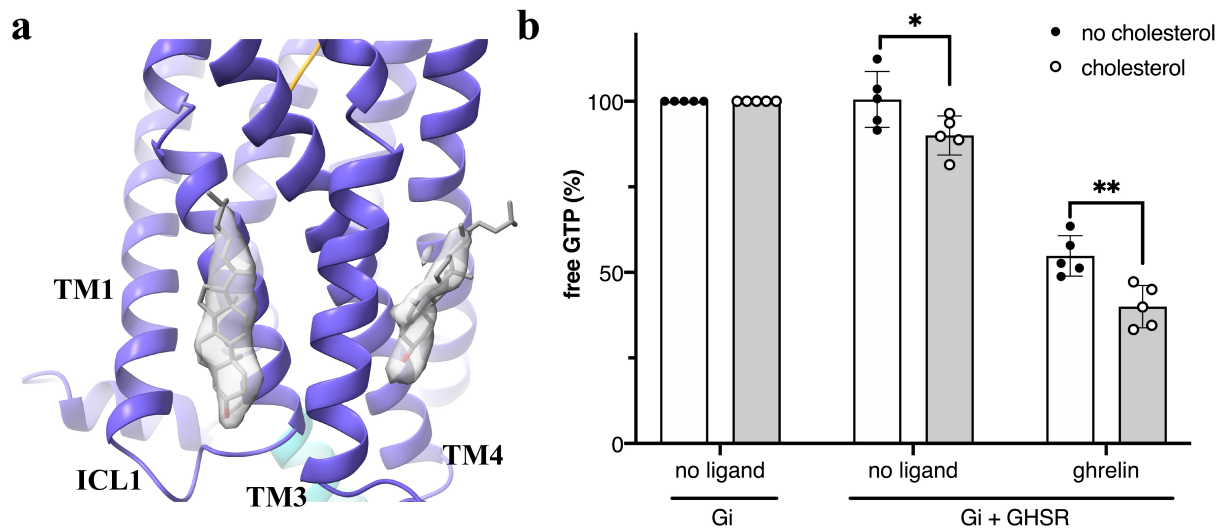
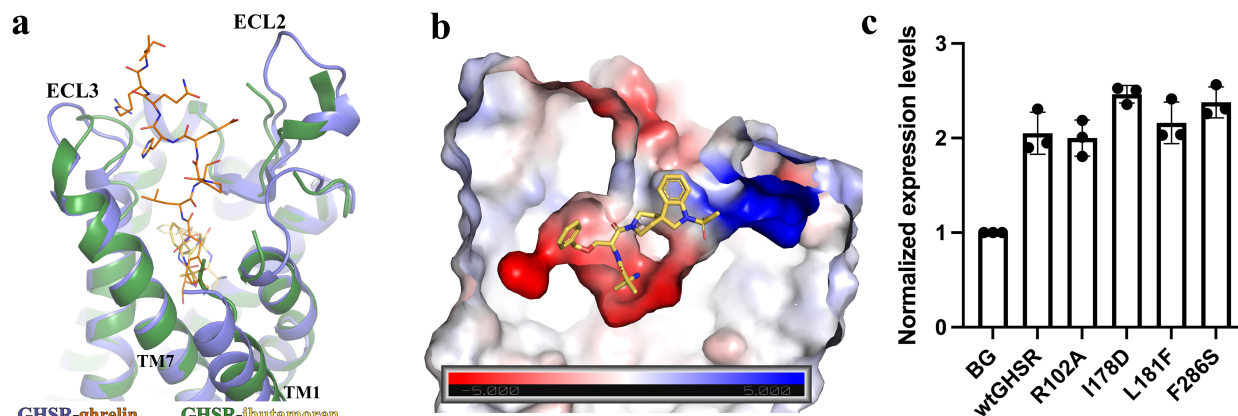


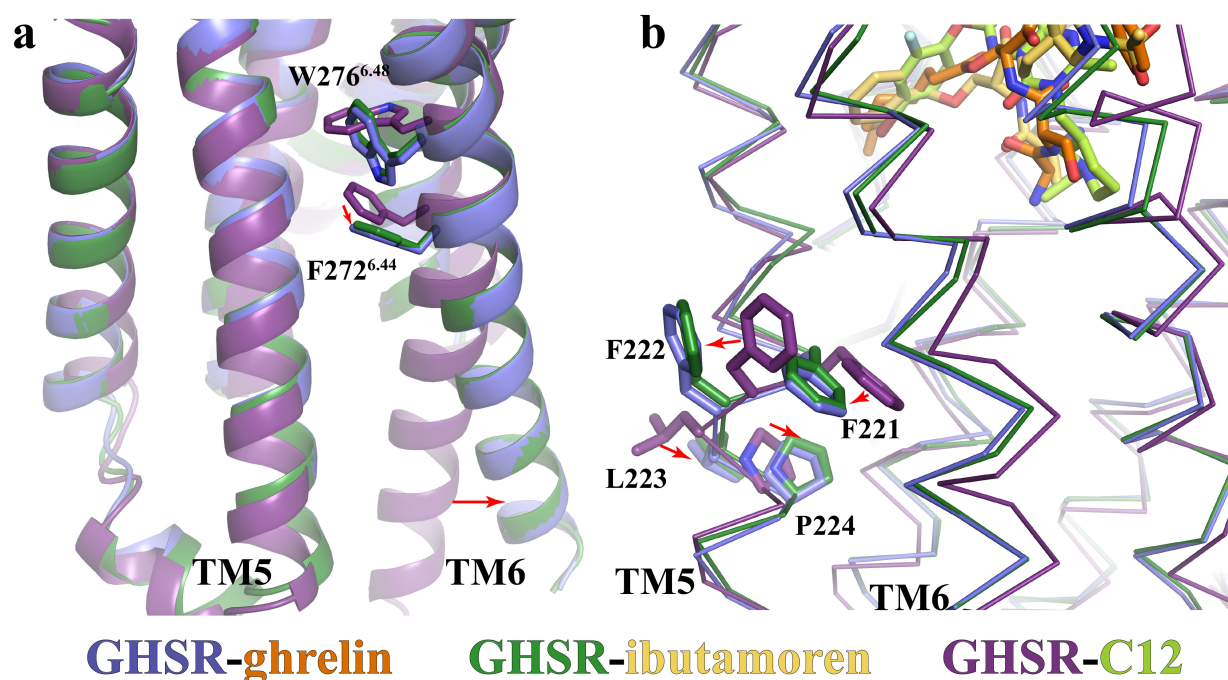
Extended Data Figure 1. Structure determination of the GHSR-G_i complexes with ghrelin and ibutamoren. (a) Chemical structures of ghrelin and ibutamoren. (b) Size exclusion profile and SDS-PAGE analysis of the purified GHSR-G_i complex with ghrelin. (c) and (d) Cryo-EM image processing workflow for the ghrelin-GHSR-G_i complex (c) and the ibutamoren-GHSR-G_i complex (d). (e) and (f) Fourier shell correlation (FSC) curves with the estimated resolution according to the gold standard with a mask excluding detergent micelles and local resolution estimates for the ghrelin-GHSR-G_i complex (e) and the ibutamoren -GHSR-G_i complex (f). In both structures, the GHSR residues from P39 to F340 except for R244-R254 in the intracellular loop 3 (ICL3) and the whole G_i heterotrimer except for the α -helical domain (AHD) of G_{αi} were modeled. In the structure of GHSR with ibutamoren, the regions Y106-R107 and N188-D191 in the extracellular loops 1 and 2 (ECL1 and 2), respectively, are not modeled due to weak maps.



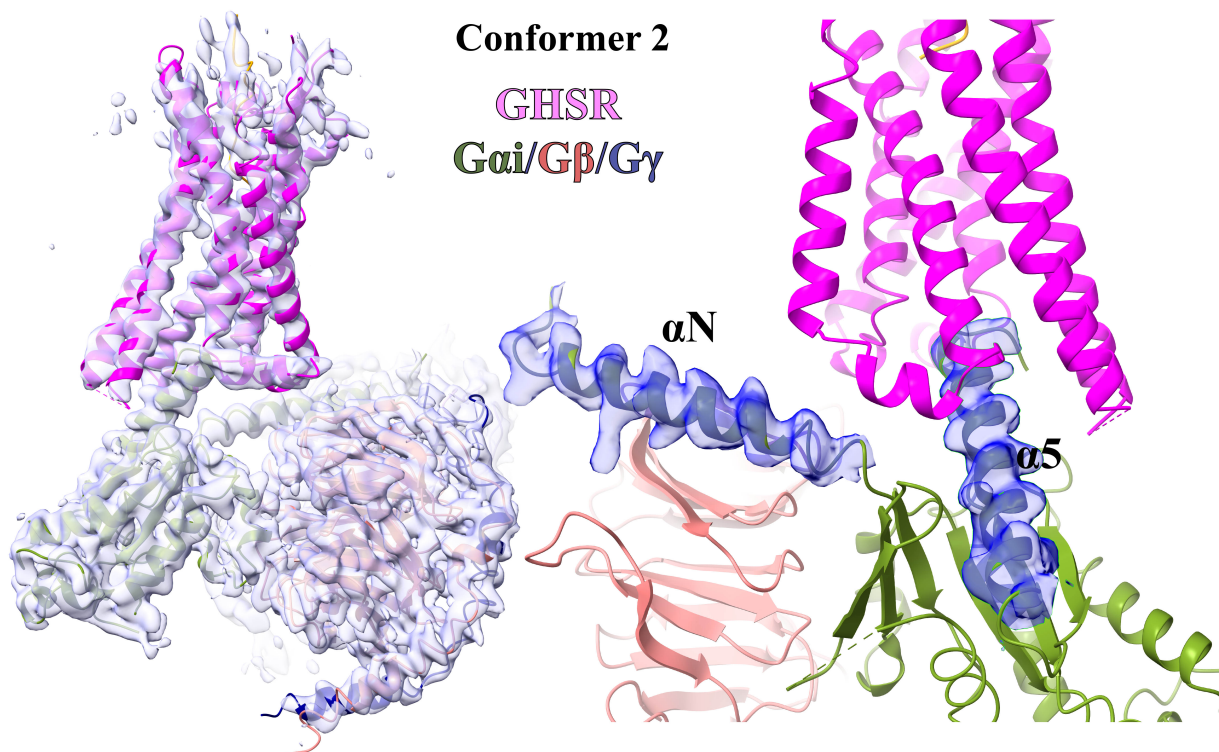
Extended Data Figure 2. Potential cholesterol binding sites and effects of cholesterol in GHSR signaling. **(a)** Cryo-EM density maps of two cholesterol molecules modeled in the structure of ghrelin-GHSR- G_i . **(b)** GTP turnover for G_i catalyzed by wild type GHSR in nanodiscs containing 10% cholesterol (molar ratio) or not, in the absence or presence of 10 μ M ghrelin. The signal was normalized to that obtained for the G protein in the absence of receptor (G_i panels), and data is mean \pm SD of five experiments. Statistical values were obtained by means of unpaired Student's t test (* $0.01 < p < 0.05$, ** $0.001 < p < 0.01$). Cholesterol showed subtle but significant positive effects in enhancing GHSR-induced G_i activation both with and without ghrelin.



Extended Data Figure 3. Ghrelin and ibutamoren binding pockets and expression of GHSR mutants. **(a)** Alignment of the extracellular regions of GHSR bound to ghrelin and ibutamoren. ECL2 and ECL3 show notable conformational differences. **(b)** Charge distribution of the ibutamoren-binding pocket. **(c)** Surface expression levels of GHSR mutants. For each GHSR mutant, the expression level was determined by measuring the fluorescence intensity of transfected HEK-293 cells stained with a fluorescent anti-FLAG antibody that could bind to the N-terminal FLAG peptide of each GHSR construct. BG means background, representing results from non-transfected HEK-293 cells. Each column represents results shown as mean \pm s.d. from 3 wells of cells transiently transfected with each GHSR mutant.



Extended Data Figure 4. Conformational changes of residues in the core region of active GHSR bound to two agonists compared to them in the inactive GHSR bound to C12. (a) Conformational changes of the highly conserved transmission switch F272^{6.44} and W276^{6.48} and accompanied conformational change of TM6. Numerous studies suggested that conformational changes of these two residues link extracellular agonist-binding events to the activation of GPCRs at cytoplasmic regions for G protein-coupling. **(b)** Conformational changes of residues in the core region of TM5. Red arrows indicate changes of residues from the inactive to the active states.



Extended Data Figure 5. Cryo-EM density maps of the ghrelin-GHSR-G_i complex in Conformer 2. The density of the entire ghrelin-GHSR-G_i complex is shown in the left panel. The density of α 5 and α N of G_i is shown in the right panel.

Extended Data Table 1. Cryo-EM data collection, structure refinement and statistics

Ghrelin-GHSR-G_i-scFV16 (EMD-24267, PDB 7NA7)		Ibutamoren-GHSR-G_i-scFV16 (EMD-24268, PDB 7NA8)
Data acquisition		
Microscope/Camera	Titan Krios/Gatan K3 Camera	
Voltage (kV)	300	
Defocus range (μM)	0.8-1.6	0.8-1.6
Pixel size (Å)	0.649	0.826
Total electron dose (e ⁻ /Å ²)	82.3	81.0
Exposure time (s)	2.1	3
Reconstruction		
Particle number	280,046	283,300
Resolution (masked, Å)	2.7	2.7
RMS deviation		
Bond length (Å)	0.006	0.006
Bond angle (°)	1.042	0.864
Ramachandran plot		
Favored (%)	98.39	96.91
Allowed (%)	1.61	3.09
Outliers (%)	0	0
MolProbity		
Clash score	6.71	7.77
Rotamer outliers (%)	0.61	0.31