SUPPLEMENTAL ITEMS

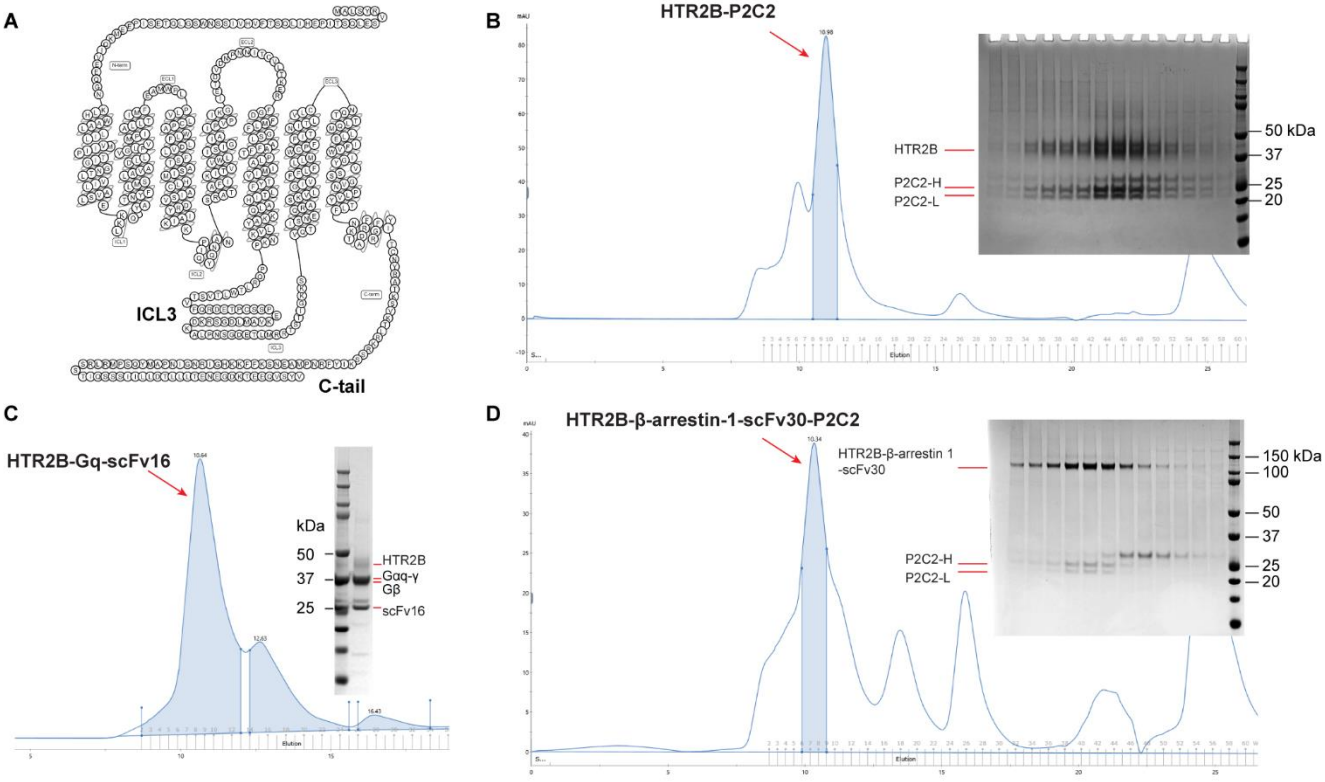

Figure S1. Purification of HTR2B complexes, related to Figure 1. (A) Snake plot of HTR2B (from GPCRDB), showing the long ICL3 and C-tail. (B) Size exclusion chromatography and Nu-PAGE gel of transducer-free HTR2B in complex with Fab P2C2. (C) Size exclusion chromatography and Nu-PAGE gel of HTR2B-Gq-scFv16 complex. (D) Size exclusion chromatography and Nu-PAGE gel of HTR2B-β-arrestin-1-scFv30 in complex with Fab P2C2.

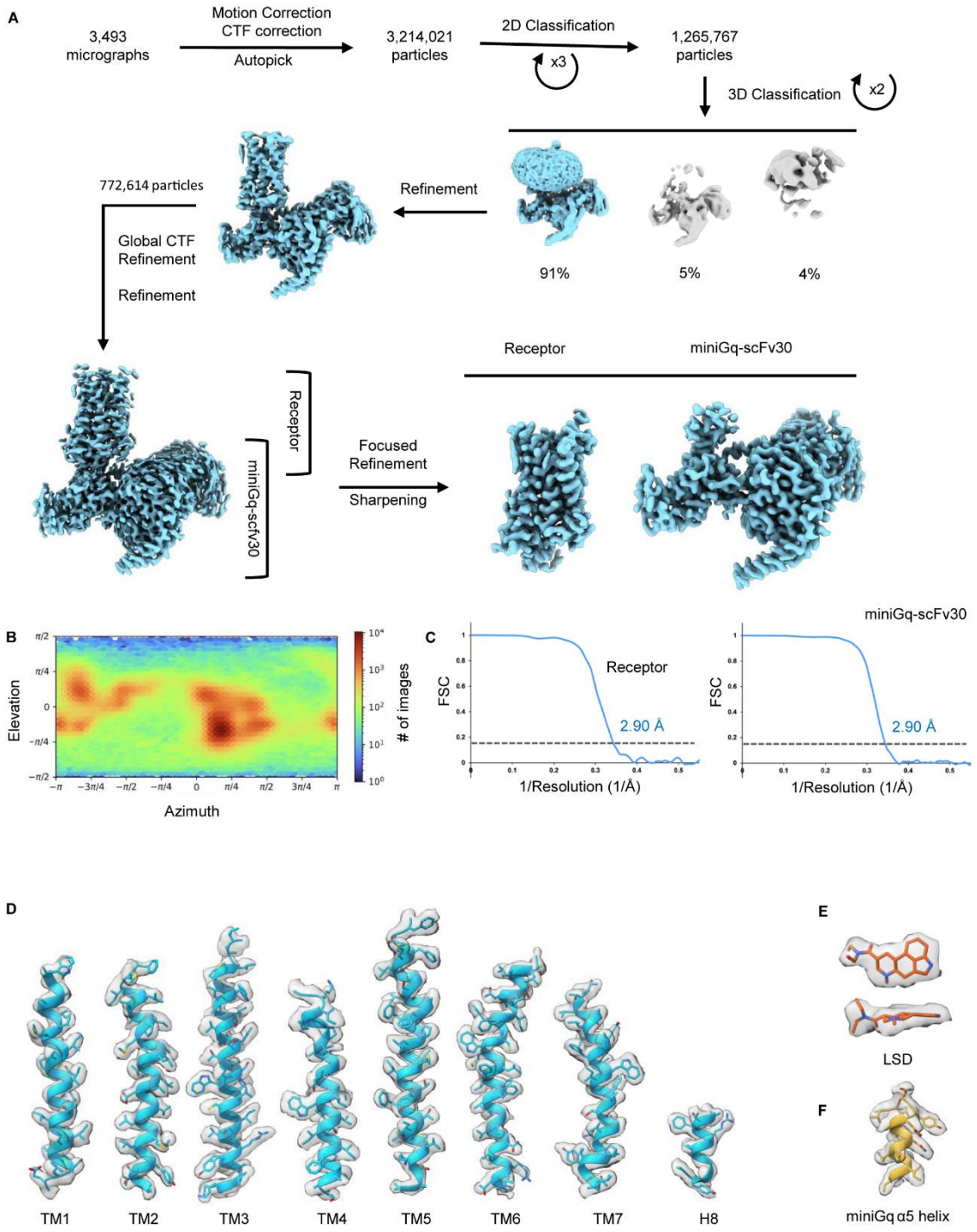

Figure S2. cryoEM Data processing of transducer-free HTR2B in complex with LSD, related to Figure 1 and Figure 2. (A) Workflow of cryo-EM data processing for HTR2B bound to LSD. (B) Angular distribution heat map of HTR2B-LSD/2B-Fab particles for the reconstruction. (C) Gold standard Fourier shell correlation (FSC) curve for the local

receptor-LSD reconstruction. Dashed line represents the overall nominal resolution at 0.143 FSC calculated by CryoSPARC. (D) Cryo-EM density for TM1-7 and helix 8 of HTR2B. (E) CryoEM density of LSD.

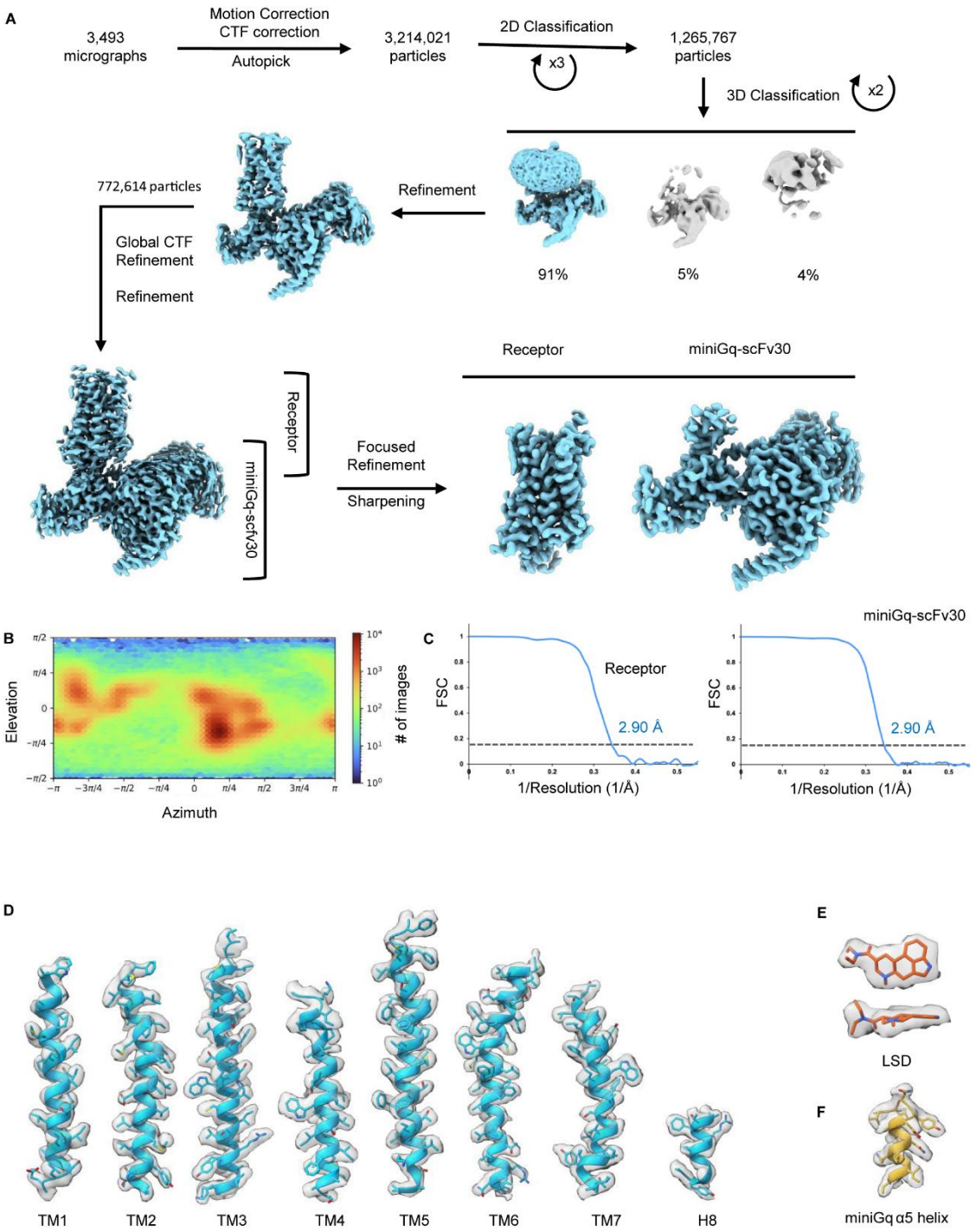

Figure S3. cryoEM Data processing of Gq-coupled HTR2B in complex with LSD, related to Figure 1 and Figure 3. (A) Workflow of cryo-EM data processing for the HTR2B/miniGq complex bound to LSD. (B) Angular distribution heat map of particles for HTR2B-LSD/miniGq reconstruction. (C) Gold standard Fourier shell correlation (FSC)

curve for receptor-LSD and miniGq/scfv local reconstructions. Dashed line represents the overall nominal resolution of each reconstruction at 0.143 FSC calculated by CryoSPARC. (D) Cryo-EM density for TM1-7 and helix 8 of HTR2B. (E-F) CryoEM density for LSD (E) andα5 helix of miniGq (F).

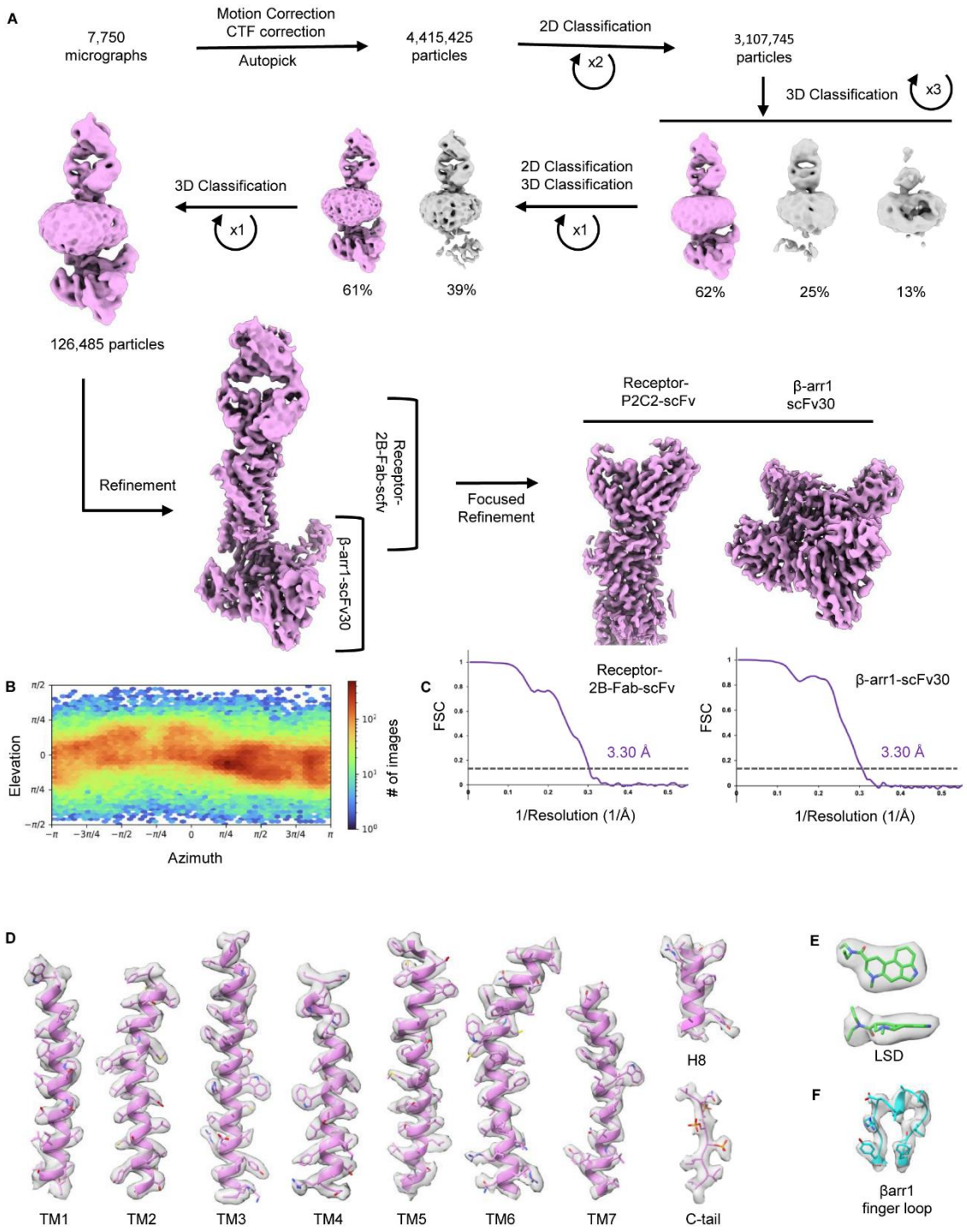

Figure S4. cryoEM Data processing of β-arrestin-1-coupled HTR2B in complex with LSD, related to Figure 1 and Figure 4. (A) Workflow of cryo-EM data processing for the HTR2B/β-arrestin-1 complex bound to LSD. (B) Angular distribution heat map of particles for HTR2B-LSD/ β-arrestin-1 reconstruction. (C) Gold standard Fourier shell correlation

(FSC) curve for receptor-LSD/P2C2-scFv and β-arrestin-1/scFv30 local reconstructions. Dashed line represents the overall nominal resolution of each reconstruction at 0.143 FSC calculated by CryoSPARC. (D) Cryo-EM density for TM1-7, helix 8 and C-terminal tail of HTR2B. (E-F) CryoEM density for LSD (E) and β-arrestin-1 finger loop (F).

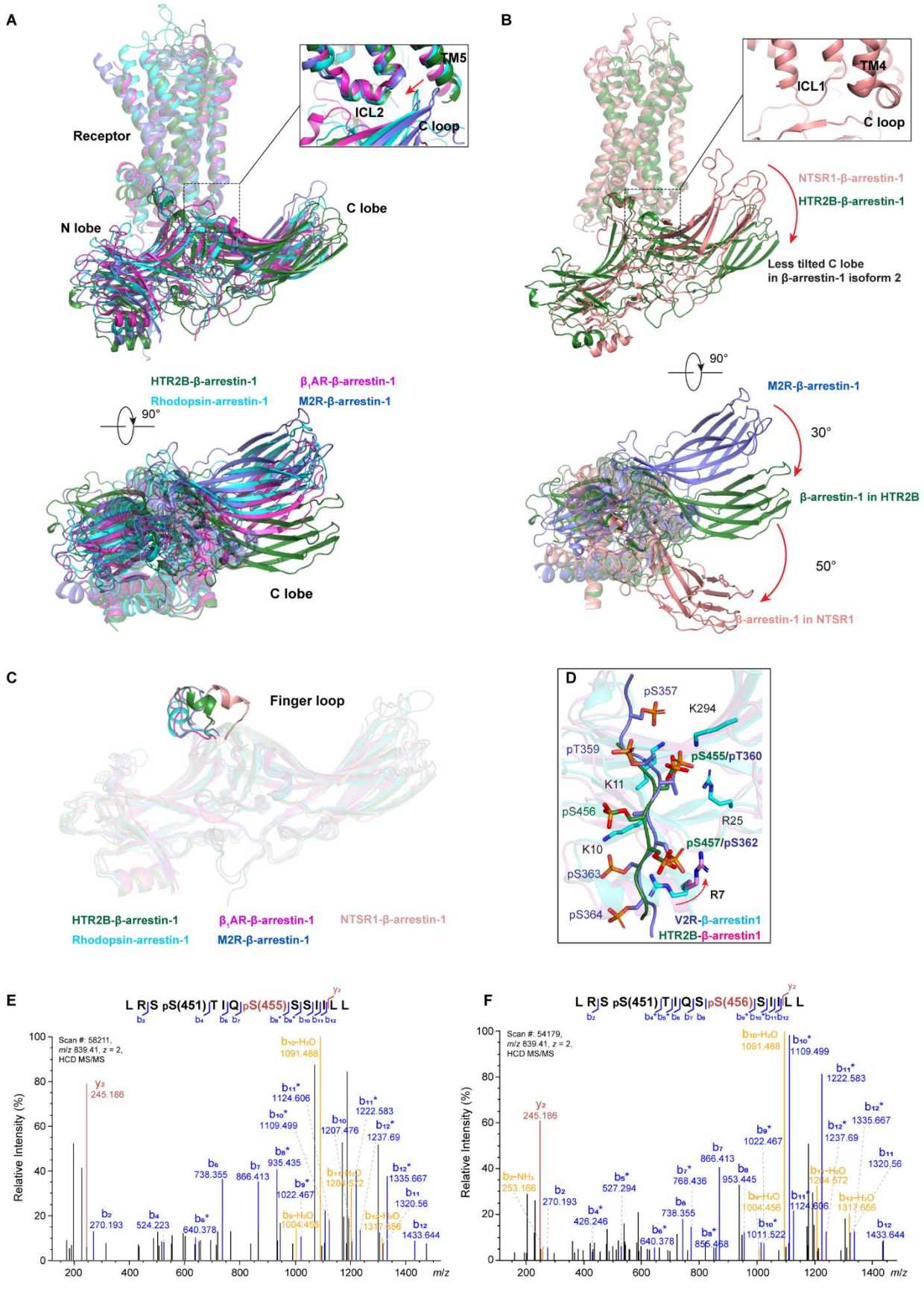
A
Receptor
TM5
ICL2
C loop
N lobe
C lobe
HTR2B-β-arrestin-1
β1AR-β-arrestin-1
Rhodopsin-arrestin-1
M2R-β-arrestin-1
90°
C lobe
B
TM4
ICL1
C loop
NTSR1-β-arrestin-1
HTR2B-β-arrestin-1
Less tilted C lobe
in β-arrestin-1 isoform 2
90°
M2R-β-arrestin-1
30°
β-arrestin-1 in HTR2B
50°
β-arrestin-1 in NTSR1
C
Finger loop
HTR2B-β-arrestin-1
β1AR-β-arrestin-1
NTSR1-β-arrestin-1
Rhodopsin-arrestin-1
M2R-β-arrestin-1
D
pS357
K294
pT359
pS455/pT360
K11
pS456
R25
K10
pS457/pS362
pS363
R7
pS364
V2R-β-arrestin1
HTR2B-β-arrestin1
E
L R S pS(451) T I Q pS(455) S S I I L L
Scan #: 58211,
m/z 839.41, z = 2,
HCD MS/MS
Relative Intensity (%)
m/z
F
L R S pS(451) T I Q S pS(456) S I I L L
Scan #: 54179,
m/z 839.41, z = 2,
HCD MS/MS
Relative Intensity (%)
m/z

Figure S5. Structural comparison of GPCR-arrestin complexes, related to Figure 4. (A) Overlay of HTR2B-β-arrestin-1 structure with structures of Rhodopsin-arrestin 1 (PDB: 5W0P), β_1AR-β-arrestin-1 (PDB: 6TKO) and M2R-β-arrestin-1 (PDB: 6U1N), showing a similar arrestin binding mode in these receptors. The inset shows an enlarged view of the interactions between the ICL2 of receptor and arrestins. The relative movement of β-arrestin-1 C loop pushed by the TM5 of HTR2B is indicted by red arrow. (B) Overlay of HTR2B-β-arrestin-1 structure with NTSR1-β-arrestin-1 structure to show a different arrestin binding mode in these two receptors. Although both structures are obtained in detergent micelle, β-arrestin-1 isoform 2 used in HTR2B has a less tilted angle. The inset shows an enlarged view of the interactions between ICL1, instead of ICL2, of NTSR1 and β-arrestin-1. M2R-β-arretin-1 structure is added in the top view of alignment to highlight the unique coupling mode of β-arrestin-1 in HTR2B. (C) Structural alignment of arrestins shows various conformations of finger loop upon coupling to different GPCRs. (D) Structural comparison of HTR2B C-tail with V2R tail (PDB: 4JQI), highlighting the difference of C-tail phosphorylation patterns. (E-F) MS spectra of HTR2B-β-arrestin-1-scFv30 fusion protein indicated that residues S455 (E) and S456 (F) were phosphorylated. See Table S5 for phosphorylation probabilities.

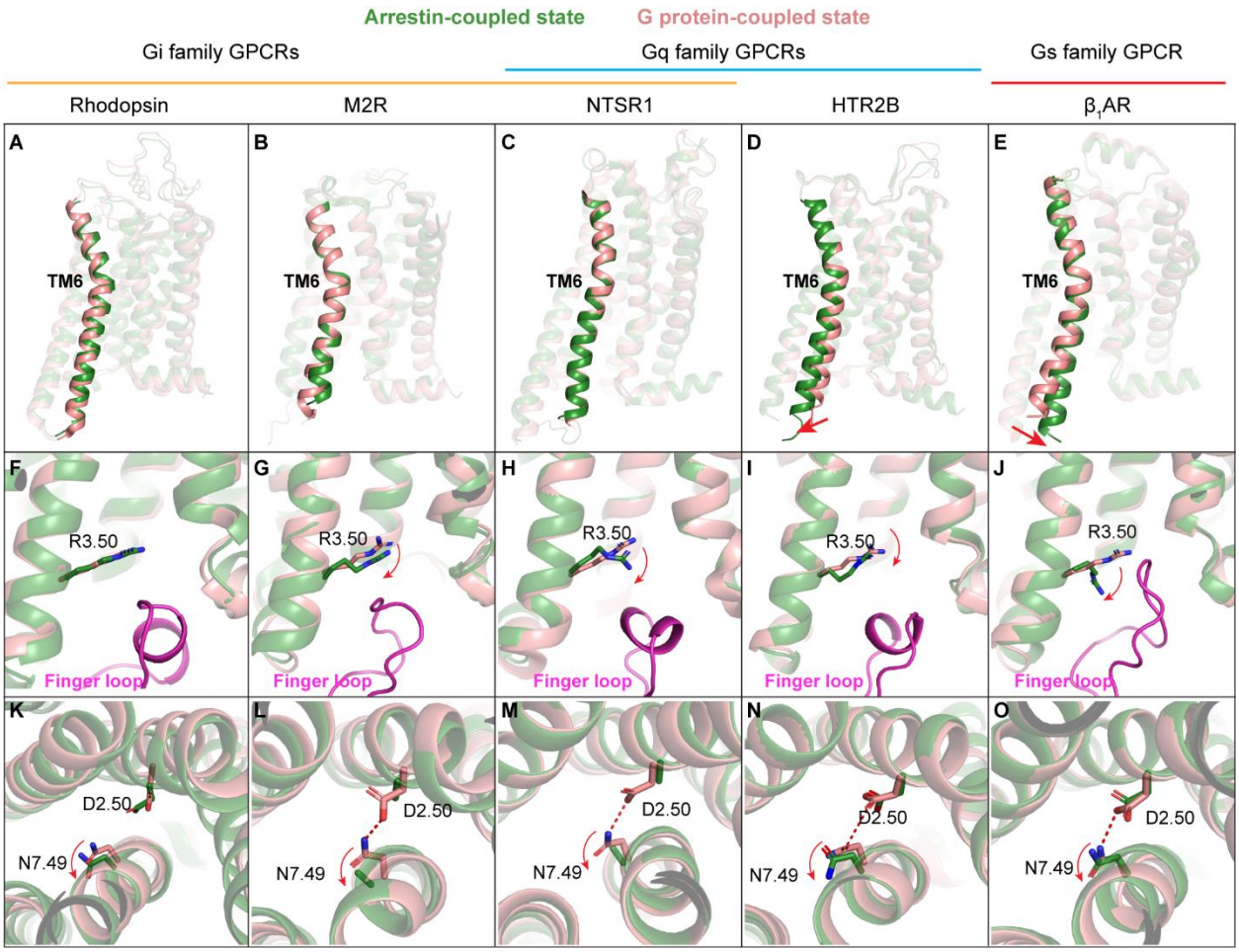

Figure S6. Structural comparison of the receptor portion of GPCR-arrestin complexes with their G protein-coupled structures, related to Figure 5 and Figure 6. (A-E) Overlay of G protein-coupled Rhodopsin (A), M2R (B), NTSR1 (C), HTR2B (D) and β_1AR (E) with their arrestin-coupled states, highlighting the conformational difference of TM6 upon coupling to different transducers. The relative movement of TM6 from G protein-coupled state to arrestin-coupled state is indicated by red arrow. Structures of Gi1-coupled Rhodopsin (PDB: 6CMO), arrestin 1-coupled Rhodopsin (PDB: 5W0P), GoA-coupled M2R (PDB: 6OIK), β-arrestin-1-coupled M2R (PDB: 6U1N), Gi1-coupled NTSR1 (PDB: 6OS9), β-arrestin-1-coupled NTSR1 (PDB: 6UP7), Gq-coupled HTR2B, β-arrestin-1-coupled HTR2B, Gs-coupled β_1AR (PDB: 7JJO) and β-arrestin-1-coupled β_1AR (PDB: 6TKO) were used. (F-J) Conformational difference of $R^{3.50}$ in G protein- and arrestin-coupled structures of Rhodopsin (F), M2R (G), NTSR1 (H), HTR2B (I) and β_1AR (J). A

downward shift of the side chain of R3.50 is observed in most receptors to accommodate the various conformation of arrestin finger loop. In contrast, $R^{3.50}$ adopts an extended conformation to engage G protein in all the receptors. (K-O) Residue $N^{7.49}$ has a counter-clockwise rotation away from $D^{2.50}$ in arrestin-coupled structures of Rhodopsin (K), M2R (L), NTSR1 (M), HTR2B (N) and β_1AR (O).

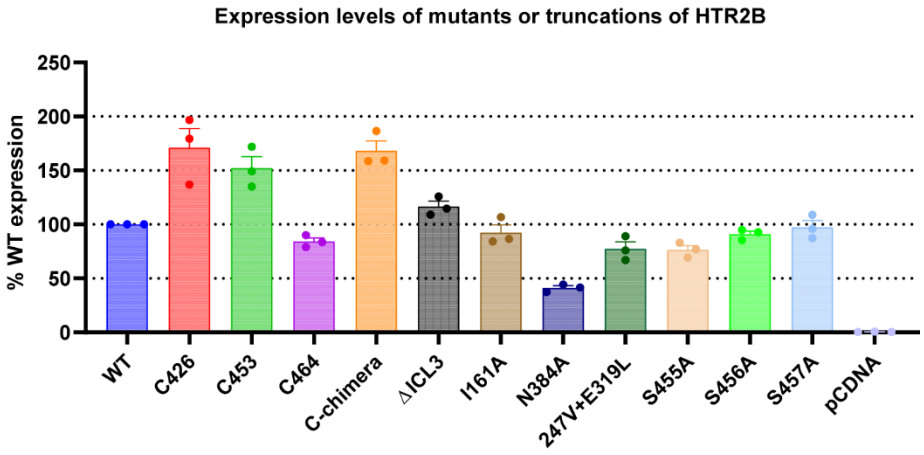

Figure S7. The expression level of HTR2B mutants relative to the WT measured by the Rluc8 counts, related to Figure 1, Figure 4 and Figure 5. Data represent mean ± SEM of n = 3 biological replicates.

Table S1. Cryo-EM data collection, model refinement and validation statistics. Related to Figure 1

Structure	**HTR2B/LSD**	**HTR2B-miniGq-LSD**	**HTR2B/β-arrestin-1**
PDB ID	**7SRQ**	**7SRR**	**7SRS**
Data collection and processing			
Magnification	55,000	55,000	57,050
Voltage (kV)	300	300	300
Dose per frame ($e^-/Å^2$)	1.23	1.07	1.36
Electron exposure ($e^-/Å^2$)	61.5	61.0	68.0
Defocus Range (⎧m)	-0.7 to -1.8	-0.8 to -1.8	-0.8 to -1.8
Pixel size (Å)	0.8677	0.8677	0.8521
Symmetry imposed	C1	C1	C1
Number of Micrographs	12,303	3,493	7,750
Initial particle images (no.)	8,491,352	3,214,021	4,415,425
Final particle images (no.)	665,475	772,614	126,485
Map resolution (Å) #	Receptor (2.7)	Receptor (2.9) G protein (2.9)	Receptor (3.3) β-arrestin-1 (3.3)
FSC threshold	0.143	0.143	0.143
Refinement Statistics*			
Model composition			
Number of chains	1	5	6
Number of residues	254	1119	1103
Total number of atoms	1960	8394	8357
Protein	1922	8370	8319
Ligand	24	24	24
R.m.s. deviations			
Bond lengths (Å)	0.014	0.007	0.008

Bond angles (°)	0.912	0.706	1.003
Ramachandran plot			
Favored (%)	97	97	97
Outlier (%)	0	0	0
Rotamer outliers (%)	1.5	0.12	0.23
Clash score	8.67	3.89	3.82
Molprobity score	1.75	1.39	1.39

[#]Reported by cryoSPARC and *Phenix comprehensive cryo-EM validation

Table S2. BRET1 β-arrestin-1 recruitment assay for C-tail truncation of HTR2B. Related to Figure 1. Emax is defined as percent WT maximum response. Data represent mean ± SEM of n = 6 biological replicates.

HTR2B construct	5-HT		
	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (% WT)
WT	8.65 ± 0.03	2.22	100.0 ± 1.2
C426	8.97 ± 0.11	1.07	22.7 ± 0.8
C453	8.61 ± 0.08	2.41	26.4± 0.7
C464	8.55 ± 0.03	2.79	154.8 ± 1.9
C-Chimera	8.87 ± 0.05	1.35	242.9 ± 5.1
ΔICL3	8.75 ± 0.04	1.78	182.7 ± 2.7

Table S3. BRET1 β-arrestin-1 recruitment assay for ionic lock mutations of HTR2B. Related to Figure 1. Emax is defined as percent WT maximum response of each agonist. Data represent mean ± SEM of n = 4 biological replicates.

HTR2B construct	Agonist	pEC_{50} ± SEM	EC_{50} (nM)	Basal (% WT Emax)	Emax ± SEM (% WT)
WT	5-HT	8.46 ± 0.02	3.43	0.0 ± 0.7	100.0 ± 0.8
K247V+E319L		9.21 ± 0.25	0.61	67.5 ± 2.9	98.5 ± 2.4
WT	LSD	8.65 ± 0.13	2.25	2.1 ± 1.1	27.6 ± 1.2
K247V+E319L		8.96 ± 0.39	1.07	71.1 ± 3.0	92.6 ± 2.8

Table S4. BRET1 β-arrestin-1 recruitment assay for β-arrestin-1 and HTR2B interface mutations. Related to Figure 4. Emax is defined as percent WT maximum response. Data represent mean ± SEM of n = 3 biological replicates.

Protein	5-HT			
	Mutations	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (% WT)
β-arrestin-1	WT	8.72 ± 0.05	1.88	100.0 ± 1.6
	R65A	8.68 ± 0.14	2.07	21.5 ± 1.1
	L71A	8.37 ± 0.19	4.30	26.9 ± 1.8
	L73A	8.49 ± 0.10	3.23	60.3 ± 2.2
HTR2B	WT	8.53 ± 0.03	2.89	100.0 ± 1.2
	I161A	8.20 ± 0.14	6.35	30.8 ± 1.7
	S455A	8.50 ± 0.08	3.10	58.2 ± 1.6
	S456A	8.55 ± 0.06	2.78	72.2 ± 1.6
	S457A	8.58 ± 0.07	2.59	51.9 ± 1.4

Table S5. Mass spectrometry analysis of GRK2 phosphorylated HTR2B-β-arrestin-1-scFv30 samples expressed in sf9 cells. Related to Figure 4. The of HTR2B C-tail residues S455, S456 and S457 in selected peptide fragments that have a phosphorylation probability (shown in the brackets) over 0.75 are highlighted in red.

Position	Phospho (STY) Probabilities	Charge	m/z
S450	LRS450(0.333)S(0.333)T(0.333)IQSSSIILL	2	799.43167
S450	S450(0.333)S(0.333)T(0.333)IQSSSIILL	2	664.839083
S450/S451	LRS450(0.414)S451(0.472)T(0.487)IQS(0.246)S(0.207)S(0.173)IILL	2	839.414836
S450/S455	LRS450(0.571)S(0.418)T(0.011)IQS455(0.575)S(0.422)S(0.004)IILL	2	839.414836
S451/S455	LRS(0.372)S451(0.495)T(0.186)IQS455(0.807)S(0.137)S(0.003)IILL	2	839.414836
S451/S455	LRS(0.383)S451(0.501)T(0.152)IQS455(0.765)S(0.112)S(0.087)IILL	2	839.414836
S451/S455	LRS(0.408)S451(0.52)T(0.363)IQS455(0.58)S(0.107)S(0.022)IILL	2	839.414836
S451/S456	LRS(0.326)S451(0.478)T(0.196)IQS(0.049)S456(0.925)S(0.026)IILL	2	839.414836
T452/S455	LRS(0.239)S(0.328)T452(0.45)IQS455(0.774)S(0.121)S(0.088)IILL	2	839.414836
T452/S455	LRS(0.265)S(0.308)T452(0.475)IQS455(0.729)S(0.12)S(0.103)IILL	2	839.414836
T452/S455	LRS(0.305)S(0.354)T452(0.406)IQS455(0.713)S(0.119)S(0.103)IILL	2	839.414836
T452/S455	LRS(0.316)S(0.378)T452(0.437)IQS455(0.695)S(0.096)S(0.079)IILL	2	839.414836
T452/S455	LRS(0.376)S(0.442)T452(0.474)IQS455(0.528)S(0.099)S(0.082)IILL	2	839.414836
S456	SST(0.001)IQS(0.066)S456(0.867)S(0.066)IILL	2	664.839083
S456	SSTIQS(0.035)S456(0.617)S(0.348)IILL	2	664.839083
S457	LRS(0.26)S(0.327)T452(0.413)IQS(0.054)S(0.418)S457(0.528)IILL	2	839.414836

Table S6. BRET1 β-arrestin-1 or miniGq recruitment assay for WT and N384A mutant of HTR2B. Related to Figure 5. Emax is defined as percent transducer maximum response of each agonist. Data represent mean ± SEM of n = 3 biological replicates.

HTR2B construct	Agonist	Transducer	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (% WT)
WT	5-HT	β-arrestin-1	8.31 ± 0.05	4.79	99.8 ± 1.9
		miniGq	8.94 ± 0.04	1.15	100.2 ± 1.3
	LSD	β-arrestin-1	8.92 ± 0.08	1.20	95.2 ± 2.6
		miniGq	9.01 ± 0.05	0.97	99.3 ± 1.4
N384A	LSD	β-arrestin-1	8.46 ± 0.02	2.04	192.9 ± 4.8
		miniGq	8.84 ± 0.07	1.45	64.6 ± 1.5