Supplementary figures

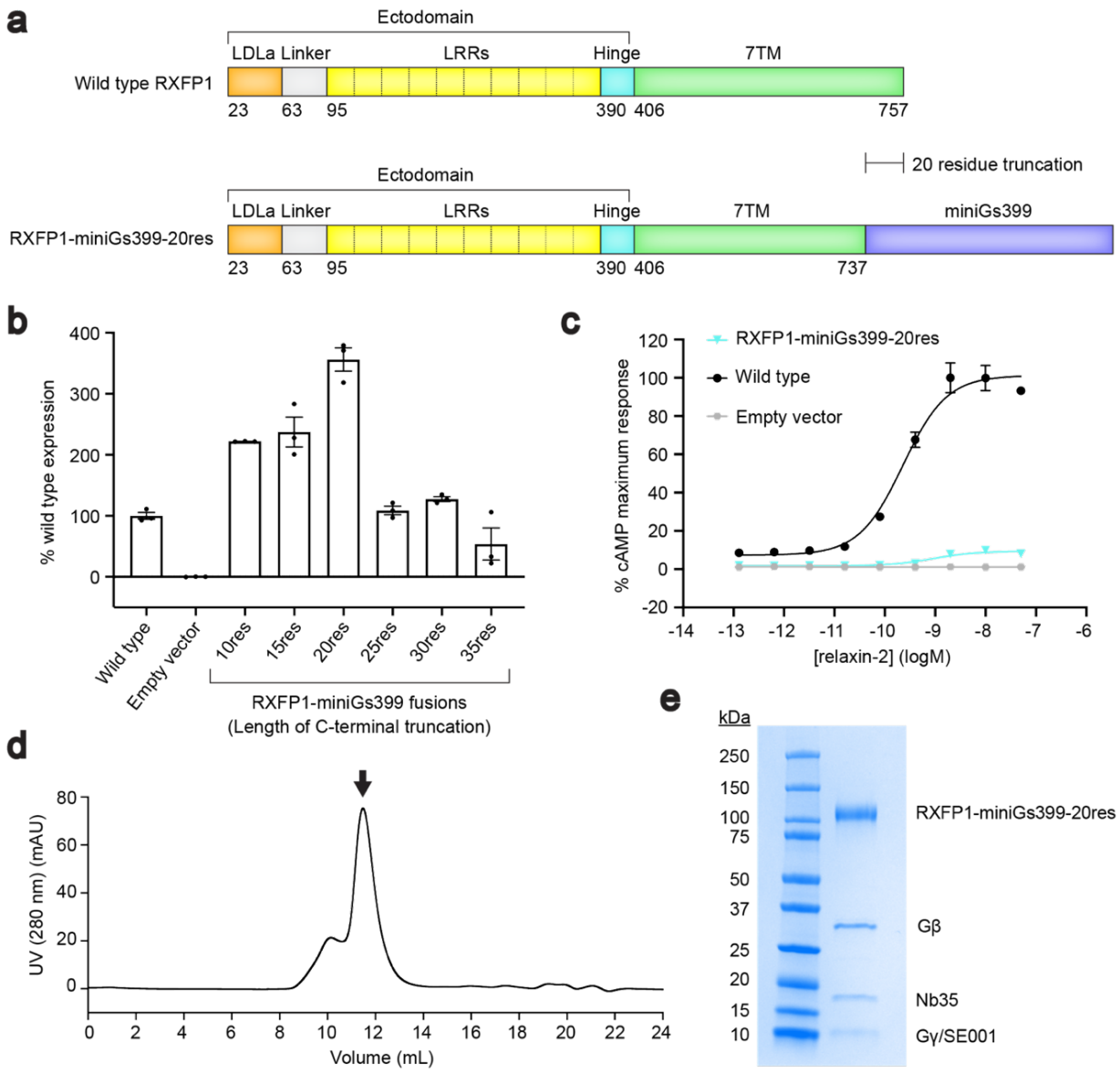

Fig. S1 | Engineering and purification of the RXFP1–G_s complex. a, Diagram of the primary structure of RXFP1 domains versus the RXFP1-miniG_s399-20res fusion construct. **b**, Flow cytometry cell surface expression tests in Expi293F tetR cells for RXFP1-miniG_s fusion constructs. Data is mean ± s.e.m., n=3 technical replicates. **c**, G_s signaling assay comparing the signaling levels of wild type RXFP1 versus RXFP1-miniG_s399-20res in response to relaxin-2. **d**, Size exclusion chromatography profile for the RXFP1–G_s complex. Arrow indicates the peak fractions pooled for RXFP1–G_s. **e**, Coomassie-stained SDS-PAGE gel for the RXFP1–G_s complex.

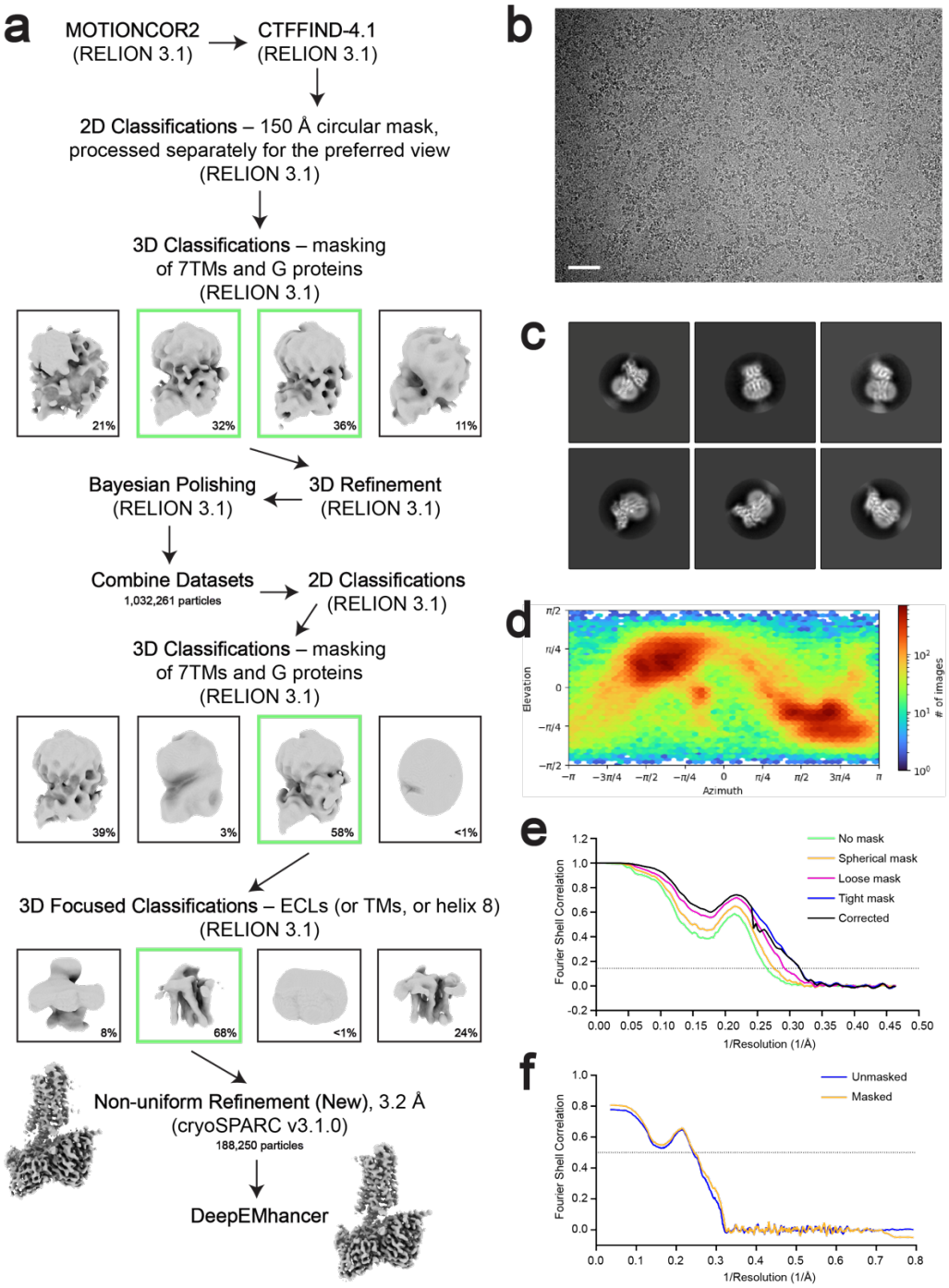

Fig. S2 | Cryo-EM data processing for the 7TM domain of RXFP1–G_s. a, Cryo-EM data processing scheme for the 7TM domain of RXFP1 in complex with G_s. Shown are representative processing steps for one of four individual datasets and the steps used for the combined datasets. **b**, Representative micrograph from the RXFP1–G_s complex datasets (Scale bar = 50 nm). **c**, Two-dimensional class averages for the 7TM domain of RXFP1 and G proteins. **d**, Angular distribution of particles in the final refinement for the 7TM domain with G proteins. **e**, Fourier shell correlation (FSC) used to determine the overall map resolution. **f**, Map to model FSC curve.

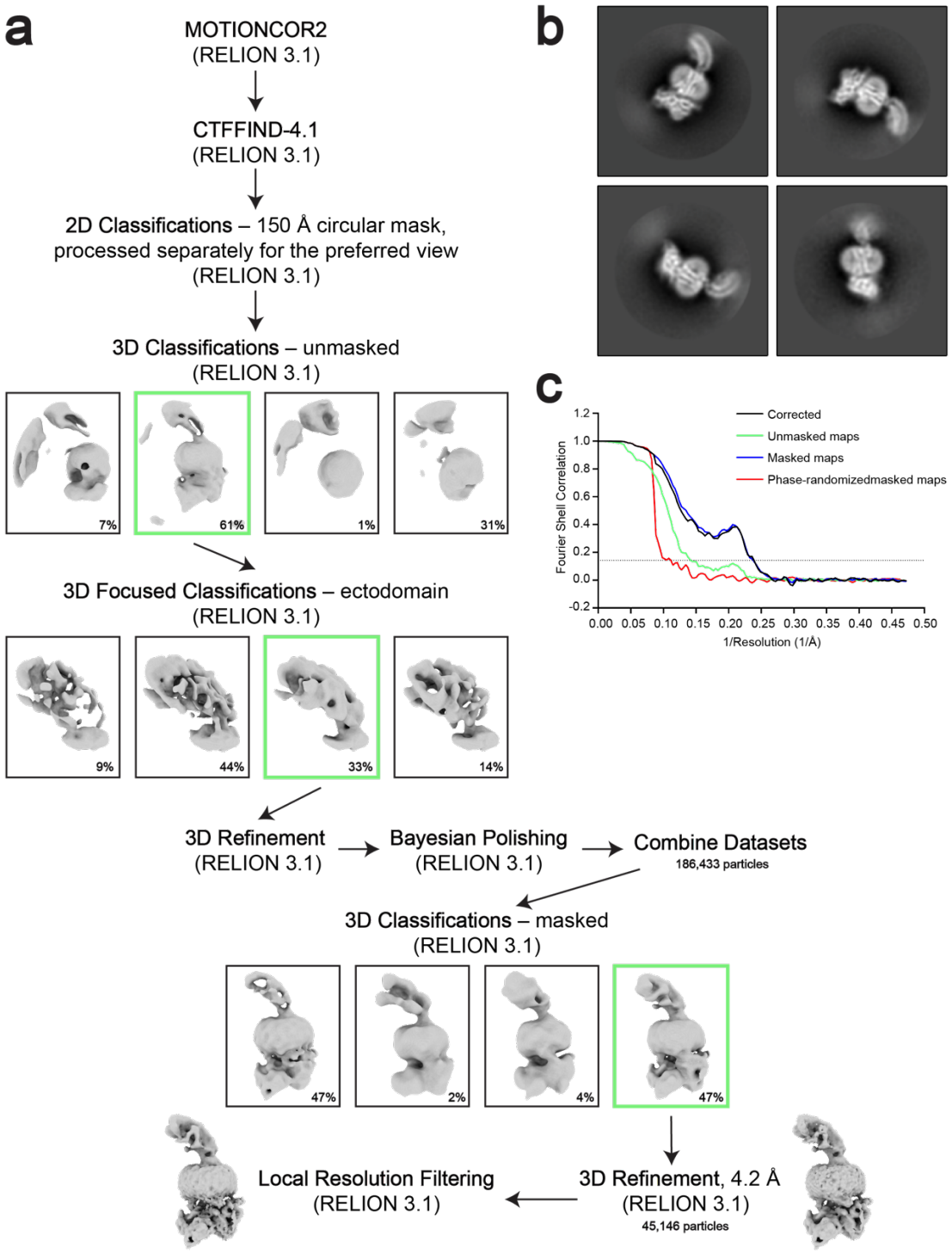

Fig. S3 | Cryo-EM data processing for full-length RXFP1–G_s. a, Cryo-EM data processing scheme for the full-length RXFP1–G_s complex. Shown are representative processing steps for one of four individual datasets and the steps used for the combined datasets. **b**, Two-dimensional class averages for the full-length RXFP1–G_s complex. **c**, FSC used to determine the overall resolution of the map.

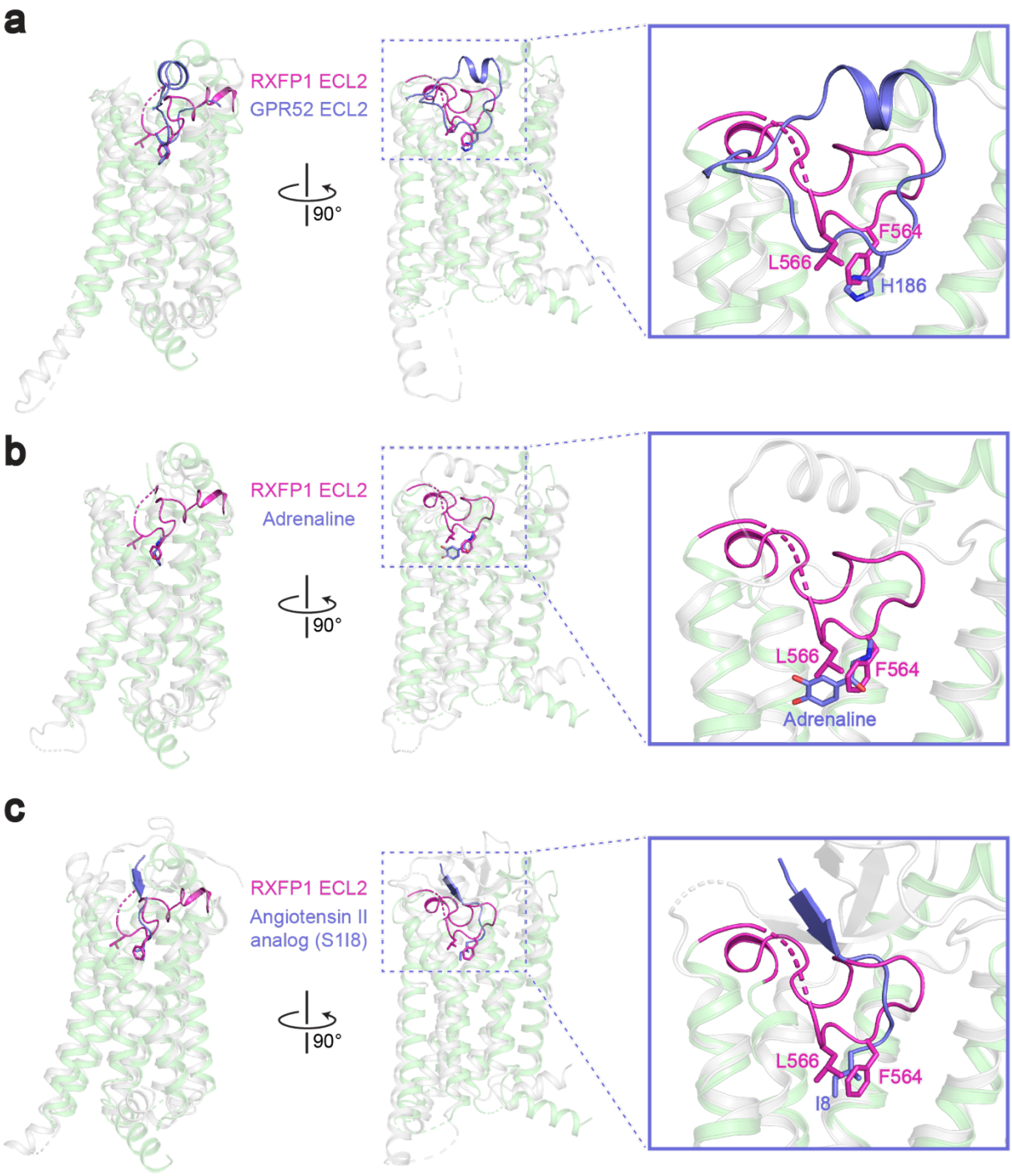

Fig. S4 | Alignments of RXFP1's ECL2 with GPR52 and family A orthosteric agonists. a-c, Alignment of active-state RXFP1 (green, with ECL2 in magenta) with GPR52 (gray with ECL2 in purple; PDB ID: 6LI3)[1] (**a**), the β_2 adrenergic receptor (gray) bound to adrenaline (purple; PDB ID: 4LDO)[2] (**b**), and the angiotensin II type I receptor (gray) bound to the angiotensin II analog S1I8 (purple; PDB ID: 6DO1)[3] (**c**).

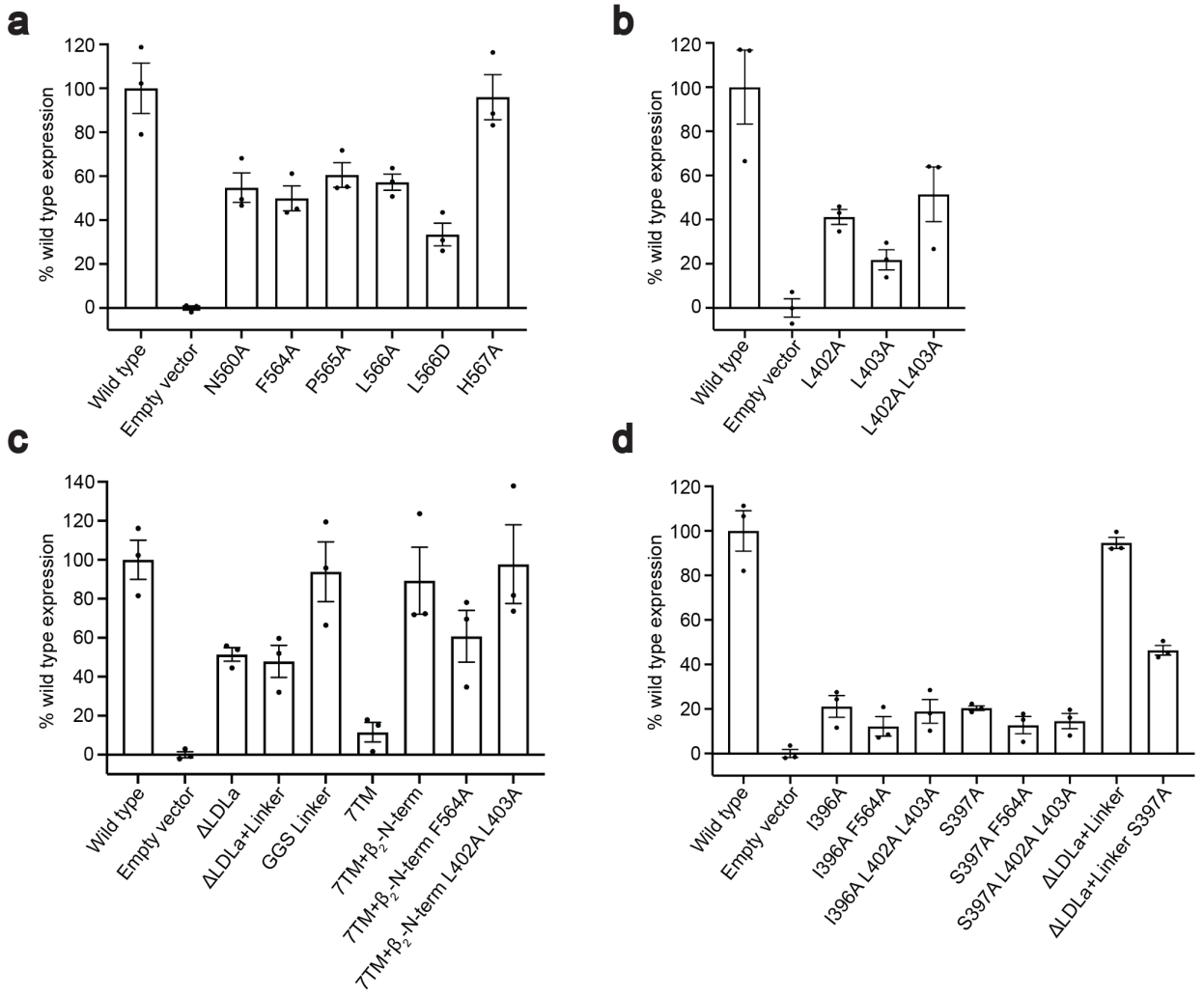

Fig. S5 | Cell surface expression of RXFP1 constructs in Figure 2.5. a-d, Flow cytometry cell surface expression tests with HEK293T cells for RXFP1 ECL2 mutants (**a**), Leu402 and Leu403 hinge region mutants (**b**), ectodomain truncation constructs (**c**), and evolutionary coupling analysis Ile396 and Ser397 hinge mutants (**d**). Data is mean ± s.e.m., n=3 technical replicates.

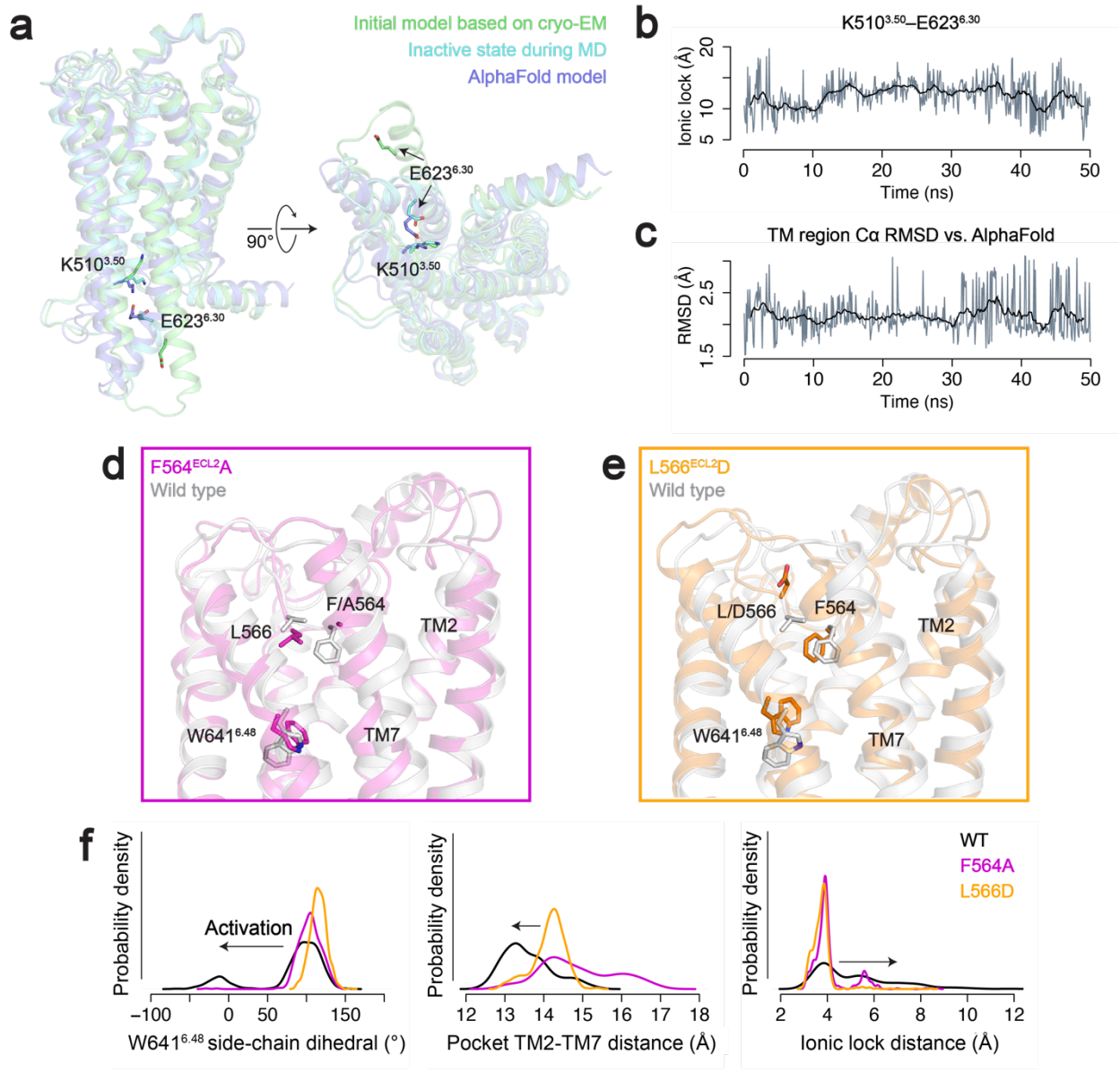

Fig. S6 | Molecular dynamics of RXFP1. a-c, The RXFP1 7TM domain alone is deactivated by adding a sodium in the conserved sodium-binding site. **d-e,** The RXFP1 7TM domain alone shows autoactivation starting from the inactive-state AlphaFold2 model. Autoactivation in these simulations is impaired by the addition of the Phe564^{ECL2} to Ala (**d**) or Leu566^{ECL2} to Asp mutations (**e**). **f**, Histograms describing activation-related differences in transmembrane conformations between WT, F564^{ECL2}A, and L566^{ECL2}D RXFP1 models, including the distance between TM2 and TM7, side-chain flips of the toggle switch residue W641$^{6.48}$, and ionic lock distance.

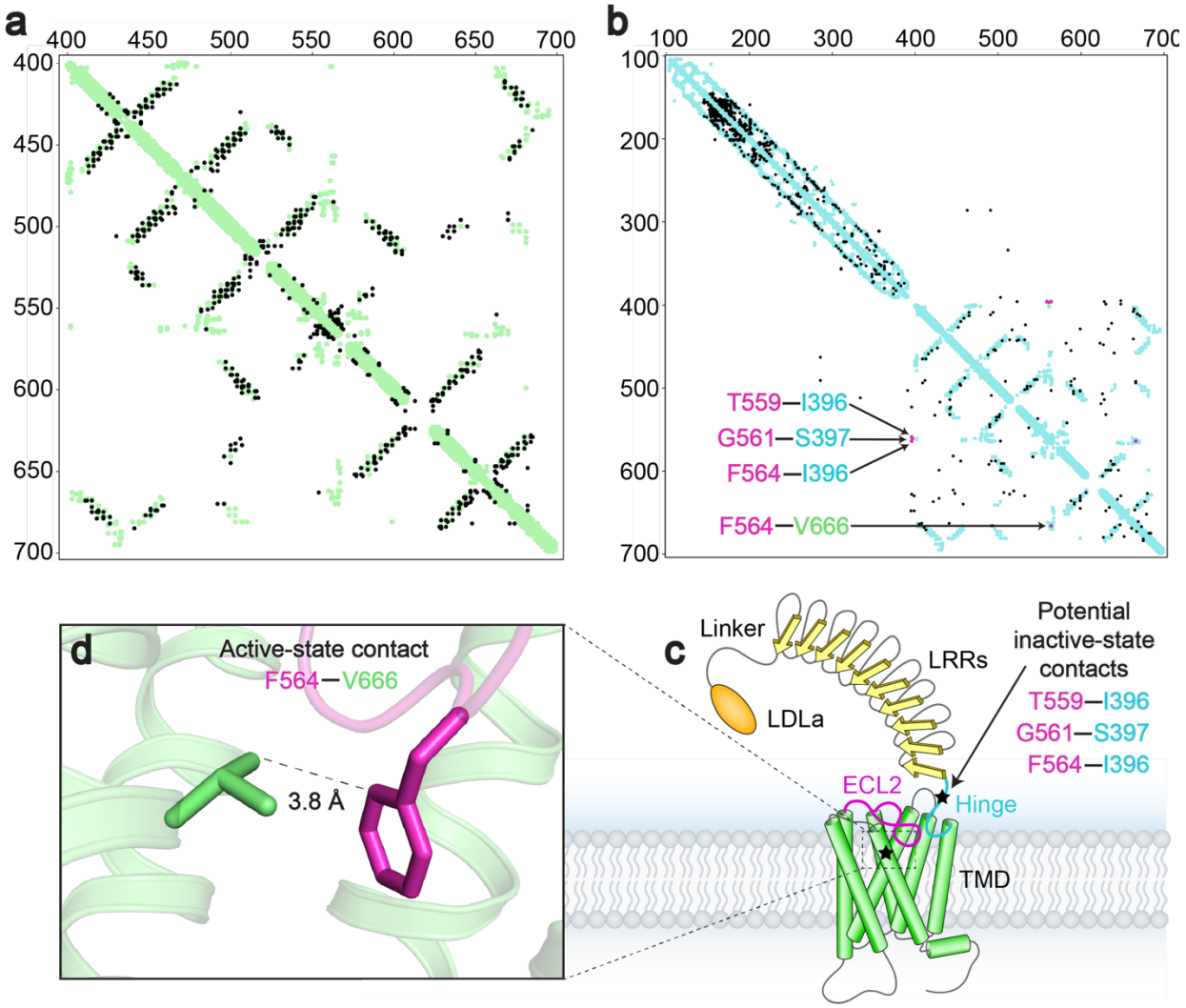

Fig. S7 | Evolutionary coupling analysis of RXFP1. a, Evolutionary couplings for RXFP1 residues 405-689 (black) compared to the active-state structure contacts (green) show close agreement between predicted contacts from ECs and the cryo-EM model. **b**, Evolutionary couplings for RXFP1 residues 120-757 (black) compared to the active-state 7TM structure and LRR AlphaFold2[4] model contacts (blue), highlighting ECL2 evolutionary couplings that provide insight into two potential loop conformations in magenta (T559^{ECL2}–Ile396, Gly561^{ECL2}–Ser397, Phe564^{ECL2}–Ile396, Phe564^{ECL2}–Val666$^{7.38}$). **c**, Diagram of RXFP1 domains. Stars indicate two regions of ECL2 predicted contacts from ECs, TM7 and the hinge region. **d**, The Phe564^{ECL2} and Val666$^{7.38}$ residues from evolutionary coupling analysis are in close contact in the RXFP1 active-state structure.

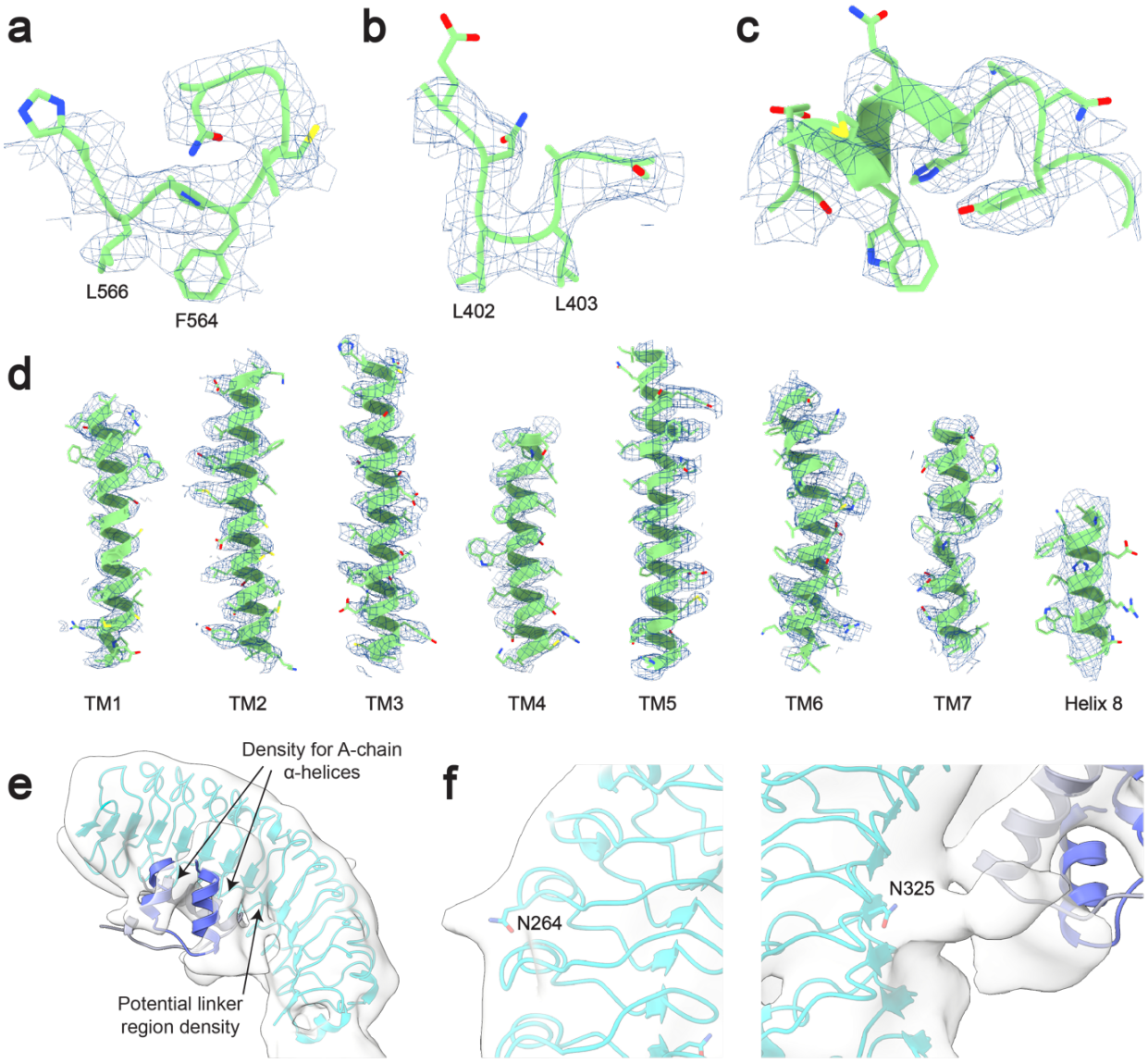

Fig. S8 | Cryo-EM map quality and additional ectodomain map features. a-c, Cryo-EM map and model for ECL2 (**a**), the hinge region (**b**), and ECL1 (**c**). **d**, Cryo-EM map and models for TMs 1-7 and Helix 8. **e**, Potential linker region density next to the relaxin-2 A-chain helices in the low-resolution ectodomain cryo-EM map. **f**, Low-resolution features in the ectodomain cryo-EM map at predicted sites of N-linked glycosylation.

Supplementary tables

Table S1 | Cryo-EM data collection, refinement, and validation statistics.

Abbreviations: 7TM, RXFP1 masking the 7TM domain and G proteins; FL, full-length RXFP1

	RXFP1–G_s-7TM (PDB 7TMW) (EMDB-26003)	**RXFP1–G_s-FL** (EMDB-26004)
Cryo-EM data collection and processing		
Magnification	81,000	81,000
Voltage (kV)	300	300
Electron exposure (e-/ Å^2)	~52	~52
Defocus range (μm)	-0.8 to -2.3	-0.8 to -2.3
Pixel size (Å)	1.06	1.06
Symmetry	C1	C1
Initial particle images (no.)	15,826,542	15,826,542
Final particle images (no.)	188,250	45,146
Map resolution (Å)	3.2	4.2
FSC threshold	(0.143)	(0.143)
Model refinement and validation		
Initial model used (PDB)	Model generated from 6GDG chains B, C, D, and E	
Model resolution (Å)	4.1	
FSC threshold	(0.5)	
Map sharpening *B* factor	DeepEMhancer	
Model composition		
Non-hydrogen atoms	7320	
Protein residues	930	
Ligands	0	
R.m.s. deviations		
Bond lengths (Å)	0.003	
Bond angles (Å)	0.701	
Validation		
MolProbity score	1.62	
Clashscore	9.24	
Poor rotamers (%)	0.00	
Ramachandran plot		
Favored (%)	97.35	
Allowed (%)	2.65	
Disallowed (%)	0.00	

Table S2 | G_S signaling and expression data for RXFP1 constructs in Figure 2c,d.

†Mean ± s.e.m., n=3 technical replicates.

ND, not determined.

Construct	pEC_{50}	E_{max} (%)	Cell surface expression (%)†
Wild type	9.8 ± 0.1	100 ± 2.3	100 ± 12
Empty vector	ND	1 ± 0.1	0 ± 1
N560A	9.3 ± 0.1	92 ± 2.6	55 ± 7
F564A	8.4 ± 0.1	16 ± 0.8	50 ± 6
P565A	8.6 ± 0.1	65 ± 3.6	61 ± 6
L566A	9.0 ± 0.1	77 ± 2.5	57 ± 4
L566D	8.0 ± 0.1	11 ± 0.4	33 ± 5
H567A	9.0 ± 0.05	96 ± 2.3	96 ± 10
Wild type	9.8 ± 0.1	100 ± 3.3	100 ± 17
Empty vector	ND	2 ± 0.1	0 ± 4
L402A	8.8 ± 0.1	32 ± 1.6	41 ± 3
L403A	8.8 ± 0.1	23 ± 0.7	22 ± 5
L402A L403A	ND	1 ± 0.05	51 ± 12

Table S3 | G_s signaling and expression data for RXFP1 constructs in Figure 2e,f.

†Mean ± s.e.m., n=3 technical replicates.

‡Mean ± s.e.m., n=9 technical replicates.

Construct	Basal signaling (%)‡	Relaxin-2 signaling (%)‡	Cell surface expression (%)†
Wild type	8 ± 0.6	100 ± 5.3	100 ± 10
Empty vector	2 ± 0.2	2 ± 0.1	0 ± 2
ΔLDLa	8 ± 0.5	9 ± 0.6	51 ± 3
ΔLDLa+Linker	10 ± 0.6	10 ± 0.7	48 ± 8
GGS Linker	12 ± 0.6	12 ± 0.7	94 ± 15
7TM	11 ± 0.5	10 ± 0.5	12 ± 5
7TM+β_2-Nterm	72 ± 3.2	70 ± 3.1	89 ± 17
7TM+β_2-Nterm F564A	1 ± 0.1	1 ± 0.1	61 ± 13
7TM+β_2-Nterm L402A L403A	6 ± 0.1	6 ± 0.2	98 ± 20
Wild type	9 ± 0.5	100 ± 2.6	100 ± 9
Empty vector	1 ± 0.1	1 ± 0.1	0 ± 2
I396A	21 ± 1	27 ± 0.7	21 ± 5
I396A F564A	1 ± 0.1	1 ± 0.1	12 ± 4
I396A L402A L403A	1 ± 0.1	1 ± 0.1	19 ± 5
S397A	55 ± 2.8	71 ± 2.9	20 ± 1
S397A F564A	1 ± 0.1	2 ± 0.1	13 ± 4
S397A L402A L403A	1 ± 0.1	1 ± 0.1	15 ± 3
ΔLDLa+Linker	13 ± 0.7	14 ± 0.5	95 ± 2
ΔLDLa+Linker S397A	77 ± 2.4	79 ± 1.5	46 ± 2

Table S4 | Binding and expression data for RXFP1 constructs in Figure 2.9e,f.

†Mean ± s.e.m., n=3 technical replicates.

Construct	Fc-relaxin fusion binding (%)†	Cell surface expression (%)†
Wild type	100 ± 8	100 ± 9
Empty vector	42 ± 7	101 ± 4
E206A	0 ± 4	0 ± 0.5

Supplementary references

1. Lin, X. *et al.* Structural basis of ligand recognition and self-activation of orphan GPR52. *Nature* **579**, 152–157 (2020).
2. Ring, A. M. *et al.* Adrenaline-activated structure of β2-adrenoceptor stabilized by an engineered nanobody. *Nature* **502**, 575–579 (2013).
3. Wingler, L. M., McMahon, C., Staus, D. P., Lefkowitz, R. J. & Kruse, A. C. Distinctive activation mechanism for angiotensin receptor revealed by a synthetic nanobody. *Cell* **176**, 479-490.e12 (2019).
4. Tunyasuvunakool, K. *et al.* Highly accurate protein structure prediction for the human proteome. *Nature* **596**, 590–596 (2021).

Table S5 | Construct sequences

Name	Sequence
SE001	MKTIIALSYIFCLVFAHHHHHHDSWMEEVIKLCGRELVRAQIAICGMSTWSDAASSHSHSSARQ LYSALANKCCHVGCTKRSLARFC
SE301	MKTIIALSYIFCLVFADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGSDSWKEEVI KLCGRELVRAQIAICGKSTASDAAGANANAGARQLYSALANKCCHVGCTKRSLARFC
WT RXFP1	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 N560A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGT**A**GVCFPLHSEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS

RXFP1 F564A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVC**A**PLHSEDTESIGAQIYSVA IFLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFL SLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPS FIWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
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RXFP1 H567A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPL**A**SEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 L402A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP

	HVRSCKPNTDGISSLENALASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 L403A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDGISSLENLAASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 ΔLDLa	MKTIIALSYIFCLVFADYKDDDDGDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPV QCLCQGLELDCDETNLRAVPSVSSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSIS IYAFRGLNSLTKLYLSHNRITFLKPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNN VLTRLPDKPLCQHMPRLHWLDLEGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQ KLDELDLGSNKIENLPPLIFKDLKELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQR MFRPLMNLSHIYFKKFQYCGYAPHVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFV ICMRPYIRSENKLYAMSIISLCCADCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGS LAILSTEVSVLLLTFLTLEKYICIVYPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYG TNGVCFPLHSEDTESIGAQIYSVAIFLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMI LAKRFFFIVFTDALCWIPIFVVKFLSLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHR FWYNYRQRKSMDSKGQKTYAPSFIWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 ΔLDLa+Linker	MKTIIALSYIFCLVFADYKDDDDCLVGSVPVQCLCQGLELDCDETNLRAVPSVSSNVTAMSLQ WNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFLKPGVFEDLHR LEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDLEGNHIHNLRN LTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLKELSQLNLSYNP

	IQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAPHVRSCKPNTDG ISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCADCLMGIYLFVIG GFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIVYPFRCVRPGKC RTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAIFLGINLAAFIII VFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLSLLQVEIPGTIT SWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSFIWVEMWPLQ EMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 GGS Linker	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSCLVGSVPVQCLCQGLELDCDETNLRAVPSVS SNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFLK PGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDLE GNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLKE LSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAPH VRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCAD CLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIVY PFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAIF LGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 TMD	MKTIIALSYIFCLVFADYKDDDDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSEN KLYAMSIISLCCADCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVL LLTFLTLEKYICIVYPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSE DTESIGAQIYSVAIFLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTD ALCWIPIFVVKFLSLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKS MDSKGQKTYAPSFIWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 TMD + β_2-Nterm	MKTIIALSYIFCLVFADYKDDDDAMGQPGNGSAFLLAPNRSHAPDHDVTQQRGISSLENLLASII QRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCADCLMGIYLFVIGGFDLKFRGEY NKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIVYPFRCVRPGKCRTITVLILIWIT GFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAIFLGINLAAFIIIVFSYGSMFYSV HQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLSLLQVEIPGTITSWVVIFILPINS ALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSFIWVEMWPLQEMPPELMKPD LFTYPCEMSLISQSTRLNSYS
RXFP1 I396A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC

	GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDG**A**SSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 S397A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLDL EGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDLK ELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYAP HVRSCKPNTDGI**A**SLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCCA DCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICIV YPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSVAI FLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKFLS LLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAPSF IWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1 E206A	MKTIIALSYIFCLVFADYKDDDDQDVKCSLGYFPCGNITKCLPQLLHCNGVDDCGNQADEDNC GDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLELDCDETNLRAVPSV SSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLNSLTKLYLSHNRITFL KPGVFEDLHRLEWLII**A**DNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDKPLCQHMPRLHWLD LEGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLGSNKIENLPPLIFKDL KELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLSHIYFKKFQYCGYA PHVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSENKLYAMSIISLCC ADCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSVLLLTFLTLEKYICI VYPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHSEDTESIGAQIYSV AIFLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFTDALCWIPIFVVKF

	LSLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRKSMDSKGQKTYAP SFIWVEMWPLQEMPPELMKPDLFTYPCEMSLISQSTRLNSYS
RXFP1-miniG$_s$399-20res	MKTIIALSYIFCLVFADYKDDDDGGSLEVLFQGPGGSQDVKCSLGYFPCGNITKCLPQLLHCNG VDDCGNQADEDNCGDNNGWSLQFDKYFASYYKMTSQYPFEAETPECLVGSVPVQCLCQGLE LDCDETNLRAVPSVSSNVTAMSLQWNLIRKLPPDCFKNYHDLQKLYLQNNKITSISIYAFRGLN SLTKLYLSHNRITFLKPGVFEDLHRLEWLIIEDNHLSRISPPTFYGLNSLILLVLMNNVLTRLPDK PLCQHMPRLHWLDLEGNHIHNLRNLTFISCSNLTVLVMRKNKINHLNENTFAPLQKLDELDLG SNKIENLPPLIFKDLKELSQLNLSYNPIQKIQANQFDYLVKLKSLSLEGIEISNIQQRMFRPLMNLS HIYFKKFQYCGYAPHVRSCKPNTDGISSLENLLASIIQRVFVWVVSAVTCFGNIFVICMRPYIRSE NKLYAMSIISLCCADCLMGIYLFVIGGFDLKFRGEYNKHAQLWMESTHCQLVGSLAILSTEVSV LLLTFLTLEKYICIVYPFRCVRPGKCRTITVLILIWITGFIVAFIPLSNKEFFKNYYGTNGVCFPLHS EDTESIGAQIYSVAIFLGINLAAFIIIVFSYGSMFYSVHQSAITATEIRNQVKKEMILAKRFFFIVFT DALCWIPIFVVKFLSLLQVEIPGTITSWVVIFILPINSALNPILYTLTTRPFKEMIHRFWYNYRQRK SMDSKGQKTYAPSFIWVEMWPLQEMPPELMKPDLNSKTEDQRNEEKAQREANKKIEKQLQKD KQVYRATHRLLLLGADNSGKSTIVKQMRIYHGGSGGSGGTSGIFETKFQVDKVNFHMFDVGG QRDERRKWIQCFNDVTAIIFVVDSSDYNRLQEALNLFKSIWNNRWLRTISVILFLNKQDLLAEK VLAGKSKIEDYFPEFARYTTPEDATPEPGEDPRVTRAKYFIRDEFLRISTASGDGRHYCYPHFTC AVDTENARRIFNDCRDIIQRMHLRQYELL
Nb35-His-PrC	MKYLLPTAAAGLLLLAAQPAMAQVQLQESGGGLVQPGGSLRLSCAASGFTFSNYKMNWVRQ APGKGLEWVSDISQSGARISYTGSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCARCPAPF TRDCFDVTSTTYAYRGQGTQVTVSSLEVLFQGPGHHHHHHHHHGSEDQVDPRLIDGK
Gβ	MHHHHHHGSSGSELDQLRQEAEQLKNQIRDARKACADATLSQITNNIDPVGRIQMRTRRTLRG HLAKIYAMHWGTDSRLLVSASQDGKLIIWDSYTTNKVHAIPLRSSWVMTCAYAPSGNYVACG GLDNICSIYNLKTREGNVRVSRELAGHTGYLSCCRFLDDNQIVTSSGDTTCALWDIETGQQTTT FTGHTGDVMSLSLAPDTRLFVSGACDASAKLWDVREGMCRQTFTGHESDINAICFFPNGNAFA TGSDDATCRLFDLRADQELMTYSHDNIICGITSVSFSKSGRLLLAGYDDFNCNVWDALKADRA GVLAGHDNRVSCLGVTDDGMAVATGSWDSFLKIWN
Gγ	MASNNTASIAQARKLVEQLKMEANIDRIKVSKAAADLMAYCEAHAKEDPLLTPVPASENPFRE KKFFCAIL

Legend:

Hemagglutinin signal sequence, His-tag, Human IgG1 Fc N297Q, FLAG tag, Linker residues (not RXFP1 domain)

β_2 adrenergic receptor N-terminus, 3C protease cleavage site, miniG$_s$399, pelB signal sequence, Protein C tag, **Mutations**