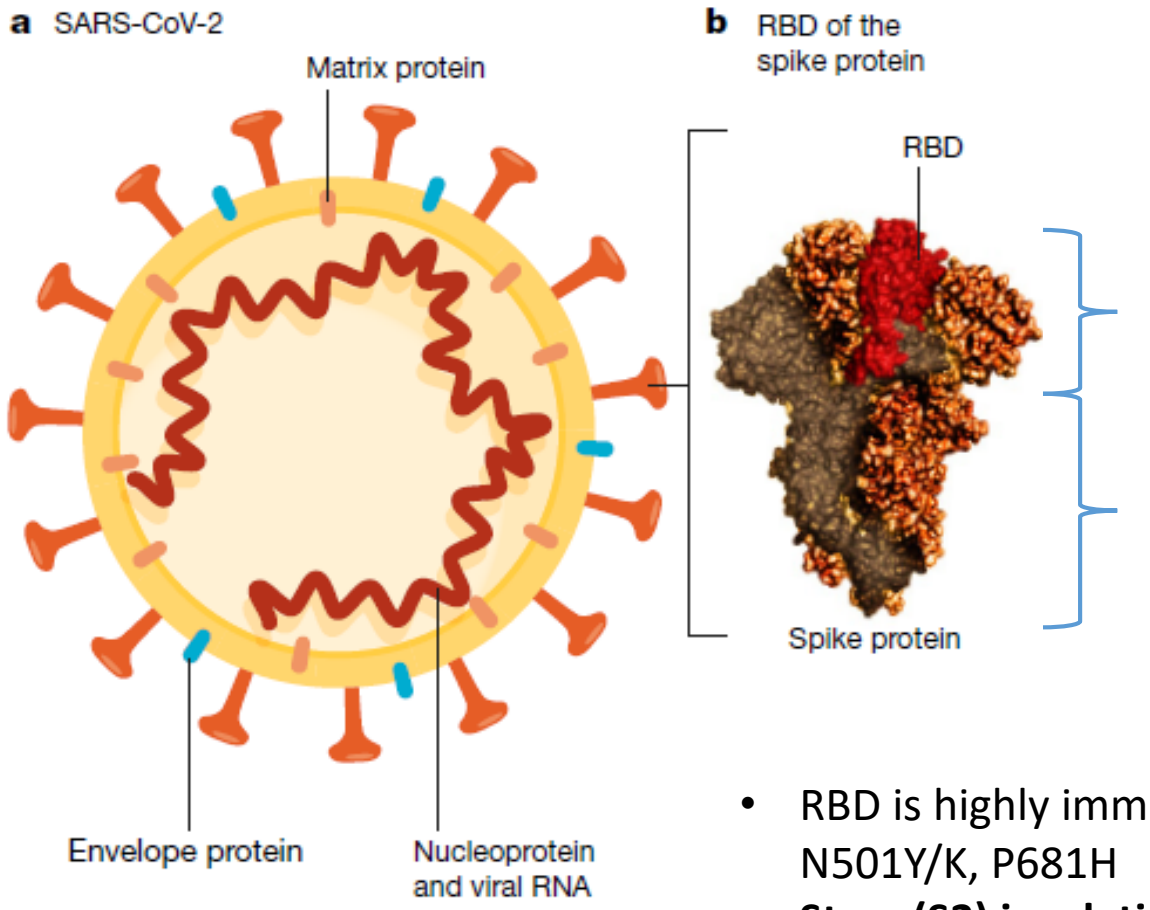
Accelerated SARS-COV2 vaccine development

Special series on Laboratory Animal Science

Regina Reimann

Structure of SARS-CoV2



3 x S1 subunit forms a “cap”
S1 contains the receptor binding domain (RBD)
Binding to the receptor angiotensin converting enzyme 2 (ACE2)

S2 subunit forms the “stem” : fusion peptide, two heptad repeats and a transmembrane peptide

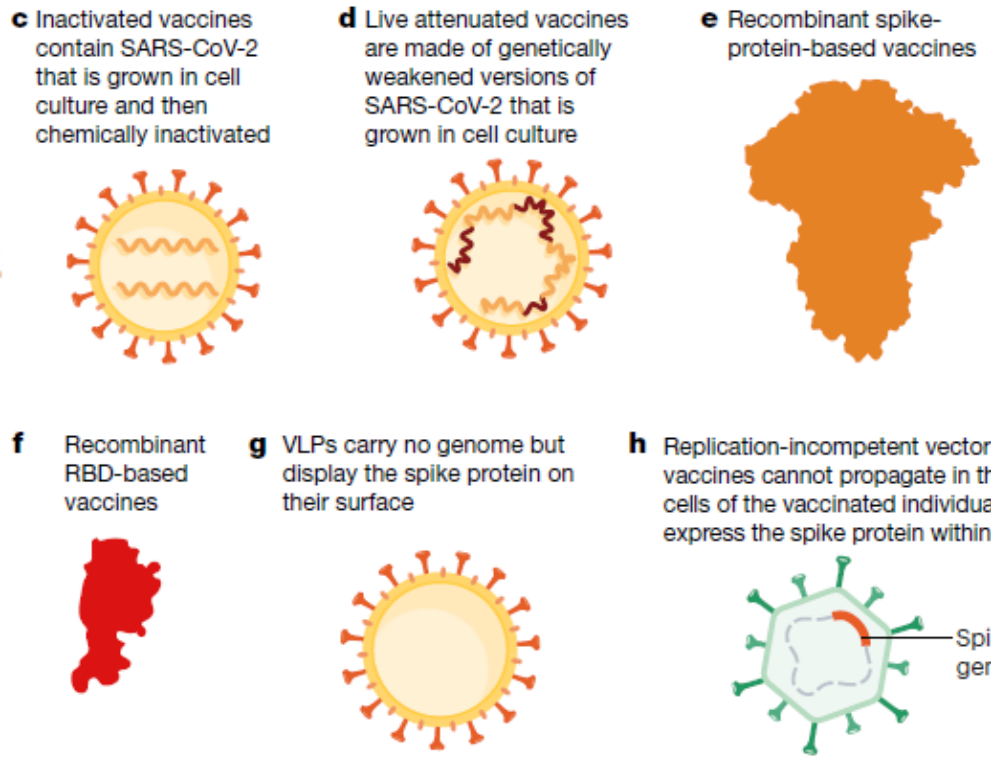
- RBD is highly immunogenic; antibody-escape mutations : K417N, E484K, N501Y/K, P681H
- **Stem (S2) is relatively conserved among coronavirus ; potent target of neutralizing antibodies**
- «Conformational masking» (by RBD) of the neutralizing epitopes

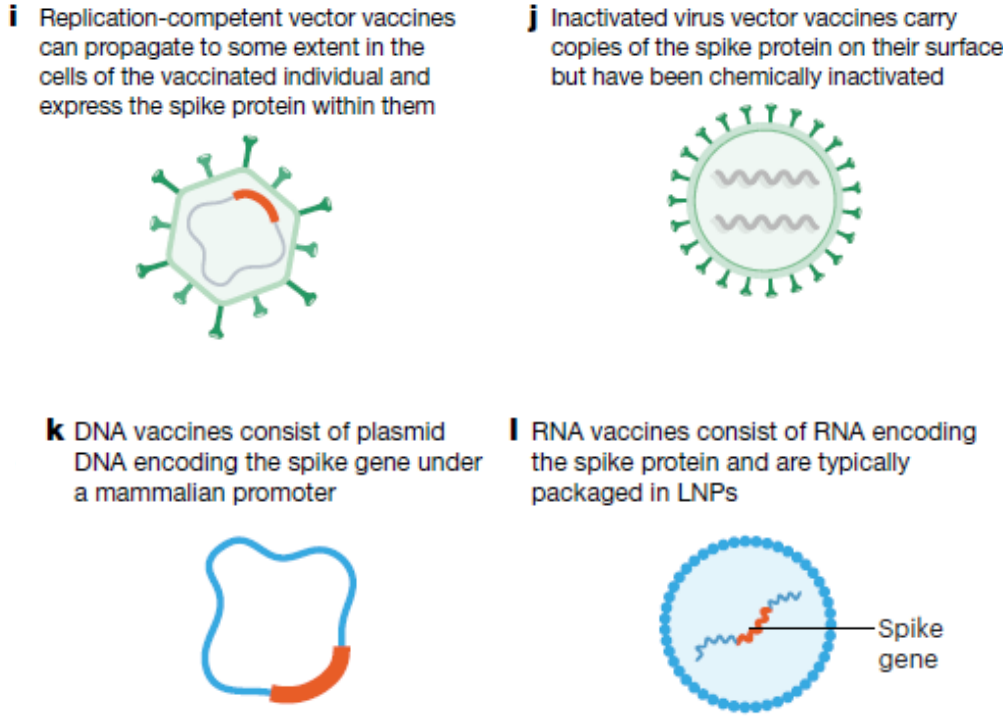
Krammer, Nature 2020

Vaccine platforms for CoV2 vaccine development

- CoronaVac (Sinovac)
- Sinopharm

- NVX CoV2372 Novavax (recombinant nanoparticle vaccine)



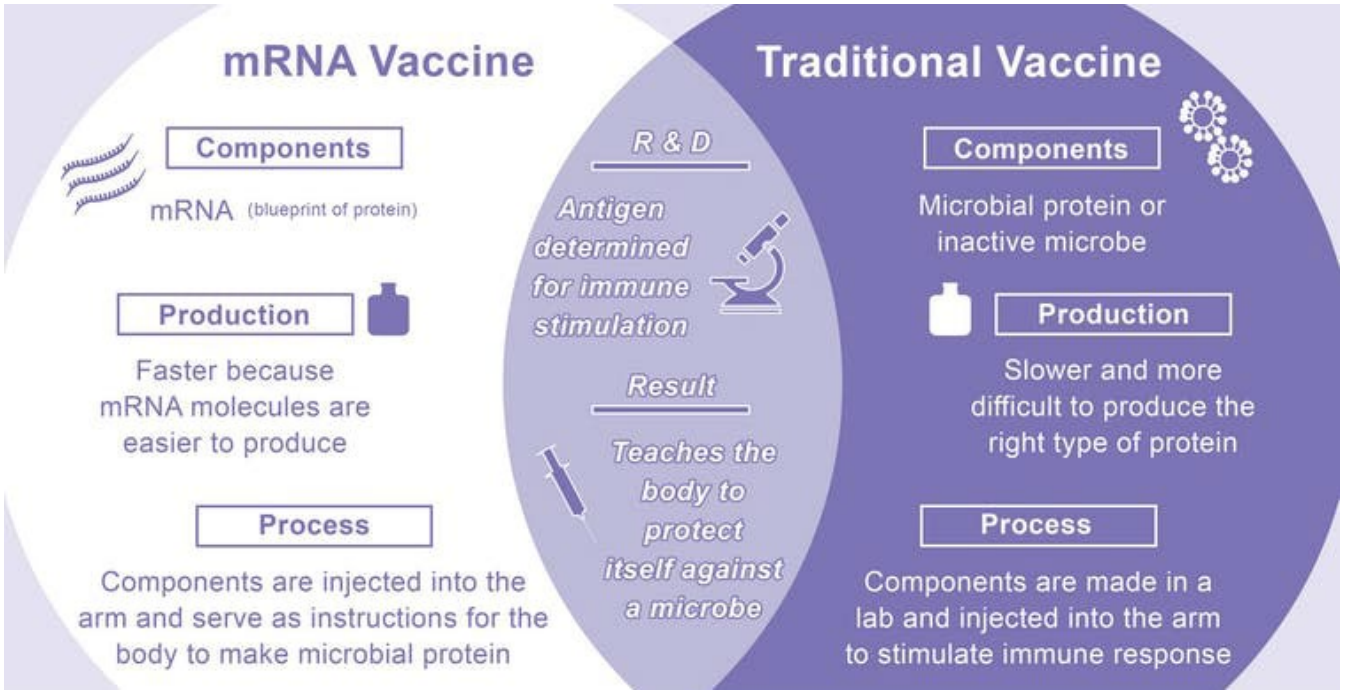


- ChAdOx nCoV-19 (AstraZeneca)
- Ad26.CoV2.S (Jannsen Vaccine)
- Ad5 nCoV (CanSino)
- Gam-COVID-Vac (Sputnik V, Gamaleya Research Institute)

- mRNA-1273 (Moderna)
- BNT162b2 (Pfizer)

Krammer, Nature 2020

mRNA Vaccines



- 1990 : first successful use of in vitro transcribed mRNA in animals
- 1992: Administration of vasopressin-encoding mRNA in the hypothalamus with a physiological response
- Progress over the last decades:
  - mRNA stability
  - Shaping innate immunogenicity
  - Efficient in vivo delivery

https://theconversation.com

Optimization of mRNA pharmacology

stabiliation

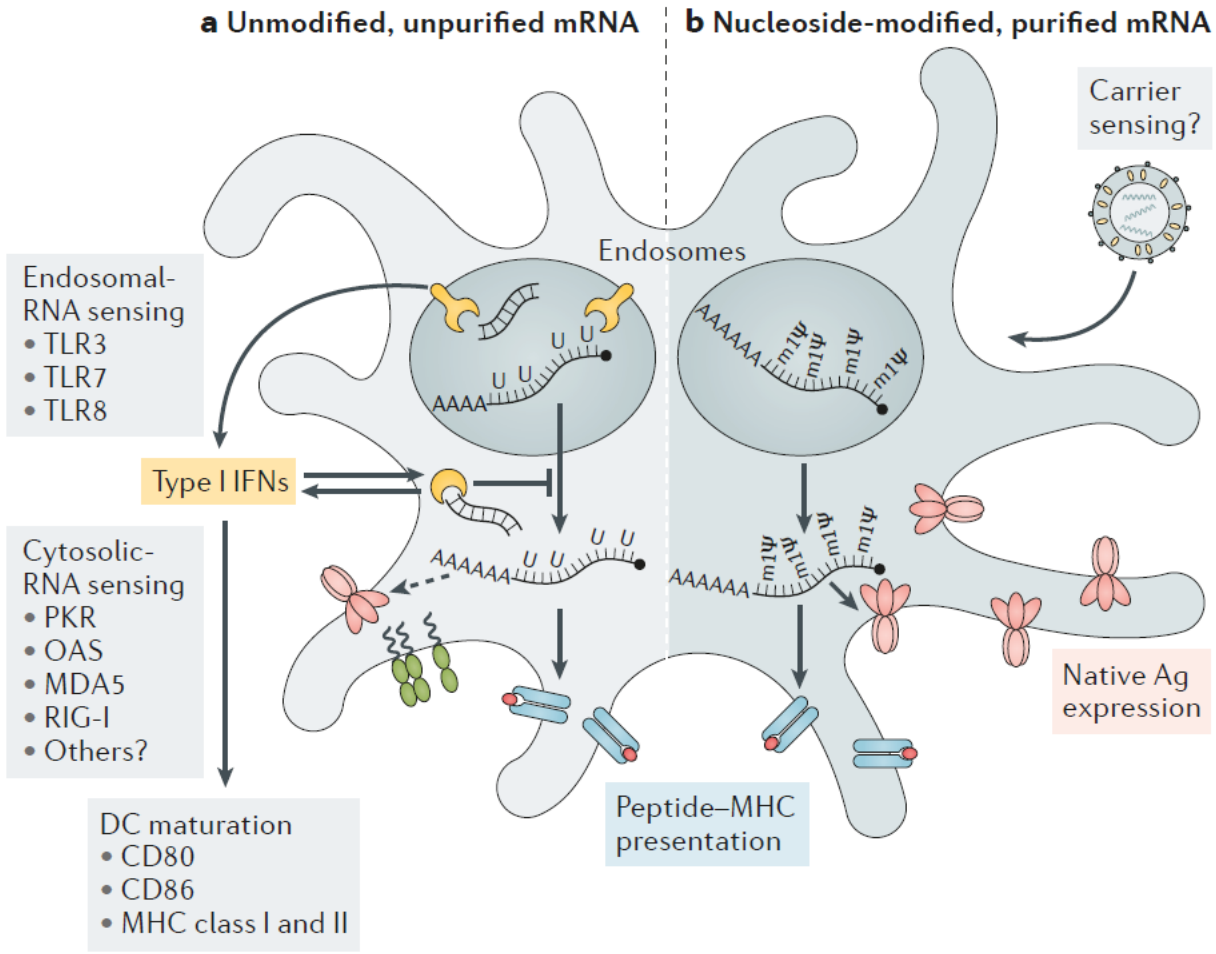
- Synthetic cap analogues and capping enzymes[26,27] stabilize mRNA and increase protein translation via binding to eukaryotic translation initiation factor 4E (EIF4E)
- Regulatory elements in the 5′-untranslated region (UTR) and the 3′-UTR[21] stabilize mRNA and increase protein translation
- Poly(A) tail[25] stabilizes mRNA and increases protein translation

Decrease innate Immune actiation

- Modified nucleosides[8,48] decrease innate immune activation and increase translation
- Separation and/or purification techniques: RNase III treatment (N.P. and D.W., unpublished observations) and fast protein liquid chromatography (FPLC) purification[13] decrease immune activation and increase translation
- Sequence and/or codon optimization[29] increase translation
- Modulation of target cells: co-delivery of translation initiation factors and other methods alters translation and immunogenicity

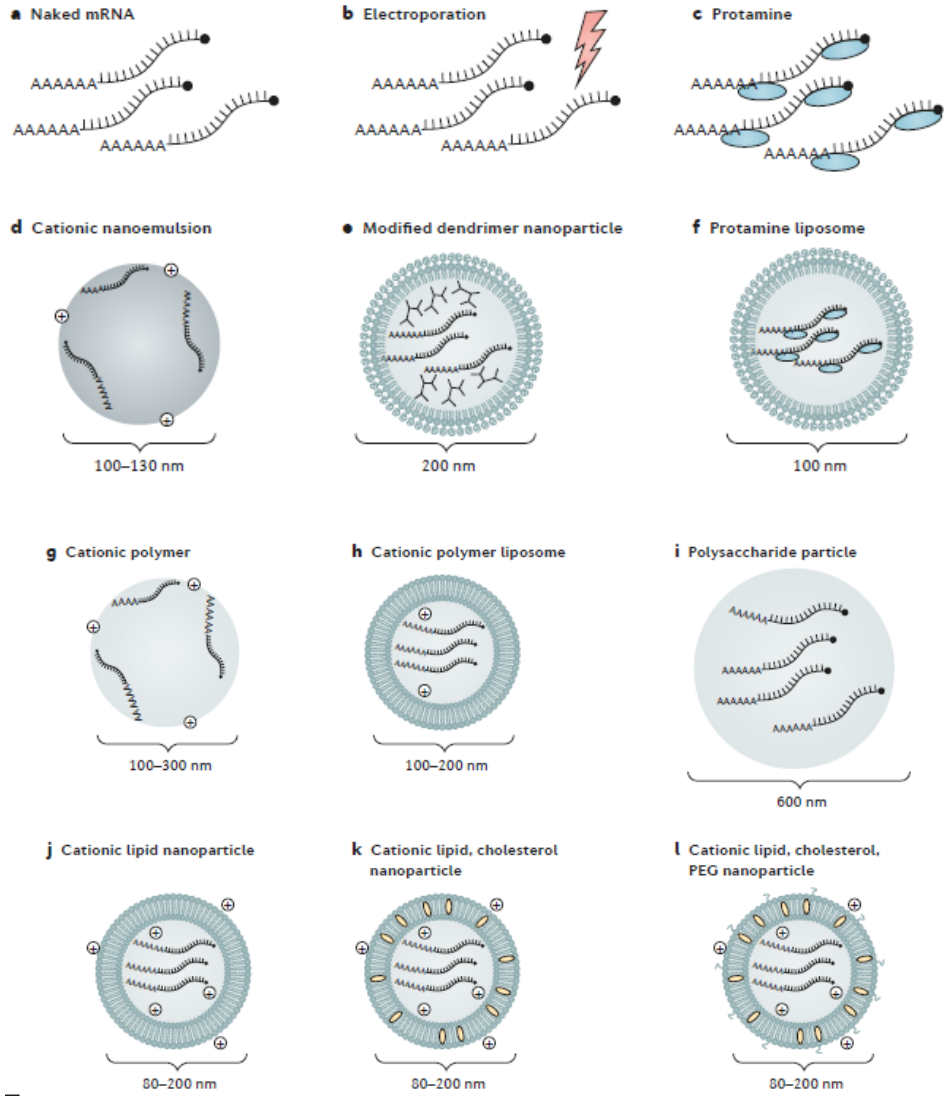
Pardi et al, Nature Reviews 2018

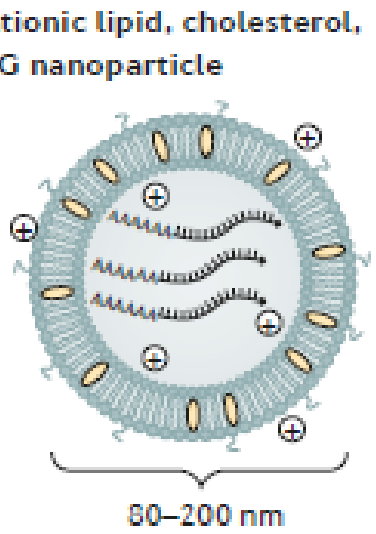
Innate immune sensing of mRNA vaccine



Pardi et al, Nature Reviews 2018

Delivery of mRNA vacine



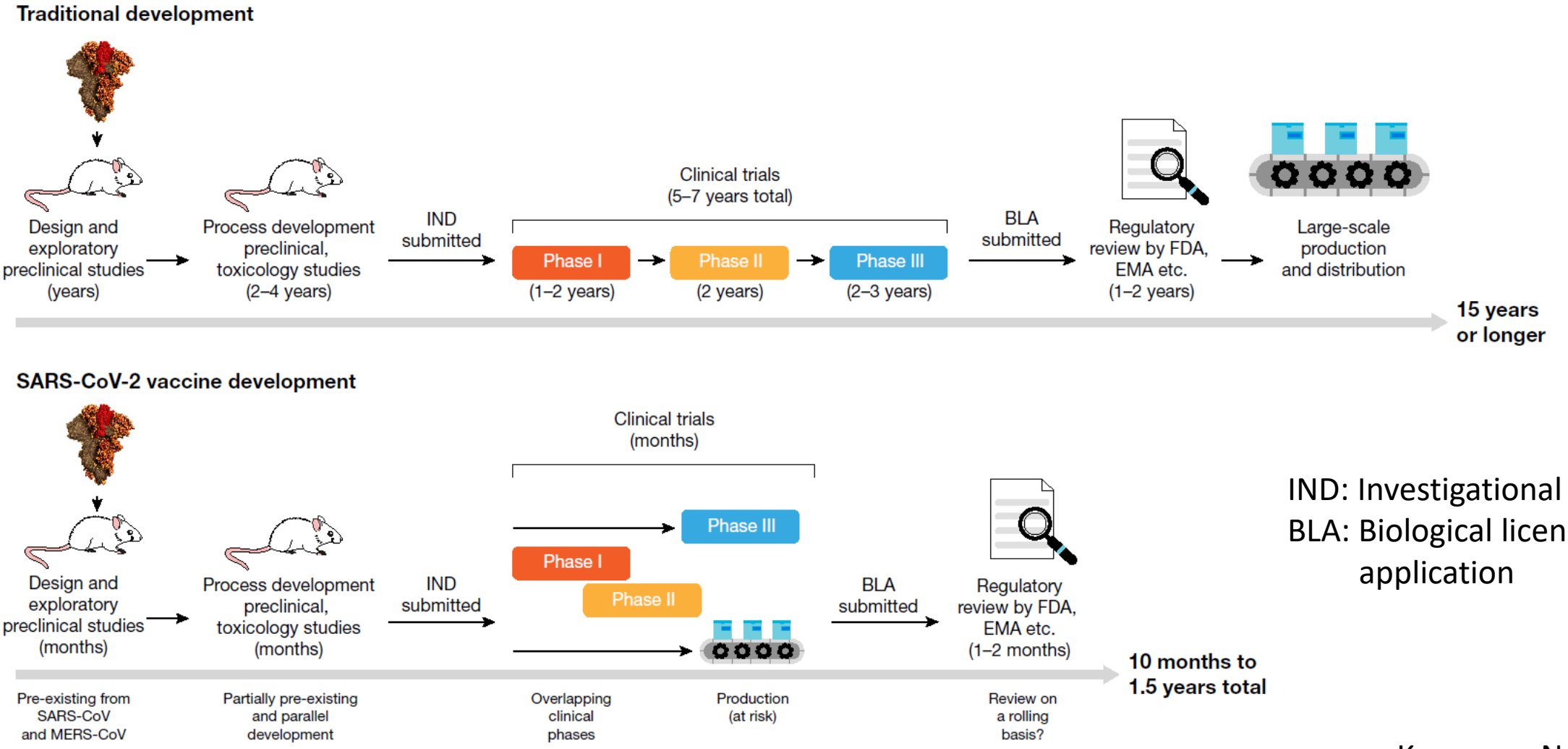


Lipid nanoparticles (LNPs):

- Lipid-linked polythylene glycol (PEG): increases half-life of formulation
- Cholesterol: Stabilizing agent
- Naturally occurring phospholipids: support lipid bilayer structure

Pardi et al, Nature Reviews 2018

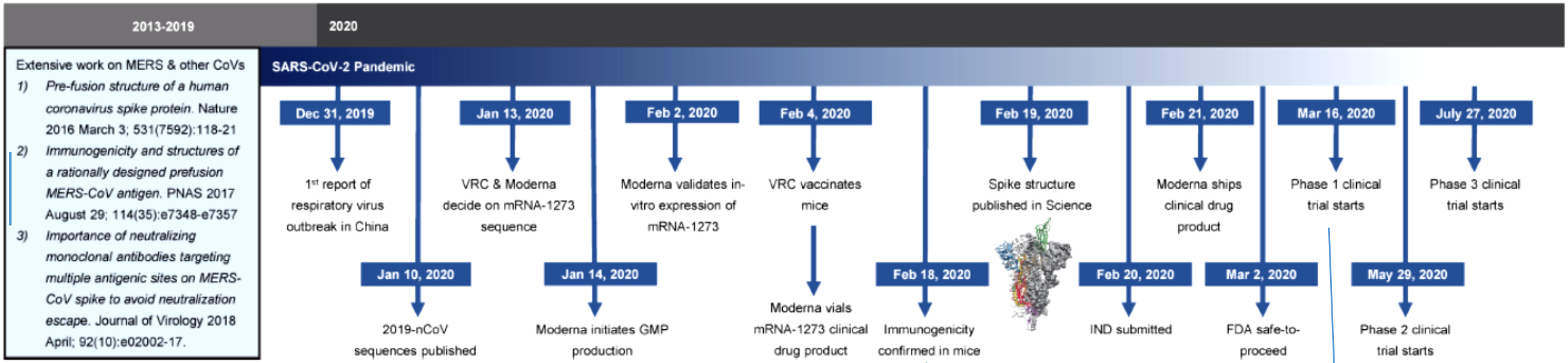
Traditional and acclerated vaccine development-pipelines



IND: Investigational new drug
BLA: Biological license application

Krammer, Nature 2020

Timeline for mRNA-1273 (Moderna) progression to clinical trial



5. 8. 2020:
mRNA-1273 vaccine design
Kizzmekia et al. , Nature

14. 07 2020
Jackson et al. , NewEngland Journal

27.8.2020:
Mouse-adapted model
SARS-COV2, Nature

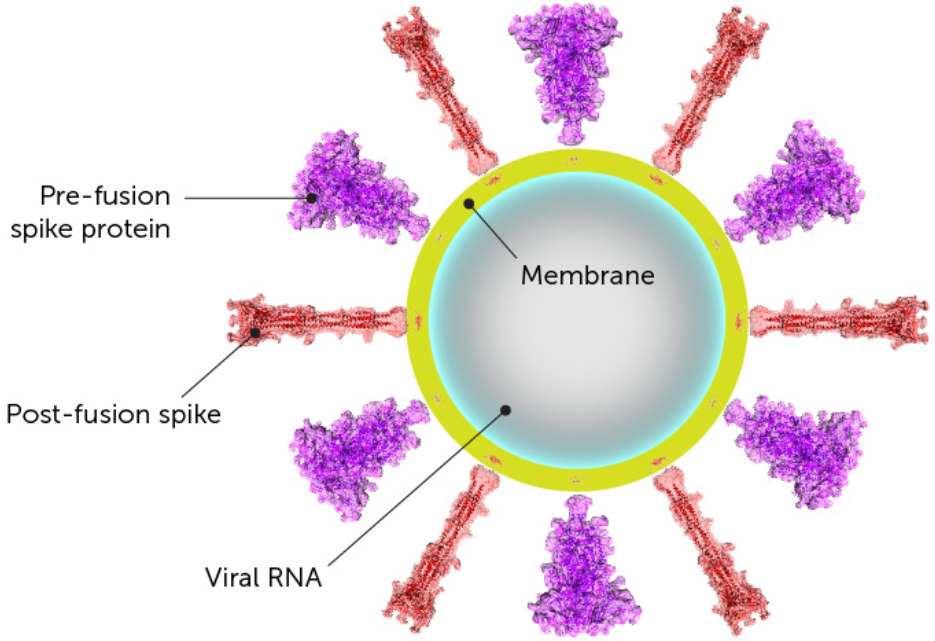
VRC: Vaccine Research Center, National Institutes of Health, Bethesda, MD, USA
IND: Investigational new drug

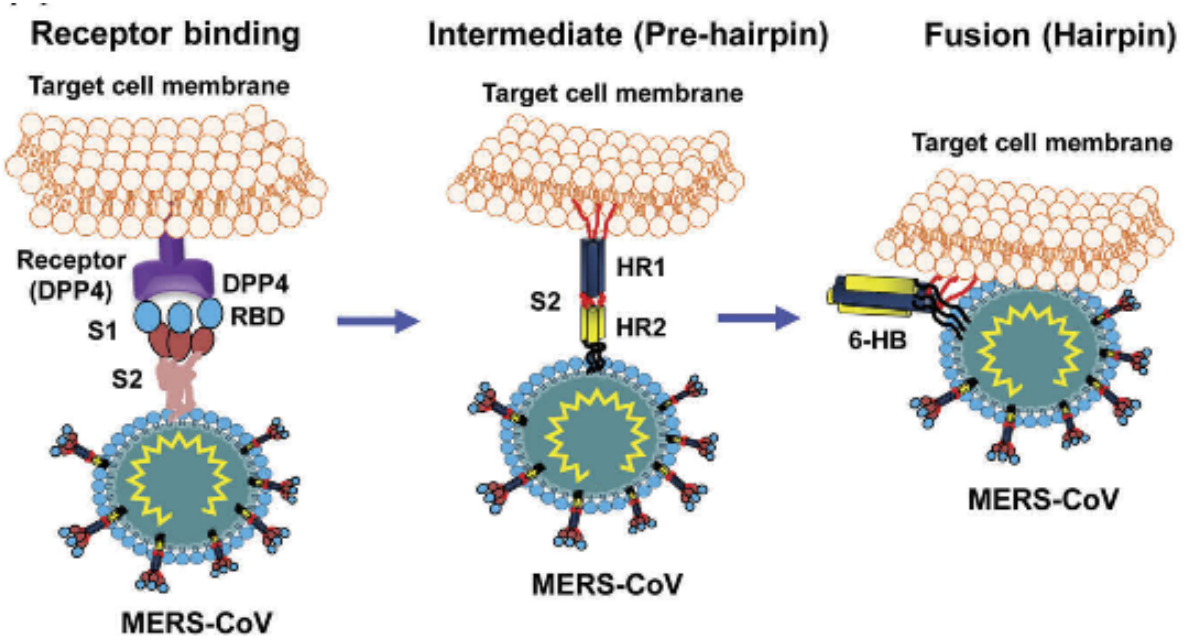
Design of prefusion MERS-CoV spike antigen

Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen

Jesper Pallesen[a,1], Nianshuang Wang[b,1,2], Kizzmekia S. Corbett[c,1], Daniel Wrapp[b], Robert N. Kirchdoerfer[a], Hannah L. Turner[a], Christopher A. Cottrell[a], Michelle M. Becker[d], Lingshu Wang[e], Wei Shi[e], Wing-Pui Kong[e], Erica L. Andres[d], Arminja N. Kettenbach[b,f], Mark R. Denison[d,g], James D. Chappell[d], Barney S. Graham[c], Andrew B. Ward[a,2], and Jason S. McLellan[b,2]

Shape shifting of the spike protein



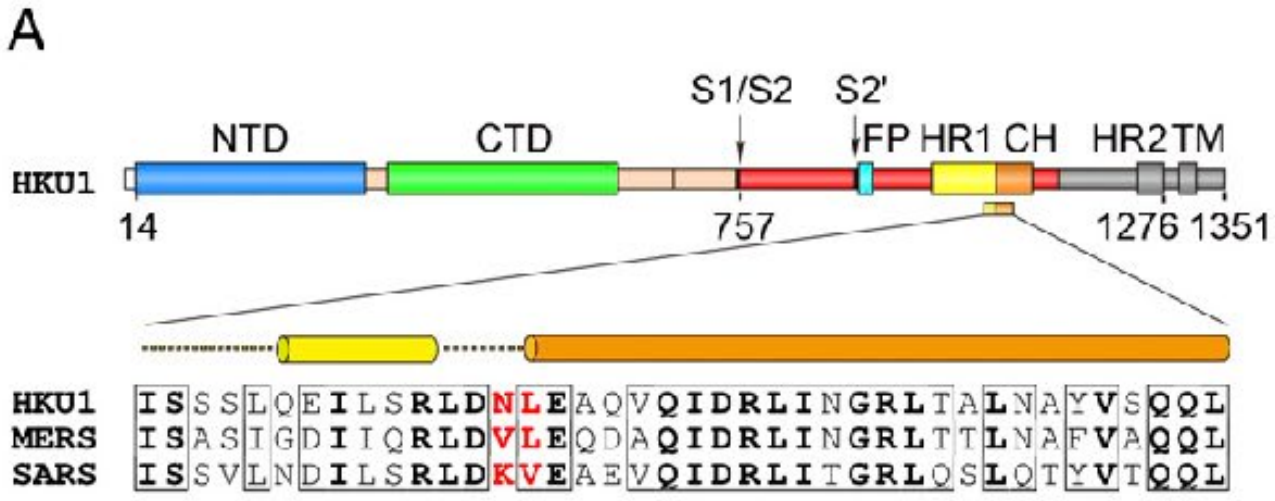


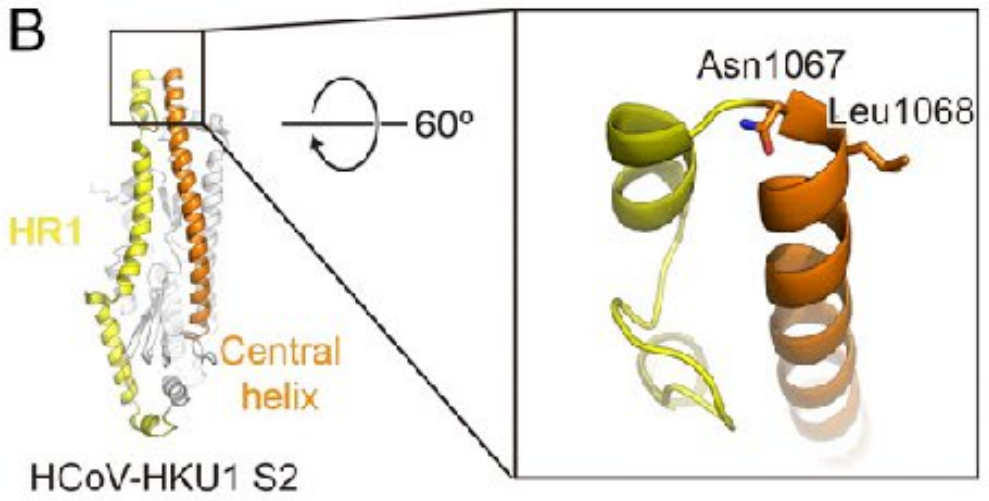
- Fusion state apposes viral and cell membrane
- Both forms of spike protein exist without the need to bind to the ACE2 receptor
- Post-fusion state is very stable and is decorated with N-linked glycans
- Protection from the environment and our immune system

Lanying et al, 2017

Two proline substitutions retain S2 in the prefusion conformation

- Based on work on the fusion protein of HIV-1 and RSV virus
- Two proline substitutions in the loop between the first heptad repeat (HR) and central helix
- Restrict triggering of the fusion protein and increases yield of prefusion ectodomains

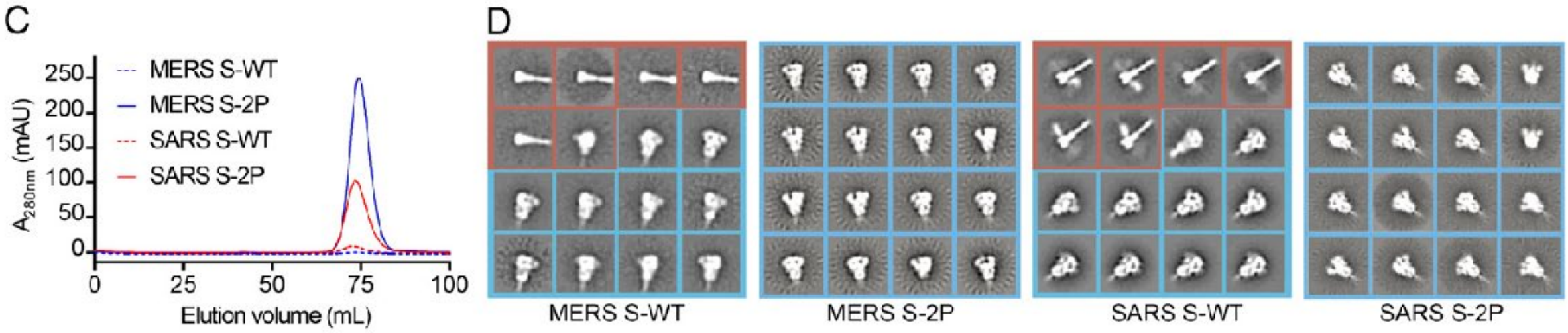




Increased expression and conformational homogenity

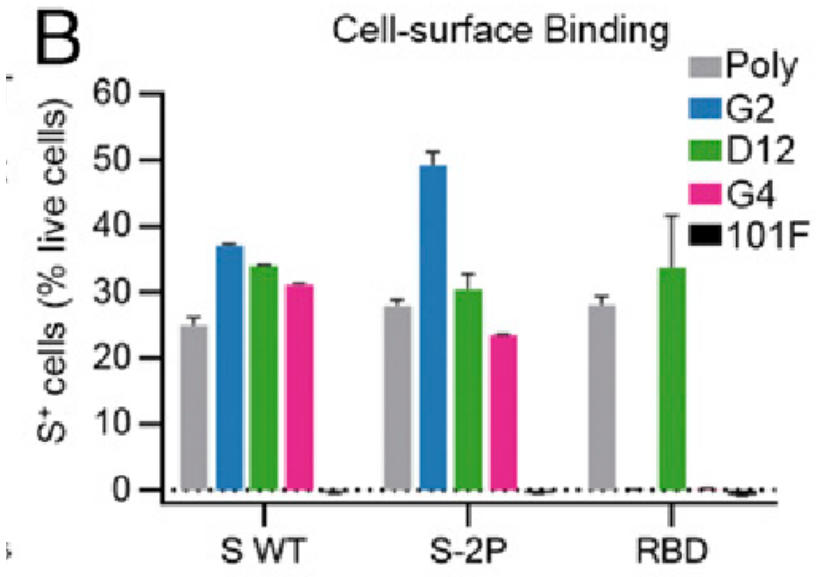
Production of S Protein Ectodomain:

- Mammalian-codon-optimized gene encoding the MERS-CoV S (England 1 strain)
- SARS-CoV1 S (Tor 2 strain)

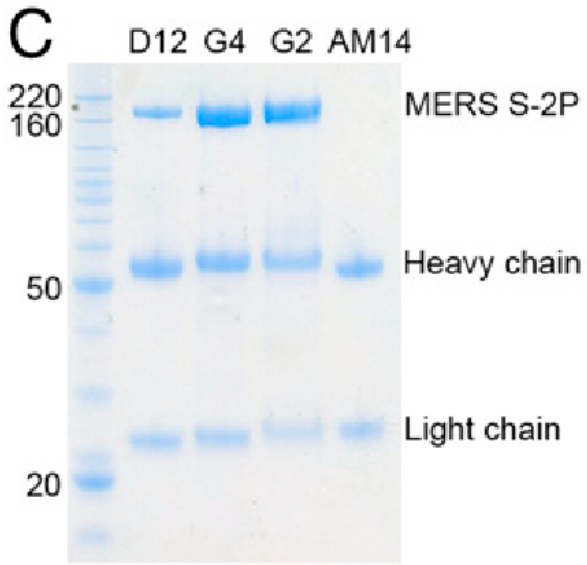


Negative-stain EM

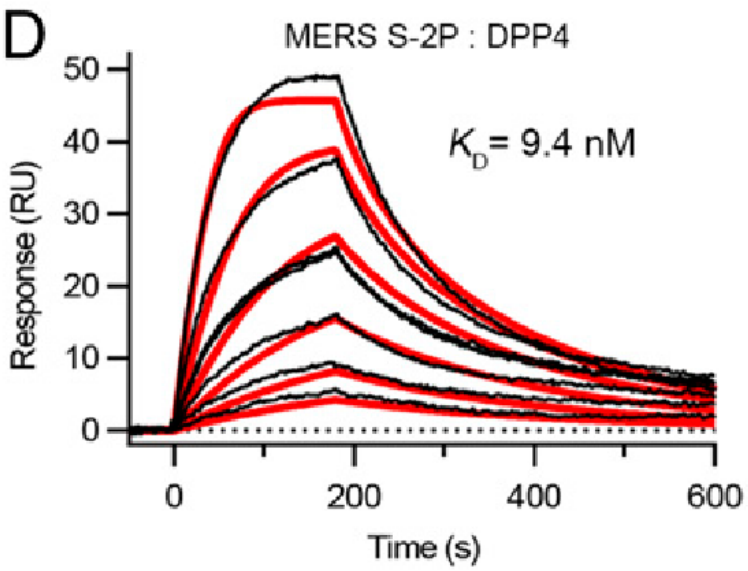
Binding to receptor and spike antibodies is conserved



Flow cytometry, S+ : Spike protein positive



Pull-down



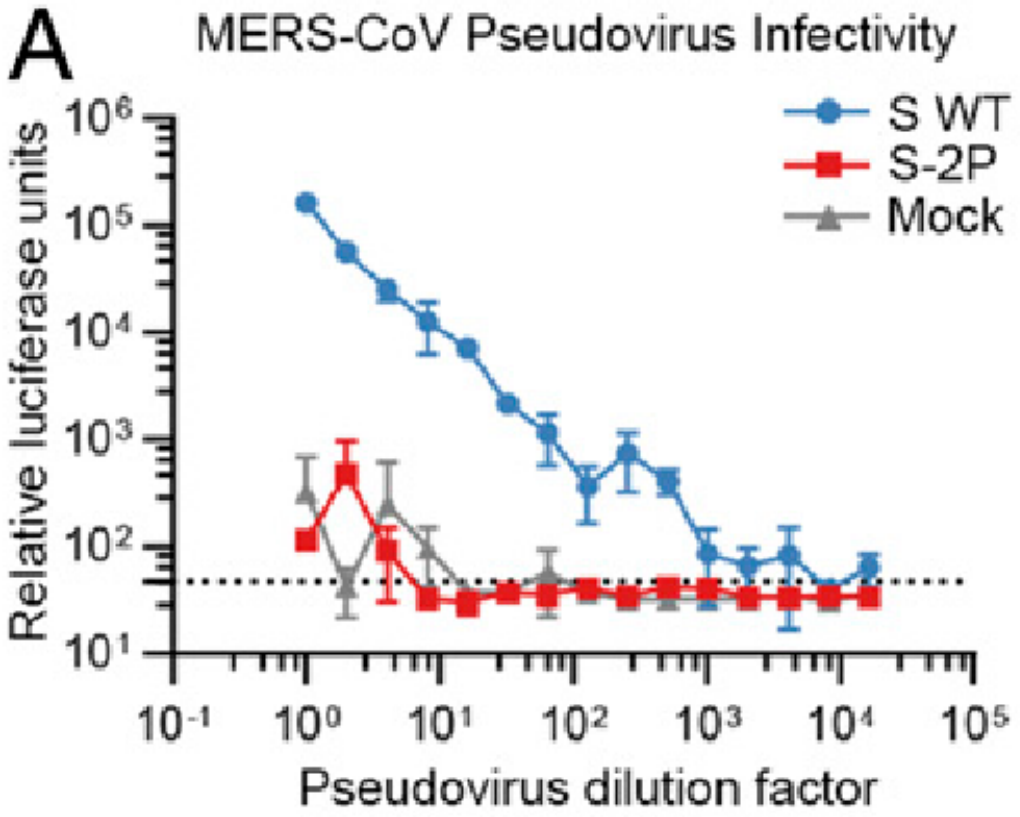
DPP4: soluble version of the receptor

Plasmon resonance

G2: anti N-terminal domain antibody
D12: anti RBD antibody
G4: anti S2 antibody
AM14: RSV F-specific antibody

MERS-CoV-S-2P is noninfectious

Infection of Huh7.5 cells with MERS-CoV pseudovirus enoding a luciferase reporter gene



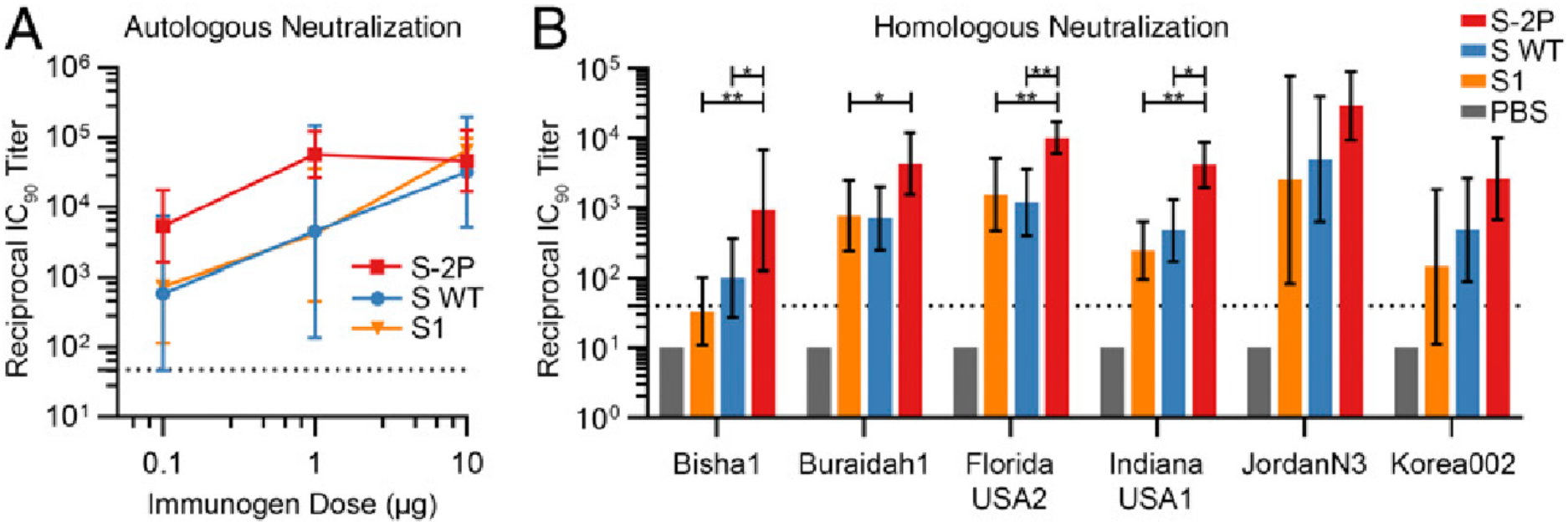
Pseudovirus:

- CMV/R-MERS-CoV
- Packaging plasmid pCMVDR8.2
- Transducing plasmid pHR’ CMV-Luc

Conclusion → 2P substitutes prevent fusion of the virus with the cell membrane

MERS-CoV-2P elict more robust neutralizing activity

- Mice vaccinated with trimers of MERS S-2P (compared to monomeric S1 protein and wt S protein) at 0 and 3 wk
- Two weeks after the final immunization, sera were collected for measurement of antibody response
- Neutralization assay : serial dilutions of mouse sera, mixed wih various pseudovirus



MERS England 1

Mouse-adapted model of SARS-CoV2

A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures

https://doi.org/10.1038/s41586-020-2708-8

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Kenneth H. Dinnon III[1,8], Sarah R. Leist[2,8], Alexandra Schäfer[2], Caitlin E. Edwards[2], David R. Martinez[2], Stephanie A. Montgomery[3], Ande West[2], Boyd L. Yount Jr[2], Yixuan J. Hou[2], Lily E. Adams[1], Kendra L. Gully[2], Ariane J. Brown[2], Emily Huang[2], Matthew D. Bryant[4], Ingrid C. Choong[4], Jeffrey S. Glenn[5,6], Lisa E. Gralinski[2], Timothy P. Sheahan[2] & Ralph S. Baric[1,2,7]✉

Departments of Microbiology and Immunology and Epidemiology, University of North Carolina

Unique divergence at position 498 of CoV2 S receptor binding domain

- First model: overexpression of human ACE2 under control of the Hfh4 (Foxj1) promoter
- Hfh4-ACE2 mice infected with CoV2 died by viral invasion of the brain, minimal lung involvement

→ Human ACE2 overexpression facilitates CoV2 infection, but the observed pathogenesis does not accurately model disease manifestation in humans

→ Instead, genetically alters the host modification of the CoV2 RBD

Mouse ACE2 as functional receptor

2B S RBD-ACE2 interacting residues

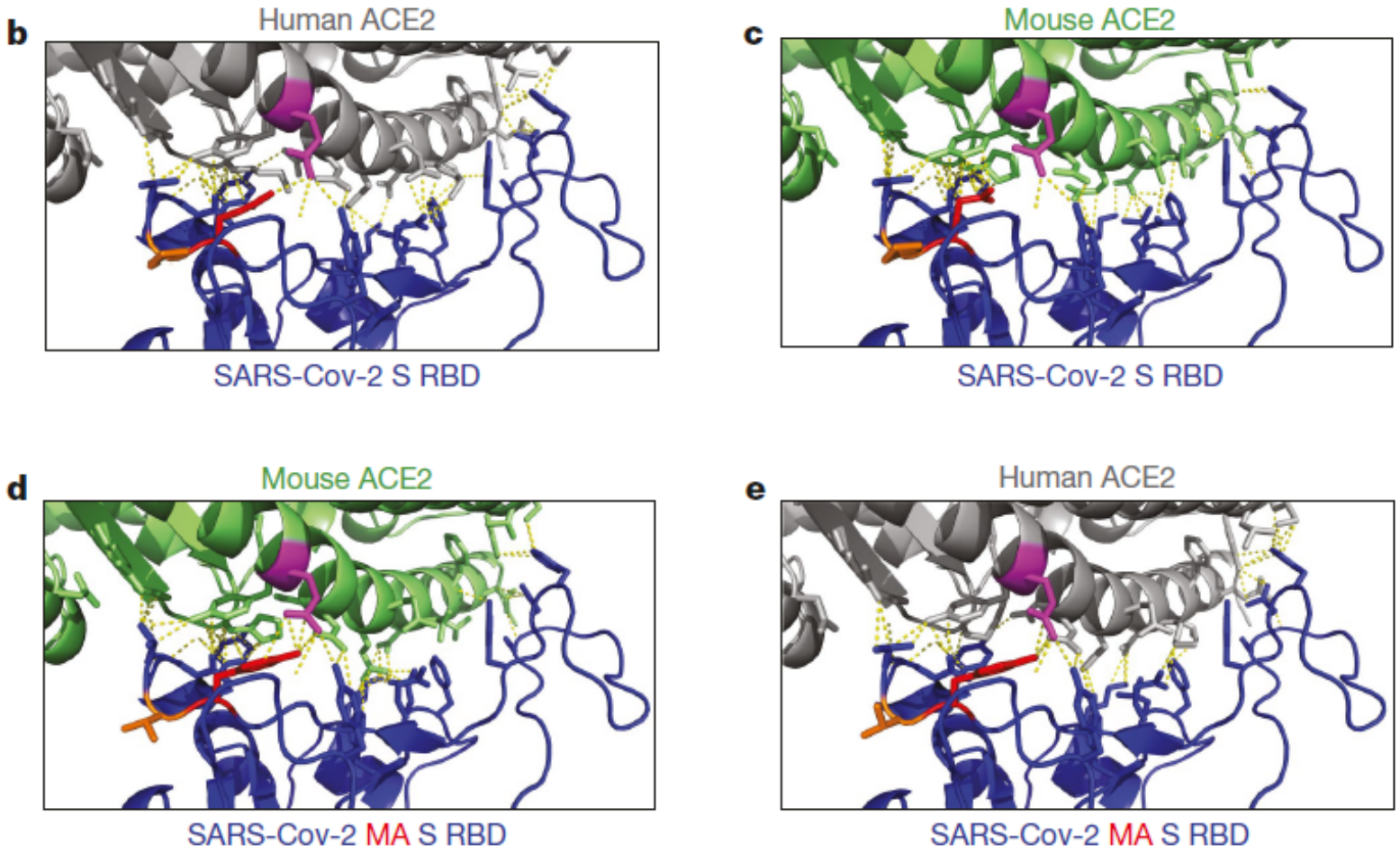
a

	402	426	436	440	442	443	462	463	472	473	475	479	482	484	486	487	488	489	491
SARS-CoV Urbani	T	R	Y	Y	Y	L	S	D	L	N	Y	N	G	Y	T	T	G	I	Y
SARS-CoV MA15	T	R	H	Y	Y	L	S	D	L	N	Y	N	G	Y	T	T	G	I	Y
WIV1	T	R	Y	Y	S	L	S	D	F	N	Y	N	G	Y	T	N	G	I	Y
SHC014	T	N	Y	Y	W	V	S	G	P	N	Y	R	G	F	T	A	G	V	H
SARS-CoV-2	T	N	Y	Y	L	F	A	G	F	N	Y	Q	G	Q	T	N	G	V	Y
	415	439	449	453	455	456	475	476	486	487	489	493	496	498	500	501	502	503	505

Green shading indicates contacts as determined by published crystal structures

Amino acids are colored by BLOSUM62 conservation score relative to S protein from SARS-CoV Urbani (red, least conserved; blue, most conserved)

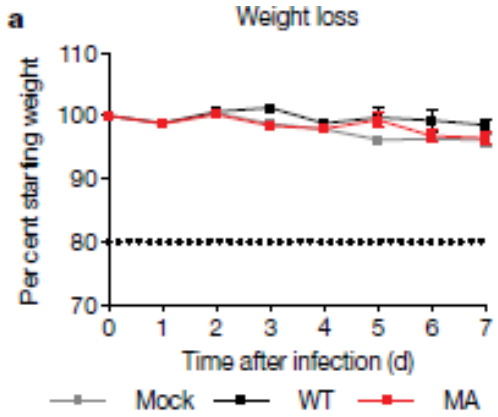
SRAS-CoV2(Q498Y/P499T) restores interaction

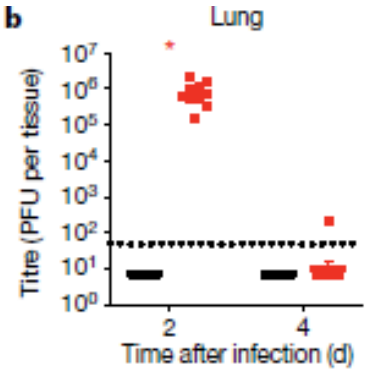


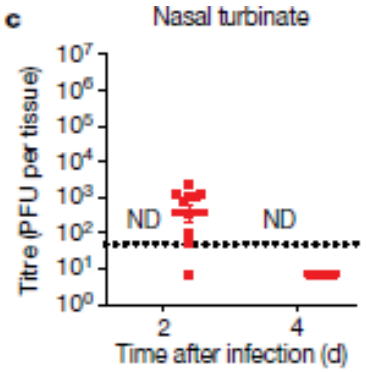
SARS-CoV-2 MA replicates in young BALB/c mice

→ Cell experiment SARS-CoV-2 MA can use mouse ACE2 for entry into cells

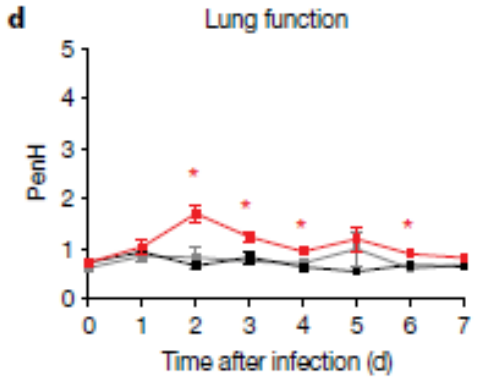
→ Intranasal ifection of mice with 10^5 PFU SARS-CoV-2 MA

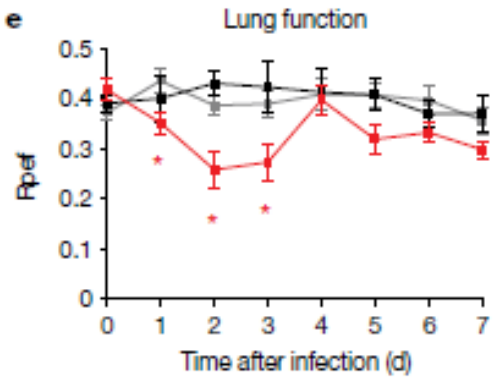


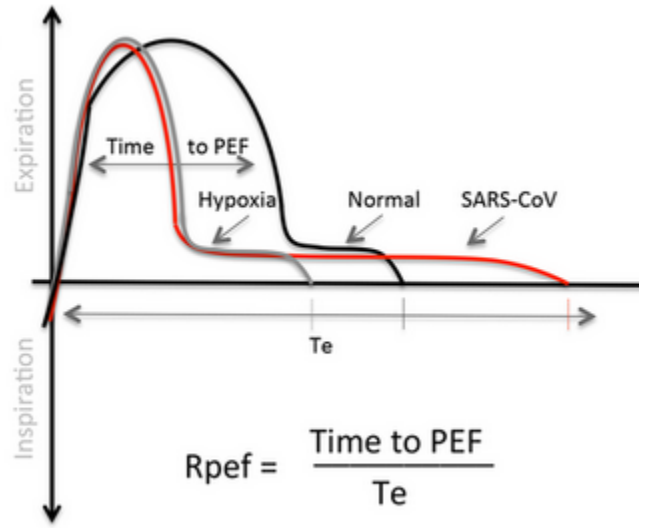




Whole-body plethysmography





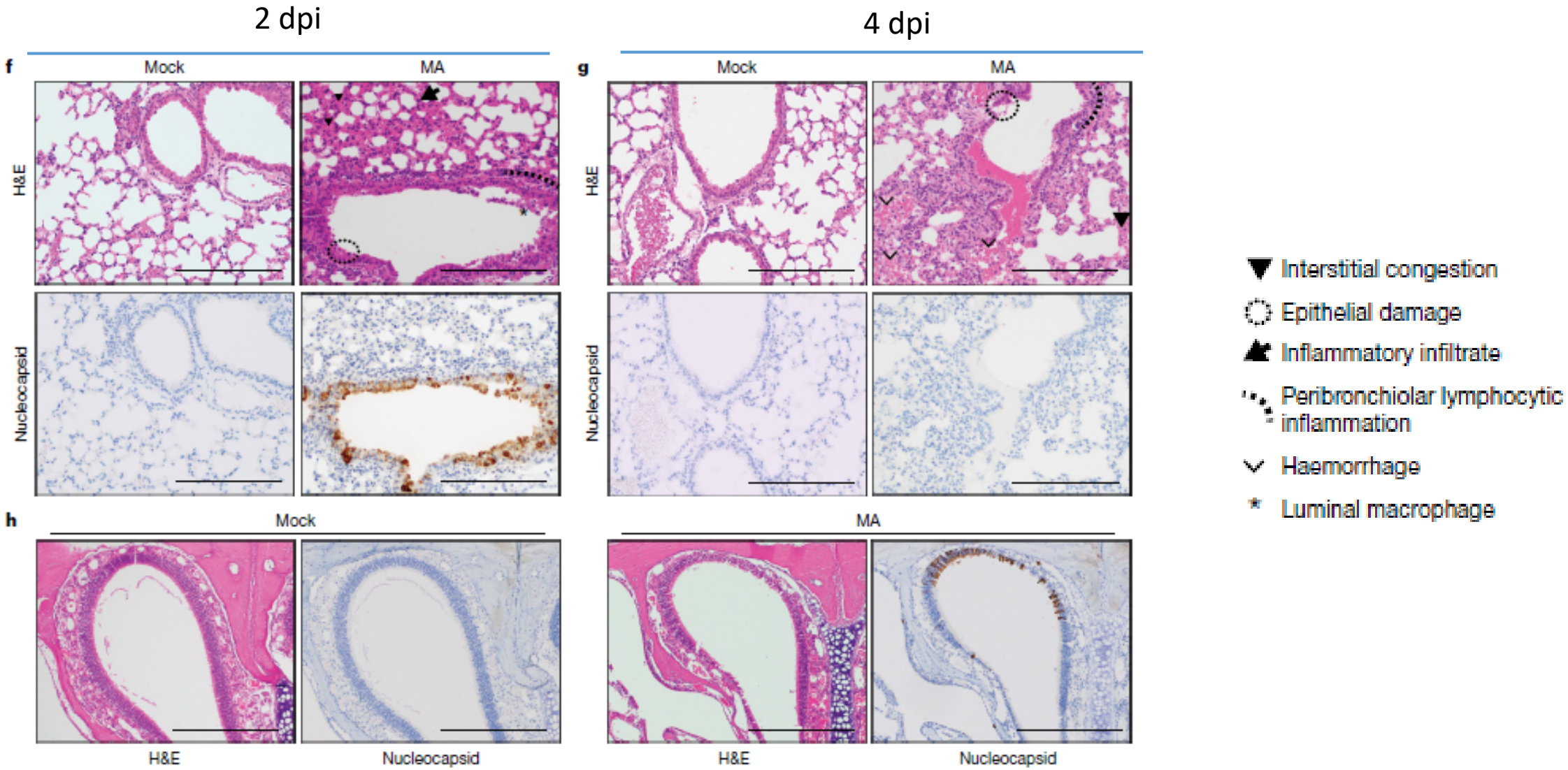


SARS-CoV-2 MA: recombinant virus Q498Y/P499T modified

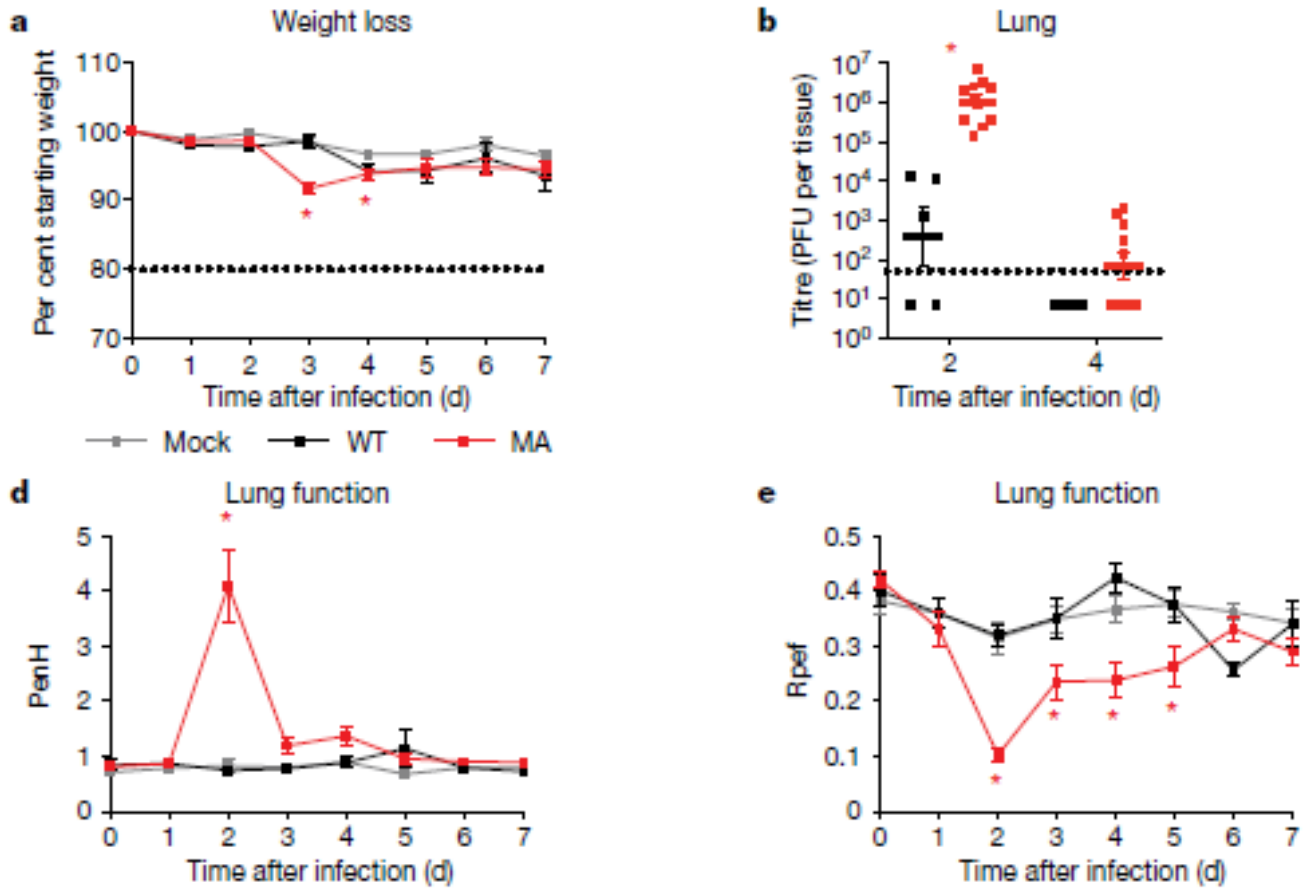
PFU: Plaque foming units

Penh: airway resistance

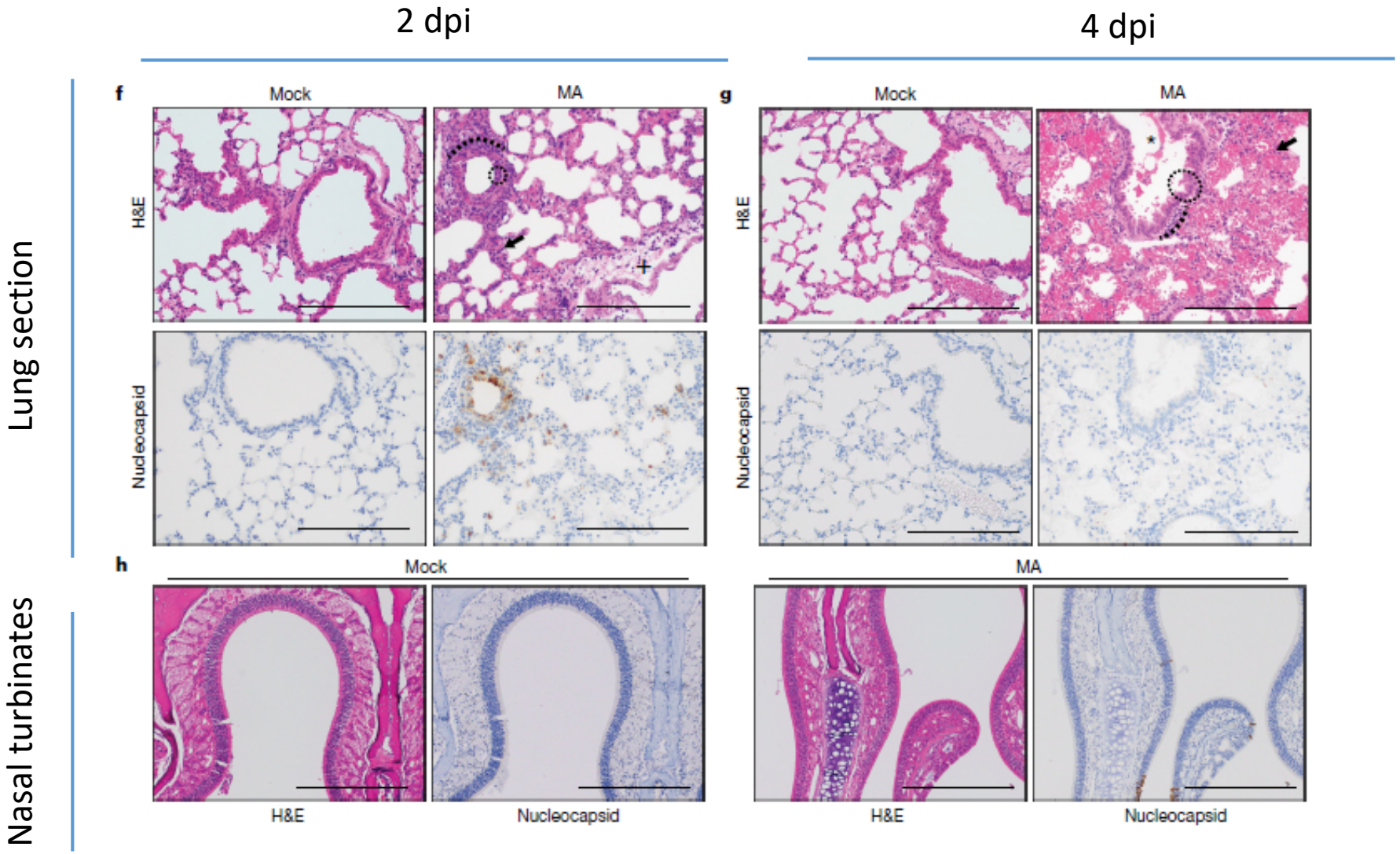
Histological analysis of mice infected with SARS-CoV-2 MA



One-year old mice show mild disease

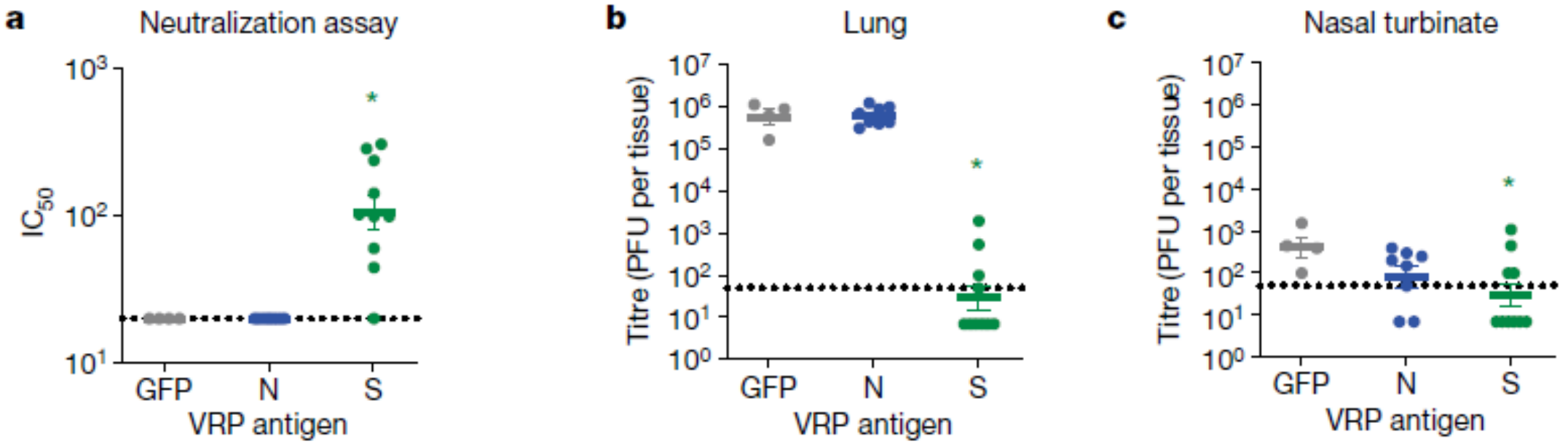


Histological analysis of old mice infected with SARS-CoV-2 MA



Evaluation of prevention against SARS-CoV-2 MA in mice

- Vaccination of 10-week old mice, boost after three weeks (GFP, N, S)
- Neutralization assay 3 with serum three weeks after boost
- Challenge four weeks after boost with SARS-CoV-2 MA



VRP: Virus replicon particle system
N : Nucleocapside

mRNA-1273 vaccine design

SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness

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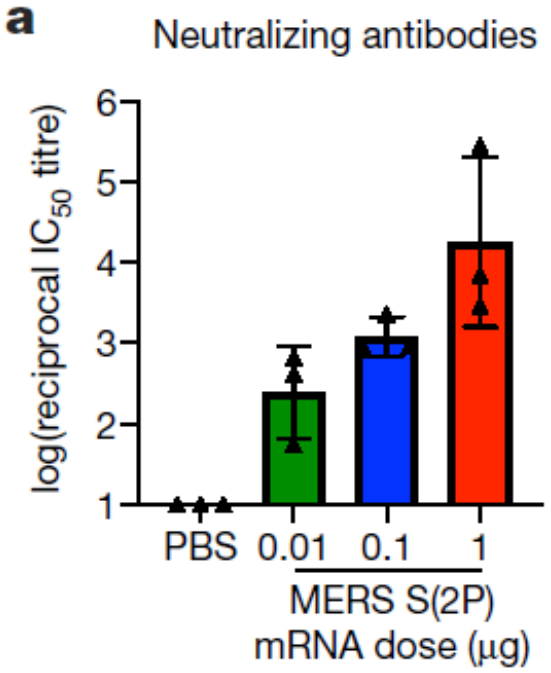
Check for updates

Kizzmekia S. Corbett[1,10], Darin K. Edwards[2,10], Sarah R. Leist[3,10], Olubukola M. Abiona[1], Seyhan Boyoglu-Barnum[1], Rebecca A. Gillespie[1], Sunny Himansu[2], Alexandra Schäfer[3], Cynthia T. Ziwawo[1], Anthony T. DiPiazza[1], Kenneth H. Dinnon[3], Sayda M. Elbashir[2], Christine A. Shaw[2], Angela Woods[2], Ethan J. Fritch[4], David R. Martinez[3], Kevin W. Bock[5], Mahnaz Minai[5], Bianca M. Nagata[5], Geoffrey B. Hutchinson[1], Kai Wu[2], Carole Henry[2], Kapil Bahl[2], Dario Garcia-Dominguez[2], LingZhi Ma[2], Isabella Renzi[2], Wing-Pui Kong[1], Stephen D. Schmidt[1], Lingshu Wang[1], Yi Zhang[1], Emily Phung[1,6], Lauren A. Chang[1], Rebecca J. Loomis[1], Nedim Emil Altaras[2], Elisabeth Narayanan[2], Mihir Metkar[2], Vlad Presnyak[2], Cuiping Liu[1], Mark K. Louder[1], Wei Shi[1], Kwanyee Leung[1], Eun Sung Yang[1], Ande West[3], Kendra L. Gully[3], Laura J. Stevens[7], Nianshuang Wang[8], Daniel Wrapp[8], Nicole A. Doria-Rose[1], Guillaume Stewart-Jones[2], Hamilton Bennett[2], Gabriela S. Alvarado[1], Martha C. Nason[9], Tracy J. Ruckwardt[1], Jason S. McLellan[8], Mark R. Denison[7], James D. Chappell[7], Ian N. Moore[5], Kaitlyn M. Morabito[1], John R. Mascola[1], Ralph S. Baric[3,4], Andrea Carfi[2]✉ & Barney S. Graham[1]✉

Optimization of vaccine design (MERS-CoV)

- 16-20 week mice immunized at week 0 and 3 with 0.01, 0.1 and 1 ug MERS-CoV S(2P)
- mRNA formulated in lipid nanoparticles (mRNA LNP)
- Collection of sera two weeks post-boost

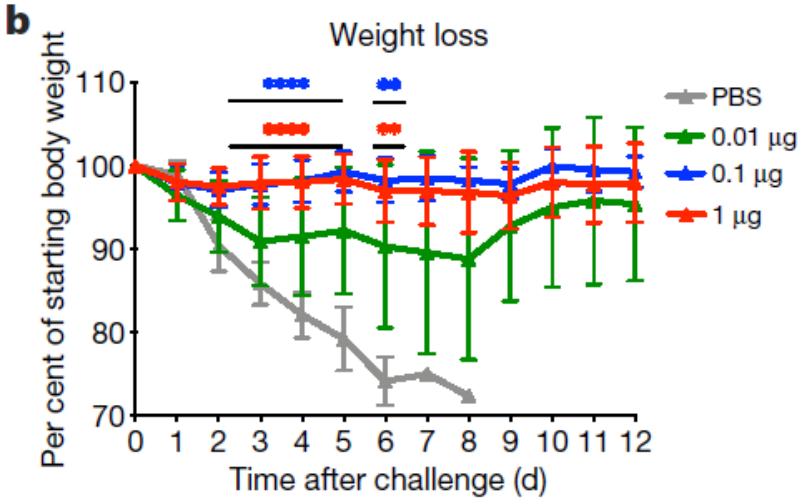


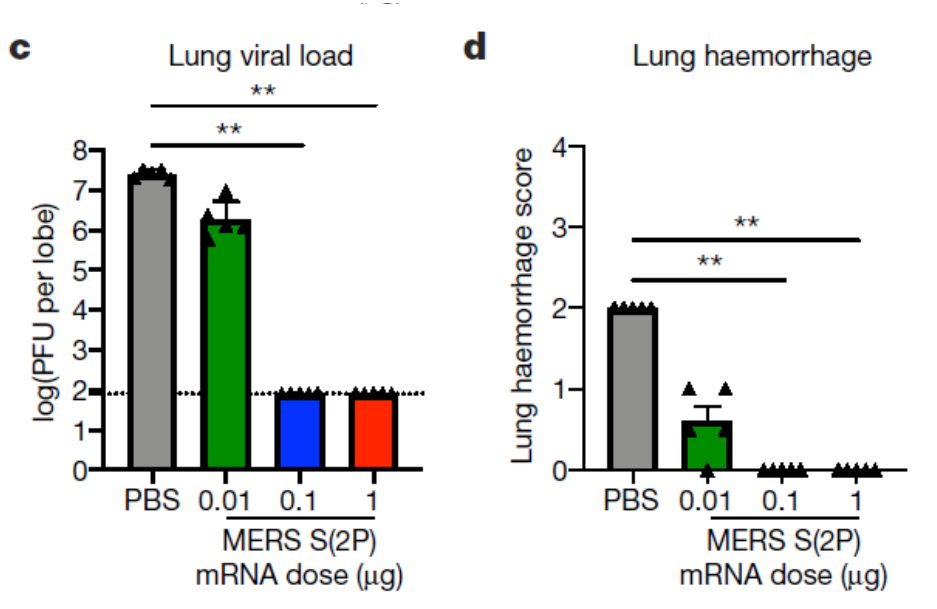
Pseudovirus (no BSL3 needed):
- Luciferase reporter
- Lentivirus backbone
- S gene

Pseudovirus-neutralization assay:
- Serum pre-incubated with pseudovirus
- ACE-2 expressing 293T cells
- 72h lysis of cell and measurement of luciferase activity

MERS-CoV S-2P mRNA protects mice from lethal challenge

- 16-20 week mice immunized at **week 0 and 3** with 0.01, 0.1 and 1 ug MERS-CoV S(2P)
- Challenge with mouse-adapted MERS-CoV **4 weeks post boost**

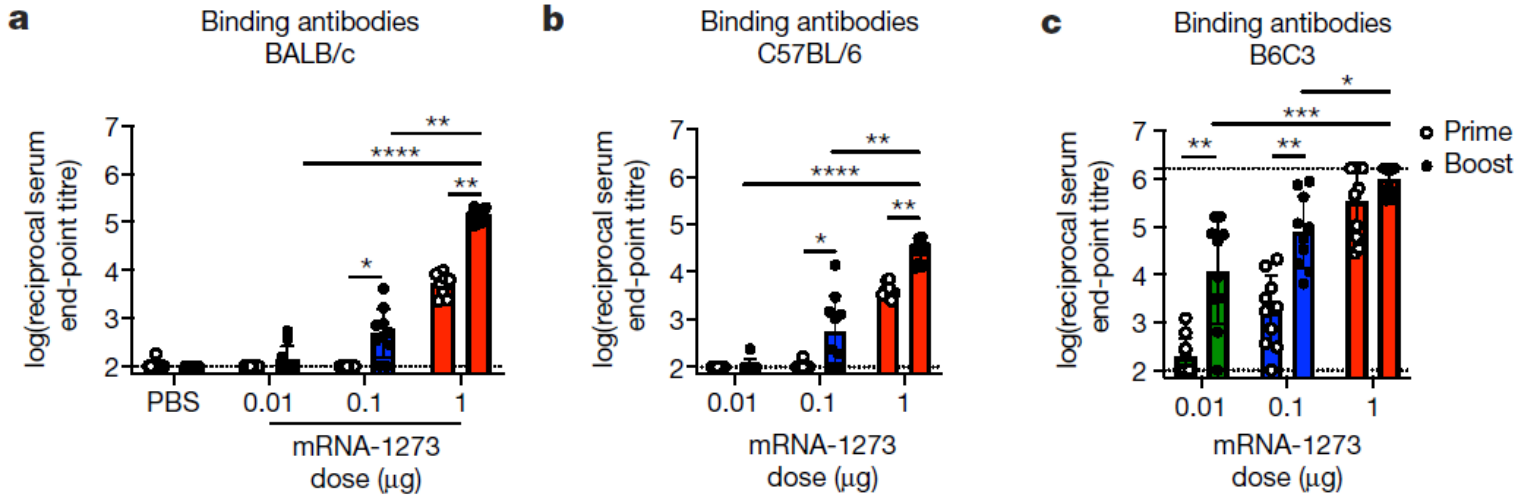




3 days post-challenge

SARS-CoV S-2P (mRNA-1273) mRNA elicts robust binding

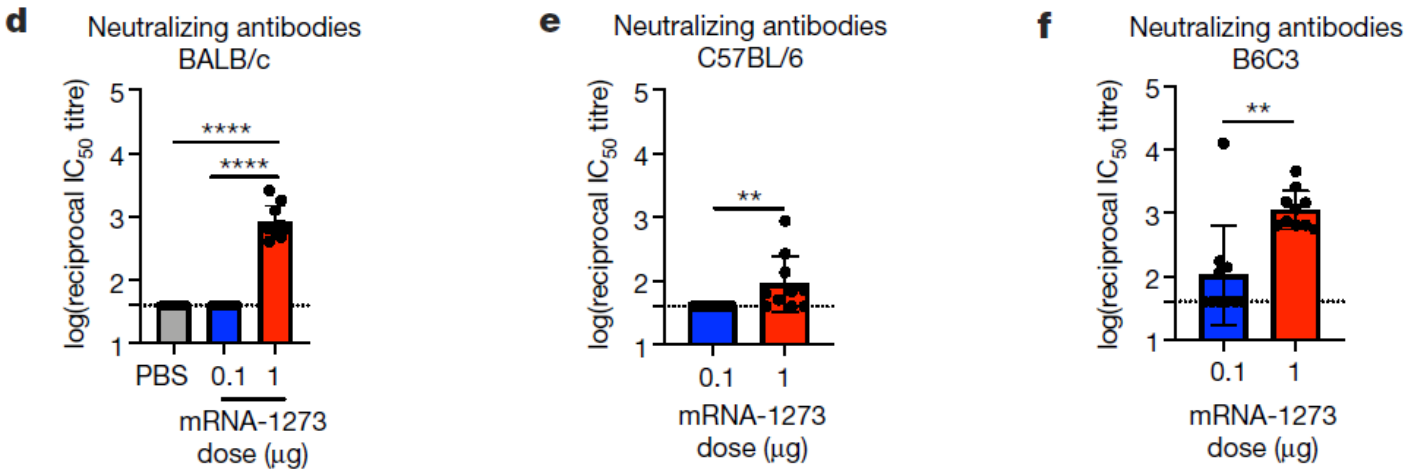
- 16-20 week mice immunized at week 0 and 3 with 0.1, 1 ug SARS-CoV S(2P)
- mRNA formulated in lipid nanoparticles (mRNA LNP)
- Sera collection : 2 weeks post-prime, 2 weeks post-boost



Sandwich ELISA end-point format

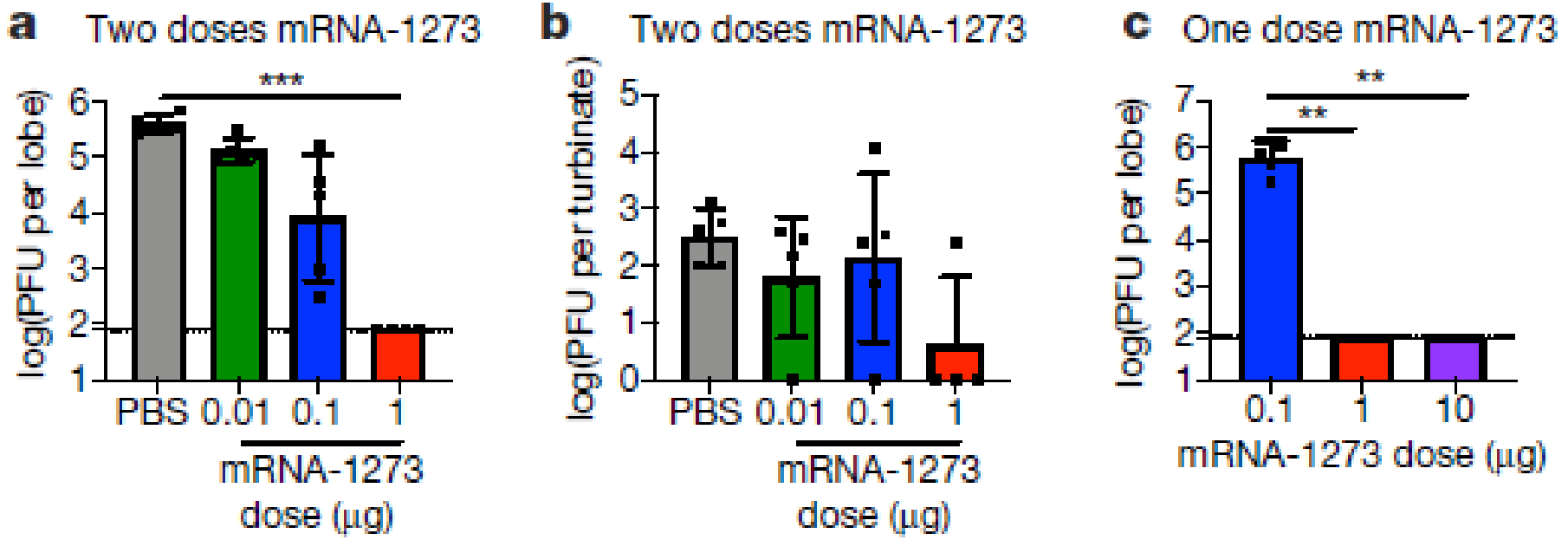
- SARS-CoV-2S(2P) coating
- End-point titres : dilution that emitted optical density exceeding 4 x background

SARS-CoV S-2P mRNA elicts robust pseudovirus neutralizing antibody response



Efficacy of mRNA-1273 from upper- and lower airway SARS-CoV2 MA infection

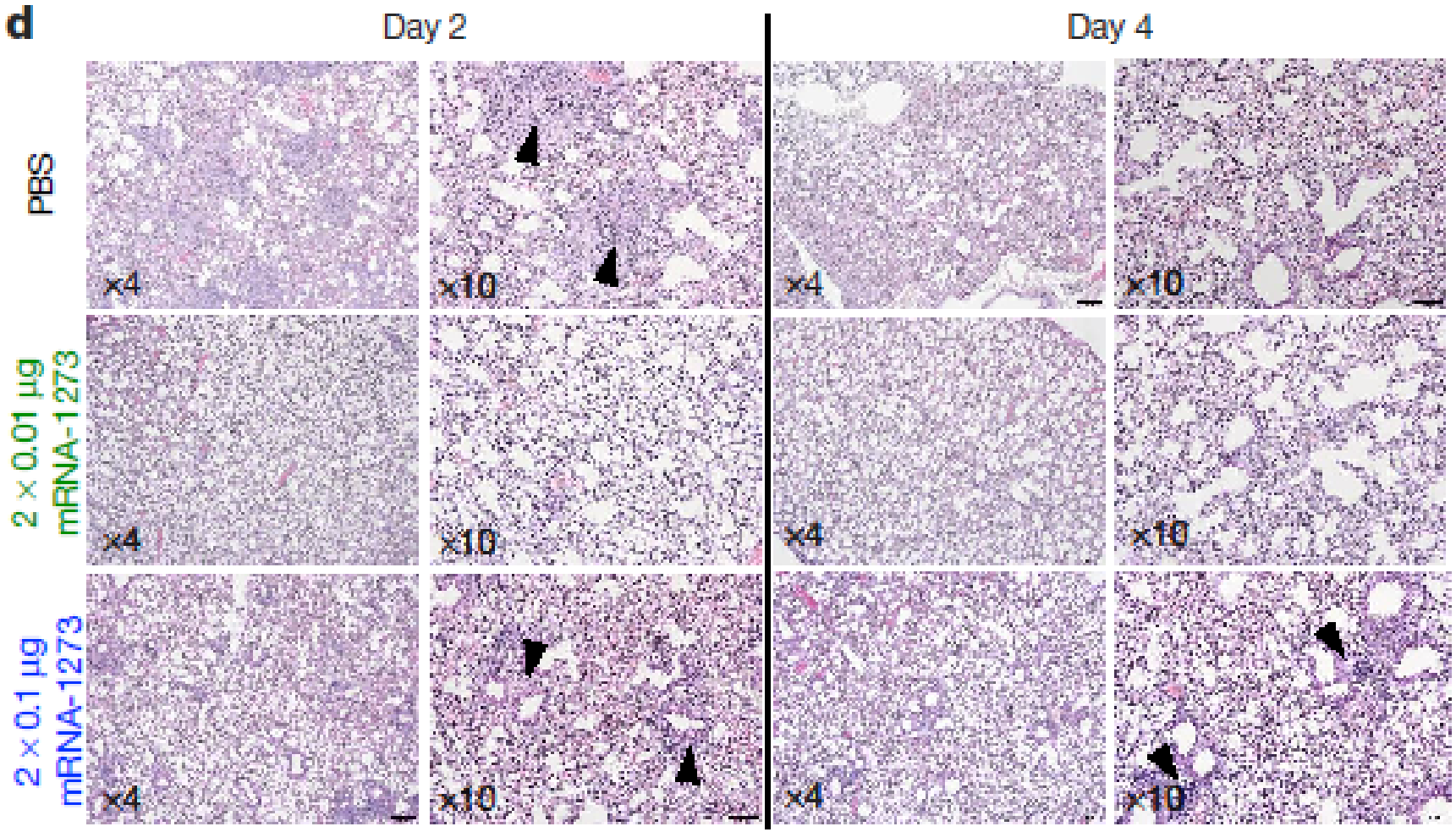
- 16-20 week mice immunized at **week 0 and 3** with 0.01, 0.1 or 1 ug mRNA-1273 or PBS
- Challenge wich mouse-adapted SARS-CoV-2 MA **5 weeks post boost**
- **Single dose** of 0.1, 1 or 10 ug mRNA-1273, challenging 7 weeks after immunization



Lung

Nasal turbinates

Histological analysis



Overview of NHP results

URT protection : important for sterilizing immunity

Company (ref.)	Vaccine candidate (type)	Dose range (route)	Neut. titre after prime	Neut. titre after boost	T cell response	Challenge dose (route)	URT protection	LRT protection	Species
Sinovac[34]	PiCoVacc (inactivated virion + aluminium hydroxide)	3–6 μg (i.m.)	None[a]	1:10 range[a] after first boost; 1:50 range[a] after second boost	ND	10^6 $TCID_{50}$ (i.t.)	Partial[b]	Partial (low dose)[b] Complete (high dose)	Rhesus macaques
Beijing Institute of Biological Products[33]	BBIBP-CorV (inactivated virion + aluminium hydroxide)	4–8 μg (i.m.)	1:100 range[a]	1:200 range[a]	ND	10^6 $TCID_{50}$ (i.t.)	Partial[b]	Complete[b]	Cynomolgus macaques
AstraZeneca[49]	ChAdOxnCoV-19 (non-replicating AdV)	2.4×10^{10} VP; 1× or 2× (i.m.)	1:5–1:40 range[a]	1:10–1:160 range[a]	Yes	2.6×10^6 $TCID_{50}$ (i.t., oral, i.n., ocular)	None (1×)[c] None (2×)[c]	Partial (1×)[c] Complete (2×)[c]	Rhesus macaques
Janssen[41]	Ad26COVS1 (non-replicating AdV)	1×10^{11} VP (i.m.)	1:100 range[d]	NA	Low	10^5 $TCID_{50}$ (i.n, i.t.)	Complete in S.PP group[c]	Complete in S.PP group[c]	Rhesus macaques
Moderna[57]	mRNA-1273 (mRNA via LNPs)	2× 10–100 μg (i.m.)	ND[e]	1:501–1:3,481 range[d]	Yes, CD4, T_{FH}	7.6×10^5 $TCID_{50}$ (i.n., i.t.)	None (10 μg)[c] Partial (100 μg)[c]	Partial (10 μg)[c] Complete (100 μg)[c]	Rhesus macaques
Novavax[79]	NVX CoV2373 (spike protein + Matrix-M)	2× 2.5–25 μg	Not reported	17,920–23,040 range[a]	ND	10^4 plaque-forming units (i.n., i.t.)	Partial (low dose)[c] Complete (higher doses)[c]	Complete[c]	Cynomolgus macaques

[a]Based on microneutralization assay with CPE as readout.
[b]Based on viral genome RNA copy number.
[c]Based on subgenomic RNA copy number.
[d]Based on microneutralization assay with a SARS-CoV-2 reporter virus; 50% reduction in relative light units as readout.
[e]Not assessed using authentic SARS-CoV-2.
Neut., neutralizing antibody; NA, not applicable; ND, not determined; i.m., intramuscular; i.n., intranasal; i.t., intratracheal; T_{FH}, T follicular helper cells.

Krammer, Nature 2020

Summary of acclerated vaccine development

- Higher neutralizing antibody titers based on prefusion spike conformation (work on MERS-CoV)
- Pandemic prepardness effort : Optimization of vaccine design using MERS-CoV as a prototypical Betacoronavirus pathogen
- Parallel studies in animal overlap with clinical trials
- Overlapping clinical trial phases

Thank you for your attention!