

1 **Mild/Asymptomatic Maternal SARS-CoV-2 Infection Leads to Immune Paralysis in Fetal**  
2 **Circulation and Immune Dysregulation in Fetal-Placental Tissues**

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## SUMMARY

Few studies have addressed the impact of maternal mild/asymptomatic SARS-CoV-2 infection on the developing neonatal immune system. In this study, we analyzed umbilical cord blood and placental chorionic villi from newborns of unvaccinated mothers with mild/asymptomatic SARS-CoV-2 infection during pregnancy using flow cytometry, single-cell transcriptomics, and functional assays. Despite the lack of vertical transmission, levels of inflammatory mediators were altered in cord blood. Maternal infection was also associated with increased memory T, B cells, and non-classical monocytes as well as increased activation. However, *ex vivo* responses to stimulation were attenuated. Finally, within the placental villi, we report an expansion of fetal Hofbauer cells and infiltrating maternal macrophages and rewiring towards a heightened inflammatory state. In contrast to cord blood monocytes, placental myeloid cells were primed for heightened antiviral responses. Taken together, this study highlights dysregulated fetal immune cell responses in response to mild maternal SARS-CoV-2 infection during pregnancy.

## KEYWORDS:

COVID-19, SARS-CoV-2, placenta, chorionic villi, umbilical cord blood, Hofbauer cells

## 1 INTRODUCTION

2 To date, over 255,000 pregnant women in the United States have been infected with  
3 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)<sup>1</sup>. Although most pregnant  
4 women experience asymptomatic or mild coronavirus disease 2019 (COVID-19), those who  
5 experience severe disease are at a significantly higher risk for admission to the intensive care  
6 unit, mechanical ventilation, and pre-term birth<sup>2-4</sup>. While some studies have described the  
7 presence of SARS-CoV-2 RNA in placental villi, including maternal macrophages and Hofbauer  
8 cells (HBC)<sup>5-7</sup>, vertical transmission is extremely rare<sup>8-11</sup>. Nevertheless, SARS-CoV-2 infection  
9 during pregnancy has been shown to alter frequencies of macrophage and effector T-cell subsets  
10 and induce a pro-inflammatory environment at the maternal-fetal interface (MFI), specifically  
11 within the maternal decidua<sup>12-14</sup>. Moreover, pregnant women with severe COVID-19 are more  
12 likely to give birth to newborns with morbidities including respiratory distress syndrome<sup>15, 16</sup>,  
13 hyperbilirubinemia<sup>16-18</sup>, sepsis<sup>16, 19-21</sup>, infections requiring antibiotic treatments<sup>22</sup>, and admission  
14 to the neonatal intensive care unit (NICU)<sup>16, 20, 23</sup>.

15 Mechanistic underpinnings explaining these adverse outcomes are only beginning to  
16 emerge. Recent studies have reported significantly elevated NK cell frequencies in UCB from  
17 neonates born to pregnant women who have recovered from SARS-CoV-2 infection compared to  
18 those born to mothers with ongoing infection at delivery<sup>24</sup>. Furthermore, umbilical cord blood  
19 (UCB) NK cells from neonates born to mothers with active SARS-CoV-2 infection or those who  
20 recovered display increased expression of DNAX accessory molecule 1 (DNAM-1)<sup>24</sup>, an NK cell-  
21 activating receptor essential for the recognition and killing of virus-infected cells<sup>25</sup>. Asymptomatic  
22 or mild maternal SARS-CoV-2 infection detected at delivery also results in an altered inflammatory  
23 milieu in fetal circulation, including increased UCB plasma levels of IL-1 $\beta$ , IL-6, IL-8, IL-18, IL-33,  
24 IFN $\gamma$ , caspase 1, nuclear factor of activated T cells (NFATC3), and CCL21<sup>26-28</sup>. Additionally, T<sub>H</sub>2  
25 responses are dampened in infants born to mothers with infection in the second and third

1 trimesters<sup>28</sup>. Cases of very high anti-SARS-CoV-2 IgG concentrations (>5871.07 U/mL) detected  
2 in UCB were associated with higher frequencies of fetal neutrophils and cytotoxic T cells<sup>27</sup>.

3 Bulk RNA sequencing of UCB revealed that mild/asymptomatic maternal SARS-CoV-2  
4 infection in the third trimester is associated with the upregulation of genes responsible for  
5 antimicrobial responses and down-regulation of genes enriched for phagocytosis, complement  
6 activation, and extracellular matrix organization<sup>26</sup>. Additionally, UCB monocytes exhibited  
7 upregulation of IFN-stimulated genes (ISG) and MHC class I and II genes<sup>26</sup>. Single-cell analysis  
8 of UCB from newborns of mothers with mild COVID-19 in the third trimester revealed  
9 transcriptional changes that correlated with activation of plasmacytoid dendritic cells (pDCs),  
10 activation and exhaustion of NK cells, and clonal expansion of fetal T cells<sup>29</sup>. While there are  
11 clear disruptions in the UCB immune landscape with maternal SARS-CoV-2 infection, the  
12 functional implications of these changes remain largely unknown.

13 Our previous studies have shown extensive remodeling of decidua (maternal placental  
14 compartment) obtained from pregnant women with mild/asymptomatic SARS-CoV-2 infection<sup>12</sup>,  
15 including altered frequencies of decidual macrophages, regulatory T cells (Tregs), and activated  
16 T cells. Furthermore, antigen presentation and type I IFN signaling were attenuated in decidual  
17 macrophages, while pathways associated with cytokine signaling and cell killing were upregulated  
18 in decidual T cells. While abnormal placental pathologies have been reported with maternal  
19 SARS-CoV-2 infection, including inflammation and necrosis<sup>30-32</sup>, few studies have addressed how  
20 maternal SARS-CoV-2 impacts the immune landscape of villous tissues (fetal placental  
21 compartment)<sup>26, 33-35</sup>. Placental SARS-CoV-2 infection is associated with the recruitment of  
22 maternal monocytes and macrophages to villous tissues and increased frequency of fetal HBCs  
23 that express PD-L1, a possible mechanism to prevent immune cell-driven placental damage<sup>36</sup>.  
24 Finally, a recent study reported a significant downregulation of genes responsible for Type 1  
25 interferon and IL-6/IL-1 $\beta$  cytokine responses in the chorionic villous regardless of maternal

1 COVID-19 severity, the gestational timing of infection, gestational age at delivery, pre-pregnancy  
2 BMI, or mode of delivery (cesarean versus vaginal delivery) <sup>34</sup>.

3         Despite these observations, our understanding of the impact of asymptomatic/mild  
4 maternal SARS-CoV-2 infection on the immune landscape of fetal placental tissues and  
5 circulation remains incomplete due to a lack of studies that examined paired samples and where  
6 transcriptional analyses were coupled with functional assays. In this study, we used a combination  
7 of single-cell RNA sequencing and functional assays to address this gap in knowledge. Our data  
8 show that mild/asymptomatic maternal SARS-CoV-2 infection leads to heightened basal  
9 activation but dysfunctional responses of both innate and adaptive branches in circulation. This  
10 dysregulation extends to the fetal placental compartment (chorionic villi), as shown by the  
11 increased infiltration of regulatory maternal monocytes/macrophages to the fetal compartment,  
12 HBC activation, and impaired responses of villous myeloid cells to antimicrobial stimulation.

13

1

## 2 **METHODS**

### 3 **Cohort characteristics**

4 This study was approved by the Institutional Ethics Review Boards of Oregon Health & Science  
5 University and the University of Kentucky. Placental chorionic villi and UCB samples from 41  
6 healthy, pregnant participants without SARS-CoV-2 infection or vaccination who had an  
7 uncomplicated, singleton pregnancy and 18 pregnant participants with asymptomatic (n=8) or  
8 mild (n=10) SARS-CoV-2 infection, but otherwise healthy pregnancies, were collected.  
9 Participants were classified as having mild SARS-CoV-2 infection if they experienced mild  
10 respiratory symptoms accompanied by a positive COVID test, while participants were classified  
11 as experiencing an asymptomatic infection if they tested positive during the mandatory COVID  
12 testing upon admission to labor and delivery and reported no symptoms. Importantly, all nasal  
13 swabs from newborns of SARS-CoV-2 infected participants as well as placental chorionic villi  
14 tissue samples tested negative for SARS-CoV-2 by qPCR. Controls were participants who did not  
15 experience COVID symptoms or report a positive COVID test at any time during their pregnancy  
16 receiving care at the same facility. The characteristics of the cohort are outlined in Table 1.

17

### 18 **Blood processing**

19 Whole blood samples were collected in EDTA vacutainer tubes. Complete blood counts were  
20 obtained by a Cell-Dyn Emerald 22 (Abbott, Abbott Park, Illinois). UCB mononuclear cells  
21 (UCBMC) and plasma were isolated after whole-blood centrifugation over LymphoPrep in  
22 SepMate tubes (STEMCELL Technologies, Vancouver, BC) following manufacturers' protocols.  
23 Plasma was stored at  $-80^{\circ}\text{C}$  until analysis. UCBMC were cryopreserved using 10% DMSO/FBS  
24 and Mr. Frosty Nalgene Freezing containers (Thermo Fisher Scientific, Waltham, Massachusetts)  
25 at  $-80^{\circ}\text{C}$  overnight and then transferred to a cryogenic unit until analysis.

26

## 1 **Placenta processing**

2 Fetal chorionic villi were separated from maternal decidua and immediately immersed in RPMI  
3 supplemented with 10% FBS, 1% penicillin-streptomycin, and 1% L-glutamine (GeminiBio,  
4 Sacramento, California). Samples were processed within 24 hours of collection. Chorionic villi  
5 were first washed thoroughly in HBSS to remove contaminating blood, then minced into  
6 approximately 0.2-0.3mm<sup>3</sup> cubes, followed by enzymatic digestion at 37°C for 1 hour in R3 media  
7 (RPMI 1640 with 3% FBS, 1% Penicillin-Streptomycin, 1% L-glutamine, and 1M HEPES)  
8 supplemented with 0.5 mg/mL collagenase IV (Sigma-Aldrich, Saint Louis, Missouri). The  
9 disaggregated cell suspension was passed through tissue strainers to eliminate large tissue  
10 chunks. Cells were pelleted and passed sequentially through 100-, 70-, and 40-µm cell sieves.  
11 Red blood cells were lysed using RBC lysis buffer (155 mM NH<sub>4</sub>Cl, 12 mM NaHCO<sub>3</sub>, 0.1 mM  
12 EDTA in double distilled water). The cell suspension was then layered on discontinuous 60% and  
13 40% percoll gradients (Sigma-Aldrich, Saint Louis, Missouri) and centrifuged for 30 minutes at  
14 930xg with the brakes off. Immune cells at the interface of 40% and 60% gradients were collected,  
15 counted, and cryopreserved as described above for UCBMC for future analysis. SARS-CoV-2  
16 viral loads were assessed in placental tissues using qPCR as previously described <sup>12</sup>.

17

## 18 **ELISA**

19 End-point titers (EPT) against the SARS-CoV-2 receptor binding domain of the spike protein  
20 (RBD) and nucleocapsid protein (NP) were determined using standard ELISA as recently  
21 described <sup>37</sup>. Plates were coated with 500 ng/mL RBD or 1 µg/mL NP (GenScript, Piscataway,  
22 New Jersey), and heat-inactivated plasma (1:50 in blocking buffer) was added in 3-fold dilutions.  
23 Responses were visualized by adding HRP anti-human IgG (BD Pharmingen, San Diego,  
24 California) followed by o-Phenylenediamine dihydrochloride (Thermo Fisher Scientific, Waltham,  
25 Massachusetts). Batch differences were minimized by normalizing to a positive control sample

1 run on each plate. EPTs were calculated using log-log transformation of the linear portion of the  
2 curve and 0.1 OD units as the cut-off.

3

#### 4 **Plasma Luminex**

5 Levels of immune mediators in plasma, cell culture supernatant following RSV or *E. coli*  
6 stimulation, and resting HBC cell culture supernatant were measured using a human, premixed  
7 45-plex panel (R&D Systems, Inc. Minneapolis, Minnesota). Immune mediators in cell culture  
8 supernatant following CD3/CD28 bead stimulation were measured using a human premixed  
9 CD8+ T Cell Human 17-plex panel (Millipore, Temecula, California). All Luminex assays were  
10 analyzed using a MAGPIX Instrument and xPONENT software (Luminex, Austin, Texas).

11

#### 12 **Phenotyping**

13 1-2 x 10<sup>6</sup> UCBMC were stained using antibodies against CD4, CD8b, CCR7, CD45RA, CD19,  
14 CD27, IgD, and KLRG1 to delineate naïve and memory T and B cell populations<sup>38</sup>. Cells were  
15 then fixed (Fixation buffer; BioLegend, San Diego, CA), permeabilized (Permeabilization wash  
16 buffer; BioLegend, San Diego, CA), and stained intracellularly for the proliferation marker Ki-67  
17 (BioLegend, San Diego, CA). A second set of samples were stained using antibodies against CD3,  
18 CD20, HLA-DR, CD14, CD11c, CD123, CD56, and CD16 to delineate monocytes, myeloid  
19 dendritic cells (mDC); plasmacytoid dendritic cells (pDC) and natural killer (NK) cell subsets<sup>39, 40</sup>.  
20 All flow cytometry samples were acquired with the Attune NxT instrument (ThermoFisher  
21 Scientific, Waltham, Massachusetts) and analyzed using FlowJo 10.5 (TreeStar, Ashland,  
22 Oregon).

23 Villous leukocytes were stained with CD45 (pan leukocyte marker), CD14, HLA-DR, FOLR2, CD9,  
24 and CCR2 to delineate HBCs (CD14<sup>+</sup>HLA-DR<sup>-</sup>FOLR2<sup>+</sup>CCR2<sup>-</sup>) and placenta associated maternal  
25 macrophages (PAMMs; PAMM1a: CD14<sup>+</sup>HLADR<sup>+</sup>FOLR2<sup>-</sup>CD9<sup>+</sup>CCR2<sup>low/int</sup>) and monocytes



1 (PAMM1b: CD14<sup>+</sup>HLADR<sup>+</sup>FOLR2<sup>-</sup>CD9<sup>-</sup>/intCCR2<sup>+</sup>) and infiltrating maternal decidual  
2 macrophages (PAMM2: CD14<sup>+</sup>HLA-DR<sup>hi</sup>FOLR2<sup>hi</sup>) as previously described <sup>41</sup>.

3

#### 4 **Ex vivo cell stimulation**

5 For T cell stimulations, 1x10<sup>6</sup> UCBMC were cultured for 24 hours at 37C in RPMI supplemented  
6 with 10% FBS in the presence or absence of anti-CD3/CD28 beads (ThermoFisher Scientific,  
7 Waltham, Massachusetts). After 24 hours, the cells were spun down and the supernatants were  
8 collected for analysis by Human T Cell 17-plex panel (Sigma-Aldrich, Saint Louis, Missouri).

9 For NK cell stimulation, 1x10<sup>6</sup> UCBMC were stimulated for 6 hours at 37°C in RPMI supplemented  
10 with 10% FBS in the presence or absence of 0.5 µg/ml PMA and 5 µg/ml ionomycin (InvivoGen,  
11 San Diego, California). CD107a antibodies were added at the beginning of stimulation; Brefeldin  
12 A (BioLegend, San Diego, CA) was added after 1 hour incubation. Cells were stained for CD3,  
13 CD20, CD16, CD56, and HLA-DR, fixed, permeabilized, and stained intracellularly for IL-2, TNFα,  
14 MIP-1β, and IFNγ.

15 For monocyte/macrophages responses, CD14<sup>+</sup> cells were FACS sorted from UCBMC or villous  
16 leukocytes and cultured for 16 h at 37°C in the absence/presence of either RSV (MOI 1) or E. coli  
17 (6x10<sup>5</sup> cfu/well). Production of immune mediators in the supernatants was assessed using a  
18 human 45-plex (R&D Systems, Inc. Minneapolis, Minnesota).

19

#### 20 **B cell purification and stimulation methods**

21 B cells were purified from UCBMC using MACS CD20<sup>+</sup> microbeads (Miltenyi Biotec, Waltham,  
22 MA). 50-100,000 B cells were plated per well and stimulated using a TLR agonist cocktail  
23 containing LPS (100 µg/µL), R848 (10 µg/mL), and ODN2216 (5µg/mL) in RP10 media. Control  
24 wells received RP10 + 0.4% DMSO. After stimulation for 24 hours, cells were surfaced stained  
25 with antibodies against CD3, CD20, HLA-DR, IgD, CD27, CD40, CD83, CD86, CD80, CD69, and  
26 IgG (BioLegend, San Diego, CA).

1

## 2 **3' multiplexed single-cell RNA sequencing**

3 Freshly thawed UCBMCs ( $1-2 \times 10^6$  cells) were stained with Ghost Violet 540 (Tonbo Biosciences,  
4 San Diego, CA) for 30 min at 4C in the dark before being incubated with Fc blocker (Human  
5 TruStain FcX, BioLegend, San Diego, California) in PBS with 1% BSA for 10 min at 4C. Cells were  
6 surface stained with CD45-FITC (HI30, BioLegend, San Diego, California) for 30 min at 4C in the  
7 dark. Samples were then washed twice in PBS with 0.04% BSA and incubated with individual  
8 CellPlex oligos (CMO) (10X Genomics, Pleasanton, California) per manufacturer's instructions.  
9 Pellets were washed three times in PBS with 1% BSA, resuspended in 300  $\mu$ L FACS buffer, and  
10 sorted on BD FACS Aria Fusion into RPMI (supplemented with 30% FBS). Sorted live CD45+  
11 cells were counted in triplicates on a TC20 Automated Cell Counter (BioRad, Hercules, California),  
12 washed, and resuspended in PBS with 0.04% BSA in a final concentration of 1500 cells/ $\mu$ L.  
13 Single-cell suspensions were then immediately loaded on the 10X Genomics Chromium  
14 Controller with a loading target of 20,000 cells.

15 Freshly thawed villous leukocytes ( $1-2 \times 10^6$  cells) were stained with Ghost Violet 540 (Tonbo  
16 Biosciences, San Diego, CA) for 30 min at 4C in the dark before being incubated with Fc blocker  
17 (Human TruStain FcX, BioLegend, San Diego, California) in PBS with 1% BSA for 10 min at 4C.  
18 Finally, cells were surface stained with HLA-DR, CD14, CCR2, and FOLR2 (BioLegend, San  
19 Diego, California) for 30 min at 4C in the dark. Samples were then washed twice and incubated  
20 with individual TotalSeq B antibodies (HTO) (BioLegend, San Diego, California) per  
21 manufacturer's instructions. Pellets were resuspended in 300  $\mu$ L FACS buffer and sorted on BD  
22 FACS Aria Fusion into RPMI (supplemented with 30% FBS). Sorted live CD14+CCR2+ and  
23 CD14+CCR2- cells were counted in triplicates on a TC20 Automated Cell Counter (BioRad,  
24 Hercules, California), washed, and resuspended in PBS with 0.04% BSA in a final concentration  
25 of 1500 cells/ $\mu$ L. Single-cell suspensions were then immediately loaded on the 10X Genomics  
26 Chromium Controller with a loading target of 20,000 cells.

1 All libraries were generated using the V3.1 chemistry for gene expression and Single Cell 3'  
2 Feature Barcode Library Kit per the manufacturer's instructions (10X Genomics, Pleasanton,  
3 California). Libraries were sequenced on Illumina NovaSeq 6000 with a sequencing target of  
4 30,000 gene expression reads and 5,000 feature barcoding reads per cell.

5

## 6 **Single-cell RNA-Seq data analysis**

7 Raw reads were aligned and quantified using Cell Ranger (version 6.0.2, 10X Genomics,  
8 Pleasanton, California) against the human reference genome (GRCh38) using the *multi-* option.  
9 Seurat (version 4.0) was used for downstream analysis. Cell doublets were removed by retaining  
10 droplets with a single CMO or HTO signal. Additionally, ambient RNA and dying cells were  
11 removed by filtering out droplets with less than 200 detected genes and greater than 20%  
12 mitochondrial gene expression, respectively. Data objects from controls and SARS+ groups were  
13 integrated using Seurat. Data normalization and variance stabilization were performed on the  
14 integrated object using the *NormalizeData* and *ScaleData* functions in Seurat, where a regularized  
15 negative binomial regression was corrected for differential effects of mitochondrial and ribosomal  
16 gene expression levels. Dimensionality reduction was performed using *RunPCA* function to obtain  
17 the first 30 principal components and clusters visualized using Seurat's *RunUMAP* function. Cell  
18 types were assigned to individual clusters using *FindAllMarkers* function with a log2 fold change  
19 cutoff of at least 0.4, FDR<0.05, and using a known catalog of well-characterized scRNA markers  
20 for human PBMC and villous leukocytes (Supplemental Table 1) <sup>42</sup>. Differential gene expression  
21 analysis was performed using MAST function in Seurat. Only statistically significant genes  
22 maintaining an FDR<0.05 and a log2 fold change  $\pm 0.25$  for UCBMC or 0.4 for villous leukocytes  
23 were included in downstream analyses. Module scores for specific pathways/gene sets were  
24 incorporated cluster-wise using the *AddModuleScores* function (Supplemental Table 2).  
25 Functional enrichment was performed using Metascape <sup>43</sup>.

26

## 1 **Statistical Analyses**

2 Data sets were first assessed for normality using Shapiro Wilk test and equality of variances using  
3 the Levene test. Group differences between datasets normally distributed were tested using an  
4 unpaired t-test (for datasets with equal variances) or an unpaired t-test with Welch's correction  
5 (for cases with unequal variances). Datasets not normally distributed were subjected to non-  
6 parametric Mann-Whitney test. All statistical analyses were conducted in Prism version 9.4.1  
7 (GraphPad)

8

1

## 2 RESULTS

### 3 ***Maternal SARS-CoV-2 infection leads to increased systemic fetal inflammation and*** 4 ***frequency of myeloid cells.***

5 UCB and placental chorionic villous tissues were collected at delivery from newborns of  
6 mothers who tested positive for SARS-CoV-2 during pregnancy (mild) or at the time of delivery  
7 (asymptomatic) or had no COVID symptoms (controls) and receiving care at OHSU. Controls  
8 were mostly participants who delivered by scheduled cesarean due to challenges associated with  
9 recruitment during the pandemic, hence the higher number of cesarean sections in the control  
10 group (67.4%,  $p < 0.0001$ ) (Table 1). Cohort characteristics can be found in Table 1. Maternal age  
11 at delivery, pre-pregnancy BMI, and fetal sex were comparable between both groups (Table 1).  
12 Gestational age at delivery was significantly lower with maternal SARS-CoV-2 infection  
13 ( $p = 0.0335$ ), consistent with findings of increased rates of early labor in SARS-CoV-2 pregnancies  
14 <sup>16, 20, 23</sup>. A greater proportion of pregnant participants with SARS-CoV-2 infection identified as  
15 Hispanic (27.8%,  $p = 0.0208$ ), in line with the increased incidence of SARS-CoV-2 in this population  
16 <sup>44</sup>.

17 All but one of the neonates of participants with SARS-CoV-2 infection had detectable IgG  
18 antibodies directed against spike protein receptor binding domain (RBD) at birth, albeit lower than  
19 maternal IgG titers (Figure 1A). Additionally, all but 2 dyads had detectable antibodies against  
20 nucleocapsid protein (NP), and maternal/neonatal titers were comparable between the two groups  
21 (Figure 1A). We observed no differences in antibody (IgG) titers against receptor binding domain  
22 (RBD) or nucleocapsid protein (NP) between participants in the mild and asymptomatic groups  
23 (Table 1). Therefore, all subsequent comparisons were performed on UCB and fetal placental  
24 tissues from newborns of SARS-CoV-2-positive (maternal SARS+) and SARS-CoV-2-naïve  
25 participants (control).

1            Interestingly, maternal SARS-CoV-2 infection altered immune mediators in cord blood  
2 (Figure 1B). Specifically, concentrations of several chemokines important for the recruitment of  
3 both innate immune cells and lymphocytes (CXCL8, CXCL9, CXCL10, CCL4, CCL3, CXCL11,  
4 and CCL11) were lower in the maternal SARS+ group (Figure 1B). Moreover, levels of several  
5 antiviral and pro-inflammatory mediators, notably IFN $\beta$ , TNF $\alpha$ , IL-23 (Th17), and IL-15 (NK cell  
6 activation), were also lower. Levels of growth factor VEGF, anti-inflammatory regulator IL-1RA,  
7 and lymphocyte survival factor IL-7 were dampened in the maternal SARS+ group. In contrast,  
8 levels of S100B, a neurobiochemical marker for CNS injury, PDGF-BB, which regulates cell  
9 growth, and IL18 were increased (Figure 1B and Table 2). Complete blood cell counts from UCB  
10 of newborns in the maternal SARS+ group show increased numbers of total white blood cells  
11 driven by elevated monocyte and granulocyte numbers (Figure 1C).

12

13 ***Maternal SARS-CoV-2 infection alters the frequency of circulating immune cells,***  
14 ***suggestive of a heightened activation state.***

15            To uncover the changes within the fetal immune compartment in response to maternal  
16 SARS-CoV-2 infection, we performed single-cell RNA sequencing (scRNA-Seq) on UCBMC. We  
17 identified 16 unique immune cell clusters (Figure 2A and Supplemental Figure 1A) that were  
18 annotated using established gene markers for adult PBMC (Figure 2B and Supplemental Table  
19 1). Within the lymphoid clusters, B cells were identified based on high expression of *MS4A1*,  
20 *CD79A*, and *IGHD*, while T cell subsets were defined based on the expression level of *CD3D*,  
21 *CD8B*, *IL7R*, and *CCR7* (Figure 2B). NK cell subsets were identified based on the high expression  
22 of *GZMA* and *NKG7* (Figure 2B). Monocyte clusters (classical, intermediate, and non-classical)  
23 were identified based on the expression of *CD14*, *HLA-DRA*, *S100A8*, *IL1B*, and *FCGR3*. Both  
24 subsets of DCs were identified – mDCs (expressing high *CD1C*) and pDCs (expressing high  
25 *IL3RA*) (Figure 2B). Additionally, stem cells (expressing *CD34*), proliferating cells (expressing

1 *MKI67*), and a cluster of contaminating erythroid cells (expressing *HBB*) were identified (Figure  
2 2B).

3 Despite the lack of differences in the total number of circulating lymphocytes (Figure 1C),  
4 maternal SARS-CoV-2 infection resulted in decreased frequencies of naïve CD4+ T cells and NK  
5 cells with high interferon signature (NK ISG) (Figure 2C). On the other hand, and in line with the  
6 increased numbers of circulating total monocytes measured by CBC (Figure 1C), the proportion  
7 of non-classical monocytes increased in the maternal SARS+ group (Figure 2C). We validated  
8 these observations using flow cytometry in a larger number of samples. This analysis confirmed  
9 the reduction of naïve CD4 T cells but also revealed a concomitant expansion of both effector and  
10 terminally differentiated effector memory (EM and TEMRA) CD4+ and CD8+ T cells (Figure 2D).  
11 Furthermore, expression of the activation marker KLRG1 was elevated in naïve and effector  
12 memory CD8+ T cells but not CD4+ T cells (Figure 2E), whereas expression of the proliferation  
13 marker Ki67 was increased in naïve CD4 and CD8 T cells as well as CM CD4 T cells in the  
14 maternal SARS+ group (Figure 2E). Similarly, a shift from naïve to unswitched memory B cell  
15 subsets was detected (Figure 2F). Finally, an expansion of immunoregulatory CD56<sup>bright</sup> NK cells,  
16 non-classical monocytes, and pDCs (Figure 2G- I) were observed in the maternal SARS+ group.  
17

### 18 ***Maternal SARS-CoV-2 infection results in aberrant activation of fetal lymphocytes.***

19 Given the observed shift toward memory for T and B cells, we used the scRNA-Seq data  
20 to interrogate gene expression patterns associated with lymphocyte activation. Within B cells,  
21 SARS-CoV-2 infection was associated with increased scores of cytokine signaling and cell  
22 migration modules (Supplemental Figure 1B and Supplemental Table 2). Differentially expressed  
23 genes (DEG) with maternal SARS-CoV-2 infection mapped to the regulation of protein kinase  
24 activity and immunoglobulin receptor binding gene ontology (GO) terms (Supplemental Figure  
25 1C) and include downregulated genes such as *FCRLA*, *MZB1*, *IGLC1/2/3*, *IGKC*, and *CD79B*  
26 (supplemental Figure 1D). Given the downregulation of these key genes, we next tested the

1 impact of maternal SARS-Cov-2 infection on functional B cell responses. Despite the increased  
2 frequency of memory subsets, B cells from the SARS+ group were less responsive to stimulation  
3 with TLR agonist cocktail <sup>45</sup>, indicated by lack of induction of CD40 and dampened expression of  
4 HLA-DR and CD83 (Supplemental Figure 1E).

5         Within CD8 T cell clusters, there was an increase in transcriptional signatures of cell  
6 migration, cytotoxicity, and cytokine signaling with maternal infection (Figure 3A and  
7 Supplemental Table 2). DEGs within the memory CD8+ T cell compartment were in line with  
8 increased potential for cytotoxicity (*IL32*, *GZMK*, *KLRC2*, *KLRD1*, *NKG7*), inflammation (*S100A4*,  
9 *S100A9*, *S100A10*), survival/differentiation of activated lymphocytes (*CD8A*, *CD27*, *CD3E*), and  
10 antiviral signaling (*IFITM1*) (Figure 3B). Within CD4+ T cell clusters, module scores associated  
11 with cell migration, cytokine signaling, Treg, and T<sub>H</sub>1 phenotype were increased in both naïve and  
12 EM subsets (Figure 3A and Supplemental Table 2). DEG analysis within naïve CD4 revealed  
13 increased transcript levels of genes associated with cell cycle (*CDK6*), consistent with elevated  
14 proliferation of naïve CD4 T cells in the maternal SARS+ group. On the other hand, EM CD4+ T  
15 cells had increased expression of genes associated with ATP synthesis and mitochondrial  
16 homeostasis (*ATP2B1*, *TSPO*), and T cell activation/signaling (*TNFRSF18*, *TRDC*, *TRAC*,  
17 *NFKBID*) (Figure 3B).

18         To interrogate the biological consequences of the changes in activation and transcriptional  
19 landscape, UCBMC from both groups were stimulated with anti-CD3/CD28 beads for 24 hours. T  
20 cells from controls generated a robust response as indicated by increased levels of canonical  
21 immune mediators (TNF $\alpha$ , sFASL, sCD137, IL-4, IL-5, IL-2, IL-13, IFN $\gamma$ , GZMB, GM-CSF) (Figure  
22 3C). On the other hand, T cells from the maternal SARS+ group responded poorly to polyclonal  
23 stimulation, indicated by the dampened secretion of both T<sub>H</sub>1 cytokines (IFN $\gamma$ , GM-CSF) and T<sub>H</sub>2  
24 cytokines (IL-5, IL-13), and cytotoxic (GZMB) mediators (Figure 3C). These data suggest that  
25 heightened maternal inflammation consequent to SARS-CoV-2 infection reprograms neonatal



1 lymphocytes leading to increased activation at baseline but their inability to respond to *ex vivo*  
2 stimulations.

3

#### 4 **Maternal SARS-CoV-2 infection enhances fetal NK cell activation.**

5 As described for T cells, maternal SARS-CoV-2 infection was associated with increased  
6 scores of modules associated with cytotoxicity, cytokine signaling, cell migration, anti-viral and  
7 bacterial pathogen responses, and inflammation in NK cell clusters (Figure 3D and Supplemental  
8 Table 2). Moreover, gene expression changes in NK cell cluster with maternal SARS-CoV-2  
9 infection enriched to GO terms associated with Fc-gamma receptor signaling, cytolysis,  
10 leukocyte-mediated cytotoxicity, regulation of NF- $\kappa$ B signaling, and viral responses (Figure 3E).  
11 This included increased expression of genes such as *GNLY*, *GZMH*, *IL32*, *IFNG*, *PRF1*, *IFITM1*,  
12 *IFI6*, *CCL5*, and *PYCARD* across the multiple NK cell subsets (Figure 3F). In line with these  
13 observations, an increase in the expression of degranulation marker CD107a by NK cells in  
14 response to PMA-ionomycin stimulation was observed in the maternal SARS+ group by flow  
15 cytometry (Figure 3G), suggesting increased NK cell activity. No significant differences were seen  
16 in the expression of MIP1 $\beta$ , IL-2, TNF $\alpha$ , or IFN $\gamma$  by NK cells in response to stimulation (data not  
17 shown).

18

#### 19 **Myeloid cells from babies born to mothers with asymptomatic/mild SARS-CoV-2 are hyper-** 20 **responsive to bacterial TLR ligands.**

21 Increased immune activation at baseline was also evident within monocytes as indicated  
22 by increased module scores for cytokine signaling in the *IL1B* and *S100A8* classical monocyte  
23 clusters (Figure 4A and Supplementary Table 2). Functional enrichment of DEG revealed an over-  
24 representation of GO terms associated with responses to cytokines and regulation of immune  
25 responses within the *IL1B* cluster (Figure 4B). While chemokine expression was increased in this  
26 subset, the expression of MHC class II molecules was reduced in the maternal SARS+ group, as

1 was the expression of several ISG (Figure 4C). These transcriptional patterns suggest a state of  
2 immune regulation in monocytes. To test this hypothesis, we assessed markers of monocyte  
3 activation using flow cytometry. While expression of CD16, TLR4, and CCR2 was increased in  
4 line with immune activation, the frequency of regulatory marker CD62L<sup>+</sup> increased while that of  
5 co-stimulatory molecules CD83 and CD86, chemokine receptor CCR7, M1-like marker TREM1,  
6 and CSF1R decreased on monocytes, indicative of immune regulation <sup>46</sup> (Figure 4D). To test this  
7 hypothesis, UCBMC were stimulated with RSV or *E. coli* overnight and secreted factors were  
8 measured using Luminex. While both groups responded to RSV, induction of RANTES, IL-12p70,  
9 GRO $\alpha$ , and Eotaxin was significantly attenuated in the maternal SARS+ group (Figure 4E and  
10 Table 3). In contrast, upon stimulation with *E. coli*, secreted levels of TNF $\alpha$  and IL1RA were  
11 significantly higher in the maternal SARS+ group (Figure 4F and Table 3). Collectively, these data  
12 suggest the rewiring of fetal monocytes towards a state of tolerance to viral antigens but enhanced  
13 responses to bacterial ligands.

14 Finally, within the stem cell cluster, module scores for cytokine signaling, cell migration,  
15 and mitosis were increased, suggesting an altered differentiation program (Supplemental Figure  
16 1I and Supplemental Table 2). Interestingly, differential gene expression analysis of the  
17 “proliferating cells” subset showed an over-representation of GO terms associated with  
18 inflammatory responses, wound healing, and regulation of viral processes (Supplemental Figure  
19 1J) with increased expression of *LYZ*, *CRIP1*, *CD52*, *LGALS1*, and *S100A8* suggesting that these  
20 cells may be myeloid in nature (Supplemental Figure 1K).

21

## 22 **Maternal SARS-CoV-2 infection is associated with increased frequency and activation of** 23 **fetal Hofbauer cells.**

24 Given the observed changes in circulating fetal immune cells and our recently described  
25 changes in decidual leukocytes with maternal SARS-CoV-2 infection <sup>12</sup>, we next interrogated the  
26 impact of maternal SARS-CoV-2 infection on the immune landscape of chorionic villi (fetal side of

1 the placenta). No viral RNA was detected in any of the villous tissue samples as measured by  
2 qPCR. Since immune cells in the villi are predominantly myeloid, we sorted CCR2+CD14+  
3 (monocytes and monocyte-derived macrophages) and CCR2-CD14+ (tissue-resident  
4 macrophages) from frozen villous leukocytes and performed scRNA-seq on multiplexed controls  
5 (n=8) and maternal SARS+ samples (n=6). Dimensionality reduction and clustering revealed 10  
6 unique cell clusters that contained cells from both groups (Figure 5A and Supplemental Figure  
7 2A). These clusters were annotated (Figure 5B and Supplemental Figure 2B) based on markers  
8 previously described for the first-trimester villous immune landscape<sup>42</sup>. HBCs were defined based  
9 on high levels of *FOLR2* and low levels of *HLA-DRA* with a proliferating HBC cluster also  
10 expressing high levels of *MKI67*. Placenta-associated maternal macrophages and monocytes  
11 (PAMM) clusters were identified based on the relative expression of *CD14*, *CCR2*, *CD9*, *HLA-*  
12 *DRA*, and *FOLR2*. In addition, other maternal infiltrating macrophages were detected and  
13 identified based on relative expression of *HLA-DRA*, *CCL4*, *APOE*, *IL1B*, *CCL20*, *CXCL10*, and  
14 *ISGs* (Figure 5B and Supplemental Figure 2B).

15 While maternal SARS-CoV-2 infection was associated with elevated frequencies of resting  
16 and proliferating HBC and PAMM-2 cells (infiltrating decidual macrophages), additional subsets  
17 of infiltrating maternal macrophages were decreased in the maternal SARS+ group (Figure 5C).  
18 The increased frequency of HBC was confirmed by flow cytometry (Figure 5D and Supplemental  
19 Figure 2C). Furthermore, module scores of gene signatures associated with cell migration,  
20 cytokine signaling, and apoptosis were elevated in HBC in the maternal SARS+ group (Figure 5E  
21 and Supplemental Table 2). Interestingly, DEG in both HBC subsets in the maternal SARS+ group  
22 mapped to pathways associated with inflammatory and cytokine responses (Figure 5F). These  
23 included both cytokines/chemokines (*CXCL8*, *CCL2*, *TNF*) and canonical transcription factors  
24 (*FOS*, *JUN*, *STAT3*, *NFKBIA*) associated with macrophage activation (Figure 5G). To test whether  
25 fetal Hofbauer cells were activated with maternal SARS-CoV-2 infection, we cultured purified HBC  
26 (CD14+FOLR2+HLA-DR-) for 16 hours and measured secreted levels of cytokines and

1 chemokines at baseline. Indeed, maternal SARS-CoV-2 infection was associated with increased  
2 secretion of immune factors associated with myeloid cell recruitment (MIP-3 $\alpha$ , MIP-3 $\beta$ ) and  
3 activation (GRO $\alpha$  and IL-1RA) (Figure 5H).

4

## 5 **Single-cell analysis of term placental villi reveals fetal macrophage adaptations to maternal** 6 **SARS-CoV-2 infection.**

7 Flow analyses of macrophage populations within placental villi revealed a decrease in the  
8 frequency of maternal macrophages (PAMM1b) in the maternal SARS+ group, while PAMM1a  
9 (maternal monocyte) frequencies remained unchanged (Figure 5D). However, both populations  
10 exhibited altered module scores for anti-viral and bacterial defenses, cell and cytokine signaling,  
11 cell migration, apoptosis, and inflammation (Supplemental Figure 2D and Supplemental Table 2).  
12 DEGs within the PAMM1a subset mapped to GO terms such as cell activation, cell  
13 death/apoptotic signaling, and vessel morphogenesis (Supplemental Figure 2E) and included an  
14 increase in the expression of *APOE*, *FN1*, *FCGR2B*, and *JUNB* (Supplemental Figure 2F). On  
15 the other hand, DEG in PAMM1b subset mapped to GO terms associated with immune activation,  
16 cytokine production, and immune effector processes (Supplemental Figure 2E). We observed up-  
17 regulation of *ATF4*, *CD55*, *EREG*, *FCN1*, *THBS1*, and class-I MHC molecules (*HLA-A*, *HLA-F*)  
18 and down-regulation of complement transcripts (*C1QA*, and *C1QB*) (Supplemental Figure 2F) in  
19 the maternal SARS+ group. Finally, while flow analyses revealed no differences in the proportion  
20 of PAMM2 cells (Figure 5D), maternal SARS-CoV-2 infection was associated with increased  
21 module scores for cell signaling, migration, and inflammation (Supplemental Figure 2D and  
22 Supplemental Table 2). Importantly, the maternal SARS+ group was associated with  
23 downregulation of *IL1B*, *HLA-DRA*, *S100A8/9*, *CXCR4*, *IFI30*, *TREM1/2* and upregulation of  
24 *C1QA*, *CCL2*, and *CSF1R* (Supplemental Figure 2F).

25 In addition to canonical macrophage populations residing in the placental chorionic villi,  
26 we identified additional clusters - a CXCL10<sup>high</sup> cluster, two maternal macrophage clusters, a

1 CCL10high monocyte cluster, and an antiviral macrophage cluster (Figure 5B and Supplemental  
2 Figure 2B). All infiltrating clusters had increased cell migration and cytokine signaling modules in  
3 the maternal SARS+ group (Supplemental Figure 3A and Supplementary Table 2). A consistent  
4 theme across these monocyte/macrophage subsets was the altered expression of genes involved  
5 in anti-microbial responses, inflammatory responses, and antigen processing and presentation  
6 (Supplemental Figure 3B, 3C, and Supplementary Table 2). Gene markers associated with  
7 immune activation were elevated in different myeloid subsets – neutrophil chemoattractant  
8 CXCL8 in infiltrating maternal macrophages, interferon-stimulated genes (IRF1, IFI6) in CCL20  
9 monocytes, and alarmins (S100A8, S100A9) in antiviral macrophage clusters. We, therefore,  
10 posit that an elevated baseline activation state might alter their functional responses to pathogens.  
11 We tested this hypothesis by purifying the CD14+ compartment from chorionic villi and stimulating  
12 them with viral and bacterial PAMPs. Our analysis of supernatants demonstrated significantly  
13 higher levels of pro-inflammatory IL-1 $\alpha$ , Flt-3L, and MCP-1 following viral TLR ligand stimulation  
14 but no differences in secreted cytokines in response to bacterial PAMPs (Supplemental Figure  
15 3D).  
16

## 1 DISCUSSION

2           The Developmental Origins of Health and Disease (DOHaD) hypothesis postulates that  
3 fetal exposure to environmental insults (such as poor nutrition, infection, chemicals, or hormonal  
4 perturbations) during critical periods of development and growth influences organ system  
5 development and susceptibility to diseases in later life <sup>47</sup>. Infectious diseases provoke the  
6 maternal immune system <sup>48</sup>, which in turn impacts the risk for disease risk in offspring <sup>49</sup>. Immune  
7 cell ontogeny in early life is particularly vulnerable to maternal infection, as shown by the higher  
8 mortality risk from infectious disease in HIV-exposed but uninfected infants <sup>50</sup> potentially due to  
9 altered neonatal Th17 and Treg immune balance <sup>51</sup>. Additionally, infection of placental HBCs by  
10 ZIKV leads to the production of type 1 interferons and pro-inflammatory cytokines and  
11 chemokines, eliciting placental inflammation, poor placental perfusion, and poor fetal outcomes  
12 <sup>52</sup>. Malaria in pregnancy is associated with dysregulation of placental development and preterm  
13 birth, with an increased risk of mortality due to complications such as acute respiratory illness and  
14 sepsis <sup>53</sup>. Similarly, SARS-CoV-2 also provokes maternal immune activation as indicated by  
15 increased levels of systemic immune mediators <sup>26, 54</sup>. While most studies of COVID-19 in  
16 pregnancy have focused on severe cases, there is growing evidence suggesting that mild  
17 maternal SARS-CoV-2 alters inflammatory responses at the MFI. Therefore, there is a critical  
18 need to understand the impact of mild/asymptomatic maternal SARS-CoV-2 infection on the  
19 immune landscape of fetal chorionic villous tissues and fetal circulation.

20           Studies included herein used UCB, a practical surrogate for newborn blood <sup>55, 56</sup>. Despite  
21 the lack of vertical transmission, levels of several chemokines and cytokines necessary for anti-  
22 microbial responses were reduced in UCB plasma in the maternal SARS+ group. These  
23 observations differ from data reported for non-gravid adult SARS-CoV-2 infection where levels of  
24 VEGF, GM-CSF, TNF $\alpha$ , IL-23, IL-4, IL-7, CXCL9, CCL2, CCL3, CCL4, and CCL11 are all elevated  
25 <sup>57-59</sup>. Our data also differ from those reported in a recent study where a lack of differences in  
26 concentration of many of these markers (except a modest increase in IFN $\alpha$ , in the SARS+ group)

1 were noted <sup>60</sup>. In contrast, S100B, IL-18, and PDGFBB were elevated in our study. These factors  
2 are linked to neurologic insults in newborns. S100B is a marker of neurological complications <sup>61</sup>  
3 while IL-18 and PDGF-BB levels are increased in circulation after traumatic spinal cord or brain  
4 injury to repair vascular dysfunction <sup>62-64</sup>. Indeed, maternal SARS-CoV-2 infection during  
5 pregnancy is linked to cases of perinatal brain injury and a greater rate of neurodevelopmental  
6 diagnoses in the first year of life <sup>65-67</sup>. Consistent with our findings, IL-18 secretion has been shown  
7 to be elevated in the circulation of neonates born to individuals with asymptomatic SARS-CoV-2  
8 infection and plays an important role in fetal cortical injury and adverse neurobehavioral outcomes  
9 <sup>28, 64, 68</sup>. These results indicate a generally immunosuppressive environment in UCB plasma in the  
10 maternal SARS+ group compared to controls, aside from elevated markers of brain injury  
11 consistent with neurodevelopmental issues reported in previous reports.

12 Here, we report an increased number of white blood cells characterized by an increased  
13 frequency of monocytes and granulocytes in UCB. These observations are in line with increased  
14 monocyte frequencies in adults with severe SARS-CoV-2 infection, driven by an expansion of  
15 intermediate and non-classical monocyte subsets <sup>69</sup> as well as infants younger than 1 year of age  
16 with mild COVID-19 <sup>70</sup>. We observed an expansion of non-classical monocytes measured by both  
17 flow cytometry and scRNA-Seq. Additionally, cytokine signaling pathways were activated in  
18 classical monocyte subsets, further reflected by increased expression of genes responsible for  
19 monocyte recruitment and cytokine signaling in the IL-1 $\beta$  classical monocyte subset. Interestingly,  
20 expression of HLA-DR and interferon-stimulated genes (ISG) was reduced in classical monocytes  
21 from the maternal SARS+ group. This is in contrast to earlier studies that reported upregulation  
22 of interferon-stimulated genes (ISG) and MHC genes in UCB monocytes with maternal SARS-  
23 CoV-2 infection <sup>71</sup>. These discrepancies in findings may be due to the emergence of the more  
24 severe delta SARS-CoV-2 variant during sample collection for this study that was not present  
25 when the prior study was completed <sup>71</sup>. Additionally, the timing of infection could potentially  
26 influence inflammation in UCB. For example, the prior study included pregnant participants that

1 were infected with SARS-CoV-2 exclusively in the third trimester, whereas this study included  
2 participants infected with the virus over the course of pregnancy <sup>71</sup>.

3 Dysregulated monocyte responses linked to COVID-19 pathogenesis and ensuing  
4 cytokine storm during infection in adults <sup>72</sup>. Our results show that frequencies of CD16+, TLR4+,  
5 and CCR2+ monocytes were increased in UCB in the maternal SARS+ group. We also noted  
6 increased expression of CD62L on UCB monocytes, consistent with reports of higher proportions  
7 of CD62L-positive monocytes in adults with COVID-19 <sup>73-75</sup>. On the other hand, decreased  
8 expression of co-stimulatory molecules CD83 and CD86 suggests a more regulatory monocyte  
9 phenotype. This hypothesis is further supported by the decreased expression of receptors  
10 important for recruiting DCs and activation of T cells (CCR7), amplification of inflammation  
11 (TREM1), and proliferation (CSF1R). Alterations in monocyte activation state may contribute to  
12 dysregulated antimicrobial responses. Indeed, UCB monocytes generated an increased response  
13 to stimulation with *E. coli*, in line with increased expression for the LPS receptor TLR4. However,  
14 monocyte responses to RSV were suppressed. We have previously shown the opposite trend  
15 with aged adults with COVID-19, where innate immune signaling was preferentially geared  
16 towards antiviral responses <sup>75</sup>, indicating that exposure to SARS-CoV-2 *in utero* has a distinct  
17 impact on innate immune responses compared to infection in later life. Minimal studies exist on  
18 the impact of maternal SARS-CoV-2 infection on newborn susceptibility to pathogens in early life.  
19 However, acute respiratory distress syndrome and pneumonia-like symptoms are more frequent  
20 in newborns of mothers with SARS-CoV-2 during pregnancy <sup>76</sup>.

21 A healthy newborn's adaptive immune system is primarily comprised of naïve lymphocytes  
22 with limited immune memory and effector function <sup>77, 78</sup>. However, our analysis of UCB revealed  
23 accelerated lymphocyte maturation indicated by the increased relative abundance of memory  
24 cells and proportionally less naïve T and B cells, increased expression of the proliferation marker  
25 Ki67, and effector marker KLRG1. Infectious exposures can broadly impact the developing T cell  
26 compartment and elicit pathogen-specific T-cell responses. For example, malaria-specific CD4+



1 T cell responses in UCB correlate with protection against malaria infection in early life <sup>79, 80</sup>,  
2 suggesting that priming of pathogen-specific CD4+ T cell responses *in utero* can confer protection  
3 later in life. Naïve T cells can also acquire memory T-cell markers and functional properties during  
4 cytokine-driven proliferation independent of antigen encounter <sup>81</sup>. Previous studies report that  
5 asymptomatic maternal SARS-CoV-2 infection resulted in dampened T<sub>H</sub>1 and T<sub>H</sub>17 responses  
6 and reduced T cell repertoire diversity that does not extend to neonatal circulation <sup>14</sup>. Here,  
7 stimulation of neonatal T cells from the maternal SARS+ group resulted in a dampened response  
8 to anti-CD3/CD28 stimulation. Poor T cell responses may result in impaired anti-microbial  
9 defenses. The mechanisms by which maternal SARS-CoV-2 infection dysregulates T cells in early  
10 life may be cell-intrinsic, as transcriptional analysis of the lymphocytes showed upregulation of  
11 mitosis, cytokine signaling, inflammation, and migration pathways. Future studies should address  
12 the molecular underpinnings (epigenetic and signaling) of these expanded yet functionally  
13 impaired fetal T cells and identify their unique phenotypes and antigen specificities.

14 Normally, early-life B cell responses to antigens are muted and exhibit distinct gene  
15 expression profiles with limited B cell activation compared to adults <sup>82</sup>. Other studies have shown  
16 that maternal SARS-CoV-2 infection in the third trimester has no significant impact on the  
17 frequency of CD19+ B cells <sup>83</sup>. However, the frequencies of memory B cell subsets and their  
18 functional capacity were not addressed. Our results show significant alterations in UCB humoral  
19 immunity in the maternal SARS+ group. Despite the increased frequency of memory subsets,  
20 UCB B cells from the maternal SARS+ group were less responsive to stimulation, as shown by  
21 decreased expression of co-stimulatory (CD40) and activation markers (CD83), indicating an  
22 early activation of UCB B cells in the SARS+ group.

23 Here, we report a decrease in the frequency of ISG-expressing NK cells but an expansion  
24 of cytokine-producing CD56<sup>Bright</sup> NK cells in UCB. These observations align with the increase in  
25 expression of genes responsible for type 2 interferon responses, cytolytic functions, and  
26 monocyte activation and recruitment. Moreover, UCB NK cells from the maternal SARS+ group

1 expressed higher levels of degranulation molecules, indicating the heightened cytotoxic potential  
2 of these cells. Our data align with other studies that reported a decrease in NK cell frequencies  
3 in adults with SARS-CoV-2 infection as well as in neonates of mothers with SARS-CoV-2 infection  
4 during pregnancy, but an activated phenotype <sup>84, 85</sup>. Increased activation of fetal NK cells is  
5 perhaps a compensatory mechanism against dampened T cell responses.

6 Maternal SARS-CoV-2 infection has been shown to compromise placental function as  
7 shown by the increased risk of pre-eclampsia <sup>86</sup>, abnormal placental histopathologic changes  
8 indicative of hypoxia <sup>87</sup>, and placental inflammation <sup>88-90</sup>. However, the impact of SARS-CoV-2  
9 infection on the immune landscape at the maternal-fetal interface has been relatively  
10 understudied. The human placenta is comprised of maternal (decidua) and fetal (chorionic villous)  
11 tissues, each with unique immune repertoires <sup>91</sup>. Studies evaluating how decidual leukocytes are  
12 altered by maternal SARS-CoV-2 infection during gestations are inconsistent. Early reports  
13 suggested that COVID-19 infection in the first trimester did not alter leukocyte frequencies within  
14 the decidua <sup>92</sup>. Other studies have demonstrated increased macrophages, NK cells, and T cells,  
15 accompanied by elevated expression of various cytokines (IL-6, IL-8, IL-10, TNF $\alpha$ ) with maternal  
16 SARS-CoV-2 infection in the first trimester <sup>92, 93</sup>. Our previous studies show significant  
17 perturbations induced by maternal SARS-CoV-2 infection in the decidua <sup>12, 38</sup>, including attenuated  
18 antigen presentation and viral signaling pathways, reduced frequencies of tissue-resident  
19 decidual macrophages, and upregulated cytokine/chemokine signaling in monocyte-derived  
20 decidual macrophages <sup>12, 38</sup>.

21 The maternal decidua is in direct and/or indirect contact with fetal membranes, placental  
22 villi, and maternal circulation. Therefore, perturbations in the maternal circulation and decidua  
23 tissues likely expand into fetal villous tissues and, thus, fetal circulation <sup>94, 95</sup>. The fetus-derived  
24 chorionic villous is comprised exclusively of macrophages, a major population being Hofbauer  
25 cells (HBC) that secrete factors important for placental angiogenesis and remodeling but also  
26 offer protection from bacterial pathogens<sup>42, 96</sup>. HBC also expand in numbers with adverse

1 pregnancy outcomes <sup>97-99</sup>. Our single cell and flow cytometry data revealed an increase in the  
2 frequency of HBCs in chorionic villi compared to uninfected negative controls, consistent with  
3 previous reports <sup>100</sup>. Furthermore, our analysis indicates increased activation of HBC in the  
4 maternal SARS+ group as suggested by the increase in the expression of genes associated with  
5 migration, cytokine signaling, and apoptosis in HBCs, as well as increased secretion of  
6 inflammatory chemoattractants (GRO $\alpha$ , MIP-3 $\alpha$ , MIP-3 $\beta$ ) and mediators of cytotoxicity (IL-1RA  
7 and GZMB). Given that HBs play a central role in pathogen sensing and host defense and the  
8 absence of active infection in the placenta, these findings suggest non-specific activation in  
9 response to inflammatory signals from the maternal compartment.

10 In addition to fetal HBC, the placental villi harbor additional maternal  
11 monocyte/macrophage subsets that assist in placental repair mechanisms and possibly the  
12 prevention of microbial transmission <sup>42, 96</sup>. Among these, are PAMM1a cells (maternal  
13 macrophages), which did not vary in numbers with maternal infection but exhibited transcriptional  
14 signatures associated with cell activation, cell death, and vessel morphogenesis. These  
15 observations suggest activation of the infiltrating maternal macrophages to repair possible  
16 placental structural damage caused by SARS-CoV-2. PAMM1b cells are less abundant in healthy  
17 villous tissues and are transcriptionally comparable to adult circulating classical monocytes <sup>42</sup>. In  
18 addition to their expansion with maternal SARS-CoV-2 infection, we observed a significant  
19 rewiring of their transcriptional states suggestive of enhanced activation and immune effector  
20 function. In this study, we also report five additional clusters of macrophages, arguably, new cell  
21 states of infiltrating maternal macrophages driven by chemokine expression (*CXCL10*, *CCL10*)  
22 that exhibited elevated expression of alarmins, ISGs, *NFKB1*, and MHC-I molecules.

23 Interestingly, frequencies of infiltrating decidual macrophage (PAMM2), the bona fide  
24 placenta-resident macrophages of maternal origin, did not vary with maternal SARS-CoV-2  
25 infection. This contrasts with their elevated frequencies in the decidual compartment, as  
26 previously described in SARS-CoV-2 infected mothers <sup>38</sup>. However, infection resulted in the

1 down-regulation of several genes involved in host defense and anti-viral immunity. This  
2 observation is in line with reports showing dampened expression of genes important for antiviral  
3 innate immunity (*IFNB*, *IFIT1*, *MXA*) and cytokine responses (*IL6*, *IL1B*) in chorionic villous tissues  
4 by qPCR regardless of gestational age during infection <sup>34, 101</sup>. Taken together, these findings  
5 suggest that maternal SARS-CoV-2 infection triggers opposing adaptations within different  
6 maternal myeloid cells residing in the fetal chorionic villi – with heightened activation and antiviral  
7 state in infiltrating maternal monocyte/macrophages and suppression of cytokine signaling and  
8 antigen-presentation pathways in rare infiltrating decidual macrophages. We argue that this  
9 enhanced antiviral state contributes to an augmented response to viral TLRs. Our findings  
10 suggest that fetal immune cells are not fully protected from inflammatory signals from  
11 mild/asymptomatic maternal SARS-CoV-2 infection. More importantly, this study highlights the  
12 unique functional adaptations within circulating and tissue-resident fetal myeloid and lymphoid  
13 cells in response to an ongoing/resolving maternal viral infection.

14

15

1 **AUTHOR CONTRIBUTIONS**

2 Conceptualization, S.S., N.E.M., and I.M.; methodology, S.S., N.E.M. and I.M.; investigation, B.D.,  
3 S.S., H.T., and N.M; writing, B.D., S.S., H.T., N.E.M, and I.M.; funding acquisition, N.E.M, and  
4 I.M.; participant enrollment, M.R. and N.E.M. All authors have read and approved the final draft  
5 of the manuscript.

6

7 **FUNDING**

8 This study was supported by grants from the National Institutes of Health 1K23HD06952 (NEM),  
9 1R01AI145910 (IM), R03AI11280 (IM), and 1R01AI142841 (IM).

10

11 **ACKNOWLEDGMENTS**

12 We are grateful to all participants in the study. We thank the MFM Research Unit at OHSU for  
13 sample collection and Allen Jankeel, Michael Z. Zulu, Gouri Ajith, Isaac Cinco, and Hannah  
14 Debray at UCI for assistance with tissue processing. We thank Dr. Jennifer Atwood at the UCIT  
15 Institute for Immunology Flow Cytometry Core for assistance with FACS sorting, and imaging flow  
16 cytometry, and Dr. Melanie Oakes at the UCI Genomics Research and Technology Hub (GRT  
17 Hub) for assistance with 10x library preparation and sequencing.

18

19 **COMPETING INTERESTS**

20 The authors declare that there is no conflict of interest regarding the publication of this article.

21

22

**1 Table 1: Description of cohort characteristics**

		Healthy	SARS	p value
# Enrolled		41	18	-
Maternal age at delivery (years)		33.65 ± 4.83	30.44 ± 6.68	0.0754
Pre-pregnancy BMI (kg/m <sup>2</sup> )		26.63 ± 6.02	29.03 ± 8.54	0.4213
Gestational age at delivery (weeks)		38.81 ± 1.97	37.46 ± 2.25	0.0335
Fetal sex (female %)		58%	65%	0.6399
COVID-19 Diagnosis	T1	-	16.7%	-
	T2	-	33.3%	-
	T3	-	5.6%	-
	Delivery	-	44.4%	-
EPT RBD [IgG]	Mild	-	8666	0.6451
	Asymptomatic	-	6717	
EPT NP [IgG]	Mild	-	2707	0.9495
	Asymptomatic	-	4098	
Mode of delivery (%)	Cesarean	67.4%	5.6%	<0.0001
	Vaginal	32.6%	88.9%	<0.0001
	Unknown	0.0%	5.6%	0.1071
Ethnicity (%)	Black	0.0%	5.6%	0.1071
	Hispanic	6.5%	27.8%	0.0208
	Caucasian (non-hispanic)	78.3%	55.6%	0.0693
	More than one race	4.3%	5.6%	0.8372
	Unknown/Declined to state	10.9%	5.6%	0.5120

2

3

- 1 **Table 2: Maternal-fetal Luminex dyads.** Concentration (pg/mL) of maternal and UCB plasma  
 2 immune mediators from controls and maternal SARS+ groups. (#=p<0.1, \*=p<0.05, \*\*=p<0.01,  
 3 \*\*\*=p<0.001, \*\*\*\*=p<0.0001)

	Maternal Plasma			UCB Plasma			Maternal:UCB SARS+
	Control	SARS+	p-value	Control	SARS+	p-value	p-value
TNF $\alpha$	2.321	2.136		4.527	2.502	**	
IL-6	0.3292	1.705		1.389	0.4739		
PDGFBB	1550	1967		1248	2358	*	
S100B	68.15	269.4	**	451.3	1102	**	*
IL-7	2.98	0.8747	***	3.03	1.339	***	*
IFN $\beta$	1.132	0.9987	*	0.7755	0.441	#	*
IL-10	0.5343	0.5599		0.5821	0.5832		
CCL2	56.71	49.64	**	361.5	37.5	**	
VEGF	3.075	7.255		85.75	42.11	**	**
CXCL13	71.21	115.9		27.68	25.76		*
IL-1RA	644.9	636		1771	2236		**
CCL3	26.52	26.91		30.44	27.64	**	
CCL4	58.41	60.32	#	104.9	65.71	*	#
IL-4	9.244	10.86	#	12.83	9.699	*	
IL-17	4.339	6.412	#	10.42	11.31		**
IL-2	3.565	3.735		4.534	3.49		
IL-15	0.8867	0.8545		0.9879	0.5705	*	*
GM-CSF	2.746	2.935		3.96	3.237	*	
IL-8	6.693	1.636	*	85.9	16.71	#	**
CXCL9	139.7	138.3		138.2	128.7	*	
IFN $\gamma$	7.096	7.247		13.6	10.21		
IL-12p70	20.69	21.27		22.61	22.91		#
IL-1 $\beta$	0.5779	1.411	*	5.837	4.098		*
CXCL11	66.2	29.16	**	44.88	16.48	#	*

CXCL10	13.71	6.819	**	10.56	4.097	**	
IL-23	118.7	63.23	**	165.9	100.7	***	**
CCL11	46.83	23.59	***	75.65	19.79	***	
IL-18	52.2	185.2	**	59.25	68.53	*	

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2



1 **Table 3: Antiviral and antimicrobial responses of UCB Monocytes.** Concentration (pg/mL) of  
 2 immune mediators from cell culture supernatant of cells from controls and maternal SARS+  
 3 groups stimulated with RSV or *E. coli*. (#=p<0.1, \*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001,  
 4 \*\*\*\*=p<0.0001)

RSV	Control			SARS+			RSV Control:SARS+
	NS	RSV	p-value	NS	RSV	p-value	p-value
VEGF	8.644	833	**	16.44	793.2	***	
TNF $\alpha$	178.9	169.7		29.82	57.4	#	#
RANTES	186.4	844.1	**	35.3	555.4	***	**
PD-L1	25.21	41.11	**	20.29	39.03	***	
PDGF-AB/BB	0.4893	7.286	**	0.08323	7.142	***	
PDGF-AA	51.88	936.7	**	50.96	938.2	***	
MIP-3 $\beta$	17.23	22.23	#	11.24	17.09	***	
MIP-1 $\beta$	698.4	932.9		181.4	600.5	*	
MCP-1	4245	5346		708.2	8343	**	
IP-10	0.4971	46.97	*	0.2228	100.1	***	
IL-6	333.9	54125	**	138.4	51648	***	
IL-1R $\alpha$	556.9	1010		533.5	1385	*	
IL-1 $\alpha$	25.5	67.27	#	2.928	63.65	***	
IL-15	0.7462	2.72	**	0.5025	2.551	***	
IL-12p70	7.992	13.44	*	6.334	11.59	***	**
IL-10	23.97	58.47	#	9.503	28.69	**	
IFN $\gamma$	30.71	32.25		26.32	29.74	*	
IFN $\beta$	0.4652	53.06	**	0.2315	51	***	
IFN $\alpha$	1.957	14.72	**	1.644	13.51	***	
GRO $\alpha$	452.5	921.5	#	46.99	326.4	**	**
GRO $\beta$	212	1104	**	32.27	847.6	***	
GM-CSF	59.65	88.57	#	10.1	48.09	**	#
G-CSF	79.49	135.5	*	3.994	26.05	***	*

Fractalkaline	90.66	140.6	*	70.89	127	***	
flt-3L	44.01	63.11	*	32.34	60.44	***	
FGF $\beta$	1.88	358.4	**	0.8137	348.3	***	
Eotaxin	5.595	12.95	*	2.704	10.27	***	*
EGF	6.887	9.028	*	6.446	8.342	***	
CD40L	785.6	992.3	#	627.7	871.7	***	

<i>E. coli</i>	Control			SARS+			<i>E. coli</i> Control:SARS+
	NS	<i>E. coli</i>	p-value	NS	<i>E. coli</i>	p-value	
TRAIL	2.534	9.728	**	0.6544	9.556	***	
TNF $\alpha$	178.9	6698	**	29.82	10915	***	#
TGF $\alpha$	2.587	7.336	*	1.968	7.375	***	
RANTES	186.4	157.7		35.3	82.93	*	
PD-L1	25.21	51.51	**	20.29	49.84	***	
PDGF-AB/BB	0.4893	4.055	**	0.08323	4.093	***	
MIP-3 $\beta$	17.23	9.119	*	11.24	9.028	*	
MIP-3 $\alpha$	3.852	85.88	**	2.903	53.48	***	
MIP-1 $\beta$	698.4	41542	**	181.4	44049	***	
MIP-1 $\alpha$	734	3252	**	190.5	3423	***	
IP-10	0.4971	4.463	**	0.2228	11.2	***	
IL-6	333.9	16161	**	138.4	15194	***	
IL-3	8.183	9.78	#	7.599	10.23	**	
IL-1R $\alpha$	556.9	659.5		533.5	1263	**	*
IL-1 $\beta$	75.76	1974	**	11.92	3538	***	
IL-1 $\alpha$	25.5	139.7	*	2.928	150.2	***	
IL-15	0.7462	2.48	**	0.5025	2.317	***	
IL-12p70	7.992	18.47	**	6.334	19.41	***	
IL-10	23.97	980.6	**	9.503	1413	***	
IFN $\gamma$	30.71	38.78	#	26.32	41.08	***	
IFN $\beta$	0.4652	1.881	**	0.2315	2.141	***	

IFN $\alpha$	1.957	5.337	**	1.644	5.187	***	
GZMB	28.72	26.92		18.3	24.45	*	
GRO $\alpha$	452.5	842.2		46.99	725.4	***	
GRO $\beta$	212	4996	**	32.27	5298	***	
GM-CSF	59.65	1334	**	10.1	2044	***	
Fractalkaline	90.66	245.5	**	70.89	247.3	***	
FLT-3L	44.01	84.86	**	32.34	82.96	***	
FGFb	1.88	10.46	**	0.8137	20.02	***	
Eotaxin	5.595	15.8	**	2.704	16.48	***	
EGF	6.887	10.43	**	6.446	10.57	***	
CD40L	785.6	1620	**	627.7	1606	***	

1

2 **Supplemental Table 1: Gene markers for human PBMC and villous leukocytes**

3

4 **Supplemental Table 2: Module scores for human PBMC and villous leukocytes**

5

6

1 **FIGURE LEGENDS**

2 **Figure 1: Maternal SARS Infection alters the frequency of circulating immune cells and**

3 **immune mediators.** (A) Maternal and umbilical cord blood (UCB) anti-RBD (left) and anti-NP

4 (right) endpoint titers (EPT) from SARS-CoV-2 infected mothers (M) and their offspring (B). All

5 samples are from mothers with a history of mild infection, except for the sample denoted by the

6 black circle, which was asymptomatic and tested positive at delivery. (B) Bubble plot comparing

7 UCB plasma immune mediators from control and maternal SARS+ group. Size represents analyte

8 concentration (pg/mL), whereas color represents statistical significance. (C) UCB complete blood

9 cell counts, including white blood cell (WBC) (top left), lymphocyte (top right), monocyte (bottom

10 left), and granulocyte (bottom right) proportions from control and maternal SARS+ groups. SVD

11 = standard vaginal delivery, CSN = cesarean section. (#=p<0.1, \*=p<0.05, \*\*\*=p<0.0001).

12

13 **Figure 2: Impact of maternal asymptomatic/mild SARS-CoV-2 infection on phenotype and**

14 **frequencies of cord blood immune cells.** (A) Uniform manifold approximation and projection

15 (UMAP) representation of 42,486 live immune cells from UCBMC of control and maternal SARS+

16 groups (N=4/group) showing 16 clusters. (B) Violin plots of marker genes used for cluster

17 identification. (C) Box and whiskers plot comparing cluster frequencies in control and maternal

18 SARS+ groups. (D) Stacked bar graphs of UCB CD4+ and CD8+ T cell subset frequencies in

19 control and maternal SARS+ groups by flow cytometry. (E) Bar graphs comparing KLRG1 and Ki-

20 67 expression within CD4+ and CD8+ T cells between control and maternal SARS+ groups. (F-I)

21 Stacked bar graphs comparing (F) B cell, (G) CD56bright/dim NK cell, (H) non-classical and

22 classical monocyte, and (I) Dendritic cell subsets between control and maternal SARS+ group.

23 (#=p<0.1, \*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001, \*\*\*\*=p<0.0001).

24

25 **Supplemental Figure 1: UCB Immune cell clusters by infection status and module scores.**

1 (A) UMAP of UCB immune cells colored by control (purple) and maternal SARS+ (blue) groups.  
2 (B) Bar graph comparing module scores within the B cell cluster for the terms indicated. (C)  
3 Bubble plot of functional enrichment of top genes within the B cell cluster. The bubble size  
4 represents the number of genes mapping to the term, whereas the color represents the level of  
5 statistical significance. (D) Violin plot of select statistically significant DEG within the B cell subset.  
6 (E) Bar graphs comparing B cell responses to stimulation with R848, ODN2216, and LPS (F) Bar  
7 graph of module scores within the stem cell cluster for the terms indicated. (G) Bubble plot of  
8 functional enrichment of top genes within the proliferating cell clusters. The bubble size represents  
9 the number of genes mapping to the term, whereas the color represents the level of statistical  
10 significance. (H) Violin plot comparing normalized transcript counts of statistically significant DEG  
11 within the proliferating cell subset. (#=p<0.1, \*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001).

12  
13 **Figure 3: The impact of maternal SARS-CoV-2 infection on fetal lymphocytes and NK**  
14 **cells.** (A) Heatmap of module scores within T cell clusters for the terms indicated. (B) Violin plot  
15 comparing normalized transcript counts of select statistically significant DEG within the indicated  
16 T cell cluster. (C) Bubble plot comparing secreted levels of immune mediators in cell culture  
17 supernatants following stimulation of UCBMC from control and maternal SARS+ groups with anti-  
18 CD3/CD28. The bubble size represents the analyte concentration (pg/mL), whereas the color  
19 represents the level of statistical significance compared to non-stimulated cells. Statistical  
20 significance between stimulated control and maternal SARS+ groups are indicated by plus signs  
21 (+=p<0.05, ++=p<0.01). (D) Heatmap of module scores within NK cell clusters for the terms  
22 indicated. (E) Bubble plot comparing functional enrichment of DEG relative to controls within ISG  
23 NK cell and NK cell clusters. The bubble size represents the number of genes mapping to the  
24 term, whereas the color represents the level of statistical significance. (F) Violin plot of select  
25 statistically significant DEG within the shown NK cell clusters. (G) Bar graph of total NK cell  
26 responses to PMA/ionomycin stimulation. (#=p<0.1, \*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001).

1  
2 **Figure 4: The impact of maternal SARS-CoV-2 infection on fetal myeloid cells.** (A) Violin plot  
3 of module scores within clusters of monocyte subsets associated with cytokine and chemokine  
4 signaling. (B) Bubble plot of functional enrichment of top genes within the IL-1B classical  
5 monocyte cluster. The bubble size represents the number of genes mapping to the term, whereas  
6 the color represents the level of statistical significance. (C) Violin plots of select statistically  
7 significant DEG within non-classical and IL-1B classical monocytes. (D) Bar graphs of significant  
8 differences in UCB monocyte activation phenotypes by maternal infection status. (E-F)  
9 Scatterplot comparing select immune mediators secreted in culture supernatants of UCBMC  
10 stimulated overnight with (E) RSV or (F) *E. coli* in control and maternal SARS+ groups. (#= $p < 0.1$ ,  
11 \*= $p < 0.05$ , \*\*= $p < 0.01$ , \*\*\*= $p < 0.001$ ).

12  
13 **Figure 5: Impact of maternal SARS-CoV-2 infection on immune cells in the villous**  
14 **compartment.** (A) Uniform Manifold Approximation and Projection (UMAP) of 48,553 immune  
15 cells within the villous compartment showing 10 clusters. (B) Violin plots of marker genes that  
16 were used for cluster annotation. (C) Box and whisker plots comparing relative cluster frequencies  
17 by infection status. (D) Bar graph comparing villous monocyte/macrophage subsets identified by  
18 flow cytometry within the live gate. (E) Bubble plot comparing module scores within HBC clusters  
19 for the terms indicated. The bubble size represents the average module score, whereas the color  
20 represents the level of statistical significance. (F) Barplot of GO terms identified in Metascape for  
21 DEG between controls and maternal SARS+ groups from the indicated cluster. The length of the  
22 bar indicates the number of genes associated with the term and the color intensity represents  
23 statistical significance. (G) Violin plots of select statistically significant DEG within the indicated  
24 cluster. (H) Bubble plot comparing secreted levels of immune factors by resting HBCs. The bubble  
25 size represents analyte concentration, whereas the color represents the level of statistical  
26 significance. (\*= $p < 0.05$ , \*\*= $p < 0.01$ , \*\*\*= $p < 0.001$ ).

1  
2 **Supplemental Figure 2: Impact of maternal SARS-CoV-2 infection on the immune**  
3 **landscape of the chorionic villous PAMMs.** (A) UMAP highlighting villous immune cells from  
4 control and maternal SARS+ groups. (B) Bubble plot of additional marker genes used for cluster  
5 identification. The bubble size represents the amount of gene expression, whereas color  
6 represents the average gene expression. (C) Gating strategy to identify villous immune cell  
7 subsets. (D) Bubble plot of module scores within PAMM clusters for the terms indicated. The  
8 bubble size represents the average module score, whereas the color represents the level of  
9 statistical significance. (E) Barplot of GO terms identified in Metascape for DEG between controls  
10 and maternal SARS+ groups from the indicated cluster. Length of the bar indicated the number  
11 of genes associated with the term and the color represents the level of statistical significance. (F)  
12 Violin plots of select statistically significant DEG within indicated clusters.

13  
14 **Supplemental Figure 3: Impact of maternal SARS-CoV-2 infection on the immune**  
15 **landscape of the infiltrating myeloid cells in chorionic villi.** (A) Bubble plot comparing module  
16 scores within infiltrating myeloid cell clusters for the terms indicated. The bubble size represents  
17 the average module score, whereas the color represents the level of statistical significance. (B)  
18 Barplot of GO terms identified in Metascape for DEG between controls and maternal SARS+  
19 groups from the indicated cluster. Length of the bar indicated the number of genes associated  
20 with the term and the color represents the level of statistical significance. (C) Violin plots of select  
21 statistically significant DEG within indicated clusters. (D) Bubble plot comparing immune  
22 mediators produced CD45+CD14+ macrophages in response to *E. coli* (top) or RSV stimulation.  
23 The bubble size represents analyte concentration, whereas the color represents the level of  
24 statistical significance.

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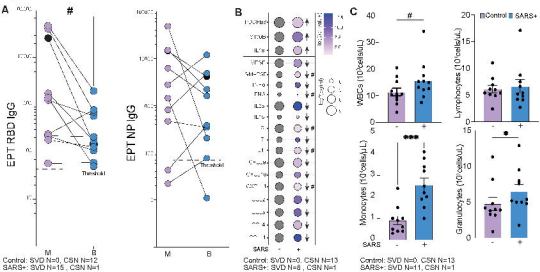
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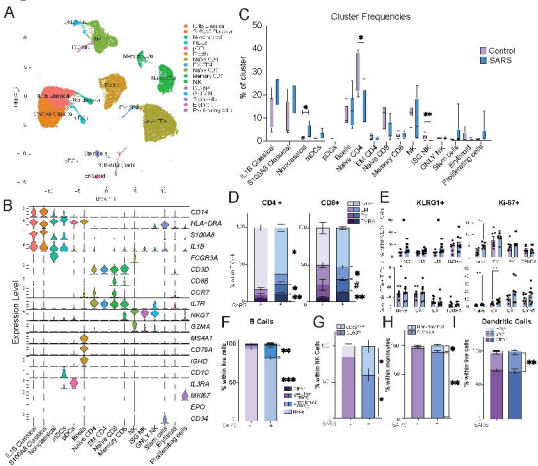
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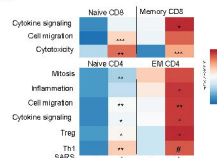
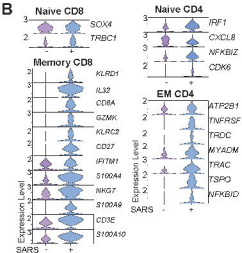
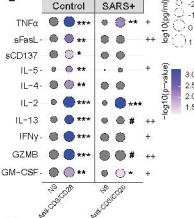
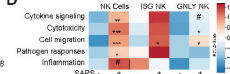
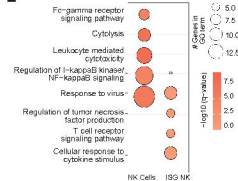
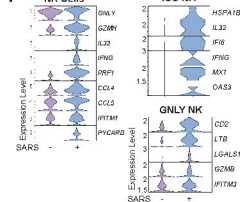
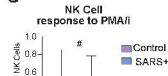
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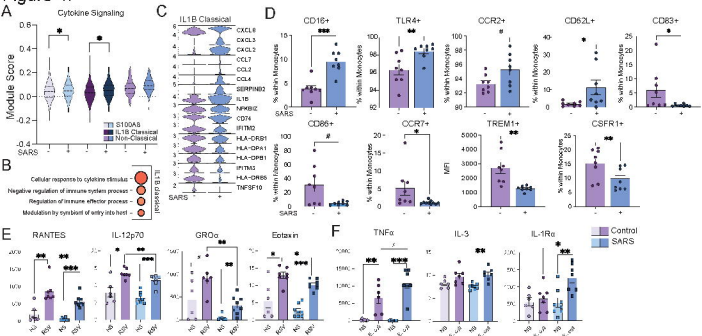
**Figure 1**

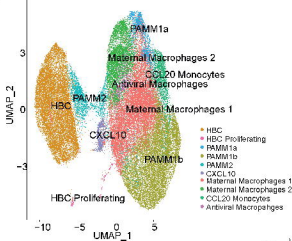
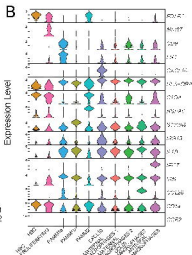
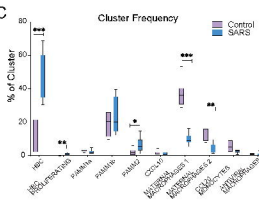
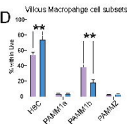
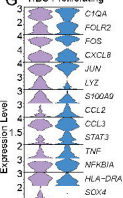
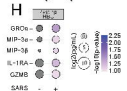
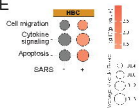
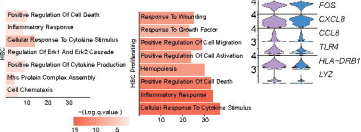
**Figure 2**



**Figure 3****A****B****C****D****E****F****G**

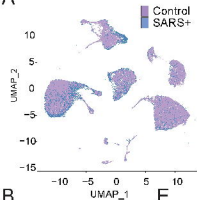
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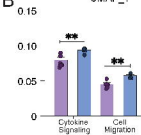
**Figure 5:**
**A**

**B**

**C**

**D**

**G** HBC Proliferating

**H**

**E**

**F**


# Supplemental Figure 1

## A



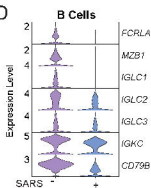
## B



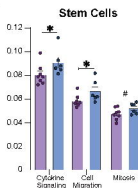
## C



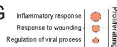
## D



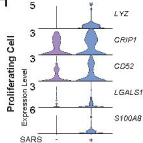
## F



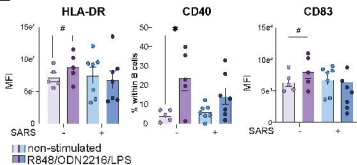
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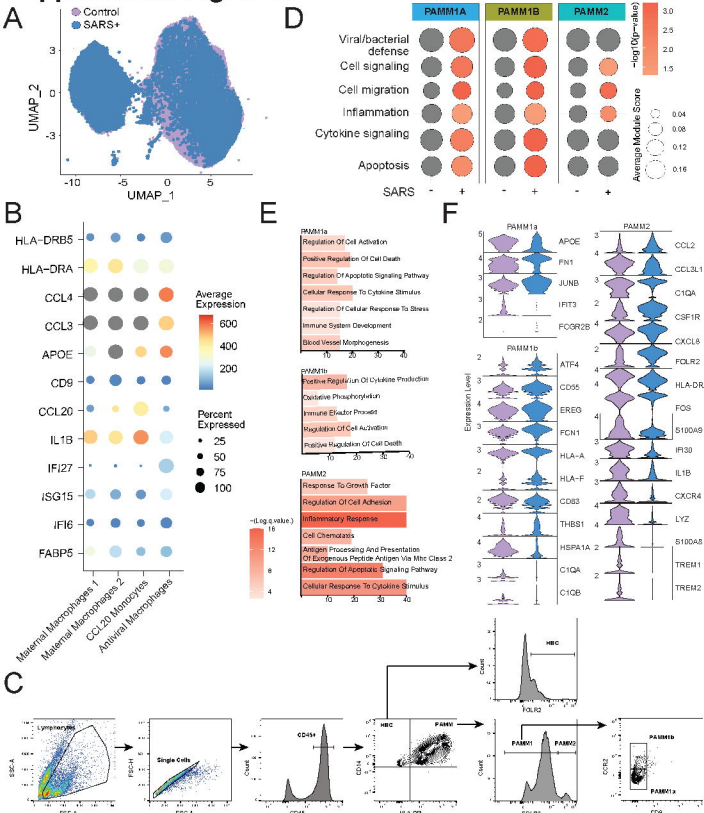
## H



## E

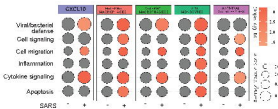


# Supplemental Figure 2:

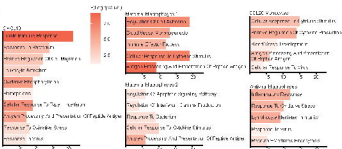


# Supplemental Figure 3:

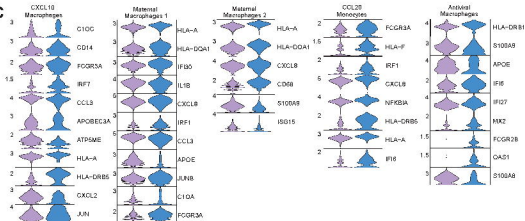
## A



## B



## C



## D

