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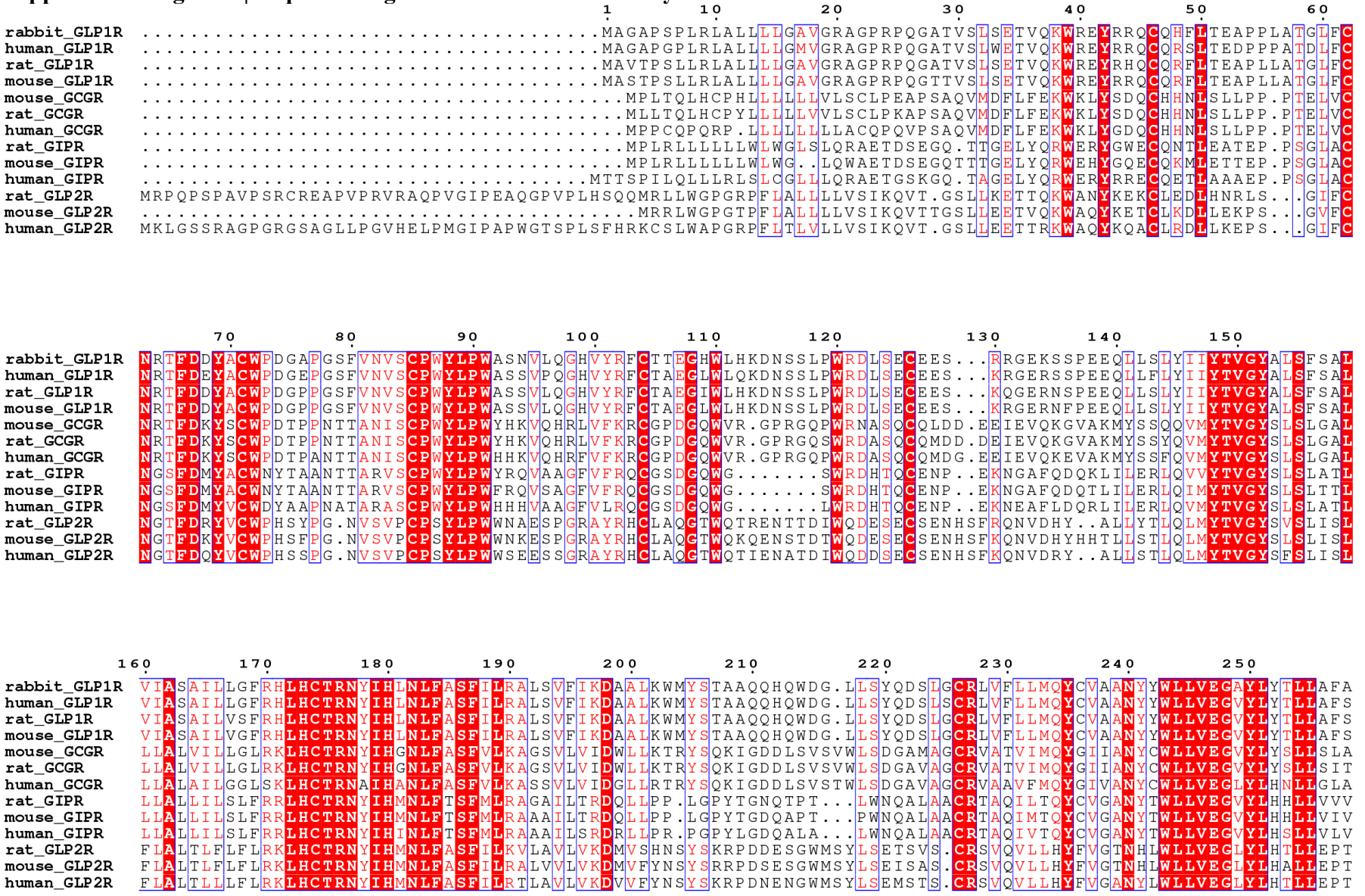
Supplementary Table 1 | Observed intermolecular interactions within the GLP-1:GLP-1R TMD interface. Superscripts refer to the Wootten residue numbering system in which the single most conserved residue among class B GPCRs is designated X.50, where X is the transmembrane helix number. References regarding mutagenesis studies for each residue are listed.

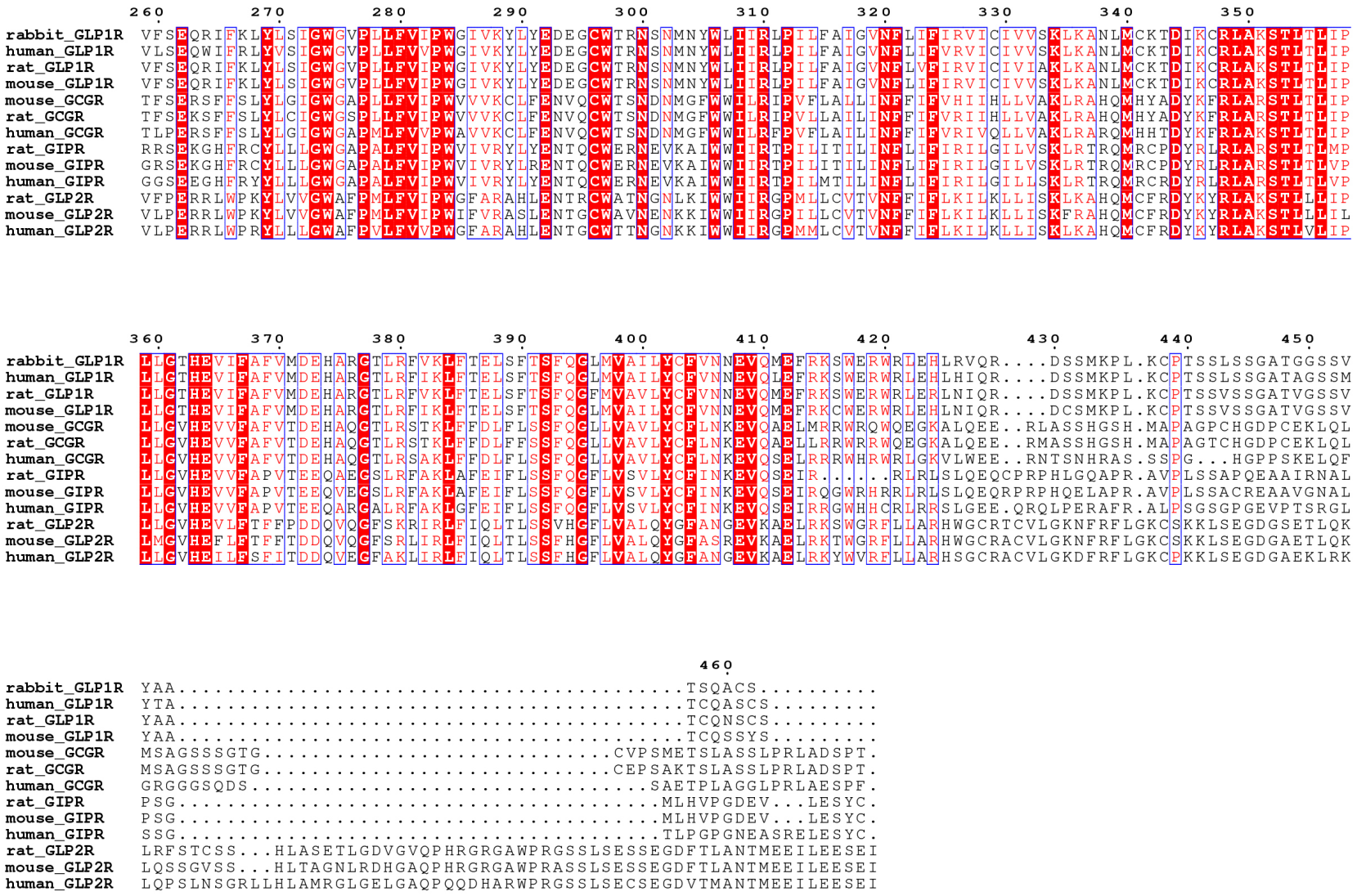
Residue in GLP-1	Residue in GLP-1R	Reference
His7	R299^{ECL2}	1-3
	W306$^{5.36b}$	1-3
Glu9	Y145$^{1.40b}$	3,4
	R190$^{2.60b}$	4-8
	L388$^{7.43b}$	4,9,10
	S392$^{7.47b}$	6,10,11
Thr11	R299^{ECL2}	1-3
Phe12	Y145$^{1.40b}$	3,4
	F385$^{7.40b}$	2,9,11,12
Thr13	K197$^{2.67b}$	5,7,8,13-15
Ser14, Ser17, Ser18 (Serine Cluster)	W297 ^{ECL2}	1,3
	T298 ^{ECL2}	1
	R299 ^{ECL2}	1-3
Leu20	L201$^{2.71b}$	9,16
	M204$^{2.74b}$	4
Trp31	Q211 ^{ECL1}	4
	H212 ^{ECL1}	4

Supplementary Table 2 | Data collection and refinement statistics.

Data collection/processing	
Voltage (kV)	300
Magnification	50,000
Defocus (μm)	-1.5 to -3.0
Pixel size (Å)	1.0
Total electron dose (e^-/Å^2)	90
Exposure time (s)	10
Number of images	17,332
Number of frames per image	50
Initial particle number	2,675,742
Particle number for 3D classification	620,626
Final particle number	139299
Resolution (core/global) (Å)	3.9/4.1
Map sharpened B-factor (Å^2)	-350
Model refinement	
Non-hydrogen atoms	9099
R. M. S. Deviations	
Bond length (Å)	0.008
Bond angle (^{o})	1.099
Ramachandran plot	
Favored (%)	5.3
Allowed (%)	4.4
Outlier (%)	0.3
Rotamer outliers (%)	0.2

Supplemental Figure 1 | Sequence alignment of GCGR subfamily GPCRs.




260 270 280 290 300 310 320 330 340 350
rabbit_GLP1R VFSEQRIFKLYLSIGWGVPLLFVIPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFIRVICIVVSKLKANLMCKTDIKCRLAKSTLTLIP
human_GLP1R VLSEQWIFRLYVSIGWGVPLLFVVPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLAKSTLTLIP
rat_GLP1R VFSEQRIFKLYLSIGWGVPLLFVIPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLVFIRVICIVIAKLKANLMCKTDIKCRLAKSTLTLIP
mouse_GLP1R VFSEQRIFKLYLSIGWGVPLLFVIPWGIVKYLYEDEGCWTRNSNMNYWLIIRLPILFAIGVNFLIFIRVICIVVSKLKANLMCKTDIKCRLAKSTLTLIP
mouse_GCGR TFSERSFFSLYLGIGWGAPLLFVIPWVVVKCLFENVQCWTSNDNMGFWWILRIPVFLALLINFFIFVHIIHLLVAKLRAHQMHYADYKFRLARSTLTLIP
rat_GCGR TFSEKSFFSLYLCIGWGSPLLFVIPWVVVKCLFENVQCWTSNDNMGFWWILRIPVLLALILINFFIFVRIIHLLVAKLRAHQMHYADYKFRLARSTLTLIP
human_GCGR TLPERSFFSLYLGIGWGAPMLFVVPWAVVKCLFENVQCWTSNDNMGFWWILRFPVFLAILINFFIFVRIVQLLVAKLRARQMHHTDYKFRLAKSTLTLIP
rat_GIPR RRSEKGHFRCYLLLGWGAPALFVIPWVIVRYLYENTQCWERNEVKAIWWIIRTPILITILINFLIFIRILGILVSKLRTRQMRCPDYRLRLARSTLTLMP
mouse_GIPR GRSEKGHFRCYLLLGWGAPALFVIPWVIVRYLRENTQCWERNEVKAIWWIIRTPILITILINFLIFIRILGILVSKLRTRQMRCPDYRLRLARSTLTLVP
human_GIPR GGSEEGHFRYYLLLGWGAPALFVIPWVIVRYLYENTQCWERNEVKAIWWIIRTPILMTILINFLIFIRILGILLSKLRTRQMRCRDYRLRLARSTLTLVP
rat_GLP2R VFPERRLWPKYLVVGWAFPMLFVIPWGFARAHLENTRCWATNGNLKIWWIIRGPMLLCVTVNFFIFLKILKLLISKLKAHQMCFRDYKYRLAKSTLLLIP
mouse_GLP2R VLPERRLWPKYLVVGWAFPMLFVIPWIFVRASLENTGCWAVNENKKIWWIIRGPILLCVTVNFFIFLKILKLLISKFRAHQMCFRDYKYRLAKSTLLLIL
human_GLP2R VLPERRLWPRYLLLGWAFPVLFVVPWGFARAHLENTGCWTTNGNKKIWWIIRGPMMLCVTVNFFIFLKILKLLISKLKAHQMCFRDYKYRLAKSTLVLIP
360 370 380 390 400 410 420 430 440 450
rabbit_GLP1R LLGTHEVIFAFVMDEHARGTLRFVKLFTELSFTSFQGLMVAILYCFVNNEVQMEFRKSWERWRLEHLRVQR....DSSMKPL.KCPTSSLSSGATGGSSV
human_GLP1R LLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQLEFRKSWERWRLEHLHIQR....DSSMKPL.KCPTSSLSSGATAGSSM
rat_GLP1R LLGTHEVIFAFVMDEHARGTLRFVKLFTELSFTSFQGFMVAVLYCFVNNEVQMEFRKSWERWRLERLNIQR....DSSMKPL.KCPTSSVSSGATVGSSV
mouse_GLP1R LLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAILYCFVNNEVQMEFRKCWERWRLEHLNIQR....DCSMKPL.KCPTSSVSSGATVGSSV
mouse_GCGR LLGVHEVVFAFVTDEHAQGTLRSTKLFFDLFLSSFQGLLVAVLYCFLNKEVQAELMRRWRQWQEGKALQEE..RLASSHGSH.MAPAGPCHGDPCEKLQL
rat_GCGR LLGVHEVVFAFVTDEHAQGTLRSTKLFFDLFFSSFQGLLVAVLYCFLNKEVQAELLRRWRRWQEGKALQEE..RMASSHGSH.MAPAGTCHGDPCEKLQL
human_GCGR LLGVHEVVFAFVTDEHAQGTLRSAKLFFDLFLSSFQGLLVAVLYCFLNKEVQSELRRRWHRWRLGKVLWEE..RNTSNHRAS.SSPG...HGPPSKELQF
rat_GIPR LLGVHEVVFAPVTEEQAESGSLRFAKLAFEIFLSSFQGFLVSVLYCFINKEVQSEIR......RLRLSLQEQCPRPHLGQAPR.AVPLSSAPQEAAIRNAL
mouse_GIPR LLGVHEVVFAPVTEEQVEGSLRFAKLAFEIFLSSFQGFLVSVLYCFINKEVQSEIRQGWRHRRLRLSLQEQRPRPHQELAPR.AVPLSSACREAAVGNAL
human_GIPR LLGVHEVVFAPVTEEQARGALRFAKLGFEIFLSSFQGFLVSVLYCFINKEVQSEIRRGWHHCRLRRSLGEE.QRQLPERAFR.ALPSGSGPGEVPTSRGL
rat_GLP2R LLGVHEVLFTFFPDDQVQGFSKRIRLFIQLTLSSVHGFLVALQYGFANGEVKAELRKSWGRFLLARHWGCRTCVLGKNFRFLGKCSKKLSEGDGSETLQK
mouse_GLP2R LMGVHEFLFTFFTDDQVQGFSRLIRLFIQLTLSSFHGFLVALQYGFASREVKAELRKTWGRFLLARHWGCRACVLGKNFRFLGKCSKKLSEGDGAETLQK
human_GLP2R LLGVHEIIFSFITDDQVEGFAKLIRLFIQLTLSSFHGFLVALQYGFANGEVKAELRKYWVRFLLARHSGCRACVLGKDFRFLGKCPKKLSEGDGAEKLRK
460
rabbit_GLP1R YAA.....TSQACS.....
human_GLP1R YTA.....TCQASCS.....
rat_GLP1R YAA.....TCQNSCS.....
mouse_GLP1R YAA.....TCQSSYS.....
mouse_GCGR MSAGSSSGTG.....CVPSMETSLASSLPRLADSPT.
rat_GCGR MSAGSSSGTG.....CEPSAKTSLASSLPRLADSPT.
human_GCGR GRGGGSQDS.....SAETPLAGGLPRLAESPF.
rat_GIPR PSG.....MLHVPGDEV...LESYC.
mouse_GIPR PSG.....MLHVPGDEV...LESYC.
human_GIPR SSG.....TLPGPGNEASRELESYC.
rat_GLP2R LRFSTCSS...HLASETLGDVGVQPHRGRGAWPRGSSLSESSEGDFTLANTMEEILEESEI
mouse_GLP2R LQSSGVSS...HLTAGNLRDHGAQPHRGRGAWPRASSLSESSEGDFTLANTMEEILEESEI
human_GLP2R LQPSLNSGRLLHLAMRGLGELGAQPQQDHARWPRGSSLSECSEGDVTMANTMEEILEESEI

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