Supplementary material

Supplementary methods

Real-Time PCR

The RNA (100 ng) extracted from TGs was reverse-transcribed using the iScript™ cDNA synthesis kit (Bio-Rad) according to the manufacturer's protocol. For relative quantification of mRNA, real time PCR was performed on Rotor Gene® Q (Qiagen). The sets of primers-probes were as follows: 18S-FW (forward): 5'-CGCGGTTCTATTTTGTTGGT-3', 18S-RE (reverse): 5'-AGTCGGCATCGTTTATGGTC-3' (NCBI Ref Seq: NR_003278.3); TRPA1-FW: 5'-CAGGATGCTACGGTTTTTTCATTACT-3', TRPA1-RE: 5'-GCATGTGTCAATGTTTGGTACTTCT-3' (NCBI Ref Seq: NM_177781.4). The chosen reference gene was the 18S. The SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad) was used for amplification, and the cycling conditions were the following: samples were heated to 95°C for 1 min followed by 40 cycles of 95°C for 10 sec, and 65°C for 20 sec. PCR reaction was carried out in triplicate.

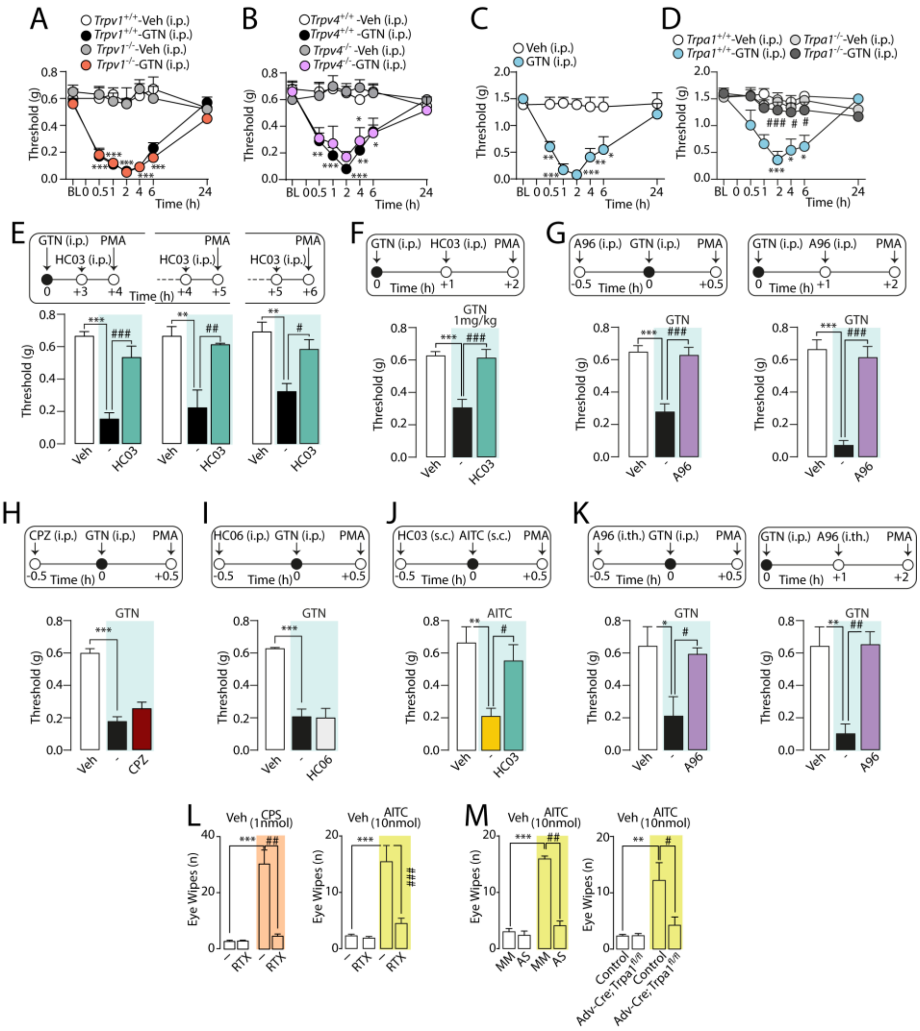
In TGs from $Adv\text{-}Cre^{+};Trpa1^{fl/fl}$ and $Adv\text{-}Cre^{-};Trpa1^{fl/fl}$, *Trpa1* was amplified and detected using the TaqMan assays (Invitrogen) (Zappia *et al.*, 2017). Relative expression of TRPA1 mRNA was calculated using the $2^{-\Delta}(^{\Delta}CT)$ comparative method, with each gene normalized against the internal endogenous reference 18S gene.

H_2O_2 assay

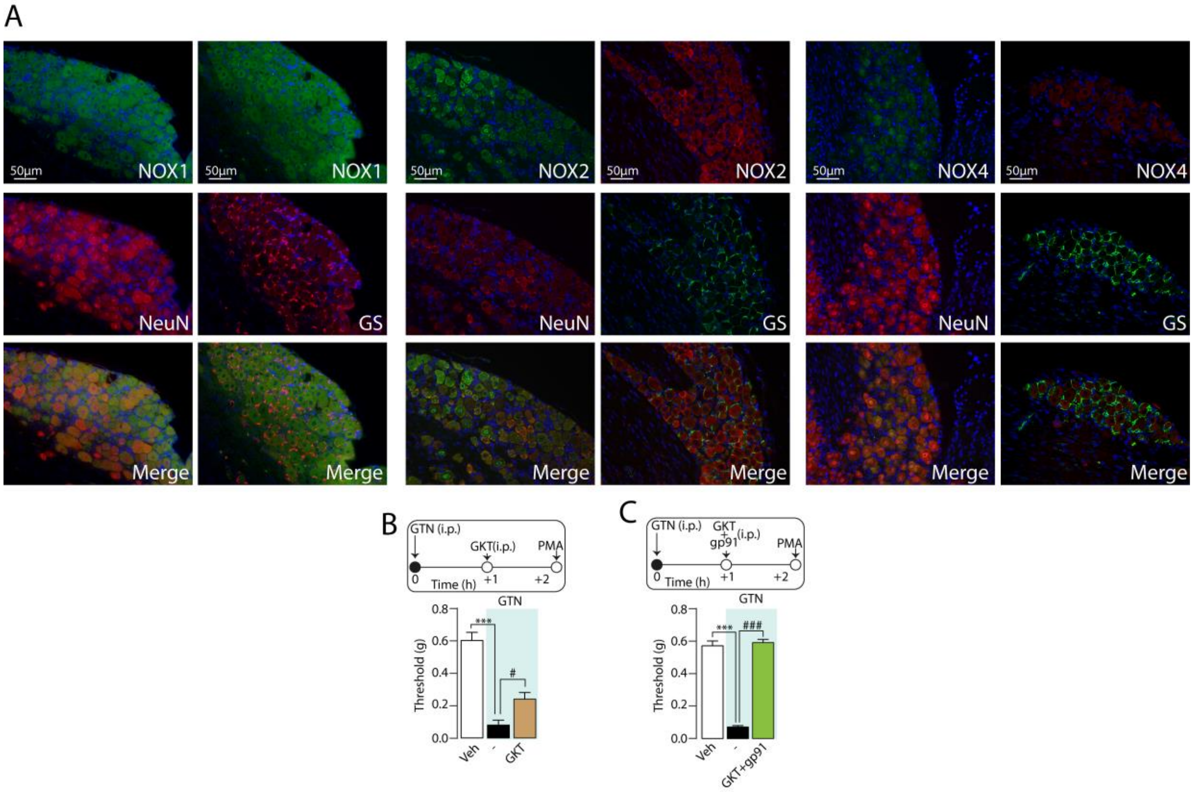
TG and brainstem tissues were removed and placed into modified Krebs/HEPES buffer [mmol/l: 99.01 NaCl, 4.69 KCl, 2.50 $CaCl_2$, 1.20 $MgSO_4$, 1.03 KH_2PO_4, 25.0 $NaHCO_3$, 20.0 Na-HEPES, and 5.6 glucose (pH 7.4)]. Samples were minced and incubated with Amplex red (100 µM) and HRP (1 U/ml) (1h, 37°C) in modified Krebs/HEPES buffer (Landmesser *et al.*, 2003). Fluorescence excitation/emission were at 540/590 nm. H_2O_2 production was expressed as µmol/l of mg of dry tissue.

For H_2O_2 assay in cultured cells, both trigeminal neuron-SGCs mixed and SGCs-enriched primary culture were plated ($5x10^5$ cells/well) in 96-well and maintained in 5% CO_2 and 95% O_2 (37°C, 48 h). The cultured medium was replaced with Krebs-Ringer phosphate (KRP) buffer [mmol/l: 2 $CaCl_2$, 5.4 KCl, 0.4 $MgSO_4$, 135 NaCl, 10 D-glucose, 10 HEPES at pH 7.4]. Both cultures were stimulated with the different stimuli added with Amplex red (50 µM) and HRP (1 U/ml) (0.5 h, room temperature, RT). Signal was detected 3 h after exposure to the stimulus. H_2O_2 release was calculated using H_2O_2 standards and expressed as nmol/l.

Supplemetary Figures



Supplementary Figure 1. (A, B) Periorbital mechanical allodynia (PMA) evoked by GTN (10 mg/kg, i.p.) in *Trpv1*$^{+/+}$ or in *Trpav4*$^{+/+}$ is unaffected in *Trpv1*$^{-/-}$ or in *Trpav4*$^{-/-}$ mice. Time-course of hind paw mechanical allodynia evoked by GTN (10 mg/kg, i.p.) in (C) C57BL/6 mice or (D) *Trpa1*$^{+/+}$ and *Trpa1*$^{-/-}$ mice. (E) TRPA1 antagonism by HC-030031 (HC03; 100 mg/kg, i.p.) transiently reverses GTN-evoked (10 mg/kg, i.p.) PMA in C57BL/6 mice. (F) PMA evoked by GTN (1 mg/kg, i.p.) is attenuated by (HC03 (100 mg/kg, i.p). (G) GTN (10 mg/kg, i.p.)-evoked PMA is inhibited by A967079 (A96; 100 mg/kg, i.p), but not by (H) capsazepine (CPZ, 4 mg/kg, i.p.) or (I) HC-067047 (HC06, 10 mg/kg, i.p.)**.** (J) PMA evoked by subcutaneous (s.c.) injection (10 µl/site) into the periorbital area of AITC (10 nmol) is inhibited by s.c. HC03 (100 µg/site). (K) GTN (10 mg/kg, i.p.)-evoked PMA is inhibited by intrathecal (i.th.) A96 (10 µg). (L) Eye wiping response evoked by ocular instillation (5 µl/drop eye) of capsaicin (CPS) or AITC in RTX desensitized C57BL/6 mice. (M) Eye wiping response evoked by ocular instillation (5 µl/drop eye) of AITC in C57BL/6 mice treated (i.th.) with TRPA1 AS/MM ODN or in *Advillin-Cre*$^{+}$;*Trpa1*$^{fl/fl}$ (*Adv-Cre*$^{+}$;*Trpa1*$^{fl/fl}$) or *Advillin-Cre*$^{-}$; *Trpa1*$^{fl/fl}$ (Control) mice. BL, baseline mechanical threshold. Veh is the vehicle of GTN. Dash (-) indicates combined vehicles of treatments. Arrows indicate time of drug administration. Error bars indicate mean ± SEM, 6-7 mice *per* group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs*. *Trpv1*$^{+/+}$-Veh, *Trpv4*$^{+/+}$- Veh, *Trpa1*$^{+/+}$-Veh, Veh. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs*. *Trpa1*$^{+/+}$-GTN, GTN, AITC, CPS; one-way or two-way ANOVA with Bonferroni post-hoc correction.



Supplementary Figure 2. (A) Representative images of staining for NOX1, NOX2 and NOX4 and neuronal (neuronal nuclei, NeuN) or satellite glial cells (glutamine synthetase, GS) in mouse trigeminal ganglion (TG) from C57BL/6 mice. (Scale bars: 100 μm). (B, C) Periorbital mechanical allodynia (PMA) evoked by GTN (10 mg/kg, i.p.) in C57BL/6 mice is reduced by the NOX1/4 inhibitor, GKT137831 (GKT, 60 mg/kg, i.p.), and completely abated by the combination of GKT and the NOX2 selective inhibitor, gp91ds-tat peptide (gp91, 10 mg/kg, i.p.). Veh is the vehicle of GTN. Dash (-) indicates combined vehicles of treatments. Error bars indicate mean ± SEM, 8 mice *per* group. ***P<0.001 *vs*. Veh. $^{\#}P$<0.05, $^{\#\#\#}P$<0.001 *vs*. GTN; one-way ANOVA with Bonferroni post-hoc correction.

Supplementary Table 1

Drugs	Target action	Dose	Administration route	Reference
HC-030031	TRPA1 antagonist	100 mg/kg	i.p.	(Eid *et al.*, 2008)
		100 µg/10 µl	s.c.	(da Costa *et al.*, 2010)
		10 µg/5 µl	i.th.	(da Costa *et al.*, 2010)
A967079	TRPA1 antagonist	100 mg/kg	i.p.	(Trevisan *et al.*, 2016)
		10 µg/5 µl	i.th.	(Wei *et al.*, 2011)
Capsazepine	TRPV1 antagonist	4 mg/kg	i.p.	(Nassini *et al.*, 2011)
HC-067047	TRPV4 antagonist	10 mg/kg	i.p.	(Materazzi *et al.*, 2012)
Disulfiram	Aldehyde dehydrogenase inhibitor	100 mg/kg	i.p.	(Kim *et al.*, 2010)
		10 µg/10 µl	s.c.	§
		5 µg/5 µl	i.th.	§
cPTIO	Nitric oxide scavenger	0.6 mg/kg	i.p.	(Lee *et al.*, 2009)
		60 µg/10 µl	s.c.	(Nozaki *et al.* 1998)
		30 µg/5 µl	i.th.	(Chen and Pan, 2003)
α-lipoic acid	Antioxidant	100 mg/kg	i.p.	(Joseph *et al.*, 2008)
		5 µg/10 µl	s.c.	(Joseph *et al.*, 2008)
		10 µg/5 µl	i.th.	§
PBN	Free-radical spin trap	100 mg/kg	i.p.	(De Logu *et al.*, 2017)
NAC	HNE sequestering agent	250 mg/kg	i.p.	(Rossato *et al.*, 2010)
		20 µg/10 µl	s.c.	(Rossato *et al.*, 2010)
		50 µg/5 µl	i.th.	(Rossato *et al.*, 2010)
L-Carnosine	HNE sequestering agent	250 mg/kg	i.p.	(Alsheblak *et al.*, 2016)
Apocynin	Non selective NOX inhibitor	100 mg/kg	i.p.	(Li *et al.*, 2013)
Gp91ds-tat	NOX2 inhibitor	10 mg/kg	i.p.	(De Logu *et al.*, 2017)
ML171	NOX1 inhibitor	60 mg/kg	i.p.	(De Logu *et al.*, 2017)
GKT137831	NOX1/4 inhibitor	60 mg/kg	i.p.	(De Logu *et al.*, 2017)
$CGRP_{8-37}$	CGRP receptor antagonist	2 µmol/kg	i.p.	(Nassini *et al.*, 2010)
		10 nmol/10 µl	s.c.	§
		5 nmol/5 µl	i.th.	(de Prado *et al.*, 2009)
BIBN4096BS	CGRP receptor antagonist	1 mg/kg	i.p.	(Doods *et al.*, 2000)
		4 nmol/10 µl	s.c.	§
		1 µg/5 µl	i.th.	(Mogil *et al.*, 2005)

§ Preliminary experiments showed that this dose did not evoke *"per se"* any behavioural response

Supplementary Table 2

		Statistical test	n		P value	DEGREES OF FREEDOM & F/t values
Fig 1 A		Two-way ANOVA			< 0.0001	F (24, 224)= 5.870
				0.5 hour		
	post hoc analysis		8 vs. 8	GTN 10mg vs. Veh	< 0.0001	t= 7.027
			8 vs. 8	GTN 5mg vs. Veh	< 0.0001	t= 5.229
				1 hour		
			8 vs. 8	GTN 10 mg vs. Veh	< 0.0001	t= 8.825
			8 vs. 8	GTN 5mg vs. Veh	< 0.0001	t= 6.864
			8 vs. 8	GTN 1mg/10ml vs. Veh	0.0447	t= 2.451
				2 hours		
			8 vs. 8	GTN 1mg vs. Veh	< 0.0001	t= 9.315
			8 vs. 8	GTN 5mg vs. Veh	< 0.0001	t= 6.537
			8 vs. 8	GTN 10mg vs. Veh	0.0176	t= 2.778
				4 hours		
			8 vs. 8	GTN 10mg vs. Veh	< 0.0001	t= 8.661
			8 vs. 8	GTN 5mg vs. Veh	< 0.0001	t= 5.72
				6 hours		
			8 vs. 8	GTN 10mg vs. Veh	< 0.0001	t= 5.393
			8 vs. 8	GTN 5mg vs. Veh	0.0006	t= 3.759
Fig 1 B		Two tailed T test				
			6 vs. 6	GTN vs. Veh	0.0018	t= 4.207 df= 10
Fig 1 C		Two-way ANOVA			0.0379	F (6, 70)= 2.377
				2 hours		
	post hoc analysis		6 vs. 6	GTN WT vs. Veh WT	0.0018	t= 4.23
			6 vs. 6	GTN KO vs. GTN WT	0.0033	t= 4.067
Fig 1 D		Two-way ANOVA			<0.0001	F (18, 196)= 7.501
				0.5 hour		
	post hoc analysis		8 vs.8	GTN WT vs. Veh WT	0.0001	t= 4.406
			8 vs.8	GTN KO vs. GTN WT	0.0006	t= 3.987
				1 hour		
			8 vs.8	GTN WT vs. Veh WT	<0.0001	t= 8.603
			8 vs.8	GTN KO vs. GTN WT	<0.0001	t= 7.554
				2 hours		
			8 vs.8	GTN WT vs. Veh WT	<0.0001	t= 10.28
			8 vs.8	GTN KO vs. GTN WT	<0.0001	t= 9.022
				4 hours		
			8 vs.8	GTN WT vs. Veh WT	<0.0001	t= 8.183
			8 vs.8	GTN KO vs. GTN WT	<0.0001	t= 6.085
				6 hours		
			8 vs.8	GTN WT vs. Veh WT	0.0001	t= 4.406
			8 vs.8	GTN KO vs. GTN WT	0.0417	t= 2.728
Fig 1 E		One-way ANOVA			< 0.0001	F (3, 20)= 19.74
	post hoc analysis		6 vs.6	GTN WT vs. Veh WT	0.0004	t= 5.027
Fig 1 F left panel						
		One-way ANOVA			< 0.0001	F (4, 25)= 4.21
	post hoc analysis		6 vs. 6	GTN vs. veh	< 0.0001	t= 6.542
			6 vs. 6	HC03 GTN vs. GTN	< 0.0001	t= 5.782
			6 vs. 6	DSF GTN vs. GTN	0.0001	t= 5.477
			6 vs. 6	cPTIO GTN vs. GTN	< 0.0001	t= 5.782
Fig 1 F right panel						
		One-way ANOVA			< 0.0001	F (4, 41) = 24.21
	post hoc analysis		6 vs. 6	GTN vs. veh	< 0.0001	t= 7.327
			6 vs. 6	GTN HC03 vs. GTN	< 0.0001	t= 7.127
Fig 1 G		One-way ANOVA			< 0.0001	F (3, 20)= 13.56
	post hoc analysis		6 vs. 6	GTN vs. veh	0.0004	t= 4.978
			6 vs. 6	GTN DSF vs. GTN	0.002	t= 4.314

	Statistical test	n	P value		DEGREES OF FREEDOM & F/t values
Fig 2 A left panel	One-way ANOVA			< 0.0001	F (4. 331) = 16.97
	post hoc analysis	78 vs. 53	veh vs. 50µM	0.0058	t= 3.212
		78 vs.83	veh vs. 100µM	< 0.0001	t= 6.216
		78 vs 54	veh vs. 300 uM	< 0.0001	t= 6.922
Fig 2 A right panel	One-way ANOVA			0.0061	F (3, 144)= 4.299
	post hoc analysis	29 vs. 79	HC03 GTN vs. GTN	0.0005	t= 4.067
Fig 2 B	One-way ANOVA			< 0.0001	F (3, 83)= 94.51
	post hoc analysis	21 vs. 20	GTN WT vs. Veh WT	< 0.0001	t= 13.5
		26 vs. 21	GTN KO vs. GTN WT	< 0.0001	t= 14.38
Fig 2 C	One-way ANOVA			< 0.0001	F (5, 452)= 121.4
	post hoc analysis	54 vs 131	GTN wt vs. Veh wt	< 0.0001	t= 17.15
		77 vs.54	GTN wt vs. GTN 3C/KQ	< 0.0001	t= 14.32
		54 vs. 131	Menthol wt vs. Veh wt	< 0.0001	t= 16.44
Fig 2 D left panel	One-way ANOVA			0.0064	F (2, 177)= 5.204
	post hoc analysis	83 vs. 47	GTN 10 µM vs. veh	0.0154	t= 2.834
	One-way ANOVA			< 0.0001	F (2, 145)= 35.45
	post hoc analysis	83 vs. 34	GTN 100 µM vs. veh	< 0.0001	t= 6.365
Fig 2 D right panel	One-way ANOVA			< 0.0001	F (3, 77)= 11.62
	post hoc analysis	22 vs. 20	SNAP vs. veh	< 0.0001	t= 5.007
		22 vs. 28	HC03 SNAP vs. SNAP	< 0.0001	t= 5.212
		22 vs. 21	cPTIO SNAP vs. SNAP	0.0037	t= 3.351
Fig 2E	Two-way ANOVA			0.0029	F (12, 100)= 2.745
	post hoc analysis		**0.5 hour**		
		6 vs. 6	GTN WT vs. Veh WT	0.0003	t= 4.272
		6 vs. 6	GTN KO vs. GTN WT	0.0046	t= 3.471
			1 hour		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 5.474
		6 vs. 6	GTN KO vs. GTN WT	< 0.0001	t= 5.741
			2 hours		
		6 vs. 6	GTN WT vs. Veh WT	0.0019	t= 3.738
		6 vs. 6	GTN KO vs. GTN WT	0.0165	t= 3.071
Fig 2F	Two-way ANOVA			< 0.0001	F (12, 140)= 7.199
	post hoc analysis		**0.5 hour**		
		8 vs. 8	SNAP WT vs. Veh WT	< 0.0001	t= 6.883
			SNAP KO vs. SNAP WT	< 0.0001	t= 5.767
			1 hour		
		8 vs. 8	SNAP WT vs. Veh WT	< 0.0001	t= 8.185
		8 vs. 8	SNAP KO vs. SNAP WT	< 0.0001	t= 7.999
			2 hours		
		8 vs. 8	SNAP WT vs. Veh WT	< 0.0001	t= 7.069
		8 vs. 8	SNAP KO vs. SNAP WT	< 0.0001	t= 6.325
Fig 2G	One-way ANOVA			0.0005	F (5, 34)= 5.880
	post hoc analysis	6 vs.6	GTN vs. veh	0.0032	t= 3.576
		6 vs.6	HC03(s.c.) GTN vs GTN	0.0382	t= 2.63
		6 vs.6	HC03(i.p.) GTN vs GTN	0.0295	t= 2.735
Fig 2 H	One-way ANOVA			< 0.0001	F (4, 27)= 9.380
	post hoc analysis	6 vs. 6	SNAP vs. veh	0.0002	t= 5.184
		6 vs. 6	HC03(s.c.) SNAP vs SNAP	0.0342	t= 3.209
		6 vs. 6	HC03(i.p.) SNAP vs SNAP	0.0007	t= 4.691
		8 vs.6	cPTIO SNAP vs. SNAP	0.0002	t= 5.146

	1 hour		
9 vs.6	GTN vs. Veh	< 0.0001	t= 6.946
9 vs.6	NAC GTN vs GTN	0.0074	t= 3.893
	2 hours		
9 vs.6	GTN vs. Veh	< 0.0001	t= 7.785
9 vs.6	NAC GTN vs GTN	< 0.0001	t= 5.906
	4 hours		
6 vs. 6	GTN vs. Veh	< 0.0001	t= 6.004
6 vs.6	NAC GTN vs GTN	< 0.0001	t= 5.269
	5 hours		
9 vs.6	GTN vs. Veh	< 0.0001	t= 6.846
9 vs.6	NAC GTN vs GTN	0.0009	t= 4.430
	6 hours		
6 vs. 6	GTN vs. Veh	0.0001	t= 4.901
6 vs.6	GTN NAC vs GTN	0.0258	t= 3.554
	8 hours		
9 vs.6	GTN vs. Veh	< 0.0001	t= 5.101
9 vs.6	GTN NAC vs GTN	0.0122	t= 3.758

	Statistical test	n		P value	DEGREES OF FREEDOM & F/t values
Fig 3 A	One-way ANOVA			< 0.0001	F (5, 31)= 8.003
	post hoc analysis	6 vs.6	GTN vs. Veh	0.0003	t= 5.006
Fig 3 B	One-way ANOVA			< 0.0001	F (5, 28)= 35.66
	post hoc analysis	6 vs.6	GTN vs. Veh	< 0.0001	t= 11.53
		8 vs. 6	GTN aLA vs. GTN	< 0.0001	t= 9.124
		6 vs.6	GTN PBN vs. GTN	< 0.0001	t= 9.732
		7 vs.6	NAC GTN vs. GTN	< 0.0001	t= 5.798
		6 vs.6	Carn GTN vs. GTN	< 0.0001	t= 5.948
Fig 3 C	One-way ANOVA			< 0.0001	F (5, 32)= 15.80
	post hoc analysis	6 vs.6	GTN vs. Veh	< 0.0001	t= 6.063
		7 vs.6	NAC GTN vs. GTN	0.0215	t= 3.073
		6 vs.6	Carn GTN vs. GTN	0.0003	t= 4.653
Fig 3 D	One-way ANOVA			< 0.0001	F (3, 28)= 67.63
	post hoc analysis	8 vs.8	GTN vs. Veh	< 0.0001	t= 12.48
Fig 3 E	One-way ANOVA			< 0.0001	F (3, 28)= 35.89
	post hoc analysis	8 vs.8	GTN vs. Veh	< 0.0001	t= 8.873
Fig 3 F	Two-way ANOVA			< 0.0001	F (14, 124)= 4.197
	post hoc analysis		**1 hour**		
		6 vs.6	GTN vs. Veh	0.0008	t= 4.311
		6 vs.6	GTN NAC vs Veh	< 0.0001	t= 5.127
			2 hours		
		6 vs.6	GTN vs. Veh	< 0.0001	t= 6.292
		6 vs.6	GTN NAC vs Veh	< 0.0001	t= 6.409
			4 hours		
		6 vs.6	GTN vs. Veh	< 0.0001	t= 5.826
		6 vs.6	GTN NAC vs Veh	< 0.0001	t= 5.826
			5 hours		
		6 vs.6	GTN vs. Veh	< 0.0001	t= 4.894
		6 vs.6	GTN NAC vs Veh	< 0.0001	t= 5.481
			6 hours		
		6 vs.6	GTN vs. Veh	0.0019	t= 4.078
			GTN NAC vs Veh	< 0.0001	t= 4.983
			8 hours		
		6 vs.6	GTN vs. Veh	0.0246	t= 3.029
			GTN NAC vs Veh	0.0246	t= 3.363
Fig 3 G	One-way ANOVA			< 0.0001	F (5, 35)= 9.520
	post hoc analysis	6 vs.6	GTN vs. Veh	0.0241	t= 2.924
		7 vs. 6	HC03 GTN vs. GTN	0.0316	t= 2.817
		7 vs 6	DSF GTN vs. GTN	0.0023	t= 3.793
		9 vs.6	cPTIO GTN vs. GTN	0.0002	t= 4.575
Fig 3 H	One-way ANOVA			< 0.0001	F (5, 42) = 41.13
	post hoc analysis	8 vs.8	GTN vs. Veh	< 0.0001	t= 9.442
		8 vs.8	GTN HC03 vs. GTN	< 0.0001	t= 8.534
		8 vs.8	GTN aLA vs. GTN	< 0.0001	t= 8.171
Fig 3 I	Two-way ANOVA			< 0.0001	F (24, 192)= 5.932
			0.5 hour		
	post hoc analysis	6 vs.6	GTN vs. Veh	< 0.0001	t= 5.098

	1 hour		
9 vs.6	GTN vs. Veh	< 0.0001	t= 6.946
9 vs.6	NAC GTN vs GTN	0.0074	t= 3.893
	2 hours		
9 vs.6	GTN vs. Veh	< 0.0001	t= 7.785
9 vs.6	NAC GTN vs GTN	< 0.0001	t= 5.906
	4 hours		
6 vs. 6	GTN vs. Veh	< 0.0001	t= 6.004
6 vs.6	NAC GTN vs GTN	< 0.0001	t= 5.269
	5 hours		
9 vs.6	GTN vs. Veh	< 0.0001	t= 6.846
9 vs.6	NAC GTN vs GTN	0.0009	t= 4.430
	6 hours		
6 vs. 6	GTN vs. Veh	0.0001	t= 4.901
6 vs.6	GTN NAC vs GTN	0.0258	t= 3.554
	8 hours		
9 vs.6	GTN vs. Veh	< 0.0001	t= 5.101
9 vs.6	GTN NAC vs GTN	0.0122	t= 3.758

	Statistical test	n		P value	DEGREES OF FREEDOM & F/t values
Fig 4 A	One-way ANOVA		**H2O2 level**	< 0.0001	F (6, 36)= 10.66
	post hoc analysis		**1 hour**		
		6 vs. 6	GTN vs. BL	0.0227	t= 3.094
			2 hours		
		6 vs. 6	GTN vs. BL	< 0.0001	t= 6.38
			3 hours		
		6 vs. 6	GTN vs. BL	0.0059	t= 3.586
			HNE level	< 0.0001	F (5, 18)= 37.64
	post hoc analysis	4 vs.4	GTN 4h vs. GTN 0.5h	< 0.0001	t= 8.475
		4 vs.4	GTN 6h vs. GTN 0.5h	< 0.0001	t= 10.53
Fig 4 C	One-way ANOVA		**H2O2 level**	< 0.0001	F (5, 26)= 39.14
	post hoc analysis	4 vs. 4	GTN vs. Veh	< 0.0001	t= 10.58
		6 vs.4	cPTIO GTN vs. GTN	< 0.0001	t= 11.95
		6 vs.4	DSF GTN vs. GTN	< 0.0001	t= 11.48
		6 vs.4	a-LA GTN vs. GTN	< 0.0001	t= 10.04
		6 vs.4	HC03 GTN vs. GTN	< 0.0001	t= 11.99
Fig 4 D	One-way ANOVA		**HNE level**	0.0003	F (4, 23)= 8.122
	post hoc analysis	6 vs.4	GTN cPTIO vs. GTN	0.0013	t= 4.208
		6 vs.4	GTN aLA vs. GTN	0.0002	t= 4.974
		6 vs.4	GTN NAC vs.GTN	0.0001	t= 5.165
		6 vs.4	GTN HC03 vs. GTN	0.0022	t= 4.017
Fig 4 E	One-way ANOVA		**H2O2 level**	< 0.0001	F (3, 20)= 29.14
	post hoc analysis	6 vs.6	GTN wt vs. Veh wt	< 0.0001	t= 7.718
		6 vs.6	GTN wt vs. GTN ko	< 0.0001	t= 7.532
	Two tailed T test		**HNE level**		t= 4.622 df= 10
		6 vs.6	GTN wt vs. GTN ko	0.0009	

	Statistical test	n	Comparison	P value	DEGREES OF FREEDOM & F/t values
Fig 5 B	Two-way ANOVA			< 0.0001	F (12, 112) = 3.908
			0.5 hour		
	post hoc analysis	6 vs. 6	GTN vs. Veh	0.0006	t= 4.349
		7 vs. 6	RTX GTN vs. GTN	< 0.0001	t= 4.851
			1 hour		
		6 vs. 6	GTN vs. Veh	< 0.0001	t= 4.892
		7 vs. 6	RTX GTN vs. GTN	< 0.0001	t= 5.303
			2 hours		
		6 vs. 6	GTN vs. Veh	< 0.0001	t= 5.762
		7 vs. 6	RTX GTN vs. GTN	< 0.0001	t= 6.205
			4 hours		
		6 vs. 6	GTN vs. Veh	0.0001	t= 4.784
		7 vs. 6	RTX GTN vs. GTN	< 0.0001	t= 5.190
			6 hours		
		6 vs. 6	GTN vs. Veh	0.0019	t= 2.718
		7 vs. 6	RTX GTN vs. GTN	0.0165	t= 2.595
	One-way ANOVA		**H_2O_2 level**	< 0.0001	F (2, 15)= 52.92
	post hoc analysis	6 vs. 6	GTN vs Veh RTX	< 0.0001	t= 9.360
		6 vs. 6	GTN RTX vs GTN	< 0.0001	t= 8.377
Fig 5 C	One-way ANOVA		**H_2O_2 level**	< 0.0001	F (5, 55)= 87.18
	post hoc analysis	8 vs. 21	Ca^{2+}-free GTN vs. GTN	< 0.0001	t= 14.81
		8 vs. 21	CPS des GTN vs. GTN	< 0.0001	t= 15.72
		6 vs. 21	HC03 GTN vs. GTN	< 0.0001	t= 13.47
	Two tailed T test				
		6 vs. 8	HC03 AITC vs. AITC	< 0.0001	t= 12.16 df= 12
	Two tailed T test				
		8 vs. 8	HC03 SNAP vs. SNAP	< 0.0001	t= 30.14 df= 14
Fig 5 E	Two tailed T test		**mRNA level**		
		6 vs. 8	AS vs. MM	< 0.0001	t= 12.16 df= 12
	Two-way ANOVA			< 0.0001	F (18, 151)= 5.566
			0.5 hour		
	post hoc analysis	7 vs. 6	MM GTN vs. MM Veh	< 0.0001	t= 6.310
		7 vs. 7	AS GTN vs. MM GTN	< 0.0001	t= 4.854
			1 hour		
		7 vs. 6	MM GTN vs. MM Veh	< 0.0001	t= 7.545
		7 vs. 7	AS GTN vs. MM GTN	< 0.0001	t= 6.996
			2 hours		
		6 vs. 6	MM GTN vs. MM Veh	< 0.0001	t= 8.093
		7 vs. 6	AS GTN vs. MM GTN	< 0.0001	t= 7.995
			4 hours		
		6 vs. 6	MM GTN vs. MM Veh	< 0.0001	t= 6.859
		7 vs. 6	AS GTN vs. MM GTN	< 0.0001	t= 5.761
			6 hours		
		6 vs. 6	MM GTN vs. MM Veh	< 0.0001	t= 5.350
		7 vs. 6	AS GTN vs. MM GTN	< 0.0001	t= 5.075
	One-way ANOVA		**H_2O_2 level**	< 0.0001	F (3, 20)= 20.11
	post hoc analysis	6 vs.6	GTN MM vs. Veh MM	< 0.0001	t= 6.497
		6 vs.6	GTN AS vs. GTN MM	< 0.0001	t= 6.264
Fig 5 F	Two tailed T test		**mRNA level**		
		6 vs. 8	AS vs. MM	< 0.0001	t= 9.625 df= 6

Two-way ANOVA			< 0.0001	F (18, 140)= 3.568
		0.5 hour		
post hoc analysis	7 vs. 6	Control GTN vs.Control Veh	0.0002	t= 4.274
	7 vs. 7	Adv-cre GTN vs Control GTN	< 0.0001	t=5.280
		1 hour		
	7 vs. 6	Control GTN vs.Control Veh	< 0.0001	t= 4.651
	7 vs. 7	Adv-cre GTN vs Control GTN	< 0.0001	t= 5.280
		2 hours		
	6 vs. 6	Control GTN vs.Control Veh	< 0.0001	t= 6.034
	7 vs. 6	Adv-cre GTN vs Control GTN	< 0.0001	t= 6.411
		4 hours		
	6 vs. 6	Control GTN vs.Control Veh	< 0.0001	t= 5.280
	7 vs. 6	Adv-cre GTN vs Control GTN	< 0.0001	t= 5.531
		6 hours		
	6 vs. 6	Control GTN vs.Control Veh	0.0002	t= 4.274
	7 vs. 6	Adv-cre GTN vs Control GTN	< 0.0001	t= 4.902
One-way ANOVA		**H2O2 level**	< 0.0001	F (3, 20)= 12.29
post hoc analysis	6 vs.6	Control GTN vs.Control Veh	0.004	t= 4.025
	6 vs.6	Adv-cre GTN vs Control GTN	0.0002	t= 5.368

	Statistical test	n		P value	DEGREES OF FREEDOM & F/t values
Fig 6 B	One-way ANOVA			0.0002	F (4, 27)= 8.044
	post hoc analysis	7 vs. 7	GTN vs. Veh	< 0.0001	t= 5.430
Fig 6 C	One-way ANOVA			< 0.0001	F (5, 42)= 32.22
	post hoc analysis	8 vs. 8	GTN vs. Veh	< 0.0001	t= 10.20
		8 vs. 8	Apo GTN vs. GTN	< 0.0001	t= 9.238
		8 vs. 8	gp91 GTN vs. GTN	< 0.0001	t= 5.196
		8 vs. 8	ML171 GTN vs. GTN	0.0002	t= 6.543
		8 vs. 8	gp91+ML171 GTN vs. GTN	< 0.0001	t= 10.58

	Statistical test	n		P value	DEGREES OF FREEDOM & F/t values
Fig 7 A left panel	One-way ANOVA			< 0.0001	F (3, 25)= 16.46
post hoc analysis		8 vs.6	GTN vs. veh	< 0.0001	t= 6.971
		7 vs.8	BIBN GTN vs GTN	0.0449	t= 2.91
Fig 7 A right panel	One-way ANOVA			< 0.0001	F (3, 25) = 51.55
post hoc analysis		8 vs.6	GTN vs. veh	< 0.0001	t= 12.29
		7 vs.8	GTN BIBN vs GTN	< 0.0001	t= 7.126
		8 vs.8	GTN CGRP837 vs. GTN	< 0.0001	t= 6.639
Fig 7 B left panel	One-way ANOVA			< 0.0001	F (3, 20)= 23.27
		6 vs.6	GTN vs. veh	< 0.0001	t= 6.635
post hoc analysis					
Fig 7 B right panel	One-way ANOVA			< 0.0001	F (3, 17)= 29.24
post hoc analysis		6 vs.6	GTN vs. veh	< 0.0001	t= 7.932
Fig 7 C	Two-way ANOVA			< 0.0001	F (15, 168)= 6.653
			0.5 hour		
post hoc analysis		8 vs. 8	CGRP 5µg vs. Veh	< 0.0001	t= 5.364
			1 hour		
		8 vs. 8	CGRP 5µg vs. Veh	< 0.0001	t= 6.954
			2 hours		
		8 vs. 8	GTN 5 µg vs. Veh	< 0.0001	t= 8.146
			4 hours		
		8 vs. 8	GTN 5 µg vs. Veh	< 0.0001	t= 5.166
Fig 7 D	One-way ANOVA			0.0014	F (3, 25)= 7.034
post hoc analysis		8 vs.7	CGRP vs. Veh	0.0007	t= 4.316
		8 vs.7	CGRP8-37 CGRP vs. CGRP	0.0462	t= 2.594
		8 vs.7	BIBN CGRP vs. CGRP	0.008	t= 3.335
Fig 7 E left panel	One-way ANOVA			< 0.0001	F (3, 20)= 25.53
post hoc analysis		6 vs.6	GTN vs. Veh	< 0.0001	t= 6.685
Fig 7 E right panel	One-way ANOVA			< 0.0001	F (3, 21)= 26.56
post hoc analysis		6 vs.6	GTN vs. veh	< 0.0001	t= 8.770
		7 vs.6	GTN BIBN vs GTN	< 0.0001	t= 5.957
		6 vs.6	GTN CGRP837 vs. GTN	0.0003	t= 5.102
Fig 7 F	One-way ANOVA			< 0.0001	F (3, 20)= 39.98
post hoc analysis		6 vs.6	GTN vs. Veh	< 0.0001	t= 8.322
		6 vs.6	BIBN GTN vs Veh	< 0.0001	t= 7.077

	Statistical test	n	P value		DEGREES OF FREEDOM & F/t values
Suppl Fig 1 A	Two-way ANOVA			< 0.0001	F (18, 140)= 5.563
			0.5 hour		
post hoc analysis		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 5.436
		6 vs. 6	GTN KO vs. Veh WT	< 0.0001	t= 5.310
			1 hour		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 6.574
		6 vs. 6	GTN KO vs. Veh WT	< 0.0001	t= 6.447
			2 hours		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 6.447
		6 vs. 6	GTN KO vs. Veh WT	< 0.0001	t= 6.574
			4 hours		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 6.700
		6 vs. 6	GTN KO vs. Veh WT	< 0.0001	t= 6.700
			6 hours		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 5.562
		6 vs. 6	GTN KO vs. Veh WT	< 0.0001	t= 6.447
Suppl Fig 1 B	Two-way ANOVA			< 0.0001	F (18, 140)= 3.628
			0.5 hour		
post hoc analysis		6 vs. 6	GTN WT vs. Veh WT	0.0031	t= 3.900
		6 vs. 6	GTN KO vs. Veh WT	0.0067	t= 3.689
			1 hour		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 5.270
		6 vs. 6	GTN KO vs. Veh WT	0.0006	t= 4.322
			2 hours		
		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 6.008
		6 vs. 6	GTN KO vs. Veh WT	< 0.0001	t= 5.060
			4 hours		
		6 vs. 6	GTN WT vs. Veh WT	0.0021	t= 4.006
		6 vs. 6	GTN KO vs. Veh WT	0.0286	t= 3.268
			6 hours		
		6 vs. 6	GTN WT vs. Veh WT	0.0403	t= 3.162
		6 vs. 6	GTN KO vs. Veh WT	0.0286	t= 3.268
Suppl Fig 1 C	Two-way ANOVA			< 0.0001	F (6, 70)= 6.154
			0.5 hour		
post hoc analysis		6 vs. 6	GTN 10mg vs. Veh	0.0022	t= 3.789
			1 hour		
		6 vs. 6	GTN 10mg vs. Veh	< 0.0001	t= 5.92
			2 hours		
		6 vs. 6	GTN 10mg vs. Veh	< 0.0001	t= 6.204
			4 hours		
		6 vs. 6	GTN 10mg vs. Veh	0.0002	t= 4.452
			6 hours		
		6 vs. 6	GTN 10mg vs. Veh	0.0022	t= 3.789
Suppl Fig 1 D	Two-way ANOVA			0.0001	F (18, 141)= 3.010
			2 hours		
post hoc analysis		6 vs. 6	GTN WT vs. Veh WT	< 0.0001	t= 5.349
		6 vs. 6	GTN KO vs. GTN WT	0.0007	t= 4.442
			4 hours		
		6 vs. 6	GTN WT vs. Veh WT	0.0013	t= 4.298
		6 vs. 6	GTN KO vs. GTN WT	0.0371	t= 3.391
			6 hours		
		6 vs. 6	GTN WT vs. Veh WT	0.0006	t= 4.489
		6 vs. 6	GTN KO vs. GTN WT	0.0392	t= 3.375
Suppl Fig 1 E	One-way ANOVA			< 0.0001	F (2, 15)= 28.47
			4 hours		
post hoc analysis		6 vs.6	GTN vs. veh	< 0.0001	t= 7.261
		6 vs.6	GTN HC03 vs. GTN	0.0002	t= 5.410
	One-way ANOVA			0.0011	F (2, 15)= 11.02
			5 hours		
post hoc analysis		6 vs.6	GTN vs. veh	0.0019	t= 4.287
		6 vs.6	GTN HC03 vs. GTN	0.0052	t= 3.8

	One-way ANOVA			0.0011	F (2, 15)= 11.16
			6 hours		
post hoc analysis		6 vs.6	GTN vs. veh	0.001	t= 4.601
		6 vs.6	GTN HC03 vs. GTN	0.0167	t= 3.233
Suppl Fig 1 F	One-way ANOVA			0.0002	F (2, 16)= 15.37
post hoc analysis		7 vs.6	GTN vs. veh	0.0005	t= 4.838
		7 vs.6	HC03 GTN vs. GTN	0.0008	t= 4.637
Suppl Fig 1G left panel	One-way ANOVA			< 0.0001	F (2, 15)= 19.68
post hoc analysis		6 vs.6	GTN vs. veh	0.0002	t= 5.578
		6 vs.6	A96 GTN vs. GTN	0.0003	t= 5.276
Suppl Fig 1G right panel	One-way ANOVA			< 0.0001	F (2, 15)= 34.16
post hoc analysis		6 vs.6	GTN vs. veh	< 0.0001	t= 7.453
		6 vs.6	GTN A96 vs. GTN	< 0.0001	t= 6.821
Suppl Fig 1H	One-way ANOVA			< 0.0001	F (2, 17)= 36.13
post hoc analysis		6 vs.6	GTN vs. veh	< 0.0001	t= 7.915
Suppl Fig 1I	One-way ANOVA			< 0.0001	F (2, 16) = 21.02
post hoc analysis		7 vs.6	GTN vs. veh	0.0001	t=5.616
Suppl Fig 1J	One-way ANOVA			0.035	F (2, 16)= 8.239
post hoc analysis		7 vs.6	AITC vs. veh	0.0042	t= 3.853
		7 vs.6	HC03 AITC vs.AITC	0.0306	t= 2.911
Suppl Fig 1K left panel	One-way ANOVA			0.016	F (2, 15)= 5.457
post hoc analysis		6 vs.6	GTN vs. veh	0.0172	t= 3.020
		6 vs.6	A96 GTN vs. GTN	0.035	t= 2.669
Suppl Fig 1K right panel	One-way ANOVA			0.0007	F (2, 15)= 12.18
post hoc analysis		6 vs.6	GTN vs. veh	0.0014	t= 4.234
		6 vs.6	GTN A96 vs. GTN	0.0012	t= 4.312
Suppl Fig 1L left panel	One-way ANOVA			< 0.0001	F (3, 20)= 28.32
post hoc analysis		6 vs.6	CAPS vs. Veh	< 0.0001	t= 7.676
		6 vs.6	RTX CPS vs.Veh RTX CPS	< 0.0001	t= 7.217
Suppl Fig 1L right panel	One-way ANOVA			< 0.0001	F (3, 20)=17.52
post hoc analysis		6 vs.6	AITC vs. Veh	< 0.0001	t= 6.099
		6 vs.6	RTX AITC vs. Veh RTX AITC	0.0003	t= 5.090
Suppl Fig 1M left panel	One-way ANOVA			< 0.0001	F (3, 20)= 91.23
post hoc analysis		6 vs.6	MM AITC vs. MM veh	< 0.0001	t= 13.56
		6 vs.6	AS AITC vs. MM AITC	< 0.0001	t= 12.47
Suppl Fig 1M right panel	One-way ANOVA			0.0018	F (3, 20)= 7.191
post hoc analysis		6 vs.6	AITC control vs.Veh control	0.0043	t= 3.989
		6 vs.6	AITC Adv-cre vs AITC control	0.0258	t= 3.220

	Statistical test	n		P value	DEGREES OF FREEDOM & F/t values
Suppl Fig 2 B	One-way ANOVA			< 0.0001	F (2, 21) = 42.56
	post hoc analysis	8 vs. 8	GTN vs. veh	< 0.0001	t= 9.007
		8 vs 8	GTN GKT vs. GTN	0.0339	t= 2.771
Suppl Fig 2 C	One-way ANOVA			< 0.0001	F (2, 21) = 186.0
	post hoc analysis	8 vs. 8	GTN vs. veh	< 0.0001	t= 16.37
		8 vs. 8	GTN GKT+gp91 vs. GTN	< 0.0001	t= 17.02

References

Alsheblak MM, Elsherbiny NM, El-Karef A, El-Shishtawy MM. Protective effects of L-carnosine on CCl4 -induced hepatic injury in rats. Eur Cytokine Netw 2016; 27:6-15.

Chen SR, Pan HL. Spinal nitric oxide contributes to the analgesic effect of intrathecal [d-pen2,d-pen5]-enkephalin in normal and diabetic rats. Anesthesiology 2003; 98:217-22.

Chen Y, Kanju P, Fang Q, Lee SH, Parekh PK, Lee W, *et al.* TRPV4 is necessary for trigeminal irritant pain and functions as a cellular formalin receptor. Pain 2014; 155:2662-72.

da Costa DS, Meotti FC, Andrade EL, Leal PC, Motta EM, Calixto JB. The involvement of the transient receptor potential A1 (TRPA1) in the maintenance of mechanical and cold hyperalgesia in persistent inflammation. Pain 2010; 148:431-7.

De Logu F, Nassini R, Materazzi S, Carvalho Goncalves M, Nosi D, Rossi Degl'Innocenti D, *et al.* Schwann cell TRPA1 mediates neuroinflammation that sustains macrophage-dependent neuropathic pain in mice. Nat Commun 2017; 8:1887.

Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, *et al.* Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. Br J Pharmacol 2000; 129:420-3.

Eid SR, Crown ED, Moore EL, Liang HA, Choong KC, Dima S, *et al.* HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol Pain 2008; 4:48.

Glowka TR, Steinebach A, Stein K, Schwandt T, Lysson M, Holzmann B, *et al.* The novel CGRP receptor antagonist BIBN4096BS alleviates a postoperative intestinal inflammation and prevents postoperative ileus. Neurogastroenterol Motil 2015; 27:1038-49.

Jeong BK, Song JH, Jeong H, Choi HS, Jung JH, Hahm JR, *et al.* Effect of alpha-lipoic acid on radiation-induced small intestine injury in mice. Oncotarget 2016; 7:15105-17.

Jiang JX, Chen X, Serizawa N, Szyndralewiez C, Page P, Schroder K, *et al.* Liver fibrosis and hepatocyte apoptosis are attenuated by GKT137831, a novel NOX4/NOX1 inhibitor in vivo. Free Radic Biol Med 2012; 53:289-96.

Joseph EK, Chen X, Bogen O, Levine JD. Oxaliplatin acts on IB4-positive nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. J Pain 2008; 9:463-72.

Kim AK, Souza-Formigoni ML. Disulfiram impairs the development of behavioural sensitization to the stimulant effect of ethanol. Behav Brain Res 2010; 207:441-6.

Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, *et al.* Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003; 111:1201-9.

Lee EJ, Hung YC, Chen HY, Wu TS, Chen TY. Delayed treatment with carboxy-PTIO permits a 4-h therapeutic window of opportunity and prevents against ischemia-induced energy depletion following permanent focal cerebral ischemia in mice. Neurochem Res 2009; 34:1157-66.

Li YQ, Li XB, Guo SJ, Chu SL, Gao PJ, Zhu DL, *et al.* Apocynin attenuates oxidative stress and cardiac fibrosis in angiotensin II-induced cardiac diastolic dysfunction in mice. Acta Pharmacol Sin 2013; 34:352-9.

Marquez de Prado B, Hammond DL, Russo AF. Genetic enhancement of calcitonin gene-related Peptide-induced central sensitization to mechanical stimuli in mice. J Pain 2009; 10:992-1000.

Materazzi S, Fusi C, Benemei S, Pedretti P, Patacchini R, Nilius B, *et al.* TRPA1 and TRPV4 mediate paclitaxel-induced peripheral neuropathy in mice via a glutathione-sensitive mechanism. Pflugers Arch 2012; 463:561-9.

Mogil JS, Miermeister F, Seifert F, Strasburg K, Zimmermann K, Reinold H, *et al.* Variable sensitivity to noxious heat is mediated by differential expression of the CGRP gene. Proc Natl Acad Sci U S A 2005; 102:12938-43.

Nassini R, Materazzi S, Andre E, Sartiani L, Aldini G, Trevisani M, *et al.* Acetaminophen, via its reactive metabolite N-acetyl-p-benzo-quinoneimine and transient receptor potential ankyrin-1 stimulation, causes neurogenic inflammation in the airways and other tissues in rodents. Faseb J 2010; 24:4904-16.

Nassini R, Gees M, Harrison S, De Siena G, Materazzi S, Moretto N, *et al.* Oxaliplatin elicits mechanical and cold allodynia in rodents via TRPA1 receptor stimulation. Pain 2011; 152:1621-31.

Nozaki-Taguchi N, Yamamoto T. Involvement of nitric oxide in peripheral antinociception mediated by kappa- and delta-opioid receptors. Anesth Analg 1998; 87:388-93.

Rossato MF, Velloso NA, de Oliveira Ferreira AP, de Mello CF, Ferreira J. Spinal levels of nonprotein thiols are related to nociception in mice. J Pain 2010; 11:545-54.

Trevisan G, Benemei S, Materazzi S, De Logu F, De Siena G, Fusi C, *et al.* TRPA1 mediates trigeminal neuropathic pain in mice downstream of monocytes/macrophages and oxidative stress. Brain 2016; 139:1361-77.

Wei H, Koivisto A, Saarnilehto M, Chapman H, Kuokkanen K, Hao B, *et al.* Spinal transient receptor potential ankyrin 1 channel contributes to central pain hypersensitivity in various pathophysiological conditions in the rat. Pain 2011; 152:582-91.

Yilmaz Z, Kalaz EB, Aydin AF, Soluk-Tekkesin M, Dogru-Abbasoglu S, Uysal M, *et al.* The effect of carnosine on methylglyoxal-induced oxidative stress in rats. Arch Physiol Biochem 2017; 123:192-98.

Zappia KJ, O'Hara CL, Moehring F, Kwan KY, Stucky CL. Sensory Neuron-Specific Deletion of TRPA1 Results in Mechanical Cutaneous Sensory Deficits. eNeuro 2017; 4:0069-16.