

Supplementary information, Table S2. Activity of pH on wild-type GPR4 or GPR65 and mutants, and inhibition of NE52-QQ57 measured by Glo-Sensor assay.

Mutants	pH ₅₀ ±s.e.m [#]	P value	E _{max} (% of WT)	P value	N	Expression (% WT) ^{\$}
GPR4-WT	7.76±0.06		100		3	100
Fusion-GPR4	7.78±0.10	NS	88.28±11.84	NS	3	113.3±4.6
C9 ^{N-term} A	7.51±0.05	*	74.47±0.59	NS	3	90.0±12.2
C258 ^{7.25} A	7.42±0.09	**	91.23±6.18	NS	3	113.9±4.6
C90 ^{3.25} A	7.83±0.02	NS	47.52±4.87	**	3	75.3±6.8
C168 ^{ECL2} A	7.60 ±0.06	NS	36.27±7.50	***	3	62.7±3.8
H165 ^{ECL2} F	7.46 ±0.02	**	126.87±16.59	NS	3	71.7±3.8
H269 ^{7.36} F	7.53 ±0.02	*	114.40±5.67	NS	3	71.4±7.5
H241 ^{6.52} F	7.31 ±0.04	*	90.20±18.07	NS	3	93.7±5.9
H165 ^{ECL2} A/H269 ^{7.36} A	6.13 ±0.09	***	29.53±5.43	***	3	35.2±1.9
H165 ^{ECL2} F/H269 ^{7.36} F	6.85 ±0.01	***	80.11±2.89	NS	3	80.7±5.1
H165 ^{ECL2} F/H269 ^{7.36} F/H241 ^{6.52} F	NA	NA	NA	NA	3	54.9±12.9
D81 ^{ECL1} N	8.07 ±0.01	**	111.20±0.90	NS	3	109.9±9.9
D81 ^{ECL1} A	8.14 ±0.03	***	108.46±3.60	NS	3	97.7±15.7
D161 ^{ECL2} N	7.80 ±0.01	NS	123.36±4.32	*	3	113.8±6.9
D161 ^{ECL2} A	7.58 ±0.02	***	165.63±11.06	***	3	105.1±9.2
D282 ^{7.49} N	8.15±0.02	***	76.13±13.70	NS	3	103.2±7.8
D63 ^{2.50} N	7.71 ±0.15	NS	71.88±2.52	*	3	93.5±12.1
L272A	7.20 ±0.14	***	98.42±2.79	NS	3	109.2±20.3
R115 ^{3.50} A	NA	NA	NA	NA	3	93.2±13.8
P193 ^{5.50} A	NA	NA	NA	NA	3	47.7±2.6
I105 ^{3.40} A	7.65 ±0.10	NS	56.84±2.34	*	3	119.7±6.5
Y98A	7.52 ±0.12	*	90.39±13.11	NS	3	96.7±13.1
F97 ^{3.32} A	7.32 ±0.04	***	94.57±12.27	NS	3	121.4±12.6

Mutants	pH ₅₀ ±s.e.m [#]	P value	E _{max} (% of WT)	P value	N	Expression (% WT) ^{\$}
GPR65-WT	6.90±0.04		100		3	100
C5 ^{N-term} A	6.47±0.03	**	44.44±4.38	***	3	80.7 ±3.0
C160 ^{ECL2} A	6.19 ±0.04	***	33.62±4.92	***	3	74.0 ±2.8
C87 ^{3.25} A	6.48±0.12	**	6.49 ±1.62	***	3	50.5 ±6.4
C170 ^{ECL2} A	6.70±0.02	NS	20.69 ±3.38	***	3	68.7 ±2.8
D172 ^{ECL2} A	NA	NA	NA	NA	3	94.6 ±8.5
R273 ^{7.36} A	NA	NA	NA	NA	3	81.6±5.2
H243 ^{6.52} F	NA	NA	NA	NA	3	53.7±4.4
R112 ^{3.50} A	NA	NA	NA	NA	3	54.9±2.9
D60 ^{2.50} A	6.67±0.09	NS	14.23±3.69	***	3	95.1±0.1
D286 ^{7.49} A	6.78 ±0.09	NS	9.28±0.93	NS	3	89.5±0.1
D78 ^{ECL1} A	6.56 ±0.05	**	62.35±10.21	**	3	154.9±12.6
H10 ^{N-term} A	6.46 ±0.05	**	40.63±7.36	***	3	113.3±1.0
F98 ^{3.36} A	6.81±0.08	NS	51.50±11.79	***	3	38.5±2.7
F239 ^{6.48} A	7.02±0.06	NS	45.01±10.06	**	3	108.6±15.1
F235A	6.95±0.06	NS	59.32±14.50	*	3	81.2±5.3
Y272A	6.34±0.16	**	36.63±7.31	**	3	113.8±6.9
F242A	6.70±0.15	**	47.41±5.90	**	3	95.3±7.7

Mutants	pIC ₅₀ ±s.e.m [#]	P value	N	Expression (% WT) ^s
GPR4-WT	7.20±0.08		7	100
F167 ^{7.36} A	6.74±0.03	*	3	93±5.2
L272 ^{7.39} A	6.43±0.19	***	3	89±7.6
Y76 ^{2.63} A	6.07±0.07	***	3	102±5.5
D81 ^{ECL1} A	6.96±0.07	ns	3	97.7±15.7
Y24 ^{1.39} F	5.42±0.19	***	3	106.9±14.8
Y24 ^{1.39} A	5.38±0.13	***	3	75.7±3.6
L70 ^{2.57} A	6.17±0.06	***	3	97.9±15.9
W73 ^{2.60} F	5.92±0.04	***	3	121.1±10.7
W73 ^{2.60} A	5.35±0.24	***	3	107.3±6.5
F77 ^{2.64} A	6.16±0.11	***	3	54.9±7.8
H269 ^{7.36} F	8.94±0.49	***	3	71.4±7.5

[#]Data are mean ± s.e.m. from at least three independent experiments. *p<0.05, **p<0.01, ***p<0.001 by one-way analysis of variance followed by Dunnett's post-test compared to the response of wild type. NS, not significant; NA (not activated) indicates that the value cannot be fitted by non-linear regression in GraphPad Prism. Data are mean ± s.e.m. from three independent experiments.

^sProtein expression levels of GPR4 or GPR65 constructs at the cell surface were determined in parallel by flow cytometry with an anti-Flag antibody (Sigma) and reported as per cent compared to the wild type from three independent measurements performed in technical duplicate.