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# **Substance P activates Mas-related G-protein coupled receptors to induce itch**

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## **Abstract**

**Background:** Substance P is linked to itch and inflammation via activation of receptors on mast cells and sensory neurons. There is increasing evidence that substance P functions through Mas-related GPCRs in addition to its conventional receptor, neurokinin-1.

## **Objective:**

As Mrgprs mediate some aspects of inflammation that had been considered mediated by NK-1R, we sought to determine if itch induced by substance P may also be mediated by Mrgprs.

**Methods:** Genetic and pharmacologic approaches were used to evaluate the contribution of Mrgprs to substance P-induced scratching behavior and activation of cultured dorsal root ganglion neurons from mice.

**Results:** Substance P-induced scratching behavior and activation of cultured dorsal root ganglion neurons was dependent on Mrgprs rather than NK-1R.

**Conclusion:** It is deduced that substance P activates MrgprA1 on sensory neurons rather than NK-1R to induce itch.

**Clinical Implications:** Mrgprs may be important target for treating histamine-independent itch.

**Capsule summary:** Mrgprs are increasingly recognized as mediating the inflammatory effects of substance P. Here, itch induced by substance P is now likewise linked to activation of Mrgprs on sensory neurons.

**Key words:** substance P, Mrgprs, dorsal root ganglion neurons, calcium imaging, receptor antagonist, knockout mice.

## Introduction

Recognition that itch is of major medical significance and poorly served by existing therapeutic options is driving research into the basic mechanisms that underlie this sensory phenomenon. Progress is being made on the identification of peripheral and central mediators, receptors and channels that contribute to the sensation of itch and scratching behavior. Substance P (SP) has long been established as an inflammatory neuropeptide and potent endogenous pruritogen in mice and humans. The classic receptor for SP is the neurokinin-1 receptor,

NK-1R. Several antagonists of this receptor have been developed to inhibit nociceptive and pro-inflammatory properties of SP. These compounds are effective in animal models of several diseases but have proved to be surprisingly ineffective with respect to inflammatory diseases in humans. With respect to itch, there are both positive<sup>1-3</sup> and negative reports<sup>4,5</sup> on the effectiveness of NK-1R antagonists. It has been proposed that nerves are the main drivers of SP-induced itch in mice<sup>6</sup>. Recent studies have demonstrated that SP activates Mrgprs in addition to NK-1R but the interaction of SP with Mrgprs on nerves has not been evaluated. Here we demonstrate that SP activates Mrgpr receptors on dorsal root ganglion neurons and not NK-1R to induce itch.

Mas-related G-protein coupled receptors (Mrgprs) on human and rodent small primary sensory neurons are implicated in nociception<sup>7</sup>. The concept of Mrgprs serving as innate environmental sensors in general, and specifically as sensors for pruritogens, is supported by a number of lines of evidence. Mrgprs first appear in tetrapods<sup>8</sup>, animals endowed with the capacity to remove exogenous agents by scratching while navigating on land. Mrgprs demonstrate high constitutive activity and respond to several ligands, many of which are pruritogens<sup>8-11</sup>. In addition, Mrgprs are expressed on peripheral terminals of sensory neurons and mast cells, consistent with a potential role in sensing<sup>8,9,12,13</sup>.

We recently identified an antagonist of human MRGPRX2 and the homologous mouse receptors MrgprA1 and MrgprB2<sup>14</sup>. This antagonist is termed QWF<sup>6</sup>. We also found that while SP-induced itch remained intact in neurokinin 1 receptor knockout mice (*NK-1R*<sup>-/-</sup> mice), QWF significantly decreased SP-induced itch in both wildtype and *NK*-

1R<sup>-/-</sup> mice. As QWF was found to inhibit SP-induced degranulation from mast cells, we suggested that SP-induced mast cell degranulation and SP-induced itch, at least in the periphery, is mediated via Mrgprs<sup>14</sup>. While an interaction of SP with mast cells in the context of inflammation has long been established, SP-induced itch is not significantly decreased in mast cell deficient mice<sup>6</sup>. A reasonable interpretation of these data is that SP interacts with a receptor on sensory nerves to evoke itch<sup>6</sup>.

Mrgprs are primarily expressed on dorsal root ganglion (DRG) neurons and are implicated in itch and nociception<sup>15</sup>. Here, we evaluated the interaction of SP with cultured murine DRG neurons. We deduce that MrgprA1 is responsible for SP-induced scratching behavior.

## Methods

**Peptides and chemicals:** SP was obtained from GenScript and dissolved in 1X PBS. The NK-1R antagonist L733060 and the dual NK-1R/Mrgprs antagonist QWF (Boc-Gln-D-Trp(Formyl)-Phe benzyl ester trifluoroacetate), were obtained from Sigma. QWF and L733060 were dissolved in DMSO (Sigma) at 10 mM and diluted into PBS for use at 500 µM or lower concentrations. In the nociceptive studies with capsaicin, where QWF was diluted in DMSO, the final concentration of DMSO was 0.01%. Capsaicin was obtained from Tocris Bioscience.

**Animals:** Male and female *Mrgpr cluster* Δ<sup>-/-</sup>, *NK-1R*<sup>-/-</sup>, *TRPV1-Cre*<sup>+/-</sup>DTA<sup>+/-</sup> (which TRPV1<sup>+</sup> sensory neurons are ablated<sup>16</sup>) or wildtype mice were used. Mice

were 2-8 months old, 20-30 g, and 4-7 included per group, depending on the experiment.

*Mrgpr cluster*  $\Delta^{-/-}$  mice on a C57BL/6 background were generously provided by Xinzhong Dong, Johns Hopkins, Baltimore, MD. *NK-1R* $^{-/-}$  mice on a C57BL/6 background were generously provided by Norma Gerard, Children's Hospital, Boston, MA. The wildtype mice used for the scratching behavior experiments are littermates of the *Mrgpr cluster*  $\Delta^{-/-}$  mice. The wildtype mice used for DRG culture and pain assessment, and the TRPV1-Cre $^{+/-}$ DTA $+/-$ <sup>16</sup> and its littermate control mice were obtained from JAX. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Massachusetts General Hospital and Boston Children's Hospital.

*Scratching behavior:* The mouse cheek model was used in this study<sup>17</sup>. The animals were habituated for 30 minutes/day for 3 days prior to and for 15 minutes on the day of the experiment. Using a BD insulin syringe with 31G needle, mice were injected intradermally (i.d.) with 10  $\mu$ l containing SP (500  $\mu$ M) alone or in combination with L733060 (500  $\mu$ M) or QWF (500  $\mu$ M) into the right cheek. Mice were not shaved prior to injections to minimize irritation. All experiments were performed at consistent times during the day (9:00 am to 2:00 pm). Mice were videotaped in a soundproof environment to minimize distraction. A blinded investigator scored the recordings for the number of scratching bouts that occur over 1 minute intervals during 25 minute observation periods. A scratching bout was initiated by lifting the hind paw to the area of injection, and ended by

returning the hind paw to the floor or to the mouth. The concentrations of SP were selected based on the literature for behavioral tests<sup>6, 14</sup>.

*Pain assessment:* Thermal latency threshold was assessed in wildtype mice using the Hargreaves' test (Plantar Test Apparatus; Ugo Basile, Comerio, Italy) 10 min after an intraplantar (i.pl.) injection of 10 µl containing capsaicin (100 µM; prepared in DMSO) into the left hind paw<sup>18</sup>. A separate group of mice received a co-injection of 10 µl containing capsaicin (100 µM) and QWF (1 µM). The control group received 10 µl of the respective vehicle (DMSO).

*DRG culture and calcium imaging:* Cervical to lumbar dorsal root ganglia from wildtype, *NK-1R*<sup>-/-</sup>, *TRPV1-Cre*<sup>+/-</sup>DTA<sup>+/-</sup> and its littermate control mice, were dissected and pooled from groups of 4 mice and maintained in DMEM (Gibco), containing 200 mM L-glutamine (Fisher Scientific), 10% heat inactivated fetal bovine serum (Gibco), 5000 U/ml penicillin and 5000 µg/ml streptomycin (Fisher Scientific). Enzymatic digestion was carried out with 1 mg collagenase in 1 ml dispase (Roche Applied Sciences), at 37°C for 70 min. The collagenase/dispase solution was removed and DRG were washed and suspended in DMEM containing 125 U DNase (Sigma) within which the ganglia were mechanically triturated using fire-polished glass pipettes. These cells were centrifuged over a 10 % bovine serum albumin solution (Sigma) gradient, pelleted at 1000 RPM and suspended in Neurobasal medium (Gibco) supplemented with B27® (Invitrogen), nerve growth factor (NGF; Invitrogen), glial cell-derived neurotrophic factor

(GDNF; Sigma) and arabinocytidine (Ara-C; Sigma). The cells were plated onto glass bottom 35 mm dishes coated with 10 µg/ml laminin (Sigma) and cultured for 24 h.

For calcium imaging experiments, neurons were loaded for 30 min with 10 µM of Fura-2 AM (Life Technologies) in Neurobasal medium, washed with Standard Extracellular Solution (SES; 145 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM glucose, 10 mM HEPES, pH 7.5), and imaged at room temperature. Cells were evaluated using a Nikon Eclipse Ti inverted microscope equipped with an Exi Aqua CCD camera (QImaging). Ca<sup>2+</sup> flux fluorescence was measured as an absorbance ratio at 340 nm and 380 nm (F340/380) (Lambda DG4, Sutter Instruments). The 340/380 ratiometric images were analyzed using Nikon Elements AR Software (Nikon). SP (10, 30, 100, 300 and 1000 nM) and QWF (1 µM) solutions were delivered directly onto neurons at a flow rate at 2 ml/min for 20 seconds using perfusion barrels followed by buffer washout and further application. 1 µM allyl isothiocyanate (AITC; Sigma), 1 µM capsaicin (Tocris Bioscience) and 40 mM KCl (Sigma) were applied at the end of each experiment.

*PCR of human dorsal root ganglion (hDRG) total RNA:* Human DRG total RNA (from 16-65 year olds) was purchased from Clontech Laboratories (cat # 636150) and converted into ss-cDNA using random hexamers and reverse transcriptase using the SuperScript III First strand Synthesis kit (Invitrogen). PCR was carried out with the cDNA as a template and primer pairs of hMRGPRX2: forward

primers F1 (CTGTGATGACCTGTGCCTACCTTGC) and F2  
(TCAGCGGTCGTGTGTGTCCTGCTCTGGG), and reverse primers R1  
(CCGCCACTGCTTCCTAAAAGAGCCC) and R2  
(GAAGAAGTAAATGATGGGGTTGGCAC). The primer pairs F1+R1 and F2+R2  
yielded the expected PCR products 551 bp and 408 bp, respectively. The PCR  
was carried out using New England Biolabs Phusion High-Fidelity DNA  
polymerase at the conditions: denaturing at 98<sup>0</sup> C, annealing at 55<sup>0</sup> C and  
extension at 72<sup>0</sup> C.

*Statistical analysis:* Group data are presented as mean ± standard error mean (S.E.M)  
of 6 to 7 animals depending on the study. This number of mice has been demonstrated  
via power calculation to generate significance in behavioral studies<sup>14</sup>. For statistical  
comparison, two-tailed paired or unpaired student's t-test or one-way analysis of  
variance (ANOVA) followed by Tukey's test was used to determine significance.  
Differences were considered statistically significant when  $P < 0.05$ . Data analysis was  
performed using Prism 6 (GraphPad).

## Results

### SP-induced itch behavior is dependent on Mrgprs

We first asked if Mrgprs played a role in SP-induced scratching behavior. We used  
the mouse cheek model<sup>17</sup> to evaluate the pruritogenic effect of SP in *Mrgpr cluster Δ<sup>-/-</sup>*  
mice in which 12 Mrgprs have been knocked out<sup>10</sup>. SP-induced itch was significantly  
decreased in these animals as compared to wildtype mice (Figure 1A). This result is

consistent with previous studies suggesting a role for DRG neurons in addition to mast cells, in SP-induced itch<sup>6, 14</sup>. To confirm these previous studies, we evaluated itch behavior in wildtype mice that were injected with SP together with L733060. L733060 is a widely used NK-1R antagonist that additionally blocks SP-induced activation of MrgprB2<sup>14</sup>. L733060 had no significant effect on SP-induced itch, implying a minimal if any role for MrgprB2 in SP-induced itch (Figure 1B). Furthermore, MrgprB2 is the only mouse Mrgpr expressed on mast cells, and is intact in *Mrgpr cluster Δ<sup>-/-</sup>* mice<sup>10, 13</sup>.

In contrast, QWF, which is an antagonist of NK-1R, MrgprB2, and MrgprA1, but not other mouse Mrgprs<sup>14</sup>, inhibited SP-induced itch (Figure 1B). MrgprA1 is expressed on sensory nerves, but not mast cells<sup>19</sup>. This result is consistent with SP-induced itch being dependent on MrgprA1 on sensory neurons.

We next sought to demonstrate that the effect of QWF is restricted to itch responses. QWF exposure to cultured DRG neurons had no effect on the relative number of capsaicin and KCl sensitive neurons or the amplitude of response induced by these stimuli (Figure 2A, Supplementary Figure 1A-C). QWF also failed to impact capsaicin-induced thermal hyperalgesia *in vivo* (Figure 2B). These findings are consistent with the activity of QWF on SP-induced itch being specific to the action of the ligand on these neurons.

#### **SP activates DRG neurons from *NK-1R<sup>-/-</sup>* mice**

The possibility that SP interacts with a receptor other than NK-1R on DRG neurons has not been reported. To explore this possibility we evaluated the effect of SP on cultured DRG neurons from wildtype and *NK-1R<sup>-/-</sup>* mice. SP activated cultured DRG

neurons from  $NK-1R^{-/-}$  mice in a concentration-dependent manner (Figure 3A-C). These activated cells were of small diameter and sensitive to capsaicin, consistent with TRPV1<sup>+</sup> sensory neurons (Figure 3D). This response was abolished in DRG cultures from TRPV1-Cre<sup>+/-</sup>DTA<sup>+/-</sup> mice from which the TRPV1 sensory neurons have been ablated<sup>16</sup> (Figure 3E). These results indicate that SP can activate pruriceptors through a receptor other than NK-1R.

#### **QWF blocks SP-induced calcium flux in $NK-1R^{-/-}$ mice DRG neurons**

To establish an *in vitro* correlate of the capacity of QWF to block SP-induced itch, we examined the capacity of QWF to prevent SP activation of DRG neurons from mice. QWF abolished SP-induced calcium flux in DRG neurons from  $NK-1R^{-/-}$  mice (Figure 4A-B). Repeated treatment with SP was used to rule out the concern that the effects observed were due to desensitization. Such treatment led to similar calcium flux amplitude and numbers of responsive neurons (Supplementary Figure 2). These data are consistent with QWF antagonism of SP-induced calcium flux in  $NK-1R^{-/-}$  mice nociceptors and we interpret this as showing that the peptide can act on these neurons independently of NK-1R.

#### **Discussion**

The limited efficacy of antihistamines in most pruritic conditions<sup>20, 21</sup> highlights the importance of histamine-independent mechanisms of itch. Mrgprs are among the most important mediators of histamine-independent itch<sup>22</sup>. This manuscript further addresses the role of Mrgprs in itch by indicating a specific role for MrgprA1 in SP-induced itch in

mice. SP has been implicated in various cutaneous and systemic pruritic disorders such as atopic dermatitis<sup>23, 24</sup> and cholestasis<sup>25</sup>. We have previously reported that wildtype and *NK-1R*<sup>-/-</sup> mice have comparable scratching behavior in response to SP and that *in vitro*, SP activates MrgprA1 in addition to NK-1R. These findings are consistent with a role for a receptor other than NK-1R in the mediation of SP-induced itch responses<sup>14</sup>.

Here we now show that SP-induced scratching behavior is dependent on Mrgprs, since *Mrgpr cluster Δ*<sup>-/-</sup> mice demonstrate a significant reduction in the number of scratching bouts compared to wildtype animals. Our previous data on *NK-1R*<sup>-/-</sup> mice were confirmed here by showing that when the NK-1R antagonist, L733060, is injected with SP into wildtype mice, itch is not reduced, reconfirming in an independent fashion that NK-1R does not play a pivotal role in SP-induced itch in mice. In contrast, scratching behavior was reduced to baseline in animals co-treated with SP and the dual NK-1R/Mrgprs antagonist QWF, consistent with SP-induced itch being dependent on Mrgprs. These results are in line with data published by our group showing that QWF is an antagonist of MrgprA1<sup>14</sup>. Since MrgprA1 is the only Mrgpr in the cluster knockout mice to which QWF binds<sup>14</sup>, this specific receptor likely mediates SP-induced itch. To further test the hypothesis that SP induces itch via a receptor that is not NK-1R, we evaluated the ability of SP to activate DRG sensory neurons lacking NK-1R. As expected, SP excited *NK-1R*<sup>-/-</sup> DRG neurons that are of small size and TRPV1<sup>+</sup> in a QWF dependent fashion .

The data presented here favor a specific role for MrgprA1 on sensory neurons in SP-induced itch. It is appreciated that availability of MrgprA1-sensory neuron conditional knockout mice would provide additional genetic-based clarity to our pharmacological

observations. As QWF appears to have selectivity for itch over nociceptive responses (Figure 2 and supplementary figure 1), it is tempting to propose that we have uncovered a new receptor that specifically mediates SP-induced itch, and in consequence antagonists to a homologous receptor in humans may be useful for treating those pruritic disorders in which SP is involved, which we consider to be MRGPRX2.

MRGPRX2 is the only human Mrgpr expressed by mast cells<sup>13</sup>. Several endogenous and exogenous molecules activate MRGPRX2 to induce mast cell degranulation implicating the receptor in inflammation and pseudoallergic drug reactions<sup>13</sup>. The diversity of ligands activating MRGPRX2 underscores its role as a sensor to detect a wide variety of stimuli. In addition to inflammation, a role for MRGPRX2 in itch has been proposed<sup>14, 26</sup>. In line with this,, MRGPRX2 is the only human Mrgpr activated by SP, an endogenous pruritogen<sup>14</sup>. Several studies have demonstrated expression of MRGPRX2 on human and primate DRG neurons by immunohistochemistry and PCR<sup>12, 27, 28</sup> although one did not<sup>29</sup>. In an effort to address this discrepancy, we used PCR and amplified MRGPRX2 from RNA extracted from human DRGs (supplementary figure 3).

We have taken advantage of the different specificities of QWF and L733060 for antagonizing MrgprA1 and MrgprB2 to evaluate the roles of these receptors in SP-induced itch. Human MRGPRX2, which is expressed on mast cells and DRG neurons, functionally resembles MrgprB2 and MrgprA1 on mouse mast cell and DRG neurons respectively. We conclude that MRGPRX2 is an important target both for treating mast cell mediated pathological actions, and for histamine-independent itch.

**Author contributions:** EA, VR and EAL conceived the overall design of the study. EA performed behavioral studies. ST and PJSP performed pain assessments. VR, ST, and PJSP performed *in vitro* studies and helped analyze the data with EA, CJW and EAL. All authors contributed to the writing and review of the manuscript.

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#### **References:**

1. Duval A, Dubertret L. Aprepitant as an antipruritic agent? N Engl J Med 2009; 361:1415-6.
2. Stander S, Siepmann D, Herrgott I, Sunderkotter C, Luger TA. Targeting the neurokinin receptor 1 with aprepitant: a novel antipruritic strategy. PloS one 2010; 5:e10968.

3. Santini D, Vincenzi B, Guida FM, Imperatori M, Schiavon G, Venditti O, et al. Aprepitant for management of severe pruritus related to biological cancer treatments: a pilot study. *Lancet Oncol* 2012; 13:1020-4.
4. Wallengren J. Topical aprepitant in clinical and experimental pruritus. *Arch Dermatol* 2012; 148:957-9.
5. Wallengren J, Edvinsson L. Topical non-peptide antagonists of sensory neurotransmitters substance P and CGRP do not modify patch test and prick test reactions: a vehicle-controlled, double-blind pilot study. *Arch Dermatol Res* 2014; 306:505-9.
6. Andoh T, Nagasawa T, Satoh M, Kuraishi Y. Substance P induction of itch-associated response mediated by cutaneous NK1 tachykinin receptors in mice. *The Journal of pharmacology and experimental therapeutics* 1998; 286:1140-5.
7. Lembo PM, Grazzini E, Groblewski T, O'Donnell D, Roy MO, Zhang J, et al. Proenkephalin A gene products activate a new family of sensory neuron--specific GPCRs. *Nat Neurosci* 2002; 5:201-9.
8. Bader M, Alenina N, Andrade-Navarro MA, Santos RA. MAS and its related G protein-coupled receptors, Mrgprs. *Pharmacol Rev* 2014; 66:1080-105.
9. Solinski HJ, Gudermann T, Breit A. Pharmacology and signaling of MAS-related G protein-coupled receptors. *Pharmacol Rev* 2014; 66:570-97.
10. Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, et al. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell* 2009; 139:1353-65.
11. Liu Q, Weng HJ, Patel KN, Tang Z, Bai H, Steinhoff M, et al. The distinct roles of two GPCRs, MrgprC11 and PAR2, in itch and hyperalgesia. *Science signaling* 2011; 4:ra45.
12. Tatemoto K, Nozaki Y, Tsuda R, Konno S, Tomura K, Furuno M, et al. Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem Biophys Res Commun* 2006; 349:1322-8.
13. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2014.
14. Azimi E, Reddy VB, Shade K-TC, Anthony RM, Talbot S, Pereira PJS, et al. Dual action of neurokinin-1 antagonists on Mas-related GPCRs. *JCI Insight*; 1.
15. Dong X, Han S, Zylka MJ, Simon MI, Anderson DJ. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell* 2001; 106:619-32.
16. Mishra SK, Tisel SM, Orestes P, Bhangoo SK, Hoon MA. TRPV1-lineage neurons are required for thermal sensation. *EMBO J* 2011; 30:582-93.
17. Shimada SG, LaMotte RH. Behavioral differentiation between itch and pain in mouse. *Pain* 2008; 139:681-7.
18. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitze KR, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 2000; 288:306-13.

19. Goswami SC, Mishra SK, Maric D, Kaszas K, Gonnella GL, Clokie SJ, et al. Molecular signatures of mouse TRPV1-lineage neurons revealed by RNA-Seq transcriptome analysis. *J Pain* 2014; 15:1338-59.
20. Thurmond RL, Kazerouni K, Chaplan SR, Greenspan AJ. Antihistamines and itch. *Handb Exp Pharmacol* 2015; 226:257-90.
21. O'Donoghue M, Tharp MD. Antihistamines and their role as antipruritics. *Dermatol Ther* 2005; 18:333-40.
22. LaMotte RH, Dong X, Ringkamp M. Sensory neurons and circuits mediating itch. *Nat Rev Neurosci* 2014; 15:19-31.
23. Borici-Mazi R, Kouridakis S, Kontou-Fili K. Cutaneous responses to substance P and calcitonin gene-related peptide in chronic urticaria: the effect of cetirizine and dimethindene. *Allergy* 1999; 54:46-56.
24. Ohmura T, Hayashi T, Satoh Y, Konomi A, Jung B, Satoh H. Involvement of substance P in scratching behaviour in an atopic dermatitis model. *Eur J Pharmacol* 2004; 491:191-4.
25. Trivedi M, Bergasa NV. Serum concentrations of substance P in cholestasis. *Ann Hepatol* 2010; 9:177-80.
26. Fujisawa D, Kashiwakura J, Kita H, Kikukawa Y, Fujitani Y, Sasaki-Sakamoto T, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol* 2014; 134:622-33 e9.
27. Robas N, Mead E, Fidock M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *The Journal of biological chemistry* 2003; 278:44400-4.
28. Zhang L, Taylor N, Xie Y, Ford R, Johnson J, Paulsen JE, et al. Cloning and expression of MRG receptors in macaque, mouse, and human. *Brain Res Mol Brain Res* 2005; 133:187-97.
29. Flegel C, Schobel N, Altmüller J, Becker C, Tannapfel A, Hatt H, et al. RNA-Seq Analysis of Human Trigeminal and Dorsal Root Ganglia with a Focus on Chemoreceptors. *PLoS One* 2015; 10:e0128951.

**Figure 1 – Mrgprs mediate SP-induced itch.** **A)** SP-induced itch (500  $\mu$ M, i.d.) is significantly decreased in *Mrgpr cluster  $\Delta^{-/-}$*  mice. **B)** Effects of treatment with the NK-1R antagonist L733060 (500  $\mu$ M, i.d.) or the dual NK-1R/Mrgpr antagonist QWF (500  $\mu$ M, i.d.) on scratching behavior caused by SP (500  $\mu$ M, i.d.). Data are expressed as mean  $\pm$  S.E.M. Unpaired Student's t test ( $n=7$  mice per group in A) and one-way ANOVA followed by Tukey's test ( $n=6$  mice per group in B). Significant differences from wildtype mice ( $^{\dagger}p<0.05$ ), baseline ( $^{\#}p<0.05$ ), SP + vehicle ( $^{*}p<0.05$ ) or SP + L733060 ( $^{\$}p<0.01$ ) are indicated.

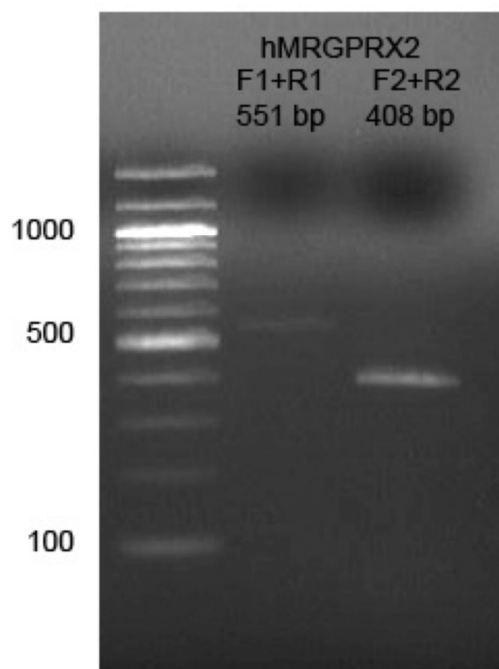
**Figure 2 – The effect of QWF on capsaicin-induced  $\text{Ca}^{2+}$  flux and thermal hyperalgesia.** **A)** QWF (1  $\mu\text{M}$ ) does not impact  $\text{Ca}^{2+}$  flux induced by capsaicin (1  $\mu\text{M}$ ) and KCl (40 mM) in cultured wildtype DRG neurons. **B)** QWF (1  $\mu\text{M}$ , i.pl.) has no effect on capsaicin-induced thermal hyperalgesia in wildtype mice. Data are expressed as mean  $\pm$  S.E.M. One-way ANOVA followed by Tukey's test (n= 6 mice per group). Statistical differences from vehicle are indicated by ### p<0.001. Total number of cells analyzed: **A)** 267.

**Figure 3 - SP activates *NK-1R*<sup>-/-</sup> nociceptors.** **A)** SP (10 - 1000 nM) induces Ca<sup>2+</sup> flux in wildtype (gray) and *NK-1R*<sup>-/-</sup> (black) cultured DRG neurons. **B)** Percentage of SP responding cells from wildtype or *NK-1R*<sup>-/-</sup> mice. **C)** Maximal Ca<sup>2+</sup> flux in DRG neurons from wildtype (gray) and *NK-1R*<sup>-/-</sup> (black) animals. **D)** Size of SP responsive cells from *NK-1R*<sup>-/-</sup> animals. **D-Insert)** Venn diagram shows overlapping populations of KCl<sup>+</sup>, capsaicin<sup>+</sup> and SP responsive neurons. **E)** Ca<sup>2+</sup> flux induced by SP (1 μM), capsaicin (1 μM) and KCl (40 mM) in DRG neurons from wildtype (gray) and TRPV1-Cre<sup>+/-</sup>DTA<sup>+/-</sup> (red) mice. Unpaired Student's t test. Significant differences from *NK-1R*<sup>-/-</sup> indicated by \*\*p<0.01 and \*\*\*p<0.001. Total number of cells analyzed: **A, B, C)** 328 (wildtype) and 1463 (*NK-1R*<sup>-/-</sup>), **D)** 1463, **D-Insert)** 314 and **E)** 787 (wildtype) and 246 (TRPV1-Cre<sup>+/-</sup>DTA<sup>+/-</sup>).

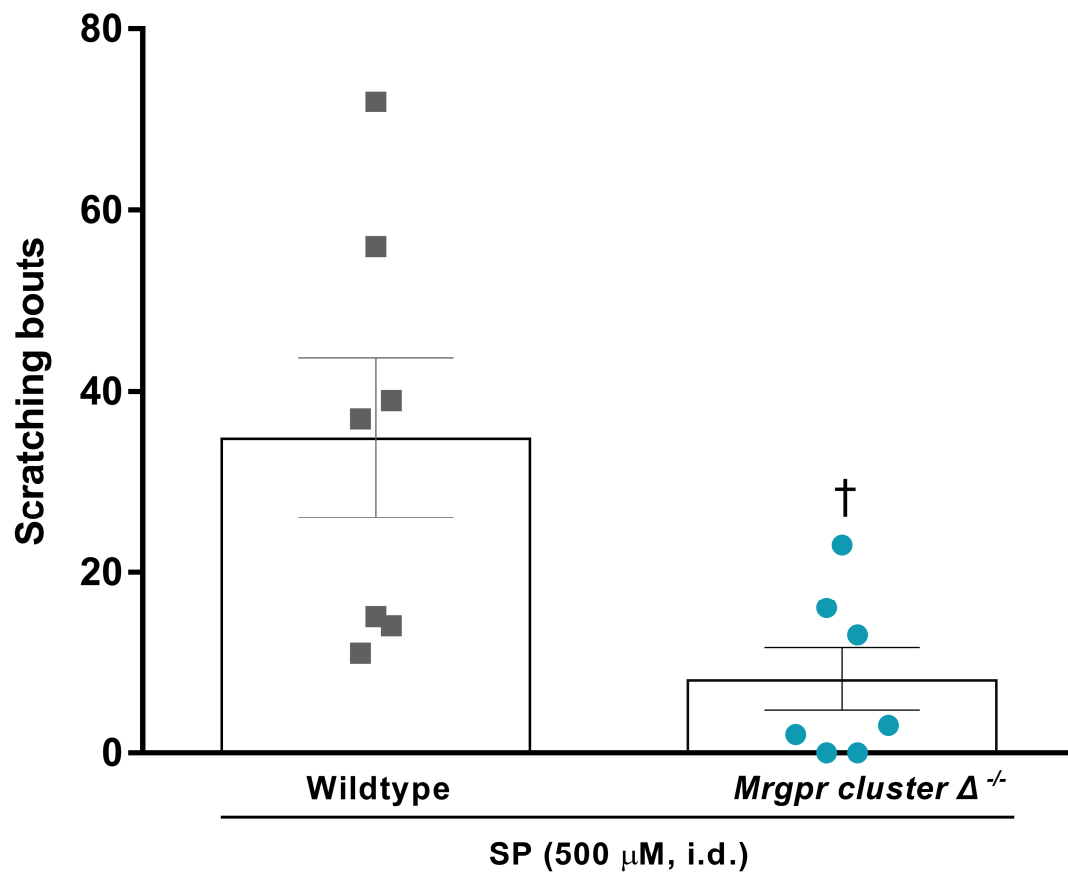
**Figure 4 – The effect of QWF on SP-induced  $\text{Ca}^{2+}$  flux in cultured DRG neurons from  $\text{NK-1R}^{-/-}$  mice. A)** QWF (1  $\mu\text{M}$ ) blocks SP-induced (300 nM)  $\text{Ca}^{2+}$  flux in DRG neurons from  $\text{NK-1R}^{-/-}$  mice. The cells blocked by QWF also respond to AITC (1  $\mu\text{M}$ ), capsaicin (1  $\mu\text{M}$ ) and KCl (40 mM). **B)** Amplitude of SP-induced  $\text{Ca}^{2+}$  flux in  $\text{NK-1R}^{-/-}$  neurons before and after treatment with QWF (1  $\mu\text{M}$ ). Data are expressed as mean  $\pm$  S.E.M. Paired Student's t-test. Statistical difference from SP (\*\*\*) $p < 0.001$  is indicated. Total number of cells analyzed: **A)** 314 and **B)** 492.

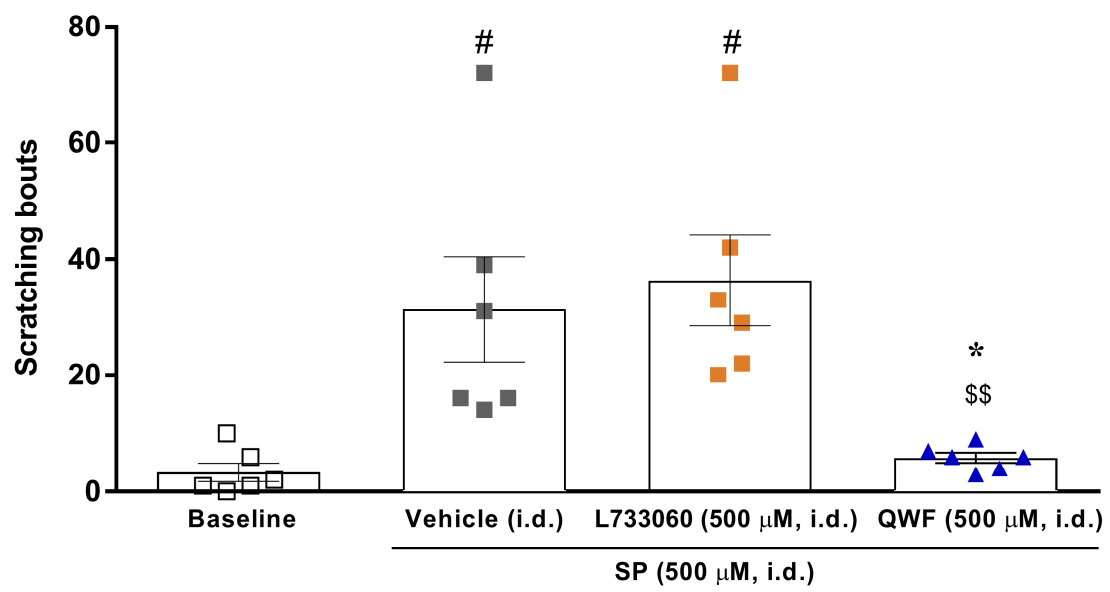
**Supplementary Figure 1 – QWF does not impact capsaicin- or KCl-induced calcium flux.**  $\text{Ca}^{2+}$  amplitude (**A**, **B**) and number of responsive cells (**C**) to capsaicin (**A** and **C**; 1  $\mu\text{M}$ ) or KCl (**B** and **C**; 40 mM) following treatment with QWF (1  $\mu\text{M}$ ) or vehicle. Data are expressed as mean  $\pm$  S.E.M. Paired Student's t-test. Significant differences from vehicle are indicated by ### $p < 0.001$ . Total number of cells analyzed: **A**, **B**, **C**) 267

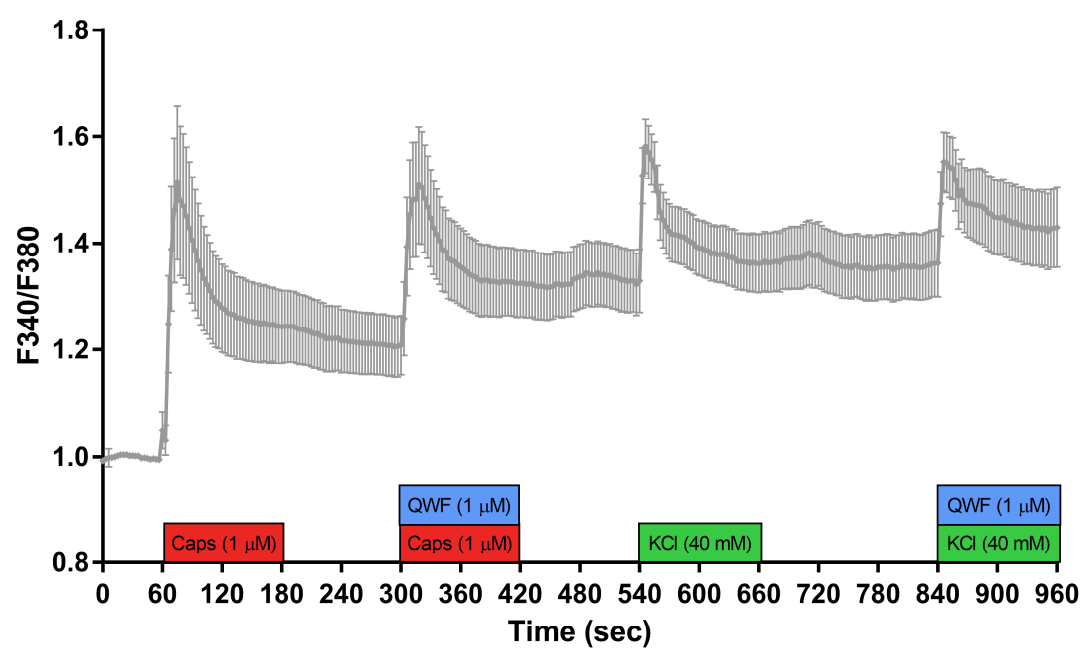
**Supplementary Figure 2 – DRG neurons from *NK-1R*<sup>-/-</sup> mice respond to subsequent SP exposure. A)  $\text{Ca}^{2+}$  response and B) maximal  $\text{Ca}^{2+}$  amplitude after repeated application of SP (300 nM), followed by KCl (40 mM). Data are expressed as mean  $\pm$  S.E.M. Paired Student's t-test. Statistical differences from vehicle are indicated by <sup>##</sup>p<0.01, <sup>###</sup>p<0.001. Total number of cells analyzed: **A and B)** 107.**

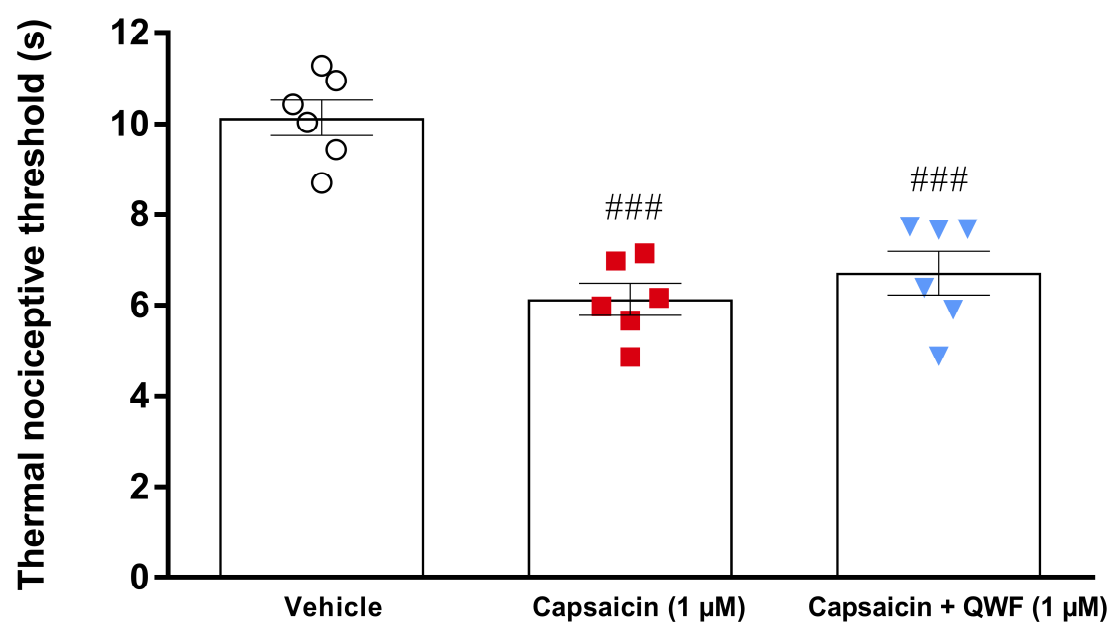


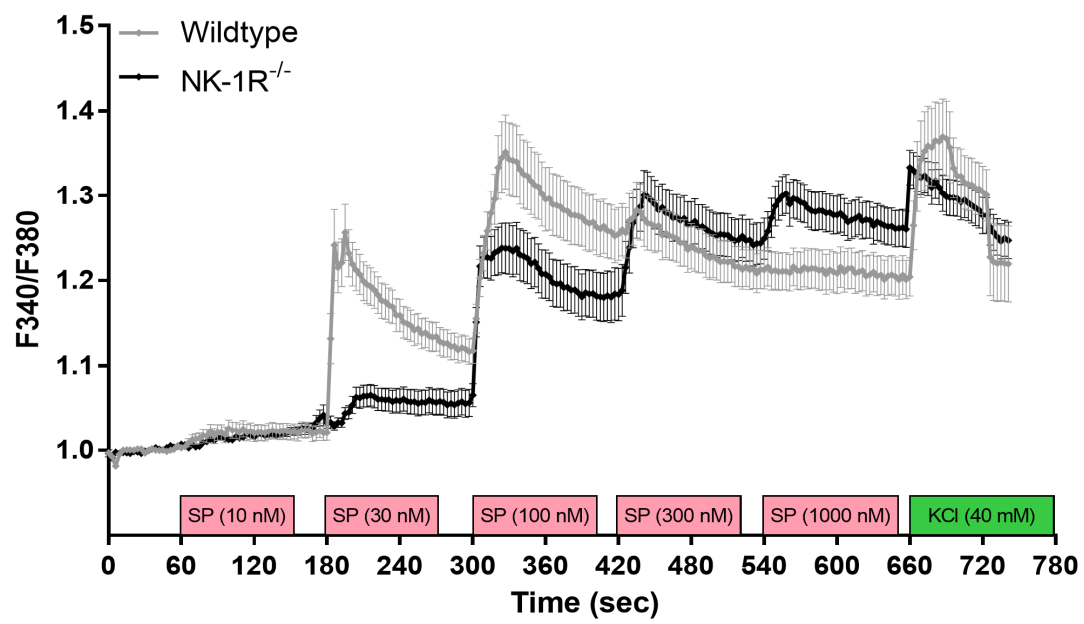
**Supplementary Figure 3. MRGPRX2 is transcribed in human DRGs as determined by PCR.** Total human DRG RNA was converted into ss-cDNA using random hexamers and reverse transcriptase. PCR was carried out with the cDNA as a template and primer pairs of hMRGPRX2: forward primers F1 (CTGTGATGACCTGTGCCTACCTTGC) and F2 (TCAGCGGTCGTGTGTGTCCTGCTCTGGG), and reverse primers R1 (CCGCCACTGCTTCCTAAAAGAGCCC) and R2 (GAAGAAGTAAATGATGGGGTTGGCAC). The primer pairs F1+R1 and F2+R2 yielded the expected PCR products of 551 bp and 408 bp, respectively. DNA markers are shown on the left.

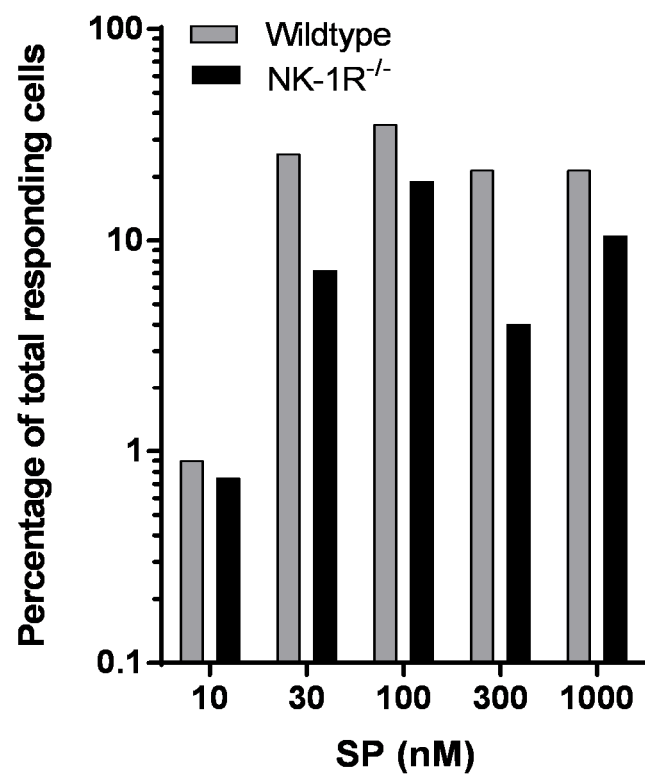


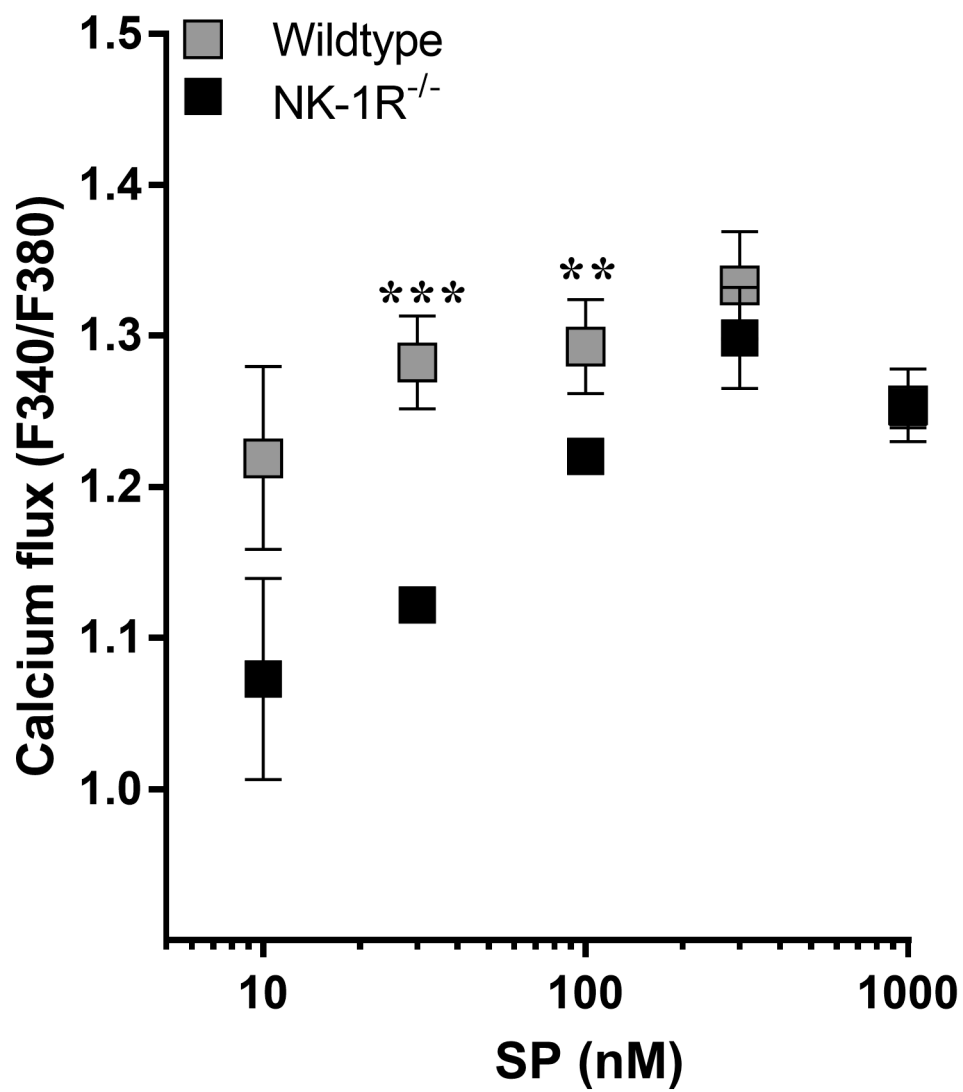


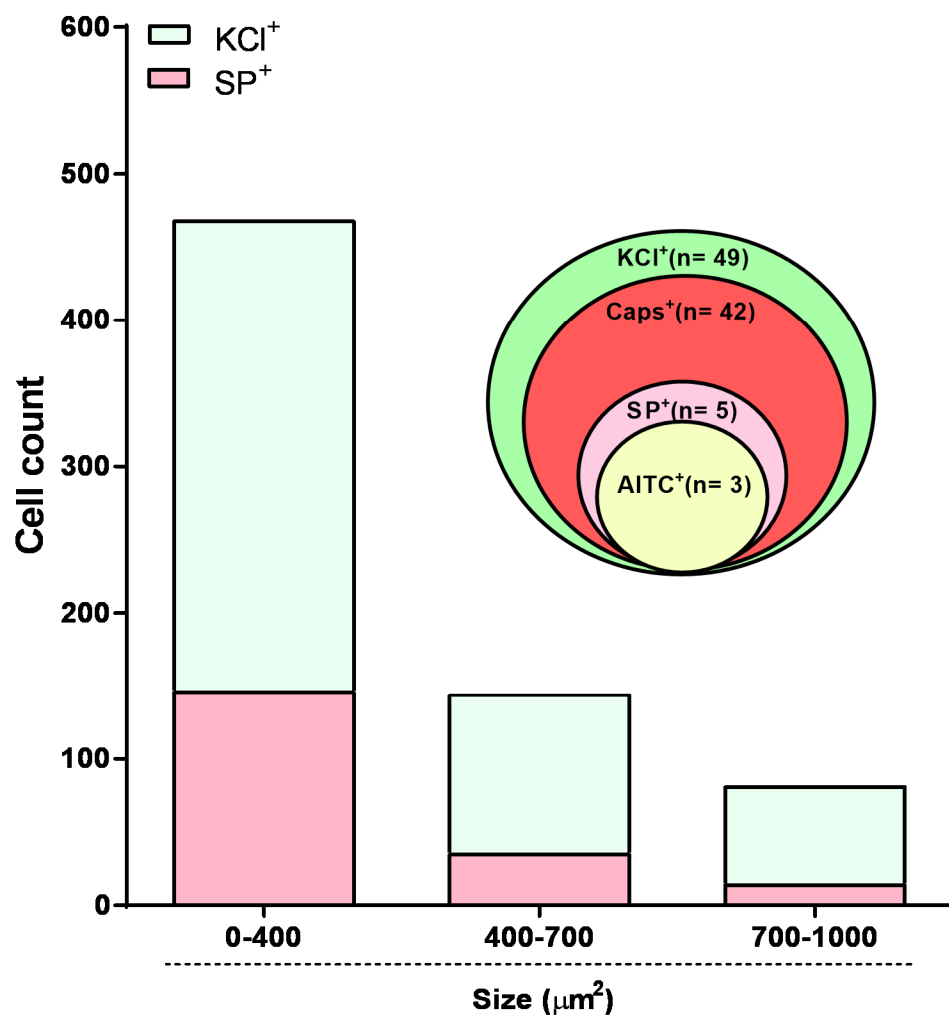


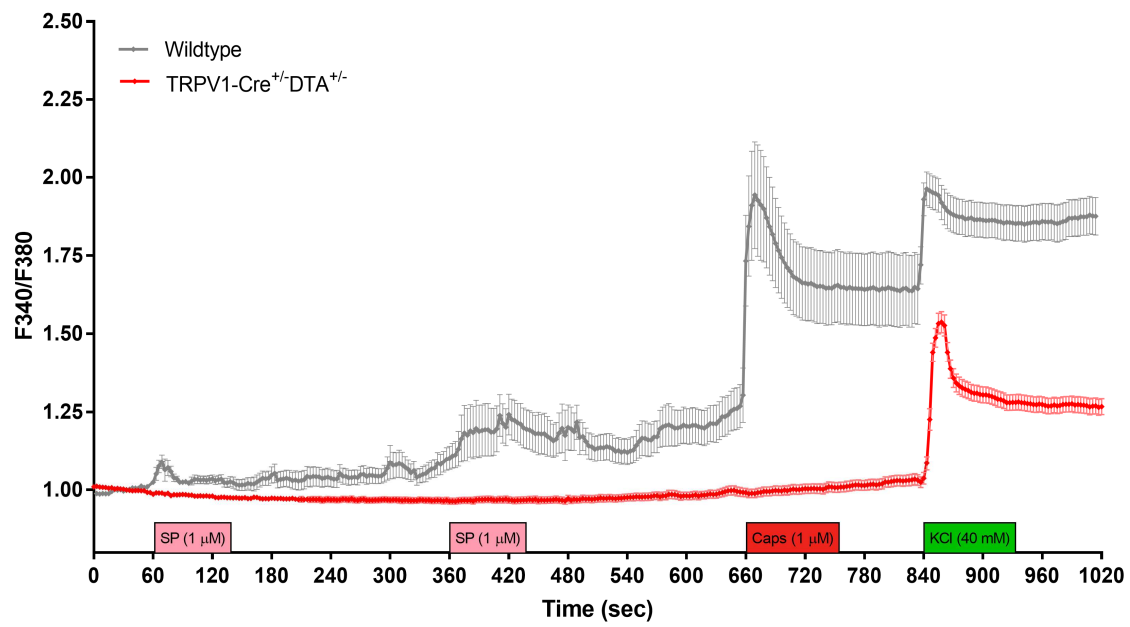


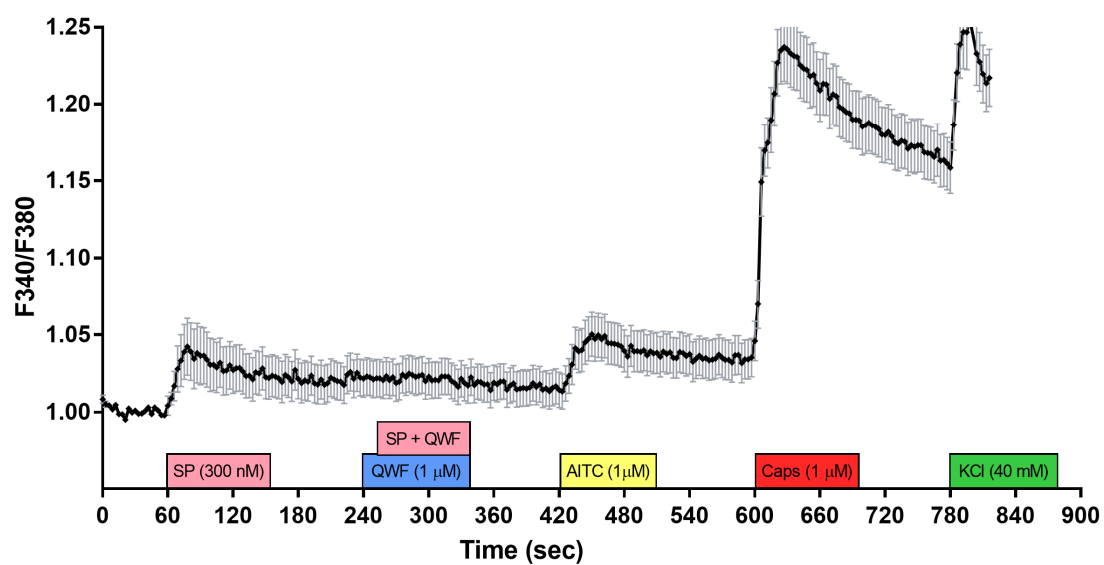


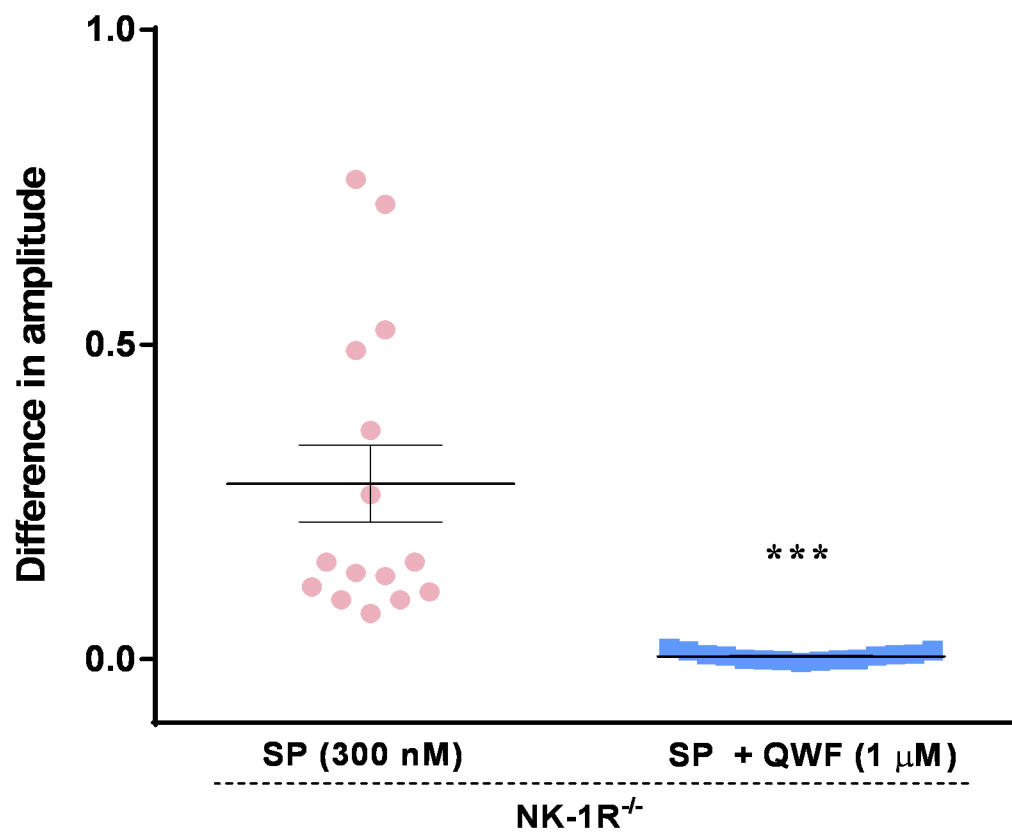












**Supplementary Figure 1 – QWF does not impact capsaicin- or KCl-induced calcium flux.**  $\text{Ca}^{2+}$  amplitude (**A, B**) and number of responsive cells (**C**) to capsaicin (**A** and **C**; 1  $\mu\text{M}$ ) or KCl (**B** and **C**; 40 mM) following treatment with QWF (1  $\mu\text{M}$ ) or vehicle. Data are expressed as mean  $\pm$  S.E.M. Paired Student's t-test. Significant differences from vehicle are indicated by  $^{###}p<0.001$ . Total number of cells analyzed: **A, B, C**) 267

**Supplementary Figure 2 – DRG neurons from *NK-1R*<sup>-/-</sup> mice respond to subsequent SP exposure.** **A)**  $\text{Ca}^{2+}$  response and **B)** maximal  $\text{Ca}^{2+}$  amplitude after repeated application of SP (300 nM), followed by KCl (40 mM). Data are expressed as mean  $\pm$  S.E.M. Paired Student's t-test. Statistical differences from vehicle are indicated by  $^{##}p<0.01$ ,  $^{###}p<0.001$ . Total number of cells analyzed: **A and B**) 107.

**Supplementary Figure 3. MRGPRX2 is transcribed in human DRGs as determined by PCR.** Total human DRG RNA was converted into ss-cDNA using random hexamers and reverse transcriptase. PCR was carried out with the cDNA as a template and primer pairs of hMRGPRX2: forward primers F1 (CTGTGATGACCTGTGCCTACCTTGC) and F2 (TCAGCGGTCGTGTGTGCTCCTGCTCTGGG), and reverse primers R1 (CCGCCACTGCTTCCTAAAAGAGCCC) and R2 (GAAGAAGTAAATGATGGGGTTGGCAC). The primer pairs F1+R1 and F2+R2 yielded the expected PCR products of 551 bp and 408 bp, respectively. DNA markers are shown on the left.

