Impaired islet function in commonly used transgenic mouse lines due to human growth hormone minigene expression

Bas Brouwers [1], Geoffroy de Faudeur [2], Anna B. Osipovich [3], Lotte Goyvaerts [2], Katleen Lemaire [2], Leen Boesmans [2], Elisa J.G. Cauwelier [1], Mikaela Granvik [2], Vincent P.E.G. Pruniau [1], Leentje Van Lommel [2], Jolien Van Schoors [4], Jennifer S. Stancill [3], Ilse Smolders [4], Vincent Goffin [5], Nadine Binart [6], Peter in't Veld [7], Jeroen Declercq [1], Mark A. Magnuson [3], John W.M. Creemers [1,*], Frans Schuit [2,*] and Anica Schraenen [2]

1 Laboratory for Biochemical Neuroendocrinology, Department of Human Genetics, KU Leuven, 3000, Belgium

2 Gene Expression Unit, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, 3000, Belgium

3 Center for Stem Cell Biology and Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

4 Center for Neurosciences, Department of Pharmaceutical Chemistry and Drug Analysis, Vrije Universiteit Brussel, Brussels, 1090, Belgium

5 INSERM U845, Research Center Growth and Signaling, PRL/GH Pathophysiology Laboratory, University Paris Descartes, Sorbonne Paris Cité, Faculty of Medicine, Paris, 75993, France

6 INSERM U693, Faculté de Médecine Paris-Sud, University Paris-Sud, Le Kremlin-Bicêtre, 94276, France

7 Diabetes Research Center, Vrije Universiteit Brussel, Brussels, 1090, Belgium

*Correspondence: Frans.Schuit@med.kuleuven.be (F.S.), John.Creemers@med.kuleuven.be (J.M.W.C)

Summary

The human growth hormone (*hGH*) minigene is frequently used in the derivation of transgenic mouse lines to enhance transgene expression. Although this minigene is present in the transgenes as a second cistron, and thus not thought to be expressed, we found that three commonly used lines; *Pdx1-Cre*^{Late}, *RIP-Cre and MIP-GFP*, each expressed significant amounts of hGH in pancreatic islets. Locally secreted hGH binds to prolactin receptors on β cells, activates STAT5 signaling, and induces pregnancy-like changes in gene expression thereby augmenting pancreatic β cell mass and insulin content. In addition, islets of *Pdx1-Cre*^{Late} mice have lower GLUT2 expression, reduced glucose-induced insulin release and are protected against the β cell toxin streptozotocin. These findings may be important when interpreting results obtained when these and other *hGH* minigene-containing transgenic mice are used.

Highlights

- hGH drives *Tph1* expression in islets from *Pdx1-Cre*^{Late}, *RIP-Cre and MIP-GFP* mice
- Locally secreted hGH activates prolactin receptors on mouse β cells
- Islets from *Pdx1-Cre*^{Late} mice have a pregnancy-like phenotype
- *hGH* minigene is used in > 20 other islet-specific Cre-driver/transgenic models

Introduction

Conditional inactivation of genes in mice by specific DNA recombination in target tissues was developed in the early nineties (Orban et al., 1992). By then, it was already known that intronic sequences and a polyadenylation signal are essential to achieve efficient expression of the transgene (Brinster et al., 1988). The entire human growth hormone (*hGH*) coding region, including introns and polyadenylation signal - also called *hGH* minigene - was oftentimes inserted downstream of coding regions, such as that of *Cre recombinase*, to generate transgenic mouse models (Orban et al., 1992), some of which are in wide use today.

Prolactin (PRL), placental lactogen (PL) and GH are homologous proteins that display overlapping structure (Goffin et al., 1996) and biological activities (Soares, 2004). The lactogens PRL and PL have a profound effect on the pancreatic islet phenotype of pregnant females (Parsons et al., 1992; Sorenson et al., 1987). During pregnancy, insulin resistance is induced in the mother, which facilitates nutrient flow towards the fetus (Freemark, 2006). To compensate for this increased metabolic demand and to prevent hyperglycemia, maternal pancreatic β cells undergo several structural and functional changes. These changes have been under investigation for several decades and involve multiple β cell parameters such as increased glucose-stimulated insulin secretion (GSIS) (Green and Taylor, 1972), enhanced β cell proliferation (Van Assche, 1974), accelerated proinsulin biosynthesis (Bone and Taylor, 1976), higher rate of glucose oxidation and glucose utilization (Green et al., 1978; Weinhaus et al., 1996), and increased gap-junctional coupling of β cells (Sheridan et al., 1988). The importance of lactogens in this phenotypic switch has been demonstrated in mouse models in which β cell-specific overexpression of PL leads to enhanced insulin secretion and increased β cell mass (Fleenor et al., 2000; Vasavada et al., 2000). These signals are mediated by prolactin receptors (PRLR), as was demonstrated in *Prlr*$^{+/-}$ mice (Huang et al., 2009). Growth hormones of primates, but not of other vertebrates, are also able to activate

PRLR, a property that is maintained in several heterologous systems (Goffin et al., 1996). Accordingly, it was shown that hGH can mimic the effects of lactogens on mouse and rat β cells (Parsons et al., 1995).

To define the molecular basis of lactogen signaling during pregnancy, we and others recently explored the changes that occur in the gene expression using genome-wide mRNA expression analysis (Kim et al., 2010; Rieck et al., 2009; Schraenen et al., 2010a; Schraenen et al., 2010b). The largest cluster of upregulated genes during pregnancy regulates β cell mass (Schraenen et al., 2010a). In addition, a strong induction of the serotonin biosynthetic pathway was found in a subset of β cells (Schraenen et al., 2010b). Genes encoding tryptophan hydroxylases-1 (TPH1) and -2 (TPH2), catalyzing the rate-limiting step of serotonin biosynthesis, were found to be vastly upregulated (Kim et al., 2010; Schraenen et al., 2010b), resulting in a more than 100-fold increase in islet serotonin content. The responsible mechanism involved PRLR and activation of its canonical Janus kinase 2 (JAK2)/signal transducer and activator of transcription 5 (STAT5) signaling pathway (Schraenen et al., 2010b). An autocrine/paracrine role for serotonin has been suggested in activating β cell proliferation via serotonin receptor 2B (Kim et al., 2010).

Here we describe unexpected functional consequences resulting from the placement of *hGH* minigene in the transgenic constructs. The principal mouse strain used for these studies was the *Tg(Pdx1-cre)1*^{Herr} mouse strain, also known as the *Pdx1-Cre*^{Late} model (Herrera, 2000). This transgenic line contains a 4.5 kb fragment of the *Pdx1* promoter, inserted upstream of the *Cre recombinase*-coding region and an *hGH* minigene in order to achieve efficient transgene expression. We studied the expression of *hGH* at the mRNA and protein level and the consequences for islets from *Pdx1-Cre*^{Late} mice such as changes in β cell mass and insulin secretion. In addition, we examined hGH expression and serotonin biosynthesis in two other lines that are frequently used for pancreatic islet research: the *C57BL/6-Tg(Ins2-cre)25Mgn/J* line (Postic et al., 1999), which is frequently used to generate β-cell conditional

gene knockouts, and the *B6.Cg-Tg(Ins1-EGFP)1Hara/J* line, which is often used to visualize and/or purify pancreatic β-cells (Hara et al., 2003), from now on referred to as *RIP-Cre* and *MIP-GFP*, respectively.

Results

Pancreata from Pdx1-Cre^{Late} mice have increased β cell mass and insulin content

While working with the *Pdx1-Cre^{Late}* driver line to generate a new β cell-specific knockout strain, we found that circulating blood glucose, both in the random fed (Figure 1A) and fasted state (Figure 1B), was lower in the *Pdx1-Cre^{Late}* mice than in littermate controls. This difference could not be explained by enhanced insulin sensitivity of *Pdx1-Cre^{Late}* mice (Figure 1C). Instead, we observed an increase in pancreatic β cell mass (Figure 1D), which was accompanied by a nearly doubled pancreatic insulin content (Figure 1E, *Pdx1-Cre^{Late}* 390±19 µg/g vs WT 204±52 µg/g, n=9-10/genotype, p=0.006).

Paracrine/autocrine PRLR activation by hGH in islets from non-pregnant Pdx1-Cre^{Late} mice

In the original description of the *Pdx1-Cre^{Late}* mouse model (Herrera, 2000), the complete coding *hGH* gene sequence, including exons, introns and its polyadenylation signal, is present downstream of the *Pdx1* promoter and *Cre recombinase* (Figure 2A). Results from Figures 2B-F demonstrate that the *hGH* minigene is specifically and highly expressed in mouse β cells of the *Pdx1-Cre^{Late}* strain. We quantified *hGH* expression both at the mRNA level (Figure 2B) and protein level (Figure 2C-E). We found significant hGH expression only in pancreatic islets but not in the other tissues (Figure 2B) including the exocrine part of the pancreas (Figure 2D). Western blots (Figure 2C) indicated that the mature hormone of the expected molecular weight (22kDa) was produced in *Pdx1-Cre^{Late}* islets. Quantification of hGH in extracts from isolated islets and in conditioned medium (Figure 2F) show that approximately 4% of cellular hGH content is released per hour from 20 mM glucose-stimulated *Pdx1-Cre^{Late}* islets. Figure 2G supports the idea that locally secreted hGH has functional effects on pancreatic islet *in vivo*. Indeed, a significant increase (3.7-fold, n=3, p=0.005) in STAT5 phosphorylation was detected in freshly isolated *Pdx1-Cre^{Late}* islets compared to control islets (Figure 2G). Because hGH can bind to GH receptors (GHR) and to PRLR (Goffin et al., 1996), and since both receptors are strongly expressed in rodent β cells

(Brelje et al., 2002; Møldrup et al., 1993), we next investigated the mechanism via which hGH causes phenotypic changes in β cells. We first used MIN6 cells as a surrogate model for mouse β cells (Miyazaki et al., 1990) and quantified *Tph1* as a marker for lactogen-mediated PRLR activation, taking ovine PL (oPL) as a positive control (Schraenen et al., 2010b). As is shown in Figure 2H, both hGH and oPL were very potent ligands in this assay as a significant induction of *Tph1* mRNA was already observed with 25 ng/ml (~1 nM). In comparison, the minimal effective concentration of mouse (m)GH, which only binds to GHR, was 500 ng/ml, indicating that synthetic hGH was at least 20-fold more potent. Similar results were obtained with primary islets: a significant induction of *Tph1* mRNA was found in islet monolayers treated with 500 ng/ml oPL or hGH, but not after treatment with mGH (Figure 2I). To confirm that hGH stimulation of *Tph1* expression is mediated by PRLR signaling, MIN6 cells stimulated or not with 25 ng/ml hGH were co-treated with increasing concentrations of the synthetic PRLR antagonist Δ1-9-G129R-hPRL (Bernichtein et al., 2003). A concentration-dependent antagonism was observed (Figure 2I). Together, our data indicate that lactogen-like increment of pancreatic β cell mass and insulin content in *Pdx1-Cre^{Late}* mice is initiated by local hGH release and mediated by activation of PRLR.

Activation of a pregnancy-related phenotypic switch in islets from Pdx1-Cre^{Late} mice

The autocrine/paracrine PRLR stimulation by local release of hGH in pancreatic islets not only enhanced the β cell mass but also upregulated more than 100 genes that are also upregulated in islets from *C57BL/6J* pregnant mice (Figure 3A). This was measured by comparison of the global islet gene expression profile in 12-week-old *Pdx1-Cre^{Late}* mice and control non-pregnant littermates as well as islet from *C57BL/6J* pregnant mice not carrying the *Pdx1-Cre^{Late}* insertion. More than 50% (121/218) of the upregulated genes in non-pregnant *Pdx1-Cre^{Late}* islets were also upregulated during pregnancy (Figure 3A). Overlap was complete for a pregnancy gene expression signature, consisting out of 12 known genes described previously to be highly upregulated during pregnancy (fold increase ≥ 3 and $P<0.001$ at pregnancy day 12.5) (Figure S1). For this strongly induced pregnancy gene

expression signature we confirmed by quantitative RT-PCR (qRT-PCR) analysis that the mRNA signal in *Pdx1-Cre^{Late}* islets was significantly higher than in control islets (Figure 3B). The two transcripts that encode the non-allelic paralogs of tryptophan hydroxylase, *Tph1* and *Tph2*, were highly induced in *Pdx1-Cre^{Late}* islets, respectively, 346.3 ± 69.4-fold (p=0.004) and 14.3 ± 3.2-fold (p=0.011). To assess the importance of the PRLR in the pregnancy switch *in vivo*, expression of the 12 genes in the gene expression signature was quantified in islets from Pdx1-Cre^{Late};PRLR$^{-/-}$ mice versus Pdx1-Cre^{Late} littermates, by qRT-PCR. A significant reduction in gene expression was observed for most of the 12 genes in the Pdx1-Cre^{Late};PRLR$^{-/-}$ mouse model as compared to Pdx1-Cre^{Late} controls (Figure 3C), again stressing the crucial role of the PRLR in the pregnancy switch.

Because DOPA decarboxylase, the second enzyme needed for serotonin biosynthesis from tryptophan, is constitutively and highly expressed in mouse pancreatic islets, the strong upregulation of the tryptophan hydroxylase step indicates that islets of *Pdx1-Cre^{Late}* mice are competent to synthesize serotonin under non-pregnant condition. This is in contrast to control mice, which only produce serotonin during pregnancy. Therefore, we quantified islet serotonin content of control and *Pdx1-Cre^{Late}* mice under non-pregnant condition and observed a striking difference (Figure 3D). This difference between mouse genotypes contrasted with islet gamma-amino butyric acid (GABA) (Figure 3E), a neurotransmitter that is constitutively produced by decarboxylation of glutamate in rodent and human β cells (Sorenson et al., 1991). Consequently, the islet serotonin/GABA molar ratio increased by at least one order of magnitude in islet extracts from the non-pregnant *Pdx1-Cre^{Late}* strain (Figure 3F). To analyze the serotonin production at the cellular level in non-pregnant *Pdx1-Cre^{Late}* mice, we performed immunostaining on pancreatic islet sections. A heterogeneous pattern of serotonin immunoreactivity was found in islet β cells of non-pregnant *Pdx1-Cre^{Late}* mice (Figure 3G), similar to the production observed in wild type *C57BL/6J* mice during pregnancy (Schraenen et al., 2010b). No serotonin immunoreactivity was detected in non-pregnant control mice. Together, these data show that the normal serotonin biosynthetic

pathway, observed in a subpopulation of β cells in the pancreas of pregnant mice, is induced independently of pregnancy in islets from *Pdx1-Cre^{Late}* mice.

Impaired glucose tolerance and reduced islet GLUT2 expression in Pdx1-Cre^{Late} mice

Overlap between genes that are downregulated in islets from pregnant wild type *C57BL/6J* mice versus non-pregnant *Pdx1-Cre^{Late}* mice was much weaker (Figure 3A). One example was the mRNA encoding the glucose transporter GLUT2, which was repressed in *Pdx1-Cre^{Late}* islets (Figure 4A) but not in islets from pregnant mice. GLUT2 acts as a glucose sensor protein in rodent pancreatic β cells and is essential for normal glucose homeostasis in mice (Guillam et al., 1997; Thorens et al., 1988). This reduction in *Glut2* mRNA expression correlated with a strong reduction in immunoreactive protein on islet β cell membranes (Figure 4B) but contrasted with that of other genes involved in GSIS which remained at the control level in islets isolated from in *Pdx1-Cre^{Late}* mice (Figure S2A). The reduction of GLUT2 expression also coincided with a partial loss of GSIS with either 20 mM glucose or 20 mM glucose plus the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) as well as higher basal release (Figure 4C). This abnormality was associated with a slightly decreased glucose tolerance (Figure 4D) and lower circulating insulin levels (Figure 4E) after 2.5 mg/g body weight (BW) intraperitoneal (i.p.) glucose injection. Glucose intolerance was even more pronounced in older *Pdx1-Cre^{Late}* mice (20-week-old) (Figure S2B). However, glycemia turned back to starting levels at the same time point as control mice. Together, these data indicate that, in addition to some pregnancy-related phenotypic changes, local release of hGH induces other changes in pancreatic islets, as exemplified by reduced GLUT2 and partial loss of GSIS in *Pdx1-Cre^{Late}* islets.

Pdx1-Cre^{Late} mice are protected against the diabetogenic toxin streptozotocin

The GLUT2 transporter on the β cell surface is also responsible for the uptake of the β cell toxin streptozotocin (STZ) (Schnedl et al., 1994). Consequently, in control mice, which have very high levels of GLUT2 expression on β cells, STZ can induce an acute β cell necrosis

after single injection of a high dose or chronically after multiple low dose injections (Lenzen, 2008). As the *Glut2* gene expression is reduced in *Pdx1-Cre*^{Late} islets, the effect of STZ might be diminished. Moreover, PRL-induced activation of the JAK2/STAT5 pathway was reported to protect mice against multiple-low doses of STZ (Holstad and Sandler, 1999; Jackerott et al., 2006). The effect of these two protective changes in islets of *Pdx1-Cre*^{Late} mice on blood glucose levels was tested in the multiple low dose STZ model (50mg/kg BW for 5 consecutive days and one month follow up of blood glucose levels - Figure 4F) as well as in the single high dose STZ model (150 mg/kg BW and 5 days of follow up - Figure 4G). *Pdx1-Cre*^{Late} mice remained normoglycemic during the whole follow-up period in both models, whereas all control animals developed diabetes. However, the protection of *Pdx1-Cre*^{Late} mice against a single high dose STZ injection was completely lost when *Pdx1-Cre*^{Late} mice were crossed to *Prlr*$^{-/-}$, further emphasizing the involvement of the PRLR in the hGH-induced phenotypic changes observed in *Pdx1-Cre*^{Late} mice (Figure 4H).

hGH and serotonin production also occurs in islets from RIP-Cre and MIP-GFP mice.

Since many transgenic mouse models, in addition to the *Pdx1-Cre*^{Late} animals, incorporated a *hGH* minigene in their design (see Table 1), we also examined whether hGH protein is produced in *MIP-GFP* and *RIP-Cre* islets, two transgenic lines that are commonly used in studies of β cell biology and cellular mechanisms of diabetes. While expression of the *hGH* mRNA was expected given the placement of the gene sequences in the transgene construct (Figure 5A) hGH protein immunoreactivity was also observed (Figure 5B and S3). Furthermore, we found evidence for a pregnancy related phenotypic switch as illustrated by a strong induction of *Tph1* mRNA (Figure 5A) and immunoreactive serotonin in islets from *MIP-GFP* and *RIP-Cre* islets (Figure 5C and quantified in Figure S4). Therefore, the presence and functional activity of hGH protein is not limited to *Pdx1-Cre*^{Late} mice but also occurs in these two other lines. Similar to what was observed in the Pdx1-Cre^{Late} mice, 9-week-old MIP-GFP mice had lower blood glucose levels after an overnight fast (Figure S5A) and lower plasma

insulin levels 30 min after start of the glucose tolerance test (Figure S5B-C), whereas insulin sensitivity was normal (Figure S5D).

Discussion

The Cre/LoxP system is a powerful tool to conditionally inactivate or overexpress genes in transgenic animals with a number of specific advantages over whole-body knockouts. However, there have been many reports describing specific limitations of some Cre driver strains. These include variegated Cre expression in target organs (Gannon et al., 2000; Ryding et al., 2001), ectopic expression in undesired tissues (Delacour et al., 2004; Song et al., 2010; Wicksteed et al., 2010), and unwanted effects related to the integration site (Cartwright and Wang, 2009). For central nervous system and pancreas-specific Cre driver lines, these limitations and their likely causes have been recently reviewed in (Harno et al., 2013; Magnuson and Osipovich, 2013). In particular for the RIP-Cre mouse model (Postic et al., 1999), a multi-center study showed glucose intolerance in these mice in the absence of genes targeted by loxP sites (Lee et al., 2006). Moreover, younger RIP-Cre mice exhibited β cell hypoplasia, whereas older mice showed β cell hyperplasia (Pomplun et al., 2007).

In the present study, we describe a previously unrecognized mechanism whereby inclusion of an *hGH* minigene as a component of the transgene construct can impair β-cell function. While use of an *hGH* minigene has long been thought to be inconsequential due to it being the second cistron in the transgene-encoded mRNA, and should not be expressed, our studies provide compelling data otherwise. Expression of hGH may have a profound influence on the interpretation of certain types of experiments, especially those pertaining to the control of pancreatic β cell mass and the regulation of insulin secretion, both of which are very active fields of diabetes research.

In islets from *Pdx1-Cre*^{Late}, the *MIP-GFP* and the *RIP-Cre* mouse models we observed expression of hGH from the *hGH* minigene sequences placed downstream of the *Cre* or *GFP* coding regions (Brinster et al., 1988). As a result, hGH of the expected molecular weight (22

kDa) is synthesized and secreted from islets of these mice, causing Cre-or GFP-independent effects by autocrine/paracrine stimulation of β cells. An insertional effect has been postulated before (Lee et al., 2006; Pomplun et al., 2007), but is unlikely since we observe *Tph1* expression and serotonin immunoreactivity in two other frequently used mouse lines (RIP-Cre and MIP-GFP). Figure 6 proposes a model in which locally secreted hGH acts as a lactogen by activating abundantly expressed PRLR on mouse β cells and initiating the JAK2/STAT5 signaling pathway. A first group of effects is therefore pregnancy-like and includes induction of serotonin biosynthesis and a doubling of β cell mass and pancreatic insulin content. The most important of pregnancy-unrelated effects is a partial loss of GSIS, decreasing glucose tolerance. This loss of function could be partially caused by downregulation of GLUT2, a transporter responsible for the rapid equilibration between extra- and intracellular glucose concentration and thus the first step of glucose sensing in rodent islets (Guillam et al., 1997). This is in contrast with the pregnancy state, as β cell *Glut2* levels have been reported to increase as part of the adaptive maternal response (Weinhaus et al., 1996). GLUT2 is also needed for rapid uptake of the β cell toxins STZ (Schnedl et al., 1994) and alloxan (De Vos et al., 1995), so it seems reasonable to attribute the protection of *Pdx1-Cre*^{Late} islets against STZ-induced diabetes to reduced toxin uptake rates caused by less GLUT2 channels (Figure 5). In addition, enhanced β cell mass and greater potential to regenerate new β cells may also be involved.

As the *hGH* minigene is frequently used to enhance expression of transgenes, we did a literature search for mouse lines generated using the same genetic strategy (Table 1). At the time of preparation of this manuscript, we listed a total of 22 mouse models. Due to the lack of information available for several other β-cell-specific mouse strains, our table may be an underestimation of the total amount of models involved. In some of the models listed in Table 1 expression of the *hGH* minigene was mentioned, either at the level of mRNA (Miyazaki et al., 2010; Postic et al., 1999; Sanvito et al., 1995) or immunoreactive protein (Klee et al., 2011). In none of them, however, the potential influence on pancreatic islet morphology, β

cell function or glucose homeostasis was considered.

The investigation of cellular and physiological mechanisms that regulate β cell mass and β cell function is currently a very active area of diabetes research that makes extensive use of genetically altered mice. Moreover, there is already a large body of experimental work that has made use of mice containing *hGH* minigene. Our current observations seem therefore to have potential implications on the interpretation of a large body of published data, in particular with respect to changes in functional β cell mass, insulin secretion and protection against the diabetogenic effect of STZ.

Experimental procedures

Mice

Pdx1-Cre^{Late} transgenic mice (Herrera, 2000)(Dr. Herrera, University of Geneva, Switzerland) were crossed with *C57BL/6J* (Janvier) for at least eight generations. PRLR knockout mice (designated *Prlr*$^{-/-}$) on a 129Sv background were described previously (Freemark et al., 2002). *Pdx1-Cre*^{late} females were bred with *Prlr*$^{+/-}$ males to generate *Pdx1-Cre*^{Late}; *Prlr*$^{+/-}$ mice, which were subsequently crossed with *Prlr*$^{+/-}$ to obtain *Pdx1-Cre*^{Late}; *Prlr* $^{-/-}$ mice. Institutional guidelines for animal welfare and experimental conduct were followed. All experiments with laboratory animals were approved by the committee for animal welfare at the KU Leuven. *RIP-Cre* mice (Postic et al., 1999) and *MIP-GFP* mice (Hara et al., 2003) were maintained on C57Bl and CD1 backgrounds respectively. All animal procedures and husbandry were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Cell cultures

MIN6 cells were incubated in DMEM (25 mmol/l glucose, 2% FCS, 4 mmol/l glutamax) (Invitrogen-Gibco) with hGH (Calbiochem), mGH or oPL (Prospec). For the experiments with

the PRLR-antagonist Δ1-9-G129R-hPRL MIN6 cells were pre-incubated for 30 min with the antagonist. Thereafter, recombinant hGH (from Vincent Goffin) was added to the medium. Twenty-four hours later RNA was extracted.

Islet isolation

Pancreatic islets were isolated after infusion and digestion of the pancreata by collagenase P (Roche) as described previously (Lemaire et al., 2009).

Islet monolayers

Islet monolayers were performed as previously described (Schraenen et al., 2010b). Isolated islets were cultured for 7 days in RPMI medium (10% [vol./vol.] decomplemented FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 4 mmol/l glutamax, 10 mM HEPES pH 7.4) to form monolayers. On day 7, they were stimulated with 0 or 500 ng/ml oPL, mGH or hGH.

Microarray expression analysis

Microarray analysis was performed on RNA of isolated islets using MoGene_1.0_ST arrays (Affymetrix). For RNA extraction, see Extended Experimental Procedures. Total islet RNA (100 ng) was used to hybridize the arrays according to manufacturer's manual 701880Rev4 as described previously (Lemaire et al., 2009). Samples were analyzed pair-wise, using $p<0.001$ and fold change≥ 1.5 as selection criteria. A list of up- and down regulated genes in islets isolated from *Pdx1-Cre*^{Late} transgenic mice versus control mice is provided as an excel table (see Table S1) and genes related to pregnancy are marked.

Quantitative RT-PCR

Following cDNA synthesis using a reverse transcriptase kit (RevertAid H Minus; Fermentas), qRT-PCR (Absolute QPCR mix; Abgene-Thermo Fisher Scientific) was performed on a Rotorgene (Corbett Research). For primers and probes see Extended Experimental

Procedures. For *Tph2* a Taqman gene expression assay (Mm00557717_m1; Applied Biosystems) was used. When a probe was used data was analyzed according to the Pfaffl method, without delta Ct was used.

Histology

Pancreata were fixed overnight in 4% formaldehyde and embedded in paraffin. Sections were rehydrated and heated for 20 minutes in Target Retrieval Solution (pH 6.1, Dako). After blocking with 20% normal goat serum (Dako) in PBS, slides were incubated with 1/1,000 anti-hGH monoclonal (ab15317, Abcam) antibody in Antibody Diluent (Dako). For double immunofluorescent labeling, 1/50,000 rabbit anti-serotonin (Immunostar, #20080) or 1/2000 polyclonal anti-GLUT2 (07-1402, Millipore) was combined with 1/10,000 diluted guinea-pig anti-insulin antibody (a gift of Dr. Van Schravendijk, VUB, Brussels) and detected with anti-rabbit Cy3 and anti-guinea-pig FITC respectively (both from Jackson Immunoresearch laboratories). For *MIP-GFP* and *RIP-Cre* mice, frozen sections were stained with 1/10,000 rabbit anti-serotonin or 1/200 mouse anti-hGH (blocking with Mouse on Mouse (M.O.M.) Basic Kit from Vector Labs) and co-stained with 1/2000 guinea pig anti-insulin in sections from *RIP-Cre* mice. For *MIP-GFP*, direct fluorescence for GFP was used to detect β cells.

Glucose (GTT) and insulin tolerance tests (ITT)

Overnight (GTT) or 6-hour-fasted (ITT) mice were injected i.p. with 2.5 mg/g BW D-glucose or 0.75 mU/g BW human insulin, respectively and glycemia was measured by tail-blood analysis using a Contour glucose meter (Bayer). For GTT, tail-blood was collected at the indicated time-points and plasma was analyzed.

Islet serotonin and GABA content

80 islets were homogenized by 3 min sonication in a buffer containing 0.01 mol/l HCl, 1 mmol/l EDTA and 4 mmol/l sodium metabisulfite. After centrifugation (20,000 g) for 15 min at

4°C and addition of 0.1% (wt/vol.) ascorbic acid (Acros), lysates were stored at −80°C. Serotonin and GABA concentrations were determined via HPLC see Extended Experimental Procedures.

Islet hGH content and release

Freshly isolated islets were incubated in batches of 100 at 37°C. Incubation was in HEPES Krebs buffer (20 mmol/l HEPES, pH7.4; 119 mmol/l NaCl; 4.75 mmol/l KCl; 2.5 mmol/l CaCl2; 1.2 mmol/l MgSO4; 1.2 mmol/l KH2PO4; 5 mmol/l NaHCO3, 0.5% [wt/vol.] BSA) containing 20 mmol/l glucose. After 1 hour, half of the medium was removed for measurement of hGH release. For the content, Triton-X-100 (final concentration: 0.5%) was added to the other half of the medium with the islets. Islets were sonicated for 3 minutes and lysates were stored at −20°C. To measure concentrations an hGH ELISA was used (Invitrogen).

Islet insulin content and release

For insulin secretion measurements, size matched islets (n = 5 per tube) were placed in glass tubes containing HEPES Krebs solution containing 0.5 % BSA, supplemented with glucose 5 mM (G5), 20mM (G20) or G20 with 250 µM IBMX. Supernatant was collected after 1 hour incubation at 37°C. The islets were sonicated for 3 min after adding Acid ethanol (final concentration: 75 % EtOH, 0.1 N HCl, 1% triton). Samples were stored at -20°C and the ELISA kit used for insulin determination was from Crystal Chem.

Total pancreas insulin content

Pancreata were dissected and acid-ethanol extracts were diluted 1/1000 in PBS and analyzed for insulin using an insulin high-range ELISA (Mercodia). Absolute insulin content per pancreas as well as relative content (corrected for pancreas weight) was quantified.

β *cell mass quantification*

Total pancreas from 24-week-old *Pdx1-Cre*^{late} and littermate control mice (4 males per group) was processed and six sections separated by 200 µm were stained for insulin as described above. The total surface area of insulin positive cells (in pixels) was quantified with Zeiss Axiovision software (Micro Imaging). The relative insulin surface area per section (total insulin area (pixels)/total pancreas area (pixels)) was multiplied by the pancreas weight (mg) to obtain the β cell mass (mg).

Western blot

Islets were isolated as described above and homogenized in lysis buffer (Cell Signaling Technology) by sonication. Protein extracts were separated by SDS-PAGE (10% [vol./vol.] Bis/Tris gel; Life Technologies), blotted on a nitrocellulose membrane, blocked in 4% [wt./vol.] milk and incubated with primary antibody (anti-hGH, ab15317, 1/1000; anti-GAPDH, 1/15,000; clone 6C5, both from Abcam). The blot was subsequently incubated with peroxidase-conjugated secondary antibody (Dako) and proteins were detected using the Western Lightning ECL system (PerkinElmer). For hGH staining on islets from *MIP-GFP* and *RIP-Cre* mice, a similar protocol was used with adjustments; 10 µg of islet protein sample in 1X Laemmli sample buffer was resolved on 15% SDS-PAGE and transferred to polyvinylidene difluoride membranes (EMD Millipore).

Statistical methods

When not differently stated in the legend or text, data are presented as mean ± SEM and significance shown on graphs as $p<0.05$*, $p<0.01$** or $p<0.001$.

Figure legends

Figure 1 Increased β cell mass and insulin content in non-pregnant *Pdx1-Cre^{Late}* mice.

(A-B) Blood glucose levels in (A) random fed and (B) fasted *Pdx1-Cre^{Late}* versus control mice. Data are represented as mean ± SEM, n=7 mice per genotype, **P<0.01.

(C) Insulin tolerance test (ITT) on 10 week-old *Pdx1-Cre^{Late}* versus control mice. Values are presented as percentage compared to starting glucose level. Data are represented as mean ± SEM, n=7-8 mice per genotype.

(D) Quantification of β cell mass in 24-week-old *Pdx1-Cre^{Late}* versus control mice, performed as described in 'Experimental Procedures'. Data are represented as mean ± SEM, n=4 mice per genotype, **P<0.01.

(E) Pancreatic insulin content in 24-week-old *Pdx1-Cre^{Late}* versus control mice. Data are represented as mean ± SEM, n=9-10 mice per genotype, **P<0.01.

Figure 2 Pregnancy-related phenotypic changes are caused by local production and secretion of hGH in islets from non-pregnant *Pdx1-Cre^{Late}* mice.

(A) Schematic representation of the *Pdx1-Cre* construct used to generate the *Pdx1-Cre^{Late}* mouse model. A 4.5 kb *Pdx1* promoter fragment was inserted upstream of the *Cre* transgene. The *hGH* minigene, containing introns, exons and polyadenylation signal, is located directly downstream of the *Cre* fragment and indicated in red. Figure adapted from (Herrera, 2000).

(B) qRT-PCR analysis of *hGH* mRNA in different tissues including isolated islets from control and non-pregnant *Pdx1-Cre^{Late}* mice. Data were quantified as delta Ct values (relative to *Gapdh*). Data are represented as mean ± SEM, n=3-6 per condition, ***P<0.001.

(C) Western blot analysis of hGH expression in islets of control and non-pregnant *Pdx1-Cre^{Late}* mice. GAPDH was used as a loading control.

(D) Immunoreactive hGH in pancreatic sections from control and non-pregnant *Pdx1-Cre*^{Late} mice. The hGH signal is most intense in pancreatic islets and not uniformly distributed over their different islet cells. Scale bar = 100 μm.

(E-F) Quantification of hGH content (panel E) and release (panel F; measured as % of cellular content per hour) in islets isolated from *Pdx1-Cre*^{Late} mice. For the release experiments, isolated islets were incubated with 20 mM D-glucose for 1 hour. Data are represented as mean ± SEM, n=3 per condition, **P<0.01.

(G) Representative immunoblot of islet phospho-STAT5 (P-STAT5) and total STAT5 protein in islets from control and non-pregnant *Pdx1-Cre*^{Late} mice. GAPDH was used as a loading control. Mean density ratios of P-STAT5/total STAT5 were 3.72 ± 0.38-fold increased in *Pdx1-Cre*^{Late} islets compared to controls. Data are represented as mean ± SEM, n=3 per condition, P=0.005.

(H-I) Induction of *Tph1* expression in MIN6 cells (H) and primary islet monolayers (I) by hGH and oPL. Cells were treated with the indicated concentrations of oPL, mGH or hGH, and islet monolayers with vehicle (control) or 500 ng/ml oPL, mGH or hGH. Expression of *Tph1* was quantified by qRT-PCR. *Polr2a* was used as a reference gene. Data was calculated via the Pfaffl method and the ratio of the control sample was normalized to 1. Data are represented as mean ± SEM, n=3-5 independent measurements per condition; *P<0.05, **P<0.01, ***P<0.001.

(J) Inhibition of hGH-induced *Tph1* expression in MIN6 cells by a specific PRLR inhibitor. MIN6 cells pre-incubated (30 min) with different concentrations of PRLR-antagonist Δ1-9-G129R-hPRL were stimulated with 0 or 25 ng/ml hGH; *Tph1* mRNA expression was used as read-out for the pregnancy signature and *Polr2a* was used as a reference gene. Data was calculated via the Pfaffl method and the average-ratio of 0 ng/ml hGH was set to 1. Data are represented as mean ± SEM, n=3 per condition *P<0.05, **P<0.01.

Figure 3 Pregnancy-related phenotypic switch in islets isolated from non-pregnant *Pdx1-Cre^{Late}* mice.

(A) Microarray analysis of islet mRNA expression. A substantial overlap of > 100 differentially expressed genes (P<0.001 and fold change of ≥ 1.5) was found when comparing pregnant versus non-pregnant mice on the one hand (left) and non-pregnant *Pdx1-Cre^{Late}* mice versus non-pregnant control mice on the other hand (right). This overlap contains almost 10-fold more up- than down-regulated genes.

(B) qRT-PCR analysis of the pregnancy gene signature in non-pregnant *Pdx1-Cre^{Late}* versus control mice. For each of the top 12 upregulated genes during pregnancy at P12.5, we observed significant upregulation in islets from non-pregnant *Pdx1-Cre^{Late}* mice. *Polr2a* was used as a reference gene. Data was calculated via the Pfaffl method and the average-ratio of each gene was set to 1 for the control mice. Two strongly induced genes in islets from *Pdx1-Cre^{Late}* mice encode the two isoforms of tryptophan hydroxylase (TPH1 and TPH2). Data are represented as mean ± SEM, n=4-6 per group, *P<0.05, **P<0.01, ***P<0.001.

(C) qRT-PCR analysis on all 12 genes from the pregnancy gene expression signature, in islets from Pdx1-Cre^{Late};PRLR-/- mice versus Pdx1-Cre^{Late} littermates n=3 mice per genotype. Data are represented as mean ± SEM, n=3 per group, *P<0.05, **P<0.01, ***P<0.001.

(D-F) Quantification of serotonin and GABA in islets isolated from non-pregnant control and non-pregnant *Pdx1-Cre^{Late}* mice. (D) Basal islet serotonin levels are near the detection limit of the assay in control mice and dramatically upregulated in non-pregnant *Pdx1-Cre^{Late}* mice. (E) In contrast, GABA is as abundant in islets of control as in islets of non-pregnant *Pdx1-Cre^{Late}* mice. (F) Consequently the molar serotonin/GABA ratio is about 100-fold higher in islets from *Pdx1-Cre^{Late}* mice compared to control islets. Data are represented as mean ± SEM, n=5-6 per group, *P<0.05, **P<0.01, ***P<0.001.

(G) Heterogeneous serotonin immunoreactivity in islets from non-pregnant *Pdx1-Cre^{Late}*

mice. No serotonin immunoreactivity is detected in control mice, while in non-pregnant *Pdx1-Cre^{Late}* mice serotonin is only present in islet β cells with marked differences between neighboring β-cells. Data are representative sections from pancreata analyzed from 5 non-pregnant control mice and 4 non-pregnant *Pdx1-Cre^{Late}* mice (scale bar = 20 μm). See also Figure S1 and Table S1.

Figure 4 Reduced islet GLUT2 expression, loss of GSIS and protection against STZ in *Pdx1-Cre^{Late}* mice.

(A) *Glut2* mRNA signal in control versus *Pdx1-Cre^{Late}* islets. *Gapdh* was used as a reference gene. Data are represented as mean ± SEM n=3-4 mice per genotype, ***P<0.001.

(B) Representative immunfluorescence staining for insulin (green signal), GLUT2 (red signal) and merge on pancreatic sections from control versus *Pdx1-Cre^{Late}* mice. Overall, the GLUT2 signal is much weaker in *Pdx1-Cre^{Late}* islets compared to controls. Scale bar = 20 μm.

(C) *Ex vivo* insulin secretion assay. Islets from *Pdx1-Cre^{Late}* and control mice were isolated and incubated for 1 hour with 5 mM (G5), 20mM (G20) or 20 mM D-glucose with the phosphodiesterase inhibitor IBMX (G20 + IBMX) Insulin release was quantified as percentage of insulin secreted compared to islet insulin content. Data are represented as mean ± SEM, n=6 mice per genotype, *P<0.05, **P<0.01.

(D) i.p. glucose tolerance test (IPGTT) on 10-week-old *Pdx1-Cre^{Late}* versus control mice, n=5 per genotype. 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 mice per genotype, ***P<0.001, repeated measures ANOVA.

(E) Circulating plasma insulin levels in blood samples obtained at indicated time points from IPGTT. Data are represented as mean ± SEM, n=5 mice per genotype. *P<0.05, ***P<0.001, repeated measures ANOVA.

(F) Multiple low-dose (MLD) treatment of the diabetogenic agent streptozotocin (STZ) in *Pdx1-Cre*^{Late} versus control mice. Mice aged 12 weeks were injected i.p. with 50mg STZ /kg BW for 5 consecutive days, and random fed blood glucose levels were measured at indicated time points. Data are represented as mean ± SEM, n=5 mice per genotype, *P<0.05, **P<0.01, ***P<0.001, repeated measures ANOVA.

(G) Single high-dose of STZ in *Pdx1-Cre*^{Late} versus control mice. Mice were injected i.p. with 150 mg STZ /kg BW, and fed blood glucose levels were measured. Data are represented as mean ± SEM, n=5-6 mice per genotype, ***P<0.001, repeated measures ANOVA.

(H) Single high-dose (150 mg/kg BW) of STZ in *Prlr*$^{-/-}$ versus *Pdx1-Cre*^{Late}; *Prlr*$^{-/-}$ mice. Data are represented as mean ± SEM, n=3-4 mice per genotype, **P<0.01, repeated measures ANOVA. See also Figure S2.

Figure 5 Growth hormone and *Tph1* expression and islet serotonin immunoreactivity in pancreatic islets from two other commonly used mouse driver strains, *MIP-GFP* and *RIP-Cre*.

(A) *hGH* and *Tph1* mRNA signal in isolated islets from *MIP-GFP* and *RIP-Cre* mice, quantified as delta Ct values relative to a housekeeping mRNA signal (*Hprt*). Data are represented as mean ± SEM, n=3-6 mice per genotype, ***P<0.001.

(B) Western blot analysis of hGH expression in islets from *MIP-GFP*, *RIP-Cre* and *C57Bl6* control mice. Predicted hGH weight = 22 kDa. GAPDH was used as a loading control.

(C) Representative immunofluorescence micrographs show serotonin immunoreactivity in islets from *MIP-GFP* and *RIP-Cre* mice, whereas no signal was observed in respective littermate controls. Scale bar = 50 µm.

Figure 6 Model of hGH-induced phenotypic changes in *Pdx1-Cre*^{Late} β cells.

Pdx1-promoter driven expression of the *hGH* minigene causes biosynthesis and secretion of hGH which exerts autocrine or paracrine effects after binding to PRLR on β cells. This causes STAT5 phosphorylation and pregnancy-like phenotypic changes like enhanced β cell mass, and serotonin (5-HT) production. In addition expression of the *hGH* minigene causes pregnancy-unrelated changes such as reduction of GLUT2 expression and partial loss of glucose-induced insulin release. Lower GLUT2-mediated uptake and higher β cell mass protect against the diabetogenic effect of the β cell toxin STZ.

Table 1: β cell specific transgenic mice generated based on a similar strategy, namely using the *hGH* minigene as transgene enhancer. The table includes the name of the strains that were identified and the reference of the first report.

Acknowledgements

The authors declare no conflict of interest. This work was financially supported by the Juvenile Diabetes Research Foundation (JDRF grant 1_2010_393), the Flemish Fund for Scientific Research (FWO grants G.0672.12 and G.A103.11 and fellowship to AS), and the Katholieke Universiteit Leuven (GOA/14/010 and GOA/12/016). BB, VP and JVS are fellows of 'IWT-Vlaanderen'. The authors thank Ria Berckmans, Gino De Smet, Florence Boutillon and Nicole Buelens for technical support, Pedro Herrera (University of Geneva) for supplying the *Pdx1-Cre*^{Late} strain, Jegan Iyyathurai for isolating bovine corneal endothelial cells and Idoya Lahortiga and Luk Cox from somersault18:24 for Figure 6.

Accession numbers

Microarray data were deposited in the Gene Expression Omnibus Database of the National Centre for Biotechnology Information under the accession number: GSE50851.

References

Bernichtein, S., Kayser, C., Dillner, K., Moulin, S., Kopchick, J.J., Martial, J.A., Norstedt, G., Isaksson, O., Kelly, P.A., and Goffin, V. (2003). Development of pure prolactin receptor antagonists. The Journal of biological chemistry *278*, 35988-35999.

Bone, A.J., and Taylor, K.W. (1976). Mitabolic adaptation to pregnancy shown by increased biosynthesis of insulin in islets of Langerhans isolated from pregnant rat. Nature *262*, 501-502.

Brelje, T.C., Svensson, A.M., Stout, L.E., Bhagroo, N.V., and Sorenson, R.L. (2002). An immunohistochemical approach to monitor the prolactin-induced activation of the JAK2/STAT5 pathway in pancreatic islets of Langerhans. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society *50*, 365-383.

Brinster, R.L., Allen, J.M., Behringer, R.R., Gelinas, R.E., and Palmiter, R.D. (1988). Introns increase transcriptional efficiency in transgenic mice. Proceedings of the National Academy of Sciences of the United States of America *85*, 836-840.

Cartwright, E.J., and Wang, X. (2009). Transgenesis. In, J.M. Walker, and R. Rapley, eds. (Cambridge: Royal Society of Chemistry), pp. 390-491.

De Vos, A., Heimberg, H., Quartier, E., Huypens, P., Bouwens, L., Pipeleers, D., and Schuit, F. (1995). Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. The Journal of clinical investigation *96*, 2489-2495.

Delacour, A., Népote, V., Trumpp, A., and Herrera, P.L. (2004). Nestin expression in pancreatic exocrine cell lineages. Mechanisms of development *121*, 3-14.

Fleenor, D., Petryk, A., Driscoll, P., and Freemark, M. (2000). Constitutive expression of placental lactogen in pancreatic beta cells: effects on cell morphology, growth, and gene expression. Pediatric research *47*, 136-142.

Freemark, M. (2006). Regulation of maternal metabolism by pituitary and placental hormones: roles in fetal development and metabolic programming. Hormone research *65 Suppl 3*, 41-49.

Freemark, M., Avril, I., Fleenor, D., Driscoll, P., Petro, A., Opara, E., Kendall, W., Oden, J., Bridges, S., Binart, N*., et al.* (2002). Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. Endocrinology *143*, 1378-1385.

Gannon, M., Herrera, P.L., and Wright, C.V. (2000). Mosaic Cre-mediated recombination in pancreas using the pdx-1 enhancer/promoter. Genesis (New York, NY : 2000) *26*, 143-144.

Goffin, V., Shiverick, K.T., Kelly, P.A., and Martial, J.A. (1996). Sequence-function relationships within the expanding family of prolactin, growth hormone, placental lactogen, and related proteins in mammals. Endocrine reviews *17*, 385-410.

Green, I.C., Perrin, D., and Howell, S.L. (1978). Insulin release in isolated islets of Langerhans of pregnant rats. Relationship between glucose metabolism and cyclic AMP. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et métabolisme *10*, 32-35.

Green, I.C., and Taylor, K.W. (1972). Effects of pregnancy in the rat on the size and insulin secretory response of the islets of Langerhans. The Journal of endocrinology *54*, 317-325.

Guillam, M.T., Hümmler, E., Schaerer, E., Yeh, J.I., Birnbaum, M.J., Beermann, F., Schmidt, A., Dériaz, N., Thorens, B., and Wu, J.Y. (1997). Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. Nature genetics *17*, 327-330.

Hara, M., Wang, X., Kawamura, T., Bindokas, V.P., Dizon, R.F., Alcoser, S.Y., Magnuson, M.A., and Bell, G.I. (2003). Transgenic mice with green fluorescent protein-labeled pancreatic beta -cells. American journal of physiology Endocrinology and metabolism *284*, E177-183.

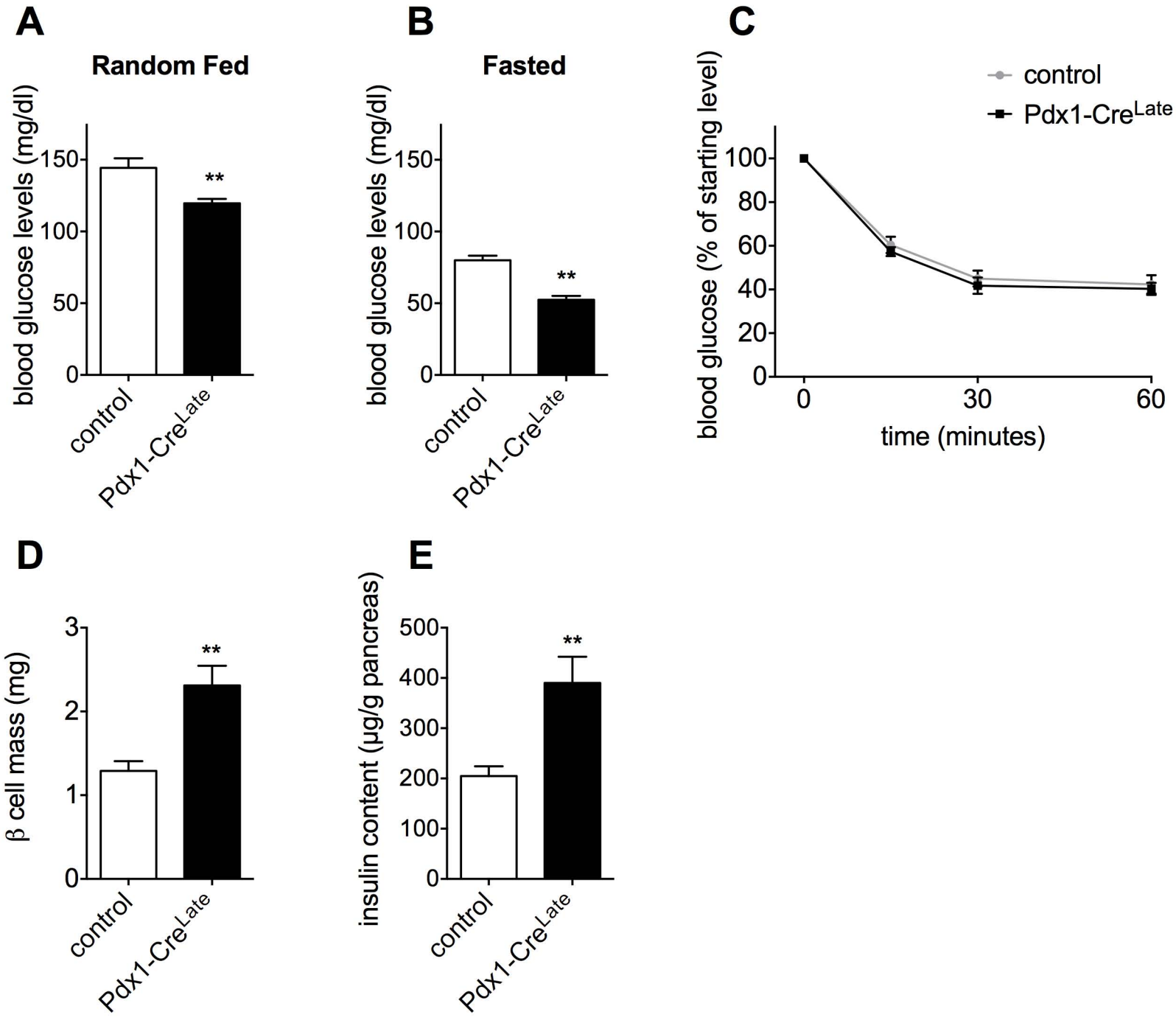
Harno, E., Cottrell, E.C., and White, A. (2013). Metabolic pitfalls of CNS cre-based technology. Cell metabolism *18*, 21-28.

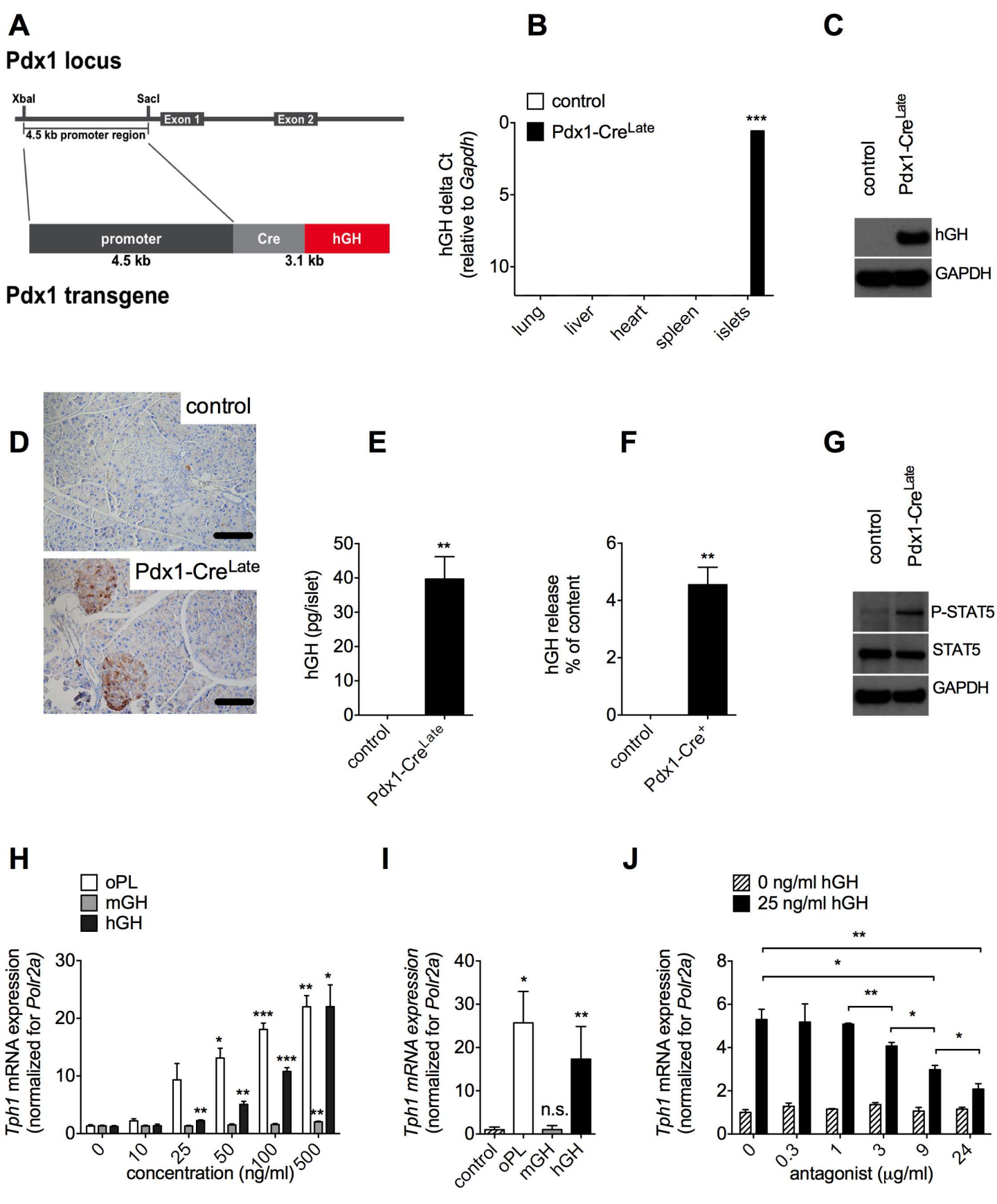
Herrera, P.L. (2000). Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. Development (Cambridge, England) *127*, 2317-2322.

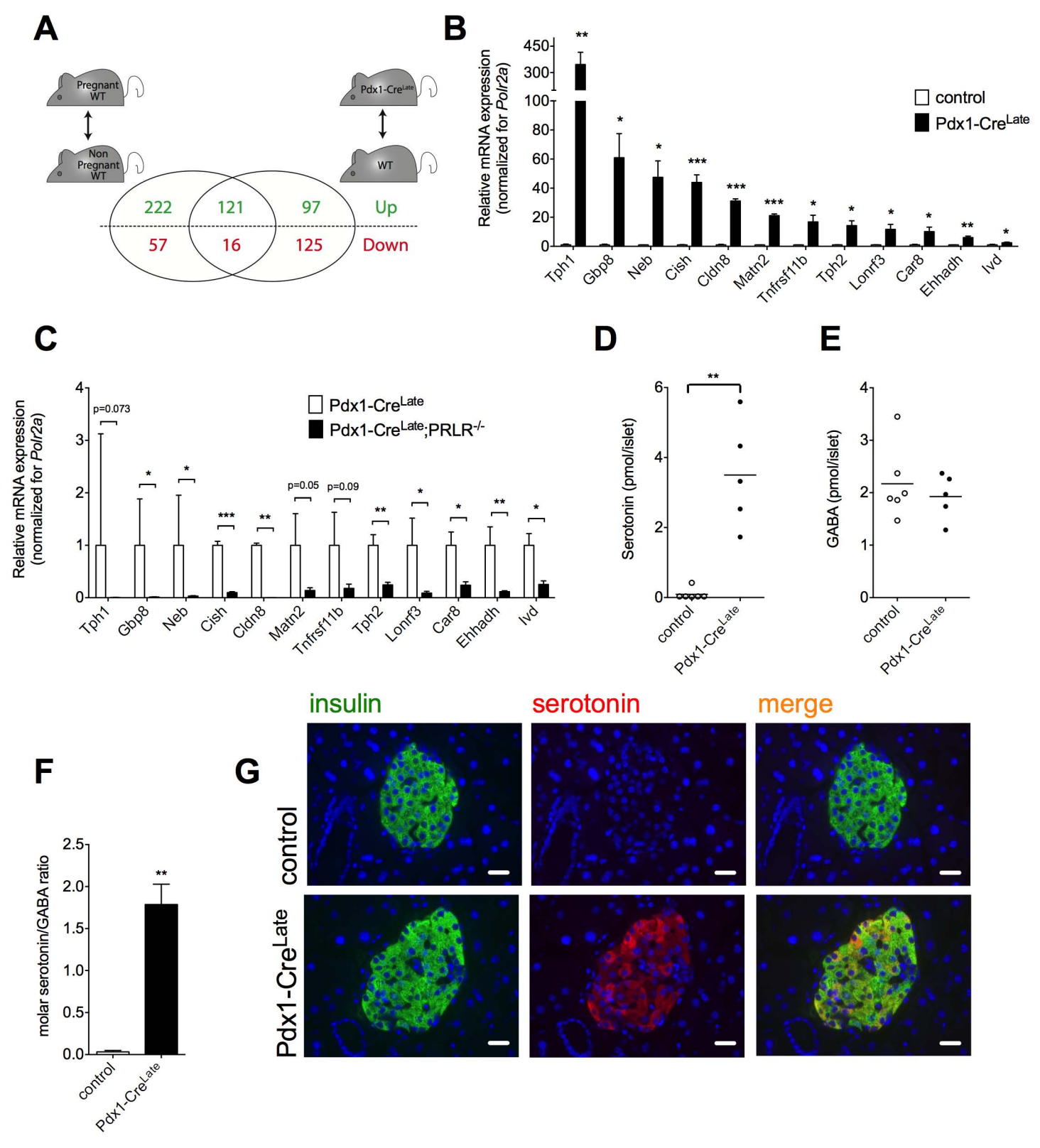
Holstad, M., and Sandler, S. (1999). Prolactin protects against diabetes induced by multiple low doses of streptozotocin in mice. The Journal of endocrinology *163*, 229-234.

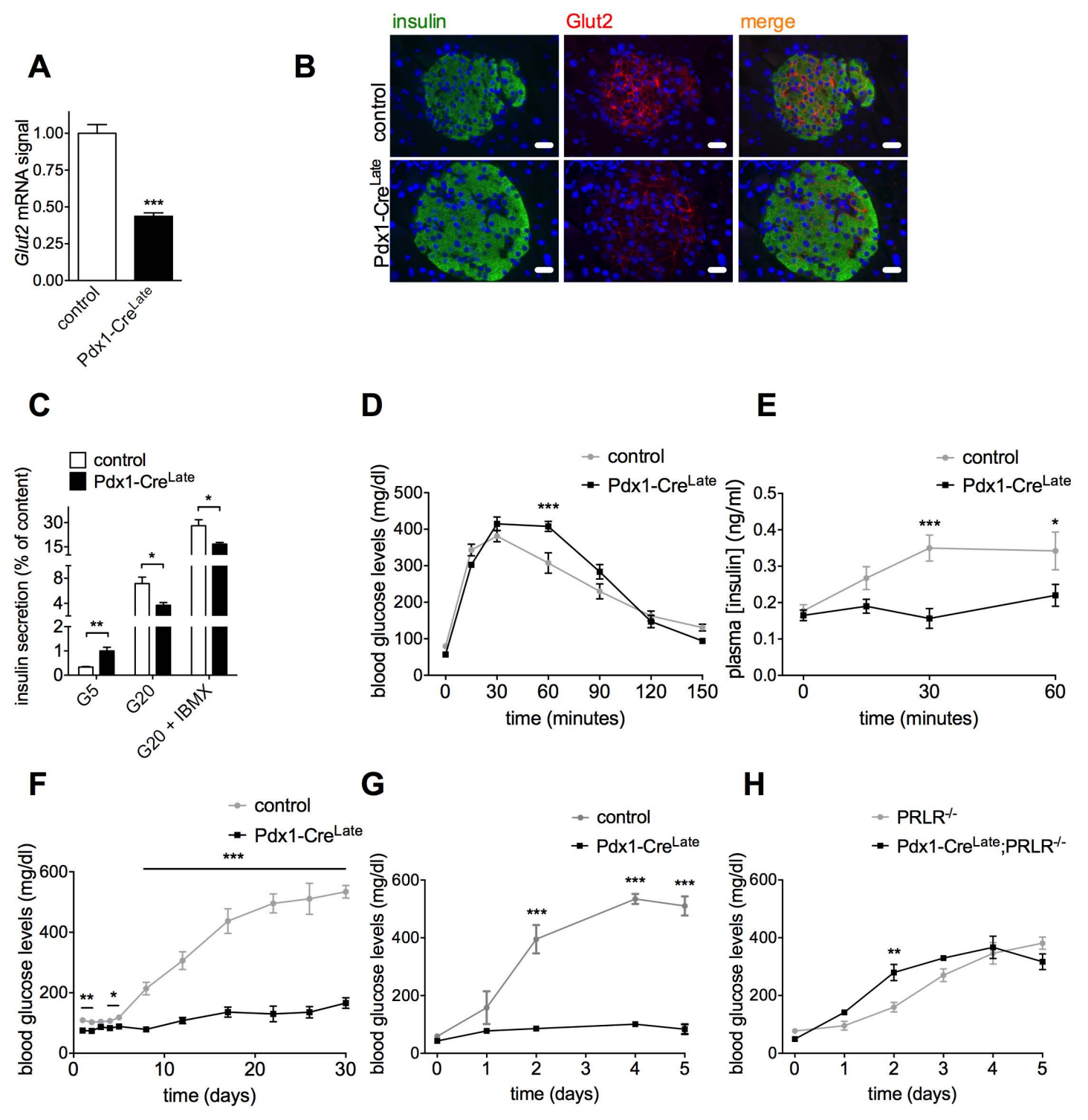
Huang, C., Snider, F., and Cross, J.C. (2009). Prolactin receptor is required for normal glucose homeostasis and modulation of beta-cell mass during pregnancy. Endocrinology *150*, 1618-1626.
Jackerott, M., Møldrup, A., Thams, P., Galsgaard, E.D., Knudsen, J., Lee, Y.C., and Nielsen, J.H. (2006). STAT5 activity in pancreatic beta-cells influences the severity of diabetes in animal models of type 1 and 2 diabetes. Diabetes *55*, 2705-2712.
Kim, H., Toyofuku, Y., Lynn, F.C., Chak, E., Uchida, T., Mizukami, H., Fujitani, Y., Kawamori, R., Miyatsuka, T., Kosaka, Y.*, et al.* (2010). Serotonin regulates pancreatic beta cell mass during pregnancy. Nature medicine *16*, 804-808.
Klee, P., Lamprianou, S., Charollais, A., Caille, D., Sarro, R., Cederroth, M., Haefliger, J.-A., and Meda, P. (2011). Connexin implication in the control of the murine beta-cell mass. Pediatric research *70*, 142-147.
Lee, J.Y., Ristow, M., Lin, X., White, M.F., Magnuson, M.A., and Hennighausen, L. (2006). RIP-Cre revisited, evidence for impairments of pancreatic beta-cell function. The Journal of biological chemistry *281*, 2649-2653.
Lemaire, K., Ravier, M.A., Schraenen, A., Creemers, J.W.M., Van de Plas, R., Granvik, M., Van Lommel, L., Waelkens, E., Chimienti, F., Rutter, G.A.*, et al.* (2009). Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proceedings of the National Academy of Sciences of the United States of America *106*, 14872-14877.
Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia *51*, 216-226.
Magnuson, M.A., and Osipovich, A.B. (2013). Pancreas-specific cre driver lines and considerations for their prudent use. Cell metabolism *18*, 9-20.
Miyazaki, J., Araki, K., Yamato, E., Ikegami, H., Asano, T., Shibasaki, Y., Oka, Y., and Yamamura, K. (1990). Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology *127*, 126-132.
Miyazaki, S., Taniguchi, H., Moritoh, Y., Tashiro, F., Yamamoto, T., Yamato, E., Ikegami, H., Ozato, K., and Miyazaki, J.-i. (2010). Nuclear hormone retinoid X receptor (RXR) negatively regulates the glucose-stimulated insulin secretion of pancreatic ß-cells. Diabetes *59*, 2854-2861.
Møldrup, A., Petersen, E.D., and Nielsen, J.H. (1993). Effects of sex and pregnancy hormones on growth hormone and prolactin receptor gene expression in insulin-producing cells. Endocrinology *133*, 1165-1172.
Orban, P.C., Chui, D., and Marth, J.D. (1992). Tissue- and site-specific DNA recombination in transgenic mice. Proceedings of the National Academy of Sciences of the United States of America *89*, 6861-6865.
Parsons, J.A., Bartke, A., and Sorenson, R.L. (1995). Number and size of islets of Langerhans in pregnant, human growth hormone-expressing transgenic, and pituitary dwarf mice: effect of lactogenic hormones. Endocrinology *136*, 2013-2021.
Parsons, J.A., Brelje, T.C., and Sorenson, R.L. (1992). Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. Endocrinology *130*, 1459-1466.
Pomplun, D., Florian, S., Schulz, T., Pfeiffer, A.F.H., and Ristow, M. (2007). Alterations of pancreatic beta-cell mass and islet number due to Ins2-controlled expression of Cre recombinase: RIP-Cre revisited; part 2. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et métabolisme *39*, 336-340.
Postic, C., Shiota, M., Niswender, K.D., Jetton, T.L., Chen, Y., Moates, J.M., Shelton, K.D., Lindner, J., Cherrington, A.D., and Magnuson, M.A. (1999). Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. The Journal of biological chemistry *274*, 305-315.
Rieck, S., White, P., Schug, J., Fox, A.J., Smirnova, O., Gao, N., Gupta, R.K., Wang, Z.V., Scherer, P.E., Keller, M.P.*, et al.* (2009). The transcriptional response of the islet to pregnancy in mice. Molecular endocrinology (Baltimore, Md) *23*, 1702-1712.

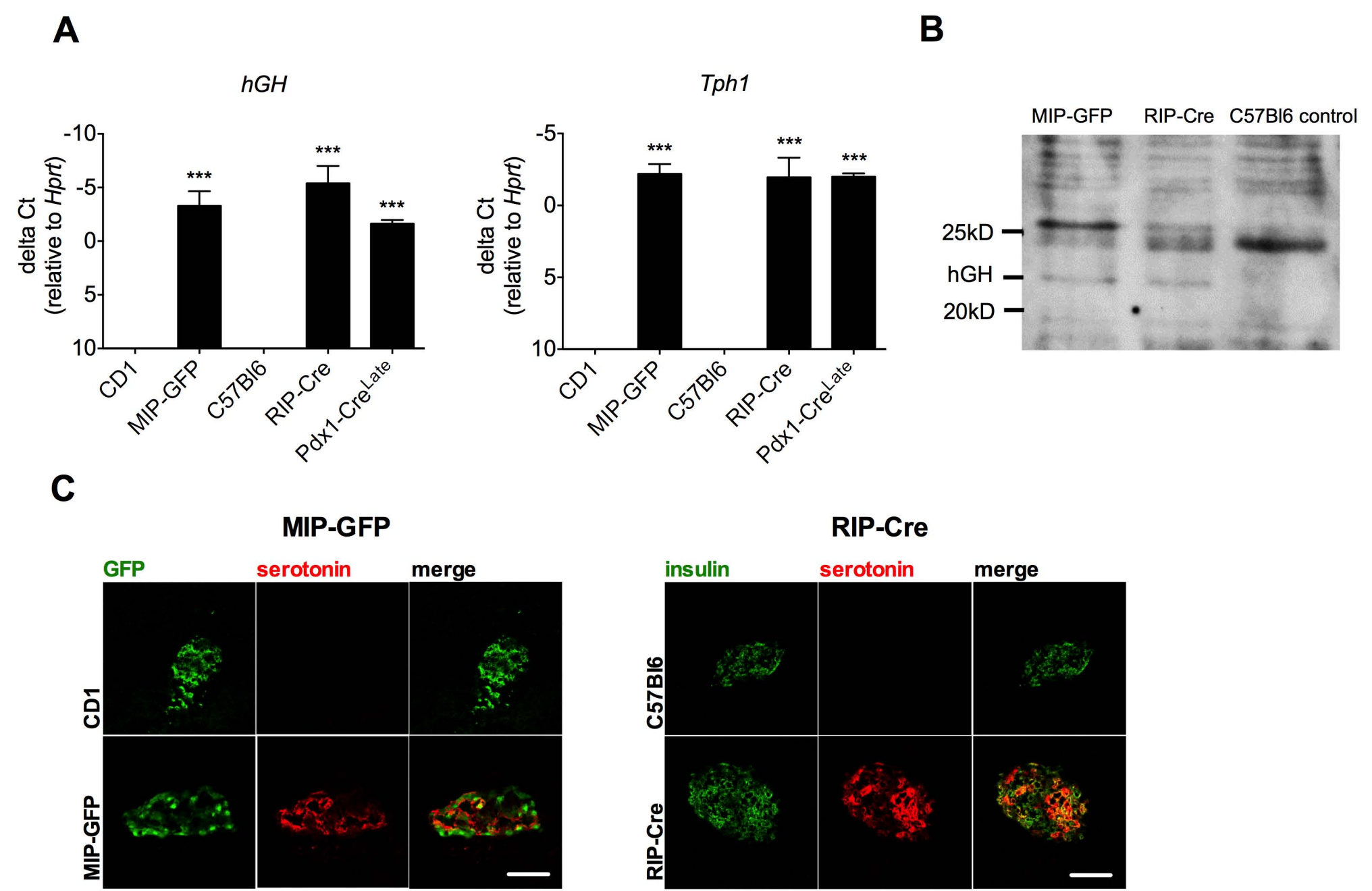
Ryding, A.D., Sharp, M.G., and Mullins, J.J. (2001). Conditional transgenic technologies. The Journal of endocrinology *171*, 1-14.
Sanvito, F., Nichols, A., Herrera, P.L., Huarte, J., Wohlwend, A., Vassalli, J.D., and Orci, L. (1995). TGF-beta 1 overexpression in murine pancreas induces chronic pancreatitis and, together with TNF-alpha, triggers insulin-dependent diabetes. Biochemical and biophysical research communications *217*, 1279-1286.
Schnedl, W.J., Ferber, S., Johnson, J.H., and Newgard, C.B. (1994). STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes *43*, 1326-1333.
Schraenen, A., de Faudeur, G., Thorrez, L., Lemaire, K., Van Wichelen, G., Granvik, M., Van Lommel, L., in't Veld, P., and Schuit, F. (2010a). mRNA expression analysis of cell cycle genes in islets of pregnant mice. Diabetologia *53*, 2579-2588.
Schraenen, A., Lemaire, K., de Faudeur, G., Hendrickx, N., Granvik, M., Van Lommel, L., Mallet, J., Vodjdani, G., Gilon, P., Binart, N.*, et al.* (2010b). Placental lactogens induce serotonin biosynthesis in a subset of mouse beta cells during pregnancy. Diabetologia *53*, 2589-2599.
Sheridan, J.D., Anaya, P.A., Parsons, J.A., and Sorenson, R.L. (1988). Increased dye coupling in pancreatic islets from rats in late-term pregnancy. Diabetes *37*, 908-911.
Soares, M.J. (2004). The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reproductive biology and endocrinology : RB&E *2*, 51.
Song, J., Xu, Y., Hu, X., Choi, B., and Tong, Q. (2010). Brain expression of Cre recombinase driven by pancreas-specific promoters. Genesis (New York, NY : 2000) *48*, 628-634.
Sorenson, R.L., Brelje, T.C., Hegre, O.D., Marshall, S., Anaya, P., and Sheridan, J.D. (1987). Prolactin (in vitro) decreases the glucose stimulation threshold, enhances insulin secretion, and increases dye coupling among islet B cells. Endocrinology *121*, 1447-1453.
Sorenson, R.L., Garry, D.G., and Brelje, T.C. (1991). Structural and functional considerations of GABA in islets of Langerhans. Beta-cells and nerves. Diabetes *40*, 1365-1374.
Thorens, B., Sarkar, H.K., Kaback, H.R., and Lodish, H.F. (1988). Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells. Cell *55*, 281-290.
Vakili, H., Jin, Y., Nagy, J.I., and Cattini, P.A. (2011). Transgenic mice expressing the human growth hormone gene provide a model system to study human growth hormone synthesis and secretion in non-tumor-derived pituitary cells: differential effects of dexamethasone and thyroid hormone. Molecular and cellular endocrinology *345*, 48-57.
Van Assche, F.A. (1974). Quantitative morphologic and histoenzymatic study of the endocrine pancreas in nonpregnant and pregnant rats. American journal of obstetrics and gynecology *118*, 39-41.
Vasavada, R.C., Garcia-Ocaña, A., Zawalich, W.S., Sorenson, R.L., Dann, P., Syed, M., Ogren, L., Talamantes, F., and Stewart, A.F. (2000). Targeted expression of placental lactogen in the beta cells of transgenic mice results in beta cell proliferation, islet mass augmentation, and hypoglycemia. The Journal of biological chemistry *275*, 15399-15406.
Weinhaus, A.J., Stout, L.E., and Sorenson, R.L. (1996). Glucokinase, hexokinase, glucose transporter 2, and glucose metabolism in islets during pregnancy and prolactin-treated islets in vitro: mechanisms for long term up-regulation of islets. Endocrinology *137*, 1640-1649.
Wicksteed, B., Brissova, M., Yan, W., Opland, D.M., Plank, J.L., Reinert, R.B., Dickson, L.M., Tamarina, N.A., Philipson, L.H., Shostak, A.*, et al.* (2010). Conditional gene targeting in mouse pancreatic ß-Cells: analysis of ectopic Cre transgene expression in the brain. Diabetes *59*, 3090-3098.

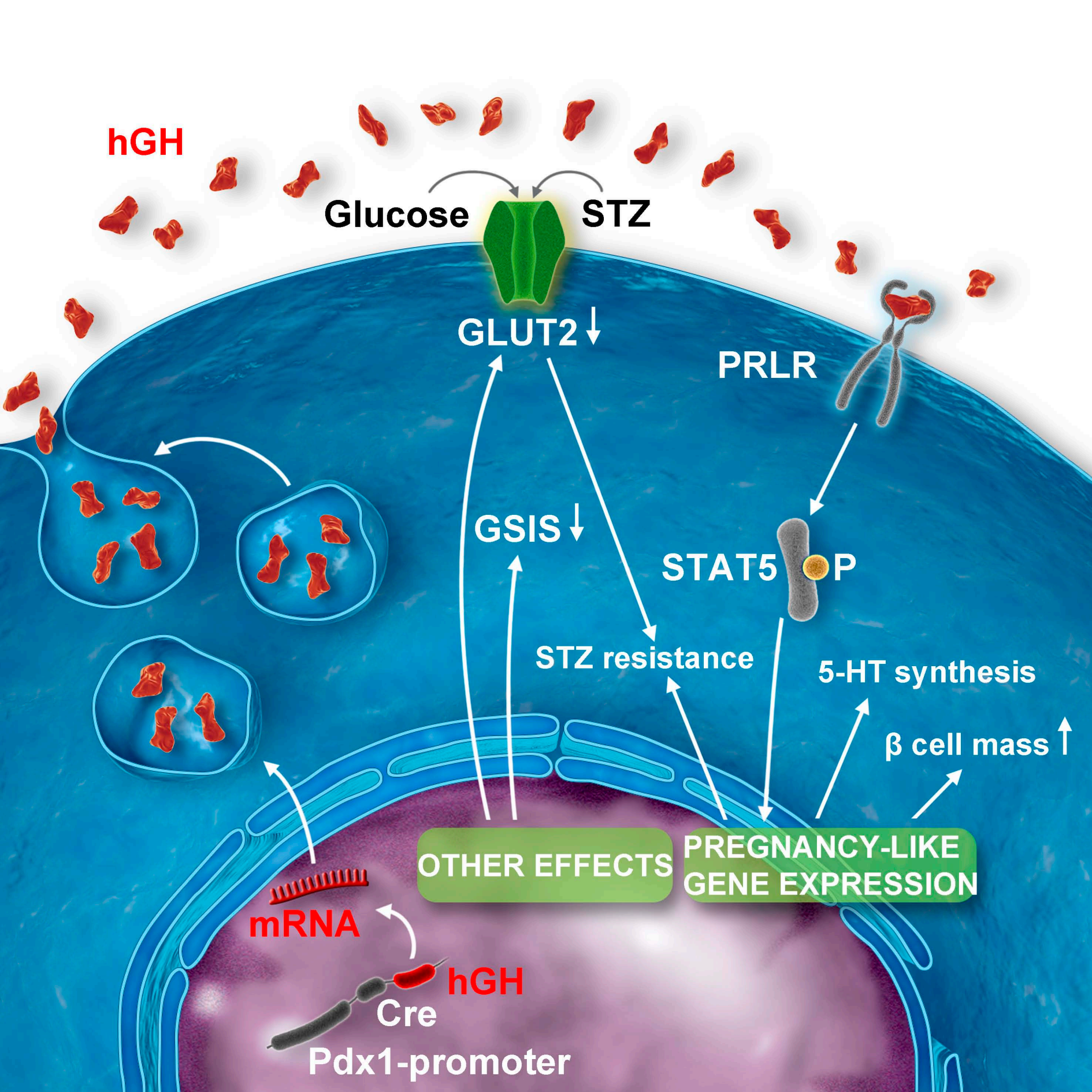

A
Random Fed
blood glucose levels (mg/dl)
150
100
50
0
**
control
Pdx1-CreLate
B
Fasted
blood glucose levels (mg/dl)
150
100
50
0
**
control
Pdx1-CreLate
C
control
Pdx1-CreLate
blood glucose (% of starting level)
100
80
60
40
20
0
0
30
60
time (minutes)
D
β cell mass (mg)
3
2
1
0
**
control
Pdx1-CreLate
E
insulin content (µg/g pancreas)
500
400
300
200
100
0
**
control
Pdx1-CreLate


A
Pdx1 locus
XbaI
SacI
Exon 1
Exon 2
4.5 kb promoter region
promoter
Cre
hGH
4.5 kb
3.1 kb
Pdx1 transgene
B
control
Pdx1-CreLate
hGH delta Ct
(relative to Gapdh)
***
lung
liver
heart
spleen
islets
C
control
Pdx1-CreLate
hGH
GAPDH
D
control
Pdx1-CreLate
E
hGH (pg/islet)
**
control
Pdx1-CreLate
F
hGH release
% of content
**
control
Pdx1-Cre+
G
control
Pdx1-CreLate
P-STAT5
STAT5
GAPDH
H
oPL
mGH
hGH
Tph1 mRNA expression
(normalized for Polr2a)
concentration (ng/ml)
I
Tph1 mRNA expression
(normalized for Polr2a)
n.s.
control
oPL
mGH
hGH
J
0 ng/ml hGH
25 ng/ml hGH
Tph1 mRNA expression
(normalized for Polr2a)
antagonist (μg/ml)


A
Pregnant WT
Non Pregnant WT
Pdx1-Cre Late
WT
222
121
97
Up
57
16
125
Down
B
Relative mRNA expression (normalized for Polr2a)
control
Pdx1-Cre Late
Tph1
Gbp8
Neb
Cish
Cldn8
Matn2
Tnfrsf11b
Tph2
Lonrf3
Car8
Ehhadh
Ivd
C
Relative mRNA expression (normalized for Polr2a)
Pdx1-Cre Late
Pdx1-Cre Late;PRLR -/-
p=0.073
p=0.05
p=0.09
D
Serotonin (pmol/islet)
control
Pdx1-Cre Late
E
GABA (pmol/islet)
control
Pdx1-Cre Late
F
molar serotonin/GABA ratio
control
Pdx1-Cre Late
G
insulin
serotonin
merge
control
Pdx1-Cre Late


A
Glut2 mRNA signal
1.00
0.75
0.50
0.25
0.00
***
control
Pdx1-CreLate
B
insulin
Glut2
merge
control
Pdx1-CreLate
C
control
Pdx1-CreLate
insulin secretion (% of content)
30
15
8
4
2
1
0
**
*
*
G5
G20
G20 + IBMX
D
control
Pdx1-CreLate
blood glucose levels (mg/dl)
500
400
300
200
100
0
***
0
30
60
90
120
150
time (minutes)
E
control
Pdx1-CreLate
plasma [insulin] (ng/ml)
0.5
0.4
0.3
0.2
0.1
0.0
***
*
0
30
60
time (minutes)
F
control
Pdx1-CreLate
***
blood glucose levels (mg/dl)
600
400
200
0
**
*
0
10
20
30
time (days)
G
control
Pdx1-CreLate
blood glucose levels (mg/dl)
600
400
200
0
***
***
***
0
1
2
3
4
5
time (days)
H
PRLR-/-
Pdx1-CreLate;PRLR-/-
blood glucose levels (mg/dl)
600
400
200
0
**
0
1
2
3
4
5
time (days)


A
hGH
Tph1
delta Ct (relative to Hprt)
-10
-5
0
5
10
***
CD1
MIP-GFP
C57Bl6
RIP-Cre
Pdx1-CreLate
B
MIP-GFP
RIP-Cre
C57Bl6 control
25kD
hGH
20kD
C
MIP-GFP
RIP-Cre
GFP
serotonin
merge
insulin
CD1
C57Bl6


hGH
Glucose
STZ
GLUT2
PRLR
GSIS
STAT5
P
STZ resistance
5-HT synthesis
β cell mass
OTHER EFFECTS
PREGNANCY-LIKE
GENE EXPRESSION
mRNA
hGH
Cre
Pdx1-promoter

Supplemental Figure Legends

Figure S1: Pregnancy gene expression signature of control and *Pdx1-cre*^{Late} islets, Related to Figure 3. 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: Reduced islet *Glut2* expression and Impaired glucose tolerance in *Pdx1-cre*^{Late} mice, Related to Figure 4. 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: 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. Related to Figure 5.

Figure S4: 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. Related to Figure 5.

Figure S5: Glucose homeostasis in MIP-GFP versus CD1 controls, Related to Figure 5.. (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: Up- and down regulated genes in islets isolated from Pdx1-Cre^{Late} transgenic mice versus control mice, determined by microarray expression analysis, Related to Figure 3.

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.

Figure S1

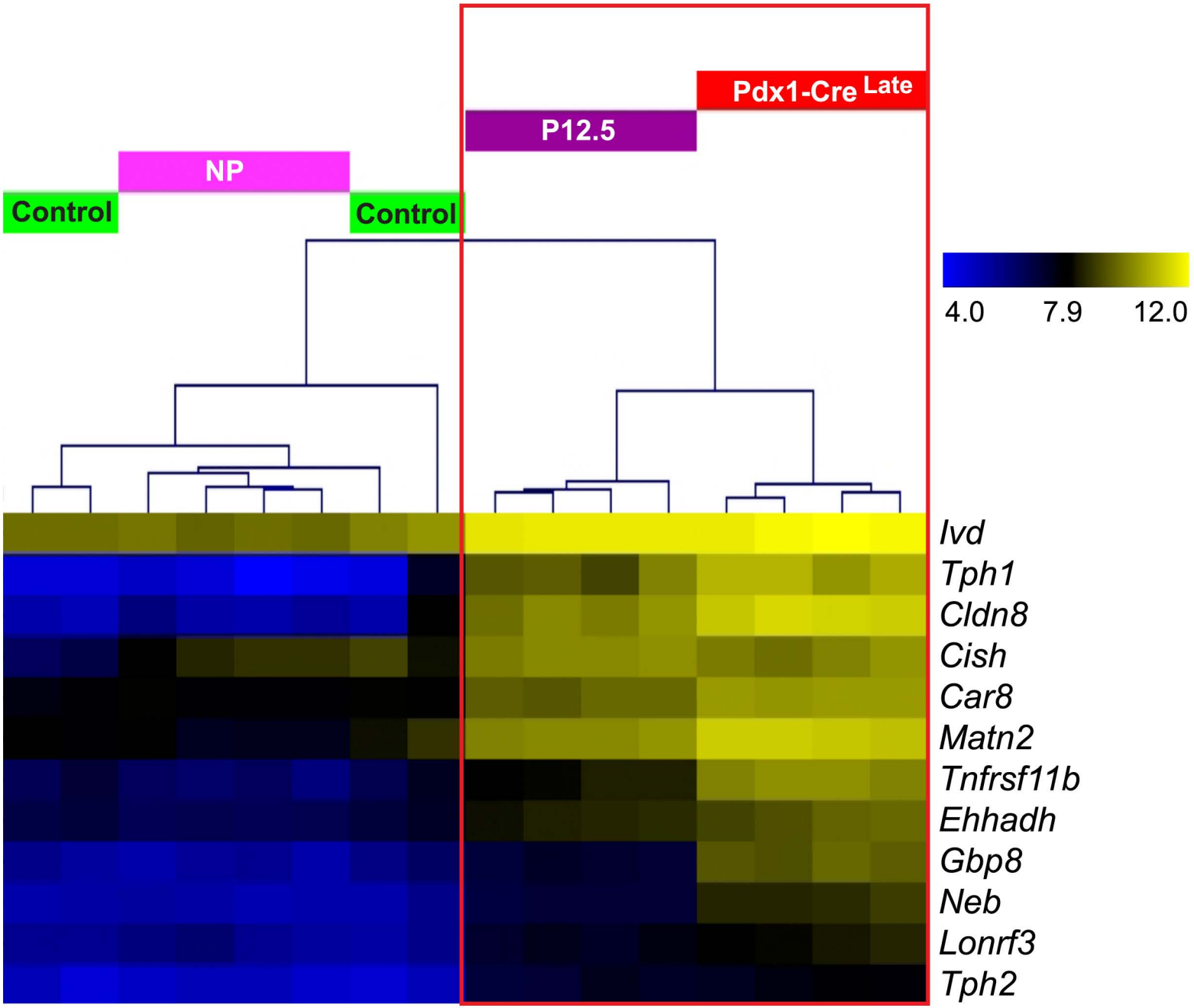
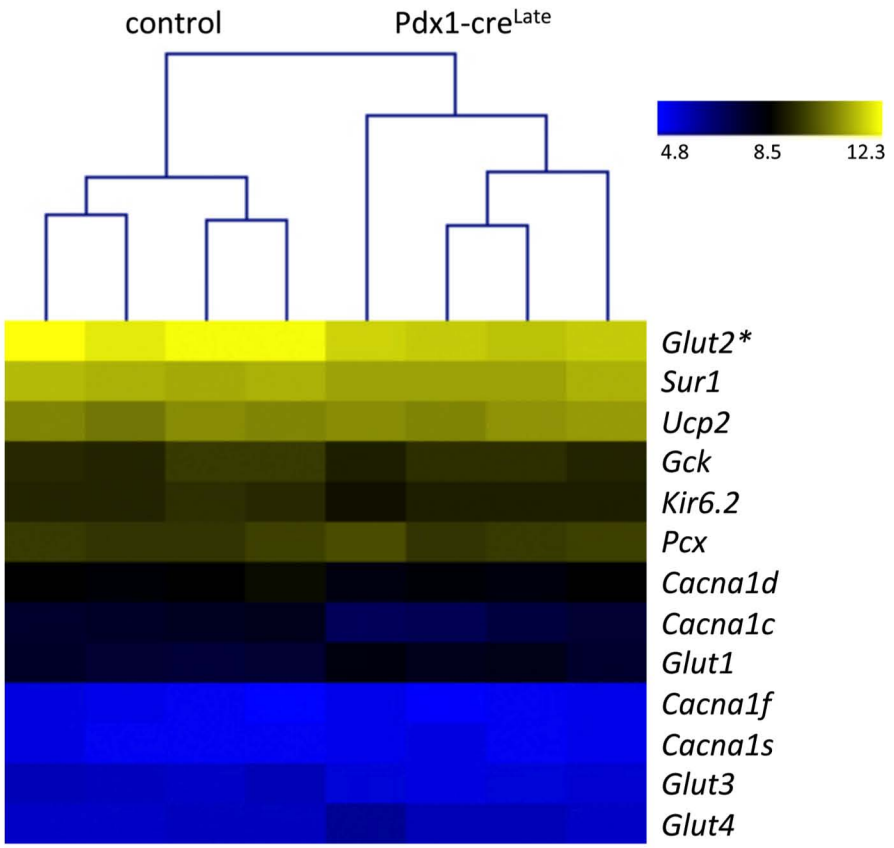

Pdx1-Cre Late
P12.5
NP
Control
Control
4.0
7.9
12.0
Ivd
Tph1
Cldn8
Cish
Car8
Matn2
Tnfrsf11b
Ehhadh
Gbp8
Neb
Lonrf3
Tph2

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

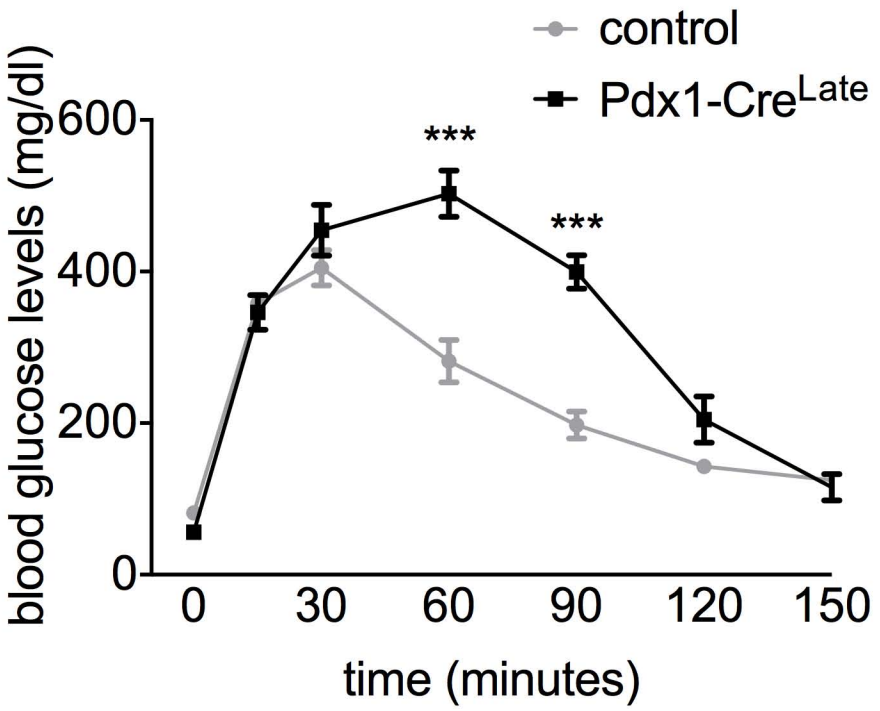

control
Pdx1-Cre^Late
blood glucose levels (mg/dl)
600
400
200
0
***
***
0
30
60
90
120
150
time (minutes)

Figure S3

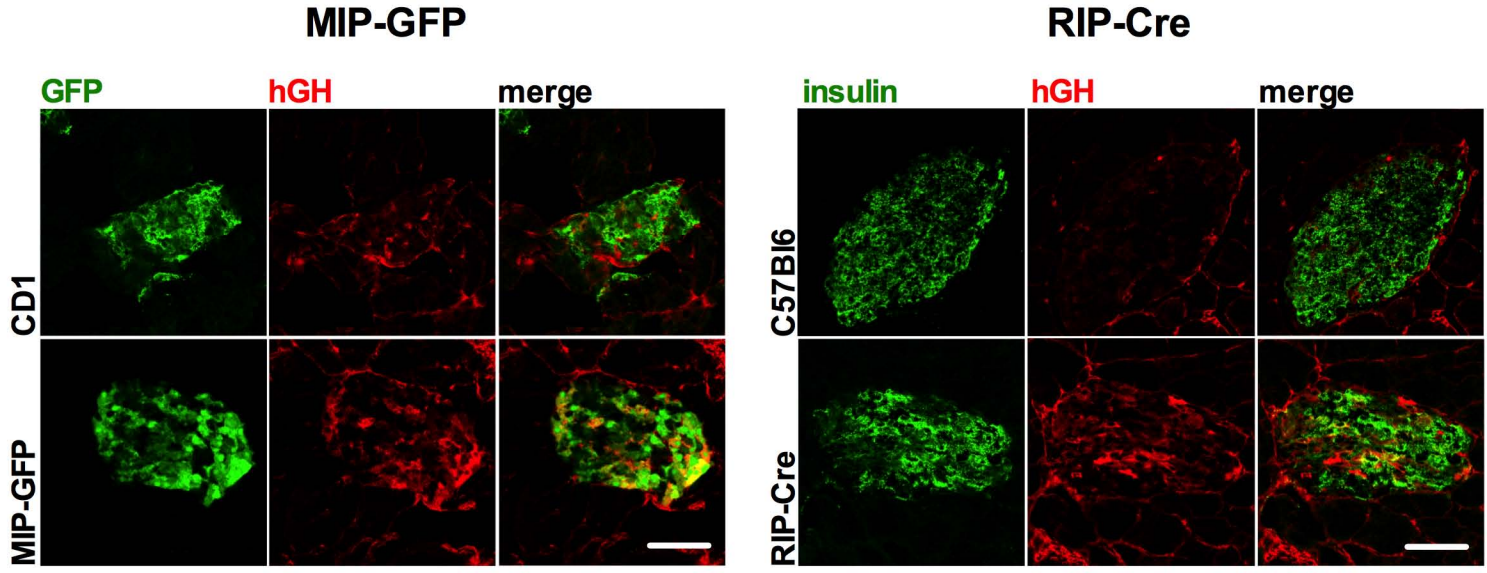

MIP-GFP
RIP-Cre
GFP
hGH
merge
insulin
hGH
merge
CD1
MIP-GFP
C57Bl6
RIP-Cre

Figure S4

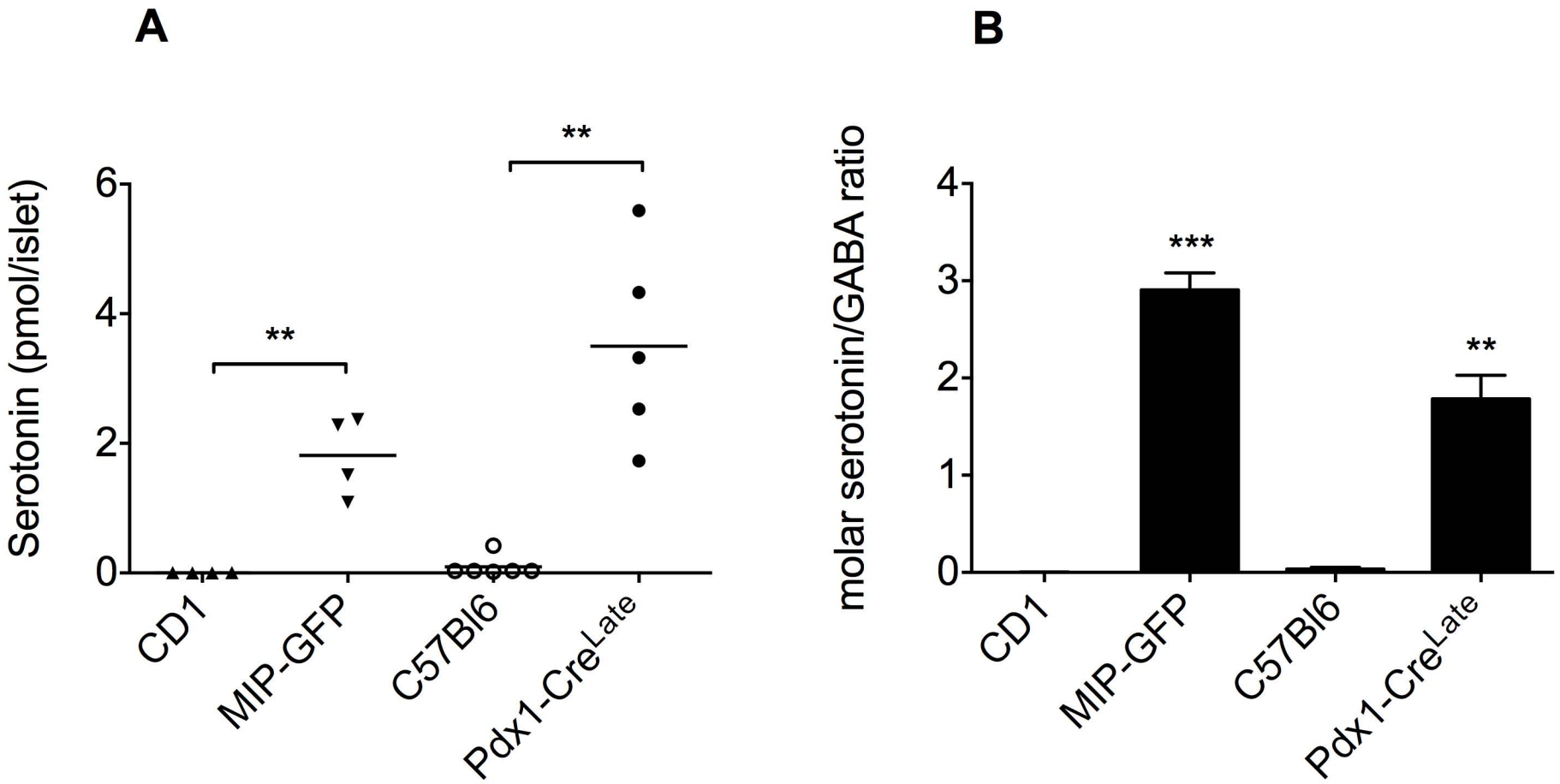
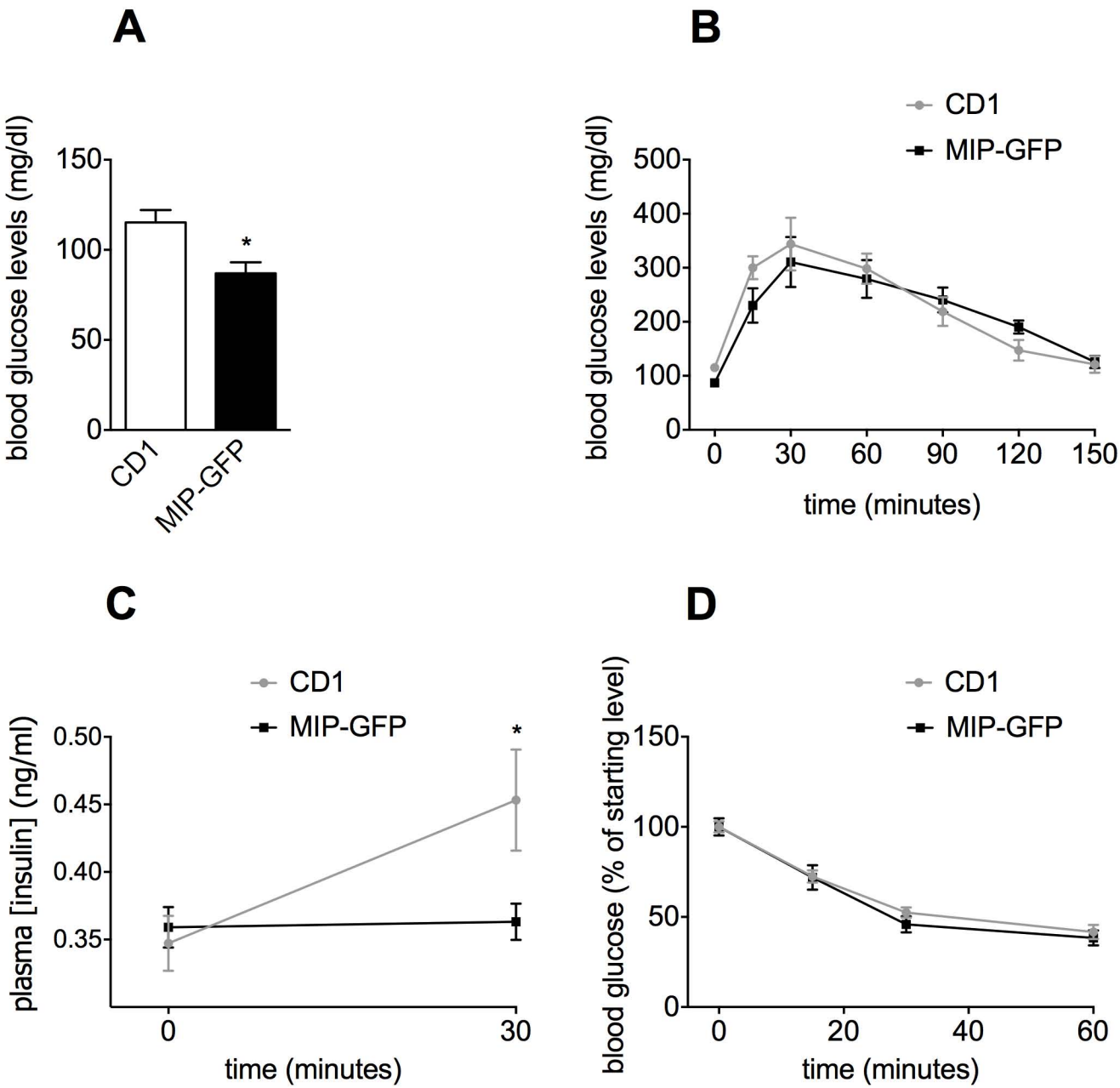

A
Serotonin (pmol/islet)
6
4
2
0
**
**
CD1
MIP-GFP
C57Bl6
Pdx1-CreLate
B
molar serotonin/GABA ratio
4
3
2
1
0
***
**
CD1
MIP-GFP
C57Bl6
Pdx1-CreLate

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)