Data S3: Analysis of Mass and Flow Cytometry, Repertoire and Proteomics, Related to STAR Methods

Related to **STAR Methods**: Mass cytometry data analysis; Flow cytometry data analysis; Repertoire analysis; Pre-processing and analysis of surface protein (ADT) data; Luminex data analysis; and Tims-TOF mass spectrometry analysis.

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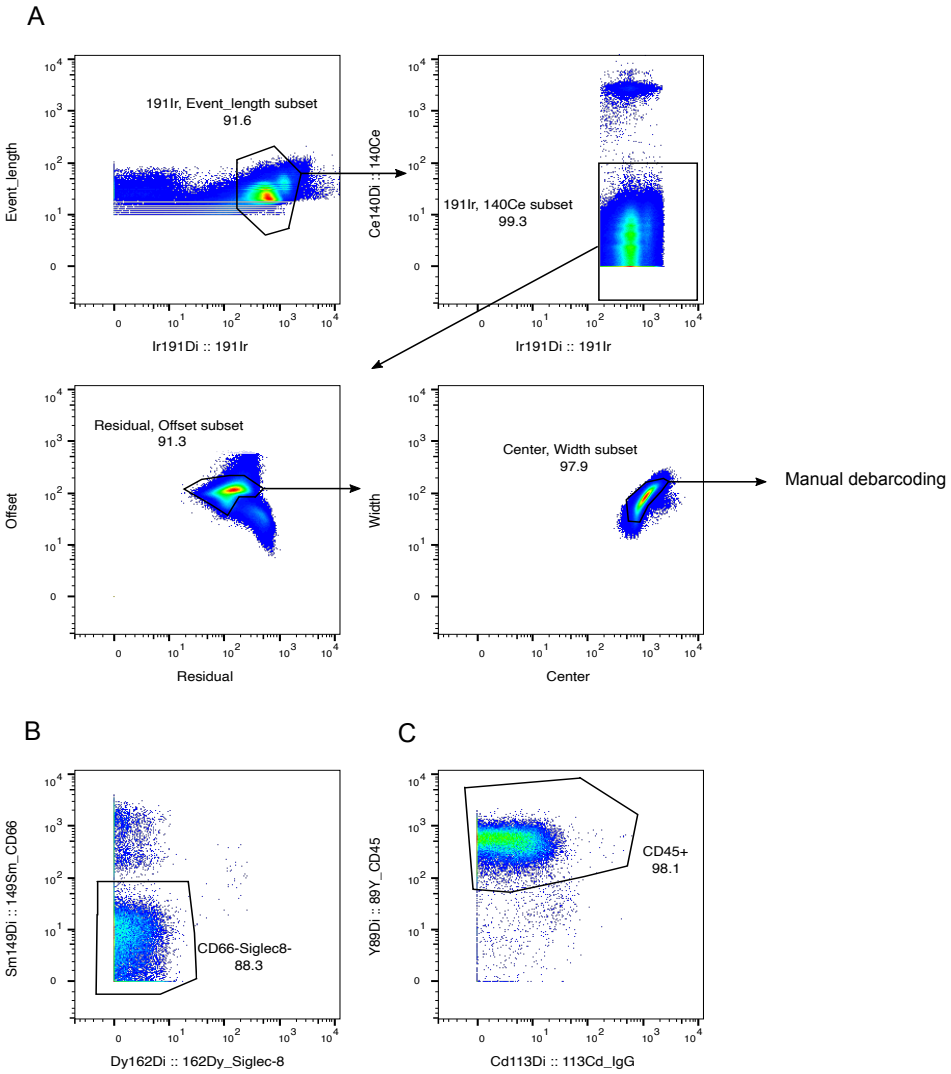
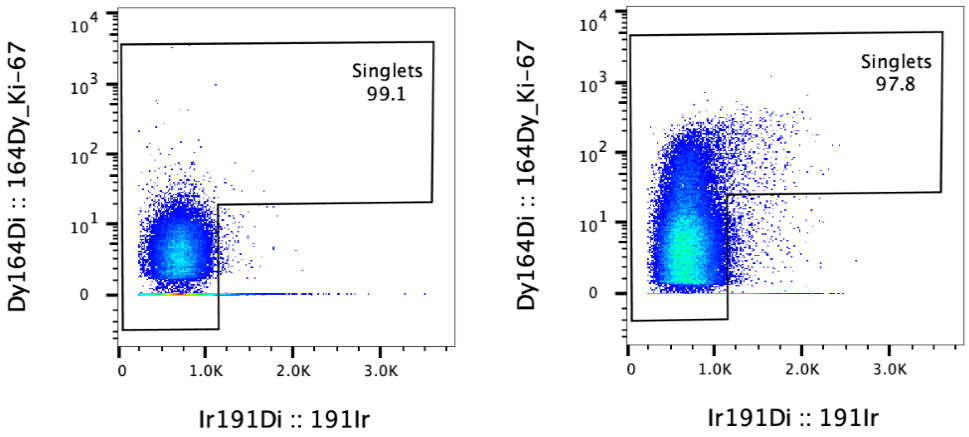
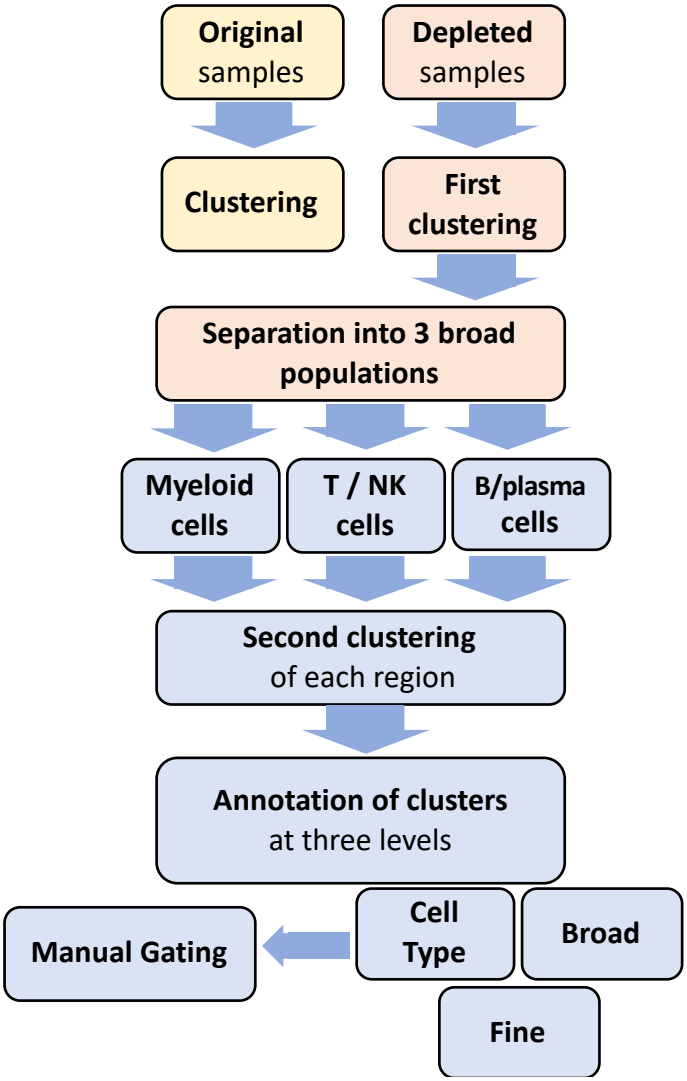
Mass cytometry



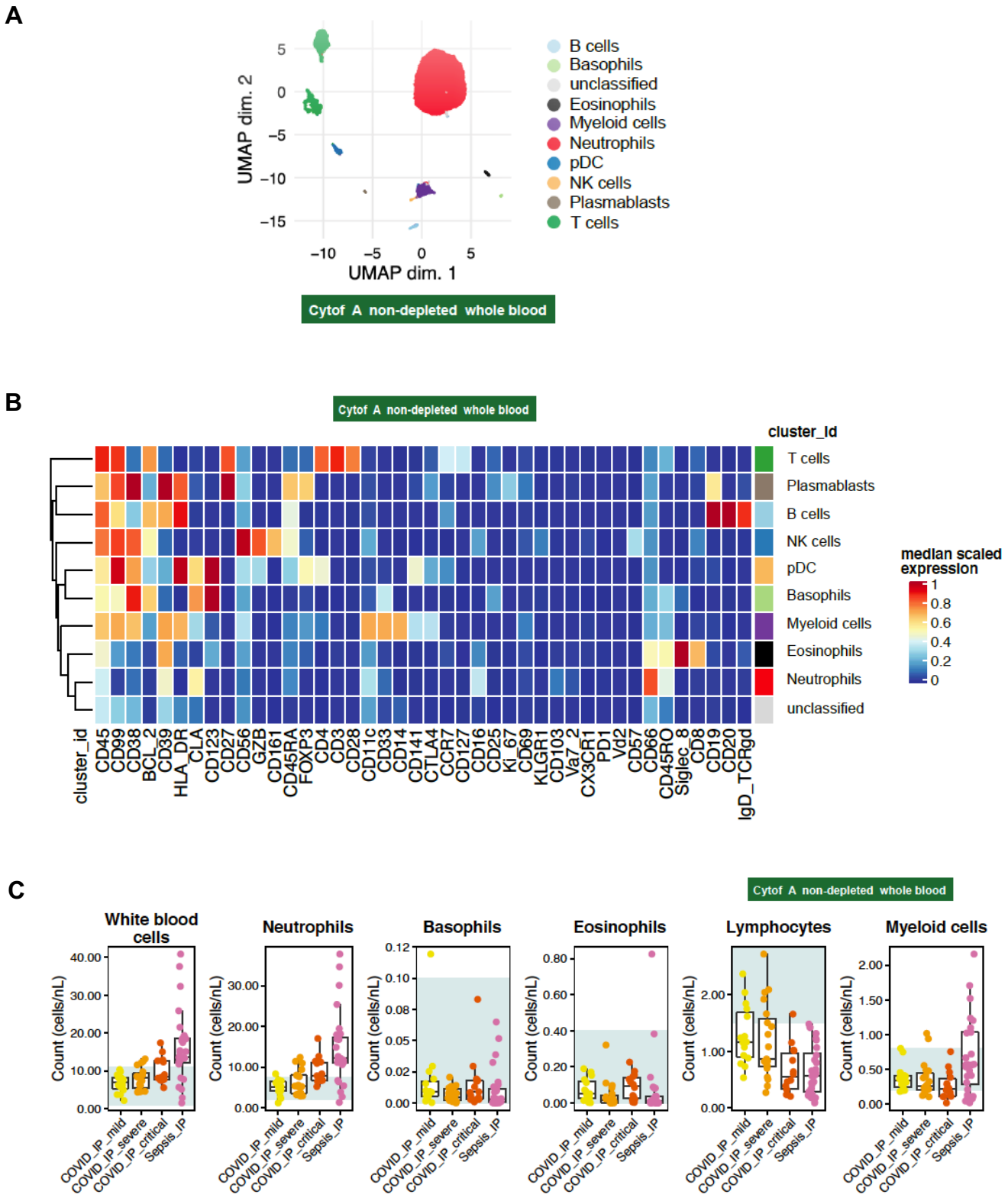
1: Gating strategy for clean-up. A Gating strategy for removal of beads, DNA negative events and for Gaussian parameters clean-up prior to Manual Debarcoding. **B** Clean up step to remove residual CD66+ granulocytes and Siglec-8 Eosinophils, in granulocytes depleted samples. This step was followed by gating of CD45+ cells. **C**. Gating of CD45+ cell for subsequent analysis. This step was performed for both depleted and non-depleted (original) samples. Related to STAR Methods: Mass cytometry data analysis**:** CyTOF data pre-processing.



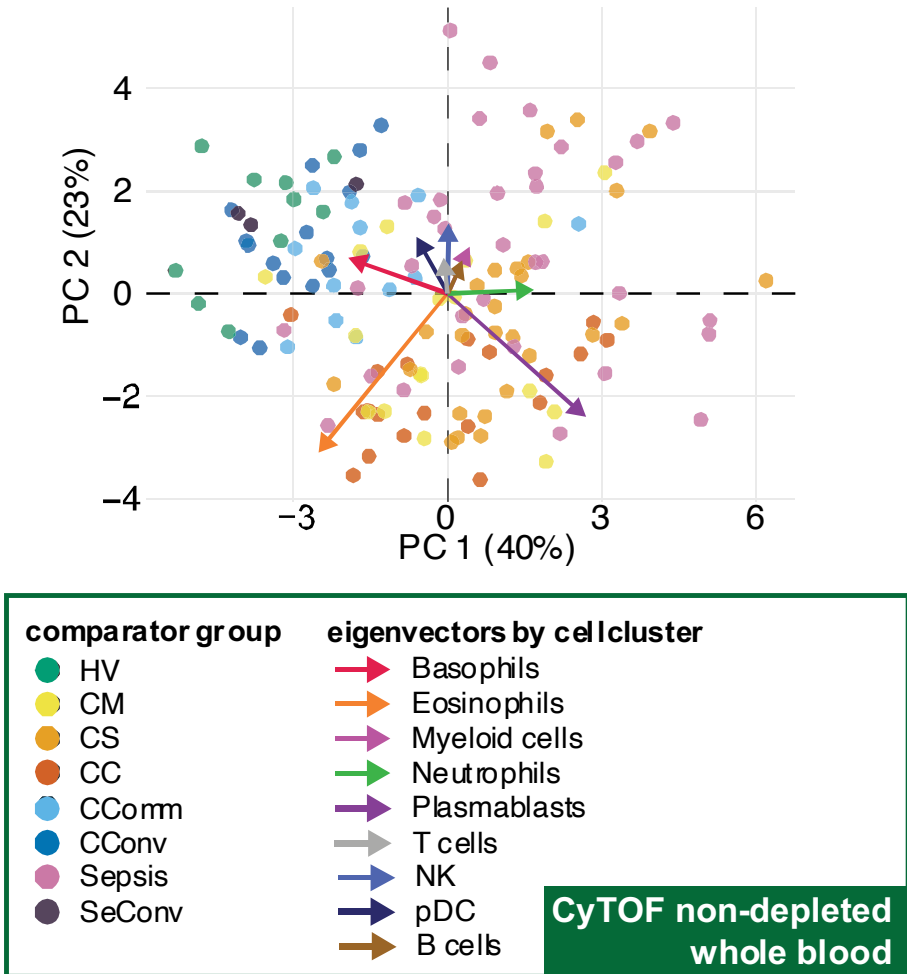
2: Gating strategy for doublets exclusion. Representative scatter plots showing DNA content on x-axis (191Ir - Iridium) versus the proliferation marker Ki67 on y-axis. The sample on the right shows a population of proliferating $Ki67^{+}$ cells absent on the sample on the left. To not exclude proliferating cells from the analysis, the gating was made excluding only $Iridium^{high}Ki67^{-}$ cells. Related to STAR Methods: Mass cytometry data analysis**:** CyTOF data pre-processing**.**



3: Flow-chart describing the analytical pipeline for the analysis of the mass cytometry data.
Related to STAR Methods: Mass cytometry data analysis: clustering.

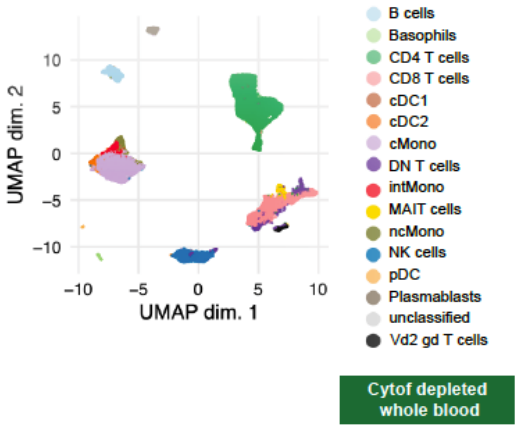


4: Whole blood (non-depleted) assayed by single cell mass cytometry (CyTOF). Non-granulocyte depleted samples. A self-organizing map algorithm (FlowSOM) resolved 25 clusters by consensus clustering for 3,893,390 cells after down sampling to a maximum of 40,000 cells. Clusters merged to identify broad immune cell populations (A) UMAP (B) clustering of major cell populations (y axis) by discriminating marker (x-axis) (C) cell counts by patient group. Related to STAR Methods: Mass cytometry data analysis: differential abundance analysis.

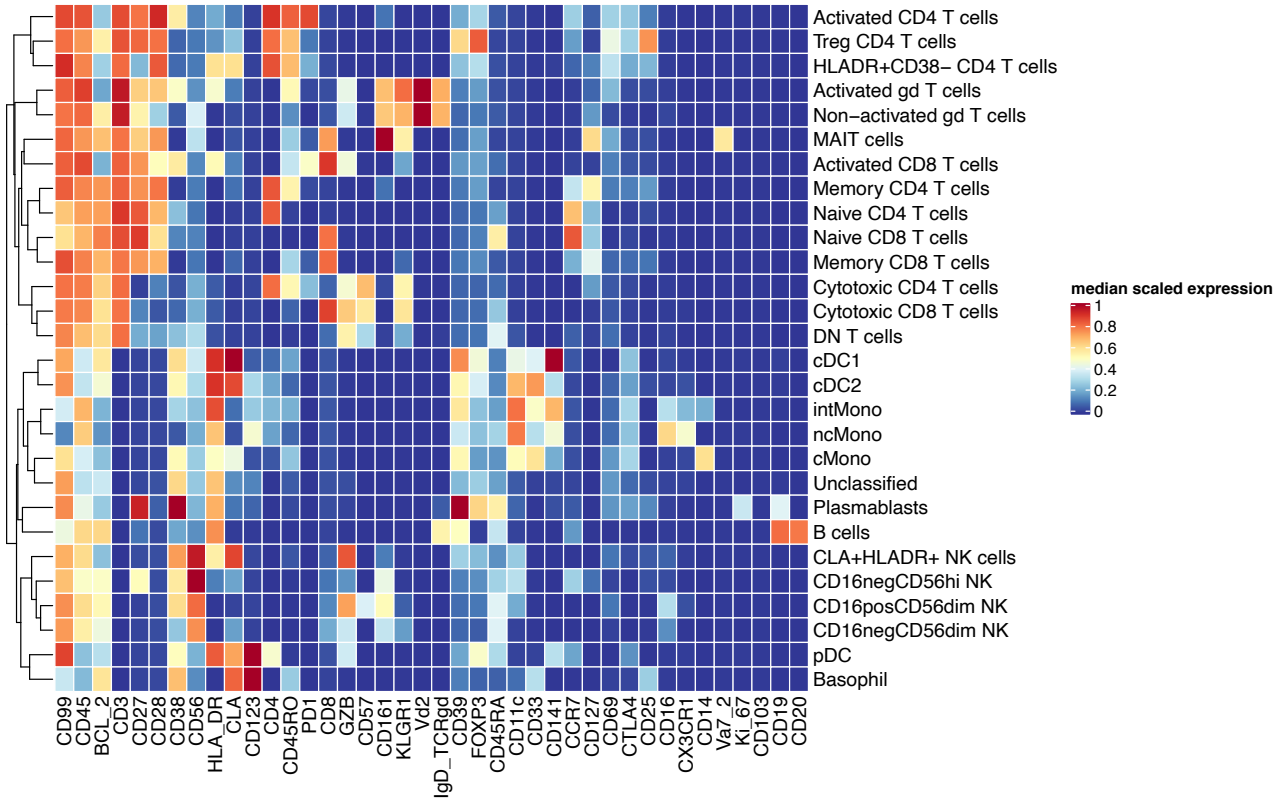


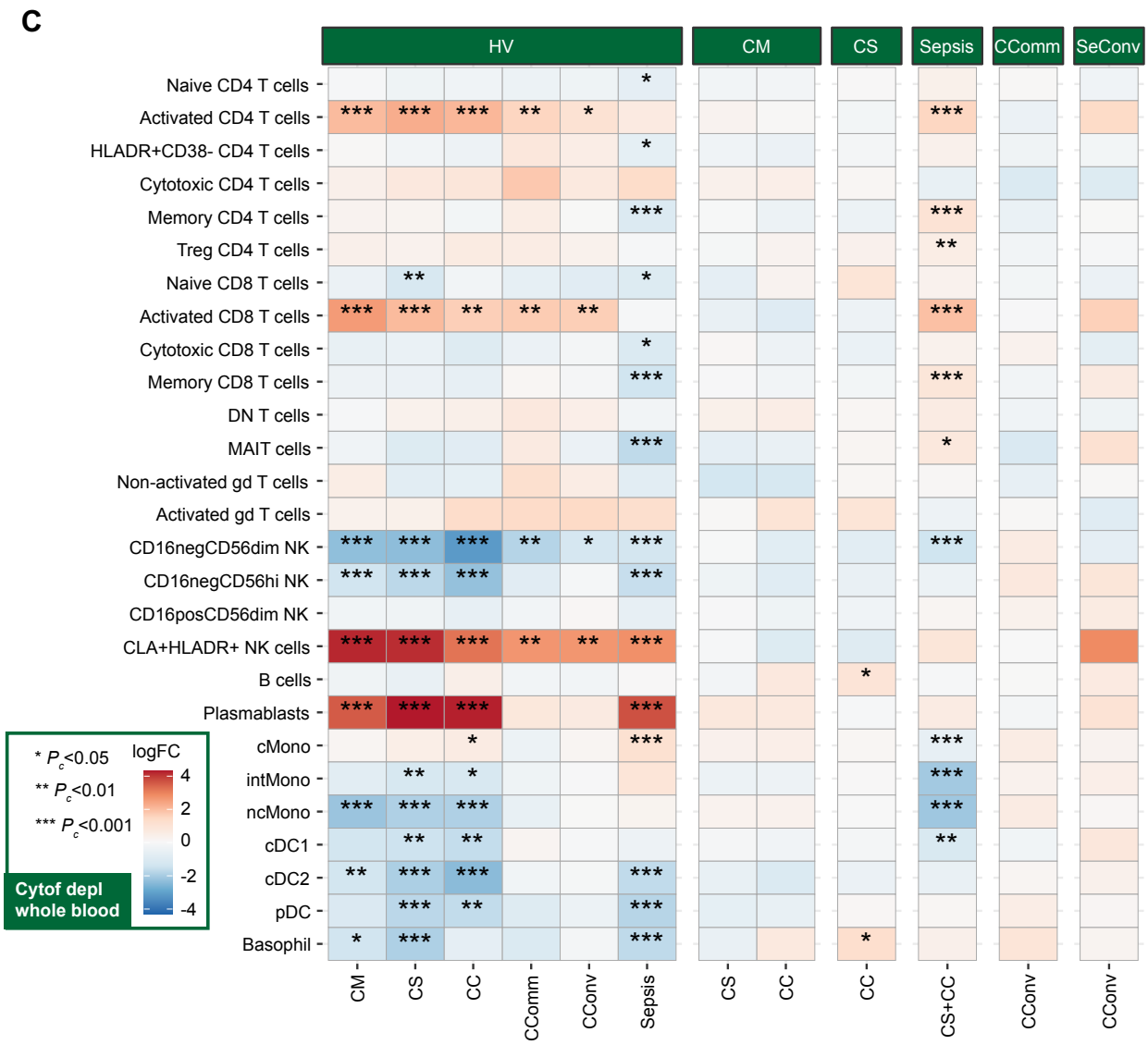
5: Principal components analysis (PCA) of whole blood (non-depleted) assayed by single cell mass cytometry (CyTOF). Major cell populations in whole blood for clinical comparator groups showing for non-granulocyte depleted samples PCA with arrows indicating drivers of variation by cell population. Related to STAR Methods: Mass cytometry data analysis: differential abundance analysis.

A

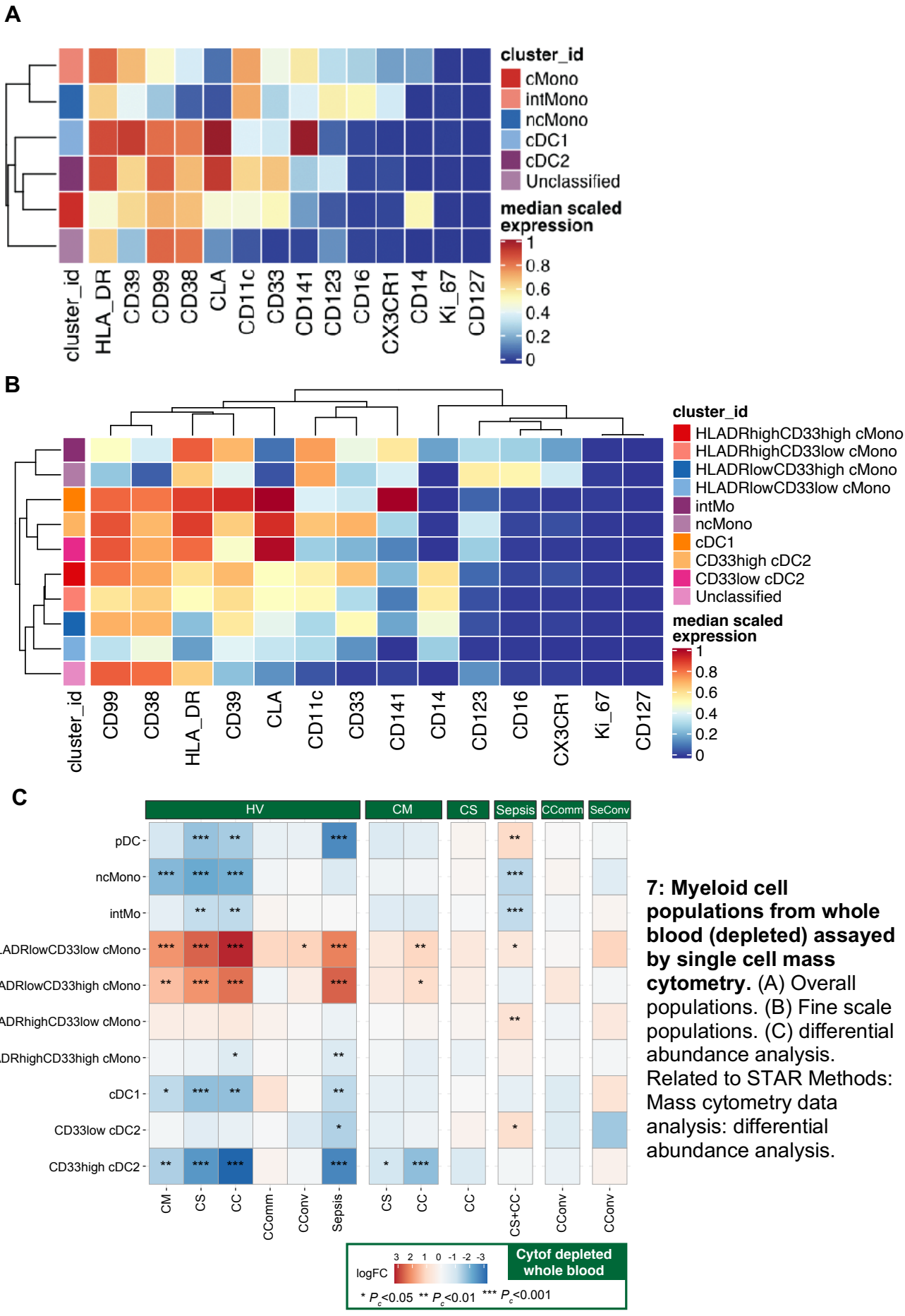

UMAP dim. 2
UMAP dim. 1
10
5
0
−5
−10
−10
−5
0
5
10
B cells
Basophils
CD4 T cells
CD8 T cells
cDC1
cDC2
cMono
DN T cells
intMono
MAIT cells
ncMono
NK cells
pDC
Plasmablasts
unclassified
Vd2 gd T cells
Cytof depleted whole blood

B

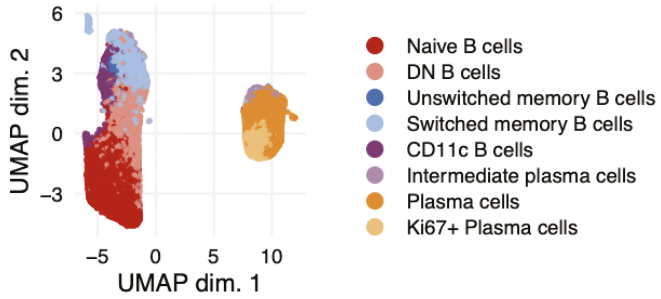

Activated CD4 T cells
Treg CD4 T cells
HLADR+CD38− CD4 T cells
Activated gd T cells
Non−activated gd T cells
MAIT cells
Activated CD8 T cells
Memory CD4 T cells
Naive CD4 T cells
Naive CD8 T cells
Memory CD8 T cells
Cytotoxic CD4 T cells
Cytotoxic CD8 T cells
DN T cells
cDC1
cDC2
intMono
ncMono
cMono
Unclassified
Plasmablasts
B cells
CLA+HLADR+ NK cells
CD16negCD56hi NK
CD16posCD56dim NK
CD16negCD56dim NK
pDC
Basophil
CD99
CD45
BCL_2
CD3
CD27
CD28
CD38
CD56
HLA_DR
CLA
CD123
CD4
CD45RO
PD1
CD8
GZB
CD57
CD161
KLGR1
Vd2
IgD_TCRgd
CD39
FOXP3
CD45RA
CD11c
CD33
CD141
CCR7
CD127
CD69
CTLA4
CD25
CD16
CX3CR1
CD14
Va7_2
Ki_67
CD103
CD19
CD20
median scaled expression
1
0.8
0.6
0.4
0.2
0

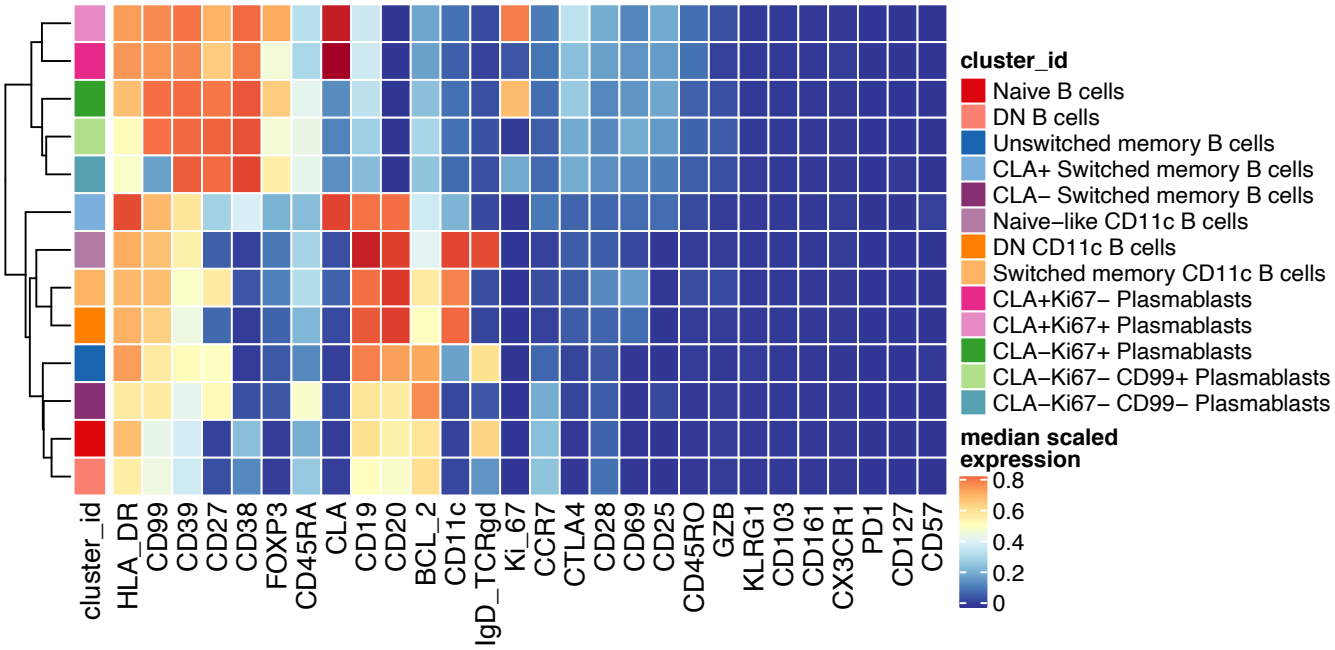


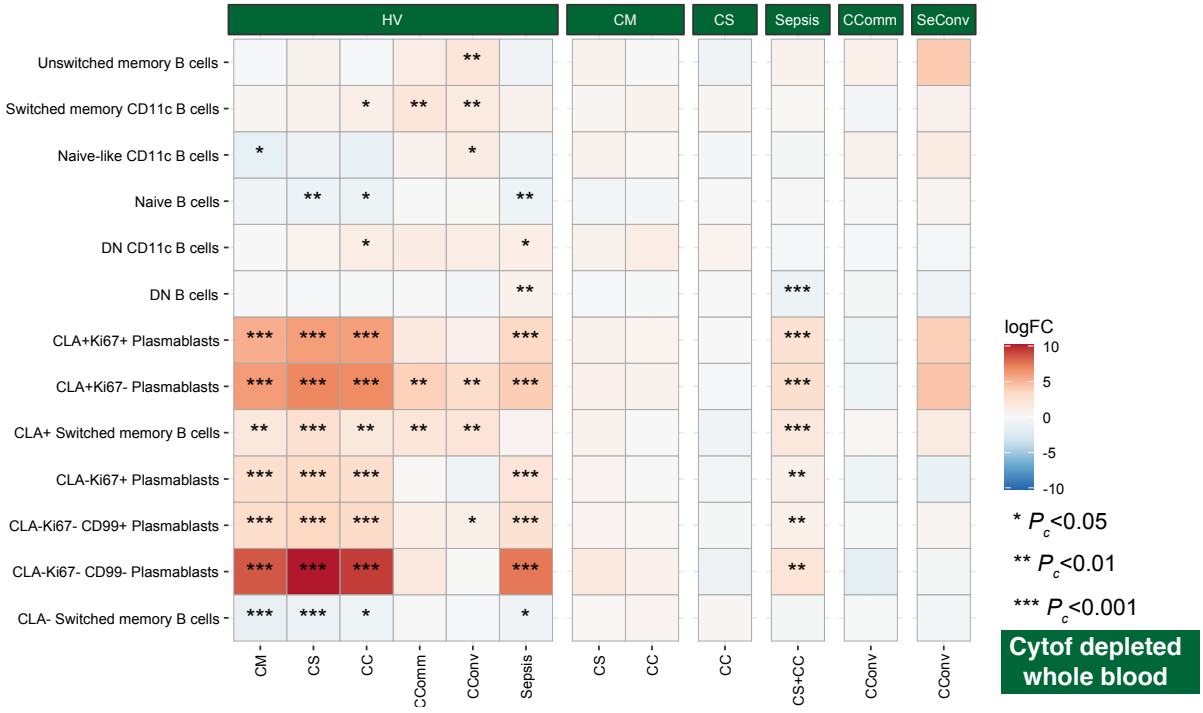
6: Whole blood (depleted) assayed by single cell mass cytometry. Heatmap showing the phenotype of broad cell populations for single cell mass cytometry (CyTOF) of granulocyte (CD66+) depleted whole blood with 7,118,158 cells assayed. (A) UMAP (B) clustering of major cell populations (y axis) by discriminating marker (x-axis) (C) differential abundance analysis. Related to STAR Methods: Mass cytometry data analysis: differential abundance analysis.

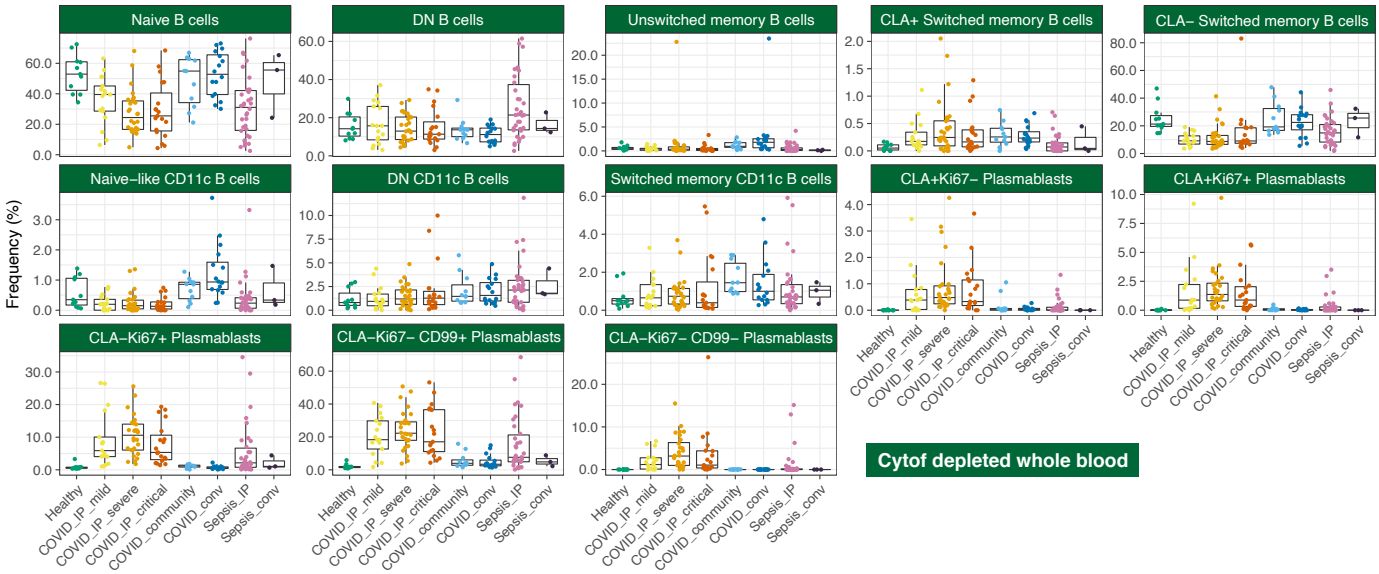


7: Myeloid cell populations from whole blood (depleted) assayed by single cell mass cytometry. (A) Overall populations. (B) Fine scale populations. (C) differential abundance analysis. Related to STAR Methods: Mass cytometry data analysis: differential abundance analysis.

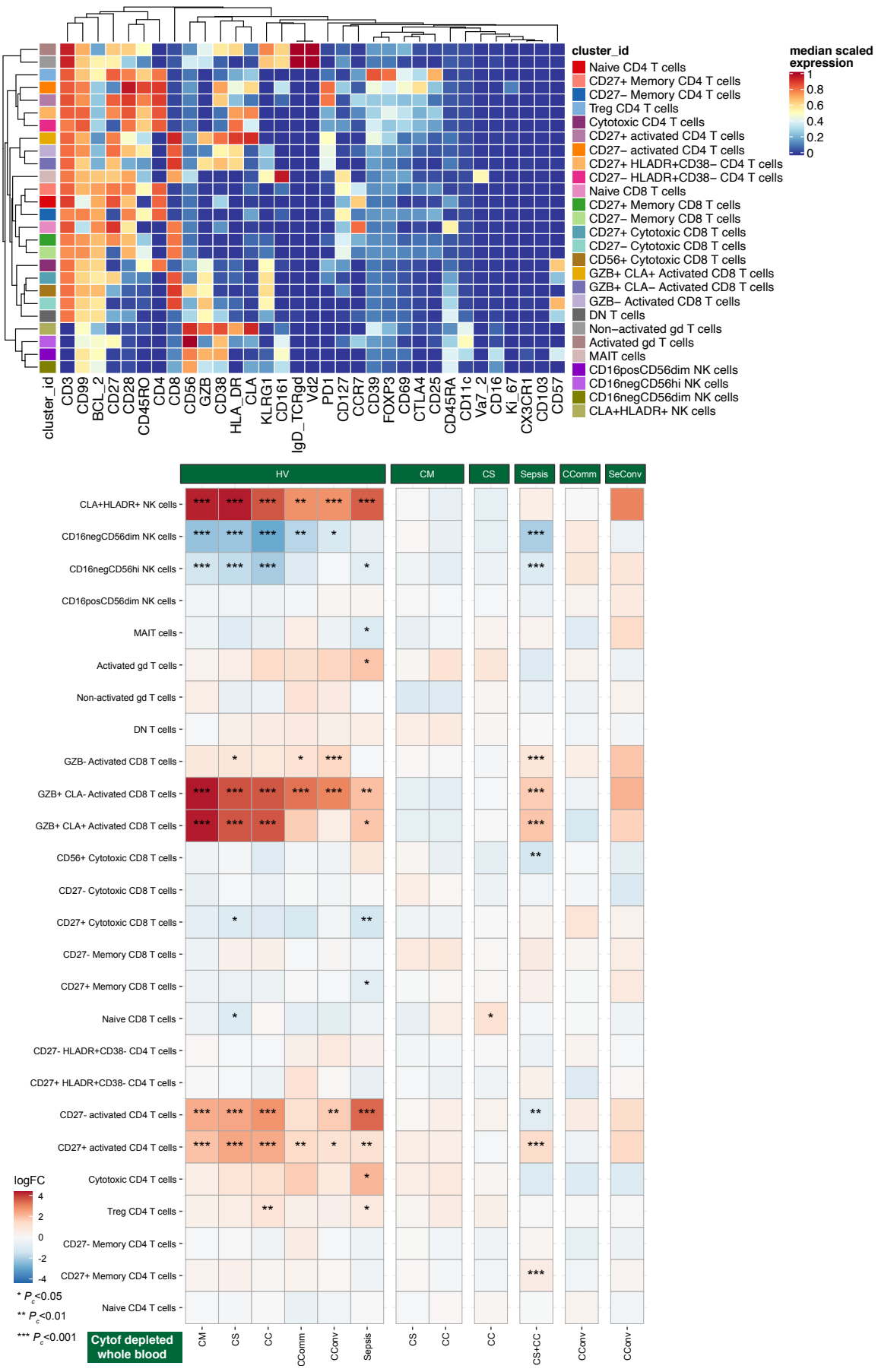

UMAP dim. 2
UMAP dim. 1
Naive B cells
DN B cells
Unswitched memory B cells
Switched memory B cells
CD11c B cells
Intermediate plasma cells
Plasma cells
Ki67+ Plasma cells


cluster_id
HLA_DR
CD99
CD39
CD27
CD38
FOXP3
CD45RA
CLA
CD19
CD20
BCL_2
CD11c
IgD_TCRgd
Ki_67
CCR7
CTLA4
CD28
CD69
CD25
CD45RO
GZB
KLRG1
CD103
CD161
CX3CR1
PD1
CD127
CD57
cluster_id
Naive B cells
DN B cells
Unswitched memory B cells
CLA+ Switched memory B cells
CLA– Switched memory B cells
Naive–like CD11c B cells
DN CD11c B cells
Switched memory CD11c B cells
CLA+Ki67– Plasmablasts
CLA+Ki67+ Plasmablasts
CLA–Ki67+ Plasmablasts
CLA–Ki67– CD99+ Plasmablasts
CLA–Ki67– CD99– Plasmablasts
median scaled expression
0.8
0.6
0.4
0.2
0


HV
CM
CS
Sepsis
CComm
SeConv
Unswitched memory B cells
Switched memory CD11c B cells
Naive-like CD11c B cells
Naive B cells
DN CD11c B cells
DN B cells
CLA+Ki67+ Plasmablasts
CLA+Ki67- Plasmablasts
CLA+ Switched memory B cells
CLA-Ki67+ Plasmablasts
CLA-Ki67- CD99+ Plasmablasts
CLA-Ki67- CD99- Plasmablasts
CLA- Switched memory B cells
CM
CS
CC
CComm
CConv
Sepsis
CS
CC
CC
CS+CC
CConv
CConv
logFC
10
5
0
-5
-10
* P_c<0.05
** P_c<0.01
*** P_c<0.001
Cytof depleted whole blood

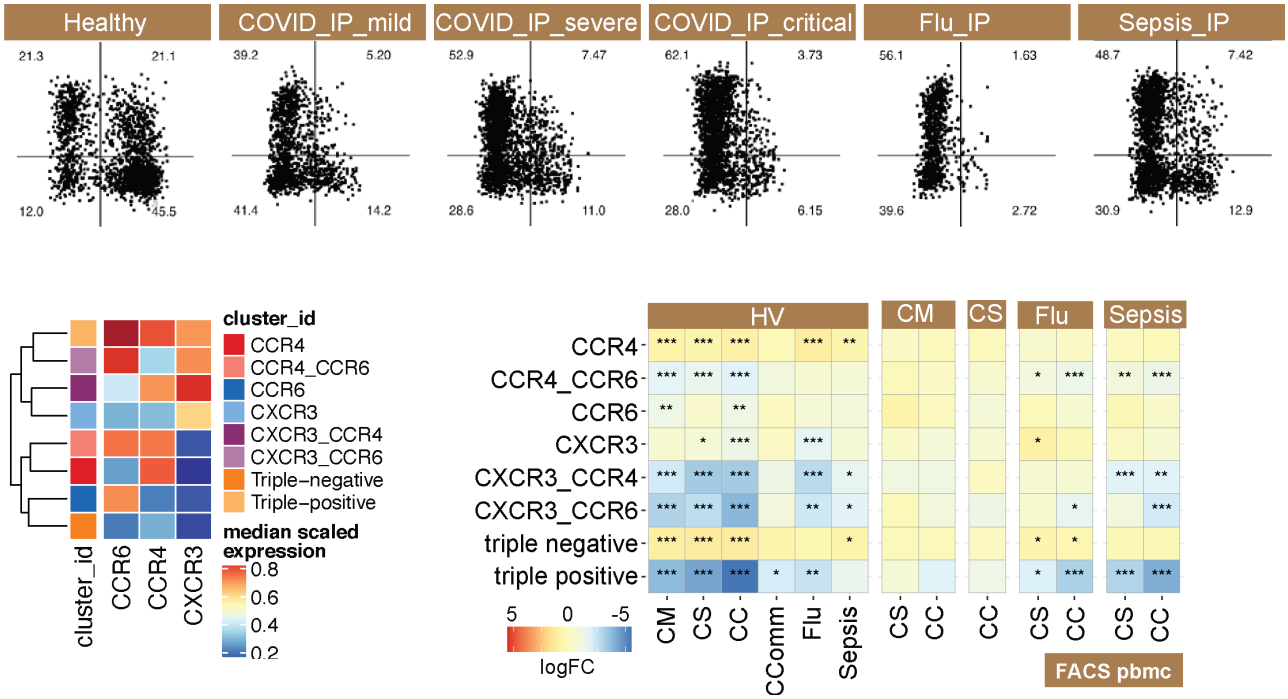


8: Single cell mass cytometry (CyTOF) compositional analysis showing UMAP, clustering and differential abundance of major B cell populations. Related to STAR Methods: Mass cytometry data analysis: differential abundance analysis.



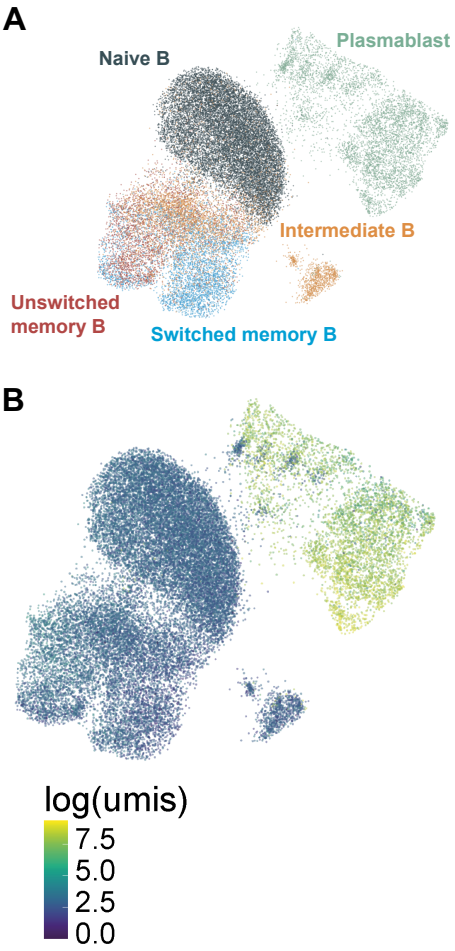
9: Phenotype of the T/NK cell populations assayed by single cell mass cytometry. (A) cell clustering (B) differential abundance analysis. Related to STAR Methods: Mass cytometry data analysis: differential abundance analysis.

Multicolor flow cytometry analysis

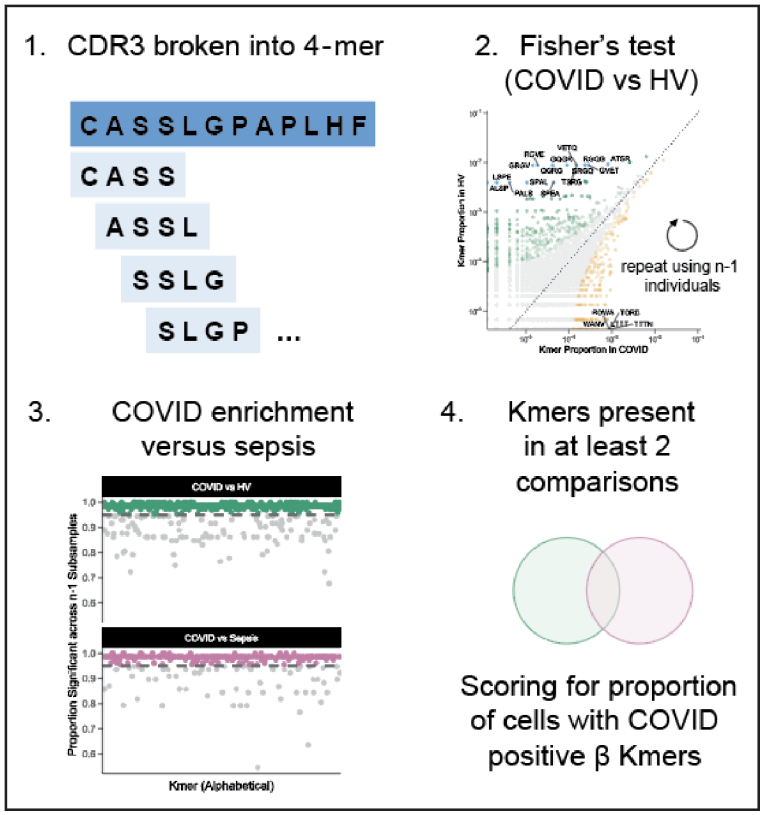


10: Phenotype and frequency of subsets of memory CD4 T cell subsets defined based on the expression of CCR4, CCR6 and CXCR3. PBMCs assayed using multicolor flow cytometry analysis. Dotplots, clustering and heatmap. Relates to Figure S4A-C. Related to STAR Method: Flow cytometry data analysis.

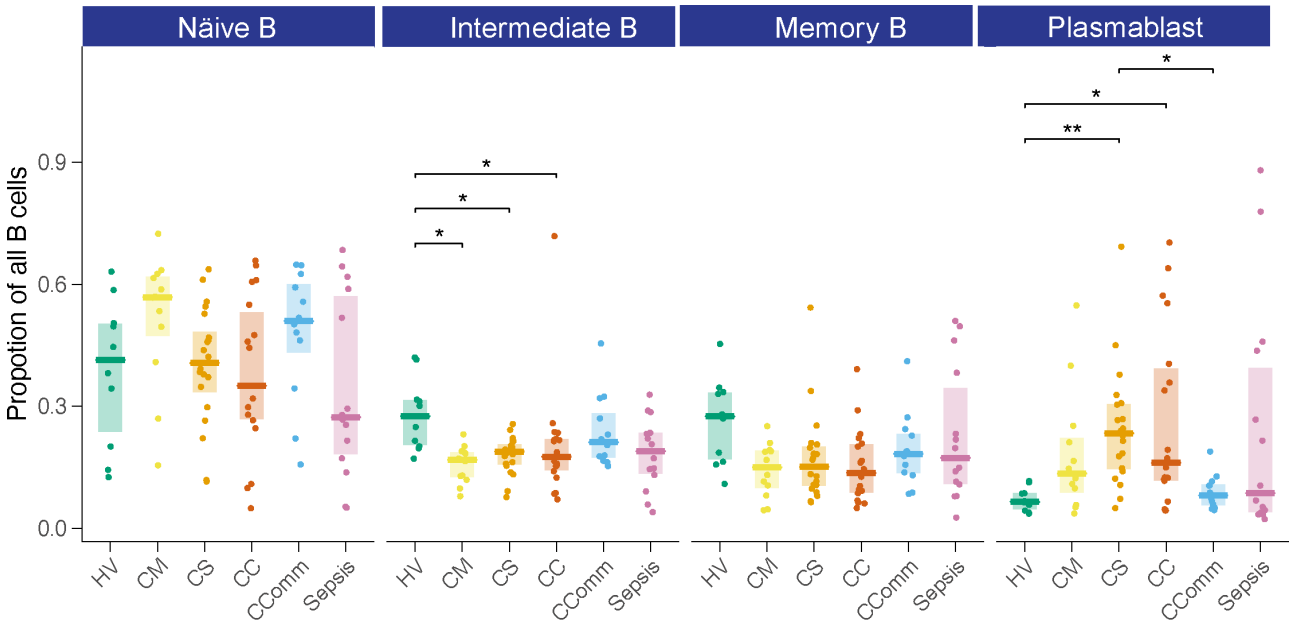
Repertoire analysis



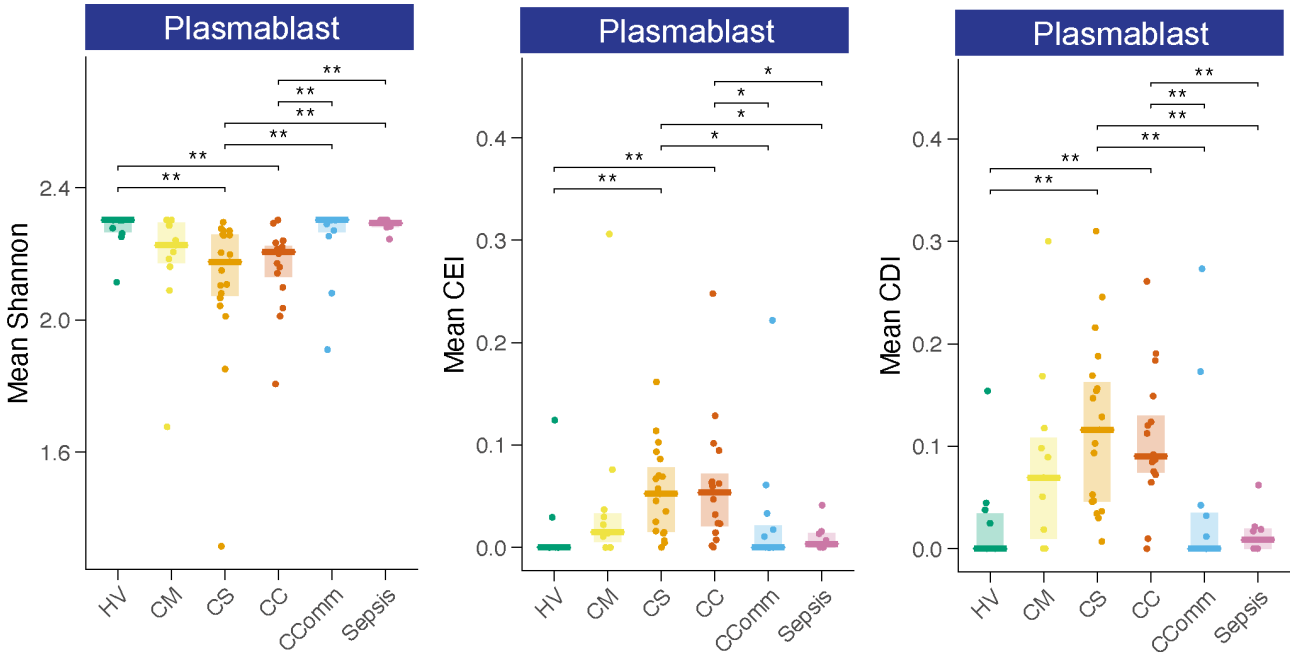
11: UMAP embedding B cell repertoire analysis (A) UMAP embedding with cluster identities from CITE-seq (B) UMAP embedding of all B cells from single cell VDJ dataset. As expected, PB showed the highest BCR expression in the CITEseq dataset. Color scale in panel B depicts log total UMIs per VDJ sequence. Related to STAR Methods: Repertoire analysis: additional pre-processing of scBCR-seq data.



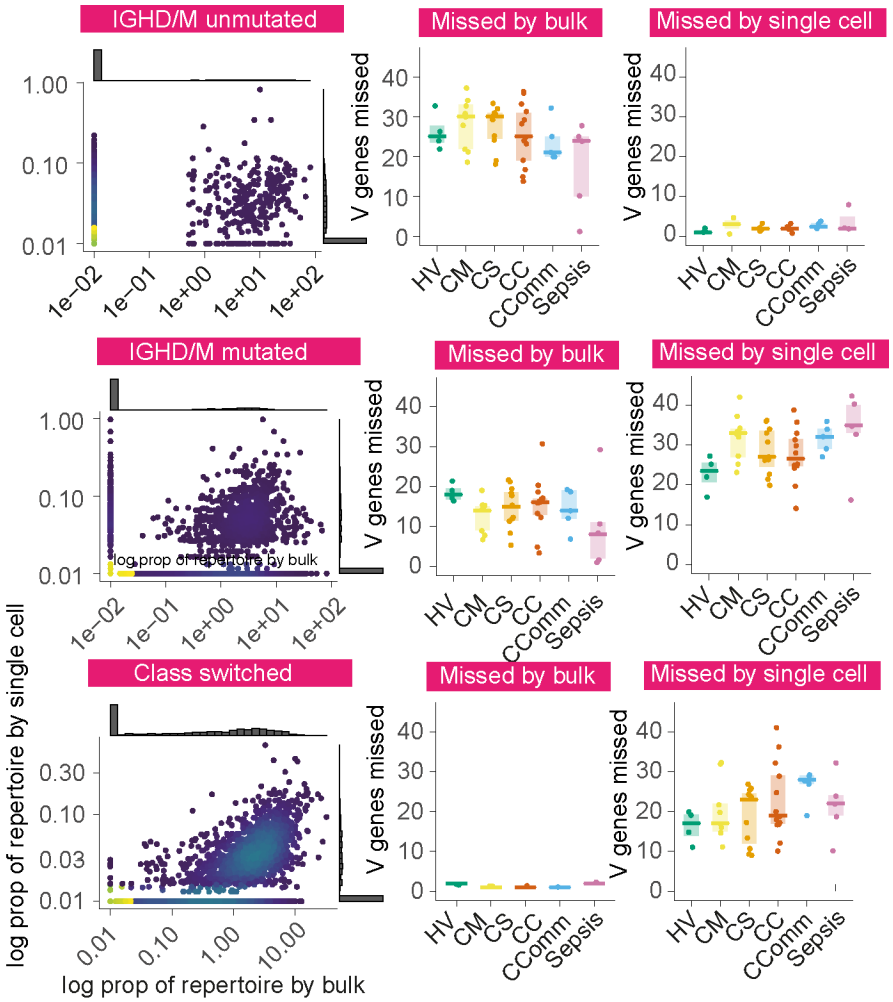
12: Illustration of the method used to identify CDR3 Kmers associated with COVID-19 compared to cells from healthy volunteers and patients with sepsis. Related to STAR Methods: Repertoire analysis: scTCR cytotoxicity and Kmer analysis.



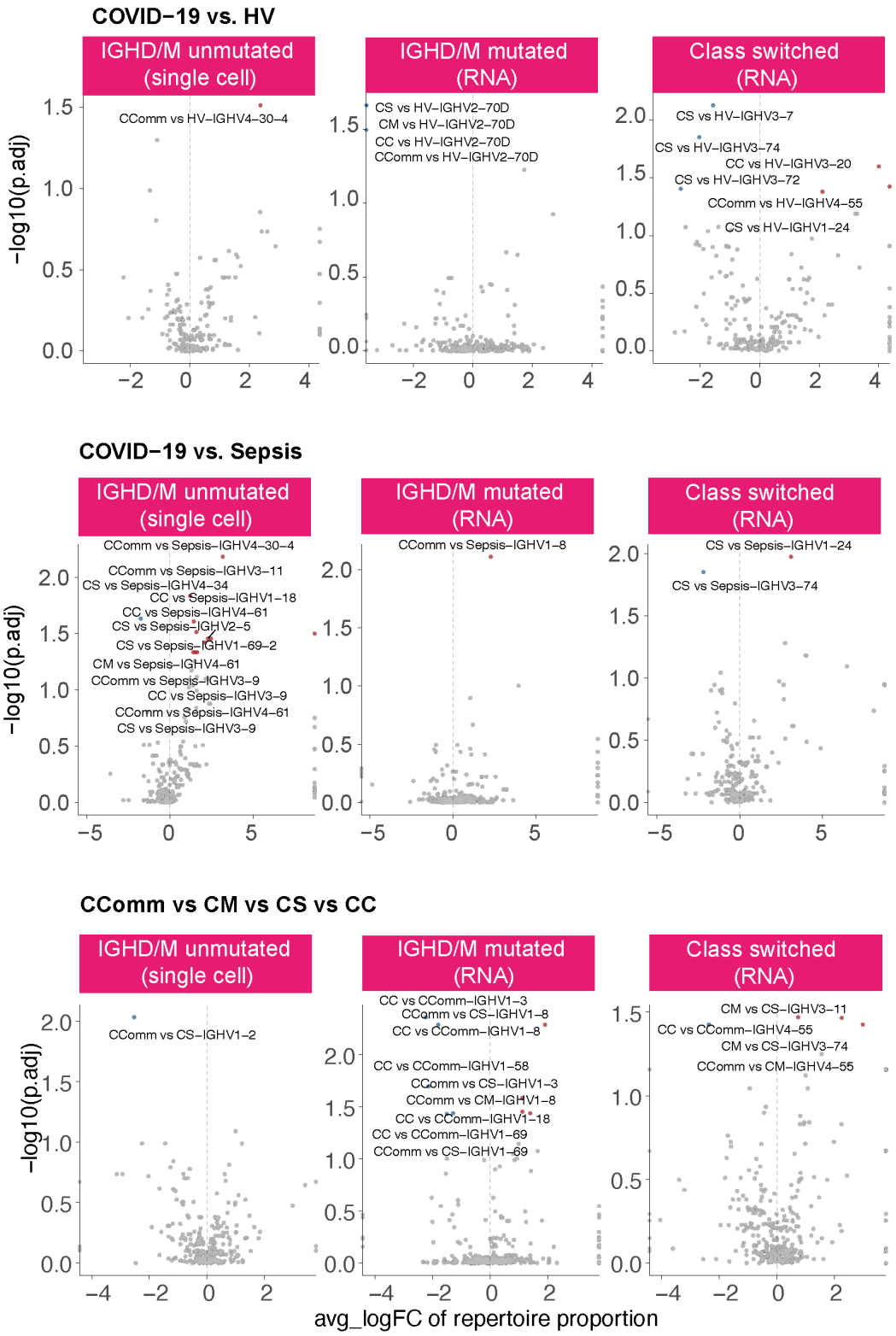
13: Cluster proportions. Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.



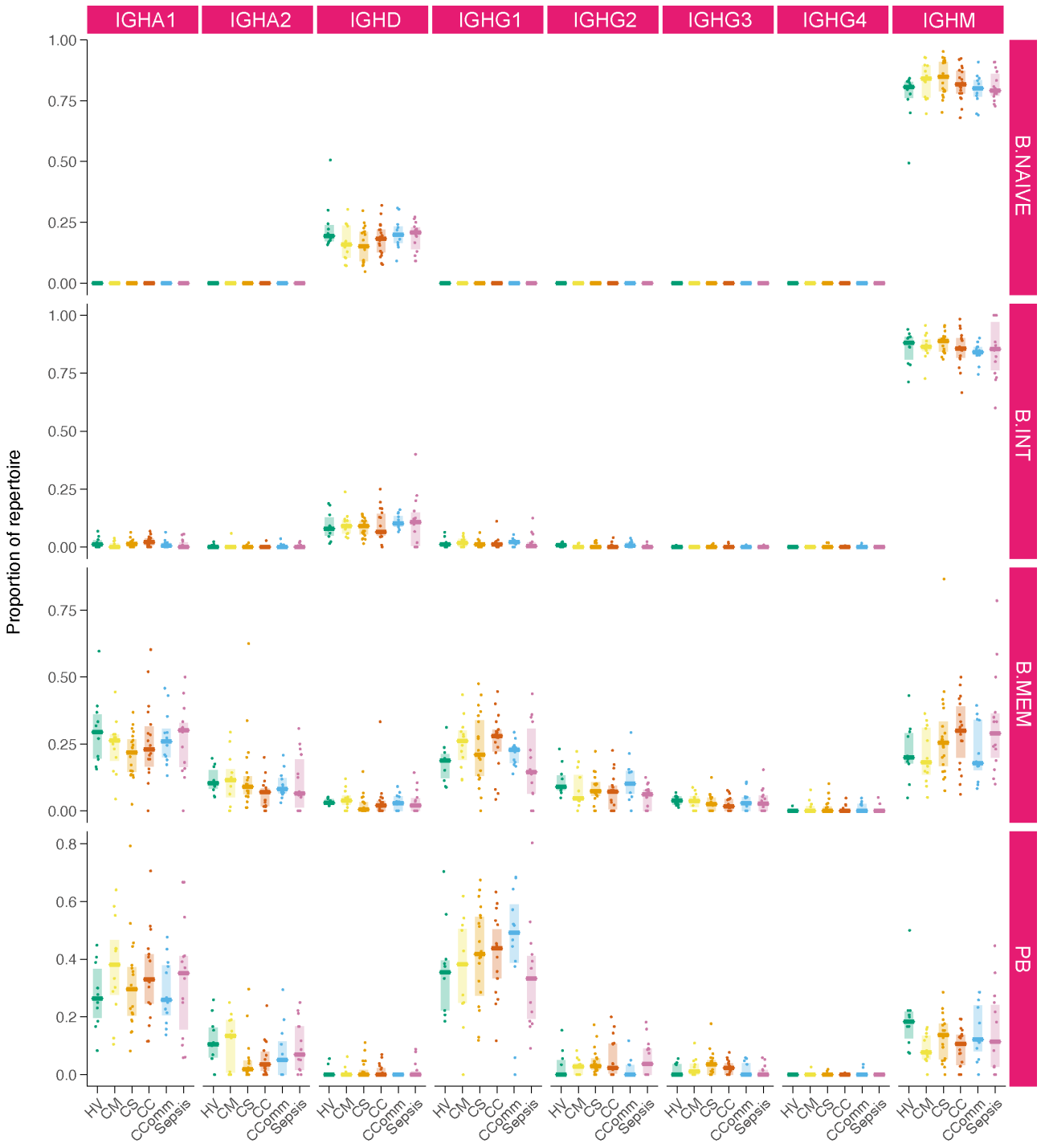
14: Mean plasmablast diversity indices by comparator group; clonal expansion index, CEI, clonal diversification index, CDI. Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.



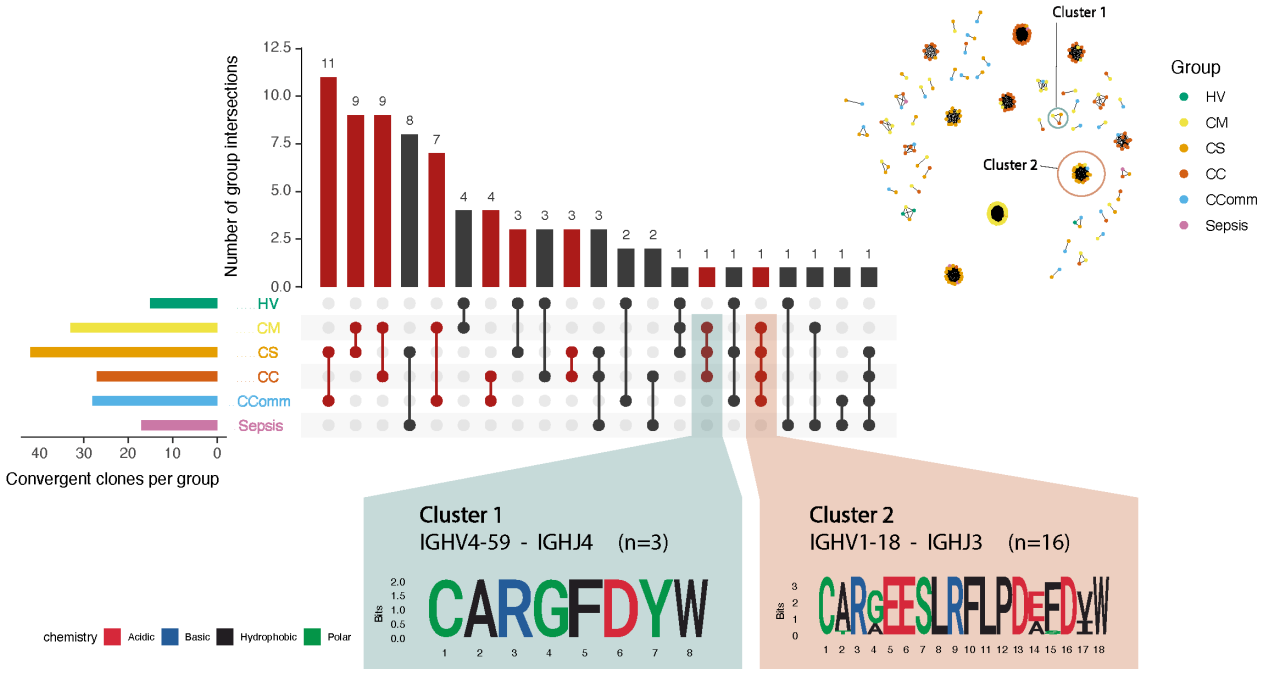
15: Correlation analysis of IGHV genes detected by single cell VDJ data and RNA VDJ data, within the (top) IGHD/M unmutated, (middle) IGHD/M mutated and (bottom) class-switched VDJ sequences. Scatter plots, with marginal histograms, depict log proportions of repertoires detected and missed by both datasets. Boxplots to the right depict number of IGHV genes missed by RNA (bulk) and single cell VDJ data. Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.



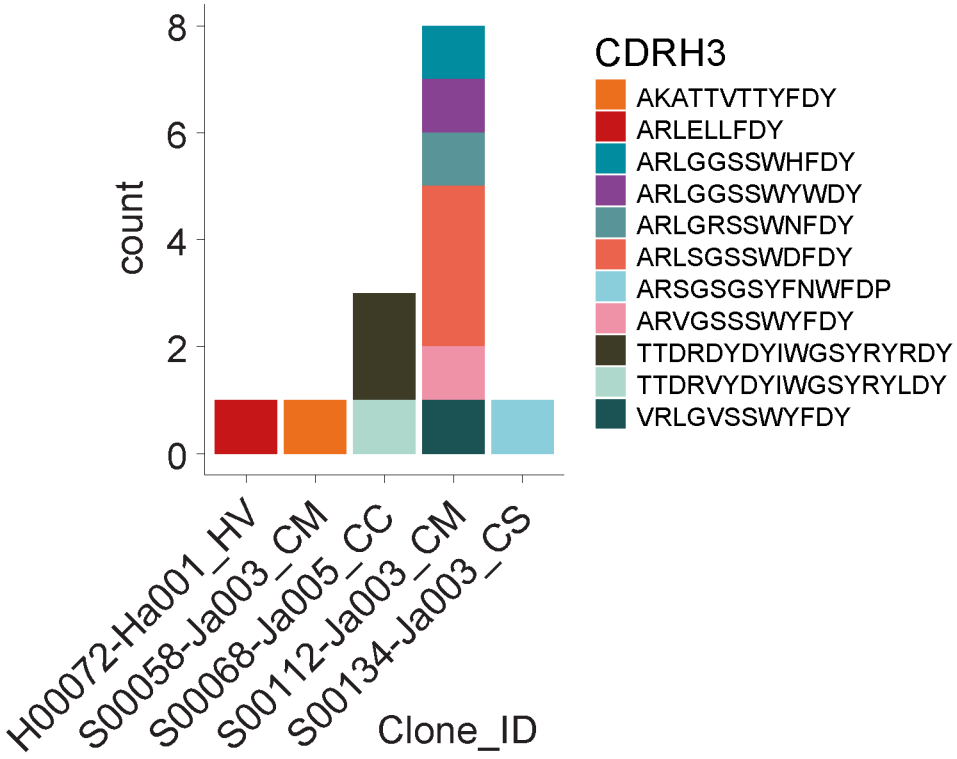
16: Volcano plots of log_2 fold change of IGHV gene usage of IGHD/M unmutated sequences (derived from single cell VDJ data), IGHD/M mutated sequences (derived from RNA VDJ data), class-switched sequences (derived from RNA VDJ data). Top panel depicts significant genes across pairwise comparisons of COVID-19 groups vs. healthy volunteers. Middle panel depicts significant genes across COVID-19 vs sepsis groups. Bottom panel depicts significant genes across COVID-19 severity groups. All significant pairwise comparisons are derived from Dunn tests post Kruskal Wallis testing. Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.



17: Proportion of constant region genes across B cell clusters per study group. Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.



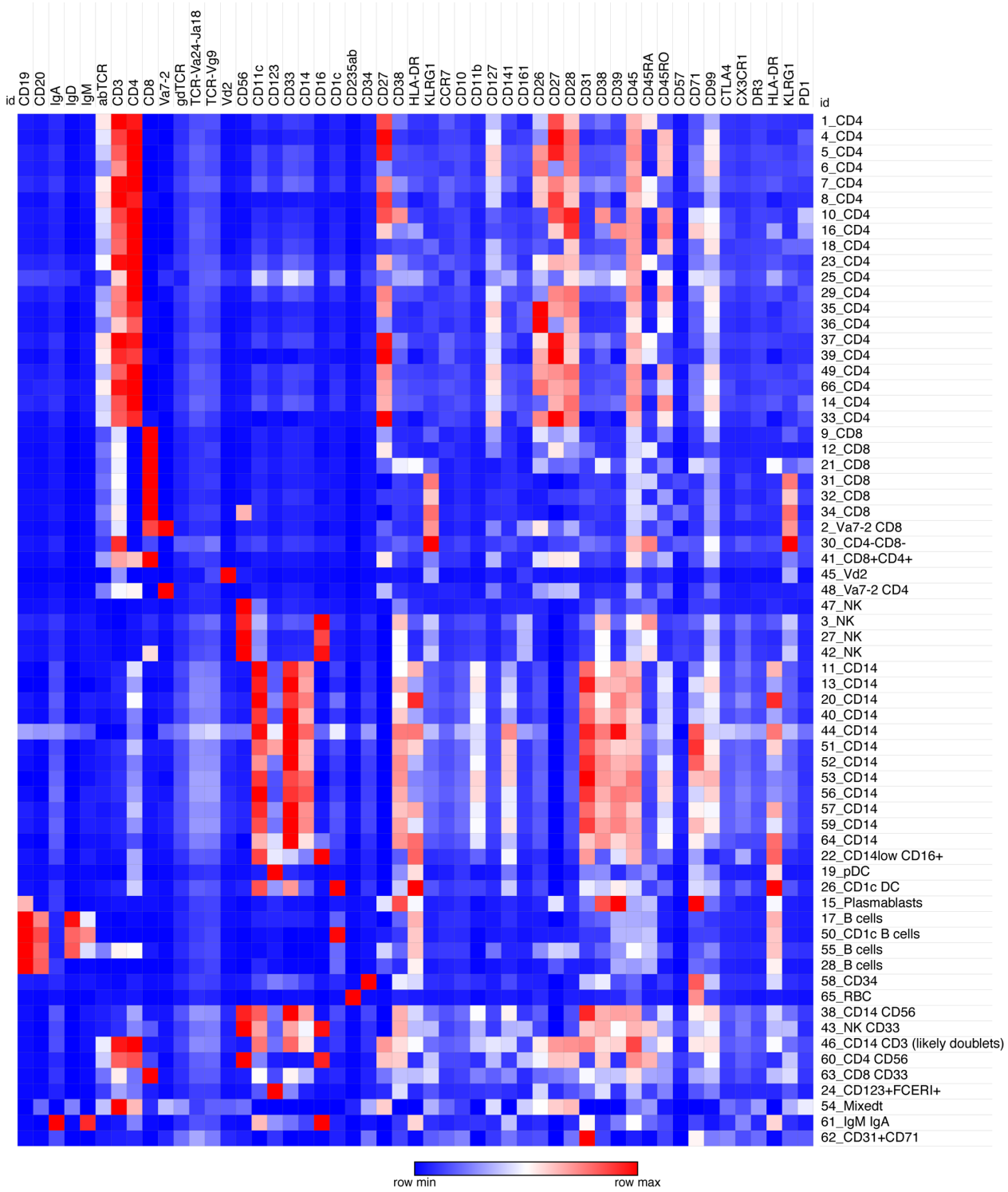
18: Clonal overlap across comparator groups with total number of convergent clones per group shown with clonal network depicting distribution of convergent clusters (inset) and sequence logos of COVID-19 exclusive clusters (with IGHV/J usage and number of members). Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.



19: Cumulative bar chart of the frequencies of known SARS-CoV-2 binding BCRs per patient group. Each section represents an individual patient. Related to STAR Methods: Repertoire analysis: RNA-velocity B cell analysis.

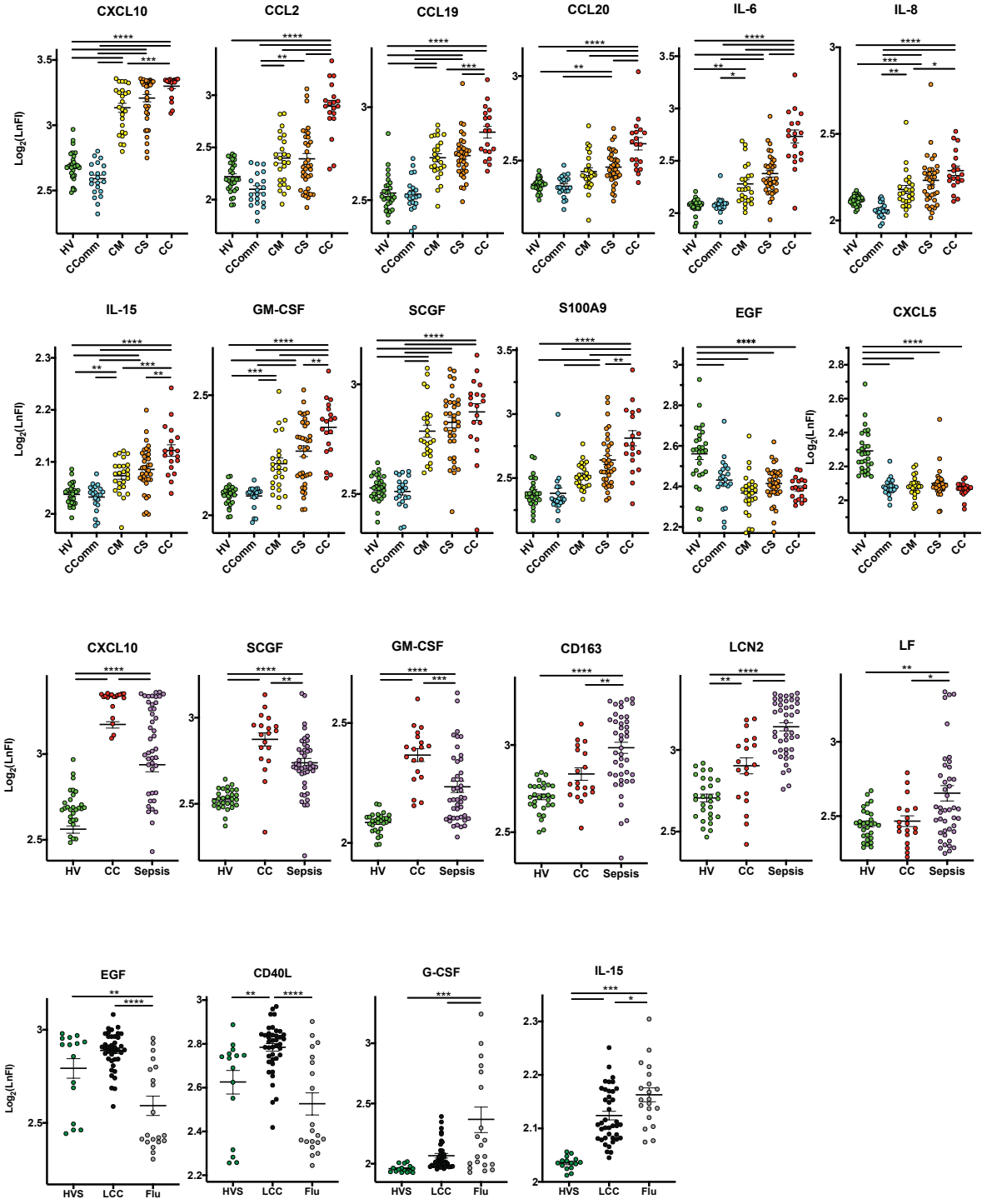
Proteomics

Clustering of surface protein (ADT) data

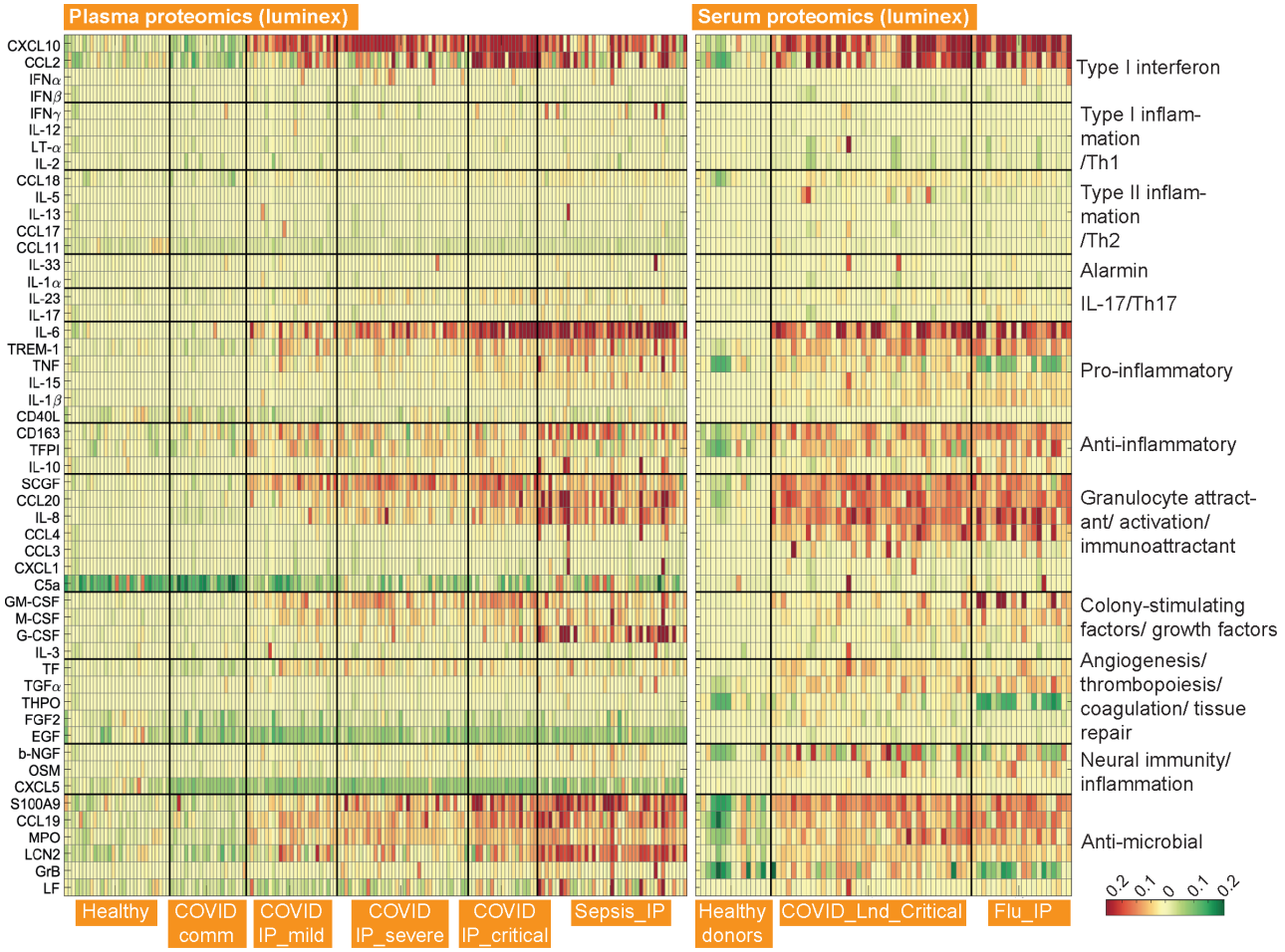


20: Clustering of surface protein (ADT) data. Initial cytosplore clustering of the CITE-seq datasaet by ADT surface phenotype based on n=192 ADT tags. Columns: a selection of informative surface proteins (top labels), rows: Cytosplore cluster numbers and assigned phenotypes (right labels). Related to Figures 1B,C; STAR Methods: CITE-seq: Pre-processing and analysis of the surface protein (ADT) data; and Data S5: Multimodal annotation of CITE-seq data.

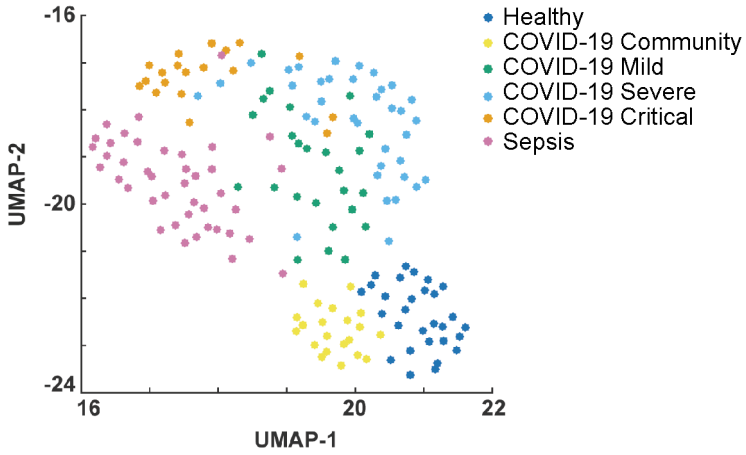
Plasma and serum proteins assayed by Luminex



21: Plasma protein abundance for specific proteins assayed by Luminex assay. Individual protein abundance across the comparator groups shown. Related to STAR Methods: Luminex data analysis: Cytokine enrichment profile analysis.

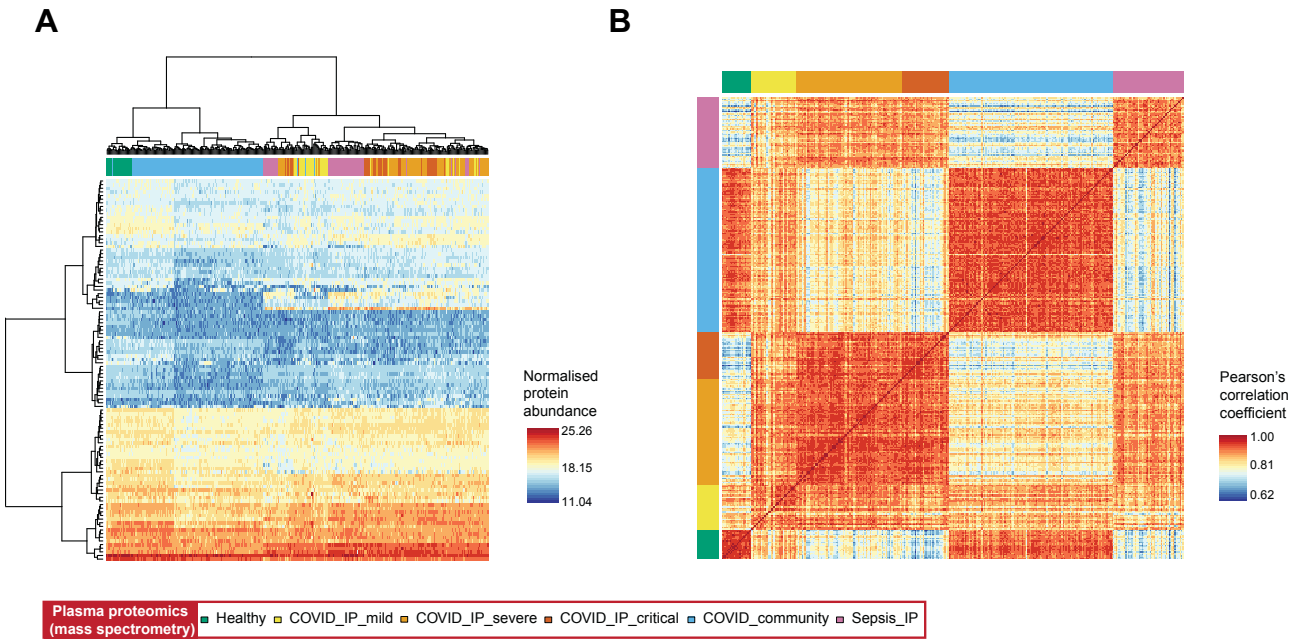


22: Heat map showing abundance of plasma and serum proteins by disease groups assayed using Luminex. Related to STAR Methods: Luminex data analysis: heatmap.

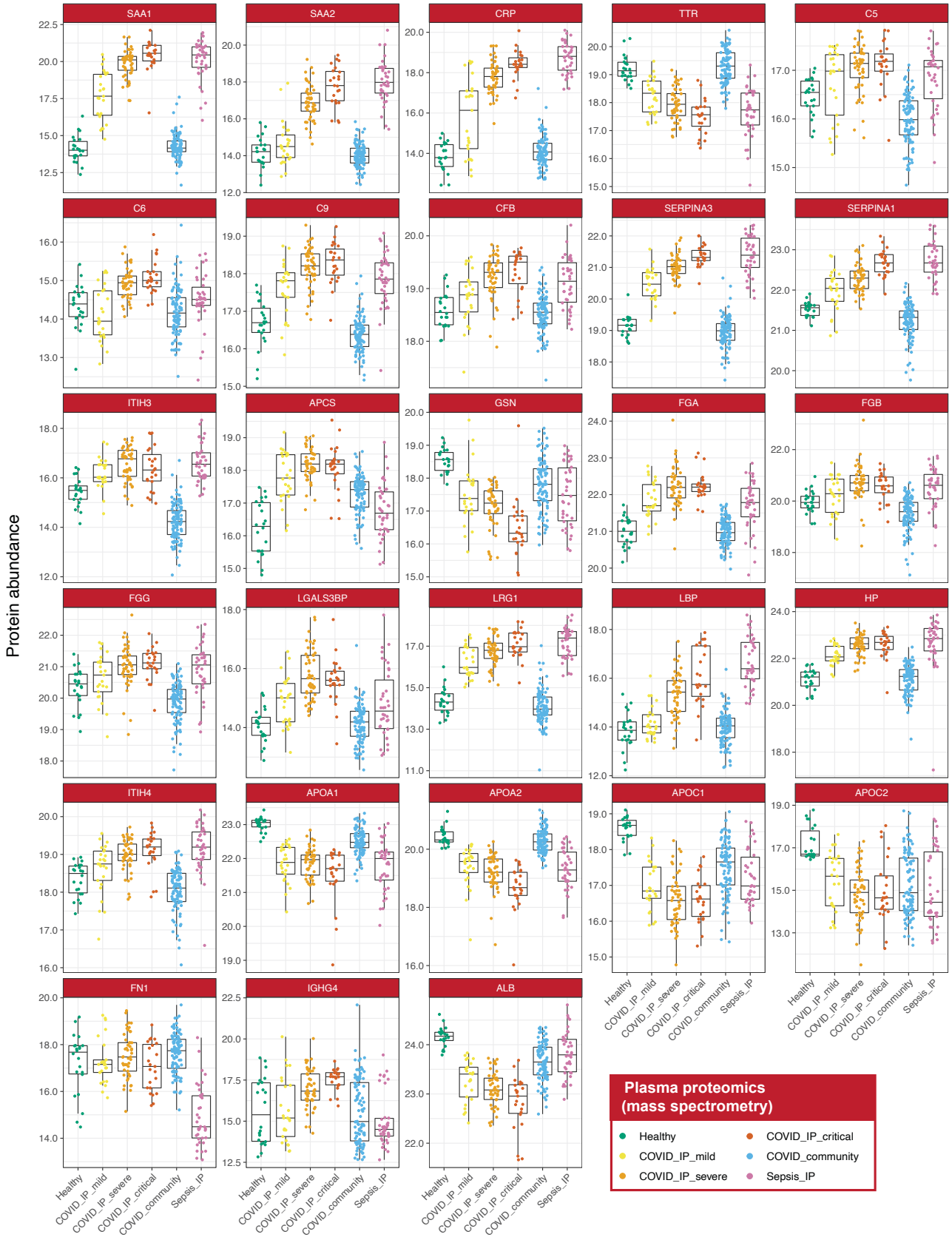


23: UMAP of plasma proteins assayed by Luminex assay, colored by comparator group. Related to STAR Methods: Luminex data analysis: uniform manifold projection.

Plasma proteins assayed by HT-LC-MS/MS mass spectrometry



24: Unsupervised hierarchical clustering and supervised correlation analysis of plasma proteins assayed by HT-LC-MS/MS mass spectrometry. (A) Unsupervised clustering analysis all samples (B) supervised correlation analysis all samples. Related to STAR Methods: Tims-TOF mass spectrometry analysis: statistical analysis.



25: Plasma protein abundance for specific proteins assayed by HT-LC-MS/MS mass spectrometry. Individual protein abundance across the comparator groups shown. Only one sample per patient at the maximal severity are plotted. Related to STAR Methods: Tims-TOF mass spectrometry analysis.