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Integrative Biochemical and Mechanistic Prioritization of Late-T2D-Associated Molecular Alterations in Human β-Cells

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Abstract: Type 2 diabetes (T2D) is associated with progressive dysfunction of β-cell but how disease-associated transcriptional changes converge into biochemical patterns remains incompletely defined. This study aimed to identify and prioritize late-T2D-associated biochemical alterations and candidate mechanisms in β-cells by integrating donor-level transcriptomics, biochemical and mechanistic prioritization, pathway analysis, independent human β-cell validation, and genetic anchoring. β-cell profiles from the human pancreatic islet dataset GSE221156 were aggregated at the donor level and analyzed across the three prespecified pairwise comparisons of non-diabetic (ND), pre-diabetic (PD), and T2D states. Transcriptional patterns were classified for their disease-stage association along the ND–PD–T2D sequence, and then biochemical and mechanistic prioritization and functional enrichment were performed. An 18-gene Core-18 subset was defined before independent validation. Genetic anchoring was performed using T2D Knowledge Portal effector genes. No significant differential expression was found between ND and PD, but 266 genes showed differential expression between PD and T2D, and 518 genes showed differential expression between ND and T2D. 154 genes were classified as Late-T2D-associated and 364 as Overall-T2D-associated. 55 candidates genes were prioritized across seven mechanistic axes, and an 18-gene Core-18 subset was identified. Functional enrichment identified 12 FDR-significant enrichment terms, involving mostly GPCR- and peptide-receptor-related signaling, β-cell development and gene regulation, corresponding to 23 unique genes in 83 pathway–gene records. Fourteen of 18 Core-18 candidates were directionally concordant, with 12 directionally supported candidates and 2 lower-confidence candidates. Genetic anchoring revealed HNF1A and SLC2A2 as shared genes between the pathway-associated gene set and the T2D Knowledge Portal effector-gene set, with significant genetic over-representation in two β-cell regulatory pathways. The study highlights a late-T2D-associated β-cell biochemical profile that includes extracellular signaling, metabolic sensing, β-cell regulatory mechanisms, and biochemical control layers. Directional and genetic evidence was integrated to prioritize candidate pathways and genes for mechanistic follow-up.

Keywords: Type 2 Diabetes Mellitus, β-cells, Transcriptomics, Biochemical Pathways, Mechanistic Prioritization, Insulin Secretion.

Research Paper

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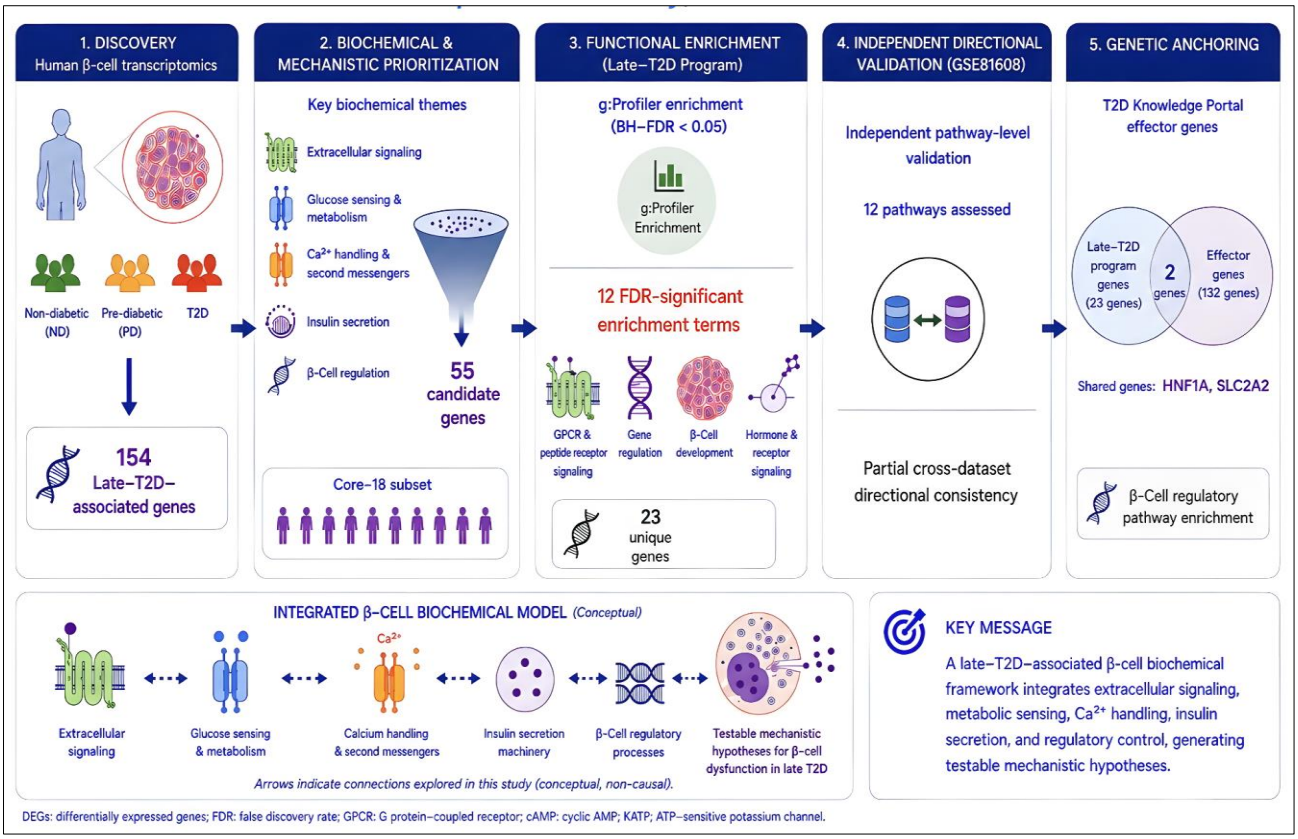

Graphical Abstract: Late-T2D-associated β-cell biochemical framework integrating transcriptomic, pathway, independent directional validation, and genetic evidence

1. INTRODUCTION

Pancreatic β-cells are the source of insulin, and their functional integrity is central to blood-glucose regulation and metabolic homeostasis. Fully functional mature human β-cells possess distinct molecular, phenotypic, structural, and functional characteristics that differentiate them from incompletely differentiated insulin-producing cells, while advanced molecular, epigenomic, histological, and functional approaches have revealed persistent gaps in human β-cell biology (Kaestner *et al*., 2021). In T2D, β-cell dysfunction is a major component of disease pathogenesis and is associated with progressive loss of β-cell function and inadequate insulin secretion (Wei *et al*., 2026).

Single-cell transcriptomic studies indicate that T2D-associated β-cell abnormalities extend beyond insulin secretion to cellular identity and broader molecular programs. In human islets from non-diabetic, pre-diabetic, and T2D donors, T2D was associated with reduced β-cell proportions, decreased β-cell identity-gene expression, increased trans differentiation markers and β-cell disallowed genes, NF-κB activation, increased SPP1 inflammatory signaling, reduced WNT activity, and involvement of the NF-κB–CDKN1C axis in β-cell identity loss (Wei *et al*., 2026). Network analysis further identified T2D-associated β-cell programs involving mitochondrial electron transport, glycolysis, cytoskeletal organization, cell proliferation, unfolded protein response, and transcription-factor networks, alongside enhanced exocytotic processes, lysosomal regulation, and programs involved in insulin translation (Martínez-López *et al*., 2025). Earlier single-cell RNA-sequencing identified cell-type-specific genes and pathways and 245 genes with disturbed expression in T2D, together with substantial species-specific differences between human and mouse α- and β-cell profiles (Xin *et al*., 2016).

Pancreatic β-cells couple nutrient metabolism to insulin secretion through glucose-stimulated insulin secretion (GSIS). In the canonical pathway, glucose metabolism increases intracellular ATP relative to ADP, promoting KATP-channel closure, membrane depolarization, and increased cytosolic Ca2+, thereby supporting insulin secretion. However, the relative contributions of impaired glucose metabolism and reduced KATP-channel responsiveness to defective GSIS in human β-cells remain unresolved (Ashcroft, 2023; Ramanadham *et al*., 2023). GSIS is further amplified and modulated by receptor-dependent signaling. Incretin receptors and other GPCRs activate cAMP-dependent signaling involving PKA and EPAC, linking membrane excitability with insulin-granule trafficking and exocytosis (Ramanadham *et al*., 2023; Stožer *et al*., 2021; Varney & Benovic, 2024). β-cells also express diverse, interconnected receptor networks whose functions and cross-talk remain incompletely characterized, underscoring the complexity of

biochemical signal integration in health and disease (Dalle & Abderrahmani, 2024; Ramanadham *et al*., 2023; Varney & Benovic, 2024).

Pancreatic islets are heterogeneous, with endocrine cell populations differing in transcriptional profiles, protein expression, and regulation of hormone release (Benninger & Kravets, 2022). In T2D, β-cell states associated with disease differ at both the transcriptional and epigenomic levels, including disease-associated heterogeneity of HNF1A (Weng *et al*., 2023). Controlled glucose exposure in human islets produces extensive time- and glucose-associated transcriptional changes and, when integrated with genetic signals, identifies candidate effector genes linked to T2D and glucose-related traits, with selected candidates affecting glucose-stimulated insulin production and secretion (Grenko *et al*., 2024). Multimodal single-β-cell analyses across non-diabetic, pre-T2D, and T2D donors have also identified β-cell subtypes whose defining chromatin is enriched for T2D risk variants and whose stress-response programs are functionally impaired during T2D progression (G. Wang *et al*., 2023).

These findings indicate that T2D β-cell dysfunction cannot be adequately represented by a single list of differentially expressed genes. Network-based, transcriptomic, epigenomic, functional, and genetic analyses have identified disease-relevant β-cell states and candidate mechanisms, including HNF1A-associated heterogeneity and distinct subtypes enriched for T2D genetic risk (Martínez-López *et al*., 2025; G. Wang *et al*., 2023; Weng *et al*., 2023). Large-scale human islet profiling has further integrated transcriptomic, genetic, proteomic, and functional evidence to prioritize candidate genes relevant to β-cell dysfunction, while the T2D Knowledge Portal provides a disease-focused genetic resource for T2D and related traits (Bandesh *et al*., 2026; Costanzo *et al*., 2023).

However, these approaches do not yet establish how disease-associated β-cell transcriptional alterations converge across biochemical pathways, independent human β-cell evidence, and genetic support. The present study therefore aimed to identify and prioritize molecular alterations associated with the T2D trajectory and to determine whether their convergence across these complementary evidence layers could define a focused set of biochemically and mechanistically relevant candidate genes and processes.

2. EXPERIMENTAL SECTION/MATERIAL AND METHODS

2.1 Data Sources, β-Cell Selection, and Donor-Level Data Preparation

The single-cell transcriptomic data of human pancreatic islets was downloaded from the NCBI Gene Expression Omnibus (GEO) dataset GSE221156 (BioProject PRJNA913127) (Bandesh *et al*., 2026). The source dataset consisted of 245,878 single-cell transcriptomes from 48 donors, comprising 17 T2D donors, 17 ND donors and 14 PD donors. Donor-and sample-level metadata were used to identify pure β-cell profiles and classify samples according to disease state. There were 42 pure β-cell donor profiles (17 ND, 11 PD, 14 T2D) after excluding mixed/paired sample records in the donor-level analysis.

The donor-level count matrix for downstream differential-expression analysis was created by summarizing gene-level count data from the retained β-cell profiles. Pure β-cell profiles were mapped to individual donors and classified into three disease-state groups: ND, PD, and T2D. The unit of biological replication was the donor, not the individual single-cell measurement.

The study aimed to describe donor-level changes in transcription of β-cells and their association with biochemical and mechanistic processes along the predetermined disease-state sequence ND → PD → T2D. Three pre-specified pairwise comparisons (ND vs PD, PD vs T2D and ND vs T2D) were evaluated for differential expression. The resulting comparison-specific differential-expression profiles were combined to classify disease-progression trajectories, prioritize biochemical and mechanistic candidates, characterize enriched biochemical pathways, assess independent directional consistency in GSE81608, and provide genetic context using T2D Knowledge Portal effector-gene data.

2.2 Differential Expression Analysis

Analysis of donor-level differential expression was performed in R/Bioconductor (Love *et al*., 2014). The donor-level β-cell count matrix was used for the pairwise analyses of ND versus PD, PD versus T2D, and ND versus T2D. Genes were defined as showing differential expression if the Benjamini–Hochberg multiple-testing-adjusted P-value (padj) was <0.05 and the log2 fold change (|log2FC|) was ≥ 0.5. The direction of differential expression was determined from the estimated log2 fold change.

In each comparison 17,664 genes were assessed. In the ND-versus-PD comparison, no genes met the predefined differential-expression criteria; in the PD-versus-T2D comparison, 266 genes met the criteria, and 518 genes met the criteria in the ND-versus-T2D comparison. Genes for which adjusted P values were not available were not deemed significant. The complete gene-level DESeq2 outputs, including effect estimates, standard errors, test statistics, nominal P values and Benjamini–Hochberg adjusted P values.

2.3 Trajectory-Based Classification

The three prespecified pairwise comparisons were combined at the gene level to describe the transcriptional changes associated with disease stage along the ND–PD–T2D sequence and to determine

patterns that are relevant to biochemical and mechanistic interpretation. Log2 fold change, adjusted P value, significance status and direction of change were kept for all three comparisons for each evaluated gene.

Late-T2D-associated genes were identified as genes that were not differentially expressed in the ND-versus-PD comparison, but were significantly altered in the PD-versus-T2D and ND-versus-T2D comparisons. Overall-T2D-associated genes were defined as genes that were significant in the ND-versus-T2D comparison, but not in either the ND-versus-PD or PD-versus-T2D comparison with the predefined significance criteria. If genes did not fit either of the patterns, they were considered to have no significant trajectory.

This classification is not a single-cell pseudotime analysis, but rather a donor-level disease-progression classification based on differential-expression patterns. It describes the changes in transcription between the predefined clinical groups, while keeping the donor as the unit of biological replication.

2.4 Biochemical and Mechanistic Prioritization

The 518 genes found in the ND vs. T2D comparison were assessed according to an integrative biochemical and mechanistic prioritization framework that included differential-expression magnitude, adjusted statistical significance, functional annotation, KEGG mapping, PD-to-T2D evidence, and assignment to predefined mechanistic axes. A composite Priority_score was defined as follows:

$$Priority\_score = |log_2FC| + [-log_{10}(padj)] + ln(GO\_term\_count + 1) + 3 \times I(KEGG_{mapped} = YES)$$

where; $I(KEGG_{mapped} = YES)$ = 1 if the gene is mapped to a KEGG pathway, and 0 otherwise

The score combined differential-expression magnitude, adjusted statistical significance, functional annotation breadth and KEGG pathway mapping, and was used as a project-defined heuristic for candidate ranking, not as a measure of statistical significance or causal evidence. This framework resulted in 55 prioritized candidates, which were spread over seven mechanistic axes. Candidates were then evaluated for consistency with PD-to-T2D transition. An 18-gene core biochemical subset was defined from the prioritized candidates as those that were significant and directionally consistent in the PD-to-T2D comparison. This set of fixed Core-18 was then carried forward for independent validation and cross-dataset evidence integration.

2.5 Functional Enrichment and Pathway-Gene Membership

To characterize biochemical signaling and regulatory processes represented by the gene sets corresponding to the Late-T2D-associated class and its directionality, functional enrichment was performed using the g:Profiler Web/API service (Kolberg *et al*., 2023). The gene identifiers were prepared for g:Profiler input and the retained enrichment resources included Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome annotations. The final analyses included the Late-T2D-associated set and its upregulated and downregulated subsets.

The set of genes associated with the Late-T2D was 154 genes, with 138 identifiers submitted to g:Profiler. The query sets for the up- and down-regulated components were 62 of 66 and 76 of 88 identifiers, respectively. g:Profiler was used to calculate enrichment significance with FDR correction and significance threshold of 0.05. The enrichment analyses were generated as a result.

The significant enrichment results were used to define the 12 enriched pathways for downstream analyses. Pathway membership analysis was corrected and 23 unique genes were found in 12 enriched pathways, resulting in 83 pathway–gene records.

2.6 Independent Validation in GSE81608

To assess cross-dataset consistency in directionality of the identified biochemical pathway set, independent validation was carried out with the GSE81608 human β-cell dataset (Xin *et al.*, 2016). The project-derived validation subset consisted of 503 β-cell records from 18 donors, of which 296 were T2D samples and 207 were non-diabetic samples. Donor-level disease-state metadata were associated with expression measurements of the identified pathway genes and prioritized candidate genes.

Donor-level expression values for the 23 genes represented in the 12 discovery pathways were calculated as the average expression across all β-cell records in each donor. Gene-level donor expression values were normalized to the sample standard deviation for each pathway, and the pathway score for each donor was determined as the average of the normalized gene expression values of the genes in each pathway that were available for validation. The validation effect was calculated as the mean pathway score difference between T2D and non-diabetic donors (T2D - non-diabetic). The two-sided Mann–Whitney U test was used to compare pathway scores between the two donor groups, and the Benjamini–Hochberg procedure was used to adjust the P values across the 12 pathways. Pathways were considered directionally supported if the sign of the validation effect was the same as the discovery direction and directionally discordant if the discovery direction was opposite the validation direction. To perform a complementary pathway-level analysis, each donor-level gene expression z-score was multiplied by the corresponding discovery direction (+1 for genes that were upregulated and −1 for genes that were

downregulated). The signed z scores were then meaned over the constituent genes of each pathway and donor. The same two-sided Mann–Whitney U test was then used to compare the signed pathway scores of the T2D and non-diabetic donors, and P values were adjusted across the 12 pathways using the Benjamini–Hochberg procedure. The 18-gene Core-18 candidate set was not redefined with the validation data set, but was evaluated separately. Candidates' evidence was categorized as directional support, lower-confidence evidence from very low expression/weak testability, or directional discordance. Since none of the 12 validated pathways had FDR <0.05 in the independent dataset, the GSE81608 analysis was considered as an indication of the directionality of the findings but not as a statistically significant replication.

2.7 Genetic Anchoring and Over-Representation Analysis

To give orthogonal genetic context to the identified biochemical pathway set, the 23 pathway genes were compared to the curated McCarthy T2D effector-gene dataset from the T2D Knowledge Portal (T2DKP) (Costanzo *et al*., 2023). The T2DKP dataset consisted of 132 T2D effector-gene records and was not a re-analysis of genome-wide association study (GWAS) loci, but rather a gene-level genetic-effector resource. The overlap between the 23 pathway genes and the T2DKP effector-gene set was calculated at the gene level, and the T2DKP prediction class and evidence category were kept for overlapping genes. The 154-gene set associated with Late-T2D was then used as the statistical universe to evaluate genetic over-representation for the 12 pathway gene sets. The number of observed T2DKP effector genes was compared to the number of expected T2DKP effector genes for each pathway in the selected universe. A one-sided Fisher's exact test was used to assess statistical enrichment with the alternative hypothesis corresponding to over-representation of T2DKP effector genes within the pathway. Enrichment ratio, odds ratio and nominal P values were computed and P values were adjusted for the 12 pathways using the Benjamini–Hochberg procedure. Pathways with the predetermined FDR <0.05 criterion were kept as genetically supported pathway signals.

2.8 Statistical Analysis and Software

All analyses were carried out using R version 4.6.1. The documented single-cell data processing workflow (Hao *et al*., 2024) was performed using Seurat version 5.5.1 and SeuratObject version 5.4.0. Donor-level differential expression was performed using DESeq2 and functional enrichment was performed using g:Profiler Web/API. Benjamini–Hochberg procedures were used for multiple-testing correction, with FDR <0.05 used as the predefined significance criterion for enrichment and adjusted P value <0.05 used for differential-expression significance together with $|\log2FC| \geq 0.5$. To validate the pathway scores, two-sided Mann–Whitney U tests were performed between T2D and non-diabetic donors with Benjamini–Hochberg correction performed across the 12 validated pathways. Genetic over-representation was assessed using one-sided Fisher's exact tests based on 2 × 2 contingency tables, using the 154-gene Late-T2D-associated set as the statistical universe. Pathway-level genetic analyses were performed and the enrichment ratio, odds ratio, nominal P values, and Benjamini–Hochberg adjusted FDR were calculated.

3. RESULTS AND DISCUSSION

3.1 Identification of Late-T2D-Associated β-Cell Transcriptional Changes

To characterize the transcriptomic changes associated with the progression of the disease, donor-level transcriptomic analysis was performed in pancreatic β-cells from the GSE221156 dataset across the non-diabetic (ND), pre-diabetic (PD), and type 2 diabetes (T2D) states (Bandesh *et al.*, 2026). The pure β-cell profiles that are retained and the donor-level disease-state mapping were used in the donor-level analysis. Three pairwise comparisons were made (ND vs PD, PD vs T2D, and ND vs T2D) and 17,664 genes were analyzed in each comparison. No genes met the predefined criteria for differential expression between ND and PD, while 266 genes showed differential expression between PD and T2D, with 100 genes being upregulated and 166 genes being downregulated. The ND vs T2D analysis, however, revealed 518 differentially expressed genes, including 182 up- and 336 down-regulated genes (Table 1).

Table 1: Summary of differential expression and trajectory classification in GSE221156

Comparison / trajectory class	Number of genes	Significant DEGs	Upregulated	Downregulated
ND vs PD	17,664	0	0	0
PD vs T2D	17,664	266	100	166
ND vs T2D	17,664	518	182	336
Overall-T2D-associated	364	—	116	248
Late-T2D-associated	154	—	66	88
No significant trajectory	17,146	—	—	—

Note: Trajectory classification partitioned the 17,664 evaluated genes into 17,146 genes with no significant trajectory, 364 Overall-T2D-associated genes, and 154 Late-T2D-associated genes. The two T2D-associated trajectory classes together accounted for the 518 genes identified in the ND-to-T2D comparison. Within the Late-T2D-associated class, 66 genes upregulation, whereas 88 genes exhibited downregulated in the PD-to-T2D comparison.

The trajectory-based classification then divided the 518 genes that had significant differential expression in the ND-to-T2D comparison into categories of genes associated with progression. Of these genes, 154 were considered as Late-T2D-associated genes, as they were also significantly differentially expressed in PD-to-T2D transition but not in the ND-to-PD comparison. The remaining 364 genes were grouped as Overall-T2D-associated, as they showed significant differential expression in the ND-to-T2D comparison but not in either the ND-to-PD or PD-to-T2D comparison. Within the Late-T2D associated group, 66 genes were up-regulated and 88 genes were down-regulated in the transition from PD to T2D (Figure 1).

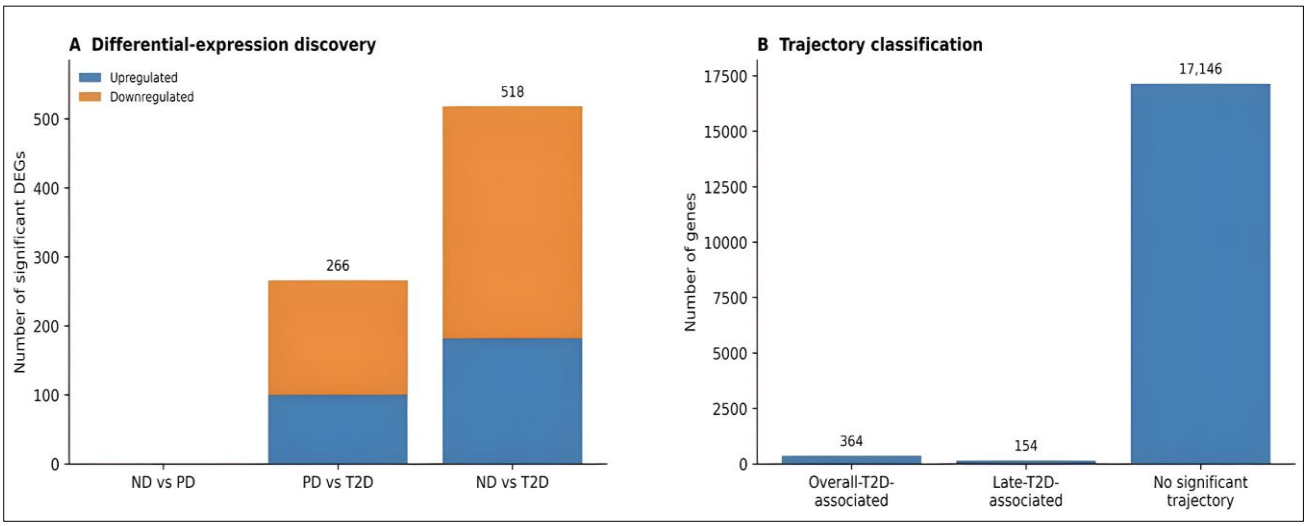

Figure 1: Differential-expression discovery and trajectory classification in GSE221156

Panel a shows significant differentially expressed genes identified in the three pairwise comparisons, with upregulated and downregulated genes displayed separately. A total of 17,664 genes were evaluated in each comparison; no significant DEGs were identified for ND versus PD, whereas 266 DEGs (100 upregulated and 166 downregulated) were identified for PD versus T2D and 518 DEGs (182 upregulated and 336 downregulated) for ND versus T2D. Panel B shows trajectory classification of the evaluated genes, comprising 364 Overall-T2D-associated genes, 154 Late-T2D-associated genes, and 17,146 genes without a significant trajectory. The Late-T2D-associated group comprised 66 upregulated and 88 downregulated genes in the PD-to-T2D transition.

These results provided the transcriptional basis for the biochemical and mechanistic prioritization, and significant differences in transcription were observed during the PD to T2D transition, but not between ND and PD.

The main transcriptomic result was the stage-specific pattern: no genes showed differential expression between ND and PD, while 266 genes showed differential expression between PD and T2D, and 518 genes between ND and T2D. The three comparisons were integrated to separate the latter set into 154 Late-T2D-associated genes and 364 Overall-T2D-associated genes. In the biochemical context of this study, this pattern aligns with coordinated changes across cellular signalling, metabolic sensing, extracellular interactions and β-cell regulatory programmes, not a single isolated molecular process. This is in line with human studies showing significant β-cell state heterogeneity and T2D-related alterations in transcriptional, epigenomic and functional programs (Benninger & Kravets, 2022; G. Wang *et al*., 2023; Weng *et al*., 2023). However, it should not be interpreted as direct evidence of a temporal molecular transition within individual donors because the present study used a cross-sectional design.

The trajectory classification offers a stage-resolved interpretation of the biochemical relevance of the differences in transcription. The 154 Late-T2D-associated genes were those that showed significant differential expression in both comparisons (PD vs. T2D and ND vs. T2D), but not in the third comparison (ND vs. PD). This pattern identifies genes preferentially linked to the late disease-stage comparison, including biochemical processes related to extracellular signal reception, metabolic sensing, intracellular signaling, and β-cell regulation. Biological plausibility of a stage-dependent change in β-cell state is supported by studies in human β-cells where disease-associated changes have been observed with respect to cell identity, stress adaptation, signaling networks, and functional heterogeneity (Benninger & Kravets, 2022; G. Wang *et al*., 2023; Weng *et al*., 2023). However, these trajectory classes represent cross-sectional analytical patterns rather than direct temporal progression within individual donors.

3.2 Biochemical and Mechanistic Prioritization of T2D-Associated β-Cell Candidates

To go from statistical differential expression to biochemical and mechanistic interpretation, the 518 genes that were found to be differentially expressed in the ND vs T2D comparison were analyzed using an integrative biochemical and mechanistic prioritization framework that included differential-effect magnitude, adjusted statistical significance, functional annotation, KEGG mapping, PD-to-T2D evidence and assignment to predefined mechanistic axes. The prioritization framework resulted in 55 candidate genes across seven mechanistic axes, the most of which were in the extracellular matrix remodeling/extracellular biochemical interactions (17 genes), followed by transcriptional/developmental regulation (10 genes), glucose sensing/transport/metabolic regulation (7 genes), inflammatory/immune-associated biochemical regulation (6 genes), cell signaling/receptor-associated regulation (5 genes), hormone/peptide secretion and β-cell function (4 genes), and a further-review category (6 genes) (Figure 2A).

The prioritized candidates were then analysed within the context of disease progression and mechanistic coherence, including the direction and significance of the PD-to-T2D effect, which was consistent with the persistence of the T2D-associated signal. An 18-gene core biochemical subset was defined as the prioritized candidates that were significant and directionally consistent in the PD-to-T2D comparison, and this fixed set was subsequently carried forward for independent validation and cross-dataset evidence integration (Figure 2B; Table 2).

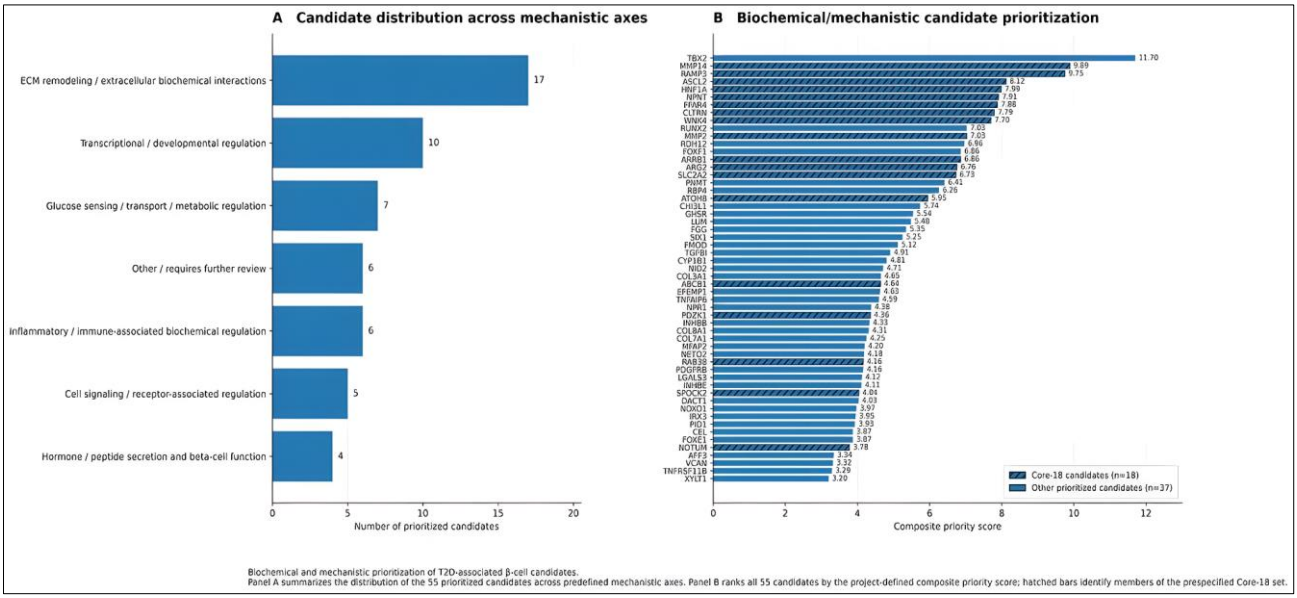

Figure 2: Biochemical and mechanistic prioritization of T2D-associated β-cell candidate genes

(A) Distribution of the 55 prioritized candidate genes across seven predefined mechanistic axes. (B) Ranking of the 55 prioritized candidates according to the project-defined composite priority score. Hatched bars indicate members of the prespecified Core-18 subset, whereas solid bars indicate the remaining 37 prioritized candidates.

Table 2: Biochemical and mechanistic prioritization of T2D-associated β-cell candidate genes

Rank	Candidate gene	Composite priority score	Core-18 status	Mechanistic axis
1	TBX2	11.7	Other prioritized	Transcriptional / developmental regulation
2	MMP14	9.89	Core-18	ECM remodeling / extracellular biochemical interactions
3	RAMP3	9.75	Core-18	Cell signaling / receptor-associated regulation
4	ASCL2	8.12	Core-18	Inflammatory / immune-associated biochemical regulation
5	HNF1A	7.99	Core-18	Glucose sensing / transport / metabolic regulation
6	NPNT	7.91	Core-18	ECM remodeling / extracellular biochemical interactions
7	FFAR4	7.88	Core-18	Glucose sensing / transport / metabolic regulation
8	CLTRN	7.79	Core-18	Hormone / peptide secretion and beta-cell function
9	WNK4	7.7	Core-18	Hormone / peptide secretion and beta-cell function
10	RUNX2	7.03	Other prioritized	Transcriptional / developmental regulation
11	MMP2	7.03	Core-18	ECM remodeling / extracellular biochemical interactions

Rank	Candidate gene	Composite priority score	Core-18 status	Mechanistic axis
12	RDH12	6.96	Other prioritized	Glucose sensing / transport / metabolic regulation
13	FOXF1	6.86	Other prioritized	Transcriptional / developmental regulation
14	ARRB1	6.86	Core-18	Cell signaling / receptor-associated regulation
15	ARG2	6.76	Core-18	Inflammatory / immune-associated biochemical regulation
16	SLC2A2	6.73	Core-18	Glucose sensing / transport / metabolic regulation
17	PNMT	6.41	Other prioritized	Hormone / peptide secretion and beta-cell function
18	RBP4	6.26	Other prioritized	Glucose sensing / transport / metabolic regulation
19	ATOH8	5.95	Core-18	Transcriptional / developmental regulation
20	CHI3L1	5.74	Other prioritized	Inflammatory / immune-associated biochemical regulation
21	GHSR	5.54	Other prioritized	Cell signaling / receptor-associated regulation
22	LUM	5.48	Other prioritized	ECM remodeling / extracellular biochemical interactions
23	FGG	5.35	Other prioritized	Inflammatory / immune-associated biochemical regulation
24	SIX1	5.25	Other prioritized	Transcriptional / developmental regulation
25	FMOD	5.12	Other prioritized	ECM remodeling / extracellular biochemical interactions
26	TGFBI	4.91	Other prioritized	ECM remodeling / extracellular biochemical interactions
27	CYP1B1	4.81	Other prioritized	ECM remodeling / extracellular biochemical interactions
28	NID2	4.71	Other prioritized	ECM remodeling / extracellular biochemical interactions
29	COL3A1	4.65	Other prioritized	ECM remodeling / extracellular biochemical interactions
30	ABCB1	4.64	Core-18	Other / requires further review
31	EFEMP1	4.63	Other prioritized	ECM remodeling / extracellular biochemical interactions
32	TNFAIP6	4.59	Other prioritized	Inflammatory / immune-associated biochemical regulation
33	NPR1	4.38	Other prioritized	Hormone / peptide secretion and beta-cell function
34	PDZK1	4.36	Core-18	Other / requires further review
35	INHBB	4.33	Other prioritized	Other / requires further review
36	COL8A1	4.31	Other prioritized	ECM remodeling / extracellular biochemical interactions
37	COL7A1	4.25	Other prioritized	ECM remodeling / extracellular biochemical interactions
38	MFAP2	4.2	Other prioritized	ECM remodeling / extracellular biochemical interactions
39	NETO2	4.18	Other prioritized	Cell signaling / receptor-associated regulation
40	RAB38	4.16	Core-18	Other / requires further review
41	PDGFRB	4.16	Other prioritized	ECM remodeling / extracellular biochemical interactions
42	LGALS3	4.12	Other prioritized	Inflammatory / immune-associated biochemical regulation
43	INHBE	4.11	Other prioritized	Other / requires further review
44	SPOCK2	4.04	Core-18	ECM remodeling / extracellular biochemical interactions
45	DACT1	4.03	Other prioritized	Transcriptional / developmental regulation
46	NOXO1	3.97	Other prioritized	ECM remodeling / extracellular biochemical interactions
47	IRX3	3.95	Other prioritized	Transcriptional / developmental regulation
48	PID1	3.93	Other prioritized	Cell signaling / receptor-associated regulation
49	CEL	3.87	Other prioritized	Glucose sensing / transport / metabolic regulation
50	FOXE1	3.87	Other prioritized	Glucose sensing / transport / metabolic regulation
51	NOTUM	3.78	Core-18	Transcriptional / developmental regulation
52	AFF3	3.34	Other prioritized	Transcriptional / developmental regulation
53	VCAN	3.32	Other prioritized	ECM remodeling / extracellular biochemical interactions
54	TNFRSF11B	3.29	Other prioritized	Transcriptional / developmental regulation
55	XYLT1	3.2	Other prioritized	Other / requires further review

Note: Composite priority scores are displayed to two decimal places for presentation. The underlying calculations are retained in the source prioritization file.

The core candidates encompassed a variety of biochemical mechanisms such as transcriptional and developmental regulation, extracellular matrix remodeling, glucose sensing and metabolic regulation, receptor-associated signaling, inflammatory/immune-associated regulation, and hormone/peptide-related β-cell functions. The 55 prioritized candidates were thus a separate layer of biochemical and mechanistic

candidates, and an 18-gene core biochemical subset was identified based on the significance and directionality of the association in the PD-T2D comparison and was then forward selected for independent validation; functional enrichment and pathway-level analyses were performed on the Late-T2D-associated gene set.

The prioritization layer narrowed down the 518 differentially expressed genes (DEGs) between ND and T2D to 55 candidates that were prioritized by effect magnitude, statistical evidence, functional annotation, KEGG mapping, PD-to-T2D evidence, and predefined mechanistic categories (Figure 2; Table 2). The distribution in extracellular matrix/extracellular biochemical interactions, transcriptional and developmental regulation, glucose sensing and metabolism, inflammatory/immune-associated regulation, receptor signaling and hormone/peptide secretion suggests that the late-T2D signal spans biochemical control layers. These categories converge conceptually on biochemical processes through which the β-cell integrates extracellular cues, maintains cellular identity, and supports stimulus–secretion coupling (Bernardo *et al*., 2025; Johansen *et al*., 2024; Qian *et al*., 2023). The categories are used to give a biochemical background to the prioritised candidates, but the specific functional consequences in the present donors require experimental confirmation. Importantly, the prioritisation framework does not imply that a prioritised gene is a causal driver, but rather biochemical plausibility and candidate priority.

3.3 Functional Enrichment Reveals Late-T2D-Associated β-Cell Signaling and Regulatory Pathways

To describe biochemical signaling and regulatory processes that were represented by mapped gene sets corresponding to the late-T2D-associated class and its directionality, functional enrichment analysis was performed. Of the 154 genes classified as Late-T2D-associated, 138 genes were included in the enrichment query, with the mapped query sets containing 62 of 66 upregulated genes and 76 of 88 downregulated genes. These query sets yielded 12, 38, and 1 FDR-significant term, respectively, after Benjamini – Hochberg correction (Table 3). Therefore, the enrichment results are not the overall number of classified genes being queried, but rather the number of genes classified successfully.

Table 3: FDR-significant enrichment terms in the Late-T2D-associated gene set

Pathway ID	Pathway	Intersection (n)	BH-FDR
KEGG:04928	Parathyroid hormone synthesis, secretion and action	7	0.003999
REAC:R-HSA-210745	Regulation of gene expression in beta cells	4	0.007539
REAC:R-HSA-419812	Calcitonin-like ligand receptors	3	0.008119
GO:0001653	peptide receptor activity	5	0.008456
GO:0008528	G protein-coupled peptide receptor activity	5	0.008456
REAC:R-HSA-388396	GPCR downstream signalling	12	0.011444
REAC:R-HSA-418555	G alpha (s) signalling events	6	0.015366
REAC:R-HSA-372790	Signaling by GPCR	12	0.015366
REAC:R-HSA-186712	Regulation of beta-cell development	4	0.016970
REAC:R-HSA-500792	GPCR ligand binding	8	0.017502
GO:0097646	calcitonin family receptor signaling pathway	3	0.047507
GO:0007186	G protein-coupled receptor signaling pathway	14	0.047507

Note: BH-FDR values are the multiple-testing-adjusted P values reported by g: Profiler for the Late-T2D-associated query, using FDR correction with a significance threshold of 0.05. Values are shown to six decimal places. The enrichment query comprised 138 mapped genes from the 154 Late-T2D-associated genes.

The 12 terms that were most significantly enriched in the Late-T2D-associated gene set were mostly associated with peptide- or G-protein coupled receptor-mediated signaling, and were also enriched for processes related to β-cell development and gene regulation. The smallest BH-FDR was seen for Parathyroid hormone synthesis, secretion and action (intersection size, 7; BH-FDR = 0.003999), followed by Regulation of gene expression in beta cells (4 genes; BH-FDR = 0.007539) and Calcitonin-like ligand receptors (3 genes; BH-FDR = 0.008119) (Table 3).

The enrichment profile was thus characterized by a broad representation of two biochemical processes: receptor-mediated signaling, especially involving GPCRs, and regulation of β-cell development and gene expression (Figure 3).

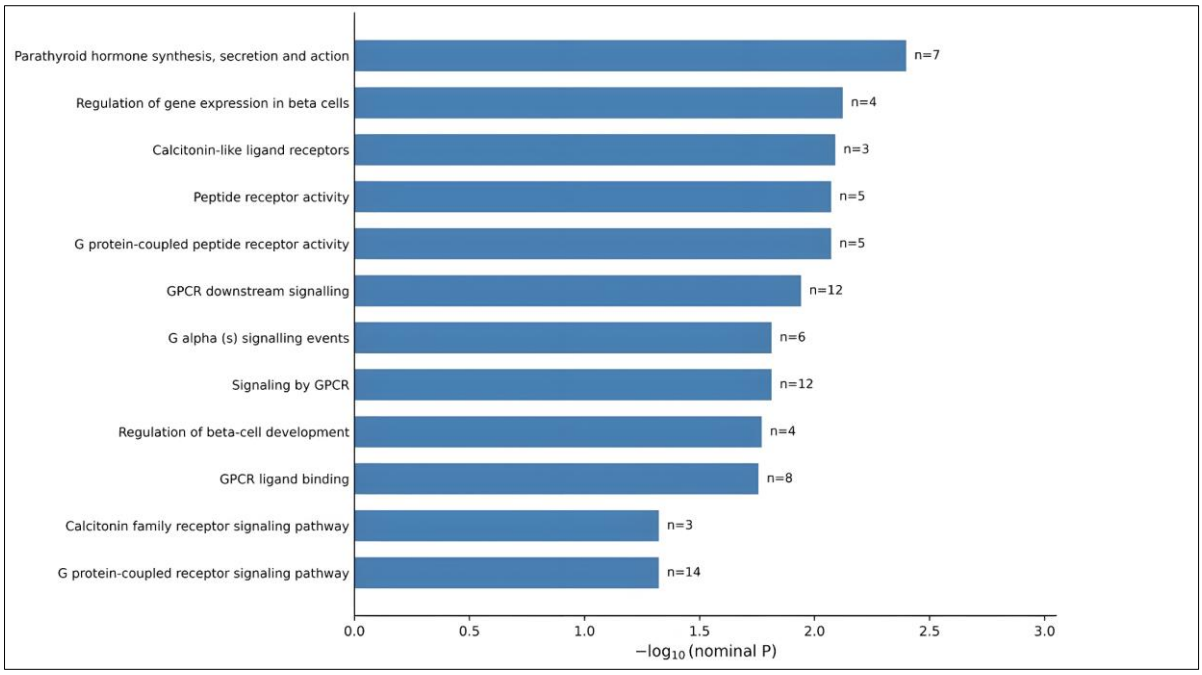

Figure 3: Functional enrichment of the Late-T2D-associated β-cell gene set

The 12 enrichment terms meeting the Benjamini–Hochberg FDR < 0.05 criterion in the mapped subset of the Late-T2D-associated gene set (138 of 154 classified genes) are shown ranked by nominal enrichment P values. Bar length represents −log10(nominal P), and n indicates the number of genes intersecting each enriched term. The enriched terms predominantly represent GPCR- and peptide-receptor-related signaling together with regulation of β-cell development and gene expression.

Concurrently, the 88 genes that were downregulated gave rise to just one pathway (Parathyroid hormone synthesis, secretion and action, intersection size 6, BH-FDR = 0.000758) that was found to be significant.

The pathway membership analysis identified 83 pathway–gene records linking the23 distinct genes to the 12 enriched pathways.

The most significant enrichment results were those related to GPCR- and peptide-receptor-related terms, which appeared repeatedly in the strongest functional theme; 12 significant terms were identified from the mapped query set of genes that are associated with Late-T2D. This finding is biochemically coherent because β-cell GPCRs represent an important link between extracellular hormonal and paracrine signals and stimulus–secretion coupling in the cell (Dalle & Abderrahmani, 2024; Stožer *et al*., 2021). Gs-, Gq-, and Gi-coupled receptors can regulate cAMP- and phospholipid-dependent signaling and, therefore, Ca2+ handling, granule trafficking and insulin secretion through the actions of PKA, EPAC, PKC and related effectors (Dalle & Abderrahmani, 2024; Ramanadham *et al*., 2023; Stožer *et al*., 2021). There is another regulatory mechanism that involves GPCR kinase/arrestin that regulates β-cell signaling and function (Varney & Benovic, 2024). The enrichment therefore identifies extracellular signal processing as a prominent biochemical component of the late-T2D-associated transcriptional changes. But transcriptomic enrichment does not prove the activity of receptors, phosphorylation status, or concentration of second messengers or functional output of signaling in the donors.

The inclusion of glucose sensing/metabolic regulation and β-cell regulatory pathways offers a second biochemical axis to interpret the late-T2D associated program. In the classical model of glucose stimulated insulin secretion, glucose metabolism causes an increase in ATP/ADP ratio, which causes the closure of ATP-sensitive K+ (KATP) channels, the depolarization of the β-cell membrane, and the opening of voltage-gated Ca2+ channels, leading to the exocytosis of insulin granules (Ramanadham *et al*., 2023). In fact, cAMP-dependent pathways enhance this metabolic trigger by regulating ion channels, granule trafficking, and fusion machinery through PKA- and EPAC-mediated mechanisms (Ramanadham *et al*., 2023; Stožer *et al*., 2021), indicating that β-cell metabolism is not only an energy-producing process, but also a signaling system that links metabolic flux with electrical activity and secretion in β-cells (Ramanadham *et al*., 2023; Y. Wang *et al*., 2026). Recent biochemical studies also suggest that glucose sensing and insulin secretion could be spatially arranged in the submembrane space, and that localized ATP and Ca2+ signals connect metabolic activity with electrical and secretory coupling (Y. Wang *et al*., 2026). The present data thus give biochemical context to the observed changes in metabolic-to-secretory coupling,

but do not show defects in ATP production, KATP-channel activity, Ca2+ influx or exocytosis.

The β-cell state at late T2D is not limited to acute signaling and secretion, but rather is characterized by the enrichment of pathways involved in β-cell development and gene expression, as well as the prioritization of transcriptional/developmental regulators. In particular, experimental suppression of HNF1A in primary human pseudoislets altered insulin output and the expression of genes associated with hormone secretion, calcium-channel biology, ATPase transport, and extracellular-matrix constituents (Qian *et al.*, 2023). More recently, HNF1A was demonstrated to regulate a β-cell transcription–splicing axis involving A1CF, and this axis was found to be suppressed in β-cells from individuals with T2D; human genetic variation affecting this pathway has also been associated with glycemic and T2D risk (Bernardo *et al*., 2025). The present analysis reveals the appearance of β-cell regulatory pathways that can be explained mechanistically based on these findings. They do not, however, prove that the entire late-T2D molecular pattern described here is downstream of HNF1A dysregulation.

3.4 Independent Directional Evaluation of Late-T2D-Associated Pathways in GSE81608

The 12 pathways identified from GSE221156 were evaluated in the independent human β-cell dataset GSE81608 (Xin *et al*., 2016) to determine the directionality of the identified biochemical pathways. The validation data set consisted of 503 β-cell samples from 18 donors, including 296 T2D samples and 207 non-diabetic samples, for which the 23 genes represented in the late-T2D-associated pathways were available for pathway-level assessment.

With the primary pathway-level analysis, 8 of the 12 pathways were directionally concordant with the discovery analysis, while 4 were directionally discordant (Table 4). The two terms related to β-cells (Parathyroid hormone synthesis and secretion and Parathyroid hormone action) had opposing directions of concordance, while the two GPCR-related terms and the two peptide-receptor related terms had predominant directions of concordance.

Table 4: Independent directional evaluation of the late-T2D-associated pathway program in GSE81608

Analysis	Pathway	Genes validated	Validation effect	P	FDR	Directional classification
Primary	Parathyroid hormone synthesis, secretion and action	7	0.1499	0.2496	0.4992	Directionally discordant
Primary	Regulation of beta-cell development	4	-0.3861	0.2129	0.4992	Directionally discordant
Primary	Regulation of gene expression in beta cells	4	-0.3861	0.2129	0.4992	Directionally discordant
Primary	G alpha (s) signalling events	5	0.0858	0.3845	0.6592	Directionally discordant
Primary	Signaling by GPCR	11	-0.2117	0.2129	0.4992	Directionally supported
Primary	GPCR downstream signalling	11	-0.2117	0.2129	0.4992	Directionally supported
Primary	GPCR ligand binding	7	-0.2681	0.0831	0.4992	Directionally supported
Primary	peptide receptor activity	4	-0.1021	0.9623	0.9623	Directionally supported
Primary	G protein-coupled receptor signaling pathway	13	-0.1047	0.6820	0.9623	Directionally supported
Primary	G protein-coupled peptide receptor activity	4	-0.1021	0.9623	0.9623	Directionally supported
Primary	calcitonin family receptor signaling pathway	3	-0.2154	0.8513	0.9623	Directionally supported
Primary	Calcitonin-like ligand receptors	3	-0.2154	0.8513	0.9623	Directionally supported
Signed	peptide receptor activity	4	0.1667	0.8871	0.9253	Directionally supported
Signed	G protein-coupled receptor signaling pathway	13	-0.1156	0.3845	0.6592	Directionally discordant
Signed	G protein-coupled peptide receptor activity	4	0.1667	0.8871	0.9253	Directionally supported
Signed	calcitonin family receptor signaling pathway	3	-0.0123	0.9253	0.9253	Directionally discordant
Signed	Parathyroid hormone synthesis, secretion and action	7	-0.2265	0.2129	0.6387	Directionally discordant
Signed	Regulation of beta-cell development	4	-0.3861	0.2129	0.6387	Directionally discordant
Signed	Regulation of gene expression in beta cells	4	-0.3861	0.2129	0.6387	Directionally discordant

Analysis	Pathway	Genes validated	Validation effect	P	FDR	Directional classification
Signed	Signaling by GPCR	11	-0.1366	0.3845	0.6592	Directionally discordant
Signed	GPCR downstream signalling	11	-0.1366	0.3845	0.6592	Directionally discordant
Signed	G alpha (s) signalling events	5	-0.2224	0.2129	0.6387	Directionally discordant
Signed	Calcitonin-like ligand receptors	3	-0.0123	0.9253	0.9253	Directionally discordant
Signed	GPCR ligand binding	7	-0.0441	0.8916	0.9253	Directionally discordant

Note: Directional classifications were assigned by comparing the validation direction with the corresponding discovery direction. No pathway achieved FDR < 0.05 in the independent dataset. Genes validated denotes the number of pathway genes available for the corresponding pathway-level analysis.

The most negative validation effects among the directionally concordant pathways were observed for GPCR ligand binding (effect = −0.2681; P = 0.0831; FDR = 0.4992), Signaling by GPCR and GPCR downstream signalling (both −0.2117; P = 0.2129; FDR = 0.4992), whereas none reached statistical significance after FDR correction (Table 4).

A complementary signed validation analysis yielded a more conservative pattern. In this method, 2 of the 12 pathways were considered directionally supported while 10 were directionally discordant. The pathways that were concordantly positively validated were peptide receptor activity (validation effect = 0.166731; P = 0.887111; FDR = 0.925342) and G protein-coupled peptide receptor activity (validation effect = 0.166731; P = 0.887111; FDR = 0.925342). The other 10 pathways were directionally discordant, such as the two β-cell regulatory pathways, the parathyroid hormone pathway, G alpha (s) signalling events, and several GPCR-related pathways. Therefore, the signed analysis revealed less directionality than the main pathway-level analysis (Figure 4).

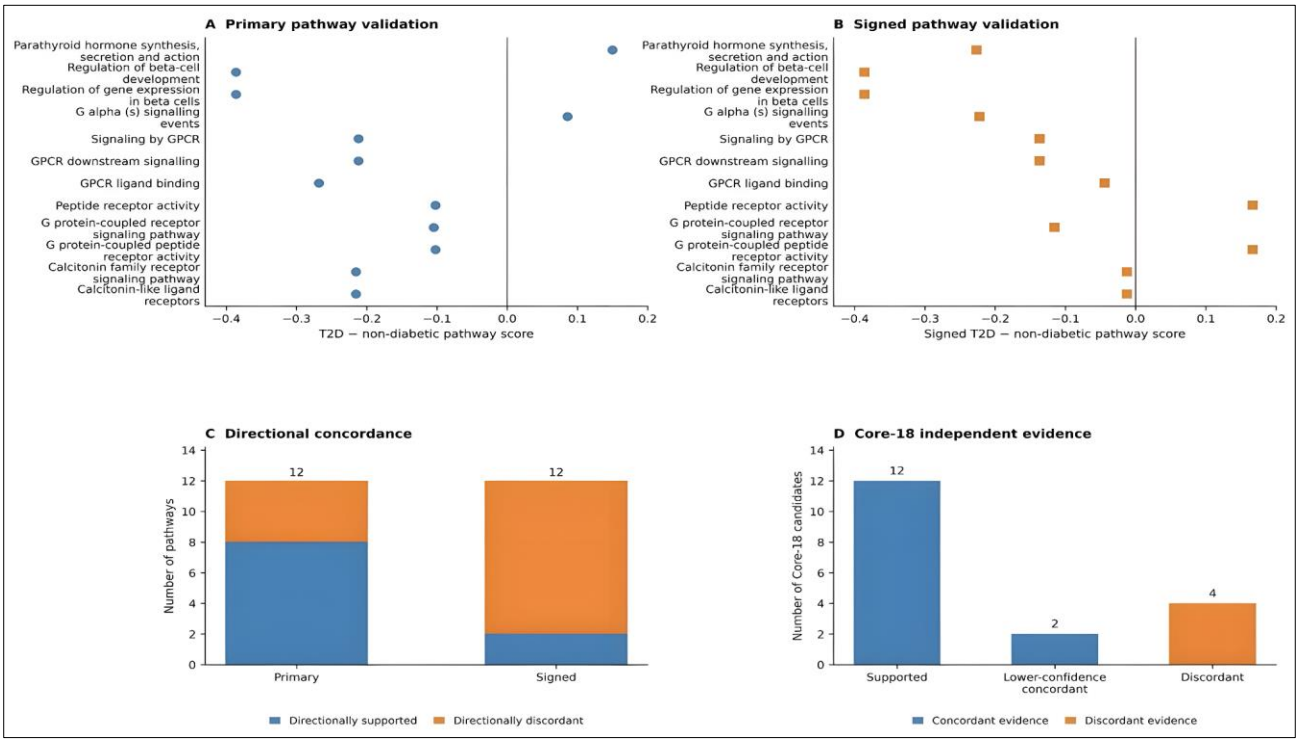

Figure 4: Independent directional evaluation of the late-T2D-associated β-cell pathways in GSE81608

(A) Primary pathway-level validation effects for the 12 pathways identified in GSE221156. (B) Signed pathway-level validation effects for the same 12 pathways. The vertical line at zero indicates no directional effect. (C) Directional concordance across the 12 pathways, showing eight directionally supported and four directionally discordant pathways in the primary analysis, compared with two supported and ten discordant pathways in the signed analysis. (D) Independent evidence for the fixed Core-18 biochemical candidate set, comprising 12 directionally supported candidates, two concordant but lower-confidence candidates, and four directionally discordant candidates. No pathway-level validation result reached FDR < 0.05.

Overall, the independent GSE81608 analysis showed partial cross-dataset directional consistency of the identified pathways associated with late-T2D, with

more directional concordance in the primary analysis than in the signed analysis. The 18-gene core biochemical candidate set was fixed and was directionally concordant for 14 of 18 candidates in the independent dataset. Twelve candidates were directionally supported, while two candidates had concordant, but lower-confidence evidence due to very low expression/weak testability, and one further candidate had lower-confidence evidence that was directionally discordant, along with three further directionally discordant candidates. Importantly, the pathway-level validation results should be viewed as evidence of directionality, not as statistically significant replications, as none of the 12 pathways had an FDR < 0.05 in the independent dataset.

The fixed Core-18 set offers a candidate-level evidence layer for biochemical interpretation following pathway-level discovery. The set was defined prior to independent validation and remained the same throughout GSE81608 analysis. Biochemically, this pattern suggests that a subset of prioritized genes could reflect the consistent biochemical aspects of the late-T2D state across independent human β-cell data, and highlights the importance of expression abundance and dataset-specific measurement limitations on candidate-level inference. The validation analysis is an external test of a prespecified candidate set, since the Core-18 was defined prior to independent validation, and was not redefined using GSE81608. Nevertheless, directional concordance remains evidence of consistency rather than proof of protein-level dysfunction or causal biochemical activity.

This biochemical model is bounded by the independent data set. The primary pathway level analysis indicated directional concordance in 8 of 12 pathways, while the signed analysis indicated concordance in only 2 of 12 pathways. No pathway achieved FDR <0.05 in GSE81608 (Table 4; Figure 4). As such, the evidence in the present study suggests partial cross-dataset directional consistency, rather than statistically significant pathway-level replication. The apparent strength of a pathway-level signal is also shown to be dependent upon the definition of the validation metric in the more conservative signed analysis. These findings argue against treating the enriched GPCR or β-cell regulatory pathways as universally reproduced molecular abnormalities in T2D β-cells. Instead, they identify candidate biochemical pathways and processes whose directionality is sufficiently consistent to justify further functional testing.

3.5 Genetic Anchoring of Late-T2D-Associated β-Cell Pathways

To give orthogonal genetic context to the identified biochemical pathway program, the 23 unique genes represented in the 12 pathway gene sets were compared with the 132 curated T2D effector genes from the T2D Knowledge Portal (McCarthy dataset) (Costanzo *et al*., 2023). There were only two genes common to both the late-T2D pathway-gene set and the T2DKP effector-gene set: HNF1A and SLC2A2. SLC2A2 was classified as prediction class B (STRONG) while HNF1A was classified as prediction class A (CAUSAL) by T2DKP. The two genes were shared by two pathways: Regulation of beta-cell development and Regulation of gene expression in beta cells. The evidence categories for the corresponding evidence were reported as CAUSAL for HNF1A and STRONG for SLC2A2.

Genetic over-representation analysis was then carried out with the 154 genes associated with Late-T2D as the statistical universe. Within this universe, three of the 132 T2DKP T2D effector genes were found, and two of the T2D regulatory pathways in the β-cell contained four genes from the universe, including two T2DKP effector genes, HNF1A and SLC2A2. The number of T2DKP effector genes found in the overlap of each pathway was observed to be 2, while the expected number of genes was 0.077922, giving an enrichment ratio of 25.666667 and an odds ratio of 149.0. The enrichment was statistically significant for both Regulation of beta-cell development and Regulation of gene expression in beta cells (P = 0.001514; Benjamini–Hochberg FDR = 0.009087) (Table 5; Figure 5).

Table 5: T2DKP genetic over-representation of the two significant β-cell regulatory pathways

Pathway	Pathway genes in universe	T2DKP effector genes in universe	Observed	Expected	Enrichment ratio	Odds ratio	P	BH-FDR	Overlap genes
Regulation of beta-cell development	4	3	2	0.077922	25.667	149.0	0.001514	0.009087	HNF1A; SLC2A2
Regulation of gene expression in beta cells	4	3	2	0.077922	25.667	149.0	0.001514	0.009087	HNF1A; SLC2A2

Note: Only pathways meeting the Benjamini–Hochberg FDR < 0.05 threshold are presented. Three T2DKP effector genes were present in the 154-gene statistical universe; two of these genes, HNF1A and SLC2A2, overlapped each of the two reported pathway gene sets. HNF1A was classified as prediction class A (CAUSAL) and SLC2A2 as prediction class B (STRONG).

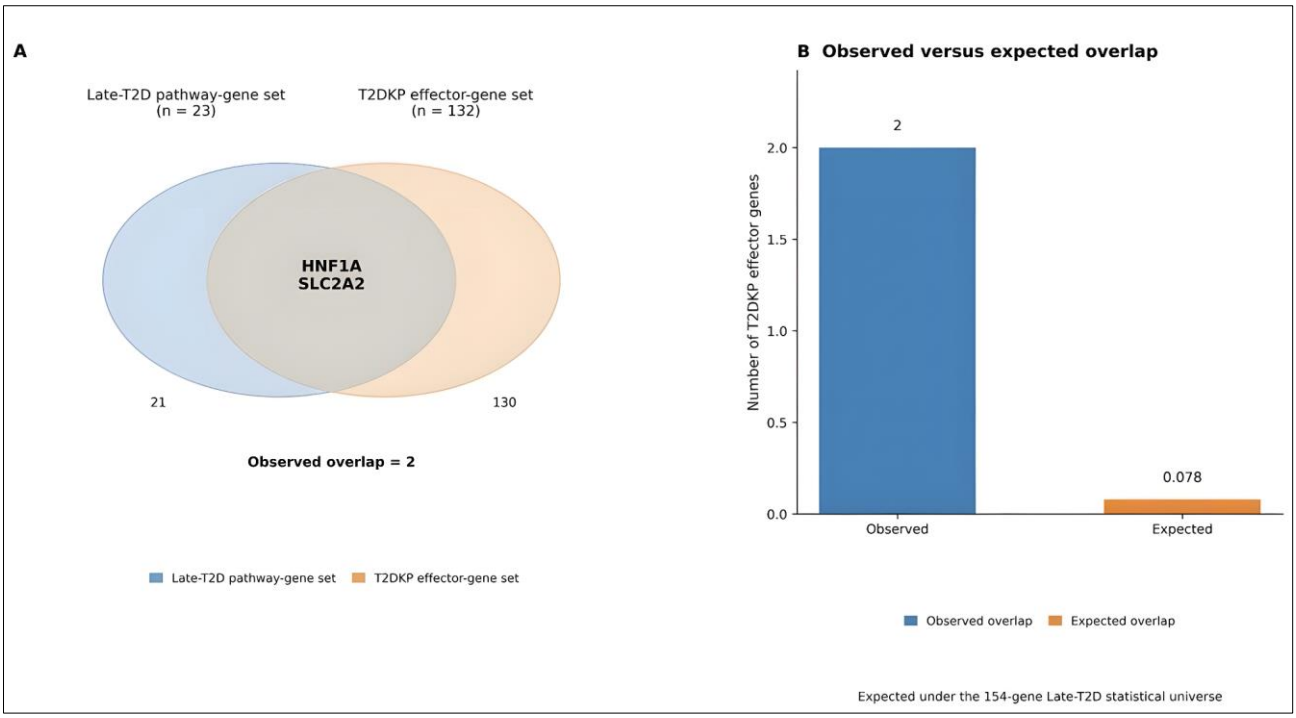

Figure 5: Genetic anchoring of prioritized β-cell biochemical pathways

(A) Overlap between the late-T2D pathway-gene set (n = 23) and the T2DKP effector-gene set (n = 132), identifying HNF1A and SLC2A2 as the two shared genes. (B) Observed versus expected overlap under the 154-gene Late-T2D statistical universe. The observed overlap was 2 genes compared with an expected overlap of 0.078 genes (enrichment ratio = 25.67; OR = 149.0; P = 0.001514; BH-FDR = 0.009087).

These results collectively offer genetic contextual support to the two β-cell regulatory pathways of the late-T2D-associated biochemical program.

The genetic analysis provides an orthogonal evidence layer, which is independent of transcriptomic enrichment. Only HNF1A and SLC2A2 overlapped the curated T2D Knowledge Portal effector-gene set, and both were present in the two β-cell regulatory pathways with significant genetic over-representation. In addition to the glucose-sensing/metabolic anchor (SLC2A2), HNF1A offers a transcriptional-regulatory anchor (Caspi *et al*., 2024). HNF1A has been shown to play important roles in human β-cell transcriptional control and insulin secretory function (Bernardo *et al*., 2025; Qian *et al*., 2023). SLC2A2/GLUT2 plays a role in glucose handling in β-cells and in glucose stimulated insulin secretion, but the relative contribution of SLC2A2/GLUT2 versus GLUT1 is different in rodents than in humans and should not be over-simplified (Bacos *et al*., 2023; Caspi *et al*., 2024). The pathway-level genetic analysis therefore provides biochemical context for the convergence of these two genes within β-cell regulatory pathways linked to glucose-responsive function (Table 5; Figure 5). The genetic enrichment should be taken as a contextual support, not as a discovery of new causal T2D genes, HNF1A or SLC2A2.

The integration of the evidence layers indicates a coherent biochemical model, where the late-T2D-associated transcriptional pattern is characterized by extracellular signal processing, receptor signaling, metabolic sensing, β-cell regulatory programs, and genetically anchored candidate genes. The principal signaling component is provided by GPCR- and peptide-receptor-related pathways, complemented by regulatory and metabolic anchors provided by HNF1A and SLC2A2. These are all connected conceptually by the ability of extracellular receptor signaling to modulate cAMP-dependent and phospholipid-dependent pathways and Ca2+ handling, while nutrient metabolism contributes to the metabolic signals that regulate β-cell electrical activity and insulin secretion (Alenkvist *et al*., 2017; Caspi *et al*., 2024; Ramanadham *et al*., 2023; Stožer *et al*., 2021). The biochemical signals that result can affect insulin-granule trafficking, priming and exocytosis (Alenkvist *et al*., 2017; Ramanadham *et al*., 2023; Stožer *et al*., 2021). The present study does not define these biochemical steps as being impaired simultaneously, but instead identifies a transcriptomic configuration where the molecular components of the process of extracellular signal integration, metabolic sensing, β-cell regulation and secretion are represented across complementary evidence layers.

3.6 Strengths, Limitations, and Experimental Implications

One of the strengths of the study is the use of donors as the biological units for differential expression,

followed by an explicit progression framework and multiple independent evidence layers. The analysis also distinguishes discovery, biochemical prioritization, functional enrichment, external validation and genetic anchoring, rather than lumping in non-equivalent evidence as a single score. The cross-sectional design, the absence of direct functional perturbation, and attrition of identifiers during enrichment analysis are limitations, and the absence of FDR-significant pathway replication in GSE81608 further limits the strength of mechanistic inference. The enrichment analysis is in particular not the result of the complete number of classified genes but of the successfully queried sub sets. The study is limited and should be considered as a biochemical prioritization and hypothesis generation tool. The next experimental step should, therefore, test the most promising candidates at the protein and functional levels such as receptor signaling, metabolic coupling, Ca2+ handling, and insulin-granule exocytosis. HNF1A and SLC2A2 are particularly interesting mechanistic anchors, because independent human evidence supports roles for HNF1A in β-cell regulatory control and SLC2A2 in insulin secretion (Bacos *et al*., 2023; Bernardo *et al.*, 2025; Qian *et al*., 2023).

4. CONCLUSION

The present study reveals late-T2D-associated β-cell transcriptional changes characterized by coordinated alterations in cellular signaling, metabolic sensing, extracellular interactions, and β-cell regulatory processes. The disease-stage pattern revealed that there was no significant differential expression between ND and PD, 266 genes were identified between PD and T2D, and 518 genes were identified between ND and T2D, with 154 genes being identified as associated with Late-T2D and 55 being prioritized biochemical and mechanistic candidates. Functional enrichment identified 12 significantly enriched pathways, predominantly involving GPCR- and peptide-receptor-related signaling and the regulation of β-cell development and gene expression. Independent evaluation in GSE81608 showed partial cross-dataset directional consistency, more so in the primary analysis than in the signed analysis, and no pathway with FDR < 0.05. Of the fixed Core-18 set, 14 of 18 candidates were directionally concordant (including 12 directionally supported candidates and two lower-confidence concordant candidates); four candidates were directionally discordant. Genetic anchoring further indicated that the genes HNF1A and SLC2A2 are part of the significantly enriched β-cell regulatory pathways. Overall, these results indicate that the molecular state of late-T2D β-cells is not a reflection of a single biochemical pathway, but rather multiple interconnected biochemical systems. The resulting biochemical candidate and pathway framework therefore provides a focused basis for subsequent mechanistic experiments to determine whether prioritized molecular components alter receptor signaling, metabolic coupling, Ca2+ handling, and insulin secretion at the protein and functional levels.

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