

Supplementary Materials for

LRP6 Mediates cAMP Generation by G Protein–Coupled Receptors Through Regulating the Membrane Targeting of G α_s

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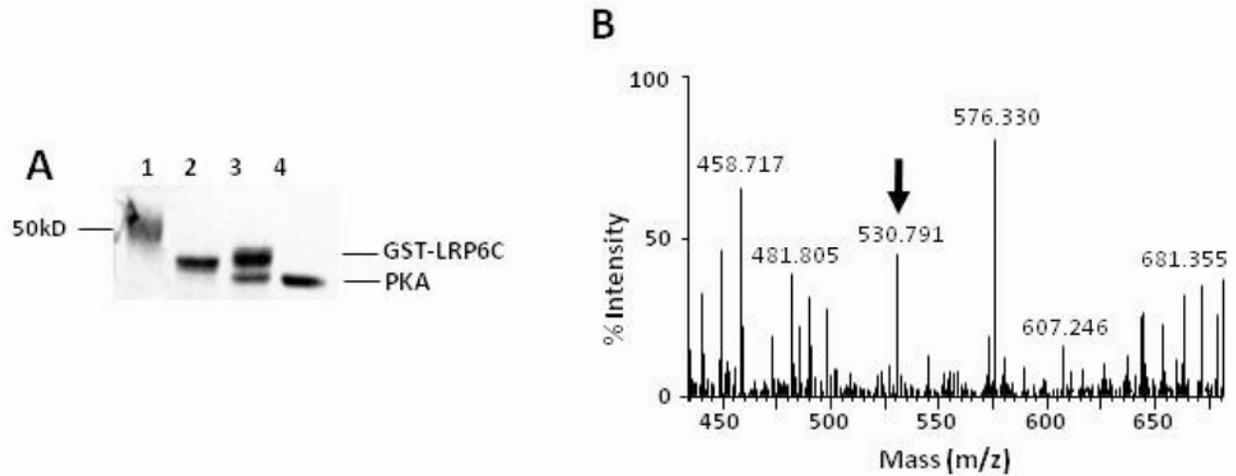


Fig. S1. Mass spectrometric identification of a PKA phosphorylation site in LRP6. **(A)** GST-6C protein was incubated with a recombinant catalytic subunit of PKA and ATP. Phosphorylated GST-6C protein was subjected to SDS-PAGE followed by staining with Coomassie brilliant blue. Lane 1, protein molecular mass marker; Lane 2, GST-6C alone; Lane 3, aliquot of the reaction mixture including GST-6C, PKA, and ATP; Lane 4, PKA alone. The upper band of lane 3 was collected for mass spectrometry analysis. **(B)** Mass spectrometry analysis showed a peak at m/z 530.791 (arrow) different from the mass of the peptide. Data are representative data of three experiments.

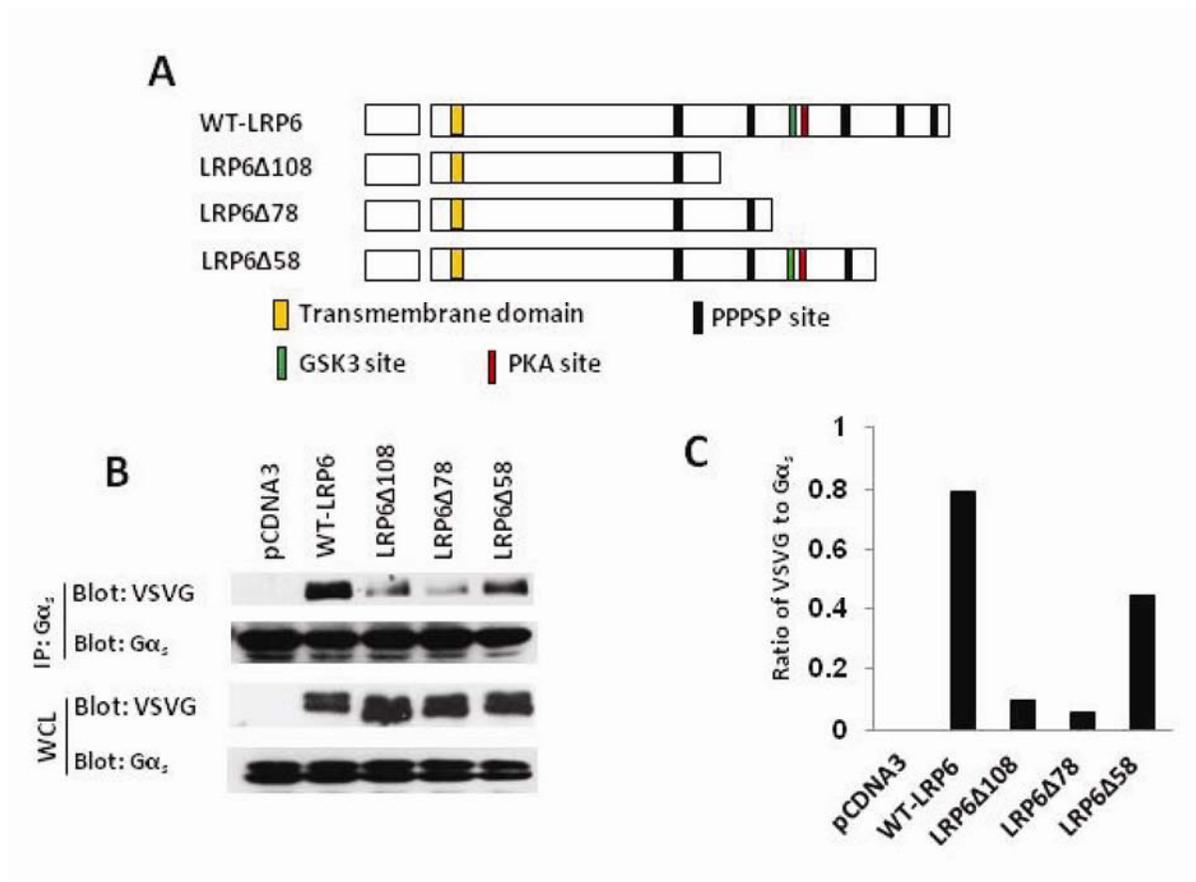


Fig. S2. Identification of the $G\alpha_s$ interaction domain in LRP6. **(A)** WT and mutant constructs used in these experiments. **(B)** HEK 293 cells were transfected with plasmids encoding VSVG-tagged WT LRP6 (WT) or a series of deletion constructs and were treated with PTH(1-34) (50 nM). $G\alpha_s$ -associated LRP6 was detected by Western blotting analysis with an antibody against VSVG. LRP6 and $G\alpha_s$ proteins in cell lysates were detected by Western blotting analysis with antibodies against VSVG and $G\alpha_s$. WCL, whole-cell lysates. **(C)** The intensities of the bands in the Western blots were quantitated by phosphorimaging and normalized to the density of the anti- $G\alpha_s$ blots, that is, the ratio of the band density in the first row to the band density in the second row. Data are representative data of three experiments.