# Profiling autoantibody signatures after SARS-CoV-2 infection or COVID-19 vaccination utilizing high-density peptide microarrays

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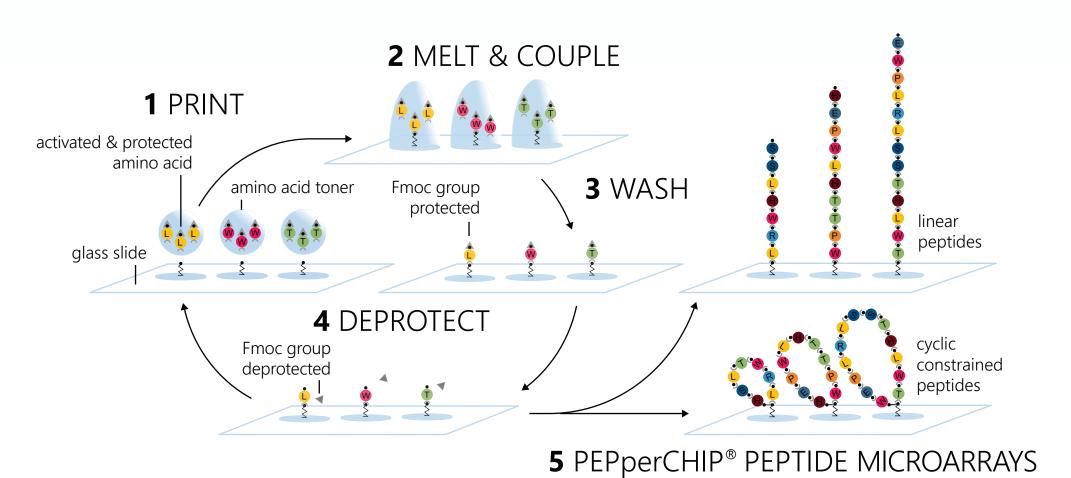[1]PEPperPRINT GmbH, Heidelberg, Germany

## KEY FINDINGS

- numerous IgG and/or IgA-specific autoantibody reactivities in severe COVID-19
- several epitopes can be assigned to nuclear autoantigens, others may be summarized as tissue-associated autoantigens
- few antibody responses to the selected 5-14mer peptides shared between SARS-CoV-2 and human proteins in infected individuals
- no antibody responses against most of the selected 5-14mer 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

## PEPperCHIP® Peptide Microarray Platform Technology

High-density PEPperCHIP® peptide microarrays are generated by digital laser printing on standard glass slides using a proprietary laser printer with 24 individual amino acid toners. For array production, amino acid toners are simultaneously printed with high precision on their respective positions on the glass slides.
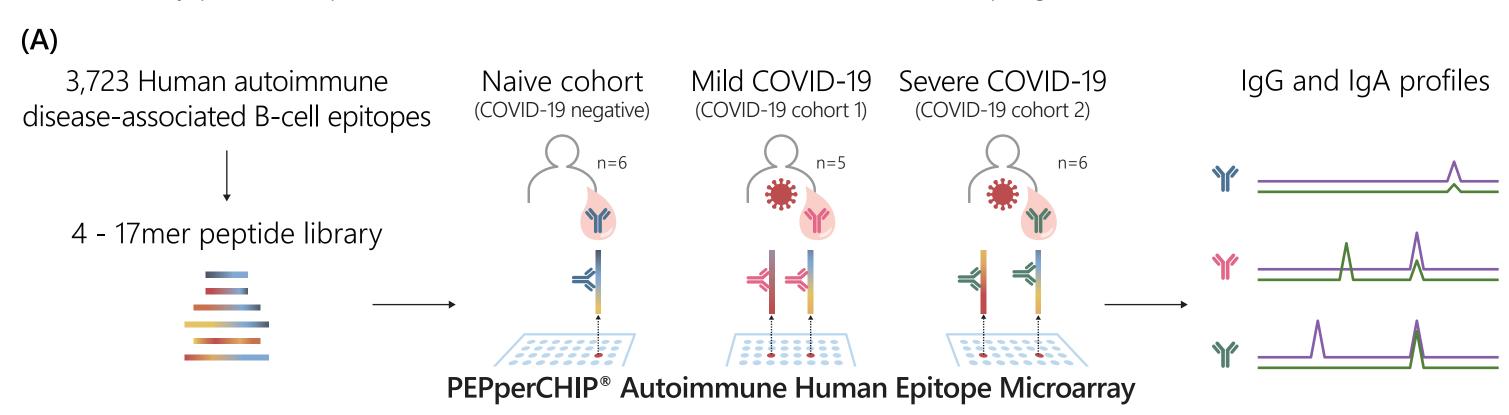


**1** PRINT
activated & protected amino acid
amino acid toner
glass slide
**2** MELT & COUPLE
Fmoc group protected
**3** WASH
linear peptides
**4** DEPROTECT
Fmoc group deprotected
cyclic constrained peptides
**5** PEPperCHIP® PEPTIDE MICROARRAYS

### Benefits
- high spot density/high content (1,200 peptides/cm²)
- digital printing flexibility
- high peptide quality with routine double couplings
- very low material consumption

**Fig. 1.** The PEPperCHIP® Peptide Microarray printing process.

## Screening of COVID-19 patient sera against human autoimmune disease-associated linear epitopes

Antibody responses are crucial to combat SARS-CoV-2 infection. However, there is considerable evidence indicating that dysregulated humoral immunity might contribute to the immunopathology of COVID-19. Intriguingly, autoimmune diseases and COVID-19 show common immunological features. Several studies reported the detection of autoantibodies in COVID-19 patients significantly correlating with the disease outcome. Here, we examined epitope-specific autoantibody profiles in patients with a mild versus severe COVID-19 disease progression.
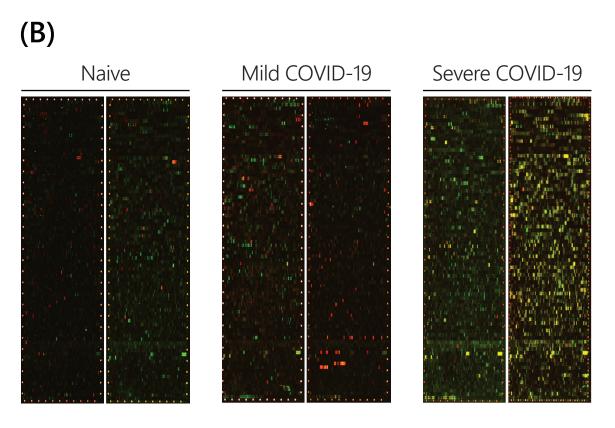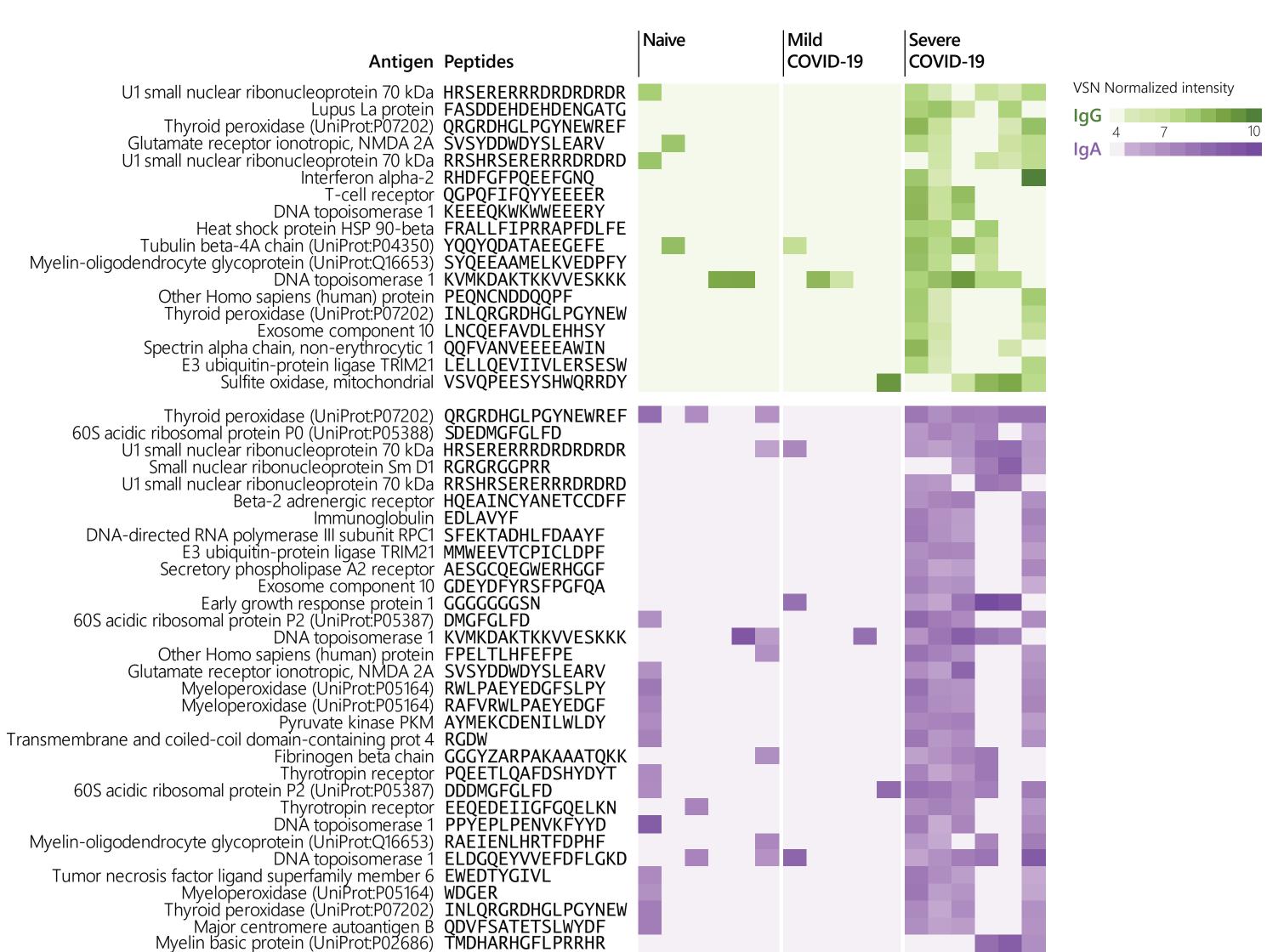
**(A)**



3,723 Human autoimmune disease-associated B-cell epitopes
4 - 17mer peptide library
Naive cohort (COVID-19 negative) n=6
Mild COVID-19 (COVID-19 cohort 1) n=5
Severe COVID-19 (COVID-19 cohort 2) n=6
IgG and IgA profiles
PEPperCHIP® Autoimmune Human Epitope Microarray

**(B)**
Naive | Mild COVID-19 | Severe COVID-19

**Fig. 2. Autoantibody screening of COVID-19 patient sera.** **(A) Study outline.** Sera from COVID-19 patients and SARS-CoV-2 negative subjects (naive cohort) were screened on PEPperCHIP® Autoimmune Human Epitope Microarrays covering 3,723 human B-cell epitopes associated with autoimmune diseases including epitopes with post translational modifications described in autoimmunity. IgG and IgA responses were analyzed simultaneously. The severe cohort comprised of patients with acute respiratory distress syndrome (ARDS). **(B) Strong autoantibody reactivity in severe COVID-19.** Representative staining images of COVID-19 patient sera (mild and severe disease outcome) and SARS-CoV-2 negative subjects (healthy control). PEPperCHIP® Autoimmune Human Epitope Microarrays were incubated with respective sera (1:100) overnight at 4°C. Detection of antibody binding was done using anti-human IgG DL680 (red) and anti-human IgA DL800 (green). Polio peptide spots served as control frame.



| Antigen | Peptides | Naive | Mild COVID-19 | Severe COVID-19 |
|---|---|---|---|---|
| U1 small nuclear ribonucleoprotein 70 kDa | HRSERERRRDRDRDRDR | | | |
| Lupus La protein | FASDDEHDEHDENGATG | | | |
| Thyroid peroxidase (UniProt:P07202) | QRGRDHGLPGYNEWREF | | | |
| Glutamate receptor ionotropic, NMDA 2A | SVSYDWDYSLEARV | | | |
| U1 small nuclear ribonucleoprotein 70 kDa | RRSHRSERERRRDRDRD | | | |
| Interferon alpha-2 | RHDFGFPQEEFGNQ | | | |
| T-cell receptor | QGPQFIFQYYEEEER | | | |
| DNA topoisomerase 1 | KEEEQKWKWEEERY | | | |
| Heat shock protein HSP 90-beta | FRALLFIPRRAPFDLFE | | | |
| Tubulin beta-4A chain (UniProt:P04350) | YQQYQDATAEEGEFE | | | |
| Myelin-oligodendrocyte glycoprotein (UniProt:Q16653) | SYQEEAAMELKVEDPFY | | | |
| DNA topoisomerase 1 | KVMKDAKTKKVVESKKK | | | |
| Other Homo sapiens (human) protein | PEQNCNDDQQPF | | | |
| Thyroid peroxidase (UniProt:P07202) | INLQRGRDHGLPGYNEW | | | |
| Exosome component 10 | LNCQEFAVDLEHHSY | | | |
| Spectrin alpha chain, non-erythrocytic 1 | QQFVANVEEEEAWIN | | | |
| E3 ubiquitin-protein ligase TRIM21 | LELLQEVIIVLERSESW | | | |
| Sulfite oxidase, mitochondrial | VSVQPEESYSHWQRRDY | | | |
| Thyroid peroxidase (UniProt:P07202) | QRGRDHGLPGYNEWREF | | | |
| 60S acidic ribosomal protein P0 (UniProt:P05388) | SDEDMGFGLFD | | | |
| U1 small nuclear ribonucleoprotein 70 kDa | HRSERERRRDRDRDRDR | | | |
| Small nuclear ribonucleoprotein Sm D1 | RGRGRGGPRR | | | |
| U1 small nuclear ribonucleoprotein 70 kDa | RRSHRSERERRRDRDRD | | | |
| Beta-2 adrenergic receptor | HQEAINCYANETCCDFF | | | |
| Immunoglobulin | EDLAVYF | | | |
| DNA-directed RNA polymerase III subunit RPC1 | SFEKTADHLFDAAYF | | | |
| E3 ubiquitin-protein ligase TRIM21 | MMWEEVTCPICLDPF | | | |
| Secretory phospholipase A2 receptor | AESGCQEGWERHGGF | | | |
| Exosome component 10 | GDEYDFYRSFPGFQA | | | |
| Early growth response protein 1 | GGGGGGGSN | | | |
| 60S acidic ribosomal protein P2 (UniProt:P05387) | DMGFGLFD | | | |
| DNA topoisomerase 1 | KVMKDAKTKKVVESKKK | | | |
| Other Homo sapiens (human) protein | FPELTLHFEFPE | | | |
| Glutamate receptor ionotropic, NMDA 2A | SVSYDWDYSLEARV | | | |
| Myeloperoxidase (UniProt:P05164) | RWLPAEYEDGFSLPY | | | |
| Myeloperoxidase (UniProt:P05164) | RAFVWLPAEYEDGF | | | |
| Pyruvate kinase PKM | AYMEKCDENILWLDY | | | |
| Transmembrane and coiled-coil domain-containing prot 4 | RGDW | | | |
| Fibrinogen beta chain | GGGYZARPAKAAATQKK | | | |
| Thyrotropin receptor | PQEETLQAFDSHYDYT | | | |
| 60S acidic ribosomal protein P2 (UniProt:P05387) | DDDMGFGLFD | | | |
| Thyrotropin receptor | EEQEDEIIGFGQELKN | | | |
| DNA topoisomerase 1 | PPYEPLPENVKFYYD | | | |
| Myelin-oligodendrocyte glycoprotein (UniProt:Q16653) | RAEIENLHRTFDPHF | | | |
| DNA topoisomerase 1 | ELDGQEYVVEFDFLGKD | | | |
| Tumor necrosis factor ligand superfamily member 6 | EWEDTYGIVL | | | |
| Myeloperoxidase (UniProt:P05164) | WDGER | | | |
| Thyroid peroxidase (UniProt:P07202) | INLQRGRDHGLPGYNEW | | | |
| Major centromere autoantigen B | QDVFSATETSLWYDF | | | |
| Myelin Basic protein (UniProt:P02686) | TMDHARPFLPRRHR | | | |

VSN Normalized intensity
**IgG** 4 7 10
**IgA**

**Fig. 3. Most differentially recognized epitopes that show a strong reactivity in severe COVID-19 patients compared to mild COVID-19 and healthy subjects.** Heat maps show VSN normalized fluorescence intensities for each peptide (vertical) and individual patient sera (horizontal). The color coding correspond to low (light) to high signal intensities (IgG: green; IgA: purple). **Several epitopes can be assigned to nuclear autoantigens, others may be summarized as tissue-associated autoantigens (e.g. from thyroid glands, CNS).**

## Antibody responses against epitopes shared between SARS-CoV-2 and human proteins in infection and vaccination

Molecular mimicry is one mechanism described for viral-induced autoimmunity. It has been suggested that the immune response induced to fight against SARS-CoV-2 may cross-react with human proteins sharing epitope sequences with viral antigens. In this context, we investigated the epitope recognition in infected and vaccinated individuals, respectively.



Naive cohort (COVID-19 negative) n=6
Mild COVID-19 (COVID-19 cohort 1) n=5
Severe COVID-19 (COVID-19 cohort 2) n=6
Pre-vaccinated n=6
Post-vaccinated n=6
INFECTION
VACCINATION
PEPperCHIP® Custom Peptide Microarray
90 SARS-CoV-2 peptides with homologous sequences in human proteins*
5-14mer peptides
IgG and IgA profiles

*Lucchese et al., 2020; Gamazza et al., 2020; Kanduc et al., 2020; Ehrenfeld et al., 2020
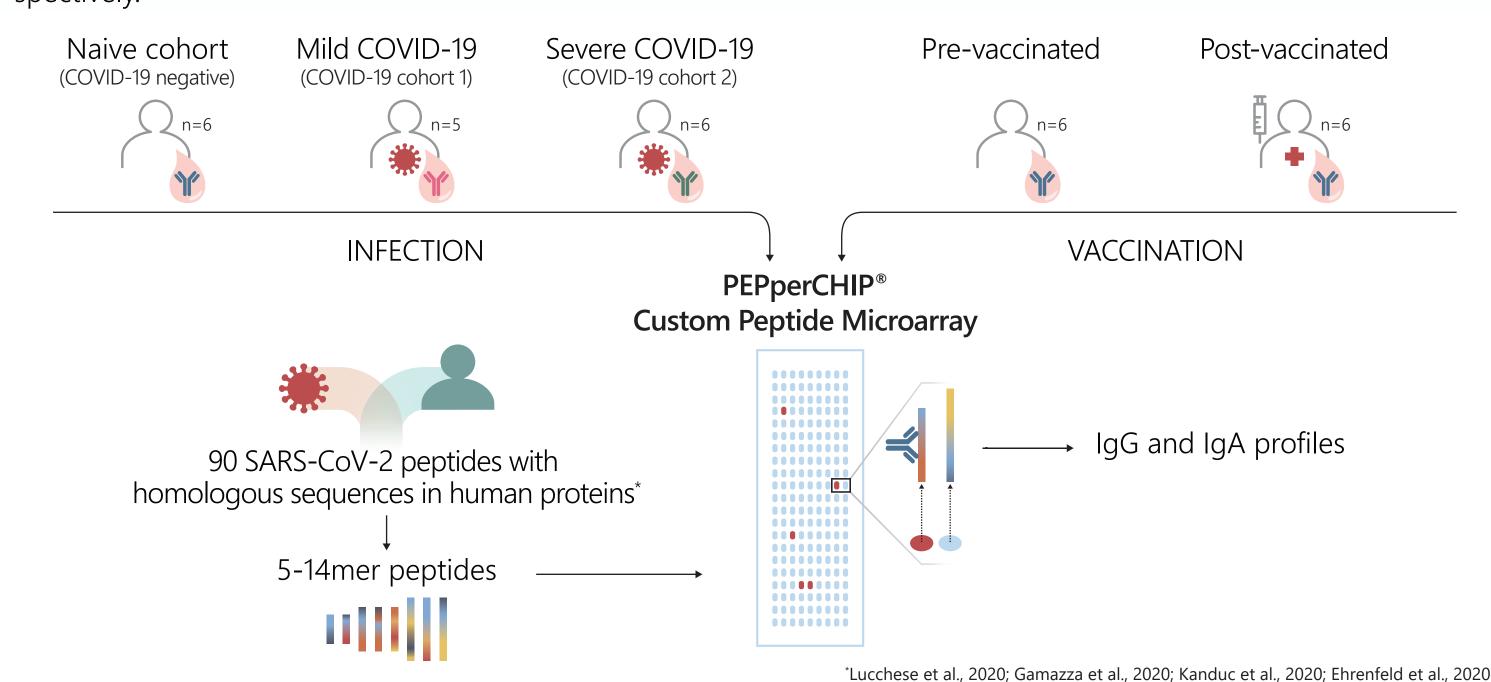
**Fig. 4. Study outline for assessing antibody responses against epitopes shared between SARS-CoV-2 and human proteins in COVID-19 infection and vaccination.** Antibody responses against described cross-reactive epitopes present in SARS-CoV-2 & human proteins were investigated. For this, a customized peptide microarray was designed displaying 5-14mer peptides described in the literature as shared sequences between SARS- CoV-2 and human proteins. In total, the peptide microarray display 90 peptide sequences shared between SARS-CoV-2 and human proteins. Dual isotype read-out was performed (anti-human IgG and anti-human IgA).
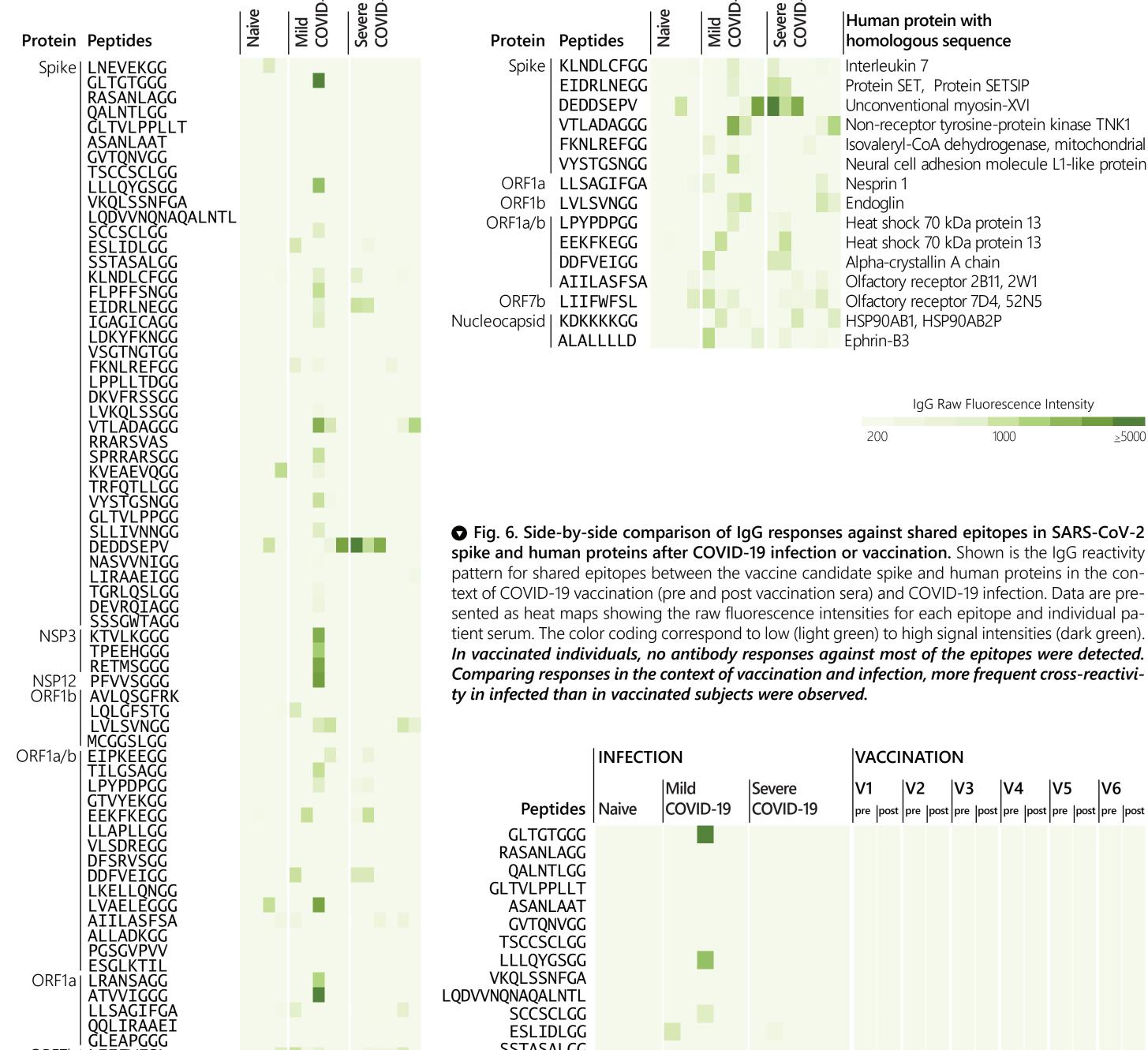
**(A)**



| Protein | Peptides | Naive | Mild COVID-19 | Severe COVID-19 |
|---|---|---|---|---|
| Spike | LNEVEKGG | | | |
| | GLTGTCGG | | | |
| | RASANLAGG | | | |
| | QALNTLGG | | | |
| | GLTVLPPLLT | | | |
| | ASANLAAT | | | |
| | GVTONVGG | | | |
| | TSCCSCLGG | | | |
| | LLLQYGSGG | | | |
| | VKQLSSNFGA | | | |
| | LQDVVNQNAQALNTL | | | |
| | SCCSCLGG | | | |
| | ESLIDLGG | | | |
| | SSTASALGG | | | |
| | KLNDLCFGG | | | |
| | FLPFFSNGG | | | |
| | EIDRLNEGG | | | |
| | IGAGICAGG | | | |
| | LDKYFKNGG | | | |
| | VSGTNGTGG | | | |
| | FKNLREFGG | | | |
| | LPPLLTDGG | | | |
| | DKVFRSSGG | | | |
| | LVKQLSSGG | | | |
| | VTLADAGGG | | | |
| | RRARSVAS | | | |
| | SPRRARSGG | | | |
| | KVEAEVQGG | | | |
| | SLLIVNNGG | | | |
| | VYSTGSNGG | | | |
| | GLTVLPPGG | | | |
| | DEDDSEPV | | | |
| | NASVVNIGG | | | |
| | LIRAAEIGG | | | |
| | TGRLQSLGG | | | |
| | DEVRQIAGG | | | |
| | SSSGWTAGG | | | |
| NSP3 | KTVLKGGG | | | |
| | TPEEHGGG | | | |
| | RETMSGGG | | | |
| NSP12 | PFVVSGGG | | | |
| ORF1b | AVLGSGFRK | | | |
| | LQLGFSTG | | | |
| | LVLSVNGG | | | |
| | MCGGSLGG | | | |
| ORF1a/b | EIPKEEGG | | | |
| | TILGSAGG | | | |
| | LPYPDPGG | | | |
| | GTVYEKGG | | | |
| | EEKFKEGG | | | |
| | LLAPLLGG | | | |
| | VLSDREGG | | | |
| | DFSRVSGG | | | |
| | DDFVEIGG | | | |
| | LKELLONGG | | | |
| | LVAELEGGG | | | |
| | AITLASFSA | | | |
| | ALLADAGG | | | |
| ORF1a | PGSGVPVV | | | |
| | ESGLKTIL | | | |
| | LLSAGIFGA | | | |
| | QQLIRAAEI | | | |
| | GLEAPGGG | | | |
| | ATVVIGGG | | | |
| ORF7b | LIIFWFSL | | | |
| | AFILFLVLIMLIIF | | | |
| ORF9b | LGSPLSLGG | | | |
| Nucleocapsid | GSQASSGG | | | |
| | SAAEASGG | | | |
| | KDKKKKGG | | | |
| | AEGSRGGSQA | | | |
| | ALALLLLD | | | |
| | ALNTPKGG | | | |
| | APSASAFF | | | |
| | AQFAPSASA | | | |
| | ATEGALNTPK | | | |
| | LLLLDRLN | | | |
| | LQLPQGGG | | | |
| | GMEVTPSGTWL | | | |
| | QASSRSSSR | | | |
| Membrane protein | FPPTEPGG | | | |
| | TSRTLSYYK | | | |
| | LWPVTLAGG | | | |
| | SFNPETGG | | | |
| | LVIGFLFL | | | |
| | ICLLQGGG | | | |

**(B)**

| Protein | Peptides | Naive | Mild COVID-19 | Severe COVID-19 | Human protein with homologous sequence |
|---|---|---|---|---|---|
| Spike | KLNDLCFGG | | | | Interleukin 7 |
| | EIDRLNEGG | | | | Protein SET, Protein SETSIP |
| | DEDDSEPV | | | | Unconventional myosin-XVI |
| | VTLADAGGG | | | | Non-receptor tyrosine-protein kinase TNK1 |
| | FKNLREFGG | | | | Isovaleryl-CoA dehydrogenase, mitochondrial |
| | VYSTGSNGG | | | | Neural cell adhesion molecule L1-like protein |
| ORF1a | LLSAGIFGA | | | | Nesprin 1 |
| ORF1b | LVLSVNGG | | | | Endoglin |
| ORF1a/b | LPYPDPGG | | | | Heat shock 70 kDa protein 13 |
| | EEKFKEGG | | | | Heat shock 70 kDa protein 13 |
| | DDFVEIGG | | | | Alpha-crystallin A chain |
| | AIILASFSA | | | | Olfactory receptor 2B11, 2W1 |
| ORF7b | LIIFWFSL | | | | Olfactory receptor 7D4, 52N5 |
| Nucleocapsid | KDKKKKGG | | | | HSP90AB1, HSP90AB2P |
| | ALALLLLD | | | | Ephrin-B3 |

IgG Raw Fluorescence Intensity
200 1000 ≥5000

**Fig. 6. Side-by-side comparison of IgG responses against shared epitopes in SARS-CoV-2 spike and human proteins after COVID-19 infection or vaccination.** Shown is the IgG reactivity pattern for shared epitopes between the vaccine candidate spike and human proteins in the context of COVID-19 vaccination (pre and post vaccination sera) and COVID-19 infection. Data are presented as heat maps showing the raw fluorescence intensities for each epitope and individual patient serum. The color coding correspond to low (light green) to high signal intensities (dark green). **In vaccinated individuals, no antibody responses against most of the epitopes were detected. Comparing responses in the context of vaccination and infection, more frequent cross-reactivity in infected than in vaccinated subjects were observed.**



| | | INFECTION | | | VACCINATION | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptides | Naive | Mild COVID-19 | Severe COVID-19 | V1 pre | V1 post | V2 pre | V2 post | V3 pre | V3 post | V4 pre | V4 post | V5 pre | V5 post | V6 pre | V6 post |
| GLTGTGGG | | | | | | | | | | | | | | | |
| RASANLAGG | | | | | | | | | | | | | | | |
| QALNTLGG | | | | | | | | | | | | | | | |
| GLTVLPPLLT | | | | | | | | | | | | | | | |
| ASANLAAT | | | | | | | | | | | | | | | |
| GVTONVGG | | | | | | | | | | | | | | | |
| TSCCSCLGG | | | | | | | | | | | | | | | |
| LLLQYGSGG | | | | | | | | | | | | | | | |
| VKQLSSNFGA | | | | | | | | | | | | | | | |
| LQDVVNQNAQALNTL | | | | | | | | | | | | | | | |
| SCCSCLGG | | | | | | | | | | | | | | | |
| ESLIDLGG | | | | | | | | | | | | | | | |
| SSTASALGG | | | | | | | | | | | | | | | |
| KLNDLCFGG | | | | | | | | | | | | | | | |
| FLPFFSNGG | | | | | | | | | | | | | | | |
| IGAGICAGG | | | | | | | | | | | | | | | |
| EIDRLNEGG | | | | | | | | | | | | | | | |
| LDKYFKNGG | | | | | | | | | | | | | | | |
| VSGTNGTGG | | | | | | | | | | | | | | | |
| FKNLREFGG | | | | | | | | | | | | | | | |
| LPPLLTDGG | | | | | | | | | | | | | | | |
| DKVFRSSGG | | | | | | | | | | | | | | | |
| LVKQLSSGG | | | | | | | | | | | | | | | |
| VTLADAGGG | | | | | | | | | | | | | | | |
| RRARSVAS | | | | | | | | | | | | | | | |
| SPRRARSGG | | | | | | | | | | | | | | | |
| KVEAEVQGG | | | | | | | | | | | | | | | |
| TRFQTLLGG | | | | | | | | | | | | | | | |
| VYSTGSNGG | | | | | | | | | | | | | | | |
| GLTVLPPGG | | | | | | | | | | | | | | | |
| SLLIVNNGG | | | | | | | | | | | | | | | |
| DEDDSEPV | | | | | | | | | | | | | | | |
| NASVVNIGG | | | | | | | | | | | | | | | |
| LIRAAEIGG | | | | | | | | | | | | | | | |
| TGRLQSLGG | | | | | | | | | | | | | | | |
| DEVRQIAGG | | | | | | | | | | | | | | | |
| SSSGWTAGG | | | | | | | | | | | | | | | |

IgG Raw Fluorescence Intensity
200 1000 ≥5000

**Fig. 5. IgG responses against shared epitopes in SARS-CoV-2 and human proteins in COVID-19 patient sera.** Shown is the IgG reactivity pattern for (A) all epitopes printed on the peptide microarray and (B) the most frequently recognized epitopes in infected individuals. Data are presented as heat maps showing the raw fluorescence intensities for each epitope and individual patient serum. The color coding correspond to low (light green) to high signal intensities (dark green). **Few antibody responses to the selected 5-14mer peptides shared between SARS-CoV-2 and human proteins.**