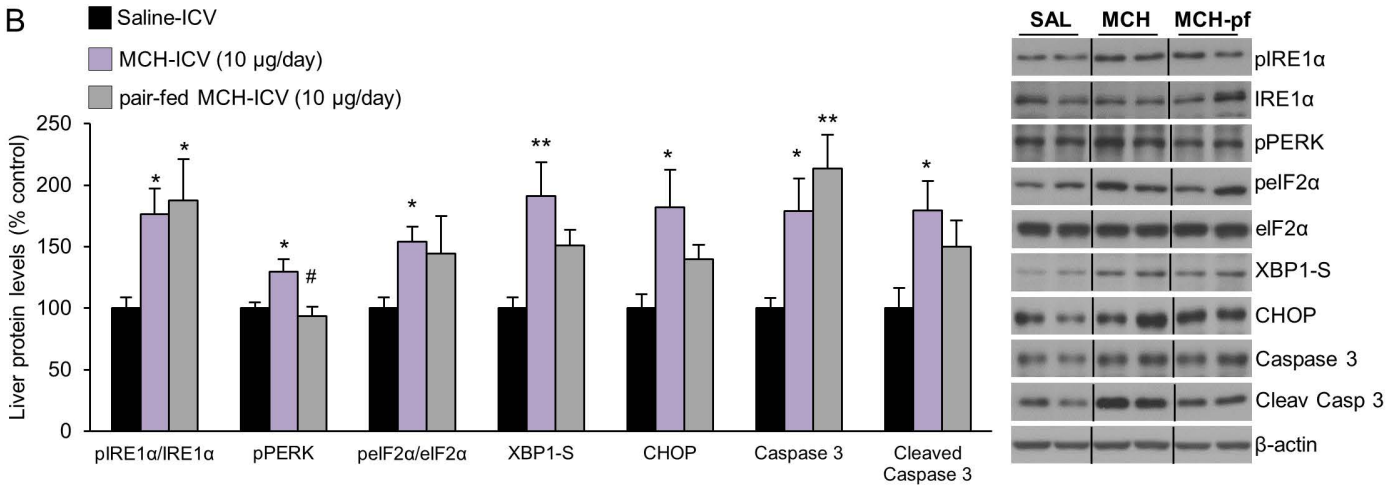
Supplementary Figure 1

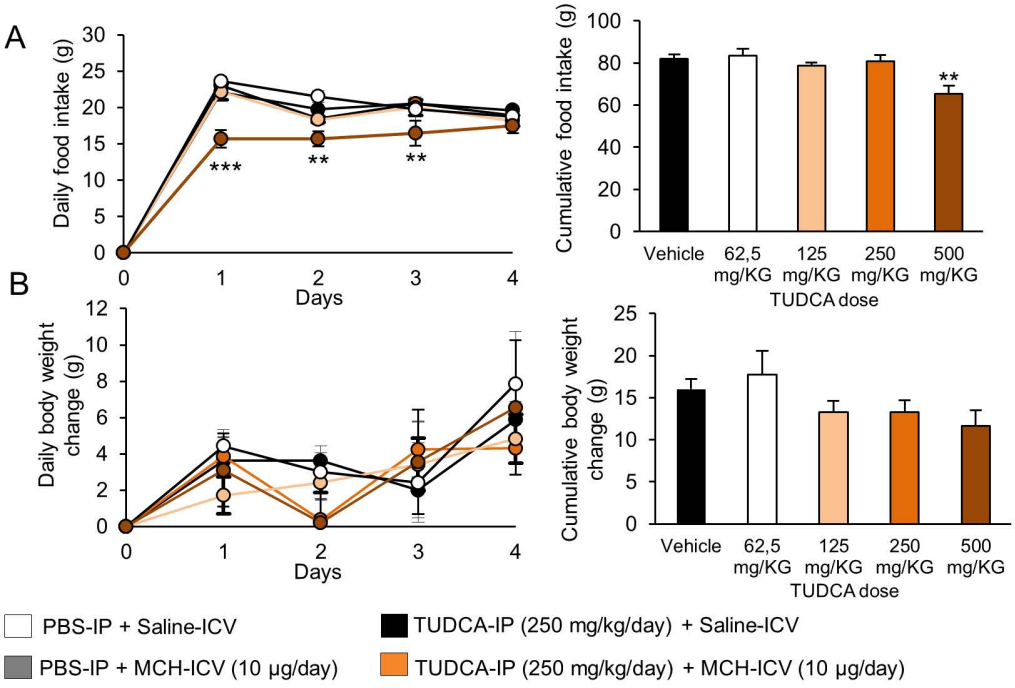
A

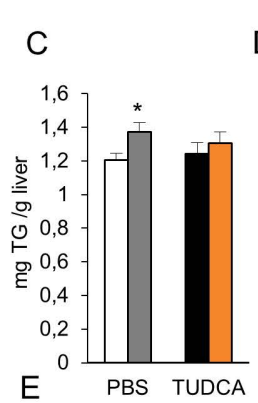
	Saline-ICV	MCH-ICV (10 µg/day)	pair-fed MCH-ICV (10 µg/day)
Body weight gain (g)	48 ± 2,31	67,6 ± 2,53 **	56,6 ± 3,84 *
Food intake (g)	208,25 ± 4,462	234,12 ± 6,156 **	208,25 ± 4,462
Liver TG (mg/g liver)	1,54 ± 0,17	2,35 ± 0,23 **	2,39 ± 0,15 **

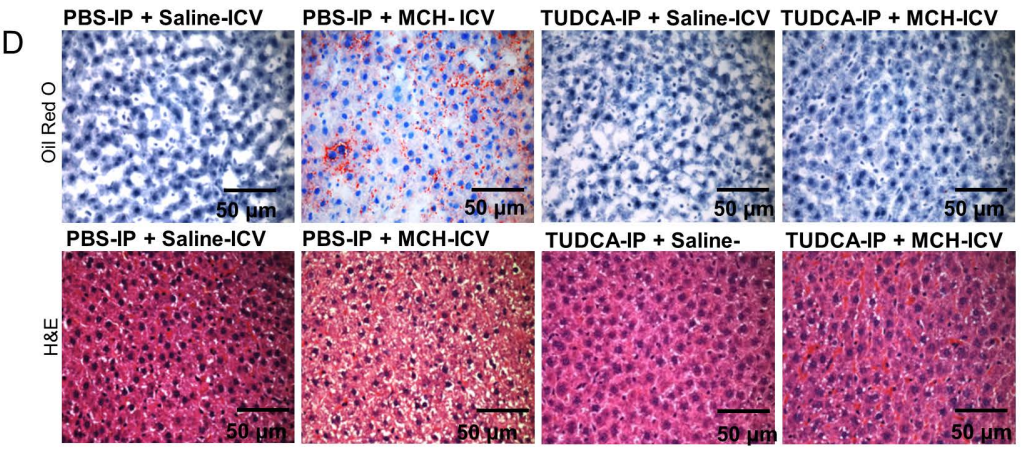
B

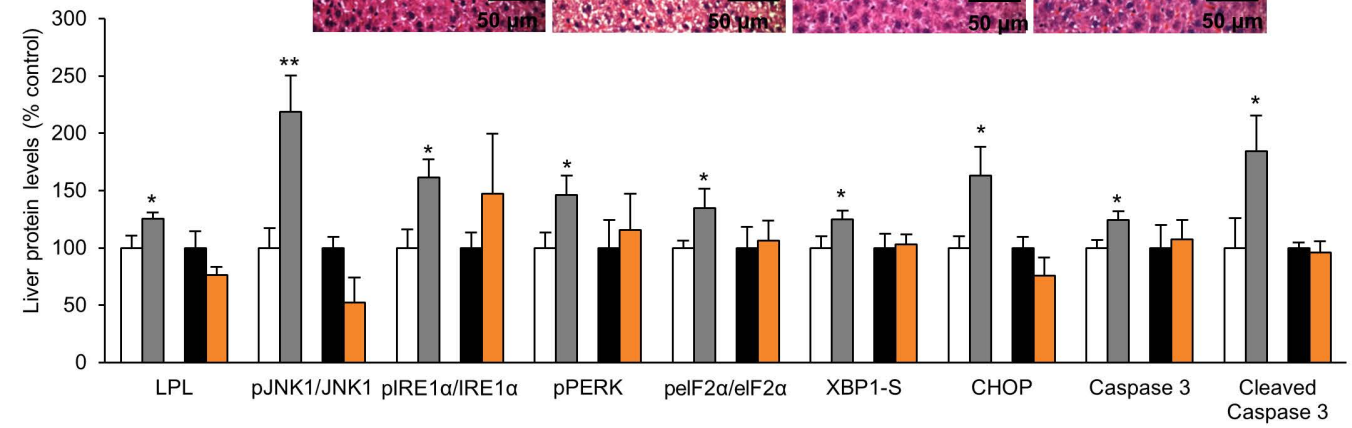


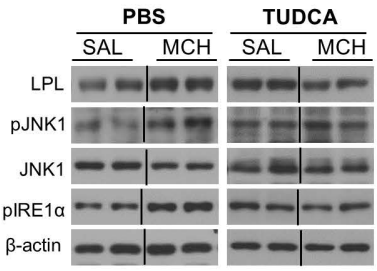
Supplementary Figure 2

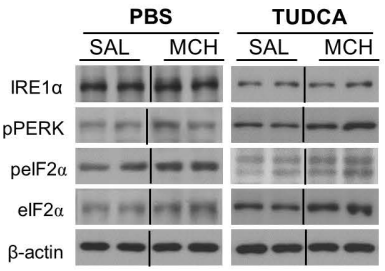


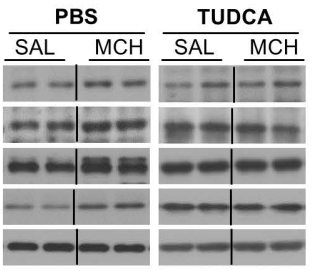




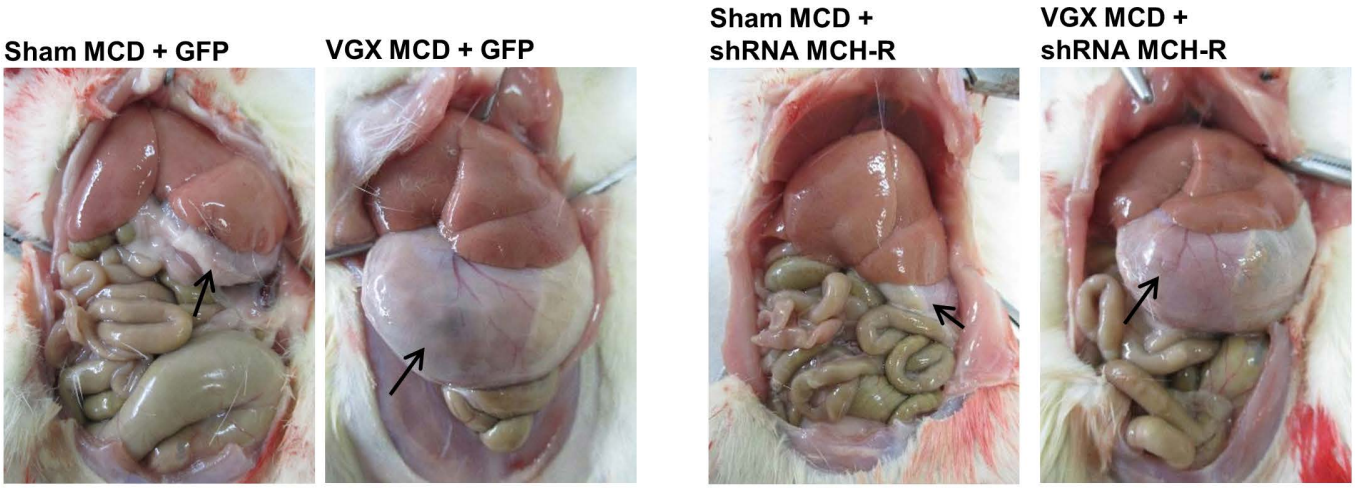




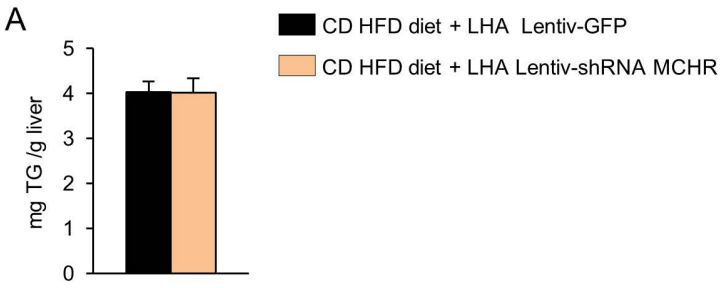


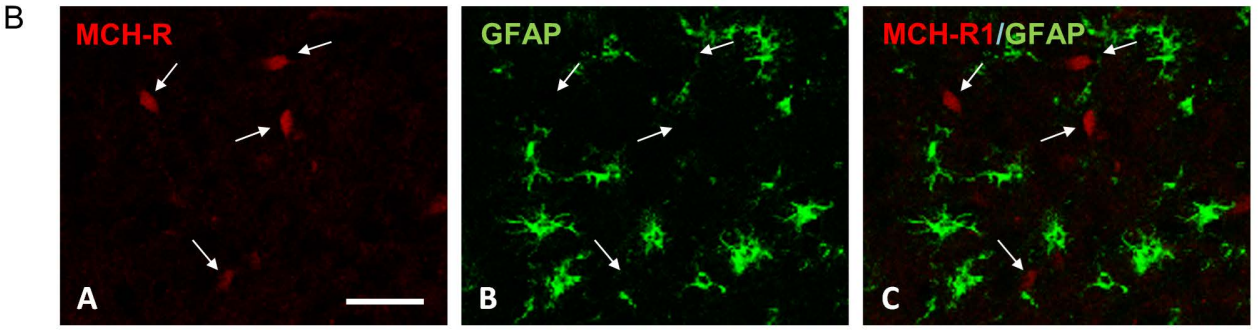


Supplementary Figure 3

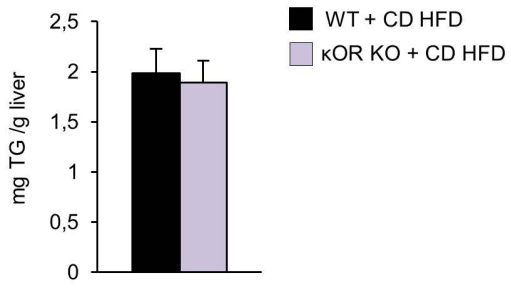


Supplementary Figure 4



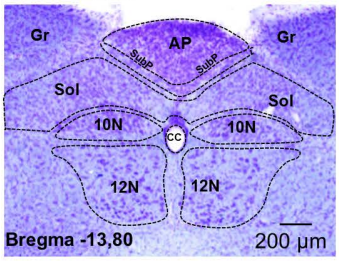


Supplementary Figure 5



Supplementary Figure 6

A



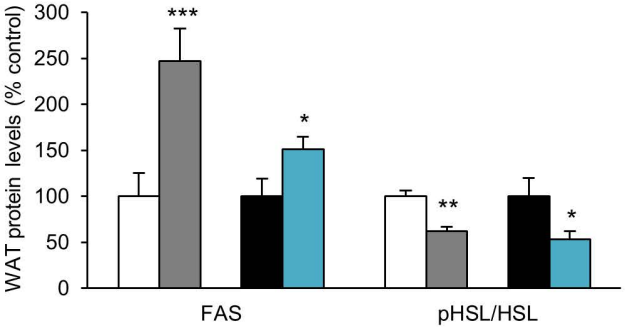
LHA AAV-GFP + Saline -CV

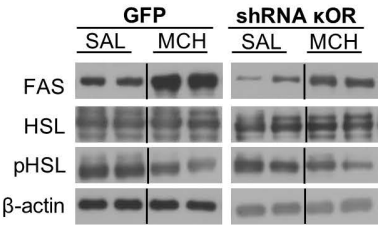
LHA AAV-GFP + MCH-ICV (10 µg/day)

LHA AAV shRNA-κOR + Saline-ICV

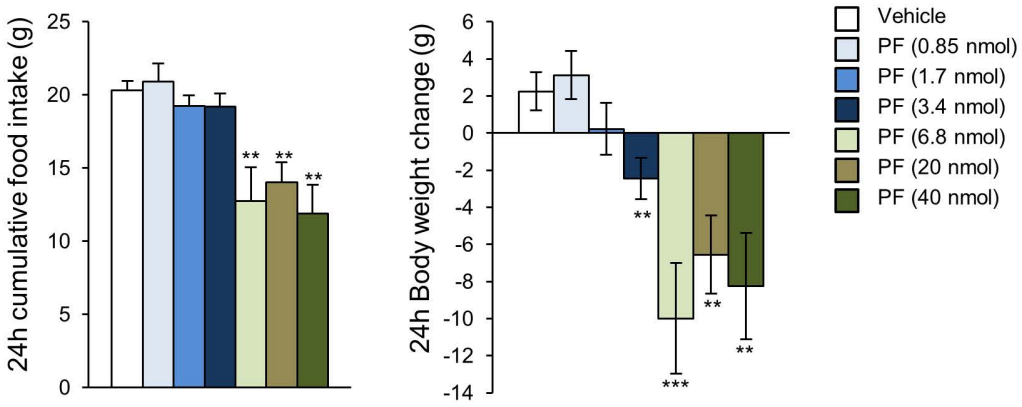
LHA AAV shRNA-κOR + MCH-ICV (10 µg/day)

B

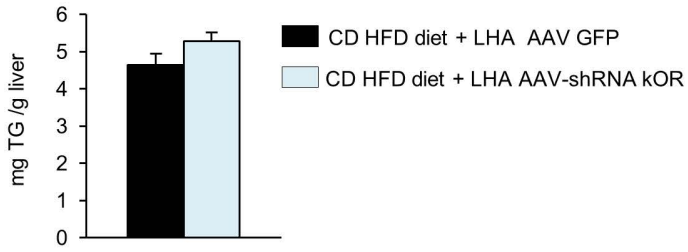




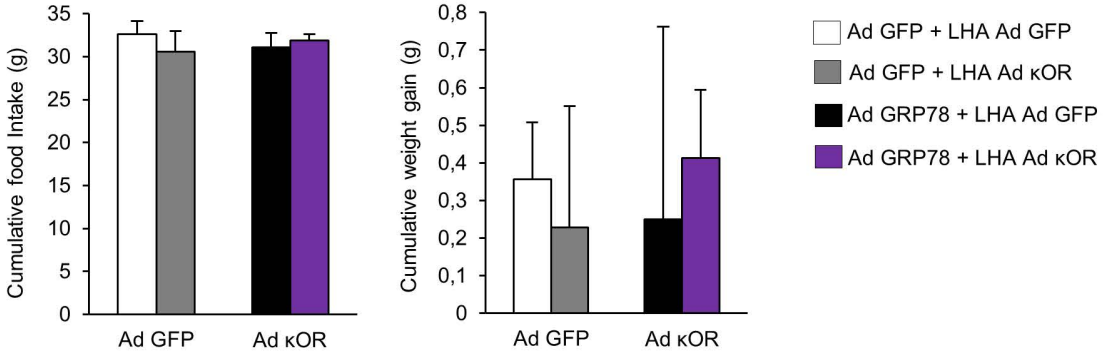
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplemental Material

Animals

For all experiments, 8- to 10-week-old (250 to 300 g) male Sprague Dawley rats were housed in individual cages under conditions of controlled temperature (23°C) and maintained on a 12:12 hr light-dark cycle. They were allowed *ad libitum* access to water and standard laboratory chow from Scientific animal Food & Engineering, unless otherwise stated (Methione-choline deficient diet (MCD) and Choline-deficient High fat diet (CD-HFD) were purchased (Research Diets USA)).

8-week-old C57BL/6 and Kappa-opioid receptor mutant mice (κOR KO) were fed *ad libitum* with free access to water in all experiments. Mice were purchased from The Jackson Laboratory (USA) and maintainined on a Specific Pathogen Free room (SPF) and fed with regular diet. κOR mutant mice were backcrossed to C57BL/6J mice, thus, they have a pure C57BL/6J genetic background.

Food intake and body weight were measured daily during the experimental phase in all experiments. All the experimental procedures involved in this study were reviewed and approved by the Ethics Committee of the USC, in accordance with EU normative for the use of experimental animals.

Implantation of intracerebroventricular cannulae and dose response.

Rats were anesthetized by an intraperitoneal injection of ketamine and xylazine (80 mg/kg and 100 mg/kg). Intracerebroventricular (ICV) cannulae were implanted stereotaxically in mice and rats, as described previously (1). After 3 day of recovery, animals received an intracerebroventricular administration of vehicle (saline) or PF04455242 at diference doses (0.85, 1.7, 3.4, 6.8, 20 and 40 nmol, Tocris) and food intake and body weight was monitoring during 24 hours.

Chronic ICV MCH infusion

Rats were anesthetized by an intraperitoneal injection of ketamine-xylacine (ketamine 80 mg/kg BW + xylazine 8 mg/kg BW) and mice were anaesthetized under ketamine-xylacine (ketamine 100 mg/kg BW + xylazine 3 mg/kg BW). Saline or MCH (10 µg/rat/day or 2,5 µg/mouse/day) were delivered via ICV infusion using osmotic minipumps for 7 days (models *2001* for rats, total volume of 200 µl, and *1007D* for mice, total volume of 100 µl, Alzet Osmotic Pumps; DURECT, CA, USA). Saline or MCH (10 µg/rat/day or 2,5 µg/mouse/day) and Saline or PF 04455242 (3,4 nmol/day/rat) were delivered via ICV infusion using osmotic minipumps for 7 days (models *2001* for rats, total volume of 200 µl, and *1007D* for mice, total volume of 100 µl, Alzet Osmotic Pumps; DURECT, CA, USA) and 14 days (model 2002, total volume of 200 µl). Intracerebroventricular cannulae were implanted at lateral ventricle as previously described (2).A catheter tube was connected from the brain infusion cannula to the osmotic minipump flow moderator. A subcutaneous pocket on the dorsal surface was created using blunt dissection and the osmotic minipumps was inserted as previously described (1). The incision was closed with sutures, and rats were kept warm until fully recovered.

Intraperitoneal TUDCA treatment

Animals were adapted for 3 days to the intraperitoneal puncture with 200 uL of PBS. Then, a dose-response experiment (62.5 125, 250 and 500 mg/Kg/day) was carried out to determine the optimal dose of TUDCA. We chose the 250 mg/Kg/day dose because as this dose did not affect food intake or body weight change. Simultaneously to the chronic ICV MCH infusion, rats received an IP dose of 250 mg/Kg/day of TUDCA (Calbiochem) administered intraperitoneally twice a day: at the beginning and at the end of the light phase, during one week. PBS was used as vehicle as previously described (3).

Adenoviral tail vein injection in mice

Either dominant positive GRP78 adenovirus or GFP control (VQ Ad mGRP 78 WT 050312 and Empty AfIII/eGFP 101311 respectively (4) (ViraQuest Inc., North Liberty, IA, USA) were administered in a volume of 100 µl 10^9 PFU/0,2 ml in the tail vein of C57BL/6 mice with a 27G x 3/8" (0.40mm x 10mm) BD Plastipak syringe.

Stereotaxic microinjection of viral vectors

Rats or mice were placed in a stereotaxic frame (David Kopf Instruments) under ketamine-xylazine anesthesics and several virus types were injected in the LHA of either rats (AP: -2,85 mm; L: ±2 mm; DV: -8,1 mm) or mice (AP: -1,3 mm; L ± 1,1 mm; DV; -5,2 mm) as previously described (Imbernon M. et al. 2013; Beiroa D. et al. 2013). Lentiviral vectors over-expressing shRNA (short-hairpin RNAs) against MCH-R (1,2X10^6 TU/ml clone ID TRCN0000028357, Sigma Aldrich), GFP controls (10^6 TU/mL Sigma-Aldrich Inc., Buchs, Switzerland), adeno-associated viral (AAV) vectors (1x10^9 genomic copies per 1 uL) encoding GFP or kappa opioid receptor shRNA (5), and adenoviral vectors encoding GFP or κOR (7.98x10^{10} PFU/mL) (Signagen, USA) were used.

Surgical vagotomy in rats

The surgical procedure was performed as previously described (6). Briefly under ketamine–xylazine anesthesia rats were placed on their backs and a midline abdominal incision was made. The liver was then carefully moved to the right exposing the esophagus. Dorsal and ventral branches of the vagus nerve were exposed and dissected from the esophagus. Each branch of the nerve was ligated with surgical suture at two points as distally as possible to prevent bleeding, and then cauterized between the sutures. The abdominal muscles and the skin were then sutured with surgical silk. Sham surgeries were also performed, in which each trunk of the nerve was exposed but not tied or cauterized. The effectiveness of the vagotomy was assessed one week after the surgery by post-mortem stomach observation (Supplemental figure 3). Only the rats

which showed an evident increase in stomach size after vagotomy (due to motoric dysfunction) were included in the analysis.

Detection of κOR in MCH1R-expressing cells by immunohistochemical_double labeling.

Double immunohistochemistry was performing over brain slides of transgenic mice expressing tdTomato Red in MCH1R-CRE positive cells. To expose the antigenic sites and reduce non-specific binding of the immunohistochemical reagents, sections were treated with 0.5% H_2O_2 (made in 0.1M; PBS; 10 min) and 10 mg/ml pepsin (made in 0.2M HCl for 5 min at 37 °C), then blocked against non-specific antibody binding with 2% bovine serum albumin (BSA; fraction V; Sigma) in PBS for 30 min. After pre-treatment, they were transferred into a mixture of goat anti-OPRK1 (2 µg/ml; Sigma-Aldrich, #SAB2501442) and rabbit anti-DSRed Express (1:1000; Clontech, #632496) antisera for 24 h. The tissue-bound primary antibodies were visualized by CY5-donkey anti-goat IgG (1:2,000; Jackson Labs) and CY3-donkey anti-rabbit IgG (1:3,000; Jackson Labs), respectively. Sections were mounted onto glass slides, coverslipped and scanned by a Nikon A1R confocal microscope.

Nissl, Oil Red O, Hematoxiline EosineMasson Trichrome and Sirius Red staining.

Nissl staining was performed following the Bielschowsky-Plien protocol with 0.1% cresyl violet. Brains were fixed by perfusion followed by immersion (12h) in 10% buffered formalin for 24 hours. Sections of 50-µm thick were cut on a Vibratome Series 1000 (Technical Products International, Inc, St. Louis, MO). Paraffin was removed and then sections were washed first with 96º alcohol and then with distilled water. The differentiation was continued in 70º alcohol. After one more washing with distilled water, the differentiation was finished with a wash in lithium carbonate solution. Then several changes of 70º alcohol where conducted and another distilled water wash was carried. Then the sample was kept 6 minutes in the 0.1% cresyl violet

solution. After several washes in 96º and 100 º alcohols, sections were rinsed with xylol and finally mounted.

In the Oil red O staining, frozen sections of livers (8 μm) were cut, fixed in 10% neutral buffered formalin, and stained in red oil for 20 minutes. Sections were washed with water and counterstained with Harris haematoxylin for 10 minutes. Sections were mounted in aqueous mountant (glycerine jelly).

In the Haematoxiline eosine staining, formalin-fixed sections of liver were paraffin-embedded, cut (3 μm) and put at 60º where then started to desparaffin, ending this process with 3 xylol baths of 10 to 15 minutes. Sections were dehydrated with several washes in 100º, 96º, 70º and 50º alcohols, then washed in distilled water, counterstained with Harris haematoxylin about 10 minutes , washed again with water, counterstained with eosin 5 minutes, dehydrated with 50, 70º, 96º and 100º alcohols, and finally rinsed with xylol and mounted.

For the *Masson trichrome* staining, paraffined sections of the liver (8 μm) were cut, desparaffined and submerged in xylol during 10 to 15 minutes. Sections were hydrated with several washes in 100º, 96º, 70º and 50º alcohols, then washed with water, counterstained with haematoxylin, red Masson, phosphomolybdic acid and aniline blue (with washes after each stain) dehydrated with alcohol and finally rinsed with xylol and mounted..

For the *Sirius Red staining*, samples were fixated in paraffin. These sections were de-waxed, hydratated and stained in picro-sirius red for one hour. After two washes with acidified water, most of the water from the slides of the sample, was removed and then dehydratated in three changes of 100% ethanol. Samples were finally cleared in xylene and mounted in a resinous medium. Collagen deposition in Sirius Red-stained sections were analyzed using the Frida software.

Tissue Triglycerides Content in Liver

Liver tissues were homogenized in ice-cold chloroform-methanol (2:1 vol/vol). Triglycerides (TG) were extracted during 3 hours shaking at room temperature. For phase separation, miliQ water was added, samples were centrifuged, and the organic bottom layer was collected. The organic solvent was dried using a Speed Vac and dissolved in chloroform. TG content of each sample was measured in duplicate after evaporation of the organic solvent with a colorimetric assay (Spinreact, Girona, Spain).

Western Blot Analysis

Western blot were performed as previously described (7, 8). Briefly, total protein lysates from liver (20 µg), epididymal WAT (30 µg) and LHA nucleus (12 µg) were subjected to SDS-PAGE, electrotransferred on a polyvinylidene difluoride membrane and probed with the indicated antibodies: ACC (Upstate, Lake Placid, NY); phospho-SAPK/JNK (Thr183/Tyr185) (pJNK1), and phospho-HSL (Ser 660), caspase 3 (8G10), cleaved caspase 3 (Asp175) GPR78, (Cell Signaling, Danvers, MA); HSL, IRE1, IRE1 alpha (pSer724), ProDynorphin (Abcam, Cambridge, UK); β-actin, κ-Opioid Receptor (Sigma-Aldrich, USA); FAS (H-300), JNK1/3 (C-17), and LPL (H-53), XBP-1S (M-186), pPERK (Thr 981), CHOP (GADD 153 (R-20)), eIF2α (FL-315), peIF2α (Ser 52), pro-MCH (H-143) (Santa Cruz Biotechnology, Santa Cruz, CA) and MCHR1 (Abnova, Taipei City, Taiwan). For protein detection we used horseradish peroxidase-conjugated secondary antibodies (Dako Denmark, Glostrup, Denmark) and chemioluminescence (Pierce ECL Western Blotting Substrate, Thermo scientific, USA). Then, the membranes were exposures to X-ray film (Super RX, Fuji Medical X-Ray Film, Fujifilm, Japan) and developed with developer and fixing liquids (AGFA, Germany) under appropriate dark room conditions. We used 7-8 samples per group and the protein levels were normalized to β-actin for each sample.

Quantitative RT-PCR procedure

RNA was extracted using Trizol® reagent (Invitrogen) according to the manufacturer's instructions and as previously described (7). Two micrograms of total RNA were used for each RT reaction and cDNA synthesis was performed using SuperScript™ First-Strand Synthesis System (Invitrogen) and random primers. For the analysis of gene expression we used real-time reverse-transcription polymerase chain reaction (RT-PCR) analyses performed in a fluorescent temperature cycler (TaqMan®; Applied Biosystems; Foster City, CA) following the manufacturer's instructions (9, 10). 500 ng of total RNA were used for each RT reaction. The PCR cycling conditions included an initial denaturation at 50ºC for 10 min followed by 40 cycles at 95°C for 15 sec; 60°C for 1min. The oligonucleotide specific primers and probes are described in Table 1. For the analysis of the data, the input value of the gene expression was standardized to the 18S value for the sample group and was expressed compared with the average value for the control group.

Supplemental figures

Supplementary Figure 1. Chronic ICV MCH infusion increases hepatic ER stress. Effect of a 7-day ICV MCH infusion (10 µg/day) on body weight gain, food intake and liver triglycerides (TG) (A), and liver protein levels of pIRE1α, IRE1α, pPERK, peIF2α, eIF2α, XBP1-S, CHOP, caspase 3 and cleaved caspase 3 (B). Protein β-actin levels were used to normalize protein levels. Dividing lines indicate splicings within the same gel. Values are mean ± SEM of 7–8 animals per group. *P < 0.05; **P < 0.01; versus controls and # P < 0.05 versus MCH group.

Supplementary Figure 2. Peripheral administration of TUDCA ameliorates MCH-induced steatosis in rats fed a chow diet. Daily and cumulative food intake (A) and body weight (B) of rats treated during four days with TUDCA at four different doses (62.5; 125; 250 and 500 IP mg/KG/day). Effect of a 7-day ICV MCH infusion (10 µg/day) combined with peripheral intraperitoneal administration of the chemical chaperone TUDCA (250 mg/kg/day) or PBS on triglyceride (TG) liver content (C). Representative photomicrographs of oil red O and hamatoxylin-eosin (H&E) staining of liver sections (40X magnification) (D). Effect of a 7-day ICV MCH infusion (10 µg/day) combined with peripheral intraperitoneal administration of TUDCA (250 mg/kg/day) or PBS on liver protein levels of LPL, pJNK, JNK, pIRE1α, IRE1α, pPERK, peIF2α, eIF2α, XBP1-S, CHOP, caspase 3 and cleaved caspase 3 (E). Protein β-actin levels were used to normalize protein levels. Dividing lines indicate splicings within the same gel. Separated photos indicate that gels were run independently. Values are mean ± SEM of 7–8 animals per group. *P < 0.05; **P < 0.01; versus controls and # P < 0.05 versus MCH group.

Supplementary Figure 3. Representatives photographs that corroborate the effect of vagotomy as indicated by stomach hypertrophy.

Supplementary Figure 4. Genetic down-regulation of MCH-R in the LHA of rats fed a CD-HFD does not change hepatic TG content; MCH-R is exclusively expressed y neural population TG liver content in rats fed a CD-HFD diet infected with a lentivirus encoding GFP or shRNA MCH-R in the LHA. (A). Values are mean ± SEM of 7–8 animals per group. Immunofluorescent detection of GFAP in LHA containing sections of mice expressing tdTomato in MCHR1-CRE positive cells (arrows) revealed, that MCHR1 does not co-localize with GFAP-immunoreactive cells. Scale bar: 20 μm (B).

Supplementary Figure 5. Global κOR knockout mice fed a CD-HFD does not change hepatic TG content. TG liver content in globlal κOR knockout mice fed a CD-HFD diet during two weeks. Values are mean ± SEM of 7–8 animals per group.

Supplementary Figure 6. Representative photomicrograph of a Nissl stain section of the brain-stem (medulla oblongata), that represents the dorsal motor nuclei (A). Gr: gracille nucleus; AP: area postrema; SubP: subpostrema area; Sol: solitary tract; 10N: vagus nerve; CC: central nerve; 12N: hypoglossal nerve. Effect of a 7-day ICV MCH infusion (10 μg/day) in either GFP of shRNA κOR AAV LHA administration on epididymal white adipose tissue of FAS, HSL and pHSL protein levels (B). β-actin were used to normalize protein levels. Values are mean ± SEM of 7–8 animals per group. *P < 0.05; **P < 0.01; ***P < 0.001 versus controls.

Supplementary Figure 7. Effects of ICV PF04455242 on food intake and body weight in rats fed a chow diet. Daily and cumulative food intake and body weight of rats 24 hours after the treatment with a single ICV injection of PF04455242 at different doses (0.85, 1.7, 3.4, 6.8, 20 and 40 nmol). Values are mean ± SEM of 7–8 animals per group. *P < 0.05; **P < 0.01; versus controls.

Supplementary Figure 8. Genetic down-regulation of κOR in the LHA of rats fed a CD-HFD does not change hepatic TG content. TG liver content in rats fed a CD-HFD diet infected with a lentivirus encoding GFP or shRNA κOR in the LHA. Values are mean ± SEM of 7–8 animals per group.

Supplementary Figure 9. 7-day effect of LHA adenoviral injection over-expressing κOR and tail vein adenoviral injection encoding GPR78 on cumulative food intake (A) and body weight (B) of mice.

Supplemental Table1: Primers and probes for real-time PCR (TaqMan®) analysis

mRNA	GeneBank Accession Number		Sequence
CD68	NM_001031638	Fw Primer	5’-TCCCTTACGGACAGCTTACCTTT-3’
		Rv Primer	5’-GCTTGTGGGAAGGACACATTGTA-3’
		Probe	5’-ATTCAAACAGGACCGACATCAGAGCCAC-3’
CD36	NM_031561	Fw Primer	5’-CGAAGGCTTGAATCCTAACGAA-3’
		Rv Primer	5’-ATGTTGACCTGCAGTCGTTTTG-3’
		Probe	5’-TGGATGTGGAACCCATAACTGGATTCACTC-3’
HPRT	NM_012583	Fw Primer	5’-AGCCGACCGGTTCTGTCAT-3'
		Rv Primer	5’-GGTCATAACCTGGTTCATCATCAC -3'
		Probe	FAM-5’- CGACCCTCAGTCCCAGCGTCGTGAT 3’-TAMRA
IL6	NM_012589	Fw Primer	5'-TGCCCTTCAGGAACAGCTATG-3'
		Rv Primer	5'-TGTCAACAACATCAGTCCCAAGAA-3'
		Probe	5'-CTCCGCAAGAGACTTCCAGCCAGTTG-3'
TNFα	NM_012675	Fw Primer	5'-CTAACTCCCAGAAAAGCAAGCAA-3'
		Rv Primer	5'-CCTCGGGCCAGTGTATGAGA-3'
		Probe	5'-CAGCCAGGCAGGTTCCGTCC-3'

Supplemental References

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