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# Mast cell-mediated hypersensitivity to fluoroquinolone is MRGPRX2 dependent

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ABSTRACT

Fluoroquinolones trigger anaphylaxis during clinical applications, affecting the safety of their administration. Mast cells are immune cells that act as sentinels during host defenses, mediating hypersensitivity and anaphylactic reactions. Mas-related G protein-coupled receptor X2 (MRGPRX2) is a mast cell-specific receptor that mediates cell degranulation in anaphylactic reactions. In this study, the mechanism underpinning the anaphylactic reactions caused by fluoroquinolones was investigated. Hypersensitivity was assessed through hindpaw swelling, tissue fluid leakage assays, in vivo and body temperature measurements assay in vivo, and cell calcium mobilization assays, and mast cell degranulation assays in vitro. Mast cell-deficient *W-sash c-kit* mutant $Kit^{W\text{-}sh/W\text{-}sh}$ mice and MrgprB2 (the orthologous receptor of MRGPRX2 in mice) knockout mice exhibited reduced fluoroquinolone-induced anaphylactic effects. Fluoroquinolones activated mast cells in a dose-dependent manner and reduced degranulation was observed following MRGPRX2 silencing. These results reveal that fluoroquinolone-induced anaphylactic reactions are mediated by mast cells through MRGPRX2.

## 1. Introduction

Mast cells are immune cells located near the boundary between the external environment and the internal milieu and play a key role in allergic inflammatory responses [1]. When stimulated, mast cells release granules and recruit neutrophils [2]. G protein-coupled receptors (GPCRs) are seven transmembrane domain receptors that convert extracellular signals into biological responses [3]. Mas-related G protein-coupled receptor-X2 (MRGPRX2) is a newly discovered MRGPRX family member [4,5]. In 2015, McNeil and his coworkers discovered that MRGPRX2 is a target for drugs associated with systemic pseudo-allergies or anaphylactic reactions. MrgprB2 is the mouse ortholog of human MRGPRX2 [6].

Fluoroquinolones are antibacterial drugs used for the treatment of Gram-negative and Gram-positive ocular infections, and have topical, intravitreal and systemic routes of administration [7,8]. However, fluoroquinolones can induce hypersensitivity reactions upon initial use, most commonly urticaria and anaphylaxis [9,10]. Ciprofloxacin, the most frequently used fluoroquinolone, induces adverse reactions in approximately 1–2% of patients [11]. McNeil and colleagues reported that ciprofloxacin can activate MRGPRX2 [6], but the detailed mechanism underlying fluoroquinolone induced hypersensitivity was not explored.

In this study, we assessed nine fluoroquinolones (Fig. 1) that induce anaphylactic reactions through MRGPRX2. Wild-type (WT) mice, mast cell-deficient W-sash c-kit mutant $Kit^{W\text{-}sh/W\text{-}sh}$ (Kit) mice and MrgprB2 knockout (MUT) mice were used as models to investigate the anaphylactic effects of the fluoroquinolones in vivo. MRGPRX2 expressing HEK293 cells and the human mast cell line LAD2 were used to elucidate the mechanism of fluoroquinolone-induced calcium ($Ca^{2+}$) mobilization and mast cell degranulation via MRGPRX2 in vitro.

## 2. Materials and methods

### 2.1. Drugs and reagents

*p*-Nitrophenyl *N*-acetyl-β-D-glucosamide and Compound 48/80 (C48/80) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fleroxacin (CAS#79660-72-3), lomefloxacin (CAS#98079-52-8), pefloxacin (CAS#149676-40-4), norfloxacin (CAS #104142-93-0),

*Abbreviations:* MRGPRX2/B2, Mas-related G protein-coupled receptor X2/B2; C48/80, Compound 48/80; LAD2 cells, Laboratory of Allergic Disease 2 cells; WT mice, Wild-type mice; Kit mice, mast cell-deficient W-sash c-kit mutant $Kit^{W\text{-}sh/W\text{-}sh}$ mice; MUT mice, MrgprB2-mutant mice; $EC_{50}$, 50% of the maximal effect

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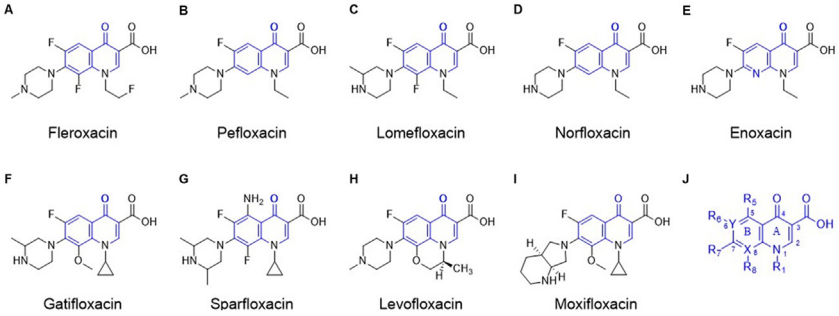


**Fig. 1.** Chemical structures of nine Fluoroquinolones. (A) Fleroxacin. (B) Pefloxacin. (C) Lomefloxacin. (D) Norfloxacin. (E) Enoxacin. (F) Gatifloxacin. (G) Sparfloxacin. (H) Levofloxacin. (I) Moxifloxacin. (J) The chemical skeleton of Fluoroquinolones.

enoxacin (CAS#74011-58-8), gatifloxacin (CAS#112811-59-3), sparfloxacin (CAS#110871-86-8), levofloxacin (CAS#177325-13-2), moxifloxacin (CAS#186826-86-8) were purchased from Dalin Meilun Biotechnology Co., LTD (Dalian, Liaoning, China). Saline was purchased from Shandong Qidu Pharmaceutical Co., Ltd. Fluo-3,AM was procured from Thermo Fisher Scientific (Waltham, MA, USA). Pluronic F-127 was procured from Biotium (SFO, CA, USA).

### 2.2. Mouse models

Young adult mice aged 6–8 weeks were used in all the experiments in this study. Animals were housed at the Experimental Animal Center of Xi'an Jiaotong University and provided food and water adlibitum. C57BL/6 (WT) mice were purchased from the Experimental Animal Center at Xi'an Jiaotong University (Xi'an, China). MrgprB2-knockout (MUT) mice on a C57BL/6 background were kindly provided by Professor Xinzhong Dong from Johns Hopkins University (Baltimore, MD, USA), and mast cell-deficient *W-sash c-kit* mutant $Kit^{W\text{-}sh/W\text{-}sh}$ (Kit) mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). The MUT mice were same as McNeil's study [6]. All experiments requiring identical treatment administration in the animals were conducted by investigators blinded to the conditions.

### 2.3. Ethics statement

The mice experimental protocol was approved by the Animal Ethics Committee of Xi'an Jiaotong University (Permit Number: XJTULAC2018-0541).

### 2.4. Cell lines

The human mast cell line, Laboratory of Allergic Disease 2 (LAD2), was kindly provided by A. Kirshenbaum and D. Metcalfe (NIH, USA), which were maintained in StemPro-34 medium with StemPro nutrient supplement, 2 mM L-glutamine and 100 ng/mL human stem cell factor (SCF) in the 37 °C incubator containing 5% $CO_2$. Culture medium was replaced every 7 days and the cells were kept at a density of $2 \times 10^6$ cells/mL. The MRGPRX2/HEK293 cells were also kindly provided by Professor Xinzhong Dong from Johns Hopkins University (Baltimore, MD, USA) and cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100× penicillin-streptomycin (HyClone, UT).

### 2.5. Mice peritoneal mast cells (MPMCs) purification assay

The MPMCs purification assay was the same as the protocol of the previous studies [12]. The process can be reformulated with more detail as follows, firstly, six to ten-month-old adult mouse was sacrificed through excess $CO_2$ inhalation. Secondly, 12 mL ice-cold mast cell dissociation media (MCDM; HBSS with 3% fetal bovine serum and 10 mM HEPES, pH 7.2) was used to obtain twice sequential peritoneal lavages. Thirdly, the cells were centrifuged at 200 ×*g* at 4 °C for 5 min, and washed with HBSS. Fourthly, the cells were resuspended in 2 mL MCDM, layered over 4 mL of Percoll suspension (2.8 mL Percoll, 320 μL 10× HBSS, 40 μL 1 M HEPES, and 830 μL MCDM) and centrifuged at 500 ×*g* and 4 °C for 20 min. Mast cells were in the pellet, the purity of which was confirmed by morphological analysis and toluidine blue staining. MPMC were resuspended in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal calf serum (FBS) 25 ng/mL recombinant mouse stem cell factor (SCF) and seeded in 96-well plates.

### 2.6. Avidin staining of MPMCs

Purified MPMCs were fixed with 4% PFA at room temperature for 10 min, and then were pre-incubated in blocking solution (PBS, pH 7.4, contained 10% normal goat serum and 0.2% Triton X-100 (v/v)) for 2 h, then incubated with FITC-avidin (1/500) for 1 h. The cells were washed three times with PBS, and observed under a fluorescence microscope using excitation wavelengths of 488 nm.

### 2.7. Intracellular calcium mobilization assay

All the fluoroquinolones were diluted to the required concentration by the calcium imaging buffer (CIB, 125 mM NaCl, 2.5 mM CaCl2, 3 mM KCl, 0.6 mM MgCl2, 20 mM glucose, 10 mM HEPES, 1.2 mM $NaHCO_3$, 20 mM sucrose, brought to pH 7.4 using NaOH [12]). For imaging, cells were incubated with incubation buffer consisted by 4 μM Fluo-3, AM and 0.1% Pluronic acid F-127 in CIB for 45 min, then washed twice in CIB, and cells were imaged under 488 nm excitation, fluoroquinolones were added to the well at 10 s after starting imaging, and responses were monitored for an additional 120 s of 1-s intervals.

### 2.8. β-Hexosaminidase release assay

The β-Hexosaminidase release assay was the same as the protocol of the previous studies [12]. LAD2 cells were seeded at the density of $2 \times 10^4$ cells per well into a 96 well plate, changed the culture medium to modified Tyrode's solution (120 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 5.5 mM glucose, 10 mM HEPES, 5 mM BSA), which contained drugs at the indicated concentrations. The cells were incubated at 37 °C for 30 min, the plate was then centrifuged at 200 ×*g* for 5 min, and the β-hexosaminidase was released into the supernatants. 50 μL cell supernatant was quantified via hydrolysis of *p*-nitrophenyl *N*-acetyl-β-D-glucosamide in 50 μL 0.1 M citric acid/sodium citrate buffer (pH 4.5) for 90 min at 37 °C. The reaction was stopped by adding 150 μL sodium carbonate buffer (0.1 M sodium carbonate/sodium bicarbonate, pH 11.0), and the samples were measured at 405 nm using a Microplate Reader. In the control group, cells were incubated by Tyrode's solution, after incubating, the cells were lysed with 0.1% Triton X-100 in modified Tyrode's solution, 50 μL of the cell supernatant and 50 μL of the cell lysate were treated by the

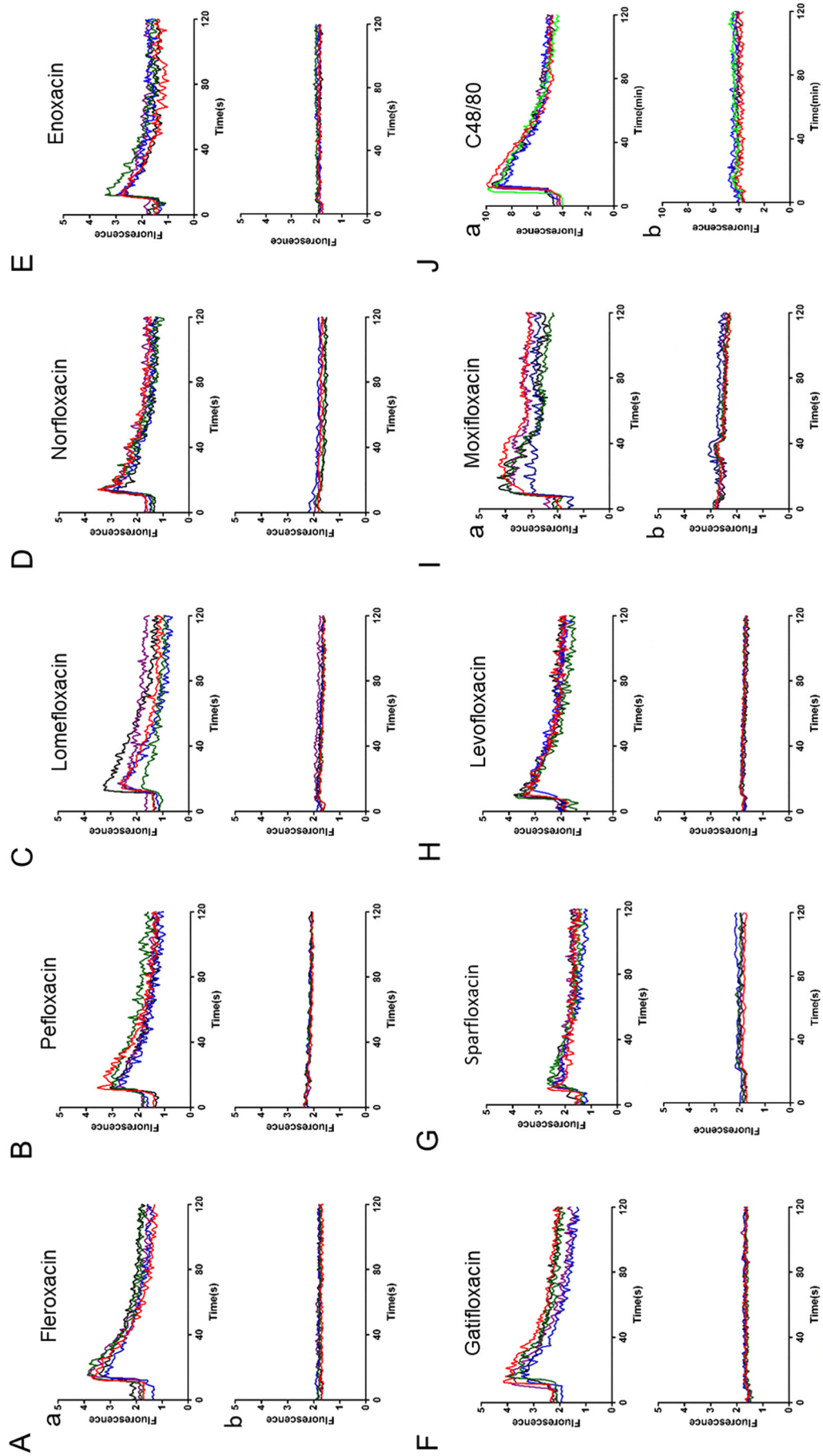


**Fig. 2.** Calcium imaging of nine Fluoroquinolones in HEK293-MRGPRX2 cells. Representative imaging traces of $Ca^{2+}$ concentrations treated by 200 μg/mL Fleroxacin. (A), Pefloxacin. (B), Lomefloxacin (C), Norfloxacin (D), Enoxacin (E), Gatifloxacin (F), Sparfloxacin (G), Levofloxacin (H), Moxifloxacin (I), and C48/80 (J) as a positive control in HEK293-MRGPRX2 cells (a) and HEK293 cells (b) (n = 3 per concentration; 0.150 cells counted per condition).

same method as the experimental group. The β-hexosaminidase release was assessed as percentage release of total β-hexosaminidase.

### 2.9. Histamine release assay

The histamine release assay was the same as the protocol of the previous studies [12]. An LCMS-8040 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used in the applied LC-ESI-MS/MS method. Histamine (HA) was purchased from Sigma, histamine·2HCl (A, A, B, B-D4, 98%) was obtained from Cambridge Isotope Laboratories, Inc. (MA, USA), and HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific (Waltham, MA, USA). LC-MS grade formic acid was obtained from Sigma. LAD2 cells were treated by fluoroquinolones at the indicated concentrations for 30 min, and the supernatant was collected. Histamine was evaluated on this system with a HILIC column (Venusil HILIC, 2.1 mm × 150 mm, 3 μm, Agela Technologies, Tianjin, China), and an isocratic elution buffer comprising a solution of acetonitrile-water containing 0.1% formic acid and 20 mM ammonium formate (77:23, v/v) was used to elute the histamine at a flow rate of 0.3 mL/min.

### 2.10. siRNA transfection of LAD2 cells assay

Specific knockdown was achieved using small interfering siRNAs as previous studies mentioned [12]. The siRNAs targeting MRGPRX2 or non-specific siRNAs were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China). The sequences of siRNA were as follows: Negative Control (NC), forward, 5′-UUCUCCGAACGUGUCACGUTT-3′, and reverse, 5′-ACGUGACACGUUCGGAGAATT-3′; MRGPRX2, forward, 5′-GUACAACAGUGAAUGGAAATT-3′, and reverse, 5′-UUUCCAUUCAC UGUUGUACTT-3′; the siRNAs were delivered at a final concentration of 1 μM using Lipofectamine® 3000 transfection reagent according to the manufacturer's instructions. LAD2 cells were cultured for 48 h for MRGPRX2 knockdown.

### 2.11. Hindpaw swelling and extravasation assay

The protocol was the same as the previous studies [12]. 6–8 weeks old mice were anesthetized with intraperitoneal injection of 70 mg/kg pentobarbital sodium. The mouse was injected intravenously with 50 μL Evans blue (12.5% in saline, w/v), and the paw thickness was measured using a Vernier caliper before test. 5 μL of fluoroquinolones at the indicated concentrations or 10 μg/mL C48/80 was injected into the subcutaneous of one paw, and saline was injected into the other paw as a negative control. 15 min later, the paw thicknesses were measured again and recorded. The mice were sacrificed, and the paw tissues were collected, dried at 50 °C, and weighed separately. Evans blue dye was extracted by adding 200 μL of a mixture of acetone-saline (7:3) to each tissue sample and incubating at 37 °C for 12 h. Tissues were then minced, disrupted for 10 min in an ultrasonic machine, and centrifuged at 5000 rpm for 10 min. The supernatant was transferred equally into 96-well plates (200 μL/well), and the OD value was measured at 620 nm using a Microplate Reader.

### 2.12. Body temperature test assay

Wild type (WT) or MrgprB2 knockout (MUT) young adult mice (aged 6–8 weeks old) were used for body temperature test by a Model FT3400 animal temperature tester (Nanjing Calvin biotechnology CO. LTD, Nanjing, Jiangsu, China). The WT and MUT mice were randomly divided into 3 groups (n = 5 each group), respectively. The mice were injected intravenously with 26 mg/kg of Lomefloxacin, Norfloxacin or Moxifloxacin (in saline, w/v), respectively. The body temperature was recorded using a biological function experimental system, in which a probe was inserted into the anus of the mice to get the rectal temperatures every 3 min continued for a total of 30 min.

### 2.13. Statistical analysis

Group data are expressed as mean ± SD. The obtained data were analyzed by SPSS 18.0 software (Chicago, IL, USA). Continuous variables were compared using *t*-test or the nonparametric Mann–Whitney test, as appropriate. One-way analysis of variance (ANOVA) was used for comparison of multiple groups. Bonfernoni method was used as post-hoc test. Differences were considered statistically significant of $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

## 3. Results

### 3.1. Fluoroquinolones induce calcium mobilization

To assess $Ca^{2+}$ mobilization, cells were treated with 200 μg/mL fleroxacin, pefloxacin, lomefloxacin, norfloxacin, enoxacin, gatifloxacin, sparfloxacin, levofloxacin or moxifloxacin. Upon analysis, all the fluorquinolones stimulated MRGPRX2-HEK293 $Ca^{2+}$ mobilization, but had little influence on naive HEK293 cells, indicating MRGPRX2 dependent effects (Fig. 2). Compound 48/80 (C48/80) is a secretagogue that activates MRGPRX2 [6] and was used as a positive control in these experiments. The concentration for 50% of maximal $Ca^{2+}$ mobilization effect ($EC_{50}$) of Lomefloxacin, Norfloxacin and Moxifloxacin on MRGPRX2/HEK293 cells were 46.22 ± 9.36 μg/mL, 24.28 ± 3.88 μg/mL or 56.88 ± 13.36 μg/mL, respectively (Fig. 3).

### 3.2. Fluoroquinolones trigger LAD2 cells degranulation

LAD2 cells closely resemble primary human mast cells [13]. To verify whether fluoroquinolones induce mast cell-mediated degranulation, the secretion of β-hexosaminidase and histamine were assessed in LAD2 cells. Both of them are pre-synthesized particulate matter released by activated mast cells. Following treatment with Fleroxacin, Pefloxacin, Lomefloxacin, Norfloxacin, Enoxacin, Gatifloxacin, Sparfloxacin, Levofloxacin, and Moxifloxacin (100 or 200 μg/mL) for 30 min, β-hexosaminidase and histamine secretion increased in a dose-dependent manner (Fig. 4). Secretion of these factors was also enhanced by treatment with 30 μg/mL C48/80, included as a positive control.

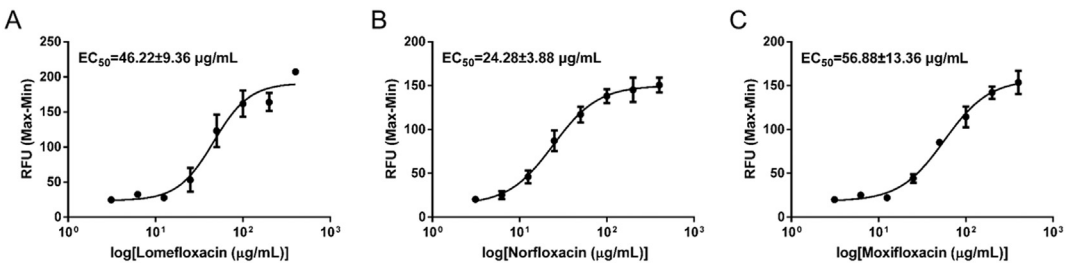


**Fig. 3.** Half-maximum effective concentration ($EC_{50}$) values of Lomefloxacin (A), Norfloxacin (B) and Moxifloxacin (C) to activate MRGPRX2-HEK293 cells. The $EC_{50}$ values were determined from dose-response studies, which were repeated three times.

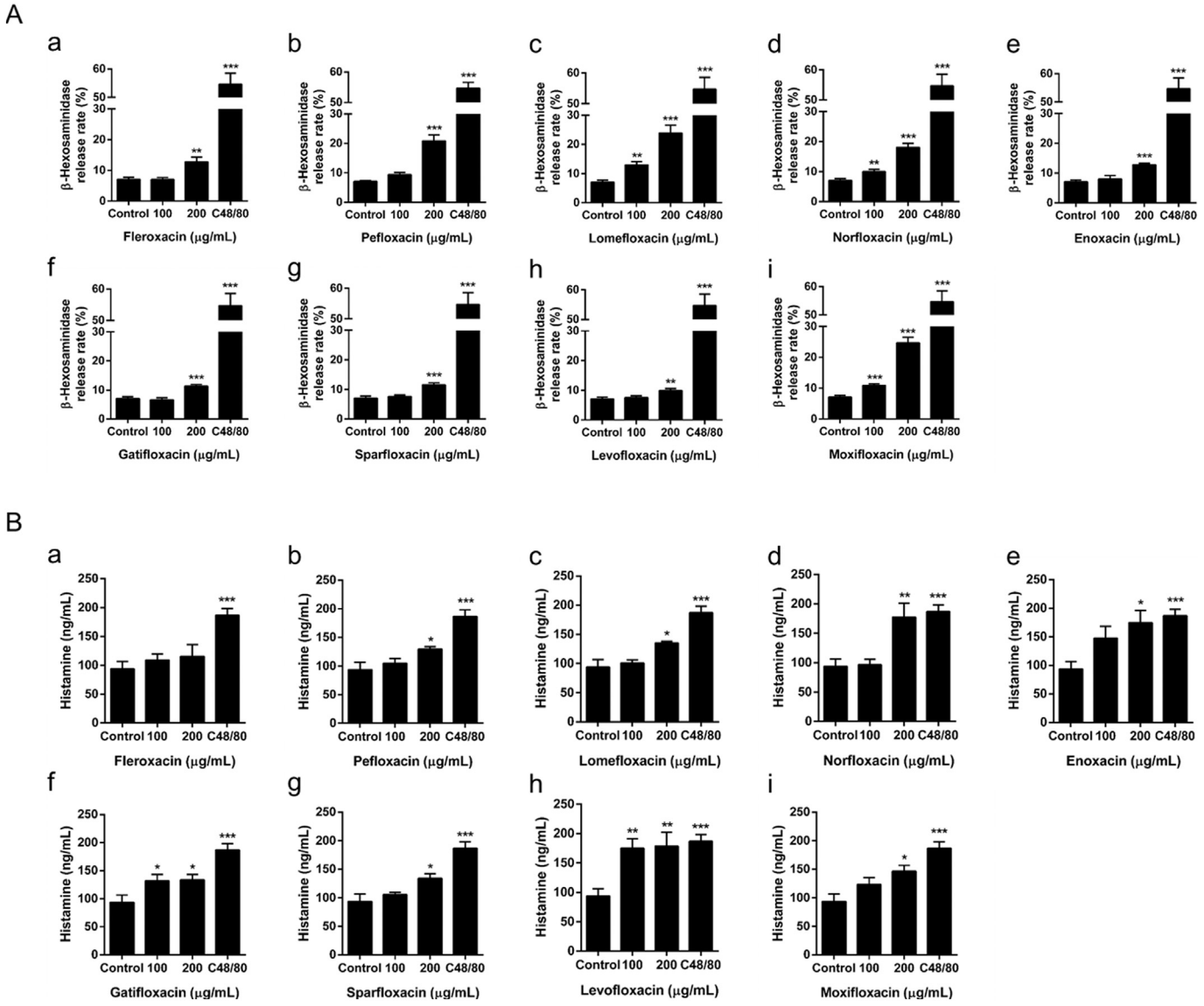


**Fig. 4.** The β-hexosaminidase release (A) and histamine release (B) of Fleroxacin (a), Pefloxacin (b), Lomefloxacin (c), Norfloxacin (d), Enoxacin (e), Gatifloxacin (f), Sparfloxacin (g), Levofloxacin (h) and Moxifloxacin (i) in LAD2 cells. Statistical significance was accepted at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$).

### 3.3. Fluoroquinolones induce LAD2 cell degranulation and $Ca^{2+}$ mobilization through MRGPRX2

We performed siRNA mediated MRGPRX2 silencing in LAD2 cells [12]. Both of the NC transfected LAD2 cells and MRGPRX2 knockdown LAD2 cells were treated with 0, 100, 200, and 400 μg/mL fluoroquinolones or 30 μg/mL C48/80. The β-hexosaminidase and histamine release were tested, which showed a significant reduction of degranulation in LAD2 cells by Lomefloxacin (Fig. 5A-a, b), Norfloxacin (Fig. 5B-a, b) or Moxifloxacin (Fig. 5C-a, b). Lomefloxacin, Norfloxacin or Moxifloxacin (200 μg/mL) could induce $Ca^{2+}$ mobilization in LAD cells transfected with scrambled siRNA, but had little effect on MRGPRX2 silenced LAD2 cells (Fig. 5A-c, d, B-c, d, C-c, d). Taken together, these data indicate that MRGPRX2 is essential for fluoroquinolone mediated LAD2 cell activation.

### 3.4. Fluoroquinolones induce $Ca^{2+}$ mobilization via MrgprB2 in mouse peritoneal mast cells (MPMCs)

MrgprB2 is the mouse ortholog of human MRGPRX2 that is expressed in MPMCs. MPMCs were washed and collected using previously described methods [6] and identified by toluidine blue and avidin staining (Fig. 6A–C). MPMC $Ca^{2+}$ mobilization was strongly stimulated by 200 μg/mL Moxifloxacin, Lomefloxacin and Norfloxacin, whilst almost no response occurred in MUT MPMCs (Fig. 6D–G). This indicated that MrgprB2 plays a crucial role in $Ca^{2+}$ mobilization in MPMCs.

### 3.5. Fluoroquinolones induce passive cutaneous anaphylaxis in mice

Hindpaw edema and exudation anaphylaxis mouse models [6] were used to explore in vivo fluoroquinolone-induced anaphylaxis. We performed intraplantar injections of Lomefloxacin, Norfloxacin, Moxifloxacin and C48/80 in the hindpaw for 15 min, and assessed extravasation leakage through Evans staining and hindpaw thickness. The results showed that all the fluoroquinolones evoked hindpaw swelling and Evans blue exudation in a dose-dependent manner. Saline injection produced no response (Fig. 7A, B, C).

### 3.6. Fluoroquinolone-induced mice pseudo-anaphylactic reactions are mediated through MrgprB2

Fluoroquinolones-evoked hindpaw inflammation (swelling and

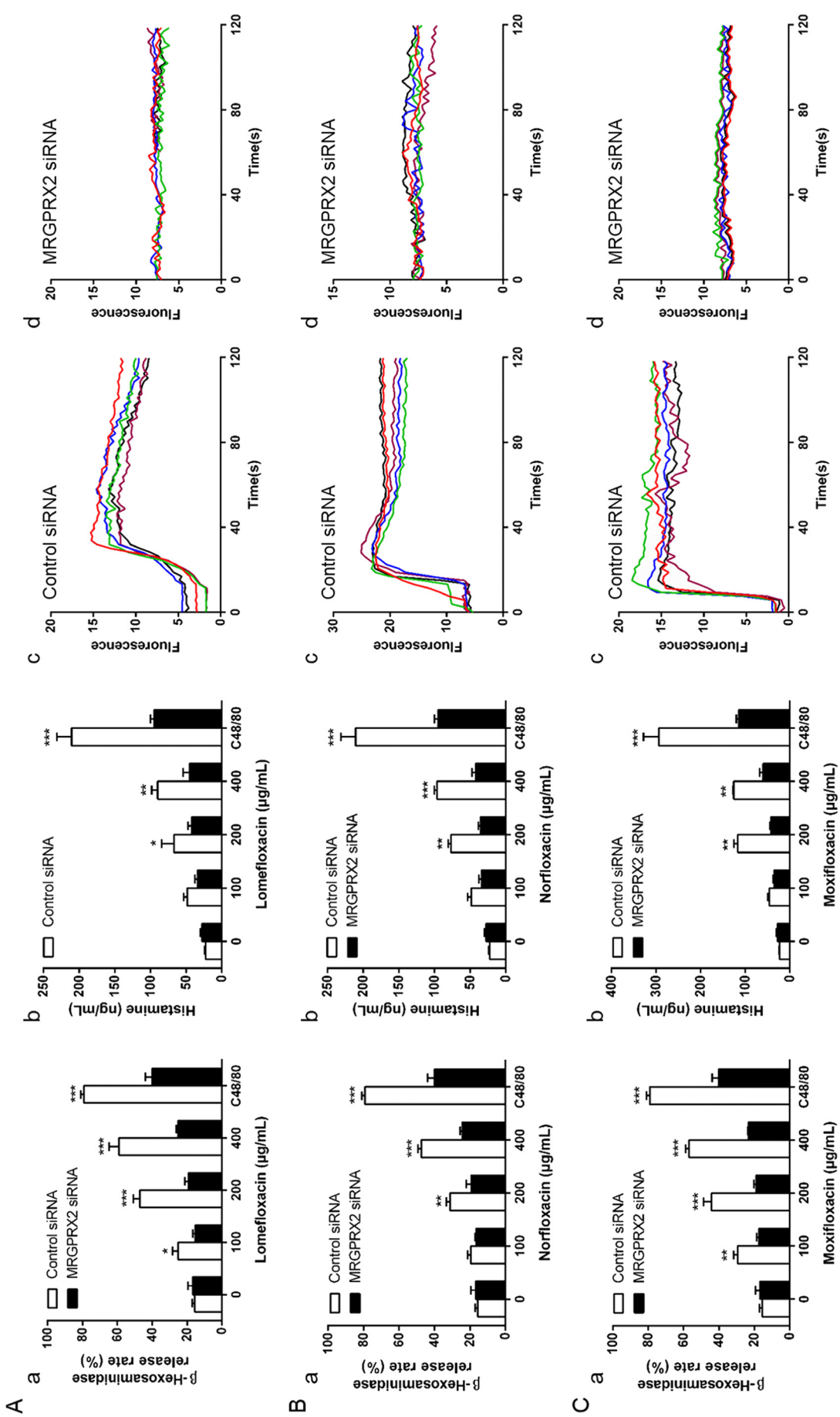


**Fig. 5.** The degranulation of mast cell triggered by Lomefloxacin (A), Norfloxacin (B) and Moxifloxacin (C) via MrpgrpX2. (a, b) The release of β-hexosaminidase (a) and histamine (b) in LAD2 cells treated with Fluoroquinolones for 30 min. (c) Representative imaging traces $Ca^{2+}$ concentrations treated by 200 μg/mL Fluoroquinolones in transfected negative control LAD2 cells. (d) Representative imaging traces $Ca^{2+}$ concentrations treated by 200 μg/mL Fluoroquinolones in transfected knockdown LAD2 cells. The date are presented as mean ± SD (n = 3). Statistical significance was accepted at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

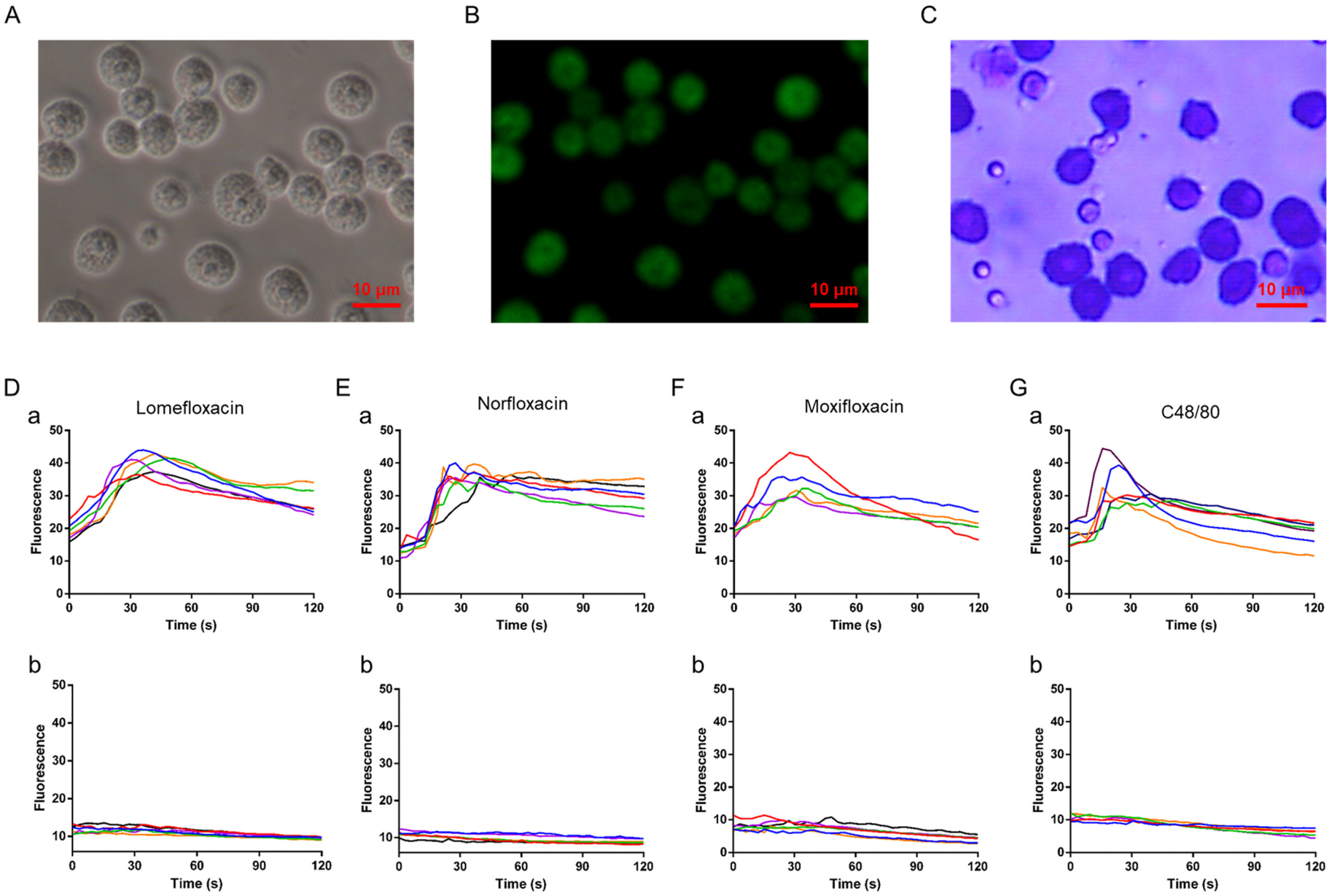


**Fig. 6.** Calcium imaging of Moxifloxacin, Lomefloxacin and Norfloxacin in MPMC. (A) Normal field of vision of MPMC. (B) Avidin staining of MPMC. (C) Toluidine blue staining of MPMC. (D, E, F, G) Representative imaging traces of $Ca^{2+}$ concentrations treated by 200 μg/mL Lomefloxacin (D), Norfloxacin (E), Moxifloxacin (F) and C48/80 (G) as a positive control in MrgprB2-WT MPMC (a) and MrgprB2-KO MPMC (b) (n = 3 per concentration, 150 cells counted per condition).

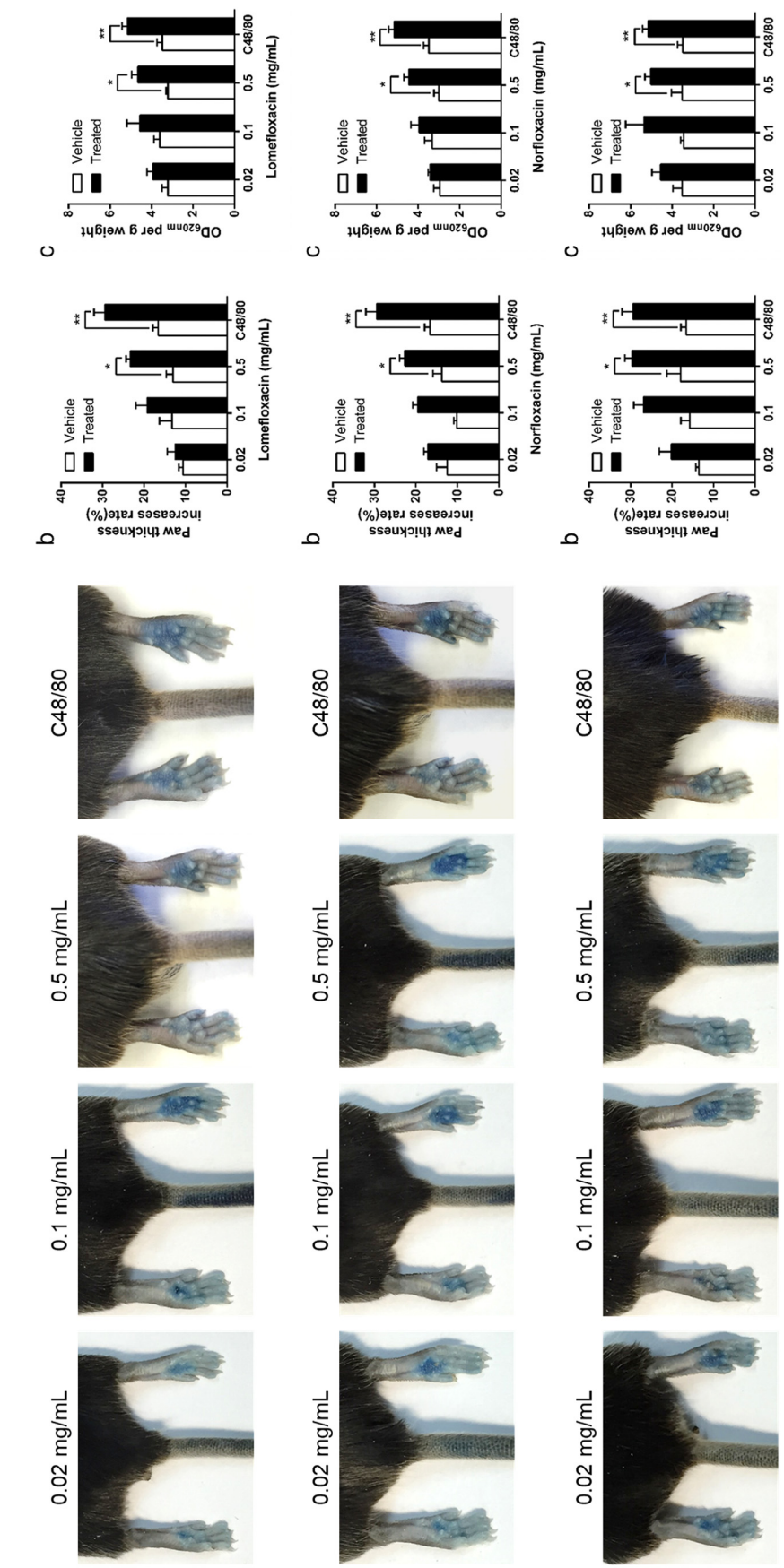


**Fig. 7.** Cutaneous flare reactions of mice treated with Lomefloxacin (A), Norfloxacin (B) and Moxifloxacin (C). (a) Representative images of Evans blue stained extravasation 15 min after intraplantar injection of Moxifloxacin (A), Lomefloxacin (B) or Norfloxacin (C) (right, 5 μL in saline) or C48/80 (right, 10 μg/mL, 5 μL in saline) as a positive control or saline (left, 5 μL). (B, C) Quantification of Evans blue leakage into the paw and paw thickness increase after 15 min. $OD_{620}$, optical density at 620 nm. Quantitative data were presented as mean ± SD. of five independent experiments. Statistical significance was accepted at $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$).

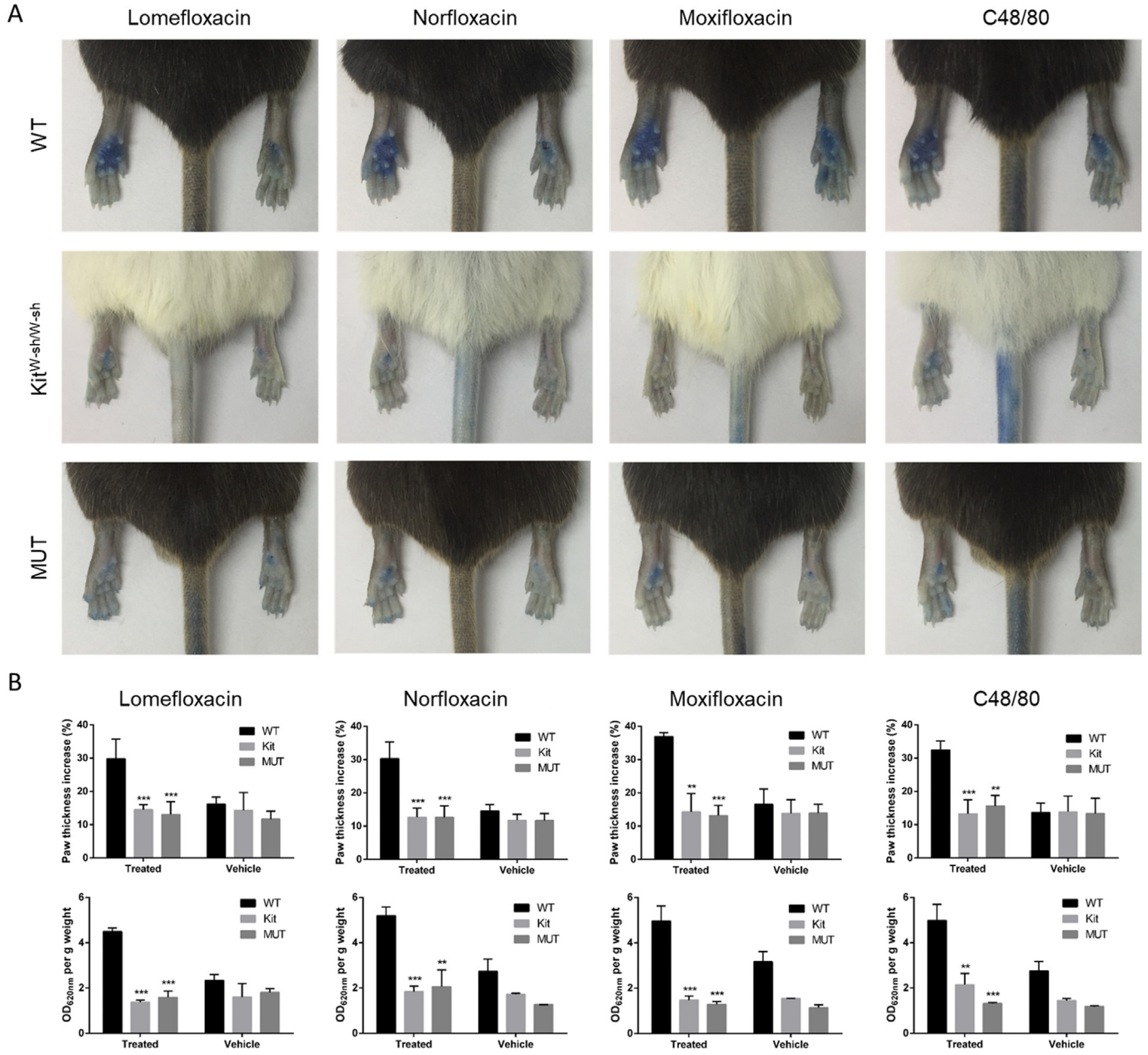


**Fig. 8.** Cutaneous flare reactions of mice treated with Lomefloxacin, Norfloxacin and Moxifloxacin in WT, Kit and MUT mice. (A) Representative images of Evans blue stained extravasation 15 min after intraplantar injection (B) Quantification of Evans blue leakage into the paw and paw thickness increase after 15 min. Quantitative data were presented as mean ± SD. of five independent experiments. Statistical significance was accepted at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$).

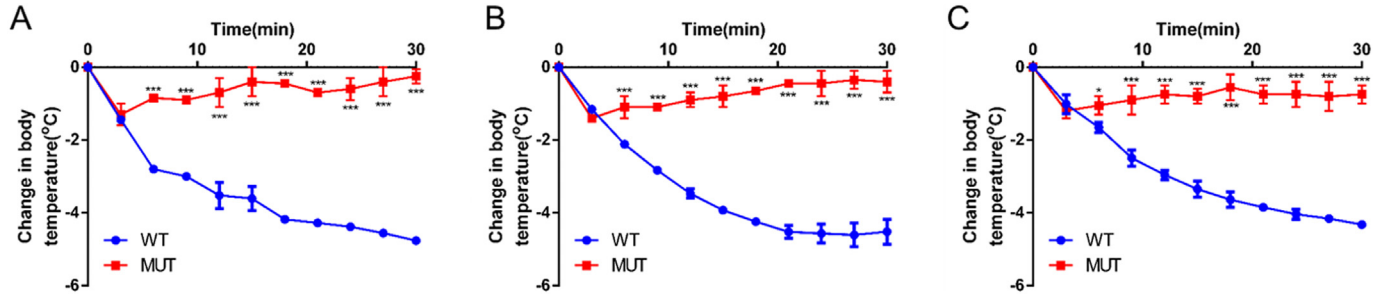


**Fig. 9.** Changes in body temperature after intravenous injection of 26 mg/kg Lomefloxacin (A), Norfloxacin (B) or Moxifloxacin (C) in saline. n = 4 mice per Fluoroquinolones. Data are presented as mean ± SD.

extravasation) test was carried out among the WT, Kit and MUT C57BL/6 mice to assess the anaphylactoid effects triggered by them. Following Lomefloxacin, Norfloxacin, Moxifloxacin or C48/80 treatment, WT mice displayed significant hindpaw inflammation, but it almost completely absented in Kit and MUT mice (Fig. 8A, B). A decreased body temperature also reflects anaphylaxis in vivo, which is likely due to blood pressure and peripheral vasodilatory changes [14]. When mice were treated with high doses of Lomefloxacin, Norfloxacin, Moxifloxacin or C48/80, the body temperature of WT mice dropped rapidly that was very slow to recover, whilst the reductions in MUT mice were more modest and were proceeded by a quick recovery (Fig. 9A, B, C). These results further explained that fluoroquinolone-induced hypersensitivity reactions target MrgprB2.

## 4. Discussion

It has been reported that fluoroquinolones induce hypersensitivity reactions through T-cell stimulation [15,16], causing immediate anaphylactoid reactions [17,18]. MRGPRX2 is a newly identified mast cell receptor that has been shown to mediate drug-induced pseudo-allergic reactions [6]. Despite the reported ability of ciprofloxacin to activate MRGPRX2, the ability of other fluorquinolones to influence this receptor has not been investigated. In this study, we investigated if fluoroquinolones mediate $Ca^{2+}$ mobilization and mast cell degranulation in LAD2 cells via MRGPRX2. We found that all the fluoroquinolones increased intracellular $Ca^{2+}$ mobilization in MRGPRX2/HEK293 cells, and enhanced β-hexosaminidase and histamine secretion in LAD2 cells. Lomefloxacin, Norfloxacin, and Moxifloxacin were selected for further exploration of the mechanism of anaphylactoid reactions. The $EC_{50}$ of $Ca^{2+}$ mobilization ranged from 20 to 60 μg/mL, which were significantly reduced following MRGPRX2 silencing. Thus, fluoroquinolones activate mast cells through MRGPRX2.

$Ca^{2+}$ mobilization plays a crucial role and regulates cell functions in mast cells [19]. The increased $Ca^{2+}$ concentration in the cytoplasm may activate STIM1, which is the endoplasmic reticulum (ER) $Ca^{2+}$ sensor, and STIM1 could act on Orai1, which is the $Ca^{2+}$ channel protein in the plasma membrane [20]. One of the mechanisms of fluoroquinolones-derived mast cells activation via MRGPRX2 and increasing intracellular $Ca^{2+}$ concentration may be accomplished by activating STIM1 and Orai1. Besides, mitochondria were also involved in regulating $Ca^{2+}$ concentration in mast cells. $Ca^{2+}$ mobilization is a ubiquitous intermediary of mast cell function in adaptive immune responses, and the subsequent degranulation of cytokines and chemokines played crucial roles in drug-induced anaphylactic reaction [21].

MrgprB2 is the mouse homolog of MRGPRX2 [6]. Fluoroquinolones were found to trigger $Ca^{2+}$ mobilization in WT C57BL/6 MPMCs, but had little effect on MrgprB2-MUT MPMCs. Fluoroquinolones also induced paw edema and Evans blue exudation into the paw of WT C57BL/6 mice in a dose-dependent manner, but did not influence Kit or MUT mice. Tail vein injections of the fluoroquinolones led to a decline in body temperature in WT but not MUT mice. These results suggested that mast cells play a significant role in fluoroquinolone induced anaphylactic shock, mediated through MrgprB2 in mice.

Different fluoroquinolones substituents were designed to withstand bacterial resistance and to obtain a wider spectrum of activity against anaerobic organisms [22]. In 2015, McNeil et al. reported the anaphylactoid reaction treated by ciprofloxacin was related to MRGPRX2 [6]. And in this study, we further investigated the degranulation characteristics of nine types of fluoroquinolones and found that all substances can activate mast cells and induce anaphylactoid reactions in mice model. Besides, it also has been reported that MRGPRX2 is a novel GPCR for cationic drugs [23,24], and McNeil et al. reported that ligand with charged nitrogen is critical for MRGPRX2 activation [6].

In this study, large amounts of tertiary amine and secondary amine groups are in those fluoroquinolones by the structural analysis, and the nitrogen atoms are easy to be charged. Hence, we proposed that easy protonation nitrogen atom may be the crucial structure for fluoroquinolones triggered anaphylactoid reaction via MRGPRX2. Meanwhile, the substituent of the nitrogen atom can affect the electrical properties of the whole compound. So we estimate that Fleroxacin induced degranulation of LAD2 cells were less than others was due to the more fluorine atoms in the substituents of which reduced its positive electric charge.

## 5. Conclusions

In summary, fluoroquinolones cause immediate hypersensitivity reactions and stimulate mast cells in a dose-dependent manner. Fluoroquinolones cause mast cell degranulation via MRGPRX2 and increase vascular permeability and anaphylactoid symptoms. Given these findings, we suggest that the symptoms of shock should be closely monitored during the clinical application of fluorquinolone.

## Conflict of interest

The authors declare no financial or commercial conflict of interest.

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