SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTARY FIGURE 1: CHRONIC DIABETES IN MICE CAN BE STABLY REVERTED BY TRANSIENT RELEASE OF EGF AND CNTF BY BETA CELL NEOGENESIS. (A) $ALX_{35d/CK}$ mice treated as indicated in Figure 2 can survive for up to 287 days (average blood glucose level of 8.2±0.4 mmol/L at day 287; n=4). **(B)** Long term follow-up of body weight of $ALX_{35d/CTR}$ and $ALX_{35d/CK}$ mice. **(C)** Fat (FM) and lean mass (LM) were analyzed at day 42 by EchoMRI in $ALX_{35d/CTR}$, $ALX_{35d/CK}$ and $NG_{35d/CTR}$ mice) **: $p<0.01$; n=8 for each condition). **(D)** Serum glucagon levels were measured by radio-immuno assay ($p>0.05$; n=5 for each condition). **(E,F)** Treatment of ALX_{35d} mice with single cytokines EGF (**E**) or CNTF (**F**) did not ameliorate hyperglycemia. **(G)** Hyperglycemia was induced in 30 mice by a single dose of the alternative beta cell toxin streptozotocin (STZ). Glycemia was measured as in **Figure** 1A. 35 days after STZ injection, osmotic mini-pumps loaded with EGF and CNTF ($STZ_{35d/CK}$, 15 mice) or vehicle ($STZ_{35d/CTR}$, 15 mice) were i.p.-implanted. **(H)** Follow-up of body weight of 15 $STZ_{35d/CTR}$ and 15 $STZ_{35d/CK}$ mice.. **(I)** Analysis of the $insulin^+$ cell area by conventional morphometry at the indicated time points after ALX injection (*: $p<0.05$, **: $p<0.01$; n=6 for each data bar). No $ALX_{35d/CTR}$ or $ALX_{35d/CK}^{unresp}$ mice survived until day 98 (N.A.). **(J)** Cycling beta cells during the hyperglycemic and treatment period were identified 7 days after pump implantation by $insulin^+Ki67^+$ antibody staining. Scale bar=10μm. **(K)** Quantification of labeled cells based on data shown in **(J).** (*: $p<0.05$, **: $p<0.01$; n=6 for each data bar)**(L)** The cumulative fraction of actively cycling beta cells was analyzed by IdU incorporation during the 7 days after pump implantation and staining of pancreas tissue sections with antibodies specific for IdU and insulin. Scale bar=20μm. **(M)** Quantification of labeled cells based on data shown in **(L).** (*: $p<0.05$; n=4 for each data bar)

SUPPLEMENTARY FIGURE 2: PRE-EXISTING DUCT CELLS CONTRIBUTE MARGINALLY TO NEW BETA-LIKE CELLS IN DIABETIC MICE TREATED WITH EGF AND CNTF. (A) $Hnf1b^{CreERT}R26^{LacZ}$ mice were treated or not with ALX and implanted with pumps as indicated (see Figure 1 for details). At 7 days after pump implantation, the pancreas of each mouse was stained with XGal, with anti-insulin, anti-amylase, anti-keratin 19 and Hoechst 33342. Scale bar=15μm. **(B)** Quantification of labeled cells based on

data shown in **(A)** (*: p>0.05; n=12)**.** Overall Xgal labeling, representing the recombination efficiency, was around 20%. in $Rip^{CreERT}R26^{LacZ}$ mice and 45% in $Ela^{CreERT}R26^{LacZ}$ mice.

SUPPLEMENTARY FIGURE 3: $PTF1A^{+}$ ACINAR CELLS GIVE RISE TO NEW BETA-LIKE CELLS IN DIABETIC MICE TREATED WITH EGF AND CNTF. (A) $Ptf1a^{CreERT}R26^{YFP}$ mice were treated or not with ALX and implanted with pumps as indicated. At 7 days after pump implantation, the pancreas of each mouse was stained with antibodies specific for YFP, insulin, and keratin 19, and DNA was stained by Hoechst 33342. Scale bar=10μm ($ALX_{35d/CTR}$), 70μm ($ALX_{35d/CK}$), 10μm ($ALX_{35d/CK}$ detail). **(B)** Quantification of labeled cells based on data shown in **(A)** (*: p<0.01; $ALX_{35d/CK}$ vs $ALX_{35d/CTR}$ and $NG_{35d/CTR}$ mice; n=12). **(C)** At day 4 (3 days prior to the end of the pump release period) pancreases of $ALX_{35d/CK}$ $Ptf1a^{CreERT}R26^{YFP}$ mice were stained with anti-insulin, anti-keratin 19 and anti-YFP and DNA was stained by Hoechst 33342. Scale bar=70μm ($ALX_{35d/CK}$), 5μm ($ALX_{35d/CK}$ detail). **(D)** From pancreases of $Ptf1a^{CreERT}R26^{YFP}$ $NG_{35d/CTR}$ (left panel) and $ALX_{35d/CK}$ (right panel) mice, $YFP^{-}TSQ^{+}$ beta cells (populations 1 and 1'), acinar cell-derived $YFP^{+}TSQ^{+}$ beta-like cells (populations 2 and 2') and $YFP^{+}TSQ^{-}$ acinar cells (populations 3 and 3') were isolated by flow cytometry:. **(E)** DNA was extracted from the cell populations sorted in **(D).** Methylation of promoter DNA that controls the expression of insulin, GLUT2 and Amylase2b was determined by bisulphite sequencing, as described in Supplemental Experimental Procedures. Circles represent know CpG methylation sites in the corresponding promoter region, white circles mark unmethylated sites, black circles marking methylated sites. Each row represents a single molecule analyzed. Data are from 5 independent mice per condition.

SUPPLEMENTARY FIGURE 4: EMBRYONIC FEATURES OF YFP^{+} CELLS FROM DIABETIC $NGN3^{YFP}$ MICE TREATED WITH EGF AND CNTF.(A) Ngn3 transcript was specifically amplified from pancreas RNA of $NG_{35d/CTR}$, $ALX_{35d/CTR}$ and $ALX_{35d/CK}$ mice (n=10 for each condition) In order to correlate the expression to an adequate reference, all tested conditions were normalized to the Ngn3 mRNA level in the duodenum (set at value 1). **(B)** YFP^{+} SSC^{low} TSQ^{-} cells, YFP^{+} SSC^{high} TSQ^{-} cells and $YFP^{-}SSC^{high}TSQ^{+}$ cells were isolated from $Ngn3^{YFP}$ $ALX_{35d/CK}$ mice, as in **Figure 3F**.

SUPPLEMENTARY FIGURE 5: NGN3+ CELLS DO NOT DIFFERENTIATE TO ALPHA CELLS IN MICE TREATED WITH EGF AND CNTF. (A) Pancreas of $Ngn3^{CreERT}R26^{YFP}$ tracer mice that allow continuous tracing of the fate of cells that expressed Ngn3 during TAM treatment (as described in **Figure 4**) was stained with anti-insulin, anti-glucagon and anti-YFP (DNA was stained by Hoechst 33342). Scale bar=10μm. **(B)** Quantification of labeled cells based on data shown in **(A)** (*: $p<0.05$; n=7).

SUPPLEMENTARY FIGURE 6: NGN3 IS REQUIRED FOR PROPER ACINAR-TO-BETA-LIKE CELL DIFFERENTIATION IN MICE TREATED WITH EGF AND CNT. The function of Ngn3 in pre-existing acinar cells was studied in mice that allow conditional Ngn3 knockout under control of the acinar-specific Ptf1a promoter ($Ptf1a^{CreERT}R26^{YFP}Ngn3^{lox/lox}$ mice). Mice lacking loxP sites flanking the endogenous Ngn3 gene were used as control ($Ptf1a^{CreERT}R26^{YFP}Ngn3^{+/+}$ mice). All mice were treated as shown in **Figure 2A**. **(A)** Overall YFP labeling, representing the recombination efficiency, was 50%. **(B)** The proportion of YFP-labeled beta-like cells represents the $insulin^+$ cells that underwent recombination and thus deleted Ngn3. (*: $p<0.01$, $ALX_{35d/CK}$ vs. $ALX_{35d/CTR}$; §: $p<0.01$, $ALX_{35d/CK\text{-}Ngn3}^{+/+}$ vs. $ALX_{35d/CK\text{-}Ngn3}^{lox/lox}$; n=4) **(C)** Effect on the total population of $insulin^+$ cells, including the ones that did not recombine and thus still express Ngn3. (*: $p<0.01$, $ALX_{35d/CK}$ vs. $ALX_{35d/CTR}$; §: $p<0.01$, $ALX_{35d/CK\text{-}Ngn3}^{+/+}$ vs. $ALX_{35d/CK\text{-}Ngn3}^{lox/lox}$; n=4) **(D)** Immune detection of YFP, insulin and Ptf1a-p48 cells on pancreas tissue sections of $ALX_{35d/CK}$ $Ptf1a^{CreERT}R26^{YFP}Ngn3^{+/+}$ and $Ptf1a^{CreERT}R26^{YFP}Ngn3^{lox/lox}$ mice. In mice with an acinar-specific deletion of Ngn3 only few $YFP^+insulin^+$ cells were present (small arrows). YFP^+ cells located in or near the islets are often $insulin^-$ (large arrows) (lower panels). Scale bar=10μm. **(E)** To selectively delete the Ngn3 gene in pre-existing acinar cells, $Ptf1a^{CreERT}R26^{YFP}Ngn3^{lox/lox}$ mice were treated with TAM before ALX injection. Where indicated, $Ptf1a^{CreERT}R26^{YFP}Ngn3^{+/+}$ were used as a control. Mice were then treated with pumps as in **Figure 2A**, and blood glucose was measured for the indicated time period. Following pump implantation, glycemia in $Ngn3^{lox/lox}$ $ALX_{35d/CK}$ (15.8±1.8 mmol/L) mice was significantly higher compared to $Ngn3^{+/+}$ $ALX_{35d/CK}$ mice (9.9±0.7 mmol/L) (*: $p<0.05$; n=4).

ONLINE METHODS

MICE

All mouse experiments were performed according to our institutional ‘‘Ethical Committee for Animal Experiments’’ following the national guidelines and regulations. All mice strains were described previously [5, 8, 19, 27, 42]: RipCre^{ERT}R26^{LacZ}, ElaCre^{ERT}R26^{LacZ}, Hnf1bCre^{ERT}R26^{LacZ}, Ngn3^{YFP}, Ptf1aCre^{ERT}R26^{YFP}, Ngn3Cre^{ERT}R26^{YFP}, Ngn3$^{lox/lox}$ and Stat3$^{lox/lox}$. The beta cell toxins alloxan (ALX) and streptozotocin (STZ) were injected in 8 and 13 week (in case of genetic lineage tracing) old male mice via the dorsal tail vein (at 70 mg ALX/kg body weight) and into the peritoneum (at 200 mg STZ/kg body weight). Tail vein blood glucose level (Glucocard X-meter, Arkray Inc.) and body weight were evaluated daily or weekly, following 2 hours of fasting. Tamoxifen (TAM, Sigma) was prepared at 10mg/ml in corn oil (Sigma). For tracing studies in adult mice, a total of 20 mg of TAM was given s.c. in 5 doses (each 4 mg) over a 2 week period, unless stated otherwise. A washout period of 24 days was maintained (unless stated otherwise) to assure TAM clearance. Of all mice rendered diabetic, 44.3±6.7% survived the diabetic period of 35 days and were included in subsequent experiments.

CYTOKINES

Human recombinant Epidermal Growth Factor (Sigma) was diluted in 10 mmol/L acetic acid solution to a final concentration of 1 mg/ml. Human recombinant Ciliary Neurotrophic Factor (BioVision) was diluted to a final concentration of 2 mg/ml. The mixture was injected into mini-osmotic pumps (Alzet 1007D) (Charles River Laboratories) to obtain a flux rate of 10 mg/kg body weight/hour for EGF and 23.8 mg/kg body weight/hour for CNTF. Pumps with cytokines or vehicle (composed of 5mmol/L acetic acid solution) were implanted intraperitoneally at 35 days post ALX or STZ injection. The manufacturer determined the pump release time to be 7 days. The exact dose used for EGF was derived from previous publications[14], while the CNTF dose was derived from a dose-response experiment using 4 doses (5.95, 11.90, 23.8 and 35.70 mg/kg body weight).

CELL SORTING

For FACS analysis, the dissected pancreas was digested with collagenase (0.8 mg/ml, Sigma), dissociated to single cells by addition of trypsin (1 mg/ml, Sigma) and DNAse (0.4 mg/ml, Sigma), passed through a 66 µm filter and resuspended in isolation medium (Lonza). Propidium Iodide (Molecular Probes, Life Sciences) (0.5 mg/ml) and Hoechst 33342 (Molecular Probes, Life Sciences) (1 mg/ml) were added for live/dead analyses. To visualize granulated beta cells, a Zn^{2+} binding dye *N*-(6-Methoxy-8-Quinolyl)-*p*-Toluenesulfonamide (TSQ) was added (1 mg/ml).

RNA ANALYSIS

Total RNA was isolated from cells (RNeasy, Qiagen; Picopure, Arcturus) or tissues (TRIzol, Invitrogen). Only RNA with RNA Integrity Number (RIN) ≥7 (2100 BioAnalyzer, Agilent) was further analyzed. cDNA synthesis and RT-PCR were done as described[48] using gene-specific primers. Primer and probe sequences are present in **Table S1**. To avoid interference from contaminating genomic DNA, all primer sets were designed to span at least one intron. All targets were amplified using 30 cycles by conventional RT-PCR (except for CycloA, 22 cycles) and 40 cycles by RT-qPCR (Applied Biosystems). Data were analyzed using the Sequence Detection Systems Software, Version 1.9.1 (Applied Biosystems).

PROTEIN ANALYSIS

Samples for immunohistochemistry (IHC) were overnight fixed in 4% formaldehyde (FA), embedded in paraffin and cut to 4–5 µm tissue sections. Primary antibodies: guinea pig polyclonal anti-insulin and rabbit polyclonal anti-glucagon (kind gift from C Van Schravendijk, Vrije Universiteit Brussel, Brussels) ; rabbit polyclonal antibodies against Ngn3[49], rabbit polyclonal antibodies against Pdx1[50]; rabbit polyclonal antibodies against Ki67 (ACK02, Novocastra)[17] and amylase (A8273, Sigma)[17], rat polyclonal antibodies against keratin-19 (TromaIII, Hybridoma Bank, Iowa)[17] and goat polyclonal anti-GFP (ab6658, Abcam)[22]. IdU incorporation was visualized using mouse anti-BrdU antibody (347580, Becton Dickinson)[7]. Antigen retrieval in paraffin sections for detection of GFP, K19, Pdx1 and Ngn3 was heat- or enzyme-mediated. Secondary antibodies for indirect fluorescent staining were Cy3-or FITC-labeled anti-

rabbit, anti-rat, anti-mouse, anti-goat and anti-guinea pig (all from Jackson ImmunoResearch Labs). Nuclei were labeled by Hoechst 33342 (4 mg/ml, Sigma). A minimum of 2000 cells was analyzed per condition.

METABOLIC STUDIES

Mice were fasted during 4 hours and injected intraperitoneally (i.p.) with glucose (2 g per kg body weight) or insulin (0.75 U per kg body weight) for glucose and insulin tolerance tests, respectively, and blood glucose concentration was measured from tail vein blood with a portable glucometer. For insulin secretion tests, mice were fasted for 6 hours, i.p. injected with glucose (2 g per kg body weight) and at 0 and 30 minutes blood was sampled from retroorbital plexus with a heparinized microvette (Sarstedt AG & Co.). Plasma insulin and glucagon concentration were determined with the Mouse Linco Insulin ELISA kit (Millipore) and the chemiluminescent Glucagon ELISA kit (Millipore), respectively. Total pancreas insulin content was determined using a mouse insulin radioimmunoassay kit (Linco Research Inc). Lean and fat mass were determined using EchoMRI. For GSIS analysis, pancreatic islets were isolated by collagenase digestion, handpicked and pooled.

BISULFITE METHYLATION ANALYSIS.

DNA was isolated by digestion buffer supplemented with proteinase K overnight, followed by phenol-chloroform extraction and ethanol precipitation. DNA subjected to bisulfite treatment performed with the EZ DNA Methylation-Direct kit (ZYMO RESEARCH) according to the manufacturer's instructions. Following treatment DNA was amplified using primer sets that encompass the promoter region of Amylase2b insulin and Glut2 genes The primers used were the following for Amy2b (-155+151), 5′ ATGGTTTTAGAGGGTTTTTAG 3′ (forward), 5' CCTCCAAATCCCTTAAAAA 3' (reverse), for Insulin (-411-168) 5′ TTTAAGTGGGATATGGAAAGAGAGATA 3′ (forward), 5′ ACTACAATTTCCAAACACTTCCCTAATA 3'(reverse), for Glut2 (-518 -290) 5' GAAAGTATATTGTTATTATTTATTGAG 3′ (forward) 5' ACTAAAAAAAACAACCTATAAAATC 3'(reverse). Reaction conditions of PCR were 5 cycles of 95 °C 1 min, 52 °C 3 min, 72 °C 3 min followed by 35 cycles of 95 °C 45 s, 52 °C 1 min, 72 °C 1 min followed by 7 min at 72 °C. Purified PCR products (Roche high pure PCR kit) were cloned into pGEM vectors (Promega) before

sequencing.

BETA CELL NUMBER, PROLIFERATION AND MASS ANALYSIS

Morphometry of the insulin area and determination of the beta cell fraction were by sectioning and analyzing 2% of the total pancreas.. Counting of IdU^+ and $Ki67^+$ beta cells were performed by serially sectioning of the entire pancreas followed by staining and counting every 30th section. A minimum of 4,000 $insulin^+$ cells were counted per pancreas.

IMAGE ANALYSIS

Images were acquired with a normal (Zeiss Axioplan 2 with Hamamatsu C10600 ORKA-R^2 camera) or confocal multiphoton (Zeiss LSM710 NLO with TiSa laser) microscopy. Images were analyzed using Smartcapture 3 (version 3.0.8) and Photoshop/Illustrator CS5. Confocal images were processed using Improvision VolocityLE and Zeiss Zen software.

DATA ANALYSIS AND BIOSTATISTICS

All values are depicted as average ± standard error of the mean (s.e.m.) from ≥4 independent experiments. Statistical significance is defined as $p<0.05$. Sample sizes are provided in the figure legends.

Blood glucose and bodyweight measurements were taken during the hyperglycemic period before treatment and at the time of cytokine treatment (data in figures 1B, 1E, 5E, S1G, S1H, S6C). Individual animals in these experiments were measured 15 times each on days -1, 0, 1, 7, 14, 21, 28, 35, 36, 37, 38, 39, 40, 41, 42. Alloxan injection was at day 0, and cytokine treatment period at day 35. For longer-term post-treatment follow-up, blood glucose levels and body weight were measured once a week.

Dynamic measurement of blood glucose levels was performed during glucose or insulin tolerance tests (data in figures 1C and 1D). Following glucose or insulin challenge performed at the end of the 7 days treatment period (day 42), every mouse was measured 7 times (0, 10, 20, 30, 60, 90, 120 minutes after glucose or insulin

injection).

Static measurement of INS^+ cell fraction, area and proliferation was on 6 animals per experimental group assayed on days 7, 16, 37, 39, 42 and 98 (data in figures 1I, S1I, S1K).

No data points were missing due to human error and no outliers were excluded. However, animals were excluded from the study when they died during the hyperglycemic period prior to cytokine-treatment or when their fasting blood glucose measurement at day 35 was <25mmol/l. All diabetic animals were randomly assigned to either control or treatment groups (no blinding was performed).

Some attrition did occur following cytokine treatment among the non-responder mice that died from hyperglycemia. The data obtained from these animals were included up to the point at which they were lost. The Linear Mixed Effects Model is able to appropriately deliberate missing data, provided they can be considered as missing at random. Overall, less than 5% of all animals included in the study were lost to attrition during the period of cytokine treatment and attrition was equal amongst different hyperglycemic groups. When mice were followed-up for extended periods after pump implantation (vehicle or cytokine treated) attrition did become a major factor because the control animals that only received vehicle treatment finally all succumbed to hyperglycemia. We therefore did not perform formal statistical analyses of these extended period data.

All data were statistically analyzed by unpaired Student t-test (Figures 1H, 2C, 2E, S2B), 1-way ANOVA (Figures 1F, 3B, 4B, 4C, S1C, S1D, S3B, S4A, S5B), 2-way ANOVA with Bonferroni post hoc test (1D, 1G, 1I, 3G, 3H, 5D, S1I, S1K, S6A, S6B, S6C) or a Linear Mixed Effects Model (Figures 1B, 1C, 1E, 5C, S1A, S1B, S1G, S1H, S6E). The Linear Mixed Effects Model included time and group as categorical predictors as well as a time by group interaction. A random mouse effect was added to explicitly account for within and between mouse variance. Time was modeled as categorical predictor, akin to repeated measures ANOVA, in order to avoid any a priori assumptions of specific temporal patterns in the outcome. Primary statistical testing was determined via detection of a statistically significant time-by-group interaction (Chi-square likelihood ratio test comparing models with and without the interaction term – both fitted by maximum likelihood). A statistically significant time-by-group interaction would indicate a difference in the temporal evolution of the

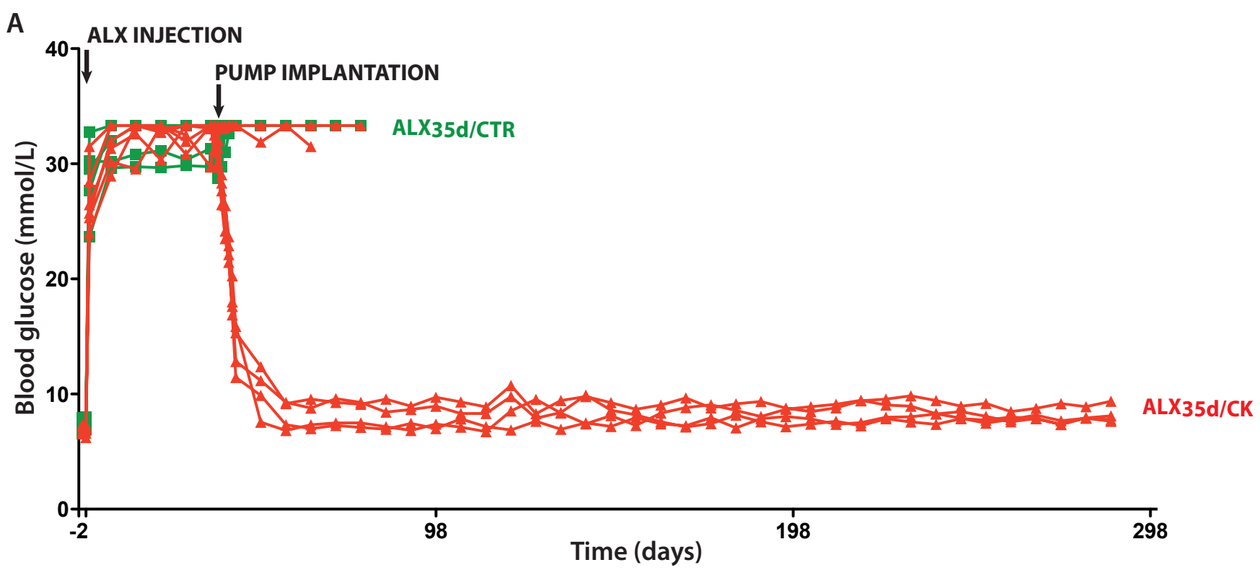
outcome between the two groups.

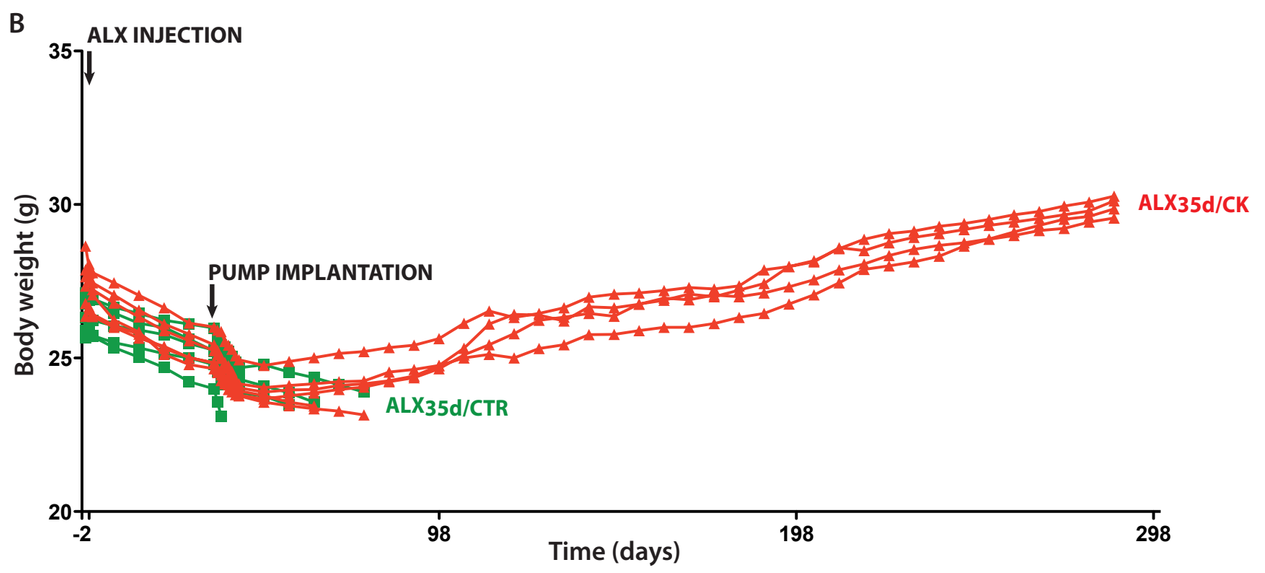
The residuals of all fitted models were assessed for approximate normality using QQ-plots. The majority of QQ-plots indicated that the residuals were approximately normal.

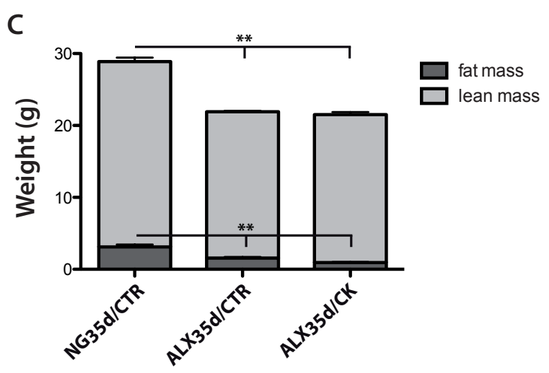
A priori power calculations: Samples size was a priori determined by power analysis to discriminate hypothetical differences of 50% or greater (type I error: 0.05; type II error: 0.01).

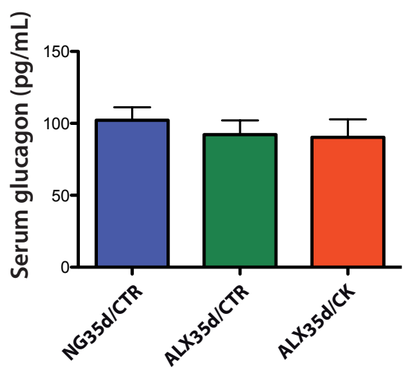
Graphpad Prism 5 for Mac OSX version 5.0f was used for ANOVA and Student t-test; R 3.0.1 (http://www.r-project.org/) and the nlme library was used for Linear Mixed Effects model

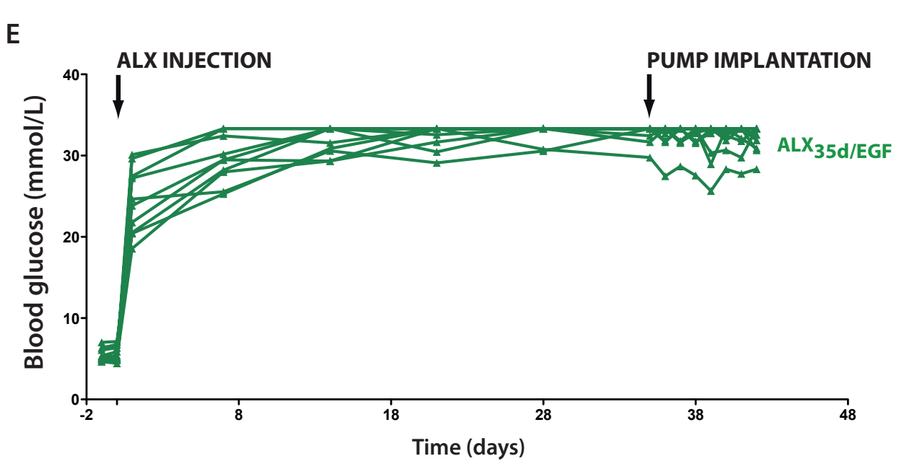
Figure S1

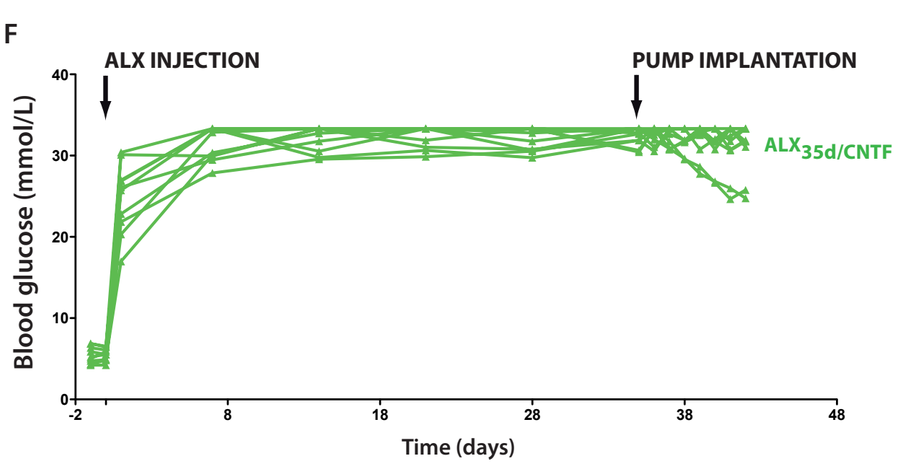

A
ALX INJECTION
PUMP IMPLANTATION
ALX35d/CTR
ALX35d/CK
Blood glucose (mmol/L)
Time (days)

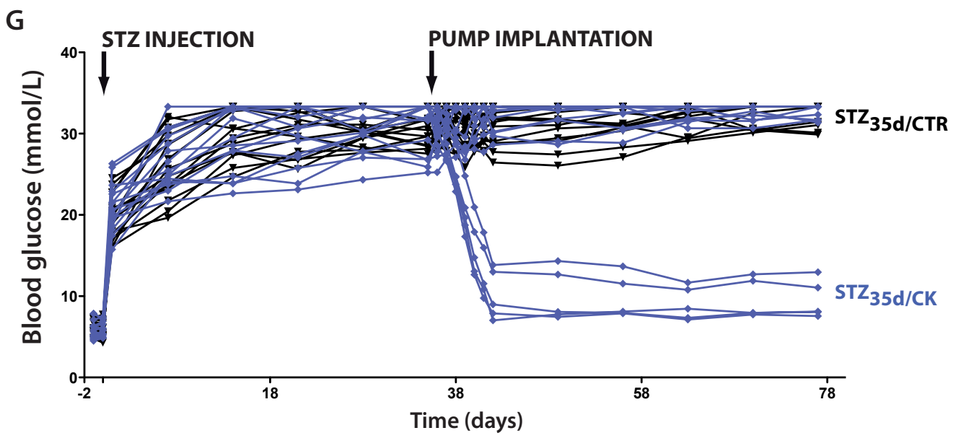

B
ALX INJECTION
PUMP IMPLANTATION
ALX35d/CK
ALX35d/CTR
Body weight (g)
Time (days)

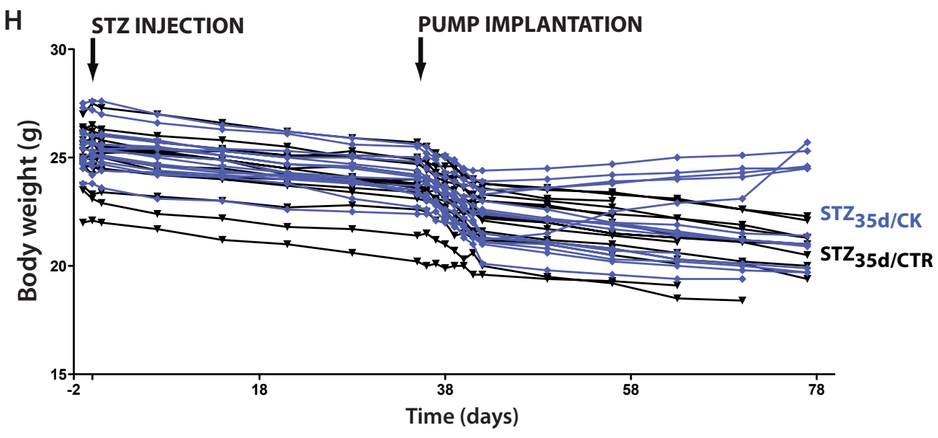

C
fat mass
lean mass
Weight (g)
NG35d/CTR
ALX35d/CTR
ALX35d/CK

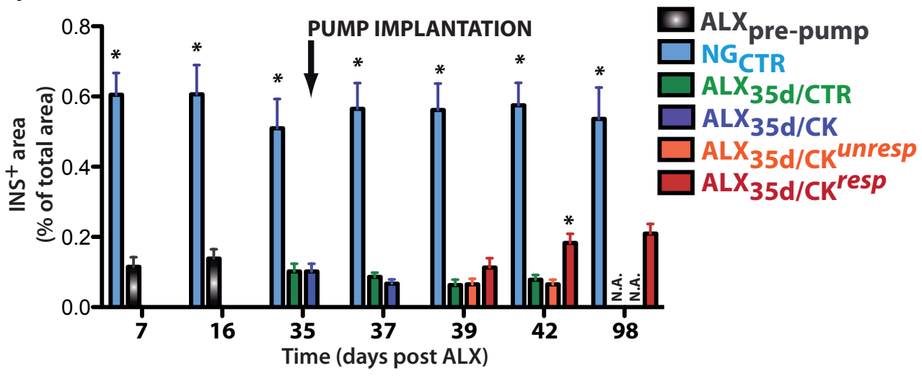

D
Serum glucagon (pg/mL)
NG35d/CTR
ALX35d/CTR
ALX35d/CK

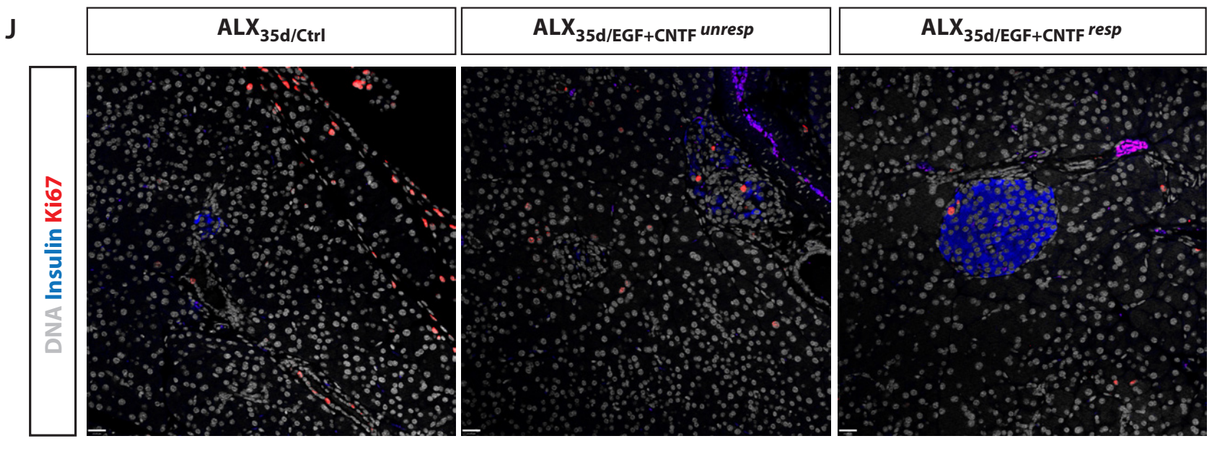

E
ALX INJECTION
PUMP IMPLANTATION
ALX35d/EGF
Blood glucose (mmol/L)
Time (days)

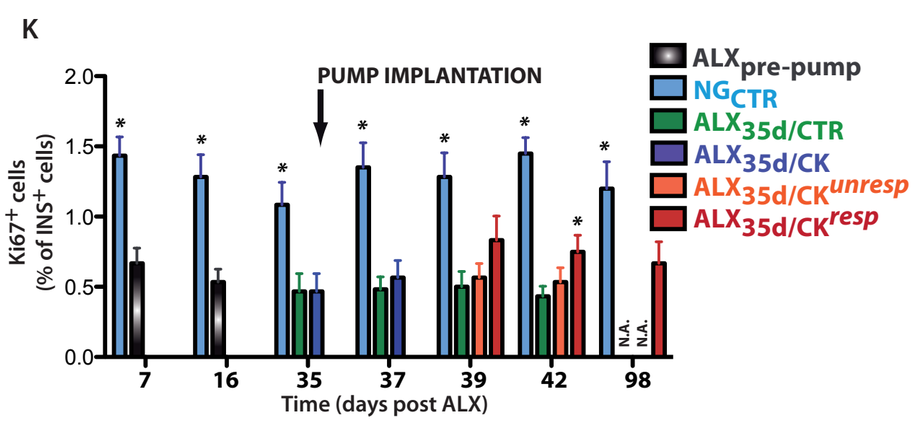

F
ALX INJECTION
PUMP IMPLANTATION
ALX35d/CNTF
Blood glucose (mmol/L)
Time (days)

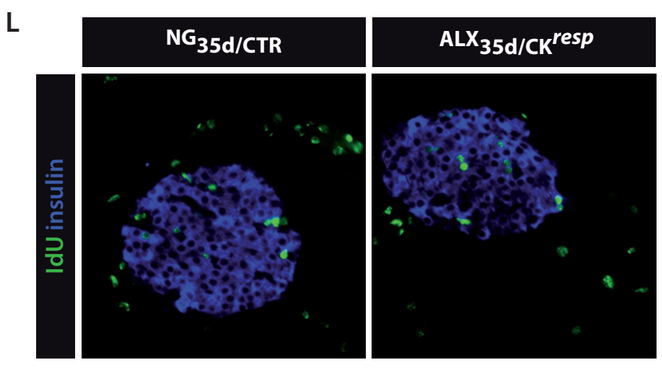

G
STZ INJECTION
PUMP IMPLANTATION
STZ35d/CTR
STZ35d/CK
Blood glucose (mmol/L)
Time (days)


H
STZ INJECTION
PUMP IMPLANTATION
STZ35d/CK
STZ35d/CTR
Body weight (g)
Time (days)


I
PUMP IMPLANTATION
ALXpre-pump
NGCTR
ALX35d/CTR
ALX35d/CK
ALX35d/CKunresp
ALX35d/CKresp
INS+ area (% of total area)
Time (days post ALX)


J
ALX35d/Ctrl
ALX35d/EGF+CNTF unresp
ALX35d/EGF+CNTF resp
DNA Insulin Ki67


K
PUMP IMPLANTATION
ALXpre-pump
NGCTR
ALX35d/CTR
ALX35d/CK
ALX35d/CKunresp
ALX35d/CKresp
Ki67+ cells (% of INS+ cells)
Time (days post ALX)


L
NG35d/CTR
ALX35d/CKresp
IdU insulin

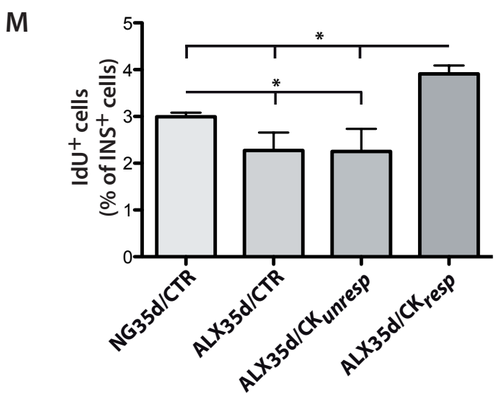
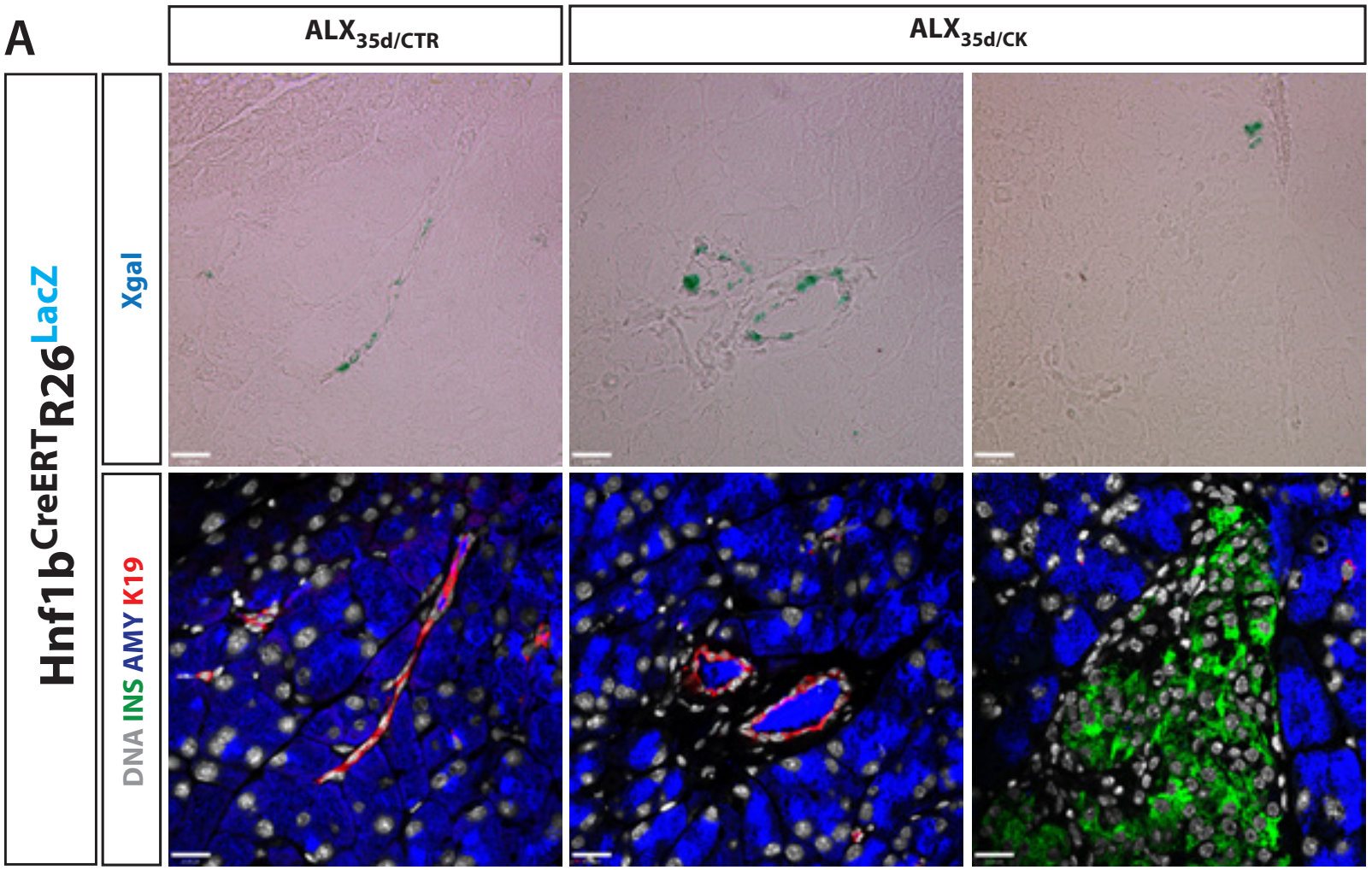

M
IdU+ cells (% of INS+ cells)
NG35d/CTR
ALX35d/CTR
ALX35d/CKunresp
ALX35d/CKresp

Figure S2


A
ALX35d/CTR
ALX35d/CK
Hnf1bCreERTR26LacZ
Xgal
DNA INS AMY K19

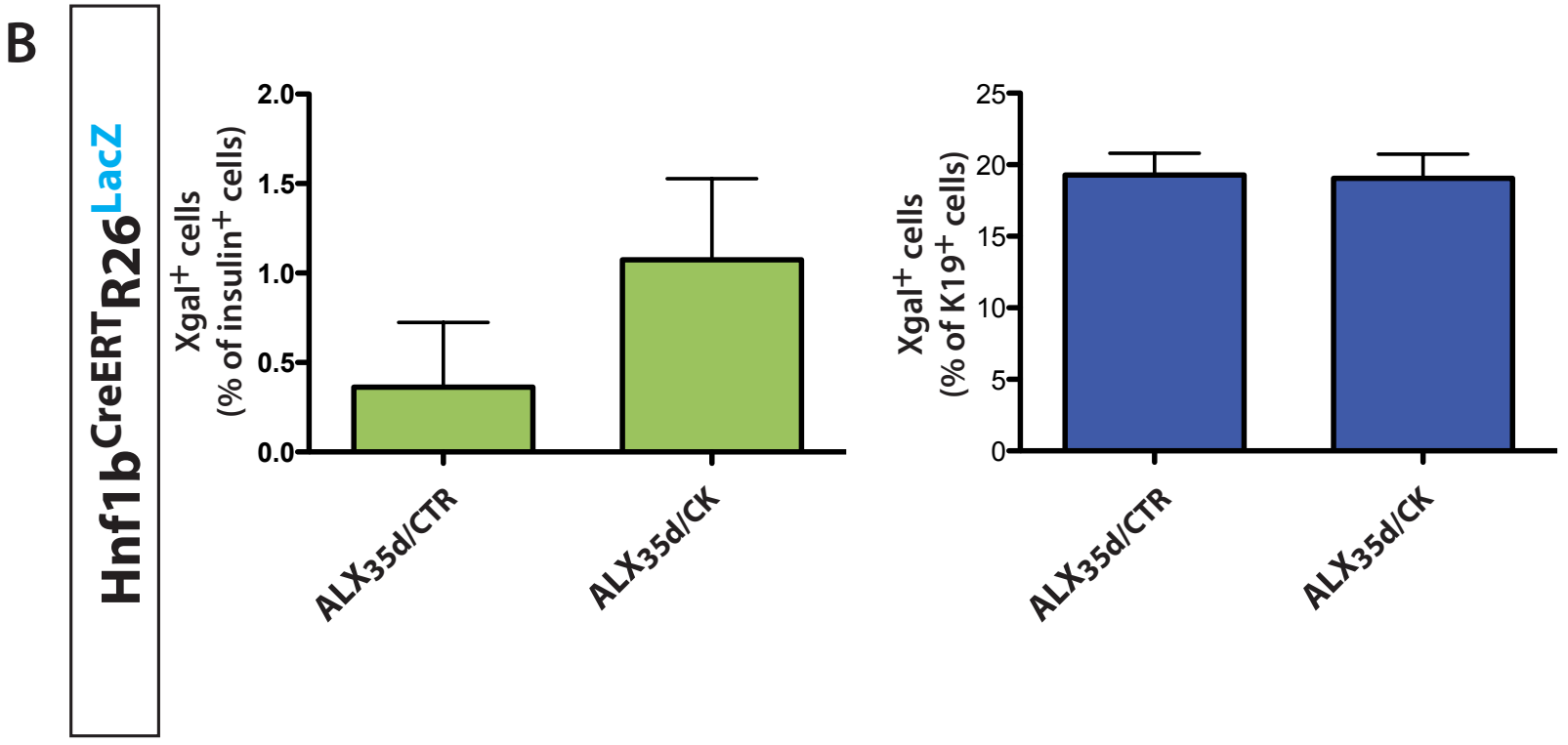
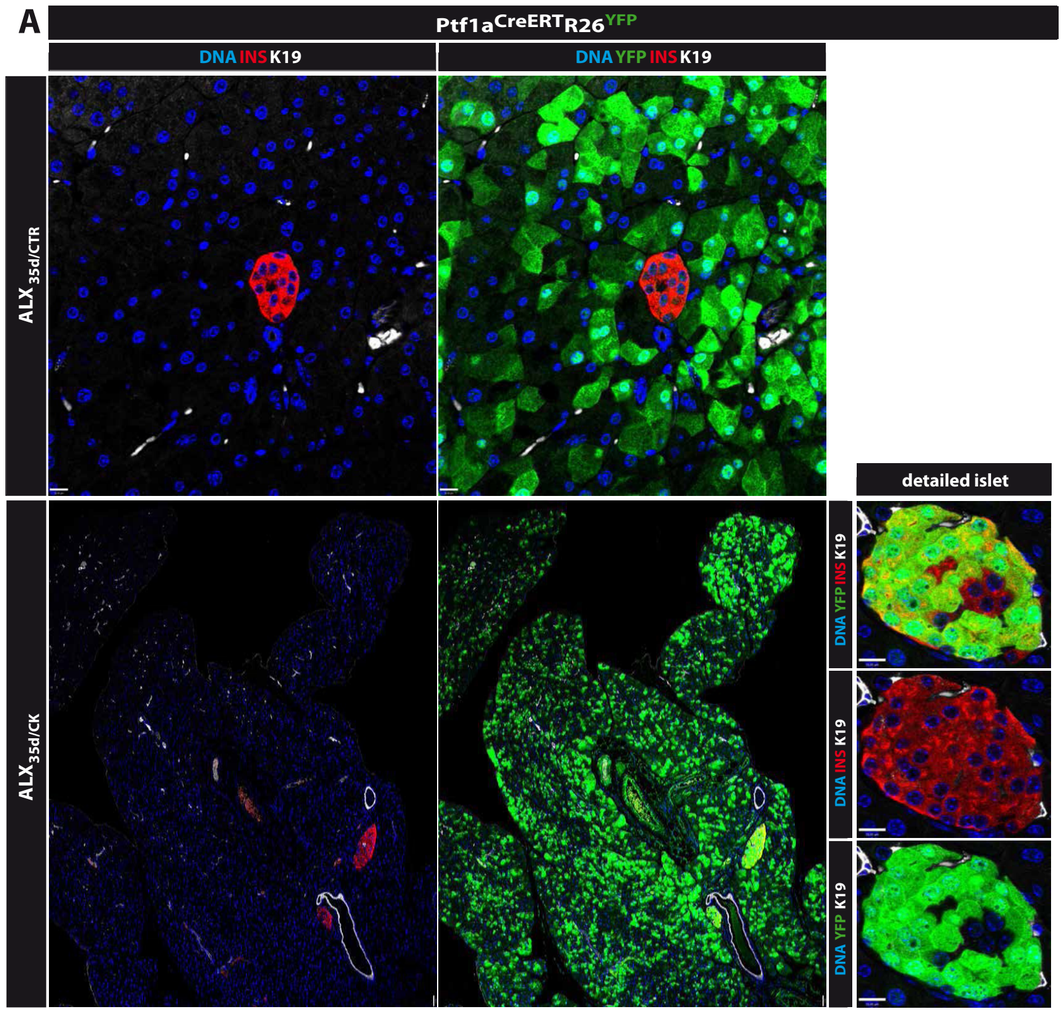
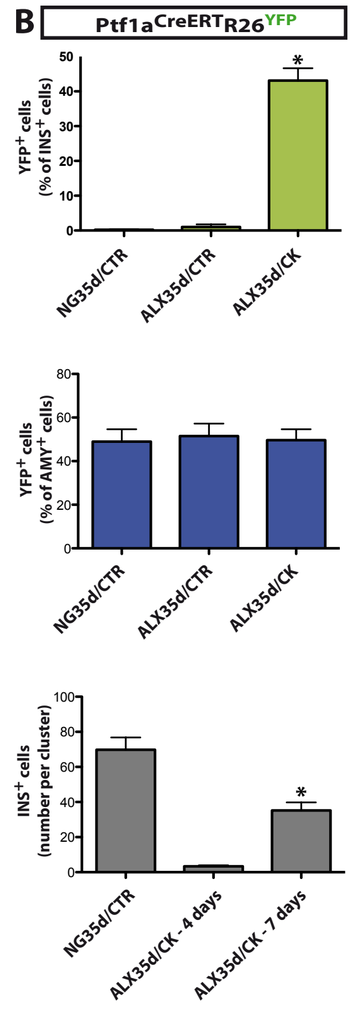
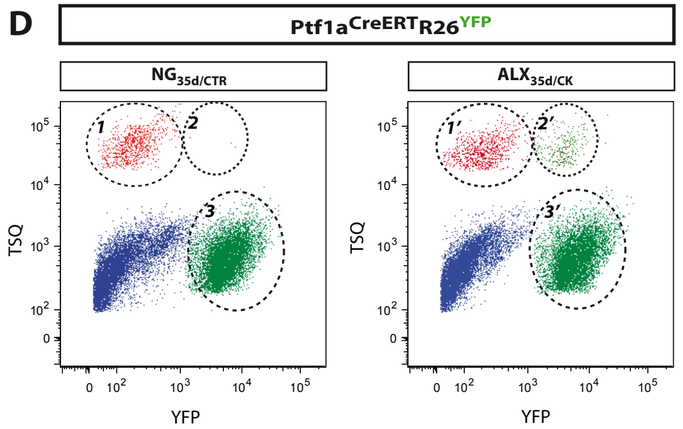
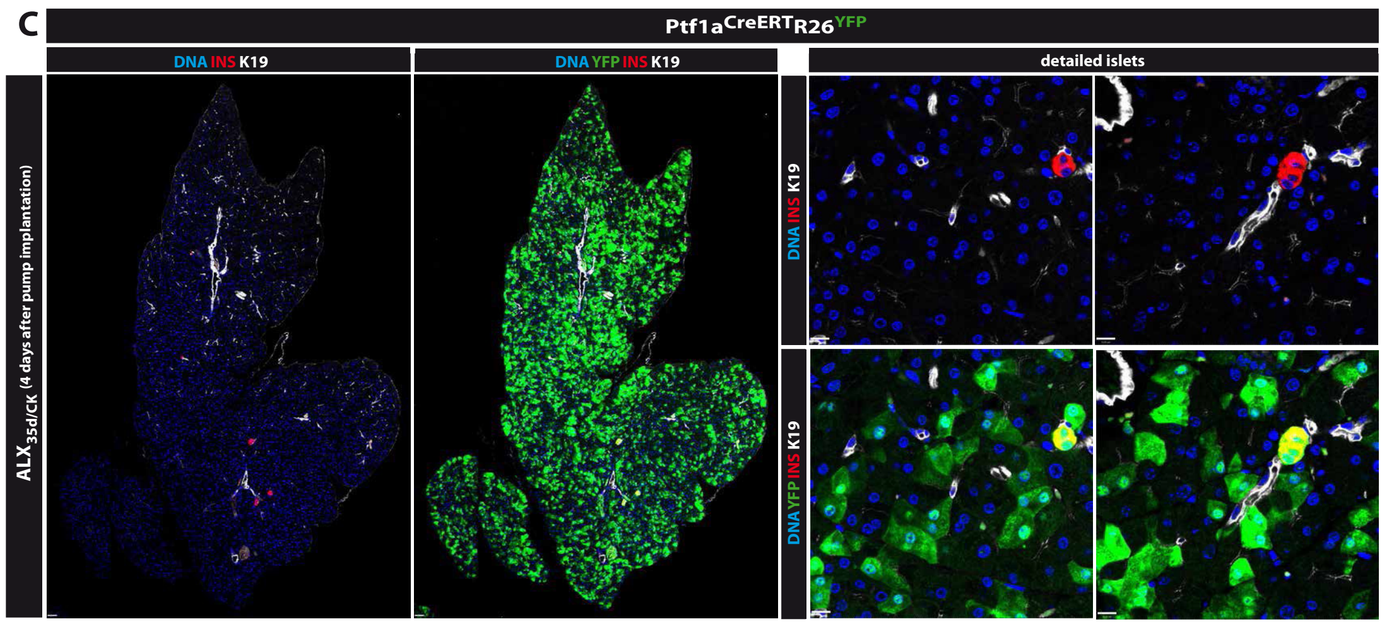

B
Hnf1bCreERTR26LacZ
Xgal+ cells
(% of insulin+ cells)
2.0
1.5
1.0
0.5
0.0
ALX35d/CTR
ALX35d/CK
Xgal+ cells
(% of K19+ cells)
25
20
15
10
5
0
ALX35d/CTR
ALX35d/CK

Figure S3


A
Ptf1a^CreERT R26^YFP
DNA INS K19
DNA YFP INS K19
ALX35d/CTR
ALX35d/CK
detailed islet
DNA YFP INS K19
DNA INS K19
DNA YFP K19


B
Ptf1a^CreERT R26^YFP
YFP+ cells (% of INS+ cells)
NG35d/CTR
ALX35d/CTR
ALX35d/CK
YFP+ cells (% of AMY+ cells)
INS+ cells (number per cluster)
NG35d/CTR
ALX35d/CK - 4 days
ALX35d/CK - 7 days


D
Ptf1a^CreERT R26^YFP
NG35d/CTR
ALX35d/CK
TSQ
YFP


C
Ptf1a^CreERT R26^YFP
DNA INS K19
DNA YFP INS K19
detailed islets
ALX35d/CK (4 days after pump implantation)
DNA INS K19
DNA YFP INS K19

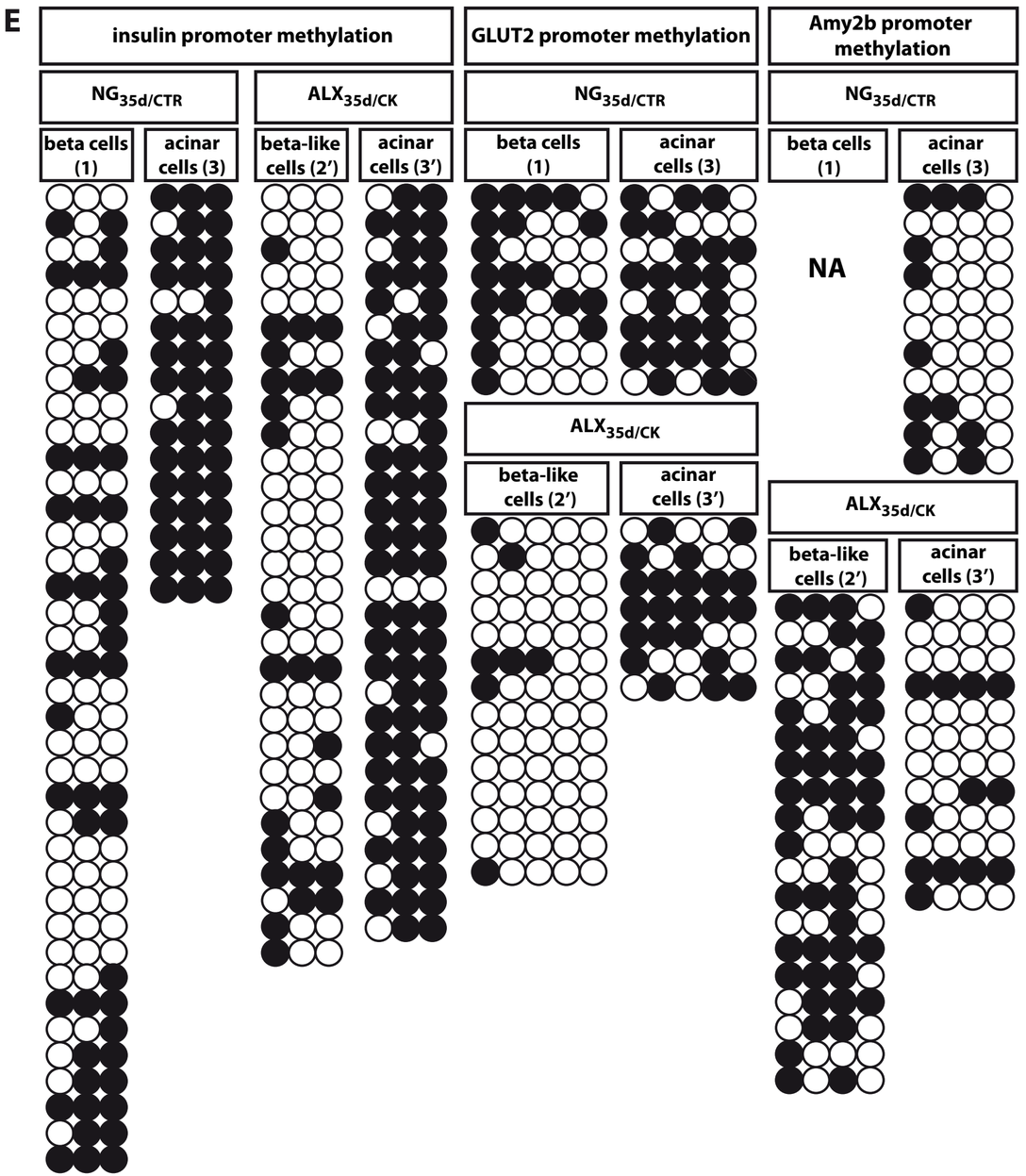
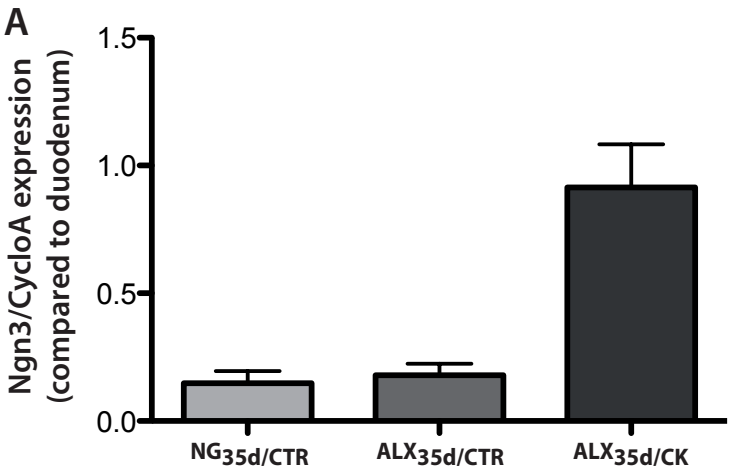

E
insulin promoter methylation
GLUT2 promoter methylation
Amy2b promoter methylation
NG35d/CTR
ALX35d/CK
beta cells (1)
acinar cells (3)
beta-like cells (2')
acinar cells (3')
NA

Figure S4


A
Ngn3/CycloA expression
(compared to duodenum)
1.5
1.0
0.5
0.0
NG35d/CTR
ALX35d/CTR
ALX35d/CK

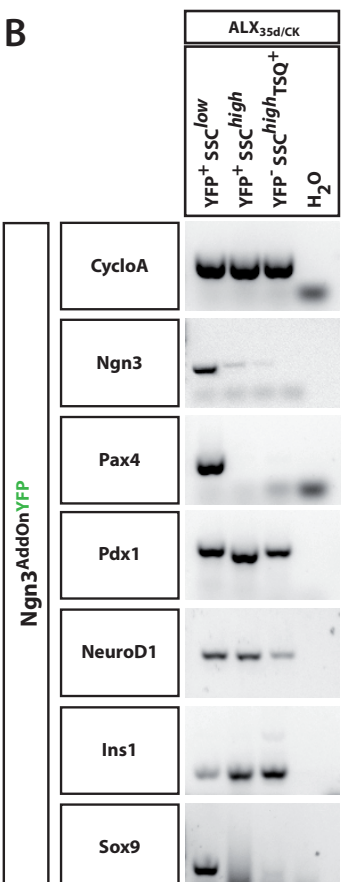
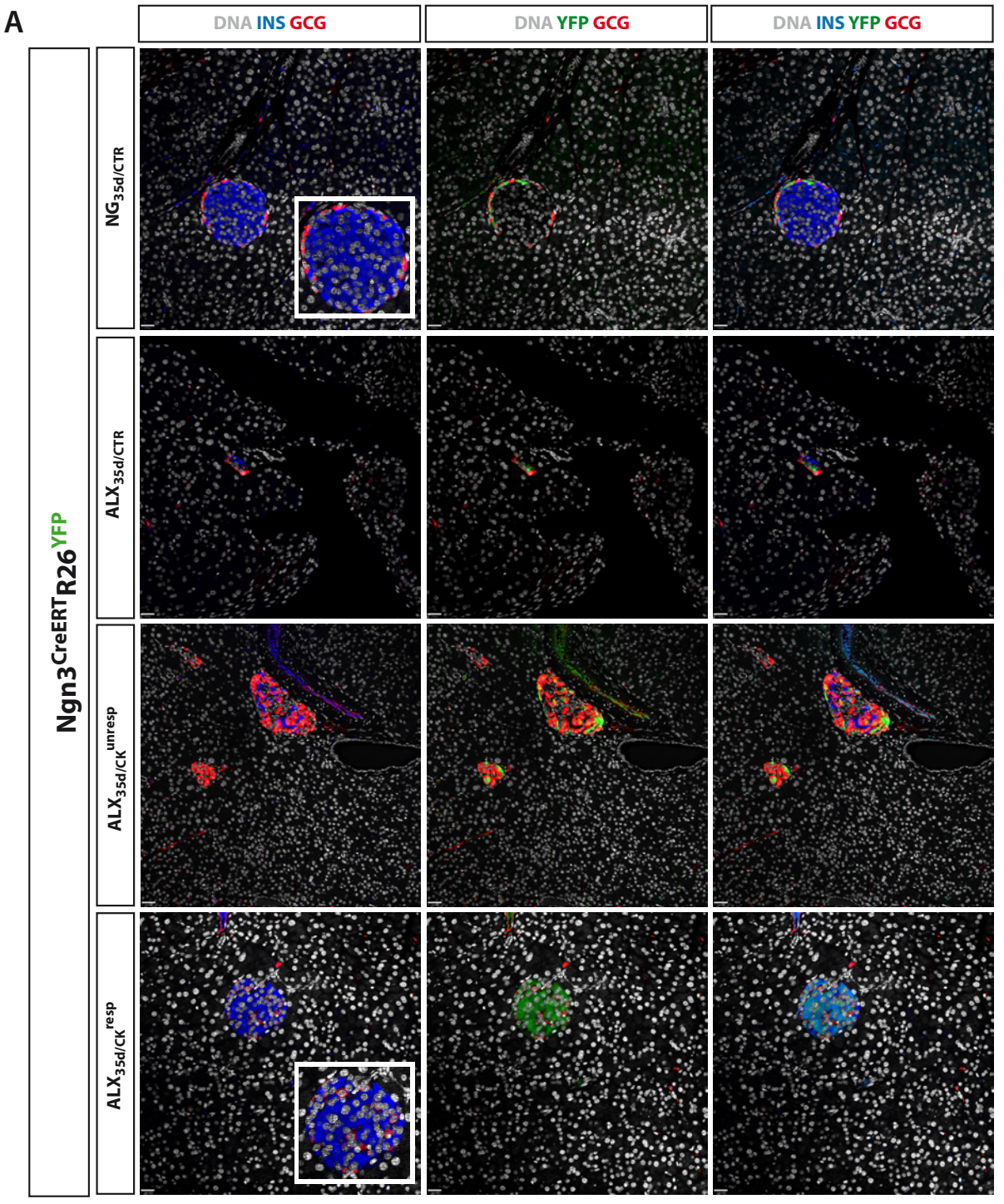

B
ALX35d/CK
YFP+ SSClow
YFP+ SSChigh
YFP- SSChighTSQ+
H2O
Ngn3AddOnYFP
CycloA
Ngn3
Pax4
Pdx1
NeuroD1
Ins1
Sox9

Figure S5


A
DNA INS GCG
DNA YFP GCG
DNA INS YFP GCG
Ngn3CreERTR26YFP
NG35d/CTR
ALX35d/CTR
ALX35d/CKunresp
ALX35d/CKresp

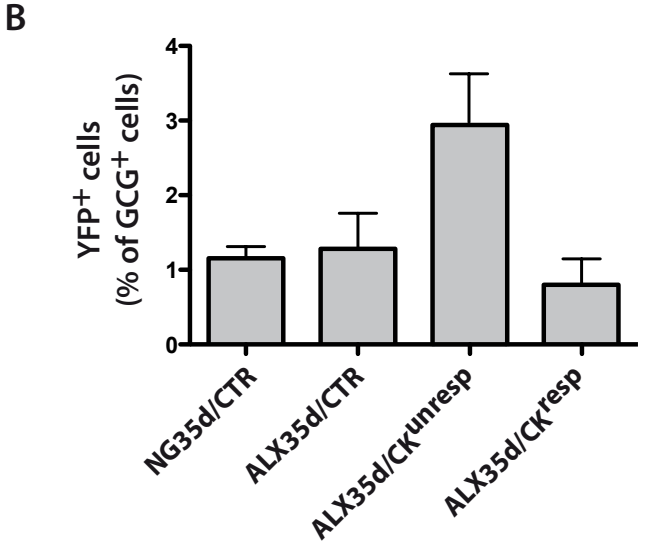

B
YFP+ cells
(% of GCG+ cells)
0
1
2
3
4
NG35d/CTR
ALX35d/CTR
ALX35d/CKunresp
ALX35d/CKresp

Figure S6

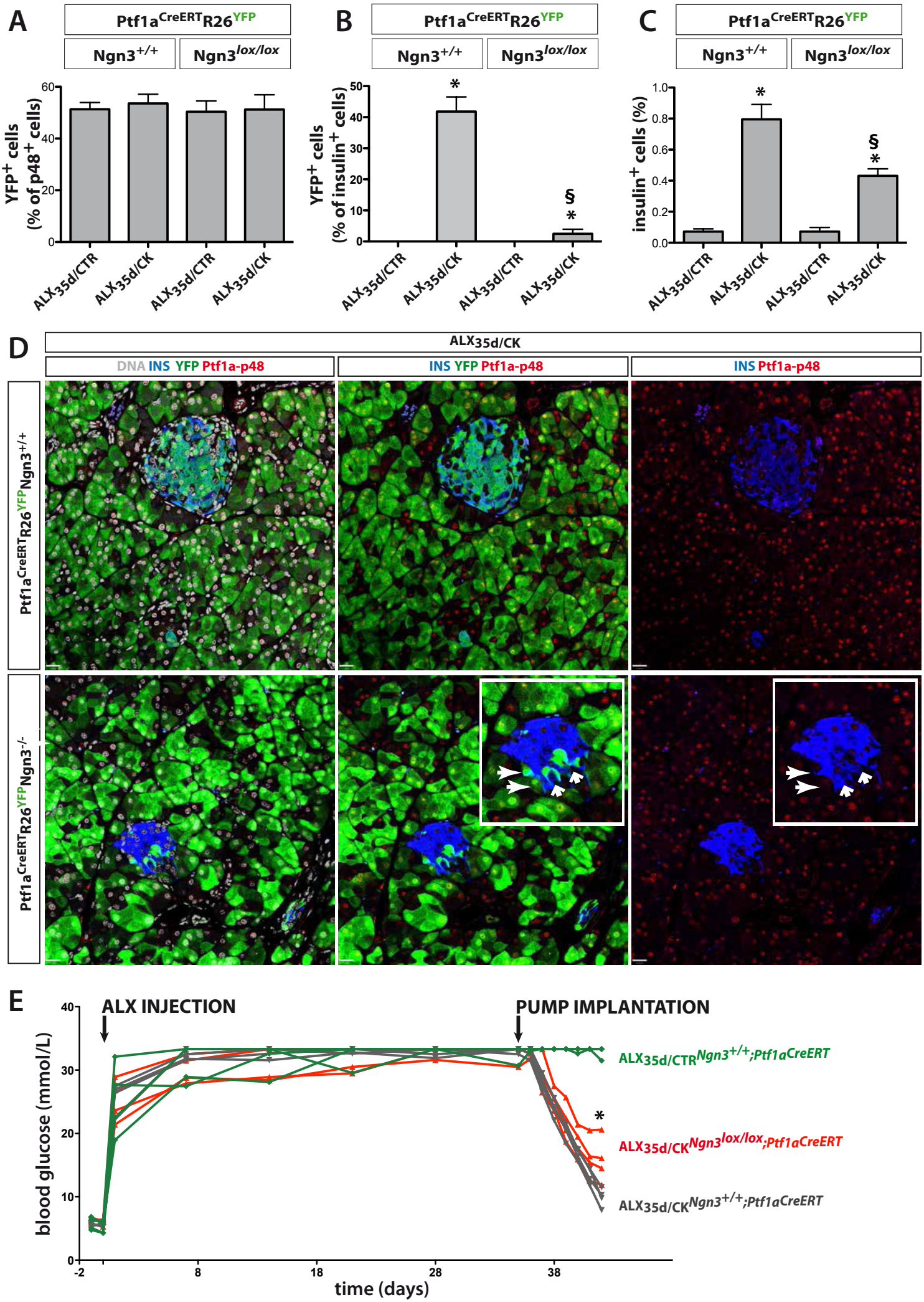

A
Ptf1a^CreERT R26^YFP
Ngn3^+/+
Ngn3^lox/lox
YFP+ cells (% of p48+ cells)
ALX35d/CTR
ALX35d/CK
B
YFP+ cells (% of insulin+ cells)
§
*
C
insulin+ cells (%)
D
ALX35d/CK
DNA INS YFP Ptf1a-p48
INS YFP Ptf1a-p48
INS Ptf1a-p48
Ptf1a^CreERT R26^YFP Ngn3^+/+
Ptf1a^CreERT R26^YFP Ngn3^-/-
E
ALX INJECTION
PUMP IMPLANTATION
blood glucose (mmol/L)
time (days)
ALX35d/CTR Ngn3^+/+;Ptf1aCreERT
ALX35d/CK Ngn3^lox/lox;Ptf1aCreERT
ALX35d/CK Ngn3^+/+;Ptf1aCreERT

TABLE S1: LIST OF PRIMERS AND PROBES.

Gene	Primer 1	Primer 2	Probe
CycloA	GCAGACAAAGTTCCAAAGACAG	CATTATGGCGTGTAAAGTCACC	TGAGCACTGGAGAGAAAGGATTTGGC
Neurog3	GTCGGGAGAACTAGGATGGC	GGAGCAGTCCCTAGGTATG	CCGGAGCCTCGGACCACGAA
Pdx1	CCCTTTCCCGTGGATGAAATC	GAATTCCTTCTCCAGCTCCAG	ACAAGAGGACCCGTACTGCCTACA
Insulin1	ATCAGAGACCATCAGCAAGC	GTTTGACAAAAGCCTGGGTG	TGTTGGTGCACTTCCTACCCCTG
Insulin2	GACTCCCAGAGGAAGAGCA	CCCTAAGTGATCCGCTACAATC	AAGCAGGAAGGTTATTGTTTCAACATGGC
Pax4	GGAGGCTGTTACAAGACCAG	GGCAAACTGAAAACGGAGAG	CCATCCAGAACCAGTCCCAAAGAGAA
NeuroD1	CTCCAGGGTTATGAGATCGTC	GTCCTGAGAACTGAGACACTC	AGCCCGCTCTCGCTGTATGATTT
Ptf1a-p48	AGGACCCCAGAAAACTCAAC	CAATATGCACAAAGACGCGG	TCTCAGGACACAAACTCAAAGGGTGG
Amylase	ACTCTGCTTGGGACTTTAACG	CACCTTGGTACGAACATAATCTTTC	ACTGGCCTTCTGGATCTTGCACTT
Sox9	CAAGACTCTGGGCAAGCTC	GGGCTGGTACTTGTAATCGG	AGGGTCTCTTCTCGCTCTCGTTCA
Hnf1b	AGCACCTTGACGAATATCCAC	AGCCACACTGTTAATGACCG	CACAATCCCCAGCAATCTCAGAACCT
Notch1	ATGTCAATGTTCGAGGACCAG	TCACTGTTGCCTGTCTCAAG	TTCACACCCTCATGATTGCCTCC