



Fig. S23: Comparison of amino acid side chains in highly conserved MC4R motifs between the inactivated and activated MC4R structures.

(a) The *CWxP* motif with W^{6.48} has been described in several GPCRs as central for the "toggle switch" activation mechanism. In MC4R W258^{6.48} undergoes a toggle switch like motion after agonist binding and is located at the pivot point of the TM6 outward movement.

(b) The conserved *NPxxY* motif of class A GPCRs is in MC4R a *DPxxY* motif. Upon activation Y302^{7.52} gets directed towards TM5 and TM7 and interacts with the Y^{5.58} in TM5, which is crucial for the active state structure stabilization.

(c) The *DRY* motif is located at the top of the G-protein binding cavity and R147^{3.50} is stabilized by Y212^{5.58} in the active state. Additionally, in Fig. 6 the TM3-TM6 lock of the inactive state and in Fig. 7 the D146^{3.49}-Y157^{IL} interaction that stabilizes IL2 are highlighted.

(d) Most class A GPCRs are characterized by a bulge and kink supporting proline in TM5. In MC4R instead M204^{5.50} leads to a regular helical conformation without any kink or bulge. This residue together with I137^{3.40} in TM3 and F254^{6.54} in TM6 forms the *MIF* motif, reminiscent to the so called "*PIF*" motif of class A GPCRs involved in regulation of the activity state.