Figure S1. Development of diabetes is not influenced by changes in body weight or insulin sensitivity . (**A-B**) No significant weight differences were found in Nkx2.2 ΔBeta vs. littermate control females (n=5-20) and males (n=3-12), by 2-tailed Student's t test. (**C-D**) Female Nkx2.2ΔBeta mice are glucose intolerant beginning at 3 weeks of age (n=6-23) and become overtly diabetic by 11 weeks of age (n=7-33). *p ≤0.05; **p ≤0.01; ***p ≤0.001, by 2-tailed Student's t test. (**E**) Insulin tolerance tests demonstrate that the Nkx2.2ΔBeta mice do not have insulin sensitivity defects compared to controls at 3 weeks of age. (n=3-5) , by 2-tailed Student's t test. (**F**) Glucose stimulated insulin secretion assays show normal insulin secretion levels in Nkx2.2ΔBeta mice at 4 weeks of

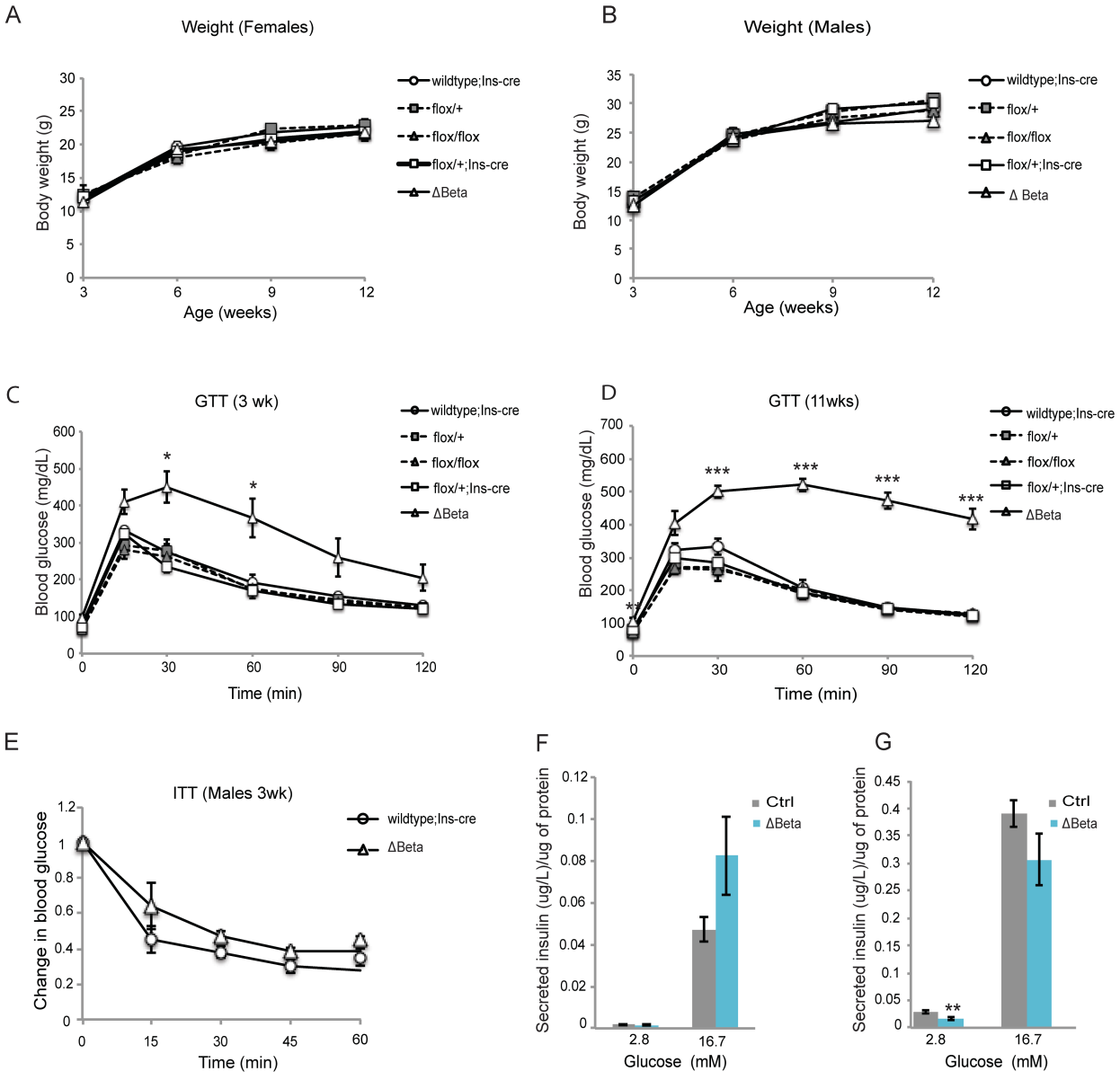
age compared to controls (n=3). (**G**) Glucose stimulated insulin secretion assay exhibits an impaired basal insulin response in female Nkx2.2ΔBeta mice compared to controls at 17 months of age (n=3).**p ≤0.01; by 2-tailed Student's t test.

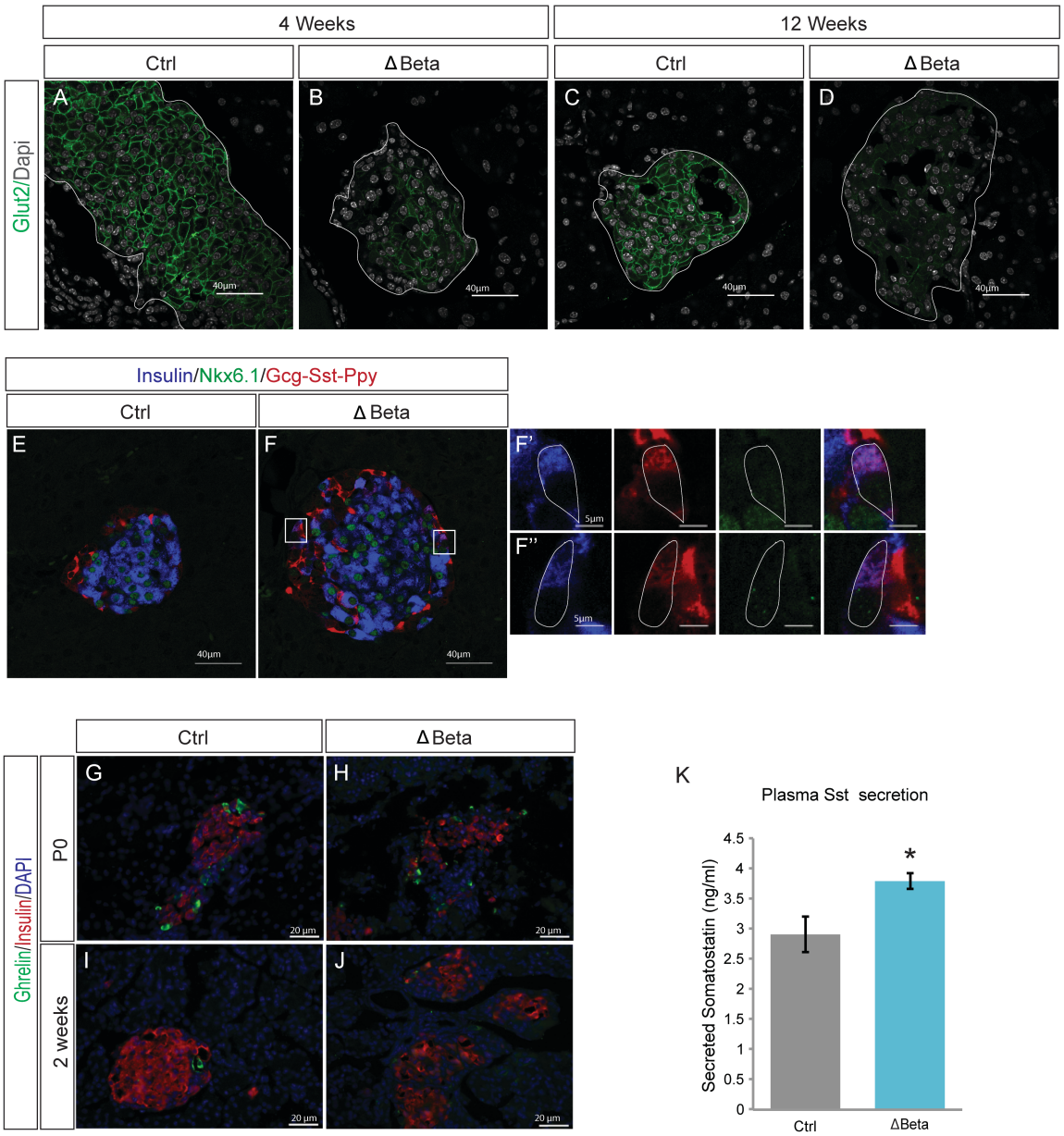
Figure S2. Loss of important beta cell protein expression in Nkx2.2ΔBeta islets correlated with their decreased RNA expression. (**A-D**) Representative images of immunostained islet sections from 4 week old (**A,B**) and 12 week old (**C,D**) Nkx2.2ΔBeta mice (**B,D**) and their littermate controls (**A,C**). Dapi (grey) marks the nuclei and Glut2 (green) is on the cell membrane. Glut2 expression is decreased at 4 weeks of age and becomes almost undetectable at 12 weeks. (**E-F**) Representative images of immunostained islet sections from 4 week old Nkx2.2ΔBeta mice (**E**) and a littermate controls (**F**). Insulin (blue), glucagon, somatostatin and pancreatic polypeptide, combined (red), and Nkx6.1 (green). **F'** and **F''** show higher magnification images of cells that are insulin+, express other hormones and are Nkx6.1 negative. (**G-J**) Immunostained islet sections from P0 (**G,H**) and 2 week old (**I,J**) Nkx2.2ΔBeta mice (**H,J**) and their littermate controls (**G,I**). Dapi (blue) marks the nuclei, Insulin (red) and Ghrelin (green). Ghrelin expressing cells are rare and the numbers decrease with age both in the control and Nkx2.2ΔBeta mice. (**K**) Elevated plasma somatostatin levels are observed 30 min after glucose stimulation by intraperitoneal injection in Nkx2.2ΔBeta 4 week old mice compared to control mice (n=5).*p≤0.05, by 2-tailed Student's t test.

Figure S3. Nkx2.2 activates and represses an equal amount of targets preferentially through active and poised enhancer binding. (**A**) Comparative analysis of ChIP-Seq

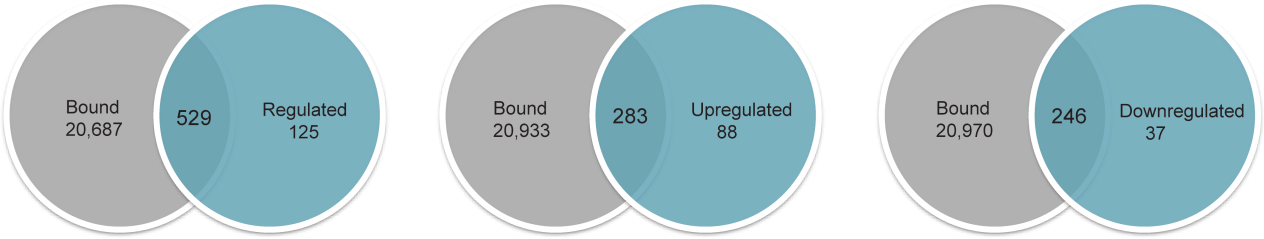
data from MIN6 cells with RNA-Seq data from Nkx2.2ΔBeta compared to control islets demonstrates that Nkx2.2 represses and activates relatively equal numbers of direct targets regulated by Nkx2.2. (**B**) Chromatin state of regions bound by Nkx2.2 shows preferential binding to active enhancers (H3k427ac+/H3k4me+) and poised enhancers (H3k4me+). Repressed enhancers (H3k4me+/H3k27me3+) constitute a minor proportion. ChIP-Seq analysis done using Nkx2.2 binding peaks present in all triplicate samples.

Figure S4. Nx2.2 activates beta cell genes and represses non beta cell islet genes during adulthood. (**A**) qRT-PCR analysis from Nkx2.2ΔAdult Beta and control mice present with gene expression changes at 5 wks after the last tamoxifen injection that continue to exacerbate 6-7months after the last tamoxifen injection.*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$, by 2-tailed Student's t test.


A
Weight (Females)
Body weight (g)
Age (weeks)
wildtype;Ins-cre
flox/+
flox/flox
flox/+;Ins-cre
ΔBeta
B
Weight (Males)
Body weight (g)
Age (weeks)
wildtype;Ins-cre
flox/+
flox/flox
flox/+;Ins-cre
Δ Beta
C
GTT (3 wk)
Blood glucose (mg/dL)
Time (min)
wildtype;Ins-cre
flox/+
flox/flox
flox/+;Ins-cre
ΔBeta
D
GTT (11wks)
Blood glucose (mg/dL)
Time (min)
wildtype;Ins-cre
flox/+
flox/flox
flox/+;Ins-cre
ΔBeta
E
ITT (Males 3wk)
Change in blood glucose
Time (min)
wildtype;Ins-cre
ΔBeta
F
Secreted insulin (ug/L)/ug of protein
Glucose (mM)
Ctrl
ΔBeta
G
Secreted insulin (ug/L)/ug of protein
Glucose (mM)
Ctrl
ΔBeta


4 Weeks
12 Weeks
Ctrl
ΔBeta
Ctrl
ΔBeta
Glut2/Dapi
A
B
C
D
40µm
Insulin/Nkx6.1/Gcg-Sst-Ppy
Ctrl
Δ Beta
E
F
F'
F''
5µm
Ctrl
ΔBeta
Ghrelin/Insulin/DAPI
P0
2 weeks
G
H
I
J
20 µm
K
Plasma Sst secretion
Secreted Somatostatin (ng/ml)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
Ctrl
ΔBeta

A

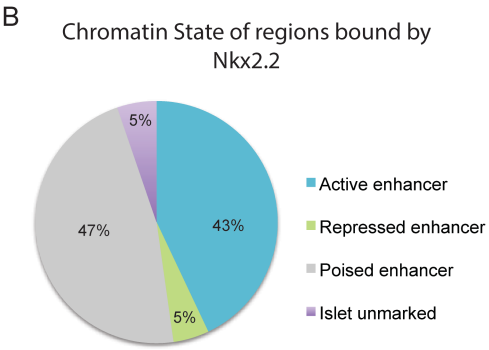


80.9% Bound and Regulated

53.5% Bound and Upregulated

46.5% Bound and Downregulated

B



A

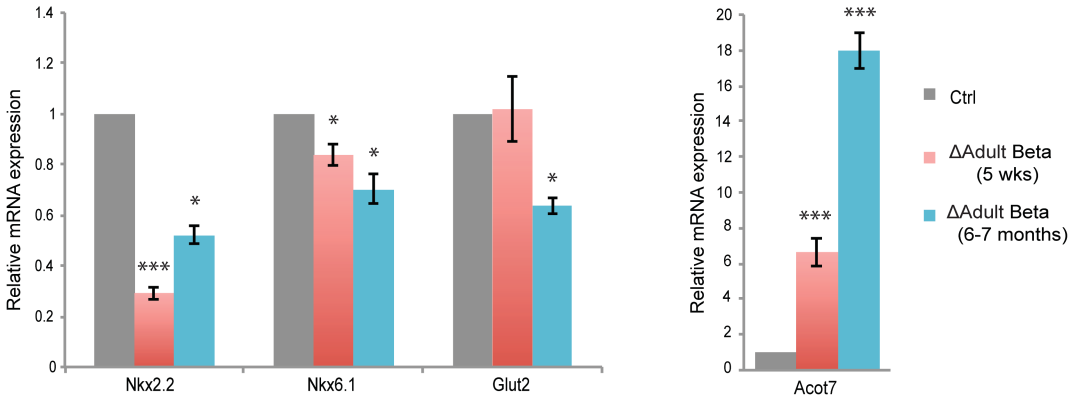

Relative mRNA expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
*
*
*
*
Nkx2.2
Nkx6.1
Glut2
Relative mRNA expression
20
18
16
14
12
10
8
6
4
2
0
***
***
Acot7
Ctrl
ΔAdult Beta (5 wks)
ΔAdult Beta (6-7 months)

Table S4. List of primary antibodies used in western blot analysis and immunohistochemistry

Primary Antibodies				
Antigen	***Host***	***Dilution***	***Source***	***Catalogue #***
Glucagon	Goat	1:200	Santa Cruz	sc-7780
Insulin	Guinea Pig	1:1000	Dako	A0564
Nkx2.2	Rabbit	1:200	Sigma	hpa003468
Pancreatic Polypeptide	Goat	1:150	Sigma	SAB2500747
RFP	Rabbit	1:1000	Rockland Immunochemicals	600-401-379
Somatostatin	Rat	1:500	Abcam	ab30788
Cleaved Caspase 3	Rabbit	1:500	Cell Signaling	9661
α-Ghrelin	Goat	1:800	Santa Cruz Biotechnologies	sc10368

Table S5. List of secondary antibodies used in western blot analysis and immunohistochemistry.

Secondary Antibodies			
Antigen	***Conjugation***	***Dilution***	***Source***
Guinea Pig/Rabbit/Goat	Alexa-488	1:500	Jackson Immunoresearch
Rabbit	Cy3	1:500	Jackson Immunoresearch
Goat/ Guinea Pig/ Rat	Alexa-647	1:500	Jackson Immunoresearch
Rabbit	HRP	1:10,000	Jackson Immunoresearch

Table S6. List of primer sequences, probes and AODs used for qRT-PCR analysis in mouse samples

Gene	**AOD**
Insulin 2	Mm00731595_gh
Slc2a2	Mm00446229_m1
Nkx6.1	Mm00454962_m1
Somatostatin	Mm00436671_m1
Insulin 1	Mm01950294_s1
Chromogranin A	Mm00514341_m1
Acot7	Mm00460107_m1

Gene	**Sybr green FWD**	**Sybr green REV**
Hhex	TCAGAATCGCCGAGCTAAAT	CTGTCCAACGCATCCTTTTT

Gene	**Probe**	**FWD**	**REV**
Nkx2.2	CCATTGACTCTGCCCCATCGCTCT	CCTCCCCGAGTGGCAGAT	GAGTTCTATCCTCTCCAAAAGTTCAAA
Cyclophilin B	TGGTACGGAAGGTGGAG	GCAAAGTTCTAGAGGGCAGGA	CCCGGCTGTCTGTCTGGT

Table S7. List of primer sequences and AODs used for qRT-PCR analysis in human samples

Gene	**AOD**
NKX2.2	Hs00159616_m1

Gene	**Sybr green FWD**	**Sybr green REV**
36B4	GGCGACCTGGAAGTCCAACT	CCATCAGCACCACAGCCTTC