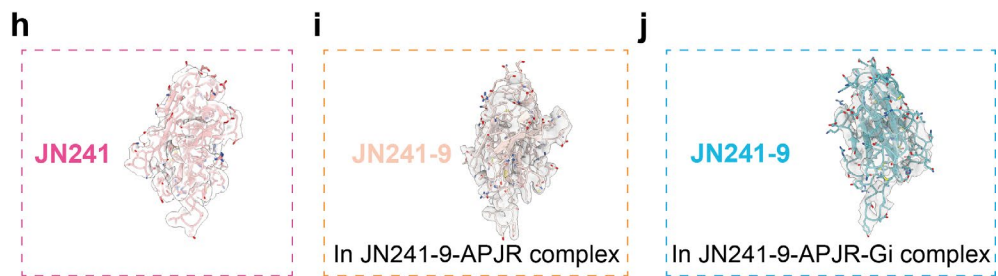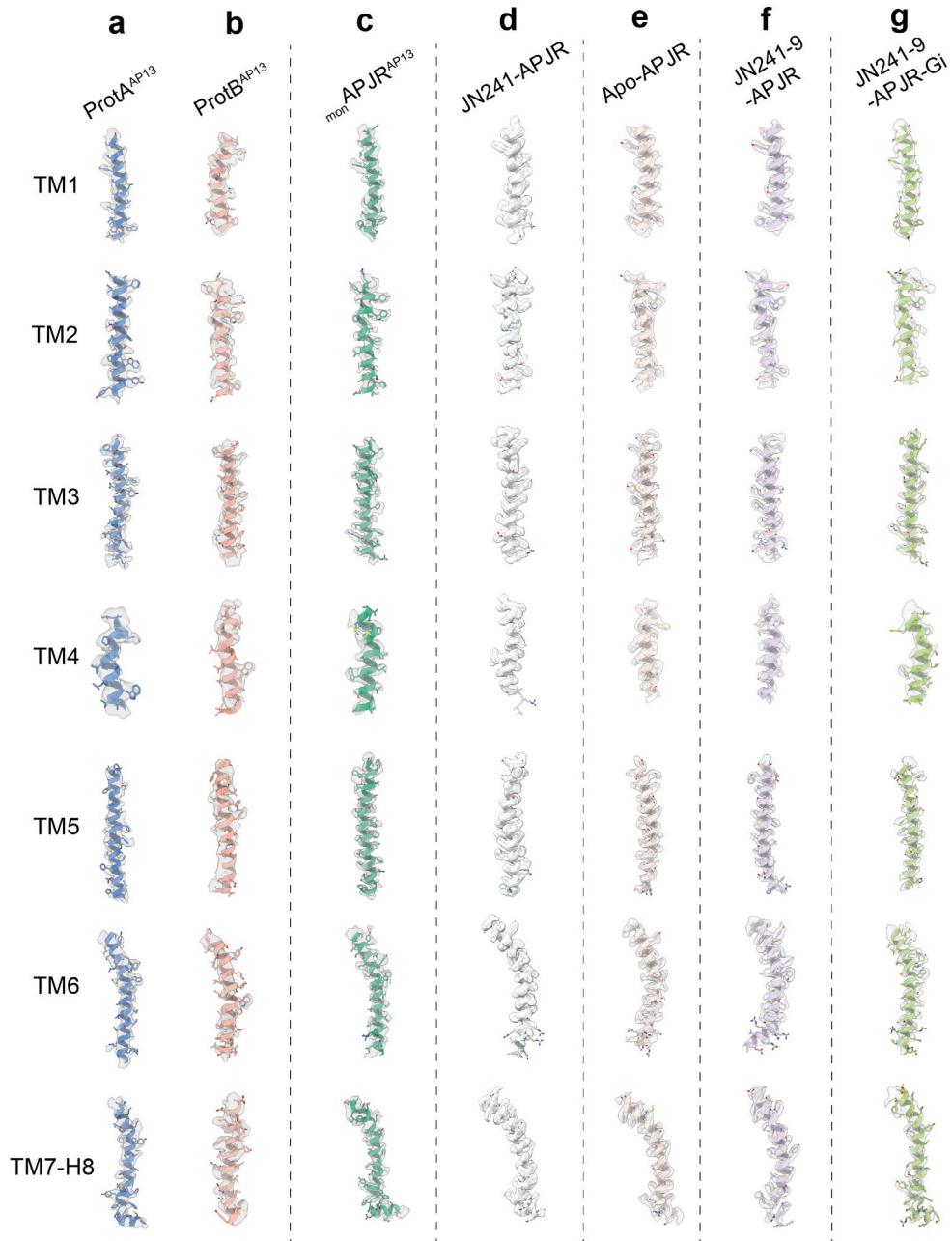Supplementary Figure 1. Characterization of APJR dimerization on cell surface by the single-molecule imaging approach. a, APJR dimer and downstream Gi-protein complex surface model from cmpd644-APJR-Gi complex (PDB ID: 7W0L). The dimer interface "FGTFF" motif is shown in sticks. **b,** Representative fluorescence time course for an individual molecule. Left, 1-step photobleaching (monomer); middle, 2-steps photobleaching (dimer) and right, 3-steps photobleaching (trimer). **c,** Histogram of photobleaching step numbers for all Snap-APJR molecules analyzed. 1867 spots from 9 movies in basal group (no-treatment); and 1234 spots from 8 movies in cmpd644 group (1 mm treatment for 30 min) were analyzed. Data are mean ± SEM from three biologically independent experiments and were analyzed using two-way ANOVA with Tukey's multiple comparisons test. Source data are provided as a Source Data file.
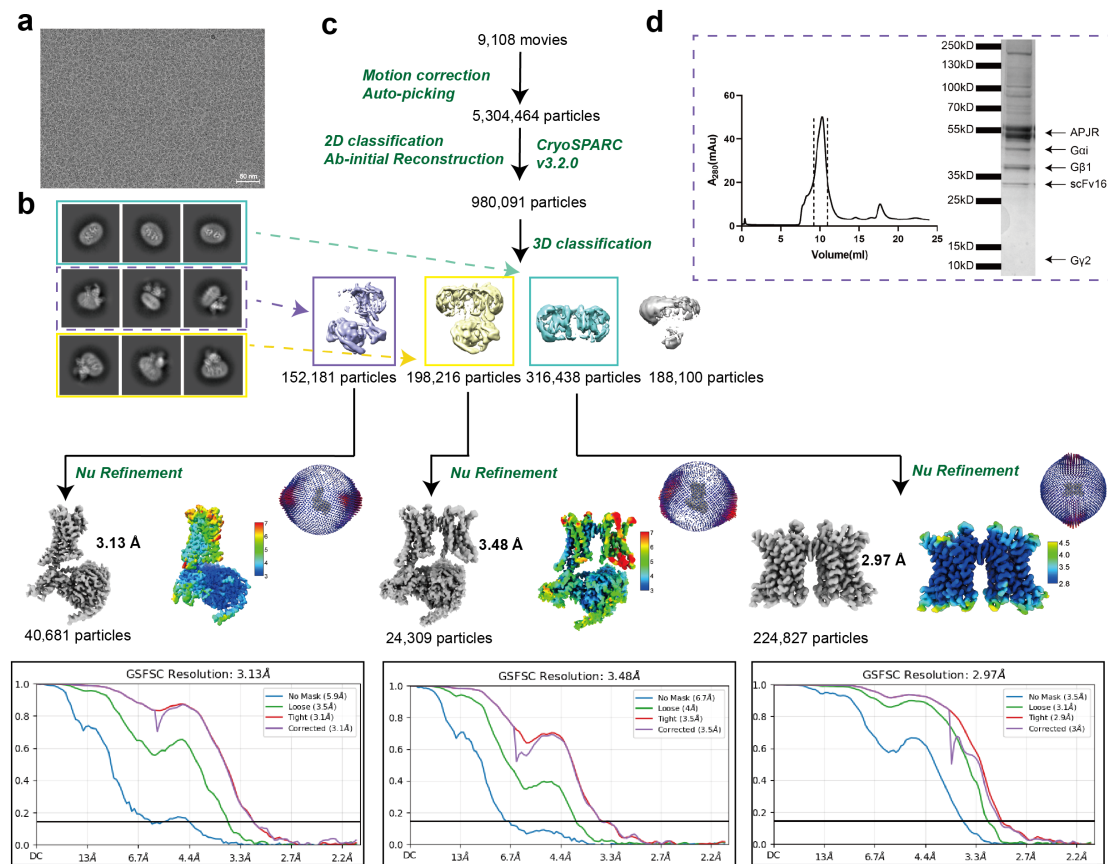
Supplementary Figure 2. Cryo-EM sample preparation, data collection and processing for APJR^{AP13}-Gi-scFv16 complex and apo-APJR. a-d, Representative cryo-EM image derived from 9,108 micrographs of the APJR^{AP13}-Gi-scFv16 complex, with a scale bar of 50 nm (**a**). Representative 2D averages showing distinct secondary structural features from various perspectives of the complex (**b**). Cryo-EM data processing workflow: The data underwent processing using CryoSPARC, and density map images were generated in UCSF Chimera. The final 3D density maps are color-coded based on local resolution and angular distribution of the particles used for the final reconstruction. Gold-standard FSC curves from Phenix indicate nominal resolutions of 3.48 Å, 3.13 Å, and 2.97 Å, employing the FSC = 0.143 criterion for the $_{dim}$APJR^{AP13}-Gi map, $_{mon}$APJR^{AP13}-Gi map, and apo-APJR, respectively (**c**). Elution profile and gel image of APJR (comprising residues 1–350), Gαi, Gβ, Gγ, and scFv16 after SEC purification. The collected fraction for cryo-EM sample were marked between dashed lines (**d**).

a ProtA^{AP13} **b** ProtB^{AP13} **c** $_{mon}$APJR^{AP13} **d** JN241-APJR **e** Apo-APJR **f** JN241-9-APJR **g** JN241-9-APJR-Gi

TM1

TM2

TM3

TM4

TM5

TM6

TM7-H8

h JN241

i JN241-9

In JN241-9-APJR complex

j JN241-9

In JN241-9-APJR-Gi complex

k FGTFF Motif

ProtB^{AP13} ProtA^{AP13} JN241-APJR JN241-9-APJR Apo-APJR

T$^{3.22}$ F$^{3.23}$ F$^{23.52}$ F$^{3.24}$ F$^{23.52}$ F$^{3.23}$ T$^{3.22}$
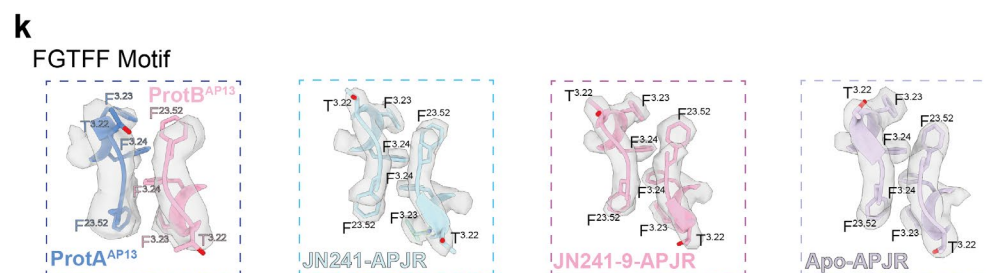
Supplementary Figure 3. Cryo-EM density maps and models of representative helices from the ${}_{dim}$APJR^{AP13}–Gi, ${}_{mon}$APJR ^{AP13}-Gi, JN241-APJR, apo-APJR, JN241-9-APJR and JN241-9-APJR-Gi structures. a-g, Cryo-EM density maps and models of TM1, TM2, TM3, TM4, TM5, TM6, TM7-H8 in the PortA (**a**) and ProtB (**b**) of ${}_{dim}$APJR^{AP13}–Gi, ${}_{mon}$APJR ^{AP13}-Gi (**c**), JN241-APJR (**d**), apo-APJR (**e**), JN241-9-APJR (**f**) and JN241-9-APJR-Gi (**g**). **h-j,** Cryo-EM density maps and models of JN241 in JN241-APJR complex (**h**), JN241-9 in the JN241-9-APJR complex (**i**) and JN241-9 in the JN241-9-APJR-Gi complex (**j**). **k,** Cryo-EM density maps and models of FGTFF motif (dimer interface) in ${}_{dim}$APJR^{AP13}–Gi, JN241-APJR, JN241-9-APJR and apo-APJR complexes, respectively.

a

AMG3054
Apelin-13

Site 2

R2 P3 Q1
H7
TM5
R4 S6 K8
F13 L5 G9
P12 M11 P10 Site 1
TM4 TM3 P10 TM2 TM1

b

Peptide ligands	Sequence													
	1~18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
ELABELA-32		Gln	Arg	Arg	Cys	Met	Pro	Ile	His	Ser	Arg	Val	Pro	Phe	Pro
Apelin-13		Gln	Arg	Pro	Arg	Leu	Ser	His	Lys	Gly	Pro	Met	Pro	Phe	-
		1	2	3	4	5	6	7	8	9	10	11	12	13	

c

ProtA^{AP13} ProtB^{AP13}
ProtA^{ELA} ProtB^{ELA}

TM2
TM7 TM1
TM5
TM3
TM6

d

ELA   Apelin-13

Site 2

TM5   TM4   F13   Site 1
M11   TM1
TM3   TM2

e

	TM2					TM3					TM6			TM7	
	Y35$^{1.39}$	F78$^{2.53}$	W85$^{2.60}$	Y88$^{2.63}$	Y93^{ECL1}	I109$^{3.32}$	F110$^{3.33}$	M113$^{3.36}$	R168$^{4.64}$	Y185^{ECL2}	W261$^{6.48}$	K268$^{6.55}$	Y271$^{6.58}$	F291$^{7.35}$	Y299$^{7.43}$
AP13-APJR-Gi	●	—	●	—	—	●	●	●	●	●	—	●	●	●	●
ELA-APJR-Gi	●	—	●	●	●	●	●	●	●	—	●	●	—	●	●
Cmpd644-APJR-Gi	●	●	—	—	●	●	●	●	—	●	●	●	●	●	●

Supplementary Figure 4. Structural comparison between $_{dim}$**APJR^{AP13}-Gi and** $_{mon}$**APJR^{AP13}-Gi/AMG3054-APJR complexes and peptide sequence alignment between apelin-13 and ELA. a,** Structural comparison of apelin-13 and AMG3054. Apelin-13 is depicted in yellow, and AMG3054 is depicted in gray. APJR in the ProtA^{AP13} structure is illustrated in blue cylinders, with site 1 and site 2 encircled by dashed lines. **b,** Sequence alignment of ELA-32 and apelin-13. Peptide numbering (upper panel for ELA-32, lower panel for apelin-13) is highlighted in brown. The 1-18 residues of ELA-32 have been omitted. **c,** Structural superposition of ProtAs in apelin-13- with ELA-bound APJR-Gi structure. ProtA^{AP13} and ProtA^{ELA} are colored in blue and purple cylinders, respectively. **d,** Structural comparison of apelin-13 and ELA. Apelin-13 and ELA are shown in yellow and red, respectively, with APJR depicted in blue cylinders. The orientation of the two peptide ligands is indicated with arrows. **e,**

Sequence alignment of key residues of APJR in the ligand binding pocket. The key residues in the ligand-binding pocket of APJR are aligned, with residues interacting with apelin-13 colored in orange, residues interacting with ELA colored in green, and residues interacting with cmpd644 colored in gray. Conserved residues are highlighted in blue.

a

b

c

3,115 movies

Motion correction
Auto-picking

3,071,733 particles

2D classification
Ab-initial Reconstruction   *CryoSPARC v3.2.0*

510,362 particles

3D classification

117,307 particles   136,217 particles   170,746 particles   860,92 particles

Nu Refinement

d

A_{280}(mAu)

Volume(ml)

170kD
130kD
95kD
72kD   ← JN241
55kD   ← APJR
43kD
34kD
26kD
17kD
10kD

2.95 Å

170,746 particles

GSFSC Resolution: 2.95Å

No Mask (3.8Å)
Loose (3.2Å)
Tight (2.9Å)
Corrected (3Å)

DC   13Å   6.7Å   4.4Å   3.3Å   2.7Å   2.2Å

e

f

45431 ptcls   41895 ptcls   39874 ptcls
7.0 A 1 ess   7.0 A 1 ess   7.0 A 1 ess
39495 ptcls   37545 ptcls   29451 ptcls
7.0 A 1 ess   7.0 A 1 ess   7.0 A 1 ess

g

4,886 movies

Motion correction
Auto-picking

2,203,592 particles

2D classification
Ab-initial Reconstruction   *CryoSPARC v3.2.0*

464,559 particles

Nu Refinement

h

A_{280}(mAu)

Volume(ml)

170kD
130kD
95kD
72kD
55kD   ← APJR
43kD
34kD
26kD
17kD
10kD   ← JN241-9

3.01 Å

488,065 particles

GSFSC Resolution: 3.01Å

No Mask (3.4Å)
Loose (3.2Å)
Tight (3Å)
Corrected (3Å)

DC   13Å   6.7Å   4.4Å   3.3Å   2.7Å   2.2Å

i

j

17623 ptcls   17030 ptcls   16365 ptcls
8.7 A 1 ess   8.7 A 1 ess   8.7 A 1 ess
13303 ptcls   13295 ptcls   13001 ptcls
8.7 A 1 ess   8.7 A 1 ess   8.7 A 1 ess

k

4,071 movies

Motion correction

1,047,133 particles

2D classification
Ab-initial Reconstruction   *CryoSPARC v3.2.0*

441,368 particles

3D classification

85,472 particles   82,405 particles   76,528 particles   69,516 particles

Nu Refinement

l

A_{280}(mAu)

Volume(ml)

170kD
130kD
95kD
72kD   ← APJR
55kD   ← Gαi
43kD   ← Gβ1
34kD   ← scFv16
26kD
17kD   ← JN241-9
10kD   ← Gγ2

3.12 Å

65,206 particles

GSFSC Resolution: 3.12Å

No Mask (4.2Å)
Loose (3.4Å)
Tight (3.1Å)
Corrected (3.1Å)

DC   13Å   6.7Å   4.4Å   3.3Å   2.7Å   2.2Å

Supplementary Figure 5. Cryo-EM sample preparation, data collection and processing for JN241-APJR. a-d, Representative cryo-EM image derived from 3,115

micrographs of the JN241-APJR complex (50 nm scale) (**a**). Representative 2D averages of complex (**b**). Cryo-EM workflow with CryoSPARC and UCSF Chimera, 3D density maps color-coded by local resolution, angular distribution of the particles used for the final reconstruction, and FSC curves show 2.95 Å resolution (**c**). Elution profile and gel image of APJR and JN241 after SEC purification. The collected fraction for cryo-EM sample were marked between dashed lines (**d**). **e-h,** Representative cryo-EM image derived from 4,886 micrographs of the JN241-9-APJR complex (50 nm scale) (**e**). Representative 2D averages of complex (**f**). Cryo-EM workflow with CryoSPARC and UCSF Chimera, 3D density maps color-coded by local resolution, angular distribution of the particles used for the final reconstruction, and FSC curves show 3.01 Å resolution (**g**). Elution profile and gel image of APJR and JN241-9 after SEC purification. The collected fraction for cryo-EM sample were marked between dashed lines (**h**). **i-l,** Representative cryo-EM image derived from 4,071 micrographs of the JN241-9-APJR-Gi complex (50 nm scale) (**i**). Representative 2D averages of complex (**j**). Cryo-EM workflow with CryoSPARC and UCSF Chimera, 3D density maps color-coded by local resolution, angular distribution of the particles used for the final reconstruction, and FSC curves show 3.12 Å resolution (**k**). Elution profile and gel image of APJR, Gαi, Gβ, Gγ, scFv16 and JN241-9 after SEC purification. The collected fraction for cryo-EM sample were marked between dashed lines (**l**).
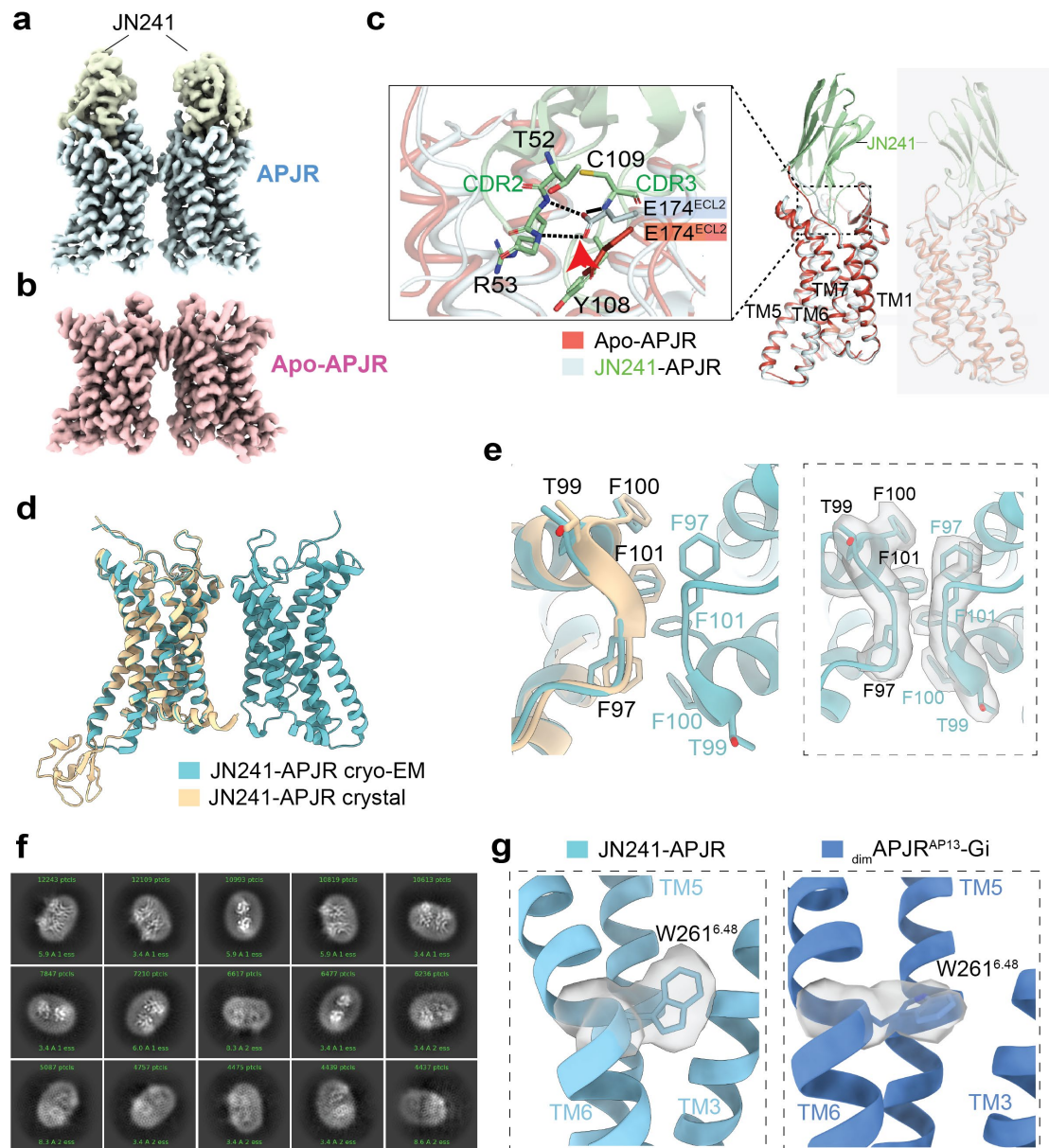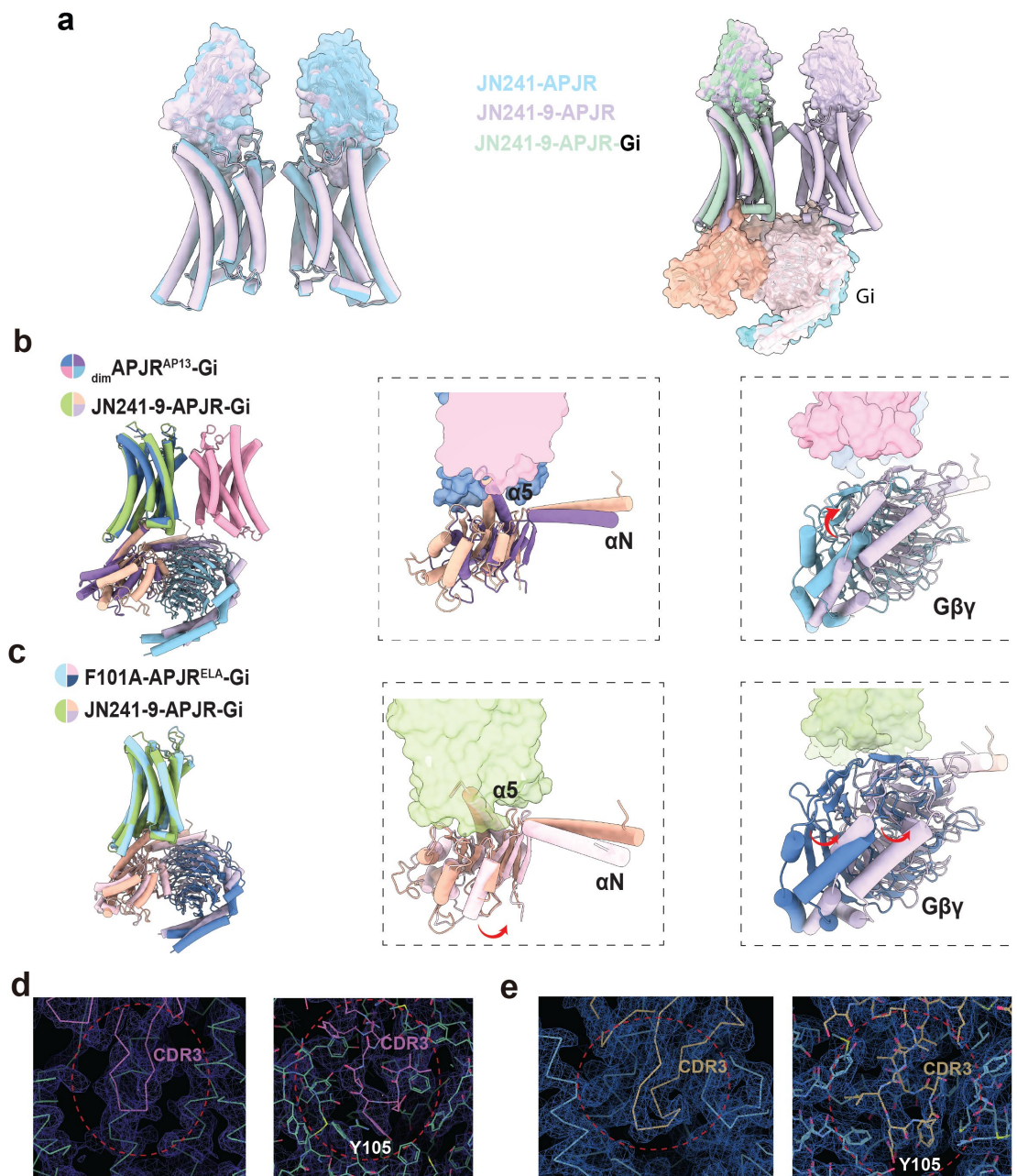
Supplementary Figure 6. Structural characterization of JN241-APJR and apo-APJR dimers, and comparison with ProtB in apelin-13-bound APJR-Gi structure.
a, Cryo-EM map of JN241-APJR complex. APJR is depicted in light cyan, and JN241 is shown in light green. **b,** Cryo-EM map of apo-APJR without G-protein coupling. Apo-APJR, separated from the dataset of the APJR^{AP13}-Gi complex, is colored in pink. **c,** Conformational comparison of E174^{ECL2} in JN241- and apo- APJR. Hydrogen bonds formed by residues T52/R53 from CDR2 of JN241 or residue C109 from CDR3 in JN241 are indicated with arrows. The symmetric partner in the JN241-APJR dimer structure is shaded in gray. **d, e,** Structural comparison between JN241-APJR cryo-EM

and crystal (PDB ID: 6KNM) structures. Overall structural comparison between JN241-APJR cryo-EM and crystal structures (**d**). Superimposition of the crystal structure of JN241-APJR onto cryo-EM structure, with a focus on the dimeric interface of FGTFF motif, shows subtle conformational changes in the side chains of three critical phenylalanine residues. Additionally, the cryo-EM density map for the amino acids comprising the FGTFF motif is shown (**e**). **f,** Representative 2D averages of the JN241-APJR complex, devoid of the Fc tag, consistently exhibit dimer formation, indicating that the presence or absence of the Fc tag does not affect the dimerization of APJR. **g,** Cryo-EM density maps for the "toggle switch" residue $W261^{6.48}$ within the JN241-APJR complex and $_{dim}APJR^{AP13}$-Gi complex structures, respectively.
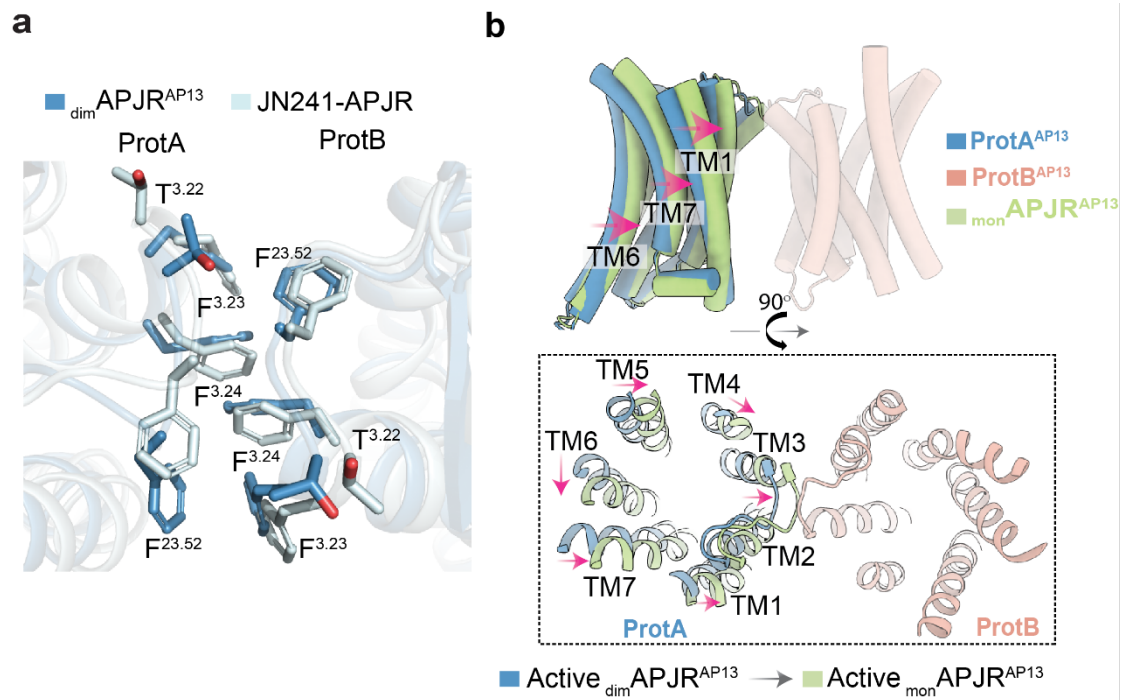
Supplementary Figure 7. Structural comparisons of the dimer interface and the structural transitions from APJR dimer to monomer. a, The "FGTFF motif" residues (shown in sticks) at the dimer interface in apelin-13-bound and antagonist-bound structures are illustrated. **b,** Structural transitions from active dimer to active monomer induced by apelin-13 are depicted. Superposition of $_{dim}$APJR^{AP13}-Gi (ProtA: blue, ProtB: pink) and $_{mon}$APJR^{AP13}-Gi (green) aligned on the Gi protein portion, with the Gi protein omitted.

Supplementary Figure 8. The dimerization modes and conformational changes in transmembrane domains of APJR in different functional states. a, Structural alignments of JN241-APJR, JN241-9-APJR, and JN241-9-APJR-Gi complexes. **b,** Structural superposition of $_{dim}$APJR^{AP13}-Gi and JN241-9-APJR-Gi complexes shows conformational shifts in Gα and Gβγ, indicated by arrows. These shifts suggest an upward displacement in the JN241-9-APJR-Gi complex towards the region that would otherwise be occupied by ProtB in the $_{dim}$APJR^{AP13}-Gi complex. **c,** Structural superposition of F101A-APJR^{ELA}-Gi (PDB ID: 7W0P) and JN241-9-APJR-Gi

complexes. The conformational changes of Gα and Gβγ were indicated as arrows. **d, e,** Cryo-EM maps of CDR3 region of JN241-9 in the orthosteric pocket, with the key residue Y105 highlighted: (**d**) CDR3 region of JN241-9 in the Gi protein-bound structure, shown as cartoon (left) and sticks (right), respectively; (**e**) CDR3 region of JN241-9 in the Gi protein-free structure, shown as cartoon (left) and sticks (right), respectively.
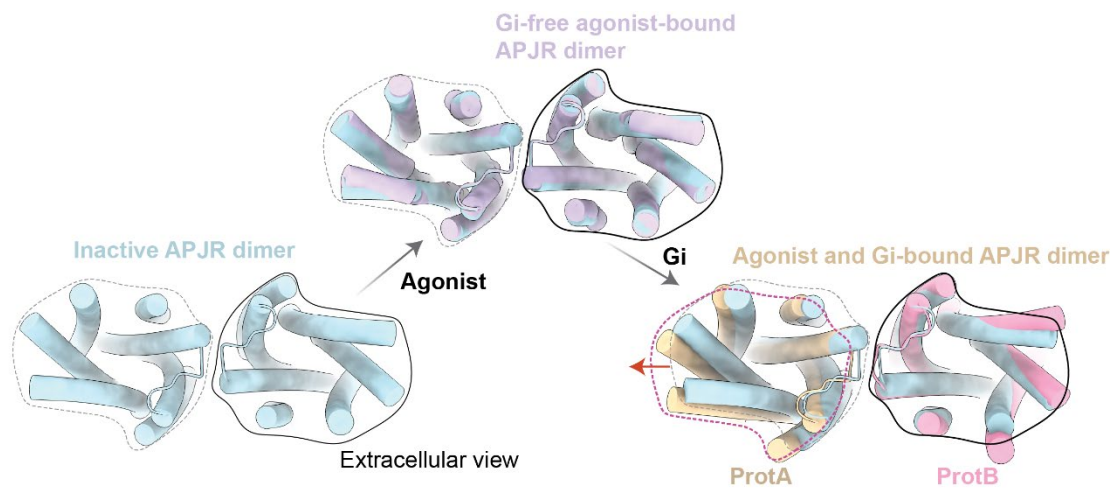
Supplementary Figure 9. The dimerization modes and conformational changes in transmembrane domains of APJR in different functional states. Various dimerization modes of the transmembrane domain of APJR during receptor activation, aligned on ProtBs. The red arrow indicates the movement of ProtA upon agonist and Gi binding.

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics of G protein coupled GPCR complexes.

	$_{dim}$APJR^{AP13}-Gi	$_{mon}$APJR^{AP13}-Gi	Apo APJR
Data collection and processing			
Magnification	105,000	105,000	105,000
Voltage (kV)	300	300	300
Electron exposure (e-/Å^2)	60	60	60
Defocus range (μm)	-0.7~-2.2	-0.7~-2.2	-0.7~-2.2
images	9108	9108	9108
Pixel size (Å)	1.04	1.04	1.04
Symmetry imposed	C1	C1	C2
Final particles	24,309	40,681	224,827
Map resolution	3.48	3.13	2.97
FSC threshold	0.143	0.143	0.143
Refinement			
Initial model used (PDB code)	7W0L	7W0M	7SUS
Map sharping B factor (Å)	61.0	64.9	96.2
Model composition			
Non-hydrogen atoms	11348	9233	4842
Protein residues	1442	1177	597
Ligand	0	0	2
B-factors			
Protein	90.62	67.71	44.89
Ligand			37.65
R.M.S. deviations			
Bond lengths (Å)	0.003	0.004	0.004
Bond angles (°)	0.752	0.677	0.664
Validation			
MolProbity score	2.21	2.08	1.89
Clash score	20.32	14.35	12.59
Poor rotamers (%)	0.00	0.00	0.00
Ramachandran plot			
Favored (%)	93.87	93.71	95.94
Allowed (%)	6.13	6.20	4.06
Disallowed (%)	0.00	0.09	0.00
EMD code	38574	38575	38578
PDB code	8XQE	8XQF	8XQI

	JN241-APJR	JN241-9-APJR	JN241-9-APJR-Gi
Magnification	105,000	105,000	105,000
Voltage (kV)	300	300	300
Electron exposure (e-/Å^2)	60	60	60
Defocus range (μm)	-0.7~-2.2	-0.7~-2.2	-0.7~-2.2
images	3115	4,886	4,071
Pixel size (Å)	1.04	1.04	1.04
Symmetry imposed	C2	C1	C1
Final particles	170,746	488,065	65,206
Map resolution	2.95	3.01	3.12
FSC threshold	0.143	0.143	0.143
Refinement			
Initial model used (PDB code)	7SUS	7SUS	7W0L
Map sharping B factor (Å)	85.6	136.8	121.4
Model composition			
Non-hydrogen atoms	6904	6709	10089
Protein residues	864	847	1293
Ligand	4	2	
B-factors			
Protein	58.62	66.42	77.45
Ligand	74.36	49.30	
R.M.S. deviations			
Bond lengths (Å)	0.003	0.003	0.003
Bond angles (°)	0.568	0.630	0.661
Validation			
MolProbity score	1.83	1.90	2.01
Clash score	10.26	13.38	16.06
Poor rotamers (%)	0.00	0.00	0.00
Ramachandran plot			
Favored (%)	95.68	96.07	95.69
Allowed (%)	4.32	3.93	4.23
Disallowed (%)	0.00	0.00	0.00
EMD code	38579	39810	39816
PDB code	8XQJ	8Z74	8Z7J

Supplementary Table 2. Mutational effects on cAMP accumulation of APJR induced by apelin-13 and ELA measured by Glo-Sensor assays.

Ligands	Apelin-13		ELA		Expression Level±SEM (% WT)
	Gi signaling				
	pEC50±SEM	Emax±SEM (% WT)	pEC50±SEM	Emax±SEM (% WT)	
WT	10.68±0.11	100±0.00	10.34±0.09	100±0.00	100±0.00
F78A	10.32±0.01[ns]	83.49±5.84[ns]	9.40±0.03*	72.46±3.79[ns]	122.07±13.23[ns]
I109A	10.59±0.06[ns]	90.86±9.24[ns]	9.56±0.05[ns]	86.49±19.63[ns]	106.40±4.28[ns]
M113A	10.67±0.13[ns]	79.24±4.12[ns]	10.49±0.05[ns]	79.11±1.81[ns]	84.50±4.41[ns]
Y185A	ND	ND	9.81±0.49[ns]	50.25±6.43[ns]	56.82±12.04*
K268A	9.39±0.09[ns]	63.36±7.01[ns]	8.82±0.23***	25.62±41.00*	124.10±16.95[ns]
F291A	10.66±0.23[ns]	73.81±12.87[ns]	10.53±0.10[ns]	72.46±9.89[ns]	100.72±18.62[ns]
Y299A	10.28±0.11[ns]	93.42±10.29[ns]	10.37±0.27[ns]	139.30±5.64[ns]	88.52±1.98[ns]
Y271A	6.07±2.43**	55.76±2.99[ns]	9.64±0.14[ns]	80.09±12.24[ns]	94.03±2.68[ns]

The data are presented as the mean ± SEM of three independent experiments performed in triplicates (n=3). ND indicates no detectable signal. [ns]$P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ by one-way ANOVA followed by Dunnett's multiple comparisons test, compared with the response of the APJR-WT.
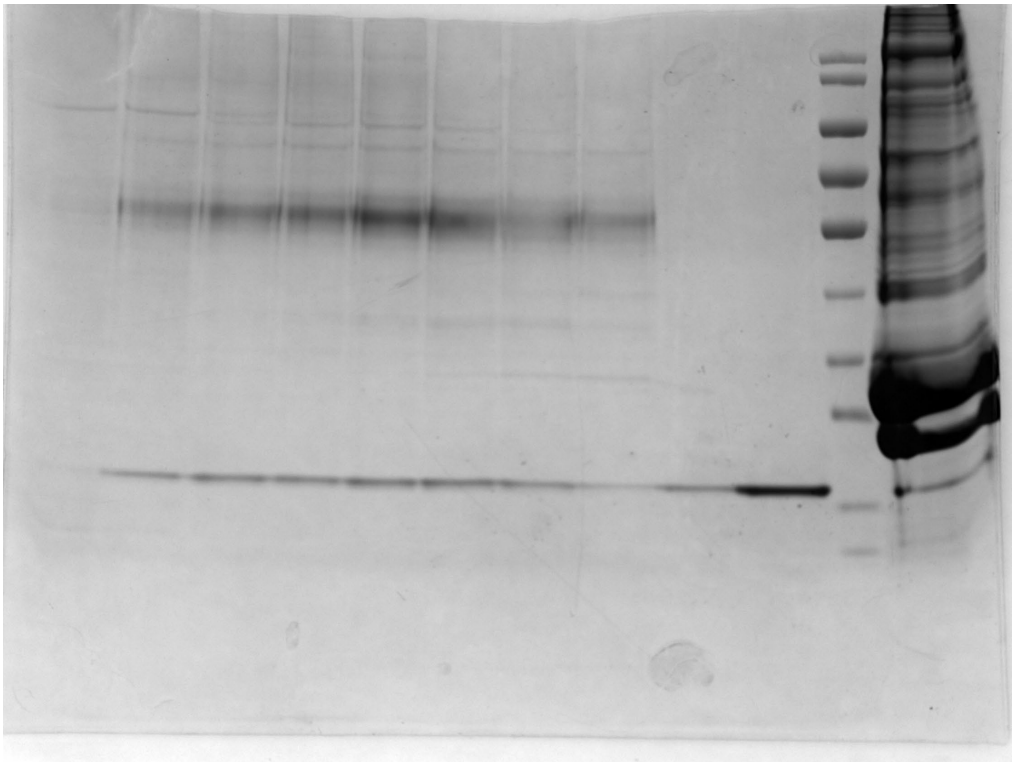
Uncropped SDS PAGE (Supplementary Figure 2d)
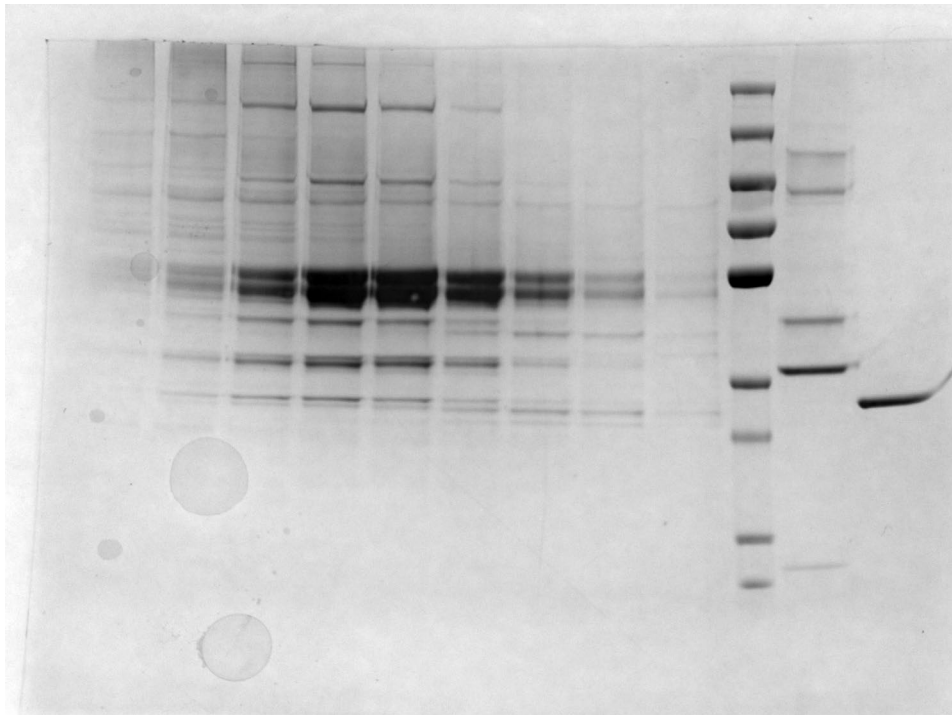
Uncropped SDS PAGE (Supplementary Figure 5d)
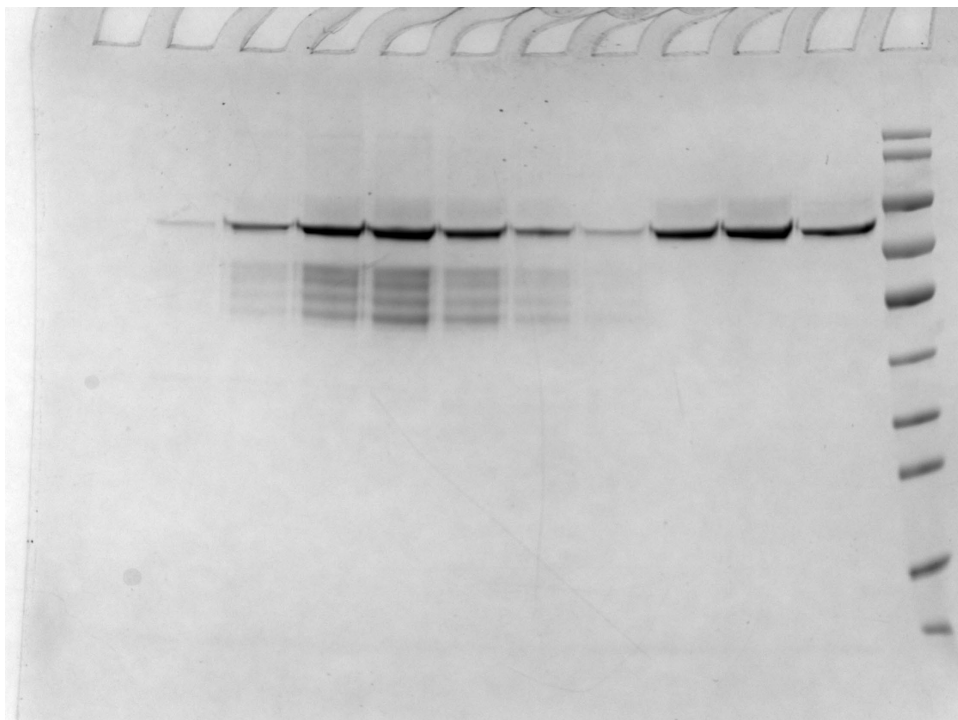
Uncropped SDS PAGE (Supplementary Figure 5h)

Uncropped SDS PAGE (Supplementary Figure 5i)