IL1R1 is required for celastrol’s leptin-sensitization and antiobesity effects

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Celastrol, a pentacyclic triterpene, is the most potent antiobesity agent that has been reported thus far. The mechanism of celastrol’s leptin-sensitizing and antiobesity effects has not yet been elucidated. In this study, we identified interleukin-1 receptor 1 (IL1R1) as a mediator of celastrol’s action by using temporally resolved analysis of the hypothalamic transcriptome in celastrol-treated DIO, lean, and db/db mice. We demonstrate that IL1R1-deficient mice are completely resistant to the effects of celastrol in leptin sensitization and treatment of obesity, diabetes, and nonalcoholic steatohepatitis. Thus, we conclude that IL1R1 is a gatekeeper for celastrol’s metabolic actions.

Increased endoplasmic reticulum (ER) stress in the hypothalamus has a central role in the development of leptin resistance and thus obesity1–3. Given these findings, we undertook in silico screens using systems biology approaches to identify new chemical chaperones that would serve as leptin sensitizers. These efforts yielded celastrol, a pentacyclic triterpene, as a potentially efficacious chemical chaperone and leptin sensitizer4. Treatment with celastrol reduces the body weight of diet-induced obese (DIO) mice by 45–50% and further ameliorates insulin resistance and type 2 diabetes (T2D), nonalcoholic steatohepatitis (NASH), hypercholesterolemia, and liver damage in these mice5.

Considering that leptin signaling in the hypothalamus is critical for regulating food intake and body weight6–8, we investigated how celastrol alters the hypothalamic transcriptome of DIO mice. Because celastrol’s leptin-sensitizing effect requires both high levels of circulating leptin and intact leptin receptor signaling9, we also investigated db/db mice, which have high circulating levels of leptin but lack intact leptin receptor signaling, as well as lean control mice, which have low levels of circulating leptin. We reasoned that inclusion of all three groups would support the elucidation of differentially expressed genes and gene networks that mediate celastrol’s leptin-sensitizing effect in DIO mice. Thus, we undertook a time course analysis of changes in hypothalamic gene expression in celastrol-treated DIO mice as well as db/db and lean mice. Hypothalamic RNA from DIO, db/db, and lean mice was used for microarray analysis after 6 h, 1 d, and 4 d of treatment with vehicle or celastrol. Genes with potential relevance for celastrol-mediated leptin sensitization were identified through a temporal-pattern-based analysis (Fig. 1).

We first grouped the genes on the basis of two threshold criteria: fold up- or downregulation in celastrol-treated mice of $|\log_2(FC)| > 0.20$ and an associated $P$ value of less than 0.05 ($=\log_{10}(P) > 1.3$) (Fig. 1a,c,e). These threshold criteria reduced the analysis to 0.99%, 2.03%, and 1.16% of the hypothalamic transcripts in DIO mice at the time points of 6 h, 1 d, and 4 d, respectively (Fig. 1b). Similar identification of celastrol-associated gene expression changes was repeated for the db/db and lean mouse groups (Fig. 1d,f).

We next grouped together the up- and downregulated genes that met the threshold criteria at each time point for all groups to identify the union and non-union sets (Fig. 1g). This analysis surprisingly revealed no genes that were up- or downregulated in common in DIO, db/db, and lean mice at 6 h, 1 d, and 4 d of treatment. Thus, we turned our attention to the temporal patterns of celastrol-mediated gene expression changes that appeared specific to DIO mice, but not db/db or lean mice (Methods).

In the pattern analysis, patterns 10 and 17 had the highest final score values for the DIO group, differentiating this group from the db/db and lean groups (Fig. 1h). Pattern 10 was characterized by no regulation of the included genes 6 h after celastrol administration but upregulated gene expression at both 1 d and 4 d. Conversely, pattern 17 was characterized by no regulation after 6 h but downregulated gene expression at both 1 d and 4 d (Extended Data Fig. 1). To identify biological pathways containing the genes in patterns 10 and 17, we employed enrichment analysis in the Gene Ontology (GO) database. We were unable to obtain enriched pathways using pattern 17, which had a small number of genes (15 genes). However, using pattern 10, we identified three pathways in which the celastrol-upregulated genes were significantly enriched (Fig. 1i,j) and found Il1r1 as the most upregulated gene within the enriched pathways at both 1 d and 4 d after celastrol treatment (Fig. 1k). A combined analysis of the scores from day 1 and day 4 further identified Il1r1 as having the highest score (Fig. 1l–n and Methods).

We additionally used a linear model combining all experimental conditions. For each gene, we performed an $F$ test for the set of seven simultaneous contrasts in the model and computed $P$ values using tools in the Bioconductor limma package (Extended Data Fig. 1). Using this approach (Methods), we found enrichment of the regulation of inflammatory response pathway, which was the same pathway we identified via the temporal-pattern-based analysis (Extended Data Fig. 1). The celastrol-associated changes in the mRNA levels of Il1r1 and other genes involved in this enriched pathway were further confirmed by qPCR (Extended Data Fig. 1).

IL1R1 is the major receptor mediating the biological function of the IL-1 cytokine family10 and has diverse roles in the maintenance of body homeostasis during virus infection and other processes10,11.

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As IL1R1 deficiency in mice results in mature-onset obesity and leptin resistance, we obtained Il1r1−/− mice and wild-type control mice (Il1r1+/+) and fed them on a high-fat diet (HFD) for 16 weeks to induce obesity. The Il1r1−/− mice gained more weight than Il1r1+/+ mice during HFD feeding (Extended Data Fig. 2). Daily food intake and lean body mass were not different between the groups, but body fat was increased in Il1r1−/− versus Il1r1+/+ mice (Extended Data Fig. 2). Until week 8 of HFD feeding, leptin levels were higher in Il1r1−/− mice; however, this difference disappeared by week 12 (Extended Data Fig. 2). Performance of a glucose tolerance test (GTT) revealed that Il1r1−/− mice were more glucose intolerant (Extended Data Fig. 2), and an insulin tolerance test (ITT) showed that Il1r1−/− mice were less insulin sensitive than controls (Extended Data Fig. 2). Il1r1−/− mice had higher glucose levels than Il1r1+/+ mice, whereas circulating insulin levels were higher in Il1r1−/− mice after 4 weeks of HFD feeding, but not thereafter (Extended Data Fig. 2).

After induction of obesity, Il1r1−/− and Il1r1+/+ mice were treated with either vehicle or celastrol. Celastrol significantly decreased the body weights of Il1r1−/− mice but failed to decrease the body weights of Il1r1+/+ mice (Fig. 2a,b). The reduction in food intake observed in celastrol-treated Il1r1−/− mice was abolished in Il1r1+/+ mice (Fig. 2c). Celastrol did not alter lean body mass in either group (Fig. 2d). Celastrol-induced reduction in body fat and leptin levels was blocked by IL1R1 deficiency (Fig. 2e–g). GTT (Fig. 2h,i) and ITT (Fig. 2j,k), as well as analyses of blood glucose and insulin levels (Fig. 2l,m), demonstrated that deficiency of IL1R1 was sufficient to abrogate enhancement by celastrol of glucose homeostasis. Furthermore, celastrol treatment failed to reduce serum aspartate transaminase (AST) and alanine transaminase (ALT) levels as well as hepatic steatosis in Il1r1−/− mice (Fig. 2n–p). However, celastrol had no effect on core body temperature in either genotype (Extended Data Fig. 3).

To investigate whether celastrol induces sickness-associated behavior to reduce food intake, we performed several behavioral tests on wild-type DIO mice. As expected, food intake and body weight were significantly lower in the celastrol-treated mice (Extended Data Fig. 4). First, we performed home-cage and open-field tests in vehicle- and celastrol-treated mice and documented that celastrol treatment was not associated with changes in innate self-maintenance behavior (Extended Data Fig. 4). In the open-field test, there was no difference in total locomotor movement, average movement velocity, or percentage of time spent moving between the groups (Extended Data Fig. 4). Moreover, time spent within the central portion of the open field did not differ significantly between vehicle- and celastrol-treated mice (Extended Data Fig. 4). Thus, celastrol-treated mice appeared to have no greater (or lesser) anxiety in the context of the open-field test than the control group.

We next performed consecutive sociability and social novelty tests. During the sociability test, time spent in the chamber containing a novel mouse (novel mouse 1) was similar for vehicle- and celastrol-treated mice, as were respective times spent in directly socializing with the novel mouse (Extended Data Fig. 4). During the social novelty test, both groups spent more time in the chamber in which a second novel mouse (novel mouse 2) was introduced (Extended Data Fig. 4). Thus, celastrol had no negative effects on sociability or normal social preferences.

We then placed separate cohorts of mice either fed ad libitum or fasted for 20 h into a (food-)conditioned place preference assay during the light period of the light/dark cycle. Mice in the vehicle- and celastrol-treated groups showed similar levels of activity and preference for food in the ad libitum-fed state (Extended Data Fig. 5). Thus, celastrol treatment that resulted in weight loss had no effect on motivation to seek food in the context of a mildly aversive side chamber when compared to sated DIO mice. The second cohort was used to assess how celastrol affects food-paired place preference after fasting. The celastrol-treated mice showed significantly lower locomotor activity and average velocity of movement, in comparison to vehicle-treated controls (Extended Data Fig. 5). In this assay, the celastrol-treated mice displayed lower motivation to seek food than vehicle-treated mice (Extended Data Fig. 5). Thus, we infer that celastrol-treated DIO mice are not food averse, but are instead specifically less motivated to seek food than fasted control mice, which explains the reduction in locomotor activity seen in the dark cycle for celastrol-treated DIO mice.

We next investigated hypothalamic phosphorylation of STAT3 at Tyr705 (p-STAT3Y705) to assess the status of leptin sensitivity. Celastrol increased the total number of positive cells and total fluorescence intensity for p-STAT3Y705 in the arcuate nucleus (ARC), ventromedial hypothalamus (VMH), and dorsomedial hypothalamus (DMH) in Il1r1−/− mice; however, this effect was lost in Il1r1+/+ mice (Extended Data Fig. 6). We next pretreated Il1r1−/− mice with either vehicle or celastrol and then administered saline or leptin to each group. Hypothalamic immunoblotting showed that celastrol

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**Fig. 1** Identification of IL1R1 as a candidate mediator of celastrol’s action. DIO, db/db, and lean mice were treated with either vehicle (Veh) or celastrol (Cel) for 6 h (250 µg/kg body weight, i.p.), 1 d (100 µg/kg body weight, i.p.), or 4 d (100 µg/kg body weight, i.p., once a day), and total RNA was extracted from the hypothalamus for transcriptome analysis. **a**, **c**, Volcano plots depicting regulation of the hypothalamic transcriptome by celastrol in DIO (**a**), db/db (**c**), and lean (**e**) mice. DIO mice: n = 3 mice (6 h, 1 d, 4 d) for vehicle-treated groups, n = 4 (6 h, 1 d) or 3 mice (4 d) for celastrol-treated groups; db/db mice: n = 4 mice (6 h, 1 d, 4 d) for vehicle-treated groups, n = 3 (6 h) or 4 mice (1 d, 4 d) for celastrol-treated groups; lean control mice: n = 4 mice (6 h, 1 d, 4 d) for vehicle-treated groups, n = 4 (6 h) 3 mice (1 d, 4 d) for celastrol-treated groups. Relative gene expression levels (fold change, FC) in the celastrol- versus vehicle-treated groups are plotted on the x axes as mean log2 ratios (log2 (FC)), while log10-transformed P values are plotted on the y axes (−log10 (P value)). **b**, **d**, **f**. The number of genes that surpassed the threshold criteria as a percentage of the transcriptome in DIO (**b**), db/db (**d**), and lean (**f**) mice at 6 h, 1 d, and 4 d of treatment. **g**, The number of up- or downregulated genes that surpassed the threshold criteria in DIO, db/db, and lean mice at 6 h, 1 d, and 4 d of treatment. **h**, Column 1 (patterns): the 26 possible temporal patterns for celastrol-regulated genes (upregulated, non-regulated, or downregulated versus vehicle treatment), identified by consideration of three time points (6 h, 1 d, and 4 d). Column 2 (number of genes): number of genes falling into each temporal pattern in DIO, db/db, and lean mice at 6 h, 1 d, and 4 d of treatment. **i**, Column 3 (fraction of total): fraction of the total number of celastrol-regulated genes in DIO, db/db, and lean mice falling into each temporal pattern. Column 4 (ratio): the ratio of DIO to db/db and DIO to lean values in column 3, showing the differential patterns in the DIO group relative to the db/db and lean groups. **j**, Column 5 (final score): multiplication of the column 4 ratio values (DIO/db/db × DIO/lean). N/A, not applicable. **k**, Identification of GO pathways significantly enriched in genes found in pattern 10 (n = 38 genes; Fisher/binomial test with Bonferroni-adjusted P value). **l**, Genes in each of the enriched pathways: (i) regulation of inflammatory response, (ii) regulation of defense response, and (iii) regulation of response to external stimulus. **m**, Fold change (Cel/Veh) in expression in DIO mice at 1 d and 4 d for the individual pattern 10 genes present in the identified GO pathways. **n**, Geometric mean of the fold change values at 1 d and 4 d of genes in the GO pathways regulation of inflammatory response (I), regulation of defense response (m), and regulation of response to external stimulus (n).
pretreatment was unable to increase either basal or leptin-induced p-STAT3 Tyr705 levels in Il1r1 c–/– mice (Fig. 3a,b).

Furthermore, pretreatment with celastrol alone or administration together with exogenous leptin did not affect the body weight or food intake of Il1r1 c–/– mice (Fig. 3c,d). We next pretreated both Il1r1 c–/– and Il1r1 c+/+ mice with vehicle or celastrol, but fed the Il1r1 c–/– mice on each day with half the amount of food consumed by celastrol-treated Il1r1 c+/+ mice (Fig. 3e). The food-restricted (FR) Il1r1 c–/– mice—whether treated with vehicle or celastrol—lost similar amounts of body weight as the celastrol-treated Il1r1 c+/+ mice (Fig. 3f), resulting in a >15% loss in body weight in all but the vehicle-treated Il1r1 c+/+ group (Fig. 3g), and had similar blood glucose levels.

| Gene Pathways                     | Fold Enrichment | P-value |
|-----------------------------------|-----------------|---------|
| Regulation of inflammatory response | 15.58           | 3.11 x 10^-4 |
| Response to external stimulus     | 9.86            | 9.86    |
| Regulation of defense response    | 9.4             | 9.4     |

| GO Pathways                     | Regulation of inflammatory response | Regulation of defense response | Regulation of response to external stimulus |
|---------------------------------|-------------------------------------|--------------------------------|------------------------------------------|
| Apobec3                         | Apobec3                             | Apobec3                         | Apobec3                                  |
| Fgl1                             | Fgl1                                | Fgl1                             | Fgl1                                     |
| Tgm2                             | Tgm2                                | Tgm2                             | Tgm2                                     |
| Bcl6                             | Bcl6                                | Bcl6                             | Bcl6                                     |
| Partners                         | Part1                               | Part1                            | Part1                                    |
| Tm6                               | Tm6                                 | Tm6                              | Tm6                                      |
| Mfia                              | Mfia                                | Mfia                             | Mfia                                     |
| Apoebc3                          | Apoebc3                             | Apoebc3                         | Apoebc3                                  |

| Column 1 Patterns | Column 2 Number of genes | Column 3 Fraction of total | Column 4 Ratio | Column 5 Final score |
|-------------------|--------------------------|-----------------------------|----------------|----------------------|
| DIO               | db/db                    | Lean                        | DIO            | db/db                |
| Up                | 133                      | 47                          | 26.9%          | 0.003                |
| Down              | 78                       | 99                          | 22.3%          | 0.000                |
| Lean              | 181                      | 105                         | 93.3%          | 0.006                |
| 1 d DIO           | db/db                    | Lean                        | 1 d DIO        | db/db                |
| Up                | 253                      | 86                          | 25.0%          | 0.005                |
| Down              | 77                       | 185                         | 30.2%          | 0.004                |
| Lean              | 191                      | 181                         | 92.9%          | 0.001                |
| 4 d DIO           | db/db                    | Lean                        | 4 d DIO        | db/db                |
| Up                | 183                      | 72                          | 22.0%          | 0.000                |
| Down              | 72                       | 175                         | 28.2%          | 0.001                |
| Lean              | 151                      | 175                         | 85.3%          | 0.007                |

| Regulation of defense response   |                         |                         |                         |
|-----------------------------------|-------------------------|-------------------------|-------------------------|
| Il1r1 c–/–                        |                         |                         |                         |
| PIK3                              |                         |                         |                         |
| PI3K                              |                         |                         |                         |
| PI3K                              |                         |                         |                         |
| PI3K                              |                         |                         |                         |

| Regulation of response to external stimulus |                         |                         |                         |
|-----------------------------------------------|-------------------------|-------------------------|-------------------------|
| Il1r1 c–/–                        |                         |                         |                         |
| PIK3                              |                         |                         |                         |
| PI3K                              |                         |                         |                         |
| PI3K                              |                         |                         |                         |

Bar graph showing various treatments: DIO vs. db/db, Lean vs. DIO, and 1 d vs. 4 d, with fold change and P-value calculations.
Fig. 2 | IL1R1 is required for celastrol’s antiobesity effects. Il1r1+/+ and Il1r1−/− mice were fed a HFD for 20 weeks and treated with vehicle (Veh) or celastrol (Cel; 100 μg/kg body weight, i.p., once a day) for 3 weeks. n = 9 for vehicle- and celastrol-treated Il1r1+/+ mice; n = 12 for vehicle-treated and n = 13 for celastrol-treated Il1r1−/− mice. a, Blood weight. Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.653 for Il1r1−/− mice. b, Percent change in body weight for Il1r1+/+ and Il1r1−/− mice during the treatment period. Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.104 for Il1r1−/− mice. c, Average 24-h food intake per mouse during the first week of vehicle or celastrol treatment. Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.9 for Il1r1−/− mice. d-f, dual-energy X-ray absorptiometry (DEXA) of Il1r1+/+ and Il1r1−/− mice treated with vehicle or celastrol for 3 weeks. d, Lean body mass. e, Fat mass. Cel vs. Veh, P = 0.005 for Il1r1+/+ mice and P > 0.99 for Il1r1−/− mice. f, Fat percentage at the end of the treatment period. Cel vs. Veh, P = 0.0005 for Il1r1+/+ mice and P = 0.175 for Il1r1−/− mice. g, Plasma leptin levels in Il1r1+/+ and Il1r1−/− mice after 3 weeks of vehicle or celastrol treatment. Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.162 for Il1r1−/− mice. h, GTT after 1 week of vehicle or celastrol treatment (i) and area under curve (AUC) analysis of GTT results (j). Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.811 for Il1r1−/− mice. k, ITT after 2 weeks of treatment (k) and AUC analysis of ITT results (l). Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.634 for Il1r1−/− mice. I, Six-hour fasting blood glucose levels of Il1r1+/+ and Il1r1−/− mice after 1 week of vehicle or celastrol treatment. Cel vs. Veh, P < 0.0001 for Il1r1+/+ mice and P = 0.88 for Il1r1−/− mice. m, Plasma insulin levels of Il1r1+/+ and Il1r1−/− mice after 3 weeks of treatment. The experiment was repeated in two independent cohorts with similar outcomes (total n = 13 for vehicle-treated and celastrol-treated Il1r1−/− mice; n = 17 for vehicle-treated and n = 18 for celastrol-treated Il1r1−/− mice). Cel vs. Veh, P = 0.04 for Il1r1+/+ mice and P > 0.99 for Il1r1−/− mice. n, Plasma ALT levels after 3 weeks of treatment. Cel vs. Veh, P = 0.02 for Il1r1+/+ mice and P > 0.99 for Il1r1−/− mice. p, H&E staining of liver sections from Il1r1+/+ and Il1r1−/− mice treated with vehicle or celastrol for 3 weeks. Values indicate averages ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant (P > 0.05).
**Fig. 3 | IL1R1 is required for celastrol’s ability to enhance the actions of exogenous leptin.** a, Il1r1–/– mice were treated with vehicle (Veh) or celastrol (Cel; 500 μg/kg body weight, i.p.). After 15 h, mice were administered either saline (Sal) or leptin (Lep; 1 mg/kg body weight, i.p.) 45 min before collection of hypothalamus. The results are a combination of two independent experiments (total n = 6 mice in each group). a, Representative immunoblots for p-STAT3 Tyr705 and total STAT3 in the hypothalamus. Representative immunoblots for p-STAT3 Tyr705 and total STAT3 in the hypothalamus. b, Quantification of the ratio of p-STAT3 Tyr705 to STAT3 signal on immunoblots. c, d, Il1r1–/– mice were treated with vehicle or celastrol (100 μg/kg body weight, i.p., once a day) for 2 d and then administered saline or leptin (1 mg/kg body weight). c, Percent change in body weight during the 16-h period after saline or leptin administration. Lep vs. Sal, P = 0.883 for vehicle-treated mice and P = 0.99 for celastrol-treated mice. d, Cumulative food intake during the 16-h period after saline or leptin treatment. The results in c and d combine data from two independent cohorts (total n = 9 mice for saline-injected groups and n = 10 mice for leptin-injected groups). e–i, Il1r1–/– mice and food-restricted (FR) Il1r1–/– mice (0.5 g per day) were injected with vehicle or celastrol (100 μg/kg body weight, i.p., once a day) for 7 d. Subsequently, mice were divided into two subgroups and administered saline or leptin (1 mg/kg body weight, i.p.) for 45 min (n = 6 mice for each group). e, Daily food intake (in grams). Cel vs. Veh, P < 0.0001 for Il1r1–/– mice. f, Body weight. Cel vs. Veh, P < 0.0001 for Il1r1–/– mice and P = 0.657 for Il1r1+/+ mice. g, Percent change in body weight over the 7-d treatment period. Cel vs. Veh, P < 0.0001 for Il1r1–/– mice and P = 0.235 for Il1r1+/+ mice. h, Fed blood glucose levels in Il1r1–/– and Il1r1+/+ mice after 7 d of treatment. Cel vs. Veh, P < 0.0001 for Il1r1–/– mice and P > 0.099 for Il1r1+/+ mice; Il1r1–/– + Veh vs. Il1r1–/– + Lep, P = 0.0001. i, Representative immunoblots of p-STAT3 Tyr705 and total STAT3 in hypothalamus for the Il1r1–/– and Il1r1+/+ mice shown in e–h. j, Ratio of p-STAT3 Tyr705 to total STAT3 signal on blots of Il1r1–/– hypothalamus. Sal vs. Lep, P = 0.01 for celastrol-treated groups and P = 0.639 for vehicle-treated groups; Veh vs. Cel, P = 0.02 for leptin-treated groups. k, Ratio of p-STAT3 Tyr705 to total STAT3 signal on blots of Il1r1–/– hypothalamus. Sal vs. Lep, P = 0.0005 for celastrol-treated groups and P = 0.006 for vehicle-treated groups; Veh vs. Cel, P = 0.833 for leptin-treated groups. l, Normalized ratio of p-STAT3 Tyr705 to total STAT3 signal on blots of hypothalamus from Il1r1–/– and Il1r1+/+ mice. Veh + Lep vs. Cel + Lep, P = 0.007, and Cel + Sal vs. Cel + Lep, P = 0.004 for Il1r1+/+ mice; Veh + Lep vs. Cel + Lep, P > 0.99, and Cel + Sal vs. Cel + Lep, P = 0.691 for Il1r1–/– mice; Il1r1–/– vs. Il1r1+/+ mice, P = 0.005 for Cel + Lep groups. Values indicate means ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant (P > 0.05).
Administration of anakinra attenuates celastrol action. a–g, DIO mice were centrally administered aCSF (Con) or anakinra (Ank; 5 μg/mouse per day, intracerebroventricular injection) in combination with celastrol (Cel; 100 μg/kg, i.p., once a day) for 7 d (n = 5 mice for each group). a, Body weight. P = 0.02. b, Percent body weight reduction. P < 0.0001. c, Daily food intake. P < 0.0001. d, Average 24-h food intake per mouse during the treatment. P = 0.005. e, Plasma leptin levels. P = 0.008. f, Six-hour fasting blood glucose levels. P = 0.267. g, Fasted plasma insulin levels after 7 d of treatment. P = 0.257. The experiment was repeated in two independent cohorts with similar outcomes (total n = 12 mice in the control group and n = 11 mice in the anakinra-treated group). h–m, DIO mice were peripherally administered anakinra (Ank; 30 mg/kg body weight, i.p., twice a day) in combination with celastrol (100 μg/kg body weight, i.p., once a day, intracerebroventricular injection) for 7 d (n = 6 mice for the Sal + Veh, Sal + Cel, and Ank + Veh groups and n = 8 mice for the Ank + Cel group). h, Body weight. Sal vs. Veh, Sal vs. Cel, and Veh vs. Cel, P = 0.0001; Veh vs. Anakinra, P = 0.0006; Sal vs. Anakinra, P = 0.0036. i, Percent body weight reduction. P < 0.0001 for each of Sal + Veh vs. Sal + Cel, Anakinra + Veh vs. Anakinra + Cel, and Sal + Cel vs. Anakinra + Cel. j, Daily food intake. P < 0.0001 for each of Sal + Veh vs. Sal + Cel, Anakinra + Veh vs. Anakinra + Cel, and Sal + Cel vs. Anakinra + Cel. k, Average 24-h food intake per mouse. Sal vs. Veh, P > 0.99 for vehicle-treated groups and P = 0.08 for celastrol-treated groups and P = 0.08 for anakinra-treated groups. Veh vs. Cel, P < 0.0001 for saline-treated groups and P = 0.0001 for anakinra-treated groups. l, Plasma leptin levels. Sal vs. Veh, P = 0.174 for vehicle-treated groups and P > 0.99 for celastrol-treated groups; Veh vs. Cel, P = 0.001 for saline-treated groups and P = 0.756 for anakinra-treated groups. m, Six-hour fasting blood glucose levels after 7 d of treatment. Sal vs. Veh, P > 0.99 for both vehicle- and celastrol-treated groups; Veh vs. Cel, P < 0.04 for saline-treated groups and P = 0.008 for anakinra-treated groups. Values indicate means ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test (a–c and h–m) or two-tailed Student’s t test (d–g). *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant (P > 0.05).
as celastrol-treated Il1r1+/+ mice (Fig. 3h). At day 7, we injected mice with saline or leptin and analyzed p-STAT3 Tyr705 levels in the hypothalamus. Celastrol treatment resulted in significantly higher levels of leptin-stimulated p-STAT3 Tyr705 in Il1r1+/+ mice (Fig. 3i,j). By contrast, although leptin clearly stimulated phosphorylation of STAT3 in FR Il1r1−/− mice, there was not a significantly higher level of p-STAT3 Tyr705 in celastrol- versus vehicle-treated Il1r1−/− mice (Fig. 3i,k,l).

Analysis of hypothalamic gene expression showed higher Agrp and Socs3 mRNA levels in the hypothalamus of celastrol-treated Il1r1+/+ mice; however, this effect was absent from Il1r1−/− mice. Npy and Pomc mRNA levels were not significantly different between the groups (Extended Data Fig. 6).

To investigate whether central or peripheral IL1R1 is involved in mediating celastrol’s antiobesity action, we used an IL1R1 antagonist (anakinra) to reduce its activity. We centrally administered either artificial cerebrospinal fluid (aCSF) as a control or anakinra for 2 d and then administered celastrol peripherally for 7 d. The body weight of the control group was reduced by around 16%, whereas that of the anakinra-treated group declined by around 10.8% (Fig. 4a,b) following celastrol treatment. Daily food intake was significantly lower in the control group (in comparison to the anakinra-treated group) (Fig. 4c,d). Furthermore, circulating leptin levels were significantly lower in the control group than in the anakinra-treated group, but blood glucose and insulin levels were not different between the groups (Fig. 4e–g).

Anakinra crosses the blood–brain barrier. To investigate whether peripheral administration of anakinra would have similar effects as central administration, we first injected DIO mice peripherally with saline or anakinra and then administered vehicle or celastrol for 7 d. Similar results to those of central administration of anakinra were obtained from these experiments (Fig. 4h–m). These data indicate that both central and peripheral administration of anakinra reduce the anorexigenic and antiobesity effects of celastrol, suggesting that celastrol reduces food intake and body weight through its effects on the central nervous system (CNS) in an IL1R1-dependent manner.

Il1r1−/− mice had lower levels of phosphorylated p38 (p-p38) than Il1r1+/+ mice in hypothalamus and lower levels of phosphorylated Erk1 and Erk2 (p-Erk1/2) in liver (Extended Data Fig. 7). Central administration of anakinra resulted in lower hypothalamic p-p38 levels in celastrol-treated DIO mice compared with the celastrol-treated control mice (Extended Data Fig. 7). Peripheral administration of anakinra likewise resulted in lower hepatic p-Erk1/2 levels in both vehicle- and celastrol-treated groups (Ank+Veh and Ank+Cel), in comparison to saline-treated control groups (Sal+Veh and Sal+Cel), although the difference was significant between saline- and anakinra-administrated group after celastrol treatment (Sal+Cel versus Ank+Cel) (Extended Data Fig. 7). These data show that anakinra treatment, similar to IL1R1 deficiency, is associated with lower MAP kinase phosphorylation in hypothalamus and/or liver, indicating that anakinra treatment was effective. We next treated Il1r1+/+, Il1r1−/−, and Il1r1−/− mice with either vehicle or celastrol and documented that haplosufficiency for Il1r1 was sufficient for response to celastrol treatment (Extended Data Fig. 8). Notably, celastrol treatment did not alter circulating IL-1β levels in Il1r1+/+, Il1r1−/−, and Il1r1−/− mice, suggesting that the antiobesity function of celastrol is independent of circulating IL-1β levels (Extended Data Fig. 8).

LepRb and IL1R1 are members of the cytokine receptor family, for which signaling activity requires dimerization. Coimmunoprecipitation of Flag-tagged LepRb with IL1R1 in cells indicates that LepRb and IL1R1 might physically interact (Extended Data Fig. 9). However, we have not yet demonstrated that this interaction occurs in mouse hypothalamus or that it underlies celastrol’s antiobesity effects.

Mammals, through evolution, have developed strong mechanisms to sense and cope with starvation by reducing energy expenditure and activating central mechanisms that promote food seeking. By contrast, chronic presence of excess energy stores in the body neither increases energy expenditure nor reduces food seeking in the modern obesogenic environment. Rather, the obese state is associated with lower energy expenditure, as well as excess food intake, which is sufficient to maintain body weight around a higher lower bound. One explanation for this phenomenon is the development of leptin resistance, and perhaps generalized cytokine resistance. Similar in its effects to leptin receptor deficiency, leptin resistance is an obesity-associated condition in which high levels of leptin fail to appropriately signal the presence of adequate energy stores within the organism.

Conserved throughout metazoan lineages but present in prototypical form even in protozoans, ER stress signaling provides an important homeostatic control for dealing with intracellular nutrient deprivation or excess. Relief of obesity-associated ER stress with chemical chaperones has been shown to reduce leptin resistance, leading to the identification of celastrol as a truly effective treatment for diet-induced obesity in mice. An important conclusion of this work is that leptin resistance from hypothalamic ER stress is not just a result of obesity but actively contributes to maintenance of this pathological state.

Our results establish that IL1R1 is required for the effects of celastrol in DIO mice to promote leptin sensitization, reduce food consumption and obesity, and restore glucose tolerance and insulin sensitivity—as well as to reduce hepatic steatosis and improve liver function. The identification of IL1R1 as a mediator of improved metabolic health (secondary to the action of celastrol) offers unique insight into obesity and its associated ailments. IL1R1 belongs to the cytokine receptor superfamily and activates inflammatory signaling pathways. The involvement of IL1R1 in increasing leptin sensitivity is against the general dogma that cytokine/inflammatory signaling pathways have a key role in aggravation of obesity and associated metabolic diseases. Leptin itself is a cytokine, and its receptor belongs to a cytokine (IL-6) receptor family. As is well known, deficiency of leptin or its receptor leads to morbid obesity. Similarly, IL1R1 deficiency—as published previously and confirmed herein—leads to a higher degree of obesity and metabolic disturbance. These data support the idea that cytokine signaling can be co-opted for beneficial metabolic purposes and that development of cytokine resistance could be one of the mechanisms underlying development of ER stress, obesity, and T2D. Further understanding the exact mechanism(s) of action by which celastrol and IL1R1 increase leptin sensitivity will take extensive efforts but will undoubtedly yield new opportunities for effective therapy of obesity and its associated diseases.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at [https://doi.org/10.1038/s41591-019-0358-x](https://doi.org/10.1038/s41591-019-0358-x).

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Methods

Animals and treatment. We performed all animal experiments according to the relevant ethical regulations and protocols approved by the Boston Children’s Hospital Institutional Animal Care and Use Committee. Wild-type C57BL/6J mice (stock number 000097), db/db mice (stock number 000097), and Il1r1 homozygous knockout mice (stock number 003245) were purchased from Jackson Laboratories. Heterozygous Il1r1-knockout mice (Il1r1+/−) were generated by crossing male Il1r1 homozygous knockout mice with female wild-type mice. Il1r1 knockout mice (Il1r1−/−) and their wild-type controls (Il1r1+/+) were generated by intercrossing Il1r1−/− mice.

To generate DIO mice, wild-type (C57BL/6J) or Il1r1−/− and Il1r1+−/+ male mice were placed on a HFD (45% of calories from fat) at age 4–6 weeks and maintained on the same diet for 16–20 weeks. The HFD was purchased from Research Diets. Eight-week-old db/db and lean mice were placed on normal chow diet (NCD; 13.5% of calories from fat) from Lab Diet. Mice were housed on a 12-h dark/12-h light cycle with the dark cycle lasting from 7 p.m. to 7 a.m. All mice had ad libitum access to food and water unless otherwise indicated. Celastrol was dissolved in sterile DMSO (25 µl) and administered to the mice i.p. 60–90 min prior to the dark cycle, unless otherwise specified. The corresponding vehicle groups were injected i.p. with a total volume of 25 µl of sterile DMSO.

Total RNA extraction and microarray analysis. DIO, lean, and db/db mice were injected i.p. with vehicle or celastrol one time (250 µg/kg body weight) and killed shortly thereafter at 6 h or were injected once per day (100 µg/kg body weight) for 1 d or 4 d and killed 6 h after the final injection. Hypothalami were extracted and stored at −80 °C for RNA extraction. To extract total RNA, 500 µl of TRIzol was added to each sample. Tissues were homogenized with a bench-top TissueLyser II (Qiagen), and hypotonic RNA was extracted according to the manufacturer’s instructions for the TRIzol lysis reagent. For microarray analysis, the extracted total RNA was cleaned using an RNeasy MinCleanup kit (74104, Qiagen) and 1 µg of total RNA was used for microarray analysis in the Molecular Biology Core Facilities of the Dana–Farber Cancer Institute–Harvard Medical School. Briefly, the total RNA was processed using the Affymetrix GeneChip WT Reagent kit. The kit generates amplified and biotinylated sense-stranded DNA targets for hybridization. Fragmented, biotinylated cDNA was hybridized to Mouse Gene 1.0 ST arrays for 16 h at 45 °C and 60 r.p.m. in an Affymetrix GeneChip Hybridization Oven 645. Mouse GeneChip ST arrays were washed and stained using the Affymetrix FS450 automated fluidics station. GeneChip arrays were scanned in an Affymetrix GCS3000 7G scanner with autoloader.

Microarray data analysis. To begin analysis of the microarray data, we performed principal-component analysis and sample–sample correlation testing as quality control to look for unexpected relationships between the individual samples. For differential analysis of gene expression between celastrol- and vehicle-treated groups, as well as between DIO and db/db mice and between DIO and lean mice, we first performed the temporal-pattern-based gene identification approach, which used liberal thresholds (P < 0.05 by two-tailed t test and log2(FC) > 0.5) and grouped up- or downregulated genes. On the basis of the three time points used (6 h, 1 d, and 4 d) and the three possible regulations in celastrol versus vehicle-injected mice at each time point (upregulated, non-regulated, or downregulated) in DIO mice, there were 27 (3 × 3 × 3) possible patterns in total. One of the 27 patterns—containing genes non-regulated at all three time points—was excluded from consideration, as it contained six genes that met the fold change and significance criteria. Thus, we examined 26 patterns of celastrol-associated gene expression changes (Fig. 1b, column 1).

For each pattern, we separately denoted the number of genes that exceeded our threshold criteria in each of the three mouse models (Fig. 1b, column 2). We then calculated the fraction of all threshold-exceeding genes in each mouse model that were accounted for by each of the 26 temporal patterns (Fig. 1b, column 3) (for example, for pattern 10, 38/921 = 0.041 in the DIO model). To highlight the temporal patterns that accounted, in the greatest part, for differences in celastrol-mediated regulation between DIO and control mice (db/db and lean), we divided each column 3 DIO value by the corresponding column 3 values for db/db and lean control. To obtain this ratio value, we multiplied each column 3 DIO value by the corresponding Rn18s value, and then divided the product by the Rn18s value of the DIO mice. For each gene, we calculated this ratio for each column and determined which temporal pattern best described the expression of each gene as a function of treatment over time.

We also used a linear model combining all experimental conditions as factors to identify celastrol-regulated genes. For each gene, we performed an F test for the set of seven simultaneous contrasts in the model and computed P values (Benjamini–Hochberg) using tools in the Bioconductor limma package.

To begin analysis of the microarray data, we performed GCS3000 7G scanner with autoloader. FS450 automated fluidics station. GeneChip arrays were scanned in an Affymetrix.

DNA synthesis and quantitative real-time PCR. Total RNA was extracted from tissues with TRIzol lysis reagent following the manufacturer’s protocol. cDNA was synthesized with 1 µg of total RNA using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's protocol. qPCR was conducted by SYBR Green on a QuantStudio 6 Flex Real-Time PCR system (Life Technologies). The relative expression of genes of interest was calculated by the comparative C method and Rn18s was used as an endogenous control. The primers used to detect the genes are as follows: Il1r1: forward: 5′-GTGCTACCTGGGGCTTATTTGT-3′, reverse: 5′-GGAGTAGAAAGGAGACCTTGGAAT-3′; Rn18s: forward: 5′-AGTTCTCTGCGTTTGCACA-3′, reverse: 5′-CGATCGAGGGGGGCTCATCA-3′; Agrp: forward: 5′-AGCTGACGTGGCCTGCTGG-3′, reverse: 5′-CAGCTATTACCTGGAAACTCCT-3′; Npy: forward: 5′-ATGGTACGAGACCGAGAATG-3′, reverse: 5′-TGTCGAGACGGGAGATGAT-3′; Pomc: forward: 5′-ATGCCGAGTTCTGCTACAGT-3′, reverse: 5′-TCCAAGAGGAGGTGATTGTT-3′; Socs3: forward: 5′-ATGTCACCACAGCGATCTTT-3′, reverse: 5′-TCCAGTGAATGCGCTCCTCT-3′.

Behavioral studies. Home-cage behavior. DIO mice were singly housed during acclimation with daily injection of vehicle. After acclimation, the mice were injected with vehicle or celastrol (100 µg/kg body weight, i.p., daily) for 3 d prior to the behavioral test. On the fourth day of treatment, 1 h after the start of the light cycle, the mice were administered vehicle or celastrol (200 µg/kg body weight, i.p.) and then placed in the testing room 1 h for acclimation. During the test, mice in the home cage were placed under the CCD camera of an Ethovision video tracking system for 20 min. Grooming frequency and grooming bouts were analyzed with blind to mouse treatment.

Open-field test. DIO mice were injected with vehicle or celastrol (100 µg/kg body weight, i.p., daily) for 3 d prior to the behavioral test. On the fourth day of treatment, 1 h after the start of the light cycle, the mice were administered a single dose of either vehicle or celastrol (200 µg/kg body weight, i.p.) and the open-field test was conducted 6 h after the last injection in the context of ad libitum feeding. Before the test, the mice were exposed to the field for 5 min for two consecutive days. During the test, the mice were placed on the side of the field and allowed to explore the apparatus for 10 min. Noldus Ethovision tracking software was used to map the central portion of the field. Locomotor activity was recorded by a CCD camera and analyzed using a Noldus Ethovision video tracking system.

Social behavior test. DIO mice were injected with vehicle or celastrol (100 µg/kg body weight, i.p., daily) for 3 d prior to the behavioral test. On the fourth day, 1 h after the start of the light cycle, the mice were treated with vehicle or celastrol (200 µg/kg body weight, i.p.) for 6 h and the behavioral test was conducted in the context of ad libitum feeding using a three-chambered apparatus, which includes a central chamber and two side chambers. The dividing walls had doorways allowing access into each chamber. Each side chamber was paired with a wire cup as the novel object, where the novel mouse would be located. In the habituation phase, mice were placed in the central chamber and allowed to freely access all chambers for 5 min without the novel mouse in the wire cup. At the end of habituation, the treated mice were returned to their home cage and a novel mouse (novel mouse 1) was placed into the wire cup located in the corner of the right side chamber. In the sociability phase, mice were placed in the central chamber again and allowed to freely move between chambers for 10 min. After the sociability test, the treated mice were returned to their home cage and novel mouse 1 remained in the right chamber. For the social novelty test, a second novel mouse (novel mouse 2) was introduced into the wire cup located in the left side chamber. The treated mice were placed in the central chamber again and allowed to freely access all the chambers for 10 min. Activities, including total movement, time spent in different chambers, and time spent with different novel mice, were recorded by a CCD camera and analyzed using Noldus Ethovision video tracking system.

Conditioned place preference test. Mice were tested for food-conditioned place preference in a three-chambered apparatus comprising side chambers and a central chamber. One side chamber had a wall paired black, a rough floor, and an inedible...
object (food mimic), while the other side chamber had a wall paired white, a smooth floor, and food. The experiment included three phases.

In the preconditioning phase (day 1), mice were placed in the central chamber and allowed to freely access the side chambers for 15 min. In the conditioning phase, the mice had free access to the food-paired side chamber on days 2, 3, and 6 and had no access to the food-paired side chamber on days 3, 4, and 7. The mice were injected with vehicle (25 µl of DMSO, i.p., daily) from day 1 through day 4. Prior to the place preference test, mice received injections of vehicle or celastrol (100 µg/kg body weight, i.p., daily at 5 p.m.) for 3 d (from day 3 through day 7). The mice were then either fasted for 20 h (fasting groups) or allowed to freely access food (ad libitum–fed groups).

The testing phase was performed during the light cycle on the fourth day of celastrol treatment following injection of vehicle or celastrol (200 µg/kg body weight, i.p., 8 a.m.) for 5 h. The mice were placed in the central chamber and allowed to freely move between the chambers for 20 min. The frequency with which the mice traveled to each chamber and the food-located zones, as well as the total and percentage of time spent in each chamber, was recorded via a CCD camera with a Noldus Ethovision video tracking system and analyzed using Noldus Ethovision software.

Blood collection. Blood was collected from the tail vein with heparinized capillary tubes, transferred to ice-cold Eppendorf tubes, and centrifuged at 3,000 r.p.m. for 30 min at 4 °C. Plasma portions were transferred to new vials and stored at −80 °C until processing.

Glucose tolerance test and insulin tolerance test. For the GTT, mice were fasted for 15 h (from 5 p.m. to 8 a.m.) and dextrose (1 g/kg body weight, i.p., 8 a.m.) was administered p.o. Blood glucose levels were measured with a glucometer using the blood obtained from the tail before dextrose administration and 15, 30, 60, 90, and 120 min after administration.

For the ITT, mice were fasted for 6 h (from 8 a.m. to 2 p.m.), Recombinant human insulin (1 IU/kg body weight) was administered i.p. Blood glucose levels were measured with a glucometer using the blood obtained from the tail before insulin administration and 15, 30, 60, 90, and 120 min after administration.

Leptin administration and food intake and body weight measurements. DIO Il1r1−/− mice were administered vehicle or celastrol (100 µg/kg body weight, i.p.) once a day for 2 d. One hour after the second injection (7 p.m.), we further divided the vehicle- and celastrol-injected groups into two subgroups. Each subgroup of mice was injected with either saline or leptin (1 mg/kg body weight, i.p.). Food intake and body weight changes were measured during the 16-h experimental period after saline or leptin administration.

Body composition measurement. We assessed total lean mass, fat mass, and fat percentage using dual-energy X-ray absorptiometry (DEXA; Lunar PIXImus2, GE Lunar Corp.).

Body temperature measurement. Body temperature was measured by inserting a probe (model BAT-12, Physiemp Instruments) into the rectum of mice after 1, 2, and 3 weeks of celastrol treatment.

Total-protein extraction and western blotting. The hypothalamus or liver was homogenized with a bench-top TissueLyser II in ice-cold tissue lysis buffer (25 mM Tris-HCl (pH 7.4), 100 mM NaCl, 50 mM Na2PO4, 10 mM Na4P2O7, 10 mM Na3VO4, 10 mM EGTA, 10 mM EDTA, and 1% NP-40; supplemented with phosphatase and protease inhibitors) and then subjected to centrifugation at 13,400g for 30 min at 4 °C. Protein concentration was quantified using a Protein Assay kit (Bio-Rad). Protein samples were mixed with 5x Laemmli buffer and boiled at 95 °C for 5 min before loading onto SDS–PAGE gels. After electrophoresis, we transferred the proteins onto PVDF membranes at 4 °C and 100 V for 2 h and blocked the membranes in TRIS–0.1% Tween-20 (TBST) with 10% blocking reagent. We incubated the membranes with primary antibodies overnight in TBST containing 10% blocking reagent. After primary antibody incubation, we probed the membranes three times in TBST each 20 min each wash with TBST and then incubated the membranes with secondary antibodies in TBST with 10% blocking reagent for 1 h at room temperature. After washing the membranes three times in TBST, we developed them using a chemiluminescence assay system and quantified band intensities using the ImageJ (NIH) analysis program.

Hormone and metabolite measurements from mouse plasma. Plasma leptin, insulin, ALT, and AST levels were measured using the corresponding ELISA or assay kit according to the manufacturer's instructions. Plasma from DIO mice was diluted 5–10 times in the leptin ELISA. We used 5 µl of plasma for the insulin ELISA and AST assay and 10 µl of plasma for the ALT assay.

Immunoprecipitation. HEK 293 cells were transfected with plasmids encoding LacZ, human IL1R1, or Flag-tagged LepRb for 24 h. Immunoprecipitation cell lysates (500 µg per sample) were incubated with anti-Flag M2 Affinity Agarose Gel (30 µl; A2220, Sigma) for 3 h at room temperature with gentle rotation. Beads were precipitated by centrifugation at 800g for 30 s and washed three times with ice-cold lysis buffer. The pellet was resuspended in 2x Laemmli buffer and incubated at 100 °C for 5 min. Supernatants were collected and used for western blotting to detect Flag and IL1R1. The expression levels of IL1R1 and HSP90 in the total-cell lysates were detected as loading controls.

Immunohistofluorescence staining. DIO Il1r1−/− or Il1r1+ mice were acclimated with 25 µl of DMSO i.p. 60 min prior to the dark cycle for 7 d. Then, mice were injected with 25 µl of either vehicle or celastrol (100 µg/kg body weight) for 3 d. Fourteen hours after the third injection, each group of mice was injected once more with vehicle or celastrol (200 µg/kg body weight) and was then fasted for 6 h. Subsequently, brains were fixed via perfusion with ice-cold 4% paraformaldehyde (PFA) through the heart after the blood was flushed out with PBS (3.2 mM Na2HPO4, 0.5 mM KH2PO4, 1.3 mM KCl, and 135 mM NaCl, pH 7.4). Following additional overnight fixation in 4% PFA, brains were incubated sequentially with 20% sucrose and 30% sucrose for 2 d and were frozen in Tissue-Tek OCT compound (Sakura Finetek) on dry ice and stored at −80 °C. A total of 48 sections (30 µm section, divided into 4 sets with 12 sections/set; from bregma −0.9 to −2.3), including the whole ARC, VMH, and DMH from each mouse, were collected using a cryostat (Leica) and stored in ice-cold PBS. One set of sections from each brain was subjected to staining for p-STAT3+/- by Flou 488 conjugated goat anti-rabbit IgG for 1 h at room temperature. After three additional washes with PBS, the sections were placed onto microscopy slides and coveredslipped and were then subjected to image processing. The images from ARC, VMH, and DMH were acquired with a Zeiss 710 confocal microscope with a 20X objective (resolution of 1,042×1,042 pixels for analysis). A representative image was acquired under the 10X objective to show the wide field sections. Sections without detectable p-STAT3 signal were not subjected to analysis. Fluorescence-positive cell numbers and fluorescence intensities representing p-STAT3 were analyzed using ImageJ software with the option of analysis particle, which determined the area of measurement and calculated the mean gray value, particle numbers, and integrated fluorescence densities. The same settings were applied for all image processing and analysis. The sum of p-STAT3-positive cell numbers (p-STAT3 cell number) and fluorescence intensities from sections of ARC, VMH, and DMH of each brain was calculated and is presented as a percentage relative to the control group (vehicle–plus saline-treated group). All images were processed and analyzed by the investigator with blinding to sample identity.

H&E staining. After treatment of Il1r1−/− or Il1r1+ mice with vehicle or celastrol (100 µg/kg body weight, i.p.) for 3 weeks, the liver was dissected and stored in 10% buffered formalin phosphate. Paraflin-embedded liver sections were stained with H&E.

Cannula placement and intracerebroventricular injection. DIO mice were anesthetized using a ketamine–xylazine combination (100 and 20 mg/kg body weight, respectively) and placed on a stereotaxic apparatus. 26-gauge guide cannulae (C315GC-SP, PlasticsOne) were implanted into the lateral ventricle using appropriate coordinates (bregma, −0.22 mm; midline, −1 mm; dorsal surface, −2.1 mm). Dental acrylic was used to secure the cannula to the skull. A dummy cannula (C315DC-SP, PlasticsOne) was twisted onto the guided cannula. Animals were allowed to recover for 10 d prior to the experiment. Body weight and food intake were measured daily.

During the intracerebroventricular injection, the dummy cannula was removed and the internal cannula (C3151-SP, PlasticsOne) was inserted into the guide cannula. The internal cannula was connected to a plastic tube containing a Hamilton syringe at its end, allowing free movement of the mice during injection. Two days prior to celastrol treatment, anakirina (5 µg in 2 µl of aCSF, daily) was implanted into the ventricle. The internal cannula was kept in the brain for 1 min before replacement, and 2 µl of aCSF was infused in the same way to the control group. During treatment of mice with celastrol, aCSF or anakirina was infused 2 h before i.p. injection with celastrol.

Statistical analysis. All data are presented as means ± s.e.m. Statistical significance was measured using Student’s t test (two-tailed) or two-way ANOVA as indicated in figure legends. P values below 0.05 were considered significant. Numbers of cohorts and n values for each experiment are indicated in figure legends. Mice that died or became sick before the end of experiments and statistical outliers (judged by Grubb’s outlier test) were excluded in the final analysis. No statistical method was used to predetermine sample size, and sample size was determined on the basis of previous experiments, our previous experience, and the literature. The variance was similar in the groups being compared.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
Data availability
Raw data from the mouse hypothalamic microarray analysis are available from the Gene Expression Omnibus (GEO) under accession numbers GSE124353 (DIO mice), GSE124355 (db/db mice), and GSE124356 (lean mice). The source images for p-STAT3 staining can be found at https://figshare.com/s/0741ab007e6f5666605. All other data that support the findings of this study are available from the corresponding author upon reasonable request.
Extended Data Fig. 1 | See figure caption on next page.
**Extended Data Fig. 1 | Microarray analysis of the hypothalamic transcriptome.**

**a.** Flow chart for microarray analysis.

**b.** Heat map depicting the average log₂ FC (Cel/Veh) for the 38 upregulated genes and 15 downregulated genes in pattern 10 and pattern 17 from Fig. 1h.

**c.** Heat map showing clustering of 74 upregulated genes and 29 downregulated genes from limma F-test analysis.

**d.** GO pathways significantly enriched in 74 upregulated genes from limma F-test analysis.

**e.** log₂-transformed fold change (log₂ FC) in the nine genes present in the GO pathway regulation of inflammatory response in DIO mice after 6 h, 1 d, and 4 d of vehicle or celastrol administration. n = 4 mice (6 h, 1 d) and n = 3 mice (4 d). Values indicate means ± s.e.m.

**f.** Heat map showing log₂-transformed expression of the nine identified genes present in the GO pathway regulation of inflammatory response in DIO, lean, and db/db mice at 6 h, 1 d, and 4 d.

**g.** Geometric mean of the fold change values at 1 d and 4 d for the identified genes in DIO, lean, and db/db mice.

**h, i.** Hypothalamic mRNA levels for the identified genes in DIO mice treated with vehicle or celastrol for 1 d (Il1r1, P = 0.0002; Ada, P = 0.0001; Nfkbia, P = 0.01; Ptgs2, P = 0.003; Tgm2, P = 0.0002; Zfp36, P = 0.01; n = 6 mice for each group) (h) and 4 d (Il1r1, P = 0.01; Ada, P = 0.03; Nfkbia, P = 0.01; Ptgs2, P = 0.009; Tgm2, P = 0.007; Zfp36, P = 0.02; n = 4 mice for each group) (i). Values indicate averages ± s.e.m. P values were determined by two-tailed Student’s t test in h and i. *P < 0.05, **P < 0.01, ***P < 0.001.
Extended Data Fig. 2 | IL1R1 deficiency leads to significantly higher levels of obesity. Il1r1<sup>−/−</sup> mice and their wild-type (Il1r1<sup>+/+</sup>) littermates were fed a HFD for 16 weeks to induce obesity. The experiments were repeated two times with similar results. **a**, PCR analysis of the Il1r1 locus in genomic DNA from Il1r1<sup>+/+</sup> and Il1r1<sup>−/−</sup> mice. **b,c**, qPCR analysis of Il1r1 mRNA levels in the hypothalamus (P < 0.0001) (b), and liver (P = 0.007) (c). n = 6 for the Il1r1<sup>−/−</sup> group and n = 4 for the Il1r1<sup>+/+</sup> group. **d**, Body weights of Il1r1<sup>−/−</sup> and Il1r1<sup>+/+</sup> mice during HFD feeding (n = 10 mice for the Il1r1<sup>−/−</sup> group and n = 9 mice for the Il1r1<sup>+/+</sup> group; P < 0.0001). **e**, Average 24-h food intake per mouse after 4, 8, 12, or 16 weeks of HFD feeding (n = 3 cages for each group). **f–h**, DEXA analysis of body composition after 16 weeks of HFD (n = 10 mice for the Il1r1<sup>−/−</sup> group and n = 9 mice for the Il1r1<sup>+/+</sup> group), including lean mass (P = 0.163) (f), fat mass (P = 0.003) (g), and fat percentage (P = 0.001) (h). **i–p**, Changes in levels of plasma leptin (i–k), blood glucose (l–p), and plasma insulin (p) after 4, 8, 12, or 16 weeks on an HFD (n = 5 mice for the Il1r1<sup>−/−</sup> group and n = 4 mice for the Il1r1<sup>+/+</sup> group; 4 weeks, P < 0.001; 8 weeks, P = 0.007; 12 weeks, P = 0.164; 16 weeks, P = 0.014). Values indicate averages ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test (d,j,l) or two-tailed Student’s t test (b,c,e,i,k,m–p). *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant (P > 0.05).
Extended Data Fig. 3 | Celastrol does not change the body temperature of Il1r1+/+ or Il1r1−/− mice. a–c, Il1r1+/+ and Il1r1−/− mice were fed a HFD for 20 weeks and treated with vehicle or celastrol (100 μg/kg body weight, i.p., once a day) for 3 weeks. Graphs show core body temperature (°C) for Il1r1+/+ and Il1r1−/− mice after 1 week (a), 2 weeks (b), or 3 weeks (c) of vehicle or celastrol treatment. Values indicate averages ± s.e.m. n = 9 for both the vehicle- and celastrol-treated groups for Il1r1+/+ mice; n = 12 for the vehicle-treated and n = 13 for the celastrol-treated groups for Il1r1−/− mice. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test; P > 0.99 between each group at 1 week, 2 weeks, and 3 weeks of vehicle or celastrol treatment.
Extended Data Fig. 4 | See figure caption on next page.
Extended Data Fig. 4 | Celastrol does not affect home-cage, anxiety, or social behavior in DIO mice. a–o, DIO mice were treated with vehicle or celastrol (100 µg/kg body weight, i.p., once a day) for 3 d. On the fourth day, home-cage behavior (grooming) (d,e), open-field tests (f-l), and social behavior (m–o) were assessed under the ad libitum–fed condition. a, Average 24-h food intake per mouse during the treatment (n = 3 cages for each group; P = 0.0001). b, c, Body weight (day 0 vs. day 4, P > 0.99 for the vehicle-treated group and P = 0.002 for the celastrol-treated group) (b) and percent body weight reduction before (day 0) and after 4 d (day 4) of vehicle or celastrol treatment (P < 0.0001) (c). d, Frequency (numbers) of grooming bouts (P = 0.572). e, Total time the mice spent grooming during the home-cage behavior test (P = 0.255). n = 10 mice for both the vehicle- and celastrol-treated groups. f, Total distance traveled by the mice (P = 0.956). g, Average velocity of movement during the open-field test (P = 0.956). h, Duration (percent of time in the assay) the mice spent moving (P = 0.999). i, Frequency (number of times during assay) that mice traveled to the central portion of the field (P = 0.331). j, Total time spent in the central portion of the field (P = 0.591). k, Latency (in seconds) until initial entry into the central portion of the open field (P = 0.913). l, Representative movement-tracking plots of vehicle- and celastrol-treated mice in the open-field assay. n = 8 mice for each group. m, Total time the mice spent in the object-paired side chamber (object) versus the novel mouse 1-paired side chamber (novel mouse 1). Object chamber vs. novel mouse 1, P = 0.007 in the vehicle-treated group and P < 0.0001 in the celastrol-treated group. n, Total time the mice spent with the object, which was in the left chamber, and with novel mouse 1, in the right chamber during the sociability test. Object vs. novel mouse, P = 0.0003 in the vehicle-treated group and P < 0.0001 in the celastrol-treated group. o, Total time the mice spent in the novel mouse 1-paired (novel mouse 1) or novel mouse 2-paired (novel mouse 2) side chamber during the social novelty test (novel mouse 1 vs. novel mouse 2, P = 0.01 in the vehicle-treated group and P = 0.02 in the celastrol-treated group). n = 8 mice for both groups during the sociability test and n = 7 for both groups during the social novelty test. Values indicate averages ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant (P > 0.05).
Extended Data Fig. 5 | See figure caption on next page.
Extended Data Fig. 5 | Celastrol treatment decreases motivation for food seeking in only fasted mice. a–f, Conditioned place preference (CPP) assay under the ad libitum feeding condition: DIO mice were treated with celastrol (100 μg/kg body weight, i.p., once a day) for 3 d. On the fourth day, 1 h after the beginning of the light cycle, the mice were administered vehicle or celastrol (200 μg/kg body weight, i.p.) and the CPP assay was performed 6 h later under the ad libitum feeding condition (n = 8 mice for both the vehicle- and celastrol-treated groups). a, Total distance that the mice traveled during the test (P = 0.941). b, Average velocity of movement during the CPP assay (P = 0.934). c, Frequency (numbers during assay) with which mice traveled to the food-paired side chamber (P = 0.688). d, Total time the mice spent in the food-paired side chamber (P = 0.641). e, Percentage of total assay time the mice spent in the food-paired side chamber (P = 0.641). f, Frequency (times during assay) that mice traveled to the food-containing zone within the food-paired chamber (P = 0.557). g–l, CPP assay under a 20-h fasting condition: DIO mice were treated with celastrol (100 μg/kg body weight, i.p.) once a day and fasted for 20 h after the third injection. On the fourth day, 1 h after the beginning of the light cycle, the mice were administered vehicle or celastrol (200 μg/kg body weight, i.p.) and the CPP assay was performed 5 h after this injection under the fasting condition (n = 7 mice for both the vehicle- and celastrol-treated groups). g, Total distance traveled by the mice during the CPP assay (P = 0.02). h, Average velocity of movement (P = 0.02). i, Frequency with which the mice traveled to the food-paired side chamber (P = 0.104). j, Total time the mice spent in the food-paired side chamber during the test (P = 0.02). k, Percent of total assay time the mice spent in the food-paired side chamber (P = 0.02). l, Frequency with which the mice traveled to the food-containing zone within the food-paired side chamber (P = 0.01). m,n, Representative traces showing the movements of individual vehicle- and celastrol-treated mice in the CPP assays conducted under the ad libitum feeding (m) or 20-h fasting (n) condition. Values indicate averages ± s.e.m. P values were determined by two-tailed Student’s t test. *P < 0.05; n.s., not significant (P > 0.05).
Extended Data Fig. 6 | See figure caption on next page.
Extended Data Fig. 6 | Celastrol fails to increase STAT3 phosphorylation and gene expression in the hypothalamus of Il1r1−/− mice. Il1r1+/+ and Il1r1−/− male mice were fed a HFD for 20 weeks and then administered either vehicle (Veh) or celastrol (Cel; 100 μg/kg body weight, i.p.) daily for 3 d. Each group of mice subsequently received a single dose of vehicle or celastrol (200 μg/kg body weight, i.p.) on the morning of the fourth day and was then fasted for 6 h prior to extraction of the hypothalamus. Phosphorylation of STAT3 (p-STAT3 Tyr705) in the medial basal hypothalamus (MBH) was analyzed by immunofluorescence staining using a phospho-specific antibody. a,c,e, Representative images of p-STAT3 Tyr705 immunostaining in the arcuate nucleus (ARC) (a), ventromedial hypothalamus (VMH) (c), and dorsomedial hypothalamus (DMH) (e) of Il1r1−/− and Il1r1+/+ mice after 4 d of vehicle or celastrol treatment. b,d,f, Quantification of total p-STAT3 Tyr705-positive cell numbers and total fluorescence intensity for p-STAT3 Tyr705 in the ARC (Il1r1−/−-Veh vs. Il1r1+/−-Veh, P = 0.0007; and Il1r1−/−-Cel vs. Il1r1+/−-Cel, P = 0.0006 for cell number; Il1r1−/−-Veh vs. Il1r1+/−-Cel, P = 0.001, and Il1r1−/−-Cel vs. Il1r1+/−-Cel, P = 0.0008 for fluorescence intensity) (b), VMH (Il1r1−/−-Veh vs. Il1r1+/−-Veh, P = 0.0003, and Il1r1−/−-Cel vs. Il1r1+/−-Cel, P = 0.0006 for cell number; Il1r1−/−-Veh vs. Il1r1+/−-Cel, P = 0.0004, and Il1r1−/−-Cel vs. Il1r1+/−-Cel, P = 0.0007 for fluorescence intensity) (d), and DMH (Il1r1−/−-Veh vs. Il1r1+/−-Veh, P = 0.002, and Il1r1−/−-Cel vs. Il1r1+/−-Cel, P = 0.01 for fluorescence intensity) (f). The experiments in a-f were repeated in two independent cohorts with similar outcomes, and the results in b, d, and f represent the combination of two independent experiments (total n = 7 mice for both vehicle- and celastrol-treated mice in the Il1r1−/− group; n = 8 for both vehicle- and celastrol-treated mice in the Il1r1+/− group). Scale bars, 100 μm. 3V, third ventricle. g,j, Expression levels of genes in the hypothalamus of Il1r1−/− and Il1r1+/− mice treated with vehicle or celastrol (100 μg/kg body weight, i.p., once a day) for 4 d, including for AgRP (Il1r1−/−-Veh vs. Il1r1+/−-Veh, P = 0.005; Il1r1−/−-Veh vs. Il1r1+/−-Cel, P > 0.99) (g), Npy (Il1r1−/−-Veh vs. Il1r1+/−-Veh, P = 0.362; Il1r1−/−-Veh vs. Il1r1+/−-Cel, P = 0.871) (h), Pmc (Il1r1−/−-Veh vs. Il1r1+/−-Cel, P = 0.757; Il1r1−/−-Veh vs. Il1r1+/−-Cel, P > 0.99) (i), and Socs3 (Il1r1−/−-Veh vs. Il1r1+/−-Veh, P = 0.03; Il1r1−/−-Veh vs. Il1r1+/−-Cel, P > 0.99) (j) (n = 6 vehicle-treated and n = 7 celastrol-treated mice in the Il1r1+/− group; n = 4 vehicle- and celastrol-treated mice in the Il1r1−/− group). Values indicate averages ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001; n.s., not significant (P > 0.05).
Extended Data Fig. 7 | IL1R1 deficiency or IL1R1 antagonist treatment reduces MAP kinase phosphorylation. a–d. Ad libitum–fed Il1r1+/+ mice and food-restricted (FR) Il1r1−/− mice (~0.5 g of food per day) were treated with vehicle (Veh) or celastrol (Cel; 100 μg/kg body weight, i.p., daily) for 7 d and the phosphorylation status of hypothalamic or hepatic MAP kinases (p38, Erk1/2) was analyzed by western blot. a, Representative immunoblots for phosphorylated p38 MAP kinase (p-p38) and total p38 MAP kinase (p38) in the hypothalamus of Il1r1+/+ and FR Il1r1−/− mice. b, Ratio of quantified p-p38 density to p38 density. n = 3 mice for each group. Il1r1+/+–Veh vs. Il1r1−/−–Veh, P < 0.0001; Il1r1+/+–Cel vs. Il1r1−/−–Cel, P < 0.0001; Il1r1+/+–Veh vs. Il1r1−/−–Cel, P > 0.99. c, Representative immunoblots for phosphorylated MAP kinase (p-Erk1/2) and Erk1/2 MAP kinase in the liver of Il1r1+/+ and FR Il1r1−/− mice. d, Ratio of quantified p-Erk1/2 density to Erk1/2 density. n = 3 mice for each group. Il1r1+/+–Veh vs. Il1r1−/−–Veh, P = 0.06; Il1r1+/+–Cel vs. Il1r1−/−–Cel, P = 0.01; Il1r1+/+–Veh vs. Il1r1+/+–Cel, P > 0.99. e,f, DIO mice were administered the IL1R1 antagonist anakinra (Ank; 5 μg/mouse per day) through intracerebroventricular injection into the lateral ventricle in combination with celastrol (100 μg/kg body weight, i.p., daily) for 7 d. e, Representative immunoblots for p-p38 and total p38 in the hypothalamus. f, Ratio of quantified p-p38 density to p38 density. n = 5 mice for each group. P = 0.03. g,h, DIO mice were treated with IL1R1 antagonist (Ank; 30 mg/kg body weight, i.p., twice a day) in combination with celastrol (100 μg/kg body weight, i.p., daily) for 7 d. g, Representative immunoblots for p-Erk1/2 and total Erk1/2 in the liver. h, Ratio of quantified p-Erk1/2 density to Erk1/2 density. n = 6 mice for each group. Sal + Veh vs. Sal + Cel, P = 0.0004; Ank + Veh vs. Ank + Cel, P > 0.99; Sal + Cel vs. Ank + Cel, P = 0.0004. Values indicate averages ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test (b, d, h) or two-tailed Student’s t test (f). *P < 0.05, ***P < 0.001; n.s., not significant (P > 0.05).
Extended Data Fig. 8 | Effect of celastrol on mice with heterozygous deletion of Il1r1. a, Hypothalamic Il1r1 mRNA levels in Il1r1<sup>+/+</sup> and Il1r1<sup>−/−</sup> mice determined by qPCR. n = 7 mice for each group. Values indicate averages ± s.e.m.; P = 0.0009, determined by two-tailed Student’s t test. b–g, Il1r1<sup>+/+</sup> and Il1r1<sup>−/−</sup> mice (with average body weight of 37–40 g) were fed on a chow diet and treated with vehicle or celastrol (100 μg/kg body weight, i.p., once a day) for 7 d. n = 7 mice for each group. b, Body weight. Il1r1<sup>+/+</sup>–Veh vs. Il1r1<sup>+/+</sup>–Cel, P = 0.0005; Il1r1<sup>+/+</sup>–Veh vs. Il1r1<sup>−/−</sup>–Cel, P < 0.0001; Il1r1<sup>+/+</sup>–Veh vs. Il1r1<sup>−/−</sup>–Cel, P = 0.576. d, Average daily food intake during treatment. Il1r1<sup>+/+</sup>–Veh vs. Il1r1<sup>+/+</sup>–Cel and Il1r1<sup>−/−</sup>–Veh vs. Il1r1<sup>−/−</sup>–Cel, P = 0.002. e, Plasma leptin levels after 7 d of treatment. Il1r1<sup>+/+</sup>–Veh vs. Il1r1<sup>+/+</sup>–Cel and Il1r1<sup>−/−</sup>–Veh vs. Il1r1<sup>−/−</sup>–Cel, P = 0.06. f, Six-hour fasting blood glucose levels after 7 d of treatment. Il1r1<sup>+/+</sup>–Veh vs. Il1r1<sup>+/+</sup>–Cel and Il1r1<sup>−/−</sup>–Veh vs. Il1r1<sup>−/−</sup>–Cel, P = 0.002. g, Plasma IL-1β levels after 7 d of treatment (P > 0.99, between each group). Values indicate averages ± s.e.m. P values were determined by two-way ANOVA with Bonferroni’s multiple-comparisons test. *P < 0.05, ***P < 0.001; n.s., not significant (P > 0.05).
Extended Data Fig. 9 | IL1R1 interacts with LepRb. HEK 293 cells were transfected with plasmids expressing LacZ, human IL1R1 (hIL1R1), Flag-tagged LepRb, or hIL1R1 and Flag-tagged LepRb together. The experiments were repeated two times with similar outcomes. Representative immunoblots depict the immunoblotting results for IL1R1 and Flag in the Flag immunoprecipitates (top). The expression levels of IL1R1 and HSP90 in the input total-cell lysates are shown in the lower panels.
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### Experimental design

1. **Sample size**
   - **Describe how sample size was determined.**
   
   The sample size was determined based on our experience with the experimental models, anticipated biological variables, previous literatures, and also sufficient for statistical power calculation using two tailed t-test and two-way ANOVA. We also ensuring no more animals than necessary were used.

2. **Data exclusions**
   - **Describe any data exclusions.**
   
   For the three weeks treatment of the mice with vehicle or celastrol, the data from the dead or sick mice before the end of experiments were excluded from final analysis. Statistical outliers, judged by Grubb’s outlier test \( P < 0.05 \) using GraphPad outlier calculator, were excluded in the final analysis.

3. **Replication**
   - **Describe the measures taken to verify the reproducibility of the experimental findings.**
   
   All the experiments have been replicated successfully and all the results reported have been reliably reproduced, such as the body-weight and glucose tolerance of \( \text{Il1r1}^{+/+} \) and \( \text{Il1r1}^{-/-} \) mice cohort, under a high-fat diet, were repeated in 3 independent cohorts, celastrol treatments of \( \text{Il1r1}^{+/+} \) or \( \text{Il1r1}^{-/-} \) mice were repeated two times and showed similar outcomes. The phospho-STAT3 staining experiment was conducted in two independent cohorts with similar outcome. Detecting of whole hypothalamic STAT3 phosphorylation with western blot was repeated in two independent cohorts with similar outcome. Treatment of the mice with IL1R1 antagonist was repeated in two different cohorts with similar outcome. RNA microarrays were performed once but at least three independent mice were analyzed and further validated by alternative approaches, such as qPCR. Western blotting data were confirmed by at least three independent samples in each group. The treatment of celastrol on \( \text{Il1r1}^{-/-} \) mice was performed once, but 7 mouses for each group was used and the result is black and white.

4. **Randomization**
   - **Describe how samples/organisms/participants were allocated into experimental groups.**
   
   Mice were randomly assigned at the time of purchase or weaning to minimize any possible bias. The animals of same genotype were also randomized into the treatment groups based on body weight that the mean body weight of each group were as close to each other group as possible without using excess number of animals.

5. **Blinding**
   - **Describe whether the investigators were blinded to group allocation during data collection and/or analysis.**
   
   In the experiment of immunostaining to detect hypothalamic STAT3 phosphorylation, the images were blindly processed and the total cell number of p-STAT3 and the fluorescence intensity were blindly analyzed. For the analysis of mice grooming frequency and grooming bouts, the investigators were blinded to group allocation. Microarray analyses were performed by service for fee that was blind to different treatment and different mouse model. All the body weight, food and blood glucose measurements during the collections, the investigators were blind to different groups of the mice.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- The exact sample size \( (n) \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided. Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present. Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Policy information about availability of computer code

Describe the software used to analyze the data in this study.

Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA).
Image J (ImageJ 1.48v, NIH, USA) was used for the density measurements of immunoblots and analysis of p-STAT3 positive cells.
R packages were used for PCA and LIMMA F-test analysis of microarray data.
Mouse behavioral experiments were recorded and analyzed by Noldus Ethovision program (EthoVision®XT, Noldus, Netherlands, Europe).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials and reagents

Policy information about availability of materials

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All the materials are available from standard commercial sources.
## 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

| Antibodies used:                                                                                                                                      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| Phospho-STAT3 (Tyr705), Cell Signaling Technology, Cat. #9131 (reactive with Human, Mouse and Rat, Lot 18, western blot), 1:1000 dilution; Cat. #9145 (Tyr705, D3A7, reactive with Human, Mouse and Rat, Lot 26 and 31 for immunostaining), 1:3000 dilution. |
| STAT3(79D7), Cell Signaling Technology, Cat. #4904, reactive with Human, Mouse and Rat, Lot 6, 1:1000 dilution for western blot.                           |
| Phospho-p38 MAPK (Thr180/Tyr182)(12F8), Cell Signaling Technology, Cat. #4631, reactive with Human, Mouse and Rat, Lot 9, 1:2000 dilution for western blot.    |
| STAT3(79D7), Cell Signaling Technology, Cat. #4904, reactive with Human, Mouse and Rat, Lot 6, 1:1000 dilution for western blot.                           |
| Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), Cell Signaling Technology, Cat. #9101, reactive with Human, Mouse and Rat, Lot 21, 1:2000 dilution for western blot. |
| P44/42 MAPK (Erk1/2), Cell Signaling Technology, Cat. #9102, reactive with Human, Mouse and Rat, Lot 18, 1:3000 dilution for western blot.                  |
| IL1R1 antibody, NovusBiologicals, Cat. NBP1-32929, reactive with Human, 1:1000 dilution for western blot.                                               |
| ANTI-FLAG® M2 antibody, Sigma-Aldrich, Cat. F1804, 1:5000 dilution for western blot.                                                                   |

**Antibody Validations:**

- Phospho-STAT3 (Tyr705) antibodies were validated by leptin, which is well-known to induce STAT3 (Tyr705) phosphorylation in the hypothalamus, treated mouse hypothalamus.
- Phospho-p38 MAPK (Thr180/Tyr182)(12F8) and phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibodies were validated by treatment of MEF cells with IL-1beta, which is known to induce the phosphorylation of downstream MAPK. IL1R1 antibody was validated in HEK293 cells infected with IL1R1 over-expressing Adenovirus.

## 10. Eukaryotic cell lines

| a. State the source of each eukaryotic cell line used.                                                                                     |
|---------------------------------------------------------------------------------------------------------------------------------------------|
| HEK293 cells were purchased from ATCC (Cat. CRL-1573)                                                                                       |
| The HEK293 cells used in this study were not authenticated                                                                                  |
| The HEK293 cells used in this study were not tested for mycoplasma contamination.                                                            |
| No commonly misidentified cell lines were used in this study.                                                                                   |

## 11. Description of research animals

| Provide all relevant details on animals and/or animal-derived materials used in the study.                                                                 |
|------------------------------------------------------------------------------------------------------------------------------------------------|
| 8-10 weeks old male C57BL/6J mice (stock number 000664) were purchased from Jackson Laboratories and fed with normal chow diet as lean mice.       |
| 3 weeks old male C57BL/6J mice (stock number 000664) were purchased from Jackson Laboratories and fed with high-fat diet for 16-20 weeks to induce obesity. |
| 8-10 weeks old male db/db mice (stock number 000697) were purchased from Jackson Laboratories and fed on normal chow diet.                      |
| 8-10 weeks old male Il1r1 homozygous knockout mice (stock number 003245) were purchased from Jackson Laboratories.                             |
| Heterozygous Il1r1 Knockout mice (Il1r1+/−) were generated by crossing 8-10 weeks old male Il1r1 homozygous knockout mice (Il1r1−/−) with 8-10 weeks old female C57BL/6J mice. |
| Experimental groups of Il1r1+− and Il1r1−/− mice were generated by intercrossing Il1r1+− mice with Il1r1−/− mice and fed on high-fat diet for 16-20 weeks to induce obesity. |
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not include human research participants.