



Supplementary information, Fig. S1 Cryo-EM structure determination of the ACTH-MC2R-G_s-MRAP1 complex. **a**. The size-exclusion chromatography elution profile (left) and SDS-PAGE analysis (right) of the ACTH-MC2R-G_s-MRAP1 complex. **b**, **c**. Representative cryo-EM micrograph (scale bar: 50 nm) and 2D class averages (scale bar: 10 nm) of the α -MSH-MC1R-G_s complex. **d**. The flowchart of the ACTH-MC2R-G_s-MRAP1 cryo-EM data analysis, the final cryo-

EM map surface is colored to show the local resolution estimated by Cryosparc. **d-f.** FSC curves at 0.143 of the final reconstruction of the initial structure, integrated structure and loose structure of the ACTH-MC2R-G_s-MRAP1 complex, unmasked (black) or masked (red).