Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions

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Mast cells are primary effectors in allergic reactions, and may have important roles in disease by secreting histamine and various inflammatory and immunomodulatory substances3–5. Although they are classically activated by immunoglobulin (IgE) antibodies, a unique property of mast cells is their antibody-independent responsiveness to a range of cationic substances, collectively called basic secretagogues, including inflammatory peptides and drugs associated with allergic-type reactions3–5. The pathogenic roles of these substances have prompted a decades-long search for their receptor(s). Here we report that basic secretagogues activate mouse mast cells in vitro and in vivo through a single receptor, Mrgrp2, the orthologue of the human G-protein-coupled receptor MRGPRX2. Secretagogue-induced histamine release, inflammation and airway contraction are abolished in Mrgrp2-null mutant mice. Furthermore, we show that Mrgrp2 and MRGPRX2 are targets of many small-molecule drugs associated with systemic pseudo-allergic, or anaphylactoid, reactions; we show that drug-induced symptoms of anaphylactoid responses are significantly reduced in knockout mice; and we identify a common chemical motif in several of these molecules that may help predict side effects of other compounds. These discoveries introduce a mouse model to study mast cell activation by basic secretagogues and identify MRGPRX2 as a potential therapeutic target to reduce a subset of drug-induced adverse effects.

Responsiveness to basic secretagogues is conserved among mammals4 and is also found in birds5, indicating an ancient, fundamental role for its mechanism. Many basic secretagogues are endogenous peptides, often linked to inflammation; however, they activate connective tissue mast cells only at high concentrations and independent of their canonical receptors, so another mechanism of stimulation must exist6. Several candidate proteins that bind polycationic compounds have been proposed as basic secretagogue receptors8–10. Among these, MRGPRX2 has been screened with the most compounds8,10–14, and short interfering RNA (siRNA) knockdown studies support at least a partial role for MRGPRX2 in activation by four non-canonical basic secretagogues11,13. However, no direct in vivo study or knockout model has been employed for any candidate. The investigation of MRGPRX2 in mice is complicated because no direct in vivo study or knockout model has been employed for any candidate.

Figure 1 | Mrgrp2 is the orthologue of human MRGPRX2. a, Diagram of mouse and human Mrgr genomic loci. Mouse Mrgrα3 and Mrgrα11 are orthologues of human MRGPRX1, determined by expression and ligand specificity11. The MRGPRX2 orthologue Mrgrp2 is described in this study. Chr., chromosome. b, Results from a stringent RT–PCR screen identifying Mrgrp2 transcript (arrow) in mouse peritoneal mast cells. The negative control (Neg.) omitted reverse transcriptase. RT–PCR for Mrgrp2 was repeated at least four times. c, Example traces of intracellular calcium concentrations [Ca2+]i, measured by ratiometric Fura-2 imaging, from Mrgrp2-HEK or MRGPRX2-HEK cells exposed to 20 μM PAMP(9–20) (duration indicated by black line). Each trace is a response from a unique cell. d, Representative confocal images from BAC transgenic mouse tissues in which tdTomato expression is controlled by enhanced green fluorescent protein (eGFP)-Cre expression from the Mrgrp2 locus (see Methods). Avidin staining was used to identify mast cells. Percentages of avidin-positive mast cells that were also tdTomato positive: glabrous skin, 97.5%; hairy skin, 90.1%; trachea, 97.2%; heart, 87.1%. Percentages of tdTomato-positive cells that were also avidin positive: glabrous skin, 99.2%; hairy skin, 100%; trachea, 98.3%; heart, 99%. n = 3 mice and >300 cells counted per tissue, except n = 2 and >100 cells counted in the heart. Scale bar, 20 μm.

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the gene cluster containing the four human MRGPRX members is dramatically expanded in mice, consisting of 22 potential coding genes, many with comparable sequence identity to MRGPRX2 (Fig. 1a). Therefore, a mouse MRGPRX2 orthologue must be determined by expression pattern and pharmacology. A stringent polymerase chain reaction with reverse transcription (RT–PCR) screen in mouse primary mast cells uncovered a band for a single family member, Mrgprb2 (Fig. 1b), whereas MRGPRX1 orthologues were not expressed at relevant levels (Extended Data Fig. 1a, b). Functionally, HEK293 cells heterologously expressing mouse Mrgprb2 (Mrgprb2-HEK) responded to the MRGPRX2 agonist proadrenomedullin amino-terminal 20 peptide, fragment 9–20 (PAMP(9–20))14 (Fig. 1c) and compound 48/80 (48/80), a classical mast cell activator and canonical basic secretagogue (Extended Data Fig. 2). Mrgprb2-HEK cells also responded to other MRGPRX2 ligands, including the basic secretagogue Substance P, but had no response to the MRGPRX1 ligand chloroquine15; no closely related family members in mice responded to any compound (Extended Data Figs 1c and 2a, c).

To determine the expression of Mrgprb2, we generated Mrgprb2 bacte-
rial artificial chromosome (BAC) transgenic mice in which the expres-
sion of eGFP-Cre recombinase was under the control of the Mrgprb2 promoter. Strikingly, Cre expression patterns indicate that Mrgprb2 expression is highly specific to connective tissue mast cells (Fig. 1d and Extended Data Figs 3, 4). Together, the pharmacological and expression data indicate that Mrgprb2 is the mouse orthologue of human MRGPRX2.

Next, we determined whether Mrgprb2 is the basic secretagogue recep-
tor in mouse mast cells. The Mrgprb2 genomic locus contains too much repetitive sequence to permit gene targeting through homologous recombination (Extended Data Fig. 5a). Therefore, we used a zinc-finger-nuclease-based strategy to generate a mouse line with a 4 base pair (bp) deletion in the Mrgprb2 coding region (Mrgprb2MUT mice), resulting in a frameshift mutation and early termination shortly after the first trans-
membrane domain (Extended Data Fig. 5b–d). The mutation was stable and inheritable (Extended Data Fig. 5c), so we regard Mrgprb2MUT as a functional null. Mast cell numbers were comparable in tissues of wild-type and Mrgprb2MUT mice, indicating that Mrgprb2 is not essential for mast cell survival or targeting to tissue (Extended Data Fig. 6a). The responsiveness of peritoneal mast cells to anti-IgE antibodies (Fig. 2a) and endothelin (Extended Data Fig. 7) was also comparable, demonstrating that Mrgprb2 mutation does not globally impair IgE or G-protein-coupled receptor (GPCR)-mediated mast cell signalling. However, 48/80-induced mast cell activation (Fig. 2a) and tissue histamine release were essentially abolished in mutant mast cells (Fig. 2b and Extended Data Fig. 6b). Furthermore, we found that 48/80-evoked tracheal contraction (Fig. 2c) and hindpaw inflammation (extravasation and swelling; Fig. 2d) were almost completely absent in an Mrgprb2MUT background, while antigen (Fig. 2c) and anti-IgE evoked responses (Extended Data Fig. 8) were comparable to wild-type mice. Finally, we found that four additional basic secretagogues, as well as the MRGPRX2 agonist PAMP(9–20) and cortistatin10, strongly activated wild-type but not Mrgprb2MUT mast cells (Fig. 2e and Extended Data Fig. 9a). HEK293 cells expressing Mrgprb2 or MRGPRX2 (MRGPRX2-HEK) also responded to these secretagogues (Extended Data Fig. 2). Taken together, we con-
clude that Mrgprb2 is the mouse mast cell basic secretagogue receptor.

It is likely that the list of small, basic peptides that activate Mrgprb2 is greater than the number in this study; indeed, dozens of such peptides have been shown to activate mast cells.6,8,46,47 Notably, human MRGPRX2 is much more sensitive to substance P than mouse Mrgprb2 (Extended Data Fig. 2c), suggesting a potential species-specific role for substance P in mast cell signalling.

We next considered whether Mrgprb2 factors in allergic-type reac-
tions. We specifically addressed drug-induced reactions because many therapeutic drugs are cationic. Up to 15% of drug-induced adverse reactions appear to be allergic in nature; however, many do not correlate well with IgE antibody titre, indicating that antibody-independent, or pseudo-allergic, mechanisms participate19. We focused first on peptidergic drugs.
because most are introduced subcutaneously or intramuscularly at millimolar concentrations (Supplementary Information), high enough for cationic peptides to activate mast cells. The most frequent allergic-type response described in the FDA labels of these drugs is an injection-site reaction (ISR), a local swelling and/or flare of variable size that can be accompanied by pain or pruritus. In a survey of FDA-approved peptidergic drugs, we found that the vast majority associated with ISRs are cationic (Supplementary Information). We found that representative members of all common, commercially available classes of these cationic drugs activated mast cells in an Mrgprb2-dependent manner, whereas the innocuous protein insulin had no effect (Fig. 3a and Extended Data Fig. 9b, c). Consistently, all of these peptides except insulin activate both Mrgprb2-HEK and MRGPRX2-HEK cells (Extended Data Fig. 2). We selected the drug icatibant for further study because it induces ISRs in nearly every patient19. Icatibant at the clinical concentration induced Mrgprb2 and MRGPRX2-HEK cells (Fig. 9b, c). Consistently, all of these peptides except insulin activate both Mrgprb2 and MRGPRX2 cells (Fig. 3b). These data lead us to anticipate that drug-induced ISRs may be alleviated by targeting MRGPRX2 or by using peptides with less potent MRGPRX2 agonist properties.

Next, we explored the possibility that Mrgprb2 mediates pseudoallergic reactions induced by small molecules. We focused on intravenous drugs because they are often administered rapidly and in high doses, and thus are more likely to achieve high blood concentrations and rapid tissue distribution than drugs administered through other routes. Symptoms of pseudo-allergic reactions after intravenous administration, which at their most severe are called anaphylactoid, include skin flushing or rash, changes in blood pressure or heart rate, and bronchospasms20. We based our initial search on the structure of 48/80. While the structure–function relationship of 48/80 as an MRGPRX2 agonist is unknown, a cyclized variant containing a tetrahydroisooquinoline (THIQ) motif (Fig. 4a) is reported to be seven times more potent than 48/80 as a mast cell degranulator21. A search of FDA-approved drugs containing a THIQ recovered members of the nicotinic receptor antagonist non-steroidal neuromuscular blocking drugs (NMBDs), including tubocurarine and atracurium (Fig. 4b). NMBDs are used routinely in surgery to reduce unwanted muscle movement and allow intratracheal intubation for mechanical ventilation. Intriguingly, NMBDs alone are responsible for nearly 60% of allergic reactions in a surgical setting22, and all except succinylcholine induce histamine release in humans23. We found that members of all NMBD families (Supplementary Information) except succinylcholine activated mast cells in an Mrgprb2-dependent manner at concentrations as low as 0.5% of the clinical injection concentration (Fig. 4c and Extended Data Fig. 9d). Interestingly, rocuronium does not contain a THIQ but has a bulky hydrophobic group with a charged nitrogen within several angstroms (Fig. 4b), reminiscent of 48/80. Therefore, we searched using modifications of the THIQ motif and the 48/80 structure, including changes in cyclization and position of the positive or polar nitrogen, limiting our assay to intravenous drugs at high injection concentrations. We identified the fluoroquinolone family of antibiotics as having a similar motif (Fig. 4d). Like NMBDs, these are associated with allergic-type reactions24,25 and can activate mast cells26,27. We found that the four members approved for intravenous use activated Mrgprb2-HEK and MRGPRX2-HEK cells (Extended Data Fig. 2), and

![Figure 3](https://example.com/figure3.png)

**Figure 3** | Mrgprb2 mediates mast cell responsiveness and side effects of peptidergic therapeutic drugs. a, Percentage of responding cells from wild-type (WT) and Mrgprb2MUT (MUT) peritoneal mast cells after drug application, assayed using Fluor-4 imaging. Concentrations of drugs (in μg ml–1): icatibant, 50; cetrotrelax, 20; leuprolide, 100; octreotide, 10; sermorelin, 60; insulin, 80. n = 3 per genotype; >150 cells counted for insulin. Difference between insulin responsiveness was not significant. b, Left, representative images of Evans blue stained extravasation 15 min after intraplantar injection of icatibant (right, arrow), 10 mg ml–1 (red line, at concentrations shown on the ordinate). Right, quantification of Evans blue leakage into the paw after 15 min. n = 6 per genotype. Difference after saline injection was not significant. c, Total histamine release from wild-type (red diamonds) and Mrgprb2MUT (black squares) mice after incubation with named substances. Note that no significant difference between wild-type and Mrgprb2MUT cells was found at any dose of anti-IgE antibody. Experiments were repeated >3 times. Data are presented as mean ± s.e.m. Two-tailed unpaired Student’s t-test: *P < 0.05, **P < 0.01. NS, not significant.
Figure 4 | Mrgprb2 mediates mast cell responsiveness and side effects of small-molecule therapeutic drugs. a, Structures of 48/80 and a cycled variant. The THIQ motif is highlighted in blue. b, Structures of representative members of all NMBD classes (see Supplementary Information). THIQ motifs are highlighted in blue. Note that only succinylcholine lacks a bulky hydrophobic group. c, Percentage of responding cells from wild-type (WT) and Mrgprb2MUT (MUT) peritoneal mast cells after application of various NMBDs, assayed using Fluo-4 imaging. Concentrations of drugs (in µM ± 17): atracurium, 50; mivacurium, 20; tubocurarine, 30; rocuronium, 500. n = 3 mice per genotype; >150 cells counted per substance, d, Structure of ciprofloxacin, with the motif common to all fluoroquinolones highlighted in blue. Note the nitrogen close to the quinolone motif. e, Percentage of responding cells from wild-type and Mrgprb2MUT peritoneal mast cells after fluoroquinolone application, assayed using Fluo-4 imaging. Concentrations of drugs (in µM ± 17): ciprofloxacin, 200; levofloxacin, 500; moxifloxacin, 160; ofloxacin, 400. n = 3 mice per genotype; >150 cells counted per substance. f, Changes in body temperature after intravenous injection of ciprofloxacin (1.5 mg in 125 µl saline) at time 0. n = 4 mice per genotype. Data are presented as mean ± s.e.m. Two-tailed unpaired Student’s t-test: *P < 0.05, **P < 0.01.

β-hexosaminidase from LAD2 cells (Extended Data Fig. 10a). 48/80 and mastoparan were used as positive controls. Importantly, MRGPRX2-siRNA-treated LAD2 cells exhibited significantly less β-hexosaminidase release evoked by these substances compared with responses in control-siRNA-treated cells, while IgE-mediated release was comparable (Extended Data Fig. 10b). The residual β-hexosaminidase release observed in MRGPRX2-siRNA-treated cells is probably due to incomplete messenger RNA and/or protein knockdown.

Knowledge of the role of MRGPRX2 in drug-induced pseudo-allergies should expand further, for two reasons. First, ligand binding requirement studies should enable more specific screens for drugs that cross-activate MRGPRX2. Second, screening orally administered drugs may uncover more MRGPRX2 ligands, since common side effects of orally administered drugs include gastrointestinal problems and headache, both of which may have a mast cell component.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** B.D.M. conceived the project, designed and performed all experiments except where noted, and wrote the paper. P.P. performed all LAD2 cell work with supervision from M.K. S.M. performed tracheal contraction and tissue histamine release experiments. L.H. assisted with BAC purification and staining techniques. B.J.U. supervised S.M. and contributed to experimental design. X.D. supervised the project and wrote the paper.

**Author Information** Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to X.D. (xdong2@jhmi.edu).
METHODS

Animal models. All experiments were performed in accordance with a protocol approved by the Animal Care and Use Committee at the Johns Hopkins University School of Medicine. All experiments involving equal treatments in wild-type and mutant samples and animals were conducted by experimenters blind to conditions.

Analysis. Group data were expressed as mean ± s.e.m. Two-tailed unpaired Student’s t-test was used to determine significance in statistical comparisons, and differences were considered significant at P < 0.05. Statistical power analysis was used to justify the sample size. We assumed the data were normally distributed since the most outcome values were symmetrically distributed around the mean value within each group. The variance is similar between groups as determined by the F test. Mast cells deemed to be damaged, either by visible lack of fibrinogen adherence or by abnormally high resting calcium levels, were excluded from analysis. Otherwise, no samples or animals subjected to successful procedures and/or treatments were excluded from the analysis. No randomization was used for animal studies since it is not applicable for the studies.

Peptides and drugs. Compound 48/80, vescipan mastoparan, rucornuron, tubocurarine, ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin were from Sigma. Cortistatin was from Tocris Biosciences. PAMP(9–20) was custom synthesized and purified to homogeneity. Atracurium, mivacurium, tubocurarine and all myospasmolytic drugs were from Aganto. Goat anti-mouse IgE (Ab9162) was from Abcam. Recombinant human insulin was from Roche.

Expression constructs. Mrgpr genes were cloned and inserted into the pcDNA3.1 mammalian expression plasmid using standard protocols. All mouse genes had a Kozak sequence at their amino terminus and also encoded a carboxy-terminal Flag tag separated from the genes by the amino acid linker DIIIL.

cDNA constructs. First-strand cDNA was prepared as described for RT–PCR screens, and amplification was performed using the Q5 HotStart High Fidelity Master Mix (New England Biolabs). At least five different clones each prepared from wild-type and mutant mice were sequenced to verify the presence of the deletion in the mutant and the absence of any other mutation from wild type.

Calcium imaging in HEK293 cells. In initial screens, HEK293 cells (not tested for mycoplasma but rapidly dividing) were transiently transfected with gene constructs including a C-terminal Flag tag, and plated on 100 μl ml −1 poly-l-lysine-coated glass coverslips 6 h after transfection. Twenty-four hours later, cells were loaded with AM esters of the calcium indicators Fura-2 or FluO-4 (Molecular Probes) along with 0.02% Pluronic F-127 (Molecular Probes) for 45 min at 37 °C. Fura-2-loaded cells were imaged during 340 and 380 nm excitation, and FluO-4 loaded cells were imaged during 488 nm excitation. Later experiments used cell lines stably expressing receptors along with transient or stable expression of the promiscuous G protein Gα13. Cells were imaged in calcium imaging buffer (CIB; NaCl 125 mM, KCl 3 mM, CaCl2 2.5 mM, MgCl2 0.6 mM, HEPES 10 mM, NaHCO3 1.2 mM, sucrose 20 mM, brought to pH 7.4 with NaOH). Unless otherwise specified, all drugs were perfused into the chamber for 45 to 60 s and responses were monitored at 5 ± 5 s intervals for an additional 60–90 s.

EG0 values. Determination. HEK293 cells stably expressing Gα13 and either Mrgprb2 or MrgprX2 were plated at 4 × 104 cells per well in 96-well plates and incubated overnight. The next day, media was removed and replaced with imaging solution from the FLIPR Calcium 5 assay kit (Molecular Devices), diluted according to manufacturer’s suggestions in Hank’s balanced salt solution (HBSS) with 20 mM HEPES, pH 7.4. Cells were incubated in 100 μl imaging solution at 37 °C for 60 min, and allowed to recover for 15 min at room temperature before imaging in a Flexstation 3 (Molecular Devices). Wells were imaged according to manufacturer’s specifications for 120 s, with 50 μl of test substances at three times the concentration added 30 s after imaging began. Responses were determined by subtracting the minimal signal from the maximum signal. Substances were tested in duplicate wells, the signals were averaged, and EC50 values were determined for each trial by normalizing to the peak response to the substance in that trial. All drugs were dissolved in HBSS plus HEPES solution, with the following exceptions due to solubility issues: cetrorelix acetate was dissolved in saline containing 2.5 mM CaCl2 and 0.6 mM MgCl2, and fluoroquinolones except ofloxacin were dissolved in the same solution except that the pH was adjusted with HCl to 3.5; oloxacin required 100 μg ml −1 of lactic acid for full solubility. We also noticed that peptides sometimes lost potency on freeze–thaw cycle, so most peptides were prepared directly from lyophilized stock.

Peritoneal mast cell purification and imaging. Adult male and female mice 2–5 months of age were killed through CO2 inhalation. A total of 12 ml of ice-cold mast cell dissociation media (MCDM; HBSS with 3% fetal bovine serum and 10 mM HEPES, pH 7.2) were used to make two sequential peritoneal lavages, which were combined and cells were spun down at 200g. The pellet from each mouse was resuspended in 2 ml MCDM, layered over 4 ml of an isotonic 70% Percoll suspension (2.8 ml Percoll, 320 μl 10% HBSS, 40 μl 1 M HEPES, 830 μl MCDM), and spun down for 20 min, 500g, 4 °C. Mast cells were recovered in the pellet. Purity was >95% in isolated cells and by morphological examination. Mast cells were resuspended at 5 × 106–1 × 107 cells ml −1 in DMEM with 10% fetal bovine serum and 25 mg ml −1 recombinant mouse stem cell factor (Sigma), and plated onto glass coverslips coated with 30 μg ml −1 fibronectin (Sigma). For counting, instead of plating, suspended mast cells were diluted 1/10 and affixed to slides by spinning at 1,000 r.p.m. for 5 min at 4 °C on a CytoSpin (Thermo Scientific).

For imaging, after 2 h of incubation at 37 °C, 5% CO2, mast cells were loaded with FluO-4 along with 0.02% Pluronic F-127 for 30 min at room temperature, washed three times in CIB and used immediately for imaging. Cells were used within 2 h of loading. Cells were identified as responding if the (Ca2+), rose by at least 1,000 nM above basal, for at least 10 s, which fell induced response from random flickering events. Average traces were calculated by taking the average response from each cell in a mouse, and averaging those.

BAC transgenic mouse generation. We purchased the BAC clone RP23-65123 from the Children's Hospital Oakland Research Institute. This clone contains the Mrgprb2
locus, ~60 kb of 5’ genomic sequence and over 100 kb of 3’ genomic sequence. Recombining in bacteria was used to introduce eGFP-Cre and a polyA signal immediately after the Mrgprb2 start codon1. The BAC was linearized with NotI (New England Biolabs) and injected into pronuclei from single-cell-fertilized C57BL/6 eggs. Eggs were implanted into pseudopregnant females. Three BAC mouse lines were established. Although mice were already in a C57BL/6 background, they were crossed for at least four generations to wild-type and tdTomato reporter mice to establish. Although mice were already in a C57BL/6 background, they were crossed for at least four generations to wild-type and tdTomato reporter mice to establish. Despite mice were already in a C57BL/6 background, they were crossed for at least four generations to wild-type and tdTomato reporter mice to establish. Although mice were already in a C57BL/6 background, they were crossed for at least four generations to wild-type and tdTomato reporter mice to establish. Although mice were already in a C57BL/6 background, they were crossed for at least four generations to wild-type and tdTomato reporter mice to establish. Although mice were already in a C57BL/6 background, they were crossed for at least four generations to wild-type and tdTomato reporter mice to establish. Wild-type and Mrgprb2MUT mouse genotyping.

Avidin labelling of tissue. Adult male and female mice up to 8 months of age were anaesthetized with pentobarbital and perfused with 20 ml 0.1 M PBS (pH 7.4, 2°C) followed by 25 ml of fixative (4% formaldehyde [vol/vol], 4°C). Heart, trachea and skin sections were dissected from the perfused mice. Tissues were post-fixed in fixative for more than 24 h and were sectioned (20 μm thickness) with an anti-fade solution. In parallel, we also stained cells in suspension with Hoechst 33342, spun the cells down, and mixed the resuspended cells directly in a PBS anti-fade solution before placing directly onto slides and mounting coverslips on the suspension. No tdTomato-positive cells were seen in any preparation using either method.

Tissue histamine release studies. Whole tracheae or segments of skin isolated from the abdominal aspect of shaved male and female mice up to 6 months of age (4–8 mg wet weight) were dissected and cleaned of connective tissue. After a 60 min incubation period in oxygenated Krebs’ bicarbonate buffer solution (37°C), the tissue was treated with either vehicle or compound 48/80 for 30 min. The supernatant solution was saved for histamine analysis. The tissue was then subjected to 8% perchloric acid in a 37°C water bath for 15 min to obtain total histamine content. Histamine was assayed by the automated fluorometric technique previously described11.

Tracheal contractions. Tracheal contractions were carried out as previously described12. For allergen (ovalbumin) responses, mice were actively sensitized by injecting 0.2 ml of an ovalbumin solution (3.75 μg ml$^{-1}$) mixed with Al(OH)$_3$ three times at an interval of 2 days. Experiments were conducted on male and female animals 8–12 weeks of age before beginning 2 weeks after the first injection. Trachea were cleaned of connective tissue and tracheal rings (whole or laterally divided in half), were suspended between two tungsten stirrups in 10 ml organ chambers filled with Krebs’ buffer that was warmed to 37°C and bubbled with 95% O$_2$–5% CO$_2$ to maintain a pH of 7.4. One stirrup was connected to a strain gauge (model FT03; Grass Instruments), and tension was recorded on a Grass Model 7 polygraph (Grass Instruments). Preparations were stretched to a resting tension of 0.2 g, and washed with fresh Krebs’ buffer at 15-min intervals during a 60-min equilibration period. After equilibration, trachea were challenged with either ovalbumin (10 μg ml$^{-1}$) or compound 48/80. At the end of each experiment, all trachea were maximally contracted with carbachol (1 μM). All results are expressed as a percentage of maximum contraction.

Hindpaw swelling and extravasation. An adult male mouse up to 8 months of age was anaesthetized with an intraperitoneal (i.p.) injection of 50 mg kg$^{-1}$ pentobarbital (Sigma). Fifteen minutes after induction of anaesthesia, mice were injected intravenously (i.v.) with 50 μl of 12.5 mg ml$^{-1}$ Evans blue (Sigma) in saline. Five minutes later, 5 μl of the test substance (or 7 μl of anti-IgE) was administered by intraplantar injection in one paw and saline was administered in the other paw. Paw thickness was measured by callipers immediately after injection. Fifteen minutes later (30 min after anti-IgE), paw thickness was measured again and mice were killed by decapitation. Paw tissue was collected, dried for 24 h at 50°C, and weighed. Evans blue was extracted by a 24 h incubation in formamide at 50°C, and the OD was read at 620 nm using a spectrophotometer. For studies using ketotifen, mice were challenged with ketotifen 30 min after 25 μl of a 10 mg ml$^{-1}$ solution of ketotifen at the same time as pentobarbital.

Systemic anaphylaxis assay. To minimize stress, animals were transported to the procedure area the day before injections. Adult male and female mice up to 8 months of age (25 to 35 g) were given an i.p. injection of 80 μg propanolol in saline (2 mg ml$^{-1}$) immediately after removal from their cages, and then placed back in their cages for 30 min before intravenous injections. The intravenous injections were performed on one mouse at a time. For each injection, a mouse was placed in a transport box and brought to a room with no other mice, to minimize stress from vocalizations during injection. The mouse was then placed in a restrainer, and the tail veins were dilated by repeated wiping of tail with a tissue soaked in 100% ethanol, followed by injection of ciprofloxacin in a 0.25 ml Hamilton syringe fit with a 30.5-gauge needle (BD Biosciences). The injection was determined to be successful only when all of the criteria were met: blood appeared in the syringe after needle insertion, all tail veins were visible after injection, and the mouse bled slightly from the injection site after needle withdrawal. The injection site was swabbed until blood stopped flowing, the mouse was placed in a separate cage from its housing cage, one mouse per cage, and returned to the room it was brought from. At least one wild-type and one mutant mouse were used for each experimental session. Body core temperature was measured with a rectal thermometer.

Mouse peritoneal mast cell histamine release assay. Mast cells were purified as described earlier and allowed to recover for 2 h in DMEM with 10% FBS and 25 ng ml$^{-1}$ mouse stem cell factor in a 37°C incubator with 5% CO$_2$. Cells were
then spun down, resuspended in CIB, counted, and plated at 300 cells per well in 75 μl CIB in 96-well plates coated with 20 μg ml⁻¹ fibronectin (Sigma). They were allowed to adhere to the substrate for 45 min at 37 °C in atmospheric conditions (that is, CO₂ levels were not adjusted) before assay. For the assays, cells were removed to room temperature and 75 μl of 2× concentrations of tested substances (all in CIB except for ciprofloxacin, which was in saline with 2.5 mM CaCl₂ and 0.6 mM MgCl₂, pH 3.5) were added. After 5 min, 40 μl of supernatant was aspirated, diluted with 40 μl CIB and frozen at −80 °C until histamine levels were determined. Anti-IgE treatment was similar, except that cells were incubated for 30 min at 37 °C after anti-IgE was added before aspiration of supernatant. Histamine content was determined by using an HTRF histamine assay kit (Cisbio Assays) according to the manufacturer’s instructions.

**Human mast cell culture.** LAD2 (Laboratory of Allergic Diseases 2) human mast cells were cultured in StemPro-34 SFM medium (Life Technologies) supplemented with 2 mM l-glutamine, 100 U ml⁻¹ penicillin, 50 μg ml⁻¹ streptomycin and 100 ng ml⁻¹ recombinant human stem cell factor (Peprotech). The cell suspensions were seeded at a density of 0.1×10⁶ cells ml⁻¹ and maintained at 37 °C and 5% CO₂, and periodically tested for the expression of CD117 and FcεRI by flow cytometry. Cell culture medium was semi-depleted every week with fresh medium.

**LAD2 degranulation assay.** LAD2 cells were sensitized for 20 h with 0.5 μg ml⁻¹ biotin-conjugated human IgE (Abbiotec). Cells were washed, resuspended in HEPES buffer (10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.38 mM Na₂HPO₄·7H₂O, 5.6 mM glucose, 1.8 mM CaCl₂·H₂O, 1.3 mM MgSO₄·7H₂O, 0.4% BSA, pH 7.4) at 0.025×10⁶ per well, and then stimulated with 0.1 μg ml⁻¹ streptavidin (Life Technologies) or other agonists at the indicated concentrations for 30 min at 37 °C/5% CO₂. The β-hexosaminidase released into the supernatants and in cell lysates was quantified by hydrolysis of p-nitrophenyl-β-D-glucosamide (Sigma-Aldrich) in 0.1 M sodium citrate buffer (pH 4.5) for 90 min at 37 °C until histamine levels were determined. Anti-IgE treatment was similar, except that cells were incubated for 30 min at 37 °C after anti-IgE was added before aspiration of supernatant. Histamine content was determined by using an HTRF histamine assay kit (Cisbio Assays) according to the manufacturer’s instructions.

**Enzyme immunoassay and ELISA.** LAD2 cells were washed with medium, suspended at 0.25×10⁶ cells per well, and incubated with compound 48/80, mastoparan, icatibant, atracurium or ciprofloxacin at the indicated concentrations for 3–24 h at 37 °C/5% CO₂. Cell-free supernatants were harvested and analysed for PGD₂ release by an enzyme immunoassay (Cayman chemical), while TNF content was quantified using an ELISA kit (eBioscience) according to the manufacturer’s instruction. The minimum detection limits were 55 pg ml⁻¹ for PGD₂ and 5.5 pg ml⁻¹ for TNF.

**Measurement of histamine release from LAD2 cells.** LAD2 cells were washed, suspended in BSA-free HEPES buffer at 0.1×10⁶ per well, and incubated with compound 48/80, mastoparan, icatibant, atracurium or ciprofloxacin at the indicated concentrations for 30 min at 37 °C/5% CO₂. A histamine (Sigma-Aldrich) stock solution of 100 μg ml⁻¹ was prepared and stored at −20 °C. The working standards of 4,000 ng ml⁻¹ to 7.8 ng ml⁻¹ were freshly prepared using twofold serial dilution. O-phthalaldehyde (OPT; Sigma-Aldrich) was dissolved in acetic acid methanol (10 mg ml⁻¹) and kept in the dark at 4 °C. Histamine standards and cell-free supernatants (60 μl) were transferred to a flat-bottom 96-black-well microplate and mixed with 12 μl 1 M NaOH and 3 μl OPT. After 4 min at room temperature, 6 μl 3 M HCl was added to stop the histamine-OPT reaction. Fluorescence intensity was measured using a 355 nm excitation filter and a 460 nm emission filter.

**siRNA transfection of LAD2 cells.** Expression of MRGPRX2 was downregulated with ON-TARGET plus SMARTpool siRNA against MRGPRX2 and control siRNA from Dharmacon. LAD2 cells were washed with medium, suspended at 0.5×10⁶ cells per well, and transfected with 100 nm MRGPRX2 siRNA and control siRNA in antibiotic-free StemPro medium using Lipofectamine 3000 (Life Technologies) according to the manufacturer’s instruction at 37 °C/5% CO₂. At 48 h, knockdown was confirmed by RT–PCR, and the cells were used for degranulation assays.

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Extended Data Figure 1 | MRGPRX1 orthologues are not expressed at relevant levels in mast cells under naive conditions. a, Results from a low-stringency RT–PCR screen (see Methods) in peritoneal mast cells for expression of the MRGPRX1 orthologues Mrgpra3 and Mrgprc11. Arrow points to expected band sizes. b, Percentages of peritoneal mast cells responding to the MRGPRX1 and Mrgprc11 agonist bovine adrenal medulla derived peptide, fragment 8–22 (BAM8–22, 500 nM). Activation was assayed by measuring rises in intracellular calcium, using imaging of the Fluo-4 dye. Differences are not significant (P = 0.39). n = 3 mice from each genotype. Group data are expressed as mean ± s.e.m. Two-tailed unpaired Student’s t-test was used to determine significance in statistical comparisons. WT, wild type; KO, knockout. c, Chart summarizing responses to MRGPRX2 ligands and the MRGPRX1 ligand chloroquine (CQ) by HEK293 cells transiently transfected with plasmids driving expression of MRGPRX2, Mrgprb2 and other mouse Mrgpr proteins (that is, Mrgprb1, Mrgprb10 and Mrgprb11) most closely related to Mrgprb2. Positive and negative responses are indicated with ticks and crosses, respectively. Responses were considered positive if at least half of the transfected cells showed a 50% increase in [Ca2+]i. No cells transfected with Mrgprb1, Mrgprb10 and Mrgprb11 responded to any listed drug.
Extended Data Figure 2 | Basic secretagogues and drugs that induce pseudo-allergic reactions activate mouse Mrgprb2 and human MRGPRX2 expressed in HEK293 cells. a, b, Example traces showing changes in $[Ca^{2+}]_i$, as measured by Fluo-4 imaging, from HEK293 cells expressing Mrgprb2 and Ga15 (a) or MRGPRX2 and Ga15 (b). Substances were perfused from the 30 to 90 s time period, except for ciprofloxacin, which was perfused between the 30 and 60 s time periods to minimize exposure to the low pH solutions it was dissolved in. Insulin was used as a negative control. c, Table of half-maximum effective concentration (EC$_{50}$) values of basic secretagogues and drugs associated with pseudo-allergic reactions to activate Mrgprb2- and MRGPRX2-expressing HEK293 cells. The EC$_{50}$ values were determined from dose–response studies, which were repeated three times. Data are expressed as mean ± s.e.m.
Extended Data Figure 3 | Multiple lines of BAC transgenic mice confirm mast-cell-specific MrgprB2 expression. Representative confocal images from two other BAC transgenic mouse lines. BAC mice expressing eGFP-Cre in the Mrgprb2 open reading frame were mated to tdTomato reporter mice and tdTomato (red) expression was compared to avidin staining (green), a marker for mast cells. Scale bar, 20 μm.
Extended Data Figure 4 | Mrgprb2 is not expressed in mucosal mast cells or peripheral white blood cells.  

**a**, Representative images of a stomach section from an Mrgprb2-tdTomato mouse stained with an anti-MCPT1 (β-chymase) antibody to label mucosal mast cells. White arrows indicate positive cells. No cells were double-labelled (296 Mcpt1-labelled cells and 275 tdTomato-positive cells counted, n = 3 mice). Scale bar, 40 μm.

**b**, Representative images of a Cytospin preparation of peripheral white blood cells from an Mrgprb2-tdTomato mouse doubly labelled with tdTomato for Mrgprb2-expressing cells (red; left image) and Hoechst 33342 nuclear staining (blue; right image). No peripheral white blood cell expressed tdTomato (n = 3 mice; >4,000 cells examined). Scale bar, 40 μm.
Extended Data Figure 5 | Mrgprb2MUT mice are functional knockouts.

a, Illustration of the genomic region in and around the Mrgprb2 locus. Note that repetitive sequences including long interspersed elements (LINEs), short interspersed elements (SINEs), and long tandem repeats (LTRs) begin immediately after the 3′ side of the Mrgprb2 gene, and in addition are present within 2.5 kb of the 5′ side. A BLASTN search in March 2014 using the 500 bases adjacent to the 3′ end of Mrgprb2 as a query turned up more than 269,000 hits in the mouse genome.

b, Comparison of the wild-type (WT) and mutant (MUT) genomic sequences shows the location of the 4bp deletion in the mutant. Numbers correspond to the Mrgprb2 open reading frame.

c, Sequencing result from wild-type and mutant complementary DNA sampled from mice born 18 months after the mutant line was established. The bases missing in the mutant are highlighted in red.

d, Amino acid translation of the Mrgprb2MUT open reading frame reveals that the deletion creates a frameshift mutation and an early termination codon (marked with an asterisk) shortly after the first transmembrane region. Mut, site of the frameshift deletion; TM1, transmembrane region 1.
Extended Data Figure 6 | The mast cell numbers and the histamine content of tracheal and skin tissue was not different between wild-type and Mrgrpb2\textsuperscript{MUT} animals. a, Top, representative pictures of avidin staining in wild-type (WT) and Mrgrpb2\textsuperscript{MUT} (MUT) mice. Scale bar, 40 \textmu m. Bottom, quantification of mast cell numbers in various tissues. Differences are not significant, using a two-tailed unpaired Student’s t-test (\(n = 3\) mice for each genotype; over 3,000 \(\mu \text{m}^2\) and 1,000 \(\mu \text{m}^2\) counted for each genotype for hairy and glabrous skin, respectively; over 10,000 peritoneal cells counted). b, The tracheal histamine content averaged \(5.9 \pm 0.9\) and \(5.5 \pm 1.6\) ng mg\(^{-1}\) (\(n = 5\) for each genotype), respectively; the skin histamine content averaged \(30.8 \pm 3.2\) and \(30.2 \pm 4.0\) ng mg\(^{-1}\) (\(n = 8\) for each genotype), respectively. Differences were not significant. Group data are expressed as mean ± s.e.m. Two-tailed unpaired Student’s t-test was used to determine significance in statistical comparisons.
Extended Data Figure 7 | Endothelin acting through the ETA GPCR34 induced comparable activation in Mrgprb2^MUT and wild-type mast cells. 
a, Representative heat map images of mouse peritoneal mast cells showing changes in [Ca^{2+}]_i, as assayed by Fluo-4 imaging, induced by bath application of endothelin (1 μM). Scale bar, 10 μm. b, Averages of [Ca^{2+}]_i imaging traces for wild-type (WT) (red line) and Mrgprb2^MUT (MUT) (black line). The [Ca^{2+}]_i traces are similar between wild-type and mutant groups. Traces were averaged as described for Fig. 2a. c, Quantification of percentage of responding cells. Group data are expressed as mean ± s.e.m. Two-tailed unpaired Student’s t-test was used to determine significance in statistical comparisons (n = 3 for each genotype; over 180 cells counted for each genotype). Endothelin-induced responses were not significantly different.
Extended Data Figure 8 | IgE-mediated inflammation does not differ between wild-type and MrgprB2\textsuperscript{MUT} mice. a, Representative images of Evans blue stained extravasation 15 min after intraplantar injection of anti-IgE antibody (right, arrow, 100 μg ml\textsuperscript{−1}, 7 μl in saline) or saline (left).

b, Quantification of Evans blue leakage into the paw after 15 min (n = 6 for wild type (WT), n = 7 for MrgprB2\textsuperscript{MUT} (MUT)). Differences after anti-IgE antibody (P = 0.49) and saline (P = 0.23) injection are not significant. Group data are expressed as mean ± s.e.m. Two-tailed unpaired Student’s t-test was used to determine significance in statistical comparisons.
Extended Data Figure 9 | Mrgrp2MUT mast cells are unresponsive to basic secretagogues and various therapeutic drugs.  

**a.** Example traces showing changes in $[\text{Ca}^{2+}]_i$, as measured by Fluo-4 imaging, from wild-type (WT) and Mrgrp2MUT (MUT) peritoneal mast cells induced by the basic secretagogues from Fig. 2e. Each trace is a response from a unique cell. 

**b.** Representative Fluo-4 images (left) and fluorescence traces (right) from wild-type (top) and Mrgrp2MUT (bottom) cultured peritoneal mast cells during application of icatibant ($50 \mu\text{g ml}^{-1}$). 

**c.** Example traces showing changes in $[\text{Ca}^{2+}]_i$, as measured by Fluo-4 imaging, from wild-type and Mrgrp2MUT peritoneal mast cells induced by selected FDA-approved cationic peptidergic drugs. Each trace is a response from a unique cell.  

**d.** Representative Fluo-4 images (left) and fluorescence traces (right) from wild-type (top) and Mrgrp2MUT (bottom) cultured peritoneal mast cells during application of atracurium ($50 \mu\text{g ml}^{-1}$). 

**e.** Representative Fluo-4 images (left) and fluorescence traces (right) from wild-type (top) and Mrgrp2MUT (bottom) cultured peritoneal mast cells during application of ciprofloxacin ($200 \mu\text{g ml}^{-1}$).
Extended Data Figure 10  |  Human mast cells are activated by basic secretagogues and drugs associated with pseudo-allergic reactions in an MRGPRX2-dependent manner. a, Human LAD2 mast cells were treated with different concentrations of compound 48/80, mastoparan, icatibant, atracurium and ciprofloxacin. The activation of mast cells in response to these substances was characterized by the release of β-hexosaminidase, TNF, PGD2 and histamine. In addition, 0.1 μg ml⁻¹ streptavidin stimulation of biotin-conjugated human IgE-sensitized LAD2 cells caused a robust release of β-hexosaminidase (71.3 ± 1.8% release), compared with untreated cells (4.1 ± 0.3% release). Group data are expressed as mean ± s.e.m. b, Knockdown of human MRGPRX2 significantly reduced mast cell activation evoked by basic secretagogues and drugs associated with pseudo-allergic reactions, but not by IgE. Human LAD2 mast cells were first transfected with siRNA against MRGPRX2 or control siRNA. Two days after the transfection, the cells were treated with compound 48/80 (0.1 μg ml⁻¹), mastoparan (5 μg ml⁻¹), icatibant (10 μg ml⁻¹), atracurium (25 μg ml⁻¹) and ciprofloxacin (75 μg ml⁻¹). The activation of mast cells in response to these substances characterized by the release of β-hexosaminidase was significantly reduced in MRGPRX2-siRNA-treated cells, compared to release in the control group. IgE-mediated mast cell degranulation was unaffected by MRGPRX2 siRNA knockdown. Group data are expressed as mean ± s.e.m. Two-tailed unpaired Student’s t-test was used to determine significance in statistical comparisons, and differences were considered significant at *P < 0.05, **P < 0.01, ***P < 0.005 (the experiments were repeated three times).