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The GIPR is predominantly localized to non-adipocyte cell types within white adipose tissue

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Abstract

The incretin hormone glucose-dependent insulinotropic polypeptide (GIP) augments glucose-dependent insulin secretion through its receptor expressed on islet β-cells. GIP also acts on adipose tissue, yet paradoxically, both enhanced and reduced GIP receptor (GIPR) signaling reduce adipose tissue mass and attenuate weight gain in response to nutrient excess. Moreover, the precise cellular localization of GIPR expression within white adipose tissue (WAT) remains uncertain. Here, we used mouse genetics to target *Gipr* expression within adipocytes. Surprisingly, targeting Cre expression to adipocytes using the Adiponectin (*Adipoq*) promoter did not produce meaningful reduction of WAT *Gipr* expression in *Adipoq-Cre:Gipr*$^{flx/flx}$ mice. In contrast, adenoviral expression of *Cre* under the control of the CMV promoter, or transgenic expression of *Cre* using non-adipocyte-selective promoters (*Ap2*/*Fabp4* and *Ubc*) markedly attenuated WAT *Gipr* expression. Analysis of single nucleus RNA-seq adipose tissue data sets localized *Gipr*/*GIPR*

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expression predominantly to pericytes and mesothelial cells rather than to adipocytes. Together, these observations reveal that adipocytes are not the major GIPR+ cell type within WAT, findings with mechanistic implications for understanding how GIP and GIP-based co-agonists control adipose tissue biology.

Keywords

Glucose-dependent insulinotropic polypeptide receptor; adipose; obesity; adiponectin; incretin; adipocyte

Incretin hormones are produced in specialized enteroendocrine cells and amplify meal-stimulated insulin release following food ingestion. The two incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) exert their actions through structurally related yet distinct receptors (1). The major target for incretin action is the islet β-cell, wherein GIP and GLP-1 potentiate insulin secretion through cyclic AMP-dependent and independent pathways (1).

The molecular cloning of incretin receptor cDNAs enabled identification of extrapancreatic expression of both incretin receptors, consistent with characterization of multiple actions for GIP and GLP-1 beyond the β-cell (1; 2). GLP-1 receptor (GLP1R) mRNA transcripts have been identified within the central and enteric nervous systems, the heart, gastrointestinal tract, kidney, blood vessels and immune cells (3–5), lending support for multiple direct actions of GLP-1 in peripheral tissues. Similarly, the tissue distribution of GIP receptor (GIPR) mRNA transcripts includes the brain, heart, gastrointestinal tract, blood vessels, and adipose tissue (6).

Among key differences in the extrapancreatic biology of incretin hormones, GIP, but not GLP-1, acts directly on adipose tissue (1; 7). These actions of GIP have been studied using differentiated adipose tissue cell lines, primary adipocyte cultures and experiments with animals and humans (8–14). Within white adipose tissue (WAT), GIP promotes both lipolysis and lipid accretion, glucose uptake, insulin sensitization, and adipokine expression (12; 13; 15–20). GIP also modifies adipose tissue biology through actions on blood vessels, and immune cells. For instance, GIP rapidly augments adipose tissue blood flow (21) and either augments or suppresses WAT inflammation through direct actions on immune cells (13; 18; 22; 23).

Interpreting the actions of GIP on adipose tissue has been complicated by paradoxical observations that both sustained GIPR agonism and attenuation of GIPR signaling produce overlapping phenotypes in animals, including reduction of WAT inflammation, reduced WAT mass, resistance to weight gain and improvement of insulin sensitivity (7; 18; 24–31). Understanding how gain or loss of GIP actions within unique adipose tissue GIPR+ cell types has been hampered in part by i) the lack of highly specific validated antisera or labelled analogues for detection of the GIPR (32; 33), and ii) a paucity of *in situ* hybridization or single cell RNA-seq data for detection of the WAT GIPR. Hence, the cellular localization of GIPR in different adipose tissue depots remains incompletely understood.

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In view of multiple studies linking expression of GIPR to adipocytes, we attempted to understand the metabolic consequences of targeting the adipocyte *Gipr* using mouse genetics. Surprisingly, although expression of Cre under control of the well characterized adiponectin promoter recombined *Gipr* genomic DNA in WAT, we did not observe meaningful reduction of *Gipr* expression in WAT depots of *Adipoq-Cre:Gipr*$^{flx/flx}$ mice. In contrast, expression of Cre recombinase under the control of the human adipocyte fatty acid binding protein (Ap2) *Fabp* promoter, or using *Ubc*-CRE^{ERT2} to direct widespread Cre expression, resulted in marked reduction of WAT *Gipr* mRNA transcripts in multiple WAT depots. Consistent with these findings, *Gipr*-directed reporter expression was not detected within the majority of adipocytes analyzed using a *Gipr*-Cre mouse to identify transcriptional domains of endogenous *Gipr* promoter activity. Finally, publicly available scRNA-seq data identified WAT *Gipr*/*GIPR* expression predominantly within pericytes and mesothelial cells (34). Taken together, these findings refine our understanding of WAT *Gipr* expression, providing insights for guiding interpretation of data linking GIP action to changes in adipose tissue biology.

Methods

Animal Models

Gipr$^{flx/flx}$ (35) *Adipoq*-Cre (36), *Adipoq*BAC-Cre (Jax Stock#: 028020, (37)), *Mip*-Cre^{ERT} (Jax Stock#: 024709, (35; 38), *αMHC*-Cre^{ERT} (32), *Ubc-Cre*^{ERT2} (Jax Stock#: 008085, (39)), *Adipoq*BAC-Cre^{ERT} (Jax Stock#: 024671, (40)), and *Fabp4(Ap2)*-Cre (Jax Stock #: 005069, (41)) mice have been previously described. *Gipr*-Cre (knock-in) mice were generated using CRISPR/Cas9 as described (42) and bred with Rosa26-LacZ (B6;129S4-Gt(ROSA)26Sortm1Sor/J; #003309, The Jackson Laboratory, Bar Harbor, ME) or Rosa29-mTmG (Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J, #007576, The Jackson Laboratory, Bar Harbor, ME) reporter mice. Male mice were used for all studies.

PCR analysis

RNA isolation and qPCR were carried out as previously described (43). PCR of genomic DNA was done using PrimeSTAR GXL DNA Polymerase (Cat# R050A). Primer sequences are described in the online supplementary Table.

In vivo studies

All animal studies were conducted under protocols approved by the Animal Care Committees of the Toronto Centre for Phenogenomics, the Duke Molecular Physiology Department, and the University of Cambridge Animal Welfare and Ethical Review Body and conformed to the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039). Briefly, intraperitoneal (ip) and oral glucose tolerance tests were performed in animals following a 5-hour fast using 1.5 g/kg glucose. For ip glucose tolerance tests, animals were given ip PBS or D-Ala GIP (4 nmol/kg, Chi Scientific) 10 minutes prior to glucose. Insulin tolerance was measured following administration of 0.7 U/kg Humalog in mice fasted for 5 hrs. High fat diet (HFD) feeding utilized a 45% fat diet from Research Diets (D12451). Body composition was measured with EchoMRI.

In vivo adenovirus treatment

An adenoviral vector containing the human adenovirus type5 (dE1/E3) viral backbone encoding both Cre recombinase and GFP through separate CMV promoters was a kind gift from Dr. Andras Nagy (Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto). The virus was used at a titer of $1x10^{10}$ PFU/ml and a total of 50 ul was injected directly into a single inguinal fat pad in mice lightly anesthetized with isoflurane. Virus containing an empty vector was injected at the same amounts into the contralateral inguinal fat pad. Mice were sacrificed 72 hrs later and both inguinal fat pads were harvested, flash frozen, and stored at -80C until used for RNA analysis.

HFD feeding of Ap2-Cre mice

Littermate, age-matched controls were weened at 3-4 weeks of age and maintained on standard rodent chow until 8-weeks of age. The diet was then switched to a 45% fat diet and the mice were maintained on this diet until study termination.

Whole-mount β-galactosidase assay:

Tissues were harvested from 11-month-old male mice that were hemizygous for *Gipr*-Cre and heterozygous for *ROSA*26-LacZ or heterozygous for *ROSA*26-LacZ (negative control), rinsed in PBS and transferred to 6-well plates where they were fixed for 2 h (calcium- and magnesium-free PBS containing 1% paraformaldehyde, 0.2% glutaraldehyde, and 0.02% Nonidet P-40) at 4°C using an orbital shaker. Samples were then washed twice (20 min each) in PBS and incubated in the dark overnight (16 h) at 37°C in β-galactosidase substrate (calcium- and magnesium-free PBS containing 5mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM magnesium chloride, 0.02% NP-40, 0.01% sodim deoxycholate, and 1 mg/ml X-gal substrate). The following day, samples were rinsed twice in PBS as above, fixed in 10% neutral buffered formalin overnight at 4°C and transferred to 70% ethanol until imaging. Whole mount tissues were imaged using a Leica MZ6 stereomicroscope with an attached MC170 HD digital camera (Leica Microsystems Inc., Concord, ON).

Whole-mount confocal microscopy

Adipose tissues were harvested from 14-week-old female mice that were hemizygous for Gipr-Cre and heterozygous for mTmG or heterozygous for mTmG (negative control), rinsed with PBS, cut into 0.5-1 cm pieces, transferred to 12-well plates and fixed in 1% paraformaldehyde for 1 h at room temperature. Tissues were then washed 3 times (10 min each) in PBS containing 0.3% Triton X-100, followed by an additional 3 washes (10 min each) in PBS and then incubated in Lipidtox Deep Red (1:1000 in PBS; #H34477, Thermo Fisher Scientific, Mississauga, ON) for 30 min at room temperature. Tissues were placed on a glass coverslip and saturated with DAPI-containing mounting media (Vectashield, #H-1200, Vector Labs Inc., Burlington, ON) and imaged using an inverted confocal laser microscope.

Single nucleus RNA-seq analysis

Detection of *Gipr*/*GIPR* and other class B GPCR mRNA transcripts within mouse and human adipose tissue depots was accomplished using publicly available data derived from single nucleus RNA-seq analyses as described (34), accessed from the Broad Institute single cell portal (https://singlecell.broadinstitute.org/single_cell).

Statistics

Data is presented as means ± SEM. Differences were determined by Student's t-test, one-way ANOVA, or two-way ANOVA, as appropriate. A Tukey's test was used for post-hoc analysis of ANOVAs, where appropriate. $P<0.05$ was set as the criteria for statistically significant difference.

Results

*Gipr*mRNA transcripts are not reduced in adipose tissues from *Adipoq-Cre:Gipr*$^{flx/flx}$mice

Several studies have reported targeting of murine *Gipr* expression in WAT, however the extent of *Gipr* knockdown within adipocytes in vivo was not described (41; 44). Nevertheless, based on reports localizing *Gipr* expression to adipocytes (44–46), we sought to inactivate adipocyte *Gipr* expression using the widely used Adiponectin-Cre system (47). Accordingly, we generated *Adipoq-Cre:Gipr*$^{flx/flx}$ mice by crossing *Gipr*$^{flx/flx}$ mice (35; 48) with mice expressing *Cre* driven by the *Adipoq* promoter (36). Surprisingly, levels of *Gipr* mRNA transcripts in WAT or brown adipose tissue (BAT) from *Adipoq-Cre:Gipr*$^{flx/flx}$ mice were not reduced (Figure 1A). We next generated a second mouse model utilizing an independently generated *Adipoq-Cre* mouse that uses a BAC transgene containing the majority of the *Adipoq* regulatory elements (*AdipoqBAC-Cre)* (37), a mouse line successfully used by multiple groups to achieve adipocyte-selective gene recombination (49), including our own lab (50). Unexpectedly, *AdipoqBAC-Cre:Gipr*$^{flx/flx}$ mice also failed to exhibit reduced *Gipr* expression in WAT and BAT (Figures 1B, C), despite expressing *Cre* and *Adipoq* at levels similar to WT and *AdipoqBAC-Cre* control mice (Figure 1B). Collectively these findings indirectly imply that the majority of Adiponectin+ adipocytes do not express the *Gipr* within adipose tissue in vivo.

We previously achieved reduction of *Gipr* expression, using the same *Gipr*$^{flx/flx}$ mice, in β-cells (35), cardiomyocytes (32) and brown adipose tissue (48) using *Mip-Cre*^{ERT}, *αMHC-Cre*^{ERT} and *Myf5-Cre* mice, respectively. To explain the lack of *Gipr* knockdown in WAT, we examined relative *Cre* expression across different mouse models. WAT *Cre* expression driven by the *AdipoqBAC* promoter was comparable to levels seen in primary islets, driven by *Mip*, but lower than levels detected in the heart driven by *αMHC*. (Figure 1D). *Gipr* mRNA transcript levels were reduced in heart and islet tissues of *αMHC-Cre:Gipr*$^{flx/flx}$ and *Mip-Cre:Gipr*$^{flx/flx}$ mice, respectively (Figure 1D), consistent with previous observations (32; 35). In contrast, *Gipr* mRNA transcripts were not reduced in adipose tissue depots from *AdipoqBAC-Cre:Gipr*$^{flx/flx}$ mice (Figure 1D).

Gipr expression can be reduced inwhite adipose tissue of *Gipr*$^{flx/flx}$ mice through non-adipocyte selective*Cre* expression

The failure to knock down WAT *Gipr* mRNA transcript levels using *Adipoq*-Cre raised several possibilities. First, we hypothesized that the adipocyte *Gipr* gene may be uniquely inaccessible to Cre within adipocytes, thereby preventing Cre-mediated recombination of the floxed alleles. Alternatively, we surmised that cellular *Gipr* expression may be inversely correlated to *Adipoq* expression, implying that the *Gipr* may not be expressed in the majority of white adipocytes. To examine these possibilities, we injected a *Cre*-expressing adenovirus directly into the inguinal adipose depot of *Gipr*$^{flx/flx}$ mice as a means of producing widespread expression of Cre within WAT (51). Mice injected with *Ad-CMV(GFP)-Cre* exhibited a 70% knockdown in inguinal adipose tissue *Gipr* expression (Figure 2A), illustrating the susceptibility of the *Gipr* allele within WAT of *Gipr*$^{flx/flx}$ mice to recombination and subsequent reduction of WAT *Gipr* mRNA transcripts.

To obtain complementary evidence supporting these observations, we used *Ubc-Cre*^{ERT2} mice, which express *Cre* under the control of the human ubiquitin C promoter in most cell types (39). Consistent with loss of the insulin-stimulating actions of GIP in *Gipr*$^{\beta cell-/-}$ mice (35), tamoxifen-treated *Ubc-Cre*^{ERT2}:*Gipr*$^{flx/flx}$ mice failed to exhibit reduction of glucose levels in response to exogenous GIP (Figure 2B). Importantly, levels of *Gipr* mRNA transcripts were markedly reduced in the islets, brain and WAT of *Ubc-Cre*^{ERT2}*:Gipr*$^{flx/flx}$ mice (Figure 2C). Thus, both *Cre*^{ERT2} and Ad-CMV-*Cre* can drive *Cre* expression enabling recombination of the *Gipr* gene and reduction of *Gipr* mRNA transcripts in WAT from *Gipr*$^{flx/flx}$ mice.

AdipoqBAC-Cre mice exhibit recombination of the genomic*Gipr* locus in WAT, without reduced adipocyte *Gipr* expression

Ubc-Cre^{ERT2} mice require tamoxifen to induce Cre recombinase activity, whereas the *AdipoqBAC-Cre* mice (Figure 1B-D) exhibit constitutive expression of Cre recombinase, including during development. We wondered whether postnatal induction of Cre expression may confer preferential recombination of floxed adipocyte *Gipr* alleles. To assess this possibility, we crossed *Gipr*$^{flx/flx}$ mice with *AdipoqBAC-Cre*^{ERT} mice (40), which require tamoxifen for conditional induction of Cre activity. Following the same tamoxifen protocol used for the *Ubc-Cre*^{ERT2} model (Figures 2B, C), treatment of *AdipoqBAC-Cre*^{ERT}*:Gipr*$^{flx/flx}$ mice with tamoxifen at 8-weeks of age failed to reduce epididymal *Gipr* expression (Figure 2D). However, PCR analysis of epididymal adipose tissue DNA using primers that span both loxP sites demonstrated that the Cre recombinase effectively induced recombination of the *Gipr* genomic DNA (Figure 2E). In control adipose tissue samples (WT or *Gipr*$^{flx/flx}$), only the full length genomic DNA PCR product was amplified (no recombination). In contrast, both the full length and Cre-generated products were amplified in genomic DNA from *AdipoqBAC-Cre*^{ERT}*:Gipr*$^{flx/flx}$ adipose tissue, and only the truncated Cre-generated PCR product was amplified in genomic DNA from *Ubc-Cre*^{ERT}*:Gipr*$^{flx/flx}$ adipose tissue (Figure 2E). Thus, the *Adipoq* promoter is capable of generating sufficient *Cre* expression to permit recombination of the *Gipr* allele, yet does not alter *Gipr* mRNA levels within WAT.

Ap2-Cre:Gipr$^{flx/flx}$ mice exhibit reduced*Gipr* expression in adipose tissue and brain

Several metabolic phenotypes were described for mice with *Gipr* knockdown in adipose tissue generated using *Ap2-Cre* mice (41), including a modest reduction in body weight after HFD feeding, together with improved glucose tolerance, and reduced hepatic steatosis. Intriguingly, the reductions in body weight were driven by reduced lean mass, not fat mass. Although *Gipr* expression was markedly reduced in visceral and subcutaneous adipose tissue from *Gipr*$^{adipo-/-}$ mice (41), *Ap2/Fabp4* expression is not limited to adipocytes, as the *Ap2/Fab4* promoter is transcriptionally active in heart, muscle, brain, macrophages, endothelium and testis (47; 52; 53). We examined *Gipr* expression in adipose tissue depots and brain regions of independently generated *Ap2-Cre:Gipr*$^{flx/flx}$ mice. *Gipr* transcripts were reduced in all adipose depots examined (Figure 3A), as well as in the hypothalamus, hippocampus, and cortex (Figure 3A). *Ap2-Cre:Gipr*$^{flx/flx}$ mice weighed less at 8-weeks of age (Figure 3B) and their weight remained below that of control mice throughout the HFD feeding period (Figure 3C). However, the rate of weight gain between groups in response to HFD feeding was similar (Figure 3D), as was body composition after 16-weeks of HFD feeding (Figure 3E). Moreover, glucose tolerance (Figure 3F), insulin tolerance (Figure 3G), energy expenditure (Figure 3H), food intake (Figure 3I), and tissue weights (Figure 3H) were similar, although liver weight trended lower (p=0.055). Thus, while reduction of *Gipr* in adipose tissue depots with *Ap2-Cre* is associated with modest changes in body weight, these findings cannot be directly attributed to reductions in adipose tissue *Gipr* expression, as the concurrent reduction in brain *Gipr* expression confounds attribution of phenotypes to adipose tissue in this mouse model.

Expression of *Gipr-Cre* demonstrates heterogeneity of reporter protein expression within adipose tissue

To reconcile our inability to reduce WAT *Gipr* expression with multiple *Adipoq-Cre* driver lines, we used *Gipr-Cre* to direct reporter protein expression. Crossing *Gipr-Cre* mice with a *Rosa26-LacZ* reporter line (54) produced abundant β-galactosidase activity in the pancreas, yet with little activity detected in WAT depots, including staining localized to blood vessels (Figure 4). We next crossed *Gipr-Cre* mice with a *Rosa26-mT/mG* reporter line (55). Confocal microscopy of tissues from these mice demonstrated *Gipr* promoter activity in the pancreas, in line with islet GIPR expression (Figure 4). *Gipr* promoter activity within WAT was much more heterogeneous, with a definitive signal seen in only a small fraction of putative adipocytes within multiple WAT depots.

Single nucleus RNA-seq localization of *Gipr/GIPR* expression in adipose tissue

Collectively, the genetic findings in mice imply that *Gipr* expression within WAT is predominantly localized to non-adipocyte cell types. To further interrogate this possibility, we analyzed *Gipr/GIPR* expression within distinct cell types of mouse and human inguinal and perigonadal adipose tissue using independently generated publicly available single nucleus RNA-seq data (34). Within mouse adipose tissue, *Gipr* mRNA was detected within *Pdgfrb*^{High} pericytes, with minimal expression detected in *Adipoq*+ adipocytes (Figure 5A-D). *Glp1r* was virtually absent in mouse adipose tissue (Figure 5E); intriguingly, *Glp2r* was found in adipose stem and progenitor cells (ASPCs) and macrophages (Figure 5F). *Gcgr*

showed an expression pattern similar to *Gipr* and was localized to pericytes (Figure 5G).) Similarly, scRNA-seq analysis of human subcutaneous and visceral adipose tissues detected *GIPR* primarily in *PDGFRB*^{High} pericytes followed by *WT1*$^{+}$ mesothelial cells, but not in *ADIPOQ*^{High} adipocytes (Figure 6A-E). Among the related class B G protein-coupled receptors (GPCRs), only *GLP2R*, but not *GLP1R* and *GCGR*, was detected in some human ASPCs and adipocytes (Figure 6F-H). Hence, the available RNA-seq data is sufficiently useful for detection of GIPR and related class B GPCR mRNA transcripts within various adipose tissue cell types, independently highlighting the lack of GIPR expression within the majority of mouse or human adipocytes

Discussion

Our current findings have implications for interpreting studies of GIP biology in adipose tissue. First, using independent mouse lines, we find that the murine *Gipr* is not expressed within the majority of adipocytes. Second, consistent with these findings, expression of Cre recombinase under control of the *Adiponectin* promoter does not meaningfully reduce *Gipr* expression in multiple adipose tissue depots. Third, interpretation of data generated using non-adipocyte-selective promoters to target adipose tissue *Gipr* expression, exemplified by *Ap2-Cre* (41), may be confounded by reduction of *Gipr* expression in multiple non-adipocyte cell types, including immune, neuronal, and endothelial cells (47). Although relative levels of *Gipr* mRNA transcripts were reported as normal in the brain of *Gipr*$^{adipo-/-}$ mice (41), our analyses using the same *Ap2* promoter to express *Cre* and inactivate the *Gipr,* reveal substantial reduction of *Gipr* mRNA transcripts in multiple regions of the murine central nervous system known to impact systemic metabolism. Taken together, these findings are consistent with a substantial proportion of adipose tissue GIPR expression arising within non-adipocyte lineages.

In agreement with interpretation of the data obtained using genetic approaches *in vivo*, the single nucleus RNA-seq data provides further support for the concept that mouse and human adipocytes are not major sites of canonical *Gipr*/*GIPR* expression. Indeed, pericytes appear to be a putative GIPR-expressing cell type in both human and mouse WAT, and additional human WAT *GIPR* expression is identified in mesothelial cells. These findings have implications for interpretation of the existing literature describing mechanisms of GIP action in adipose tissue and may generate new hypotheses surrounding the actions of GIPR within WAT cell types that contribute to the biology of adipose tissue development and function.

The importance of understanding the biology of the adipose tissue GIPR and its impact has accelerated in part due to translational interest in targeting the GIPR for the treatment of obesity and diabetes (7). Indeed, GIPR agonism reduces food intake, body weight and fat mass in HFD-fed mice, through mechanisms requiring central nervous system GIP receptor activation (56). A GIP-GLP-1 co-agonist LY3298716, subsequently renamed tirzepatide, robustly stimulated cAMP accumulation in adipocyte-like cells derived from progenitors differentiated ex vivo, reduced food intake and adipose tissue mass, and produced substantial weight loss in both preclinical and clinical studies (57). Moreover, tirzepatide augmented adipose tissue glucose uptake and enhanced insulin sensitivity in a GLP-1R-independent

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manner in mice (58). Remarkably, GIPR blockade with antibodies directed against the mouse or human GIPR also reduced fat (WAT) mass, blocked the actions of exogenous GIP on human adipocytes ex vivo, and attenuated weight gain, without changes in lean mass in mice and non-human primates (27; 31). Reconciliation of how both gain and loss of function at the GIPR produce overlapping effects on body weight, WAT mass and function requires a more detailed understanding of how GIP controls metabolism and adipose tissue biology.

The results of several previous studies examining GIP action in adipose tissue have yielded conflicting results, with some studies demonstrating that GIP acts directly on WAT, yet other experiments invoke a role for GIP as an insulin sensitizer on adipocytes, using cells differentiated from adipocyte progenitors ex vivo (11; 59; 60). Indeed, the very slow kinetics of the adipose tissue response to GIP (60) have prompted the suggestion that GIP might act indirectly on adipocyte lipid metabolism, through one or more downstream mediators such as insulin or resistin (35; 61; 62). Nevertheless, substantial data suggest that adipocyte-like cells studied *ex vivo* express a functional GIPR coupled to cAMP accumulation and fatty acid uptake (44; 58).

Previous studies using mouse genetics to interrogate the role of the adipocyte GIPR have been partially inconclusive. For example, transgenic targeting of GIPR expression to WAT of $Gipr^{-/-}$ mice using the *Ap2/Fabp4* promoter produced weight gain independent of changes in fat mass, without any meaningfully evident metabolic phenotypes (63). Conversely, reduction of WAT *Gipr* mRNA transcripts using the Ap2*/Fabp4* promoter to direct *Cre* expression to several cell types, including adipocytes, reduced WAT *Gipr* expression in mice with lower body weight and decreased lean body mass, yet without change in fat mass (41). More recent studies using *Adipoq-Cre* to target the mouse adipocyte *Gipr* revealed loss of *Gipr* expression in adipocytes differentiated ex vivo, together with reduced GIP-stimulated cAMP accumulation, and decreased fatty acid uptake (44). Notably, however, the levels of *Gipr* mRNA within WAT depots from $Gipr^{Adipo-/-}$ mice were not reported.

The growing importance in understanding the actions of GIP in WAT is further augmented by interest in the mechanisms of action of tirzepatide (64). Administration of tirzepatide or a long-acting GIPR agonist improved insulin sensitivity, associated with enhanced glucose uptake into white adipose tissue (58). Interestingly however, RNA-seq analysis of WAT from mice treated with tirzepatide or a long-acting GIPR agonist showed no changes in metabolic gene expression within WAT depots, whereas a GIPR agonist and tirzepatide differentially regulated >1,000 genes within brown adipose tissue (58). These latter findings are consistent with a functional role for the canonical murine GIPR in regulation of genes important for thermogenesis, lipid metabolism, and cytokine expression in brown adipose tissue (48; 62).

Limitations and future perspectives

Our data requires interpretation with caution due to a number of important limitations. First, we focused almost entirely on mRNA expression, due to the lack of suitably validated antisera for detection of the mouse GIPR (32; 33). Data from experiments using reporter genes to infer expression has caveats, as the readouts may reflect activation of transcriptional sequences in one or more early adipose tissue lineages that subsequently give rise to differentiated adipocytes. Hence, whether reporter gene expression within a few adipocytes

coincides with simultaneous co-expression of the *Gipr* mRNA transcript or protein in the same differentiated cell remains uncertain. Although the scRNA-seq and gene targeting data align with the concept that mouse adipocyte *Gipr* expression is uncommon, we did not study adipose tissue depots from a wide range of mice with metabolic perturbations. For example, animals with diabetes, insulin deficiency or resistance, or obesity, might exhibit upregulation of adipocyte *Gipr* expression, scenarios that require additional investigation.

It is also worth noting that low level adipocyte expression of class B GPCRs such as the *Glp1r* or *Gipr* might not easily be detected using thresholds set for scRNA-seq, although the same analyses successfully detected *Gipr* and *Glp2r* mRNAs in non-adipocyte cell types within adipose tissue (34). Finally, our data does not rule out an important role for GIPR activity in adipose tissue. The small fraction of mature GIPR+ adipocytes identified by scRNAseq could represent a key subset of cells that contribute to regulation of overall adipose tissue function through paracrine or endocrine processes. Alternatively, the colocalization of *Gipr/GIPR* with *Pdgfrb/PDGFRB* populations may represent early precursor cells, potentially suggesting a role for GIP in preadipocyte function. Finally, there is much less information available on the cellular localization of GIPR in human adipose tissue depots across the lifespan, in people living with diabetes or obesity, hence the putative importance of adipocyte expression of the human GIPR requires greater scrutiny. In summary, our data introduce further complexity in conceptualizing how gain or loss of GIPR signaling impacts adipose tissue biology and adipocytes in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Daniel J. Drucker is the guarantor for this manuscript and takes full responsibility for the authenticity of the data presented herein.

Data Resource Sharing and Availability

The original data in this paper is available upon reasonable request from the authors. The *Gipr* floxed mice are available for collaborative sharing from the authors (DJD). All of the other mouse lines and reagents described herein are available from commercial sources.

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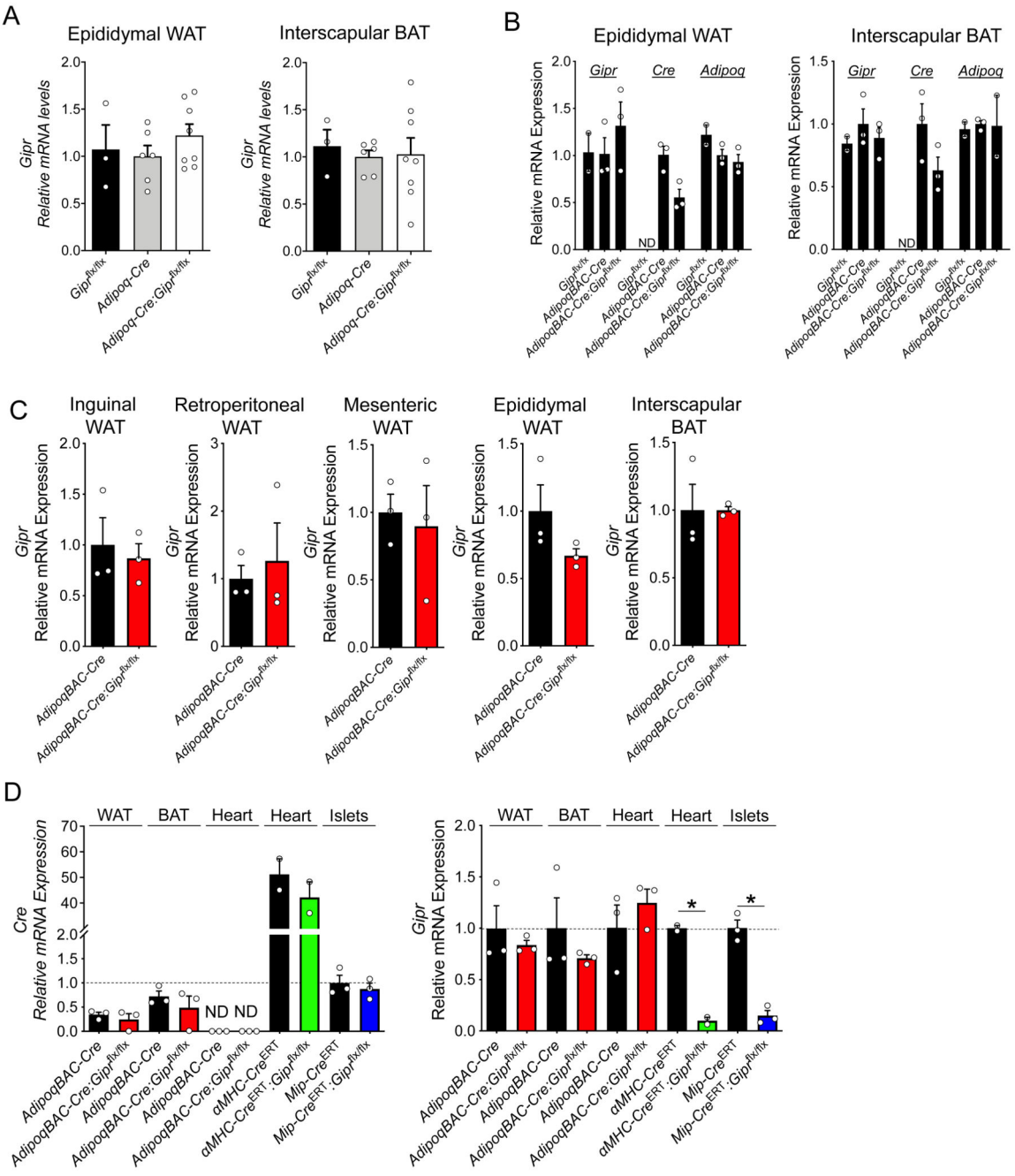

Figure 1. Cre recombinase expression driven by the adiponectin promoter does not reduce *Gipr* mRNA transcripts in adipose tissue.

A) *Gipr* expression in epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT). *Cre* expression is driven by transgenic expression of *Adipoq-Cre* (36). (n=3-8). B) *Gipr, Cre,* and *Adipoq* expression in epididymal WAT and interscapular BAT. For reference, the average cycle threshold (ct) values for *Gipr* and *Adipoq* were 26.28 and 18.77, respectively, in WAT and 28.13 and 20.73, respectively, in BAT. *Cre* expression is driven by the *AdipoqBAC* promoter (*AdipoqBAC-Cre;* (37)). (n=3). C) *Gipr* expression in various adipose tissue depots in mice with the *AdipoqBAC-Cre* transgene. (n=3). D) *Cre*

and *Gipr* expression in tissues, including epididymal WAT from various Cre recombinase models crossed with *Gipr*$^{flx/flx}$ mice. (n=2-3). For relative RNA expression values, in panels A-C, the values are normalized to expression in the Cre controls (A – *Adipoq-Cre*, B,C – *AdipoqBAC-Cre*). For panel D, the values are normalized to levels for *MIP-Cre*^{ERT}* - $p<0.05$ vs control.

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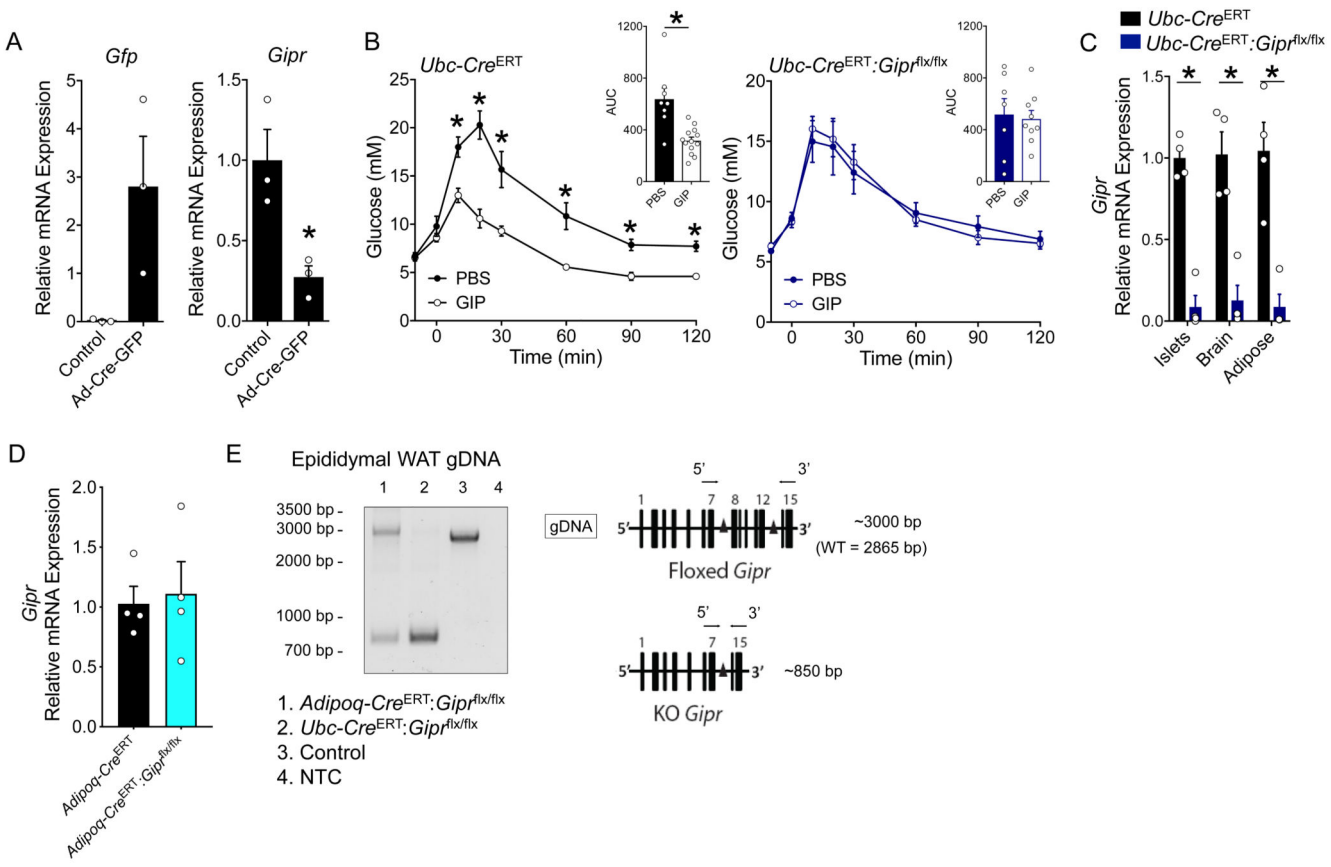

Figure 2. Complementary genetic strategies enable reduction of*Gipr* expression in adipose tissue.

A) An adenoviral vector expressing *Cre* and *Gfp* under control of the CMV promoter was injected into an inguinal adipose depot of *Gipr*$^{flx/flx}$ mice; empty vector was administered into the contralateral depot as a control. qPCR was used to determine the expression of *Gfp* and *Gipr* in both depots. (n=3 mice.) B) Blood glucose levels during an intraperitoneal glucose tolerance test where PBS or GIP (4 nmol/kg) was administered 10 minutes before glucose in control (*Ubc-Cre*^{ERT2}, n=8 -13) or *Ubc-Cre*^{ERT2}*:Gipr*$^{flx/flx}$ (n=7-9) mice. C) *Gipr* expression in primary islets, whole brain, and epididymal adipose tissue. (n=4). D) *Gipr* expression in epididymal adipose tissue from *Adipoq-Cre*^{ERT} (control) and *Adipoq-Cre*^{ERT}:*Gipr*$^{flx/flx}$ mice harvested two-weeks following tamoxifen treatment, when mice were 10 weeks of age. (n=4). E) PCR analysis of genomic DNA from epididymal WAT from different genetic mouse models. Non-recombined DNA produces a 2,865 bp product in WT mice, and an approximate 3,000 bp product in *Gipr*$^{flx/flx}$ mice. Recombination of the loxP alleles produces an 850-bp product. NTC – no template control. For relative mRNA values, expression in panel A was normalized to control, for panel C, values were normalized relative to *Ubc-Cre*^{ERT} and for Panel D, levels were normalized to *Adipo-Cre*^{ERT} * - $p<0.05$ vs control.

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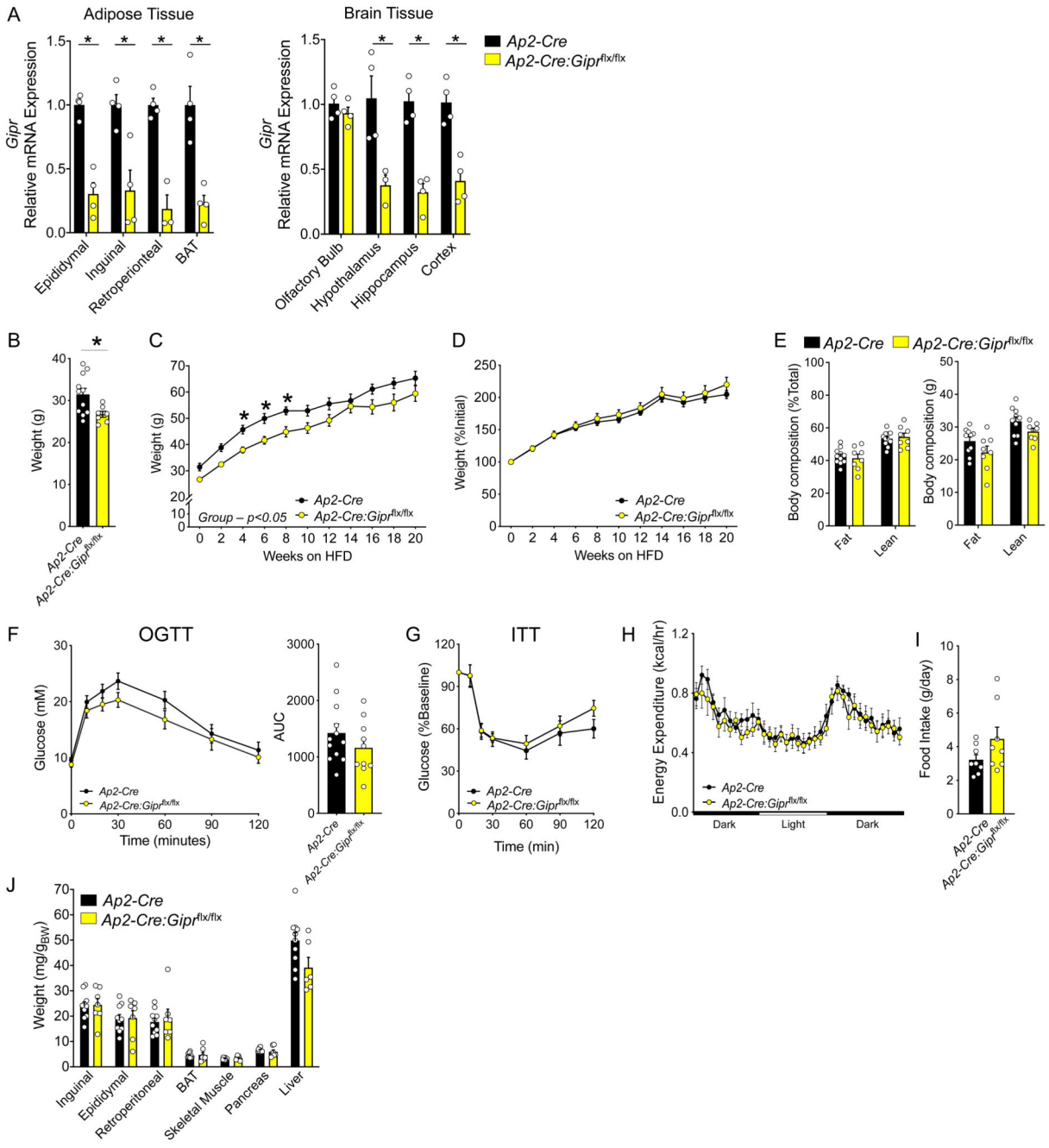

Figure 3. *Ap2-Cre* expression results in *Gipr* knockdown in adipose and brain tissues.

A) *Gipr* expression in adipose depots and brain tissues. (n=4). B) Body weights in 8-week-old mice prior to initiation of HFD feeding. (n=7-11). Absolute (C) and percent increases (D) in body weights of mice fed a 45% HFD diet for several weeks. (n=7-11). E) Body composition expressed as a percent of total (left) or absolute (right) weight in mice after 16 weeks of HFD feeding. (n=8-11). F) Blood glucose levels and area under the glucose curve (AUC) during an oral glucose tolerance test (OGTT) after 17 weeks of HFD feeding. (n=10-12). G) Blood glucose levels during an insulin tolerance test (ITT) after 6 weeks of

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HFD feeding. (n=10-12). H) Oxygen consumption after 20 weeks of HFD feeding. (n=8). I) 24 hour food intake afte 20 weeks of HFD feeding. (n=8). J) Tissues weights after 20 weeks of HFD feeding. (n=7-10). For relative mRNA expression in panel A, values were normalized to levels detected in RNA isolated from the same adipose tissue depots of *Ap2-Cre* mice. * - $p<0.05$ vs control.

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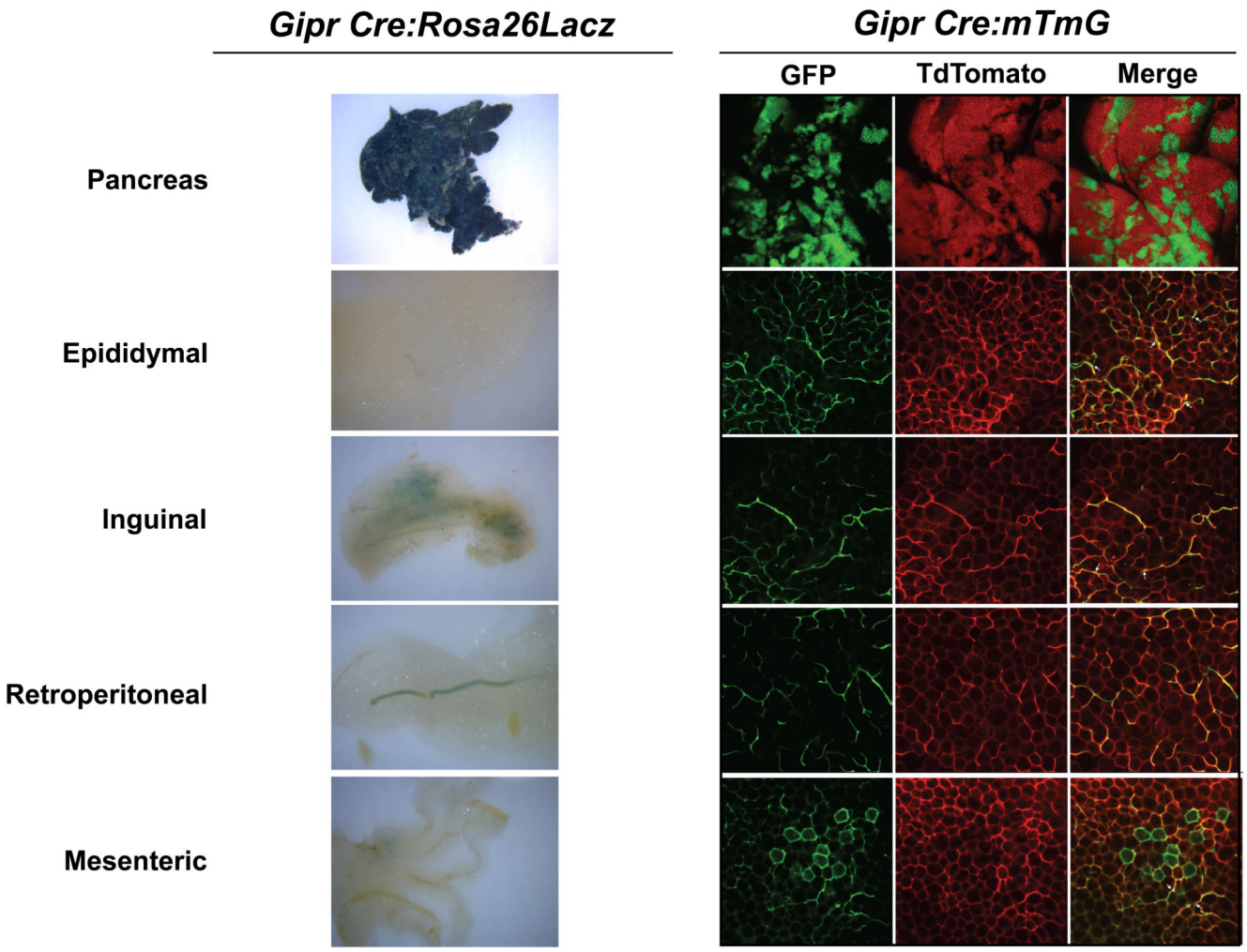

Figure 4. Heterogeneous distribution of *Gipr* promoter activity in adipose depots.

Representative whole mount images of β-galactosidase activity (left panels) and confocal fluorescence imaging (right panels) of pancreas and adipose tissues from *Gipr Cre:Rosa26Lacz* and *Gipr Cre:mTmG* mice, respectively. GFP; Cre positive green fluorescent protein images. TdTomato; Cre negative tomato red fluorescent protein images.

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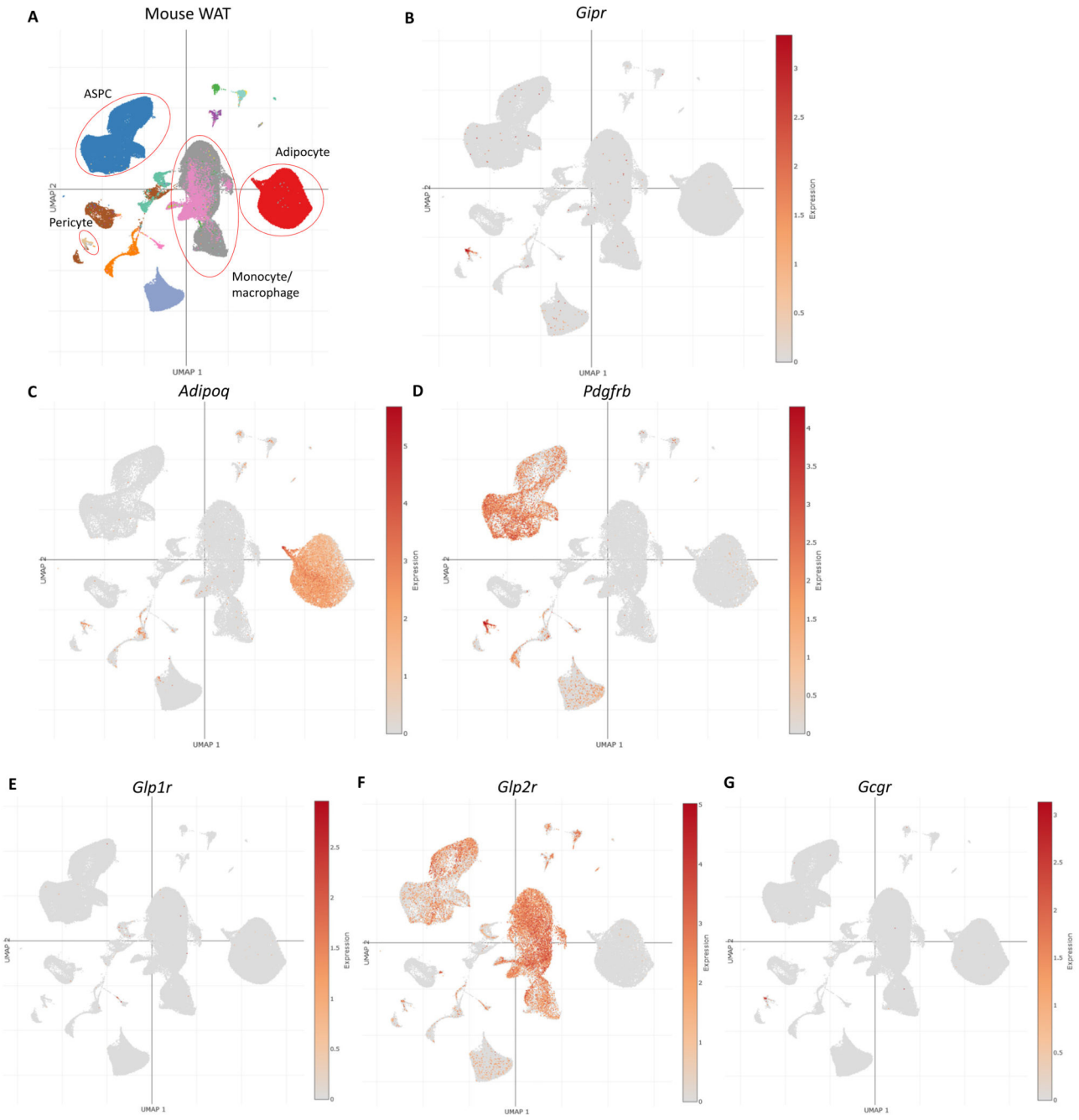

Figure 5. Mouse single nucleus RNA-seq data localizes WAT*Gipr* expression to pericytes

A) Single cell nucleus RNA-seq data (34) from a range of mouse adipose tissue cell types was analyzed for expression of (B) *Gipr*, (C) *Adipoq*, (D) *Pdgfrb,* a gene expressed in endothelial/pericyte and adipose tissue progenitors, (E) *Glp1r*, (F) *Glp2r*), and (G) *Gcgr*. ASPC, adipocyte stem progenitor cells.

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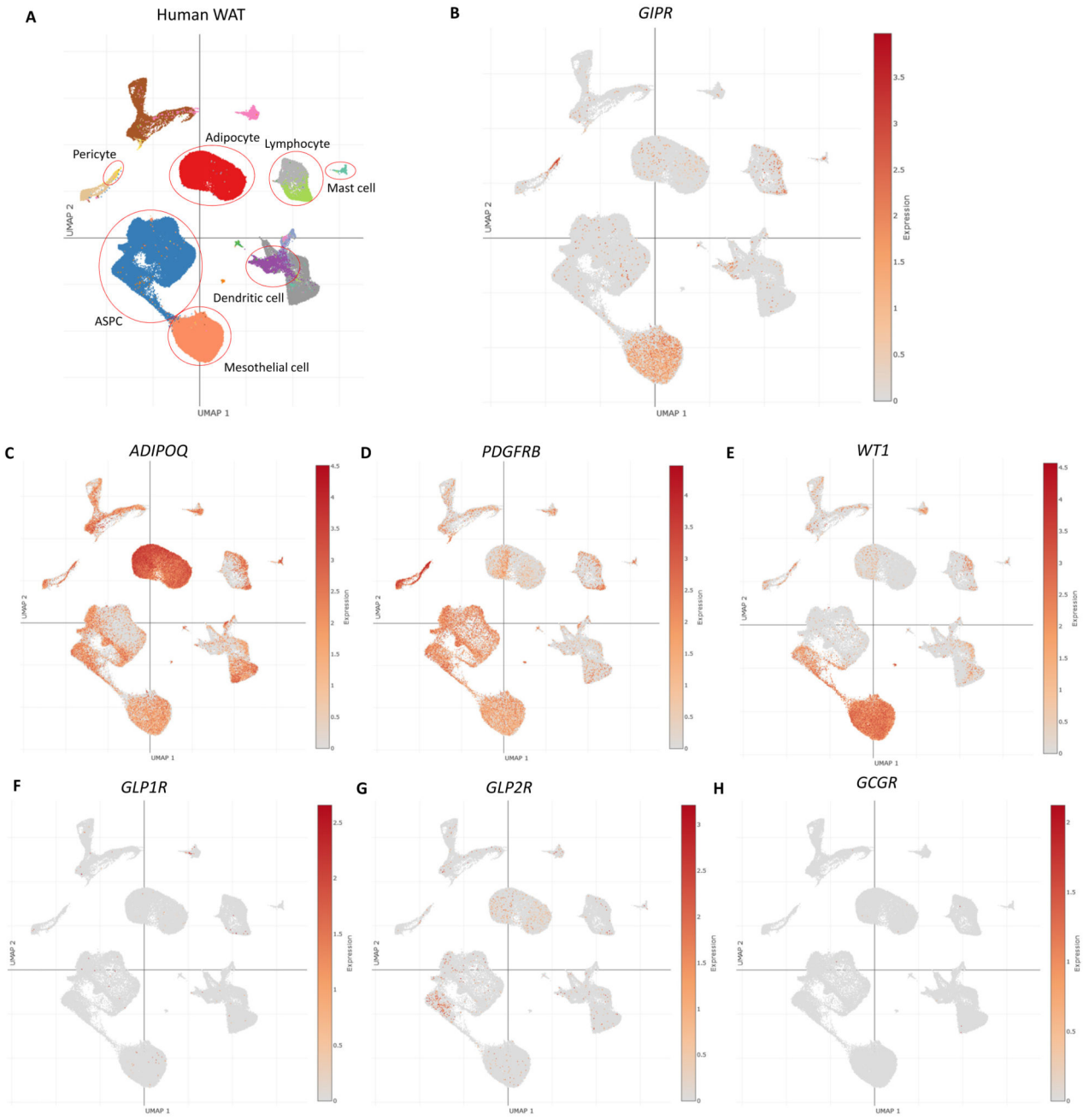

Figure 6. Human single nucleus RNA-seq data localizes white adipose tissue *GIPR* expression to pericytes and mesothelial cells.

A) Single cell nucleus RNA-seq data (34) from a range of human adipose tissue cell types was analyzed for expression of (B) *GIPR*, (C) *ADIPOQ*, (D) *PDGFRB*, (E) *WT1*, a marker for the mesothelial cell lineage, (F) *GLP1R*, (G) *GLP2R*, and (G) *GCGR* (G). ASPC, adipocyte stem progenitor cells.