

CORONAVIRUS EMERGENTES: VACUNAS ESTERILIZANTES

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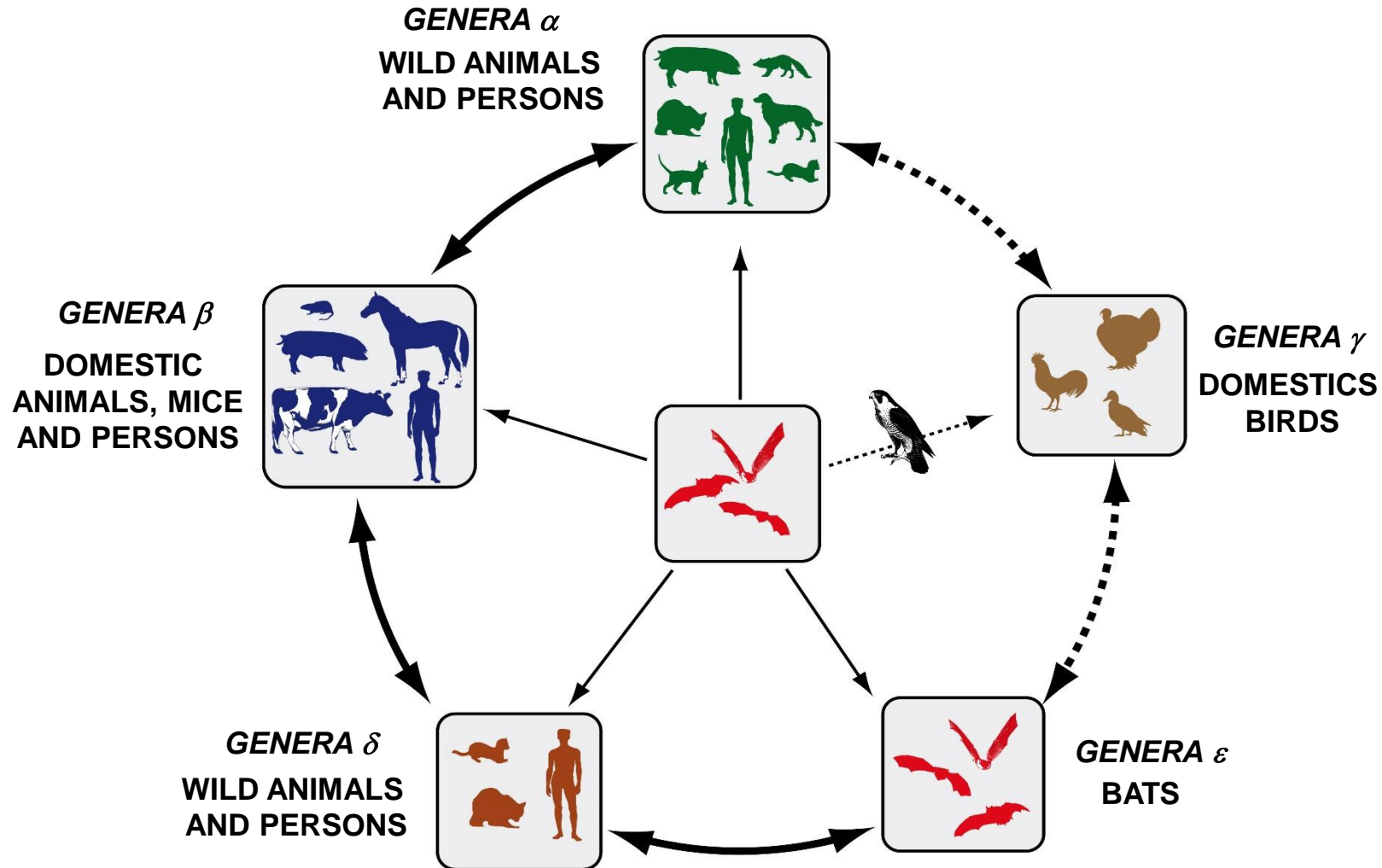
SOCIEDAD ESPAÑOLA DE FARMACIA

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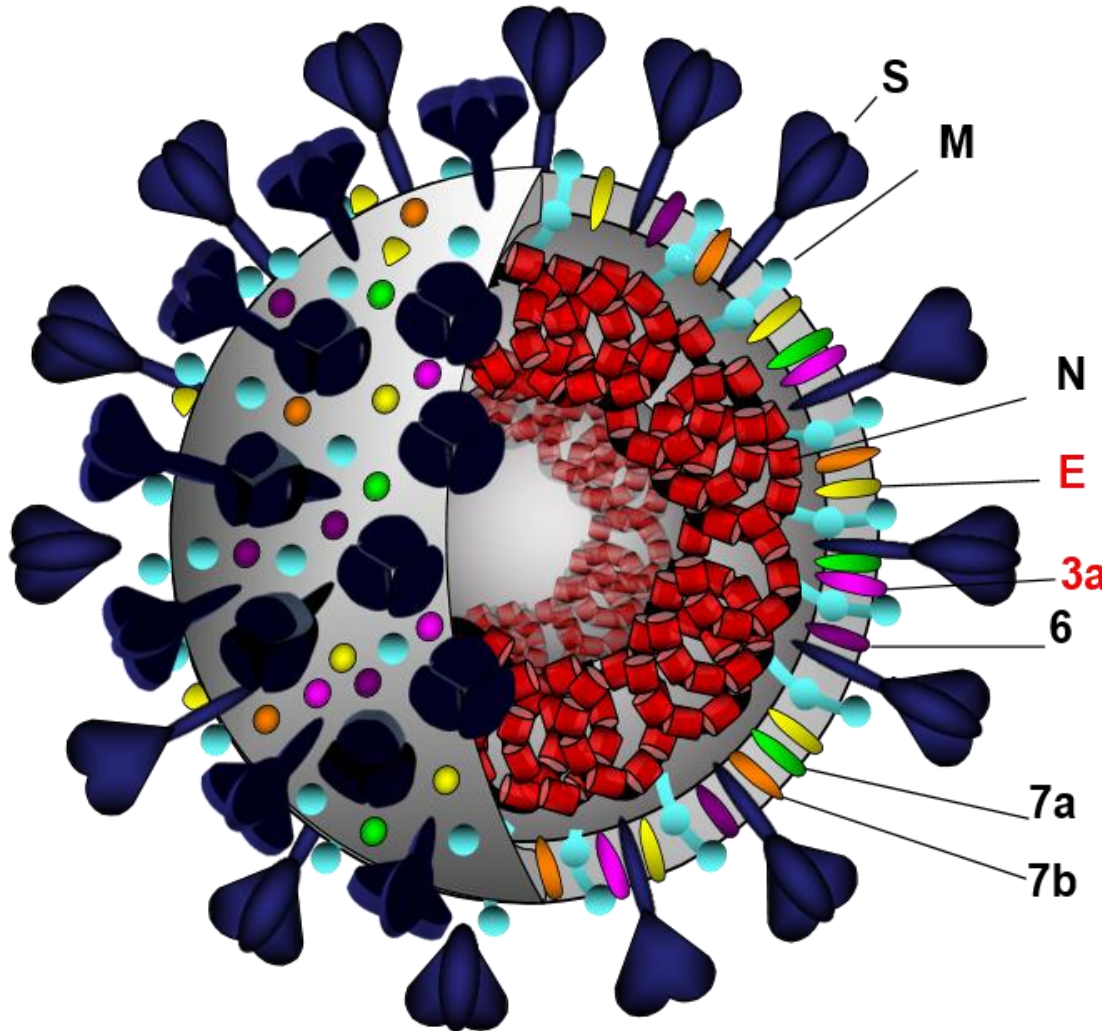
NOVEMBER 25th, 2022



POSTULATED CORONAVIRUS ECOLOGY



HUMAN CORONAVIRUSES



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

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	643x10 ⁶	6.6 x10 ⁶	<2%	235

WHO, NOVEMBER 21st, 2022

GEOGRAPHICAL ORIGIN OF SARS-CoV



SARS-CoV VECTORS

RACCOON
MAPACHE



CIVET CAT



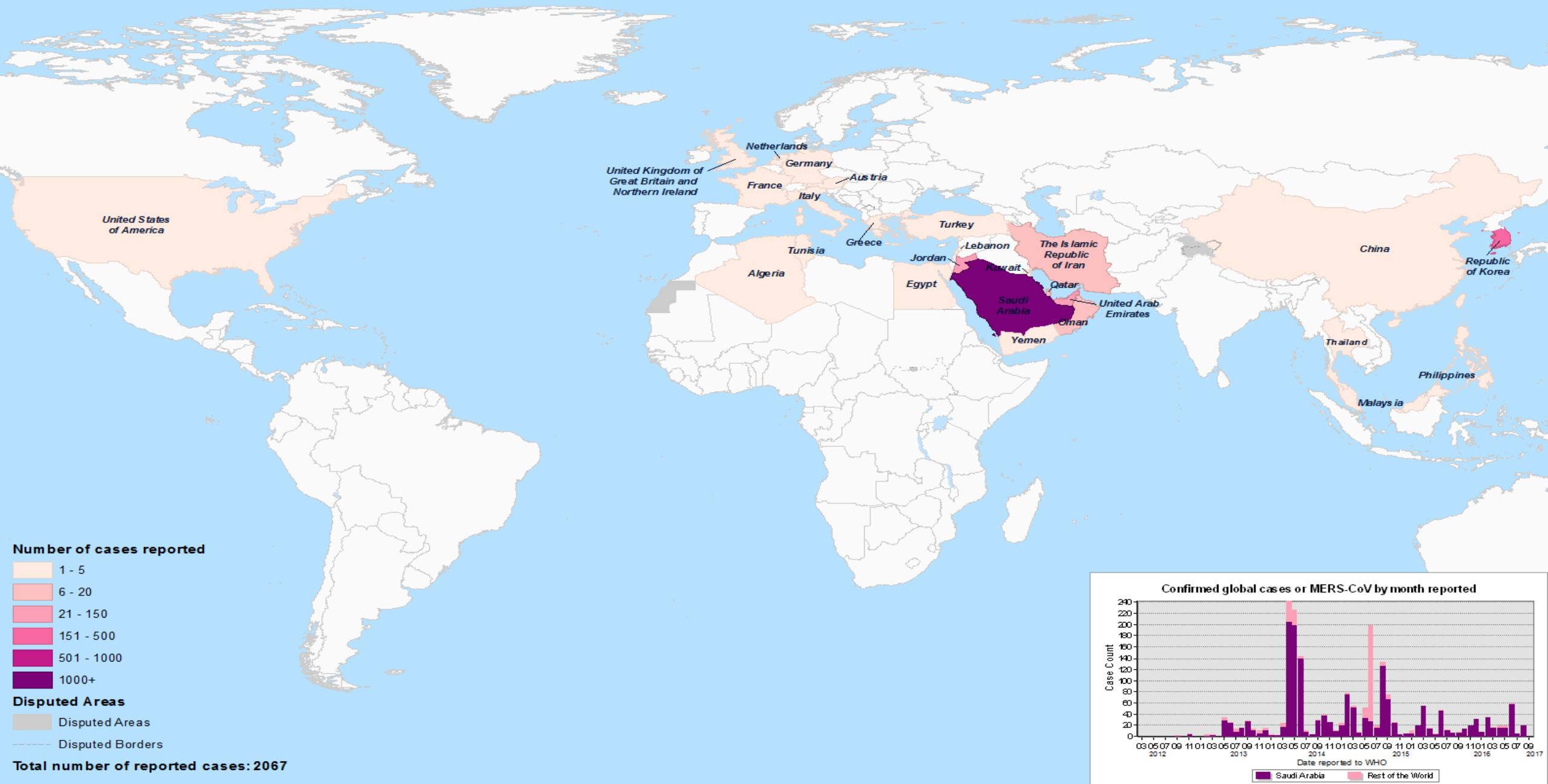
FERRET
VISON



BAT



MERS-CoV EMERGING IN 2012

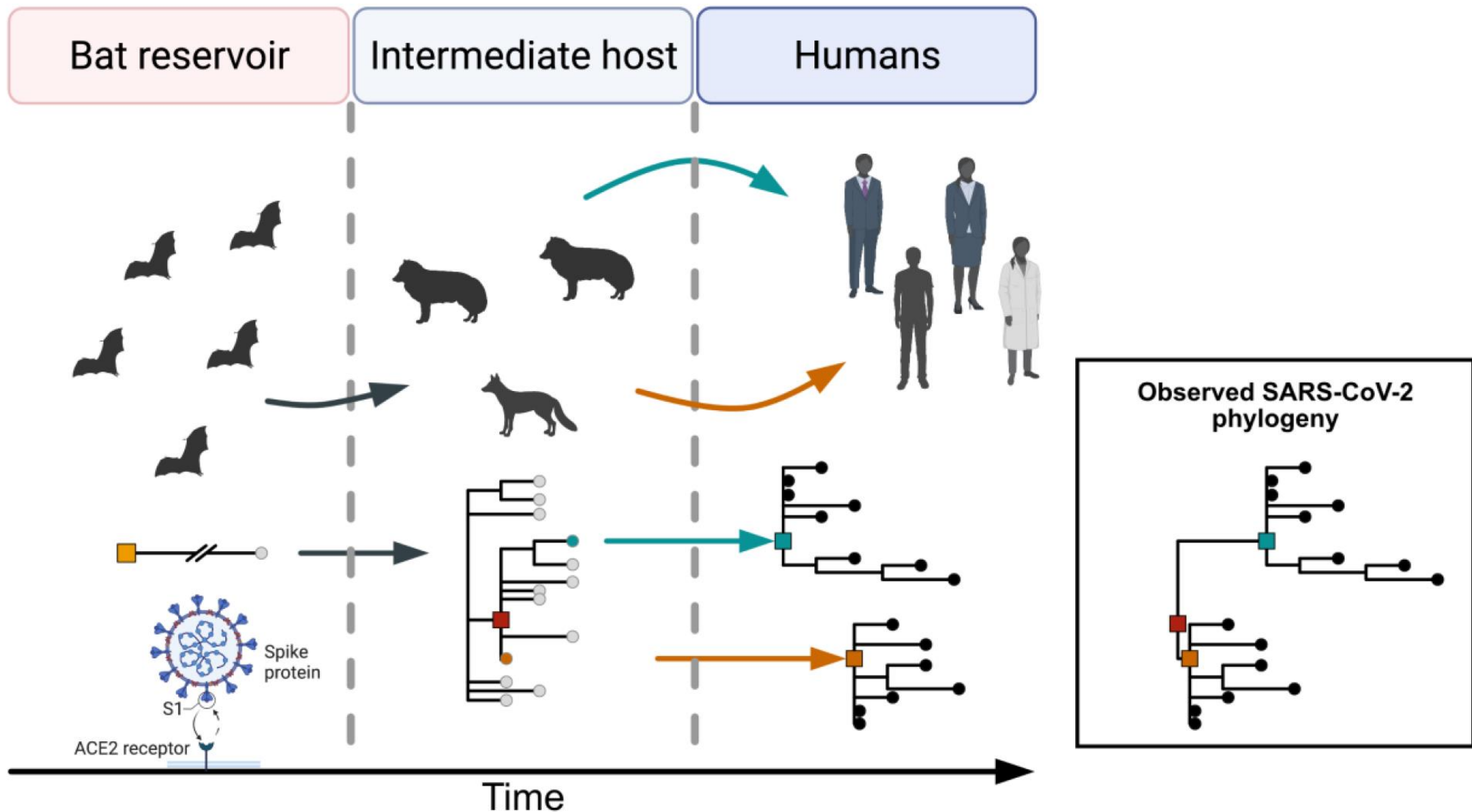


MERS-CoV IS TRANSMITTED BY CAMEL

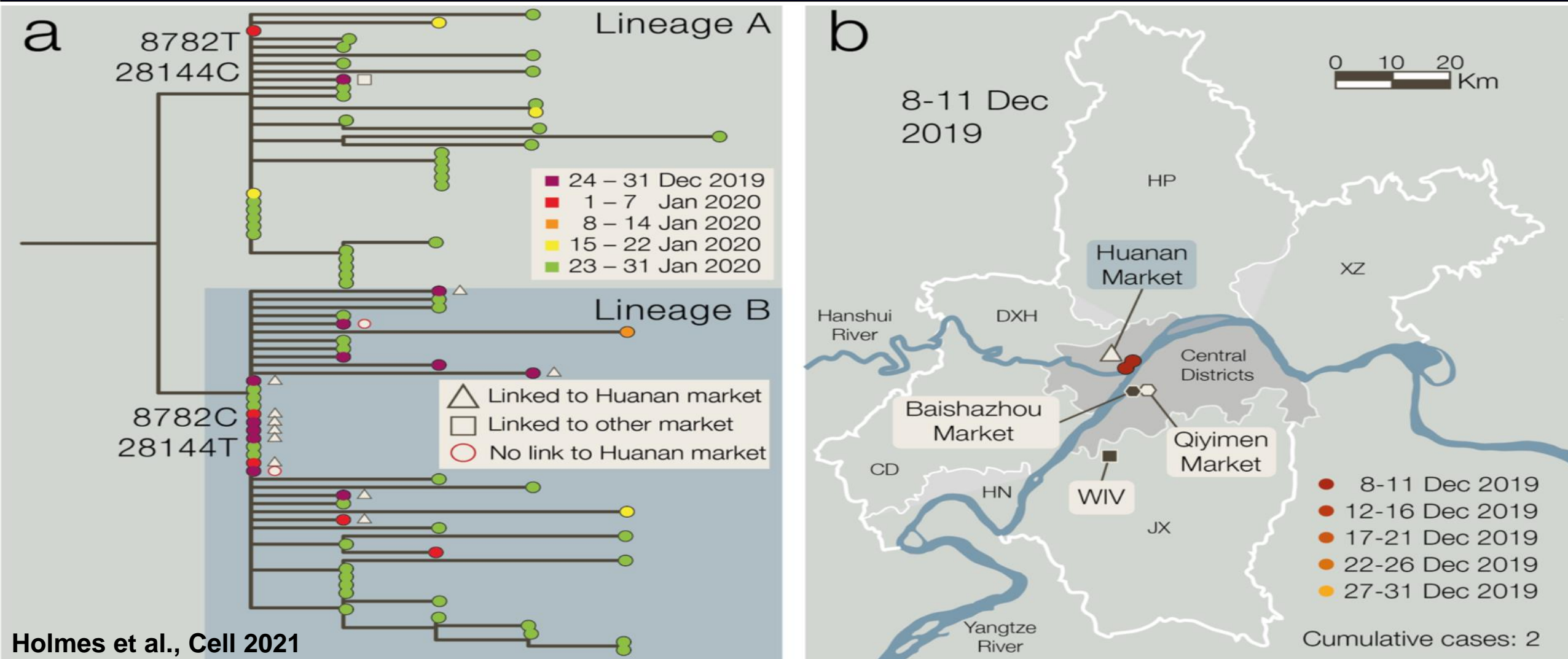


SARS-CoV-2 ORIGIN

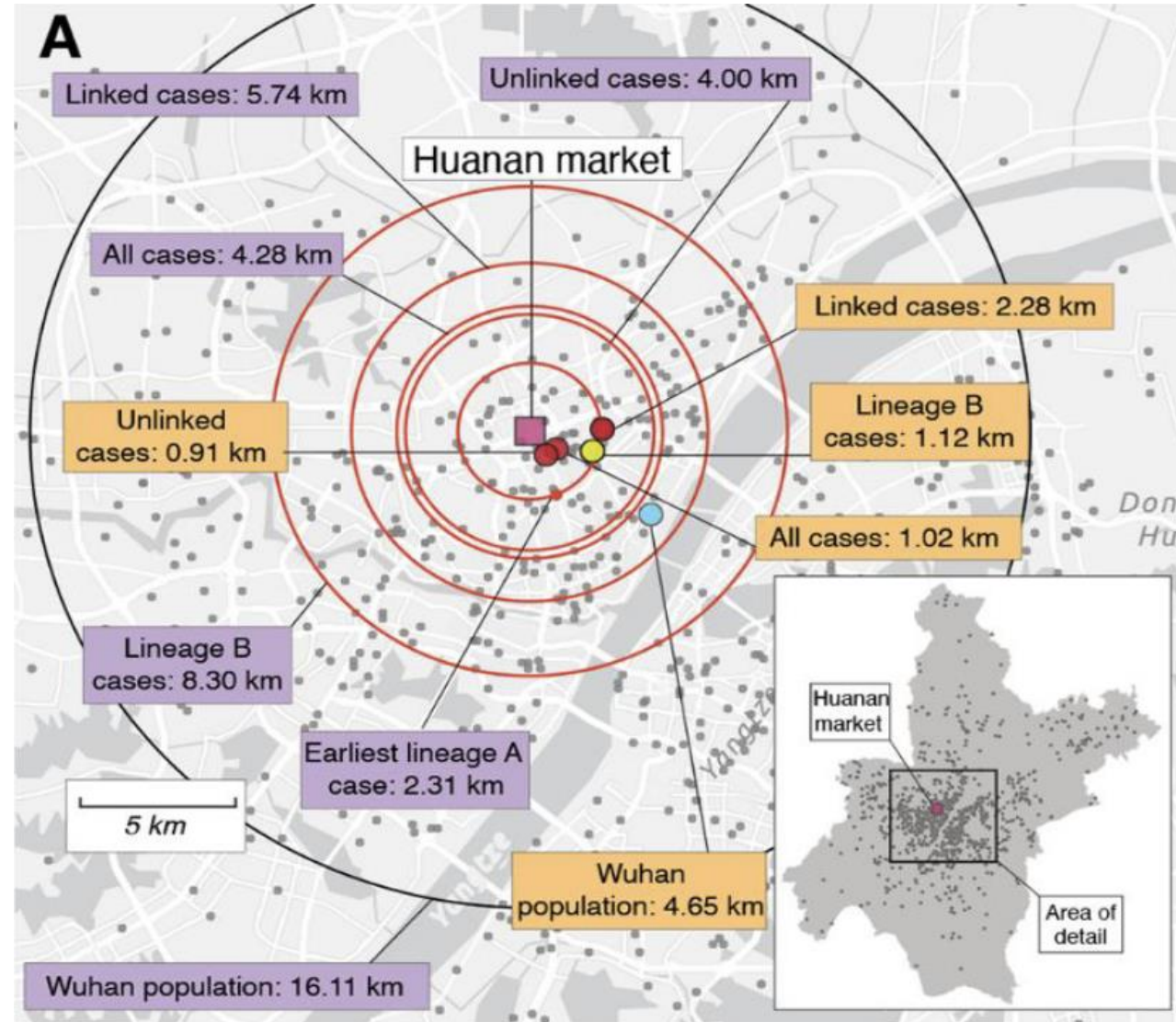
EVOLUTION AND INTERMEDIATE HOST OF SARS-CoV-2



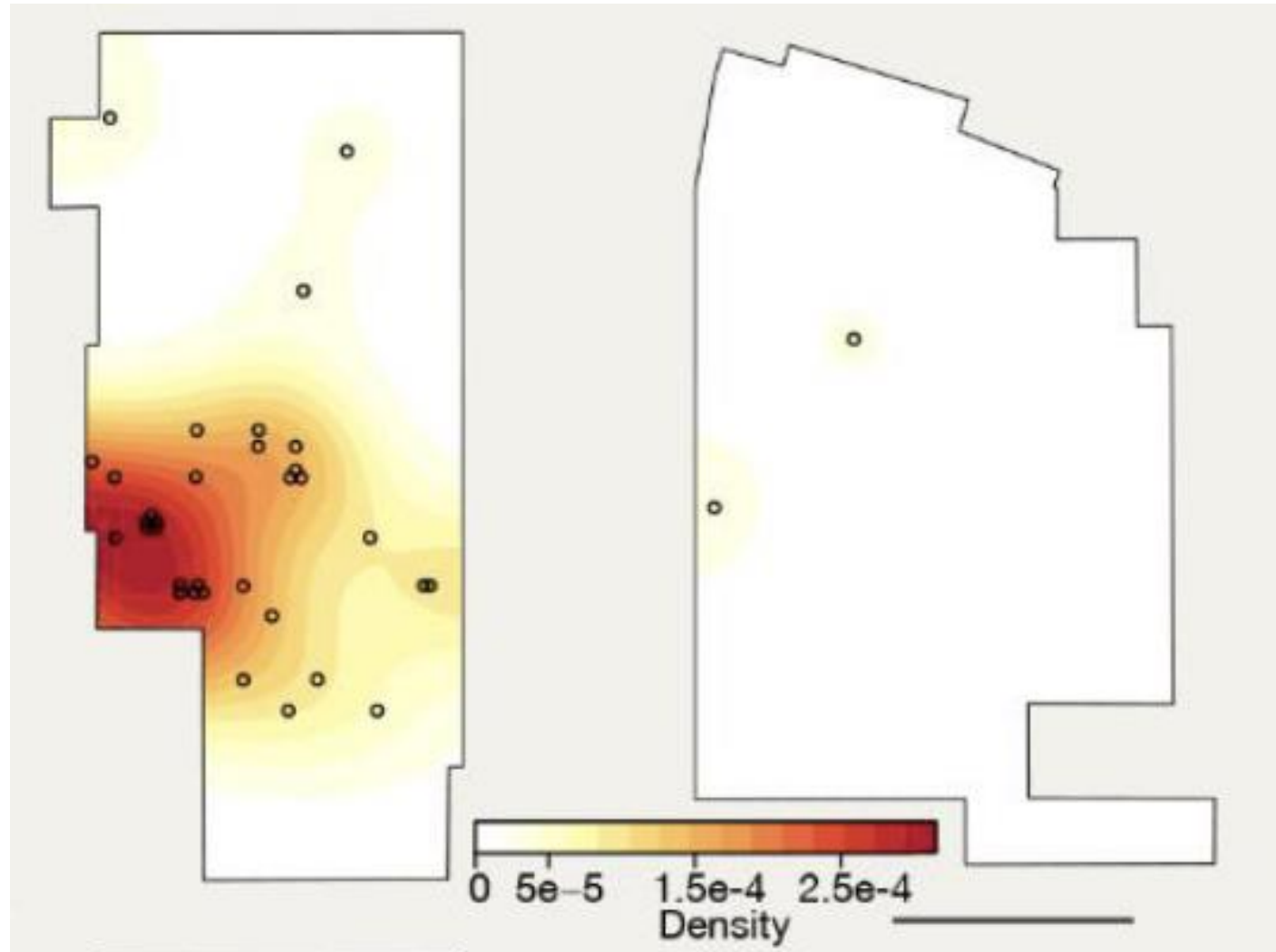
INITIAL DISSEMINATION OF SARS-CoV-2 IN WUHAN



DISTRIBUTION OF THE POSITIVE ENVIRONMENTAL SAMPLES IN HUANAN SEEFOOD MARKET



DISTRIBUTION OF THE POSITIVE ENVIRONMENTAL SAMPLES IN HUANAN SEEFOOD MARKET



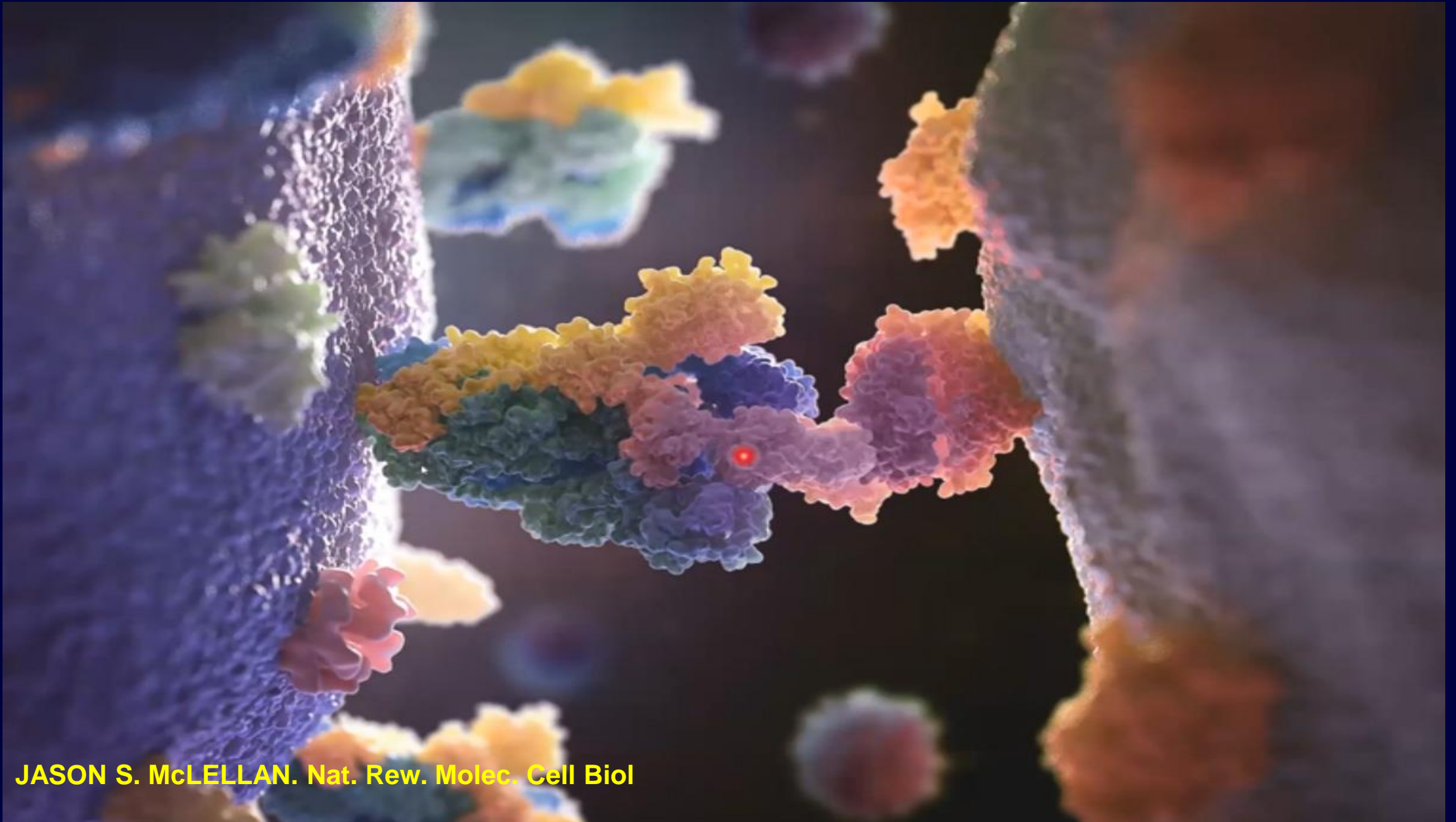
SARS-CoV-2 ORIGIN: RACCOON DOGS



CONCLUSION ON SARS-CoV-2 ORIGIN

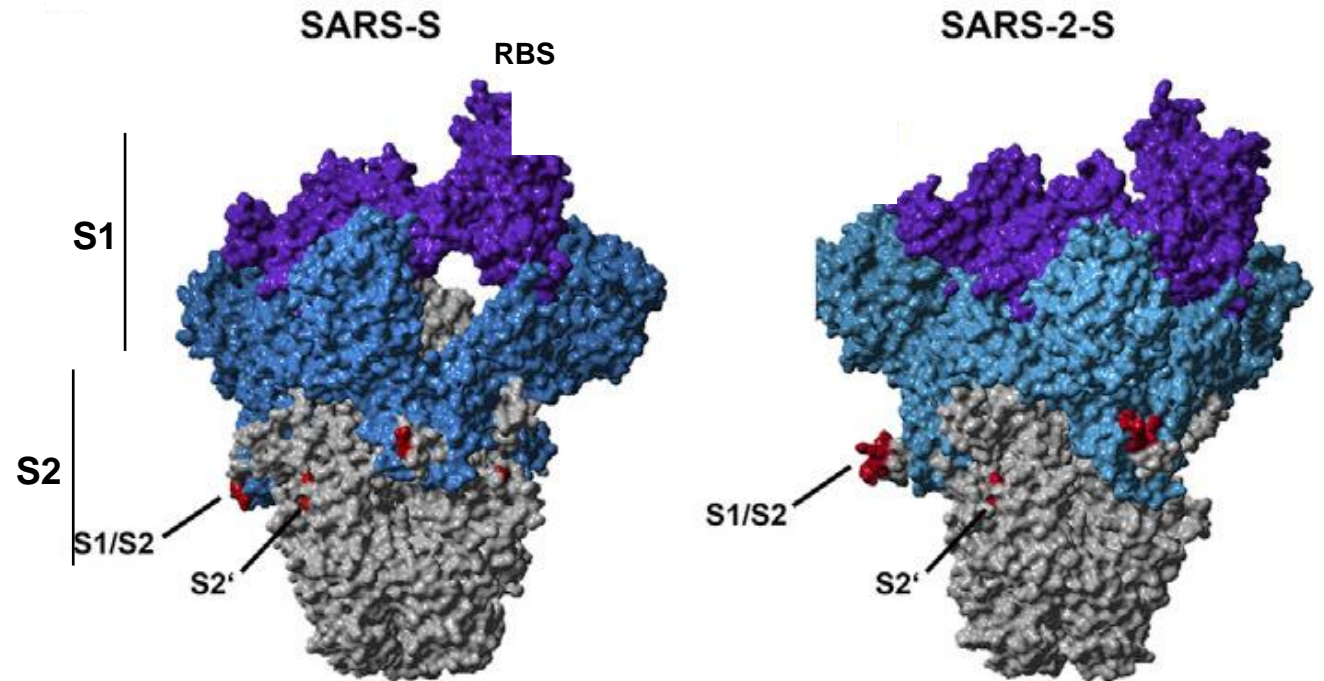
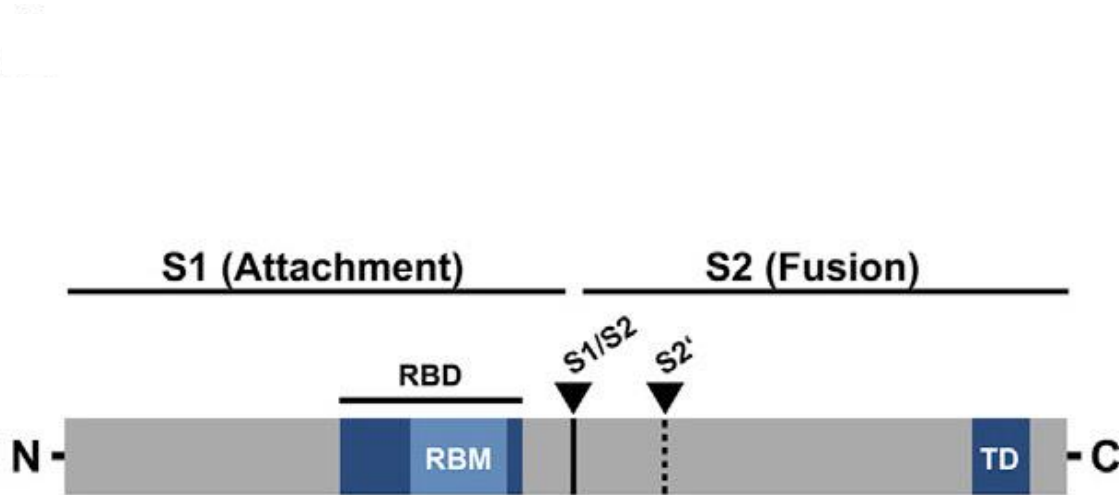
- THE MOST DIRECT EXPLANATION FOR THE ORIGIN OF SARS-CoV-2 IS A ZOO NOTIC EVENT
- THERE IS CURRENTLY NO EVIDENCE THAT SARS-CoV-2 HAS A LABORATORY ORIGIN
- BIOLOGICAL MATERIAL FROM HUANAN MARKET RACOONS INCLUDED CoVS WITH A **SEQUENCE 99.993 IDENTICAL** TO THAT OF HUMAN SARS-CoV-2, INDICATING THAT MOST LIKELY THEY TRANSMITTED THE VIRUS TO HUMANS

SARS-CoV-2 CELL INTERACTION



JASON S. McLELLAN. Nat. Rev. Molec. Cell Biol

SARS-CoV-2 PROTEOLYTIC CLEAVAGES



- THE **S1/S2 SITE** IN SARS-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

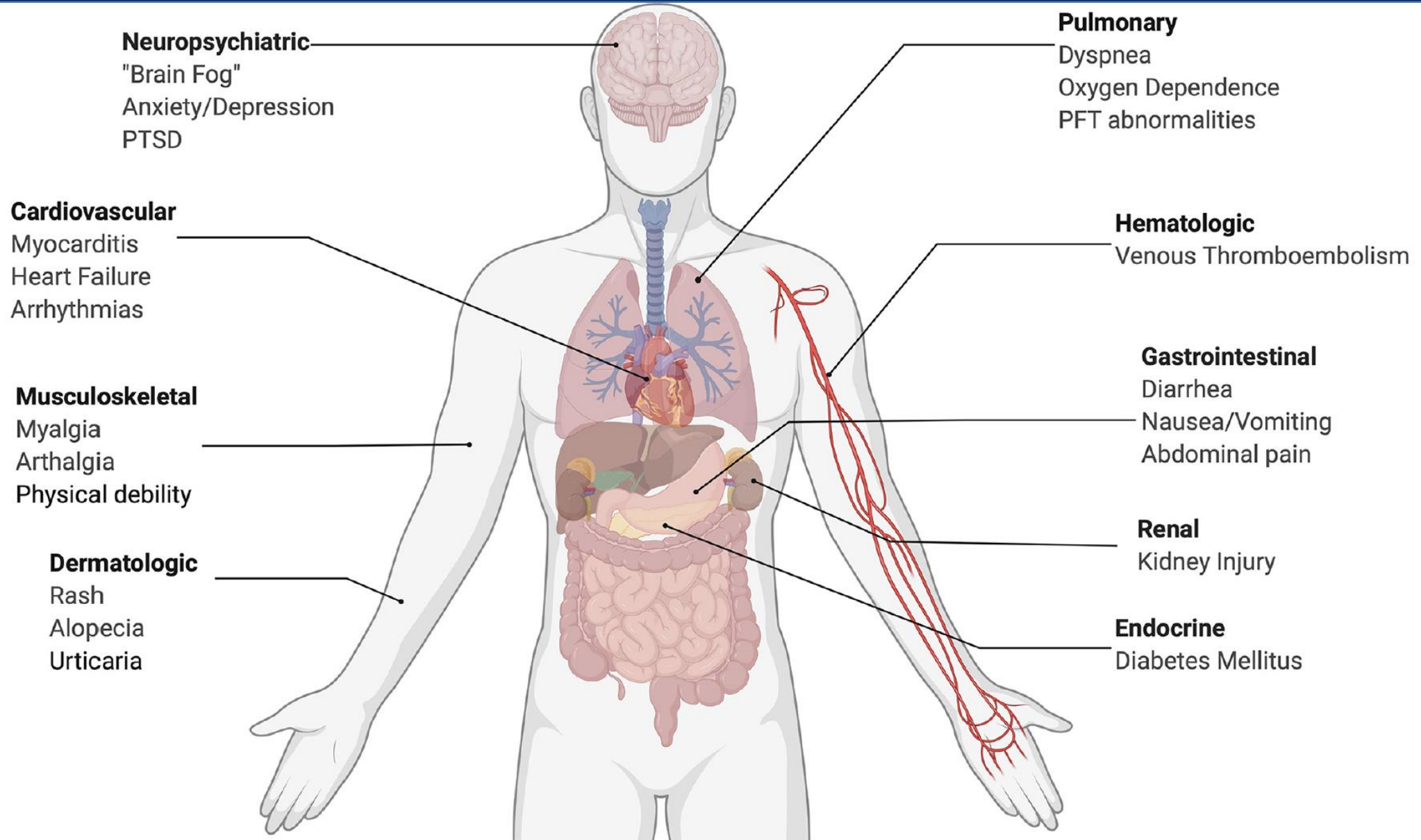
SARS-CoV-2 INDUCED HIGH PATHOLOGY: MANY ORGANS AFFECTED

- **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 ARRHYTHMIAS INFLAMMATION MYOCARD
- **VEINS** EXTENSIVELY CLOTHING. PLATELETS, STROKES
- **SMELL LOSS** NASAL EPITHELIUM HIGHEST ACE-2
- **TONG** KERATINOCYTES
- **PANCREAS** IN SEVERE DISEASE

LONG-TERM COMPLICATIONS AFTER SARS-CoV-2 INFECTION

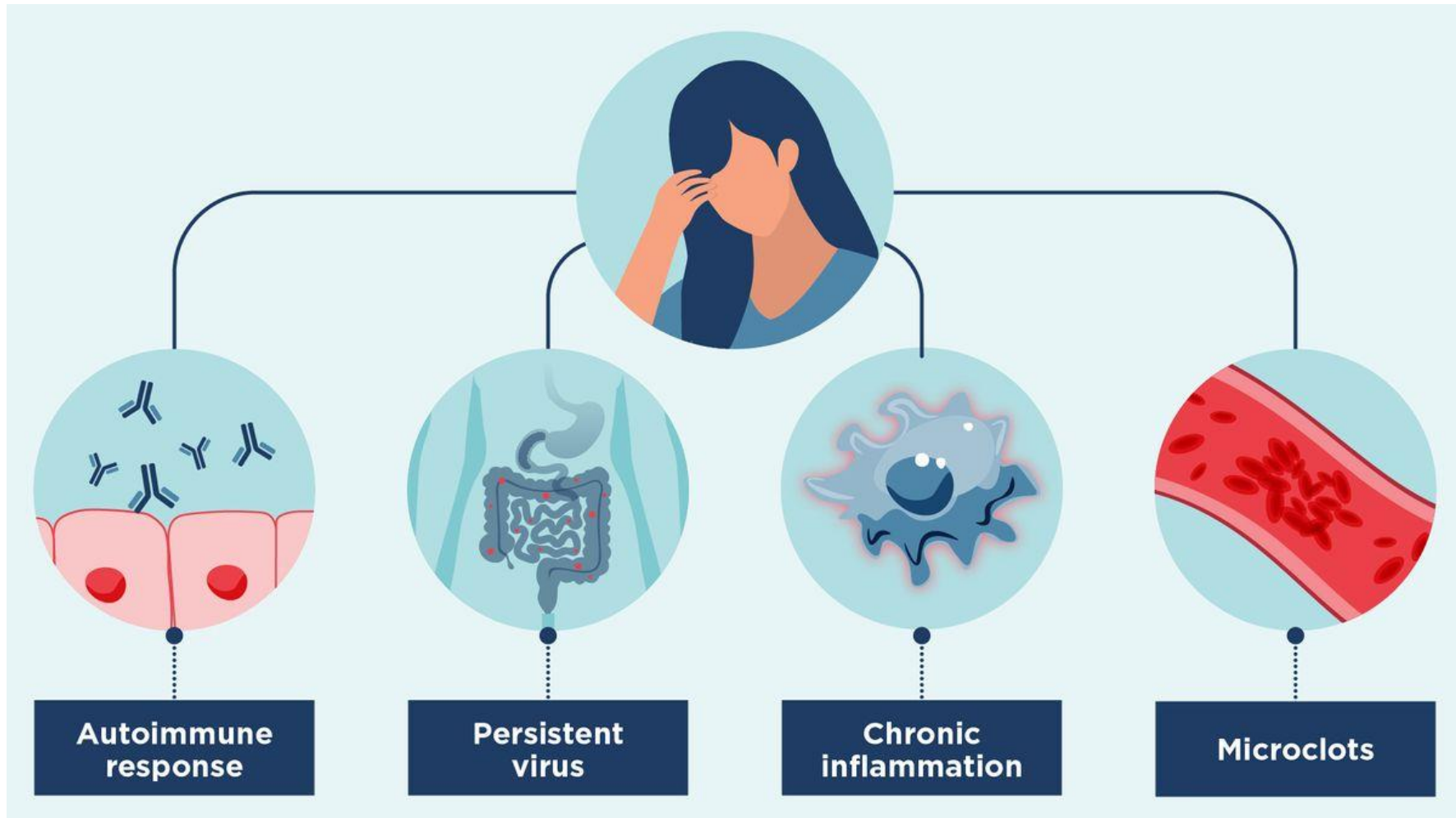
- CARDIOVASCULAR**
- NEUROLOGICAL**
- PSYCHOLOGICAL**
- HEMATOLOGICAL**
- PULMONARY**
- DERMATOLOGICAL**
- OTHER INJURIES**

CONSEQUENCES OF LONG-TERM COMPLICATIONS



POTENTIAL MECHANISMS OF LONG-TERM COVID

USA
PERSISTENT
SYMPTOMS
ARISE IN 20%
OF INFECTED
PEOPLE

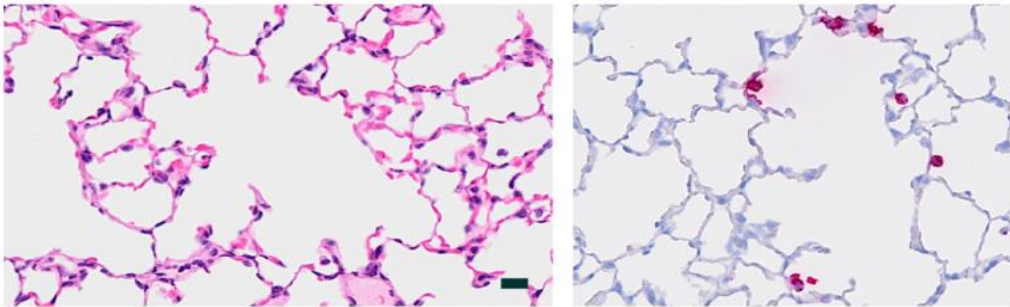


LUNG OF SARS-CoV-2 INFECTED HAMSTERS

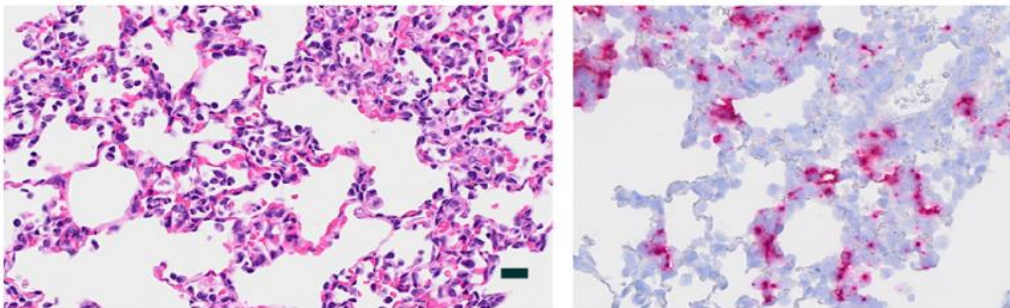
INFECTED WITH DELTA VIRUS

Alveoli

Day 3

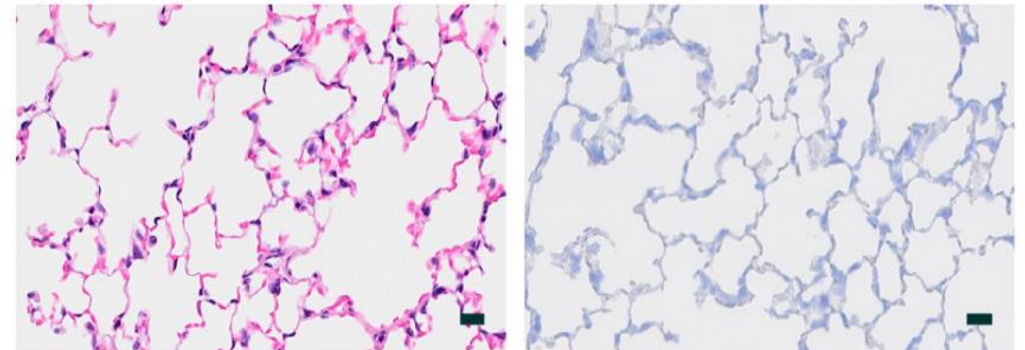


Day 6

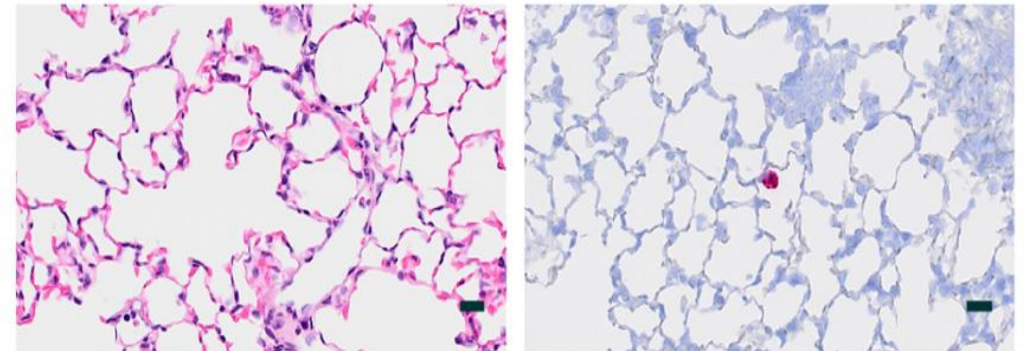


INFECTED WITH OMICRON VIRUS

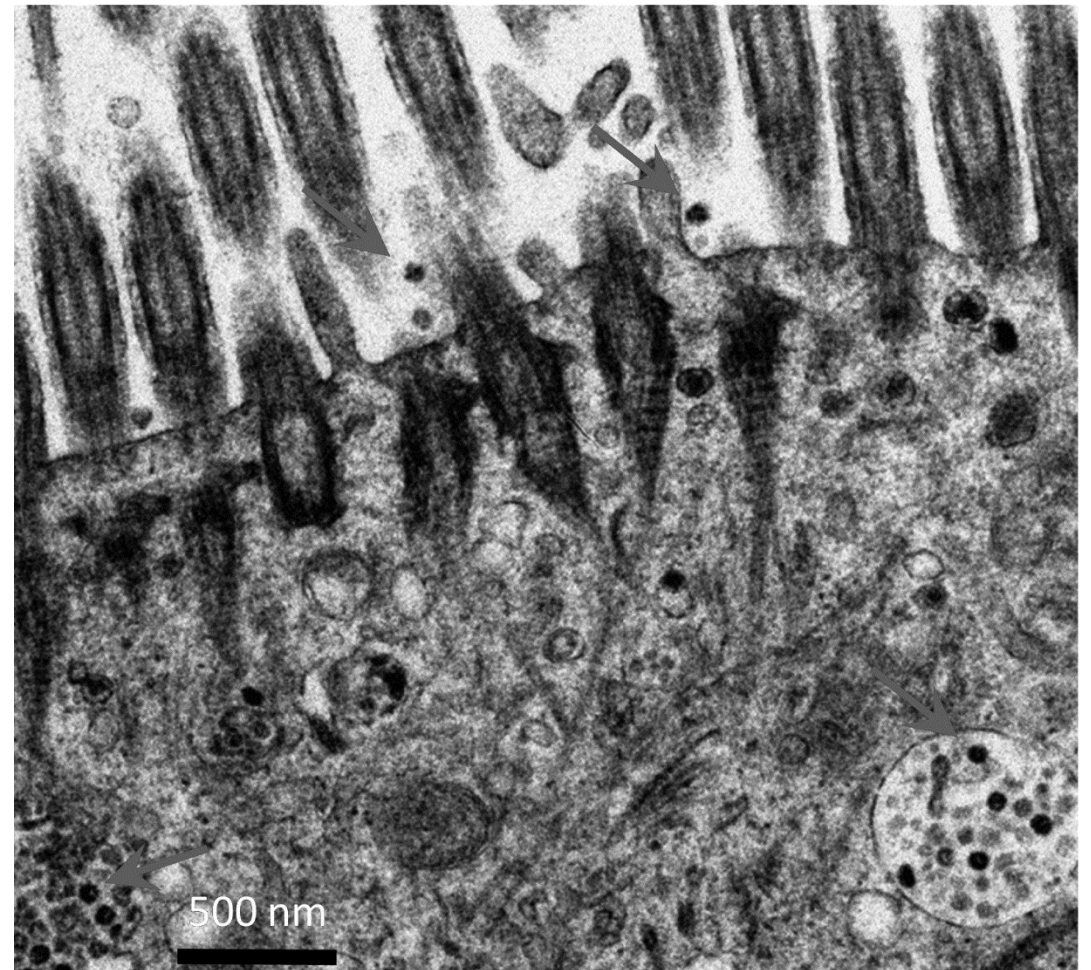
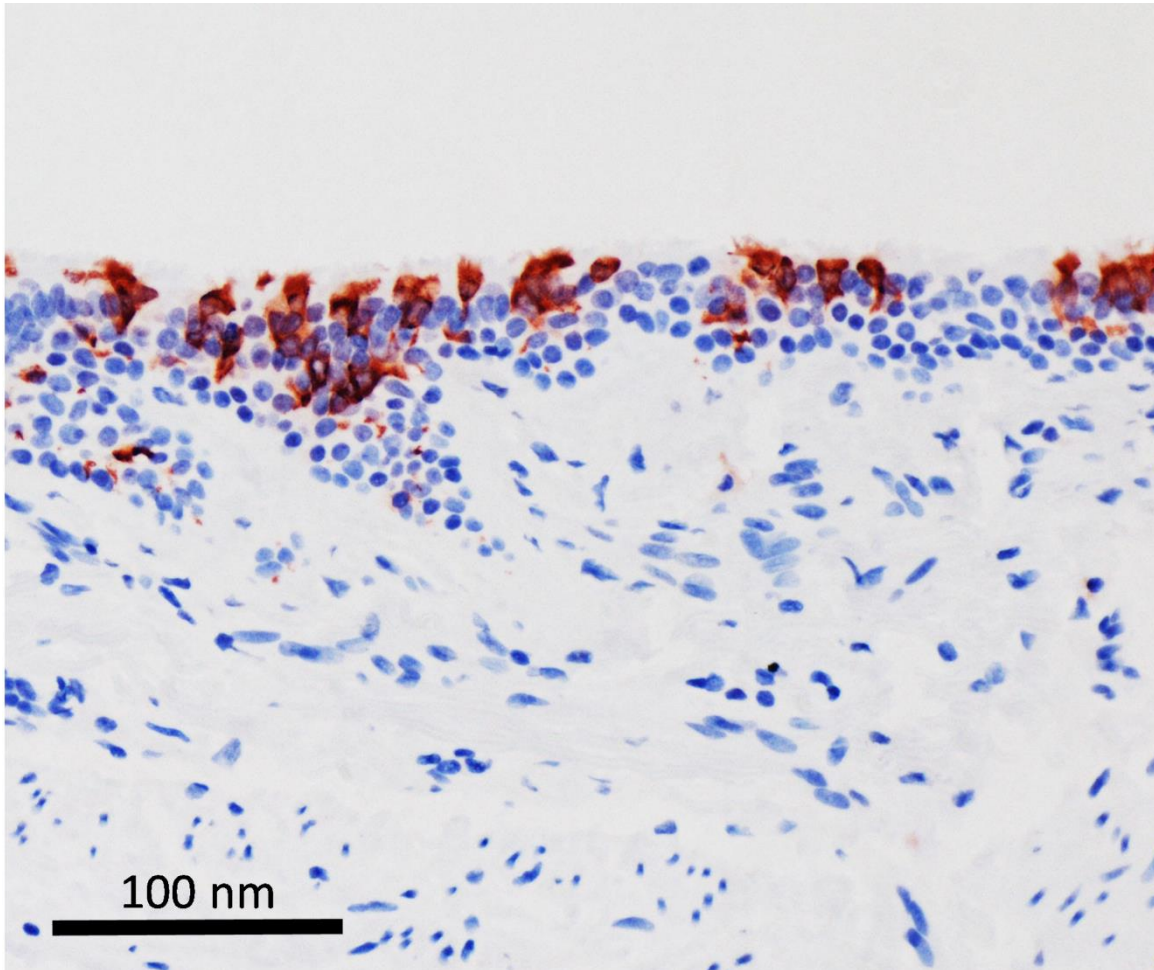
Day 3



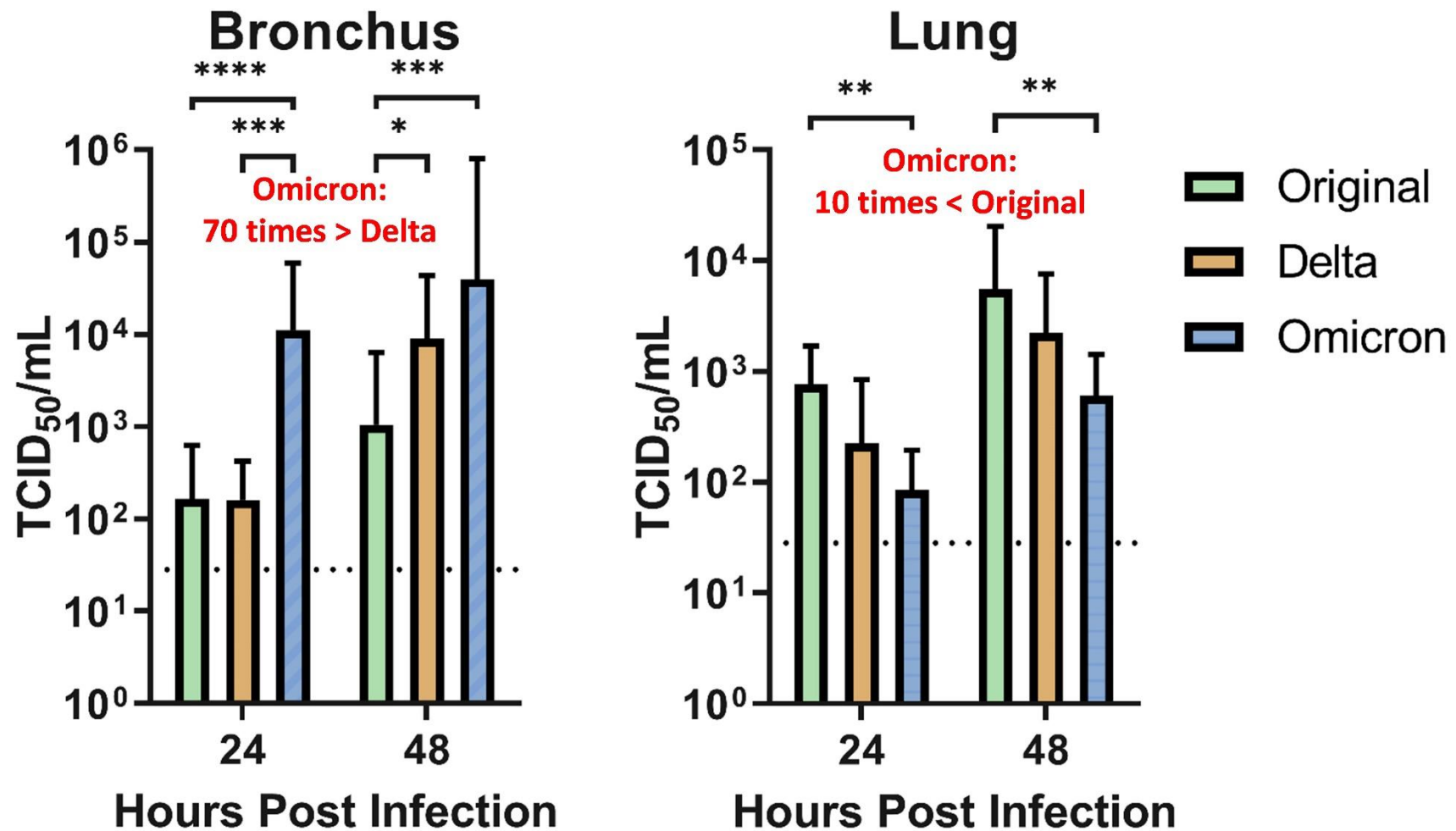
Day 6



OMICRON INFECTION OF HUMAN BRONCHUS



GROWTH OF OMICRON CoV IN HUMAN RESPIRATORY TRACT



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



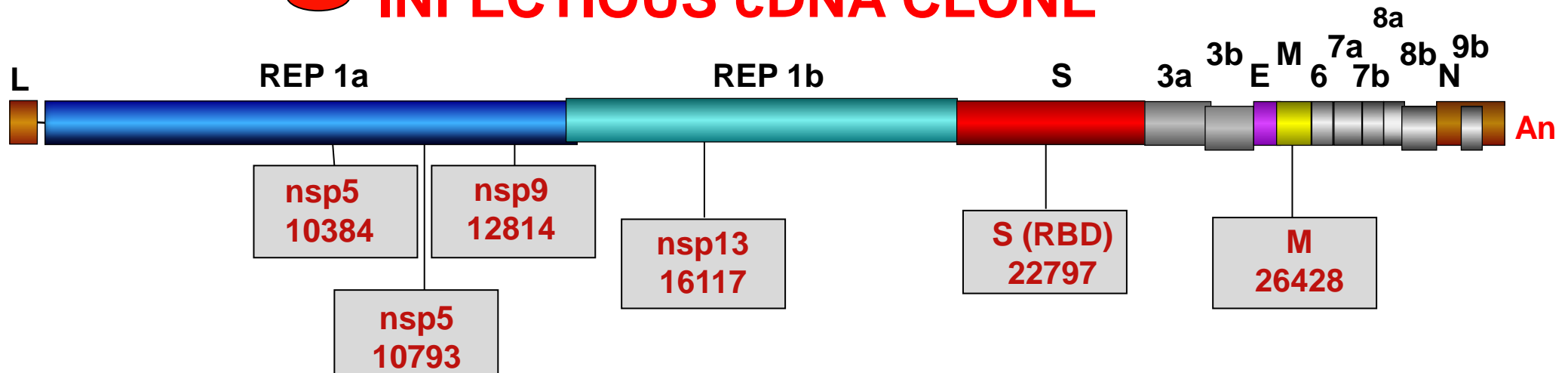
SELF-AMPLIFYING: STRONG IMMUNE RESPONSE
PROPAGATION DEFICIENT: **SAFE**

OBJECTIVES

- **MOLECULAR BASES OF VIRULENCE: VACCINES**

TOOLS

● INFECTIOUS cDNA CLONE



● ANIMAL MODEL

- The virus produces pulmonar pathology
- Death induction

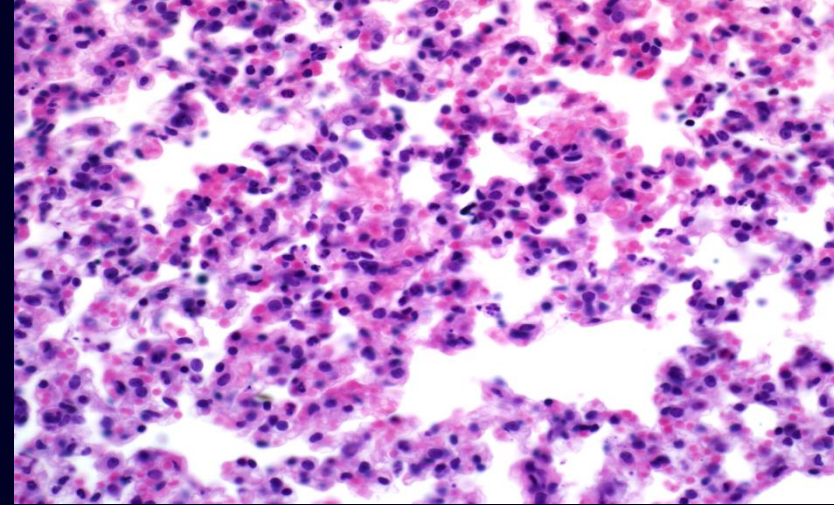
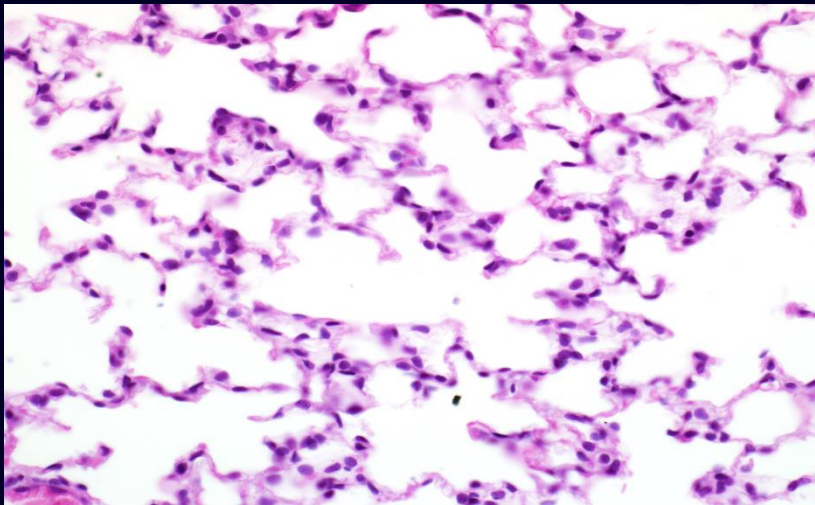


LUNG PATHOLOGY ASSOCIATED TO MICE INFECTION BY SARS-CoV-MA15

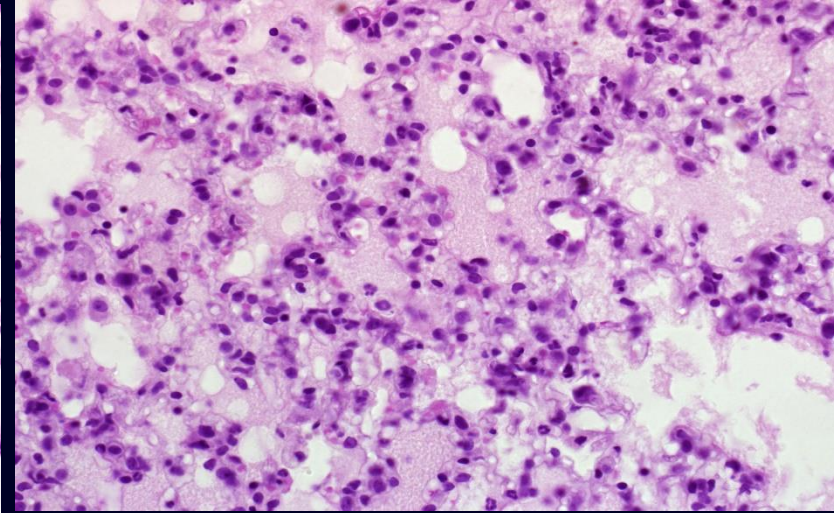
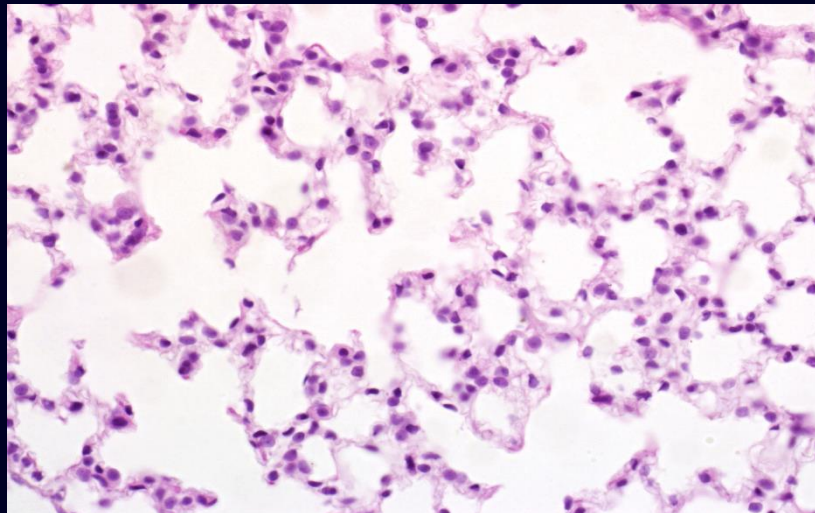
ΔE

WT-E⁺

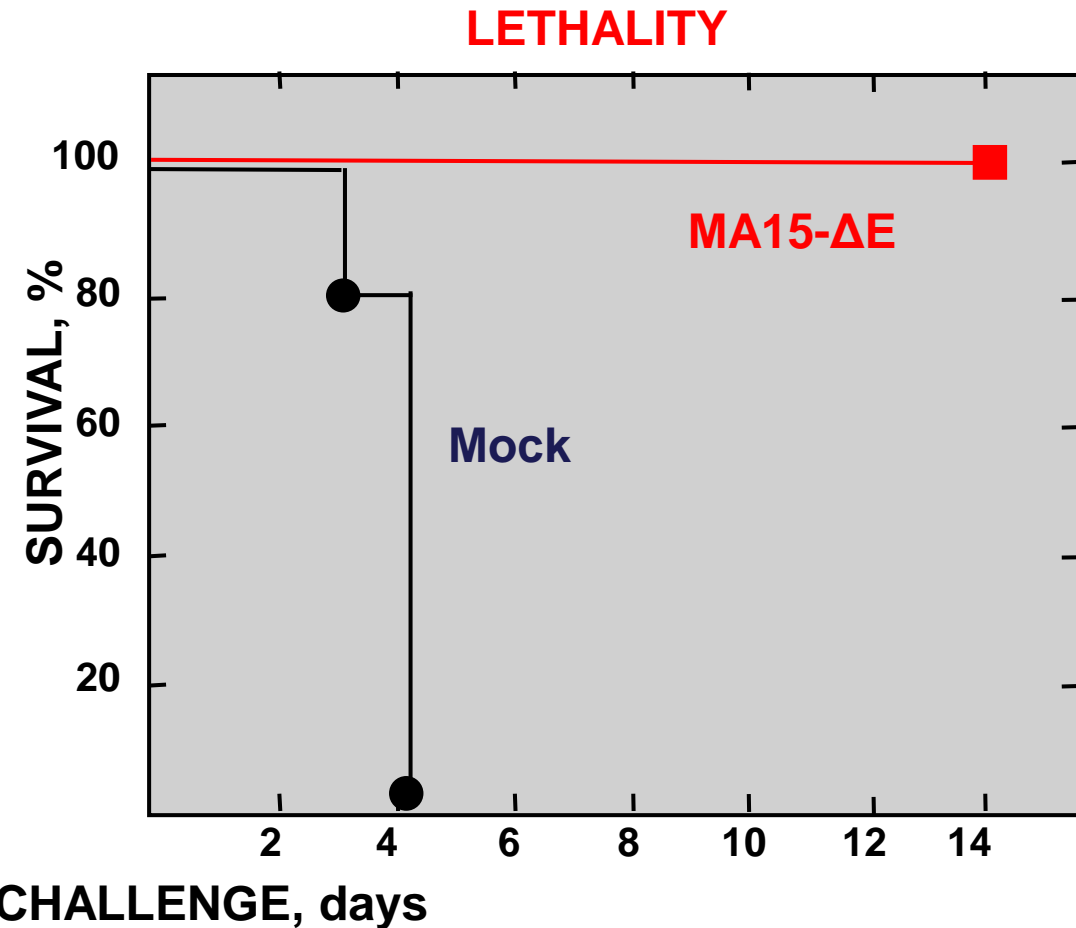
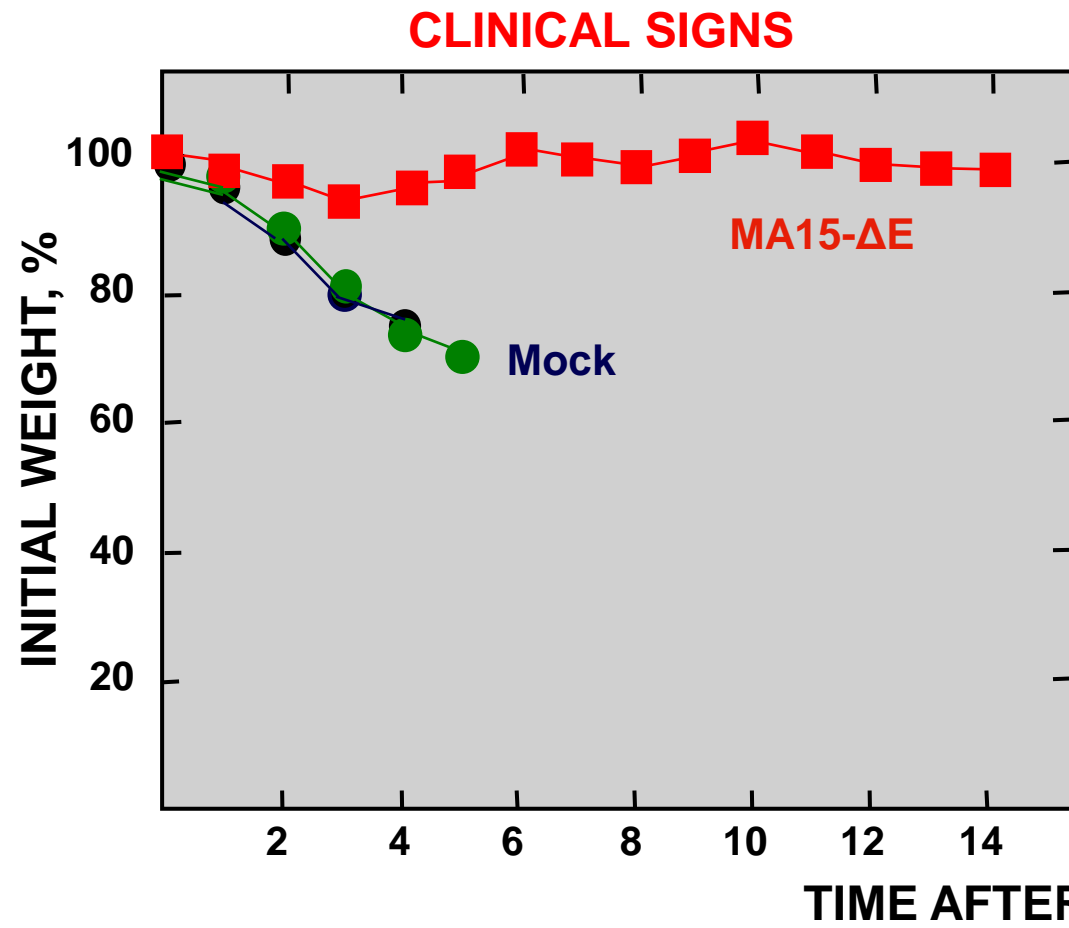
4 DDI



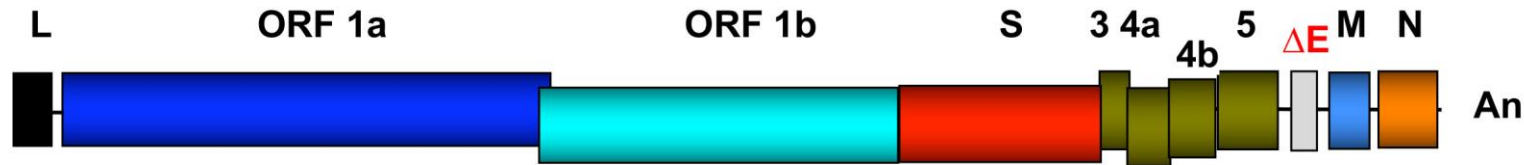
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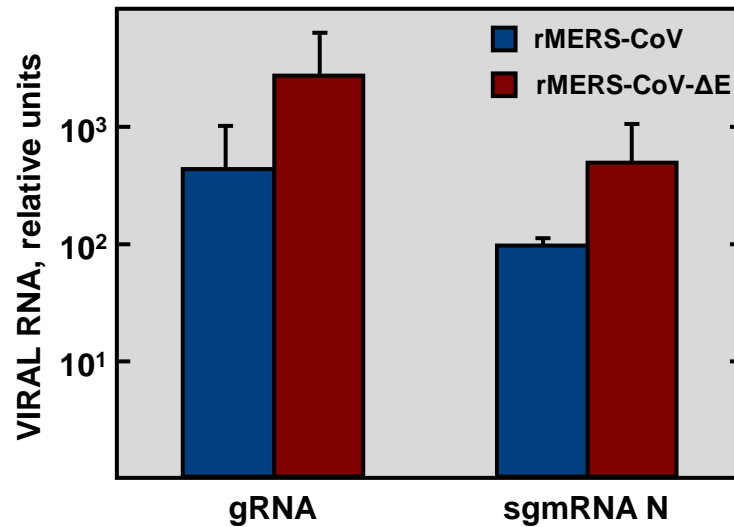
PROTECTION PROVIDED BY AN ATTENUATED SARS-CoV E PROTEIN DELETION MUTANT



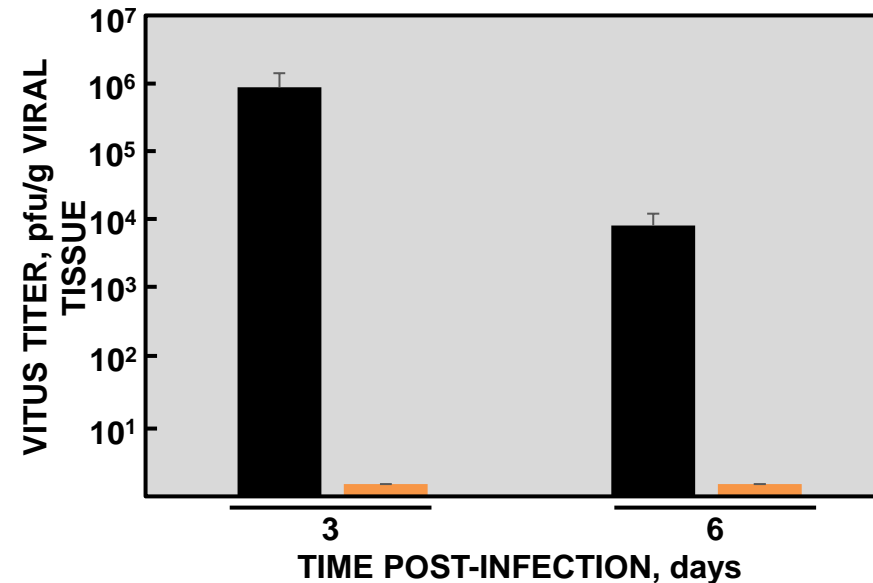
MERS-CoV- ΔE REPLICATION-COMPETENT DISSEMINATION-DEFICIENT



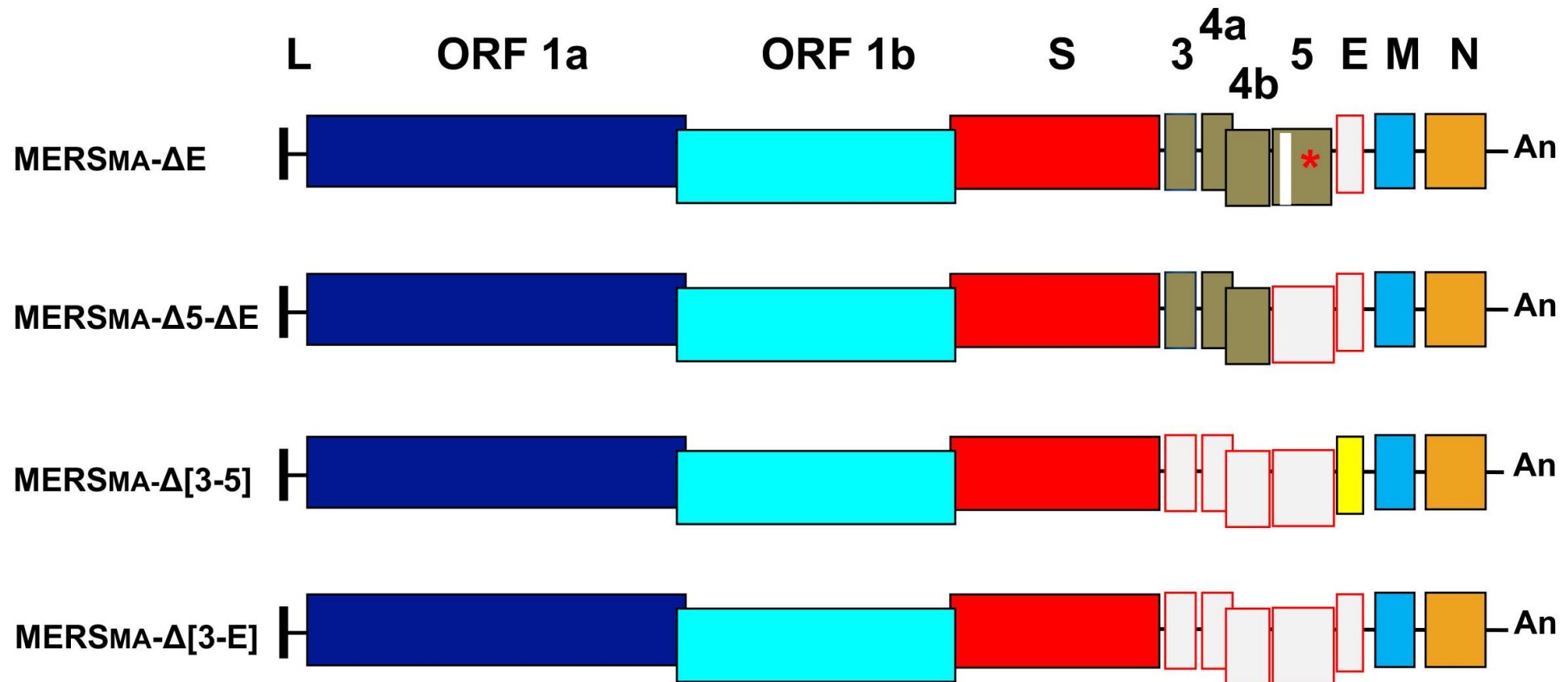
REPLICATION



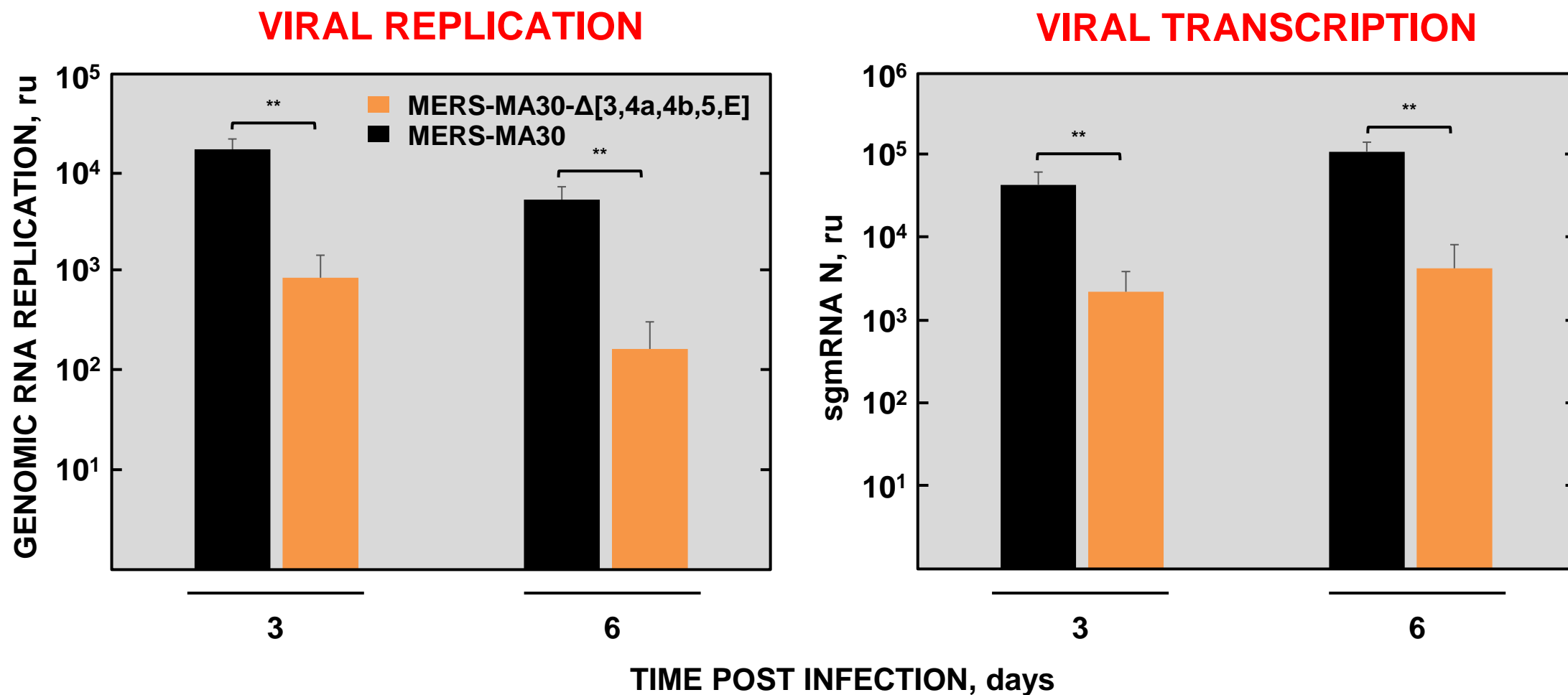
DISSEMINATION



ENGINEERING A MERS-MA REPLICON DERIVED FROM AN ATTENUATED VIRUS

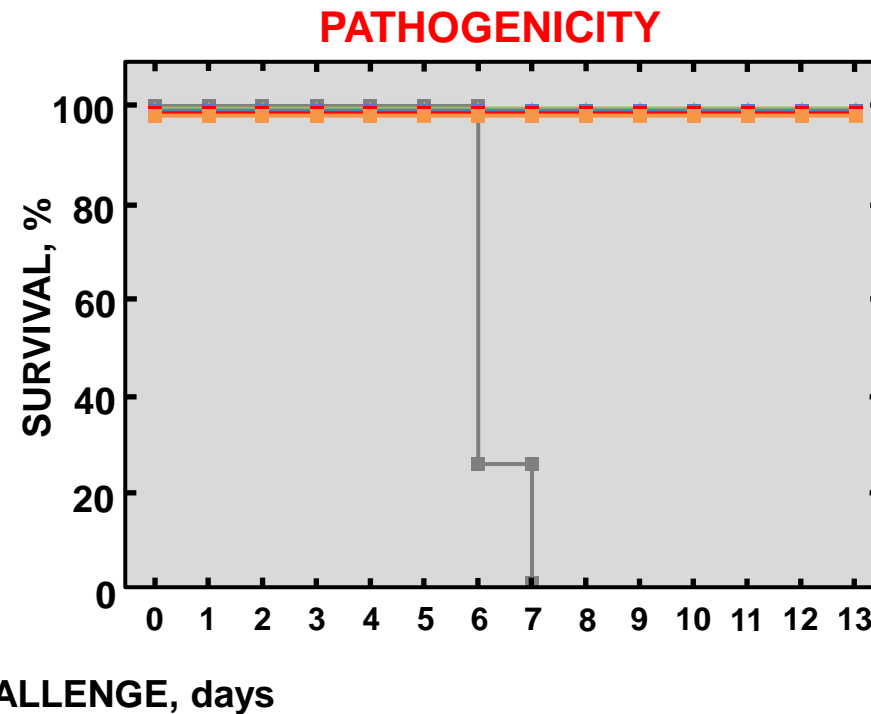
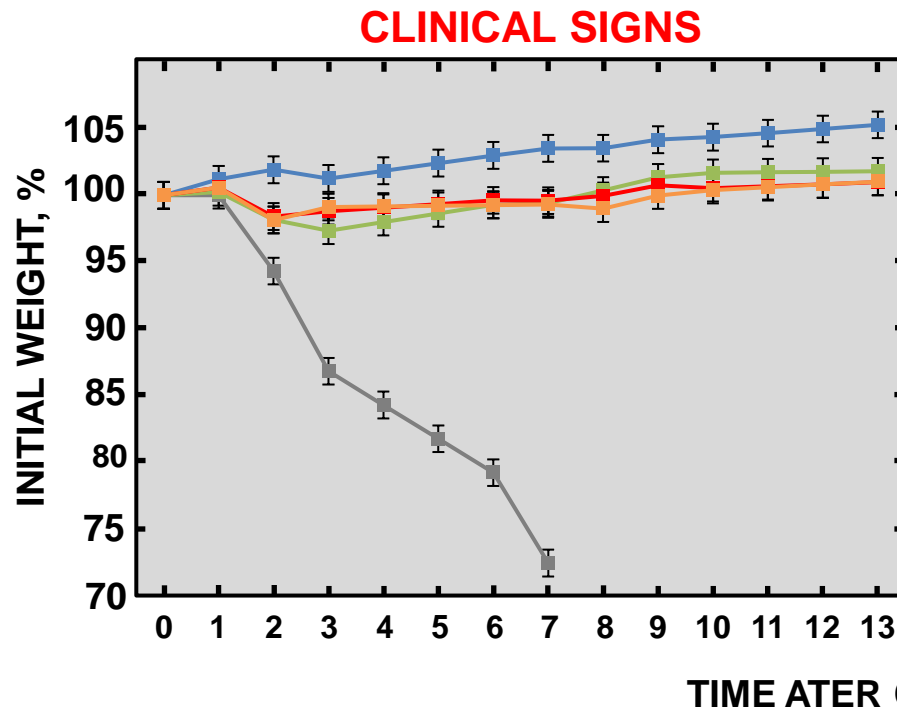


MERS-MA30- Δ [3,4a,4b,5,E] REPLICON IS AMPLIFIED IN KI MICE LUNG



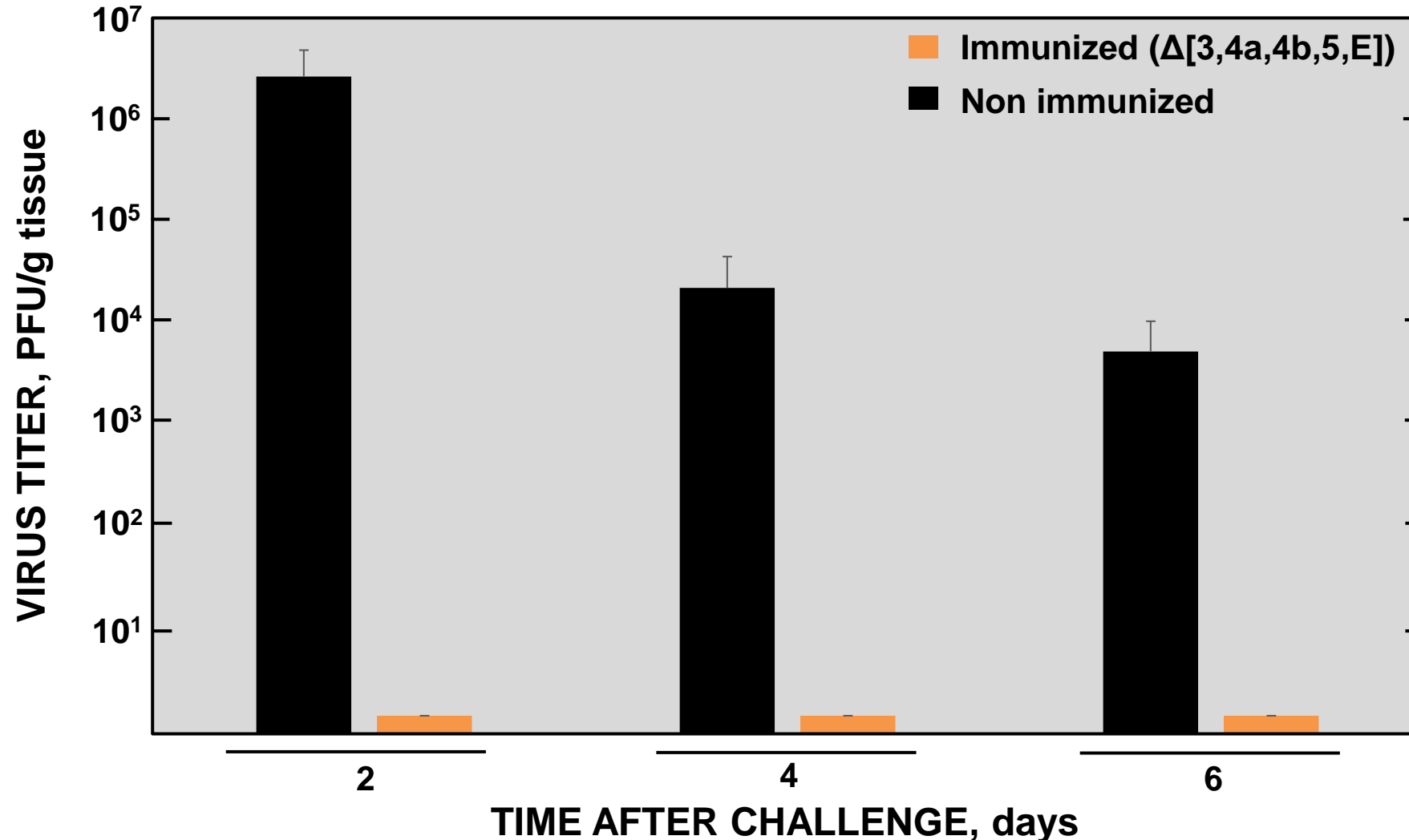
MERS-MA30 RNA REPLICON INDUCED PROTECTION IN KI MICE

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



■ NON INFECTED ■ MERS-MA30-ΔE ■ MERS-MA30-Δ[3,4a,4b,5] ■ MERS-MA30-Δ[5,E] ■ MERS-MA30-Δ[3,4a,4b,5,E]

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

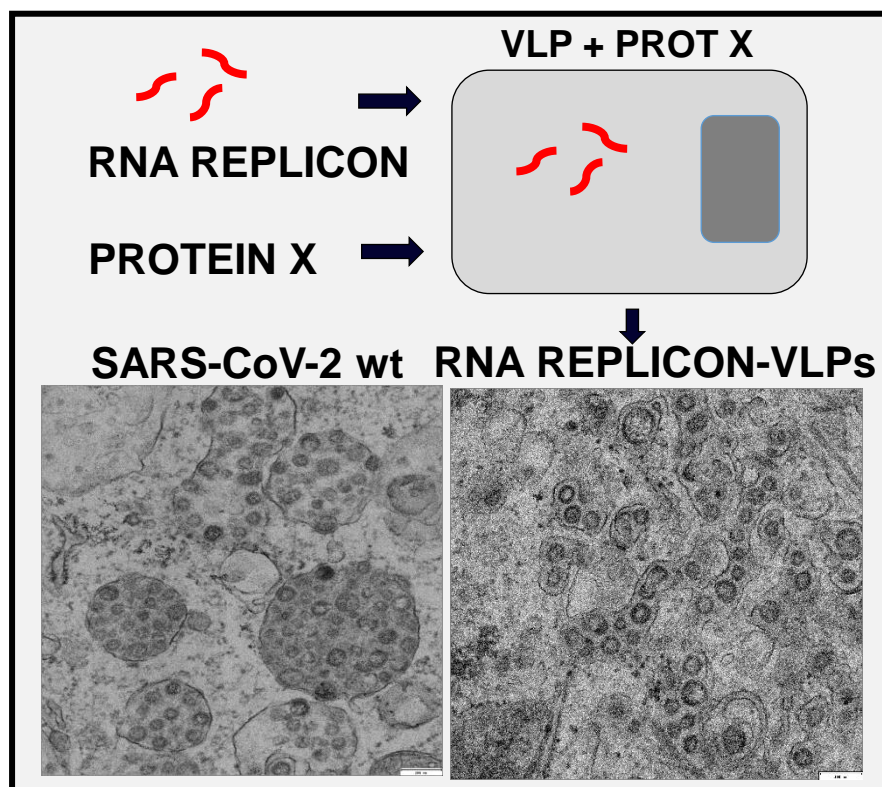


SARS-CoV-2 VLP BASED VACCINES: PROOF OF PRINCIPLE

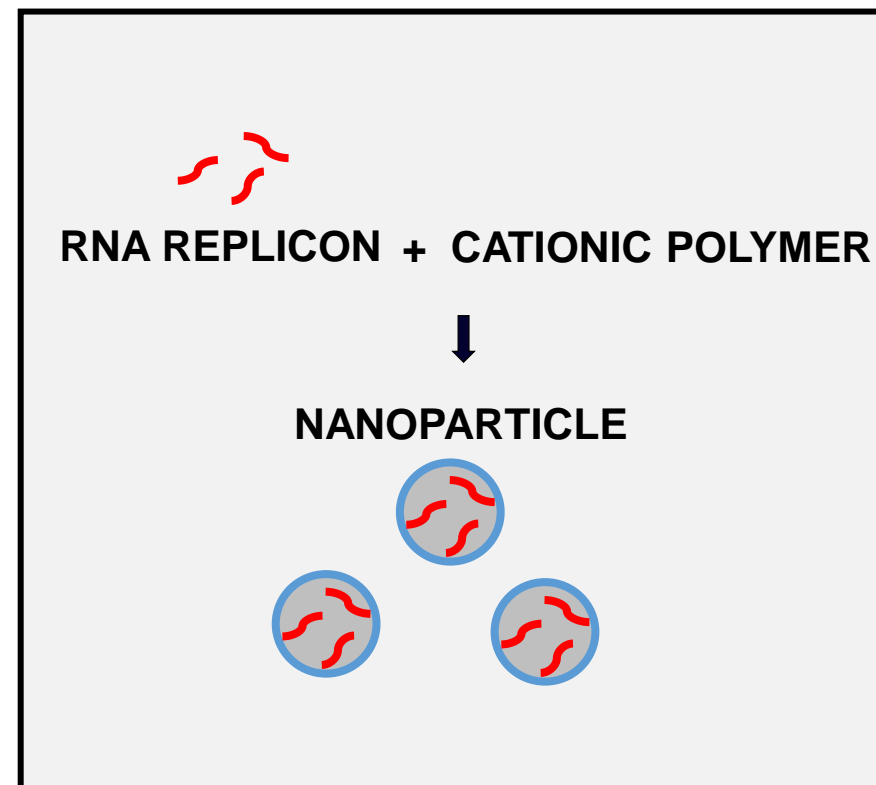
INTRANASAL RNA REPLICON DELIVERY

TWO VERSIONS

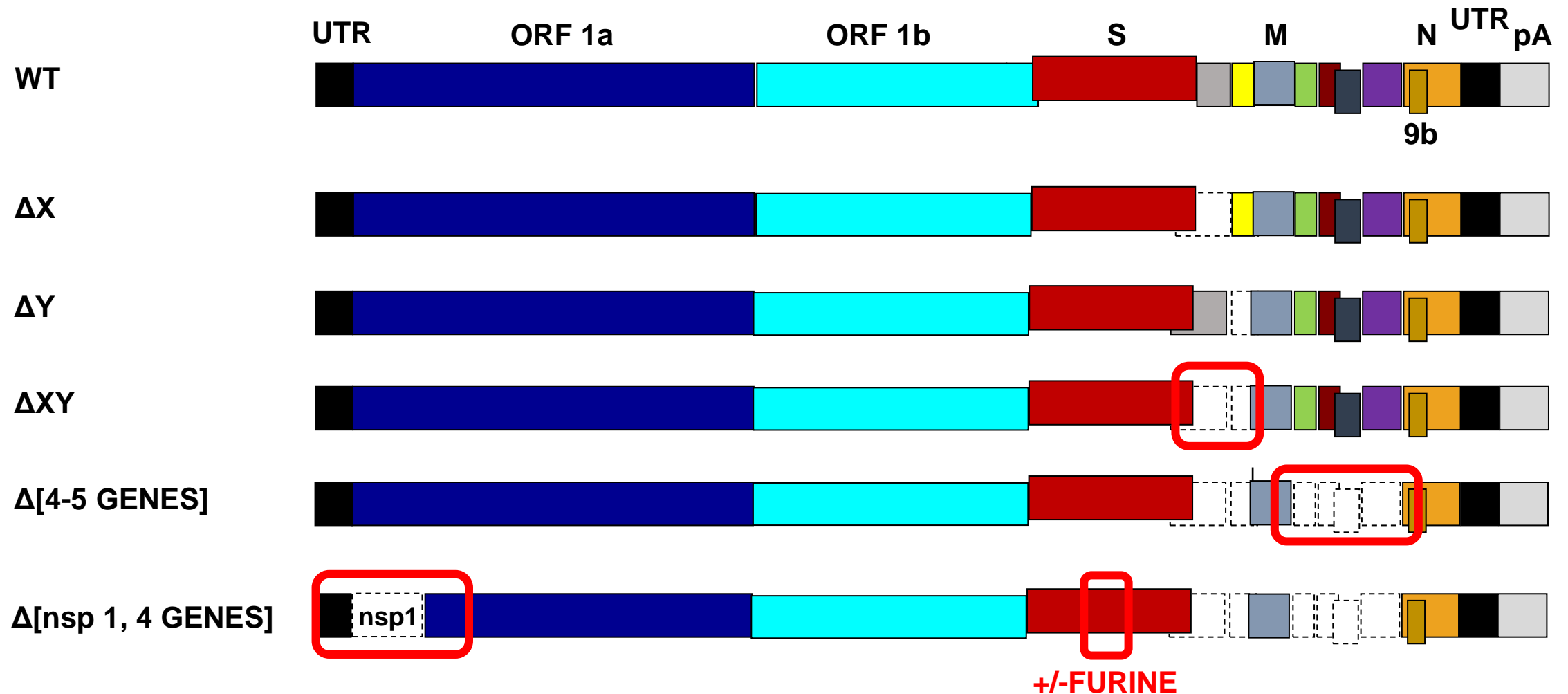
VLP FORMATION



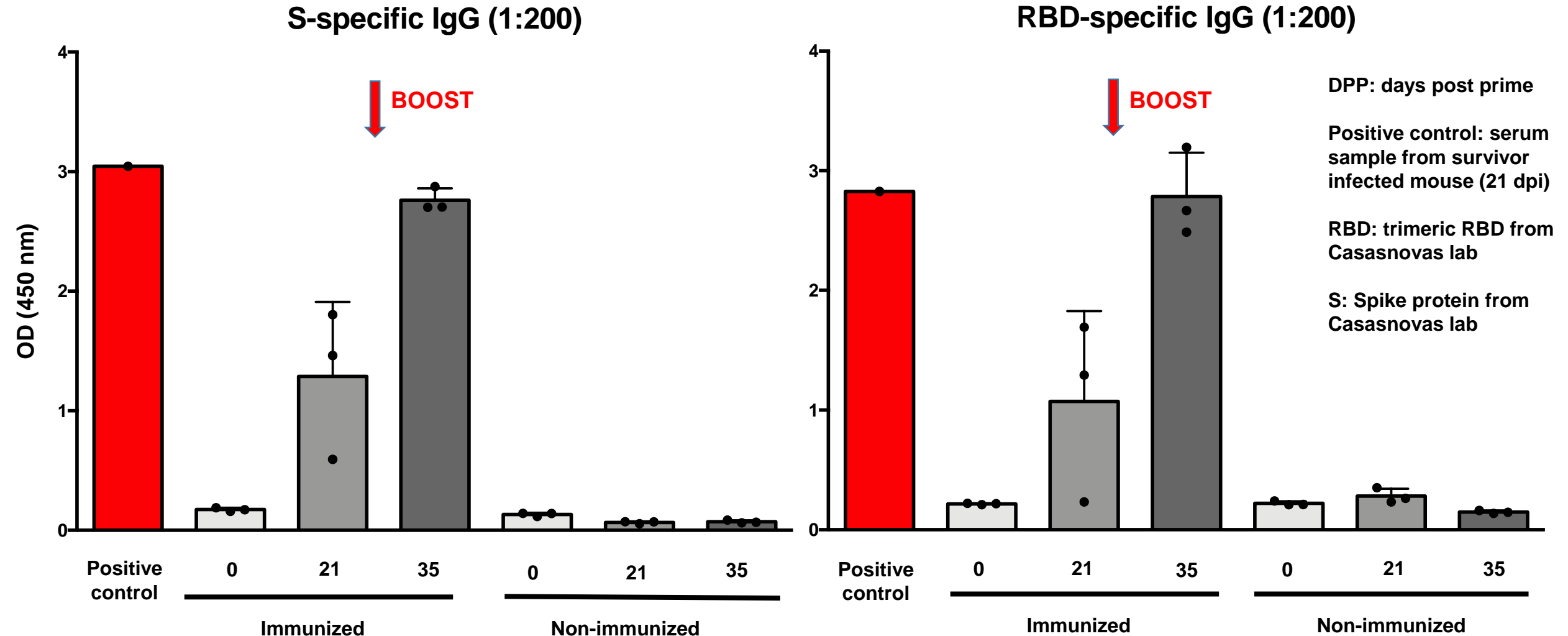
CHEMICALLY DEFINED



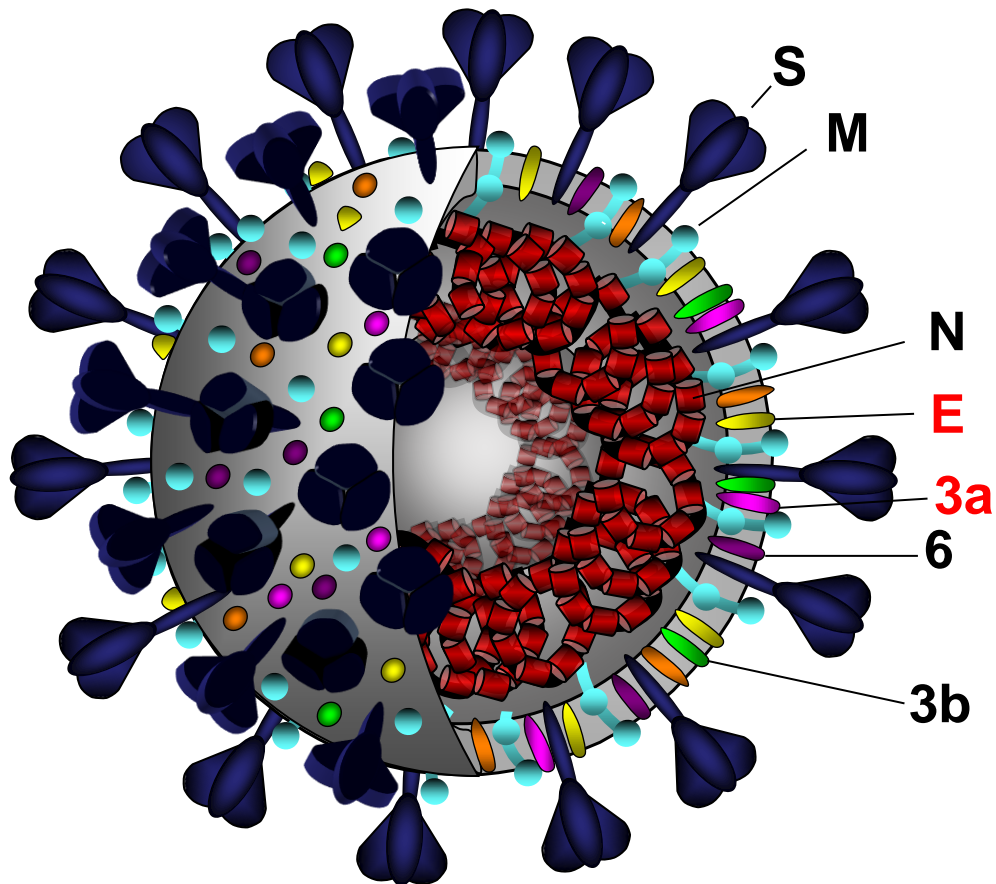
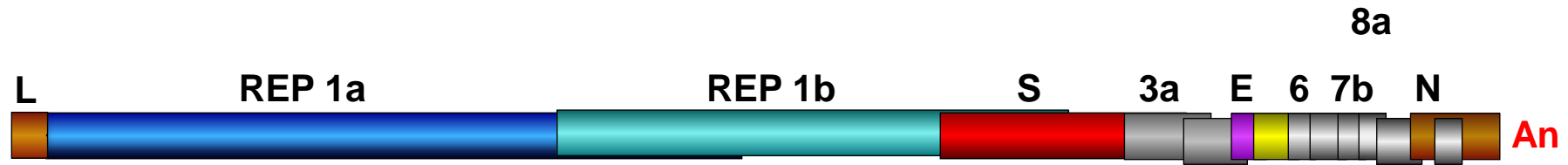
SARS-CoV-2 DELETION MUTANTS FOR REPLICON-VLP BASED VACCINE



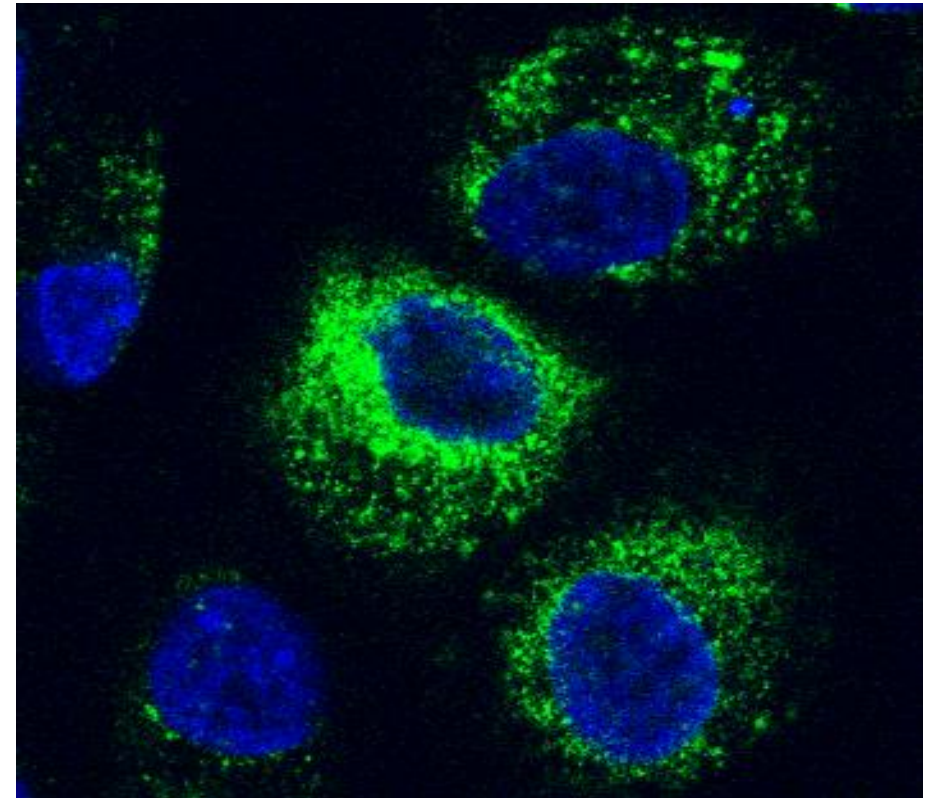
INTRANASAL IMMUNIZATION OF MICE WITH TWO DOSES OF RNA REPLICON VLPs INDUCED RBD NEUTRALIZING ANTIBODIES



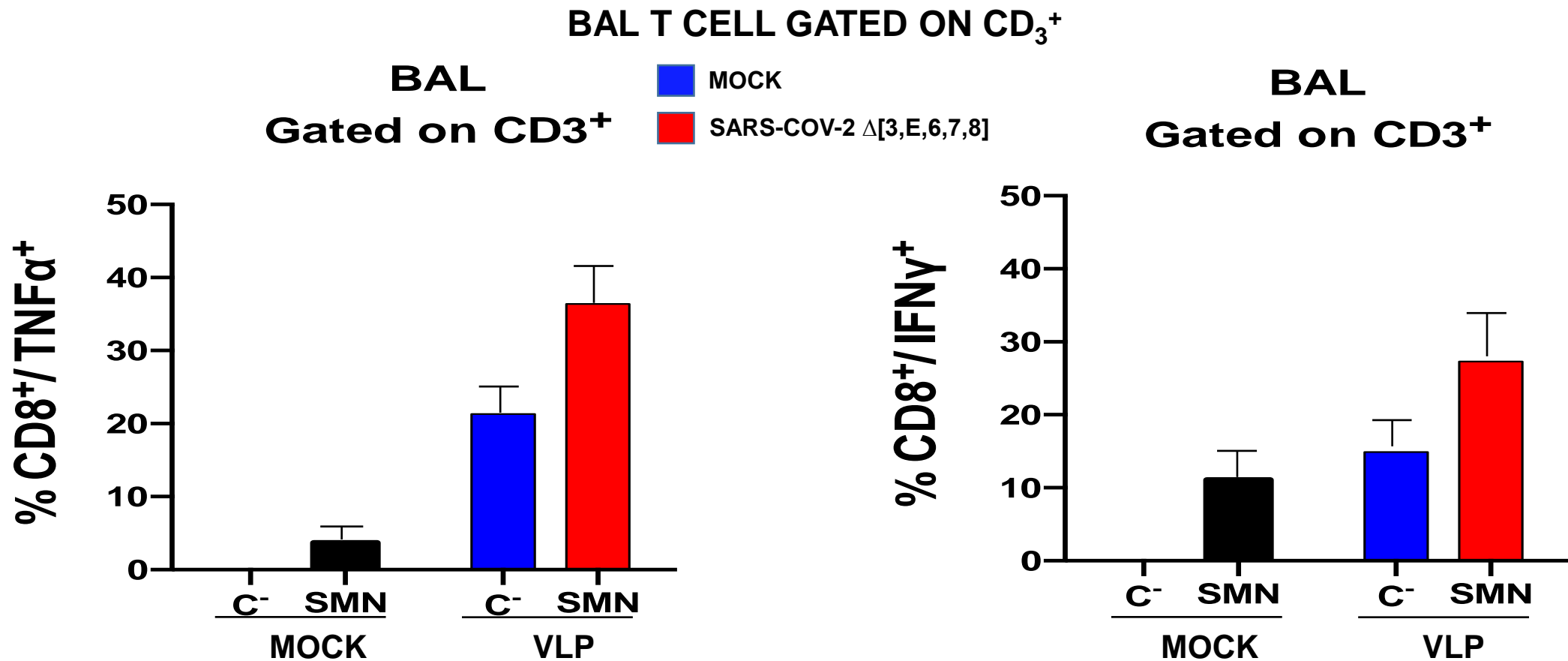
SARS-CoV



mAb α E

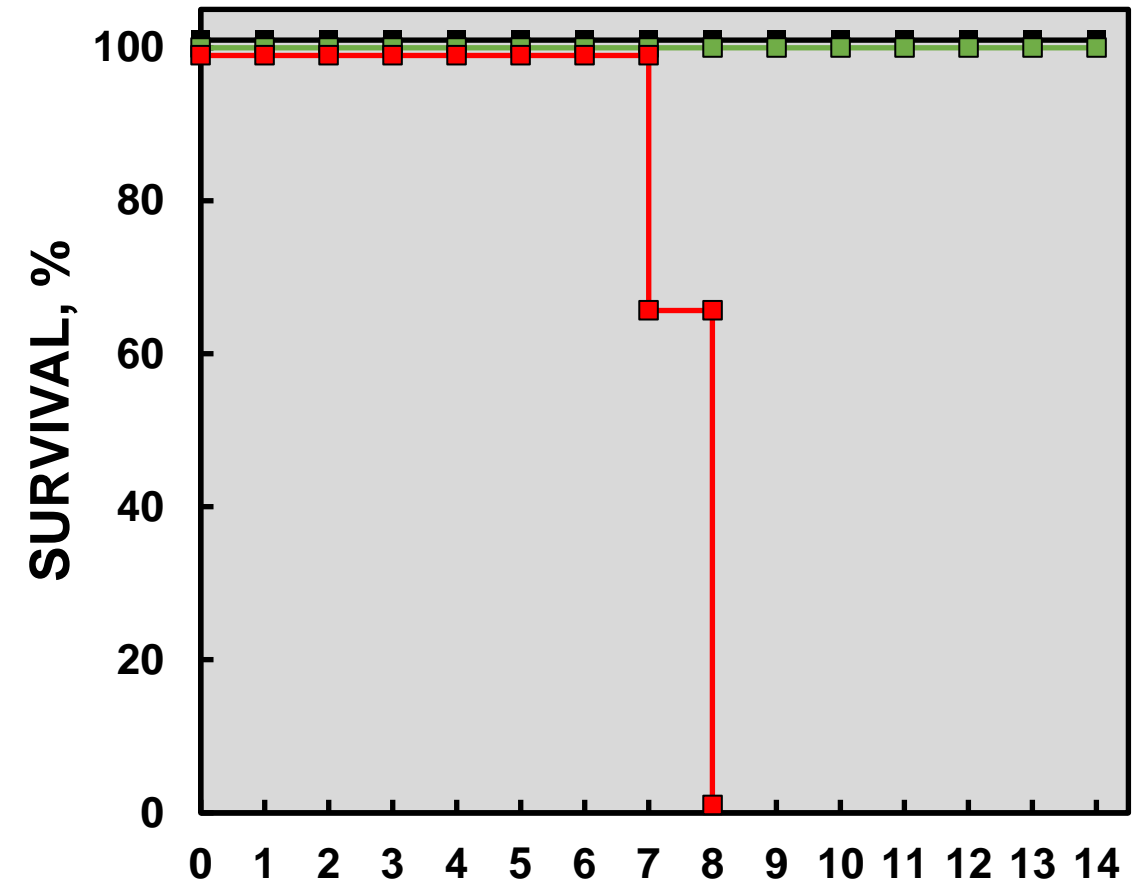
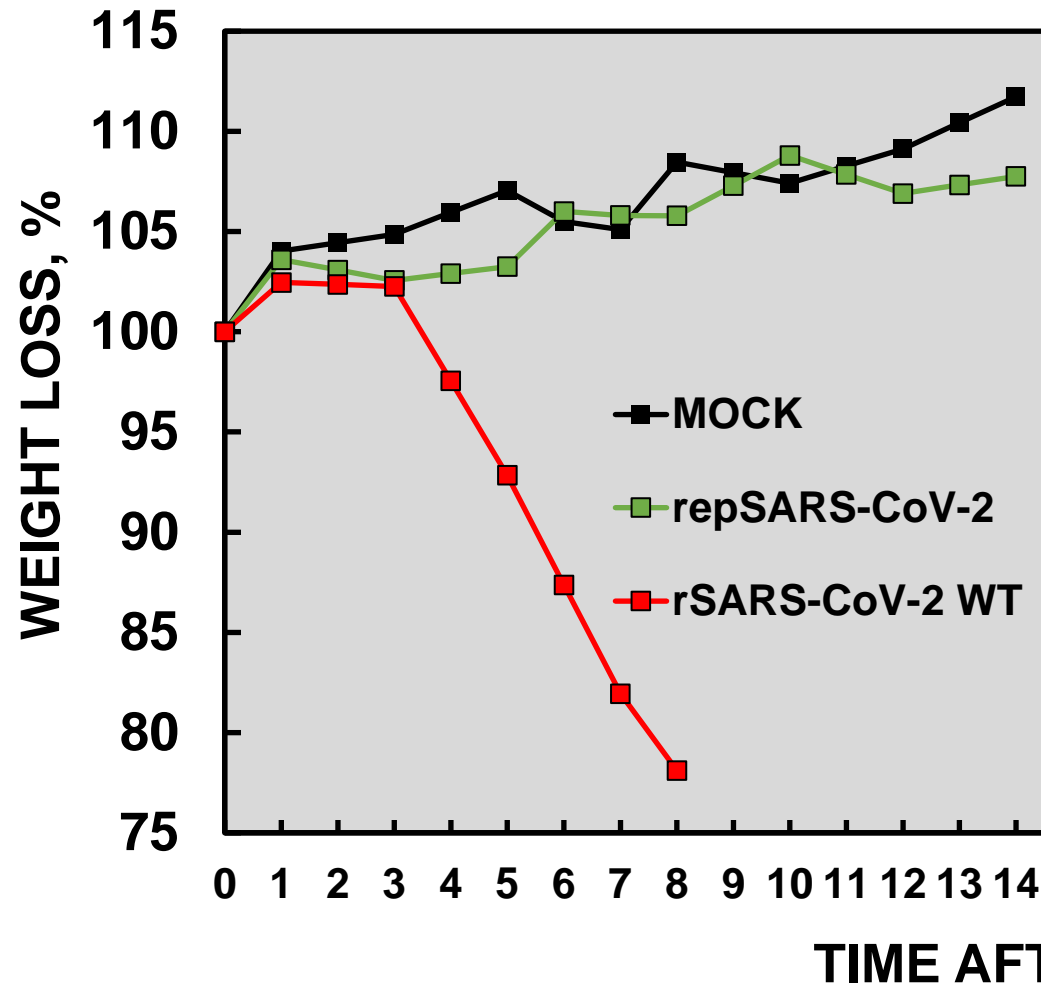


T CELL RESPONSE TO IMMUNIZATION WITH rSARS-CoV-2-Δ[5 GENES] IN MICE



SMN, MIXTURE OF T CELL STIMULATING PEPTIDES FROM THESE PROTEINS

PROTECTION BY rSARS-CoV-2- Δ [SEVERAL GENES] IN MICE



SARS-CoV-2 VACCINE

- **BASED IN A REPLICATION-COMPETENT PROPAGATION-DEFICIENT RNA**
- **EXPRESSED SEVERAL VIRUS PROTEINS**
- **INDUCES NEUTRALIZING ANTIBODIES AND T CELL RESPONSES**
- **INTRANASAL ADMINISTRATION**
- **PROVIDES STERILIZING IMMUNITY IN HUMANIZED MICE MODEL**

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POTENTIAL MECHANISMS OF LONG-TERM COVID

● AUTOIMMUNE RESPONSE

A response related to an autoimmune disease eliciting antibodies destroying the body's own tissues by targeting **self-antigens** including phospholipids, transcriptional and **nuclear proteins, interferons, CD8+ T cells**.

● PERSISTENT INFECTION

Implying that the virus never left. In fact, virus **S protein** has been found in the blood of **70% of study participants**, a clear indication of for a **persistent viral infection** circulating in some tissues (lung, gut, or other)

Continued presence of the virus or **viral particles in the gut** may cause long-lasting inflammation (around 5% of infected patients with long lasting covid are associated with fecal shedding of SARS-CoV-2 RNA seven months post infection and show GI symptoms)

● INFLAMMATION AND IMMUNE DYSFUNCTION

COVID-19 may cause an **uncontrol higher immune system triggering a long-lasting inflammatory response** on multiple organ systems. Multiple studies found **elevated cytokines and interleukins**. Due to superantigens, or **highly active T cells**. **Autoimmune disease linked to activation of Epstein-Barr virus**, systemic sclerosis, cell proteins that resemble viral proteins, and other causes.

● MICROCLOTS

Evidence is overwhelming that **microclots are responsible for many long COVID symptoms**

WHAT IS NEXT FOR SARS-CoV-2 EVOLUTION

Nature 2022

- The **new pathogen will not be eradicated**: Seasonal CoVs
- CoV evolution guided by:
 - Increase of virus infectivity
 - Evasion of previous immunity as HCoV-229E – Jesse Bloom, Washington
- **SARS-CoV-2 could evade current vaccines by recombination** with other CoVs currently circulating in animal reservoirs (mink, white-tailed deer) to escape immune response

DISTRIBUTION OF THE POSITIVE ENVIRONMENTAL SAMPLES IN HUANAN SEEFOOD MARKET

