Conversion of pancreatic α cells into insulin-producing cells modulated by β-cell insufficiency and supplemental insulin administration

Takehiro Katahira¹, Takeshi Miyatsuka¹,² *, Masaki Miura¹, Luka Suzuki¹,², Miwa Himuro¹, Yuya Nishida¹, Hiroaki Satoh¹, Hirotaka Watada¹,²,³

¹ Department of Metabolism and Endocrinology, ² Center for Identification of Diabetic Therapeutic Targets, ³ Center for Therapeutic Innovations in Diabetes, Juntendo University Graduate School of Medicine, Tokyo, Japan

*Corresponding author: Takeshi Miyatsuka

Juntendo University Graduate School of Medicine
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
Tel: +81-3-5802-1579 / Fax: +81-3-3813-5996
E-mail: miyatsuka-takeshi@umin.net
Abstract

The emergence of bihormonal (BH) cells expressing insulin and glucagon has been reported under diabetic conditions in humans and mice. Whereas lineage tracing studies demonstrated that glucagon-producing α cells can be reprogrammed into BH cells, the underlying dynamics of the conversion process remain poorly understood. In the present study, we investigated the identities of pancreatic endocrine cells by genetic lineage tracing under diabetic conditions. When β-cell ablation was induced by alloxan (ALX), a time-dependent increase in BH cells was subsequently observed. Lineage tracing experiments demonstrated that BH cells originate from α cells, but not from β cells, in ALX-induced diabetic mice. Notably, supplemental insulin administration into diabetic mice resulted in a significant increase in α-cell-derived insulin-producing cells that did not express glucagon. Furthermore, lineage tracing in Ins2^Akita diabetic mice demonstrated a significant induction of α-to-β conversion. Thus, adult α cells have plasticity, which enables them to be reprogrammed into insulin-producing cells under diabetic conditions, and this can be modulated by supplemental insulin administration.

Keywords: bihormonal cell, glucagon, insulin, α-to-β conversion, diabetes
1. Introduction

Adult pancreatic islets consist of four types of endocrine cells ($\alpha$-, $\beta$-, $\delta$-, and PP-cells), which express glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. It has been reported that glucagon-producing $\alpha$-cells can transdifferentiate into insulin-producing $\beta$-like cells under some experimental conditions, such as extreme $\beta$-cell ablation [1-3], ectopic expression of $\beta$-cell-specific transcription factors [4, 5], and administration of small molecules [6]. Such reprogrammed insulin-producing cells are expected to lead to the development of future cell therapies for curing diabetes [7].

Ablation of insulin-producing cells has been shown to cause the emergence of bihormonal (BH) cells expressing both insulin and glucagon in the islets [8], and lineage tracing studies have shown that pancreatic $\alpha$-cells can be reprogrammed into BH cells as well as into $\beta$-cells that terminate glucagon expression [1, 3]. Although these studies have shown the plasticity of pancreatic $\alpha$-cells, it remains unclear as to what types of cellular circumstances affect $\alpha$-cell identity in these experimental conditions.

In the present study, diabetic mouse models, such as alloxan (ALX)-induced diabetic mice and Akita mice, were used to trace the cell fate of endocrine cells. Our results demonstrate that BH cells originate from $\alpha$-cells, but not from $\beta$-cells. Interestingly, exogenous insulin treatment in ALX-induced diabetic mice significantly increased the number of $\alpha$-cell-derived insulin-producing cells that did not express glucagon, suggesting that supplemental insulin treatment affects cell-fate specification of the $\alpha$-cell lineage. Understanding and regulating such cellular plasticity of $\alpha$-cells may lead to a novel approach to induce $\beta$-cell neogenesis from other cell types within the pancreas.
2. Methods

2.1. Animals.

C57BL/6J male mice were purchased from Sankyo Labo Service Corporation, INC. (Tokyo, Japan). MIP-Cre\textsuperscript{ER}, Gcg-Cre\textsuperscript{ER}, and ROSA26-lacZ reporter mice (R26R) were generated as previously described [9-11]. At 6 or 7 weeks of age, alloxan (ALX; Sigma Aldrich, St. Louis, MO, USA) or saline was administered into the mice as a single intravenous injection at a dose of 100 mg/kg body weight through the tail vein. For insulin treatment experiments, the mice were treated with insulin 2 days after ALX administration using osmotic minipump (ALZET, Cupertino, CA, USA). Tamoxifen (Sigma-Aldrich) was prepared at 20 mg/mL in corn oil. For induction of Cre-mediated recombination, mice were subcutaneously injected with 4 mg of tamoxifen at the age of 4 weeks, 3 times over a week period.

Nondiabetic C57BLKS-Lepr\textsuperscript{db/+} heterozygous (db/+) mice, and Ins\textsuperscript{2}Akita mice were purchased from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan). Gcg-Cre\textsuperscript{ER}; R26R mice were backcrossed onto the C57BLKS/J background. Gcg-CreER; R26R; db/+ mice were crossed with db/+ mice to generate Gcg-Cre\textsuperscript{ER}; R26R; db/db mice.

Blood glucose levels of each mouse were measured using a portable glucose meter (Sanwa Kagaku Co., Ltd., Nagoya, Japan). Mice were housed on a 12-hour light-dark cycle in a controlled climate. The study protocol was reviewed and approved by the Animal Care and Use Committee of Juntendo University.

2.2. Histology and immunostaining

Tissues were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) at 4\textdegree{}C, washed in PBS, immersed in sucrose solution, and embedded in Tissue-Tek O.C.T
Compound (Sakura, Tokyo, Japan), or processed routinely for paraffin embedding. Sections were separated by at least 100 μm and blocked with 1% horse serum, incubated with primary antibodies overnight at 4°C, and then visualized by incubation with secondary antibodies for 30 min at room temperature. The primary antibodies used in this study were the following: guinea pig anti-insulin (1:5; Dako, Carpinteria, CA, USA), rat anti-insulin (1:200; R&D Systems, Minneapolis, MN, USA), rabbit anti-glucagon (1:1000; Dako), guinea pig anti-glucagon (1:1000; TAKARA BIO, Shiga, Japan), rabbit anti-β-galactosidase (1:200; MBL, Nagoya, Japan). Cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories Inc., Woodinville, WA, USA). The secondary antibodies used were Alexa Fluor 633-conjugated anti-rat IgG, Alexa Fluor 633-conjugated anti-rabbit IgG, Alexa Fluor 568-conjugated anti-rat IgG, Alexa Fluor 555-conjugated anti-rabbit IgG, Alexa Fluor 488-conjugated anti-guinea pig IgG, Alexa Fluor 488-conjugated anti-rabbit IgG, Alexa Fluor 488-conjugated anti-rabbit IgG (all at 1:200; Invitrogen, Carlsbad, CA, USA). After washing in PBS, sections were mounted in Vectashield mounting medium (Vector Laboratories). Slides were imaged on a Leica TCS SP5 confocal laser scanning microscope (Wetzlar, Germany).

2.3. Statistical Analyses

Statistical analyses were performed using the GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA, USA). Comparisons of two samples were performed by unpaired two-tailed t tests. Multiple groups were analyzed by one-way ANOVA with a multiple comparison test. A p-value of less than 0.05 was considered to indicate a statistically significant difference between 2 groups. Data are presented as the mean ± SE.
3. **Results**

3.1. Emergence of BH cells in the islets of ALX-induced diabetic mice

Whereas ALX was shown to induce BH cells expressing both insulin and glucagon 4 and 12 weeks after its injection [8], the temporal dynamics of BH cells remained unclear. To address this point, *C57BL/6J* mice were injected with ALX or saline, and were sacrificed at the age of 8 weeks, which was a week or 2 weeks after ALX administration (Fig. 1A). Immunofluorescence staining for insulin and glucagon demonstrated that insulin/glucagon double-positive cells were observed at both one and 2 weeks after ALX administration, but not in the control mouse (Fig. 1B). The percentage of the islets that contain BH cells in ALX-2w mice was significantly higher than those in ALX-1w mice (Fig. 1C, *P* < 0.05), whereas the control mice injected with saline showed no BH cells in the islets. These findings show that ALX treatment leads to the emergence of BH cells and the number of BH cells increases with time after ALX injection.

3.2. Quantification of BH cells after insulin treatment in ALX-induced diabetic mice

As BH cells emerged after ALX-induced hyperglycemia (Fig. 1C), accompanied by insulin deficiency, we investigated whether an improvement of glycemic control by insulin treatment may affect the emergence of BH cells after ALX administration (Fig. 2A). When insulin was continuously administered into ALX-induced diabetic mice via an osmotic minipump, blood glucose levels in the insulin-treated diabetic mice (ALX + ins) were significantly improved compared with ALX-induced diabetic mice (Fig. 2B and 2C). Whereas the number of BH cells was significantly increased in ALX-induced diabetic mice compared with nondiabetic mice, there was no significant difference in the percentage of islets containing BH cells between ALX-induced diabetic mice and insulin-
treated mice (Fig. 2D).

3.3. BH cells originate from α cells, but not from β cells

There are several possibilities as to the origin of BH cells; α cells, β cells, or some other cell types. Lineage tracing studies have shown that α cells can be reprogrammed into BH cells after β-cell ablation [1]. To clarify whether pre-existing α-cells differentiate into BH cells in our experimental conditions, α-cell lineage tracing was conducted using Gcg-CreER, ROSA26laxZ (R26R) mice (Fig. 3A), in which only the cells in α-cell lineage are irreversibly labeled as β-galactosidase (β-gal)-positive cells after Cre-mediated recombination induced by the administration of tamoxifen. Immunostaining against insulin, glucagon, and β-gal demonstrated that most β-gal-expressing cells coexpressed glucagon without insulin expression, whereas some β-gal-expressing cells were positive for both glucagon and insulin, that is, α-to-BH conversion had occurred (Fig. 3B). Furthermore, some β-gal/insulin double-positive cells were negative for glucagon, showing that α cells can be reprogrammed into β cells (α-to-β conversion), with the loss of glucagon expression.

To further investigate whether pre-existing β cells differentiate into BH cells, β-cell lineage tracing was conducted using Mouse ins1 promoter (MIP)-CreER, R26R mice, in which only the cells in β-cell lineage are irreversibly labeled as β-gal-positive cells after Cre-mediated recombination induced by the administration of tamoxifen (Supplementary Fig. 1A). Immunostaining against insulin, glucagon, and β-gal demonstrated that neither BH cells nor glucagon-expressing cells were positive for β-gal (Supplementary Fig. 1B), showing that no β cells can be reprogrammed into α-cell lineage in ALX-induced diabetic mice.
3.4. Insulin treatment enhances α-to-β conversion in ALX-induced diabetic mice

To clarify whether improvement of hyperglycemia affects the cell fate of α cells in ALX-induced diabetic mice, Gcg-CreER, R26R diabetic mice were treated with insulin via an osmotic minipump. Immunostaining against insulin, glucagon, and β-gal resulted in no significant difference in the number of β-gal/insulin double-positive cells between with or without insulin treatment in ALX-induced diabetic mice (Fig. 3C). Notably, diabetic mice treated with insulin showed a significant increase in the number of β-gal/insulin double-positive cells without glucagon expression, with a tendency of a slight decrease in the number of α-cell-derived BH cells, compared with nontreated diabetic mice (Fig. 3D and 3E). These findings suggest that insulin treatment in ALX-induced diabetic mice alters the cell fate of α cells, enhancing α-to-β conversion.

3.5. Cellular plasticity in α cells of Ins2Akita and db/db mice

To further investigate whether α-cell fate can be affected in other diabetic mouse models, α-cell lineage was traced by crossing Gcg-CreER, R26R mice with Ins2Akita diabetic mice, which is a lean diabetic mouse model with profound insulin deficiency (Fig. 4A and 4B). When Gcg-CreER, R26R; Ins2Akita mice were injected with tamoxifen at the age of 4 weeks and sacrificed 16 weeks later, β-gal/insulin coexpressing cells that did not express glucagon were observed in the islets of these diabetic mice, but rarely observed in control nondiabetic littermates. Quantification of α-cell-derived insulin-expressing cells resulted in a significant increase in the number of β-gal/insulin double-positive, glucagon-negative cells, compared with control littermates, whereas there was no significant difference in the number of β-gal/insulin/glucagon triple-positive cells between the two groups (Fig.
On the other hand, when the α-cell lineage was traced in db/db obese diabetic mice, there was no significant difference in the number of α-cell-derived insulin-expressing cells between db/db mice and nondiabetic control mice (Supplementary Fig. 2).
BH cells expressing both insulin and glucagon have been observed not only in the fetal pancreas [12], but also in the adult pancreas of diabetic patients and rodent models [8, 13]. In this study, we confirmed the emergence of BH cells in the pancreata of ALX-induced diabetic mice, and further demonstrated a time-dependent increase in BH cells after ALX injection, which is consistent with a previous study using a transgenic model of near-total β-cell ablation induced by diphtheria toxin (DT) [1]. In addition, we performed lineage-tracing experiments to clarify the origin of BH cells, and demonstrated that BH cells originate from α cells, but not from β cells, in ALX-induced diabetic mice (Fig. 3). It has been reported that α cells can be reprogrammed into BH cells in diabetic mice through targeted ablation of β cells by DT or streptozotocin, thus showing no evidence of β-to-BH conversion [1, 14]. These findings, together with the data in our study, suggest that α-to-BH conversion in islets is a common feature of acute and extreme β-cell ablation.

The increase in BH cells in ALX-induced diabetic mice prompted us to hypothesize that continuous hyperglycemia itself triggers α-to-BH conversion and that improvement of hyperglycemia may suppress α-to-BH or α-to-β conversion. Unexpectedly, continuous insulin administration enhanced α-to-β conversion (Fig. 3E). Although insulin administration in this study improved the glucose profiles of ALX-induced diabetic mice, we were unable to normalize their hyperglycemia (Fig. 2B). A more complete normalization of hyperglycemia may lead to different results regarding the number of α-cell-derived insulin-expressing cells that have terminated glucagon expression.

It has recently been reported that suppression of insulin signaling predisposes α-cells to insulin production [3], which is consistent with part of our data showing that
ALX-induced insulin insufficiency induces α-cell-derived insulin-expressing cells. On the other hand, our data demonstrated that the subsequent administration of insulin to diabetic mice enhanced α-to-β cell conversion with an increased number of α-cell-derived insulin-positive, glucagon-negative cells (Fig. 3E). These findings suggest that the optimal level of insulin signaling at the precise time may efficiently induce the cellular reprogramming of α cells into mature insulin-producing cells, which may be utilized in future therapies for diabetes by modulating such cellular plasticity of α cells.

Conflicts of interest
The authors declare that they have no conflicts of interest associated with this study.

Acknowledgements
We thank Hiroko Tsujimura, Sumie Ishikawa, and Yasuko Hirakawa for their excellent technical assistance. We also acknowledge the support of the mouse facility and the cell imaging core, Division of Molecular and Biochemical Research, and Research Support Center at Juntendo University.
References


[7] T. Miyatsuka, Chronology of endocrine differentiation and beta-cell neogenesis,
Endocr J, 63 (2016) 205-211.


Figure legends

**Fig. 1. Emergence of bihormonal cells in the islets of alloxan-induced diabetic mice.**

(A) Experimental design of ALX-induced diabetic mice. *C57BL/6J* mice were injected with ALX (100 mg/kg) or vehicle at the age of 6 (ALX-2w model) or 7 weeks (ALX-1w model), and sacrificed at 8 weeks of age. (B) Immunostaining for insulin (green) and glucagon (red) was performed in ALX-induced diabetic mice. Nuclei were stained with DAPI (blue). Insulin/glucagon double-positive cells were observed in the islets of ALX-induced mice, but not in the control group. Scale bars, 50 μm. (C) The percentage of islets containing BH cells was higher in ALX-2w mice than in ALX-1w mice and the control group (*n* = 3–4 mice for each group). Data are presented as the mean ± SE. *, *P* < 0.05 and **, *P* < 0.01.

**Fig. 2. Quantification of bihormonal cells in the islets of insulin-treated diabetic mice.**

(A) Experimental design of ALX-induced diabetic mice and subsequent insulin administration. *C57BL/6J* mice were injected with ALX (100 mg/kg) or vehicle at the age of 7 weeks. Two days after ALX injection, the mice were continuously administered with insulin using osmotic minipumps, and sacrificed at 9 weeks of age. (B) Blood glucose levels were daily monitored after ALX injection for 2 weeks. (C) Area under the curve (AUC) of blood glucose levels between 7 and 9 weeks of age was calculated (*n* = 3–5 mice for each group). (D) The percentage of islets containing BH cells was calculated in 3 groups. Data are presented as the mean ± SE. *, *P* < 0.05 and **, *P* < 0.01.

**Fig. 3. Lineage tracing demonstrates α-to-β conversion in ALX-induced diabetic**
mice.

(A) Experimental design of α-cell lineage tracing in ALX-induced diabetic mice. Gcg-CreER, ROSA26luxZ (R26R) mice were treated with tamoxifen at the age of 4 weeks, and injected with ALX or vehicle at the age of 7 weeks. Mice in the insulin-treated group were administered with insulin 2 days after ALX injection. All the mice were sacrificed 2 weeks after ALX injection. (B) Immunostaining for β-galactosidase (β-gal, green), insulin (red), and glucagon (white) was performed. Nuclei were stained with DAPI (blue). Arrows indicate insulin, glucagon, and β-gal triple-positive cells, and arrowheads indicate insulin and β-gal double-positive cells that were negative for glucagon. Scale bars, 50 μm.

(C-E) The percentage of islets containing insulin and β-gal double-positive cells with/without glucagon expression (C), insulin, glucagon, and β-gal triple-positive cells (D), and insulin and β-gal double-positive cells without glucagon expression (E) was calculated in the 3 groups (n = 5 mice for each group). Data are presented as the mean ± SE. *, P < 0.05 and **, P < 0.01.

Fig. 4. Lineage tracing demonstrates α-to-β conversion in Ins2Akita diabetic mice.

(A) Experimental design of α-cell lineage tracing in Ins2Akita diabetic mice. Gcg-CreER; R26R; Ins2Akita mice were treated with tamoxifen at the age of 4 weeks, and sacrificed at the age of 16 weeks. (B) Blood glucose levels of Gcg-CreER, R26R; Ins2Akita mice were monitored at the age of 6, 10 and 14 weeks. (C) Immunostaining for β-galactosidase (β-gal, green), insulin (red), and glucagon (white) was performed. Nuclei were stained with DAPI (blue). Arrows indicate insulin and β-gal double-positive cells that were negative for glucagon. Scale bars, 50 μm. (D-F) The percentage of islets having insulin and β-gal double-positive cells with/without glucagon expression (D), insulin, glucagon, and β-gal
triple-positive cells (E), and insulin and β-gal double-positive cells without glucagon expression (F) was calculated in Ins2\textsuperscript{Akita} diabetic mice and nondiabetic control mice (n = 3–4 mice for each group). Data are presented as the mean ± SE. *, P < 0.05.

**Supplementary Fig. 1. Lineage tracing demonstrated no evidence of β-to-α conversion**

(A) Experimental design of β-cell lineage tracing. Mouse insl promoter (MIP)-Cre\textsuperscript{ER}; R26R mice were treated with tamoxifen at the age of 4 weeks, injected with ALX or vehicle at the age of 7 weeks, and sacrificed 2 weeks after ALX injection. (B) Immunostaining for β-galactosidase (β-gal, green), glucagon (red), and insulin (white) was performed. Nuclei were stained with DAPI (blue). Arrows indicate insulin and glucagon double-positive cells that were negative for β-gal. Scale bars, 50 μm.

**Supplementary Fig. 2. Lineage tracing demonstrates α-to-β conversion in db/db obese diabetic mice.**

(A) Experimental design of α-cell lineage tracing in db/db obese diabetic mice. Gcg-Cre\textsuperscript{ER}; R26R; db/db mice were treated with tamoxifen at the age of 4 weeks, and sacrificed at the age of 16 weeks. (B) Blood glucose levels of Gcg-Cre\textsuperscript{ER}; R26R; db/db mice were monitored at the age of 6, 10 and 14 weeks. (C) Immunostaining for β-galactosidase (β-gal, green), insulin (red), and glucagon (white) was performed. Nuclei were stained with DAPI (blue). Scale bars, 50 μm. (D, E) The percentage of islets containing insulin and β-gal double-positive cells with/without glucagon expression. (D) and insulin and β-gal double-positive cells without glucagon expression (E) was in db/db diabetic mice and nondiabetic control mice (n = 3 mice for each group). Data are presented as the mean ± SE.
Figure 1

A

![Diagram showing timeline for C57BL/6J mouse with ALX-1w and ALX-2w stages, and histological analysis at week 8.]

B

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Glucagon</th>
<th>Merge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>ALX-1w</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

C

![Bar graph showing percentage of islets containing BH cells across Control, ALX-1w, and ALX-2w groups.]

*Control vs. ALX-1w, p < 0.05
**Control vs. ALX-1w, p < 0.01
**Figure 2**

A. Diagram showing the experimental timeline with C57BL/6J mice receiving Alloxan, followed by ALX or ALX + ins, and subsequent histological analysis.

B. Graph showing blood glucose levels (mg/dL) over time (day) with ALX and ALX + ins treatment compared to control.

C. Bar graph showing AUC (mg·day/dL) with differences marked by asterisks (***, **).

D. Bar graph showing islets containing BH cells (%) with differences marked by asterisks (*).
Figure 3

A  

\[ \text{Gcg-Cre}^{\text{ER}}, R26R \]

\[ \text{4} \quad \text{tamoxifen} \]

\[ \text{7} \quad \text{ALX} \]

\[ \text{9} \quad \text{Histological analysis} \]

\[ \text{Alloxan} \]

\[ \text{ALX + ins} \]

\[ \text{Insulin pump} \]

B

<table>
<thead>
<tr>
<th></th>
<th>β-gal</th>
<th>Insulin</th>
<th>β-gal/insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>ALX</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td>ALX+ins</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
</tbody>
</table>

C

D

E

Islets containing α-to-Ins(+) cells (%)

Control ALX ALX + ins

Islets containing α-to-Ins(+) and Gcg(+) cells (%)

Control ALX ALX + ins

Islets containing α-to-Ins(+) and Gcg(−) cells (%)

Control ALX ALX + ins

* p < 0.05

** p < 0.01
**Figure 4**

**A**

![Diagram showing the timeline of Gcg-CreER; R26R Akita and Control with Tamoxifen administration](image)

**B**

![Graph showing blood glucose levels (mg/dL) over weeks](image)

**C**

<table>
<thead>
<tr>
<th></th>
<th>β-gal</th>
<th>Insulin</th>
<th>β-gal/Insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image" alt="Control β-gal" /></td>
<td><img src="image" alt="Control Insulin" /></td>
<td><img src="image" alt="Control β-gal/Insulin" /></td>
<td><img src="image" alt="Control Glucagon" /></td>
</tr>
<tr>
<td>Akita</td>
<td><img src="image" alt="Akita β-gal" /></td>
<td><img src="image" alt="Akita Insulin" /></td>
<td><img src="image" alt="Akita β-gal/Insulin" /></td>
<td><img src="image" alt="Akita Glucagon" /></td>
</tr>
</tbody>
</table>

**D**

<table>
<thead>
<tr>
<th>Gcg(+)</th>
<th>Ins(-)</th>
<th>Gcg(-)</th>
<th>Ins(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Islets containing α-to-Ins(-) cells (%)" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**E**

<table>
<thead>
<tr>
<th>Gcg(+)</th>
<th>Ins(-)</th>
<th>Gcg(-)</th>
<th>Ins(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Islets containing α-to-Ins(-) cells (%)" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**F**

<table>
<thead>
<tr>
<th>Gcg(+)</th>
<th>Ins(-)</th>
<th>Gcg(-)</th>
<th>Ins(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Islets containing α-to-Ins(-) cells (%)" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Fig. 1

**A**

MIP-Cre\(^{ER}\); R26R

![Timeline diagram](image)

**B**

<table>
<thead>
<tr>
<th></th>
<th>β-gal</th>
<th>Glucagon</th>
<th>β-gal/glucagon</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td><strong>ALX</strong></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
</tbody>
</table>
**Supplementary Fig. 2**

**A**

![Diagram showing the timeline for Gcg-CreER;R26R db/db and Tamoxifen treatments with 16 weeks of histological analysis.]

**B**

![Graph showing blood glucose levels for db/db and db/m mice over 16 weeks.]

**C**

<table>
<thead>
<tr>
<th>β-gal</th>
<th>Insulin</th>
<th>β-gal/insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="db/m" /></td>
<td><img src="image" alt="db/m" /></td>
<td><img src="image" alt="db/m" /></td>
<td><img src="image" alt="db/m" /></td>
</tr>
<tr>
<td><img src="image" alt="db/db" /></td>
<td><img src="image" alt="db/db" /></td>
<td><img src="image" alt="db/db" /></td>
<td><img src="image" alt="db/db" /></td>
</tr>
</tbody>
</table>

**D**

![Diagram showing the distribution of islets containing α-to-BH cells.]

**E**

![Diagram showing the distribution of islets containing α-to-Ins(+) cells.]

**Legend**

- Gcg(+): Green
- Ins(-): Blue
- Ins(+): Red
- Gcg(-): Black

**Supplementary Information**

- Gcg-CreER; R26R
- db/db
- Gcg(+): Ins(-)
- Gcg(+): Ins(+)
- Gcg(-): Ins(+)
- db/m
- db/db
1 Conflicts of interest

2 The authors declare that they have no conflicts of interest associated with this study.