

1 **SUPPLEMENTARY INFORMATION to**

2 **SARS-CoV-2 Niches in Human Placenta Revealed by Spatial Transcriptomics**

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18 **Figure S1.** Quality control metrics of placental spatial transcriptomes, related to Figure 2.

19 **Figure S2.** Visualization of viral transcription in placentae, related to Figures 2-5.

20 **Figure S3.** Annotation of macrophage polarization subpopulations, related to Figure 6.

21 **Figure S4.** Spatial resolution of fetal sex differences in gene expression responses to SARS-CoV-2, related to Figure 7.

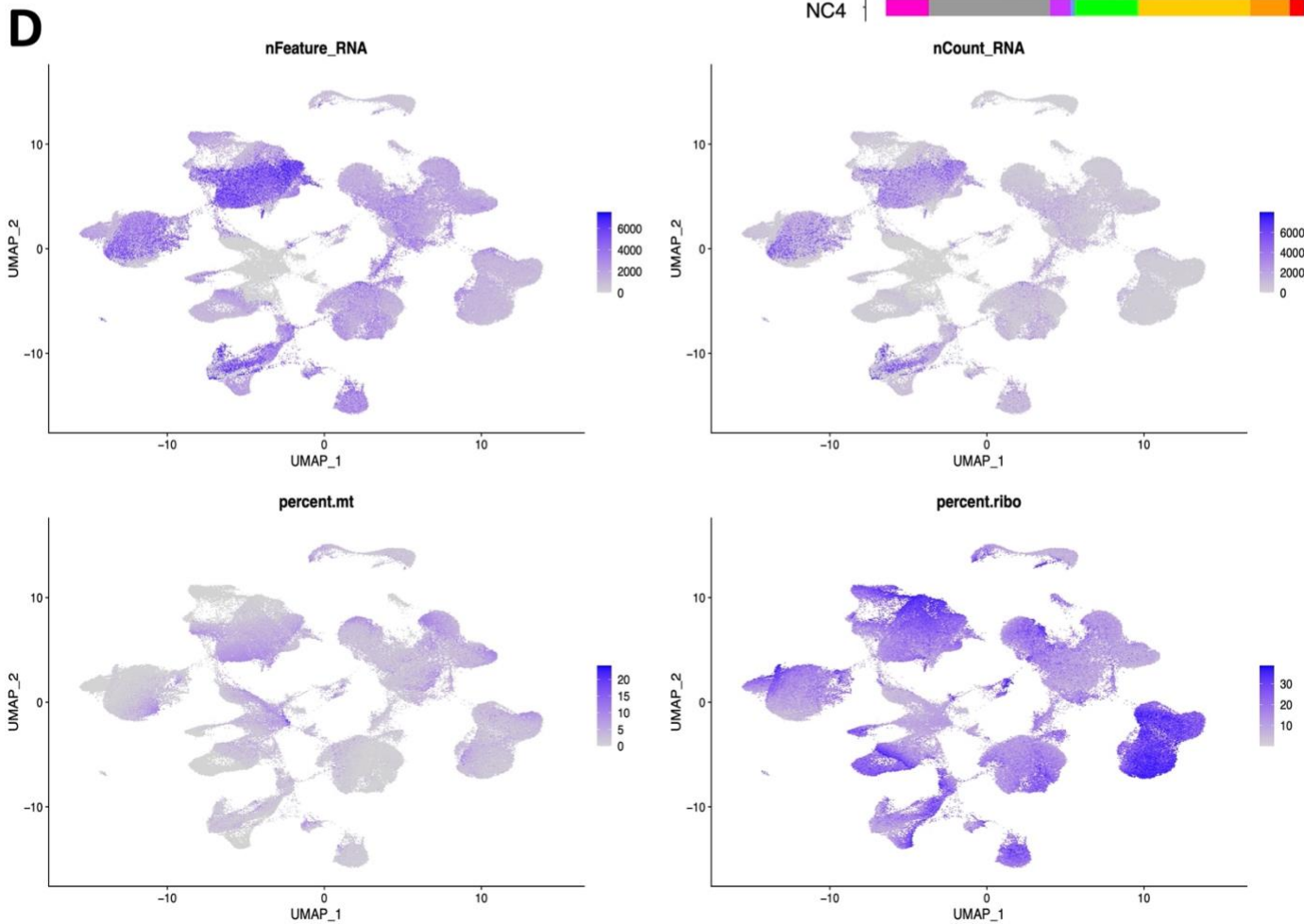
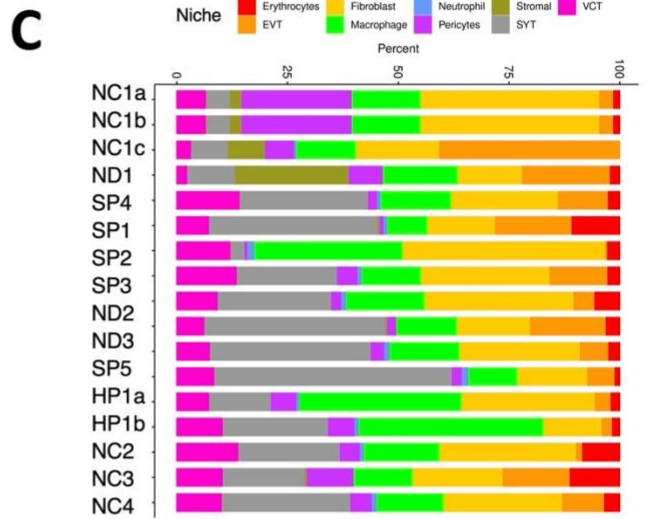
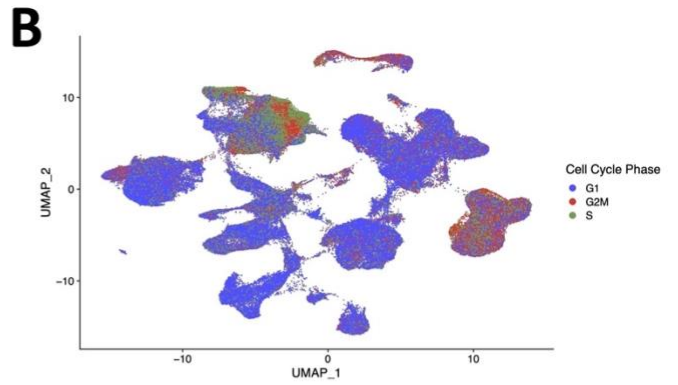
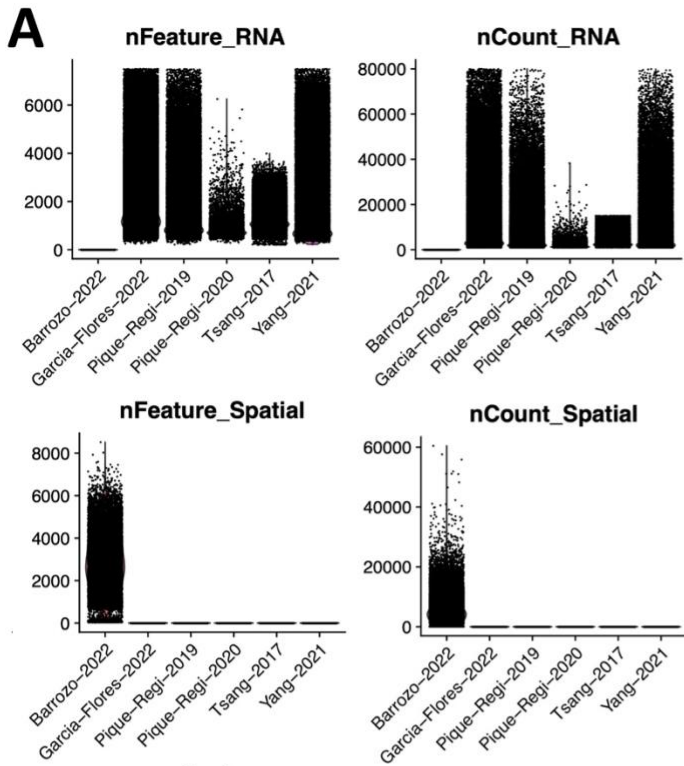
22 **Table S1.** Compiled differential expression tables from the spatial transcriptomics analyses, related to Figures 1-7.

23 Uploaded separately.

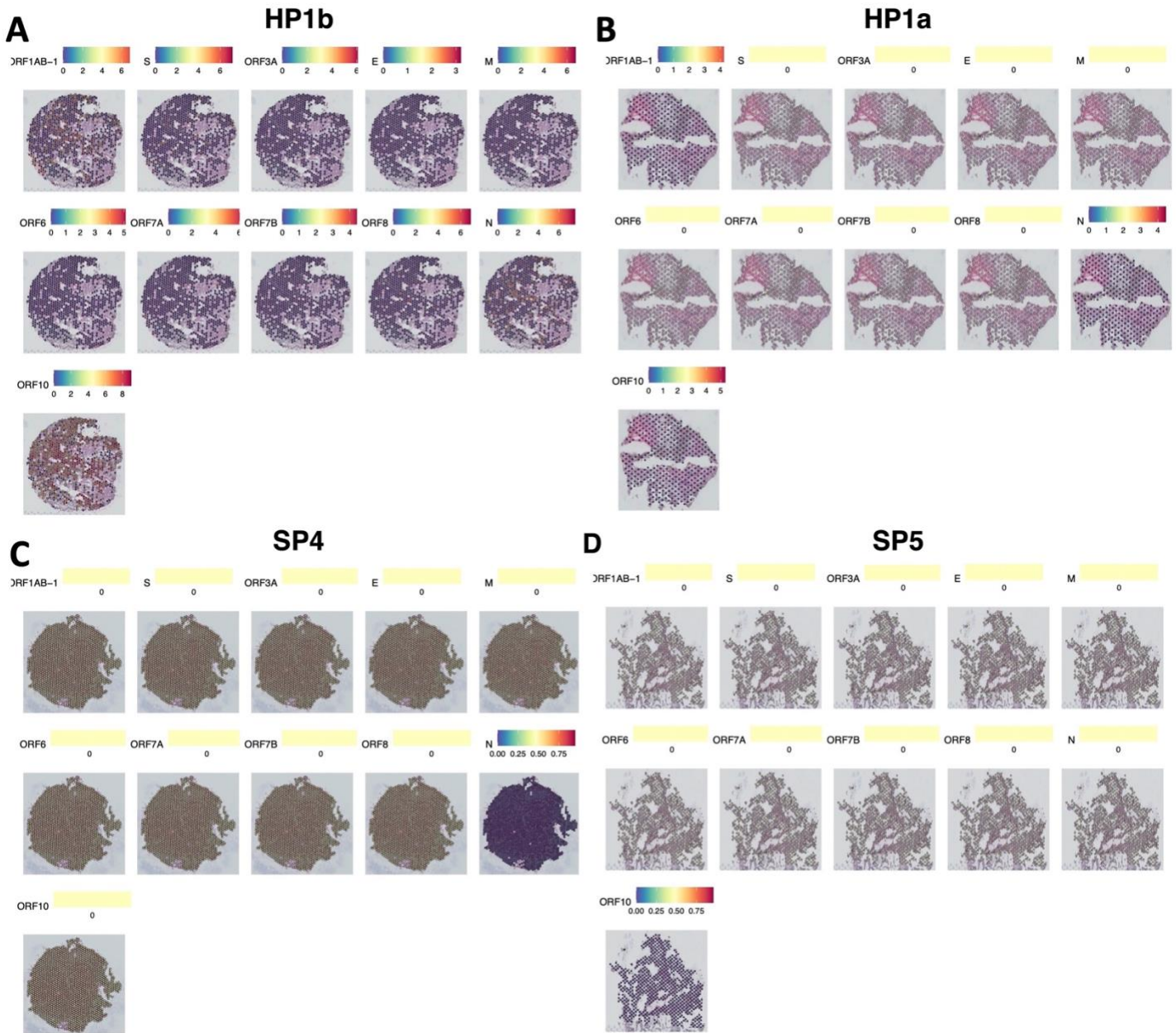
24 Table S2. **Study population characteristics.** Significance ($*P<0.05$) was determined by unpaired two-way T-tests.

25 Abbreviations: VBAC= vaginal birth after previous Cesarean section, NA=not applicable, ND= data not determined or not
26 reported, SEM= standard error of the mean).

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29 **Figure S1. Quality control metrics of placental spatial transcriptomes, related to Figure 2.** (A) Using standard quality
30 control metrics, 18,245 spatial transcriptomes were iteratively filtered for each individual library to remove outliers,
31 resulting in 17,927 high-quality transcriptomes being retained (98%). (B) Cell cycle phase assignments for the term
32 placenta atlas. (C) The proportion of placental niches per sample. (D) Feature plots of quality control metrics for the term
33 placenta atlas. Abbreviations: nFeature= number of unique transcripts, nCount= number of unique molecule identifiers
34 (UMIs), EVT= extravillous trophoblast, VCT= villous cytotrophoblast, SYT= syncytiotrophoblast, percent.mt= the
35 percentage of mitochondrial RNAs per transcriptome, and percent.ribo= the percentage of ribosomal RNAs per
36 transcriptome.



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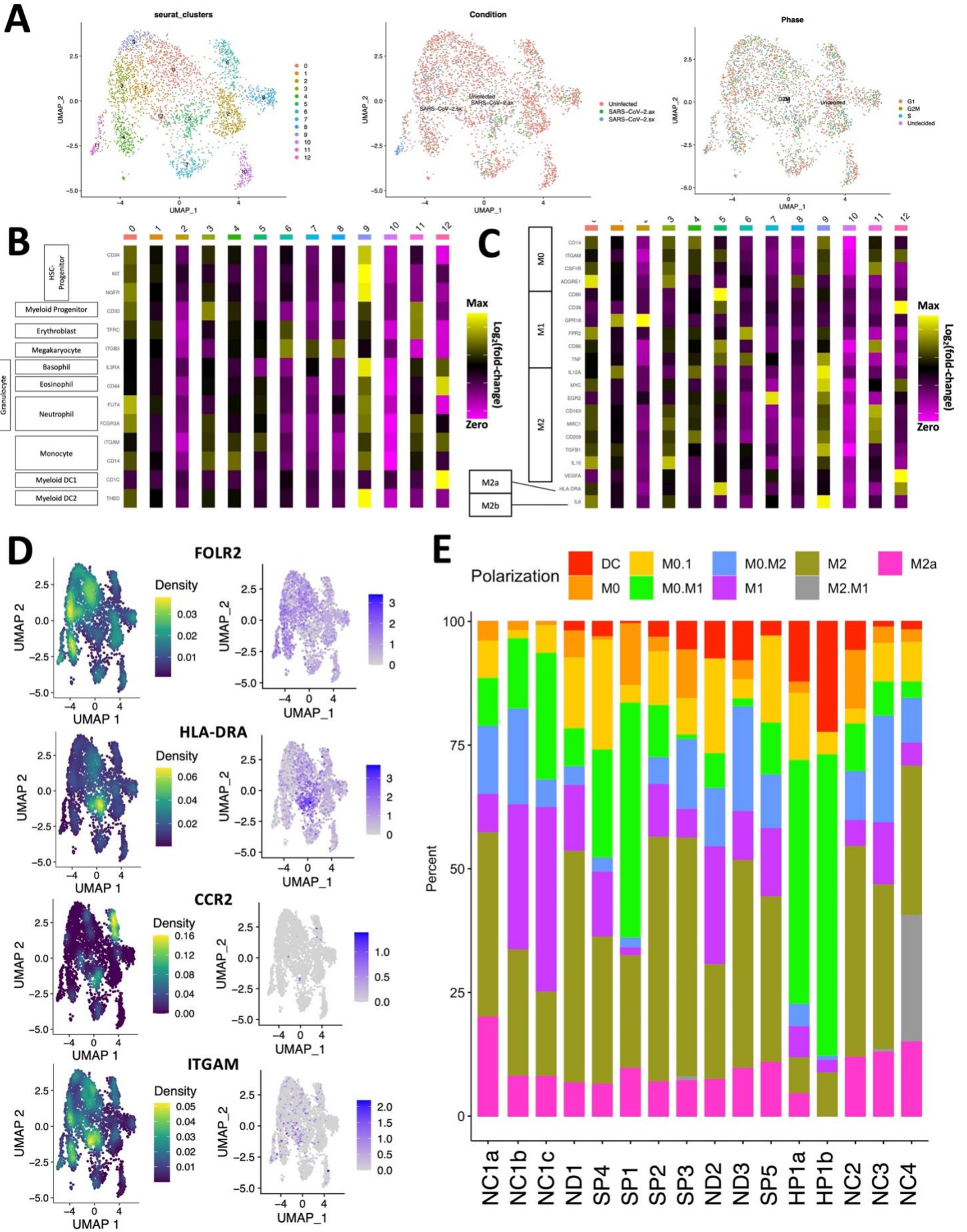
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Figure S2. Visualization of viral transcription in placentae, related to Figures 2-5. (A-D) SpatialDimPlots of SARS-CoV-2 transcripts for each placenta positive for SARS-CoV-2 by spatial transcriptomics. Note- a scale of 0 means the viral transcript was not detected in that sample and the scales for each transcript are modified to include the ranges of all datapoints by default. Abbreviations: HP= highly positive, and SP= sparsely positive.



44 **Figure S3. Annotation of macrophage polarization subpopulations, related to Figure 6.** (A) UMAPs of macrophage spatial
45 transcriptomes color annotated by cluster, condition, or cell-cycle phase. Sx and Ax refer to clinical maternal symptomatic
46 or asymptomatic SARS-CoV-2 infections; all subjects were confirmed as SARS-CoV-2 positive by nasopharyngeal PCR
47 testing. (B-C) Heatmaps with top markers for myeloid lineages (B) and macrophage polarization (C). (D) Density and
48 FeaturePlots visualizing expression of FOLR2 marking fetal macrophages (Hofbauer) and HLA expression marking placenta-
49 associated maternal macrophages (PAMMs). Expression of CCR2 and ITGAM expression mark PAMM1 and PAMM2,
50 respectively. (E) Cell counts of macrophage polarization subpopulations displayed as percentages relative to total
51 macrophages.

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A All SARS-CoV-2 Placentae Analyzed by Spatial Transcriptomics

B scRNA-seq

C Spatial Transcriptomics

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54 **Figure S4. Spatial resolution of SARS-CoV-2 differential transcripts by fetal sex (male, m; female, f), related to Figure 7.**

55 (A) Heatmap with the differential expression of previously identified SARS-CoV-2 genes reported to vary by fetal sex

56 stratification (Bordt, *et al.*, *Sci Transl Med.* (2021)) with the SARS-CoV-2 spatial transcriptomics data plotted by fetal sex

57 ($n=4$ male [m] and 5 female [f]). (B) Heatmap of the top 50 genes with expression differences relative to fetal sex in SARS-

58 CoV-2 scRNA-seq data (147,906 transcriptomes; $n=15$ female and 30 male; (Garcia-Flores *et al.* **Nat Comms.** (2022))

59 plotted by placental niche. (C) Heatmap of the top 50 genes with expression differences relative to fetal sex in SARS-CoV-

60 2 spatial transcriptomics data plotted by placental niche.

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62 **Supplementary Table 2. Study population characteristics, related to Figures 1-7.** Significance (* $P<0.05$) was determined by unpaired two-way T-tests.
 63 Abbreviations: VBAC= vaginal birth after previous Cesarean section, NA=not applicable, ND= data not determined or not reported, SEM= standard error of the
 64 mean.

Variable	Uninfected Control	Maternal SARS-CoV-2 Asymptomatic	Maternal SARS-CoV-2 Symptomatic	Control vs. SARS-CoV-2 <i>P</i> -value	Control vs. Asymptomatic <i>P</i> -value	Control vs. Symptomatic <i>P</i> -value	Asymptomatic vs. Symptomatic <i>P</i> -value
Maternal age, mean years (SEM)	28 (1.58)	27.33 (3.06)	26.8 (2.53)	0.922	0.856	0.952	0.917
Gravidity, mean (SEM)	4 (0.71)	3.5 (0.65)	2.2 (0.25)	0.342	0.620	0.101	0.216
Parity, mean (SEM)	2 (0.71)	1 (0.41)	0.83 (0.52)	0.296	0.267	0.206	1.000
Gestational age, mean weeks (SEM)	39.6 (0.34)	39.8 (0.49)	34.2 (2.81)	0.424	0.842	0.164	0.157
Birthweight, mean grams (SEM)	3361 (31.3)	3498 (179.2)	3228 (327.7)	0.973	0.724	0.770	0.523
Placental weight, mean grams (SEM)	495 (37)	509 (17.7)	412 (70.9)	0.681	0.550	0.318	0.192
Delivery Mode (%)				0.129	*0.030	0.109	0.356
Vaginal	3 (75)	0 (0)	3 (50)				
Cesarean section	1 (25)	3 (75)	3 (50)				
VBAC	0 (0)	1 (25)	0 (0)				
Ethnicity (%)				0.774	0.537	1.000	0.537
Non-Hispanic	3 (75)	2 (50)	4 (66.6)				
Hispanic	1 (25)	2 (50)	1 (20)				
Nondisclosed	0 (0)	0 (0)	1 (20)				
Fetal Sex (%)				0.48	0.537	0.356	0.798
Male	3 (75)	2 (50)	2 (33.3)				
Female	1 (25)	2 (50)	3 (50)				
Not disclosed			1 (16.6)				
COVID-19 Severity (%)				NA	NA	NA	*0.001
NA	4 (100)						
Asymptomatic		4 (100)	0 (0)				
Maternal pneumonia		0 (0)	4 (66.6)		NA	*0.035	*0.001
Maternal respiratory failure		0 (0)	1 (16.6)				
Intrauterine fetal demise		0 (0)	2 (33.3)				

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Days between onset of symptoms and delivery (SEM)	NA	0	7 (2.2)	NA	NA	NA	*0.026
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