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The nuclear receptor FXR is expressed in pancreatic β-cells and protects human islets from lipotoxicity

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Keywords: FXR, type 2 diabetes, islets, lipotoxicity

Abbreviations: BA, bile acids; BSA, bovine serum albumin; FGF, fibroblast growth factor; GSCN, guanidinium thiocyanate; FXR, farnesoid X receptor; GSIS, glucose-stimulated insulin secretion; IAPP, islet amyloid polypeptide; IPGTT, intraperitoneal glucose tolerance test; LXR, liver X receptor; PDX1, pancreatic duodenal homeobox gene-1; PPAR(α,δ,γ), peroxisome proliferator-activated receptor (α,δ,γ); SHP, small heterodimer partner; SST, somatostatin; TG, triglycerides; VLDL-R, very low density lipoprotein-receptor.

Abstract

FXR is highly expressed in liver and intestine where it controls bile acid, lipid and glucose homeostasis. Here we show that FXR is expressed and functional, as assessed by target gene expression analysis, in human islets and β-cell lines. FXR is predominantly cytosolic-localized in the islets of lean mice, but nuclear in obese mice. Compared to FXR+/+ mice, FXR-/- mice display a normal architecture and β-cell mass but the expression of certain islet-specific genes is altered. Moreover, GSIS is impaired in the islets of FXR-/- mice. Finally, FXR activation protects human islets from lipotoxicity and ameliorates their secretory index.

1. Introduction

Type 2 diabetes is characterised by the progressive deterioration of β-cell secretory function and its capacity to compensate for increased peripheral insulin resistance [1].

Nuclear receptors are transcription factors that regulate glucose and lipid metabolism in a variety of metabolically active tissues, including the pancreatic β-cell [2]. The Farnesoid X Receptor (FXR; NR1H4), which is highly expressed in liver, intestine and adrenal glands, is a receptor of bile acids (BA) [3-5]. BA-activated FXR protects the liver from BA-overload by controlling the transcription of genes responsible for their synthesis, biotransformation and cellular excretion [6]. FXR also modulates hepatic lipid and lipoprotein metabolism. Recent data identified a role for FXR in glucose homeostasis [7]. FXR gene expression is induced by glucose in rat hepatocytes, whereas insulin reversed this effect [8]. Moreover, FXR mRNA levels are increased in livers of diabetic db/db mice [9]. Treatment of type 2 diabetic dyslipidaemic patients, with intestinal-acting bile acid sequestrants, such as cholestyramine or colesevelam, resulted in lower plasma glucose and HbA1C concentrations [10]. Several authors suggested that BA decrease hepatic glucose production by the liver and therefore improve hyperglycemia by regulating enzymes of gluconeogenesis [11-13]. In addition, FXR-/- mice display impaired glucose and insulin tolerance due to blunted insulin signaling pathways in skeletal muscle and white adipose tissue [13, 14]. Moreover, treatment with GW4064 enhances insulin sensitivity in db/db [13] and ob/ob mice [14], suggesting that FXR agonists could be promising therapeutic agents for type 2 diabetes treatment.

In our previous studies, we have speculated that the decreased clearance of plasma glucose observed in FXR-/- mice upon IPGTT could be explained, besides the peripheral insulin resistance, by an impaired insulin secretion of the pancreas [14]. In order to investigate this hypothesis, we studied whether FXR is expressed in pancreatic β-cells and whether it participates in the regulation of glucose homeostasis by the β-cell. Our results show that FXR is expressed and active in β-cells and pancreatic islets, both in rodents and humans. Using islets isolated from FXR-/- mice or human

islets treated with FXR ligands, we show that FXR controls the proper response of β-cells to a glucose challenge and that FXR activation protects human islets under lipid-induced metabolic stress.

2. Materials and Methods

2.1 Cell culture

Rat INS1E and mouse βTC6 pancreatic β-cells were routinely maintained in RPMI1640 medium (Gibco, Invitrogen) supplemented with 1mM sodium pyruvate, 2mM L-glutamine, 100mM gentamycine, 50μM β-mercaptoethanol, 10% FCS and incubated at 37°C in 5% CO_2.

2.2. Protein extraction and Western blotting – see the Supplementary Data.

2.3 Animals

All studies performed with laboratory animals were approved by the institutional review boards for the care and use of experimental animals. Lean C57Bl6/J wild type and ob/ob male mice were obtained from Charles Rivers Laboratory (France). Homozygous FXR-/- and sex- and age-matched FXR+/+ littermates, bred on the C57Bl6/J genetic background, housed in a pathogen-free barrier facility with 12h light/12h dark cycle, were maintained on a standard laboratory chow diet (A03/R03).

2.4 Mouse and human islet isolation

Mouse pancreatic islets were obtained by the collagenase digestion method [15], adapted as described in *Supplementary Data*. Human islet isolation and culture conditions were described elsewhere [16]. The human islets used in this study were isolated from clinical grade pancreata which, for quantitative reasons (yield < 200,000), could not be liberated for clinical transplantation and thus, in agreement with the French law, were used for scientific research purposes. Mean time between donor death and pancreas harvesting was 5.6 days.

2.5 Islet insulin secretion and extraction- see the *Supplementary Data.*

2.6 Islet morphometry was determined as described in [16]. Measurement of the area of pancreatic islets, as well as that of total pancreatic sections, was manually performed using *Image J* software [17].

2.7 Immunodetection of FXR was performed according to the method of Higashiyama et al.[18], modified as described in *Supplementary Data***.**

2.8 Gene expression

Total RNA was isolated from pancreatic tissue using the GSCN/phenol/chloroform extraction method and from cells using the Trizol reagent (EuroBio) and subsequently reverse-transcribed into cDNA with the Superscript II kit (Applied Bioscience). mRNA levels of the analyzed genes were quantified by real-time quantitative PCR using the DNA Master Mix Syber Green II kit and specific primers listed in *Table 1 (Supplementary Data)*. mRNA levels were normalized to house keeping genes as indicated in the figures.

2.9 Statistics

Results are expressed as indicated in the figure legend. Unpaired Student's t-test was used for measuring the statistical significance of differences between two groups. $P < 0.05$ was considered of statistical significance (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

3. Results

3.1. FXR is expressed and active in pancreatic islets and β-cell lines

FXR mRNA was detected in mouse pancreas and isolated mouse islets (average Ct=28 and 26.5, respectively) (Fig.1A-B) as well as in βTC6 (average Ct=27.8) and INS1E cells (average Ct=27.3) (Fig.1C). Interestingly, unlike in the liver, FXR protein is localized in the extranuclear compartment of islets of lean mice (FXR+/+, OB/OB), whereas in obese mice (FXR+/+, ob/ob), FXR protein is nuclear (Fig.1D). FXR protein was also detected by Western blot in INS1E β-cells (Fig.1E).

To determine whether FXR is active in the pancreas, the expression of SHP and VLDL-R, two positively regulated FXR target genes in the liver [7], was analysed by Q-PCR in the pancreas of FXR-/- vs. FXR+/+ mice (Fig.2A). VLDL-R mRNA was significantly lower in the pancreas of FXR -/- mice, whereas SHP mRNA tended to be lower in FXR-/- mice (p=0.06). To determine whether FXR is active in β-cells, INS1E cells were treated with the specific FXR agonists GW4064 and CDCA. Both agonists induced the expression of SHP and VLDL-R mRNA (Fig.2B).

3.2. FXR-deficiency does not modify the architecture and morphometry of pancreatic islets

FXR-/- mice display no apparent morphological abnormalities of the pancreas. However, at 20 weeks of age, basal (after 6h of fasting) blood glucose and insulin levels of FXR-/- mice were lower compared with FXR+/+ mice (117±10 vs 176±7mg/dL and 1.00±0.1 vs 1.83±0.35µg/L, respectively). Although the hypoglycaemia of FXR-/- mice can explain their lower basal insulinemia, a modified architecture or a β-cell mass defect of the pancreas in FXR-/- mice could also contribute to this. To verify this hypothesis, islet morphometric analysis was performed in FXR-/- vs +/+ mice (Fig.3A). Islet surface was not different between FXR-/- and FXR+/+ mice. The β-cell mass, calculated as the number of islets per pancreatic surface, was also equivalent between FXR-/- and FXR+/+ mice. In addition, staining with antibodies against glucagon and insulin evidenced that

the organisation of α- and β-cells was preserved in FXR-/- compared to FXR+/+ islets (Fig.3B).

3.3 GSIS is impaired in islets isolated from FXR-/- mice

To study the functionality of the islets of FXR-/- mice, independent of any systemic influences, size-matched islets isolated from 20-25 weeks-old FXR-/- and FXR+/+ mice were incubated in the presence of low (2.8mM) and high (20mM) glucose concentrations (Fig.4A). The insulin response of FXR-/- islets to high glucose was significantly reduced compared to that of FXR+/+ mice ($p<0.01$). Consequently, the capacity of individual islets to secrete insulin upon glucose stimulation was lower in FXR-/- vs. FXR+/+ islets (insulinogenic index=2.66 vs. 4.57, respectively). The total insulin content of non-stimulated FXR-/- islets was also significantly lower compared with that of FXR+/+ islets (Fig.4B).

3.4 The expression of β-cell markers is altered in the pancreas of FXR-/- mice

The defect in GSIS of FXR-/- islets suggested that β-cells lacking FXR display abnormalities in glucose-sensing and/or in the cascade of glucose metabolism. mRNA levels of two β-cell specific hormones, insulin and IAPP, were lower in FXR-/- mice (Fig.5A). A significant decrease of SST mRNA was also observed in the FXR-/- mice, but no alteration in the expression of glucagon was seen (data not shown).

Therefore, we examined the expression of β-cell transcription factors that are necessary for glucose-sensing and stimulation of insulin secretion by the mature, differentiated β-cell (Fig.5B). MafA and PDX-1 mRNA expression, two master transcription factors regulating the insulin gene and GSIS in the mature β-cell [19], were decreased in FXR-/- pancreas. mRNA levels Beta2/NeuroD1, a key transcription factor of islet cells [20], were also reduced in FXR-/- pancreas, while mRNA level of Nkx6.1, a transcription factor primarily expressed in adult β-cells [21] was not modified.

3.5 FXR activation protects human pancreatic islets from lipotoxicity

FXR mRNA was found to be expressed in human islets isolated from 3 brain-dead donors at a level similar to that of the nuclear receptor PPARα, but higher than PPARγ (Fig.6A). When human islets were exposed *in vitro* to the FXR agonists GW4064 (5μM) or CDCA (50μM), the expression of FGF-19 mRNA, a positively regulated FXR target gene in humans [22], increased significantly in the presence of both agonists, albeit to a larger extent with the synthetic ligand GW4064 (Fig.6B). This result suggests that FXR is also active in human islets.

Incubation of the islets with GW4064 (5μM) or CDCA (50μM) decreased triglyceride accumulation and improved the viability of islets exposed to palmitate (Fig.6C-D). Consistently, activation of FXR with GW4064 or CDCA improved the stimulation index of the human islets (Fig. 6E). Interestingly, this effect was not due to an increase of insulin secretion after high glucose stimulation (20mM), but rather to a normalization of insulin secretion at low glucose (2.8mM) (Fig.6E). In the islets not exposed to palmitate, activation of FXR did not influence these parameters.

4. Discussion

In the present study we show that FXR mRNA and protein is expressed in mouse and human islets and in β-cell lines. Q-PCR analysis revealed that C57Bl6 mice significantly express FXR in the pancreas. FXR expression in isolated islets, although less important than in the liver, is comparable to that in adipose tissue (average Ct=29.01), where FXR plays a regulatory role [14]. Recently, Chuang et al. screened the expression of nuclear receptors in the endocrine pancreas and found the presence of FXR mRNA [23]. While our study was in progress, Renga et al. reported the expression of FXR in βTC6 cells and human islets where its activation has positive effects on insulin secretion [24]. Interestingly, we found that in mouse islets, FXR seems to be localized in the cytoplasmic compartment, whereas it translocates, under metabolic stress conditions such as insulin resistance

and obesity, to the nucleus. FXR-/- mice do not display on altered islet area or β-/α-cell distribution, suggesting that FXR is not required for the maintenance of islet architecture or β-cell mass in mice under normal diet conditions. However, analysis of the expression of several specific islet hormones and transcription factors revealed decreased levels in FXR-/- pancreases. PDX-1, MafA and Beta2/NeuroD1 contribute to both β-cell specific and glucose-responsive insulin gene transcription by interacting with cis-regulatory elements located in the promoter of the (pro)insulin gene [19] and interact with each other to regulate β-cell function [25].

Whole body FXR-/- mice display complex alterations in glucose and lipid metabolism since FXR-deficiency influences metabolism in several organs (i.e liver, adipose tissue) [7]. To study the tissue-autonomic function of FXR in the endocrine pancreas independently of other systemic influences, GSIS tests were performed on isolated islets of both genotypes. The secretory capacity of the islets (insulinogenic index) was decreased in FXR-/- vs. FXR+/+ islets. In addition, the total insulin content of isolated FXR-/- islets was also reduced, suggesting that the observed hypoinsulinaemia of FXR-/- mice after 6 hours of fasting is not solely due to reduced liver glucose output and plasma glucose levels in these mice.

Since FXR is nuclear localised in the endocrine cells of ob/ob mice, FXR could play a role in β-cell function under lipid-stress conditions. Indeed, treatment of human islets with FXR agonists protects islets from palmitate-induced TG accumulation and apoptosis. In addition, we found that FGF-19, a member of the FGF-family, is expressed and induced by FXR agonists in human islets. Indeed, FGF signalling is required for a proper glucose sensing in mouse β-cells [26] and in human HepG2 cells, where FGF-19 has an inhibitory effect on fatty acid synthesis induced by insulin [27]. Whether this mechanism occurs also in human β-cells, remains to be established.

In conclusion, our study identifies a role for FXR in the control of islet function and opens promising new perspectives for the prevention of type 2 diabetes.

5. Acknowledgments

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7. Figure legends

Fig. 1 FXR is expressed in pancreatic islets and β-cells. ***A,B***. FXR mRNA levels in the pancreases and islets of 20-26 week-old mice (n=3-5) vs. liver set at 1 (mean±SD) ***C***. FXR mRNA levels measured in INS1E and βTC6 cells vs. HepG2 hepatoma cells, set at 1 (mean±SD; n=3) ***D***. Immunohistochemical staining of FXR protein in pancreatic islets of lean (OB/OB) and obese (ob/ob) mice vs. FXR-/- mice as negative control. Liver sections of OB/OB and ob/ob mice were analysed as positive controls. Pancreatic sections obtained from lean mice were also stained only with the secondary antibody and compared with anti-FXR stained sections, in order to verify the specificity of the antibody ("Antibody control") - see *Supplementary Data* for details. Images are representative of 3 independent experiments. ***E*.** Immunodetection of FXR in total (T) or nuclear protein fraction (N) of INS1E and HepG2 cells.

Fig. 2 Endogenous FXR is transcriptionally active in the pancreas and β-cells. ***A.*** SHP and VLDL-R mRNA levels in the pancreas of FXR-/- and FXR+/+ mice (mean±SE; n=9). ***B.*** SHP and VLDL-R mRNA regulation in INS1E cells after 48h incubation with GW4064 (5μM) and CDCA (50μM) (mean±SD; n=3).

Fig. 3 FXR-deficiency does not alter islet morphometry and architecture. ***A.*** Mean islet area relative to cumulative pancreatic section area and islet density were calculated for pancreases of FXR-/- and FXR+/+ mice (mean±SE; n=7). ***B***. Islet architecture assessed by immunofluorescence in FXR-/- and FXR+/+ mice.

Fig. 4 FXR-deficiency results in lower islet glucose-stimulated insulin secretion and lower insulin content. ***A.*** GSIS performed in islets isolated from FXR+/+ and FXR-/- mice. ***B***. Total islet insulin content of FXR+/+ and FXR-/- islets (mean±SD; n=5). See *Supplementary Data* for details.

Fig. 5 FXR-deficiency results in altered expression of genes encoding islet hormones and β-cell transcription factors. Gene expression of islet hormones ***(A)*** and β-cell transcription factors ***(B)*** was analysed by Q-PCR in the pancreas of FXR-/- vs. FXR+/+ mice (mean±SE; n=9). The level in

FXR+/+ mice was set at 1.

Fig. 6 FXR is expressed and functional in human islets. *A*. Expression profile of several nuclear receptors in human pancreatic islets, compared with PDX1 mRNA expression, set at 1. The values were normalized to TFIIb mRNA level (mean±SE; n=3). The average Ct values are: 28.8 (PPARα), 31.8 (PPARγ), 27.6 (PPARδ), 29.2 (FXR), 27.9 (LXRβ), 26 (PDX1). ***B.*** FGF-19 mRNA level in human islets incubated for 48h with RPMI containing GW4064 or CDCA (mean±SE; n=3). The average Ct values obtained for FGF-19 are: 27.5 (DMSO), 24 (GW4064) and 26 (CDCA). TG content ***(C)***, apoptosis ***(D),*** low- and high-GSIS and the stimulation index ***(E***) of human islets incubated with FXR agonists and palmitate 0.33 mM (black bars) or fatty acid-free BSA (white bars) for 48h. Results correspond to 40IE (islet equivalent) and to 1h of static incubation (mean±SE; n=3). *P<0.05, ***P<0.001.

Figure(s)

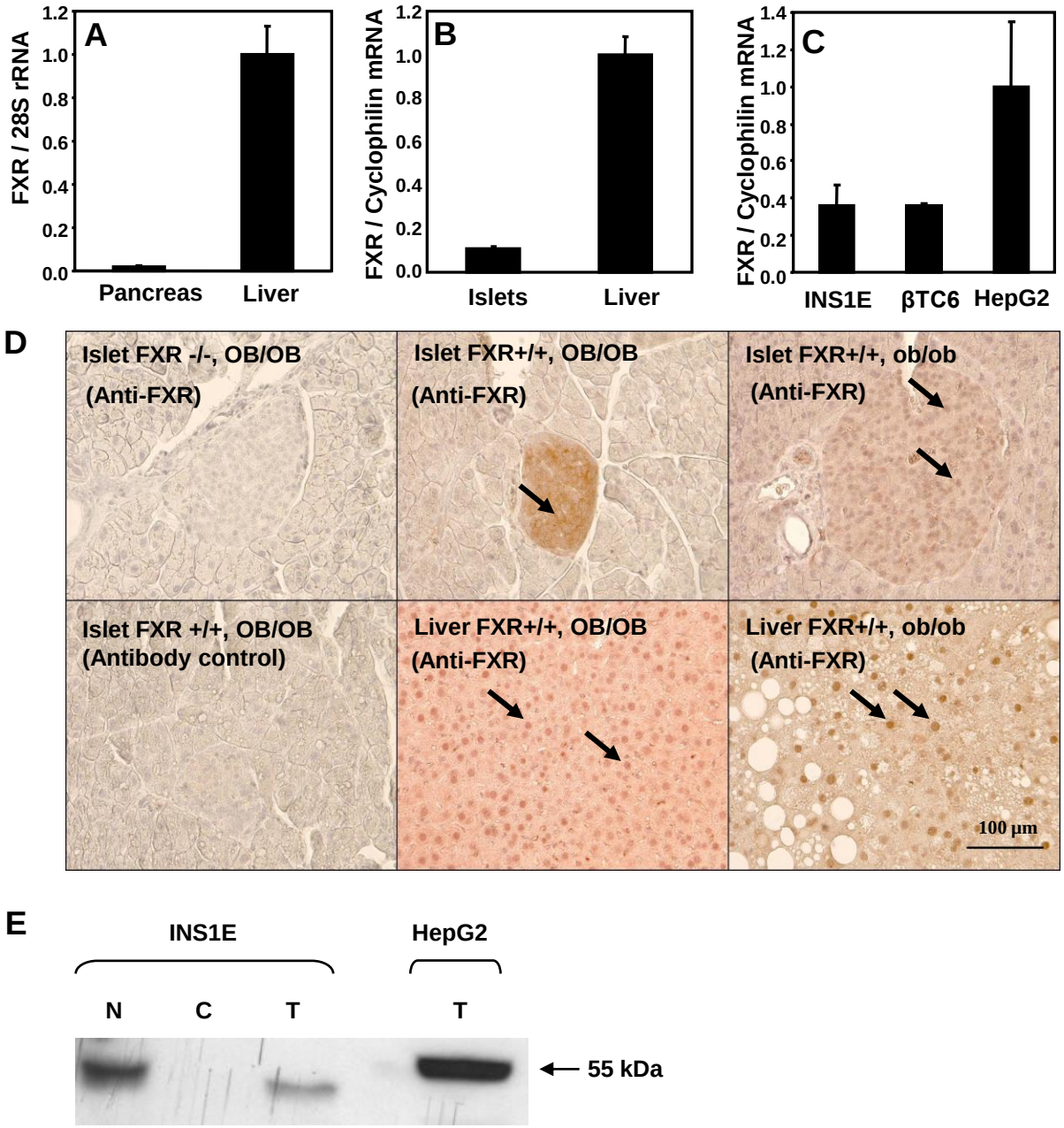
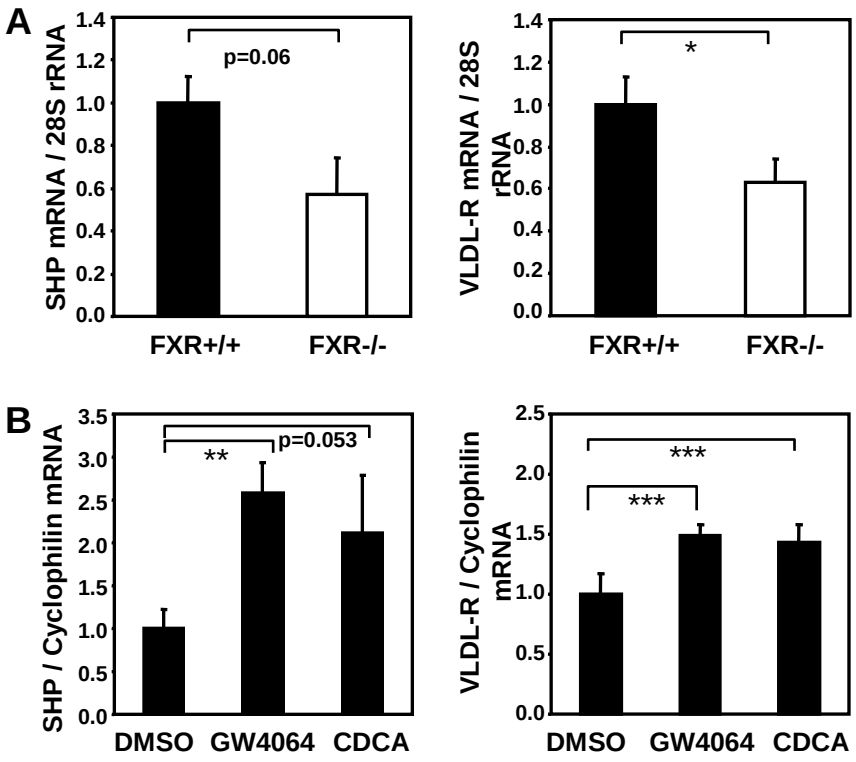


Fig. 1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1.2
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0.8
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p=0.06
FXR+/+
FXR-/-
VLDL-R mRNA / 28S rRNA
*
FXR+/+
FXR-/-
B
SHP / Cyclophilin mRNA
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2.0
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DMSO
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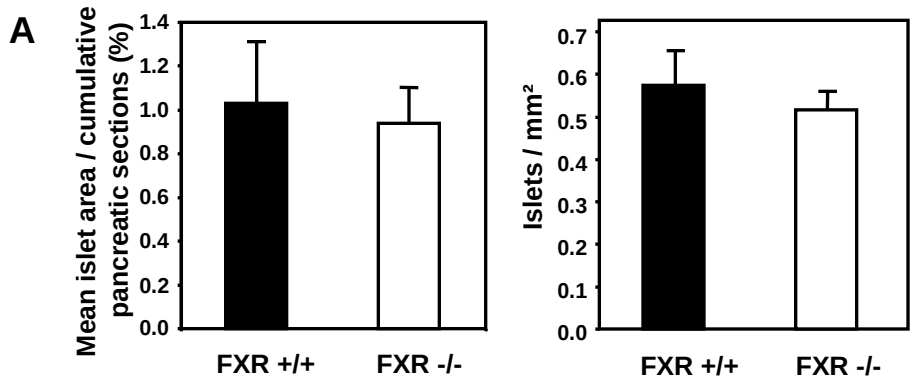
Fig. 2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Mean islet area / cumulative pancreatic sections (%)
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1.2
1.0
0.8
0.6
0.4
0.2
0.0
FXR +/+
FXR -/-
Islets / mm²
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
FXR +/+
FXR -/-

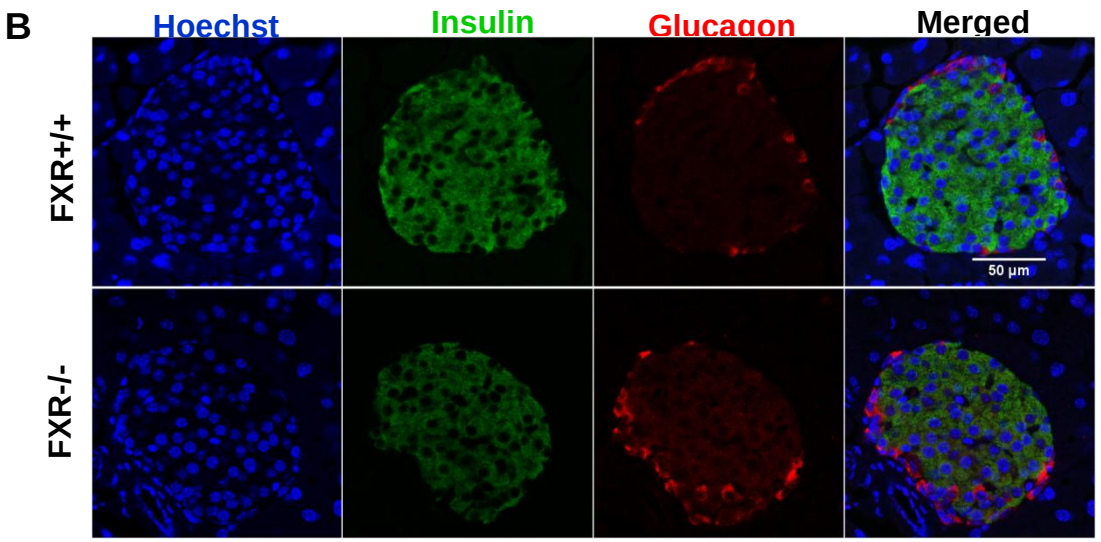
B
Hoechst
Insulin
Glucagon
Merged
FXR+/+
FXR-/-
50 µm

Fig. 3

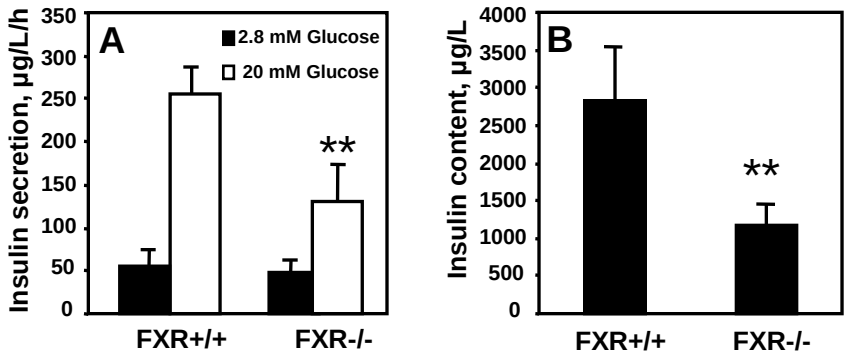
A
2.8 mM Glucose
20 mM Glucose
Insulin secretion, µg/L/h
350
300
250
200
150
100
50
0
**
FXR+/+
FXR-/-
B
Insulin content, µg/L
4000
3500
3000
2500
2000
1500
1000
500
0
**
FXR+/+
FXR-/-

Fig. 4

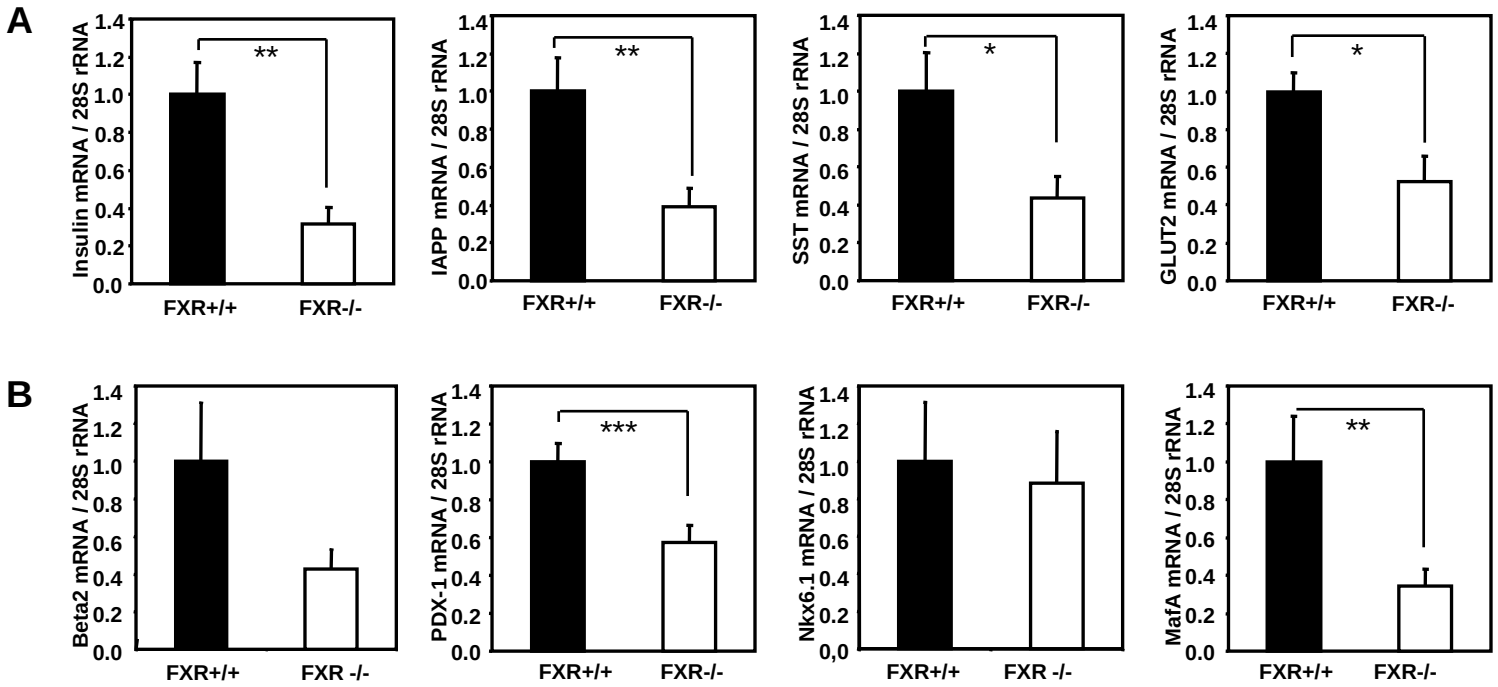
A
Insulin mRNA / 28S rRNA
IAPP mRNA / 28S rRNA
SST mRNA / 28S rRNA
GLUT2 mRNA / 28S rRNA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
*
*
FXR+/+
FXR-/-
B
Beta2 mRNA / 28S rRNA
PDX-1 mRNA / 28S rRNA
Nkx6.1 mRNA / 28S rRNA
MafA mRNA / 28S rRNA
***
**
FXR+/+
FXR -/-
FXR-/-

Fig. 5

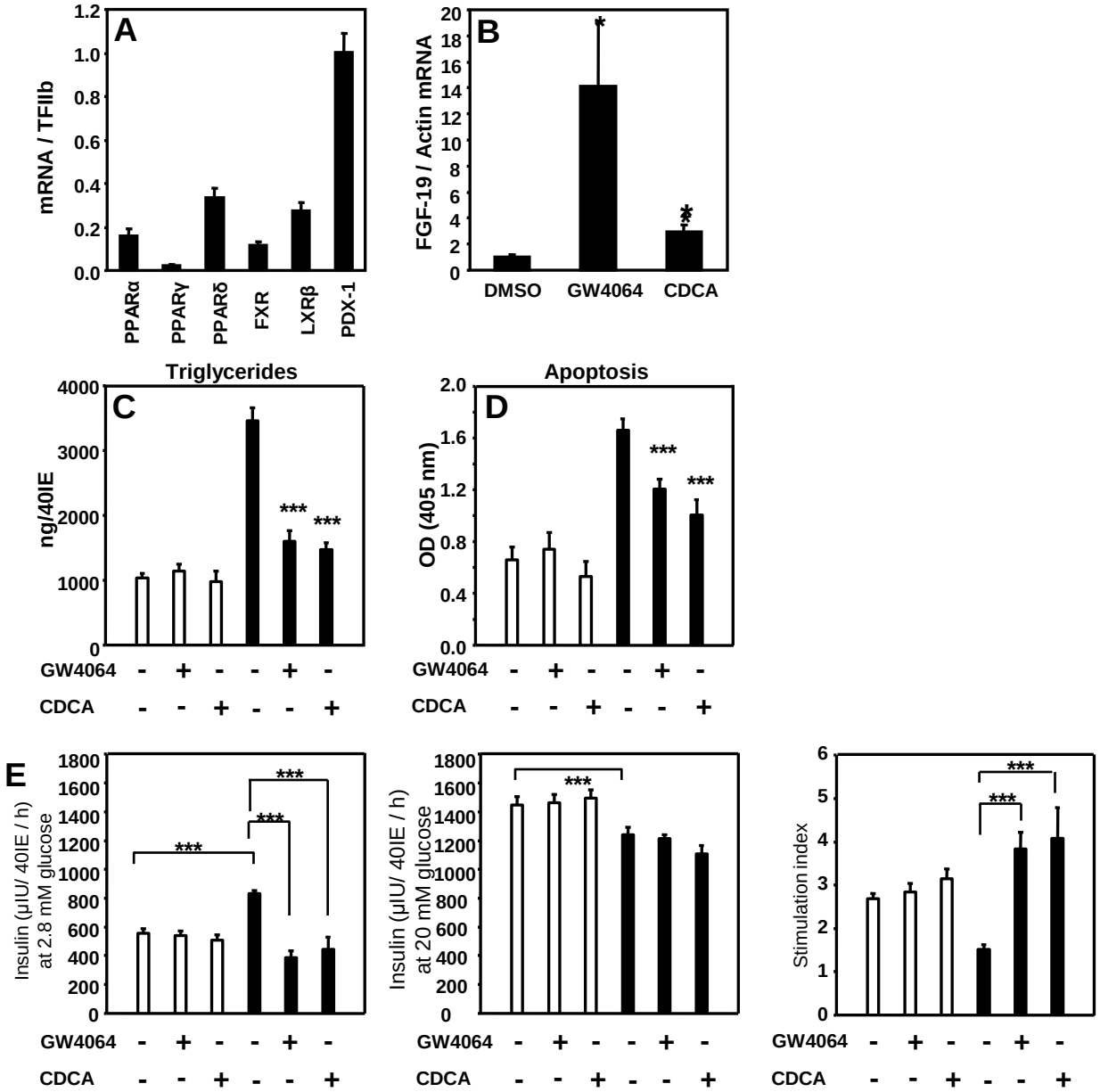
A
mRNA / TFIIb
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PPARα
PPARγ
PPARδ
FXR
LXRβ
PDX-1
B
FGF-19 / Actin mRNA
20
18
16
14
12
10
8
6
4
2
0
*
*
DMSO
GW4064
CDCA
Triglycerides
C
ng/40IE
4000
3000
2000
1000
0
***
***
GW4064 - + - - + -
CDCA - - + - - +
Apoptosis
D
OD (405 nm)
2.0
1.6
1.2
0.8
0.4
0.0
***
***
GW4064 - + - - + -
CDCA - - + - - +
E
Insulin (µIU/ 40IE / h) at 2.8 mM glucose
1800
1600
1400
1200
1000
800
600
400
200
0
***
***
***
GW4064 - + - - + -
CDCA - - + - - +
Insulin (µIU/ 40IE / h) at 20 mM glucose
1800
1600
1400
1200
1000
800
600
400
200
0
***
GW4064 - + - - + -
CDCA - - + - - +
Stimulation index
6
5
4
3
2
1
0
***
***
GW4064 - + - - + -
CDCA - - + - - +

Fig. 6