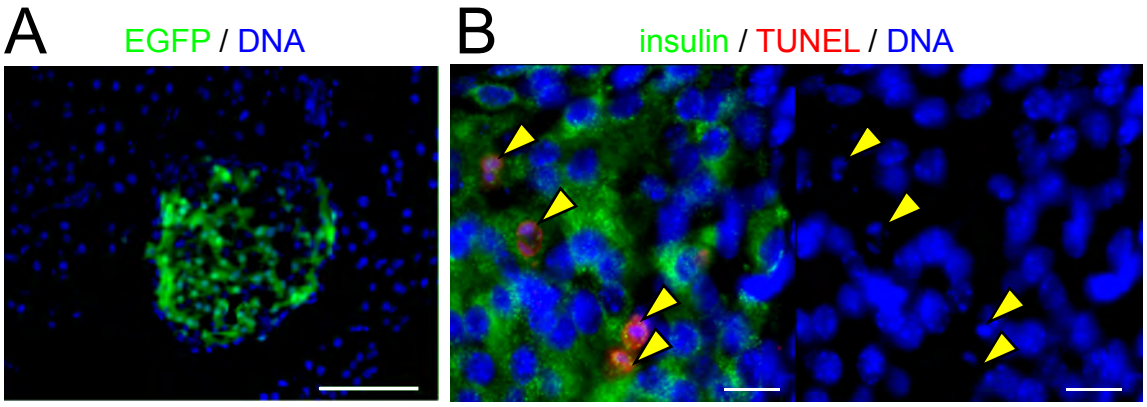


Fig s1

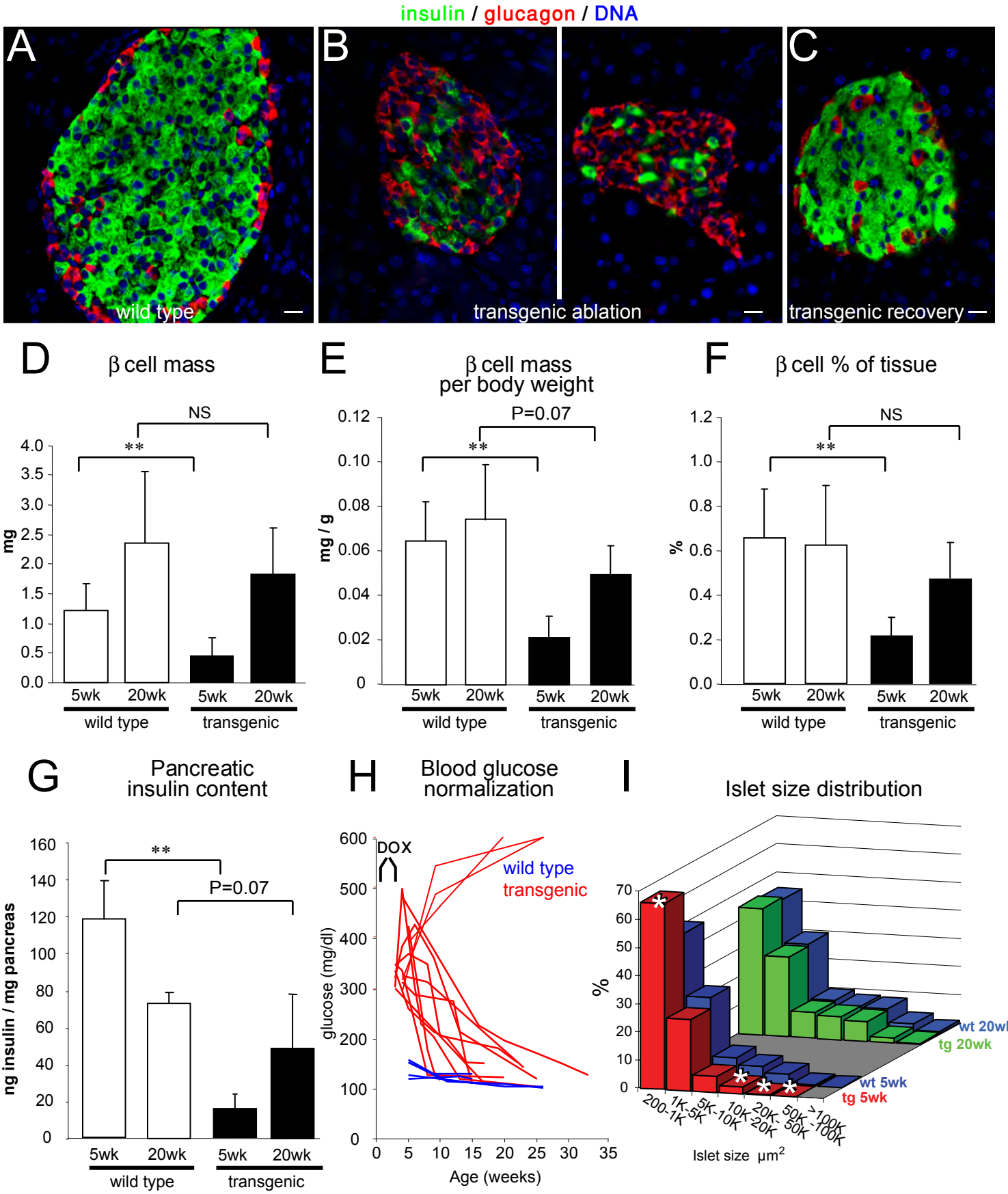
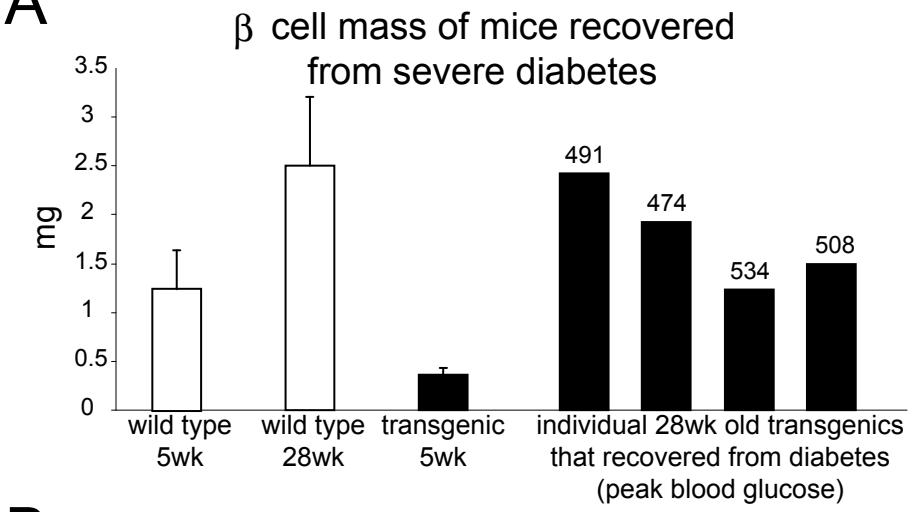

insulin / glucagon / DNA
A
wild type
B
transgenic ablation
C
transgenic recovery
D
β cell mass
mg
NS
**
5wk
20wk
5wk
20wk
wild type
transgenic
E
β cell mass
per body weight
mg / g
P=0.07
**
F
β cell % of tissue
%
NS
**
G
Pancreatic
insulin content
ng insulin / mg pancreas
**
P=0.07
H
Blood glucose
normalization
DOX
wild type
transgenic
glucose (mg/dl)
Age (weeks)
I
Islet size distribution
%
wt 20wk
tg 20wk
wt 5wk
tg 5wk
200-1K
1K-5K
5K-10K
10K-20K
20K-50K
50K-100K
>100K
Islet size µm2

Fig s2

A


β cell mass of mice recovered from severe diabetes
mg
3.5
3
2.5
2
1.5
1
0.5
0
491
474
534
508
wild type 5wk
wild type 28wk
transgenic 5wk
individual 28wk old transgenics that recovered from diabetes (peak blood glucose)

B

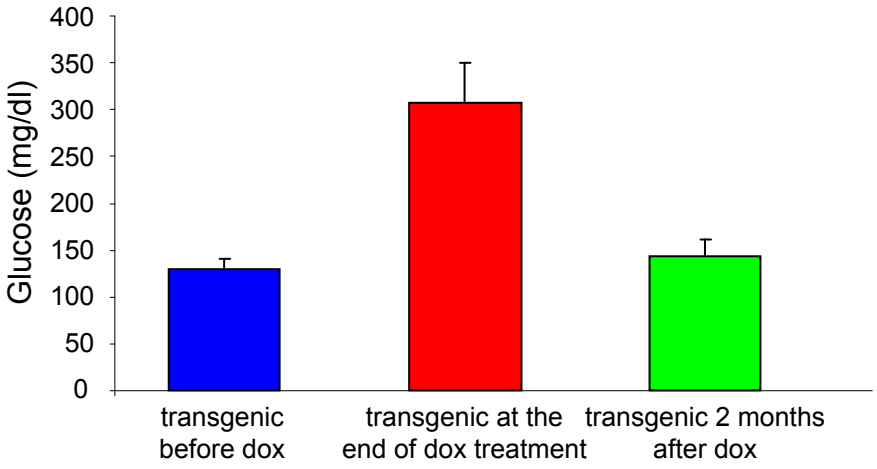
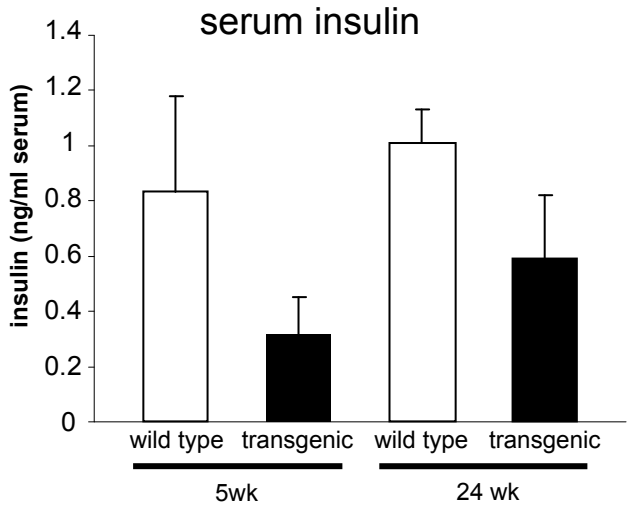
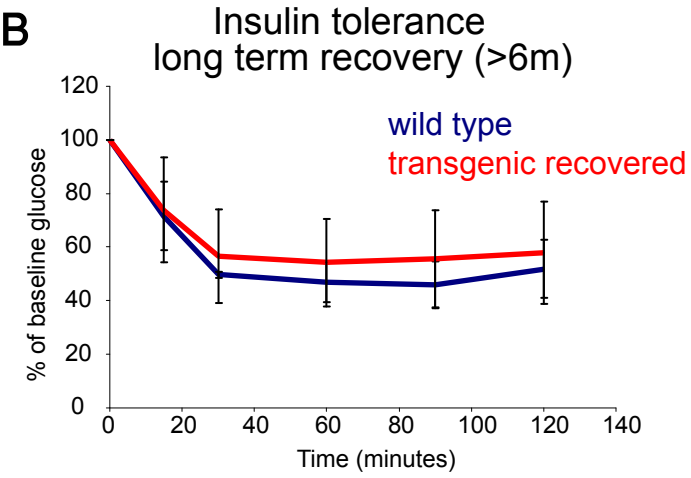

Late onset diabetes (4-5 month) and recovery
Glucose (mg/dl)
400
350
300
250
200
150
100
50
0
transgenic before dox
transgenic at the end of dox treatment
transgenic 2 months after dox

fig s3

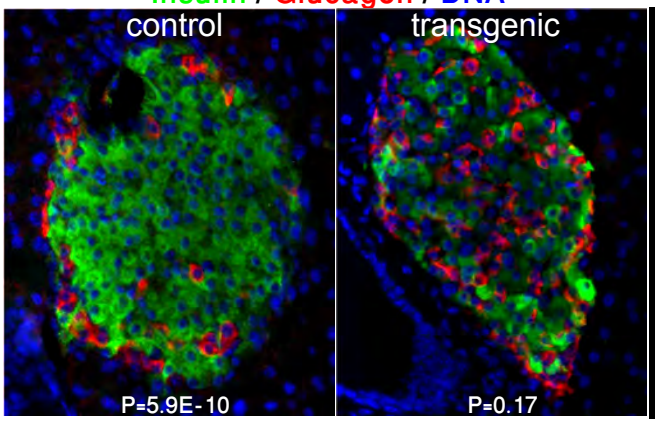
A



B

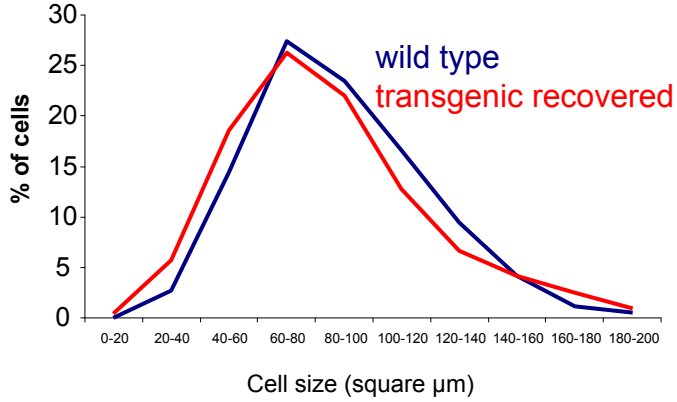


C

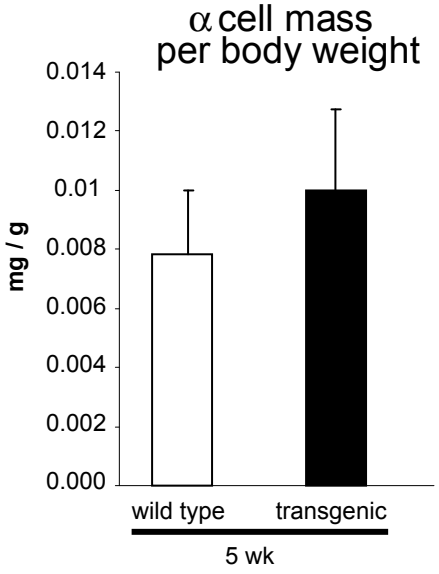


Mouse	Blood glucose mg/dl	P value
wild type1	96	9.14E-27
wild type2	149	7.4E-7
wild type3	143	0.00043
ablated transgenic1	600	0.11
ablated transgenic2	600	0.99
ablated transgenic3	600	0.04
recovered transgenic1	600>>175	1.2E-65
recovered transgenic2	600>>169	4.3E-37
recovered transgenic3	369>>124	4.1E-40
recovered transgenic4	294>>145	1.2E-81
recovered transgenic5	252>>129	7.5E-16

D



E



F

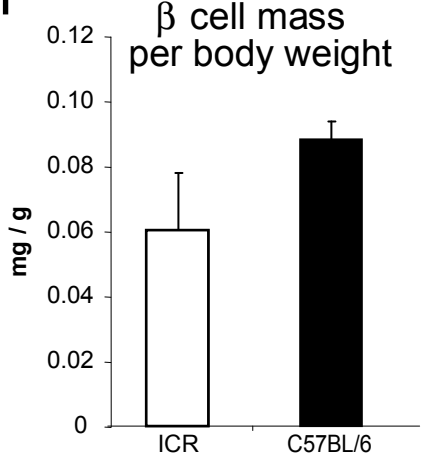


Fig s4

F4/80

Control 5 weeks

Transgenic 5 weeks

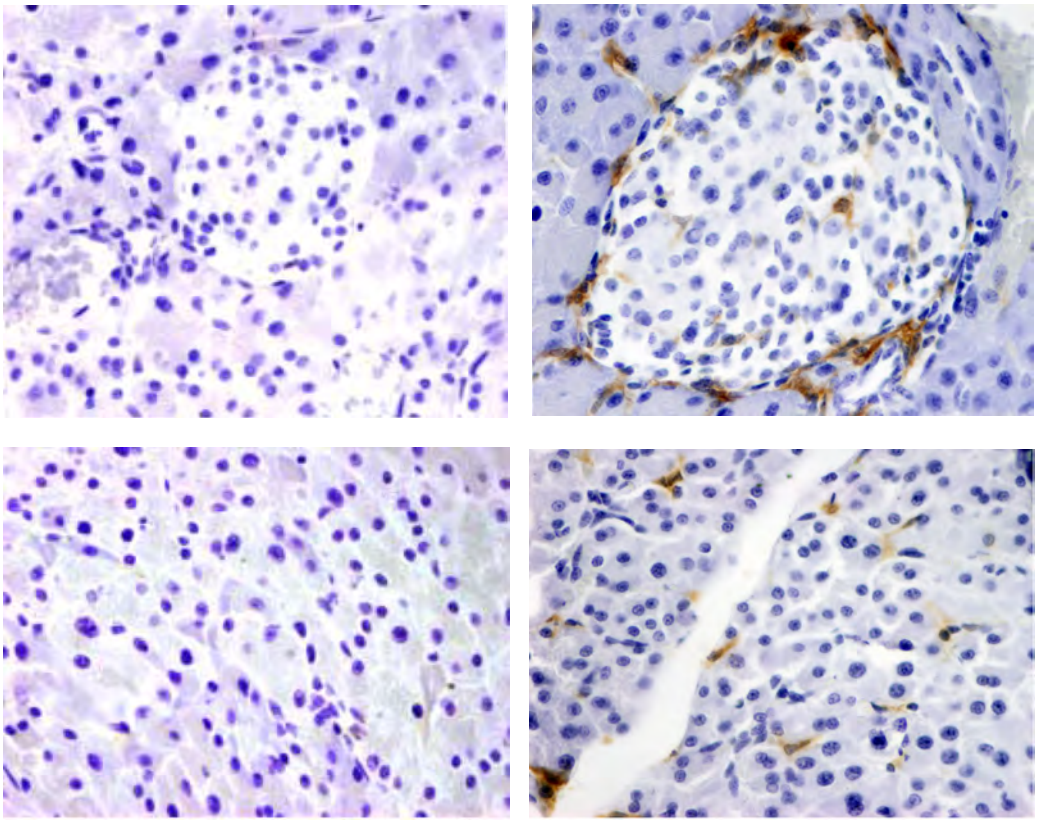
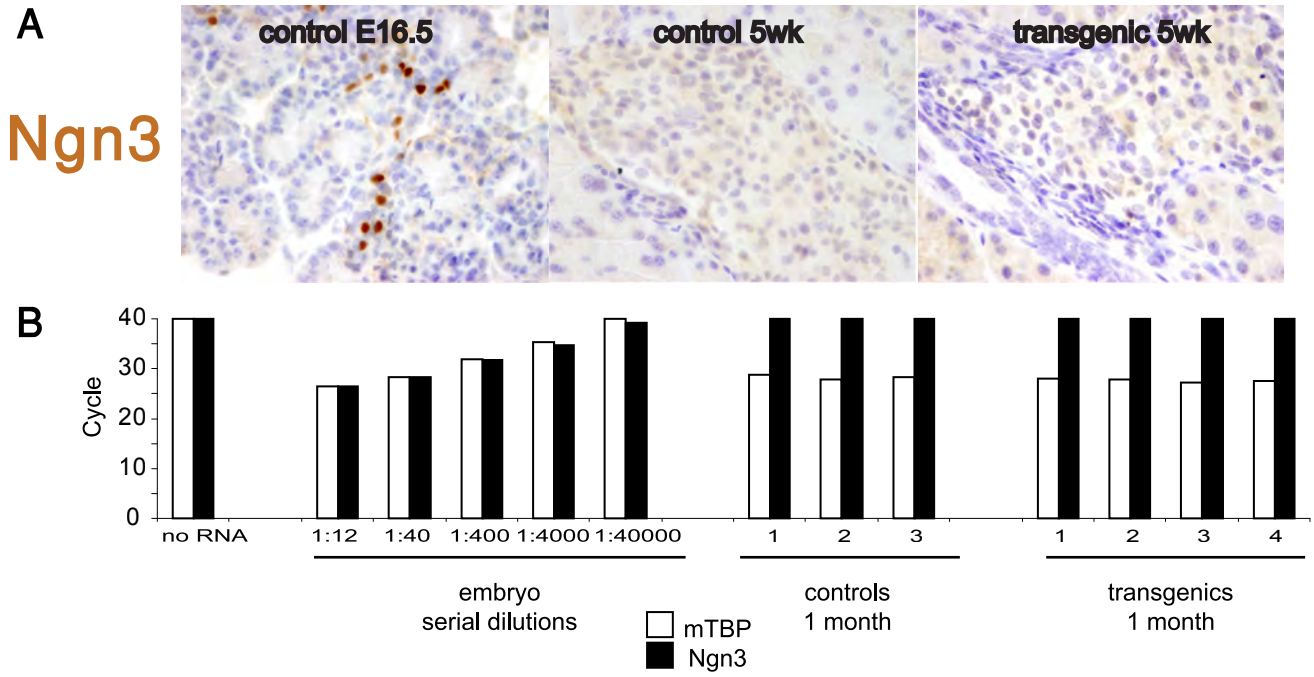
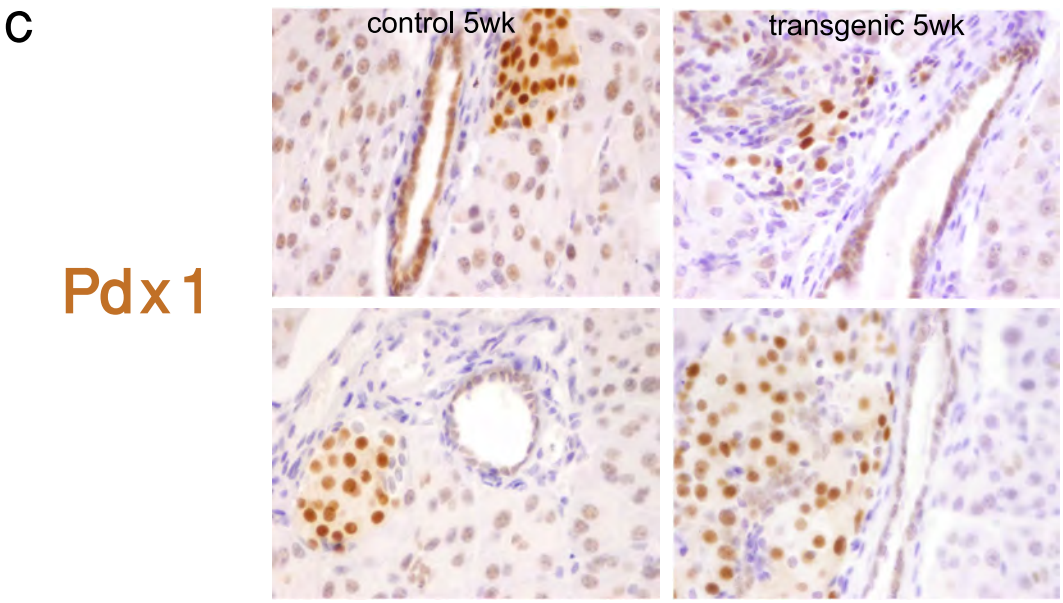


Fig s5


A
Ngn3
control E16.5
control 5wk
transgenic 5wk
B
Cycle
40
30
20
10
0
no RNA
1:12
1:40
1:400
1:4000
1:40000
1
2
3
1
2
3
4
embryo
serial dilutions
controls
1 month
transgenics
1 month
mTBP
Ngn3


C
Pdx1
control 5wk
transgenic 5wk

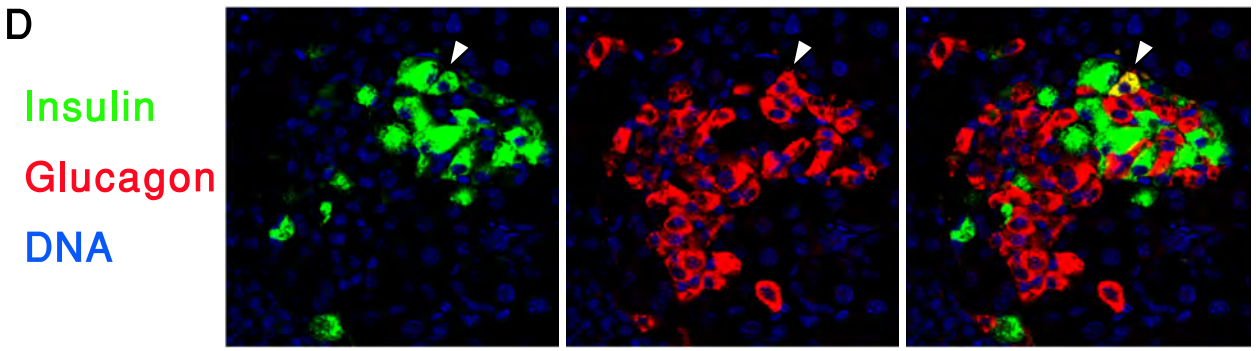

D
Insulin
Glucagon
DNA

Fig s6

Supplementary figure s1: β cell specificity of Insulin-rtTA; TET-DTA transgenic mice.

(A) After the administration of doxycycline for 2 weeks to adult Insulin-rtTA;TET-EGFP compound transgenic mice, only islet cells express the EGFP reporter transgene (green). Note that EGFP expression is localized to the center of the islet, where beta cells reside.

(B) Apoptotic beta cells after doxycycline treatment. Confocal image of a section from an insulin-rtTA; TET-DTA adult mouse treated with doxycycline for 48hrs. TUNEL+ cells are always insulin+, confirming beta cell specificity of cell killing. Note pycnotic nuclei of these TUNEL+ cells (arrows). No insulin- TUNEL+ cells are seen.

Supplementary figure s2: beta cell ablation and regeneration when doxycycline is administered from birth to 5 weeks of age.

For the experiments shown here, mice received doxycycline from birth to 5 weeks. Some mice were sacrificed at 5 weeks of age, while others were allowed to recover in the absence of doxycycline for additional ~15 weeks and were sacrificed at ~20 weeks of age.

(A-C) Islet architecture of wild type (**A**), 5 week old diabetic transgenic mice (**B**) and 20 week old transgenic mice that recovered from diabetes (**C**).

(D-F) Beta cell mass changes during ablation and recovery, represented as beta cell mass (**D**), beta cell mass normalized to body weight (**E**) and the fraction of pancreas tissue area covered by insulin-expressing cells (**F**). Each bar represents the average + standard deviation values from >5 mice.

(G) Pancreatic insulin content at 5 weeks of age (doxycycline still present in water) and at 20 weeks of age (15 weeks after doxycycline withdrawal).

(H) Blood glucose levels after doxycycline withdrawal in wild type (blue) and transgenic (red) mice. Only mice which normalized blood glucose levels

were used for the analysis shown in D-F, G, and I.

(I) Changes in islet size distribution after beta cell ablation and regeneration. The distribution of islet sizes is shifted towards smaller islets during doxycycline administration, and is normalized after doxycycline withdrawal. wt, wild type; tg, transgenic; *, $P < 0.05$ for the difference between age-matched wild type and transgenic mice.

Supplementary figure s3: recovery from severe or late onset diabetes.

(A) Beta cell mass in a subgroup of mice whose blood glucose levels upon doxycycline administration rose to >450mg/dL. The bars showing beta cell mass for wild type mice and for transgenic mice at 5 weeks of age (after doxycycline administration between weeks 4 and 5) represent the average + standard deviation of multiple mice. Individual bars represent beta cell mass of mice that were severely diabetic at 5 weeks of age, and recovered to blood glucose <150 mg/dL after doxycycline withdrawal. The number above each bar represents blood glucose level measured in that particular mouse at the end of ablation. Note that even within this severely affected group, beta cell mass regenerates spontaneously.

(B) Normalization of blood glucose levels in transgenic mice that were treated with doxycycline for ~1 week at 4-5 months of age, then left to recover without doxycycline. Each bar represents the average + standard deviation from 5 mice.

Supplementary figure s4: Further characterization of beta cell ablation and regeneration.

(A) Serum insulin levels in 5 week old mice treated for 1 week with doxycycline (between week 4 and 5), and in ~3 month old mice (2 months after doxycycline withdrawal). Bars represent the average+ standard deviation from 3-5 mice.

(B) Peripheral sensitivity to insulin is similar in wild type (n = 3) and in transgenic (n = 9) mice that recovered from diabetes (mice ~8 months of age; doxycycline administered between week 4 and 5 of age).

(C) Quantitative assessment of changes in islet architecture upon beta cell ablation and regeneration. P value represents the likelihood that the observed distribution of beta cells and alpha cells in a given islet is random. Left, representative images of control and ablated islets and calculated p values for islet organization. Right, p values from several individual islets, and blood glucose levels measured and the time of sacrifice. For diabetic mice that recovered, shown are blood glucose levels at the end of ablation and at sacrifice. Note that normal as well as regenerated islets are more ordered compared with ablated islets (i.e. their architecture is unlikely to be random). For these experiments, doxycycline was administered from birth to 4 weeks of age, after which mice were either sacrificed or allowed to recover in the absence of doxycycline.

(D) Normal beta cell size in mice that recovered from diabetes. Beta cell size was calculated by measuring the area of individual insulin+ cells. Cell boundaries were determined by immunostaining for E-cadherin. Each graph represents the measurements from 3 individual mice; for each mouse, the size of 300 individual beta cells was measured.

(E) Morphometric quantification of alpha cell mass after beta cell ablation (mice sacrificed at 5 weeks of age; doxycycline administered from birth to 5 weeks). Each bar represents the average+ standard deviation of 3 individual mice. Alpha cell mass was calculated as described for beta cell mass.

(G) Interindividual variation in beta cell mass in ICR and C57/B6 mice. Variation in beta cell mass per body weight among littermates is smaller is isogenic C57/B6 (n = 4) mice compared with ICR (n = 9) mice. This

indicates that the observed variation in our measurements of beta cell mass in transgenic mice on ICR background largely reflects normal variation rather than experimental errors.

Supplementary figure s5: Detection of macrophages concurrent with beta cell ablation.

Immunostaining for the macrophage marker F4/80 on paraffin sections of 5-week old control and transgenic mice exposed to doxycycline between 4 and 5 weeks of age. While F4/80+ cells are not detected in the normal pancreas, beta cell ablation results in accumulation of F4/80+ macrophages in the periphery of islets as well as in the exocrine pancreas. F4/80, brown; hematoxilin, blue.

Supplementary figure s6: Little evidence for the presence of embryonic-type endocrine progenitor cells in the regenerating pancreas.

(A) Immunostaining for the embryonic endocrine progenitor marker Neurogenin3 (Ngn3). While Ngn3+ progenitor cells are readily detected in the embryonic pancreas (left panel), pancreases from either control (middle panel) or regenerating transgenic (right panel) adult mice show no evidence for the presence of Ngn3+ cells, in islets or elsewhere in the pancreas. Ngn3, brown; hematoxilin, blue.

(B) Quantitative PCR using TaqMan does not detect expression of Ngn3 in total pancreatic RNA prepared from control or transgenic adult mice. By contrast, Ngn3 expression is readily detected in RNA of the embryonic pancreas, even after 1:40,000 dilution. mTBP serves as a reference mRNA,

(C) Immunostaining for Pdx1 shows a similar expression pattern in adult pancreases of control and transgenic mice. In both cases Pdx1 is expressed strongly in beta cells. Low level of Pdx1 expression is detected in acinar cells and in some but not all ducts. Pdx1, brown; hematoxilin, blue.

(D) Detection of rare cells co-expressing insulin and glucagons in isleta. Such cells were detected at about 1:5,500 of beta cells in normal mice, and 1:1,000 in regenerating transgenics. Insulin, green; glucagon; red; nuclei, blue.