Supplementary Figure 1

Time–course of FAAH inhibition after administration of a single dose of URB937 (1 mg kg\(^{-1}\), i.p.) in Swiss Webster mice. Circles, liver tissue; squares, brain tissue. Results are expressed as mean ± s.e.m.; \(n = 4–6\). *** \(P < 0.001\) vs vehicle.
Supplementary Figure 2

Effects of vehicle (open bars) or URB937 (1 mg kg\(^{-1}\), i.p., closed bars) on (a) anandamide levels, and (b) 2-arachidonoylglycerol (2-AG) levels in various tissues of Swiss Webster mice. Results are expressed as mean ± s.e.m.; \(n = 4–6\). * \(P < 0.05\), ** \(P < 0.01\) and *** \(P < 0.001\) vs vehicle.
Supplementary Figure 3

Effects of vehicle (V, open bars) or URB937 (1 mg kg$^{-1}$, i.p., closed bars) in the hot-plate test. Injections were performed 1 h before the test. Results are expressed as mean ± s.e.m.; $n = 6$. 

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Supplementary Figure 4

Effects of PPAR–α antagonist MK886 in the murine sciatic nerve ligation model. (a) Mechanical hyperalgesia, (b) thermal hyperalgesia, and (c) mechanical allodynia. Shaded bars: effects of vehicle (V) or MK886 (1 mg kg⁻¹, i.p.) administered alone; closed bars: effects of vehicle (V) or MK886 (1 mg kg⁻¹, i.p.) administered in combination with URB937 (1 mg kg⁻¹, i.p.). BL, baseline (measured before ligation). Results are expressed as mean ± s.e.m.; n = 6. * P < 0.05 and ** P < 0.001 vs baseline; # P < 0.05 and ## P < 0.01 vs vehicle.
Supplementary Figure 5

The brain–permeant FAAH inhibitor URB597 attenuated pain behavior elicited by inflammation in mice. Effects of URB597 (1 mg kg\(^{-1}\), i.p.) on carrageenan–induced responses: (a) mechanical hyperalgesia, (b) thermal hyperalgesia, (c) mechanical allodynia, and (d) paw edema. Mechanical and thermal hyperalgesia were measured immediately before carrageenan injection (0 h) or 4 h and 24 h after injection. Mechanical allodynia was measured 0 h and 24 h after carrageenan. Results are expressed as mean ± s.e.m.; \( n = 6 \). * \( P < 0.05 \), ** \( P < 0.01 \) and *** \( P < 0.001 \) vs vehicle.
Supplementary Figure 6

Effects of URB937 (1 mg kg\(^{-1}\), i.p., closed bars), administered alone or in combination with rimonabant (R, 1 mg kg\(^{-1}\), i.p.) or AM630 (1 mg kg\(^{-1}\), i.p.) on (a) mechanical hyperalgesia, (b) thermal hyperalgesia, and (c) mechanical allodynia in non–injected paws of Swiss Webster mice that received an injection of carrageenan into the contralateral paw. Results are expressed as mean ± s.e.m.; \(n = 6\).
Supplementary Figure 7

The PPAR–α antagonist MK886 (1 mg kg⁻¹, i.p.) did not consistently inhibit the analgesic effects of URB937 (1 mg kg⁻¹, i.p.) in the mouse carrageenan model. Effects of single administration of vehicle (open bars) or URB937 (closed bars; 1 mg kg⁻¹, i.p.) on (a) mechanical hyperalgesia, (b) thermal hyperalgesia, (c) mechanical allodynia, and (d) paw edema induced by carrageenan injection. Mechanical and thermal hyperalgesia were measured immediately before carrageenan injection (0 h) or 4 h and 24 h after injection. Mechanical allodynia was measured 0 h and 24 h after carrageenan. Results are expressed as mean ± s.e.m; n = 6. * P < 0.05 and ** P < 0.01 vs vehicle; # P < 0.05 vs URB937.
Supplementary Figure 8

Effects of repeated treatment with URB937 (1 mg kg\(^{-1}\), i.p., once daily for 3 days) in the mouse carrageenan model. Effects of vehicle (V, shaded bars) or URB937 (closed bars) on (a) mechanical hyperalgesia, (b) thermal hyperalgesia, (c) mechanical allodynia, and (d) paw edema. BL, baseline (measured before carrageenan injection); IL, ipsilateral (injected) paw; CL, contralateral (non–injected) paw. Results are expressed as mean ± s.e.m.; \(n = 6\). ** \(P < 0.01\) and *** \(P < 0.001\) vs vehicle.
Supplementary Figure 9

URB937 attenuated formalin–induced pain responses in rats. Effects of vehicle (open bars) or URB937 (1 mg kg$^{-1}$, i.p., closed bars) on area under the curve (AUC) for (a) the total formalin response and (b) Phase 1 of the formalin response. Results are expressed as mean ± s.e.m.; $n = 5–7$. * $P < 0.05$, all groups vs URB937.
Supplementary Figure 10

Effects of vehicle (open squares) or URB937 (1 mg kg\(^{-1}\), i.p., closed squares) on (a) cumulative free feeding, (b) latency to the first meal (min), (c) meal frequency (number of meals per h), (d) satiety ratio [intermeal interval (min) divided by normalized meal size (g kg\(^{-1}\))], and (e) cumulative locomotor activity (arbitrary units) in Swiss Webster mice. Results are expressed as mean ± s.e.m.; \(n = 12\).
Supplementary Table 1

Percent of FAAH inhibition after URB937 (1 mg-Kg⁻¹, i.p.)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mice</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td>-3.0 ± 8.0</td>
<td>0.2 ± 6.7</td>
</tr>
<tr>
<td>Colon</td>
<td>84.7 ± 0.3***</td>
<td>90.8 ± 2.0**</td>
</tr>
<tr>
<td>Duodenum</td>
<td>84.3 ± 1.4***</td>
<td>89.4 ± 0.1***</td>
</tr>
<tr>
<td><strong>Hypothalamus</strong></td>
<td><strong>18.4 ± 13.8</strong></td>
<td><strong>10.2 ± 5.8</strong></td>
</tr>
<tr>
<td>Illeum</td>
<td>86.8 ± 2.4***</td>
<td>91.5 ± 2.8***</td>
</tr>
<tr>
<td>Jejunum</td>
<td>90.0 ± 1.4***</td>
<td>90.3 ± 0.7***</td>
</tr>
<tr>
<td>Kidney</td>
<td>88.3 ± 1.0***</td>
<td>91.4 ± 0.5***</td>
</tr>
<tr>
<td>Liver</td>
<td>91.7 ± 0.7***</td>
<td>92.1 ± 0.3***</td>
</tr>
<tr>
<td>Lungs</td>
<td>Not Detectable</td>
<td>93.5 ± 1.8**</td>
</tr>
<tr>
<td><strong>Spinal cord</strong></td>
<td><strong>17.7 ± 10.6</strong></td>
<td><strong>19.2 ± 15.7</strong></td>
</tr>
<tr>
<td>Spleen</td>
<td>72.5 ± 0.7***</td>
<td>86.1 ± 2.1**</td>
</tr>
</tbody>
</table>

**P < 0.01; ***P < 0.001; n = 3
 Supplementary Methods

**Synthesis of FAAH inhibitors**

URB597 was synthesized following a published procedure\(^3\). The compound was obtained in five steps starting from 3–bromo–4–hydroxybenzaldehyde, which was benzylated (BzCl, DMF, CsCO\(_3\), rt, 3 h), then oxidized and hydrolyzed (\(m\)-CPBA, CH\(_2\)Cl\(_2\), 40 °C, 72 h; NaOMe, EtOH, rt, 1 h) to 4–benzlyoxy–3–bromophenol; the latter was elaborated by Suzuki coupling [3–carbamoylphenylboronic acid, toluene, Pd(PPh\(_3\))\(_4\), Na\(_2\)CO\(_3\)/H\(_2\)O, reflux, 2 h], carbamation (c–C\(_6\)H\(_{11}\)CNO, Et\(_3\)N, toluene/CH\(_3\)CN 1:1, reflux, 18 h) and hydrogenative deprotection to the desired compound. \(N\)–cyclohexyl–O–biphenyl–3–yl carbamates (Table 1, compounds 3–5) were synthesized by reaction of the opportune 3′–carbamoyl–4–substituted phenol with cyclohexyl isocyanate, while compound 6 was obtained by a Pd/C catalyzed hydrogenation of the corresponding nitrocarbamate precursor, which results from the suitable phenol derivative. All biphenols were synthesized by a Suzuki cross–coupling reaction between 3–carbamoylphenylboronic acid and the corresponding 3–bromo–4–substituted phenols (in the case of the precursors of compounds 3 and 4) or 3–chloro–4–fluorophenol (for those of compound 5). Detailed synthetic procedures for all the compounds will be reported elsewhere.

**Synthesis of cyclohexylcarbamic acid 3′–carbamoyl–6–hydroxybiphenyl–3–yl ester (URB937).** To a stirred suspension of cyclohexylcarbamic acid 3′–carbamoyl–6–benzlyoxybiphenyl–3–yl ester (222 mg; 0.5 mmol) in EtOAc (2.5 ml) and EtOH (2.5 ml), 10% Pd/C (22 mg) was added. The mixture was hydrogenated at 4 atm at 50 °C for 4 h, cooled, filtered on Celite and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 1:9) and recrystallization gave URB937 as a white solid. Yield: 92% (0.163 g). Mp: 128–130 °C (CH\(_2\)Cl\(_2\)/n–hexane). MS (ESI) \(m/z\): 355.2
(M+H\(^+\)). \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\): = 1.13–2.02 (m, 10H), 3.55 (m, 1H), 5.13 (br d, 1H), 5.85 (br s, 1H), 6.59 (br s, 1H), 6.74–6.95 (m, 3H), 7.07 (s, 1H), 7.34–7.41 (m, 1H), 7.56 (m, 1H), 7.68–7.75 (m, 2H) ppm. IR (Nujol) \(\nu_{\text{max}}\): 3333, 1701, 1655 cm\(^{-1}\).

**Other chemicals**

\([^{3}\text{H}]\)-Anandamide was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). \(^2\)–[^2]H\(_8\)–AG, AM630, AM251 and MK886 were from Cayman Chemical. Anandamide, \([^{4}\text{H}]\)-anandamide and PEA were synthesized in the laboratory\(^4\)\(^\text{3}\). Rimonabant and \(N\)-cyclohexyl biphenyl–3–ylacetamide were kind gifts of the National Institute on Drug Abuse and Kadmus Pharmaceuticals Inc., respectively. Probenecid, DCNP and Rifampicin were purchased from Sigma. Ko143 was purchased from Tocris. Verapamil was purchased from Biomol Research Labs.

**Animals**

We used male Swiss Webster mice (Charles River, 20–30 g), male C57Bl/6 (Jackson Laboratory, 20–25 g), male Wistar rats (Charles River, 250–300 g) and male Sprague–Dawley (SD) rats (Harlan Laboratories, 275–350 g). In some experiments, we also used male FAAH–deficient mice and PPAR–\(\alpha\)–deficient mice (25–35 g) back crossed more than 10 times on a C57Bl/6 background; wild–type littermates were used as controls. Animals were group–housed, unless otherwise stated, in standard cages at room temperature on a 12:12 h light:dark cycle with unlimited access to water and standard chow pellets. All experiments met the National Institutes of Health guidelines for the care and use of laboratory animals, were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine, and the University of Georgia, Athens, and were in compliance with the European Community Council Directive 86 (609) EEC and the experimental protocol was carried out in compliance with Italian regulations (DL 116/92).

**Tissue extractions**
Mice were sacrificed with isoflurane and tissues were collected and immediately frozen in liquid nitrogen. Frozen tissues were weighed and homogenized in methanol (1 ml) containing [\(^2\)H$_4$]–anandamide, [\(^2\)H$_4$]–PEA, [\(^2\)H$_8$]–2–AG, and N–cyclohexyl biphenyl–3–ylacetamide as internal standards. Analytes were extracted with chloroform (2 vol) and washed with water (1 vol). Organic phases were collected and dried under nitrogen. The non–fractionated organic extract was used for quantification of URB937. For other analyses the organic extract was fractionated by open–bed silica gel column chromatography, as described$^{44}$. Briefly, the extract was dissolved in chloroform and loaded onto small glass columns packed with Silica Gel G (60 ~ A 230–400 Mesh ASTM; Whatman). Anandamide, PEA and 2–AG were eluted with chloroform/methanol (9:1, vol/vol).

**Serum extractions**

Blood was collected from either decapitated mice or by cardiac punction in rats, allowed to clot and placed on ice. The clotted blood was centrifuged at 1800 x g for 10 min at 4 °C and the serum was transferred to glass vials and diluted with distilled water to 1 ml. Proteins were precipitated with ice–cold acetone (1 ml) containing N–cyclohexyl biphenyl–3–ylacetamide as an internal standard, and the precipitate was removed by centrifugation at 3000 x g for 10 min at 4 °C. The samples were dried under nitrogen to remove acetone, and extracted with chloroform/methanol as described above.

**Liquid chromatography/mass spectrometry (LC/MS)**

Tissue levels of anandamide, PEA, 2–AG and URB937 were determined using an 1100–LC system coupled to a 1946A–MS detector (Agilent Technologies, Inc.) equipped with an electrospray ionization interface. URB937 and N–cyclohexyl biphenyl–3–ylacetamide (m/z = 294) were eluted on an XDB Eclipse C18 column (50 x 4.6–mm inner diameter, 1.8 m, Zorbax) using a linear gradient of 60% to 100% of A in B over 3 min at a flow rate of 1.0 ml min$^{-1}$. Mobile phase A consisted of methanol containing 0.25% acetic acid, 5
mM ammonium acetate; mobile phase B consisted of water containing 0.25% acetic acid, 5 mM ammonium acetate. Anandamide, 2–AG and PEA were eluted with a gradient of methanol in water (from 85% to 90% methanol in 2.5 min) at a flow rate of 1 ml min\(^{-1}\). Column temperature was kept at 40 °C. MS detection was in the positive ionization mode, capillary voltage was set at 3 kV, and fragmentor voltage was varied from 120 to 140 V. Nitrogen was used as drying gas at a flow rate of 13 L min\(^{-1}\) and a temperature of 350 °C. Nebulizer pressure was set at 60 psi. Na\(^+\) adducts ([M+Na\(^+\)]) of analytes and internal standards were monitored in the selective ion–monitoring mode. Limit of quantification was 0.4 pmol.

**Drug transport assays**

Transport assays were conducted at Cerep Inc. (Remond, WA). Cellular permeability was determined in the apical (A) to basal (B) and the B to A direction in the presence of 10 µM compound in Hanks’ buffered salt solution containing 1% dimethylsulfoxide (DMSO) plus 5 mM HEPES (Sigma) in 96–well plates (Millipore). Apparent permeabilities were calculated as follows:

\[
P_{app}(\text{cm s}^{-1}) = V_R \times C_{Rt} / \Delta t \times 1 / A \times (C_{D,mid} - C_{R,mid})
\]

where \(V_R\) is the volume of the receiver chamber. \(C_{Rt}\) is the concentration of the test compound in the receiver chamber after incubation time, \(\Delta t\) is the incubation time (60 minutes for the A–B permeability, 40 minutes for the B–A permeability) and \(A\) is the surface area of the cell monolayer. \(C_{D,mid}\) is the calculated mid–point concentration of the test compound in the donor side, which is the mean value of the donor concentration at time 0 and the donor concentration at time \(t\). \(C_{R,mid}\) is the mid–point concentration of the test compound in the receiver side, which is one half of the receiver concentration at time \(t\). Concentrations of the test compound are expressed as peak areas of the test compound. Efflux ratios were calculated as the ratio of B–A to A–B permeabilities.

The recovery of the test compound was calculated as follows:
Recovery (%) = \[ \frac{(V_D \times C_{Dt} + V_R \times C_{Rt})}{V_D \times C_{D0}} \times 100 \]

were \( V_D \) and \( V_R \) are the volumes of the donor and receiver chambers, respectively. \( C_{Dt} \) is the concentration of the test compound in the donor sample at time \( t \). \( C_{Rt} \) is the concentration of the test compound in the donor sample at time \( t \). \( C_{D0} \) is the concentration of the test compound in the donor sample at time zero. Concentrations of the test compound are expressed as peak areas of the test compound.

**Fos immunohistochemistry**

Two hours after formalin injection into the dorsal paw, rats received a lethal dose of Nembutal (50 mg ml\(^{-1}\)). They were perfused through the heart with 100 ml of 1% heparinized phosphate buffered saline (PBS) followed by 300 ml of ice–cold 4% paraformaldehyde. The lumbar–sacral region of the spinal cord was collected from each rat. Spinal cords were post fixed in 4% paraformaldehyde at 4 °C for 24 h and then cryoprotected in 30% sucrose at 4 °C for 24–48 hours. Spinal cords were cryostat–cut into 40 µm transverse sections at the level of the lumbar enlargement (L4/L5). Free floating alternating sections were kept in a series of wells filled with PBS. Every fourth section was processed for Fos immunoreactivity to ensure that the same cell would not be counted twice. Endogenous peroxidases were inactivated by incubation in hydrogen peroxide. Sections were blocked with goat serum to prevent non–specific binding and were incubated in the presence of Fos primary antibody (1:20,000, Abcam) diluted in PBS containing 0.4% Triton (for 1 h at 37 °C and then 48 h at 4 °C). Tissue was incubated in the presence of biotinylated goat anti–rabbit IgG (1:600, Vector Laboratories) secondary antibody for 1 h at 37 °C. Subsequently, sections were incubated in a Vectastain Elite ABC reagent (1:200, Vector Laboratories) for 1 h, followed by a 5 min incubation in 2% nickel intensified diaminobenzidine. Sections were mounted onto glass slides, air dried, dehydrated in ascending concentrations of ethanol, cleared in xylene, and protected by a glass coverslip affixed with Permount. All
conditions in the same experiment were processed concurrently, to control for variance in immunostaining across different runs. Immunostaining specificity was established by primary antibody omission from the immunostaining protocol and by demonstration that preadsorption with the peptide antiserum blocked specific staining\(^3\).

**Immunoreactivity quantification**

Under a microscope, three L4/L5 sections that qualitatively exhibited the greatest number of Fos–positive cells were selected for quantification. Selection of sections and quantification of the number of Fos–positive cells was performed by an observer blinded to experimental conditions. Sections were captured at 5x magnification using a DMLB light microscope and a 1,300 digital camera under similar brightness/contrast settings to demonstrate comparable background staining. Laminar subdivisions were drawn on all sections using the Image J software (U. S. National Institutes of Health, Bethesda, MD). Subdivisions of the spinal gray matter were defined as the superficial laminae (laminae I and II), *nucleus proprius* (laminae III and IV), neck of the dorsal horn (laminae V and VI), and ventral horn (laminae, VII, VIII, IX, and X)\(^4\). Using Image J, Fos expressing cells were counted in each subdivision, regardless of staining intensity, by an observer blinded to experimental treatments. The intra–rater reliability ranged from 93% in the superficial lamina to 81% across all lamina subdivisions.

**Drug preparation for in vivo experiments**

Drugs were dissolved in polyethylene glycol 400/Tween–80/saline (1/1/18; by volume) and administered by i.p. (5–10 ml kg\(^{-1}\)) or s.c. injection (10 ml kg\(^{-1}\)). For lateral cerebral ventricle injections, URB937 was dissolved in 100% DMSO and injected in a volume of 5 µl.

**Surgeries**

All surgeries were conducted under aseptical conditions. *Implantation of cannulae for intracerebroventricular (i.c.v.) drug administration*. SD rats were anesthetized using a
mixture of ketamine (70 mg kg\(^{-1}\), i.p.) and xylazine (9.33 mg kg\(^{-1}\), i.p.). They were placed in a stereotaxic frame and stabilized by ear bars (David Kopf Instruments, Tujunga, CA) with the incisor bar set 2.4 mm below the horizontal plane. A 22–gauge stainless–steel guide cannula was implanted into the right lateral cerebral ventricle 7 days prior to experiments. Coordinates for implantation (–0.9 mm antero/posterior and –1.5 mm medio/lateral relative to bregma, and 3.8 mm below the surface of the skull) were determined using the Paxinos and Watson rat brain atlas\(^{46}\). Guide cannulae were anchored to the skull with 3 stainless–steel screws and dental cement, and were kept patent until injection by insertion of a dummy stylet. For injections, the stylet was removed and drug or vehicle were infused in a volume of 5 µl with a 10 µl Hamilton microsyringe connected to a 28 gauge stainless–steel injector, which protruded 1 mm beyond the tip of the guide cannula, by a PTFE 24G catheter (Small parts Inc.) filled with PBS. A small air bubble (3 µl) was drawn at the distal end of the PTFE 24G catheter to separate the injected solution from the PBS and for visual inspection of the injection. Injections were performed over a 1–min period and the injector was kept in place for an additional 1 min to prevent back flow leakage. Placement of the cannulae was verified at the end of the experiments by injection of trypan blue (5 µl) before the rats were euthanized. Only animals with proper placements were included in the study. Rats were allowed to recover for 7–10 days before experiments. *Sciatic nerve ligation* was performed in Swiss Webster mice, using an adaptation of the method of Bennett and Xie\(^{22}\). Mice were anesthetized with xylazine (10 mg kg\(^{-1}\), i.p.) and ketamine (100 mg kg\(^{-1}\), i.p.), the left thigh was shaved and scrubbed with Betadine\(^{®}\), and a small incision (2 cm in length) was made in the middle of the left thigh to expose the sciatic nerve. The nerve was loosely tied at two distinct sites (spaced at a 2–mm interval) around the entire diameter of the nerve using silk sutures (7–0). The surgical area was dusted with streptomycin powder and closed with a single muscle suture and two skin clips and
finally scrubbed with Betadine®. In sham–operated animals, the nerve was exposed but left untied. The animals were placed under a heat lamp until they awakened.

**Behavioral tests**

The *hot–plate test* was performed as described⁴⁷, with minor modifications. The hot–plate apparatus (Ugo Basile, Varese, Italy) was maintained at 55.5 ± 1 °C. Animals were placed into a glass cylinder of 24 cm diameter on the heated surface, and the time between placing of the animal on the hot–plate and the occurrence of licking of hind paws or jumping off the surface was recorded as response latency (s). Each animal was tested before administration of drugs in order to obtain a baseline (BL) value, and mice displaying BL latencies between 0 and 20 s were excluded from the study. A 60 s cut–off was used to prevent tissue damage. One h after i.p. treatments, animals were placed on the heated surface and response latency recorded as described above. *Acetic acid–induced writhing* was measured in Swiss Webster mice or C57Bl/6 mice (wild–type and FAAH–deficient)⁴¹. The mice were acclimated to the experimental room for 2 h. Each animal was injected with acetic acid (150 µl, 0.6% in saline) and placed into a glass cylinder. Abdominal stretches (extension of body and hind limbs) were counted for 20 min, starting 5 min after acetic acid injection. URB937, rimonabant and AM630 were administered by s.c. injection 1 h before acetic acid. Behavior was scored by an observer blinded to the treatment conditions. *Formalin–induced nociception* was assessed in Sprague–Dawley rats, as described⁴². The rats were singly housed and kept in a shared holding room under a 12:12 h light:dark cycle. They were given free access to food and water, and allowed to acclimate to the facility for a week before testing. One h prior to formalin administration, the rats received i.p. injections of vehicle, URB937 (1 mg kg⁻¹, i.p.), rimonabant (2 mg kg⁻¹i.p.) or a combination of URB937 and rimonabant. They were acclimated to the observation container (clear plexiglas box, 29 x 29 x 25 cm) for 15 min before receiving an injection of formalin (50 µl, 5% in saline) into
the dorsal surface of the right hind paw. Immediately after formalin injection, the rats were returned to the observation container and nocifensive behavior was recorded for 60 min with a video camera. Recordings were analyzed by observers blinded to treatment conditions. Nocifensive behavior was measured continuously for 60 min\(^42\). The total time spent by the animals in three different behavioural categories (0, 1, 2) was recorded in 5–min bins where: (0) the rat exhibits normal posture, (1) the injected paw is raised, or (2) the injected paw is licked, shaken or bitten. Each 5–min bin was analyzed for time spent (1) lifting and (2) licking or biting the injected paw. Nocifensive behavior was analyzed using the composite pain score weighted scores technique (CPS–WST1,2) calculated for the entire time of observation (0–60 min) and, separately, for the first (0–10 min) and second phase (10–60 min) of the behavioral response\(^48\). The area under the curve (AUC) corresponding to CPS–WST1,2 was calculated using the trapezoidal rule. 

*Paw edema* was induced in mice by injection into the right hind paws of 50 µl of sterile saline containing 1% λ–carrageenan. Paw volumes were measured using a plethysmometer (Ugo Basile, Milan, Italy). Vehicle or URB937 (1 mg kg\(^{-1}\), i.p.) were injected immediately before carrageenan. Rimonabant and AM630 (1 mg kg\(^{-1}\), i.p.) were injected 30 min before carrageenan. *Mechanical hyperalgesia* was assessed by measuring the latency (s) to withdraw the paw from a constant mechanical pressure exerted onto its dorsal surface. A 15–g calibrated glass cylindrical rod (diameter = 10 mm) chamfered to a conical point (diameter = 3 mm) was used to exert the mechanical force. The weight was suspended vertically between two rings attached to a stand and was free to move vertically. A cutoff time of 3 min was used. *Thermal hyperalgesia* was assessed as described\(^49\), measuring the latency to withdraw the hind paw from a focused beam of radiant heat (thermal intensity: infrared 3.0) applied to the plantar surface, using a commercial apparatus (Ugo Basile). The cutoff time was set at 30 s. *Mechanical allodynia* was assessed by applying a graded force to the plantar hind paw.
surface with a Von Frey filament, using a Dynamic Plantar Anesthesiometer (Ugo Basile). The cut–off force was set at 5 g. Food intake and motor activity were measured in Swiss Webster mice using an automated monitoring system, as previously described\textsuperscript{60}.

\textbf{Statistical Analyses}

Results are expressed as the mean ± s.e.m. Statistical significance was determined by Student’s \( t \) test, one–way, or two–way analysis of variance (ANOVA) followed by Bonferroni \textit{post hoc} test when appropriate. A separate univariate analysis of variance was performed to determine the effects of experimental treatment on formalin–induced nocifensive behavior as measured by area under the curve. A repeated measures (Treatment x Time [repeated factor]) analysis of variance was performed on formalin–induced composite pain score. The Greenhouse–Geisser correction was applied to all repeated factors. For each laminar subdivision at L4/L5 of the spinal cord, a univariate analysis of variance was performed to determine the effects of experimental treatment on the number of Fos–expressing cells. Fisher’s LSD and Tukey \textit{post hoc} tests were performed on behavioral and Fos immunostaining data, respectively. \textit{Post hoc} comparisons that did not meet the equal variance assumption were corrected by fractional adjustment of the degrees of freedom. Analyses were performed using SPSS statistical software (version 17.0; SPSS Inc.).


