IDOL regulates systemic energy balance through control of neuronal VLDLR expression

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Liver X receptors limit cellular lipid uptake by stimulating the transcription of inducible degrader of the low-density lipoprotein receptor (IDOL), an E3 ubiquitin ligase that targets lipoprotein receptors for degradation. The function of IDOL in systemic metabolism is incompletely understood. Here we show that loss of IDOL in mice protects against the development of diet-induced obesity and metabolic dysfunction by altering food intake and thermogenesis. Unexpectedly, analysis of tissue-specific knockout mice revealed that IDOL affects energy balance, not through its actions in peripheral metabolic tissues (liver, adipose tissue, endothelium, intestine, and skeletal muscle) but by controlling lipoprotein receptor abundance in neurons. Single-cell RNA sequencing of the hypothalamus demonstrated that IDOL deletion altered gene expression linked to the control of metabolism. Finally, we identified very low-density lipoprotein receptor (VLDLR) rather than low-density lipoprotein receptor (LDLR) as the primary mediator of the effects of IDOL on energy balance. These data identify a role for the neuronal IDOL–VLDLR pathway in metabolic homeostasis and diet-induced obesity.

Lipoprotein receptors are key determinants of cardiovascular disease owing to their pivotal roles in regulating blood cholesterol levels. Assessment of the function and regulation of members of the low-density lipoprotein receptor (LDLR) superfamily has advanced our understanding of fundamental processes such as receptor-mediated endocytosis, neuronal development, and lipid-responsive transcription. Cells maintain optimal cholesterol levels, in part by regulating the uptake of cholesterol from circulating lipoproteins via LDLR. Sterol regulatory element binding proteins (SREBPs) are transcription factors that are activated by low cholesterol levels and stimulate the expression of genes that drive cholesterol synthesis and uptake, including LDLR. Conversely, when cells accumulate excess cholesterol, activation of the liver X receptors (LXRs) promotes the expression of genes that restore homeostasis by increasing cholesterol efflux and transport. The LXR and SREBP pathways also exert negative feedback on each other.

One LXR-mediated feedback mechanism is to limit the influx of lipoprotein cholesterol into cells via the inducible degrader of the LDLR (IDOL), an E3 ubiquitin ligase that targets members of the LDLR family for degradation. Previous work elucidated the molecular mechanisms underlying IDOL-dependent degradation of its lipoprotein receptor targets and addressed its role in the species-specific regulation of hepatic cholesterol metabolism. IDOL and its targets are expressed in a number of metabolically active tissues other than the liver, but the physiological role of IDOL in systemic lipid metabolism remains poorly understood. Furthermore, the lipoprotein receptor (or receptors) through which IDOL regulates metabolism are unknown.

Here we report that mice globally deficient in IDOL expression are protected against diet-induced obesity and metabolic dysfunction. Unexpectedly, loss of IDOL in individual metabolic tissues, including the liver, skeletal muscle, adipose tissue, and intestine, failed to recapitulate the phenotype of the whole-body knockouts, prompting us to search for an alternative mechanism. Ultimately, we traced this metabolic phenotype to loss of IDOL in the central nervous system (CNS), and to the consequent dysregulation of VLDLR protein levels. Deletion of IDOL from mouse neurons mimics the phenotype of the whole-body IDOL knockout mice. Our data identify the IDOL–VLDLR axis in neurons as a regulatory pathway that affects systemic energy balance.

Results
Global IDOL knockout mice are protected from diet-induced metabolic dysfunction. Previous efforts to characterize the physiological role of IDOL have focused primarily on its ability to regulate cholesterol metabolism. Unexpectedly, in the course of these studies, we noted that 18-month-old chow-fed IDOL knockout mice (developed at the University of California, Los Angeles (UCLA),...
USA) demonstrated that deletion of IDOL protected against age-induced obesity, even when the mice were fed a standard chow diet (Extended Data Fig. 1a,b). We then proceeded to challenge mice with obesogenic diets. Group-housed IDOL knockout mice fed a western diet enriched in fat and cholesterol for 15 weeks were 22% lighter than wild-type controls (Fig. 1a). Body composition analysis by magnetic resonance imaging (MRI) revealed that IDOL knockout mice were leaner, averaging 11% body fat compared to 32% in wild-type mice (Fig. 1b). This was accompanied by reduced hepatic lipid accumulation and smaller adipocytes in the inguinal white adipose tissue depot (Fig. 1c). As the gene encoding IDOL is an LXR target, we postulated that the differential response to diet may require dietary cholesterol to stimulate LXR activity; however, the IDOL knockout mice were also leaner when fed a high-fat diet (HFD) that contained comparatively little cholesterol (Extended Data Fig. 1c).

We also analysed a second, independently derived IDOL knockout mouse line generated by crossing Idol floxed mice developed at AstraZeneca, denoted IDOL(AZ), with a Rosa26-Cre transgenic line, denoted Cre26b (Supplementary Fig. 1). When maintained on a low-fat, low-cholesterol diet (LFD), these Cre26b/Idolf/f mice also showed a lean phenotype after 20 weeks of age (Extended Data Fig. 1d). The phenotype was exaggerated when the mice were challenged with a high-fat, high-cholesterol (HFHC) diet (Extended Data Fig. 1e).

We found no evidence of a generalized developmental growth defect in either strain of IDOL knockout mice. There were no differences in lean body mass (Extended Data Fig. 1f), nasal to anal length (Extended Data Fig. 1g), or body temperature attributable to genotype (Extended Data Fig. 1h). The reduced adiposity in the western diet-fed IDOL knockout mice was associated with improved glucose clearance (Fig. 1d, 9 weeks of western diet) and insulin tolerance (Fig. 1e, 14 weeks of western diet). Although we did not detect differences in food intake in the short term (<7 d), analysis of mice housed singly over the course of weeks (wild type, n = 14; IDOL knockout, n = 12) revealed reduced food intake in the genetic absence of IDOL (Fig. 1f), associated with reduced diet-induced adipose expansion (Fig. 1g,h).

Deletion of IDOL from peripheral metabolic tissues does not protect against obesity. We postulated that the metabolic phenotype of IDOL-deficient mice resulted from the primary actions of the IDOL pathway in one or more metabolic tissues. To test this idea, we crossed Idol floxed mice to a range of tissue-specific Cre transgenic mouse lines to generate tissue-selective IDOL knockouts. We first analysed the contribution of the liver to the IDOL knockout phenotype using Alb-Cre-IDOL(AZ)-floxed mice (Idol(AZ)/f; Cre26b) generated at AstraZeneca. Hepatic deletion of IDOL had no effect on the obesity of mice challenged with the obesogenic HFHC diet (Fig. 2a,f), nor on glucose metabolism (Extended Data Fig. 2a,b), suggesting that the liver is not the primary driver of the global IDOL knockout phenotype.

Given the reduced adiposity in IDOL knockout mice, we next considered that a direct effect of the IDOL pathway on adipose tissue might explain the phenotype. We crossed AdipoQ-Cre transgenic mice to Idol floxed mice to generate mice lacking IDOL expression in both white and brown fat (Idol(AZ)/f; Cre26). We challenged the mice with 15 weeks of western-diet feeding but saw no effect on mass or adiposity (Fig. 2b,g), or on glucose or insulin tolerance (Extended Data Fig. 2c,d). In a complementary approach, we developed a transgenic mouse expressing a dominant active form of human IDOL under the control of the Fabp4 promoter (aP2-Tg). This line showed nearly complete ablation of VLDR protein levels in both white and brown adipose depots, confirming the activation of the IDOL pathway (Extended Data Fig. 3a,b). Despite this change in VLDR levels, we observed no differences in mass between the

aP2-IDOL Tg mice and their littermate controls fed a western diet for 12 weeks (Extended Data Fig. 3c). Accordingly, neither adiposity nor responses to glucose or insulin challenges were affected by the transgene (Extended Data Fig. 3d–f). Collectively the data collected from both gain-of-function and loss-of-function mice lead us to conclude that adipose-intrinsic actions of IDOL cannot explain the phenotype of global IDOL knockout mice.

We next considered the possibility that IDOL-dependent changes in LDLR or VLDR protein levels may act in the vasculature to alter lipid delivery and metabolism in adipocytes secondarily. This would be consistent with studies showing that VLDR affects lipoprotein lipase activity20,21. We crossed Idolf/f mice with a line expressing Cre from the cadherin 5 promoter (commonly referred to as VE cadherin-Cre)22 to generate an endothelial-specific IDOL knockout line (Idol(AZ); Cre26b). Loss of IDOL activity in the endothelium had no effect on the initiation or progression of obesity after challenge with a western diet for 15 weeks (Fig. 2c,h). There was also no difference in glucose or insulin tolerance between groups (Extended Data Fig. 2e,f).

An alternative possibility was that altered caloric intake from the diet could cause the change in weight gain. Idol is highly expressed in the intestine23, and it is plausible that altering lipoprotein receptor expression could affect dietary lipid absorption or efflux. We crossed the Idol floxed mice with Villin-Cre transgenic mice to generate intestine-specific knockout mice (Idol(AZ); Cre26). However, loss of IDOL activity in the intestine did not affect mass gain or adiposity (Fig. 2d,i) and did not affect glucose or insulin tolerance (Extended Data Fig. 2g,h). Prompted by the high level of expression of Vldlr in muscle, we also generated muscle-specific IDOL knockouts (Idol(AZ); Cre26a) by crossing the Idol floxed mice to MCK-Cre transgenic mice24. Again, the tissue-selective knockout failed to recapitulate the effects of whole-body IDOL loss (Fig. 2e,j and Extended Data Fig. 2i,j).

IDOL regulates systemic energy balance through VLDR. IDOL controls the abundance of three proteins (LDLR, VLDR, and ApoER2) via ubiquitination, which targets the proteins for lysosomal degradation25. We therefore postulated that the metabolic phenotype of the global IDOL knockout was due to changes in levels of one or more of these lipoprotein receptors. To test this idea, we developed two double-knockout mouse lines in which IDOL and either LDLR or VLDR were deleted simultaneously. We did not pursue an ApoER2 compound mutant line as global ApoER2 knockout mice are sterile26 and have severe neurological defects at baseline27, and floxed-Apoer2 mice are not available. We challenged cohorts of single and double IDOL knockout mice with a HFHC diet. Consistent with the results of prior cohorts, the mass (Fig. 3a,b) and adiposity (Fig. 3c,f) of global IDOL knockout mice diverged rapidly from the wild-type group, reaching statistical significance after 2–4 weeks on the HFHC diet (P < 0.05 by repeat measures two-way ANOVA). Throughout the study, the IDOL knockout and IDOL–LDLR double-knockout groups were indistinguishable with respect to body mass and adiposity (Fig. 3a,c). LDLR is the only IDOL target expressed in the liver, further supporting the conclusion that the liver is not the primary driver of the global IDOL knockout phenotype.

Whole-body deletion of VLDR has previously been shown to be protective against diet-induced obesity28,29,30. To further understand the contributions of IDOL to body weight we compared the responses of wild-type, VLDR knockout, IDOL knockout, and IDOL–VLDR double-knockout mice to HFHC diet feeding (Fig. 3b,f). The VLDR single-knockout mice and IDOL–VLDR double-knockout mice showed comparable protection against adipose expansion following HFHC feeding, confirming previous reports that deletion of VLDR is protective against diet-induced obesity. We further showed that deletion of IDOL, which leads to an
Fig. 1 | Global IDOL knockout mice are protected from diet-induced metabolic dysfunction. a, Growth curve for wild-type versus IDOL knockout (KO) mice fed a western diet from 5–6 weeks of age. The mean masses are shown ± the s.e.m., n = 12 wild-type mice, n = 9 knockout mice. *P < 0.05 by repeat measures two-way ANOVA. b, Adiposity reported as body fat percentage ± the s.e.m., as measured by MRI after 15 weeks of western diet feeding. n = 5 wild-type mice, n = 7 knockout mice. ****P < 0.0001 by two-tailed t-test. c, Haematoxylin- and eosin-stained sections of liver and epididymal white adipose tissue (eWAT) depots from mice maintained on a western diet for 15 weeks; these images are representative of tissues analysed from both the UCLA-produced and AstraZeneca-produced lines. d, Intraperitoneal glucose tolerance test (1 mg kg⁻¹) administered after 9 weeks of western diet feeding. The mean blood glucose levels are shown ± the s.e.m., n = 10 wild-type mice, n = 9 knockout mice. *P < 0.05 by two-tailed t-test of the AUC. e, Intraperitoneal insulin tolerance test (1 U kg⁻¹) administered after 14 weeks of western diet feeding. The mean blood glucose levels are shown ± s.e.m. n = 7 wild-type mice, n = 6 knockout mice. *P < 0.05 by two-tailed t-test of the AUC. f–h, Single-housed IDOL knockout mice consume less food than wild-type littermates and are protected from diet-induced adipose expansion. n = 14 wild-type mice, n = 12 knockout mice. **P < 0.01, ****P < 0.0001 wild-type versus knockout by repeat measures ANOVA. f, The mean food consumed per mouse is labelled ± s.e.m. g, The mean mass gained per mouse after being placed in single housing ± s.e.m. h, Adiposity reported as body fat percentage ± s.e.m., as measured by MRI. The precise n, P values, and details of the statistical testing are provided in the source data file.
over-abundance of VLDLR protein (Extended Data Fig. 4a,b), has an even more pronounced protective effect on wild-type mice, but has no additional effect on the background of VLDLR deficiency. These data strongly suggest that altered abundance of VLDLR and not LDLR is an important contributor to systemic energy balance.

We assessed metabolic fitness in the compound mutant mice by performing a glucose tolerance test after 6 weeks on a HFHC diet (Fig. 3c) and an insulin tolerance test after 10 weeks on this diet (Fig. 3d). Both the IDOL knockout and IDOL–LDLR double-knockout groups had improved glucose clearance and enhanced response to a bolus of insulin relative to the wild-type group; however, the IDOL–VLDLR double-knockout group was indistinguishable from the wild-type group. These data were analysed by comparing the areas under the curves (AUCs) using a one-way analysis of variance (ANOVA) (Fig. 3g,h). Collectively, these data provide strong evidence for the idea that regulation of VLDLR protein levels is important for the maintenance of metabolic homeostasis.

**Acute knockdown of IDOL in the central nervous system increases energy expenditure.** VLDLR is most highly expressed in the brain, muscle, fat, and endothelium. Having ruled out the peripheral metabolic tissues as the source of the IDOL–VLDLR effect on energy balance, we considered the CNS as a potential site of action. Previous studies showed that the IDOL pathway in the CNS affects the function of both microglia and neurons; however, the possibility that central actions of IDOL affect metabolism has not been addressed. To test whether IDOL was acting centrally, we developed a CNS-optimized antisense oligonucleotide (ASO) targeting Idol. Dose–response studies determined that 40 μg given by intracerebroventricular injection was the minimum dose required for maximal suppression of Idol expression in whole-brain homogenates collected 2 weeks after the injection (Extended Data Fig. 5a). Using this optimized protocol, we knocked down Idol expression in 5-week-old C57Bl/6J mice and initiated a western diet-induced obesity study 1 week after injection. At the end of the 7-week western diet study, Idol expression was suppressed by 65% in the hypothalamus of ASO-injected mice relative to vehicle-treated control mice (Fig. 4a). As expected, Idol knockdown in the CNS was associated with increased VLDLR protein in the hypothalamus (Fig. 4c). The mice treated with the IDOL ASO weighed less than their control counterparts after 6 weeks on the western diet (Fig. 4b; P < 0.01 by repeat measures two-way ANOVA). These findings were confirmed in a second cohort of mice using a CNS-optimized negative control ASO (Extended Data Fig. 5b). Consistent with the whole-body knockout, the Idol ASO-treated mice were leaner by MRI analysis (Fig. 4d) and had smaller fat pads on dissection (Extended Data Fig. 5c,d).

Although IDOL ASO-treated mice mimicked the phenotype of the whole-body IDOL knockout mice, we did not observe changes in food intake. We postulated that the body weight difference may involve changes in energy expenditure. We performed indirect calorimetry on the cohorts after 2–3 weeks on the western diet (before their body mass and adiposity diverged, Extended Data Fig. 5e). The ASO-treated mice exhibited increased energy expenditure (Fig. 4f), determined with two different data analysis methods: the NIDDK Mouse Metabolic Phenotyping Centers energy expenditure analysis (https://www.mmpc.org/shared/regression.aspx), and the BWH-Harvard web application for indirect calorimetry analysis (https://calrapp.org)11. The treatment effect was significant (P < 0.05 by analysis of covariance (ANCOVA)) when either the overall mean total body mass or the mean lean body mass of each treatment group was used as the covariate. The increased energy expenditure could not be explained by changes in locomotor activity (Fig. 4g,h).

The livers of the IDOL ASO-treated mice had less lipid deposition, similar to the global IDOL knockout mice (Extended Data Fig. 5f). The IDOL knockout mice also had smaller white adipose
tissue depots containing smaller adipocytes, but we did not find an obvious increase in multi-locular cells resembling beige adipocytes (Fig. 4c). Histological analysis of brown adipose tissue revealed smaller cells with more abundant lipid droplets (Fig. 4c), consistent with increased energy expenditure. Increased brown adipose tissue activity is associated with alterations in glucose catabolism,

whereas acute pharmacological stimulation of brown adipose tissue can reduce circulating lipids by driving fatty acid oxidation.

The ASO-treated mice appeared to have increased carbohydrate metabolism, but we did not observe a consistent change in fat oxidation (Extended Data Fig. 5g,h). ASO treatment increased the phosphorylation of hormone sensitive lipase (HSL) in the subcutaneous white adipose depot (Fig. 4i), a canonical downstream target of adrenergic receptor-mediated protein kinase A (PKA) stimulation;

we also noted an increased abundance of tyrosine hydroxylase in the brown adipose tissue (Fig. 4i), consistent with increased catecholamine production by sympathetic nerves. These observations linked acute knockdown of Idol in the CNS to systemic changes in energy expenditure; however, they did not provide insight into the brain cell type (or types) responsible for the phenotype.

**IDOL deletion alters hypothalamic gene expression linked to energy balance.** The increased energy expenditure seen when CNS expression of Idol was knocked down suggests that IDOL regulates one or more energy homoeostasis circuits in the brain. We used Drop-seq, a single-cell RNA-sequencing technique, to obtain an overview of the changes in the transcriptional landscape of individual cell populations in the hypothalamus provoked by IDOL deletion (Fig. 5a). We placed wild-type and IDOL knockout mice on a HFHC for 2 weeks and then analysed hypothalamic gene expression. Clustering algorithms successfully identified 26 populations of cells in the hypothalamus, including 11 non-neuronal populations and 15 distinct populations of neurons (Fig. 5b and Extended Data Fig. 6a–c). We analysed the dataset for Vldlr gene expression and found two clusters in which significant numbers of cells had high Vldlr expression: oligodendrocytes and neurons (Extended Data Fig. 6d). When the genotype of the neurons was unmasked, the clustering analysis revealed several populations of neurons that had incompletely overlapping clusters of wild-type and IDOL knockout cells (Fig. 5c).

Such separations indicate altered global transcriptional profiles in these populations. We assessed two populations of cells that have been extensively studied for their contributions to energy homoeostasis: Agouti-related peptide (AGRP) neurons and pro-opiomelanocortin (POMC) neurons. A third population of cells, histaminergic neurons expressing Slc18a2, was also profiled because of its apparent reduction in the IDOL knockout mice; however, our analysis of transcriptional changes did not reveal any reason for the difference in this population. It is likely that the observed changes in cell abundance are an artefact of this cell population being a relatively under-represented cluster even in the wild-type mice.

Analysis of transcript abundance in the AGRP neurons revealed that loss of IDOL altered the expression of a range of genes linked to energy balance. Each of the labelled genes in each of the volcano plots in Figure 5d is annotated in genome-wide association studies (GWAS), OMIM, or PubMed as being associated with energy homoeostasis (Supplementary Table 2). The differentially expressed...
Fig. 4 | Acute knockdown of IDOL in the CNS reduces adiposity by increasing energy expenditure. a, Intracerebroventricular injection of a CNS-optimized ASO targeting Idol reduced Idol expression by 65% in the hypothalamus measured at study termination. The mean Idol expression ± s.e.m. is shown. P < 0.0001 by two-tailed t-test, n = 8. b, Growth curve showing the mean mass ± s.e.m. for male mice placed on western diet 1 week after injection of Idol-targeting ASO or vehicle control (VEH). **P < 0.01 by repeat measures ANOVA, n = 10. c, Increased VLDLR protein levels in the hypothalamus of IDOL knockout mice 8 weeks after ASO injection. The blots are representative of two independent analyses. d, Adiposity reported as body fat percentage ± s.e.m. measured by MRI. n = 10, ****P < 0.0001 by repeat measures ANOVA. e, Haematoxylin- and eosin-stained subcutaneous white adipose tissue (sWAT) and interscapular brown adipose tissue (BAT) sections from mice after 7 weeks of western diet feeding. A ×20 objective lens was used for sWAT and a ×40 objective lens was used for BAT. These images are representative of five independent biological replicates. f, Energy expenditure measured by indirect calorimetry in mice after 2–3 weeks on a western diet, showing the mean value per h ± s.e.m. n = 10, *P < 0.05 VEH versus ASO by ANCOVA using either total body mass or lean body mass as covariates. g, Locomotor activity as measured when a mouse crossed multiple infrared beams during the calorimetry experiment. Mean values ± s.e.m. are shown for ten mice. h, No difference was seen in the overall locomotion of mice as quantified by the cumulative number of beam breaks in a 48 h period ± s.e.m. P = 0.4772 by two-tailed t-test, n = 10. i, Western blot analysis of the phosphorylation of HSL in sWAT. This blot is representative of two independent analyses. j, Western blot analysis of tyrosine hydroxylase abundance in the BAT. This blot is representative of two independent analyses. The precise n, P values, and details of the statistical testing are provided in the source data file.
**Fig. 5** | The single-cell transcriptional landscape of the hypothalamus is affected by deletion of IDOL. 

**a.** Simplified schematic of the single-cell RNA-seq experimental design. 5-week-old mice were placed on a HFHC diet for 2 weeks. Hypothalamic tissues from six mice (n = 3 per group) were dissected and dissociated into single-cell suspension. Single cells and barcoded beads were captured into droplets, and this was followed by complementary DNA synthesis, amplification, and library preparation. The library was sequenced with an Illumina HiSeq 4000 next-generation instrument using the Drop-seq custom read 1B primer.

**b.** Global gene expression relationships in the 11,453 single cells projected onto two dimensions using tSNE. The clusters were defined using shared nearest neighbour graph-based clustering. VSMCs, vascular smooth muscle cells; VLMCs, vascular and leptomeningeal cells; vascular smooth muscle cells (VSMCs); OligoPC, oligodendrocyte precursor cells.

**c.** tSNE plot demonstrating the effect of IDOL knockout on the clustering of single cells in 15 different neuron clusters. n = 11,453 cells.

**d.** Volcano plot of the differentially expressed genes in AGRP neurons. Expression data calculated as counts per 10,000 unique molecular identities (UMIs). The P values were calculated using two-tailed Wilcoxon rank sum tests from n = 56 wild-type and n = 45 Idol−/− neurons identified within the AGRP cluster. Labelled genes are linked to whole-body metabolic homoeostasis (see Extended Data Table 1 for details).

**e.** Violin plots demonstrating that IDOL deletion reduced gene expression of Agrp and Npy in AGRP neurons. Individual data points indicate the magnitude of gene expression in a single cell. These are superimposed on a probability density plot for the distribution of the data. Statistics were calculated using two-tailed Wilcoxon rank sum tests on non-Ln transformed data. n = 56 wild-type and n = 45 IDOL knockout neurons identified within the AGRP cluster.
genes identified in AGRP neurons showed an enrichment of these genes (Fig. 5d). Modest differences were also observed between genotypes in POMC and histaminergic neurons (Extended Data Fig. 5e,f). Interestingly, two key orexigenic genes were downregulated in the AGRP neurons of IDOL knockout mice: the genes encoding AGRP and neuropeptide Y (Fig. 5e). AGRP neurons have also been associated with the negative regulation of energy expenditure36–38. Collectively, these data reveal that deletion of IDOL in the CNS altered the transcriptional regulation of several key neural circuits known to regulate energy balance.

**IDOL deletion in neurons regulates energy balance.** We followed up on the ASO and single-cell RNA-sequencing results by creating pan-neuron conditional knockout mice by crossing the Idolf/Cre−/− mice with SynI-Cre transgenic mice (Idolf/Cre−/−). Similar to the global knockout mice, the Cre−/−/Idolf/Cre−/− mice had reduced mass and adiposity when challenged with the western diet (Fig. 6a,b, n = 18). Again, we conducted long-term food intake measurements to maximize our ability to detect small changes in food intake. As shown in Figure 6c, the Cre−/−/Idolf/Cre−/− mice consumed less food per week than their Idolf/Cre−/− littermates. This finding shows that the changes in food intake in IDOL-deficient mice are the result of primary IDOL effects in neurons. We assessed energy expenditure by indirect calorimetry but did not detect any statistically significant differences between the two groups. Western blot analysis of subcutaneous white adipose tissue showed enhanced phosphorylation of HSL in Cre−/−/Idolf/Cre−/− mice (Fig. 6d). The activation of HSL clearly indicates that deletion of IDOL from neurons affected peripheral energy homoeostasis; however, if changes in whole-body energy expenditure were present they were too subtle for the calorimeter to detect.

To distinguish the relative contributions of reduced food intake and increased energy expenditure to the IDOL knockout phenotype, we endeavoured to minimize the energy expenditure effects by housing the mice at thermoneutrality (30 °C for 12 weeks). Relative
to wild-type mice, global IDOL knockout mice gained less mass and adiposity on the obesogenic HFHC diet, suggesting that changes in food intake were sufficient to affect body weight in the absence of differences in energy expenditure (Fig. 6e,f).

We endeavoured to identify a specific hypothalamic locus driving the IDOL knockout phenotype; however, viral-Cre-mediated deletion of IDOL in neurons in the arcuate nucleus, or the paraventricular nucleus of the hypothalamus was not sufficient to recapitulate the Cre<sup>Idol<sup>Cre<sup>Idol<sup> mouse phenotype (Extended Data Fig. 7). Although the results of our viral experiments did not allow us to identify a precise neuron population responsible for the IDOL knockout phenotype, our work with the SynI-Cre mice clearly demonstrated that deletion of IDOL from neurons protected mice against diet-induced obesity by reducing food intake while enhancing energy expenditure pathways. These results further identified IDOL-mediated regulation of neuronal VLDLR as a previously unrecognized modulator of whole-body energy homoeostasis.

**Discussion**

The ability of an organism to adapt to changing nutritional availability is fundamental to its survival. A multitude of mechanisms for maintaining whole-body energy homoeostasis have been selected for over the course of mammalian evolution. In this study, we identify the IDOL–VLDLR pathway in neurons as a previously unknown modulator of whole-body energy homoeostasis. Two independent lines of global IDOL knockout mice (one derived at UCLA and one at AstraZeneca) exhibited a highly reproducible phenotype of resistance to diet-induced obesity. Despite the abundant expression of IDOL and its targets in adipose tissue, tissue-specific studies suggested that the CNS is the primary site of IDOL action in the regulation of energy balance. Loss of VLDLR but not LDLR expression on an IDOL knockout background abolished the protective effects of IDOL deficiency on the development of obesity.

Previous studies have established the molecular mechanisms by which IDOL regulates the abundance of its lipoprotein receptor targets. IDOL forms a homodimer that recognizes a specific conserved amino acid sequence in its membrane-bound targets for recognition and ubiquitin transfer. So far only three proteins have been identified that meet this stringent criterion: LDLR and its two most closely related family members, VLDLR and ApoER2. Although unexpected, our finding that VLDLR expression is required for the effects of IDOL on whole-body metabolism is consistent with prior work demonstrating that VLDLR is important in metabolic homoeostasis. One of the initial observations made by Herz and colleagues when they developed the VLDLR knockout mice was that the mice exhibited a reduction in mass and body-mass index relative to wild-type controls. The acute nature of the ASO studies allowed us to detect increased energy expenditure at a whole-organism level, linking IDOL in the CNS to peripheral energy homoeostasis. We did not detect changes in food intake during a 2-week-long evaluation, perhaps due to differences between chronic deletion of IDOL and acute knockdown, or due to the duration of the food intake study.

Based on the Vldlr expression pattern seen in our single-cell RNA-seq data, we focused our attention on IDOL in neurons. The complete loss of IDOL from neurons provided evidence that IDOL regulates food intake, consistent with the reduced expression of Agrp and Npy seen in our single-cell RNA-seq data of whole-body IDOL knockout mice. The relative importance of altered food intake to the phenotype was confirmed by thermoneutrality studies showing that body weight differences persisted when the role of thermogenesis was minimized.

Unfortunately, we have not yet been able to identify specific hypothalamic regions involved in the effects of IDOL on energy balance. We acknowledge that the viral-Cre approach used here suffers from the inherent weakness that the SynI promoter-driven Cre will be expressed in all infected neurons, which might obscure the role of specific neuronal populations. Individual populations of neurons within a given hypothalamic nucleus may have opposing effects, as with POMC and AGRP neurons in the arcuate nucleus.

Collectively, our studies identify the IDOL–VLDLR pathway as a previously unrecognized mode of regulating whole-body energy homoeostasis. We expect that this observation will prompt further investigation into the specific cellular mechanism (or mechanisms) by which cell-surface VLDLR levels regulate energy balance. The CNS contains a specific set of lipoproteins. Both ApoE and ApoJ (also called clusterin), two apolipoproteins found in cerebrospinal fluid, are known ligands for VLDLR<sup>20,21</sup>. Several non-apolipoprotein ligands have also been proposed for VLDLR, including reelin and F-spondin<sup>19</sup>. Although there is evidence that ApoJ can bind to leptin and modulate the leptin response in cell culture<sup>19</sup>, so far none of these ligands has conclusively been shown to regulate whole-body energy homoeostasis. In the periphery, VLDLR has a clear role in facilitating the lipolytic activity of lipoprotein lipase<sup>20,21</sup>, and thereby in enhancing the release of free fatty acids. Free fatty acid sensing by the hypothalamus is a well-established modulator of neuronal activity<sup>27</sup>, affecting peripheral metabolism<sup>26,66</sup>, but the role of central VLDLR in this process has not been explored. Future studies will be required to define precisely how IDOL activity in the brain conveys a VLDLR-dependent signal to the periphery to regulate energy balance and which specific neuronal populations are involved.
Methods

Contact for reagent and resource sharing. Further information and requests for resources and reagents should be directed to, and will be fulfilled by, the lead contact, Peter Tontonoz (PPTontonoz@mednet.ucla.edu). The antisense oligonucleotides were provided by Ionis Pharmaceuticals and are subject to a material transfer agreement between Ionis Pharmaceuticals and UCLA.

Experimental models. The UCLA line of IDOL knockout mice was generated with a gene trap cassette inserted in intron 1, which could be released with FRT recombination to create conditional knockout mice in which exon 2 was floxed\(^6\). All UCLA mice were studied after ten backcrosses to C57Bl/6J mice. The AstraZeneca line of IDOL knockout mice was bred to a pure C57Bl/6N strain such that exon 2 was flanked by loxP sites. Whole-body IDOL knockout mice were derived from this strain by crossing the IDOL\(^\text{−}\) mice with Rosa26-Cre transgenic mice. This strain was also used to create hepatocyte-specific IDOL\(^\text{−}\) mice by crossing with Albumin-Cre transgenic mice.\(^5\) The pA2- IDOL transgenic mice were generated at the UCLA core facility. We cloned a dominant active form of human IDOL (K293R, K309R, K310R, K320R) into a pBluescript vector containing the 5-4 kb Fabp4 enhancer-promoter (a gift from Ronald Kahn, Joslin Diabetes Center) followed by bovine growth hormone polyA. The linearized construct was gel purified and microinjected into C57Bl/6J mice fertilized zygotes. Founders were identified using the following PCR primers (forward, GCCAGGGGCTGTCTGACA; reverse, GATAGATGACATCTACGACAAAT). The mouse studies conducted at UCLA were reviewed and approved by the Chancellor’s Animal Research Committee. The mouse studies conducted by AstraZeneca were approved by Gothenburg Ethics Committee for Experimental Animals, license number 38-2011.

Mouse studies. All studies were performed with male mice in climate-controlled facilities maintained on a 12 h light–dark cycle at a constant temperature of 22 °C with group-housed mice, unless otherwise noted. Pilot studies used both male and female mice; however, female mice are resistant to diet-induced obesity and required prolonged challenges with more severe diets to elicit changes in adiposity. Consequently, male mice were used for all subsequent studies. For LFD and HFHC studies with the AstraZeneca mouse lines, dietary challenge was initiated in 8-week-old littermate mice. Body weights were recorded on a weekly basis. Body length (nose to base of the tail) was assessed at 4 and 20 weeks of age. Food intake in AstraZeneca mice was measured at 15 weeks of age by fasting mice for 12 h during the dark period before single-housing them in cages (23 × 16 cm) with pre-weighed aliquots of food for 48 h. Body composition was assessed in 20-week-old mice by dual energy X-ray absorptiometry (DEXA, GE Lunar) in isoflurane-anesthetized mice. Metabolic fitness was determined by oral glucose tolerance tests (2 g kg\(^{-1}\)) performed at 22 weeks of age. The energy content of the faeces was determined using a bomb calorimeter (C 5000, IKA Werke). For the preliminary western diet and HFHD studies (Fig. 1 and Extended Data Fig. 1), we placed alternate littermates aged 5–6 weeks old on test diets for 15 weeks and measured body mass weekly. We separated the mice to measure food intake at week 12 before returning them to group housing. Metabolic fitness was determined by administering an intraperitoneal glucose tolerance test (1 g kg\(^{-1}\)) at week 9, and an intraperitoneal insulin tolerance test (1 U kg\(^{-1}\)) at week 14. We measured body composition by MRI after 15 weeks on the western diet (EchoMRI, 3-in-1). For western diet-fed conditional knockout studies, we placed littermate mice aged 5–6 weeks old on the diet for 15 weeks and measured their body mass weekly. We measured adiposity by MRI at baseline, then every four weeks until study termination. Adiposity, and metabolic fitness as described for the western diet conditional knockout studies above. Each cohort in the double-knockout studies was arranged to maximize the number of littermate controls. Double-knockout mice had single-knockout littermates, whereas single-knockout had wild-type littermate controls. For the western diet ASO study, we performed a pilot dose–response study in 5-week-old male C57Bl/6J mice to determine the minimum dose of ASO given by intracerebroventricular injection required for the maximal suppression of IDOL expression in whole-brain homogenates relative to vehicle-control-treated mice (Extended Data Fig. 5A). For the adiposity studies, 5-week-old male C57Bl/6J mice ordered from The Jackson Laboratory were administered 40 µg ASO or 10 µL vehicle control by intracerebroventricular injection. The study was repeated using 40 µg IDOL-targeting ASO or 40 µg control ASO. The mice recovered for 1 week before we initiated the dietary challenge. We measured energy expenditure by indirect calorimetry after 2–3 weeks of western diet feeding (Oxymax CLAMS, Columbus). Instrumental energy expenditure was measured by MRI at baseline, immediately before the calorimetry experiments, at 4 weeks on the diet, and at 7 weeks on the diet, just before study termination. Mice were singly housed from the time they were moved to the metabolic chambers until the end of the study. We monitored food intake from week 4 to week 7.

For the food intake studies, littermate mice were singly housed in standard housing and fed a western diet ad libitum. The mass of diet administered and consumed was recorded weekly.

For the thermoneutrality studies, littermate mice were group housed in monitored climate-controlled facilities maintained on a 12 h light–dark cycle at a constant temperature of 30 °C. For the vireogenetic studies 10-week-old littermate UCLA-derived IDOL\(^\text{−}\) male mice were anesthetized with isoflurane and received analgesics (0.01 mg ml\(^{-1}\) buprenorphine and 0.58 mg ml\(^{-1}\) carprofen) before and after surgery. The AAV-

hSyn-GFP or AAV-hSyn-GFP-Cre (UNC Vector Core, titre 2–6 × 10\(^{12}\) vg ml\(^{-1}\)) was injected bilaterally into the following brain regions with the listed coordinates and volumes: arcuate nucleus, anterior–posterior (A–P) −1.57 mm from bregma, lateral ± 0.2 mm from bregma, dorsal–ventral (D–V) 5.8 mm from the cortex, 125 nl per side; paraventricular nucleus of the hypothalamus, A–P −0.33 mm from bregma, lateral ± 0.25 mm from bregma, D–V 4.4 mm from the cortex, 250 nl per side; ventromedial hypothalamus, A–P −1.6 mm from bregma, lateral ±0.45 mm from bregma, D–V 5.4 mm from the cortex, 200 nl per side. Mice were allowed to recover for 1 week before starting on a western diet for 1 week; energy expenditure was then measured using the OxMax CLAMS system. Following completion of the energy expenditure study (12), the mice were singly housed and food intake was monitored climate-controlled facilities maintained on a 12 h light–dark cycle at a constant temperature of 30 °C. For the ASO production. ASOs targeting IDOL (5’-TTCCTTTTTTTCCACAGCGCA-3’) were provided by Ionis Pharmaceuticals. Complete ASO chemistry information is as follows. IDOL ASO (Tes Tes mCes mCes Tes Tds Tds Tds Tds mCds mGds Ads mGds Ads mCes mCes mCes Ae) and control ASO (mCes mCes Tes Tds Tds Tds Tds mCds mGds mGds Ads Adm Adm Adm Adm mCes Ae) were made by addition of capping monomers to the 3’ end. Equal masses of proteins were separated by electrophoresis through Bis-Tris gels before being transferred to PVDF membrane. The membranes were probed using the antibodies listed in the key resources table.

Western blot analysis. Proteins were isolated from frozen tissue using a Dounce homogenizer on ice with RIPA buffer (Boston Bioproducts) containing phosphatase and protease inhibitors (Roche). Equal masses of proteins were separated by electrophoresis through Bis-Tris gels before being transferred to PVDF membrane. The membranes were probed using the antibodies listed in the key resources table.

Histology. Tissues were fixed in 4% paraformaldehyde and stored in ethanol before being mounted in paraffin. Sections (10µm) were cut and stained with haematoxylin and eosin by the UCLA Translational Pathology Core.

Single-cell RNA-seq. Single-cell preparation. Single-cell suspensions were generated at a final concentration of 100 cells per µL in 0.1% BSA (in PBS) at 200 μl per well. The final cell suspension solution was passed through a 40 μm strainer (Thermo Fisher Scientific) to discard debris, followed by cell counting.

Drop-seq single-cell barcoding, library preparation, and sequencing. Drop-seq was performed using standard methodology, with the following modifications: the number of beads in a single PCR tube was increased to 4,000 per tube, the number of PCR cycles was increased to 4–11 cycles, and multiple PCR tubes were pooled before size selection and purification with AMPure XP (Beckman Coulter). The libraries were then sequenced on a TapeStation (Agilent) for library quality, average size, and estimation of the concentration. The samples were then tagedmental using the Nextera DNA Library Preparation kit (Illumina) and multiplexed indices.
were identified using the JackStraw permutation-based approach. These significant genes were used to scale and centre the data. Principal components analysis for each sample was generated in which each row is the read count of a gene and each column a unique single cell. The transcript counts of each cell were normalized by the total number of unique molecular identities for that cell. These values were then multiplied by 10,000 and ln transformed. Digital gene expression matrices from the six samples (three wild-type and three knockout samples) were combined to create a pooled digital gene expression matrix. Single cells were identified from background noise using a threshold of at least 250 genes and 500 transcripts, resulting in a dataset of 6,600 cells from IDOL knockout samples and 4,800 cells from wild-type samples.

Identification of cell clusters. The Seurat R package was used to project all sequenced cells onto two dimensions using t-distributed stochastic neighbour embedding (tSNE) and shared nearest neighbour graph-based clustering. To further refine the cell clusters, known cell type-specific markers from previous studies were pooled and re-analysed separately in a similar manner, considering only this neuronal subset. Briefly, the most highly variable genes were identified using the mean and dispersion (variance/mean), which were used to scale and centre the data. Principal components analysis was performed on this normalized data and significant principal components were identified using the jackStraw permutation-based approach. These significant principal components were used in tSNE to project the data onto two dimensions and graph-based clustering was used to identify cell clusters.

Resolving cell identities of the cell clusters. To resolve the identities of the cell clusters, known cell type-specific markers from previous studies were curated and checked for expression patterns within the cell clusters. A cluster showing high expression levels of a known marker gene specific for a particular cell type was considered to carry the identity of that cell type. Known markers for major hypothalamic cell types and neuronal subtypes were retrieved from (2009), Chen et al., and Campbell et al.. These markers are sufficient to define all major cell types as well as neuronal subpopulations.

Identification of differentially expressed genes between the wild type and knockout. Within each identified cell type, single cells from wild-type and knockout samples were compared for differential gene expression using a Wilcoxon rank sum test. To be considered in the analysis, the gene had to be expressed in at least 25% of the single cells from one of the two groups within that cell type and there had to be at least 0.25 log fold change in gene expression between the groups.

Data quantification and statistical analysis. Data from the energy expenditure data were analysed using GraphPad Prism with a predetermined α value of 0.05. The values reported in the text and figures are the group means ± s.e.m. where n refers to the number of animals per group. The data were analysed for the assumptions of parametric statistical methods (equal variance and normal distribution) and log-transformed if required. If the log-transformed data did not meet the assumptions of parametric statistical methods, the non-transformed data were analysed using a non-parametric method, including the Mann–Whitney U test and the Wilcoxon rank sum test, as appropriate. The significance of the difference between two groups was determined by t-tests and comparisons of multiple groups with a single independent factor were done by one-way ANOVA. If a parameter was measured over time, then data were analysed by repeat measures two-way ANOVA with time as one independent factor. The glucose- and insulin-tolerance tests were analysed by calculating the AUC for each mouse and then analysing the AUC data set by t-test or one-way ANOVA, as appropriate.

The energy expenditure data generated by the indirect calorimetry experiment were analysed using two methods: the NIDDK Mouse Metabolic Phenotyping Centers method, using their energy expenditure analysis page (http://www.mmpc.org/shared/regression.aspx); and the R programming language with CaR, a custom package for analysis of indirect calorimetry using analysis of covariance with a graphical user interface (https://calr.bwh.harvard.edu). Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data Availability. The data that support the findings of this study are available from the corresponding author upon request and the Reporting Summary is available from the Nature Metabolism website. The single-cell RNA-seq data has been deposited in the NCBI Gene Expression Omnibus, accession number GSE119960. Source data for Figs. 1–6 and Extended Data Figs. 1 and 3–7 are available online.

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References
1. Goldstein, J. L. & Brown, M. S. A century of cholesterol and coronaries: from plagues to genes to statins. Cell 161, 161–172 (2015).
2. Trommsdorff, M. et al. Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 97, 689–701 (1999).
3. Goldstein, J. L., DeBose-Boyd, R. A. & Brown, M. S. Protein sensors for membrane sterols. Cell 124, 35–46 (2006).
4. Lee, S. D. & Tontonoz, P. Liver X receptors at the intersection of lipid metabolism and atherogenesis. Atherosclerosis 242, 29–36 (2015).
5. Hong, C. & Tontonoz, P. Liver X receptors in lipid metabolism: opportunities for drug discovery. Nat. Rev. Drug. Discov. 13, 433–444 (2014).
6. Rayner, K. J. et al. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 328, 1570–1573 (2010).
7. Sallam, T. et al. Feedback modulation of cholesterol metabolism by the lipid-responsive non-coding RNA LeXis. Nature 534, 124–128 (2016).
8. Zelcer, N., Hong, C., Boyadjian, R. & Tontonoz, P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 325, 100–104 (2009).
9. Hong, C. et al. The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2. J. Biol. Chem. 285, 19720–19726 (2010).
10. Scotti, E. et al. IDOL stimulates clathrin-independent endocytosis and microvascular body-mediated lysosomal degradation of the low-density lipoprotein receptor. Mol. Cell. Biol. 33, 1503–1514 (2013).
11. Zhang, L., Xu, M., Scotti, E., Chen, Z. J. & Tontonoz, P. Both K63 and K48 ubiquitin linkages signal lysosomal degradation of the LDL receptor. J. Lipid Res. 54, 1410–1420 (2013).
12. Zhang, L. et al. The IDOL-UBE2D complex mediates sterol-dependent degradation of the LDL receptor. Genes Dev. 25, 1262–1274 (2011).
13. Calkin, A. C. et al. FERM-dependent E3 ligase recognition is a conserved mechanism for targeted degradation of lipoprotein receptors. Proc. Natl Acad. Sci. USA 108, 20107–20112 (2011).
14. Hong, C. et al. The LXR-Idol axis differentially regulates plasma LDL levels in primates and mice. Cell Metab. 20, 910–918 (2014).
15. Teslovich, T. M. et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466, 707–713 (2010).
16. Sorrentino, V. et al. Identification of a loss-of-function inducible degrader of the low-density lipoprotein receptor variant in individuals with low circulating low-density lipoprotein. Eur. Heart J. 34, 1292–1297 (2013).
17. Weissglas-Volkov, D. et al. The N342S MYLIP polymorphism is associated with high total cholesterol and increased LDL receptor degradation in humans. J. Clin. Invest. 121, 3062–3071 (2011).
18. Calkin, A. C. et al. Transgenic expression of dominant-active IDOL in liver causes diet-induced hypercholesterolemia and atherosclerosis in mice. Circ. Res. 115, 442–449 (2014).
19. Eguchi, J. et al. Transcriptional control of adipose lipid handling by IRS4. Cell Metab. 13, 249–259 (2011).
20. Yagyu, H. et al. Very low density lipoprotein (VLDL) receptor-deficient mice have reduced lipoprotein lipase activity. Possible causes of hypertriglyceridemia and reduced body mass with VLDL receptor deficiency. J. Biol. Chem. 277, 10037–10043 (2002).
21. Garcia-Arcos, I. et al. Adipose-specific lipoprotein lipase deficiency more profoundly affects brown than white fat biology. J. Biol. Chem. 288, 14046–14058 (2013).
22. Goudriaan, J. R. et al. The VLDL receptor plays a major role in chylomicron metabolism by enhancing LP-Lmediated triglyceride hydrolysis. J. Lipid Res. 45, 1475–1481 (2004).
23. Obunike, J. C. et al. Transcytosis of lipoprotein lipase across cultured endothelial cells requires both heparan sulfate proteoglycans and the very low density lipoprotein receptor. J. Biol. Chem. 276, 8934–8941 (2001).
24. Alva, J. A. et al. VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. Dev. Dyn. 235, 759–767 (2006).
25. Brüning, J. C. et al. A muscle-specific insulin receptor knockout exhibits profound effects on triglyceride metabolism and atherogenesis. Mol. Cell. 2, 559–569 (1998).
26. Andersen, O. M. et al. Essential role of the apolipoprotein E receptor-2 in sperm development. J. Biol. Chem. 278, 23989–23995 (2003).
27. Frykman, P. K., Brown, M. S., Yamamoto, T., Goldstein, J. L. & Herz, J. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc. Natl Acad. Sci. USA* 92, 4853–4857 (1995).

28. Goudriaan, J. R. et al. Protection from obesity in mice lacking the VLDL receptor. *Arterioscler. Thromb. Vasc. Biol.* 21, 1488–1493 (2001).

29. Choi, J. et al. The E3 ubiquitin ligase Idol controls brain LDL receptor expression, ApoE clearance, and AGE amyloidosis. *Sci. Transl. Med.* 7, 31ra184 (2015).

30. Gao, J. et al. The E3 ubiquitin ligase IDOL regulates synaptic ApoER2 levels and is important for plasticity and learning. *eLife* 6, 1741 (2017).

31. Mina, A. I. et al. CaIR: a web-based analysis tool for indirect calorimetry experiments. *Cell Metab.* 28, 656–666 (2018).

32. Cannon, B. & Nedergraaf, J. Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359 (2004).

33. Berbée, J. F. P. et al. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. *Nat. Commun.* 6, 6536 (2015).

34. Gautron, L., Elfmqist, J. K. & Williams, K. W. Neural control of energy balance: translating circuits to therapies. *Cell 161*, 133–145 (2015).

35. Tschop, M. H. et al. A guide to analysis of mouse energy metabolism. *Nat. Methods* 9, 57–63 (2012).

36. Caron, A., Lee, S., Elfmqist, J. K. & Gautron, L. and adipose crosstalks. *Nat. Rev. Neurosci.* 19, 153–165 (2018).

37. Kong, D. et al. GABAergic RIC-cre neurons in the arcuate nucleus selectively regulate energy expenditure. *Cell 151*, 645–658 (2012).

38. Ruan, H.-B. et al. O-GlcNAc transferase enables AgRP neurons to suppress the hypothalamic and feeding. *Cell Metab.* 22, 962–970 (2015).

39. Wahl, T., D'Amour, K. L. & Masurovic, D. A. Neuronal regulation of energy homeostasis: beyond the hypothalamus and feeding. *Cell Metab.* 22, 1001–1008 (2015).

40. Waterson, M. J. & Horvath, T. L. Neuronal regulation of energy homeostasis: translating circuits to therapies. *Cell 170*, 429–442.e11 (2017).

41. Williams, K. W. & Elfmqist, J. K. From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. *Nat. Neurosci.* 15, 1350–1355 (2012).

42. Chechi, K., Carpenter, A. C. & Richard, D. Understanding the brown adipocyte as a contributor to energy homeostasis. *Trends Endocrinol. Metab.* 24, 408–420 (2013).

43. Barnshad, M., Song, C. K. & Bartness, T. J. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *Am. J. Physiol. 276*, R1569–R1578 (1999).

44. Friedrich, J. 20 years of leptin: leptin at 20: an overview. *Nat. Rev. Endocrinol.* 11, 618–622 (2015).

45. Xu, Y., O’Malley, B. W. & Elfmqist, J. K. Brain nuclear receptors and body weight regulation. *J. Clin. Invest.* 127, 1172–1180 (2017).

46. López, M. et al. The hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat. Med.* 16, 1001–1008 (2010).

47. Alvarez-Crespo, M. et al. Essential role of UCPI modulating the central effects of thyroid hormones on energy balance. *Mol. Metab.* 5, 271–282 (2016).

48. Mancini, G. & Horvath, T. L. Viral vectors for studying brain mechanisms that control energy homeostasis. *Cell Metab.* 27, 1168–1173 (2018).

49. Schwartz, M. W., Woods, S. C., Porte, D., Seeley, R. J. & Baskin, D. G. Central nervous system control of food intake. *Nature 404*, 661–670 (2000).

50. Leeb, C., Eresheim, C. & Nimpf, J. Clusterin is a ligand for apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR) and signals via the Reelin-signaling pathway. *J. Biol. Chem.* 289, 4161–4172 (2014).

51. Poulichamp, T., Wadsworth, R. R. & Herz, J. Functional roles of the interaction of APP and lipoprotein receptors. *Front Mol. Neurosci.* 10, 54 (2017).

52. Zisman, S. et al. Proteolysis and membrane capture of F-spondin generates combinatorial guidance cues from a single molecule. *J. Cell Biol.* 178, 1237–1249 (2007).

53. Bajari, T. M., Strasser, V., Nimpf, J. & Schneider, W. J. A model for modulation of leptin activity by association with clusterin. *FASEB J.* 17, 1505–1507 (2003).

54. Oomura, Y., Nakamura, T., Sugimori, M. & Yamada, Y. Effect of free fatty acid on the rat lateral hypothamnic neurons. *Physiol. Behav.* 14, 483–486 (1975).

55. Lam, T. K. T. et al. Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. *Nat. Med.* 11, 320–327 (2005).

56. Lam, T. K. T., Schwartz, G. J. & Rossetti, L. Hypothalamic sensing of fatty acids. *Nat. Neurosci.* 8, 579–584 (2005).

57. Yue, J. Y. T. et al. A fatty acid-dependent hypothalamic-DVC neurocircuity that regulates hepatic secretion of triglyceride-rich lipoproteins. *Nat. Commun.* 6, 5970 (2015).

58. Scotti, E. et al. Targeted disruption of the idol gene alters cellular regulation of the low-density lipoprotein receptor by sterols and liver x receptor agonists. *Mol. Cell. Biol.* 31, 1883–1893 (2011).

59. Postic, C. et al. Dual role for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. *J. Biol. Chem.* 274, 305–315 (1999).

60. Swayne, E. E. et al. Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. *Nucleic Acids Res.* 20, 867–870 (2002).

61. Seth, P. P. et al. Synthesis and biophysical evaluation of 2‘-constrained 2‘-O-methoxyethyl and 2‘-O-constrained 2‘-O-ethyl nucleic acid analogues. *J. Org. Chem.* 75, 1569–1581 (2010).

62. Ostergaard, M. E. et al. Rational design of antisense oligonucleotides targeting single nucleotide polymorphisms for potent and allele selective suppression of signal transduction. *J. Nucl. Acid. Res.* 41, 9634–9650 (2013).

63. Brewer, G. J. & Torricelli, J. R. Isolation and culture of adult neurons and neurophers. *Nat. Protoc.* 2, 1490–1498 (2007).

64. Poon, K., Barson, J. R., Fagan, S. E. & Leibowitz, S. F. developmental changes in embryonic hypothalamic neurons during perinatal fat exposure. *Am. J. Physiol. Endocrinol. Metab.* 303, E432–E441 (2012).

65. Macosko, E. Z. et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell 161*, 1202–1214 (2015).

66. Wiltman, L. & van Eck, N. J. A small local moving algorithm for large-scale modularity-based community detection. *Eur. Phys. J. B* 86, 75 (2013).

67. Ohung, N. C. & Storey, J. D. Statistical significance of variables driving systematic variation in high-dimensional data. *Bioinformatics* 31, 545–554 (2015).

68. Romanov, R. A. et al. Molecular interrogation of hypothalamic organization reveals distinct dopamine neuronal subtypes. *Nat. Neurosci.* 20, 176–188 (2017).

69. Chen, R., Wu, X., Jiang, L. & Zhang, Y. Single-cell RNA-seq reveals hypothalamic cell diversity. *Cell Rep.* 18, 3227–3241 (2017).

70. Campbell, J. N. et al. A molecular census of arcuate hypothalamus and median eminence cell types. *Nat. Neurosci.* 20, 484–496 (2017).
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Metabolic phenotype of IDOL-deficient mice. **a**, 18-month-old IDOL KO mice are protected against age-induced adiposity. **a**, Mean mass and standard error of the mean; n = 9 WT, n = 10 KO mice, ****p < 0.0001 vs. WT by two-tailed t-test. **b**, Mean body fat percentage +/− the standard error of the mean; n = 9 WT, n = 10 KO mice, ****p < 0.0001 vs. WT 2-tailed t-test. **c**, Growth curve for littermate male mice fed a 60% kcal high fat diet (HFD) starting when the mice were 6 weeks old. The mean values are shown +/− the standard error of the mean; n = 5 WT and n = 6 KO mice *p < 0.05 by repeat measures ANOVA. **d**, Growth curve for littermate male IDOL(AZ)f/f mice with or without whole-body Rosa26 Cre fed a low-fat diet from 8 weeks of age. The mean mass is shown +/− SEM; n = 10 mice per group. **e**, Growth curve for littermate male IDOL(AZ)f/f mice with or without whole-body Rosa26 Cre fed a high fat high cholesterol diet from 8-weeks of age; n = 8 Idol(AZ)f/f mice n = 9 CreR26+Idol(AZ)f/f mice; *p < 0.05 by repeat measures ANOVA. **f**, Unchanged lean body mass in male IDOL knockout mice administered test diets despite adiposity changes measured by MRI. Mean lean mass is shown +/− SEM; n = 9 WT and n = 10 KO mice fed Chow, n = 5 WT and n = 7 mice fed western diet, n = 5 WT and n = 6 KO mice fed the 60% HFD, p-values calculated by two-way ANOVA with Sidak post hoc tests. **g**, Body length measured from nose to anus at 20 weeks of age for male mice fed either a low-fat diet (LFD) or a high-fat high-cholesterol diet (HFHC) for 12 weeks. The mean values are shown +/− the standard error of the mean. Low-fat diet: n = 10 mice per genotype. High Fat High Cholesterol diet: n = 8 Idol(AZ)f/f mice and n = 9 CreR26+Idol(AZ)f/f mice. P-values calculated by repeat measures ANOVA. **h**, Mean body temperature +/− SEM measured rectally in response to fasting in mice fed a high-fat high-cholesterol diet; n = 8 WT and n = 9 IDOL KO mice. The precise n-number, p-value, and details of all statistical testing are provided in the source data file.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Glucose and insulin tolerance tests conducted on Idol−/− and conditional knockouts with tissue-specific deletion of IDOL. a–b. Mean blood glucose levels +/− SEM for liver-specific conditional IDOL KO mice challenged with an oral glucose tolerance test (2 g/kg) at 22 weeks of age fed either a, the low-fat diet; n=12 mice per genotype or b, the high-fat high-cholesterol diet for 16 weeks; n=11 mice per genotype. c–j, Mean blood glucose levels +/− SEM for male mice fed a western diet challenged with an intraperitoneal glucose tolerance test (1 g/kg; shown on the left) after six weeks on diet and an intraperitoneal insulin tolerance test (1 U/kg; shown on the right) after ten weeks on diet. c, d, Adipose-specific conditional IDOL KO; n=11 Idol−/−, n=9 CreAdipoQIdol−/− mice. e, f, Endothelium-specific conditional IDOL KO; n=13 Idol−/−, n=10 CreCdh5Idol−/− mice. g, h, Intestine-specific conditional IDOL KO; n=8 Idol−/−, n=4 CreVilIdol−/− mice. i, j, Muscle-specific conditional IDOL KO; n=10 mice per genotype for the GTT and n=10 Idol−/−, n=9 CreMckIdol−/− mice for the ITT.
Extended Data Fig. 3 | Adipose-specific transgenic IDOL mice are not protected from diet-induced obesity. a, Ablation of VLDLR protein levels in the subcutaneous inguinal white adipose tissue of the aP2-IDOL transgenic mice. This blot is representative of many independent experiments conducted by two independent researchers. b, Reduced VLDLR protein in the interscapular brown adipose tissue of the aP2-IDOL transgenic mice. This blot is representative of many independent experiments conducted by two independent researchers. c, Growth curve for WT and aP2-IDOL transgenic mice fed a western diet from 5 weeks of age showing the mean mass +/− SEM; n = 13 WT and n = 9 aP2-IDOL transgenic mice. d, Mean body composition measured by MRI +/− SEM for n = 13 WT and n = 9 aP2-IDOL transgenic mice after 12 weeks of western diet feeding. e, Intraperitoneal glucose tolerance test (1g/kg) administered after six weeks of western diet feeding; n = 13 WT and n = 9 aP2-IDOL transgenic mice. f, Intraperitoneal insulin tolerance test (1U/kg) administered after ten weeks of western diet feeding n = 13 WT and n = 9 aP2-IDOL transgenic mice.
Extended Data Fig. 4 | IDOL regulates systemic energy balance through the VLDL receptor. 100μg of RIPA isolate from the hypothalamus of individual wild-type (WT), Idol-- (KO), Idol--/Ldlr-- (LDLR DKO), or Idol--/Vldlr-- (VLDLR DKO) mice was loaded per lane of a Tris-Acetate NuPAGE gel. The image is representative of many independent experiments. These samples were repeated twice to assess reproducibility. a, Western blot analysis of VLDLR protein levels in the hypothalamus at study termination. b, Western blot analysis of LDLR protein levels in the hypothalamus at study termination.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Acute knockdown of Idol in the central nervous system increases energy expenditure. a, Optimization of the dose of ASO required to suppress Idol expression in whole-brain homogenates measured 8-weeks post-injection. The values represent the mean expression of Idol in a whole brain homogenate 8-weeks post-injection +/− SEM; n = 4 mice per time point. b, Growth curve for male mice placed on western diet one week after intracerebroventricular injection of Idol-targeting antisense oligonucleotide (IDOL ASO) or CNS-optimized control (CTRL ASO). The mean masses are shown +/− SEM; *p < 0.05, **p < 0.01, ***p < 0.001 by repeat measures two-way ANOVA, n = 10 mice treated with CTRL and n = 5 mice treated with IDOL ASO. c, Macroscopic view of interscapular brown adipose tissue depots after seven weeks on western diet. These images are representative of the ten mice per group in the ASO study. d, Macroscopic view of subcutaneous (inguinal) white adipose tissue depots after seven weeks on western diet. These samples are representative of the ten mice per group in the ASO study. e, No statistically significant differences in body composition at the onset of CLAMS experiment. The mean total body mass, lean body mass, and fat body mass are shown +/− SEM; statistical significance determined by two-way ANOVA to account for multiple testing, n = 10 mice per group. The precise n-number, p-value, and details of all statistical testing are provided in the source data file. f, Reduced lipid accumulation in the livers of ASO treated mice evident with hematoxylin and eosin staining of 5 μm sections of liver (10x objective). These images are representative of three mice per treatment group that were analysed for histology. g, Calculation of carbohydrate metabolism in the n = 10 mice per group. The mean energy expenditure derived from carbohydrate metabolism for the mice in Fig. 4f is shown +/− s.e.m. for each time point. h, Calculation of lipid oxidation in the n = 10 mice per group. The mean energy expenditure derived from lipid metabolism for the mice in Fig. 4f is shown +/− s.e.m. for each time point.
Extended Data Fig. 6 | Single cell RNA sequencing examination of the transcriptional landscape of the hypothalamus with Drop-seq. Clustering analysis combined with expression profiling of a panel of marker genes allowed us to discriminate 26 unique clusters of cells in the hypothalamus. **a**, Violin plots demonstrate the expression patterns of the 38 marker genes used to identify the cell clusters. Individual data points indicating the magnitude of gene expression in a single cell are superimposed on a probability density plot for the distribution of the data; the expression analysis is based on the data collected from n = 11,453 single cells. **b**, Global gene expression relationships in the 11,453 single cells isolated from the hypothalamic tissues of six mice projected onto two dimensions using t-distributed Stochastic Neighbour Embedding (tSNE). The clusters were defined using shared nearest neighbour graph-based clustering. **c**, tSNE plot of the neuronal cells identified in the Drop-seq experiment (n = 3369 single cells). **d**, Violin plot demonstrating that Vldlr is only appreciably expressed in neuron and oligodendrocyte cell populations. Individual data points indicating the magnitude of gene expression in a single cell; the expression analysis is based on the unique molecular identities (UMI) data collected from n = 11,453 single cells. **e-f**, Volcano plots of the differentially expressed genes analyzed by two-sided Wilcoxon rank sum tests in **e**, POMC+ (n = 24 WT and n = 26 idol−/− cells) and **f**, Histaminergic neurons (n = 23 WT and n = 11 idol−/− neurons. Labelled genes are linked to whole body metabolic homeostasis – see Supplemental Data Table 2 for details.
Extended Data Fig. 7 | Neuron-specific virogenetic deletion of IDOL from individual hypothalamic nuclei is insufficient to protect against diet-induced obesity. a–c, Adeno-associated virus (AAV) expressing either GFP-Cre or GFP regulated by the Synapsin I (SynI) promoter were injected into the arcuate nucleus (ARC; panels a–c) or the paraventricular nucleus of the hypothalamus (PVH; panels d–f). a–c, Deletion of IDOL from neurons in the ARC had no effect on body mass or food intake for mice fed a western diet for 12 weeks; n = 8 mice injected with AAV-expressing GFP (GFPARC-SynI), n = 8 mice injected with AAV expressing Cre-GFP (CreARC-SynI). a, An image of the ARC showing GFP-positive cells to demonstrate successful infection of neurons; the image is representative of the sixteen mice injected in the ARC study. b, Growth curve showing the mean mass of the mice from each treatment group +/− one standard deviation. c, The mean cumulative mass of food consumed per mouse +/− one standard deviation. d–f, Deletion of IDOL from neurons in the PVH had no effect on body mass or food intake for mice fed a western diet for 12 weeks; n = 8 mice injected with AAV expressing GFP (GFPPVH-SynI), n = 9 mice injected with AAV expressing Cre-GFP (CrePVH-SynI). d, An image of the PVH showing GFP-positive cells to demonstrate successful infection of neurons; the image is representative of the seventeen mice injected in the PVH experiment. e, Growth curve showing the mean mass of the mice from each treatment group +/− one standard deviation. f, The mean cumulative mass of food consumed per mouse +/− one standard deviation.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: N/A

Data analysis:

The energy expenditure data generated by the indirect calorimetry experiment were analyzed using two methods: 1. the NIDDK Mouse Metabolic Phenotyping Centers using their energy expenditure analysis page (http://www.mmpc.org/shared/regression.aspx) and 2. the R programming language with CaR, a custom package for analysis of indirect calorimetry using analysis of covariance with a graphical user interface (https://car.bwh.harvard.edu)

The single cell RNA sequencing data were analyzed using several open source software packages, as described in detail with links to the source code in the methods section.

- Drop-seq sequencing data were processed into a digital gene expression matrix using the dropSeqR package (https://github.com/Hochoh/ dropSeqPipe) SnakeMake wrapper for Drop-seq tools version 1.13.
- The Seurat R package (version 2.3.1; https://github.com/satijalab/seurat) was used to project all sequenced cells onto two dimensions using t-Distributed Stochastic Neighbor Embedding (tSNE) and shared nearest neighbor graph-based clustering 57 was used to define clusters.
- The R programming language was used to analyze differential gene expression with Wilcoxon Rank Sum Testing

All other data were analyzed for statistical significance using GraphPad Prism v8.2.0

For manuscripts utilizing custom algorithms or software that are not central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

We deposited the single-cell RNA sequencing data in the NCBI Gene Expression Omnibus. Its accession number is GSE119960.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size Sample sizes were determined by doing a sample size estimate based on the differences and variability seen in data collected during preliminary pilot studies. We chose an alpha value of 0.05 and a beta value of 0.2 for these calculations.

Data exclusions No data were excluded

Replication The data in this manuscript were obtained from two groups [UCLA and AstraZeneca]. The two lines of iDO1 knockout mice were made independently, maintained in different vivariums on different continents. The robust iDO1 knockout phenotype observed despite these factors speaks to the reproducibility of the results.

Randomization Mice were randomly assigned to cages at weaning and the cages were allocated to experimental groups based on the sample size estimates.

Blinding The studies were conducted without any notation on the cages of which mouse belonged which genetic group. The data were unblinded for analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☐   | Palaeontology         |
| ☒   | Animals and other organisms |
| ☐   | Human research participants |
| ☐   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChIP-seq              |
| ☒   | Flow cytometry        |
| ☒   | MRI-based neuroimaging |

Antibodies

| Antibodies used | Validation |
|-----------------|------------|
| Rabbit anti-VDL/ - ProteinTech #19493-1 |
| Rabbit anti-LDLR - Cayman Chemicals #1007665 |
| Mouse anti-Tyrosine Hydroxylase - Millipore #MA3118 |
| Rabbit anti-Phospho-HSL(Ser660) - Cell Signaling Technologies #4126 |
| Rabbit anti-HSL - Cell Signaling Technologies #4107 |
| Rabbit anti-GAPDH - ProSci #E781 |
| Rabbit anti-Actin - Sigma #A2066 |

The LD/L and VDLR antibodies were validated using protein extracts obtained from knockout mice lacking the appropriate gene.

The Tyrosine Hydroxylase antibody has been extensively validated by Fischer et al [DOI: 10.1038/nm.4316] The Phospho-HSL(Ser660) antibody was validated by the manufacturer by Western blot analysis of extracts from differentiated
NIH/3T3-L1 cells treated with isoproterenol
The HSL antibody was validated by the manufacturer by Western blot analysis of extracts from NIH/3T3 and differentiated 3T3-L1 cells
The GAPDH antibody was validated by the manufacturer by Western blot analysis of extracts from NIH/3T3 and 3T3-L1 cells
The Actin antibody was validated by the manufacturer by doing a Western blot titration analysis on Chicken Gizzard Extract on the affinity isolated purified antibody

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals
For all studies, male mice were used. The diets were initiated when the mice were 5-8 weeks old, as indicated in the appropriate figure legend.
Mouse Strains:
CS7B6/1 (JAX #000864)
VE Cadherin Cre (JAX #006317)
Albumin Cre (JAX #003574)
Mck Cre (JAX #006475)
Villin Cre (JAX #004586)
Adiponectin Cre (JAX #028020)
Synapsin Cre (JAX #003966)
Rosa26 Cre (AstraZeneca)
Idol knock out (UCLA - Peter Tontonoz’s Laboratory)
Idol +/- (UCLA - Peter Tontonoz’s Laboratory)
Idol+/+ (AstraZeneca)
VLDLR knockout (JAX #002529)
LDLR knockout (JAX #002207)

Wild animals
N/A

Field-collected samples
N/A

Ethics oversight
The mouse studies conducted at UCLA were reviewed and approved by the Chancellor’s Animal Research Committee (DHHS OLAW #A3196-01, UCLA approvals #99-131 and #03-166). The mouse studies conducted by AstraZeneca were approved by Gothenburg Ethics Committee for Experimental Animals, license nr: 38-2011.

Note that full information on the approval of the study protocol must also be provided in the manuscript.