GLP-1 Val8: A biased GLP-1R agonist with altered binding kinetics and impaired release of pancreatic hormones in rats

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Table S-1 | Pharmacology in rat and human GLP-1R

	cAMP accumulation				β-arrestin 2 recruitment	
	rat GLP-1R (n=3)		human GLP-1R (n=3)		rat GLP-1R (n=3)	
Ligand	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)
GLP-1	10.2 ± 0.1	100 ± 0.0	10.2 ± 0.1	100 ± 0.0	8.2 ± 0.5	100 ± 0.0
GLP-1 Val8	10.1 ± 0.2	100 ± 11.2	9.7 ± 0.1	96 ± 3.6	8.0 ± 0.1	49 ± 5.4*

Human GLP-1R was tested in parallel with the rat GLP-1R. All data were fitted with the three-parameter logistic curve to obtain pEC_{50} and E_{max}. pEC_{50} represents the negative logarithm of agonist concentration in molar that produces half the maximal response. E_{max} is characterized as the maximal response normalized to the GLP-1 response. Data represent the mean ± s.e.m. of n independent experiments performed duplicate. Statistical significance was assessed using a two-tailed paired *t*-test for E_{max} (*$P < 0.05$; as compared to GLP-1 response).

Table S-2 | Relaxation of the *in-silico* homology model

Stage	Ensemble	Lipids (positional)	Lipids (dihedral)	Protein (bb)	Protein (sc)
Minimization	St. descent	1000	1000	4000	2000
Stage 1	NVT	1000	1000	4000	2000
Stage 2	NVT	1000	400	2000	1000
Stage 3	NPT	400	200	1000	500
Stage 4	NPT	200	200	500	200
Stage 5	NPT	40	100	200	50
Stage 6	NPT	0	0	50	0
Production run	NPT	0	0	0	0

Summary of the ensemble and restraints applied in the six-stage relaxation procedure of the molecular dynamics simulations of the GLP-1R. Units are In kJ/mol. bb is backbone and sc is side chain.

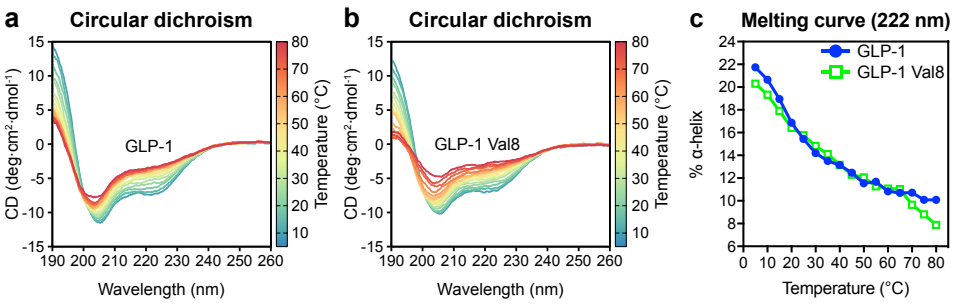

Figure S-1 | Secondary structure characterization through circular dichroism. Measured mean residue molar ellipticity ($[\theta]_{mr}$) for both GLP-1 (**a**) and GLP-1 Val8 (**b**) for the temperature range 5 to 80 °C (colorbar). A clearly distinguishable double valley appeared, indicating that both GLP-1 and GLP-1 Val8 display an α-helical nature [84]. In addition, we observed a loss of this distinct absorbance upon heating of the sample, indicating the transition from helical to a disordered state. (**c**) The corresponding melting curve obtained by plotting the $[\theta]_{mr}$ at 222 nm as a function of temperature. This value gives an indirect measure of the peptide's helical propensity, which can be estimated using equation (6).

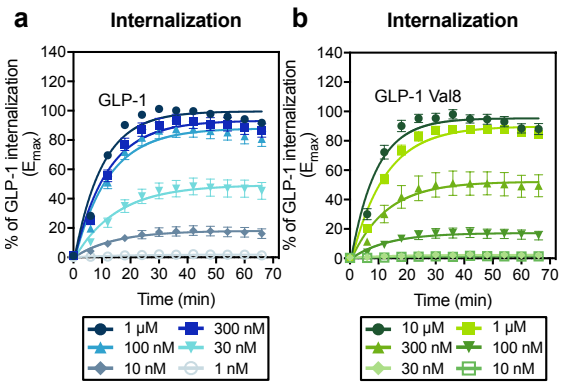

Figure S-2 | Internalization of GLP-1R by GLP-1 and GLP-1 Val8.

TR-FRET internalization (**a**) for GLP-1 and (**b**) GLP-1 Val8 in HEK 293A cells expressing SNAP-tagged human GLP-1R. Data represent the mean ± s.e.m. of 6 independent experiments performed in duplicate and triplicate.

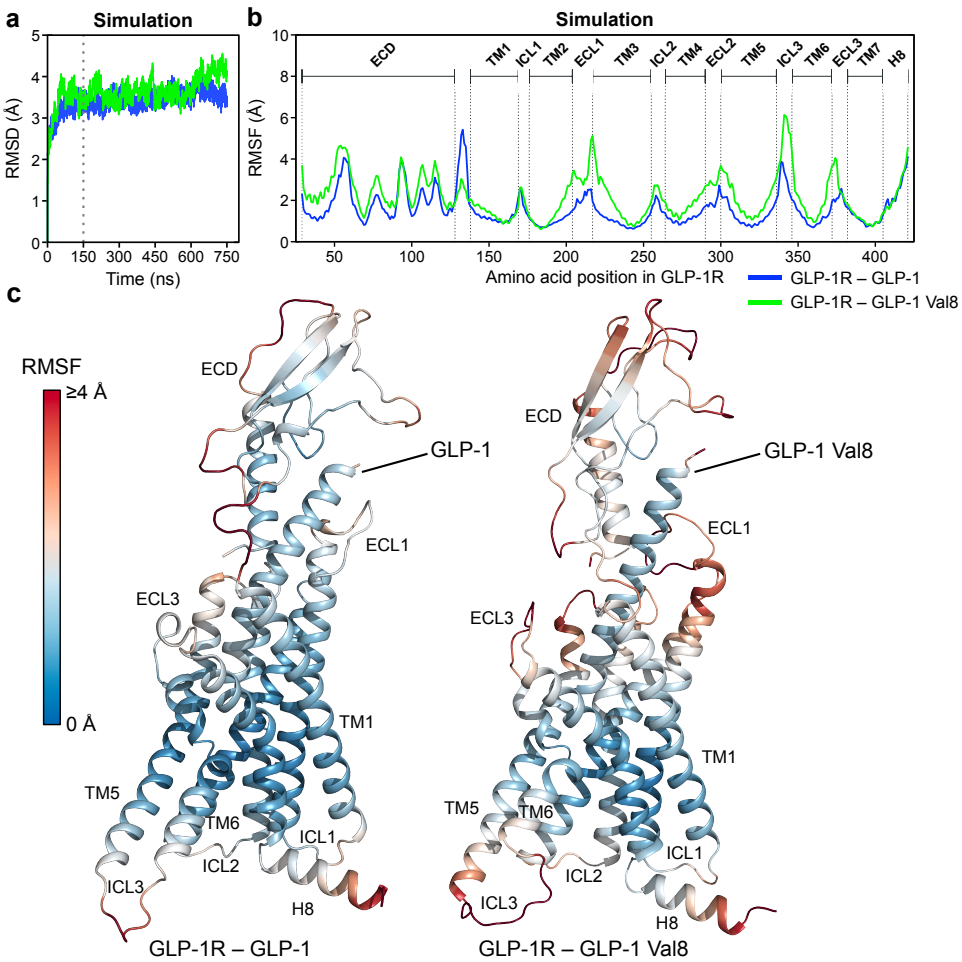

Figure S-3 | Molecular dynamic simulations of GLP-1R (PDB: 5VAI) with GLP-1 and GLP-1 Val8.

Different parameters to determine the stability and mobility of the simulated receptor. (**a**) Root-mean-square deviation (RMSD) of GLP-1R in complex with GLP-1 or GLP-1 Val8, relative to the starting structure. Root-mean-square fluctuation (RMSF) of regions in GLP-1R when bound to GLP-1 or GLP-1 Val8 displayed as graph (**b**) and visualized in structure of GLP-1R (**c**).

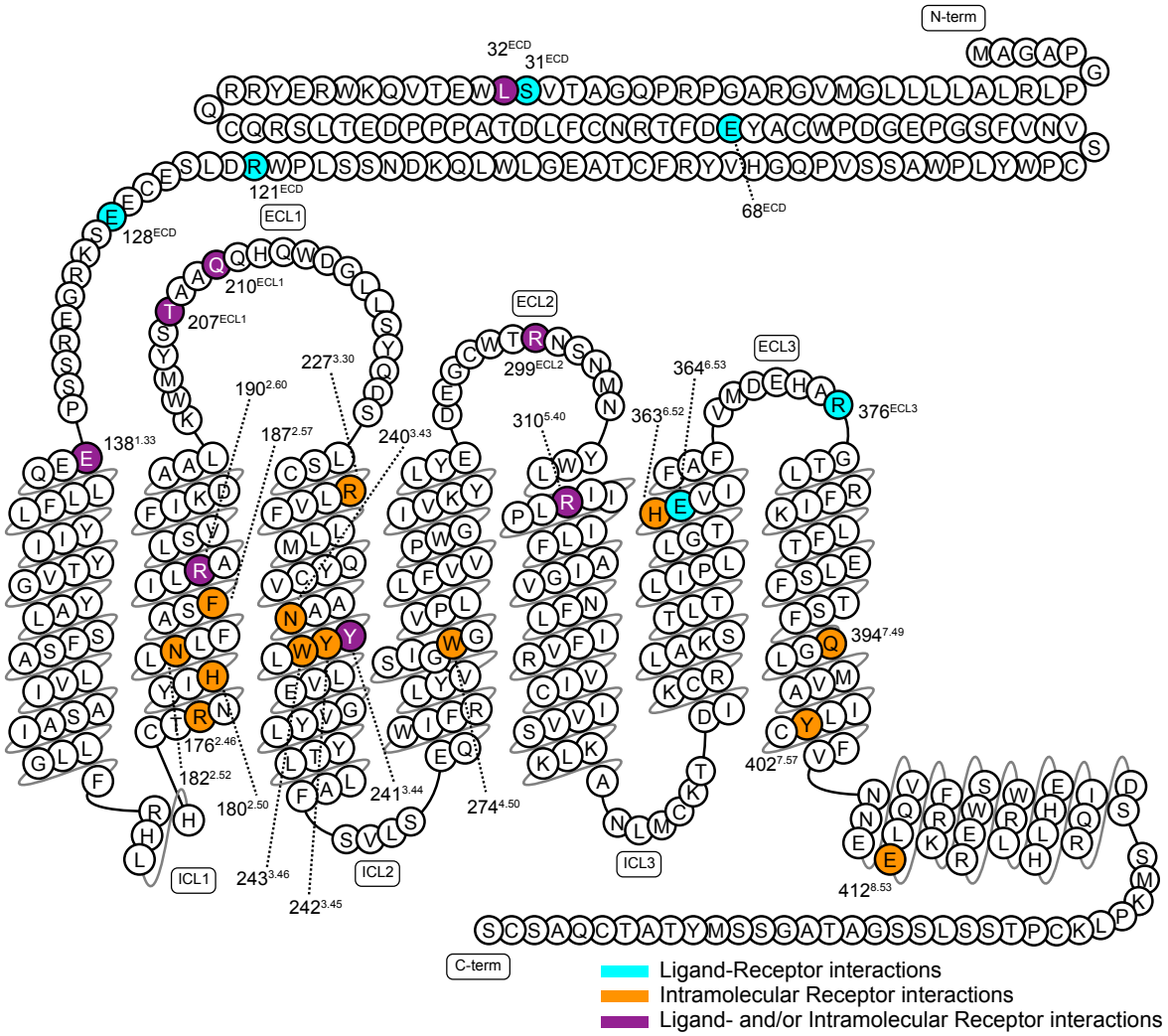

Figure S-4 | Serpentine model of GLP-1R.

The 27 substitutions to alanine are colored based on their interaction pattern: Ligand-receptor, Intramolecular receptor and Ligand and/or intramolecular.

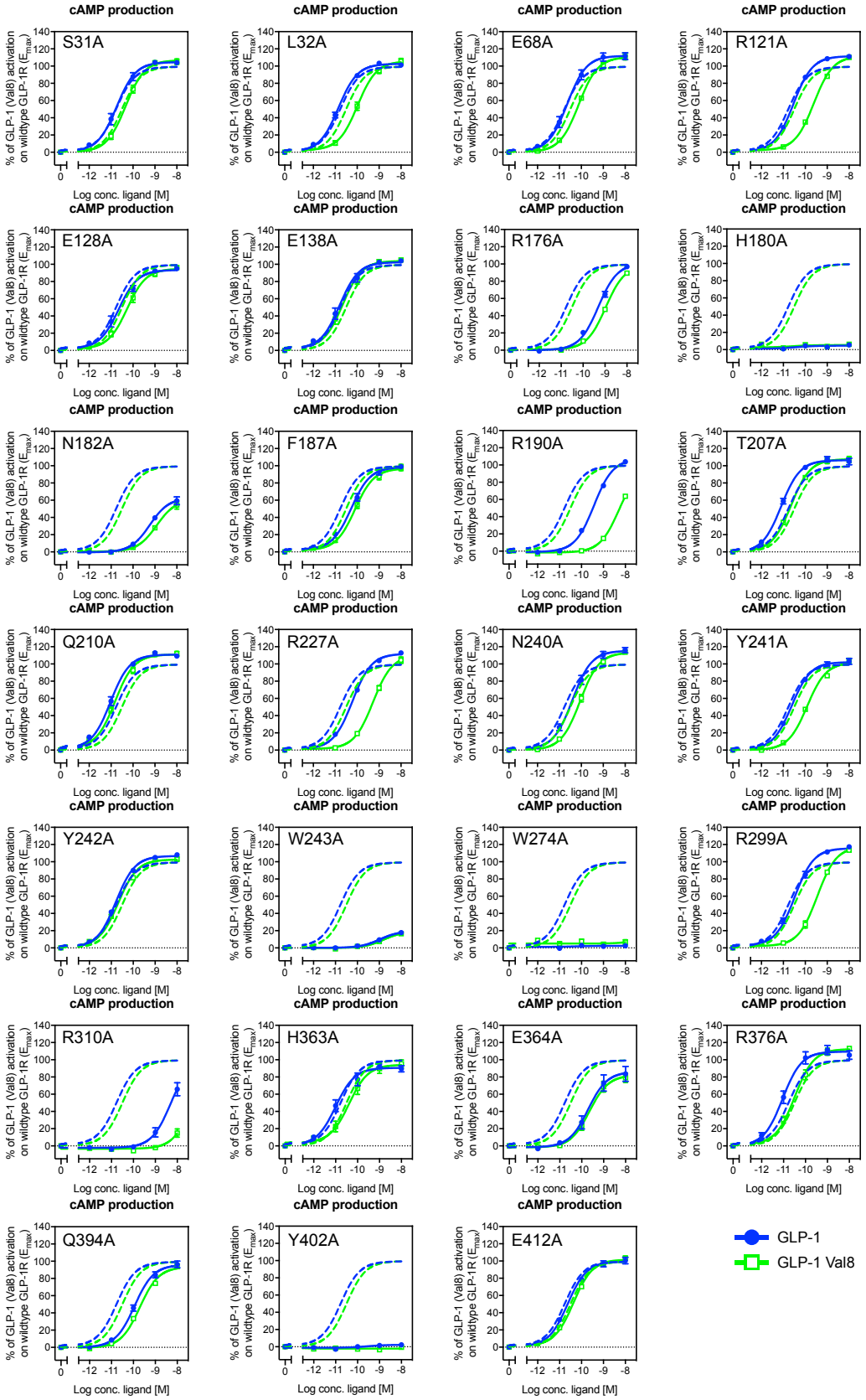

Figure S-5 | Dose-response curves of cAMP production on 27 alanine mutations in GLP-1R.

Data were normalized to GLP-1R WT for both GLP-1 and GLP-1 Val8. Wildtype curves are presented as dashed lines. Data represent the mean ± s.e.m. of 18 independent experiments performed in duplicate on WT, and 3–4 on the mutants.