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Ligand recognition and allosteric modulation of the human MRGPRX1 receptor

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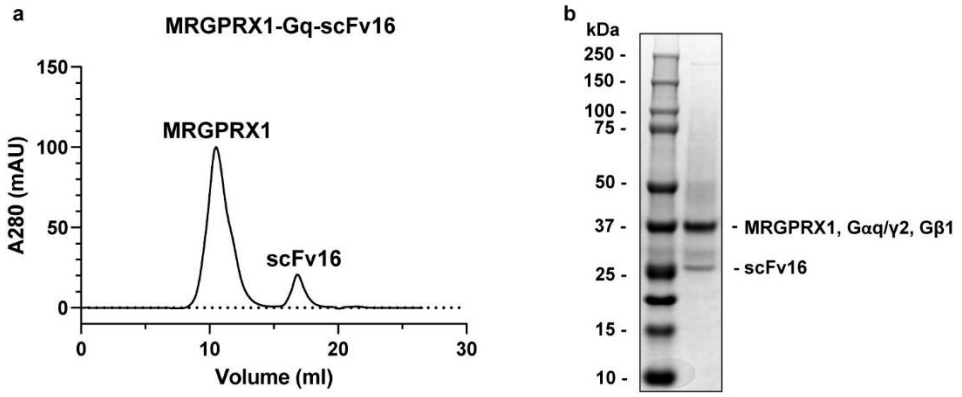
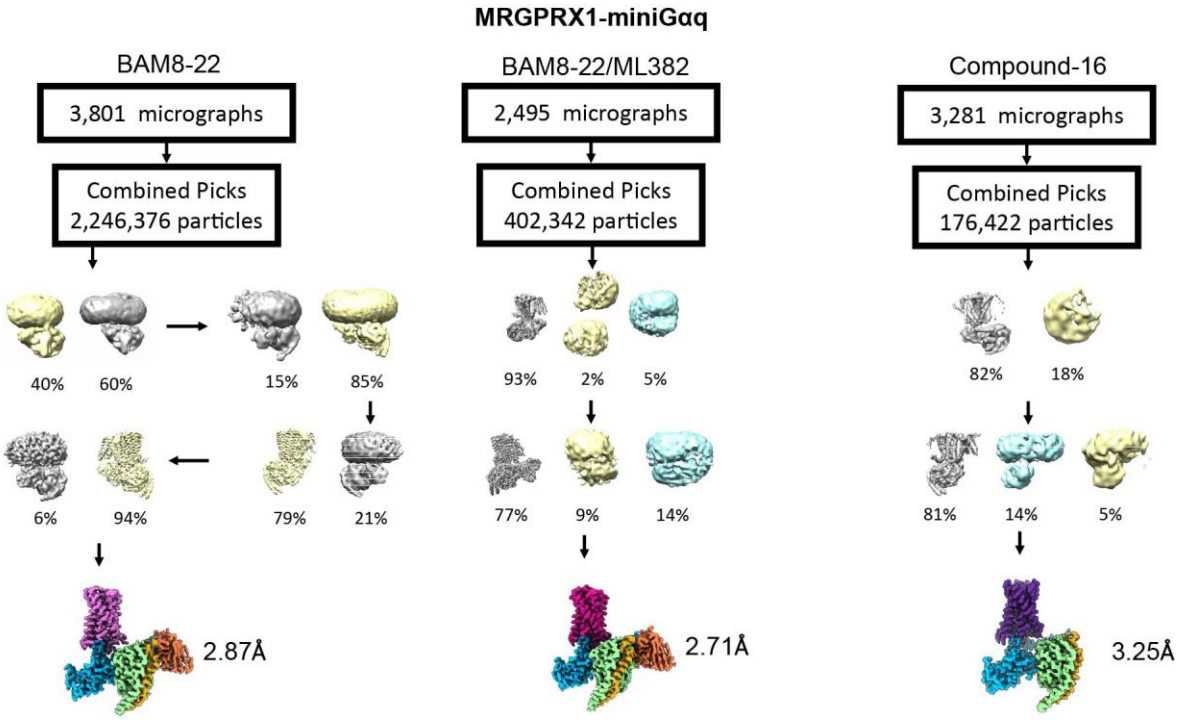
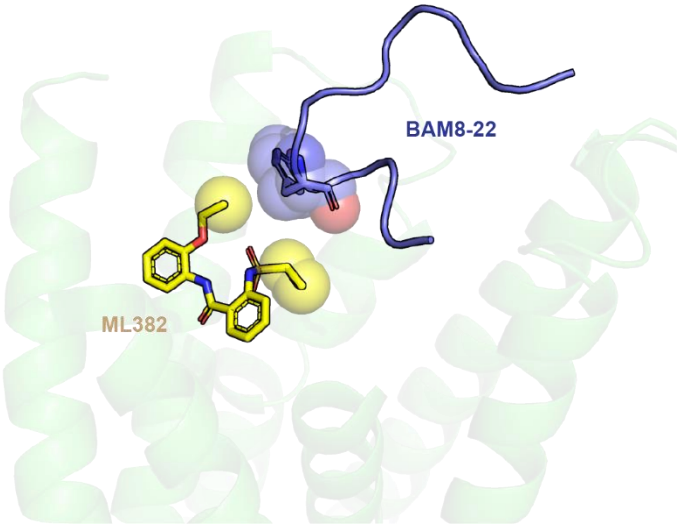


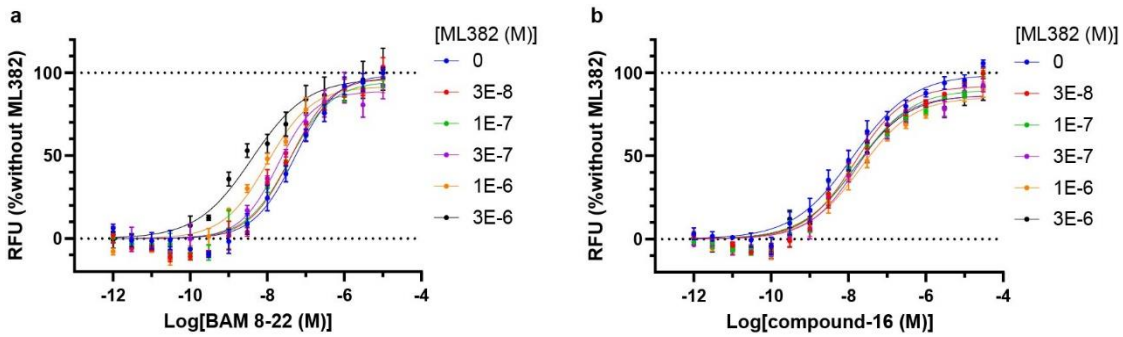
Supplementary Figure 1. Purification of the MRGPRX1-Gq complex. a, Representative size exclusion chromatography elusion profile of the MRGPRX1-Gq complex. **b,** SDS-PAGE of samples of vitro reconstituted MRGPRX1-Gq complex. Full SDS-PAGE scans were shown at the end of supplementary information. Experiments were repeated three times with similar results.



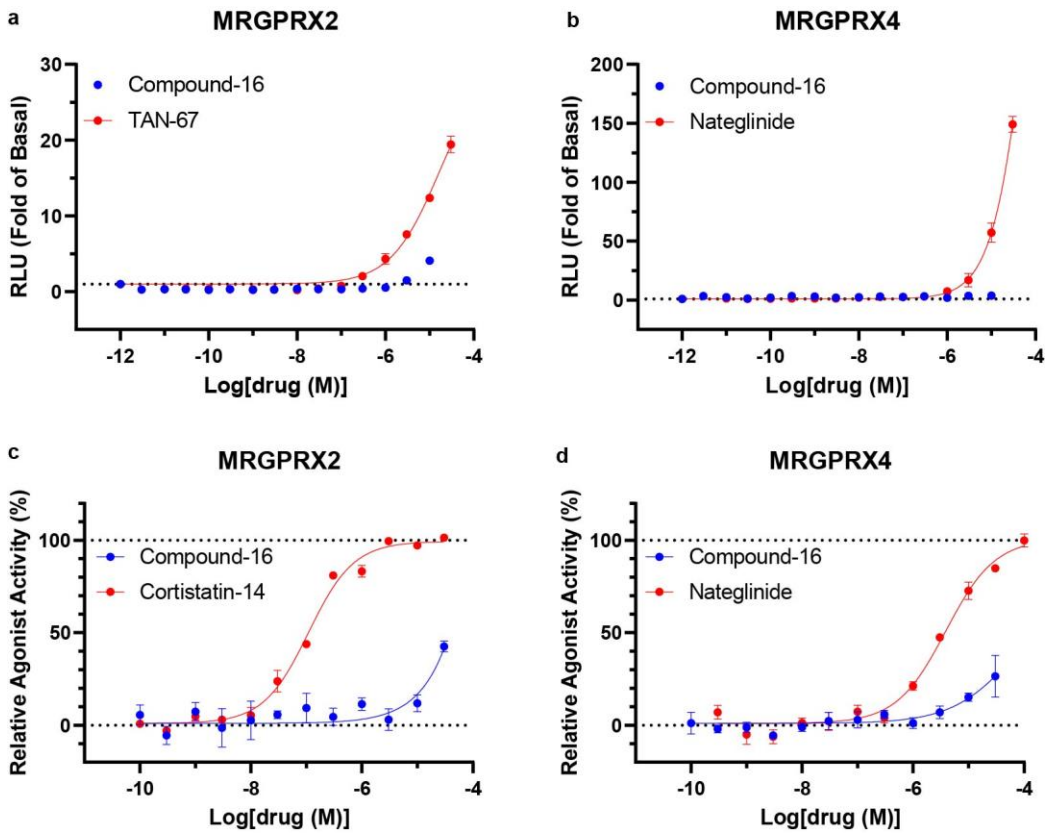
Supplementary Figure 2. Cryo-EM data processing flowcharts of MRGPRX1-Gq complex bound to BAM8-22, BAM8-22/ML382 and compound-16. The number of micrographs, particles and 3D classification of MRGPRX1-Gq complex bound to BAM8-22, BAM8-22/ML382 and compound-16 have been shown.



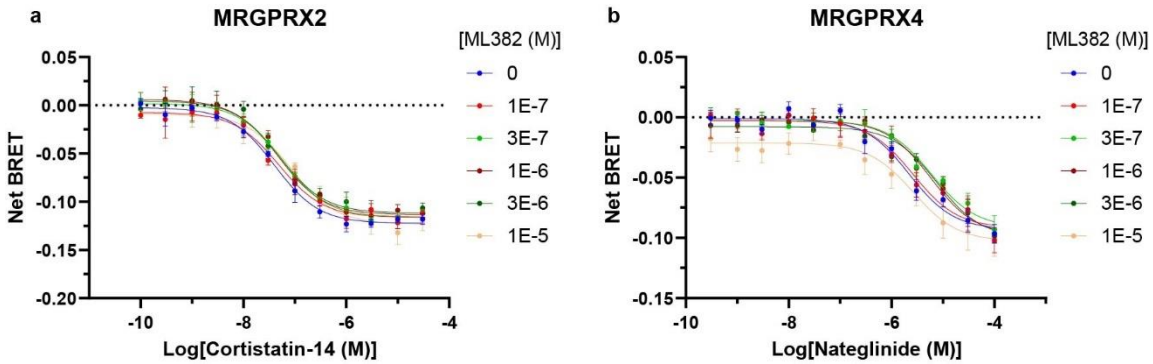
Supplementary Figure 3. The interactions between ML382 and the Tyr17 of BAM8-22. The upper part of 2-ethoxyphenyl and cyclopropyl sulfonamide groups of ML382 and Tyr17 of BAM8-22 are shown as spheres to highlight the hydrophobic interactions.



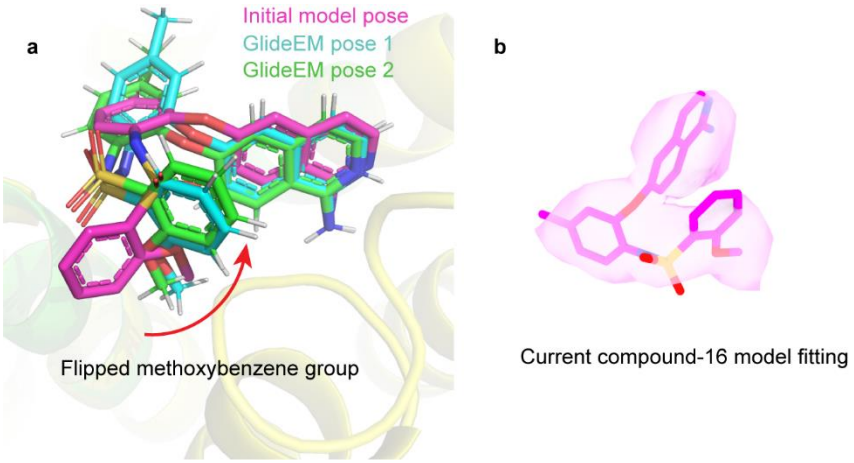
Supplementary Figure 4. Dose-response curves of MRGPRX1 with graded concentrations of ML382 using BAM8-22 and compound-16 as orthosteric ligands. Calcium mobilization assay was performed to assess ML382 allosteric regulation of BAM8-22 (**a**) and compound-16 (**b**), respectively. The data were presented as mean ± SEM of n=3 biological replicates.



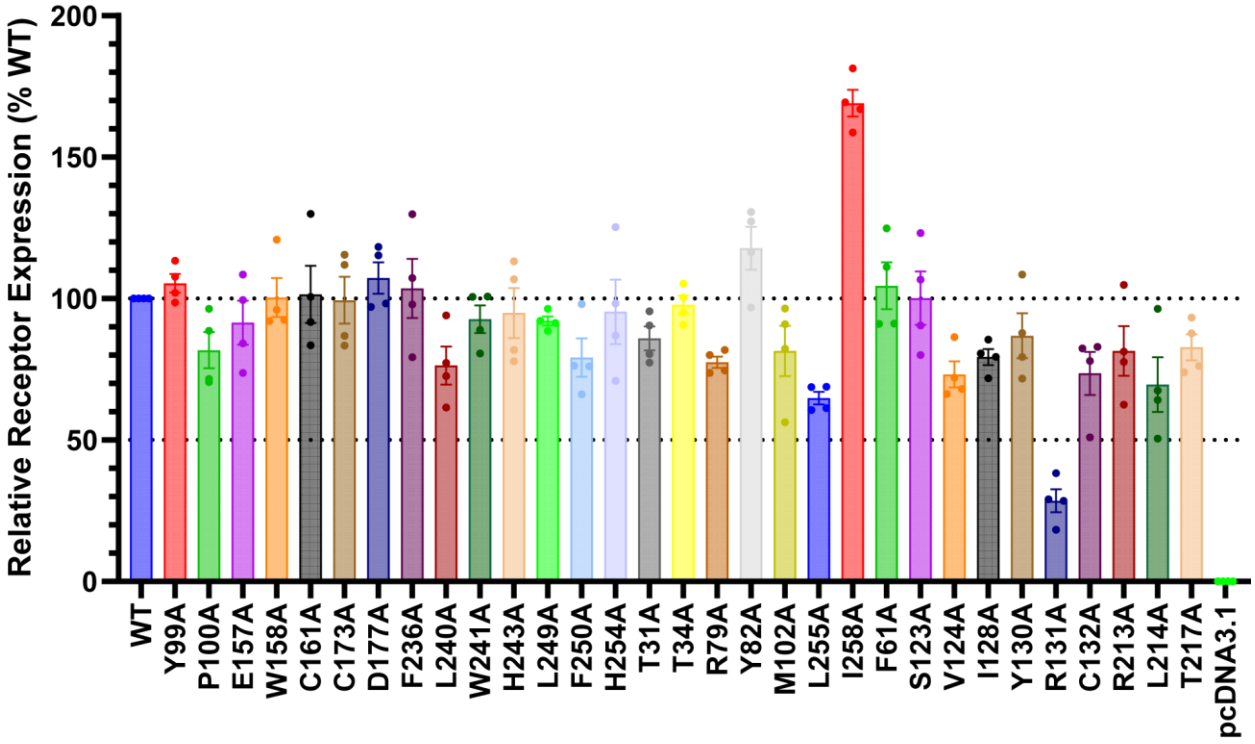
Supplementary Figure 5. Weak agonist activity of compound-16 with MRGPRX2 and MRGPRX4 receptors. Representative dose response curves for the MRGPRX2 and MRGPRX4 receptors in Tango assay (**a-b**) and BRET2 assay (**c-d**). Known agonists for MRGPRX2 (TAN-67 and cortistatin-14) and MRGPRX4 (nateglinide) were used as positive controls. The data were presented as mean ± SEM of n=3 biological replicates.



Supplementary Figure 6. Allosteric activity of ML382 with other MRGPRX subtypes. In presence of Graded concentrations of ML382**,** Gq dissociate BRET2 assay was performed to assess ML382 allosteric regulation of cortistatin-14 with MRGPRX2 (**a**) or nateglinide with MRGPRX4 (**b**), respectively. The data were presented as mean ± SEM of n=3 biological replicates.



Supplementary Figure 7. Validation of compound-16 pose. a, GlideEM docking result suggested compound-16 poses with the methoxybenzene group flipped are much more preferred in the current cryoEM map and MRGPRX1 model. **b,** The updated compound-16 pose could fit the ligand density well.



Supplementary Figure 8. Measurement of the cell surface expression level of WT and mutants of MRGPRX1 by ELISA. Data represent mean ± SEM of n = 4 biological replicates.

Supplementary Table 1. Gαq dissociation (BRET2) parameter estimates for MRGPRX1 pocket mutations. EC_{50} and E_{max} represents the average and standard error of the mean (SEM) from three independent experiments performed in duplicate. E_{max} is defined as percent WT maximum response. N.D., no detected activity as equilibrium could not be achieved at maximum agonist concentration for a reliable curve fitting.

MRGPRX1 Constructs	BAM8-22			Compound-16		
	pEC_{50} ± SEM	EC_{50} (μM)	*E*max ± SEM, % WT	pEC_{50} ± SEM	EC_{50} (μM)	E_{max} ± SEM, % WT
WT	6.30 ± 0.04	0.50	100 ± 2	6.67 ± 0.07	0.21	100 ± 3
Y99A	5.14 ± 0.12	7.33	82 ± 9	N.D.	N.D.	<50
P100A	5.62 ± 0.07	2.38	119 ± 5	6.26 ± 0.06	0.54	100 ± 3
E157A	N.D.	N.D.	<50	N.D.	N.D.	<50
W158A	5.83 ± 0.05	1.49	101 ± 3	6.63 ± 0.07	0.23	101 ± 3
C161A	N.D.	N.D.	<50	N.D.	N.D.	<50
C173A	N.D.	N.D.	<50	N.D.	N.D.	<50
D177A	N.D.	N.D.	<50	N.D.	N.D.	<50
F236A	N.D.	N.D.	<50	N.D.	N.D.	N.D.
L240A	N.D.	N.D.	<50	6.11 ± 0.08	0.77	75 ± 3
W241A	N.D.	N.D.	<50	4.95 ± 0.24	11.1	66 ± 15
H243A	5.68 ± 0.07	0.61	98 ± 4	6.33 ± 0.07	0.46	96 ± 3
L249A	N.D.	N.D.	<50	5.10± 0.14	7.91	80 ± 9
F250A	N.D.	N.D.	<50	5.52 ± 0.12	2.99	70 ± 5
H254A	N.D.	N.D.	<50	5.92 ± 0.15	1.22	71 ± 6

Supplementary Table 2. Allosteric effect on WT and mutant MRGPRX1 using BRET2 dissociation assay. BRET dissociation assays were performed in the presence of increasing concentrations of BAM8-22 and ML382. Allosteric parameter (pK_B) obtained by fitting dose response curves to "Allosteric EC_{50} shift" function of Graphpad Prism 9.0. Data are presented as mean ± SEM with n = 3 biological replicates. Greek letter delta (Δ) for the difference (ΔpEC_{50}) or affinity (ΔpK_B) when compared with the wild-type (WT) receptor values.

MRGPRX1 Constructs	BAM8-22		BAM8-22/10 μM ML382		Allosteric	
	pEC_{50} ± SEM	ΔpEC_{50}	pEC_{50} ± SEM	ΔpEC_{50}	pK_B	ΔpK_B
WT	6.33 ± 0.05	0	8.28 ± 0.07	0	4.86 ± 0.09	0
T31A	6.00 ± 0.09	-0.36	7.20 ± 0.06	-1.08	4.41 ± 0.09	-0.45
T34A	5.63 ± 0.11	-0.73	7.06 ± 0.09	-1.22	4.46 ± 0.13	-0.40
Y82A	6.11 ± 0.07	-0.25	6.63 ± 0.11	-1.65	3.62 ± 0.10	-1.24
Y99A	5.40 ± 0.10	-0.96	6.23 ± 0.09	-2.05	3.81 ± 0.11	-1.05
M102A	5.70 ± 0.09	-0.66	7.24 ± 0.08	-1.04	4.55 ± 0.11	-0.31
H254A	N.D.	N.D.	6.53 ± 0.09	-1.75	4.34 ± 0.06	-0.52
I258A	5.85 ± 0.07	-0.51	6.21 ± 0.08	-2.07	3.24 ± 0.09	-1.62

Supplementary Table 3. The MRGPRX1 and Gq protein interface mutations tested in BRET2 dissociation assay. EC_{50} and E_{max} estimates represent the average and standard error of the mean (SEM) from three independent experiments performed in triplicate. E_{max} is defined as percent WT maximum response. N.D; no detected activity as equilibrium could not be achieved at maximum agonist concentration for a reliable curve fitting.

MRGPRX1 Constructs	Compound-16			BAM8-22			BAM8-22 + 1 µM ML382		
	pEC_{50} ± SEM	EC_{50} (µM)	E_{max} ± SEM, % WT	pEC_{50} ± SEM	EC_{50} (µM)	E_{max} ± SEM, % WT	pEC_{50} ± SEM	EC_{50} (µM)	E_{max} ± SEM, % WT
WT	6.68 ± 0.05	0.21	100 ± 2	6.23 ± 0.04	0.59	100 ± 2	7.15 ± 0.03	0.07	100 ± 1
F61A	6.09 ± 0.06	0.81	94 ± 3	5.57 ± 0.08	2.70	91 ± 5	6.78 ± 0.05	0.17	99 ± 2
S123A	6.83 ± 0.05	0.15	104 ± 2	6.07 ± 0.05	0.85	101 ± 3	6.96 ± 0.04	0.11	99 ± 2
V124A	5.84 ± 0.07	1.46	76 ± 3	5.42 ± 0.10	3.83	77 ± 6	6.64 ± 0.05	0.23	88 ± 2
I128A	4.66 ± 0.25	22.1	58 ± 17	N.D.	N.D.	<50	N.D.	N.D.	<50
Y130A	6.50 ± 0.05	0.31	98 ± 2	5.96 ± 0.07	1.09	93 ± 5	7.00 ± 0.05	0.10	86 ± 2
R131A	N.D.	N.D.	<50	N.D.	N.D.	<50	N.D.	N.D.	<50
C132A	5.25 ± 0.14	5.60	63 ± 7	5.23 ± 0.13	5.92	60 ± 7	6.47 ± 0.08	0.34	63 ± 3
R213A	7.38 ± 0.03	0.04	105 ± 2	6.66 ± 0.04	0.22	116 ± 2	7.32 ± 0.03	0.05	106 ± 2
L214A	5.84 ± 0.07	1.44	68 ± 3	5.56 ± 0.10	2.78	67 ± 6	6.68 ± 0.05	0.21	89 ± 2
T217A	7.11 ± 0.04	0.08	105 ± 2	6.45 ± 0.04	0.36	123 ± 2	7.23 ± 0.04	0.06	106 ± 2

Supplementary Table 4. The sequences of primers that used for generating the MRGPRX1 mutations.

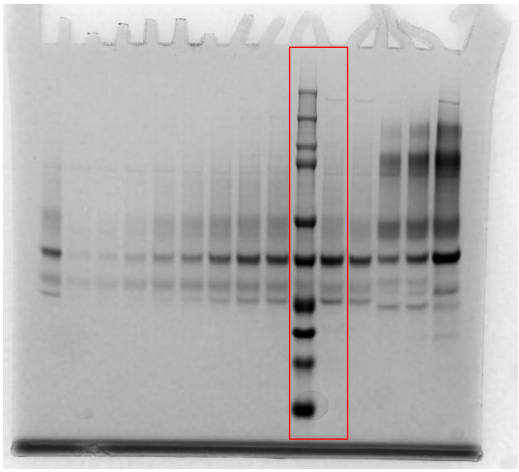
Name	5'-Sequence-3'
MRGPRX1_T31A_Fwd	GTCCTTGgCTGTCCTCACCTGTATTGTAAGTCTGGTG
MRGPRX1_T31A_Rev	GAGGACAGcCAAGGACAGTGTTTGCTTGTAACAAAG
MRGPRX1_T34A_Fwd	GTCCTCgCCTGTATTGTAAGTCTGGTGGGGCTGAC
MRGPRX1_T34A_Rev	CAATACAGGcGAGGACAGTCAAGGACAGTGTTTGCTTG
MRGPRX1_F61A_Fwd	GAATGCTgcCTCCATTTACATACTGAACCTCGCCG
MRGPRX1_F61A_Rev	AATGGAGgcAGCATTCCGTCGCATCCGGCAACCCAG
MRGPRX1_R79A_Fwd	CTCAGGGgcACTTATCTACAGCCTTTTGTCTTTTATC
MRGPRX1_R79A_Rev	GATAAGTgcCCCTGAGAGGAAAAGGAAATCAGCAG
MRGPRX1_Y82A_Fwd	CTTATCgcCAGCCTTTTGTCTTTTATCAGCATTC
MRGPRX1_Y82A_Rev	AAGGCTGgcGATAAGTCTCCCTGAGAGGAAAAG
MRGPRX1_Y99A_Fwd	GATCCTGgcCCCGGTCATGATGTTTTCTTACTTC
MRGPRX1_Y99A_Rev	GACCGGGgcCAGGATCTTGGAAATTGTATGTGGAATG
MRGPRX1_P100A_Fwd	CTGTACgCGGTCATGATGTTTTCTTACTTCGCC
MRGPRX1_P100A_Rev	CATGACCGcGTACAGGATCTTGGAAATTGTATG
MRGPRX1_M102A_Fwd	CCGGTCgcGATGTTTTCTTACTTCGCCGGCCTGTC
MRGPRX1_M102A_Rev	GAAAACATCgcGACCGGGTACAGGATCTTGGAAATTG
MRGPRX1_S123A_Fwd	TGTCTGgcTGTCCTCTGGCCGATTTGGTATAGGTG
MRGPRX1_S123A_Rev	GAGGACAgcCAGACATCTTTCCGTGGAGACTGCAC
MRGPRX1_V124A_Fwd	CTGTCTGcCCTCTGGCCGATTTGGTATAGGTGTC
MRGPRX1_V124A_Rev	CCAGAGGgCAGACAGACATCTTTCCGTGGAGAC
MRGPRX1_I128A_Fwd	TGGCCGgcTTGGTATAGGTGTCATAGGCCGACTC
MRGPRX1_I128A_Rev	ATACCAAgcCGGCCAGAGGACAGACAGACATCTTTC
MRGPRX1_Y130A_Fwd	GATTTGGgcTAGGTGTCATAGGCCGACTCACCTG
MRGPRX1_Y130A_Rev	GACACCTAgcCCAAATCGGCCAGAGGACAGACAG
MRGPRX1_R131A_Fwd	TGGTATgcGTGTCATAGGCCGACTCACCTGAGTG
MRGPRX1_R131A_Rev	ATGACACgcATACCAAATCGGCCAGAGGACAGAC
MRGPRX1_C132A_Fwd	GTATAGGgcTCATAGGCCGACTCACCTGAGTGCCG
MRGPRX1_C132A_Rev	CCTATGAgcCCTATACCAAATCGGCCAGAGGACAG
MRGPRX1_E157A_Fwd	ATACTGGcGTGGATGCTGTGTGGATTTCTCTTTAGTG
MRGPRX1_E157A_Rev	CATCCACgCCAGTATAGACCGCAGAAGGGAGAGG
MRGPRX1_W158A_Fwd	CTGGAGgcGATGCTGTGTGGATTTCTCTTTAGTG
MRGPRX1_W158A_Rev	CAGCATCgcCTCCAGTATAGACCGCAGAAGGGAG
MRGPRX1_C161A_Fwd	ATGCTGgcTGGATTTCTCTTTAGTGGTGCCGACTC
MRGPRX1_C161A_Rev	GAAATCCAgcCAGCATCCACTCCAGTATAGACC
MRGPRX1_C173A_Fwd	GCATGGgcTCAGACCAGTGATTTTATCACTGTC
MRGPRX1_C173A_Rev	GGTCTGAgcCCATGCAGAGTCGGCACCACTAAAGAG
MRGPRX1_D177A_Fwd	ACCAGTGcTTTTATCACTGTCGCCTGGCTTATTTTC
MRGPRX1_D177A_Rev	GATAAAAgCACTGGTCTGACACCATGCAGAGTCGGCAC
MRGPRX1_R213A_Fwd	CTGACAgcCCTGTACGTGACCATCCTTCTGACGG
MRGPRX1_R213A_Rev	GTACAGGgcTGTCAGTGGGATTTTGCGGCTGCCAC
MRGPRX1_L214A_Fwd	GACACGCgcGTACGTGACCATCCTTCTGACGGTA
MRGPRX1_L214A_Rev	CACGTACgcGCGTGTCAGTGGGATTTTGCGGCTG
MRGPRX1_T217A_Fwd	GTACGTGgCCATCCTTCTGACGGTATTGGTGTTC
MRGPRX1_T217A_Rev	GAAGGATGGcCACGTACAGGCGTGTCAGTGGGATTTTG
MRGPRX1_F232A_Fwd	TTGCCTgcCGGGATCCAATTCTTCCTCTTCCTCTG
MRGPRX1_F232A_Rev	GATCCCGgcAGGCAAACCACACAGGAGGAACACC
MRGPRX1_F236A_Fwd	GATCCAAgcCTTCCTCTTCCTCTGGATACACGTG
MRGPRX1_F236A_Rev	GAGGAAGgcTTGGATCCCGAAAGGCAAACCACAC
MRGPRX1_L240A_Fwd	CTCTTCgcCTGGATACACGTGGATCGGGAAGTG
MRGPRX1_L240A_Rev	GTATCCAGgcGAAGAGGAAGAATTGGATCCCGAAAG
MRGPRX1_W241A_Fwd	CTTCCTCgcGATACACGTGGATCGGGAAGTGCTTTTC
MRGPRX1_W241A_Rev	GTGTATCgcGAGGAAGAGGAAGAATTGGATCCCGAAAG
MRGPRX1_L249A_Fwd	GAAGTGgcTTTCTGCCACGTTCACCTGGTGAGTATC
MRGPRX1_L249A_Rev	GCAGAAAgcCACTTCCCGATCCACGTGTATCCAG

MRGPRX1_F250A_Fwd	GTGCTTgcCTGCCACGTTCACCTGGTGAGTATCTTTC
MRGPRX1_F250A_Rev	GTGGCAGgcAAGCACTTCCCGATCCACGTGTATCCAG
MRGPRX1_H254A_Fwd	CACGTTgcCCTGGTGAGTATCTTTCTGTCCGCACTG
MRGPRX1_H254A_Rev	CACCAGGgcAACGTGGCAGAAAAGCACTTCCCGATC
MRGPRX1_L255A_Fwd	GTTCACgcGGTGAGTATCTTTCTGTCCGCACTG
MRGPRX1_L255A_Rev	ACTCACCgcGTGAACGTGGCAGAAAAGCACTTC
MRGPRX1_I258A_Fwd	GTGAGTgcCTTTCTGTCCGCACTGAATAGCAGCGC
MRGPRX1_I258A_Rev	CAGAAAGgcACTCACCAGGTGAACGTGGCAGAAAAG

Supplementary Table 5. Cryo-EM data collection, refinement, and validation statistics.

	MRGPRX1-Gq BAM8-22 (EMD-27752) (PDB 8DWC)	MRGPRX1-Gq BAM8-22/ML382 (EMD-27753) (PDB 8DWG)	MRGPRX1-Gq Compound-16 (EMD-27754) (PDB 8DWH)
Data collection and processing			
Magnification	45,000	45,000	45,000
Voltage (kV)	200	200	200
Electron exposure (e–/Å^2)	44.5	44.5	43.8
Number of movies used	3801	2495	3218
Defocus mean (SD) μm[1]	1.4 (0.5)	1.3 (0.3)	1.5 (0.5)
Range	0.1-3.3	0.2-2.4	0.1-3.6
Pixel size (Å)	0.88	0.88	0.88
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	2,246,376	402,342	176,422
Final particle images (no.)	652,843	290,962	116,978
Map resolution (Å)[2]	2.87	2.71	3.25
FSC threshold	0.143	0.143	0.143
Map resolution range (Å)	2.5-4.7	2.3-5.1	2.8-6.6
Refinement			
Initial model used (PDB code)	7S8N	7S8N	7S8N
Model resolution (Å)	3.01	2.88	3.58
FSC threshold	0.5	0.5	0.5
Map sharpening B factor (Å2)	110.5	76.6	89.4
Model composition			
Non-hydrogen atoms	8298	8350	6444
Protein residues	1102	1101	858
Ligands	/	1	1
B factors (Å^2)			
Protein	52.92	50.63	60.77
Ligand	/	49.92	61.66
R.m.s. deviations			
Bond lengths (Å)	0.008	0.005	0.004
Bond angles (°)	0.637	0.590	0.551
Validation			
MolProbity score	1.73	1.68	1.64
Clashscore	7.22	6.56	7.56
Poor rotamers (%)	0.00	0.00	0.00
Ramachandran plot			
Favored (%)	0.00	0.00	0.00
Allowed (%)	4.81	4.53	3.44
Disallowed (%)	95.19	95.47	96.56

1 underfocus positive 2 Resolution estimates from cryoSPARC auto-corrected GSFSC



The unprocessed scan of the SDS-PAGE gel. The Supplementary Figure 1b is shown in the red frame.