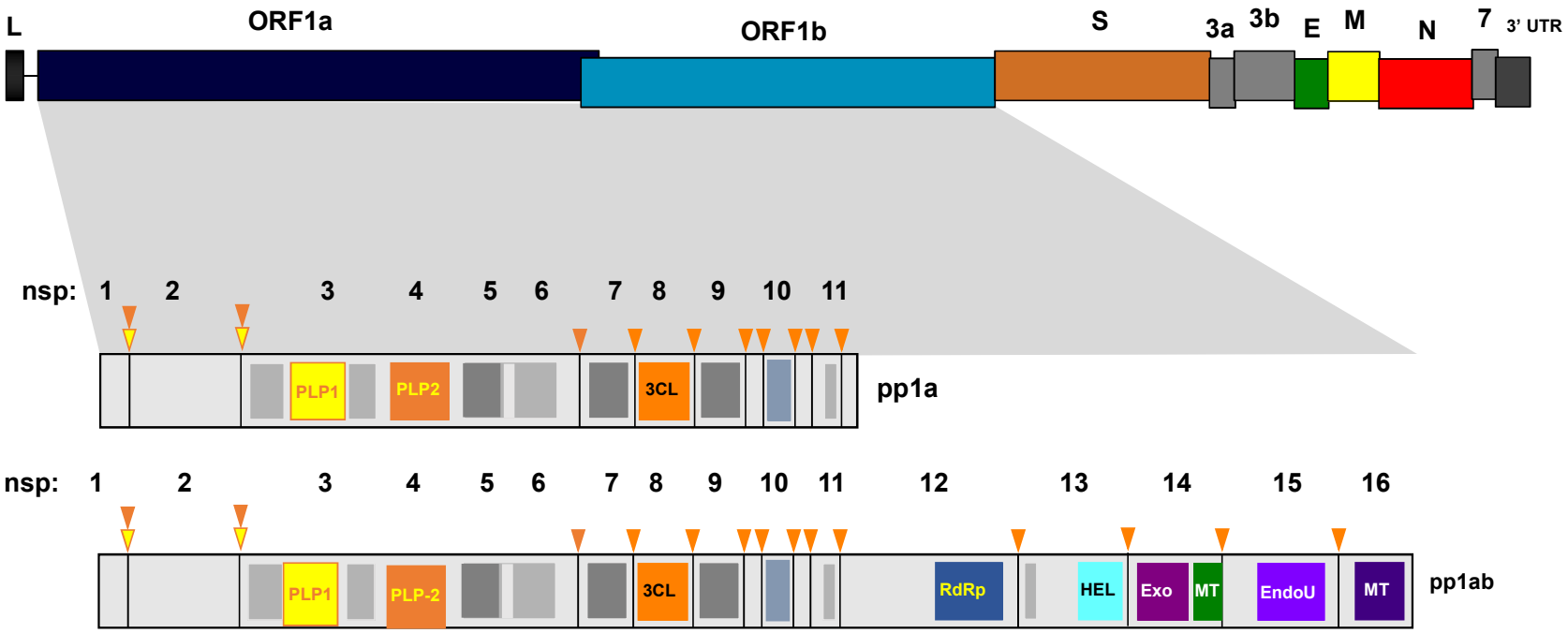
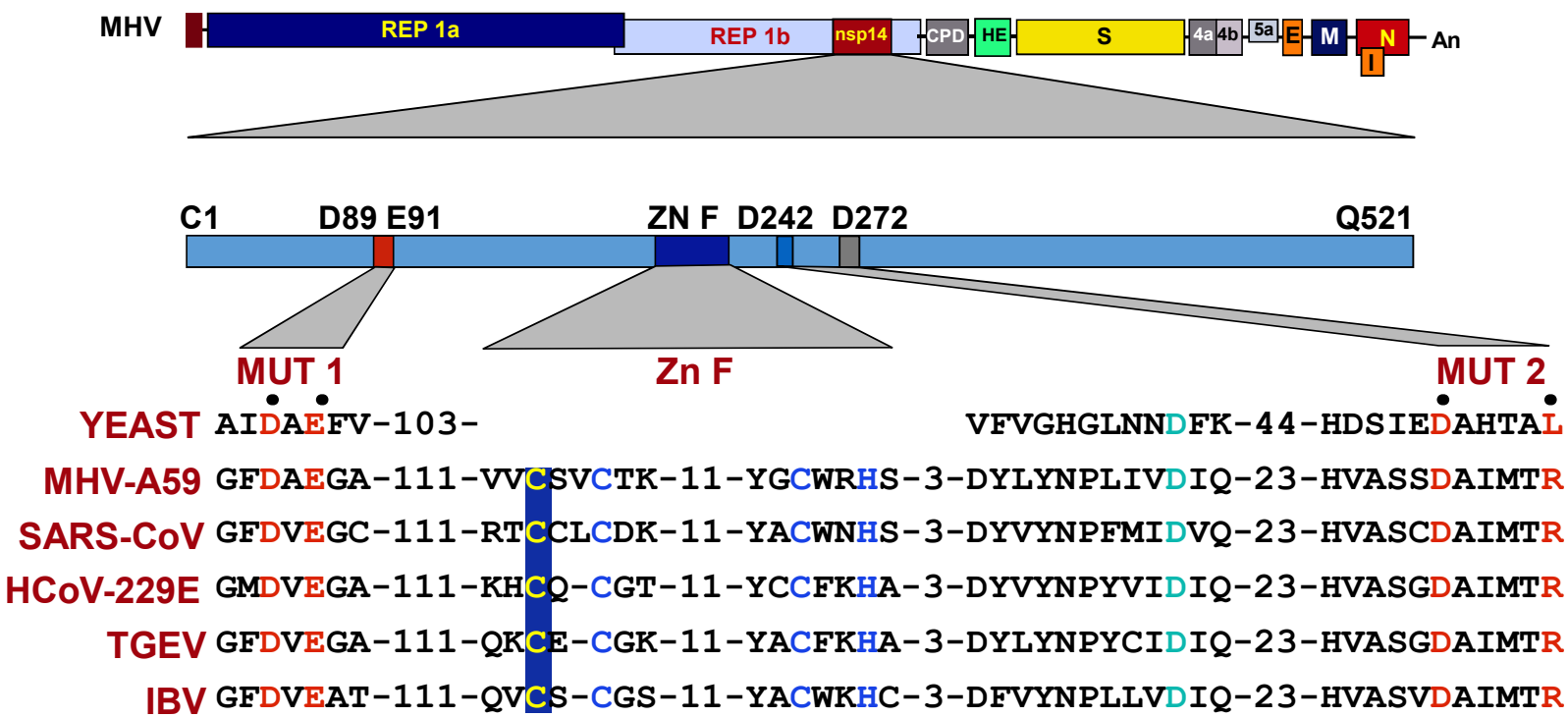
CORONAVIRUS STRUCTURE AND GENE EXPRESSION



TRANSCRIPTION MECHANISM

- TEMPLATE SWITCH
- A HIGH FREQUENCY RECOMBINATION

MUTATION OF CORONAVIRUS EXON MOTIFS



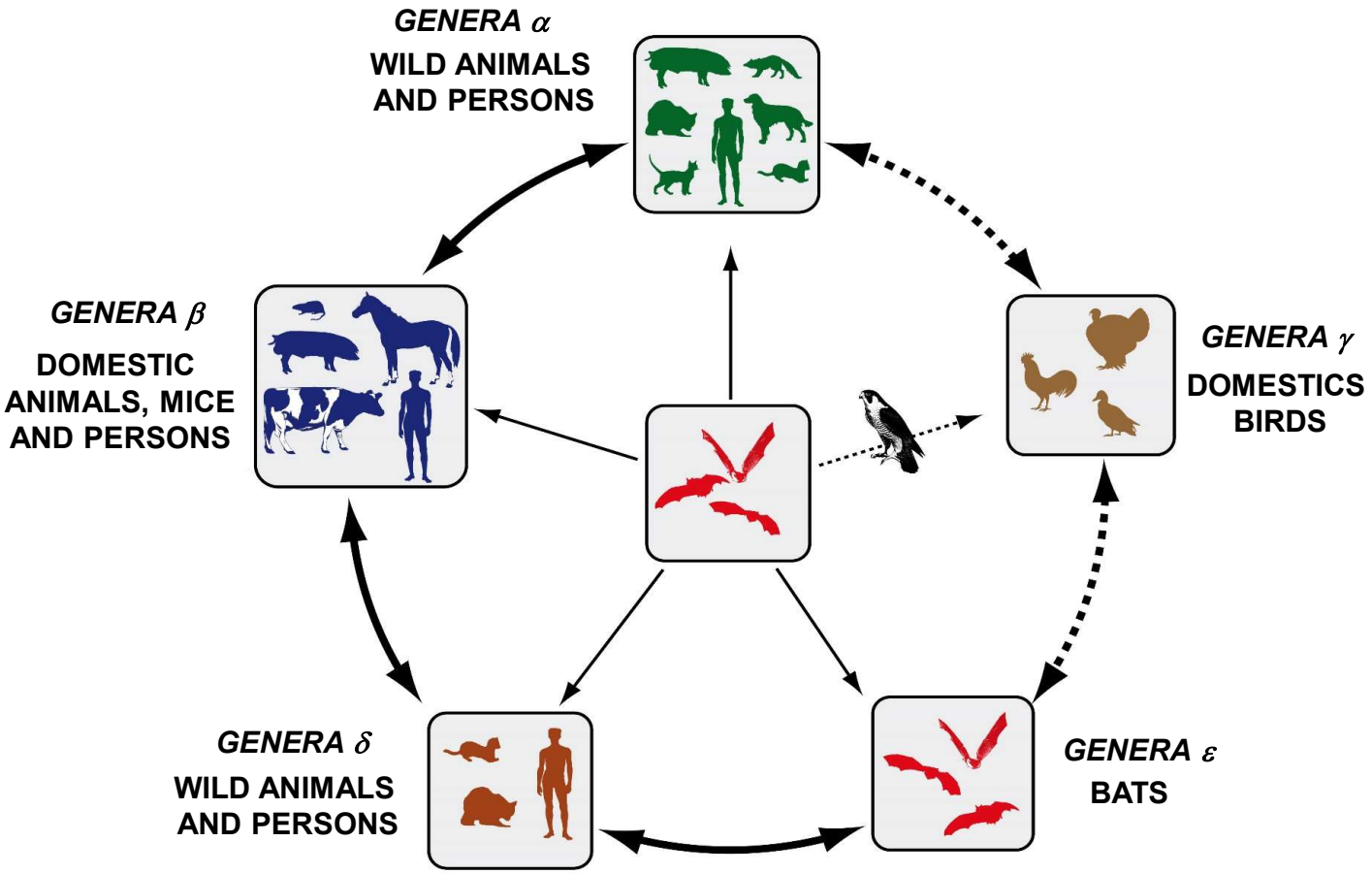
Eckerle et al., 2007, 2010

VIRUS DISSEMINATION ROUTES

- MOSQUITO TRANSMITTED VIRUSES: BTV
  ZIKA
  CHIKUNGUNYA
- MIGRATORY BIRDS AND MOSQUITO: WNV
- DIRECT PERSON TO PERSON TRANSMISSION: EBOLA
- AIR TRANSMISSION BETWEEN PERSONS: CoV

VIRUSES TRANSMITED BETWEEN BATS AND PERSONS AND FROM PERSON TO PERSON

POSTULATED CORONAVIRUS ECOLOGY

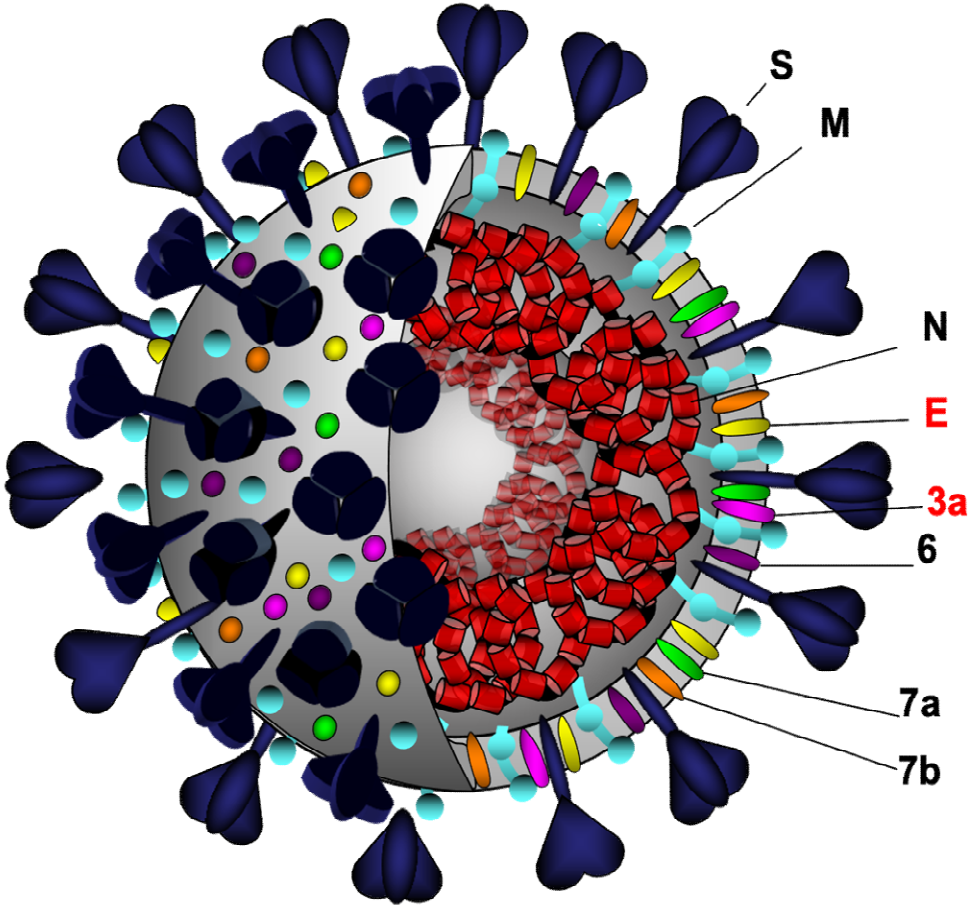


HUMAN PATHOGENIC CORONAVIRUSES

VIRUS	YEAR	INFECTED	DEATHS	MORTALITY	COUNTRIES
SARS-CoV	2002	8 098	774	10%	29
MERS-CoV	2012	2650	858	37%	27
SARS-CoV-2	2019	518×10^6	6.3×10^6	2%	235

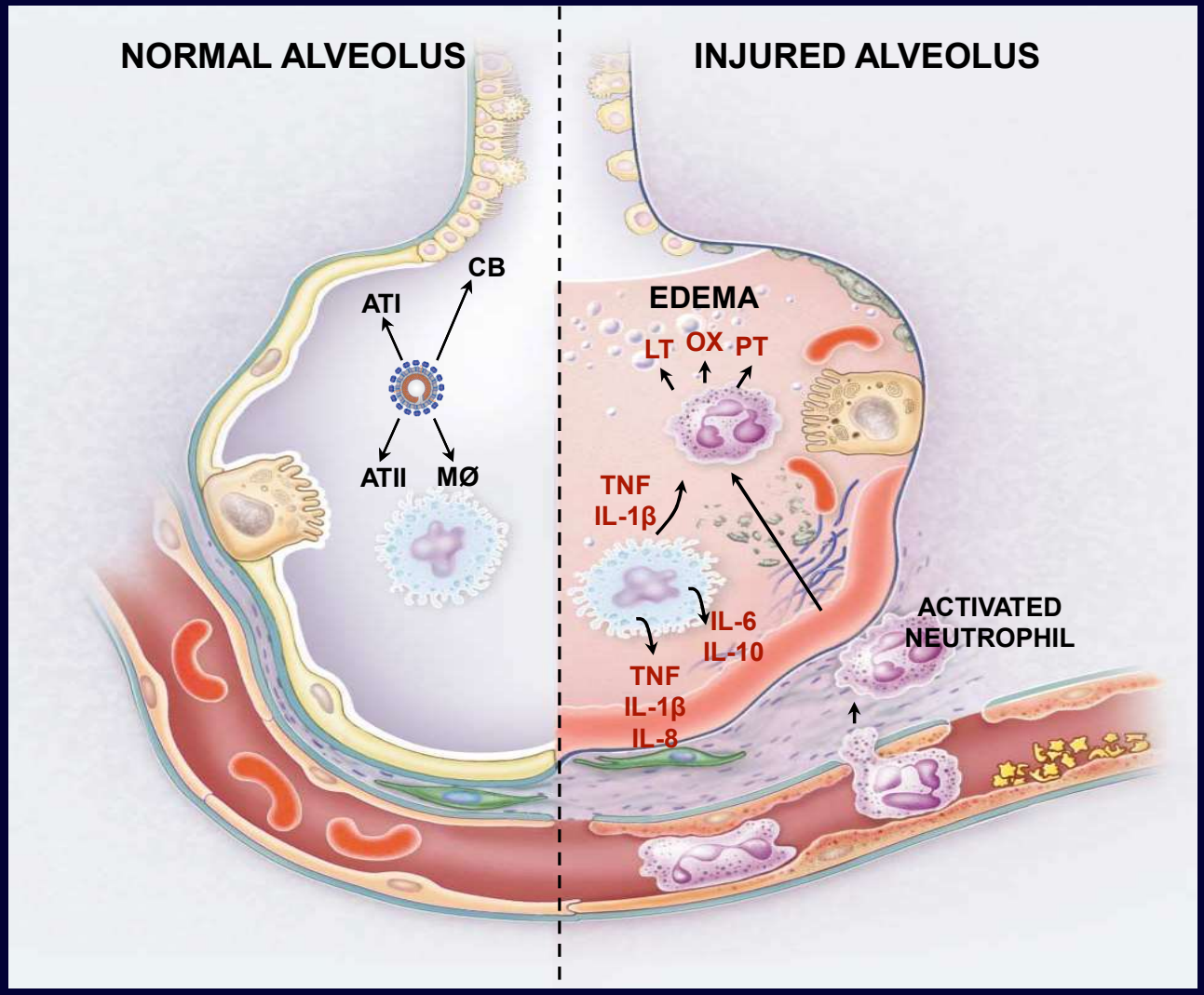
WHO, MAY 12th, 2022

HUMAN CORONAVIRUSES

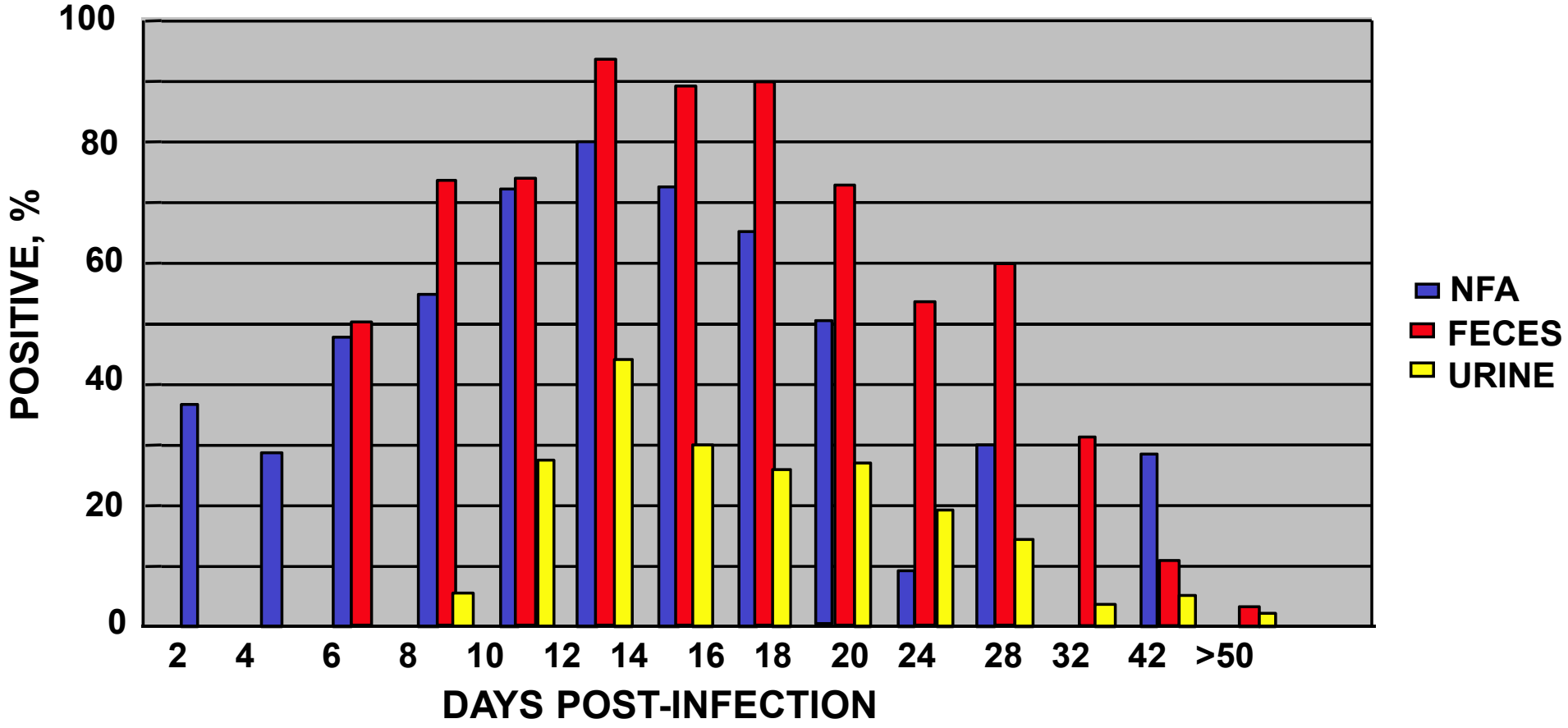


- HCoV-OC43
- HCoV-229E
- HCoV-NL-63
- HCoV-HKU1
- SARS-CoV
- MERS-CoV
- SARS-CoV-2

SARS-CoV AND MERS-CoV CAUSE ARDS

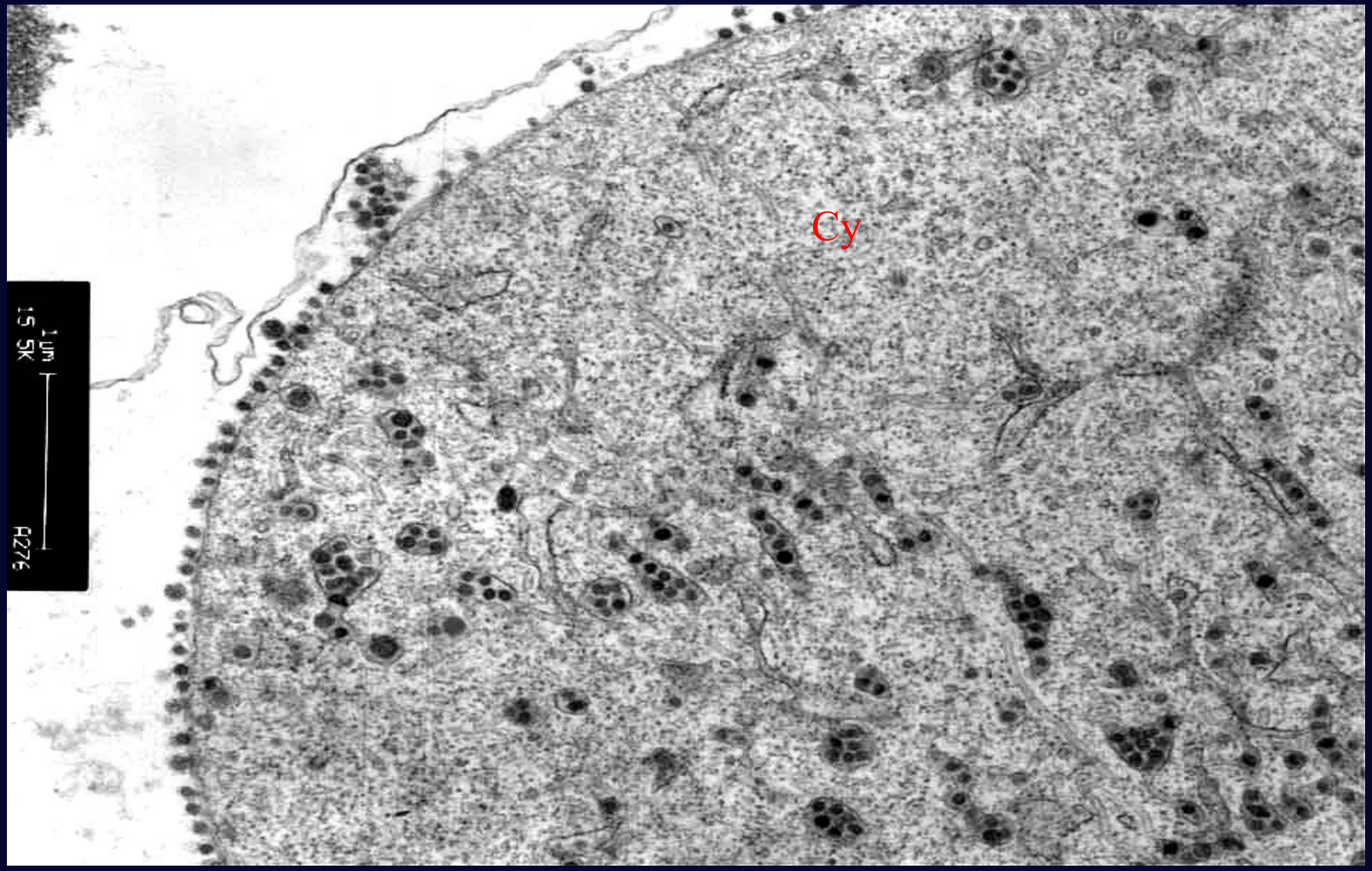


RT-PCR EVALUATION OF CLINICAL SAMPLES FROM SERO-POSITIVE PATIENTS



Chan KH et al., 2004 EID 10: 294

HUMAN ENTERIC TRACT INFECTED WITH SARS-CoV



GEOGRAPHICAL ORIGIN GRAPHIC

SARS-CoV-1 VECTORS

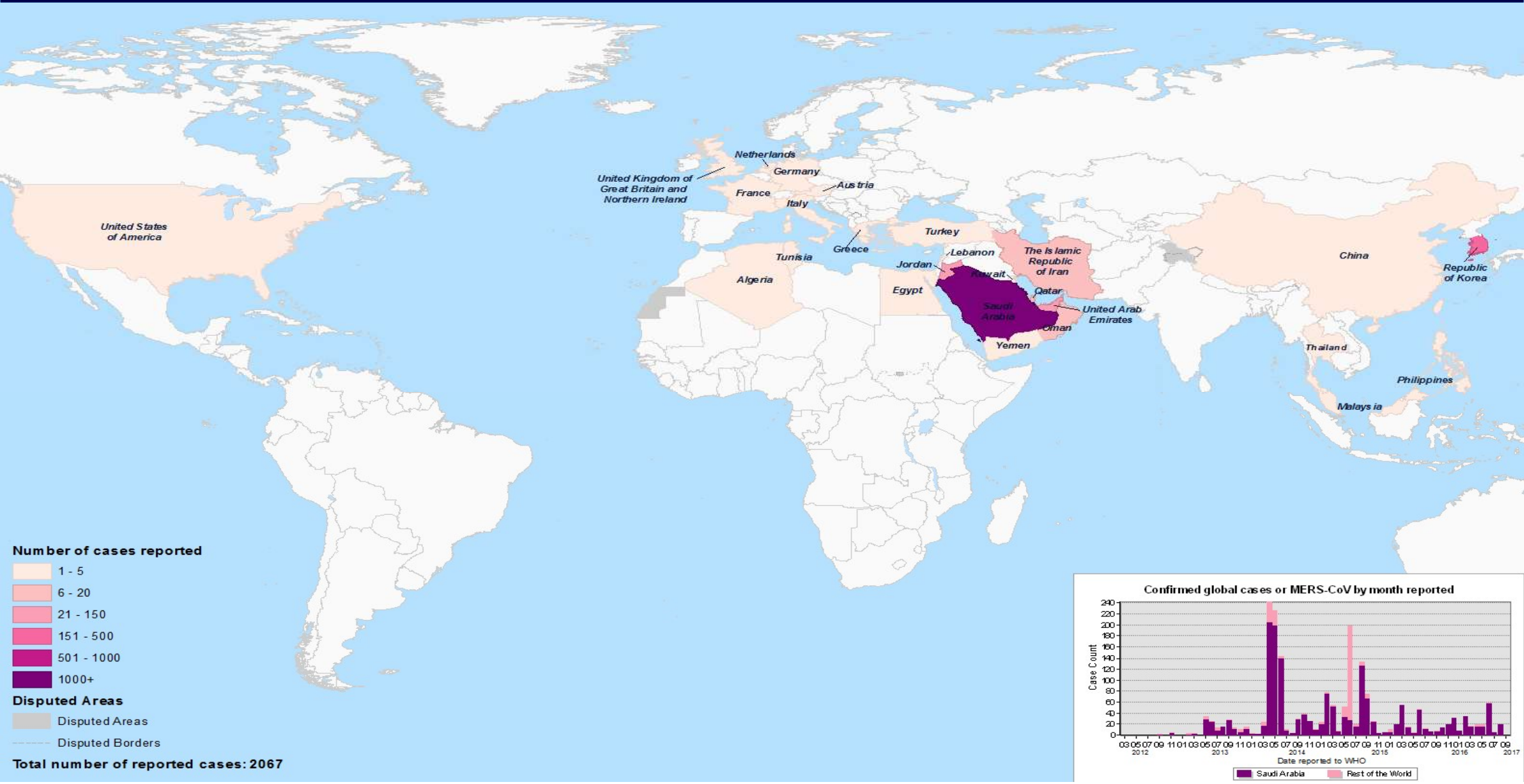
RACOON
MAPACHE

CIVET CAT

FERRET
VISON

BAT

MERS-CoV EMERGING IN 2012

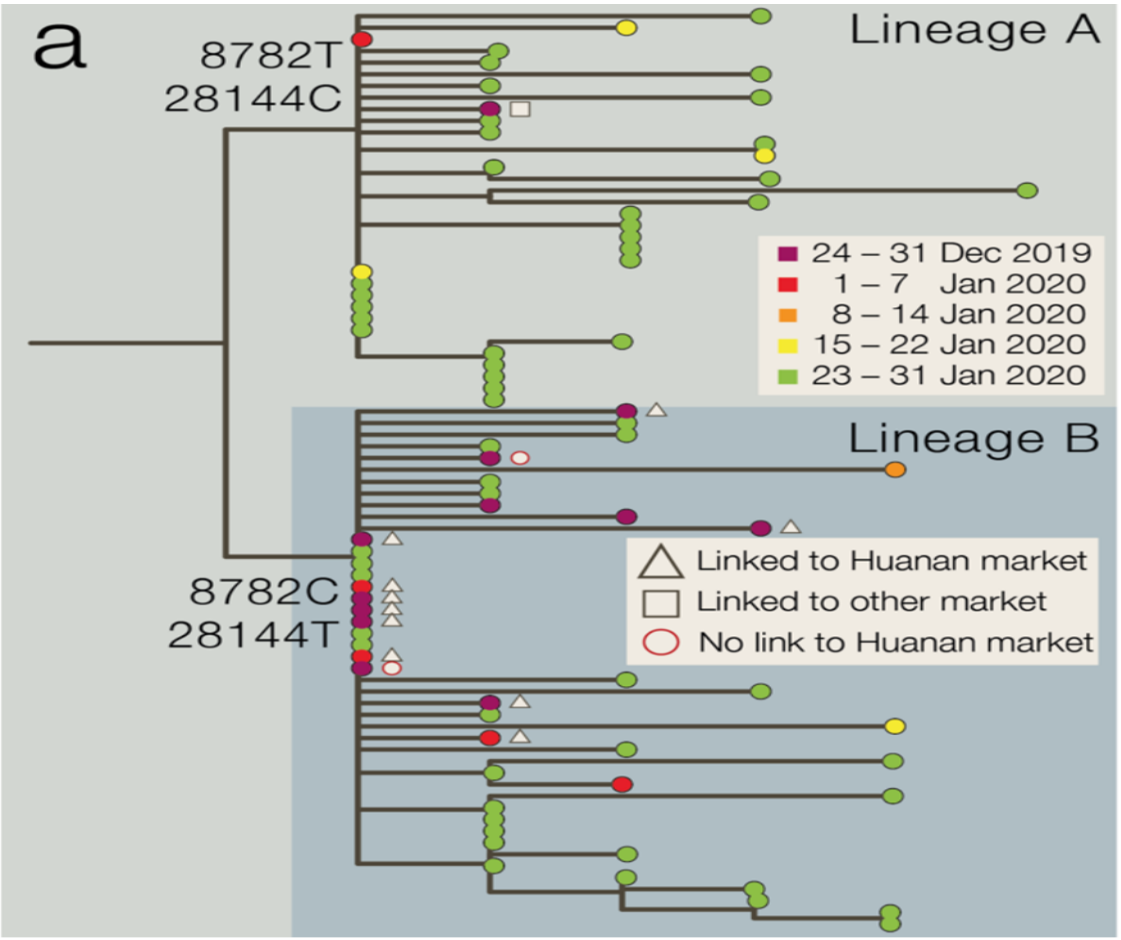


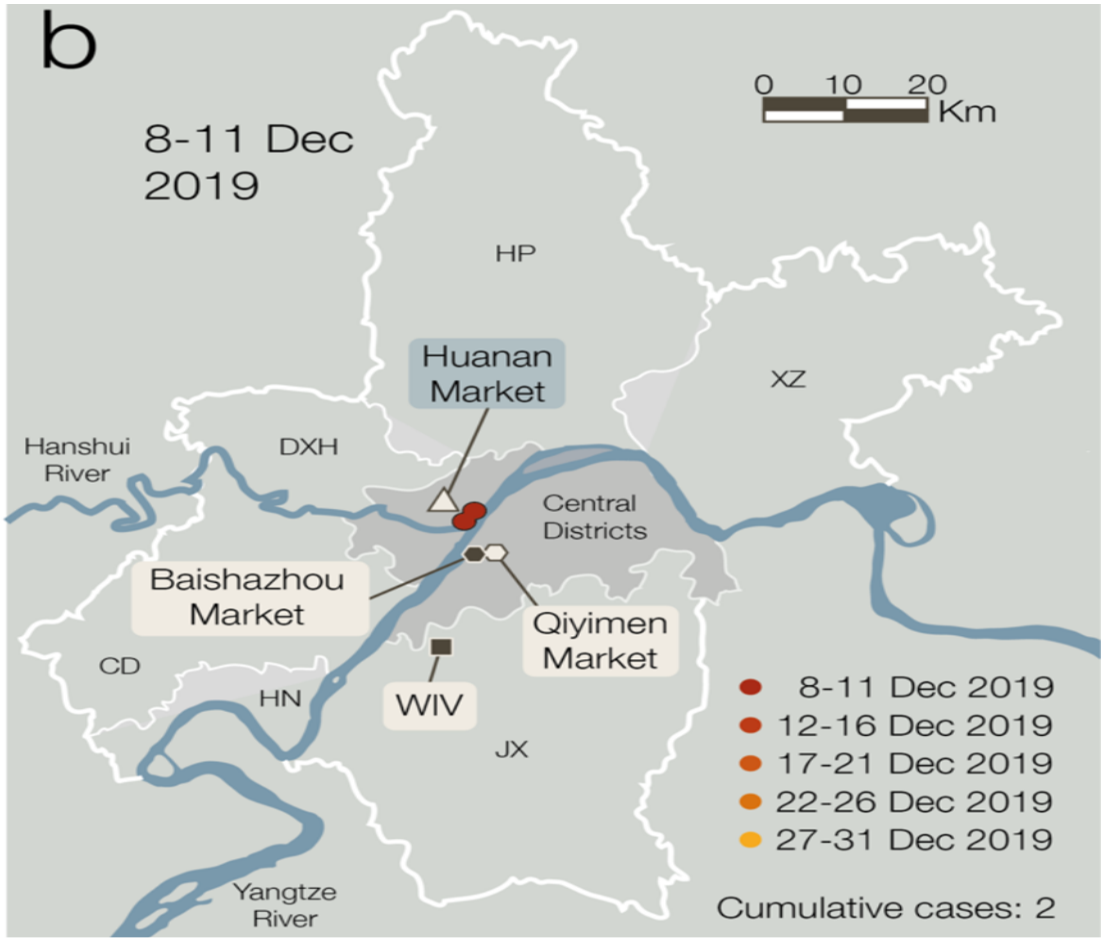
MERS-CoV IS TRANSMITTED BY CAMEL

DISSEMINATION OF SARS-CoV-2

SARS-CoV-2 ORIGIN

INITIAL DISSEMINATION OF SARS-CoV-2 IN WUHAN



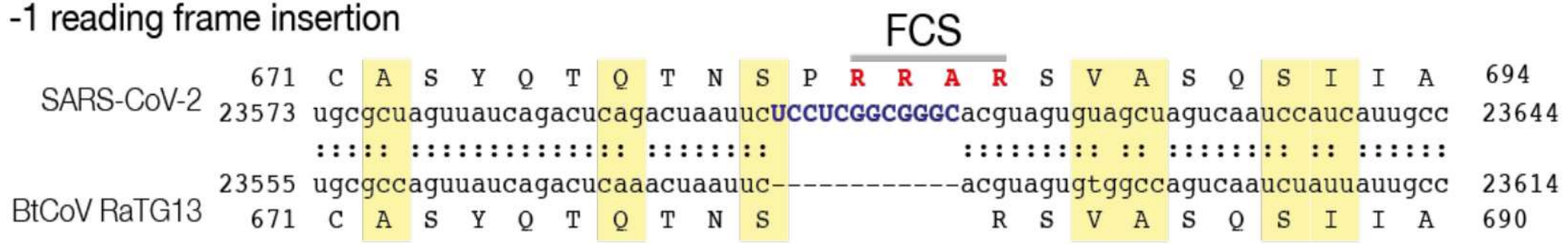


INITIAL SARS-CoV-2 INFECTIONS

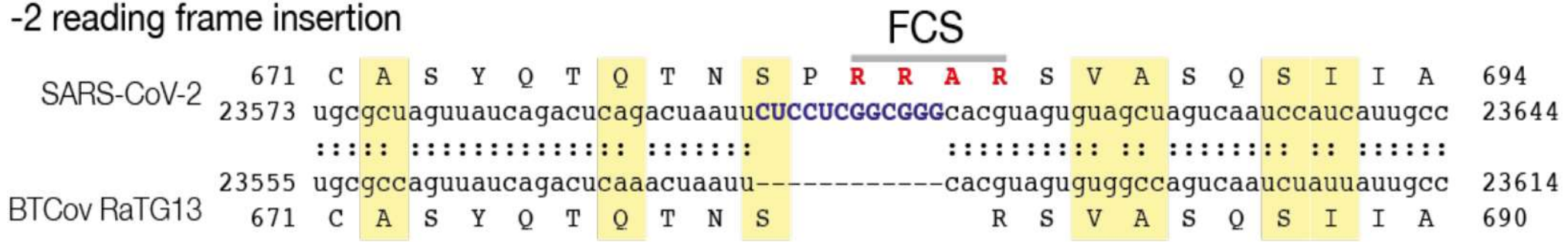
- All previous human coronaviruses have zoonotic origins, as have the vast majority of human viruses
- Locations of early cases shows that most were located around the Huanan market, located north of the Yangtze River
- Wuhan Institute of Virology is located South => unlike the virus origin
- Viruses closely related to SARS-CoV-2 have been documented in bats and pangolins in multiple localities in South-East Asia, including in China, Thailand, Cambodia, and Japan. Importantly, in the metal cages of racoons viruses with a genetic identity of 99.99% with SARS-CoV-2 were found, indicating that they transmitted the virus to humans

INSERTION OF A FURIN SITE OF FOUR AMINO ACIDS IN SARS-CoV-2 S PROTEIN

- -1 reading frame insertion

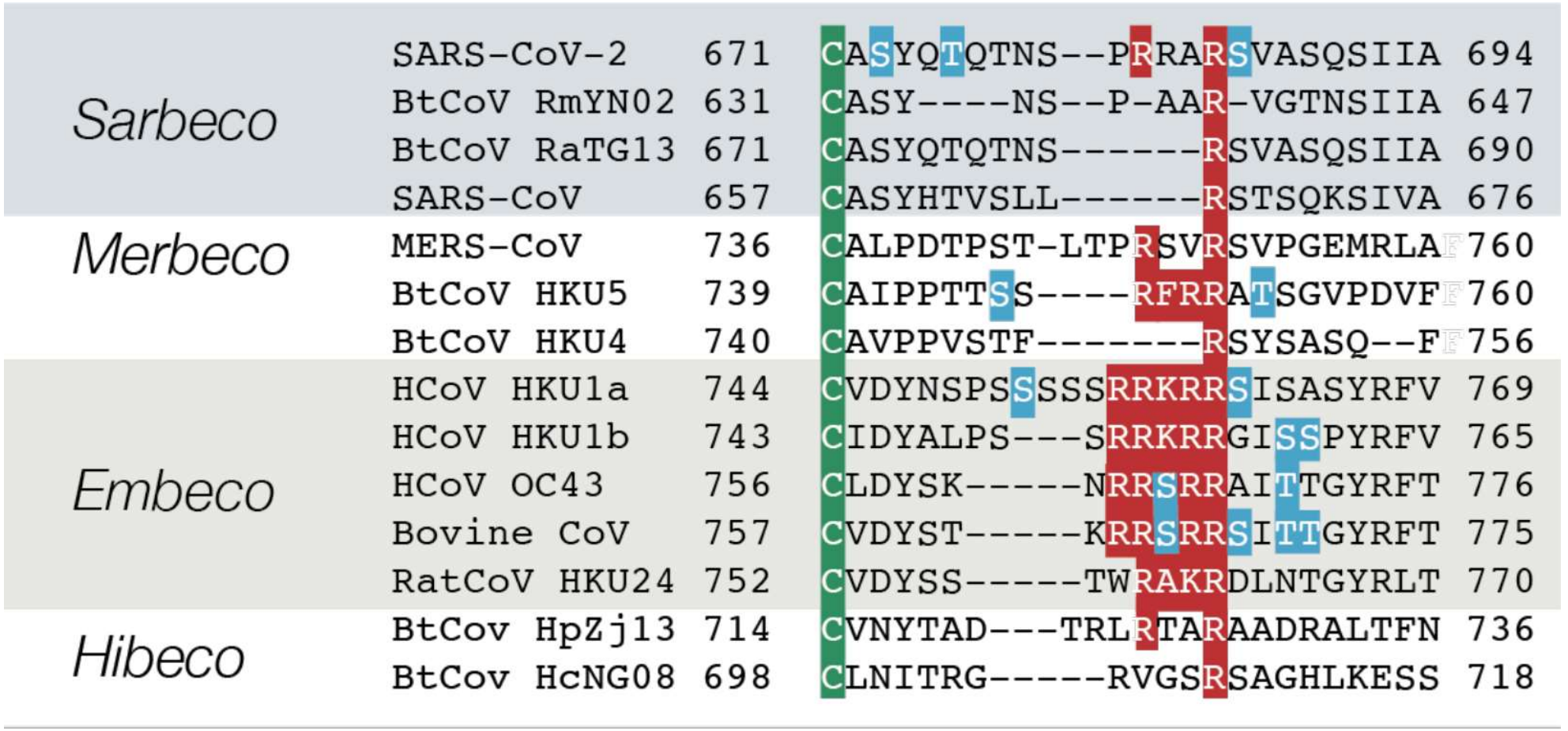


- -2 reading frame insertion



Holmes et al., Cell 2021

PRESENCE OF FURIN CLEAVAGE SITE IN DIFFERENT CoVs



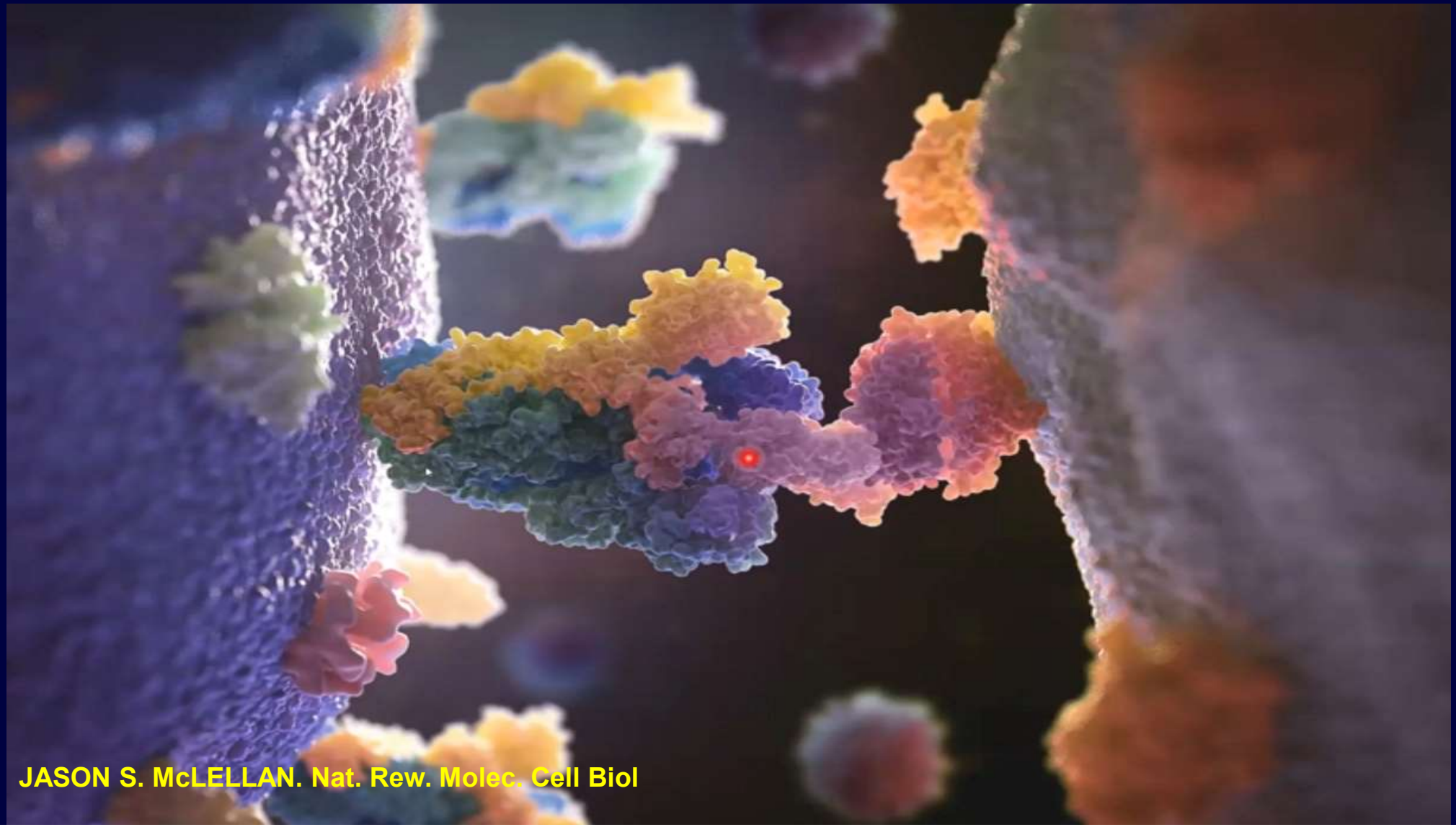
Evidence from genomic structure and ongoing evolution of SARS ORIGIN-CoV-2

CONCLUSION ON SARS-CoV-2 ORIGIN

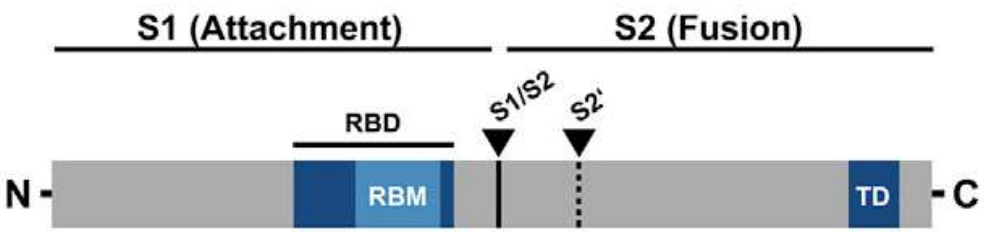
- THE MOST DIRECT EXPLANATION FOR THE ORIGIN OF SARS-COV-2 IS A ZOONOTIC EVENT
- THERE IS CURRENTLY NO EVIDENCE THAT SARS-CoV-2 HAS A LABORATORY ORIGIN
- BIOLOGICAL MATERIAL FROM HUANAN MARKET RACOONS INCLUDED CoVS WITH THE SAME SEQUENCE THAN HUMAN SARS-CoV-2 INDICATING THAT THEY TRANSMITTED THE VIRUS TO HUMANS

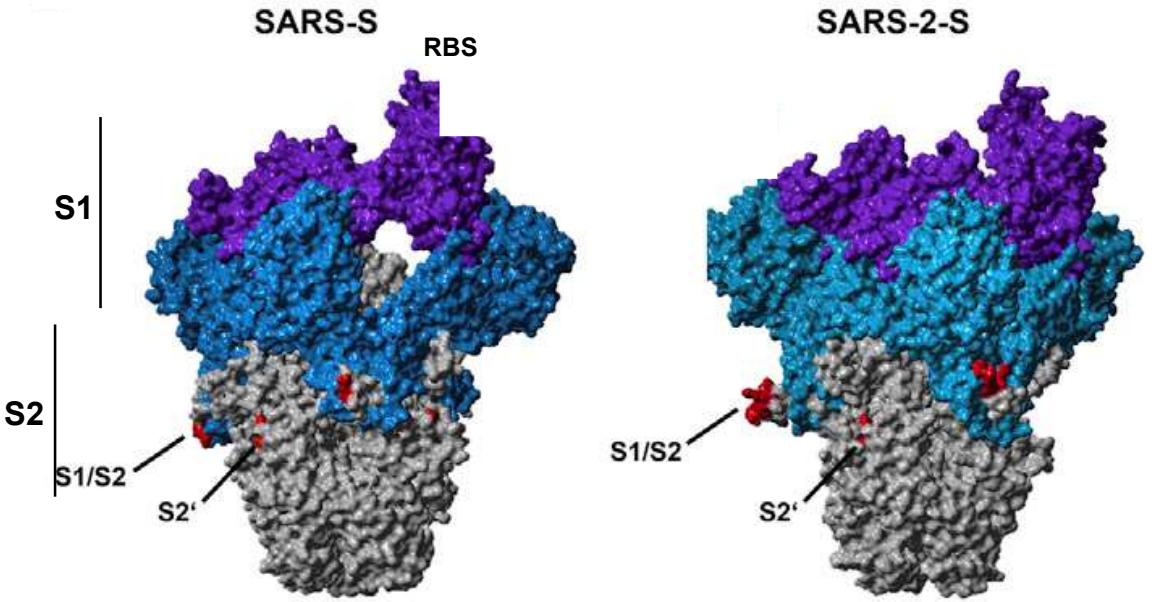
SARS-CoV-2 ORIGIN: RACOON [MAPACHES]

SARS-CoV-2 CELL INTERACTION



SARS-CoV-2 PROTEOLITIC CLEAVAGES





- THE S1/S2 SITE IN SASRS-CoV-2 SPIKE IS CLEAVED BY FURIN IN INFECTED CELLS
- S2' CLEAVAGE BY TMPRSS2^{HIGH} OR CATHEPSIN^{LOW} IS ESSENTIAL FOR VIRAL ENTRY IN LUNG CELLS

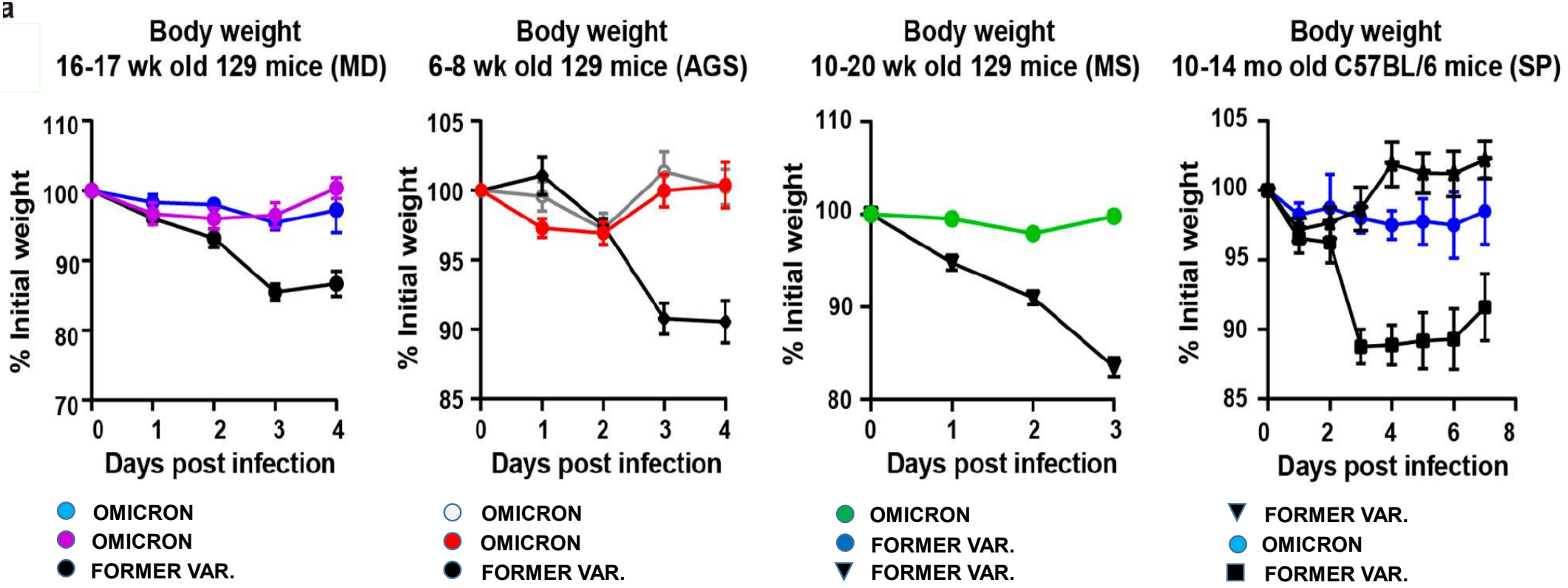
HOFFMANN ET AL., Molec. Cell 2020

SARS-CoV-2 INDUCED PATHOLOGY: ORGANS AFFECTED

LUNGS	ARDS ➡ LUNG EDEMA
GUT	LARGE AND SMALL INTESTINE ENTEROCYTES
KIDNEY	OBSERVED IN INTENSE CARE UNIT
BRAIN:	VIRUS PRESENCE IN BRAIN DETECTED
HEART	ARREST AND ARRHYMIAS INFLAMMATION MYOCARD
VEINS	EXTENSIVELY CLOTHING. PLATELETS, STROKES
SMELL LOSS	NASAL EPITHELIUM HIGHEST ACE-2
TONG	KERATINOCYTES
PANCREAS	IN SEVERE DISEASE

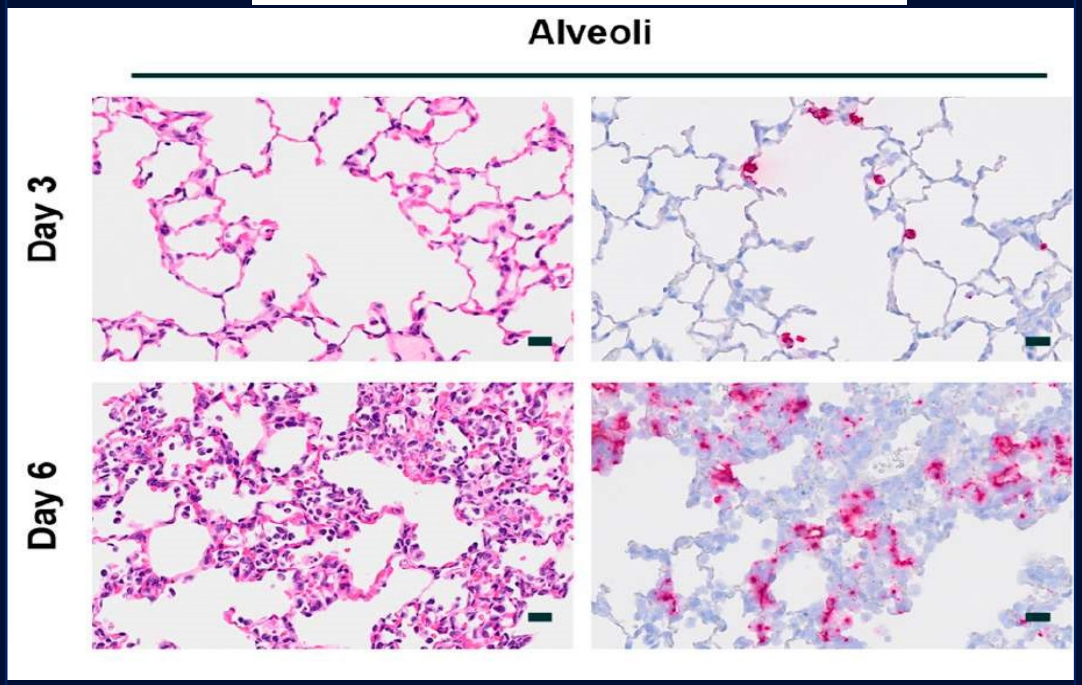
BODY WEIGHT LOSSES CAUSED BY INFECTING WITH OMICRON MICE STRAINS

a

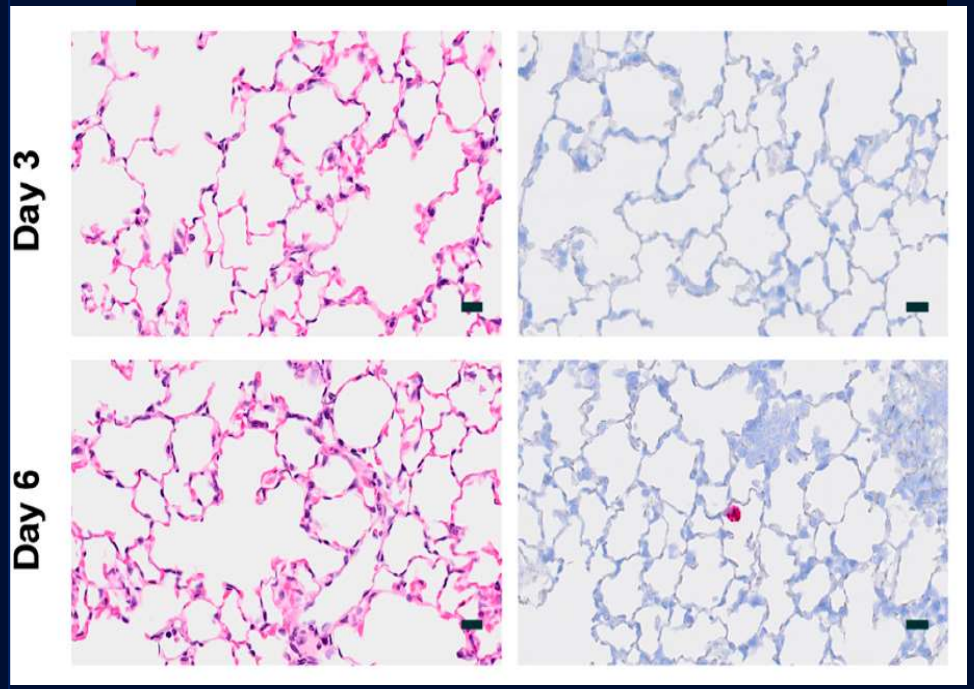


LUNG OF SARS-CoV-2 INFECTED HAMSTERS

INFECTED WITH DELTA VIRUS

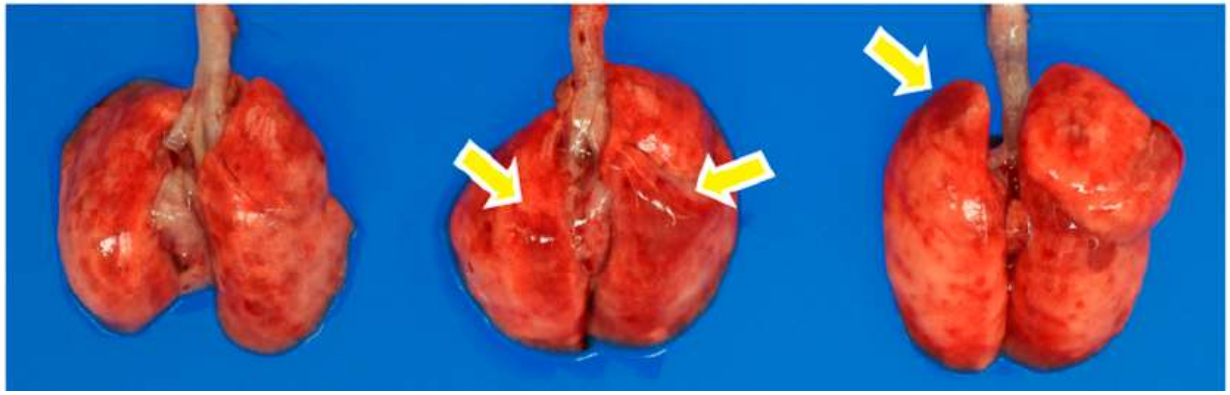


INFECTED WITH OMICRON VIRUS

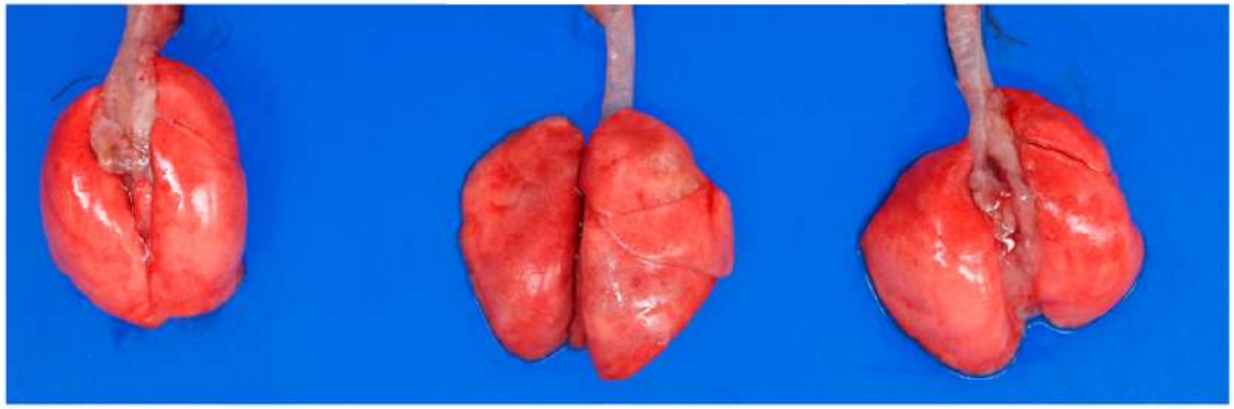


PATHOLOGY OF LUNGS INFECTED WITH DELTA OR OMICRON VIRUSES

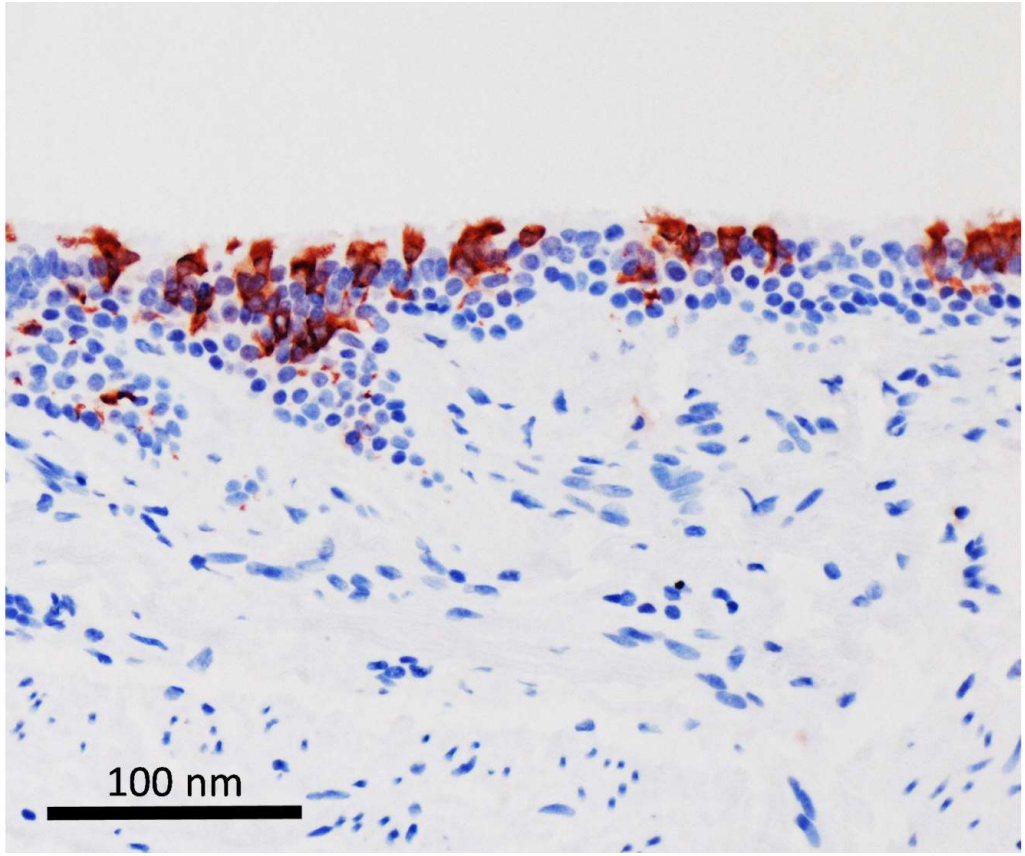
DELTA

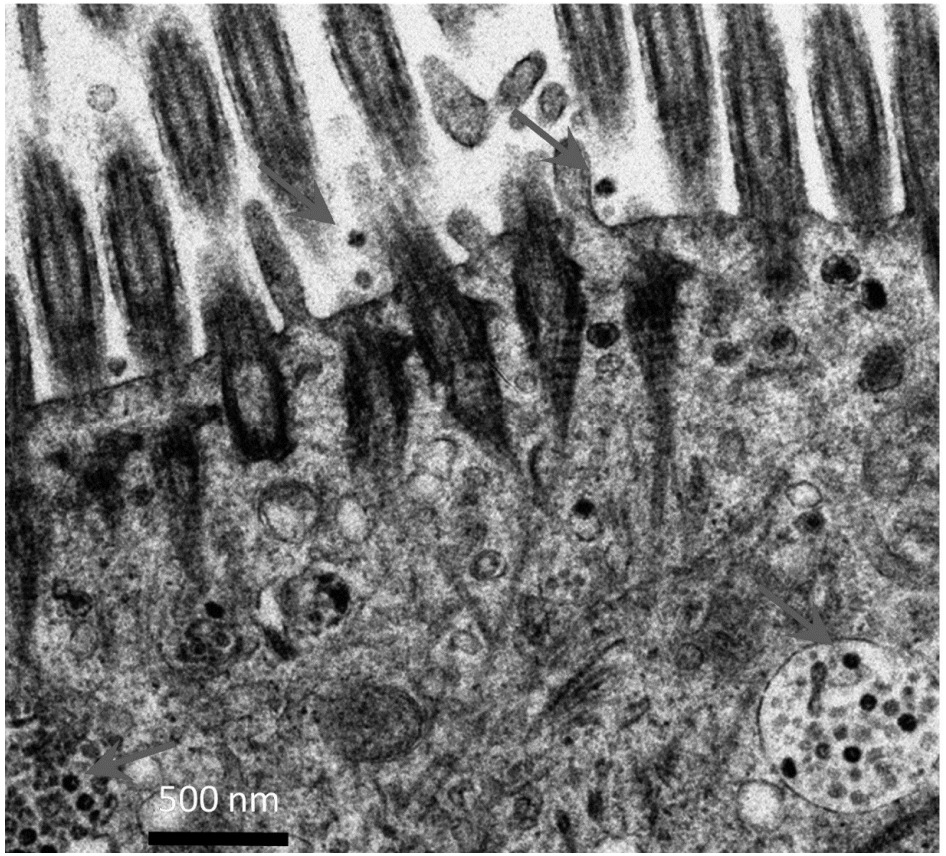


OMICRON

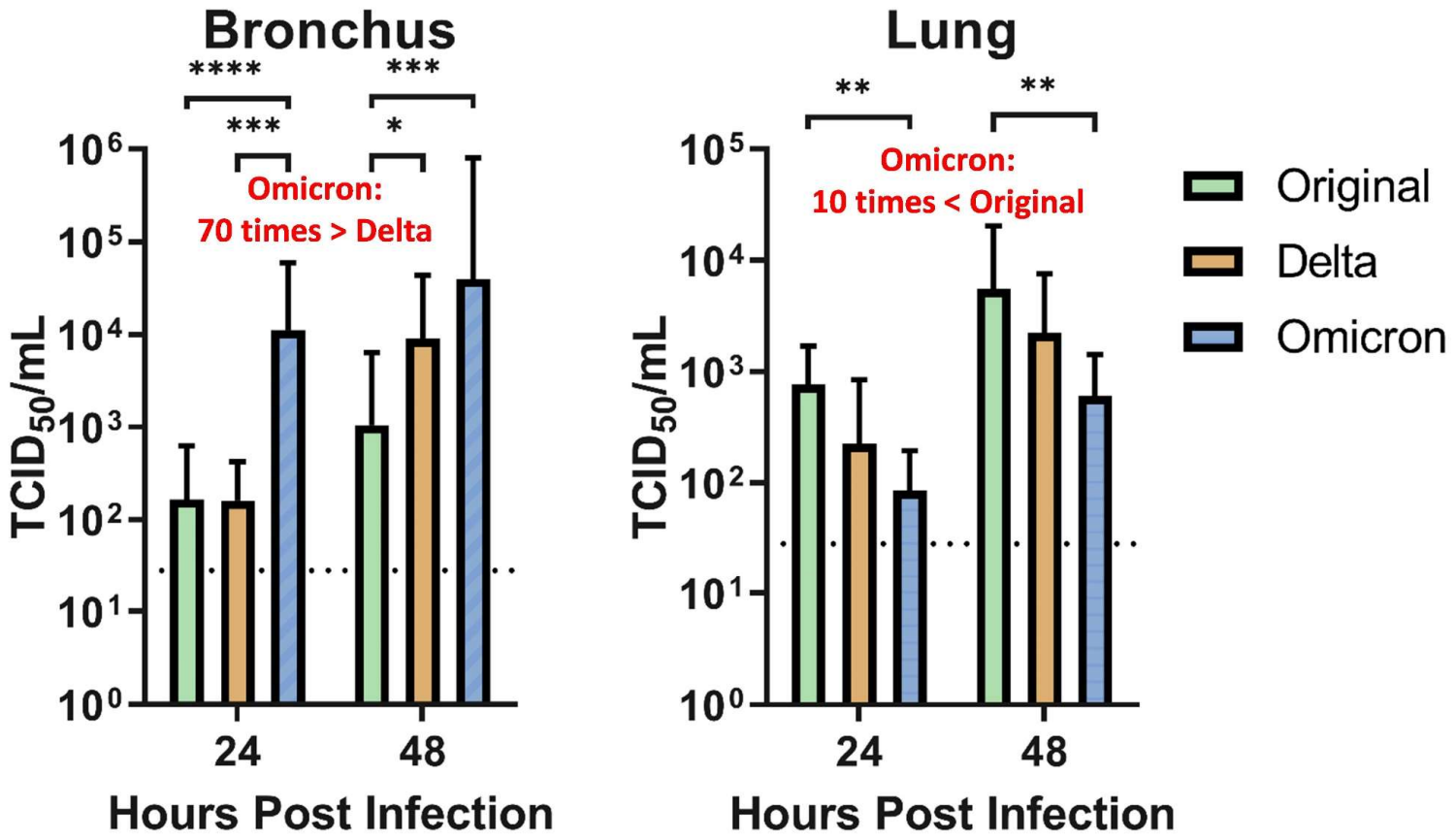


OMICRON INFECTION OF HUMAN BRONCHUS





GROWTH OF OMICRON CoV IN RESPIRATORY TRACT



WHAT IS NEXT FOR SARS-CoV-2 EVOLUTION. Nature Dec 22

- The new pathogen will not be eradicated. Seasonal CoVs as potential models for SARS-CoV-2: HCoV-229E Since 1980. The virus is scaping immune response, as Omicron and Delta (Jesse Bloom, Washington)

- CoV evolution guided by:
  - Increase of virus infectivity
  - Evasion of previous immunity

- CoV must balance its ability to replicate to high levels with the need to keep healthy the new new host (Fred Hutchinson. Washington)

- SARS-CoV-2 could become more severe or evade current vaccines by recombination with other CoVs currently circulating in animal reservoirs (mink (visones), white-tailed deer [ciervo], etc) to scape immune response (UK government advisory group)

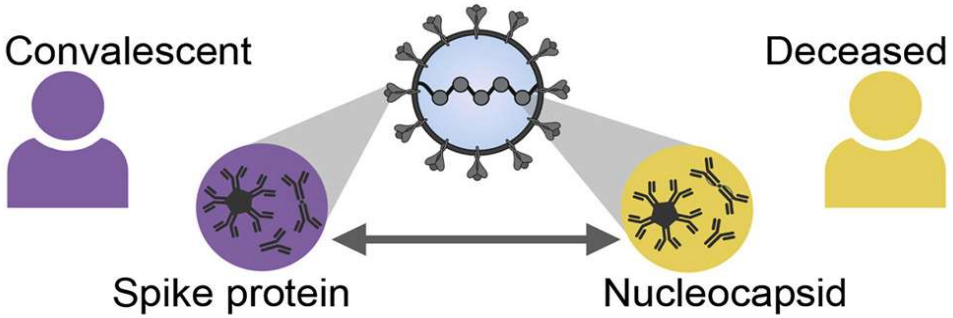
INNATE IMMUNE RESPONSE IN SARS-CoV-2 PATIENTS

- **REDUCED INNATE ANTIVIRAL DEFENSES**
  - **LOW IFN-I & IFN-III : ANTI-IFN I Auto Abs; Genetic defects**
- **HUGE INFLAMMATORY CYTOKINE PRODUCTION**
  - **HIGH LEVELS CXCL8 → NEUTROFIL CHEMOATTRACTANT**
  - **HIGH SERUM LEVELS CYTOKINE IL6**
  - **HIGH LEVELS CHEMOKINES CXCL9, CXCL16→ CHEMO-ATTRACTANTS OF T AND NK CELLS**
  - **HIGH LEVELS CCL8 AND CCL2 → RECRUIT MONOCYTES AND MACROPHAGES**

Zhang et al. Science. 2020. 10.1126/science.abd4570 Blanco-Melo et al. Cell 181, 1036–1045. 2020 Bastard et al. Science. 2020. 10.1126/science.abd4585

DYSFUNTION OF SARS-CoV-2 ELICITED ANTIBODIES

- Abnormalities of the adaptive immune system driven by the dysregulated cytokine response
- Similar quantity of antibodies in survivors and those who died
  Differences in their function: viral proteins targeted

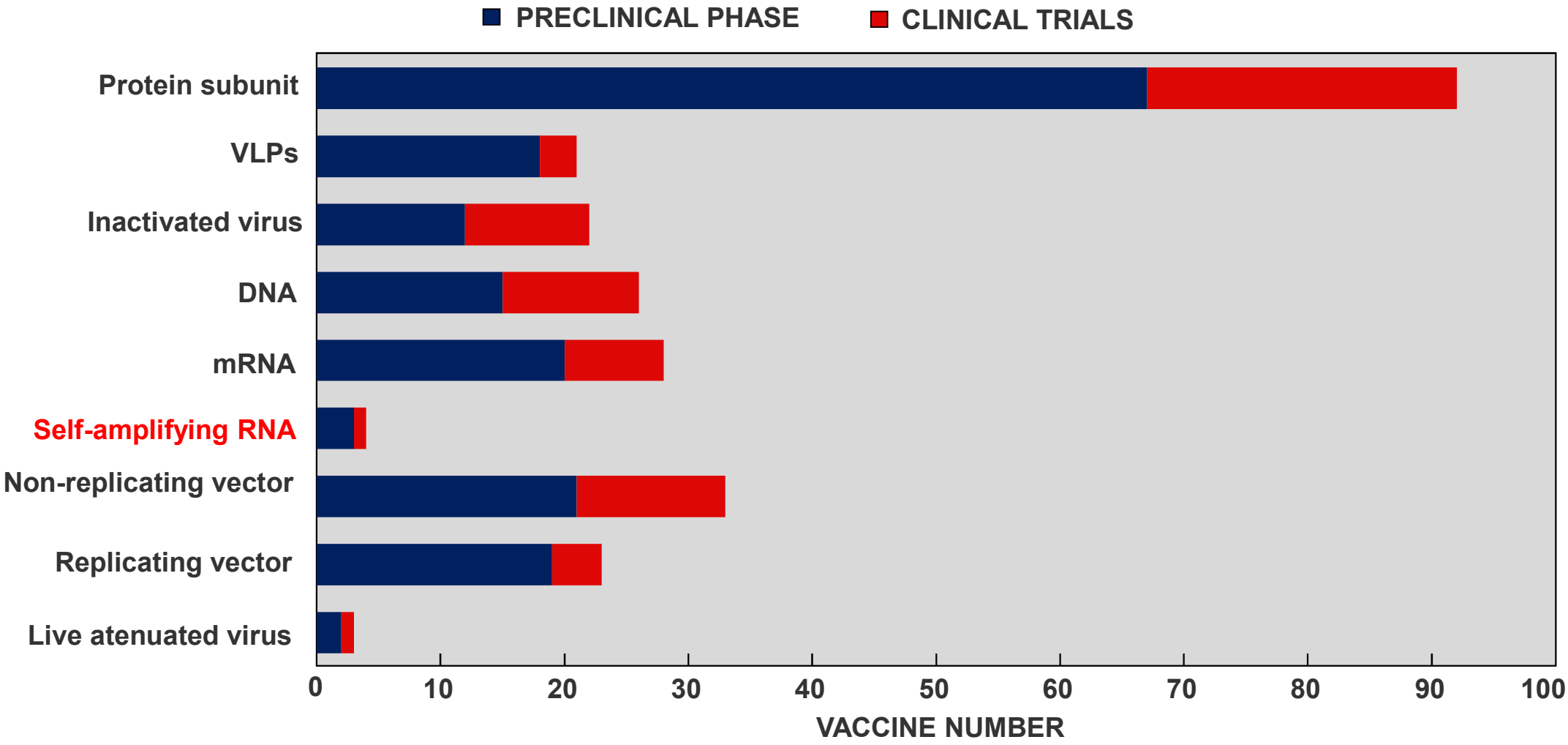


- Absence of germinal centers: Defects in Ab maturation, long-lasting plasma cells, and memory B cells
- Low neutralizing activity

TYPES OF VACCINES

INACTIVATED	LOW MUCOSAL IMMUNITY NON-LASTING MEMORY EOSINOPHILIA & ADEI
LIVE-ATTENUATED	MIMIC THE NATURAL ROUTE OF INFECTION HIGHLY IMMUNOGENIC LONG LASTING MEMORY BIOSAFETY CONCERS
BASED ON VIRAL VECTORS	FAST DEVELOPMENT SAFETY DOCUMENTED EFFICIENT
mRNA	FAST DEVELOPMENT HIGH AMOUNTS AND TWO DOSES REQUIRED
RNA REPLICONS	SELF-AMPLIFYING: STRONG IMMUNE RESPONSE PROPAGATION DEFICIENT: SAFE

SARS-CoV-2 VACCINE DEVELOPMENT

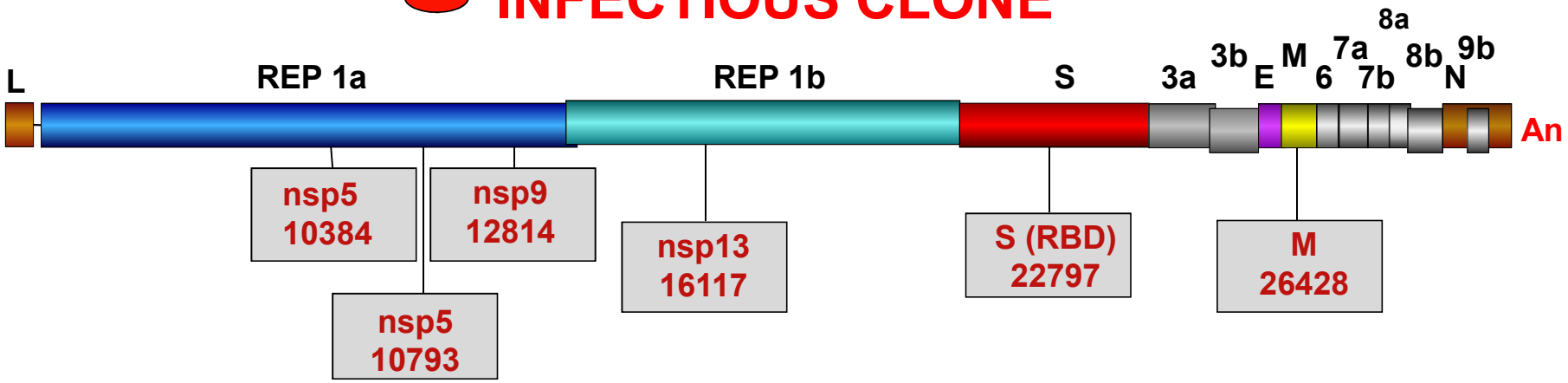


OBJETIVES

- MOLECULAR BASES OF VIRULENCE: VACCINES

TOOLS

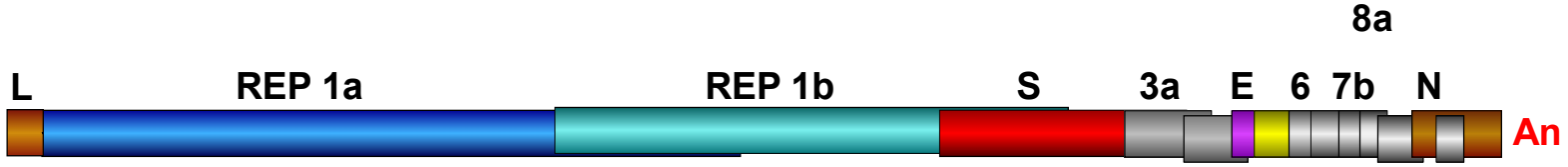
INFECTIOUS CLONE

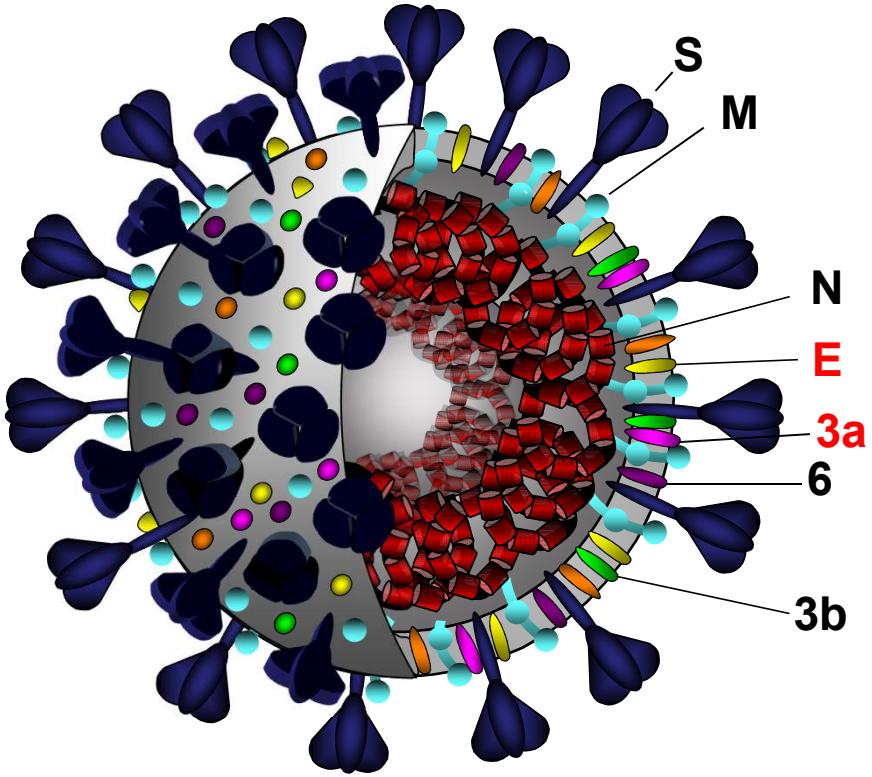


ANIMAL MODEL

- The virus produces pulmonar pathology
- Death induction

SARS-CoV





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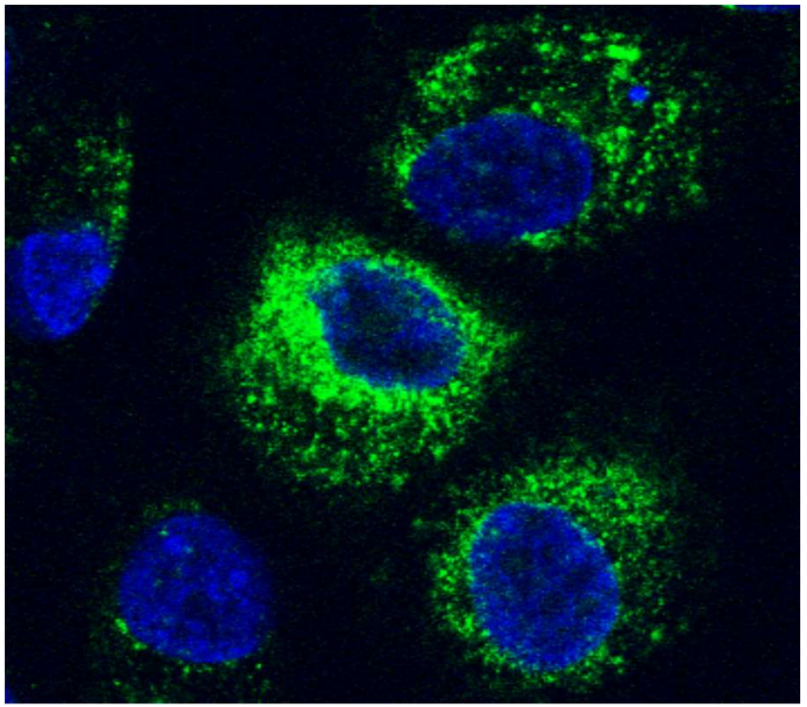
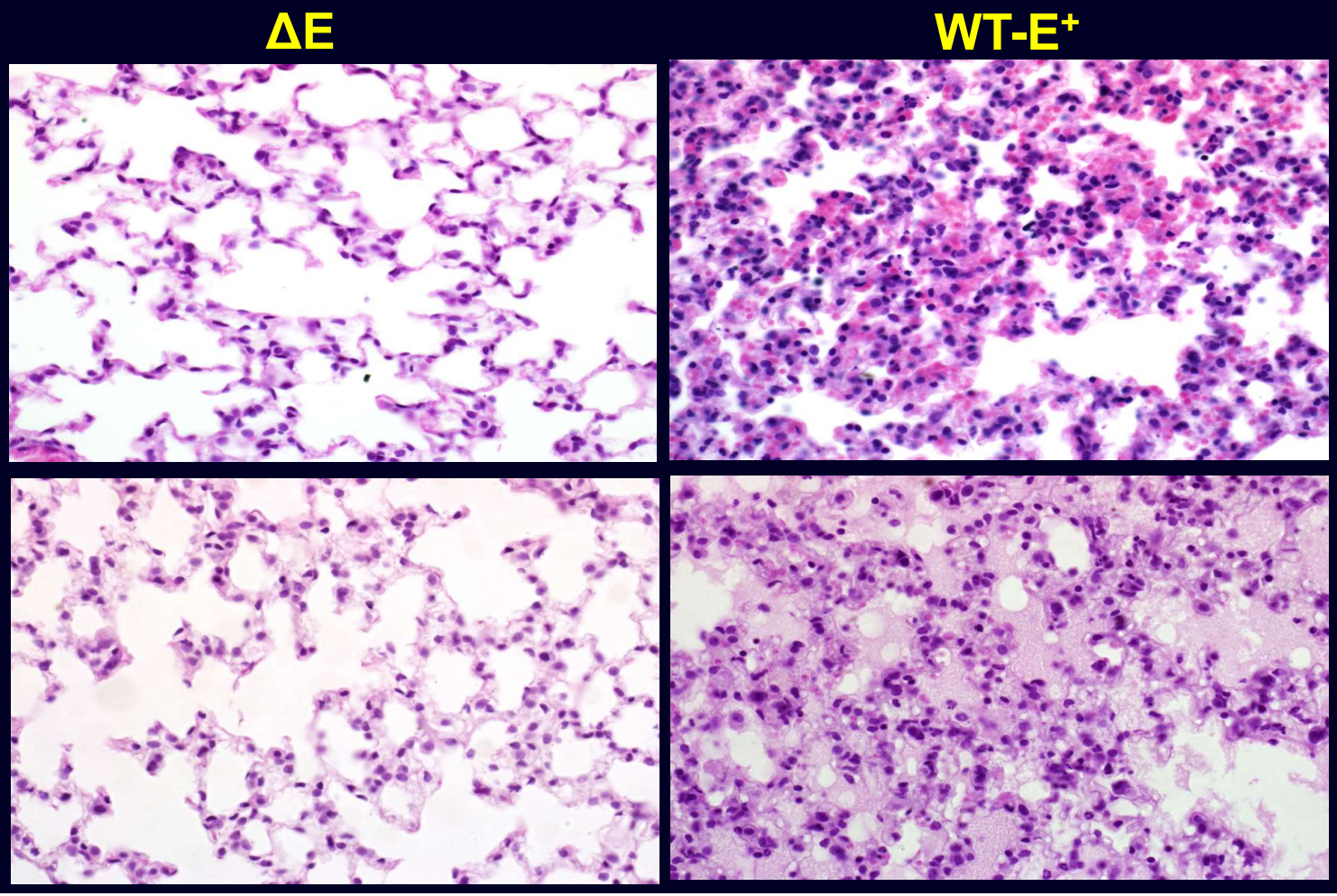
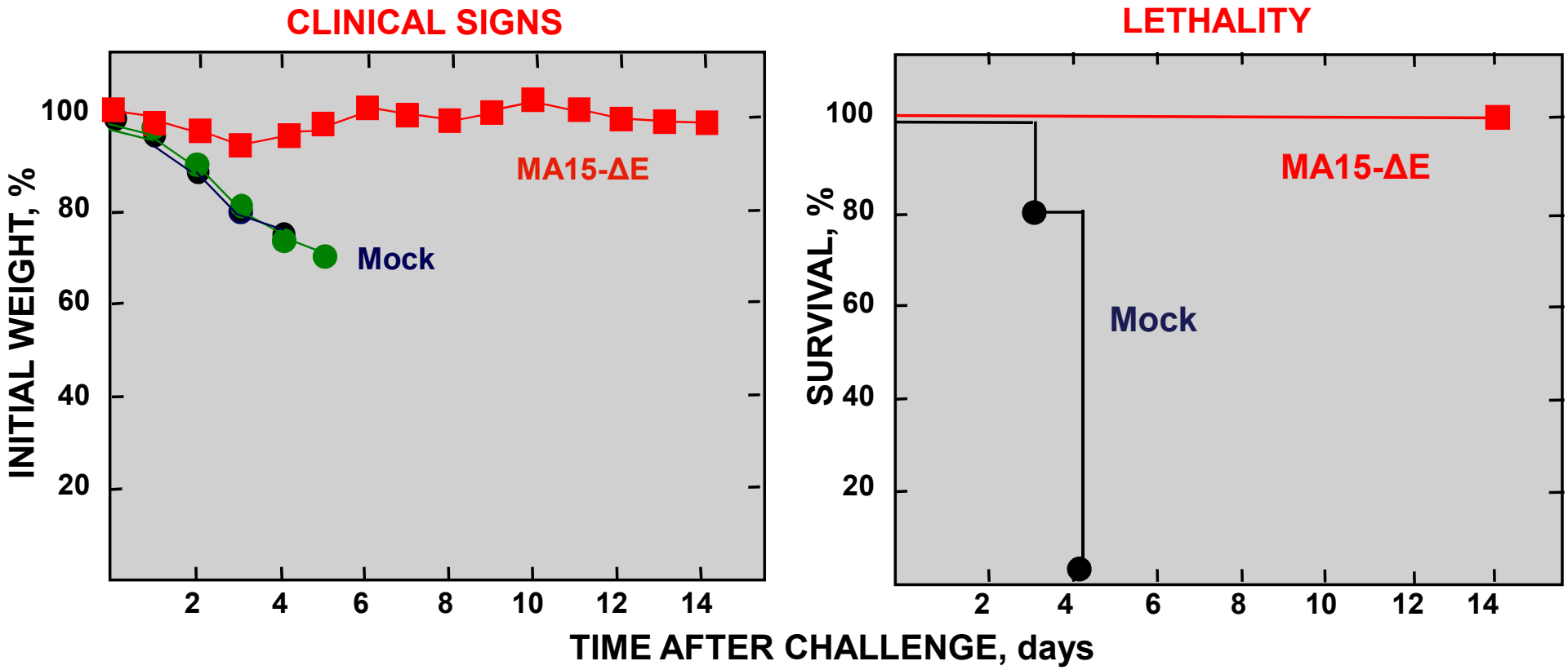


BSL-3 LABORATORY AT CNB. CSIC MADRID

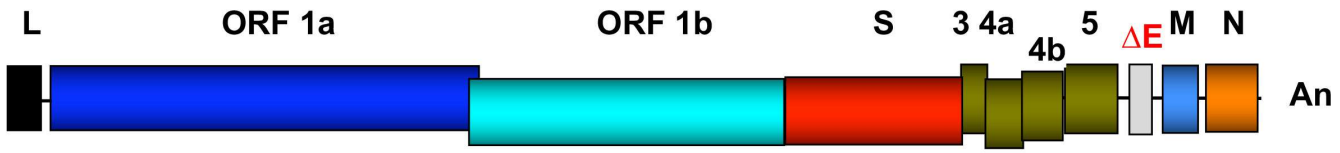
LUNG PATHLOGY ASSOCIATED TO MICE INFECTION BY SARS-CoV-MA15

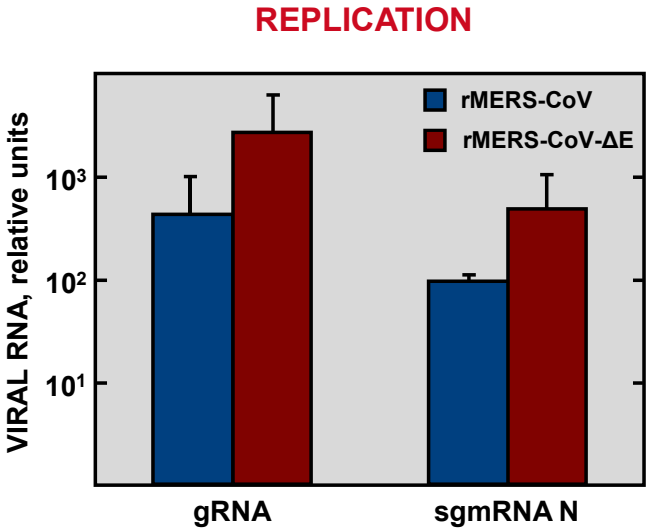


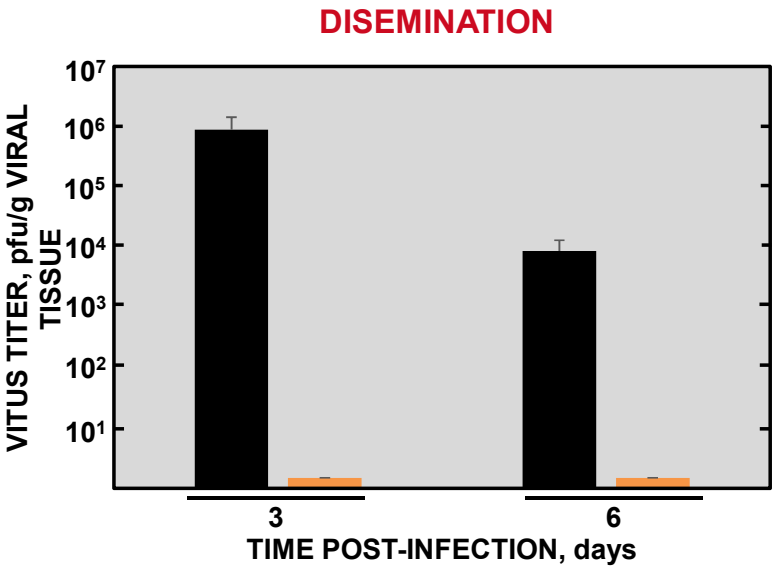
PROTECTION PROVIDED BY AN ATTENUATED SARS-CoV E PROTEIN DELETION MUTANT



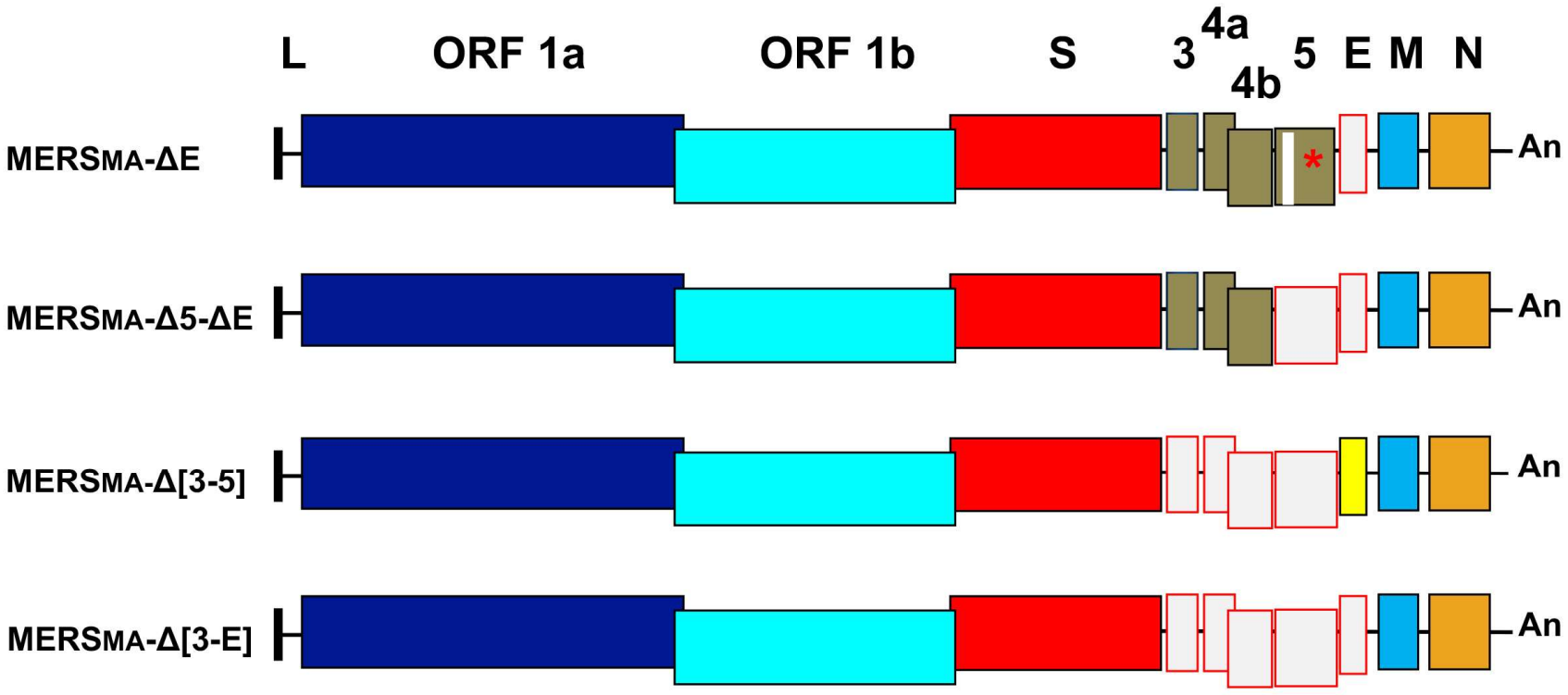
MERS-CoV-ΔE REPLICATION-COMPETENT DISSEMINATION-DEFICIENT





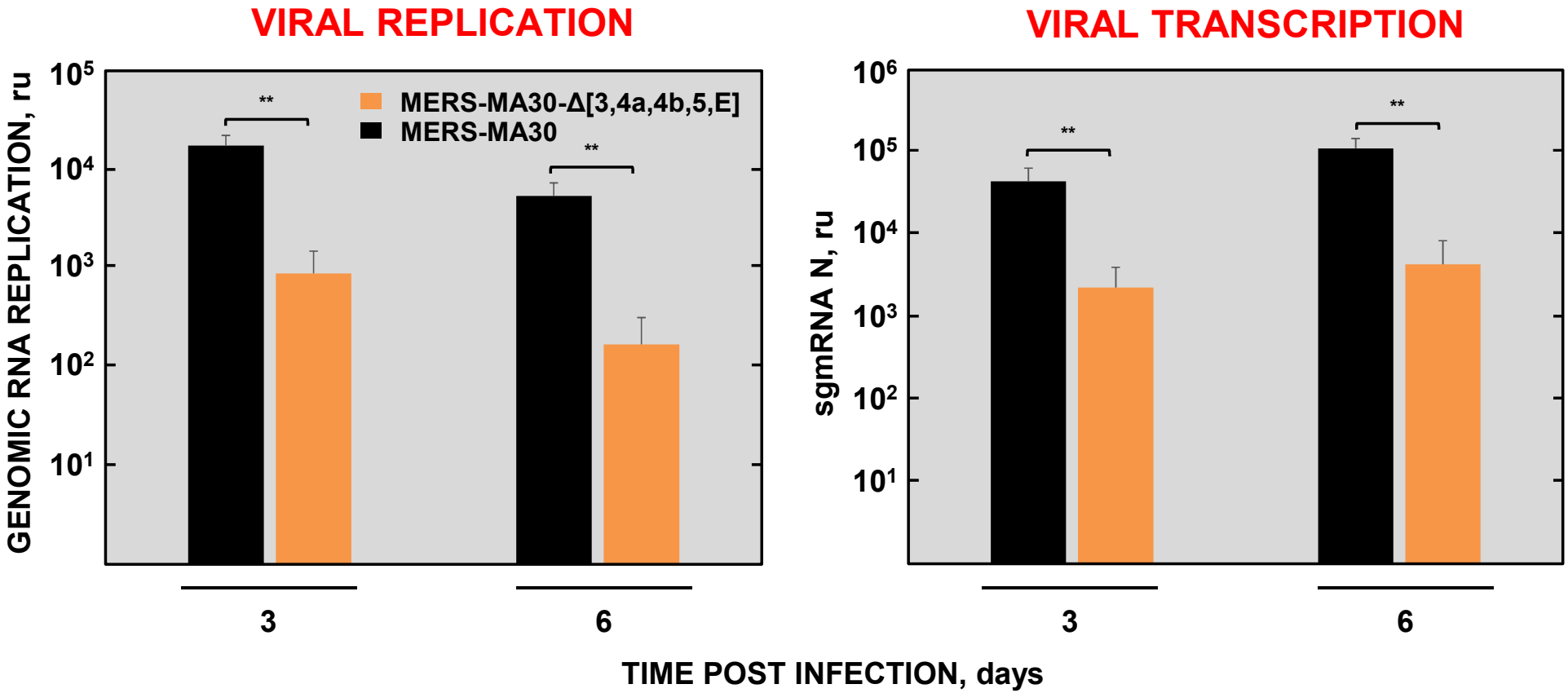


ENGINEEREING A MERS-MA REPLICON DERIVED FROM AN ATTENUATED VIRUS

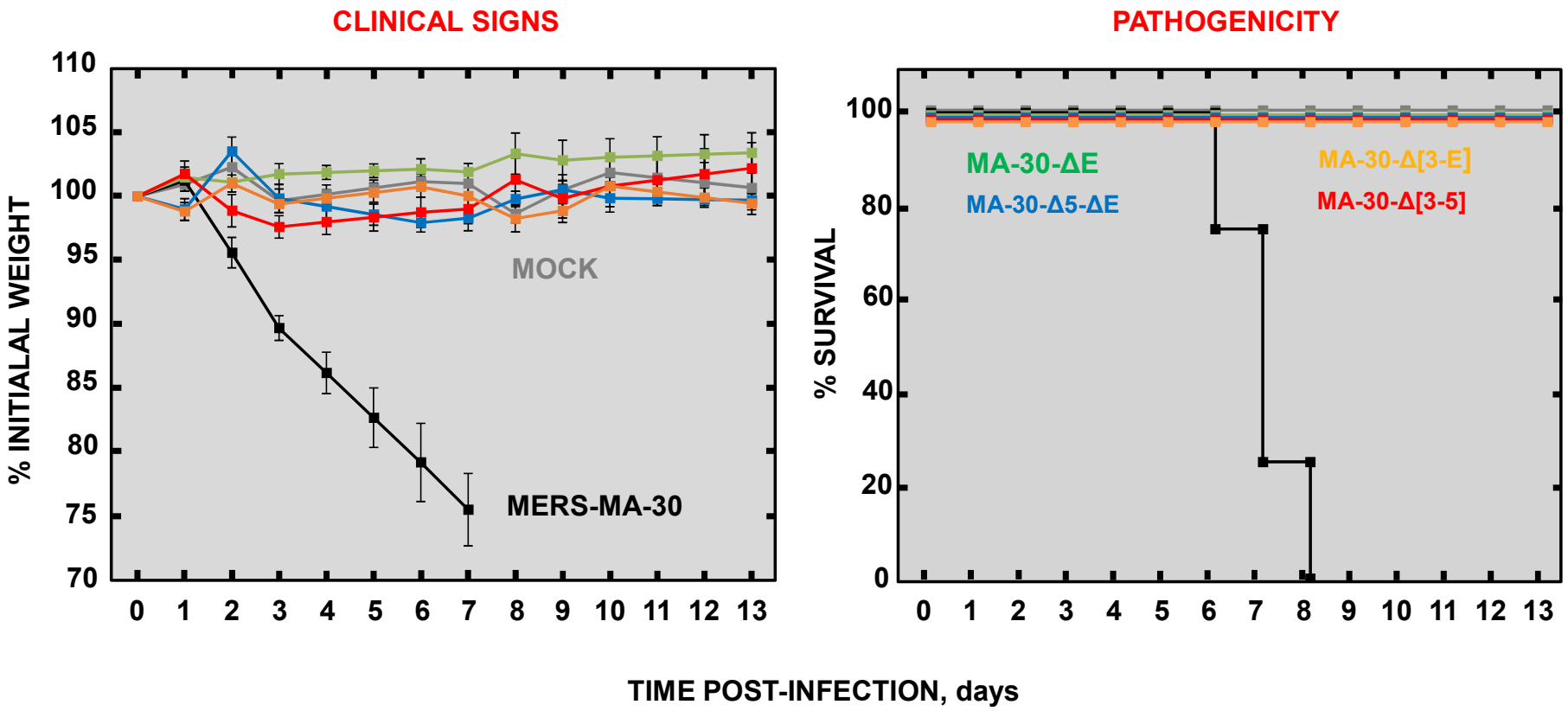


J. Gutierrez[1], J. M. Honrubia, Li Wang, S. Zuniga, I. Sola, L. Enjuanes. PNAS 2021

MERS-MA30-Δ[3,4a,4b,5,E] REPLICON IS AMPLIFIED IN KI MICE LUNG

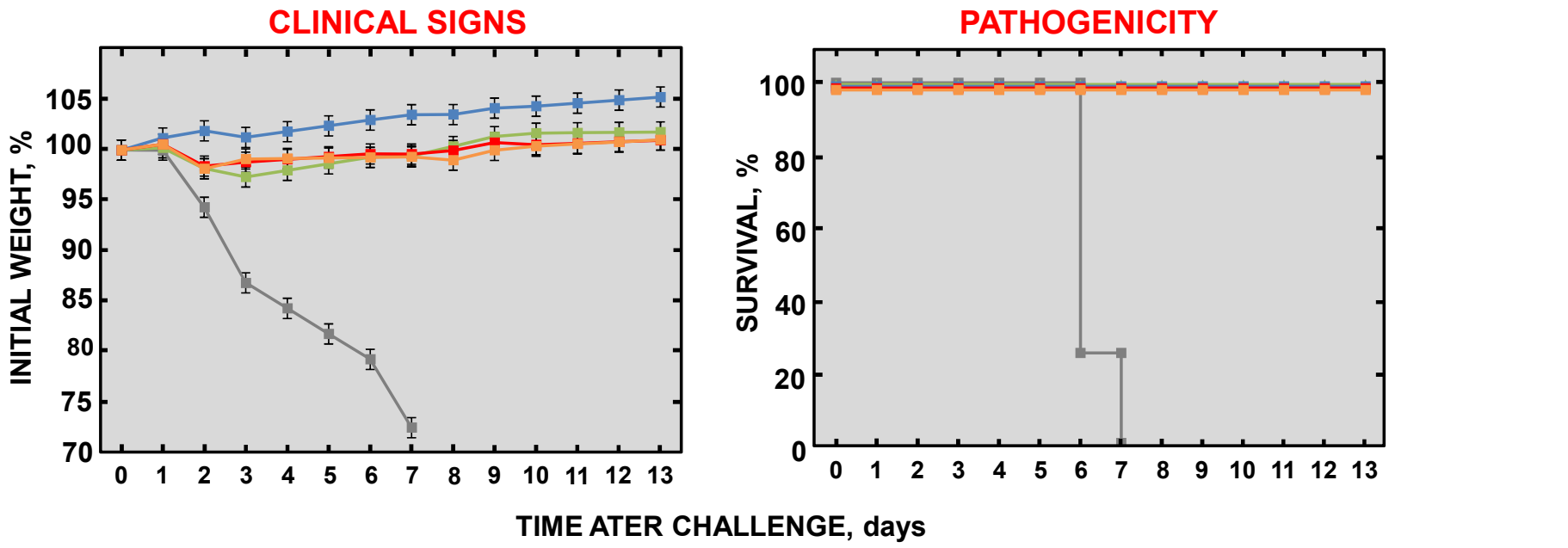


ATTENUATION OF MERS-CoV RNA REPLICONS

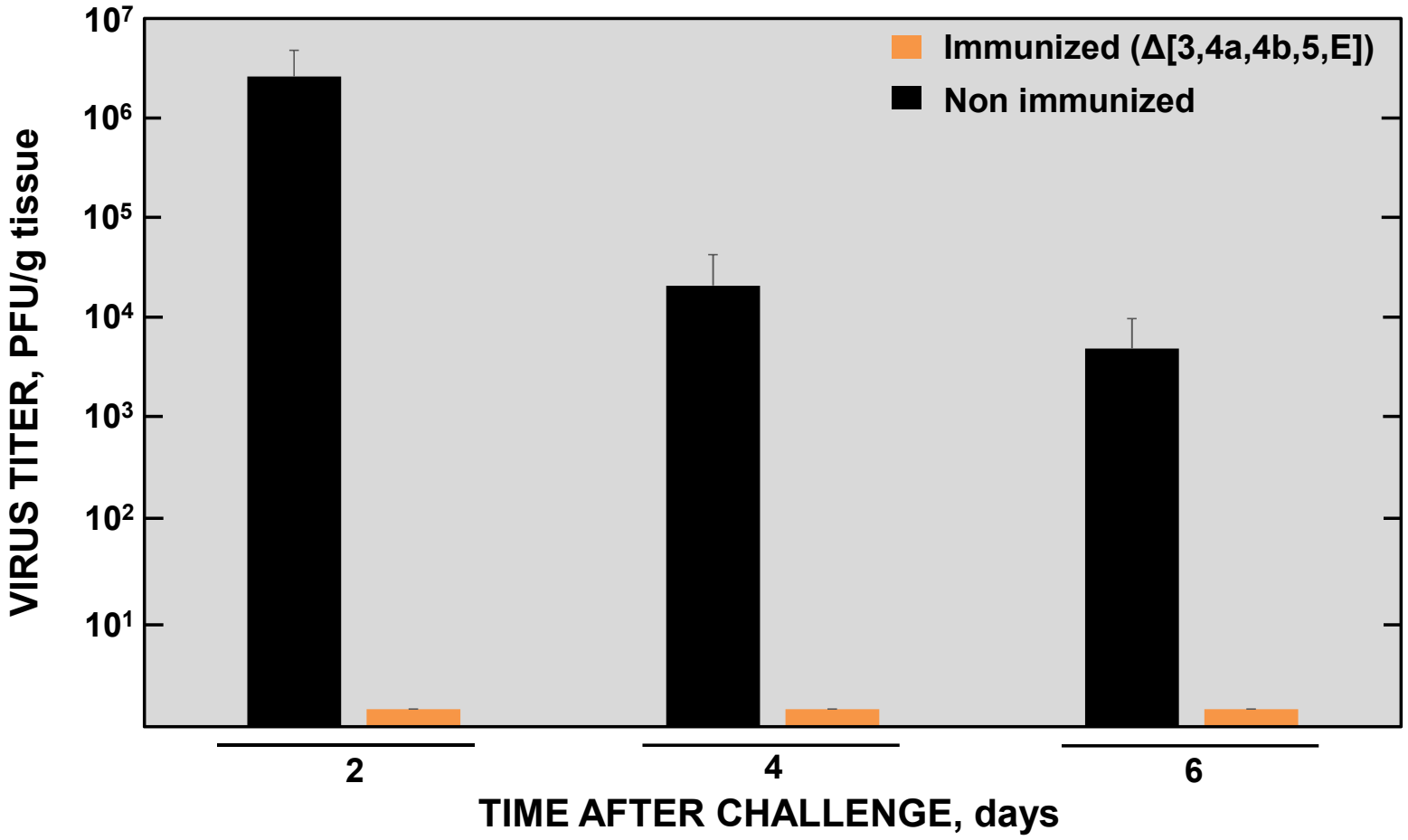


MERS-MA30 RNA REPLICON INDUCED PROTECTION IN KI MICE

CHALLENGE WITH 1 x 10^5 PFU/MOUSE IN IMMUNIZATION



MERS-MA30-Δ[3,4a,4b,5,E] REPLICON CONFERRED STERALIZING IMMUNITY IN KI MICE



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