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SUPPLEMENTARY INFORMATION

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Pancreatic islet-autonomous insulin and smoothened-mediated signalling modulate identity changes of glucagon$^+$ α-cells

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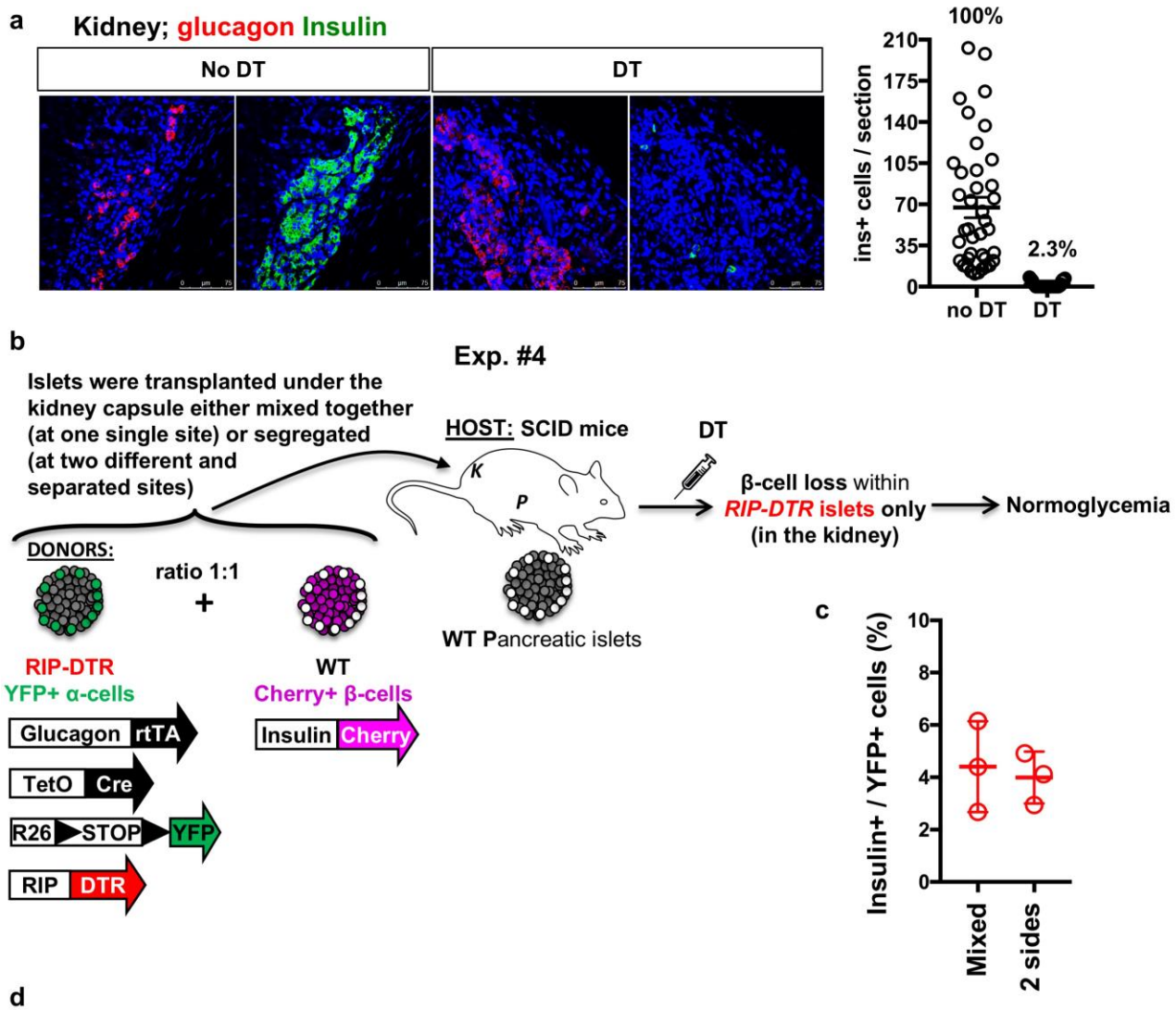
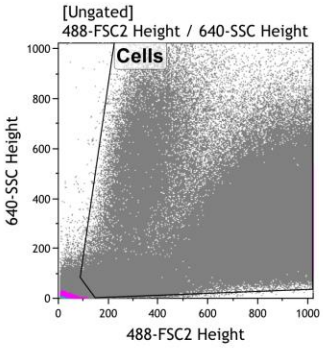

a
Kidney; glucagon Insulin
No DT
DT
100%
210
175
140
105
70
35
0
ins+ cells / section
2.3%
no DT
DT
b
Exp. #4
Islets were transplanted under the kidney capsule either mixed together (at one single site) or segregated (at two different and separated sites)
HOST: SCID mice
K
P
DT
β-cell loss within RIP-DTR islets only (in the kidney)
Normoglycemia
DONORS:
ratio 1:1
+
WT Pancreatic islets
RIP-DTR
YFP+ α-cells
WT
Cherry+ β-cells
Glucagon rtTA
TetO Cre
R26 STOP YFP
RIP DTR
Insulin Cherry
c
Insulin+ / YFP+ cells (%)
10
8
6
4
2
0
Mixed
2 sides
d
mixed RIP-DTR : WT (1:1) islets
Exp. #4
mCherry
YFP
insulin
RIP-DTR islets
W-T islet (mCherry+)
W-T islet
merged
mCherry
YFP
insulin
merged

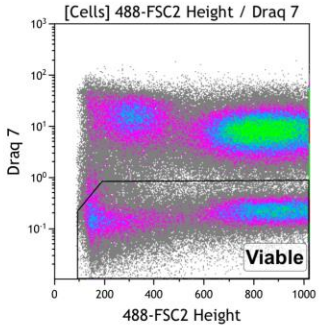
Supplementary Figure 1

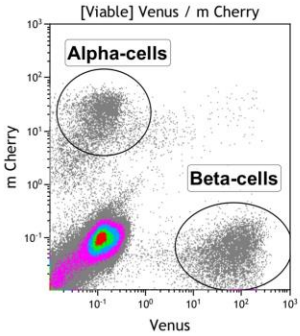
Insulin production by α-cells after β-cell ablation in islets transplanted under the kidney capsule.

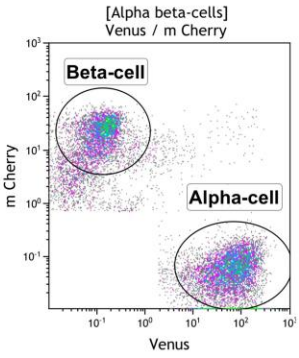
(a) Immunofluorescence staining of insulin (green) and glucagon (red) in *RIP-DTR* islets transplanted under the kidney capsule, either before ("no DT") or after ("DT") β-cell ablation. The scatter graph reports a quantification of the β-cell ablation efficiency, measured as the number of insulin-containing cells remaining per islet section in non-treated and DT-treated animals, 10 days later. (mean ± s.e.m., n=40 islet sections per condition. **(b)** Experimental design of Exp. #4, where islets were transplanted in the kidney capsule of immunocompromised mice (SCID) either mixed together or at 2 separated sites. **(c)** Proportion of YFP-traced α-cells expressing insulin 1-month post-DT in mixed *RIP-DTR*+WT islets compared to *RIP-DTR* islets from 2-sides graft. Data are shown as mean ± s.d.; n=3 biologically independent animals per condition. **(d)** Immunofluorescence staining of mCherry (magenta), YFP (green), insulin (red) in transplanted mixed *RIP-DTR*+WT islets 1-month post-DT. Scale bars: 20 µm. Experiment repeated independently 3 times. See Supplementary Table 1 as source data.

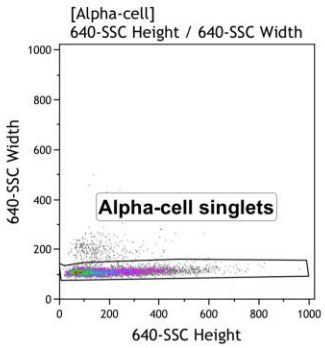
α- and β- cell sorting from Gcg-Venus / RIP-mCherry transgenic mice.

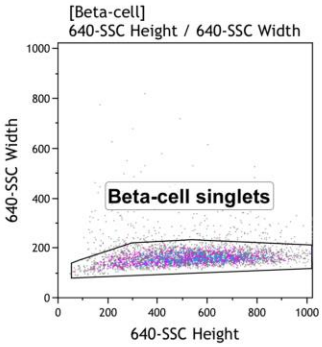






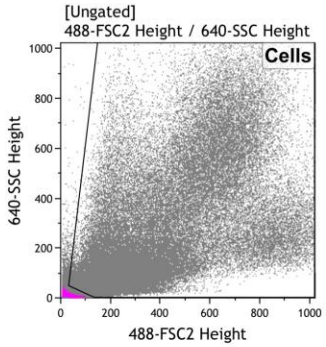


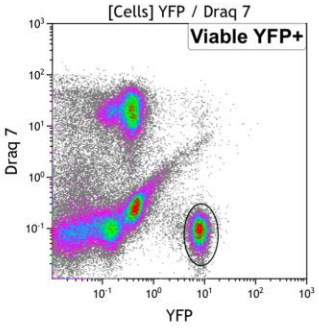




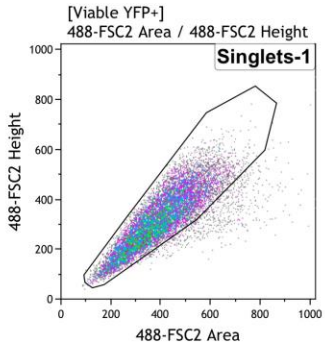
Gate	%Total	%Gated
All	100.00	100.00
Cells	21.54	21.54
Viable	7.99	37.07
Alpha beta-cells	0.75	9.40
Alpha-cell	0.36	47.93
Alpha-cell singlets	0.34	94.38
Beta-cell	0.25	33.68
Beta-cell singlets	0.24	93.39

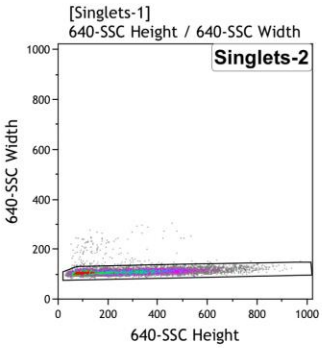
α-cell sorting from transgenic mice allowing Gcg⁺ cell-lineage tracing through DOX administration.





Gate	%Total	%Gated
All	100.00	100.00
Cells	17.89	17.89
Viable YFP+	2.41	13.45
Singlets-1	2.30	95.40
Singlets-2	2.24	97.69

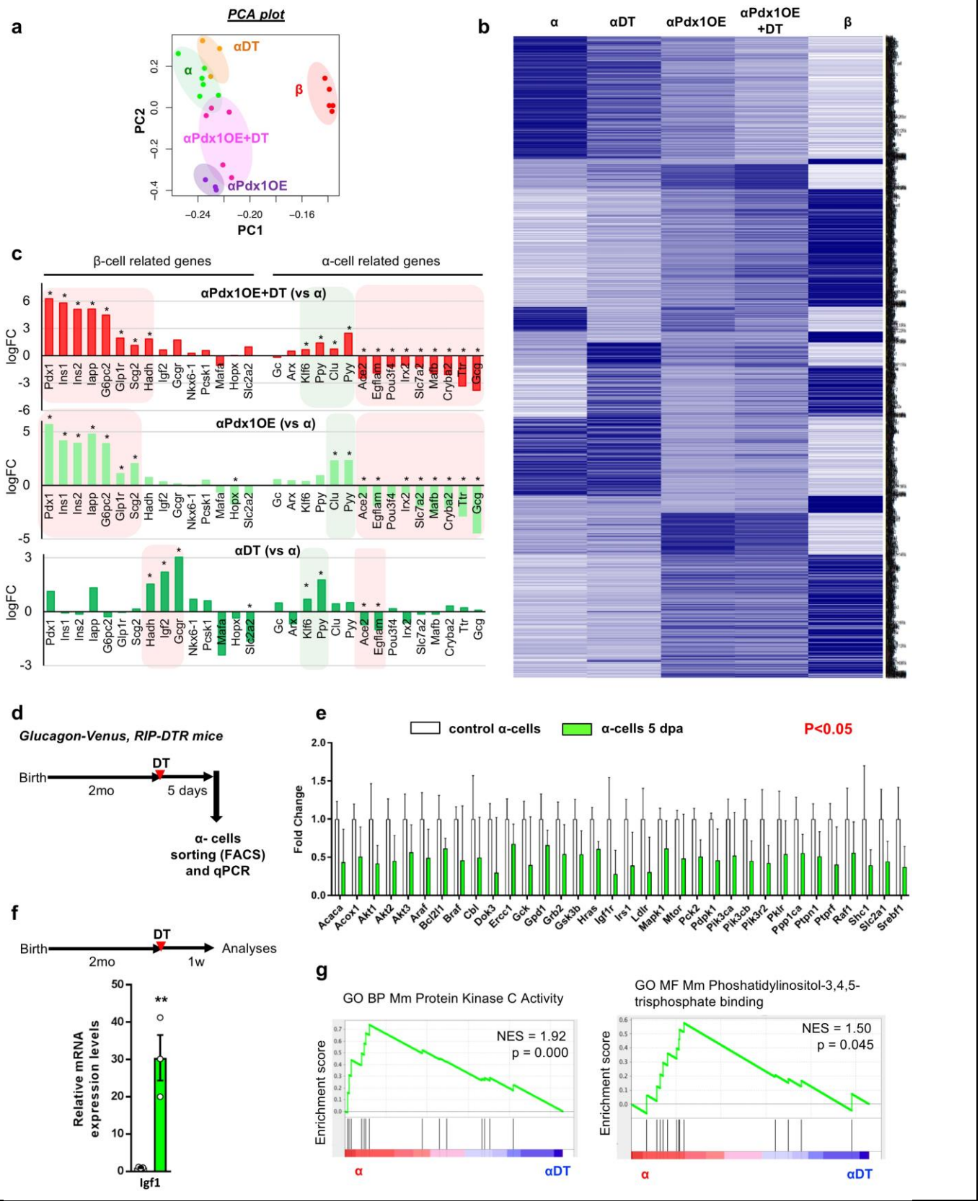




Supplementary Figure 2

FACS sorting.

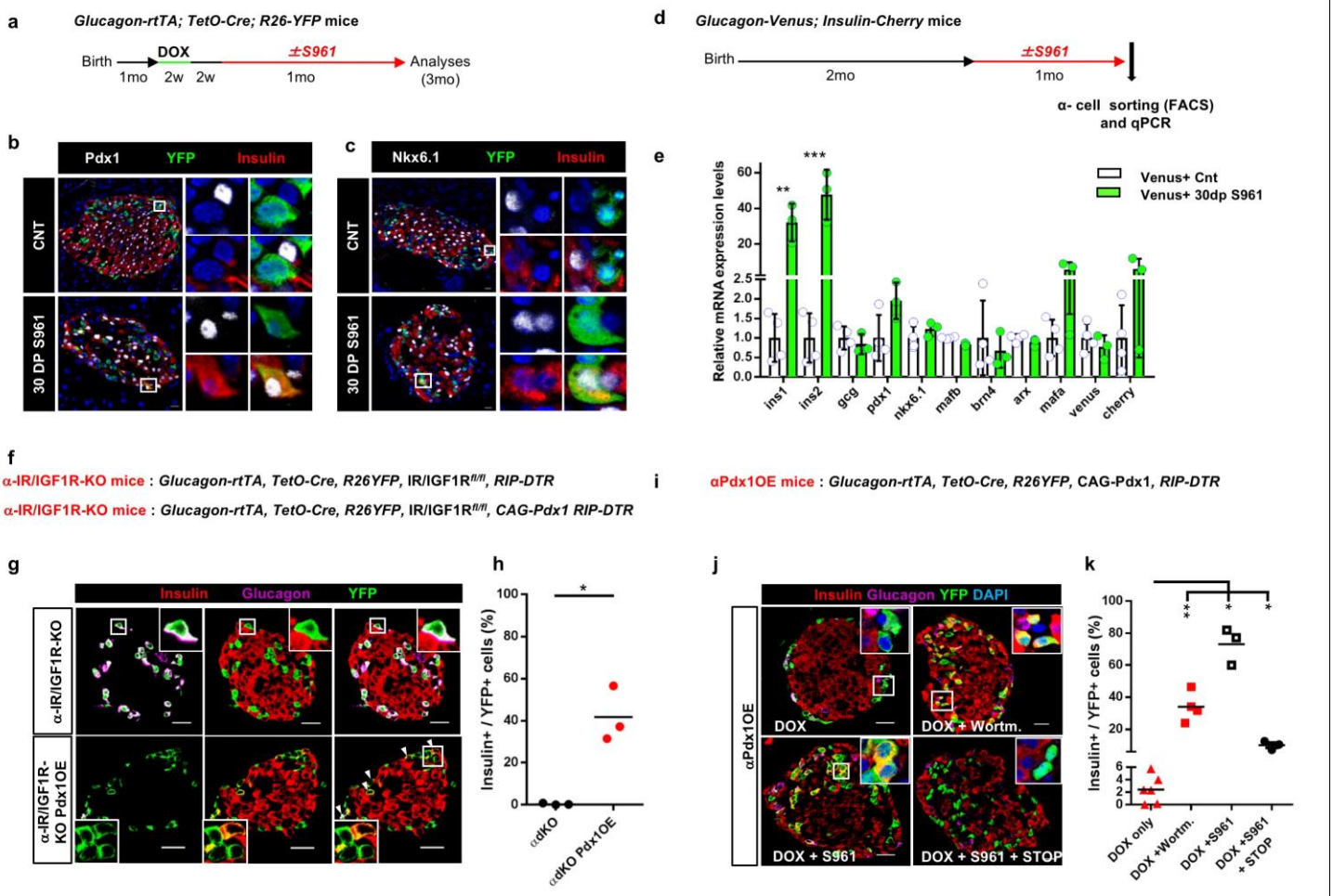
Gating strategy used for FACS sorting the different islet cell types used for RNA seq analyses. Mice used to purify the different islet cell types are listed in the methods.


a
PCA plot
αDT
α
β
αPdx1OE+DT
αPdx1OE
PC2
PC1
b
α
αDT
αPdx1OE
αPdx1OE +DT
β
c
β-cell related genes
α-cell related genes
αPdx1OE+DT (vs α)
αPdx1OE (vs α)
αDT (vs α)
logFC
Pdx1 Ins1 Ins2 Iapp G6pc2 Glp1r Scg2 Hadh Igf2 Gcgr Nkx6-1 Pcsk1 Mafa Hopx Slc2a2 Gc Arx Klf6 Ppy Clu Pyy Ace2 Egflam Pou3f4 Irx2 Slc7a2 Mafb Cryba2 Ttr Gcg
d
Glucagon-Venus, RIP-DTR mice
Birth
DT
2mo
5 days
α- cells sorting (FACS) and qPCR
e
control α-cells
α-cells 5 dpa
P<0.05
Fold Change
Acaca Acox1 Akt1 Akt2 Akt3 Araf Bcl2l1 Braf Cbl Dok3 Ercc1 Gck Gpd1 Grb2 Gsk3b Hras Igf1r Irs1 Ldlr Mapk1 Mtor Pck2 Pdpk1 Pik3ca Pik3cb Pik3r2 Pklr Ppp1ca Ptpn1 Ptprf Raf1 Shc1 Slc2a1 Srebf1
f
Birth
DT
Analyses
2mo
1w
Relative mRNA expression levels
**
Igf1
g
GO BP Mm Protein Kinase C Activity
NES = 1.92
p = 0.000
GO MF Mm Phoshatidylinositol-3,4,5-trisphosphate binding
NES = 1.50
p = 0.045
Enrichment score
α
αDT

Supplementary Figure 3

α-cell transcriptome analysis and gene expression changes after β-cell loss.

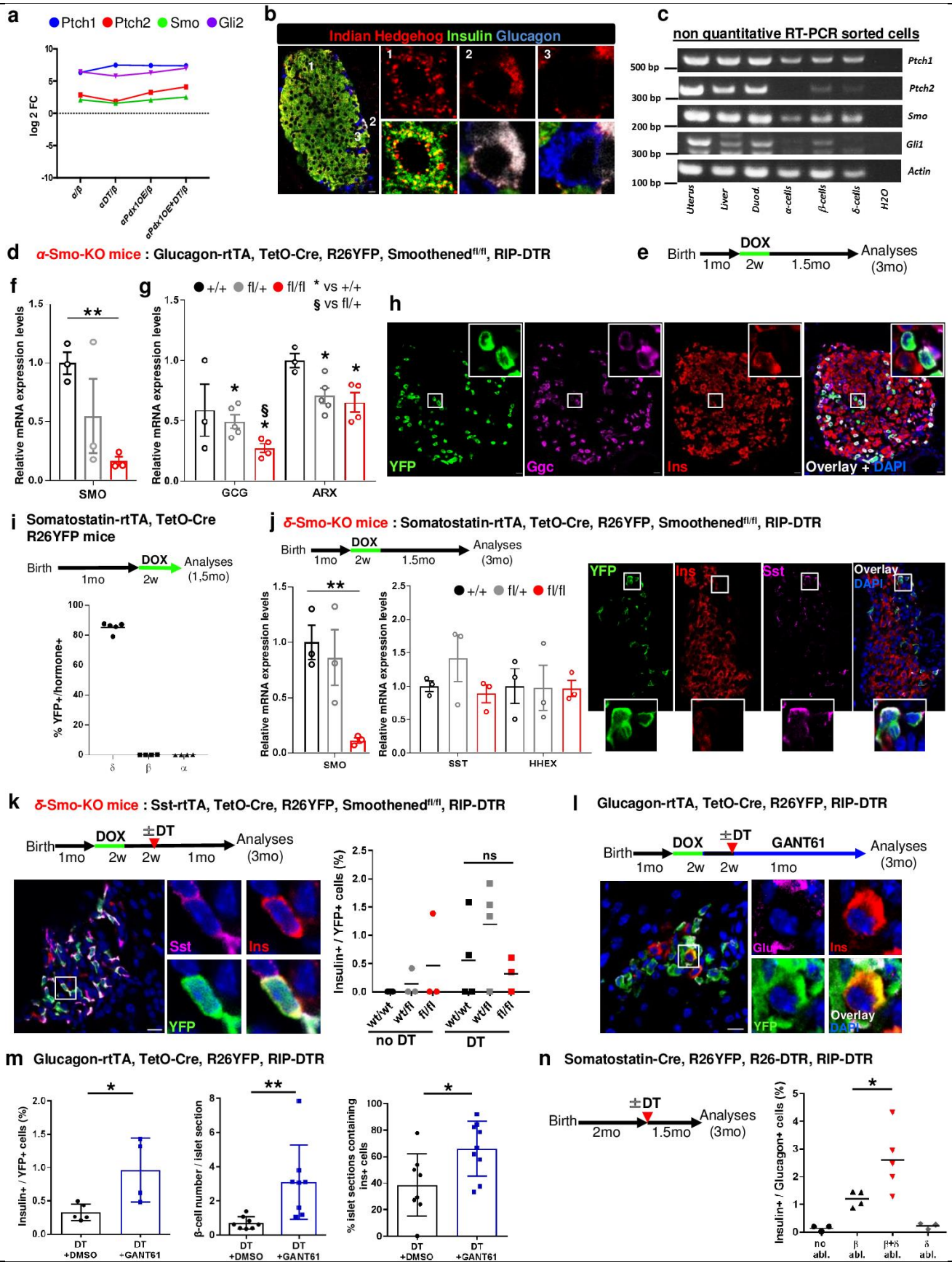
(a) PCA plot of all samples analyzed by RNA-Seq. Native β-cells ("β", n=5 mice) show a different profile compared to α-cell groups including native α-cells ("α", n=6 mice), α-cells 1 month after DT-induced β-cell ablation ("αDT", n=3 mice), α-cells overexpressing Pdx1 ("αPdx1OE", n=3 mice), and α-cells overexpressing Pdx1 combined with β-cell ablation ("αPdx1OE+DT", n=5 mice). **(b)** Heat map showing scaled expression (blue, high; white, low) of α- / β-cell-enriched genes differentially expressed in αDT, αPdx1OE, αPdx1OE+DT conditions compared to native α-cells. **(c)** Gene expression changes (log2 value) in αPdx1OE+DT (top), αPdx1OE (middle) and αDT (bottom) compared to native α-cells. DEGs indicated by red-colored background show induced β-cell signature upon the conditions, while DEGs indicated by green-colored background show refractory responses. * FDR < 0.05. n = 6 mice in α, n = 5 mice in αPdx1OE+DT, n = 3 mice in αPdx1OE, and n = 3 mice in αDT. **(d)** Transgenes required for constitutive labeling α-cells with the reporter *Venus* and experimental design for α-cell labeling and purification after β-cell loss**. (e)** RT-qPCR analyses for different components of the insulin/IGF1-R signaling pathway. Components of the pathway are downregulated in α-cells 5 days post-DT. Center indicates the mean. qPCR were performed in triplicates and data processing and statistical analyses were performed using the web-based software "RT2 Profiler PCR Array Data Analysis version 3.5", from Qiagen. Quantification of the gene expression changes were performed using the $2^{-\Delta\Delta Ct}$ method. *P*-values were calculated using a Student's t-test (two-tail distribution and equal variances between the two samples) on the $2^{-\Delta Ct}$ values. Genes with $P<0.05$ are shown. n=3 mice. qPCR was performed once. **(f)** Experimental design for DT-mediated β-cell loss, islet isolation and qPCR. Igf1 mRNA is upregulated in islets after β-cell loss, likely as an attempt to sustain insulin signaling. Data shown as mean ± sem; n=3 mice with 3 technical replicates each. Center indicates the mean. Two-tailed unpaired t-test $P=0.0086$. **(g)** Gene set enrichment analysis (GSEA) from RNA-Seq showing enrichment of the insulin signaling pathway components PKC (left) and PI3K (right) in α-cells after DT-mediated β-cell ablation (αDT) compared to native α-cells (α). *P*-values were calculated by an empirical phenotype-based permutation test. n = 6 mice in α, n = 3 mice in αDT. NES: normalized enrichment score. See Supplementary Table 2 as source data.



Supplementary Figure 4

Insulin production in α-cells under insulin signaling blockade.

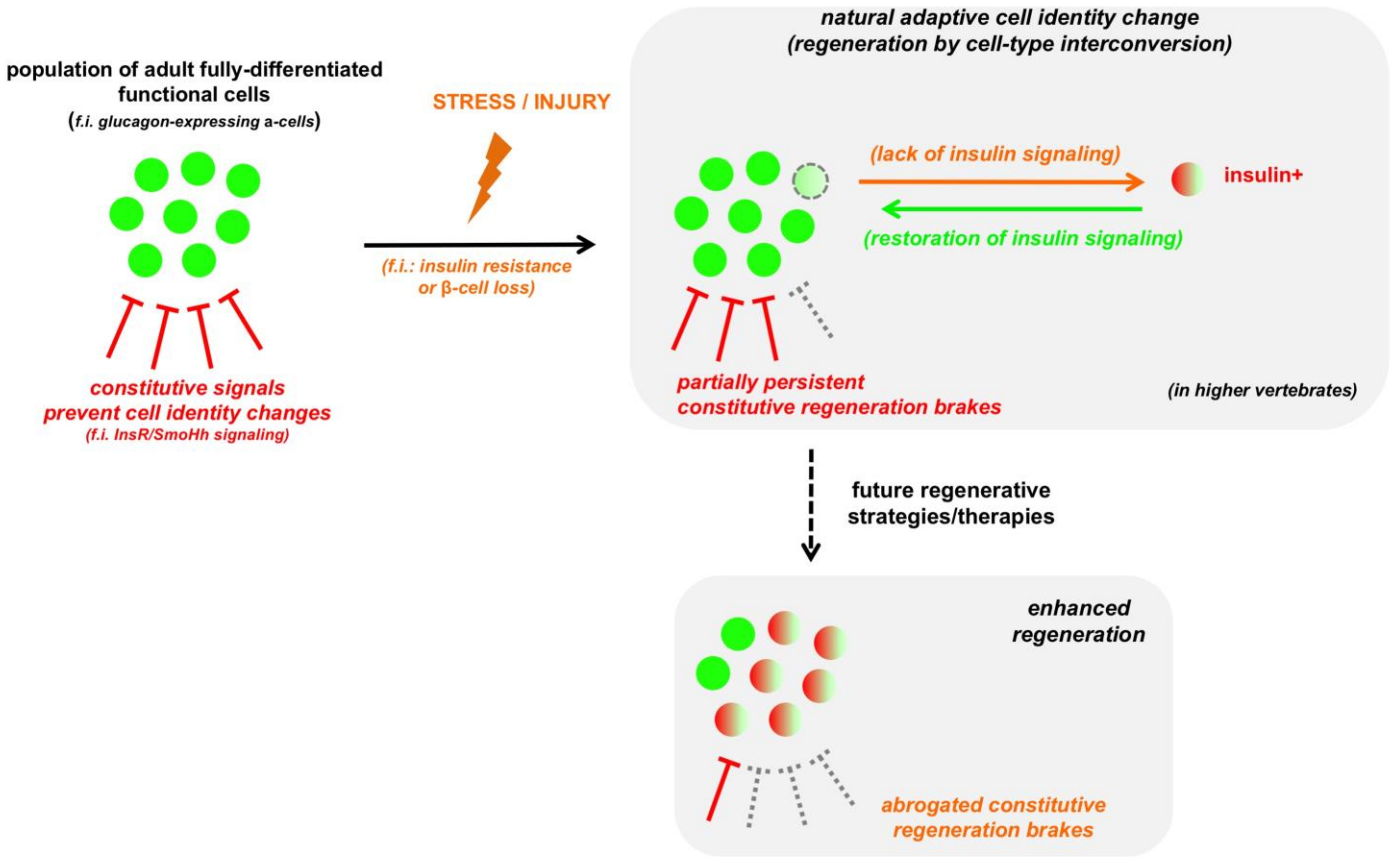
(a) Transgenes for inducible α-cell lineage tracing and experimental design for α-cell lineage tracing and S961 treatment. **(b-c)** Immunofluorescence staining for the β-cell-specific transcription factors Pdx1 and Nkx6.1 in islets of mice treated for 1 month with S961. Experiments were repeated once, with n=3 mice treated asynchronously according to their availability. **(d)** Mice and experimental design for α-cell labeling and sorting by FACS. **(e)** β-cell-specific genes are upregulated in α-cells upon S961 treatment. qPCRs were performed in triplicates. Data shown as mean ± sem; n=3 mice. Experiment performed once. Two-tailed unpaired t-test (*P*=0.0017 INS1; *P*=0.0010 INS2**)** **(f)** Transgenes required for inducible α-cell lineage tracing, simultaneous insulin receptor (IR) and IGF-1 receptor (IGF1R) downregulation, and ectopic Pdx1 induction in adult α-cells. **(g)** Impaired insulin/IGF1 signaling in α-cells through IR and IGF1R downregulation predisposes α-cells to insulin gene expression in mice with normal β-cell mass. **(h)** Fractions of α-cells expressing insulin after IR/IGF1R downregulation in α-cells and Pdx1OE. *n* = 3 mice. Two-tailed Mann Whitney test**,** P=0.05. **(i)** Transgenes required for inducible Pdx1 expression in adult α-cells. **(j)** Wortmannin-mediated PI3K inhibition or S961-mediated insulin antagonism trigger insulin production in Pdx1OE α-cells. Insulin production in Pdx1OE α-cells is reversed when islets are analyzed 1 month after ending S961 treatment. **(k)** Fraction of converted α-cells found upon insulin signaling antagonism/inhibition. Black and red symbols: control and αPdx1OE mice, respectively. *n* = 6, 4, 3, 3 mice in DOX only, DOX+Wort, DOX+S961 and DOX+S961+STOP, respectively. Two-tailed Mann Whitney test**,** *P***=**0.0061 Pdx1 only vs Pdx1+wort, *P*= 0.0167 Pdx1 only vs Pdx1+S961, *P*= 0.0333 Pdx1 only vs Pdx1+S961then stop. All experiments were performed once with animals treated in an asynchronous way depending on their availability. Scale bars: 20 μm. See Supplementary Table 1d as source data.


a
Ptch1 Ptch2 Smo Gli2
log 2 FC
αβ
αDT/β
αPdx1OE/β
αPdx1OE+DT/β
b
Indian Hedgehog Insulin Glucagon
c
non quantitative RT-PCR sorted cells
500 bp
300 bp
200 bp
300 bp
100 bp
Ptch1
Ptch2
Smo
Gli1
Actin
Uterus
Liver
Duod.
α-cells
β-cells
δ-cells
H2O
d α-Smo-KO mice : Glucagon-rtTA, TetO-Cre, R26YFP, Smoothened fl/fl, RIP-DTR
e
Birth
DOX
1mo
2w
1.5mo
Analyses (3mo)
f
Relative mRNA expression levels
SMO
g
+/+ fl/+ fl/fl * vs +/+ § vs fl/+
GCG
ARX
h
YFP
Ggc
Ins
Overlay + DAPI
i Somatostatin-rtTA, TetO-Cre R26YFP mice
Birth
1mo
DOX
2w
Analyses (1,5mo)
% YFP+/hormone+
δ
β
α
j δ-Smo-KO mice : Somatostatin-rtTA, TetO-Cre, R26YFP, Smoothened fl/fl, RIP-DTR
Birth
1mo
DOX
2w
1.5mo
Analyses (3mo)
Relative mRNA expression levels
SMO
+/+ fl/+ fl/fl
SST
HHEX
YFP
Ins
Sst
Overlay DAPI
k δ-Smo-KO mice : Sst-rtTA, TetO-Cre, R26YFP, Smoothened fl/fl, RIP-DTR
Birth
1mo
DOX
2w
±DT
2w
1mo
Analyses (3mo)
Sst
Ins
YFP
Insulin+ / YFP+ cells (%)
ns
wt/wt
wt/fl
fl/fl
no DT
DT
l Glucagon-rtTA, TetO-Cre, R26YFP, RIP-DTR
Birth
1mo
DOX
2w
±DT
2w
GANT61
1mo
Analyses (3mo)
Glu
Ins
YFP
Overlay DAPI
m Glucagon-rtTA, TetO-Cre, R26YFP, RIP-DTR
Insulin+ / YFP+ cells (%)
β-cell number / islet section
% islet sections containing ins+ cells
DT +DMSO
DT +GANT61
n Somatostatin-Cre, R26YFP, R26-DTR, RIP-DTR
Birth
2mo
±DT
1.5mo
Analyses (3mo)
Insulin+ / Glucagon+ cells (%)
no abl.
β abl.
β+δ abl.
δ abl.

Supplementary Figure 5

Hedgehog signaling modulation in α- and δ-cells.

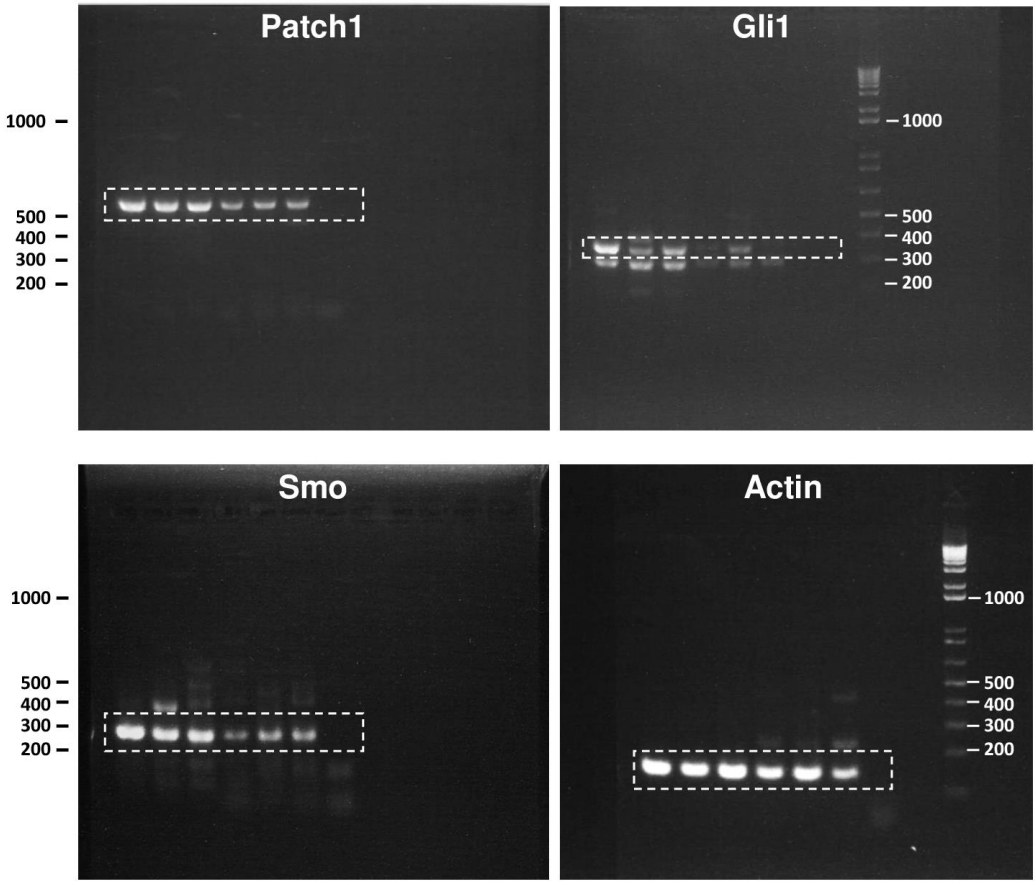
(a) Hh signaling components are enriched in α- *vs* β-cells and their expression is unchanged 30 days upon DT-induced β-cell ablation, Pdx1OE or both. Log2FC are taken from the RNA-Seq presented in Fig.3. **(b)** Indian Hedgehog ligand expression in islets of control mice. Immunofluorescence was repeated two times with similar results. **(c)** Non-quantitative RT-PCR of Hedgehog pathway's components in sorted α-, δ- and β-cells. Liver, duodenum and uterus were used as controls. Experiments were repeated three times with similar results. Unprocessed scans of the blots are provided in Suppl. Fig.7. **(d)** Transgenes required for simultaneous α-cell lineage tracing, *Smo* co-receptor downregulation and DT-induced β-cell ablation. **(e)** Experimental design. **(f)** *Smo* and **(g)** α-cell genes Arx and Glucagon are downregulated in α-cells with reduced Smo activity upon DOX treatment. n=3 mice. qPCR was performed once. Two-tailed unpaired t-test; SMO: *P*=0.0012, GCG: *P*=0.0325 *fl*/+ vs +/+ *P*=0.0141 *fl/fl* vs +/+ and *P*=0.0177 *fl/fl* vs *fl*/+, ARX: *P*=0.0214 *fl*/+ vs +/+, 0.0245 *fl/fl* vs +/+). Center indicates the mean. **(h)** *Smo* inactivation in α-cells in absence of β-cell loss or insulin signaling impairment does not lead to insulin production in intact islets. Immunofluorescence was repeated once, on 2 slides of 3 different mice. **(i)** Transgenes required to lineage trace δ-cells, experimental design and characterization of labeling specificity in the *Sst-rtTA* line. Only δ-cells express YFP. *n* = 5, 4, 4 for δ-, β- and α-cells, respectively. **(j)** Transgenes required for lineage tracing and *Smo* coreceptor inactivation in δ-cells, and for DT-induced β-cell ablation and experimental design. *Smo* is downregulated in purified δ-cells after DOX treatment. n=3 mice. qPCRs were performed once. Two-tailed unpaired t-test, *P*=0.0048. Downregulation of *Smo* in δ-cells does not alter the expression of the δ-cell genes Somatostatin and Hhex. n=3 mice. qPCR was performed once. Center indicates the mean. Insulin protein is not expressed in δ-cells upon *Smo* inactivation in intact islets. Immunofluorescence was repeated once, on 3 slides of 3 different mice. **(k)** Experimental design for δ-cell lineage tracing and DT-induced β-cell loss in δ-*Smo*-KO mice. A fraction of δ-cells express insulin after β-cell ablation in δ-*Smo*-KO mice, but their percentage is not increased when compared to mice with intact *Smo* expression. *n* = 3, 3, 3 mice in no DT *wt/wt*, *wt/fl, fl/fl* respectively and *n* = 4, 4, 3 mice in DT *wt/wt*, *wt/fl*, *fl/fl* respectively. Immunofluorescence was repeated once, on 3 to 5 slides per mouse. Center in the graph indicates the mean. Two-tailed unpaired t-test. (**l**) Transgenes required for α-cell tracing and DT-induced β-cell ablation, experimental design and immunofluorescence staining of mouse islets 1 month after DT+GANT61 treatment. Immunofluorescence was repeated once, on 3 to 5 slides of *n* = 8 mice treated with DT+GANT61. A representative islet is shown. (**m)** The % of α-cells expressing insulin (*n* = 5 and 4 mice for DT+DMSO and DT+GANT61, respectively), the number of insulin-expressing cells per islet section (*n* = 8 mice for DT+DMSO and DT+GANT61), and the % of islets containing insulin$^+$ cells (*n* = 8 mice and n=9 mice for DT+DMSO and DT+GANT61, respectively) are increased in mice treated with GANT61 after DT-induced β-cell loss. Two-tailed unpaired t-test (*P*=0.0236 panel left**,** *P*=0.0085 middle, *P*=0.0213 right graph. **(n)** Transgenes required for simultaneous β- and δ-cell ablation, experimental design and percentage of cells coexpressing insulin and glucagon after β- and δ-cell loss. *n* = 4, 5, 3 mice for β-, β+δ and δ-cell ablation, respectively. Center indicates the mean. Two tailed unpaired t-test, *P*=0.0494. Scale bars: 10 μm. See Supplementary Table 1m,n,q as source data.



Supplementary Figure 6

Constitutive signals ensure maintenance of cell identity.

In physiological conditions, the identity of fully differentiated cells (such as α-cells) is maintained through a variety of constitutive signals preventing cell identity changes. Most of these brakes (f.i. insulin signaling) are preserved upon stress and injury (f.i. β-cell loss). However, few cells may undergo changes in cell identity as an attempt to replenish the compromised cell population (f.i. α-cells produce insulin to cope with insulin insufficiency). Due to the persistence of most constitutive brakes, this kind of natural cell interconversion events are rare and help explaining the poor regenerative capacity observed in higher vertebrates. Insulin-expressing α-cells may reacquire their initial fate upon restoration of physiological conditions (reversibility). Future therapies aimed at identifying and abrogating constitutive regeneration brakes should improve the intrinsic regenerative capacity of likely any organ.



Supplementary Figure 7

Unprocessed blots.

Unprocessed scans of the non-quantitative PCR blots provided in Supplementary Fig. 6.

Supplementary Table 1. Statistics Source Data.

a) Statistics Source Data for for Figure 1 and Supplementary Figure 1
Proportion of α-cells that engage in insulin expression after β-cell ablation in islets transplanted under the kidney capsule of SCID host mice.

b) Statistics Source Data for Figure 2
Percentage of YFP+ α-cells expressing glucagon or insulin in control and αPdx1OE mice in presence of intact β-cell mass.

c) Statistics Source Data for for Figure 2
Pancreatic content in control and αPdx1OE mice.

d) Statistics Source Data for Fig.2 and Supplementary Figure 4
Conversion rate of YFP-labeled α-cells to insulin production in Pdx1OE mice.

e) Statistics Source Data for Figure 4
Conversion rate of YFP-labeled α-cells to insulin production after insulin signaling blockade and administration of insulin pellets.

f) Statistics Source Data for Figure 5
Conversion rate of YFP-labeled α-cells to insulin production in α-Smo-KO mice.

g) Statistics Source Data for Supplementary Fig.5
In vivo GSIS (1 month after DT in α-Smo-KO mice).

h) Statistics Source Data for Supplementary Fig.5
α-cells FACS-sorting from Smo-KO mice for pseudoislets reconstruction.

i) Statistics Source Data for Supplementary Fig.5
% of Smo-KO α-cells producing insulin in pseudoislets.

l) Statistics Source Data for Supplementary Fig.5
In vitro GSIS of composed of Smo-KO α-cell pseudoislets.

m) Statistics Source Data Supplementary Figure 5
Labeling of δ-, β- and α-cells in the Somatostatin-rtTA mouse line.

n) Statistics Source Data for Figure 6 and Supplementary Figure 6
Conversion rate of YFP-labeled δ-cells to insulin production in δ-Smo-KO mice.

o) Statistics Source Data for Figure 6
% of cells coexpressing glucagon and insulin in δ-Smo-KO mice.

p) Statistics Source Data for Figure 6
Conversion rate of YFP-labeled α-cells to insulin production in α+δ-Smo-KO mice.

q) Statistics Source Data for Supplementary Figure 6
Cells coexpressing glucagon and insulin after β-, δ+β- and δ-cell ablation.

Supplementary Table 2: RNA analyses

a) DEGs between α- vs β-cells at a fold change (FC) >2 (log2FC > 1) and false discovery rate (FDR) <0.01.
b) Data of all comparisons in αDT, αPdx1OE and αPdx1OE+DT compared to native α-cells.
c) DEG lists in α-Pdx1OE (vs α)
d) DEG lists in αDT (vs α)
e) DEG lists in αPdx1OE+DT (vs α)
f) Gene lists in Venn diagram shown in Fig. 2d
g) Gene set list significantly modulated upon DT-induced β-cell loss in α-cells (related to GSEA in Suppl. Fig. 6f)

Supplementary Table 3: Primers
List of primers used for qPCR analyses.

Supplementary Table 4: Antibodies
List of antibodies used for Immunofluorescence.