



Fig. S15: Functional data of MC4R variants.

(a-d) Cell surface expression of wild-type MC4R (WT) and mutants shown as fold over WT. (e-h) Maximal Gs-protein signaling determined as cAMP accumulation of MC4R WT and mutants after addition of 1 μ M α -MSH or NDP- α -MSH indicated as fold of MC4R WT max. signaling [%] (left y-axis). Basal cAMP accumulation of MC4R WT and mutants are depicted as fold over WT basal (right y-axis). (i) Maximal Gs-protein signaling determined as cAMP accumulation of MC4R WT and mutants after addition of 1 μ M setmelanotide indicated as fold of MC4R WT max. signaling [%] (left y-axis). Basal cAMP accumulation of MC4R WT and mutants are depicted as fold over WT basal (right y-axis).

(j) Maximal Gq/11-protein signaling determined by NFAT reporter gene assay (downstream transducer of PLC) of MC4R WT and mutants after addition of 1 μ M setmelanotide or NDP- α -MSH indicated as fold of MC4R WT max. signaling [%] (left y-axis). Basal PLC activation of MC4R WT and mutants are depicted as fold over WT basal (right y-axis).

(k-l) Concentration-response curves of **(k)** Gs-protein signaling determined as cAMP accumulation and **(l)** Gq/11 signaling determined by the activation of PLC of MC4R WT upon setmelanotide and NDP- α -MSH stimulation. Data is plotted as fold over WT basal.

(m-s) Concentration-response curves of Gs-protein signaling determined as cAMP accumulation of WT and indicated mutants after addition of α -MSH and NDP- α -MSH shown as fold of MC4R WT max. signaling [%]. Cell surface expression data, E_{MAX} and EC_{50} values are summarized in Tables S7-S9.