Cell Reports Medicine, Volume *4*

Supplemental information

Single-cell RNA-sequencing of PBMCs from SAVI patients reveals disease-associated monocytes with elevated integrated stress response

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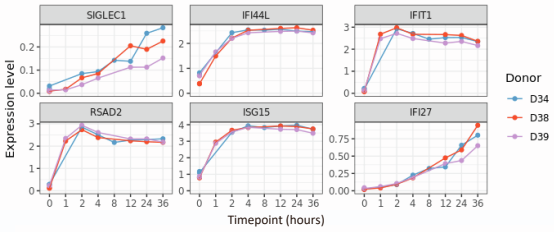
A

Patient description

Patient	Correspondance in Frémond *et al* JACIP, 2021	Other descriptions
P1	P1	P1 in Frémond *et al* JACI, 2016 Patient III-2 in Jeremiah et al JCI, 2014
P2	P3	Patient II-6 in Jeremiah *et al* JCI, 2014
P4	P9	N/A
P5	P8	P2 in Frémond *et al* JACI, 2016, Munoz et al JAMA Dermatol, 2015
P6	P5	P3 in Frémond *et al* JACI, 2016, Case 1 in Picard *et al* Chest, 2016

B



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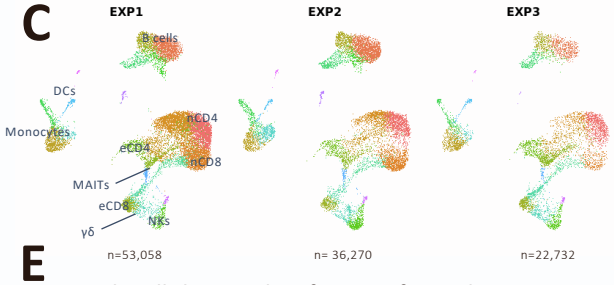

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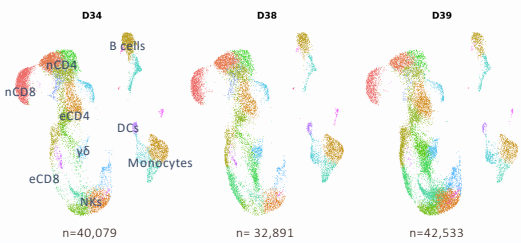

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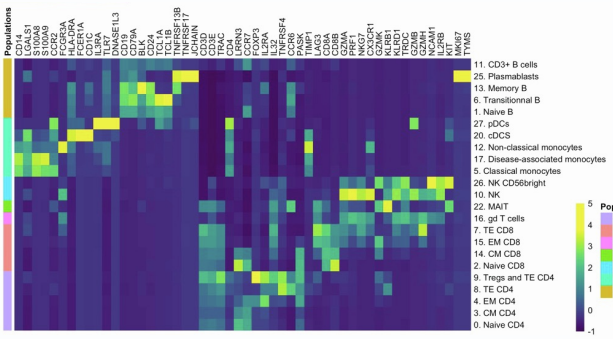

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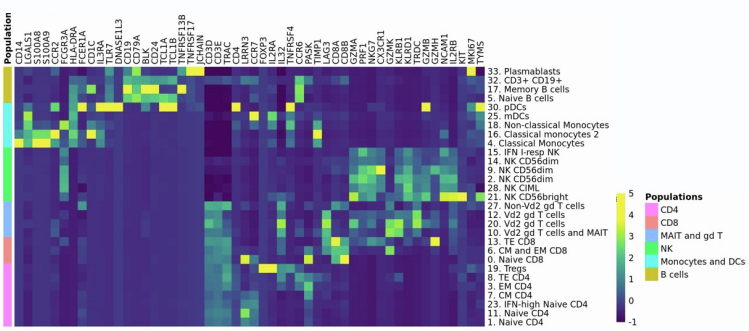

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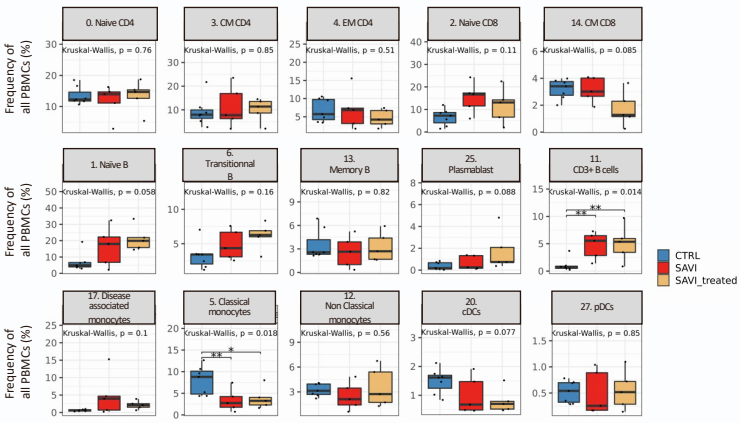

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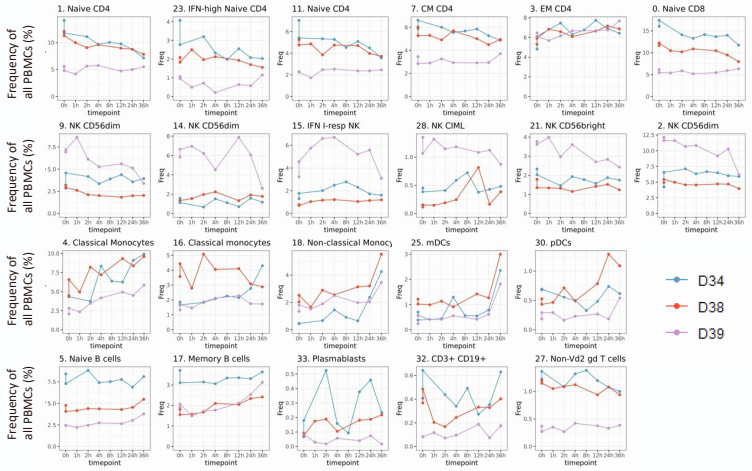

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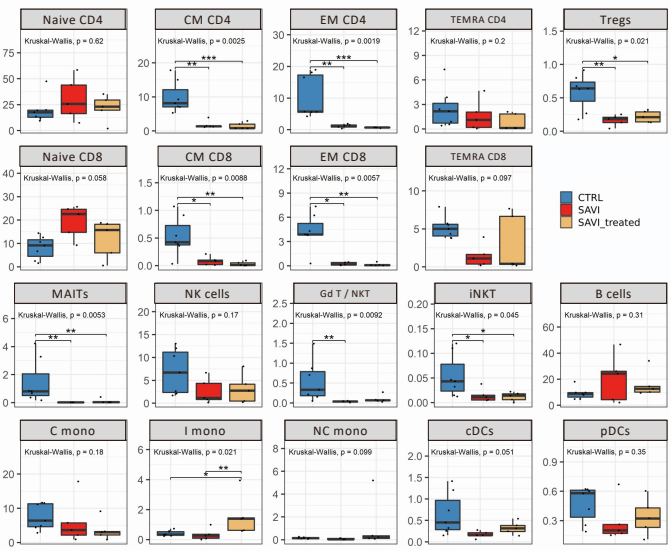


J

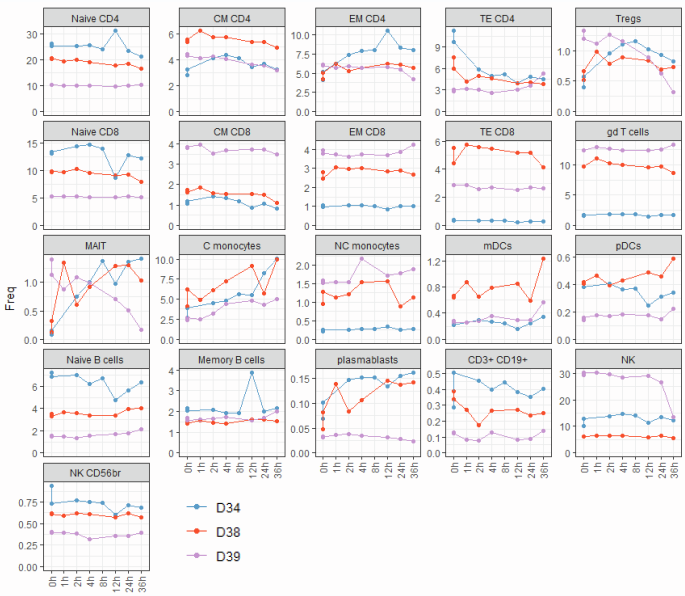


Figure S1

Figure S1: scRNA-seq and phenotyping of the SAVI dataset and the IFN-β dataset. Related to Figure 1.

A. The identification number of the SAVI patients in Frémond *et al*, 2021
B. IFN-β dataset: Validation of the IFN-β stimulation using the mRNA levels measured by scRNA-seq of 6 well known IFN-stimulated genes
C. UMAP of the SAVI dataset separated by batch. The number of cells in each group is indicated below the UMAP. nCD4: naïve CD4; eCD4: effector CD4; nCD8: naïve CD8; eCD8: effector CD8; γδ: γδ T cells
D. UMAP of the IFN-β dataset separated by donor. The number of cells in each group is indicated below the UMAP. nCD4: naïve CD4; eCD4: effector CD4; nCD8: naïve CD8; eCD8: effector CD8; γδ: γδ T cells
E. Heatmap of the average expression of cell type markers in each cluster of the SAVI dataset. These markers were used to manually assign a cell type to each cluster
F. Heatmap of the average expression of cell type markers in each cluster of the IFN-β dataset. These markers were used to manually assign a cell type to each cluster.
G. Boxplot of the proportion of PBMCs found each cluster of the SAVI dataset, by scRNA-seq. *p*-values are calculated by Kruskal-Wallis test for multiple comparisons, followed by a post hoc Dunn's test. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$)
H. Evolution of the proportion of PBMCs found in each cluster of the IFN-β dataset over the time course of IFN-β stimulation, by scRNA-seq
I. Boxplot of the proportion of PBMCs found each cell population of the SAVI dataset, by flow cytometry. *p*-values are calculated by Kruskal-Wallis test for multiple comparisons, followed by a post hoc Dunn's test. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$). C mono: classical monocytes; I mono : intermediary monocytes; NC mono : non-classical monocytes.
J. Evolution of the proportion of PBMCs found in each cluster of the IFN-β dataset over the time course of IFN-β stimulation, by Cytometry by Time of Flight

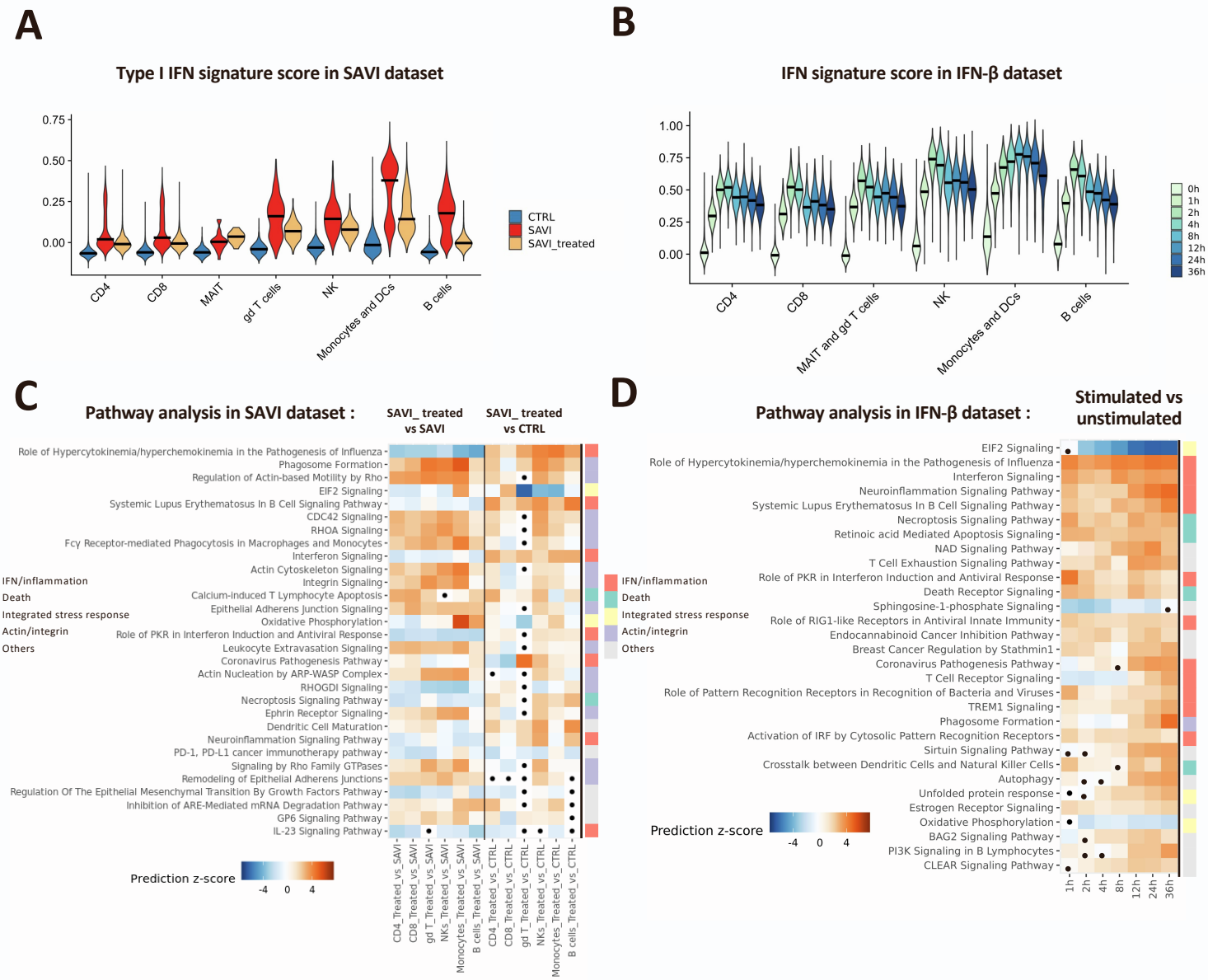

A
Type I IFN signature score in SAVI dataset
0.75
0.50
0.25
0.00
CD4
CD8
MAIT
gd T cells
NK
Monocytes and DCs
B cells
CTRL
SAVI
SAVI_treated
B
IFN signature score in IFN-β dataset
1.00
0.75
0.50
0.25
0.00
CD4
CD8
MAIT and gd T cells
NK
Monocytes and DCs
B cells
0h
1h
2h
4h
8h
12h
24h
36h
C
Pathway analysis in SAVI dataset :
SAVI_ treated vs SAVI
SAVI_ treated vs CTRL
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza
Phagosome Formation
Regulation of Actin-based Motility by Rho
EIF2 Signaling
Systemic Lupus Erythematosus In B Cell Signaling Pathway
CDC42 Signaling
RHOA Signaling
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes
Interferon Signaling
Actin Cytoskeleton Signaling
Integrin Signaling
Calcium-induced T Lymphocyte Apoptosis
Epithelial Adherens Junction Signaling
Oxidative Phosphorylation
Role of PKR in Interferon Induction and Antiviral Response
Leukocyte Extravasation Signaling
Coronavirus Pathogenesis Pathway
Actin Nucleation by ARP-WASP Complex
RHOGDI Signaling
Necroptosis Signaling Pathway
Ephrin Receptor Signaling
Dendritic Cell Maturation
Neuroinflammation Signaling Pathway
PD-1, PD-L1 cancer immunotherapy pathway
Signaling by Rho Family GTPases
Remodeling of Epithelial Adherens Junctions
Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway
Inhibition of ARE-Mediated mRNA Degradation Pathway
GP6 Signaling Pathway
IL-23 Signaling Pathway
IFN/inflammation
Death
Integrated stress response
Actin/integrin
Others
Prediction z-score
-4
0
4
CD4_Treated_vs_SAVI
CD8_Treated_vs_SAVI
gd T_Treated_vs_SAVI
NKs_Treated_vs_SAVI
Monocytes_Treated_vs_SAVI
B cells_Treated_vs_SAVI
CD4_Treated_vs_CTRL
CD8_Treated_vs_CTRL
gd T_Treated_vs_CTRL
NKs_Treated_vs_CTRL
Monocytes_Treated_vs_CTRL
B cells_Treated_vs_CTRL
D
Pathway analysis in IFN-β dataset :
Stimulated vs unstimulated
EIF2 Signaling
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza
Interferon Signaling
Neuroinflammation Signaling Pathway
Systemic Lupus Erythematosus In B Cell Signaling Pathway
Necroptosis Signaling Pathway
Retinoic acid Mediated Apoptosis Signaling
NAD Signaling Pathway
T Cell Exhaustion Signaling Pathway
Role of PKR in Interferon Induction and Antiviral Response
Death Receptor Signaling
Sphingosine-1-phosphate Signaling
Role of RIG1-like Receptors in Antiviral Innate Immunity
Endocannabinoid Cancer Inhibition Pathway
Breast Cancer Regulation by Stathmin1
Coronavirus Pathogenesis Pathway
T Cell Receptor Signaling
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses
TREM1 Signaling
Phagosome Formation
Activation of IRF by Cytosolic Pattern Recognition Receptors
Sirtuin Signaling Pathway
Crosstalk between Dendritic Cells and Natural Killer Cells
Autophagy
Unfolded protein response
Estrogen Receptor Signaling
Oxidative Phosphorylation
BAG2 Signaling Pathway
PI3K Signaling in B Lymphocytes
CLEAR Signaling Pathway
IFN/inflammation
Death
Integrated stress response
Actin/integrin
Others
Prediction z-score
-4
0
4
1h
2h
4h
8h
12h
24h
36h

Figure S2

Figure S2: Pathway analysis in the SAVI dataset and the IFN-β dataset. Related to Figure 2.

A. Violin plot of a type I IFN response signature of 272 genes, in the SAVI dataset. Dark lines indicate medians
B. Violin plot of a type I IFN response signature of 272 genes, in the IFN-β dataset. Dark lines indicate medians
C. Heatmap of the pathway enrichment analysis, performed in IPA, between SAVI_treated and SAVI and between SAVI_treated and CTRL in each cell compartment. Dots indicate non-significant pathways (Bonferroni-Hochberg corrected p-values > 0.05). Side color bar indicate groups of pathways based on broader functions. Color scales indicate direction of prediction with orange for predicted activation and blue for predicted inhibition
D. Heatmap of the pathway enrichment analysis, performed in IPA, between PBMCs stimulated with IFN-β for each timepoint and unstimulated PBMCs. Dots indicate non-significant pathways (Bonferroni-Hochberg corrected p-values > 0.05). Side color bar indicate groups of pathways based on broader functions. Color scales indicate direction of prediction with orange for predicted activation and blue for predicted inhibition

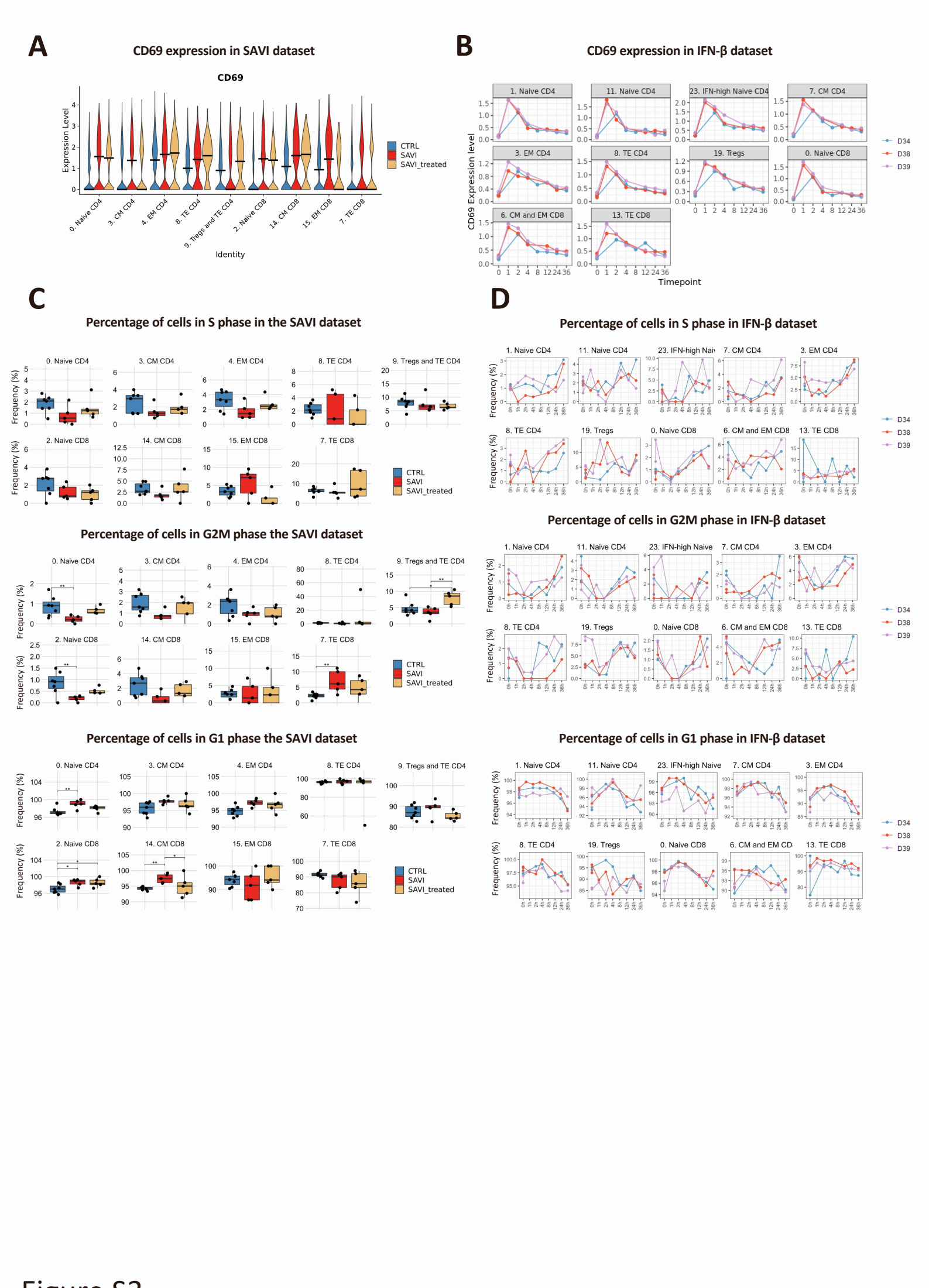

A
CD69 expression in SAVI dataset
CD69
Expression Level
Identity
0. Naive CD4
3. CM CD4
4. EM CD4
8. TE CD4
9. Tregs and TE CD4
2. Naive CD8
14. CM CD8
15. EM CD8
7. TE CD8
CTRL
SAVI
SAVI_treated
B
CD69 expression in IFN-β dataset
CD69 Expression level
Timepoint
1. Naive CD4
11. Naive CD4
23. IFN-high Naive CD4
7. CM CD4
3. EM CD4
8. TE CD4
19. Tregs
0. Naive CD8
6. CM and EM CD8
13. TE CD8
D34
D38
D39
C
Percentage of cells in S phase in the SAVI dataset
Percentage of cells in G2M phase the SAVI dataset
Percentage of cells in G1 phase the SAVI dataset
Frequency (%)
D
Percentage of cells in S phase in IFN-β dataset
Percentage of cells in G2M phase in IFN-β dataset
Percentage of cells in G1 phase in IFN-β dataset

Figure S3

Figure S3: *CD69* expression and cell cycle phase at the transcriptomic level in $CD4^+$ and $CD8^+$ T cells the SAVI dataset and the IFN-β dataset. Related to Figure 3.

A. Violin plot of *CD69* expression, in $CD4^+$ and $CD8^+$ T cells of the SAVI dataset. Dark lines indicate medians
B. Evolution of *CD69* expression in $CD4^+$ and $CD8^+$ T cells of the IFN-β dataset over the time course of IFN-β stimulation. Each dot is the average score of the signature for a sample
C. Boxplot of the proportion of cells in the S phase (top), the G2M phase (middle), and the G1 phase (bottom) of the cell cycle, in $CD4^+$ and $CD8^+$ T cells of the SAVI dataset
D. Evolution of the proportion of cells in the S phase (top) the G2M phase (middle), and the G1 phase (bottom) of the cell cycle in $CD4^+$ and $CD8^+$ T cells of the IFN-β dataset over the time course of IFN-β stimulation

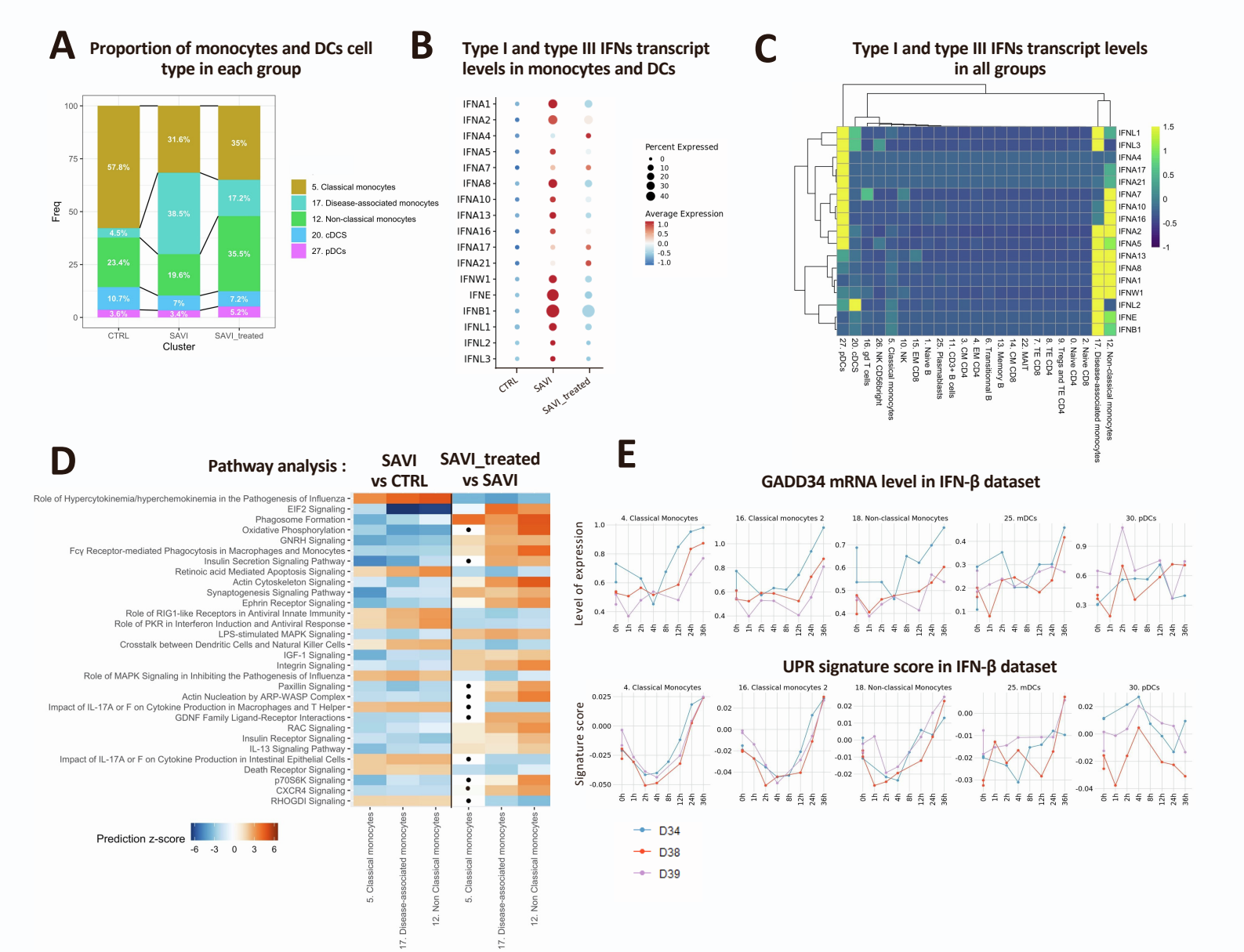

A
Proportion of monocytes and DCs cell type in each group
Freq
Cluster
CTRL
SAVI
SAVI_treated
57.8%
4.5%
23.4%
10.7%
3.6%
31.6%
38.5%
19.6%
7%
3.4%
35%
17.2%
35.5%
7.2%
5.2%
5. Classical monocytes
17. Disease-associated monocytes
12. Non-classical monocytes
20. cDCS
27. pDCs
B
Type I and type III IFNs transcript levels in monocytes and DCs
IFNA1
IFNA2
IFNA4
IFNA5
IFNA7
IFNA8
IFNA10
IFNA13
IFNA16
IFNA17
IFNA21
IFNW1
IFNE
IFNB1
IFNL1
IFNL2
IFNL3
Percent Expressed
Average Expression
C
Type I and type III IFNs transcript levels in all groups
27. pDCs
20. cDCS
16. gd T cells
26. NK CD56bright
5. Classical monocytes
10. NK
15. EM CD8
1. Naive B
25. Plasmablasts
11. CD3+ B cells
3. CM CD4
4. EM CD4
6. Transitional B
13. Memory B
14. CM CD8
22. MAIT
7. TE CD8
8. TE CD4
9. Tregs and TE CD4
0. Naive CD4
2. Naive CD8
17. Disease-associated monocytes
12. Non-classical monocytes
D
Pathway analysis :
SAVI vs CTRL
SAVI_treated vs SAVI
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza
EIF2 Signaling
Phagosome Formation
Oxidative Phosphorylation
GNRH Signaling
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes
Insulin Secretion Signaling Pathway
Retinoic acid Mediated Apoptosis Signaling
Actin Cytoskeleton Signaling
Synaptogenesis Signaling Pathway
Ephrin Receptor Signaling
Role of RIG1-like Receptors in Antiviral Innate Immunity
Role of PKR in Interferon Induction and Antiviral Response
LPS-stimulated MAPK Signaling
Crosstalk between Dendritic Cells and Natural Killer Cells
IGF-1 Signaling
Integrin Signaling
Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza
Paxillin Signaling
Actin Nucleation by ARP-WASP Complex
Impact of IL-17A or F on Cytokine Production in Macrophages and T Helper
GDNF Family Ligand-Receptor Interactions
RAC Signaling
Insulin Receptor Signaling
IL-13 Signaling Pathway
Impact of IL-17A or F on Cytokine Production in Intestinal Epithelial Cells
Death Receptor Signaling
p70S6K Signaling
CXCR4 Signaling
RHOGDI Signaling
Prediction z-score
5. Classical monocytes
17. Disease-associated monocytes
12. Non Classical monocytes
E
GADD34 mRNA level in IFN-β dataset
Level of expression
4. Classical Monocytes
16. Classical monocytes 2
18. Non-classical Monocytes
25. mDCs
30. pDCs
UPR signature score in IFN-β dataset
Signature score
D34
D38
D39

Figure S4

Figure S4: Further characterization of a disease-associated cluster of monocytes at the transcriptomic level. Related to Figure 4.

A. Proportion of cells from each monocyte and dendritic cell clusters in each group in the SAVI dataset
B. Dot plot of the scaled average expression of each type I and type III IFN in all monocytes and DCs clusters in each group of the SAVI dataset. Dot size indicates the percentage of cells expressing the gene and color scale indicates the average expression of the gene in each group.
C. Heatmap of the scaled expression levels of all type I and type III IFN, in each cluster, in all cells of the SAVI dataset. Hierarchical clustering based on Euclidian distances.
D. Heatmap of the pathway enrichment analysis, performed in IPA, between SAVI and CTRL, and between SAVI_treated and SAVI in the clusters of the monocyte compartment. Dots indicate non-significant pathways (BH > 0.05)
E. Evolution of the expression of *PPP1R15A* which codes for GADD34 (left) and of an unfolded protein response signature of 85 genes (right) in each monocyte or dendritic cell cluster of the IFN-β dataset over the time course of IFN-β stimulation. Each dot is the average score for a sample

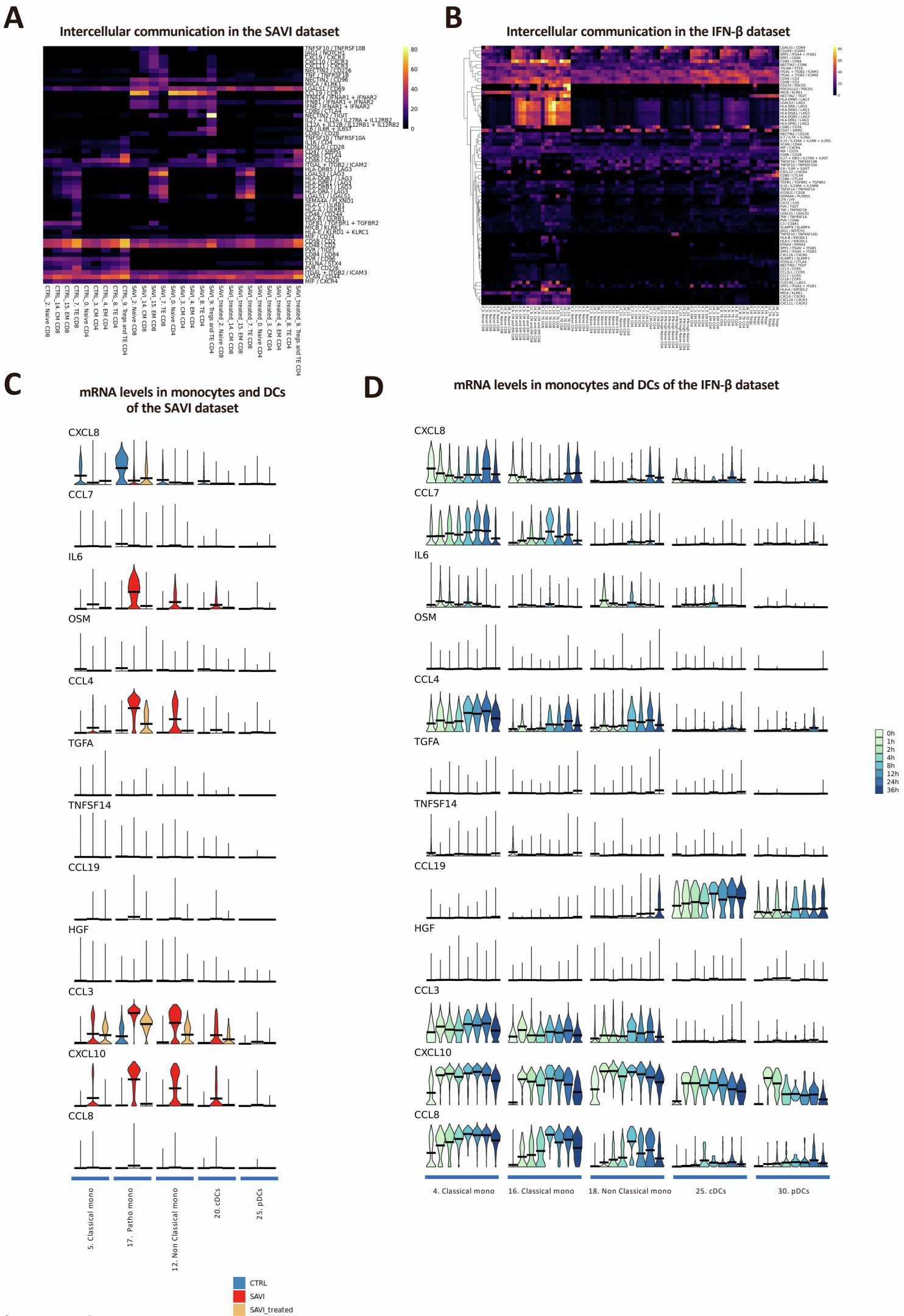
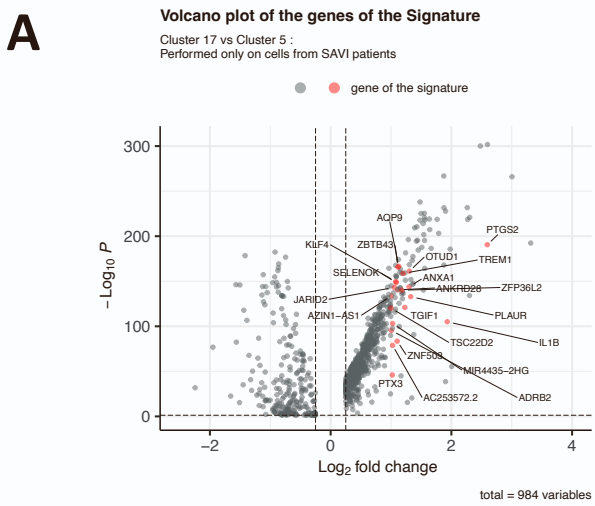
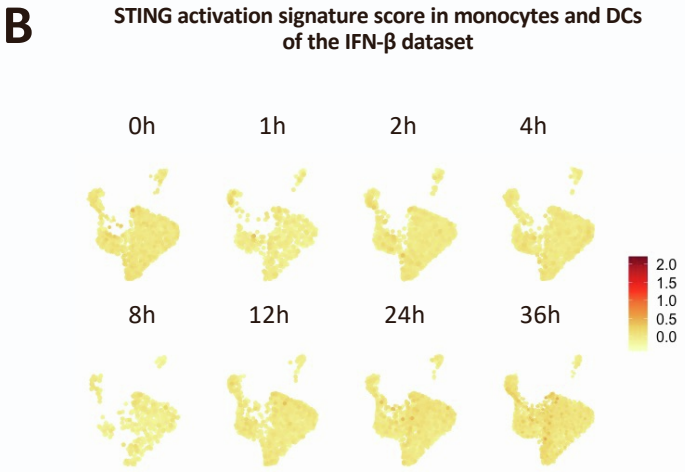

A
Intercellular communication in the SAVI dataset
B
Intercellular communication in the IFN-β dataset
C
mRNA levels in monocytes and DCs of the SAVI dataset
D
mRNA levels in monocytes and DCs of the IFN-β dataset
CXCL8
CCL7
IL6
OSM
CCL4
TGFA
TNFSF14
CCL19
HGF
CCL3
CXCL10
CCL8
5. Classical mono
17. Patho mono
12. Non Classical mono
20. cDCs
25. pDCs
4. Classical mono
16. Classical mono
18. Non Classical mono
25. cDCs
30. pDCs
CTRL
SAVI
SAVI_treated
0h
1h
2h
4h
8h
12h
24h
36h

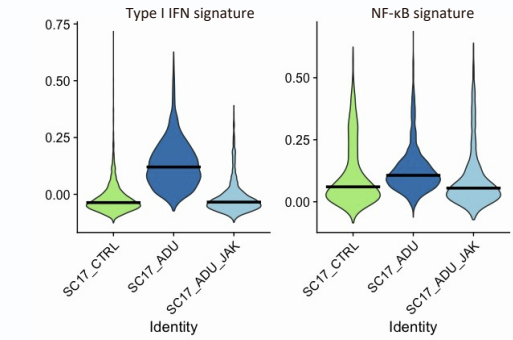
Figure S5

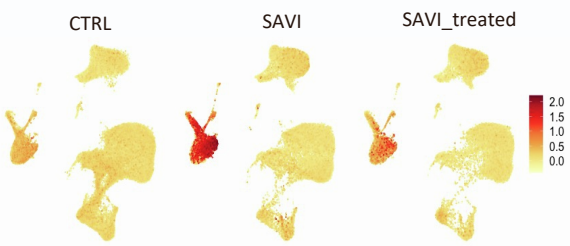
Figure S5: Ligand/receptors prediction and mRNA levels of secreted proteins. Related to Figure 5.

A. Heatmap of the score of each ligand/receptor pair between each T cell cluster and the monocytes, either in the SAVI, the treated_SAVI or the CTRL group
B. Heatmap of the score of each ligand/receptor pair between each T cell cluster and the monocytes, for each timepoint of IFN-β stimulation. Hierarchical clustering based on Pearson correlation
C. Violin plot of the mRNA levels of the 12 proteins found upregulated in the blood of SAVI patients in Figure 5F, in each monocyte and DC clusters of the SAVI dataset
D. Violin plot of the mRNA levels of the 12 proteins found upregulated in the blood of SAVI patients in Figure 5F, in each monocyte and DC clusters of the IFN-β dataset


A
Volcano plot of the genes of the Signature
Cluster 17 vs Cluster 5 :
Performed only on cells from SAVI patients
gene of the signature
AQP9
PTGS2
KLF4
ZBTB43
OTUD1
TREM1
SELENOK
ANXA1
ANKRD28
ZFP36L2
JARID2
AZIN1-AS1
TGIF1
PLAUR
TSC22D2
IL1B
ZNF503
MIR4435-2HG
PTX3
AC253572.2
ADRB2
−Log10 P
Log2 fold change
total = 984 variables


B
STING activation signature score in monocytes and DCs of the IFN-β dataset
0h
1h
2h
4h
8h
12h
24h
36h
2.0
1.5
1.0
0.5
0.0


C
Score of type I IFN signature and NF-κB signature scores in all PBMCs of the ADUS100 dataset
Type I IFN signature
NF-κB signature
SC17_CTRL
SC17_ADU
SC17_ADU_JAK
Identity


D
STING activation signature score in all PBMCs of the SAVI dataset
CTRL
SAVI
SAVI_treated
2.0
1.5
1.0
0.5
0.0

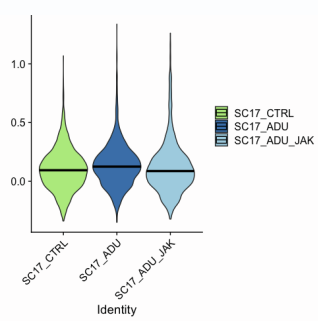

E
STING activation signature score in all PBMCs of the ADUS100 dataset
SC17_CTRL
SC17_ADU
SC17_ADU_JAK
Identity

Figure S6

Figure S6: Design of a STING-activation signature, independent of type I IFN response. Related to Figure 6.

A. Volcano plot of the DEGs between cluster 17 and cluster 5, in SAVI, with the 21 genes of the STING activation signature highlighted in red.
B. Feature plot of the signature score of the 21 genes of the STING activation signature in the monocytes and DCs of each timepoint of the IFN-β dataset
C. Violin plots of the score of a type I IFN response signature of 272 genes and of an NF-κB activation signature of 200 genes in all PBMCs of CTRL, ADUS100 and ADUS100+JAK-inhibitor in the ADUS100 dataset. Dark lines indicate medians
D. Feature plot of the signature score of the 21 genes of the STING activation signature in all PBMCs of CTRL, SAVI and SAVI_treated in the SAVI dataset
E. Violin plot of the signature score of the 21 genes of the STING activation signature in all PBMCs of CTRL, ADUS100 and ADUS100+JAK-inhibitor in the ADUS100 dataset. Dark lines indicate medians

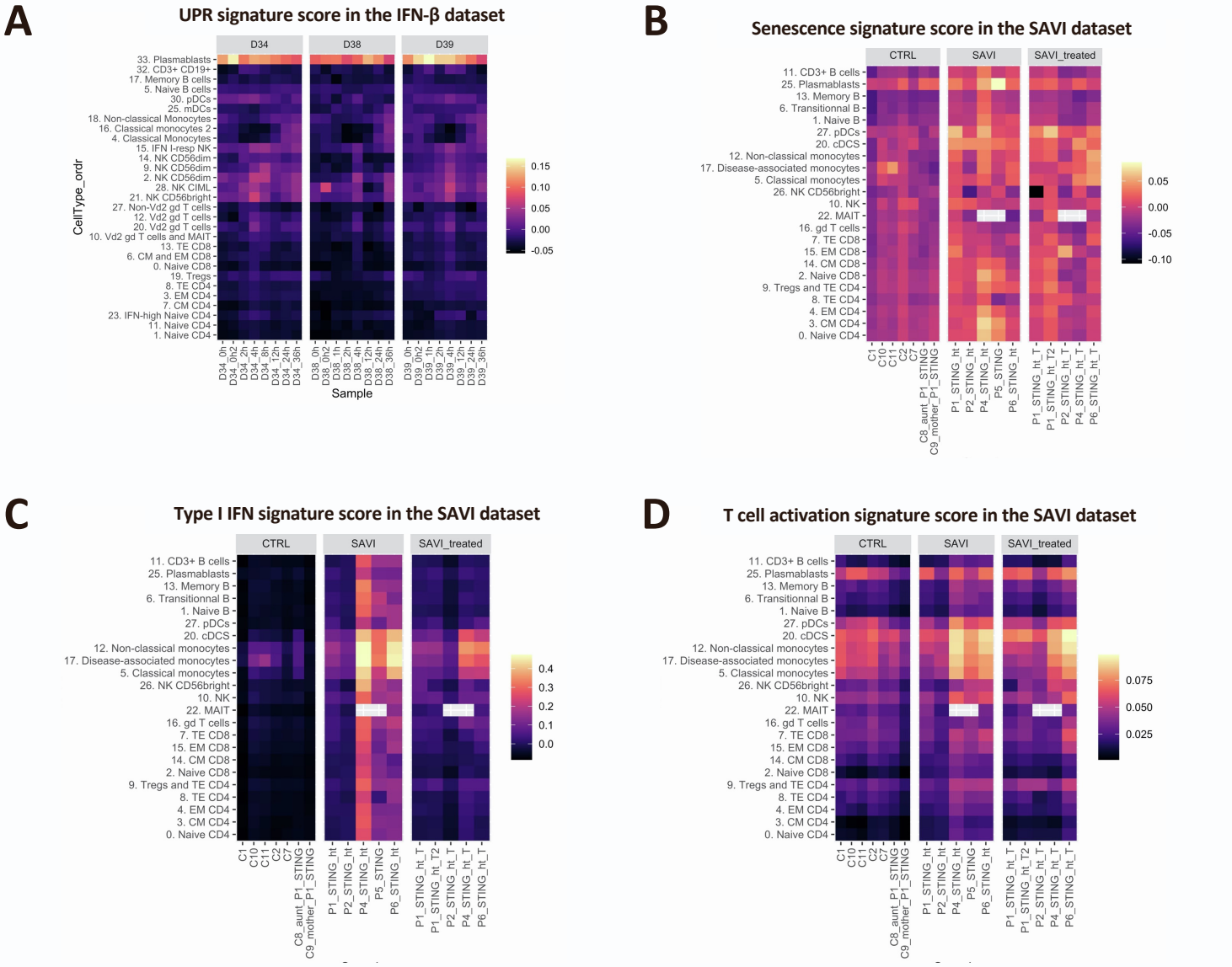

A
UPR signature score in the IFN-β dataset
D34
D38
D39
CellType_ordr
Sample
B
Senescence signature score in the SAVI dataset
CTRL
SAVI
SAVI_treated
C
Type I IFN signature score in the SAVI dataset
D
T cell activation signature score in the SAVI dataset
E
Genealogic tree of P1 and P2
C9
P2
C8
P1
Healthy woman
Healthy man
SAVI patient
STING1 p.V155M mutation
EIF2AK3 p.P649 mutation
F
Sanger sequencing of PERK
Healthy donor
P1

Figure S7

Figure S7: Transcriptomic profiles of signatures relevant to P1. Related to Figure 6.

A. Heatmap of a UPR signature of 85 genes in each sample and each cluster of the IFN-β dataset
B. Heatmap of a senescence signature of 50 genes in each sample and each cluster of the SAVI dataset. Grey squares are used when no cells from a sample in found in a cluster.
C. Heatmap of a type I IFN response signature of 272 genes in each sample and each cluster of the SAVI dataset. Grey squares are used when no cells from a sample in found in a cluster.
D. Heatmap of a T cell activation signature of 4917 genes in each sample and each cluster of the SAVI dataset. Grey squares are used when no cells from a sample in found in a cluster.
E. Genealogic tree of P1's family. Squares represent male individuals and rounds represent female individuals.
F. Sanger sequencing of genomic DNA from SV40-fibroblasts of a healthy control and P1. The « M » stands for a heterozygous C>A variation.