**Supplemental Figure 1:** Constitutive activity of WT and mutant MC4Rs was assessed using a luciferase-based assay that is described in *Materials and Methods*. Bar graphs represent the level of cAMP accumulation when CHO-K1 cells were transiently transfected with MC4R constructs. Results are expressed as mean ± SEM of pooled data from atleast three independent experiments in which each variant was run in triplicate. $p < 0.05$(*), $p < 0.01$(**), $p < 0.001$(***), $p < 0.0001$(****).

|	EC$_{50}$ (nM)	
	α-MSH	des-acetyl-α-MSH
WT	3.52E-10^{ns}	1.85E-10^{ns}
D37V	5.85E-10^{ns}	2.58E-10^{ns}
V50M	1.25E-09^{ns}	6.44E-10^{ns}
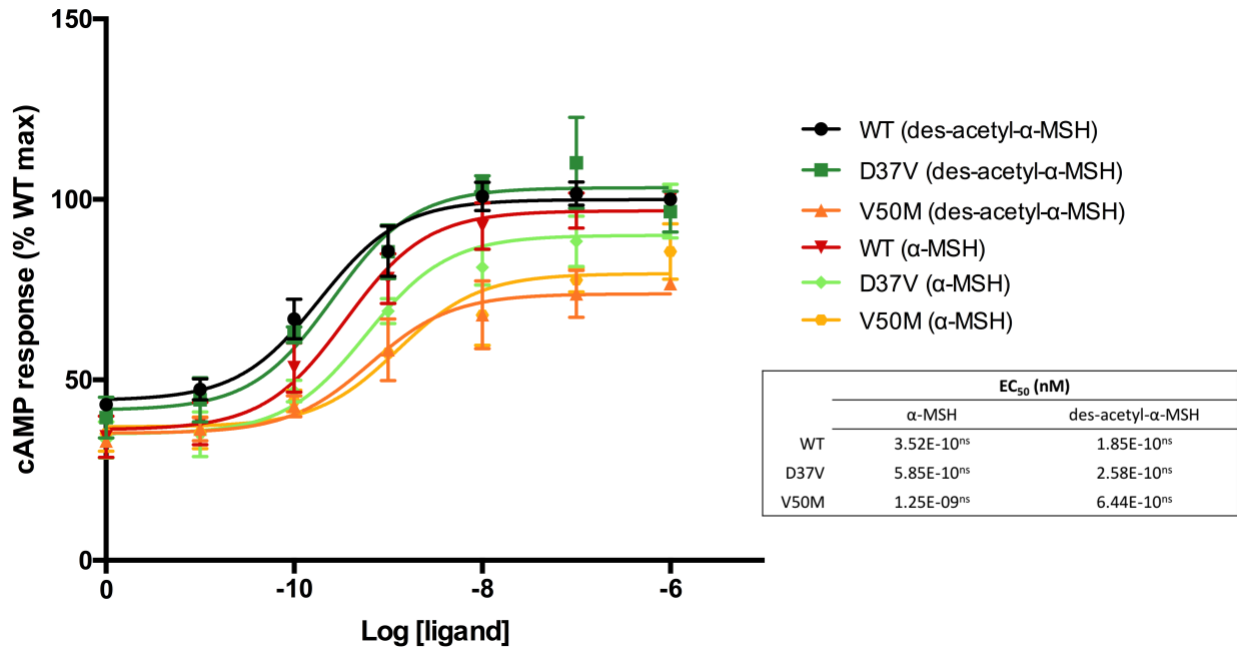
Supplemental Figure 2:

Accumulation of intracellular cAMP in CHO-K1 cells transiently co-transfected with WT or mutant MC4R. Transfected cells were treated with α-MSH or des-acetyl-α-MSH and intracellular cAMP was measured using a luciferase-based assay described in *Materials and Methods*. All variants were run in triplicate and experiments were performed at least three times. Results are expressed as mean ± SEM.
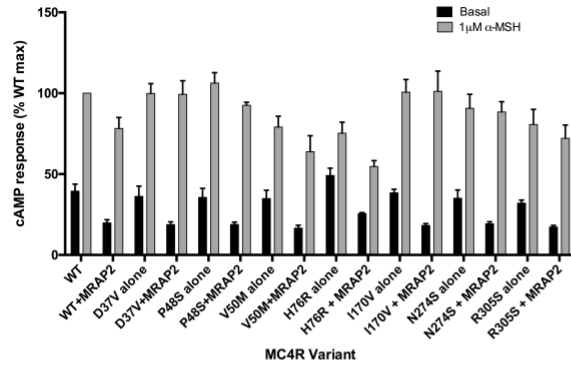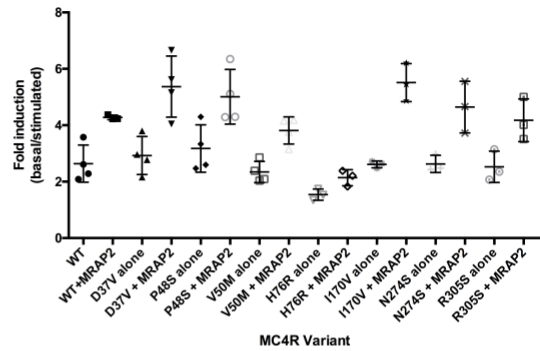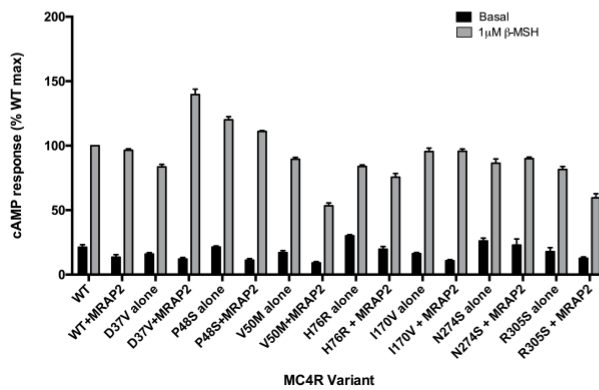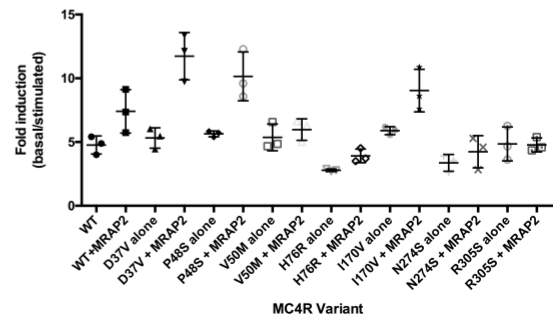
Supplemental Figure 3:
Accumulation of intracellular cAMP in CHO-K1 cells transiently co-transfected with WT or mutant MC4R with or without MRAP2. Transfected cells were treated with α-MSH (A,B) or β-MSH (C,D) and intracellular cAMP was measured using a luciferase-based assay described in *Materials and Methods*. All experiments were run in triplicate and performed at least three times. Results are expressed as mean ± SEM.
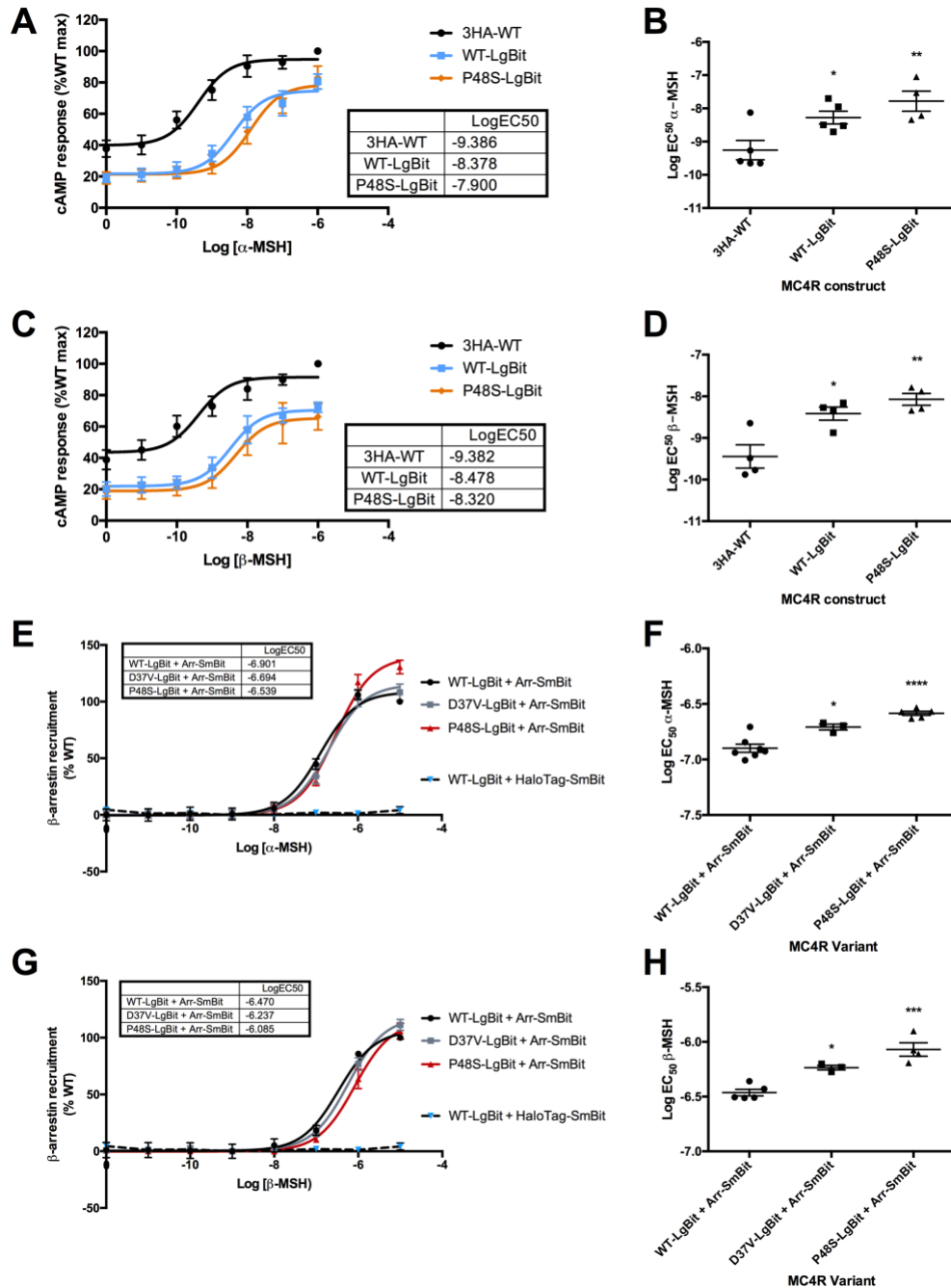
Supplemental Figure 4:

(A-D) Accumulation of intracellular cAMP in CHO-K1 cells transiently transfected with 3HA-WT or LgBit-fused MC4R in response to MSH α-MSH (A,B) or β-MSH (C,D). (E-F) HaloTag protein fused to SmBit (Promega) does not cause ligand-induced β-arrestin recruitment at MC4R-LgBit in response to α-MSH (E,F) or β-MSH (G,H). Results are expressed as mean ± SEM of pooled data from atleast three independent experiments in which each variant was run in triplicate. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****).