## **Supporting Information**

Fortin et al. 10.1073/pnas.0900149106

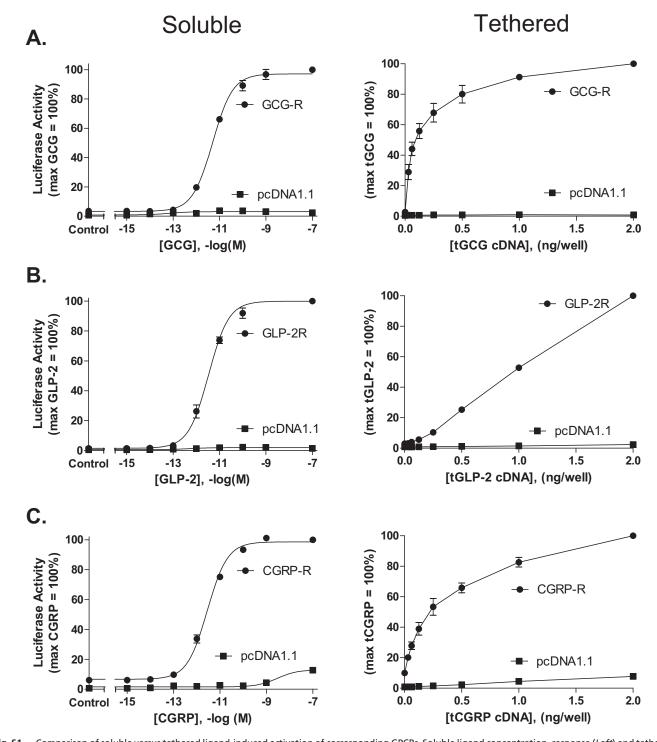


Fig. S1. Comparison of soluble versus tethered ligand-induced activation of corresponding GPCRs. Soluble ligand concentration—response (*Left*) and tethered ligand cDNA—activity curves (*Right*) are compared. HEK293 cells expressing receptors (*A*) GCG-R, (*B*) GLP-2R, and (*C*) CGRP-R were stimulated with either soluble or tethered ligands. CGRP-R requires coexpression of the calcitonin-like receptor and RAMP1 [Poyner DR, et al. (2002) *Pharmacol Rev* 54:233–246]. Each graph represents data (mean ± SEM) from 3 independent experiments performed in triplicate.

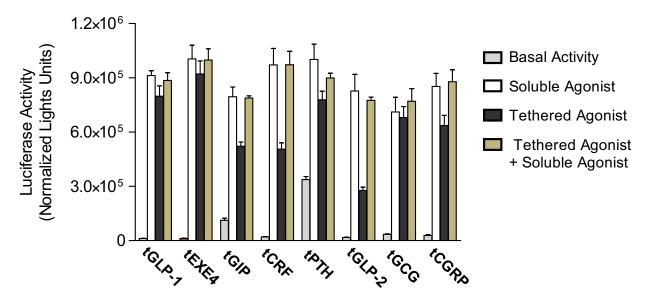


Fig. 52. Comparison of receptor-mediated signaling induced by soluble and tethered ligands. HEK293 cells were transfected with cDNA encoding a class B1 GPCR and a CRE<sub>6x</sub> luciferase reporter gene construct. Where indicated, cDNA encoding a tethered ligand (2 ng per well) was also included. Eighteen hours after transfection, cells were incubated in the presence or absence of a saturating concentration (10<sup>-8</sup> M) of soluble agonist for 6 h. Basal activity corresponds to receptor-mediated signaling in the absence of soluble and tethered ligand. Signaling resulting from the combination of soluble and tethered agonists was comparable to the maximal activity observed with the soluble hormone alone. For tethered peptides that are not fully active relative to the corresponding soluble ligand (tGIP, tCRF, tPTH, tGLP-2, and tCGRP), this finding suggests that such constructs do not induce complete receptor desensitization. The ratio between activities induced by tethered versus corresponding soluble ligand ranged from 0.35 to 1.00. Each bar represents data (mean ± SEM) from 4 independent experiments performed in triplicate.