

ALDOB influences β -cell insulin secretion through modulating GLP-1R signaling in type 2 diabetes

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Abstract

Prolonged exposure to elevated levels of blood glucose impairs β -cell insulin secretion in type 2 diabetes (T2D) but the underlying mechanisms remain incompletely defined. Herein we find that fructose-1,6-bisphosphate aldolase B (ALDOB) is the most significantly upregulated aldolase in islets of T2D humans and T2D mice. Paradoxically, β cell-specific ALDOB knockout mice exhibits impaired insulin secretion via attenuated cAMP-PKA pathways independent of ALDOB enzymatic function, whereas ALDOA exerts enzymatic roles in regulating insulin secretion. Mechanistically, ALDOB interacts with insulin-like growth factor binding protein 5 (IGFBP5) to modulate IGFBP5 nuclear migration and transcription of inhibitory G_{ai2} subunit (GNAI2), thereby tuning the glucagon-like peptide-1 receptor (GLP-1R)-cAMP-PKA pathway. Furthermore, GNAI2 inhibition synergizes the efficacy of GLP-1R agonists and overcomes GLP-1R desensitization due to a long-term exposure. Collectively, our findings support a mechanism by which ALDOB modulates β -cell insulin secretion in T2D through tuning GLP-1R-cAMP-PKA signaling pathways, highlighting a potential therapeutic strategy for T2D.

Main Text

Type 2 diabetes (T2D) is a chronic metabolic disorder with insulin resistance and/or inadequate insulin production of islet β cells^{1,2}. As the major threat to global public health, T2D is projected to affect up to 690 million people in year 2045 with a global healthcare cost around US \$850 billion³. Current therapeutic strategies for T2D include increasing pancreatic insulin production, decreasing insulin resistance or preventing reabsorption of glucose into the blood by the kidney. In particular, agonists for the glucagon-like peptide-1 receptor (GLP-1R) achieves effective reduction of glycemia in T2D patients by stimulating insulin secretion and inhibiting glucagon secretion, which have fundamentally changed the landscape of T2D and weight management for recent years^{4,5}.

Although year 2021 marked the 100th year anniversary of the discovery of insulin, the underlying mechanisms of glucose stimulated insulin secretion (GSIS) from β -cells remain incompletely understood⁶. In the “canonical” model of GSIS, ATP production from glycolysis and mitochondrial oxidative phosphorylation leads to the closure of ATP sensitive potassium channel, influx of Ca^{2+} , and insulin secretion from β cells⁷. Autocrine insulin signaling through insulin receptor (IR) or insulin like growth factor 1 receptor (IGF1R) is important for GSIS, while impaired insulin secretion may lead to T2D^{8–10}. Upon binding to insulin or IGF1, IR and IGF1R form heterodimers to activate downstream signaling events for inducing glucose uptake and glycolysis^{11–13}. Interestingly, IGF1R signalling can be further regulated by various IGF binding proteins (IGFBPs) although it is poorly studied how IGFBPs affect insulin secretion in β -cells¹⁴.

G-protein coupled receptors (GPCRs) are also important in GSIS and cross-talks between IR/IGFR and GPCR signaling have received growing interest with enormous therapeutic implications for T2D^{15–19}. However, it remains poorly defined how coupling of IR/IGF1R and GPCR signaling in β -cells regulates

insulin secretion in the context of T2D. The incretin hormones, GLP-1 and gastric inhibitory polypeptide (GIP), potentiate GSIS by binding to GPCRs, resulting in stimulation of adenylate cyclase (AC) to convert ATP into the second messenger cyclic adenosine monophosphate (cAMP), which activates protein kinase A (PKA) or the guanine nucleotide exchange protein 2A^{20,21}. GLP-1R agonists, such as semaglutide and liraglutide, have emerged as an important therapy for T2D by effective control of blood glucose and body weight^{4,22–25}. Recent studies have indicated better clinical benefits in T2D patients by dual agonist targeting GLP-1 and GIP receptors^{5,26}. On the other hand, GPCRs in β -cells can also couple to the inhibitory subunit of the heterotrimeric G proteins, G_{ai}, to attenuate insulin secretion^{17,27,28}. Thus, the overall insulin secretion from β -cells upon GPCR activation may be dictated by the balance between stimulatory and inhibitory GPCRs, converging on the cAMP signaling^{29,30}. Furthermore, GPCR desensitization due to prolonged activation, including GLP-1R and GIPR agonists, has been implicated by regulation of the G_{ai2} (GNAI2)^{31–33}.

The inadequate insulin secretion by islet β -cells in T2D can be attributed to various factors, such as oxidative stress, endoplasmic reticulum stress, autophagy, apoptosis, dedifferentiation, and metabolic dysregulation^{34–37}. Among these factors, the metabolic impairment of β -cells on insulin secretion remains poorly understood². Comprehensive genomic analyses have revealed a notable upregulation of metabolic enzymes involved in glycolysis and the pentose phosphate pathway in the islets of both T2D patients and mouse models^{38–41}. The increased expression of certain metabolic enzymes in islets is primarily associated with the heightened demand for glucose metabolism under high glucose conditions. Emerging data support a critical role of fructose-1,6-bisphosphate aldolase in glucose sensing, AMPK activating and calcium homeostasis, but its role on β -cell insulin secretion remain elusive^{42,43}. Aldolase catalyzes the breakdown of fructose-1,6-bisphosphate (FBP) into glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP)⁴⁴. Among the three isoforms of aldolases, ALDOA is expressed at much higher levels in β cells compared to ALDOB and ALDOC³⁸. Notably, previous study showed that ALDOB was significantly upregulated and emerged as the most significantly altered glycolytic gene in hyperglycemic patients⁴⁰. A case-control Japanese study also identified a significant association between a single nucleotide polymorphism (SNP) located in intron 2 of ALDOB and T2D⁴⁵. We observed decreased circulating insulin levels in global ALDOB knockout mice and hepatic ALDOB deficiency activates IR signaling to promote *de novo* fatty acid synthesis⁴⁶. However, the mechanism by which islet ALDOB regulates insulin secretion in T2D remains to be determined.

In this study, utilizing human and mouse primary islets, we validated that ALDOB is the most significantly upregulated aldolase isoform in T2D. Paradoxically, however, β cell-specific ALDOB knockout mice demonstrated a notable impairment in GSIS and exacerbated T2D progression by inhibiting cAMP-PKA pathways. Furthermore, multi-omics and single cell sequencing (scRNA-seq) identified a significantly increased β -cell subset which exhibited depleted ALDOB but upregulated IGFBP5 in T2D mouse and human islets. Further mechanical study showed that ALDOB interacts with the nuclear localization sequence (NLS) of IGFBP5 to dictate IGFBP5 nuclear translocation and transcription of GNAI2, whereas

ALDOB deficiency in β -cells increased nuclear IGFBP5 and GNAI2, leading to decreased GSIS through inhibiting cAMP-PKA-ion channel signaling pathway in β cells. Furthermore, GNAI2 inhibition synergizes insulin secretion of the GLP-1R agonists and overcomes the desensitization of GPCR signaling.

Results

ALDOB is the most significantly upregulated aldolase in diabetic human and mouse islets, but ALDOB deficiency in mouse β -cells impairs insulin secretion

Prolonged exposure of pancreatic islets to elevated glucose levels in T2D can result in islet metabolic dysfunction with aberrant expressions of genes associated with glycolysis metabolism, such as aldolase^{38,47}. However, the functional roles of these metabolic alterations on β -cell insulin secretion in T2D remain largely unknown. To bridge this knowledge gap, we performed Immunofluorescence analysis on pancreatic islets from nondiabetic and T2D patients (**Table S1**) and revealed a notable elevation of aldolase levels in T2D patient islets compared to the nondiabetic controls (Fig. 1a,b). Interestingly, further qPCR results showed that among the three isoforms, *Aldob* was elevated to the largest extent in T2D patient islets (Fig. 1c). Similar observations were made in diabetic db/db mouse islets compared to control (Fig. 1d,f). To further confirm the upregulation of ALDOB during the progression of T2D, we fed wild-type (WT) mice with a high-fat high-sucrose diet (HFHS) for 25 weeks to mimic contemporary human dietary patterns to induce diabetes⁴⁸. HFHS-fed mice exhibited progressively increased body weights compared to Chow Diet (CD) mice (**Extended Data Fig. 1a**). Moreover, fasting blood glucose level was significantly increased after feeding HFHS diet for 25 weeks (**Extended Data Fig. 1b**). Consistently, in HFHS-induced diabetic mouse model, *aldob* mRNA level was also significantly elevated to the largest extent in the mouse islets fed HFHS for 25 weeks (**Extended Data Fig. 1c-e**).

To investigate a functional consequence of aldolase expressions in islets on insulin secretion, we knocked down aldolase in human primary islets (**Table S2 and Extended Data Fig. 1f,g**) and found that knocking down aldolase significantly decreased GSIS with 16.7 mM glucose stimulation (Fig. 1g). This phenotype was also confirmed in both human induced pluripotent stem cells (iPSC)-derived islets and rat endoderm cell-induced islets, respectively (Fig. 1h,i and **Extended Data Fig. 1h-k**). To pinpoint the role of ALDOB on insulin secretion, we generated islet β cell-specific ALDOB knockout mice (ALDOB islet KO, ALDOB^{Ins2-/-}) by crossing floxed ALDOB animals with Ins2^{Cre} mice. As expected, ALDOB was significantly knocked down in the islets of KO mice without affecting the expressions in other tissues, including liver, kidney, muscle, brain, epididymal white adipose tissue (eWAT), and brown adipose tissue (BAT) (**Extended Data Fig. 2a,b**). Interestingly, feeding male ALDOB^{Ins2-/-} mice with Chow Diet for 3 months significantly impaired glucose tolerance (Fig. 1j) and decreased C-peptide in the blood (**Extended Data Fig. 2c**) without affecting the insulin sensitivity in the insulin tolerance test (ITT) (Fig. 1k). Interestingly, no differences in body weights and levels of Triglycerides (TG) and Total Cholesterol (TC) in the blood were observed (**Extended Data Fig. 2d-f**). Similar phenotypes were also observed in female mice with Chow Diet (**Extended Data Fig. 2g,h**). Moreover, there were no differences in numbers of β cells per islet (**Extended Data Fig. 3a,b**), islet size (**Extended Data Fig. 3c,d**), or insulin vesicle numbers

(**Extended Data** Fig. 3e,f), and total insulin (**Extended Data** Fig. 3g) between ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice, suggesting that ALDOB in β -cells plays a primary role on insulin secretion without affecting islet differentiation or insulin production.

To determine whether the impaired insulin secretion of ALDOB^{Ins2-/-} mice was an islet cell autonomous effect, we evaluated GSIS in cultured islets isolated from 12-week-old ALDOB^{Ins2-/-} mice and their control littermates, and found that Phase I and Phase II insulin secretion in ALDOB^{Ins2-/-} islets was substantially lower than those from ALDOB^{fl/fl} islets (Fig. 1l,m). Similarly, serum insulin levels were significantly reduced after injection of 30% glucose in ALDOB^{Ins2-/-} mice (Fig. 1n). All these results suggest that ALDOB-null β -cell *per se* is defective in GSIS.

To further substantiate that ALDOB expressions dictate insulin secretion in β -cells, we overexpressed ALDOB in the islets of ALDOB^{Ins2-/-} mice by employing an adeno-associated virus (AVV) with an Ins2-specific promoter (**Extended Data** Fig. 3h). After ALDOB restoration in ALDOB^{Ins2-/-} mice, the impaired glucose tolerance and decreased GSIS were all rescued (Fig. 1o,p). Together, these results underscore a direct role of ALDOB in regulating insulin secretion in islet β -cells.

ALDOB deletion in β -cells exacerbates impaired insulin secretion in mouse model of T2D

Although ALDOB is significantly upregulated in diabetic human and mouse islets, ALDOB knockout in β -cells paradoxically impaired insulin secretion even with Chow Diet. Next, we set out to investigate the effects of ALDOB disruption on insulin secretion in the context of T2D. To do this, we fed ALDOB^{Ins2-/-} and their control littermates with HFHS diet for 19 weeks (Fig. 2a **and** **Extended Data** Fig. 4a). Despite the fact that HFHS feeding did not significantly change the body weights of ALDOB^{Ins2-/-} mice and control mice (**Extended Data** Fig. 4b), ALDOB deficiency led to severe impairment of glucose tolerance and significantly decreased insulin levels in ALDOB^{Ins2-/-} mice (Fig. 2b,c) without affecting insulin sensitivity in ITT (**Extended Data** Fig. 4c). Consistently, primary islets isolated from ALDOB^{Ins2-/-} mice with HFHS feeding also demonstrated significantly impaired GSIS *in vitro* (Fig. 2d). Moreover, the number of β -cells per islet (*Insulin*⁺) and size of islets slightly decreased in ALDOB^{Ins2-/-} mice without affecting the number of α cells (*glucagon*⁺) per islet (Fig. 2e-h), consistent with more pronounced β -cell failure in ALDOB^{Ins2-/-} mice. We observed manifestations of other severe diabetic phenotypes in ALDOB^{Ins2-/-} mice with HFHS feeding. Lipid accumulation in the liver of ALDOB^{Ins2-/-} mice was more apparent compared to control mice (**Extended Data** Fig. 4d). The ALDOB^{Ins2-/-} mice also exhibited severe insulin resistance in the liver as indicated by the dysregulation of IR signaling pathways with decreased phosphorylation of IR and AKT (**Extended Data** Fig. 4e,f). Moreover, ALDOB^{Ins2-/-} mice had more severe kidney fibrosis (**Extended Data** Fig. 4g,h). These data indicate that ALDOB plays an important role in regulating insulin secretion in β -cells in T2D.

ALDOB expression in islet cells significantly increases with diabetes mellitus progression, yet β cell-specific ALDOB KO impaired insulin secretion in mouse model of T2D, suggesting that ALDOB upregulation in T2D is likely a compensatory response due to the heightened demand of glycolysis with high glucose levels. To confirm this hypothesis, we knocked down ALDOB in the β cells during T2D progression after 8-week HFHS feeding, and then continued feeding these mice HFHS diet for 11 weeks (Fig. 2i and **Extended Data** Fig. 4i,j). ALDOB deficiency in β -cells after the established T2D still exhibited worsened glucose tolerance (Fig. 2j) and reduced GSIS (Fig. 2k) without significantly altering body weights, insulin sensitivity, or islet morphology (**Extended Data** Fig. 4k-n).

Together, these results demonstrate that ALDOB expressions in β -cells dictate insulin secretion in T2D, and ALDOB upregulation during T2D progression is likely an adaptive response to the elevated blood glucose level in T2D.

ALDOB in β -cells regulates insulin secretion independent of its enzymatic activity

Aldolase is a key glycolytic enzyme, responsible for facilitating the breakdown of FBP into GAP and DHAP. Among different aldolase isoforms, ALDOA is the predominant isoform in pancreatic β -cells, yet ALDOB increased to the largest extent in T2D, suggesting ALDOB modulates insulin secretion beyond its enzymatic function. To investigate the role of aldolase-mediated glycolysis on β -cell insulin secretion, we knocked out ALDOA in β -cells using AAV (**Extended Data** Fig. 5a-c), and observed that loss of ALDOA in β -cells led to impaired glucose tolerance in mice and significantly reduced GSIS in primary islets (**Extended Data** Fig. 5d,e). We performed stable isotope tracing experiment using 16.7 mM of universally labeled ^{13}C -glucose in isolated primary islets and revealed decreased glycolytic metabolites, such as F6P, F1,6P, and GAP, in primary mouse islets of ALDOA KO mice compared to the control mice (**Extended Data** Fig. 5f). Consistently, ATP levels also significantly decreased in islets from β -cell ALDOA KO mice with slightly decreased glucose uptake (**Extended Data** Fig. 5g,h). Importantly, however, there were no alterations of major glycolytic metabolites in islets of ALDOB KO mice after treated with 16.7 mM ^{13}C -labeled glucose (**Extended Data** Fig. 7i). All data demonstrate that ALDOA rather than ALDOB exerts metabolic role in ATP production for insulin secretion in β -cells.

Our previous studies identified multiple critical amino acid residues in the active sites of ALDOB that are important for its enzymatic activities⁴⁹. Among these mutants, mutation of arginine at 304 to alanine (R304A) in ALDOB reduced its catalytic efficiency to 50%. To further substantiate the non-enzymatic roles of ALDOB in β -cell insulin secretion, we generated β cell-specific ALDOB R304A mutant mice by crossing ALDOB-R304A knock in mice by CRISPR-Cas9 with Ins2-cre mice, and observed no significant differences in glucose tolerance (GTT), insulin sensitivity (ITT), or GSIS of primary islets compared to the control littermates (**Extended Data** Fig. 5j-l). All results showed that ALDOB in β -cells modulates insulin secretion independent of its enzymatic functions.

ALDOB in β -cells regulates GSIS through cAMP signaling pathway

To investigate the underlying mechanisms by which ALDOB regulates GSIS beyond its enzymatic activity, we performed proteomic and transcriptomic analyses using primary islets isolated from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice. By overlapping these two datasets, we identified 62 genes differentially expressed at both mRNA and protein levels (Fig. 3a). After further validation with qPCR of those genes associated with islet function including insulin secretion, we identified that cAMP signaling pathway ranks as the most significantly altered pathways in ALDOB^{Ins2^{-/-}} mice islets (Fig. 3b,c). These data prompted us to hypothesize that ALDOB in β -cells regulates insulin secretion primarily through cAMP signaling pathway.

Next, we developed a Liquid chromatography-Mass spectrometry (LC/MS) method to detect cAMP levels in Ins1 cells and found that knocking down ALDOB significantly decreased intracellular cAMP levels following 16.7 mM glucose treatment (**Extended Data Fig. 6a**), consistent with a significant reduction of the phosphorylated PKA substrates without affecting the expression of Epac1 and Epac2 (**Extended Data Fig. 6b,c**). Moreover, PKA downstream genes including ATP-sensitive potassium channel (K_{ATP}), calcium-activated potassium channel (Kcnn), voltage-gated calcium channel (VDCC), and chloride channel genes, all decreased in ALDOB^{Ins2^{-/-}} mice islets (Fig. 3d). Consistent with the downregulation of cAMP pathway, calcium flux was also significantly reduced in ALDOB^{Ins2^{-/-}} mice islets under 16.7 mM glucose stimulation (Fig. 3e and **Extended Data Fig. 6d**). Treatment with Forskolin, an adenylate cyclase AC agonist, and IBMX, a phosphodiesterase PDE inhibitor, resulted in increased cAMP content. Notably, Forskolin and IBMX significantly enhanced insulin secretion by approximately fourfold under high glucose stimulation, counteracting the decreased insulin secretion in ALDOB^{Ins2^{-/-}} mice (Fig. 3f).

Together, these data demonstrate that ALDOB regulates insulin secretion through cAMP-PKA signaling pathway in β -cells, and ALDOB deficiency impairs GSIS in T2D mice.

scRNA-seq identified a significant increase of a subset of Igfbp5⁺ β cells with downregulated cAMP-PKA and impaired GSIS in ALDOB^{Ins2^{-/-}} mice and T2D patients

Pancreatic islet cells comprise heterogeneous cell populations, including β , α , and δ cells, each with distinct functional roles⁵⁰. The cAMP signaling pathway is a critical regulator of secretory function in these cell types. In α cells, cAMP signaling promotes glucagon release, while in β cells, it stimulates insulin secretion⁵¹. Even among the heterogeneous populations of β -cells in human islets, about 75% of β cells respond to glucose challenge and insulin secretion, and single cells transcriptomics (scRNA-seq) has enabled to identify these heterogeneous cell populations⁵⁰. Next, we performed scRNA-seq to explore β -cell populations with lower ALDOB levels associated with impaired insulin secretion and downregulated cAMP-PKA pathways in T2D islets. To do this, we utilized primary islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice fed with 12-week Chow Diet or HFHS diet for scRNA-seq. Our scRNA-seq analysis successfully identified 10 cell types in the mouse islets, among which β -cells represent a major cell type with β cell-specific markers, such as *ins1*, *ins2*, *g6pc2* (**Extended Data Fig. 7a,b**). Gene ontology (GO)

enrichment analysis of differential genes in β -cells identified that cAMP signaling pathway was significantly downregulated in ALDOB^{Ins2-/-} mice (Fig. 4a). Next, we performed unsupervised clustering analysis and classified β -cells into five sub-clusters (Cluster 0,4,1,2 3) according to their distinctive gene expressions (Fig. 4b **and Extended Data Fig. 7c**). Among these five sub-clusters of β -cells, Cluster 0 was significantly decreased while Cluster 4 significantly increased from 1.9% to 42.6% in Chow Diet, from 3.1% to 34.4% in HFHS diet in ALDOB KO compared to the control (Fig. 4c). Slingshot analysis showed that sub-cluster 4 of β cells was a functional immature cluster in the pseudo-time analysis (**Extended Data Fig. 7d**). Further analysis showed that β cell-marker genes and function genes, including the cAMP-PKA pathways, were significantly downregulated in Cluster 4 compared with Cluster 0 (Fig. 4d **and Extended Data Fig. 7e**). These data suggest that Cluster 4 is predominantly responsible for impaired insulin secretion associated with ALDOB depletion in β -cells of mouse islets.

Furthermore, we constructed a volcano plot to analyze differential genes between Cluster 4 and 0 of β -cells, and identified that *Igfbp5* was the most significantly upregulated gene in Cluster 4 (Fig. 4f,g). Interestingly, *Igfbp5* also significantly increased in β -cells of ALDOB KO mice compared to the control group (**Extended Data Fig. 7f**). Importantly, after analyzing scRNA-seq data from normal and T2D human islets, the number of *Igfbp5*⁺ β -cells increased around seventeen folds in T2D human β -cells (1.1% VS 19.5%, Fig. 4g **and Extended Data Fig. 7g-i**). Notably, *Igfbp5*⁺ β -cells in T2D humans manifested downregulated β -cell marker genes and functional genes, reminiscent of gene expression patterns of Cluster 4 in β cell-specific ALDOB mice (Fig. 4h).

Together, these data demonstrate that *Igfbp5*⁺ β -cells are primarily responsible for impaired insulin secretion through downregulated cAMP-PKA pathways in T2D.

ALDOB deficient β -cells decreases GSIS through upregulating IGFBP5

Next, we investigated the role of IGFBP5 expressions on GSIS in β -cells. Consistent with scRNA-seq data, IGFBP5 was the only significantly increased isoforms within the family of five IGFBPs in the islets of ALDOB^{Ins2-/-} mice (Fig. 5a). IGFBP5 is a secreted protein that influences IGF1R signaling pathway through modulating half-life of IGF1 upon binding to the circulating IGF1⁵². Surprisingly, upregulated IGFBP5 in primary islets from ALDOB^{Ins2-/-} mice did not appear to affect IGF1R signaling (Fig. 5b,c). Moreover, although HFHS feeding led to elevated levels of IGFBP5 in the mouse serum compared to the Chow Diet, there was no difference in serum of ALDOB^{Ins2-/-} mice compared to the control (Fig. 5d). Interestingly, IGFBP5 expression in primary islets of ALDOB^{Ins2-/-} mice was significantly increased without affecting the levels of secreted IGFBP5 in the supernatant of islets, and the amount of IGFBP5 secreted from islets is minimal (Fig. 5e **and Extended Data Fig. 8a**), suggesting that ALDOB primarily modulates intracellular IGFBP5 in β cells. Moreover, IGFBP5 overexpression in Ins1 cells inhibited insulin secretion with 16.7 mM glucose treatment, whereas IGFBP5 knocking down increased GSIS (Fig. 5f,g

and Extended Data Fig. 6b-e). In addition, IGFBP5 overexpression also led to a marked reduction in PKA substrate phosphorylation (Fig. 5h,i).

Next, we employed AAV virus to specifically knock down IGFBP5 levels in the β -cells of ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice, and found that IGFBP5 knockdown significantly rescued impaired GSIS in ALDOB^{Ins2-/-} mice islets compared to ALDOB^{fl/fl} mice (Fig. 5j). Moreover, IGFBP5 knockdown largely improved the impaired glucose tolerance in ALDOB^{Ins2-/-} mice without affecting insulin sensitivity (Fig. 5k,l). Notably, ALDOB^{Ins2-/-} mice with IGFBP5 knockdown did not completely reverse the decreased insulin secretion compared to IGFBP5 knockdown in ALDOB^{fl/fl} mice, which can be explained by the higher IGFBP5 expression in ALDOB^{Ins2-/-} mice islets compared to the control even after knocking down IGFBP5 (**Extended Data Fig. 8f,g**).

Collectively, these data suggest that ALDOB deficiency in β -cells impairs insulin secretion largely through upregulating IGFBP5.

ALDOB/IGFBP5 interactions dictate IGFBP5 nuclear translocation to regulate GNAI2 transcription and cAMP signaling critical for GSIS

Next, we set out to study the mechanisms by which ALDOB in β -cells affects GSIS *via* IGFBP5-cAMP pathways. The IGFBP5 comprises three domains: N-terminal conserved globular domain with a signal peptide and IGF binding region, central non-conserved linker domain with a proteolytic cleavage site, and C-terminal conserved globular domain containing a nuclear localization sequence (NLS)⁵³. UniProt predicts that IGFBP5 can be distributed in extracellular, cytoplasmic, and nuclear locations⁵⁴. Next, we performed cellular fractionation experiments and found that IGFBP5 localized both in the cytoplasm and nucleus of Ins1 cells (**Extended Data Fig. 9a,b**), consistent with immunofluorescence results (**Extended Data Fig. 9c**). However, we found that IGFBP5 predominantly localized in the nucleus in primary islets. Moreover, ALDOB deficiency in primary mouse islets of ALDOB^{Ins2-/-} or Ins1 cells significantly increased nuclear IGFBP5 (Fig. 6a and **Extended Data Fig. 9d,e**). Because our data suggest that IGFBP5 regulates insulin secretion in β -cells through a non-canonical mechanism, with no apparent changes in the IGF1R signaling pathway, we hypothesized that IGFBP5 affects GSIS likely by modulating the transcription of genes involved in the cAMP–PKA pathway in β -cells, and ALDOB affects the nuclear translocation of IGFBP5.

To test whether ALDOB and IGFBP5 physically interact with each other, we performed co-immunoprecipitation (Co-IP) experiments with various mutants of the three domains (M1: N terminus, M2: C terminus, M3: N + C) and (**Extended Data Fig. 9f**). Interestingly, C-terminal domain mutation alone (M2) or with N terminus (M2 and M3) of IGFBP5 significantly decreased the nuclear IGFBP5, whereas N terminus mutation did not affect the nuclear levels of IGFBP5 (**Extended Data Fig. 9g,h**), suggesting that C terminus is critical for ALDOB/IGFBP5 interaction. Notably, molecular dynamic simulations predicted that ALDOB interacts with sites close to NLS sequence on the C terminus of IGFBP5 (**Extended Data Fig. 9i**). We further performed Co-IP experiments with mutants of IGFBP5, and found that the interaction

of ALDOB and IGFBP5 was significantly attenuated after IGFBP5 C-terminal domain was mutated (**Extended Data Fig. 9j-m**). These results suggest that ALDOB interacts with the NLS of IGFBP5 to affect the nuclear translocation of IGFBP5, and ALDOB deficiency led to the increase of nuclear IGFBP5.

Although a few studies suggested that IGFBP5 can migrate into nucleus to function as a regulator for gene transcription in cardiomyocytes⁵³, the similar roles of IGFBP5 in β -cells to affect gene transcription remains to be determined. Next, we performed chromatin immunoprecipitation sequencing (ChIP-seq) to explore the target genes and motifs bound by IGFBP5 using Ins1 cells overexpressing IGFBP5 (overIGFBP5) and control (overNC). ChIP-seq was performed in parallel for RNA Polymerase II (Pol II) phosphorylated at 5th Serine position of the C-terminal domain (CTD) (Pol II S5P), preferentially associated with the gene promoters and transcription start site (TSS). Strikingly, IGFBP5 peaks were highly enriched near TSSs in Ins1 cells (**Extended Data Fig. 10a**), suggesting that IGFBP5 possibly regulates gene transcription. *De novo* consensus motif discovery showed that IGFBP5-enriched DNA sequences shared common motifs and common transcriptional factors (**Table S3**), such as NRF1, highlighting an extremely high specificity of the IGFBP5 chromatin association. Notably, KEGG pathway enrichment analysis of IGFBP5-enriched genes showed that cAMP pathway is one of the most significantly altered pathways (Fig. 6b). Among these target genes of IGFBP5 that may be involved in cAMP-PKA pathways, the *Gai2* (*GNAI2*) was significantly enriched in the *GNAI2* promoter region in the OE-IGFBP5 group compared to the NC group (**Extended Data Fig. 10b**), and is also the only GPCR-related genes which significantly increased in ALDOB^{Ins2^{-/-}} mice islet in proteomics and transcriptomic analysis (Fig. 3b). To further validate that *gnai2* is the target genes regulated by IGFBP5, we designed sixteen primers including the 2 kb upstream of *gnai2* promoter to confirm the binding of IGFBP5 and *GNAI2* genome. ChIP-PCR experiments showed that IGFBP5 significantly binds to primers 10 and 11, which covered 1126 to 1400 bp upstream of *gnai2* promoter (Fig. 6c and **Table S4**). Correlation analysis using a public data database shows a statistically significant positive correlation between IGFBP5 and *GNAI2* in β -cells (**Extended Data Fig. 10c**). Furthermore, knock down IGFBP5 in Ins1 cells also decreased *GNAI2* expressions, whereas overexpression of IGFBP5 upregulated *GNAI2* expressions (Fig. 6d,e and **Extended Data Fig. 10d-f**). Moreover, *GNAI2* overexpression in Ins1 cells exhibited significantly reduced cAMP levels after 16.7 mM glucose treatment (**Extended Data Fig. 10g-i**), suggesting that IGFBP5 possibly inhibits cAMP-PKA pathway and GSIS through increasing *GNAI2* expressions in ALDOB KO β -cells.

To further interrogate how ALDOB in β -cells is involved in IGFBP5-regulated *GNAI2* expression, we first measured *GNAI* expressions in primary mouse islet and found that only *GNAI2* expression was specifically increased in ALDOB^{Ins2^{-/-}} islets (Fig. 6f,g and **Extended Data Fig. 11a**). Interestingly, both *GNAI2* and IGFBP5 expressions were significantly upregulated in Cluster 4 of β cells in ALDOB^{Ins2^{-/-}} compared to the control mice (Fig. 4e and **Extended Data Fig. 11b**). Consistently, ALDOB knockdown led to more IGFBP5 enrichments in the upstream of *gnai2* promoter region (1126-140kb) in Ins1 cells (**Extended Data Fig. 11c**). Next, to test the functional consequence of ALDOB deficiency-induced upregulation of *GNAI2* on GSIS through cAMP-PKA pathways, we inhibited *GNAI2* activity and expressions by using a *GNAI* inhibitor 0990CL and *GNAI2* siRNA, respectively. On one hand, treatment of

mouse islets with 0990CL significantly rescued the decreasing PKA substrate phosphorylation in ALDOB^{Ins2^{-/-}} mouse islets (Fig. 6h,i). On the other hand, genetic knockdown of *gnai2* in mouse islets using two *gnai2* siRNA sequences improved GSIS in ALDOB^{Ins2^{-/-}} mouse islets *in vitro* (**Extended Data Fig. 11d-f**). Importantly, knocked down GNAI2 in β -cells *in vivo* via intraperitoneal injection of shGNAI2 AAV virus significantly improved glucose tolerance in ALDOB^{Ins2^{-/-}} mice and GSIS of primary islets, without affecting insulin sensitivity (Fig. 6j-k and **Extended Data Fig. 11g-i**).

All these data demonstrate that ALDOB/IGFBP5 interactions regulate nuclear translocation of IGFBP5 to bind to the promoter of target gene *gnai2*, thereby modulating the insulin secretion via cAMP-PKA signaling pathways. Consistently, β cell-specific ALDOB deficiency inhibits GSIS by upregulating GNAI2 expression.

GLP-1 couples to G_{as} and G_{ai2}, and GNAI2 inhibition synergizes insulin-stimulating efficacy of GLP-1R agonists for T2D

The GLP-1R agonists, such as Semaglutide and Liraglutide, have become the widely used medication for T2D. However, clinical studies have found that long-term use of these medications can lead to reduced efficacy due to the desensitization of GPCR signaling, requiring a period of drug withdrawal in therapy⁵⁵. In our scRNA-seq data, *glp1r* is also a highly expressed GPCRs in Cluster 4, accompanied by upregulation of *gnai2* and *igfbp5* (**Extended Data Fig. 12a**), consistent with the hypothesis that ALDOB-IGFBP5-GNAI2 axis may affect the therapeutic response to GLP-1R agonists. Next, we performed Bioluminescence Resonance Energy Transfer (BRET) assay to examine G proteins of GLP-1R and found that 10nM GLP1 treatment activated G_{ai2} in addition to G_{as} (Fig. 7a and **Extended Data Fig. 12b**), indicating that GLP-1R and G_{ai2} are also coupled. Next, we treated Ins1 cells with 10 nM semeglutide, and found a significant increase in GNAI2 expression after 24-hour exposure (Fig. 7b,c). In primary islet cells, expression of GNAI2 was significantly increased after 48h of semeglutide treatment (**Extended Data Fig. 12c**). Furthermore, primary islet cells were pretreated *in vitro* with 10nM GLP-1 or 10nM semeglutide for 48h, followed by treatment with 16.7 mM high glucose and 10nM GIP incretin for 1 h to induce insulin secretion. The results showed that islet sensitivity to glucose and GIP was reduced after GLP-1 or semeglutide pretreatment compared with control group (Fig. 7d). Furthermore, we tested GSIS ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mouse islets with liraglutide. Interestingly, the insulin secretion was significantly impaired in ALDOB KO compared to the ALDOB^{fl/fl} (Fig. 7e), suggesting that ALDOB depletion-induced GNAI2 expression leads to reduced efficacy of GLP-1R agonists.

To test whether GNAI2 inhibition improves therapeutic efficacy of GLP-1R agonists, we used shGNAI2-AAV to specifically knockdown GNAI2 in mouse β cells under a HFHS condition and treated these mouse islets with semaglutide *in vitro* (**Extended Data Fig. 12e-g**). Compared to control mice, GNAI2 knocking down significantly improved the anti-diabetic functions of liraglutide on GSIS and glucose tolerance without affecting insulin sensitivity (Fig. 7f and **Extended Data Fig. 12h,i**). To provide evidence that GNAI2 inhibition synergizes β -cell insulin secretion, we treated mouse and healthy human primary islets

with 0990CL and semaglutide. Compared to semaglutide alone, combination of 0990CL and semaglutide increased GSIS in mouse and human primary islets (Fig. 7g,h).

Together, all these data strongly suggest that GNAI2 inhibition synergizes the efficacy of GLP-1R agonists on insulin secretion from β -cells.

Discussion

Islet β -cells play a pivotal role in maintaining metabolic homeostasis as the exclusive endocrine cells responsible for insulin secretion, and impairment in insulin secretion may lead to diabetes^{1,2}. Insulin secretion from β -cells is achieved by integrating IR/IGF1R and GPCR-cAMP-PKA signaling pathways upon exposure to various nutrients and hormonal stimuli^{15–19,30}. However, it remains poorly defined how glucose metabolism couples with various GPCRs to affect insulin secretion in T2D. In this study, we identified a novel mechanism by which a glycolytic enzyme, ALDOB, exerts non-enzymatic function to modulate insulin secretion (Fig. 7i). In this model, the major aldolase isoform ALDOA fulfils its canonical enzymatic function to generate ATP from glycolysis and TCA cycle. On the other hand, ALDOB interacts with IGFBP5 to modulate its nuclear translocation to control the transcription of GNAI2. In turn, this non-enzymatic function of ALDOB is critical for tuning the inhibitory (G_{ai2}) and stimulatory (G_{as}) G proteins upon GLP-1 activation, thereby converging on the cAMP-PKA pathways to potentiate insulin release. In addition, GNAI2 inhibition synergizes insulin secretion stimulated by GLP-1R agonists, highlighting a potential therapeutic approach for the treatment of T2D, and overcoming desensitization of GLP-1R signaling.

First, this study identifies a novel mechanism by which interactions between stimulatory and inhibitory GPCRs play an important role in insulin secretion from pancreatic β -cells with tremendous therapeutic implications in T2D. Although β -cells express around 300 GPCRs on the cell surface, only a few has been successfully targeted to increase insulin production in T2D^{4,26,56}. For recent years, GLP-1R agonists have dominated the therapeutic landscape for T2D and body weight control^{4,22–25}. Recent clinical data have indicated better clinical benefits in T2D patients by dual agonist targeting GLP-1 and GIP receptors^{5,26}. Quintuple agonists with GLP-1R, GIPR and PPAR α /g/d have shown efficacy in the treatment of obesity and T2D⁵⁶. On the other hand, GPCRs in β -cells can also couple to the inhibitory subunit of the heterotrimeric G proteins, Gai, to decrease insulin secretion and β cell expansion^{17,27,28}. For example, catecholamines and somatostatin inhibit insulin secretion in β cells by acting on α 2A adrenergic receptor (ADRA2A) and Somatostatin Receptor 3 (SSTR3), respectively^{16,57,58}. Thus, the overall insulin secretion from β -cells may be dictated by the interplays between stimulatory GPCRs and inhibitory GPCRs, converging on the cAMP signaling^{29,30}. Furthermore, desensitization of GPCR signaling due to prolonged exposure of GLP-1R agonists has been implicated as a potential concern^{31–33}. In this study, we uncovered that ALDOB exerts non-enzymatic function to regulate insulin secretion through IGFBP5-GNAI2-cAMP axis. Pharmacological or genetic inhibition of GNAI2 synergizes insulin secretion by GLP-1R agonists, and potentially overcome the desensitization of GPCR signalling due to long-term exposure.

Furthermore, validation of this target by improving the specificity of GNAI2 and optimizing pharmacokinetics/pharmacodynamic properties of these inhibitors are expected to be a promising therapeutic strategy for T2D.

Second, our study has uncovered an under-appreciated intrinsic metabolic factor, ALDOB, affecting the β -cell insulin secretion. Utilizing the human primary islet in conjunction with both db/db mice and the HFHS-induced diabetic mouse model, this study validated a significant upregulation of ALDOB during diabetes progression. Although ALDOB is the most significantly upregulated aldolase in the human and mouse islets of T2D, disruption of ALDOB in mouse β -cells impaired insulin secretion under physiological (Chow Diet feeding) and diabetic conditions (HFHS feeding). Similarly, knockdown of ALDOB in healthy human islets and rat islets also inhibited insulin secretion. On the other hand, the deletion of ALDOB in ALDOB^{fl/fl} mice exacerbated T2D, resulting in impaired glucose tolerance and diminished insulin secretion. This seemingly contradictory observation reflects a compensatory response of increased ALDOB expression in T2D due to high metabolic demands. Furthermore, our scRNA-seq analysis identified heterogeneous populations of β -cells among which a subset of *Igfbp5*⁺ mouse β -cells (Cluster 4) manifest ALDOB downregulation with impaired GSIS in ALDOB^{Ins2-/-} mice and T2D patients. Importantly, these *Igfbp5*⁺ β cells significantly increased around 15 folds in T2D compared to healthy human islets. Nonetheless, the precise mechanism underlying the compensatory upregulation of ALDOB expression warrants future investigation. Previous research indicated that hepatocyte nuclear factor 4 α (HNF4 α) can directly interact with multiple sites within the ALDOB promoter region, thereby playing a role in *aldob* transcriptional regulation⁵⁹. Moreover, HNF1 α has been implicated in the regulation of metabolic enzyme genes, including ALDOB⁶⁰. Fructose has been shown to trigger the translocation of carbohydrate response element-binding protein (ChREBP) from the cytoplasm to the nucleus, consequently activating *Aldob* gene expression^{48,61}. Our findings demonstrate that a diabetic microenvironment characterized by elevated glucose and insulin levels can stimulate increased ALDOB expression. Furthermore, downstream of cAMP-PKA signaling, ChREBP phosphorylation facilitates its nuclear translocation, suggesting a potential role for ChREBP in ALDOB transcriptional regulation within islet cells⁶².

Third, the IGFBP family comprises six members that exhibit high affinity for IGF and modulate IGF half-life, thereby influencing its interaction with the IGFR⁶³. While circulating IGFBP5 extends the half-life of IGF and enhances IGFR phosphorylation, locally expressed IGFBP5 exerts the opposite effect⁶⁴. Notably, in mice with β cell-specific Akt1 overexpression, IGFBP5 knockout resulted in increased mass and size of islet β cells, indicating that IGFBP5 may not be essential for the IGF1-Akt pathway to stimulate islet β -cell proliferation¹⁴. The secretion of IGFBP5 from islet cells into the extracellular environment is minimal, with a predominant intracellular function beyond canonical IGF signaling. While previous studies have suggested a nuclear role for IGFBP5, particularly in its interaction with the NFAT4 transcription factor to modulate the expression of microfibril-associated protein 5 (MFAP5) and its involvement in cardiac fibrosis, the precise binding motifs of IGFBP5 and its comprehensive downstream regulatory effects remain unexplored⁵³. To address this gap, we conducted Chip-seq experiments on IGFBP5 and identified

potential binding motifs. However, further investigations employing IP proteomics of IGFBP5 are necessary to identify its interacting transcription factors and integrate this information with the predicted motifs. Our findings also reveal that IGFBP5 downstream targets include GNAI2, impacting the cAMP signaling pathway. To confirm the direct transcriptional regulation of GNAI2 by IGFBP5, additional luciferase reporter gene assays are warranted in conjunction with Chip-PCR experiments. Furthermore, the influence of IGFBP5 on the cAMP signaling pathway through GNAI2 raises the question of whether IGFBP5 regulates the transcription of other genes within this pathway, necessitating further validation studies.

Loss of ALDOB in islet cells not only promoted IGFBP5 entry into the nucleus but also upregulated IGFBP5 transcriptional and protein level expression, but how ALDOB affects IGFBP5 expression remains unclear. IGFBP5 expression is subject to modulation by systemic hormones, local factors, and various transcriptional regulators¹⁴. Notably, the IGFBP5 promoter region contains binding sites for AP2⁶⁵, cAMP-responsive element binding protein (CREB), c-myc⁶⁶, C/EBP, NF-1, and a progesterone response element⁶⁷. Studies have suggested that Akt-responsive elements within the IGFBP5 promoter can facilitate the upregulation of IGFBP5 expression via the p-IR-PI3K-Akt pathway⁶³. Given our previous findings demonstrating ALDOB interaction with Akt and the promotion of Akt phosphorylation upon ALDOB loss⁶⁸, it is conceivable that ALDOB may influence IGFBP5 expression by directly modulating Akt phosphorylation. It warrants future studies to explore the regulatory role of ALDOB on IGFBP5 expression within islet cells.

In summary, we present novel findings demonstrating the direct impact of ALDOB on insulin secretion in β -cells through GLP-1R-cAMP-PKA pathways. ALDOB deficiency in mouse β -cells releases IGFBP5 to enter the nucleus, resulting in an IGF-independent nuclear function that augments transcriptional repression of cAMP signaling pathways through upregulating GNAI2, thereby diminishing insulin secretion. Interestingly, genetic or pharmacological inhibition of GNAI2 synergizes insulinotropic effectiveness of GLP-1R agonists, highlighting a potential therapeutic strategy for T2D.

Methods

Cells

HEK293 cells (ATCC CRL-1573) were maintained in DMEM (Gibco 12430112) supplemented with 10% fetal bovine serum (FBS; Gibco A3160801). Rat INS-1E cells (ATCC HB-8065) MIN6 cells (a kind gift from Zhuoxian Meng Laboratory, Zhejiang University) were maintained in RPMI 1640 medium (Gibco 21875059) supplemented with 10% FBS + 0.05mM β -mercaptoethanol.

Human islets and pancreatic tissue

Human pancreas and human islets for research were collected from the islet transplantation center of Changzheng Hospital, Shanghai, China. Islet isolation was approved by the Human Research Ethics

Board at the Changzheng Hospital (Pro00013094). All donors' families gave informed consent for the use of pancreatic tissue in research. The pancreas batches used and donor characteristics are summarized in Supplementary Table 1. Human primary islets were derived from four female and two male donors (Supplementary Table 2). Donors had a body mass index of between 19.5 and 31. Human pancreatic islets from nondiabetic donors were cultured in human complete medium composed of CMRL-1066 (Sigma C0422) supplemented with 50 µg/ml streptomycin, 50 U/ml penicillin, 10% FBS, 10 U/ml heparin sodium, and 90 U/ml insulin.

siRNA-mediated gene silencing in human and primary islets

Human islets and primary islets isolated from rat were for ALDOB gene silencing. 300 IEQ islets were transiently transfected with 100 nM of Control siRNA or ALDOB siRNA (sense-GGCGCUGUGUGCUAAAGAUA dTdT anti-sense AUCUUUAGCACACAGCGCCdTdT) using Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific) according to the manufacturer's instruction. At 48 h after transfection, islets were harvested for protein extraction and Western blotting analysis of ALDOB protein levels or perfusion study to evaluate GSIS function. Primary islets were isolated from C57BL6/J wild-type mice and ALDOB^{Ins2-/-} mice for IGFBP5 and GNAI2 gene silencing. 300 IEQ islets were transiently transfected with 100 nM of Control siRNA or IGFBP5 siRNA (sense-GUGACCGCAAAGGAUUCUATT anti-sense UAGAAUCCUUUGCGGU CACTT) or GNAI2 siRNA (sense1-GAGGACCTGAATAAGCGCAAA anti-sense-1 TTTGCGCTT ATTCAGGTCCTC; sense2-GCTGCCTACTACCTGAACGA anti-sense-2 TCGTTCAGGTAGTA GGCAGC) using Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific, 13778075).

Mouse models

All the mice used in this study were raised in SPF (Specific-pathogen Free) animal house of Shanghai Institute of Nutrition and Health (SINH), and the mice of physiological model were fed chow diet. DIO or T2D mice were fed Sucrose and 40kcal% Fat (HFHS, D12327). ALDOB-floxed (ALDOB^{fl/fl}) mice were generated as previously described⁶⁹. Ins2-Cre mice were used to generate β-cell-specific ALDOB KO (ALDOB^{Ins2-/-}) mice. All the above mice were crossed with C57BL/6J mice for at least eight generations and maintained on the C57BL/6J genetic background. db/db diabetic mice and their lean control m+/db mice were originally obtained from the Jackson Laboratory and maintained on the C57BLKS genetic background. Our experimental animals included the following: ALDOB control mice (ALDOB^{fl/fl} mice), ALDOB islet beta cell specific knockout mice (ALDOB^{Ins2-/-} mice), ALDOB islet beta cell specific R304A mutant mice (ALDOB-R304A), ALDOA control mice (ALDOA^{fl/fl} mice), ALDOA islet beta cell specific knockout mice (ALDOA^{Ins2-/-} mice). Mice were numbered and genotyped on day 14 of life. PCR identification using primers Aldob-flox-F and Aldob-flox-R, if only one band and size of 351bp, Neo delete

positive homozygote (flox/flox). On this basis, Ins2-cre primer was used to identify whether cre gene was carried, and cre band size was 203bp. ALDOB-R304A mice need to be identified by using 3 pairs of primers. If the result is that the product band size of primer 1 is 546bp, the product band size of primer 2 is 600bp, and the product band size of primer 3 is 244bp, the mouse is ALDOB-R304A KI/KI mouse. On this basis, Ins2-cre primer was used to identify whether cre gene was carried, and the band size was 203bp. (ALDOB-flox-F: CTGGAGACAATGAGTATCTG CTC, ALDOB-flox-R: TGGATTAAGTAG TGCTTGCACT, Ins2-cre-F: ACTCCAAGTGGAGGCT GAGA, Ins2-cre-R: TCCTTCCA CAAACCCATAGC, R304A-F1: CTAAAGCCAAACATGGTGAC TGC, R304A-R1: CCGT AGCTCCAATCCTTCCATTTC, R304A-F2 GAAGCAGCACGACT TCTT CAAG, R304A-R2: TTAGGAAAGGACAGTGGGAGTGGR, 304A-F3: CTAATGTCCCAGAGAC CATCATCC).

Isolation of primary islet

Mouse islet isolations were performed essentially by the procedure described by Carter et al⁷⁰. Briefly, in anesthetized mice, the common bile duct was clamped off near the junction with the small intestine and cannulated. The pancreas was perfused with 2 mL of 0.5 mg/mL collagenase P (Roche, Cat. No. 11213857001) solution (10 mM HEPES, 1 mM MgCl₂ and 5 mM glucose in Hank's Balanced Salt Solution without CaCl₂, MgCl₂, and phenol) and then removed from the mouse and kept with 1 mL of collagenase P solution on ice. Pancreatic digestion was performed in 50 mL tubes at 37°C for 14 min, and facilitated with hand-shaking for approximately 30 times to complete the separation of tissue. The reaction was stopped by dilution with 15 mL wash buffer (1 mM CaCl₂·2H₂O, 10 mM HEPES, 1 mM MgCl₂ and 5 mM glucose in Hank's Balanced Salt Solution without CaCl₂, MgCl₂, NaHCO₃ and phenol, 10%FBS) on ice, and the digested pancreas was transferred into a 50 mL Falcon tube for washing three times with wash buffer. After the final wash, the pancreas suspension was filtered through a 70 µm cell strainer, and the filtrate maintained in complete RPMI (Gibco, Cat. No. 11875-085) media (10% FBS, 2% Pen/Strep and 2 mM GlutaMax in RPMI 1640 basal media). Individual islets were then picked up manually under the stereomicroscope, and cultured in RPMI 1640 complete media overnight for further experiments.

Measurement of insulin secretion in mouse islets

Krebs–Ringer Bicarbonate HEPES (KRBH) buffer (NaCl 140 mM, KCl 3.6 mM, NaHCO₃ 2 mM, NaH₂PO₄ 0.5 mM, MgSO₄ 0.5 mM, CaCl₂ 1.5 mM and HEPES 10 mM; pH 7.4) containing 0.1% BSA supplemented with 2.8 mM or 16.7 mM glucose. Islets incubated overnight are picked into Krebs buffer (2.8 mM Glucose) and washed 1–2 times to remove medium. The islets were then cultured in Krebs buffer (2.8 mM Glucose) and incubated at 37°C for 1 h. According to the experimental design, islets were treated with different drugs and then picked into twelve-well plates at 30 IEQ per well. Add 1 mL Krebs buffer (2.8 mM Glucose) to each well and incubate in incubator for 1 h. Islets are gathered in the middle of the well by centripetal force, and the culture medium is collected from the periphery, that is, the supernatant stimulated by low glucose. Then, 1 mL Krebs buffer (16.7 mM Glucose) was added to each well and

incubated for 1 h. Supernatants stimulated by high glucose were collected in the same way. Islets were picked for protein quantification. The supernatant was diluted 3–10 times and then the insulin content was detected by ELISA kit (Beyotime Biotechnology, PI602).

Dynamic insulin secretion perifusion analysis

Primary murine islets were cultured overnight after isolation before perifusion assays. For each assay, perifusion chambers were loaded with 150 IEQ islets. Islets were first perifused with Krebs's buffer (135 mM NaCl, 3.6 mM KCl, 0.5 mM NaH₂PO₄, 1.5 mM CaCl₂, 2 mM NaHCO₃, 10 mM Hepes, and 0.1% BSA, pH 7.4) containing 2.8 mM glucose for 30 min, followed by perifusion with Krebs's buffers containing 2.8 mM glucose, 16.8 mM glucose, and 2.8 mM glucose for 25, 30, and 20 min, respectively, under 1 ml/min flow rate. Fractions were collected every minute. Islets were recollected from the perifusion chamber after assay to obtain total protein content via BCA protein assay. Insulin secretion at each time point was quantified using HTRF insulin assay kit (62INSPEC; Cisbio) according to manufacturer's instructions and normalized to total protein content. To quantify the biphasic insulin secretion, the phase I and phase II of insulin secretion were manually separated based on the plateau formation of the II phase and quantified by calculating the area under curve (AUC). Insulin levels were normalized to the average baseline insulin secretion in buffer containing low glucose (2.8 mM) and presented as fold insulin secretion⁷¹.

Measurements of intracellular ATP and cAMP

To measure the intracellular ATP levels, ten islets with similar size per condition were incubated in the KRBH buffer with 2.8 mM glucose for 30 min, followed by treatment with glucose (16.7 mM) or pyruvate (10 mM) for 10 min. Islets were lysed to release ATP and the released ATP was measured using the ATP Determination kit (Thermo Fisher Scientific A22066) and normalized with the islet numbers. Ins1 cells were cultured in 10cm dish and Ins1 cells were collected under 5 x 10⁶ different conditions. Ins1 cells were first incubated in Krebs buffer (2.8 mM Glucose) for 1 h, then Krebs buffer (2.8 mM Glucose) and Krebs buffer (16.7 mM Glucose) for 1 h, respectively, and washed 3 times with 1x PBS. Cells were scraped with cells by adding 1 mL of 1x PBS (with 0.5 mM IBMX) and 100 µL was taken for cellular protein quantification. The remaining 900 µL cell fluid, 800g, was centrifuged at 4°C for 3 min and the supernatant was removed. 400 µL solvent (4:4:2 = methanol: acetonitrile: water), 5 µL internal standard (10 mM ¹³C glutamine), 0.5 mM IBMX were added and sonicated in ice bath for 5 min. Centrifuge at 14000r for 20 min at 4°C, take supernatant and dry sample with nitrogen blowing instrument, add 70 µL mobile phase A for redissolution. Centrifuge at 14000r, 4°C for 10 min, take 60 µL of supernatant and put it into the sample. Mobile phase A: 1.54 g ammonium acetate + 950 mL water + 2.8 mL 25% ammonia + 50 mL acetonitrile, adjust pH to 9.0; mobile phase B: 100% pure acetonitrile. Injection volume 10 µL, XBridge Amide column (186004868), Thermo Q Exactive Plus.

Calcium imaging and analysis

For calcium imaging, isolated islets from ALDOB^{fl/fl} or ALDOB^{Ins2-/-} global deficient mice were incubated at room temperature for 30 min with 4 mM Fluo-4 AM (Thermofisher, Cat. No. F14201) prior to live cell imaging performed with an LSM880 two-photon inverted microscope (Carl Zeiss) using a heated stage-top incubator (Pecon GmbH). Relative fluorescence was captured after exposure to 2 mM or 16.7 mM glucose for multiple islets from each group, and images were analyzed using ImageJ software.

scRNA-seq and data analysis

Pancreatic islets were isolated and cultured in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin. Then, islets were dissociated with 0.05% trypsin (Corning) for 10 min at 37°C. If the islet pellets were slightly agglomerated, the islets were digested with Tryple at room temperature for 10min and resuspended in 1× PBS + 0.05% BSA. Cellular viability was higher than 95% as determined by using Trypan Blue assay, and doublets represented less than 5% of total cells. Single-cell suspensions were sequenced by the Chromium Single Cell 3' Reagent Kit v3 (10x Genomics) and 10x Genomics' Chromium Controller at the Shanghai OE Biotech.

The FASTQ files were processed and aligned to GRCh38 human reference genome using Cell Ranger software (version 8.0.1) from 10x Genomics, with unique molecular identifier (UMI) counts summarized for each barcode. The UMI count matrix was then analyzed using Seurat (version 4.0.0) R package⁷². To remove low-quality cells and likely multiplet captures, a set of criteria were conducted: Cells were filtered by (1) gene numbers (gene numbers < 200), (2) UMI (UMI < 1000), (3) log10GenesPerUMI (log10GenesPerUMI < 0.7), (4) percentage of mitochondrial RNA UMIs (proportion of UMIs mapped to mitochondrial genes > 20%) and (5) percentage of hemoglobin RNA UMIs (proportion of UMIs mapped to hemoglobin genes > 5%). Subsequently, the DoubletFinder⁷³ package (version 2.0.3) was used to identify potential doublets. Top 2000 highly variable genes (HVGs) were calculated using the Seurat function FindVariableGenes (mean.function = FastExpMean, dispersion.function = FastLogVMR). Principal-component analysis (PCA) was performed to reduce the dimensionality with RunPCA function. The FindAllMarkers function (test.use = presto) was used to identify marker genes of each cluster. Differentially expressed genes (DEGs) were selected using the function FindMarkers (test.use = presto). P value < 0.05 and |log2foldchange| > 0.58 was set as the threshold for significantly differential expression. GO enrichment and KEGG pathway enrichment analysis of DEGs were respectively performed using R (version 4.0.3) based on the hypergeometric distribution. To find major subclusters of beta cells that are transcriptionally distinct and not rare (< 1% of beta cells), we set the resolution parameter to 0.4.

Single-cell RNA-seq data was analyzed using SCENIC to identify transcription factor (TF)-target gene regulatory networks (regulons). SCENIC infers co-expression modules linking TFs to potential target genes, then assesses regulon activity per cell using AUCell. This yields a binary regulon activity matrix,

indicating activated regulons in each cell. The results were used for downstream analyses, including clustering and trajectory inference. Single cell pseudotime trajectories were inferred using Slingshot. Starting from user-defined clusters, Slingshot constructs smooth lineage curves and assigns cells pseudotime values along branching paths. Differential expression analysis was performed across trajectories.

Immunoblotting

200 IEQ islets per sample were washed in PBS twice and then lysed and sonicated in RIPA buffer (10 mM HEPES, 50 mM β -glycerol phosphate, 1% Triton X-100) supplemented with inhibitor cocktail (Selleck, B14002). Proteins were separated by SDS-PAGE (Tsingke, TSP024-15) and transferred to PVDF membranes (Millipore, #IPVH00010). After blocking with 5% milk, membranes were incubated with primary antibodies overnight at 4°C, followed by secondary antibodies. Protein bands were visualized using ECL and detected by imaging system (Tanon, 5200Multi). The following antibodies were as follows: ALDOB (1:1000, Proteintech #18065-1-AP), GNAI2 (1:1000, ABclonal #9624S), IGFBP5 (1:1000, Abcam # Ab254324), Phospho-PKA Substrate (1:1000, CST # A20930), FLAG (1:1000, Proteintech #20543-1-AP), Myc (1:1000, ABclonal #1 AE070), α -Tubulin (1:1000, Proteintech #14555-1-AP), Lamin B1 (1:1000, Proteintech #12987-1-AP), IGF-I Receptor β (1:1000, Selleck #F0332), IGF-I Receptor β (Tyr980 (1:1000, Proteintech # F1451), INSR- β subunit (1:1000, CST #3025), p-IR Y1146 (1:1000, CST #3021), Phospho-AKT-S473 (1:1000, CST #4060), AKT (1:1000, CST #2938), G6PD (1:1000, Proteintech # 66373-1-Ig), GLUT1 (1:1000, CST #12939S), PC (1:1000, Proteintech #116588-1-AP). Protein detection was performed by the use of the HRP-conjugated secondary antibodies: anti-HRP-labeled Streptavidin (A0303, Beyotime, 1:1,000 for IB); anti-PARP (#9542, CST, 1:1,000 for IB); HRP-conjugated Goat Anti-Rabbit (SA00001-2, Proteintech, 1:5,000 for IB); HRP-conjugated Goat Anti-Mouse (SA00001-1, Proteintech, 1:5,000 for IB).

Quantitative RT-PCR analysis

Total RNA was extracted using Trizol reagent (Thermo Fisher Scientific, 15596018) and reverse transcribed using HiScript II Q RT SuperMix (Vazyme Biotech, R223-01). qPCR was performed using Taq Pro Universal SYBR qPCR Kit (Vazyme Biotech, Q712-03) with acta1 as internal control, as previously described. Primer sequences are as follows: mouse-Aldob, 5'- GAAACCGCCTGCAAAGGATAA-3' and 5'- GAGGGTCTCGTGGA AAA GGAT-3'; mouse-Aldoa, 5'-CGTGTGAATCCCTGCATTGG-3' and 5'- CAGCCCCTGGGT AGTTGTC-3'; mouse-Aldoc, 5'- AGAAGGAGTTGTCCGATATTGCT-3' and 5'-TTCTCCA CCCCAATTTGGCTC-3'; mouse-Igfbp5, 5'-CCCTGCGACGAGAAAGCTC-3' and 5'- GCTCTTTTCGTTGAGGCAAACC-3'; mouse-Igfbp1, 5'-AAGGCACAGGAGACATCAG-3' and 5'- TATCTGGCAGTTGGGGTCTC-3'; mouse-Igfbp2, 5'-AGCATGGCCGGTACAACCT-3' and 5'- CGCGCTGTCCGTT CAGA-3'; mouse-Igfbp3, 5'-ACCTGCTCCAGGAAACAT CAGT-3' and 5'- TTTCCACACTCCCAGCATT G-3'; mouse-Igfbp4, 5'-CAGAGCCGTACC CACGAAGA-3' and 5'-

GCCGTTGCGGTCACAGTT-3'; mouse-Gnai2, 5'-TGCCTTGAGT GTGTCTGCGTG-3' and 5'-CTCAGTGACGTTGGCAGTT G-3'; mouse-Gnai1, 5'-AAGCT GACTCGCCTTCCCAG-3' and 5'-GTAGTTTACAGTTCTCCACA CG-3'; mouse-Gnai3, 5'-GTGCAGTCCGTGTACAAGAG-3' and 5'-GATGAATGGATCCGAGCC AC-3'; mouse-Abcc4, 5'- TCCCTTGTCTGGCGAAGAC-3' and 5'-CGAAGACGATGACTCCCT CG-3'; mouse-Abcc8, 5'-TGAGCATTGGAAGACCCTCAT-3' and 5'-CAGCACCGAAGATAA GTTGTC-3'; mouse- Kcnj11, 5'- CTTGGAAGGCGTGGTAGAAA-3' and 5'-TAGAATCT CGTCA GCTAGGTAGG-3'; mouse-Kcnj8, 5'- GGCACACAAGAACATCCGAGAG-3' and 5'-TGCAGAGG AAGGACATGGTGA-3'; mouse-Kcnn1, 5'-AGCCAGCAGCCCACAAATT C-3' and 5'-GTCCAAG GGGCTCTGGAAAC-3'; mouse-Kcnn2, 5'-CCTCTCTCGCACCAC CAC-3' and 5'- AGAATACAG CGACGCCTTGT-3'; mouse-Kcnn3, 5'-CCAAGCGGATCA AGAATGCTGC-3' and 5'- GACGCT CCTCAACTGGTGGATA-3'; mouse-Kcnn4, 5'-CGG GGCACCTCACAGACACACT-3' and 5'-CGCCGCTGACTCCTTCATCTCT-3'; mouse-Cacna1a, 5'- AGGCTGCTCAAGTTTTCTCG-3' and 5'-CAGATTGGTCCTTTGGCACT-3'; mouse-Cacna1b, 5'-GATGGAAGAAGCAGCCAATC-3' and 5'-ACGCAGATTCTGGAGC CTTA-3'; mouse-Cacna1c, 5'-ACAGAAGGGAACCTAGAGC AAG-3' and 5'-GGATCAGG AAGGTGGCATAGAA-3'; mouse-Cacna1d, 5'-ACACCTTGATGTG GTCACCTCTGCT-3' and 5'-TGTCCCATCACTGTTGAGAGGCAT-3'; mouse- Cacna1s, 5'-TTTG GAGATCCTT GGAATGTG-3' and 5'-AGTCTCATGACCCGGAACAG-3'; mouse-Cngb, 5'-GCTG GTCCCAGCCTTCAAGAGA-3' and 5'-CTCTTCTGAGATGAGTTTTTGTTCGGTC-3'; mouse-Cftr, 5'-CATCTTTGGTGTTCCTATGATG-3' and 5'-GTAAGGTCTCAGTTAGAATTGAA-3'; mouse-18s, 5'-AGCCCCTGCCCTTTGTACACA-3' and 5'-CGATCCGAGGGCCTCAC TA-3'.

Construction and interfering of AAV

ComplementaryDNA encoding EGFP-tagged mouse ALDOB was amplified from a pHBAAV-Insulin2-ZsGreen plasmid obtained from Hanheng biotech using primers (AAV-m-Aldob-KpnI-F: AGTAACCCCCAGCCCTAAGGTACCGCCACCATGGCTCACCGATT TC; AAV-m-Aldob-KpnI-R: ATCCTTG TAGTCGTTAATTAAGGTACCATAGGTGTAGGA GGCTGTGAAG) and then cloned into pShuttle–CMV vector (Agilent 240007) using KpnI recognition sites. AAV vector encoding GFP (AAV–Ins2–GFP) or Cre (AAV–Ins2–cre) under the control of rat insulin promoter was generated as previously described⁷⁴. To generate recombinant AAV particles, HEK293 cells were co-transfected with the AAV vector, pAAV2 rep cap and pAAV helper plasmids. AAVs were purified by polyethylene glycol/aqueous two-phase partitioning method and were titrated by real-time PCR analysis. The 12-week-old male ALDOA^{fl/f} mice were randomly assigned to i.p. injection with AAV encoding GFP or Cre (1×10^{12} genomic copy number per mouse). shRNAs targeting mouse IGFBP5 gene and scramble shRNA were inserted into the lentiviral GV248 vector to generate knockdown or control constructs, followed by validation with DNA sequencing. The following shRNAs were used in the experiments: shIGFBP5-F: GAAGCTGACCCAGTCCAA G; shIGFBP5-F: CTTGGACTGGGTCAGCTTC; shGNAI2-F: CCGGGCCGCTTACTACCTGAAT GATCTCGAGATCATT CAGGTAGTAAGCGGCTTTTTG; sh-Gnai2-R2: AATTCAAAAAGCCGC T TACTACCTGAATGATCTCGA GATCATTCA GGTAGTAAGCGGC.

H&E and immunofluorescence staining.

Each group of 6 mice aged 12 weeks was anesthetized with isoflurane and blood was drawn from the heart. Open the abdominal cavity and find the spleen. The pancreatic tissue connected with the spleen is the tail of the pancreas. The pancreatic tail was removed and placed in 4% paraformaldehyde fixative. After 24 hours of fixation at room temperature, paraffin embedment and tissue sectioning were performed. For staining insulin, glucagon and ALDOB in mouse islets, sections were incubated with corresponding primary antibodies (mouse anti-insulin monoclonal antibody, HyTest 2IP10-D6C4, 1:200 dilution; rabbit anti-glucagon polyclonal antibody, Sigma SAB4501137, 1:100 dilution; goat anti-ALDOB polyclonal antibody, Abcam ab153828, 1:100 dilution) overnight at 4°C. Panoramic MIDI laser confocal high-resolution panoramic imaging was used, and image analysis was performed with CaseViewer scanning software.

Metabolic flux analysis and Analysis of Intracellular Metabolites

Freshly isolated islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice were maintained in RPMI 1640 medium supplemented with 10% FBS for 12 h. Labelled ¹³C-glucose assay was performed and analyzed using GC-MS following established protocols. 400 IEQ islets with similar size from each mouse were selected, washed with warm PBS buffer and resuspended in 1 ml KRBH buffer for 30 min at 37°C with 5% CO₂. The medium was replaced with 1 ml fresh KRBH buffer containing 16.7 mM ¹³C-glucose. After 2 h, the reaction was stopped. 400 µL of pre-cooled 60% methanol aqueous solution containing 100 µM Norvaline internal standard was added to each tube and the cells were disrupted by repeated freeze-thaw. Add 300 µL chloroform into each tube, shake with shaker for 30s, centrifuge at 12,000 rpm for 10 min at 4°C, take the upper aqueous phase into a new centrifuge tube, freeze concentrate and dry. Add 70 µL of 20 mg/mL O-Isobutylamine Hydrochloride into each tube and place in a metal bath at 55°C for 20 min. Add 30 µL of derivatizing agent BSMFT (N-tert-Butyldimethylsilyl-N-Methyltrifluoroacetamide) into each tube, blow and mix well, and place in a metal bath at 55°C for 60 min. Centrifuge at 12000 rpm for 10 min at 4°C, and transfer the supernatant to the machine for detection. A 30 m × 0.25 mm × 0.25 µm HP-5ms GC column was used for metabolite separation. GC-MS data were analyzed to determine isotope labeling patterns and metabolite quantities. The data analysis, including isotope fractionation and quantification, was implemented in MATLAB. Metabolites were quantified based on total ion count peak areas, normalized to internal standards and protein concentrations. Natural isotope correction was applied to account for isotopic contributions during quantification. Retention times were verified using standards within the same batch of samples, ensuring accurate identification and quantification.

Measurement of Mouse Plasma Parameters

Mouse plasma parameters were measured using the following kits according to the manufacturers' protocols: plasma Insulin (Beyotime, PI602), blood C-peptid (Crystal Chem, 90050), HbA1c (Mlbio, ml401824), and IGFBP5 (Shanghai Ciobo, CB12085).

ChIP seq and ChIP-qpcr

ChIP assays were performed in INS-1E β -cells infected with adenoviruses for overexpression of IGFBP5 or GFP for 48 h (ABclonal, RK20258). Then, 8×10^6 cells were first cross-linked using 8 ml 1% formaldehyde in PBS for 10 min at room temperature, followed by washing with cold PBS and resuspension in 250 μ l of ChIP lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0 and protease inhibitor cocktails) through sonication (30% maximum output for 15 s with 3 min pause, four cycles) in an ice bath. A 5% portion of the lysates was used for extraction of total DNA for input gene quantification. The remaining lysates were mixed with 10 μ g rabbit anti-IGFBP5 polyclonal antibody (Abcam, Ab254324) and incubated at 4°C for 12 h. The immunocomplexes were precipitated using protein A agarose and washed sequentially with the following buffers: low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1 and 150 mM NaCl); high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1 and 500 mM NaCl); LiCl wash buffer (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA and 10 mM Tris-HCl, pH 8.1); and TE solution (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA). DNA–protein complexes were dissociated by heating at 65°C for 12 h, followed with treatment of Proteinase K (NEB P8107S) to degrade proteins. DNA was purified by ABclonal AFTSpin multifunctional DNA recovery and purification kit (RK30100). Chip Library construction was performed using the Novitzan VAHTS Universal DNA Library Prep Kit for Illumina V3. DNA was subjected to real-time PCR analysis with primers spanning the promoter of murine Gnai2 gene (Supplementary Table 3).

BRET experiments

Bioluminescence resonance energy transfer (Bret) assay was used to detect the level of G protein activation. All G protein particles were obtained from Trupath kit (Addgene, 100000163). 293T cells were inoculated in 6cm dish overnight and then transfected. Human-GLP 1R-PCDH plasmid, α plasmid, β plasmid and γ plasmid were added with 1ug each. 400 μ L opti-MEM and 15 μ L TransIT-2020 recommended in literature were added and mixed for 30 min. Gently added to the cells, 8 hours after transfection, the medium was changed. 96-well plates were prepared with 0.1 mg/mL poly-L-Lysine one night in advance. Inoculate at 50000/well and adhere overnight. Prepare Assay buffer (250 mL HBSS, 5 mL HEPES(1M), adjust pH to 7.4 with KOH). The Assay buffer was configured to a concentration of 10 nM GLP1. Add 20 μ L of corresponding drug and 80 μ L of substrate to each well. The substrate does not need to be incubated. The cycle detection method is used. The excitation wavelength is 515 nm and the emission wavelength is 395 nm. The detection cycle is 13 cycles.

Statistics

Statistics. Data were calculated and analyzed using GraphPad (GraphPad Prism v.8.4.3) and Microsoft Excel. Values are shown as mean $\pm$ s.e.m. as specified in each figure. Statistical significance was determined by two-tailed Student's t-test (for comparison of two experimental conditions) or one/two-way ANOVA (for comparison of three or more experimental conditions). The detailed individual statistical test is provided in each figure legend. In all statistical comparisons a P value < 0.05 was considered to indicate a statistically significant difference. P and n values are given in the figures or corresponding legends.

Declarations

Ethical statement:

All mouse experiments were approved by the Institutional Animal Care Committees of Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai, China.

Data availability

[Retain the original Methods text and topical subheadings here.] All raw data for scRNA-seq and 10x Visium generated in this study are deposited in the Genome Sequence Archive⁶⁸ at the National Genomics Data Center⁶⁹, the National Center for Bioinformation/Beijing Institute of Genomics and the Chinese Academy of Sciences (GSA for Human: HRA007511), which are available at <https://ngdc.cncb.ac.cn/gsa-human>. The spatial metabolomic data have been deposited in the OMIX, the China National Center for Bioinformation/Beijing Institute of Genomics and the Chinese Academy of Sciences under accession no. OMIX009098 (<https://ngdc.cncb.ac.cn/omix>). The processed scRNA-seq and Visium ST data are available from OMIX under accession no. OMIX010136. The spatial proteomic data is also available from

OMIX under accession no. OMIX009117. All processed data can be visualized and downloaded at the HMSMA website (<https://db.genomics.cn/stomics/hmsma>). Source data are provided with this paper.

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Author contributions

H.-Y.Y., S.-S.Z. and S.-X.L. conceived and supervised the study. H.-M.J. and Y.-F.F. designed and performed the experiments, analyzed and interpreted the data. H.-M.J. prepared the initial draft of the manuscript. X.-Y.Z. analyzed the single cell RNA-seq data. Y.-Q.W., G.-J.L., J.-W.L., R.L. and H.-R.M. contributed to the experimental work, data collection and data analysis. Z.-X.M. and Z.-F.C. provided scientific expertise, research resources and critical input into the interpretation of the results. H.-Y.Y., S.-S.Z. and S.-X.L. acquired funding and revised the manuscript. H.-M.J. and Y.-F.F. contributed equally to this work. All authors reviewed, edited and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Declaration of AI Assistant in scientific writing

The authors used Nature Research Assistant (<https://natureresearchassistant.com/>) for final checking of the manuscript and will take full responsibility for all the data presented in the manuscript.

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Figures

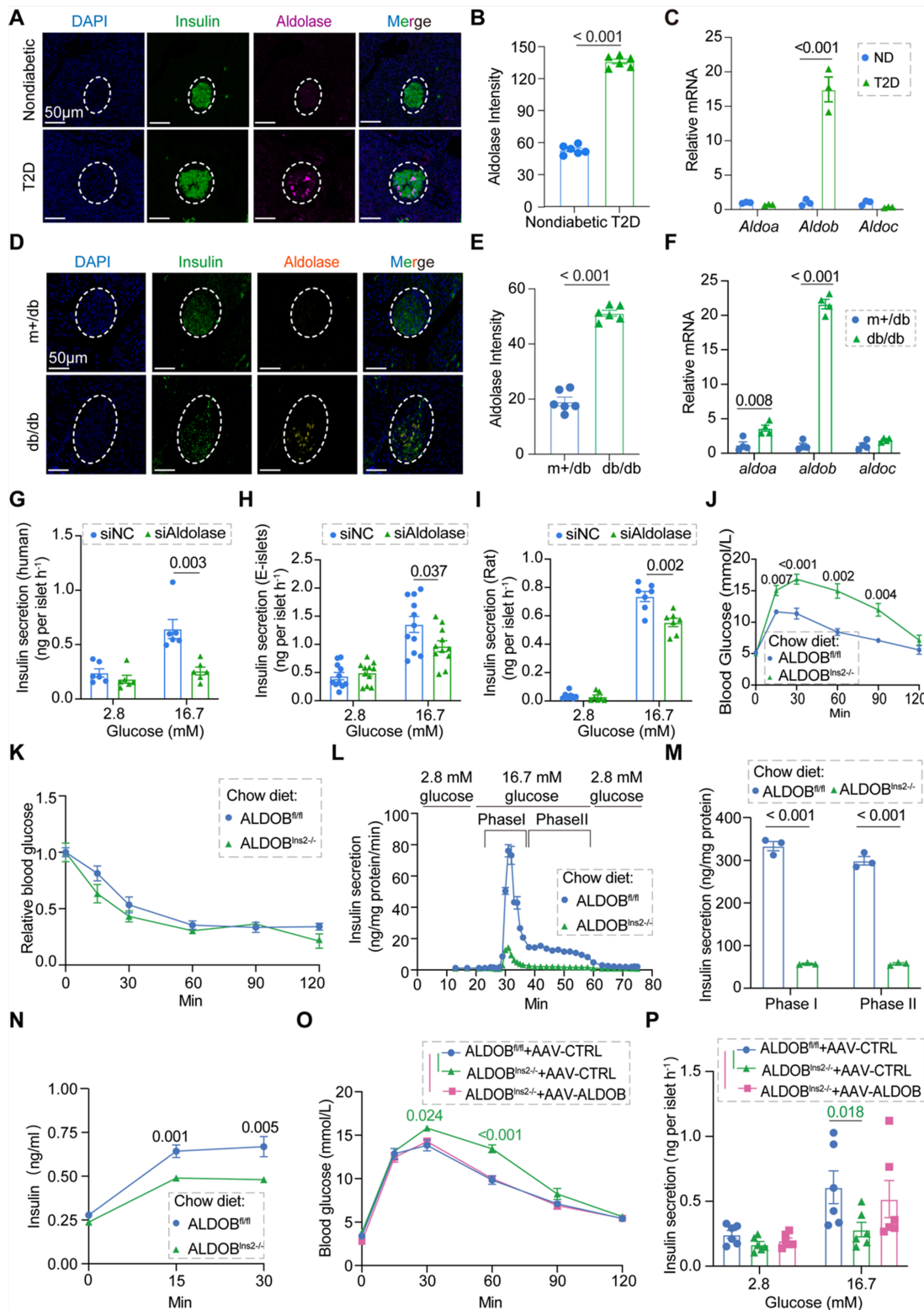


Figure 1

ALDOB significantly increases among the three aldolase isoforms in T2D human and mouse islets but ALDOB knockout in b cells impairs insulin secretion.

a-b, Representative images (a) and quantifications (b) of immunofluorescence of nuclear staining (DAPI, blue), insulin (green), and aldolase (purple) expressions in primary islets of nondiabetic and T2D

humans. Scale bar, 50 mm. **c**, Relative RNA gene expressions of different *Aldolase* isoforms in islets from nondiabetic (ND) and T2D patients. Each gene expression is normalized to the average of its expressions in ND group. **d-e**, Representative images (d) and quantification (e) of immunofluorescence analysis of nuclear staining (DAPI, blue), insulin (green) and aldolase (yellow) expression in control (m+/db) and diabetes (db/db) mouse primary islets. Scale bar, 50 mm. **f**, Relative RNA gene expressions of different *Aldolase* isoforms in islets from control (m+/db) and diabetes (db/db) mice. Each gene expression is normalized to the average of its expressions in m+/db group. **g-i**, Insulin secretion with glucose treatment (2.8 and 16.7 mM) on primary islets from nondiabetic human subjects (g), human iPSC-derived islets (h) and rat endoderm cell induced islets (i) with control (siNC) and knocking down aldolase (siAldolase). **j**, Glucose tolerance test (GTT) in ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice with Chow Diet. **k**, Insulin tolerance test (ITT) in ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice with Chow Diet. **l-m**, GSIS with islet perfusion analyses of dynamic glucose-stimulated phase I and phase II insulin secretion in islets from ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice. **n**, Insulin concentrations in serum of ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice fed with Chow Diet after intraperitoneal injecting 30% glucose 0, 15 and 30 min (n=5-6). **o**, Glucose tolerance test (GTT) in ALDOB^{fl/fl} mice with control AAV injection, ALDOB^{Ins2-/-} mice with control AAV injection and ALDOB^{Ins2-/-} with overexpressed ALDOB AAV injection mice (n=6). **p**, GSIS in primary islets of ALDOB^{fl/fl} mice with control AAV, ALDOB^{Ins2-/-} mice injection with control AAV injection and overexpressed ALDOB AAV. Results are presented as mean ± SEM. Statistical analysis was performed using two-sided unpaired t-tests (b, c, e, f, g-n) and two-way ANOVA (o and p).

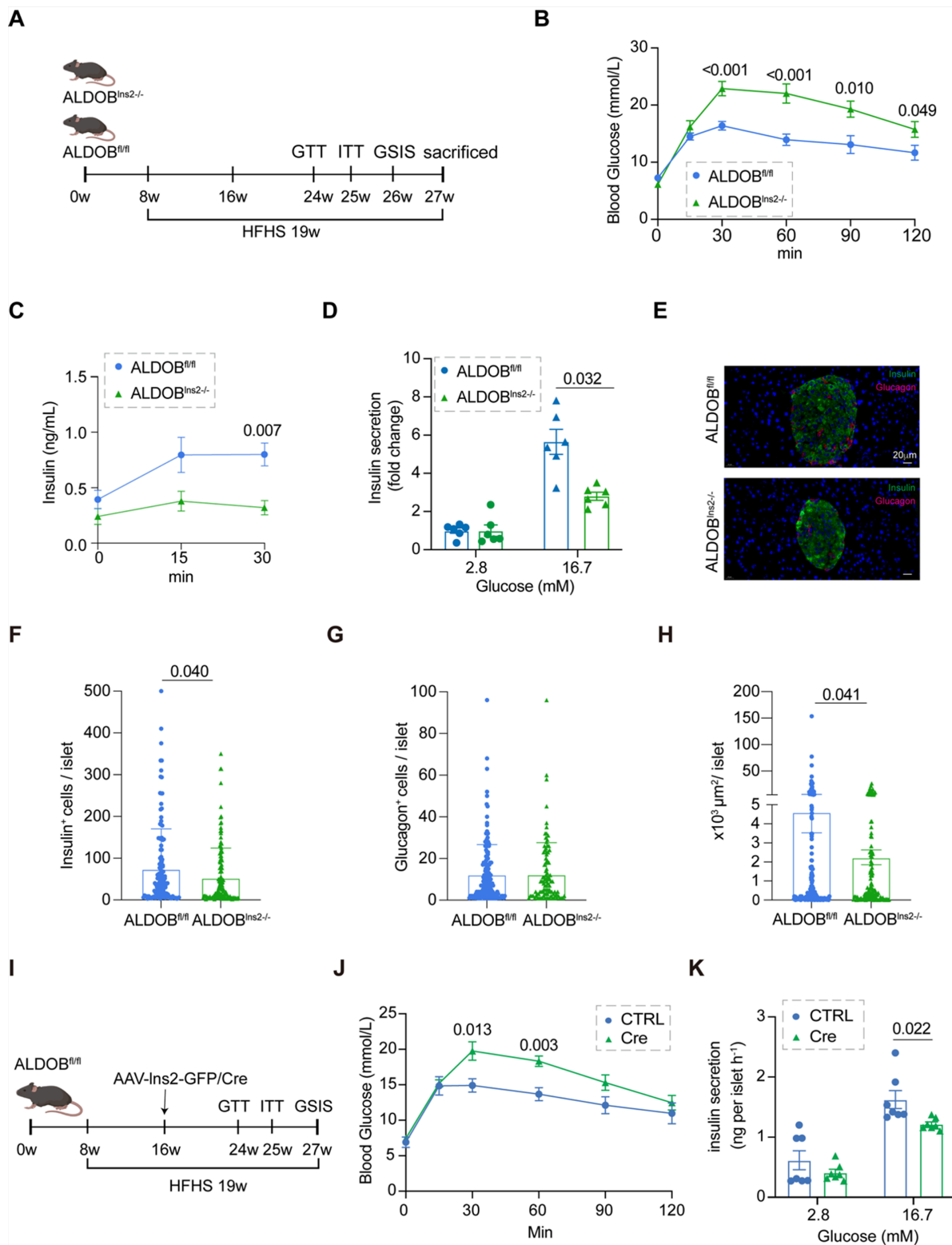


Figure 2

β cell-specific ALDOB knockout impairs glucose tolerance and insulin secretion in HFHS mice.

a, Diagram of experimental design of control (ALDOB^{fl/fl}) mice and β cell specific ALDOB knockout (ALDOB^{Ins2-/-}) mice with high fat high sucrose (HFHS) treatment. **b**, Glucose tolerance test (GTT) on 24-week-old ALDOB^{fl/fl} and ALDOB^{Ins2-/-} male mice fed with HFHS diet for 16 weeks (n=9-10). **c**, Serum

insulin levels in 26-week-old ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} male mice fed with 18-week HFHS diet after intraperitoneal injecting 30% glucose 0, 15 and 30 min (n=5-6). **d**, Insulin secretion of Isolated primary islets from 27-weeks-old ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} male mice fed 19w HFHS diet. Secreted insulin normalized by islet equivalent (IEQ). **e-g**, Representative image of islet morphology (e) and quantification of number of insulin positive cells per islet (f), glucagon positive cells per islet (g) and size of islet. **h**, from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} male mice fed 19w HFHS diet. **i**. Diagram of experimental design of knocking out ALDOB in islets from 8-week HFHS diet ALDOB^{fl/fl} mice through intraperitoneal injection with Cre recombinase AAV driven by insulin promoter (AAV–Ins2–Cre). Age-matched ALDOB^{fl/fl} mice injected with AAV encoding GFP (AAV–Ins2–GFP) were included as a control group. **j**. Glucose tolerance test (GTT) in ALDOB^{fl/fl} mice with AAV-GFP injection (CTRL) and ALDOB^{fl/fl} mice with AAV-Cre injection (Cre) fed with HFHS diet (n=6). **k**. GSIS of primary islets from ALDOB^{fl/fl} mice with AAV-GFP injection (CTRL) and ALDOB^{fl/fl} mice with AAV-Cre injection (Cre) fed with HFHS diet. Data are presented as mean $\pm$ SEM. Statistical analysis was performed using two-sided unpaired t-tests.

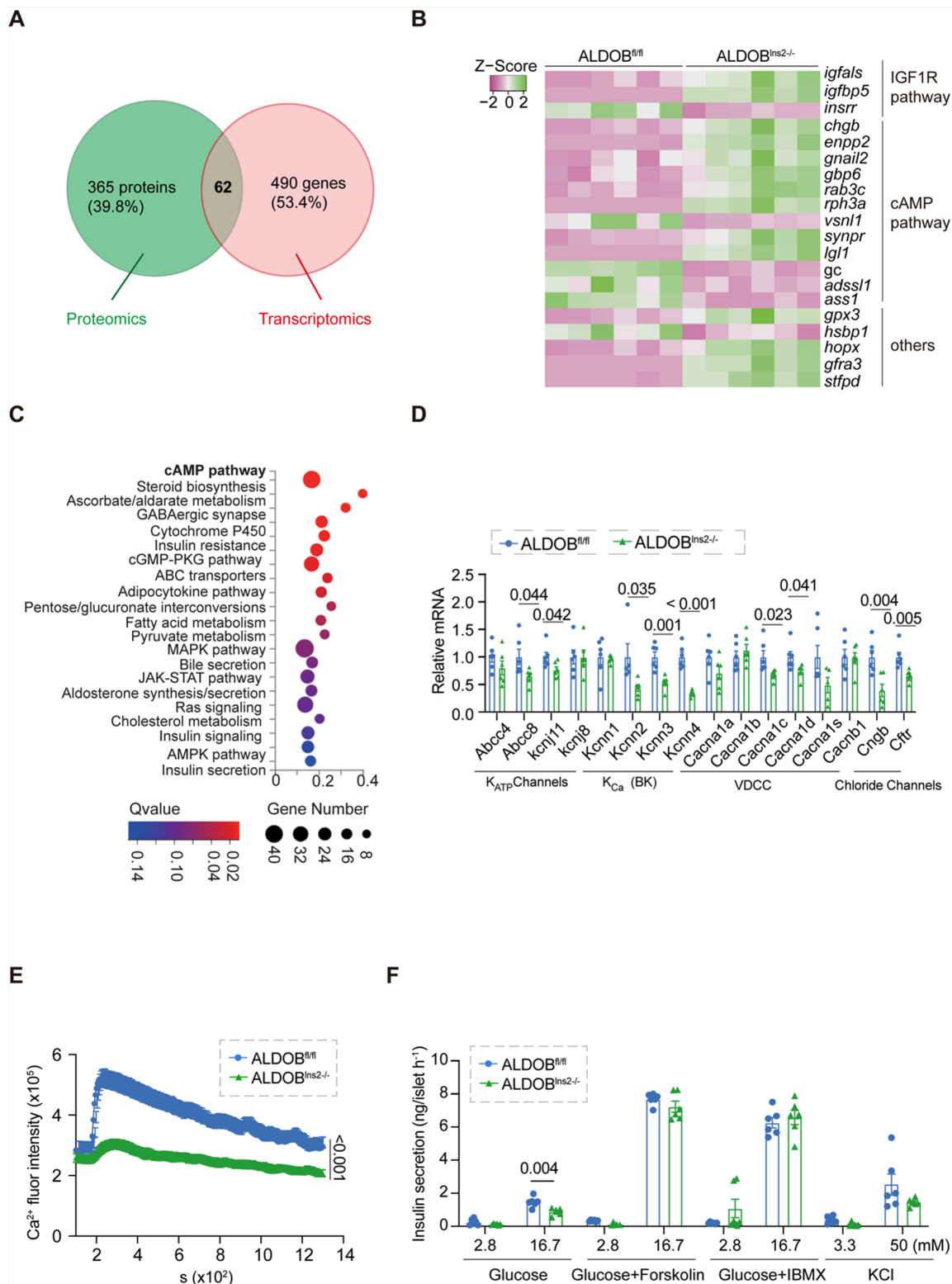


Figure 3

ALDOB knockout reduces GSIS by inhibiting cAMP-PKA pathway in β cells.

a, Venn diagram of 62 differential expressed genes from proteomics and bulk RNA-seq from primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice (n=6). **b**, Heatmap showed qPCR validation of 20 islet functions

related differential expressed genes in both proteomics and bulk RNA-seq from primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice.

c, KEGG analysis of differential expressed genes in bulk RNA-seq from primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice. **d**, Relative expression of PKA downstream genes mRNA in primary islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice. Each gene expression is normalized to the average of its expressions in ALDOB^{fl/fl} group. **e**, Quantitative analysis of calcium in primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice under 2.8 mM and 16.7mM glucose stimulation (n=22). **f**, Insulin secretion levels under 2.8 and 16.7mM glucose stimulation in ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mouse primary islet cells treated with Forskolin and IBMX. Results are presented as mean $\pm$ SEM. Statistical analysis was performed using two-sided unpaired t-tests (d and e) and 2-way ANOVA (f).

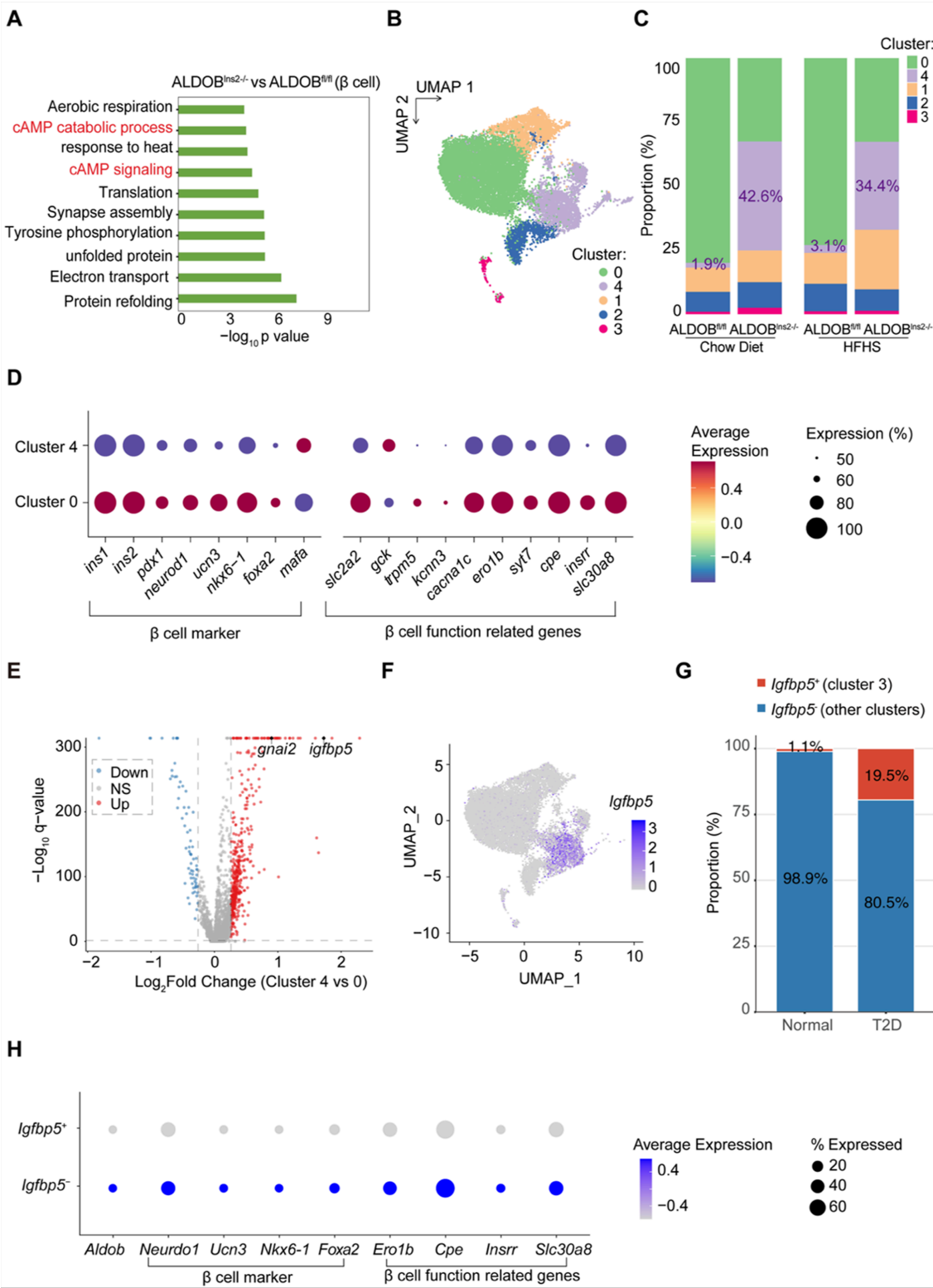


Figure 4

A subset of dysfunctional β cells (*Aldob*^{low} *Igfbp5*^{high}) significantly increased in ALDOB^{Ins2-/-} mice and T2D populations.

a, GO Pathway enrichment analysis of differential expressed genes in single cell sequencing β cells from ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice. **b**, Sub-clustering of mice β cells, which are defined according to k-

nearest neighbors (kNN)-based clusters. **c**, Distribution of β cell sub-clusters in ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice islet. **d**, Relative expression levels of specific genes that characterize the identity and function of β -cells in mice β cell sub-cluster 0 and 4. **e**, Volcano plot analysis of differential expressed genes between mice β -cell subpopulations cluster 4 and 0. **f**, t-SNE visualization of *igfbp5* expression in mice β -cell subclusters (Figure 4B). **g**, The proportion of *igfbp5*⁺ β cells in normal and type 2 diabetes (T2D) human populations. **h**, Relative expression levels of specific genes that characterize the identity and function of β -cells in normal and type 2 diabetes (T2D) human *igfbp5*⁺ and *igfbp5*⁻ β cells.

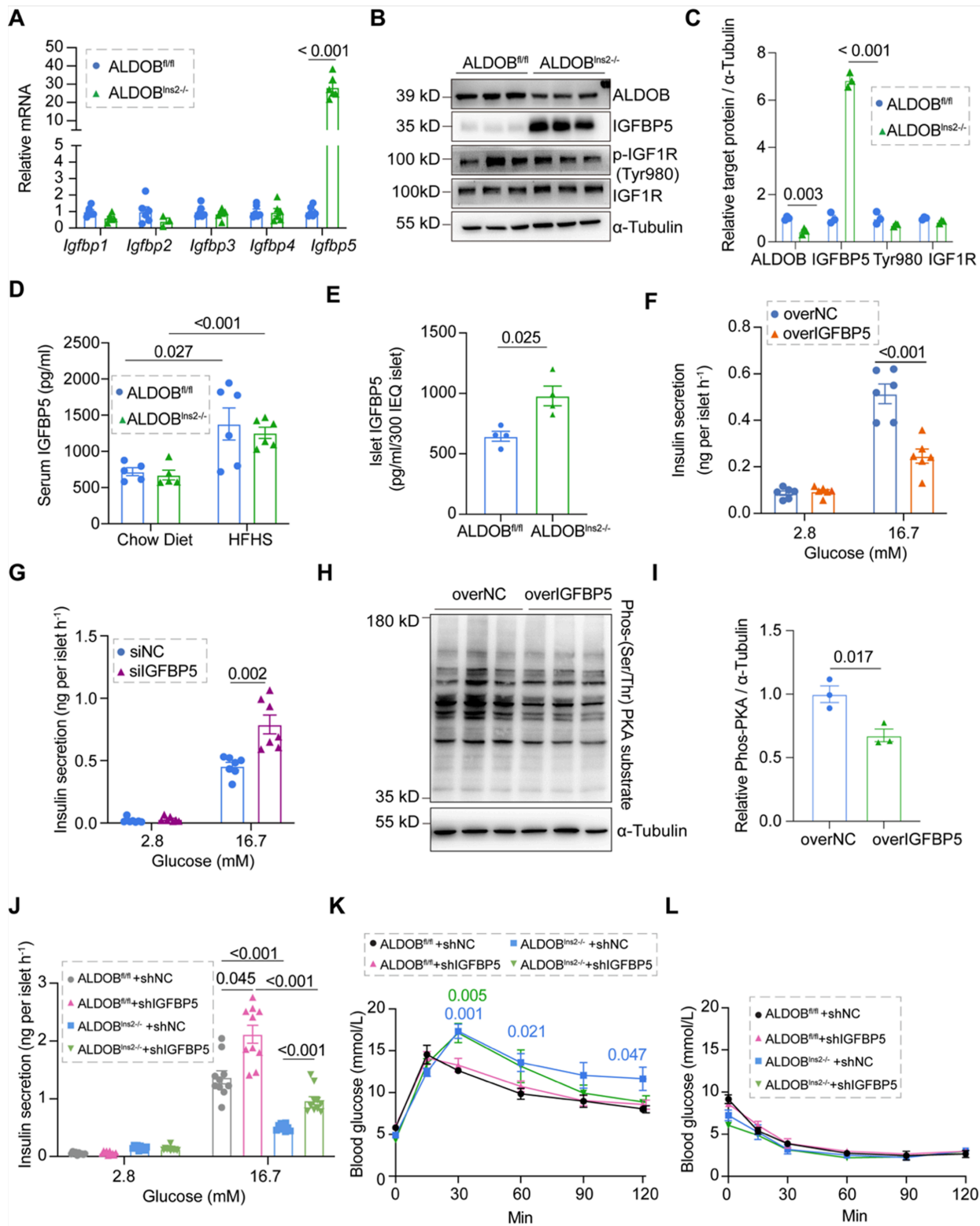


Figure 5

IGFBP5 knockdown rescues the decreased GSIS in ALDOB^{Ins2-/-} mice.

a, Relative mRNA levels of IGFBP family genes in primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice (n=6). Each gene expression is normalized to the average of its expressions in ALDOB^{fl/fl} group. **b-c**, Protein levels (b) and qualifications (c) of ALDOB, IGF1R and phosphorylated IGF1R in primary islets of ALDOB^{fl/fl}

and ALDOB^{Ins2-/-} mice. Each protein expression is normalized to the average of its expressions in ALDOB^{fl/fl} group. **d**, Serum IGFBP5 level in ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice with chow diet and HFHS diet accordingly. **e**, Intracellular IGFBP5 content in primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice. **f**, Insulin secretion in overexpressed control (overNC) and overexpressed IGFBP5 (overIGFBP5) Ins1 cells under 2.8 mM and 16.7 mM glucose stimulation. **g**, Insulin secretion in control (siNC) and IGFBP5 knocking down (siIGFBP5) Ins1 cells under 2.8 mM and 16.7 mM glucose stimulation. **h-i**, PKA substrate phosphorylation (H) and quantification (I) in overexpressed control (overNC) and overexpressed IGFBP5 (overIGFBP5) Ins1 cells. Each protein expression is normalized to the average of its expressions in overexpressed control (overNC) group. **j**, Insulin secretion levels of primary islets from ALDOB^{fl/fl} mice with control AAV, ALDOB^{fl/fl} mice with shIGFBP5 AAV, ALDOB^{Ins2-/-} mice with control AAV, ALDOB^{Ins2-/-} mice with shIGFBP5 AAV, after stimulated with 2.8 mM and 16.7 mM glucose (n=10).

k-i, Glucose tolerance test (GTT, K) and insulin tolerance test (ITT, L) in ALDOB^{fl/fl} mice with control AAV, ALDOB^{fl/fl} mice with shIGFBP5 AAV, ALDOB^{Ins2-/-} mice with control AAV, ALDOB^{Ins2-/-} mice with shIGFBP5 AAV (n=10). Results are presented as mean ± SEM. Statistical analysis was performed using two-sided unpaired t-test (a, b, e-g, i) and 2-way ANOVA (d, j-l).

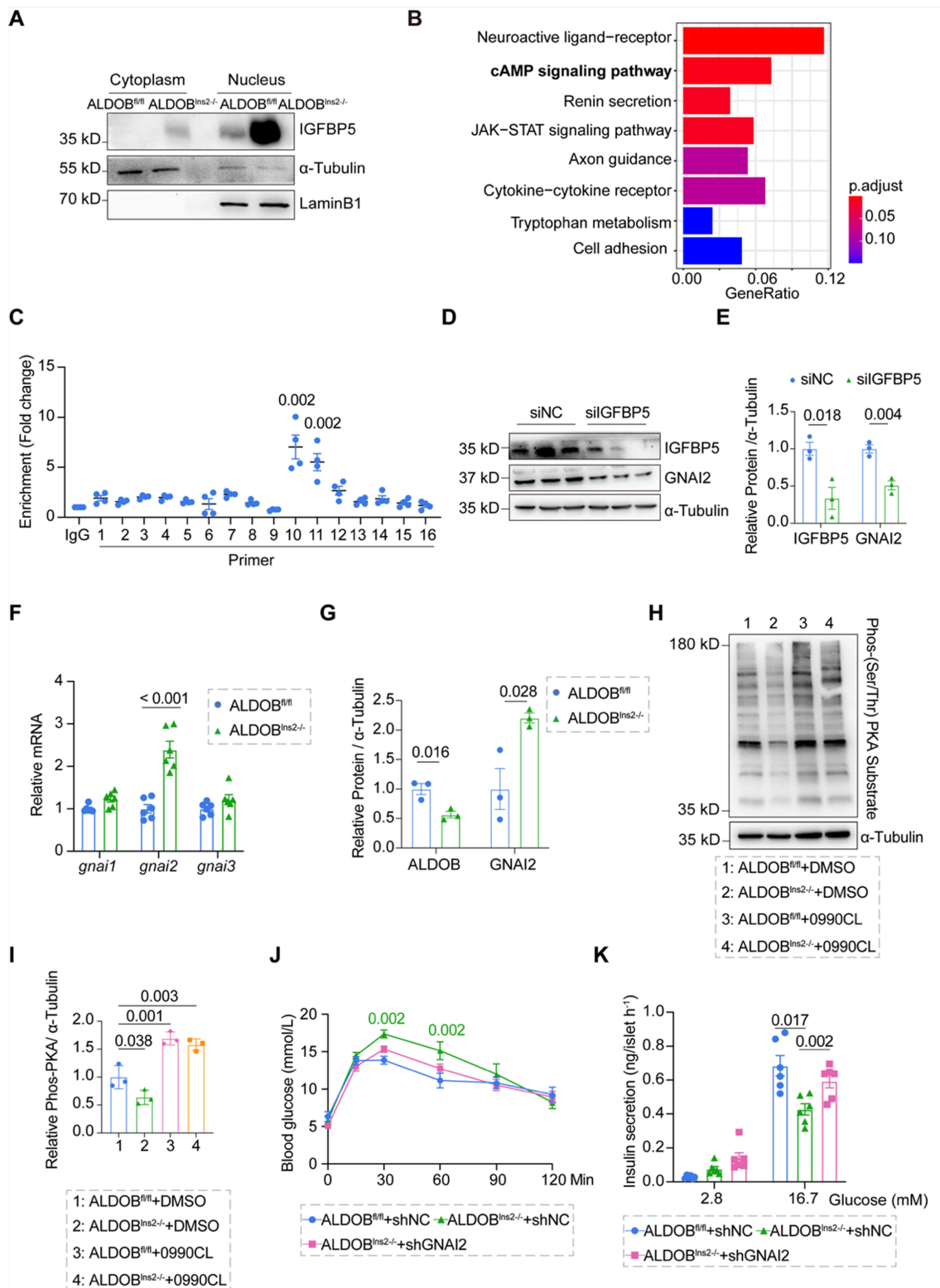


Figure 6

IGFBP5 regulates the transcription of GNAI2 to inhibit cAMP signaling pathway.

a, Nuclear and cytoplasm fractionation of IGFBP5 in primary islet of ALDOB^{fl/fl} and ALDOB^{Ins2-/-} mice. **b**, KEGG pathway enrichment of differential target genes enriched by IGFBP5 in ChIP-seq experiments. **c**, ChIP-qPCR results of IGFBP5 binding GNAI2 transcriptional region. Primer 1 to 16 are designed to cover

0-2kb upstream of GNAI2 transcriptional start site (TSS). **d-e**, Representative immunoblots (d) and quantification (e) of GNAI2 expression in Ins1 cells transfected with siNC and siGFBP5 for 48 h. Each protein expression is normalized to the average of its expressions in siNC group. **f**, Relative mRNA levels of different *gnai* isoforms in primary islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice. Each gene expression is normalized to the average of its expressions in ALDOB^{fl/fl} group. **g**, Quantifications of ALDOB and GNAI2 expression in primary islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice. Each protein expression is normalized to the average of its expressions in ALDOB^{fl/fl} group. Immunoblot image in Figure S13A. **h-i**, Representative immunoblots (h) and quantifications (i) for phosphorylated PKA substrate proteins in primary islets of ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice treated with control (DMSO) and a GNAI2 inhibitor 0990CL. **j**, Glucose tolerance test in ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice with control (shNC) and specific GNAI2 knocking down (shGNAI2) in β cells (n=6). **k**, GSIS assay of primary islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice with control (shNC) and specific GNAI2 knocking down (shGNAI2) in β cells. For all data, values are presented as mean $\pm$ SEM. Statistical analysis was performed using two-sided unpaired t-test (b, d, f, g) and 2-way ANOVA (i-k).

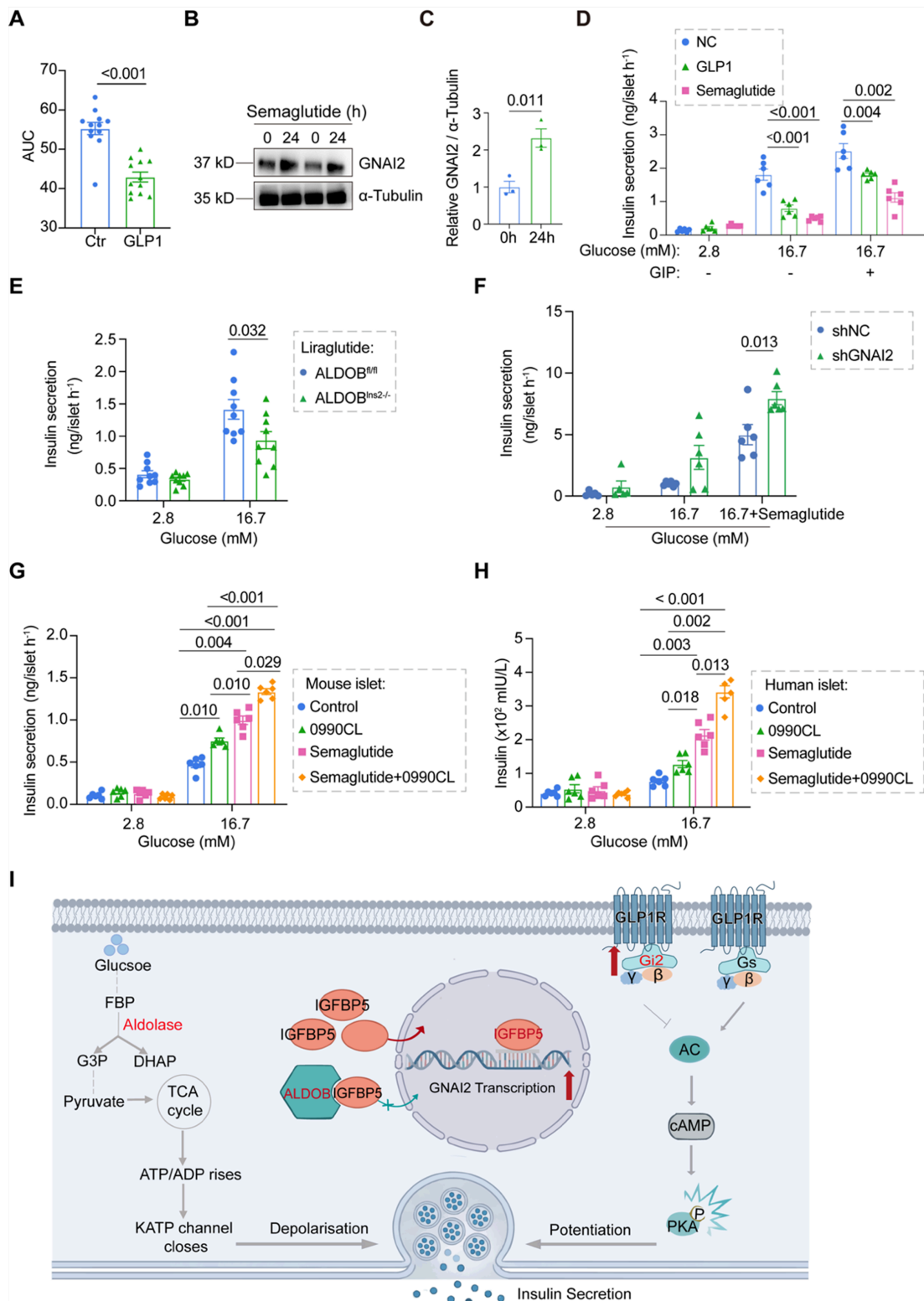


Figure 7

GNAI2 inhibition synergizes Insulin-stimulating efficacy of GLP-1R agonists.

a, Quantification of AUC area in bioluminescence resonance energy transfer (BRET). **b-c**, Representative immunoblots (b) and quantifications (c) of GNAI2 in mouse primary islets treated with 10 nM semaglutide for 0 and 24 hours. **d**, GSIS assay to analyze insulin secretion of mouse primary islets pre-

incubated with 10 nM GLP1 or 10nM semaglutide for 48h. **e**, GSIS assay to analyze insulin secretion of primary islets from ALDOB^{fl/fl} and ALDOB^{Ins2^{-/-}} mice with 10nM liraglutide treatment. **f**, GSIS assay to analyze insulin secretion of primary islets from 24w shNC and shGNAI2 male mice fed with HFHS diet under 2.8 mM glucose, 16.7 mM glucose, and 16.7 mM glucose plus 10mM semaglutide stimulation. **g-h**, Insulin secretion of mouse primary islets (**g**) and normal human primary islets (**h**) with 10nM semaglutide and 100nM 0990CL treatment accordingly. **i**, Working model: ALDOB and IGFBP5 interactions modulate the insulin secretion in b cells through tuning the inhibitory (Gi2) and stimulatory (Gs) of GLP-1R and downstream cAMP-PKA pathways. For all data, values are presented as mean $\pm$ SEM. Statistical analysis was performed using two-sided unpaired t-test (c-f) and 2-way ANOVA (a, g and h).

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