Paired box 6 programs essential exocytotic genes in the regulation of glucose-stimulated insulin secretion and glucose homeostasis

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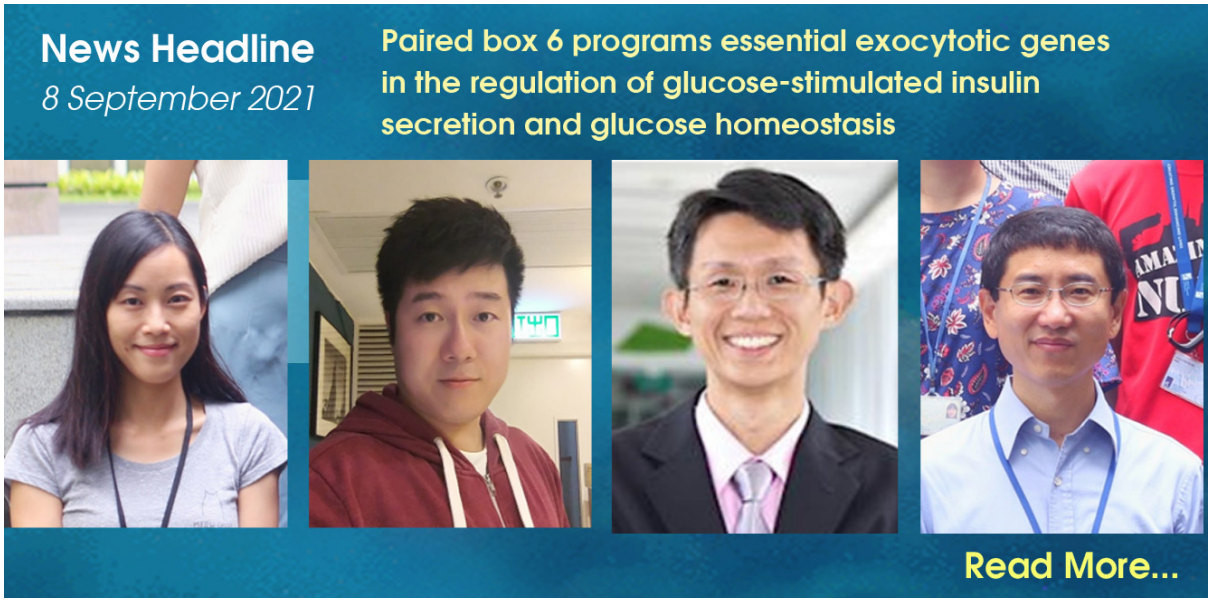

Authors

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Abstract

The paired box 6 (PAX6) transcription factor is crucial for normal pancreatic islet development and function. Heterozygous mutations of *PAX6* are associated with impaired insulin secretion and early-onset diabetes mellitus in humans. However, the molecular mechanism of PAX6 in controlling insulin secretion in human beta cells and its pathophysiological role in type 2 diabetes (T2D) remain ambiguous. We investigated the molecular pathway of PAX6 in the regulation of insulin secretion and the potential therapeutic value of PAX6 in T2D by using human pancreatic beta cell line EndoC-βH1, the *db/db* mouse model, and primary human pancreatic islets. Through lossand gain-of-function approaches, we uncovered a mechanism by which PAX6 modulates glucose-stimulated insulin secretion (GSIS) through a cAMP response element–binding protein (CREB)/Munc18-1/2 pathway. Moreover, under diabetic conditions, beta cells and pancreatic islets displayed dampened PAX6/CREB/Munc18-1/2 pathway activity and impaired GSIS, which were reversed by PAX6 replenishment. Adeno-associated virus–mediated PAX6 overexpression in *db/db* mouse pancreatic beta cells led to a sustained amelioration of glycemic perturbation in vivo but did not affect insulin resistance. Our study highlights the pathophysiological role of PAX6 in T2D-associated beta cell dysfunction in humans and suggests the potential of *PAX6* gene transfer in preserving and restoring beta cell function.

Figure:

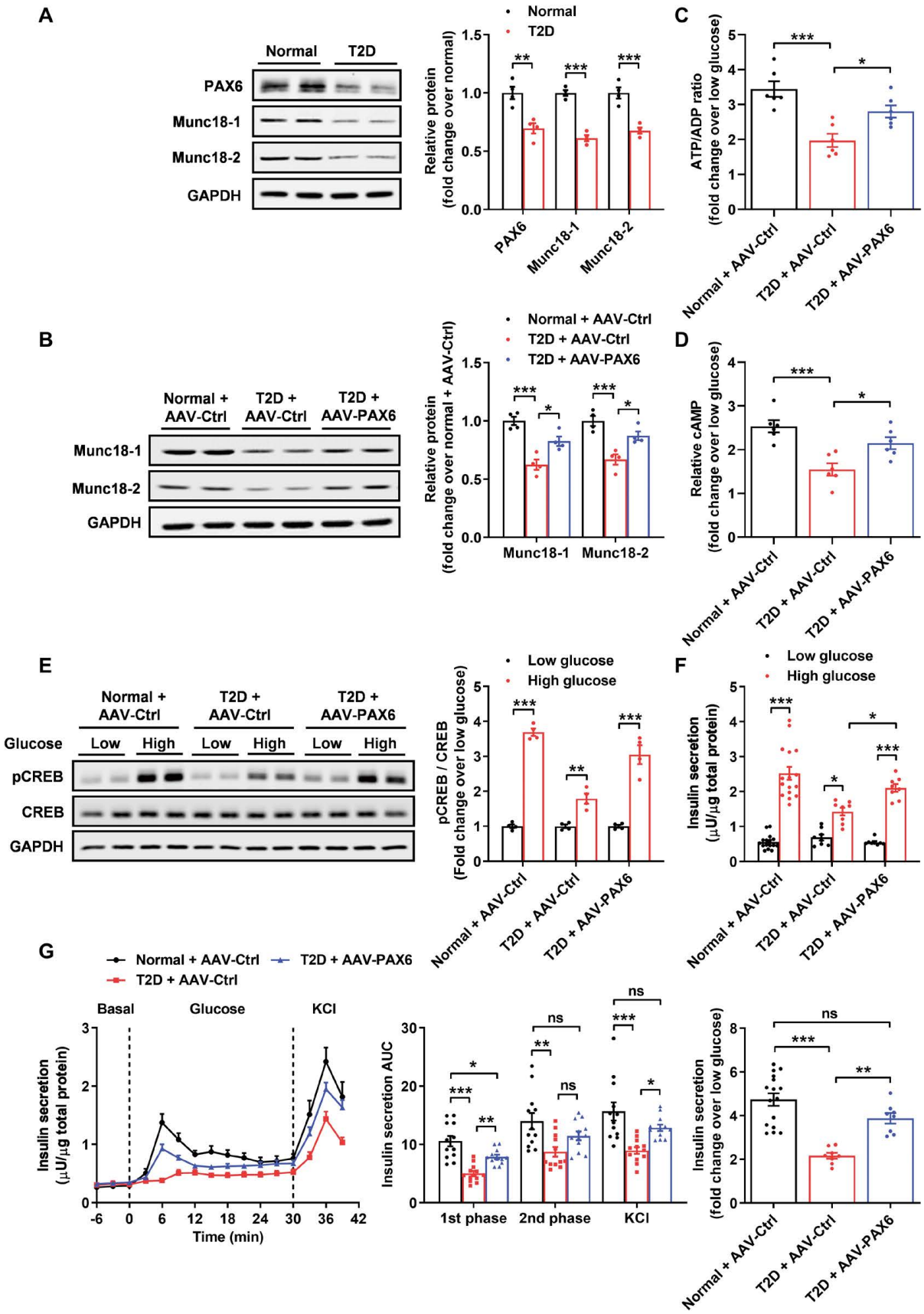
A
Normal
T2D
PAX6
Munc18-1
Munc18-2
GAPDH
Relative protein (fold change over normal)
PAX6
Munc18-1
Munc18-2
C
ATP/ADP ratio (fold change over low glucose)
Normal + AAV-Ctrl
T2D + AAV-Ctrl
T2D + AAV-PAX6
B
Normal + AAV-Ctrl
T2D + AAV-Ctrl
T2D + AAV-PAX6
Relative protein (fold change over normal + AAV-Ctrl)
D
Relative cAMP (fold change over low glucose)
E
Glucose
Low
High
pCREB
CREB
pCREB / CREB (Fold change over low glucose)
F
Insulin secretion (μU/μg total protein)
G
Basal
Glucose
KCl
Time (min)
Insulin secretion AUC
1st phase
2nd phase
KCl
ns

Figure Legend: Beta cell–specific PAX6 overexpression restores CREB/Munc18-1/2 signaling and GSIS in T2D human pancreatic islets. (**A**) Protein expression of PAX6 and Munc18-1/2 in normal and T2D human islets (*n* = 4). Two-tailed Student's t test. (**B**) Protein expression of Munc18-1/2 in human islets with AAV-Ctrl or AAV-PAX6 transduction (*n* = 4). One-way ANOVA. (**C**) ATP/ ADP ratio (*n* = 6) and (**D**) cAMP (*n* = 6) in dispersed human islet cells after 15-min glucose (2.5 or 20 mM) stimulation. One-way ANOVA. (**E**) Phosphorylated and total CREB in human islets after 15-min glucose (2.5 or 20 mM) stimulation (*n* = 4). Two-tailed Student's *t* test. (**F**) Static insulin secretion of human islets expressed as absolute amount (μU/μg total protein) and fold change (*n* = 16 for Normal +AAV-Ctrl; n = 8 for T2D + AAV-Ctrl/ AAV-PAX6). One-way and two-way ANOVA. (**G**) Dynamic insulin secretion of human islets in response to glucose (16.7 mM) and KCl (20 mM) stimulation (*n* = 12). One-way ANOVA. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Data are means ± SEM.