

Supplementary Materials for **Hedgehog-Stimulated Chemotaxis Is Mediated by Smoothed Located Outside the Primary Cilium**

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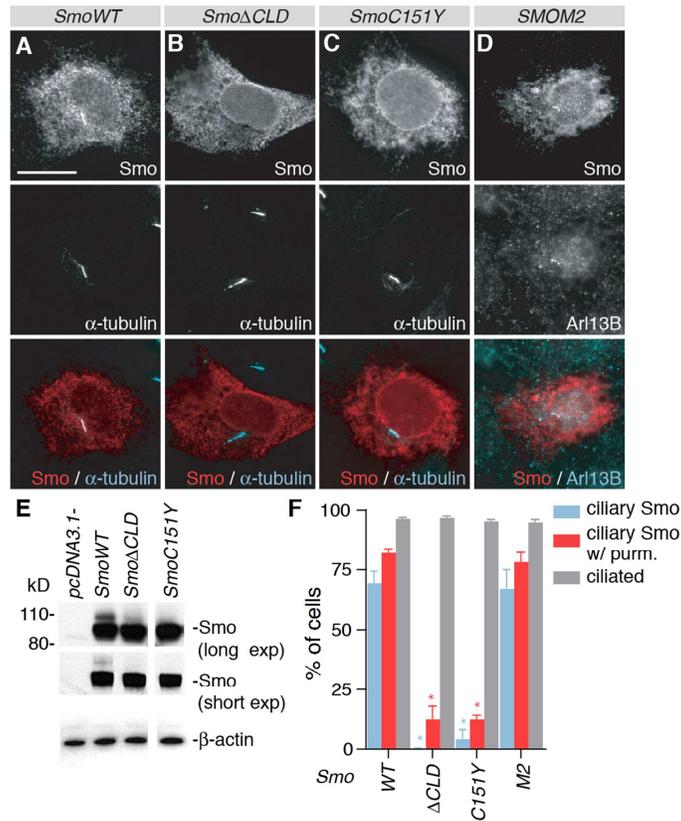


Fig. S1. Smo mutants fail to localize to the primary cilium. **(A-D)** *Smo*^{-/-} MEFs were transfected with mutant forms of *Smo* and localization of Smo was visualized by immunofluorescence for the Myc tag on Smo or the gD tag on SMOM2. Primary cilia were visualized by staining for acetylated α-tubulin or Arl13B. Scale bar, 20 μm. **(E)** The abundance of Smo was assessed by Western blot. Both a short and long exposure of the blot are shown. **(F)** The fraction of cells with primary cilium (gray bars) was determined by staining for acetylated α-tubulin. Ciliary localization of Smo following DMSO control or 2 μM purmorphamine for 16h was quantified by staining for Smo. Data are the mean ± SEM of 50 cells measured in 3 experiments. Statistically significant differences compared to *Smo*^{WT} are indicated, *P , 0.05 by Mann-Whitney statistical test.

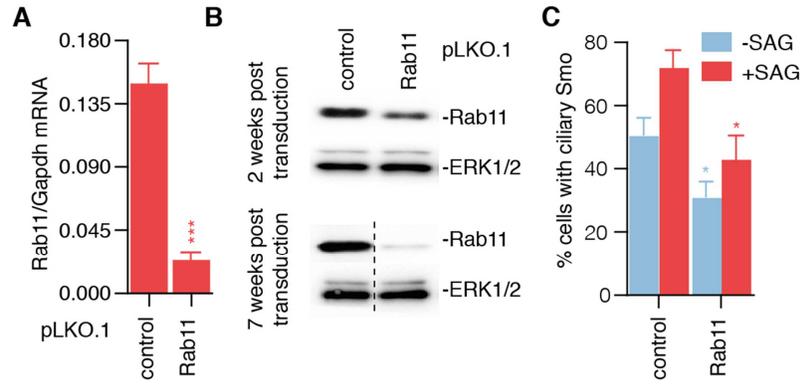


Fig. S2. Rab11 functions in the trafficking of Smo to the primary cilium. (A) *Smo*^{-/-} MEFs were transduced with control pLKO.1 virus or TRC clone 100344 shRNA against Rab11, target sequence; CAGAGATATACCGCATTGTTT. Two weeks after transduction and selection with 1 μ g/mL puromycin, quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for *Rab11* and *Gapdh* was performed to assess knockdown. (B) Two and 7 weeks after transduction, cells were lysed and Western blot analysis for the abundance of extracellular-regulated kinase 1 and 2 (ERK1/2) (Cell Signaling Technologies) and Rab11 (BD Biosciences) was performed. Dashed line indicates part of membrane not shown. Bands shown are from the same membrane and exposure. (C) Stable knockdown cells were transfected with SmoWT, and ciliary localization was assessed by colocalization with acetylated tubulin, following water control or 500 nM SAG treatment for 4 hours. Quantification of at least 50 cells over 3 experiments is shown, mean \pm SEM. *P < 0.05, ***P < 0.005 by Mann-Whitney statistical test.

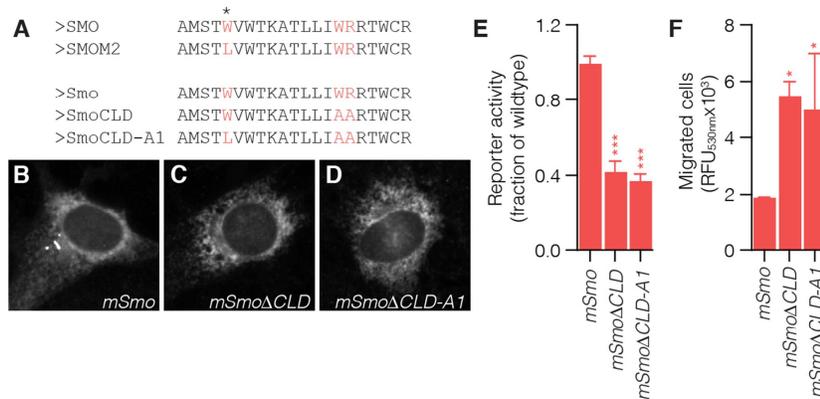


Fig. S3. Activating mutations in Smo do not rescue the transcriptional response phenotype or ciliary localization defect of Smo Δ CLD. (A) Amino acid sequence alignment of human SMO, activated SMOM2, and indicated mouse forms of Smo generated by site-directed mutagenesis (SmoCLD-A1). The SMOM2 mutation is marked with an asterisk. (B-D) *Smo*^{-/-} MEFs were transfected with indicated forms of Smo and immunofluorescence was performed for Smo. (E) *Smo*^{-/-} MEFs were transfected with together with a Gliuciferase reporter construct. Activation of the transcriptional response pathway was measured and is shown as mean fraction of wild-type mSmo (set to 1), \pm SEM, n=3. (F) *Smo*^{-/-} MEFs were transfected and chemotaxis to 2 μ M of purmorphamine was assessed. Data are shown as the mean migration \pm SEM, n \geq 3. Statistically significant differences compared to wild-type mSmo are indicated in panels E and F. *P < 0.05; ***P < 0.005, by Student's t statistical test.

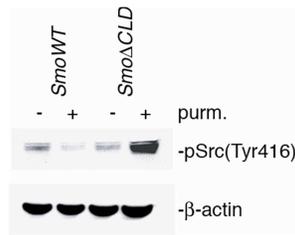


Fig. S4. Activation of nonciliary Smo results in activation of Src. *Smo*^{-/-} MEFs were transfected with SmoWT or SmoΔCLD, serum starved, and treated with 2 μM purmorphamine or DMSO control for 10 min. Cells were subsequently lysed in Laemmli buffer and Western blotting was performed with an antibody recognizing phosphorylated Src (Cell Signaling Technologies) at 1:1,000 or an antibody recognizing β-actin (Santa Cruz Biotechnologies) at 1:1,2,000. Blot shown is representative of 3 experiments.

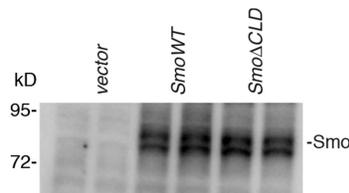


Fig. S5. SmoWT and SmoΔCLD are expressed at similar amounts after transfection of *Smo*^{+/-} ES cells. *Smo*^{+/-} ES cells were transfected with vector, SmoWT, or SmoΔCLD. After 48 hours, cells were subsequently lysed in RIPA buffer, and protein concentration was measured by BCA protein assay (Pierce, Rockford, IL). Equal amounts of protein were mixed with Laemmli buffer and Western blotting was performed with an antibody recognizing the Myc tag on Smo. Shown is a blot from a single experiment.