



**Fig. S19: Cryo-EM map differences of the NDP- $\alpha$ -MSH–MC4R–Gs–Nb35 and setmelanotide–MC4R–Gs–Nb35 complexes at Q156<sup>IL2</sup> in the IL2–Gs interface.**

(a) Close-up view on the cryo-EM maps for Q156<sup>IL2</sup> in the NDP- $\alpha$ -MSH–MC4R–Gs–Nb35 and

(b) setmelanotide–MC4R–Gs–Nb35 complex and

(c) the superposition of both complexes.

By comparison, both agonist-bound MC4R complexes display two different rotamers for Q156<sup>IL2</sup>, which results in the case of the NDP- $\alpha$ -MSH–MC4R–Gs–Nb35 complex in a hydrogen bond distance between Q156<sup>IL2</sup> and D201<sup>s2s3</sup>. This subtle difference among setmelanotide and NDP- $\alpha$ -MSH activated MC4R indicates that MC4R agonists modulate G-protein binding at IL2.

NDP- $\alpha$ -MSH–MC4R, the corresponding Gs-protein, setmelanotide–MC4R and its Gs-protein are colored orange, dark green, yellow, and slate, respectively. Amino acids are shown as sticks. The protein is depicted as ribbon. Cryo-EM maps are displayed as mesh and volume, contoured at 4  $\sigma$  level and colored corresponding to the displayed proteins.