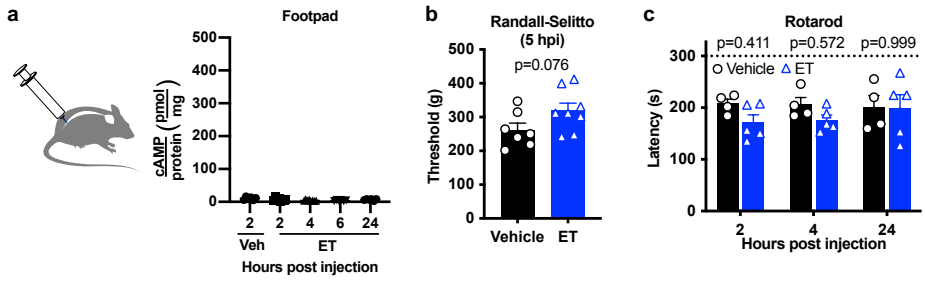
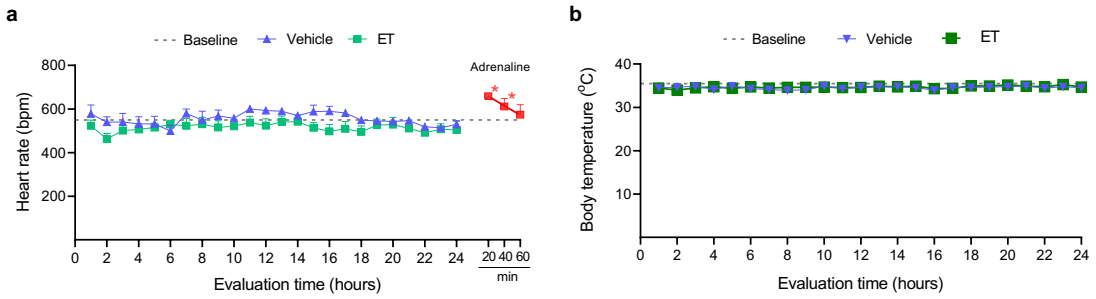
Supplementary Figure 1. Intrathecal ET injection does not affect cAMP in the footpad, noxious mechanoreception at 5 hours post-injection or motor function.



(a) cAMP levels in the footpad after intrathecal administration of vehicle (PBS) or ET (2 µg PA + 2 µg EF) (n = 4 mice/group).
(b) Mice received intrathecal administration of vehicle (PBS; n=7 mice) or ET (2 µg PA + 2 µg EF; n=8 mice). Responses to the Randall-Selitto test was measured at 5 hours post-injection.
(c) Motor function and coordination assessed by the rotarod test after intrathecal administration of vehicle (PBS; n=4 mice) or ET (2 µg PA + 2 µg EF; n=5 mice).

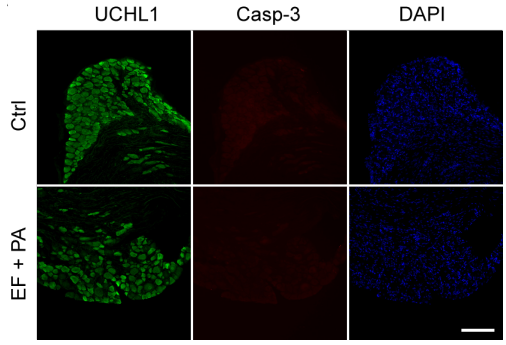
Statistical significance was assessed by one-way ANOVA with Dunnett's post hoc test **(a)**, two-tailed unpaired t-test **(b)** or two-way RM ANOVA with Sidak's post hoc test **(c)**. Data represent the mean ± s.e.m. For detailed statistical information, see Supplementary Table 2.

Supplementary Figure 2. Intrathecal administration of Edema Toxin does not affect heart rate or body temperature.



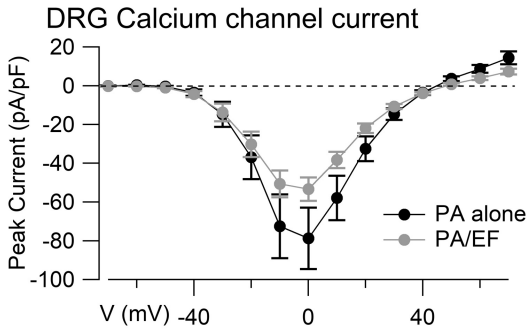
(a) Heart rate in C57/Bl6 male mice over a 24 hour period after intrathecal injection of ET (2 µg PA + 2 µg EF; n=8 mice) or vehicle (n=5 mice). Each point indicates the mean ± SEM over a 1 h period. In red, the heart rate of adrenaline treated mice (0.2 mg/kg iv; n=3 mice), which were injected 24 hours after ET and monitored for 60 minutes. The dashed line represents the heart rate mean of all points over 24 hours prior to ET injection. *$p<0.05$, multiple t-tests assuming no consistent SD and with Holm Sidak's post hoc test. All other data points are not significant. For detailed statistical information, see Supplementary Table 2.
(b) Body temperature in C57/Bl6 male mice over a 24 hour period after intrathecal injection of ET (2 µg PA + 2 µg EF; n=8 mice) or vehicle (n=5 mice). Each point indicates the mean ± SEM over a 1 h period. The dashed line represents the body temperature mean of all points over 24 hours prior to ET injection.

Supplementary Figure 3. Intrathecal administration of Edema Toxin does not induce cleaved caspase-3 in the DRG.



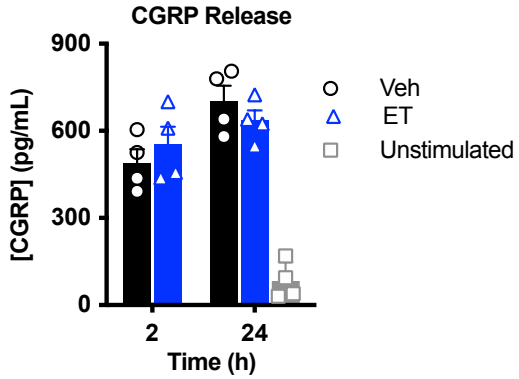
Representative images of frozen L3 - L6 DRG sections at 2 h post intrathecal injection of vehicle (PBS) or ET (2 µg PA + 2 µg EF) stained for cleaved caspase-3. Multiple sections were analyzed across 3 mice. Scale bar, 100 µm.

Supplementary Figure 4. Edema Toxin does not affect DRG neuron calcium currents.



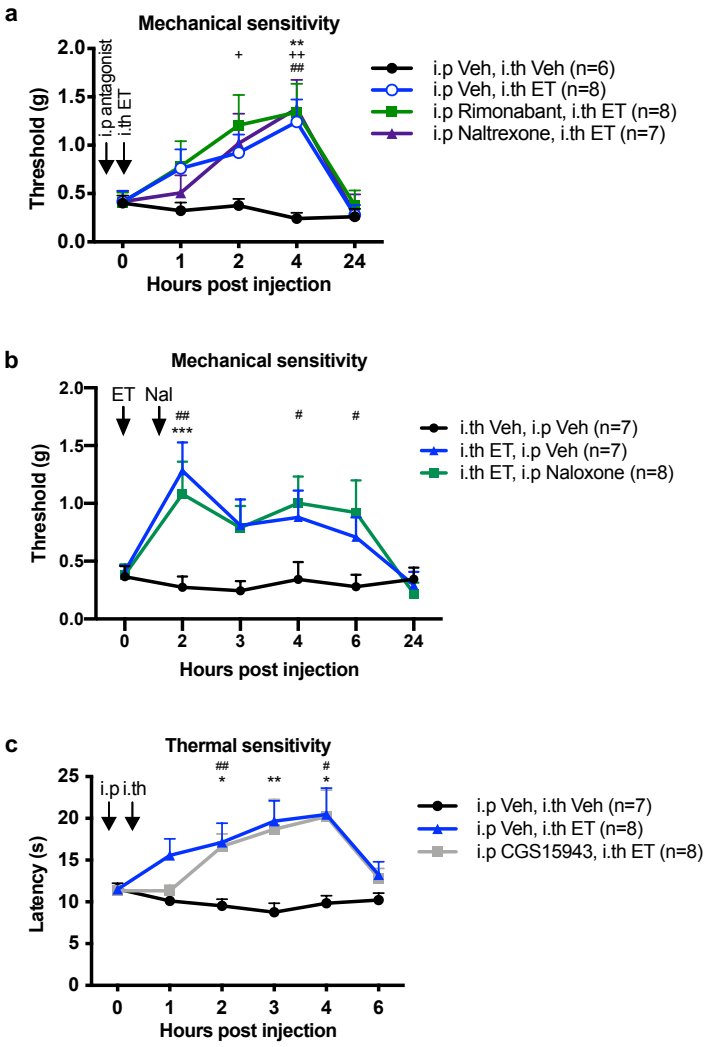
Voltage-dependent calcium channel current in mouse DRG neurons incubated at 37 ºC for at least two hours with Edema Toxin (ET, 10 nM EF + 10 nM PA) compared to PA alone (10 nM). Voltage-dependent calcium channel current carried by 5 mM Ba^{2+} was activated by 100-ms steps from a holding voltage of -70 mV. Mean ± SEM, n=7 cells for each condition. For detailed statistical information, see Supplementary Table 2.

Supplementary Figure 5. Edema Toxin does not affect CGRP release in DRG cultures.



DRG cultures were treated with ET (10 nM PA + 10 nM EF) for 2 or 24 hours (n=4 wells), then stimulated with Krebs-Ringer solution supplemented with 75 mM KCl. CGRP release in the supernatant was measured by EIA. The unstimulated group was treated with Krebs-Ringer solution without any added KCl. Data represent the mean ± s.e.m.

Supplementary Figure 6. ET induced analgesia is unaffected by pharmacological inhibition of endogenous opioid, endocannabinoid or adenosine signaling.

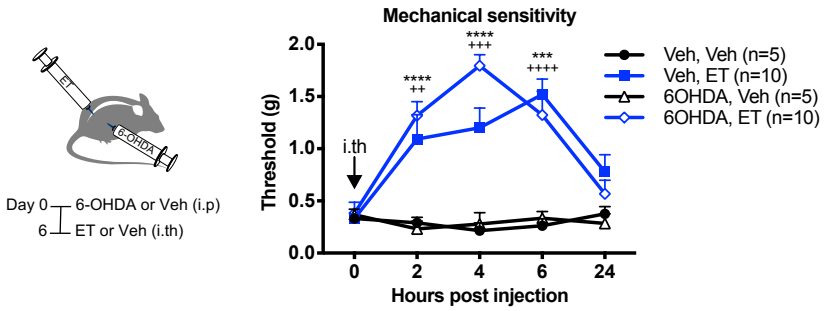


Supplementary Figure 6. ET induced analgesia is unaffected by pharmacological inhibition of endogenous opioid, endocannabinoid or adenosine signaling.

(a) The opioid receptor antagonist Naltrexone (10 mg/kg), the CB1 receptor antagonist/inverse agonist Rimonabant (5 mg/kg) or Vehicle (4% DMSO and 1% Tween-80 in saline) was administered intraperitoneally 15 min prior to intrathecal administration of ET (2 µg PA + 2 µg EF). Mechanical pain sensitivities were measured using von Frey filaments. (n=6 mice for i.p Veh, i.th Veh; n=8 mice for i.p Veh, i.th ET and i.p Rimonabant, i.th ET; n=7 mice for i.p Naltrexone, i.th ET.) The '*' symbol compares i.p Veh, i.th Veh vs. i.p Veh, i.th ET. The '+' symbol compares i.p Veh, i.th Veh vs. i.p Rimonabant, i.th ET. The '#' symbol compares i.p Veh, i.th Veh vs. i.p Naltrexone, i.th ET.
(b) ET (2 µg PA + 2 µg EF) or Vehicle (PBS) was administered intrathecally, followed by Naloxone (2 mg/kg, i.p) or Vehicle (0.9% saline) 15 min prior to the first behavioral measurement. Mechanical pain sensitivities were measured using von Frey filaments. (n=7 mice for i.th Veh, i.p Veh and i.th ET, i.p Veh; n=8 mice for i.th ET, i.p Naloxone.) The '*' symbol compares i.th Veh, i.p Veh vs. i.th ET, i.p Veh. The '#' symbol compares i.th Veh, i.p Veh vs. i.th ET, i.p Naloxone.
(c) Thermal sensitivity thresholds following intraperitoneal administration of CGS15943 (10 mg/kg) or Vehicle (5% DMSO, 0.3% Tween-80 in 0.9% saline), and intrathecal administration of vehicle (PBS) or ET (2 µg PA + 2 µg EF). (n=7 mice for i.p Veh, i.th Veh; n=8 mice for i.p Veh, i.th ET and i.p CGS15943, i.th ET.) The '*' symbol compares i.p Veh, i.th Veh vs. i.p Veh, i.th ET. The '#' symbol compares i.p Veh, i.th Veh vs. i.p CGS15943, i.th ET.

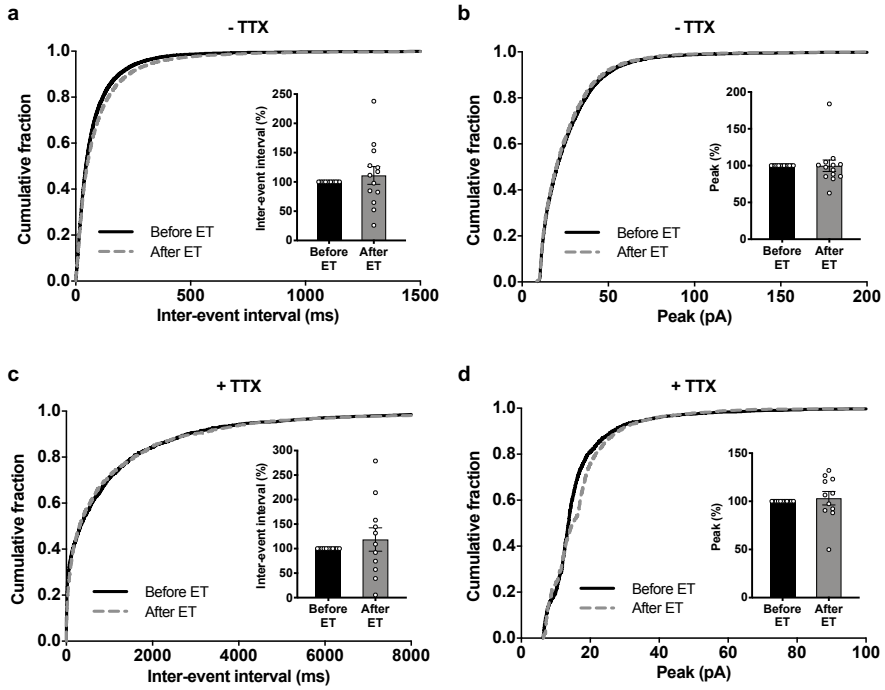
Statistical significance was assessed by two-way RM ANOVA with Tukey's post hoc test **(a-c)**. *p<0.05, **p<0.01, ***p<0.001, +p<0.05, ++p<0.01, #p<0.05, ##p<0.01. Data represent the mean ± s.e.m. For detailed statistical information, see Supplementary Table 2.

Supplementary Figure 7. Chemical sympathectomy does not affect ET induced analgesia.



Chemical sympathectomy was performed by single i.p injection of 6-OHDA (100 mg/kg) or Vehicle (0.1% ascorbic acid in 0.9% saline). After 1 week of rest, mice received intrathecal administration of ET (2 μg PA + 2 μg EF) or Vehicle (PBS). Mechanical pain sensitivities were measured using von Frey filaments. (n=5 mice for Veh, Veh and 6-OHDA, Veh; n=10 mice for Veh, ET and 6-OHDA, ET.) ++p<0.01, +++p<0.001, ++++p<0.0001, Veh, Veh vs. Veh, ET. ***p<0.001, ****p<0.0001, 6-OHDA, Veh vs. 6-OHDA, ET. Two-way RM ANOVA with Tukey's post hoc test. Data represent the mean ± s.e.m. For detailed statistical information, see Supplementary Table 2.

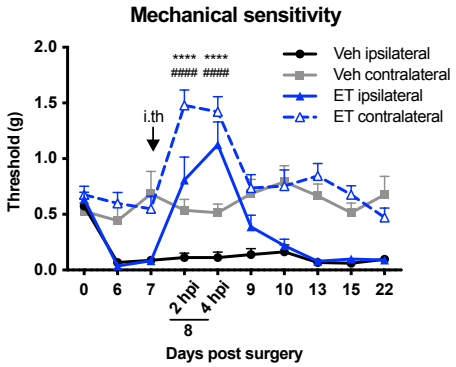
Supplementary Figure 8. Edema Toxin treatment produces minor effects on spinal dorsal horn mEPSCs.



mEPSCs were recorded from Lamina I neurons in horizontal spinal cord slices before and after treatment with ET (10 nM PA + 10 nM EF). Insets display percent changes for each cell before and after ET. **(a-b)** Recordings in the absence of tetrodotoxin (TTX) (n=13 cells). **(a)** Cumulative fraction of inter-event intervals, $p<0.0001$; Inset bar graphs, Percent change in median inter-event interval, n.s. **(b)** Cumulative fraction of amplitudes, $p<0.01$; Inset bar graphs, Percent change in median amplitude, n.s. **(c-d)** Recordings in the presence of 1 μM tetrodotoxin (TTX) (n=11 cells). **(c)** Cumulative fraction of inter-event intervals, $p<0.0001$; Inset bar graphs, Percent change in median inter-event interval, n.s. **(d)** Cumulative fraction of amplitudes, $p<0.0001$; Inset bar graphs, Percent change in median amplitude, n.s.

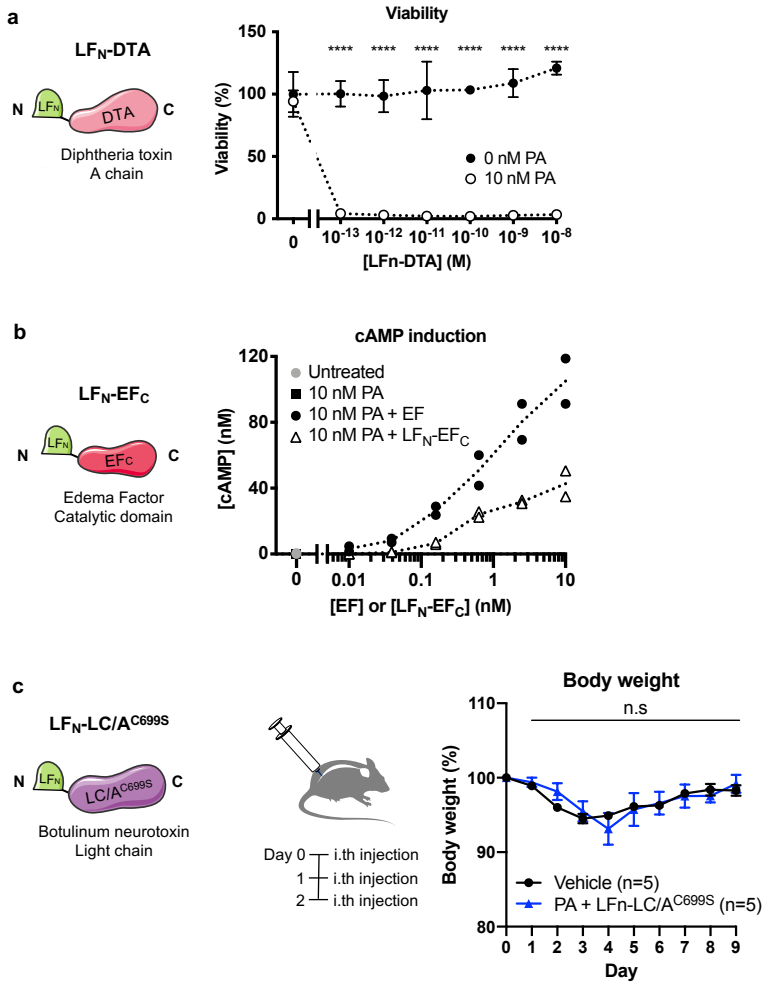
Statistical significance was assessed by Kolmogorov-Smirnov test **(a-d, cumulative distributions)** or two-tailed Wilcoxon matched-pairs signed rank test **(a-d, insets)**. n.s, not significant. Data represent the mean ± s.e.m for inset bar graphs. For detailed statistical information, see Supplementary Table 2.

Supplementary Figure 9. Edema Toxin elevates mechanical sensitivity thresholds in ipsilateral and contralateral sides of SNI-operated mice.



Mechanical sensitivity in the ipsilateral or contralateral hind paw of SNI mice following intrathecal administration of vehicle (PBS) or ET (2 µg PA + 2 µg EF) (n=9 mice/group). ****p<0.0001, Vehicle ipsilateral vs. ET ipsilateral. ####p<0.0001, Vehicle contralateral vs. ET contralateral. Two-way RM ANOVA with Tukey's post hoc test. Data represent the mean ± s.e.m. For detailed statistical information, see Supplementary Table 2.

Supplementary Figure 10. Engineered anthrax toxins deliver distinct molecular cargoes into DRG neurons.



(a) (Left) Design of LF_N-DTA linking the N terminal domain of LF (LF_N) to the A chain of diphtheria toxin (DTA). **(Right)** Viability of DRG cultures following 7 day treatment with the indicated concentrations of LF_N-DTA + PA (10 nM) (n=3 wells). ****p<0.0001, two-way ANOVA with Sidak's post hoc test.
(b) (Left) Design of LF_N-EF_C linking the N terminal domain of LF (LF_N) to the catalytic domain of EF (EF_C). **(Right)** cAMP levels in DRG cultures treated with 10 nM PA + EF or 10 nM PA + LF_N-EF_C for 2 h (n=2 wells). Dotted lines connect the means. The EF and LF_N-EF_C preps for this experiment were produced recombinantly in an avirulent strain of *B. anthracis.*
(c) (Left) Design of LF_N-LC/A^{C699S} linking the N terminal domain of LF (LF_N) to a mutated light chain (LC) of type A botulinum neurotoxin (LC/A^{C699S}). **(Right)** Body weight of mice following three daily intrathecal injection of vehicle (PBS) or PA + LF_N-LC/A^{C699S} (500 ng + 200 ng) (n=5 mice/group). n.s, not significant.

Data represent the mean ± s.e.m. For detailed statistical information, see Supplementary Table 2.

Supplementary Figure 11. Protein sequences

EF

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LF_N-DTA

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KALSVELEINFETRGKRGQDAMYEYMAQASAGNR

LF_N-EF_C

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EVFAKAFAYYIEPQHRDVLQLYAPEAFNYMDKFNEQEINLSTRDRIDVLKGEKALKASGLVPEHADAFKKI
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LF_N-LC/A^{C699S}

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EKALNVYYEIGKILSRDILSKINQPYQKFLDVLNTIKNASDSDGQDLLFTNQLKEHPTDFSVEFLEQNSNEVQ
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CKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAI
NPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKAKSIVGTTA
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PKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKEN
LYFQ

Supplementary Table 1. Human DRG donor information.

Donor #	DRG	Sex	Age	Surgery/Cause of Death
1	L5	Female	48	Head trauma
2	L5	Male	28	Anoxia/Asphyxiation
3	L5	Male	55	Anoxia/Seizure