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http://hdl.handle.net/10026.1/16051

10.1038/s41467-019-08737-6
Nature Communications
Springer Science and Business Media LLC

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Steroid receptor coactivator-1 modulates the function of Pomc neurons and energy homeostasis

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Hypothalamic neurons expressing the anorectic peptide Pro-opiomelanocortin (Pomc) regulate food intake and body weight. Here, we show that Steroid Receptor Coactivator-1 (SRC-1) interacts with a target of leptin receptor activation, phosphorylated STAT3, to potentiate Pomc transcription. Deletion of SRC-1 in Pomc neurons in mice attenuates their depolarization by leptin, decreases Pomc expression and increases food intake leading to high-fat diet-induced obesity. In humans, fifteen rare heterozygous variants in SRC-1 found in severely obese individuals impair leptin-mediated Pomc reporter activity in cells, whilst four variants found in non-obese controls do not. In a knock-in mouse model of a loss of function human variant (SRC-1L1376P), leptin-induced depolarization of Pomc neurons and Pomc expression are significantly reduced, and food intake and body weight are increased. In summary, we demonstrate that SRC-1 modulates the function of hypothalamic Pomc neurons, and suggest that targeting SRC-1 may represent a useful therapeutic strategy for weight loss.
Transcriptional coactivators and corepressors regulate the ability of nuclear hormone receptors (NRs) and transcription factors (TFs) to enhance/suppress the expression of target genes by facilitating the assembly of the transcription complex at target gene promoters. Understanding the molecular mechanisms by which coactivators and corepressors alter gene expression to modulate physiological processes may provide insights into disease mechanisms and highlight potential therapeutic targets.

Steroid receptor coactivator (SRC)-1 belongs to a family of coactivators (SRC-1, -2, and -3) that mediate NR-dependent or TF-dependent transcription. Global deletion of SRC-1 in mice leads to obesity; however, to date, the molecular mechanisms involved are incompletely understood. SRC-1 is abundantly expressed in the hypothalamus, including neurons within the arcuate nucleus of the hypothalamus (ARH), which play a key role in mediating the weight-reducing effects of the adipocyte-derived hormone leptin. Leptin is a signal of nutrient deprivation, with a fall in leptin levels triggering a set of responses that seek to restore energy homeostasis by increasing food intake and decreasing energy expenditure.

In the fed state, an increase in leptin levels leads to the activation of neurons expressing the anorectic peptide Pro-opiomelanocortin (POMC) leading to a reduction in food intake. Specifically, leptin binding to its receptor phosphorylates the transcription factor STAT3 which dimerizes and translocates to the nucleus where it stimulates the expression of POMC.

Leptin-induced STAT3 activation also stimulates expression of Socs3 (suppressor of cytokine signaling-3) which acts to inhibits leptin signaling.

In this study, we sought to investigate the central mechanisms by which SRC-1 modulates energy homeostasis. SRC family members bind to STAT transcription factors in cells. Thus, we first examined the effects of SRC-1 on STAT3 transcriptional activity and POMC expression. We then characterized metabolic phenotypes in mice lacking SRC-1 in POMC neurons and explored the underlying mechanisms. Additionally, we examined the potential functional consequences of rare human variants in SRC-1 identified in severe childhood-onset obesity. Finally, we generated a knock-in mouse model of the most severe loss of function human SRC-1 variant and characterized the metabolic consequences of these mutant mice.

**Results**

SRC-1 interacts with pSTAT3 to stimulate POMC expression. We found that global SRC-1-KO mice had lower POMC but normal Socs3 mRNA levels in the hypothalamus compared to control littermates (Fig. 1a). Using Chromatin-immunoprecipitation (ChIP) assays, we found that leptin-stimulated pSTAT3 binding to POMC promoters was decreased.
**Fig. 2** SRC-1 mediates leptin signaling. Numbers of mice/experiments/neurons are indicated; data are presented as mean ± SEM and compared using T-tests or one- or two-way ANOVA followed by post hoc Sidak tests (#). a Serum leptin levels 42 days after HFD feeding (n = 5/8); *P < 0.05. b Time course of hypothalamic SRC-1-pSTAT3 interaction in C57Bl6 wild type mice that received i.p. injections of leptin (5 mg/kg). c Quantification of the hypothalamic SRC-1-pSTAT3 interaction in C57Bl6 wild type mice that received i.p. injections of leptin (5 mg/kg). d Two-hour fasted mice (12 weeks of age) received i.p. injections of saline or leptin (5 mg/kg) 15 min prior to refeeding and food intake was recorded for 1 h afterwards (n = 7/9); *P < 0.05. e Representative pSTAT3 immunohistochemical staining in the ARH and VMH of control and pomcSRC-1-KO mice receiving a single bolus i.p. injection of leptin (0.5 mg/kg, 90 min). Scale bar = 50 μm. f The 3rd ventricle, ARH arcuate nucleus, VMH ventromedial hypothalamic nucleus. g Representative traces of mIPSC in untreated mature Pomc neurons from control mice vs. from MpomcSRC-1-KO mice. h Quantification of amplitude (Δ) of mIPSC in two groups (n = 39/43); ***P < 0.001. i Representative traces of action potentials in untreated mature Pomc neurons from control mice vs. from MpomcSRC-1-KO mice. j Quantification of firing frequency (f) and resting membrane potential (V) in two groups (n = 29–36); *P < 0.05. k Quantitative traces of mIPSC in untreated mature Pomc neurons from control mice vs. from MpomcSRC-1-KO mice. l Quantification of the amplitude of mIPSC in two groups (n = 13/14); ***P < 0.001. Source data are provided as Source Data Fig. 2.
in the hypothalamus of SRC-1-KO mice compared to control mice (Fig. 1b). In keeping with these findings, SRC-1 overexpression potentiates STAT3-induced Pomc transcription but had no effect on Socs3 transcription in Neuro2A cells and HEK293 cells (Fig. 1c, d; Supplementary Figure 1a-b). Similar effects of SRC-1 were observed in SRC-1-KO MEFs cells, although STAT3 alone could stimulate Pomc expression in these cells devoid of endogenous SRC-1 (Supplementary Figure 1c-d). These results indicate that SRC-1, while not required for STAT3 transcriptional activity, can facilitate STAT3-induced Pomc expression.

SRC-1 in Pomc neurons regulates energy homeostasis. To test whether SRC-1 in Pomc neurons plays a functionally significant role in energy homeostasis, we crossed SRC-1lox/lox mice with Pomc-Cre mice to generate mice lacking SRC-1 selectively in Pomc lineage cells (pomcSRC-1-KO, Supplementary Figure 1e). On a standard chow diet, the body weight of male pomcSRC-1-KO mice was comparable to control littersmates (SRC-1lox/lox) (Supplementary Figure 1f), whilst female pomcSRC-1-KO mice showed significant weight gain (Supplementary Figure 1g). This sexual dimorphism may be explained by our earlier observations that global SRC-1 deficiency blunts the weight-reducing effects of estrogen4. On a high fat diet (HFD), male pomcSRC-1-KO mice gained significantly more weight compared to control littersmates (Fig. 1e) due to an increase in fat mass (Fig. 1f). In weight-matched mice, we observed a significant increase in HFD intake in pomcSRC-1-KO mice vs. controls (Fig. 1g, h); measurements of energy expenditure were comparable (Supplementary Figure 1h–j).

A caveat of the regular Pomc-Cre mouse line is that, during the early development, Cre recombinase is transiently expressed in a broader population of neurons and some of these Pomc lineage cells mature into orexigenic Npy/Agrp neurons with opposing effects on food intake16. To address this concern, we crossed a Pomc-CreERT transgene17 onto the SRC-1lox/lox mouse allele. Tamoxifen induction at 9 weeks of age resulted in the deletion of SRC-1 in mature Pomc neurons (MpmMsrc-1-KO; Supplementary Figure 1k-l). When fed with a HFD, MpmMsrc-1-KO mice displayed increased weight gain and fat mass, associated with increased food intake compared to control littersmates (Fig. 1i–k), which recapitulated the phenotypes observed in pomcSRC-1-KO mice. Collectively, these results indicate that SRC-1 in mature Pomc neurons is required to defend against diet-induced obesity.

SRC-1 in Pomc neurons is required for the anorectic effects of leptin. Several studies have shown that STAT3 signaling is a mediator of leptin’s effects on body weight10,18. In HFD-fed pomcSRC-1-KO mice, we observed a 5–6-fold increase in circulating leptin levels in HFD-fed pomcSRC-1-KO mice (Fig. 2a), whilst adiposity only increased 2-fold (Fig. 1f). Thus, we hypothesized that SRC-1 is downstream of leptin action and loss of SRC-1 in Pomc neurons may impair leptin signaling. Supporting this possibility, we found that intra-peritoneal administration of leptin to control mice rapidly increased the hypothalamic SRC-1-
pSTAT3 interaction (Fig. 2b, c). Leptin administration significantly reduced 1-hour (1h) food intake in control mice but not in pomcSRC-1-KO mice (Fig. 2d), despite increased leptin-induced pSTAT3 in the arcuate nucleus (Fig. 2e, f). These results suggest that the SRC-1-pSTAT3 interaction is downstream of leptin-MPomc signaling, and contributes to the acute anorectic effects of leptin. Notably, the effects of leptin on 4 and 24 h food intake were not significantly altered in pomcSRC-1-KO mice (Supplementary Figure 2a, b), presumably because the anorectic effects of leptin after the first hour are mediated by other leptin-responsive neurons or other signaling pathways19, 20.

Leptin also depolarizes a subset of Pomc neurons to exert its anorectic effects9, although recent fiber photometry studies failed to detect acute effects of leptin on calcium dynamics in Pomc neurons21. Thus, we examined leptin-induced depolarization in TOMATO-labeled mature Pomc neurons from MpomcSRC-1-KO mice and tamoxifen-treated controls after 1-week HFD feeding. We recorded leptin-induced changes in resting membrane potential (RM) in the presence of tetrodotoxin (TTX), which blocks action potentials, and a mixture of fast synaptic inhibitors which block the majority of presynaptic inputs. We found that 26/39 (67%) of Pomc neurons from control mice were depolarized (>2 mV elevations in resting membrane potential) (RM) by leptin (Fig. 2g, h). In contrast, only 14/43 (33%) of Pomc neurons from MpomcSRC-1-KO mice were depolarized by leptin (P = 0.002) and the amplitude of leptin-induced depolarization was significantly reduced in these Pomc neurons (Fig. 2g–i). Interestingly, in the absence of TTX and synaptic inhibitors, leptin-induced depolarization and increases in firing frequency were comparable between the two groups (Supplementary Figure 2c–f), suggesting...
that indirect effects of leptin through presynaptic terminals were not affected by the loss of SRC-1 in Pomc neurons. Notably, the baseline firing frequency was significantly decreased in mature Pomc neurons from MmpomcSRC-1-KO mice compared to those from control mice, whereas the baseline RM remained unchanged (Fig. 2j–l). We found that the amplitude, but not the frequency, of miniature inhibitory postsynaptic currents (mIPSC) was significantly higher in mature Pomc neurons from MmpomcSRC-1-KO mice than those from control mice (Fig. 2m–o). The frequency of mIPSC is thought to reflect presynaptic events (e.g., GABA release), while mIPSC amplitude is largely determined by the responsiveness of postsynaptic neurons. Thus we suggest that SRC-1 also regulates the responsiveness of Pomc neurons to GABA-ergic inputs via a leptin-independent mechanism.

Rare SRC-1 variants found in obese humans impairs SRC-1 functions. We next investigated the potential role of SRC-1 in humans by interrogating exome sequencing and targeted resequencing data on 2548 European ancestry individuals with severe, early-onset obesity (mean body mass index [BMI] standard deviation score = 3; age of onset <10 years) and 1117 ancestry-matched controls. Eleven rare heterozygous variants in SRC-1 were identified; another 8 variants were identified in an earlier data release (total n = 19). Fifteen SRC-1 variants were identified only in obese cases (N1212K was found in two unrelated obese individuals); the other 4 variants were found in controls (Fig. 3a). Compared to WT SRC-1, six of seven randomly selected SRC-1 mutants found in obese cases (except for S738L) were significantly impaired in their interaction with pSTAT3 in leptin-treated HEK293 cells (Fig. 3b, c, Supplementary Figure 3a–c). To test whether heterozygous SRC-1 variants exerted a dominant negative effect to inhibit the interaction between WT SRC-1 and pSTAT3, we overexpressed SRC-1 mutants in HEK293 cells which endogenously express SRC-1. After leptin treatment, an anti-pSTAT3 antibody was used to pull down the immunocomplex from cell lysates, followed by immunoblotting with an anti-SRC-1 antibody to examine the interaction between pSTAT3 and total SRC-1. Overexpression of SRC-1 mutants found in obese cases (6 of 7 tested mutants) significantly decreased the interaction between pSTAT3 and the total SRC-1, suggesting that these SRC-1 mutations can impair the ability of WT SRC-1 to interact with pSTAT3 (Fig. 3d, e and Supplementary Figure 3d–e). This dominant negative effect was not seen when testing the 4 mutants found in controls (Fig. 3e and Supplementary Figure 3d). We used a POMC-luciferase reporter assay to examine the effects of leptin on POMC expression. We found that WT SRC-1 significantly enhanced leptin-induced POMC-luciferase reporter activity, but co-expression of a dominant negative form of STAT3 abolished this effect (Supplementary Figure 3f–g), suggesting that the interaction with STAT3 is required for the observed effects of SRC-1 on POMC transcription. Fourteen of fifteen SRC-1 mutants found in severely obese cases (except for S738L) significantly impaired leptin-induced POMC expression, whereas the 4 control mutants exhibited WT-like responses in this assay (Fig. 3f). Interactions with estrogen receptor-α, vitamin D receptor, glucocorticoid receptor, thyroid hormone receptor-β, and peroxisome proliferator-activated receptor γ (PPARγ) were comparable to those seen for WT SRC-1 (Supplementary Figure 4) in co-immunoprecipitation assays.

A mouse model of the human SRC-1 variant L1376P is obese. To directly test whether rare human SRC-1 variants contribute to Pomc neuron function and/or energy homeostasis, we generated a knock-in mouse model of a human variant which results in a severe loss of function in cells, SRC-1_L1376P (Fig. 4a). Heterozygous mutant mice (SRC-1_L1376P+/−) fed a HFD exhibited increased weight gain, adiposity and food intake, associated with reduced Pomc mRNA levels compared to WT controls (Fig. 4b–e). We recorded leptin-induced depolarization in Pomc neurons in control vs. SRC-1_L1376P+/− mice 1 week after HFD feeding. In control mice, 13/19 (68%) Pomc neurons were depolarized by leptin, whilst only 5/18 (26%) Pomc neurons from SRC-1_L1376P+/− mice were depolarized by leptin (P = 0.022) and the amplitude of leptin-induced depolarization was significantly reduced in these Pomc neurons (Fig. 4f–h). Baseline firing frequency and resting membrane potential were both significantly decreased in Pomc neurons from SRC-1_L1376P+/− mice compared to those from control mice (Fig. 4i–k). Further, the amplitude, but not the frequency, of the mIPSC was significantly higher in Pomc neurons from SRC-1_L1376P+/− mice than those from control mice (Fig. 4l–n). Thus, these data indicate that the SRC-1_L1376P variant causes obesity in mice, associated with decreased Pomc expression and decreased Pomc neuron excitability through both leptin-dependent and independent mechanisms.

Discussion

In this study, we demonstrated that in the hypothalamus, the coactivator SRC-1 modulates the ability of leptin to regulate the expression of the anorectic peptide POMC by directly interacting with phosphorylated STAT3, a known product of leptin-receptor activation. In mice, disruption of SRC-1 in Pomc neurons led to increased food intake, weight gain on a HFD and impaired the acute anorectic response to leptin administration demonstrating the physiological relevance of this molecular interaction. The modest degree of obesity in these mice was comparable to that seen with inactivation of STAT3 in Pomc neurons and studies demonstrating that direct leptin action on Pomc neurons accounts for a proportion of leptin’s effects on body weight. The obesity seen in SRC-1 deletion or mutant mice was less severe than that seen in mice deficient in POMC or melanocortin 4 receptor in keeping with SRC-1’s role as a modulator of Pomc expression. Additionally, leptin-responsive Agrp neurons have been shown to play a major role in energy homeostasis.

We identified 15 rare heterozygous variants in SRC-1 in 16 severely obese individuals and 4 rare variants in controls. Notably, there are several low frequency and many rare variants in this gene in publically available databases (http://gnomad.broadinstitute.org/). Some of these low frequency variants have been shown to have functional consequences, for example, P1272S (MAF: 3.16% in cases, 3.45% in controls; 1.66% in gnomAD) disrupts a putative glycogen synthase 3 (GSK3)β phosphorylation site and has been shown to exhibit reduced ability to co-activate Estrogen Receptor in multiple cell lines. Genetic studies in larger numbers of cases and controls with functional studies of all variants identified will be needed to establish whether variants that result in a loss of function when tested in cells are more likely to be found in severely obese individuals than in controls. In this study, the variants found in obese individuals, but not those found in controls, were associated with impaired interaction with pSTAT3 and reduced POMC reporter activity in cells, predominantly through a dominant negative effect. Given the challenges associated with studying such rare variants, and to directly test whether rare human SRC-1 variants contribute to Pomc neuron function and/or energy homeostasis, we generated a knock-in mouse model of a human variant which results in a severe loss of function in cells, SRC-1_L1376P. The increased food intake and weight gain in heterozygous knock-in mice carrying a severe loss of function human SRC-1 variant supports the
potential importance of the mechanism identified here in humans.

Recent evidence indicates that loss of leptin receptors in Pomc neurons does not affect body weight in chow-fed mice32,39. In line with these reports, we show that loss of SRC-1 in Pomc neurons produced minor effects on energy balance in chow-fed male mice. These suggest that the physiological consequences of disrupting this interaction in normal weight animals are small and/or may be compensated for by increased signaling through non-POMC expressing leptin-responsive neurons30 and/or signaling via phosphoinositide-3-kinase (PI3K)31, mtTOR/S6K32 and/or AMPK pathways33,34. We showed that SRC-1 deletion in Pomc neurons attenuated the acute anorectic response (1 h) to leptin but not the late phase (4–24 h). Cumulatively, these findings indicate that leptin-mediated POMC expression (modulated by the SRC-1-pSTAT3 interaction) primarily contributes to the acute anorectic response to leptin. In keeping with this finding, we demonstrated that the hypothalamic SRC-1-pSTAT3 interaction was enhanced by leptin. Consumption of HFD leads to sustained positive energy balance and an increase in leptin levels. The resulting increase in pSTAT3 would be expected to stimulate POMC expression and reduce food intake, a response that we have shown is modulated by the interaction between pSTAT3 and SRC-1. We suggest that in the absence of functional SRC-1, pSTAT3 is less effective at stimulating POMC expression, which manifests as a relative increase in food intake and weight gain when mice are challenged with HFD. In this way, we conclude that SRC-1 acts as a positive regulator of leptin sensitivity in hypothalamic Pomc neurons.

Our findings suggest that SRC-1 facilitates but is not required for pSTAT3 to regulate POMC expression and that this effect is target-specific as SRC-1 does not modulate the ability of pSTAT3 to regulate Socs3. The mechanisms underlying such specificity remain unclear at present. The molecular interaction between SRC-1 and pSTAT3 enhances pSTAT3-mediated transcriptional activity, presumably by stabilizing pSTAT3 binding to the POMC promoter, although we cannot exclude the possibility that recruitment of other co-activators or histone acetyltransferase activity of SRC-1 also may be involved35. Further studies of the molecular mechanisms that modulate leptin signaling are emerging36–41. For example, Chen et al showed that the nuclear receptor Nur77 facilitates STAT3 acetylation by recruiting acetylase p300 and dissociating deacetylase histone deacetylase 1 (HDAC1) to enhance the transcriptional activity of STAT342. In findings that parallel our studies, they showed that Nur77 deficiency reduced the expression of Pmc in the hypothalamus and attenuated the response to leptin in mice fed on a HFD42.

Transcriptional coactivators such as SRC-1 facilitate the signaling mediated by multiple NRs and/or TFs factors2. Several NRs/TFs have been shown to affect energy homeostasis through their actions in the brain43, including FoxO144–47, ERα48,49, PPARγ30,51, and THR52 and thus could contribute to the body weight phenotype seen with SRC-1 disruption in mice and loss of function variants in humans. In addition to the central actions of SRC-1 on energy homeostasis, SRC-1 is expressed in brown adipose tissue, where it appears to compete with SRC-2 to interact with the PPARγ-PGC1α complex. Picard et al showed that SRC-1-KO mice had reduced rectal temperatures upon cold exposure and reduced oxygen consumption although they did not quantify food intake in this study3. Notably, we did not observe any changes in energy expenditure in mice lacking SRC-1 in Pomc neurons, consistent with the notion that SRC-1 in other tissues may also contribute to the regulation of energy expenditure3. Whilst we found that SRC-1 variants detected in obese patients did not affect the interactions with a number of NRs, these results do not exclude the potential impact of SRC-1 variants on the signaling of these NRs which need to be explored in more detail using tissue-specific conditional knockout mouse models.

Targeting specific coactivator-mediated interactions has emerged as a potential therapeutic strategy to enhance signaling in some tissues while inhibiting signaling in others33,34. For example, Selective Estrogen Receptor Modulators (SERMs) are effective in modulating the growth of hormone-responsive tumors (e.g., Tamoxifen in breast cancer) by impacting on coactivator stability and activity35. As such, compounds that target the interaction between SRC-1 and STAT3 at specific sites may potentially be used to modulate (i.e., enhance) leptin signaling. Could this approach be efficacious in the treatment of obesity? Studies in mice and humans have consistently demonstrated that leptin sensitivity is greatest in those with no/very low endogenous circulating leptin levels56,57. Whether enhancing leptin sensitivity in the context of common obesity, which is associated with elevated leptin levels, may be clinically beneficial, is the subject of much debate3,8,80. The finding that some compounds (e.g., the amylin derivative pramlintide) can augment the effect of leptin61,62 suggests that it may be possible to increase the sensitivity of some individuals to therapeutic leptin administration and that this approach may lead to weight loss. These observations and our findings on SRC-1 suggest that pharmacological approaches based on the modulation of leptin sensitivity could represent a potential therapeutic strategy for the treatment of obesity-associated metabolic disease.

Methods
Contact for reagent and resource sharing. Further information and requests for resources and reagents should be directed to and will be fulfilled by Yong Xu (yongx@bcm.edu) and Sadaf Farooqi (isf20@cam.ac.uk).

Experimental model and subject details. Mice: We crossed regular Pomc-Cre transgenic mice26 and SRC-1lox/lox mice63. This cross produced pomicSRC-1-KO mice (those that are homozygous for SRC-1lox/lox and also carry the Pomc-Cre transgene) and control mice (those that are homozygous for SRC-1lox/lox but do not carry the Pomc-Cre transgene). These littersmates were used to characterize the metabolic profile.

In addition, we also crossed inducible Pomc-CreER mice32 with SRC-1lox/lox mice to generate MpmomcSRC-1-KO mice (those that are homozygous for SRC-1lox/lox and also carry the Pomc-CreER transgene) and control mice (those that are homozygous for SRC-1lox/lox but do not carry the Pomc-CreER transgene). These littersmates were used to characterize the metabolic profile.

For electrophysiological recordings, we crossed the inducible Pomc-CreER and the Rosa26-tdTOMATO mouse alleles onto SRC-1lox/lox mice, to produce MpmomcSRC-1-KO mice with mature Pomc neurons labeled by TOMATO; as controls, we crossed inducible Pomc-CreER mice and Rosa26-tdTOMATO mice to generate Pomc-CreER/Rosa26-tdtOAMTO mice. In parallel, we also crossed the Npy-GFP mouse allele32 and the Rosa26-tdtOMATO allele onto inducible Pomc-CreER mice. This cross produced Pomc-CreER/Rosa26-tdtOAMTO/Npy-GFP mice, which were subjected to histology validation for the inducible Pomc-CreER mice.

To generate the SRC-1L1378Y knock-in mice, a single-guide RNA (sgRNA) sequence was selected over lap amino acid residue L1382 (equivalent to human L1376) in SRC-1 (sgRNA 5′-CATCTGGCTTCTGTTTGAGAGG chr12:4235665-4253687; GRCm38/mm10) using the CRISPR Design Tool (Ran et al. 2013). A DNA template for in vitro transcription of the sgRNA was produced using overlapping oligonucleotides in a high-fidelity PCR reaction44, and sgRNA was transcribed using the MEGASHortscript T7 kit (ThermoFisher, Waltham, MA). Cas9 mRNA was purchased from ThermoFisher. The donor DNA template to introduce the L1382P point mutation, as well as a silent mutation D1381D to introduce a novel restriction site for SnaBI, was purchased as an Ultradimer from IDT (Corvallel, IA). The sequence of ssODN is as follows (complementary to non-target strand): 5′-TGAATATCTG CTCTTTGATGT TATCCTTAAAT AGTGAATT A TCCAGACTG AGACAGACAG GCTCTACTG CAACCAGCTC TCGTCCA ATGCGCTCAT AAAAGGGCCA GGTCAGTAA GAAA, where the homology arms are in bold. The mutations introduced in the donor sequence disrupt base 20 of the sgRNA and the PAM site to prevent additional mutations. The BCM Genetically Engineered Mouse (GEM) Core microinjected Cas9 mRNA (100 ng/μl), Ultradimer ssDNA (100 ng/μl), and sgRNA (20 ng/μl) into the cytoplasm of 20 pronucleus stage C57BL/6 embryos. Cytoplasmic injections were performed using a microinjection needle (1 mm outer and 0.75 mm inner) with a tip diameter of 0.25–0.5 μm, an Eppendorf Femto Jet 4i to set pressure and time to control injection volume (0.5–1 pl per embryo). Injections were performed...
under a 200–400× magnification with Hoffman modulation contrast for visualizations. Founder animals (F0) were identified by PCR-based restriction digestion to detect the CRIPSR generated point mutations in SRC-1. PCR product was first amplified with the primer pairs: 5’-CCTACTCT GGTGCAATTGTGA and 5’-TGTTGGGCTCTGCTGATGAC; and then amplified with 2nd pairs: 5’-CAGTGGACCAACAGGGCTCTC and 5’-ATGGAACGTTCGAGCCTTC. The 121 bp PCR products were then digested with SacII and 31 bp after digest could be detected only for the mutated SRC-1 PCR products. Three independent lines were sequenced for the further confirmation of the point mutation. One of these lines was cross to C57Bl/6 to produce cohorts comprised of SRC-1 ΔTMD5- and wild-type control mice. In some breedings, the Pomc-CreER/Rosa26 tdTOMATO alleles were introduced to allow specific labeling of Pomc neurons. In parallel, we crossed heterozygous SRC-1 KO mice to heterozygous SRC-1 KO mice to produce homozygous SRC-1 KO and wild-type littermates. All the breeders were backcrossed to C57Bl/6 background for more than 12 generations. In addition, some C57Bl6 mice were purchased from the mouse facility of Bay College of Medicine. Care of all animals and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Bay College of Medicine Animal Facility, and all experimental procedures in animals complied with all relevant ethical regulations. Mice were housed in a temperature-controlled environment in groups and all experimental procedures in animals complied with all relevant ethical regulations. Food intake and energy expenditure: To further characterize the food intake and energy expenditure of pomSRC-1 KO mice, an independent male cohort (pomSRC-1 KO mice and their control littermates) was weaned on the standard chow. At the age of 12 weeks, these mice were acclimated into the Comprehensive Laboratory Animal Monitoring System (CLAMS). Mice were housed individually in cages with an infrared camera, and metabolic data were collected daily using CLAMS software. A neuron was considered depolarized or hyperpolarized if a change in membrane potential was larger than 0.1 mV and lasted for at least 2 min. Leptin-induced anorexia: Male pomSRC-1 KO mice and their control littermates (chow-fed) were firstly fasted for 2 h prior to the onset of dark cycle. These mice received intraperitoneal injections of saline or leptin (5 mg/kg in saline in a volume of 0.1 ml/15 g body weight) at 15 min prior to the dark cycle. The standard chow was provided at the onset of dark cycle. Food intake was measured 1, 4, and 24 h after food provided. Each mouse was fasted with saline injection, administered in a balanced order, with 4-day interval between the treatments.

**Histology**
To validate specificity of the indicator of Pomc-CreER transgene, Pomc-CreER/Rosa26 tdTOMATO/Npy-GFP mice received tannoxin injections (0.2 mg/g, i.p., twice) at 9 weeks of age, and then were perfused 1 week later. Brain sections were cut at 25 μm (1.5 series) and subjected to direct visualization of GFP and TOMATO signals using a Leica DMS500 fluorescence microscope with OptiGrid structures and to further characterize the food intake and energy expenditure of pomSRC-1 KO mice and their control littermates.
amplification. Results were normalized against the expression of housekeeping gene-Cyclophilin. Primer sequences were listed in Supplementary Table 1.

Immunoprecipitation (Co-IP) and immunoblotting

The N-terminal HA tag was added using the Q5 site-directed mutagenesis kit (Origene) KpnI and XhoI sites. SRC-1 mutant constructs were generated using the rosiglitazone (at 50 \( \mu \)M, 15 min, HARBOR-UCLA Research And Education Institute), or chromatin input. The assays were repeated independently 3 times. The short form of SRC-1 was generated using the Q5 site-directed mutagenesis kit immunoprecipitated using anti-Flag conjugated beads for 1 h which were total chromatin (1%) was saved as an protein were pre-cleared and incubated with the Pierce Protein A/G Magnetic Beads (at 4 °C). Subsequently, cross-linking was reversed by overnight incubation at 65 °C. DNAs were purified by phenol/chloroform extraction, ethanol precipitation and the enriched promoter fragments were measured by qPCR (primer sequences provided in Supplementary Table 1). Relative STAT3 promoter occupancy was adjusted to the background content of the negative control, and the initial chromatin input. The assays were repeated independently 3 times.

Generation of SRC-1 constructs and expression plasmids: The long form of SRC-1 containing a C-terminal Flag MYC tag was produced from ORG5 (RC224812). The short form of SRC-1 was generated using the Q5 site-directed mutagenesis kit (NEB) using primers containing the sequence specific to the short form of SRC-1. The N-terminal HA tag was used in the Q5 site-directed mutagenesis kit (NEB) using primers containing the HA tag sequence. The short and long forms of SRC-1 was then cloned into the pCDNA3.1(-) vector using KpnI and XhoI restriction sites after PCR amplification of SRC-1 using primers flanking the Origene KpnI and XhoI sites. SRC-1 mutant constructs were generated using the Quickchange II XL site-directed mutagenesis kit (Agilent).

In vitro protein interaction: HEK293 (Human embryonic kidney 293) cells were transfected with either Flag-tagged transcriptional factor (bSTAT3 or hPpARY), Flag-tagged human hormone receptor (EhA, VDR, THR or GR) or empty vector using lipofectamine 2000 (Invitrogen). Before harvest, cell were treated with leptin (at 200 ng/ml, 15 min, HARBOR-UCLA Research And Education Institute), or rosiglitazone (at 50 \( \mu \)M, ADIPOGEN), 17β-estradiol (at 0.2 \( \mu \)g/ml, Sigma, E2758), Vitamin D3 (Calcitrol at 0.2 \( \mu \)M, TOCRIS), dexamethasone (at 10 \( \mu \)M, Sigma, D4902) for 30 min. Cells were collected and lysed with cell lysis buffer: 50 mM Tris, 150 mM NaCl, 10 mM KCl, 1% NP-40, supplemented with protease inhibitors (Complete, Roche) and phosphatase inhibitor cocktail A (Santa Cruz). The lysates were incubated with proper amount of anti-phospho-STAT3 sepharose beads (Cell Signaling, #4074) or anti-Flag beads (Sigma) for 4 h at 4 °C. After wash, beads were aliquoted equally and incubated with comparable amounts of SRC-1 protein (wt or mutants) overnight, and the interacting protein was detected by Western-Blot. SRC-1 WT or mutants were expressed in HEK293 cells and the amount of the SRC-1 expressed was determined using Western-Blot. The minimal sample size was pre-determined for the nature of experiments. The actual sample size was indicated in each figure legend. The data are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism to evaluate normal distribution and variations within and among groups. Methods of statistical analyses were chosen based on the design of each experiment and are indicated in figure legends. P < 0.05 was considered to be statistically significant.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All relevant data are available from the authors. The source data underlying Figs. 1-4 and Supplementary Figs. 1-4 are provided as Source Data files. A Reporting Summary for this Article is available as a Supplementary Information file.

Received: 26 September 2018 Accepted: 21 January 2019
Published online: 12 April 2019

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The authors thank BCM Mouse Embryonic Stem Cell Core and Genetically Engineered Mouse Core for their assistance in generating the SRC-111576 knock-in mouse. The authors acknowledge the expert assistance of Mr. Firoz Vohra and the MMRU Core Director, Dr. Marta Fierotto. Studies in humans were supported by the Wellcome Trust (A.A. v d K., T.M.C., I.B., I.S.F.) (098497/Z/12/Z; WT098051; 203513/Z/16/Z), NIHR Cambridge Biomedical Research Centre (I.S.F., I.B., S. O’R), Bernard Wolfe Health Neuroscience Endowment (I.S.F.). T.M.C. was supported by a Philip Greenwood Clinical Research Fellowship and a Wellcome Trust Research Training Fellowship. The authors are indebted to the participants and their families for their participation and to the Physicians involved in the Genetics of Obesity Study (GOOS) (www.goos.org.uk); whole-exome sequencing was performed as part of the UK10K Consortium (a full list of investigators who contributed to the generation of the human genetics data as part of the UK10K Consortium is available from www.UK10K.org.uk).

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Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-019-08737-6.

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