

### Supporting Information

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Integrating Spatial and Single-Nucleus Transcriptomic Data to Assess the Effects of Intrauterine Hyperglycemia on Fetal Pancreatic Development

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Supporting Information

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(A-E) Random blood glucose levels of pregnant mice, body weights of pregnant mice and fetal mice, and live fetuses per litter at different developmental time points (E0.5, E12.5, E14.5, E16.5 and E18.5) between the PGDM and Con group. Data represented as the mean  $\pm$  SD. *P* values were calculated by t-tests. \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001 versus the Con group. Abbreviation: E0.5, embryonic day E0.5; E12.5, embryonic day E12.5; E14.5, embryonic day E14.5; E16.5, embryonic day E16.5; E18.5, embryonic day E18.5; ns, not significant.



Figure S2. The sample collection of single nuclei from the fetal pancreas (E16.5 and E18.5), related to Figure 1.

(A) Representative images of E16.5 fetuses and placentas from the PGDM and control (Con) group. Pancreatic tissues were stored in RNAlater, showing some degree of dehydration. (B) Distribution of 38237 single nucleus in each cluster from the fetal pancreas (E16.5). (C) Representative images of E18.5 conceptuses (upper panel), fetuses (lower panel), and placentas (lower panel) from the PGDM and control (Con) group. (D) Distribution of 21657 single nucleus in each cluster from the fetal pancreas (E18.5). (E) PCA of all samples used in this study. Twenty-one samples included in analysis were summarized in left panel. Abbreviation: PCA, principal component analysis.



Figure S3. Global single-nucleus transcriptome map of fetal pancreas (E16.5).

(A) Uniform manifold approximation and projection (UMAP) representation of the fetal pancreas (E16.5). (B)Bar graph showing the cluster composition of the PGDM and control (Con) groups at E16.5 in this study. (C)Dotplot showing the expression levels of specific marker genes in each cell cluster at E16.5.



#### Figure S4. The PAGA and pseudo-time trajectory analysis among acinar and beta subpopulations.

(A) PAGA trajectory analysis among acinar and beta subpopulations. Each node represented a cell partition (cluster) and thicker edges indicated stronger connectedness between clusters. (B-C) Pseudo-time trajectory analysis among acinar and beta subpopulations using Monocle2. Abbreviations: PAGA, partition-based graph abstraction.



Figure S5. Heatmap showing the metabolic activity analysis of all clusters by scMetabolism R package, revealing the heterogeneity of metabolic pathways in the fetal pancreas (E18.5).



Figure S6. Further analysis of the metabolism pathways among acinar\_1 cells, acinar\_2 cells, beta\_1 cells, and beta\_2 cells in the fetal pancreas (E18.5) by scMetabolism R package.

The statistical difference was compared by wilcox.test.



Figure S7. Comparison of the glycolysis/gluconeogenesis pathway in all clusters between PGDM (E18.5) and Con groups (E18.5).

The statistical difference was compared by wilcox.test.





(A) Violin plots showing expression levels of select up-regulated DEGs between PGDM and control (E18.5) groups across clusters. Clusters were indicated on the right side of the panel. The gene name was indicated below the violin plots. (B) GeneMANIA visualization of select up-regulated DEGs. PPI networks were generated using GeneMANIA (<u>https://genemania.org/</u>, accessed May 2024). Abbreviation: DEGs, differentially expressed genes; PPI, Protein-protein interaction.





(A-D) GO enrichment analysis of differentially upregulated genes ( $avg_log2FC > 1$  and  $P_val_adj < 0.05$ ) between PGDM and control (E18.5) groups across clusters (the acinar\_1 cell, acinar\_2 cell, beta\_1 cell and beta\_2 cell cluster). Bar plot (left panels) and cneplot (right panels) were used to visualize the GO terms.



## Figure S10. The kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of significantly upregulated genes between PGDM and control (E18.5) groups, related to Figure 2F.

(A-B) KEGG pathway analysis of differentially upregulated genes ( $avg_log2FC > 1$  and  $P_val_adj < 0.05$ ) between PGDM and control (E18.5) groups across clusters (beta\_1 cell and beta\_2 cell cluster). Bar plot (left panels) and cneplot (right panels) were used to visualize the KEGG pathway terms.



Figure S11. The GO enrichment analysis of significantly upregulated genes between PGDM and control (E16.5) groups, related to Figure 2F.

(A-C) GO enrichment analysis of differentially upregulated genes ( $avg_log2FC > 1$  and  $P_val_adj < 0.05$ ) between PGDM and control (E16.5) groups across clusters (the acinar\_1 cell, acinar\_2 cell and acinar\_3 cell cluster). Bar plot (left panels) and cneplot (right panels) were used to visualize the GO terms.



### Figure S12. The GO enrichment analysis of significantly upregulated genes between PGDM and control

#### (E16.5) groups, related to Figure 2F.

(A-B) GO enrichment analysis of differentially upregulated genes ( $avg_log2FC > 1$  and  $P_val_adj < 0.05$ ) between PGDM and control (E16.5) groups across clusters (the beta\_1 cell and beta\_2 cell cluster). Bar plot (left panels) and cneplot (right panels) were used to visualize the GO terms.



Figure S13. The KEGG pathway analysis of significantly upregulated genes between PGDM and control (E16.5) groups, related to Figure 2F.

(A-E) KEGG pathway analysis of differentially upregulated genes ( $avg_log2FC > 1$  and  $P_val_adj < 0.05$ ) between PGDM and control (E16.5) groups across clusters (the acinar\_1 cell, acinar\_2 cell, acinar\_3 cell, beta\_1 cell and beta\_2 cell cluster). Bar plot (left panels) and cneplot (right panels) were used to visualize the KEGG pathway terms.



#### Figure S14. Analysis of the cell type-specific regulons in the fetal pancreas (E18.5), related to Figure 3.

(A) Identification of cell type-specific regulons across clusters. Identified regulons were ranked based on the regulon specificity score (rss, y-axis). (B) SCENIC analysis revealed significant changes in the expression levels of regulons in the PGDM group within each cluster compared with the control group. (C) Feature plots depicting TF gene expression levels, including of *Pdx1*, *Nkx6.2*, *Mafb*, *Mafa*, and *Nkx6.1* between the PGDM and controls. Abbreviation: SCENIC, single-cell regulatory network inference and clustering; TF, transcription factor.





(A) The PCA of 12 samples used for proteomic analysis of the fetal pancreas tissue. (B-E) The KEGG pathway analysis of differentially expressed proteins (fold change cutoff = 1.2 and *P*-value < 0.05) between PGDM and control (E16.5 and E18.5) groups. The bar plot was used to visualize the KEGG pathway terms.



#### Figure S16. Metabolite analysis of plasma samples from 12 pregnant mice at E18.5.

(A) The PCA of 12 samples used for untargeted plasma metabolome. (B) OPLS-DA score plot of untargeted plasma metabolome. (C) The hierarchical clustering heatmap of the top 50 differential plasma metabolites of pregnant mice between PGDM and control (E18.5) groups. The differential metabolites were determined according to the VIP (VIP > 1) and *P*-value (*P*-value < 0.05, Student's t test). Abbreviation: OPLS-DA, orthogonal partial least-squared discriminant analysis; VIP, variable importance in projection.

Top 30 positive correlation of proteome and plasma metabolome



Figure S17. Correlation of differentially expressed proteins of fetal pancreas (E18.5) and differential plasma metabolites of pregnant mice (E18.5).



## Figure S18. Cell–cell interaction analysis between acinar cells and multiple cell types in the presence of maternal PGDM (E16.5), related to Figure 4G.

(A) CellChat analysis showed that the ligand-receptor pairing signaling network based on snRNA-seq data was enhanced in the PGDM group (E16.5) compared to the control group (E16.5). (B) Circle network plots, derived from snRNA-seq data (E16.5), displaying the enhanced signaling pathways in the PGDM group compared to the control group, including PARs, PTN, CDH, NOTCH, IGF, and SPP1 signaling pathway. Abbreviations: PARs, protease-activated receptors; PTN, pleiotrophin; CDH, cadherin; IGF, insulin-like growth factor; SPP1, secreted phosphoprotein 1.



## Figure S19. Cellular communication features between acinar cells and multiple cell types, related to Figure 4.

(A) ST data based on spots (bin 50,  $50 \times 50$  nanoballs aggregated) showing the gene expression level of *Irf3, Try5, Try4, Cela2a, Fau, Pard3, Prss2, Spink1, and Gm10334* in the PGDM (E16.5) and control (E16.5) group. (B) Comparison of the overall information flow of each signaling pathway between the PGDM (E18.5) and control (E18.5) group. Significantly enriched signaling pathways in the PGDM group were colored in red text, and signaling pathways with blue text were significantly enriched in the control group.



## Figure S20. Spatial distribution analysis of Try5, Try4 and Pard3 based on E16.5 ST data, related to Figure 4.

(A-B) The ST data based on spots (bin 20, 20 × 20 nanoballs aggregated) showing Try5 (red) and Try4 (red) were located in close proximity to the Pard3 (purple). Abbreviations: ST, spatial transcriptomics.



Figure S21. Spatial distribution analysis of Vegfa, Kdr, Dlk and Notch2 based on E16.5 ST data, related to Figure 5.

(A) The ST data based on spots (bin 20, 20 × 20 nanoballs aggregated) showing Vegfa (red) was located in close proximity to the Kdr (purple). (B) The ST data based on spots (bin 20, 20 × 20 nanoballs aggregated) showing Dlk (red) was located in close proximity to the Notch2 (purple).



#### Figure S22. Cell neighborhood analysis, related to Figure 6.

(A-D) Integration of spatial and single-nucleus transcriptome information in the PGDM group (A) and control group (B) at E16.5, and the PGDM group (C) and control group (D) at E18.5. Cell types in stereo-seq chips were annotated with snRNA-seq data by using "TransferData" function. The x-coordinate axis represented cell clusters identified by ST analysis and the y-coordinate axis represented cell clusters identified by snRNA-seq analysis.

| Table S1. Cluster annotations based on top differentially expressed genes in single | nucleus RNA |
|---|-------------|
| sequencing analysis (E18.5).  |             |

| Clusters (E18.5)           | Marker genes (E18.5)  |
|----------------------------|---|
| Acinar_1 cell              | <i>Rbpjl</i> <sup>[1]</sup> and <i>Selll</i> <sup>[2]</sup>                                     |
| Acinar_2 cell              | Cpb1 <sup>[3]</sup> , Amy2a1 <sup>[4]</sup> , and Pnliprp1 <sup>[5]</sup>                       |
| Alpha cell                 | Gcg <sup>[6]</sup> and $Nxph1$ <sup>[7]</sup>   |
| B cell                     | Blnk [8], Pou2af1 [9], and Slamf6 [10]  |
| Beta_1 cell                | <i>Trpm3</i> <sup>[11]</sup> , <i>Kcnip1</i> <sup>[12]</sup> , and <i>Robo2</i> <sup>[13]</sup> |
| Beta_2 cell                | Mlxipl <sup>[14]</sup> and $Pax6$ <sup>[15]</sup>   |
| Delta cell                 | Sst <sup>[6a]</sup>   |
| Ductal cell                | <i>Bicc1</i> $^{[16]}$ and <i>Erbb4</i> $^{[17]}$   |
| Fibroblast cell            | Sulf1 <sup>[18]</sup> and $Wt1$ <sup>[19]</sup>   |
| General endothelial cell   | <i>Vwf</i> and <i>Pecam1</i> <sup>[20]</sup>  |
| Lymphatic endothelial cell | <i>Flt4</i> <sup>[21]</sup> and <i>Mmrn1</i> <sup>[22]</sup>                                    |
| Macrophage cell            | <i>F13a1</i> <sup>[23]</sup> and <i>Mrc1</i> <sup>[24]</sup>                                    |
| Mesenchymal_1 cell         | Col3a1 <sup>[25]</sup> , Vcan <sup>[26]</sup> , and Adam12 <sup>[27]</sup>                      |
| Mesenchymal_2 cell         | Acta2, Actg2 <sup>[28]</sup> , and Col8a1 <sup>[29]</sup>                                       |
| Neuronal cell              | Nrg3 <sup>[30]</sup> , Syt1 <sup>[31]</sup> , and Nrxn1 <sup>[32]</sup>                         |
| T cell                     | <i>Il7r</i> <sup>[33]</sup> , <i>Il18r1</i> <sup>[34]</sup> , and <i>Itga4</i> <sup>[35]</sup>  |
| Vascular endothelial cell  | <i>Flt1</i> <sup>[36]</sup> and <i>Eng</i> <sup>[37]</sup>                                      |

 Table S2. Cluster annotations based on top differentially expressed genes in single nucleus RNA
 sequencing analysis (E16.5).

| Clusters (E16.5)   | Marker genes (E16.5)  |
|--------------------|---|
| Acinar_1 cell      | <i>Try5</i> <sup>[38]</sup> , <i>Try10</i> and <i>Prss1</i> <sup>[39]</sup> |
| Acinar_2 cell      | Rbpjl <sup>[1]</sup> and $Esrrg$ <sup>[40]</sup>                            |
| Acinar_3 cell      | Rap1gap <sup>[41]</sup>   |
| Alpha cell         | $Nxph1^{[7]}$ and $Wnk3^{[7]}$  |
| Beta_1 cell        | <i>Mlxipl</i> <sup>[14]</sup> and <i>Tmem163</i> <sup>[42]</sup>            |
| Beta_2 cell        | <i>Kcnb2</i> <sup>[43]</sup> and <i>Rfx6</i> <sup>[44]</sup>                |
| Delta cell         | <i>Sst</i> <sup>[6a]</sup>  |
| Ductal cell        | Bicc1 $^{[16]}$ and Pkhd1 $^{[45]}$   |
| Endothelial cell   | Flt1 $^{[36]}$ , Pecam1 $^{[20]}$ , Ptprm $^{[20]}$ and Eng $^{[46]}$       |
| Fibroblast cell    | Sulf1 $^{[18]}$ , Wt1 $^{[19]}$ and Bnc2 $^{[47]}$                          |
| Immune cell        | F13a1 <sup>[48]</sup> and $Ptprc$ <sup>[49]</sup>                           |
| Mesenchymal_1 cell | <i>Slit2</i> <sup>[50]</sup> and <i>Epha3</i> <sup>[51]</sup>               |
| Mesenchymal_2 cell | <i>Tshz2</i> <sup>[52]</sup> and <i>Pde3a</i> <sup>[53]</sup>               |
| Neuronal cell      | Nrg3 <sup>[30]</sup> , Syt1 <sup>[31]</sup> , and Nrxn1 <sup>[32]</sup>     |
| Proliferating cell | <i>Top2a</i> <sup>[54]</sup> and <i>Cenpp</i> <sup>[55]</sup>               |
| Schwann cell       | Slc35f1 [56], Csmd1 [56] and Cdh19 [25]                                     |

| Sample name                   | Estimated<br>number<br>of cells | Mean<br>reads<br>per cell | Median genes<br>per cell | s Number of<br>reads | Total<br>genes<br>detected | Median UMI<br>counts per<br>cell |
|-------------------------------|---------------------------------|---------------------------|--------------------------|----------------------|----------------------------|----------------------------------|
| PGDM-(NO.1-3)<br>(E16.5)      | 13832                           | 30059                     | 1213                     | 415,774,567          | 22232                      | 1880                             |
| Control-(NO.4-5)<br>(E16.5)   | 12117                           | 31629                     | 1314                     | 383,248,596          | 21750                      | 2051                             |
| Control-(NO.6-7)<br>(E16.5)   | 13687                           | 27114                     | 1548                     | 371,112,138          | 21738                      | 2508                             |
| PGDM-(NO.8-9)<br>(E18.5)      | 4515                            | 78978                     | 648                      | 356,584,488          | 21085                      | 1077                             |
| PGDM-(NO.10-11)<br>(E18.5)    | 6056                            | 62983                     | 724                      | 381,423,828          | 22688                      | 1310                             |
| Control-(NO.12-13)<br>(E18.5) | 4931                            | 64906                     | 807                      | 320,053,230          | 22995                      | 1180                             |
| Control-(NO.14-15)<br>(E18.5) | 7889                            | 53477                     | 838                      | 421,881,113          | 23633                      | 1265                             |

Table S3. The data quality of single nucleus RNA sequencing.

#### Table S4. The data quality of stereo-seq chips.

| Sample name                | Total reads   | Unique reads | Clean<br>reads | Genes<br>under<br>tissue | Mean<br>MID<br>per<br>bin200 | Mean<br>gene<br>type per<br>bin200 | Mean<br>MID<br>per<br>bin50 | Mean gene<br>type per<br>bin50 |
|----------------------------|---------------|--------------|----------------|--------------------------|------------------------------|------------------------------------|-----------------------------|--------------------------------|
| PGDM-(NO.16)<br>(E16.5)    | 432,303,946   | 67,947,252   | 82.6%          | 21632                    | 39142                        | 6701                               | 3087                        | 1498                           |
| Control-(NO.17)<br>(E16.5) | 1,383,198,944 | 49,025,248   | 88.9%          | 21149                    | 36358                        | 4673                               | 2880                        | 967                            |
| PGDM-(NO.18)<br>(E18.5)    | 272,821,647   | 13,054,552   | 78.3%          | 26276                    | 9998                         | 2078                               | 769                         | 277                            |
| PGDM-(NO.19)<br>(E18.5)    | 1,148,147,467 | 11,711,868   | 81.7%          | 21780                    | 12857                        | 1636                               | 1031                        | 233                            |

| Control-(NO.20)<br>(E18.5) | 262,443,323   | 9,220,156  | 66.4% | 27782 | 4958 | 2163 | 412 | 243 |
|----------------------------|---------------|------------|-------|-------|------|------|-----|-----|
| Control-(NO.21)<br>(E18.5) | 1,078,043,868 | 18,045,573 | 82.7% | 25535 | 8612 | 1699 | 684 | 224 |

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