Leptin receptor-expressing neuron Sh2b1 supports sympathetic nervous system and protects against obesity and metabolic disease

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Leptin stimulates the sympathetic nervous system (SNS), energy expenditure, and weight loss; however, the underlying molecular mechanism remains elusive. Here, we uncover Sh2b1 in leptin receptor (LepR) neurons as a critical component of a SNS/brown adipose tissue (BAT)/thermogenesis axis. LepR neuron-specific deletion of Sh2b1 abrogates leptin-stimulated sympathetic nerve activation and impairs BAT thermogenic programs, leading to reduced core body temperature and cold intolerance. The adipose SNS degenerates progressively in mutant mice after 8 weeks of age. Adult-onset ablation of Sh2b1 in the mediobasal hypothalamus also impairs the SNS/BAT/thermogenesis axis; conversely, hypothalamic overexpression of human SH2B1 has the opposite effects. Mice with either LepR neuron-specific or adult-onset, hypothalamic-specific ablation of Sh2b1 develop obesity, insulin resistance, and liver steatosis. In contrast, hypothalamic overexpression of SH2B1 protects against high fat diet-induced obesity and metabolic syndromes. Our results unravel an unrecognized LepR neuron Sh2b1/SNS/BAT/thermogenesis axis that combats obesity and metabolic disease.
Adipose hormone leptin critically regulates body weight and metabolism, and disruption of leptin/leptin receptor (LepR) signaling results in morbid obesity and severe metabolic disease. LepR is widely expressed in the hypothalamus, including the preoptic area (POA), lateral hypothalamus, dorsomedial hypothalamus (DMH), ventromedial hypothalamus, and arcuate nucleus (ARC). Leptin exerts its anti-obesity action by activating LepR signaling in hypothalamic energy balance circuits, but it remains elusive whether leptin regulates energy expenditure vs energy intake by similar or discrete pathways. Leptin signaling is mediated by tyrosine kinase JAK2 that interacts with long-form LepR. Of note, a number of negative regulators of JAK2, including SOCS3, PTP1B, RPTPε, and TCPTP, have been reported to promote obesity, supporting the notion that JAK2 inhibitory molecules increase risk for leptin resistance, obesity, and metabolic disease. Interestingly, we identified a JAK2-binding protein Sh2b1 as a potent positive regulator of JAK2 (refs. 13,14); however, JAK2/Sh2b1 pathways in LepR neurons are lost in Sh2b1 conditional knockout mice. JAK2-Sh2b1 binding protein Sh2b1 as a potent positive regulator of kinase activity. Sh2b1 also binds to IRS1 and IRS2 and links it to JAK2 via its SH2 domain and robustly enhances JAK2 kinase activity. Sh2b1 also binds to IRS1 and IRS2 and links JAK2 to IRS1/2-mediated activation of the PI 3-kinase pathway in cell cultures. Aside from JAK2, Sh2b1 also binds to receptor tyrosine kinases, including insulin receptors, platelet-derived growth factor receptors, nerve growth factor receptor TrkA, and brain-derived neurotrophic factor (BDNF) receptor TrkB.

To examine Sh2b1 function in vivo, we generate and characterize global Sh2b1 knockout mice. Sh2b1-null mice develop severe leptin resistance, obesity, and type 2 diabetes. We and other groups further demonstrate that the metabolic function of Sh2b1 has conserved from flies to humans. Deletion of Sh2b1 results in fat accumulation in Drosophila. In human genome-wide association studies, numerous SH2B1 single-nucleotide polymorphisms (SNPs) have been identified to link to obesity, type 2 diabetes, and cardiovascular diseases. Deletion of chromosomal 16p11.2, which encompasses the SH2B1 gene, is associated with severe obesity in humans. Human SH2B1 missense mutations are cosegregated with the obesity and metabolic disease traits. Thus, SH2B1 is emerging as a critical regulator of body weight and metabolism in both animals and humans; however, SH2B1 target cell types remain poorly understood.

We report that neuron-specific restoration of Sh2b1 expression reverses the obesity phenotypes of Sh2b1-null mice, indicating that neurons mediate Sh2b1 actions on body weight and metabolism. Given that Sh2b1 augments LepR/JAK2 signaling in cell cultures, we postulate that Sh2b1 might cell-autonomously increase the ability of LepR neurons to control energy balance and body weight, perhaps by directly enhancing leptin signaling. In this study, we generate and characterize LepR cell-specific Sh2b1 knockout (Sh2b1<sup>−/−</sup>) mice. Sh2b1<sup>−/−</sup> mice, like global Sh2b1 knockout mice, develop obesity, insulin resistance, and liver steatosis. Remarkably, Sh2b1 deficiency in LepR neurons abrogates the ability of leptin to stimulate sympathetic nerves innervating brown adipose tissue (BAT), leading to BAT dysfunction and reduced core body temperature in Sh2b1<sup>−/−</sup> mice. Collectively, our results unveil an unrecognized leptin/Sh2b1/sympathetic nerve/adipose thermogenesis axis that combats obesity, type 2 diabetes, and liver steatosis.

**Results**

**Sh2b1<sup>ΔLepR</sup> mice spontaneously develop obesity.** To determine the role of Sh2b1 in LepR neurons, we generated Sh2b1<sup>ΔLepR</sup> mice (Sh2b1<sup>ΔLepR</sup>/LepR-Cre<sup>+/−</sup>) by crossing Sh2b1<sup>−/−</sup> mice with LepR-Cre drivers. LepR-Cre mice were characterized previously. Mice were in a C57BL/6J background and fed a standard chow diet. Sh2b1<sup>ΔLepR</sup> male and female mice progressively became heavier than sex/age-matched Sh2b1<sup>+/−</sup> and LepR-Cre mice (Fig. 1a). Fat content was dramatically higher in Sh2b1<sup>ΔLepR</sup> males and females relative to sex/age-matched Sh2b1<sup>+/−</sup> and LepR-Cre mice (Fig. 1b).

Both gonadal and inguinal white adipose tissue (WAT) depots were significantly larger in Sh2b1<sup>ΔLepR</sup> relative to Sh2b1<sup>+/−</sup> and LepR-Cre mice (Supplementary Fig. 1a). Individual white adipocyte size was substantially larger in Sh2b1<sup>ΔLepR</sup> than in LepR-Cre mice (Fig. 1c). Lean mass was not significantly different between Sh2b1<sup>ΔLepR</sup> and LepR-Cre mice (Supplementary Fig. 1b). To gain insight into the underlying mechanism, we analyzed energy balance. Food intake was relatively normal (Fig. 1d). O<sub>2</sub> consumption and CO<sub>2</sub> production (per mouse) were also not significantly different between Sh2b1<sup>ΔLepR</sup> and Sh2b1<sup>+/−</sup> mice (Fig. 1e, Supplementary Fig. 1c). Of note, O<sub>2</sub> consumption and CO<sub>2</sub> production, after normalization to body weight, were significantly lower in Sh2b1<sup>ΔLepR</sup> males and females relative to sex/age-matched Sh2b1<sup>+/−</sup> mice (Supplementary Fig. 1d, e). Core body temperature was significantly lower in Sh2b1<sup>ΔLepR</sup> males and females relative to sex/age-matched LepR-Cre or Sh2b1<sup>+/−</sup> mice (Fig. 1f, g).

We further confirmed that Sh2b1<sup>ΔLepR</sup> mice had lower body temperature using E-Mitters, and Sh2b1<sup>ΔLepR</sup> locomotor activity was relatively normal (Fig. 1h). These data indicate that Sh2b1 in LepR neurons is indispensable for the maintenance of both body weight and core body temperature.

**Sh2b1<sup>ΔLepR</sup> mice develop insulin resistance and liver steatosis.** Obesity promotes type 2 diabetes and nonalcoholic fatty liver disease (NAFLD), prompting us to assess insulin sensitivity and hepatic lipid content. Sh2b1<sup>ΔLepR</sup> males and females developed hyperglycemia and hyperinsulinemia compared to sex/age-matched LepR-Cre mice at 19–20 weeks of age (Fig. 2a). In glucose (GTG) or insulin (ITT) tolerance tests, blood glucose levels were markedly higher in Sh2b1<sup>ΔLepR</sup> males and females relative to sex/age-matched LepR-Cre or Sh2b1<sup>+/−</sup> mice (Fig. 2b).

Consistently, insulin-stimulated phosphorylation of Akt (pThr308 and pSer473) in liver and skeletal muscle was substantially lower in Sh2b1<sup>ΔLepR</sup> than in LepR-Cre mice (Fig. 2c). Sh2b1<sup>ΔLepR</sup> mice also developed severe liver steatosis, as demonstrated by markedly increased lipid droplet number and size and triacylglycerol (TAG) levels in the liver (Fig. 2d, e). These results suggest that Sh2b1 in LepR neurons combats against insulin resistance, type 2 diabetes, and NAFLD.

**Adult-onset ablation of hypothalamic Sh2b1 results in obesity.** We recently reported that neuronal Sh2b1 promotes brain development. To distinguish between brain development-dependent and -independent actions of Sh2b1 on body weight and metabolism, we generated adult-onset, hypothalamus-specific Sh2b1 knockout mice by bilaterally microinjecting AAV1-hSyn-Cre vectors into the mediobasal hypothalami (MBH) of Sh2b1<sup>+/−</sup> males at 10 weeks of age. AAV1-hSyn-green fluorescent protein (GFP) vectors were used as control. Bilateral MBH injections were histologically verified (Supplementary Fig. 2a). MBH-specific ablation of Sh2b1 substantially increased body weight and fat content (Fig. 3a, b). As an additional control, AAV1-hSyn-Cre vectors were bilaterally injected into the MBH of wild-type C57BL/6 mice. There was no difference in body weight and fat content between the AAV1-hSyn-Cre and AAV1-hSyn-GFP groups (Supplementary Fig. 2b–c). O<sub>2</sub> consumption and CO<sub>2</sub> production (per mouse) were not significantly different between the AAV1-hSyn-Cre and AAV1-hSyn-GFP groups (Fig. 3c). Nonetheless, O<sub>2</sub> consumption and CO<sub>2</sub> production, after normalization to body weight, were significantly lower in AAV1-hSyn-Cre relative to AAV1-hSyn-GFP groups in the dark.
Fig. 1 Sh2b1ΔLepR mice develop obesity. a Growth curves. Male: ΔLepR-Cre: n = 19, Sh2b1ΔLepR: n = 25; female: ΔLepR-Cre: n = 15, Sh2b1ΔLepR: n = 16. b Fat content. Male (22 weeks): ΔLepR-Cre: n = 8, Sh2b1ΔLepR: n = 8; females (20 weeks): ΔLepR-Cre: n = 8, Sh2b1ΔLepR: n = 5. c Representative H&E staining of epididymal WAT sections at 22 weeks of age (3 pairs). Scale bar: 200 μm. d Food intake of males at 10 weeks of age. LepR-Cre: n = 7, Sh2b1ΔLepR: n = 8. e O2 consumption and CO2 production at 10 weeks of age. Male: ΔLepR-Cre: n = 5, Sh2b1ΔLepR: n = 7. f-g Rectal temperature at 20 weeks of age. Male: Cre: n = 4, Sh2b1ΔLepR: n = 6; female: ΔLepR-Cre: n = 8, Sh2b1ΔLepR: n = 8. h Core body temperature and locomotor activity in male mice (10 weeks) recorded using pre-implanted E-Mitters. LepR-Cre: n = 4, Sh2b1ΔLepR: n = 6. Data are presented as mean ± SEM. *p < 0.05, two-tailed unpaired Student’s t-test (b female, d-g), two-way ANOVA (a), or one-way ANOVA (b male). Source data are provided as a Source Data file.

Insulin-stimulated phosphorylation of hepatic Akt was lower in AAV1-hSyn-Cre-transduced than in AAV1-hSyn-GFP-transduced Sh2b1ΔLepR mice (Fig. 3h). MBH-specific Sh2b1 knockout mice also developed severe liver steatosis, as demonstrated by elevated levels of hepatocyte lipid droplets (Oil Red O staining of liver sections) and high levels of liver TAG (Fig. 3i). These data indicate that Sh2b1 in

Phase (Supplementary Fig. 2d). Core body temperature was significantly lower in the AAV1-hSyn-Cre mice in the dark cycle (Fig. 3d). Notably, MBH-specific ablation of Sh2b1 significantly increased food intake (Fig. 3e). Like Sh2b1ΔLepR mice, adult-onset and MBH-specific Sh2b1 knockout mice developed hyperinsulinemia, glucose intolerance, and insulin resistance (Fig. 3f, g).
the MBH regulates body weight, metabolism, food intake, and/or body temperature independently of its action on brain development.

**Hypothalamic overexpression of SH2B1 ameliorates obesity.** To determine whether MBH-specific overexpression of human SH2B1 protects against obesity, AAV9-CAG-SH2B1 or AAV9-CAG-GFP (control) vectors were bilaterally injected into the MBH of C57BL/6J males. Mice were fed an HFD to induce obesity. Recombinant SH2B1 was detected in AAV9-CAG-SH2B1-transduced but not AAV9-CAG-GFP-transduced mice (Supplementary Fig. 3a, b). Body weight and fat content were significantly lower in the AAV9-CAG-SH2B1 group relative to...
Overnight fasting plasma insulin levels in 10 weeks after transduction. Liver extracts were immunoblotted with the indicated antibodies. Overexpression of SH2B1 in the hypothalamus also blocked HFD-induced insulin resistance and glucose intolerance, as assessed by ITT, GTT, and NAFLD in adult mice. Perhaps in LepR neurons, protects against obesity, type 2 diabetes, and NAFLD in adult mice.

**LepR neuron Sh2b1 is required for brown fat thermogenesis**.

Given that BAT and beige fat promote adaptive thermogenesis, energy expenditure, and weight loss, we examined the impact of hypothalamic Sh2b1 on BAT activity. Ablation of Sh2b1 in either LepR neurons (Sh2b1<sup>ΔLepR</sup> mice) or the MBH (AAV1-hSyn-Cre-transduced Sh2b1<sup>ΔLepR</sup> mice) caused whitening of BAT (e.g. enlarged lipid droplets) and dramatic downregulation of uncoupling protein 1 (Ucp1) (Fig. 5a). Ucp1 protein and mRNA were barely detectable in Sh2b1<sup>ΔLepR</sup> mice at 22 weeks of age (Fig. 5b, c). Ucp1 expression in inguinal WAT markedly decreased in Sh2b1<sup>ΔLepR</sup> mice (Supplementary Fig. 1g). Of note, absolute expression levels of Ucp1 was markedly higher in BAT than in WAT. Conversely, MBH-specific overexpression of SH2B1 reversed HFD-induced

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**Fig. 3 Adult-onset, MBH-specific ablation of Sh2b1 results in obesity, insulin resistance, and liver steatosis.** AAV1-hSyn-Cre or AAV1-hSyn-GFP vectors were bilaterally injected into the MBH of Sh2b1<sup>fl/fl</sup> males at 10 weeks of age. a Growth curves. b Fat content in 10 weeks post transduction. c Energy expenditure in 10 weeks after transduction. d Rectal temperature in 10 weeks following transduction. e Food intake at 10 weeks post transduction. f Overnight fasting plasma insulin levels in 10 weeks after transduction. g GTT and ITT in 10-11 weeks post transduction. h Mice were stimulated with insulin in 11 weeks post transduction. Liver extracts were immunoblotted with the indicated antibodies (n = 3 mice per group). i Representative Oil Red O staining of liver sections and liver TAG levels (normalized to liver weight) in 11 weeks post transduction. a, b, e, g, i AAV1-hSyn-GFP: n = 7, AAV1-hSyn-Cre: n = 7; AAV1-hSyn-GFP: n = 7, AAV1-hSyn-Cre: n = 5; f: AAV1-hSyn-GFP: n = 5, AAV1-hSyn-Cre: n = 5. Scale bar: 100 μm. Data are presented as mean ± SEM. *p < 0.05, two-tailed unpaired Student’s t-test. Source data are provided as a Source Data file.
whitening of BAT and downregulation of Ucp1 (Fig. 5a). Ucp1 protein and mRNA levels were substantially higher in AAV9-CAG-SH2B1-transduced relative to AAV9-CAG-GFP-transduced mice (Fig. 5b, c). Ucp1 mediates adaptive thermogenesis and energy expenditure42. Accordingly, Sh2b1ΔLepR mice displayed markedly lower core body temperature compared to LepR-Cre mice upon cold exposure (on a chow diet), and mice with MBH-specific overexpression of SH2B1 (on a HFD) had the opposite effects (Fig. 5d). Thus, we uncovered an unrecognized hypothalamic Sh2b1/BAT axis that critically regulates adaptive thermogenesis and core body temperature.

LepR neuron Sh2b1 mediates leptin stimulation of the SNS. Considering the pivotal role of the sympathetic nervous system (SNS) in BAT activation, we assessed the impact of Sh2b1 deficiency on the ability of leptin to stimulate sympathetic nerve transmissions in BAT. Both male and female Sh2b1ΔLepR mice developed hyperleptinemia (Fig. 6a). Leptin stimulated phosphorylation of hypothalamic Stat3 to a lower degree in Sh2b1ΔLepR relative to Sh2b1f/f mice (Fig. 6b, c). Conversely, MBH-specific overexpression of SH2B1 augmented leptin-stimulated phosphorylation of hypothalamic Stat3 (Fig. 6b). To directly assess sympathetic nerve activity (SNA), we electrophysiologically recorded BAT SNA in Sh2b1ΔLepR mice at both 6 weeks (prior to the onset of obesity) and 12 weeks of age. Baseline SNA was significantly lower in Sh2b1ΔLepR relative to Sh2b1f/f mice (Fig. 6d, e). In agreement with the previous reports43, central injection of leptin progressively and markedly increased SNA in wild type (i.e. Sh2b1f/f) mice (Fig. 6d, f). It is likely that multiple synaptic modifications and/or polysynaptic transmissions contribute to a delayed onset of the leptin action on SNA. Strikingly, deletion of
Sh2b1 in LepR neurons completely abrogated the ability of leptin to stimulate SNA in Sh2b1ΔLepR mice (Fig. 6f). These results indicate the Sh2b1 branch of LepR signaling pathways is required for leptin to stimulate the SNS.

We next set out to examine neuronal activity (c-Fos expression as a surrogate marker) in the central sympathetic network, focusing on the POA, DMH, ARC, paraventricular hypothalamus (PVH), and rostral raphe pallidus (rRPa). These regions are known to control sympathetic outflows to BAT14. Cold exposure rapidly and robustly increased the number of c-Fos neurons in Sh2b1f/f mice (Fig. 6g, h). Sh2b1 deficiency substantially suppressed cold-stimulated neuronal activation in the POA, DMH, and rRPa of Sh2b1ΔLepR mice (Fig. 6g, h). In the ARC, neural activity was significantly lower in Sh2b1ΔLepR relative to Sh2b1f/f mice at both 22 and 4 °C (Fig. 6h).

To further confirm the role of LepR neuron Sh2b1 in regulating the SNS, we measured the levels of tyrosine hydroxylase (TH), a sympathetic nerve marker, in BAT. We previously validated that anti-TH antibody specifically recognizes TH in immunostaining45. Immunoreactivity to TH was dramatically lower in Sh2b1ΔLepR relative to LepR-Cre mice (Fig. 5a). Likewise, TH levels in BAT were also markedly reduced by MBH-specific ablation of Sh2b1 (AAV-Cre vs AAV-GFP groups) (Fig. 5a). Conversely, MBH-specific overexpression of SH2B1 increased BAT TH levels (AAV-SH2B1 vs AAV-GFP) (Fig. 5a). Collectively, these results unveil an
unrecognized Sh2b1/hypothalamic sympathetic network/SNS/BAT energy expenditure axis.

LepR neuron Sh2b1 preserves adipose SNS integrity in aging. Sh2b1ΔLepR mice develop obesity in an age-dependent manner, prompting us to examine age-associated SNS degeneration. BAT TH levels were normal in young Sh2b1ΔLepR mice prior to 8 weeks of age (Fig. 7a, b), indicating that Sh2b1 in LepR neurons is dispensable for the development of adipose SNS. TH levels decreased progressively post 8 weeks of age and were barely

| Plasma leptin (ng/ml) | Male | Female |
|-----------------------|------|--------|
| PBS                   | 5    | 10     |
| Leptin                | 20   | 25     |

Plasma leptin (ng/ml) | Male | Female |
|----------------------|------|--------|
| PBS                  | 5    | 10     |
| Leptin               | 20   | 25     |

f/f | PBS | Leptin |
|-----|-----|--------|
| Stat3 | f/f | Stat3 |
| PBS | Leptin | PBS | Leptin |

f/f | PBS | Leptin |
|-----|-----|--------|
| Blot | f/f | Blot |
| PBS | Leptin | PBS | Leptin |

f/f | PBS | Leptin |
|-----|-----|--------|
| pStat3 | f/f | pStat3 |
| Blot | f/f | Blot |
| PBS | Leptin | PBS | Leptin |

f/f | PBS | Leptin |
|-----|-----|--------|
| G0 | f/f | G0 |
| PBS | Leptin | PBS | Leptin |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |

f/f | PBS | Leptin |
|-----|-----|--------|
| POA | f/f | POA |
| rRPa | f/f | rRPa |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |

f/f | PBS | Leptin |
|-----|-----|--------|
| DMH | f/f | DMH |
| Arc | f/f | Arc |

f/f | PBS | Leptin |
|-----|-----|--------|
| POA | f/f | POA |
| rRPa | f/f | rRPa |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |

f/f | PBS | Leptin |
|-----|-----|--------|
| POA | f/f | POA |
| DMH | f/f | DMH |
| Arc | f/f | Arc |
| rRPa | f/f | rRPa |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |

f/f | PBS | Leptin |
|-----|-----|--------|
| POA | f/f | POA |
| DMH | f/f | DMH |
| Arc | f/f | Arc |
| rRPa | f/f | rRPa |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |

f/f | PBS | Leptin |
|-----|-----|--------|
| POA | f/f | POA |
| DMH | f/f | DMH |
| Arc | f/f | Arc |
| rRPa | f/f | rRPa |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |

f/f | PBS | Leptin |
|-----|-----|--------|
| POA | f/f | POA |
| DMH | f/f | DMH |
| Arc | f/f | Arc |
| rRPa | f/f | rRPa |

f/f | PBS | Leptin |
|-----|-----|--------|
| 22 °C | 4 °C | 22 °C | 4 °C |
| 22 °C | 4 °C | 22 °C | 4 °C |
detectable in Sh2b1ΔLepR mice at 22 weeks of age (Fig. 7a, b). To confirm sympathetic degeneration, we assessed the levels of class III β-tubulin, a neuronal marker, using antibody TUJ1. TUJ1 immunoreactivity in BAT was abundant in Sh2b1ΔLepR but not Sh2b1ΔLep mice at 22 weeks of age (Fig. 7c). Of note, Ucp1 downregulation followed the course of SNS deterioration in Sh2b1ΔLep mice (Fig. 7d).

Next, we asked whether Sh2b1 deficiency in LepR neurons worsens SNS degeneration in white adipose tissue (WAT). Because sympathetic innervation of WAT is sparse and difficult to be detected,54,55, we assessed phosphorylation of hormone-sensitive lipase (HSL), a surrogate marker for SNS activation. HSL phosphorylation (pSer563 and pSer660) was normal or slightly higher in Sh2b1ΔLep mice at 3 weeks of age; thereafter, HSL phosphorylation decreased progressively and became barely detectable at 22 weeks of age (Fig. 7e). Collectively, these results suggest that Sh2b1 in LepR neurons is involved in preserving the SNS in both BAT and WAT during aging.

Deletion of POMC neuron Sh2b1 is unable to induce obesity

We next aimed to further map Sh2b1 target neurons. Brain sections were prepared from wild-type and global Sh2b1 knockout (negative control) mice and stained with anti-Sh2b1 antibody. Sh2b1 was detected in hypothalamic cells in wild-type mice (Supplementary Fig. 4a). To confirm these results, we generated Sh2b1-Cre knockin mice by inserting an IRES-eGFP-2A-Cre cassette into the Sh2b1 locus 3′ to the STOP codon (Supplementary Fig. 4b). GFP levels in Sh2b1-Cre mice were below detection thresholds. To facilitate detection of Sh2b1 neurons, Sh2b1-Cre drivers were crossed with Rosa-mTmG reporter mice to genetically label Sh2b1 neurons with mGFP in Sh2b1-Cre;Rosa-mTmG mice. We found that mGFP (a marker for expression of endogenous Sh2b1) was expressed in most of hypothalamic cells in Sh2b1-Cre;Rosa-mTmG but not Rosa-mTmG mice (Supplementary Fig. 4c). In line with these findings, Sh2b1 protein is detected in the entire brain by immunohistochemistry.16 To confirm that proopiomelanocortin (POMC) and AgRP neurons express Sh2b1, hypothalamic sections were prepared from Sh2b1-Cre;Rosa-mTmG mice and immunostained with antibodies to POMC and AgRP. Both POMC and AgRP neurons expressed mGFP (Supplementary Fig. 4d).

To explore the role of Sh2b1 in POMC neurons, we generated POMC neuron-specific Sh2b1 knockout (Sh2b1ΔPOMC) mice (Sh2b1ΔLepR;POMC-Cre+/−) by crossing Sh2b1ΔLepR mice with POMC-Cre drivers. Unlike Sh2b1ΔLepR mice, Sh2b1ΔPOMC mice were grossly normal when fed a chow diet (Supplementary Fig. 5a). We placed Sh2b1ΔLepR mice on HFD for 10 weeks. Body weight, fat content, GTT, and ITT were comparable between Sh2b1ΔLepR, POMC-Cre, and Sh2b1ΔLep mice (Supplementary Fig. 5b–e). Together, these data suggest that POMC neuron-specific ablation of Sh2b1 is insufficient to induce obesity and metabolic disease.

Discussion

We herein identify LepR neurons as key Sh2b1 targets that mediate Sh2b1 protection against obesity, type 2 diabetes, and NAFLD. We demonstrated that LepR neuron-specific deletion of Sh2b1, or adult-onset deletion of Sh2b1 in the hypothalamus (containing LepR neurons), resulted in severe obesity, insulin resistance, and liver steatosis. Conversely, MBH-specific overexpression of SH2B1 ameliorated HFD-induced obesity and metabolic syndromes. Leptin stimulation of the hypothalamic JAK2/Stat3 pathway was impaired in Sh2b1ΔLepR mice, supporting the notion that Sh2b1 is an endogenous sensitizer for leptin action, perhaps by enhancing JAK2 activation. Remarkably, ablation of Sh2b1 in LepR neurons abrogated the ability of leptin to stimulate sympathetic nerves projecting to BAT. Likewise, adult-onset, MBH-specific ablation of Sh2b1 also impaired sympathetic transmissions in BAT. BAT became whitening and impaired in adaptive thermogenesis in both Sh2b1ΔLepR mice and mice with MBH-specific ablation of Sh2b1, presumably owing to adipose SNS-deficits. Consequently, core body temperature was low and cold tolerance was impaired in both Sh2b1ΔLepR mice and MBH-specific Sh2b1 knockout mice. These findings define LepR neuron Sh2b1 as a critical central regulator of thermogenesis and body temperature. Thus, we unveil an unrecognized leptin/LepR neuron Sh2b1 as a critical central regulator of thermogenesis and body temperature axis. However, we cannot exclude the possibility that hypothermic Sh2b1 may increase thermogenesis and body temperature by an additional leptin-independent mechanism. For instance, Sh2b1 may enhance the ability of interleukin-6, a well-known pyrogenic cytokine, to increase thermogenesis and body temperature through enhancing the JAK2/Stat3 pathway. Furthermore, hypothermic Sh2b1 may increase body temperature by a SNS-independent mechanism, perhaps by enhancing the ability of hypothalamic–pituitary–thyroid axis to increase thermogenesis and body temperature. Given that the SNS/BAT pathway increases energy expenditure and weight loss, the LepR neuron Sh2b1/SNS/BAT/thermogenesis/body temperature axis. However, we cannot exclude the possibility that hypothermic Sh2b1 may increase thermogenesis and body temperature by an additional leptin-independent mechanism. For instance, Sh2b1 may enhance the ability of interleukin-6, a well-known pyrogenic cytokine, to increase thermogenesis and body temperature through enhancing the JAK2/Stat3 pathway. Further-
not required for adipose SNS development; rather, it plays an important role in preserving the adipose SNS against degeneration. Given that Sh2b1 is expressed broadly in the hypothalamus, it is not surprising that the neuronal activity of the central sympathetic network, particularly in the POA, DMH, and rRPa, is inhibited in \( \text{Sh2b1}^{\Delta \text{LepR}} \) mice. These results suggest that hypothalamic Sh2b1 preserves adipose SNS integrity and sympathetic transmissions by a top-down mechanism, perhaps by enhancing leptin and/or other hormone and neuropeptide signaling in the central sympathetic network. We acknowledge that our data do not exclude the possibility that adipose SNS deterioration may be secondary to obesity in \( \text{Sh2b1}^{\Delta \text{LepR}} \) mice. Additional studies are warranted to further characterize hypothalamic Sh2b1 circuits that protect against adipose SNS degeneration.

**Fig. 7 Sh2b1 in LepR neurons supports the maintenance of adipose SNS.** a, b BAT sections were prepared from male mice at 8, 12, and 22 weeks of age and stained with anti-tyrosine hydroxylase (TH) antibodies. a Representative images (\( n = 3 \) mice per group). b TH areas were quantified and normalized to total areas (\( n = 3 \) mice per group). c BAT sections were prepared at 22 weeks of age and stained with anti-TUJ1 antibody. TUJ1 areas were normalized to total areas. \( \text{Sh2b1}^{\Delta \text{LepR}} \): \( n = 6 \), \( \text{Sh2b1}^{\text{f/f}} \): \( n = 4 \). d, e BAT (d) and epididymal WAT (e) extracts were prepared from male mice at 3, 12, and 22 weeks of age and immunoblotted with the indicated antibodies. Each lane represents an individual mouse. Data are presented as mean ± SEM. *\( p < 0.05 \), two-tailed unpaired Student’s t-test. Source data are provided as a Source Data file.
The hypothalamic PI 3-kinase pathway was reported to mediate thereby masking difference between these two groups. Notably, the hypothalamic PI 3-kinase pathway was reported to mediate leptin stimulation of the SNS against degeneration. The Sh2b1/SNS/fat axis may act as a potential therapeutic target for the treatment of obesity and metabolic disease. The Sh2b1/SNS/fat axis may serve as a potential therapeutic target for the treatment of obesity and metabolic disease.

Animals. Sh2b1f/f mice were euthanized and organs were harvested and weighted. Liver samples were homogenized in 1% acetic acid and extracted using chloroform:methanol (2:1). The organic phase was dried via evaporation and dissolved in isopropanol. Tissue extracts were immunblotted with the indicated antibodies (Supplemental Table 1). Images were visualized using a BX51 Microscope (Olympus, Tokyo, Japan) and a DP72 digital camera (Olympus, Tokyo, Japan).

Immunostaining. Brain and BAT sections were prepared using a Leica cryostat (Leica Biosystems Nussloch GmbH, Nussloch, Germany), and immunostained with the indicated antibodies (Supplemental Table 1). Images were analyzed using the BD FACSCanto II flow cytometer (BD Biosciences) and the FlowJo software (TreeStar, Ashland, OR).

Statistical analysis. Data were presented as means ± SEM. Differences between two groups were analyzed by two-tailed Student’s t-test, and differences between more than two groups were analyzed using one-way and two-way analysis of variance (ANOVA) and Bonferroni posttest using GraphPad Prism 7. A P value less than 0.05 was considered significant.
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**Author contributions**

L.J., H. Su, X.W., H. Shen, and M.-H.K. conducted the experiments, L.J. and L.R. designed the experiments and wrote the paper, and L.J., H. Su, X.W., H. Shen, M.-H.K., Y.L., M.G.M., C.O., and L.R. performed data analyses and edited the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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