Supplementary Information

Article Title

Allosteric modulation of endogenous metabolites as an avenue for drug discovery

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Journal Title

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Supplemental Fig. 1. Metabolic breakdown of endogenous ligands to their metabolites and structures of the allosteric ligands used in this study. (A) Acetylcholine is metabolised by acetylcholinesterases to choline and acetate. (B) Adenosine is metabolised by adenosine deaminase to inosine. (C) The peptide GLP-1(7-36)NH_2 is degraded by dipeptidyl peptidase IV to the inert metabolite GLP-1(9-36)NH_2 (the primary amino acid sequences are shown). (D) Structures of the four allosteric ligands used in this study.

Supplemental Fig. 2. The allosteric agonist LY2033298 displays positive allosteric modulation of the metabolite choline in GTPγS binding in membranes expressing M2 mAChR. Interaction studies between LY2033298 and ACh (A) or Ch (B) GTPγS binding assays. All values are mean ± SEM of three independent experiments performed in duplicate.

Supplemental Fig. 3. The allosteric agonist LY2033298 shows weak positive allosteric modulation of the metabolite choline in competition binding assays in membranes expressing M2 mAChR. Interaction studies between LY2033298 and Ch in a competition radioligand binding assay using the radioligand [^{3}H]NMS. Curves were fitted using a one site modulator plus allosteric ligand model. The logα for NMS was fitted to 0.5 as determined in Valant *et al* 2012. All values are mean ± SEM of three independent experiments performed in duplicate.

Supplemental Fig. 4. Small molecule ligands of the GLP-1R do not modulate binding affinity of th**e GLP-1(7-36)NH_2 or its metabolite GLP-1R(9-36)NH_2 in competition binding experiments in intact cells expressing human GLP-1R.** Effects of increasing concentrations of

either Compound 2 (A and B) or BETP (C and D) on the inhibition of ^{125}I-exendin(9-39) binding by GLP-1(7-36)NH_2 (A and C) or GLP-1(9-36)NH_2 (B and D). Data are normalised to specific radioligand binding. Nonspecific binding was determined by inhibition of ^{125}I-exendin(9-39) by 1 μM exendin(9-39). All values are mean ± SEM of four independent experiments performed in duplicate.

Supplemental Fig. 5. Ex vivo and in vivo studies reveal allosteric modulation of the GLP-1 metabolite at the GLP-1R leads to insulin secretion. (A) Insulin concentrations from cultures of SD rat islets incubated in media containing low glucose (2.8mM), high glucose (11.2 mM), GLP-1(7-36)NH_2 (100 nM), BETP (1 μM) and GLP-1(9-36)NH_2 (1 and 10 μM) in the presence and absence of BETP (1 μM). Islet treatments were performed for 90 min. (B) Timecourse of plasma insulin concentrations in fasted, anaesthetised animals treated with either vehicle, GLP-1(7-36)NH_2 (3 nmol/kg), GLP-1(9-36)NH_2 (400 nmol/kg), BETP (10 mg/kg) or co-administration of BETP and GLP-1(9-36)NH_2, immediately prior to intravenous administration of a glucose bolus (0.5 g/kg). Inset, AUC_{020min} of the insulin secretion for the various treatment groups. All results are expressed as mean ± SEM of five experiments, (* = $p < 0.05$ as determined using a one way anova followed by Dunnett's comparison to vehicle group).

Supplemental Fig. 6. In vivo studies reveal BETP does not alter the pharmacokinetics of GLP-1(9-36)NH_2. Time course of total plasma GLP-1 levels in fasted anaesthetized animals treated with either vehicle, GLP-1(7-36)NH_2 (3 nmol/kg), GLP-1(9-36)NH_2 (150 nmol/kg), BETP (5 mg/kg) or GLP-1(9-36)NH_2 (150 nmol/kg) in the presence of BETP (5 mg/kg) immediately prior to intravenous administration of a glucose bolus (0.5 g/kg). Inset.Same data set with smaller y axis. Results are expressed as mean ± SEM of six experiments (* = $p < 0.05$ as determined using a one way anova followed by Dunnett's comparison to vehicle group).

Supplementary Figure 1.

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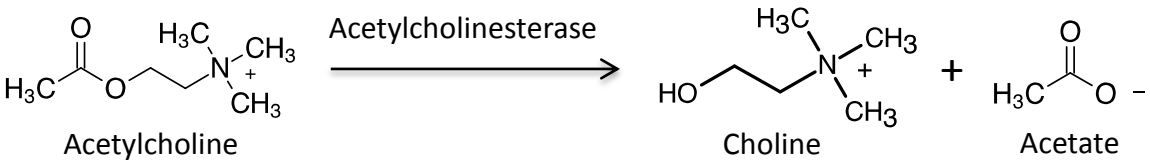

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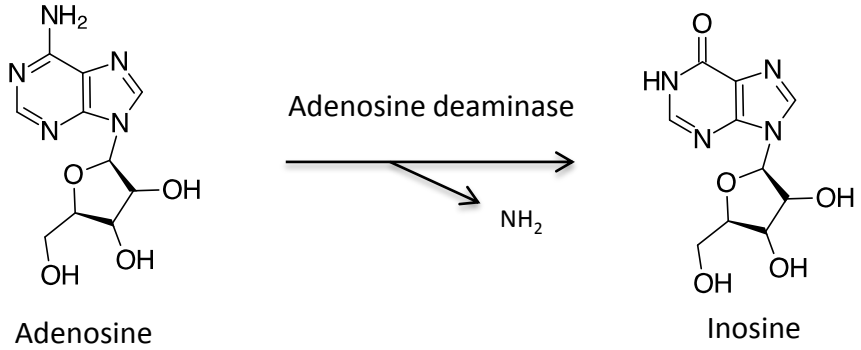

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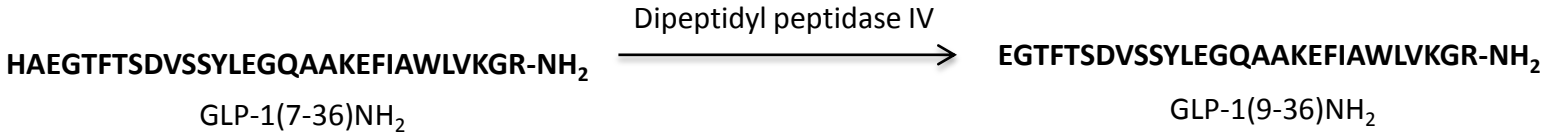

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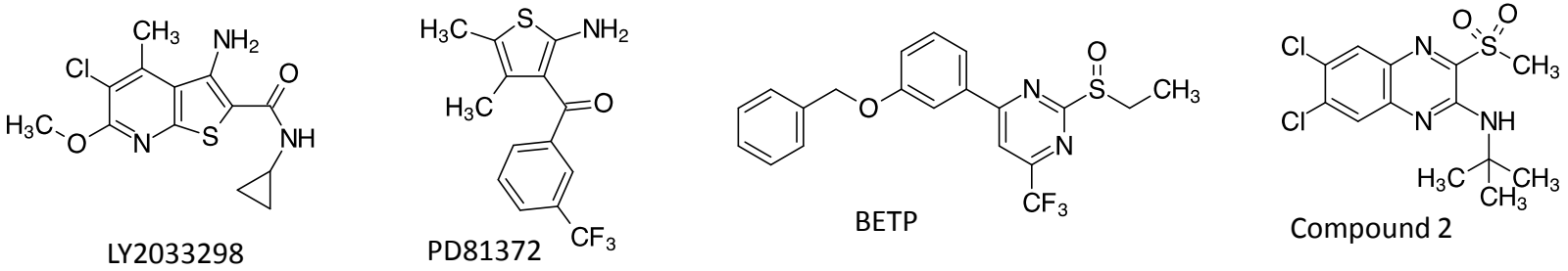

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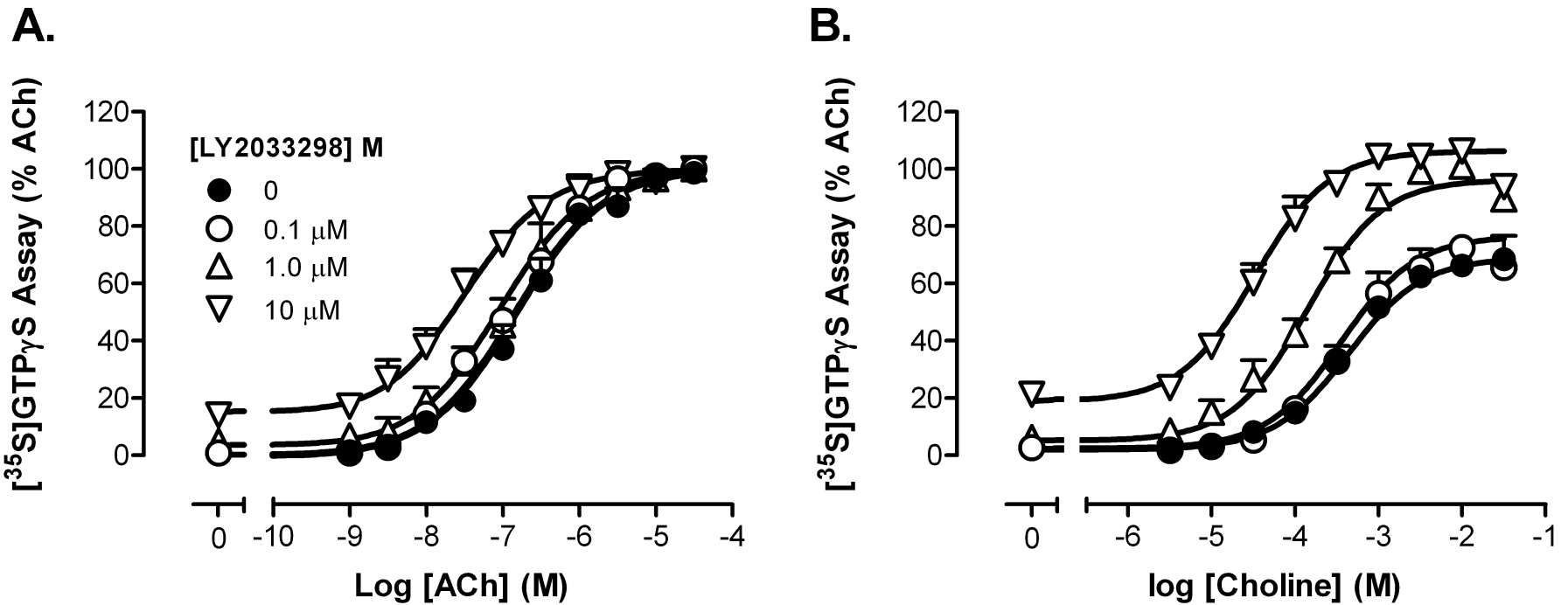

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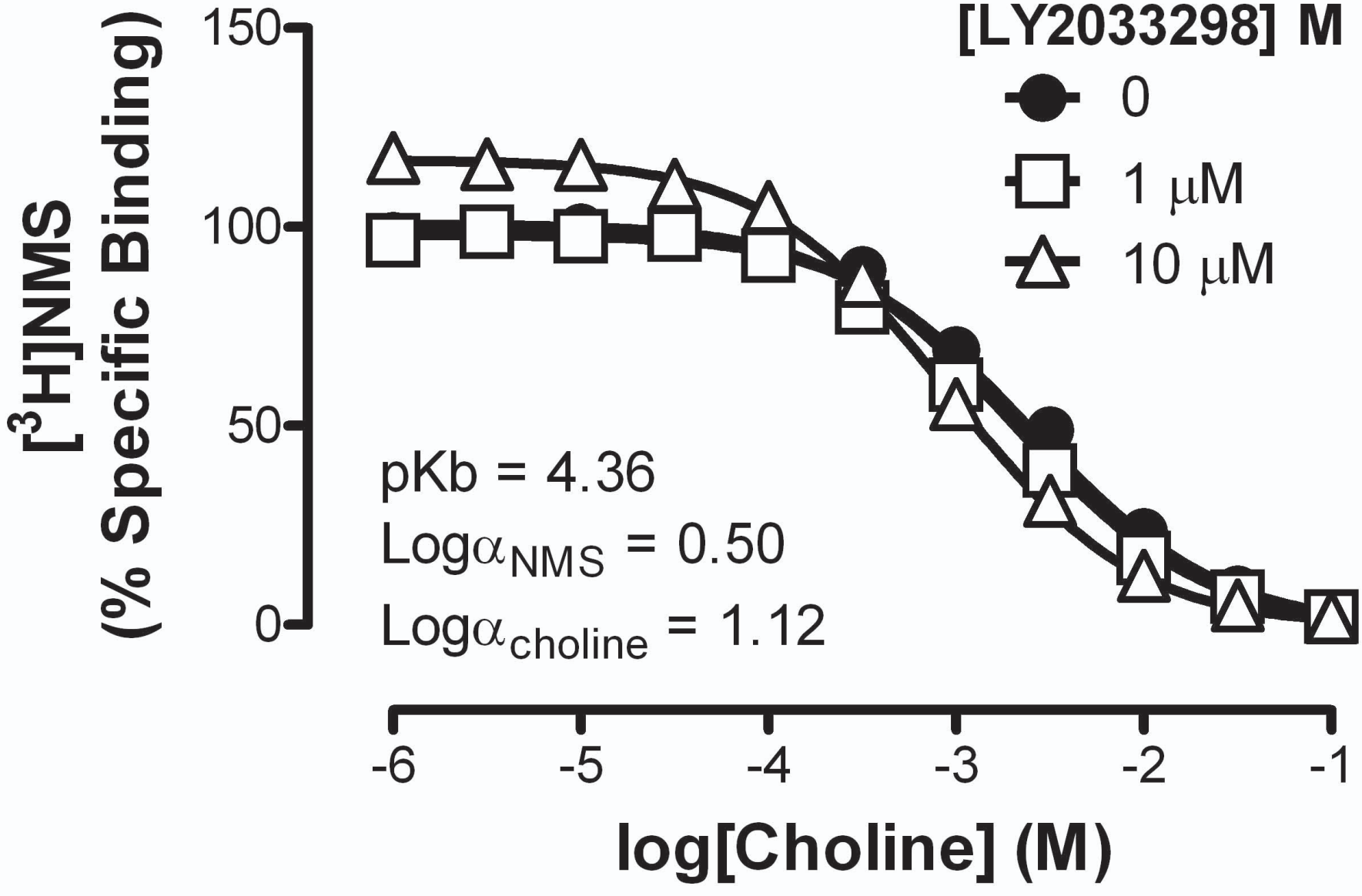

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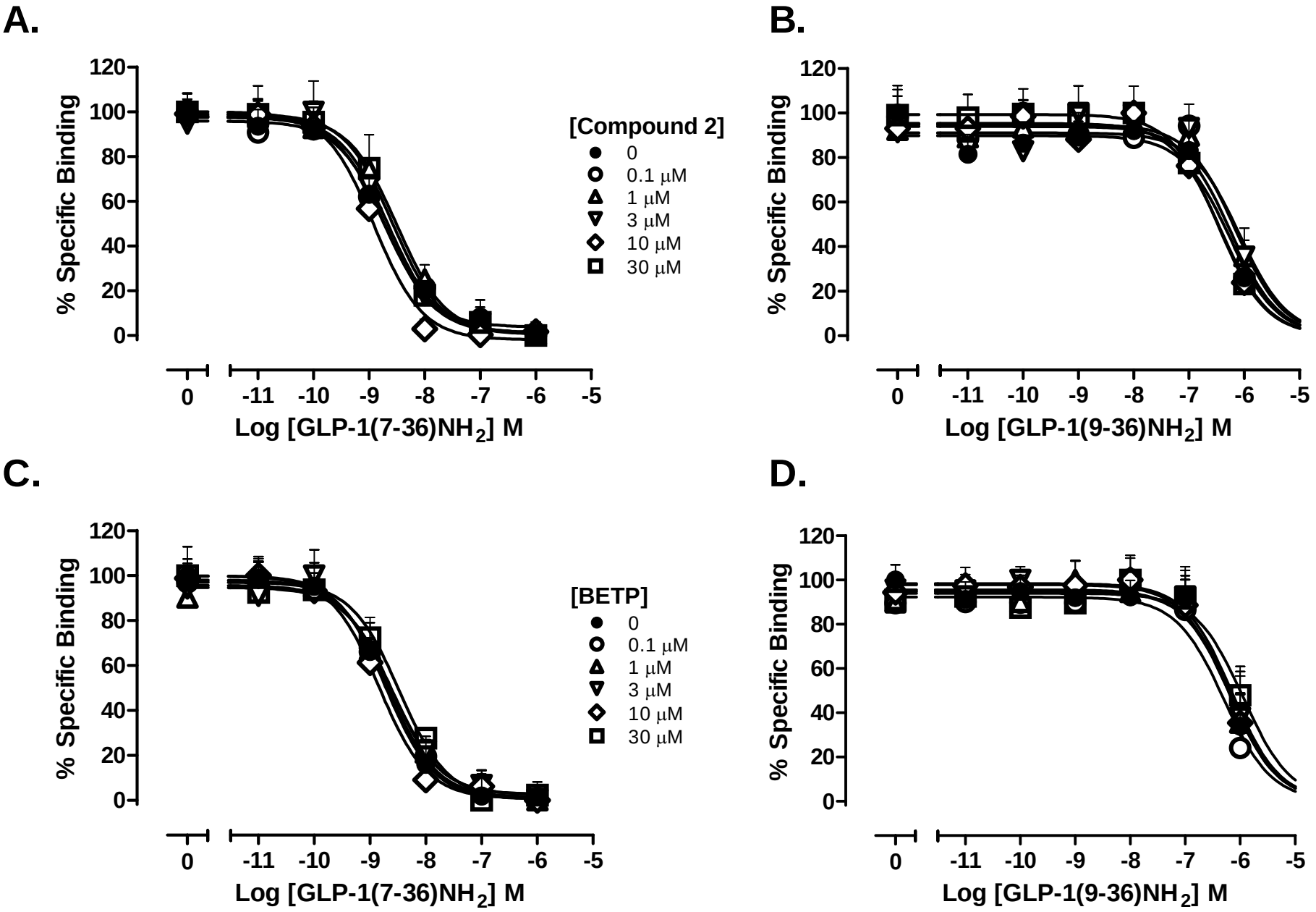
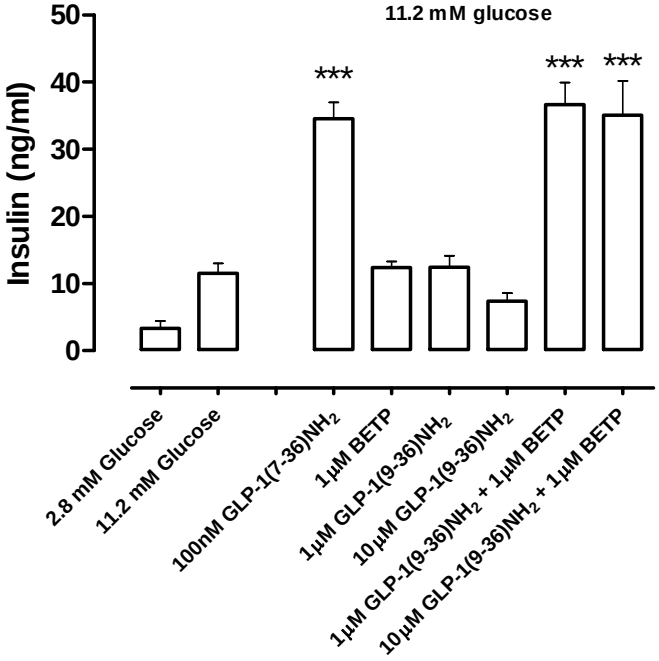


Supplemental Figure 5.

A.



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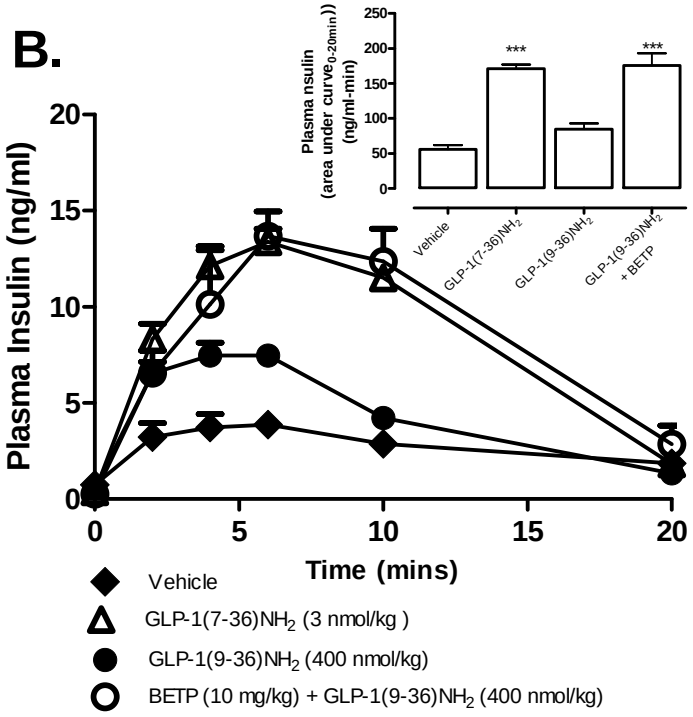

Supplementary Fig. 6