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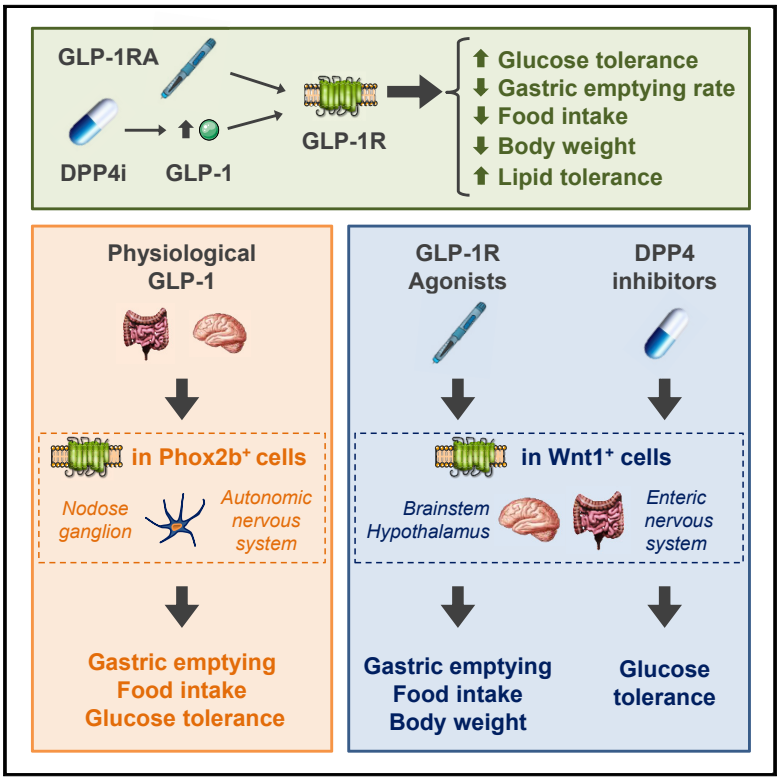
Distinct Neural Sites of GLP-1R Expression Mediate Physiological versus Pharmacological Control of Incretin Action

Graphical Abstract



Authors

Elodie M. Varin, Erin E. Mulvihill, Laurie L. Baggio, Jacqueline A. Koehler, Xiemin Cao, Randy J. Seeley, Daniel J. Drucker

Correspondence

drucker@lunenfeld.ca

In Brief

Varin et al. use *Wnt1-Cre2* and *Phox2b-Cre* to target distinct GLP-1R^{+} neuronal populations with different roles in physiological versus pharmacological control of food intake, glucose homeostasis, gastric emptying, and the gut-brain glucoregulatory axis.

Highlights

- Loss of the neural *Glp1r* in *Glp1r*$^{\Delta Wnt1-/-}$ mice does not impair basal metabolism
- The glucoregulatory actions of GLP-1R agonists are preserved in *Glp1r*$^{\Delta Wnt1-/-}$ mice
- *Phox2b-Cre*-targeted GLP-1R controls glucose homeostasis and gastric emptying
- *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice exhibit impairments in the gut-brain axis

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Distinct Neural Sites of GLP-1R Expression Mediate Physiological versus Pharmacological Control of Incretin Action

Elodie M. Varin,[1,2,4] Erin E. Mulvihill,[1,2,4,5] Laurie L. Baggio,[1] Jacqueline A. Koehler,[1] Xiemin Cao,[1] Randy J. Seeley,[3] and Daniel J. Drucker[1,2,6,*]

[1]Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, ON M5G 1X5, Canada
[2]Department of Medicine, University of Toronto, Toronto, ON M5S 2J7, Canada
[3]Department of Surgery, University of Michigan, Ann Arbor, MI 48109, USA
[4]These authors contributed equally
[5]Present address: University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, ON H32 28A, Canada
[6]Lead Contact
*Correspondence: drucker@lunenfeld.ca
https://doi.org/10.1016/j.celrep.2019.05.055

SUMMARY

Glucagon-like peptide 1 (GLP-1) receptors are widely distributed throughout the nervous system, enabling physiological and pharmacological control of glucose and energy homeostasis. Here we elucidated the importance of *Glp1r* expression within cellular domains targeted by expression of *Wnt1-Cre2* or *Phox2b-Cre*. Widespread loss of neural *Glp1r* in $Glp1r^{\Delta Wnt1-/-}$ mice had no effect on basal food intake, gastric emptying, and glucose homeostasis. However, the glucoregulatory actions of GLP-1R agonists, but not gut-selective DPP-4 inhibition, were preserved in $Glp1r^{\Delta Wnt1-/-}$ mice. Unexpectedly, selective reduction of *Glp1r* expression within neurons targeted by *Phox2b-Cre* impaired glucose homeostasis and gastric emptying and attenuated the extent of weight loss achieved with sustained GLP-1R agonism. Collectively, these studies identify discrete neural domains of *Glp1r* expression mediating GLP-1-regulated control of metabolism and the gut-brain axis and reveal the unexpected importance of neuronal $Phox2b^{+}$ cells expressing GLP-1R for physiological regulation of gastric emptying, islet hormone responses, and glucose homeostasis.

INTRODUCTION

Glucagon-like peptide 1 (GLP-1) is a 30-amino acid peptide hormone produced by enteroendocrine cells and subsets of neurons within the caudal hindbrain (Campbell and Drucker, 2013; Jin et al., 1988). GLP-1 exerts a wide range of metabolic actions that regulate energy homeostasis through a single GLP-1 receptor (GLP-1R) (Thorens, 1992). The GLP-1R is widely distributed in peripheral tissues, including the endocrine pancreas, lungs, stomach, intestine, kidneys, heart, and some blood vessels (Bullock et al., 1996; Campos et al., 1994; Thorens, 1992). Hence, current concepts of GLP-1 biology invoke direct actions of GLP-1 acting as a circulating hormone to enable the metabolic activities ascribed to GLP-1R signaling (Campbell and Drucker, 2013).

The classical endocrine concept of GLP-1 biology has been challenged by observations that plasma concentrations of GLP-1 are extremely low, raising questions about whether the metabolic actions of GLP-1 are mediated via its envisioned role as a circulating hormone (Chambers et al., 2017; D'Alessio, 2016). Indeed, the GLP-1R is widely expressed in neurons, supporting a role of GLP-1 as a neurotransmitter, locally within the enteric nervous system (ENS) and centrally within the brain, regulating neural circuits controlling metabolic homeostasis (Drucker, 2018b). A combination of pharmacological studies, optogenetics, chemogenetics, cell ablation, and cell-specific knockdown of the GLP-1R within the CNS implicates multiple neural GLP-1R circuits in the control of food intake, reward, and body weight (Adams et al., 2018; Burmeister et al., 2017; Hayes et al., 2011; Holt et al., 2019; Kanoski et al., 2011; Liu et al., 2017; López-Ferreras et al., 2018; Sisley et al., 2014). GLP-1R signaling also controls glucose homeostasis and regulates post-prandial lipoprotein production and secretion through incompletely defined central and/or peripheral neural GLP-1R networks (Farr et al., 2015; Kooijman et al., 2015).

Interpretation of the sites and mechanisms underlying GLP-1 action requires consideration of the differences in biology of native GLP-1, produced predominantly in the gut and brain, versus the actions emanating from systemic pharmacological administration of GLP-1R agonists. Indeed, GLP-1R agonists are often resistant to enzymatic degradation and circulate at much higher levels with extended pharmacokinetic profiles relative to the low circulating levels of native GLP-1 (Meier, 2012). Moreover, GLP-1R agonists developed for clinical use exhibit considerable structural heterogeneity, ranging from small peptides to higher-molecular-weight molecules containing GLP-1 epitopes covalently linked to larger proteins (Andersen et al., 2018; Meier, 2012). These unique structures may contribute to differences in GLP-1R activation or preferential access to

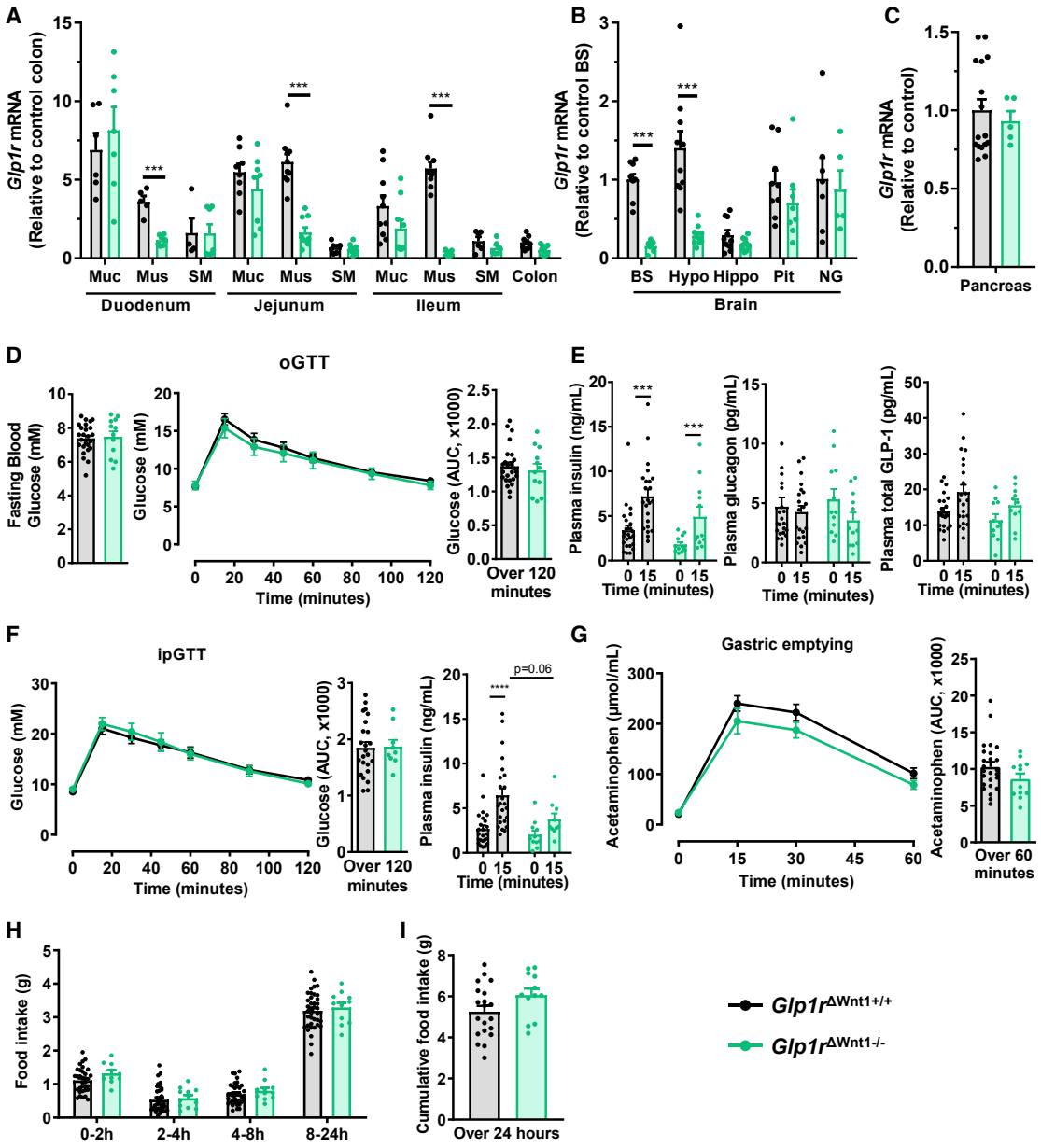

Figure 1. Reduction of *Glp1r* Levels in Wnt1^{+} Cells within the Intestine (ENS) and the Brain Does Not Affect Glucose Tolerance, Gastric Emptying, or Food Intake

(A–C) *Glp1r* mRNA abundance (relative to levels of *Ppia*) was measured in 10- to 15-week-old female *Glp1r*$^{\Delta Wnt1+/+}$ versus *Glp1r*$^{\Delta Wnt1-/-}$ mice within various ENS-containing intestinal compartments (mucosa [Muc], muscle [longitudinal and circular muscle and myenteric plexus (Mus)], and submucosa (muscularis mucosa and submucosal plexus [SM]) in different regions of the digestive tract (duodenum, jejunum, ileum, and colon; expressed relative to *Glp1r* mRNA abundance detected in the colon of control animals [*Glp1r*$^{\Delta Wnt1+/+}$]) (A), in different regions of the brain (brain stem [BS], hypothalamus [Hypo], hippocampus [Hippo], pituitary [Pit], and nodose ganglion [NG]; expressed relative to the BS of control animals [*Glp1r*$^{\Delta Wnt1+/+}$]) (B), and in the pancreas (expressed relative to control animals [*Glp1r*$^{\Delta Wnt1+/+}$]) (C) (n = 5–10/group).

(D and E) Fasting blood glucose, blood glucose excursion, and area under the curve (AUC) over 2 h (D) and plasma levels of total GLP-1, insulin, and glucagon before and 15 min after glucose administration (2 g/kg body weight [BW]) (E) during an oral glucose tolerance test (oGTT) in 6- to 10-month-old *Glp1r*$^{\Delta Wnt1+/+}$ versus *Glp1r*$^{\Delta Wnt1-/-}$ male mice after a 5-h fast (n = 12–25 animals/group).

(F) Blood glucose excursion, AUC over 2 h, and plasma insulin before and 15 min after glucose administration (2 g/kg BW) during an i.p. GTT (ipGTT) in 6- to 10-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ versus *Glp1r*$^{\Delta Wnt1-/-}$ mice after a 5-h fast.

(G) Plasma acetaminophen levels and AUC over 1 h as a measure of gastric emptying in 8- to 10-month-old *Glp1r*$^{\Delta Wnt1+/+}$ versus *Glp1r*$^{\Delta Wnt1-/-}$ male mice after a 5-h fast and oral administration of glucose (2 mg/kg) and 100 mg/kg BW acetaminophen (1% solution) (n = 12–25 animals/group).

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subsets of CNS GLP-1Rs, potentially contributing to the differential efficacy observed in glycemic control, body weight reduction, and cardiovascular outcomes (Andersen et al., 2018; Drucker, 2018a; Meier, 2012).

To elucidate the importance of specific sites of GLP-1 action relevant for the control of glycemia and food intake, multiple studies have utilized mice and rats treated with GLP-1R antagonists or subjected to cell- and tissue-specific knockdown of the *Glp1r* in islets and in different regions of the nervous system (Adams et al., 2018; Drucker, 2018b; Hayes et al., 2009; Jessen et al., 2017; Kanoski et al., 2011; Liu et al., 2017; López-Ferreras et al., 2018; Reiner et al., 2018; Sisley et al., 2014; Smith et al., 2014). Here we interrogated the metabolic actions of a small peptide GLP-1R agonist, exendin-4, and two larger GLP-1R agonists, albiglutide and dulaglutide, as well as different doses of the DPP-4 inhibitor, sitagliptin in $Glp1r^{\Delta Wnt1-/-}$ mice with broad neural inactivation of the *Glp1r* in cells targeted by expression of the *Wnt1-Cre2* promoter within the hypothalamus (Hypo), brain stem (BS), and ENS. Given the potential contribution of neural GLP-1R signaling outside of the brain for control of glycemia and food intake (Grasset et al., 2017; Iwasaki et al., 2018; Kanoski et al., 2011; Krieger et al., 2016), we also assessed the importance of neural sites for the actions of these incretin agents within regions of the autonomic nervous system targeted by *Phox2b*, including the dorsal motor nucleus of the vagus (DMV) in parasympathetic visceral and branchial motor neurons, the nodose sensory ganglia, and in cells within the nucleus of the solitary tract (NTS). Simultaneously, we interrogated the importance of these GLP-1R$^+$ brain regions for basal control of glycemia, food intake, and gastric emptying in $Glp1r^{\Delta Wnt1-/-}$ and $Glp1r^{\Delta}$Phox2b$-/-$ mice. Finally, we investigated the importance of the GLP-1Rs targeted by *Wnt1* and *Phox2b* for the acute control of basal and GLP-1-regulated lipid metabolism.

RESULTS

Loss of the *Glp1r* within the *Wnt1*$^+$ Cellular Domain Does Not Impair Glucose Tolerance or Gastric Emptying

Central and peripheral neural GLP-1R circuits control food intake and body weight and have been proposed to control glucose homeostasis (D'Alessio, 2016; Drucker, 2018b). Less well studied is the extent to which structurally distinct small peptide versus higher-molecular-weight GLP-1R agonists similarly engage GLP-;1Rs within the nervous system to control metabolism. Accordingly, we examined the actions of exendin-4 (a 39-amino acid peptide) and two larger GLP-1R agonists, albiglutide (a cleavage-resistant GLP-1 dimer within the N-terminal domain of human albumin) and dulaglutide (GLP-1(7–37) covalently linked to an Fc fragment of human IgG4). We selected doses of these agents to achieve similar metabolic activities (glucose control, food intake, and lipid tolerance) in acute studies (Figures S1A–S1D).

To evaluate the combined contribution of CNS and ENS GLP-1R$^+$ circuits to the acute metabolic actions of GLP-1R agonists, we crossed $Glp1r^{Flox/Flox}$ with *Wnt1-Cre2* mice, known to target neurons within the CNS and ENS (described in the STAR Methods; Zhang et al., 2017), to generate $Glp1r^{\Delta Wnt1-/-}$mice. *Glp1r* expression was consistently reduced within the muscle layer but not the submucosa or the epithelial mucosa of the small bowel of $Glp1r^{\Delta Wnt1-/-}$ mice (Figure 1A), with no reduction of *Glp1r* mRNA transcripts detected in the large bowel (Figure 1A). *Glp1r* mRNA transcripts were also markedly reduced in the BS and hypothalamus; the levels of *Glp1r* mRNA transcripts within the hippocampus (Hippo), pituitary (Pit), nodose ganglion (NG), and pancreas were unaffected (Figures 1B and 1C).

Basal Control of Glucose Tolerance, Food Intake, and Gastric Emptying in $Glp1r^{\Delta Wnt1-/-}$ Mice

Distinct GLP-1R$^+$ neuronal circuits are important for the physiological control of metabolism and also mediate the pharmacological responses to GLP-1R agonism (Drucker, 2018b; Kanoski et al., 2016); we therefore analyzed mice in the basal state and following administration of structurally distinct incretin therapies. Reduction of *Glp1r* expression within *Wnt1-Cre2*-expressing cells had no effect on fasting blood glucose, oral glucose tolerance (Figure 1D), or plasma levels of GLP-1, insulin, or glucagon (Figure 1E). Similarly, intraperitoneal (i.p.) glucose tolerance and the associated levels of insulin were not different, although insulin levels trended lower in $Glp1r^{\Delta Wnt1-/-}$ mice (Figure 1F). Surprisingly, the rates of gastric emptying (Figure 1G), food intake over 24 h (Figures 1H and 1I), and body weight (Figure S2J) were not different in $Glp1r^{\Delta Wnt1-/-}$mice. Hence, simultaneous reduction of hypothalamic, BS, and ENS *Glp1r* expression is not associated with perturbations in the basal control of glucose homeostasis, gastric emptying, or food intake.

Acute Metabolic Responses to Exendin-4, Albiglutide, and Dulaglutide in $Glp1r^{\Delta Wnt1-/-}$ Mice

We next assessed the importance of GLP-1R expression within the Wnt1$^+$ domain for the acute metabolic responses arising from pharmacological administration of structurally distinct GLP-1R agonists. The small peptide exendin-4 as well as the higher-molecular-weight GLP-1R agonists albiglutide and dulaglutide improved oral glucose tolerance to a similar extent in $Glp1r^{\Delta Wnt1+/+}$ and $Glp1r^{\Delta Wnt1-/-}$ mice (Figure 2A and 2B). Similarly, exendin-4 enhanced glucose clearance in both genotypes following i.p. administration of glucose (Figures S3A and S3C), consistent with functional preservation of GLP-1R within pancreatic β cells (Lamont et al., 2012; Smith et al., 2014). Interestingly, glucose-stimulated insulin levels were lower in $Glp1r^{\Delta Wnt1-/-}$ versus $Glp1r^{\Delta Wnt1+/+}$ mice following exendin-4 administration (Figure 2C; Figures S3B and S3D) despite similar glycemic excursion profiles; however, the basal levels of glucagon were not different, and all three GLP-1R agonists reduced plasma glucagon levels to a similar extent following glucose challenge in $Glp1r^{\Delta Wnt1+/+}$ versus $Glp1r^{\Delta Wnt1-/-}$ mice (Figure 2D).

(H and I) 24-h food intake after refeeding following an overnight fast (H, non-cumulative over different time periods; I, cumulative over 24 h) in 6- to 10-month-old $Glp1r^{\Delta Wnt1+/+}$ versus $Glp1r^{\Delta Wnt1-/-}$ male mice (n = 12–25 animals/group).

Data are expressed as means ± SEM. ***p < 0.001 and ****p < 0.0001 by t test (A and B) or two-way ANOVA (E and F).

See also Figure S2.

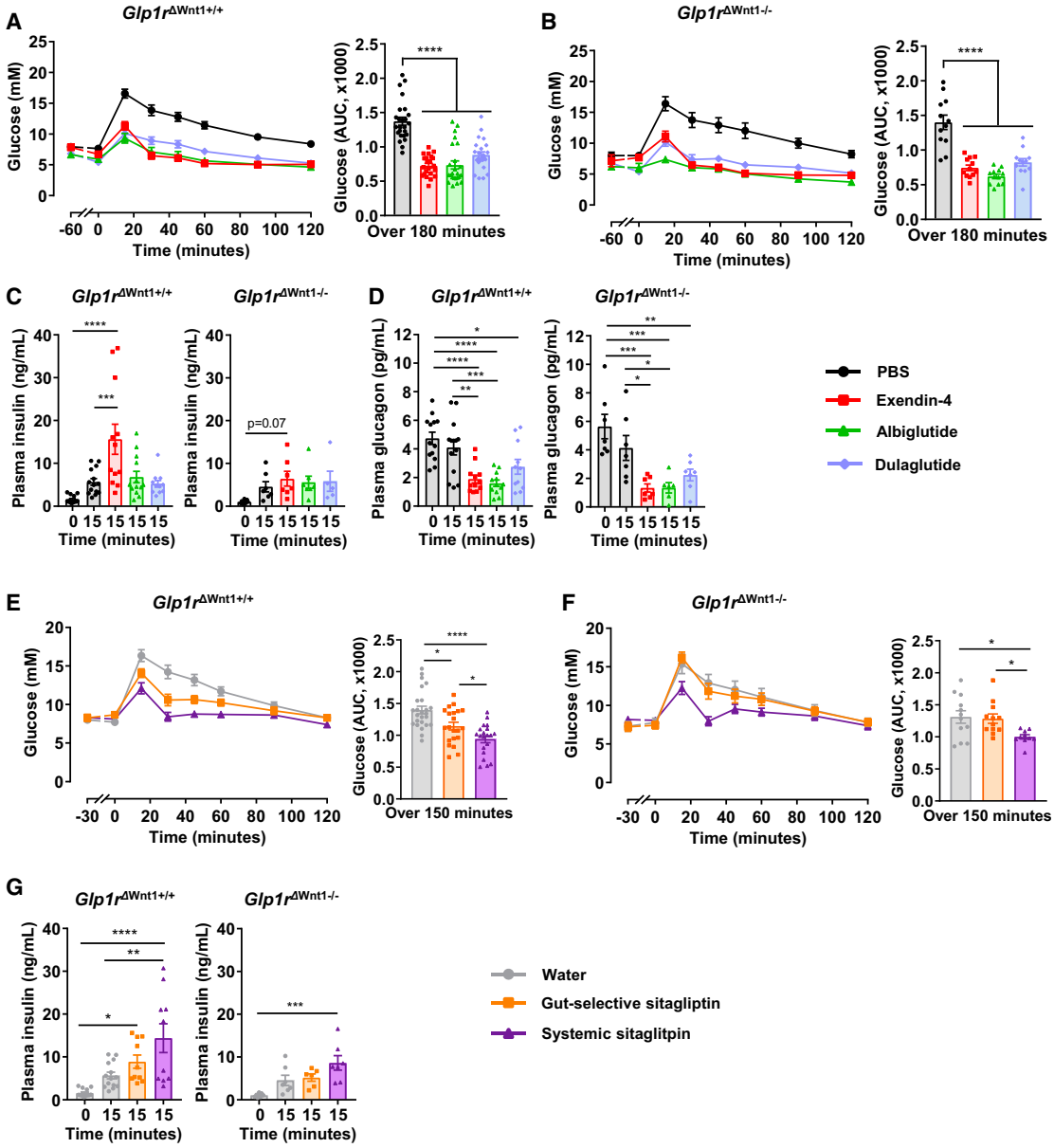


Figure 2. Assessment of Glucose Tolerance in *Glp1r*$^{\Delta Wnt1-/-}$ Mice

(A–D) Blood glucose and AUC over 3 h (A and B), plasma insulin (C), and glucagon (D) before and 15 min after glucose gavage (2 g/kg BW) during an oGTT in 6- to 10-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ (n = 21–23/group in A and n = 12–14/group in C and D) and *Glp1r*$^{\Delta Wnt1-/-}$ (n = 12/group in B and n = 6–7/group in C and D) mice after a 5-h fast and in response to PBS, exendin-4 (1 nmol/kg body BW), albiglutide (2 mg/kg BW), or dulaglutide (0.05 mg/kg BW) given just before (exendin-4) or 1 h before (albiglutide and dulaglutide) glucose gavage.

(E–G) Blood glucose and AUC over 2.5 h (E and F) and plasma insulin (G) before and 15 min after glucose gavage (2 g/kg BW) during an oGTT in 6- to 10-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ (n = 21–23/group in E and n = 12–14/group in G) and *Glp1r*$^{\Delta Wnt1-/-}$ (n = 12/group in F and n = 6–7/group in G) mice administered water or 14 μg/mouse (gut-selective dose) or 10 mg/kg (systemic dose) of sitagliptin 30 min prior to glucose gavage.

Data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-way ANOVA.

See also Figures S1 and S3.

Loss of GLP-1R within Wnt1^{+} Cells Impairs the Glycemic Response to Low-Dose Sitagliptin

Considerable pharmacological evidence supports the existence of a gut-brain axis important for transduction of glucoregulatory signals arising from endogenous GLP-1 or gut-selective dosing of DPP-4 inhibitors (Mulvihill et al., 2017; Vahl et al., 2007; Varin et al., 2019; Waget et al., 2011). Nevertheless, few studies have interrogated the simultaneous importance of GLP-1Rs within the ENS and CNS for control of the gut-brain axis. Although both gut-selective and systemic DPP-4 inhibition with sitagliptin

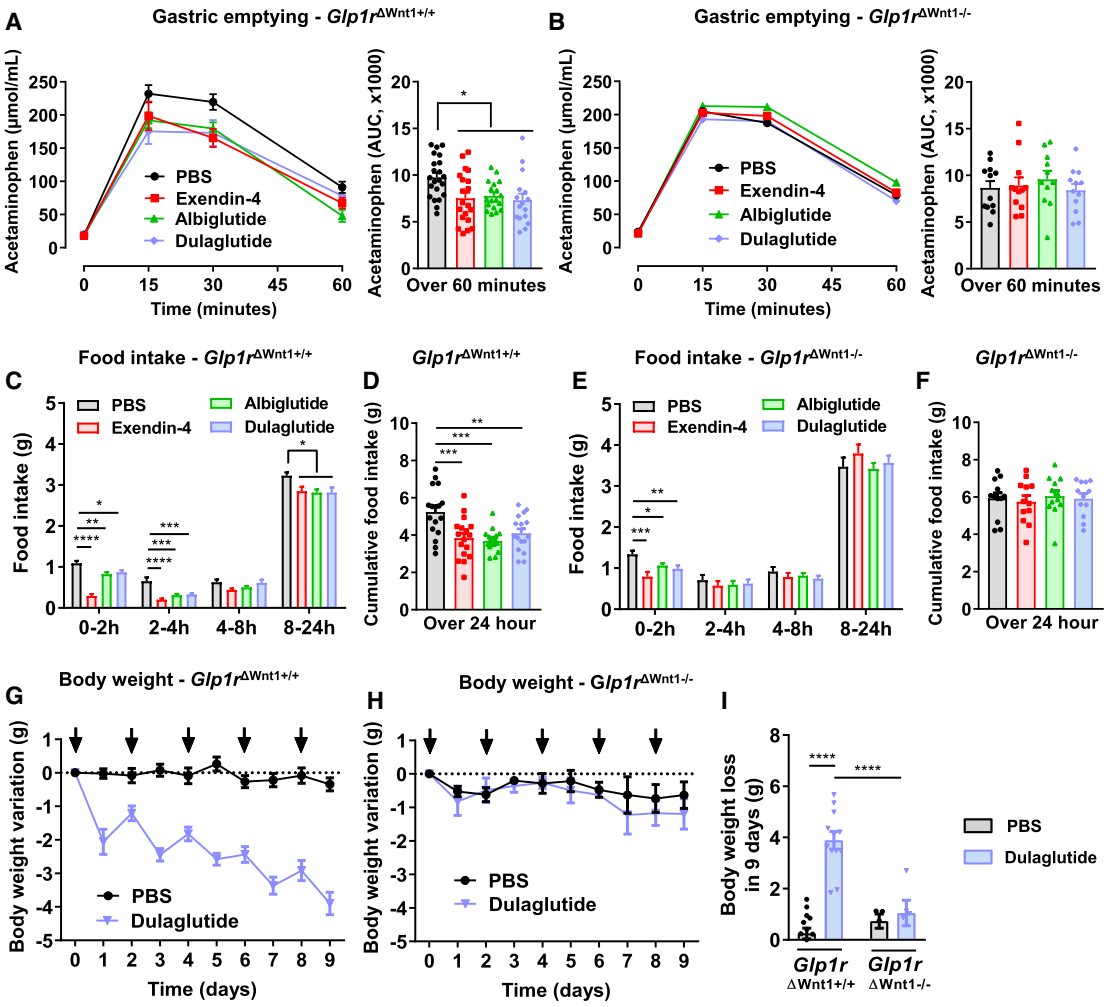


Figure 3. Gastric Emptying, Food Intake, and Weight Loss in *Glp1r*$^{\Delta Wnt1-/-}$ Mice

(A and B) Plasma acetaminophen levels (as a measure of gastric emptying) and AUC over 1 h during oGTT and after oral gavage of 100 mg/kg BW acetaminophen (1% solution) in 6- to 10-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ (A; n = 17–20/group) and *Glp1r*$^{\Delta Wnt1-/-}$ (B; n = 11–12/group) mice after a 5-h fast and in response to PBS, exendin-4 (1 nmol/kg BW), albiglutide (2 mg/kg BW), or dulaglutide (0.05 mg/kg BW) given just before (exendin-4) or 1 h before (albiglutide and dulaglutide) glucose gavage.

(C–F) Non-cumulative food intake (C and E) and 24-h cumulative food intake (D and F) following an overnight fast and i.p. administration of exendin-4 (10 nmol/kg BW just before refeeding), albiglutide (2 mg/kg BW just before fasting), or dulaglutide (0.05 mg/kg BW just before fasting) in 6- to 10-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ (n = 16/group) and *Glp1r*$^{\Delta Wnt1-/-}$ (n = 12/group) mice.

(G–I) Body weight variation (G and H) and cumulative body weight loss (I) during 9 days of treatment with PBS or dulaglutide (0.5 mg/kg BW) every other day in 8- to 10-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ (n = 12–14/group) and *Glp1r*$^{\Delta Wnt1-/-}$ (n = 4–5/group) mice. Arrows represent i.p. injections of PBS or dulaglutide.

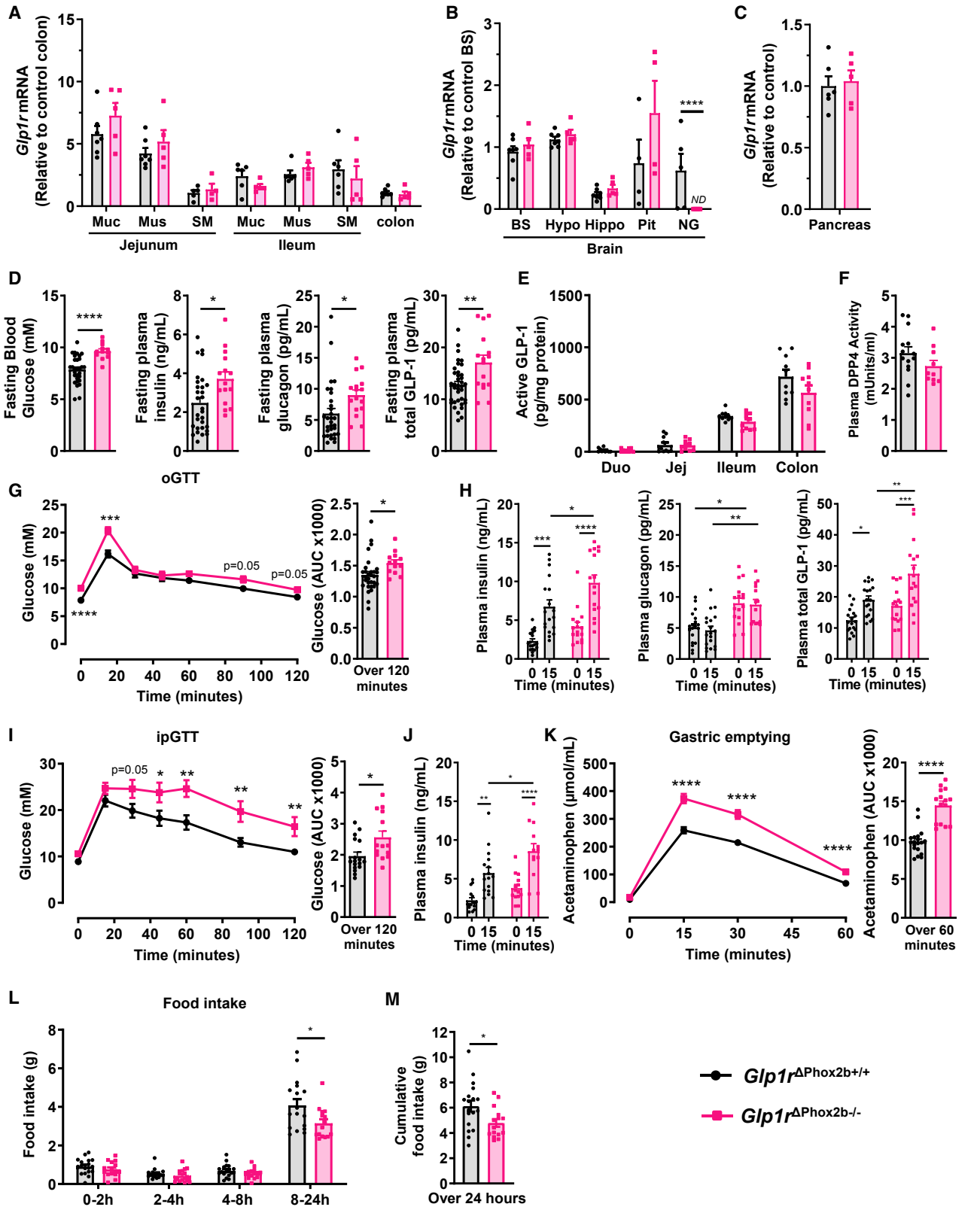
Data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 by one-way ANOVA (A and C–E) or two-way ANOVA (I).

See also Figure S1.

lowered glycemia and increased plasma insulin levels following oral glucose administration in *Glp1r*$^{\Delta Wnt1+/+}$ mice (Figures 2E and 2G), the glycemic and insulin responses to gut-selective sitagliptin were abolished in *Glp1r*$^{\Delta Wnt1-/-}$ mice (Figures 2F and 2G).

GLP-1R Agonism Does Not Reduce Food Intake or Gastric Emptying in *Glp1r*$^{\Delta Wnt1-/-}$ Mice

Inhibition of gastric emptying following GLP-1R agonism is thought to involve neural transmission of GLP-1R-dependent signals, in part through the vagus nerve (Charpentier et al., 2018; Plamboeck et al., 2013). Acute treatment with exendin-4, albiglutide, or dulaglutide reduced gastric emptying in *Glp1r*$^{\Delta Wnt1+/+}$ but not in *Glp1r*$^{\Delta Wnt1-/-}$ mice (Figures 3A and 3B). Furthermore, all three GLP-1R agonists decreased food intake over 24 h in *Glp1r*$^{\Delta Wnt1+/+}$ mice (Figures 3C and 3D) but not in *Glp1r*$^{\Delta Wnt1-/-}$ mice (Figures 3E and 3F). To examine whether GLP-1Rs within the Wnt1 domain were similarly required for weight loss following more sustained GLP-1R agonism, we evaluated the response to dulaglutide administration over 9 days. *Glp1r*$^{\Delta Wnt1+/+}$ mice



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lost ~4 g over 9 days (Figures 3G and 3I). However, dulaglutide failed to reduce body weight in *Glp1r*$^{\Delta Wnt1-/-}$ mice (Figures 3H and 3I).

Glp1r$^{\Delta Phox2b-/-}$ Mice Exhibit Impaired Glucose Homeostasis and Gastric Emptying

Complementary lines of evidence invoke vagal GLP-1Rs as a component of signals necessary for transduction of gut-brain GLP-1 communication and control of glucose homeostasis (Charpentier et al., 2018; Krieger et al., 2016; Plamboeck et al., 2013). Nevertheless, whether GLP-1Rs within the Phox2b expression domain contribute to basal and GLP-1RA-regulated glucose control and food intake remains uncertain (Sisley et al., 2014). Accordingly, we used *Phox2b-Cre* mice, known to target *Phox2b*$^{+}$ cells within the NG, midbrain, and hindbrain, including the DMV (Vianna et al., 2012), to selectively attenuate expression of the *Glp1r* within visceral sensory neurons (Rossi et al., 2011). No reduction of *Glp1r* mRNA transcripts was detected in the jejunum, ileum, colon, BS, Hypo, Hippo, Pit, or pancreas from *Glp1r*$^{\Delta Phox2b-/-}$ mice; however, *Glp1r* mRNA transcripts were not detectable in the NG of *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figures 4A–4C).

We next interrogated the physiological consequences of disrupting GLP-1 action within cellular domains targeted by Phox2b. Remarkably, *Glp1r*$^{\Delta Phox2b-/-}$ mice exhibited increased fasting levels of glucose, plasma insulin, glucagon, and GLP-1 (Figure 4D). No differences were detected in GLP-1 concentration within segments of the small and large bowel (Figure 4E), and plasma DPP4 activity was similar in *Glp1r*$^{\Delta Phox2b+/+}$ versus *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figure 4F). Moreover, *Glp1r*$^{\Delta Phox2b-/-}$ mice exhibited a modest impairment in glycemic excursion following oral glucose (Figure 4G) and a marked deterioration in glucose clearance following i.p. glucose challenge despite comparable effects of glucose on the levels of plasma insulin, glucagon, and GLP-1 in both genotypes (Figures 4H–4J). Furthermore, gastric emptying was accelerated (Figure 4K), and cumulative 24-h food intake was modestly reduced in *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figures 4L and 4M). However, the total body weight was not different (Figure S2K). Hence, selective reduction of basal GLP-1R signaling within Phox2b-targeted cells, including peripheral sensory neurons, leads to dysregulation of the enteroinsular axis, impaired glucose homeostasis, and gastric motility.

The *Phox2b*-Targeted *Glp1r* Is Not Required for the Glucoregulatory Actions of GLP-1R Agonists or the DPP-4 Inhibitor Sitagliptin

To ascertain whether Phox2b-targeted GLP-1Rs were similarly important for the metabolic actions of pharmacological GLP-1R agonists, we assessed the actions of exendin-4, albiglutide, and dulaglutide in *Glp1r*$^{\Delta Phox2b-/-}$ mice. All three GLP-1R agonists reduced glycemic excursion to similar levels following oral glucose administration in *Glp1r*$^{\Delta Phox2b+/+}$ versus *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figures 5A and 5B), accompanied by increased levels of plasma insulin and reduced levels of circulating glucagon (Figures 5C and 5D). Similarly, exendin-4 improved glucose tolerance and increased insulin levels in both genotypes in response to i.p. administration of glucose (Figures S3E–S3H), consistent with a functional GLP-1R in pancreatic β cells. Furthermore, both gut-selective and systemic inhibition of DPP-4 activity with sitagliptin reduced glycemic excursion following oral glucose administration in *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figures 5E–5G). Hence, the GLP-1R within the Phox2b-targeted domain, including the NG, is not required for glucoregulatory responses to GLP-1R agonists or the DPP-4 inhibitor sitagliptin.

Glp1r$^{\Delta Phox2b-/-}$ Mice Exhibit Selectively Impaired Responses to GLP-1R Agonists

We next examined whether the GLP-1R within domains targeted by Phox2b was required for the reduction of gastric emptying following acute administration of GLP-1R agonists. Exendin-4, albiglutide, and dulaglutide reduced the rate of gastric emptying in *Glp1r*$^{\Delta Phox2b+/+}$ mice (Figure 6A). Basal gastric emptying was accelerated in *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figure 4K) and reduced by albiglutide and dulaglutide but not exendin-4 (Figure 6B). In contrast, exendin-4 reduced food intake in both *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figures 6C–6F), whereas albiglutide and dulaglutide failed to reduce 24-h cumulative food intake in *Glp1r*$^{\Delta Phox2b-/-}$ mice (Figures 6E and 6F). Consistent with these findings, chronic administration

Figure 4. *Glp1r*$^{\Delta Phox2b-/-}$ Mice Exhibit Altered Glucose Tolerance, Gastric Emptying, and Food Intake

(A–C) *Glp1r* mRNA abundance (relative to *Ppia*) in tissues from 10- to 15-week-old female *Glp1r*$^{\Delta Phox2b+/+}$ versus *Glp1r*$^{\Delta Phox2b-/-}$ mice in various enteric nervous system-containing intestinal compartments (Muc, Mus, and SM) in different regions of the digestive tract (duodenum, jejunum, ileum, and colon) expressed relative to *Glp1r* mRNA abundance detected in the colon of control animals (*Glp1r*$^{\Delta Phox2b+/+}$) (A), in different regions of the brain (BS, Hypo, Hippo, Pit, and NG) expressed relative to the BS of control animals (*Glp1r*$^{\Delta Phox2b+/+}$) (B), and in the pancreas (expressed relative to levels in control animals (*Glp1r*$^{\Delta Phox2b+/+}$) (C). n = 5–10/group.

(D–F) Blood glucose and plasma levels of insulin, glucagon and total GLP-1 (D); levels of active GLP-1 within the different regions of the gut (duodenum [Duo] and jejunum [Jej] (E); and plasma DPP-4 activity (F) in 8- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice after a 5-h fast (n = 8–18/group).

(G and H) Blood glucose and AUC over 2 h (G) and plasma levels of insulin, glucagon, and total GLP-1 before and 15 min after glucose administration (2 g/kg BW) (H) during an oGTT in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice after a 5-h fast (n = 8–18/group).

(I and J) Blood glucose and AUC over 2 h (I) and plasma insulin before and 15 min after glucose administration (2 g/kg BW) (J) during an ipGTT in 8- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice after a 5-h fast (n = 8–18/group).

(K) Plasma acetaminophen levels (as a measure of gastric emptying) and AUC over 1 h during oGTT and after oral gavage of 100 mg/kg BW acetaminophen (1% solution) in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice after a 5-h fast (n = 15/group).

(L and M) Non-cumulative (L) and cumulative (M) food intake over 24 h after refeeding following an overnight fast in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice (n = 15/group).

Data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by t test (B, D, G, I, and K–M) or two-way ANOVA (H and J).
See also Figure S2.

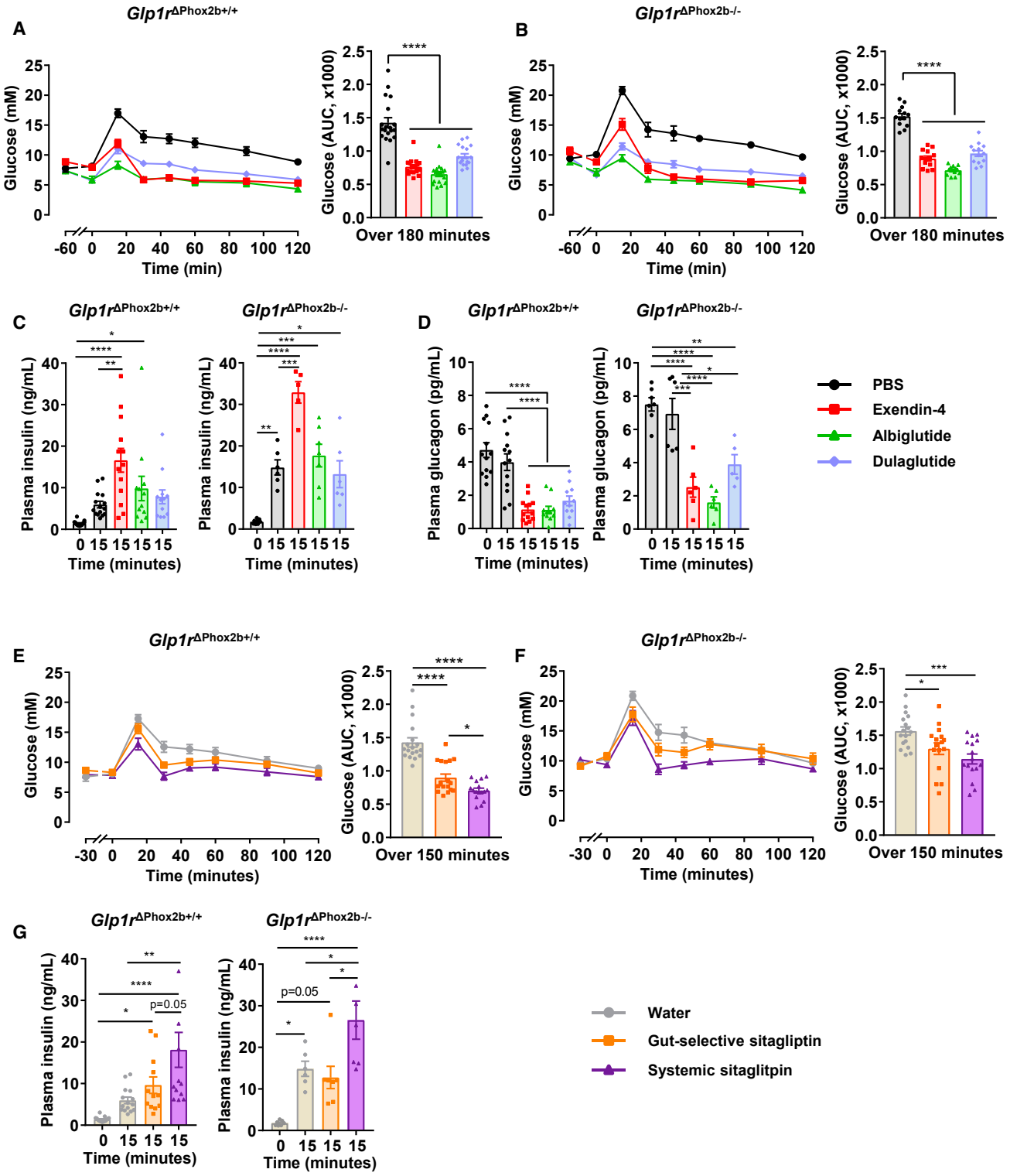

Figure 5. Glucose-Lowering Effects of GLP-1R Agonists and the DPP-4 Inhibitor Sitagliptin Are Preserved in *Glp1r*$^{\Delta Phox2b-/-}$ Mice

(A–D) Blood glucose and AUC over 3 h (A and B, n = 12–18/group), plasma insulin (C, n = 6–15/group), and glucagon (D, n = 6–15/group) before and 15 min after glucose administration (2 g/kg BW) during an oGTT in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice after a 5-h fast and in response to i.p. administration of PBS, exendin-4 (1 nmol/kg BW), albiglutide (2 mg/kg BW), or dulaglutide (0.05 mg/kg BW) given just before (exendin-4) or 1 h before (albiglutide and dulaglutide) glucose gavage.

(legend continued on next page)

of dulaglutide was associated with attenuated weight loss in *Glp1r*$^{\Delta Phox2b-/-}$ versus *Glp1r*$^{\Delta Phox2b+/+}$ mice (Figures 6G–6I).

Glp1r$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ Mice Exhibit Normal Lipid Tolerance and Preserved Hypolipidemic Responses to GLP-1R Agonism

GLP-1R agonists have been postulated to inhibit post-prandial lipoprotein production and secretion through central and/or peripheral neural GLP-1R networks (Farr et al., 2015; Kooijman et al., 2015). We therefore evaluated triglyceride excursion in response to an oral gavage of olive oil in the presence or absence of acute GLP-1R agonists. Triglyceride excursions following oral olive oil administration were not different in *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ versus littermate control mice (Figures 7A and 7B). Moreover, all three structurally distinct GLP-1R agonists reduced triglyceride excursions in *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice and their respective controls (Figures 7C–7F). Hence, GLP-1R expression within the broad neuronal domains targeted by *Wnt1-Cre2* and GLP-1R^{+} neurons targeted by *Phox2b-Cre* is not required for GLP-1R-dependent inhibition of triglycerides following oral lipid challenge.

DISCUSSION

GLP-1 exerts a broad range of metabolic activities, encompassing control of glycemia, reduction of food intake, and inhibition of gut motility, actions supporting the development of GLP-1R agonists for the treatment of type 2 diabetes and obesity (Drucker et al., 2017; Sandoval and D'Alessio, 2015). The CNS actions of small peptide GLP-1R agonists have been associated with direct access to hypothalamic and hindbrain GLP-1R^{+} neurons (Knudsen and Lau, 2019; Secher et al., 2014). Although we initially hypothesized that larger GLP-1R agonists such as albiglutide and dulaglutide might exhibit slightly less effective activation of neural GLP-1R^{+} circuits regulating gastric emptying or food intake, our experimental pharmacology results did not reveal broad differences in the acute metabolic actions of structurally distinct GLP-1R agonists.

The extent to which basal GLP-1R signaling within distinct cell compartments is physiologically essential for acute versus long-term control of glucose homeostasis and body weight is more controversial and depends on the experimental context. *Glp1r*$^{-/-}$ mice exhibit impaired glucose tolerance (Scrocchi et al., 1996), and selective deletion or restoration of the GLP-1R within murine β cells of *Glp1r*$^{-/-}$ mice reveals an essential role for the β cell GLP-1R in glucose homeostasis (Lamont et al., 2012; Smith et al., 2014). The relative importance of the GLP-1R for control of murine body weight, as revealed through genetic approaches, reflects the experimental paradigm. Mice with germline deletion of the *Glp1r* in the C57BL/6 background resist development of diet-induced obesity and insulin resistance, in part because of upregulation of locomotor activity and energy expenditure (Ayala et al., 2010; Hansotia et al., 2007; Scrocchi et al., 1996). In contrast, glucose tolerance and body weight were not different in *Glp1r*$^{\Delta Nestin-/-}$ mice with widespread reduction of CNS *Glp1r* expression (Sisley et al., 2014), and selective targeting of hypothalamic *Glp1r* expression did not identify a subset of hypothalamic GLP-1R^{+} neurons essential for the control of body weight or glucose homeostasis (Burmeister et al., 2017).

On the other hand, transient central or peripheral pharmacological antagonism of the GLP-1R led to increased food intake and more significant weight gain in rats and mice (Meeran et al., 1999; Patterson et al., 2011). Similarly, postnatal inactivation of the *Glp1r* within the hypothalamus increased food intake and weight gain (Liu et al., 2017), and selective reduction of GLP-1R activity within different regions of the rat brain impaired the control of either food intake, meal size, body weight, energy expenditure, or adiposity (Alhadeff et al., 2017; Lee et al., 2018; López-Ferreras et al., 2018). Our current findings of normal glucose tolerance, food intake, and gastric emptying in *Glp1r*$^{\Delta Wnt1-/-}$ mice provide further evidence that germline reduction of hypothalamic, BS, and ENS *Glp1r* expression in mice does not markedly perturb basal control of glucose or food intake or the acute glucoregulatory response to exogenous GLP-1R agonists. Nevertheless, exogenous administration of GLP-1R agonists failed to reduce food intake (and gastric emptying) in *Glp1r*$^{\Delta Wnt1-/-}$ mice. These findings are consistent with the marked simultaneous reduction of hypothalamic and BS *Glp1r* mRNA transcripts and reflect the importance of multiple neural hypothalamic and BS GLP-1R^{+} populations for the pharmacological control of GLP-1-regulated feeding (Burmeister et al., 2017; Hayes et al., 2011; Sisley et al., 2014).

A considerable body of evidence also supports the functional importance of a gut-brain axis, whereby intestinal GLP-1 activates neural circuits both external to and within the CNS to control glucose metabolism (Burcelin et al., 2014). Nevertheless, the precise cellular location of GLP-1R^{+} neurons critical for transduction of these physiological signals remains uncertain. Previous studies have revealed that gut-selective potentiation of endogenous incretin action, achieved through reduction of intestinal but not systemic DPP-4 activity, is partially attenuated following GLP-1R pharmacological blockade or whole-body inactivation of the *Glp1r* (Mulvihill et al., 2017; Waget et al., 2011). Indeed, high-fat diet feeding to reduce ENS *Glp1r* expression, subdiaphragmatic vagotomy, and administration of *cis*-platinum to induce ENS neuropathy were all associated with relative GLP-1 resistance and impairment of the GLP-1-gut-brain axis (Grasset et al., 2017; Kanoski et al., 2011). Here we further localize the GLP-1R^{+} populations transducing the glucoregulatory actions of intestine-selective DPP-4 inhibition to those targeted by *Wnt1-Cre2*, as the metabolic effects of low-dose intestine-selective sitagliptin were completely attenuated in *Glp1r*$^{\Delta Wnt1-/-}$ mice. These findings are consistent with

(E–G) Glucose and AUC over 2.5 h (E and F, n = 12–18/group) and insulin (G, n = 6–15/group) before and 15 min after glucose administration (2 g/kg BW) during an oGTT (5-h fast) in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice administered water or 14 μg/mouse (gut-selective dose) or 10 mg/kg (systemic dose) of sitagliptin 30 min prior to glucose gavage.

Data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-way ANOVA.

See also Figures S1 and S3.

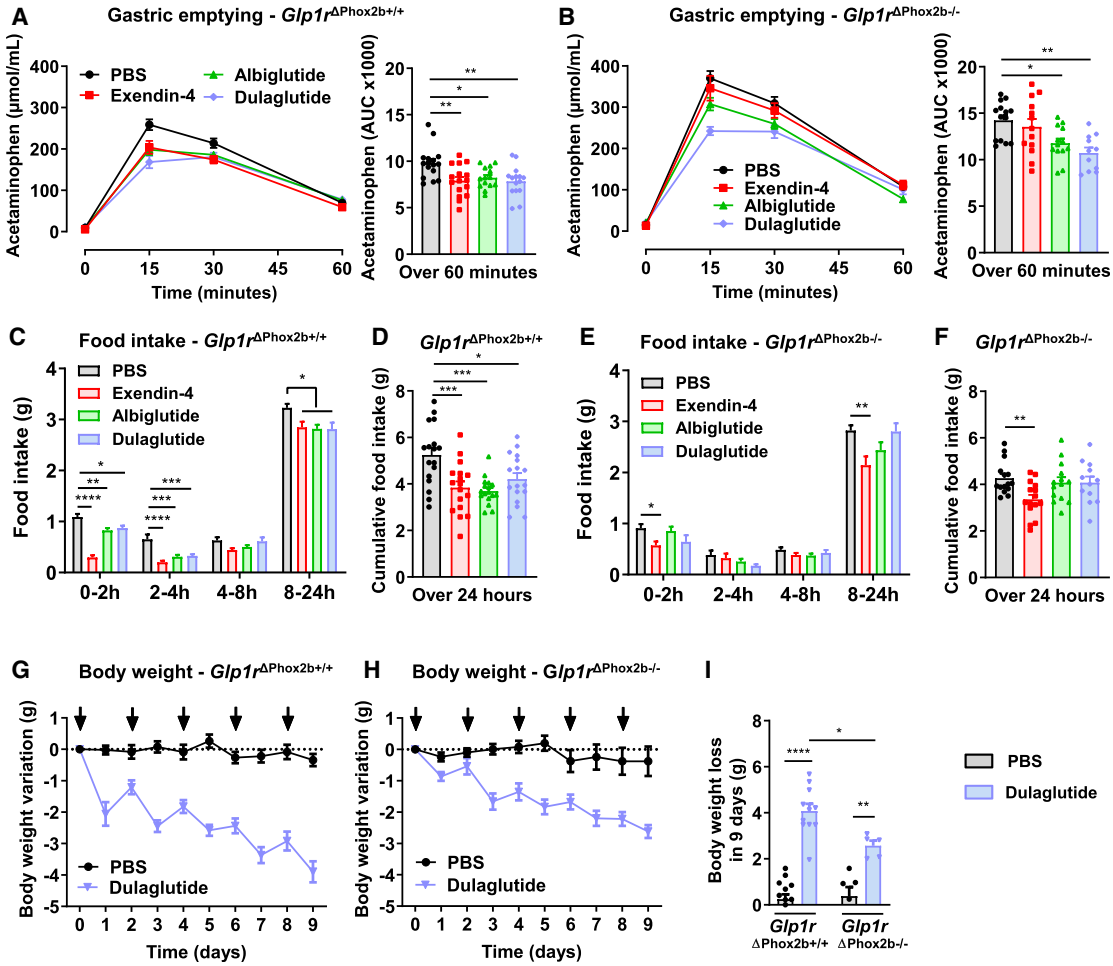

Figure 6. Gastric Emptying, Food Intake, and Weight Loss Responses to GLP-1R Agonists in *Glp1r*$^{\Delta Phox2b-/-}$ Mice

(A and B) Plasma acetaminophen levels (as a measure of gastric emptying) and AUC over 1 h during oGTT and after oral gavage of 100 mg/kg BW acetaminophen (1% solution) in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ (A, n = 16/group) and *Glp1r*$^{\Delta Phox2b-/-}$ (B, n = 13/group) mice after a 5-h fast and in response to i.p. administration of PBS, exendin-4 (1 nmol/kg BW), albiglutide (2 mg/kg BW), or dulaglutide (0.05 mg/kg BW) given just before (exendin-4) or 1 h before (albiglutide and dulaglutide) glucose gavage.

(C–F) Non-cumulative food intake (C and E) and 24-h cumulative food intake (D and F) after re-feeding following an overnight fast in response to i.p. administration of exendin-4 (10 nmol/kg BW just before refeeding), albiglutide (2 mg/kg BW just before fasting), or dulaglutide (0.05 mg/kg BW just before fasting) in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice (n = 15–16/group).

(G–I) Change in body weight (G and H) and cumulative body weight loss (I) during 9 days of treatment with PBS or dulaglutide (0.5 mg/kg BW) every other day in 6- to 10-month-old male *Glp1r*$^{\Delta Phox2b+/+}$ (n = 12–14/group) and *Glp1r*$^{\Delta Phox2b-/-}$ (n = 5–6/group) mice. Arrows represent i.p. injections of PBS or dulaglutide.

Data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-way ANOVA.

See also Figure S1.

the marked reduction in small bowel *Glp1r* expression within the muscular layer of *Glp1r*$^{\Delta Wnt1-/-}$ mice, an anatomical site with ENS localization of GLP-1Rs (Grasset et al., 2017; Wismann et al., 2017). In contrast, selective DPP-4 inhibition with low-dose sitagliptin maintained glucoregulatory activity in *Glp1r*$^{\Delta Phox2b-/-}$ mice.

Surprisingly, selective loss of the GLP-1R in *Glp1r*$^{\Delta Phox2b-/-}$ mice resulted in increased levels of fasting glycemia, impaired glucose tolerance, accelerated gastric emptying, and increased levels of GLP-1, insulin, and glucagon. Indeed, these striking findings are consistent with observations in rats with lentiviral vector-mediated knockdown of the NG *Glp1r*, resulting in elevated post-meal glycemia and accelerated gastric emptying (Krieger et al., 2016). Notably, increased circulating levels of GLP-1, insulin, C-peptide, and glucagon and increased rates of gastric emptying were also observed in humans following truncal vagotomy (Plamboeck et al., 2013). Equally unexpected was our finding that the extent of dulaglutide-induced weight loss was diminished in *Glp1r*$^{\Delta Phox2b-/-}$ mice, implicating the GLP-1R within the *Phox2b* expression domain as yet another contributor to signals regulating the pharmacological GLP-1R-dependent control of body weight. These results are consistent with data demonstrating diminished reduction of food intake in rats with subdiaphragmatic vagal deafferentation (Kanoski et al., 2011) and loss of

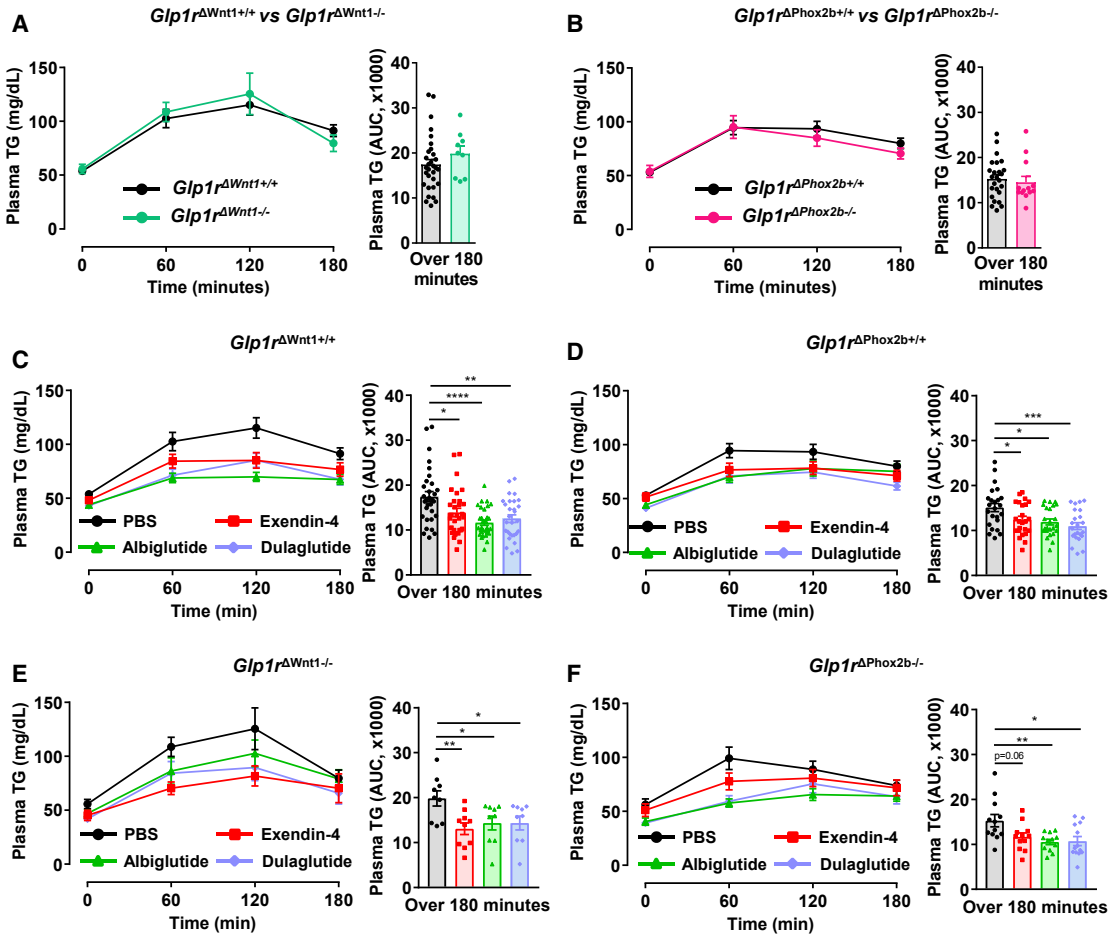

Figure 7. GLP-1R Agonists Reduce Triglyceride Excursions in *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ Mice

(A–F) Plasma triglycerides (TGs) over a 3-h period after oral gavage with olive oil (200 μL/mouse) during a lipid tolerance test in 5-h-fasted, 9- to 13-month-old male *Glp1r*$^{\Delta Wnt1+/+}$ versus *Glp1r*$^{\Delta Wnt1-/-}$ (A, C, and E) or *Glp1r*$^{\Delta Phox2b+/+}$ versus *Glp1r*$^{\Delta Phox2b-/-}$ (B, D, and F) mice basally (A and B) or in response to i.p. administration of PBS, exendin-4 (1 nmol/kg BW just before olive oil gavage), albiglutide (2 mg/kg BW 16 h before olive oil gavage), or dulaglutide (0.05 mg/kg BW just before fasting) (C–F), (n = 9–25 animals / group).

Data are expressed as means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by one-way ANOVA.

See also Figure S1.

the inhibitory actions of GLP-1 on food intake in human subjects after vagotomy (Plamboeck et al., 2013). Collectively, these findings reveal essential physiological roles of GLP-1R expression within *Phox2b*$^{+}$ vagal sensory afferents, including the NG, in the control of GLP-1 levels, islet hormones, and glucose tolerance and establish the GLP-1Rs within the Phox2b domain as essential for a component of the weight loss response ensuing following sustained GLP-1R agonism.

GLP-1R agonists also rapidly reduce plasma levels of postprandial triglycerides in mice and humans, actions mediated by the canonical GLP-1R (Hsieh et al., 2010; Xiao et al., 2012). Indeed, plasma levels of TG-rich lipoproteins are higher after olive oil challenge in *Glp1r*$^{-/-}$ mice (Hsieh et al., 2010), consistent with the importance of basal GLP-1R signaling for either triglyceride production, secretion, and/or clearance. Although the precise GLP-1R^{+} cell types mediating the hypolipidemic actions of GLP-1 remain unclear, several studies implicated neural GLP-1 receptors in GLP-1R-dependent control of triglyceride-rich lipoproteins (Burmeister et al., 2012; Farr et al., 2015; Kooijman et al., 2015). Nevertheless, we did not observe any perturbation of triglyceride excursion following oral olive oil administration in *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice. Furthermore, although GLP-1R agonists lost their ability to reduce gastric emptying in *Glp1r*$^{\Delta Wnt1-/-}$ mice, the reduction of acute triglyceride excursion following administration of exendin-4, albiglutide, or dulaglutide was preserved in *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice. Hence, the GLP-1R^{+} cell type(s) essential for transduction of the rapid (within minutes) triglyceride-lowering actions of GLP-1R agonist administration are distinct from those controlling food intake and gastric emptying and not targeted by *Wnt1-Cre2* or *Phox2b-Cre*. The putative identity and importance of neural sites transducing the acute hypolipidemic actions of GLP-1R agonists remain uncertain.

Our studies have a number of important limitations. First, we assessed the phenotypes of *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$

mice with germline inactivation of the *Glp1r* in multiple neuronal populations and, hence, cannot rule out modification of one or more metabolic phenotypes as a result of adaptation to embryonic or developmental loss of the GLP-1R. Second, the majority of our assessments, principally of glucose homeostasis, gastric emptying, food intake, and triglyceride excursion, were acute and may not reflect the biology of the GLP-1R within these neural circuits over more prolonged periods of assessment. Third, we studied regular chow-fed mice without experimental diabetes or obesity, and resistance to GLP-1 action under these circumstances (Grasset et al., 2017) might alter future conclusions from studies conducted under different metabolic conditions. Fourth, we chose doses of exendin-4, albiglutide, and dulaglutide based on their equivalent pharmacodynamic activity in acute glucose tolerance tests, and it remains possible that a different dosing paradigm would yield experimental results with alternative conclusions. Fifth, *Wnt1* and *Phox2b* direct *Cre* expression to multiple GLP-1R$^+$ sites, precluding definitive assignment of specific cellular GLP-1Rs to individual phenotypes described here. Nevertheless, our studies are in agreement with but extend concepts invoking multiple central and peripheral neuronal GLP-1R$^+$ populations required for GLP-1R agonism to control food intake and body weight. Furthermore, our current findings elevate the importance of the GLP-1R within the *Phox2b* domain as an essential sensor of physiological peptidergic signals, linking basal GLP-1R signaling to the control of L cell and islet hormone secretion, gastric emptying, and glucose homeostasis.

In summary, our findings provide evidence that germline disruption of the *Glp1r* within the cellular domains (including the enteric and central nervous system) targeted by *Wnt1-Cre2* does not impair basal control of food intake, body weight, gastric emptying, or glucose homeostasis. Nevertheless, these same GLP-1R$^+$ populations are essential for transducing the pharmacological responses to small and larger GLP-1R agonists in control of food intake, body weight, and gastric emptying but not required for GLP-1R-dependent regulation of glucose homeostasis. Furthermore, neural GLP-1Rs targeted by *Wnt1-Cre2* are essential for transduction of a glucoregulatory and islet response to low dose sitagliptin, further highlighting the importance of the gut-brain axis for conveying metabolic signals originating from gut-selective potentiation of incretin action.

Equally compelling are insights gleaned from our studies of *Glp1r*$^{\Delta Phox2b-/-}$ mice, demonstrating that GLP-1Rs within the *Phox2b* expression domain are essential for basal control of gastric emptying, food intake, glucose tolerance, and circulating levels of insulin and glucagon in response to glucose challenge. In contrast, these *Phox2b*$^+$ GLP-1R$^+$ cellular domains are not required for the pharmacological actions of GLP-1R agonists to control glucose or lipid homeostasis. Collectively, these findings further emphasize the divergence in functional GLP-1R$^+$ populations controlling physiological actions of endogenous GLP-1 versus pharmacological responses to GLP-1R agonism and identify key neuronal populations essential for the transduction of gut-derived GLP-1R signals important for control of glucose homeostasis.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Mouse housing and treatments
  - Mouse line generation
  - Mouse line characterization
- METHOD DETAILS
  - Gastric Emptying
  - Food Intake and Body Weight
  - Lipid Tolerance Test
  - Glucose Tolerance Test
  - Metabolic measurements
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.05.055.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.M.V., E.E.M., and D.J.D.; Investigation, E.M.V., E.E.M., L.L.B., J.A.K., and X.C.; Formal Analysis and Visualization, E.M.V., J.A.K., and E.E.M.; Resources, R.J.S.; Writing – Original Draft, E.E.M., E.M.V., and D.J.D.; Writing – Review & Editing, E.M.V., E.E.M., L.L.B., J.A.K., R.J.S., and D.J.D.; Funding Acquisition and Project Administration, D.J.D.; Supervision, D.J.D.

DECLARATION OF INTERESTS

D.J.D. has served as an advisor or consultant to Intarcia, Forkhead Biopharmaceticals Inc., Kallyope Inc., Merck Research Laboratories, Pfizer Inc., Novo Nordisk Inc., Sanofi Inc., and Zafgen Inc. E.E.M. has received speaker's honoraria from Merck Canada. R.J.S. is a consultant to Ironwood Pharmaceuticals Inc., Kallyope Inc., Novo Nordisk Inc., Scohia, and Zafgen and has received research support from Kallyope Inc., MedImmune Inc., Novo Nordisk Inc., and Zafgen Inc.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Sitagliptin (JANUVIA 100mg tablets)	Merck Laboratories	N/A
Albiglutide (TANZEUM 30mg/0.5ml pen)	GSK	N/A
Dulaglutide (TRULICITY 0.75mg/0.5ml pen)	Eli Lilly	N/A
Exendin-4	Chi Scientific	Custom synthesis
Acetaminophen	Sigma	Cat#A7085
Olive oil	Sigma	Cat#O1514
Critical Commercial Assays		
Ultrasensitive Mouse Insulin ELISA	Alpco Diagnostics	Cat#80-INSMSU-E01; RRID: AB_2792981
Total GLP-1 (ver. 2) kit	Mesoscale	Cat#K150JVC-2; RRID: AB_2801383
Active GLP-1 (ver. 2) kit	Mesoscale	Cat#K150JWC-2
Glucagon ELISA - 10μl	Mercodia	Cat#10-1281-01; RRID: AB_2783839
Triglyceride assay kit	Roche Diagnostics	Cat#11877771 216
Triglyceride calibrator	Wako	Cat#464-01601
Acetaminophen-L3K Assay kit	Sekisui Diagnostics	Cat#506-30
H-Gly-Pro-AMC HBr (for DPP4 activity assay)	Bachem	Cat#I-1225
AMC (for DPP4 activity assay)	Bachem	Cat#Q-1025
Experimental Models: Organisms/Strains		
Mouse: *Wnt1-Cre2:* B6.Cg-E2f1$^{Tg(Wnt1\text{-}cre)2Sor}$/J	Jackson Laboratories	IMSR Cat#JAX:022501; RRID: IMSR_JAX:022501
Mouse: *Phox2b-Cre:* B6(Cg)-Tg(*Phox2b*-cre)3Jke/J	Jackson Laboratories	IMSR Cat#JAX:016223; RRID: IMSR_JAX:016223
Mouse: *Glp1r*$^{Flox/Flox}$	R. Seeley lab (Wilson-Pérez et al., 2013)	N/A
Oligonucleotides		
Cyclophilin *(ppia)*	Applied Biosystems	Cat#Mm02342430_g1
Glucagon-Like Peptide-1 receptor *(Glp1r)* (ex5-6)	Applied Biosystems	Cat#Mm00445292_m1
Software and Algorithms		
GraphPad Prism, version 7	GraphPad Prism Software	https://www.graphpad.com; RRID:SCR_002798
Others		
Regular Chow Diet (RC)	Harlan Teklad	Cat No: 2018

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Daniel J. Drucker (drucker@lunenfeld.ca).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mouse housing and treatments

All experiments involving mice were approved (Animal Use Protocol (AUP) approval number 20-0045H) by the Animal Care and Use Subcommittee at the Toronto Centre for Phenogenomics (TCP) at Mt. Sinai Hospital. Mice were housed (2 to 5 mice per cage), under a 12-h light/12h-dark cycle in the TCP and maintained on regular chow (RC; 18% kcal from fat, 2018, Harlan Teklad, Mississauga, ON) with food and water access *ad libitum* unless fasted for metabolic studies. Room temperature is maintained at 20-22°C. Experiments were started in mice at 8-12 weeks of age, as indicated in figure legends. Age- and sex-matched littermate C57BL/6 mice were randomly assigned to experimental groups, and all metabolic tests were performed in males, each mouse being its own control (saline versus treatment) on sequential experiments. Mice were given either one week (exendin-4 or sitagliptin treatment) or two

weeks (albiglutide or dulaglutide treatment) recovery between each experiment. Doses of GLP-1R agonists and the DPP-4 inhibitor sitagliptin were based on previous studies, and pilot experiments in our laboratory. Acute activation of GLP-1R for assessment of glucose and lipid tolerance and food intake was achieved using 1 or 10 nmol/kg (equivalent to 0.17 mg/kg or 1.7 mg/kg) body weight (BW) exendin-4 (Baggio et al., 2004); 2 mg/kg BW albiglutide (Baggio et al., 2004; Glaesner et al., 2010) or 0.05 mg/kg BW dulaglutide (Byrd et al., 2015; Glaesner et al., 2010). For assessment of weight loss, a dose of 0.5 mg/kg BW dulaglutide was utilized (Byrd et al., 2015; Glaesner et al., 2010). Acute gut-selective inhibition of DPP-4 activity was achieved with sitagliptin at a dose of 14 μg/mouse (~300-400 μg/kg BW), whereas 10 mg/kg BW achieved systemic inhibition of DPP-4 activity (Mulvihill et al., 2017; Waget et al., 2011).

Mouse line generation

To generate *Glp1r*$^{\Delta Wnt1-/-}$ and *Glp1r*$^{\Delta Phox2b-/-}$ mice, *Wnt1-Cre2* B6.Cg-E2f1$^{Tg(Wnt1-cre)2Sor}$/J mice (Lewis et al., 2013) (#022501, Jackson Laboratories, also known as B6 *Wnt1*-Cre2) and *Phox2b-Cre* B6(Cg)-Tg(*Phox2b*-cre)3Jke/J mice (Rossi et al., 2011) (#016223, Jackson Laboratories, also known as *Phox2b-Cre*) were bred with *Glp1r*$^{Flox/Flox}$ mice (Wilson-Pérez et al., 2013). *Wnt-1* (and *Wnt1*-Cre) is expressed very early and transiently during embryonic neural development where expression has been demonstrated in the midbrain, in a band across the midbrain-hindbrain boundary, as well as anteriorly into the diencephalon, and is expressed in progenitor cells giving rise to the mossy fiber neurons of the cerebellum as well as midbrain dopamine (MbDA) neurons (Nichols and Bruce, 2006; Rodriguez and Dymecki, 2000; Wurst and Prakash, 2014; Yang et al., 2013). *Wnt-1* is also expressed in neural crest precursors primarily at the dorsal midline region which include neural crest cells in the branchial arches, dorsal root ganglion, cranial ganglia and enteric nervous system (Danielian et al., 1998; Echelard et al., 1994; Lewis et al., 2013; Wilkinson et al., 1987).

The *Phox2b*-Cre mouse line targets the nodose ganglion, as well as neuronal components of the autonomic nervous system within the brainstem, classically the dorsal motor complex of the vagus nerve and sensory vagal afferents. Phox2b is expressed in all epibranchial placode-derived ganglia but is not expressed in neural crest–derived ganglia (Pattyn et al., 1997). The Phox2b expression domains relevant for studies of metabolism have been described by Elmquist and colleagues (Rossi et al., 2011; Vianna et al., 2012).

Male mice were obtained from Jackson Laboratories (Bar Harbor, ME) and bred according to previous reports on controlling gene dosage of *Cre. Cre* expression was restricted to male breeders for the *Phox2b* line and female breeders for the *Wnt1* line. All mice were born at the expected Mendelian ratios and appeared healthy. Intercrossing *Cre*-positive and *Cre*-negative *Glp1r loxP* heterozygotes from these 2 lines resulted in 6 genotypes: wild-type mice with no *Cre* (WT), mice homozygous for the *LoxP Glp1r* gene (*Glp1r*$^{Flox/Flox}$), wild-type mice expressing Cre recombinase (*Wnt1*-Cre2 or *Phox2b*-Cre) and *Glp1r*$^{Flox/Flox}$ mice expressing Cre recombinase (*Glp1r*$^{\Delta Wnt1-/-}$ or *Glp1r*$^{\Delta Phox2b-/-}$). As no significant differences in glucose or lipid homeostasis and food intake were observed in any of the control lines (Figure S2A-I), data from control lines were combined; *Glp1r*$^{\Delta Phox2b+/+}$ correspond to WT, *Glp1r*$^{Flox/Flox}$, and *Phox2b-Cre* mice generated from the *Phox2b* line, and *Glp1r*$^{\Delta Wnt1+/+}$ correspond to WT, *Glp1r*$^{Flox/Flox}$, and *Wnt1-Cre2* mice generated from the *Wnt1* line.

Mouse line characterization

The small intestine was divided into three sections: duodenum, jejunum and ileum. Each section was further sub-divided into the mucosa, the muscle layer (longitudinal and circular muscle and myenteric plexus) and the submucosa layer (muscularis mucosa and submucosal plexus). Regions of the brain were also isolated: Brainstem (BS), Hypothalamus (Hypo), Hippocampus (Hippo), Pituitary (Pit) and the Nodose Ganglion (NG). Total RNA was extracted from tissues using Tri Reagent (Molecular Research Center Inc., Cincinnati, OH). cDNA was synthesized from DNase I-treated (Thermo-Fisher Scientific, Markham, ON) total RNA (0.5-3 μg) using random hexamers and Superscript III (Thermo-Fisher Scientific, Markham, ON). Real-time PCR was carried out using a QuantStudio 5 System and TaqMan Gene Expression Assays (Thermo-Fisher Scientific, Markham, ON). Primer-probe sets were manufactured by Taqman® Assays-on-Demand (Applied Biosystems) to measure Exons 5-6 of *Glp1r*. Relative mRNA expression was normalized to levels of cyclophilin (*Ppia*) mRNA, and relative gene expression was expressed relative to *Glp1r* mRNA levels in BS (brain regions) or colon (gut regions).

Our data demonstrating diminished *Glp1r* expression in the hypothalamus and brainstem in *Glp1r*$^{\Delta Wnt1-/-}$ mice is consistent with *Wnt-1*-expression in the developing midbrain/hindbrain including the diencephalon (hypothalamus, thalamus, epithalamus), mesencephalon (midbrain), and myelencephalon (medulla oblongata), whereas *Glp1r* expression remains intact in other regions of the CNS including the hippocampus and pituitary, as well as in the nodose ganglia. Our data demonstrating reduction in *Glp1r* expression in the nodose ganglion in *Glp1r*$^{\Delta Phox2b-/-}$ mice is consistent with the established *Phox2b* expression pattern.

METHOD DETAILS

Gastric Emptying

Male mice were subjected to an acetaminophen (paracetamol) absorption test after fasting for 5-6 h (8-9am to 1-2pm) to assess the rate of gastric emptying. Vehicle (PBS), exendin-4 (1 nmol/kg BW), dulaglutide (0.05 mg/kg BW), or albiglutide (2 mg/kg BW) was administered via intraperitoneal (*i.p.*) injection just before (exendin-4) or 1h prior (albiglutide, dulaglutide) to oral administration of a glucose solution (2 g/kg BW) containing 1% (w/v) acetaminophen (#A7085, Sigma, Oakville, ON) administered at a dose of 100 mg/kg. Blood was collected from the tail vein into heparin-coated tubes before and 15, 30 and 60 minutes after acetaminophen

administration as described (Maida et al., 2008). Acetaminophen levels were measured in plasma using an enzymatic-spectrophotometric assay as a measure of gastric emptying (Acetaminophen-L3K, #506-30, Sekisui Diagnostics, Lexington, MA). This method is based on the principle that paracetamol absorption occurs completely in the small intestine, with negligible absorption in the stomach (Balkan et al., 1999). Therefore, the rate of gastric emptying is directly related to the appearance of paracetamol in the blood (Willems et al., 2001)

Food Intake and Body Weight

After overnight fasting (~16 h, from 5-6pm to 9-10am), mice were weighed, singly housed and given a pre-weighed amount of food (RC; 18% kcal from fat, 2018, Harlan Teklad, Mississauga, ON) with free access to water. Mice were *i.p.* injected with vehicle (PBS) or exendin-4 (10 nmol/kg BW) just before refeeding (9-10 am), or dulaglutide (0.05 mg/kg BW) or albiglutide (2 mg/kg BW) administered just before fasting (~5-6 pm). Food was then weighed 2, 4, 8 and 24 h after refeeding. For chronic treatment, vehicle (PBS) or dulaglutide (0.5 mg/kg BW) was administered by i.p. injections every other morning (9 am) and body weight monitored every day (9 am).

Lipid Tolerance Test

Mice were gavaged with 200 μL of olive oil after 5-6 h of fasting (from 8-9am to 1-2pm) and blood collected in heparin-coated capillary tubes before and 1, 2, and 3 hours post gavage for measurement of triglycerides (TG). Vehicle (PBS), exendin-4 (1nmol/kg BW), dulaglutide (0.05 mg/kg BW), or albiglutide (2 mg/kg BW) was administered via *i.p.* injection just before olive oil administration (exendin-4) or the night before the experiment (~5-6 pm, albiglutide, dulaglutide).

Glucose Tolerance Test

Mice were fasted for 5-6 h (from 8-9am to 1-2pm). For GLP-1R agonists, mice were *i.p.* injected with either vehicle (PBS), exendin-4 (1 nmol/kg BW, just before glucose administration), dulaglutide (0.05 mg/kg BW, 1 hour before glucose administration), or albiglutide (2mg/kg BW, 1 hour before glucose administration). Separate groups of mice were given vehicle (water) or a gut-selective (14 μg/mouse; ~300-400 μg/kg BW) or systemic dose (10 mg/kg BW) of sitagliptin (Mulvihill et al., 2017) by oral gavage 30 minutes before glucose administration. Oral or intraperitoneal glucose tolerance tests (OGTT and IPGTT, respectively) were carried out using 2 g/kg BW of glucose (20% solution). Blood glucose levels were assessed in tail vein blood using a hand-held glucometer (Contour glucometer, Bayer Healthcare, Toronto, ON) and blood was collected at 0 and 15 minutes after glucose administration in heparin-coated capillary microvette tubes. For measurement of total GLP-1, insulin and glucagon, blood was mixed with 10% TED (vol/vol) (5,000 KIU/ml Trasylol, 1.2 mg/ml EDTA and 0.1 nmol/l Diprotin A) and plasma was isolated after centrifugation (13,000 rpm 5 min 4°C) and stored at −80°C until further analysis.

Metabolic measurements

Plasma triglycerides (TG) were measured using an enzymatic assay (# 11877771 216, Roche Diagnostics, Indianapolis, IN) and the calibrator 464-01601 (Wako, Mountain View, CA). Insulin (80-INSMSU-E01, Alpco, Salem, NH), glucagon (10-1281-01, Mercodia, Winston Salem, NC), and total GLP-1 (K150JVC-2, Mesoscale, Rockville, MD) were measured in plasma samples obtained before and 15 minutes after glucose administration. Active GLP-1 was measured in gut homogenates from fasted mice using a Mesoscale assay (K150JWC-2) and normalized to protein content (Bradford assay). Plasma DPP-4 activity was measured in 10 μl of plasma from fasted animals using a fluorometric assay (substrate: 10 mM H-Gly-Pro-AMC HBr (Bachem #I-1225, Torrence, CA); standard: AMC (Bachem #Q-1025, Torrence, CA)).

QUANTIFICATION AND STATISTICAL ANALYSIS

Results are expressed as the mean ± SEM. Statistical comparisons were made by ANOVA followed by a Dunnet or Tukey post hoc, by a 2-way ANOVA followed by a Sidak post hoc, or by Student t test (when only 2 conditions) using GraphPad Prism 7. Statistically significant differences are indicated as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$. No method was used to determine whether the data met assumptions of the statistical approach. Statistical parameters and number of values per group can be found in the figure legends.

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Supplemental Information

Distinct Neural Sites of GLP-1R Expression Mediate Physiological versus Pharmacological Control of Incretin Action

Elodie M. Varin, Erin E. Mulvihill, Laurie L. Baggio, Jacqueline A. Koehler, Xiemin Cao, Randy J. Seeley, and Daniel J. Drucker

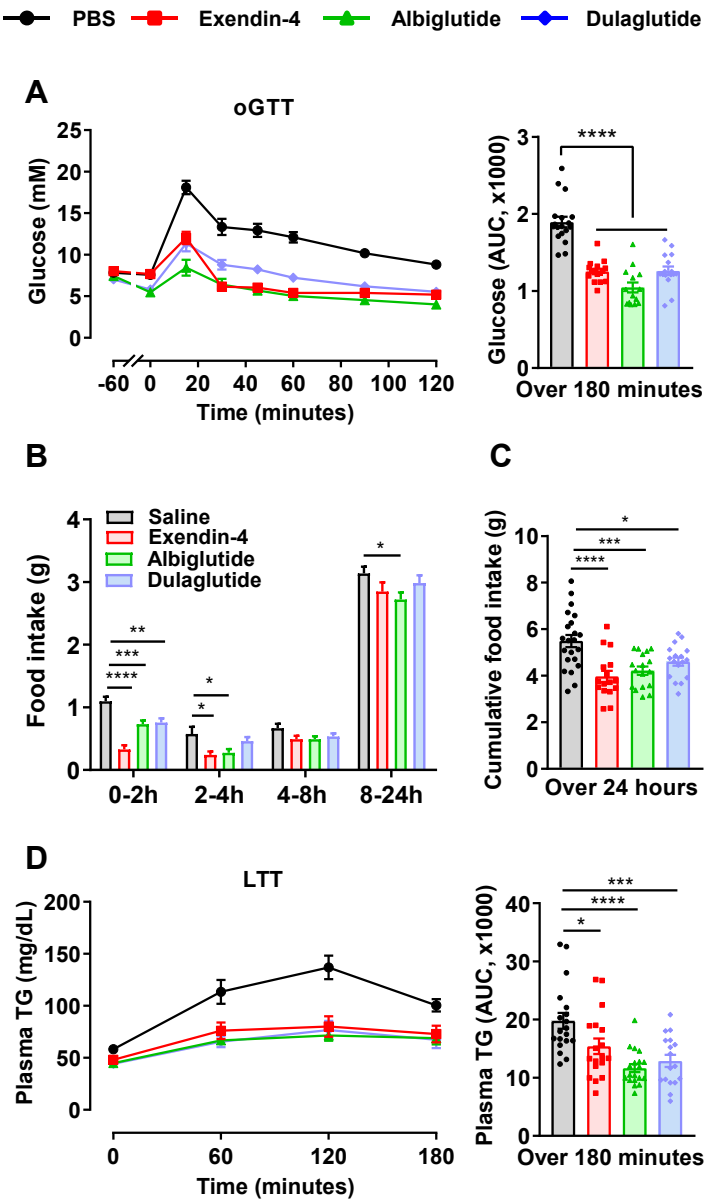

Figure S1. Both small and large molecule GLP-1R agonists improve glucose control, and reduce food intake and postprandial triglycerides excursion in WT mice, Related to Figures 2, 3, 5, 6 and 7. A: Blood glucose and AUC over 3 hours during an oral glucose tolerance test (oGTT) performed in 8-10 month-old chow diet fed wild-type (WT) male mice after a 5 hour fast and oral administration of 2mg/kg body weight (BW) glucose. Ex-4 (1 nmol/kg BW) was administered by ip injection right before glucose gavage, albiglutide (2 mg/kg BW) and dulaglutide (0.05 mg/kg BW) were administered by ip injection 1h before glucose gavage (n=16-18 animals / group). **B-C**: Non-cumulative food intake (B) and cumulative food intake over 24 hours of refeeding (C) following an overnight fast in response to ip administration of Exendin-4 (10 nmol/kg, just before refeeding), albiglutide (2 mg/kg, just before fasting), or Dulaglutide (0.05 mg/kg, just before fasting) in 8-10 month-old chow diet fed WT mice (n=16-18 animals / group). **D:** Plasma triglycerides (TG) and AUC before and after oral gavage of olive oil (200µl per mouse) in 5h fasted 8-10 month-old chow diet fed WT male mice. Treatments were similar to the glucose study in A (n=16-18 animals / group). Data are presented as mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001 by one-way ANOVA.

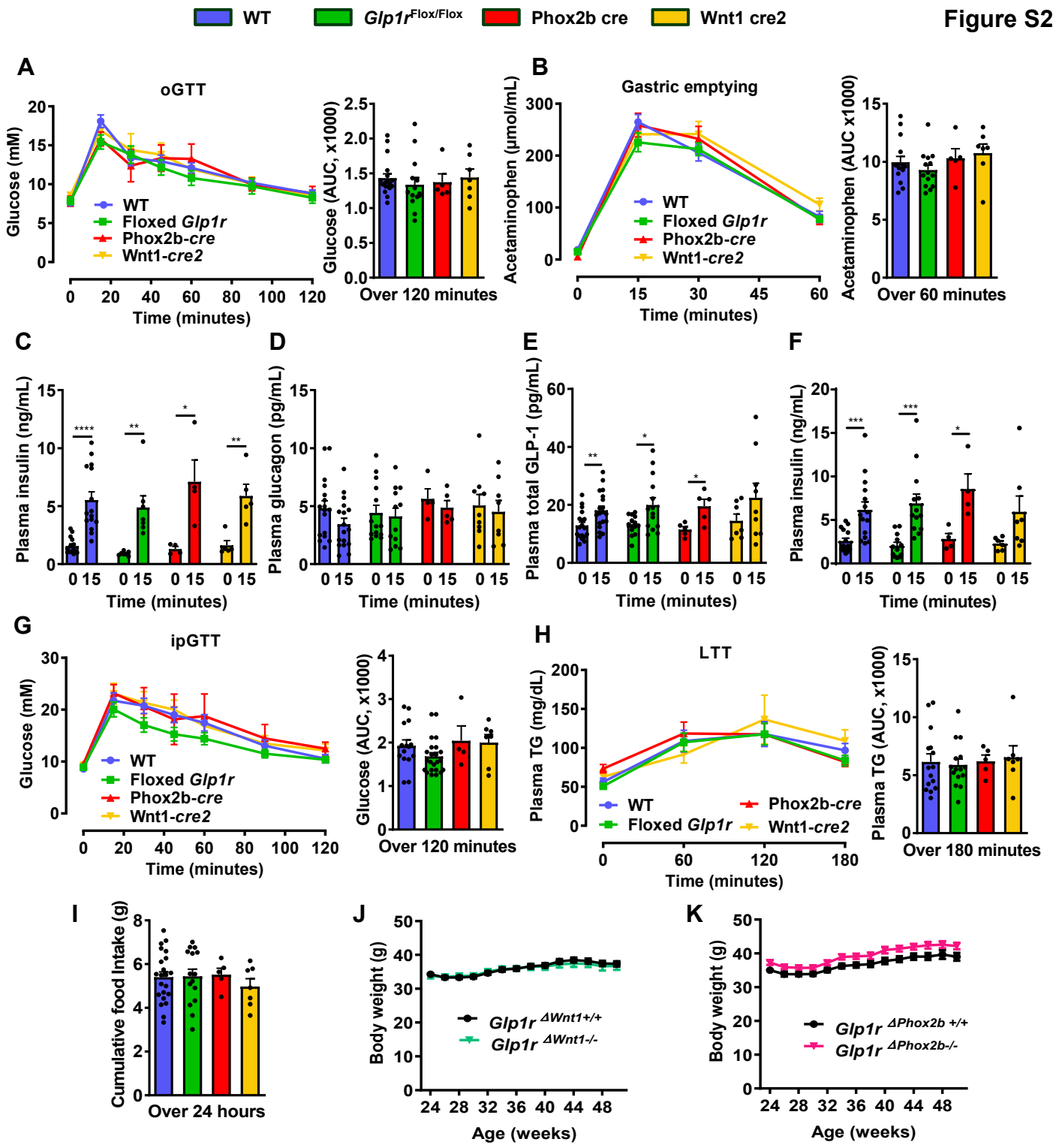

Figure S2. Comparable metabolic phenotype of WT, *Glp1*$^{Flox/Flox}$, *Wnt1-cre2* and *Phox2B-cre* mice, Related to Figures 1 and 4 and STAR Methods. A-E: Blood glucose and AUC (A), plasma acetaminophen (as a measure of gastric emptying) and AUC (B), plasma insulin (C), glucagon (D) and total GLP-1 (E) before and 15min after glucose administration during oral glucose tolerance test (oGTT) (2 g/kg body weight (BW)) in wild-type (WT), *Glp1r*$^{flox/flox}$, Phox2b-cre and Wnt1-cre2 8-10 month-old male mice after a 5h fast. **F-G:** Plasma insulin levels before and 15 min after glucose administration (F) and blood glucose and AUC (G) and during an ip glucose tolerance test (ipGTT) (2 g/kg BW) in WT, *Glp1r*$^{flox/flox}$, Phox2b-cre and Wnt1-cre2 8-10 month-old male mice after a 5h fast. **H:** Plasma triglyceries (TG) and AUC before and after oral gavage of olive oil (200 µl/mouse) during a lipid tolerance test (LTT) in WT, *Glp1r*$^{flox/flox}$, Phox2b-cre and Wnt1-cre2 8-10 month-old male mice after a 5h fast. **I:** Cumulative food intake over 24 hours after refeeding (after an overnight fast). **J-K:** Body weight overtime in *Glp1r*$^{\Delta Wnt1+/+}$ vs *Glp1r*$^{\Delta Wnt1-/-}$ and in *Glp1r*$^{\Delta Phox2b+/+}$ vs *Glp1r*$^{\Delta Phox2b-/-}$. Data are presented as mean ± SEM for n=15 WT, n=7 *Glp1r*$^{Flox/Flox}$, n=4 Phox2b-cre, n=5 Wnt1-Cre2.

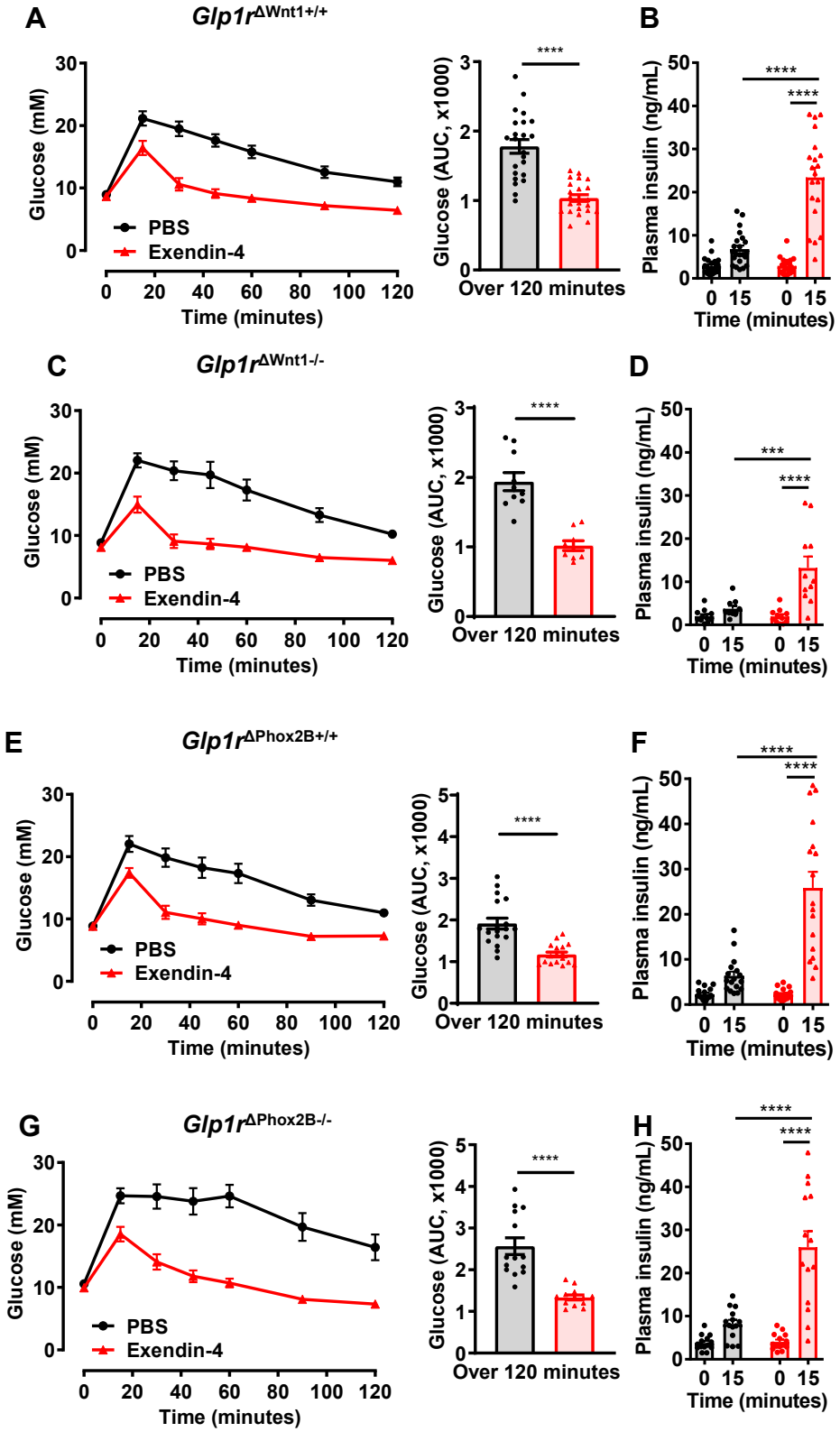

Figure S3. Both *Glp1r*$^{\Delta Phox2b-/-}$ and *Glp1r*$^{\Delta Wnt1-/-}$ mice have functional GLP-1R in β-pancreatic cells, Related to Figures 2 and 5. Blood glucose and AUC (A,B,E,G) and plasma insulin (B,D,F,H) before and 15 minutes after glucose administration (2 g/kg body weight (BW)) during an intraperitoneal GTT (ipGTT) in 5-hour fasted *Glp1r*$^{\Delta Wnt1+/+}$ (A,B), *Glp1r*$^{\Delta Wnt1-/-}$ (C,D), *Glp1r*$^{\Delta Phox2b+/+}$ (E,F) and *Glp1r*$^{\Delta Phox2b-/-}$ (G,H) 8-10 month-old male mice treated with water or Exendin-4 (1 nmol/kg BW) (n=10-13 animals / group). Data are expressed as means ± SEM. *** p<0.001 and ****p<0.0001 by t-test (A,C,E,G) or by two-way ANOVA (B,D,F,H).