Supporting Information

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SI Materials and Methods

Immunohistochemical Localization of Prostaglandin E Receptors and Double Labeling. ***Tissue preparation.*** For the preparation of lumbar dorsal root ganglia (DRGs) and spinal cord rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.). They received 0.5 mL heparin i.p. [5,000 international units per 1 mL United States Pharmacopeia (USP) units/10 mL] and were perfused intracardially with 100 mL 0.1 M sodium cacodylate buffer (Sigma), pH 7.2, containing 0.4% sodium nitrite and 2% (wt/vol) polyvinyl-pyrrolidone (Sigma) for 5 min, followed by 2% (vol/vol) paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 15 min. Sections of DRGs and spinal cord were postfixed in 4% (vol/vol) paraformaldehyde in phosphate buffered saline (PBS) for 24 h. For the studies of peripheral nerve bundles in human joints, material was resected from osteoarthritic human knee joints of patients (seven female, one male; 58–80 y old) with primary idiopathic osteoarthritis who underwent surgical replacement of knee joints using standardized surgical technique under general anesthesia. The tissue was fixed in 4% (vol/vol) paraformaldehyde in PBS for at least 48 h. All these materials were embedded in Histosec (Merck) and then cut into 6-μm sections. For the studies of nerve bundles in rat knee joint, in rat skin over the knee, and in rat dura, the tissues were resected and fixed at least 24 h in 4% (vol/vol) paraformaldehyde in PBS, then embedded in Technovit 9100, and then cut into 4-μm sections.

For labeling of DRG neurons supplying the knee joint, rats were briefly anesthetized with 2.5% isoflurane (vol/vol), and 20 μL of the tracer FAST-DiI (1,1′-dilinoleyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; Invitrogen) at 0.1 μg/μL were injected into a knee joint. After 3 d, the rats were perfused (see above), and the lumbar and thoracic DRGs were removed and postfixed in 4% paraformaldehyde in PBS for 24 h. The DRGs were embedded in agarose (ultra-low gelling, A2576; Sigma), 3.5% (wt/vol) in PBS, and cut into 50-μm sections using a vibratome (Leica VT1000S). We analyzed DRGs from six rats, and DiI-labeled neurons were only detected in lumbar DRGs but not in thoracic DRGs excluding unspecific spreading of the tracer.

Immunohistochemistry. Dewaxed sections were rinsed with ddH_2O and 50 mM Tris·HCl buffer containing 12.6% (wt/vol) NaCl (TBS buffer, pH 7.4), and then placed in retrieval medium consisting of 10 mM citrate buffer (pH 6.0). Sections were heated three times for 5 min each in a microwave oven (Supratronic M 752; 80 W, 80 °C). After cooling down, the DRG and spinal cord sections were brought into protein containing TBS (with 1% gelatin from fish skin + 1% Triton X-100], the sections from human joint material were washed and brought into protein containing TBS [with 10% (vol/vol) goat serum + 1% Triton X-100], both for 30 min at 20 °C. Then DRG and spinal cord sections were incubated overnight at 4 °C in corresponding buffer containing polyclonal antibodies (rabbit, all from Cayman Chemical) against the prostaglandin E (EP) 1 receptor (1:50 or 1:3,000; different batches), or the EP2 receptor (1:200), or the EP3 receptor (1:1,000), or the EP4 receptor (1:100). Human joint material was incubated in corresponding buffer containing the EP3 receptor antibody (1:500).

After treatment with primary antibody, sections were incubated for 40 min in biotinylated secondary antibody (1:200; DAKO) at 20 °C, and then the avidin-biotin peroxidase complex (Vectastain-Elite ABC kit; Vector Laboratories) was applied for 40 min. The ABC complex was visualized using the fluorochrome *(E)*-2-[2-(4-hydroxystyryl)]quinoline in 0.05 M phosphate buffer (pH 8.0) including 0.005% Tween 20, 0.075% CHAPS (Fluka), and 0.005% H_2O_2. Finally, the sections were dehydrated rapidly one time in absolute ethanol and three times in xylene/ethanol 1:1 and then mounted in Entellan.

To localize EP receptor sites in the spinal cord, colocalization was assessed. Glial cells in the white (radial glia) and gray matter were detected with monoclonal antibody against glial fibrillary acidic protein (GFAP) (Sigma; 1:100 in TBS, 20 °C, 40 min). Neurons were labeled using monoclonal neuronal nuclei (NeuN) antibody (Chemikon; 1:100 in TBS, 20 °C, 40 min). Visualization was performed by using Alexa Fluor 568 GAM-IgG (Mobitec; 1:50 in TBS, 20 °C, 30 min).

In DRG sections colocalization of the EP3 receptor with either CGRP, IB4, or neurofilament was assessed. After antigen retrieval DRG sections were incubated in protein containing TBS [with 10% (vol/vol) goat serum + 1% Triton X-100], both for 30 min at 20 °C. Then DRG sections were incubated overnight at 4 °C in corresponding buffer containing polyclonal antibodies against calcitonin gene-related peptide (CGRP) (1:100; Acris Antibodies) or neurofilament (1:100; Invitrogen). After washing in TBS the sections were incubated with goat anti-mouse immun globulin G (IgG) conjugated with Alexa Fluor 488 (1:100; Invitrogen). Thereafter the EP3 receptor antibody (1:100) was applied to the sections and then labeled with goat anti-rabbit IgG conjugated with Alexa Fluor 568 (1:200; Invitrogen). For double labeling of EP3 receptor and I isolectin B4 (IB4), the sections were first incubated with the EP3 receptor antibody and then IB4 conjugated with fluorescein isothiocyanate 1 (FITC) (1:20; Sigma) was applied for 45 min. For the anti-CGRP and the antineurofilament antibodies we performed controls by omitting the primary antibody and did not detect unspecific labeling.

For the study of colocalization of FAST DiI and EP3 receptor-like IR DRG, sections were incubated with PBS plus 2% (vol/vol) goat serum and 1,000 μg/mL digitonin (Fluka) for 30 min at 20 °C. Then sections were incubated in corresponding buffer containing the EP3 receptor antibody (1:200). As the second antibody, goat anti-rabbit IgG conjugated with Alexa Fluor 488 (1:200) was used. For staining of cell nuclei, sections were incubated with Hoechst 34580 (1:1,000; Invitrogen) for 5 min.

For microscopy we used confocal laser scanning microscopes (LSM 710, LSM 310; Zeiss and Leica TCS SP5). *(E)*-2-[2-(4-hydroxystyryl)]quinoline and Alexa Fluor 488 were excited with the Ar^+ laser at 488 nm, the emission signals were detected between 500 nm and 560 nm or using the long-pass optical filter LP 525 nm. Alexa Fluor 568 was irradiated with a He-Ne laser at 543 nm or a DPSS laser at 561 nm, and signals were recorded using the long-pass optical filter CP560 and were detected at 565–650 nm.

Membrane-associated EP receptor expression sites in cultured rat DRG neurons. Unfixed cultured DRG neurons (see below) were washed three times for 10 min at 4 °C in 0.1 M Hepes buffer [pH 7.4; containing 10% (vol/vol) culture medium, see below, and 10% (wt/vol) sucrose]. They were then treated for 4 h at 4 °C in corresponding incubation buffer that contained 1% fish gelatin and 1% Triton X-100 with either EP2, or EP3, or EP4 receptor primary antibody (1:100). Thereafter the cultures were treated with Hepes incubation buffer with secondary anti-rabbit antibodies labeled with Alexa Fluor 488 (1:400; Invitrogen) for 2 h at 4 °C. Finally, the incubated specimens were washed three times in Hepes buffer for 10 min, coverslipped with buffer, and viewed in the LSM.

Part of the cultures used for EP3 receptor expression were exposed to either PGE_2 (500 nM), or the EP2 receptor agonist ONO-AE-248 (500 nM), or the EP4 receptor agonist ONO-AE1-329 (500 nM). The latter were added to the buffer containing the EP3 receptor antibody, and cultures were kept in this

solution for 4 h at 4 °C. Further treatment with Alexa Fluor 488-labeled secondary antibody was carried out as described above.

PCR for Identification of EP3 Isoforms in DRGs. Total RNA was prepared from total DRGs using the Absolutely RNA Miniprep kit (Stratagene). RNA concentrations were measured, and 1 μg total RNA was used for reverse transcription using the RevertAidFirst Strand cDNA Synthesis kit (Thermo Scientific). Using the oligo$(dT)_{18}$ primer, all mRNA templates were transcribed into cDNA. The final concentration was adjusted to 15 ng/μL.

We used the following primers: EP3 forward, ccagcttatgggggatcatgtg (before the splicing site common to all EP3 isoforms) and EP3A reverse, ggcaaggaggtagagctggaagc [predicted PCR products: 276 bp (EP3A), BamHI restriction 178 + 98 bp, TaqI restriction 217 + 59 bp]; EP3B reverse, gcaatgaaactccgcttcagg [predicted PCR products: 264 bp (EP3B), BamHI restriction 178 + 86 bp, TaqI restriction 217 + 47 bp; 353 bp (EP3A), BamHI restriction 178 + 175 bp, TaqI restriction 217 + 136 bp; 284 bp (EP3D), BamHI restriction 178 + 106 bp, TaqI restriction 217 + 67 bp]; EP3C reverse, gcagctggagacagcgtttgc [predicted PCR products: 256 bp (EP3C), BamHI restriction 178 + 78 bp, TaqI restriction 217 + 39 bp]; EP3D reverse, catcatctgttaaaacagagagagaac [predicted PCR products: 258 bp (EP3D), BamHI restriction 178 + 80 bp, TaqI restriction 217 + 41 bp, BseNI restriction 229 + 29 bp]. For PCR of the EP3B splice variant, different numbers of base pairs were used to differentiate it from EP3A and EP3D splice variants. The specificity of all PCR products was confirmed by restriction analysis using the endonucleases BamHI and TaqI (Fermentas). The EP3D PCR products had additionally a BseNI restriction site.

PCR was performed using the Platinum Taq DNA polymerase (Invitrogen). For the standard amplification, the following final concentrations were chosen: 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.5 μM primer mix, PCR buffer, and 1.5 units Platinum Taq DNA polymerase per 50 μL PCR mixture. For the amplification of EP3A, -B, or -C, a final cDNA template concentration of 0.5 ng/μL was used. For EP3D, 2.5 ng/μL cDNA was used, respectively. The PCR was performed using the thermocycler TGradient (Biometra). Reaction conditions for the PCR were as follows: step 1, 5 min at 95 °C; step 2, 30 s at 95 °C; step 3, 20 s at 62 °C; step 4, 30 s at 72 °C; step 5, 3 min 72 °C (steps 2–4 repeated 35 times).

Induction of Inflammation in the Knee Joint. In anesthetized rats, acute inflammation was induced by injection of suspensions of 4% (wt/vol) kaolin (70 μL) and 2% (wt/vol) lambda-carrageenan (70 μL) into the knee (1). Kaolin was injected first and then the joint was slowly moved for 15 min. Thereafter carrageenan was injected into the knee, and the joint was moved for another 15 min. For induction of chronic antigen-induced arthritis (AIA), rats were immunized by two s.c. injections of 500 μg antigen [methylated bovine serum albumin (mBSA)] in saline emulsified with 500 μL Freund's complete adjuvant (supplemented with *Mycobacterium tuberculosis* strain H37RA) at an interval of 7 d. After another 14 d, monoarticular AIA was induced by injection of mBSA into the left knee joint cavity (2).

Inflammation was documented by measurement of the swelling of the knee joint using a vernier caliper (Mitutoyo). In antigen-induced arthritis also histology of the knee joints was assessed. Sections (5 μm) of fixed, decalcified knee joints were stained with hematoxylin-eosin and scored taking into account the number and density of granulocytes and mononuclear leukocytes in synovial membrane and joint space, the degree of synovial hyperplasia, and cartilage and bone destruction (2).

Intrathecal Application of ONO-AE-248 in Behavioral Experiments. In behavioral experiments in Lewis rats the effect of the EP3 receptor agonist ONO-AE-248 on joint nociception was assessed. During isoflurane anesthesia (3–4%) intrathecal catheters for application of the EP3 agonist were implanted 8–12 d before induction of acute inflammation. Mechanical pain thresholds at the knee joints were assessed using a dynamometer (Correx). To measure the threshold at the knee, the rats were gently placed in a piece of cloth leaving the hindlimbs outside the cloth. In this position (which rats like due to their instinct to go toward the dark) the rats are completely quiet. While holding (not restraining) the animal in this position in one hand, the dynamometer was placed to the knee (lateral side between femur and tibia) with the other hand, and pressure was increased at the knee until rats withdrew their legs or vocalized. The weight force to elicit this response was read out in grams. To prevent tissue damage, a cutoff value of 250 g was defined. Static motor behavior was assessed using an incapacitance tester (Linton Instrumentation). Animals were placed in a plastic cage with both hindpaws resting on scales. After accommodation to the device when the animal was sitting calmly, the weight force resting on the two scales was obtained and averaged during 3 s. From these values, the relative weight (in percent) resting on the inflamed hindlimb was calculated (weight on inflamed hindlimb × 100%/weight on the inflamed + the noninflamed hindlimb).

Mechanical threshold and weight distribution were measured 2 d before induction of kaolin/carrageenan inflammation. To induce inflammation, kaolin and carrageenan were injected into the knee joint during brief isoflurane anesthesia (2.5%). After 6 h, mechanical threshold and weight distribution was assessed at hourly intervals. In the first approach rats received repeated intrathecal applications of either saline or one of the ONO-AE-248 concentrations (1 ng/μL, 10 ng/μL, or 100 ng/μL; each injection had a volume of 10 μL) at 6.5, 7.5, 8.5, and 9.5 h after induction of arthritis. One group without inflammation received 100 ng/μL ONO-AE-248. In the second approach, rats received just one intrathecal application of 100 ng/μL ONO-AE-248 or saline at 6.5 h after induction of arthritis.

Application of ONO-AE-248 in Electrophysiological Experiments. Adult male rats were anesthetized with 100 mg/kg sodium thiopentone i.p. and supplemental doses (20 mg/kg i.p.) as necessary to maintain areflexia. Through the cannulated trachea the animals breathed spontaneously during surgery and recording protocol. Mean arterial blood pressure was continuously measured to control depth of anesthesia. Body temperature was kept at 37 °C using a temperature constanter.

For extracellular recordings from afferent C fibers of the knee joint, the knee joint and the saphenous nerve, which contains joint afferents, were exposed. A pool was formed with skin flaps and filled with oil. The femur was fixed, allowing rotation of the lower leg, fixed in a shoe-like holder, against the femur at innocuous and noxious intensity (20 and 40 mNm, 15 s each). The saphenous nerve was split into small bundles using watchmaker forceps, and nerve fibers from the knee joint were identified by mechanical stimulation of the joint using a glass rod and electrical stimulation of the receptive field (stimulus parameters 1–10 V, 0.5-ms pulse width, C fibers conducting at ≤1.25 m/s were selected). Responses of fibers to rotation were repeatedly tested before and after injection of the EP3 agonist or vehicle into the joint. Knee joints were normal or acutely inflamed with kaolin and carrageenan 7–11 h before recordings.

For recordings from spinal cord neurons with knee joint input, a laminectomy exposed the lumbar segments L1–L4. A trough with about a 30-μL capacity, sealed with silicone gel onto the spinal surface over the recording region, allowed the spinal application of the EP3 agonist. Using glass-insulated carbon filaments, we recorded extracellularly from individual dorsal horn neurons, which were identified by spike shape and height. Neurons were selected that encoded innocuous and noxious pressure applied to the knee joint by their discharge rates but did not

respond to brushing or squeezing of the skin over the knee. For testing, pressure (each 15 s) was applied in the mediolateral axis to the knee using a mechanical device at innocuous intensity (1.9 N/40 mm^2) and noxious intensity (7.8 or 5.9 N/40 mm^2). ONO-AE-248 was spinally applied at increasing concentrations (1, 10, 100 ng/μL; each for 50 min). The knee joint was normal or acutely or chronically inflamed.

After monitoring baseline responses, the EP3 agonist was applied and mechanical stimulation was repeated at regular intervals (*Results*). In each experiment the number of impulses of baseline responses was averaged and subtracted from the number of impulses of each response. Changes of responses within groups were tested for significance with the Wilcoxon matched pairs signed rank test, differences between groups with the Mann–Whitney *U* test. Significance was accepted at $P < 0.05$.

Culturing of DRG Neurons. DRG were dissected from all spinal segments of male rats killed with ether. DRG neurons were isolated with 215 units/mg collagenase type II (Gibco BRL) dissolved in Hamś F-12 medium (Sigma) for 100 min, followed by DMEM (Sigma), containing 10,000 units/mL trypsin (Sigma) for 11 min. After dissociation by gentle agitation and by triturating through a fire-polished Pasteur pipette, dispersed cells were collected by centrifugation (500 × *g*, 5 min), and then the neuron pellets were suspended in Hamś F-12 medium containing 10^{-3} M L-glutamine (Sigma), 10% (vol/vol) heat inactivated horse serum, 100 units/mL penicillin, 100 μg/mL streptomycin (all Gibco BRL), and 10 ng/mL nerve growth factor (NGF 7S; Boehringer). After a further centrifugation, cells were plated on glass coverslips precoated with poly-L-lysine (50 μg/mL; Sigma), and kept at 37 °C in a humidified incubator gassed with 3.5% or 5% CO_2 in air. Cultures were daily fed with Hamś F-12 culture medium.

Whole-Cell Patch Clamp Recordings from Cultured DRG Neurons. In single neurons (12–48 h in culture) tetrodotoxin (TTX)-resistant Na^+ currents were measured in the whole-cell configuration (Axopatch 200B amplifier; Axon Instruments). The cells were continuously superfused with standard solution (in millimoles): 35 NaCl, 72.5 choline-Cl, 5 KCl, 30 TEA-Cl, 2 $CaCl_2$, 2 $MgCl_2$, 0.1 $CdCl_2$, 10 glucose, 10 Hepes, pH 7.4. We added 500 nM TTX. Recording electrodes (1.0–3.0 MΩ) contained (in millimoles): 140 CsCl, 10 NaCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 2 Na_2-ATP, 5 EGTA, 10 Hepes, pH 7.2. Stimuli were controlled with pCLAMP 7.01 software (Axon Instruments) using 80–90% series resistance compensation. Na^+ currents were elicited by 40-ms pulses in increments of 5 mV to potentials between −40 mV and +40 mV from a holding potential of −70 mV (interpulse interval 2.0 s). The voltage protocol was applied before and after application of the test compound(s) every minute for about 10 min after compound application. Testing compounds were: PGE_2, dissolved in 0.07% ethanol, final bath concentrations 0.5, 1.0, and 2.5 μM; the EP2 receptor agonist ONO-AE1-259–01, dissolved in 0.9% NaCl, final concentration 1.0 μM; the EP3 receptor agonist ONO-AE-248, dissolved in 0.5% DMSO and 0.9% NaCl, final concentration 2.0 μM; the EP4 receptor agonist ONO-AE1-329, dissolved in 5% (vol/vol) DMSO and 0.9% NaCl, final concentration 1.0 μM; and the EP3 receptor antagonist ONO-AE3-240, dissolved in 5% (vol/vol) DMSO and 0.9% NaCl, final concentration 0.004 μM. In some experiments, cells were preincubated with pertussis toxin (100 ng/mL) for 30 min to 2 h.

Current signals were filtered at 1 kHz (4-pole Bessel) and sampled at 5 kHz with the interface (Digidata 1200; Axon Instruments). For presentation, data were filtered with a Gaussian filter at 500 or 250 Hz. The data were analyzed using the pCLAMP 7.01 software (Axon Instruments) and Origin 6.1 or 7.5 (MicroCal) software programs. Current densities were calculated by dividing the peak current (I_{peak}) evoked at each membrane potential (V_m) by the cell capacitance (C_m). The peak conductance (G) of Na^+ currents at each potential was calculated from the corresponding peak current by using the equation $G = I/(E - E_{Rev})$ (E_{Rev}: reversal potential of Na^+ current; I: peak current amplitude of Na^+ current; E: membrane potential). Normalized peak conductance (G/G_{max}) was fitted with a Boltzmann function $G/G_{max} = [1 + \exp((V_{1/2} - V_m)/k)]^{-1}$, where $V_{1/2}$ is the membrane potential generating half maximal current or conductance, V_m is the prepulse membrane potential, and k is the slope of the function. Time constants for activation (τ_{act}) and inactivation (τ_{inact}) were obtained by fitting the activating and inactivating phase of TTX-R Na^+ currents with a single exponential function and found to be in the typical range. All data are expressed as means ± SEM unless otherwise stated. For the display of I/V curves the average peak currents at each voltage test were used. For statistical comparison of current densities before and after treatment the maximal negative peak currents were taken from each neuron irrespective of shifts of maximum currents with respect to voltage, and the paired *t* test was used (after testing for normality). Groups of neurons were compared using Fisher's exact test. Significance was accepted at $P < 0.05$. Individual neurons were considered PGE_2- or EP-agonist responsive when the maximal peak Na^+ currents were changed by at least 10% after PGE_2 or EP receptor agonist application. The size of the neurons was calculated from capacitance that was read out using the specific membrane capacitance (1 $\mu F/cm^2$).

1. Neugebauer V, Lücke T, Schaible H-G (1993) N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists block the hyperexcitability of dorsal horn neurons during development of acute arthritis in rat's knee joint. *J Neurophysiol* 70(4):1365–1377.

2. Boettger MK, et al. (2008) Antinociceptive effects of TNF-α neutralization in a rat model of antigen-induced arthritis. *Arthritis Rheum* 58:2368–2378.

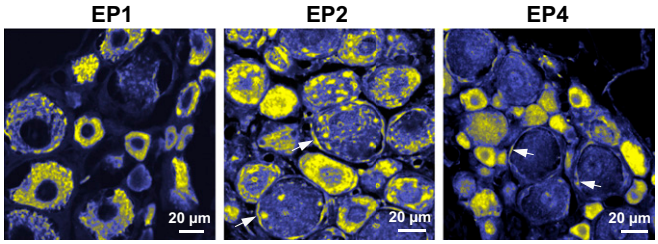

Fig. S1. Localization of EP1, EP2, and EP4 receptor-like IR (yellow) in neurons in DRG sections. Arrows indicate satellite cells. EP1 receptor-like (IR) in 87 ± 7%, EP2 receptor-like IR in 87 ± 12%, and EP4 receptor-like IR in 53 ± 12% of the neurons.

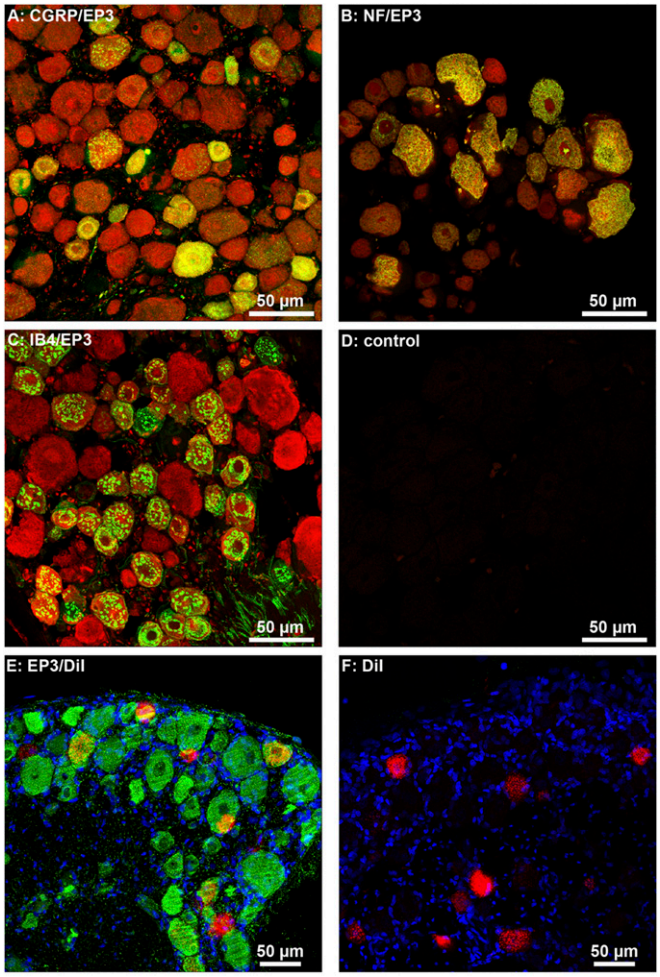

Fig. S2. Representative lumbar DRG sections showing the colocalization of EP3 receptor-like IR with neuronal markers in rat DRG neurons. (*A*) Colocalization with CGRP. Red shows EP3 receptor-like IR, yellow shows double-labeled neurons (CGRP-positive neurons without EP3 receptor-like IR would appear green). (*B*) Colocalization with neurofilament (NF). Red shows EP3 receptor-like IR, yellow shows double-labeled neurons. (*C*) Colocalization with IB4. Red shows EP3 receptor-like IR, green shows IB4-positive neurons and yellow, double-labeled neurons. (*D*) Control: in this section no primary antibody was used but both secondary antibodies (conjugated with either Alexa Fluor 488 or Alexa Fluor 568) were administered: no unspecific staining. (*E*) Colocalization with the tracer FAST DiI (injected into the knee joint). Green shows EP3 receptor-like IR; red shows FAST DiI-labeled neurons; yellow, double-labeled neurons; blue, Hoechst 34580 labeling for all cell nuclei. (*F*) Control section without primary antibody against EP3 receptor. Red shows FAST DiI-labeled neurons; blue, Hoechst 34580 labeling for all cell nuclei.

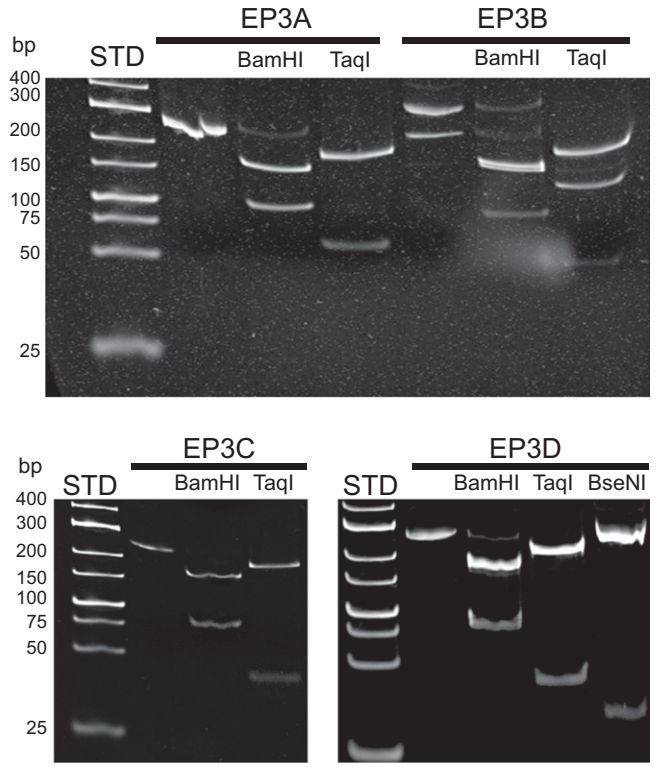

Fig. S3. Gel electrophoresis of PCR products obtained using EP3A, -B, -C, and -D primer combinations (*SI Materials and Methods*) and their restriction fragments after treatment with BamHI, TaqI, or BseNI. The BseNI restriction products of EP3D PCR product show the correct amplification of EP3D. STD, standard.

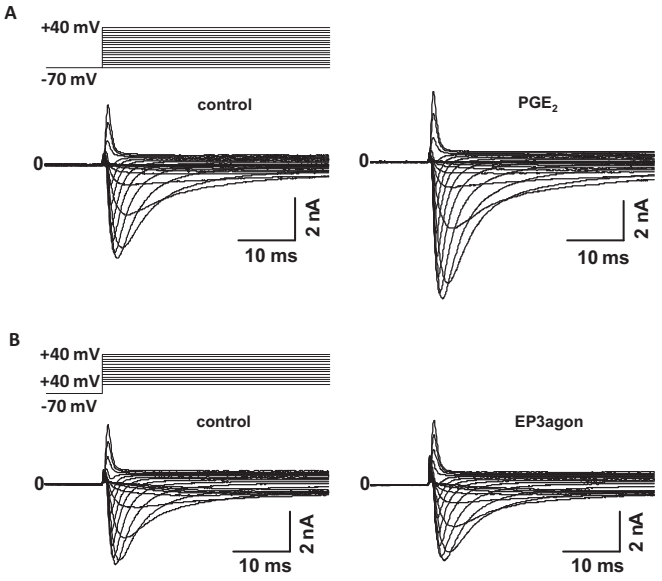

Fig. S4. Different effects of PGE_2 and the EP3 agonist ONO-AE-248 on TTX-R Na^+ currents. (*A*) Voltage clamp protocol before and 3 min after administration of 2.5 μM PGE_2. (*B*) Voltage clamp protocol before and 3 min after 2.0 μM ONO-AE-248. From the holding potential of −70 mV, the neurons were depolarized to potentials between −40 mV and +40 mV.

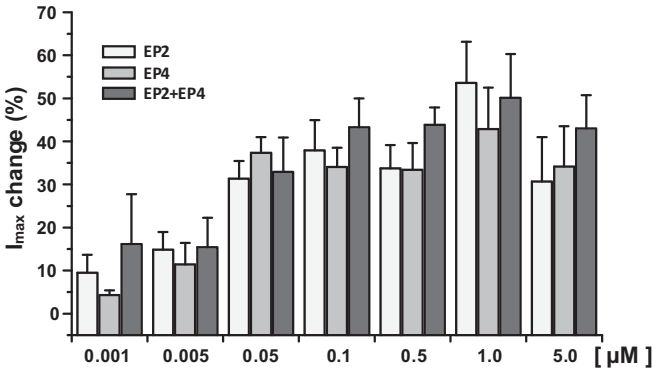

Fig. S5. Maximal increases of TTX-R Na^+ currents after bath application of either the EP2 agonist ONO-AE1-259–01 or the EP4 agonist ONO-AE1-329 or of both agonists at different concentrations.

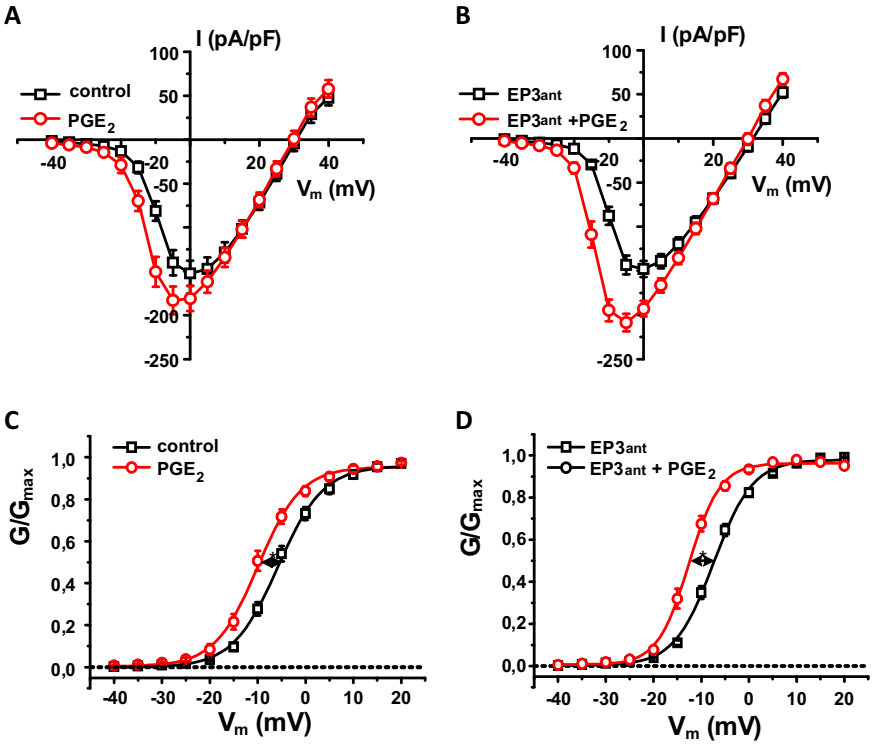

Fig. S6. Effect of PGE_2 on TTX-R Na^+ currents and change by the coadministration of the EP3 antagonist ONO-AE3-240. (*A*) Current/Voltage (I/V) curve before and 3–5 min after 0.5 µM PGE_2. (*B*) I/V curve before and 3–5 min after 0.5 µM PGE_2, in the presence of the EP3 antagonist (4 nM). (*C*) Conductance (G/G_{max}) before and 3–5 min after 0.5 µM PGE_2. (*D*) Conductance before and 3–5 min after PGE_2, in the presence of the EP3 antagonist (4 nM). *Significant difference ($P < 0.05$), paired t-test, followed by Fisher's exact test.

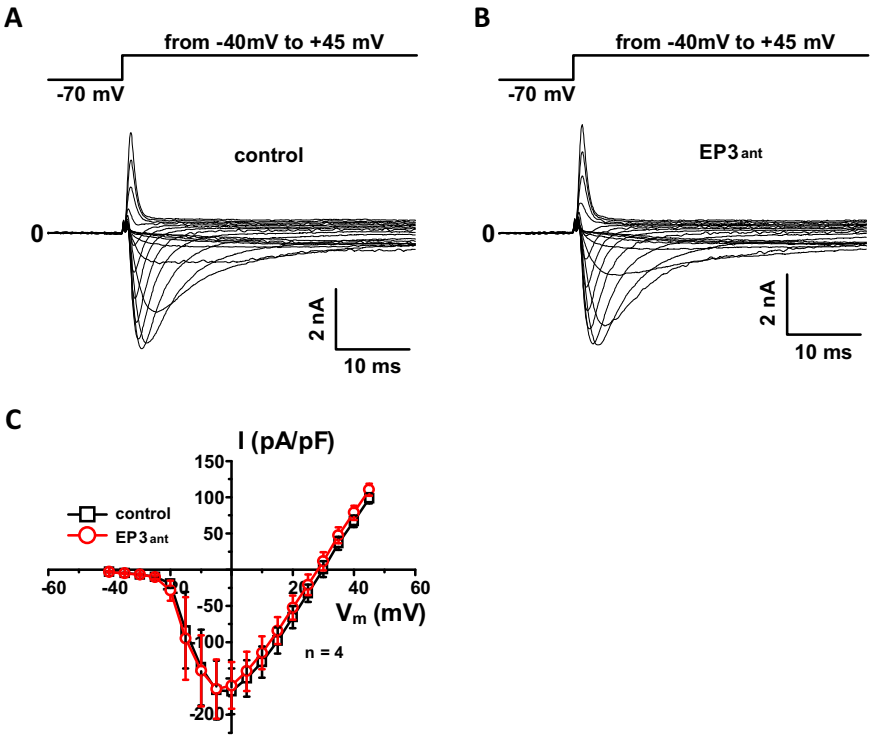

Fig. S7. No change of TTX-R Na^+ currents of cultured DRG neurons by administration of the EP3 receptor antagonist ONO-AE3-240. (*A*) Voltage-gated Na^+ currents (using voltages from −40 mV to +45 mV and in 5-mV increments) of a neuron before and (*B*) 5 min after 4 nM ONO-AE3-240 application to the bath. (*C*) Averaged I/V curves of Na^+ currents before (control) and about 5–7 min after 4 nM ONO-AE3-240 ($n = 4$ neurons).

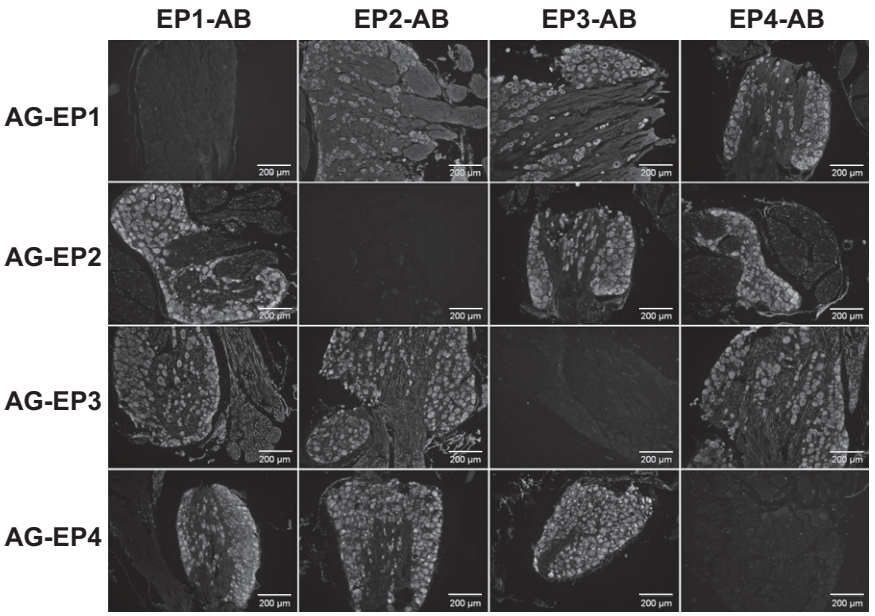

Fig. S8. Preabsorption experiments showing the specificity of antibodies to EP receptors in lumbar DRG sections, and localization of EP1, EP2, EP3, and EP4 receptor-like IR in rat DRG sections. The antibody for the EP1 receptor (EP1-AB, first column) did not label sections after preabsorption with the blocking peptide of the EP1 receptor (AG-EP1), but labeled neurons after preabsorption with the blocking peptides of the EP2 (AG-EP2), EP3 (AG-EP3), and EP4 (AG-EP4) receptors. Columns 2–4 show the testing of antibodies against the EP2 (EP2-AB), EP3 (EP3-AB), and EP4 (EP4-AB) receptors. Labeling of sections was only prevented by preabsorption with the specific blocking peptide.