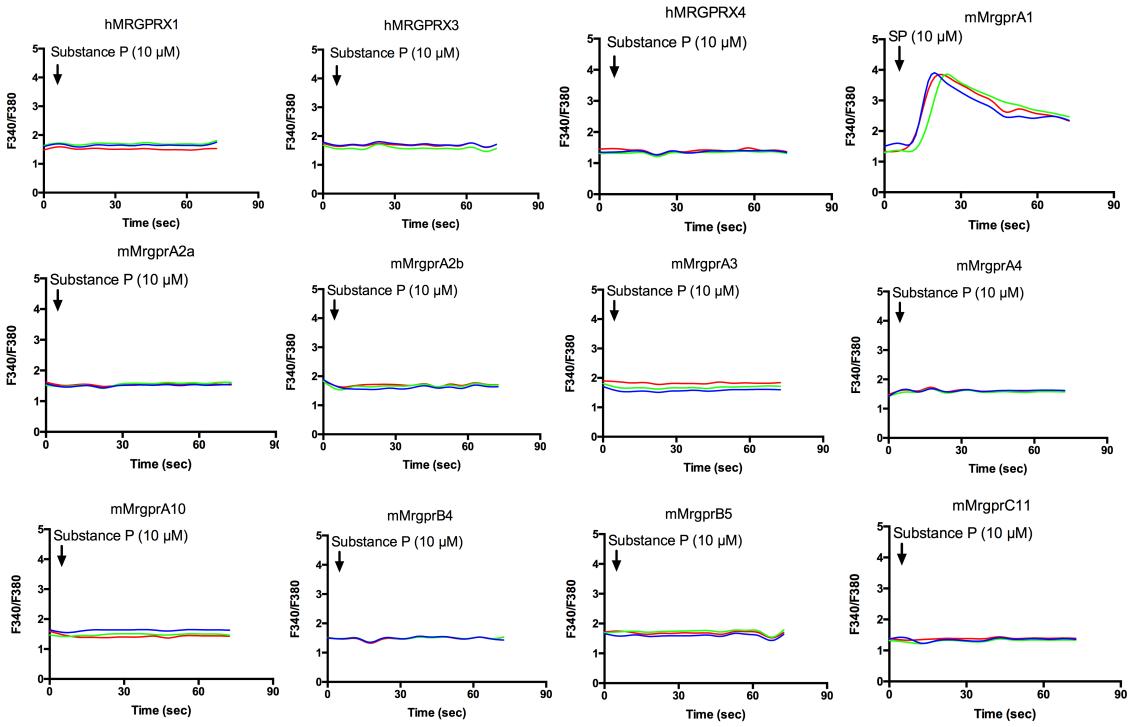
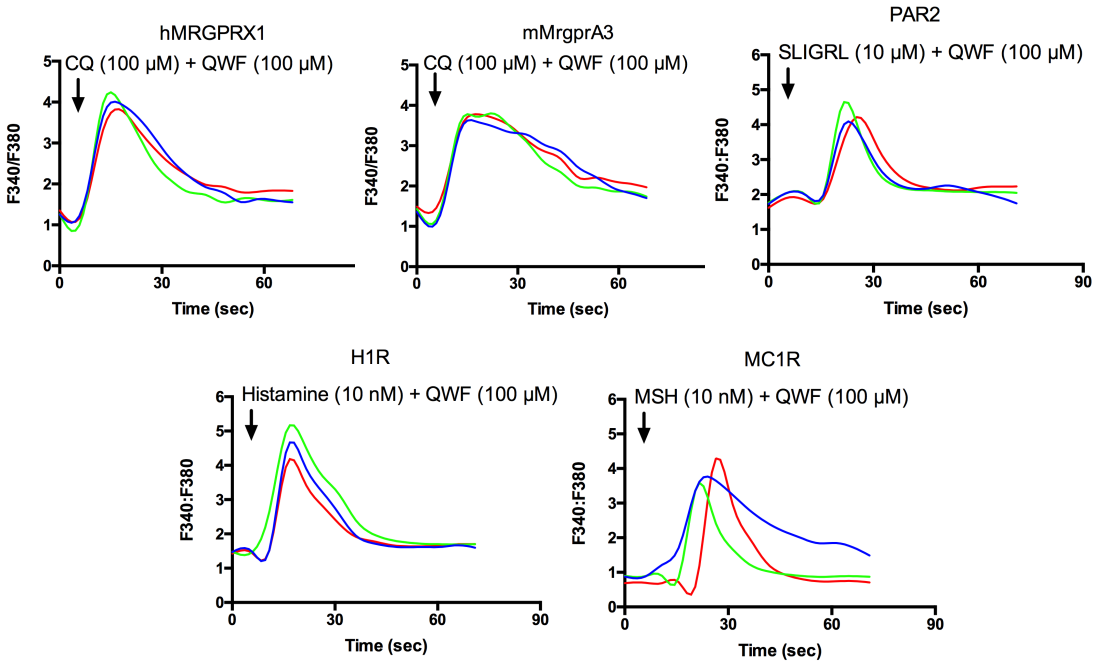
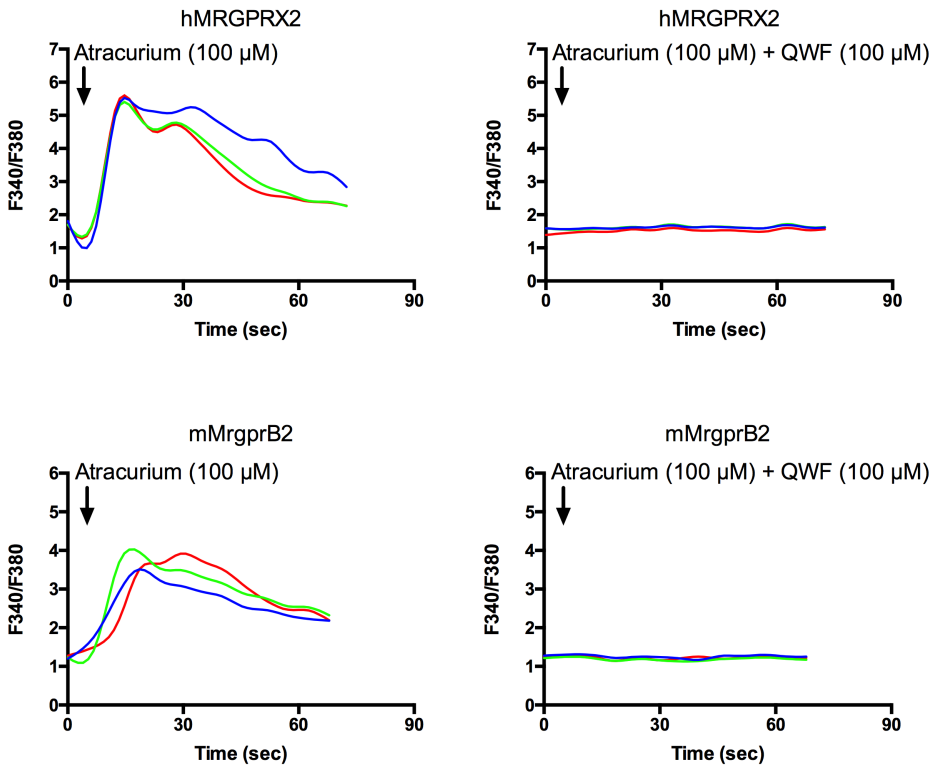


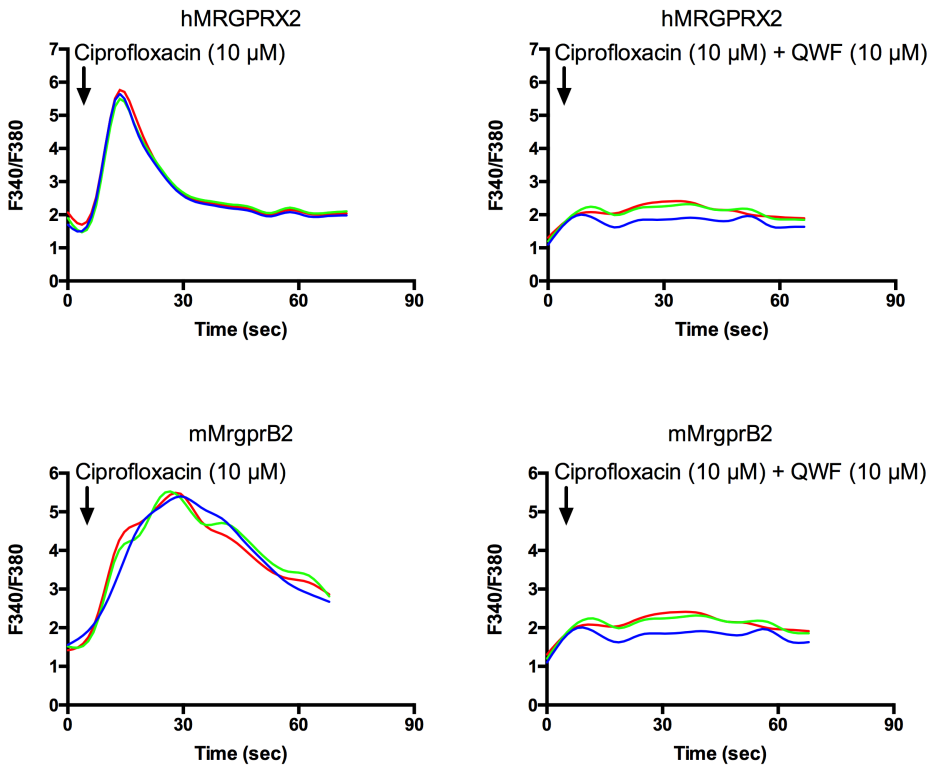
Supplementary figure 1. SP activates HeLa cells transfected with mouse MrgprA1 but not other Mrgprs. HeLa cells were transfected with cDNAs encoding mouse and human Mrgprs. Intracellular calcium $[Ca^{2+}]i$ was determined by ratiometric Fura-2 imaging after addition of SP. Each trace represents the response of a different cell. The studies in the individual panels were performed at least twice.



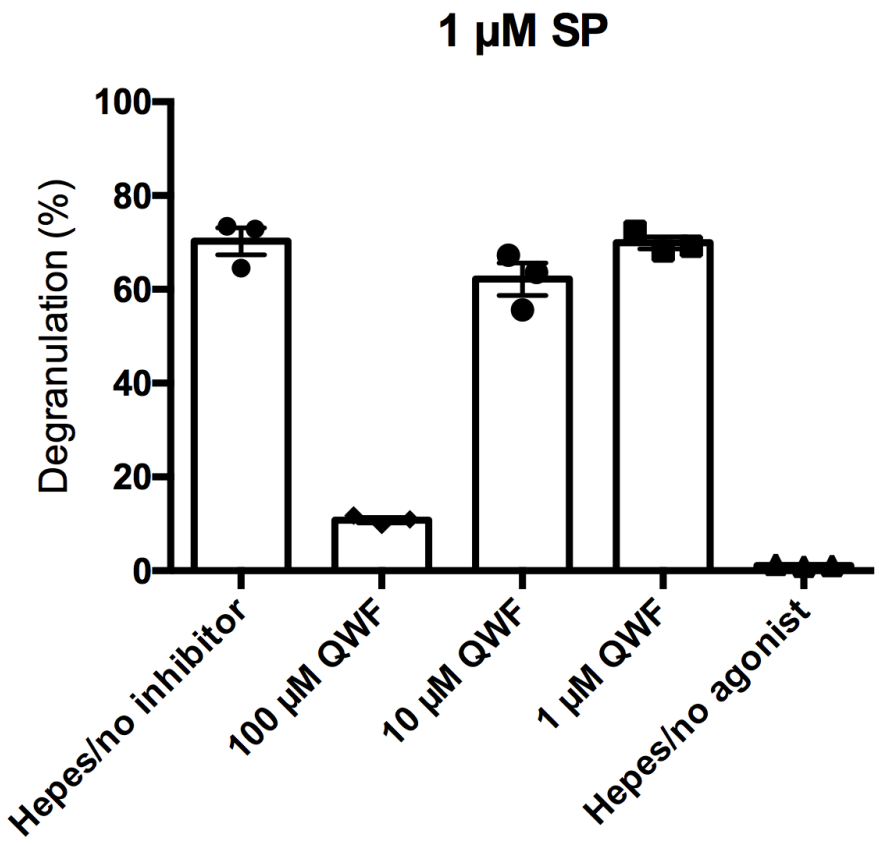
Supplementary figure 2. Specificity of QWF. QWF has no effect on other receptors involved in itch, including chloroquine (CQ) activation of MRGPRX1 or MrgprA3, SLIGRL activation of PAR2 or melanocyte stimulating hormone (MSH) on melanocortin-1, a non-itch receptor. HeLa cells were transfected with cDNAs of human MRGPRX1, mouse MrgprA3, PAR2, histamine-1 receptor, and melanocortin-1 receptor. Intracellular calcium $[Ca^{2+}]i$ was determined by ratiometric Fura-2 imaging. Each trace represents the response of a different cell. The studies in the individual panels were performed at least twice.



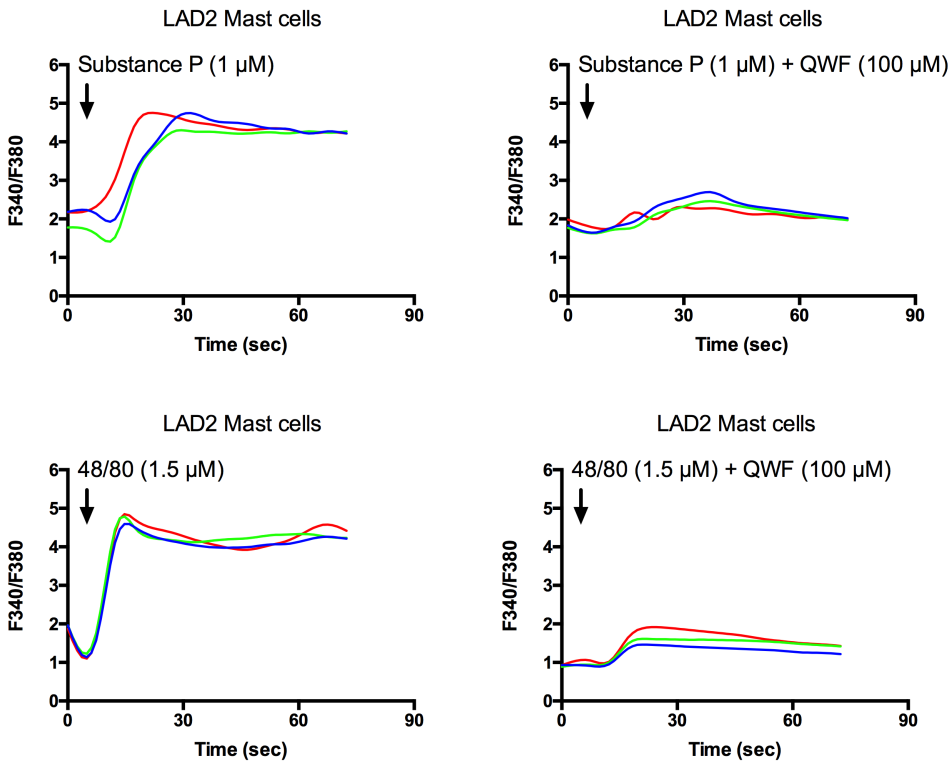
Supplementary figure 3. QWF inhibits activation of human MRGPRX2 and mouse MrgprB2 by atracurium. HeLa cells were transfected with cDNAs encoding mouse MrgprB2. A stably transfected HEK-293 cell line was used for human MRGPRX2. Intracellular calcium $[Ca^{2+}]i$ was determined by ratiometric Fura-2 imaging after addition of atracurium and QWF. Each trace represents the response of a different cell and the studies in the individual panels were performed at least twice.



Supplementary figure 4. QWF inhibits activation of human MRGPRX2 and mouse MrgprB2 by ciprofloxacin. HeLa cells were transfected with cDNAs encoding mouse MrgprB2. A stably transfected HEK-293 cell line was used for human MRGPRX2. Intracellular calcium $[Ca^{2+}]i$ was determined by ratiometric Fura-2 imaging after addition of ciprofloxacin and QWF. Each trace represents the response of a different cell and the studies in the individual panels were performed at least twice.



Supplementary figure 5. The effect of QWF on mast cell degranulation. The level of mast cell degranulation was assessed by the release of β-hexosaminidase in mast cell granules, quantified by the level of its substrate *p*-nitrophenyl *N*-acetyl-β-$_D$-glucosamide (PNAG) digested in a colorimetric assay that was performed three times.



Supplementary figure 6. QWF inhibits SP and compound 48/80 induced calcium [Ca^{2+}]i responses in human LAD2 mast cells. Intracellular calcium [Ca^{2+}]i was determined by ratiometric Fura-2 imaging of human LAD2 mast cells. Each trace represents the response of a different cell and the studies in the individual panels were performed at least twice.