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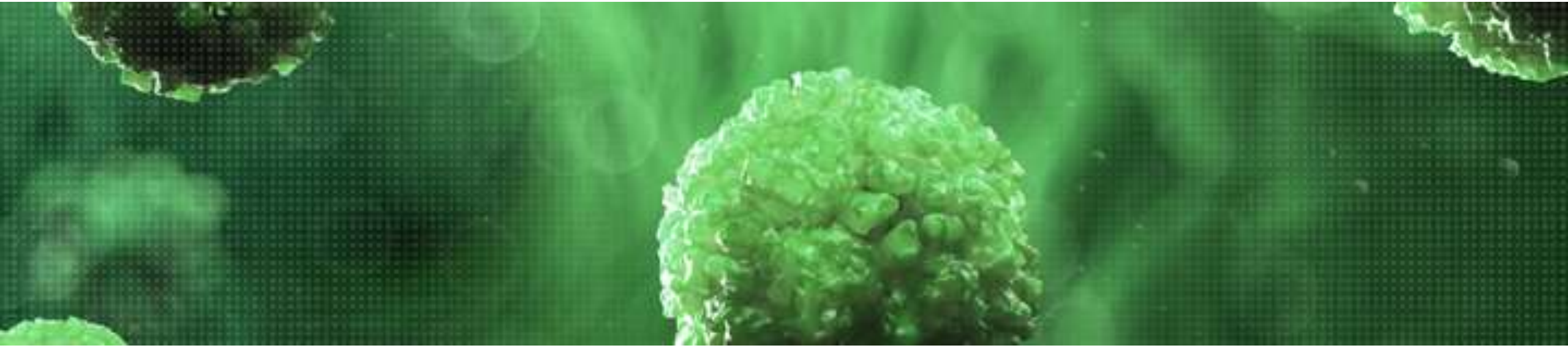
Viroscience lab
WHERE SKILLS MEET TO STUDY & PROTECT



SARS-CoV-2-specific T-cell responses in COVID-19 patients

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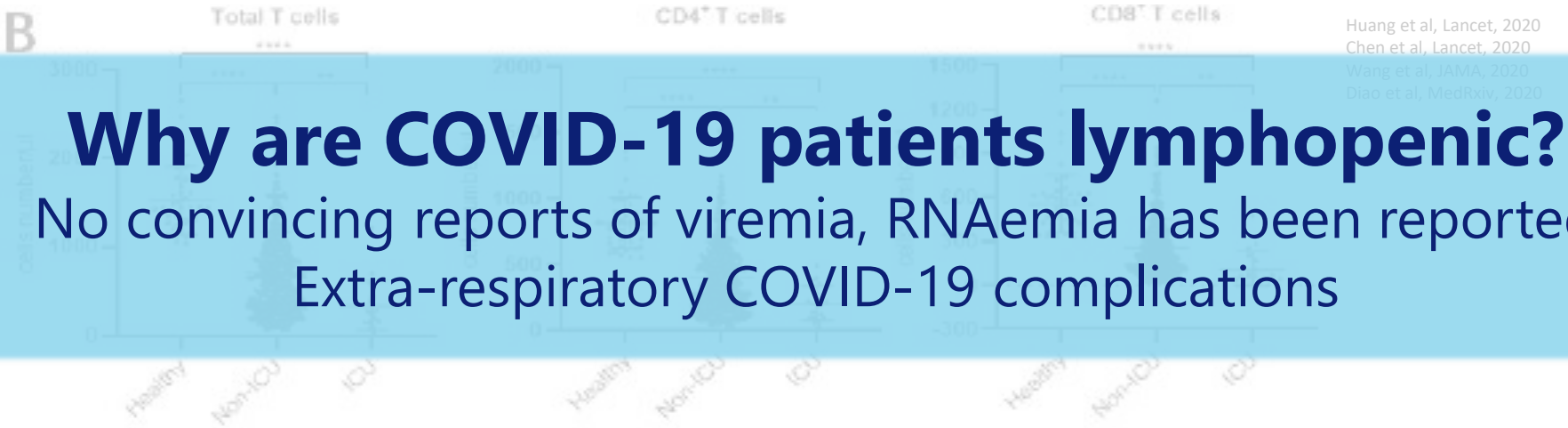
Department of Viroscience
Erasmus MC, Rotterdam
the Netherlands

Role for T-cells in COVID-19

- COVID-19 patients present with low $CD3^+$, $CD4^+$ and $CD8^+$ T cell counts



Huang et al, Lancet, 2020
Chen et al, Lancet, 2020
Wang et al, JAMA, 2020
Diao et al, MedRxiv, 2020

Why are COVID-19 patients lymphopenic?

No convincing reports of viremia, RNAemia has been reported

Extra-respiratory COVID-19 complications

- Lymphopenia is associated with disease severity

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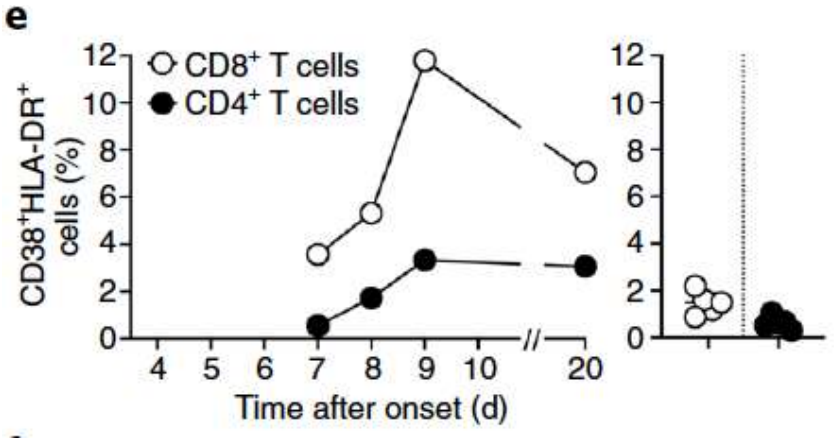
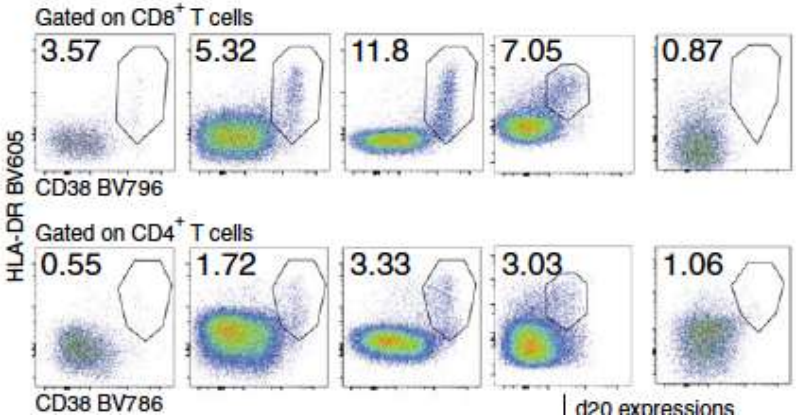
T-cell activation in COVID-19

- **Increase in activated T-cells in PBMC fraction during COVID-19**





Thevarajan et al, Nat Med, 2020

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SARS-CoV-2-specific T-cells

- What do we know about SARS-CoV-2-specific T-cells?
  - ±10 published papers out, many more prepints
  - Role of SARS-CoV-2-specific T-cells in disease is not yet understood
    - Clearance vs immunopathology
- $CD4^+$ T-cell responses were correlated to positive outcomes in SARS-CoV

Lymphopenia and immune hyperresponsiveness
complex interaction between SARS-CoV-2 and the immune system
that is not fully understood

Channappanavar et al, Imm Res, 2014
Li et al, J Immunol, 2008

- Immune hyperreactivity and high levels of cytokines observed in severe COVID-19
  - IL-6, IL-10, IP10, etc

Zhou et al, Lancet, 2020
Evangelos et al, Cell, 2020

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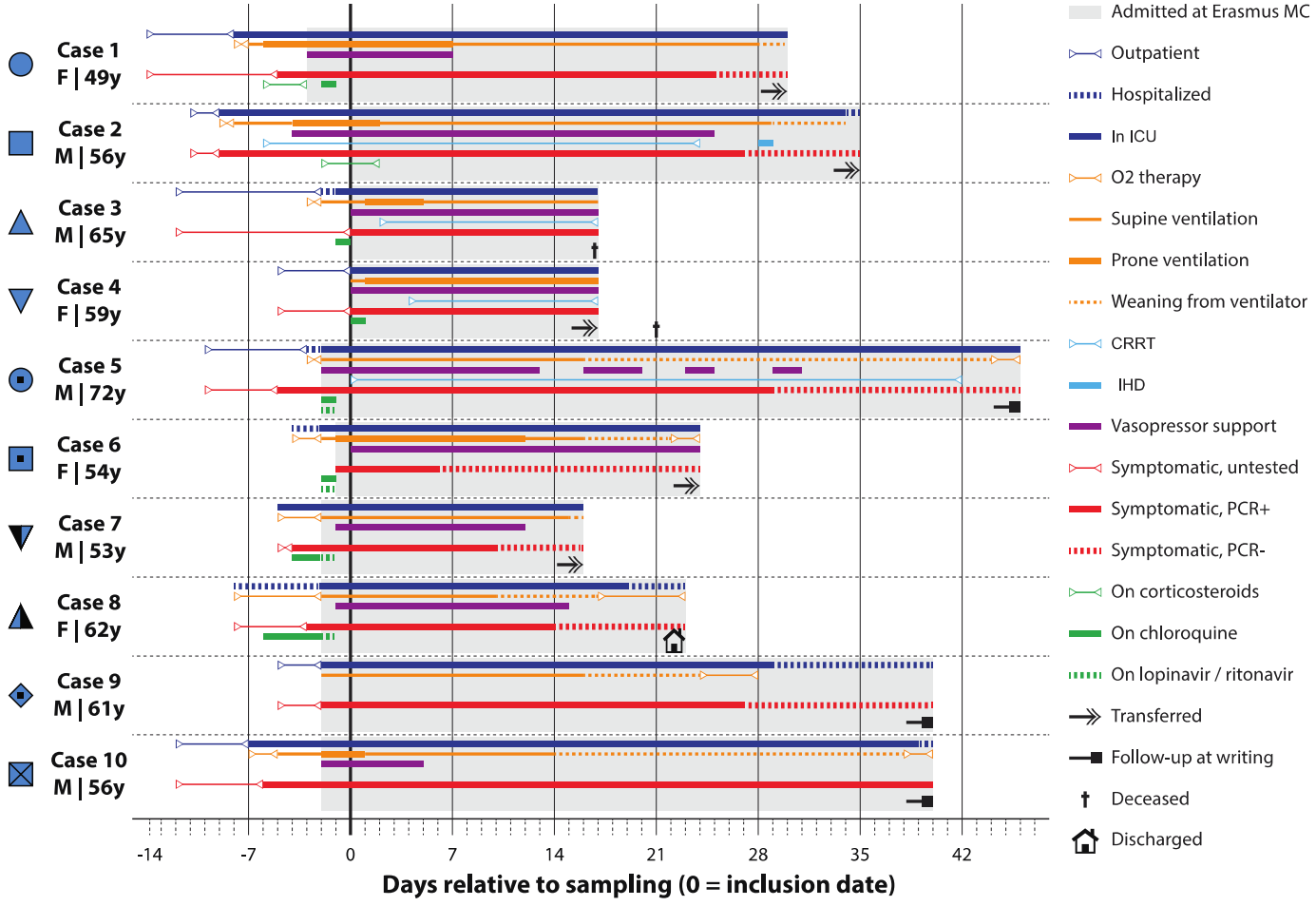
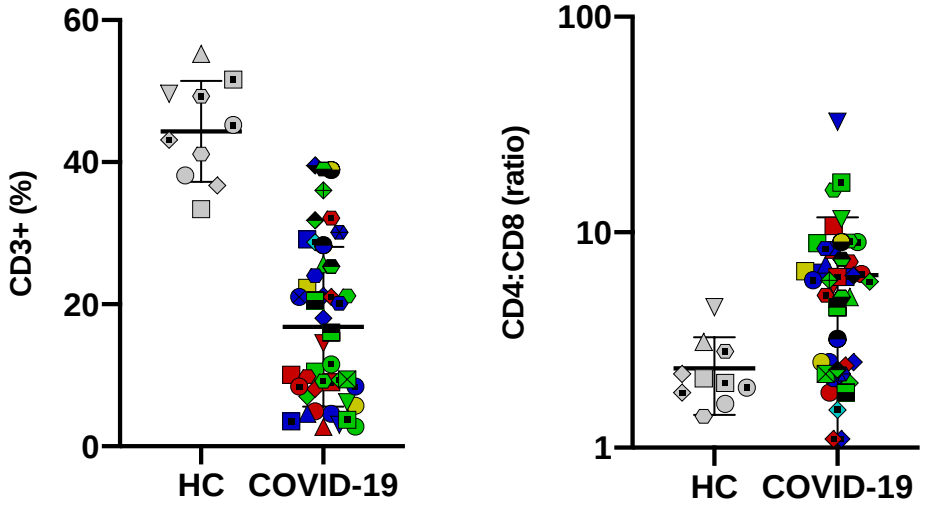
Study cohort (Acute Respiratory Distress Syndrome)



- **Study cohort**
  - N=10 COVID-19 ARDS patients (expanding)
  - N=10 age-matched healthy controls



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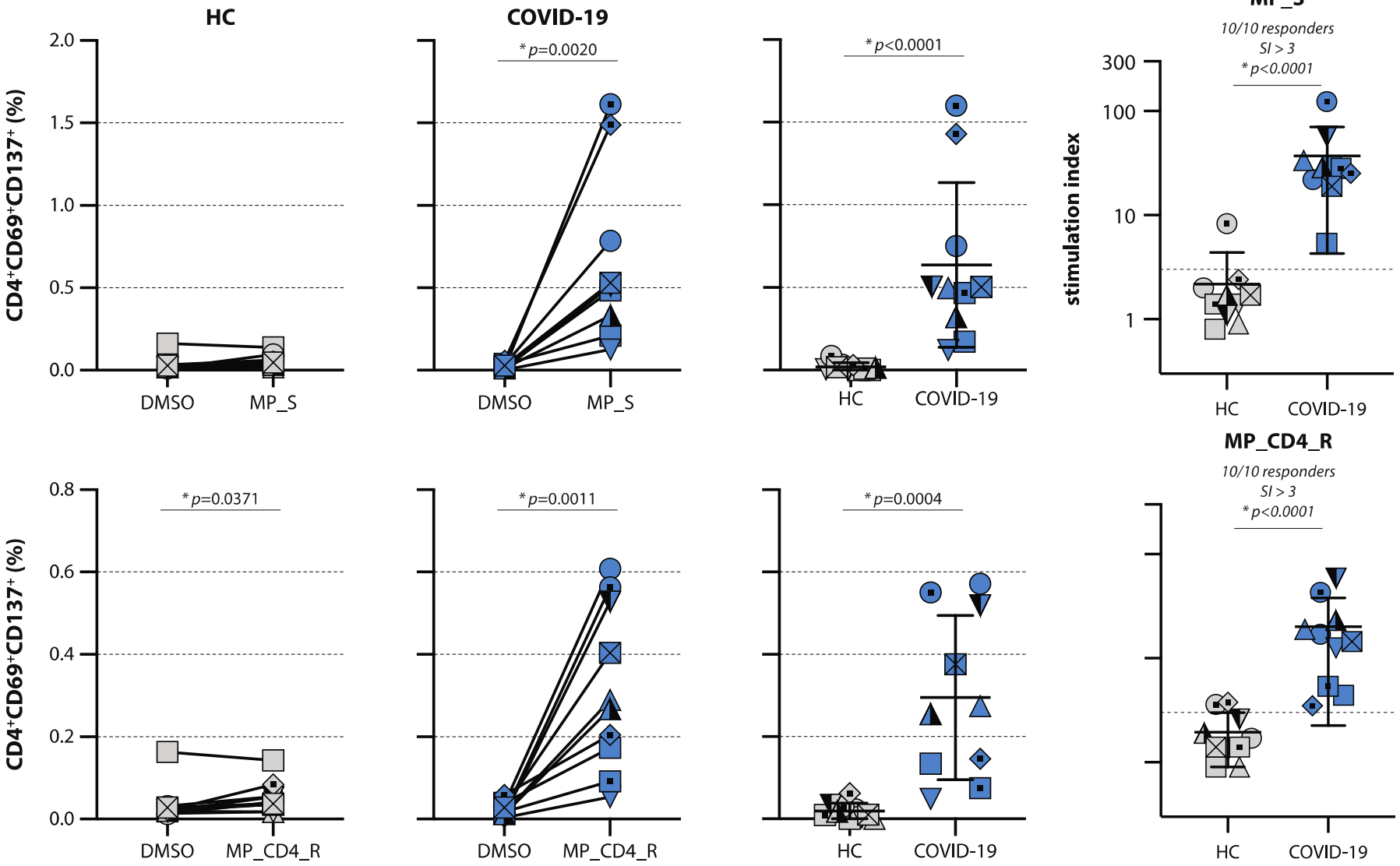
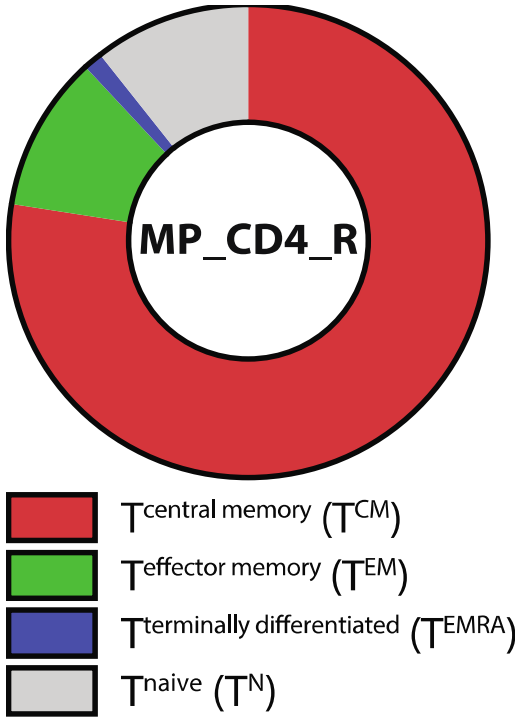
SARS-CoV-2-specific CD4$^+$ T-cells





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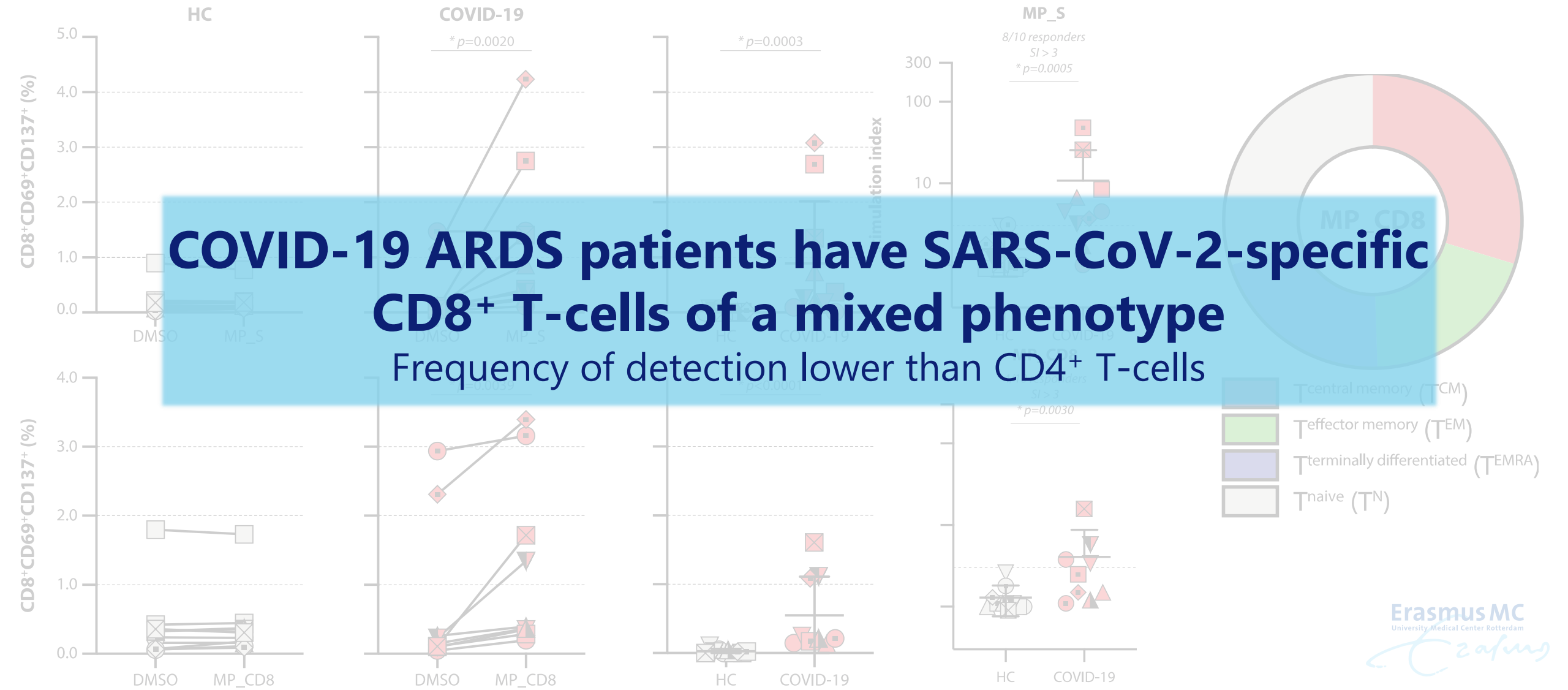
SARS-CoV-2-specific CD8+ T-cells



COVID-19 ARDS patients have SARS-CoV-2-specific CD8+ T-cells of a mixed phenotype

Frequency of detection lower than CD4+ T-cells

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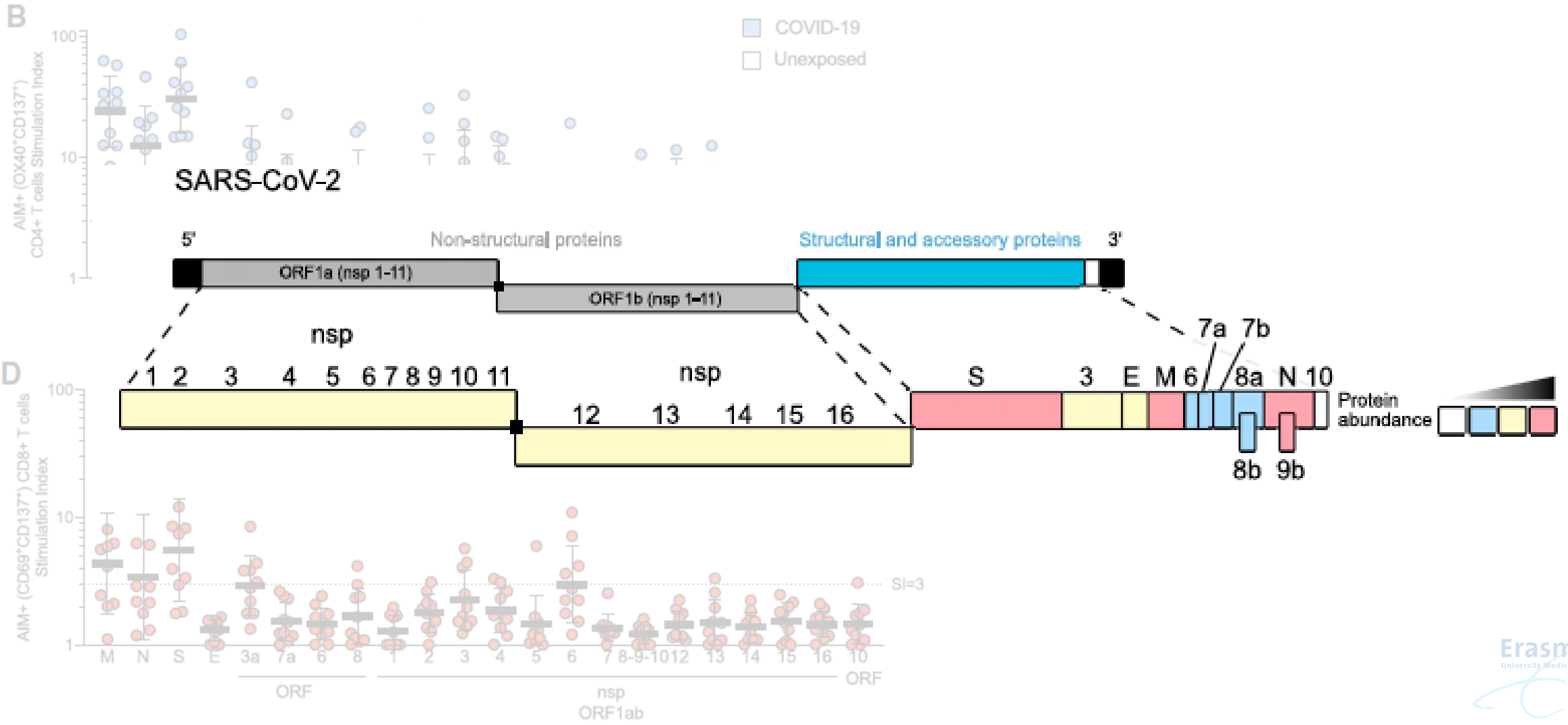
S, M and N are the prominent targets



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SARS-CoV-2-specific T-cells - OVERVIEW

	$CD4^+$ MP_S	$CD4^+$ MP_CD4_R	$CD8^+$ MP_S	$CD8^+$ MP_CD8
Weiskopf et al, Sci Imm, 2020				
HC	1/10 (10%)	2/10 (20%)	1/10 (10%)	0/10 (0%)
COVID-19	40/45 (89%)	30/42 (71%)	27/45 (60%)	15/41 (37%)
Braun et al, Nature, 2020				
HC	24/68 (35%)			
COVID-19	15/18 (83%)			
Grifoni et al, Cell, 2020				
HC	1/11 (9%)	4/11 (36%)		1/10 (10%)
COVID-19	10/10 (100%)	10/10 (100%)		7/10 (70%)

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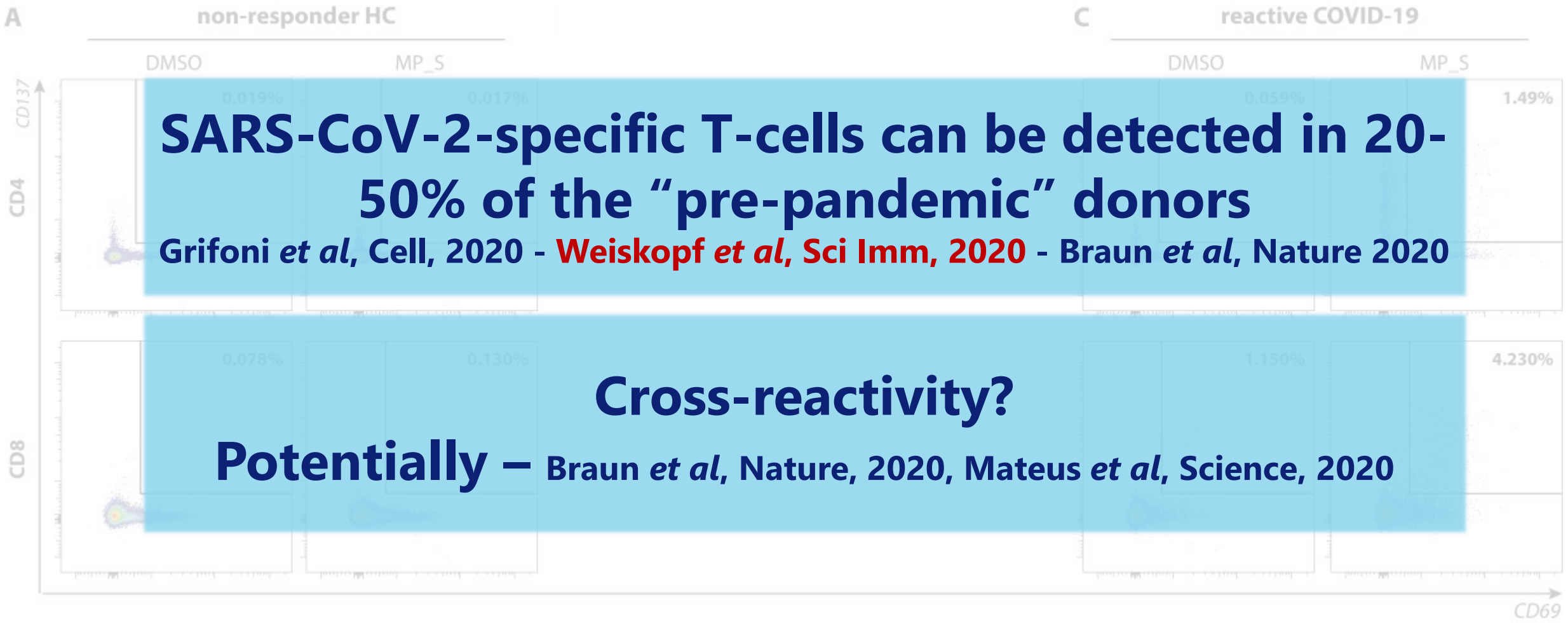
Cross-reactive T-cells in HC



SARS-CoV-2-specific T-cells can be detected in 20-50% of the "pre-pandemic" donors

Grifoni *et al*, Cell, 2020 - Weiskopf *et al*, Sci Imm, 2020 - Braun *et al*, Nature 2020

Cross-reactivity?

Potentially – Braun *et al*, Nature, 2020, Mateus *et al*, Science, 2020

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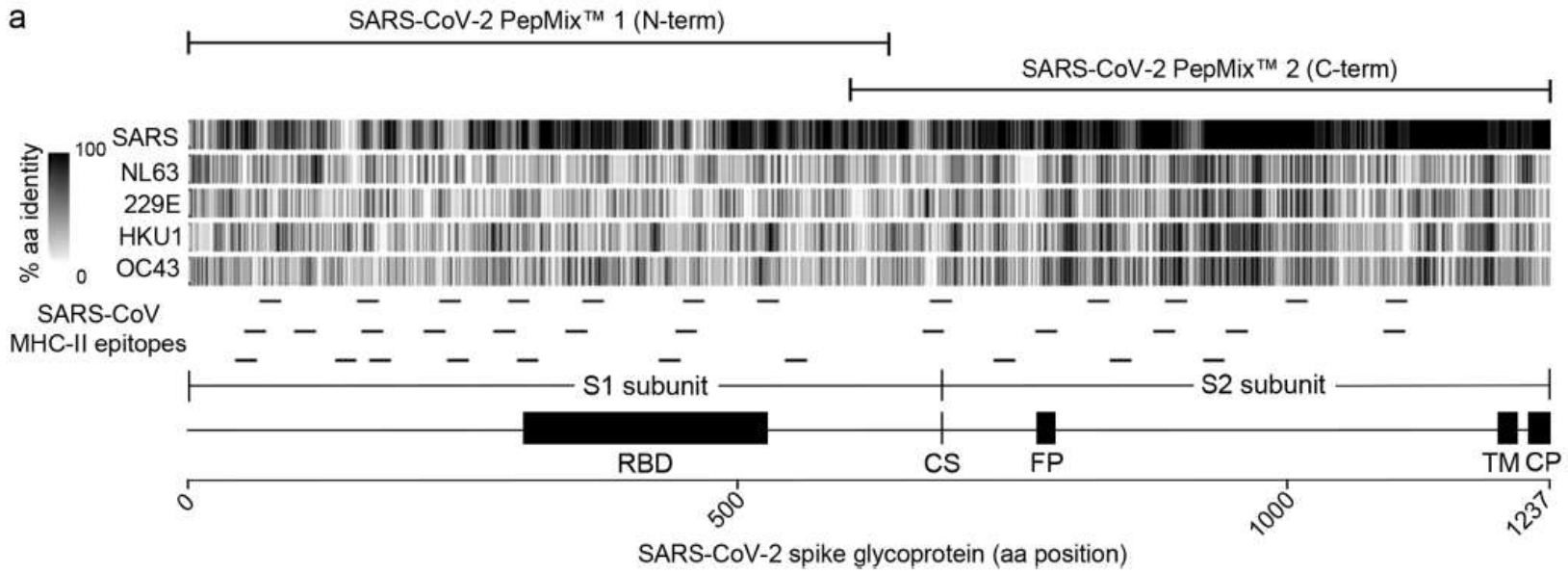
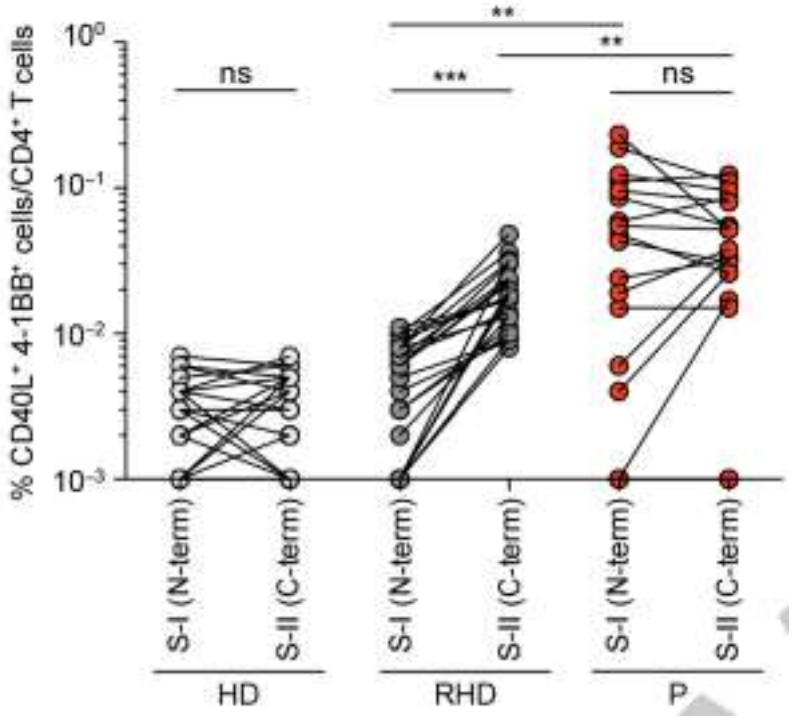
Cross-reactive T-cells in HC





Braun et al, Nature, 2020

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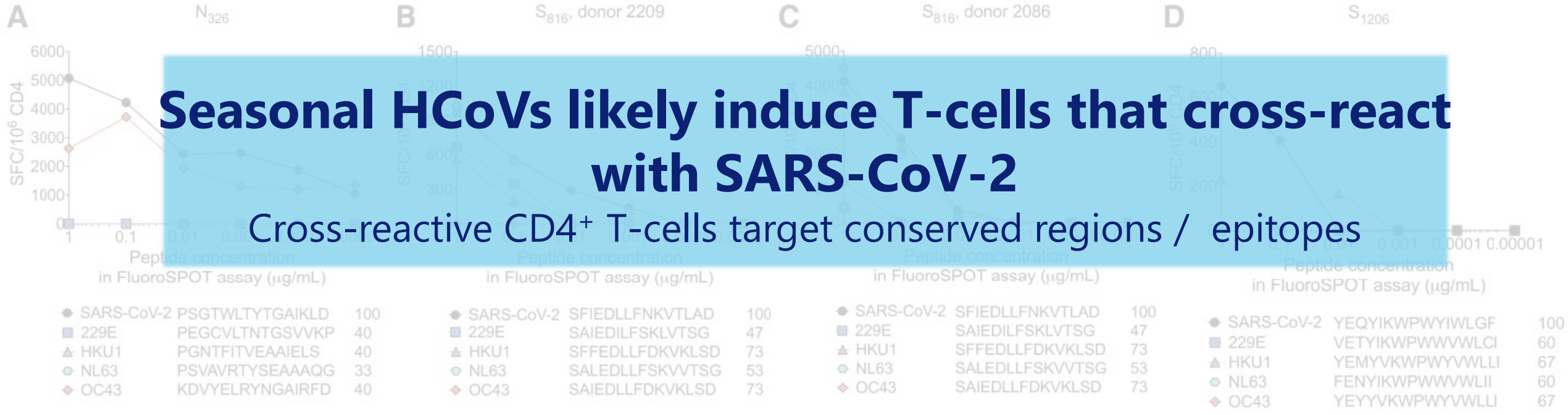
Cross-reactive T-cells in HC

- T-cell lines generated after peptide stimulation of PBMC from pre-pandemic donors



Seasonal HCoVs likely induce T-cells that cross-react with SARS-CoV-2

Cross-reactive CD4$^+$ T-cells target conserved regions / epitopes

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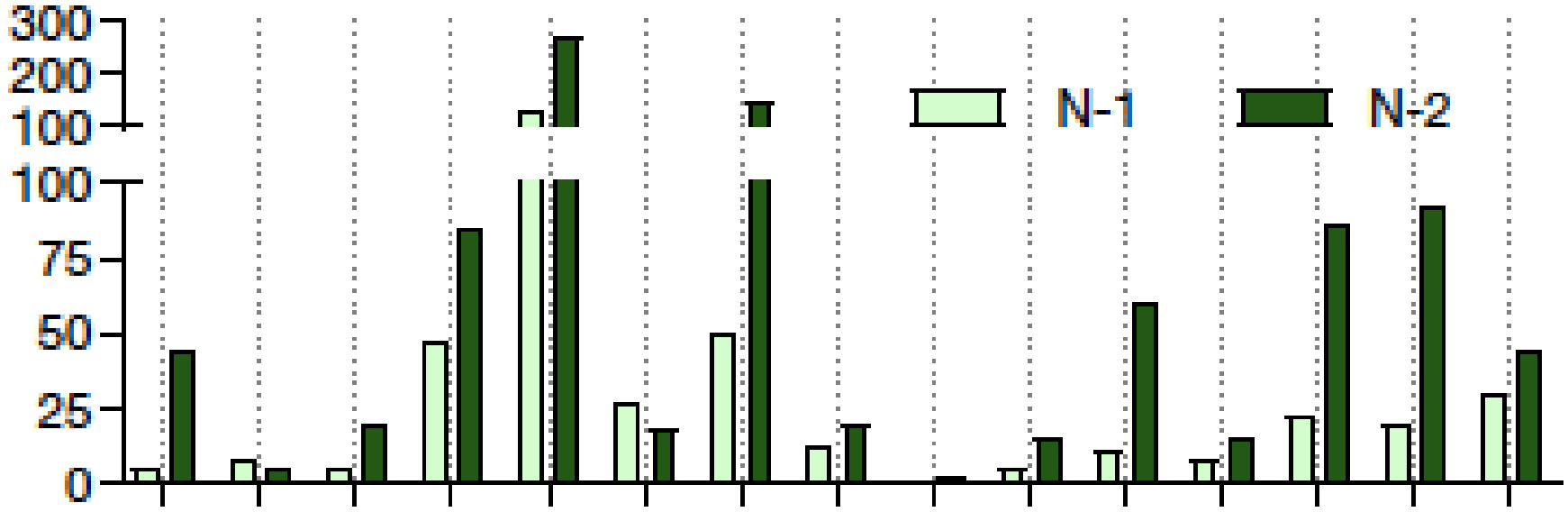
SARS-CoV-specific T-cells are long-lived

- **PBMC isolated from SARS recovered individuals 17 years after disease**
  - Stimulated with N peptide pool



Le bert et al, Nature, 2020

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Summary and Discussion

- SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cells detected in blood of COVID-19 patients
  - S, M and N are the dominant targets
- (Dominant effector and Th1) cytokine production in response to viral antigen
  - No detection of production of IL-6 in PBMC stimulated with peptide pools due to high background production in controls
- Reactive T cells were detected in HC after MP stimulation → cross-reactivity
  - Also reported by Grifoni *et al* (US), Braun *et al* (Germany), Le Bert *et al* (Singapore) and Meckiff *et al* (UK)
  - Possible induction by circulating seasonal coronaviruses (HCoV-viruses) (Mateus *et al*)
- SARS-CoV-specific T-cells are long-lived
- Detection of SARS-CoV-2-specific T-cells is an accurate measure of exposure

What is the role of (cross-reactive) T-cells in (prevention of) disease??

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- Katharina Schmitz
- Matthijs Raadsen
- Nisreen Okba
- Richard Molenkamp
- Marion Koopmans
- Bart Haagmans
- Eric van Gorp
- Rik de Swart

Erasmus MC, ICU

- Henrik Endeman
- Johannes vd Akker

LJI, San Diego, USA

- Daniela Weiskopf
- Alba Grifoni
- Alessandro Sette

MegaPool (MP) stimulations

- **Method:** stimulate PBMC with peptide pools, detect T cell activation

- **Specific peptide pools used (MegaPools, MPs)**
  - MPs are pools with large numbers of peptides generated by sequential pooling and lyophilization.
  - Can incorporate **overlapping peptides**, or **predicted** or **experimentally validated epitopes**

Activation-Induced Markers (AIM)

- **Method:** stimulate PBMC with peptide pools, detect T cell activation
  - Upregulation of activation markers **CD69** and **CD137** (20h stimulation)
  - Intracellular detection of IFNγ and TNFα (8h stimulation)
  - Cytokines measured in culture sup with multiplex beads assay (20h stimulation)

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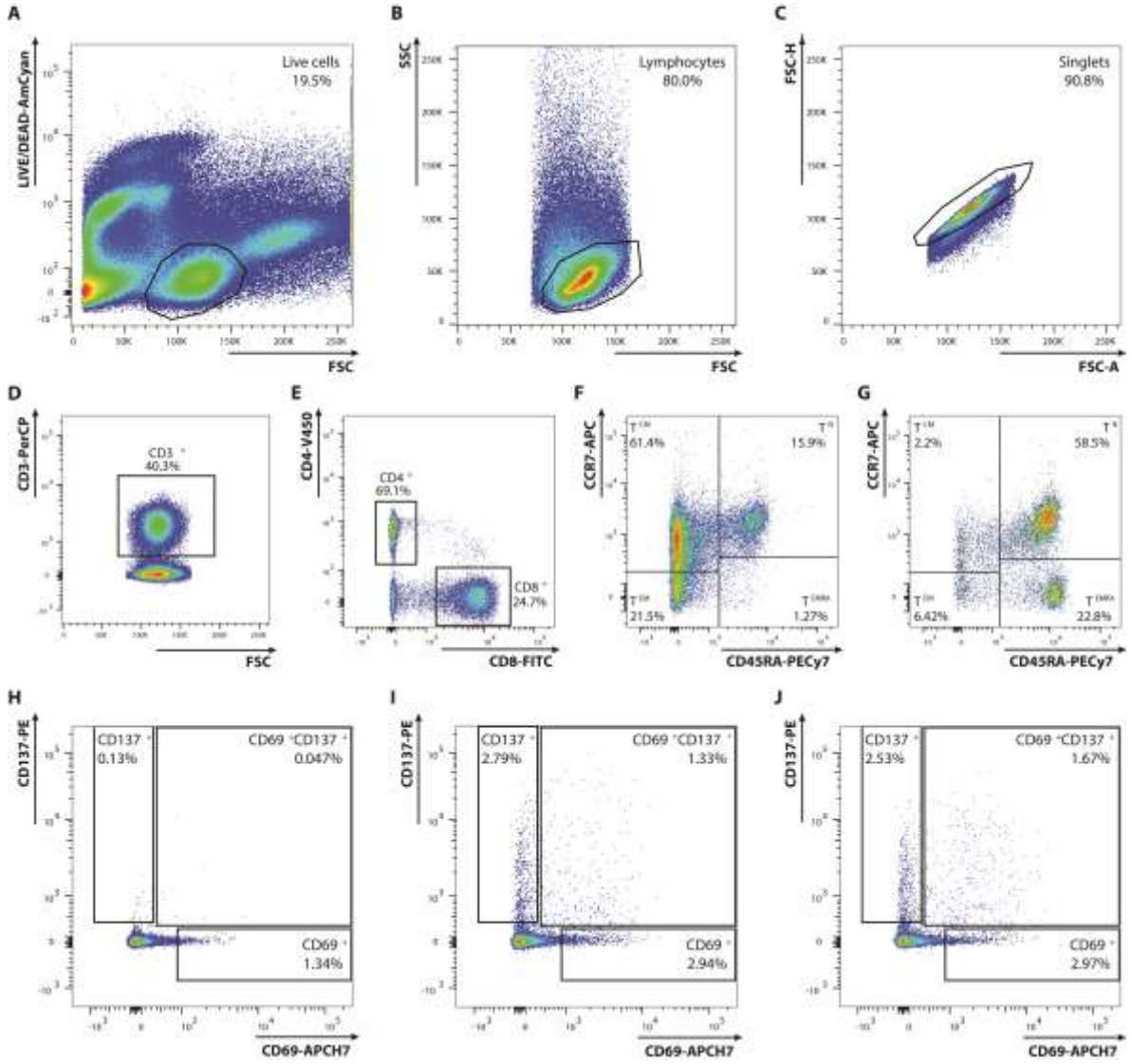
Activation-Induced Markers (AIM)



- Gating strategy:
  - A: LIVE cells
  - B: Lymphocytes FSC / SSC
  - C: Single cells
  - D: $CD3^+$ cells
  - E: $CD4^+$ / $CD8^+$ cells
  - F/G: Memory phenotyping on basis of CD45RA / CCR7
  - H: CD69 vs CD137 **DMSO (- control)**
  - I: CD69 vs CD137 **CMV (+ control)**
  - J: CD69 vs CD137 **MP_S**

- **Double positive cells in J used in graphs**

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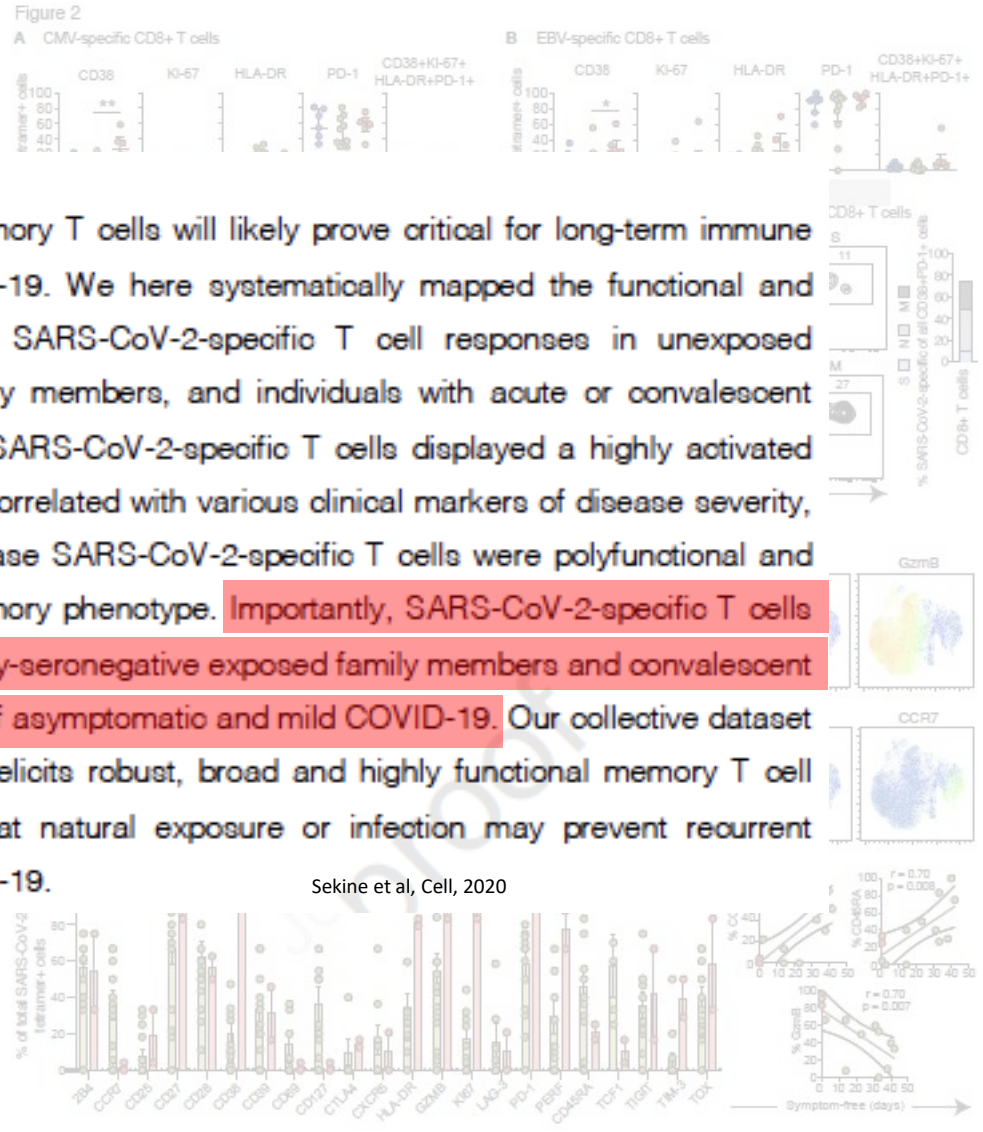
T-cells in absence of seroconversion



SUMMARY

SARS-CoV-2-specific memory T cells will likely prove critical for long-term immune protection against COVID-19. We here systematically mapped the functional and phenotypic landscape of SARS-CoV-2-specific T cell responses in unexposed individuals, exposed family members, and individuals with acute or convalescent COVID-19. Acute phase SARS-CoV-2-specific T cells displayed a highly activated cytotoxic phenotype that correlated with various clinical markers of disease severity, whereas convalescent phase SARS-CoV-2-specific T cells were polyfunctional and displayed a stem-like memory phenotype. Importantly, SARS-CoV-2-specific T cells were detectable in antibody-seronegative exposed family members and convalescent individuals with a history of asymptomatic and mild COVID-19. Our collective dataset shows that SARS-CoV-2 elicits robust, broad and highly functional memory T cell responses, suggesting that natural exposure or infection may prevent recurrent episodes of severe COVID-19.

Sekine et al, Cell, 2020

Summary and Discussion

ZonMw

The Netherlands Organisation for Health Research and Development
Programme: Off Road 2016-2017

Project summary

Coronaviruses are endemic in humans and often cause a self-limiting infection with common cold-like symptoms. Some of the zoonotic high-threat emerging viruses are also coronaviruses: MERS-coronavirus (CoV) has recently been identified as novel zoonotic agent and is continuing to spill over to humans. Virus-specific cytotoxic T-cells, considered crucial for viral clearance, have not yet been detected for human coronaviruses. The overall aim of this proposal is to detect T-cells specific for endemic- and MERS-CoV and determine whether these T-cells can be cross-reactive. This will be addressed by probing blood samples from unique MERS-CoV-infected patients from the Middle East for endemic coronavirus-specific, MERS-CoV-specific and cross-reactive T-cells. As a proof of principle, I will develop a mouse model to prove that endemic coronaviruses can protect from MERS-CoV-related disease.

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