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Incretins play an important role in FFA4/GPR120 regulation of glucose metabolism by GW-9508

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Abstract

Aims: To assess the role of GPR120 in glucose metabolism and incretin regulation from enteroendocrine L- and K-cells with determination of the cellular localisation of GPR120 in intestinal tissue and clonal Glucagon-Like Peptide-1 (GLP-1)/Gastric Inhibitory Polypeptide (GIP) cell lines.

Main methods: Anti-hyperglycaemic, insulinotropic and incretin secreting properties of the GPR120 agonist, GW-9508 were explored in combination with oral and intraperitoneal glucose tolerance tests (GTT) in lean, diabetic and incretin receptor knockout mice. Cellular localisation of GPR120 was assessed by double immunofluorescence.

Key findings: Compared to intraperitoneal injection, oral administration of GW-9508 (0.1μmol/kg body weight) together with glucose reduced the glycaemic excursion by 22-31% ($p<0.05$-$p<0.01$) and enhanced glucose-induced insulin release by 30% ($p<0.01$) in normal mice. In high fat fed diabetic mice, orally administered GW-9508 lowered plasma glucose by 17-27% ($p<0.05$-$p<0.01$) and augmented insulin release by 22-39% ($p<0.05$-$p<0.001$). GW-9508 had no effect on the responses of GLP-1 receptor knockout mice and GIP receptor knockout mice. Consistent with this, oral GW-9508 increased circulating total GLP-1 release by 39-44% ($p<0.01$) and total GIP by 37-47% ($p<0.01$-$p<0.001$) after 15 and 30 min in lean NIH Swiss mice. Immunocytochemistry demonstrated GPR120 expression on mouse enteroendocrine L- and K-cells, GLUTag cells and pGIP/Neo STC-1 cells.

Significance: GPR120 is expressed on intestinal L- and K-cells and stimulates GLP-1/GIP secretory pathways involved in mediating enhanced insulin secretion and improved glucose tolerance, following oral GW-9508. These novel data strongly support the development of potent and selective GPR120 agonists as an effective therapeutic approach for diabetes.

1. Introduction

GPR120, an long-chain fatty acid receptor, plays a key role in maintaining metabolic homeostasis. GPR120 is a rhodopsin-like GPCR which belongs to a class of receptors including GPR40, GPR41, GPR43 and GPR84 that are activated by free fatty acids (1-4). The GPR120 gene is located on chromosome 10q23.3 and the protein exhibits only 10% homology to GPR40 (5). GPR120 has high affinity for saturated long chain fatty acids (14-18 carbons) and unsaturated fatty acids (16-22 carbons) (6,7). Rodent GPR120 genes encodes for a 361 amino acid protein while two isoforms of the human GPR120 exist including a 361 amino acid isoform (GPR120 short) and the longer isoform consisting of 377 amino acids (GPR120 long) (6, 8).

Activation of GPR120 results in coupling to Gαq, stimulating the protein kinase C (PKC) pathway and enhancing intracellular Ca^{2+} concentrations (3,6,9). GPR120 cell signalling was tissue specific, for example adipocytes require the $G_{q/11}$ protein whereas macrophages utilise the β-arrestin adaptor proteins for GPR120 activation (10). In addition, GPR120 can also stimulate ERK1/2, phosphoinositide-3 kinase and serine/threonine protein kinase Akt pathways in cell lines expressing GPR120 (11-13).

Expression of GPR120 has been found predominantly in the intestines, adipose tissue, lungs, spleen and pro-inflammatory macrophages (3, 14). GPR120 was also specifically co-localised on insulin secreting beta cells (15, 16) and somatostatin secreting delta-cells (17). Additionally, studies using GPR120 knockout mice displayed an integral role in islet function and glucose homeostasis involving regulation of glucagon secretion from alpha cells (18). Intestinal expression of GPR120 was confirmed on GLP-1 secreting L-cells including STC-1 cells (6) and GLUTag cells (19). GPR120 expression was identified on GIP secreting enteroendocrine K-cells and played a pivotal role in lipid-induced GIP secretion (20). GPR120 has a role in the secretion of gastrointestinal hormones including GLP-1 (21, 22), GIP (23, 24), cholecystokinin (CCK) (24, 25) and GLP-2 (26). Acute oral

administration of alpha linolenic acid (ALA) increased plasma GLP-1, and 28-day treatment of ALA augmented plasma GLP-1, insulin and pancreatic beta cell proliferation (21). Both omega-3 fatty acids EPA and DHA are also potent stimulators of GLP-1 secretion (27).

GW-9508 is a chemically synthetic dual agonist for GPR40 and GPR120 (7, 28) while synthetic antagonist GW-1100 is specific for GPR40 with no affinity for GPR120 (7). GW-9508 treatment increased insulin secretion through Ca^{2+} dependent pathways in pancreatic beta cells (7, 16). Conversely, a study found that GPR120 agonist GW-9508 inhibited insulin secretion from clonal beta cells (29). GW-9508 attenuated norepinephrine-induced ghrelin secretion from ghrelin cell lines and plasma ghrelin in mice via GPR120 (30). Additionally, GW-9508-induced active GLP-1 secretion in primary human colonic cultures (31). Furthermore, acute and long-term administration of GW-9508 displayed anti-hyperglycaemic and insulinotropic properties with improved insulin sensitivity in high fat fed mice (28, 32).

Few studies have evaluated the effects of GW-9508 on intestinal enteroendocrine cells and the secretion of the incretin hormones; gastric inhibitory polypeptide (GIP) and glucagon like peptide-1 (GLP-1). In this study, we assessed the effects of GW-9508-induced activation of GPR120 on glucose homeostasis, insulin and incretin hormones in lean, diabetic and incretin receptor knockout mouse models. Furthermore, we determined the cellular localisation of GPR120 in GLP-1 secreting GLUTag cells, GIP secreting pGIP/neo STC-1 cells and jejunum tissue from NIH Swiss mice. These studies indicate therapeutic potential of multi-acting GPR120 agonists for type 2 diabetes.

2. Materials and Methods

2.1 Materials

GW-9508 was purchased from Sigma-Aldrich (Poole, UK). GIP (1-42) and GLP-1 (7-36) were purchased from GL Biochem (Shanghai, China). Total GLP-1 and total GIP ELISA kits were obtained from Millipore (Watford, UK). Rabbit anti-GPR120 (H-155), goat anti-GLP-1 (F-15) and goat anti-GIP (Y-20) polyclonal IgG antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2 Animals

All animal experiments were carried out in accordance with the UK Animal (Scientific Procedures) Act 1986, the ARRIVE guidelines for reporting experiments involving animals (33) and approved by the local Ulster Animal Welfare and Ethical Review Body (AWERB) committee (PPL 2902, approved on 26/04/2021). Experimental animals were individually housed in an air-conditioned room at $22 \pm 2°C$ with 12 hr light: 12 hr darkness cycles. Drinking water and standard rodent maintenance diet (10% fat, 30% protein and 60% carbohydrate; Trouw Nutrition, Cheshire, UK) were supplied *ad libitum.* To induce diabetes, male NIH Swiss mice (8-10 wk) purchased from Harlan (Blackthorne, UK) were fed a high fat diet (35% carbohydrate, 20% protein, 45% fat (26.15 kJ/g), Special Diet Service, Essex, UK) for 16 weeks. GIP receptor and GLP-1 receptor knockout mice maintained on a C57/BL6 genetic background (courtesy of Prof B Thorens, Lausanne, Switzerland and Prof. DJ Drucker, Toronto, Canada respectively) and age-matched control wild-type C57/BL6 mice were obtained from an in-house breeding colony.

2.3 Acute effects of GW-9508 in-vivo

Non-fasted lean NIH Swiss mice (n=6) received an intraperitoneal or oral injection of glucose alone (18mmol/kg body weight) or in combination with intraperitoneal or orally administered GW-9508

(0.1μmol/kg body weight). Fasted (18 hr) high fat fed mice (n=6) received an oral injection of glucose alone (18mmol/kg body weight) or in combination with orally administered GW-9508 (0.1μmol/kg body weight). Age-matched, non-fasted GIP receptor knockout, GLP-1 receptor knockout and wild type C57/BL6 mice (n=6) received an oral injection of glucose alone (18mmol/kg body weight) or in combination with oral GW-9508 (0.1μmol/kg body weight). Confirmation of functional incretin receptor knockout was achieved by demonstration of the lack of effects with GIP (1-42) or GLP-1 (7-36) (25nmol/kg body weight) when administered in combination with glucose administered orally (18mmol/kg body weight).

2.4   *Biochemical analysis*

Blood samples were obtained by cut tip from tail vein of conscious mice at the time points indicated in the Figures and centrifuged at 13,000 rpm for 3 min at 4˚C. Plasma glucose was measured by an automated glucose oxidase procedure using a Beckman glucose analyser and insulin determined by radioimmunoassay (34). Total GIP and GLP-1 were measured using ELISA assay kits according to the manufacturers' instructions (Millipore, Watford, UK).

2.5 *Histology*

GLUTag cells (courtesy Prof. DJ Drucker, Toronto, Canada) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5.5mmol/l glucose and 2mmol/l L-glutamine, 10% (v/v) fetal bovine serum and antibiotics (penicillin (100U/ml), streptomycin (0.1mg/l)) as previously described (35). pGIP/neo STC-1 cells (courtesy of Dr B Wice, Washington, USA) were cultured in monolayers with DMEM media containing antibiotics (400μg/ml) and 10% foetal calf serum at 37˚C in an atmosphere of 95% air and 5% carbon dioxide (36). Cells were detached using trypsin/EDTA and allowed to attach overnight to polylysine-coated slides and fixed using paraformaldehyde/PBS for 20 min. Antigen retrieval was achieved by incubation in sodium citrate (50mmol/l) at 90˚C for 20 min. Small intestinal tissues from lean NIH Swiss mice were fixed in 4% PFA/PBS, embedded in paraffin

wax and cut at 10μm. Sections were mounted onto polylysine-coated slides and dried on a hot plate. Jejunum sections were dewaxed and following antigen retrieval, incubated overnight at 4˚C with goat anti-GLP-1 (1:50), goat anti-GIP (1:50) and rabbit anti-GPR120 (1:100). After washing in PBS, sections were incubated with Alexa Fluor 488nm fluorescein donkey anti-goat and Alexa 594nm goat anti-rabbit (1:400; Molecular Probes (Life Technologies Ltd, Paisley, UK)) for 45 min at 37˚C and DAPI nuclear stain for 15 min at 37˚C. Finally, slides were washed in PBS, mounted and analysed using a BX51 Olympus microscope equipped with an Olympus XM10 digital camera. Negative staining in the absence of antibody was carried out on each tissue sample, as used in previous studies [16]

2.6 Acute hormone secretion from intestinal cell lines

GLUTag and pGIPneo STC-1 cells were seeded into 24-well plates and incubated overnight in 1ml of culture medium at 37°C and 5% CO_2. After overnight incubation and preincubation with 1.1mM glucose for 40 min, acute incubations were carried out at 37°C 5% CO_2 for 2 hrs in 2mM glucose in KRBB buffer supplemented with GW9508. Test solutions were added both alone and in combination with the GPR120 antagonist, AH7614 (10^{-5}M). After incubations, total GIP and GLP-1 were measured using ELISA assay kits according to the manufacturers' instructions (Millipore, Watford, UK).

2.7 Statistical analyses

Data are expressed as the mean ± the standard error of the mean (SEM). Results were compared using the Student's t-test or one-way ANOVA on Prism graph pad version 5.0. Differences in data were considered to be statistically significant for $p < 0.05$.

3. Results

3.1 Effects of intraperitoneal and oral GW-9508 on plasma glucose and insulin in lean mice

Oral and intraperitoneal glucose tolerance tests were utilised to determine the anti-hyperglycaemic and insulinotropic potential of GW-9508 at 0.1μmol/kg in non-fasted NIH Swiss mice by examining its effect when bypassing the gastrointestinal tract (Fig. 1). Compared with intraperitoneal glucose, oral glucose decreased the glycaemic excursion by 27% ($p<0.05$) after 15 min and by 28% ($p<0.01$) after 30 min (Fig. 1A). Similarly, oral administration of GW-9508 reduced plasma glucose by 28% ($p<0.01$) after 15 min, by 31% ($p<0.05$) after 30 min and by 22% ($p<0.05$) after 105 min when compared to intraperitoneal injection of GW-9508 (Fig. 1B). The reduced glycaemic excursion was confirmed by AUC values for oral administration of glucose ($p<0.05$) and GW-9508 ($p<0.01$) (Fig. 1C). Additionally, oral administration of GW-9508 moderated the glycaemic excursion ($p<0.05$) compared with oral glucose (Fig. 1C). Consistent with these observations, oral administration of glucose enhanced insulin release by 27% ($p<0.05$) after 30 min when compared to intraperitoneal injection of glucose (Fig. 1D). Orally administrated GW-9508 increased glucose-stimulated insulin release by 30% ($p<0.01$) after 30 min when compared to intraperitoneal injection of GW-9508 (Fig. 1E). This increased glucose-induced insulin secretion was confirmed by AUC values for oral administration of GW-9508 ($p<0.001$) (Fig. 1F). Additionally, intraperitoneal injection of GW-9508 augmented glucose-stimulated insulin release ($p<0.01$) when compared to intraperitoneal glucose alone (Fig. 1F). Finally, oral administration of GW-9508 enhanced glucose-induced insulin release ($p<0.001$) when compared to oral glucose alone (Fig. 1F).

3.2 Effects of GW-9508 on plasma glucose and insulin in diet-induced diabetic mice

The anti-diabetic potential of GW-9508 was assessed in insulin resistant high fat fed NIH Swiss mice (Fig. 2). GW-9508 attenuated plasma glucose by 27% ($p<0.01$) after 30 min, by 17% ($p<0.01$) after 60 min and by 20% ($p<0.05$) after 105 min when compared to oral glucose alone (Fig. 2A). This

reduction in plasma glucose was confirmed by AUC values for GW-9508 (p<0.01) (Fig. 2C). Insulinotropic effects of GW-9508 were enhanced by 39% (p<0.001) after 30 min and by 22% (p<0.05) after 60 min when compared to oral glucose alone (Fig. 2B). GW-9508 increased insulin secretion (p<0.001) was confirmed by the AUC values (Fig. 2D).

3.3 Effects of GW-9508 on plasma glucose and insulin in wild-type C57 BL/6 mice and incretin receptor knockout mice

As expected, intraperitoneal administration of GIP or GLP-1 had no biological effects in GIP or GLP-1 receptor knockout mice respectively (Fig. 3). Conversely, in wild-type C57/BL6 mice, GIP (25nmol/kg body weight) reduced the glycaemic excursion by 18-24% (p<0.05-p<0.01) and increased glucose-induced insulin secretion by 23-28% (p<0.05) (Fig. 3A, D). Similarly, GLP-1 (25nmol/kg body weight) reduced plasma glucose by 23-32% (p<0.05-p<0.01) and stimulated insulin release by 34-41% (p<0.01) (Fig. 3A, D). GW-9508 also decreased plasma glucose by 28% (p<0.01) after 15 min and by 13% (p<0.05) after 30 min (Fig. 3A). Similarly, glucose-stimulated insulin release was increased by GW-9508 by 34% (p<0.01) after 15 min and by 30% (p<0.05) after 30 min (Fig. 3D). Overall assessment based on the AUC, GW-9508 lowered the glycaemic excursion by 14% (p<0.05) (Fig. 3A) and augmented insulin release by 24% (p<0.01) in C57/BL6 wild-type mice (Fig. 3D). In both GLP-1 and GIP receptor knockout mice, the glucose lowering and insulinotropic actions of GW-9508 were abolished (Fig. 3).

3.4 Effects of GW-9508 on plasma glucose, insulin, total GLP-1 and total GIP in lean mice

Further experiments were performed in lean NIH Swiss mice to directly evaluate involvement of GIP and GLP-1 secretion (Fig. 4). As expected, oral administration of GW-9508 (0.1μmol/kg body weight) to non-fasted mice decreased the glycaemic excursion by 21% (p<0.05) and increased glucose-stimulated insulin release by 45% (p<0.001) after 30 min (Fig. 4A, B). Interestingly, GW-9508 augmented total GLP-1 release by 44% (p<0.01) after 15 min and by 39% (p<0.01) after 30 min

(Fig. 4C). GW-9508 stimulated total GIP release by 47% (p<0.001) after 15 min and 37% (p<0.01) after 30 min (Fig. 4D).

3.5 Distribution of GPR120 in GLUTag, pGIP/neo STC-1 cells and mouse jejunum

Cellular localisation of GPR120, GIP, GLP-1 and DAPI nuclear stain were determined in GLUTag and pGIP/neo STC-1 cells by double immunofluorescence (Fig. 5-6). DAPI stained the nuclei of GLUTag cells (blue), GLP-1 was distributed across the cells (green) with a similar staining pattern to GPR120 (red) (Fig. 5C). Merge of GLP-1 and GPR120 identified areas of co-localisation indicated by arrows (yellow) (Fig. 5D). In pGIP/neo STC-1 cells DAPI stained the nuclei (Blue), GIP was dispersed across the cells (green) with a similar staining pattern to GPR120 (red) (Fig. 6C). Merge of GIP and GPR120 identified areas of co-localisation indicated by arrows (yellow) (Figure 6D). Similarly, in normal mouse jejunum DAPI stained the nuclei (blue, Fig. 7A, B) and GPR120 (red, Fig. 7E, F) was expressed throughout the tissue with a similar staining pattern to GIP (green, Figure 7C), and GLP-1 (green, Fig. 7D). Merge of GIP with GPR120 (Fig. 7G) and GLP-1 with GPR120 (Fig. 7H) indicated that GIP secreting K-cells and GLP-1 secreting L-cells express GPR120.

3.6 Effects of GW9508 and GPR120 antagonist AH7614 on GLP-1 secretion in intestinal GLUTag cells and GIP secretion in intestinal pGIP/neo STC-1 cells

The effects of agonist GW9508 in the presence and absence of the GPR120 antagonist AH7614 were investigated on GLP-1 secretion from GLUTag cells in 2mM glucose. GW9508 increased GLP-1 secretion 1.8-fold (p<0.001) from GLUTag cells at 10^{-6}M in 2mM glucose (Fig. 8). GLUTag cells were treated with GPR120 agonist and antagonist AH7614 (10^{-5}M). AH7614 reduced the agonistic action on GLP-1 secretion (p<0.05-p<0.01). In the presence of AH7614, GW9508 secretory action was reduced from 1.5 to 1.7-fold (10^{-8}M to 10^{-6}M, p<0.01).

The effects of GPR120 agonists on GIP secretion were investigated in intestinal pGIPneo STC-1 cells. GW9508 demonstrated a 2.0-fold (p<0.05) increase in GIP secretion from pGIPneo STC-1 cells at 10^{-6}M (Fig 8B). Treatment of pGIPneo STC-1 cells with both GPR120 agonist GW9508 (10^{-6}M) and antagonist AH7614 (10^{-5}M) reduced GIP secretion to basal levels (1.9-fold decrease) demonstrating the selectivity of GW9508 for GPR120.

4. Discussion

FFA4/GPR120 pharmacology suggests beneficial anti-diabetic, anti-inflammatory, neuroprotective and anti-proliferative properties (3, 37, 38). GPR120 deficient mice are more susceptible to a high fat diet resulting in increased glucose intolerance, insulin resistance, fatty liver and reduced adipocyte differentiation (39). In human islets, GPR120 expression was downregulated in diabetic or hyperglycaemic individuals and GPR120 gene knockdown affected fatty-acid induced apoptosis (40). These observations plus the potential of GPR120 agonists to enhance the secretion of GLP-1 (21), GIP (20) and CCK (25) have resulted in significant pharmaceutical interest in drugs targeting GPR120. GPR40/FFA1 has been targeted therapeutically and in human clinical trials to assess whether activation of this receptor could have beneficial effects in the treatment of type 2 diabetes (7, 28). GPR120/FFA4, is of interest as a potential therapeutic target for diabetes and other metabolic disorders however data lags behind studies on FFA1. This study has shown that using specific GPR120 antagonism reduces the effect of GPR120 to basal levels in GLP-1 and GIP secreting cells, confirming a significant role of GPR120 on incretin hormone secretion. Both GPR40 and GPR120 are activated by long chain fatty acids so development of novel dual agonists for both GPCRs could have therapeutic potential in the future (29, 41). Synthetic ligands of FFAR4, such as TUG-891 and GW9508, have been indicated as therapeutic agents of diabetes and obesity. Schilperoort et al. 2018 reported that TUG-891 increases fat oxidation and reduces body weight and fat mass of C57Bl/6J mice, while increasing BAT activity (42). Recently, a new synthetic dual agonist of GPR120/GPR40

was shown to significantly delay receptor endocytosis compared to other known GPR120 agonists and improve glucose homeostasis in mice (22). Further studies are essential for the development of FFAR4/GPR120 agonist-based drugs, and understanding the mechanism of GW-9508. Synthetic agonist GW-9508 has a high affinity for both GPR40 and GPR120 with no effect on fatty acid receptors GPR43 and GPR41 (7, 43).

The present study assessed the glucose lowering and both the insulin and incretin releasing properties of GW-9508 in lean, obese high fat fed and incretin receptor knockout mouse models. Administration of GW-9508 orally to normal mice resulted in enhanced glucose lowering and insulinotropic abilities when compared to the intraperitoneal route of administration. This suggests that the direct effects of GW-9508 on pancreatic beta cells are significantly augmented by stimulation of GLP-1 and GIP secretion from GPR120-expressing enteroendocrine cells. The enhanced effects of oral GW-9508 are therefore mediated by direct stimulation of insulin release in beta cells, combined with stimulation of GLP-1 and GIP secretion from GPR120-expressing enteroendocrine cells.

To further explore whether these findings, translated to an obesity-diabetes setting, effects of oral GW-9508 were also assessed in high fat fed mice. This confirmed similar glucose lowering and insulinotropic actions of oral GW-9508 as observed in normal mice. These data are in harmony with the observation that GW-9508 attenuated blood glucose and increased glucose-induced insulin release in high fat fed mice (28). Furthermore, acute oral administration of GPR120 agonists including GW-9508 have previously been shown to lower plasma glucose and augment glucose stimulated insulin release in lean NIH Swiss mice (16, 41).

To confirm the suspected role for GW-9508 in the incretin pathway, we studied incretin receptor knockout mice together with wildtype C57/BL6 controls. These experiments showed that the glucose lowering and insulinotropic abilities of oral GW-9508 were curtailed in GIP as well as GLP-1 receptor knockout mice, suggesting a role of combined incretin action in stimulation of beta cell function. Additionally, our data indicate that GW-9508 stimulates GLP-1 and GIP secretion via

enhanced insulin release which may contribute to the beneficial effects of oral GW-9508 on glucose homeostasis. To investigate the ability of GW-9508 to stimulate the release of GIP and GLP-1, further studies were performed using lean NIH Swiss mice. In harmony with the incretin receptor knockout data, oral GW-9508 administration had significantly enhanced total plasma GLP-1 and GIP concentrations. Previous studies found that GW-9508 increased GLP-1 secretion in human L-cells (44) and primary human colonic cultures (31) but had no effect on GLP-1 release from the GLUTag cell line (44). Limited studies have assessed the role of GW-9508 in GIP secretion from enteroendocrine K-cells, however GPR120 expression was identified on GIP secreting K-cells and regulated lipid-induced GIP secretion (20). Further studies are clearly warranted to assess the exact mechanism underlying GPR120 induced GLP-1 and GIP secretion.

In keeping with our functional data, immunocytochemical evaluation of GPR120 in GLUTag, pGIP/neo STC-1 cells and mouse jejunum revealed that GPR120 was co-expressed with GIP and GLP-1. Expression of GPR120 is not confined to the gastrointestinal tract and has been found in adipose tissue, pancreas, lungs, spleen, taste buds and pro-inflammatory macrophages (14, 45). GPR120 mRNA has been identified in rat ileum and proximal colon, almost all epithelial cells lining the villi (2). Expression of GPR120 has been identified on GLP-1 secreting L-cells including GLUTag cells (19, 46) and STC-1 cells (26). GPR120 mRNA has also been identified on mouse enteroendocrine K-cells (23) and accumulating data supports a role in GIP secretion (20).

5. Conclusions

In conclusion, oral GW-9508 administration improves glucose tolerance and stimulates glucose-induced insulin release mediated in part by activation of the incretin pathway and secretion of both GIP and GLP-1. GPR120 is highly expressed in the gastrointestinal tract, raising interest in GPR120 as a target for new pharmaceutical agents. These results also implicate GPR120 in regulation of glucose

homeostasis and incretin secretion, suggesting that specific targeting of GPR120 may lead to novel anti-diabetic therapies in the future.

Author contributions

AMK and PRF conceived the idea and conceptualised the study. MGM and BMM collected and analysed the data. AMK and BMM drafted the manuscript. AMK, MGM PRF BMM reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest Statement

The authors declare that there no conflicts of interest.

Acknowledgements

References

1. Burns RN & Moniri NH. Agonism with the omega-3 fatty acids α-linolenic acid and docosahexaenoic acid mediates phosphorylation of both the short and long isoforms of the human GPR120 receptor. *Biochem Biophys Res Commun* (2010) 396:1030-1035.

2. Paulsen SJ, Larsen LK, Hansen G, Chelur S, Larsen PJ & Vrang N. Expression of the Fatty Acid Receptor GPR120 in the Gut of Diet-Induced-Obese Rats and Its Role in GLP-1 Secretion. *PLoS ONE* (2014) 9:e88227.

3. Carullo G, Mazzotta S, Vega-Holm M, Iglesias-Guerra F, Vega-Perez JM, Aiello F, Brizzi A GPR120/FFAR4 Pharmacology: Focus on agonists in type 2 diabetes mellitus drug discovery. *J Med Chem* (2021) 64(8):4312-4332.

4. Al Mahri S, Malik SS, Al Ibrahim M, Haji E, Dairi G, Mohammad S. Free Fatty Acid Receptors (FFARs) in Adipose: Physiological Role and Therapeutic Outlook. Cells (2022) 11(4):750-760.

5. Hirasawa A, Hara T, Katsuma S, Adachi T & Tsujimoto G. Free fatty acid receptors and drug discovery. *Biol Pharm Bull* (2008) 31:1847-1851.

6. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, *et al.* Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* (2005) 11**:**90–94.

7. Briscoe CP, Peat AJ, McKeown SC, Corbett DF, Goetz AS, Littleton TR, *et al*. Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small agonist. *Br J Pharmacol* (2006) 148:619-628.

8. Fredriksson R, Hoglund PJ, Gloriam DE, Lagerstrom MC & Schioth HB. Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. *FEBS Lett* (2003) 554:381-388.

9. Moore K, Zhang Q, Murgolo N, Hosted T & Duffy R. Cloning, expression, and pharmacological characterization of the GPR120 free fatty acid receptor from cynomolgus monkey: Comparison with human GPR120 splice variants. *Comp Biochem Physiol-B* (2009) 154:419-426.

10. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM & Olefsky JM. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* (2010) 142:687-698.

11. Katsuma S, Hatae N, Yano T, Ruike Y, Kimura M, Hirasawa A & Tsujimoto G. Free fatty acids inhibits serum deprivation-induced apoptosis through GPR120 in a murine enteroendocrine cell line STC-1. *J Biol Chem* (2005) 280**:**19507-19515.

12. Hara T, Hirasawa A, Sun Q, Sadakane K, Itsubo C, Iga T, *et al*. Novel selective ligands for free fatty acid receptors GPR120 and GPR40. *N-S Arch Pharmacol* (2009) 380:247-255.

13. Song T, Zhou Y, Peng J, Tao YX, Yang Y, Xu T, *et al*. GPR120 promotes adipogenesis through intracellular calcium and extracellular signal-regulated kinase 1/2 signal pathway. *Mol Cell Endocrinol*. (2016) 434:1-13.

14. Miyauchi S, Hirasawa A, Iga T, Liu N, Itsubo C, Sadakane K, *et al.* Distribution and regulation of protein expression of the free fatty acid receptor GPR120. *N-S Arch Pharmacol* (2009) 379**:**427-434.

15. Dhayal S, Welters HJ & Morgan NG. Structural requirements for the cytoprotective actions of mono-unsaturated fatty acids in the pancreatic β-cell line, BRIN-BD11. *Br J Pharmacol* (2008) 153:1718-1727.

16. Moran BM, Abdel-Wahab YH, Flatt PR & McKillop AM. Evaluation of the insulin releasing and glucose lowering effects of GPR120 activation in pancreatic beta cells. *Diabetes Obes Metab* (2014) 11:1128-1139.

17. Stone VM, Dhayal S, Brocklehurst KJ, Lenaghan C, Sorhede Winzell M, Hammar M, Xu X, Smith DM & Morgan NG. GPR120 (FFAR4) is preferentially expressed in pancreatic delta cells and regulates somatostatin secretion from murine islets of Langerhans. *Diabetologia* (2014) 57**:**1182-1191.

18. Suckow AT, Polidori D, Yan W, Chon S, Ma JY, Leonard J & Briscoe CP. Alteration of the glucagon axis in GPR120 (FFAR4) knockout mice: a role for GPR120 in glucagon secretion. *J Biol Chem* (2014) 289:15751-15763.

19. Kamakura R, Raza GS, Prasannan A, Walkowiak J, Herzig KH. Dipeptidyl peptidase-4 and GLP-1 interplay in STC-1 and GLUTag cell lines. *Peptides* (2020) 134:170419. doi: 10.1016/j.peptides.2020.170419.

20. Iwasaki K, Harada N, Sasaki K, Yamane S, Iida K, Suzuki K, *et al.* Free fatty acid receptor GPR120 is highly expressed in enteroendocrine K cells of the upper small intestine and has a critical role in GIP secretion after fat ingestion. *Endocrinology* (2015) 156:837-846.

21. Tanaka T, Yano T, Adachi T, Koshimizu T, Hirasawa A & Tsujimoto G. Cloning and characterization of the rat free fatty acid receptor GPR120: in vivo effect of the natural ligand on GLP-1 secretion and proliferation of pancreatic beta cells. *N-S Arch Pharmacol* (2008) 377: 515-522.

22. Bianchini G, Nigro C, Sirico A, Novelli R, Prevenzano I, Miele C, *et al*. A new synthetic dual agonist of GPR120/GPR40 induces GLP-1 secretion and improves glucose homeostasis in mice. *Biomed Pharmacother*. (2021) 139:111613-111626.

23. Parker HE, Habib AM, Rogers GJ, Gribble FM & Reimann F. Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* (2009) 52:289-298.

24. Murata Y, Harada N, Kishino S, Iwasaki K, Ikeguchi-Ogura E, Yamane S, *et al.* Medium-chain triglycerides inhibit long-chain triglyceride-induced GIP secretion through GPR120-dependent inhibition of CCK. *iScience* (2021) 24(9):102963-102985.

25. Tanaka T, Katsuma S, Adachi T, Koshimizu TA, Hirasawa A & Tsujimoto G. Free fatty acids induce cholecystokinin secretion through GPR120. *N-S Arch Pharmacol* (2008) 377**:**523-527.

26. Tsukahara T, Watanabe K, Watanabe T, Yamagami H, Sogawa M, Tanigawa T, *et al.* Tumor necrosis factor α decreases glucagon-like peptide-2 expression by up-regulating G-protein-coupled receptor 120 in Crohn disease. *Am J Pathol* (2015) 185:185-196.

27. Morishita M, Tanaka T, Shida T & Takayama K. Usefulness of colon targeted DHA and EPA as novel diabetes medications that promote intrinsic GLP-1 secretions. *J Control Release* (2008) 132**:**99-104.

28. Ou HY, Wu HT, Hung HC, Yang YC, Wu JS & Chang CJ. Multiple mechanisms of GW-9508, a selective G protein-coupled receptor 40 agonist, in the regulation of glucose homeostasis and insulin sensitivity. *Am J Physiol Endocrinol Metab* (2013) 304**:**668-676.

29. Zhao Y, Wang L, Zha D, Qiao L, Lu L, Yu J, *et al.* GW9508 inhibits insulin secretion by activating ATP-sensitive potassium channels in rat pancreatic β-cells. *J Mol Endocrinol* (2013) 51:69-77.

30. Gong Z, Yoshimura M, Aizawa S, Kurotani R, Zigman JM, Sakai T & Sakata I. G protein-coupled receptor 120 signaling regulates ghrelin secretion *in vivo* and *in vitro*. *Am J Physiol Endocrinol Metab* (2014) 306**:**28-35.

31. Habib AM, Richards P, Rodgers CJ, Reimann F & Gribble FM. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. *Diabetologia* (2013) 56:1413-1416.

32. Guoxia J, Qinghua G, Qidi X, Ruifang K, Shiben W, Kang L, *et al*. Novel GPR120 Agonists with Improved Pharmacokinetic Profiles for the Treatment of Type 2 Diabetes. *Molecules* (2021) 26(22):6907-6922.

33. Kilkenny C, Browne W, Cuthill IC, Emerson M & Altman DG. Animal research: Reporting *in vivo* experiments: the ARRIVE guidelines. *Br J Pharmacol* (2010) 160:1577–1579.

34. Flatt PR & Bailey CJ. Abnormal plasma glucose and insulin responses in heterozygous lean (ob/+) mice. *Diabetologia* (1981) 20:573–577.

35. Drucker DJ, Jin T, Asa SL, Young TA & Brubaker PL. Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. *Mol Endocrinol* (1994) 8:1646-1655.

36. Hand KV, Giblin L & Green BD. Hormone profiling in a novel enteroendocrine cell line pGIP/neo: SCT-1. *Metabolism* (2012) 61:1683-1686.

37. Kimura, I.; Ichimura, A.; Ohue-Kitano, R.; Igarashi, M. Free Fatty Acid Receptors in Health and Disease. *Physiol Rev* (2020) 100:171−210.

38. Stuttgen GM, Sahoo D. FFAR4: A New Player in Cardiometabolic Disease? *Endocrinology* (2021) 162(8) 1-39.

39. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, *et al*. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and humans. *Nature* (2012) 483:350-357.

40. Taneera J, Lang S, Sharma A, Fadista J, Zhou Y, Ahlqvist E, *et al*. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. *Cell Metab* (2012) 16**:**122-134.

41. Ya-Qin D, Xue-Ying S, Jie C, Jin W, Jing-Yu L, Wen-Tao A, *et al*. Endogenous Lipid-GPR120 Signaling Modulates Pancreatic Islet Homeostasis to Different Extents. *Diabetes* (2022) 71(7):1454-1471.

42. Schilperoort M, van Dam AD, Hoeke G, Shabalina IG, Okolo A, Hanyaloglu AC, *et al*. The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat. *EMBO Mol Med* (2018) 10:e8047.

43. Shimpukade B, Hudson BD, Hovgaard CK & Milligan G. Discovery of a potent and selective GPR120 agonist. *J Med Chem* (2012) 55:4511-4515.

44. Lauffer LM, Iakoubov R & Brubaker PL. GPR119 is essential for oleoylethanolamide–induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cells. *Diabetes* (2009) 58 1058-1066.

45. Hara T, Kimura I, Inoue D, Ichimura A, Hirasawa A. Free fatty acid receptors and their role in regulation of energy metabolism. *Rev Physiol Biochem Pharmacol* (2013) 164:77–116

46. Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ & Gribble FM. Glucose sensing in L-cells: a primary cell study. *Cell Metab* (2008) 8**:**532-539

Legends for Figures

Fig. 1: Effects of glucose and GW-9508 administered orally or by intraperitoneal injection (A, B) glucose tolerance and (D, E) plasma insulin in response to glucose. AUC values for 0-105 min post injection for (C) glucose and (F) insulin are also shown. Glucose (18mmol/kg body weight) in combination with GW-9508 (0.1µmol/kg body weight) was administered by oral or intraperitoneal injection to non-fasted NIH Swiss mice (n=6). Values are mean ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to intraperitoneal injection. + $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ compared to glucose alone.

Fig. 2: Effects of GW-9508 on (A) oral glucose tolerance (B) plasma insulin in response to glucose. AUC values for 0-105 min post injection for (C) glucose and (D) insulin are also shown. Glucose (18mmol/kg body weight) in combination with GW-9508 (0.1µmol/kg body weight) was administered by oral injection to 18 h fasted high fat fed NIH Swiss mice (n=6). Values are mean ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to glucose alone.

Fig. 3: Effects of GW-9508 and incretin hormones on glucose tolerance and insulin response to glucose in (A, D) C57/BL6 wild-type mice, (B, E) GIP receptor knockout mice and (C, F) GLP-1 receptor knockout mice. Plasma glucose (A-C) and insulin (D-F) were determined prior to and after oral administration of glucose (18mmol/kg body weight) and in combination with GW-9508 (0.1µmol/kg body weight) or intraperitoneal injection of GIP or GLP-1 (both at 25nmol/kg body weight). AUC values for 0-105 min post injection for (A-C) glucose and (D-F) insulin are also shown. Values are mean ± SEM for 6 mice. * $p<0.05$, ** $p<0.01$ compared to glucose alone.

Fig. 4: Effects of GW-9508 on (A) plasma glucose, (B) plasma insulin, (C) total plasma GLP-1 and (D) total plasma GIP at 0-30 min post injection with glucose are shown. Glucose (18mmol/kg body weight) alone and in combination with GW-9508 (0.1µmol/kg body weight) was administered orally

to non-fasted NIH Swiss mice (n=6). Values are mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001 compared to glucose alone.

Fig. 5: Distribution in GLUTag cells of (A) DAPI nuclear stain, (B) GLP-1, (C) GPR120 and (D) merge of GPR120 co-localised with GLP-1. Examples of co-localisation indicated by arrows.

Fig. 6: Distribution in pGIP/neo STC-1 cells of (A) DAPI nuclear stain, (B) GIP, (C) GPR120 and (D) merge of GPR120 co-localised with GIP. Examples of co-localisation indicated by arrows.

Fig. 7: Distribution in mouse jejunum tissue of (A, B) DAPI nuclear stain, (C) GIP, (D) GLP-1, (E, F) GPR120, (G) Merge of GPR120 co-localised with GIP and (H) merge of GPR120 co-localised with GLP-1. Examples of co-localisation indicated by arrows.

Fig. 8: Effects of GPR120 agonist GW9508 (10^{-10}-10^{-6}M) and antagonist AH7614 (10^{-5}M) on (A) GLP-1 secretion in GLUTag cells and (B) GIP Secretion in pGIPneo STC-1 cells at 2mM glucose. Results are mean ± SEM (n=3). **p<0.01 and ***p<0.001 compared to basal glucose control. $^{\Delta\Delta}$p<0.01 compared to antagonist treatment at same concentration of agonist.

Fig. 1:

A

B

C

D

E

F
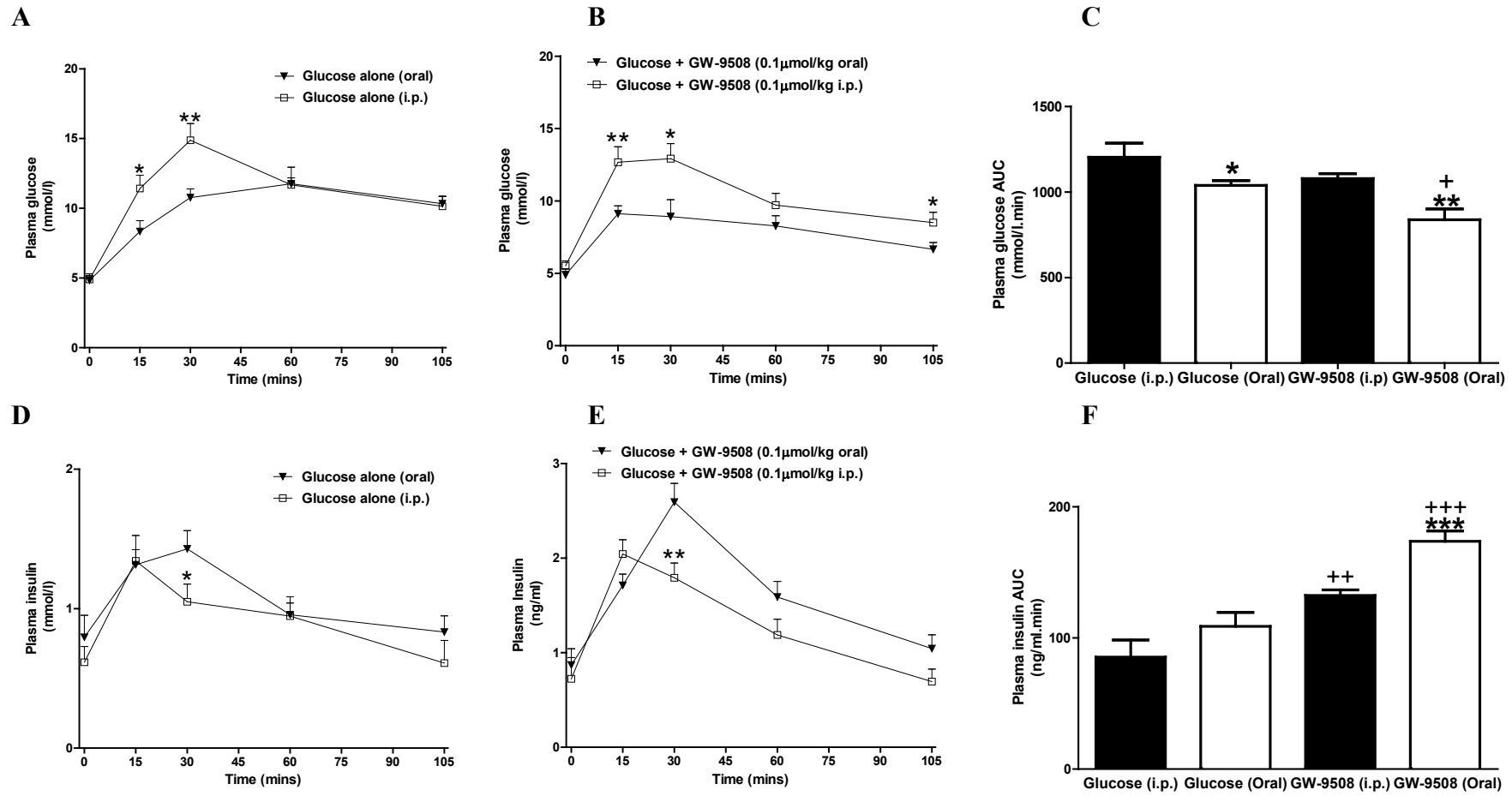
Fig. 2:

A
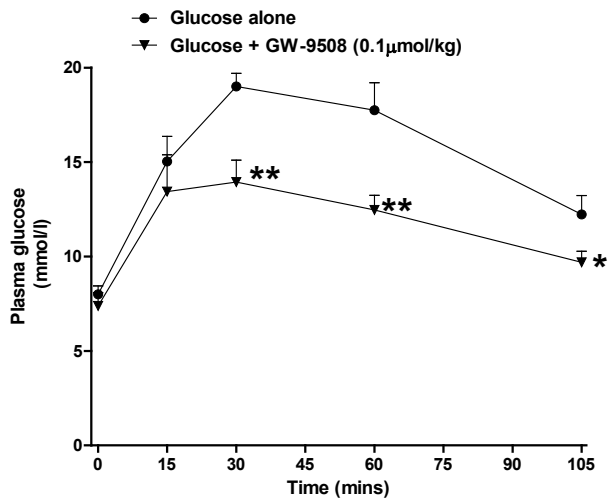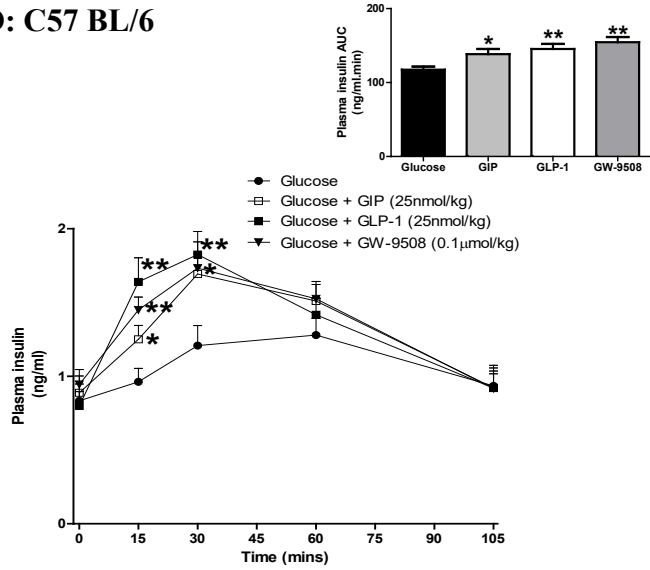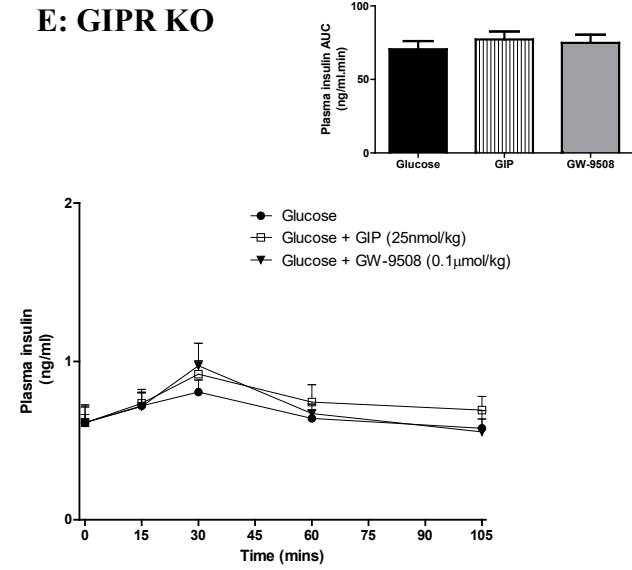

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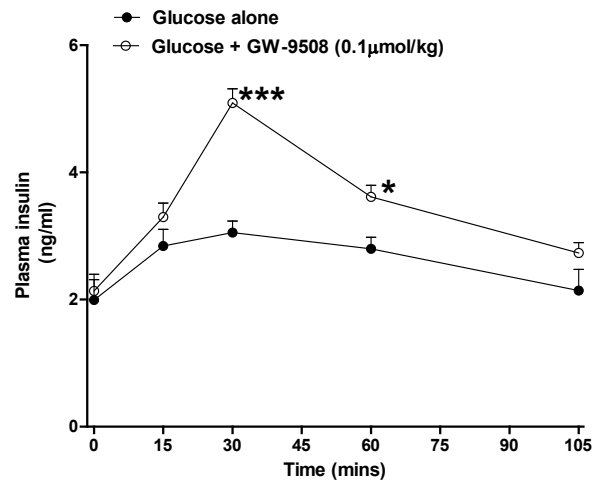
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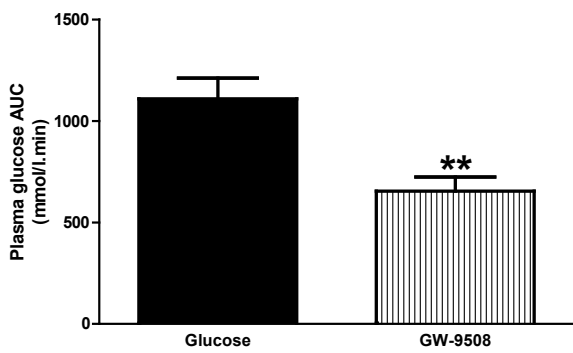
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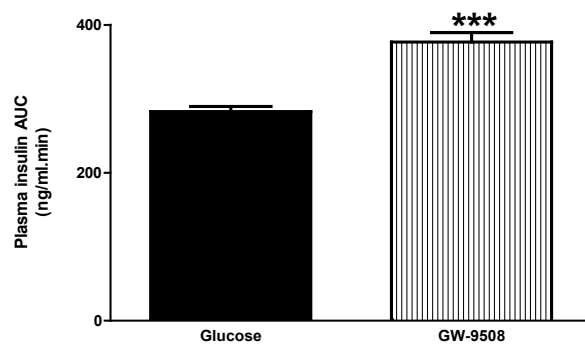
Fig. 3:

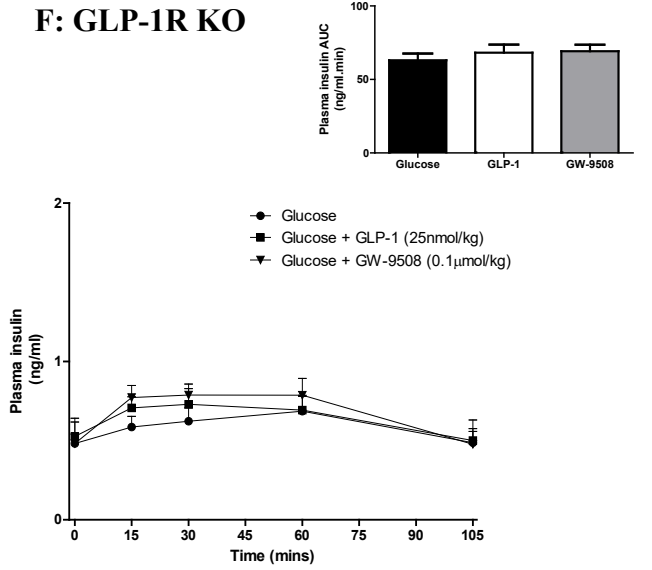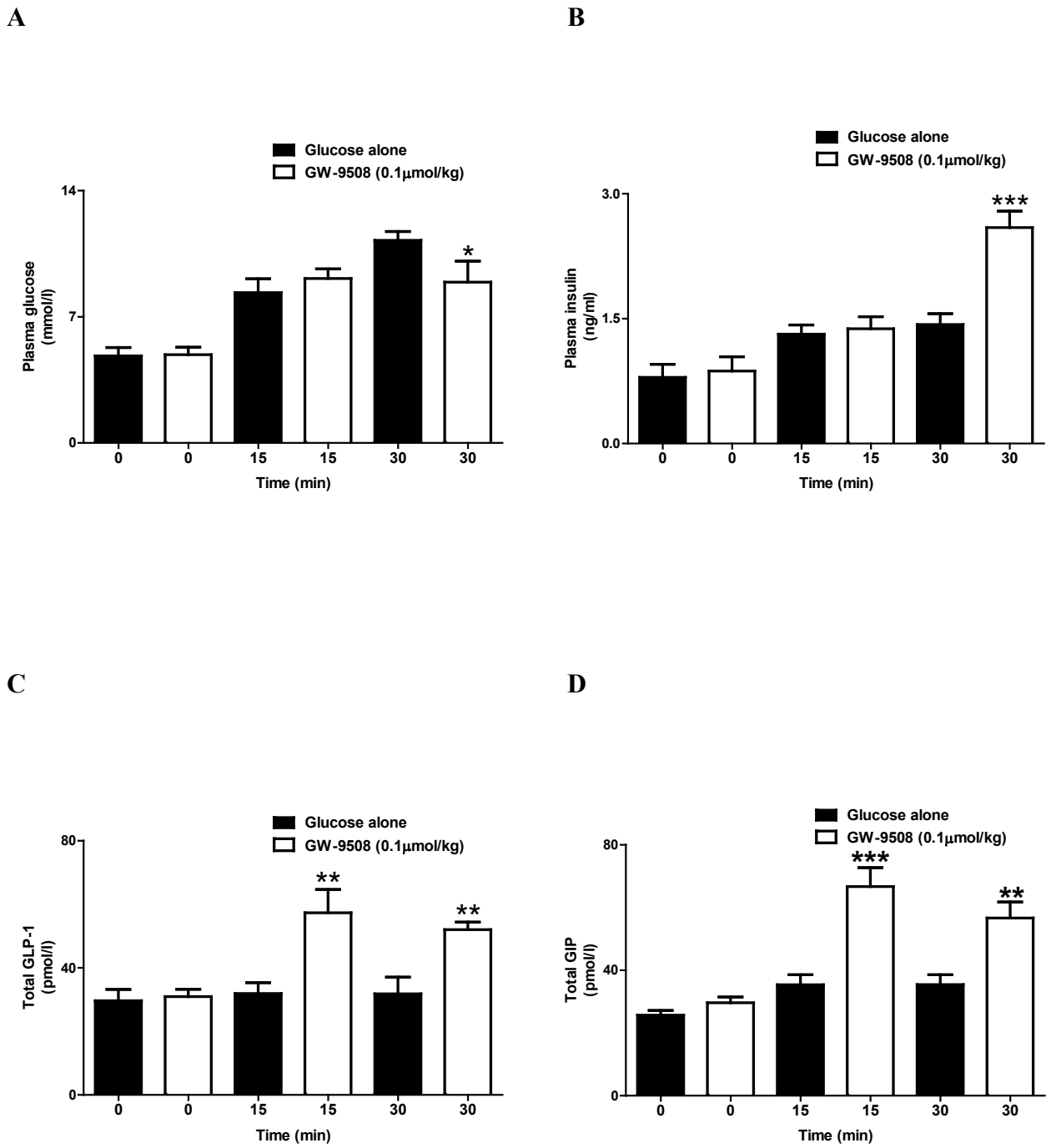

A: C57 BL/6
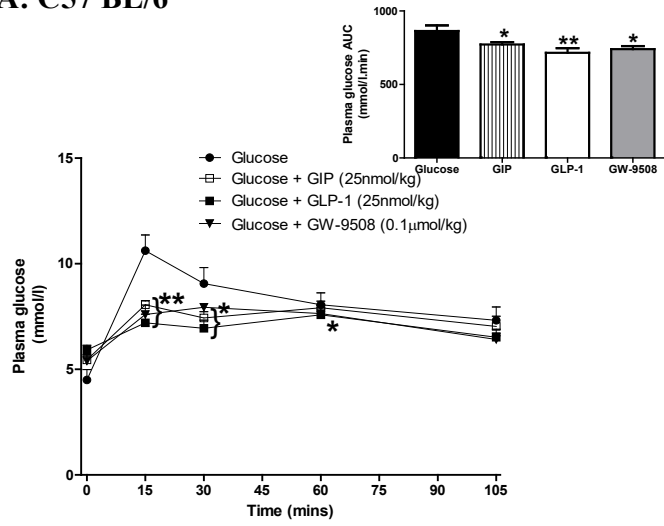
B: GIPR KO
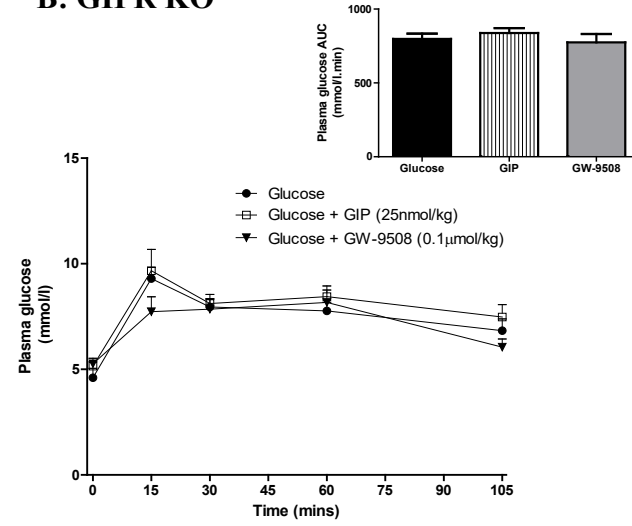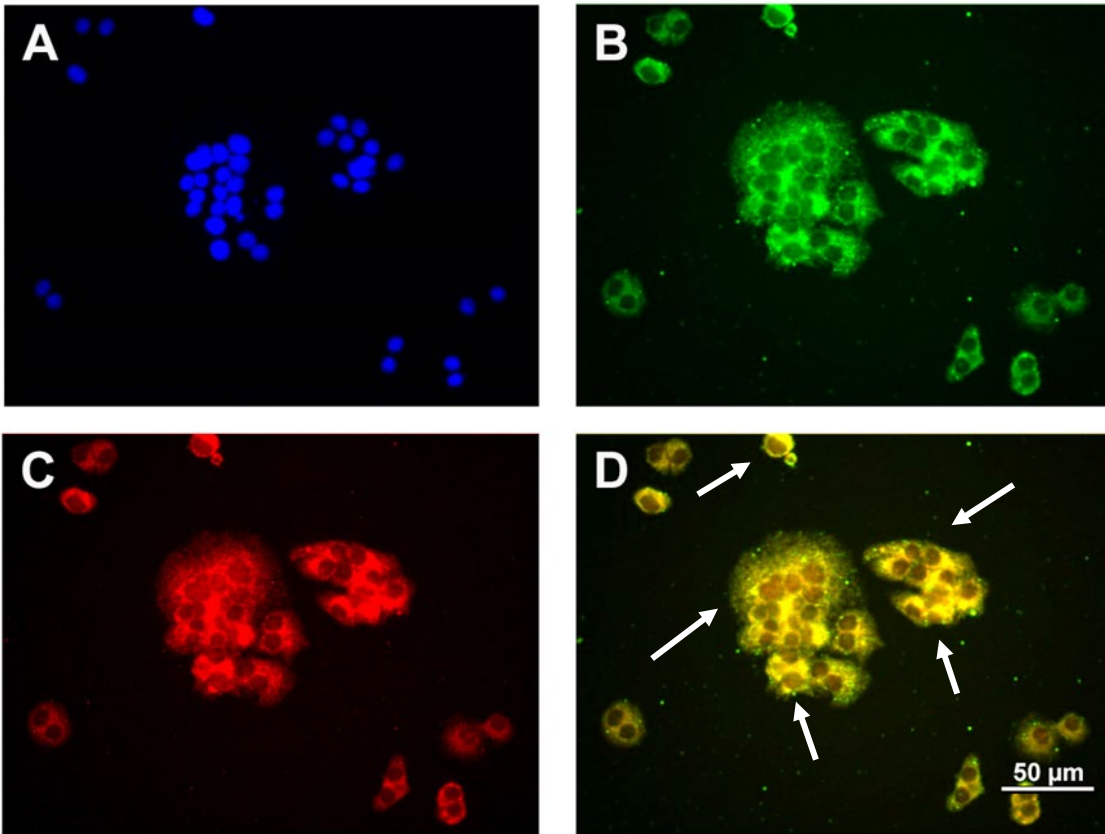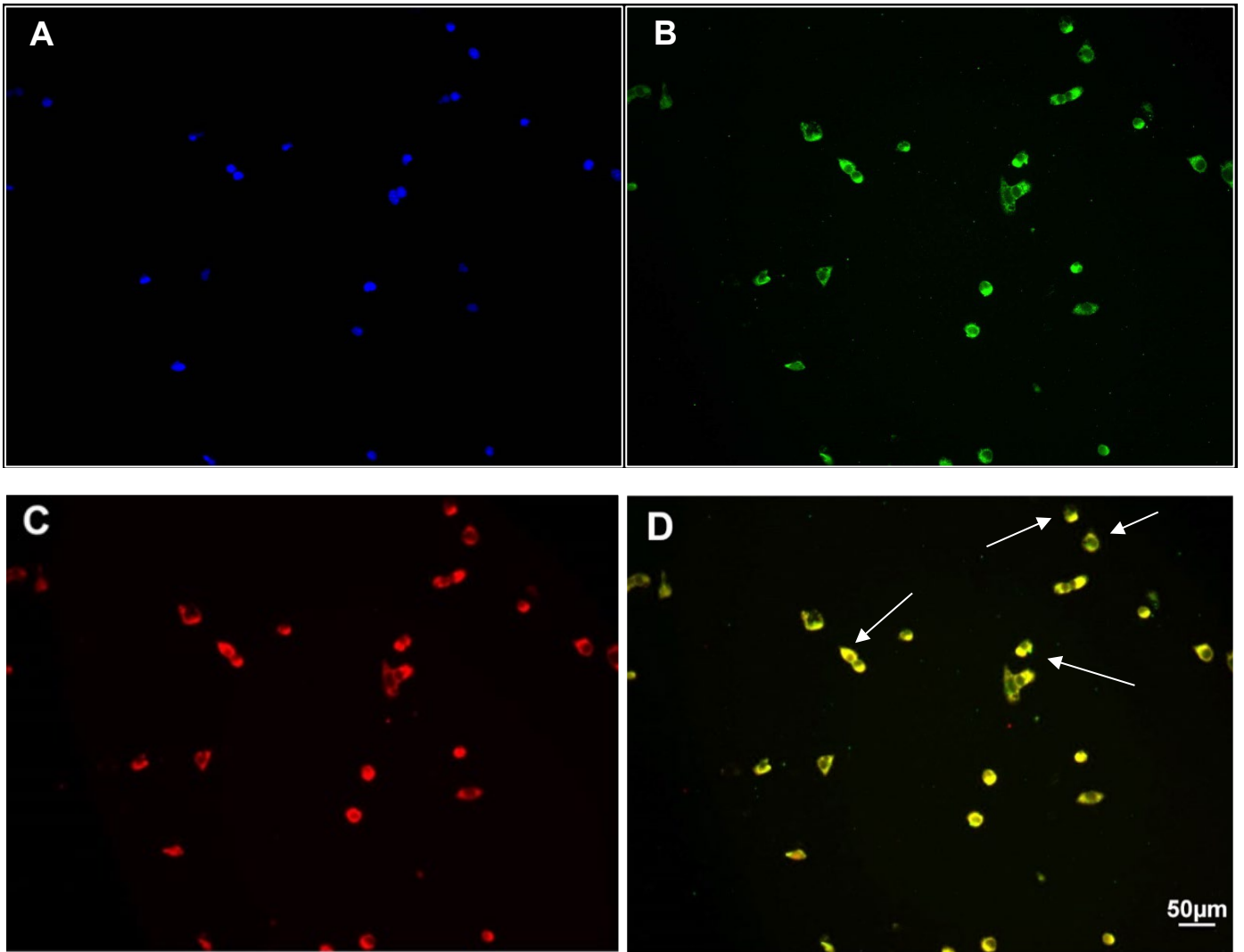
C: GLP-1R KO
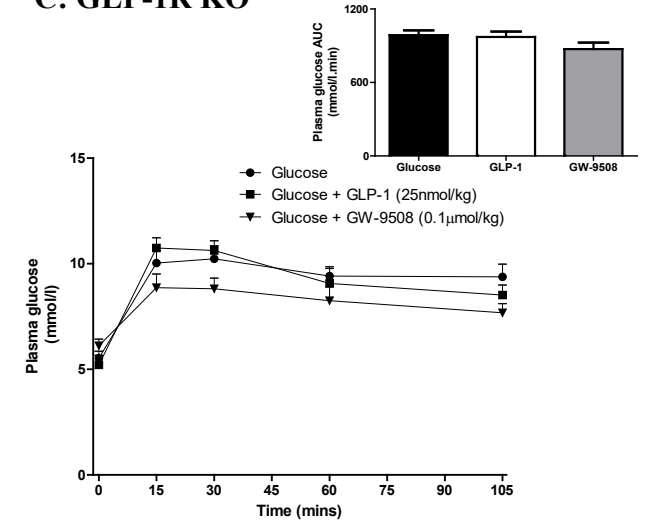
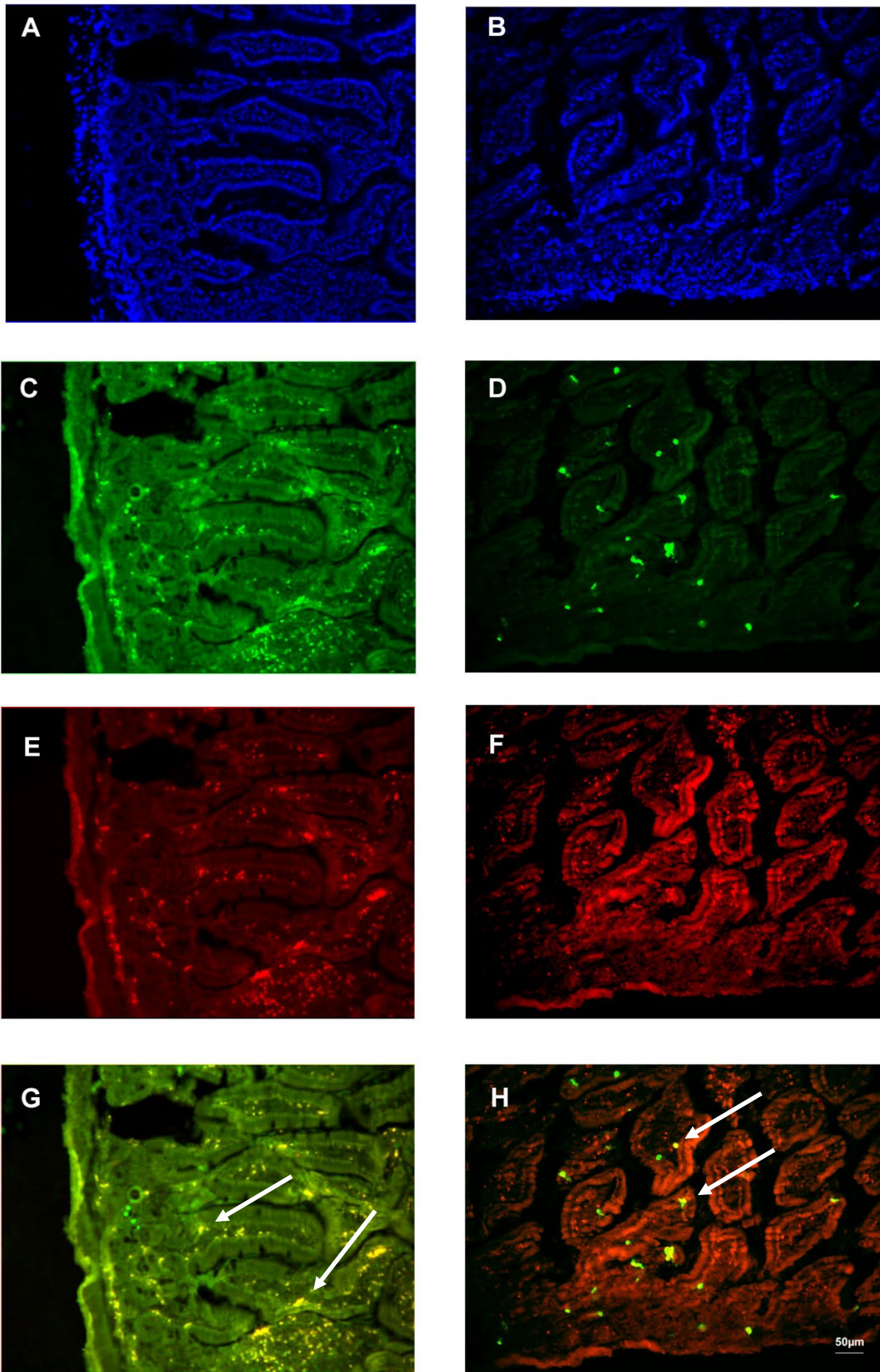**D: C57 BL/6**

E: GIPR KO

F: GLP-1R KO
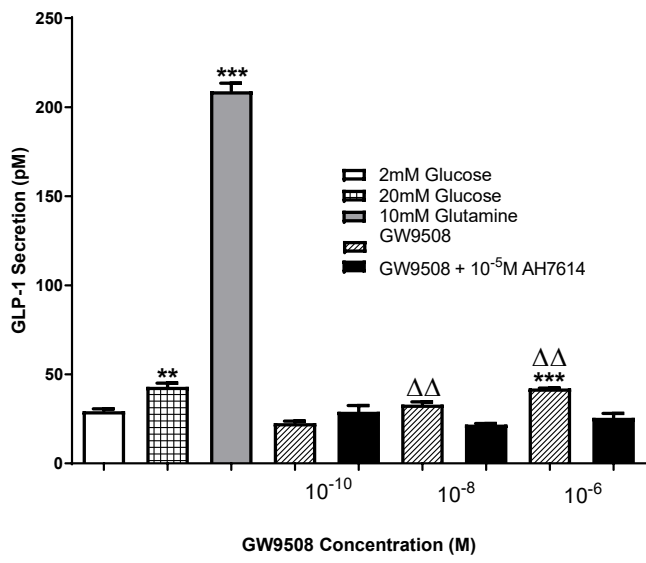
Fig. 4:

A

B

C

D

Fig. 5:

Fig. 6:

Fig. 8:

(A)

(B)