Supplemental Information

Phosphoinositides Regulate Ciliary Protein Trafficking to Modulate Hedgehog Signaling

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SUPPLEMENTAL FIGURES

Figure S1 (related to Fig.1).

**PI(4)P and PI(4,5)P2 localize to distinct ciliary compartments.** (A) Ciliated IMCD3 cells were stained with anti-PI(4)P (green) and anti-Arl13b (red) antibodies and their nuclei marked with DAPI (blue). Scale bar, 5µm. (B) Purified cilia from sea urchin gastrulae were stained with anti-PI(4)P (red) and anti-detyrosinated tubulin (Glu-Tub, green) antibodies. Scale bar, 5µm. (C) Live IMCD3 cells were imaged for the ciliary marker 5HT6-CFP (red) and the PI(4,5)P2 sensor EYFP-PH<sup>PLCδ1</sup> (green). Scale bar, 5µm. (D) XZ optical section of live IMCD3 cells expressing 5HT6-CFP (red) and EYFP-PH<sup>PLCδ1</sup> (green). Scale bar, 5µm. (E) Live imaging of NIH-3T3 cells cotransfected with plasmids expressing the PI(4,5)P2 sensor mCerulean3-PH<sup>PLCδ1</sup> (green) and the indicated ciliary fusion proteins (red) containing the catalytically active and inactive forms of Inp54p, a yeast PI(4,5)P2 5-phosphatase, and PIPK, a mouse PI(4)P 5-kinase. Scale bars, 5µm. (F) Quantitation of the extension of the mCerulean3-PH<sup>PLCδ1</sup> fluorescence relative to ciliary length. The catalytically active phosphatase and kinase decreased and increased, respectively, the extent of ciliary mCerulean3-PH<sup>PLCδ1</sup> fluorescence. Data are means ± standard error of the mean (SEM). Asterisks indicate p<0.05 in unpaired t-tests.

Figure S2 (related to Fig.2).

**Inpp5e and Tctn1 affect ciliary PI(4,5)P2 levels.** (A) MEFs derived from littermate Inpp5e<sup>+/−</sup> and Inpp5e<sup>−/−</sup> embryos were stained for Tub<sup>Ac</sup> (red), Inpp5e (green), γ-Tub (cyan) and DNA (blue). Scale bar, 5µm. (B) Inpp5e<sup>+/−</sup> and Inpp5e<sup>−/−</sup> MEFs were starved for 48 hours and stained for Tub<sup>Ac</sup> (green), Arl13b (red), and DNA (blue). Scale bar, 5µm. (C) Quantitation
of the proportion of \textit{Inpp5e}^{+/−} and \textit{Inpp5e}^{−/−} MEFs that possessed cilia. Error bars depict standard deviations (SDs). (D) Live imaging of \textit{Inpp5e}^{−/−} MEFs cotransfected with plasmids expressing the PI(4,5)P\textsubscript{2} sensor mCerulean3-PH\textsuperscript{PLC\textgamma{1}} (green) and the indicated ciliary fusion proteins (red) of catalytically inactive (D281A) or wild type Inp54p, a yeast PI(4,5)P\textsubscript{2} 5-phosphatase. Scale bars, 5\textmu m. Quantitation of the extent of ciliary mCerulean3-PH\textsuperscript{PLC\textgamma{1}} fluorescence (PI(4,5)P\textsubscript{2} length) relative to the cilium length in \textit{Inpp5e}^{−/−} MEFs. Data are means ± SEM. Asterisks indicate p<0.01 in unpaired t-tests. (E) Live \textit{Tctn1}^{+/+} and \textit{Tctn1}^{−/−} MEFs were imaged for the ciliary marker 5HT\textsubscript{6}-CFP (red) and the PI(4,5)P\textsubscript{2} sensor EYFP-PH\textsuperscript{PLC\textgamma{1}} (green). Scale bars, 5\textmu m. (F) Quantitation of the extent of ciliary EYFP-PH\textsuperscript{PLC\textgamma{1}} fluorescence (PI(4,5)P\textsubscript{2} length) relative to the extent of 5HT\textsubscript{6}-CFP fluorescence (Cilium length) in \textit{Tctn1}^{+/+} and \textit{Tctn1}^{−/−} MEFs. Data are means ± SEM. Asterisks indicate p<0.01 in unpaired t-tests.

**Figure S3** (related to Fig.3).

\textbf{Inpp5e regulates Hh signaling.} (A) qRT-PCR quantitation of \textit{Gli1} expression by \textit{Inpp5e}^{+/−} and \textit{Inpp5e}^{−/−} MEFs treated with vehicle (DMSO), SAG or ShhN. Data are means ± SDs from triplicates of one experiment. (B) qRT-PCR quantitation of \textit{Ptch1} expression by \textit{Inpp5e}^{+/−} and \textit{Inpp5e}^{−/−} MEFs treated with vehicle (DMSO), SAG or ShhN. Data are means ± SDs from triplicates of one experiment. (C) Fold induction of \textit{Ptch1} and \textit{Gli1} expression by SAG relative to vehicle (DMSO) in \textit{Inpp5e}^{+/−} and \textit{Inpp5e}^{−/−} MEFs. Asterisks indicate p<0.01 in unpaired t-tests. (D) \textit{Inpp5e}^{+/−} and \textit{Inpp5e}^{−/−} MEFs were stained for Tub\textsuperscr{Ac} (red), Ptch1 (green) and DNA (blue). Arrows indicate ciliary Ptch1. Insets depict magnified view of a single cilium. Scale bar, 5\textmu m. (E) \textit{Inpp5e}^{+/−} and \textit{Inpp5e}^{−/−} MEFs were stained for Tub\textsuperscr{Ac} (red),
Polycystin-2 (green) and DNA (blue). Arrows indicate ciliary Polycystin-2. Insets depict magnified view of a single cilium. Scale bar, 5µm. (F) Fold increase in ciliary Smo by SAG relative to vehicle in Inpp5e+/− and Inpp5e−/− MEFs. (G) Fold increase in Gli3 at the ciliary tip by SAG relative to vehicle in Inpp5e+/− and Inpp5e−/− MEFs. Asterisk indicates p<0.05 in unpaired t-test. (H) Fold reduction in ciliary Gpr161 levels by SAG relative to vehicle in Inpp5e+/− and Inpp5e−/− MEFs.

Figure S4 (related to Fig.4).

**Inpp5e regulates IFT-A but not IFT-B ciliary localization.** (A) Inpp5e+/− and Inpp5e−/− MEF lysates were blotted for Tulp3 (top) and α-Tubulin (bottom). Molecular weight markers are shown in the left. (B) Inpp5e+/− and Inpp5e−/− MEFs were stained for TubAc (green), Ift139 (red), γ-Tub (cyan), and DNA (blue). Scale bar, 5µm. Insets depict magnified view of a single cilium. (C) Ventral neural tube sections of E9.5 Inpp5e+/− and Inpp5e−/− mouse embryos were stained for Arl13b (red), γ-Tub (cyan), Ift88 (green), and DNA (blue). Ventral is down. Insets depict magnified view of cilia. Scale bar, 5µm. (D) Tctn1+/+ and Tctn1−/− MEFs were stained for TubAc (green), Tulp3 (red), γ-Tub (cyan), and DNA (blue). Scale bar, 5µm. (E) Tctn1+/+ and Tctn1−/− MEFs were stained for TubAc (green), Gpr161 (red), γ-Tub (cyan), and DNA (blue). Scale bar, 5µm.

Figure S5 (related to Fig.5).

**Ciliary PI(4,5)P2 synthesis increases ciliary Gpr161 levels.** (A) IMCD3 cells expressing wild type (WT) or catalytically inactive (D253A) 5-HT6-EYFP-PIPK were stained for TubAc (cyan), EYFP (green) and Gpr161 (red). Arrowheads indicate 5-HT6-EYFP-PIPK-containing
cilia. Scale bar, 5µm. (B) Quantification of the fluorescence intensity of Gpr161 in cilia expressing 5-HT$_6$-EYFP-PIPK WT or D253A. Data are means ± SEM. Asterisk indicates p<0.05 in unpaired t-test.

**Figure S6 (related to Fig.6).**

**Inhibiting Tulp3 or Gpr161 increases Hh signaling in Inpp5e$^{-/-}$ cells.** (A) Inpp5e$^{-/-}$ MEFs transfected with Tulp3 siRNA (siTulp3), Gpr161 siRNA (siGpr161) or scrambled control siRNA (siCtrl) were stained for Tulp3 or Gpr161 (green), Tub$^\text{Ac}$ (red), γTub (cyan) and nuclei (blue). Scale bars, 5µm. (B) Lysates of Inpp5e$^{-/-}$ MEFs transfected with either a scrambled control (siControl) or Tulp3 (siTulp3) siRNAs were blotted for Tulp3 (top) and α-Tubulin (bottom). Molecular weight markers are shown in the left. (C) Quantification of the fluorescence intensity of Tulp3 and Gpr161 in cilia of siTulp3 or siGpr161-transfected Inpp5e$^{-/-}$ MEFs relative to siCtrl-transfected Inpp5e$^{-/-}$ MEFs. Data are means ± SEM. One asterisk indicates p<0.05 and two indicates p<0.01 in unpaired t-tests. (D) Inpp5e$^{+/-}$ and Inpp5e$^{-/-}$ MEFs transfected with siTulp3, siGpr161 or siCtrl were treated with SAG or vehicle and expression of Ptch1 was measured by qRT-PCR. Error bars represent SDs of three independent experiments. One asterisk indicates p<0.05 and two represents p<0.01 in unpaired t-tests. (E) Fold induction of Ptch1 expression by SAG relative to vehicle in Inpp5e$^{+/-}$ and Inpp5e$^{-/-}$ MEFs. Data are means ± SEM. One asterisk indicates p<0.05 and two indicates p<0.01 in unpaired t-tests.
Figure S1. Garcia-Gonzalo et al. 2015

A) PI(4)P

B) PI(4)P

C) Cilium

D) Cilium

E) 5HT-EYFP-Inp54p (D281A)

F) Histogram showing PI(4,5)P₂ length / Cilia length for different conditions:

- Inp54p (D281A)
- Inp54p (WT)
- PIPK (D253A)
- PIPK (WT)
Figure S2. Garcia-Gonzalo et al. 2015

A) Tub Ac
Inpp5e
Merge γTub
DNA

B) Tub Ac
Arl13bAcAc
PI(4,5)P₂
5HT₆-EYFP-Inp54p
(WT)

C) Inpp5e

D) Inpp5e

Ciliation (%)

Inpp5e

E) Cilium
Tctn1

F) PI(4,5)P₂ length / Cilium length (%)

Tctn1

* *
Gli1 mRNA level (A.U.)

Inpp5e+/–  Inpp5e–/–

Ptch1 mRNA level (A.U.)

DMSO  SAG  ShhN

Fold induction by SAG

Ptch1  Gli1

Inpp5e

Fold Smo increase by SAG

Fold Gli3 increase by SAG

Fold Gpr161 reduction by SAG
Figure S5. Garcia-Gonzalo et al. 2015

A

Tub\textsuperscript{Ac}  
5HT\textsubscript{6}-EYFP-PIPK-D253A  
Gpr161  
Merge DNA

B

Gpr161 Ciliary Intensity (A.U.)

\begin{tabular}{l l}
  & PIPK-D253A & PIPK \\
  \textbf{PIPK D253A} & 1 & * \textbf{PIPK} \\
\end{tabular}