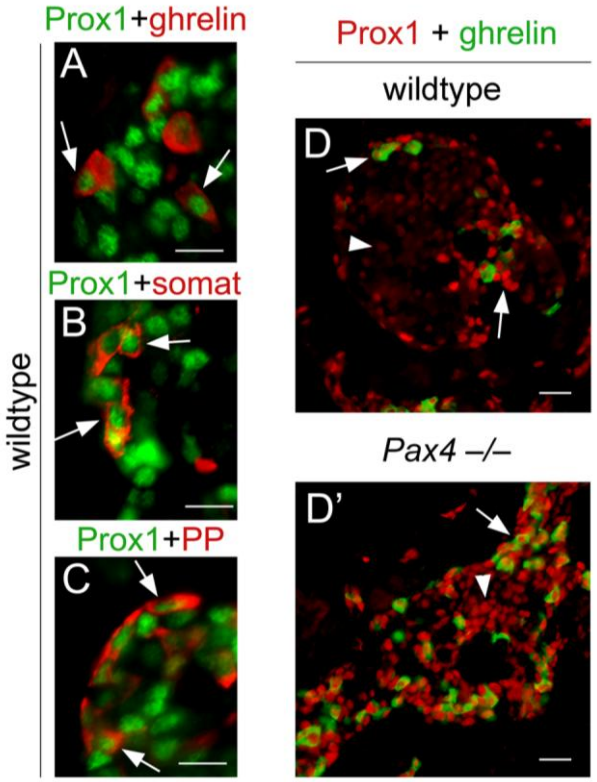
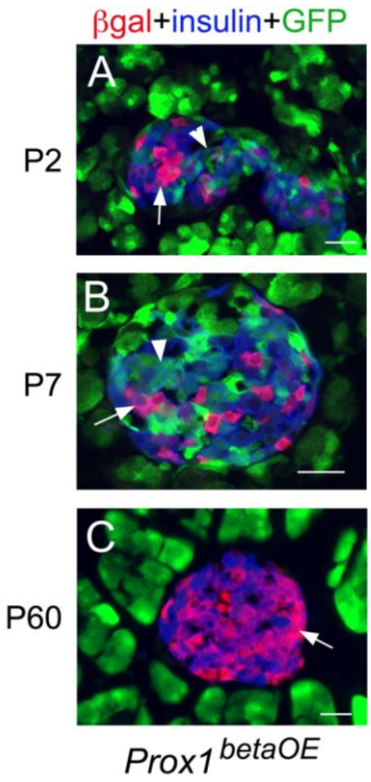
SUPPLEMENTARY DATA

Supplementary Figure 1. Prox1 levels are high in peripheral islet cells and low in core islet cells. A-C: Prox1 (green) was expressed in ε-cells (ghrelin$^+$, arrows in **A**), δ-cells (somatostain$^+$, arrows in **B**), and PP cells (pancreatic polypeptide$^+$, arrows in **C**) of pancreata of wild-type mice. **D**: Prox1 expression was high in peripheral islet cells (arrows) that include the small ghrelin$^+$ cell population (green) and low in core islet cells (arrowhead) in pancreata of wild-type mice. **D'**, Prox1 expression (red) was high in most islet cells in pancreata of *Pax4*-null mice. (Note that ghrelin$^+$ cells [green] are overabundant in islets of *Pax4* mutant mice [44].) Scale bars: 12.5 μm (**A-C**) or 25 μm (**D, D'**).



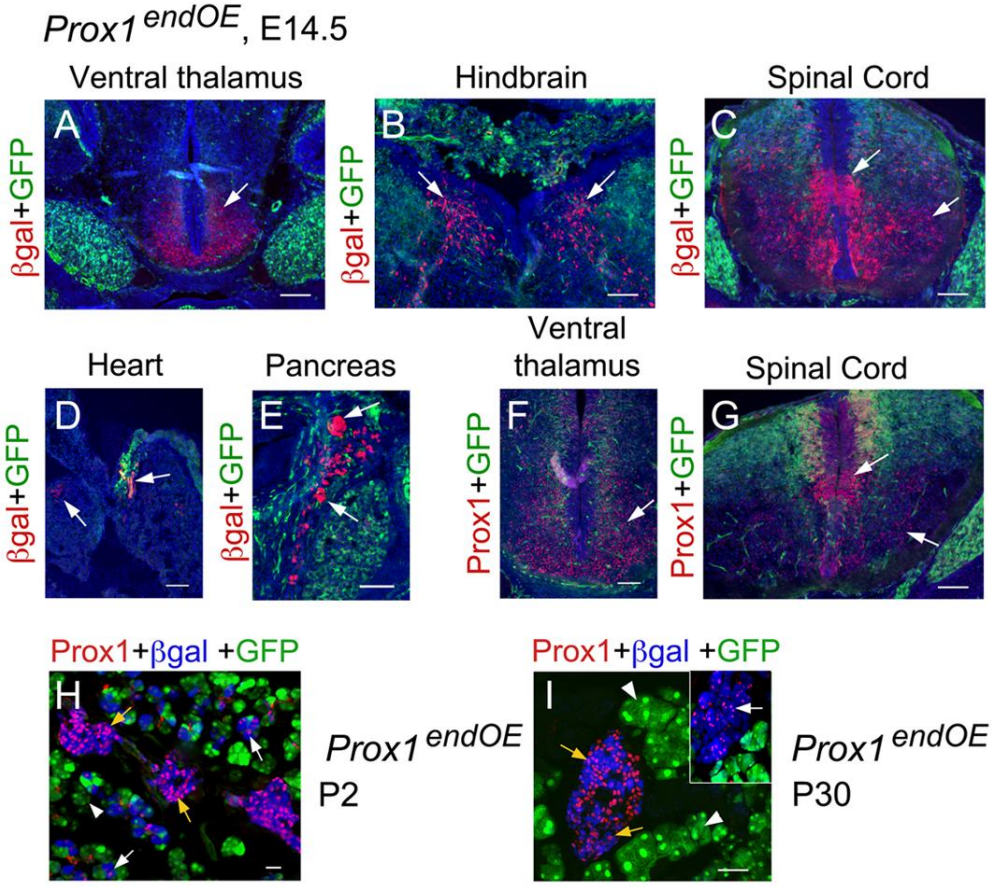
SUPPLEMENTARY DATA

Supplementary Figure 2. β-gal expression is different in neonatal and adult insulin^{+} cells in the pancreas of *Prox1^{betaOE}* mice. A, B: βgal expression (red and arrows) was very limited and GFP expression (green and arrowheads) was very extensive in pancreatic insulin^{+} cells (blue) of *Prox1^{endOE}* mice at P2 (**A**) and P7 (**B**). Notice that GFP and βgal immunoreactivities were mutually exclusive. **C:** βgal (red and arrow) colocalized extensively with insulin (blue) in the pancreas of *Prox1^{endOE}* adult mice. Scale bars: 25 μm.



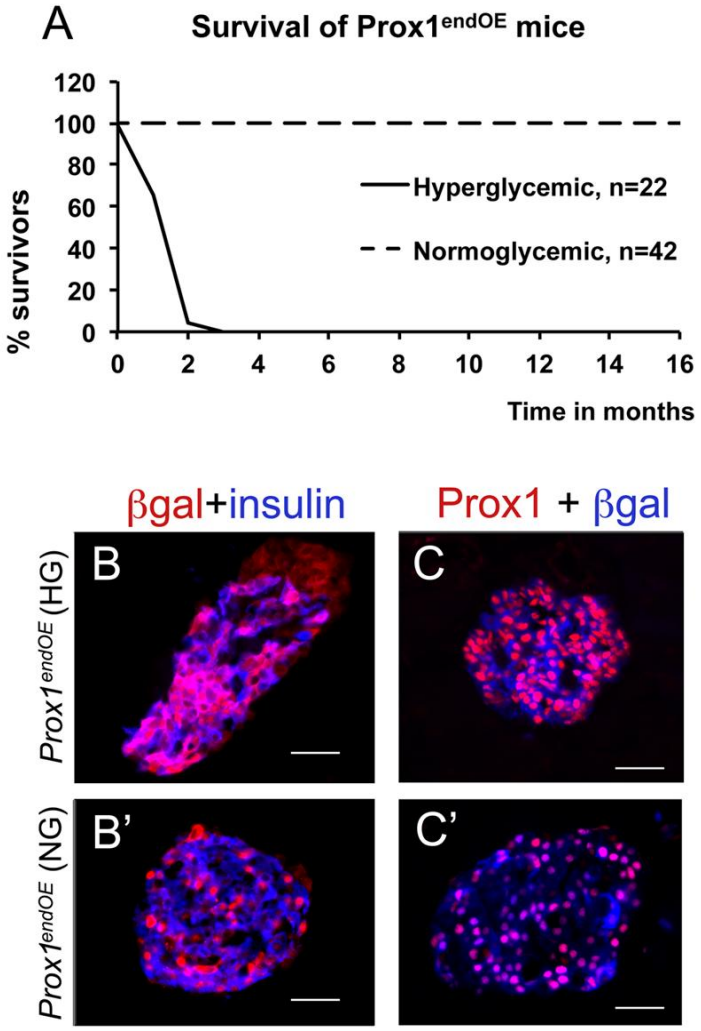
SUPPLEMENTARY DATA

Supplementary Figure 3. β-gal is expressed in pancreatic and non-pancreatic tissues of *Prox1*^{endOE} embryos at E14.5. A-E: E14.5 *Prox1*^{endOE} coronal sections stained for βgal uncovered expression of this protein (red and arrows) in numerous cells in the ventral thalamus (**A**), hindbrain (**B**) and spinal cord (**C**); in a few cell clusters in the developing heart (**D**); and in numerous cells in the pancreas (**E**). **F,G**: Prox1 (red and arrows) had similar expression to βgal (**A,C**) in the ventral thalamus and spinal cord of E14.5 *Prox1*^{endOE} embryos. **H, I**: Prox1$_{HIGH}$ (red and yellow arrows) colocalized extensively with βgal (blue) in pancreata of newborn (**H**) and adult (**I**) *Prox1*^{endOE} mice. **I [inset]:** The expression of βgal in some acini indicated that they originate from Neurog3^{+} precursors, and these cells also expressed ectopic Prox1 (arrow). Scale bars: 25 μm (**H, I**) or 100 μm (**A-G**).



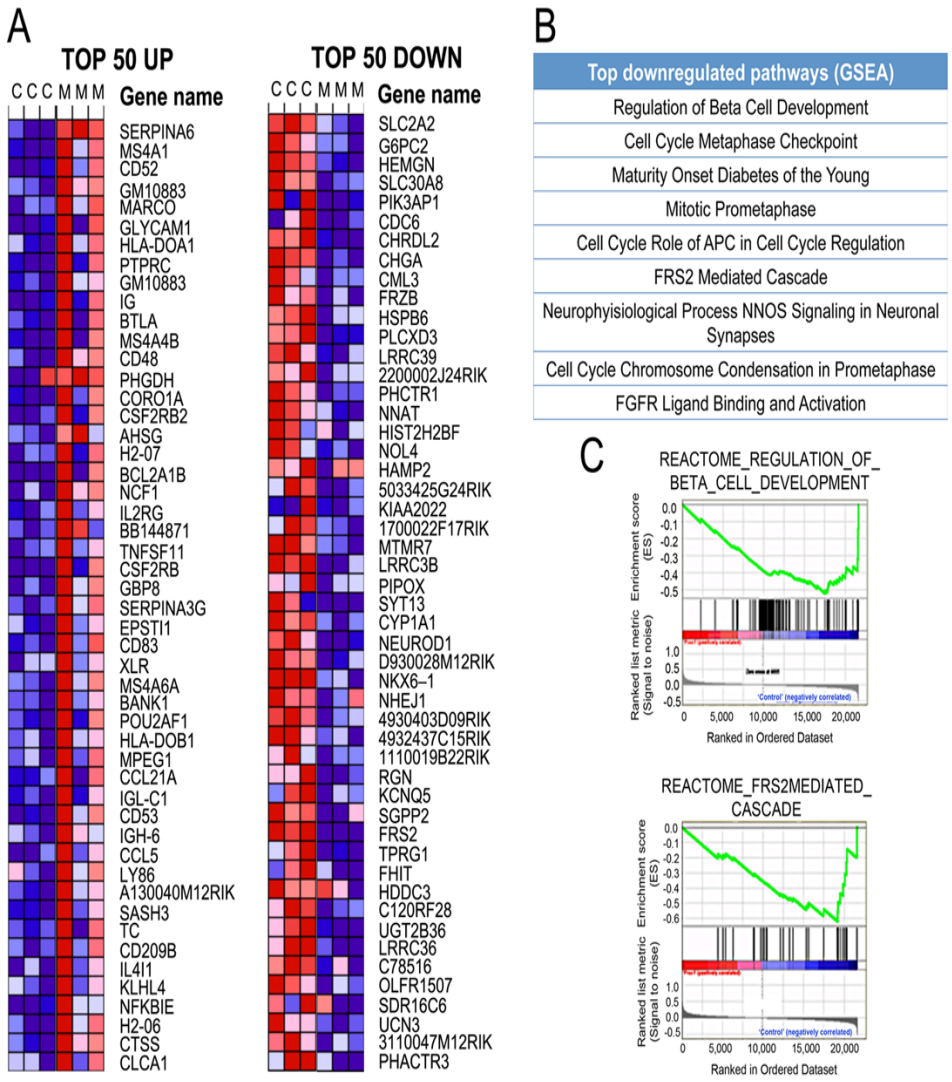
SUPPLEMENTARY DATA

Supplementary Figure 4. Extensive Prox1 and β-gal immunoreactivity is detected in the islets of *Prox1*^{endOE} adult mice. A: Prox1 overexpression decreased survival in *Prox1*^{endOE} (HG) mice but not in *Prox1*^{endOE} (NG) mice. **B, B'**: Insulin (blue) and β-gal (red) colocalized extensively in the islets of *Prox1*^{endOE} (HG) adult mice (**B**) and partially in the islets of *Prox1*^{endOE} (NG) adult mice (**B'**). **C, C':** Cells expressing both βgal^{+} (red) and high Prox1 (red) were abundant in pancreata of *Prox1*^{endOE} *(HG)* mice (**C**) and moderate in pancreata of *Prox1*^{endOE} *(NG)* mice (**C'**). Scale bars: 25 μm.



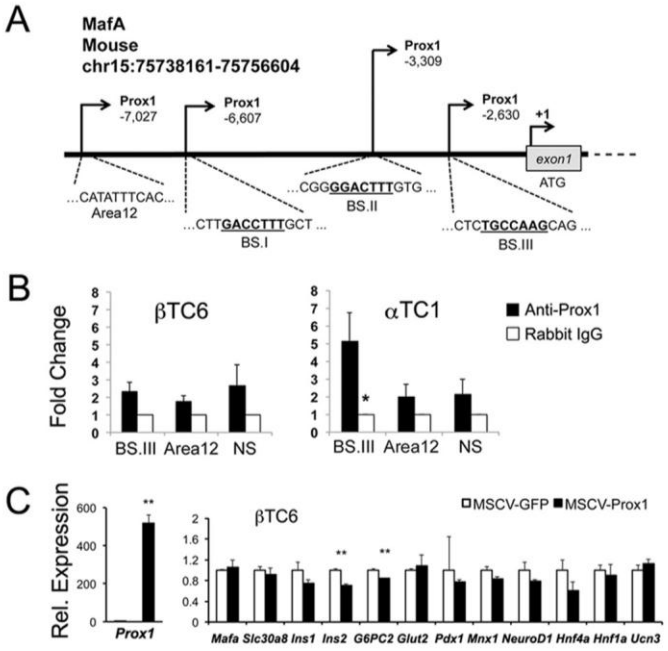
SUPPLEMENTARY DATA

Supplementary Figure 5. Prox1 misexpression in β-cells changes gene expression profiles. A: Heat maps showing the 50 most upregulated transcripts (left) and the 50 most downregulated transcripts (right), in pancreata of *Prox1*^{endOE} mice at P15. ("C" are control [*Neurog3-cre*] triplicates and "M" are *Prox1*^{endOE} triplicates.) **B:** Top downregulated pathways identified by Gene set enrichment analysis (GSEA) in *Prox1*^{endOE} pancreata. **C**: GSEA showed that β-cell development and FGF signaling were amongst the top downregulated pathways in pancreata of *Prox1*^{endOE} mice.



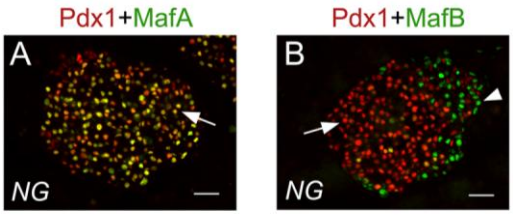
SUPPLEMENTARY DATA

Supplementary Figure 6. Prox1 upregulation in murine β-TC6 cells does not affect *MafA* transcript levels. A: Schematic representation of an approximately 10 kb mouse *MafA* upstream fragment containing putative Prox1-binding sites (+1 refers to the mouse *MafA* Transcription Start Site [TSS] reported by Raum et al [45]). The conserved site 12 in MafA region 3 (*Area 12*) has been reported to bind an ~80 kb activator that is different from Prox1 (46). The TGCCAAG (BS.III) Prox1-binding motif was conserved in *MafA* upstream sequences of rodents. **B:** Chromatin immunoprecipitation results showed significant enrichment of Prox1 to the predicted binding site 3 (*BS.III*) of *MafA* in the chromatin of αTC-1 cells and lack of enrichment to the predicted *MafA* upstream binding sites in the chromatin of β-TC6 cells. Also, there was no significant enrichment of Prox1 in *MafA* area 12 or a distant area (–30 Kb) lacking putative Prox1-binding sites (*NS* or nonspecific). **C**: QPCR analysis of β-TC6 cells that were transduced with MSCV-GFP (control) or MSCV-Prox1 viruses and harvested 48 hours post-transduction to compare the expression of transcripts associated with β-cell function or transcripts encoding β-cell TFs. Data represent the mean (±SEM) of 3 independent experiments. (**P*<0.05, ***P*<0.01.)

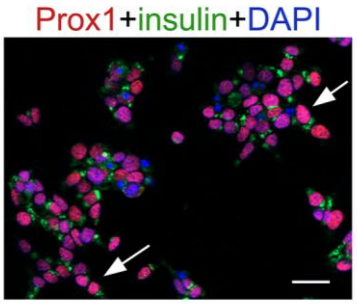


SUPPLEMENTARY DATA

Supplementary Figure 7. Prox1 overexpression in β-cells of *Prox1^{endOE}(NG)* mice does not affect the expression of MafA and MafB. A: MafA (green and arrow) colocalized normally with Pdx1 (red) in core islet cells of *Prox1^{endOE}(NG)* adult mice. **B:** MafB (green and arrowhead) was expressed in peripheral islet cells and excluded from Pdx1^{+} (red and arrow) core islet cells in pancreata of *Prox1^{endOE}(NG)* mice. Scale bars: 25 μm.



Supplementary Figure 8. PROX1 is broadly expressed in human EndoC-βH1 cells. Immunodetection of PROX1 (red) and insulin (green) in cultures of human EndoC-βH1 cells (DAPI [blue] was used to stain the cell nucleus). Scale bar: 25 μm.



SUPPLEMENTARY DATA

Supplementary Table 1. Antibodies used in this study.

Antibody	Species	Source	Dilution	Application
Prox1	Goat	R&D Systems	1:50	IHC Frozen
Prox1	Guinea Pig	Rockland	1:200	IHC Frozen
Prox1	Rabbit	Millipore	1:1,000	IHC Frozen
Prox1	Rabbit	Rockland custom antibody	1μg	ChIP
Insulin	Guinea Pig	Dako	1:250	IHC Frozen
Glucagon	Rabbit	Abcam	1:500	IHC Frozen
Glucagon	Guinea Pig	Linco	1:500	IHC Frozen
Somatostatin	Rabbit	Zymed	1:100	IHC Frozen
Ghrelin	Rabbit	Phoenix Pharmaceuticals	1:300	IHC Frozen
Pancreatic Polypeptide	Rabbit	Zymed	1:50	IHC Frozen
β-galactosidase	Chicken	Abcam	1:500	IHC Frozen
β-galactosidase	Rabbit	ICN	1:5,000	IHC Frozen
MafA	Rabbit	Bethyl Labs	1:500	IHC Frozen
MafB	Rabbit	Bethyl Labs	1:200	IHC Frozen
Glut-2	Rabbit	Alpha Diagnostics	1:200	IHC Frozen
Pdx1	Rabbit	Abcam	1:2,000	IHC Frozen
Pdx1	Goat	Chris Wright lab	1:1,000	IHC Frozen
Ki-67	Rabbit	Neomarkers	1:500	IHC Frozen
Synaptophysin	Rabbit	Zymed	1:1,000	IHC Frozen
E-Cadherin	Rat	Sigma	1:1,000	IHC Frozen

SUPPLEMENTARY DATA

Supplementary Table 2. Mouse Primers used for qPCR and ChIP experiments.

Gene	Forward primer	Reverse primer
Prox1	CGCAGAAGGACTCTCTTTGTC	GATTGGGTGATAGCCCTTCAT
Actb	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
bgal	GCGTGGATGAAGACCAGC	CGAAGCCGCCCTGTAAAC
Ins1	CAGAGAGGAGGTACTTTGGACTATAAA	GCCATGTTGAAACAATGACCT
Ins2	GAAGTGGAGGACCCACAAGTG	CTGAAGGTCCCCGGGGCT
Gcg	CACGCCCTTCAAGACACAG	GTCCTCATGCGCTTCTGC
MafA	CTCCAGAGCCAGGTGGAG	GTACAGGTCCCGCTCCTTG
MafB	TGAAAGCCCAGTGTTCTGC	AGGGCTACCGGATGAGAAAC
Slc30a8	GCTGCTTCAGCAATATGCTTC	CAGACTCCCAGCAACGTGT
Slc2a2/Glut2	GGGCCATCAACATGATCTTC	AATCATCCCGGTTAGGAACA
G6pc2	TGCCCTAAGCTACACCATCA	AAAGGACCAGGTCAGTCTGTG
Pdx1	GAAATCCACCAAAGCTCACG	CGGGTTCCGCTGTGTAAG
Neurod1	CGCAGAAGGCAAGGTGTC	TTTGGTCATGTTTCCACTTCC
Nkx6-1	CTGCACAGTATGGCCGAGATG	CCGGGTTATGTGAGCCCAA
Nkx2-2	GAGTCACCGGACAATGACAAG	TAGGTCTGCGCTTTGGAGAAG
Hnf4a	CTACGGAGCCTCGAGCTGT	CCACACATTGTCGGCTAAAC
Hnf1a	CGCCTCCACCCTGGTTAT	ACTCCCCATGCTGTTGATG
Rbp4	AAGGGACGAGTCCGTCTTCT	TGAAAGTGCCCACCATGTC
UCN3	CCAGAGCAAAGTCCACTTACAG	GCTTGTCCTTGGACCTCCT
Mnx1	GAACACCAGTTCAAGCTCAACA	GCTGCGTTTCCATTTCATTCG
UCN3	CCAGAGCAAAGTCCACTTACAG	GCTTGTCCTTGGACCTCCT
Rbp4	AAGGGACGAGTCCGTCTTCT	TGAAAGTGCCCACCATGTC
Dnmt3a	ATTCCTTCTCACAACCCGC	TACTTCCAGAGCTTCAGGGC
CyclinD1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
FRS2	AGCTGTCCAGATAAAGACACTGT	ATTTTACCGAGTCCCGTTTCC
Fgf2	GCGACCCACACGTCAAACTA	CCGTCCATCTTCCTTCATAGC
Fgf4	TGGGCCTCAAAAGGCTTCG	CGTCGGTAAAGAAAGGCACAC
Fgf7	TGGGCACTATATCTCTAGCTTGC	GGGTGCGACAGAACAGTCT
MafA-BSIII	ACTCTGCCAAGCAGTCCCTA	AGGGTGATCCCTGAAAGCAG
MafA-Area12	TTGCGACCATACGGCTATCA	TGCTCAGTGGGGCTGTTAGA
MafA-NS	TATCTGTGGCCACCCTGAGA	CAACAAACAAGGAGCCTCGC

SUPPLEMENTARY DATA

Human Primers used for qPCR in EndoC-βH1 cells

Gene	Forward primer	Reverse primer
Prox1	CTTCACTATCCAGCTTGCAG	CTACATTCAGATGGAGAAGTACG
MafA	TGAGCGGAGAACGGTGATTTCTAAGG	GGAACGGAGAACCACGTTCAACGTA
Slc30a8	GATCCAGGCGACTGTGATGAT	TGGCTTGTACTTCCTTGTGATTG
G6pc2	GCAGGGCTTTATGGGCTATT	AGTTCATTTCCTCCAAGGTCAG
NeuroD	ATTGCACCAGCCCTTCCTTTGATC	TCGCTGCAGGATAGTGCATGGTAA
Glut1	GGACAGGCTCAAAGAGGTTAT	AGGAGGTGGGTGGAGTTAAT
Glut2	CTAGTTGGGAGTCCTGTCAATTC	CTAGGCAGAGCTGCGAATAAA
MafB	ACCTTGGCTAAGGCGAGAGTAG	CTTCAGCCTGGAGAGAAGTTACTC
Ins	AGAGGCCATCAAGCAGATCACTGT	AGGTGTTGGTTCACAAAGGCTG
Hnf4a	CCCATCAGAAGGCACCAACC	AGCGGCACTGGTTCCTCTTG
Hnf1a	GAGCAAGAGGCACTGATCC	CTCCAGCTCTTTGAGGATGG
Nkx6-1	ATTCGTTGGGGATGACAGAG	CGAGTCCTGCTTCTTCTTGG
Mnx1	AGAAGGCGGAAACCCACAGTGTT	CAGCAGTTTGAACGCTCGTGACA
HK1	GCTCTCCGATGAAACTCTCATAG	GGACCTTACGAATGTTGGCAA
Aldo B	CAAGGCTGCAAACAAGGAG	CCCGTGTGAACATACTGTCCT
GP1	CCAATGGCCAGCATGCTTTT	CCTCTGTCTGGGCCAAGAAG
TPI1	ACTGCCTATATCGACTTCGCC	AAGCCCCATTAGTCACTTTGTAG
Dnmt3a	ATTCCTTCTCACAACCCGC	TACTTCCAGAGCTTCAGGGC
LDHa	GGAGATCCATCATCTCTCCC	GGCCTGTGCCATCAGTATCT
UCN3	AGATACGTGTCCCAAGCACA	TTCTTCCTCCCAATTTGCGC
GCK	CCTTCTTCAGGTCCTCCTCC	GATGGATCTCACAAGGAGCC
FGF7	AAAGGCTCACACACACACAC	TCCATGTCTGTTGTCTGCCT
FRS2	TCCAGGATTTGCTGCTCAGA	TTTCCGCTCTTCTTGCACAC
FGF4	GTTTCCCCTATGTGCAAGTCC	GCGCTGCTGCGGTCCATGT
CCND1	GCACAGCTGTAGTGGGGTTCTAGGC	CAGGCGCAAAGGACATGCACACGGC
CCND3	GCAGCGCCTTTCCCAACT	TCAAAAGGAATGCTGGTGTATGTATC