**Detailed Methodology:**

**PRESTO-Tango GPCR β-arrestin recruitment assay**

HTLA cells, (an HEK293 cell line stably expressing a tTA-dependent luciferase reporter and a β-arrestin2-TEV fusion gene) were maintained in DMEM supplemented with 10% FBS, 2 μg/ml puromycin and 100 μg/ml hygromycin B in a humidified atmosphere at 37°C in 5% CO2. For the screening with P17, cells were seeded at poly-L-lysine coated and rinsed 96-well white cell culture plates (PerkinElmer) with 2 x 104 cells per well in 100 μl (day 1). The following day (day 2) cells were transfected with (0.2 μg) Tango-constructs using Lipofectamine 2000). 100 nM final concentration of P17 and SCT (positive control for Tango-SCTR construct) were prepared in filter-sterilized assay buffer, which consisted of 20 mM HEPES and 1x HBSS at pH 7.4, and 10 μl added to each well (day 3). Medium and drug solutions were removed from the wells (by aspiration) and 30 μl luciferase substrate solution (consists 500 mM DTT, 10 mM CoA, 100 mM ATP, 80 mg/ml luciferin and triton lysis buffer) were added to each well (day 4). After incubation for 10 min at room temperature, luminescence was counted in PerkinElmer Victor X4. To measure constitutive basal activity, no ligand was added on day 3.

For Tango-MRGPRX2 construct in vitro assays, cells were plated at 1 x 106 cells per 100 mm cell culture dish (day 1). The following day (day 2), cells were transfected using Lipofectamine 2000 with (2 μg) Tango-MRGPRX2 construct. On day 3, transfected cells were transferred at 20,000 – 30,000 cells per well in 90 μl of medium into poly-L-lysine coated and rinsed 96-well white cell culture plates (PerkinElmer). On day 4, 10x drug stimulation solutions (P17, CST-14, compound 48/80 or other P17 analogues) were prepared in filter-sterilized assay buffer, which consisted of 20 mM HEPES and 1x HBSS at pH 7.4, and 10 μl added to each well. For antagonistic study, 100 μM quercetin was added 1 hr before adding the agonists. On day 5, medium and drug solutions were removed from the wells (by aspiration) and 30 μl luciferase substrate solution (consists 500 mM DTT, 10 mM CoA, 100 mM ATP, 80 mg/ml luciferin and triton lysis buffer) were added to each well. After incubation for 10 min at room temperature, luminescence was counted in PerkinElmer Victor X4. Results in the form of RLU (relative luminescence units) were exported into Excel spreadsheets and percentage luminescence were calculated, and GraphPad Prism 8 was used for analysis of data. To measure constitutive basal activity, no ligand was added on day 4.

**Intracellular calcium assay**

Chinese hamster ovary (CHO-K1) cells from the American Type Culture Collection (ATCC) (Manassas, VA) were propagated in Minimum Essential Medium (MEM) (Gibco®, Life Technologies, Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco®) and 1% (v/v) antibiotic- antimycotic solution (penicillin, 100 U/mL; streptomycin, 100 μg/mL, (P/S)) (Gibco®). The cells were maintained in a 5% (v/v) CO2 humidified chamber at 37 ̊C and passaged twice per week using 0.25% (v/v) Trypsin-EDTA (TE) (Gibco®) on NuncTM Surface (Nunc, Denmark) tissue culture flasks. For all assays, CHO- K1 cells were seeded at a density of 2.0 x 105 per well onto six-well plates (Nunc) using MEM supplemented with 10% (v/v) FBS and 1% (v/v) P/S 24 hr prior transfection. CHO-K1 cells were used for transient expression of MRGPRX2 receptor constructs in pcDNA3.1 for Fluo4-NW calcium assay and confocal microscopy. Transient transfection was achieved using Lipofectamine 2000 Transfection Reagent (Invitrogen) according to the manufacturer’s protocol. After 24 hr post transfection, the cells were lifted using Gibco® Versene Solution and 5 x 104 cells were transferred to 35 mm glass-bottom petri dishes (MatTek Corporation) in overnight for confocal microscopy. 3 x 104 cells per well were seeded in clear bottom black 96 well plate (Part No. 6005182, PerkinElmerTM, USA) for the calcium assay. On the day of measurement, the clear bottom was covered with black seal (Part No. 6005189, PerkinElmerTM, USA) and fluorescence were counted in PerkinElmer Victor X4. Fluo4-NW assay kit (Thermo Fisher Scientific) is used for detecting the intracellular calcium as specified by the manufacturer.

**Real-time quantitative reverse transcription PCR (real-time qPCR)**

Harvested cells (CHO-K1, HTLA, LAD2, THP-1, differentiated THP-1 (MDMs) were used for RNA isolation. Total RNA from the harvested cells were obtained using Trizol. Total RNA was precipitated for 30 min in 20°C with isopropanol and glycogen. the precipitated RNA was then washed by 75% ethanol and resuspended in RNase-free water for first strand cDNA synthesis following the protocol from HiScript®II Q RT SuperMix (Vazyme biotech co., ltd, China), followed by quantitative PCR (7300 Real-Time PCR System, Applied Biosystems, Forster City, CA, USA). The expression of transcripts was examined using ChamQTM SYBR Color qPCR Master Mix as specified by the manufacturer. 2-ΔΔCt method (1) was employed for data analysis with the internal control, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*). qPCR primers used were tabulated and attached with supplementary documents.

**The β-hexosaminidase release assay**

LAD2 cells were suspended in modified Tyrode’s solution (consists of 120 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 10 mM HEPES, 5.5 mM glucose, 5 mM BSA) and seeded at 2 x 104 cells per well in 96 well plate. Either P17 or compound 48/80 at the indicated concentrations diluted in modified Tyrode’s solution into each well and incubated for 30 min. For antagonistic study, quercetin was added 30 min before adding the agonists. The plate was centrifuged at 200g for 5 min and the 50 μl of supernatant is removed and added into another 96 well plate. Then, 100 μl of 0.1% Triton X-100 in modified Tyrode’s solution is added on to lyse the cells. To analyze total β-hexosaminidase content in the cells, the β-hexosaminidase that was released into the supernatants and cell lysates were quantified via hydrolysis of p-nitrophenyl N-acetyl-β-d-glucosamide in 0.1 M citric acid/sodium citrate buffer (pH 4.5) for 90 min at 37 °C. The reaction was halted by adding 100 μl stop solution buffer (consists of 0.1 M sodium carbonate/sodium bicarbonate, pH 11.0), and the samples were measured at 405 nm using a Microplate Reader.  Percent (%) degranulation was calculated using the following equation: (Experimental β-hexosaminidase release – vehicle control β-hexosaminidase release) / (Experimental β-hexosaminidase release + (Triton-X-100 β-hexosaminidase release – vehicle control β- hexosaminidase release)) x 100. compound 48/80 was used as a positive control (2).

**Lentivirus production and MRGPRX2 silencing in LAD2 cells**

MRGPRX2-targeted Mission short hairpin RNA (shRNA) lentiviral plasmids were purchased from Sigma-Aldrich. We used shRNA for lentiviral production and a non-target shRNA vector was used as a control. SHC016**,** TRCN0000357640, TRCN0000357571, TRCN0000357641, TRCN0000357642, TRCN0000009176 were used to produce lentivirus, according to the manufacturer’s instructions. Cell transduction was conducted by mixing 2 ml viral supernatant with 3 ml LAD2 (5 x 106) cells. Eight hr post infection, medium was changed to virus-free complete medium, and antibiotic (puromycin, 2 μg/ml; Sigma-Aldrich) selection was initiated 16 hr later. Cells were analyzed for MRGPRX2 knockdown by qPCR (3). Stable knocked down of MRGPRX2 in LAD2 cells were obtained from TRCN0000357642 and TRCN0000009176 shRNA constructs.

**Quantification of Evans blue**

Mice were given an intraperitoneal injection of anaesthetics. Young adult mice (C57BL/6N aged 6–8 weeks old, n=6/group) were intravenously (i.v.) injected with 50 μl (irrespective of the bodyweight) of 12.5% Evans blue in saline. Before any test substance to be injected, a Vernier calliper was used to measure the thickness of the paws. Five min later, 5 μl of 10 μM P17 or CST-14 were injected into one paw, and saline was injected into the other paw as a negative control. Fifteen minutes later, the paw thicknesses were measured again and documented. Mice were euthanized by cervical dislocation under anaesthesia and the paw tissues were collected. These paws were dried at 50 °C, and weighed separately. Evans blue dye was extracted by adding 500 μl of a mixture of acetone-saline (7:3) to each tissue sample and incubating at 37°C for 12 hr. Tissues were minced and centrifuged at 3000 rpm for 20 min. The supernatant were aliquoted equally into 96-well plates (100 μl/well), and the OD value at 620 nm was measured using a Microplate Reader.

**Identification of P17 binding site on MRGRX2 receptor**

MRGPRX2 receptor belongs to opioid receptor family and all “Class A” GPCRs are generally known to have their active site at the transmembrane domain, largely adapted as a binding site for small compound agonists, while P17 is a 13-aa long peptide. In order to obtain the interaction sites between the ligand and the receptor, we used smaller fragments of the peptide, P17(5-13) and P17(5-11). For MD simulation, the homology model of receptor and peptide were merged and corrected for abnormalities by protein preparation wizard (PPW) and was later embedded in hydrolyzed 8×8 nm 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid bilayer structure. The system was submitted to steepest-descent energy minimization up to a tolerance of 1000 KJ.mol-1.nm-1 and was evaluated by protein-ligand root RMSD analysis for the 200 ns of simulation time. The RMSD of protein-ligand provided insight on the structural configuration showing that the simulation has equilibrated and fluctuations throughout the simulation are around the thermal average. We have performed a preliminary study of single unbiased MD simulation of 200 ns in which the P17 fragments start the interaction process with the MRGPRX2 model using GTX 1080Ti GPU based Linux platform with i7 8700s processor. Additionally, we have performed virtual docking algorithm also to identify the interaction sites of the ligand and receptor.

**Site directed mutagenesis**

We performed site directed mutagenesis on Tango-MRGPRX2 GPCR for aa 172 and aa 172-175 using the Q5 Site-Directed Mutagenesis Kit (BioLabs Inc.). The primers were generated using NEBaseChanger (BioLabs Inc.). To obtain MRGPRX2\*F172A, the forward primer used was 5’-TGGGTTCCTGGCAAGCGACGGGG-3’ and the reverse primer used was 5’-CAGAATTTTCCCTCCAGGATG-3’. For MRGPRX2\*172-175, the forward primer used was 5’- GCAGCAGACTCCGGCTGGTGTCAG-3’ and the reverse primer used was 5’-TGCTGCCAGGAACCCACAGAATTTTCC-3’. These mutants were verified with Sanger sequencing. The Mutant Tango-MRGPRX2 receptors were used for PRESTO-Tango GPCR β-arrestin recruitment assay.

**Pharmacophore region and alanine scanning of peptide P17**

In an attempt to locate the pharmacophore region of P17, we have synthesized 8 truncated P17: P17(3-13), P17(5-13), P17(7-13), P17(9-13), P17(1-5), P17(1-7), P17(1-9) and P17(1-11), and 12 peptides by replacing each aa of P17 with alanine. All these compounds were functionally tested for standard degranulation β-hexosaminidase assays in LAD2 cells. The sequence of the P17 peptide, P17 fragments and P17 alanine mutants were characterized by *de novo* sequencing using mass spectrometry and Edman degradation (4). P17, downsized and alanine-substituted analogues were synthesized by Fmoc solid phase methodology on a Liberty microwave assisted automated peptide synthesizer (CEM, Saclay, France) using the standard manufacturer’s procedures at 0.1 mmol scale as previously described (10, 28). All Fmoc-amino acids (0.5 mmol, 5 eq.) were coupled on preloaded Fmoc-Leu-, Fmoc-Ile-, Fmoc-Glu(O*t*Bu)- or Fmoc-Ala-Wang resin by *in situ* activation with HBTU (0.5 mmol, 5 eq.) and DIEA (1 mmol, 10 eq.) and Fmoc removal was performed with a 20% piperidine solution in DMF. After completion of the chain assembly, peptides were deprotected and cleaved from the resin by adding 10 mL of the mixture TFA/TIS/H2O (9.5:0.25:0.25) for 180 min at room temperature. After filtration, crude peptides were washed thrice by precipitation in TBME followed by centrifugation (4500 rpm, 15 min). The synthetic peptides were purified by reversed-phase (RP) HPLC on a 21.2 x 250 mm Jupiter C18 (5 µm, 300 Å) column (Phenomenex, Le Pecq, France) using a linear gradient (10-50% or 10-40% over 45 min) of acetonitrile/TFA (99.9:0.1) at a flow rate of 10 ml/min. The purified peptides were then characterized by MALDI-TOF mass spectrometry on a UltrafleXtreme (Bruker, Strasbourg, France) in the reflector mode using α-cyano-4-hydroxycinnamic acid as a matrix. Analytical RP-HPLC, performed on a 4.6 x 250 mm Jupiter C18 (5 µm, 300 Å) column, indicated that the purity of the peptides was >99.%.

**Cytokine Microarray study**

LAD2 cells and/or the MRGPRX2 impaired LAD2 cells were treated with water or P17 for 30 min in 1 ml serum free media for cytokine release. After 30 mins, only the supernatant is harvested for cytokine array. Array membranes were blocked with 2 ml blocking buffer for 30 mins at RT. Blocking buffer is removed and harvested supernatants were added to the membrane and incubated overnight at 4 °C. Supernatants were aspirated and washed for 5 mins at RT with 2 ml wash buffer I for 3 times. Then, the membranes were washed for 5 mins at RT with 2 ml wash buffer II for 2 times. 1 ml Biotinylated antibody cocktail was added into the membrane and incubated at RT for 2 hours. Upon removal of the antibody, membranes were washed as mentioned above 3 times with wash buffer I and 2 times with wash buffer II. 2 ml HRP-Conjugated Streptavidin were added to the membrane and incubated for 2 hours at RT then washed as mentioned above with wash buffer I and wash buffer II. Excess wash buffer is removed and 500 μl of detection buffer is added on the membrane and incubated for 2 mins before imaging using UVITEC Cambridge Alliance. Results from microarray were normalized with the positive control and analyzed with NIH ImageJ software.

**Human monocyte isolation**

Whole blood was collected from subjects in EDTA tubes (4 ml). Blood is then added to Ficoll-Paque Plus (4 ml) (Cytiva) containing tubes. Tube is then centrifuged at 400*g*, 30 min, 20 °C; brake off, the plasma was removed from each tube and the buffy coat was collected. The buffy coat was then washed with 40 ml serum free RPMI media and tubes were centrifuged (400*g*, 10 min) and the supernatants were discarded. The wash was repeated twice, and the final spin was followed by removal of the supernatant and resuspension of the cells in serum containing RPMI 1640 media and plated in 100 mm culture dish for 4 hours. The media is removed by pipetting up and down, and fresh RPMI 1640 media is added to the adhered monocytes at the culture disk until it is used for experiments (5). Isolated human monocytes were verified with flow cytometry analysis with markers CD11b and CD14.

**In vitro cell invasion and migration assays**

In vitro cell invasion assays were performed in 12-mm diameter and 3 μm pore polycarbonate filter trans-well plates (Corning® Transwell® polycarbonate membrane cell culture inserts). THP-1 cells (2 × 105 cells in 300 μL RPMI-1640) were seeded onto the upper well of the chamber, and the lower well was filled to the top (700 μL) with LAD2 cell supernatants (serum free StemPro-34) after the treatments of LAD2 cells (wildtype and knockdowns) with P17, compound 48/80 or water as chemoattractant. After incubation for 16-18 hr at 37°C in the presence of 5% CO2, the cells were fixed for 15 min in methanol and stained for 5-10 min with crystal violet. The non-migrating cells were then carefully removed from the upper surface (inside) of the trans-well with a wet cotton swab. Cells that had migrated or invaded to the bottom surface of the filter were captured using Nikon Eclipse Ni-U upright microscope (Nikon H550L) and counted using NIH ImageJ software (6).

**Cell culture and differentiation assay**

RPMI-1640 medium supplemented with 10% FBS, 2 mmol/L, L-glutamine, 5 mmol/L sodium pyruvate, and antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/mL, (P/S)) were used to culture the human monocytic THP-1 cells in culture flasks. Cells were maintained at 37°C in a humidified 5% CO2 atmosphere (6). For all experiments, THP-1 cells were cultured at an initial density of 5 × 105 cells/ml (1ml) and treated with Stempro-34 supernatants (1ml) collected from LAD2 cells (1 × 106) treated with P17 (10 μM), compound 48/80 (10 μM), or water for 24 - 48 hr in 6-well plate. These cells were then collected for qPCR and flow cytometry assays.

**Flow cytometry assay**

THP-1 cells were plated in 6-well tissue culture treated plates as described above. Media was collected in a 14 ml falcon tube and 2 ml versene (ThermoFisher Scientific, USA) were added to the cells for 10 min at 37°C. Cells were harvested and centrifuged at 400 g for 5 min. Cells were pelleted and resuspended in FACS buffer (0.05% sodium azide, 0.5 g bovine serum albumin in 100 ml PBS). 1 x 106 cells/ml were added in FACS buffer and blocked with 1 μg of Fc receptors binding reagent Human BD Fc Block™ was used (BD Pharmingen™, USA) for 10 min. 20 μl of PE conjugated CD11b (Cat. No. 30455X, BD Pharmingen™, USA), FITC conjugated CD14 (Cat. No. 555397, BD Pharmingen™, USA), PE conjugated IgG1, κ (Cat. No. 33815X, BD Pharmingen™, USA) and FITC conjugated IgG2a (Cat. No. 33034X, BD Pharmingen™, USA) per 100 μl cells were added and incubated for 30 min in dark at 4°C. For double staining PE conjugated CD11b and FITC conjugated CD14 were added together in cells, and the isotype controls (PE conjugated IgG1, κ and FITC conjugated IgG2a) were added together in cells. Stained cells were washed with FACS buffer for three times and sorted using BD FACSAriaTM III cell sorter. Analysis was done using FlowJo V10 software and/or WinList™.

**Immunohistochemistry**

Mice were given an intraperitoneal injection of anesthetics. Young adult mice (C57BL/6N aged 6–8 weeks old, n=6/group) were intradermally injected with P17 (10 μM), saline, saline (DMSO), P17 (DMSO) and P17 (10 μM) co-treated with quercetin (100 μM). Ears were isolated from mice and fixed in 10% formaldehyde, dehydrated with graded ethanol, embedded in paraffin, and sectioned (10 μm). Immunostaining was performed with a Leica Bond-Max automatic immunostainer (Leica Bannockburn, IL, USA) according to the recommended procedure using rabbit anti-CD11b antibody (1:1000 dilution; Abcam, USA). Images were captured with Nikon Eclipse Ni-U upright microscope (Nikon H550L)(7). Statistical comparisons were made between the number of CD11b positive cells in these ear areas in control and treatment groups using Student’s t-test.

**Hematoxylin and Eosin (H&E) staining**

HE staining was performed using Leica ST5020 Multistainer according to recommended procedures. Briefly, the paraffin sections (10 μm) were dewaxed and rehydrated in graded ethanol. After washing with distilled water (diH2O), the sections were then stained with  hematoxylin for 10 min, followed by rinsing with tap water for 1 min and staining with eosin for another 1 min. H&E-stained sections were washed by diH2O and allowed to air dry before mounted with Histomount (Invitrogen). Images of ear tissues were captured using Nikon Eclipse Ni-U upright microscope (Nikon H550L) (7).

**Immunofluorescence staining**

Paraffin sections (10 μm) were dewaxed and rehydrated in degraded ethanol. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Microwave antigen retrieval was performed with 10 mM sodium citrate buffer at pH 6.0 for 10 min, followed by blocking of non-immunological binding with 5% bovine serum albumin (BSA) for 2 hr. Sections were then incubated overnight at 4°C with rabbit anti-CD11b (1:500 dilution; Abcam, USA). After several washes with PBS, sections were incubated with Alexa Fluor 488 donkey anti-rabbit IgG (1:500 dilution; Invitrogen, USA) (7). For double staining, rabbit anti-CD11b (1:500 dilution; Abcam, USA) was incubated overnight at 4°C followed by 3 washes with PBS, then Rat anti-F4/80 [CI:A3-1] (1:200 dilution, Abcam, USA) was incubated overnight at 4°C followed by 3 washes with PBS. Sections were incubated with Alexa Fluor 488 donkey anti-rabbit IgG (1:500 dilutions; Invitrogen, USA) and Alex Fluor 594 Goat Anti-Rat IgG (1:500 dilution; Abcam, USA). Sections were counterstained with Hoechst 33258 (Invitrogen, USA). Images were captured using confocal microscope.

**Quantification and Statistical Analysis**

All data are shown as means ± standard error of the mean (S.E.M) unless specified. The graphs between groups were plotted using Prism 8.0 software (GraphPad Software Inc.). The data were analyzed using Student’s t-tests, analysis of variance (two way ANOVA) followed by Tukey’s multiple comparisons test throughout this study. A value of *p* ≤ 0.05 was considered to be significant.

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