DOI:
10.1111/dom.13529

Document Version
Peer reviewed version

Short chain fatty acids stimulate insulin secretion and reduce apoptosis in mouse and human islets in vitro: role of FFAR2

Pingitore A.[1], Gonzalez-Abuin N.[2], Ruz-Maldonado I.[1], Huang G.C.[1], Frost G.[2] and Persaud S.J.[1] *

1. Department of Diabetes, Faculty of Life Sciences & Medicine, King's College London (UK)

2. Division of Human Nutrition, Faculty of Medicine, Imperial College London (UK)

Short running title: ScFAs act via FFAR2 in islets of Langerhans

Abstract word count: 247

Main body of the text word count: 3589

Number of references: 35

Number of figures: 5

Abstract

Aims: The aims of this study were to evaluate the role of free fatty acid receptor 2 (FFAR2/GPR43) in mediating the effects of the short chain fatty acids (ScFAs) sodium acetate (SA) and sodium propionate (SP) on islet function in vitro, and to identify the intracellular signalling pathways utilised in ScFA-induced potentiation of glucose-induced insulin secretion.

Material and methods: Islets of Langerhans were isolated from WT and FFAR2$^{-/-}$ mice and from non-diabetic human donors. The effects of SA and SP on dynamic insulin secretion from perifused islets were quantified by radioimmunoassay, signalling downstream of ScFAs was profiled by single-cell calcium microfluorimetry, and measurement of cAMP by fluorescence assay. Islet apoptosis was induced by exposure to cytokines or sodium palmitate and the effects of SA and SP to regulate islet apoptosis were assessed by quantification of caspase 3/7 activities.

Results: Deletion of FFAR2 did not affect islet morphology or insulin content. SA and SP reversibly potentiated insulin secretion from mouse islets in a FFAR2-dependent manner. ScFA-induced potentiation of insulin secretion was coupled to Gq activation of phospholipase C and protein kinase C, with no evidence of Gi-mediated signalling. SA and SP protected human and mouse islets from apoptosis and these pro-survival properties were dependent on islet expression of FFAR2.

Conclusions: Our results indicate that FFAR2 directly mediates both the stimulatory effects of SA and SP on insulin secretion and their protection against islet apoptosis. We have also shown that ScFA coupling in islets occurs via Gq-coupled intracellular signalling.

Introduction

Diet-derived short-chain fatty acids (ScFAs) originate from the intestinal fermentation of fibre and undigested carbohydrates by bacteria residing in the colonic lumen [1]. They consist of fatty acid chains of up to 6 carbons, with acetate (C2), propionate (C3) and butyrate (C4) representing the main circulating forms in the human body [2]. In recent years it has become apparent that ScFAs are the endogenous ligands for two G-protein coupled receptors, namely FFAR2 (GPR43) and FFAR3 (GPR41) [3], and that these receptors are expressed in organs playing a role in glucose homeostasis. In particular, we and others have identified that FFAR2 and FFAR3 are expressed by islets of Langerhans [4-7], opening up the possibility that ScFAs contribute to the regulation of insulin secretion through activation of these receptors. However, the data published so far do not provide a consensus role for ScFAs and their receptors in islets: the first paper published in this area reported that acetate inhibited insulin release in vitro and that deletion of FFAR2 and FFAR3 improved glucose tolerance [7], while there have been more recent reports of ScFAs stimulating insulin secretion and deletion of FFAR2 being associated with insufficient β-cell mass expansion in obesity and gestational diabetes [5, 6, 8-12]. Acetate is the most abundant circulating ScFA, reaching plasma concentrations of approximately 200μM [13], while propionate concentrations are approximately 10-fold lower [2], and these are the two ScFAs that are used most commonly for experimental studies. They can couple to both inhibitory and stimulatory signalling through activation of FFAR2 and/or FFAR3, which differ in their downstream signalling cascades [3, 14]. Thus, while FFAR3 couples only to Gi to decrease intracellular cyclic AMP levels, FFAR2 is able to recruit both Gi and Gq to decrease cyclic AMP and increase diacylglycerol and inositol trisphosphate generation. We have previously demonstrated that cannabinoid receptor agonists that signal through both Gi and Gq increase insulin secretion, as the stimulatory input outweighs the reduction in cyclic AMP production [15,16], and it has recently been reported that a Gq-biased FFAR2-selective agonist potentiates glucose-induced insulin secretion [12]. Consistent with this, we have reported that propionate reversibly stimulates insulin secretion from human islets through a Gq/protein kinase C-dependent pathway [5].

Observations of stimulatory signalling in β-cells via FFAR2 contrast with the report that transgenic mice with global FFAR2 deletion show enhanced insulin secretion, which implicates pre-eminence of the Gi signalling pathway in mouse β-cells [7]. In the current paper we have used islets isolated from wildtype and FFAR2$^{-/-}$ mice to quantify the effects of acetate and propionate on dynamic insulin secretion and determine the roles of intracellular calcium and PKC activation in ScFA signalling in mouse islets. We have also determined whether ScFAs can protect mouse islets from apoptosis induced by lipo- and cyto-toxic assault, and the role of FFAR2 in this. Key experiments exploring the role of acetate on insulin secretion and islet survival were also carried using isolated human islets.

MATERIAL AND METHODS

Materials

Culture media and supplements, general laboratory chemicals and genotyping primers were from Merck (Dorset, UK); tissue culture flasks, coverslips and ThermoPrime 2x ReddyMix PCR Master Mix were from Thermo Fisher Scientific (Paisley, UK); Cell Titer-Glo 3D assay and Caspase3/7 Glo assay were from Promega (Hampshire, UK); cyclic AMP HiRange assay was from Cisbio (Codolet, France); TNFα, IL-1β and IFNγ were from PeproTech (London, UK); the high capacity reverse transcriptase kit was from Applied Biosystems (Woolston, UK) and RNeasy RNA extraction kits and Quantitect qPCR primers were from Qiagen (Manchester, UK). Anti-insulin, anti-glucagon and anti–somatostatin antibodies were from DAKO (Cambridge, UK) and Alexa-fluor secondary antibodies were from GE Healthcare Life Sciences (Little Chalfont, UK).

Mice

All animal procedures were approved by the UK Home Office Animals (Scientific Procedures) Act 1986 (project licence number: 70/8068). FFAR2 global knockout (FFAR2$^{-/-}$) mice were obtained from Professor McKay at the Garven Institute, Australia. FFAR2 deletion in C57Bl/6J mice was obtained by

homologous recombination which substitutes a 55bp of Ffar2 exon 1 with the β-gal-neo cassette, shifting the downstream amino acid sequence out of the reading frame and the progenies were generated through cross breeding of mice heterozygous for FFAR2 ($FFAR2^{+/-}$). Genotypes of offspring were determined by standard PCR as described earlier [17]. All mice were housed at 21-23°C on a 12h light/dark cycle at Imperial College London, with food and water provided *ad libitum*.

Islet isolation

Islets were isolated from $FFAR2^{-/-}$ mice (aged 8-10 weeks) and age-matched wildtype (WT) mice, essentially as described [18]. Islets were maintained overnight at 37°C in RPMI supplemented with 10% FBS, 2mM glutamine and 100U/mL/0.1mg/mL penicillin/streptomycin. Human islets were isolated from the pancreases of seventeen non-diabetic donors (BMI: 28.8±1.3; donor age: 41.6±3.1 years; 8M, 9F) at the King's College Hospital Islet Transplantation Unit, with appropriate ethical approval (LREC 01-082) [19] and maintained for up to 48 hours in CMRL supplemented with 2% human albumin, 4mM glutamine, 2mM HEPES (pH 7.2-7.4), and 10mM nicotinamide. When used for mRNA extraction, islets were snap frozen in liquid nitrogen immediately after isolation.

Quantification of FFAR3 mRNA expression

Total RNA was extracted from groups of 200 islets isolated from either WT or $FFAR2^{-/-}$ mice using the Qiagen RNeasy Minikit [4], then mRNA was reverse-transcribed into cDNA, which was quantified using a NanoDrop spectrophotomoter. Quantitative PCR reactions were performed on a Lightcycler 480 using 1µg of cDNA and specific primers for *FFAR3*, and expression levels were normalised to *Gapdh* mRNA expression in the same samples [4].

Fluorescence immunohistochemistry

5µm sections of paraffin-embedded fixed pancreases from WT or $FFAR2^{-/-}$ mice were boiled in 0.01M citric acid buffer (pH 6.0) for 2.5 minutes for antigen retrieval, blocked with 0.1% donkey serum in

PBS/0.02% triton X-100 for 1 hour then incubated at 4°C overnight with guinea pig anti-insulin (1:250), rabbit anti-glucagon (1:250) and rabbit anti-somatostatin (1:250) antibodies. Sections were exposed to Alexa-fluor secondary antibodies (1:250) for 1 hour at room temperature and fluorescence images were acquired using a Nikon Eclipse TE2000-U microscope.

Insulin secretion and content

Groups of 40 mouse or 50 human islets were perifused at 0.5mL/min with a physiological salt solution [20] supplemented with agents of interest and perifusate samples were collected every two minutes for quantification of secreted insulin [21]. In some experiments islet PKC was down-regulated by maintenance of islets for 20 hours in the presence of 200nM 4β phorbol 12-myristate 13-acetate (PMA) and control islets were treated with the inactive phorbol ester 4α phorbol 12,13 didecanoate (PDD) [22], and in other experiments islets were exposed to 100ng/mL of pertussis toxin (PTX) for 18 hours to block Gi-dependent signalling [23]. For measurements of insulin content, groups of 10 islets were sonicated in 100μL of acidified ethanol (absolute ethanol: deionised water: concentrated HCl in the ratio of 52:17:1v/v) and insulin was quantified by radioimmunoassay following 1/10,000 dilution of samples in assay buffer [21].

Calcium microfluorimetry

Groups of 100,000 mouse islet cells adherent on UV-sterilised acetic acid-washed glass coverslips were incubated for 15 minutes with 5μM Fura-2 AM. The coverslips were placed in a steel chamber on a heating platform and cells were perifused with a physiological salt solution [20] containing test agents. Real-time changes in $[Ca^{2+}]_i$ were determined by illuminating cells alternately at 340nm and 380nm, and light emitted from groups of cells in the field of view with a x20 objective was filtered at 510nm and recorded with a CCD camera [21].

Cyclic AMP quantification

Groups of 5 mouse or human islets were incubated for 1 hour at 37°C in a physiological salt solution [20] containing test agents of interest and supplemented with 2mM IBMX to inhibit phosphodiesterases. Islets were lysed and cAMP levels were quantified using a fluorescence assay, according to the manufacturer's instructions. Data were acquired using a PHERAstar FS microplate reader (100μs delay time, 100μs integration time, 50 flash read time).

ATP generation

Groups of 3 islets were incubated for 1 hour at 37°C and ATP levels were determined by chemoluminescence with the Promega® 3D Cell Titer-Glo assay, according to the manufacturer's instructions [24].

Apoptosis

Mouse or human islets were pre-cultured in complete medium in the absence or presence of propionate or acetate for 24h, then groups of 5 islets were exposed to RPMI with 2% FBS (mouse) or CMRL with 0.2% albumin (human) supplemented either with 0.5mM sodium palmitate or a cytokine cocktail (5U/μl TNFα, 0.5U/μl IL-1β, 5U/μl IFNγ) in the continued absence or presence of acetate or propionate for a further 20h. Palmitate was initially made as a 100mM stock in 50% ethanol, diluted to 5mM in 10% fatty acid-free BSA in RPMI, then further diluted in RPMI (2% FBS) to 0.5mM for experimental use. Islet cell apoptosis was determined using the Caspase3/7 Glo assay according to the manufacturer's instructions [5]. In this assay activation of the effector caspases 3 and 7 leads to cleavage of a proluminescent substrate, resulting in generation of a luminescent signal that was quantified using a Veritas luminometer at 450nm.

Statistical Analysis

Static incubation data were analysed with Prism® Graphpad using one way ANOVA followed by Dunnet multiple comparison test or Student's t-tests, as appropriate. Perifusion secretion data were analysed

by one or two way ANOVA with repeated measures. Differences were considered statistically significant when $p<0.05$.

RESULTS

FFAR2 deletion does not impair mouse islet physiological parameters.

Immunohistochemical analysis of pancreas sections indicated that global deletion of FFAR2 did not have any effect on islet morphology, with a similar proportion of insulin, glucagon and somatostatin immunostaining observed in islets of WT and FFAR2$^{-/-}$ mice (Figure 1a), as determined by Image J analysis of individual image acquisitions. Similarly, loss of FFAR2 did not induce any significant changes in the amplitude of glucose-induced insulin secretion from isolated islets (Figure 1b), nor in the islet insulin content (Figure 1c), and no differences in islet size or yield were identified during the isolation procedure. In addition, FFAR2 deletion was not associated with any changes in expression of the other ScFA receptor, FFAR3 in islets (Figure 1d).

Acetate and propionate potentiate glucose-stimulated insulin secretion from mouse islets in a FFAR2-dependent manner.

Quantification of dynamic insulin secretion from mouse islets revealed that both SA and SP reversibly potentiated glucose-induced insulin secretion in a FFAR2- dependent manner. Thus, islets responded within two minutes to exposure to 1mM SA (Figure 2a) or 1mM SP (Figure 2b) such that the plateau phase of secretion in response to 20mM glucose was significantly enhanced by both fatty acids. The effect of SA was of lower amplitude and less sustained than that of SP, which induced a potentiation that was maintained for the duration of exposure and readily reversible upon its removal. The requirement of FFAR2 for the stimulatory effects of SA and SP was evident from their inability to potentiate insulin release when islets isolated from FFAR2$^{-/-}$ mice were used (Figures 2a and 2b, open circles).

Effects of acetate and propionate on islet second messenger generation.

We have previously shown that SP increases $[Ca^{2+}]_i$ in human islets [5], consistent with Gq-mediated signalling. Microfluorimetry measurements were made from Fura-2-loaded mouse islet β-cells, which were identified by their characteristic biphasic response to an elevation in extracellular glucose concentration (Figures 3a and 3b). These experiments indicated that SA elevated β-cell $[Ca^{2+}]_i$, with a transient potentiation of the elevation induced by 20mM glucose (Figure 3a). This effect of SA was completely dependent on the presence of FFAR2, because it did not increase $[Ca^{2+}]_i$ in islets from FFAR2$^{-/-}$ mice, whereas the response to 20mM glucose was not impaired by FFAR2 deletion (Figure 3b).

The potential contribution of Gi-mediated signalling in the effects of SA and SP in islets was investigated by the quantification of mouse islet cAMP levels by FRET. Elevations in islet cAMP were induced by exposure to 1μM forskolin in the presence of the phosphodiesterase inhibitor IBMX, and the Gi-coupled α_2-adrenergic agonist clonidine completely suppressed cAMP production in response to forksolin (Figure 3c). However, under the same conditions neither 1mM SA nor 1mM SP had any significant effects on islet cAMP generation, and no significant differences in cAMP levels were observed in the responses of islets from WT and FFAR2$^{-/-}$ mice (Figure 3c).

As ScFAs may regulate intracellular signalling secondary to their mitochondrial metabolism their effects on islet ATP generation were also investigated. These experiments indicated that elevation in glucose concentration from 2mM to 20mM stimulated ATP levels, as expected (Figure 3d), but 1mM SA or SP did not elevate basal or glucose-stimulated ATP production. Similar to the experiments in which cAMP was quantified, deletion of FFAR2 did not influence glucose-induced ATP generation in mouse islets, and nor did it modify the levels of ATP observed in the presence of SA or SP (Figure 3d).

Acetate potentiates insulin secretion from human islets via PLC/PKC-dependent pathways, but does not activate Gi-coupled signalling.

Our earlier experiments investigating ScFA signalling in human islets focused on the role of SP [5] so we extended these studies here to determine the concentration-dependent effects of the shorter ScFA, SA, on insulin secretion from isolated human islets, and the signalling pathways utilised. It can be seen from Figure 4a that SA stimulated a concentration-dependent reversible potentiation of glucose-induced insulin release, which was statistically significant at concentrations as low as 1μM SA (Figure 4b). We have previously reported that SP-induced insulin secretion from human islets involves the recruitment of PLC/PKC-dependent pathways [5]. To verify whether SA utilises similar signalling cascades to SP, isolated human islets were pre-perifused for 10 minutes with 10μM of the PLC inhibitor U73122 (open circles) or vehicle (closed circles), and dynamic perifusion indicated that the potentiation of glucose-induced insulin secretion by 100μM SA was lost following PLC inhibition (Figure 4c). In parallel experiments human islets were pre-incubated for 20 hours with PMA to deplete PKC [25] prior to perifusion. As shown in Figure 4d, 100μM SA induced a large potentiation of insulin secretion in islets that had been pre-treated with vehicle (solid circles) or with the inactive phorbol ester PDD (open squares), and this secretory response to SA was significantly reduced after PKC depletion (open circles).

The stimulation of insulin release from human islets by SA is in contrast to a previous report of its Gi-mediated inhibition of glucose-stimulated insulin secretion from human islets in static incubations [8]. We therefore investigated whether SA acted via Gi in human islets by pre-treating them with 100ng/mL PTX to ADP-ribosylate Gi and thus abolish signalling via this pathway. As can be seen from Figure 4e, PTX-treated islets (open circles) responded to SA with a similar profile to the vehicle-treated islets (closed circles), indicating that inhibition of Gi-dependent signalling did not unveil any further potentiation of insulin secretion by SA. Further demonstration that SA does not act via Gi in human

islets was evident from the lack of inhibitory effect of SA on intracellular cAMP levels (1μM forskolin: 120.8±11.9nM; +1mM SA: 144.8±7.6nM, $p>0.2$; +10μM clonidine: 79.6±6.8nM, $p<0.001$, n=6).

ScFAs protect islets from apoptosis

The effects of ScFAs on isolated islets are not limited to improving their insulin secretory performance. Previously we established that overnight exposure of human islets to SP protects against both cytokine and palmitate-induced apoptosis [5], and we observed that SA also has an anti-apoptotic effect in human islets in the current study (Figure 5a). To determine whether ScFAs also reduce apoptosis in mouse islets, and the role of FFAR2 in this effect, apoptosis was induced in islets isolated from WT and FFAR2$^{-/-}$ mice by exposure to a cytokine cocktail (Figure 5b, black bars) or 0.5mM palmitate (Figure 5b, grey bars). Incubation of islets with SA or SP provided significant protection against the elevation in islet caspase 3/7 activities induced by cytokines or palmitate (Figure 5b, upper panel), and these anti-apoptotic effects of the ScFAs were significantly reduced or abolished when the experiments were carried out using islets from FFAR2$^{-/-}$ mice (Figure 5b, lower panel).

Discussion

The beneficial effects of diets rich in fibre and fermentable carbohydrates on glucose homeostasis in humans has been evident for several years [1, 2, 5, 26-30], and it is now recognised that dietary fermentable precursors lead to elevations in SA and SP both in the colonic lumen and in the general circulation [5, 14]. The G-protein coupled receptor FFAR2 has been implicated in the effects of ScFAs in colonic L-cells, adipocytes and immune cells to promote secretion of incretin hormones, reduce lipolysis and regulate production of immune mediators [4, 27-32]. The demonstration of FFAR2 involvement in GLP-1 and PYY secretion from the intestine suggests that beneficial effects of ScFAs on islets could be, at least in part, secondary to the indirect stimulatory and protective effects of these incretins on islet β-cells [27, 28, 33]. However, the presence of FFAR2 on mouse and human β-cells [4-

7] supports a direct role in islets for those ScFAs originating from colonic fermentation of dietary fibre and undigested carbohydrates.

Although FFAR2 and FFAR3 deletion is reported to improve glucose tolerance in obese mice [7], it has also been reported that FFAR2$^{-/-}$ mice fed a high fat diet show no changes in glucose tolerance [8] and that obese [6] and pregnant [9] FFAR2$^{-/-}$ mice develop glucose intolerance, so it is not clear from published studies whether FFAR2 improves or impairs glucose homeostasis. The observation that FFAR2$^{-/-}$ mice have reduced β-cell mass at birth suggests a role for FFAR2 in β-cell development, and the reduced β-cell area was also observed in adulthood [11]. However, β-cell proliferation is elevated in FFAR2$^{-/-}$ mice [11] and deletion of FFAR2 does not impair glucose tolerance in mice fed normal chow [6, 8, 9], suggesting that FFAR2 is not essential for normal glucose homeostasis. Consistent with this, our immunohistochemical analysis of islets from 8-10 week old FFAR2$^{-/-}$ mice and quantification of insulin secretion and content demonstrate that there was no impairment of functional β-cell mass following FFAR2 deletion. Since in vivo measurements on global FFAR2$^{-/-}$ mice do not specifically identify the role of this receptor in regulating β-cell function, in the current study we have used isolated mouse and human islets to identify direct functional effects of SA and SP, distinct from any influence of circulating mediators released from other cells expressing FFAR2.

Our data with isolated islets build on our earlier study in which we demonstrated direct potentiation of glucose-induced insulin secretion from human islets by SP [5], and our use here of islets isolated from FFAR2$^{-/-}$ mice has now allowed us to identify that ScFAs directly stimulate insulin secretion and protect against apoptosis in a FFAR2-dependent manner. In addition, we have identified that SA and SP signal in mouse and human islets via stimulatory Gq-dependent pathways, such that they activate PLC and stimulate FFAR2-dependent elevation in $[Ca^{2+}]_i$. Furthermore, experiments in which islet PKC was down-regulated indicate that this kinase is important in mediating SA potentiation of insulin secretion, most likely secondary to PLC-mediated diacylglycerol generation and increased $[Ca^{2+}]_i$.

However, the stimulatory effects of SA on insulin release were not abolished following PKC depletion, implicating a PKC-independent mode of action, perhaps via another Ca^{2+}-dependent pathway such as activation of Ca^{2+}-calmodulin-dependent kinase II [34].

The capacity of ScFAs to bind to and activate both FFAR2 and FFAR3 has led to some inconsistencies in published data, because FFAR3 couples only to Gi whereas FFAR2 couples to both Gi and Gq [14]. Inhibitory effects of ScFAs on insulin secretion have been reported, most likely a consequence of Gi-mediated reductions in intracellular cAMP levels [7]. Our results indicate that ScFAs do not reduce cAMP production by mouse or human islets, and the effects of SA to potentiate insulin secretion were not modified by PTX-induced inactivation of Gi signalling in human islets. The data that we obtained with PTX treatment of human islets also indicate that its abolition of the cAMP inhibitory tone does not result in an increase in basal or glucose-stimulated insulin secretion, as is observed with PTX inactivation of Gi signalling in mouse islets [23]. The reasons for this difference is not clear, but these observations suggest that tonic cAMP inhibition is more important in mouse than human islets.

It has recently become apparent that co-expression of FFAR2 and FFAR3 by cells can result in formation of FFAR2/FFAR3 heteromers in which ScFA-induced inhibition of cAMP generation is impaired and Ca^{2+} signalling is enhanced [35]. It is thus possible that FFAR2/FFAR3 heteromers in β-cells allow ScFAs to preferentially couple to PLC activation to promote stimulatory cascades.

In addition to promoting insulin secretion we found that SA and SP also exerted anti-apoptotic actions in islets under both cytotoxic and lipotoxic conditions that mimic the physiological stresses to which islets are exposed during type 1 and type 2 diabetes. The use of islets from $FFAR2^{-/-}$ mice has allowed us to identify the important role of this receptor in transducing not only the stimulatory effects of SA and SP on insulin release, but also their protective effects against apoptotic stimuli. These data are in agreement with earlier reports that FFAR2 is required for the adaptive expansion of β-cell mass during

pregnancy [28], and that the loss of FFAR2 is linked to impaired islet mass and β-cell survival [29]. Nevertheless, more studies are needed to elucidate the intracellular pathways that are activated by FFAR2 to sustain islet viability and mass expansion.

In conclusion, our work provides important data to support a re-evaluation of FFAR2 in mediating stimulatory effects of the ScFAs SA and SP in islets. The SA- and SP-induced reversible potentiation of glucose-induced insulin secretion and protection against apoptosis are typical of an effective therapy for type 2 diabetes, and the FFAR2-dependence of these effects suggest that FFAR2 represents a potential pharmacological candidate for the treatment of impaired glucose homeostasis and type 2 diabetes.

Acknowledgements

We thank the families of pancreas donors that have made human islets available for this study. This work was funded by the European Commission [PIEF-GA-2013-622612-BRiDiT to AP] and the Society for Endocrinology [Early Career Award to AP].

References

1. Cani P, Everard A, Duparc T. Gut microbiota, enteroendocrine functions and metabolism Curr Opin Pharmacol. 2013;13:935-940.

2. Canfora E, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity Nature Rev Endocrinol 2015;11:577-591.

3. Brown AJ, Goldsworthy SM, Barnes AA et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 2003 Mar 28;278(13):11312-9

4. Amisten S, Salehi A, Rorsman P et al. An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans. Pharmacol Ther. 2013 Sep;139(3):359-91

5. Pingitore A, Chambers ES, Hill T et al. The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. Diabetes Obes Metab, 2017: 19(2):257-265

6. McNelis JC, Lee YS, Mayoral R et al. GPR43 Potentiates β-Cell Function in Obesity. Diabetes 2015, 64 :3203-3217.

7. Tang C, Ahmed K, Gille A, et al. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes Nature Med. 2015; 21:173-177.

8. Priyadarshini M, Villa SR, Fuller M, et al. An Acetate-Specific GPCR, FFAR2, Regulates Insulin Secretion. Mol Endocrinol. 2015;29:1055-1066.

9. Fuller M, Priyadarshini M, Gibbons SM, et al. The short-chain fatty acid receptor, FFA2, contributes to gestational glucose homeostasis. Am J Physiol Endocrinol Metab. 2015;309:E840-E851.

10**.** Fuller M, Li X, Fisch R, et al. FFA2 Contribution to gestational glucose tolerance is not disrupted by antibiotics. PLoS One. 2016 Dec 13;11(12):e0167837. doi: 10.1371/journal.pone.0167837

11. Villa SR, Priyadarshini M, Fuller MH, et al. Loss of Free Fatty Acid Receptor 2 leads to impaired islet mass and beta cell survival. Sci Rep. 2016; 6:28159.

12. Villa SR, Mishra RK, Zapater JL, et al. Homology modeling of FFA2 identifies novel agonists that potentiate insulin secretion. J Investig Med. 2017 Dec;65(8):1116-1124. doi: 10.1136/jim-2017-000523.

13. Pomare EW, Branch WJ, Cummings JH. Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. J Clin Invest. 1985 May;75(5):1448-54.

14. Le Poul E, Loison C, Struyf S, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem 2003;278:25481–25489

15. Li C, Jones PM, Persaud SJ. Cannabinoid receptors are coupled to stimulation of insulin secretion from mouse MIN6 beta-cells Cell Physiol Biochem 2010; 26:187-196.

16. Li C, Bowe JE, Huang GC, et al. Cannabinoid receptor agonists and antagonists stimulate insulin secretion from isolated human islets of Langerhans. Diabetes Obes Metabol 2011;13:903-910.

17. Maslowski et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 2009; 461-1282-1286

18. Papadimitriou A, King AJF, Jones PM, Persaud SJ. Anti-Apoptotic Effects of Arachidonic Acid and Prostaglandin E2 in Pancreatic β-Cells. Cell Physiol Biochem 2007 20:607-616.

19. Huang GC, Zhao M, Jones P, et al. The development of new density gradient media for purifying human islets and islet-quality assessments. Transplantation 2004, 77:143-145.

20. Gey GO, Gey MK. The maintenance of human normal cells and tumor cells in continuous culture. Am J Cancer. 1936;27:45-76.

21. Jones PM, Salmon DM, Howell SL. Protein phosphorylation in electrically permeabilized islets of Langerhans. Effects of Ca^{2+}, cyclic AMP, a phorbol ester and noradrenaline. Biochem J. 1988;254:397-403.

22. Persaud SJ, Liu B, Jones PM. Functional analysis of human islets of Langerhans maintained in culture. Methods Mol Biol. 2012;806:55-71.

23. Persaud SJ, Jones PM, Howell S. Effects of Bordetella pertussis toxin on catecholamine inhibition of insulin release from intact and electrically permeabilized rat islets. Biochem J. 1989 Mar 15;258(3):669-75.

24. Pingitore A, Ruz-Maldonado I, Liu B, et al. Dynamic Profiling of Insulin Secretion and ATP Generation in Isolated Human and Mouse Islets Reveals Differential Glucose Sensitivity. Cell Physiol Biochem. 2017;44(4):1352-1359

25. Persaud SJ, Jones PM, Sugden D, Howell S. The role of protein kinase C in cholinergic stimulation of insulin secretion from rat islets of Langerhans. Biochem J. 1989 Dec 15;264(3):753-8.

26. Byrne CS, Chambers ES, Morrison DJ, Frost G. The role of short chain fatty acids in appetite regulation and energy homeostasis. Int J Obes (Lond). 2015 Sep;39(9):1331-1338

27. Psichas A, Sleeth ML, Murphy KG, et al. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. Int. J. Obesity 2015, 39:424-429

28. Tolhurst G, Heffron H, Lam YS, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 2012, 61:364-371

29. Salmeron J, Manson JE, Stampfer MJ, et al. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women JAMA 1997, 277:472-477

30. Sleeth ML, Thompson EL, Ford HE, Zac-Varghese SE, Frost G. Free fatty acid receptor 2 and nutrient sensing: a proposed role for fibre,fermentable carbohydrates and short-chain fatty acids in appetite regulationNutr. Res. Rev. 2010;23:135-145

31. Ge H, Li X, Weiszmann J, et al. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology 2008;149:4519-4526.

32. Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunology. 2016 Apr 22;5(4):e73. doi: 10.1038/cti.2016.17

33. Franklin ZJ, Tsakmaki A, Fonseca Pedro P, et al. Islet neuropeptide Y receptors are functionally conserved and novel targets for the preservation of beta-cell mass. Diabetes Obes Metab. 2018 Mar;20(3):599-609. doi: 10.1111/dom.13119

34. Jones PM, Persaud SJ. Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic beta-cells. Endocr Rev. 1998 Aug;19(4):429-61.

35. Ang Z, Xiong D, Wu M, Ding JL. FFAR2-FFAR3 receptor heteromerization modulates short-chain fatty acid sensing. FASEB J. 2018 Jan;32(1):289-303. doi: 10.1096/fj.201700252RR.

Figure Legends

Figure 1: Effects of FFAR2 deletion on islet physiological parameters.

a) Islet hormone immunofluorescence in pancreas sections from WT (upper panel) and FFAR2$^{-/-}$ (lower panel) mice. Sections were co-stained for insulin (red) and glucagon (green) or insulin (red) and somatostatin (green), as indicated. Images are representative of 10-15 sections from each of 4 WT and 4 FFAR2$^{-/-}$ mice.

b) Dynamic profiling of glucose-dependent insulin secretion from islets isolated from WT (solid circles) and FFAR2$^{-/-}$ (open circles) mice. Data are expressed as mean+SEM of 6 independent isolations, with 4 independent replicates per experiment for each genotype. p>0.1: WT vs FFAR2$^{-/-}$, insulin secretion.

c) Insulin content of islets isolated from WT (white bar) and FFAR2$^{-/-}$ (black bar) mice. Data are expressed as mean+SEM of 3 independent isolations. p>0.1: WT vs FFAR2$^{-/-}$, islet insulin content.

d) Expression of *FFAR3* mRNA in islets isolated from WT and FFAR2$^{-/-}$ mice relative to *Gapdh* mRNA. Data are expressed as mean+SEM of 4 independent isolations. p>0.1: WT vs FFAR2$^{-/-}$, FFAR3 mRNA expression.

Figure 2: Role of FFAR2 in sodium acetate and sodium propionate potentiation of glucose-induced insulin secretion from mouse islets.

Dynamic profiling of sodium acetate (SA) (a) and sodium propionate (SP) (b) potentiation of glucose-dependent insulin secretion from islets isolated from WT (solid circles) and FFAR2$^{-/-}$ (open circles) mice. Data are expressed as mean±SEM of n=8 experiments with 4 independent replicates per experiment for each genotype. p<0.0001: WT vs FFAR2$^{-/-}$, insulin secretion in response to SA; **p<0.01: WT vs FFAR2$^{-/-}$, insulin secretion in response to SP.

Figure 3: Effects of sodium acetate and sodium propionate on generation of islet second messengers.

Dynamic profiling of the effects of sodium acetate (SA) on intracellular Ca^{2+} levels in dispersed islet cells from WT (a) and FFAR2$^{-/-}$ (b) mice. Data are expressed as mean±SEM of 26 WT cells and 31 FFAR2$^{-/-}$ cells and the traces are representative of 4 independent experiments for each genotype.

c) Cyclic AMP generation in islets isolated from WT (white bars) and FFAR2$^{-/-}$ (black bars) mice. Data are expressed as mean+SEM, n=6, representative of 3 independent experiments for each genotype. ***p<0.001.

d) ATP generation in islets isolated from WT (white bars) and FFAR2$^{-/-}$ (black bars) mice. Data are expressed as mean+SEM, n=4-6, representative of 4 independent experiments for each genotype. *p<0.05: ATP vs levels at 2mM glucose of the relative group.

Figure 4: Sodium acetate reversibly potentiates insulin secretion from human islets via PLC- and PKC-dependent pathways.

a) Dynamic profiling of insulin secretion from human islets exposed to different concentrations of sodium acetate (SA) in the presence of 20mM glucose. Data are expressed as mean±SEM of n=6 independent experiments, with 4 replicates per SA concentration.

b) Area under the curve (AUC) data were calculated for all perifusion conditions shown in panel a). **p<0.01, ***p<0.001, ^{ns}p>0.1: insulin secretion in response to SA vs control.

c) Dynamic profiling of insulin secretion from human islets exposed to sodium acetate (SA) in the absence (solid circles) or presence (white circles) of the selective PLC inhibitor U73122. Data are expressed as mean±SEM of n=3 independent experiments, with 3 replicates per condition. p<0.05: control vs U73122, insulin secretion in response to SA.

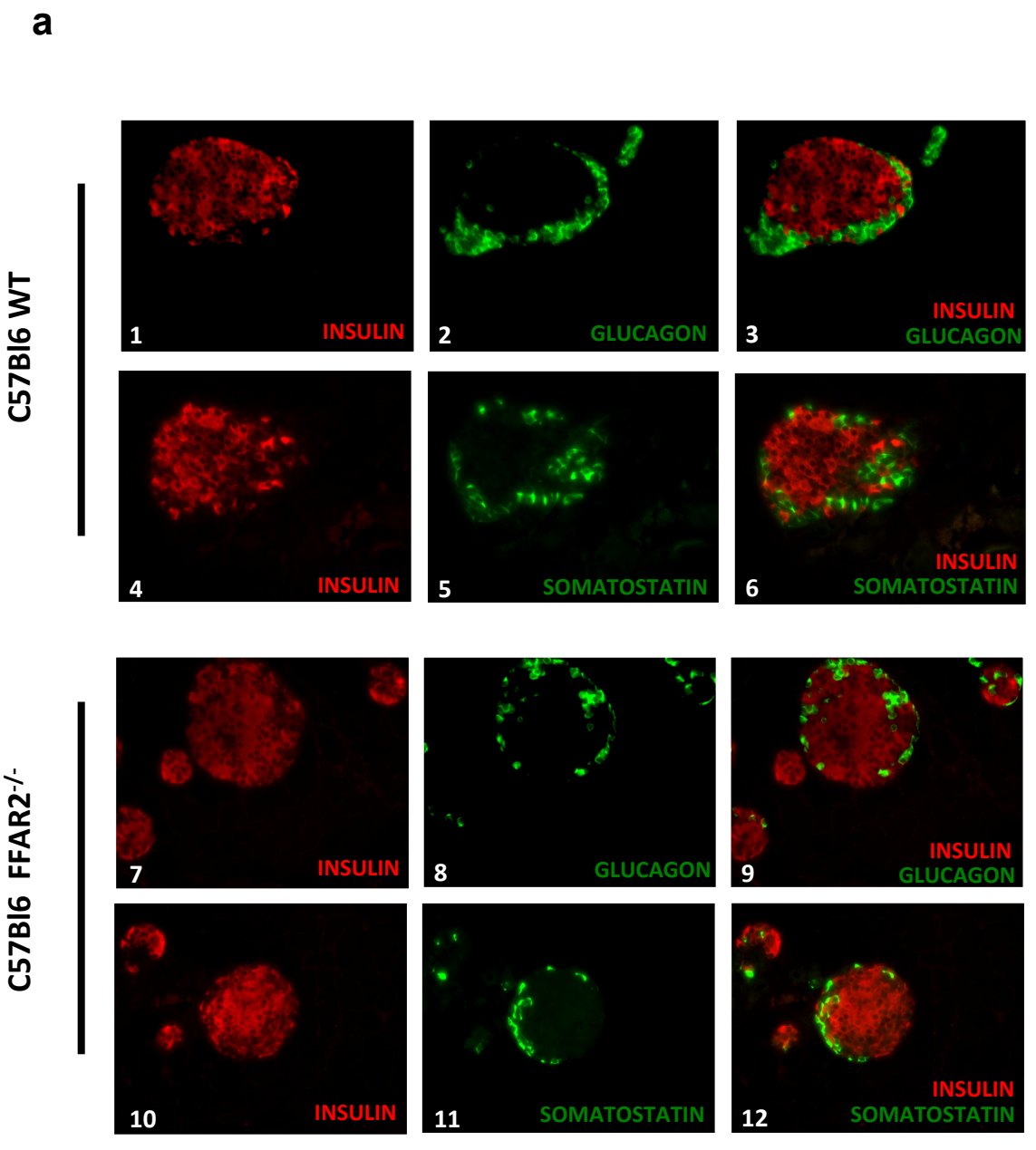
d) Dynamic profiling of insulin secretion from human islets incubated for 20 hours with 200nM PMA to down-regulate PKC (white circles), 200nM PDD, an inactive phorbol ester (open squares), or under standard culture conditions (solid circles). Data are expressed as mean±SEM of n=3 independent experiments, with 4 replicates per treatment. p<0.001: control vs PMA, insulin secretion in response to SA.

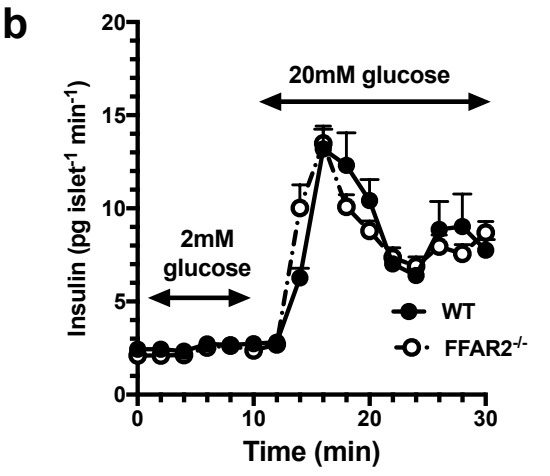
e) Dynamic profiling of glucose-dependent insulin secretion from human islets pre-exposed for 18 hours to 100ng/mL PTX (open circles) or vehicle (solid circles). Data are expressed as mean±SEM of n=2 independent experiments, with 4 replicates per treatment.

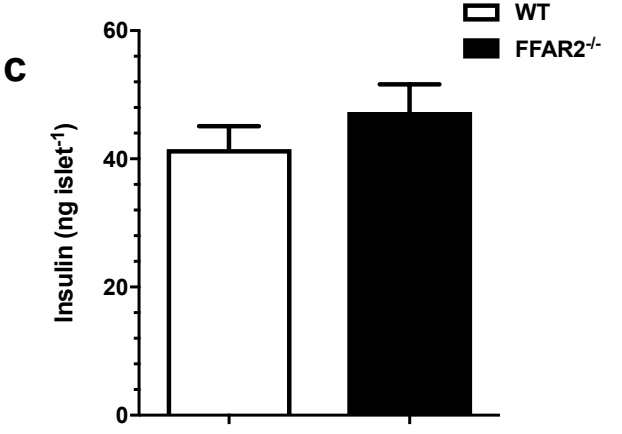
Figure 5: Sodium acetate and sodium propionate protect islets from apoptosis in a FFAR2-dependent manner.

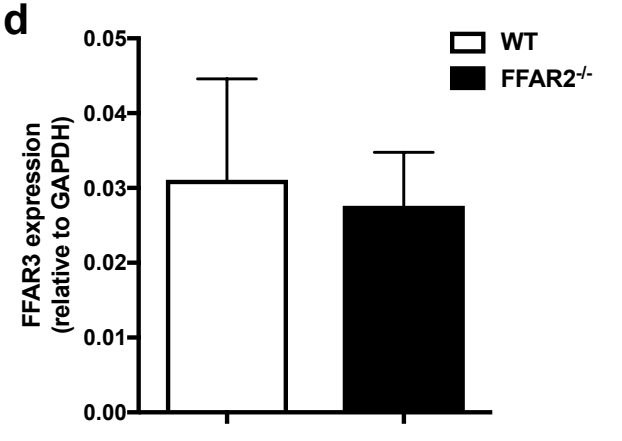
a) Effects of sodium acetate (SA) on human islet apoptosis. Human islets were maintained for 20 hours in the presence of RPMI containing mixed cytokines (black bars) or cytokine-free RPMI (white bars) in the absence or presence of 1mM SA, and apoptosis was determined by luminescence assay of caspase 3/7 activities. Data are expressed as mean+SEM, n=8, representative of 3 independent experiments. *p<0.05, **p<0.01.

b) Contribution of FFAR2 to the anti-apoptotic effects of sodium acetate (SA) and sodium palmitate (SP). Islets isolated from WT (upper panel) or FFAR2$^{-/-}$ (lower panel) mice were maintained for 20 hours in the presence of RPMI containing mixed cytokines (black bars), sodium palmitate (grey bars) or RPMI containing 0.25% ethanol/0.5% BSA as a control for palmitate treatment (white bars) in the absence or presence of 1mM SA or SP. Apoptosis was determined as in (a) and data are expressed as mean+SEM of 6 independent experiments, each consisting of 6-8 replicates. **p<0.01, ***p<0.001, ^{ns}p>0.1.

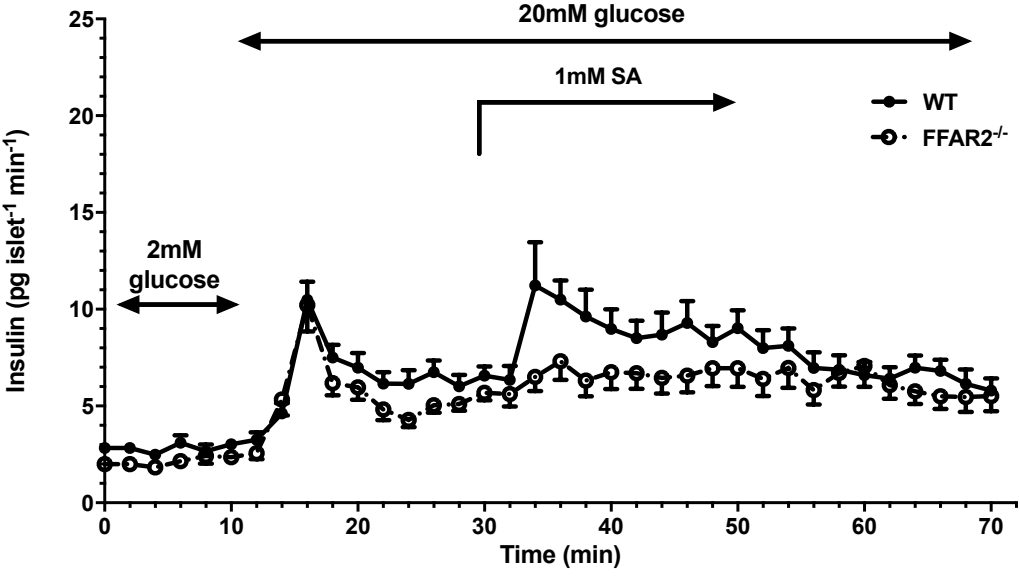

a
C57Bl6 WT
C57Bl6 FFAR2-/-
1
INSULIN
2
GLUCAGON
3
INSULIN
GLUCAGON
4
INSULIN
5
SOMATOSTATIN
6
INSULIN
SOMATOSTATIN
7
INSULIN
8
GLUCAGON
9
INSULIN
GLUCAGON
10
INSULIN
11
SOMATOSTATIN
12
INSULIN
SOMATOSTATIN


b
20mM glucose
2mM
glucose
Insulin (pg islet-1 min-1)
Time (min)
WT
FFAR2-/-
0
5
10
15
20
0
10
20
30

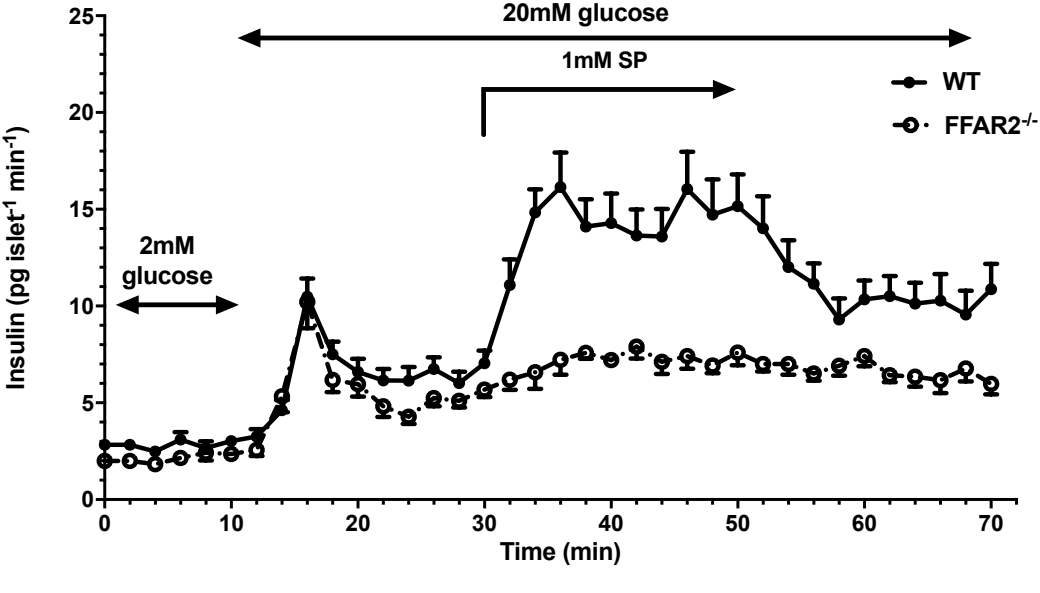

c
WT
FFAR2-/-
Insulin (ng islet-1)
0
20
40
60

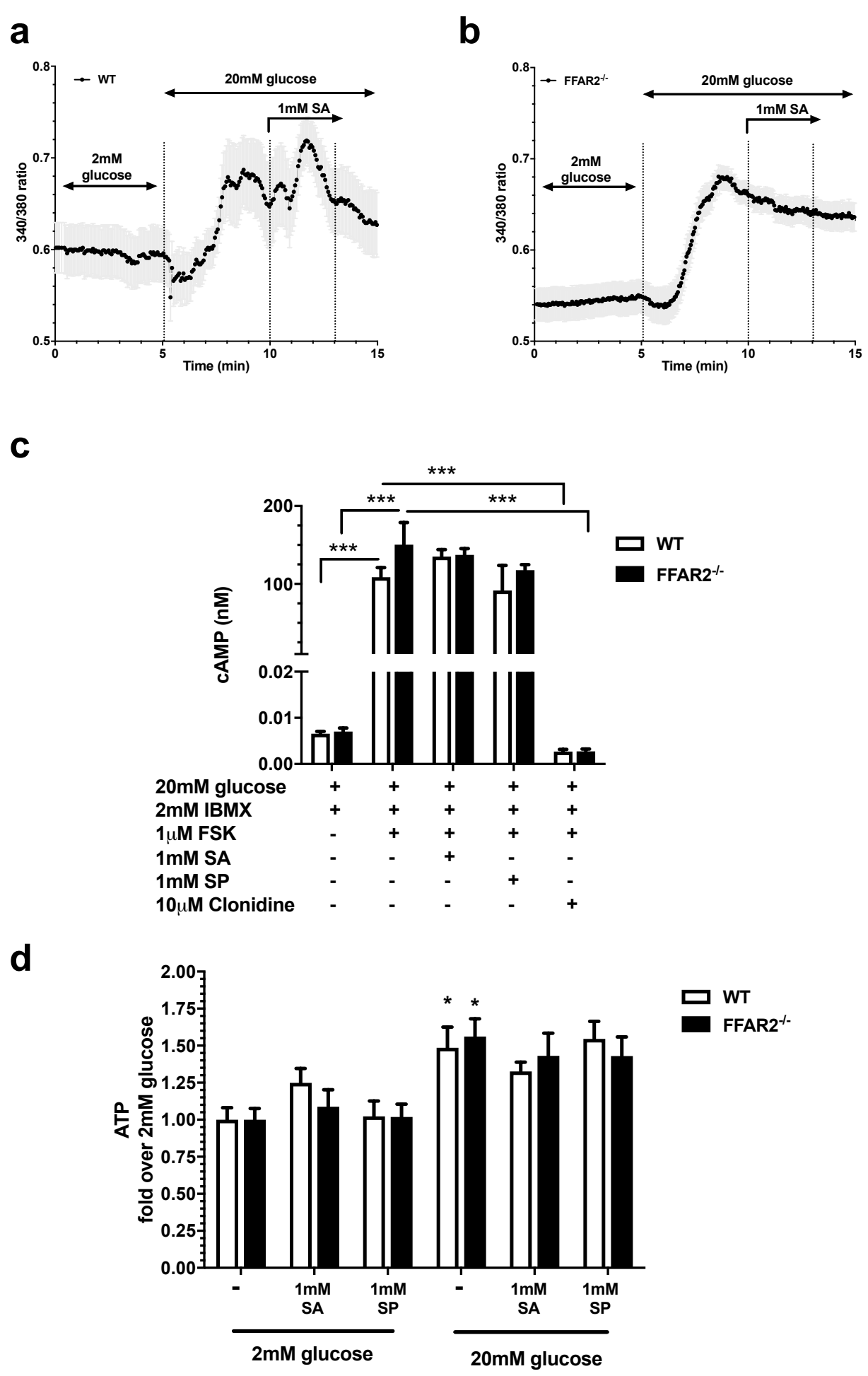

d
WT
FFAR2-/-
FFAR3 expression
(relative to GAPDH)
0.00
0.01
0.02
0.03
0.04
0.05

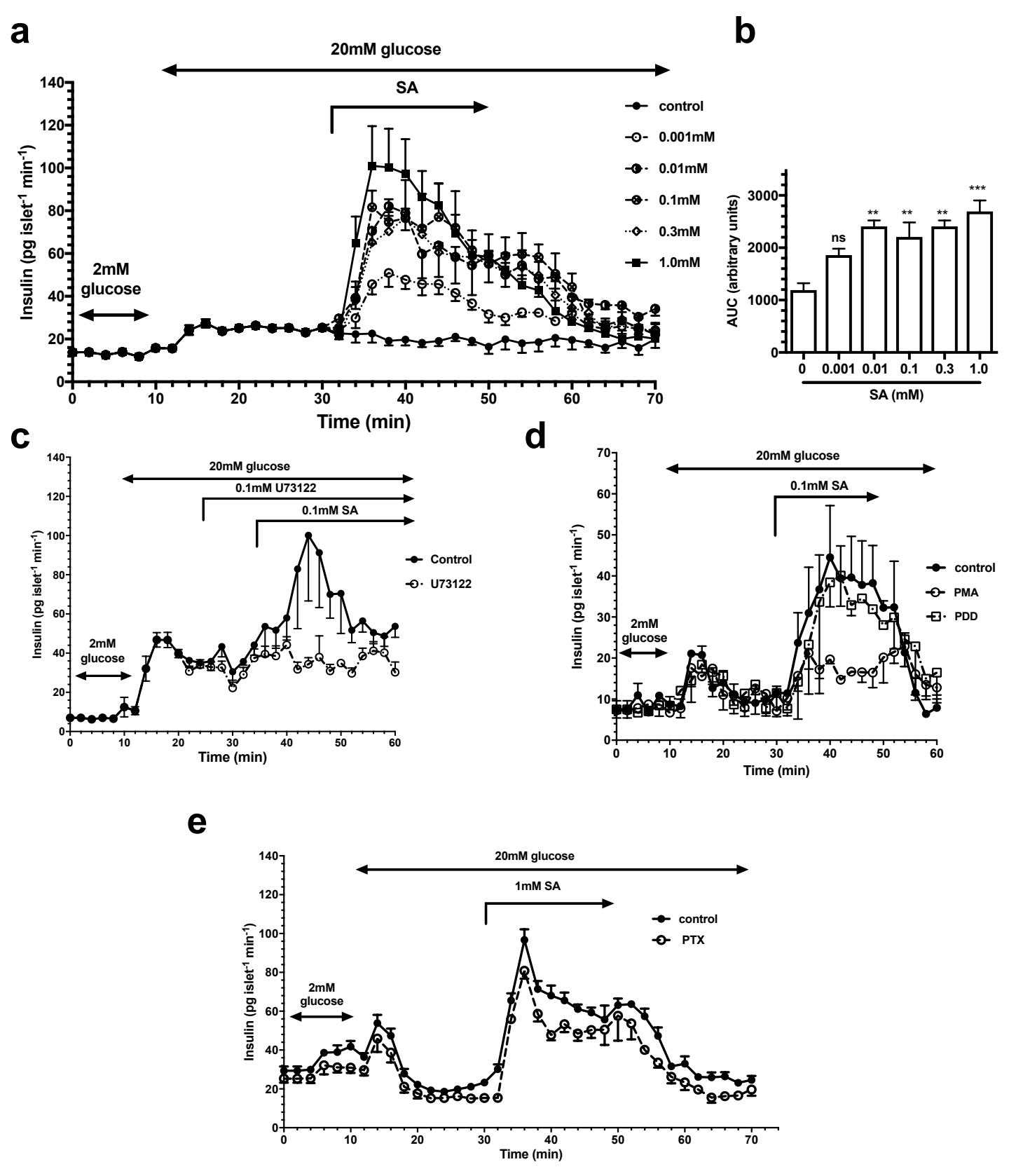
a

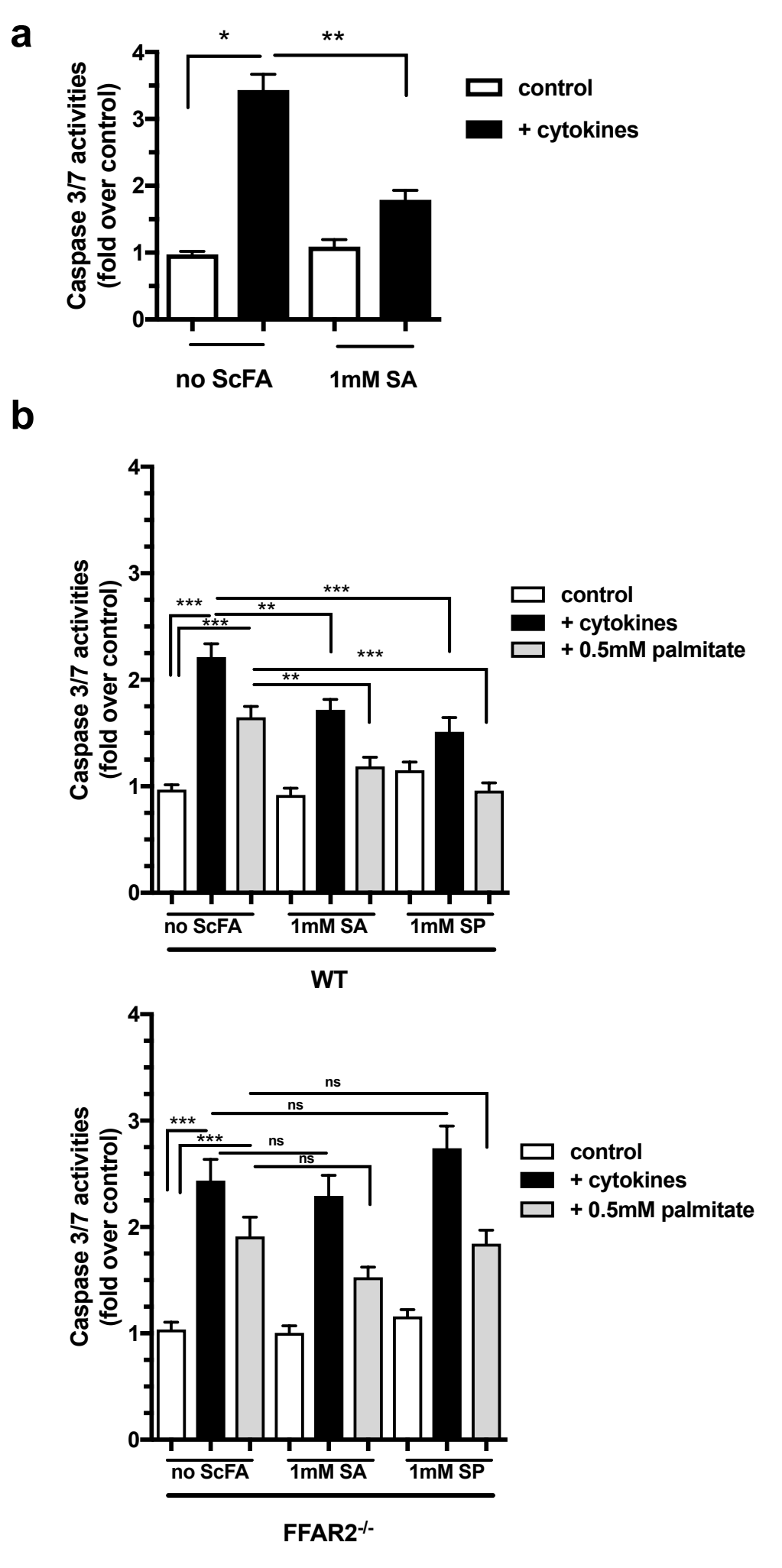

20mM glucose
1mM SA
WT
FFAR2-/-
2mM glucose
Insulin (pg islet-1 min-1)
Time (min)
0
5
10
15
20
25
0
10
20
30
40
50
60
70

b


20mM glucose
1mM SP
WT
FFAR2-/-
2mM glucose
Insulin (pg islet-1 min-1)
Time (min)
0
5
10
15
20
25
0
10
20
30
40
50
60
70


a
WT
20mM glucose
1mM SA
2mM glucose
340/380 ratio
Time (min)
b
FFAR2-/-
20mM glucose
1mM SA
2mM glucose
340/380 ratio
Time (min)
c
***
***
***
***
WT
FFAR2-/-
cAMP (nM)
20mM glucose
2mM IBMX
1μM FSK
1mM SA
1mM SP
10μM Clonidine
d
WT
FFAR2-/-
ATP
fold over 2mM glucose
1mM SA
1mM SP
2mM glucose
20mM glucose


a
20mM glucose
SA
2mM glucose
control
0.001mM
0.01mM
0.1mM
0.3mM
1.0mM
Insulin (pg islet-1 min-1)
Time (min)
b
AUC (arbitrary units)
ns
**
**
**
***
0
0.001
0.01
0.1
0.3
1.0
SA (mM)
c
20mM glucose
0.1mM U73122
0.1mM SA
2mM glucose
Control
U73122
Insulin (pg islet-1 min-1)
Time (min)
d
20mM glucose
0.1mM SA
2mM glucose
control
PMA
PDD
Insulin (pg islet-1 min-1)
Time (min)
e
20mM glucose
1mM SA
2mM glucose
control
PTX
Insulin (pg islet-1 min-1)
Time (min)


a
Caspase 3/7 activities
(fold over control)
control
+ cytokines
no ScFA
1mM SA
b
Caspase 3/7 activities
(fold over control)
control
+ cytokines
+ 0.5mM palmitate
no ScFA
1mM SA
1mM SP
WT
Caspase 3/7 activities
(fold over control)
control
+ cytokines
+ 0.5mM palmitate
no ScFA
1mM SA
1mM SP
FFAR2-/-