THE α SUBUNIT OF THE G PROTEIN G₁₃ REGULATES THE ACTIVITY OF ONE OR MORE GLI TRANSCRIPTION FACTORS INDEPENDENTLY OF SMOOTHENED* Andrew E. Douglas¹, Jennifer A. Heim², Feng Shen¹, Luciana L. Amada², Natalia A. Riobo³, Martin E. Fernández-Zapico², and David R. Manning¹

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Running head: Activation of Gli by G_{13}

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Supplemental Material

Supplemental Figure Legend

<u>Fig S1.</u> Smoothened couples to Gα_i but does not couple to Gα₁₃. *A*) HEK293 cells were transfected with vector for Smo or the 5-HT_{1A} receptor, or with empty vector (pcDNA). At 48 h, membranes were isolated and [³⁵S]GTPγS binding to endogenous Gα_i was evaluated with or without 10 µM purmorphamine (Smo) or 1µM 8-OH-DPAT (5-HT_{1A} receptor). *B*) HEK293 cells were transfected with vector for Smo or TPα, or with empty vector, and [³⁵S]GTPγS binding to endogenous Gα₁₃ in subsequently prepared membranes was evaluated with or without 10 µM purmorphamine (Smo) or 10 µM U46619 (TPα). *C*) HEK293 cells were transfected with vector, and [³⁵S]GTPγS binding to Prα·Gα₁₃, or with empty vector, and [³⁵S]GTPγS binding was evaluated in subsequently prepared membranes with or without agonists. Data in each panel represent a typical experiment performed in triplicate.

Supplemental Figures

Figure S1.



A. Coupling to endogenous $\mbox{G}\alpha_{i}$

B. Coupling to endogenous $\text{G}\alpha_{13}$



C. Coupling to fused $\text{G}\alpha_{13}$

