Differential roles of extracellular Histidine residues of GPR68 for proton-sensing and allosteric modulation by divalent metal ions

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Supplementary Table 1. Pharmacological parameters of metal ions and small molecule PAMs (ogerin and lorazepam) at GPR68 WT and mutant receptors.

Ligand	Receptor	E_{max}[a]	pEC_{50}	Hill
Cd^{2+}	WT	2.70 (S) 0.85 (I)	7.52 4.15	17.13 -6.84
	H17A	1.8 (S) 0.40 (I)	6.28 3.74	16.22 -11.20
	H20A	1.87 (S) 0.97 (I)	5.48 3.40	16.16 -26.25
	H84A	1.86 (S) 0.61 (I)	7.21 3.98	18.01 -7.51
	H89A	1.55 (S) 0.58 (I)	7.01 4.12	14.12 -8.45
	H159A	1.90 (S) 0.44 (I)	7.38 3.86	22.81 -5.94
	H169A	0.00 (I)	3.91	-0.68
	H175A	1.90 (S) 0.78 (I)	7.43 4.12	18.63 9.62
	H245A	2.55 (S) 0.36 (I)	7.57 4.09	21.88 -10.00
	H269A	2.48 (S) 0.65 (I)	6.97 3.98	19.05 -6.90
	C13A	4.32 (S) 0.56 (I)	5.78 3.20	10.40 -9.16
	C258A	3.76 (S) 0.14 (I)	5.94 3.01	11.52 -8.13

	4HA	0.00 (I)	3.69	-0.75
Co^{2+}	WT	3.01 (S)	7.36	1.21
	H17A	3.02 (S)	6.09	0.75
	H20A	2.83 (S)	4.31	0.60
	H84A	2.90 (S)	4.88	0.50
	H89A	1.65 (S)	5.77	0.48
	H159A	2.03 (S)	7.06	1.35
	H169A	1.99 (S)	4.39	0.71
	H175A	2.50 (S)	6.93	0.82
	H245A	3.19 (S)	7.19	0.92
	H269A	2.79 (S)	6.70	0.98
	C13A	6.60 (S)	4.99	0.59
	C258A	5.64 (S)	5.14	0.58
	4HA	0.00 (I)	2.57	-0.82
Cu^{2+}	WT	1.40 (S) 0.14 (I)	7.15 5.51	0.79 -2.52
	H17A	0.09 (I)	5.15	-4.02
	H20A	0.04 (I)	4.83	-1.41
	H84A	0.11 (I)	5.11	-3.41
	H89A	0.08 (I)	4.72	-1.86
	H159A	0.07 (I)	4.67	-1.92
	H169A	0.05 (I)	5.34	-1.69
	H175A	0.10 (I)	4.75	-2.25
	H245A	0.18 (I)	6.04	-6.08
	H269A	0.07 (I)	4.69	-1.58
	C13A	ND		

	C258A	ND		
	4HA	ND		
Fe^{2+}	WT	2.98 (S)	5.80	0.80
	H17A	2.24 (S)	5.10	0.82
	H20A	1.66 (S)	5.37	0.28
	H84A	2.24 (S)	4.61	0.59
	H89A	1.50 (S)	5.91	0.65
	H159A	2.01 (S)	5.53	1.04
	H169A	2.34 (S)	2.31	0.34
	H175A	2.26 (S)	5.60	0.83
	H245A	2.93 (S)	5.57	1.04
	H269A	2.37 (S)	5.28	0.96
	C13A	6.15 (S)	4.02	0.62
	C258A	5.62 (S)	3.87	0.58
	4HA	1.55 (S)	2.99	0.56
Mn^{2+}	WT	6.18 (S)	3.81	0.36
	H17A	4.61 (S)	3.94	0.80
	H20A	3.96 (S)	2.80	0.56
	H84A	5.43 (S)	3.79	0.58
	H89A	3.16 (S)	3.59	0.59
	H159A	4.19 (S)	4.02	0.46
	H169A	2.83 (S)	3.39	0.84
	H175A	6.16 (S)	3.16	0.34
	H245A	8.26 (S)	3.60	0.48
	H269A	4.49 (S)	4.02	0.72
	C13A	10.09 (S)	3.22	0.79

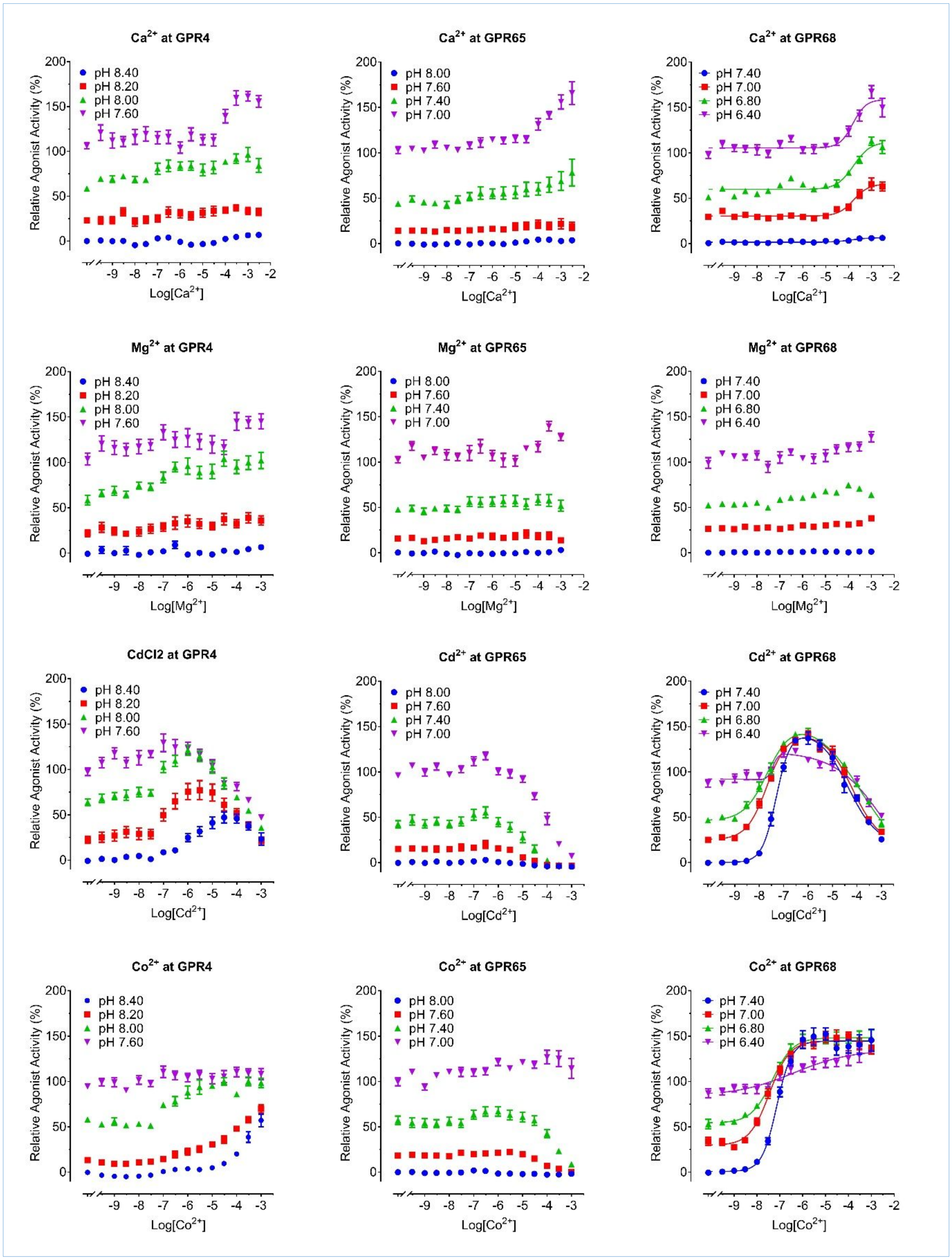
	C258A	8.51 (S)	3.32	0.82
	4HA	1.45 (S)	3.78	1.07
Ni^{2+}	WT	2.98 (S)	6.55	0.51
	H17A	2.77 (S)	5.84	0.66
	H20A	2.13 (S)	5.47	1.70
	H84A	2.55 (S)	4.47	0.48
	H89A	1.71 (S)	5.02	0.48
	H159A	2.83 (S)	5.82	0.39
	H169A	1.83 (S)	4.95	0.87
	H175A	2.52 (S)	5.98	0.39
	H245A	2.77 (S)	6.59	0.69
	H269A	2.93 (S)	5.89	0.41
	C13A	6.28 (S)	5.47	0.82
	C258A	5.22 (S)	5.46	0.81
	4HA	0.22 (I)	3.20	-1.36
Zn^{2+}	WT	2.44 (S) 0.30 (I)	6.98 4.47	21.39 11.08
	H17A	0.06 (I)	4.52	-1.89
	H20A	0.10 (I)	3.84	-1.37
	H84A	1.40 (S) 0.10 (I)	6.79 4.63	21.29 -4.63
	H89A	1.35 (S) 0.14 (I)	6.69 4.12	21.69 -10.29
	H159A	1.75 (S) 0.01 (I)	6.75 4.28	22.93 -10.55
	H169A	-0.02 (I)	5.68	-1.38

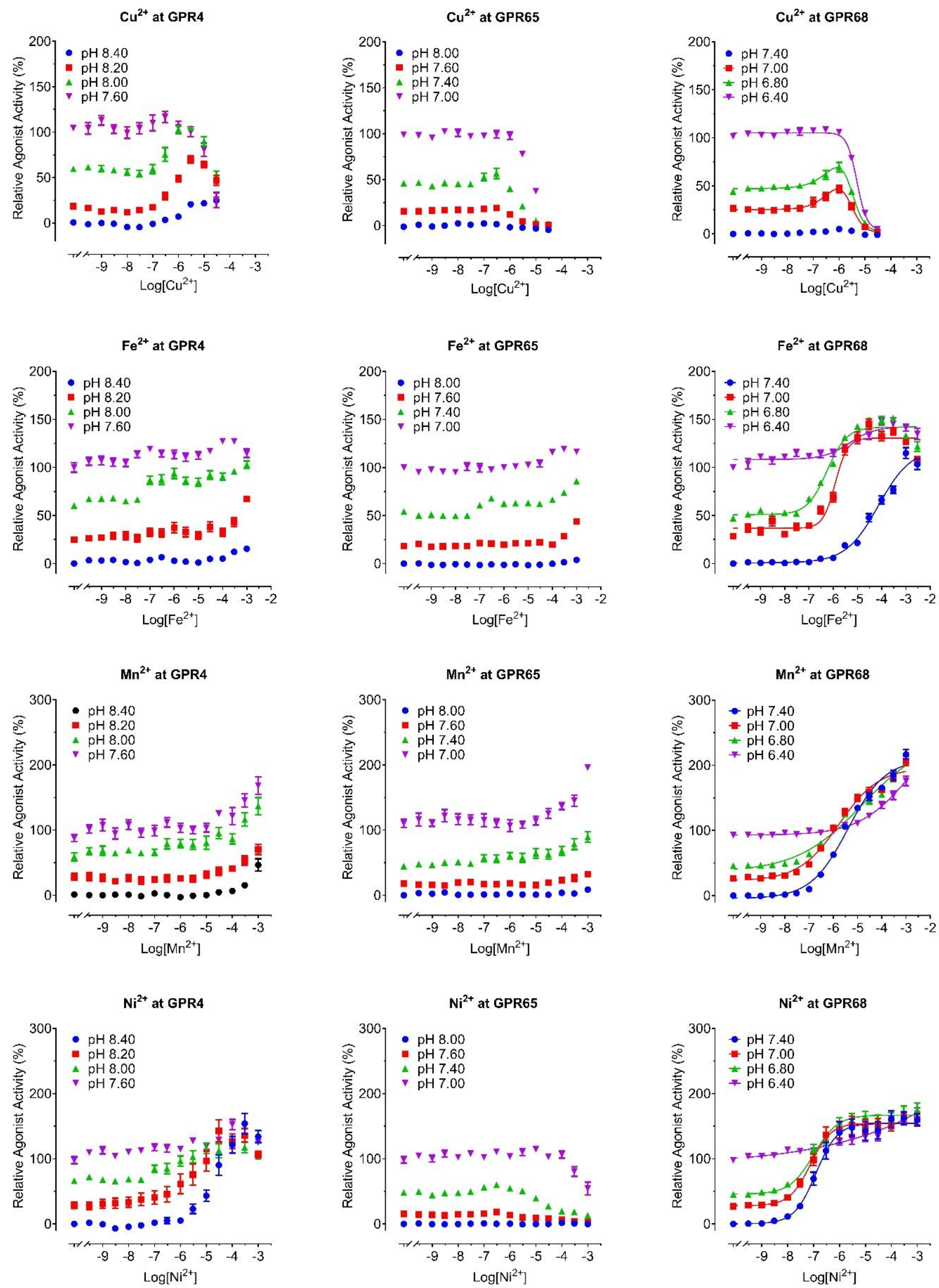
	H175A	1.60 (S) 0.30 (I)	6.80 4.32	20.73 -10.14
	H245A	2.20 (S) 0.14 (I)	6.91 4.70	19.83 -14.20
	H269A	1.60 (S) 0.40 (I)	6.59 4.24	16.66 -16.20
	C13A	1.71 (S) 0.10 (I)	5.81 4.25	15.12 -16.82
	C258A	1.45 (S) 0.13 (I)	6.14 4.28	23.41 -16.08
	4HA	0.04 (I)	5.05	-1.00
Lorazepam	WT	2.78 (S)	5.28	0.87
	H17A	2.40 (S)	5.79	0.80
	H20A	2.53 (S)	5.22	0.97
	H84A	2.26 (S)	5.87	0.88
	H89A	1.73 (S)	5.78	0.70
	H159A	1.83 (S)	5.51	1.23
	H169A	2.44 (S)	5.40	0.59
	H175A	2.79 (S)	4.86	0.56
	H245A	1.94 (S)	6.03	1.48
	H269A	2.38 (S)	5.66	1.16
	C13A	3.35 (S)	5.40	1.15
	C258A	2.93 (S)	5.29	0.95
	4HA	3.21 (S)	5.34	1.20
	WT	2.74 (S)	5.52	1.23
	H17A	2.21 (S)	5.55	1.02

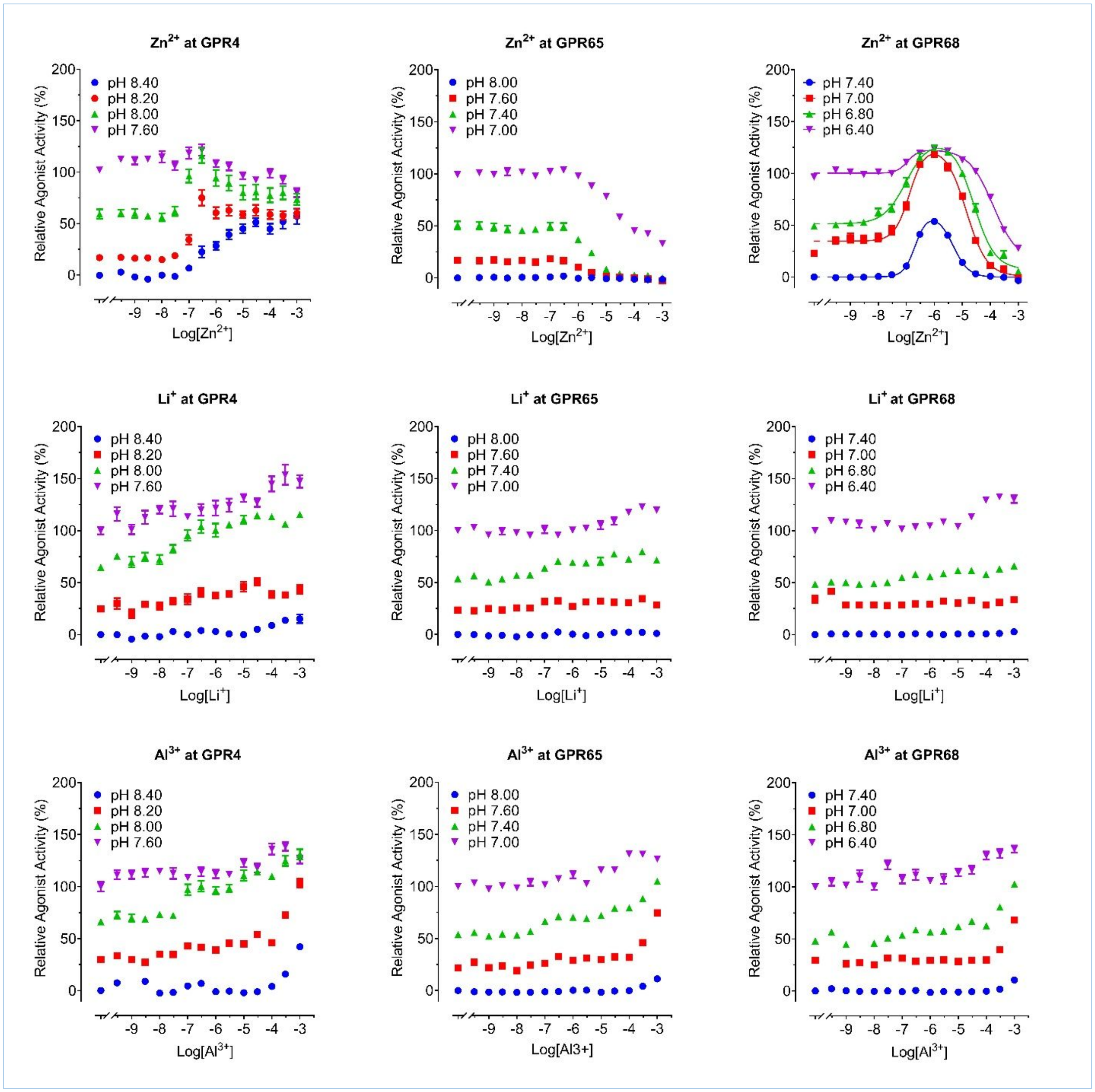
Ogerin	H20A	2.28 (S)	5.27	1.00
	H84A	2.26 (S)	5.56	1.03
	H89A	1.97 (S)	5.41	0.88
	H159A	1.71 (S)	5.34	1.43
	H169A	2.16 (S)	5.36	0.78
	H175A	1.42 (S)	5.33	1.83
	H245A	1.53 (S)	5.43	1.56
	H269A	2.38 (S)	5.54	1.31
	C13A	3.17 (S)	5.40	1.13
	C258A	2.71 (S)	5.07	0.99
	4HA	2.30 (S)	5.06	1.28

Concentration responses are presented in Figure 7 and Figure 9. Parameters were retrieved from fitted curves in Prism 8.4. [a] (S) for stimulation and (I) for inhibition. ND, not determined.

Supplementary Figure 1. Metal ion concentration responses at GPR68 (right), GPR65 (middle) and GPR4 (left) under different pH conditions – pH 8.40, 8.20, 8.00, and 7.60 at GPR4; pH 8.00, 7.80, 7.40, and 7.00 at GPR65; and pH 7.40, 7.00, 6.80, and 6.40 at GPR68. G_s-cAMP production was measured in transiently transfected HEK293T cells using the GloSensor cAMP assays. Metal ion stock solutions were made in water and diluted in pH pre-adjusted Ca^{2+}/Mg^{2+} free HBSS based buffers. Results were normalized (0% at pH 8.40 and 100% at pH 7.60 for GPR4, 0% at pH 8.00 and 100% at pH 7.00 for GPR65, 0% at pH 7.40 and 100% at pH 6.40 for GPR68) and represented means ± SEM from a minimum of 3 independent assays, each in quadruplicate. Curves were analyzed in the Prism 8 using the built-in 4-parameter logistic unction or Bell-shape function. 3D-mesh figures are presented in Figure 3. Pharmacological parameters were listed in the Table 2.


Ca2+ at GPR4
Ca2+ at GPR65
Ca2+ at GPR68
Mg2+ at GPR4
Mg2+ at GPR65
Mg2+ at GPR68
CdCl2 at GPR4
Cd2+ at GPR65
Cd2+ at GPR68
Co2+ at GPR4
Co2+ at GPR65
Co2+ at GPR68
pH 8.40
pH 8.20
pH 8.00
pH 7.60
pH 7.40
pH 7.00
pH 6.80
pH 6.40
Relative Agonist Activity (%)
Log[Ca2+]
Log[Mg2+]
Log[Cd2+]
Log[Co2+]


Cu2+ at GPR4
pH 8.40
pH 8.20
pH 8.00
pH 7.60
Relative Agonist Activity (%)
Log[Cu2+]
Cu2+ at GPR65
pH 8.00
pH 7.60
pH 7.40
pH 7.00
Relative Agonist Activity (%)
Log[Cu2+]
Cu2+ at GPR68
pH 7.40
pH 7.00
pH 6.80
pH 6.40
Relative Agonist Activity (%)
Log[Cu2+]
Fe2+ at GPR4
pH 8.40
pH 8.20
pH 8.00
pH 7.60
Relative Agonist Activity (%)
Log[Fe2+]
Fe2+ at GPR65
pH 8.00
pH 7.60
pH 7.40
pH 7.00
Relative Agonist Activity (%)
Log[Fe2+]
Fe2+ at GPR68
pH 7.40
pH 7.00
pH 6.80
pH 6.40
Relative Agonist Activity (%)
Log[Fe2+]
Mn2+ at GPR4
pH 8.40
pH 8.20
pH 8.00
pH 7.60
Relative Agonist Activity (%)
Log[Mn2+]
Mn2+ at GPR65
pH 8.00
pH 7.60
pH 7.40
pH 7.00
Relative Agonist Activity (%)
Log[Mn2+]
Mn2+ at GPR68
pH 7.40
pH 7.00
pH 6.80
pH 6.40
Relative Agonist Activity (%)
Log[Mn2+]
Ni2+ at GPR4
pH 8.40
pH 8.20
pH 8.00
pH 7.60
Relative Agonist Activity (%)
Log[Ni2+]
Ni2+ at GPR65
pH 8.00
pH 7.60
pH 7.40
pH 7.00
Relative Agonist Activity (%)
Log[Ni2+]
Ni2+ at GPR68
pH 7.40
pH 7.00
pH 6.80
pH 6.40
Relative Agonist Activity (%)
Log[Ni2+]


Zn2+ at GPR4
Zn2+ at GPR65
Zn2+ at GPR68
Li+ at GPR4
Li+ at GPR65
Li+ at GPR68
Al3+ at GPR4
Al3+ at GPR65
Al3+ at GPR68
Relative Agonist Activity (%)
pH 8.40
pH 8.20
pH 8.00
pH 7.60
pH 7.40
pH 7.00
pH 6.80
pH 6.40
Log[Zn2+]
Log[Li+]
Log[Al3+]
Log[Al3+]