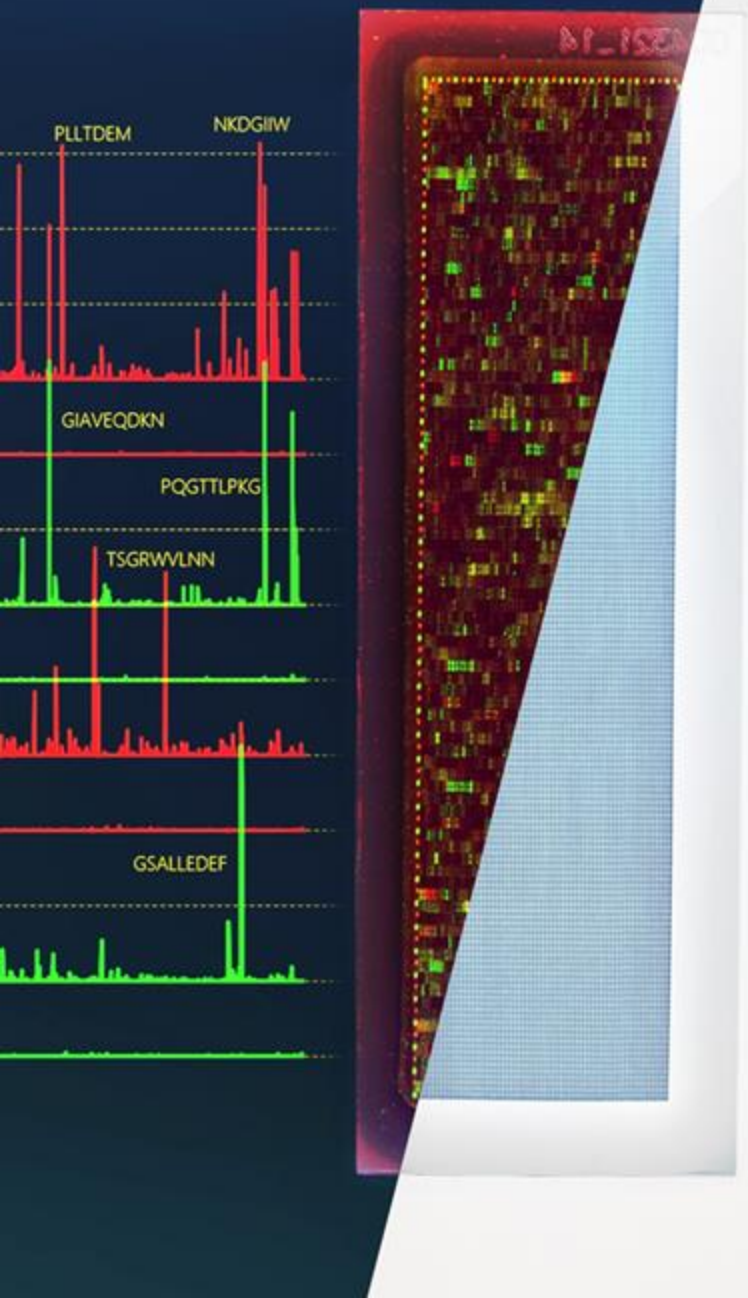

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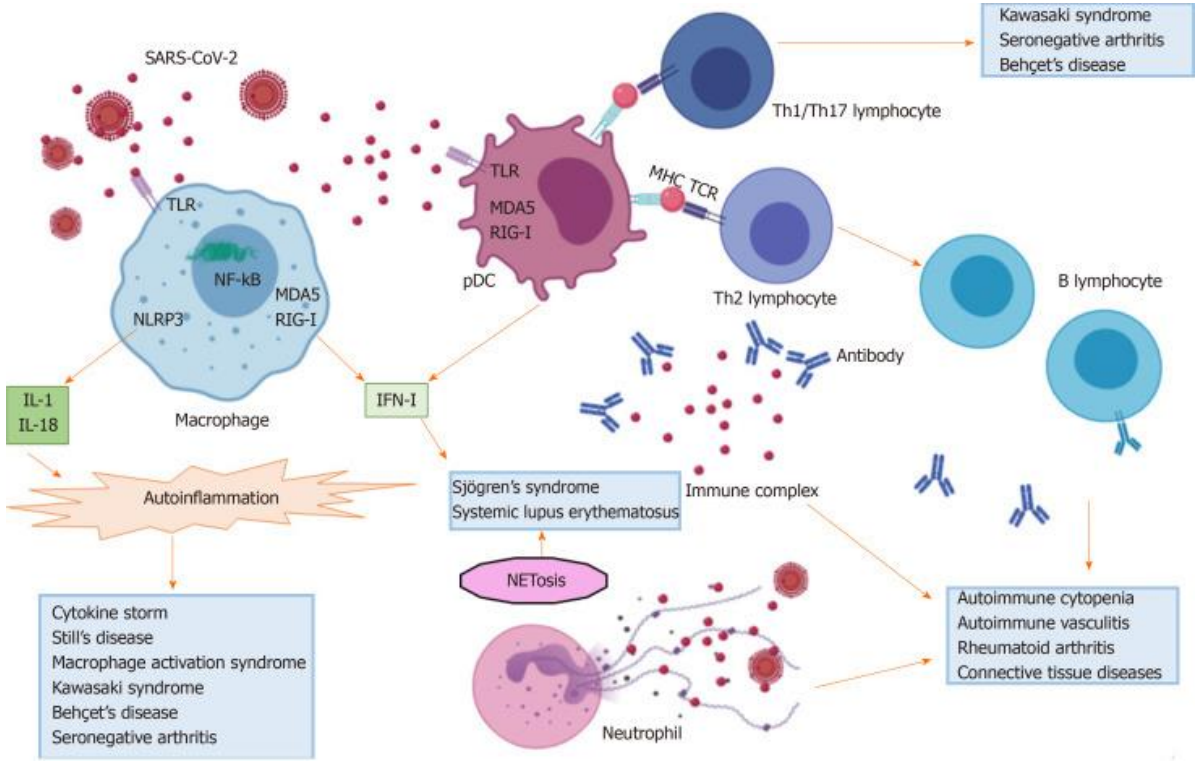
Autoantibody signatures in SARS-CoV-2 infected and vaccinated individuals

Dr. Kirsten Heiss
Research & Development
PEPperPRINT GmbH

November 2021

Coronavirus disease 2019 (COVID-19)



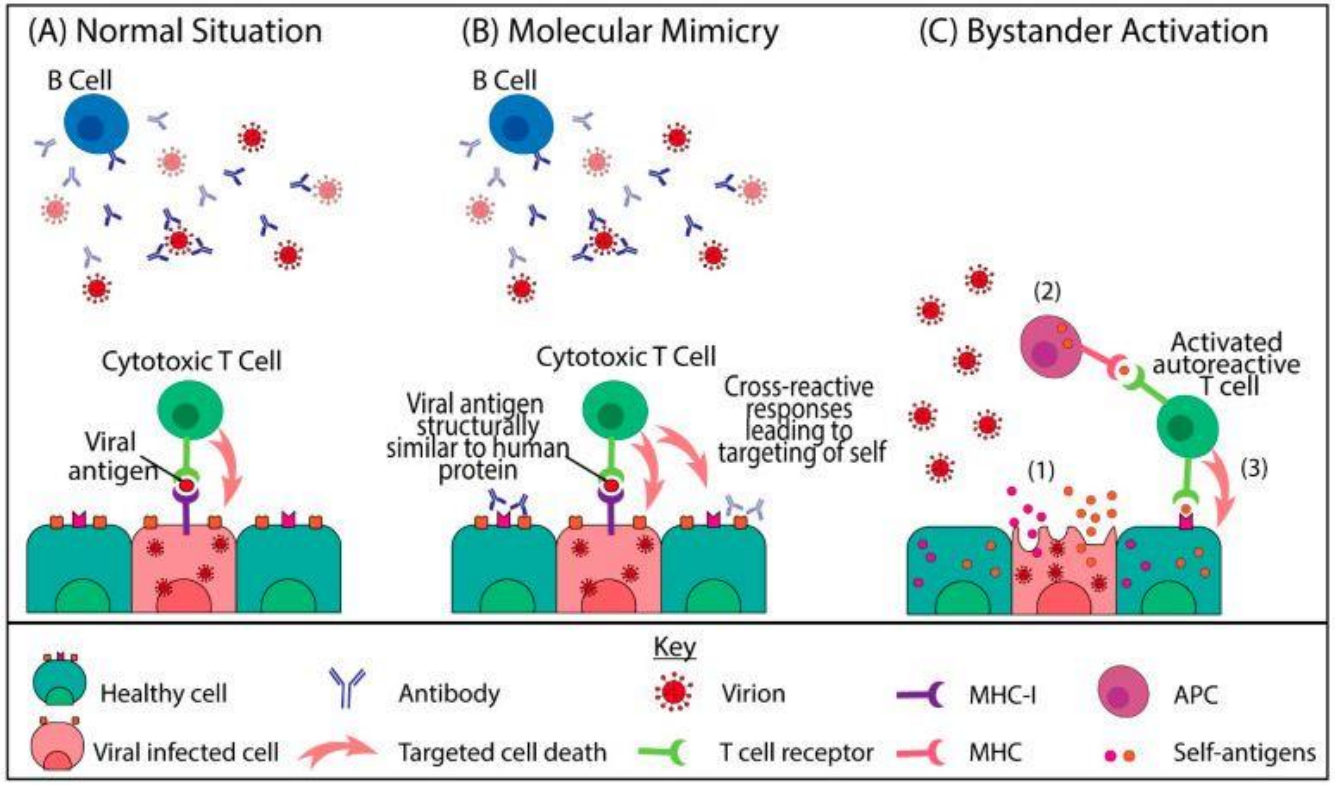
Source: Talotta R. *et al.*, *World J Clin Cases*, 2020 Sep 6;8(17):3621-3644

- 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

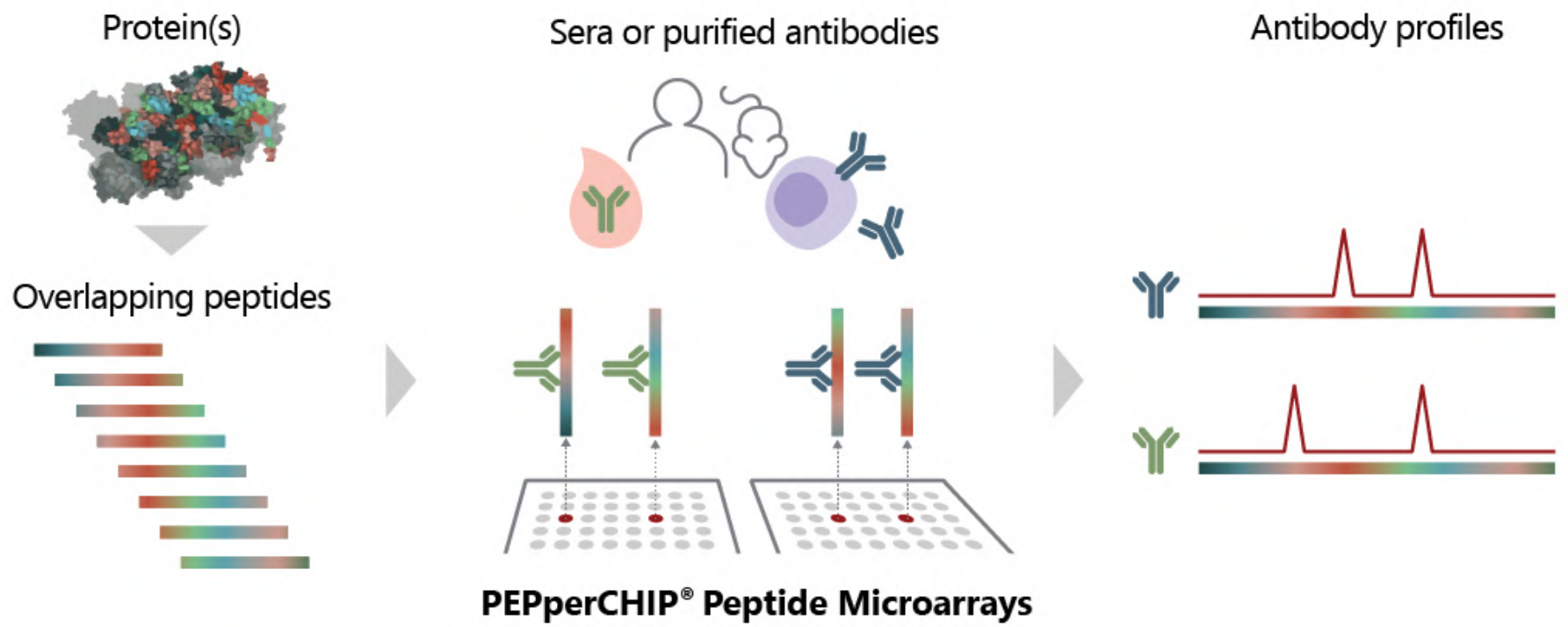
A list of autoimmune diseases and autoantibodies associated with COVID-19 infection.

Autoimmune disease/syndromes 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) antibodies
Immune thrombocytopaenic purpura	Anti-MDA5 antibodies
systemic lupus erythematosus (SLE)	Anti RBC antibodies (direct anti globulin)
Kawasaki disease	LAC –lupus anticoagulant
Cold agglutinin disease & autoimmune 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	pANCA AND cANCA
Psoriasis	Anti-CCP antibodies
Subacute thyroiditis	
Graves' disease	
Sarcoidosis	
Inflammatory arthritis	

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

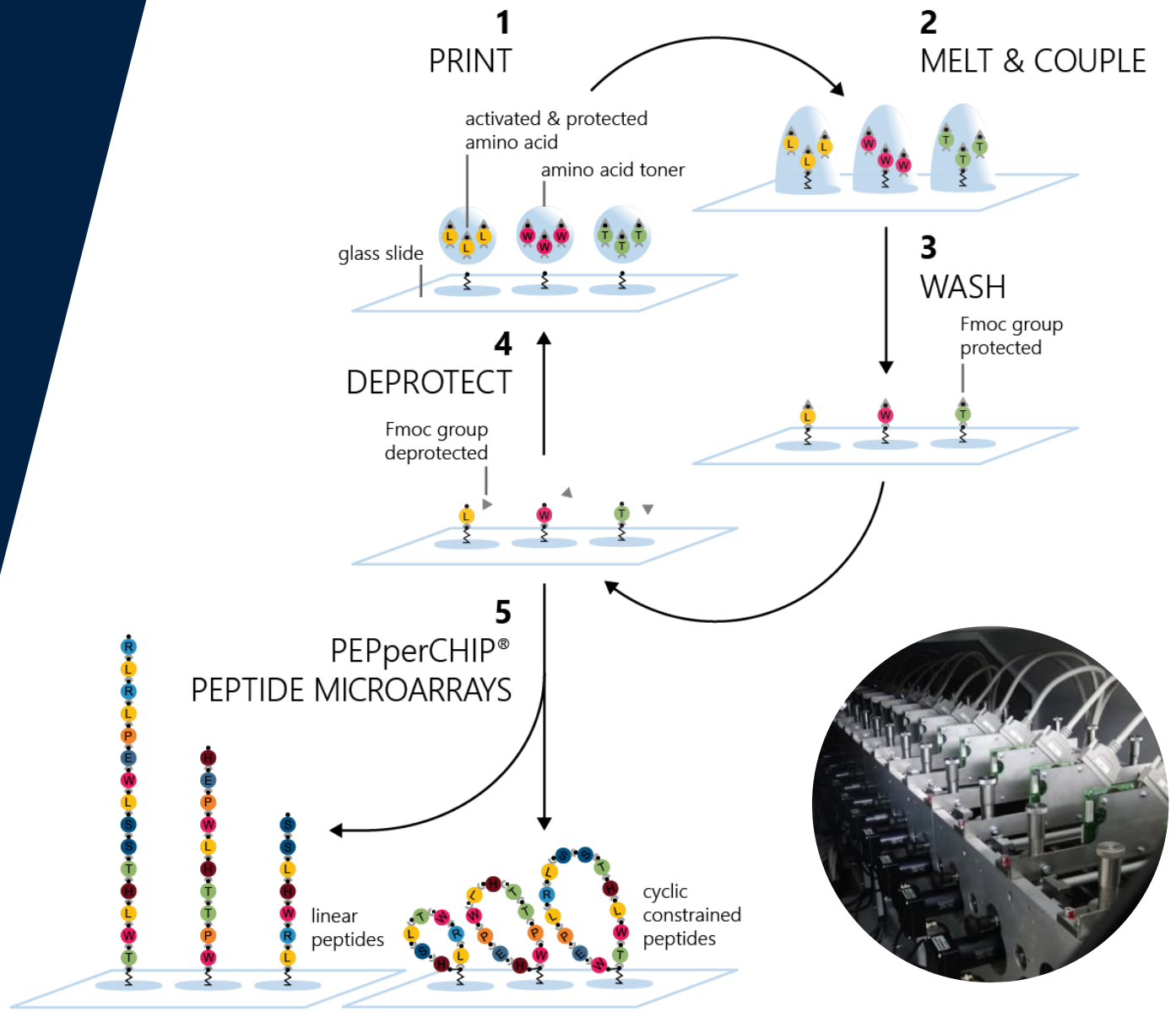
- Various autoantibodies were found in COVID-19 patients
- (Possible) association with life-threatening SARS-CoV-2 infection / poor prognosis for some of the autoantibodies
- For others, clinical significance is unclear so far
- Potentially there are more autoantibody reactivities existing which are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

Screening for epitope-specific antibody responses



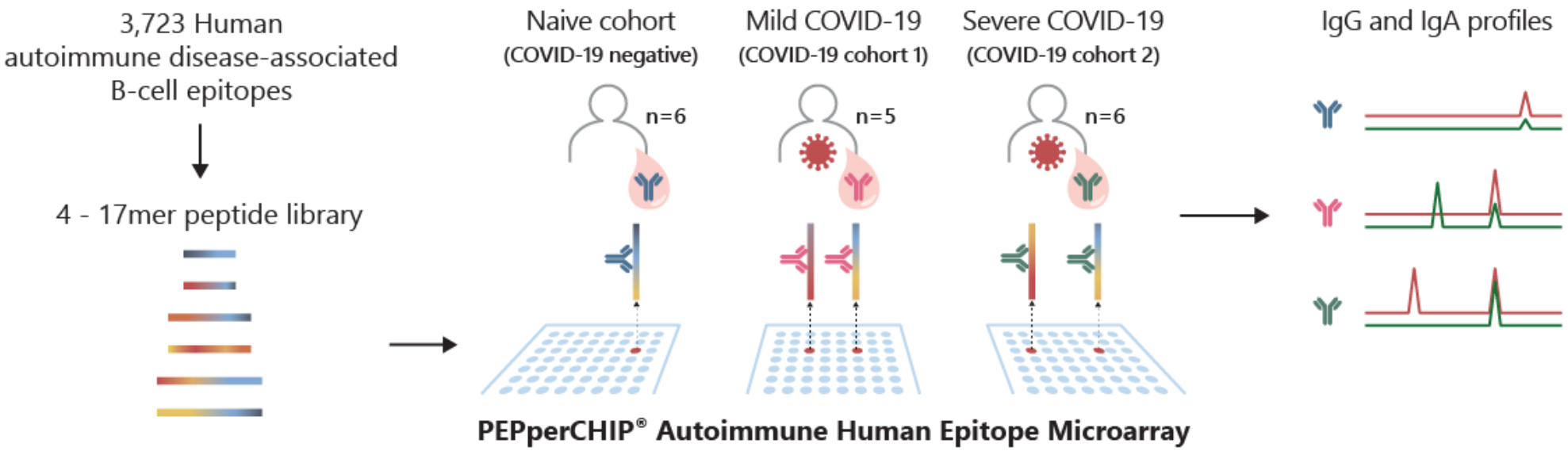
Our Platform Technology

- High spot density (1,200 peptides/cm^2)
- Digital printing flexibility: multiple formats with high scalability
- Fast production times
- High peptide quality by routine double coupling
- Very low material consumption



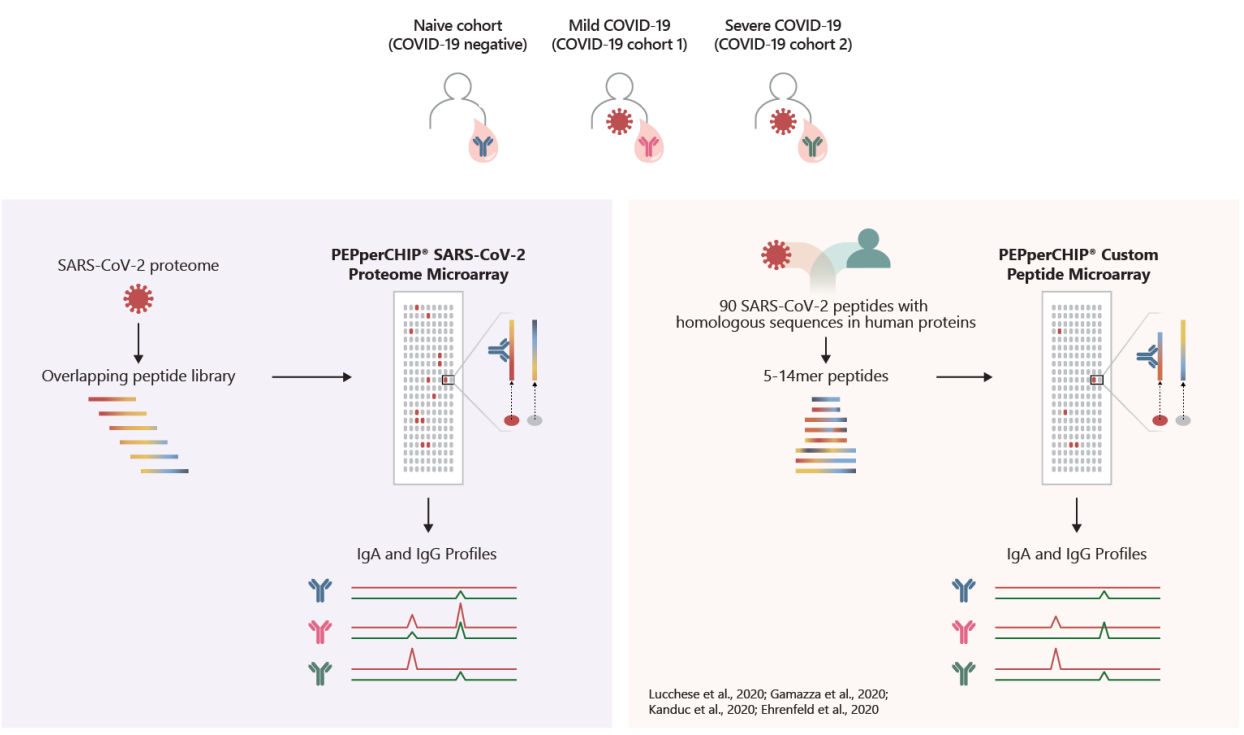
Study outline

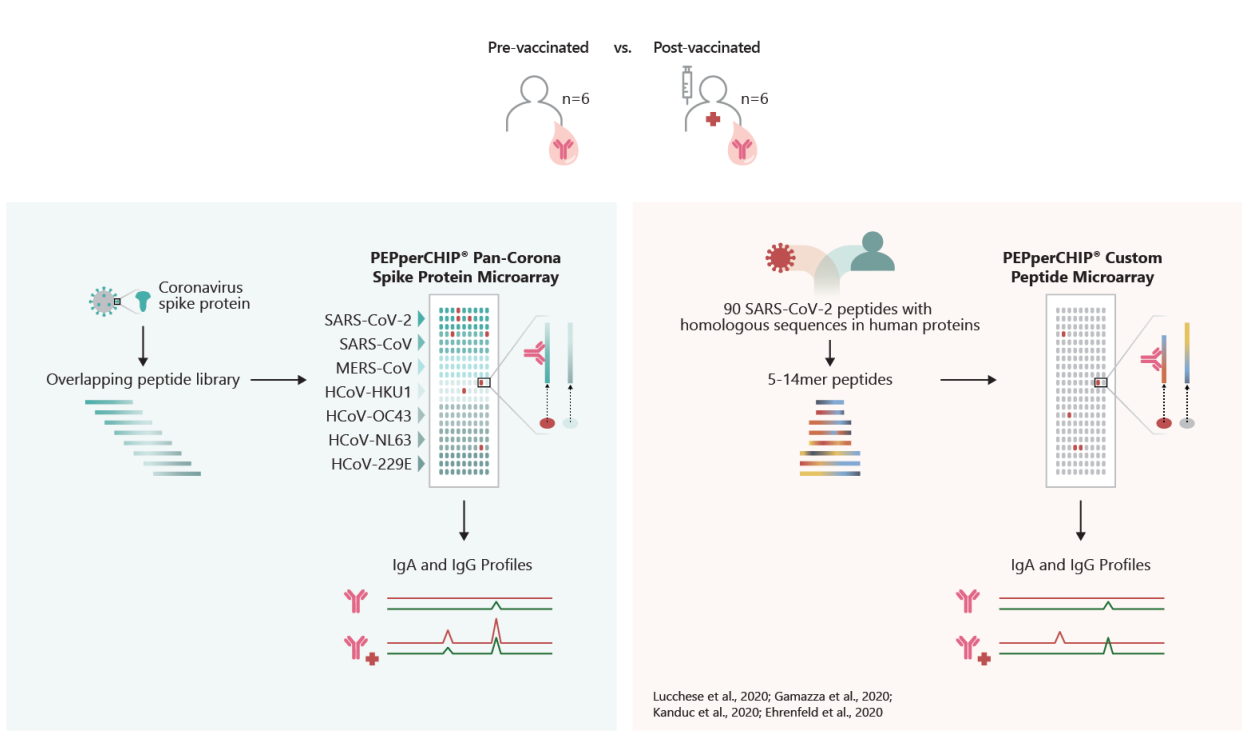
Study 1: Screening of COVID-19 patient sera with different disease outcomes against human autoimmune disease-associated linear epitopes



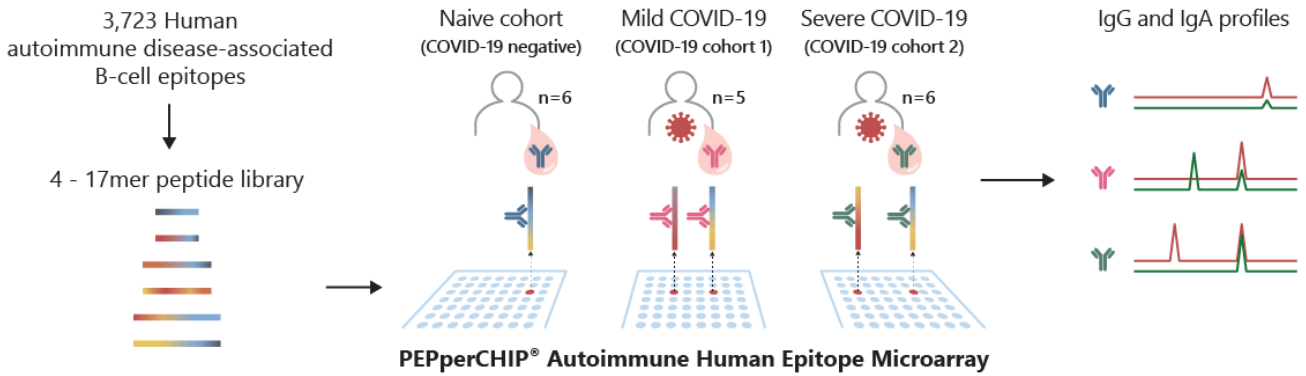
Study outline

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

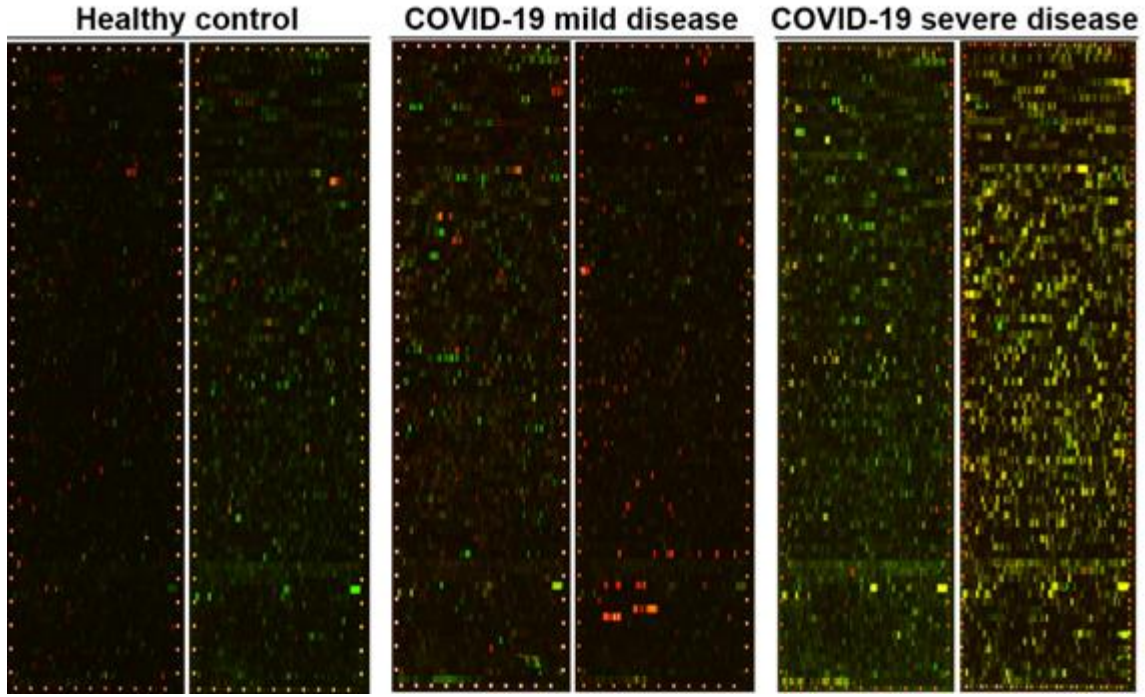




Autoantibody signatures in COVID-19 patients



Epitope-resolved view of autoantibody responses



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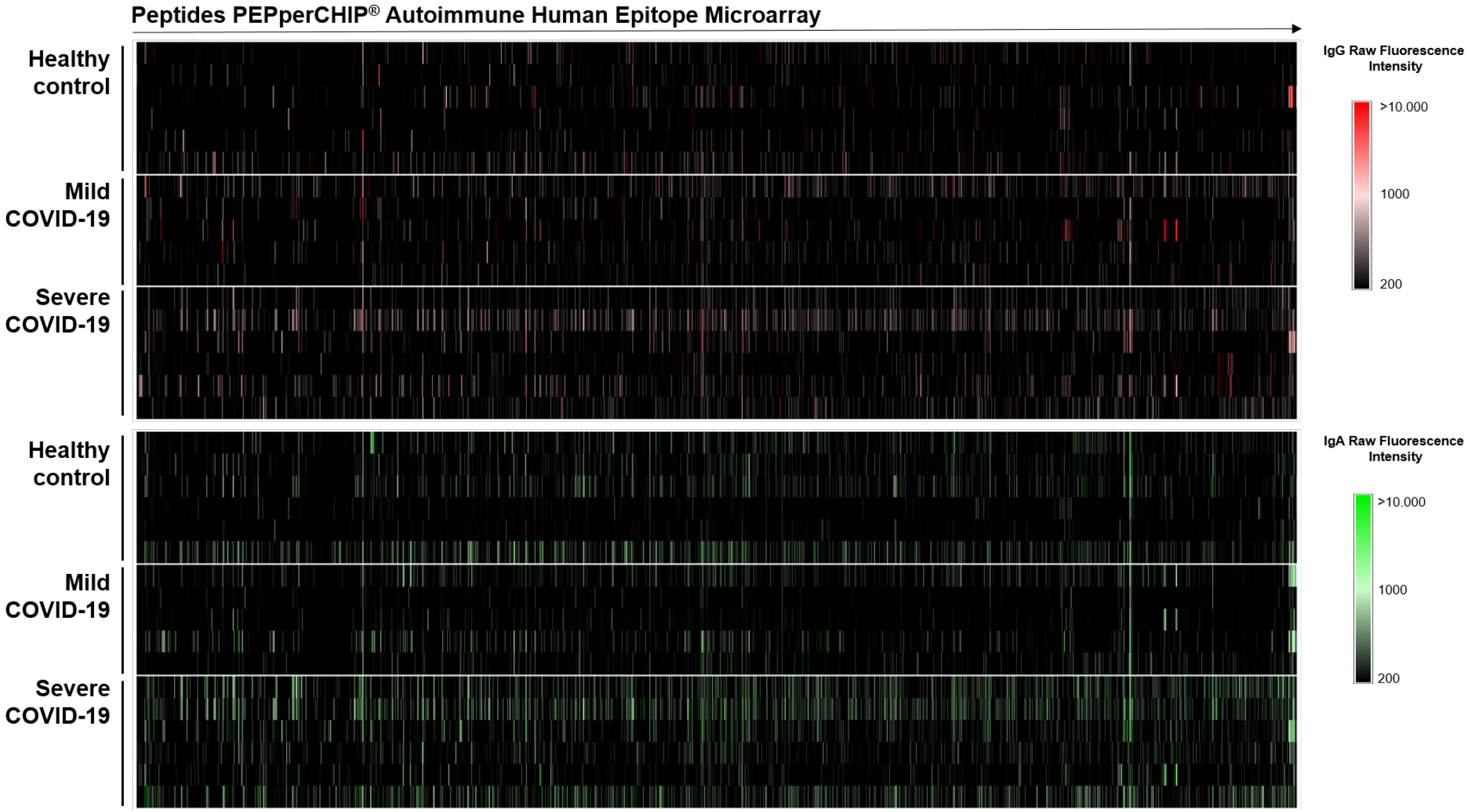

Epitope signatures in severe COVID-19

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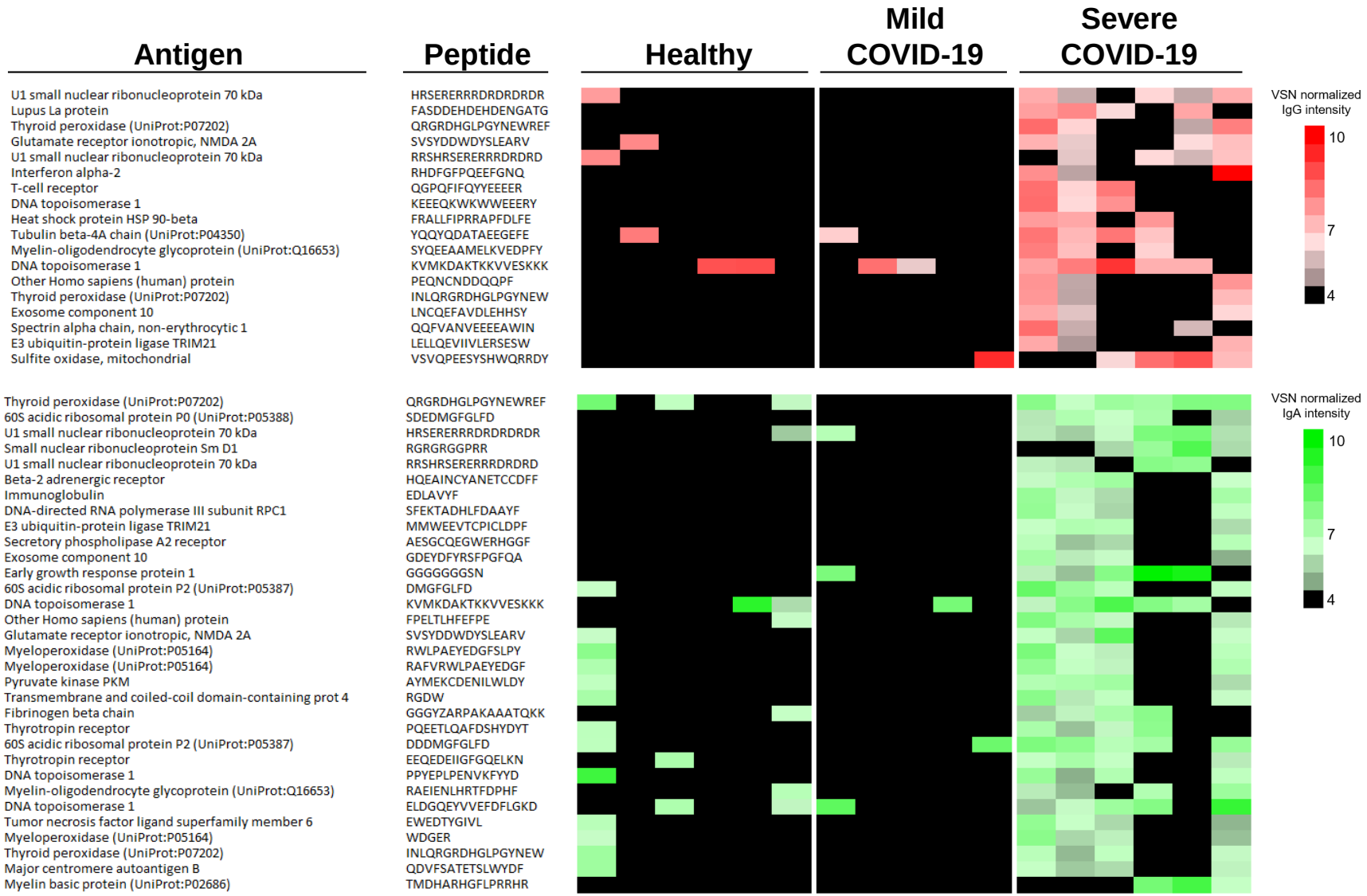
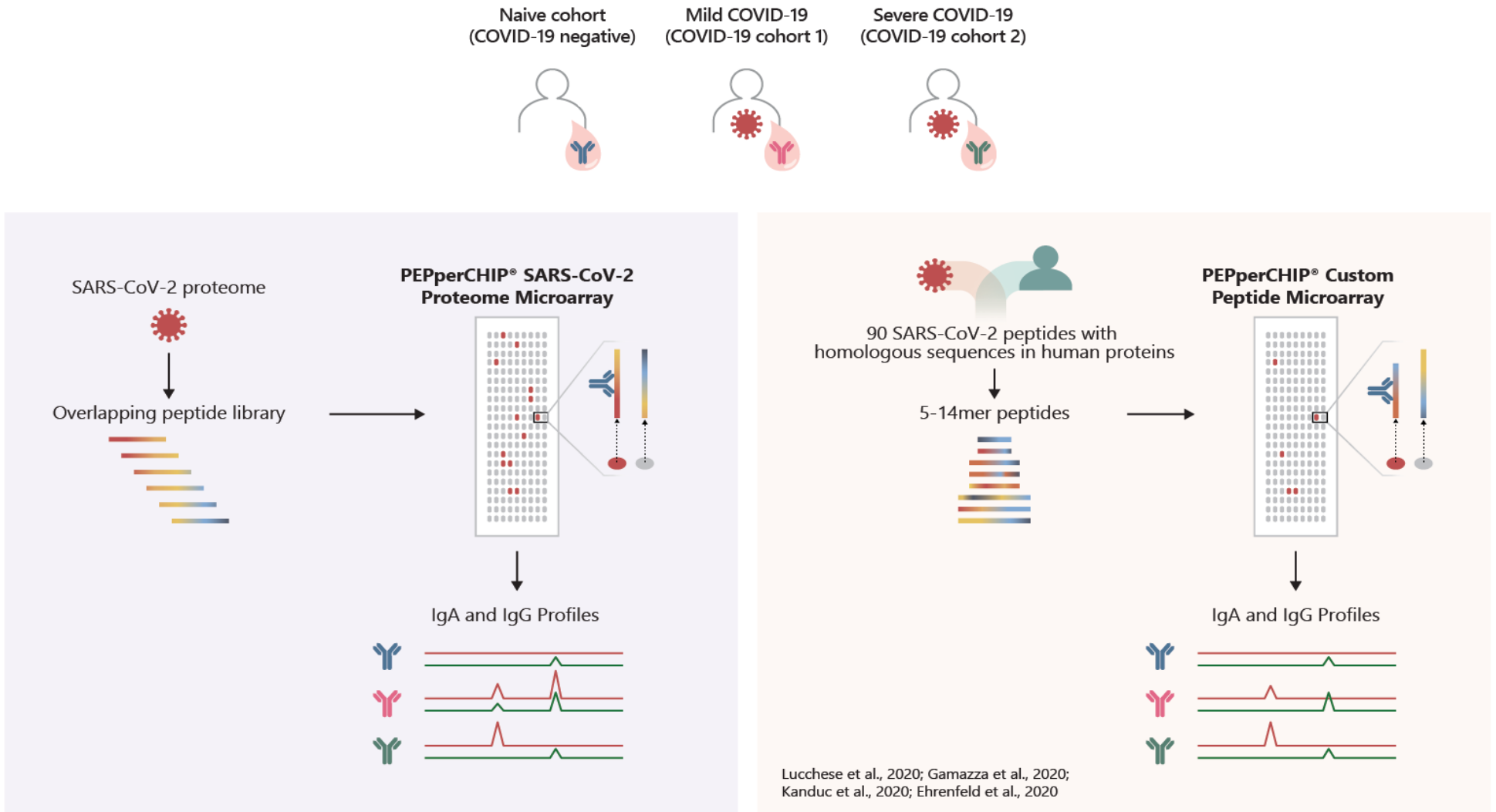
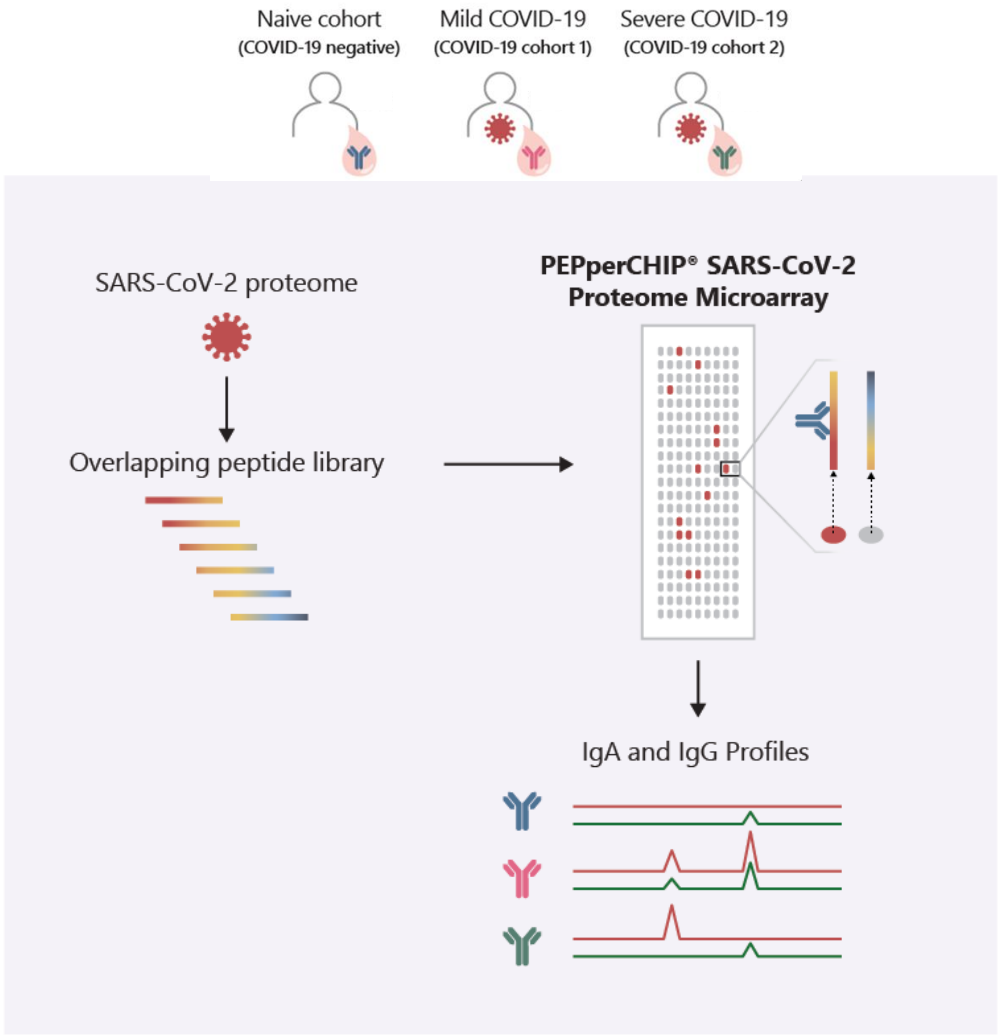


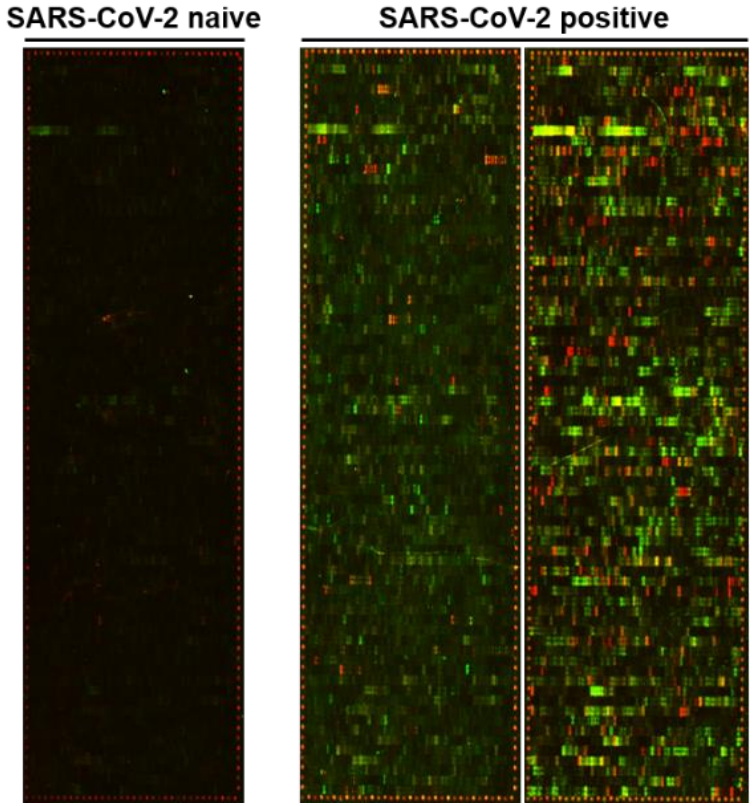
Antibody responses against shared epitopes



Lucchese et al., 2020; Gamazza et al., 2020;
Kanduc et al., 2020; Ehrenfeld et al., 2020

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





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

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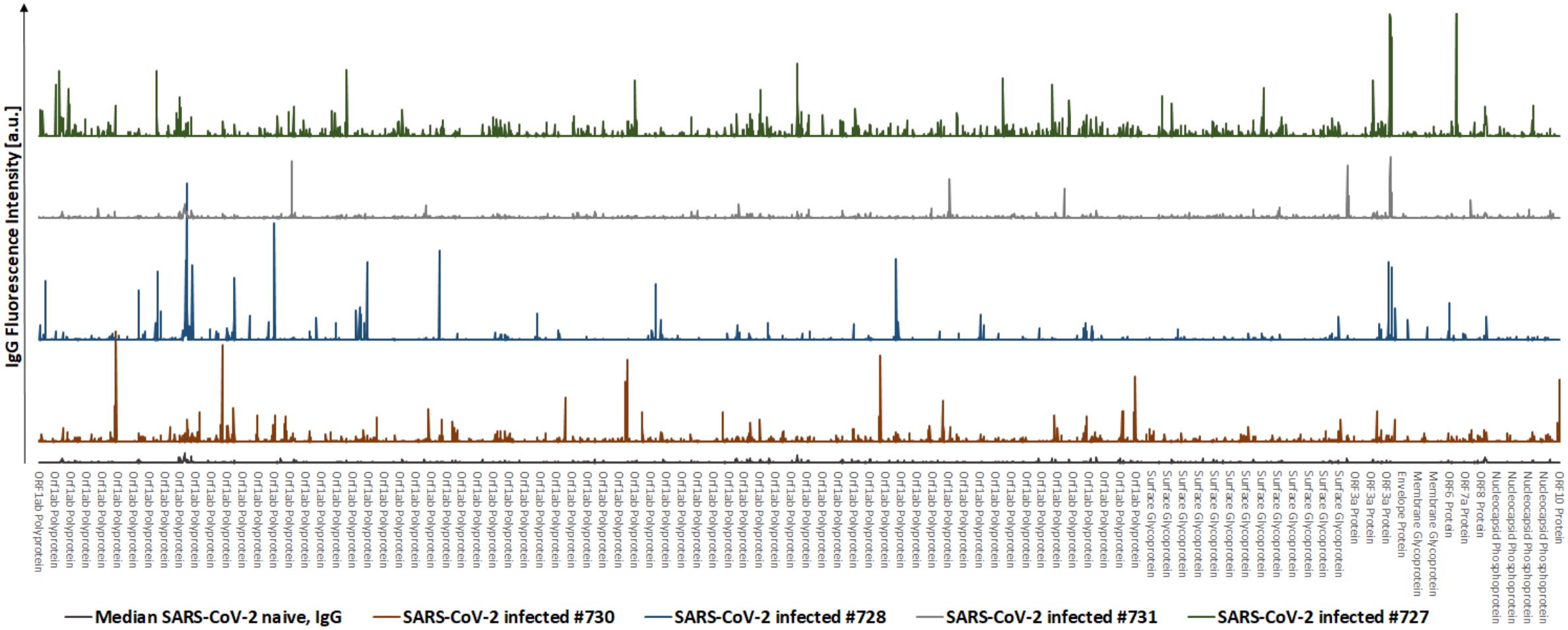
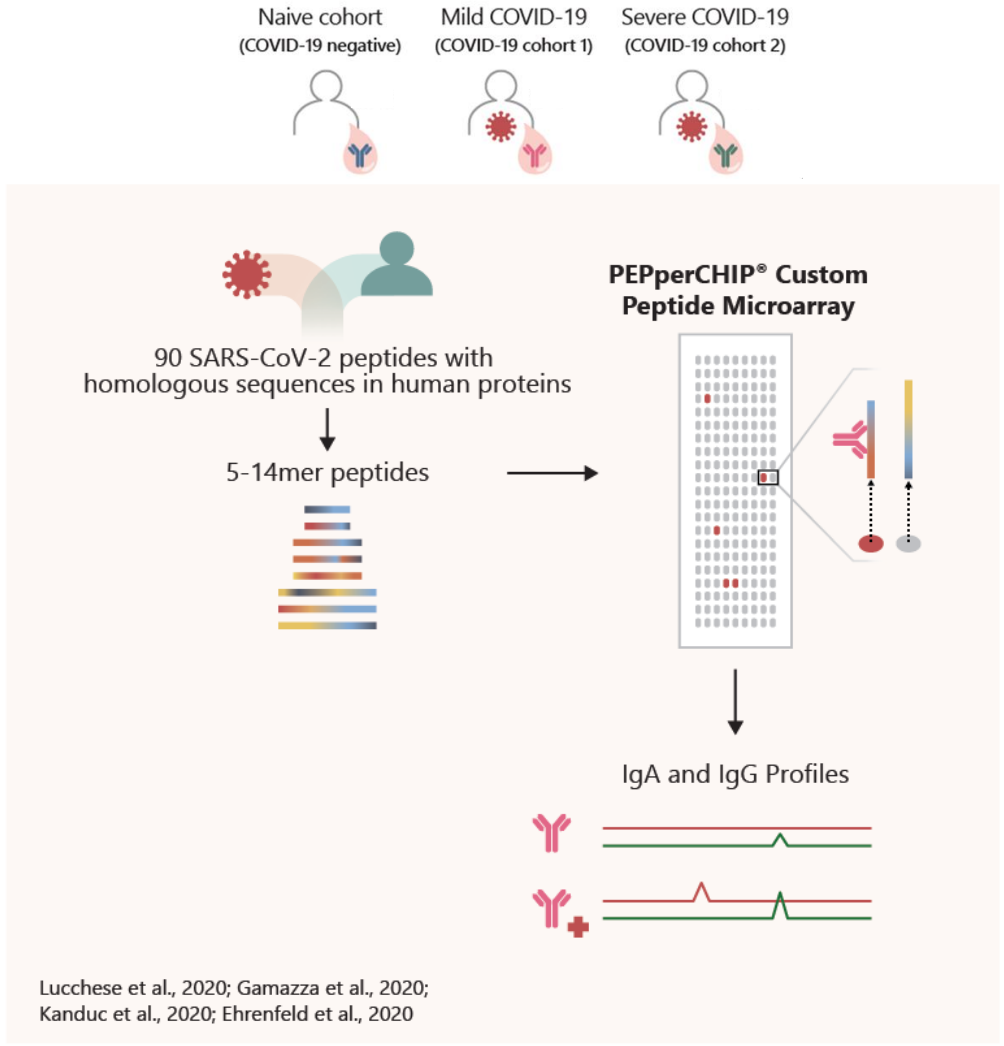
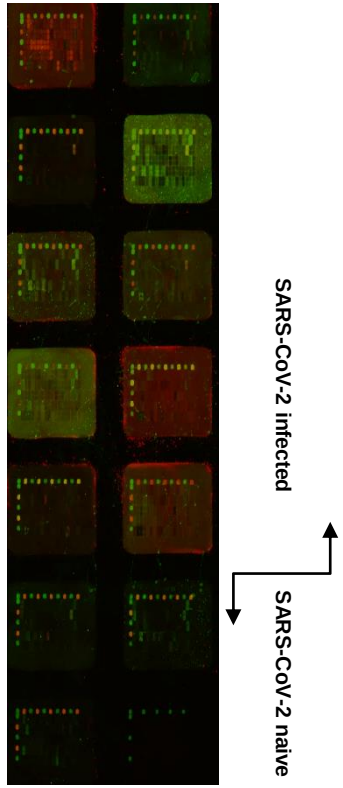
IgG responses



Antibody responses against shared epitopes





Antibody responses against shared epitopes

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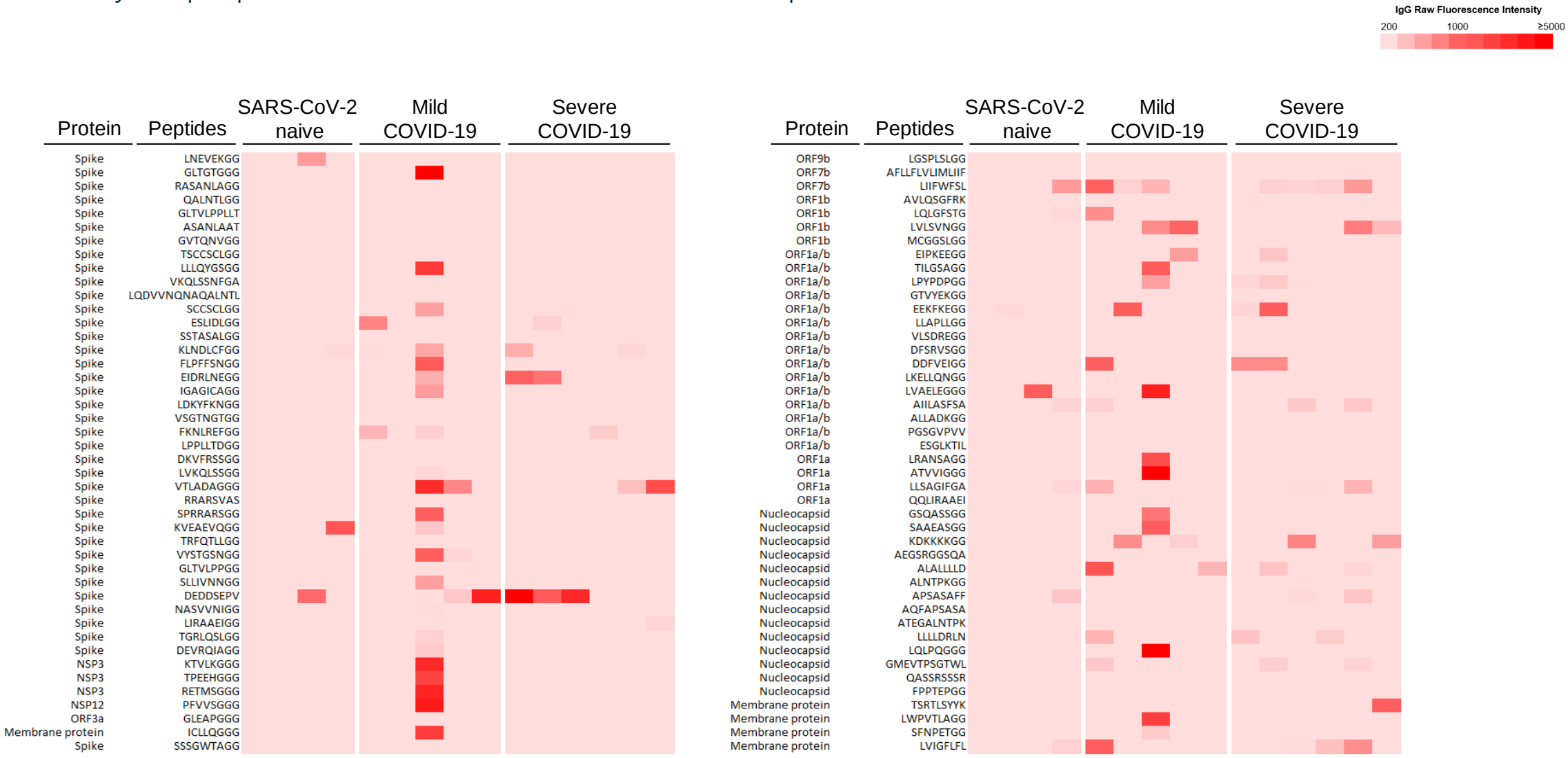
IgG reactivity to epitopes shared between SARS-CoV-2 & human proteins in SARS-CoV-2-infected individuals



Antibody responses against shared epitopes

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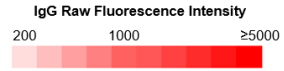

SARS-CoV-2 protein	Peptide sequence	Human protein with homologous sequence
Spike	KLNDLCFGG	Interleukin 7
Spike	EIDRLNEGG	Protein SET, Protein SETSIP
Spike	DEDDSEPV	Unconventional myosin-XVI
Spike	VTLADAGGG	Non-receptor tyrosine-protein kinase TNK1
Spike	FKNLREFGG	Isovaleryl-CoA dehydrogenase, mitochondrial
Spike	VYSTGSNGG	Neural cell adhesion molecule L1-like protein
ORF7b	LIIFWFSL	Olfactory receptor 7D4, 52N5
ORF1b	LVLSVNGG	Endoglin
ORF1a/b	LPYPDPGG	Heat shock 70 kDa protein 13
ORF1a/b	EEKFKEGG	Heat shock 70 kDa protein 13
ORF1a/b	DDFVEIGG	Alpha-crystallin A chain
ORF1a/b	AIILASFSA	Olfactory receptor 2B11, 2W1
ORF1a	LLSAGIFGA	Nesprin 1
Nucleocapsid	KDKKKKGG	HSP90AB1, HSP90AB2P
Nucleocapsid	ALALLLLD	Ephrin-B3

Antibody responses against shared epitopes

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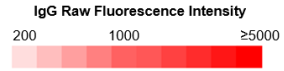

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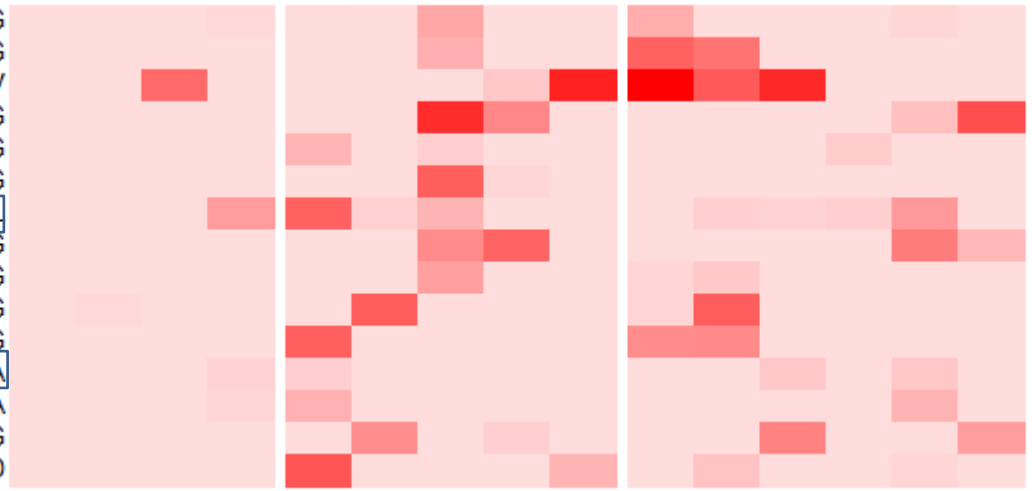
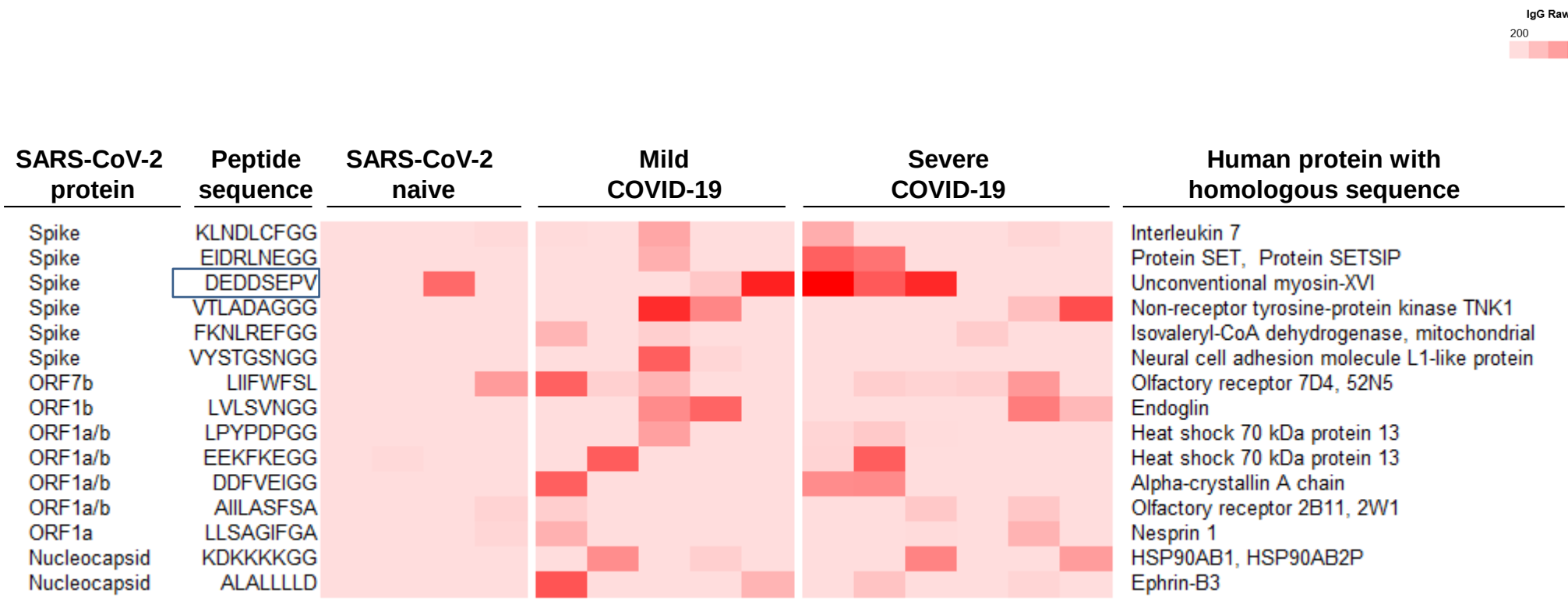
ORF1a/b	EEKFKEGG	Heat shock 70 kDa protein 13
ORF1a/b	DDFVEIGG	Alpha-crystallin A chain
ORF1a/b	AIILASFSA	Olfactory receptor 2B11, 2W1
ORF1a	LLSAGIFGA	Nesprin 1
Nucleocapsid	KDKKKKGG	HSP90AB1, HSP90AB2P
Nucleocapsid	ALALLLLD	Ephrin-B3



Antibody responses against shared epitopes

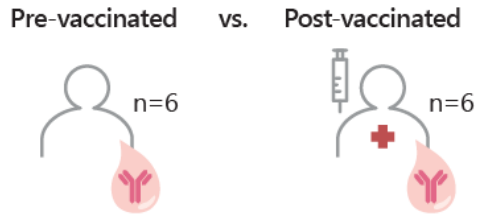


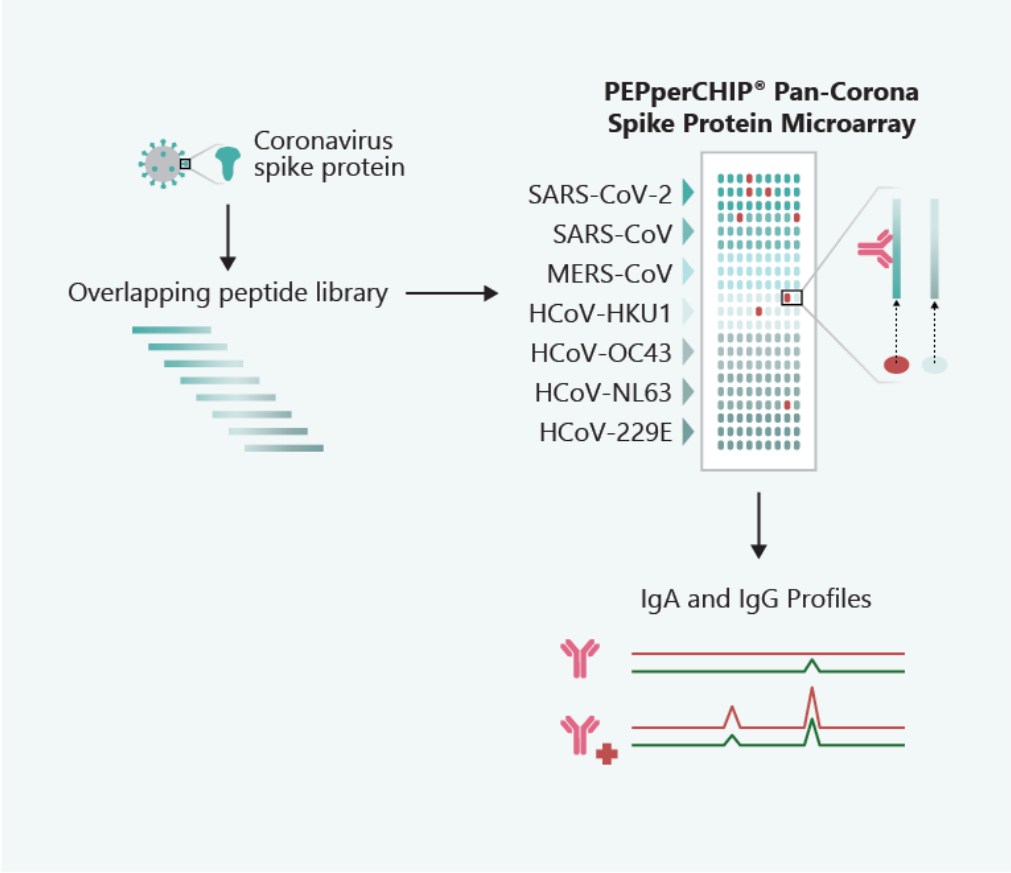
DEDDSEPV:

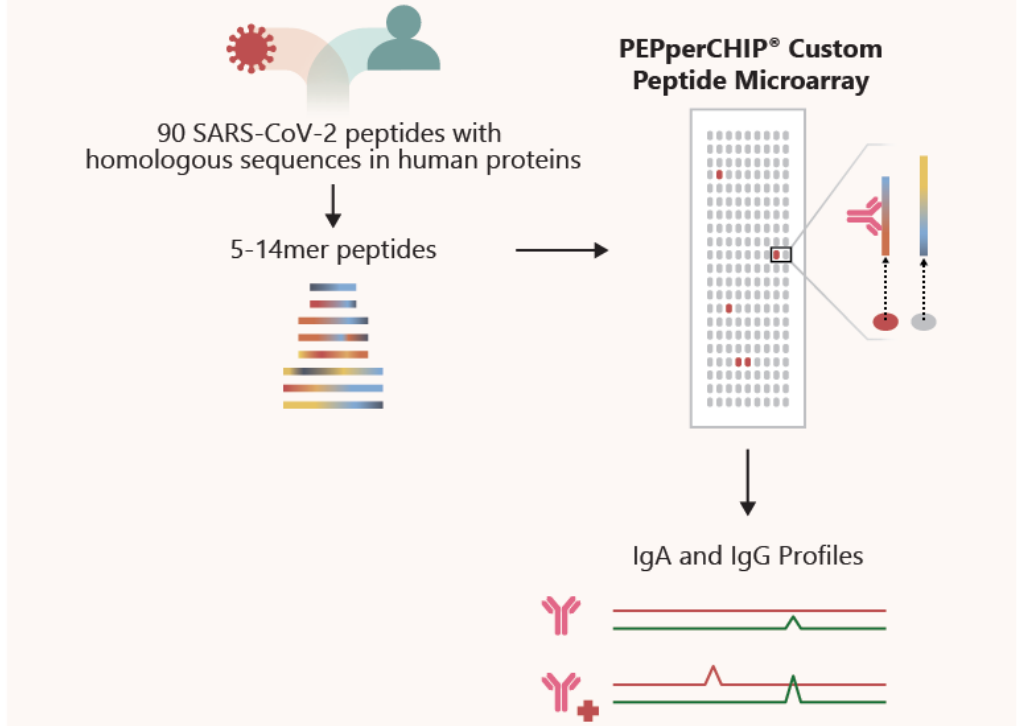
- conserved B cell epitope in SARS-CoV and SARS-CoV-2 Spikes
- also found in a T cell epitope of SARS-CoV-2 Spike
- bind to various HLA's including those associated with autoimmune disease

(Fath MK *et al.*, 2021)

Vaccination-induced antibody responses

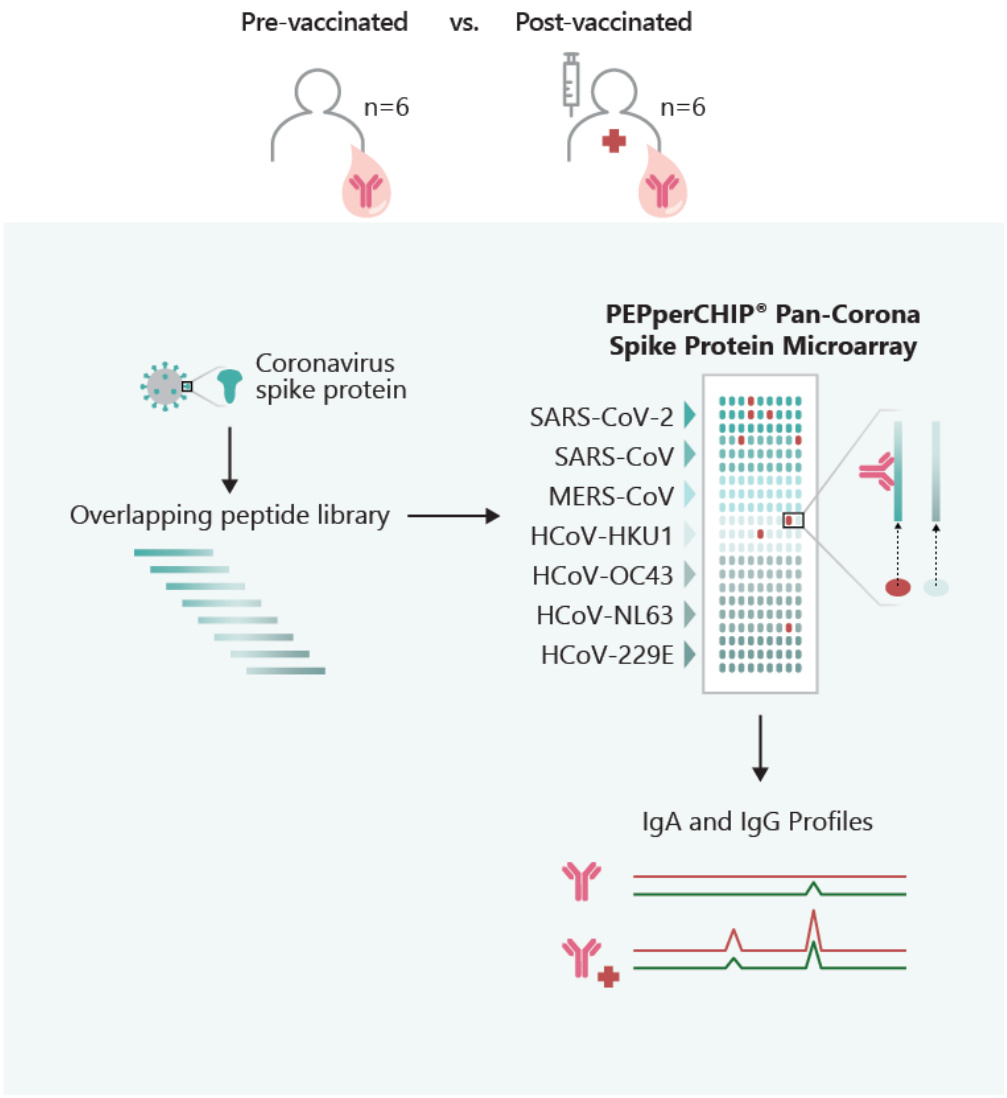


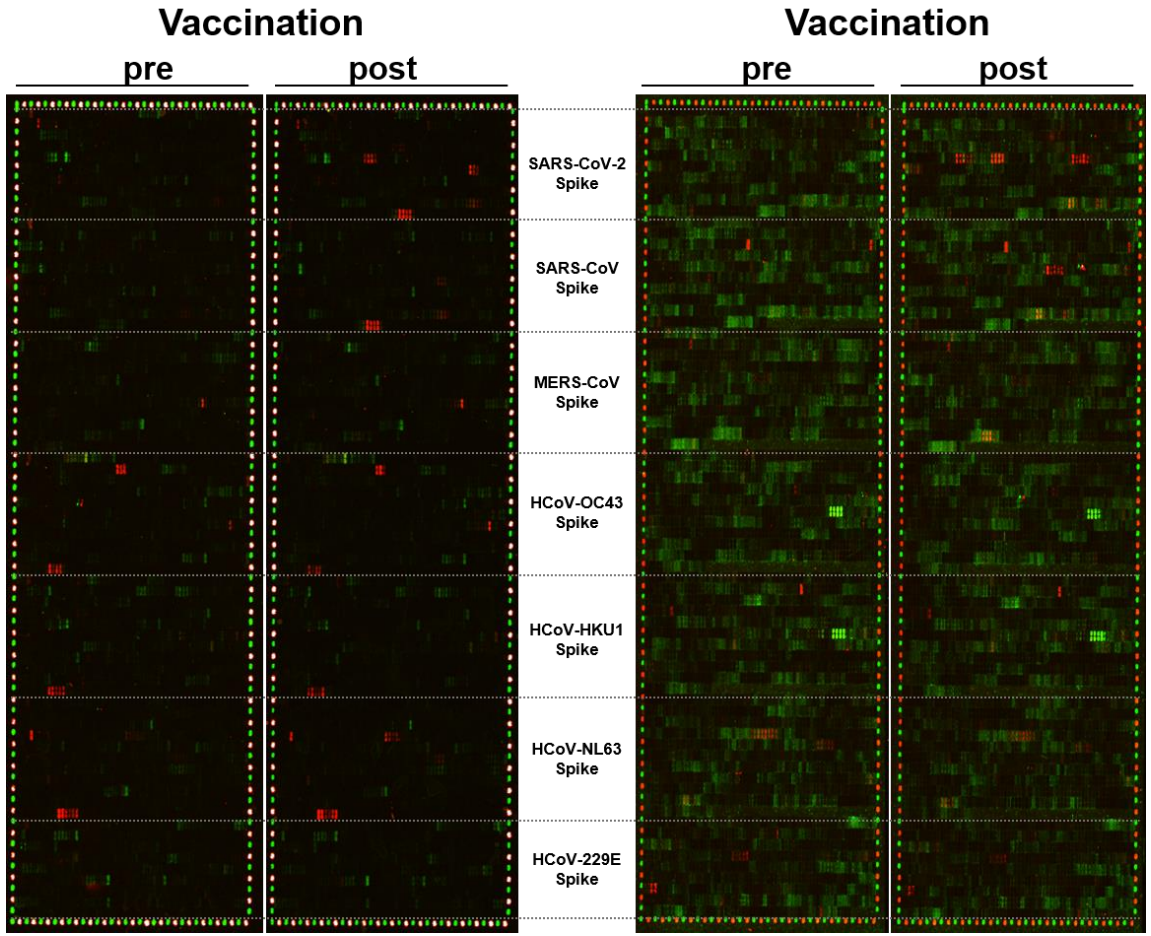




Lucchese et al., 2020; Gamazza et al., 2020; Kanduc et al., 2020; Ehrenfeld et al., 2020

Vaccination-induced antibody responses





Vaccination-induced antibody responses

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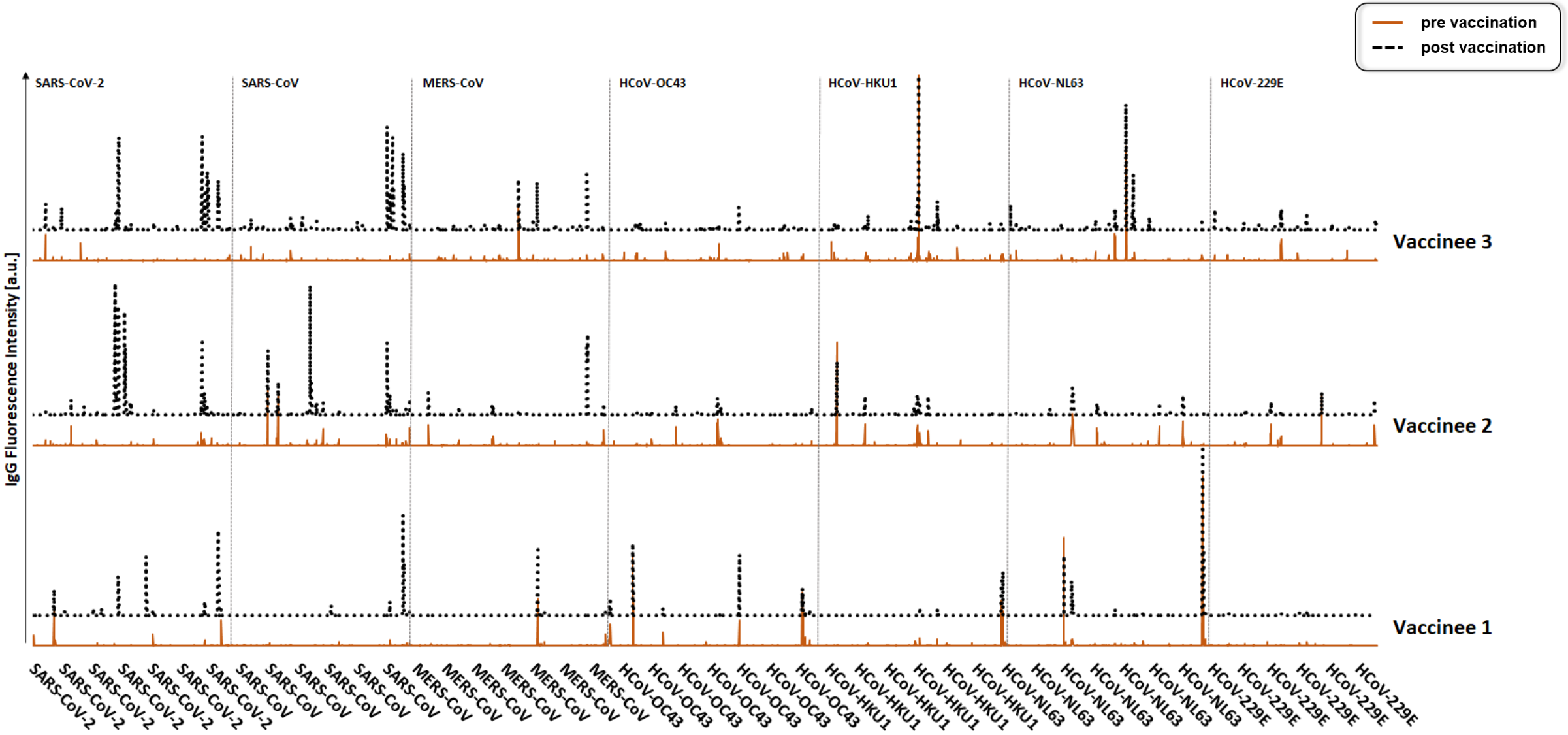
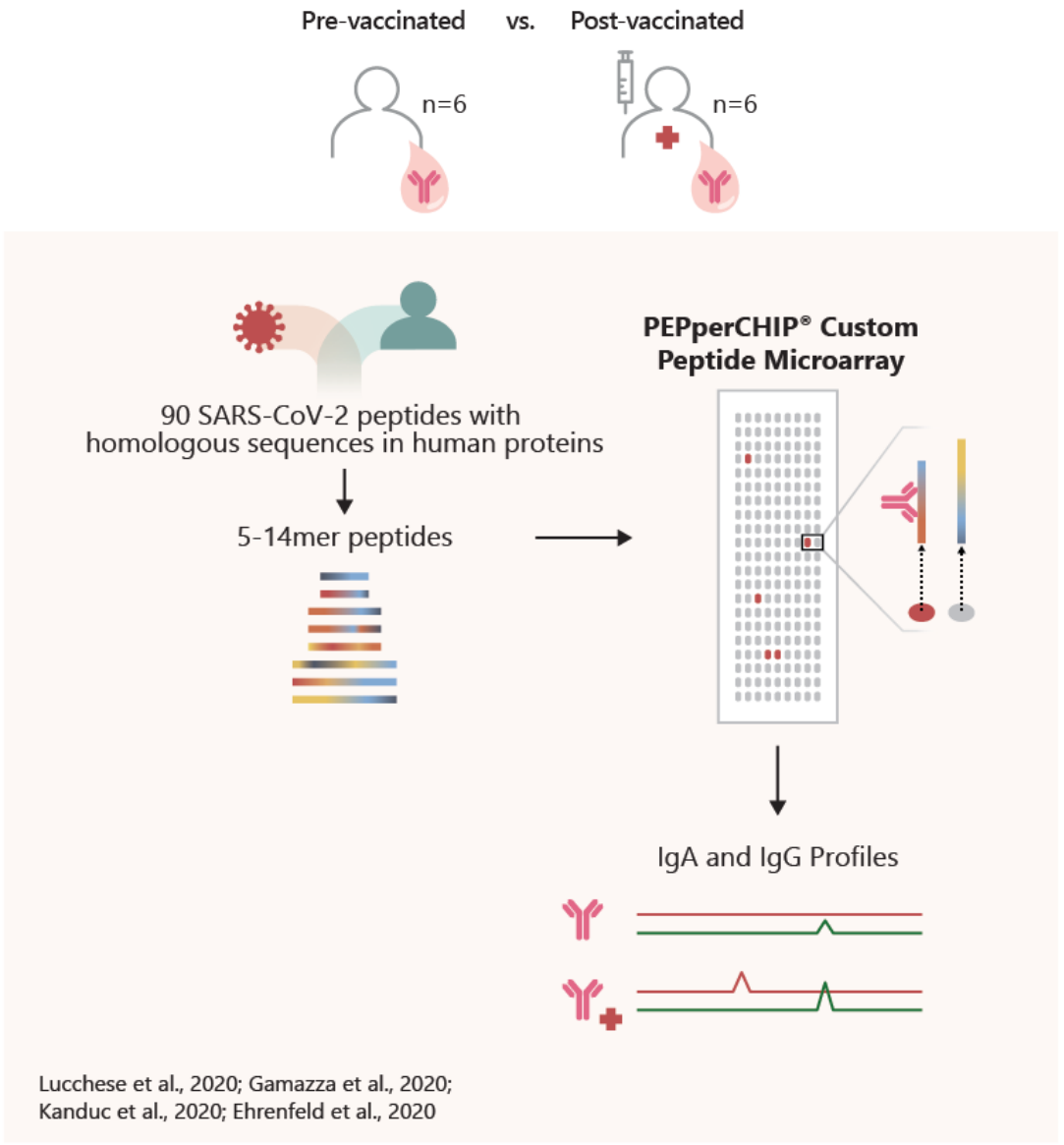
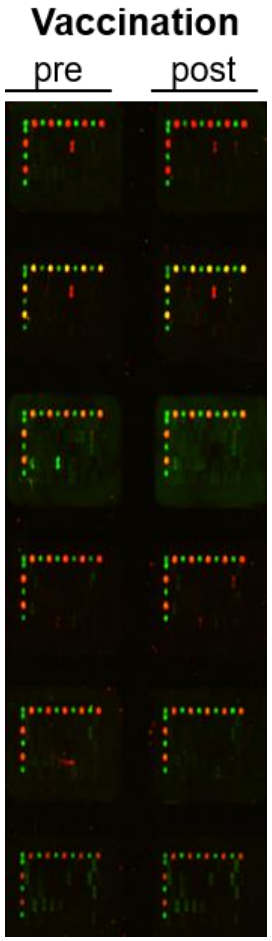
IgG responses



Antibody responses against shared epitopes



Lucchese et al., 2020; Gamazza et al., 2020; Kanduc et al., 2020; Ehrenfeld et al., 2020



Antibody responses against shared epitopes

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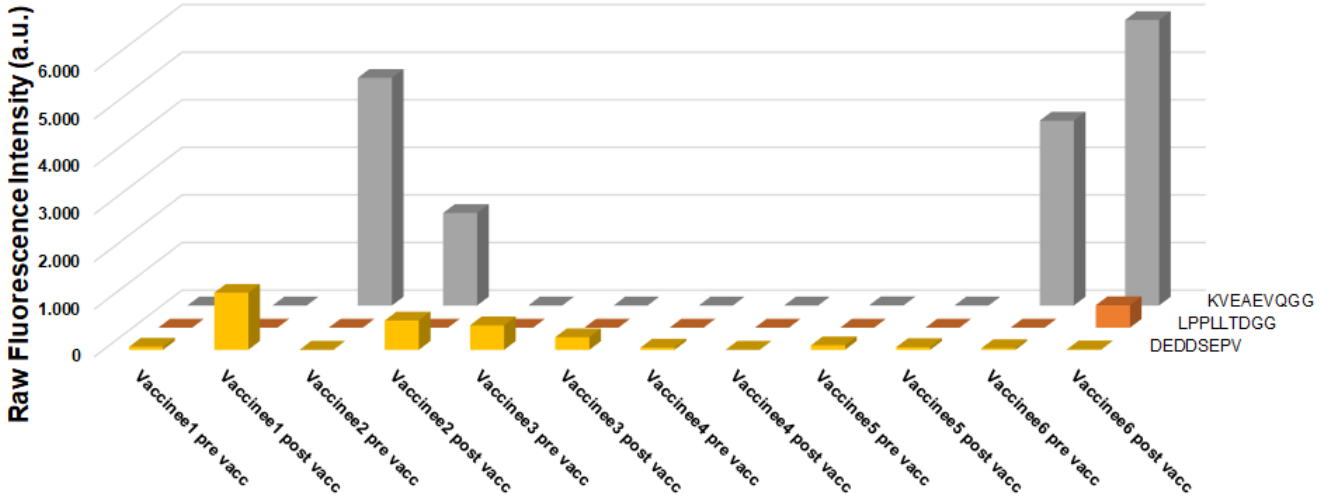
IgG reactivity to epitopes shared between SARS-CoV-2 Spike & human proteins in COVID-19-vaccinated individuals



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

Antibody responses against shared epitopes

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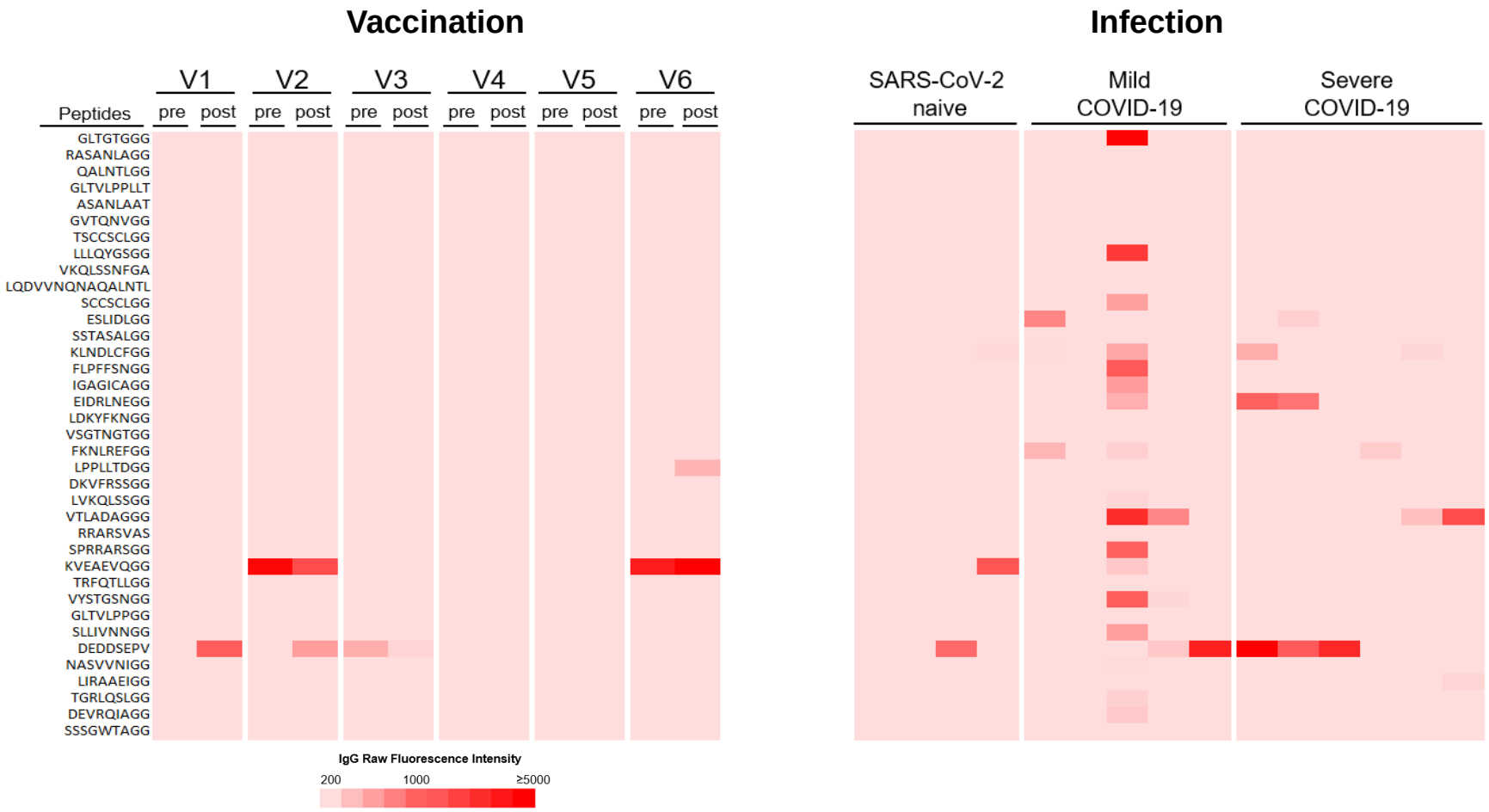
IgG reactivity to epitopes shared between SARS-CoV-2 Spike & human proteins



- 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

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Thank you for joining the webinar

Contact

Dr. Kirsten Heiss

Research & Development

PEPperPRINT GmbH
Tullastrasse 2
69126 Heidelberg, Germany

Visit us: www.pepperprint.com

Get in touch: info@pepperprint.com

pepperprint-gmbh @pepperchip pepperprint.gmbh