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Supplementary Materials for

Plasma from patients with bacterial sepsis or severe COVID-19 induces suppressive myeloid cell production from hematopoietic progenitors in vitro

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The PDF file includes:

Figs. S1 to S11

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S7
Data files S1 to S4

SUPPLEMENTARY FIGURES

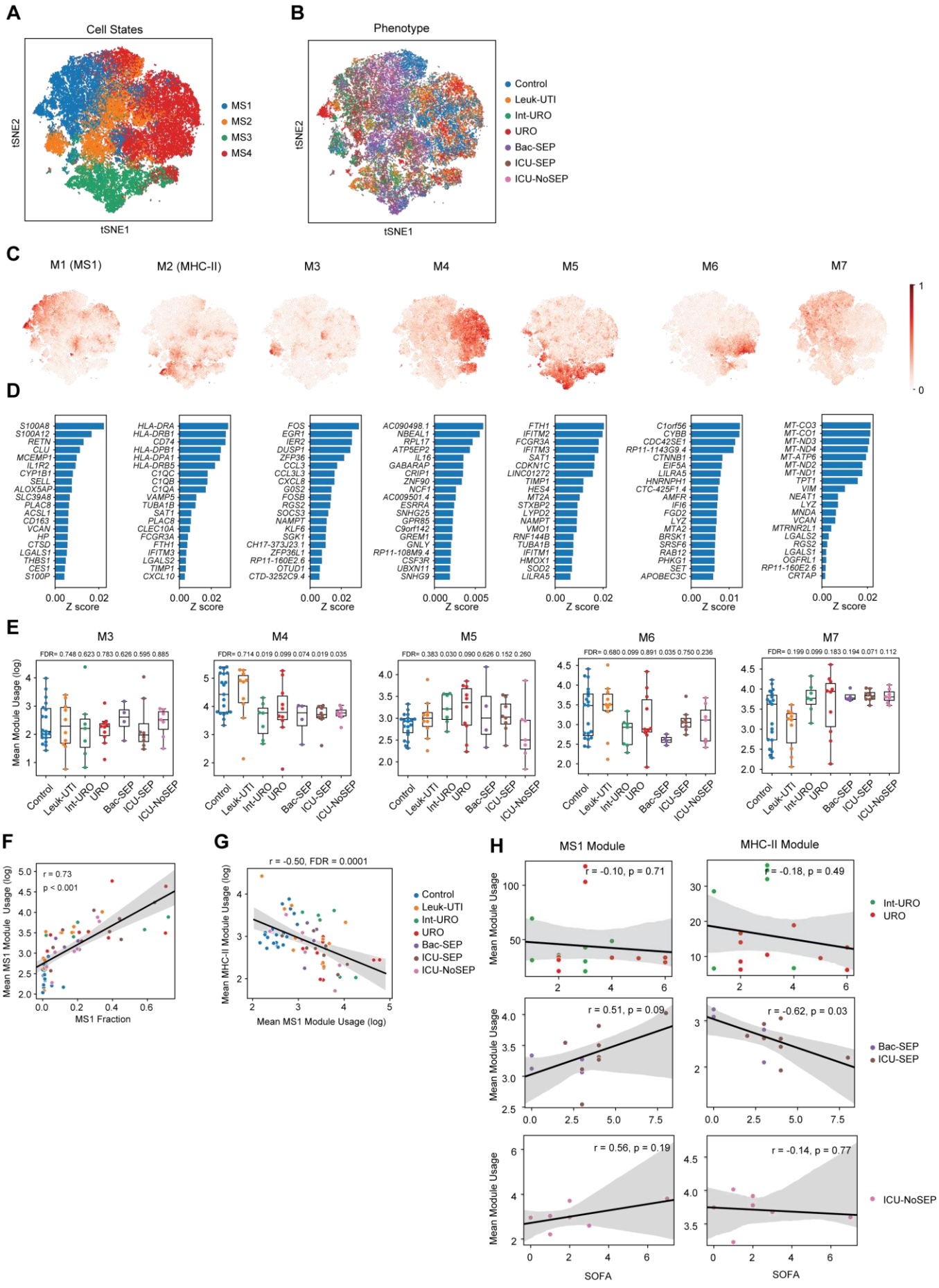

Figure S1. **Characterization of gene expression modules in sepsis monocytes.** (**A-B**) tSNE plot of monocytes from sepsis patients and controls colored by (**A**) cell state and (**B**) cohort. (**C**) Relative usage of gene expression module unbiasedly derived from the monocyte scRNA-seq data. (**D**) Genes with the top 10 loading in each gene expression module. (**E**) Mean usage of each gene module in monocytes for each patient type. FDR values are shown when comparing each disease state with healthy controls (two-tailed Wilcoxon rank-sum test, corrected for testing of multiple modules). Boxes show the median and IQR for each patient cohort, with whiskers extending to 1.5 IQR in either direction from the top or bottom quartile. (**F**) Scatterplot showing correlation between mean MS1 gene module usage in monocytes and MS1 fractions for each patient. Line and shadow indicate linear regression fit and 95% confidence interval, respectively. Significance of the correlations (Pearson r) were calculated with a two-sided permutation test. (**G**) Scatterplot showing correlation between mean MS1 and MHC-II gene module usage in monocytes for each patient. Line and shadow indicate linear regression fit and 95% confidence interval, respectively. Significance of the correlations (Pearson r) were calculated with a two-sided permutation test and corrected for multiple comparison of modules. (**H**) Scatter plots showing the correlation between mean module usage in monocytes and SOFA scores for each subject. Line and shadow indicate linear regression fit and 95% confidence interval, respectively. Significance of the correlations (Pearson *r*) were calculated with a two-sided permutation test. Detailed description of the patient cohorts and number of cells and patient groups are outlined in Reyes, et al *(23)*. Control, healthy controls; Leuk-UTI, urinary tract infection with leukocytosis; Int-URO, intermediate urosepsis; URO, urosepsis; Bac-SEP, sepsis with confirmed bacteremia; ICU-SEP, intensive care with sepsis; ICU-NoSEP, intensive care without sepsis; SOFA, sequential organ failure assessment.

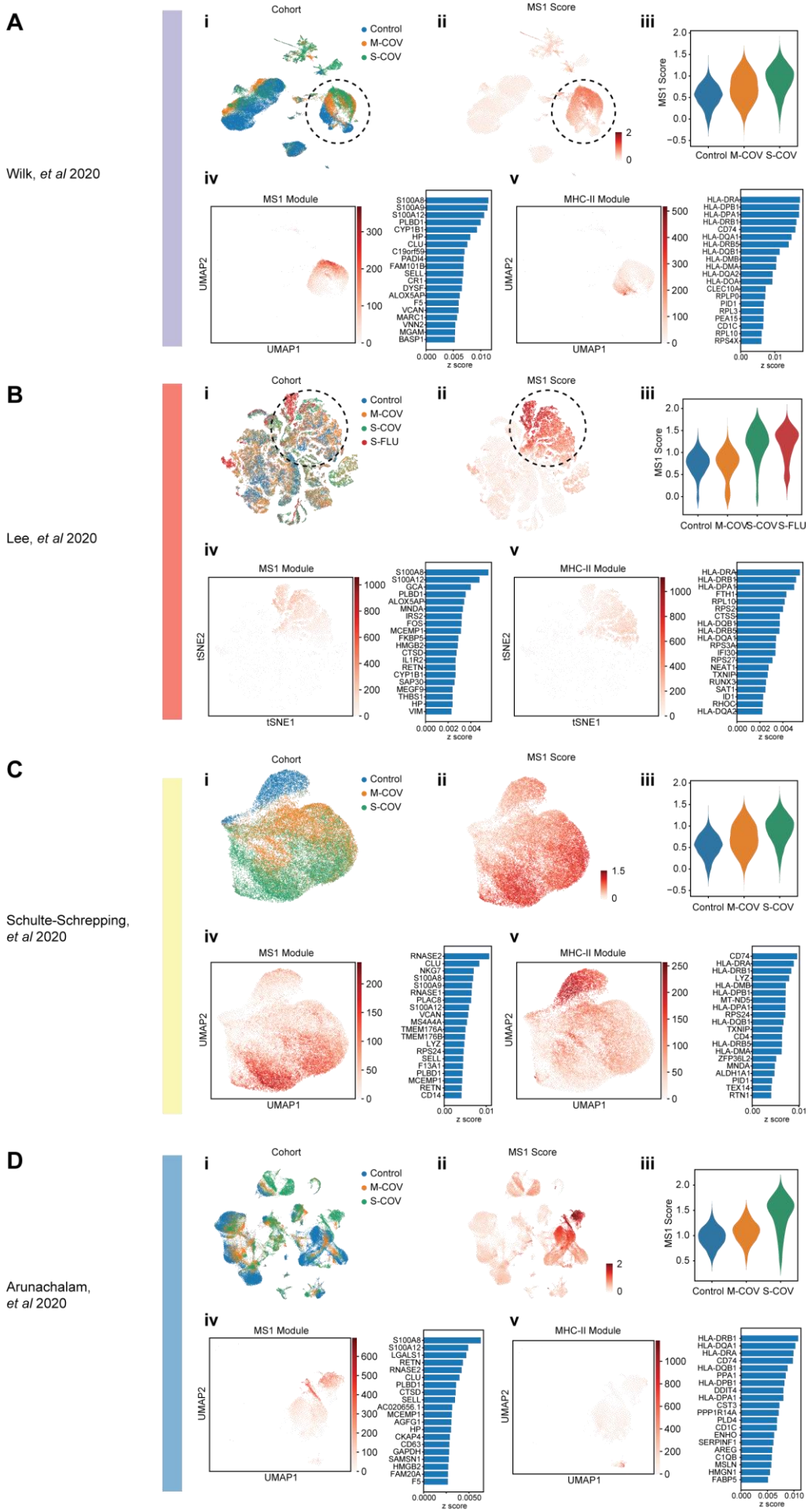
A
Wilk, et al 2020
i
Cohort
Control
M-COV
S-COV
ii
MS1 Score
2
0
iii
MS1 Score
Control M-COV S-COV
iv
MS1 Module
UMAP2
UMAP1
S100A8
S100A9
S100A12
PLBD1
CYP1B1
HP
CLU
C19orf59
PADI4
FAM101B
SELL
CR1
DYSF
ALOX5AP
F5
VCAN
MARC1
VNN2
MGAM
BASP1
z score
v
MHC-II Module
HLA-DRA
HLA-DPB1
HLA-DPA1
HLA-DRB1
CD74
HLA-DQA1
HLA-DRB5
HLA-DQB1
HLA-DMB
HLA-DMA
HLA-DQA2
HLA-DOA
CLEC10A
RPLP0
PID1
RPL3
PEA15
CD1C
RPL10
RPS4X
B
Lee, et al 2020
Control
M-COV
S-COV
S-FLU
tSNE2
tSNE1
C
Schulte-Schrepping, et al 2020
RNASE2
CLU
NKG7
S100A8
S100A9
RNASE1
PLAC8
S100A12
VCAN
MS4A4A
TMEM176A
TMEM176B
LYZ
RPS24
SELL
F13A1
PLBD1
MCEMP1
RETN
CD14
CD74
HLA-DRA
HLA-DRB1
LYZ
HLA-DMB
HLA-DPB1
MT-ND5
HLA-DPA1
RPS24
HLA-DQB1
TXNIP
CD4
HLA-DRB5
HLA-DMA
ZFP36L2
MNDA
ALDH1A1
PID1
TEX14
RTN1
D
Arunachalam, et al 2020
S100A8
S100A12
LGALS1
RETN
RNASE2
CLU
PLBD1
CTSD
SELL
AC020656.1
MCEMP1
AGFG1
HP
CKAP4
CD63
GAPDH
SAMSN1
HMGB2
FAM20A
F5
HLA-DRB1
HLA-DQA1
HLA-DRA
CD74
HLA-DQB1
PPA1
HLA-DPB1
DDIT4
HLA-DPA1
CST3
PPP1R14A
PLD4
CD1C
ENHO
SERPINF1
AREG
C1QB
MSLN
HMGN1
FABP5

Figure S2. Analysis of MS1 and MHC-II gene expression modules in COVID-19 blood scRNA-seq datasets. Independent analysis of four datasets: (**A**) Wilk, *et al* 2020, (**B**) Lee, *et al* 2020, (**C**) Schulte-Schrepping, *et al* 2020, and (**D**) Arunachalam, *et al* 2020. For each dataset: (i-ii) UMAP or tSNE projection of the cells colored by (i) patient condition and (ii) MS1 scores. (iii) Violin plot of MS1 scores for CD14-expressing cells from each condition. (iv-v) Left, UMAP or tSNE plot, showing usage of the MS1 and MHC-II gene expression modules unbiasedly derived from each dataset. Right, Barplots of the top 20 genes with the highest z-score loading in each module. Detailed description of the patient cohorts and number of cells and patients for each dataset in (**D,E**) are outlined in the corresponding publications *(7, 28, 29, 31)*. M-COV, mild COVID-19; S-COV, severe COVID-19, S-FLU, severe influenza A.

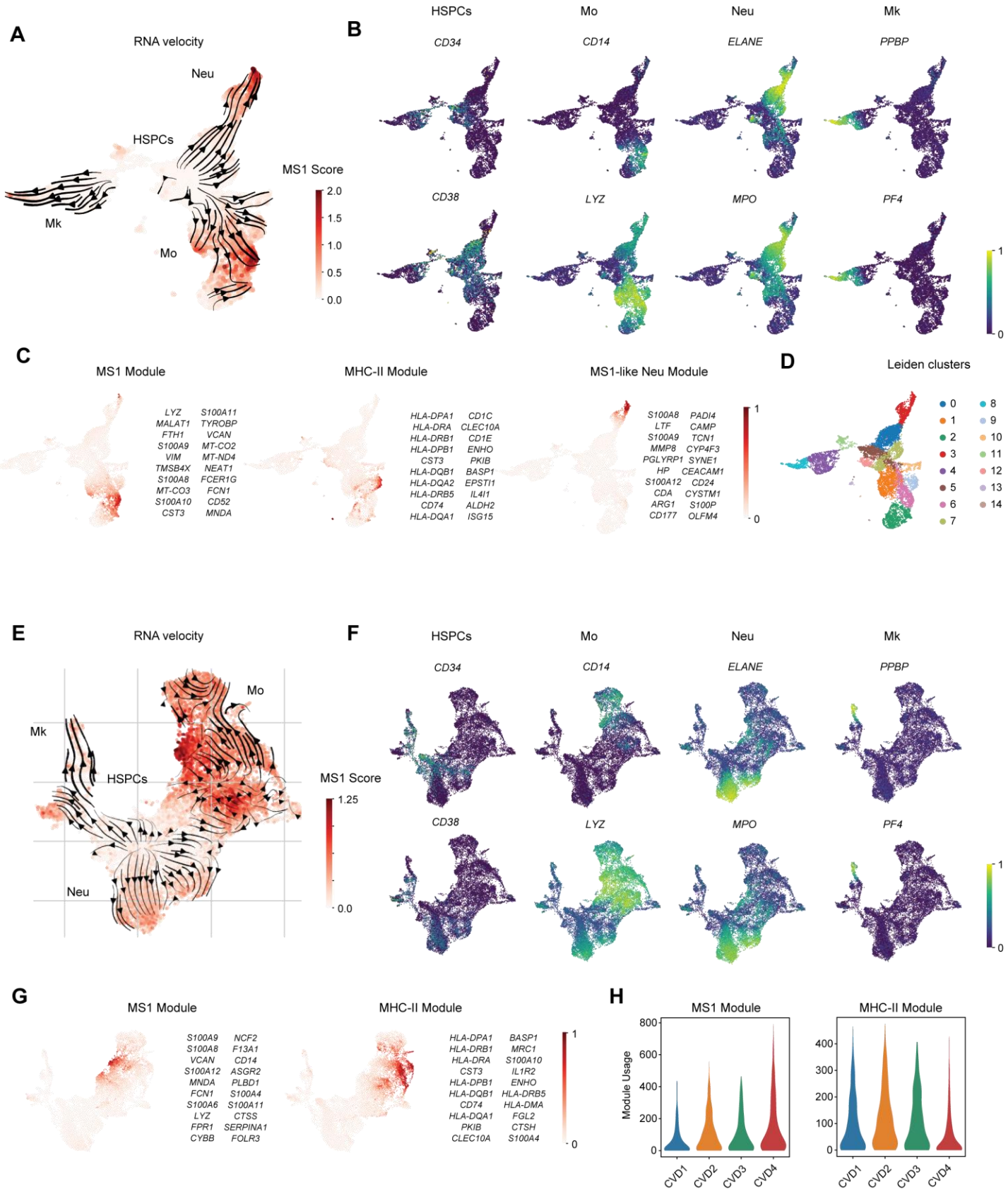
A
RNA velocity
Neu
HSPCs
Mk
Mo
MS1 Score
2.0
1.5
1.0
0.5
0.0
B
HSPCs
Mo
Neu
Mk
CD34
CD14
ELANE
PPBP
CD38
LYZ
MPO
PF4
1
0
C
MS1 Module
LYZ S100A11
MALAT1 TYROBP
FTH1 VCAN
S100A9 MT-CO2
VIM MT-ND4
TMSB4X NEAT1
S100A8 FCER1G
MT-CO3 FCN1
S100A10 CD52
CST3 MNDA
MHC-II Module
HLA-DPA1 CD1C
HLA-DRA CLEC10A
HLA-DRB1 CD1E
HLA-DPB1 ENHO
CST3 PKIB
HLA-DQB1 BASP1
HLA-DQA2 EPSTI1
HLA-DRB5 IL4I1
CD74 ALDH2
HLA-DQA1 ISG15
MS1-like Neu Module
S100A8 PADI4
LTF CAMP
S100A9 TCN1
MMP8 CYP4F3
PGLYRP1 SYNE1
HP CEACAM1
S100A12 CD24
CDA CYSTM1
ARG1 S100P
CD177 OLFM4
1
0
D
Leiden clusters
0 8
1 9
2 10
3 11
4 12
5 13
6 14
7
E
RNA velocity
Mo
Mk
HSPCs
Neu
MS1 Score
1.25
0.0
F
HSPCs
Mo
Neu
Mk
CD34
CD14
ELANE
PPBP
CD38
LYZ
MPO
PF4
1
0
G
MS1 Module
S100A9 NCF2
S100A8 F13A1
VCAN CD14
S100A12 ASGR2
MNDA PLBD1
FCN1 S100A4
S100A6 S100A11
LYZ CTSS
FPR1 SERPINA1
CYBB FOLR3
MHC-II Module
HLA-DPA1 BASP1
HLA-DRB1 MRC1
HLA-DRA S100A10
CST3 IL1R2
HLA-DPB1 ENHO
HLA-DQB1 HLA-DRB5
CD74 HLA-DMA
HLA-DQA1 FGL2
PKIB CTSH
CLEC10A S100A4
1
0
H
MS1 Module
MHC-II Module
Module Usage
800
600
400
200
0
400
300
200
100
0
CVD1
CVD2
CVD3
CVD4

Figure S3. **scRNA-seq of plasma-treated CD34+ HSPCs.** (**A-G**) UMAP projection of differentiated cells from CD34+ HSPCs incubated with (**A-D**) sepsis or (**E-G**) COVID-19 plasma and corresponding controls. Cells are colored by (**A,E**) MS1 scores, (**B,F**) relative expression of HSPC, monocyte (Mo), neutrophil (Neu), and megakaryocyte (Mk) marker genes, (**C,G**) relative usage of the MS1 and MHC-II modules, and (d) Leiden clusters. Stream plots showing inferred trajectories from RNA velocity are overlaid in (**A,E**). Top 20 genes in each module are listed in (**C,G**). (**H**) Violin plots showing the usage of the MS1 (left) and MHC-II (right) modules in CD14-expressing cells across the different plasma treatment conditions. The experiment in (**A-D**) was performed on 2 healthy bone marrow donors with 2 plasma donors for each condition; a total of 3,039 and 5,254 cells were profiled for Control and URO plasma treatment, respectively. The experiment in (**E-H**) was performed on 2 healthy bone marrow donors with pooled plasma for each COVID-19 patient group; a total of 4,449, 4,591, 3,129 and 3,711 cells were profiled for CVD1-CVD4 plasma, respectively.

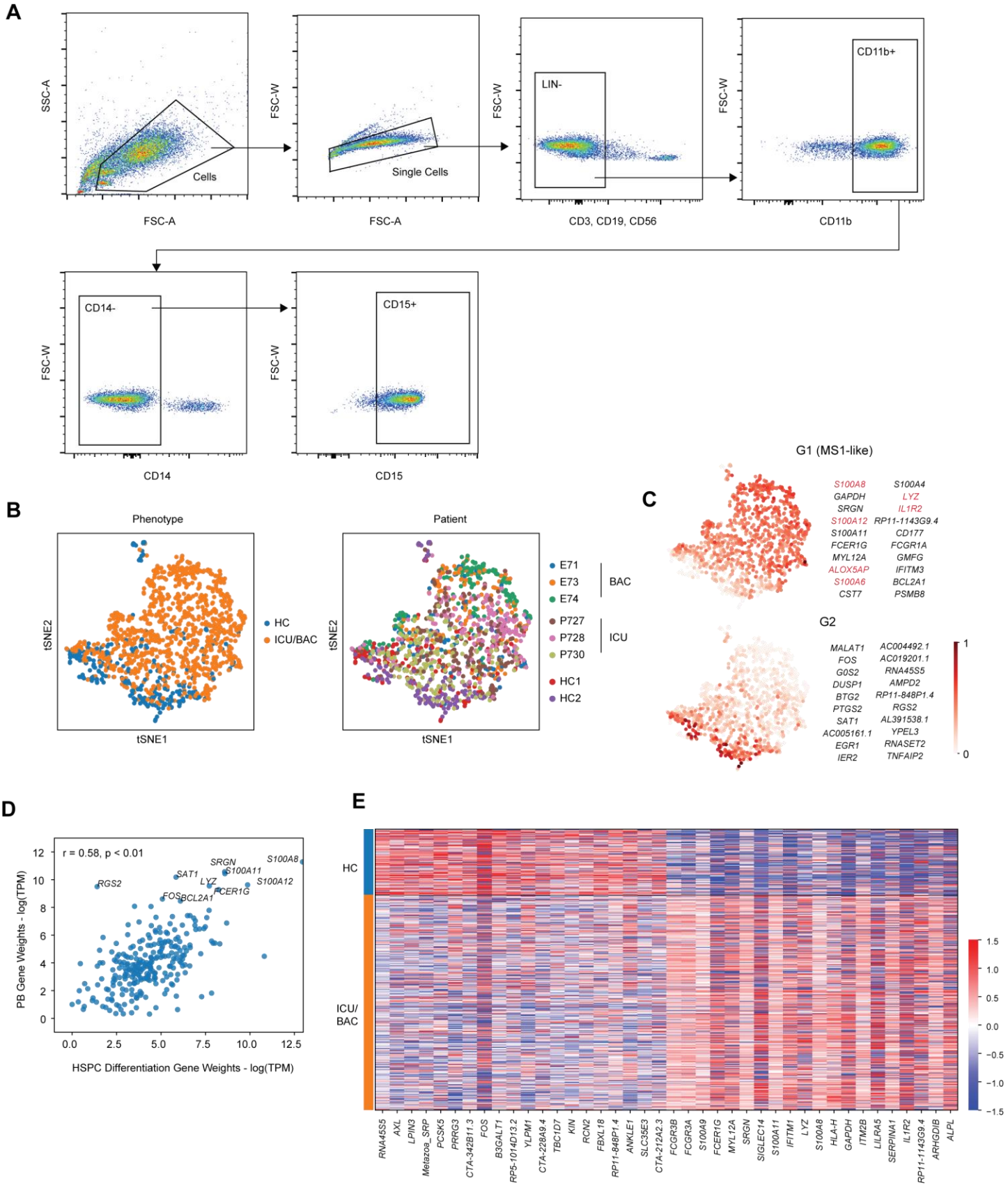
A
SSC-A
FSC-A
Cells
FSC-W
FSC-A
Single Cells
FSC-W
CD3, CD19, CD56
LIN-
FSC-W
CD11b
CD11b+
FSC-W
CD14
CD14-
FSC-W
CD15
CD15+
B
Phenotype
tSNE2
tSNE1
HC
ICU/BAC
Patient
tSNE2
tSNE1
E71
E73
E74
BAC
P727
P728
P730
ICU
HC1
HC2
C
G1 (MS1-like)
S100A8 S100A4
GAPDH LYZ
SRGN IL1R2
S100A12 RP11-1143G9.4
S100A11 CD177
FCER1G FCGR1A
MYL12A GMFG
ALOX5AP IFITM3
S100A6 BCL2A1
CST7 PSMB8
G2
MALAT1 AC004492.1
FOS AC019201.1
G0S2 RNA45S5
DUSP1 AMPD2
BTG2 RP11-848P1.4
PTGS2 RGS2
SAT1 AL391538.1
AC005161.1 YPEL3
EGR1 RNASET2
IER2 TNFAIP2
1
0
D
r = 0.58, p < 0.01
PB Gene Weights - log(TPM)
HSPC Differentiation Gene Weights - log(TPM)
S100A8
SRGN
S100A11
S100A12
SAT1
LYZ
RGS2
FCER1G
FOS
BCL2A1
E
HC
ICU/
BAC
RNA45S5
AXL
LPIN3
Metazoa_SRP
PCSK5
PRRG3
CTA-342B11.3
FOS
B3GALT1
RP5-1014D13.2
YLPM1
CTA-228A9.4
TBC1D7
KIN
RCN2
FBXL18
RP11-848P1.4
ANKLE1
SLC35E3
CTA-212A2.3
FCGR3B
FCGR3A
S100A9
FCER1G
MYL12A
SRGN
SIGLEC14
S100A11
IFITM1
LYZ
S100A8
HLA-H
GAPDH
ITM2B
LILRA5
SERPINA1
IL1R2
RP11-1143G9.4
ARHGDIB
ALPL
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5

Figure S4. **scRNA-seq of neutrophils sorted from critically-ill and bacteremic patients.** (**A**) Gating strategy for single cell sorting of neutrophils. (**B**) tSNE plot (*n* = 2,168 cells) of neutrophil scRNA-seq data colored by cohort (left) and individual patients (right). (**C**) Relative usage of two gene modules unbiasedly derived from the neutrophil data (by cNMF). The top 20 genes in each module are listed. Genes in module N1 that are also among the top 30 genes in the MS1 module are highlighted in red. (**D**) Gene weight correlation between the MS1-like modules detected in neutrophils generated from incubation of HSPCs with sepsis plasma (x-axis) and neutrophils isolated from sepsis patients (y-axis). Significance of the correlations (Pearson *r*) are calculated with a permutation test. (**E**) Heatmap showing the top 20 differentially expressed genes (Wilcoxon rank-sum test, FDR < 0.01) between neutrophils sorted from critically-ill patients and healthy controls. BAC, bacteraemic patient; ICU, patient in intensive care.

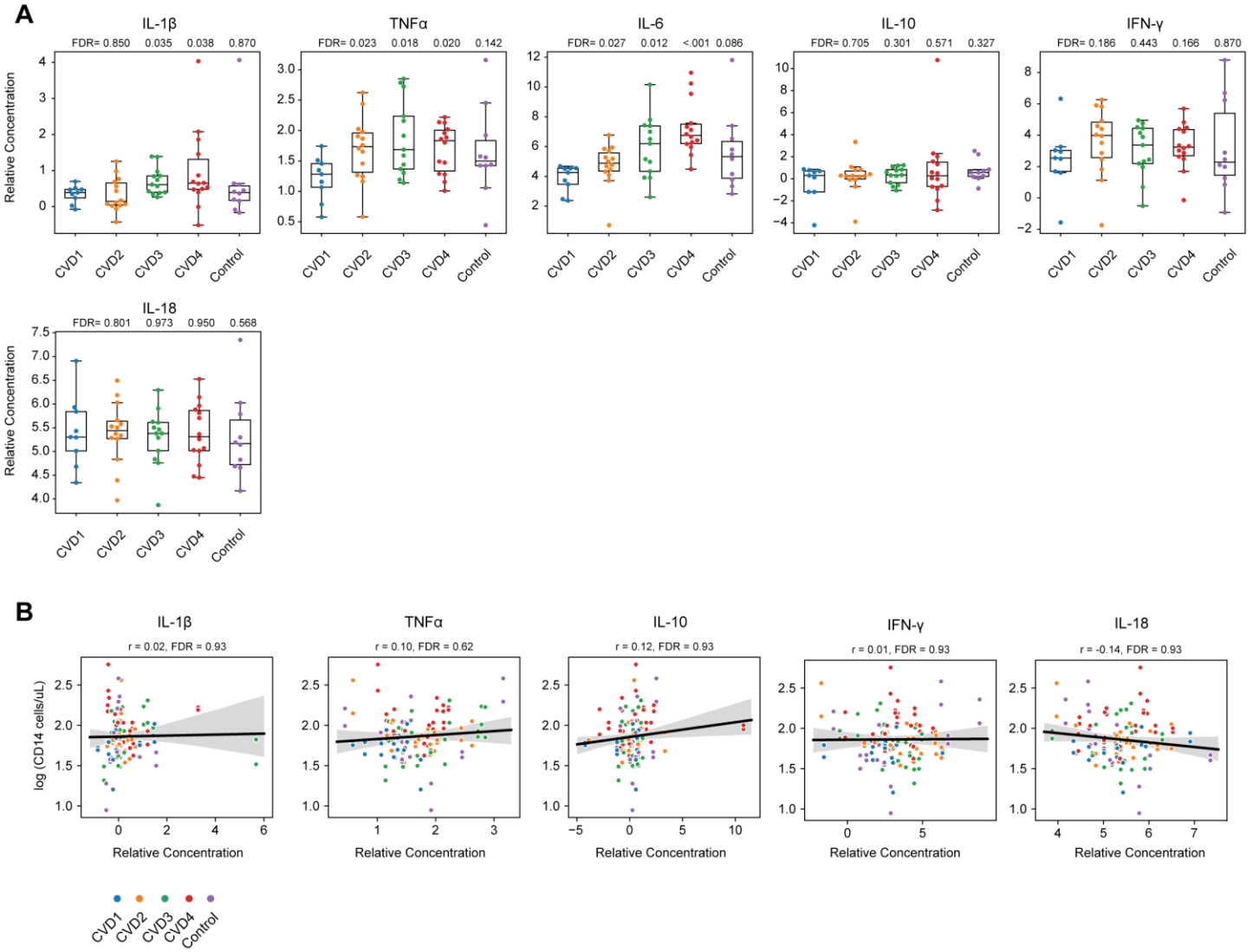

Figure S5. Plasma inflammatory cytokine levels in COVID-19 patients and correlation with cellular output. (**A**) Boxplots showing NPX (normalized protein expression) levels of inflammatory cytokines in plasma for each patient (*n* = 61). FDR values are shown when comparing each disease state with healthy controls (two-tailed Wilcoxon rank-sum test, corrected for testing of multiple cytokines). Boxes show the median and IQR for each patient cohort, with whiskers extending to 1.5 IQR in either direction from the top or bottom quartile. (**B**) Correlation between cytokine levels and CD14 cell output after CD34+ HSPCs are incubated with plasma for 7 days. Line and shadow indicate linear regression fit and 95% confidence interval, respectively. Significance of the correlations (Pearson r) were calculated with a two-sided permutation test.

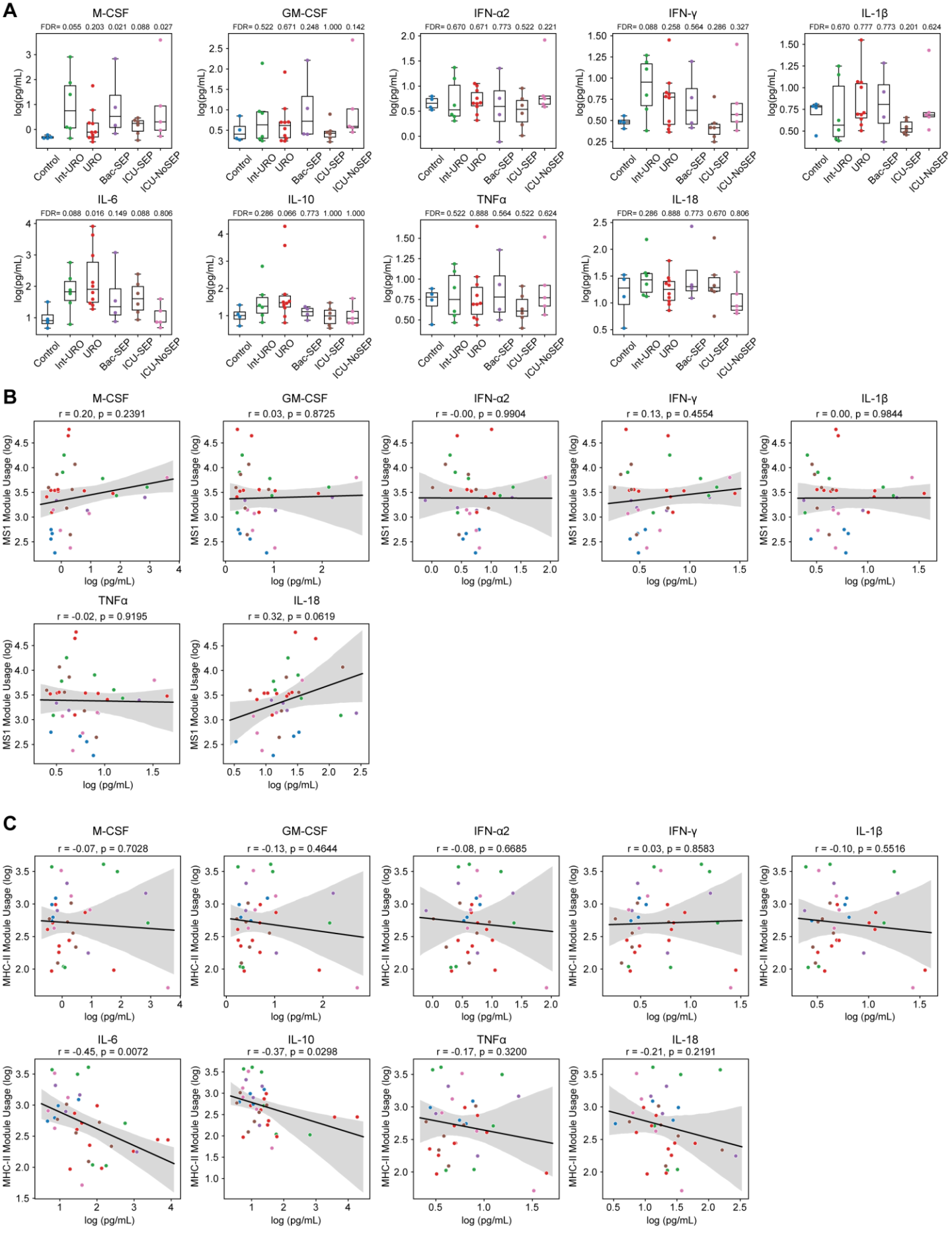
A
M-CSF
FDR= 0.055 0.203 0.021 0.088 0.027
GM-CSF
FDR= 0.522 0.671 0.248 1.000 0.142
IFN-α2
FDR= 0.670 0.671 0.773 0.522 0.221
IFN-γ
FDR= 0.088 0.258 0.564 0.286 0.327
IL-1β
FDR= 0.670 0.777 0.773 0.201 0.624
IL-6
FDR= 0.088 0.016 0.149 0.088 0.806
IL-10
FDR= 0.286 0.066 0.773 1.000 1.000
TNFα
FDR= 0.522 0.888 0.564 0.522 0.624
IL-18
FDR= 0.286 0.888 0.773 0.670 0.806
log(pg/mL)
Control
Int-URO
URO
Bac-SEP
ICU-SEP
ICU-NoSEP
B
M-CSF
r = 0.20, p = 0.2391
GM-CSF
r = 0.03, p = 0.8725
IFN-α2
r = -0.00, p = 0.9904
IFN-γ
r = 0.13, p = 0.4554
IL-1β
r = 0.00, p = 0.9844
TNFα
r = -0.02, p = 0.9195
IL-18
r = 0.32, p = 0.0619
MS1 Module Usage (log)
log (pg/mL)
C
M-CSF
r = -0.07, p = 0.7028
GM-CSF
r = -0.13, p = 0.4644
IFN-α2
r = -0.08, p = 0.6685
IFN-γ
r = 0.03, p = 0.8583
IL-1β
r = -0.10, p = 0.5516
IL-6
r = -0.45, p = 0.0072
IL-10
r = -0.37, p = 0.0298
TNFα
r = -0.17, p = 0.3200
IL-18
r = -0.21, p = 0.2191
MHC-II Module Usage (log)
log (pg/mL)

Figure S6. Plasma inflammatory cytokine levels in sepsis patients and correlations with monocyte gene expression modules. (**A**) Boxplots showing levels of indicated inflammatory cytokines in plasma for each patient (*n* = 40 total). FDR values are shown when comparing each disease state with healthy controls (two-tailed Wilcoxon rank-sum test, corrected for testing of multiple cytokines). Boxes show the median and IQR for each patient cohort, with whiskers extending to 1.5 IQR in either direction from the top or bottom quartile. (**B-C**) Correlations between level of each indicated cytokine and MS1 (**B**) or MHC-II (**C**) module usage in each patient (*n* = 40 total). Line and shadow indicate linear regression fit and 95% confidence interval, respectively. Significance of the correlations (Pearson r) were calculated with a two-sided permutation test.

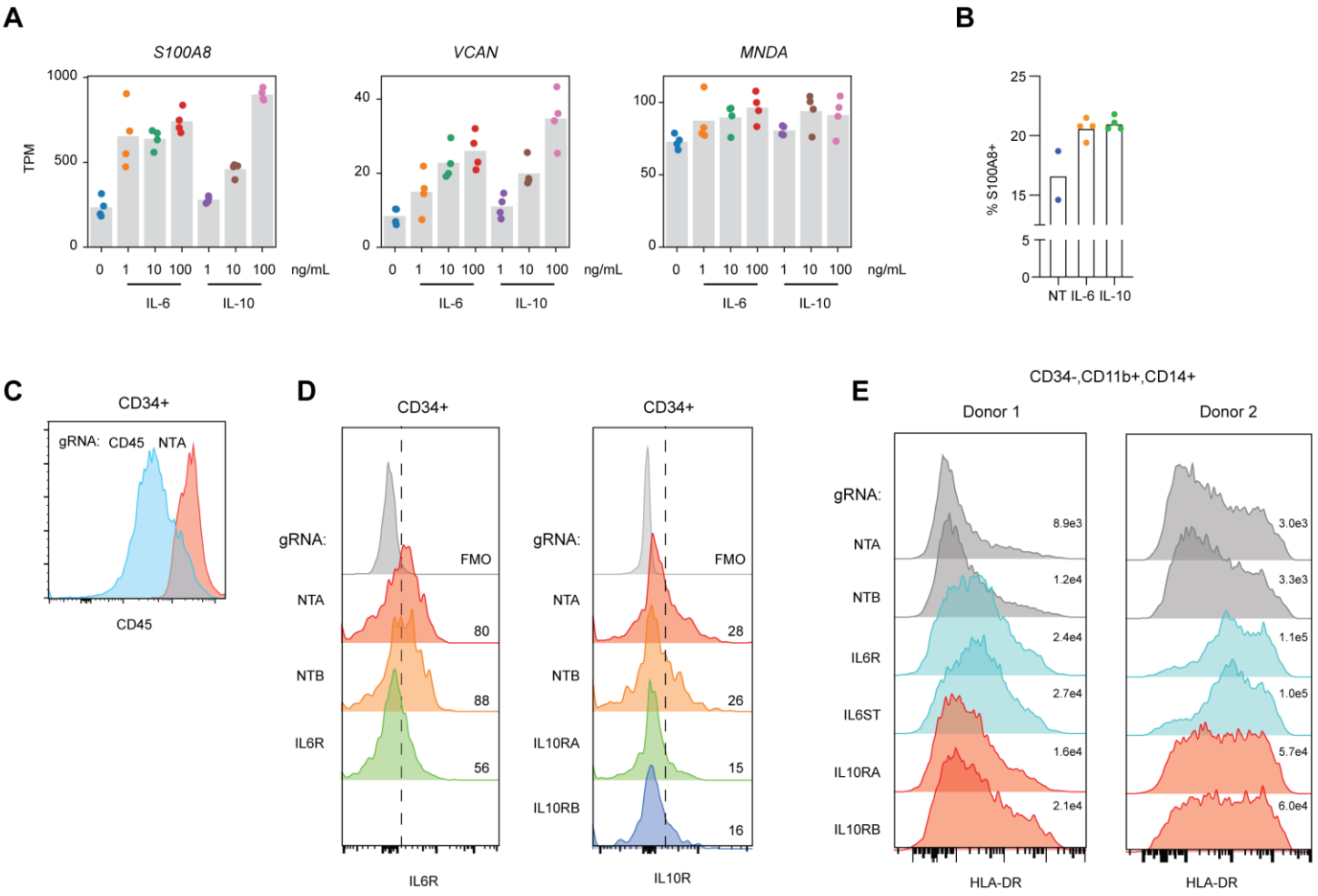

Figure S7. Short term stimulation and Cas9 RNP-based knockouts of CD34+ HSPCs. (**A**) Expression of 'early' MS1 genes in CD34+ HSPCs after treatment with IL-6 or IL-10 for 24 h. Experiments were performed with *n* = 4 for each condition (2 bone marrow donors with 2 technical replicates). (**B**) S100A8 intracellular staining of CD34+ HSPCs treated with 100 ng/mL IL-6 or IL-10 for 24 h. (**C**) CD45 surface expression levels in CD34+ HSPCs electroporated with either CD45 or NTA RNPs. (**D**) IL6R (left) and IL10R (right) expression in CD34+ HSPCs electroporated with the indicated gRNA-RNPs. Numbers indicate the percentage of cells that are IL6R+ or IL10R+, determined by gating on the full-minus-one (FMO) control. Results are representative of 2 independent experiments in different bone marrow donors. (**E**) Expression of HLA-DR in CD14+ cells after differentiation of RNP-electroporation HSPCs with 20% sepsis plasma for 2 bone marrow donors. Median fluorescence intensities are indicated for each sample.

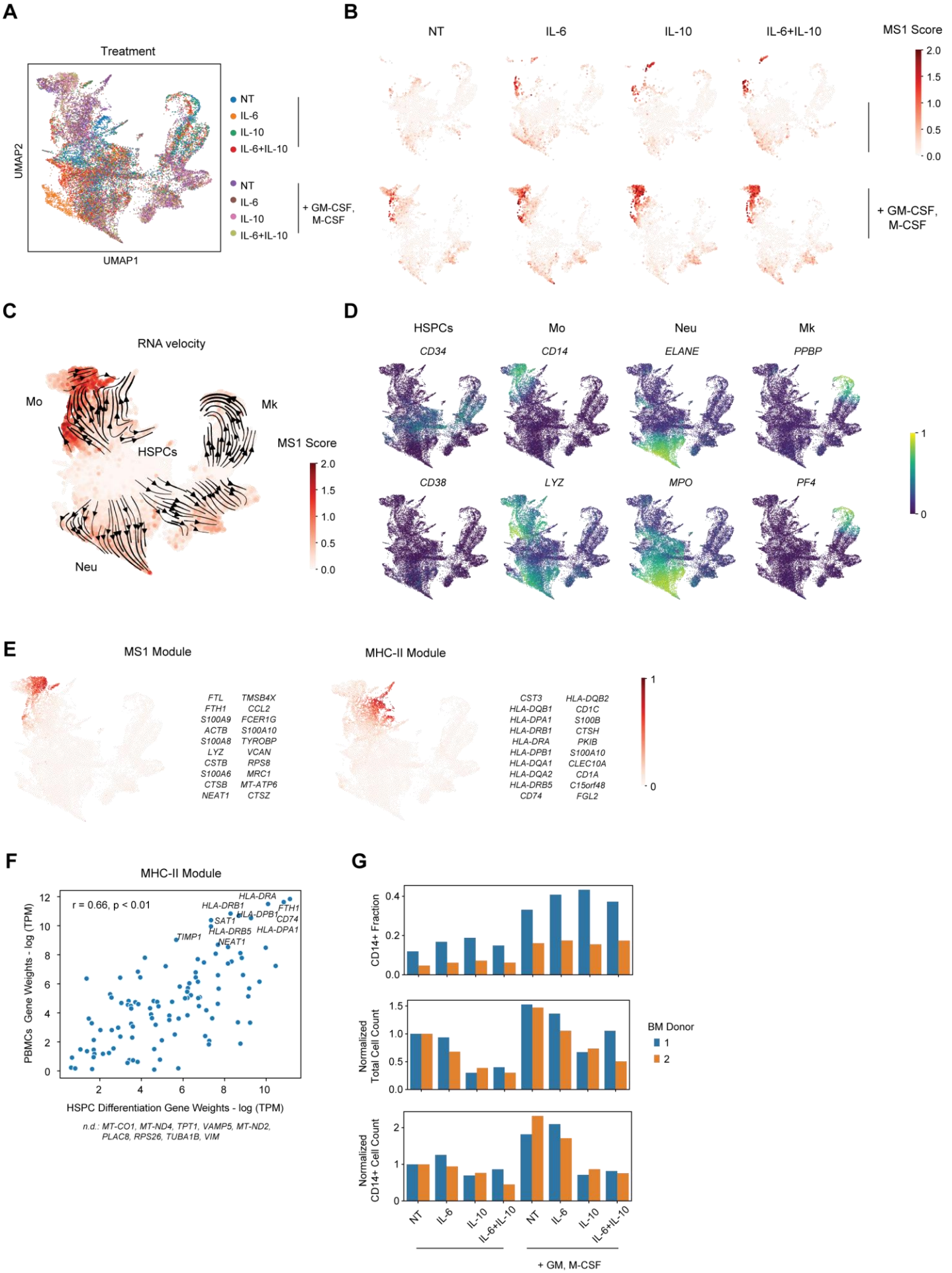
A
Treatment
UMAP2
UMAP1
NT
IL-6
IL-10
IL-6+IL-10
NT
IL-6
IL-10
IL-6+IL-10
+ GM-CSF, M-CSF
B
NT
IL-6
IL-10
IL-6+IL-10
MS1 Score
2.0
1.5
1.0
0.5
0.0
+ GM-CSF, M-CSF
C
RNA velocity
Mo
Mk
HSPCs
Neu
MS1 Score
2.0
1.5
1.0
0.5
0.0
D
HSPCs
Mo
Neu
Mk
CD34
CD14
ELANE
PPBP
CD38
LYZ
MPO
PF4
1
0
E
MS1 Module
FTL TMSB4X
FTH1 CCL2
S100A9 FCER1G
ACTB S100A10
S100A8 TYROBP
LYZ VCAN
CSTB RPS8
S100A6 MRC1
CTSB MT-ATP6
NEAT1 CTSZ
MHC-II Module
CST3 HLA-DQB2
HLA-DQB1 CD1C
HLA-DPA1 S100B
HLA-DRB1 CTSH
HLA-DRA PKIB
HLA-DPB1 S100A10
HLA-DQA1 CLEC10A
HLA-DQA2 CD1A
HLA-DRB5 C15orf48
CD74 FGL2
1
0
F
MHC-II Module
r = 0.66, p < 0.01
HLA-DRA
HLA-DRB1
FTH1
HLA-DPB1
CD74
SAT1
HLA-DRB5
HLA-DPA1
TIMP1
NEAT1
PBMCs Gene Weights - log (TPM)
HSPC Differentiation Gene Weights - log (TPM)
n.d.: MT-CO1, MT-ND4, TPT1, VAMP5, MT-ND2, PLAC8, RPS26, TUBA1B, VIM
G
CD14+ Fraction
Normalized Total Cell Count
Normalized CD14+ Cell Count
BM Donor
1
2
NT
IL-6
IL-10
IL-6+IL-10
NT
IL-6
IL-10
IL-6+IL-10
+ GM, M-CSF

Figure S8. scRNA-seq of cytokine-differentiated HSPCs. (**A**) UMAP projection of differentiated cells from CD34+ HSPCs incubated with cytokines (all at 100 ng/mL). Cells are colored by cytokine conditions. (**B**) Individual UMAP projections of each cytokine condition with cells colored by MS1 scores. (**C**) Combined UMAP projection of all cytokine conditions with cells colored by MS1 scores. Stream plots showing inferred trajectories from RNA velocity are overlaid. Mo, monocytes; Neu, neutrophils; Mk, megakaryocytes. (**D**) Relative expression of HSPC, monocyte, neutrophil, and megakaryocyte marker genes in (**A**). (**E**) Relative expression of the MS1 and MHC-II modules. The top 20 genes in each module are listed. (**F**) Gene weight correlation between the MHC-II modules detected in the cytokine treatment (x-axis) and patient PBMC datasets (y-axis). Significance of the Pearson correlation (r) is calculated with a permutation test. Genes which are not detected (n.d.) in the cytokine treatment module but are among the top 30 for the corresponding module in the PBMC dataset are indicated. (**G**) Fraction of CD14+ cells (top), total number of cells (middle), and total number of CD14+ cells (bottom) after incubation of HSPCs in the indication cytokines. Cell counts were normalized to the NT condition (without GM/M-CSF) for each bone marrow donor. The experiment was performed on 2 bone marrow donors for each treatment condition; a total of 3,365, 2,986, 2,550, 3,025, 3,194, 3,061, 2,850, and 2,918 cells for each cytokine treatment condition indicated on the plots, respectively, were profiled.

Liao, *et al* 2020 (BALF)

Chua, *et al* 2020 (NS)

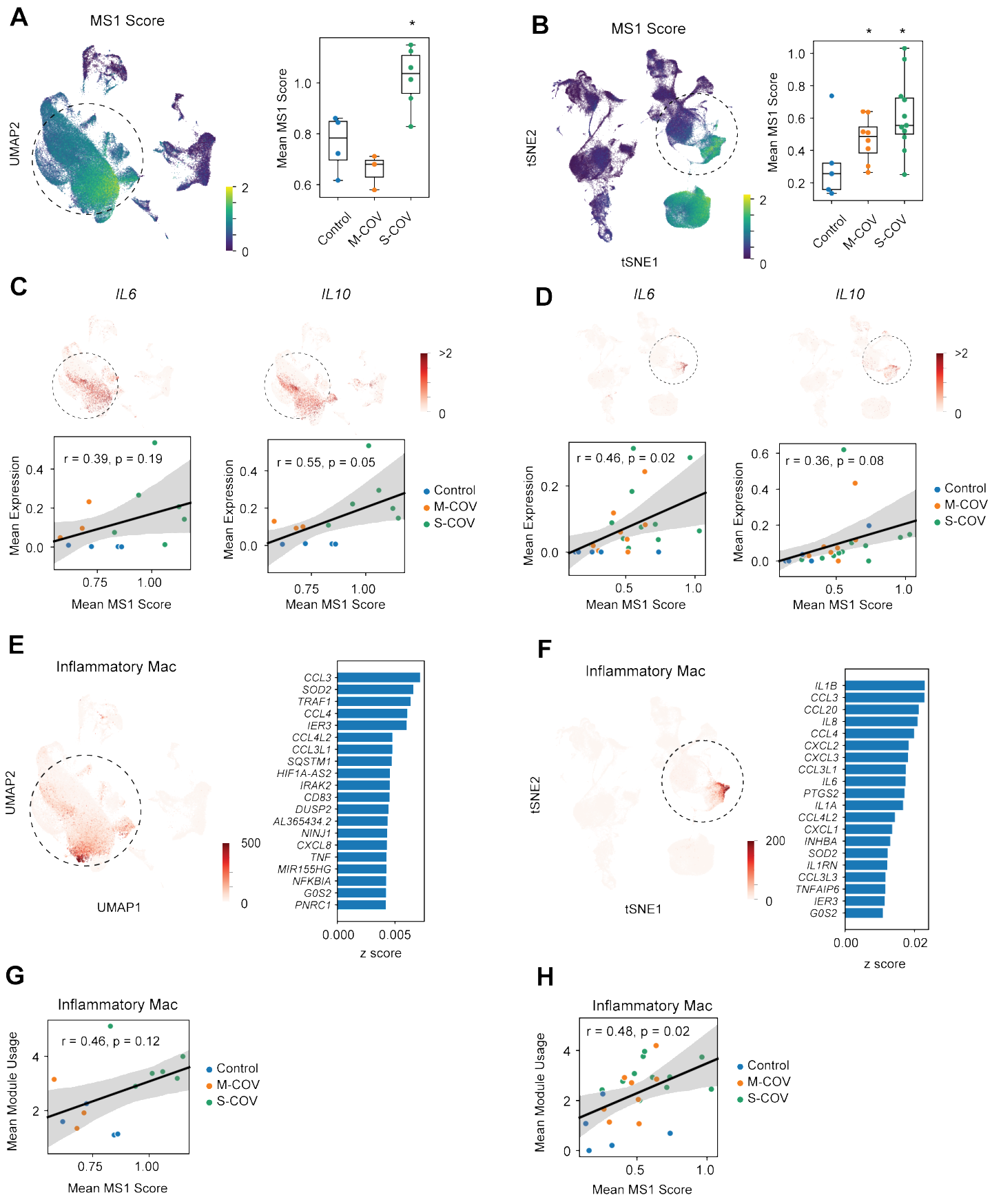

Figure S9. Expression of the MS1 program in tissues is associated with inflammatory macrophage activation. Single cell gene expression analysis of bronchoalveolar lavage fluid (BALF) and nasopharyngeal swabs (NS) from published COVID-19 datasets *(44, 45)*. (**A,B**) UMAP/tSNE projection of cells colored by MS1 score. MS1 scores were calculated based on the top 30 genes from the MS1 module. The clusters corresponding to monocytes/macrophages are indicated by a dotted circle. Mean MS1 scores in monocytes/macrophages for each patient across patient groups for each dataset are shown on the right. Asterisks indicate $p < 0.05$, computed by comparing each disease state with healthy controls (two-tailed Wilcoxon rank-sum test). Boxes show the median and IQR for each patient cohort, with whiskers extending to 1.5 IQR in either direction from the top or bottom quartile. (**C,D**) UMAP/tSNE projection (top) of cells colored by expression of *IL6* (left) or *IL10* (right). Scatterplots (bottom) showing the correlation between mean expression of *IL6* (left) or *IL10* (right) with MS1 scores in each patient. Line and shadow indicate linear regression fit and 95% confidence interval, respectively. Significance of the correlation (Pearson *r*) was calculated with a two-sided permutation test. (**E,F**) UMAP/tSNE projection of cells colored by expression of an inflammatory macrophage module detected by cNMF. Genes with the top 10 loadings in the inflammatory macrophage module are shown on the right. (**G,H**) Correlation between mean expression of *IL6* (left) or *IL10* (right) with MS1 scores in each patient.

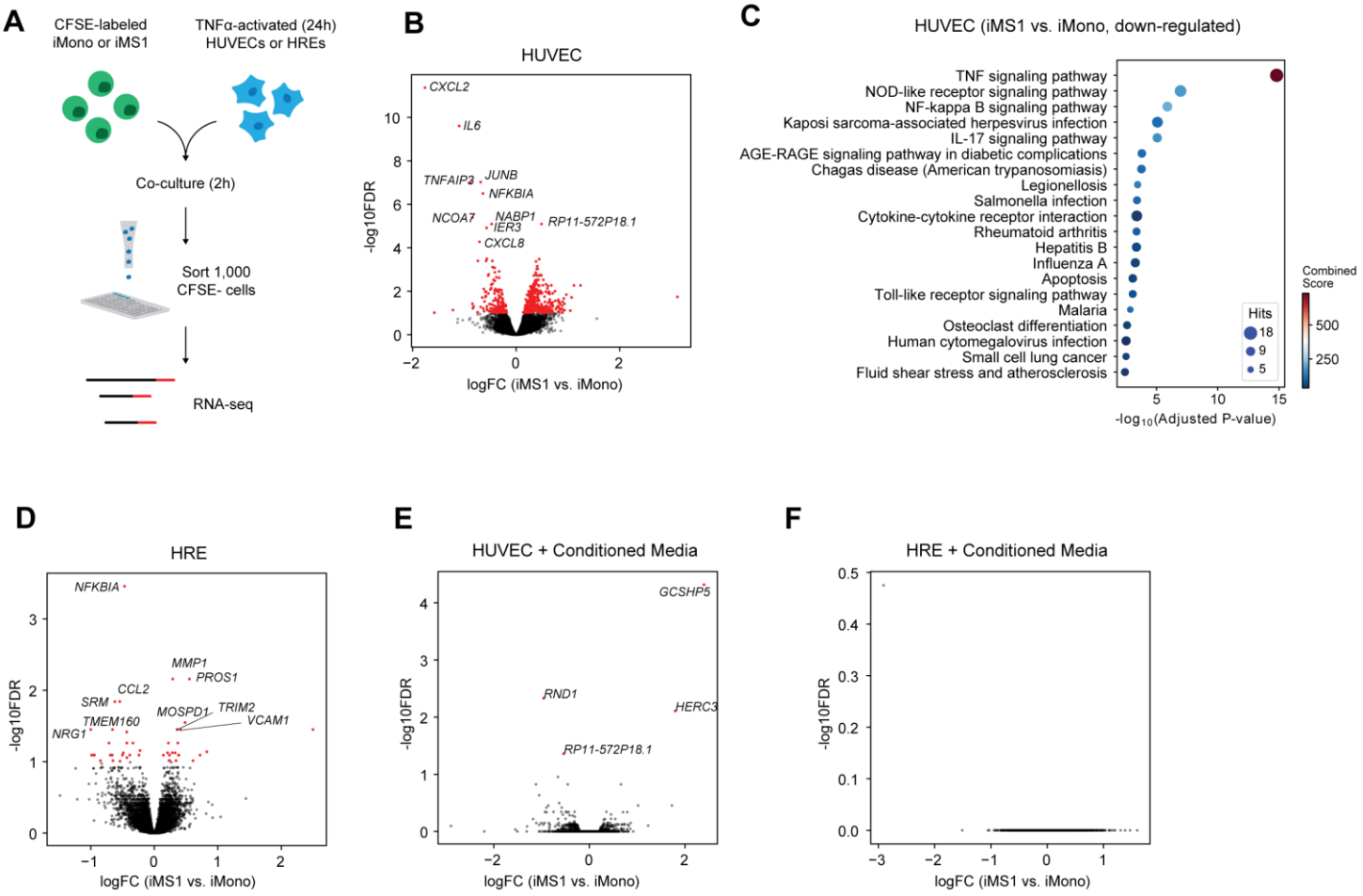

Figure S10. iMS1 co-incubation experiments with structural cells. (**A**) Experimental schematic of the co-incubation of iMS1 or iMono with primary human umbilical vein endothelial cells (HUVECs) or human renal epithelial cells (HREs). (**B**) Volcano plot showing differential expression analysis results (exact test) between TNFα-activated HUVECs co-incubated with either iMono or iMS1 cells generated from CD34+ HSPCs. Genes with FDR < 0.1 are highlighted in red, and the top 10 genes with lowest FDR values are shown. (**C**) Dotplot showing enrichment of pathways (KEGG database) for down-regulated genes in HUVECs co-incubated with iMS1 vs. iMono (FDR < 0.1, edgeR exact test). Sizes of circles are proportional to the number of gene hits in a set, whereas color represents the enrichment score of each gene set. (**D**) Volcano plot showing differential expression analysis results (exact test) between TNFα-activated HREs co-incubated with either iMono or iMS1 cells generated from CD34+ HSPCs. Genes with FDR < 0.1 are highlighted in red, and the top 10 genes with lowest FDR values are shown. (**E-F**) Volcano plot showing differential expression analysis results (exact test) between TNFα-activated HUVECs (**E**) or HREs (**F**) incubated with iMono or iMS1 conditioned media. Genes with FDR < 0.1 are highlighted in red. In (**B-D**) and (**E-F**), *n* = 8 experiments were performed for each condition (2 bone marrow donors with 2 biological and 2 technical replicates)

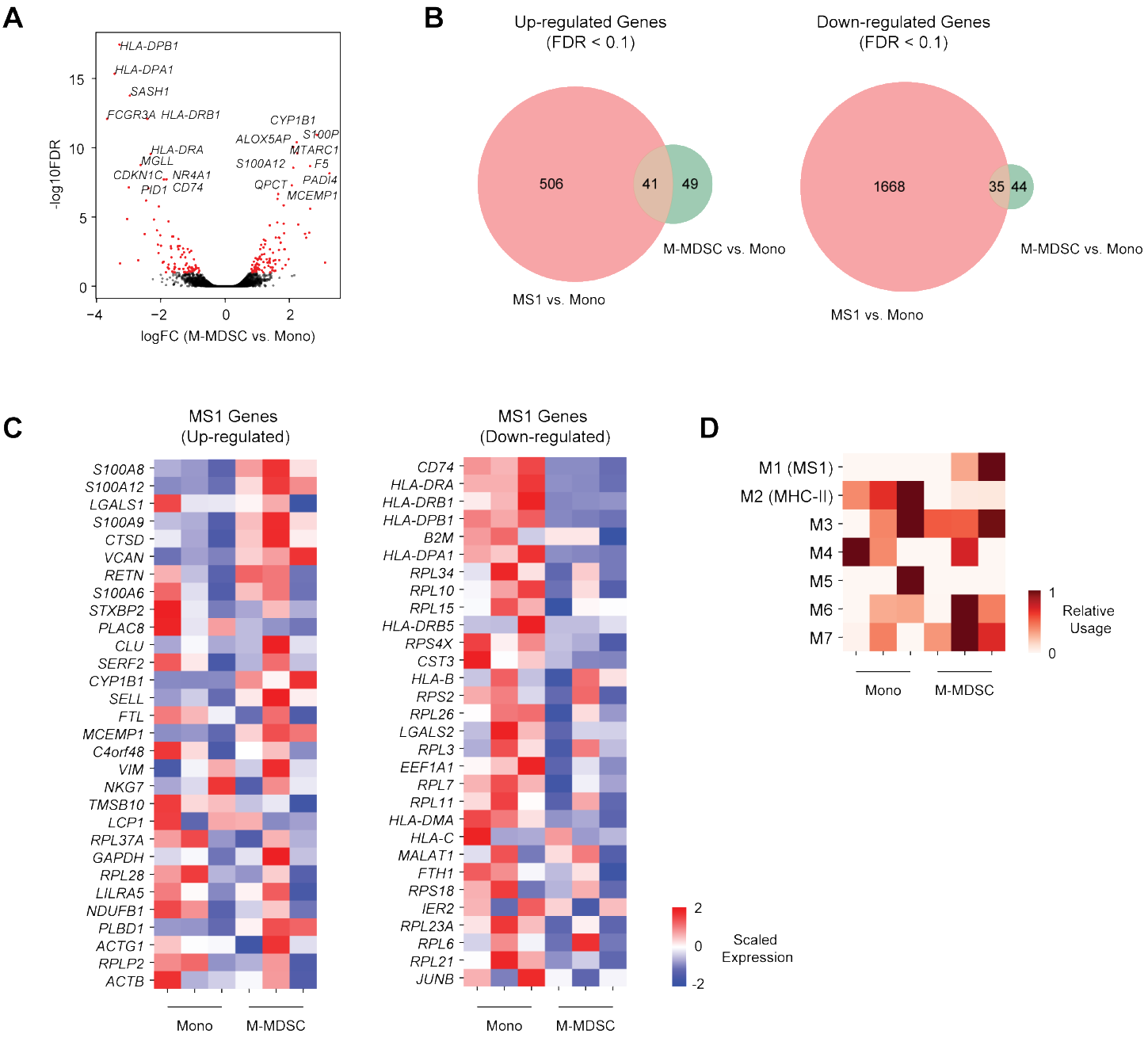

Figure S11. Comparison of MS1 with M-MDSCs. (**A**) Volcano plot showing differential expression analysis results (exact test) between M-MDSCs and monocytes (*n* = 3 for each) in lung cancer patients from Mastio, *et al (54)*. Genes with FDR < 0.1 are highlighted in red. (**B**) Venn diagrams showing overlap of differentially expressed genes (FDR < 0.1, exact test) for MS1 vs. monocytes and M-MDSCs vs. monocytes. (**C**) Heatmaps showing the expression of the top 30 up-regulated (left) or down-regulated (right) MS1 genes in bulk RNA-seq of monocytes and M-MDSC s (*n* = 3 patients for each). (**D**) Relative usage of monocyte modules in monocytes and M-MDSCs.