Supplementary Table S1. Summary of the oligonucleotides used in this study.

Oligonucleotides were designed for use with the QuikChange site-directed mutagenesis kit (Stratagene) to generate mutations into the human GLP-1R. Bold italicized characters denote nucleotides at which the mutations were introduced.

Mutation	Oligonuceotide orientation	Oligonucleotide sequence (5'-3')
A^{149}	forward	CTCTACATCATCTAC***GCC***GTGGGCTACGCACTC
	reverse	GAGTGCGTAGCCCAC***GGC***GTAGATGATGTAGAG
C^{149}	forward	CCTCTACATCATCTAC***TGC***GTGGGCTACGCACTC
	reverse	GAGTGCGTAGCCCAC***GCA***GTAGATGATGTAGAGG
F^{149}	forward	CCTCTACATCATCTAC***TTC***GTGGGCTACGCACTCTC
	reverse	GAGAGTGCGTAGCCCAC***GAA***GTAGATGATGTAGAGG
I^{149}	forward	CTACATCATCTACA***TC***GTGGGCTACGCACTC
	reverse	GAGTGCGTAGCCCAC***GA***TGTAGATGATGTAG
S^{149}	forward	CTACATCATCTACA***GC***GTGGGCTACGCACTC
	reverse	GAGTGCGTAGCCCAC***GC***TGTAGATGATGTAG
V^{149}	forward	CTCTACATCATCTAC***GT***GGTGGGCTACGCAC
	reverse	GTGCGTAGCCCACC***AC***GTAGATGATGTAGAG
Y^{149}	forward	CCTCTACATCATCTAC***TAC***GTGGGCTACGCACTCTC
	reverse	GAGAGTGCGTAGCCCAC***GTA***GTAGATGATGTAGAGG
A^{333}	forward	CTGCATCGTGGTA***G***CCAAACTGAAGGC
	reverse	GCCTTCAGTTTGG***C***TACCACGATGCAG
V^{333}	forward	GTCATCTGCATCGTGGTA***GTG***AAACTGAAGGCCAATCTC
	reverse	GAGATTGGCCTTCAGTTT***CAC***TACCACGATGCAGATGAC

Supplementary Table S2. Functional pK_A values derived from operational fitting of concentration-response data for each pathway. Data were analyzed using an operational model of agonism (Equation 2). pK_A values represent the negative logarithm of the concentration of agonists that produces the functional affinity associated with the individual pathway under analysis. Values are expressed as mean ± S.E.M. of four to seven independent experiments, conducted in duplicate. Data were analyzed with one-way analysis of variance and Dunnett's post test.

	cAMP				**$_iCa^{2+}$**		**pERK1/2**			
	GLP-1(1-36)NH_2	GLP-1(7-36)NH_2	Exendin-4	Oxyntomodulin	GLP-1(7-36)NH_2	Exendin-4	GLP-1(1-36)NH_2	GLP-1(7-36)NH_2	Exendin-4	Oxyntomodulin
Wildtype (T^{149}, S^{333})	6.8 ± 0.1	8.5 ± 0.2#	9.7 ± 0.2#^	7.4 ± 0.2	7.2 ± 0.2#	6.9 ± 0.2#§	7.2 ± 0.3	7.9 ± 0.2	7.9 ± 0.3^§	7.2 ± 0.2
A^{149}	**N.D.**	6.9 ± 0.5	**8.4 ± 0.3***	**N.D.**	**N.D.**	**N.D.**	6.3 ± 0.7	6.4 ± 0.7	7.5 ± 0.4	6.9 ± 0.3
C^{149}	6.7 ± 0.4	7.9 ± 0.2	9.3 ± 0.2#^	6.7 ± 0.2	7.0 ± 0.2	7.0 ± 0.4#	6.1 ± 0.7	7.1 ± 0.4	7.4 ± 0.3^	6.7 ± 0.3
F^{149}	**N.D.**	**N.D.**	**7.3 ± 0.4***	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	7.4 ± 0.9	7.0 ± 0.6
I^{149}	**N.D.**	**N.D.**	**7.9 ± 0.5***	**N.D.**	**N.D.**	**N.D.**	6.3 ± 0.8	**N.D.**	**N.D.**	7.3 ± 0.6
S^{149}	5.9 ± 0.3	8.6 ± 0.2^#	8.9 ± 0.5#^	7.4 ± 0.2	6.7 ± 0.2#	6.7 ± 0.2#	6.8 ± 0.3	7.3 ± 0.3^	7.6 ± 0.3^	7.0 ± 0.2
V^{149}	**N.D.**	7.3 ± 0.5	8.5 ± 0.3	**N.D.**	**N.D.**	**N.D.**	**N.D.**	7.4 ± 0.9	**N.D.**	6.9 ± 0.6
Y^{149}	**N.D.**	**N.D.**	**7.9 ± 0.5***	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
A^{333}	6.8 ± 0.1	8.6 ± 0.2^#	9.7 ± 0.2#^	7.6 ± 0.2^	7.2 ± 0.1#	6.8 ± 0.2#	6.8 ± 0.2	7.2 ± 0.2^	7.3 ± 0.2^	6.4 ± 0.4^
V^{333}	6.9 ± 0.1	9.1 ± 0.2^#	9.9 ± 0.2#^	8.3 ± 0.2^	7.3 ± 0.1#	7.0 ± 0.2#	6.8 ± 0.3	7.5 ± 0.2^	7.7 ± 0.2^	6.9 ± 0.2^

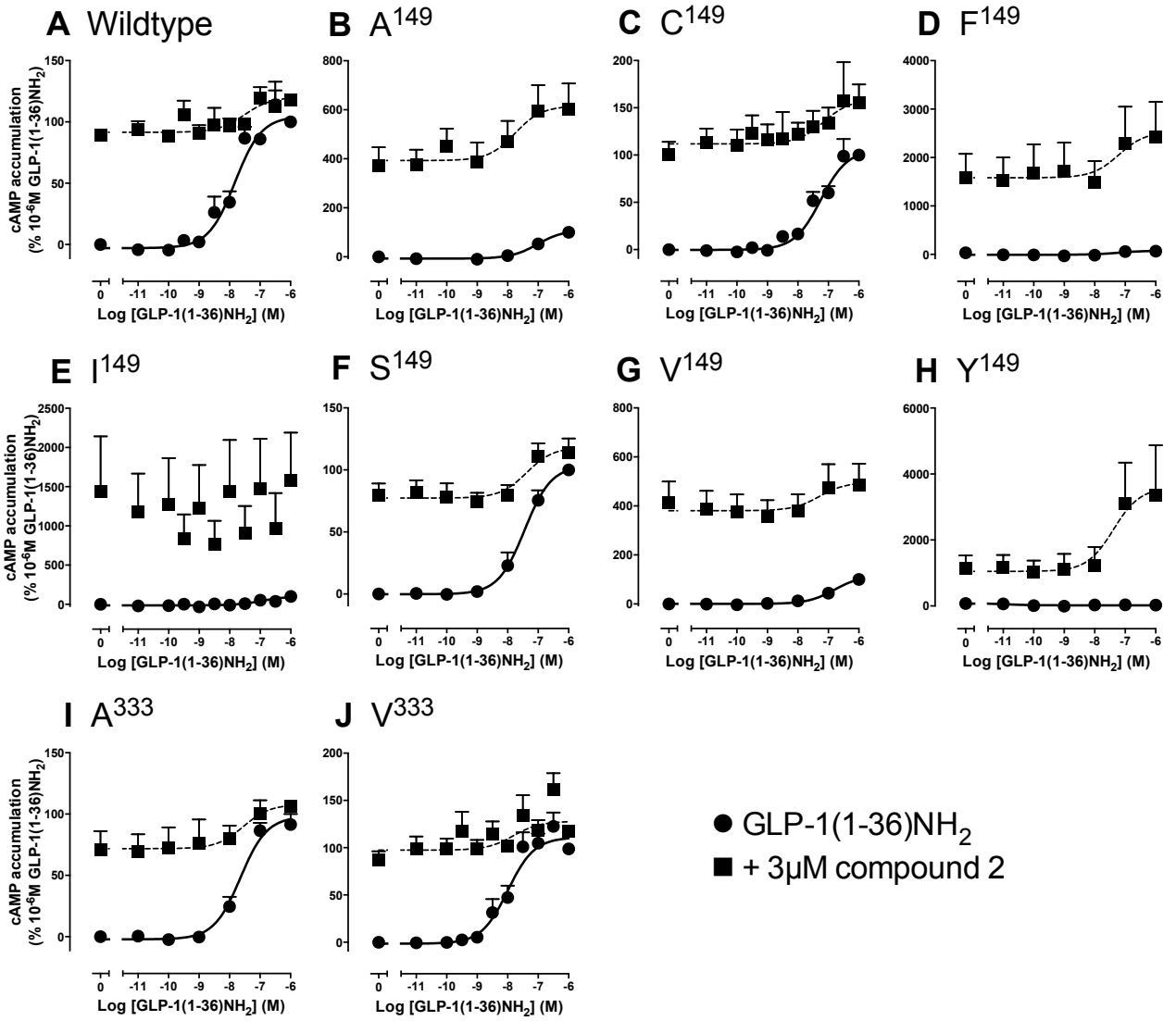
N.D., data unable to be experimentally defined or with incomplete curves

* statistically significant at $p < 0.05$, one-way analysis of variance and Dunnett's post test in comparison to wildtype control

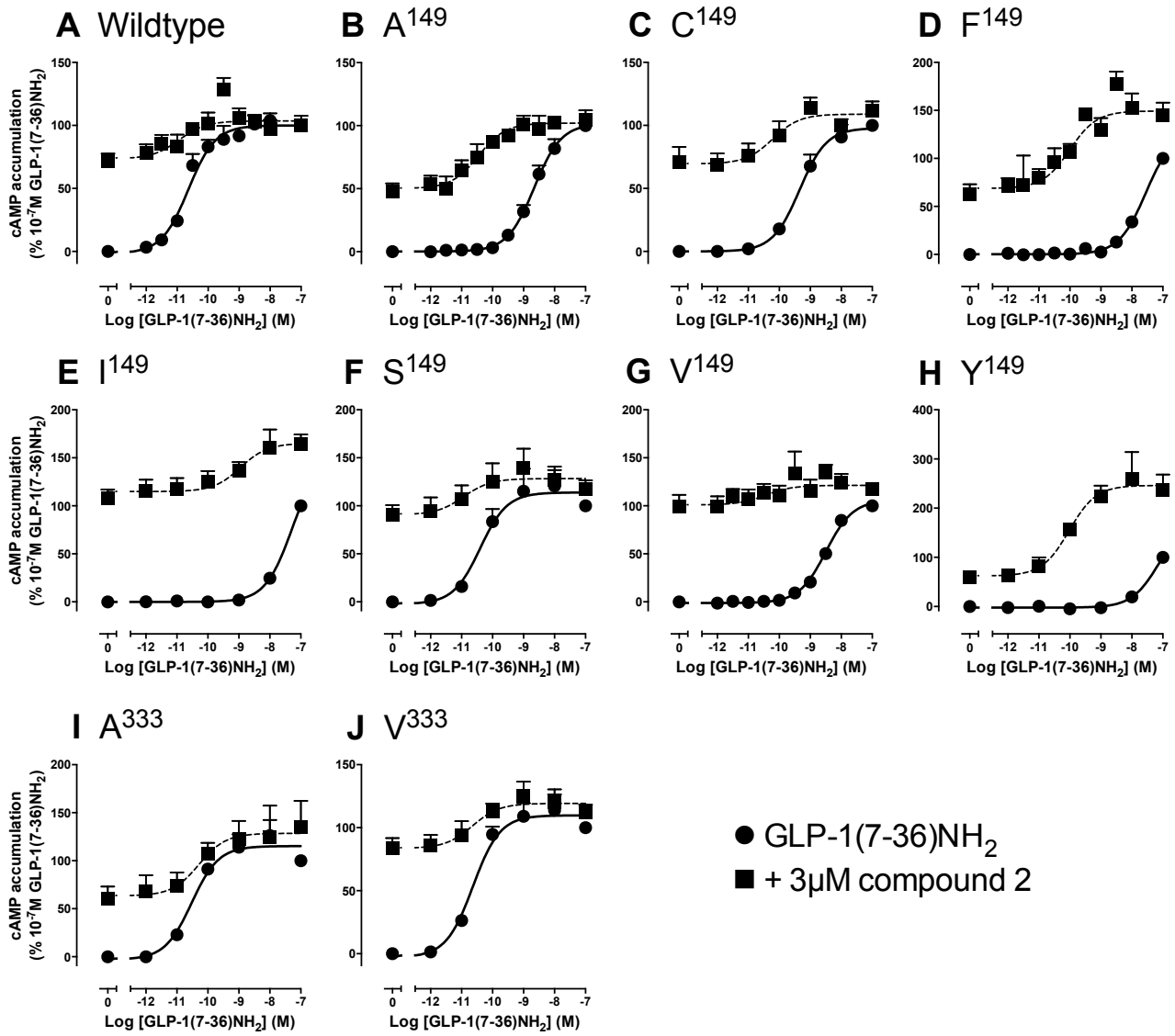
statistically significant between cAMP and $_iCa^{2+}$ at $p < 0.05$, t-test or one-way analysis of variance and Tukey's post test as appropriate

^ statistically significant between cAMP and pERK1/2 at $p < 0.05$, t-test or one-way analysis of variance and Tukey's post test as appropriate

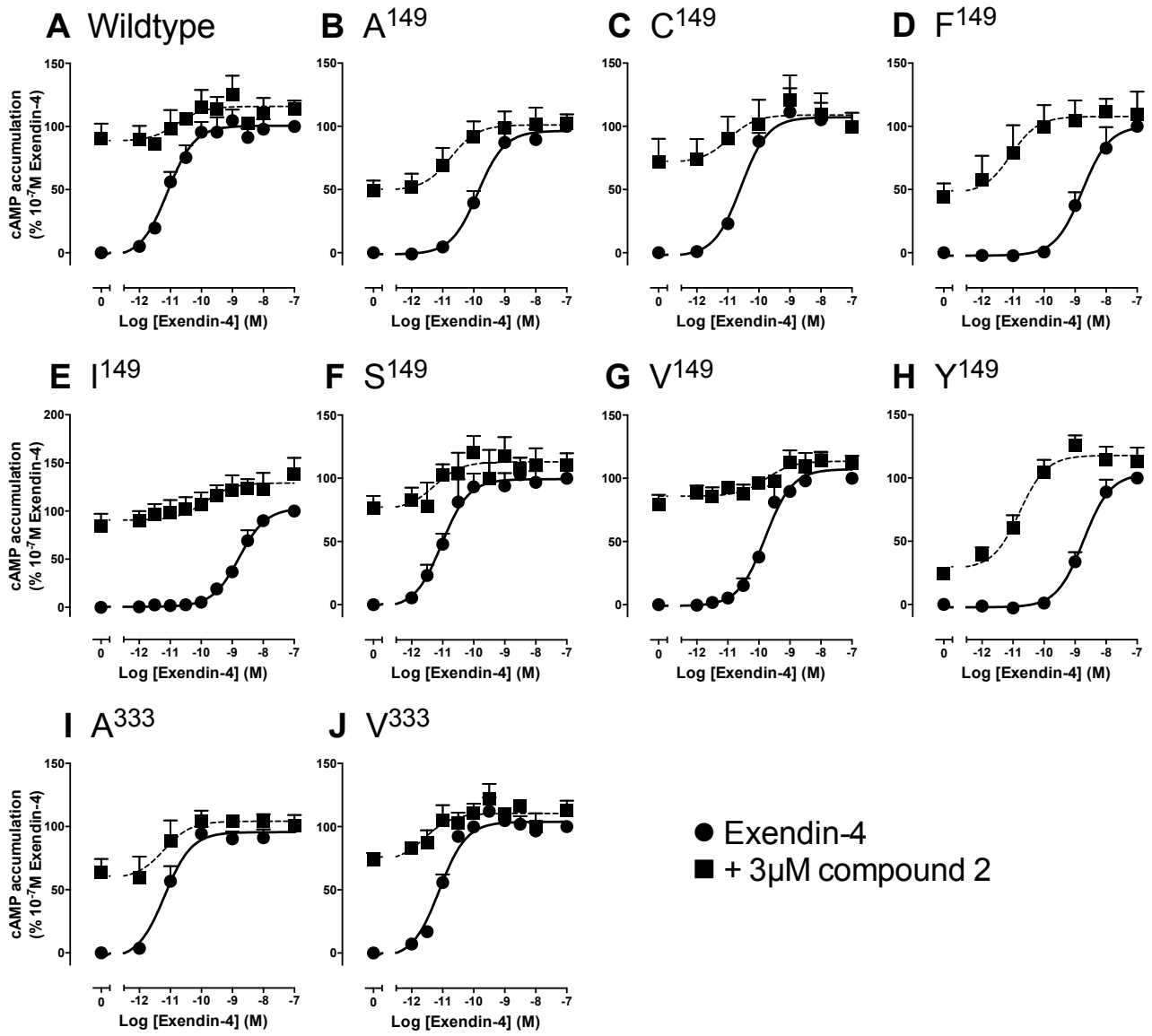
§ statistically significant between $_iCa^{2+}$ and pERK1/2 at $p < 0.05$, t-test or one-way analysis of variance and Tukey's post test as appropriate



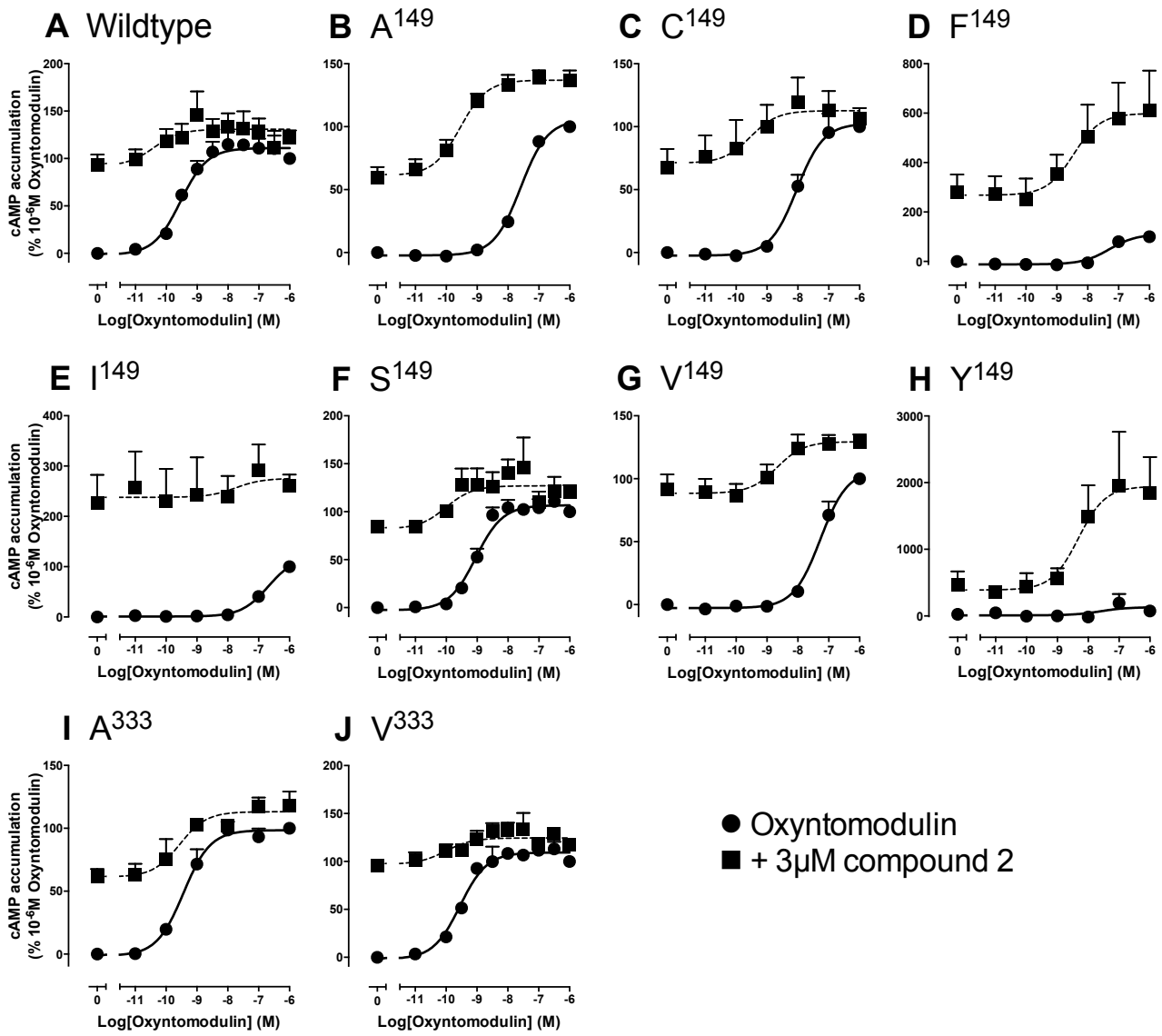
Supplementary Figure S1. Characterization of cAMP accumulation in the presence of GLP-1(1-36)NH_2 at wildtype (A), A149 (B), C149 (C), F149 (D), I149 (E), S149 (F), V149 (G), Y149 (H), A333 (I) and V333 (J) GLP-1Rs in FlpInCHO cells in the presence (■) or absence (●) of 3 μM compound 2. Data are normalized to the response elicited by 100 μM forskolin followed by the response elicited by 1 μM GLP-1(1-36)NH_2, and analyzed with a three parameter logistic equation as defined in Equation 1. All values are means ± S.E.M. of four to nine independent experiments, conducted in duplicate.



Supplementary Figure S2. Characterization of cAMP accumulation in the presence of GLP-1(7-36)NH_2 at wildtype (A), A149 (B), C149 (C), F149 (D), I149 (E), S149 (F), V149 (G), Y149 (H), A333 (I) and V333 (J) GLP-1Rs in FlpInCHO cells in the presence (■) or absence (●) of 3 μM compound 2. Data are normalized to the response elicited by 100 μM forskolin followed by the response elicited by 100 nM GLP-1(7-36)NH_2, and analyzed with a three parameter logistic equation as defined in Equation 1. All values are means ± S.E.M. of four to nine independent experiments, conducted in duplicate.



Supplementary Figure S3. Characterization of cAMP accumulation in the presence of Exendin-4 at wildtype (A), A149 (B), C149 (C), F149 (D), I149 (E), S149 (F), V149 (G), Y149 (H), A333 (I) and V333 (J) GLP-1Rs in FlpInCHO cells in the presence (■) or absence (●) of 3 μM compound 2. Data are normalized to the response elicited by 100 μM forskolin followed by the response elicited by 100 nM Exendin-4, and analyzed with a three parameter logistic equation as defined in Equation 1. All values are means ± S.E.M. of four to nine independent experiments, conducted in duplicate.



Supplementary Figure S4. Characterization of cAMP accumulation in the presence of Oxyntomodulin at wildtype (A), A149 (B), C149 (C), F149 (D), I149 (E), S149 (F), V149 (G), Y149 (H), A333 (I) and V333 (J) GLP-1Rs in FlpInCHO cells in the presence (■) or absence (●) of 3 μM compound 2. Data are normalized to the response elicited by 100 μM forskolin followed by the response elicited by 1 μM Oxyntomodulin, and analyzed with a three parameter logistic equation as defined in Equation 1. All values are means ± S.E.M. of four to nine independent experiments, conducted in duplicate.