

Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing

Wen et al.

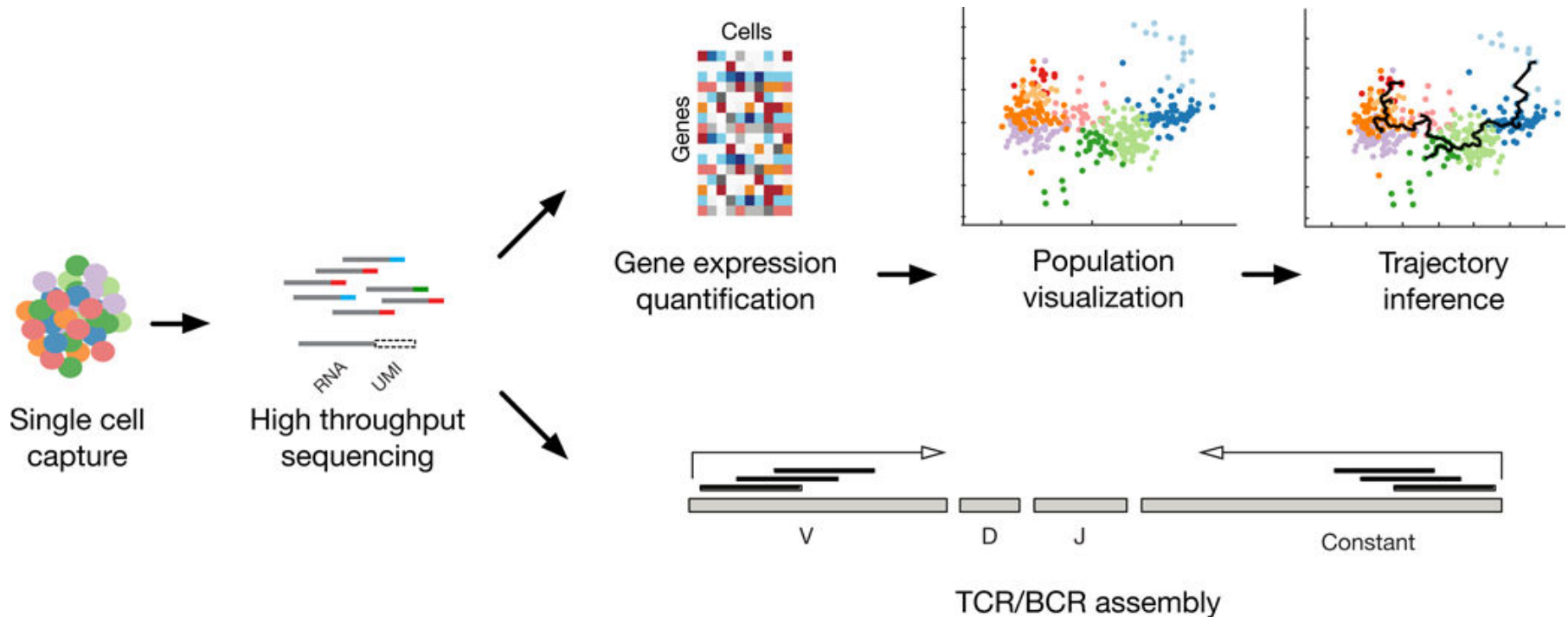
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Applications for single-cell RNAseq in immunology



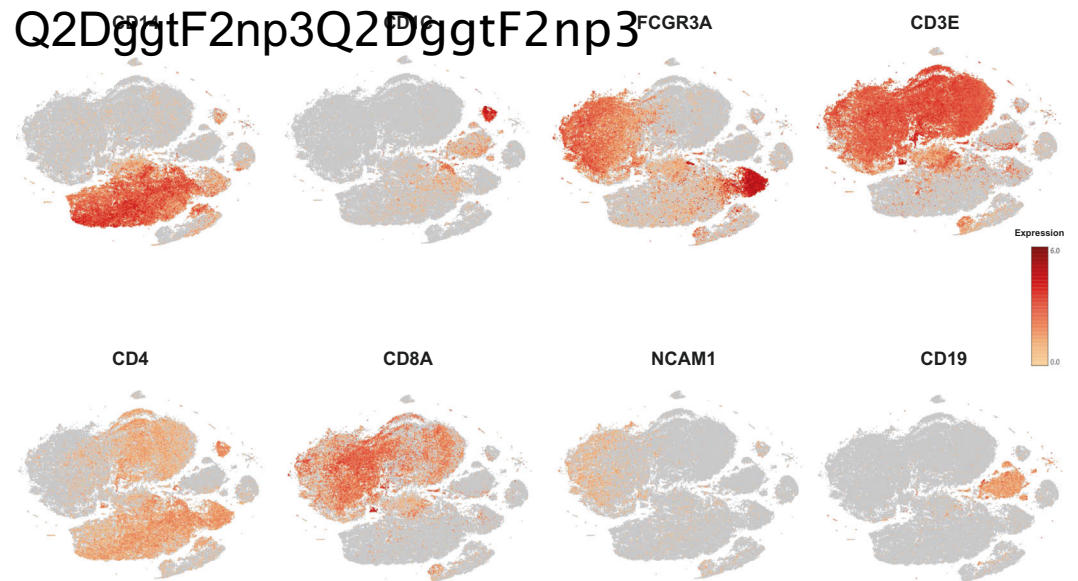
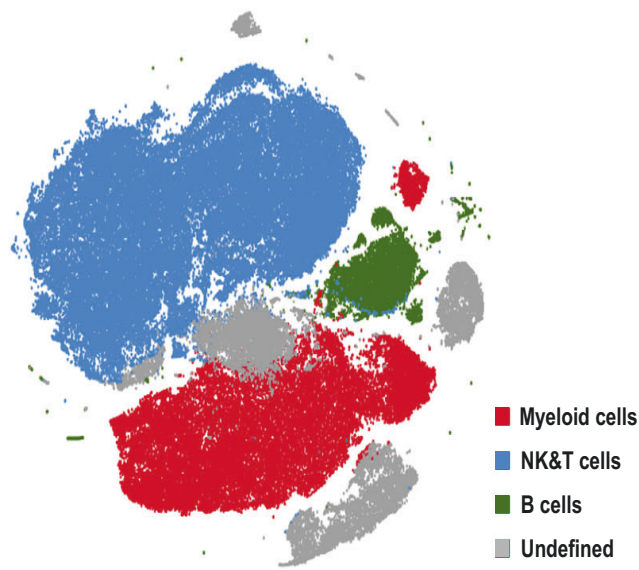
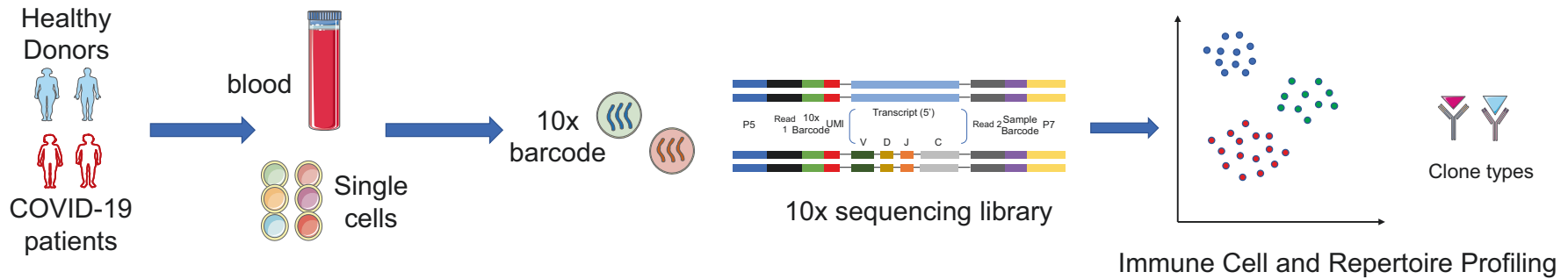
Identify different cell population/cell states
Characterization of TCR/BCR receptor clonality/specificity

Neu et.al Trends Immunol. 2017 February ; 38(2): 140-149. doi:10.1016/j.it.2016.12.001.

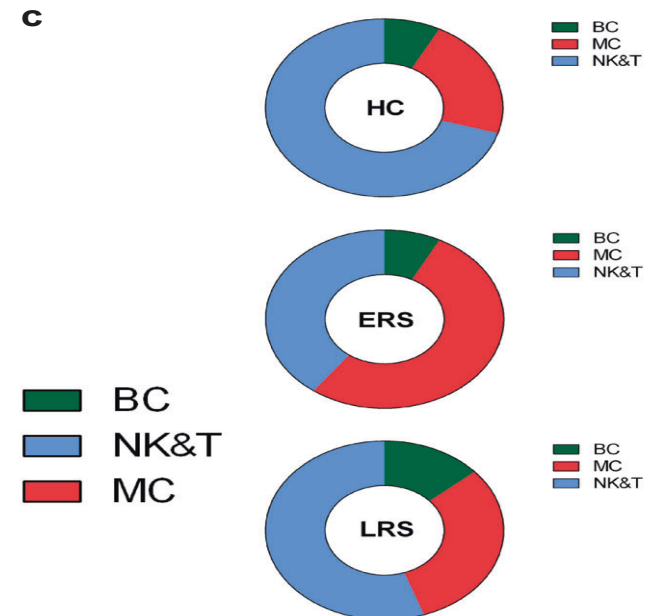
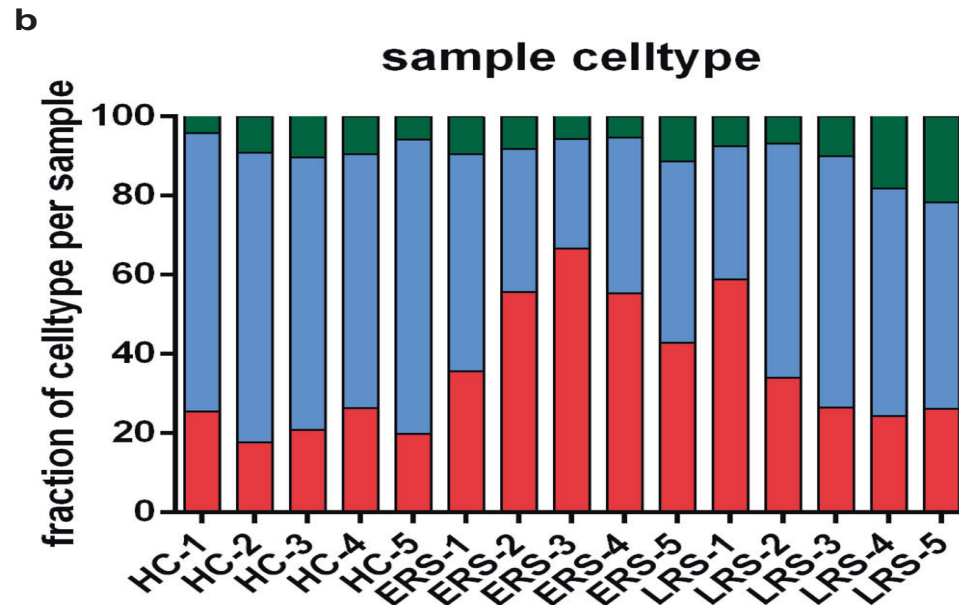
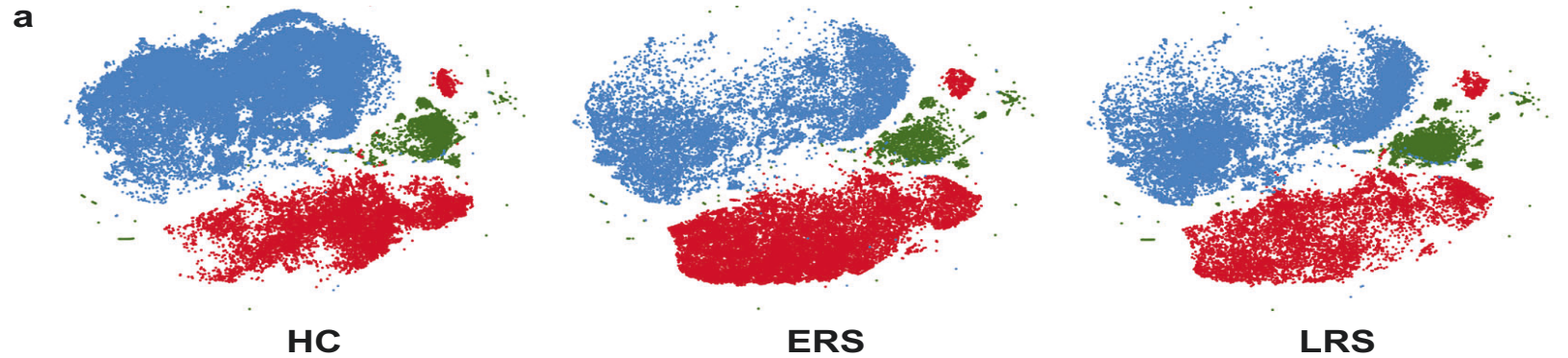
Material and Methods

- Blood samples from 10 COVID-19 patients
Early recovery stage (ERS) → time to neg. PCR < 7 days
Late recovery stage (LRS) → time to neg PCR > 14 days
- Preparation of single cell libraries bioinformatic processing with Seurat v3 in R (integration, dimensional reduction, clustering, differential gene expression ..)
- GO – Term analysis and KEGG pathway analysis (Metascape webtool)
- TCR/BCR V(D)J sequencing and analysis
- Cell – cell interaction analysis

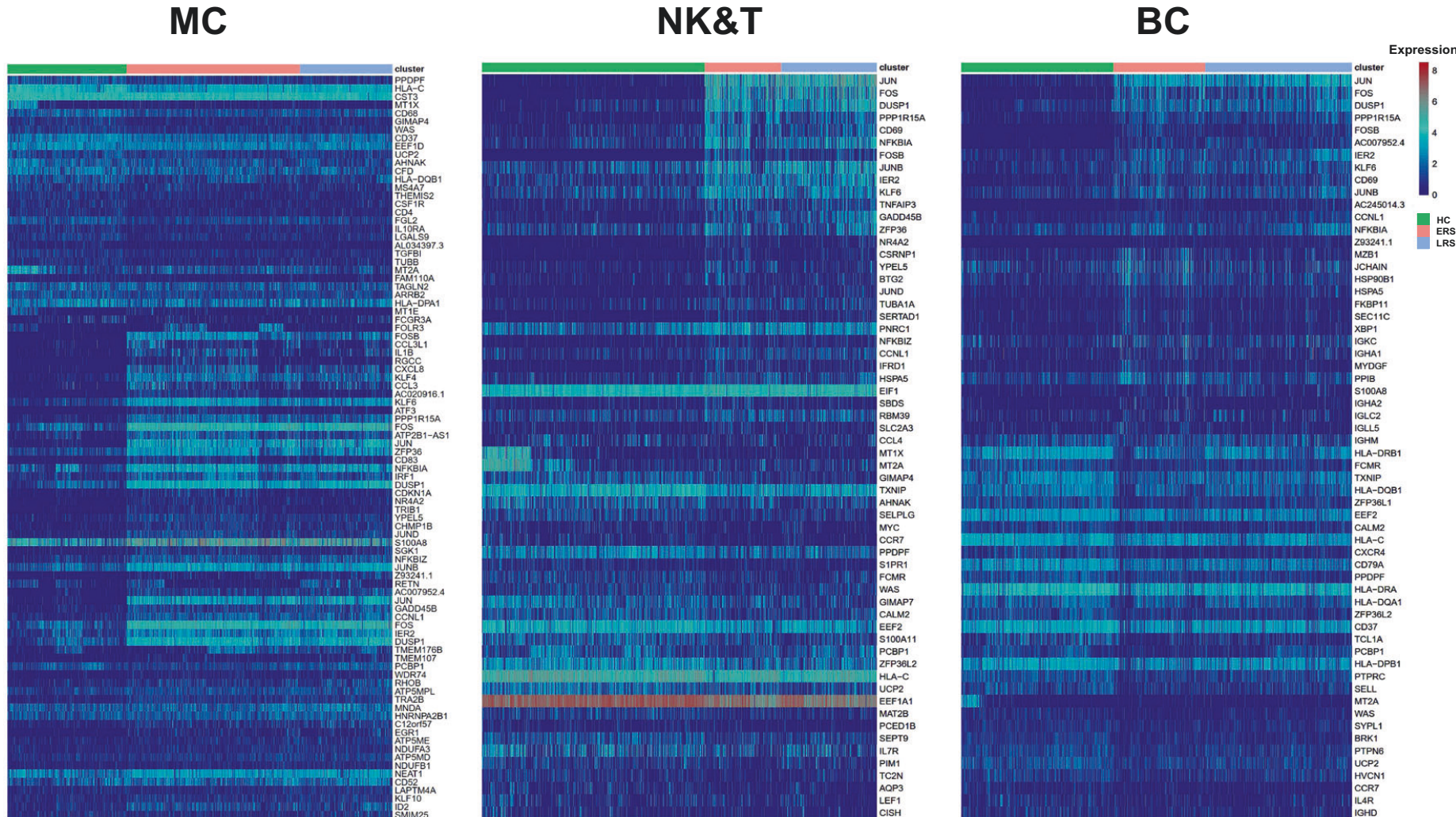
Study design and analysis of single immune cell profiling in COVID-19 patients.



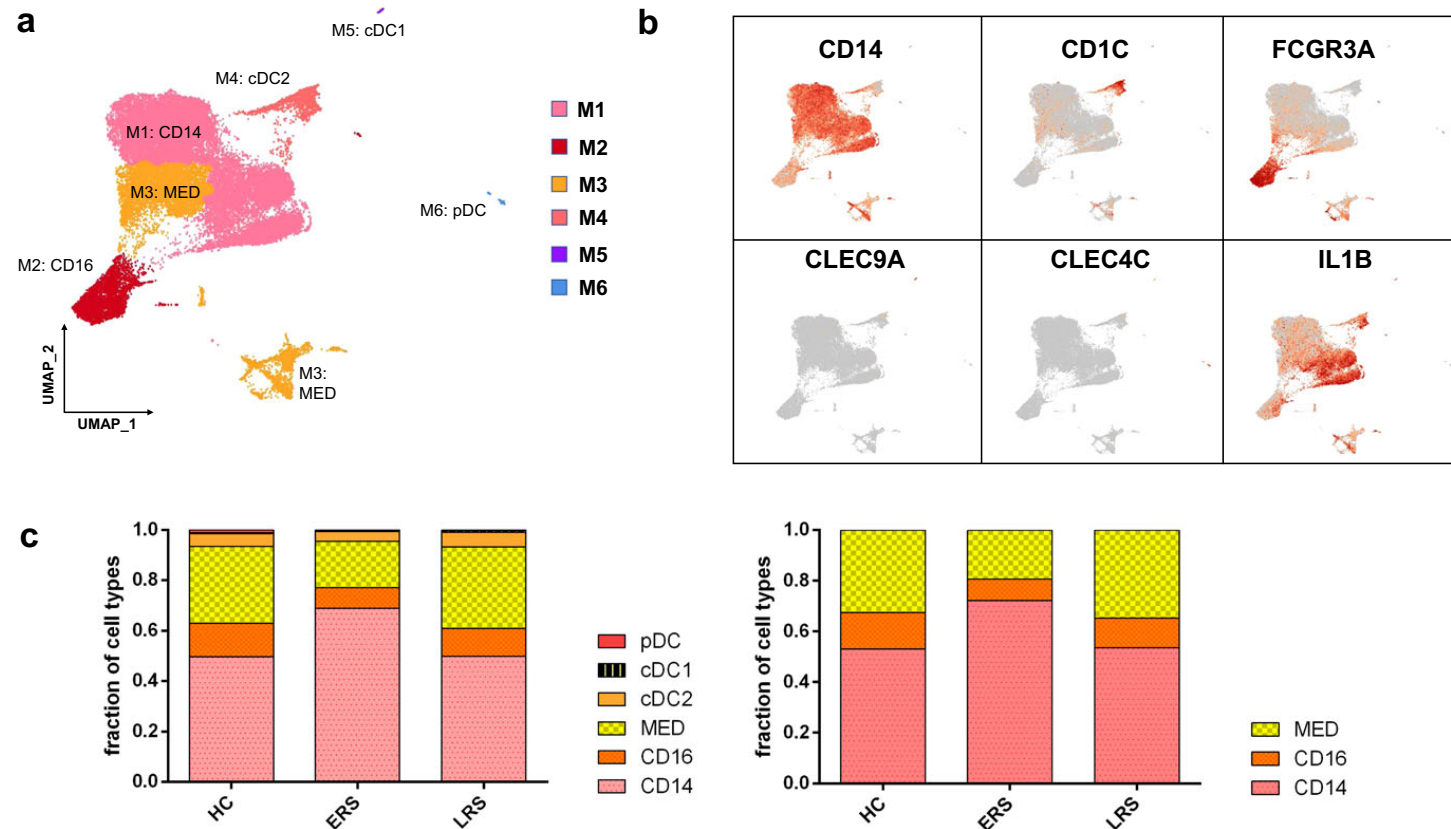
Distribution of NK and T, B, and myeloid cells in the blood of convalescent patients with COVID-19.



Differences in gene expression for the three main clusters between groups

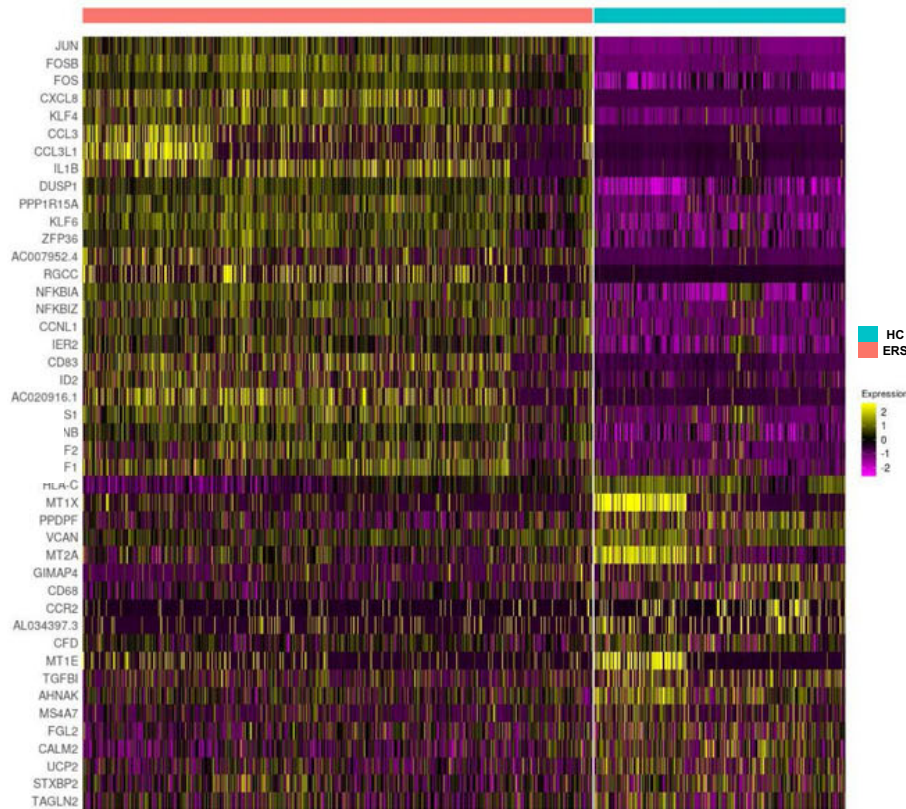


Myeloid cell subsets and their states in the blood of convalescent patients with COVID-19



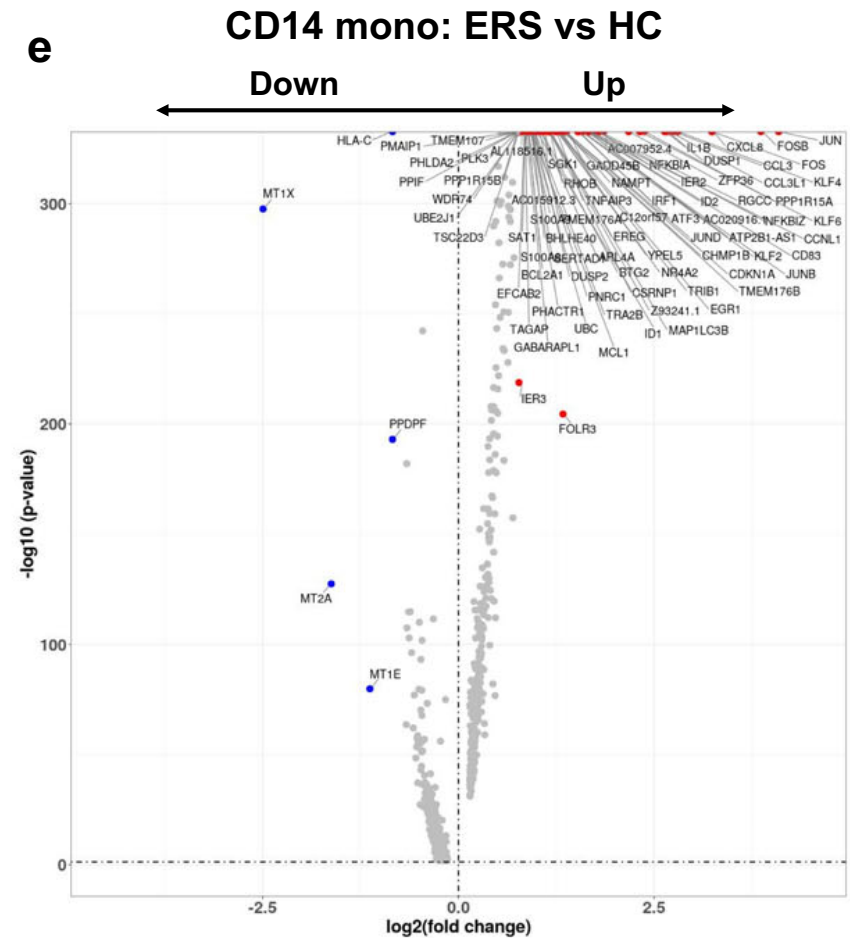
Top DEGs between COVID-19 patients and HCs in CD14++ monocytes.

d

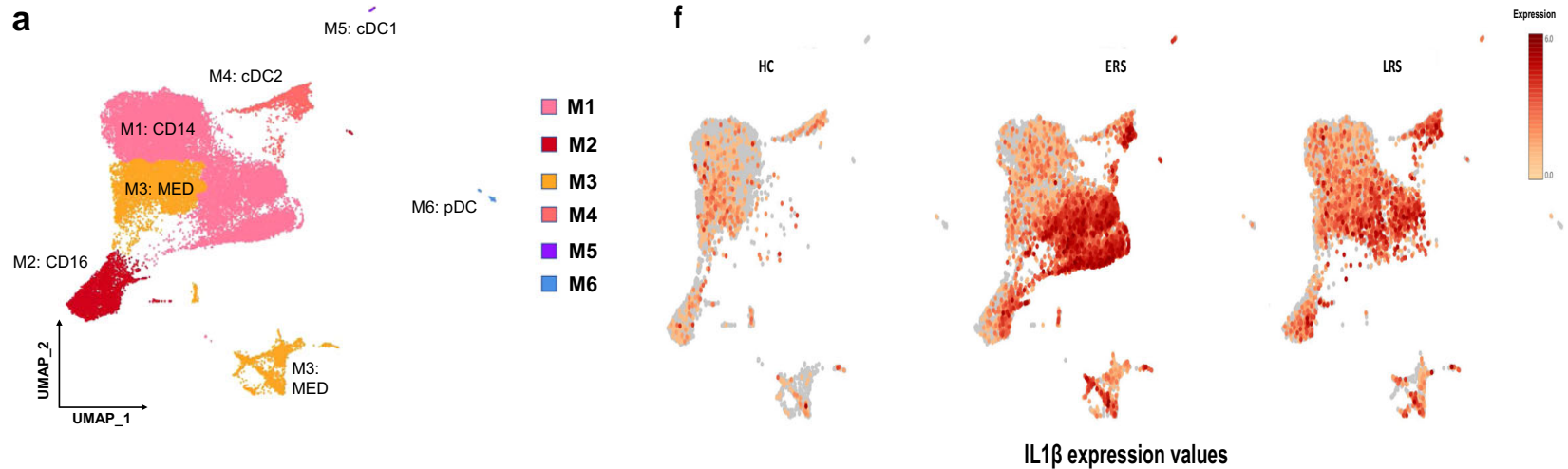


CD14 mono heatmap

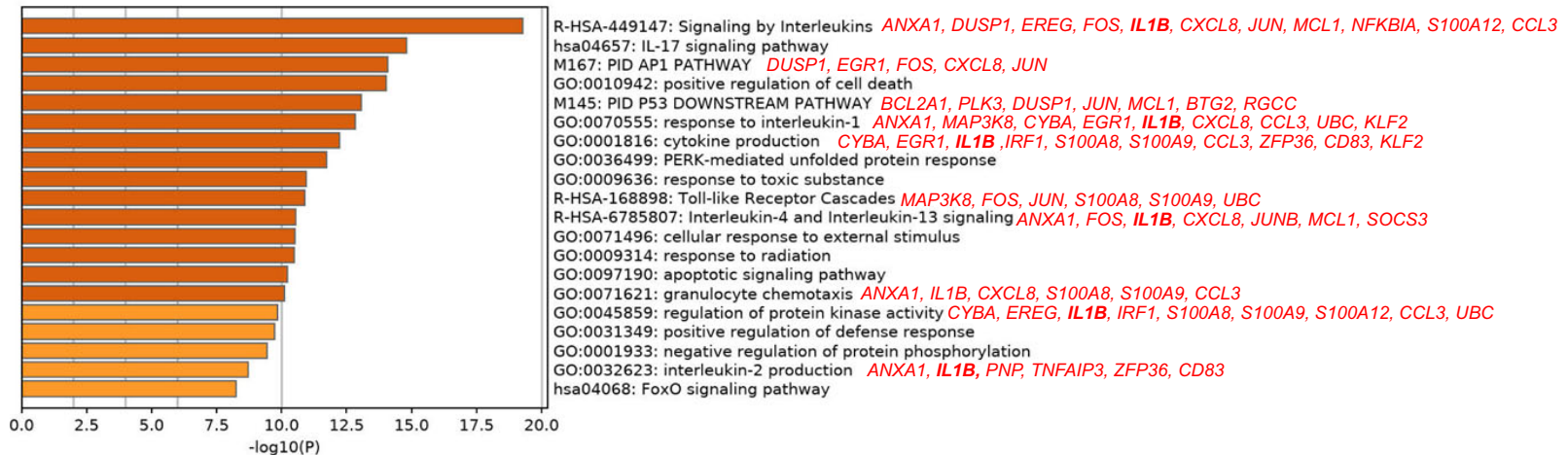
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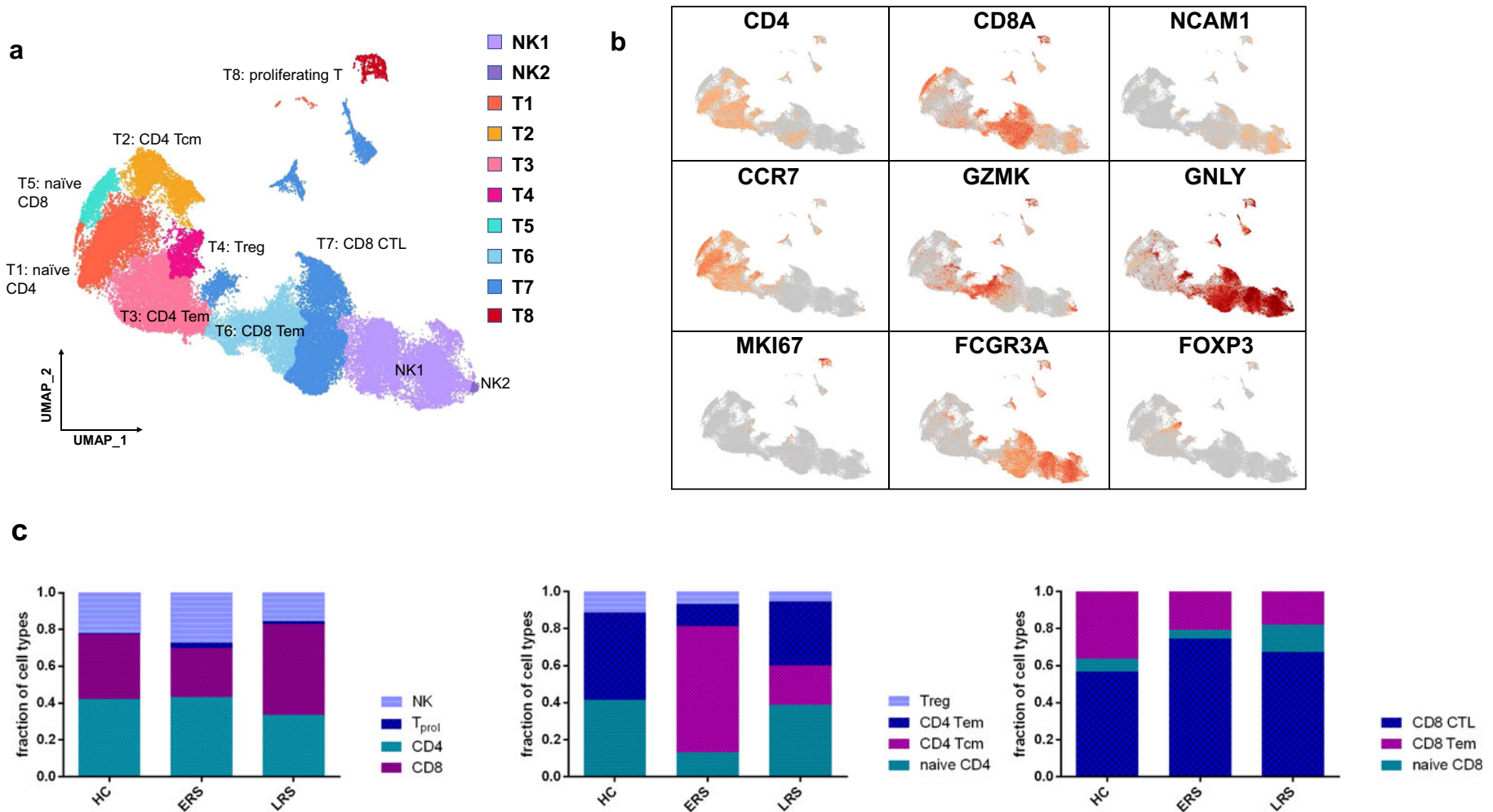
GO BP enrichment analysis of the DEGs of CD14++ monocytes upregulated in COVID-19 patients.



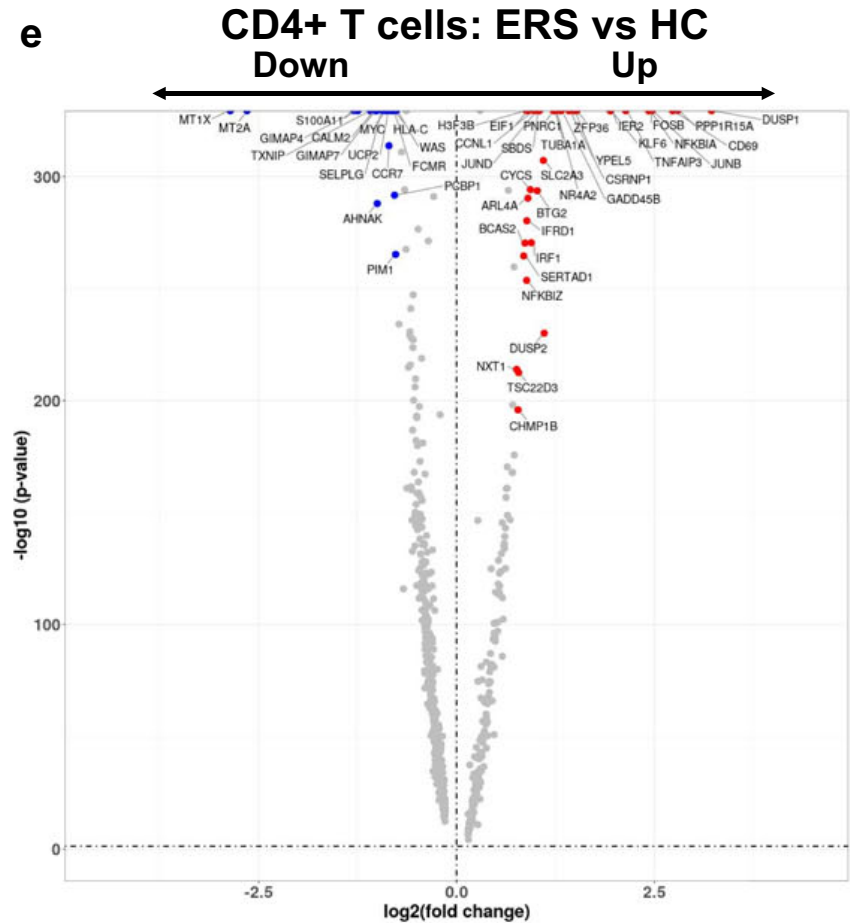
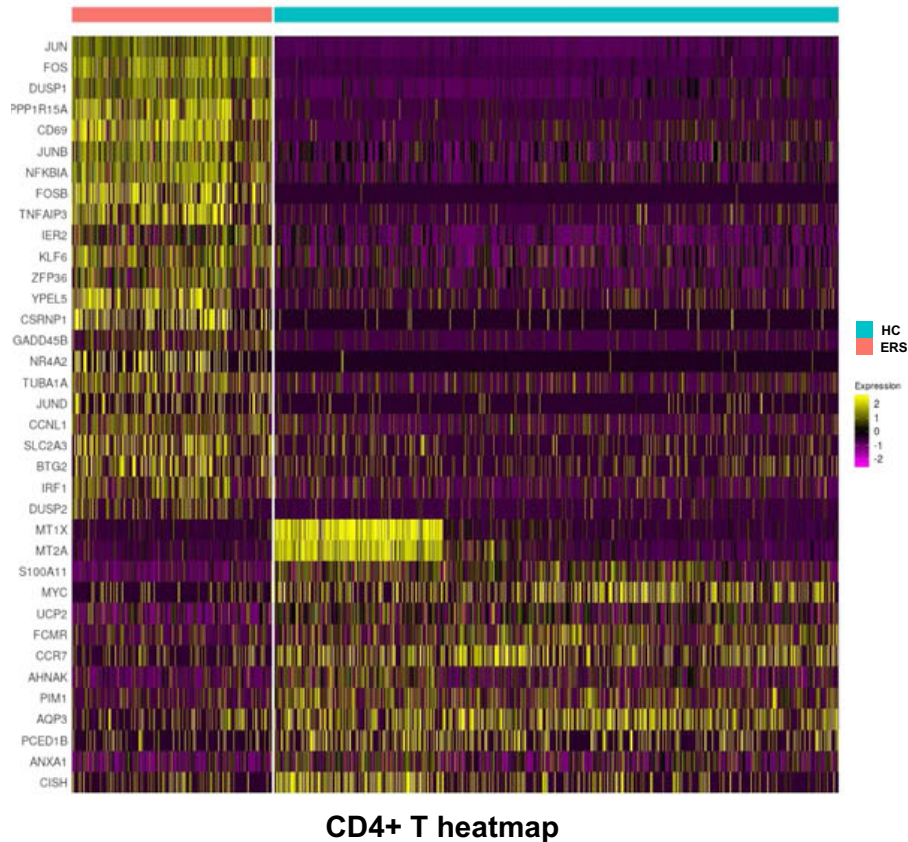
g Up in CD14 mono of ERS



Characterization of T and NK cell responses in the blood of recovered COVID-19 patients

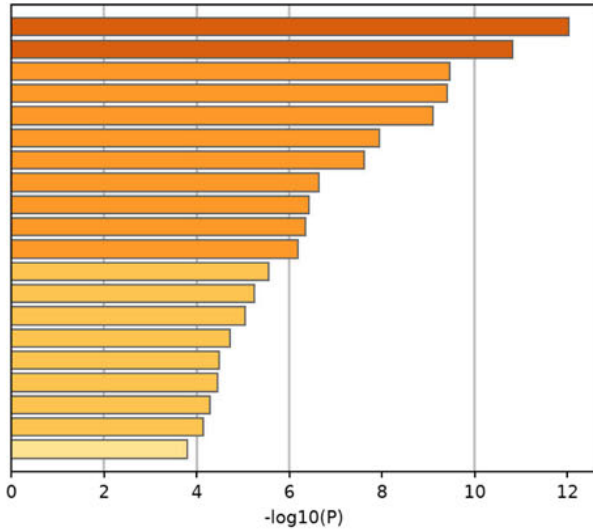


Top DEGs between COVID-19 patients and HCs in CD4+ T cells



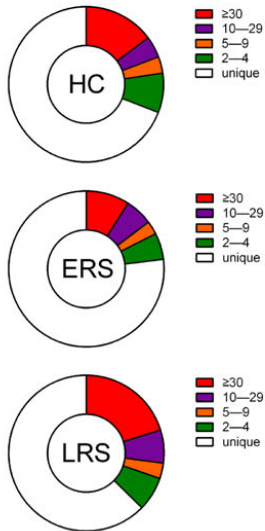
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Up in CD4+ T cells of ERS

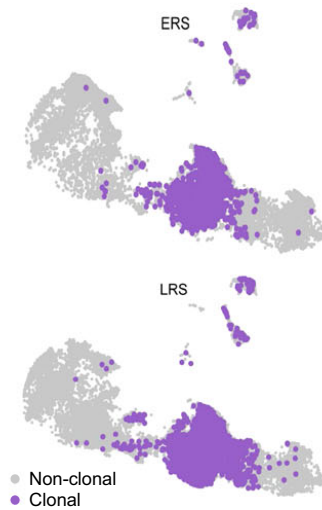


M167: PID AP1 PATHWAY *DUSP1, FOS, FOSB, HLA-A, JUN, JUNB, MYC*
 GO:0019221: cytokine-mediated signaling pathway *CD4, KLF6, FOS, HLA-A, HLA-C, IRF1, JUNB, MYC, STAT3, UBC, CXCR4,*
 GO:0002764: immune response-regulating signaling pathway *SOCS1, PELI1*
 M183: PID IL6 7 PATHWAY *FOS, IRF1, JUN, JUNB, MYC, STAT3*
 GO:0007159: leukocyte cell-cell adhesion
 GO:0046649: lymphocyte activation *AIF1, CD4, KLF6, IRF1, PTGER4, RELB, STAT3, WAS, PELI1, NFKBIZ*
 GO:0002819: regulation of adaptive immune response
 GO:0002683: negative regulation of immune system process
 M45: PID CD40 PATHWAY *JUN, MYC, NFKBIA, TNFAIP3*
 GO:0050900: leukocyte migration
 GO:0009636: response to toxic substance
 GO:0002573: myeloid leukocyte differentiation
 GO:0009611: response to wounding
 GO:0048545: response to steroid hormone
 GO:0045859: regulation of protein kinase activity
 R-HSA-2173796: SMAD2/SMAD3:SMAD4 heterotrimer regulates transcription
 GO:0014072: response to isoquinoline alkaloid
 M196: PID IL23 PATHWAY *CD4, NFKBIA, STAT3*
 GO:0042035: regulation of cytokine biosynthetic process
 GO:0032663: regulation of interleukin-2 production *CD4, TNFAIP3, ZFP36*

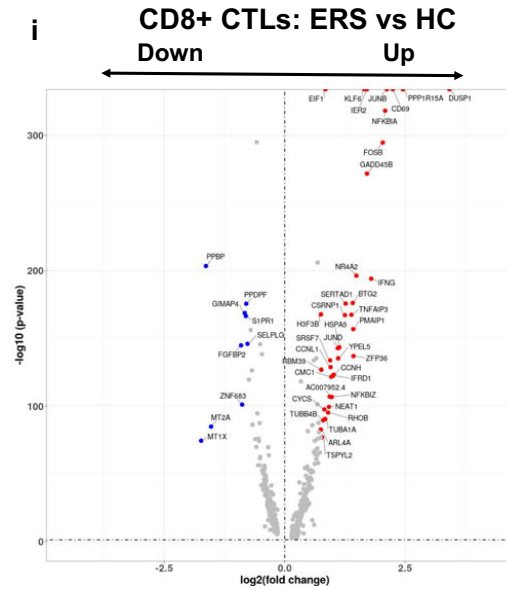
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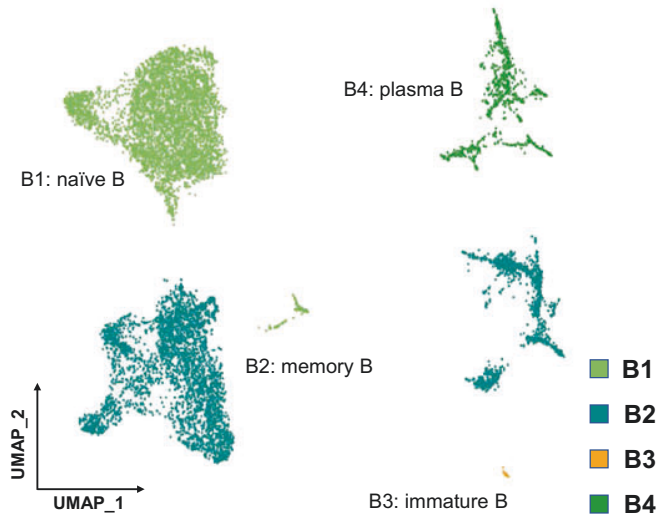


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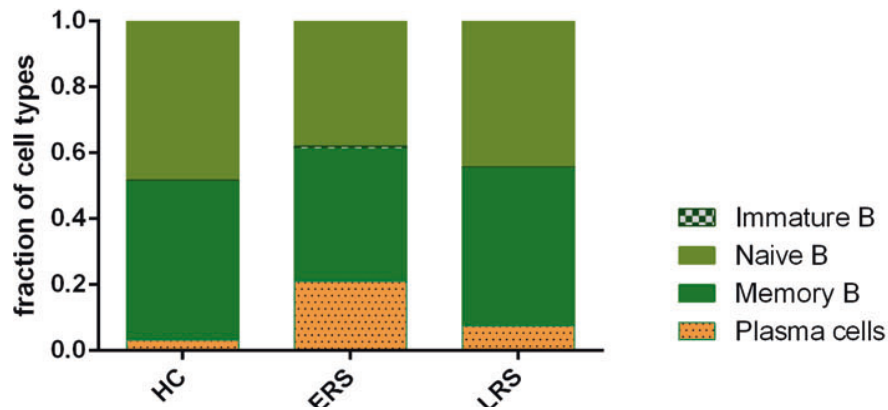
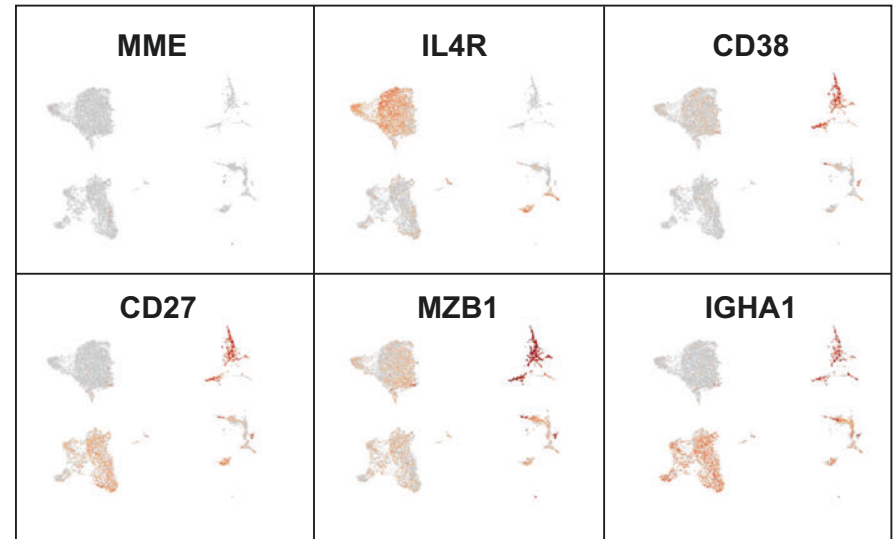


Characterization of single-cell B cells in COVID-19 patients

a

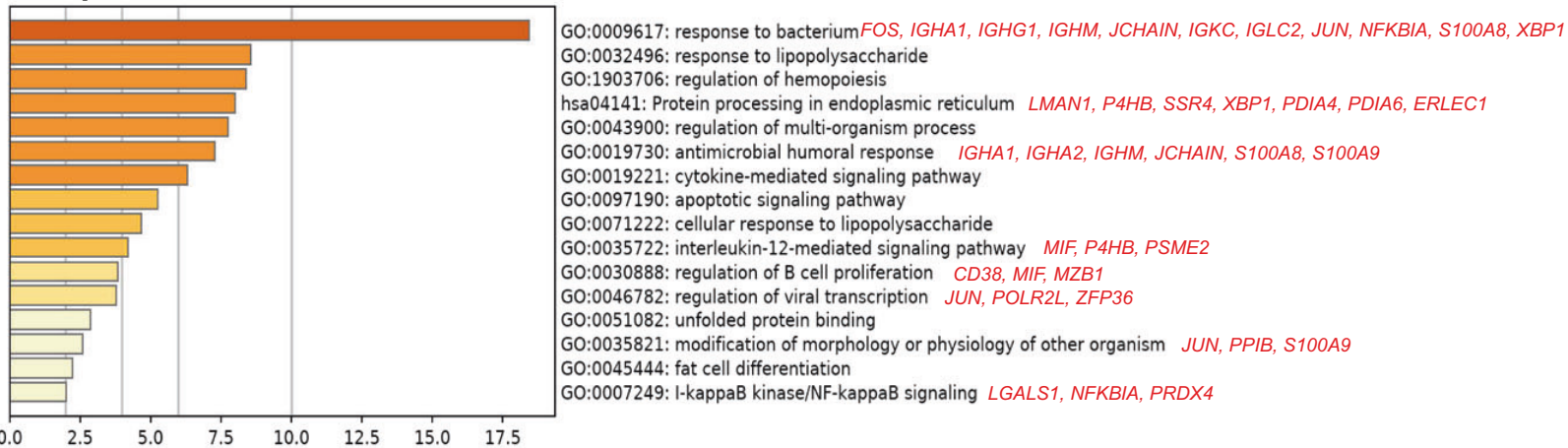


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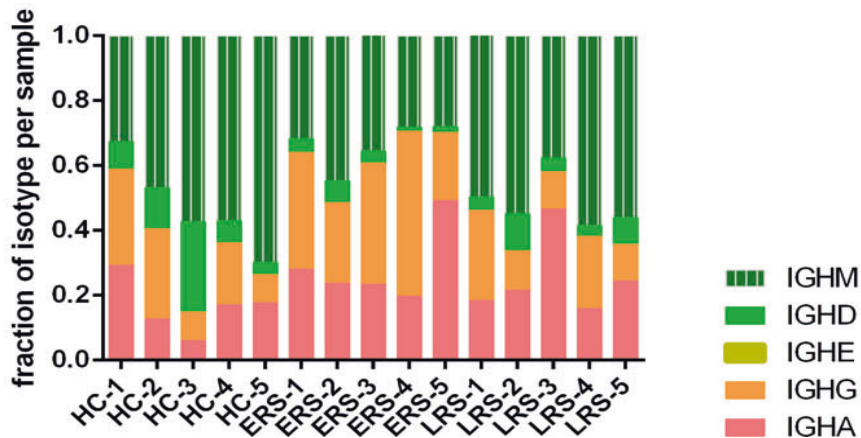


GO BP enrichment analysis of the DEGs of MPB cells between the COVID-19 patients vs. the HCs.

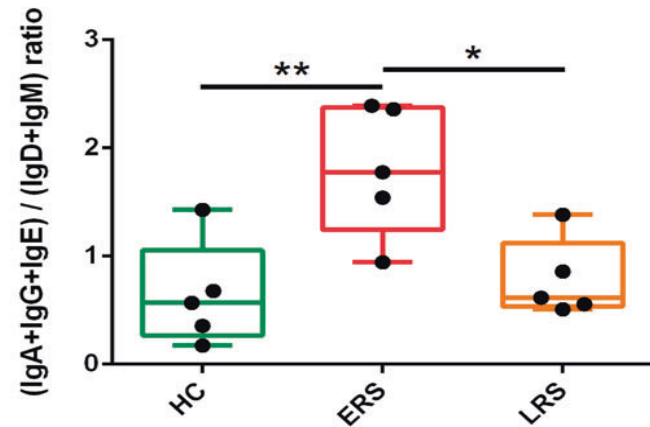
Up in MPBs of ERS



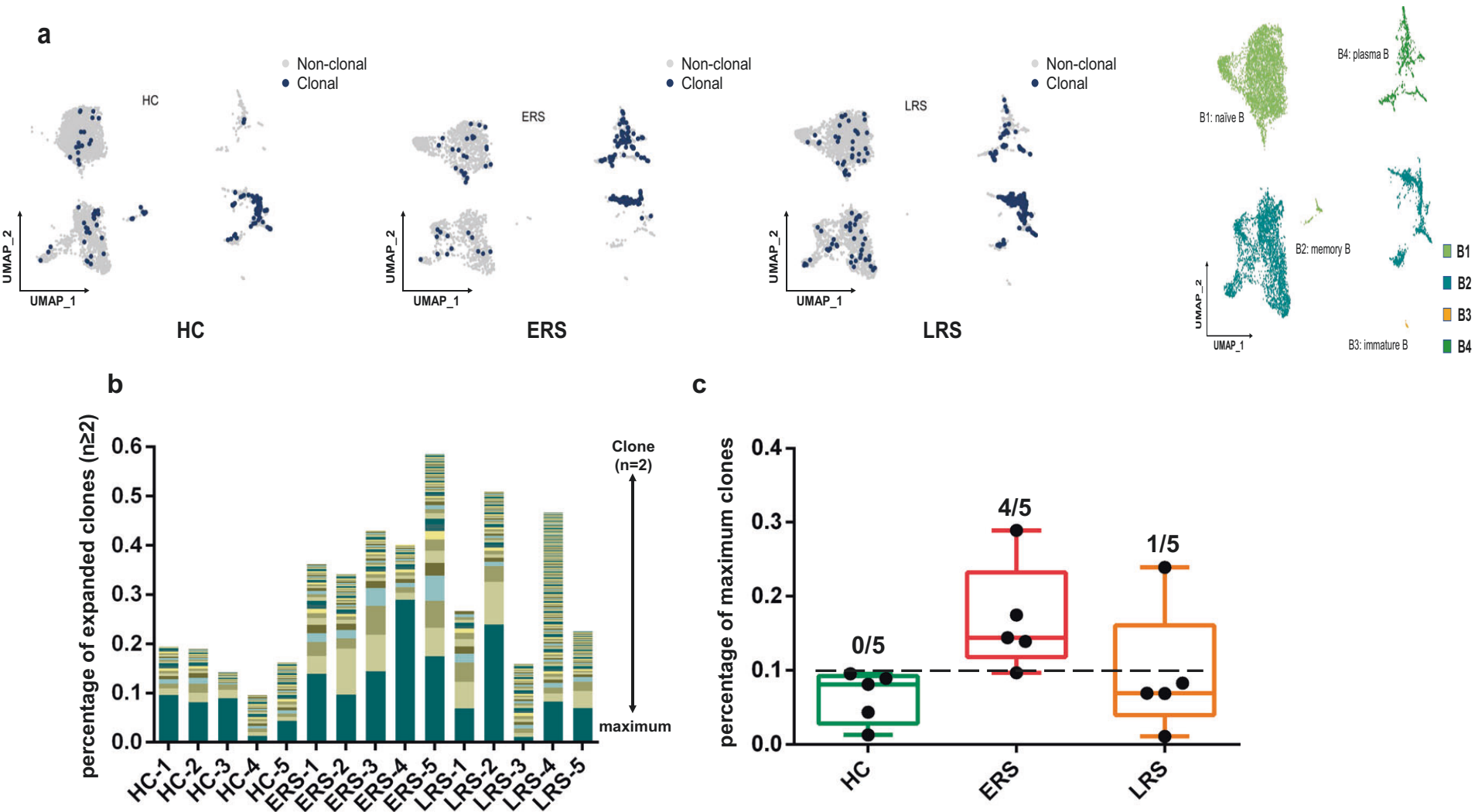
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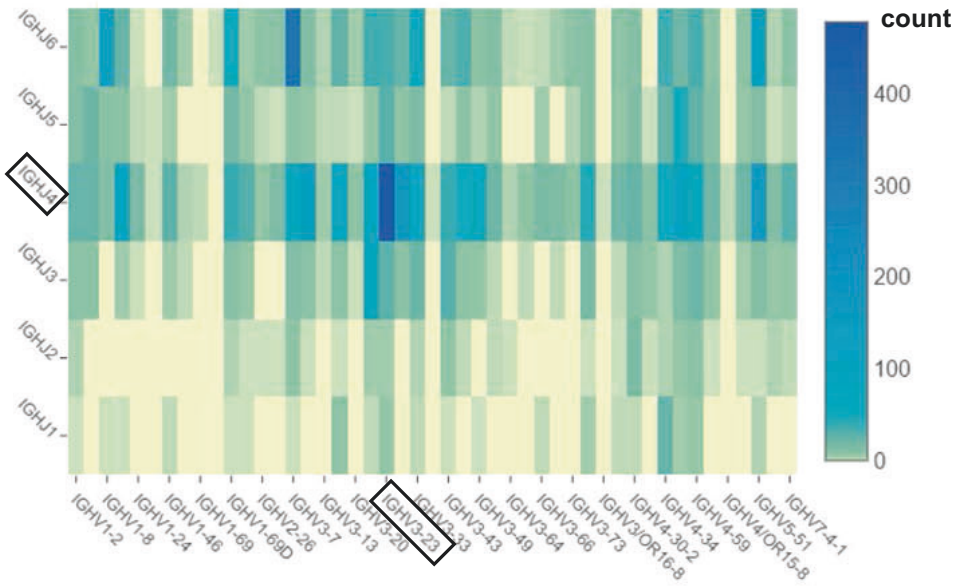


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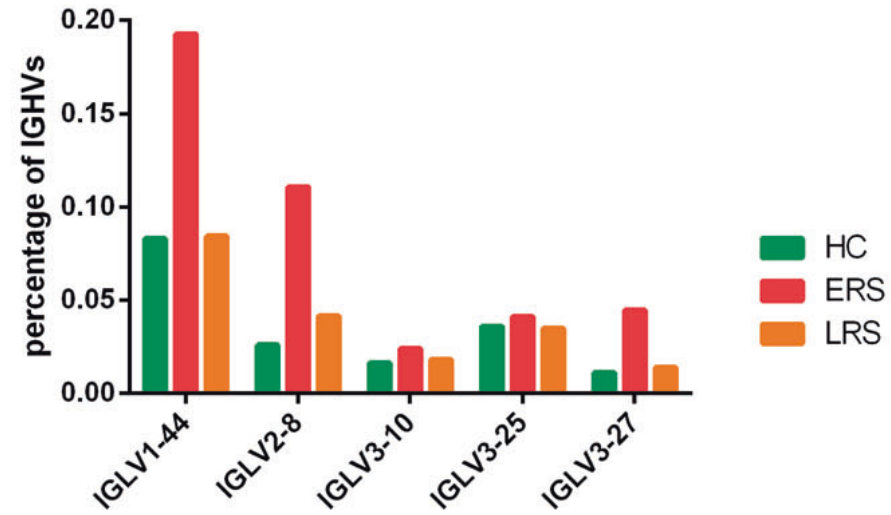


Expanded BCR clones and biased usage of VDJ genes observed in the COVID-19 patients



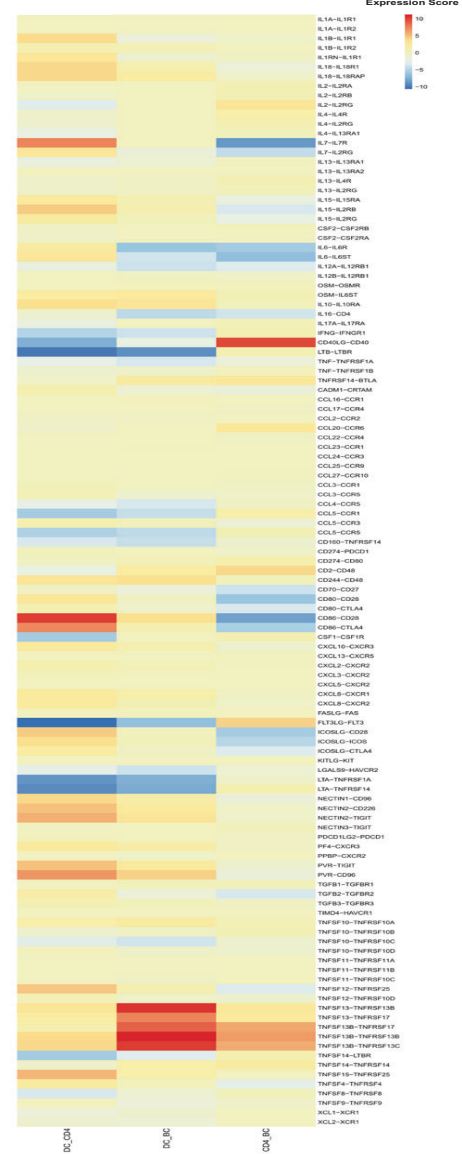
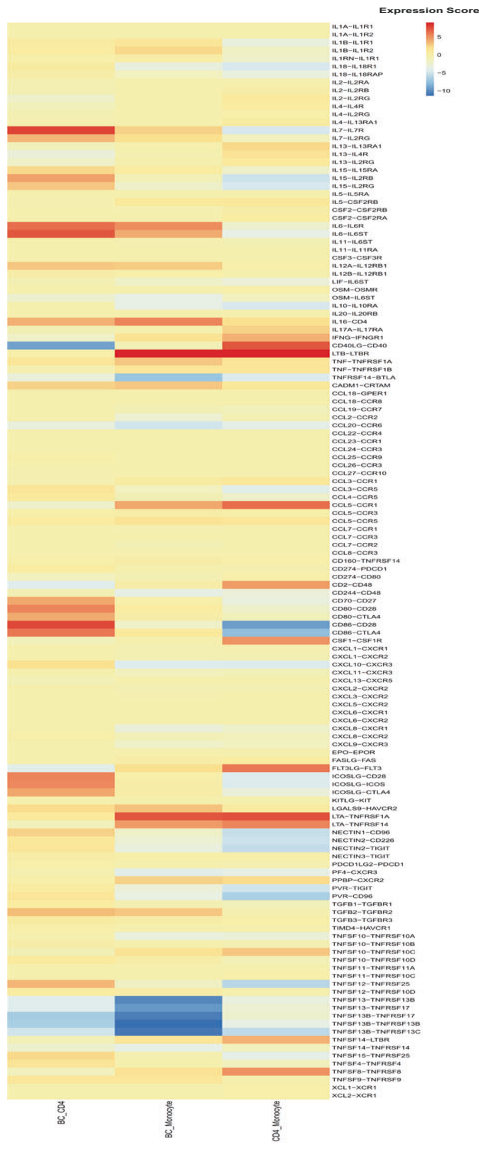


The heatmap of V-J gene in the IGH of ERS group



ERS

LRS

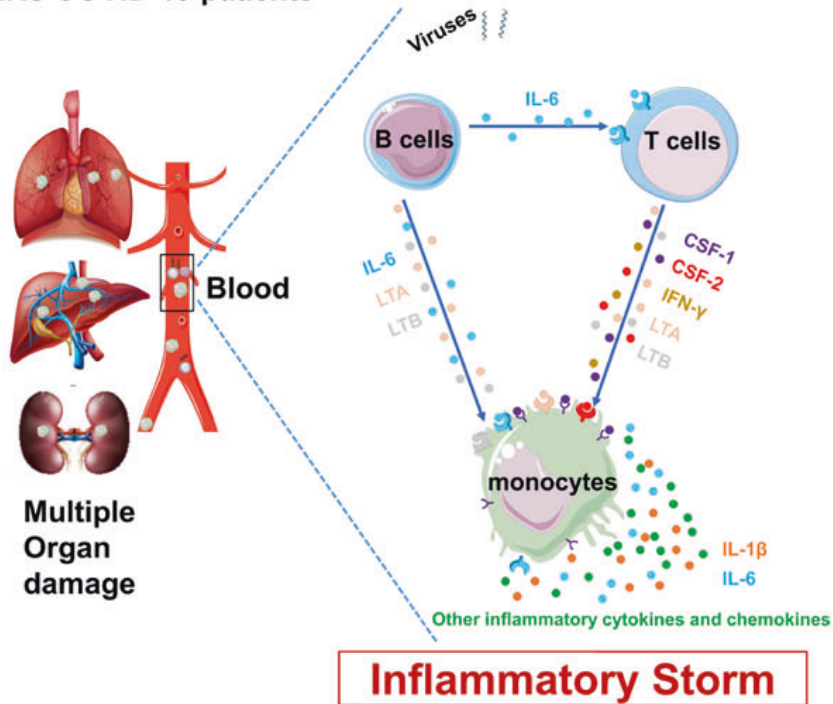


Given are expression scores of cytokines and respective receptors on pairs of cells between different cell types.

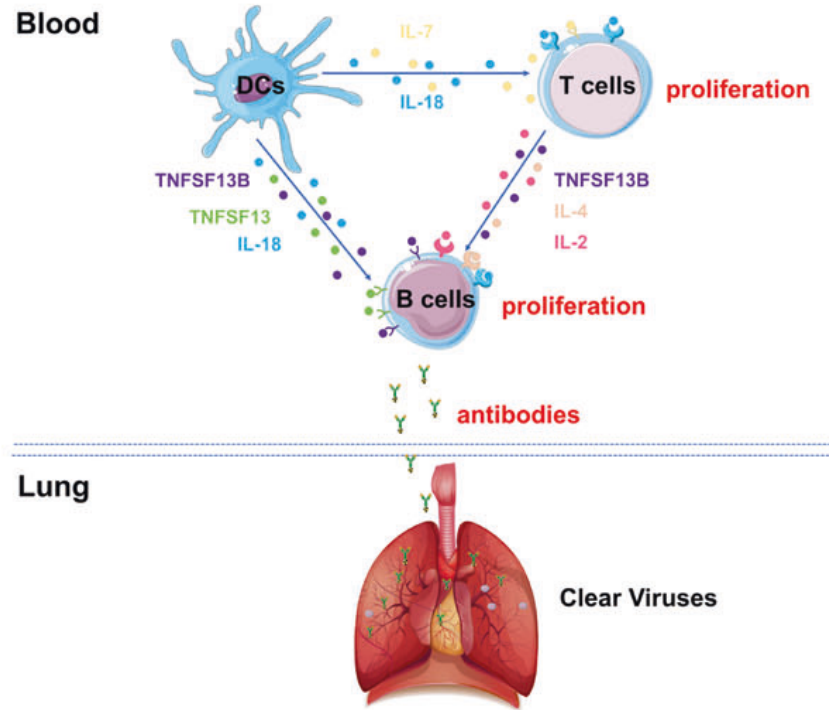
In ERS: adaptive signals involved in monocyte activation, inflammatory signalling IL1 β , CSF1, IL6, and CSF2 may be associated with cytokine storm.

In LRS: DC ligands were predicted to interact B and T cell receptors involved in cell proliferation and the production of antibodies

ERS COVID-19 patients



LRS COVID-19 patients



schematics illustrating the key innate and adaptive immune cell functional alterations and main differences in cell-cell communications in the ERS (1) and LRS (2) COVID-19 patients.

Summary

- Identified a hyper-inflammatory response in ERS patients
- Unique signatures of myeloid, NK and T, and B cells and pin-pointed the changes in the epitopes of TCR and BCR
→ Offering promising opportunities for developing immunotherapies using vaccines and neutralizing antibodies
- Propose CD14+ IL1 β + monocytes to become an important detection marker for monitoring COVID-19 disease recovery
- ERS patients who recovered less than 7 days have a lower ratio of T and NK cells, and these patients' T cells express higher levels of inflammatory genes, such as JUN, FOS, JUNB, and KLF6

Conclusion

- This study provided the first immune atlas of patients who have recovered from COVID-19
- Identified adaptive immune dysregulation after discharge
- Longitudinal studies of recovered patients in a larger cohort might help to understand the long term consequences of the disease

Thank you for your
attention