NORTHWESTERN UNIVERSITY





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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection had increased the overall numbers of morbidity and mortality worldwide. Despite the advances in the production of COVID-19 vaccine peptides, there is still a need for an effective approach for patient response predictions (especially among immunocompromised patients) for better patient management and reduction of mortality.

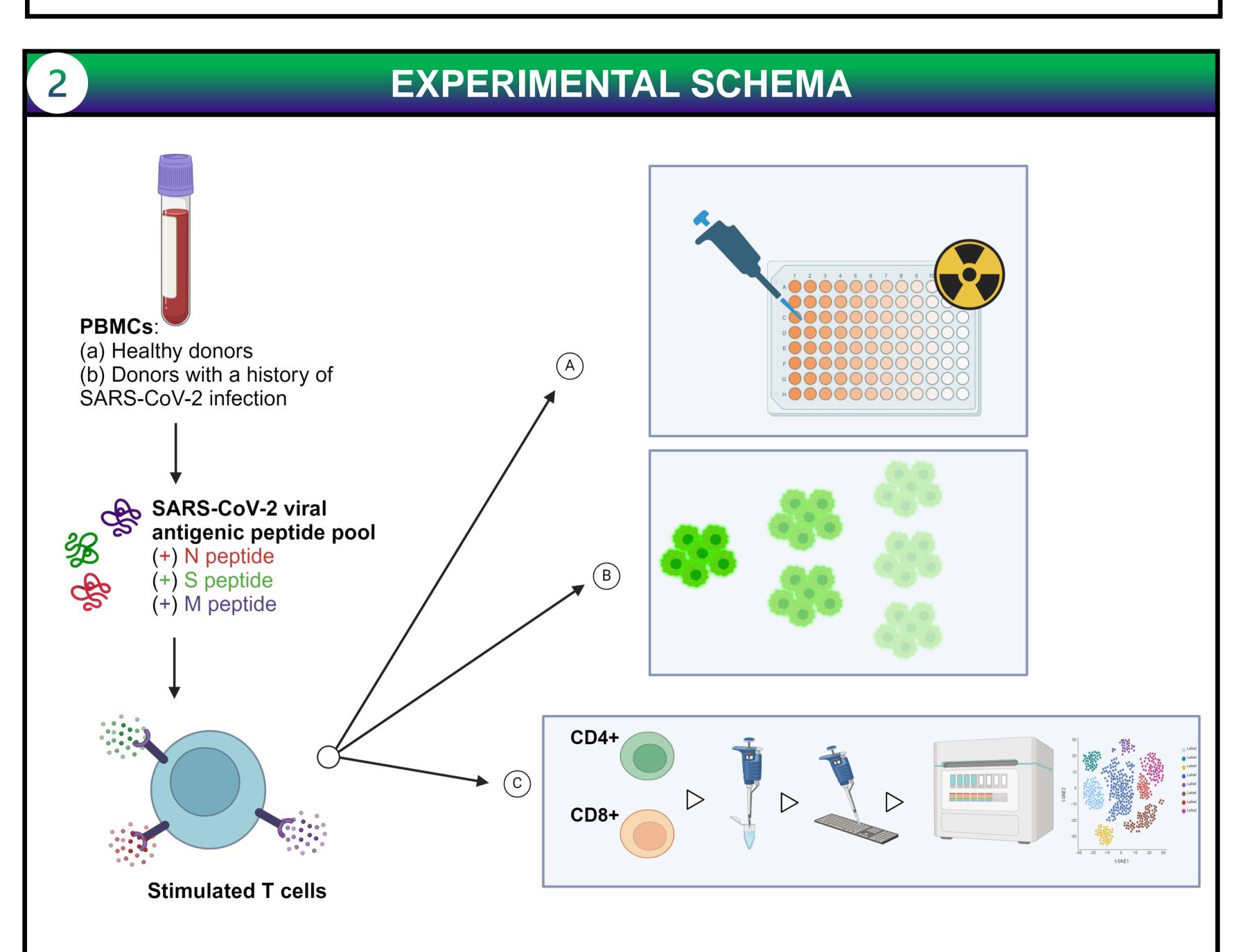


Figure 1(A-C). Experimental schema for the IsoLight assay. Peripheral Blood Mononuclear Cells (PBMCs) from human subjects with and without history of SARS-CoV-2 infection were stimulated with SARS-CoV-2 viral antigenic (S/M/N) peptide pool. The viral-specific responding cell proliferation was measured using (A) 3H-thymidine (3H-TdR) incorporation assay and the specific cell subsets that responded to SARS-CoV-2 antigenic stimulation were defined using the (B) carboxyfluorescein succinimidyl ester (CFSE) dilution assay. (C) Responder CD69+, CD4+ and CD8+ T cells were also isolated from the stimulated PBMCs, stained with fluorochrome-conjugated antibodies, and loaded onto an IsoCode chip for single cell deep functional analysis using the IsoPlexis' IsoLight technology.

SINGLE CELL DEEP FUNCTIONAL ANALYSIS: **A BETTER APPROACH TO PREDICT COVID-19 PATIENT RESPONSE & MANAGEMENT?**

DONORS WITH A HISTORY OF SARS-CoV-2 INFECTION SHOWED STRONG PROLIFERATIVE RESPONSES (A) COVID-19 SURVIVORS

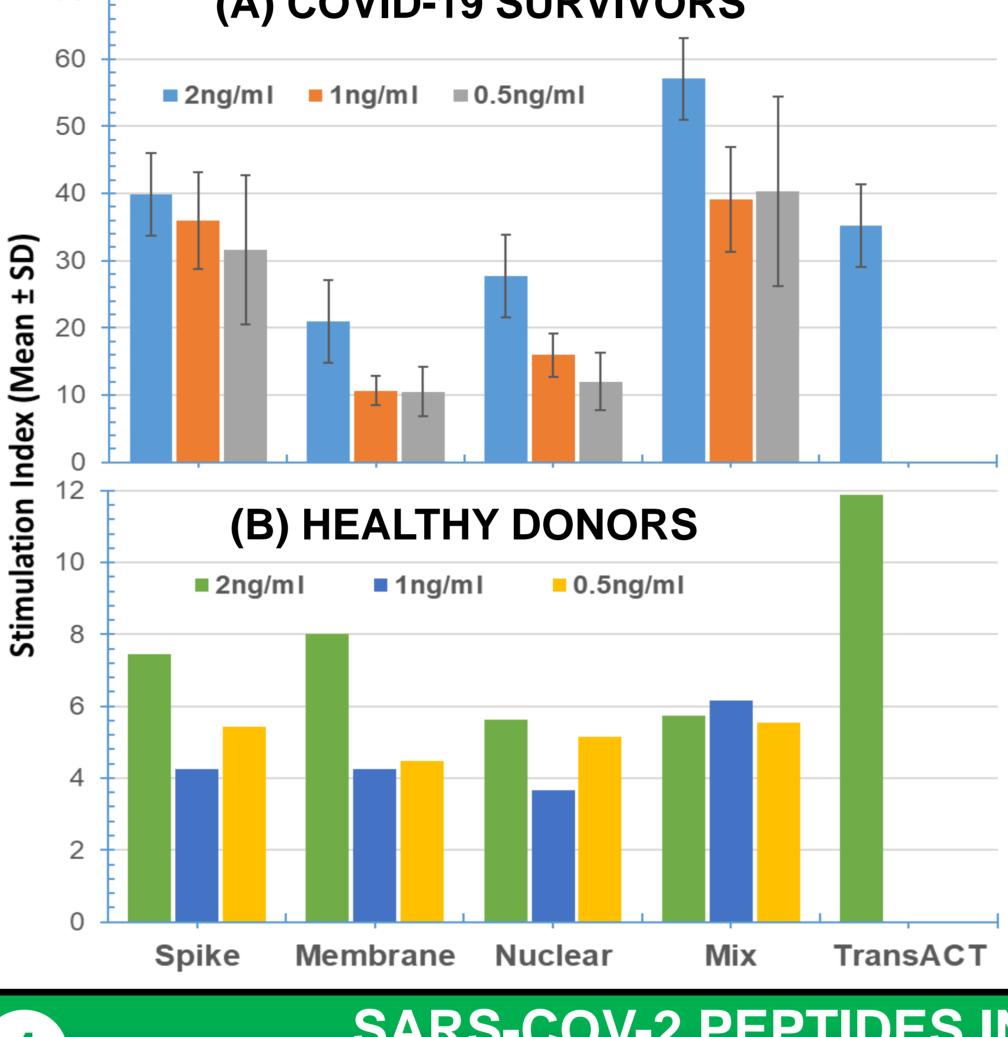


Figure 3. CFSE dilution assays were performed with PBMC from COVID-19 survivors and the responding T cell subsets were enumerated by flow cytometric analysis. The single cells were gated on live cells using ViaKrome 808 fixable live dead dye (Beckman-Coulter) prior to gating for CFSE diluting CD3 cells (lower bottom). A robust proliferation of CD3 cells (CD4 and CD8 subsets) and a minor population of non-T cells were observed.

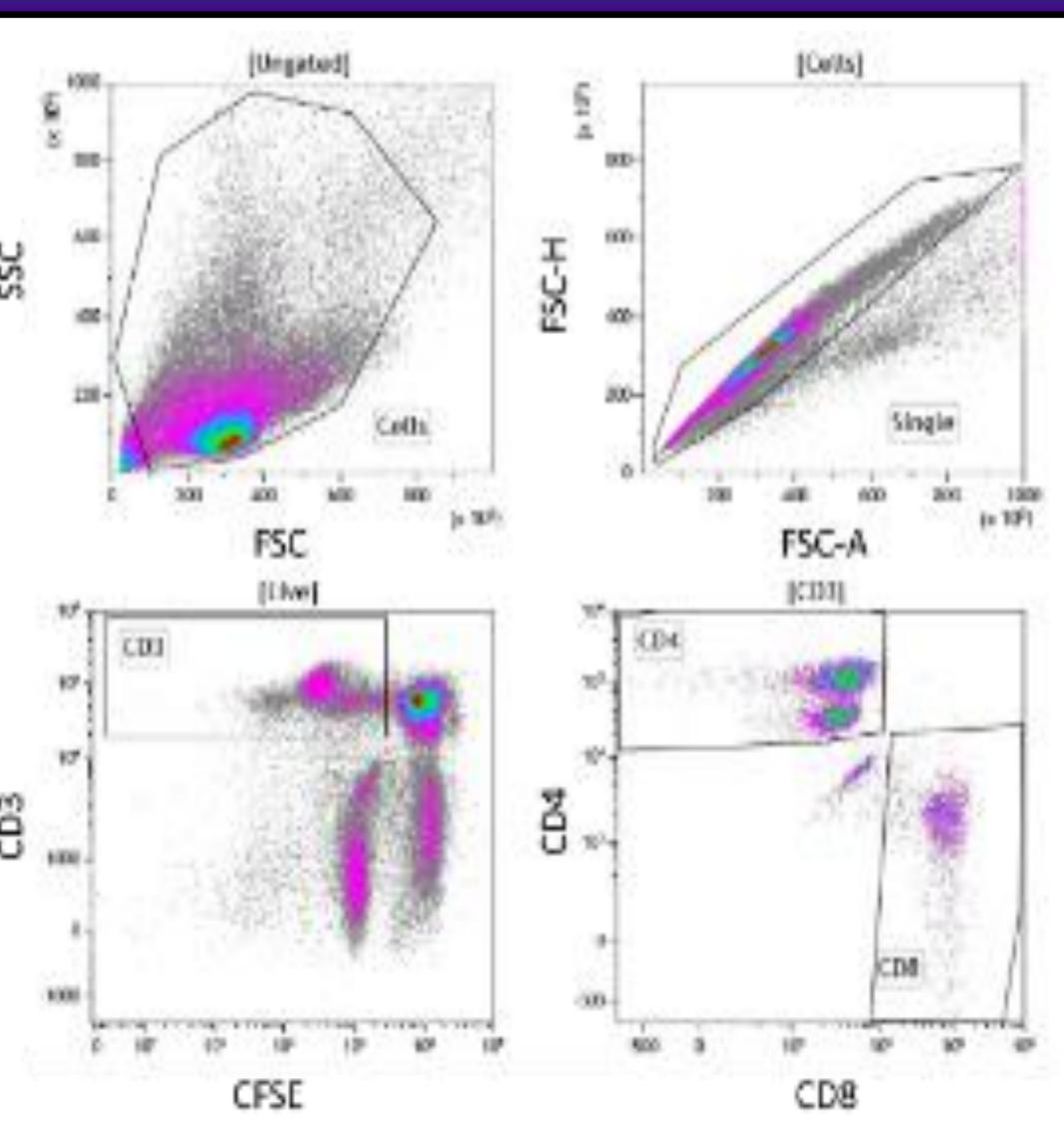
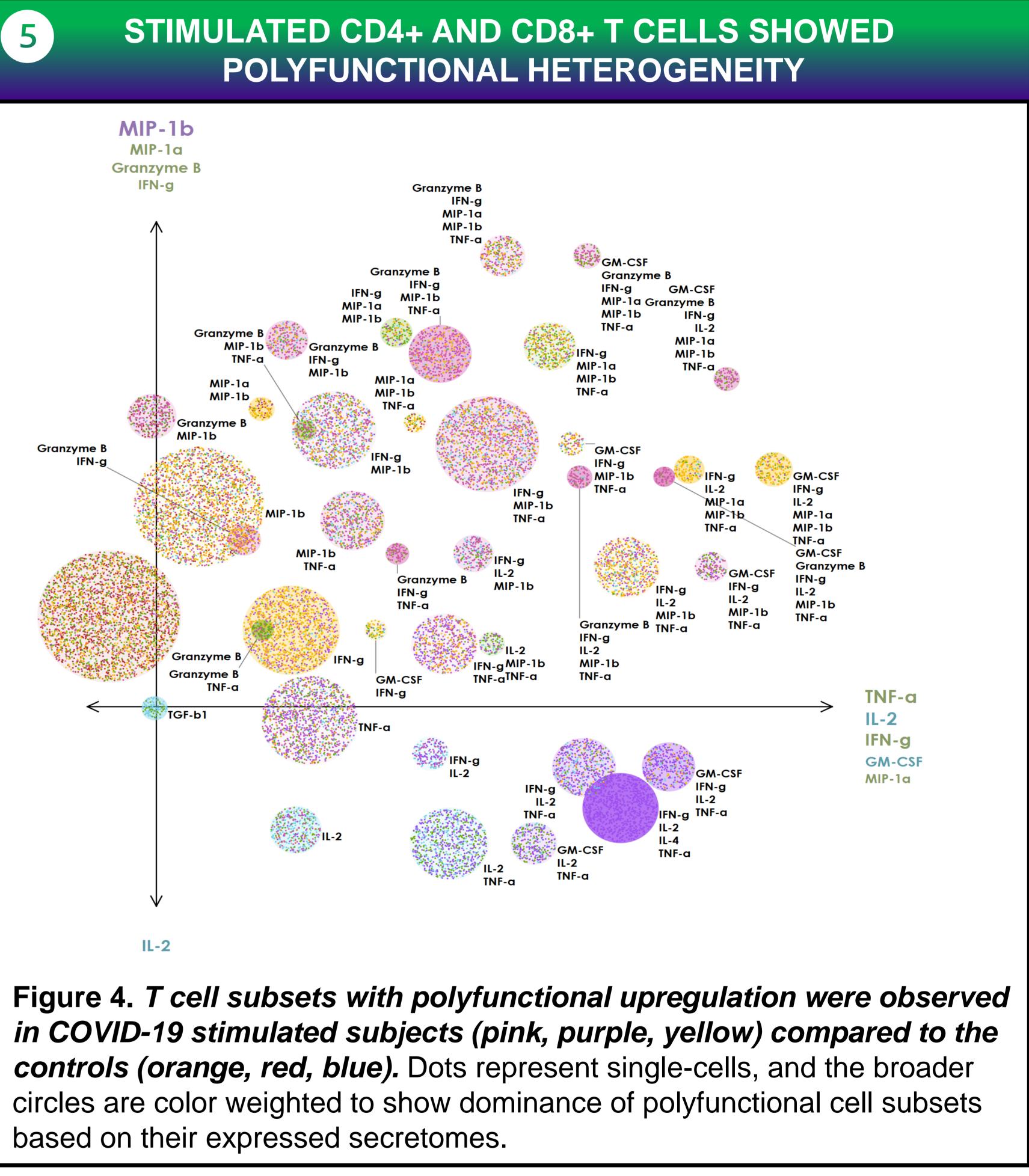


Figure 2(A-B). Proliferative **Responses to SARS-CoV-2** Peptides. 3H-Thymidine incorporation assays were performed, and the data were calculated as SI. Please note the differences in the Y-axis scale between COVID-19 survivor (top) and uninfected (bottom). Compared to the control, COVID-19 subjects with a history of infection had a very strong proliferative response, particularly to the spike protein, which even surpassed the positive control polyclonal stimulation.

SARS-COV-2 PEPTIDES INDUCED THE PROLIFERATION OF CD3 CELLS





Single cell deep functional analysis on T cells from COVID-19 responders based on the released secretomes/ proteins as compared to the conventional bulk cell analysis, would pave the path to ensure improved patient response prediction and management. This can be a stepping-stone to provide clear indication if immunosuppressed transplant patients will require modifications to the vaccination scheme.



CONCLUSION