

Supplemental Information, Bohnacker et al.

Supplementary Methods

Primer sequences used for qPCR:

Human Primer	Forward Sequence	Reverse Sequence
<i>ACE2</i>	GGGATCAGAGATCGGAAGAAGAAA	AGGAGGTCTGAACATCATCAGTG
<i>ACOD1</i>	TGCCAAAGAGATTCCATCCCC	CTCGGCACTTTGTCGAGCTA
<i>ACTB</i>	GGATGCAGAAGGAGATCACT	CGATCCACACGGAGTACTTG
<i>ALOX5</i>	GATTGTCCCCATTGCCATCC	AGAAGGTGGGTGATGGTCTG
<i>CMPK2</i>	CACCCGGTGGTGCCAG	GAGGTACACTCCTCCAAAACG
<i>CXCL8</i>	GAAGTTTTTGAAGAGGGCTGAGA	TGCTTGAAGTTTCACTGGCAT
<i>EIF2AK2</i>	GCATGGGCCAGAAGGATTTT	GCGGCCAATTGTTTTGCTTC
<i>GAPDH</i>	GAAGGTGAAGTCCGGAGT	GAAGATGGTATGGGATTTT
<i>HPRT1</i>		
<i>IDO1</i>	ACAGACCACAAGTCACAGCG	TTGGCAAGACCTTACGGACA
<i>IFIH1</i>	AGATGCAACCAGAGAAGATCCA	TGGCCATTGTTTCATAGGGT
<i>IL1B</i>	AGAAGTACCTGAGCTCGCCA	CTGGAAGGAGCACTTCATCTGT
<i>IL6</i>	ACATGTGTGAAAGCAGCAAAG	GGCAAGTCTCCTCATTGAATCC
<i>IRF7</i>	CATCTTCAAGGCCTGGGCTG	GCAGCGGAAGTTGGTTTTCC
<i>ISG15</i>	CAGCGAACTCATCTTTGCCAG	GGACACCTGGAATTCGTTGC
<i>ISG20</i>	GTGGCCAGGCTAGAGATCC	GGAAGTCGTGCTTCAGGTCA
<i>LTC4S</i>	GACGGTACCATGAAGGACGA	GGAGAAGTAGGCTTGCAGCAG
<i>OAS2</i>	TCAACGCTCTGAGCTTAAATGA	CAAACCTACCAGGACTGGCA
<i>OAS3</i>	ACTACAACGCCAAGGACAAGA	GATGATAGGCCTGGGCTTCTG
<i>OASL</i>	GGACATCGTTGCTCAGAGGG	CTCGTGCCCTCTTCACGTT
<i>PTGDS</i>	CCCAGGGCTGAGTTAAAGGA	AGAGCAGAGACATCCAGAGC
<i>PTGES</i>	TCAAGATGTACGTGGTGGCC	GAAAGGAGTAGACGAAGCCCAG
<i>PTGS2</i>	GCTGGAACATGGAATTACCCA	CTTTCTGTACTGCGGGTGGAA
<i>TFRC</i>	GGACGCGCTAGTGTCTTCT	CATCTACTTGCCGAGCCAGG
<i>TMPRSS2</i>	AGAGTGCGACTCCTCAGGTA	AGGCGAACACACCGATTCTC
<i>USP18</i>	GGCTCCTGAGGCAAATCTGT	CAACCAGGCCATGAGGGTAG

HPRT1: QuantiTect Primer Assay Cat.No. QT00059066 (Qiagen)

Flow cytometry

Human MDM from either seronegative or seropositive patients were stained with antibodies against EP2 (PE), HLA-DR (BV650), CD206 (BB515) (all from BD Biosciences) as well as CD11b (BV421) (Biolegend). All samples were acquired on a BD LSRFortessa (BD Biosciences) and analyzed by using FlowJo v10 software (FlowJo LLC).

Data analysis and statistics

For LC-MS/MS (lipid mediator) and ELISA (cytokines/ chemokines) data, missing values below the lower limit of detection were interpolated using $\frac{1}{4}$ of the minimum value for each metabolite. For the lipid mediator dataset outliers were excluded using the ROUT (robust regression and outlier removal) method for each stimulation separately in Graph Pad Prism (version 9). Metabolites with more than 60% of values below the media blank levels were excluded. Statistical analysis of two group comparisons was performed either using Mann-Whitney (unpaired) or Wilcoxon test (paired). For multiple comparisons, Kruskal-Wallis test with Dunn correction was chosen. Principal component analyses (PCA) were generated using MetabolAnalyst 5.0.¹ The Morpheus software (Broad Institute) was used for the design of heatmaps.

RNAseq data were analyzed in R. Genes with fewer than 100 counts in the RNA-seq data set were filtered out. Only the genes with more than 10 counts in at least one sample were kept. Next, the vst^2 normalization implemented in the DESeq2 package³ was used to normalize the filtered RNA-seq counts. Combat⁴ was used for batch correction implemented in the sva package⁵ in order to remove the batch effects of gender and age. During the batch correction, age was categorized into groups of 5 years intervals. Principal component analysis (PCA) on the normalized and batch-corrected data was performed and the ggplot2 package⁶ was used to plot the samples in the plane on the 1st and 2nd principal components. The DESeq2 package was used to calculate the fold change, base mean and adjusted p-values of the differentially genes for the following comparisons: seronegative without any treatment vs post CoV without any treatment; seronegative treated with spike vs post CoV treated with spike; and seronegative treated with LPS vs post CoV treated with LPS. The pheatmap package⁷ (<https://CRAN.R-project.org/package=pheatmap>) was used to plot heatmaps of the top 50 genes with the largest log₂ fold change with at least 50 base counts for the above-mentioned comparisons. The genes in rows of the heatmaps were not clustered, while the samples in the columns of the heatmaps were clustered based Euclidean distance. The EnhancedVolcano package⁸ (<https://github.com/kevinblighe/EnhancedVolcano>) was used to create volcano plots of the differentially expressed genes with at least 50 base counts.

Supplementary Figures

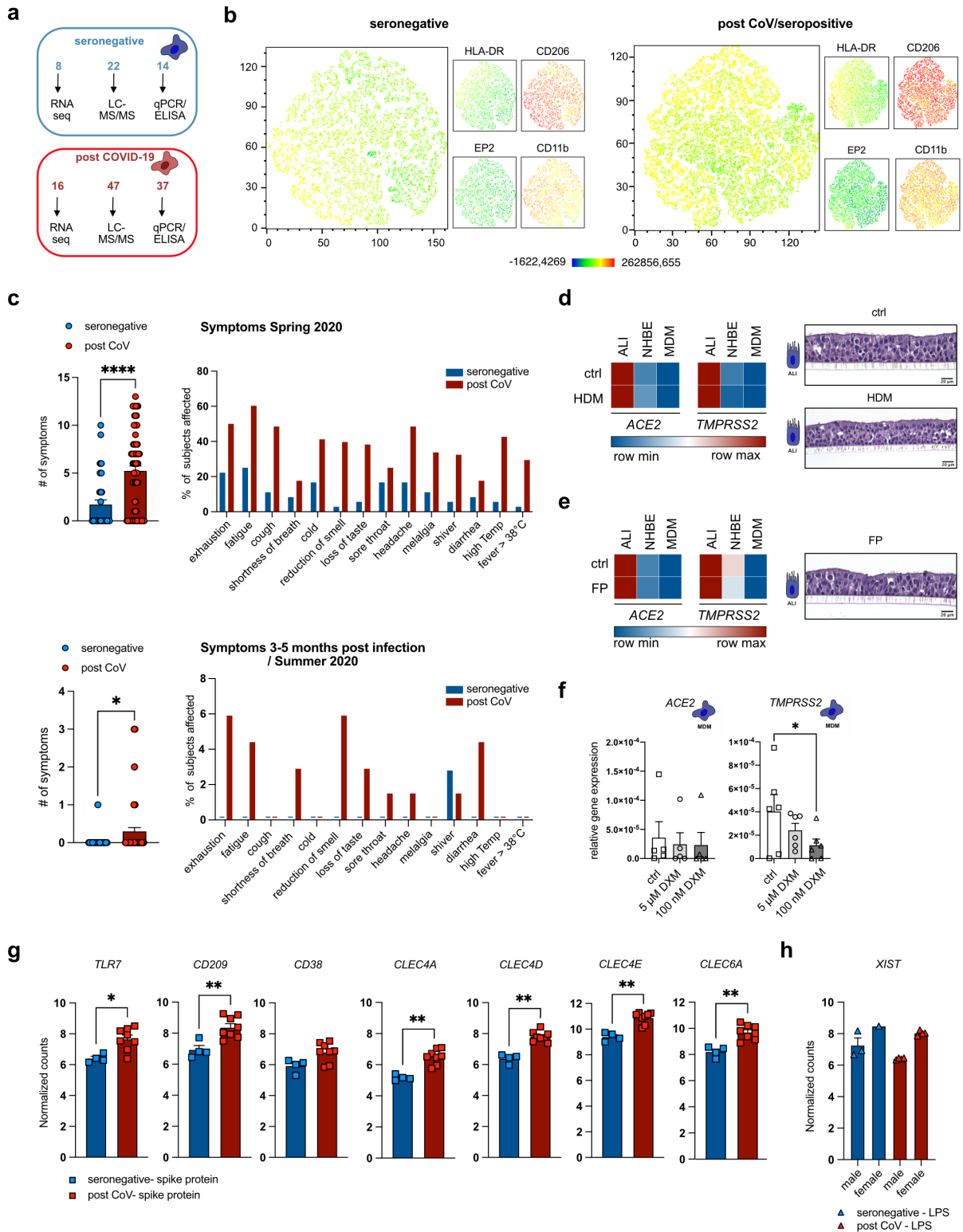


Figure S1: Monocyte-derived macrophages express low levels of ACE2 and TMPRSS2, but high levels of C-type lectin receptors involved in SARS-CoV-2 recognition

(a) Overview of samples from the post COVID cohort and the corresponding seronegative patients that were subjected to each type of analysis. (b) Representative FACS data (tsne plots) for MDM from seronegative ($n=2$) or post CoV/seropositive subjects ($n=4$). (c) Symptom load of seronegative (healthy) ($n=36$) and SARS-CoV-2 seropositive (post CoV) ($n=68$) individuals in Spring 2020 or at 3-5 months post infection (Summer 2020). (d) Relative gene

expression (shown as average in the heatmap) of *ACE2* and *TMPRSS2* at baseline and upon house dust mite extract (HDM) stimulation (10 $\mu\text{g}/\text{mL}$) in ALI (n=5), NHBE (n=3) and MDM (n=4) measured by qPCR. Hematoxylin & eosin staining of ALI cells after stimulation with HDM (10 $\mu\text{g}/\text{mL}$). (e) Relative gene expression (shown as average in the heatmap) of *ACE2* and *TMPRSS2* in ALI (n=5), NHBE (n=3) and MDM (n=4) after fluticasone propionate (FP) treatment (1 μM). (f) Effect of dexamethasone (DXM) on *ACE2* and *TMPRSS2* expression in healthy MDM. Healthy volunteers n=4 included in panels d-f were recruited in a cohort separate from the post COVID cohort. (g) Expression of pathogen recognition receptors (RNAseq) upon spike protein stimulation in MDM from seronegative (n=4) or post COVID (post CoV, n=8) individuals. Bar graphs are depicted as mean + SEM. Statistical significance was determined by Mann-Whitney test (g) or Kruskal-Wallis test followed by Dunn's multiple comparison test (f,h). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

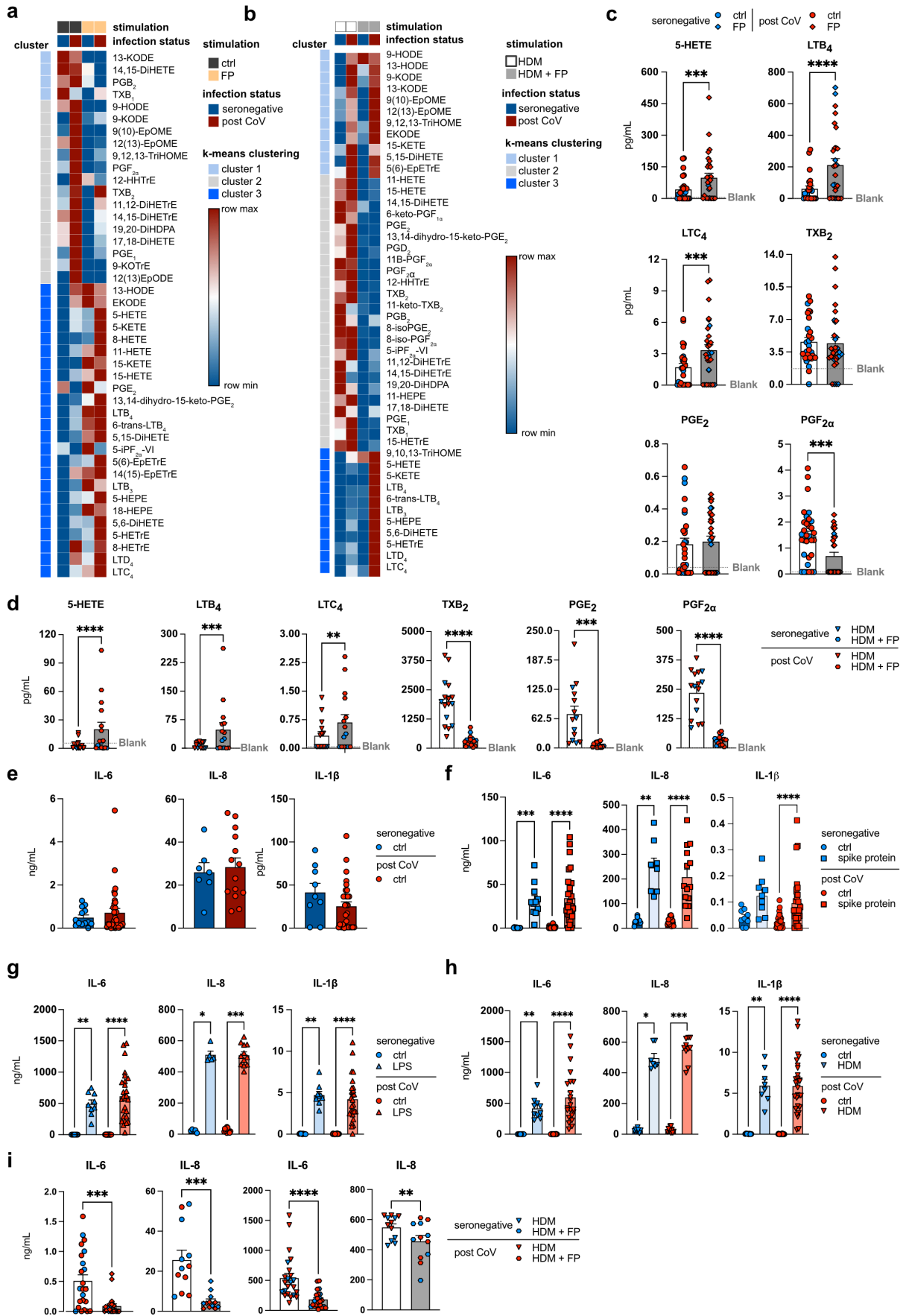


Figure S2: Corticosteroids differentially affect prostanoid, leukotriene and cytokine production in post COVID-19 macrophages

(a,b) Heatmap of lipid mediators quantified by LC-MS/MS in supernatant of MDM stimulated +/- fluticasone propionate (FP) (1 μ M), house dust mite extract (HDM) (10 μ g/mL) and HDM+FP. Rows are clustered with k-means using Pearson correlation. Data are shown as mean of seronegative (n=5) or SARS-CoV-2 seropositive (n=11) individuals. (c-d) 5-LOX and COX metabolites produced by MDM (seronegative or post CoV) stimulated with +/- fluticasone propionate FP (1 μ M), HDM (10 μ g/mL) or HDM+FP. (e-i) IL-6, IL-8 and IL-1 β secretion in supernatant of MDM from seronegative or SARS-CoV-2 seropositive (post CoV) individuals; (e) Baseline (ctrl), (f) after stimulation with S-protein (20 nM), (g) LPS (100 ng/mL), (h) house dust mite extract (HDM) (10 μ g/mL), (i) FP (1 μ M) or HDM+FP quantified by ELISA. Data are shown as mean + SEM. Seronegative individuals (n=8-14), previously SARS-CoV-2 infected individuals (n=13-37). Statistical significance was determined by Mann-Whitney test (c-e; i) or Kruskal-Wallis test followed by Dunn's multiple comparison test (f-h). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

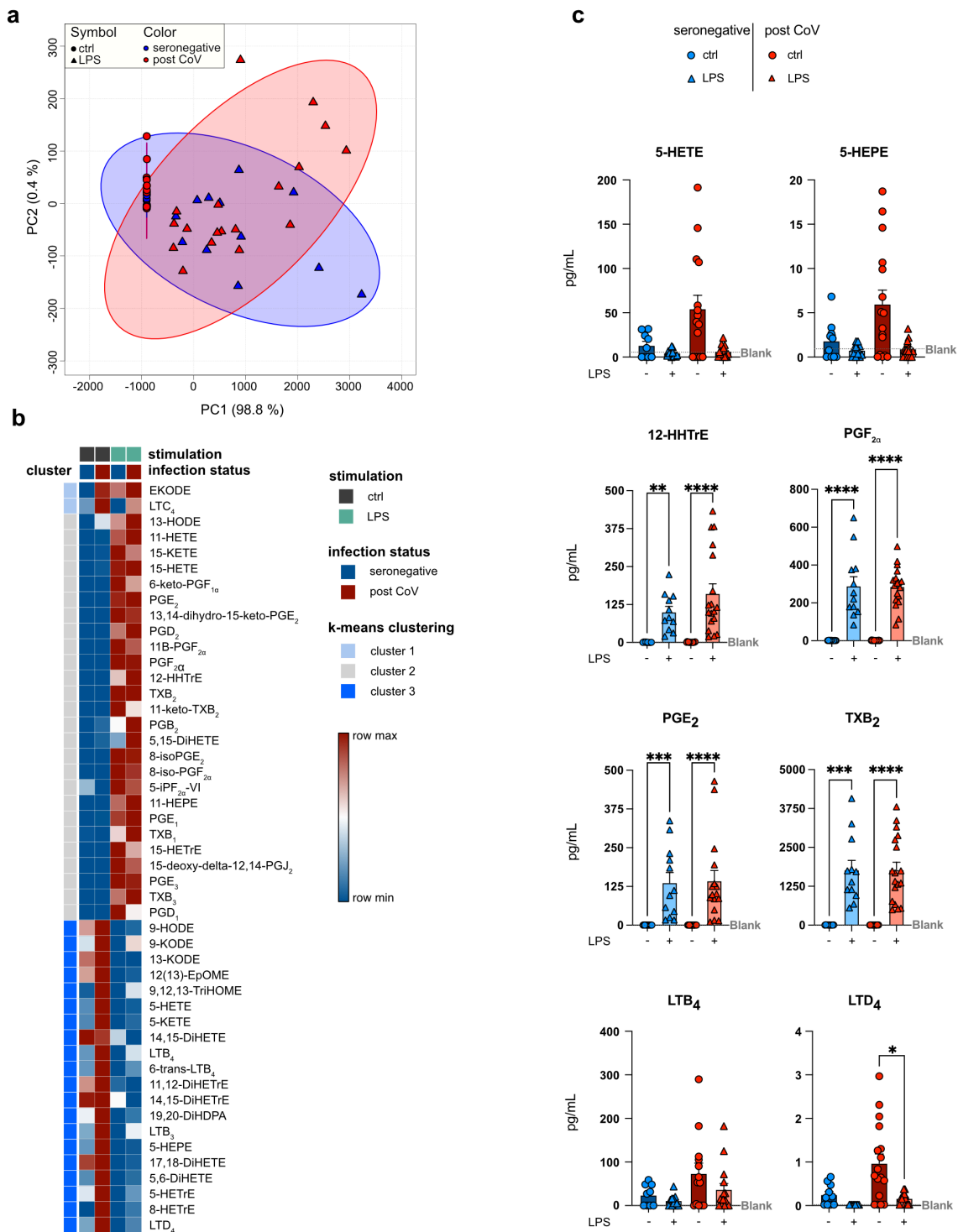


Figure S3: LPS-induced eicosanoid responses are largely unaltered in post COVID-19 MDM

(a) PCA of lipid mediators quantified in MDM supernatant (SN) of seronegative ($n=12$) or SARS-CoV-2 seropositive ($n=18$) individuals +/- LPS (100 ng/mL) stimulation. Red and blue circles depict the 95% CI. (b) Heatmap of lipid mediators measured in MDM SN stimulated +/- LPS. Rows are clustered with k-means using Pearson correlation. Data are shown as mean for MDM from seronegative ($n=12$) or SARS-CoV-2 seropositive ($n=18$) individuals. (c) Concentrations of major COX and 5-LOX metabolites in MDM SN after 24h stimulation with LPS quantified by LC-MS/MS, depicted as mean + SEM of $n=12$ (seronegative) or $n=18$ (post

CoV). Statistical significance was determined by Kruskal-Wallis test followed by Dunn's multiple comparison test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Supplementary Tables:

Table S1: Patient characterization and differentially expressed genes in MDM from seropositive and seronegative individuals +/- stimulation with LPS or S-protein; subjects characteristics for each figure separately; metabolites included in the LC-MS/MS panel

References

1. Chong, J., Wishart, D. S. & Xia, J. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. *Current Protocols in Bioinformatics* **68**, (2019).
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8. Blighe K *EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling*. (2019).at <<https://github.com/kevinblighe/EnhancedVolcano>>