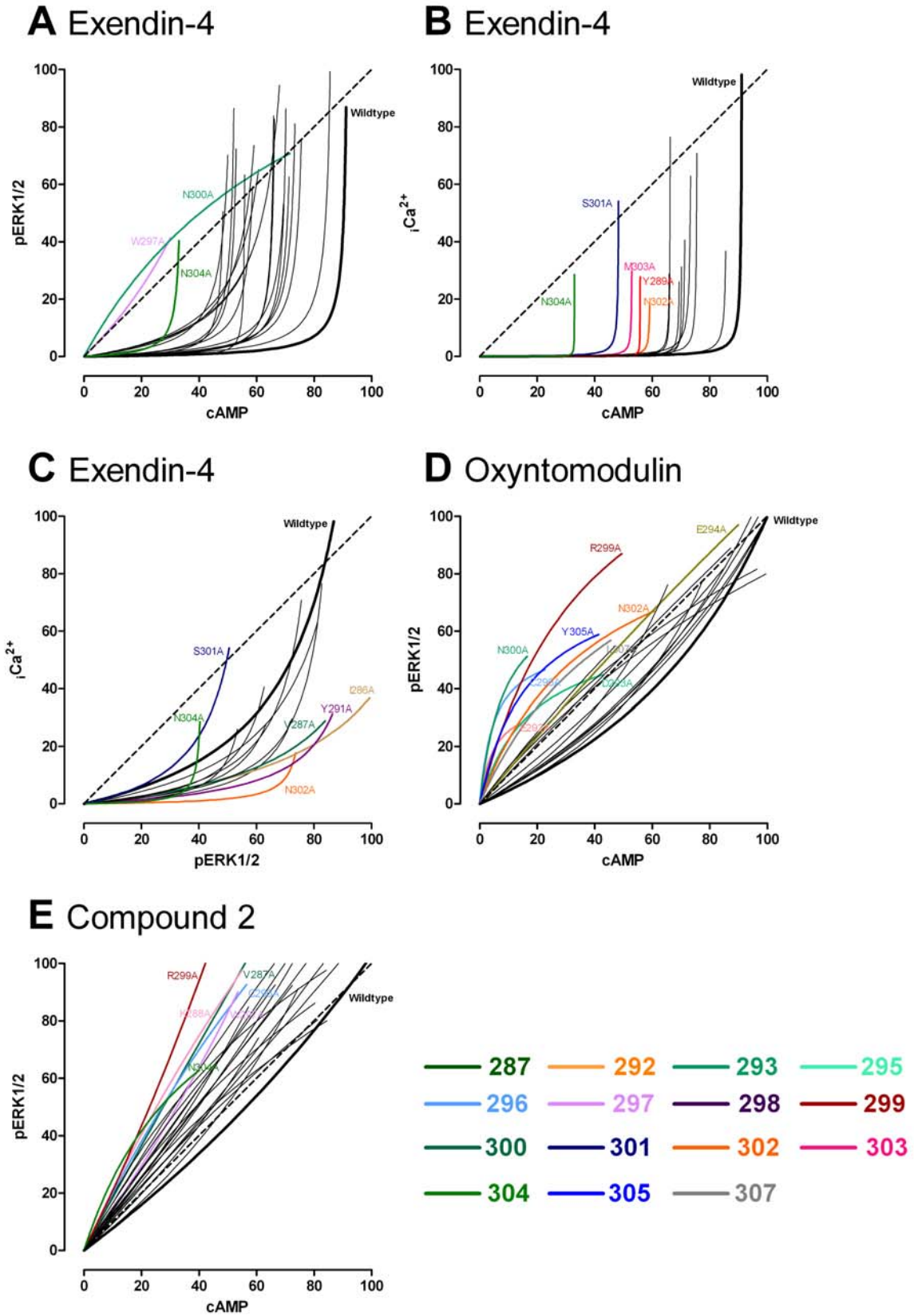
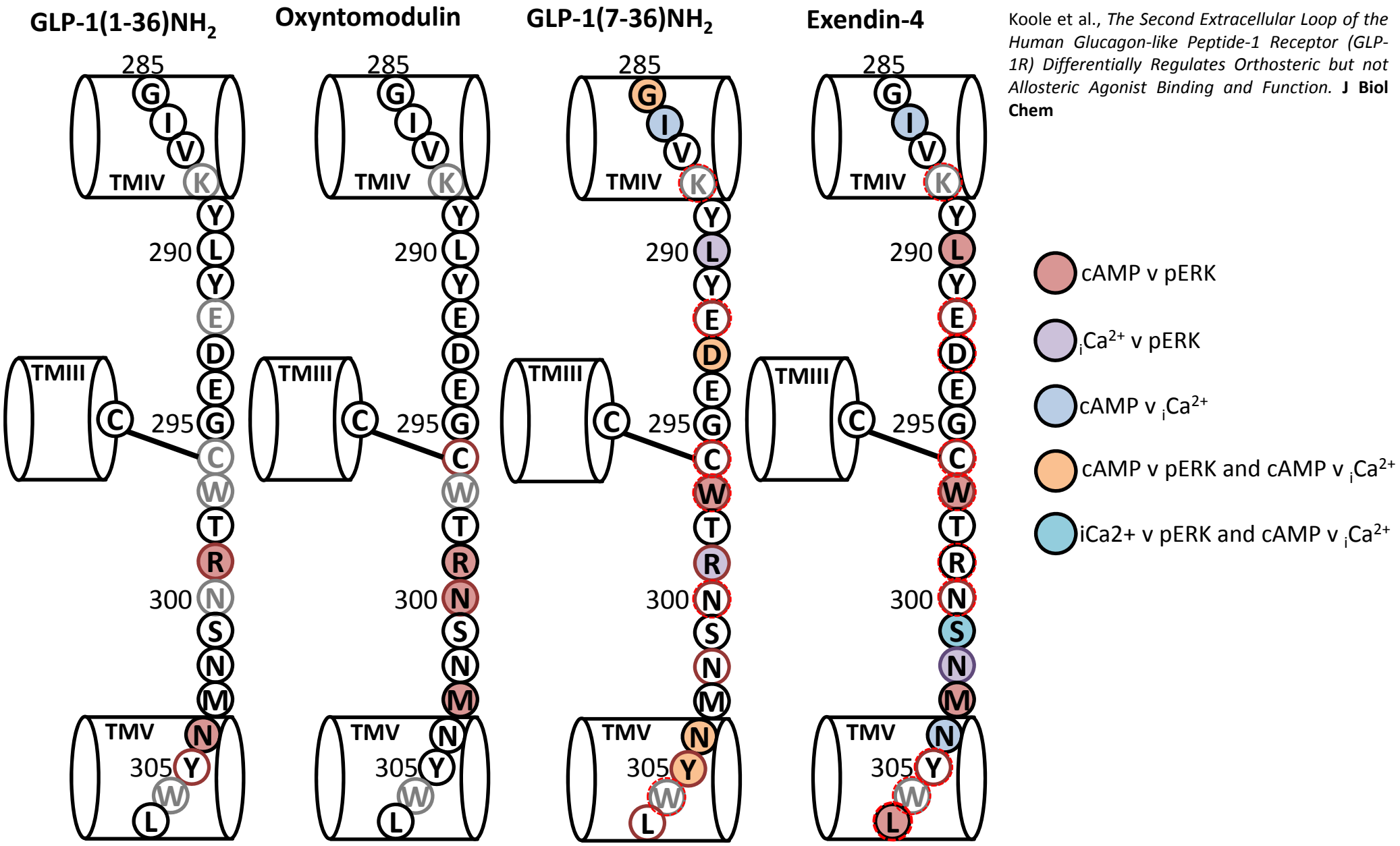
SI Figure S1.



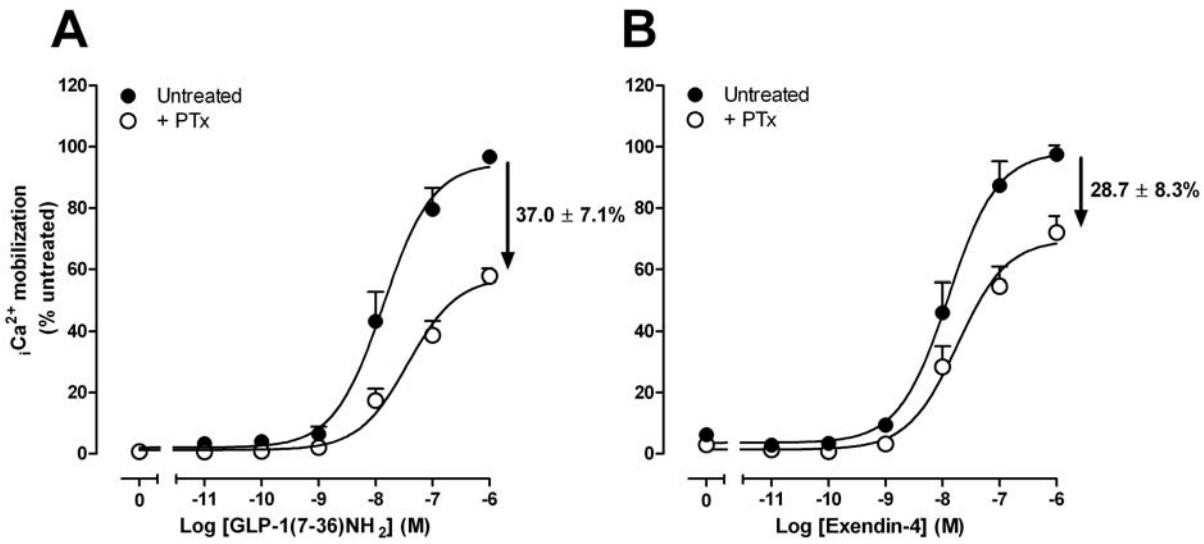
Supplementary Figure S1. Bias plots of human GLP-1R ECL2 alanine mutants. (A) cAMP v pERK1/2 for Exendin-4, (B) cAMP v $_{i}Ca^{2+}$ for Exendin-4, (C) pERK1/2 v $_{i}Ca^{2+}$ for Exendin-4, (D) cAMP v pERK1/2 for Oxyntomodulin and (E) cAMP v pERK1/2 for Compound 2. Data for each pathway are normalized to the maximal response elicited by peptide at the wildtype human GLP-1R, and analyzed with a three-parameter logistic equation as defined in equation 1, with at least 140 points defining the curve.



Koole et al., *The Second Extracellular Loop of the Human Glucagon-like Peptide-1 Receptor (GLP-1R) Differentially Regulates Orthosteric but not Allosteric Agonist Binding and Function.* **J Biol Chem**

Supplementary Figure S2. Schematic representation of ECL2 and adjacent amino acids identifying residues that selectively alter receptor signaling in response to peptide ligands. Filled circles are those that demonstrated non-correlative behaviour in assessment of changes to delta Logtauc (from Figure 6 and Figure 7 from [18]). Those with coloured circle outlines display bias factors that were significantly different from wildtype receptor (Table 6 and Table 5 from [18]). No data are available for correlations with $_{i}Ca^{2+}$ for the oxyntomodulin and GLP-1(1-36)NH_2 peptides due to lack of, or limited response in this assay. Grey circles and letters are those residues for which no cAMP response could be measured. Red-dashed over-circles identify residues for which no $_{i}Ca^{2+}$ response could be measured for the exendin-4 or GLP-1(7-36)NH_2 peptides.

SI Figure S3.



Supplementary Figure S3. PTx inhibition of $_iCa^{2+}$ mobilization at the wildtype human GLP-1R. Characterization of PTx inhibition (A) GLP-1(7-36)NH_2-mediated and (B) Exendin-4-mediated $_iCa^{2+}$ mobilization in FlpInCHO cells stably expressing the wildtype human GLP-1R. Cells were pretreated with 100 ng ml^{-1} PTx overnight in FBS-free DMEM prior to peptide addition and $_iCa^{2+}$ mobilization detection. Data are normalized to the maximal response elicited by peptide alone and analyzed with a three-parameter logistic equation as defined in equation 1. All values are mean ± S.E.M. of six to eight independent experiments, conducted in duplicate.