SUPPLEMENTARY MATERIAL

Figure S1: Overview of modelling procedure. See Methods for narrative.

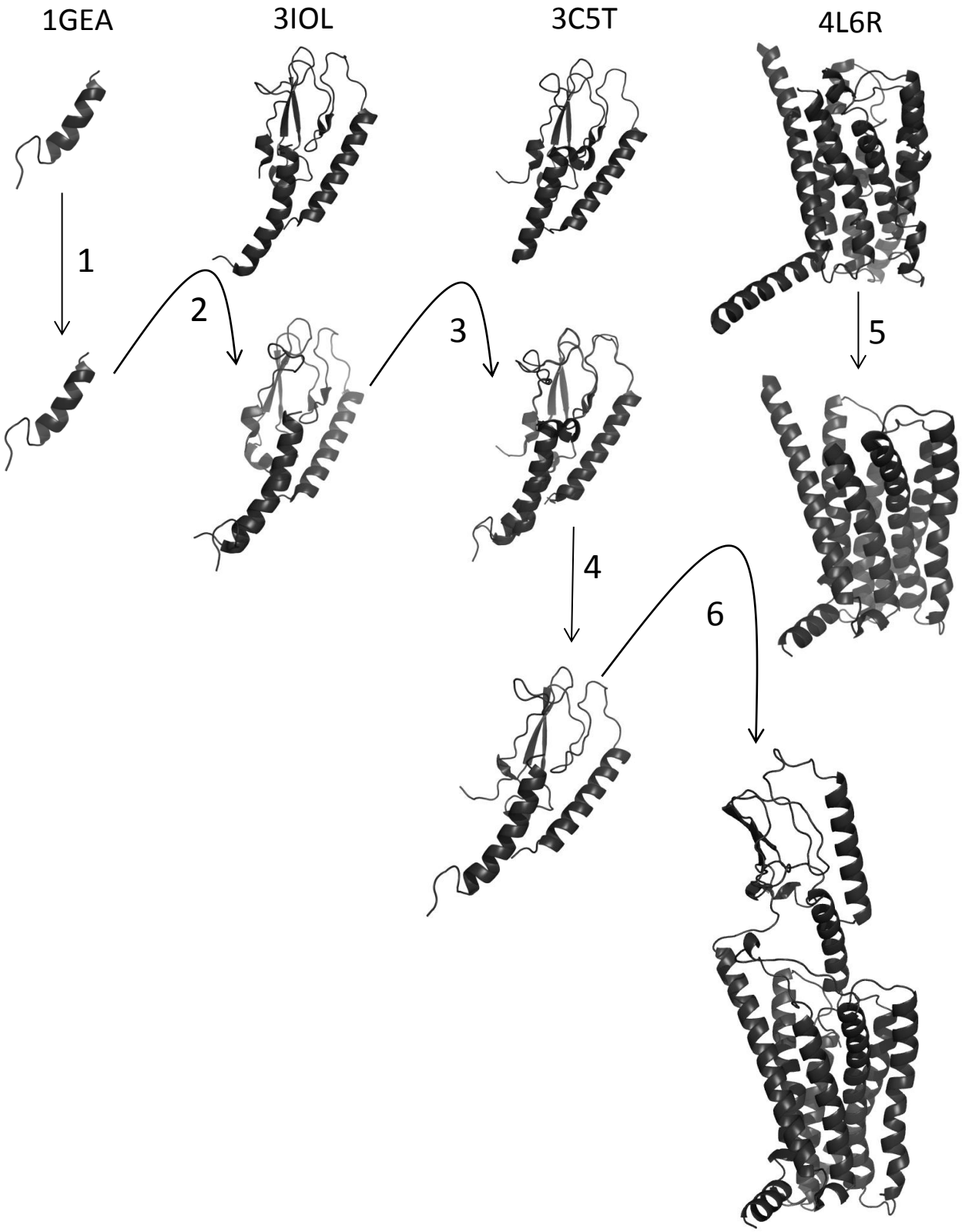

Figure S2: Summary of effects on GLP-1 pharmacology (affinity and ability to activate cAMP pathway) in published site-directed mutagenesis studies focussed on residues in the transmembrane helices and loops of the 7TM domain of GLP-1R. See Table S1 for details.

Key:

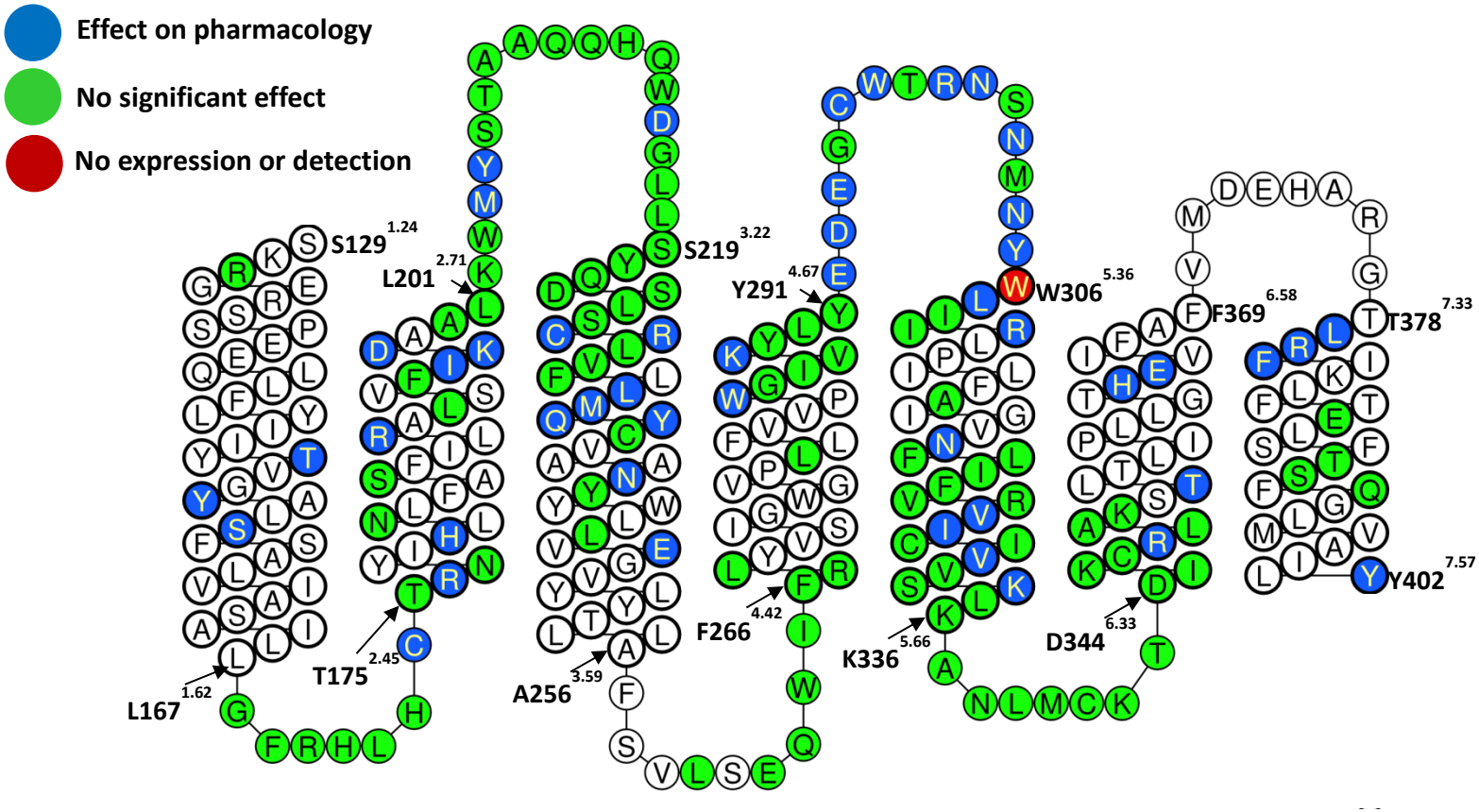

Table S1: Summary of effects on GLP-1 pharmacology (affinity and ability to activate cAMP pathway) in published site-directed mutagenesis studies focussed on residues in the transmembrane helices and loops of the 7TM domain of GLP-1R. **WT** refers to mutations which resulted in <5-fold or no statistically significant change from wild type GLP-1R. **ND** refers to a property that was measured but for which a value could not be reliabley determined. **Blank cells** mean that the assays to estinate that particular pharmacological property were not carried in the cited work. Residues with symbol † refer to data from rat GLP-1R. E_{max} and EC_{50} values from Wootten *et al*. (2013) were a personal communication from Dr Denise Wootten.

Residue	Mutated to…	-fold reduction in affinity	-fold reduction in potency	Comments and/or other effects	Reference source
Arg131$^{1.26}$	Asn	WT	WT		Koole et al. 2011
Thr149$^{1.44}$	Met	60	30		Beinborn et al. 2005
Thr149$^{1.44}$	Met*	250	160	E_{max} = ND	Koole et al. 2011 *For additional residue substiutions, see Koole et al. 2015
Tyr-152$^{1.47}$	Ala	30	ND	low B_{max}	Coopman et al. 2011
Ser-155$^{1.50}$	Ala	WT	10	38% E_{max} ΔLog τ_c = 0.75	Wootten et al. 2013
Gly-168^{ICL1}	Ser	WT	WT		Koole et al. 2011
†Phe-169^{ICL1}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Arg-170^{ICL1}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†His-171^{ICL1}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Leu-172^{ICL1}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†His-173^{ICL1}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Cys-174^{ICL1}	Ala	WT		37% cAMP 10^{-7}M GLP-1 but low B_{max}	Mathi et al. 1997
†Thr-175$^{2.45}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Arg-176$^{2.46}$	Ala	WT	13	26% cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Asn-177$^{2.47}$	Ala	WT		43% cAMP 10^{-7}M GLP-1 but low B_{max}	Mathi et al. 1997
†His-180$^{2.50}$	Arg	21		Authors' quote "50% decrease in cAMP production"	Heller et al. 1996
His-180$^{2.50}$	Ala	ND	12	30% E_{max} ΔLog τ_c = 0.86	Wootten et al. 2013
†Asn-182$^{2.52}$	Ala	WT		low B_{max}	Xiao et al. 2000
Ser-186$^{2.56}$	Ala	WT	WT		Wootten et al. 2013
†Arg-190$^{2.60}$	Ala	>20		27% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
Arg-190$^{2.60}$	Ala	32	270	low B_{max}	Coopman et al. 2011
Arg-190$^{2.60}$	Ala	20	34	56% E_{max} ΔLog τ_c = 0.53	Wootten et al. 2013
Leu-192$^{2.62}$	Ser		WT		Underwood et al. 2011
Phe-195$^{2.65}$	Leu		WT		Underwood et al. 2011
†Ile-196$^{2.66}$	Ser	WT	ND		Moon et al. 2012
Lys-197$^{2.67}$	Ala	28	630		Coopman et al. 2011
†Lys-197$^{2.67}$	Ala	5		25% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†Asp-198$^{2.68}$	Ala	10		20% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†Asp-198$^{2.68}$	Ala	63	44		Lopez & Donnelly 2002
†Asp-198$^{2.68}$	Asn	8			Lopez & Donnelly 2002
Asp-198$^{2.68}$	Ala	43	977		Coopman et al. 2011

Residue	Mutated to…	-fold reduction in affinity	-fold reduction in potency	Comments and/or other effects	Reference source
†Ala200-Leu201	Val, Ala	WT	WT		Lopez et al. 2004
†Lys-202^{ECL1}	Ala	WT		71% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†Lys202-Trp203	Ala, Ala	WT	WT		Lopez et al. 2004
†Met204-Tyr205	Ala, Ala	37	51		Lopez et al. 2004
†Met204-Tyr205	Val, Ala	23	32		Lopez et al. 2004
†Met204-Tyr205	Ala, Val	29	87		Lopez et al. 2004
†Met204^{ECL1}	Ala	WT	WT		Lopez et al. 2004
†Tyr-205^{ECL1}	Ala	WT	WT		Lopez et al. 2004
†Ser206-Thr207	Ala, Ala	WT	WT		Lopez et al. 2004
†Ala208-Ala209	Val, Val	WT	WT		Lopez et al. 2004
†Gln210-Gln211	Ala, Ala	WT	WT		Lopez et al. 2004
†His-212^{ECL1}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†His212-Gln213	Ala, Ala	WT	WT		Lopez et al. 2004
†Asp-215^{ECL1}	Ala	WT		57% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†Trp214-Asp215	Ala, Ala	WT	WT		Lopez et al. 2004
†Gly216-Leu217	Ala, Ala	WT	WT		Lopez et al. 2004
†Leu218-Ser219	Ala, Ala	WT	WT		Lopez et al. 2004
†Tyr220-Gln221	Ala, Ala	WT	WT		Lopez et al. 2004
†Asp-222$^{3.25}$	Ala	WT		82% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†Asp222-Ser223	Ala, Ala	WT	WT		Lopez et al. 2004
†Leu224-Gly225	Ala, Ala	WT	WT		Lopez et al. 2004
†Cys-226$^{3.29}$ *	Ala	25	38		Mann et al. 2010 *also included in double mutations
Cys-226$^{3.29}$	Ala		>90		Underwood et al. 2013
†Arg-227$^{3.30}$	Ala	>20		90% cAMP 10^{-7}M GLP-1	Xiao et al. 2000
†Arg227-Leu228	Ala, Ala	WT	WT		Lopez et al. 2004
†Val229-Phe230	Ala, Ala	WT	WT		Lopez et al. 2004
†Leu232-Met233	Val, Thr	10	100		Moon et al. 2012
Gln-234$^{3.37}$	Ala	13	45		Coopman et al. 2011
Tyr-235$^{3.38}$	Ala	24	23		Coopman et al. 2011
Cys-236$^{3.39}$	Ala		WT		Underwood et al. 2013
†Asn-240$^{3.43}$	Ala	>20		8% cAMP 10^{-7}M GLP-1 but low B_{max}	Xiao et al. 2000
Asn-240$^{3.43}$	Ala	WT	7	78% E_{max} ΔLog τ_c = 0.67	Wootten et al. 2013
Tyr-241$^{3.44}$	Ala		WT		Underwood et al. 2011
Glu-247$^{3.50}$	Ala	ND	14	19% E_{max} ΔLog τ_c = 0.99	Wootten et al. 2013
Phe-260^{ICL2}	Leu	WT	WT		Koole et al. 2011
†Glu-262^{ICL2}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Gln-263^{ICL2}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Arg-264^{ICL2}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Ile-265^{ICL2}	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Phe-266$^{4.42}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Lys-267$^{4.43}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Leu-268$^{4.44}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
Leu-278$^{4.54}$	Met		WT		Underwood et al. 2011
Trp-284$^{4.60}$	Ala	32	1349		Coopman et al. 2011
Gly-285$^{4.61}$	Ala	WT	WT		Koole et al. 2012
Ile-286$^{4.62}$	Ala	WT	WT		Koole et al. 2012

Residue	Mutated to…	-fold reduction in affinity	-fold reduction in potency	Comments and/or other effects	Reference source
Val-287$^{4.63}$	Ala	WT	WT		Koole et al. 2012
Lys-288$^{4.64}$	Ala	126	ND		Koole et al. 2012
†Lys-288$^{4.64}$	Ala	79	251		Al-Sabah & Donnelly 2003
†Lys-288$^{4.64}$	Leu	63	79		Al-Sabah & Donnelly 2003
†Lys-288$^{4.64}$	Arg	WT	WT		Al-Sabah & Donnelly 2003
Tyr-289$^{4.65}$	Ala	WT	WT		Koole et al. 2012
Leu-290$^{4.66}$	Ala	WT	WT		Koole et al. 2012
Tyr-291$^{4.67}$	Ala	WT	WT		Koole et al. 2012
†Leu290-Tyr291	Ala, Ala	WT	WT		Mann et al. 2010
Glu-292^{ECL2}	Ala	100	126	ΔLog τ_c = 0.57	Koole et al. 2012
Asp-293^{ECL2}	Ala	25	16		Koole et al. 2012
†Glu292-Asp293	Ala, Ala	8	79		Mann et al. 2010
Glu-294^{ECL2}	Ala	WT	WT	ΔLog τ_c = 0.65	Koole et al. 2012
Gly-295^{ECL2}	Ala	WT	WT		Koole et al. 2012
†Glu294-Gly295	Ala, Ala	WT	WT		Mann et al. 2010
†Cys-296^{ECL2} *	Ala	18	WT		Mann et al. 2010 *also included in double mutations
Cys-296^{ECL2}	Ala	13	126		Koole et al. 2012
Trp-297^{ECL2}	Ala	63	316	ΔLog τ_c = 1.00	Koole et al. 2012
Thr-298^{ECL2}	Ala	WT	WT		Koole et al. 2012
†Trp297-Thr298	Ala, Ala	100	50		Mann et al. 2010 Donnelly 2012
Arg-299^{ECL2}	Ala	32	85	ΔLog τ_c = 0.46	Koole et al. 2012
Asn-300^{ECL2}	Ala	126	501	ΔLog τ_c = 0.80	Koole et al. 2012
†Arg299-Asn300	Ala, Ala	251	>3000		Mann et al. 2010 Donnelly 2012
Ser-301^{ECL2}	Ala	WT	WT		Koole et al. 2012
Asn-302^{ECL2}	Ala	25	16	ΔLog τ_c = 0.53	Koole et al. 2012
†Ser301-Asn302	Ala, Ala	WT	WT		Mann et al. 2010
Met-303^{ECL2}	Ala	WT	WT		Koole et al. 2012
Asn-304^{ECL2}	Ala	WT	WT	ΔLog τ_c = 0.74	Koole et al. 2012
†Met303-Asn304	Ala, Ala	WT	WT		Mann et al. 2010
Tyr-305$^{5.35}$	Ala	79	40		Koole et al. 2012
Trp-306$^{5.36}$	Ala	ND	ND	No receptor expression	Koole et al. 2012
†Tyr305-Trp306	Ala, Ala	316	50		Mann et al. 2010 Donnelly 2012
Leu-307$^{5.37}$	Ala	13	25	ΔLog τ_c = 0.49	Koole et al. 2012
†Leu307-Ile308	Ala, Ala	251	6		Mann et al. 2010 Donnelly 2012
Arg-310$^{5.40}$	Ala	10	1259		Coopman et al. 2011
†Ile309-Arg310	Ala, Ala	50	>3000		Mann et al. 2010 Donnelly 2012
Ala-316$^{5.46}$	Thr	WT	WT		Koole et al. 2011
Asn-320$^{5.50}$	Ala	18	10	ΔLog τ_c = 0.50	Wootten et al. 2013
†Phe-321$^{5.51}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Leu-322$^{5.52}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Ile-323$^{5.53}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Phe-324$^{5.54}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Ile-325$^{5.55}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Phe-326$^{5.56}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997

Residue	Mutated to…	-fold reduction in affinity	-fold reduction in potency	Comments and/or other effects	Reference source
†Val-327$^{5.57}$	Ala	WT	15		Mathi et al. 1997
†Ile-328$^{5.58}$	Ala	WT	9		Mathi et al. 1997
†Cys-329$^{5.59}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Ile-330$^{5.60}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Val-331$^{5.61}$	Ala	WT	14		Mathi et al. 1997
†Ile-332$^{5.62}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Ala-333$^{5.63}$	Leu	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
Ser-333$^{5.63}$	Cys	WT	WT		Koole et al. 2011 *For additional residue substiutions, see Koole et al. 2015
†Lys-334$^{5.64}$	Ala	WT		28% cAMP 10^{-7}M GLP-1	Mathi et al. 1997 Takar et al. 1996
†Leu-335$^{5.65}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
†Lys-336$^{5.66}$	Leu	WT		WT cAMP 10^{-7}M GLP-1	Mathi et al. 1997
Lys334-Lys351	Deletions	WT		See paper for etail	Takar et al. 1996
Cys-347$^{6.36}$	Ala		WT		Underwood et al. 2013
†Arg-348$^{6.37}$	Gly	12	ND		Heller et al. 1996
†Arg-348$^{6.37}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Takar et al. 1996
†Leu-349$^{6.38}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Takar et al. 1996
†Ala-350$^{6.39}$	Glu	ND		Low B_{max}	Takar et al. 1996
†Ala-350$^{6.39}$	Lys	WT		Low B_{max}	Takar et al. 1996
†Lys-351$^{6.40}$	Ala	WT		WT cAMP 10^{-7}M GLP-1	Takar et al. 1996
Thr-353$^{6.42}$	Ala	ND	22	42% E_{max} ΔLog τ_c = 0.84	Wootten et al. 2013
His-363$^{6.52}$	Ala	98	ND		Coopman et al. 2011
His-363$^{6.52}$	Ala	23	4	17% E_{max} ΔLog τ_c = 1.71	Wootten et al. 2013
Glu-364$^{6.53}$	Ala	58	15		Coopman et al. 2011
Leu-379$^{7.33}$	Arg	12	141		Moon et al. 2015
Leu-379$^{7.33}$	Glu	11	165		Moon et al. 2015
Arg-380$^{7.34}$	Asp	21	1853		Moon et al. 2015
Arg-380$^{7.34}$	Gly	4	40		Moon et al. 2015
Phe-381$^{7.35}$	Arg	WT	WT		Moon et al. 2015
Phe-381$^{7.35}$	Glu	>200	234		Moon et al. 2015
Glu-387$^{7.41}$	Ala	WT	WT		Coopman et al. 2011
Thr-391$^{7.45}$	Ala	WT	WT		Coopman et al. 2011
Thr-391$^{7.45}$	Ala		WT		Underwood et al. 2011
Ser-392$^{7.46}$	Ala	WT	WT		Wootten et al. 2013
Gln-394$^{7.49}$	Ala	WT	WT	ΔLog τ_c = 0.36	Wootten et al. 2013
Tyr-402$^{7.57}$	Ala	ND	10	10% E_{max} ΔLog τ_c = 1.59	Wootten et al. 2013

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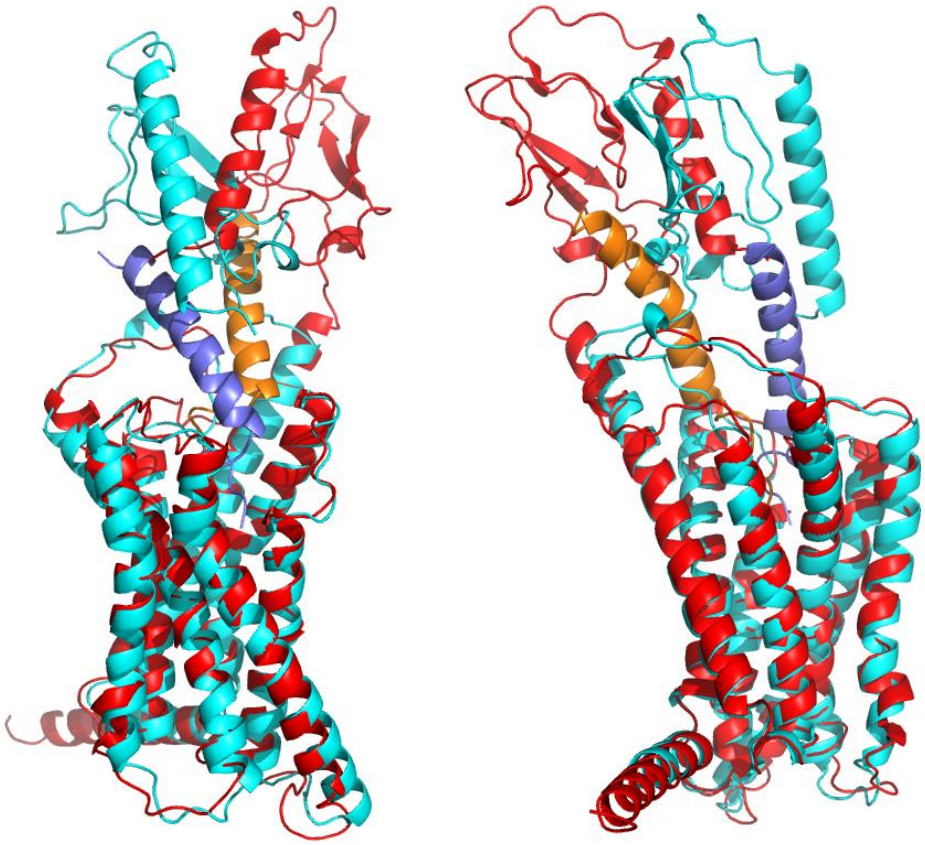
Table S2: Pharmacological properties of wild-type and ECL2/TM5 interface double mutant receptors.

Mutant	GLP-1 (7-36)-NH_2 pEC_{50}	Specific Binding (%)
Wild type GLP-1R	**10.81 ± 0.29**	**100**
IV 286 287 AA	10.94 ± 0.10	84.6 ± 2.0
KY 288 289 AA	**7.47 ± 0.43 (2188)****	**28.3 ± 2.5*****
LY 290 291 AA	11.33 ± 0.36	99.0 ± 0.1
ED 292 293 AA	9.41 ± 0.24 (25)*	75.3 ± 4.0
EG 294 295 AA	10.86 ± 0.51	97.2 ±0.2
WT 297 298 AA	9.47 ± 0.26 (22)*	57.1 ±8.6**
RN 299 300 AA	**8.29 ± 0.36 (331)****	**41.1 ± 6.2****
SN 301 302 AA	11.06 ± 0.28	85.8 ± 2.5
MN 303 304 AA	11.49 ± 0.16	90.8 ± 1.1
YW 305 306 AA	**8.39 ± 0.25 (263)****	**60.1 ± 1.5*****
L307A	10.67 ± 0.11	87.2 ± 1.9
I308A	10.47 ± 0.06	77.7 ± 1.1
IR 309 310 AA	**6.68 ± 0.33 (13490)*****	**13.4 ± 5.9*****
LP 311 312 AA	10.19 ± 0.20	90.7 ± 0.4

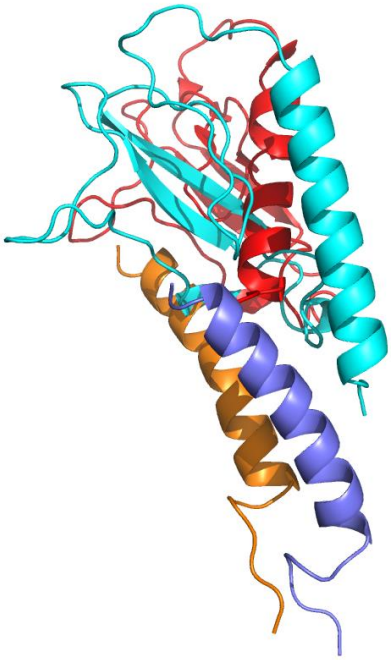
Following observed differences between the published pharmacological properties of ECL2 mutants in the rat [**17, 29**] and human GLP-1Rs [**30**], our previous double mutant rat GLP-1R screen was repeated, and slightly extended, in human GLP-1R as shown in the table. As we were initially unable to generate the LI 307 308 AA double mutant via QuikChange, we made the individual single mutants directly. The most interesting residues were identified using a low resolution pharmacological screen: pEC_{50} values were obtained from a 3-parameter logistic analysis of LANCE cAMP assays, with the fold difference in potency compared with the wild type hGLP-1R shown in brackets; effects upon affinity were estimated by calculating specific binding, as a percentage of total binding, using ^{125}I-GLP-1 (7-36) at 75 pM against non-radiolabelled GLP-1 at 4 x 10^{-6} M. Values illustrate the mean ± SEM, significantly different from wild-type value, *p<0.05, **p<0.01, ***p<0.001. The profile of ECL2 matched that seen in rat GLP-1R, with residue pairs 292/293, 297/298, 299/300, 305/306 and 309/310 having lower potency. An additional double mutation, KY 288 289 AA, displayed reduced potency and specific binding. Over all, there were four ECL2 double mutants (KY 288 289 AA; RN 299 300 AA; YW 305 306 AA; and IR 309 310 AA) with a particularly large and significant decrease in both potency and specific binding compared with wild-type, and were therefore mutated individually and analysed in more detail (see body of main paper). The ED 292 293 AA and WT 297 298 AA mutations were not pursued further in this study, despite some affect upon the pharmacology. Glu292, Asp293 and Trp306 have been shown to be important in GLP-1 pharmacology [**30**].

Figure S3: Comparison of GLP-1 model described this paper (light blue receptor, purple ligand) with the glucagon receptor model of Siu *et al.* (2013) (red receptor, orange ligand). **A** Two perpendicular views of the models superimposed via their TM domains to highlight the different relative orientations of the NTD and ligand. In the Siu *et al* model, the helix of the ligand is much closer to the stalk region, while in our model it is translated towards TM5 and TM6. **B** The same structural alignment as **A,** but with the TM domains removed to show the N-terminus of ligands. The different conformations mean that they would interact with different residues in the TMD. **C** The aligned ligands, showing the PACAP21-based conformation in purple and the more extended conformation of Siu *et al.* in orange.

A



B



C

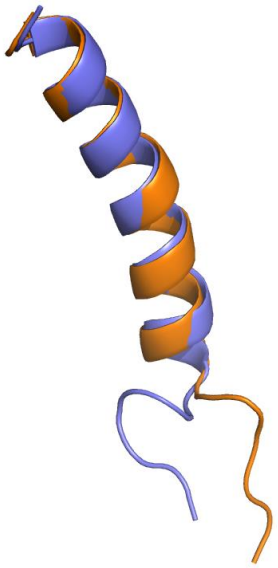
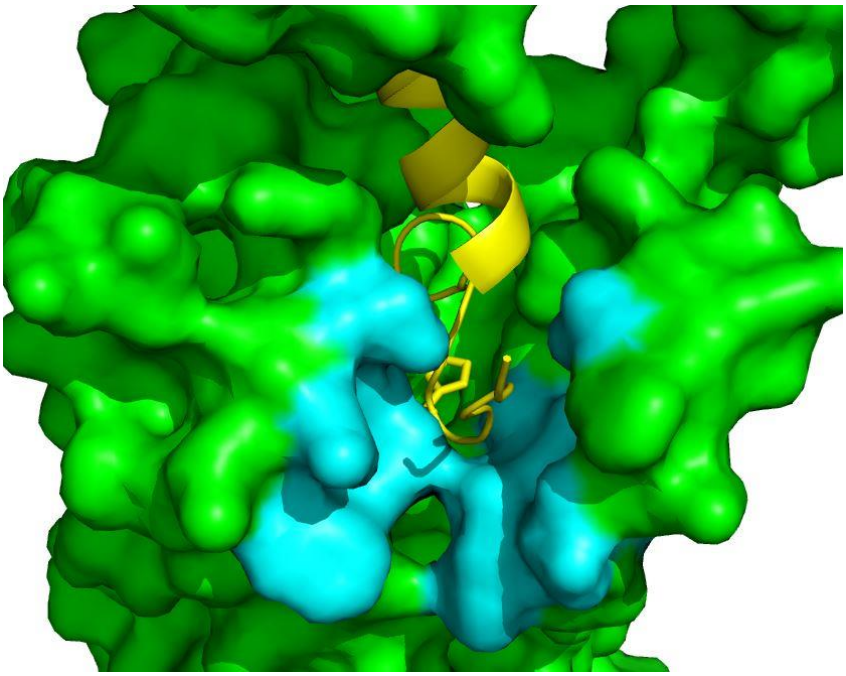


Figure S4: Hypothetical binding of GLP-1(1-36) to GLP-1R to explain how the additional 6 N-terminal resiudes can be accomodated in the binding cavity. The ligand is shown in cartoon form in yellow, with the receptor surface shown in green or cyan (residues lining the "residual pocket"). **A** View from above, showing the side chain of His-7* at the base of the cavity, with the residues 1*-6* coming towards the page and making use of the residual pocket and gap between TM5/ECL2 (left) and TM6/ECL3 (right). **B** View from side.

A



B

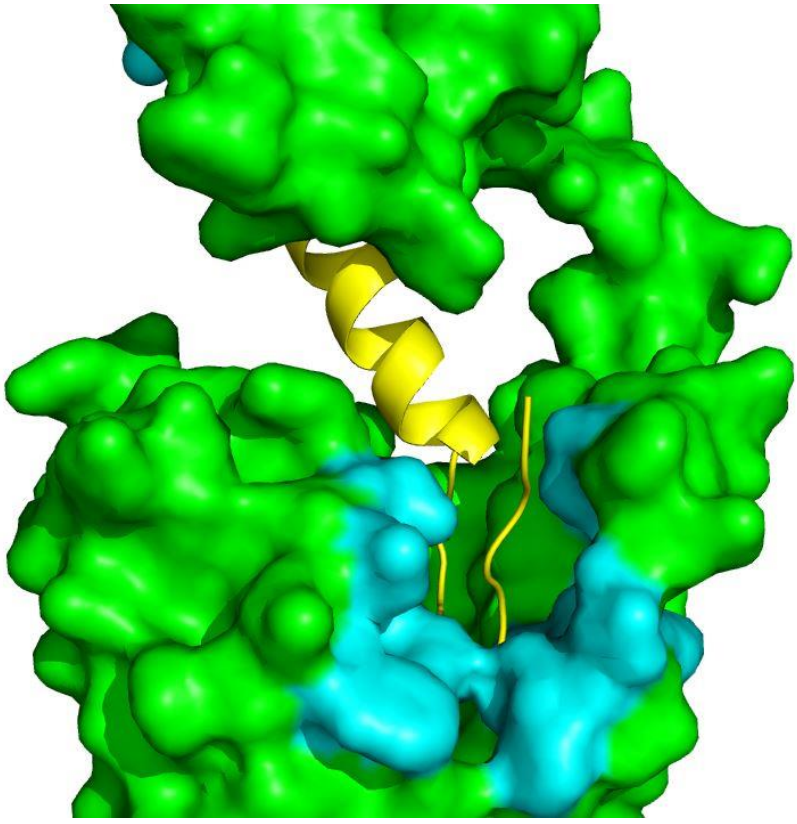


Figure S5: Schematic of hypothetical conformational states involving domain movement and ligand binding (see Discussion).

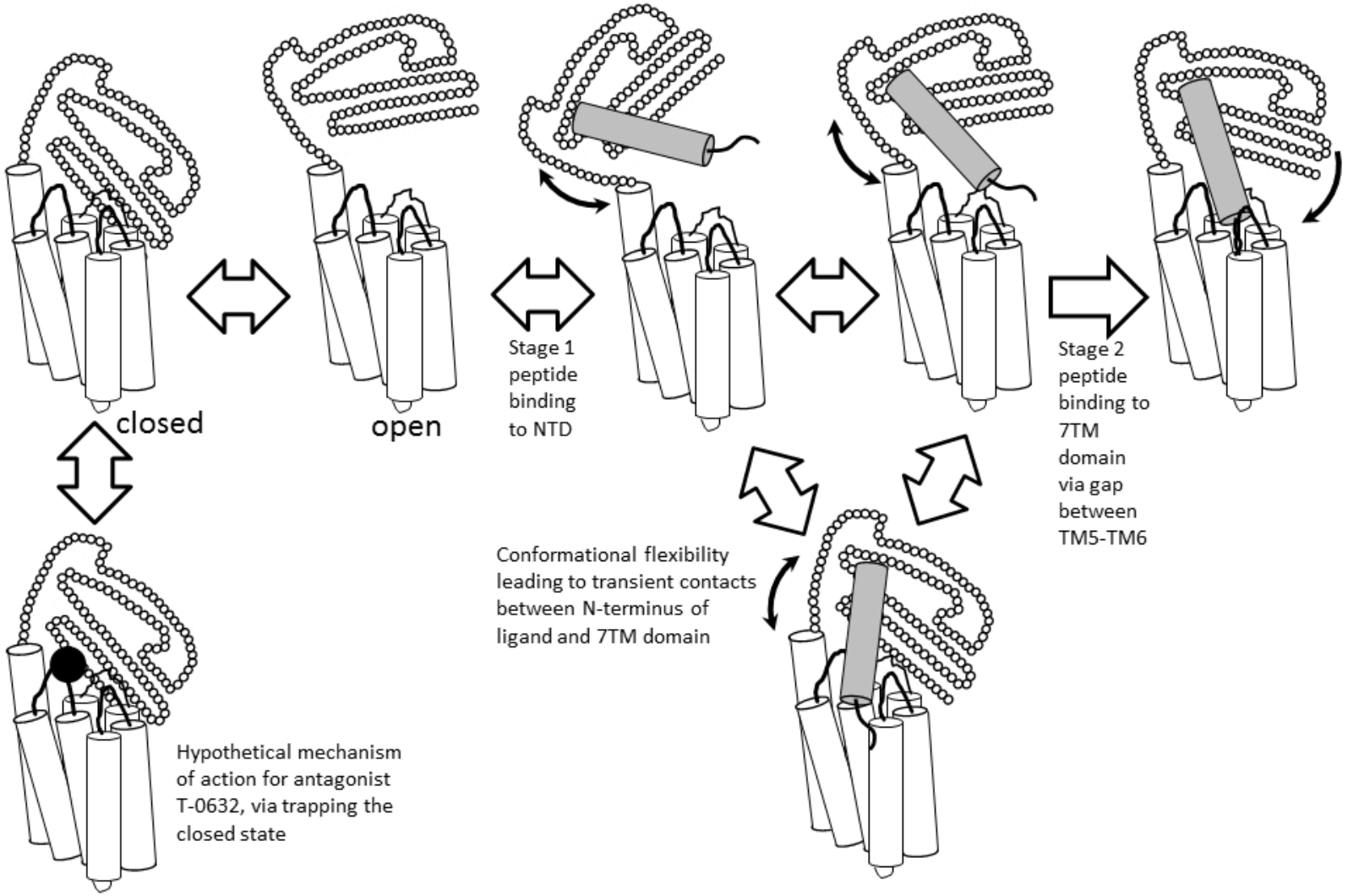


Figure S6: A-C Surface representations of glucagon receptor 4L6R (**A**), CRF1 receptor 4K5Y (**B**) and the model of GLP-1R (**C**). A gap can been seen between TM5 (green) and TM6 (orange) and between ECL2 (red) and ECL3 (cyan) which could allow entry of the N-terminal region of the ligand (purple) into the binding pocket from the side.

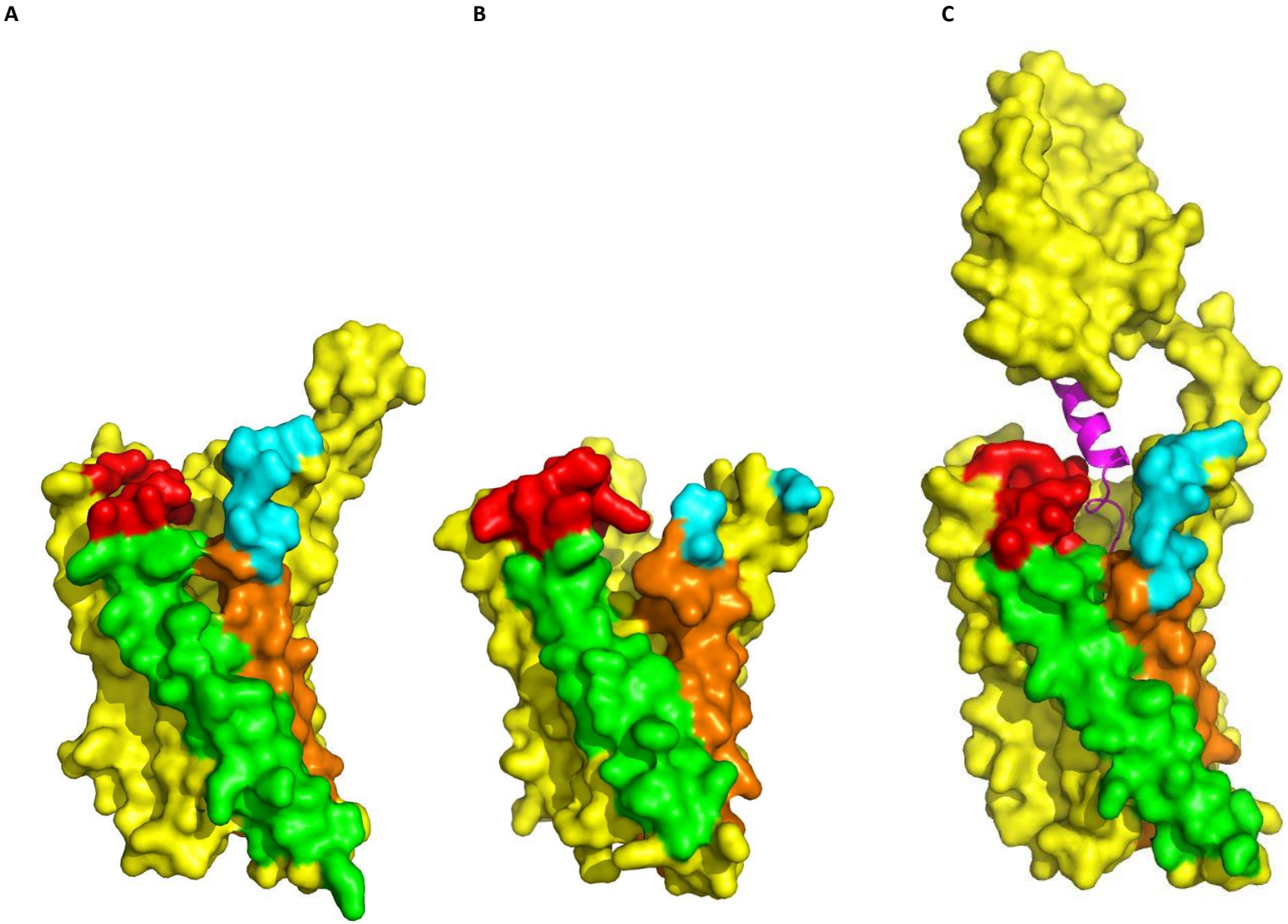


Figure S7: A-C The 7TM domain of the glucagon receptor 4L6R (green) with residues 5.44, 6.53, 6.56 and 6.57 mutated to Cys and shown as spheres (**A**- side view; **B**. top view; **C** top view with binding cavity as surface. In PTH_1, all four residues (mutated individually [49]) could be disulphide linked to an analogue of PTH with Cys at residue position 1. While the three TM6 residues line the binding cavity and would be expected to be accessible to the N-teminus of the ligand, residue 5.44 is lipid facing and hence more difficult to explain. However, entry of the ligand's N-terminus via the TM5-TM6 gap (Figure S6) would allow transient contact between the cysteine residues and disulphide formation.

A **B** **C**