Supplemental Information

Supplemental Data:

Figure S1 is related to Figure 1:

Details regarding design and validation of the *Glp1r* $^{f/f}$ line and the global knockout (*Glp1r*^{CMVKO}) derived from the line. Characterization of the MIPcreER including documentation of the lack of an impact of the presence of MIPcreER on glucose tolerance and weight gain, the absence of hypothalamic induction after crossing with a double reporter line, and relative expression Cre mRNA.

Figure S2 is related to Figure 1:

Documentation of β-cell specific *Glp1r* knockdown by MIPcreER, expression of *Gipr* and the effect of a GLP1R agonist on weight gain.

Figure S3 is related to Figure 2:

Additional documentation of the tamoxifen effect on glucose metabolism in MIPcreER; *Glp1r* $^{f/f}$ animals, effect of inhibition with Ex-9 and the impact on glucose tolerance of *Glp1r* $^{f/f}$ animals crossed with a RIPcre line.

Figure S4 is related to Figure 4:

Effects of GLP1R agonist on glucose tolerance in RIPcre; *Glp1r* $^{f/f}$ animals.

Supplemental Experimental Procedures

Generation of a *Glp1r*$^{f/f}$ and *Glp1r*^{CMVKO} mouse lines:

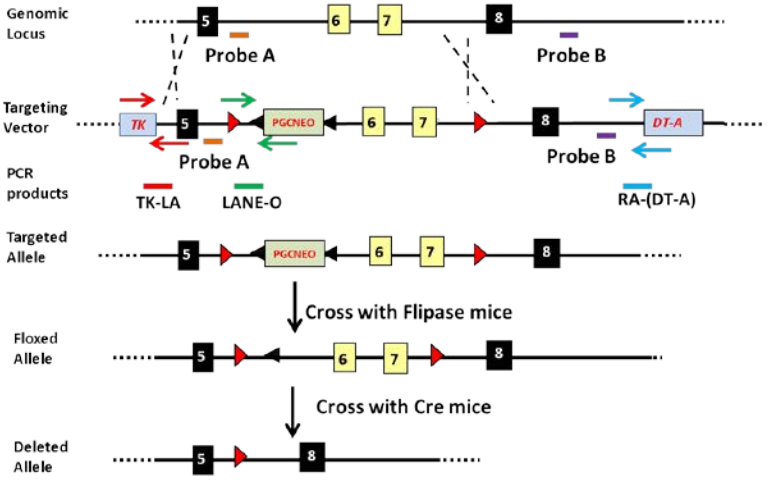
Validation of MIPcreER line:

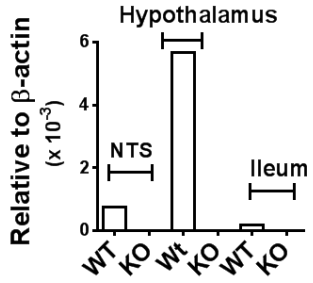
Generation of a MIPcreER; *Glp1r*$^{\Delta/f}$ mouse line:

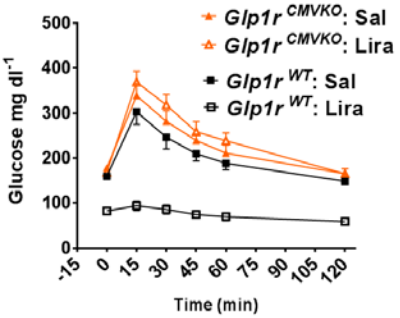
Genotyping and documentation of *Glp1r* exon 6/7 deletion:

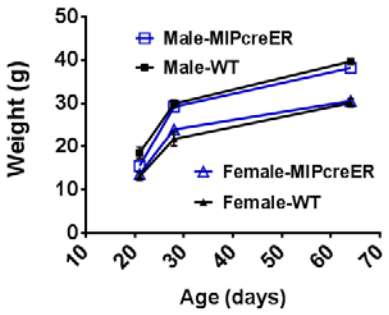
Mixed nutrient OGTT:

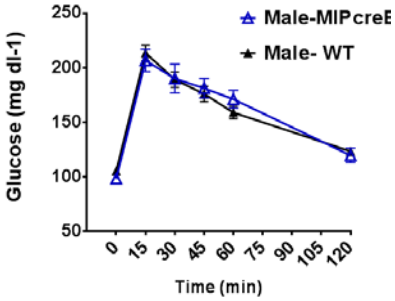
Figure S1

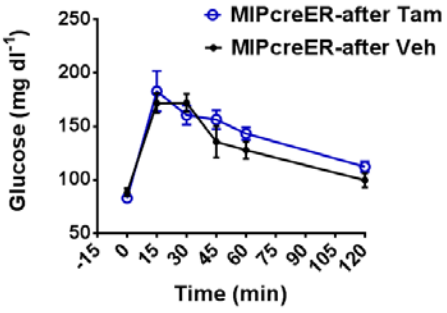

Genomic Locus
Probe A
Probe B
Targeting Vector
TK
PGCNEO
DT-A
PCR products
TK-LA
LANE-O
RA-(DT-A)
Targeted Allele
Cross with Flipase mice
Floxed Allele
Cross with Cre mice
Deleted Allele

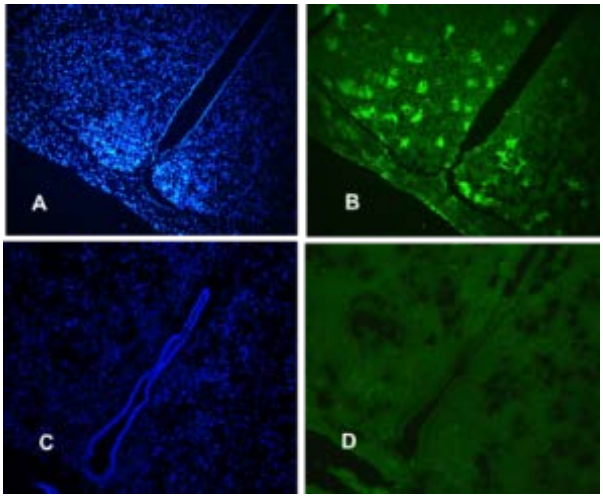

Hypothalamus
Relative to β-actin
(x 10-3)
NTS
Ileum
WT
KO
Wt
KO
WT
KO


Glp1r CMVKO: Sal
Glp1r CMVKO: Lira
Glp1r WT: Sal
Glp1r WT: Lira
Glucose mg dl-1
Time (min)


Male-MIPcreER
Male-WT
Female-MIPcreER
Female-WT
Weight (g)
Age (days)


Male-MIPcreE
Male- WT
Glucose (mg dl-1)
Time (min)


MIPcreER-after Tam
MIPcreER-after Veh
Glucose (mg dl-1)
Time (min)


A
B
C
D

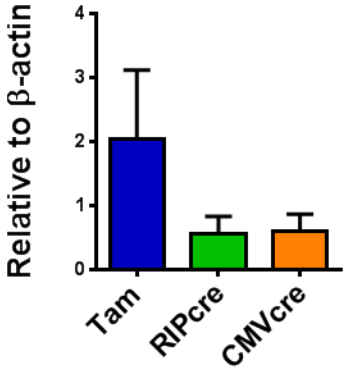

Relative to β-actin
Tam
RIPcre
CMVcre

Figure S1 related to Figure 1: Generation of *Glp1r*$^{f/f}$ line and validation of *Glp1r*^{CMVKO} and MIPcreER lines. (A) Schematic diagram of WT *Glp1r* gene, targeting vector containing a phosphoglycerol kinase (PGK) promoter/a neomycin (NEO) resistance selection cassette (light green box) flanked by flippase (FLP) recognition target (FRT) sites (black triangles), and loxP sites (red triangles) on either side exons 6 and 7 of the *Glp1r* gene, targeted allele, floxed allele following flippase recombination and final allele with exons 6 and 7 deleted. (B) Documentation by qPCR of absent *Glp1r* mRNA expression in *Glp1r*^{CMVKO} compared with *Glp1r*^{WT}. (C) IPGTT (1.5g/kg, 20% glucose) in *Glp1r* ^{CMVKO} and *Glp1r*^{WT} mice with and without IP liraglutide (200µg/kg) administered 4 hours prior to glucose injection. (D) Longitudinal weight measurements of MIPcreER males and females and their litter mate controls. (E) OGTT (1.5 g/kg, 44% glucose) in male MIPcreER vs. WT male littermate controls. (F) OGTT (1.5 g/kg, 44% glucose) on MIPcreER male animals after tamoxifen or vehicle treatment. (G) Hypothalamic sections from RIPcre (A,B) and tamoxifen treated MIPcreER (**C,D**) lines crossed with a "double reporter" (DR) line; comparison of images captured under a Cy5 filter (**B, D**) or for detection of DAPI (**A, C**) of hypothalamic sections. Sections showed marked activation of EGFP in the RIPcre double reporter crosses (**B**) with no evidence of EGFP expression in the tamoxifen (**D**) treated MIPcre double reporter crosses. (H) qPCR expression of Cre recombinase mRNA in isolated islets from tamoxifen treated MIPcreER; *Glp1r* $^{f/f}$, RIPcre; *Glp1r* $^{f/f}$ and *Glp1r*^{CMVKO} mice; expression detected using Sybr green. All data shown are as mean ± S.E.M

Figure S2

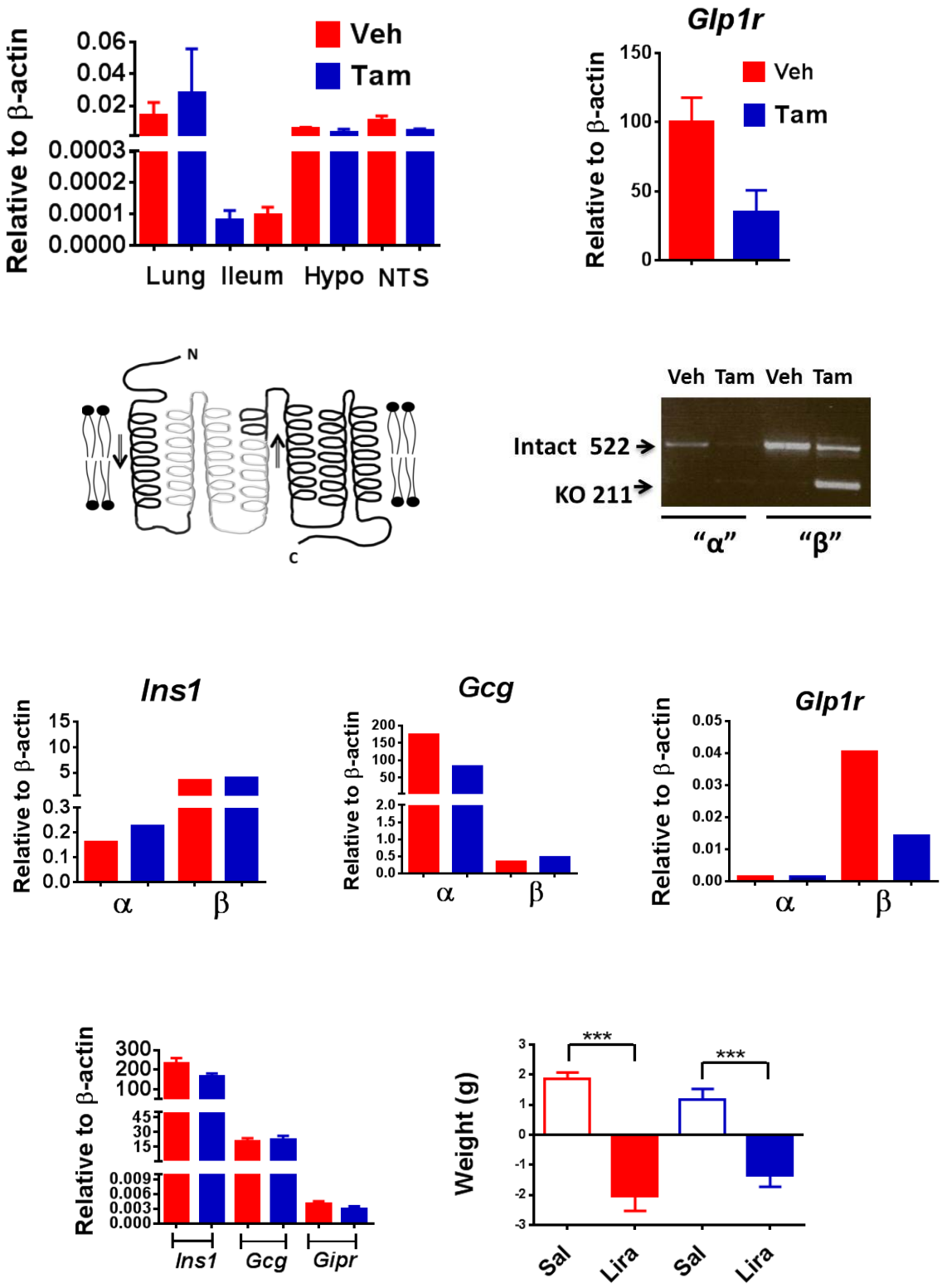

Relative to β-actin
0.06
0.04
0.02
0.0003
0.0002
0.0001
0.0000
Veh
Tam
Lung
Ileum
Hypo
NTS
Glp1r
150
100
50
0
N
C
Veh Tam Veh Tam
Intact 522
KO 211
"α"
"β"
Ins1
15
10
5
0.3
0.2
0.1
0.0
α
β
Gcg
200
150
100
50
2.0
1.5
1.0
0.5
0.0
Glp1r
0.05
0.04
0.03
0.02
0.01
0.00
300
200
100
45
30
15
0.009
0.006
0.003
0.000
Ins1
Gcg
Gipr
Weight (g)
***
***
3
2
1
0
-1
-2
-3
Sal
Lira
Sal
Lira

Figure S2 related to Figure 1: Validation of β-cell specific knock down of *Glp1r* by MIPcreER. (A) Survey of *Glp1r* mRNA expression in tissues from MIPcreER; *Glp1r*$^{f/f}$ animals (3/group) treated with tamoxifen or vehicle. There are no statistically significant differences in expression between tamoxifen and vehicle treated animals, with highest expression of the *Glp1r* in the brain and lung. (B) Islets from MIPcreER*; Glp1r*$^{f/f}$ animals previously treated with tamoxifen or vehicle (2/group) were isolated, RNA extracted and processed for qPCR. mRNA abundance was normalized to β-actin. (C) Predicted deletion of peptide sequenced within the GLP1R deleted after Cre recombination (gray). (D though G) Islets from MIPcreER*; Glp1r*$^{f/f}$ animals (2/group) treated with tamoxifen (blue) or vehicle (red) were digested into single cell suspensions (two from each group pooled) and were FACS sorted to enrich for β or non-β (predominantly α-) cells. (D) Agarose gel electrophoresis of PCR products from primers generating an amplicon spanning exons 6 and 7 in *Glp1r* gene; cDNAs are from the FACS sorting of islets (pool of 2 separate animals for each condition) used to generate the qPCR depicted in E-G. (E) qPCR for insulin (*Ins1*) and (F) proglucagon (*Gcg*) expression in vehicle or tamoxifen -treated α or β cell enriched samples. (G**)** qPCR of G*lp1r* mRNA expression in α or β cell enriched samples from either tamoxifen or vehicle-treated animals. (H) Relative expression by qPCR of insulin, glucagon and GIP receptor genes in islets from six tamoxifen- (blue) and six vehicle-treated (red) mice. (I) Cumulative weight loss in vehicle and tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice treated with daily injections of liraglutide for 10 days. Both groups of mice lost comparable amounts of weight demonstrating that β-cell *Glp1r* knockdown does not affect the satiety response to liraglutide. All data shown are as mean ± S.E.M except ± SD for panel B.

Figure S3

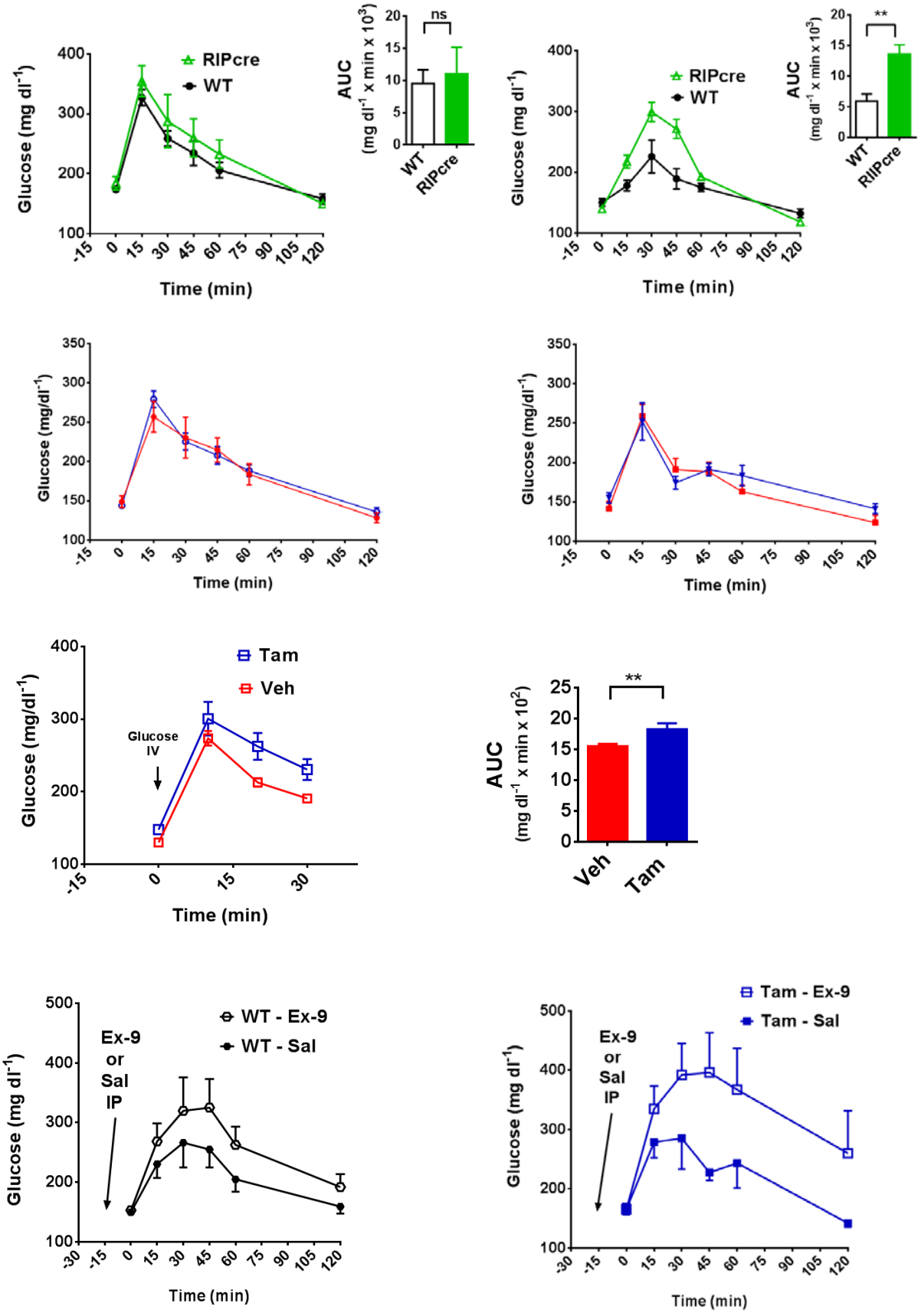

RIPcre
WT
Glucose (mg dl-1)
Time (min)
AUC
(mg dl-1 x min x 10-3)
ns
**
Glucose (mg/dl-1)
Tam
Veh
Glucose IV
(mg dl-1 x min x 10-2)
WT - Ex-9
WT - Sal
Tam - Ex-9
Tam - Sal
Ex-9 or Sal IP

Figure S3 related to Figure 2: Effects of β-cell GLP1R signaling on oral, IP and IV glucose tolerance. (A) Oral and **(B)** IPGTTs in RIPcre; *Glp1r*$^{f/f}$ (green) and control (black) mice. Similar to tamoxifen-treated MIPcreER; *Glp1r* $^{f/f}$ animals, there is intolerance to IP, but not oral, glucose. (C) Oral glucose tolerance tests in separate cohorts of vehicle and tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice (Vehicle, red; Tamoxifen, blue; 8 animals per group). (D) IV GTTs tests in vehicle- and tamoxifen-treated mice (Vehicle, red; Tamoxifen, blue). Similar to the results with IP glucose loading, the β-cell *Glp1r* knockdowns have impaired clearance of IV glucose. (E and F) Oral glucose tolerance tests in WT and tamoxifen-treated MIPcreER; *Glp1r*$^{f/f}$ mice with and without the GLP1R antagonist Ex-9 (WT, black; Tamoxifen, blue; 8 animals per group). In both lines, with and without β-cell *Glp1r* knockdown, pharmacologic blockade of the Glp1r caused glucose intolerance to an oral challenge. Data are as mean ±S.E.M. ** $P \leq 0.01$

Figure S4

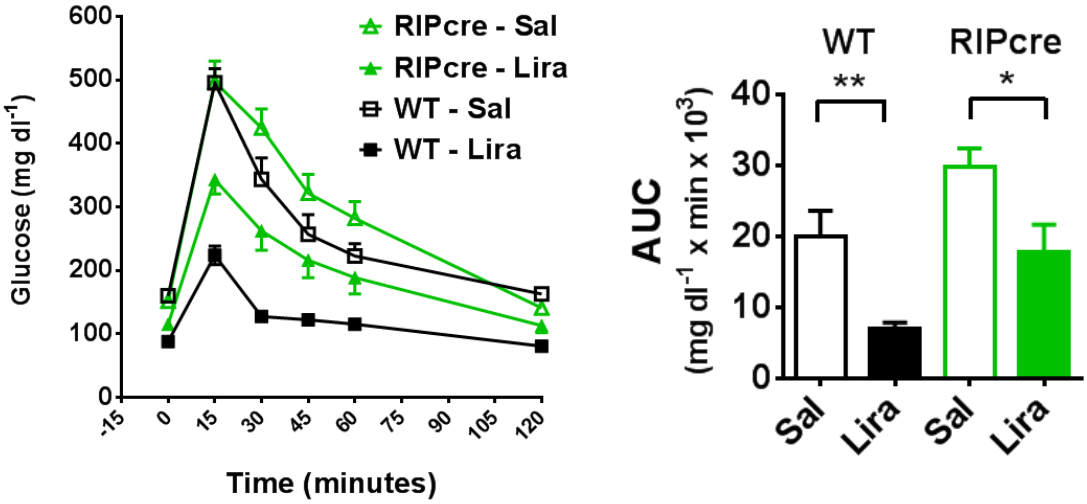


Figure S4 related to Figure 4: Impact of β-cell specific knock down on response to GLP1R agonist. Effects of the GLP1R agonist liraglutide on glucose tolerance following an IP glucose challenge in WT and RIPcre; *Glp1r* $^{f/f}$ mice (WT, black; RIPcre, green). In both lines liraglutide treatment increased glucose clearance, but the effect was greater in the WT animals. This result is comparable to the relative effects of liraglutide on IP glucose tolerance in vehicle and tamoxifen-treated MIPcreER;*Glp1r*$^{f/f}$ mice Data are as mean ±S.E.M. ** $P \leq 0.01$ and * $P \leq 0.05$

Generation of a *Glp1r*$^{f/f}$ and *Glp1r*^{CMVKO} mouse lines: A construct was designed with insertion of loxP sites 5' to exon 6 and 3' to exon 7 within nonregulatory domains of introns 5 and 8. This construct, modified to include loxP sites, was used to insert a vector containing a phosphoglycerol kinase neomycin resistance selection cassette (PGKNEO) flanked by flippase (FLP) recognition target (FRT) sites (Figure S1A). The *Glp1r* targeting vector was electroporated into C57Bl6 embryonic stem (ES) cells, and clones containing the construct were identified by Southern blotting. Positive ES clones were screened by PCR using 3 sets of primers. Correctly targeted alleles were positive for the left arm-neo (LANEO) junction, but not those junctions with TK (TKLA) or DTA (RADTA) sequence. From 287 clones, 66 positives were obtained; 32 of the PCR-positive clones were analyzed by Southern blot, and all had confirmed integration of the full length construct indicated by a 7.7 kb band diagnostic for the targeted allele, and 6.4 kb band consistent for the wild type allele. Positive ES cells were injected into albino C57BL/6 blastocysts to generate chimeras and these were implanted into pseudopregnant dams giving 8 chimeric offspring. The chimeric animals were crossed with wild type C57BL/6 mice and 6 of the 8 founders produced litters, with 3 showing germ-line transmission. Agouti mice with germ-line transmission were bred to Flp-recombinase mice (Jackson labs, stock number 0033800, C57BL/6) to remove the neomycin resistance *cassette*. They were then bred to wild-type C57BL/6 mice to identify offspring lacking the Flp-recombinase transgene to generate a line for *Glp1r* knockout or knockdown experiments. Mice with *Glp1r*$^{f/f}$ were crossed with animals expressing Cre ubiquitously under the control of a cytomegalovirus (CMV) promoter, (BALB/c-Tg(CMV-cre)1Cgn/J). Two lines were generated with either mixed or pure C57Bl/6J background. For the mixed line, the *Glp1r* $^{f/f}$ mice (see above), C57Bl/6J background, were bred with CMV-Cre mice (Jackson labs, stock number

003465, BALB/c-Tg(CMV-cre)1Cgn/J BALB/c) to generate *CMVcre;Glpr1r* $^{\Delta/f}$ in a mixed balbc/C57 background with global knockout of *Glp1r* in one allele (Schwenk et al., 1995). The animals were then back crossed with *Glp1r* $^{f/f}$ animals to generate *Glpr1r* $^{\Delta/f}$ animals without CMV-Cre. Finally, these animals were crossed together to generate the *Glpr1r* ^{CMVKO} line. *Glp1r* expression assessed by qPCR was undetectable in the CMVcre-*Glp1r*$^{\Delta/\Delta}$ mice (*Glp1r*^{CMVKO}) using primers specific for deleted exons 6 and 7 (Figure S1B). Similar to published findings in a previously established *Glp1r* null mouse line (Scrocchi et al., 1996) with deletion of the same exons by homologous recombination, our mice with Cre-loxP deletion of the receptor had mild glucose intolerance when challenged with oral or intraperitoneal (IP) glucose (Figure 2A and 2H). Moreover, these animals had no response to the glucose lowering or anorectic effects of the GLP1R agonist, liraglutide (Figure S1C and Figure 1D, upper panel).

Validation of MIPcreER line: The MIP-creER^{TM}-hGH (MIPcreER) transgene was constructed using an 8.3-kb fragment of mouse insulin I promoter (MIP; nucleotides -8,280 to +12 relative to the transcriptional start site) (Hara et al., 2003), a cDNA for Cre recombinase and hormone-binding domain of a mutant mouse estrogen receptor, ER^{TM} (Danielian et al., 1998; Hayashi and McMahon, 2002) and, to augment expression level, a 2.1-kb fragment of the human growth hormone (hGH). Six founder lines were identified and all six, on crossing with a *Gt(ROSA)26Sort*^{m1Sor} line (Jackson Labs, stock 003309) revealed β-cell specific recombination after tamoxifen treatment without significant leakiness following vehicle and without CNS recombination..

To demonstrate islet-specific disruption of *Glp1r*, islets were isolated from tamoxifen and vehicle treated MIPcreER*; Glp1r* $^{f/f}$ mice and by qPCR demonstrated a ~70% knockdown of the

native *Glp1r* (Figure S2B). Survey of other relevant tissues did show knockdown as expected from the specificity of the MIPcreER promoter (Figure S2A). Cell-specific *Glp1r* expression was assessed by enriching the β-cell population through fluorescence activated cell sorting (FACS) (Figures 2SD-G). RNA was extracted followed by PCR of cDNA using primers generating a product spanning the deleted exons 6 and 7 (Figure S1A). WT mice had a transcript of 511 bp that defined the intact *Glp1r* gene. RNA from the islets of tamoxifen treated MIPcreER*; Glp1r*$^{f/f}$ had predominantly the truncated transcript in this mixed population of endocrine cells, and effective sorting was confirmed by selective expression of insulin in the FluoZin-3-AM staining "β" cells (Figure S2E) and greater expression of proglucagon in the non-β-cell population that had relatively lower FluoZin-3-AM uptake (Figure S2F). qPCR *Glp1r* in revealed knock down similar to what was observed with the isolated islets prior to sorting (Figure S2G). To demonstrate the impact of β-cell specific knockdown of GLP-1 signaling, isolated islets from MIPcreER*; GLP-1r*$^{f/f}$ mice treated with tamoxifen or vehicle were incubated in IMBX and 15 mM glucose in the presence or absence of 10 nM Exendin-4 for 15 minutes. GLP-1 agonist treatment increased levels of intracellular cAMP significantly in islets from vehicle treated, but not tamoxifen-treated, mice (Figure 1C. upper panel); similar insulin secretion derived from the same islet experiment showed no insulin secretory response in the tamoxifen-treated islets (Figure 1C, lower panel).

Generation of MIPcreER; *Glp1r*$^{f/f}$ and MIPcreER; *Glp1r*$^{\Delta/f}$ mouse lines: The primary MIPcreER;*Glp1r*$^{f/f}$ line was a mixed background cross of the MIPcreER and *Glp1r*$^{f/f}$ lines. Following a breeding strategy described by Feil (Feil et al., 2009), a heterozygous global knockout of the *Glp1r*, i.e. *Glp1r*$^{\Delta/f}$, was generated by crossing our *Glp1r*^{CMVKO} line with WT

animals. Mice with a single intact WT *Glp1r* allele (*Glp1r* $^{\Delta/WT}$) were then crossed with MIPcreER; *Glp1r* $^{WT/WT}$ to generate a breeder that is MIPcreER; *Glp1r* $^{\Delta/WT}$. Breeding this animal with *Glp1r* $^{f/f}$ line generated mice the following experimental genotypes:

MIPcreER; *Glp1r* $^{\Delta/f}$ (tamoxifen treatment induces further cell specific deletion of *Glp1r* in the β-cell and is compared with animals with the same genotype treated with vehicle);
MIPcreER; *Glp1r*$^{f/WT}$ (tamoxifen treatment induces heterozygous deletion of β-cell *Glp1r*)
WT; *Glp1r* $^{\Delta/f}$ (global heterozygous deletion of *Glp1r*)
WT: *Glp1r* $^{f/WT}$ (controls for the floxed allele).

Genotyping and documentation of *Glp1r* exon 6/7 deletion: The MIPcreER and CMV lines employed the following primer combinations: Cre-for: 5'-gat ttc gac cag gtt cgt tc -3' and Cre-rev: 5'-ttg ccc ctg ttt cac tat cc-3' generating amplicon of 583 bp and TSH beta1 for: 5'-tcc tca aag atg ctc att ag-3'; TSH beta1 rev: 5'-gta act cac tca tgc aaa gt-3' generating amplicon of 386 bp. PCR protocol: 94 ºC for 2 min; 35 cycles, 94 ºC, 30 sec; 53 ºC 30 sec; 72 ºC, 1 min then 72 ºC for 5 min final elongation. The *Glp1r* $^{f/f}$ and *Glp1r* ^{CMVKO} lines use the following primers combinations: **1**) to demonstrate presence of floxed alleles Glp1r-F2: TGAGCCATCTCCTCAGCTCT and Glp1r-R2: AGGCATGTATCCACCTCTGG generating WT amplicon of 356 bp and targeted amplicon of 450 bp. **2**) To document Cre recombination with either CMV, RIPcre or tamoxifen treated MIPcreER using forward primer Glp1r-F3 (on intron 5): CTCTGCCCCAGAGAACAGTC and Glp1r-R3 (on intron 7): CCCTGCACTGGGTATGAAGT generated a 387 bp amplicon. PCR conditions were same as

for MIPcreER except annealing temperature was 55C. 3) Finally, to demonstrate a truncated *Glp1r* cDNA the following primers were used: mGLP1R-420F: CCTGAGGAACAGCTCCTGTC (on exon 5) and mGLP1R-944R: CAATCGGATGATGAGCCAGT (on exon 9) generating an amplicon of 525 bp with the intact gene and 211 bp following deletion. The annealing temperature for this reaction was 60C.

Mixed nutrient OGTT:

Mixed nutrient OGTT was performed by training mice to consume 0.5 ml of Ensure supplemented with additional glucose to approximate 2.5 g of glucose per kg if a 35 g mouse consumed all the Ensure. At the onset of the dark phase, the GTT was performed under red lights after an overnight fast. Animals were excluded from the analysis if Ensure was not fully consumed after ~10 minutes.

Supplemental references:

Danielian, P.S., Muccino, D., Rowitch, D.H., Michael, S.K., and McMahon, P. (1998). Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. Curr. Biol. *8*, 1323–1326.

Hara, M., Wang, X., Kawamura, T., Bindokas, V.P., Dizon, R.F., Alcoser, S.Y., Magnuson, M., and Bell, G.I. (2003). Transgenic mice with green fluorescent protein-labeled pancreatic beta -cells. Am. J. Physiol. Endocrinol. Metab. *284*, E177–83.

Hayashi, S., and McMahon, A.P. (2002). Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. Dev. Biol. *244*, 305–318.

Schwenk, F., Baron, U., and Rajewsky, K. (1995). A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. Nucleic Acids Res. *23*, 5080–5081.

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