Supplementary Materials for

SARS-CoV-2 Spike protein co-opts VEGF-A/Neuropilin-1 receptor signaling to induce analgesia

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Materials and Methods

Animals: Pathogen-free, adult male (250g) or female (225g) Sprague–Dawley rats (Envigo) were housed in temperature (23 ± 3 °C) and light (12-h light/12-h dark cycle; lights on 07:00–19:00) controlled rooms with standard rodent chow and water available ad libitum. The Institutional Animal Care and Use Committee of the College of Medicine at the University of Arizona approved all experiments. All procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain. Animals were randomly assigned to treatment or control groups for the behavioral studies. Animals were initially housed three per cage but individually housed after the intrathecal cannulation on a 12 h light-dark cycle with food and water ad libitum. All behavioral experiments were performed by experimenters who were blinded to the experimental groups and treatments.

Preparation of cultured dorsal root ganglia neurons: Female Sprague–Dawley rats (100 g; Envigo) were deeply anaesthetized with isoflurane overdose (5% in air) and sacrificed by rapid decapitation. Following laminectomy, dorsal root ganglia (DRG) were quickly removed, trimmed at their roots, and enzymatically digested in 3 mL bicarbonate-free, serum-free, sterile DMEM (Cat# 11965, Thermo Fisher Scientific, Waltham, MA) solution containing neutral protease (3.125 mg/mL, Cat#LS02104; Worthington, Lakewood, NJ) and collagenase type I (5 mg/mL, Cat# LS004194, Worthington, Lakewood, NJ). Subsequently, the isolated DRGs were incubated with the enzyme cocktail for 60 minutes at 37˚C under gentle agitation. The digested DRGs were then mechanically separated by gently passing them through the tip of a 1 mL pipette until a single cell suspension was obtained. The fully dissociated DRG neurons were then gently centrifuged to collect the cells (~1.5 x 10^6) as a pellet and the supernatant was discarded. The cells were resuspended and washed with DRG media (DMEM containing 1% penicillin/streptomycin sulfate from 10,000 μg/mL stock, and 10% fetal bovine serum (Hyclone)) before plating onto poly-D-lysine and laminin-coated 12-mm glass coverslips. All whole-cell electrophysiology experiments were performed within 48 h of plating DRG neurons since electrophysiological profiles change during this period.

Whole-cell electrophysiological recordings of sodium and calcium currents in cultured rat DRG neurons: All recordings were obtained from acutely dissociated DRG neurons from Sprague Dawley rats, using procedures adapted from our prior work [64; 67; 69]. For sodium current recordings the internal pipette solution consisted of (in mM): 140 CsF, 10 NaCl, 1.1Cs-EGTA, and 15 HEPES (pH 7.3, mOsm/L = 290-310) and external solution contained (in mM): 140 NaCl, 30 tetraethylammonium chloride, 10 D-glucose, 3 KCl, 1 $CaCl_2$, 0.5 $CdCl_2$, 1 $MgCl_2$, and 10 HEPES (pH 7.3, mOsm/L = 310-315). DRG neurons were interrogated with current-voltage (I-V) and activation/inactivation voltage protocols as described previously [63; 65]. The voltage protocols were as follows: (a) I-V protocol: from a holding potential of −60 mV, cells were depolarized with 150-millisecond voltage steps over a range of −70 to +60 mV in +5-mV increments. This permitted acquisition of current density values such that the activation of sodium channels, occurring between ~0 to 10 mV, could be analyzed as a function of voltage, from which peak current density was inferred (normalized to cell capacitance (in picofarads, pF)); (b) inactivation protocol: from a holding potential of −60 mV, cells were subjected to hyperpolarizing/repolarizing pulses for 1 second over a range of −120 to 0 mV in +10 mV steps. This incremental increase in membrane potential conditioned various proportions of sodium

channels into a state of fast-inactivation – in this case the 0-mV test pulse for 200 milliseconds revealed fast inactivation when normalized to maximum sodium current [63].

Recordings of N-type (CaV2.2) voltage-gated calcium currents were obtained using recording solutions and protocols described earlier [71]. The intracellular pipette solution was composed of (in mM): 150 $CsCl_2$, 10 HEPES, 5 Mg-ATP, and 5 BAPTA (pH 7.3, mOsm/L=290-310) and the external solution contained (in mM): 110 NMDG, 10 $BaCl_2$, 30 TEA-Cl, 10 HEPES, 10 glucose and 1 μM TTX (pH 7.3, mOsm/L = ~ 310). To isolate N-type specific calcium currents, the following blockers were used: SNX482 (200 nM, R-type Ca^{2+} channel blocker), TTA-P2 (1 μM, T-type Ca^{2+} channel blocker), ω-agatoxin (200 nM, P/Q-type Ca^{2+} channel blocker), and nifedipine (10 μM, L-type Ca^{2+} channel blocker). Activation of I_{Ca} was measured from a holding voltage of -60 mV for 5 ms followed by 200-ms depolarizing voltage steps from -70 mV to +60 mV in 10-mV increments. Whole-cell currents were normalized to cellular capacitance for analysis of channel activation profiles as a function of voltage in addition to peak current density. Steady-state inactivation of I_{Ca} was determined by applying a 1500 ms conditioning prepulse (−100 to +30 mV in +10 mV increments) after which, the voltage was stepped to +10 mV for 200-ms. There were 15-s intervals separating each acquisition to allow channels to revert to their basal state.

Pipettes with 1 to 3 MΩ resistance were used for all recordings and pulled from borosilicate capillaries on a Flaming/Brown P-97 puller (Sutter Instruments, California).

Hind paw injection procedures: PBS vehicle (NaCl 137 mM, KCl 2.5 mM, Na_2HPO_4 10 mM and KH_2PO_4 1.8 mM), $VEGF\text{-}A_{165}$ (10 nM), Spike (1 μM) and EG00229 [68] (30 μM, Cat. No. 6986, Tocris Bioscience) were injected subcutaneously, alone or in combination, in the dorsum of the left hind paw. Rats were gently restrained under a fabric cloth, and 50 μL were injected using 0.5 mL syringes (27-G needles).

Preparation of Spinal Cord slices: Pathogen-free, male Sprague-Dawley rat pups (10-15 days old; Envigo) were deeply anesthetized with isoflurane (4% for induction and 2% for maintaining). For spinal nerve block, 0.3 mL of 2% lidocaine was injected to both sides of L4 to 5 lumbar vertebrae. Laminectomy was performed from mid-thoracic to low lumbar levels, and the spinal cord was quickly removed to cold modified ACSF oxygenated with 95% O2 and 5% CO2. The ACSF for dissection contained the following (in millimolar): 80 NaCl, 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2.2H_2O, 3.5 MgCl2.6H_2O, 25 NaHCO3, 75 sucrose, 1.3 ascorbate, 3.0 sodium pyruvate, with pH at 7.4 and osmolarity at 310 mOsm. Transverse 380-mm thick slices were obtained by a vibratome (VT1200S; Leica, Nussloch, Germany). Slices were then incubated for at least 40 min at 37°C and then for 1h at RT in an oxygenated recording solution containing the following (in millimolar): 125 NaCl, 2.5 KCl, 2 CaCl2.2H_2O, 1 MgCl2.6H_2O, 1.25 NaH2PO4, 26 NaHCO3, 25 D-glucose, 1.3 ascorbate, 3.0 sodium pyruvate, with pH at 7.4 and osmolarity at 320 mOsm. The slices were then positioned in a recording chamber and continuously perfused with oxygenated recording solution at a rate of 3 to 4 mL/min before electrophysiological recordings at RT.

Electrophysiological recordings in spinal cord slices by whole-cell patch clamp: *Substantia gelatinosa* neurons (lamina I/II) were visualized and identified in the slices by means of infrared differential interference contrast video microscopy on an upright microscope (FN1; Nikon, Tokyo, Japan) equipped with a 3.40/0.80 water-immersion objective and a charge-coupled device camera. Patch pipettes with resistance at 6 to 10MΩ were made from borosilicate glass

(Sutter Instruments, Novato, CA) on a four-step micropipette puller (P-90; Sutter Instruments, Novato, CA). The pipette solution contained the following (in millimolar): 120 potassium gluconate, 20 KCl, 2 MgCl2, 2Na2-ATP, 0.5 Na-GTP, 20 HEPES, 0.5 EGTA, with pH at 7.28 and osmolarity at 310 mOsm. The membrane potential was held at − 60 mV using a PATCHMASTER software in combination with a patch clamp amplifier (EPC10; HEKA Elektronik, Lambrecht, Germany). The whole-cell configuration was obtained in voltage-clamp mode. To record spontaneous excitatory postsynaptic currents (sEPSCs), bicuculline methiodide (10 μM) and strychnine (2 μM) were added to the recording solution to block γ-aminobutyric acid-activated (GABA) and glycine-activated currents. VEGFA (1nM), NRP-1 inhibitor (EG00229, 30 μM) and Spike protein were added directly to the recording solution as indicated.

Hyperpolarizing step pulses (5 mV in intensity, 50 milliseconds in duration) were periodically delivered to monitor the access resistance (15–25 MΩ), and recordings were discontinued if the access resistance changed by more than 20%. For each neuron, sEPSCs were recorded for a total duration of 2 min. Currents were filtered at 3 kHz and digitized at 5 kHz. Data were further analyzed by the Mini-Analysis Program (Synatosoft Inc., NJ) to provide spreadsheets for the generation of cumulative probability plots. The amplitude and frequency of sEPSCs were compared between neurons from animals in control and the indicated groups.

Synapse enrichment and fractionation: Adult rats were killed by isofluorane overdose and decapitation, the spinal cords dissected, the lumbar region isolated and separated into contralateral and ipsilateral sides. Only the dorsal horn of the spinal cord was used as this structure contains the synapses arising from the DRG. Synaptosomes isolation was done according to [70]. Fresh tissues were homogenized in ice-cold Sucrose 0.32M, HEPES 10 mM, pH 7.4 buffer. The homogenates were centrifuged at 1000xg for 10 min at 4°C to pellet the insoluble material. The supernatant was harvested and centrifuged at 12000xg for 20 min at 4°C to pellet a crude membrane fraction. The pellet was then re-suspended in a hypotonic buffer (4 mM HEPES, 1 mM EDTA, pH 7.4) and the resulting synaptosomes pelleted by centrifugation at 12000xg for 20 min at 4°C. The synaptosomes were then incubated in 20 mM HEPES, 100 mM NaCl, 0.5% triton X, pH= 7.2) for 15 min on ice and centrifuged at 12000xg for 20 min at 4°C. The supernatant was considered as the non-postsynaptic density (non-PSD) membrane fraction, sometimes referred to as the triton soluble fraction. All buffers were supplemented with protease (Cat#B14002) and phosphatase (Cat#B15002) inhibitor cocktails (Bimake).

Implantation of intrathecal catheter: For intrathecal drug administration, rats were chronically implanted with catheters as described (Yaksh and Rudy, 1976). Rats were anesthetized (ketamine/xylazine anesthesia, 80/12 mg/kg i.p) and placed in a stereotaxic head holder, the occipital muscles were separated from their occipital insertion and retracted caudally to expose the cisternal membrane at the base of the skull, the cisterna magna was exposed and incised, an 8-cm catheter (PE10 polyethylene tubing) was passed caudally from the cisterna magna to the level of the lumbar enlargement. Catheters were sutured (3-0 silk suture) into the deep muscle and externalized at the back of the neck; skin was closed with auto clips. Animals were allowed to recover and were examined for evidence of neurologic injury. Animals with evidence of neuromuscular deficits were excluded.

Spared nerve injury (SNI): After a recovery period of 7 days after implantation of intrathecal catheter, the spared nerve injury was induced. Under isoflurane anesthesia (5% induction, 2.0% maintenance in 2 L/min air), skin on the lateral surface of the left hind thigh was incised. The

biceps femoris muscle was bluntly dissected to expose the three terminal branches of the sciatic nerve [62]. Briefly, the common peroneal and tibial branches were tightly ligated with 5-0 silk, 2–3 mm of the nerves was removed below the ligations, with special care taken to avoid any damage to the sural nerve. Closure of the incision was made in two layers. The muscle was sutured once with 3-0 silk suture; skin was auto-clipped. Animals were allowed to recover for 12-14 days before the drug testing.

Tactile sensory thresholds: The assessment of tactile allodynia (i.e., a decreased threshold for paw withdrawal after probing with normally innocuous mechanical stimuli) consisted of testing the withdrawal threshold of the paw in response to probing with a series of calibrated fine (von Frey) filaments. Each filament was applied perpendicularly to the plantar surface of the paw of rats held in suspended wire mesh cages. We determined the withdrawal threshold by sequentially increasing and decreasing the stimulus strength (the 'up and down' method), and we analyzed data using the nonparametric method of Dixon (as described by Chaplan et al.) with results expressed as the mean withdrawal threshold

Thermal sensory thresholds: Paw withdrawal latencies were determined as described by Hargreaves et al.[66] was used. Rats were acclimated within Plexiglas enclosures on a clear glass plate for 30 minutes. A radiant heat source (high-intensity projector lamp) was focused onto the plantar surface of the hind paw. A motion detector halted the stimulus and a timer when the paw was withdrawn. To prevent tissue damage, a maximal cutoff of 33.5 sec was used.

Statistical analyses: All data was first tested for a Gaussian distribution using a D'Agostino-Pearson test (Prism 8 Software, Graphpad, San Diego, CA). The statistical significance of differences between means was determined by a parametric ANOVA followed by Tukey's post hoc or a non-parametric Kruskal Wallis test followed by Dunn's post-hoc test depending on if datasets achieved normality. Behavioral data with a time course were analyzed by Two-way ANOVA with Sidak's post hoc test. Differences were considered significant if $p \leq 0.05$. Error bars in the graphs represent mean ± SEM. Full statistical analyses are described in Table 1. All data were plotted in Prism 8.

Table S1. Statistical analyses of experiments.

Figure panel	Assay	Statistical test; findings	Post-hoc analysis (adjusted p-values)	Number of subjects	Number of subjects excluded (ROUT test)
Figure 1A	multiwell microelectrode arrays (MEAs) on dorsal root ganglion sensory neurons – mean firing rate (Hz)	One-way ANOVA p <0.0001	Holm- Sidak's multiple comparisons test PBS vs. Sema 3A p = 0.5439; PBS vs. VEGF-A165 p<0.0001; PBS vs. VEGF-B p = 0.5439; VEGF-A165 vs. Spike protein 100nM p<0.0001; VEGF-A165 vs. EG p<0.0001	PBS n = 56; Sema 3A n = 42; VEGF-B n = 37; VEGF-A n = 42; VEGF-A + Spike protein n = 95; VEGF-A + EG00229 n = 90	None
Figure 2A	Naïve male rats – paw withdrawal threshold	Two-way ANOVA p <0.0001	Sidak's multiple comparisons test Time after injection: 0.5 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.9581 PBS vs. VEGF-A165 + Spike p>0.9999 1 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p=0.997 PBS vs. EG00229 p=0.9997 PBS vs. EG00229 + VEGF-A165 p=0.0273 PBS vs. VEGF-A165 + Spike p=0.9891 2 h PBS vs. VEGF-A165 p=<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGF-A165 + Spike p>0.9999 3 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGF-A165 + Spike p=0.0586 4 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	None

			PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0002 PBS vs. VEGF-A165 + Spike p=0.0066 5 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGF-A165 + Spike p=0.0001 6 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p <0.0001 PBS vs. VEGF-A165 + Spike p= 0.0009 7 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p= 0.0029 PBS vs. VEGF-A165 + Spike p <0.0001 9 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p= 0.0018 PBS vs. VEGF-A165 + Spike p= 0.0003		
Figure 2B	Naïve male rats – paw withdrawal threshold: Area over the curve	One-way ANOVA p <0.0001	Sidak's multiple comparisons test PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p=0.9997 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGFA165 p<0.0001 PBS vs. VEGFA165 + Spike p=0.0111 VEGF-A165 vs. EG00229 + VEGFA165 p=0.0004 VEGF-A165 vs. VEGFA165 + Spike p<0.0001	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	None
Figure 2C	Naïve male rats – paw withdrawal latency	Two-way ANOVA p <0.0001	Sidak's multiple comparisons test Time after injection: 0.5 h	PBS n = 12; VEGF-A n = 12;	None

			PBS vs. VEGF-A165 p=0.8005 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.9999 PBS vs. VEGFA165 + Spike p=0.9996 1 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p= 0.1568 PBS vs. VEGFA165 + Spike p>0.9999 2 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p= 0.0017 PBS vs. VEGFA165 + Spike p= 0.04 3 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p=0.9993 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0001 PBS vs. VEGFA165 + Spike p=0.0087 4 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGFA165 + Spike p=0.3511 5 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0002 PBS vs. VEGFA165 + Spike p=0.1723 6 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999	Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	

			PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0052 PBS vs. VEGFA165 + Spike p=0.4168 7 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p= .9993 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGFA165 + Spike p=0.0774 9 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0012 PBS vs. VEGFA165 + Spike p=0.0202		
Figure 2D	Naïve male rats – paw withdrawal latency: Area over the curve	One-way ANOVA p <0.0001	Sidak's multiple comparisons test PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p=0.9776 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGFA165 p<0.0001 PBS vs. VEGFA165 + Spike p=0.0039 VEGF-A165 vs. EG00229 + VEGFA165 p=0.4728 VEGF-A165 vs. VEGFA165 + Spike p<0.0001	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	
Figure 2E	Naïve female rats – paw withdrawal threshold	Two-way ANOVA p <0.0001	Sidak's multiple comparisons test Time after injection: 0.5 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.9902 PBS vs. VEGF-A165 + Spike p>0.9999 1 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.9709 PBS vs. VEGF-A165 + Spike p=0.1298 2 h	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	None

			PBS vs. VEGF-A165 p=<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0038 PBS vs. VEGF-A165 + Spike p=0.2143 3 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.1133 PBS vs. VEGF-A165 + Spike p>0.9999 4 h PBS vs. VEGF-A165 p=0.0004 PBS vs. Spike p>0.9999 PBS vs. EG00229 p=0.9993 PBS vs. EG00229 + VEGF-A165 p=0.0012 PBS vs. VEGF-A165 + Spike p=0.6620 5 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGF-A165 + Spike p=0.5423 6 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p<0.0001 PBS vs. VEGF-A165 + Spike p=0.1275 7 h PBS vs. VEGF-A165 p=0.0113 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0039 PBS vs. VEGF-A165 + Spike p>0.9999 9 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999		

			PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0311 PBS vs. VEGF-A165 + Spike p=0.1095		
Figure 2F	Naïve female rats – paw withdrawal threshold: Area over the curve	One-way ANOVA p <0.0001	Sidak's multiple comparisons test PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p=0.9516 PBS vs. EG00229 p=0.8341 PBS vs. EG00229 + VEGFA165 p=0.0066 PBS vs. VEGFA165 + Spike p=0.0810 VEGF-A165 vs. EG00229 + VEGFA165 p=0.2626 VEGF-A165 vs. VEGFA165 + Spike p=0.0320	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	None
Figure 2G	Naïve female rats – paw withdrawal latency	Two-way ANOVA p <0.0001	Sidak's multiple comparisons test Time after injection: 0.5 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p=0.9984 PBS vs. EG00229 + VEGF-A165 p=0.8339 PBS vs. VEGFA165 + Spike p=0.9984 1 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p>0.9999 PBS vs. VEGFA165 + Spike p=0.3044 2 h PBS vs. VEGF-A165 p<0.0001 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.1905 PBS vs. VEGFA165 + Spike p=0.1617 3 h PBS vs. VEGF-A165 p=0.0848 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.3859 PBS vs. VEGFA165 + Spike p=0.1404 4 h	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	None

			PBS vs. VEGF-A165 p=0.3047 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0471 PBS vs. VEGFA165 + Spike p=0.9958 5 h PBS vs. VEGF-A165 p=0.1732 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0349 PBS vs. VEGFA165 + Spike p=0.9126 6 h PBS vs. VEGF-A165 p=0.0111 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0412 PBS vs. VEGFA165 + Spike p=0.0943 7 h PBS vs. VEGF-A165 p=0.0088 PBS vs. Spike p=0.9998 PBS vs. EG00229 p=0.9996 PBS vs. EG00229 + VEGF-A165 p=0.0761 PBS vs. VEGFA165 + Spike p=0.0536 9 h PBS vs. VEGF-A165 p=0.0348 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGF-A165 p=0.0011 PBS vs. VEGFA165 + Spike p=0.3535		
Figure 2H	Naïve female rats – paw withdrawal latency: Area over the curve	One-way ANOVA p <0.0001	Sidak's multiple comparisons test PBS vs. VEGF-A165 p=0.0080 PBS vs. Spike p>0.9999 PBS vs. EG00229 p>0.9999 PBS vs. EG00229 + VEGFA165 p=0.1992 PBS vs. VEGFA165 + Spike p>0.9999 VEGF-A165 vs. EG00229 + VEGFA165 p>0.9999 VEGF-A165 vs. VEGFA165 + Spike p0.8178	PBS n = 12; VEGF-A n = 12; Spike protein n = 6; EG00229 n = 6; VEGF-A + EG00229 n = 6; VEGF-A + Spike protein n = 6;	

Figure 3C	Whole cell patch clamp electrophysiology – Peak sodium currents	One-way ANOVA p = 0.0087	Holm-Sidak's multiple comparison post hoc test: Control (0.1% PBS) vs. VEGFA 1nM p = 0.0247; Control (0.1% PBS) vs. Spike protein 100nM p = 0.6984; Control (0.1% PBS) vs. Spike protein + VEGFA p = 0.6272; VEGFA 1nM vs. Spike protein 100nM p = 0.0034; VEGFA 1nM vs. Spike protein + VEGFA p = 0.0009; Spike protein 100nM vs. Spike protein + VEGFA p = 0.7683	PBS vehicle n = 19; VEGF-A n = 20; Spike protein n = 18; VEGF-A + Spike protein n = 21	None
Figure 3H	Whole cell patch clamp electrophysiology – Peak sodium currents	One-way ANOVA p = 0.0006	Dunn's multiple comparison post hoc test: PBS vehicle vs. VEGF-A p = 0.0108l; PBS vehicle vs. VEGF-A + EG00229 p>0.9999; VEGF-A vs. VEGF-A + EG00229 P = 0.0160	PBS vehicle n = 12; VEGF-A n = 12; EG00229 n = 11; VEGF-A + EG00229 n = 16	None
Figure 4C	Whole cell patch clamp electrophysiology – Peak N type currents		Holm-Sidak's multiple comparison post hoc test: Control (0.1% PBS) vs. VEGFA 1nM p = 0.0115; Control (0.1% PBS) vs. Spike protein 100nM p = 0.9926; Control (0.1% PBS) vs. Spike protein + VEGFA p = 0.9926; VEGFA 1nM vs. Spike protein 100nM p = 0.0134; VEGFA 1nM vs. Spike protein + VEGFA p =0.0353; Spike protein 100nM vs. Spike protein + VEGFA p = 0.9926	PBS vehicle n = 20; VEGF-A n = 15; Spike protein n = 18; VEGF-A + Spike protein n = 14	None
Figure 4H	Whole cell patch clamp electrophysiology – Peak N type currents		Holm-Sidak's multiple comparison post hoc test: Control (0.1% DMSO) vs. VEGFA 1nM p = 0.0021; Control (0.1% DMSO) vs. Spike protein 100nM p = 0.9898; Control (0.1% DMSO) vs. Spike protein + VEGFA p = 0.9898; VEGFA 1nM vs. Spike protein 100nM p = 0.0041; VEGFA 1nM vs. Spike protein + VEGFA p =0.0050; Spike protein 100nM vs. Spike protein + VEGFA p = 0.9898	0.1% DMSO n = 27; VEGF-A n = 32; EG00229 n = 16; VEGF-A + EG00229 n = 18	None
Figure 5B	Slice electrophysiology –	One-way ANOVA p = 0.1044	Tukey's multiple comparison post hoc test: Control vs. VEGF-A p = 0.9781;	Control n = 16; VEGF-A n = 14;	None

	Amplitude of EPSCs		Control vs. VEGF-A + EG00229 p = 0.3770; Control vs. EG00229 p = 0.9731; Control vs. VEGF-A + Spike p = 0.2744; Control vs. Spike p = 0.8701; VEGF-A vs. VEGF-A + EG00229 p= 0.8322; VEGF-A vs. VEGF-A + Spike protein p= 0.7486; VEGF-A + EG00229 vs. VEGF-A + Spike protein p >0.9999	VEGF-A + EG00229 n = 11; EG00229 n = 13 VEGF-A + Spike n = 15 Spike n=11	
Figure 5C	Slice electrophysiology – Frequency of EPSCs	One-way ANOVA p = 0.0009	Holm-Sidak's multiple comparison post hoc test: Control vs. VEGF-A p =0.0002; Control vs. VEGF-A + EG00229 p = 0.9582; Control vs. EG00229 p = 0.9957; Control vs. VEGF-A + Spike p = 0.8877; Control vs. Spike p = 0.9966; VEGF-A vs. VEGF-A + EG00229 p = 0.0012; VEGF-A vs. VEGF-A + Spike p = 0.0135; VEGF-A + EG00229 vs. VEGF-A + Spike p = 0.9966	Control n = 16; VEGF-A n = 14; VEGF-A + EG00229 n = 11; EG00229 n = 13 VEGF-A + Spike n = 15 Spike n=11	None
Figure 6B	Pre-synaptic fractionation – western blot	Kruskal-Wallis test P=0.0008	Dunn's multiple comparisons test pVEGFR2 : contra PBS vs. Contra spike p=0.0777 ipsi PBS vs. ipsi spike p=0.0347 contra PBS vs. ipsi PBS p>0.9999 VEGFR2 : contra PBS vs. Contra spike p=0.6204 ipsi PBS vs. ipsi spike p>0.9999 contra PBS vs. ipsi PBS p>0.9999 NRP1 : contra PBS vs. Contra spike p>0.9999 ipsi PBS vs. ipsi spike p>0.9999 contra PBS vs. ipsi PBS p>0.9999		None
Figure 6C	Spared nerve injury in male rats – paw withdrawal threshold	Two-way ANOVA p <0.0001	Dunnet's multiple comparisons test 60 min PBS vs. Spike 2.14 µg in 5µl p=0.0069 PBS vs. Spike 0.214 µg in 5µl p<0.0001 PBS vs. Spike 0.0214 µg in 5µl p=0.0017 PBS vs. Spike 0.00214 µg in 5µl p>0.9999 120min	PBS n = 12 Spike 2.14 µg in 5µl n = 9 Spike 0.214 µg in 5µl n=10 Spike 0.0214 µg in 5µl n=6 Spike 0.00214 µg in 5µl n=6	None

			PBS vs. Spike 2.14 µg in 5µl p<0.0001 PBS vs. Spike 0.214 µg in 5µl p<0.0001 PBS vs. Spike 0.0214 µg in 5µl p=0.0038 PBS vs. Spike 0.00214 µg in 5µl p>0.9999 180 min PBS vs. Spike 2.14 µg in 5µl p<0.0001 PBS vs. Spike 0.214 µg in 5µl p<0.0001 PBS vs. Spike 0.0214 µg in 5µl p=0.0951 PBS vs. Spike 0.00214 µg in 5µl p=0.9999 240 min PBS vs. Spike 2.14 µg in 5µl p=0.0035 PBS vs. Spike 0.214 µg in 5µl p=0.1417 PBS vs. Spike 0.0214 µg in 5µl p=0.993 PBS vs. Spike 0.00214 µg in 5µl p=0.9987 300 min PBS vs. Spike 2.14 µg in 5µl p=0.0744 PBS vs. Spike 0.214 µg in 5µl p=0.3308 PBS vs. Spike 0.0214 µg in 5µl p=0.9917 PBS vs. Spike 0.00214 µg in 5µl p=0.9984		
Figure 6D	Spared nerve injury in male rats – paw withdrawal threshold: Area over the curve	Kruskal-Wallis test	Dunn's multiple comparisons test PBS vs. Spike 2.14 µg in 5µl p=0.0059 PBS vs. Spike 0.214 µg in 5µl p=0.0019 PBS vs. Spike 0.0214 µg in 5µl p=0.3467 PBS vs. Spike 0.00214 µg in 5µl p>0.9999	PBS n = 12 Spike 2.14 µg in 5µl n = 9 Spike 0.214 µg in 5µl n=10 Spike 0.0214 µg in 5µl n=6 Spike 0.00214 µg in 5µl n=6	None
Figure 6E	Spared nerve injury in female rats – paw withdrawal threshold	Two-way ANOVA p <0.0001	Sidak's multiple comparisons test PBS vs. Spike 2.14 µg in 5µl 60 min p<0.0001 120 min p<0.0001 180 min p <0.0001 240 min p=0.9798 300 min p>0.9999	PBS n = 8 Spike 2.14 µg in 5µl n = 7	None
Figure 6F	Spared nerve injury in female rats –	Kruskal-Wallis test	Mann Whitney test	PBS n = 8	None

	paw withdrawal threshold: Area over the curve		PBS vs. Spike 2.14 µg in 5µl: p=0.0003	Spike 2.14 µg in 5µl n = 7	
Figure 6G	Spared nerve injury – paw withdrawal threshold	Two-way ANOVA p <0.0001	Sidak's multiple comparisons test PBS vs EG00229: time after injection 60 min p=0.0007 120 min p<0.0001 180 min p=0.0009 240 min p=0.0008 300 min p=0.0235	PBS n = 6 EG00229 n = 5	None
Figure 6H	Spared nerve injury – paw withdrawal threshold: Area over the curve	Mann Whitney test	PBS vs EG00229: p=0.087	PBS n = 6 EG00229 n = 5	None

Table 1. Gating properties of sodium and calcium currents recorded from DRG neurons[a]

	Sodium	Calcium (CaV2.2)
Control (0.1%PBS)		
Activation		
$V_{1/2}$	-19.9±0.6(19)	-0.5±0.8(20)
k	5.5±0.5(19)	6.1±0.7(20)
Inactivation		
$V_{1/2}$	-42.3±3.9(19)	-20.3±9.1(20)
k	-14.5±4.0 (19)	-14.8±7.3(20)
VEGF-A (1 nM)		
Activation		
$V_{1/2}$	-22.1±0.4(20)	0.3±0.7(15)
k	4.2±0.4(20)	5.3±0.6(15)
Inactivation		
$V_{1/2}$	-40.1±2.4(20)	-19.3±5.7(15)
k	-13.4±2.4 (20)	-14.4±4.7(15)
Spike protein (100 nM)		
Activation		
$V_{1/2}$	-19.8±0.5(18)	1.1±0.6(18)
k	5.0±0.5(18)	5.4±0.6(18)
Inactivation		
$V_{1/2}$	-46.4±3.5(18)	-21.1±4.6(18)
k	-13.1±3.6 (18)	-15.0±4.0(18)
VEGF-A (1 nM) + Spike protein (100 nM)		
Activation		
$V_{1/2}$	-17.1±1.6(19)	-0.8±0.7(14)
k	5.7±0.6(19)	6.0±0.6(14)
Inactivation		
$V_{1/2}$	-45.3±3.3(14)	-24.4±4.5(14)
k	-13.6±3.2(14)	-13.0±4.0(14)
Control (0.1% DMSO)		
Activation		
$V_{1/2}$	-19.9±2.2(12)	-2.0±0.6(27)
k	6.3±1.4(12)	5.6±0.6(27)
Inactivation		
$V_{1/2}$	-40.6±2.1(12)	-22.7±4.1(27)
k	-14.2±3.5(12)	-16.1±3.7(27)
VEGF-A (1 nM)		
Activation		
$V_{1/2}$	-24.8±1.4(12)	1.5±0.7(32)
k	4.3±0.9(12)	5.6±0.6(32)
Inactivation		
$V_{1/2}$	-42.0±1.6(12)	-22.8±2.8(32)
k	-11.4±2.3(12)	-13.4±2.8(32)
EG00229 (30 μM)		
Activation		
$V_{1/2}$	-20.7±1.3(11)	3.2±0.5(16)
k	3.5±1.4(11)	5.0±0.4(16)
Inactivation		
$V_{1/2}$	-40.9±3.1(11)	-18.1±5.5(16)

k	-15.1±3.8(11)	-15.9±4.4(16)
VEGF-A (1nM) + EG00229 (30 μM)		
Activation		
$V_{1/2}$	-19.0±1.1(16)	2.5±0.6(18)
k	5.0±1.1(16)	5.1±0.5(18)
Inactivation		
$V_{1/2}$	-50.2±2.6(16)[b]	-24.6±4.3(18)
k	-13.6±2.4(16)	-16.3±4.3(18)

[a]Values are means ± S.E.M. calculated from fits of the data from the indicated number of individual cells (in parentheses) to the Boltzmann equation; $V_{1/2}$ midpoint potential (mV) for voltage-dependent activation or inactivation; *k*, slope factor. These values pertain to Fig. 2 of the main manuscript. Only statistically significant differences are indicated within the table. Data were analyzed with one-way ANOVA with Dunnett's post hoc test.
[b]p=0.0165 comparing Control (0.1% PBS) vs. VEGF-A + EG00229 (one-way ANOVA with Dunnett's post hoc test)

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