

Table S8: Determination of half maximal effective concentration EC_{50} via cAMP accumulation using the AlphaScreen® assay after addition of agonists α -MSH, NDP- α -MSH and setmelanotide. Data are given as the result of four to eight independent experiments performed in triplicates \pm SEM. Statistics were done by one-way ANOVA with Kruskal-Wallis test. Wild-type MC4R (WT) was tested against all mutants stimulated with the indicated ligand. a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$; d: $p < 0.0001$; n.d. = not determined due to either too low E_{max} or due to severely shifted concentration-response curve which do not allow proper EC_{50} calculation.

Substitution	α-MSH EC_{50} [nM]	NDP-α-MSH EC_{50} [nM]	Setmelanotide EC_{50} [nM]
MC4R WT	17.6 \pm 4.21	1.17 \pm 0.26	1.24 \pm 0.39
E100N	n.d.	383 \pm 139 ^c	n.d.
T101A	49.4 \pm 6.12	0.9 \pm 0.05	-
D122S	n.d.	9.74 \pm 1.61 ^b	n.d.
N123A	33.3 \pm 5.97	0.93 \pm 0.23	6.40 \pm 2.82
D126S	n.d.	166 \pm 34.2 ^c	n.d.
C130A	136 \pm 21.9 ^c	2.20 \pm 0.71	-
L133A	94.7 \pm 1.58	2.07 \pm 0.26	-
L133F	76.1 \pm 17.0	3.79 \pm 0.77	-
I137A	19.7 \pm 5.97	1.15 \pm 0.08	-
T150A	n.d.	n.d.	1.09 \pm 0.08
T150I	86.2 \pm 34.9	1.41 \pm 0.35	-
T150F	91.9 \pm 16.9	2.06 \pm 0.58	-
T150S	36.2 \pm 9.10	0.76 \pm 0.16	-
H158A	7.59 \pm 3.83	0.58 \pm 0.14	2.4 \pm 0.82
S188A	101 \pm 30.2	0.97 \pm 0.19	-
M204A	6.34 \pm 1.17	1.06 \pm 0.44	-
L205F	7.18 \pm 1.84	1.38 \pm 0.45	-
W258A	70.7 \pm 17.7	2.53 \pm 0.34	-
W258F	2.60 \pm 0.53	0.59 \pm 0.2	-
F261V	118 \pm 14.5 ^c	2.96 \pm 1.44	28.80 \pm 7.70
H264A	n.d.	179 \pm 32.5 ^d	n.d.