Inventory of Supplementary Materials:

Figure S1, related to Figure 1. PI(4)P and PI(4,5)P2 localize to distinct ciliary compartments.

Figure S2, related to Figure 2. Inpp5e and Tctn1 affect ciliary PI(4,5)P_2 levels.

Figure S3, related to Figure 3. Inpp5e regulates Hh signaling.

Figure S4, related to Figure 4. Inpp5e regulates IFT-A but not IFT-B ciliary localization.

Figure S5, related to Figure 5. Ciliary PI(4,5)P_2 synthesis increases ciliary Gpr161 levels.

Figure S6, related to Figure 6. Lowering Tulp3 levels rescues Hh signaling in *Inpp5e*$^{-/-}$ cells.

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). (**B**) Purified cilia from sea urchin gastrulae were stained with anti-PI(4)P (red) and anti-detyrosinated tubulin (Glu-Tub, green) antibodies. (**C**) Live IMCD3 cells were imaged for the ciliary marker 5HT6-CFP (red) and the PI(4,5)P_2 sensor EYFP-PH^{PLCd1} (green). (**D**) XZ confocal scan of live IMCD3 cells imaged as in C. (**E**) Live imaging of NIH-3T3 cells cotransfected with plasmids expressing the PI(4,5)P2 sensor mCerulean3-PH^{PLCd1} (green) and the indicated ciliary fusion proteins (red) containing the catalytically active and inactive forms of Inp54p, a yeast PI(4,5)P_2 5-phosphatase, and PIPK, a mouse PI(4)P 5-kinase. Scale bars, 5μm. (**F**) Quantitation of the extension of the mCerulean3-PH^{PLCd1} flurorescence relative to ciliary length. The catalytically active phosphatase and kinase decrease and increase, respectively, the extent of ciliary mCerulean3-PH^{PLCd1} flurorescence. Asterisks indicate $p<0.05$ in unpaired t-tests.

Figure S2 (related to Fig.2).

Inpp5e and Tctn1 affect ciliary PI(4,5)P_2 levels. (**A**) MEFs derived from littermate *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ embryos were stained for Tub^{Ac} (red), Inpp5e (green), γ-Tub (cyan) and DNA (blue). (**B**) *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs were starved for 48 hours and stained for Tub^{Ac} (green), Arl13b (red), and DNA (blue). (**C**) Quantitation of the proportion of *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs that possessed cilia. Error bars depict

standard deviations. (**D**) Live imaging of *Inpp5e*$^{-/-}$ MEFs cotransfected with plasmids expressing the PI(4,5)P_2 sensor mCerulean3-PH^{PLCd1} (green) and the indicated ciliary fusion proteins (red) of catalytically inactive (D281A) or wild type Inp54p, a yeast PI(4,5)P_2 5-phosphatase. (**E**) Live *Tctn1*$^{+/+}$ and *Tctn1*$^{-/-}$ MEFs were imaged for the ciliary marker 5HT6-CFP (red) and the PI(4,5)P_2 sensor EYFP-PH^{PLCd1} (green). (**F**) Quantitation of the extent of ciliary EYFP-PH^{PLCd1} fluorescence (PI(4,5)P_2 length) relative to the extent of 5HT_6-CFP fluorescence (Cilium length) in *Tctn1*$^{+/+}$ and *Tctn1*$^{-/-}$ MEFs. Data are means±SEM. Asterisks indicate $p<0.01$ in unpaired t-tests.

Figure S3 (related to Fig.3).

Inpp5e regulates Hh signaling. (**A**) qRT-PCR quantitation of *Gli1* expression by *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs treated with vehicle (DMSO), SAG or ShhN. Data are means±SDs from triplicates of one experiment. (**B**) qRT-PCR quantitation of *Ptch1* expression by *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs treated with vehicle (DMSO), SAG or ShhN. Data are means±SDs from triplicates of one experiment. (**C**) Fold induction of *Ptch1* and *Gli1* expression by SAG relative to vehicle (DMSO) in *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs. Asterisks indicate $p<0.01$ in unpaired t-tests. (**D**) *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs were stained for Tub^{Ac} (red), Ptch1 (green) and DNA (blue). Arrows indicate ciliary Ptch1. (**E**) *Inpp5e*$^{+/-}$ and *Inpp5e*$^{-/-}$ MEFs were stained for Tub^{Ac} (red), Polycystin-2 (green) and DNA (blue). Arrows indicate ciliary Polycystin-2. (**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 Tub^{Ac} (green), Ift139 (red), γ-Tub (cyan), and DNA (blue). Scale bar, 5μm. (**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. (**D**) *Tctn1*$^{+/+}$ and *Tctn1*$^{-/-}$ MEFs were stained for Tub^{Ac} (green), Tulp3 (red), γ-Tub (cyan), and DNA (blue). (**E**) *Tctn1*$^{+/+}$ and *Tctn1*$^{-/-}$ MEFs were stained for Tub^{Ac} (green), Gpr161 (red), γ-Tub (cyan), and DNA (blue).

Figure S5 (related to Fig.5).

Ciliary PI(4,5)P_2 synthesis increases ciliary Gpr161 levels. (**A**) IMCD3 cells expressing wild type (WT) or catalytically inactive (D253A) 5-HT_6-EYFP-PIPK were stained for Tub^{Ac} (cyan), EYFP (green) and Gpr161 (red). Arrowheads indicate 5-HT_6-EYFP-PIPK-containing cilia. (**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^{Ac} (red), γTub (cyan) and nuclei (blue). (**B**) Lysates of *Inpp5e*$^{-/-}$ MEFs transfected with either a scrambled control (*siControl*) or Tulp3 (*siTulp3*) siRNAs were blotted for Tulp3 (top) and a-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 $p<0.01$ 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 standard deviations of three independent experiments. One asterisk indicates $p<0.05$ and two $p<0.01$ 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 $p<0.01$ in unpaired t-tests.

Figure S1. Garcia-Gonzalo et al. 2015

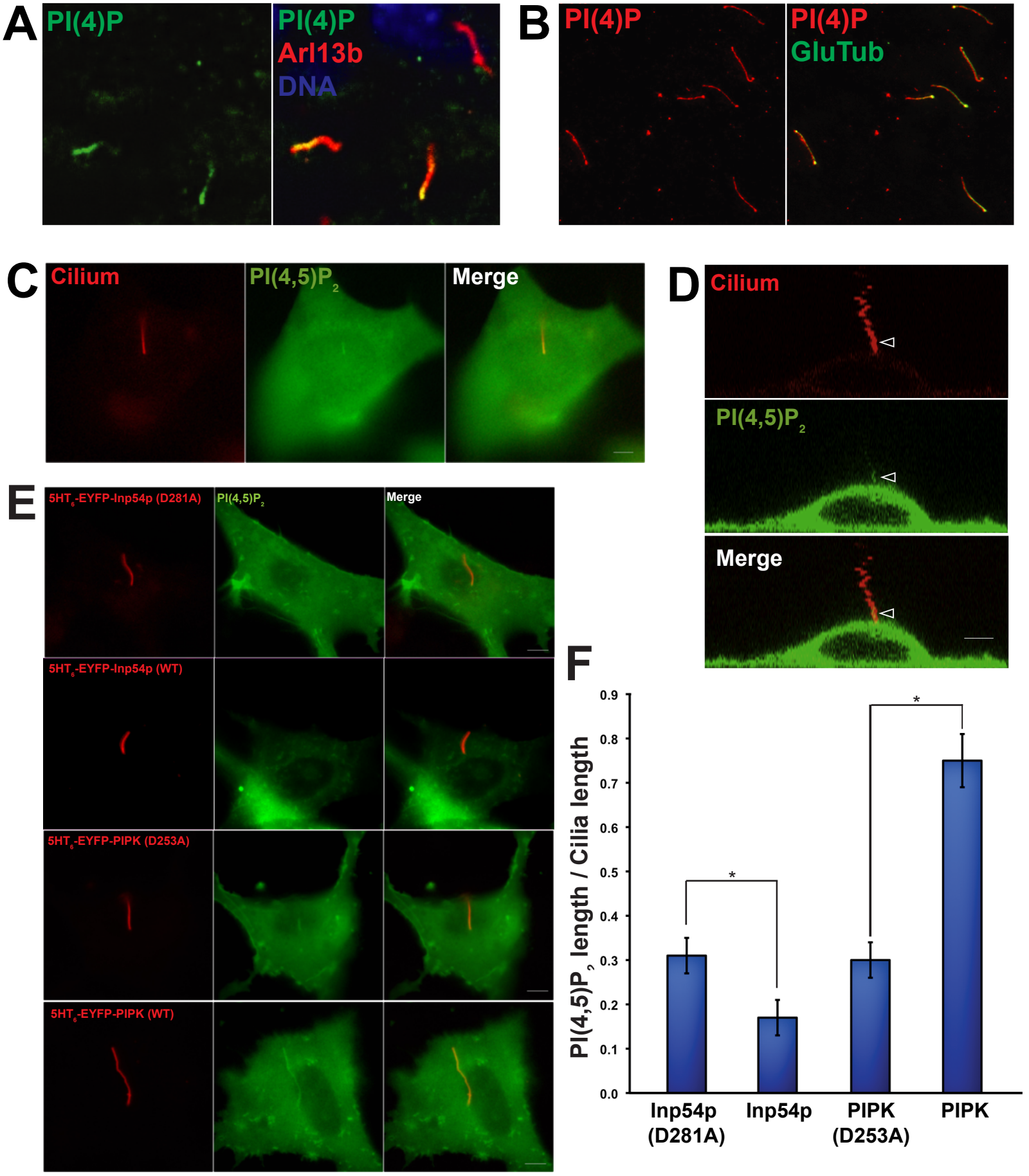


Figure S2. Garcia-Gonzalo et al. 2015

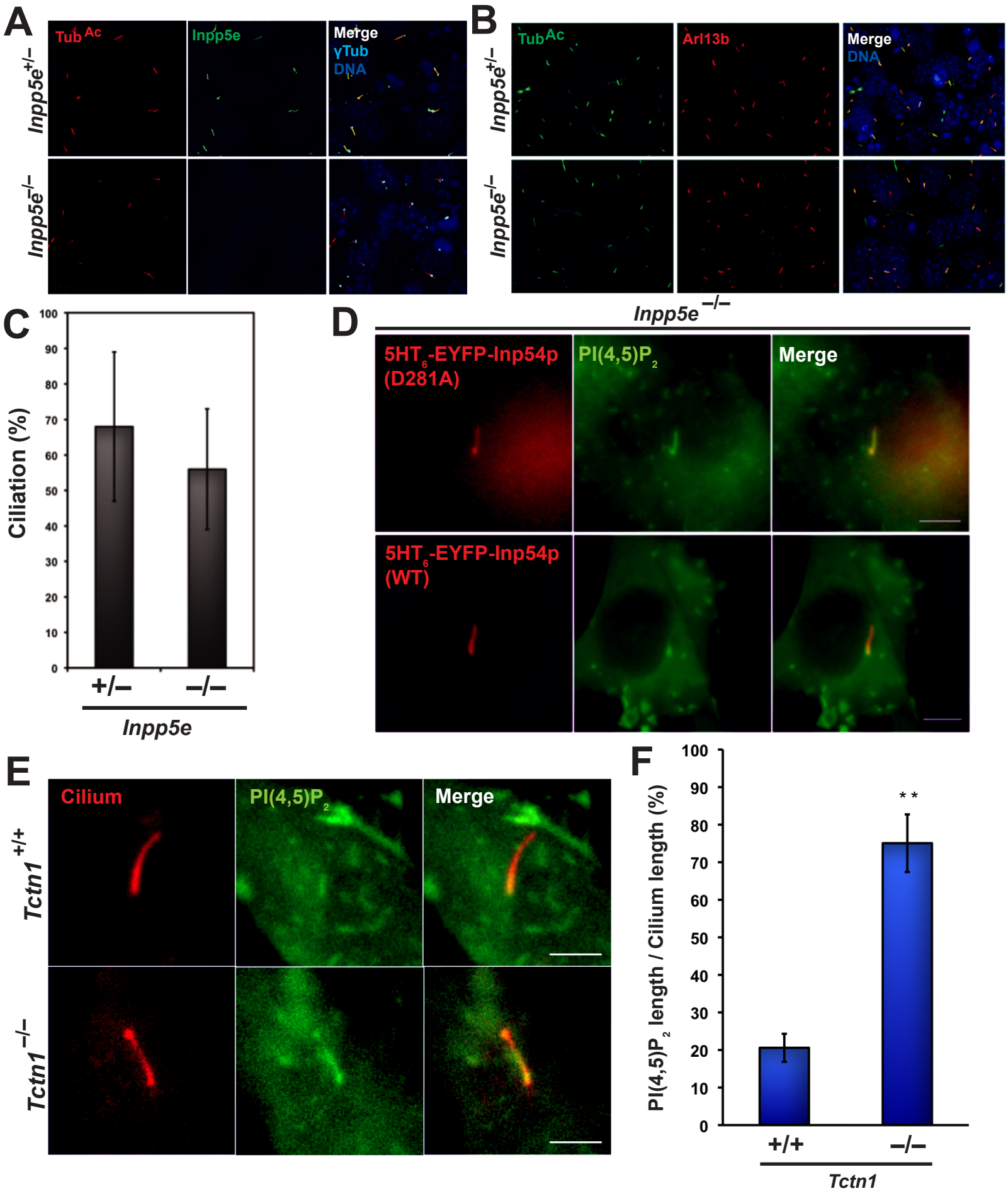
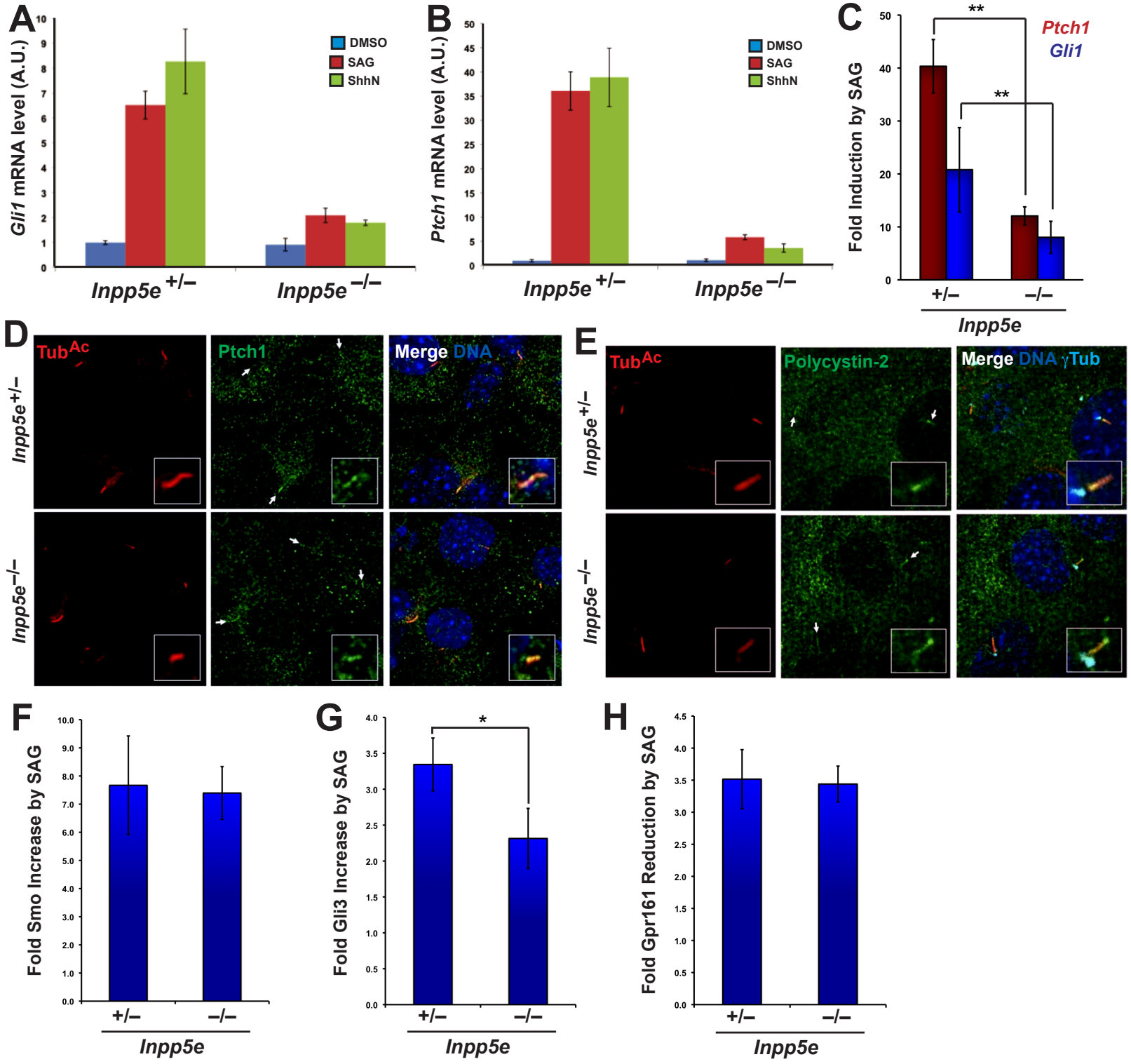


Figure S3. Garcia-Gonzalo et al. 2015



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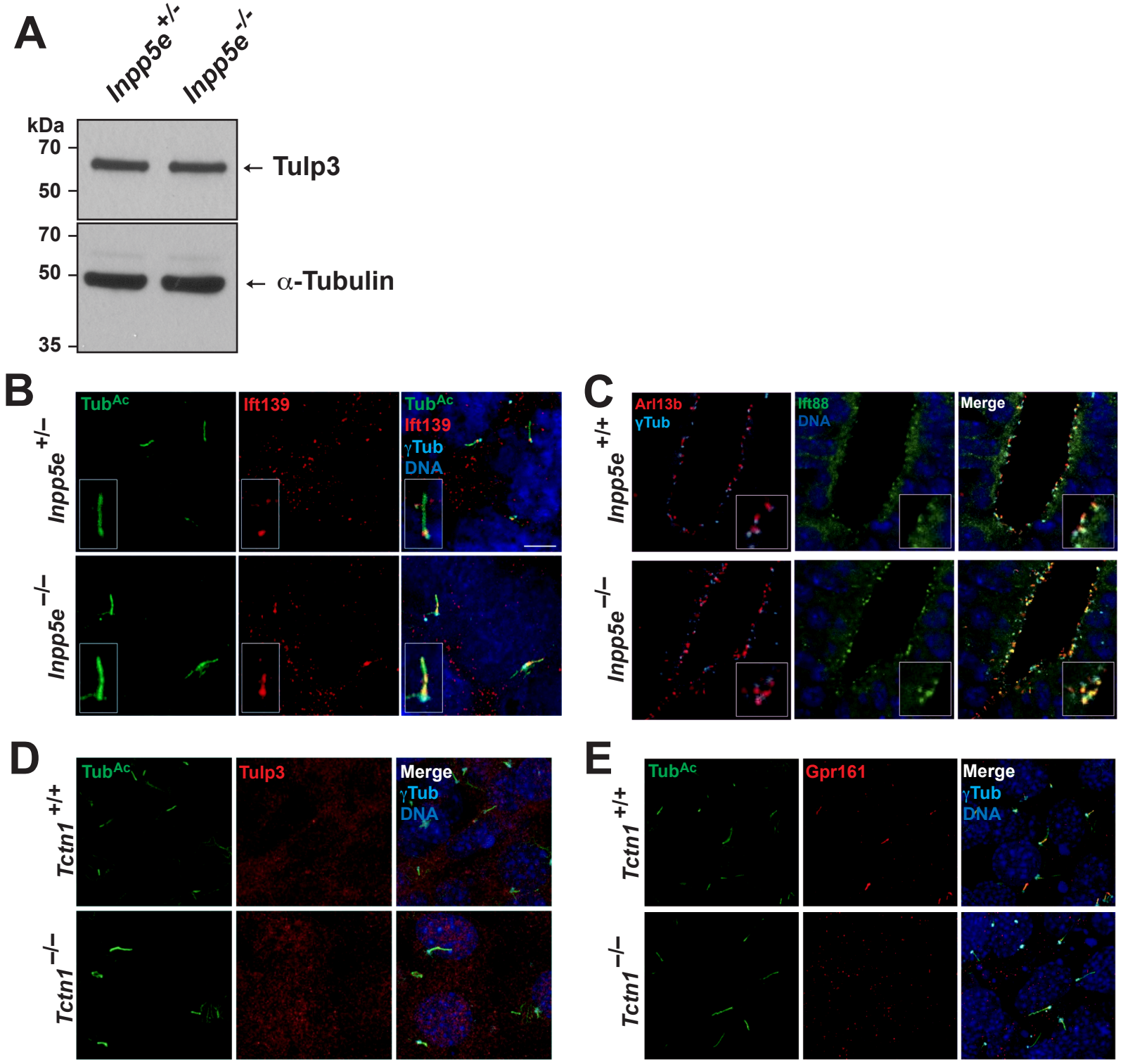
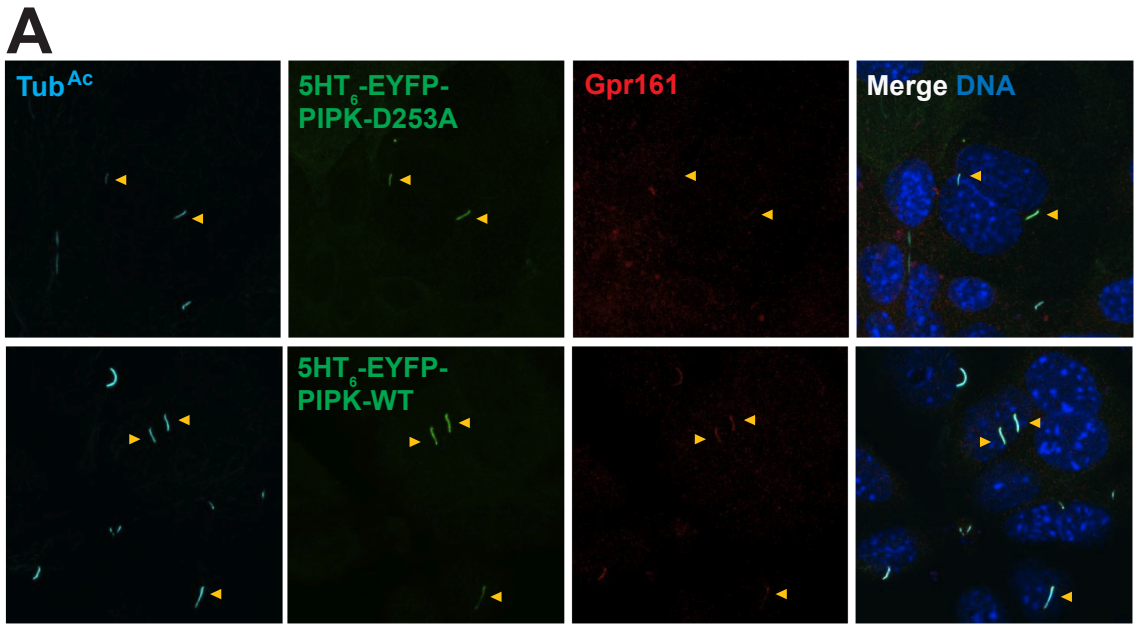


Figure S5. Garcia-Gonzalo et al. 2015



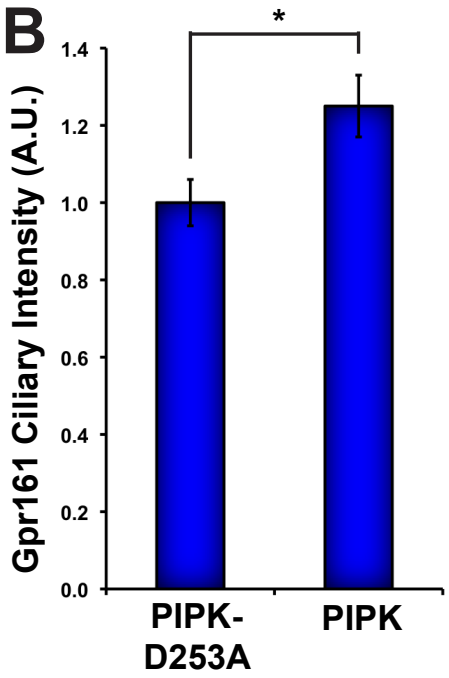


Figure S6. Garcia-Gonzalo et al. 2015