# EMERGENT CORONAVIRUSES: GENOME ENGINEERING AND VACCINE DEVELOPMENT

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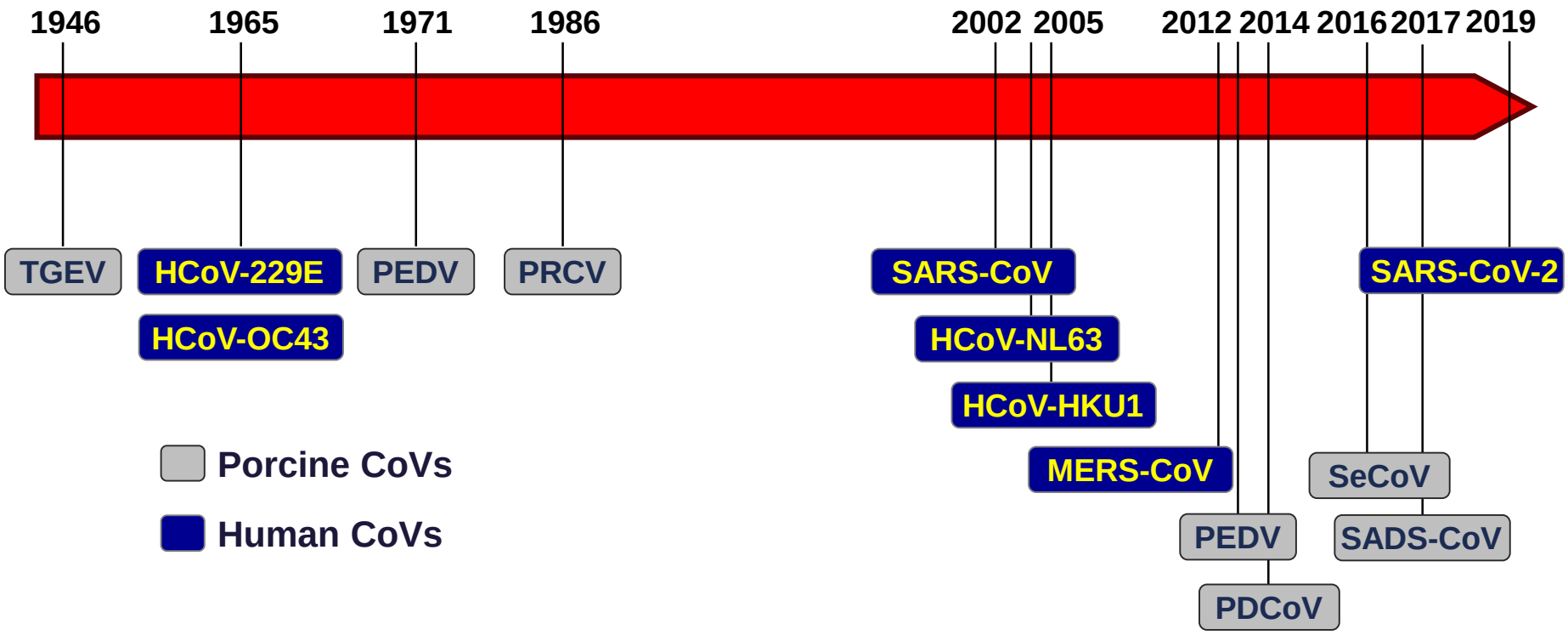
16DEC20

CNB-CSIC

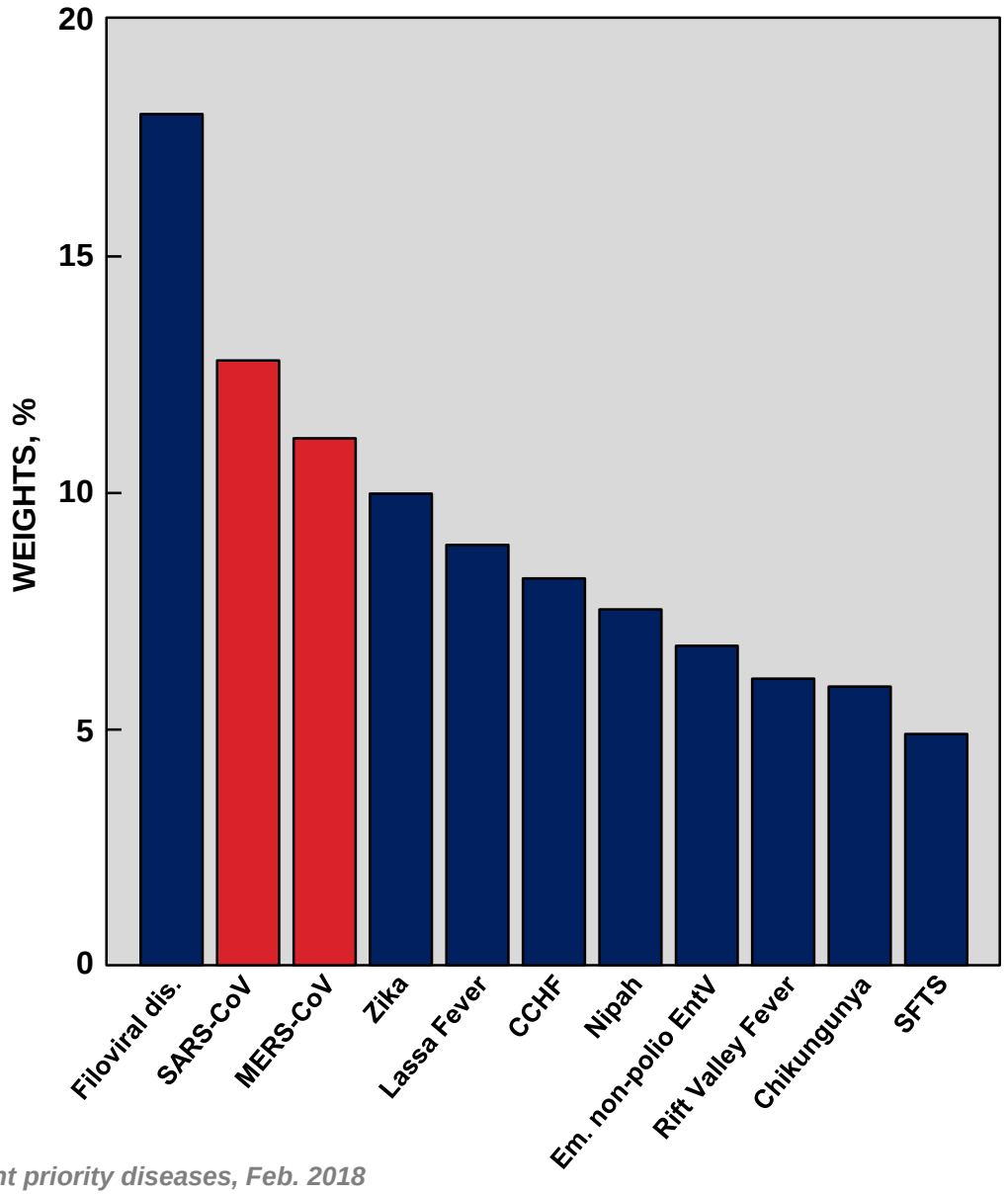
# CoVs ARE EMERGENT VIRUSES



# WHO 2018 DISEASES PRIORITIZATION

**PRIORITIZATION CRITERIA:**

- **Human transmission**
- **Severity or case fatality rate**
- **Human-animal interface**
- **Other factors (i.e., geographic range, epidemic threat, absence of robust protective immunity, high risk of occupational exposure, connections with biological weapons programs)**
- **Public health context in the affected area**
- **Potential societal impacts**
- **Evolutionary potential**

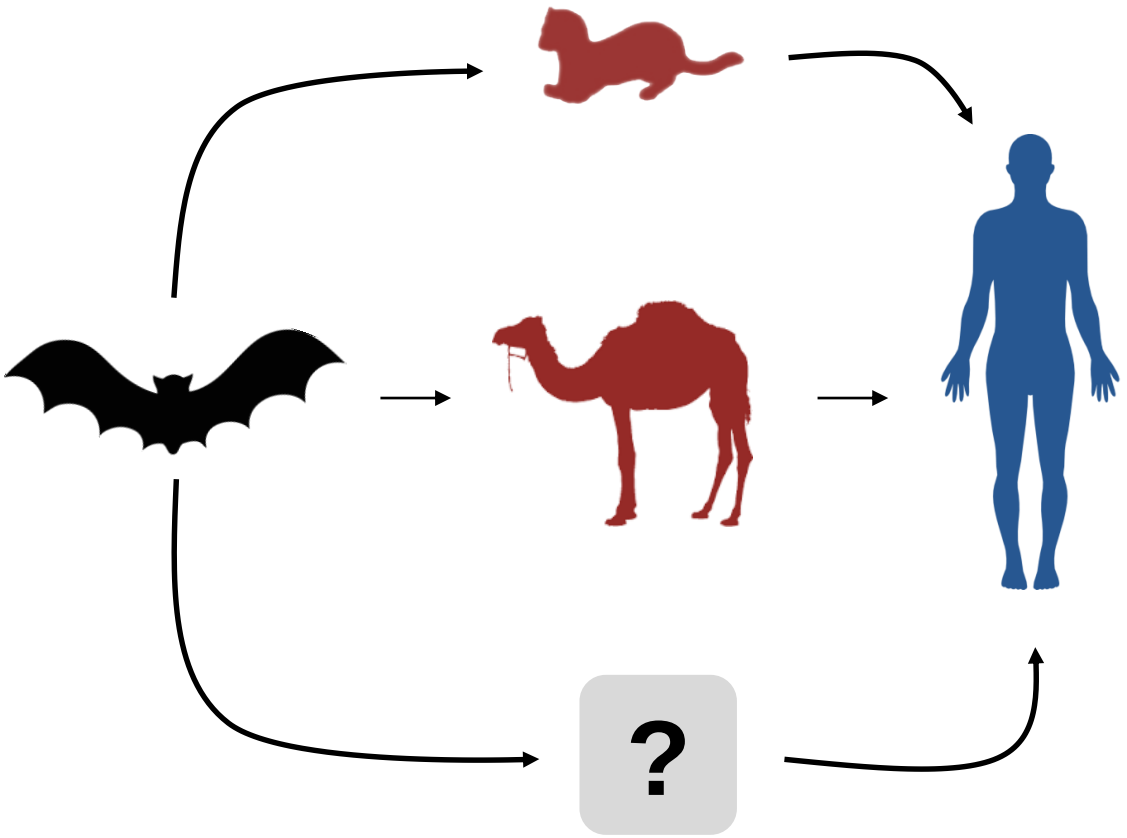



*Adapted from WHO 2nd Annual Review Report on R&D Blueprint priority diseases, Feb. 2018*

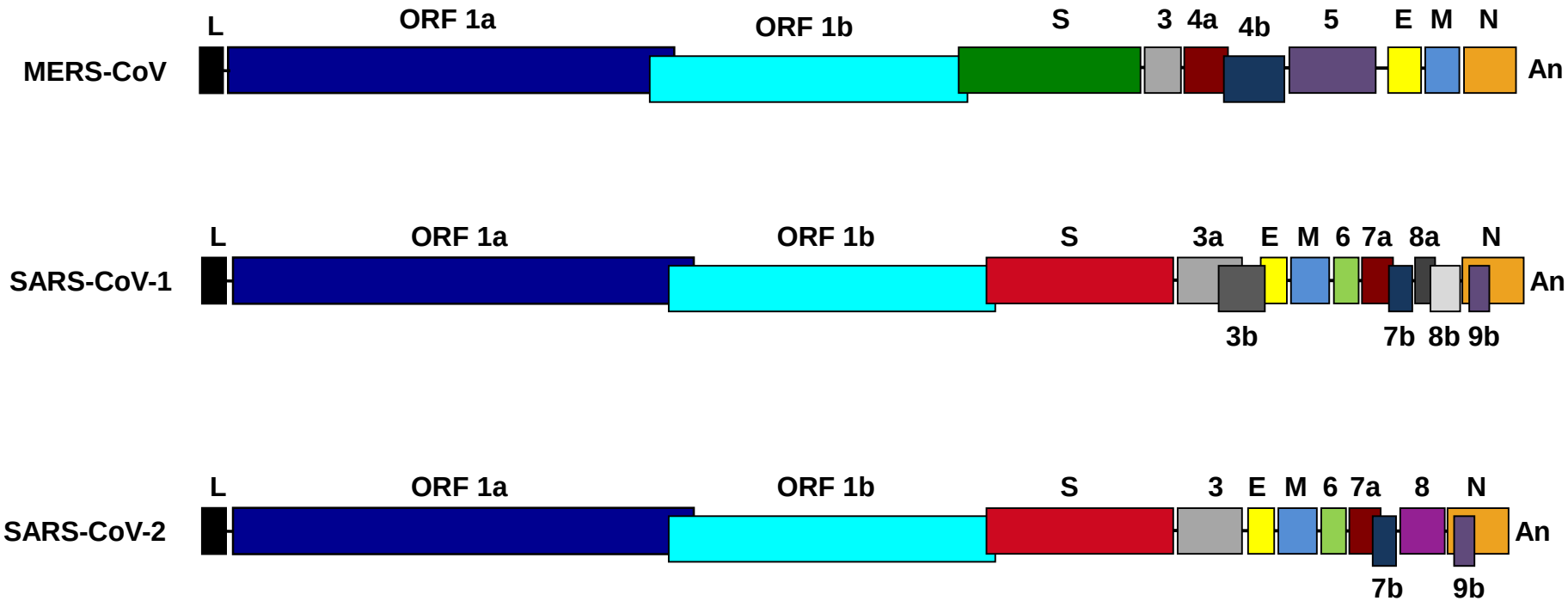
# HUMAN CORONAVIRUSES

- HCoV-229E
- HCoV-OC43
- HCoV-HKU1
- HCoV-NL63

- SARS-CoV, 2003
- MERS-CoV, 2012
- SARS-CoV-2, 2019

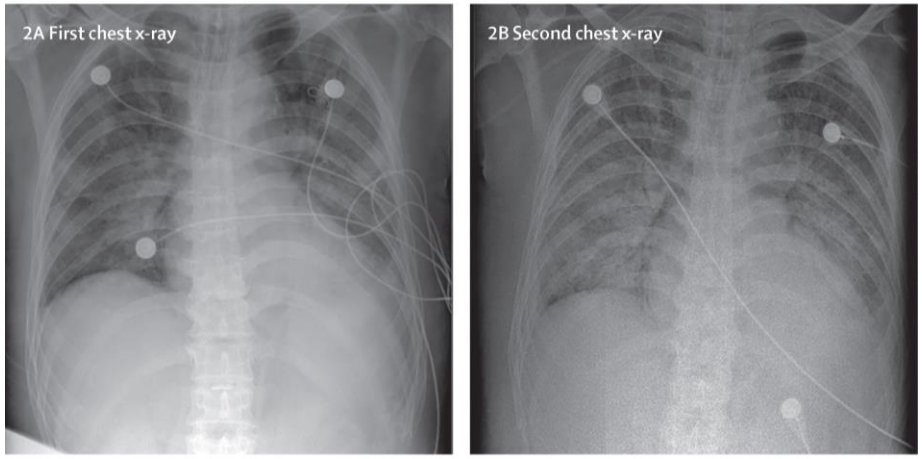


# CoVs GENOME STRUCTURE

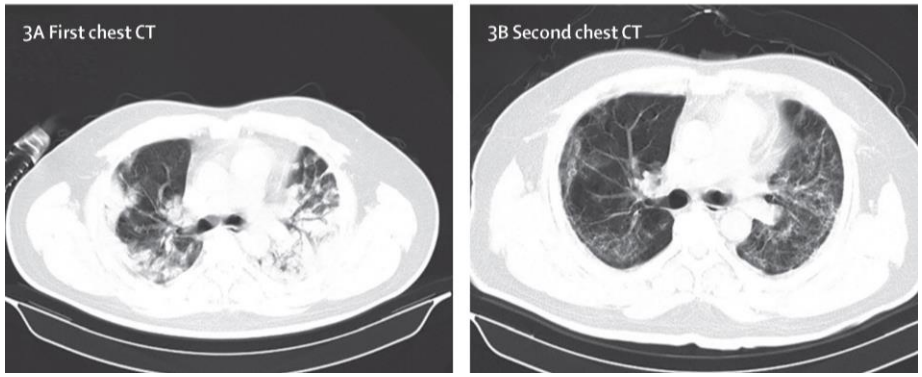


# SARS-CoV-2

Case 2



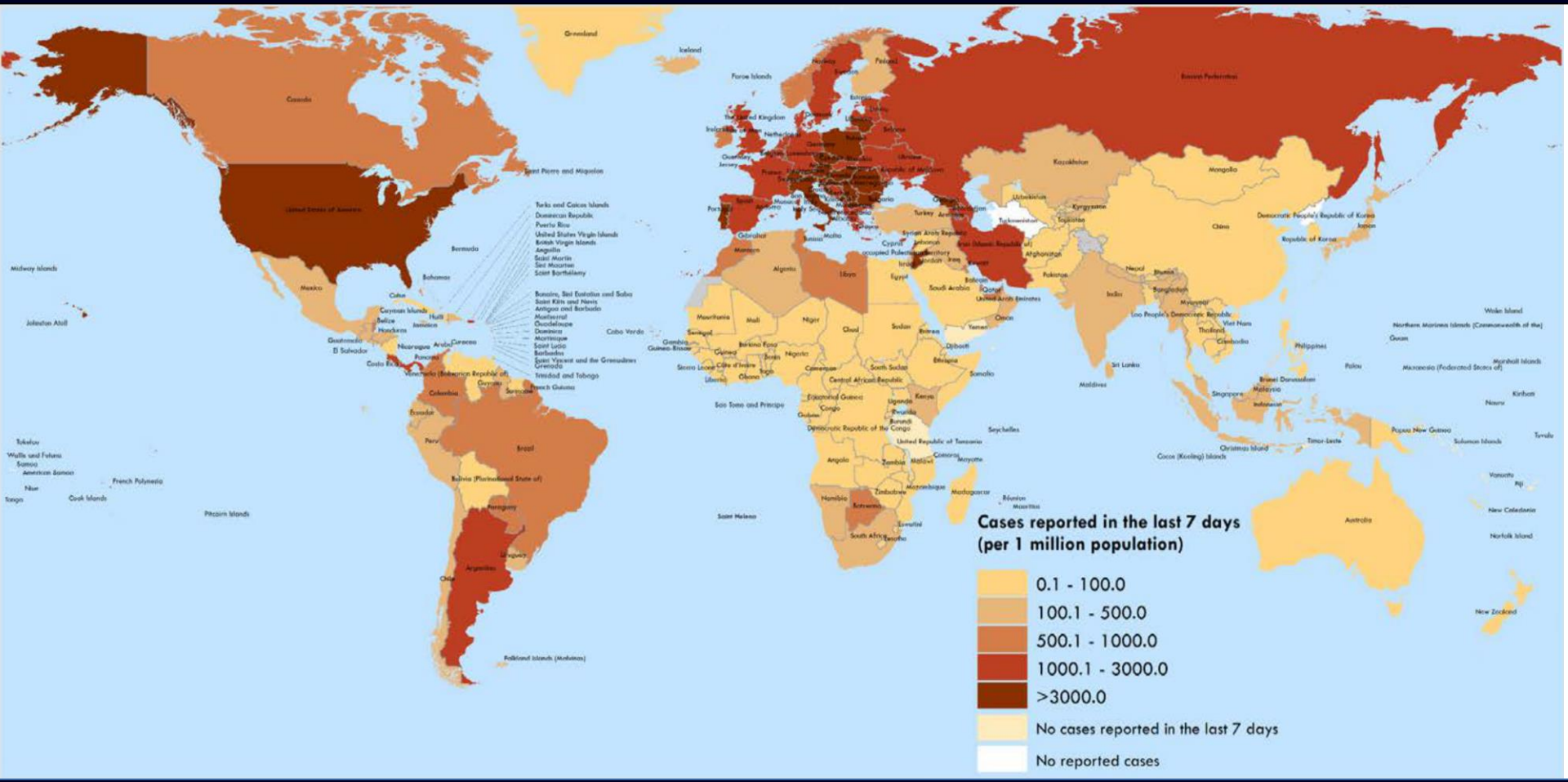

Case 3




- **ISOLATED IN CHINA JANUARY 2020**
- **ASSOCIATED WITH ARDS**
- **BAT ORIGIN**

*Chen N. et al, 2020, Lancet 395:507-513*

# SARS-CoV-2 (COVID-19) CASES MAP
# UPDATED 16 – 22 NOVEMBER 2020




**Total cases 13 December 2020: 72,207,546 (99.5 % mild)**
**Deaths: 1,613,689 Recovered: 50,594,965**

# HUMAN CoVs INFECTION AND DISEASE

HCoV-229E, HCoV-OC43,
HCoV-NL63, HCoV-HKU1

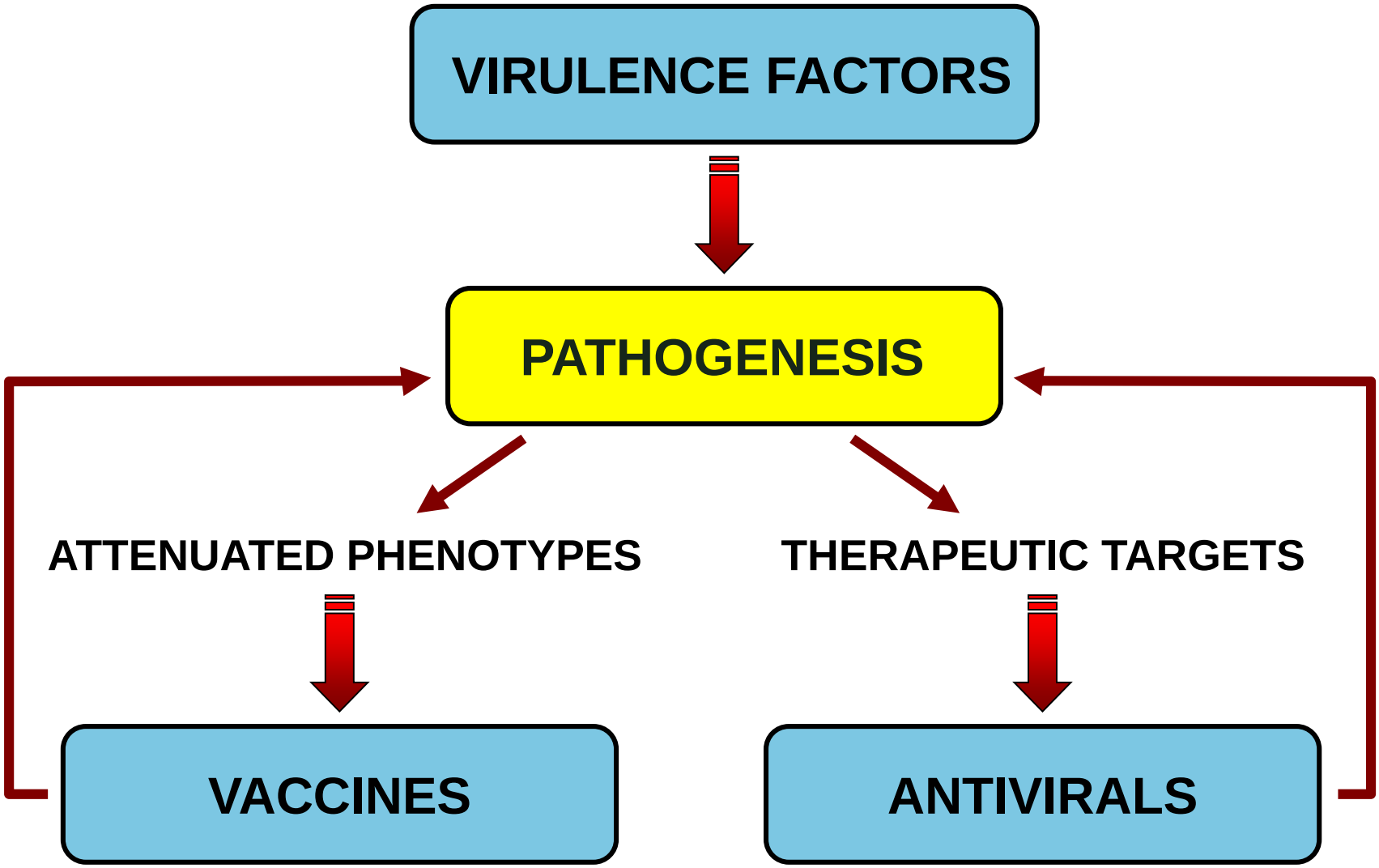
SARS-CoV-2

SARS-CoV
MERS-CoV

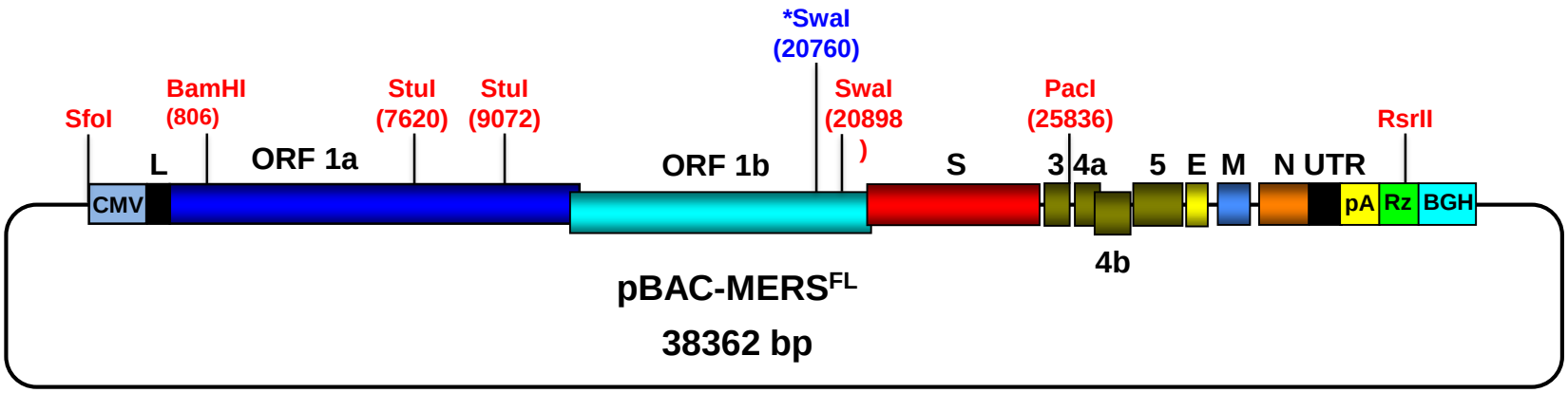
*Adapted from Sariol A. and Perlman S., 2020, Immunity 2:248-263*

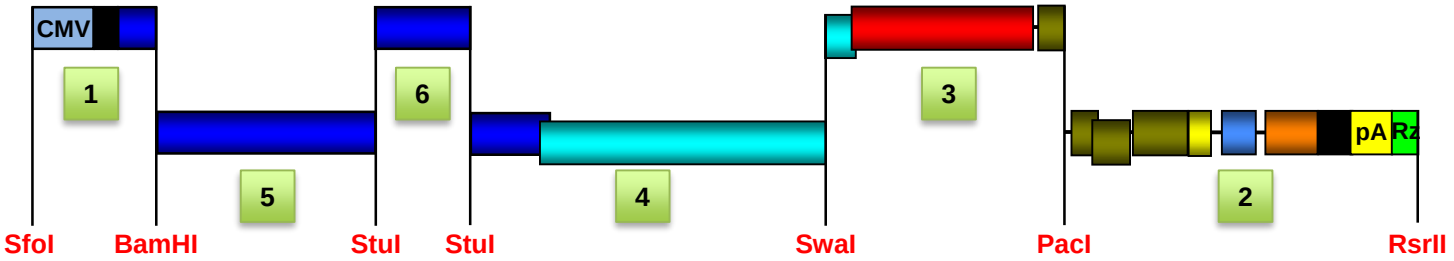
# CoV REVERSE GENETICS AND VIRUS-HOST INTERACTION

# CoVs – HOST INTERACTION

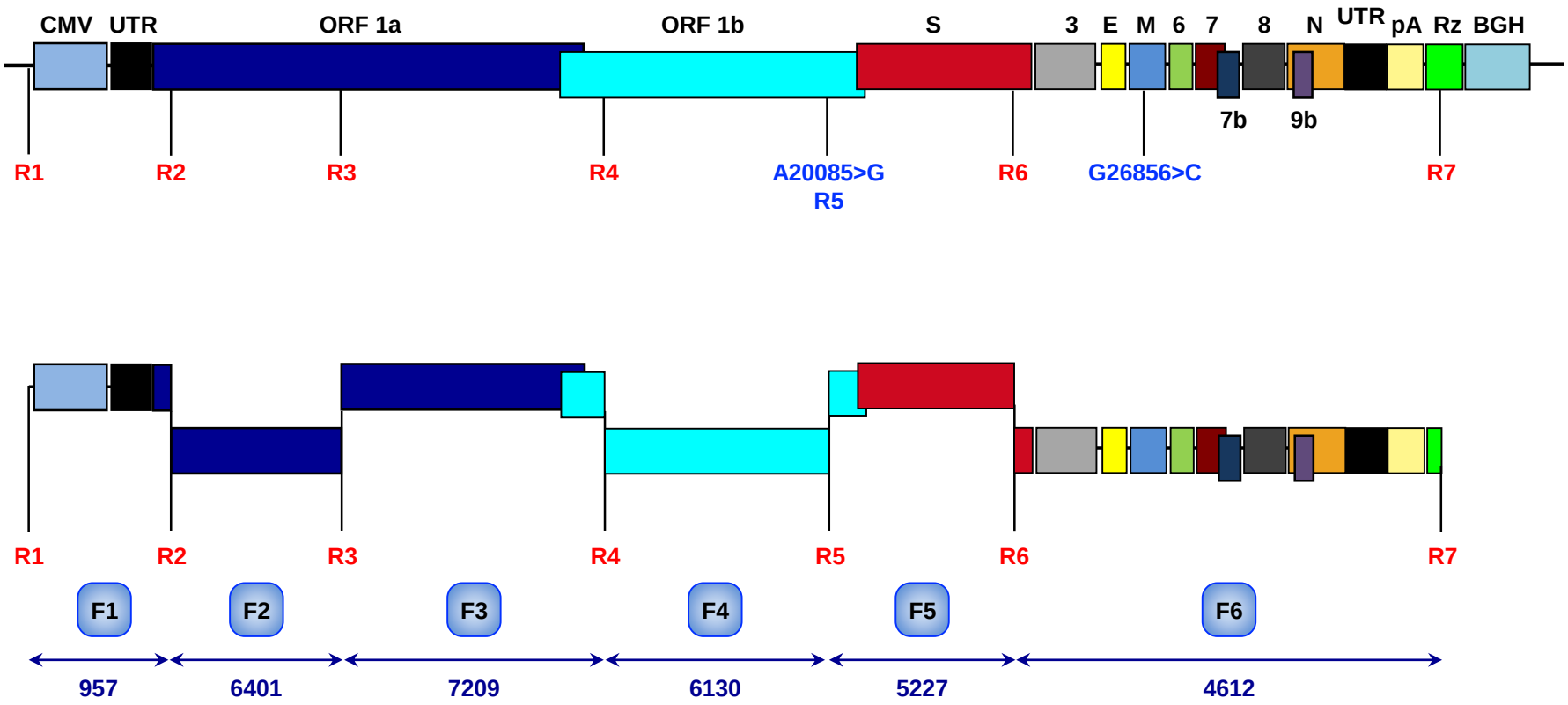


# INFECTIOUS cDNAs IN BACs



# SARS-CoV-2 cDNA ENGINEERING



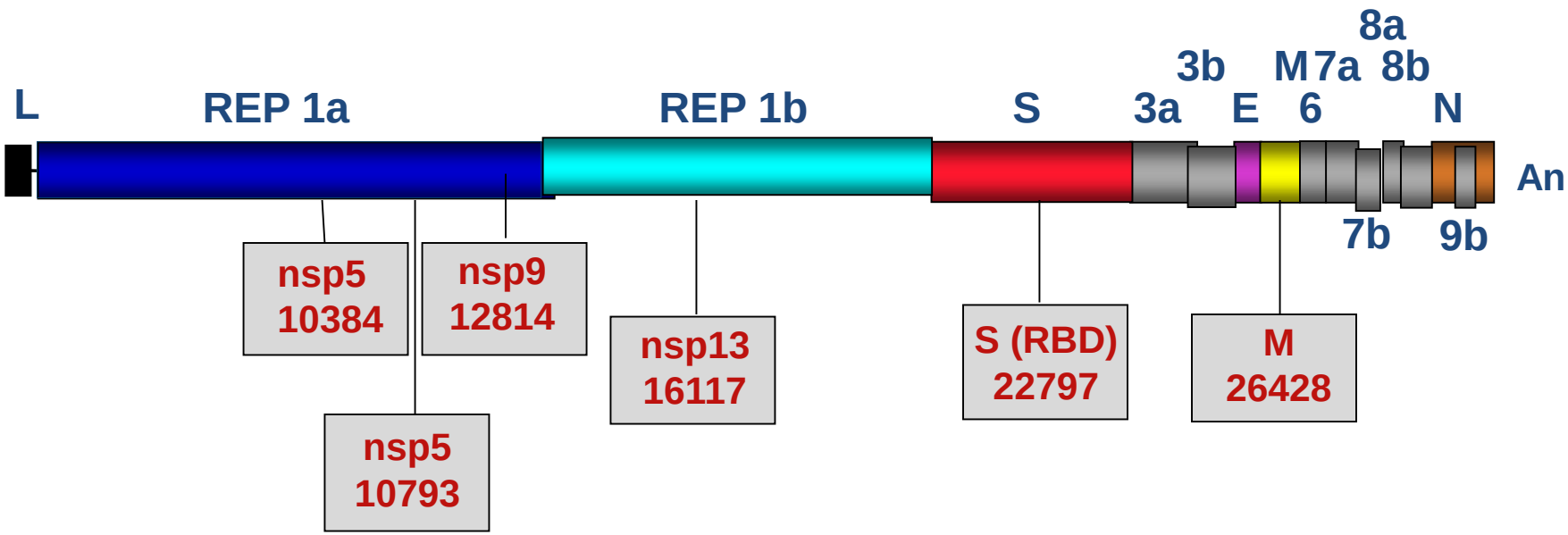
# MOUSE ADAPTED SARS-CoV

## SARS-CoV-MA15

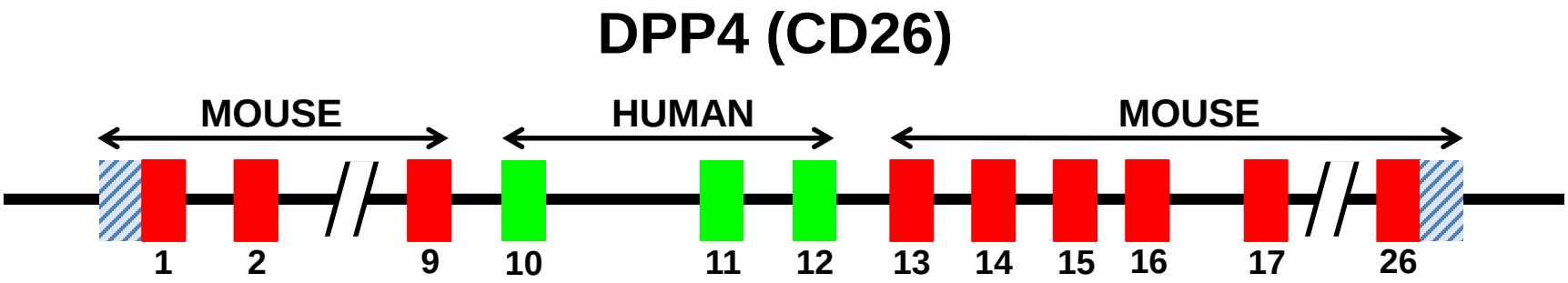
- High titers in lungs
- Viremia, extrapulmonary spread
- Neutrophilia
- Pathological changes in lungs
- Death

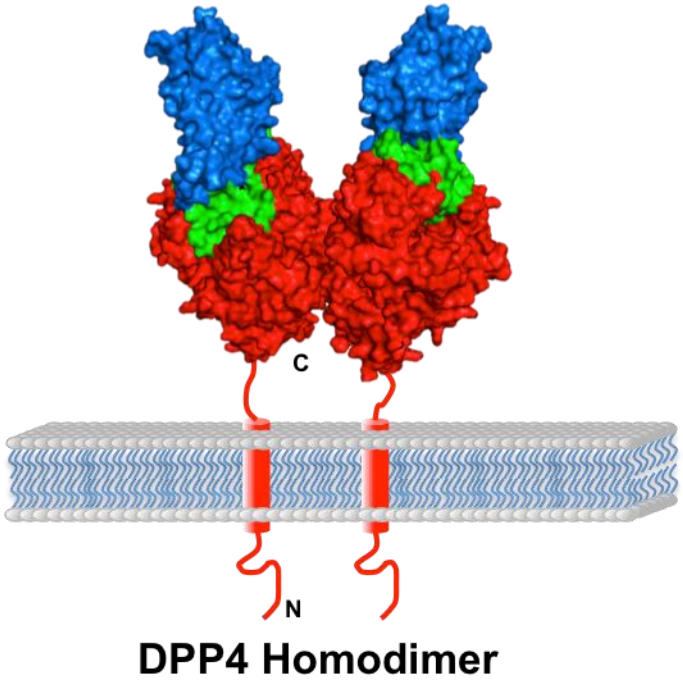
K. SUBBARAO AND R. BARIC LABS

# CONSTRUCTION OF A MOUSE ADAPTED SARS-CoV

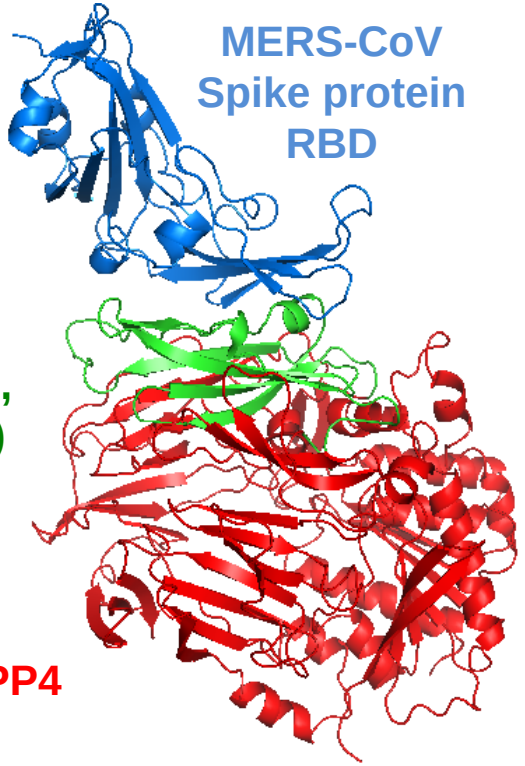


# MERS-CoV MOUSE KNOCK-IN MODEL

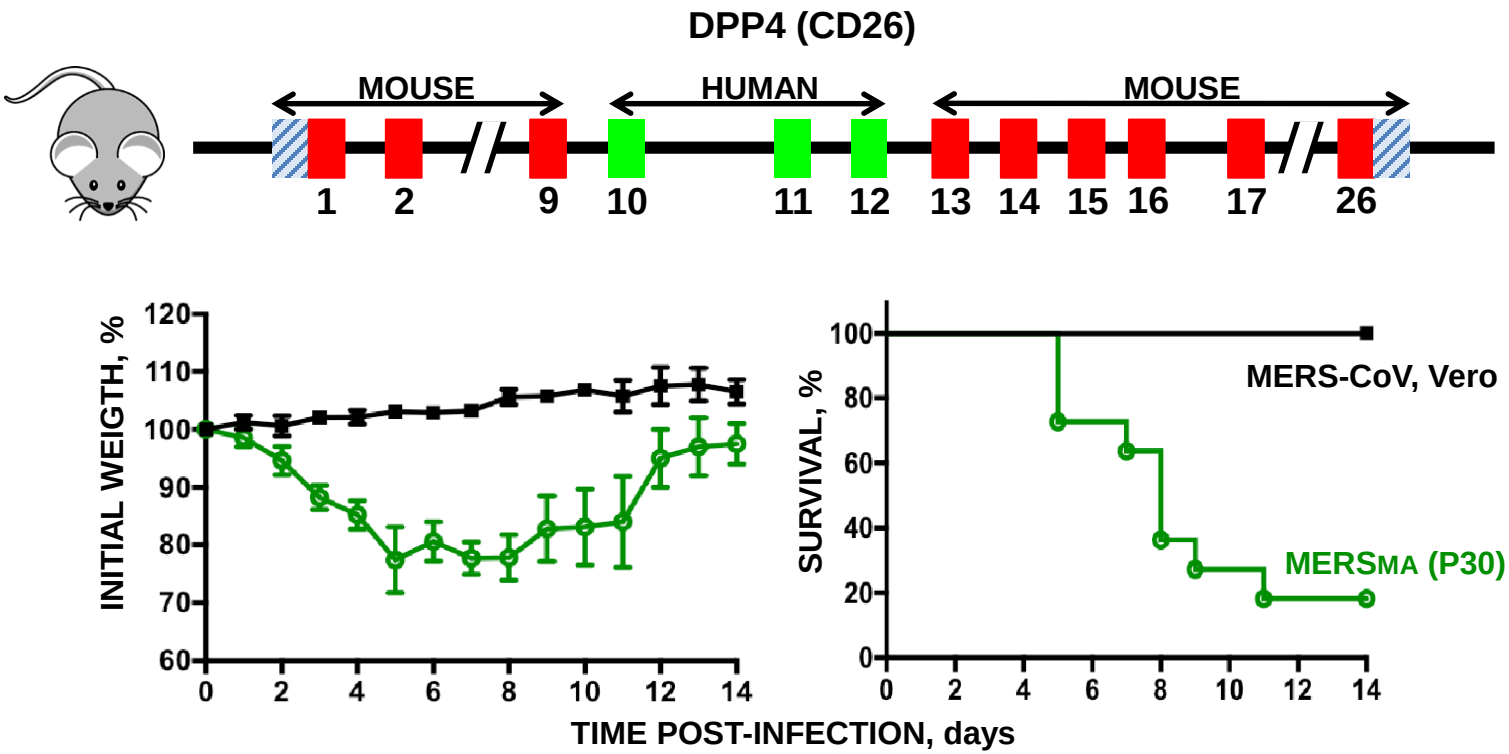



DPP4 Homodimer

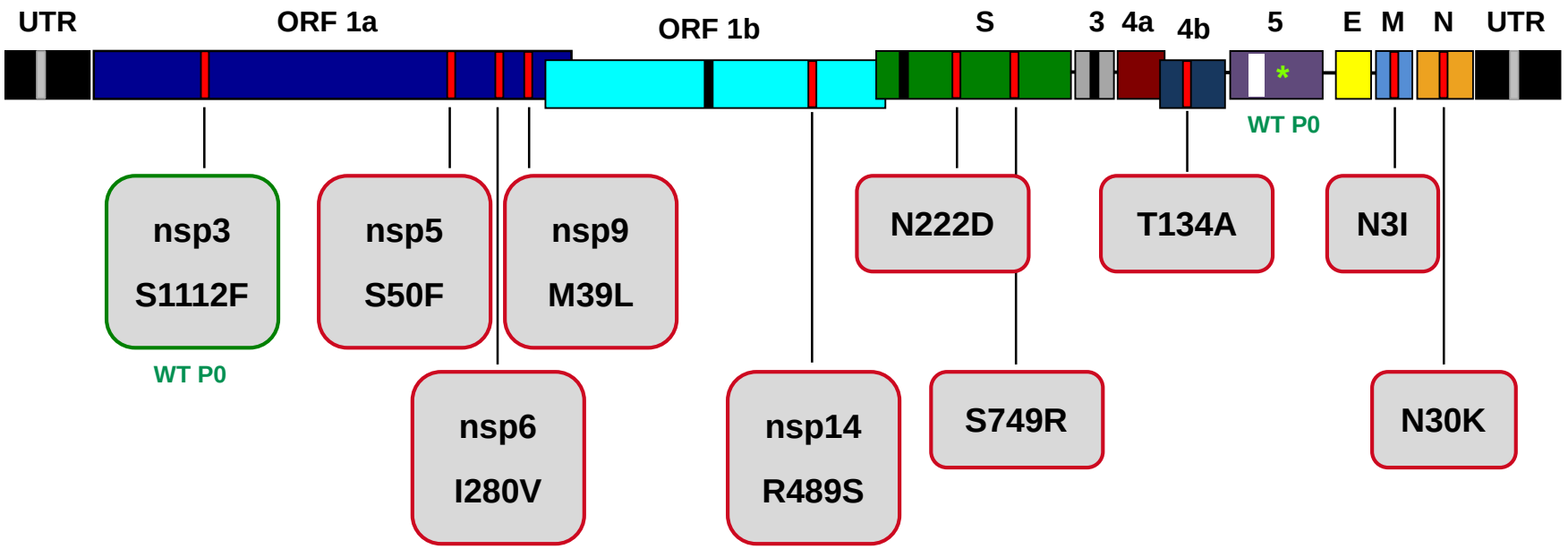


# MERS-CoV MOUSE KNOCK-IN MODEL




*Li K. et al, 2017, PNAS 114:E3119-E3128*

# ENGINEERING MERS-CoV-MA cDNA




Mutation in UTR

Missense mutation

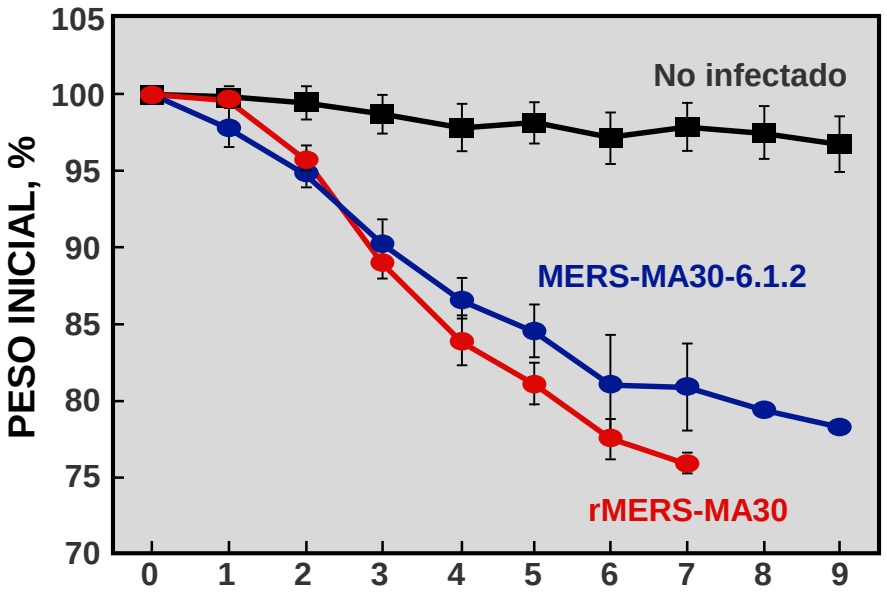
Synonymous mutation

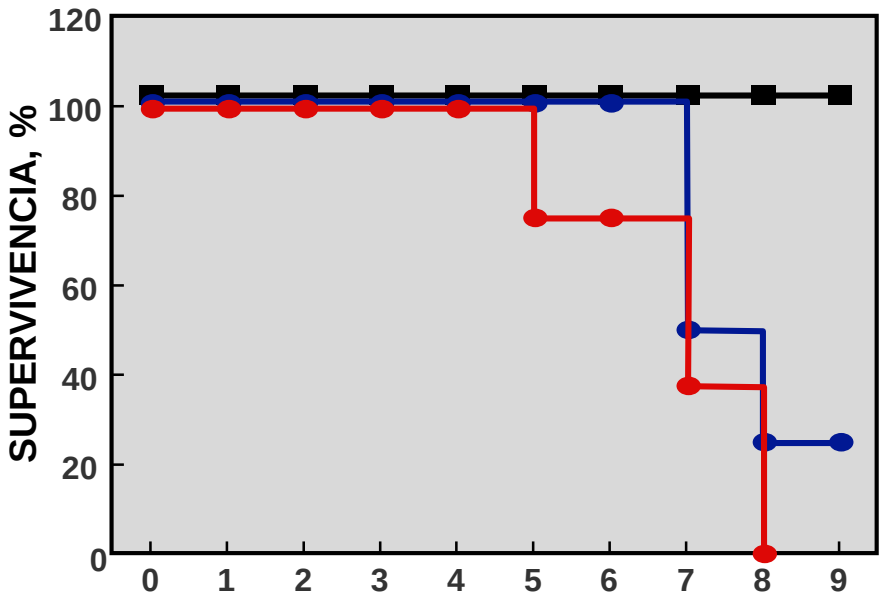
Δ(42-58) nt. deletion with frameshift

* STOP codon

# VIRULENCIA DEL rMERS-MA30 EN RATONES KI

**Ratones KI de 16 semanas**
**1 x $10^5$ UFP por ratón intranasalmente**

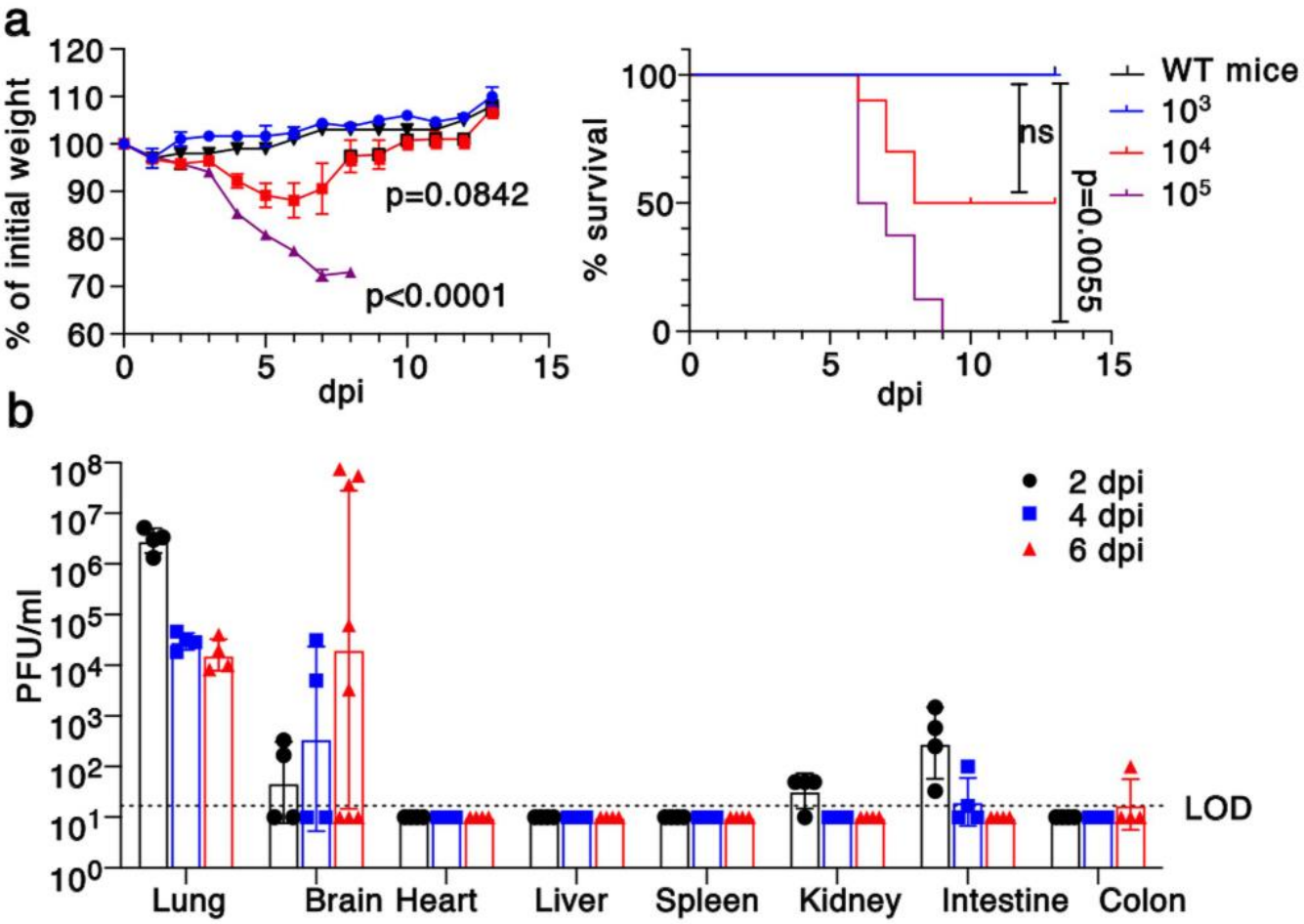



**TIEMPO DESPUÉS DE LA INFECCIÓN, días**

*Gutierrez-Alvarez J., ….. Zuñiga S. and Enjuanes L., 2020, J. Virol. doi:10.1128/JVI.01172-20*

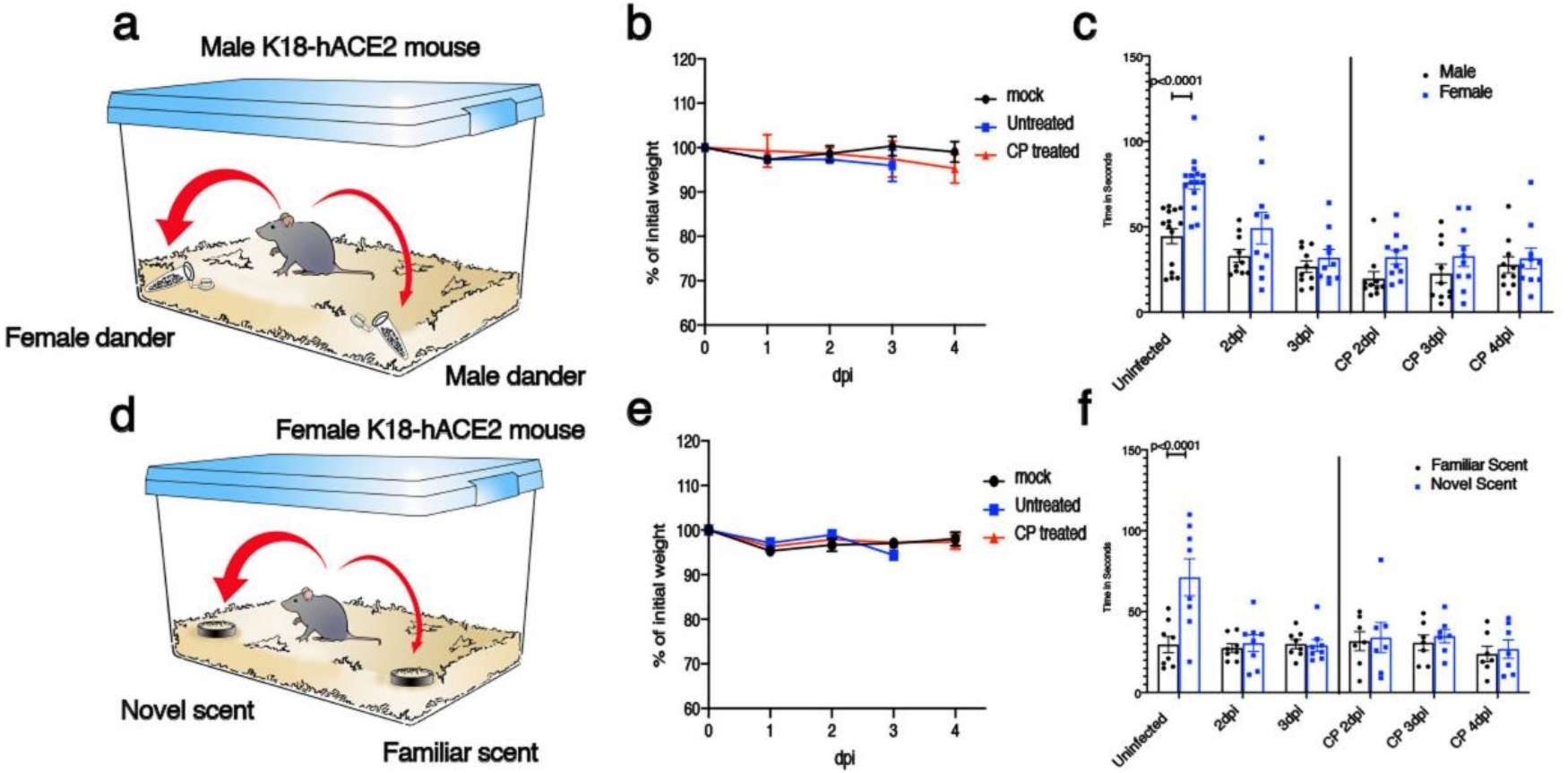
# SARS-CoV-2 K18TghACE2 MOUSE MODEL




*Zheng J. et al, 2020, Nature doi:10.1038/s41586-020-2943-z*

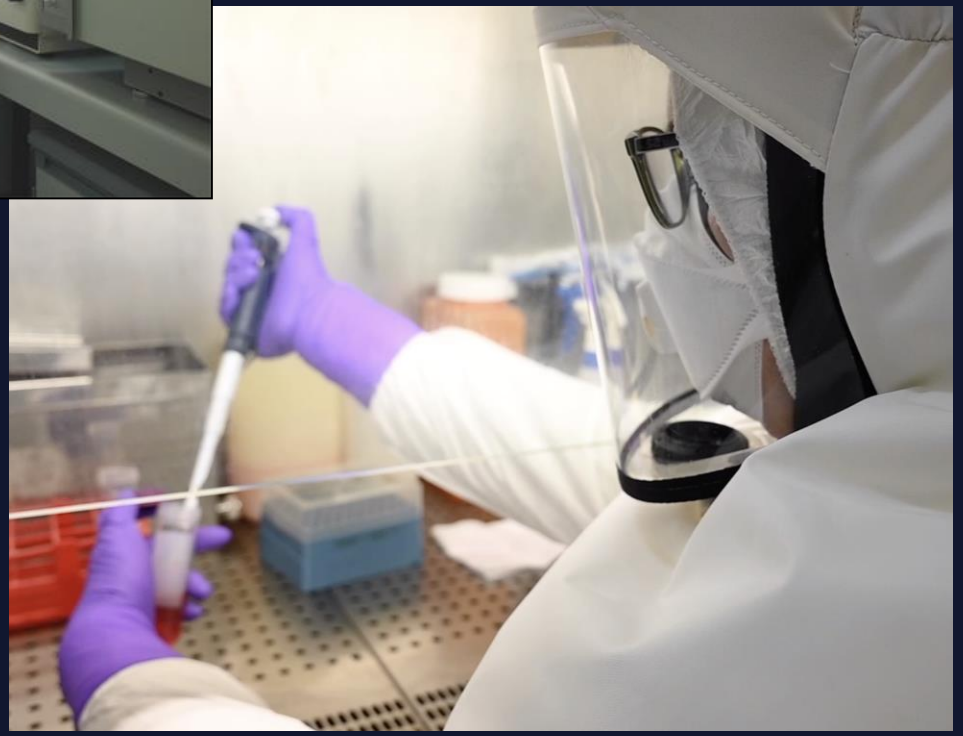
# SARS-CoV-2 K18TghACE2 MOUSE MODEL

## SARS-CoV-2 ANOSMIA MODEL



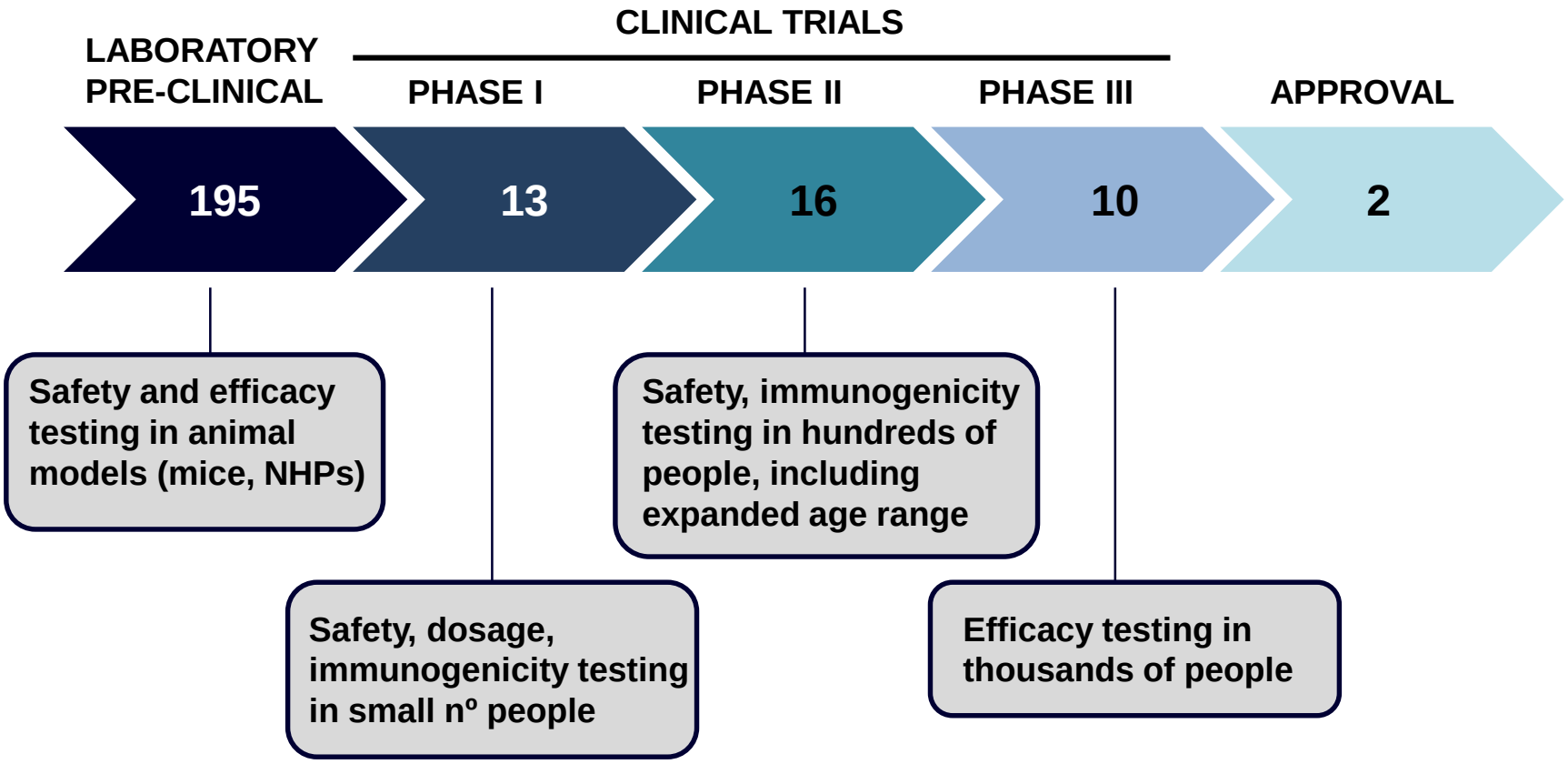

*Zheng J. et al, 2020, Nature doi:10.1038/s41586-020-2943-z*

# BSL-3 CNB-CSIC. MADRID



# ENGINEERING BIOSAFE VACCINES

# STEPS IN VACCINE DEVELOPMENT




*Updated 16NOV20*

# EFFECTIVE VACCINE PROPERTIES

- HIGH IMMUNOGENICITY
- LONG-TERM IMMUNITY (IMMUNE MEMORY)
- GENETICALLY AND THERMALLY STABLE
- BIOSAFE

- SUBUNIT VACCINES
- INACTIVATED VIRUSES
- VECTOR-BASED VACCINES

**ADVANTAGES**
FAST DEVELOPMENT
STABILITY

**LIMITATIONS**
SHORT-TERM IMMUNITY
EOSINOPHILIA
Ab DEPENDENT ENHANCEMENT

# SARS-CoV-2 OMS VACCINE LIST

| TYPE | Nº VACCINES PRE-CLINICAL | CLINICAL TRIALS |
|---|---|---|
| Protein subunit | 56 | 16 |
| VLPs | 16 | 2 |
| Inactivated virus | 15 | 7 |
| mRNA | 19 | 5 |
| DNA | 14 | 6 |
| → Self-amplifying RNA | 3 | 1 |
| Non-replicating vector | 19 | 10 |
| Replicating vector | 18 | 5 |
| Live attenuated virus | 2 | 1 |

*WHO, 10DEC20*

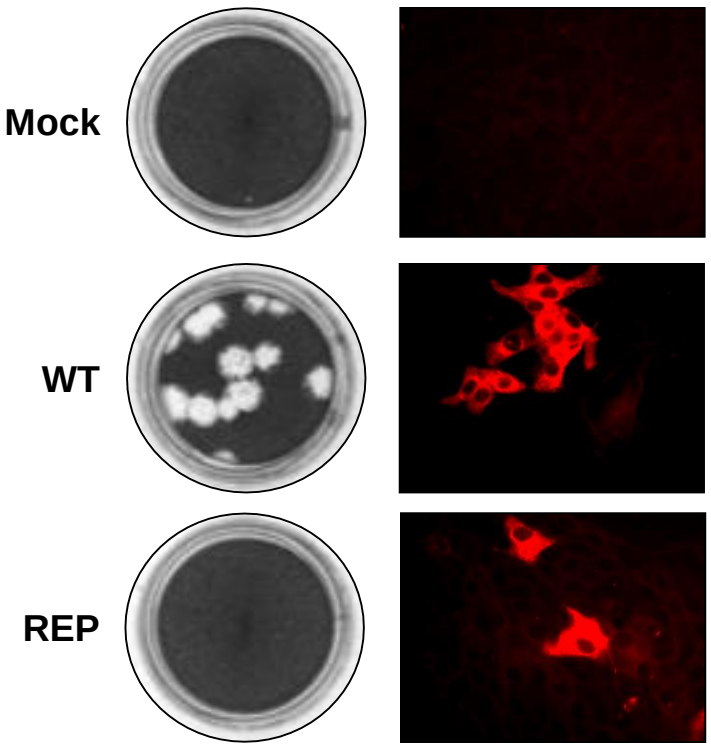
# SARS-CoV-2 VACCINES DATA, AUG20 (I)

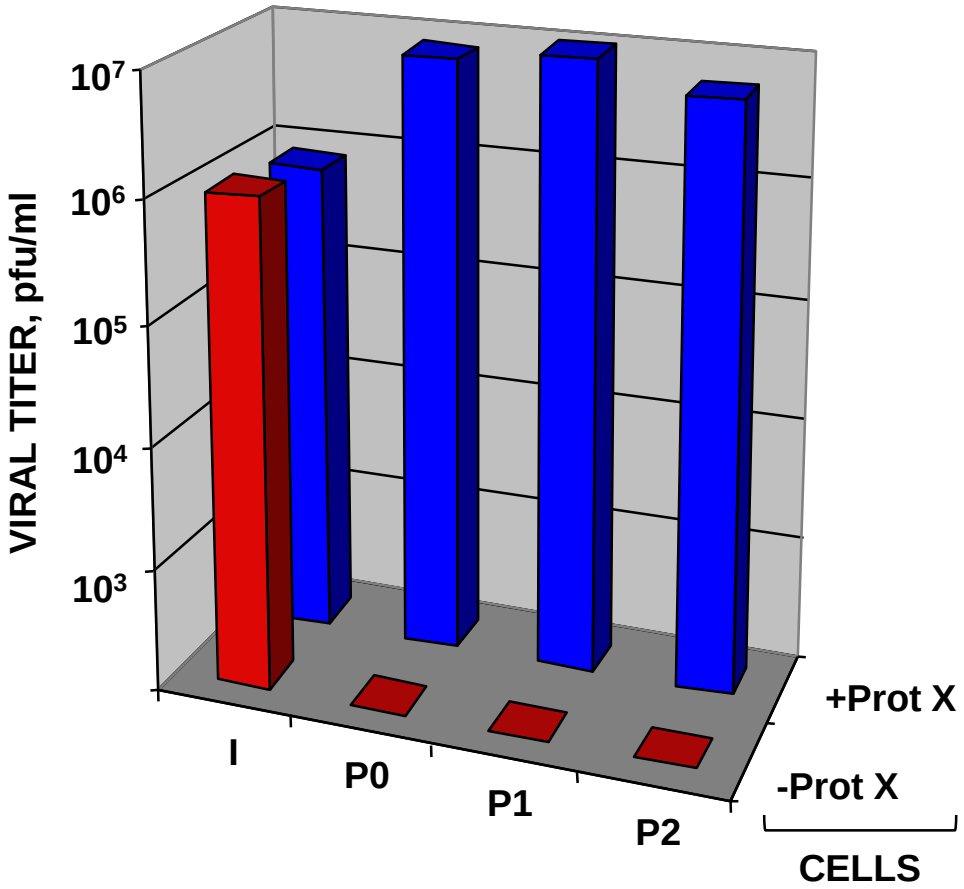
| | | PRECLINICAL | | | | CLINICAL TRIALS | |
|---|---|---|---|---|---|---|---|
| | | MICE | | NHPs | | | |
| VACCINE | TYPE | IMMUNOGENICITY | EFFICACY | IMMUNOGENICITY | EFFICACY | DESIGN | IMMUNOGENICITY |
| West China Hospital | RBD Baculovirus | ≠ protocols 1-40 µg<br>NAbs $IC_{50}$ 1:2800 | Sera yes<br>T cells no | 2 doses 40 µg<br>NAbs $IC_{50}$ 1:200 | No gRNA lung<br>↓ 100000 fold throat<br>↓ 1000 fold anal swabs<br>No sgRNAs<br>No lung damage | | |
| University of Washington, WA | Nanop 60xRBD Mamm. cells | 2 doses 0.9 or 5 µg<br>NAbs $IC_{50}$ 1:500, p – 1:7000, b | 7wpboost, MA<br>No virus lung nasal turbinates | | | | |
| Inovio | DNA c.opt. S | 2 doses 25 µg<br>NAbs $IC_{50}$ 1:97-1:340<br>Th1 | | | | | |
| Moderna/NIH | mRNA pre-fusion S | 2 doses 0.01-1µg<br>NAbs $IC_{50}$ PV 1:1000 (1µg)<br>Th1/Th2 | 5wpboost, MA<br>No gRNA lung<br>↓ >200-fold nasal turbinates<br>↓ Lung damage | 2 doses 10 or 100 µg<br>NAbs $IC_{50}$ 1:501-1:3481 (pb)<br>CD4 Th1 | No gRNA in BALF<br>↓ >1000-fold nasal swabs<br>No lung damage | 45 (15x3g)<br>2 doses<br>25, 100, 250 µg | $VNT_{80}$ 1:340 (25 µg)<br>1:654 (100 µg)<br>CD4 Th1 |
| BioNTech/Pfizer | mRNA trimerized RBD | | | | | 60 (12x5g)<br><br>45 (12x3g +9 placebo) | $VNT_{50}$ 1:578 (2x50 µg)<br>CD4CD8 Th1<br><br>$VNT_{50}$ 1:267 (2x30 µg |
| Imperial College | s.a. mRNA pre-fusion S | 2 doses 0.01-1 µg<br>NAbs $IC_{50}$ PV 1:5000, p – 1:100000, b<br>T cell responses | | | | | |

# SARS-CoV-2 VACCINES DATA, AUG20 (II)

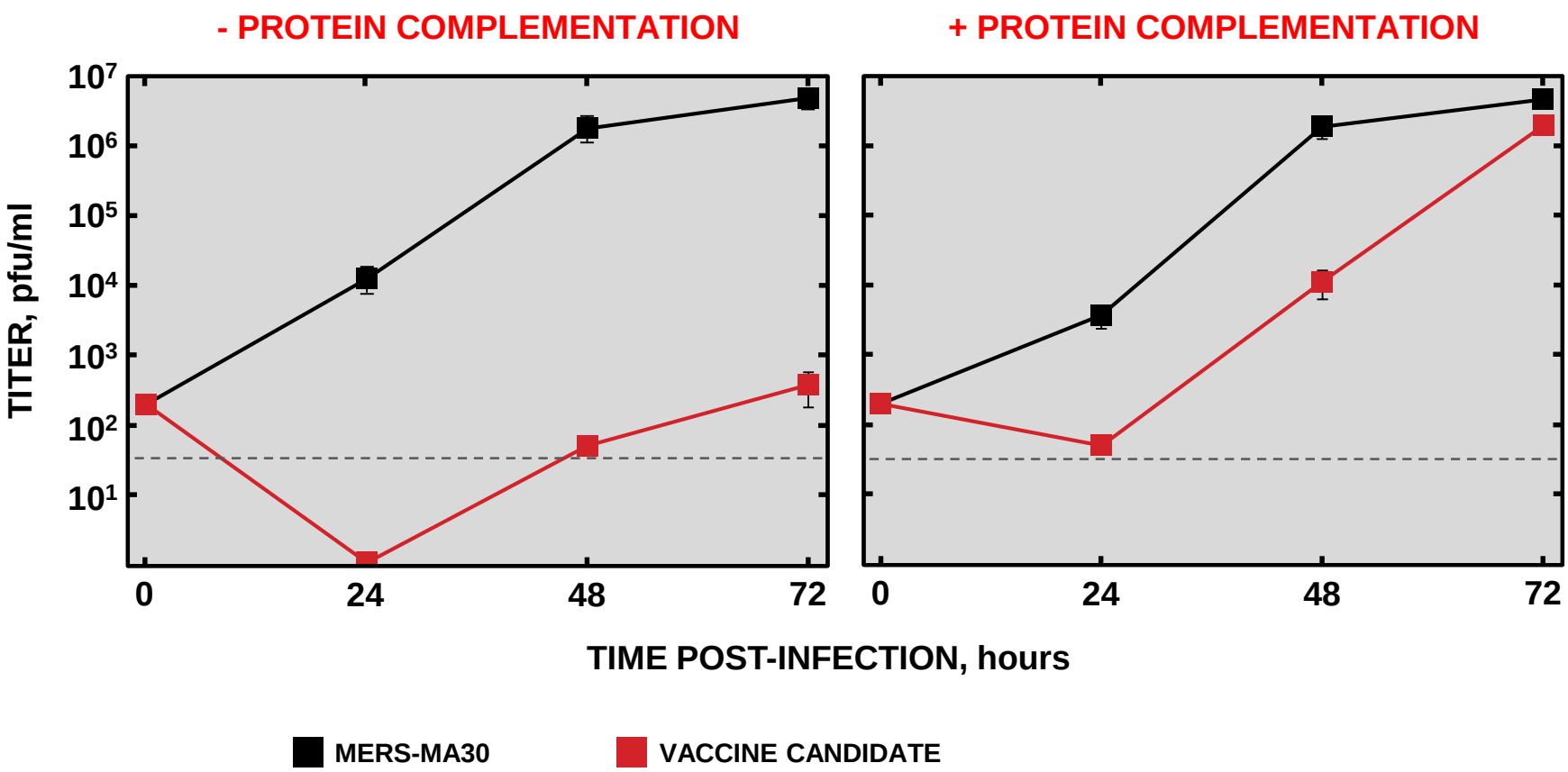
| | | PRECLINICAL | | | | CLINICAL TRIALS | |
|---|---|---|---|---|---|---|---|
| | | MICE | | NHPs | | | |
| VACCINE | TYPE | IMMUNOGENICITY | EFFICACY | IMMUNOGENICITY | EFFICACY | DESIGN | IMMUNOGENICITY |
| CanSino /Beijing Inst. Biotechnology | Ad5<br>c.opt S | | | | | 108 (36x3g)<br>$5x10^{10}$, $10^{11}$, $1.5x10^{11}$<br>508<br>253 $10^{11}$, 129 $5x10^{10}$, 126 placebo | NAbs 1:14 – 1:34<br>T CD4CD8<br><br>NAbs 1:19<br>T cell response |
| Oxford/AstraZeneca | CHAdOx1<br>c.opt. S | $6x10^{9}$ single dose<br>NAbs 1:80<br>Th1 | | $2.5x10^{10}$ 1 or 2 doses<br>NAbs 1:40 (pp)-1:160 (pb)<br>T cell response | = gRNA nasal swabs<br>↓ 100-fold BALF, lung<br>No lung damage (control 3/6) | 543 (10 prime-boost) $5x10^{10}$ dose | $VNT_{100}$ 1:316 (35, p)<br>1:34 (10, b)<br>T cell responses |
| Harvard/Janssen Vaccines | Ad26<br>S pre-fusion+furin cl.mut. | | | $10^{11}$<br>NAbs $IC_{50}$ 1:113<br>Th1 | No gRNA in BALF nasal swabs<br>Minim. disease model | | |
| City of Hope National Medical Center, CA | MVA<br>S, N, S+N | 2 doses $2.5x10^{7}$ or $5x10^{6}$<br>NAbs $NT_{90}$ 1:200, p – 1:1000, b<br>CD4 CD8 Th1 | | | | | |
| Sinovac Biotech | Inactivated PiCoVacc | 1 dose 1.5, 3 or 6µg<br>NAbs $IC_{50}$ 1:3000 | | 3 doses 3 or 6 µg<br>NAbs $IC_{50}$ 1:12800<br>= CD4CD8 | No gRNA lung, anal swabs<br>↓ 100-10000 fold throat<br>Mild focal lung damage | | |
| Beijing Inst. Biological Products Ltd. | Inactivated BBIBP-CorV | ≠ protocols 2-8 µg<br>NAbs $IC_{50}$ 1:1024-1:30000 | | 2 doses 2 or 8 µg<br>NAbs $IC_{50}$ 1:1700 | No gRNA lung<br>↓ 1000 fold throat, anal swabs (8µg)<br>No lung damage | | |

# ENGINEERING REPLICATION-COMPETENT PROPAGATION-DEFECTIVE CoVs



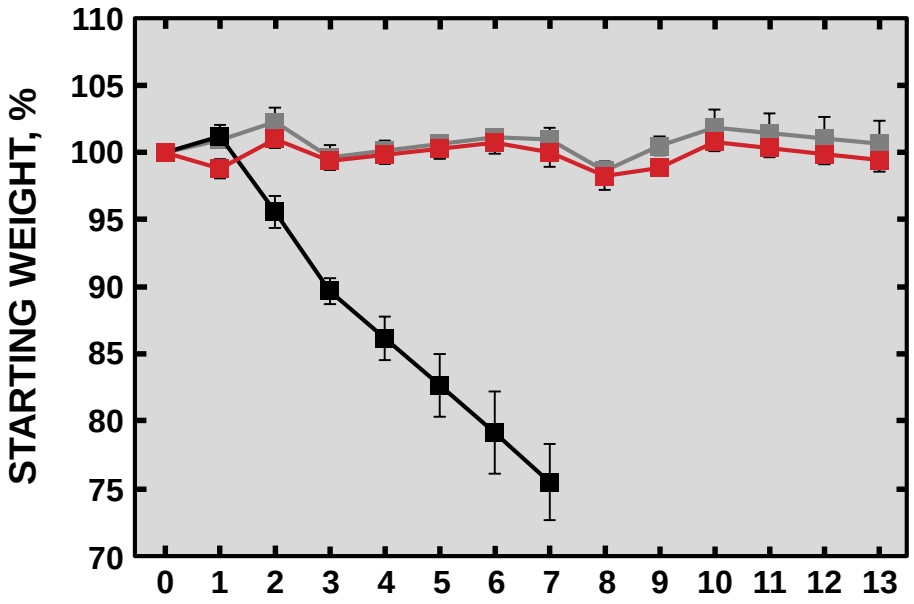



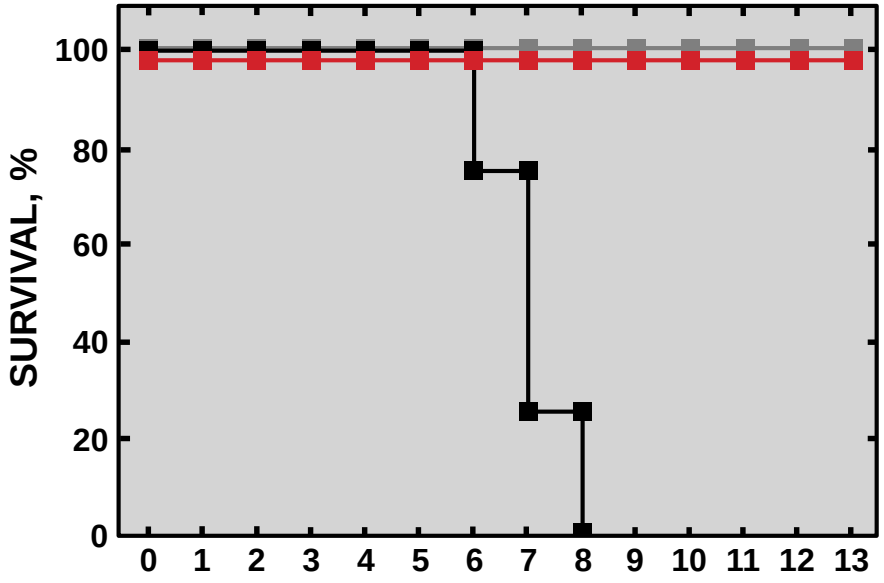
# MERS-CoV VACCINE CANDIDATE GROWTH KINETICS IN CELL CULTURES



# MERS-CoV VACCINE CANDIDATE ATTENUATION IN KI MICE

**16-week-old mice**
**$10^4$ pfu/mice intranasally**

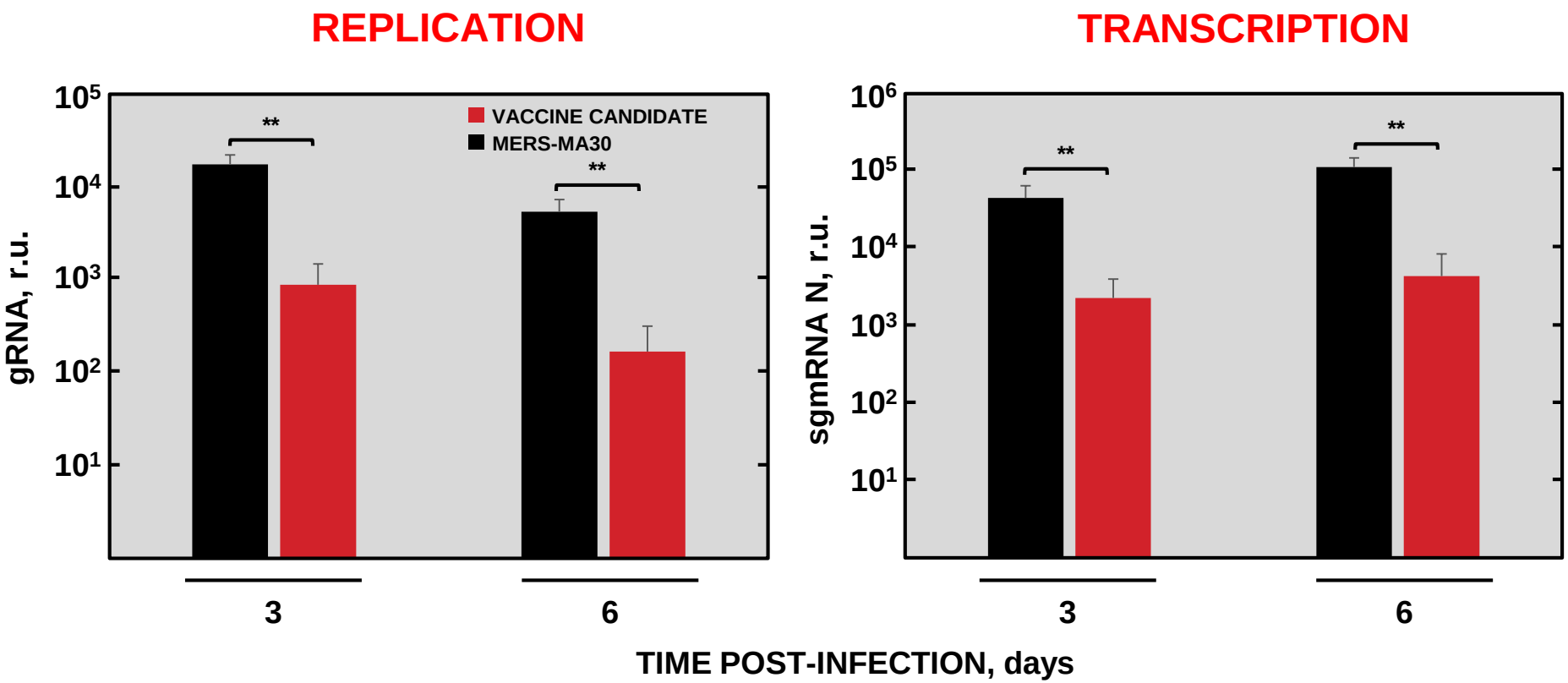


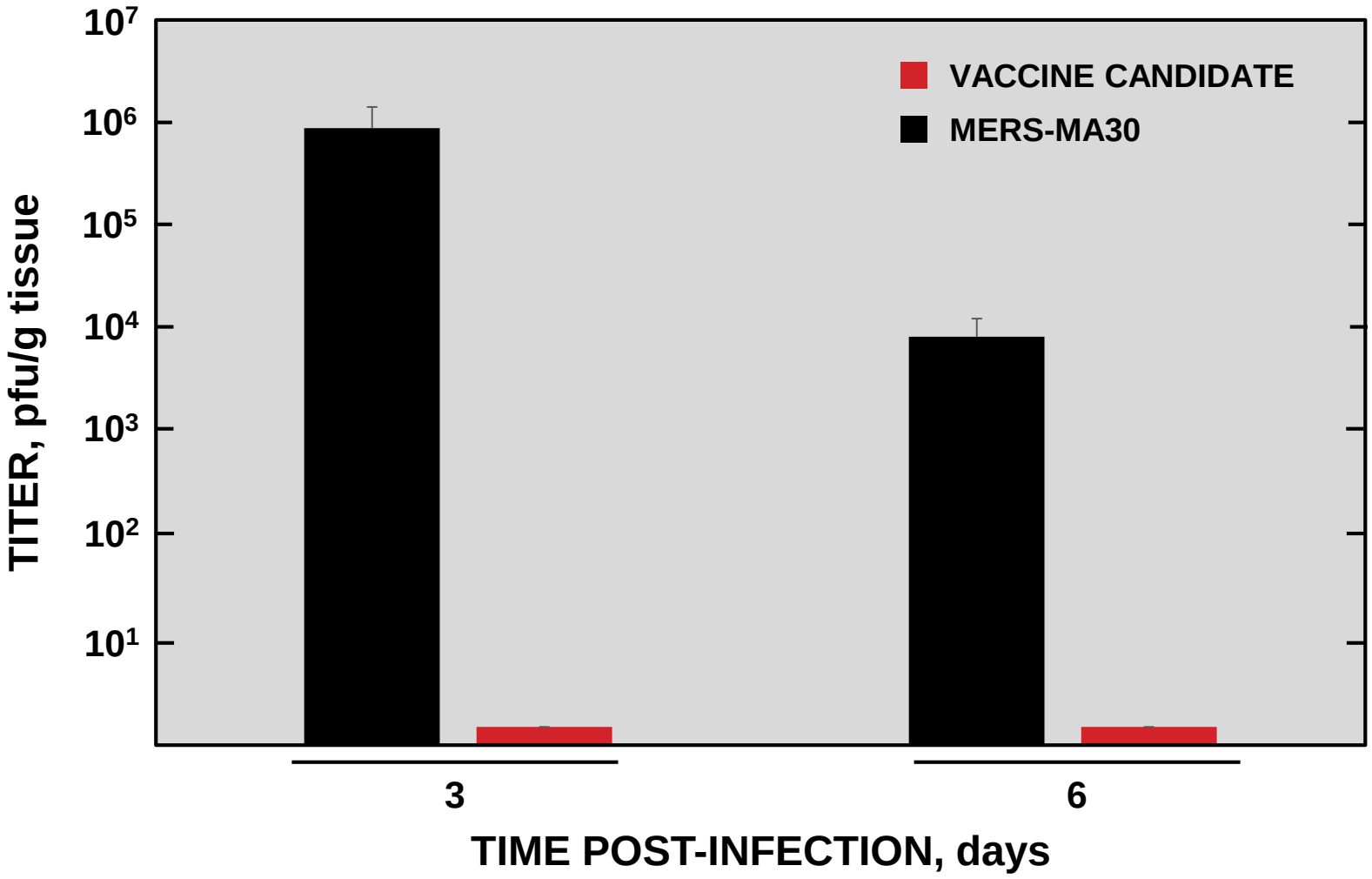

TIME POST-INFECTION, days

MOCK  MERS-MA30  VACCINE CANDIDATE

# MERS-CoV VACCINE CANDIDATE REPLICATION IN THE LUNG OF KI MICE

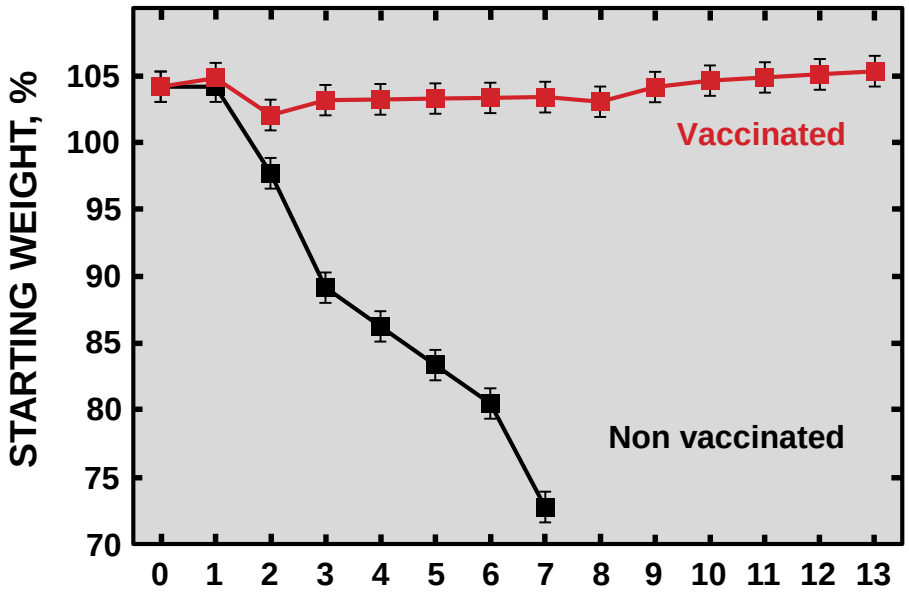


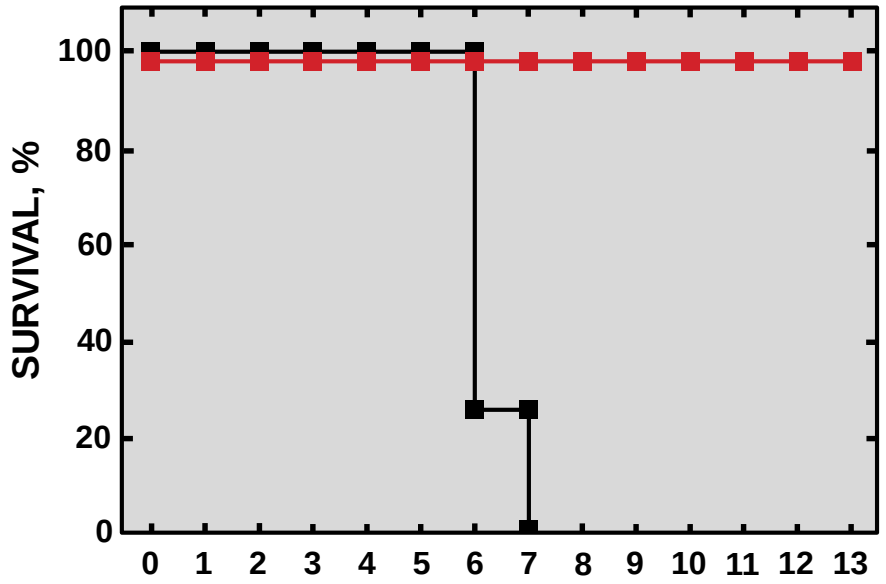
# MERS-CoV VACCINE CANDIDATE GROWTH IN THE LUNG OF KI MICE



# PROTECTION INDUCED BY MERS-CoV VACCINE CANDIDATE IN KI MICE

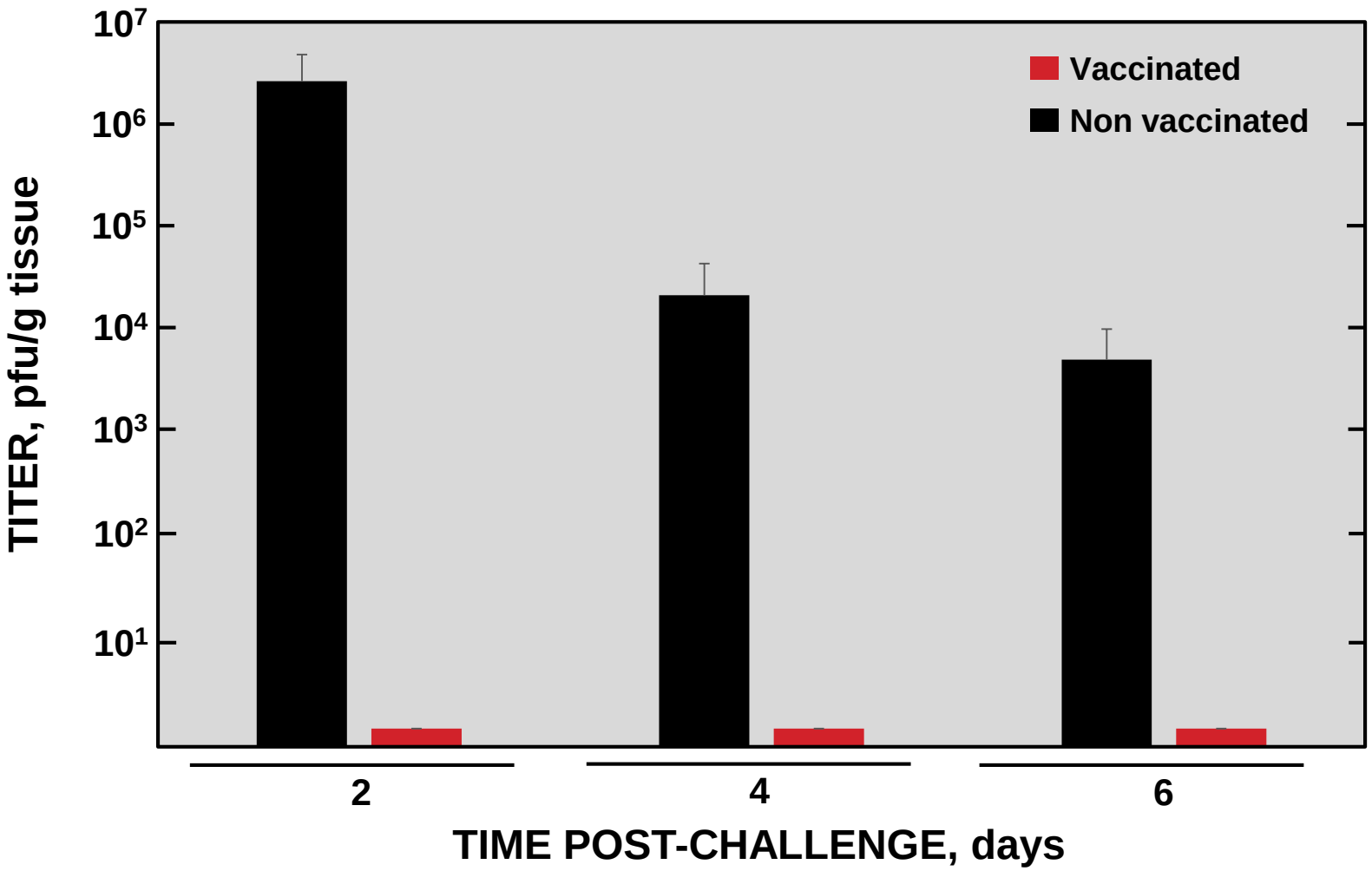
**16-week-old mice challenge with**
**$10^5$ pfu/mice of MERS-MA intranasally**




**TIME POST-CHALLENGE, days**

# MERS-CoV VACCINE CANDIDATE CONFERRED STERILIZING IMMUNITY IN KI MICE



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**THANK YOU !!**

**QUESTIONS?**