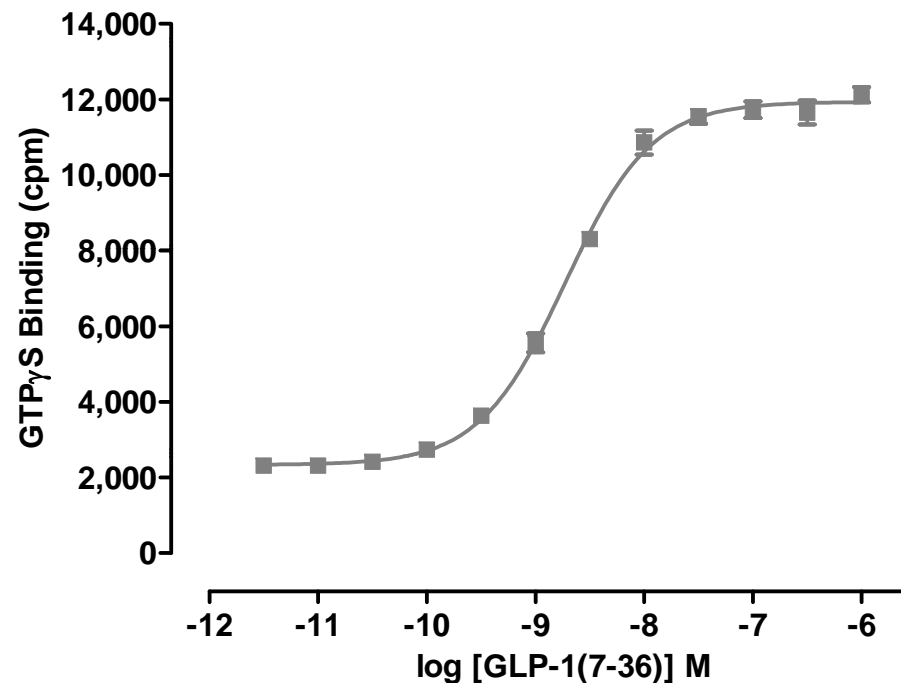


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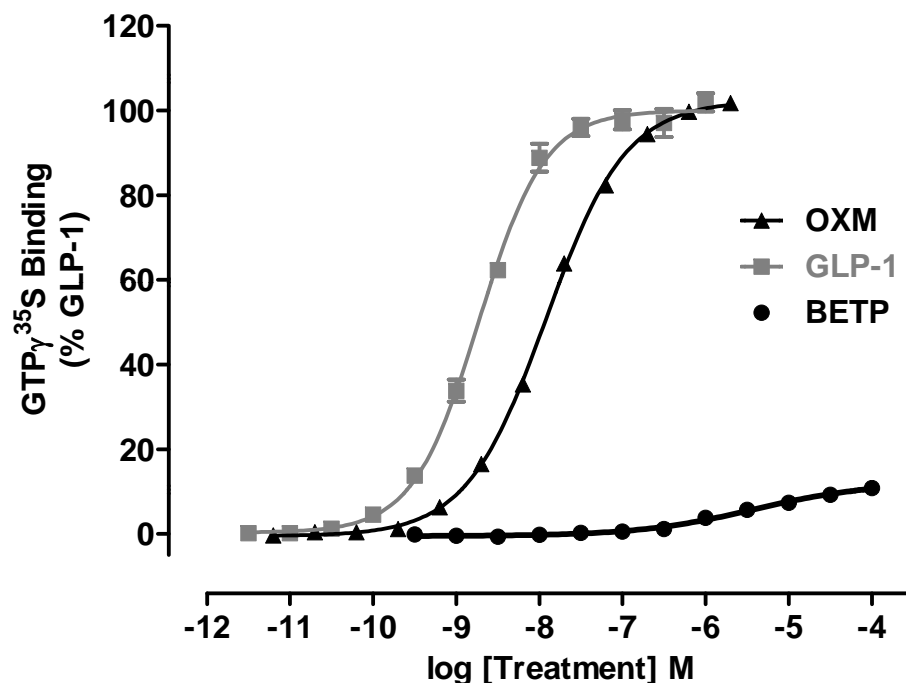
Supplementary Figure 1

Ligand mediated [35 S]GTP γ S binding to endogenous $G\alpha_s$ protein in GLP-1 receptor expressing membranes is measured by antibody capture scintillation proximity. Saturating GLP-1 concentrations induced a 5.2-fold increase in GTP γ S binding as measured in raw cpm values. Data are fit to the four parameter logistic equation. EC_{50} of GLP-1 (7-36)NH $_2$ was 1.9 nM. Data are the mean \pm S.E.M. of a single experiment conducted in triplicate and are representative of three independent experiments.

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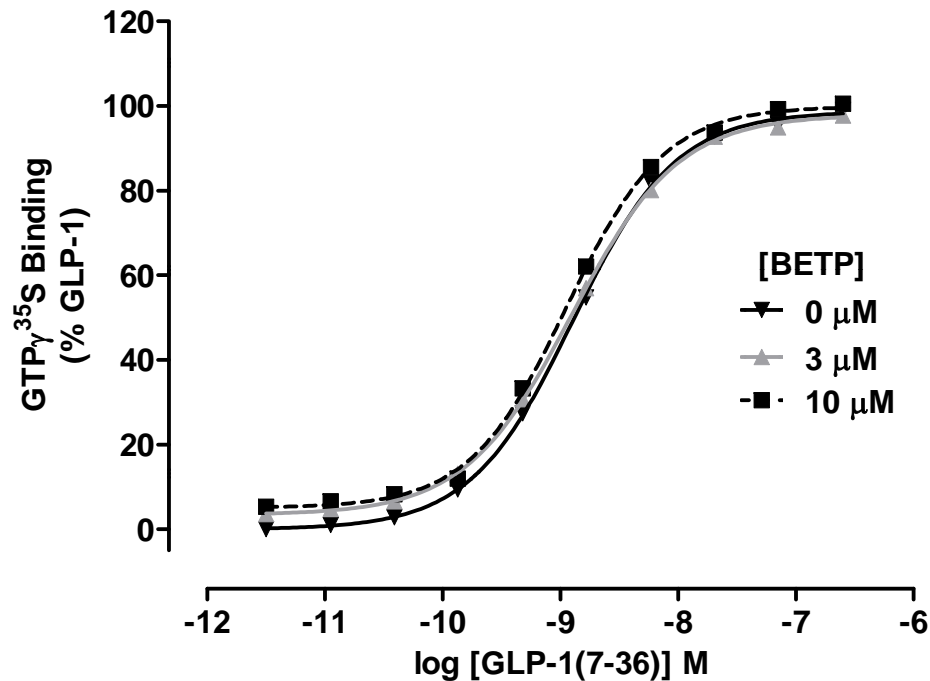
Supplementary Figure 2

Intrinsic efficacy of BETP, oxyntomodulin (OXM), and GLP-1 (7–36)NH₂ for G protein activation. Ligand mediated [³⁵S]GTP γ S binding to endogenous G α_s protein in GLP-1 receptor expressing membranes is measured by antibody capture scintillation proximity. Data are normalized to control values measured from saturating GLP-1 (7–36)NH₂ concentrations. Data are fit to the four parameter logistic equation. EC₅₀ and E_{MAX} values (% of maximal GLP-1 (7–36)NH₂ effect): BETP, 3.7 μ M, 12%; OXM, 12 nM, 103%; GLP-1 (7–36)NH₂, 1.9 nM, 100 %. Data are the mean \pm S.E.M. of a single experiment conducted in triplicate and are representative of three independent experiments.

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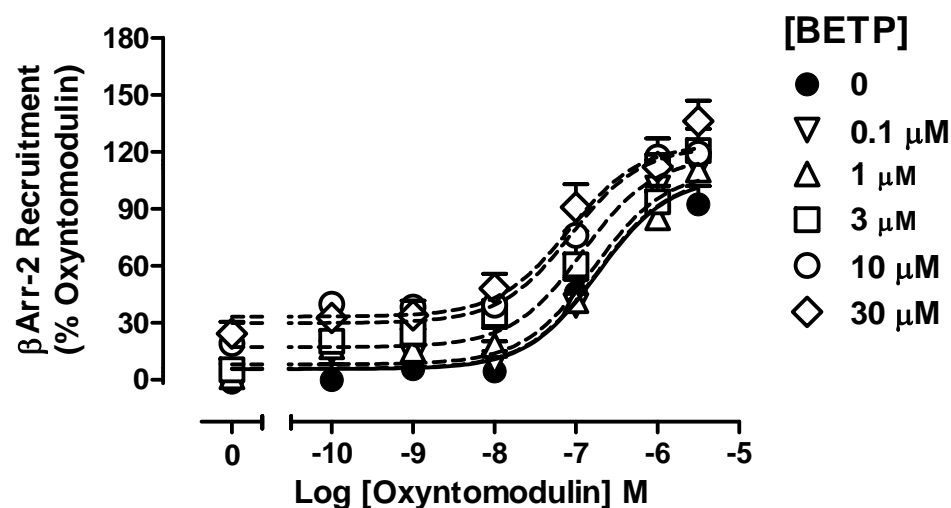
Supplementary Figure 3

The effect of BETP on GLP-1 (7–36)NH₂ mediated G protein activation. Ligand mediated [³⁵S]GTP_γS binding to endogenous Gα_s protein in GLP-1 receptor expressing membranes is measured by antibody capture scintillation proximity. Data are normalized to control values measured from saturating GLP-1 (7–36)NH₂ concentrations. Data are fit to the four parameter logistic equation. EC₅₀ and E_{MAX} values (% of maximal GLP-1 (7–36)NH₂ effect): 0 μM BETP, 1.3 nM, 99%; 3 μM BETP, 1.3 nM, 95%; 10 μM 1.1 nM, 95 %. Data are the mean ± S.E.M. of a single experiment conducted in triplicate and are representative of three independent experiments.

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Supplementary Figure 4

BETP modulation of oxyntomodulin stimulated GLP-1 receptor mediated activation of β -arrestin2. The dose-dependent effects of BETP on oxyntomodulin mediated activation of β -arrestin2 recruitment are measured by bioluminescence resonance energy transfer. Data are fit to the operational model of allosteric agonism to quantify biased signaling; resultant calculated parameters are in Table 1.

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Assay	pIC ₅₀		pEC ₅₀		Emax (% GLP-1(7-36)NH ₂)	
	- BETP	+ BETP	- BETP	+ BETP	- BETP	+ BETP
Binding	7.92 ± 0.10	8.82 ± 0.13	-	-	-	-
GTPγS	-	-	7.78 ± 0.03	8.40 ± 0.04	95 ± 1	100 ± 1
cAMP	-	-	8.48 ± 0.07	9.44 ± 0.11	98 ± 2	94 ± 3
pERK1/2	-	-	7.34 ± 0.17	6.93 ± 0.17	95 ± 8	83 ± 4
Ca ²⁺	-	-	6.34 ± 0.19	6.51 ± 0.22	105 ± 12	144 ± 9
β-Arr1	-	-	7.13 ± 0.16	7.48 ± 0.25	105 ± 7	147 ± 9
β-Arr2	-	-	6.92 ± 0.13	7.28 ± 0.18	101 ± 6	124 ± 7

Supplementary Table 1

Oxyntomodulin binding and signalling parameters in the absence and presence of 30 μM BETP. Data were analysed using a four parameter logistic equation as defined in materials and methods. pIC₅₀ values represent the negative logarithm of the concentration of ligand that inhibits binding of half the total concentration of radiolabelled antagonist, ¹²⁵I-exendin(9-39). pEC₅₀ values represent the negative logarithm of the concentration of agonist that produces half the maximal response. Emax values represent the maximal response normalised to that elicited by 100 nM GLP-1(7-36)NH₂.