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The role of β-cell GLP-1 signaling in glucose regulation and response to diabetes drugs

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SUMMARY

Glucagon-like peptide-1 (GLP-1), an insulinotropic peptide released from the intestine after eating, is essential for normal glucose tolerance (GT). To determine whether this effect is mediated directly by GLP-1 receptors (GLP1R) on islet β-cells, we developed mice with β-cell specific knockdown of *Glp1r*. β-cell *Glp1r* knockdown mice had impaired GT after intraperitoneal (IP) glucose, and did not secrete insulin in response to IP or intravenous GLP-1. However, they had normal GT after oral glucose, a response that was impaired by a GLP1R antagonist. β-cell *Glp1r* knockdown mice had blunted responses to a GLP1R agonist, but intact glucose lowering with a DPP-4 inhibitor. Thus, in mice, β-cell Glp1r are required to respond to hyperglycemia and exogenous GLP-1, but other factors compensate for reduced GLP-1 action on the β-cell during meal ingestion. These results support a role for extra-islet GLP1R in oral glucose tolerance and paracrine regulation of β-cells by islet GLP-1.

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AUTHOR CONTRIBUTIONS

E.P.S, R.J.S, D.A.D designed the study and co-wrote the paper. E.P.S. performed most of the experiments. Z.A. designed and preformed a subset of the glucose tolerance tests. B.L. helped characterize the $Glp1r^{CMVKO}$ and $Glp1r^{f/f}$ lines. A.G.L performed islet cell perfusions, contributed to study design and assisted with writing. C.W. prepared islets and islet cell suspensions and designed the *in vitro* experiments. E.B.C. performed peptide assays. P.M performed pPCR assays. D.S and D.P-T assisted with data interpretation. L.H.P and N.T. created the MIPcreER mouse line, and D.A.S. contributed to development of the $Glp1r^{f/f}$ line.

DISCLOSURE SUMMARY

RJS: consults for Johnson & Johnson, and Roche; is on scientific advisory boards for Lilly, J & J, Zafgen and Merck; is a speaker for Lilly, Novo Nordisk, and Merck; has stock options in Zafgen; and research grants from J & J, Zafgen, Roche, and Mannkind.
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INTRODUCTION

GLP-1, a peptide produced by mucosal endocrine cells in the distal intestine, is released from the gut into the circulation after nutrient ingestion. GLP-1 is generally thought to signal as a hormone, directly activating β-cell GLP1R to enhance glucose-stimulated insulin secretion, i.e. the incretin effect (Campbell and Drucker, 2013; Kieffer and Habener, 1999). In addition, GLP-1 has a broad range of actions that contribute to glucose regulation including inhibition of glucagon secretion and gastrointestinal motility, suppression of hepatic glucose production, and reduction of appetite (Barrera et al., 2011a; Campbell and Drucker, 2013). Based on these physiologic actions, the GLP1R is a logical pharmacologic target, and there are now two classes of drugs for type 2 diabetes, GLP1R agonists and inhibitors of dipeptidyl peptidase 4 (DPP-4i), that act through this receptor (Drucker and Nauck, 2006)

There are several reasons to question the conventional endocrine model proposed for GLP-1 action, a view recently expressed by several groups (D'Alessio, 2011; Holst and Deacon, 2005). First, GLP-1 circulates in relatively low concentrations and post-prandial changes in plasma levels are modest compared to other gut hormones (Baggio and Drucker, 2007; Vilsbøll et al., 2003). Second, GLP-1 is rapidly inactivated by dipeptidyl peptidase 4 resulting in a very short plasma half-life limiting availability to target cells (Deacon et al., 1995). It has been estimated that ~ 90% of secreted GLP-1 is metabolized by DPP-4 before reaching the central venous circulation (Hansen et al., 1999; Holst and Deacon, 2005). Finally, there is growing evidence that GLP-1 regulates glucose metabolism indirectly via GLP1R expressed on peripheral and central neurons (Donath and Burcelin, 2013; Vahl et al., 2007; Waget et al., 2011). This study was designed to determine whether GLP-1 mediates insulin secretion and glucose lowering as a hormone acting directly on islet β-cells.

RESULTS and DISCUSSION

β-cell GLP1R are not necessary for normal oral glucose tolerance

To address the role of β-cell GLP1R on glucose homeostasis, a Cre-loxP strategy was used to create a mouse line, *Glp1r*$^{f/f}$, permitting tissue-specific knockdown of the *Glp1r* gene (Figure 1A, upper panel, and Figures S1A and S1B and Supplemental text). Mice with *Glp1r*$^{f/f}$ were crossed with animals expressing Cre recombinase ubiquitously under the control of a cytomegalovirus (CMV) promoter to create CMVcre;*Glp1r*$^{\Delta/\Delta}$ mice (*Glp1r*$^{\mathrm{CMVKO}}$) that are functionally global knockouts (Figure 1D, upper panel, and Figure S1C). The *Glp1r*$^{f/f}$ mice were also crossed with lines expressing Cre in the β-cell either under constitutive control with a rat insulin promoter (RIP) or under tamoxifen inducible regulation using a mouse insulin promoter (MIPcreER) (Kaihara et al., 2013; Wicksteed et al., 2010); (Figures S1D-S1F). To demonstrate β-cell specific disruption of *Glp1r*, islets were isolated from *Glp1r*^{WT}, *Glp1r*^{CMVKO}, RIPcre;*Glp1r*$^{f/f}$, and tamoxifen or vehicle treated MIPcreER;*Glp1r*$^{f/f}$ mice. RNA was extracted followed by PCR of cDNA using primers that generated a product spanning the deleted exons 6 and 7 (Figure 1A, upper panel). WT mice had a transcript of 522 bp that defined the intact *Glp1r* gene. Islets from *Glp1r*^{CMVKO} expressed exclusively a truncated cDNA of 211 bp due to deletion of the floxed portion of the *Glp1r* (Figure 1A, lower panel). MIPcreER;*Glp1r*$^{f/f}$ mice treated with tamoxifen, and RIPcre; *Glp1r*$^{f/f}$ mice, expressed both WT and truncated products. Islet Cre expression under the control of the CMV, RIP and MIP promoters was comparable (Figure S1H). Fidelity of Cre expression in both the RIPcre and MIPcreER lines was confirmed by crossing each with a "double reporter" Gt(ROSA)26Sortm4 (ACTB-tdTomato,-EGFP)Luo/J line (Figure 1B). RIPcre mice (Figure 1B: panel A and D), and MIPcreER mice treated with tamoxifen (Figure 1B: B and E), demonstrated robust islet-specific recombination, while MIPcreER mice treated with vehicle showed minimal recombination (Figure 1B: C and F). In contrast to the RIPcre construct, MIPcreER did not induce recombination in the hypothalamus (Figure S1G). Isolated islets, and β-cells sorted from islet cell digests, demonstrated 70–80% knockdown of *Glp1r* mRNA expression after tamoxifen treatment respectively (Figures S2A–S3G). Consistent with the RNA results, isolated islets from tamoxifen treated mice did not increase cytosolic cAMP (Figure 1C, upper panel), or secrete insulin (Figure 1C, lower panel), in response to the GLP1R agonist exendin-4. However, in contrast to in animals with a global deletion of *Glp1r* (Figure 1D, upper panel), food intake was suppressed in mice with β-cell knockdown of the *Glp1r* in response to GLP1R agonists (Figure 1D, lower panel), and they lost weight with chronic liraglutide treatment (Figure S2I). β-cell specific *Glp1r* knockdown did not affect body weight (Veh 32 ± 0.7 and Tam 31 ± 0.5 g; Figure 1E, upper panel) but caused a small, significant increase in fasting blood glucose (Veh 143 ±2 and Tam 157 ± 2.6 mg/dl, $p < 0.001$; Figure 1E, lower panel). Expression of proinsulin and proglucagon mRNA was similar in tamoxifen and vehicle treated MIPcreER;*Glp1r*$^{f/f}$ mice (Figure S2H).

The effect of β-cell specific GLP1R signaling on GT was examined by comparing WT, MIPcreER;*Glp1r*$^{f/f}$, RIPcre;*Glp1r*$^{f/f}$, and *Glp1r*^{CMVKO} mice. Compared to *Glp1r*^{WT}, animals with a global deletion of the *Glp1r* had impaired oral GT (Figure 2A). In contrast, the glycemic response to oral glucose loading did not differ between RIPcre;*Glp1r*$^{f/f}$ and controls (Figure S3A), or between tamoxifen- and vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ mice (Figure 1B), results that were repeatable in multiple separate cohorts (Figure S3C). Moreover, in response to oral glucose mice with β-cell *Glp1r* knockdown had similar insulin secretion (Figure 2C), comparable postprandial GLP-1 levels and diminished plasma GIP (Figure 2D) compared to controls. To further test the question of whether mealinduced GLP-1 acts directly on β-cells, glucose levels were measured in tamoxifen- and vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ mice that had been trained to spontaneously ingest a fixed amount of a mixed liquid nutrients. Similar to the GTTs with gastric gavage of glucose, postprandial glucose excursions were almost identical in the two groups (Figure 2E). These findings demonstrate that during enteral glucose absorption, the setting under which GLP-1 levels increase in the circulation, β-cell GLP1R are not necessary for normal glycemia.

To determine whether extra-islet GLP1R are important for normal oral glucose tolerance, WT mice or animals with β-cell specific knockdown of the *Glp1r* had oral glucose tolerance tests with and without the GLP1R antagonist Ex-9. Blockade of the Glp1r caused glucose intolerance in both WT mice and animals with β-cell *Glp1r* knockdown (Figure 1F, Figure S3E and S3F), implicating non-β-cell GLP1R in the incretin effect and regulation of postprandial glucose. Recent evidence suggests that GLP-1 has direct effects on islet α-cells

(De Marinis et al., 2010), and we cannot rule out the possibility that worsening of glucose tolerance during acute GLP1R blockade is due to interference with glucagon suppression.

β-cell GLP1R are necessary for a normal response to IP glucose

In contrast to the results with oral glucose, β-cell *Glp1r* knockdown with either RIPcre (Figure S3B) or MIPcreER (Figure 2G) caused significant glucose intolerance in mice receiving IP glucose; this is similar to the response in *Glp1r*$^{\mathrm{CMVKO}}$ animals (Figure 2H). Mice heterozygous for deletion of the GLP-1 receptor, MIPcreER;*Glp1r*$^{\Delta/f}$ mice (See supplemental text) treated with vehicle had similar IP glucose tolerance to controls with a full complement of *Glp1r* (Figure 2I). MIPcreER;*Glp1r*$^{\Delta/f}$ mice treated with tamoxifen, a more complete β-cell-specific knockdown, had impaired IP glucose tolerance. To determine whether the abnormal IPGTT was due to a lack of islet *Glp1r*, MIPcreER;*Glp1r*$^{f/f}$ mice had IP glucose tolerance tests with and without Ex-9. Acute blockade of the Glp1r caused glucose intolerance in vehicle treated mice but had no effect in animals with β-cell *Glp1r* knockdown (Figure 2J). These results support the importance of β-cell GLP1R in the correction of IP glucose-induced hyperglycemia. Consistent with these results, tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice had higher glucose levels following IV glucose administration than vehicle-treated controls (Figure S3D). Since glucose administered IP or IV causes hyperglycemia but does not affect the release of gastrointestinal hormones or the neural activation that contribute to insulin secretion after meals (Thorens, 2011), these results suggest that β-cell GLP1R are needed for normal β-cell sensitivity to hyperglycemia, independent of acute changes in circulating GLP-1.

β-cell specific knockdown of *Glp1r* eliminates the insulin responses to IV and IP GLP-1

To analyze the role of exogenous GLP-1 on glucose tolerance in the absence of β-cell GLP1R, tamoxifen- and vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ were given IP or IV GLP-1 during an IP glucose tolerance test. Vehicle-treated mice had substantial improvement in IP glucose tolerance when given parenteral GLP-1, and this was associated with a significant increase in insulin secretion (Figures 3A–C). In contrast, the tamoxifen-treated animals had a modest reduction of glycemia when given IP GLP-1, but no increase in plasma insulin. This muted effect on glycemia is presumably the result of insulin-independent actions of GLP-1. In response to IV GLP-1, there was no effect on plasma glucose or insulin in tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice (Figures 3D–3F). Similar to MIPcreER;*Glp1r*$^{f/f}$ mice, mice heterozygous for deletion of the GLP-1 receptor, MIPcreER;*Glp1r*$^{\Delta/f}$, and treated with tamoxifen, had no response to IV GLP-1. (Figures 2G and 2F). Taken together with the results of IP GLP-1 administration, the lack of an insulin response to IV GLP-1 in the setting of a robust response in vehicle-treated controls, confirms a reduction of β-cell *Glp1r* in tamoxifen-treated MIPcreER *Glp1r*$^{f/f}$ mice to a degree that eliminates detectable effects *in vivo*. Moreover, the differential effects of IP and IV GLP-1, with a partial glucose response to the former but complete absence glucose lowering with the latter, suggests that the GLP-1 system is compartmentalized, with some gluco-regulatory GLP1R sequestered from peptide in the circulation but available to peptide in the peritoneal cavity.

Our studies of the physiologic role of GLP1R during oral and IP glucose challenges have similarities and differences with a recent report of glucose tolerance in global *Glp1r* null animals with transgenic expression of a human GLP1R construct specifically in β-cells (Lamont et al., 2012). Mice with islet rescue of the GLP1R also recovered an insulinotropic response to an exogenous GLP1R agonist, similar to the results described here. However, these animals had improved oral glucose tolerance compared to *Glp1r* null mice, supporting a direct effect of circulating GLP-1 on β-cells, a finding that is at odds with the normal oral glucose tolerance we have observed repeatedly in mice with β-cell specific *Glp1r* knockdown. This discrepancy could be explained either by non-physiologic expression of the hGLP1R construct in the rescue model or insufficient knockdown of *Glp1r* in our inducible Cre-loxP model. Based on significant knockdown of *Glp1r* expression in islets and β-cells, and the inability of GLP1R agonists *in vitro* and *in vivo* to stimulate insulin release, there do not appear to be a sufficient numbers of β-cell *Glp1r* to mount functional responses in tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ animals. Moreover, the normal oral glucose tolerance in this line is very reproducible, reducing the likelihood that this is an underpowered observation.

The action of long-acting GLP-1 agonists but not DPP4i are impaired with β-cell knockdown of *Glp1r*

To address the mechanisms by which GLP-1 signaling contributes to diabetes therapeutics, we determined the impact of β-cell *Glp1r* knockdown on the response to GLP-1-based drugs. Tamoxifen and vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ (Figures 4A and 4B), and RIPcre;*Glp1r*$^{f/f}$ or WT (Figures S4) were given the long-acting GLP1R agonist liraglutide 30 minutes prior to an IP glucose load. The glucose profile after liraglutide was nearly flattened in the mice retaining β-cell *Glp1r*. Treatment with liraglutide improved glucose tolerance in mice with β-cell *Glp1r* knockdown, through either RIPcre or MIPcreER, but the effect was blunted compared to the controls. Quite distinct from the response to liraglutide, administration of the DPP-4i vildagliptin lowered blood glucose equivalently in vehicle and tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice challenged with either oral or IP glucose (Figures 4C–F). These findings indicate that liraglutide exerts glucose lowering actions, in part, through β-cell GLP1R. The intact glucose lowering by DPP-4 inhibition may be explained by GLP-1 effects on non-β-cell GLP1R populations, or may result from compensation by other factors that are also DPP-4 substrates.

The results reported here indicate that glucose control following oral administration of carbohydrate does not require direct signaling through the β-cell Glp1r in the mouse. A major implication of these findings is that GLP-1 released into the circulation after meals does not stimulate insulin secretion through an endocrine mechanism. Rather our findings are compatible with a model in which GLP-1 acts indirectly to mediate the incretin effect, possibly through neural GLP1R. There is experimental support for neural GLP1R in the hepatic portal vein to mediate glucose tolerance (Rüttimann et al., 2009; Vahl et al., 2007) and recent evidence supports a similar mechanism in the splanchnic bed to mediate the effects DPP-4 inhibitors (Waget et al., 2011). Moreover, intra-cerebral administration of GLP-1 transiently lowers blood glucose in freely-fed rats and reduces hepatic glucose production (Barrera et al., 2011b; Burmeister et al., 2012; Sandoval et al., 2008). In this context a strong case can be made that neural GLP1R are the extra-β-cell receptors mediating glucose lowering in the present study. That β-cell GLP1R are not necessary in the setting of hyperglycemia induced by meals, but are needed for a normal response to parenteral glucose administration, may be explained by redundancy and overlap of insulinotropic signals initiated by glucose ingestion and absorption.

Our data indicate that β-cell GLP1R are necessary for the normal clearance of IP and IV glucose loads and for the insulinotropic response to exogenous GLP1R agonists. While these are experimental manipulations, a case can be made that the results have both physiologic and pharmacologic relevance. The relative responses of our knockdown and control mice to IP and IV glucose is consistent with previous work indicating that GLP-1 signaling is essential for β-cells to maintain glucose competence, or sensitivity to changes in ambient glycemia (Flamez et al., 1998; Holz et al., 1993). This effect has also been demonstrated in humans whereby Ex-9 reduces glucose-stimulated insulin release during fasting when plasma GLP-1 is low and unchanging (Salehi et al., 2010; Schirra et al., 1998). Important in this context is recent work suggesting that GLP-1 produced by α-cells in the pancreatic islet is important for local regulation of insulin secretion (Ellingsgaard et al., 2011; Kilimnik et al.; Nie et al., 2000). Our results are compatible with this model of paracrine actions of α-cell GLP-1 on β-cell GLP1R that enhance glucose-stimulated insulin release.

On the basis of the studies reported here, we conclude that the incretin role of GLP-1 cannot be explained by an endocrine mechanism of action, and that extra-islet GLP1R pathways must be invoked to explain the GLP-1 contribution to the incretin effect (See Graphical Abstract). Direct signaling through GLP1R is necessary for clearance or IV and IP glucose induced hyperglycemia, possibly by promoting glucose competence, and it is plausible that this is mediated by the actions of locally produced GLP-1. However, the role of circulating GLP-1 acting on β-cell receptors is limited to circumstances where plasma GLP-1 is substantially elevated, pharmacologically or otherwise, in a sustained manner. These findings suggest distinct levels of β-cell regulation by GLP-1 with a component of direct, paracrine or neuracrine action, and indirect mediation by extra-islet GLP1R. Moreover, our findings suggest that long-acting GLP1R agonists co-opt the paracrine system, and DPP-4 inhibitors act through non-β-cell GLP1R, to exert their anti-diabetic actions.

EXPERIMENTAL PROCEDURES

Reagents

GLP-1[7–36NH2] (21st Century Biochemicals, Marlboro, MA), vildagliptin, liraglutide, exendin-4 (Amylin, La Jolla, CA) and exendin-(9–39) (Ex-9) (21^{st} Century Biochemicals) were reconstituted in saline containing 0.1% (w/v) bovine serum albumin, aliquotted and stored at −20 °C. Liraglutide was kindly provided by Dr. Lotte Bjerre-Nielsen, Novo Nordisk.

Animal husbandry and glucose tolerance tests

Mice were housed in a temperature-controlled room under a 12 h light-dark cycle (lights on 0600–1800 h), and fed standard chow with water available *ad libitum*. Oral and IP GTT’s, unless otherwise stated, were performed using 2.5g /kg, 44% glucose and 2.5 g/kg, 25% glucose, respectively, by standard methods; blood was sampled from the tail vein. Mixed nutrient OGTT was performed by training mice to consume 0.5 ml of Ensure (see Supplemental text). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Transgenic lines

The MIPcreER line generation has been previously described (Kaihara et al., 2013; Wicksteed et al., 2010) with more characterization in the Supplemental Information (see also Figure S2 and Figure S2 legend). The targeting strategy and validation of the $Glp1r^{f/f}$ line and the global knockout ($Glp1r^{CMVKO}$) is described in Supplemental text (see also Figure S1 legend). β-cell specific *Glp1r* knockdowns $Glp1r^{f/f}$ mice were generated by crossing with either RIPcre (Jackson Labs, $Tg(Ins2\text{-}cre)^{25Mgn}$, stock number 003573) or MIPcreER lines as described in the Supplemental text. A secondary MIPcreER;$Glp1r^{\Delta/f}$ mouse line was generated following the breeding strategy of Feil (Feil et al., 2009) Supplemental Information). The MIPcreER crosses were treated at approximately 2 months of age with 1 mg IP of tamoxifen (free base, Sigma, T5648) in ethanol/sunflower oil (Feil et al., 2009) for 5 days, and experiments were started 4 weeks later. Lack of response to IV GLP-1 during an IPGTT was used to test for effective β-cell knockdown.

Food intake studies

To test the anorectic effects of exendin-4 or liraglutide, animals were placed in cages with fresh bedding, and chow removed 4 h before lights off. Mice were injected IP at the beginning of the dark phase and food intake measured after 1, 2, 4, 6 and 24 h. To assess chronic effects of liraglutide on body weight, mice were given IP liraglutide (1.0 mg/kg, SQ/day) for 14 days with food and body weight measured daily.

RNA extraction and real-time PCR

For RNA extraction, samples were processed using an RNA mini kit (QIAGEN Inc, Valencia, CA) for whole tissues, the RNA aqueous mini kit (Ambion/Life Technologies, Grand Island, NY) for mouse islets, and the RNA aqueous micro kit (Ambion) for sorted cells. cDNAs were synthesized with SuperScript® III First-Strand Synthesis kit (Invitrogen, Life Technologies). PCR primers were: β-actin, 4352341E; Glp1r KO, Mm00445292.m1; Glp1r intact, Mm0045290.m1; *Gipr* Mm01316344, proglucagon, Mm00801712.m1; and *Ins1*, Mm01259683.m1. Messenger RNA expression was calculated from the C_T of target genes and β-actin using standard methods.

Assays

The following assays were performed: Insulin, ultra-sensitive rat/mouse insulin ELISA (Crystal Chem, Inc.), GIP, EMD ELISA (Millipore, Inc), cAMP, acetylated version of the cAMP ELISA kit (Cell Biolabs), and total GLP-1, ELISA (Meso Scale, Rockville, MD) as previously described (Jessen et al., 2012).

Islet cell isolation, cAMP stimulation and FACS

Islets were isolated by standard procedures (Carter et al., 2009). For cAMP experiments, 40 equally sized islets were incubated in HEPES balanced salt solution (HBSS) and 0.1%

bovine serum albumin containing 3 mM glucose for 1 hour in 5% CO_2 at 37°C, and then in HBSS containing 15mM glucose and 100mM IBMX, with or without 10 nM exendin-4 for 15 minutes. Islets were lysed and cAMP measured. For FACS analysis, islets were dissociated by standard methods at the *Research Flow Cytometry Core* at Cincinnati Children's Hospital (Jayaraman, 2011).

Statistical Analysis

Data are presented as mean ± SEM. Analyses were performed using GraphPad Prism, version 5.01 (GraphPad Software, Inc., San Diego, CA). Comparisons of 2 samples were done with unpaired two-tailed t-tests. Analysis of multiple groups used one-way ANOVA with a multiple comparison test and two-way ANOVA was used to compare different treatments in two mouse strains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- β-cell Glp1r are not necessary for normal oral glucose tolerance in lean mice.
- The incretin effect of GLP-1 is not mediated through an endocrine mechanism.
- β-cell Glp1r are required for normal responses to hyperglycemia and GLP1R agonists.
- DPP-4 inhibitors improve glucose tolerance through non-β-cell Glp1r.

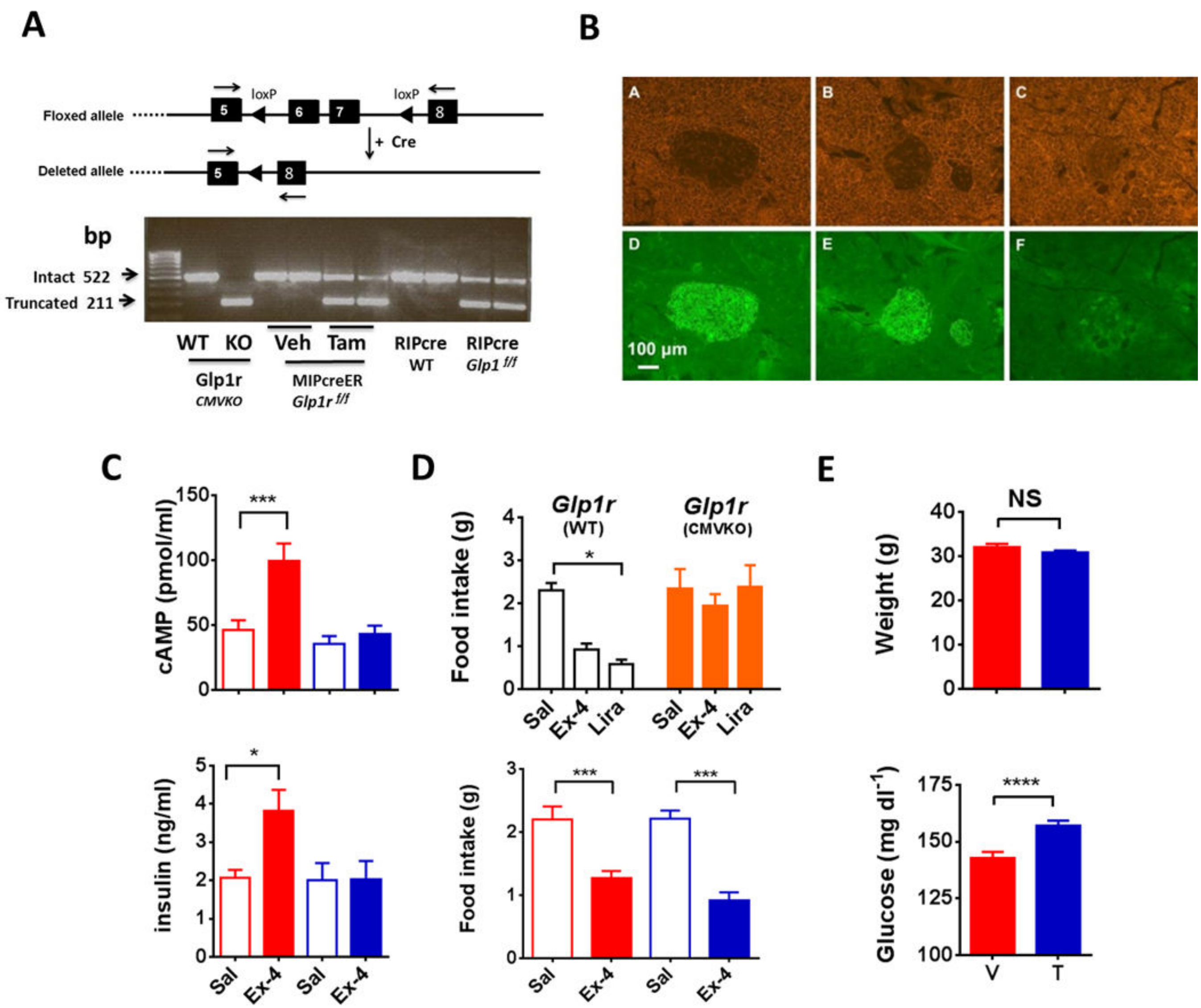

Figure 1. Description and validation of *Glp1r*$^{f/f}$ and Cre lines

(A) Upper panel: schematic depicting the location of loxP sites inserted within *Glp1r* gene and the result of exons 6 and 7 deletion. Lower panel: agarose gel electrophoresis of PCR products from primers designed to generate amplicons spanning exons 6 and 7 in the *Glp1r* gene; the WT band is 522 bp and the truncated band 211 bp. (B) Pancreatic sections from cross of Cre lines with a "double reporter" (DR). RIPcre × DR (**A, D**) and MIPcreER × DR treated with tamoxifen (**B, E**) show diffuse EGFP in the islet under a Cy5 filter, while MIPcreER × DR given vehicle (**C, F**) have minimal fluorescence in β-cells. (C) Upper panel: cAMP accumulation in isolated islets (40 islets/sample, 8 mice per group, 4 separate isolations) incubated for 15 minutes in media containing IBMX with 10 nM Ex-4 or control (Vehicle red; Tamoxifen blue, *** $p \leq 0.001$). (C) Lower panel: insulin concentrations in media from the islet studies described for top panel (Vehicle, red; Tamoxifen, blue, * $p \leq 0.05$). (D) Upper panel: no effect of exendin-4 or liraglutide on cumulative 4 hour food intake in *Glp1r*^{CMVKO} compared with *Glp1r*^{WT} mice (8 per group); Lower panel: food intake in tamoxifen or vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ mice (8 per group) in the 6 hours after administration of 2.5 ug Ex-4 IP or saline (*** $P \leq 0.001$). (E) Upper panel: body weight in vehicle and tamoxifen treated MIPcreER;*Glpr1*$^{f/f}$ animals (78 veh and 95 tam treated mice); Lower panel: fasting glucose in vehicle and tamoxifen treated MIPcreER;*Glpr1*$^{f/f}$ animals (95 veh and 107 tam treated mice; *** $P \leq 0.001$). (See also Figure S1 and Figure S2)

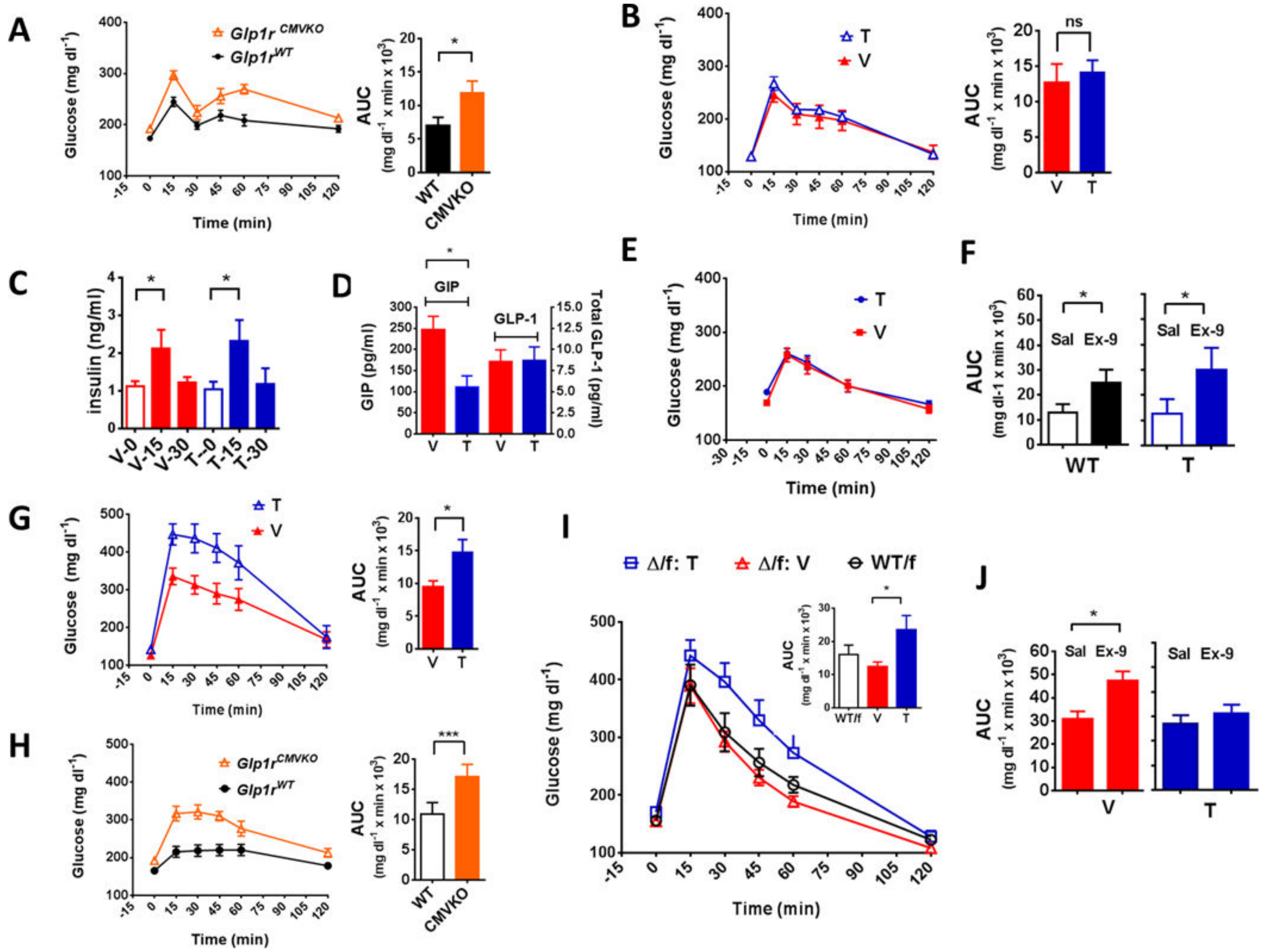

Figure 2. Effects of global or selective *Glp1r* disruption on glucose tolerance

(A) Blood glucose during OGTT in *Glp1r*^{CMVKO} and *Glp1r*^{WT} mice with corresponding AUC. (B) Blood glucose (2.0 g/kg, 20% glucose) during OGTT in MIPcreER; *Glp1r*$^{f/f}$ mice treated with tamoxifen or vehicle (see also Figure S3C). (C) Insulin concentrations from GTT depicted in (one-tailed t test: $p \leq 0.05$). (D) GIP and total GLP-1 concentrations obtained at 15 minutes following an OGTT in MIPcreER;*Glp1r*$^{f/f}$ mice treated with tamoxifen or vehicle. (E) Blood glucose following voluntarily ingested mixed liquid meal in MIPcreER;*Glp1r*$^{f/f}$ mice treated with tamoxifen (T) or vehicle (V). (F) AUC of blood glucose during OGTT in *Glp1r*$^{WT/f}$ and tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice given saline or the GLP1R antagonist Ex-9 (100 μg/kg) (see Figure S3E and F for corresponding glucose curves). (G) Blood glucose (2.0 g/kg, 20% glucose) during IPGTT in MIPcreER;*Glp1r*$^{f/f}$ mice treated with tamoxifen or vehicle. (H) Blood glucose during IPGTT in *Glp1r*^{CMVKO} and *Glp1r*^{WT} mice. (I) IPGTT in mice with a heterozygous global *Glp1r* knockout, (Δ/f: Tam), β-cell-specific deletion of *Glp1r* (tamoxifen-treated MIPcreER;*Glp1r*$^{\Delta/f}$, Δ/f: Veh) and a full complement of *Glp1r* (WT; *Glp1*$^{WT/f}$). (J) AUC of blood glucose following IPGTT in vehicle- and tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice with and without Ex-9 (vehicle, red; tamoxifen, blue). Experiments used 7–11 mice per group; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. (See also Figure S3B for IPGTT results in RIPcre; *Glp1r*$^{f/f}$ mice)

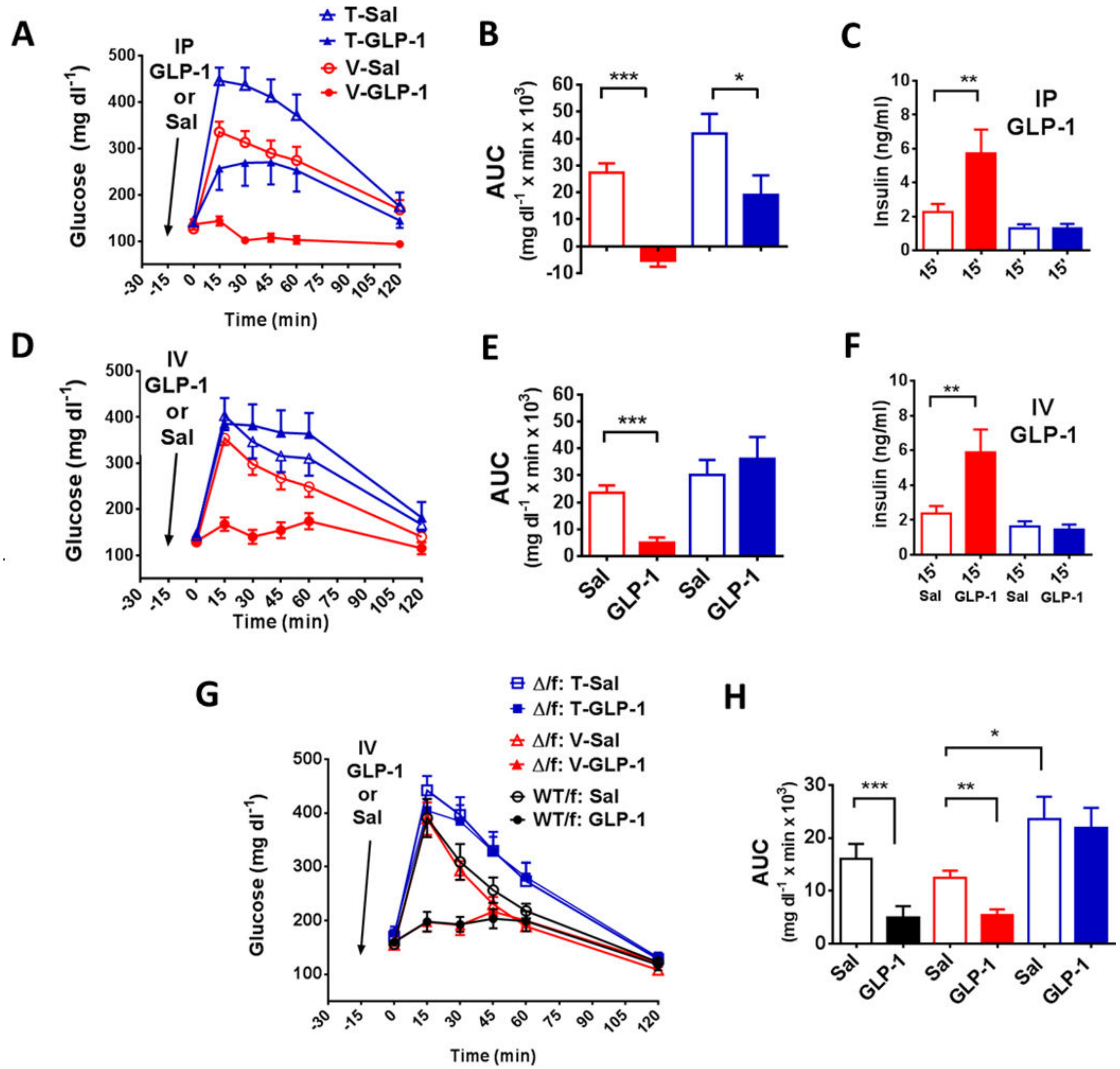

Figure 3. Effects of GLP-1 administration on glucose tolerance in MIPcreER;*Glp1r$^{f/f}$* mice

(A) Blood glucose (2.0 g/kg, 20% glucose) during IPGTT in tamoxifen- and vehicle-treated MIPcreER;*Glp1r$^{f/f}$* mice given IP saline or GLP-1 (10 ug) 15 minutes prior to glucose injection. (B) AUC of glucose, and (C) insulin concentrations at 15 minutes, from GTTs depicted in panel A. (D) Blood glucose (2.0 g/kg, 20% glucose) during IPGTT in tamoxifen and vehicle-treated MIPcreER;*Glp1r$^{f/f}$* given IV saline or GLP-1 (10 ug) 15 minutes prior to glucose injection. (E) AUC of glucose, and (F) insulin concentrations at 15 minutes, from mice in panel D. (G) Blood glucose during IPGTT in mice with a heterozygous global *Glp1r* knockout, (Δ/f: Tam), β-cell-specific deletion of *Glp1r* (tamoxifentreated MIPcreER;*Glp1r*Δ/*f*, Δ/f: Veh) and a full complement of *Glp1r* (WT; *Glp1$^{WT/f}$*), with or without GLP-1 (10 ug, IV) given 15 min. prior to glucose. (H) AUC of glucose tolerance depicted in panel G. Experiments used 8–12 mice per group, * $p \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

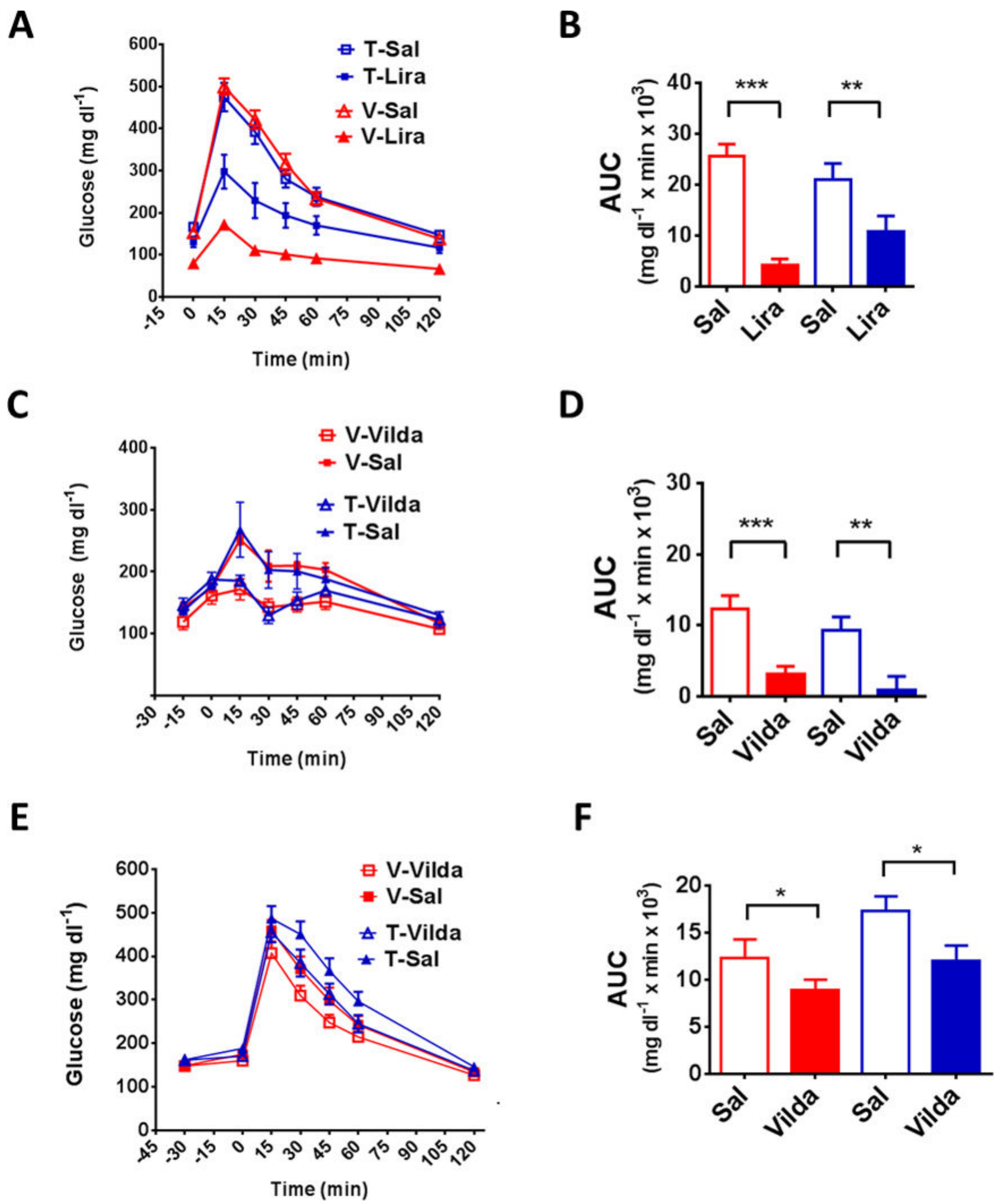

Figure 4. Effect of β-cell-specific knockdown on responses to GLP1R agonist and DPP4i

(A) Blood glucose during IPGTT in tamoxifen or vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ (Tam, blue or Veh, red) mice given liraglutide (200 ug/kg) or saline 4 hours prior to glucose injection. (B) AUC from GTT in A. (C) OGTT in tamoxifen and vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ mice given IP vildagliptin (150 ug) or saline (100 ul) 15 minutes before the glucose challenge. (D) AUC from GTT in C. (E) Blood glucose during IPGTT in tamoxifen- and vehicle-treated MIPcreER;*Glp1r*$^{f/f}$ mice given IP saline or vildagliptin (150 ug) 30 minutes prior to glucose injection. (F) AUC from GTT in E. Experiments used 8–16 mice per group, with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. (See Figure S4)