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Supplemental Information

Impaired Islet Function in Commonly Used Transgenic Mouse Lines Due to Human Growth Hormone Minigene Expression

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Figure S1

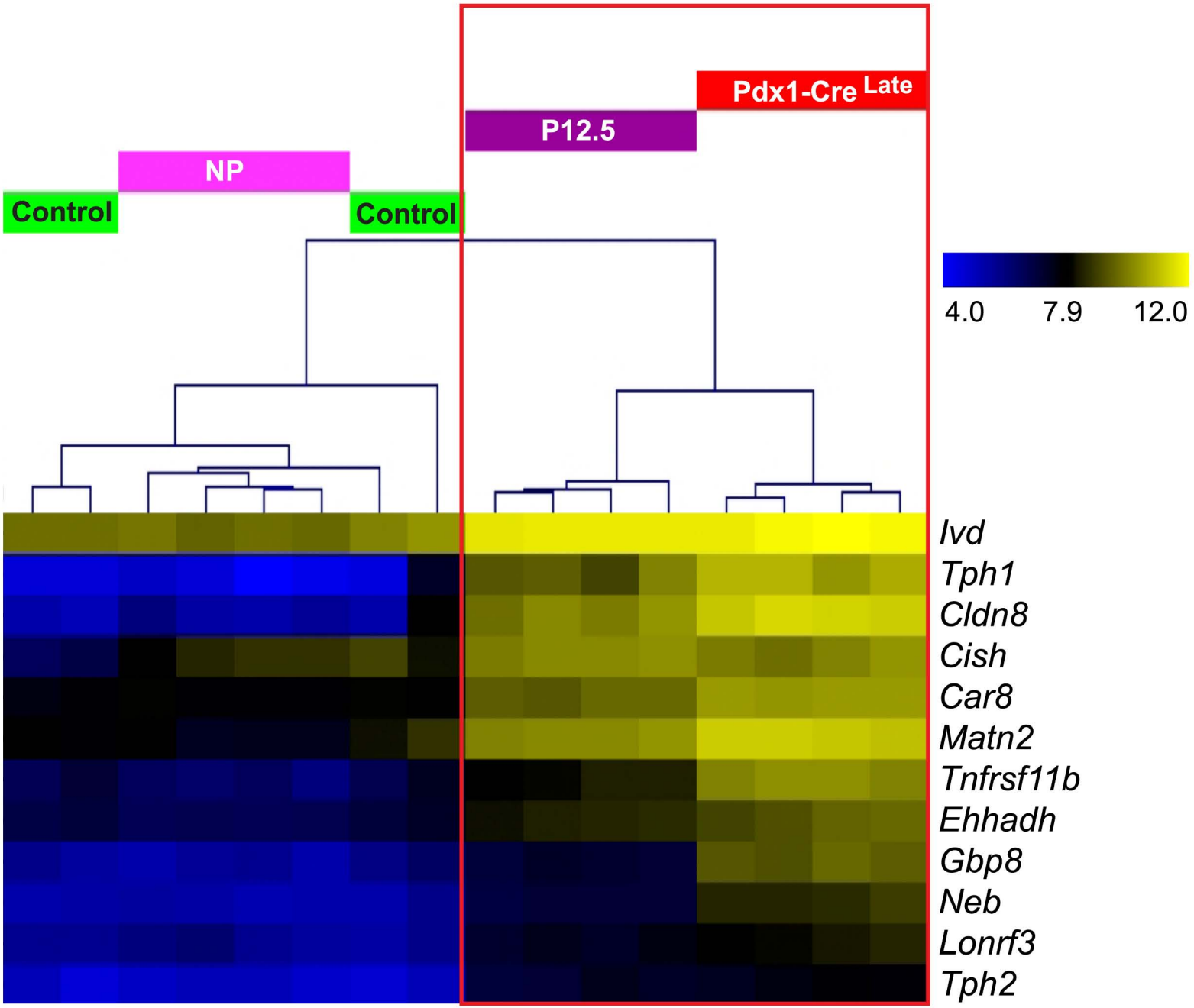
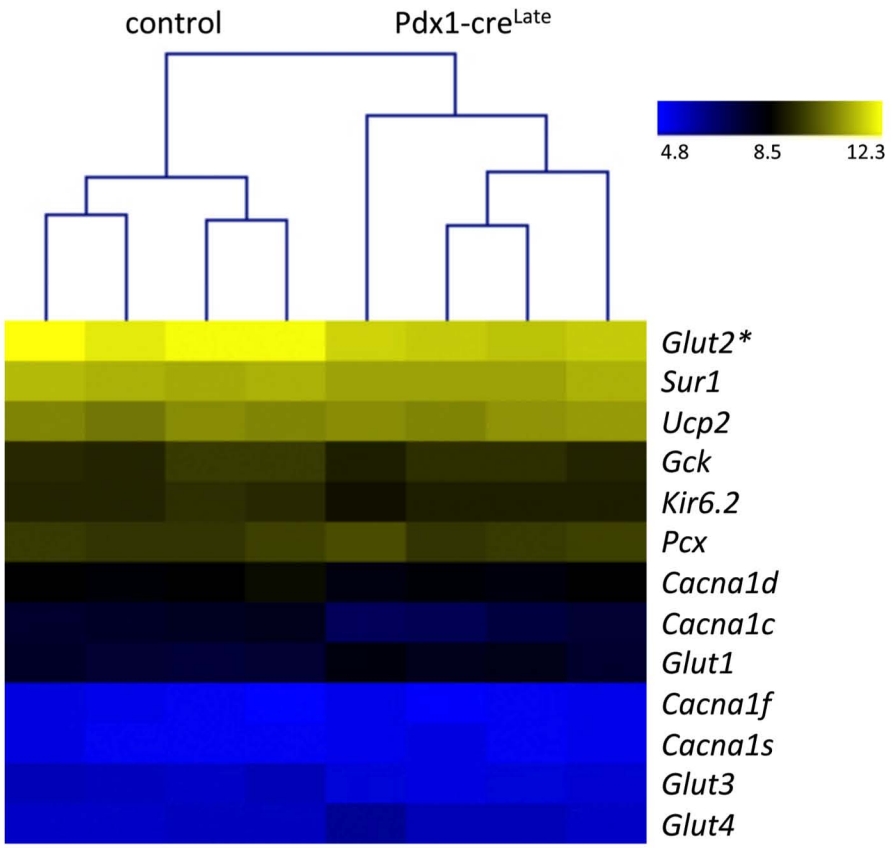

Pdx1-Cre Late
P12.5
NP
Control
Control
4.0
7.9
12.0
Ivd
Tph1
Cldn8
Cish
Car8
Matn2
Tnfrsf11b
Ehhadh
Gbp8
Neb
Lonrf3
Tph2

Figure S2

A


control
Pdx1-cre^Late
4.8
8.5
12.3
Glut2*
Sur1
Ucp2
Gck
Kir6.2
Pcx
Cacna1d
Cacna1c
Glut1
Cacna1f
Cacna1s
Glut3
Glut4

B

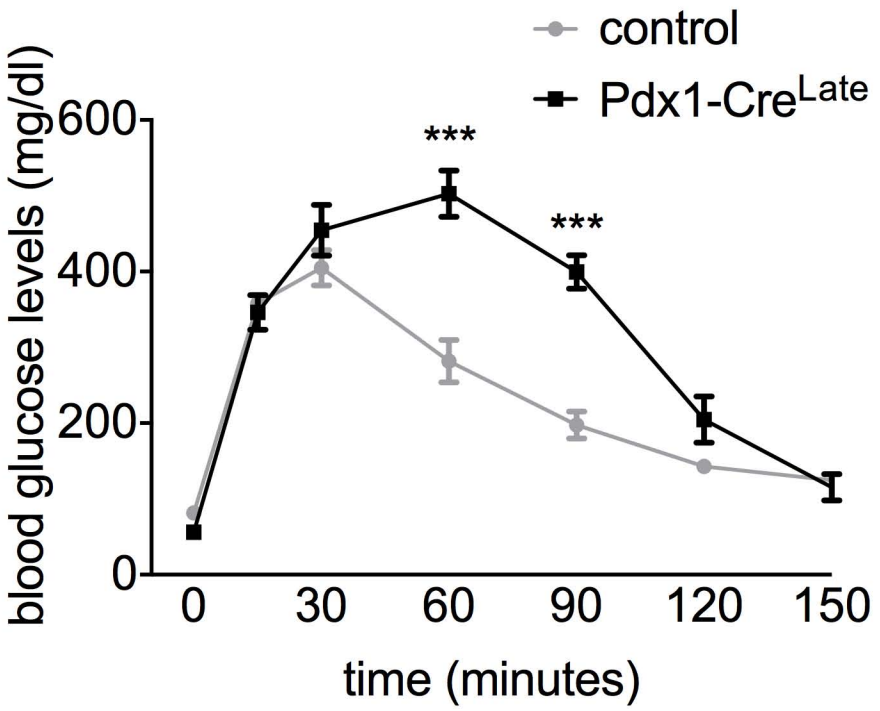

control
Pdx1-Cre^Late
blood glucose levels (mg/dl)
600
400
200
0
***
***
0
30
60
90
120
150
time (minutes)

Figure S3

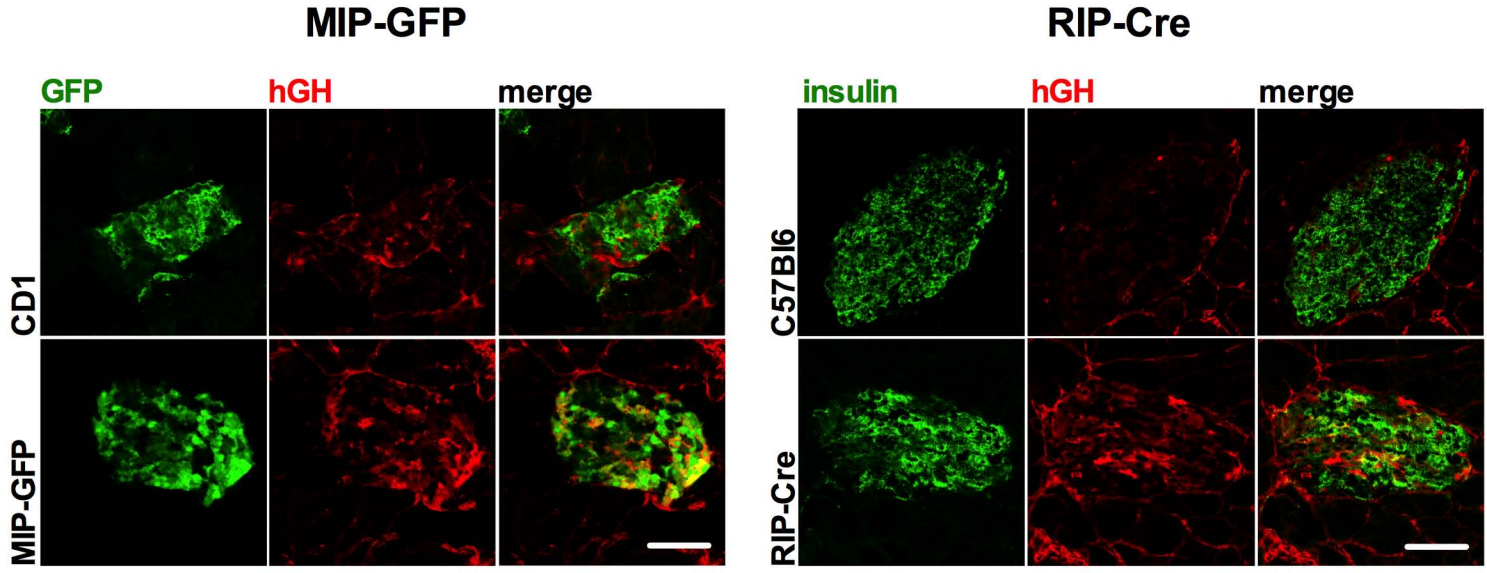

MIP-GFP
RIP-Cre
GFP
hGH
merge
insulin
hGH
merge
CD1
MIP-GFP
C57Bl6
RIP-Cre

Figure S4

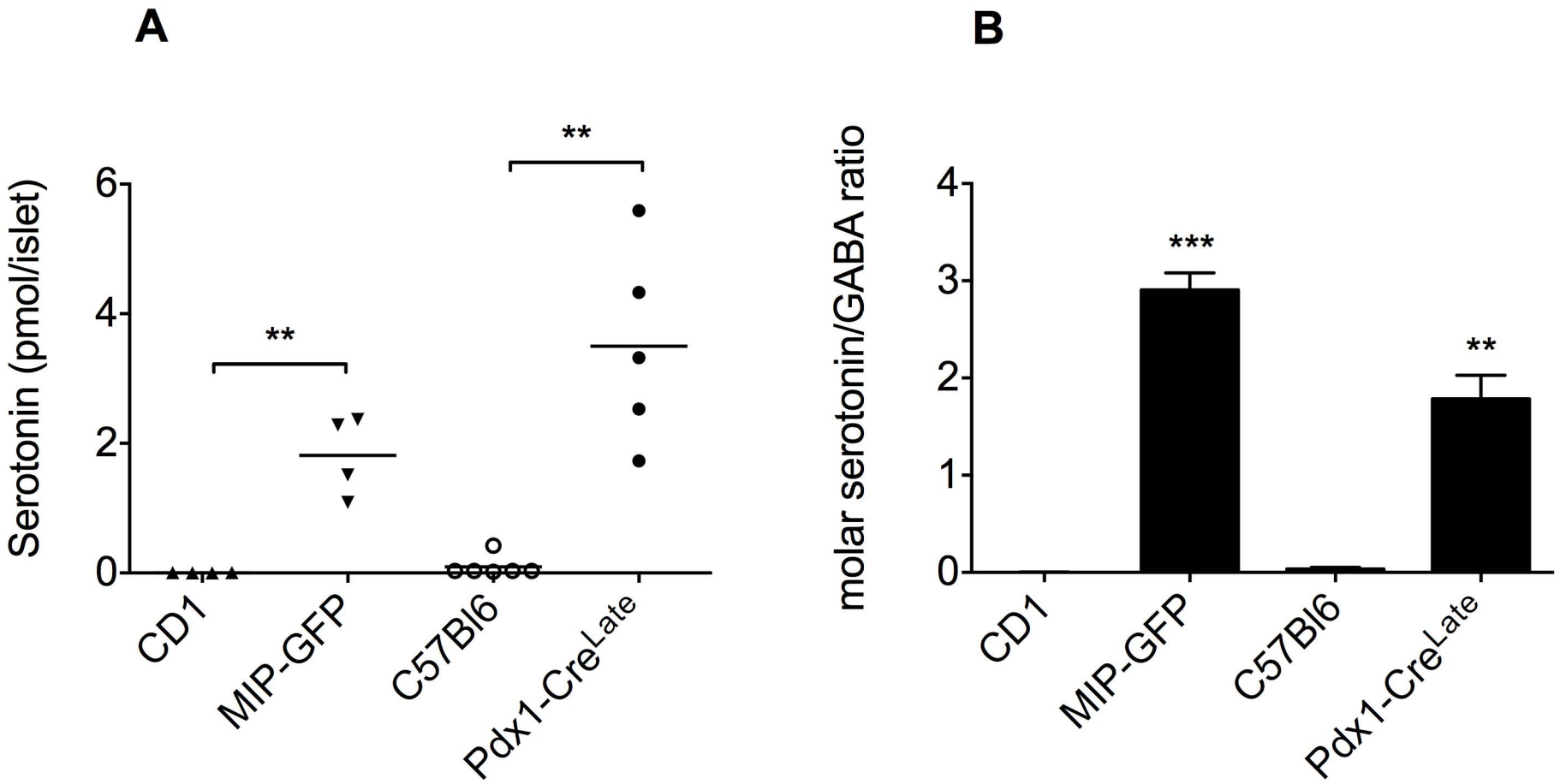
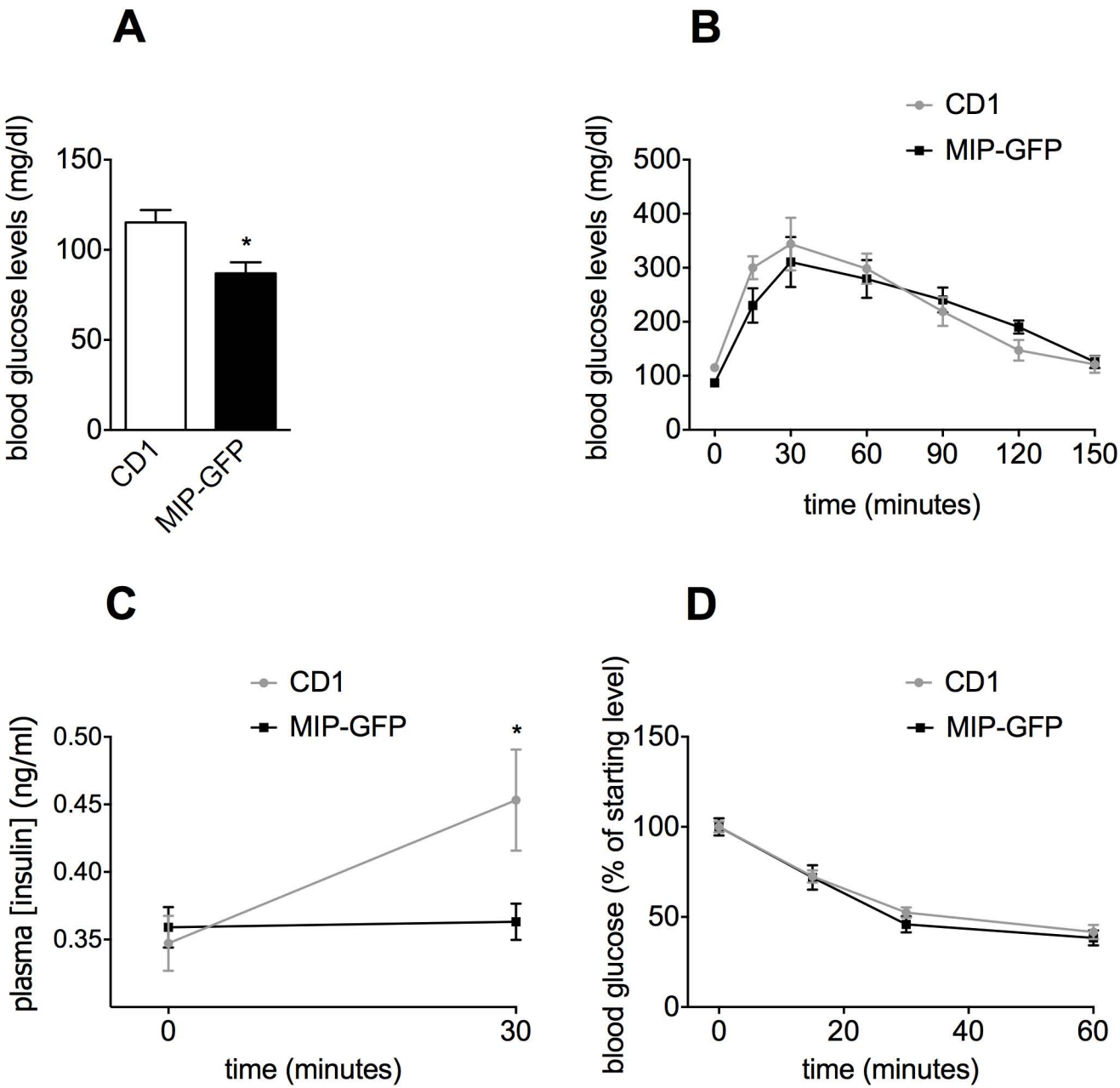

A
Serotonin (pmol/islet)
6
4
2
0
**
**
CD1
MIP-GFP
C57Bl6
Pdx1-CreLate
B
molar serotonin/GABA ratio
4
3
2
1
0
***
**
CD1
MIP-GFP
C57Bl6
Pdx1-CreLate

Figure S5


A
blood glucose levels (mg/dl)
150
100
50
0
*
CD1
MIP-GFP
B
CD1
MIP-GFP
blood glucose levels (mg/dl)
500
400
300
200
100
0
0
30
60
90
120
150
time (minutes)
C
CD1
MIP-GFP
plasma [insulin] (ng/ml)
0.50
0.45
0.40
0.35
*
0
30
time (minutes)
D
CD1
MIP-GFP
blood glucose (% of starting level)
150
100
50
0
0
20
40
60
time (minutes)

Supplemental Figure Legends

Figure S1, related to Figure 3: Pregnancy gene expression signature of control and *Pdx1-cre*^{Late} islets. Hierarchical clustering of islets samples from control, *Pdx1-cre*^{Late}, non-pregnant C56BL/6J (NP) and pregnant (day 12.5, P12.5) C57BL/6J mice together with a heat map of the log2 values of the 12 genes of the pregnancy gene expression signature obtained by microarray. A color scale for the log2 values is shown at the right. See Extended Experimental Procedures.

Figure S2, related to Figure 4: Reduced islet *Glut2* expression and Impaired glucose tolerance in *Pdx1-cre*^{Late} mice. Hierarchical clustering of control and *Pdx1-cre*^{Late} islets together with a heat map of the log2 values obtained by microarray for genes involved in GSIS. A color scale for the log2 values is shown at the right. See Extended Experimental Procedures. (B) i.p. glucose tolerance test (IPGTT) on 20-week-old *Pdx1-Cre*^{Late} versus control mice. Overnight fasted mice were injected i.p. with 2.5 mg/g BW D-glucose and blood glucose levels were measured at indicated time points. Data are represented as mean ± SEM, n=5-7 mice per genotype, ***P<0.001, repeated measures ANOVA.

Figure S3, related to Figure 5: Representative immunofluorescence micrographs show hGH immunoreactivity in islets from MIP-GFP and RIP-Cre mice, whereas no signal was observed in respective littermate controls. Scale bar = 50μm.

Figure S4, related to Figure 5: HPLC quantification of serotonin expressed in pmol/islet (Figure S4A) and molar serotonin/GABA ratios (Figure S4B) in islets from MIP-GFP, Pdx1-Cre^{Late} and their respective controls. Data are represented as mean ± SEM, n=4-6 mice per genotype, **P<0.01, ***P<0.001.

Figure S5, related to Figure 5: Glucose homeostasis in MIP-GFP versus CD1 controls. (A) Blood glucose levels from nine-week-old animals after an overnight fast. (B) MIP-

GFP and control animals were subjected to an IPGTT by injecting with 2.5 mg/g BW D-glucose and blood glucose was measured at indicated time points. (B) During this IPGTT, plasma was taken at 0 and 30 minutes after glucose injection and insulin was quantified by ELISA. (C) Insulin tolerance test (ITT) on the same animal group. Data are represented as means ± SEM, n=5 per genotype, *P<0.05.

Table S1, related to Figure 3: Up- and down regulated genes in islets isolated from Pdx1-Cre^{Late} transgenic mice versus control mice, determined by microarray expression analysis.

Supplemental Experimental Procedures

Primers and probes for quantitative RT-PCR

GENE_ID	Primer/probe	Sequence
Car8	Forward primer	5′-AGGATATTCAATATAAGGGAA-3′
	Probe	5′-(6-FAM)TAATCCTAACACTTTATTACCAGACCCTCT(TAMRA)-3′
	Reverse primer	5′-CTTCATAGACCCAGTAAT-3′
Cish	Forward primer	5′- AAGGTGCTAGACCCTGA -3′
	Probe	5′-(6-FAM)ATAGCCAAGACGTTCTCCTACCTTCGGGAAT(TAMRA)-3′
	Reverse primer	5′- CTCGCTGGCTGTAATAGAA-3′
Cldn8	Forward primer	5′-TGGTGGATGTGGCCCTAAA-3′
	Probe	5′-(6-FAM)GAGGGCTTCTCCCAGCTCGCG(TAMRA)-3′
	Reverse primer	5′-CGCTGTGGTCCAGCCTATGT-3′
Ehhadh	Forward primer	5′-AAGCTAGTTTGGACCATACG-3′
	Probe	5′-(6-FAM)AGCAAATGACAACTTCTGTGCAGGTGCTGA(TAMRA)-3′
	Reverse primer	5′-CTTCTGGTATCGCTGTATTTC-3′
Gapdh	Forward primer	5’- CCCCAATGTGTCCGTCGTG-3′
	Reverse primer	5’- GCCTGCTTCACCACCTTCT-3′
Gbp8	Forward primer	5′-AAGAACGACTTGTGGAT-3′
	Probe	5′-(6-FAM)CATGATTCCCTGGAGAAACTACATTATGTC(TAMRA)-3′

	Reverse primer	5′-GGATTTGGTGAAGACTTT -3′
Glut2	Forward primer	5’ - ATCCCTTGGTTCATGGTTGCTG-3′
	Reverse primer	5’ - TCCGCAATGTACTGGAAGCAG-3′
Ivd	Forward primer	5′-ATGTGTTGGTAATGGAAGAGA-3′
	Probe	5′-(6-FAM)ACTCCAACCTCTGCGTCAACCAGATTGTTC(TAMRA)-3′
	Reverse primer	5′-CGATGAACTCACCACTGAT-3′
hGH	Forward primer	5’ - CCAGGAGTTTGAAGAAGCCT-3′
	Reverse primer	5’ - GGAGGTCATAGACGTTGCTGT-3′
Hprt	Forward primer	5’- TACGAGGAGTCCTGTTGATGTTGC-3’
	Reverse primer	5’- GGGACGCAGCAACTGACATTTCTA-3’
Lonrf3	Forward primer	5′- AATGCCAGAGAAGGACGAAGA-3′
	Probe	5′-(6-FAM)ACACTGGCGGTTCTTCCTCTGGAAAGCAGA(TAMRA)-3′
	Reverse primer	5′- AGGATACGCCGAATACCATTCAAT-3′
Matn2	Forward primer	5′-CAACACACCTGGCTCGTA-3′
	Probe	5′-(6-FAM)AGCACGGATCAGAAGACTTGCAGAATCCA(TAMRA)-3′
	Reverse primer	5′-AGACAAAGGAACCCAGCA-3′
Neb	Forward primer	5′-AAAGGGACAGCCATAC-3′
	Probe	5′-(6-FAM)ATACTCCAGAACTTCGCAGAATCAAGAAAG(TAMRA)-3′
	Reverse primer	5′- ATCCATTCGATACTTAACCT -3′
Polr2a	Forward primer	5′-GCACCACGTCCAATGATATTGTG-3′
	Probe	5′-(6-FAM)CTTCCGCACAGCCTCAATGCCCAGT(TAMRA)-3′
	Reverse primer	5′-GGAGATGACATGGTACAGTTCTCG-3′
Tnfrsf11b	Forward primer	5′- CATCCAAGACATTGACCTCTGTG-3′
	Probe	5′-(6-FAM)AGCAGCTTCGTGCCTTGATGGAGAGCCTG(TAMRA)-3′
	Reverse primer	5′- CTTCTGGGCTGATCTTCTTCC-3′
Tph1	Forward primer	5′-TTCCAGGAGAATCATGTGAGC-3′
	Probe	5′-(6-FAM)TCAACTGTTCTCGGCTGATGTCGCAGTCA(TAMRA)-3′
	Reverse primer	5′-CATAACGTCTTCCTTCGCAGT-3′

RNA extraction

Total RNA from islets was isolated using an Absolutely RNA microprep kit (Stratagene), RNA from other tissues was isolated using RNA L kit (Macherey-Nagel) and RNA from

MIN6 cells with the PureLink RNA Mini kit (Invitrogen). All extractions were performed according to manufacturer's protocol. RNA quantity and quality were determined using a spectrophotometer (ND-1000; NanoDrop Technologies) and a bioanalyser (2100; Agilent), respectively.

HPLC

A 1 mmol/l stock standard solution of serotonin hydrochloride (Sigma-Aldrich, Steinheim, Germany) was prepared in a solution of 10 mmol/l hydrochloric acid (Merck, Darmstadt, Germany), 5.26 mmol/l sodium metabisulfite (Sigma-Aldrich) and 0.27 mmol/l EDTA (Sigma-Aldrich). Further dilutions were made in a mixture of four parts of a modified Ringer's solution (147 mmol/l sodium chloride (Sigma-Aldrich), 4 mmol/l potassium chloride (Merck) and 2.3 mmol/l calcium chloride (Sigma-Aldrich)) and one part of an antioxidant solution (100 mmol/l acetic acid (Fisher Scientific, Loughborough, United Kingdom), 3.3 mmol/l L-cysteine (Sigma-Aldrich), 0.27 mmol/l EDTA and 12.5 µmol/l ascorbic acid (Sigma-Aldrich) (Thorré et al., 1997). A 2.5 mmol/l stock standard solution of GABA (Sigma-Aldrich) was prepared in 100 mmol/l hydrochloric acid and further dilutions in purified water.

The method for serotonin was based on the microbore LC-ECD method described in (Sarre et al., 1997), using a chromatographic system with a FAMOS miocroautosampler of LC Packings/Dionex (Amsterdam, The Netherlands), a Gilson 307 piston pump (Villiers-le-Bel, France), a Dionex DEGASYS DG-1210 degasser and a DECADE II electrochemical detector equipped with a µ-VT03 flow cell of Antec (Zoeterwoude, The Netherlands). The mobile phase was a mixture of 87% (vol./vol.) aqueous buffer solution at pH 5.5 (100 mmol/l sodium acetate (Sigma-Aldrich), 20 mmol/l citric acid (Sigma-Aldrich), 2 mmol/l sodium decanesulfonate (Sigma-Aldrich), 0.5 mmol/l EDTA) and 13%

(vol./vol.) acetonitrile HPLC grade (Fisher Scientific). The flow rate of the mobile phase was 60 µl/min, the temperature of the autosampler tray 15°C and the injection volume 10 µl. The stationary phase was a microbore UniJet C8 column (100 x 1.0 mm, 5 µm) from Bioanalytical Systems (West Lafayette, Indiana, United States). The separation and detection temperature were set at 35°C and the detection potential was + 450 mV vs. Ag/AgCl.

The chromatographic determination of GABA in islets was based on the microbore LC-ECD method described in (Smolders et al., 1995). The chromatographic system consisted of a 307 piston pump, a 231 XL sampling injector, a 402 syringe pump, all from Gilson (Villiers-le-Bel, France), and an LC-4C electrochemical detector equipped with an amperometric flow cell, supplied by Bioanalytical Systems (West Lafayette, Indiana, United States). The mobile phase was a mixture of 56% (vol./vol.) aqueous buffer solution at pH 5.0 (100 mmol/l sodium acetate, 0.1 mmol/l EDTA) and 44% (vol./vol.) acetonitrile, pumped with a flow rate of 100 ml/min. A two-step derivatization procedure was carried out at 4°C. In the first step, 3 µl working reagent A (mixture of 500 µl 15 mmol/l o-phtaldialdehyde (Sigma-Aldrich) and 1.2 µl tert-butylthiol (Janssen Chimica, Geel, Belgium)) was added to 15 µl diluted sample or standard solution. 3 µl working reagent B (92.5 mg iodoacetamide (Sigma-Aldrich) in 500 µL methanol (Fisher Scientific)) was added in a second step and subsequently 10 µl was injected on a microbore UniJet C8 column (100 x 1.0 mm, 5 µm) from Bioanalytical Systems (West Lafayette, Indiana, United States). Amperometric detection was carried out at room temperature, applying a potential of 700 mV vs. Ag/AgCl.

Data acquisition was performed by Clarity chromatography software version 3.0.2 from Data Apex (Prague, The Czech Republic).

Hierarchical clustering with heat map

MultiExperiment Viewer (MEV) which is part of the TM4 Microarray Software Suite was used to perform hierarchical clustering and to generate heat maps with the Log2 values for each gene (Saeed et al., 2006). The parameters used for the hierarchical clustering were the Euclidean distance and the average linkage method.

Supplemental References

Saeed, A.I., Bhagabati, N.K., Braisted, J.C., Liang, W., Sharov, V., Howe, E.A., Li, J., Thiagarajan, M., White, J.A., and Quackenbush, J. (2006). TM4 microarray software suite. Methods in enzymology *411*, 134-193.

Sarre, S., Thorré, K., Smolders, I., and Michotte, Y. (1997). Microbore liquid chromatography analysis of monoamine transmitters. Methods in molecular biology (Clifton, NJ) *72*, 185-196.

Smolders, I., Sarre, S., Michotte, Y., and Ebinger, G. (1995). The analysis of excitatory, inhibitory and other amino acids in rat brain microdialysates using microbore liquid chromatography. Journal of neuroscience methods *57*, 47-53.

Thorré, K., Pravda, M., Sarre, S., Ebinger, G., and Michotte, Y. (1997). New antioxidant mixture for long term stability of serotonin, dopamine and their metabolites in automated microbore liquid chromatography with dual electrochemical detection. Journal of chromatography B, Biomedical sciences and applications *694*, 297-303.