Methods

Mice.

All animals were housed and handled according to the guidelines established by the Direction Générale de la Santé of the canton of Geneva. *RIP-DTR* and *Glucagon-rtTA* [1], *TetO-Cre* [2], *R26-EYFP* [3], *R26-dTomato* [4], *Ngn3-YFP* [5], *RIP-Cre* [6], *Ngn3-tTA* and *Tre-Ngn3* [7], *R26-iDTR* [8], and *Ngn3-CreERT* [9] mice were previously described. F. Reimann and F. M. Gribble generated the *Somatostatin-Cre* mice. This line bears an *Sst-mCherry-2A-iCre* transgene. The Sst promoter was cloned from BAC bQ73b10, with NOD background; initially a rpsLneo sequence (Genebridges) providing kanamycin resistance and streptomycin sensitivity was introduced after the STOP codon in Sst-exon2 and subsequently all sequence between the Sst-START codon in exon1 and the rpsLneo sequence was replaced by the mCherry-2A-iCre sequence. In the resulting mice, no mCherry-fluorescence can be detected on tissue sections; however, when combined with the *R26-EYFP* or *R26-dTomato* transgenes, strong fluorescence can be detected in about 80% of pancreatic δ-cells as well as gastric D cells. The *Insulin-mCherry* mice were generated by one of us (G. Gu); in these animals, more than 95% of insulin-expressing cells are mCherry^{+}. For *Ngn3CreERT* mice, as previously reported, no Ngn3 or YFP expression can be detected in postnatal islets. In juvenile (i.e. prepubescent) mice, islet Ngn3 expression is upregulated exclusively after β-cell ablation as confirmed by both qPCR (on isolated islets) and with a second transgenic line, the *Ngn3-YFP* knock-ad on mice. The age-specific Cre labeling efficiencies for the different lines used in the manuscript are presented in the following table. For each experiment mice were selected according to gender, general health and genotype. Mice fulfilling these conditions were randomly selected for treatments/controls. No blinding was possible due to regular glycaemia check-up and the obvious phenotype.

***Somatostatin-Cre / R26-YFP* line**	**YFP-labeled Sst^{+} cells (%)**	**mice (n)**	**scored Sst^{+} cells**
Juvenile	82.6 ± 6.9	7	3081
Adult	80.3 ± 6.2	4	1400
Aged (not analysed)	-	-	-
***Glucagon-rtTA / TetO-Cre / R26-YFP* line**	**YFP-labeled Glu^{+} cells (%)**	**mice (n)**	**scored Glu^{+} cells**
Juvenile	88.6 ± 3.6	5	3711
Adult	86.9 ± 3.3	3	1850
Aged	85.6 ± 3.2	4	4852
***RIP-Cre / R26-YFP* line**	**YFP-labeled Ins^{+} cells (%)**	**mice (n)**	**scored Ins^{+} cells**
Juvenile	79.7 ± 4.2	3	7516
Adult (not analysed)	-	-	-
Aged (not analysed)	-	-	-

Diphtheria toxin, tamoxifen, doxycycline, streptozotocin, FoxO1 inhibitor (AS1842856*)* and insulin treatments.

DT (Sigma) was given in 3 intraperitoneal (i.p.) injections (126 ng of DT per injection, on days 0, 3 and 4), or as single intraperitoneal (i.p.) injection to 2-week-old pups. Injected middle-aged and aged mice were always males; pups of both genders were given DT, however only the males were used in the experiments presented in the manuscript, for homogeneity.

Tamoxifen (TAM) was freshly prepared (Sigma) and administered i.p. (2 doses of 5 mg, 2 days apart). TAM (20 mg) was diluted in 50 µl 100% ethanol and 950 µl corn oil.

DOX (1 $mg.ml^{-1}$) (Sigma) was added to drinking water for 2 weeks.

Streptozotocin (Sigma) was administrated by a single intra-peritoneal (i.p.) injection (200 mg/kg) to 2-week-old pups as previously described [10].

2-month-old mice were i.p. injected daily, for 5 days, either 30 mg/kg of AS1842856 (a FoxO1 inhibitor; Calbiochem [11,12]) or the vehicle (DMSO).

Mice received subcutaneous implants of insulin (Linbit) when hyperglycemic (>20 mM) in the long-term regeneration experiments.

Immunofluorescence.

Cryostat sections were 10µm-thick. The following antibodies were used:

Primary antibody	Dilution	Company
guinea-pig anti-porcine insulin	1/400	Dako
mouse anti-porcine glucagon	1/1,000	Sigma
rabbit anti-human somatostatin	1/200	Dako
mouse anti-human somatostatin	1/200	BCBC (Ab1985)
goat anti-human somatostatin	1/200	SantaCruz
rabbit anti-human PP	1/200	Bachem
mouse anti-Ki67	1/200	BD Transduction Laboratory
rabbit anti-GFP	1/200	Molecular Probes
chicken anti-GFP	1/400	Abcam
mouse anti-mCherry	1/500	Abcam

Because of known technical difficulties, we were unable to perform Ngn3 immunodetection on adult pancreas sections.

The secondary antibodies were coupled with Alexa 488, 555, 546, 598 or 647 (Molecular Probes, 1:500), or TRITC (Southern Biotech, 1:500). Wherever necessary the secondary detection was performed in two sequential stages (we detected firstly the primary antibody raised in goat by using a donkey anti-goat AlexaFluor secondary antibody then, following extensive washings, we performed a second round of detection using a cocktail of the goat-raised secondary antibodies).

Secondary antibody	Dilution	Company
goat anti-mouse TRITC (IgG1 –γ1)	1/500	Southern Biotech
goat anti-mouse 555 (IgG1 –γ1)	1/500	Molecular Probes
goat anti-mouse 647 (IgG1 –γ1)	1/500	Molecular Probes
goat anti-rabbit 488 (highly cross-adsorbed)	1/500	Molecular Probes
donkey anti-rabbit 594	1/500	Molecular Probes
goat anti-chicken 488	1/500	Molecular Probes
goat anti-guinea pig 488 (highly cross-adsorbed)	1/500	Molecular Probes
goat anti-guinea pig 568 (highly cross-adsorbed)	1/500	Molecular Probes
goat anti-guinea pig 647 (highly cross-adsorbed)	1/500	Molecular Probes
donkey anti-goat 647	1/500	Molecular Probes

For the double tracing experiment the following two different combinations of antibodies were used:

FIRST COMBINATION		
Primary antibodies:	**Dilution**	**Source**
guinea-pig anti-porcine insulin	1/400	Dako
chicken anti-GFP	1/400	Abcam
mouse anti-mCherry	1/500	Abcam
rabbit anti-human somatostatin	1/200	Dako
Secondary antibodies:	**Dilution**	**Source**
goat anti-guinea pig 647 (highly cross-adsorbed)	1/500	Molecular Probes
goat anti-chicken 488	1/500	Molecular Probes
goat anti-mouse 555 (IgG1 –γ1)	1/500	Molecular Probes
donkey anti-rabbit 594	1/500	Molecular Probes

SECOND COMBINATION		
Primary antibodies:	**Dilution**	**Source**
guinea-pig anti-porcine insulin	1/400	Dako
rabbit anti-GFP	1/200	Molecular Probes
mouse anti-human somatostatin	1/200	BCBC (Ab1985)
Secondary antibodies:	**Dilution**	**Source**
goat anti-guinea pig 647 (highly cross-adsorbed)	1/500	Molecular Probes
goat anti-rabbit 488 (highly cross-adsorbed)	1/500	Molecular Probes
goat anti-mouse TRITC (IgG1 –γ1)	1/500	Southern Biotech

In the second combination, δ-cells were traced directly with the endogenous fluorophore, without further antibody amplification, since its intensity was high enough (1 hour 5% PFA for sample fixation).

Sections were analyzed with Leica TCS SPE, SP2 AOBS, Leica TCS SP5 STED CW confocal microscopes and Leica M205FA binocular equipped with a Leica DFC360FX camera, when appropriate. Section area quantifications were performed with *Imaris, Volocity* or *ImageJ* programs. Additionally, in a few images the original color of each channel was changed to colors that allow a better visualization of the overlay colocalization (like Extended Data Fig. 8k). No specific feature of the original data was obscured, eliminated or misrepresented.

Physiological studies.
Glucose tolerance tests and insulin dosages (immunoassay, ELISA kit mouse insulin ultrasensitive Mercodia) were performed as described [1]. Animals (4 males per group, 5-month-old) were fasted overnight for 12 hours before starting the experiment.
Insulin tolerance test was performed as described [13]. Animals (7 males for control and 10 males for DT-treated, 1.5-year-old) were fasted for 5 hours before the experiment. 0.75 U/kg per mice of Novorapid insulin was injected.

Transplantations.
Islet transplantations under the kidney capsule were performed as described [14].

Total RNA extraction, cDNA synthesis and qPCR.
Adult and pup islets (n≥3) were isolated as described [15] and the samples were either directly processed for RNA extraction (1 sample = 1 mouse) or incubated in accutase (Invitrogen) for 12 min. at 37°C to prepare a single-cell suspension, followed by sorting on a FACSAria2 (BD Biosciences) or Moflo Astrios (Beckman Coulter) system (for β-cell sorting, 1 sample = 1 mouse; for δ-cell sorting, 1 sample = pool of 3 mice). For all samples the total RNA was isolated with the Qiagen RNeasy Micro kit (Qiagen #74004). The subsequent cDNA synthesis, qPCR reaction and data analysis, were performed either as described [1] or by using the RT^2 Profiler PCR Array combined to RT² SYBR Green ROX FAST Mastermix (QIAGEN) according to the manufacturer's

instructions. Each individual sample (mouse) was run in triplicate, in 3 independent qPCR reactions.

Gene	Primer Sequence
β-actin	**F**5'AAGGCCAACCGTGAAAAGAT 3' **R**5'GTGGTACGACCAGAGGGATAC 3'
18S	**F**5'CAGATTGATGGCTCTTTCTCG 3' **R**5'AGACAAATCGCTCCACCAAC 3'
AKT2	**F**5'AGGTAGCTGTCAACAAGGCA3' **R**5'CTTGCCGAGGAGTTTGAGAT3'
AR	**F**5'CGAAGTGTGGTATCCTGGTG3' **R**5'GGTACTGTCCAAACGCATGT3'
ARX	**F**5'TTTTCTAGGAGCAGCGGTGT3' **R**5'AGTGGAAAAGAGCCTGCCAA3'
BRN4	**F**5'CATCGAGGTGAGTGTCAAGG3' **R**5'CAGACACGCACCACTTCTTT3'
CDK2	**F**5**'**GGACTAGCAAGAGCCTTTGG3' **R**5'AAGAATTTCAGGTGCTCGGT3'
CDKN1a	**F**5'AGTCTCATGGTGTGGTGGAA3**'** **R**5'GACATCACCAGGATTGGACA3'
CDKN1b	**F**5'AGTGTCCAGGGATGAGGAAG3' **R**5'CTTCTGTTCTGTTGGCCCTT3'
CDKN1c	**F**5'AATCAGCCAGCCTTCGAC3' **R**5'ATCACTGGGAAGGTATCGCT3'
CKS1b	**F**5'TCCATGAACCAGAACCTCAC3' **R**5'GGCTTCATTTCTTTGGCTTC3'
ESR1	**F**5'GCCTCAATGATGGGCTTATT3' **R**5'AAAGCCTGGCACTCTCTTTG3'
FOXO1	**F**5'GAGAAGAGGCTCACCCTGTC3' **R**5'ACAGATTGTGGCGAATTGAA3'
GADPH	**F**5'TCCATGACAACTTTGGCATTG3' **R**5'CAGTCTTCTGGGTGGCAGTGA3'
GCG	**F**5'GAGGAGAACCCCAGATCATTCC3' **R**5'TGTGAGTGGCGTTTGTCTTCA3'
GLUT2	**F**5'CTCGTGGCGCTGATGCT3' **R**5'CTGGTTGAATAGTAAAATATCCCATTGA3'
Insulin2	**F**5'TCAACATGGCCCTGTGGAT3' **R**5'AAAGGTGCTGCTTGAAAAAGC3'
MafA	**F**5'GGAGGTCATCCGACTGAAACA3'

	R5'GCACCTCTCGCTCTCCAGAAT3'
MafB	**F**5'TGAGCTAGAGGGAGGAAGGA3' R5'CCGGGTTTCTCTAACTCTGC3'
Ngn3	**F**5'GTCGGGAGAACTAGGATGGC 3' **R**5'GGAGCAGTCCCTAGGTATG 3'
Nkx6.1	**F**5'AGAGAGCACGCTTGGCCTATTC3' **R**5'GTCGTCAGAGTTCGGGTCCAG3'
Pax4	**F**5'GGACAAGGCTCCCAGTGTGT3' **R**5'GCAAGCTCTGGTCTTCCTTGAA3'
PC1/3	**F**5'TGGAGTTGCATATAATTCCAAAGTT3' **R**5'CTAGCCTCAATGGCATCAGTT3'
Pdx1	**F**5'GCCCGGGTGTAGGCAGTAC3' **R**5'CAGTGGGCAGGAGGTGCTTA3'
PDK1	**F**5'TAAAAGTTCAGACCTTTGGGCC3' **R**5'TCCCGGCTCTGAATGGTG3'
SST	**F**5'CTCTCCCCCAAACCCCATAT3' **R**5'TTTCTAATGCAGGGTCAAGTTGAG3'
SKP2	**F**5'GAAAGCTTCAGCTCTTTCCG 3' **R**5'AGGCCTTCCAGGCTTAGATT 3'
Smad3	**F**5'GCACAGCCACCATGAATTAC3' **R**5'GGAGGTAGAACTGGCGTCTC3'

For the second method, briefly, the relative expression of 84 genes of either the Hedgehog or the BMP/TGFβ pathways was evaluated using the PAMM-078Z (for the Hedgehog signaling pathway) and PAMM-035Z (for BMP/TGFβ Pathway). Samples were aliquot in the discs using the CorbettRobotics4 robot and the PCR reaction was performed in the CorbettResearch6000 series cycler using the RT² SYBR Green ROX FAST Mastermix (QIAGEN). CT values were exported from the qPCR instrument and analyzed with the ΔΔCt method using the online software provided by the manufacturer (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php). Five control genes, B2M, Hsp90ab1, Gusb, GAPDH, and β-actin present on the PCR array were used for normalization. For gene expression comparison between different age groups, the expression levels were always normalized to the appropriate age-matched controls, the difference in the expression levels reflecting solely the DT-effect on each age group.

Histological and morphometric analyses.

Histological and morphometrical analyses were performed as previously described [15,16].

Statistical analyses.

All mice used in experiments were males of mixed genetic background. Whenever possible, littermates of appropriate genotype were used as age-matched controls. The number of mice per experiment was limited by the availability of the required genotype and age. Criteria of exclusion were: (1) inadequate transgene combination set, (2) gender, (3) evident signs of disease, including hyperglycemia before DT-administration and (4) spontaneous natural death during an experiment. Sample size (scored numbers of mice, islets and cells) is within the range of published literature in the field. Islets and cells were counted on multiple (at least 10) non-consecutive slides. All error bars represent s.d. (standard deviation) except for Extended Data Figures 2f,g & 10d,e, where they indicate the s.e.m. (standard error of mean). P-values are given within figures or figure legends; n values are indicated in supplemental tables or within figures. Each graph corresponds to a supplemental table, which contains the number of mice employed, scored islets/cells and numerical values (average + s.d. or s.e.m.). When indicated, we tested data for normality with the Shapiro-Wilk Normality Test; for large data sets the unpaired t-test with Welch's correction (Welch's test) was used. Statistical analyses were assessed using *Prism v6.0* software, and are summarized in the table below.

For Extended Data Figure 4 we used the χ-squared test with 1 degree of freedom to compare observed and estimated data. For qPCR studies, the statistic analyses were performed with either the in-build program mentioned above or with the *RT-PCR analysis macro* (provided by the Genomics Platform, University of Geneva).

Graph	Corresponding Table	Error bars	Statistical tests
Extended Data Fig1f	Supp. Table S2	s.d.	No comparison preformed
Extended Data Fig1g	Supp. Table S3	Individual points	One way Anova
Extended Data Fig1i	Supp. Table S4	s.d.	One way Anova
Extended Data Fig1j	Supp. Table S5	s.d.	One way Anova
Extended Data Fig2f	Supp. Table S8	s.e.m.	No comparison performed
Extended Data Fig2g	Supp. Table S9	s.e.m.	Welch's test
Extended Data Fig2h	Supp. Table S10	s.d.	Welch's test, one way Anova, Mann-Whitney
Extended Data Fig3a	Supp. Table S11	s.d.	Welch's test, one way Anova, Mann-Whitney
Extended Data Fig3d	Supp. Table S12	Individual points	No comparison performed
Figure 2b	Supp. Table S13	s.d.	Welch's test and Mann Whitney
Figure 2d	Supp. Table S15	s.d.	Welch's test and Mann Whitney
Figure 2e	Supp. Table S16	s.d.	Welch's test and Mann Whitney
Extended Data Fig5b	Supp. Table S18	s.d.	Welch's test and Mann Whitney
Extended Data Fig5c	Supp. Table S19	s.d.	Welch's test
Extended Data Fig5d	Supp. Table S20	s.d.	unpaired t-test, two-tailed
Extended Data Fig5g	Supp. Table S21	Individual points	unpaired t-test, two-tailed
Extended Data Fig5h	Supp. Table S22	s.d.	unpaired t-test, two-tailed
Extended Data Fig8b	Supp. Table S24	s.d.	Welch's test
Extended Data Fig8c	Supp. Table S25	s.d.	Welch's test and one way Anova
Extended Data Fig8e	Supp. Table S26	s.d.	No comparison performed

Extended Data Fig8f	Supp. Table S27	s.d.	No comparison performed
Extended Data Fig8i	Supp. Table S28	s.d.	Welch's test and Mann-Whitney
Extended Data Fig8j	Supp. Table S28	s.d.	Welch's test and one way Anova
Extended Data Fig10b	Supp. Table S30	s.d.	No comparison performed
Extended Data Fig10c	Supp. Table S31	s.d.	Welch's test
Extended Data Fig10d	Supp. Table S32	s.e.m.	No comparison performed
Figure 3f	Supp. Table S33	s.d.	Welch's test and Mann-Whitney
Figure 3g	Supp. Table S34	s.d.	Welch's test, Mann Whitney
Figure 3h	Supp. Table S35	s.d.	Welch's test
Extended Data Fig10e	Supp. Table S36	s.e.m.	No comparison performed
Extended Data Fig10f	Supp. Table S37	s.d.	No comparison performed
	Supp. Table S38		
	Supp. Table S39		

Supplementary references.

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Chera et al., Supplementary Tables:

Table S1. **Evolution of β-cell mass (µg) after β-cell ablation in 2-month-old adults as well as 1-year-old and 1.5 year-old aged animals:**

Cells [text]	Age at DT-administration	Time point	# of mice	Mean ± SD
β-cell mass (µg):	2-month-old	0.5 mpa	4	5.3±05 (0.30%)
		1 mpa	4	21.2±4.0 (1.21%)
		7 mpa	4	83.9±13.0 (4.7%)
	1-year-old	0.5 mpa	5	23±2.6 (0.34%)
		1 mpa	5	85.3±6.3 (1.26%)
		7 mpa	5	299±25.4 (4.40%)
		14 mpa	8	COMBINED: 748.39±203.80 (11.05%)
				RECOVERED: 855.10±54.12 (12.63%)
				DIABETIC: 428.25±52.99 (6.32%)
	1.5-year-old	0.5 mpa	3	28.8±0.3 (0.37%)
		1 mpa	3	94.1±12.4 (1.21%)
		7 mpa	3	338±25.6 (4.79%)
		14 mpa	8	COMBINED: 784.24±219.09 (10.04%)
				RECOVERED: 901.07±33.38 (11.54%)
				DIABETIC: 433.74±53.15 {5.55%)

Table S2. **Proliferating insulin+ cells in 1.5-year-old mice, before and after β-cell ablation:**

Cells [Figure S1f]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
Ki67+/Ins+ (%)	**1.5-year-old**	no DT	8	39,790	1.51 ± 0.6
		0.5 mpa	6	938	0.23 ± 0.2

Table S3. **Glucagon+/Insulin+ bihormonal cells in adult (2-month-old) and aged animals (1- and 1.5-year-old) before and after β-cell ablation:**

Cells [Figure S1g]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
Glu+/Ins+ (%)	**2-month-old**	no DT	3	3,021	0.13 ± 0.14
		0.5 mpa	5	70	20.36 ± 4.98
		1 mpa	4	136	42.65 ± 6.08
		7 mpa	5	622	22.1 ± 3.49
	1-year-old	no DT	3	3,994	0.14 ± 0.13
		0.5 mpa	5	93	15.07 ± 3.63
		1 mpa	6	197	40.84 ± 8.40
		7 mpa	5	450	20.8 ± 4.73
	1.5-year-old	no DT	3	5,791	0.21 ± 0.06
		0.5 mpa	6	151	15.43 ± 9.94
		1 mpa	4	224	39.97 ± 14.2
		7 mpa	6	733	17.93 ± 3.72

Table S4. **Conditional α-cell lineage tracing (*Glucagon-rtTA, TetO-Cre, R26-YFP, RIP-DTR* mice) in adult (2-month-old) and aged animals (1- and 1.5-year-old). DOX administered BEFORE DT-induced ablation:**

Cells [Figure S1i]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/Ins+ (%)	2-month-old	no DT	3	6,917	0.26 ± 0.07
		1 mpa	5	354	62.26 ± 16.56
		7 mpa	5	1,273	82.45 ± 12.90
	1-year-old	no DT	3	8,887	0.27 ± 0.08
		1 mpa	3	307	59.36 ± 13.04
		7 mpa	5	1,724	80.74 ± 11.74
	1.5-year-old	no DT	3	21,308	0.28 ± 0.06
		1 mpa	3	357	57.14 ± 12.53
		7 mpa	5	2,031	80.61 ± 11.42

Table S5. **Conditional α-cell lineage tracing (*Glucagon-rtTA, TetO-Cre, R26-YFP, RIP-DTR* mice) aged animals (1- and 1.5-year-old). DOX administered AFTER DT-induced ablation:**

Cells [Figure S1k]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/Ins+ (%)	1-year-old	no DT	5	5,117	0.26 ± 0.04
		7 mpa	5	1,970	16.15 ± 6.43
	1.5-year-old	no DT	5	42,500	0.27 ± 0.07
		7 mpa	5	2,179	15.62 ± 5.5

Table S6. **Evolution of β-cell mass (µg) after β-cell ablation in 2-week-old pups, and in unablated controls (no DT) of the corresponding age:**

Cells [Figure 1c]	Age at DT-administration	Time point	# of mice	Mean ± SD
β-cell mass (µg):	2-week-old	no DT (1mo)	5	948.13 ± 204.36
		0.5 mpa	5	13.3 ± 2.25
		no DT (4.5mo)	5	2,707.5 ± 587.01
		4 mpa	5	608.26 ± 61.4
		no DT (15.5mo)	4	6917.05 ± 669.6
		15 mpa	4	3232.79 ± 1119.34

Table S7. **Conditional α-cell lineage tracing (*Glucagon-rtTA, TetO-Cre, R26-YFP, RIP-DTR* mice) in pups (2-week-old) treated with DOX before DT-induced ablation:**

Cells [text, Fig1d]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/Ins+ (%)	2-week-old	no DT	4	7541	0.25 ± 0.1
		1.5 mpa	5	854	0.23 ± 0.3

Table S8. **Bihormonal glucagon+/insulin+ cells after 2 sequential episodes of massive β-cell ablation:**

Cells [Figure S2f]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SEM
Glu+/Ins+ (%)	2-week-old AND 5-month-old	1 mpa (2^{nd} DT)	2	40	30.6 ± 2.8

Table S9. **Proliferating insulin+ cells (insulin+ cells with Ki67+ nuclei) before and after β-cell ablation at 2 weeks of age:**

Cells [Figure S2g]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SEM
Ki67+/Ins+ (%)	2-week-old	no DT (1mo)	3	6,006	2.05 ± 0.67
		0.5 mpa	5	412	0.40 ± 0.24
		No DT (2mo)	3	6,358	1.80 ± 0.5
		1.5 mpa	3	675	0

Table S10. **Number of Ki67+ cells per islet section before and after β-cell ablation in pups (2-week-old), adults (2-month-old) and aged (1.5-year-old) mice:**

Cells [Figure S2h]	Age at DT-administration	Time point	# of mice	# of scored islets	Mean ± SD
Ki67+ (#)	2-week-old	no DT (1mo)	3	95	2.5 ± 0.91
		0.5 mpa	6	333	8.8 ± 0.97
		no DT (1.5mo)	3	94	2.1 ± 1.19
		1 mpa	3	91	4.0 ± 0.93
		no DT (2mo)	3	90	1.97 ± 1.23
		1.5 mpa	3	90	1.12 ± 0.25
	2-month-old	no DT (2.5mo)	3	89	1.90 ± 0.95
		0.5 mpa	3	76	1.53 ± 0.52
		no DT (3mo)	3	91	1.56 ± 0.98
		1 mpa	3	77	1.8 ± 0.71
		no DT (3.5mo)	3	93	2.11 ± 0.8
		1.5 mpa	3	81	1.23 ± 0.21
	1.5-year-old	no DT (18.5mo)	3	83	1.40 ± 0.52
		0.5 mpa	3	74	0.9 ± 0.29
		no DT (19mo)	3	83	1.16 ± 0.58
		1 mpa	3	81	0.35 ± 0.2
		no DT (19.5mo)	3	88	1.1 ± 0.21
		1.5 mpa	3	77	0.61 ± 0.31

Table S11. **Number of somatostatin positive cells per islet section during the first 1.5 months post-ablation in pups (2-week-old):**

Cells [Figure S3a]	Age at DT-administration	Time point	# of mice	Scored islets	Mean ± SD
Sst+ (#)	**2-week-old**	no DT	7	255	13.19 ± 4.76
		3 dpa	5	240	11.53 ± 4.63
		5 dpa	5	228	11.93 ± 4.19
		7 dpa	5	251	9.85 ± 4.53
		0.5 mpa	6	267	2.9 ± 1.83
		1 mpa	5	266	7.1 ± 3.07
		1.5 mpa	5	206	11.39 ± 4.0

Table S12. **Specificity of YFP labeling in the *Sst-Cre, R26-YFP, RIP-DTR* transgenic line:**

Cells [Figure S3d]	# of mice	# of scored YFP+ cells	Mean ± SD
Sst+/YFP+ (%)	4	1,263	80.63 ± 6.2
Ins+/YFP+ (%)		1,263	0.95 ± 0.91
Glu+/YFP+ (%)		1,263	0.2 ± 0.14
YFP only		1,263	17.94 ± 3.38

Table S13. **YFP-labeled cells with Ki67+ nuclei (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) before and after β-cell ablation:**

Cells [Figure 2b]	Age at DT-administration	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Ki67+/YFP+ (%)	**2-week-old**	no DT	6	2,754	2.83 ± 0.4
		0.5 mpa	6	3,146	80.25 ± 14.34

Table S14. **Ki67+ cells labeled with YFP (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) after β-cell ablation:**

Cells [text]	Age at DT-administration	Time point	# of mice	# of scored Ki67+ cells	Mean ± SD
YFP+/Ki67+ (%)	**2-week-old**	0.5 mpa	6	2,947	85.3 ± 7.65

Table S15. **Insulin+ cells labeled with YFP (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) before and after β-cell ablation:**

Cells [Figure 2d]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	2-week-old	no DT	3	6,480	0.03 ± 0.03
		1.5 mpa	7	1,592	89.4 ± 6.8

Table S16. **Cell fate of YFP-traced cells (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) before and after β-cell ablation:**

Cells [Figure 2e]	Age at DT-administration	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Sst+/YFP+ (%)	2-week-old	no DT	3	1,673	99.94 ± 0.1
		1.5 mpa	5	2,295	54.81 ± 3.4
Ins+/YFP+ (%)	2-week-old	no DT	3	1,673	0.06 ± 0.1
		1.5 mpa	5	2,295	44.23 ± 4.03

Table S17. **Somatostatin+/insulin+ bihormonal cells in pups (2-week-old) at 1.5 months post-ablation:**

Cells [text]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SEM
Sst+/Ins+ (%)	2-week-old	1.5 mpa	7	1,592	0.83 ± 0.33

Table S18. **Number of β-cells per islet section at 0.5 months after either streptozotocin (STZ) treatment (200µg/g) or diphtheria toxin (DT) treatment in pups:**

Cells [Figure S5b]	Treatment	Age at ablation	Time point	# of mice	Scored islets	Mean ± SD
β-cells (#)	STZ	2-week-old	0.5 mpa	3	87	14.08 ± 5
	DT			4	361	0.94 ± 0.84

Table S19. **Number of YFP+Ins+ cells per islet section at 0.5 months, following either streptozotocin (STZ) treatment (200µg/g) or DT treatment in pups:**

Cells [Figure S5c]	Treatment	Age at ablation	Time point	# of mice	Scored islets	Mean ± SD
YFP^+Ins^+ (#)	STZ	2-week-old	1.5 mpa	3	88	5.80 ± 2.87
	DT			7	193	7.41 ± 2.06

Table S20. **Number of somatostatin+ cells per islet section during the first month post-ablation in adults (2-month-old):**

Cells [Figure S5d]	Age at DT-administration	Time point	# of mice	Scored islets	Mean ± SD
Sst+ (#)	2-month-old	no DT	3	174	8.87 ± 2.01
		0.5 mpa	4	140	10.19 ± 4.16
		1 mpa	3	86	8.38 ± 2.62

Table S21. **Insulin+ cells labeled with YFP (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) before and after β-cell ablation:**

Cells [Figure S5g]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	2-month-old	no DT	4	9,562	0.14 ± 0.15
		1.5 mpa	8	149	17.08 ± 14.54

Table S22. **Cell fate of YFP-traced cells (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) before and after β-cell ablation:**

Cells [Figure S5h]	Age at DT-administration	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Sst+/YFP+ (%)	2-month-old	no DT	4	1,263	80.63 ± 6.2
		1.5 mpa	8	1,342	97.53 ± 1.95
Ins+/YFP+ (%)	2-month-old	no DT	4	1,263	0.95 ± 0.91
		1.5 mpa	8	1,342	17.08 ± 14.54

Table S23. Insulin+ cells with Ki67+ nuclei after β-cell ablation:

Cells [Figure S7]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SEM
Ki67+/Ins+ (%)	2-week-old	3 mpa	1	162	53.7%
		3.3 mpa	1	89	4.5%
		4 mpa	1	175	73.7%

Table S24. Insulin+ cells labeled with YFP (*Ngn3-YFP, RIP-DTR* mice) before and after β-cell ablation:

Cells [Figure S8b]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	2-week-old	no DT	3	6,358	0
		1.5 mpa	3	675	86.4 ± 4.8

Table S25. YFP-labeled cells positive for insulin (*Ngn3-YFP, RIP-DTR* mice) after β-cell ablation:

Cells [Figure S8c]	Age of DT-administration	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
ins+/YFP+ (%)	2-week-old	0.5 mpa	3	31	9.97 ± 0.31
		1 mpa	3	123	33.3 ± 9.7
		1.5 mpa	3	729	80.9 ± 15
		2 mpa	3	47	8.17 ± 5.35

Table S26. Insulin+ cells labeled with YFP (*Ngn3-CreERT, R26-YFP, RIP-DTR* mice) before and after β-cell ablation:

Cells [Figure S8e]	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	2-week-old	no DT	3	3,472	0
		1.5 mpa	3	489	90.76 ± 1.8

Table S27. **YFP-labeled cells positive for insulin (*Ngn3-CreERT, R26-YFP, RIP-DTR* mice) after β-cell ablation:**

Cells [Figure S8f]	Age at DT-administration	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
ins+/YFP+ (%)	2-week-old	1.5 mpa	3	478	92.9 ± 5.74

Table S28. **Number of insulin+ cells per islet section, with or without DOX treatment, in *Ngn3-tTA, Tre-Ngn3, RIP-DTR* pups at 1.5 months post DT-ablation:**

Cells [Figure S8i]	Treatment	Age at DT-administration	Time point	# of mice	Scored islets	Mean ± SD
Ins^+ (#)	+ DOX	2-week-old	1.5 mpa	4	167	0.71 ± 0.85
	no DOX			3	266	8.29 ± 2.8

Table S29. **Glucagon+/ Insulin+ bihormonal cells in *Ngn3-tTA, Tre-Ngn3, RIP-DTR* pups (2-week-old), with or without DOX treatment, at 1.5 months post DT-ablation:**

Cells [Figure S8j]	Treatment	Age at DT-administration	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
Glu+/Ins+ (%)	+ DOX	2-week-old	No DT	3	9,233	0.16 ± 0.05
	no DOX		1.5 mpa	3	1,385	0
	+ DOX		1.5 mpa	4	141	40.71 ± 12.88

Table S30. Cell fate of YFP-traced cells (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) in adults with or without FoxO1 inhibitor treatment:

Cells [Figure S10b]	Age	Treatment	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Sst+/YFP+ (%)	2-month-old	-	No DT	4	1,224	92.84 ± 3.86
		AS1842856	No DT	3	1,347	65.92 ± 3.45
Ins+/YFP+ (%)		-	No DT	4	1,224	0.91 ± 0.85
		AS1842856	No DT	3	1,347	5.23 ± 3.45
YFP+ only (%)		-	No DT	4	1,224	6.25 ± 3.24
		AS1842856	No DT	3	1,347	28.85 ± 6.17

Table S31. Insulin+ cells labeled with YFP (*Somatostatin-CRE, R26-YFP, RIP-DTR* mice) in adults with or without transient FoxO1 inhibitor (AS1842856) treatment:

Cells [Figure S10c]	Age	Treatment	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	2-month-old	-	No DT	4	9,562	0.14 ± 0.15
		AS1842856		3	3,249	2.45 ± 1.82

Table S32. YFP-labeled cells positive for glucagon (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) in adults transiently treated with FoxO1 inhibitor (AS1842856):

Cells [Figure S10d]	Age at DT-administration	Treatment	Time point	# of mice	# of scored YFP+ cells	Mean ± SEM
Glu+/YFP (%)	2-month-old	AS1842856	No DT	2	728	0.69±0.21

Table S33. Number of insulin+ cells per islet section at in adults at 1 month after β-cell ablation and transient FoxO1 inhibitor (AS1842856) treatment:

Cells [Figure 3f]	Age at DT-administration	Treatment	Time point	# of mice	Scored islets	Mean ± SD
Ins+ (#)	2-month-old	-	1 mpa	3	95	0.41 ± 0.49
		AS1842856		4	190	4.71 ± 2.9

Table S34. **Insulin+ cells labeled with YFP (*Somatostatin-CRE, R26-YFP, RIP-DTR* mice) in adults at 1 month after β-cell ablation with or without transient FoxO1 inhibitor (AS1842856) treatment:**

Cells [Figure 3g]	Age at DT-administration	Treatment	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	2-month-old	-	1 mpa	6	370	4.95 ± 3.31
		AS1842856		4	894	93.49 ± 2.73

Table S35. **Cell fate of YFP-traced cells (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) in adults at 1 month after β-cell ablation with or without FoxO1 inhibitor treatment:**

Cells [Figure 3h]	Age at DT-administration	Treatment	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Sst+/YFP+ (%)	2-month-old	-	1 mpa	6	2,559	94.30±4.31
		AS1842856	1 mpa	4	3,538	64.22±1.49
Ins+/YFP+ (%)		-	1 mpa	6	2,559	0.39±0.24
		AS1842856	1 mpa	4	3,538	23.57±1.25
YFP+ only (%)		-	1 mpa	6	2,559	5.31±4.53
		AS1842856	1 mpa	4	3,538	12.21±1.15

Table S36. **YFP-labeled cells positive for glucagon (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) in adults at 1 month after β-cell ablation and transient FoxO1 inhibitor (AS1842856) treatment:**

Cells [Figure S10e]	Age at DT-administration	Treatment	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Glu+/YFP+ (%)	2-month-old	AS1842856	1 mpa	2	986	0.71±0.06

Table S37. **Number of insulin+ cells per islet section at in adults at 2 month after β-cell ablation with transient FoxO1 inhibitor (AS1842856) treatment during the 5th week of regeneration:**

Cells [Figure S10f]	Age at DT-administration	Treatment	Time point	# of mice	Scored islets	Mean ± SD
Ins+ (#)/ mouse	**2-month-old**	**AS1842856**	2 mpa	3	71	3.75±1.57

Table S38. **Insulin+ cells labeled with YFP (*Somatostatin-CRE, R26-YFP, RIP-DTR* mice) in adults at 2 month after β-cell ablation with transient FoxO1 inhibitor (AS1842856) treatment during the 5th week of regeneration:**

Cells [Figure S10f]	Age at DT-administration	Treatment	Time point	# of mice	# of scored insulin+ cells	Mean ± SD
YFP+/ins+ (%)	**2-month-old**	**AS1842856**	2 mpa	3	300	94.32 ± 2.82

Table S39. **Cell fate of YFP-traced cells (*Somatostatin-Cre, R26-YFP, RIP-DTR* mice) in adults at 1 month after β-cell ablation with transient FoxO1 inhibitor (AS1842856) treatment during the 5th week of regeneration:**

Cells [Figure S10f]	Age at DT-administration	Treatment	Time point	# of mice	# of scored YFP+ cells	Mean ± SD
Sst+/YFP+ (%)	**2-month-old**	**AS1842856**	2 mpa	3	1,216	66.37±3.67
Ins+/YFP+ (%)		**AS1842856**	2 mpa	3	1,216	20.89±7.36
YFP+ only (%)		**AS1842856**	2 mpa	3	1,216	12.74±3.91