Supplemental Table S1: Single cell qRT-PCR of trigeminal ganglion neurons retrogradely labeled from the conjunctiva or cornea.

Conjunctiva	Receptors/channels					Neuropeptides/cytokines			
Sample	*Ntrk2* (TrkB)	*Ntrk3* (TrkC)	*Mrgprd*	*Mrgpra3*	*Trpm8*	*Sst*	*Calca* (CGRP*)	*Tac1* (SP)	*Fam19a1*
CJ28	0	10.23184082	0	0	0	0	64.30677702	0	14.09304737
CJ18	0	5.707343085	0	0	0	0	0	0	0
CJ10	0.085879066	1.016409221	0	0	0	0	0	0.087395318	0.002279768
CJ16	0	0.722126268	1.048730198	0	0	0	2.919881624	0.074420034	0
CJ25	0.225820536	0.411268104	0	0	0	0	2.552619146	0.021943142	0
CJ24	0.266985867	0.335063475	0	0	0	0	3.827902159	0.079665958	0
CJ21	0	0	2.209927591	0	0	0	2.251399601	0.126659545	0
CJ19	0	0.095979986	2.051702233	0	0	0	1.150941178	0.042902195	0
CJ26	0	0	1.307184847	0	0	0	0	0	0.181684938
CJ1	0	0	0.879209912	0.329880379	0	0.064804958	0.83359204	0.065648145	0
CJ34	0	0	0	1.915913132	0	0	0	0.047044501	0.013859186
CJ27	0	0.776110815	0	1.188974449	0	0	9.057395288	0	0
CJ6	0	0	0	0	0	0	75.71783945	3.010820199	0
CJ15	0	0	0	0	0	0	53.92762435	1.117258955	0
CJ3	0	0	0	0	0	0	17.85245451	0.191096007	0.609192181
CJ9	0	0	0	0	0	0	11.95441468	0.22554216	0
CJ4	0	0	0	0	0	0	0	0	2.178015846
CJ12	0	0	0	0	0	0	0.035270372	0	0.383370638
CJ11	0	0	0	0	0	0.012138585	0.023256321	0	0.29206771
CJ32	0	0.123281494	0.094475535	0	0.3450744	0	0.003112141	0.18018701	0
CJ17	0.001171559	0	0	0	0	0	2.183398506	0.095114821	0
CJ7	0	0.043961776	0	0	0	0	1.267141406	0.006361483	0
CJ13	0	0	0	0	0	0.059047597	0	0	0
CJ23	0	0	0	0	0	0	0.002872462	0.005782444	0
CJ29	0	0	0	0	0	0	0	0.042729831	0
CJ30	0	0	0	0	0	0	0	0	0
CJ31	0	0.010926249	0.068497651	0	0	0	0	0.216181027	0
CJ33	0	0	0	0	0	0.017376418	0	0.014233937	0
CJ35	0.063617439	0	0	0	0	0	0	0	0
CJ36	0	0	0	0	0	0	0	0	0
CJ37	0	0	0.036092427	0	0	0	0	0.106970986	0
CJ38	0	0	0	0	0	0	0	0.123055312	0

Cornea	Receptors/channels					Neuropeptides/cytokines			
Sample	*Ntrk2* (TrkB)	*Ntrk3* (TrkC)	*Mrgprd*	*Mrgpra3*	*Trpm8*	*Sst*	*Calca* (CGRP*)	*Tac1* (SP)	*Fam19a1*
CR26	1.269327821	0	0	0	0	0.053758214	4.257599384	87.56103496	0
CR23	1.036381834	0	0	0	0	0	24.74093381	995.4859374	0
CR36	0	0.140035463	0.013344142	0	4.17915	0.175379508	0	0.306099437	0
CR31	0.005992392	0	0	0	0.9649502	0	0	3.846688128	0.004487782
CR22	0	0.188712405	0	0	0.7313721	0	1.873590399	20.75629208	0.826478946
CR30	0.105119278	0	0	0	0	0.044922038	0.427916188	30.58461784	2.182437476
CR24	0	0.277049977	0	0	0	0	0.404154245	19.60114128	0.910380782
CR1	0	0.254008931	0	0	0	0	0	0.325270763	0
CR13	0	0	0	0	0	0.018989119	2.599649059	27.65350729	0.001410002
CR12	0	0	0	0	0	0.028444021	1.206485922	32.59720082	0
CR15	0	0	0	0	0	0	0.526719791	18.31495313	0.27027379
CR29	0	0	0	0	0	0	0.528509691	10.2540089	0.734153397
CR28	0	0	0	0	0	0.029363732	0.042180505	1.423224156	0
CR10	0	0	0	0	0	0.000180839	0.0229345	0.737879265	0
CR3	0	0	0	0	0	0.12424425	0	0	0
CR4	0	0	0	0.003793388	0	0	0	0	0.003870113
CR5	0	0	0	0	0	0.159347505	0	0	0
CR7	0	0	0	0	0	0	0	0	0
CR8	0	0	0	0	0	0.038742951	0	0	0
CR19	0	0	0	0	0	0	0.008447362	0	0
CR32	0	0	0	0	0	0	0.006106275	0	0
CR33	0.003148709	0	0	0	0	0	0	0	0
CR34	0.006134872	0	0	0	0	0	0	0.144837325	0.013036401
CR35	0.052027298	0	0	0	0	0	0	0.209803181	0
CR37	0	0	0	0	0	0	0	0	0.014634097

Note:

***CGRP has two forms in mice: α- and β-CGRP, which are encoded by *Calca* and *Calcb*, respectively.**

Expression level is presented as folds of *Gapdh* expression, calculated using 2^-(Ct(target Gene)-Ct(Gapdh)).

Numbers marked in red stand for data points significantly higher than others in the group shown in Fig. 1s

Supplemental Table S2: The axonal projection of various populations of primary sensory neurons to the conjunctiva, cornea and skin

Table 1: Mouse lines	Conjunctiva	Cornea	Back Skin
Pirt $^{GCaMP3/+}$	+++++	+++++	+++++
Nav1.8: Scn10a$^{cre/+}$*; Rosa26* $^{tdT/+}$	+++	+++++	++++
Trpv1 $^{ALPP/+}$	+++	++++	+++
Trpm8 $^{gfp/+}$	+	++++	++
Mrgprd$^{egfp/+}$	+	absent	+++
Mrgpra3 $^{cre/+}$*; Rosa26* $^{tdT/+}$	+++	absent	+++
Sst $^{cre/+;}$ *Rosa26* $^{tdT/+}$	absent	absent	+++
Vglut3: Slc17a8 $^{cre/+}$*; Rosa26* $^{tdT/+}$	absent	++	+++
CGRP (immunofluorescence)	+++	++++	+++

Supplemental Figures

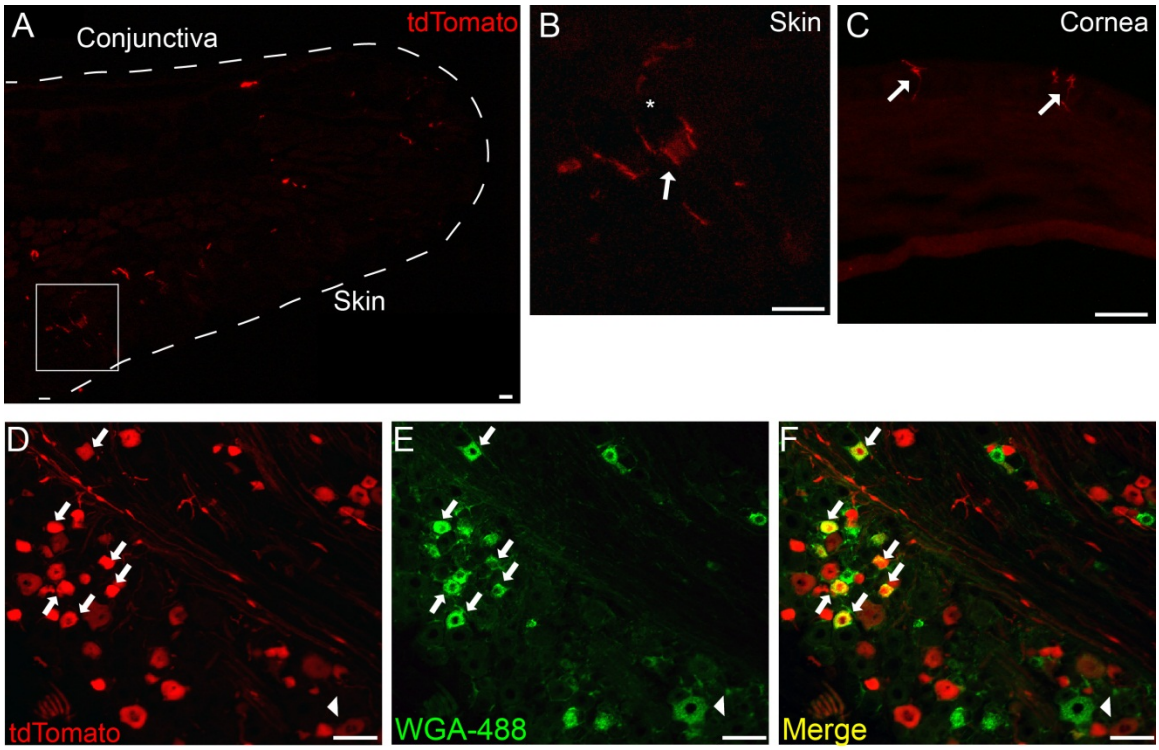

Fig. S1: Low-threshold mechanosensitive C fiber neurons that express vesicular glutamate transporter 3 (VGLUT3, gene *Slc17a8*) do not innervate the conjunctiva (**A**) *Slc17a8*$^{cre/+}$*; Rosa26*$^{tdTomato/+}$ (*Slc17a8*$^{tdTomato/+}$) sensory fibers in a section of an eyelid. (**B)** High magnification view of boxed area in (**A**). Asterisk indicates a hair follicle. Arrow indicates *Slc17a8*$^{tdTomato/+}$ sensory fibers (**C**) *Slc17a8*$^{tdTomato/+}$ sensory fibers in a section of cornea. Arrows indicate VGLUT3^{+} sensory fibers. (**D**-**F**) WGA-mediated retrograde labeling of corneal afferent neurons in *Slc17a8*$^{tdTomato/+}$ mice. Arrows indicate co-localization of tdTomato^{+} neurons (red) and WGA (green) in the section of trigeminal ganglion. Arrowhead indicates a large diameter neuron labelled only by WGA. Representative images shown were chosen from 3 trigeminal ganglia imaged from three mice. Scale bars: 50 μm.

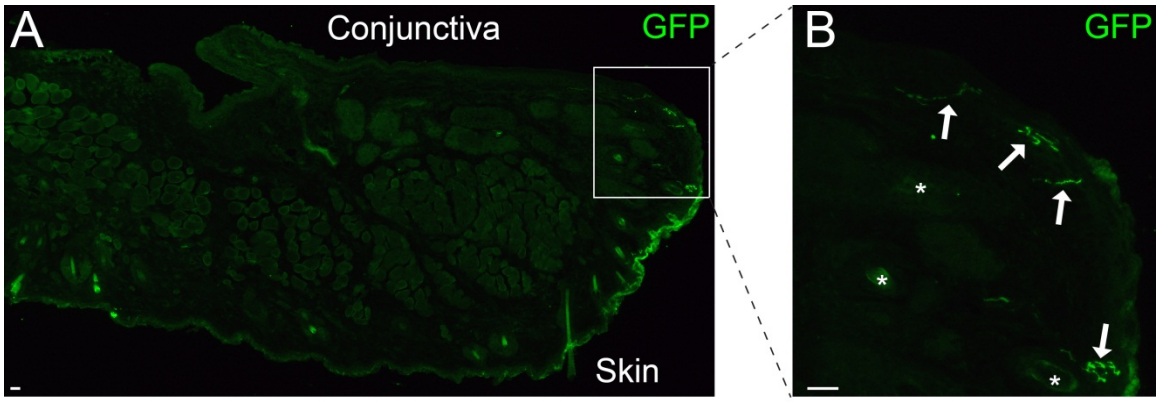

Fig. S2: Sparse innervation of TRPM8^{+} sensory fibers in the conjunctiva (**A**) *Trpm8*$^{gfp/+}$ sensory fibers in a section of an eyelid. (**B**) High magnification view of boxed area in (**A**). Arrows indicate GFP^{+} fibers. Asterisks indicate hair follicles in the eyelid skin. Representative images were chosen from 6 conjunctivae imaged from three mice. Scale bars: 50 μm.

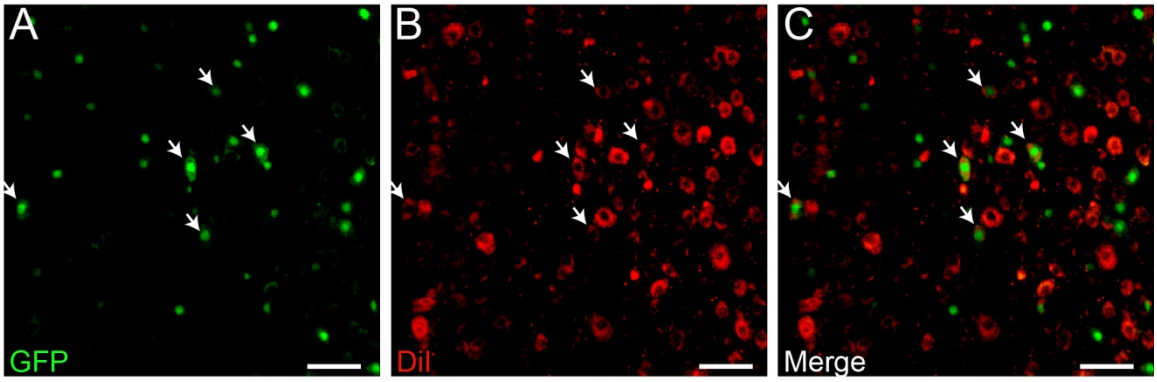

Fig. S3: A group of conjunctival afferent neurons express MrgprA3. (**A-C**) DiI-mediated retrograde labeling of conjunctival afferent neurons in *MrgprA3*$^{gfp\text{-}cre}$ mice. Arrows indicate co-localization of GFP^{+} neurons (green) and DiI (red) in the section of trigeminal ganglion. Representative images shown were chosen from 3 trigeminal ganglia imaged from 3 mice. Scale bars: 50 μm.

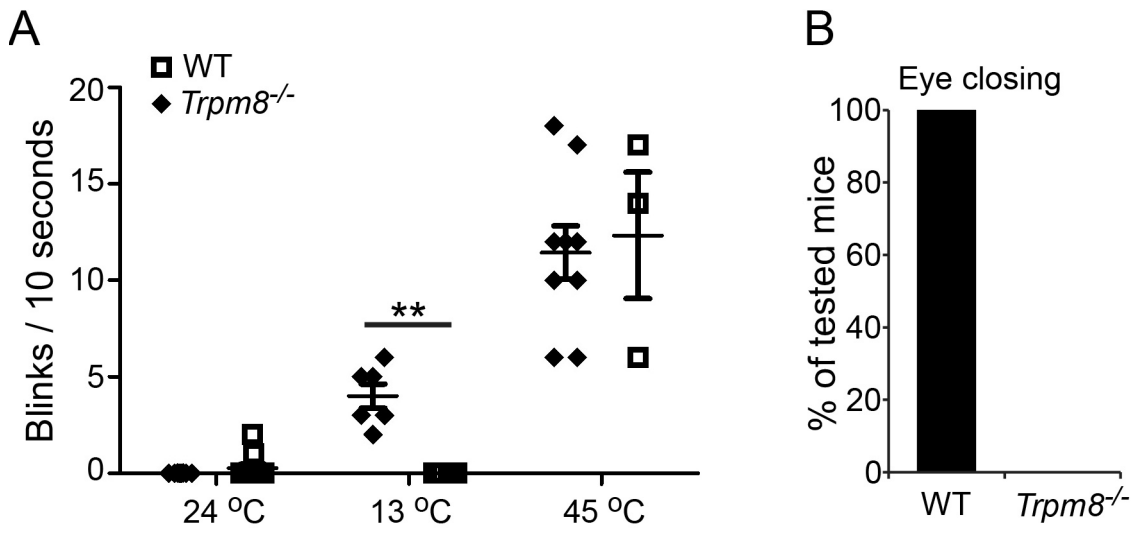

Fig. S4: TRPM8 mediates ocular pain induced by the cold temperature. (**A**) Blinking responses to air flow (0.5 L/min) at different temperatures in WT (n=11, 6, 9, respectively) and *Trpm8*$^{-/-}$ mice (n=11, 8, 3, respectively). Data are expressed as mean±s.e.m Statistical analysis by two tailed Student's t-test (**P=0.0015). (**B**) Eye-closing behavior to air flow (0.5 L/min) at different temperature in WT (n=6) and *Trpm8*$^{-/-}$ mice (n=3).

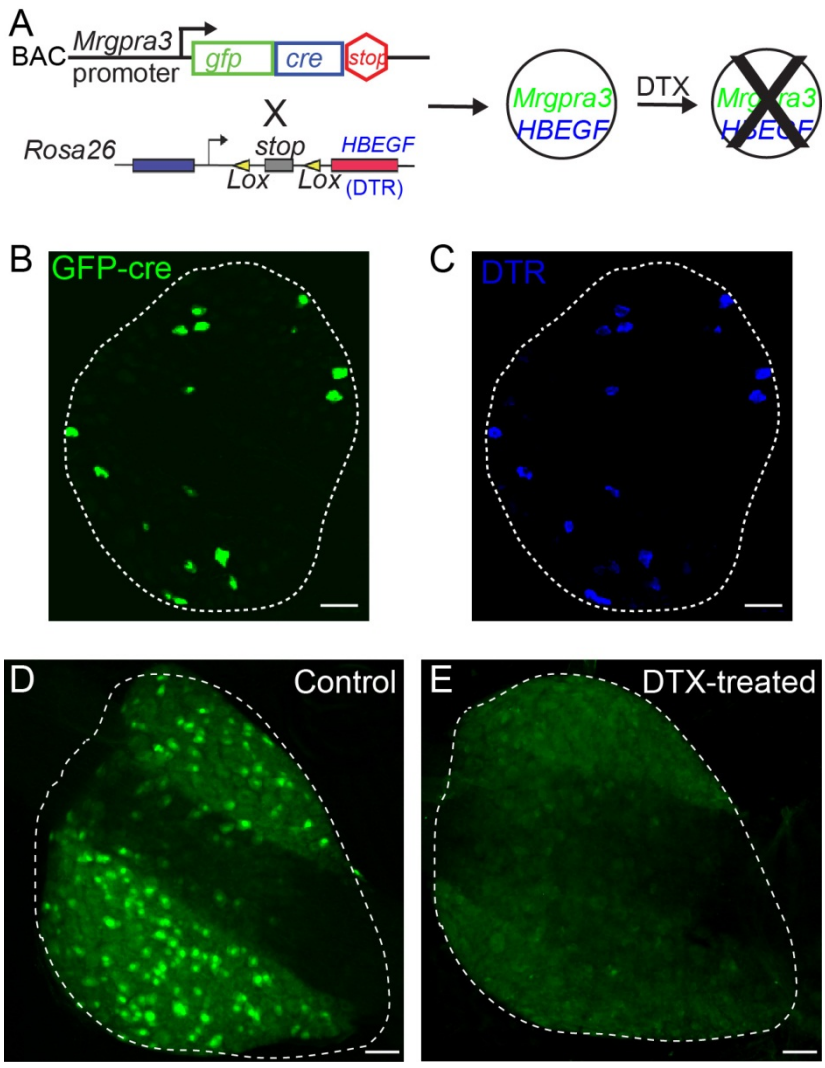

Fig. S5: Genetic- and temporal-specific ablation of MrgprA3^{+} neurons. (**A**) Combining Cre-Lox and diphtheria toxin receptor (DTR, gene *HBEGF*) approaches to ablate MrgprA3^{+} neurons. (**B-C**) All *Mrgpra3*$^{gfp\text{-}cre}$ neurons (green) express DTR (blue). No ectopic DTR expression was detected. (**D-E**) Stacked confocal microscopy images of the whole DRG from *Mrgpra3*$^{gfp\text{-}cre}$*; Rosa*$^{HBEGF/+}$ mice before (**D**) and after (**E**) diphtheria toxin treatment. Representative images were chosen from DRGs imaged from 3 mice. Scale bars: 50 μm.

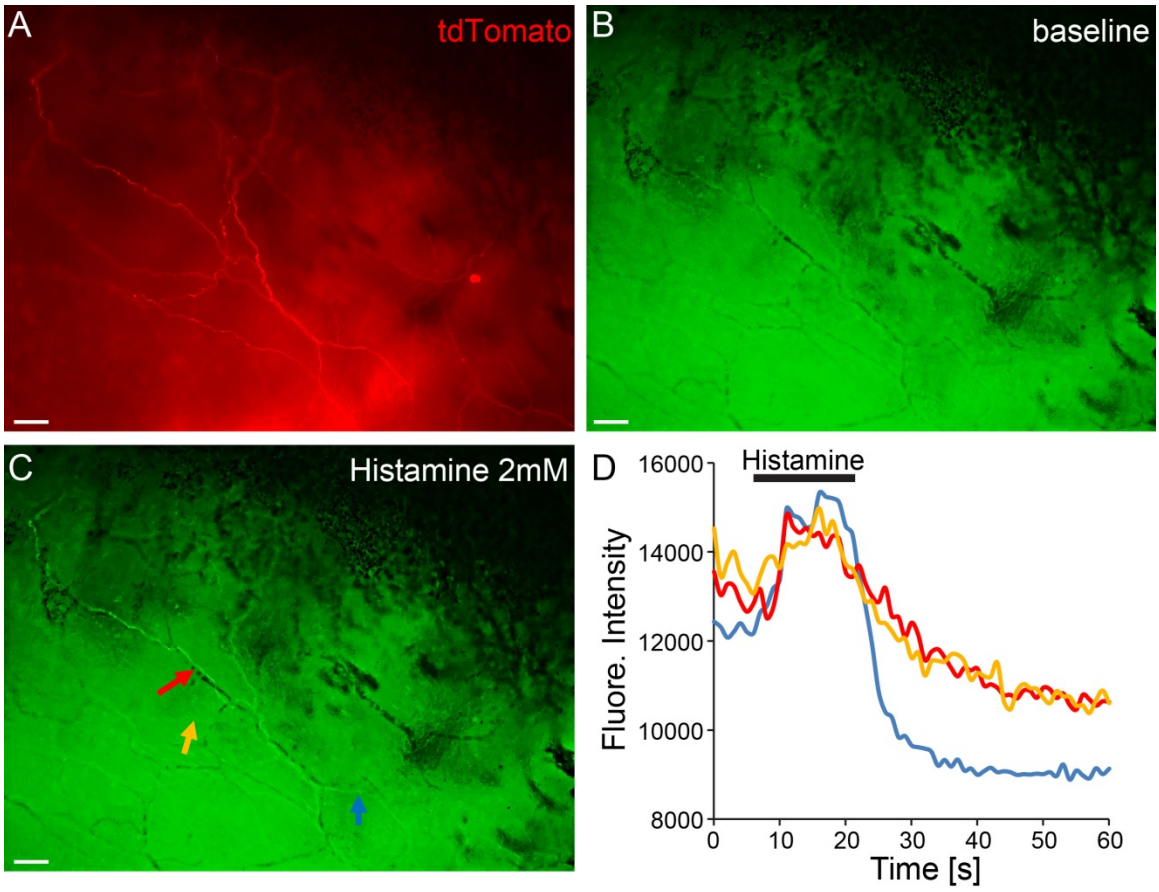

Fig. S6: MrgprA3^{+} conjunctival fibers response to histamine. (**A**) *Mrgpra3*$^{gfp\text{-}cre/+}$; *Rosa26*$^{tdTomato/+}$; *Pirt*$^{GCaMP3/+}$ sensory fibers (red) in the conjunctiva. (**B**) Baseline of GCaMP3 fluorescence in the conjunctiva explant. (**C**) Increased GCaMP3 fluorescence upon 2 mM histamine stimulation. Arrows indicate activated MrgprA3^{+} sensory fibers. (**D**) Dynamics of GCaMP3 fluorescence upon histamine stimulation in MrgprA3^{+} sensory fibers from (**C**). Representative images were chosen from 5 conjunctival explants imaged from 3 mice. Scale bars: 50 μm.

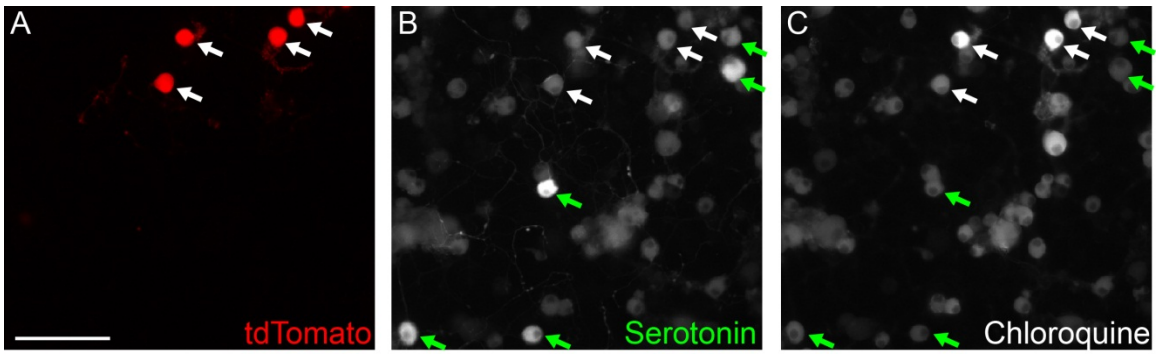

Fig. S7: MrgprA3^{+} DRG neurons respond to chloroquine but not serotonin. (**A**) DRG neurons cultured from *Mrgpra3*$^{cre/+}$; *Rosa26*$^{tdTomato/+}$; *Pirt*$^{GCaMP3/+}$ mice. White arrows indicate MrgprA3^{+} DRG neurons. (**B**) Increased GCaMP3 fluorescence upon 10 μM serotonin stimulation. (**C**) Increased GCaMP3 fluorescence upon 1 mM chloroquine stimulation. Green arrows indicate serotonin-sensitive neurons, which do not express MrgprA3 and are insensitive to chloroquine. The experiment was repeated independently three times with similar results. Scale bars: 100 μm.

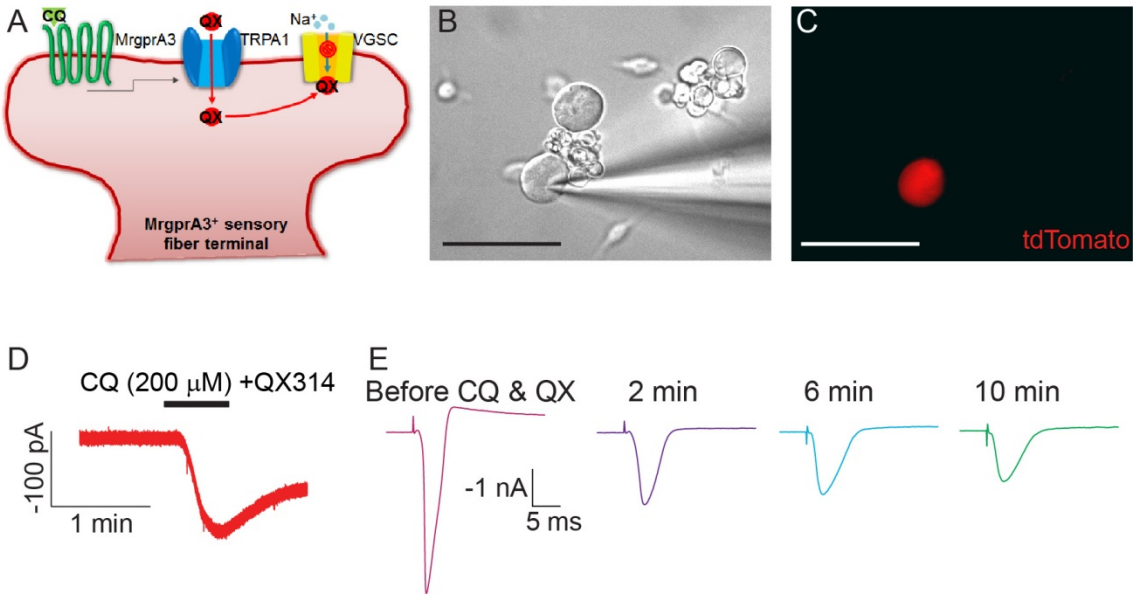

Fig. S8: Chloroquine-mediated entry of QX-314 decreases inward sodium current in MrgprA3^{+} neurons. (**A**) Diagram depicting the action model of QX-314 in MrgprA3^{+} neurons. (**B**) Representative photomicrograph of a recorded sensory neuron from *Mrgpra3*$^{gfp\text{-}cre/+}$*; Rosa26*$^{tdTomato/+}$ (*Mrgpra3*$^{tdTomato/+}$) mice. (**C**) The presence of tdTomato (red) indicates that the recorded neuron is MrgprA3^{+}. Scale bars: 100 μm. (**D**) Inward currents triggered by application of chloroquine and QX-314 (**E**) Representative traces of inward sodium current recorded before and after application of CQ and QX-314.

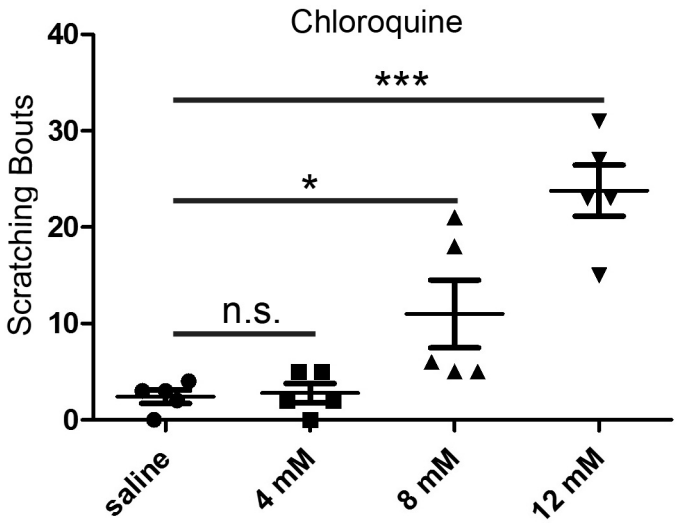

Fig. S9: Dose-dependent ocular scratching behavior evoked by topical chloroquine application in the conjunctiva sac of WT mice (n=5/group). All data are expressed as mean±s.e.m. Statistical analysis by two tailed Student's *t*-test (4 mM vs. saline, P=0.744; 8 mM vs. saline, *P=0.04; 12 mM vs. saline, ***P=0.0009).

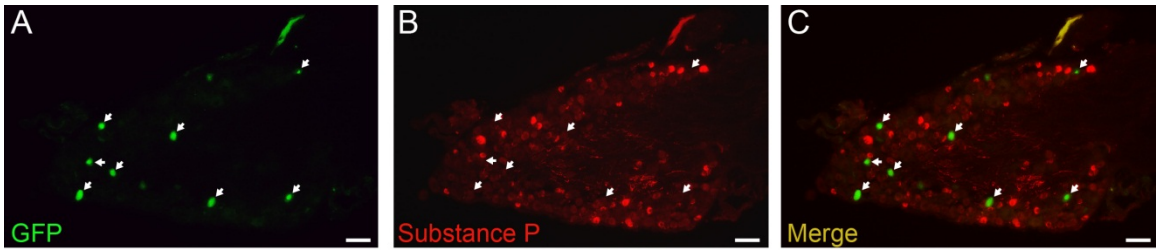

Fig. S10: MrgprA3^{+} neurons do not express substance P. (**A**) DRG section of *Mrgpra3*$^{gfp\text{-}cre/+}$ mouse. (**B**) Immunofluorescence of substance P. (**C**) Merged image of (**A**) and (**B**). Arrows indicate MrgprA3^{+} DRG neurons, which do not overlap with substance P immunofluorescence signals. Representative images were chosen from DRGs imaged from 3 mice. Scale bars: 50 μm.