

Table S9: Determination of Gq/11 activation by determination of phospholipase C activation via NFAT reporter gene assay. Data are given as the result of four to six independent experiments performed in triplicated \pm SEM. The stimulation of wild-type MC4R (WT) as fold over WT basal is 7.56 ± 0.87 for NDP- α -MSH challenge and 10.91 ± 0.88 for stimulation with setmelanotide at a concentration of 1 μ M. Statistics were done by one-way ANOVA with Kruskal-Wallis test. 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.

Variant	Basal	NDP- α -MSH		Setmelanotide	
	[fold over MC4R WT basal]	E_{max} at 1 μ M [fold of MC4R WT at 1 μ M]	EC_{50} [nM]	E_{max} at 1 μ M [fold of MC4R WT at 1 μ M]	EC_{50} [nM]
MC4R WT	1	100	5.81 ± 0.88	100	6.29 ± 0.76
E100N	1.17 ± 0.21	22.94 ± 3.20^c	n.d.	46.72 ± 10.23^a	n.d.
D122S	1.07 ± 0.18	194 ± 17	85.5 ± 12.4	132 ± 37	401 ± 59.6^a
D126S	0.97 ± 0.09	30.53 ± 4.33^b	n.d.	25.72 ± 6.94^d	n.d.
T150A	0.73 ± 0.08^b	129 ± 14	11.7 ± 1.98	126.75 ± 13.19	92.1 ± 24.7
H158A	1.01 ± 0.15	51.66 ± 9.35	10.00 ± 4.47	57.45 ± 7.56^a	20.6 ± 6.58