



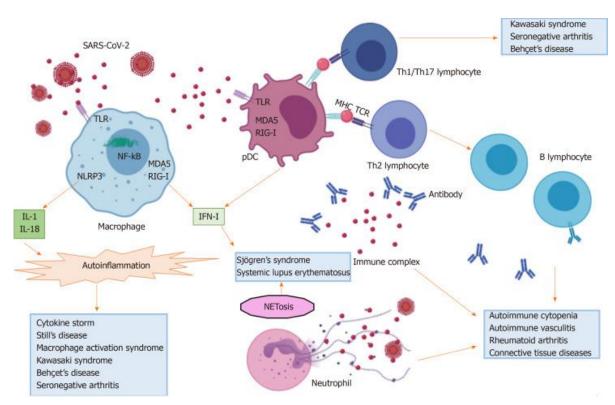
# Autoantibody signatures in SARS-CoV-2 infected and vaccinated individuals

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## Coronavirus disease 2019 (COVID-19)





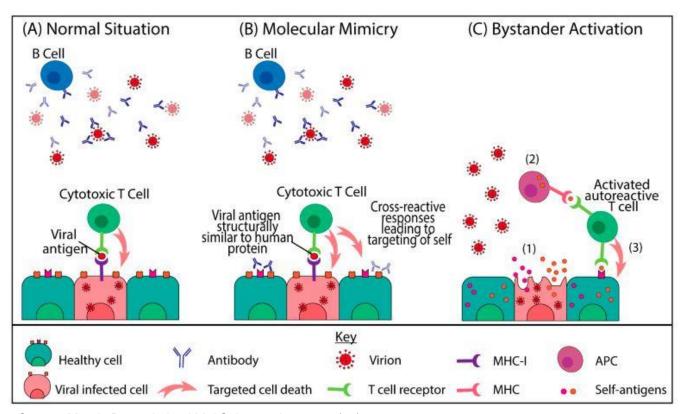
Source: Talotta R. et al., World J Clin Cases, 2020 Sep 6;8(17):3621-3644

- Respiratory illness caused by SARS-CoV-2
- Course of disease varies in symptoms and severity
- Immune system presents as double-edged sword

  Viral clearance or excessive inflammation and autoimmunity
- Several immune-mediated disorders have been described in SARS-CoV-2-infected individuals
- Presence of autoantibodies in COVID-19 patients

#### Mechanisms for viral-induced autoimmunity





Source: Moody R. et al., Int J Mol Sci, 2021 Aug 20;22(16)

#### Molecular mimicry:

Immune responses towards epitopes that are shared between virus and human proteins

#### Bystander activation

Autoreactive immune cell activation due to the release of self antigens

#### Autoantibodies associated with COVID-19



#### A list of autoimmune diseases and autoantibodies associated with COVID-19 infection.

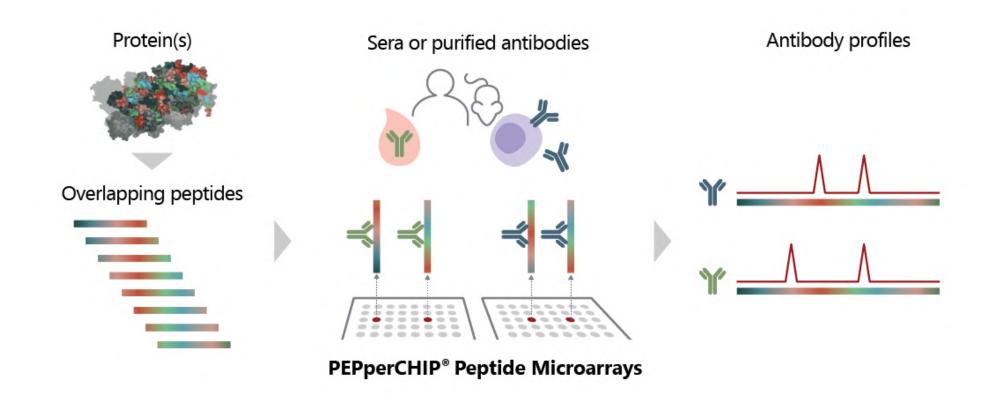
| Autoimmune disease/syndromes<br>secondary to COVID-19 infection | Circulating autoantibodies reported in COVID-19 patients |
|---|--|
| Guillain-Barré syndrome   | Anti-nuclear antibodies (ANA)                            |
| Miler Fisher Syndrome (MFS)                                     | Anti-cardiolipin (aCL) antibodies                        |
| Antiphospholipid syndrome                                       | Anti-β2 glycoprotein 1 (aβ2GP1)<br>antibodies            |
| Immune thrombocytopaenic purpura                                | Anti-MDA5 antibodies                                     |
| systemic lupus erythematosus (SLE)                              | Anti RBC antibodies (direct anti<br>globulin)            |
| Kawasaki disease  | LAC –lupus anticoagulant                                 |
| Cold agglutinin disease & autoimmune<br>hemolytic anemia        | Antiprothrombin IgM                                      |
| Neuromyelitis optica  | Antiphosphatidylserine IgM/IgG                           |
| NMDA-receptor encephalitis                                      | Antiannexin V IgM/IgG                                    |
| Myasthenia gravis   | Anti-GD1b antibodies                                     |
| Type I diabetes   | Anti-heparin PF4 complex antibody                        |
| Large vessel vasculitis & thrombosis                            | pANGA AND cANGA  |
| Psoriasis   | Anti-CCP antibodies                                      |
| Subacute thyroiditis  |  |
| Graves' disease   |  |
| Sarcoidosis   |  |
| Inflammatory arthritis  |  |
|   |  |

Source: Halpert G. and Shoenfeld Y., Autoimmun Rev., 2020

- Various autoantibodies were found in COVID-19 patients
- (Possible) association with life-threatening SARS-CoV-2 infection / poor prognosis for some of the autoantibodies
- For others, clinical significance is unclear so far
- Potentially there are more autoantibody reactivities existing which are unknown so far

#### Screening for epitope-specific antibody responses

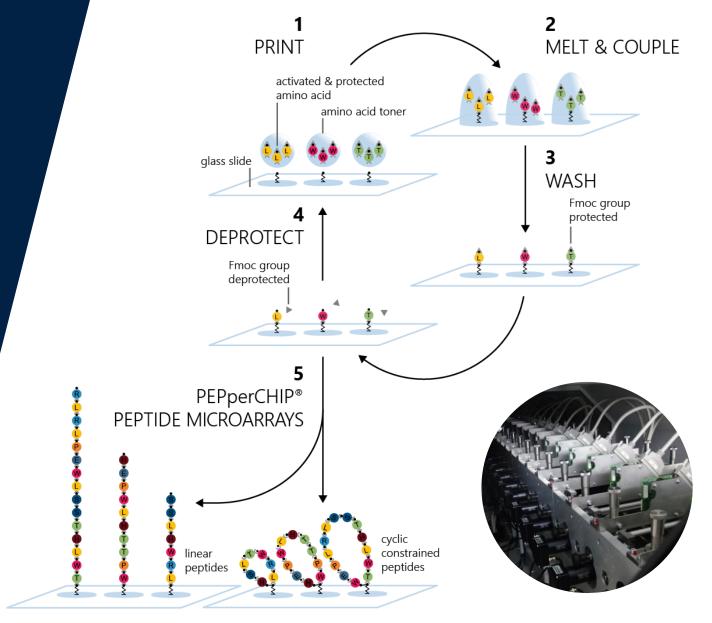




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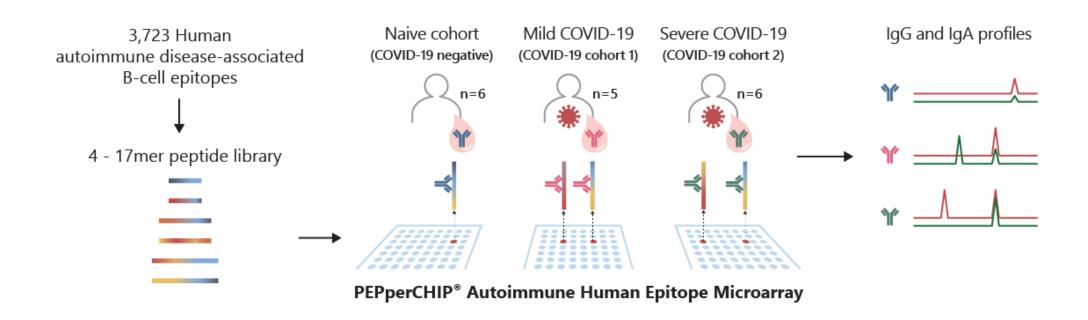




### Study outline



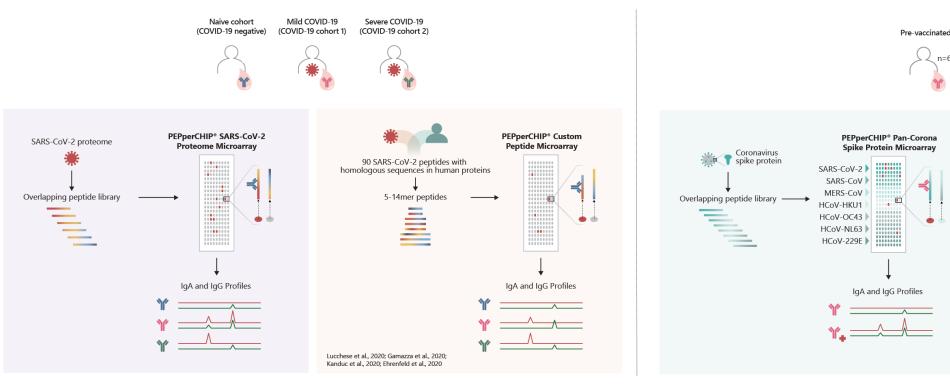
Study 1: Screening of COVID-19 patient sera with different disease outcomes against human autoimmune disease-associated linear epitopes

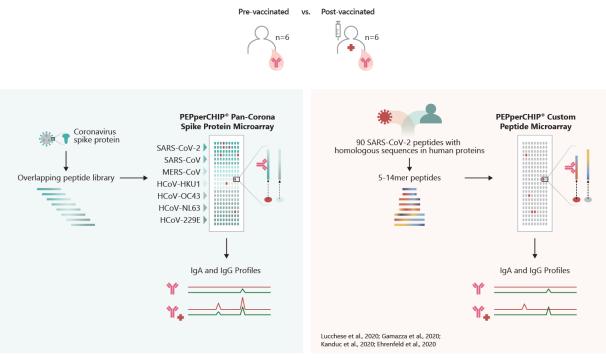


### Study outline



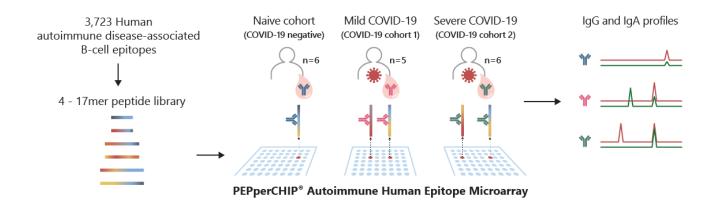
Study 2: Investigating antibody responses against epitopes shared between SARS-CoV-2 & human proteins in infected subjects **and** pre- and post-COVID-19 vaccination



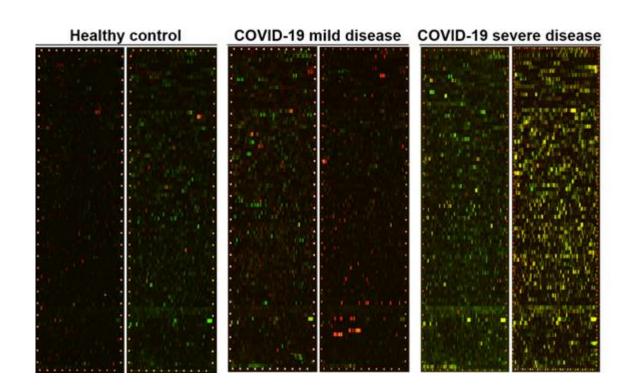


## Autoantibody signatures in COVID-19 patients



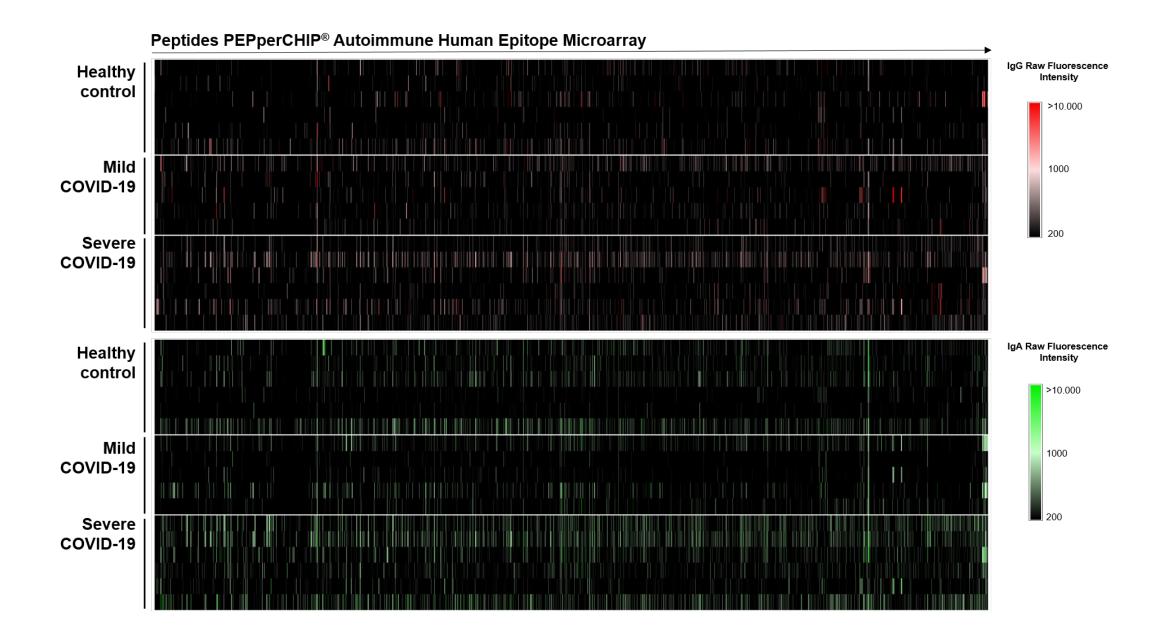


Epitope-resolved view of autoantibody responses



## Autoantibody signatures in COVID-19 patients





#### Epitope signatures in severe COVID-19



#### **Antigen**

U1 small nuclear ribonucleoprotein 70 kDa

Spectrin alpha chain, non-erythrocytic 1

E3 ubiquitin-protein ligase TRIM21

Sulfite oxidase, mitochondrial

Lupus La protein
Thyroid peroxidase (UniProt:P07202)
Glutamate receptor ionotropic, NMDA 2A
U1 small nuclear ribonucleoprotein 70 kDa
Interferon alpha-2
T-cell receptor
DNA topoisomerase 1
Heat shock protein HSP 90-beta
Tubulin beta-4A chain (UniProt:P04350)
Myelin-oligodendrocyte glycoprotein (UniProt:Q16653)
DNA topoisomerase 1
Other Homo sapiens (human) protein
Thyroid peroxidase (UniProt:P07202)
Exosome component 10

Thyroid peroxidase (UniProt:P07202) 60S acidic ribosomal protein P0 (UniProt:P05388) U1 small nuclear ribonucleoprotein 70 kDa Small nuclear ribonucleoprotein Sm D1 U1 small nuclear ribonucleoprotein 70 kDa Beta-2 adrenergic receptor Immunoglobulin DNA-directed RNA polymerase III subunit RPC1 E3 ubiquitin-protein ligase TRIM21 Secretory phospholipase A2 receptor Exosome component 10 Early growth response protein 1 60S acidic ribosomal protein P2 (UniProt:P05387) DNA topoisomerase 1 Other Homo sapiens (human) protein Glutamate receptor ionotropic, NMDA 2A Myeloperoxidase (UniProt:P05164) Myeloperoxidase (UniProt:P05164) Pyruvate kinase PKM Transmembrane and coiled-coil domain-containing prot 4 Fibrinogen beta chain Thyrotropin receptor 60S acidic ribosomal protein P2 (UniProt:P05387) Thyrotropin receptor DNA topoisomerase 1 Myelin-oligodendrocyte glycoprotein (UniProt:Q16653) DNA topoisomerase 1 Tumor necrosis factor ligand superfamily member 6 Myeloperoxidase (UniProt:P05164) Thyroid peroxidase (UniProt:P07202) Major centromere autoantigen B Myelin basic protein (UniProt:P02686)

#### **Peptide**

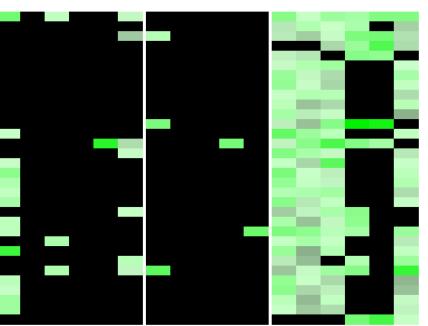
HRSERERRRDRDRDRDR FASDDEHDEHDENGATG QRGRDHGLPGYNEWREF SVSYDDWDYSLEARV RRSHRSERERRRDRDRD RHDFGFPQEEFGNQ QGPQFIFQYYEEEER KEEEQKWKWWEEERY FRALLFIPRRAPFDLFE YQQYQDATAEEGEFE SYQEEAAMELKVEDPFY KVMKDAKTKKVVESKKK PEQNCNDDQQPF INLQRGRDHGLPGYNEW LNCQEFAVDLEHHSY QQFVANVEEEEAWIN LELLQEVIIVLERSESW VSVQPEESYSHWQRRDY

QRGRDHGLPGYNEWREF SDEDMGFGLFD HRSERERRRDRDRDRDR RGRGRGGPRR RRSHRSERERRRDRDRD HQEAINCYANETCCDFF **EDLAVYF** SFEKTADHLFDAAYF MMWEEVTCPICLDPF **AESGCQEGWERHGGF GDEYDFYRSFPGFQA** GGGGGGSN DMGFGLFD KVMKDAKTKKVVESKKK **FPELTLHFEFPE** SVSYDDWDYSLEARV RWLPAEYEDGFSLPY RAFVRWLPAEYEDGF AYMEKCDENILWLDY RGDW GGGYZARPAKAAATQKK PQEETLQAFDSHYDYT DDDMGFGLFD EEQEDEIIGFGQELKN PPYEPLPENVKFYYD RAEIENLHRTFDPHF ELDGQEYVVEFDFLGKD EWEDTYGIVL WDGER INLQRGRDHGLPGYNEW QDVFSATETSLWYDF **TMDHARHGFLPRRHR** 

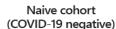
# Healthy COVID-19 COVID-19 VSN normalized IgG intensity 10 VSN normalized IgA intensity VSN normalized IgA intensity

Severe

Mild







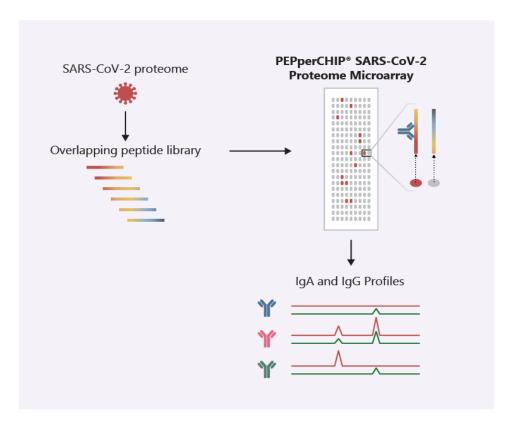
Mild COVID-19 (COVID-19 cohort 1)

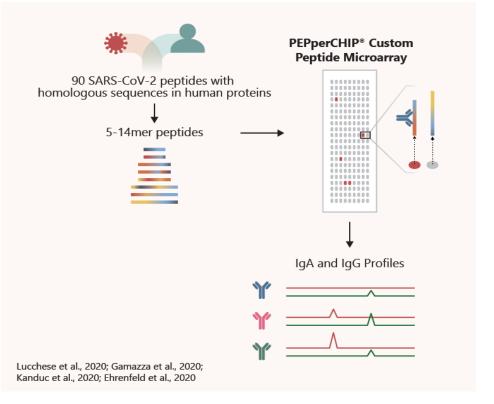
Severe COVID-19 (COVID-19 cohort 2)





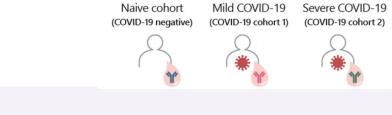


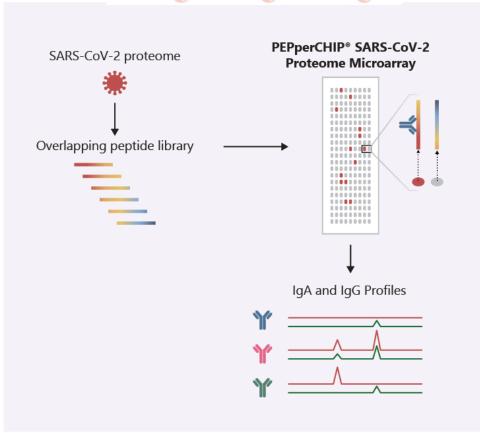


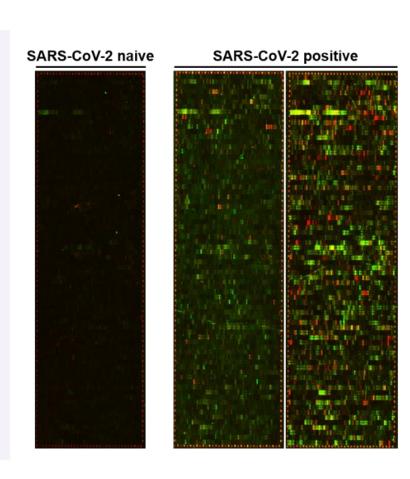


### Proteome-wide anti-SARS-CoV-2 antibody responses





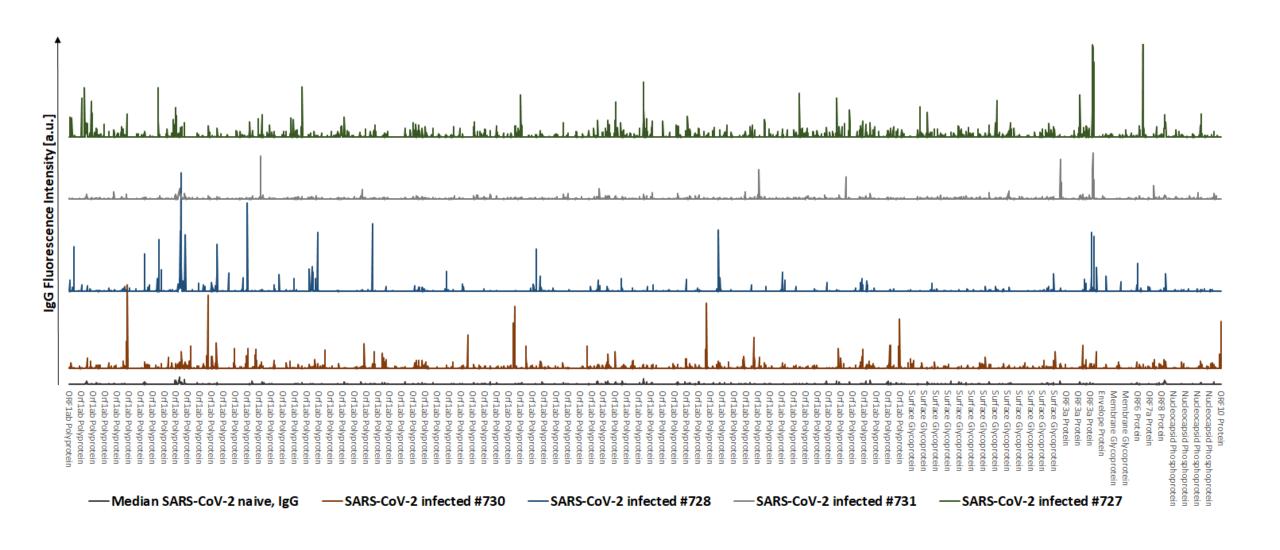




## Proteome-wide anti-SARS-CoV-2 antibody responses



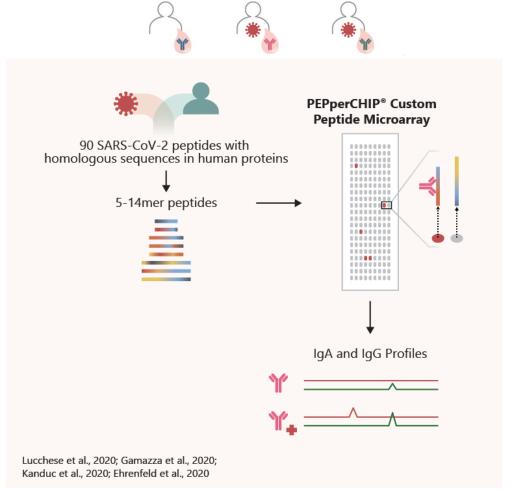
IgG responses



Naive cohort

(COVID-19 negative)



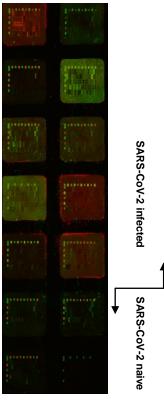


Mild COVID-19

(COVID-19 cohort 1)

Severe COVID-19

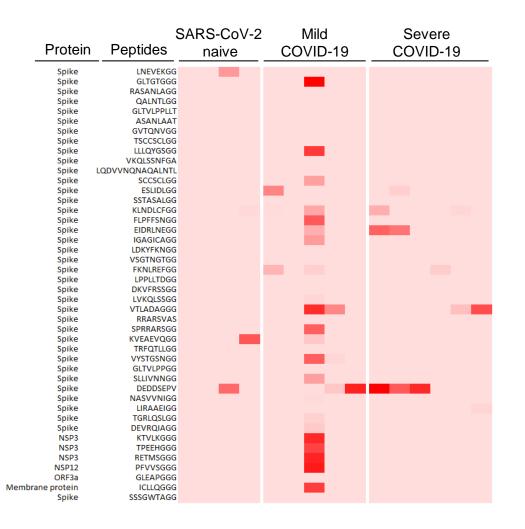
(COVID-19 cohort 2)





IgG reactivity to epitopes shared between SARS-CoV-2 & human proteins in SARS-CoV-2-infected individuals

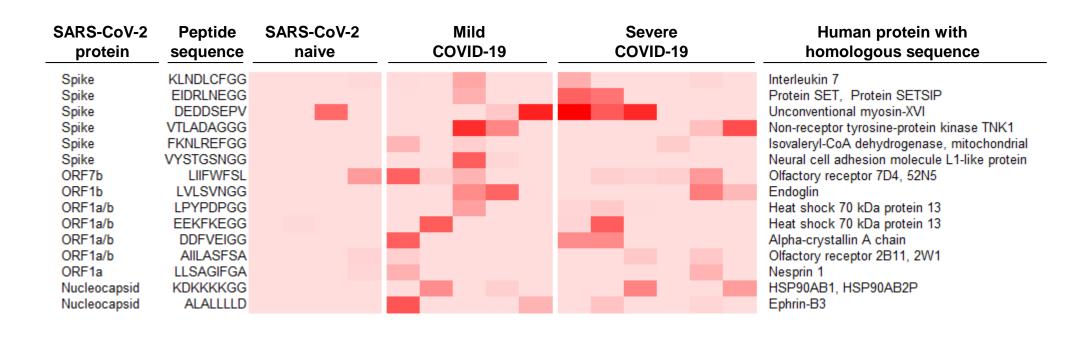




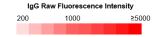
| Protein          | S<br>Peptides        | ARS-CoV-2 | Mild<br>COVID-19 | Severe<br>COVID-19 |
|------------------|----------------------|-----------|------------------|--------------------|
| - ODES!          | <del></del>          |           |                  |                    |
| ORF9b            | LGSPLSLGG            |           |                  |                    |
| ORF7b            | AFLLFLVLIMLIIF       |           |                  |                    |
| ORF7b            | LIIFWFSL             |           |                  |                    |
| ORF1b            | AVLQSGFRK            |           | _                |                    |
| ORF1b            | LQLGFSTG             |           |                  | _                  |
| ORF1b<br>ORF1b   | LVLSVNGG<br>MCGGSLGG |           |                  |                    |
| ORF1b<br>ORF1a/b | EIPKEEGG             |           |                  |                    |
| ORF1a/b          | TILGSAGG             |           | _                |                    |
| ORF1a/b          | LPYPDPGG             |           |                  |                    |
| ORF1a/b          | GTVYEKGG             |           |                  |                    |
| ORF1a/b          | EEKFKEGG             |           | _                |                    |
| ORF1a/b          | LLAPLLGG             |           | _                |                    |
| ORF1a/b          | VLSDREGG             |           |                  |                    |
| ORF1a/b          | DFSRVSGG             |           |                  |                    |
| ORF1a/b          | DDFVEIGG             |           | _                |                    |
| ORF1a/b          | LKELLQNGG            |           | _                |                    |
| ORF1a/b          | LVAELEGGG            |           | _                |                    |
| ORF1a/b          | AIILASFSA            |           | _                |                    |
| ORF1a/b          | ALLADKGG             |           |                  |                    |
| ORF1a/b          | PGSGVPVV             |           |                  |                    |
| ORF1a/b          | ESGLKTIL             |           |                  |                    |
| ORF1a            | LRANSAGG             |           |                  |                    |
| ORF1a            | ATVVIGGG             |           | _                |                    |
| ORF1a            | LLSAGIFGA            |           | _                |                    |
| ORF1a            | QQLIRAAEI            |           |                  |                    |
| Nucleocapsid     | GSQASSGG             |           |                  |                    |
| Nucleocapsid     | SAAEASGG             |           |                  |                    |
| Nucleocapsid     | KDKKKKGG             |           |                  |                    |
| Nucleocapsid     | AEGSRGGSQA           |           | _                |                    |
| Nucleocapsid     | ALALLLLD             |           |                  |                    |
| Nucleocapsid     | ALNTPKGG             |           |                  |                    |
| Nucleocapsid     | APSASAFF             |           |                  |                    |
| Nucleocapsid     | AQFAPSASA            |           |                  |                    |
| Nucleocapsid     | ATEGALNTPK           |           |                  |                    |
| Nucleocapsid     | LLLLDRLN             |           |                  |                    |
| Nucleocapsid     | LQLPQGGG             |           |                  |                    |
| Nucleocapsid     | GMEVTPSGTWL          |           | _                |                    |
| Nucleocapsid     | QASSRSSSR            |           |                  |                    |
| Nucleocapsid     | FPPTEPGG             |           |                  |                    |
| Membrane protein | TSRTLSYYK            |           |                  |                    |
| Membrane protein | LWPVTLAGG            |           |                  |                    |
| Membrane protein | SFNPETGG             |           |                  |                    |
| Membrane protein | LVIGFLFL             |           |                  |                    |

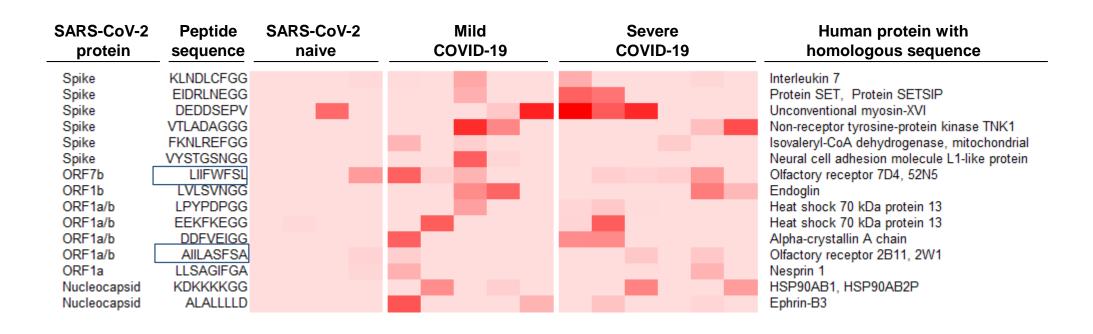






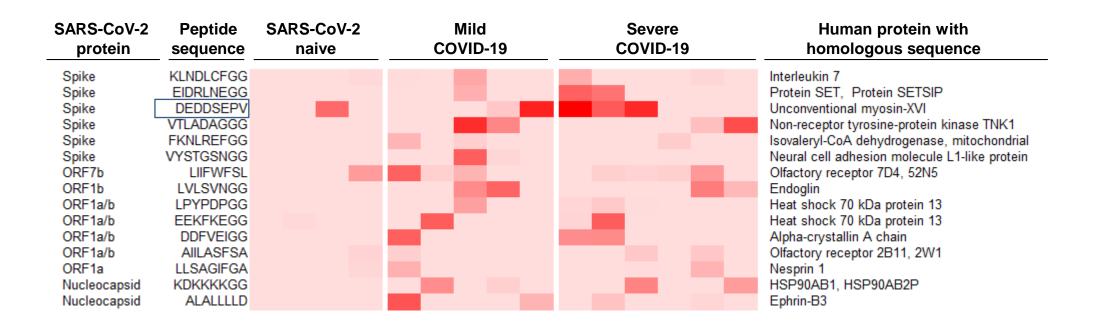












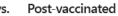
#### **DEDDSEPV**:

- conserved B cell epitope in SARS-CoV and SARS-CoV-2 Spikes
- also found in a T cell epitope of SARS-CoV-2 Spike
- bind to various HLA's including those associated with autoimmune disease (Fath MK *et al.*, 2021)

#### Vaccination-induced antibody responses

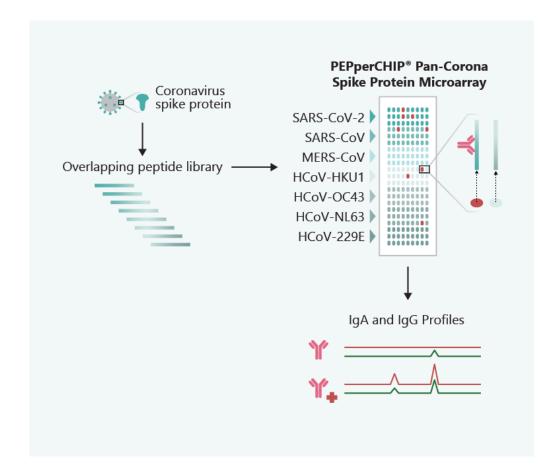


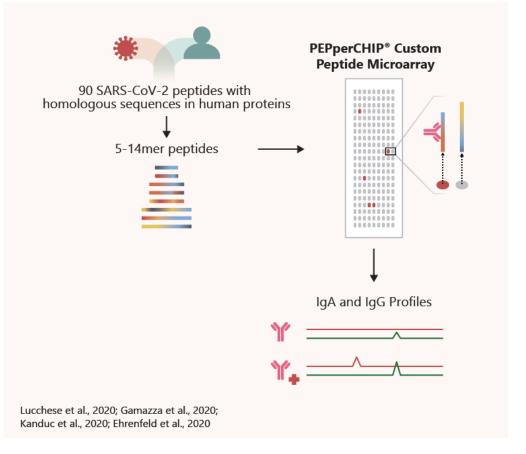






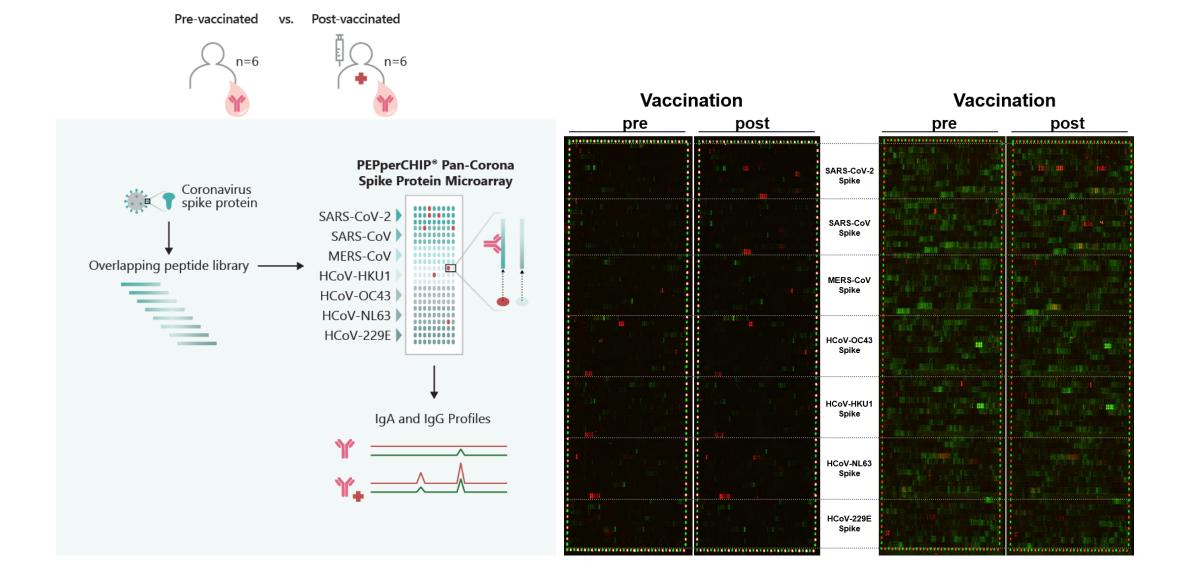






#### Vaccination-induced antibody responses

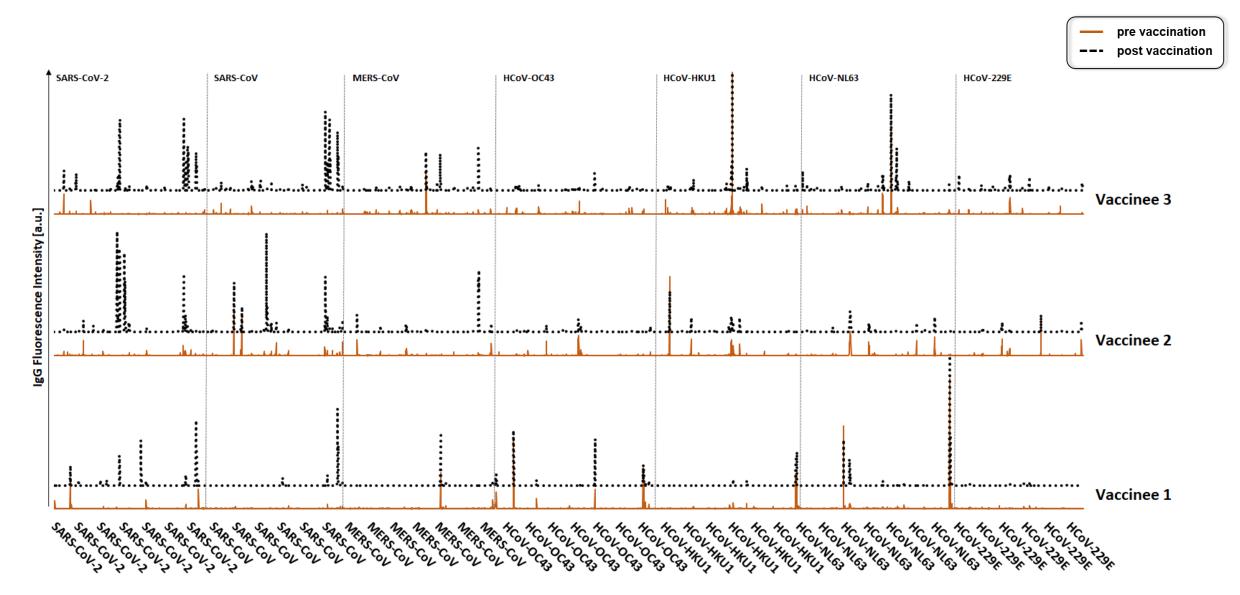




## Vaccination-induced antibody responses

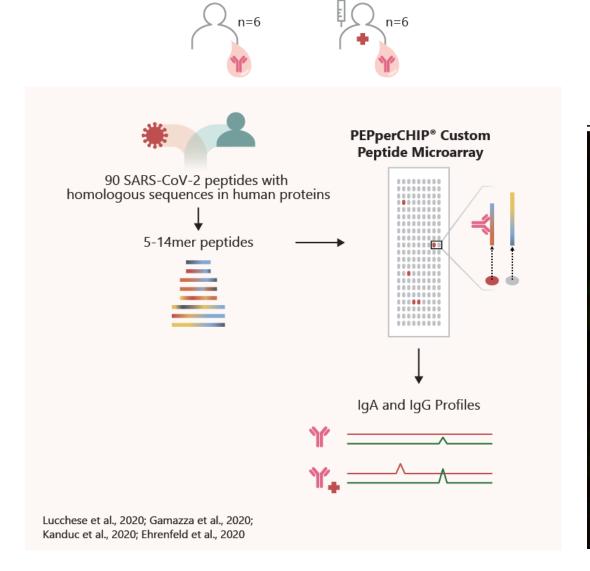


IgG responses



Pre-vaccinated





Post-vaccinated

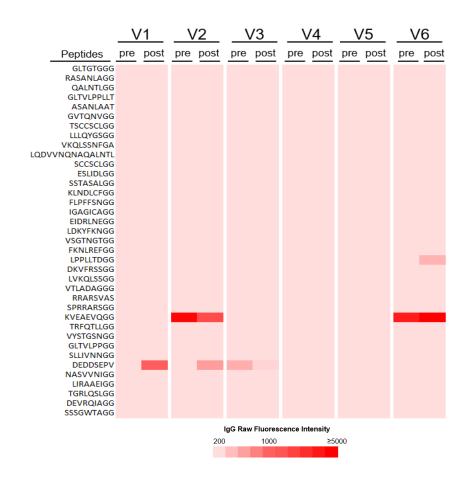
#### Vaccination pre post

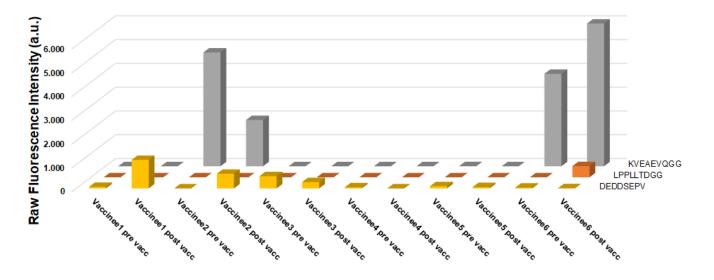
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IgG reactivity to epitopes shared between SARS-CoV-2 Spike & human proteins in COVID-19-vaccinated individuals

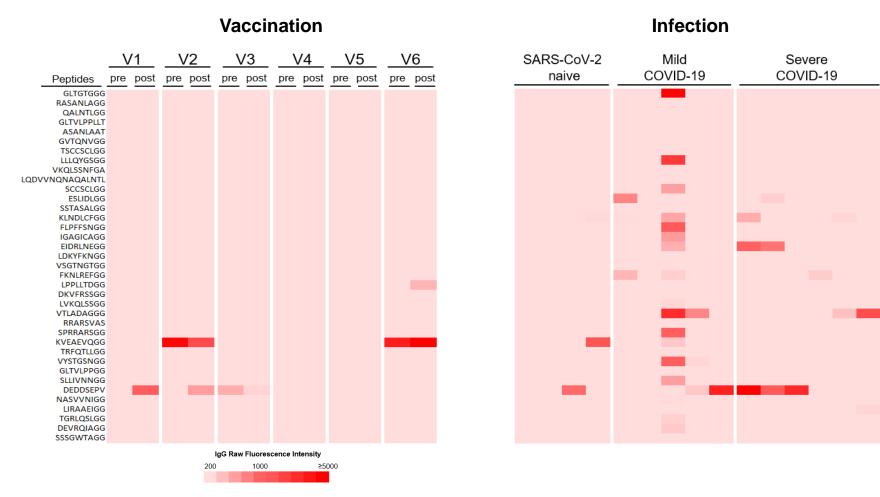




| SARS-CoV-2 Antigen | Human protein with homologous sequence                      | Peptide sequence |
|--------------------|---|------------------|
| Spike              | Maestro heat-like repeat-containing protein family member 9 | LPPLLTD          |
| Spike              | EMILIN-3  | KVEAEVQ          |
| Spike              | Unconventional myosin-XVI                                   | DEDDSEPV         |



IgG reactivity to epitopes shared between SARS-CoV-2 Spike & human proteins



More frequent cross-reactivity in infected than in vaccinated subjects

#### Conclusions



#### Epitope-specific autoantibody screening in COVID-19 patients with different disease outcomes revealed:

- Heterogeneous epitope reactivity patterns between study groups with far stronger autoantibody responses in severely ill subjects
- Severe COVID-19: numerous IgG and/or IgA-specific autoantibody reactivities
- Several epitopes can be assigned to nuclear autoantigens, others may be summarized as tissue-associated autoantigens
- Studies with larger cohorts are needed to confirm identified epitopes (and clinical relevance)

#### Antibody responses recognizing linear epitopes shared between SARS-CoV-2 & human proteins revealed:

- SARS-CoV-2 infected subjects: few antibody responses to the selected 6-14mer peptides shared between SARS-CoV-2 and human proteins were detected
- No antibody responses against most of the selected 6-14mer peptides shared between SARS-CoV-2 Spike and human proteins detected after COVID-19 vaccination
- Infection versus vaccination: cross-reactive antibodies against peptides shared between SARS-CoV-2 Spike and human proteins were detected more frequently after infection
- Whether or not antibodies recognizing DEDDSEPV have a clinical relevance remains to be determined



## Thank you for joining the webinar

Contact

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