Bariatric Surgery Induces Pancreatic Cell Transdifferentiation as Indicated by Single-Cell Transcriptomics in Zucker Diabetic Rats

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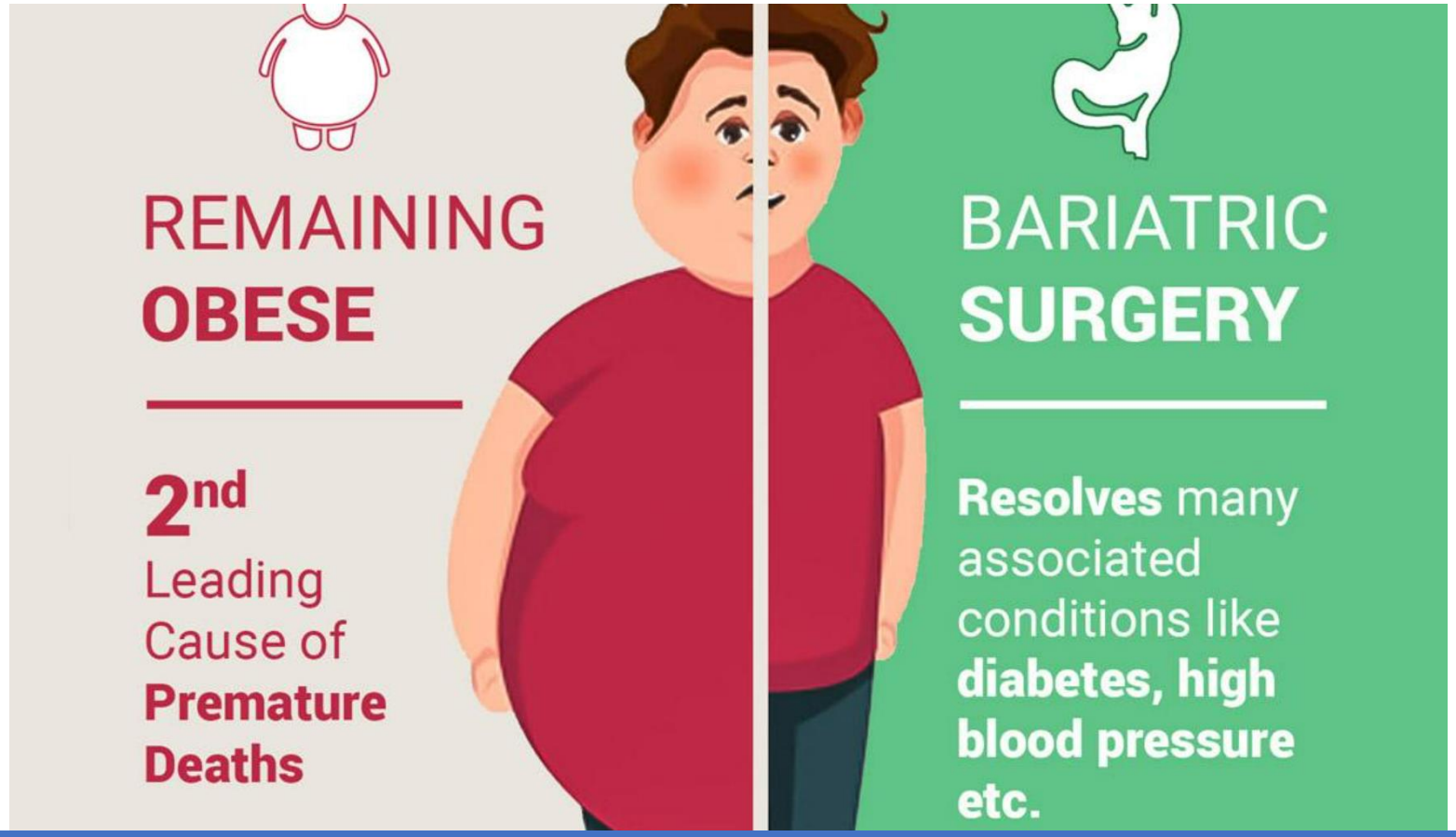
CONFLICT OF INTEREST DISCLOSURE

No Disclosure

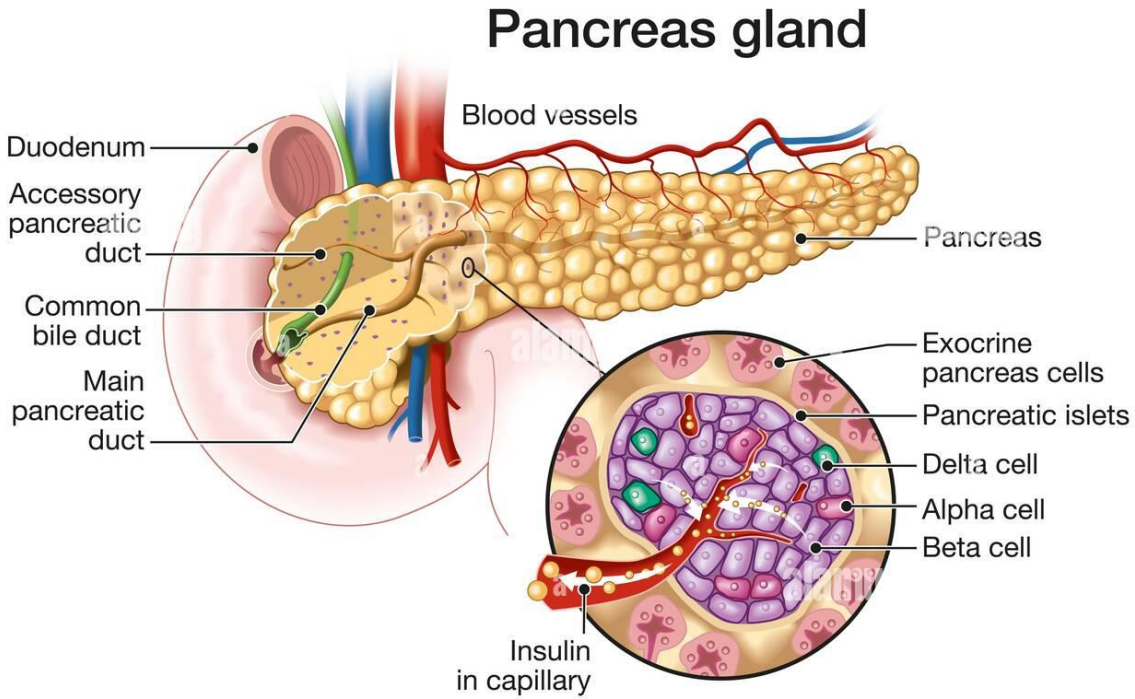
Bariatric surgery results in rapid recovery of glucose control in the subjects with type 2 diabetes mellitus (T2DM)



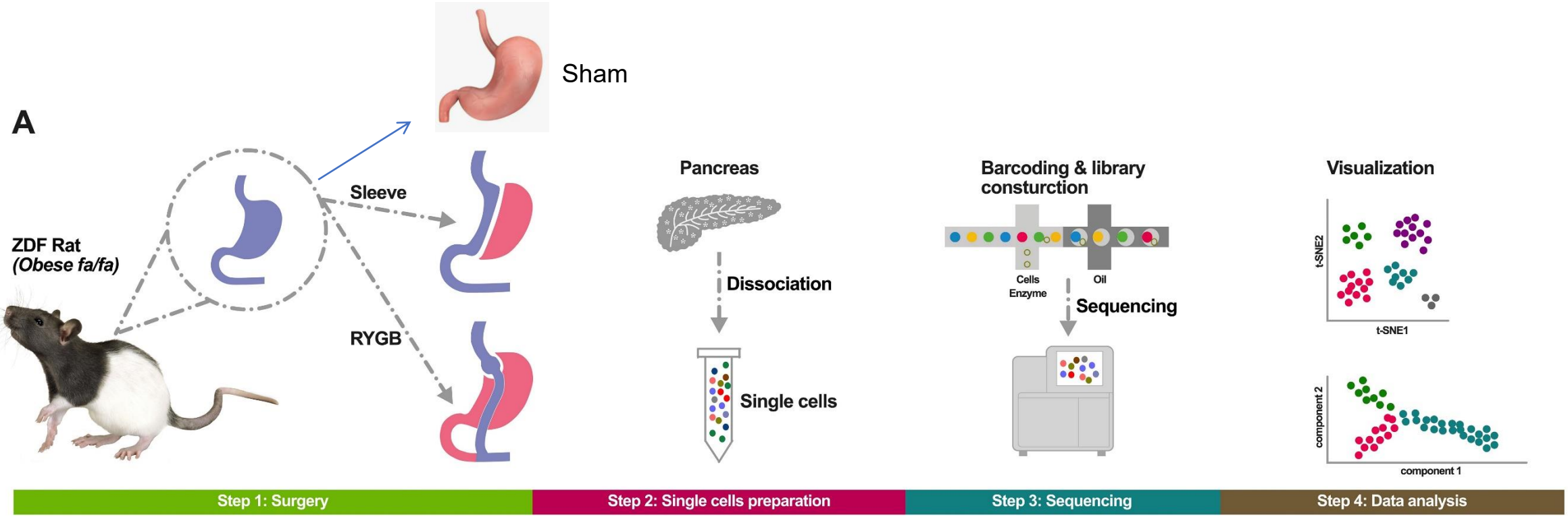
The underlying mechanisms are still largely unknown

Aim

- To clarify how bariatric surgery modifies pancreatic cell subgroup differentiation and transformation



Workflow of the study



8-week-old, male, Zucker Diabetic Fatty (ZDF) rats with obese and diabetic phenotypes

Two weeks after surgery

Single-cell RNA-sequencing (scRNA-seq)

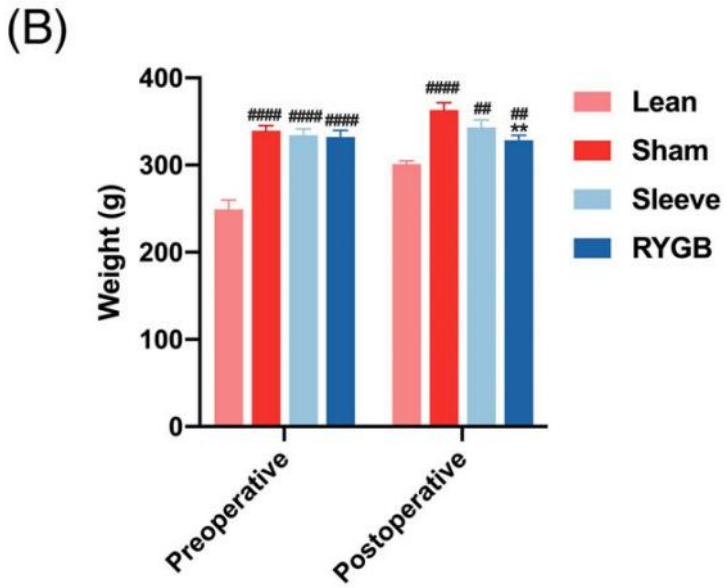
Methods

- Diabetes phenotype identification of ZDF rats and surgical procedures
- Rat pancreas procurement for islet isolation and single-cell preparation
- Single-cell RNA library preparation and sequencing
- Single-cell RNA-seq data analysis
- Pseudo time trajectory with monocle
- SCENIC analysis
- Cell–cell communication analysis

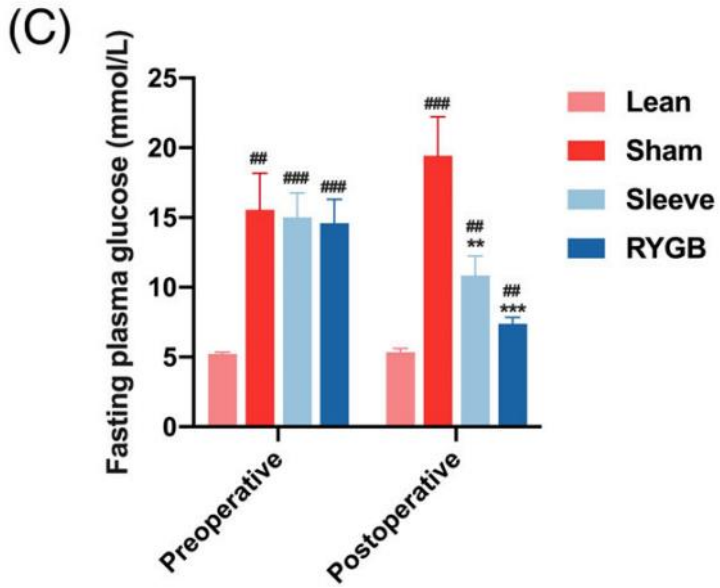
Study 1

Bariatric surgery effects on weight loss an DM control

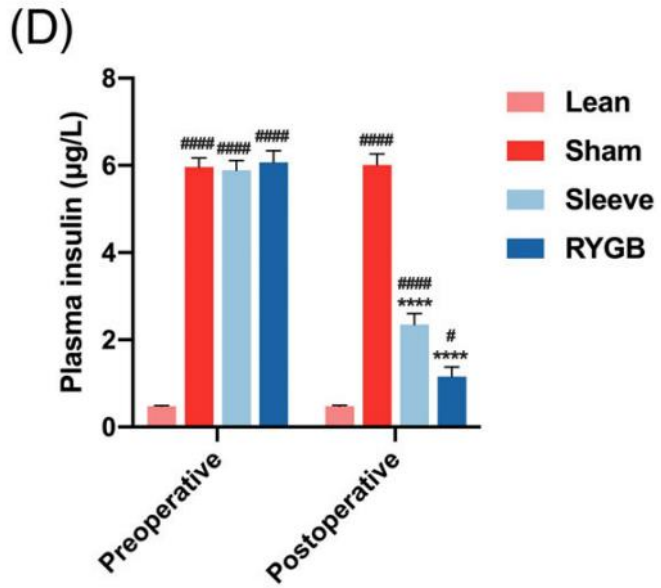
2 weeks Post-Op



Weigh



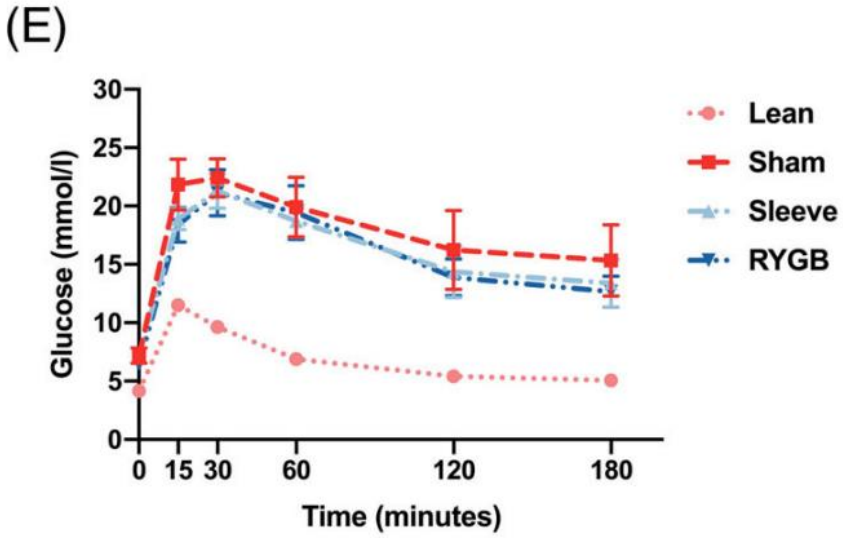
FBG



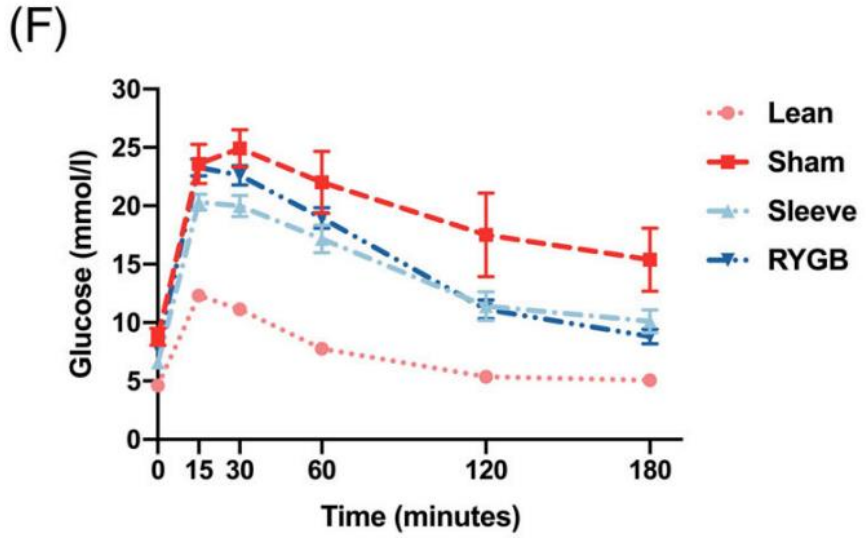
plasma insulin

Result 1

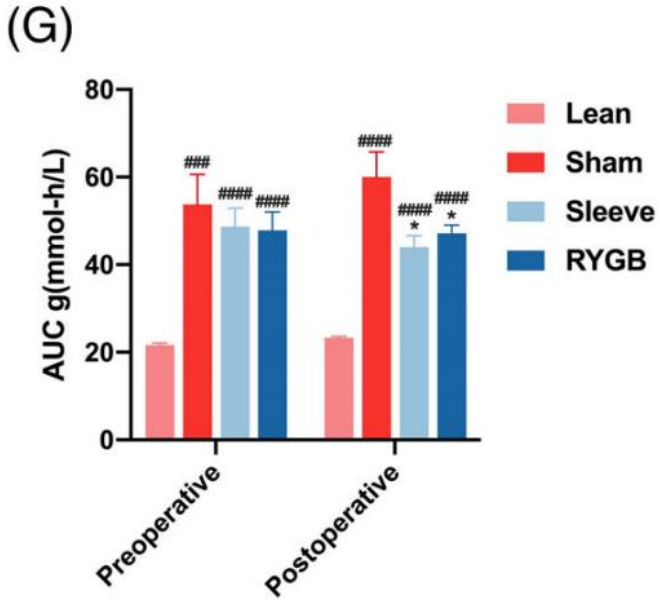
Surgical Outcomes：
Metabolic surgery ameliorates diabetes phenotypes



OGTT before surgery



OGTT after surgery



Area under curve (AUC) of OGTT glucose excursion

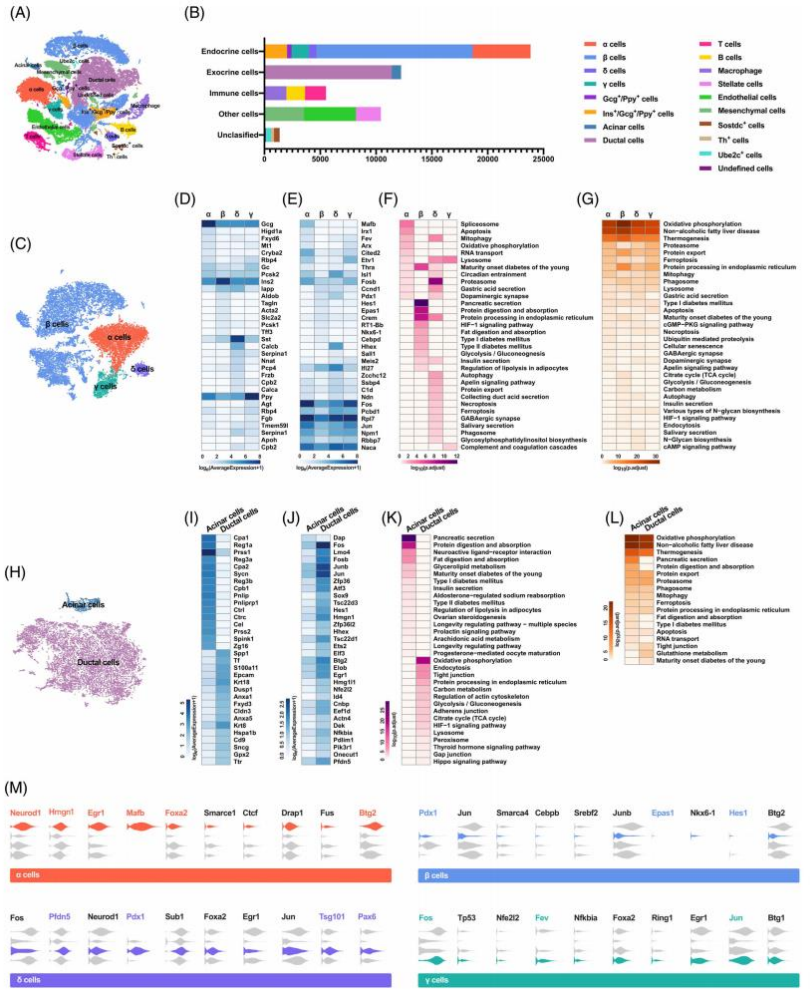
Study 2

Single-cell transcriptome analyses of rat pancreas

- t-SNE （t-distributed stochastic neighbor embedding） map：Analyzing gene expression variation of the all-filtered cells , with t-SNE the single-cell transcriptome data was visualized)

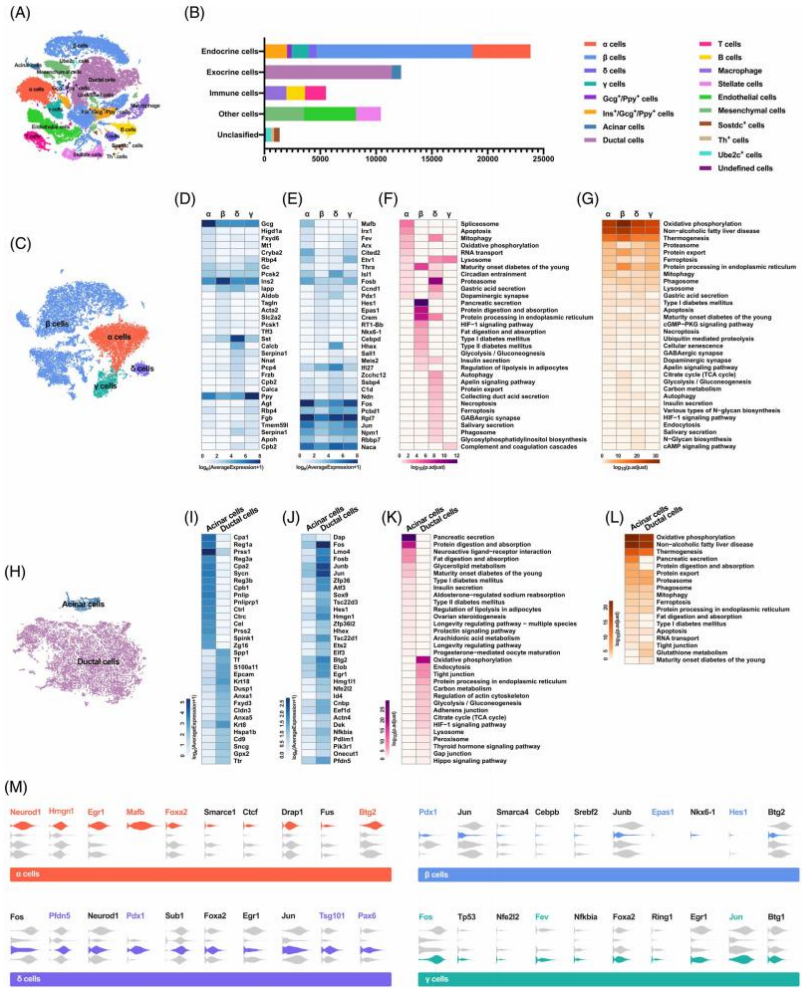
- Heatmaps with expression distributions for enriched genes and TFs

- Ingenuity pathway analysis (IPA) was conducted.



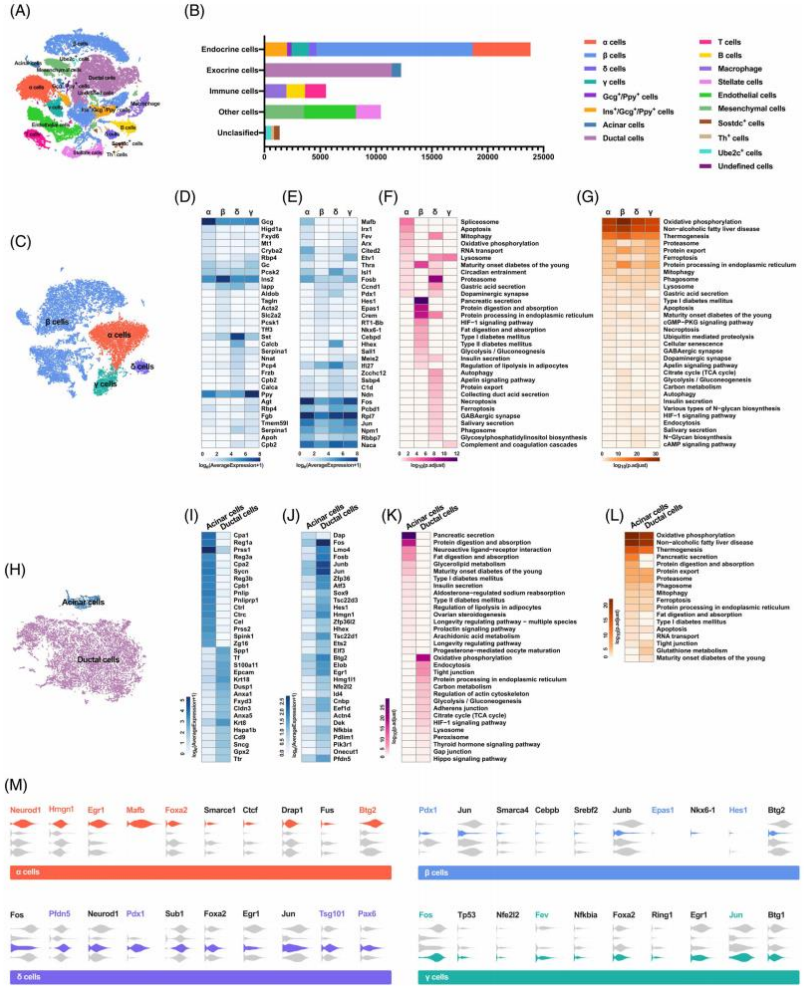
Result 2

- The transcriptomes of the four types of endocrine cells are significantly distinct. For β cells, there is a strong internal heterogeneity with several subclusters
- Compared with the other cell types,δ cells had the largest number of enriched genes; however, most of their functions were unclear.
- α and δ cells had more enriched transcription factors (TFs), CITED2 and THRA were two newly identified TFs restricted in α cell
- New PDX1 and NKX6-1, CCND1,HES1, EPAS1, CREM, RT1-BB,and CEBPD were detected in β cells



Result 2

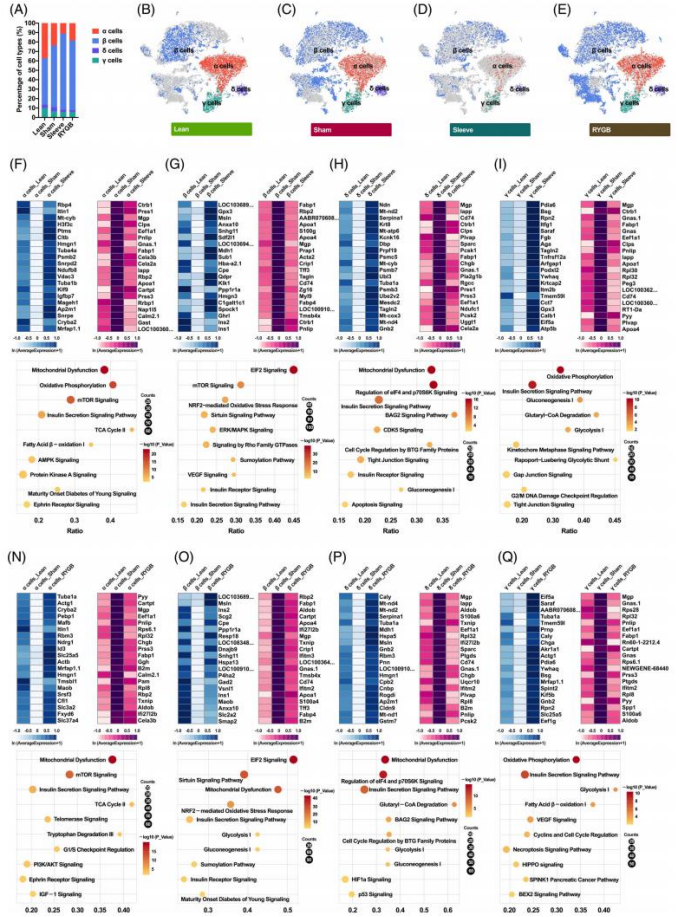
- Ingenuity pathway analysis (IPA):
  - β cells, including glucose sensing and insulin secretion enriched genes were involved in pathways of insulin secretion, glycolysis/gluconeogenesis, pancreatic secretion, T2DM, etc., contributing to metabolic homeostasis
  - α cell-specific genes demonstrated that the pathways related to cell proliferation, apoptosis, metabolism, and secretion were enriched
  - δ cells had the most enriched genes,the pathways associated also covered the most functions.Some of them overlapped with α cells related to diabetes,and the rest were specific to δ cells
- As endocrine and exocrine organs,the function of acinar and duct cells is also crucial for the pancreas to maintain metabolic homeostasis.
  - Other than Cpa1, Reg1a,Prss1, Reg3a, Cpa2, Pnlip, and Spink1, the rest were brand new signature genes meriting further verification and investigation
  - Ductal cells,Spp1, Krt18, Krt8, and Cd9, were known genes with specificity,t expression in ductal cells
  - There were hundreds of enriched TFs in ductal cells, such as ID4, HHEX, SOX9 etc. but almost none in acinar cells
  - IPA analysis revealed that the enriched genes of acinar cells contributed to pathways relevant to digestive enzyme secretion or metabolism



Study 3

Metabolic surgery resulted in multiple changes within the pancreatic endocrine cells

- Compared with the Sham group,RYGB and Sleeve groups increased the proportion of β cells and reduced the ratio of α cells.
- In addition to the quantitive changes,the characteristics of endocrine cells also changed after surgery,especially β cells.suggesting that the heterogeneity of β cells became more obvious under T2DM or surgical intervention.
- Four up-regulated genes (Cryba2,Itln1, Mrfap1.1, and Hmgn1) and nine downregulated genes (Cartpt,Mgp, Eef1a1, Pnlip,Prss3,Fabp1, Calm2.1,Rbp2,and Cela3b) were both detected in pancreatic cells after Sleeve and RYGB surgery
- For pancreatic β cells,eight upregulated genes (LOC103689940,Msln,Ins2,Cpe,Ppp1r1a,Snhg11,Ins1,and Anxa10) and 10 downregulated genes (Rbp2,Fabp1,Apoa4,Mgp,Crip1,Tmsb4x,Cd74,Apoa1,Tff3,and Fabp4) were both detected after Sleeve and RYGB surgery



Changes in transcriptome and function of endocrine cells after metaboli csurgery

Study 4

Changes in transcriptome and function of endocrine cells after metabolic surgery.

- Clustering analysis was performed, and robust separation was observed in α and β cells but not in δ and γ cells.
  - Six cell subsets were introduced in α cells, with subsets 1, 2, and 3 accounting for nearly 90%. The subsets 4, 5, and 6 were more separated from the main body of the cell population on t-SNE map, which suggests that they were relatively more heterogeneous
  - Five cell subsets were introduced in β cells, with subset 1 and subset 2 accounting for 82%
- T2DM significantly reduced the overall proportion of subset 1 and subset 2 of β cells which responsible for the impaired insulin secretion during T2DM progression. After bariatric surgery, subset 1 and subset 2 of β cells were elevated and restored.
- In general, under different metabolic stress, the α and β cell subpopulations will undergo adaptive changes in the cell distribution. bariatric surgery could robustly restored the disordered proportions of α and β cell subsets during T2DM.

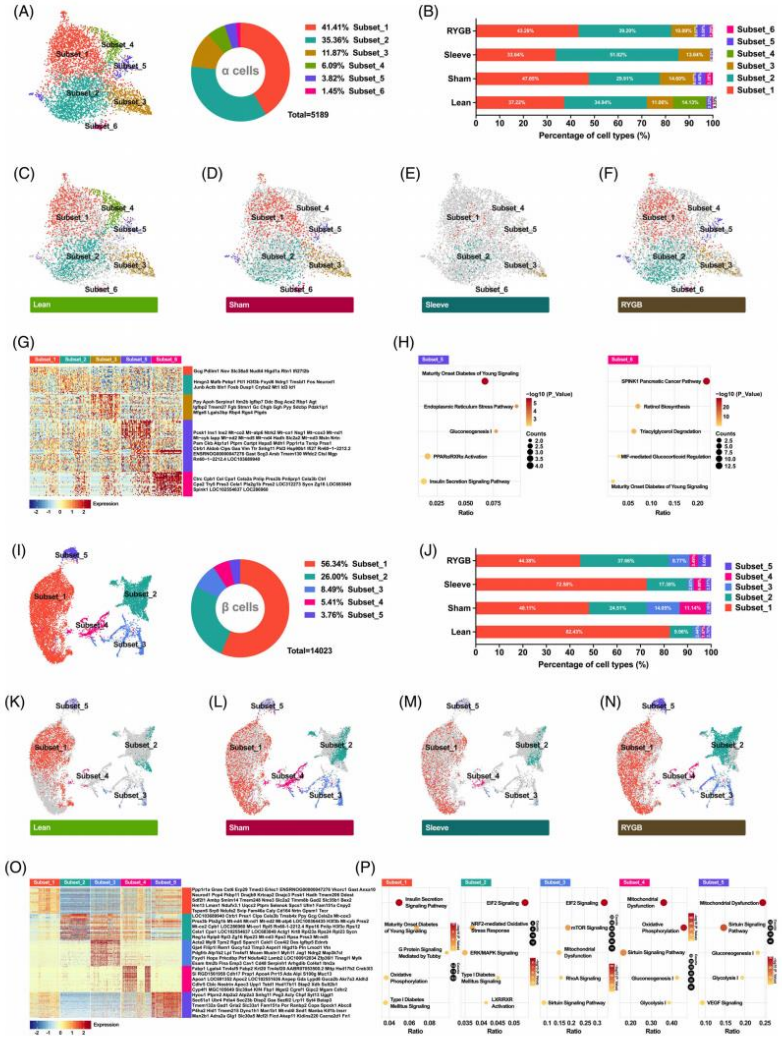
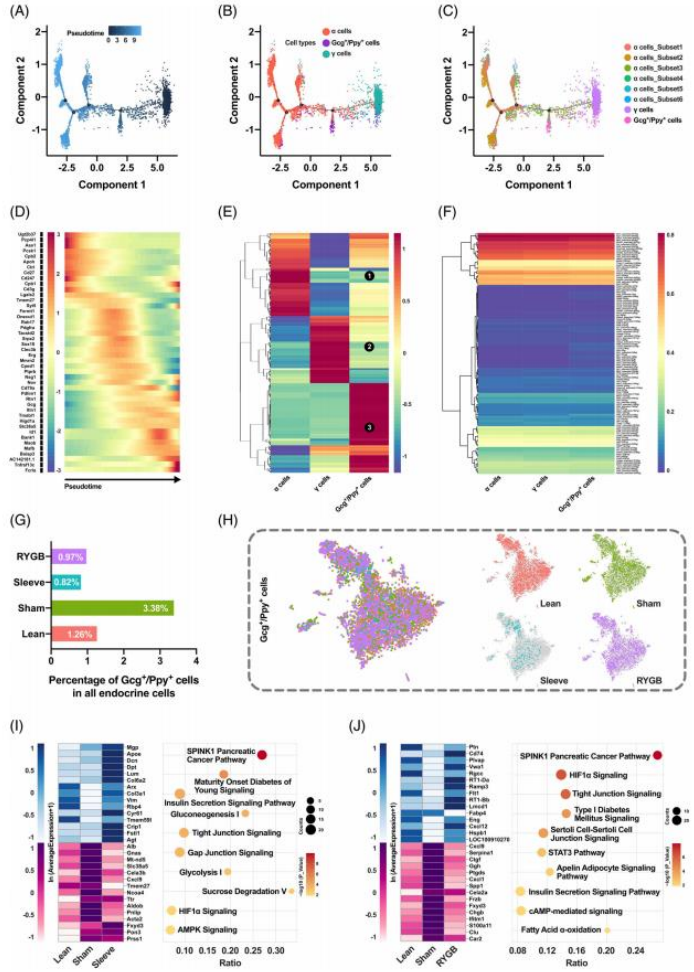


FIGURE 4 Legend on next page.

Study 5

Characterization of pancreatic Gcg+/Ppy+cells and functional change under surgical intervention

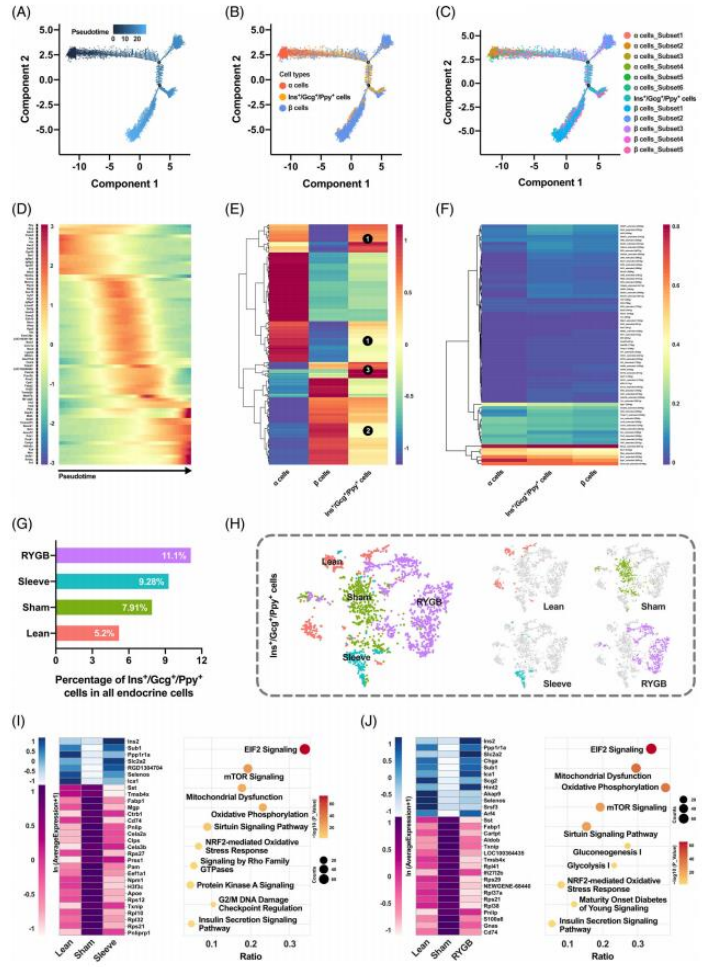
- Pseudotime analysis was performed,mapping the cell types onto the trajectory found that γ cells gathered at the initial point, α cells formed the terminal branches, whereas the Gcg+/Ppy + cells were located between the two.
- The transdifferentiation trajectory may be γ cells-Gcg+/Ppy+ cells-subset 3 of α cells.
- γ -cells show a functional cell plasticity similar to α and δ cells.
- The proportion of Gcg+/Ppy + cells in all pancreatic endocrine cells increased during T2DM but decreased significantly after bariatric surgery
- Transdifferentiation of γ cells into insulin-producing β cell is possible underlying mechanism for DM remission after MBS



Study 6

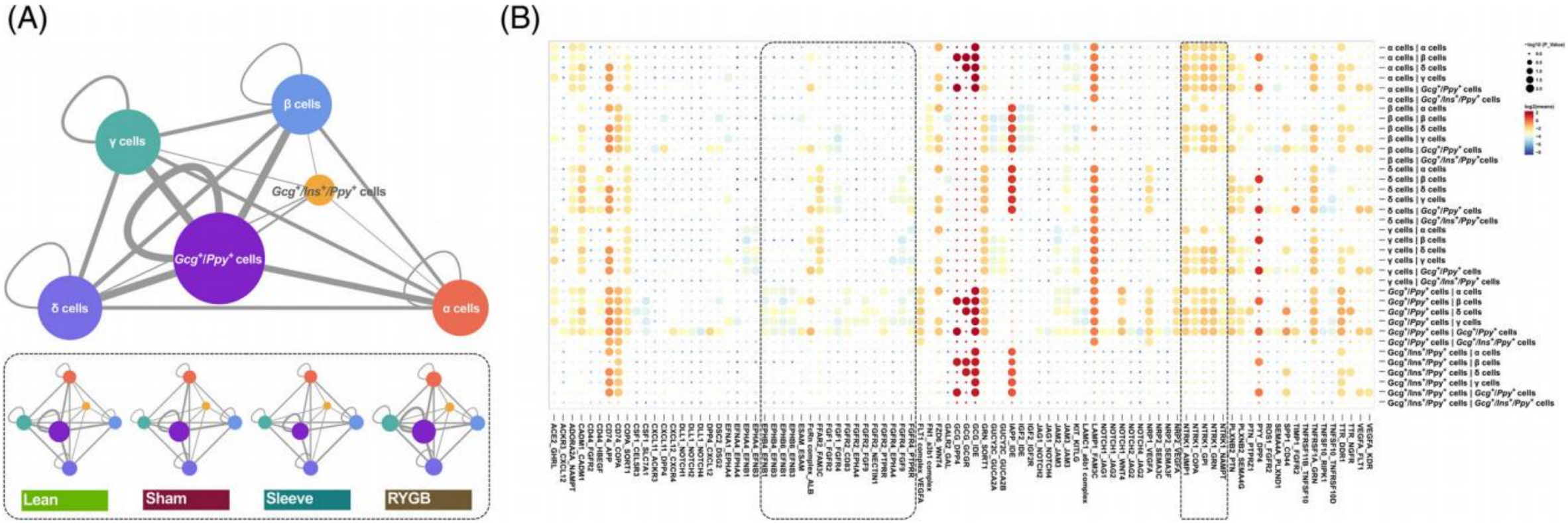
Characterization and functional survey of pancreatic Ins+/Gcg+/Ppy +cells

- Pancreatic Ins+/Gcg+/Ppy+ cells founded for the first time through single-cell transcriptome study.
- The Ins+/Gcg+/Ppy + cells overlapped the most with subset 5 of α cells. In contrast, the distribution of the triple-positive cells highly tended to subset 3 and subset 4 of β cells, which had stronger plasticity in transdifferentiation, dedifferentiation, redifferentiation, and endothelial mesenchymal transition.
- The heatmaps displaying the gene expression and the dot plots presenting pathways annotated by the differentially expressed genes of Ins+/Gcg+/Ppy+ cells in bariatric surgery groups suggest that the Ins+/Gcg+/Ppy + cells participated in the recovery of metabolic homeostasis through improvements in the distribution and function after surgery.



Study 7

Dynamic reciprocal interaction between pancreatic endocrine cells



Interaction network of pancreatic endocrine cells were analyzed by CellPhoneDB and projected with Cytoscape
The potential ligand-receptor pairs in different cell types were predicted and the cell–cell communications were visualized

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Result 7

- A total of 634 ligand-receptor pairs were included in this network,The communication patterns of the Gcg+/Ppy + cells were the most complicated, and the Ins+/Gcg+/Ppy + cells were the simplest.
- For β cells, its functions were finely regulated by α cells, Gcg+/Ppy + cells, and Ins+/Gcg+/Ppy. Similarly, α cells received multiplex signals from β cells，δ cells，Gcg+/Ppy + cells , and Ins +/Gcg+/Ppy + cells .
- The endocrine cell had similar patterns under different metabolic stresses including diabetes or surgical intervention,but the number of interacting pairs was slightly different,with the RYGB group having the most

Conclution

- First:The single-cell transcriptome map of ZDF rats' pancreatic endocrine cells after bariatric surgery
- An increased ratio of pancreatic β cells, which is associated with other pancreatic endocrine cell types transdifferentiated into β cells, was observed following bariatric surgery.
- For the first time, we identify the elevation of cells in the pancreas following bariatric surgery, which is indirect evidence to support the existence of transdifferentiation.


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