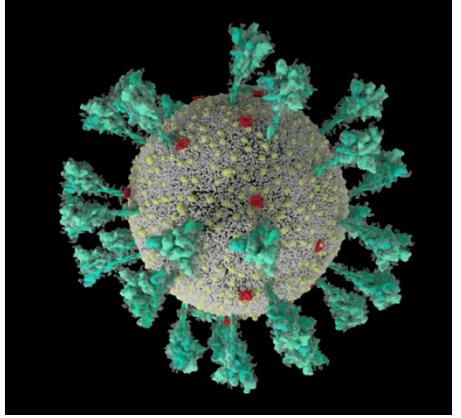
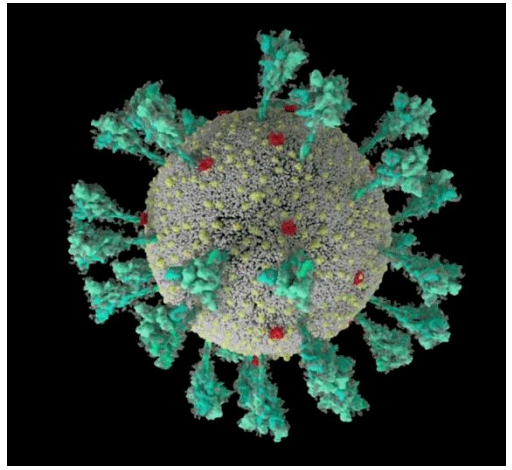
Εθνικόν και Καποδιστριακόν Πανεπιστήμιον Αθηνών
ΙΔΡΥΘΕΝ ΤΟ 1837



COVID-19 immunobiology and autoantibodies

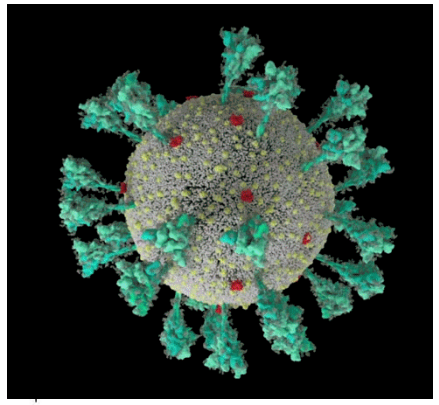
Panayiotis G. Vlachoyiannopoulos MD
National and Kapodistrian University of Athens
Athens, Greece
Chalkidiki 2021

Immune responses to SARS-CoV-2



- ✓ Life cycle of SARS-CoV-2: From Inhalation to COVID-19
- ✓ Innate Immune response to SARS-CoV-2
- ✓ Adaptive immune response against SARS-CoV-2
- ✓ COVID-19 Immunobiology
    - From Normal to Aberrant Host Immune Response
    - Deregulated Interferon responses
    - Cytokine deregulation
- ✓ Drivers of Cytokine deregulation
    - Immune mediators
    - Cellular stress-Response mediators
    - Uncontrolled inflammatory Cell Death-PANoptosis
- ✓ Consequences of Cytokine Deregulation
    - Immune sequelae
        - Deregulated Innate immunity
        - Deregulated adaptive immunity
    - Disease sequelae
        - Endothelial activation and endotheliitis
        - Immunothrombosis
        - Autoantibodies and autoimmunity

The receptor of SARS-CoV-2 on human cells is the enzyme ACE-2



ACE2 exists in:

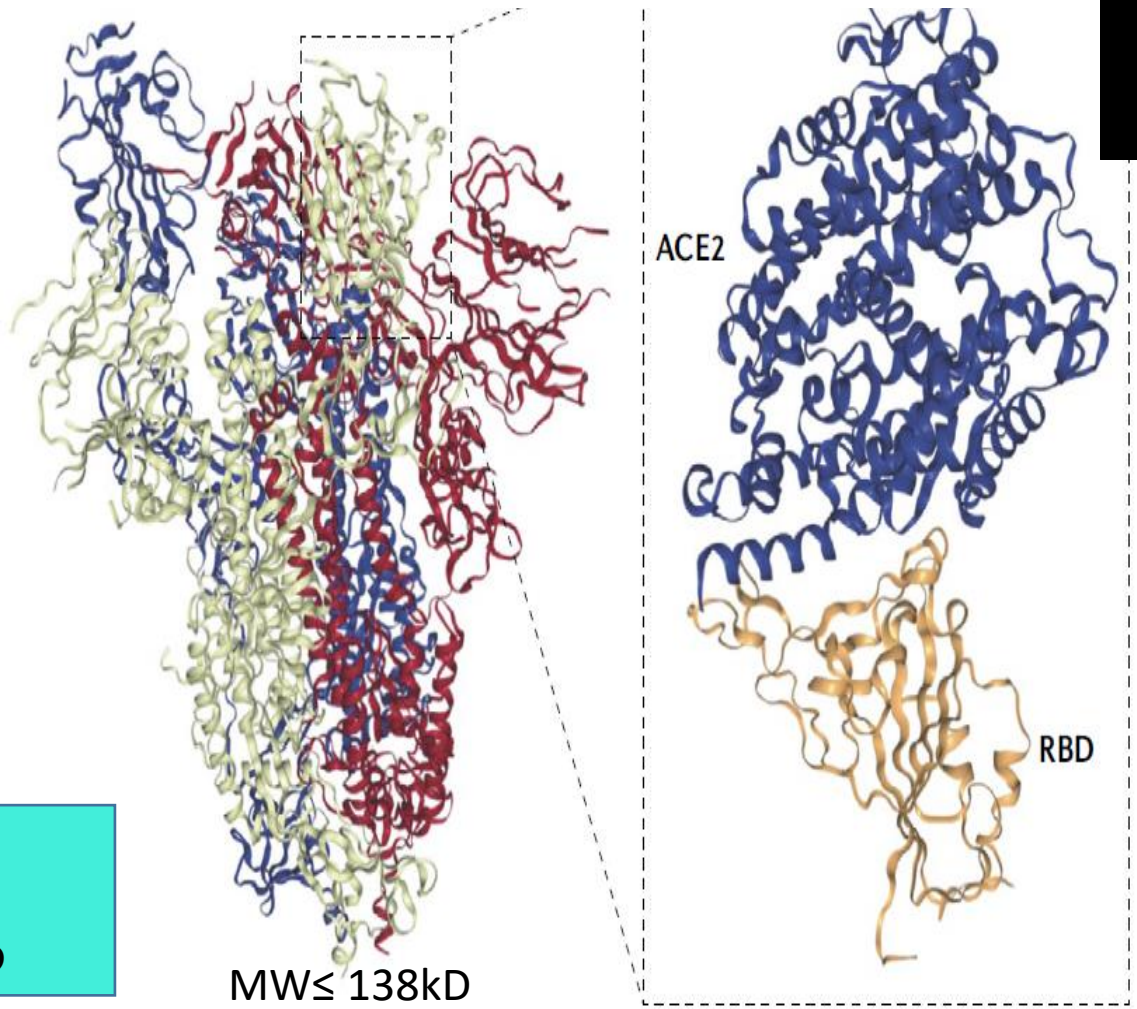
- ✓Bronchial epithelial cells
- ✓Alveolar epithelial cells
- ✓Endothelial cells
- ✓Lung macrophages

The structure of Spike protein

S = S1+S2 $\xrightarrow{S2}$ FP + HR1 + HR2

↓

S1= Aminoterminal domain + RBD



FP: Fusion peptide

HR: Heptad repeat

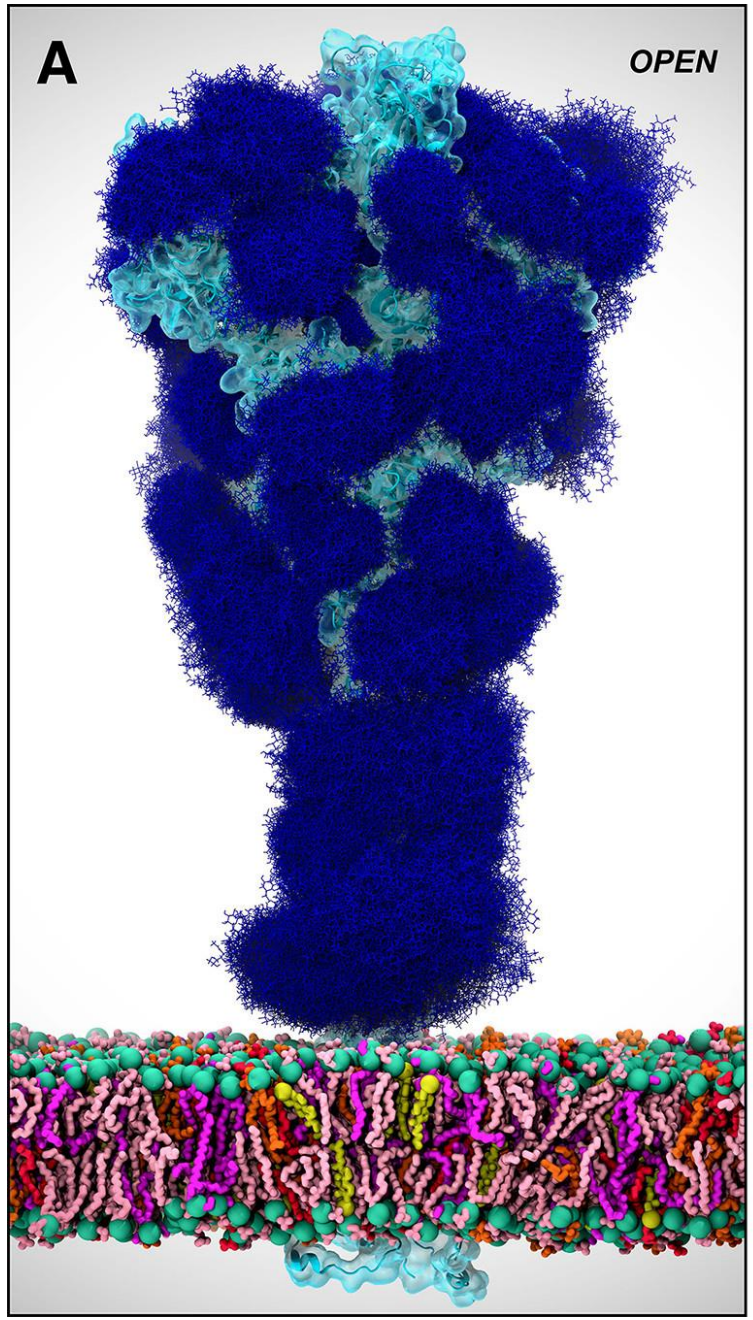
The heptad repeat is an example of a structural motif that consists of a repeating pattern of seven amino acids

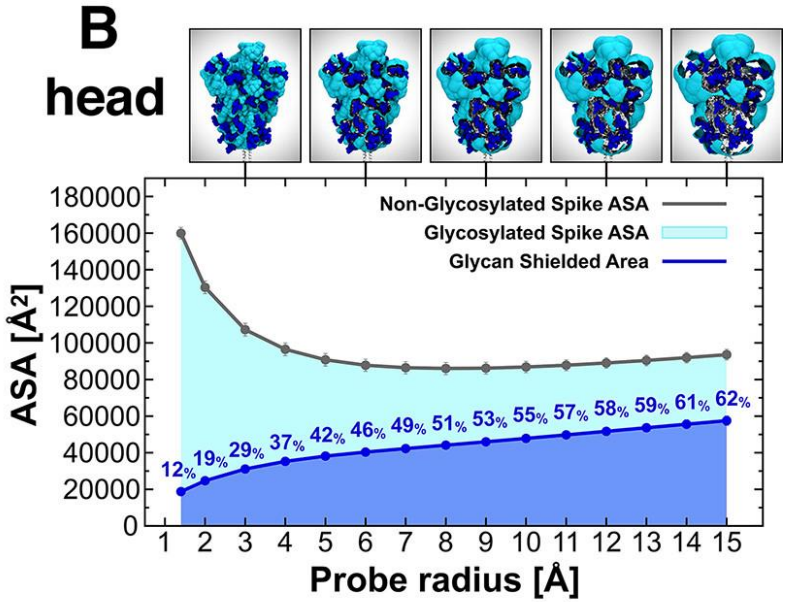
RBD: Receptor binding domain
ACE: Angiotensin converting enzyme

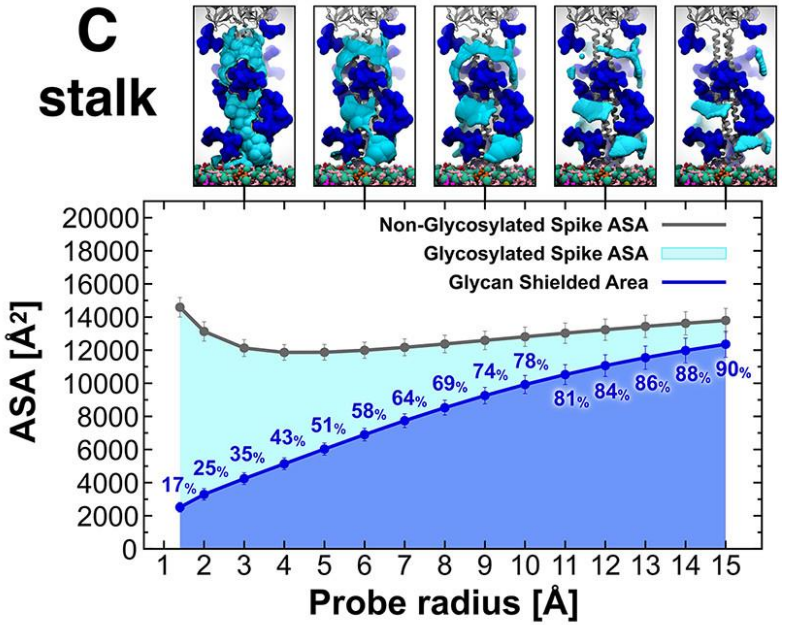
Matthew Zirui Tay et al, Nature Reviews Immunology https://doi.org/10.1038/s41577-020-0311-8

Sugar-coated shield of spike protein of SARS-CoV-2

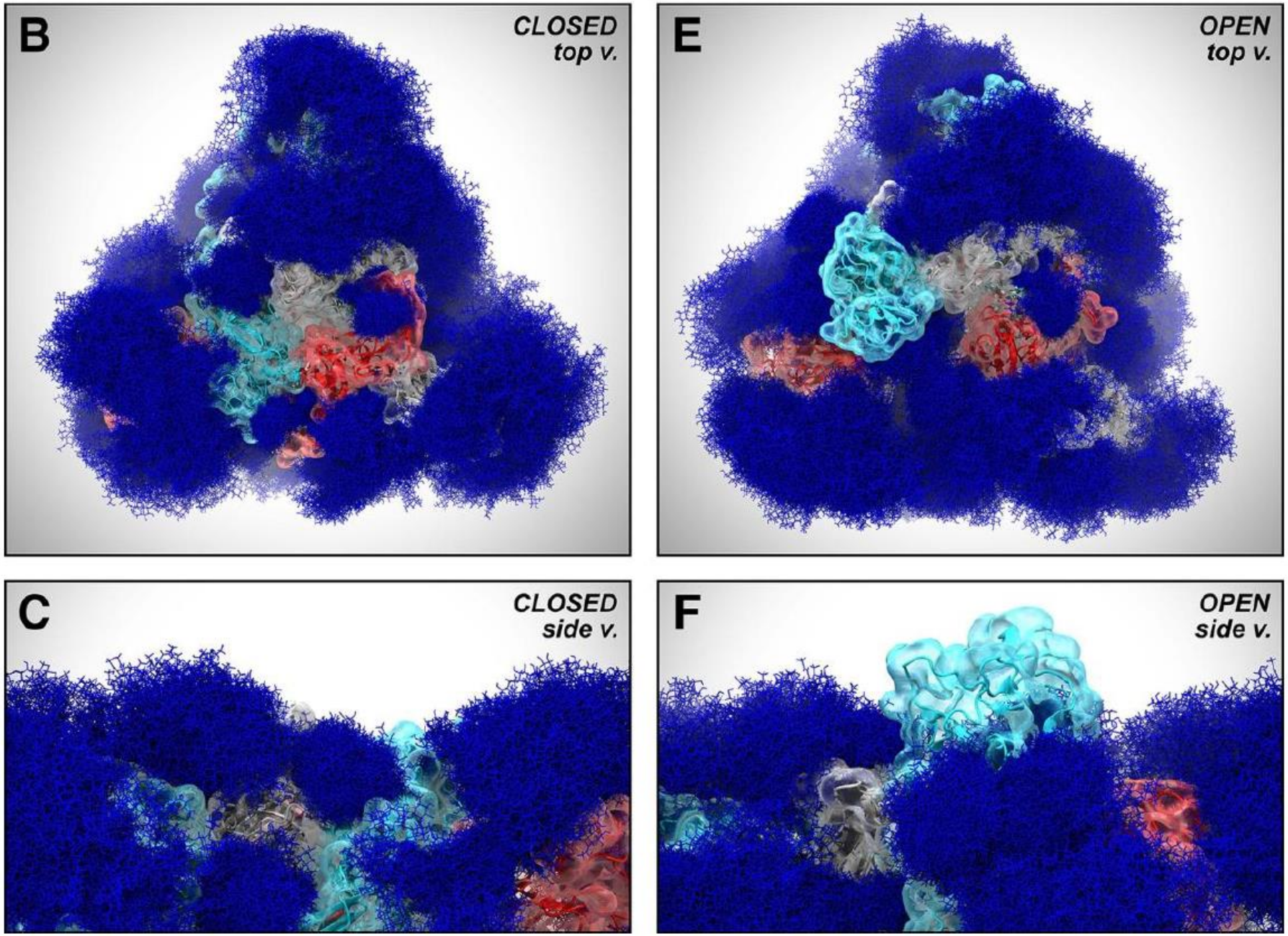
Once SARS-CoV-2 infects someone's body, it becomes covered in that person's unique glycans, making it difficult for the immune system to recognize the virus as something it needs to fight. Those glycans also play an important role in activating the virus

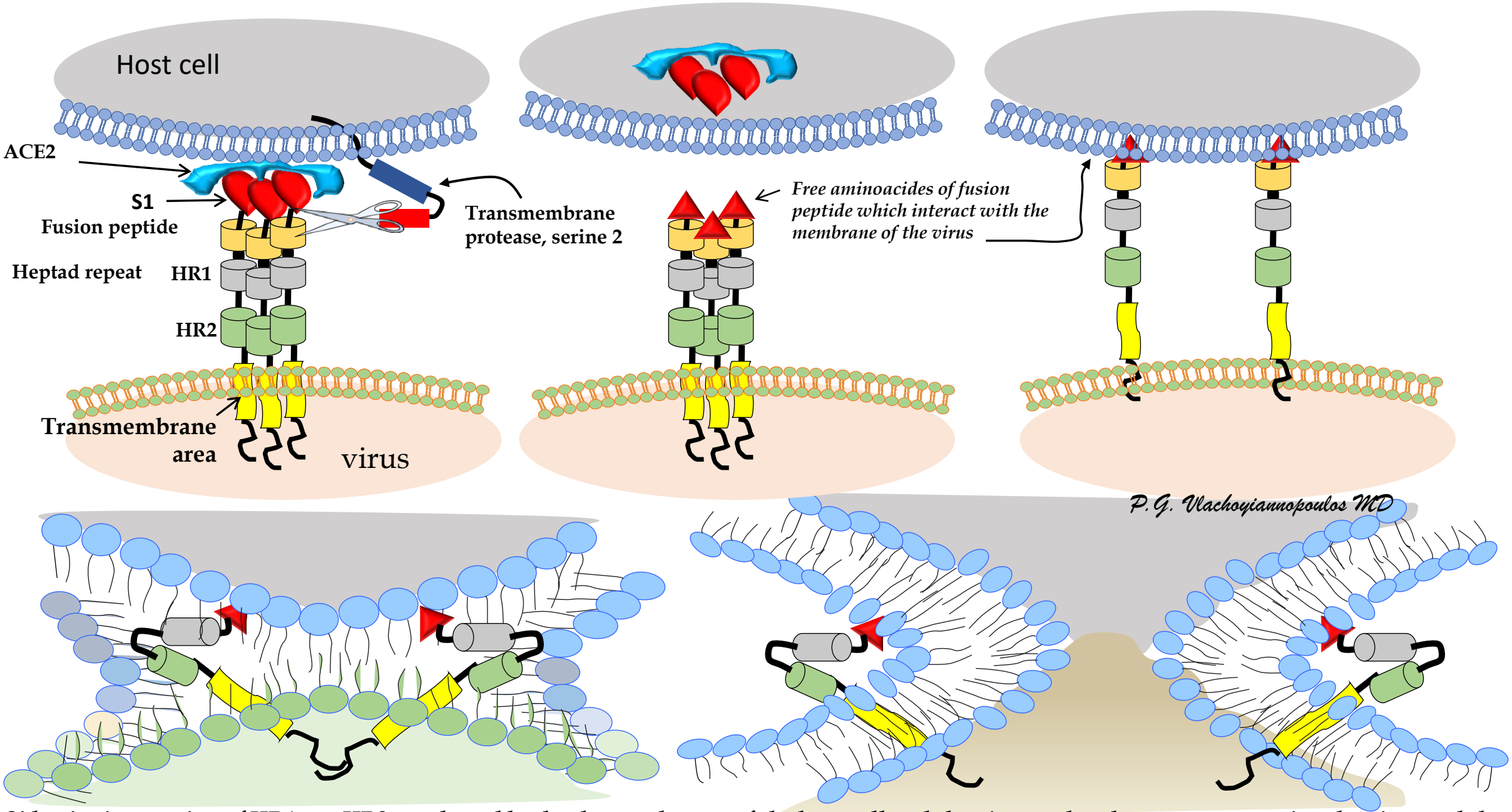






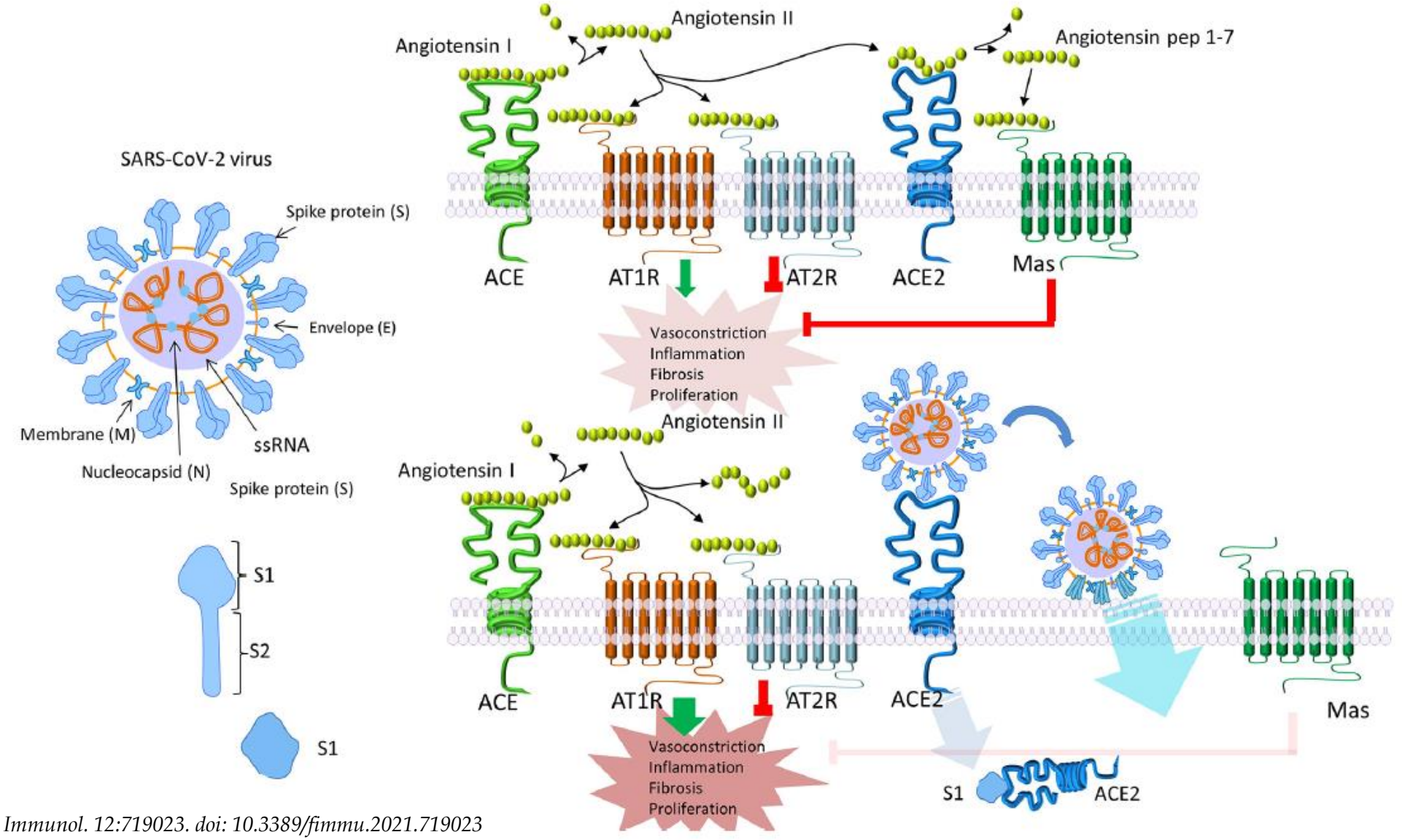
Glycan shield of SARS-CoV-2's spike protein protects virus from the host's immune system





Sidewise interaction of HR1 και HR2 angle and brake the membranes of the host cell and the virus and make a pore connecting the virus and the host cell

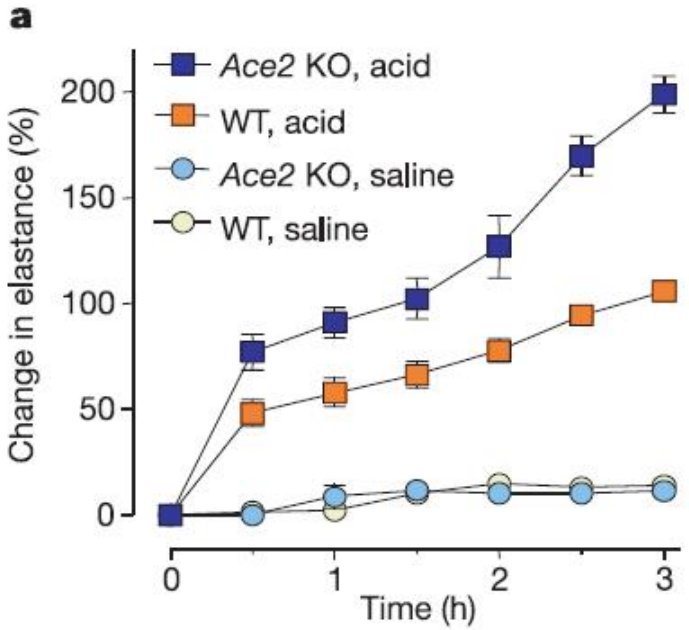
SARS-CoV-2 endocytosis alters angiotensin II signaling, leading to vasoconstriction, inflammation, fibrosis and proliferation

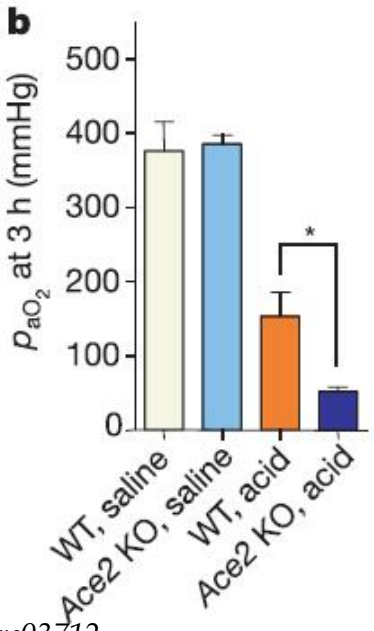


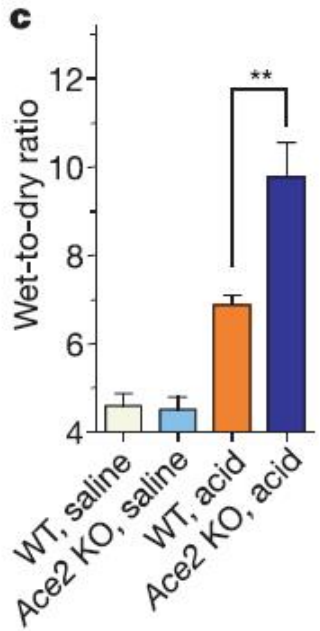
Kaklamanos A, et al, (2021)Front. Immunol. 12:719023. doi: 10.3389/fimmu.2021.719023

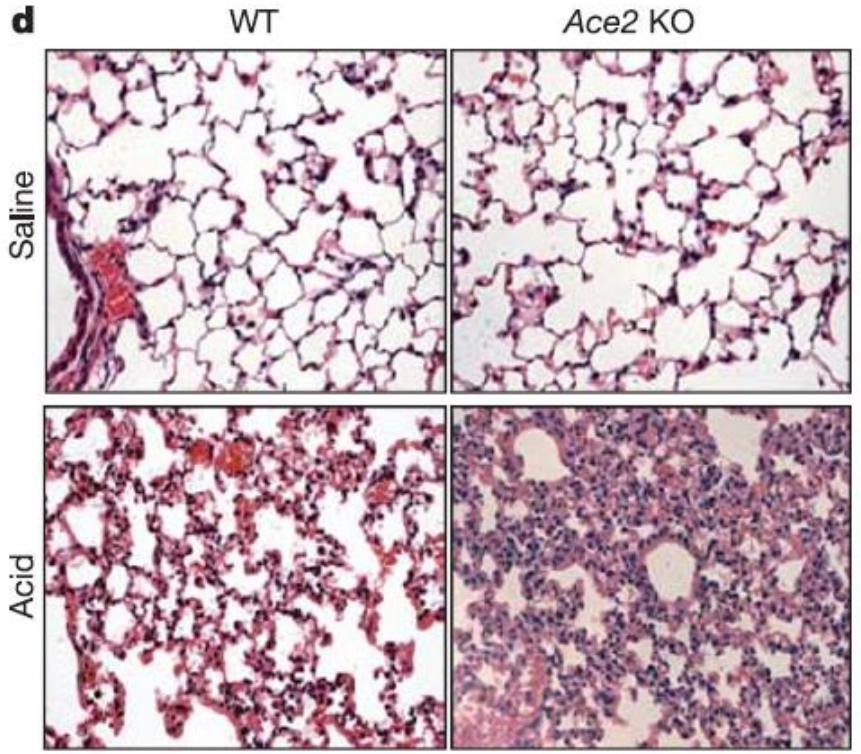
Absence of ACE2 decreases the elastance of lung after acid inhalation

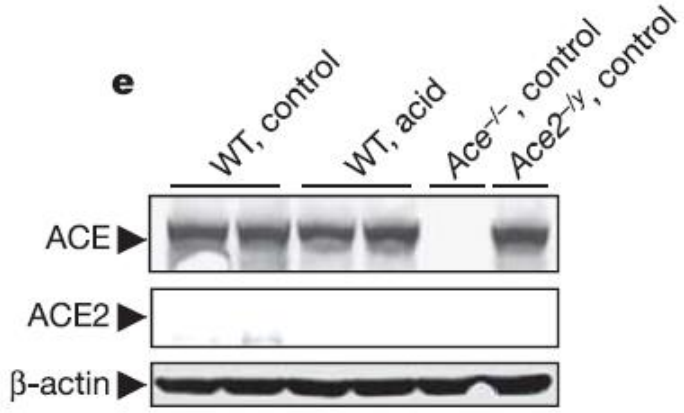
Ace2 KO = mice knockuot for ACE2
WT = wild type mice





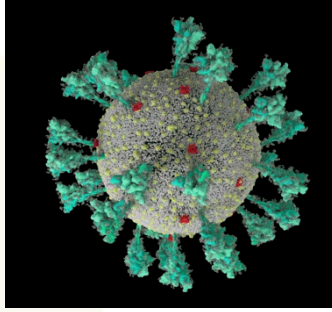


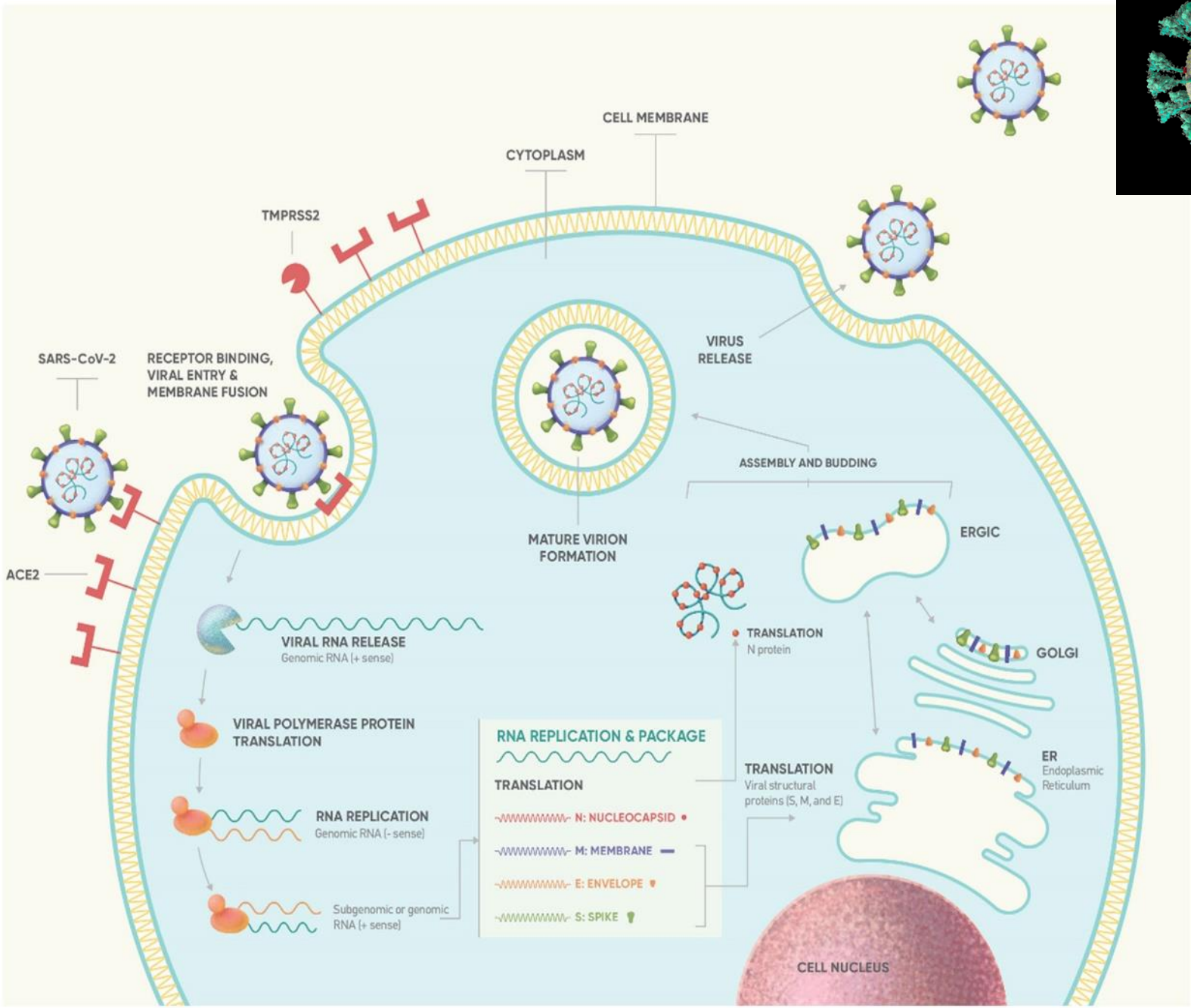




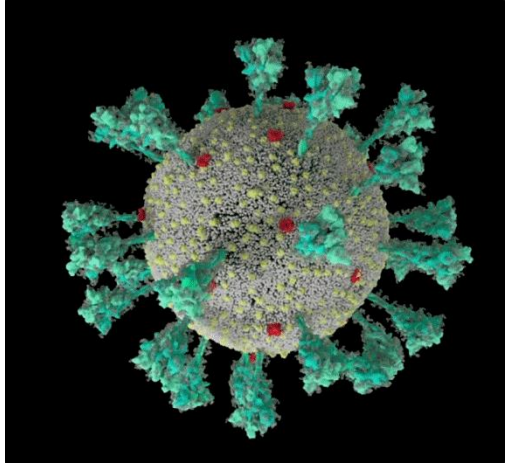
Imai Y, et al, Nature, Vol 436 | 7 July 2005 | doi:10.1038/nature03712

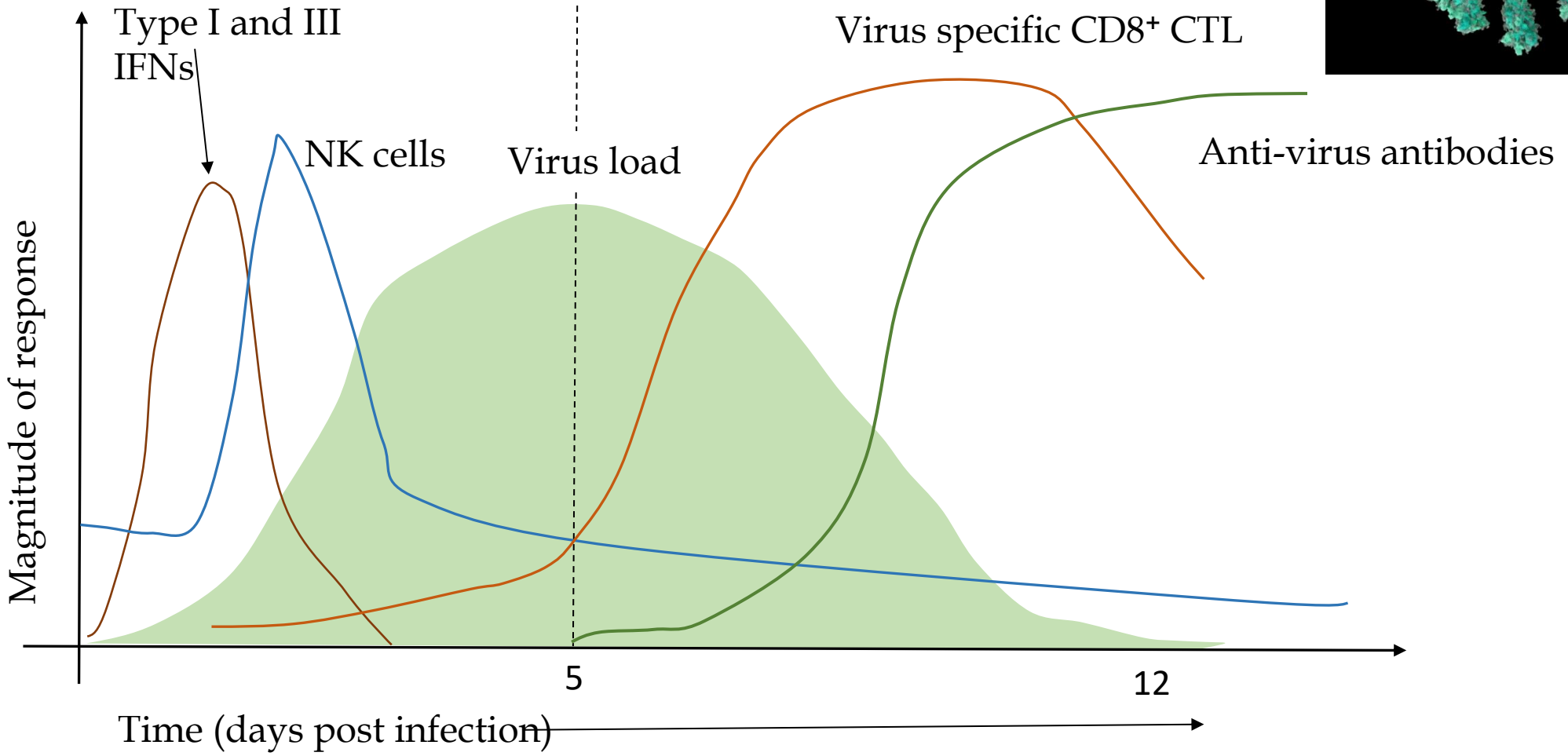
Life cycle of SARS-CoV-2



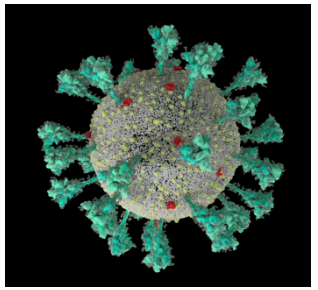


Kinetics of natural and adaptive immunity to viruses





Type I and III interferons (IFN) and NK cells are the first lines of defense against viruses



virus

virus RNA

Plasmacytoid dendritic cell

Type I IFN

MHC class I

Virus

Epithelial cell

NK cell

Activating receptor

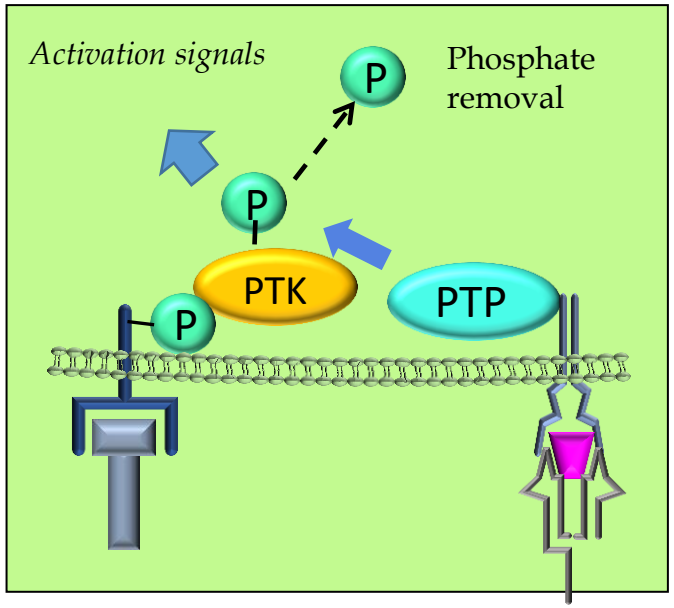
Suppressing receptor

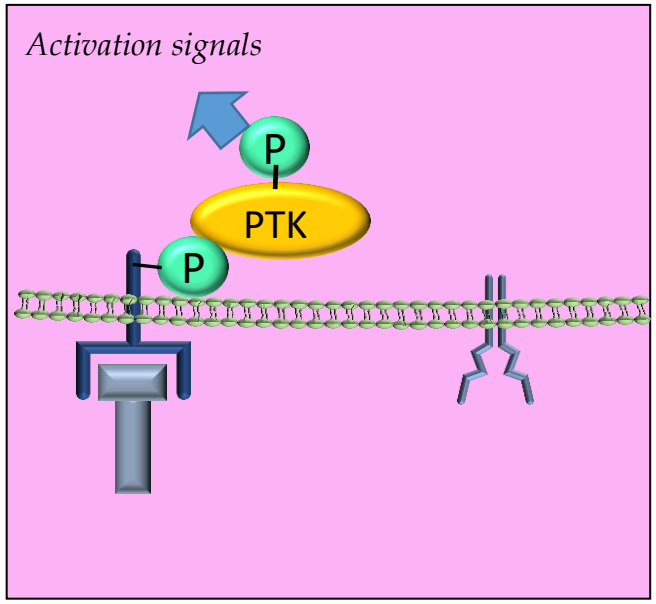
Ligand for activating NK receptor

Virus

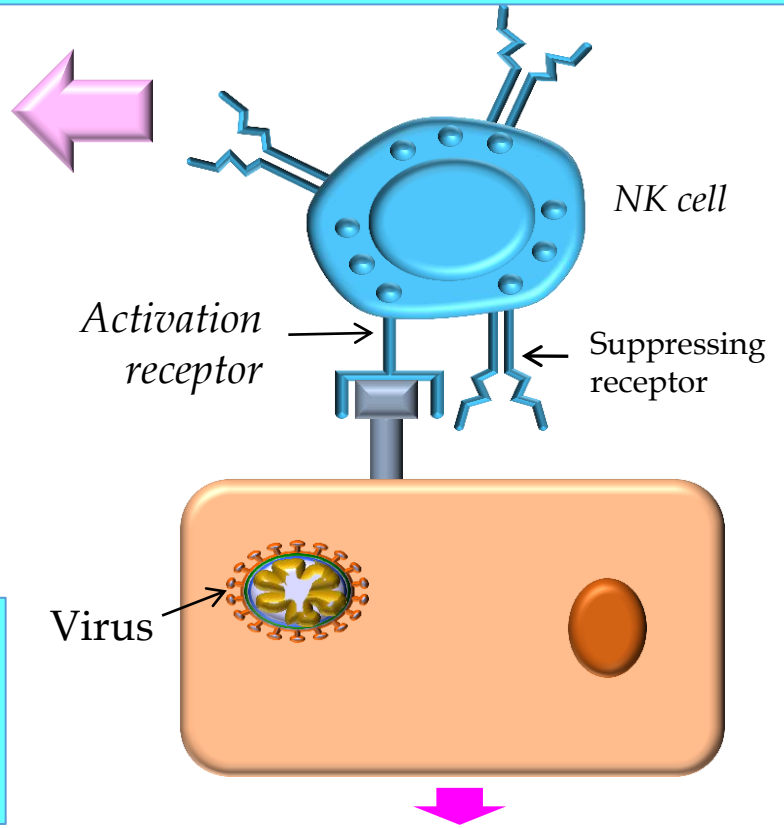
Apoptosis

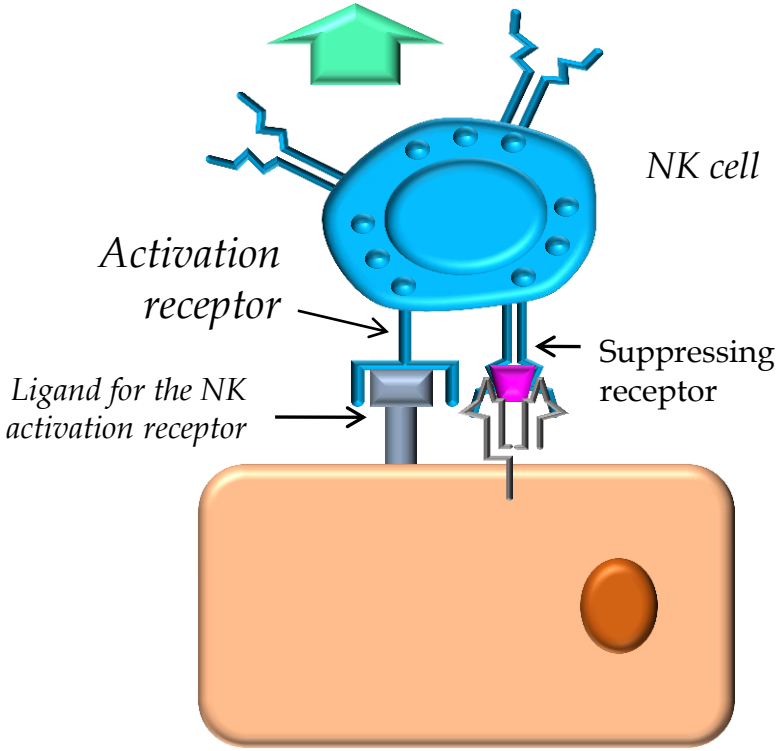
P.Vlachoyiannopoulos





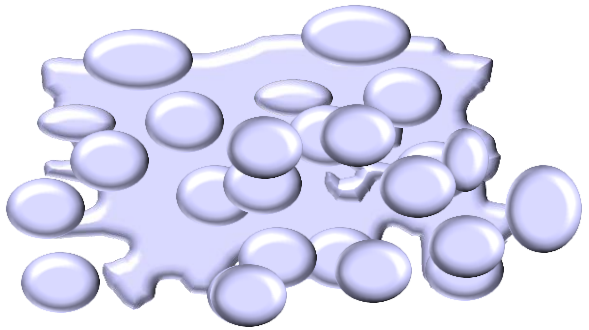
The absence of Ligand for the suppressing receptor of NK cell (self MHC-I with autologus peptides) contributes to the activation of the NK cell and apoptotic death of the infected cell



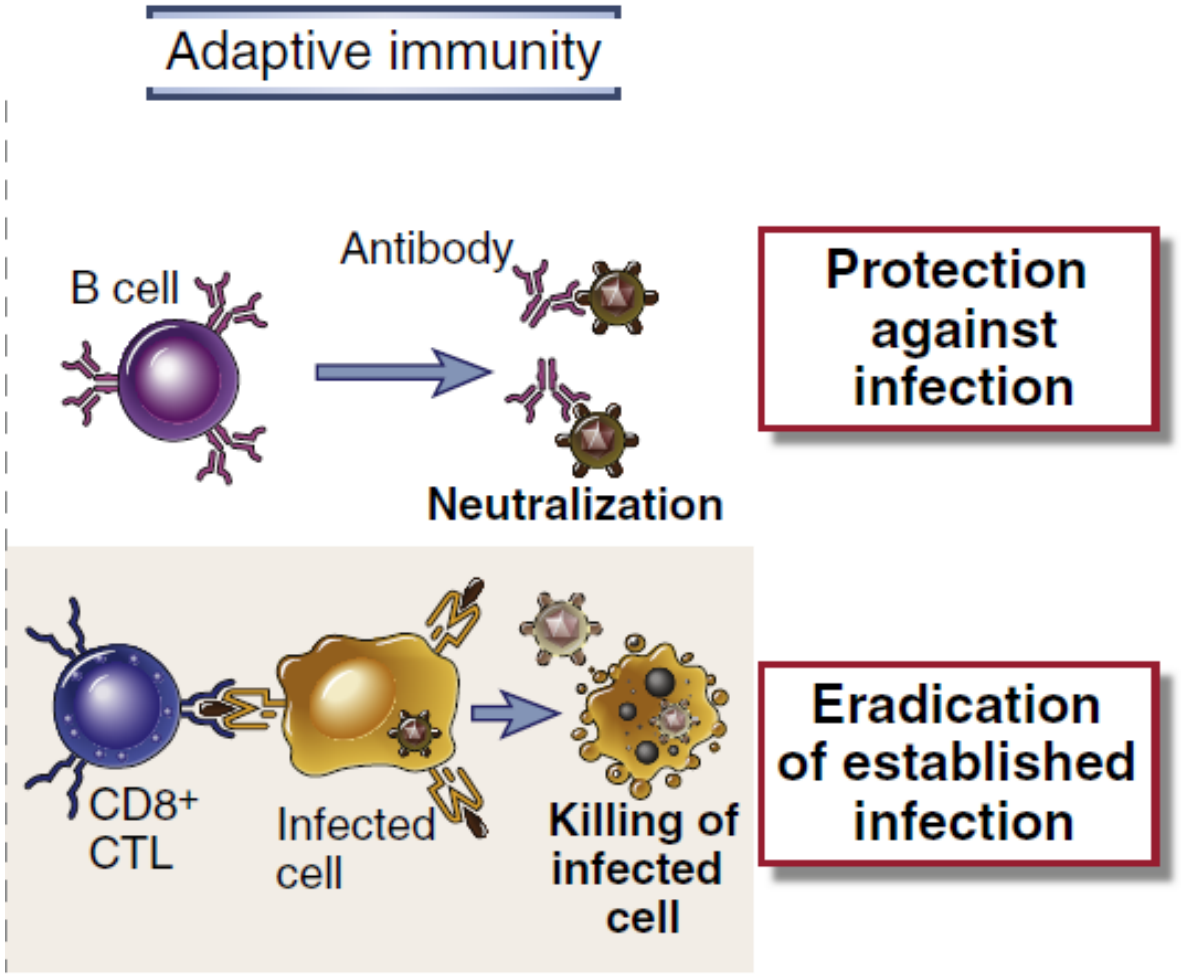


- PTK: tyrosine protein kinases
- PTP:tyrosin protein phosphatases

Apoptotic death of the infected cell



CD8+ Cytotoxic T lymphocytes and antibodies are the components of adaptive immunity to viruses



Abbas, Abul K., Lichtman, Andrew H., Pillai, Shiv.,

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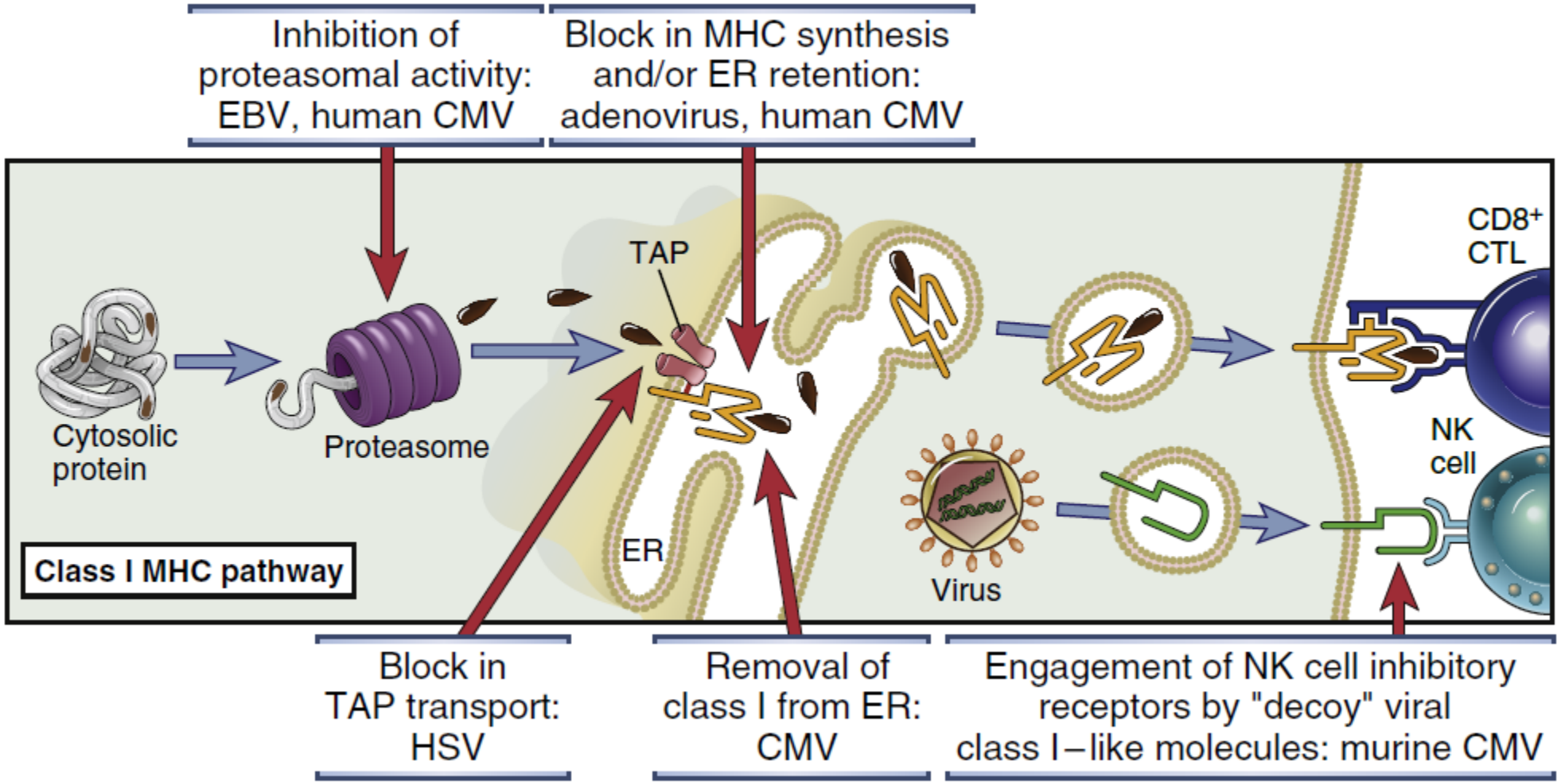
Mechanisms by which viruses inhibit antigen processing and presentation.



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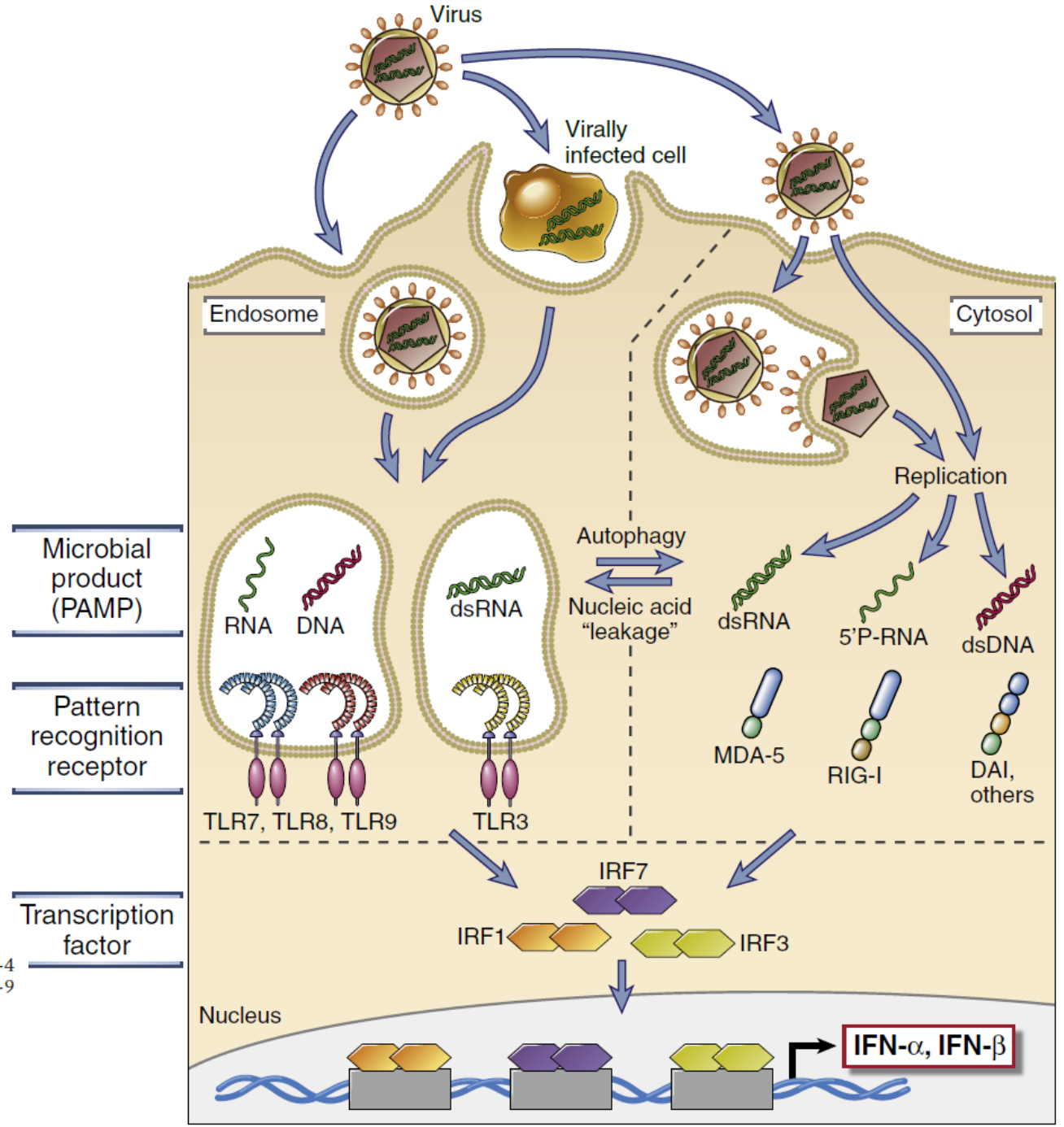
Mechanisms of induction of type I interferons by viruses.



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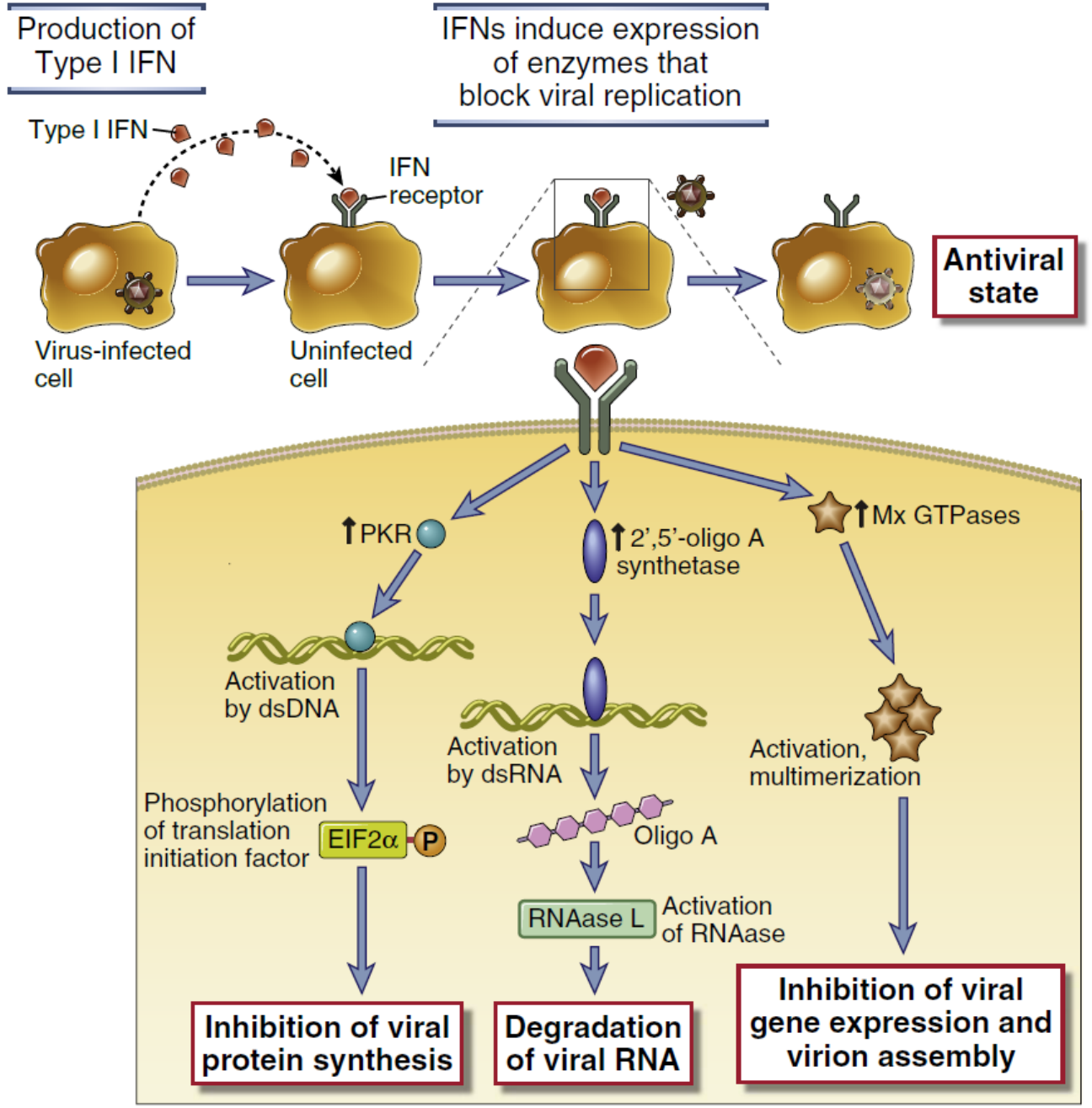
Biologic actions of type I interferons

PKR: protein kinase R
(interferon-induced, double-stranded RNA-activated protein kinase)

EIF-2alfa: Eukaryotic Initiation Factor 2a

Mx: Interferon-induced GTP-binding protein Mx1



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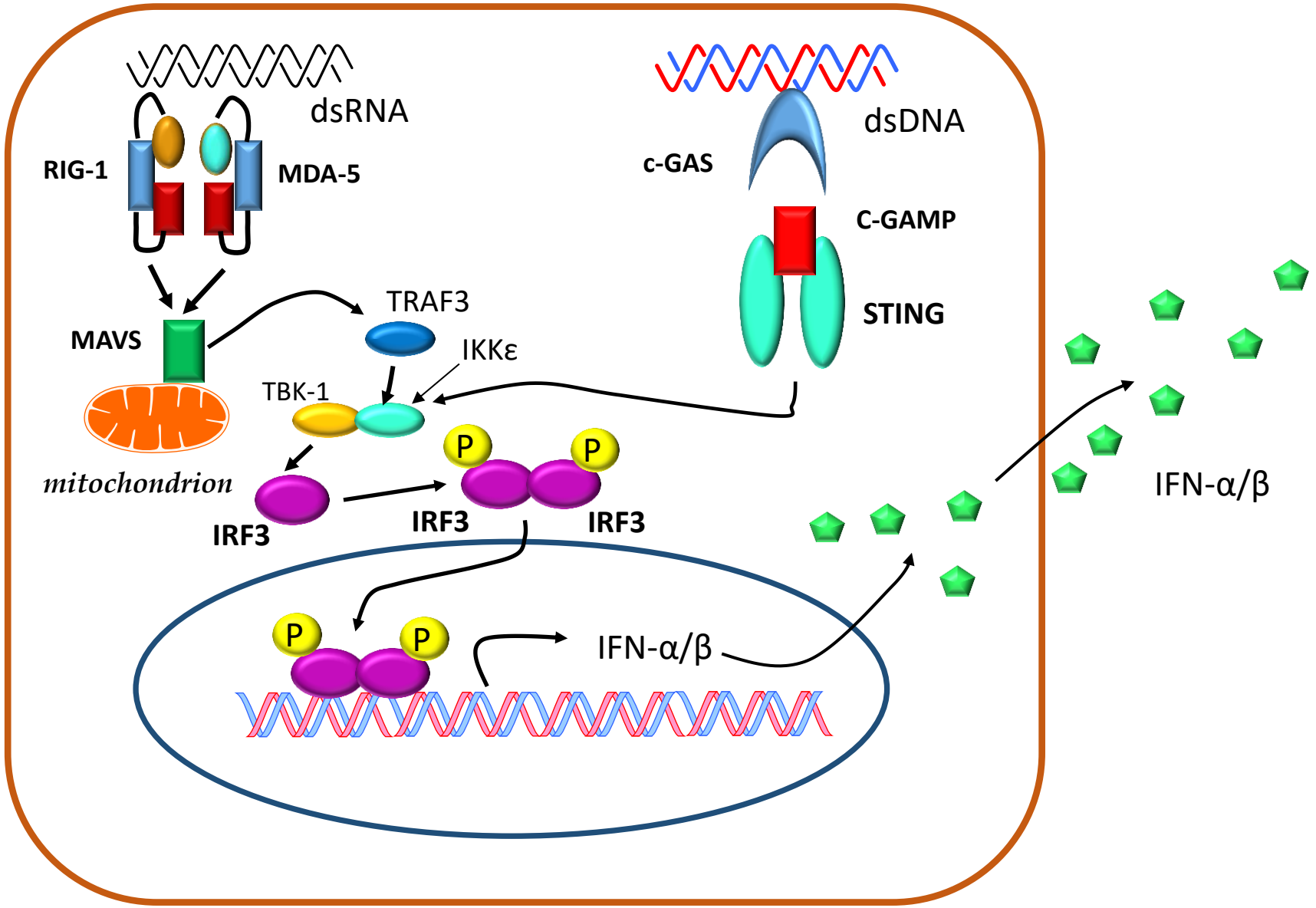
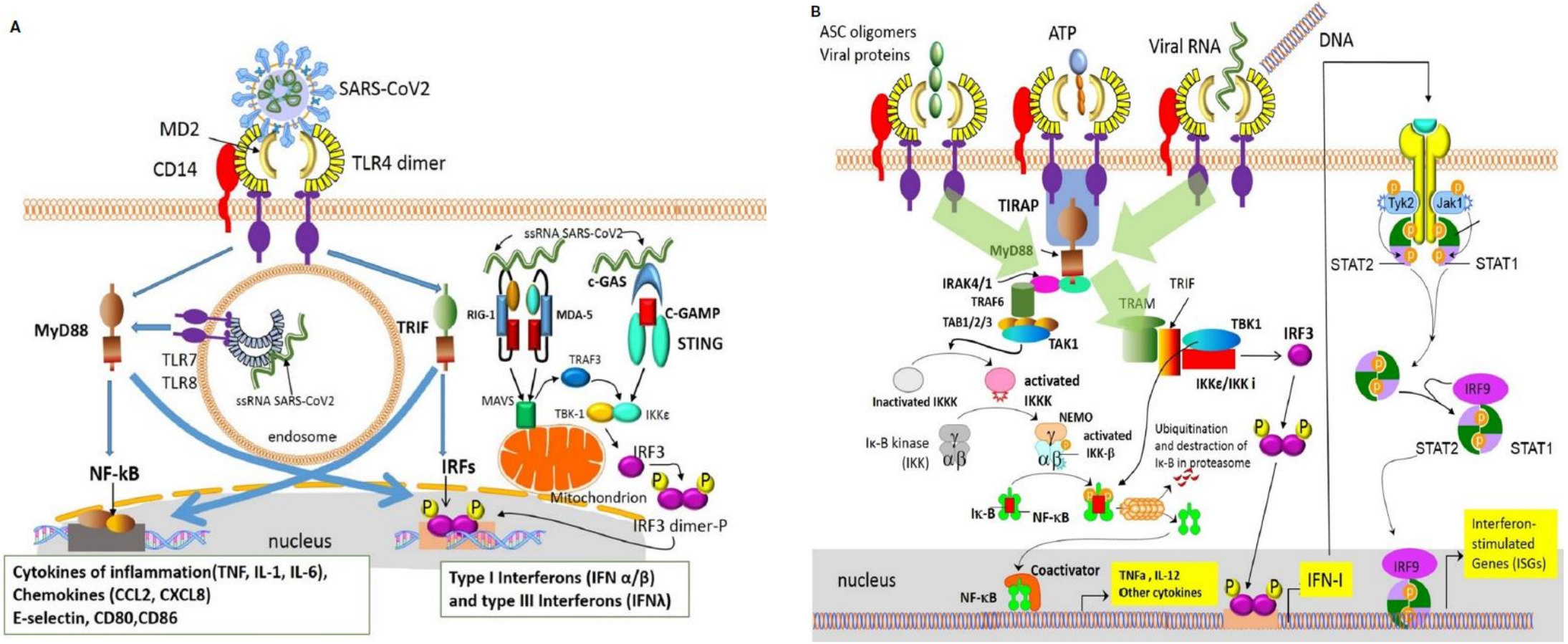
Censors of the cytosol which trigger the production of type I Interferons

IFN, interferon;
IKKε, IκB kinase-ε;
IRF, IFN regulatory factor;
MAVS, mitochondrial antiviral signaling protein;
MDA5, melanoma differentiation -associated gene 5;
P, phosphate;
RIG-I, retinoic acid-inducible gene 1;
TBK1, TANK-binding kinase 1;
TRAF3, tumor necrosis factor receptor-associated factor 3;
TANK, TRAF family member-associated NF-kappa-B activator



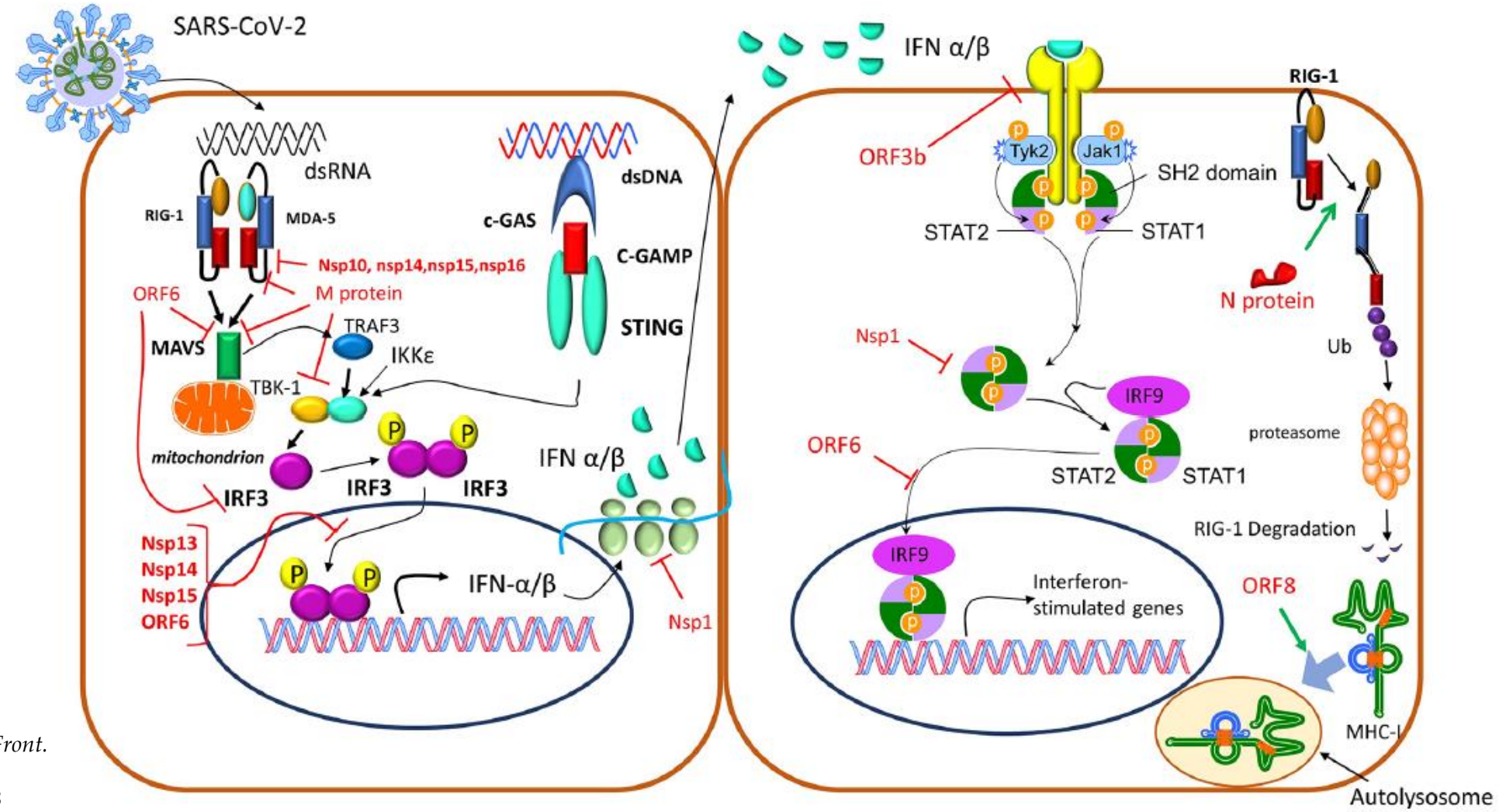
P.G. Vlachoyiannopoulos MD

Innate immune detection of SARS-CoV-2 results in the production of interferons, cytokines and chemokines



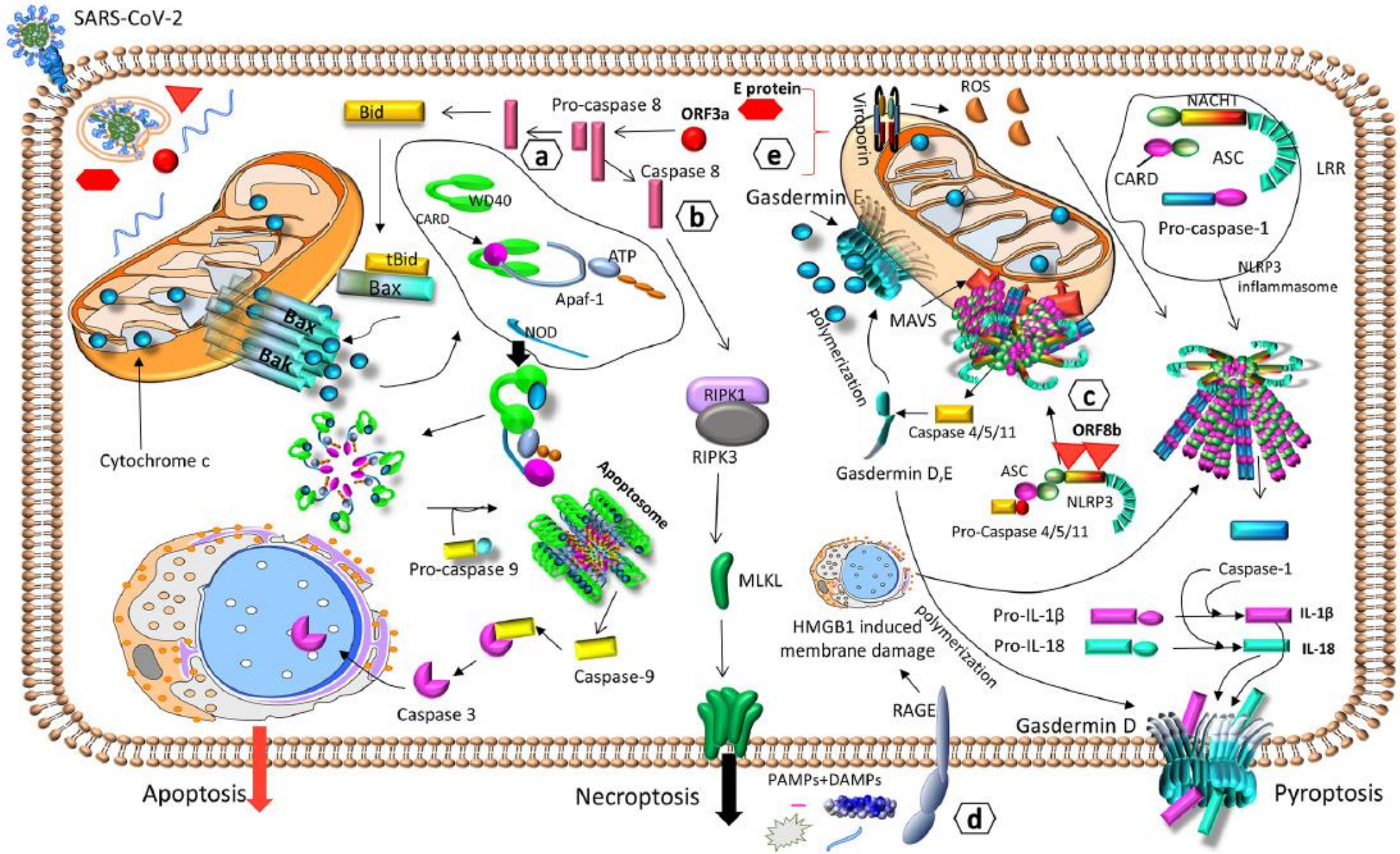
Kaklamanos A, et al, (2021)Front. Immunol. 12:719023. doi: 10.3389/fimmu.2021.719023

Corona viruses exploit different mechanisms to avoid immune detection and supress interferon production

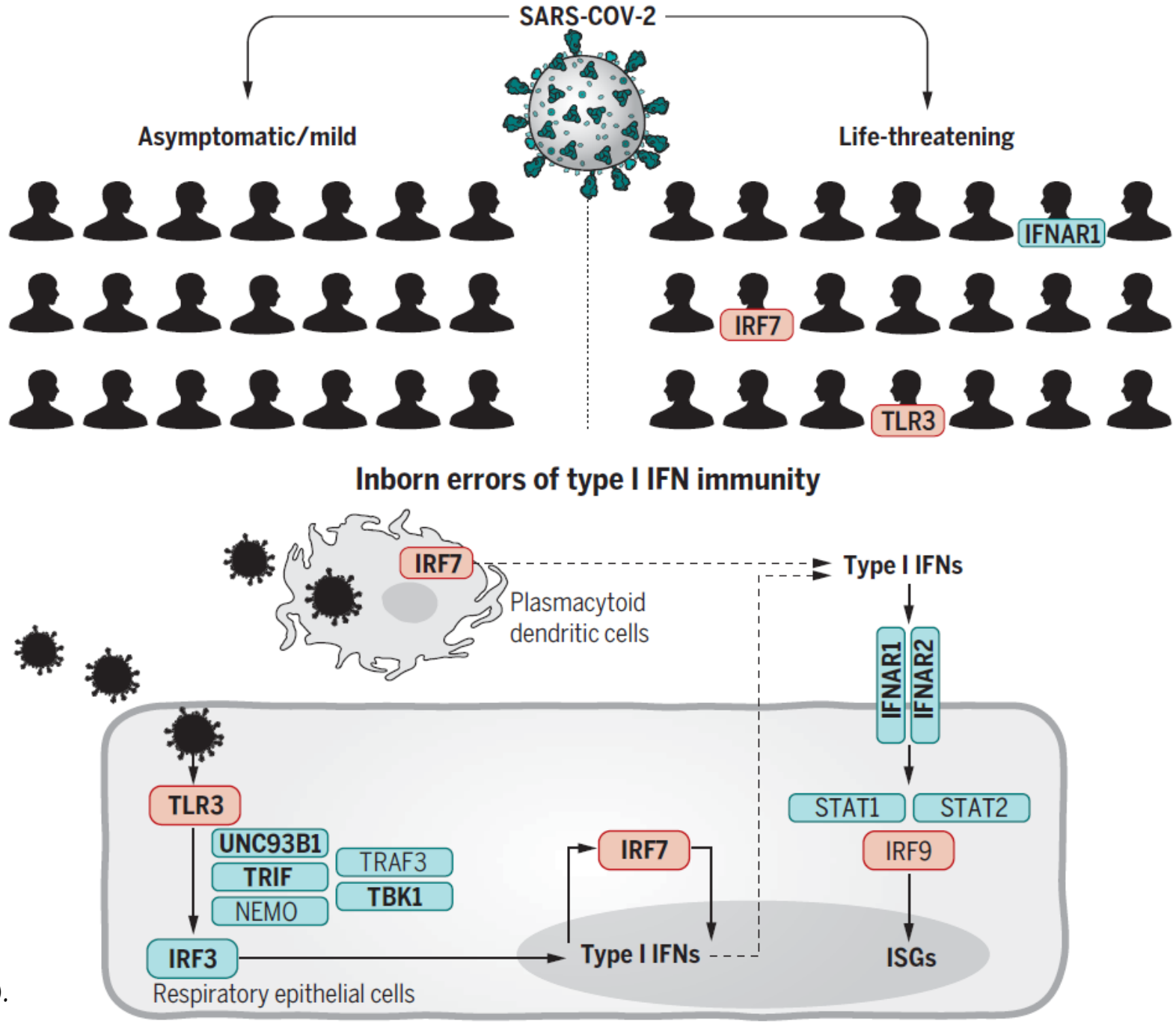


Kaklamanos A, et al, (2021)Front. Immunol. 12:719023. doi: 10.3389/fimmu.2021.719023

SARS-CoV-2 can trigger different types of cell death



Inborn errors of TLR3- and IRF7-dependent type I IFN production and amplification underlie life-threatening COVID-19 pneumonia



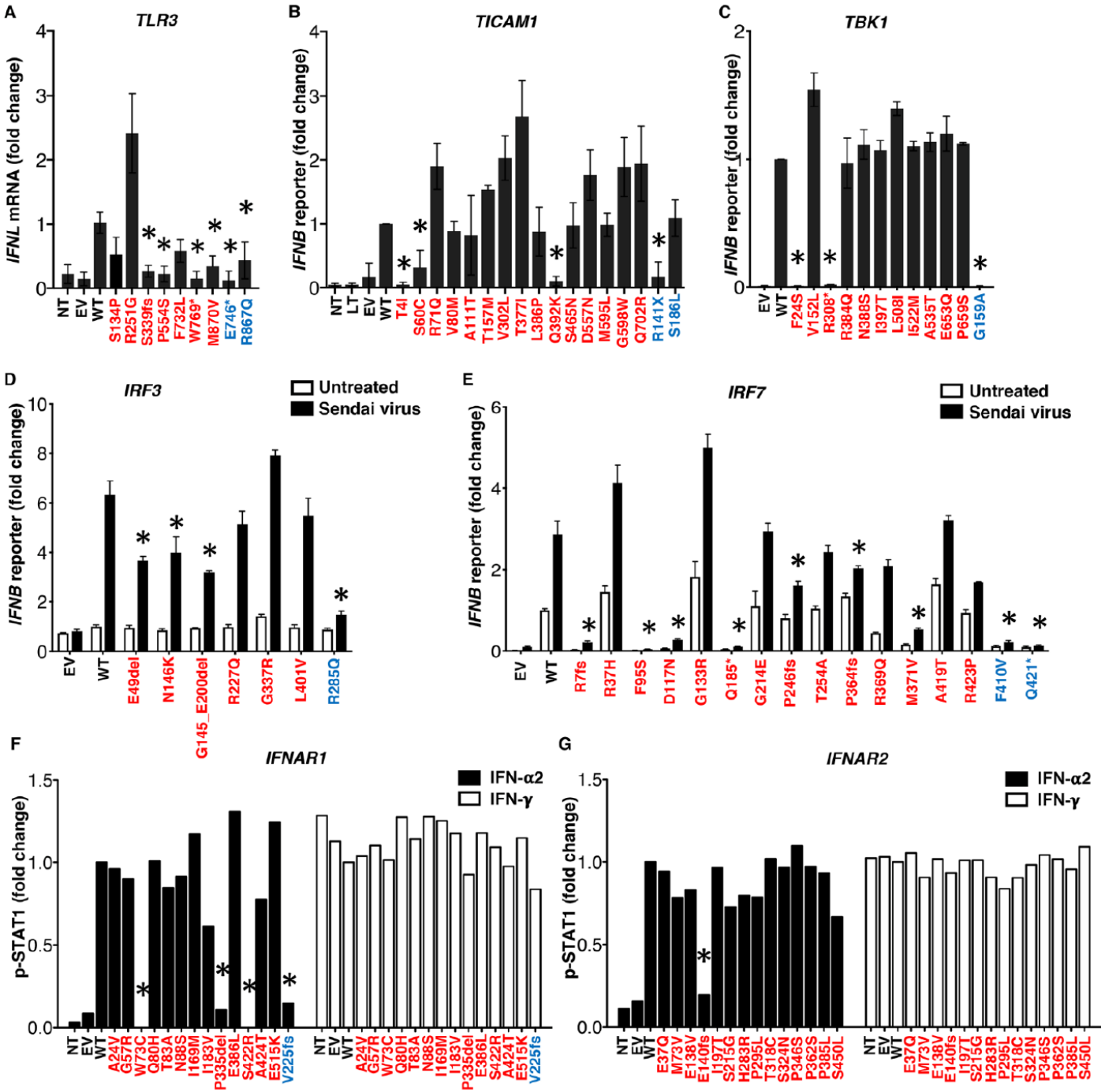
Q. Zhang et al., Science 10.1126/science.abd4570 (2020).

Inborn errors of TLR3- and IRF7-dependent type I/III IFN production in patients with severe COVID-19

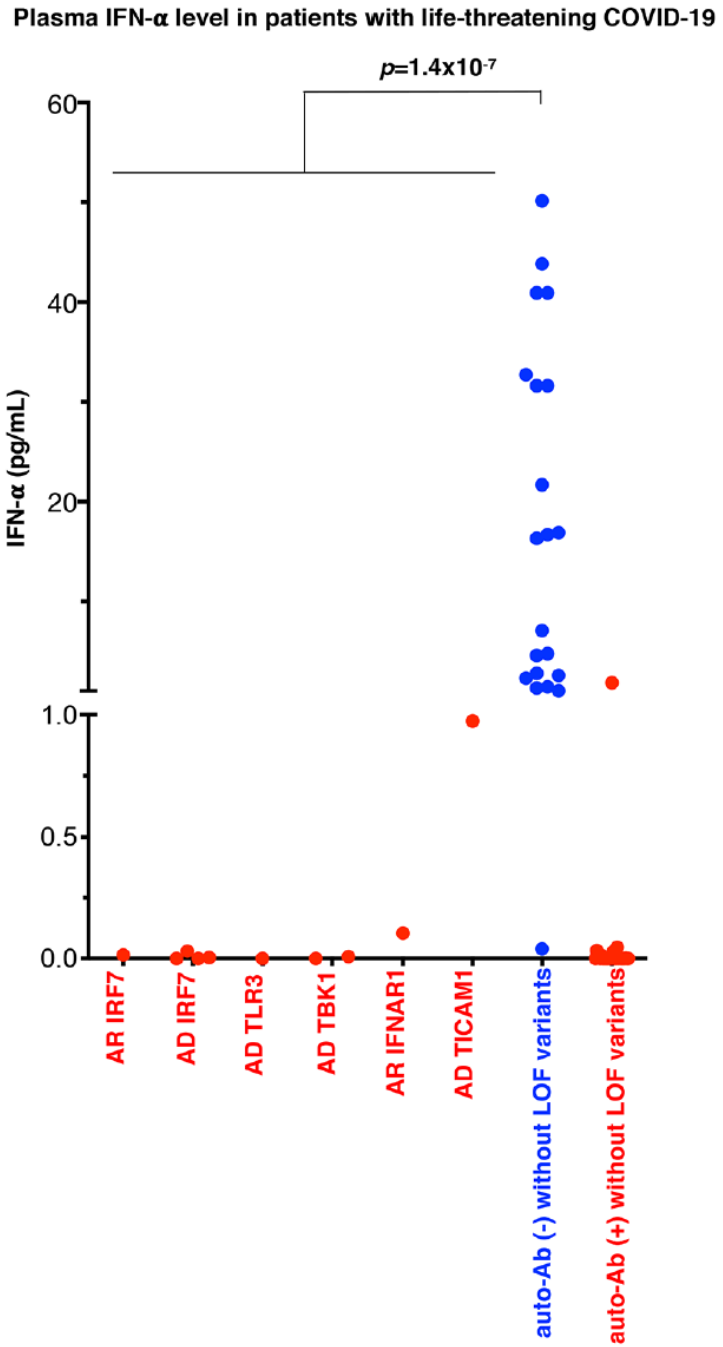
Experimental protocol

- Mutated genes affecting IFN-I/III production from patients with severe influenza pneumonia were identified
- Cells knocked out for IFN-I/III producing genes were transfected with plasmids containing wilde type or mutatant forms of genes related to IFN-I/III production
- Study of IFN-I/III production from these cells spontaneously or after viral stimulation

Q. Zhang et al., Science 10.1126/science.abd4570 (2020).

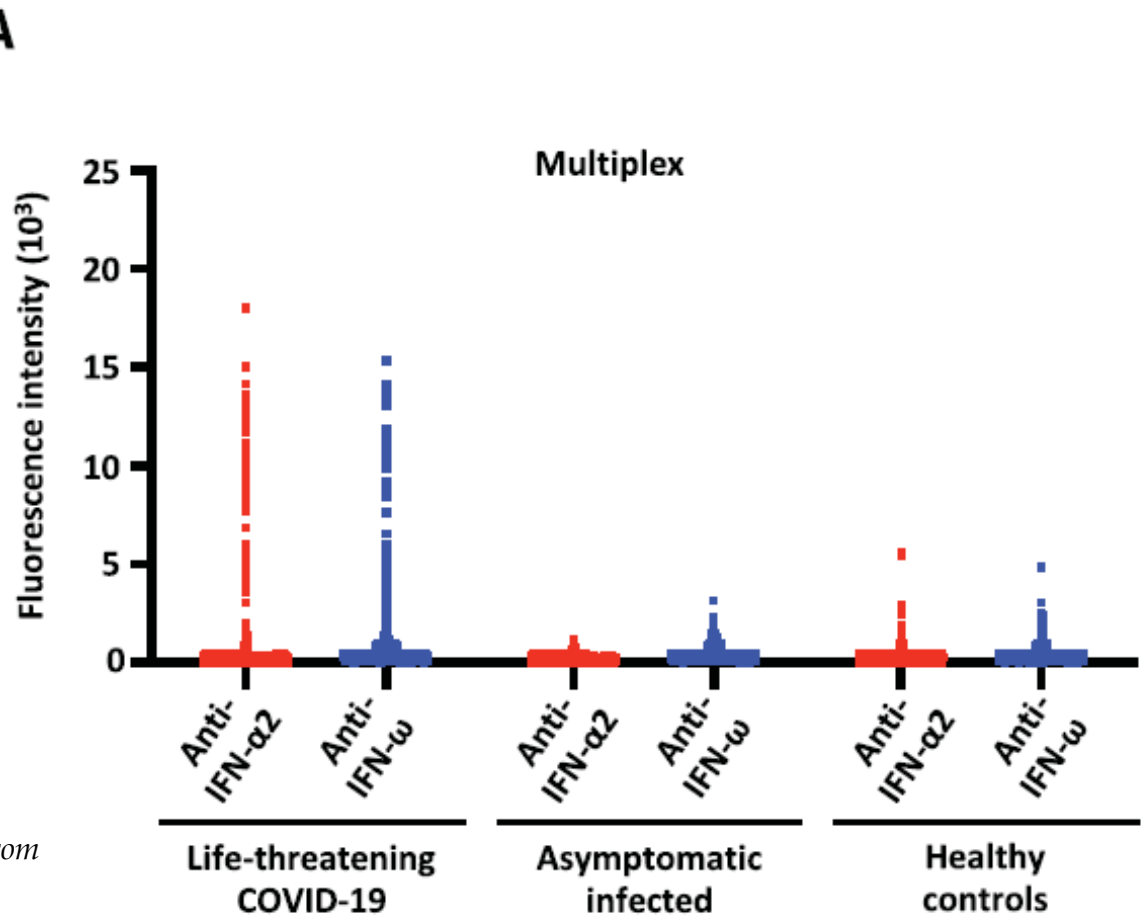


In vivo type I IFN responses to SARS-CoV-2 infections: patients with severe COVID-19 disease possess los of function (LOF) variants of IFN-α inducing genes or autoantibodies to IFN-α

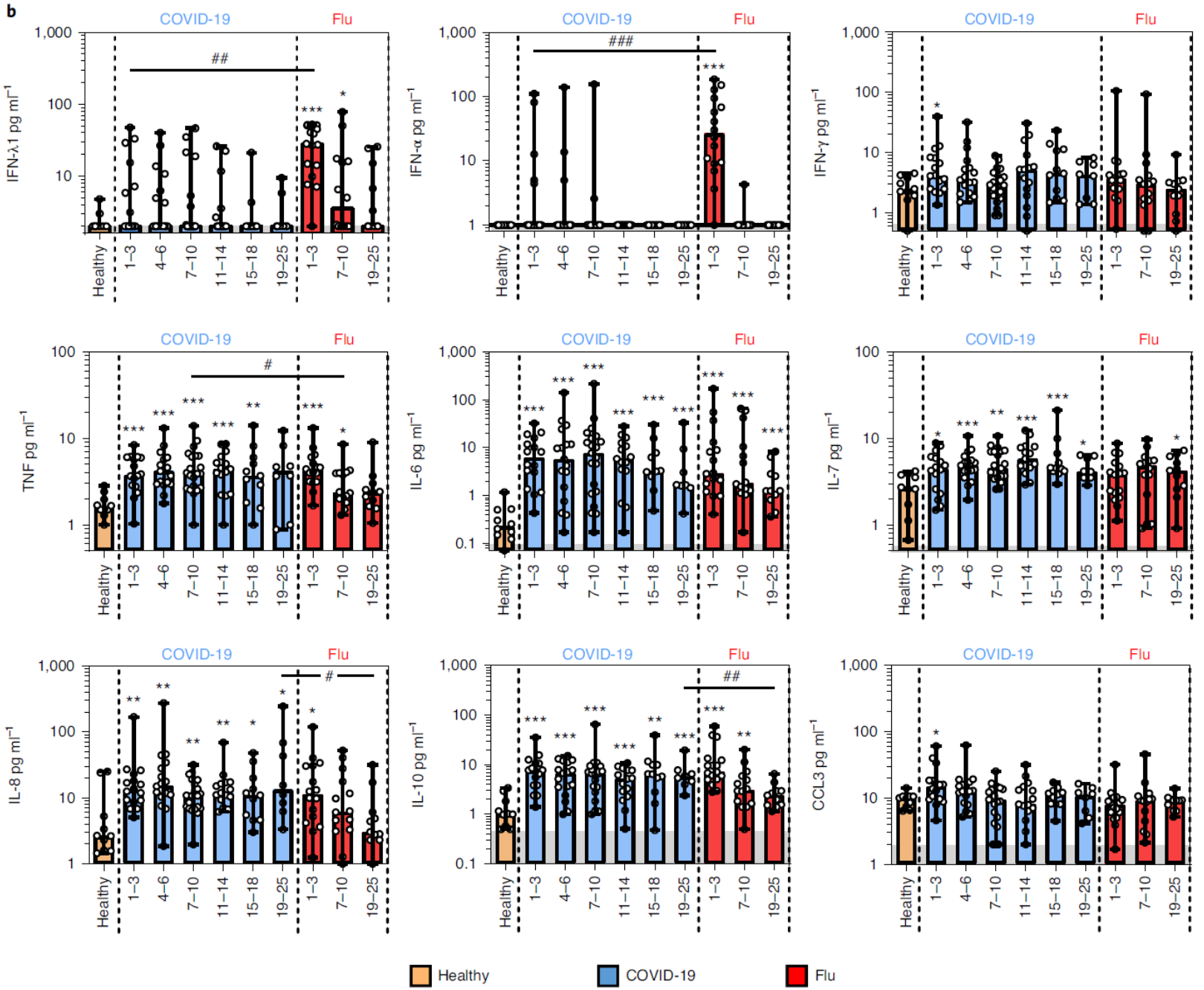


Q. Zhang et al., Science 10.1126/science.abd4570 (2020).

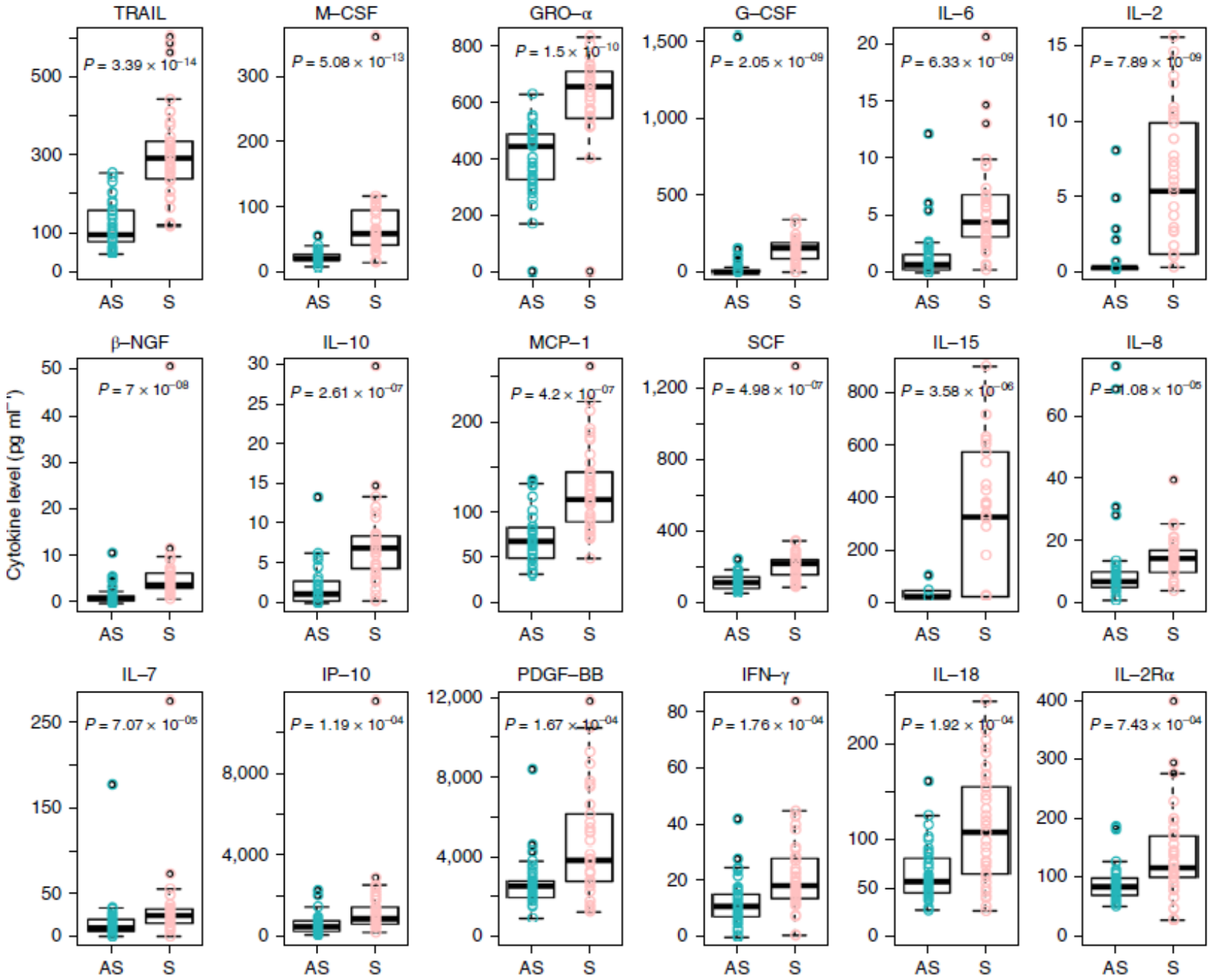
Patients with severe COVID-19 express autoantibodies to IFN-α and IFN-ω



Untuned antiviral response in COVID-19, contributing to persistent viral presence, hyperinflammation and respiratory failure.

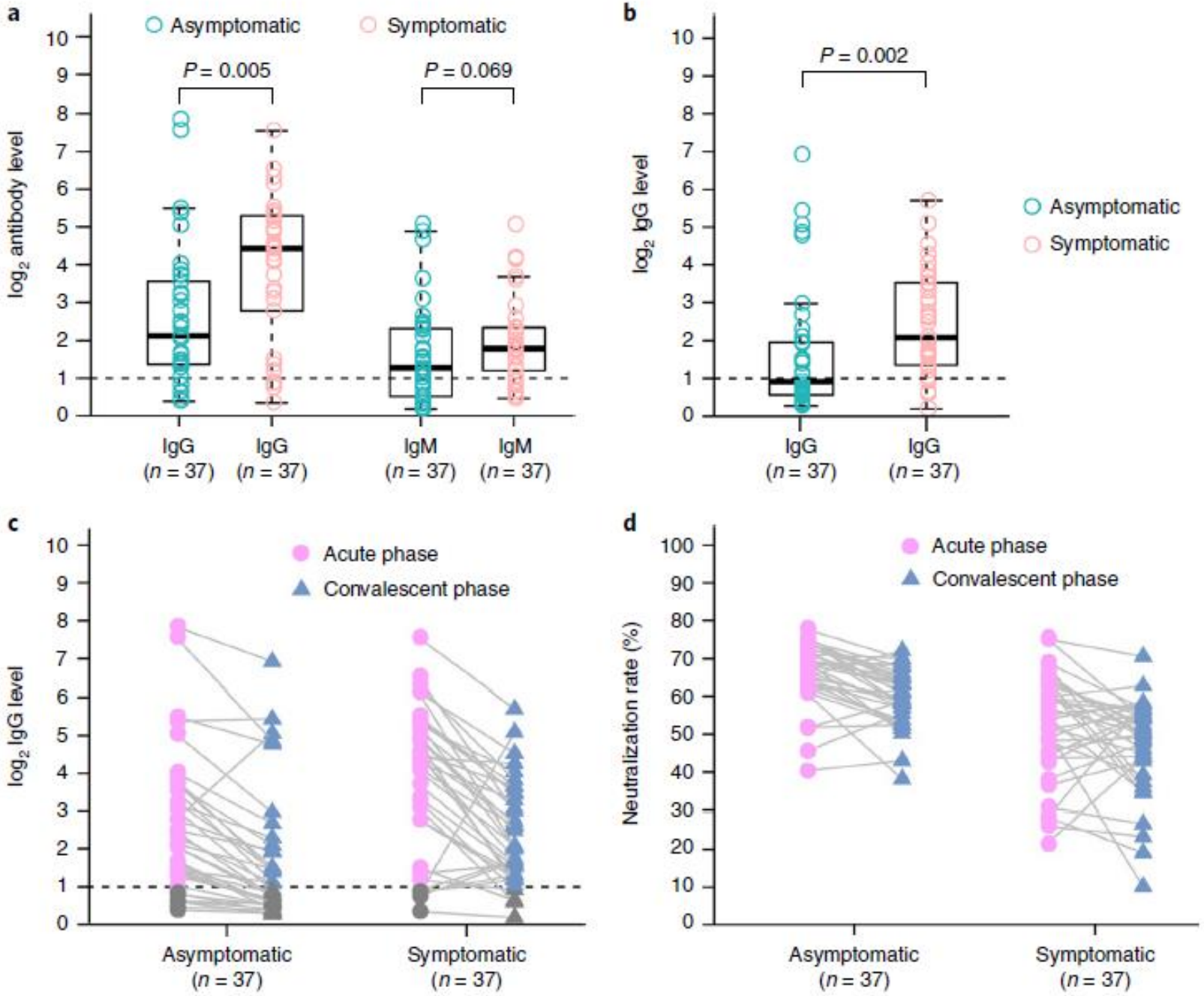


Serum cytokine/chemokine concentrations are higher in symptomatic than in asymptomatic groups of COVID-19 patients.



Long Q-X et al, Nature Medicine | VOL 26 1200 | August 2020 | 1200–1204 | www.nature.com/naturemedicine

Asymptomatic patients with COVID-19 express lower anti-SARS-CoV-2 antibody titers than the symptomatic ones



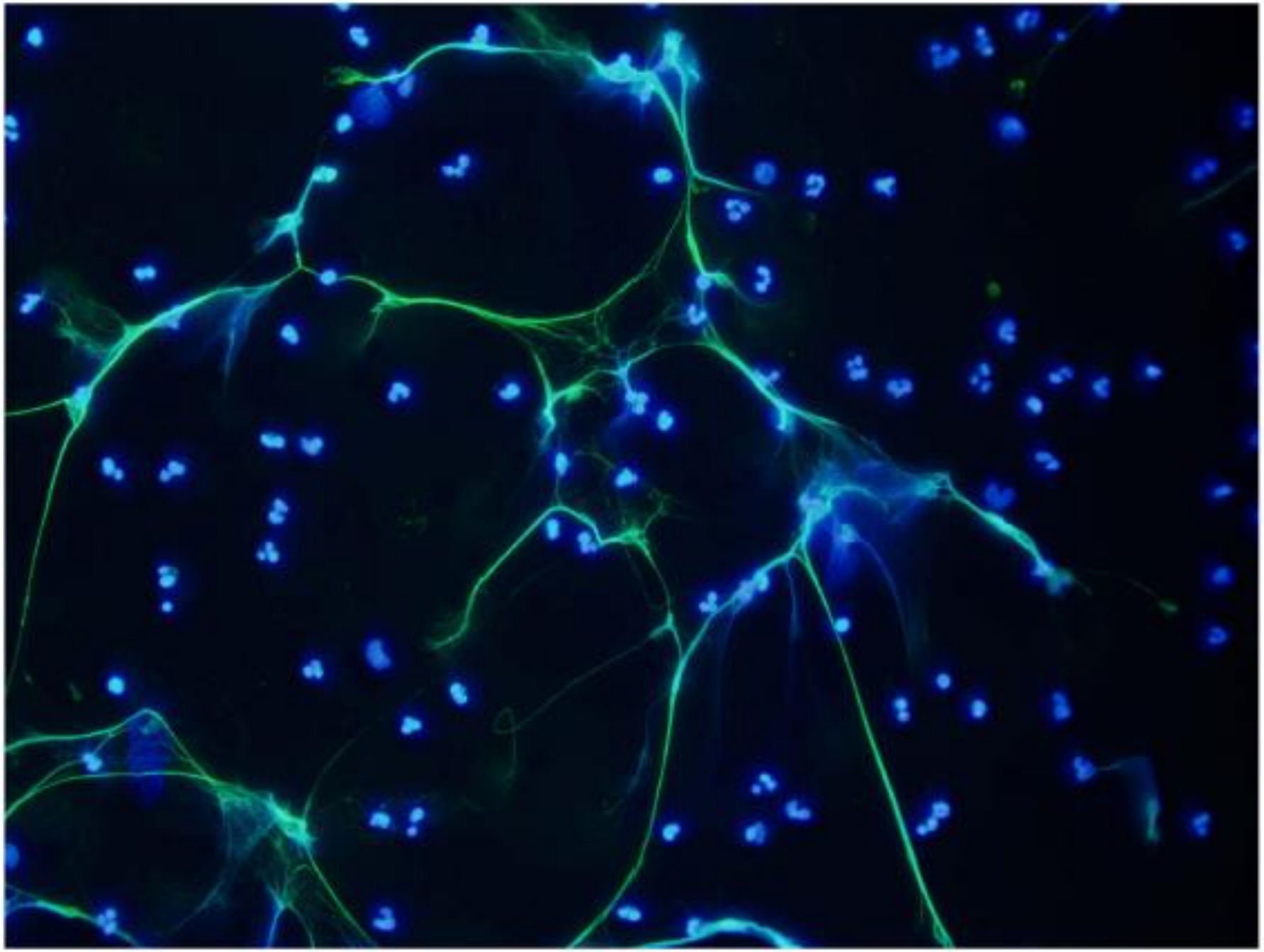
Long Q-X et al, Nature Medicine | VOL 26 1200 | August 2020 | 1200–1204 | www.nature.com/naturemedicine

Representative images of NETs produced in vitro

Blue: DAPI stain: Nuclei of Neutrophils

Green: Elastase (NETs)

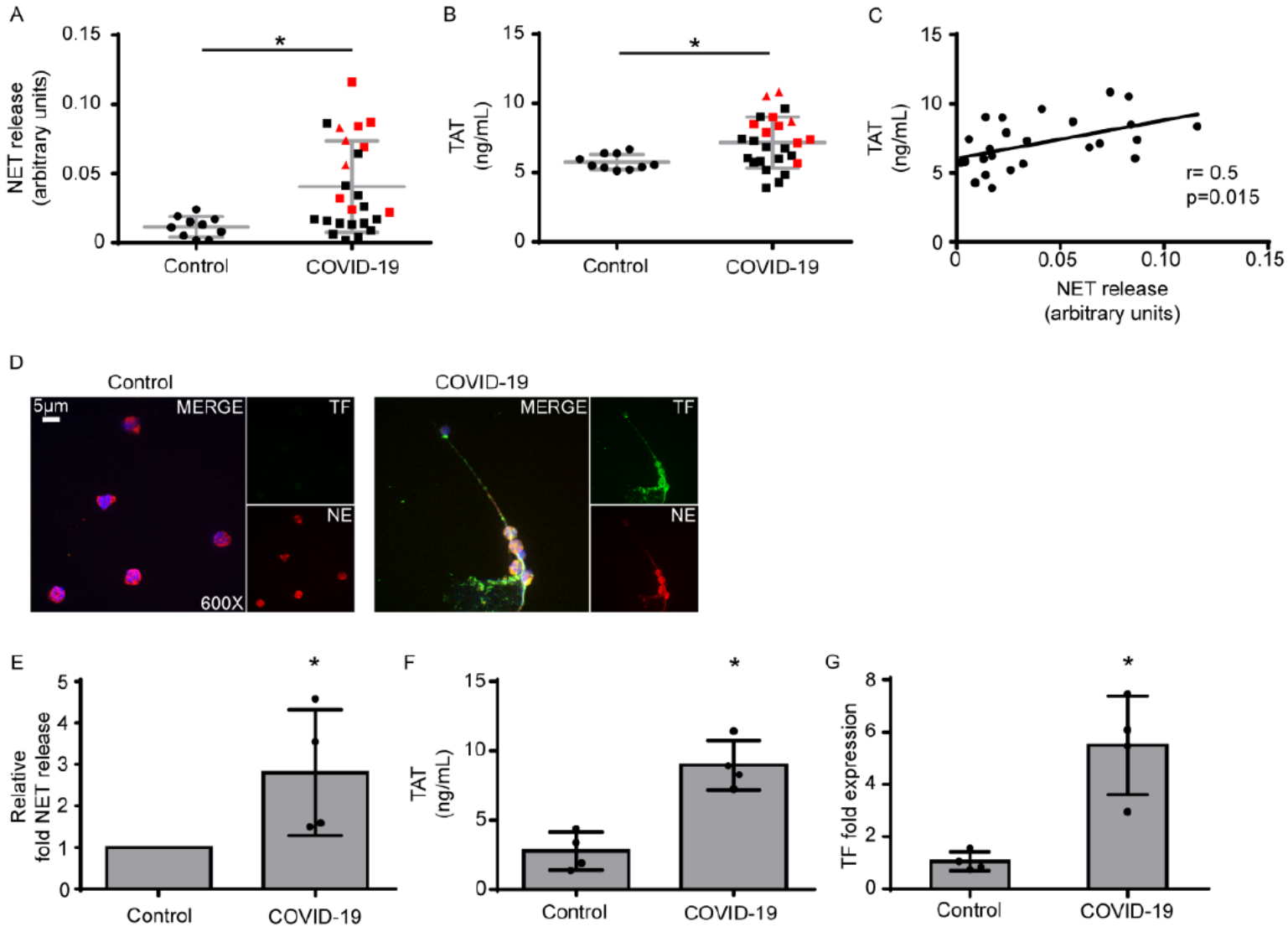
NETs: Neutrophil extracellular traps



Kaplan M, and Radic M. J Immunol. 2012 September 15; 189(6): 2689–2695. doi:10.4049/jimmunol.1201719.

Neutrophil extracellular traps in patients with COVID-19 are decorated with complement and Tissue factor and probably induce thrombosis

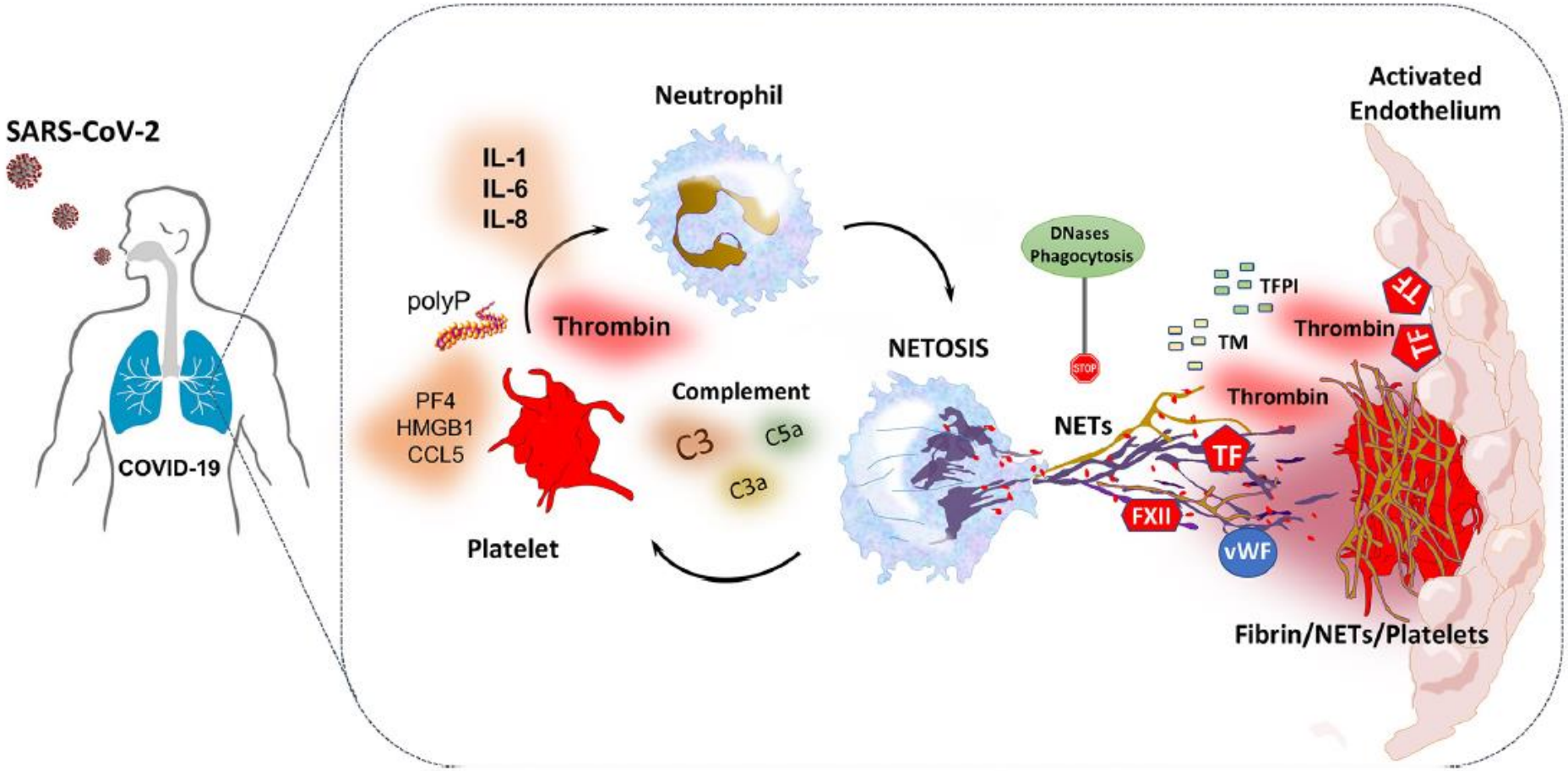
- Myeloperoxidase (MPO)-DNA complex levels representing NET release (detected by ELISA)
- TAT: Thrombin-antithrombin level
- (TF)/neutrophil elastase (NE) staining



Neutrophil extracellular traps (NETs) in the coagulopathy of COVID- 19.

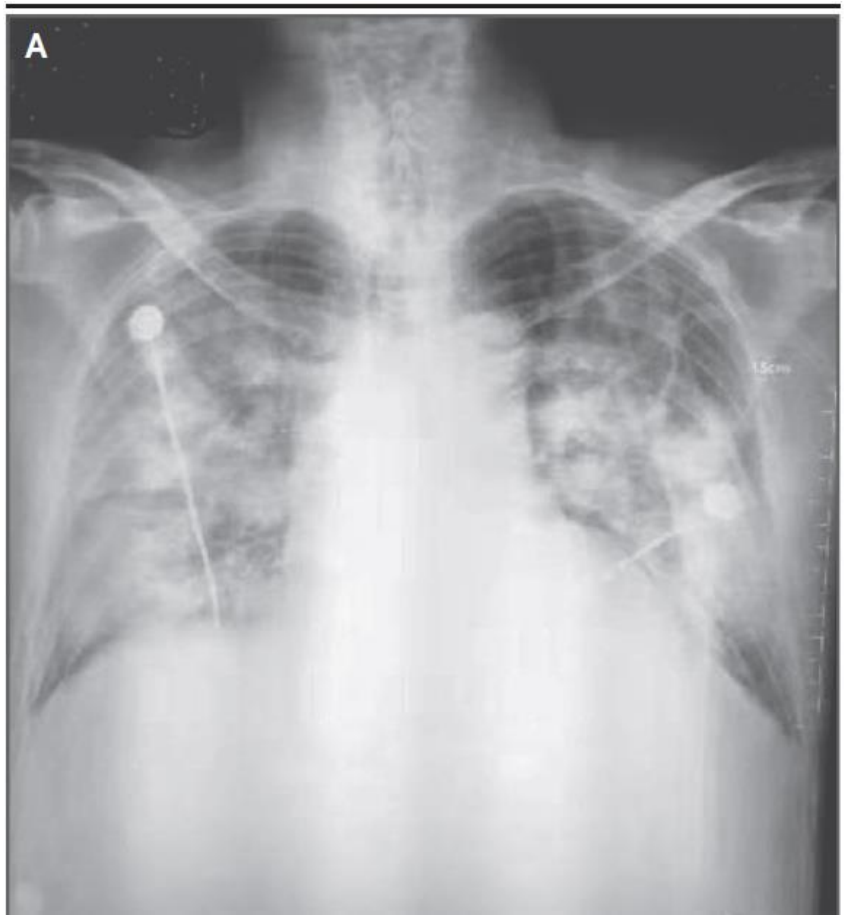
Skendros P et al, J Clin Invest. 2020. https://doi.org/10.1172/JCI141374

SARS-CoV-2-triggered NETs induce thromboinflammation through interactions with complement system, platelets and the coagulation cascade

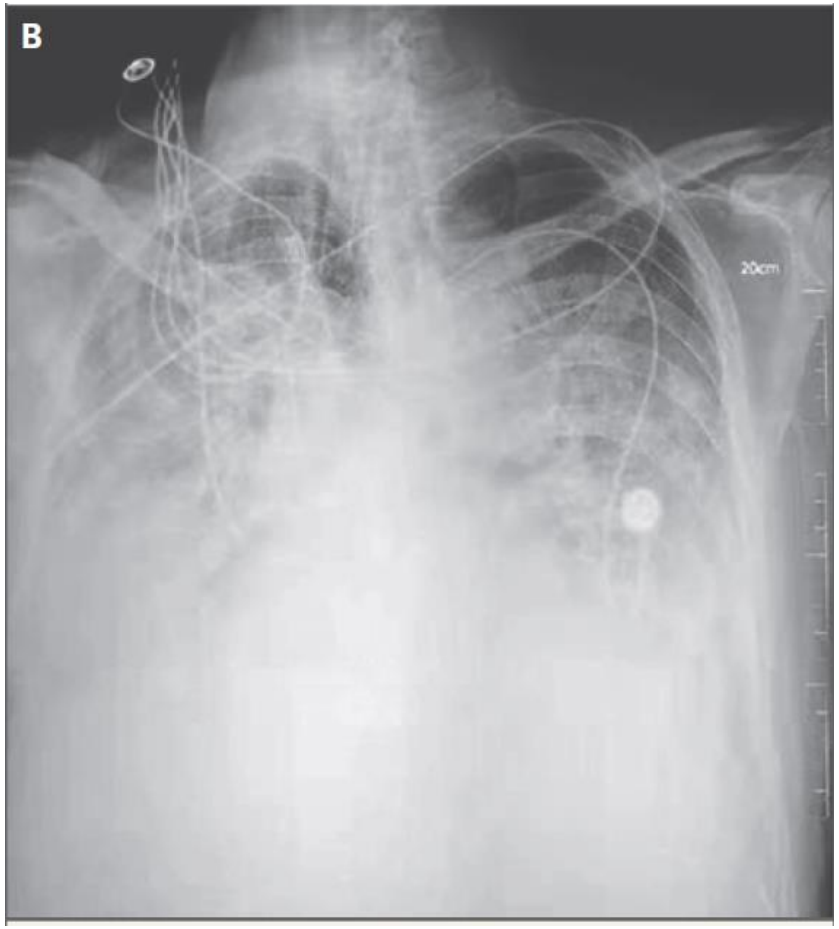


Kaklamanos A, et al, (2021)Front. Immunol. 12:719023. doi: 10.3389/fimmu.2021.719023

ARDS in COVID-19 disease: rather vasculopathy than edema



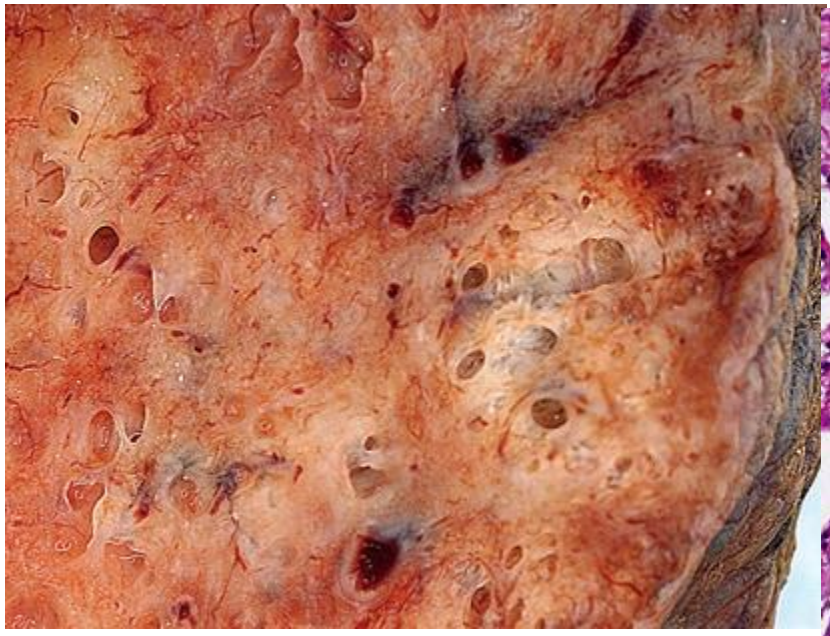
8 days after symptom onset

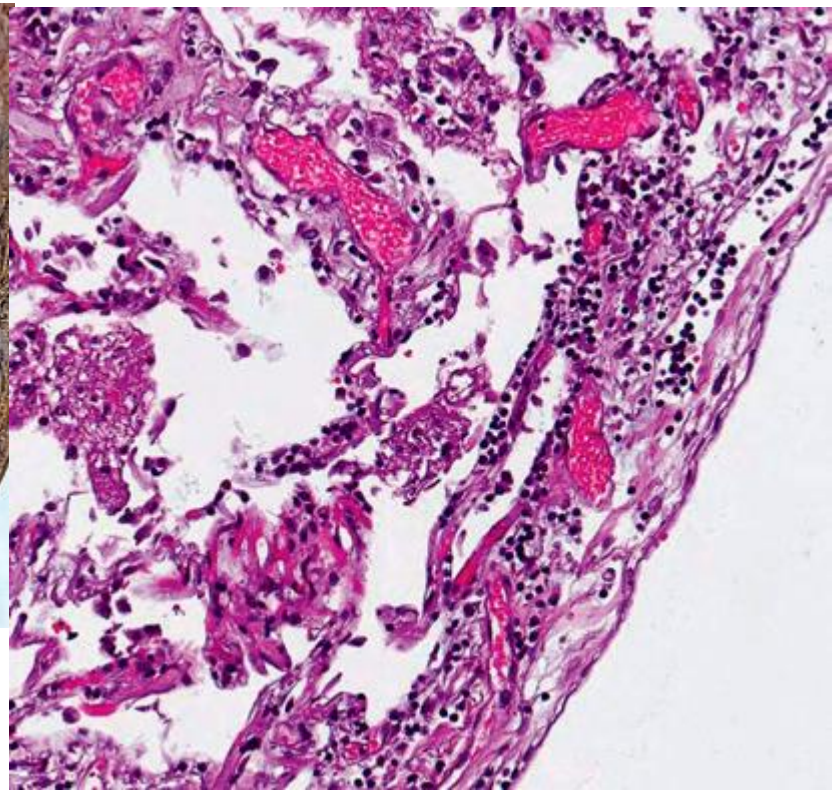


11 days after symptom onset

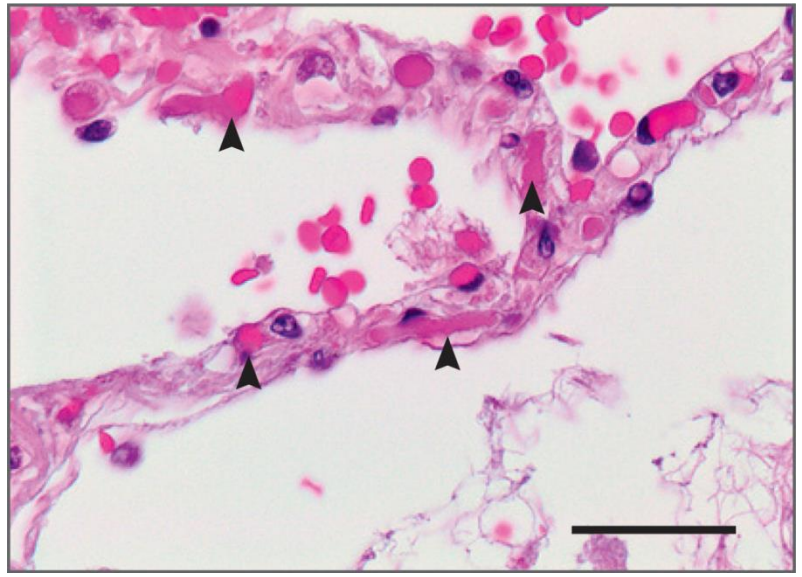
Zhu N. et al, N Engl J Med 2020;382:727-33.

Endotheliitis of pulmonary vessels in patients with severe COVID-19





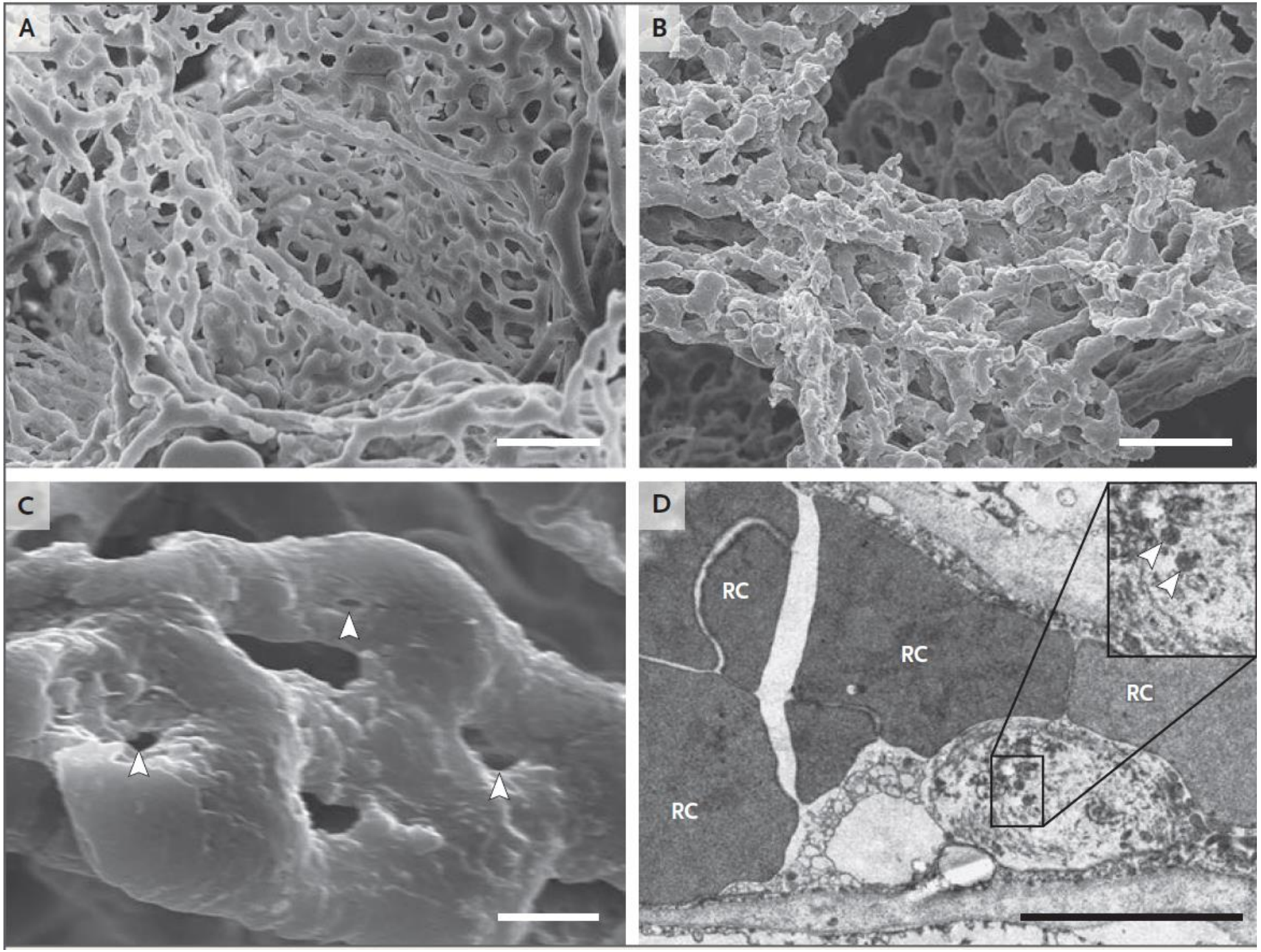
Lymphocytic inflammation in a Lung from a Patient Who Died from Covid-19.
The gross appearance of a lung from a patient who died from coronavirus disease 2019 (Covid-19) is shown in Panel A (the scale bar corresponds to 1 cm). The histopathological examination, shown in Panel B, revealed interstitial and perivascular predominantly lymphocytic pneumonia with multifocal endothelialitis (hematoxylin–eosin staining; the scale bar corresponds to 200 μm).



Microthrombi in the interalveolar Septa of a Lung from a Patient Who Died from Covid-19.
The interalveolar septum of this patient (Patient 4 in Table S1A in the Supplementary Appendix) shows slightly expanded alveolar walls with multiple fibrinous microthrombi (arrowheads) in the alveolar capillaries. Extravasated erythrocytes and a loose network of fibrin can be seen in the intraalveolar space (hematoxylin–eosin staining; the scale bar corresponds to 50 μm).

Ackermann M. et al, ,N Engl J Med 2020;383:120-8.DOI:10.1056/NEJMoa2015432

COVID-19: Defective angiogenesis

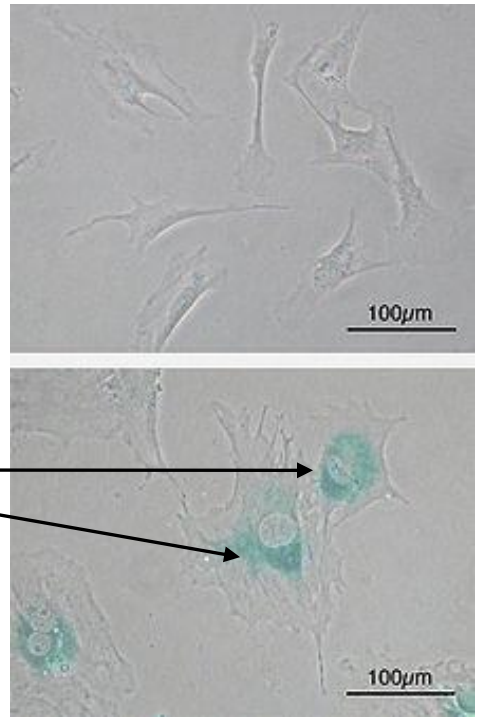


Ackermann M. et al, ,N Engl J Med 2020;383:120-8.DOI:10.1056/NEJMoa2015432

Senescent cells activate the immune system

The average cell will divide around 50 times before reaching a stage known as senescence.

Senescence- associated β-galactosidase, (SABG) (blue)



Natural killer cells directly kill senescent cells, and produce cytokines which activate macrophages which remove senescent cells. Senescent cells can be phagocytized by neutrophils as well as by macrophages

Leonard Hayflick and Paul , 1960

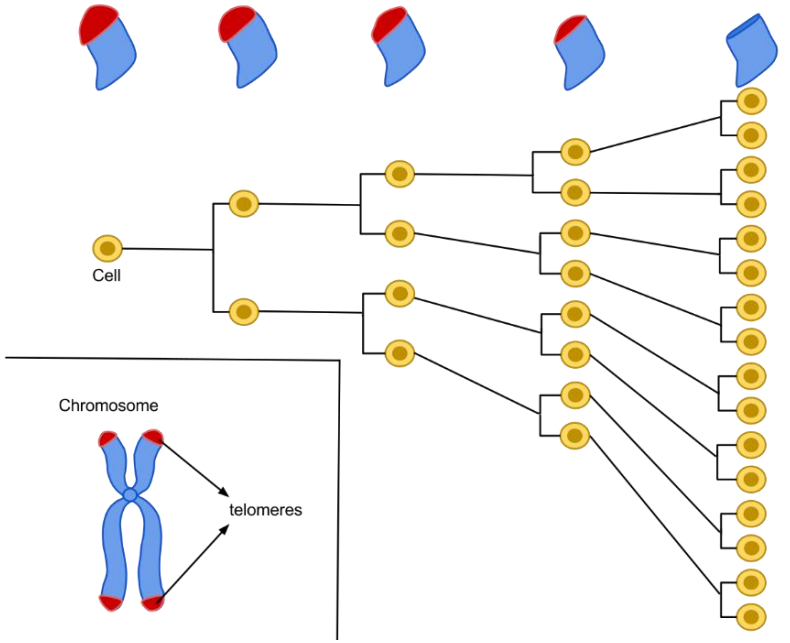
Replicative senescence can be triggered by:

✓a DNA damage response due to the shortening of telomeres

✓DNA damage in response to elevated reactive oxygen species (ROS),

✓Activation of oncogenes,

✓Cell-cell fusion



As the cell divides, the telomeres on the end of a linear chromosome get shorter. The telomeres will eventually no longer be present on the chromosome. This end stage is the concept that links the deterioration of telomeres to aging.

Inflammaging

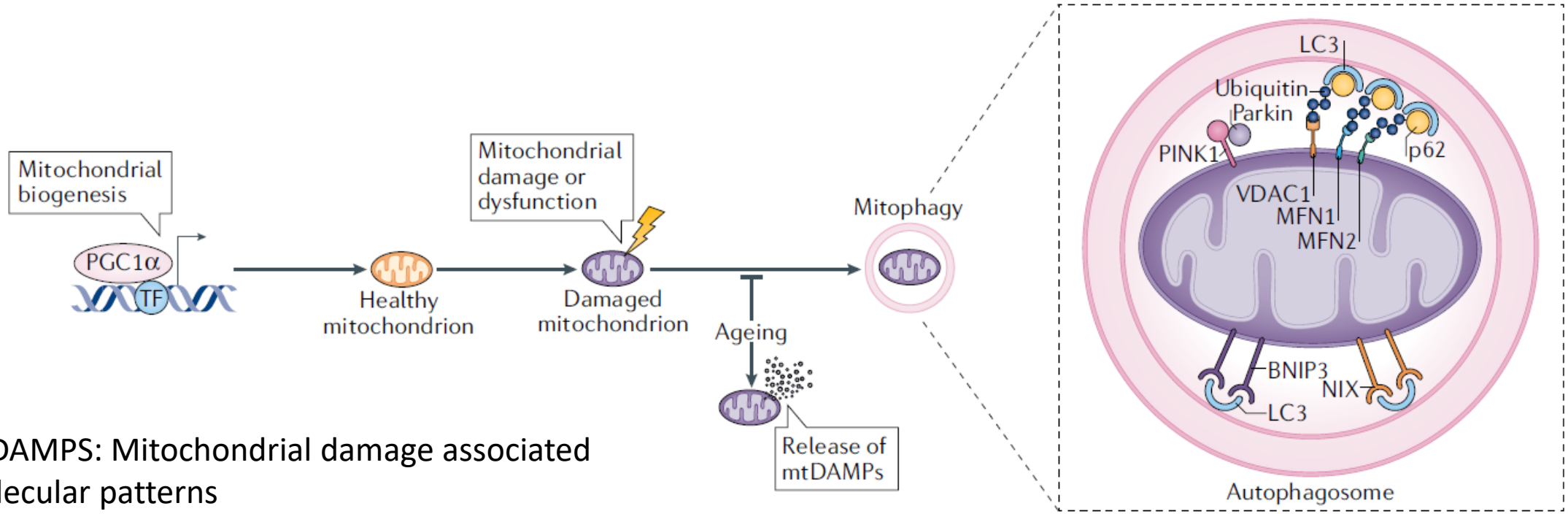
- People> 60 years express findings of “sterile” inflammation
  - ➢Increased CRP
  - ➢Increased IL-6
  - ➢Increased IL-8
- This continuous “sterile” inflammation is a biomarker of reduced survival

C. Franceschi, P. Garagnani, G. Vitale, M. Capri, S. Salvioli, Trends Endocrinol. Metab. 28, 199 (2017).

- Senescence
  - ➢Decreased mitochondrial function
  - ➢Increase IL-6
  - ➢Increased myeloid differentiation
  - ➢Increased atherosclerosis

R. P. H. De Maeyer et al., Nat. Immunol. 21, 615 (2020).

Senescence is associated with cumulative accumulation of mitochondrial injuries mainly due to the reduction of reperative *mtDNA helicase Twinkle* and decreased autophagy of injured mitochondria



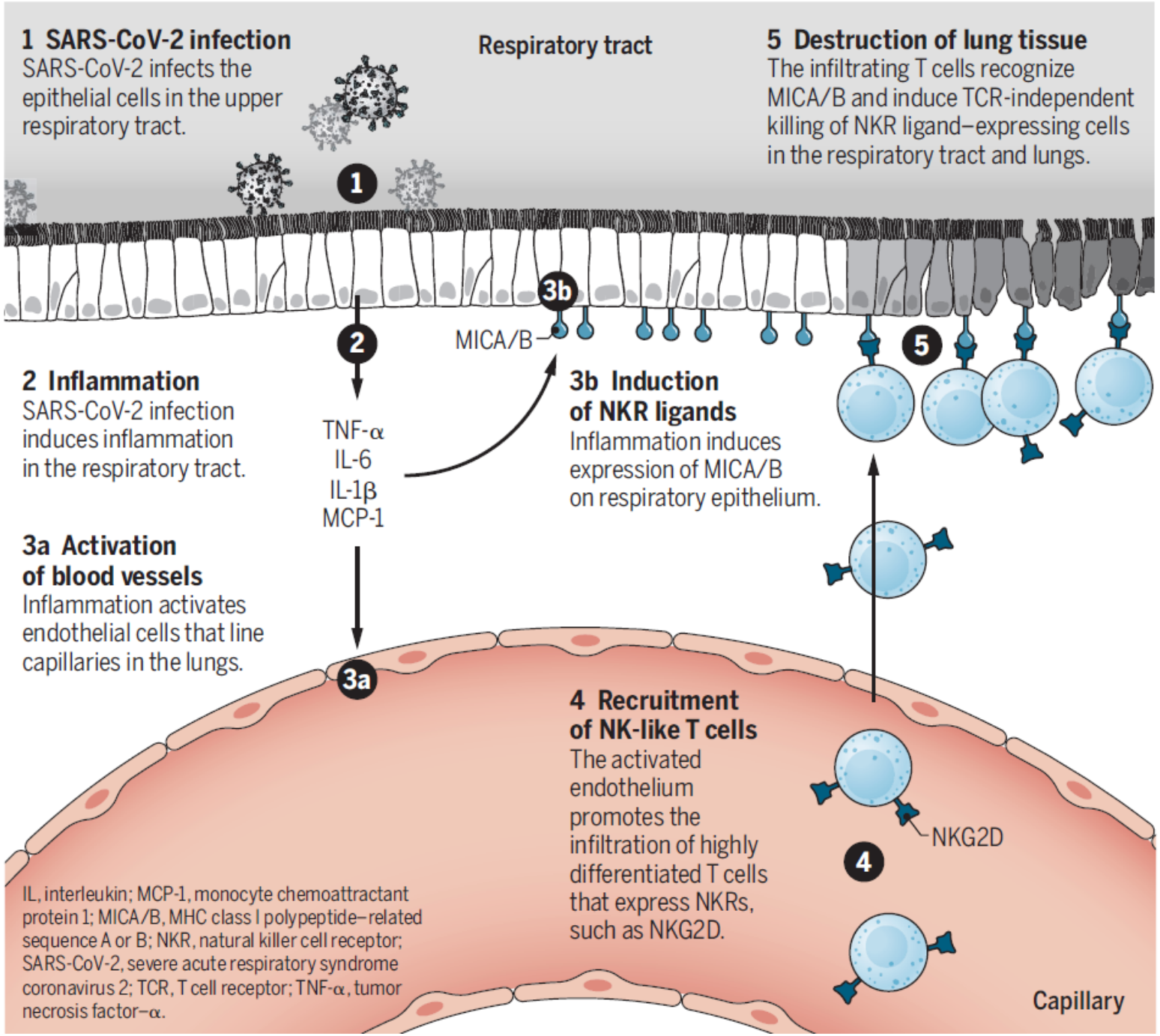
mtDAMPS: Mitochondrial damage associated molecular patterns

Tyrrel GJ, Goldstein DR, Nature Reviews Cardiology https://doi.org/10.1038/s41569-020-0431-7

Immune responses to SARS-CoV-2 in ageing results in destruction of lung tissue

- Major histocompatibility complex (MHC) class I chain-related protein A (**MICA**) and MHC class I chain-related protein B (**MICB**)

- NK-like T cells: CD8(+) T which express receptors of NK cells, such as CD56, KIR, NKG2A and NKG2C (CD159a and c), and CD94



Arne N. Akbar and Derek W. Gilroy Science 369 (6501), 256-257
DOI: 10.1126/science.abb0762

Patients which improve in terms of lung indexes but they do not recover have anti-SARS-CoV-2 antibodies production, blood-brain barrier disturbances and neurodegeneration indexes

Table 1 Anti-SARS-CoV-2 antibodies in serum and CSF, intrathecal synthesis, blood brain barrier disturbance and neurologic status of patients

Patient samples	Serum IgG (1:100 dilution) >1.1[a]	CSF 1: 100 >1.1	CSF 1: 10 >1.1	IgG index normal <0.77	Albumin index >20 × 10^{-3}	14-3-3	Autoimmune encephalitis	Main CNS clinical findings	Outcome
1	**9.81**	**2.87**	**8.64**	**1.85**	4×10^{-3}	**POS**	NEG	Coma: GCS 4	Death
2	**9.45**	**1.50**	**5.35**	0.36	**24×10^{-3}**	**POS**	NEG	Coma: GCS 9	ICU
3	NEG	NEG	NEG	0.41	5×10^{-3}	NEG	NEG	Under sedation	ICU
3′ (12 d later)	**8.62**	**1.53**	**6.42**	0.39	**25×10^{-3}**	**POS**	NEG	—	—
4	**9.68**	NEG	**2.39**	0.46	3×10^{-3}	NEG	NEG	Somnolence: GCS 15	ICU
5	**8.33**	NEG	**2.10**	0.29	4×10^{-3}	NEG	NEG	Somnolence: GCS 6	Death
6	**7.95**	NEG	NEG	0.27	4×10^{-3}	NEG	NEG	Under sedation	ICU
6′ (7 d later)	**8.62**	NEG	**1.83**	0.27	4×10^{-3}	NEG	NEG	—	—
7	**7.17**	NEG	**1.56**	0.44	3×10^{-3}	**POS**	NEG	Somnolence: GCS 13	ICU
8	**7.80**	**2.23**	**5.13**	0.37	**25×10^{-3}**	NEG	NEG	Coma: GCS 7	Death

Abbreviations: GCS = Glasgow Coma Scale; ICU = intensive care unit; IgG = immunoglobulin G; NEG = negative; POS = positive; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
Numbers in bold indicate above the normal range.
[a] Index is calculated by dividing absorbance of samples @450 nm (1:100 or 1:10 dilution) to absorbance of calibrator @450 nm according to the manufacturer's instructions. The cutoff for antibody positivity is >1.1.

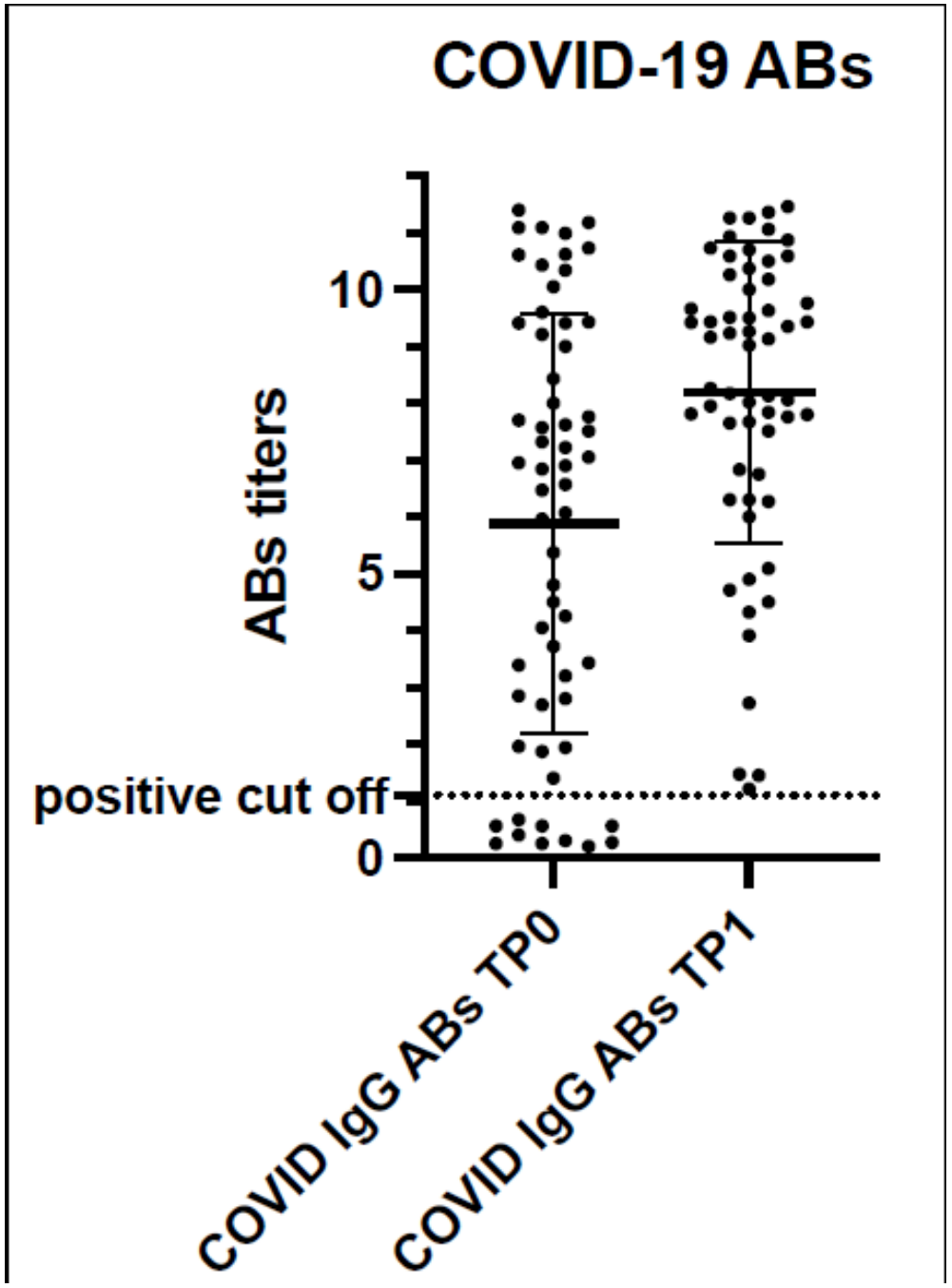
Alexopoulos H et al, Neurol Neuroimmunol Neuroinflamm 2020;7:e893. doi:10.1212/NXI.0000000000000893

Severely ill patients with COVID-19 express plethora of autoantibodies

Patients (n=29)	N, (%)
Male , n, (%)	21 (72,4%)
Female, n, (%)	8 (27,6%)
Age, (min, max, median), (years)	43 -65- 64,2
Patients positive for autoantibodies , n, (%)	**20, (68,7%)**
Anti-nuclear antibodies (ANA), n, (%)	10, (34,5%)
P- ANCA,n , (%)	2, (6,9%)
c-ANCA, n, (%)	2, (6,9%)
Anti-cardiolipin antibodies, n, (%)	7, (24%)
Anti-β2GPI antibodies, n, (%)	10, (34%)
Αντι-CCP, n, (%)	1, (3,5%)
History of a previous autoimmune disease	Ουδείς

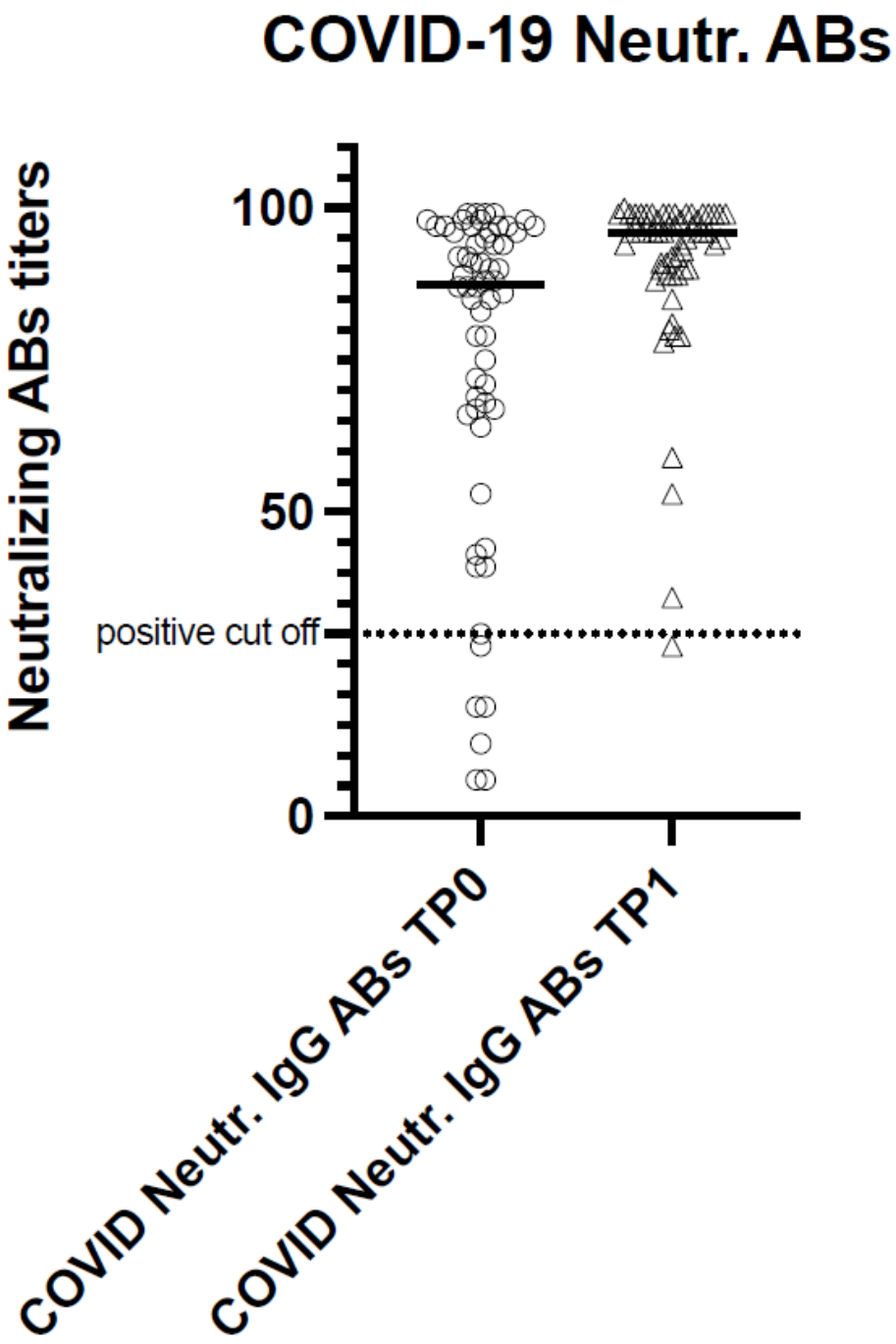
Vlachoyiannopoulos PG, Magira E, Alexopoulos H, et al. Ann Rheum Dis Epub ahead of print: [please include Day Month Year]. doi:10.1136/ annrheumdis-2020-218009

The prevalence of antibodies to SARS-CoV-2 in severe COVID-19 patients and their titers increase with time



Bakasis A, Bitzogli K, et al, unpublished

The prevalence and the titers of anti-SARS-CoV-2 neutralizing antibodies in severe COVID-19 patients increase with time



Bakasis A, Bitzogli K, et al, unpublished

Severe COVID-19: The prevalence of autoantibodies to extractable nuclear antigens in two time points

Autoantibodies to ENAs	First evaluation	Second evaluation
nRNP/Sm, N (%)	1/60 (1,66)	0/60 (0)
Sm, N (%)	0/60 (0)	0/60 (0)
SS-A, N (%)	1/60 (1,66)	0/60 (0)
Ro-52, N (%)	5/60 (8,33)	6/60 (10)
SS-B, N (%)	1/60 (1,66)	2/60 (3,33)
Scl-70, N (%)	0/60 (0)	1/60 (1,66)
Jo-1, N (%)	0/60 (0)	0/60 (0)
ANCA, N (%)	18/60 (30)	18/60 (30)
ANA, N (%)	35/60 (58,33)	43/60 (71,66)

Bakasis A, Bitzogli K, et al, unpublished

Severe COVID-19 patients: The prevalence of autoantibodies to soluble proteins increases with time

Antibody type	Initial evaluation	15-30 days later
SARS-CoV-2 ABs IgG, N(%)	50/60 (83,33)	60/60 (100)
SARS-CoV-2 RBD Neutralizing ABs, N(%)	54/60 (90)	59/60 (98,33)
a-CCP, N (%)	5/60 (8,33)	5/60 (8,33)
a-TPO, N(%)	17/60 (28,33)	20/60 (33,33)
a-TG, N (%)	3/60 (5)	6/60 (10)
a-β2GPI IgG, N (%)	13/60 (21,66)	17/60 (28,33)
a-β2GPI IgM, N (%)	6/60 (10)	8/60 (13,33)
a-CL IgG, N (%)	26/60 (43,33)	38/60 (63,33)
a-CL IgM, N (%)	17/60 (28,33)	19/60 (31,66)
a-dsDNA IgG, N (%)	7/60 (11,66)	11/60 (18,33)

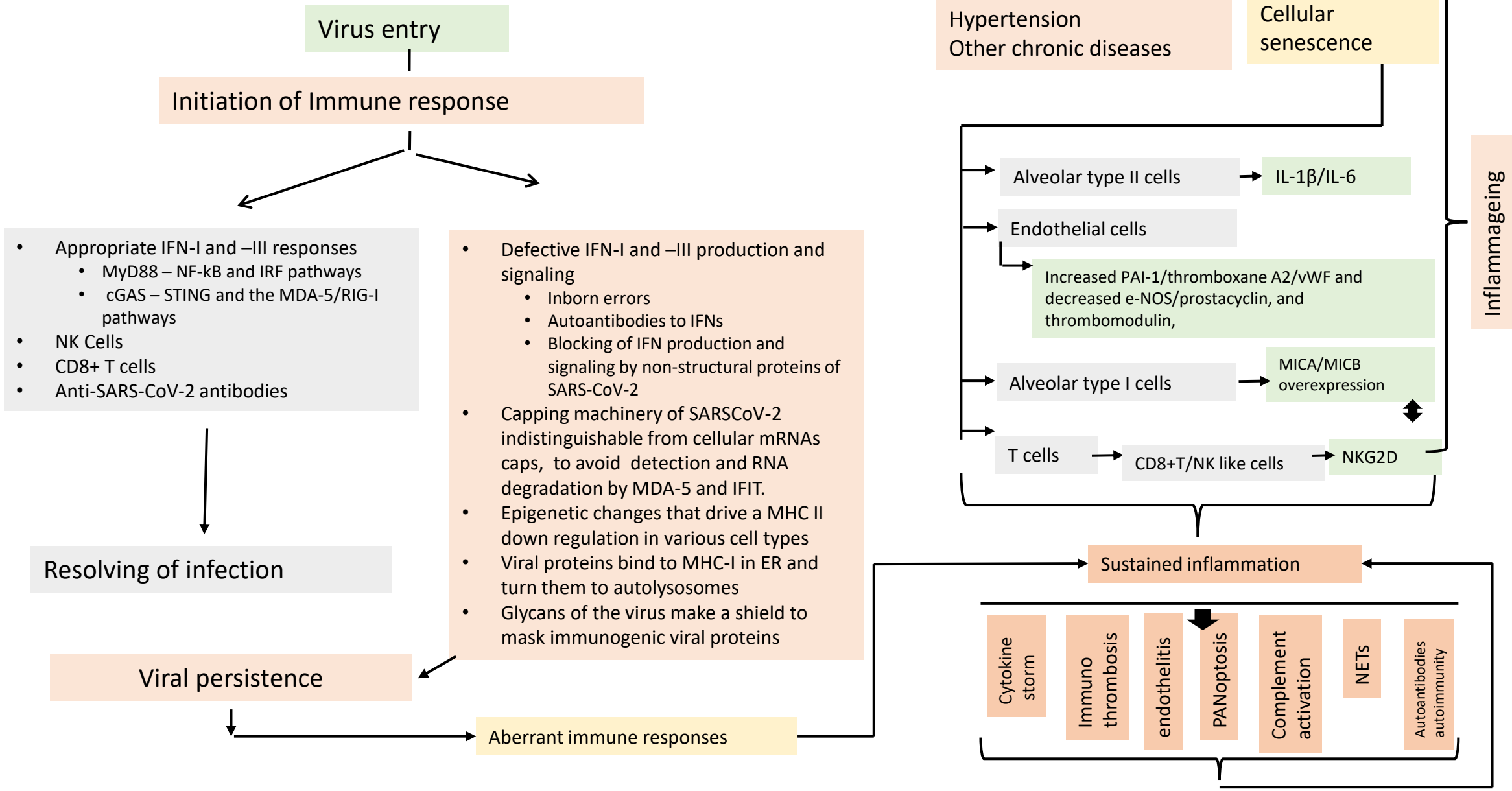
Bakasis A, Bitzogli K, et al, unpublished

COVID-19: the prevalence of autoantibodies relative to myositis remains stable or increases with time

Antibody type	Initial evaluation	15-30 days later
Mi-2α, N (%)	1/60 (1,66)	4/60 (6,66)
Mi-2β, N (%)	4/60 (6,66)	8/60 (13,33)
TIF1γ, N (%)	3/60 (5)	7/60 (11,66)
MDA5, N (%)	3/60 (5)	2/60 (3,33)
NXP2, N (%)	3/60 (5)	3/60 (5)
SAE1, N (%)	3/60 (5)	3/60 (5)
Ku, N (%)	8/60 (13,33)	10/60 (16,66)
PM-Scl100, N (%)	2/60 (3,33)	4/60 (6,66)
PM-Scl75, N (%)	5/60 (8,33)	5/60 (8,33)
Jo-1, N (%)	0/60 (0)	0/60 (0)
SRP, N (%)	1/60 (1,66)	9/60 (15)
PL-7, N (%)	5/60 (8,33)	9/60 (15)
PL-12, N (%)	3/60 (5)	9/60 (15)
EJ, N (%)	0/60 (0)	1/60 (1,66)
OJ, N (%)	4/60 (6,66)	7/60 (11,66)
Ro-52, N (%)	7/60 (11,66)	2/60 (3,33)

Bakasis A, Bitzogli K, et al, unpublished

Conclusions



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