## **Charest-Morin et al.: Supporting Information**

 $PTH_{1-34}-EGFP$  vector

ACCATGGCCCTGTGGATGCGCCTCCTGCCCGCTGCTG

MetAlaLeuTrpMetArgLeuLeuProLeuLeu

preproinsulin signal peptide

GCGCTGCTGGCCCTCTGGGGACCTGACCCAGCCGCA AlaLeuLeuAlaLeuTrpGlyProAspProAlaAla

GCGTCTGTGAGTGAAATACAGCTTATGCATAACCTG
AlaSerValSerGluIleGlnLeuMetHisAsnLeu
PTH<sub>1-2,4</sub>

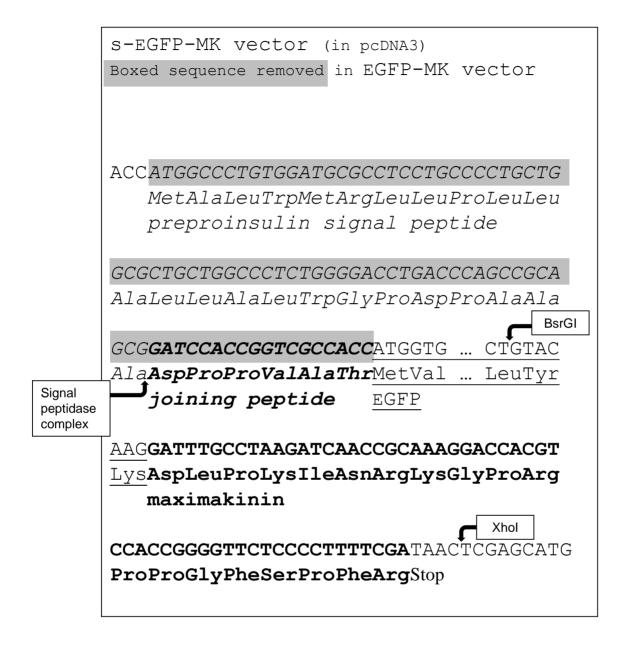
Signal peptidase complex

GGAAAACATCTGAACTCGATGGAGAGAGTAGAATGGCTG GlyLysHisLeuAsnSerMetGluArgValGluTrpLeu

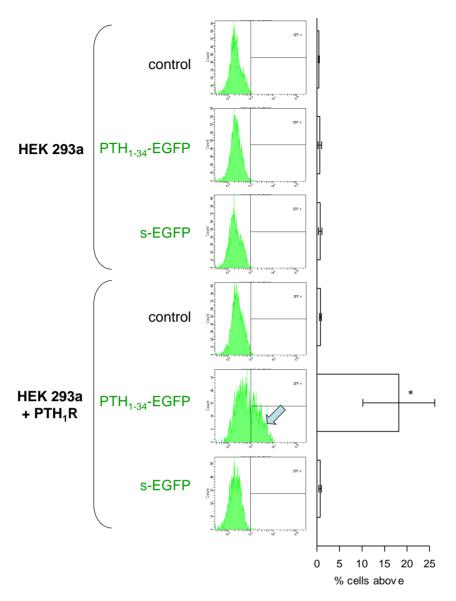
CGTAAGAAGCTGCAGGATGTGCACAATTTT ArgLysLysLeuGlnAspValHisAsnPhe

GATCCACCGGTCGCCACCATGGTG...AAGTAA
AspProProValAlaThrMetVal...Lys Stop
joining peptide EGFP →

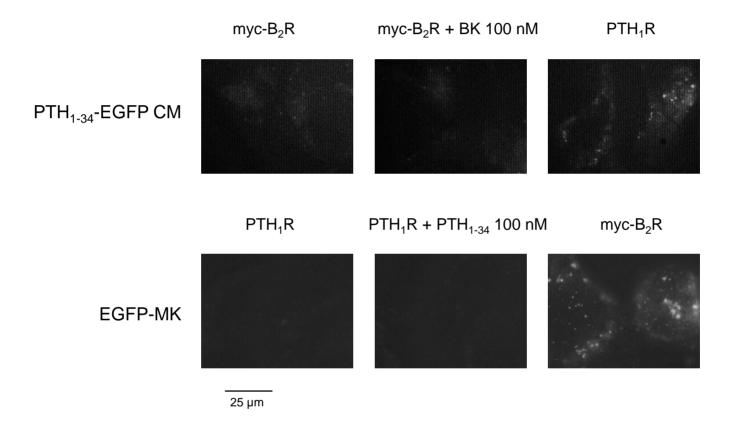
Supplementary Figure 1. Partial coding sequence of the  $PTH_{1-34}$ -EGFP vector. See text for description.



Supplementary Figure 2. Partial coding sequence of the s-EGFP-MK and the EGFP-MK vectors, the second being obtained following the deletion of the boxed sequence (signal peptide + joining peptide). See text for description.



Supplementary Figure 3. Cytofluorometry of HEK 293a cells that optionally expressed PTH<sub>1</sub>R (3 conditions at bottom), sequentially detached and stained for 30 min (37°C) with the undiluted conditioned medium of other cells producing s-EGFP or PTH<sub>1-34</sub>-EGFP, as indicated. Left: distributions based on the counting of 10000 cells. A threshold of autofluorescence was defined using control cells with no fluorophore. It was surpassed only under one set of experimental conditions (arrow). Right: proportion of cells above the threshold under each experimental condition in 3 separate experiments (means  $\pm$  s.e.m.). ANOVA indicated that the values were heterogeneous (P<0.02). \* P<0.05 vs. controls with or without receptors (Tukey-Kramer multiple comparison test). s-EGFP did not significantly stain cells.



Supplementary Figure 4. Lack of stimulated uptake of fluorescent probes by cells expressing the non-corresponding receptor. HEK 293a cells expressing the indicated receptor were incubated for 30 min with one of the EGFP fusion protein and optionally stimulated with a non-fluorescent peptide, as indicated.