Supporting Information

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SI Materials and Methods

Plasmids. We amplified the full-length coding sequence of rat NAAA by PCR using High Fidelity PCR Master (Roche) and rat brain cDNAs as templates. We designed 2 primers using sequences obtained from the National Center for Biotechnology Information (NCBI) database: 5′rNAAA (5′-ATGGGGACCCCAGCCATCCGG-3′) and 3′rNAAA (5′-TCAGCTTGGGTTTCTGATCATGGT-3′). The PCR product was subcloned into a pCMV-Flag vector (Stratagene) by HindIII and XhoI (Roche) sites to construct a mammalian expression vector encoding Flag-tagged rNAAA. A series of site-directed mutants was generated with the QuikChange II XL Kit (Stratagene) using primers designed as follows. C131A: forward: 5′-GCCTATGAGGCTTCCGCATTCGCCACCAGTATTGTGGCCCAA-3′; reverse: 5′-TTGGGCCACAATACTGGTGGCGAATGCGGAAGCCTCATAGGC-3′; C131S: forward: 5′-CTATGAGGCTTCCGCATTCTCCACCAGTATTGTGGCCCAAG-3′; reverse: 5′-CTTGGGCCACAATACTGGTGGAGAATGCGGAAGCCTCATAG-3′; N292A: forward: 5′-TGGTTCCGAGTCGAGACCGCTTATGACCACTGGGAGCCTG-3′; reverse: 5′-CAGGCTCCCAGTGGTCATAAGCGGTCTCGACTCGGAACCA-3′; Y151A: forward: 5′-TACCATGGCCGGAACCTGGACGCTCCTTTTGGAAATGCCTTA-3′; reverse: 5′-TAAGGCATTTCCAAAAGGAGCGTCCAGGTTCCGGCCATGGTA-3′; Y151F: forward: 5′-TACCATGGCCGGAACCTGGACTTTCCTTTTGGAAATGCCTTAC-3′; reverse: 5′-GTAAGGCATTTCCAAAAGGAAAGTCCAGGTTCCGGCCATGGTA-3′; D150E: forward: 5′-TTACCATGGCCGGAACCTGGAATATCCTTTTGGAAATGCC-3′; reverse: 5′-GGCATTTCCAAAAGGATATTCCAGGTTCCGGCCATGGTAA-3′; A176G; forward: 5′-CGGGCAGATTGTATTCACAGGGACCACTTTTGTTGGCTATG-3′; reverse: 5′-CATAGCCAACAAAAGTGGTCCCTGTGAATACAATCTGCCCG-3′.

Similarly, we amplified rat acid ceramidase (AC) sequence from a rat brain cDNA library using primers based on the sequence obtained from the NCBI database: 5′rAC (5′-GACCATGCTGGGCCGTAGT-3′) and 3′rAC (5′-CCAGCCTATACAAGGGTCT-3′). The PCR product was subcloned into a pEF6-V5/His vector (Invitrogen) to construct a mammalian expression vector encoding V5/His-tagged rAC.

mRNA Extraction and Real-Time PCR. We extracted total RNA using TRIzol (Invitrogen). cDNA was synthesized with 0.2 μg of total RNA and oligo $(dT)_{12–18}$ primer using SuperScript II RNase H-reverse transcriptase (Invitrogen). Quantitative PCR was performed with an Mx3000P Real-Time PCR System (Stratagene). We designed primer/probe sets using Primer Express software according to the gene sequences available from the GenBank database. Primers and fluorogenic probes were synthesized at TIB (Adelphia, NJ). The primer/probe sequences for mouse genes were as follows. *TNF-α*: forward: 5′-ACAGAAAGCATGATCCGCG-3′, reverse: 5′-GCCCCCCATCTTTTGGG-3′; probe: 5′-CGTGGAACTGGCAGAAGAGGCACTCT-3′. iNOS: forward: 5′-GGTGGGTGGCCTCGAATT-3′, reverse: 5′-CCAATCTC GGTGCCCATG-3′; probe: 5′-CCAGCCTGCCCCTTCAATGGTTG-3′. GAPDH: forward: 5′-TCACTGGCATGGCCTTCC-3′, reverse: 5′-GGCGGCACGTCAGATCC-3′; probe: 5′-TTCCTACCCCCAATGTGTCCGTCG-3′. RNA levels were normalized using GAPDH as an internal standard.

Expression of Recombinant Proteins. We transfected HEK293 cells with pCMV-Flag-rNAAA or pEF6-rAC-V5/His using SuperFect reagent (Qiagen) and screened with G418 (0.3 mg/mL). We harvested and washed cells stably expressing rNAAA or rAC, sonicated them in 20 mM Tris·HCl (pH 7.5) with 0.32 M sucrose, and centrifuged them at 800 × *g* for 15 min at 4 °C. The supernatant was ultracentrifuged at 12,000 × *g* for 30 min at 4 °C. The pellet was suspended in PBS and subjected to 2 freeze–thaw cycles at −80 °C. The suspension was centrifuged at 105,000 × *g* for 1 h at 4 °C, and the supernatant containing rNAAA or rAC was kept at −80 °C until use.

Protein Analyses. Protein concentration was measured using the BCA protein assay (Pierce). Proteins were separated by SDS-PAGE and transferred to PVDF membranes (Amersham Biosciences). Overnight incubation in antiflag antibody (Sigma; 1:5,000) at 4 °C was followed by incubation with HRP-conjugated anti-mouse IgG antibody (Sigma; 1:10,000) for 1 h at room temperature. Protein bands were visualized using an ECL Plus kit (Amersham Biosciences).

NAAA Assay. Recombinant NAAA or native rat lung NAAA was incubated at 37 °C for 30 min in 0.2 mL of sodium hydrogen phosphate buffer (50 mM, pH 5.0) containing 0.1% Triton X-100, 3 mM DTT, and 50 μM heptadecenoylethanolamide as substrate. The reaction was terminated by the addition of 0.2 mL cold methanol containing 1 nmol of heptadecanoic acid (HDA; NuChek Prep). Samples were analyzed by LC/MS. HDA was eluted on an XDB Eclipse C18 column isocratically at 2.2 mL/min for 1 min with a solvent mixture of 95% methanol and 5% water, both containing 0.25% acetic acid and 5 mM ammonium acetate. The column temperature was 50 °C. Electrospray ionization (ESI) was in the negative mode, capillary voltage was 4 kV, and fragmentor voltage was 100 V. N_2 was used as drying gas at a flow rate of 13 L/min and a temperature of 350 °C. Nebulizer pressure was set at 60 psi. We monitored $[M\text{-}H]^-$ in the selected-ion monitoring (SIM) mode using HDA as internal standard. Calibration curves were generated using commercial HDA (Nu-Chek Prep; $m/z = 267$). For measuring ex vivo NAAA activity, RAW264.7 cells were treated with (*S*)-OOPP or (*R*)-OOPP (10 μM each) for 30 min, followed by a 6-h treatment with LPS. Immediately after treatment, the cells were harvested and lysed. These cell lysates (100 μg) were assayed for NAAA activity.

PPAR-α Transactivation Assays. Transactivation assays were performed as described in ref. 1. Briefly, we generated plasmids containing the ligand-binding domain of human PPAR-α (nucleotides 499–1,407) fused to the DNA-binding domain of yeast GAL4 under control of the human cytomegalovirus promoter and to a neomycin resistance gene to provide stable selection with 0.2 mg/mL G418. We cultured HeLa cells in DMEM supplemented with FBS (10%), transfected them with 3 μL of Fugene 6 (Roche) containing 1 μg of pFR-luc plasmid (Stratagene), and replaced the media after 18 h with DMEM containing 0.1 mg/mL hygromycin (Calbiochem). After 4 weeks we isolated surviving clones, analyzed them by luciferase assay, and selected for transfection a cell line that showed the highest luciferase activity. Cells were maintained in DMEM containing hygromycin and G418. For transactivation assays we seeded cells in 6-well plates and incubated them for 7 h in DMEM containing hygromycin and G418, plus appropriate concentrations of test

compounds. We used a dual-luciferase reporter assay system (Promega) and an MIX Microtitera plate luminometer (Dynex) to determine luciferase activity in cell lysates.

Lipid Extractions. Lipids were extracted using a chloroform/methanol mixture (2:1, vol/vol, 3 mL) containing internal standards. The organic phases were collected, dried under N_2, and dissolved in methanol/chloroform (3:1, vol/vol) for LC/MS analyses.

LC/MS Analyses. ***PEA.*** We used an Agilent 1100-LC system coupled to a 1946A-MS detector equipped with an ESI interface (Agilent Technologies). PEA was separated on an XDB Eclipse C18 column (50 × 4.6 mm i.d., 1.8 μm; Zorbax, Agilent Technologies) with a gradient of methanol in water (from 85% to 90% methanol in 2.0 min and 90% to 100% in 3.0 min) at a flow rate of 1.5 mL/min. Column temperature was kept at 40 °C. MS detection was in the positive ionization mode, capillary voltage was 3 kV, and fragmentor voltage was 120 V. N_2 was used as drying gas at a flow rate of 13 L/min and a temperature of 350 °C. Nebulizer pressure was set at 60 psi.

Arachidonic acid. We eluted an XDB Eclipse C18 column with a linear gradient from 90% to 100% of methanol in water (both containing 0.25% acetic acid and 5 mM ammonium acetate) in 2.5 min at 1.5 mL/min. The column temperature was 40 °C. ESI was in the negative mode, capillary voltage was set at 4 kV, and fragmentor voltage was 100 V. N_2 was used as drying gas at a flow rate of 13 L/min and a temperature of 350 °C. Nebulizer pressure was set at 60 psi. Heptadecanoic acid (m/z = 269; Nu-Chek Prep) was used as internal standard, monitoring deprotonated molecular ions $[M\text{-}H]^-$ in the SIM mode. Calibration curves were generated using commercial arachidonic acid (Nu-Chek Prep).

Ceramides. Ceramides were analyzed by LC/MSn, using a 1100-LC system (Agilent Technologies) equipped with an Ion Trap XCT (Agilent Technologies). They were separated on a Poroshell 300 SB C18 column (2.1 × 75 mm i.d., 5 μm; Agilent Technologies) maintained at 30 °C. A linear gradient of methanol in water containing 5 mM ammonium acetate and 0.25% acetic acid (from 80% to 100% of methanol in 3 min) was applied at a flow rate of 1 mL/min. Detection was in the positive mode, capillary voltage was 4.5 kV, skim1 −40 V, and capillary exit −151 V. N_2 was used as drying gas at a flow rate of 12 L/min, temperature of 350 °C, and nebulizer pressure of 80 psi. Helium was used as collision gas. Tissue-derived ceramides were identified by comparison of their LC retention times and MSn fragmentation patterns with those of authentic standards (Avanti Polar Lipids). Extracted ion chromatograms were used to quantify stearoyl ceramide (m/z > 566.5 > 548.3 > 264.3) and standard lauroyl ceramide (m/z 482.5 > 464.5 > 264.3). Detection and analysis were controlled by Agilent/Bruker Daltonics software version 5.2. MS spectra were processed using MS Processor from Advanced Chemistry Development.

Other Enzyme Assays. AC activity was measured by incubating 25 μg of protein derived from AC-overexpressing HEK293 cells with 100 μM *N*-lauroyl ceramide (Nu-Chek Prep) as substrate in assay buffer (100 mM sodium phosphate buffer, 0.1% Nonidet P-40, 150 mM NaCl, and 3 mM DTT, pH 4.5) for 30 min at 37 °C. Reactions were stopped by addition of a mixture of chloroform/methanol (2:1, vol/vol). The organic phases were collected, dried under N_2, and analyzed by LC/MS in the negative-ion mode using HDA as internal standard (m/z = 199 for lauric acid, m/z = 269 for HDA). FAAH and NAPE-PLD assays were performed as described in ref. 2. Briefly, for FAAH activity, 50 μg of proteins from rat brain membranes were incubated at 37 °C for 30 min in Tris buffer (50 mM, pH 8.0) containing fatty acid-free BSA (0.05%) and [ethanolamine-^{3}H] anandamide (10,000 dpm, specific activity 20 Ci/mmol). After stopping the reaction with a mixture of chloroform/methanol (1:1, vol/vol), we measured radioactivity in the aqueous layers by liquid scintillation counting. NAPE-PLD activity was measured at 37 °C for 30 min in Tris·HCl buffer (50 mM, pH 7.4) containing 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, protein (100 μg), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-heptadecenoyl (100 μM) as substrate. Reactions were stopped by adding chloroform/methanol (2:1, vol/vol) containing $[^2H_4]$oleoylethanolamide as internal standard. After centrifugation at 1500 × *g* at 4 °C for 5 min, the organic layers were collected and dried under N_2. The residues were suspended in 50 μL of chloroform/methanol (1:3, vol/vol) and analyzed by LC/MS, monitoring the $[M + Na]^+$ ions of m/z = 334 for *N*-heptadecanoylethanolamide and m/z = 352 for $[^2H_4]$oleoylethanolamide. Monoacylglycerol lipase (MGL) activity was measured by incubating 50 μg of protein derived from the cytosolic fraction of MGL-overexpressing HeLa cells in assay buffer [50 mM Tris (pH 8.0) and 0.05% fatty acid-free BSA] and substrate ([^{3}H]2-oleoylglycerol, 10,000 cpm per reaction). After incubation at 37 °C for 30 min, reactions were stopped with chloroform/methanol (1:1, vol/vol), and product in the aqueous phase was quantified by scintillation counting. Triacylglycerol lipase activity was measured by incubating rat adipose homogenate with [^{3}H]triolein (Nu Chek Prep) in 0.1 M potassium phosphate buffer at pH 7.4. After 30 min at 37 °C, reactions were stopped by adding methanol/chloroform/heptane (1.21:1.25:1, vol/vol/vol) followed by 0.1 M potassium carbonate and borate buffer (pH 10.5). Radioactivity in the aqueous phase was measured by liquid scintillation counting. Phospholipase A_2 activity was determined by incubating 2 units of *Apis mellifera* PLA_2 (Sigma) in 100 mM borate buffer (pH 8.9) with 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids). After shaking at room temperature for 10 min, reactions were stopped by adding chloroform/methanol (2:1, vol/vol), and the organic layers were extracted and analyzed by LC/MS in the negative-ion mode. PLD activity was measured by incubating 0.2 U of *Streptomyces chromofuscus* PLD in 50 mM Tris·HCl buffer (pH 7.4) and 0.32 M sucrose with 0.2 mM *N*-palmitoyl phosphatidylethanolamine synthesized in the laboratory as described in ref. 2. After 30 min at 37 °C, reactions were stopped by adding chloroform/methanol (2:1, vol/vol), and lipids in the organic layers were analyzed by LC/MS in the positive-ion mode. Diacylglycerol lipase activity was measured by harvesting Neuro-2a cells overexpressing DGL-α in 50 mM Tris·HCl (pH 7.0, 1 mL per dish) and homogenizing them with a Dounce homogenizer on ice. Homogenates were centrifuged at 800 × *g* for 5 min at 4 °C, and the resulting supernatants were used for the assay. DGL activity was measured at 37 °C for 30 min in 50 mM Tris·HCl (pH 7.0) containing 0.1% Triton X-100, tissue homogenate protein (100 μg), and diheptadecanoyl-*sn*-glycerol (50 μM) as substrate. The reactions were stopped by adding chloroform/methanol (1:1) containing $[^2H_8]$-2-AG. After centrifugation at 1500 × *g* at 4 °C for 5 min, the organic layers were collected and dried under N_2. The residues were suspended in chloroform/methanol (1:3, 50 μl) and analyzed by LC/MS. For quantification purposes, we monitored the reaction product HDG $[M+Na]^+$ (m/z 367) along with standard $[^2H_8]$2-AG (m/z 409). Cyclooxygenase-2 and 5-lipoxygenase activities were measured using commercial kits (Cayman Chemicals).

Immunohistochemical Localization of iNOS, Par, Nitrotyrosine, FAS-Ligand, Bax, and Bcl-2. Twenty-four hours after SCI, nitrotyrosine, a specific marker of nitrosative stress, was measured by immunohistochemical analysis in the spinal cord sections to determine the localization of "peroxynitrite formation" and/or other nitrogen derivatives produced during SCI. The tissues were fixed in 10% (wt/vol) PBS-buffered formaldehyde, and 8-mm sections were prepared from paraffin-embedded tissues. After deparaf-

finization, endogenous peroxidase was quenched with 0.3% (vol/vol) hydrogen peroxide in 60% (vol/vol) methanol for 30 min. The sections were permeabilized with 0.1% (wt/vol) Triton X-100 in PBS for 20 min. Nonspecific adsorption was minimized by incubating the section in 2% (vol/vol) normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with biotin and avidin (DBA), respectively. Sections were incubated overnight with anti-PAR (Alexis; 1:500 in PBS, vol/vol), anti-iNOS antibody (1:500 in PBS, vol/vol), anti-nitrotyrosine rabbit polyclonal antibody (Upstate; 1:500 in PBS, vol/vol), with anti-FAS-ligand antibody (Abcam; 1:500 in PBS, vol/vol), anti-Bax antibody (Santa Cruz Biotechnology; 1:500 in PBS, vol/vol), or with anti-Bcl-2 polyclonal antibody (Santa Cruz Biotechnology; 1:500 in PBS, vol/vol). Sections were washed with PBS and incubated with secondary antibody. Specific labeling was detected with a biotin-conjugated goat anti-rabbit IgG and avidin–biotin peroxidase complex (DBA). To verify the binding specificity for nitrotyrosine, Par, iNOS, Bax, and Bcl-2, some sections were also incubated with only the primary antibody (no secondary) or with only the secondary antibody (no primary). In these situations no positive staining was found in the sections, indicating that the immunoreactions were positive in all of the experiments carried out.

TUNEL Assay. TUNEL assay was conducted by using a TUNEL detection kit according to the manufacturer's instructions (Apotag, HRP kit; DBA). Briefly, sections were incubated with 15 μg/mL proteinase K for 15 min at room temperature and then washed with PBS. Endogenous peroxidase was inactivated by 3% H_2O_2 for 5 min at room temperature and then washed with PBS. Sections were immersed in terminal deoxynucleotidyltransferase (TdT) buffer containing deoxynucleotidyl transferase and biotinylated dUTP in TdT buffer, incubated in a humid atmosphere at 37 °C for 90 min, and then washed with PBS. The sections were incubated at room temperature for 30 min with anti-HRP-conjugated antibody, and the signals were visualized with diaminobenzidine. The numbers of TUNEL-positive cells per high-power field were counted in 5 to 10 fields for each coded slide.

Light Microscopy. Spinal cord tissues were taken at 24 h after trauma. Tissue segments containing the lesion (1 cm on each side of the lesion) were paraffin embedded and cut into 5-μm-thick sections. Tissue sections (thickness, 5 μm) were deparaffinized with xylene, stained with H&E and with methyl green pyronin staining (used to simultaneously stain DNA and RNA), and studied using light microscopy (Dialux 22; Leitz).

The segments of each spinal cord were evaluated by an experienced histopathologist. Damaged neurons were counted, and the histopathologic changes of the gray matter were scored on a 7-point scale (3): 0, no lesion observed; 1, gray matter contained 1 to 5 eosinophilic neurons; 2, gray matter contained 5 to 10 eosinophilic neurons; 3, gray matter contained >10 eosinophilic neurons; 4, small infarction (less than one third of the gray matter area); 5, moderate infarction (one third to one half of the gray matter area); 6, large infarction (more than half of the gray matter area). The scores from all of the sections from each spinal cord were averaged to give a final score for individual mice. All of the histologic studies were performed in a blinded fashion.

Grading of Motor Disturbance. The motor function of mice subjected to compression trauma was assessed once per day for 10 days after injury. Recovery from motor disturbance was graded using the modified murine Basso, Beattie, and Bresnahan (4) hind limb locomotor rating scale (5). The following criteria were considered: 0, no hind limb movement; 1, slight (<50% range of motion) movement of 1 to 2 joints; 2, extensive (>50% range of motion) movement of 1 joint and slight movement of 1 other joint; 3, extensive movement of 2 joints; 4, slight movement in all 3 joints; 5, slight movement of 2 joints and extensive movement of 1 joint; 6, extensive movement of 2 joints and slight movement of one joint; 7, extensive movement of all 3 joints; 8, sweeping without weight support or plantar placement and no weight support; 9, plantar placement with weight support in stance only or dorsal stepping with weight support; 10, occasional (0–50% of the time) weight-supported plantar steps and no coordination (front/hind limb coordination); 11, frequent (50–94% of the time) to consistent (95–100% of the time) weight-supported plantar steps and no coordination; 12, frequent to consistent weight-supported plantar steps and occasional coordination; 13, frequent to consistent weight-supported plantar steps and frequent coordination; 14, consistent weight-supported plantar steps, consistent coordination, and predominant paw position are rotated during locomotion (lift off and contact) or frequent plantar stepping, consistent coordination, and occasional dorsal stepping; 15, consistent plantar stepping and coordination, no/occasional toe clearance, paw position is parallel at initial contact; 16, consistent plantar stepping and coordination (front/hind limb coordination) and frequent toe clearance, and predominant paw position is parallel at initial contact and rotated at lift off; 17, consistent plantar stepping and coordination and frequent toe clearance, and predominant paw position is parallel at initial contact and lift off; 18, consistent plantar stepping and coordination and consistent toe clearance, and predominant paw position is parallel at initial contact and rotated at lift off; 19, consistent plantar stepping and coordination and consistent toe clearance, and predominant paw position is parallel at initial contact and lift off; 20, consistent plantar stepping, coordinated gait, consistent toe clearance, and predominant paw position is parallel at initial contact and lift off and trunk instability; and 21, consistent plantar stepping, coordinated gait, consistent toe clearance, predominant paw position is parallel at initial contact and lift off and trunk stability.

Statistics. Results are expressed as mean ± SEM of n observations. They were analyzed by 1-way ANOVA followed by the Bonferroni post hoc test for multiple comparisons. P values <0.05 were considered significant.

1. Fu J, et al. (2003) Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature* 425:90–93.
2. Fu J, et al. (2007) Food intake regulates oleoylethanolamide formation and degradation in the proximal small intestine. *J Biol Chem* 282:1518–1528.
3. Sirin BH, et al. (2002) Ischaemic preconditioning reduces spinal cord injury in transient ischaemia. *Acta Cardiol* 57:279–285.
4. Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 12:1–21.
5. Joshi M, Fehlings MG (2002) Development and characterization of a novel, graded model of clip compressive spinal cord injury in the mouse: Part 2. Quantitative neuroanatomical assessment and analysis of the relationships between axonal tracts, residual tissue, and locomotor recovery. *J Neurotrauma* 19:191–203.

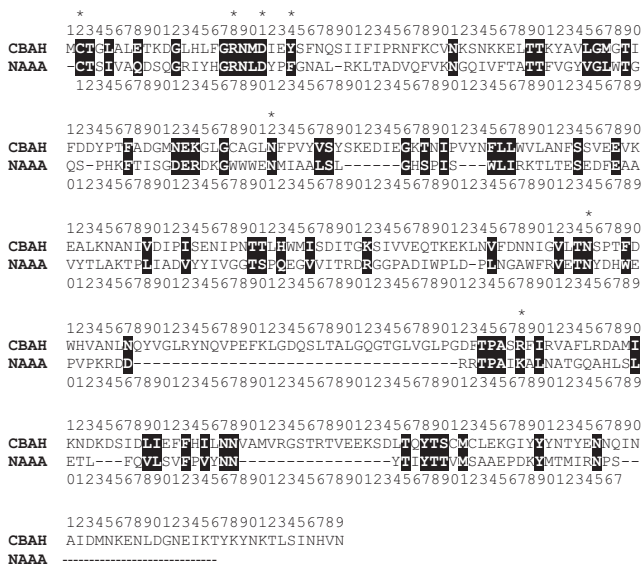


Fig. S1. Sequence alignment between CBAH and the mature form of rat NAAA (amino acids 131–362). Numbering of CBAH residues is reported at the top lines and numbering of NAAA amino acids at the bottom lines. C1 corresponds to C131 in the unprocessed precursor. The active site residues are marked with an asterisk.

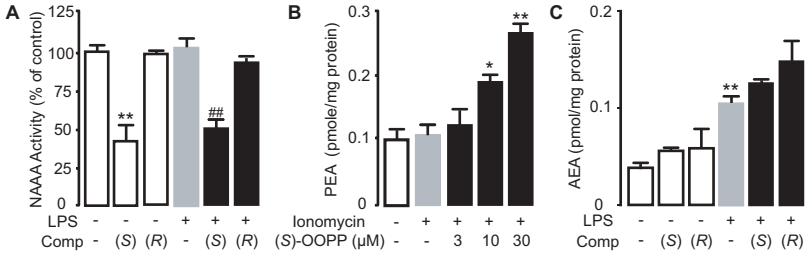


Fig. S2. Effect of (*S*)-OOPP on NAAA activity and PEA levels in RAW264.7 cells and HEK293 cells overexpressing NAAA. The effect of (*S*)-OOPP (10 μM) on NAAA activity (*A*) in RAW264.7 macrophages stimulated with LPS. (*S*)-OOPP (3–30 μM) increases PEA levels in HEK293 cells that overexpress rat NAAA stimulated with ionomycin (1 μM, 30 min) (*B*) but does not affect LPS-induced changes in anandamide (*C*). (*S*)-OOPP exerted no detectable effect in nonstimulated cells. *, $P < 0.05$ vs. vehicle/vehicle; **, $P < 0.01$ vs. vehicle/vehicle; ##, $P < 0.01$ vs. LPS/vehicle ($n = 3$).

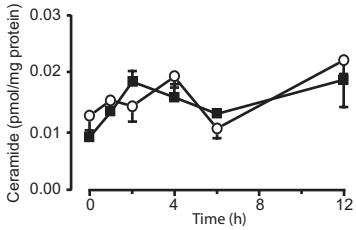


Fig. S3. Time-course of the effects of (*S*)-OOPP (10 μM, filled squares) or vehicle (DSMO, 0.1%, open circles) on endogenous levels of *N*-stearoylceramide in RAW264.7 macrophages stimulated with LPS (100 ng/mL). Cells were lysed, lipids extracted, and *N*-stearoylceramide quantified by LC/MS, as described in *SI Materials and Methods*.

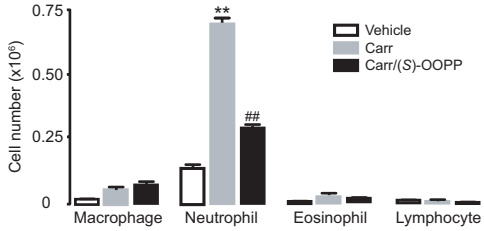


Fig. S4. H&E staining of infiltrating cells in s.c. sponges. Number of macrophages, neutrophils, eosinophils, and lymphocytes. The s.c. sponges were instilled with vehicle (0.1 mL saline, open bars), carrageenan (Carr, 1%, gray bars), or carrageenan plus (*S*)-OOPP (25 μg per sponge, filled bars). **, $P < 0.01$ vs. vehicle; ##, $P < 0.01$ vs. carrageenan ($n = 5–6$).

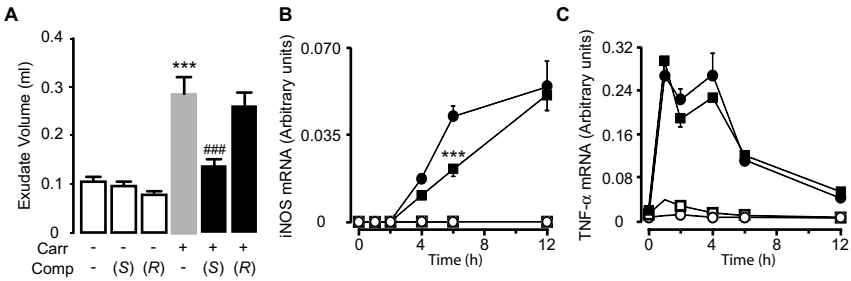


Fig. S5. Effects of (*S*)-OOPP on carrageenan (Carr)-induced plasma extravasation in mice and proinflammatory gene expression in RAW264.7 macrophages. (*S*)-OOPP but not (*R*)-OOPP (each at 25 μg per sponge) inhibits plasma extravasation (*A*) elicited by carrageenan (0.1 mL, 1%) in s.c. sponges in mice. Effects of vehicle (filled circles) or (*S*)-OOPP (filled squares) on expression of iNOS mRNA (*B*) and TNF-α mRNA (*C*) elicited by LPS (100 ng/mL). Open symbols, iNOS and TNF-α mRNA levels without LPS. ***, $P < 0.001$ vs. vehicle/vehicle or LPS/vehicle; ###, $P < 0.001$ vs. carrageenan/vehicle ($n = 3–6$).

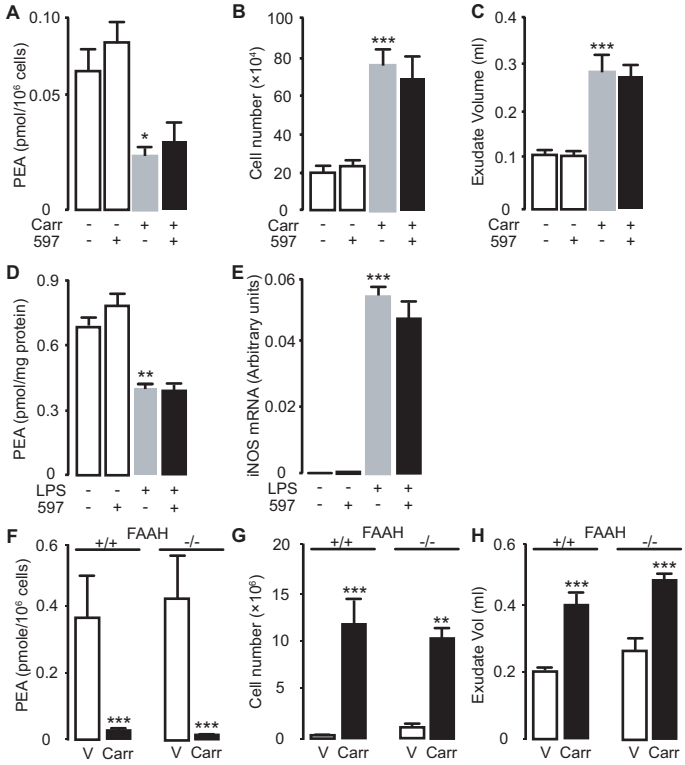


Fig. S6. The FAAH inhibitor URB597 does not alter PEA levels and inflammatory responses of RAW264.7 macrophages in vitro or inflammatory cells collected from s.c. sponges in vivo. (*A–C*) Effects of URB597 (597, 0.25 μg per sponge, 72 h) on PEA levels (*A*), leukocyte infiltration (*B*), and plasma extravasation (*C*). (*D* and *E*) Effects of URB597 (597, 0.1 μM, 6 h) on RAW264.7 cells treated with LPS (100 ng/mL) on PEA levels (*D*) and iNOS mRNA expression (*E*). (*F*) PEA levels in inflammatory cells, leukocyte infiltration (*G*), and plasma extravasation (*F*) in s.c. sponges instilled with vehicle (sterile saline, 0.1 mL) or carrageenan (Carr, 1%) in wild-type C57BL/6J (+/+) and FAAH$^{-/-}$ (−/−) mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. vehicle ($n = 6–10$).

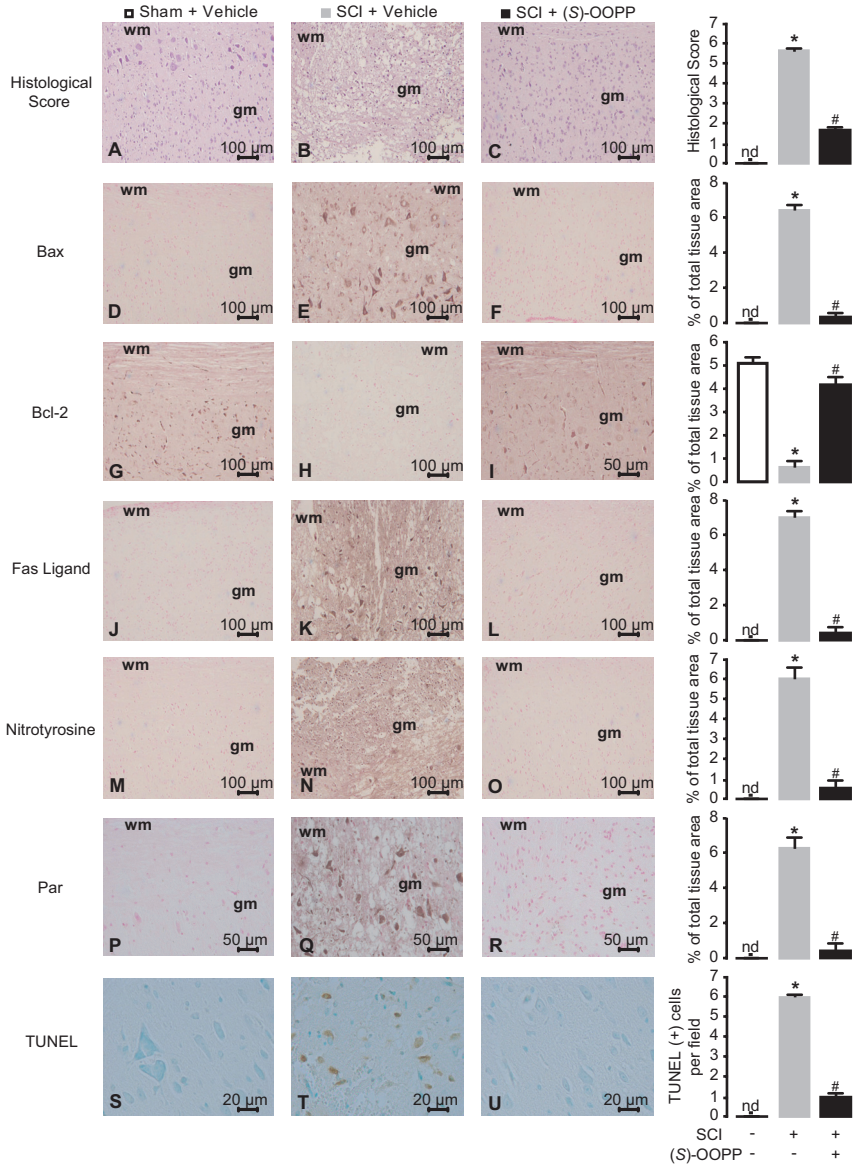
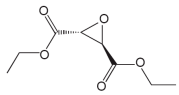
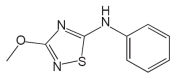
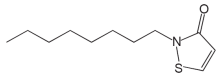
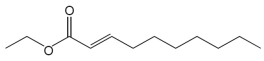
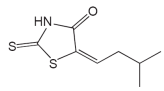
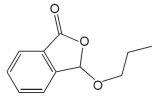
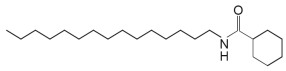


Fig. S7. Effect of (*S*)-OOPP on histologic score (*A–C*), expression of Bax (*D–F*), Bcl-2 (*G–I*), Fas ligand (*J–L*), nitrotyrosine (*M–O*), Par (*P–R*), and TUNEL staining (*S–U*) in SCI. *, $P < 0.05$ vs. sham/vehicle; #, $P < 0.05$ vs. SCI/vehicle ($n = 10$); nd, not detectable.

Table S1. Structures of representative compounds screened in the present study

Compound no.	Name		Inhibition (at 0.1 mM) (%)
1	Diethyl (2R,3R)-(-)-2,3-epoxysuccinate		17 ± 8
2	5-anilino-3-methoxy-1,2,4-thiadiazole		18 ± 7
3	2-octyl-4-isothiazolin-3-one		61 ± 8
4	Ethyl trans-2-decenoate		53 ± 9
5	5-(3-methylbutylidene)-2-thioxo-1,3-thiazolidin-4-one		44 ± 4
6	3-propoxyphthalide		31 ± 13
7	*N*-cyclohexanecarbonyl-pentadecylamine (CCP)		33 ± 9

Compounds were tested on recombinant rat NAAA under standard assay conditions (see *SI Materials and Methods*).

Table S2. Effects of (*S*)-OOPP on various enzyme activities

Enzyme	Source	IC_{50} (μM)
Monoacylglycerol lipase	Rat brain	>100
Triacylglycerol lipase	Rat adipose tissue	>100
Phospholipase A_2	*A. mellifera*	>100
Phospholipase D	*S. chromofuscus*	>100
Cyclooxygenase-2	Human recombinant	>100
5-lipoxygenase	*S. tuberosum*	>100
NAPE-PLD	Rat brain	>100
Diacylglycerol lipase-α	Human recombinant	>100

Assay conditions are described in *SI Materials and Methods*.