Intranasal Targeting of Hypothalamic PTP1B and TCPTP Reinstates Leptin and Insulin Sensitivity and Promotes Weight Loss in Obesity

Graphical Abstract

Highlights

- Elevated ARC PTP1B and TCPTP contribute to the maintenance of DIO
- Deletion of ARC PTP1B and TCPTP in DIO reinstates leptin and insulin sensitivity
- Deletion of ARC PTP1B and TCPTP represses feeding and promotes WAT browning in DIO
- Intranasal targeting of ARC PTP1B and TCPTP promotes weight loss in DIO

Authors

Garron T. Dodd, Chrysovalantou E. Xirouchaki, Matthew Eramo, ..., Belinda A. Henry, Michael A. Cowley, Tony Tiganis

Correspondence

tony.tiganis@monash.edu

In Brief

Dodd et al. report that in obesity heightened hypothalamic levels of PTP1B and TCPTP repress insulin and leptin responses and contribute to the maintenance of obesity. The combined intranasal targeting of PTP1B and TCPTP increases leptin and insulin sensitivity and promotes weight loss by repressing feeding and increasing energy expenditure.
Intranasal Targeting of Hypothalamic PTP1B and TCPTP Reinstates Leptin and Insulin Sensitivity and Promotes Weight Loss in Obesity

Garron T. Dodd,1,2 Chrysovalantou E. Xirouchaki,1,2 Matthew Eramo,1,2 Christina A. Mitchell,1,2 Zane B. Andrews,1,3 Belinda A. Henry,1,3 Michael A. Cowley,1,3 and Tony Tiganis1,2,4,5,6,*

1Metabolism, Diabetes and Obesity Program, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia
2Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800, Australia
3Department of Physiology, Monash University, VIC 3800, Australia
4Monash Metabolic Phenotyping Facility, Monash University, VIC, Australia
5Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia
6Lead Contact
*Correspondence: tony.tiganis@monash.edu
https://doi.org/10.1016/j.celrep.2019.08.019

SUMMARY

The importance of hypothalamic leptin and insulin resistance in the development and maintenance of obesity remains unclear. The tyrosine phosphatases protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP) attenuate leptin and insulin signaling and are elevated in the hypothalami of obese mice. We report that elevated PTP1B and TCPTP antagonize hypothalamic leptin and insulin signaling and contribute to the maintenance of obesity. Deletion of PTP1B and TCPTP in the hypothalami of obese mice enhances CNS leptin and insulin sensitivity, represses feeding, and increases browning, to decrease adiposity and improve glucose metabolism. The daily intranasal administration of a PTP1B inhibitor, plus the glucocorticoid antagonist RU486 that decreases TCPTP expression, represses feeding, increases browning, promotes weight loss, and improves glucose metabolism in obese mice. Our findings causally link heightened hypothalamic PTP1B and TCPTP with leptin and insulin resistance and the maintenance of obesity and define a viable pharmacological approach by which to promote weight loss in obesity.

INTRODUCTION

Obesity is a leading factor in world-wide mortality and a major driver of chronic diseases, including type 2 diabetes, cardiovascular disease, liver disease, and cancer. In developed countries such as the United States, roughly two-thirds of all adults and one-third of all children are overweight or obese. Educational and lifestyle intervention approaches have done little to curb the escalating trend toward the overweight and obese state (Price et al., 2013; Wadden et al., 2011). Several drugs that act peripherally or in the CNS have been approved for the treatment of severe obesity, but they invariably have adverse side effects and/or have limited efficacy in the absence of exercise, which remains challenging for individuals that are severely obese (Danschvar et al., 2016; Jones and Bloom, 2015). At present, non-surgical therapeutic approaches for combating the obesity epidemic remain limited (Jones and Bloom, 2015).

Obesity is an outcome of perturbations in energy balance, where food intake exceeds energy expenditure. Intuitively, approaches aimed at promoting weight loss in severely obese individuals should both repress food intake and increase energy expenditure without the need for increased physical activity (Pan and Myers, 2018). One approach by which to increase energy expenditure is through the differentiation and activation of brown and beige adipocytes and the induction of non-shivering thermogenesis (Rosen and Spiegelman, 2014; Wang and Seale, 2016). Brown and beige adipocytes contain a high density of mitochondria with high amounts of uncoupling protein-1 (UCP-1) (Rosen and Spiegelman, 2014; Wang and Seale, 2016). UCP-1 allows for the uncoupling of the electrochemical gradient across the inner mitochondrial membrane from ATP production and the expenditure of energy in the form of heat (Rosen and Spiegelman, 2014; Wang and Seale, 2016). In humans, brown and beige adipocytes are found in varied fat depots, including those in the cervical, supraclavicular, axillary, intercostal, mediastinal, ventral spinal, and perirenal areas (Cypess et al., 2009; Rosen and Spiegelman, 2014; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Wang and Seale, 2016). Although mice subjected to persistent cold exposure or chronic β-adrenergic agonist exposure have similar brown and beige fat depot distributions (Zhang et al., 2018), brown adipocytes in mice normally predominate in classical interscapular brown adipose tissue (BAT), whereas beige adipocytes are found interdispersed among white adipocytes, predominantly in inguinal white adipose tissue (WAT) (Seale et al., 2011; Wang and Seale, 2016; Wu et al., 2012). Beige adipocytes exhibit remarkable plasticity and have the capacity to convert from a beige to a white state and then reinstate their thermogenic program (WAT browning) when re-stimulated (Altshuler-Keylin et al., 2016; Rosenwald...
Figure A: Shows the effects of leptin on p-STAT3 expression in AAV-GFP and AAV-Cre-GFP transgenic mice on Chow and HFF diets.

Figure B: Demonstrates the effect of insulin on p-AKT expression in AAV-GFP and AAV-Cre-GFP transgenic mice on Chow and HFF diets.

Figure C: Illustrates the expression of Arc mRNA (fold) and Arc protein (fold) on Chow and HFF diets after 2 h of leptin treatment.

Figure D: Displays the expression of c-Fos protein (fold) in the PVH of AAV-GFP and AAV-Cre-GFP transgenic mice on Chow and HFF diets after 2 h of leptin treatment.

Figure E: Shows the food intake (g) in AAV-Cre-GFP mice on Chow and HFF diets after leptin administration for 3 days.

Figure F: Illustrates the expression of GFAP in the ARC of AAV-GFP and AAV-Cre-GFP transgenic mice on Chow and HFF diets after Dextran-TMR injection.

Figure G: Demonstrates the ARC GFAP positive cell number in AAV-GFP and AAV-Cre-GFP transgenic mice on Chow and HFF diets.
et al., 2013; Wang et al., 2013). Increasing brown and/or beige adipocyte abundance and activity in rodents can prevent diet-induced obesity (DIO) (Dempersmier et al., 2015; Kopecky et al., 1995; Seale et al., 2011), whereas their genetic ablation promotes obesity (Cohen et al., 2014; Feldmann et al., 2009; Lowell et al., 1993). Notably the abundance of brown and/or beige adipocytes is diminished in overweight individuals (Cypress et al., 2009; Ouellet et al., 2011; van Marken Lichtenbelt et al., 2009) and there is considerable interest in harnessing the thermogenic activity of brown and/or beige adipocytes to combat the obesity epidemic.

Beyond their role in the defense against cold, beige adipocytes are also instrumental in feeding-induced thermogenesis and fundamental for the maintenance energy balance and body weight (Dodd et al., 2017; Ruan et al., 2014). We and others have shown that feeding promotes (Dodd et al., 2017), whereas fasting represses WAT browning (Dodd et al., 2017; Ruan et al., 2014). The coordination of WAT browning with feeding is mediated by the hypothalamus and TCPTP, the insulin receptor (IR) tyrosine phosphatase that dephosphorylates the IR to suppress downstream phosphatidylinositol (PI3K)/AKT signaling (Dodd et al., 2015, 2017, 2018b; Tiganis, 2013). Insulin acts in the arcuate nucleus (ARC) of the hypothalamus to activate proopiomelanocortin (POMC) and inhibit agouti-related peptide (AgRP)/neuropeptide Y (NPY)-expressing neurons to promote the melanocortin response, which among other things, represses feeding, increases browning and energy expenditure, and improves glucose metabolism (Dodd et al., 2018b; Könner et al., 2007; Qiu et al., 2014; Ruud et al., 2017; Varela and Horvath, 2012). TCPTP expression is regulated by the metabolic state, whereby fasting increases and feeding represses TCPTP, to coordinate insulin signaling in POMC and AgRP neurons (Dodd et al., 2017, 2018b). In this manner, TCPTP plays a critical role in the hypothalamic control of not only WAT browning and energy expenditure (Dodd et al., 2017) but also glucose metabolism (Dodd et al., 2018b). Additionally, the response of POMC neurons to the adipokine leptin, which is produced by white adipocytes in proportion to fat mass (Maffei et al., 1995), acts in concert with insulin to promote WAT browning (Dodd et al., 2015). Leptin action in the brain additionally serves to repress feeding, increase ambulatory activity, and promote glucose homeostasis (Pan and Myers, 2018; Varela and Horvath, 2012). Leptin signaling is negatively regulated by the tyrosine phosphatase PTP1B, which dephosphorylates Janus-activated kinase (JAK)-2 to suppress the downstream tyrosine phosphor-

ylation of signal transducer and activator of transcription (STAT)-3 (Bence et al., 2006; Dodd et al., 2015; Myers et al., 2001; Tsou et al., 2014; Zabolotny et al., 2003). However, unlike TCPTP, PTP1B levels are not altered by feeding (Dodd et al., 2018a). TCPTP also attenuates leptin signaling by dephosphorylating STAT-3 (Loh et al., 2011). Importantly, in obesity, TCPTP and PTP1B protein levels in the ARC are elevated, and this is thought to repress the response to leptin and insulin (Bence et al., 2006; Loh et al., 2011; Zhang et al., 2019). Although TCPTP is primarily responsible for the repression of insulin signaling, PTP1B can also contribute, at least in AgRP neurons (Balland et al., 2019). Similarly, although PTP1B primarily mediates the repression of leptin signaling, TCPTP can also contribute (Loh et al., 2011). Here we demonstrate that the combinatorial target-
ing of ARC PTP1B and TCPTP in obese mice is sufficient to re-stimulate leptin and insulin sensitivity and promote weight loss through the repression of feeding and promotion of brown and/or beige adipocyte thermogenesis.

RESULTS

Deleting PTP1B and TCPTP Alleviates Leptin and Insulin Resistance

To assess the degree to which ARC leptin and insulin signaling are diminished in obesity and the extent to which this might be attributable to increases in PTP1B (encoded by Ptpn1) and TCPTP (encoded by Ptpn2), we deleted PTP1B and TCPTP in the ARC of adult chow-fed lean mice versus 12-week high-fat-fed (HFF; 23% total fat) obese mice. To this end, recombinant adeno-associated viruses (rAAVs) expressing GFP alone (AAV-GFP) or GFP and Cre recombinase (AAV-Cre-GFP) were injected bilaterally into the ARC of aged-matched Chow-fed versus 12-week HFF Ptpn1fl/flPtpn2fl/fl mice (Figures 1A and 1B). Post-mortem immunohistochemical analyses monitoring for GFP confirmed efficient ARC targeting, whereas immunoblotting mediobasal hypothalamic (MBH) homogenates from a separate cohort of chow-fed mice confirmed the effective deletion of PTP1B and TCPTP within 5 days of AAV-Cre-GFP administration (Figures 1A and 1B; Figure S1A). We monitored for effects on basal versus leptin-induced STAT-3 Y705 phosphorylation (p-STAT-3), or basal versus insulin-induced AKT Ser-473 phosphorylation (p-AKT), as measures of leptin and insulin signaling, respectively, as these pathways mediate many of the CNS effects on energy balance and glucose homeostasis. We found that within 5 days of targeting and deleting PTP1B and TCPTP (prior to overt effects

Figure 1. PTP1B and TCPTP Deletion Reinstates ARC Leptin and Insulin Sensitivity in Obesity

(A–D) Ptpn1fl/flPtpn2fl/fl male mice were chow fed or HFF for 12 weeks and bilaterally injected AAV-GFP or AAV-Cre-GFP into the ARC. 5 days post rAAV injection, mice were administered vehicle, leptin, or insulin and brains processed for immunohistochemistry monitoring for (A) leptin-induced p-STAT3, (B) insulin-induced p-AKT, (C) leptin-induced hypothalamic POMC and Npy gene expression, or (D) leptin-induced PVH c-Fos immunoreactivity. ARC targeting was confirmed by the post-mortem analysis of hypothalamic GFP immunofluorescence (inserts in A and B).

(E) 12-week HFF Ptpn1fl/flPtpn2fl/fl male mice were bilaterally injected AAV-GFP or AAV-Cre-GFP into the ARC and after 3 days administered leptin (2.5 μg/g/day, intraperitoneally [i.p.]) for 3 consecutive days and daily food intake determined.

(F and G) Ptpn1fl/flPtpn2fl/fl male mice were fed a chow or HFF for 12 weeks and bilaterally injected AAV-GFP or AAV-Cre-GFP into the ARC. 8 weeks post rAAV injection, mice were either (F) administered dextran-TMR and 30 min later brains fixed and processed for fluorescent TMR integration in the ARC or (G) processed for ARC GFP immunohistochemistry.

Results are means ± SEM; significance determined using (F) one-way ANOVA with Dunnett’s multiple comparisons, (C, D, and G) two-way ANOVA with Tukey multiple comparisons, or (E) repeated-measures and (A and B) three-way ANOVA with Tukey multiple comparisons. In (B), # represents chow + vehicle + AAV-GFP versus chow + vehicle + AAV-Cre-GFP using a Student’s t test. Scale bar represents 100 μm.
on body weight; data not shown), basal p-STAT-3 and p-AKT were enhanced in the ARC of fasted chow-fed mice, consistent with the promotion of the endogenous leptin and insulin response (Figures 1A and 1B; Figure S1A).

To specifically determine whether PTP1B and TCPTP influence the amplitude of leptin and insulin signaling, we first determined the maximal ARC response to exogenous leptin and insulin (intraperitoneal) in chow-fed mice (Figures S1B and S1C). Maximal leptin-induced p-STAT3 in the ARC was achieved at 2 μg leptin/g body weight, with higher doses promoting p-STAT3 in other hypothalamic nuclei, including the dorsomedial hypothalamus (DMH) and ventromedial hypothalamus (VMH), but not in the ARC (Figure S1B). Maximal insulin-induced p-AKT in the ARC was achieved at 2 mU insulin/g body weight; there was no obvious p-AKT staining in other hypothalamic nuclei even at significantly higher doses of insulin (Figure S1C).

Having established the maximal ARC response to leptin or insulin, we next assessed the influence of PTP1B and TCPTP deletion. PTP1B and TCPTP deletion in fasted chow-fed mice did not increase ARC p-STAT-3 and p-AKT in response to saturating concentrations of leptin and insulin (Figures 1A and 1B) but enhanced responses at submaximal doses (Figures S1D and S1E). This is consistent with PTP1B and TCPTP influencing the amplitude of ARC leptin and insulin signaling in chow-fed lean mice without affecting maximal responses achieved at saturating concentrations of leptin and insulin.

We next determined the influence of PTP1B/TCPTP ARC deletion on leptin and insulin responses in HFF obese mice. In vehicle-treated HFF obese mice, basal p-STAT-3 was elevated (Figure 1A), and this was accompanied by increased circulating leptin levels (Figure S1F). However, the level of basal ARC p-STAT-3 in HFF obese mice was well below that achieved with saturating doses of exogenous leptin in chow-fed lean mice (Figure 1A). By contrast, basal ARC p-AKT signaling in vehicle-treated mice was not elevated in HFF mice (Figure 1B), despite increased circulating levels of insulin (Figure S1G). At exogenous concentrations of leptin or insulin that were saturating in fasted chow-fed mice, hypothalamic p-STAT-3 and p-AKT were induced in HFF mice, but not to levels seen in chow-fed mice (Figures 1A and 1B). These results indicate that ARC leptin and insulin signaling are attenuated in DIO and are consistent with the development of hypothalamic cellular leptin and insulin resistance. Notably, PTP1B/TCPTP ARC deletion reinstated the exogenous leptin- and insulin-induced STAT-3 and PI3K/AKT signaling in HFF obese mice, so that p-STAT-3 and p-AKT levels approximated those in chow-fed mice (Figures 1A and 1B); circulating leptin and insulin levels were not affected by the deletion of PTP1B and TCPTP (Figures S1F and S1G). Moreover, the deletion of PTP1B and TCPTP was accompanied by the promotion of leptin-induced hypothalamic responses; this was evident within 5 days of AAV-Cre-GFP administration. In particular, we found that the ability of leptin to promote Pomc expression and repress Npy expression, which was readily evident in chow-fed (Figures S1H and S1I) but not HFF mice, was reinstated by the deletion of PTP1B and TCPTP (Figure 1C); PTP1B/TCPTP deletion did not affect leptin-induced Pomc and Npy expression in chow-fed mice treated with saturating leptin concentrations (Figures S1H and S1I). Consistent with this, PTP1B and TCPTP deletion enhanced the basal and leptin-induced c-Fos staining (a surrogate marker of neuronal activation) in second-order neurons of the paraventricular hypothalamus (PVH) in HFF mice (Figure 1D). Most importantly, consistent with the promotion of melanocortin signaling, we found that the deletion of PTP1B and TCPTP in HFF mice reinstated the leptin-induced repression of food intake (assessed 3–6 days post rAAV administration) that was otherwise evident in chow-fed mice (Figure S1J) but not in HFF obese mice (Figure 1E). Taken together, these results are consistent with PTP1B and TCPTP regulating the amplitude of ARC leptin and insulin signaling and for elevated ARC PTP1B and TCPTP in obesity contributing to the development of cellular leptin and insulin resistance.

**Deleting PTP1B and TCPTP Does Not Alter Gliosis or Blood-Brain Barrier Permeability**

Previous studies have reported that cellular leptin and insulin resistance might in part be attributed to decreased blood-brain barrier (BBB) permeability (Balland et al., 2014; Caro et al., 1996; Schwartz et al., 1996) and the increased glial ensheathment of hypothalamic neurons (Horvath et al., 2010; Thaler et al., 2012), limiting access to circulating nutrient and hormonal stimuli such as leptin and insulin (El-Haschimi et al., 2000; Pan and Myers, 2018). As the administration of AAV-Cre-GFP to the ARC of Ptpn1fl/fl,Ptpn2fl/fl mice would uniformly result in PTP1B/TCPTP deletion in the ARC, including in glial cells, we determined if PTP1B/TCPTP ARC deletion might also influence the ability of leptin and insulin to access the hypothalamus. As noted previously, BBB permeability, as assessed by the ability of circulating tetramethylrhodamine (TMR)-dextran (molecular weight [MW] 70,000) to cross the BBB and enter the ARC, was attenuated in HFF obese mice (Figure 1F; Figures S2A and S2B), but this was unaffected by PTP1B/TCPTP ARC deletion (Figure 1F). Similarly, gliosis, as measured by the number of glial fibrillary acidic protein (GFAP)-positive cells in the ARC, was increased in 12-week HFF obese mice, but this was unaffected by PTP1B/TCPTP ARC deletion (Figure 1G). These results are consistent with the deletion of PTP1B and TCPTP in the ARC alleviating cellular leptin and insulin resistance in 12-week HFF mice, despite persistent obesity-associated defects in BBB permeability and increased gliosis.

**Deleting PTP1B and TCPTP Promotes Weight Loss in Obesity**

Next, we assessed whether reinstating ARC leptin and insulin sensitivity by deleting PTP1B and TCPTP might promote weight loss in obese mice. Ptpn1fl/fl,Ptpn2fl/fl mice were HFF for 12 weeks and injected bilaterally with AAV-Cre-GFP or AAV-GFP into the ARC; as a control, we also deleted PTP1B or TCPTP alone (Figure 2). In each case, post-mortem analyses monitoring for the presence of GFP in the ARC confirmed efficient targeting (Figures S3A–S3C), whereas immunoblotting MBH homogenates from a separate cohort of Ptpn1fl/fl,Ptpn2fl/fl mice confirmed that PTP1B and TCPTP were effectively deleted (Figure S3D). We found that PTP1B or TCPTP deletion promoted significant weight loss despite the ongoing consumption of a high-fat diet, and that the combined deletion of PTP1B and
TCPTP resulted in even greater reductions in weight (Figure 2A). The decreased weight could be attributed to decreases in adiposity without changes in lean mass or bone density (Figure 2B; Figures S4A–S4D). Although no effects were noted on ambulatory activity (Figure S4E), weight loss in mice where PTP1B, but not TCPTP, had been deleted in the ARC was accompanied by a reduction in daily food intake (Figure 2C); the reduction in food intake was also seen in PTP1B/TCPTP ARC deleted mice (Figure 2C). This is consistent with studies showing that the deletion of PTP1B in the brain using the Nestin-Cre transgene (Bence et al., 2006) or AgRP neurons (Balland et al., 2019) promotes leptin signaling and/or the repression of feeding. By contrast, weight loss in TCPTP and PTP1B/TCPTP ARC deleted mice, but not PTP1B ARC deleted mice, was accompanied by increased oxygen consumption and energy expenditure (as assessed by indirect calorimetry) without changes in ambulatory activity (Figure 2D; Figures S4E and S4F). The increased energy expenditure in TCPTP ARC deleted mice is consistent with our previous studies showing that TCPTP deficiency alone in AgRP neurons (Dodd et al., 2017) or combined PTP1B and TCPTP deficiencies in POMC neurons (Dodd et al., 2015) drive energy expenditure by promoting BAT activity and the sympathetic nervous system (SNS)-dependent browning of inguinal WAT. Consistent with this, interscapular BAT Ucp-1 gene expression was elevated in TCPTP and PTP1B/TCPTP ARC deleted mice (Figure 2E). Moreover, ARC TCPTP, or PTP1B/TCPTP, but not PTP1B deletion, was accompanied by an increase in inguinal WAT browning, as assessed by (1) histology, monitoring for the presence of small adipocyte clusters with multilocular lipid droplets (Figure 2F); (2) immunohistochemistry, monitoring for UCP-1 positive adipocytes (Figure 2F); and (3) the increased expression of thermogenic genes (Figure 2G) including Ucp-1 as well as Tmem26 and Cd137 that are specific to beige adipocytes (Wu et al., 2012); the unaltered WAT browning and energy expenditure in PTP1B ARC deleted mice is consistent with studies showing that PTP1B deletion alone in POMC neurons (Dodd et al., 2015) or AgRP neurons (Balland et al., 2019) has no effect on WAT browning and/or energy expenditure. Importantly, the weight loss resulting from the combined deletion of PTP1B and TCPTP in the ARC was accompanied by a marked improvement in glucose homeostasis, as assessed by decreased glucose excursions in insulin (Figure 2H) and glucose (Figure S4G) tolerance tests and decreased fasted blood glucose levels (Figure S4H). Therefore, the combined deletion of PTP1B and TCPTP in the ARC leads to decreased feeding and increased BAT activity and/or WAT browning resulting in weight loss and improved glucose homeostasis in obesity, despite the ongoing consumption of a high-fat diet. These findings are consistent with the elevated hypothalamic PTP1B and TCPTP in obesity decreasing ARC leptin and insulin sensitivity and contributing the maintenance of obesity and the development of type 2 diabetes.

**Figure 2.** PTP1B and TCPTP Deletion in the ARC Promotes Weight Loss in Obesity

(A–H) Ptpn1fl/fl, Ptpn2fl/fl and Ptpn1fl/fl,Tptn2fl/fl male mice were HFF for 12 weeks and bilaterally injected AAV-GFP or AAV-Cre-GFP into the ARC. (A) Body weights and body weight change at 18 weeks of high-fat feeding and (B) subcutaneous (Sub) and epididymal (Epi) WAT weights, (C) food intake, (D) energy expenditure, (E) BAT Ucp-1 mRNA expression, (F) inguinal WAT (ingWAT) histology and UCP-1 immunohistochemistry, (G) ingWAT mRNA expression, and (H) insulin tolerances at 18 weeks of high-fat feeding were assessed. Results are means ± SEM and are representative of at least two independent experiments. Significance determined using (C, E, and H) t test, (A) one-way ANOVA with multiple comparisons, and two-way ANOVA with (B, D, and G) Sidak multiple comparisons or (A) repeated-measures. Scale bar represents 50 μm.
In this way, hypothalamic TCPTP fluctuations ensure that feeding rhythms are matched by commensurate changes in energy expenditure and endogenous glucose production so that energy balance and glucose homeostasis are maintained. Importantly, we have shown that in obesity TCPTP expression is increased and sustained after feeding and that this contributes to the repression of WAT browning and energy expenditure and the development of hyperglycemia and insulin resistance (Dodd et al., 2017, 2018a). As glucocorticoids drive ARC TCPTP expression (Dodd et al., 2017) and glucocorticoid levels (corticosterone in rodents or cortisol in humans) are elevated in obesity (Jackson et al., 2017; van Rossum, 2017), we determined whether the heightened glucocorticoids might be responsible for the increase in hypothalamic TCPTP and thereby the maintenance of obesity. First, we measured corticosterone levels in chow-fed lean versus HFF obese C57BL/6J mice. Corticosterone levels were elevated in fasted chow-fed mice and repressed in fed and satiated mice (4 h after start of dark cycle; Dodd et al., 2017; Figure S6A). By contrast, corticosterone levels were elevated in fed HFF obese mice and approximated those seen in fasted chow-fed mice; fasting further increased corticosterone levels in HFF mice (Figure S6A). As expected, leptin levels were also elevated in HFF obese mice, but this was not significantly affected by feeding (Figure S1F). To determine if the heightened corticosterone levels in HFF mice might contribute to the sustained and/or elevated TCPTP, we intracerebroventricularly (ICV) administered HFF obese mice the glucocorticoid antagonist RU486 daily for 2–10 days and assessed hypothalamic TCPTP levels in MBH extracts. RU486 repressed the heightened TCPTP protein levels in HFF mice within 2 days and reduced TCPTP protein and mRNA levels to those seen in chow-fed lean mice within 10 days of administration (Figures 4A and 4B). Therefore, the heightened glucocorticoid levels in obesity drive and/or sustain hypothalamic TCPTP expression.

### Targeting TCPTP and PTP1B in the CNS Promotes Weight Loss in Obesity

As the sustained and/or elevated hypothalamic PTP1B and TCPTP in DIO can contribute to cellular leptin and insulin resistance and the development and/or maintenance of obesity, we determined whether pharmacological targeting PTP1B and/or TCPTP in HFF obese mice might promote weight loss. To this end, we first determined whether the ICV administration of RU486, which corrects the increase in hypothalamic TCPTP in obesity, and/or claramine, a highly selective PTP1B inhibitor (Qin et al., 2015), might promote weight loss in obese mice. ICV administered RU486 dose-dependently decreased body weight, and this was attributed solely to decreases in adiposity (Figures 4C and 4D; Figure S6B). As in mice in which TCPTP had been deleted in the ARC (Figure 2C), RU486 had no effect on feeding (Figure 4E) or ambulatory activity (Figures S6C and S6D) but increased energy expenditure (Figure 4F; Figure S6E), and this was accompanied by the promotion of WAT browning (as assessed by gross morphology, histology, immunohistochemistry, and gene expression; Figures 4K–4M; Figure S6F) and BAT Ucp-1 expression (Figure 4N). The weight loss, decreased adiposity (especially in the inguinal fat pad where browning occurs), and increased energy expenditure could be largely, but not completely, prevented by chemically denervating the inguinal fat pad to prevent browning (Figures 4G–4M; Figures S6F–S6L). By contrast, although ICV administered claramine also promoted dose-dependent weight loss and reduced fat mass (Figure S7A–S7C), this was associated with decreased food intake (Figure S7D) but not changes in energy expenditure; other behaviors including drinking, grooming, and locomotor activity were not affected by claramine administration (Figures S7E–S7I). Strikingly the combinatorial administration of suboptimal doses of claramine and RU486 resulted in synergistic weight loss and reductions in adiposity accompanied by increases in energy expenditure and reductions in food intake (Figures 5A–5D; Figures S8A–S8D). Importantly, the effects of claramine and RU486 could be ascribed to the specific targeting of PTP1B and TCPTP in ARC, as they had no additional effect in mice in which PTP1B and TCPTP had been deleted in the ARC (Figures 5E–5H; Figures S8E–S8H). Taken together with our genetic studies, these results indicate that the pharmacological targeting of PTP1B and TCPTP in the CNS elicits synergistic effects to promote weight loss in obese mice through the repression of feeding and induction of energy expenditure and define PTP1B and TCPTP as therapeutic targets in obesity.

### Intranasal Targeting of PTP1B and TCPTP in the ARC Promotes Weight Loss in Obesity

An important challenge in targeting CNS circuits to combat obesity is the transport of drugs across the BBB. This challenge can be overcome by targeting the brain via the intranasal route, which allows for drugs to be delivered directly to the brain parenchyma via the olfactory or trigeminal nerves (Born et al., 2002). Thus, we determined whether the intranasal administration of RU486 plus claramine might alleviate cellular leptin and insulin resistance and promote weight loss in obese mice by targeting PTP1B and TCPTP in the hypothalamus (Figure 6; Figures S9 and S10). RU486 plus claramine administered intranasally...
A

B

C

D

E

F

G

H

I

J

K

L

M

N

(legend on next page)
enhanced leptin-induced p-STAT3 (Figure 6A) and insulin-induced p-AKT (Figure 6B) in the hypothalami of 12-week HFF obese mice. These findings are consistent with the reinstatement of ARC leptin and insulin sensitivity in obese mice. Moreover, the intranasal administration of RU486 plus claramine increased hypothalamic c-Fos staining, including that in the PVH and DMH, consistent with increased hypothalamic neuronal activity and the engagement of second-order neurons of the melanocortin circuit (Figure 6C). Importantly, the enhanced hypothalamic response in 12-week HFF mice was accompanied by significant weight loss (Figure 6D; Figure S9A), specifically attributable to reductions in adiposity (Figure 6E; Figures S9C, S9D, S10A, and S10B), and improved glucose metabolism, as assessed in insulin and glucose tolerance tests and reflected by reductions in fasted blood glucose levels (Figure 6F; Figures S10C and S10D); intranasal RU486 plus claramine also promoted weight loss in 20-week HFF mice (Figure S9E). The reduction in body weight was reliant on PTP1B and TCPTP in the ARC, as the intranasal delivery of RU486 plus claramine had no additional effect on body weight when PTP1B and TCPTP were deleted in the ARC (Figures 6I and 6J; Figures S10H–S10K). The intranasal targeting of PTP1B and TCPTP in the ARC was associated with reduced food intake (Figures 6G and 6J; Figures S9B and S9F). However, no effects were seen in locomotor activity (Figures S10G and S10K) or in preference for saccharin-sweetened water (Figure S9G); the preference for sweet taste is used to assess anhedonia in models of depression (Harkin et al., 2002; Valenstein et al., 1967). The intranasal targeting of PTP1B and TCPTP in the ARC and reduction in body weight and adiposity was also associated with increased BAT Ucp-1 expression (Figure 6K), inguinal WAT browning (Figure 6L), and energy expenditure (Figure 6H; Figures S10E, S10F, S10I, and S10J). Strikingly the effects on BAT and browned inguinal WAT thermogenic activity in obese mice treated with RU486 plus claramine was readily evident by infrared thermal imaging that measures topographic temperature (Figures 6N–6P; Figure S9H). Notably, the increase in temperature in inguinal WAT approximated that seen in lean-fed, but not food-restricted, mice (Figures 6N and 6O), consistent with RU486 plus claramine treatment reinstating feeding-induced thermogenesis, which we have shown previously is regulated by the glucocorticoid-mediated regulation of TCPTP in the ARC AgRP neurons (Dodd et al., 2017). Taken together, these results are consistent with the targeting of PTP1B and TCPTP in the ARC with intranasal RU486 plus claramine reinstating hypothalamic leptin and insulin sensitivity and promoting weight loss through the repression of feeding and induction of adipose tissue thermogenesis.

**DISCUSSION**

The extent to which cellular leptin resistance and CNS insulin resistance are causal in the development and/or maintenance of obesity and the associated perturbations in systemic glucose metabolism has remained unclear (Jais and Brüning, 2017; Myers et al., 2012; Pan and Myers, 2018). Moreover, although various mechanisms, including inflammation (Jais and Brüning, 2017; Thaler et al., 2012, 2013; Zhang et al., 2008), endoplasmic reticulum (ER) stress (Ozcan et al., 2004, 2006, 2009; Williams et al., 2014), gliosis (Horvath et al., 2010; Thaler et al., 2012), defects in BBB permeability (Balland et al., 2014; Caro et al., 1996; El-Haschimi et al., 2000; Schwartz et al., 1996), and increased negative feedback (Bence et al., 2006; Bjorbaek et al., 1998, 1999; Loh et al., 2011; Mori et al., 2004; Pan and Myers, 2018; Zabolotny et al., 2008; Zhang et al., 2015), have been proposed to interfere with CNS leptin and insulin action in obesity, their relative contribution to the diminished CNS leptin and insulin responses remains unknown. In this study, we demonstrate that targeting the tyrosine phosphatases PTP1B and TCPTP, which are elevated in the hypothalami of obese mice and negatively regulate leptin and insulin signaling (Zhang et al., 2015), reinstates ARC leptin and insulin sensitivity to promote weight loss. Thus, our findings establish the principal importance of heightened PTP1B and TCPTP in the development of cellular leptin and insulin resistance and the maintenance of obesity. Moreover, our studies demonstrate that increased circulating glucocorticoids in obesity are primarily responsible for the elevated hypothalamic TCPTP. Importantly, we demonstrate that PTP1B and TCPTP can be effectively targeted in the CNS via the intranasal route, which allows for the rapid delivery of neuropeptides, hormones, and drugs to the brain (Born et al., 2002; Crowe et al., 2018).

As in peripheral tissues, it is now widely appreciated that hypothalamic neurons develop insulin resistance (Anthony et al., 2006; Hirvonen et al., 2011; Tschritter et al., 2006). However, the extent to which this contributes to the systemic perturbations in glucose metabolism in obese humans has remained contentious (Deem et al., 2017; Ruud et al., 2017). In support of the central role of insulin signaling, intranasal insulin can elicit effects in the hypothalamus of lean humans and suppress hepatic glucose production and promote glucose uptake (Heni et al., 2014, 2017). Similarly, in rodents, insulin regulates ARC POMC and AgRP neuronal activation to influence hepatic glucose production and whole-body glucose homeostasis (Dodd et al., 2018a, 2018b; Könner et al., 2007). These CNS insulin effects are defective in overweight individuals (Heni et al., 2014, 2017), as well as in obese rodents, where CNS insulin resistance can
contribute to the development of hyperglycemia (Dodd et al., 2018b; Gelling et al., 2006; Obici et al., 2002; Ruud et al., 2017). However, beyond effects on glucose metabolism, insulin also functions with leptin to maintain energy balance (Pan and Myers, 2018; Ruud et al., 2017; Varela and Horvath, 2012). Our previous studies have shown that the insulin-mediated inhibition of AgRP neurons is instrumental in promoting WAT browning and energy expenditure in response to feeding (Dodd et al., 2017). In insulin also acts together with leptin to activate disparate POMC neurons in the ARC to promote BAT activity and WAT browning (Dodd et al., 2015). While both TCPTP and PTP1B can negatively regulate insulin and leptin signaling, our studies point toward TCPTP predominating in the insulin pathway and PTP1B in the leptin pathway in POMC and AgRP neurons (Balland et al., 2019; Dodd et al., 2015, 2017, 2018a; Banno et al., 2010; Bence et al., 2006; Loh et al., 2011; Tsou et al., 2014). We have demonstrated previously that TCPTP deletion alone in AgRP neurons is sufficient to drive WAT browning through the enhancement of IR signaling (Dodd et al., 2017). Similarly, in this study we have shown that the deletion of TCPTP, but not PTP1B, in the ARC was sufficient to promote browning and energy expenditure. We have shown previously that fluctuations in TCPTP help coordinate the insulin response in the ARC and thereby WAT browning; in the fed state, low TCPTP facilitates insulin signaling to drive browning, whereas the glucocorticoid-mediated induction of TCPTP in the fasted state represses browning and energy expenditure (Dodd et al., 2015). Consistent with this, we found that the administration of RU486 and the repression of TCPTP alone in the ARC was sufficient to induce brown and/or beige adipocyte thermogenesis and weight loss. Although PTP1B deletion alone in the ARC had no effect on WAT browning, the combined deletion of PTP1B and TCPTP and the promotion of both leptin and insulin signaling at least in POMC neurons (Dodd et al., 2015) might also contribute to brown and/or beige adipocyte thermogenesis. In obesity, inflammation, ER stress, and hyperleptinemia increase the expression of PTP1B in the hypothalamus (White et al., 2009; Williams et al., 2014; Zabolotny et al., 2008). Although hyperleptinemia in obesity can also induce TCPTP (Loh et al., 2011), our studies demonstrate that it is sustained glucocorticoids (corticosterone) that maintain elevated ARC TCPTP in the fed state to repress insulin signaling in POMC and AgRP neurons and inhibit WAT browning. Importantly we demonstrate that the resultant defective feeding-induced browning might be fundamentally important for the maintenance of DIO (Dodd et al., 2015, 2017). Recent studies have substantiated the importance of brown and/or beige adipocyte thermogenesis decreased in older and overweight individuals (Cypess et al., 2009; Ouellet et al., 2011; van Marken Lichtenbelt et al., 2009). Our findings indicate that reinstating brown and/or beige adipocyte thermogenesis by targeting

Figure 5. ICV RU486 and Claramine Synergistically Promote Weight Loss in Obesity

(A–D) 12-week HFF C57BL/6J male mice received RU (1 μg/animal/day, ICV) and/or the selective PTP1B inhibitor, claramine (CA: 1 μg/animal/day, ICV) for 10 consecutive days, and effects on (A) body weights, (B) food intake, (C) adiposity, and (D) energy expenditure were determined. 12-week HFF Ptpn1fl/fl; Ptpn2fl/fl male mice were bilaterally injected AAV-GFP or AAV-Cre-GFP into the ARC.

(E–H) 1 week post AAV administration, mice were co-administered RU (1 μg/animal/day, ICV) and CA (1 μg/animal/day, ICV) for 10 consecutive days, and effects on (E) body weights, (F) food intake, (G) adiposity, and (H) energy expenditure were determined.

Results are means ± SEM; significance determined using (C and G) one-way ANOVA with Tukey multiple comparisons and two-way ANOVA with (D) Tukey or (H) Dunnett’s multiple comparisons, or (A, B, E, and F) repeated-measures. In (A), # represents RU versus CA + RU. In (E) and (F), * represents AAV-GFP-Vehicle versus AAV-GFP-CA+RU, # represents AAV-GFP-Vehicle versus AAV-Cre-GFP-Vehicle, and $ represents AAV-GFP-CA+RU versus AAV-Cre-GFP-CA+RU.
PTP1B and TCPTP might be sufficient to promote energy expenditure independent of changes in ambulatory activity in obesity. Obese humans and rodents with DIO generally have markedly increased circulating leptin levels commensurate with the degree of adiposity (Myers et al., 2012; Pan and Myers, 2018). The high circulating leptin and the inability of exogenous leptin to repress food intake and promote weight loss in obese humans and rodents has given rise to the concept of cellular leptin resistance (El-Haschimi et al., 2000; Frederick et al., 1995; Münzberg et al., 2004; Myers et al., 2012; Pan and Myers, 2018). However, recent studies from Balland et al. (2019) have shown that basal p-STAT3 is increased in the ARC of HFF obese mice when compared to that in chow-fed mice. Therefore, leptin signaling per se may not necessarily be defective in DIO. Rather, leptin resistance may reflect a defect in the amplitude of leptin signaling, so that the heightened leptin levels in obesity are unable to elicit commensurate leptin-induced responses sufficient to prevent weight gain (Pan and Myers, 2018). Consistent with this notion, we demonstrate that the basal p-STAT3 in the ARC of HFF obese mice is significantly lower than that induced by leptin in chow-fed lean or HFF obese mice and that exogenous leptin-induced ARC p-STAT3 in HFF obese mice is diminished when compared to chow-fed age-matched controls. Importantly we demonstrate that the attenuated leptin-induced response can be attributed at least in part to the heightened expression of PTP1B and TCPTP, as their deletion in the ARC increased basal ARC p-STAT-3 and reinstated leptin-induced p-STAT-3 in HFF obese mice to that in chow-fed mice. Moreover, we demonstrate that PTP1B/TCPTP deletion in the ARC reinstated downstream leptin responses, including the repression of Npy and induction of Pomc expression, the activation of secondary-order PVH neurons, and the repression of feeding in HFF obese mice. Although PTP1B, but not TCPTP, deletion alone repressed feeding, we cannot exclude that the combined deletion of PTP1B and TCPTP might yield synergistic effects, as PTP1B and TCPTP can act in concert to antagonize leptin signaling through the dephosphorylation of JAK-2 and STAT-3, respectively (Loh et al., 2011). Nonetheless, we show that decreased food intake after PTP1B/TCPTP deletion in the ARC of obese mice is a key contributing factor for the accompanying weight loss. Therefore, beyond driving brown and/or beige adipocyte thermogenesis and energy expenditure, the inhibition and/or suppression of hypothalamic PTP1B and TCPTP may allow for combinatorial repression of feeding to synergistically ameliorate the positive energy balance in DIO.

Even though previous studies have indicated that decreased BBB permeability and giosis (Balland et al., 2014; Caro et al., 1996; El-Haschimi et al., 2000; Horvath et al., 2010; Schwartz et al., 1996; Thaler et al., 2012) also contribute to cellular leptin resistance in obesity, we found that neither was altered by PTP1B/TCPTP ARC deletion. Suppressor of cytokine signaling (SOCS)-3 has also been reported to negatively regulate ARC leptin and insulin signaling (Bjørbaek et al., 1998; Kievet et al., 2006; Mori et al., 2004; Münzberg et al., 2004). Hypothalamic SOCS-3 is increased in obesity and might to contribute to the development of CNS leptin and insulin resistance (Bjørbaek et al., 1998; Kievet et al., 2006; Mori et al., 2004; Münzberg et al., 2004). Despite this, we found that the deletion of SOCS-3 had no additional impact on body weight, feeding, WAT browning, or glucose homeostasis beyond that achieved already by deleting PTP1B and TCPTP in the ARC (unpublished data). Although we have not explored the contributions of other mediators of leptin and insulin resistance, such as inflammation and ER stress (Pan and Myers, 2018), it is important to note that such mediators can elicit their effects at least in part via the induction of PTP1B (Williams et al., 2014; Zabolotny et al., 2008). Irrespective, our findings indicate that the deletion of PTP1B and TCPTP in the ARC is sufficient to markedly alleviate cellular leptin and insulin resistance.

PTP1B has been a long-standing therapeutic target for obesity and type 2 diabetes. Mice that are globally deficient for PTP1B have improved systemic insulin sensitivity attributable to enhanced insulin signaling in liver and muscle and resistance to DIO due to enhanced brain leptin sensitivity (Bence et al., 2006; Delibegovic et al., 2007, 2009; Eichelby et al., 1999; Kåman et al., 2000). However, recent studies have shown that PTP1B deficiency in myeloid cells can result in the development of leukemia and shorten lifespan (Le Sommer et al., 2018). On the other hand, global TCPTP deficiency is accompanied by the development of systemic inflammation and morbidity and mortality (Wiede et al., 2012; You-Ten et al., 1997), whereas its deletion in hematopoietic cells, or T cells, results in the onset of leukemia and shorten lifespan (Le Sommer et al., 2018).
of inflammatory disease and autoimmunity (Wiede et al., 2011, 2017). Consistent with this, genome-wide association studies have shown that loss of function PTPN2 single nucleotide polymorphisms are associated with autoimmunity, including type 1 diabetes, rheumatoid arthritis, and Crohn’s disease (Long et al., 2011; Smyth et al., 2008; Todd et al., 2007). The potential for the development of hematological malignancies and inflammation and autoimmunity precludes the use of systemic pharmacotherapies targeting PTP1B and TCPTP in obesity.

The intranasal route has garnered significant attention as a viable option for the specific delivery of hormones and drugs to the brain. In humans, intranasal insulin delivery promotes systemic insulin sensitivity in lean, but not obese, insulin resistant participants (Heni et al., 2014, 2017). Intranasal insulin might avoid the hypoglycemia that can result from peripheral insulin administration (Cryer, 2008). Moreover, beyond its effects in glucose metabolism, the intranasal administration of insulin can enhance cognitive function and might be effective in combating neurodegenerative diseases such as Alzheimer’s disease (de la Monte, 2012; Reger et al., 2006). Therefore, the intranasal route might afford an effective means by which to specifically and safely target PTP1B and TCPTP in the brain without systemic side effects. In our studies we took advantage of the PTP1B inhibitor, claramine. Claramine is highly specific for PTP1B and an analog of the natural compound trodusquemine (MSI-1436) (Qin et al., 2015) that has undergone phase Ib clinical trials for obesity and type 2 diabetes and shown to be well tolerated. To target TCPTP, we took advantage of the non-steroidal glucocorticoid antagonist activity of mifepristone (RU486). Glucocorticoids promote feeding and repress energy expenditure in response to stressors such as fasting but chronically promote weight gain and the metabolic syndrome in mice and humans (Gray et al., 1992; Hardwick et al., 1989; Pasquale et al., 2006; Razzoli and Bartolomucci, 2016; Strack et al., 1995; van den Beukel et al., 2014). We have shown previously that fasting-associated increases in corticosterone drive TCPTP expression in the ARC to repress insulin signaling and sympathetic nerve activity (SNA)-dependent brown and/or beige thermogenic activity and energy expenditure (Dodd et al., 2017). This response would appear to be specific to the hypothalamus as similar increases in TCPTP were not evident in other peripheral tissues, including liver and muscle, upon fasting (unpublished data). As such, glucocorticoid antagonism might selectively repress TCPTP in the hypothalamus. In this study, we have shown that the sustained and/or elevated TCPTP in the ARC of obese mice could be attributed largely to heightened corticosterone levels, as the sustained and/or elevated TCPTP expression could be corrected with the daily administration of RU486. In humans, an emerging body of evidence indicates that excess adiposity and obesity are accompanied by increased cortisol levels, at least in a subset of patients (Jackson et al., 2017; van Rossum, 2017) such as night shift workers (Manschijn et al., 2011), raising the possibility that dysregulated glucocorticoids might contribute the development and/or maintenance of obesity. Mifepristone has shown significant clinical and metabolic benefits in patients with Cushing’s syndrome (Fleseriu et al., 2012) and has been shown to reduce weight gain in patients treated with antipsychotic agents (Gross et al., 2009, 2010). Repurposing mifepristone for intranasal delivery to repress TCPTP might afford an effective approach for increasing energy expenditure in obesity. However, the intranasal delivery of specific TCPTP inhibitors or dual PTP1B/TCPTP inhibitors might ultimately be more effective. The systemic administration of the natural compound celastrol promotes weight loss in obese mice (Liu et al., 2015), and this is reliant on the inhibition of PTP1B and TCPTP in the hypothalamus (Kyriakou et al., 2018). Although celastrol is not specific for PTP1B and TCPTP and has a growing list of targets and biological activities (Cascão et al., 2017; Hu et al., 2017; Ma et al., 2015), derivatives that may be more specific are under development (Cascão et al., 2017).

As sustained changes in lifestyle and diet remain a seemingly insurmountable challenge for many overweight and obese individuals, there is an unmet need for pharmacotherapeutic approaches that can independently increase energy expenditure and repress feeding. Our findings define an approach by which to reinstate hypothalamic leptin and insulin sensitivity, increase non-shivering thermogenesis and energy expenditure, and repress feeding to combat the obesity epidemic.

STAR METHODS

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

- **KEY RESOURCES TABLE**
- **LEAD CONTACT AND MATERIALS AVAILABILITY**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Intra-ARC rAAV injections
  - Immunohistochemistry
  - Functional Immunohistochemistry
  - Lateral ventricle cannulations
  - Intranasal drug administration
  - Double blinded experimental paradigm
  - Metabolic measurements
  - Pair feeding paradigm
  - Immunoblotting
  - Sympathetic denervation
  - Quantitative PCR
  - Blood brain barrier permeability
  - Leptin sensitivity
  - Thermography
  - Saccharin preference test
  - Feeding behavioral analysis
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
- **DATA AND CODE AVAILABILITY**

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.08.019.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Health and Medical Research Council (NHMRC) of Australia to T.T. (1102240), T.T. (1103037), M.A.C. (1079422), and Z.B.A. (1154974) are NHMRC Research Fellows.
AUTHOR CONTRIBUTIONS

T.T. and G.T.D. conceived, conceptualized, and designed the study; wrote the manuscript; and interpreted the data. Other authors performed and/or analyzed experiments and/or contributed to the reviewing and editing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


Tsu, R.C., Rak, K.S., Zimmer, D.J., and Bence, K.K. (2014). Improved metabolic phenotype of hypothalamic PTP1B deficiency is dependent upon the leptin receptor. Mol. Metab. 3, 301–312.


