Supplementary data

Supplementary Table 1. *GLP1R* variants that were functionally analyzed *in vitro*.

***GLP1R* variants (NM_002062.5)**	**MAF ExAC**	**Included in UKB**
c.128G>A/p.Arg43Gln	0.000017	x
c.131G>A/p.Arg44His	0.0037	x
c.251G>C/p.Ser84Thr		
c.287C>T/p.Pro96Leu		x
c.410C>T/p.Pro137Leu	0.000051	x
c.513C>A/p.His171Gln	0.0000084	
c.518A>G/p.His173Arg	0.000042	x
c.518A>C/p.His173Pro	0.0000083	
c.524C>T/p.Thr175Ile	0.0000083	
c.526A>T/p.Arg176Trp	0.000017	x
c.538C>T/p.His180Tyr	0.0000082	
c.569G>A/p.Arg190Gln	0.0000082	x
c.580G>A/p.Val194Ile	0.00048	x
c.622G>A/p.Ala208Thr		
c.679C>T/p.Arg227Cys	0.0000082	x
c.680G>A/p.Arg227His	0.0000082	x
c.692T>C/p.Leu231Pro	0.0000082	
c.707G>A/p.Cys236Tyr		
c.715G>A/p.Ala239Thr	0.00016	x
c.730C>T/p.Leu244Phe	0.0000082	
c.745G>A/p.Val249Met	0.000091	x
c.773C>T/p.Ser258Leu	0.0000082	x
c.781T>G/p.Ser261Ala	0.0000082	
c.790T>C/p.Trp264Arg	0.0000082	
c.802C>T/p.Leu268Phe	0.00024	x
c.808G>A/p.Val270Met	0.000025	x
c.860T>A/p.Val287Asp	0.0000082	
c.868C>T/p.Leu290Phe		
c.872A>G/p.Tyr291Cys	0.0000082	x
c.875A>T/p.Glu292Val	0.0000082	x
c.877G>T/p.Asp293Tyr	0.000016	x
c.880G>A/p.Glu294Lys	0.000091	x
c.928C>T/p.Arg310Trp	0.0000082	x
c.929G>A/p.Arg310Gln	0.0000082	x
c.946G>A/p.Ala316Thr	0.010	x
c.950T>C/p.Ile317Thr	0.000058	x
c.958A>T/p.Asn320Tyr	0.0000082	
c.976C>T/p.Arg326Trp	0.0000082	x
c.991G>A/p.Val331Met	0.000016	x
c.1014T>A/p.Asn338Lys		
c.1021T>A/p.Cys341Ser	0.0000082	x
c.1032C>A/p.Asp344Glu	0.00027	x
c.1064C>T/p.Thr355Ile	0.000017	
c.1069A>T/p.Ile357Phe	0.0000083	
c.1081G>A/p.Gly361Arg	0.0000083	
c.1087C>T/p.His363Tyr	0.000017	
c.1099T>C/p.Phe367Leu	0.0000082	
c.1126C>T/p.Arg376Trp	0.000033	x
c.1127G>A/p.Arg376Gln	0.0010	x
c.1138C>T/p.Arg380Cys	0.0000082	x
c.1199T>G/p.Ile400Arg		
c.1201T>A/p.Leu401Ile	0.0000083	
c.1223A>G/p.Glu408Gly	0.000017	
c.1250G>T/p.Trp417Leu	0.000016	x
c.1249T>G/p.Trp417Gly	0.0000082	
c.1256G>A/p.Arg419His	0.0000082	x
c.1261C>T/p.Arg421Trp	0.00039	x
c.1262G>A/p.Arg421Gln	0.0020	x
c.1304C>T/p.Pro435Leu	0.0000082	
c.1334G>C/p.Ser445Thr	0.0020	x

MAF, minor allele frequency; UKB, UK biobank

Supplementary Table 2a. Cell surface expression of WT and mutant GLP1R in HEK293T cell (ELISA).

Variant	Surface exp (% WT)	Variant	Surface exp (% WT)	Variant	Surface exp (% WT)	Variant	Surface exp (% WT)
R43Q	93 ± 3	R227H	66 ± 8*	D293Y	23 ± 4***	H363Y	36 ± 14***
R44H	97 ± 6	L231P	3 ± 1***	E294K	62 ± 7**	F367L	63 ± 17
S84T	91 ± 5	C236Y	31 ± 6***	R310Q	61 ± 17*	R376Q	87 ± 4
P96L	93 ± 6	A239T	75 ± 10	R310W	13 ± 2***	R376W	74 ± 7
P137L	89 ± 7	L244F	50 ± 10***	A316T	48 ± 8***	R380C	105 ± 21
H171Q	91 ± 11	V249M	89 ± 13	I317T	37 ± 11***	I400R	2 ± 0***
H173P	4 ± 2***	S258L	29 ± 7***	N320Y	4 ± 2***	L401I	92 ± 9
H173R	108 ± 12	S261A	74 ± 8	R326W	52 ± 11***	E408G	18 ± 4***
T175I	71 ± 16	W264R	138 ± 11*	V331M	55 ± 17*	W417G	17 ± 2***
R176W	8 ± 2***	L268F	71 ± 12*	N338K	93 ± 9	W417L	74 ± 4
H180Y	3 ± 1***	V270M	95 ± 5	C341S	94 ± 9	R419H	75 ± 6
R190Q	24 ± 2***	V287D	5 ± 1***	D344E	98 ± 13	R421Q	60 ± 9**
V194I	95 ± 6	L290F	70 ± 8*	T355I	100 ± 17	R421W	70 ± 11
A208T	104 ± 8	Y291C	39 ± 8***	I357F	20 ± 5***	P435L	78 ± 12
R227C	33 ± 3***	E292V	11 ± 3***	G361R	3 ± 2***	S445T	99 ± 5

Supplementary Table 2b. Cell surface expression of WT and mutant GLP1R in INS-1 823/3 (*Glp1r* KO) (ELISA).

Variant	Surface exp (% WT)	Variant	Surface exp (% WT)	Variant	Surface exp (% WT)	Variant	Surface exp (% WT)
R43Q	58 ± 17	R227H	53 ± 13*	D293Y	26 ± 7**	H363Y	52 ± 11*
R44H	109 ± 15	L231P	3 ± 2***	E294K	45 ± 11	F367L	100 ± 39
S84T	52 ± 14	C236Y	6 ± 3***	R310Q	45 ± 4*	R376Q	117 ± 28
P96L	93 ± 16	A239T	46 ± 13*	R310W	5 ± 2***	R376W	101 ± 24
P137L	82 ± 17	L244F	57 ± 13	A316T	13 ± 5***	R380C	44 ± 4**
H171Q	141 ± 24	V249M	99 ± 37	I317T	25 ± 9***	I400R	ND
H173P	11 ± 7***	S258L	27 ± 11**	N320Y	ND	L401I	58 ± 18
H173R	143 ± 48	S261A	91 ± 14	R326W	50 ± 7	E408G	18 ± 11**
T175I	97 ± 31	W264R	134 ± 19	V331M	78 ± 12	W417G	10 ± 6***
R176W	3 ± 1***	L268F	12 ± 5***	N338K	76 ± 20	W417L	89 ± 31
H180Y	10 ± 5***	V270M	91 ± 8	C341S	127 ± 32	R419H	77 ± 9
R190Q	21 ± 2***	V287D	11 ± 5***	D344E	78 ± 10	R421Q	125 ± 12
V194I	113 ± 45	L290F	80 ± 9	T355I	104 ± 22	R421W	76 ± 19
A208T	88 ± 17	Y291C	10 ± 5***	I357F	33 ± 13*	P435L	55 ± 12
R227C	39 ± 11	E292V	6 ± 1***	G361R	2 ± 1***	S445T	183 ± 13**

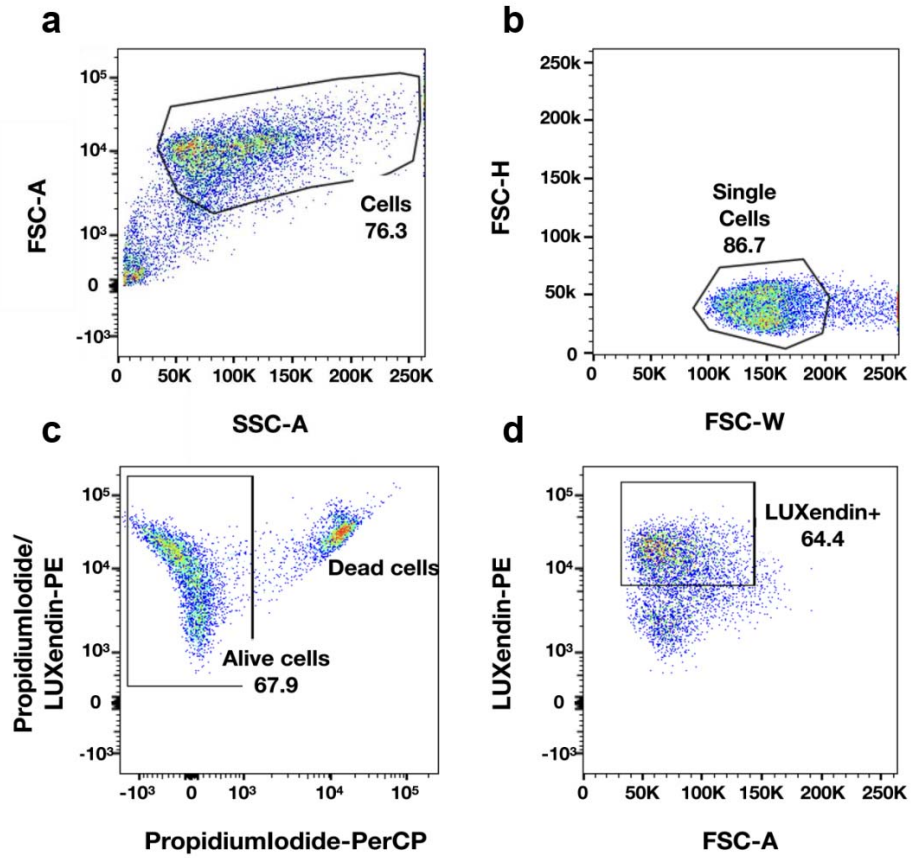
All values are expressed as means ± SEM of at least three independent experiments. Statistical significance of differences was determined by one-way analysis of variance and Dunnett's post-test *P < 0.05, **P < 0.01, ***P < 0.0001. exp, expression.

Supplementary Table 3a. Summary of the functional profiling of cAMP accumulation by WT and mutant GLP1R. (see Excel file).

Supplementary Table 4. Null *GLP1R* variants detected in the UK Biobank

***GLP1R* variants (NM_002062.5)**		MAF UKB
c.32dup/p.Leu12AlafsTer41		0.0000025
c.76del/p.Gln26ArgfsTer25		0.0000053
c.76dup/p.Gln26ProfsTer27		0.0000053
c.79-2A>C		0.0000027
c.118C>T/p.Arg40Ter		0.000013
c.126C>G/p.Tyr42Ter		0.0000025
c.127C>T/p.Arg43Ter		0.0000050
c.152_155del/p.Thr51ArgfsTer30		0.0000076
c.245dup/p.Asn82LysfsTer26		0.0000075
c.284-2A>G		0.0000025
c.391C>T/p.Arg131Ter		0.0000025
c.402+1G>A		0.0000078
c.568C>T/p.Arg190Ter		0.0000050
c.670_671del/p.Leu224GlufsTer38		0.0000025
c.722dup/p.Tyr241Ter		0.0000025
c.823+1del		0.0000025
c.824-1G>C		0.0000025
c.1117G>T/p.Glu373Ter		0.0000025
c.1337del/p.Gly446GlufsTer76		0.0000025

MAF, minor allele frequency; UKB, UK biobank



Supplementary Fig. 1 Sequence of Gating Steps in Flow Cytometry Experiments. **a,** Selection of cells; **b,** Selection of single cell; **c,** Selection of live cells; **d,** selection of LUXendin+cells. Numbers represent the % of selected cells.

Supplementary data analysis of signaling profiles of GLP1R mutants:

cAMP pathway

The E_{max} of Ex-4 to promote cAMP production was significantly diminished for eight mutants of which the five most severely affected ones (p. R176W, p. H180Y, p.N320Y, p.G361R and p.I400R) could be explained by severely impaired cell surface expression (Supplementary Table 2a and Supplementary Table 3a and Fig. 2a and Extended Data Fig. 6a). Twelve mutants showed a GoF phenotype with a significant, up to 200%, increase in E_{max} (ten from the 'cAMP GoF' category (Fig. 2f and Extended Data Fig. 6f, Supplementary Table 3a) and p.V287D (Extended Data Fig. 6a) and p.A316T (Fig. 2g)). The cell surface expression levels of these mutants ranged from 31 to 95% of WT GLP1R (Supplementary Table 2a). Twenty mutants showed significantly lower potencies with seven of them being severely affected (EC_{50} right-shifted by 2 logs). Among these, five mutants belong to the category of mutants of 'Severely surface exp defective' (p. R176W, p.L231P, p.V287D, p.E292V, p.R310W) and two (p.R190Q, p.R380C) to the category of 'All pathways defective'.

The correlation curve between cell surface expression and cAMP signaling (Extended Data Fig. 4b) allowed us to define mutants with less than 10 ± 3 % cell surface expression compared to WT GLP1R as an independent category of 'Severely surface exp defective' (Fig. 2a and Extended Data Fig. 6a). This category comprises variants encoding p.H173P, p.R176W, p.H180Y, p.L231P, p.V287D, p.E292V, p.R310W, p.N320Y, p.G361R and p.I400R. The severely impaired cell surface expression of these mutants is consistent with absence of any measurable Ex-4 binding in the TR-FRET-based ligand competitive binding assay for six members of this category (p.H173P, p.H180Y, p.L231P, p.N320Y, p.G361R and p.I400R) (Extended Data Fig. 7). The variant encoding p.R380C shows normal surface expression but severely impaired Ex-4 binding consistent with previous results obtained with an alanine substituted receptor at this position (Wootten et al, 2016). GoF mutants show increased E_{max} values despite normal surface expression.

Ca^{2+} mobilization

Similar to cAMP accumulation, the Ca^{2+} mobilization responsiveness was also differentially affected, with 21 LoF or GoF mutants each (Supplementary Table 3b). All mutants of the 'Severely surface exp defective' category were also defective in Ca^{2+} mobilization, similar to the cAMP pathway (Fig. 2a and Extended Data

Fig. 6a). In contrast to the cAMP pathway, only E_{max} values were affected without any change in EC_{50} (Supplementary Table 3b). To compensate for the impact of differences in cell surface expression of mutants, correlation curves with EC_{50} and E_{max} were generated (Extended Data Fig. 5a). Whereas E_{max} values increased linearly (Extended Data Fig. 5b), EC_{50} values were unaffected by the cell surface expression level (Extended Data Fig. 5c). After normalization to cell surface expression, ten mutants belonged to the 'Ca^{2+} GoF' category (Fig. 2g and Extended Data Fig. 6g). For all of them only E_{max} values were affected (up to 235% increase over WT GLP1R). Interestingly, three GoF variants encoding p.S258L, p.E408G and p.W417G exhibited clearly increased Ca^{2+} mobilization in the context of normal cAMP production and significantly decreased cell surface expression (Extended Data Fig. 6g and Supplementary Table 2a). Five mutants were GoF for both cAMP and Ca^{2+} pathways (p.Y291C, p.E294K, p.A316T, p.N338K and p.C341C) (Fig. 2g and Extended Data Fig. 6f). In conclusion, Ca^{2+} mobilization is affected in 2/3 of the studied GLP1R mutants with both LoF and GoF in E_{max}.

ERK1/2 activation by GLP1R mutants

Ex-4 induced a rapid increase in ERK1/2 activation for the WT GLP1R reaching a plateau at 5 minutes that was maintained for up to 1 hour (Extended Data Fig. 3e). At 5 minutes, the ERK1/2 activation was fully blocked by the PKA inhibitor H89 but not by β-arr1/2 silencing (Extended Data Fig. 3k-n) indicating that the Gs/cAMP/PKA pathway is the predominant input pathway at 5 minutes of stimulation in HEK293T cells. Ex-4-induced ERK1/2 activation of GLP1R mutants was tested at 5 minutes. Twenty-seven mutants showed significantly impaired ERK1/2 activation and one mutant (p.S258L) increased ERK1/2 activation (Supplementary Table 3c). Similar to Ca^{2+} mobilization, only E_{max} values, but not EC_{50} values, were affected. A linear correlation was observed for E_{max} values and cell surface expression and no influence of the latter on EC_{50} values (Extended Data Fig. 5d-f). Three mutants (p.L231P, p.E292V, and p.R310W) highlighted the robustness of the ERK1/2 pathway compared to the other pathways as the ERK1/2 pathway was the only one maintaining a notable level of activation upon Ex-4 stimulation at low receptor cell surface expression (Extended Data Fig. 6a). In contrast, three other variants encoding p.H173R, p.A239T, and p.D344E showed an ERK-specific defect (Fig. 2e, Extended Data Fig. 6e and Supplementary Table 3c).

β-arr2 recruitment by GLP1R mutants

With 41 defective mutants, the β-arr2 recruitment was the most affected pathway in our study. For 17 mutants β-arr2 recruitment was completely undetectable including all 10 mutants of the 'Severely surface exp defective' category and all four mutants of the 'All pathways defective' category (Fig. 2, Extended Data Fig. 6 and Supplementary Table 3d). Correlation curves of EC_{50} and E_{max} for surface expression were generated (Extended Data Fig. 5g) and showed a similar behavior as the correlation curves for the Ca^{2+} and ERK pathways; whereas E_{max} values increased linearly with increased cell surface expression (Extended Data Fig. 5h), EC_{50} values were unaffected (Extended Data Fig. 5i). After normalization to cell surface expression, 16 additional mutants showed decreased E_{max} values and eight variants a modest decrease in EC_{50} values. With nine mutants (p.P96L, p.H171Q, p.A208T, p.L268F, p.I317T, p.I357F, p.H363Y, p.R376Q, and p.S445T), the 'β-arr2 specific defective' category represented the largest pathway specific category of this study (Fig. 2d, Extended Data Fig. 6d and Supplementary Table 3d). A distinctive feature of this category is the predominant defect in EC_{50} values for all members with an additional defect in E_{max} for p.I317T, p.357F, p.H363Y, most likely because of diminished cell surface expression (Fig. 2d, Extended Data Fig. 6d and Supplementary Table 3d). Of note, p.I317T and p.I357F are the most pathway-selective mutants with a complete (p.I357F) or almost complete (p.I317T) absence of β-arr2 recruitment and normal signaling on the other pathways. In conclusion, the β-arr2 pathway represents the pathway for which most of the mutants are affected with diminished EC_{50} and/or E_{max}. GoF was not observed for this pathway.