Dyson et al., https://doi.org/10.1083/jcb.201511055

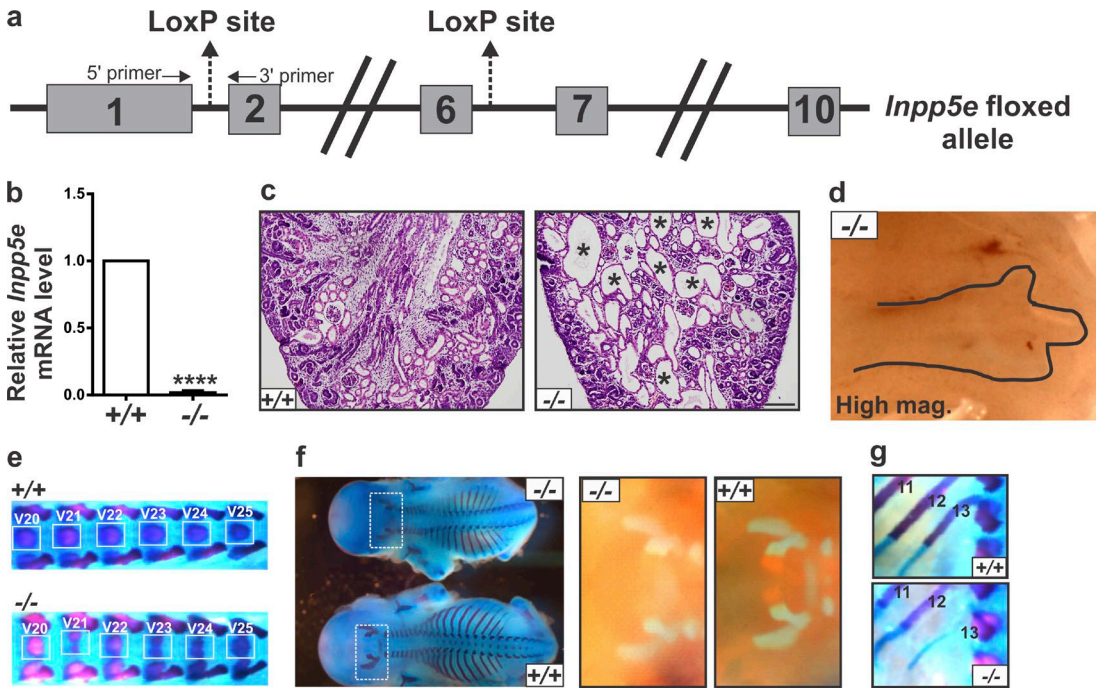

Figure S1. **Germline deletion of *Inpp5e* leads to developmental abnormalities, aberrant Hh-dependent patterning, reduced Hh signaling, and normal cilia number.** (a) Schematic representation of the mouse *Inpp5e* floxed allele; gray box represents an exon (numbered), and // represents exons not included in scheme. LoxP sites are indicated by dotted arrow, flanking exons 2 and 6. (b) RNA extracted from *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs was subjected to quantitative RT-PCR and normalized to *Gapdh*. The relative *Inpp5e* mRNA level was determined in which wild-type levels are 1. Bar represents mean ± SEM, *n* = 3 pMEF lines per genotype and performed in triplicate. ****, P < 0.0001. (c) Hematoxylin and eosin–stained *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) E18.5 kidney sections. Asterisk indicates renal cyst. Bar, 100 µm. (d) E15.5 *Inpp5e*$^{-/-}$ (−/−) embryo showing cleft palate (outlined). (e–g) Alcian blue (cartilage) and alizarin red (bone) staining of *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) embryos at E18.5 (e) or E15.5 (f and g). (e) Vertebral column shown with vertebrae 20–25 (V20-V25) boxed. (f) Boxed area shows occipital bone that is also shown at higher magnification to the right and pseudocolored for clarity. (g) Ribs 11–13 are shown and numbered.

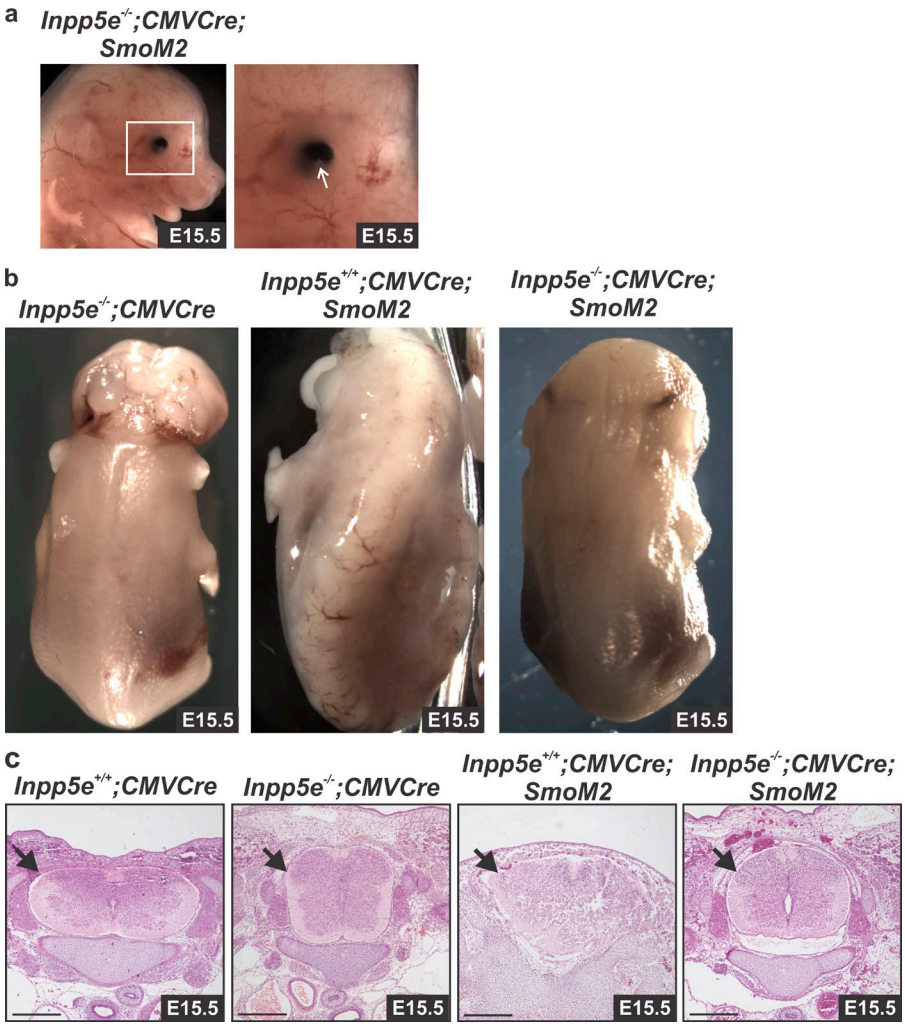

Figure S2. **Expression of SmoM2 restores *Inpp5e* null phenotypes in vivo.** (a) *Inpp5e*$^{-/-}$*;CMVCre;SmoM2* embryo at E15.5. Boxed region is shown at higher magnification on the right. Arrow indicates coloboma. (b) *Inpp5e*$^{-/-}$*;CMVCre*, *Inpp5e*$^{+/+}$*;CMVCre;SmoM2* and *Inpp5e*$^{-/-}$*;CMVCre;SmoM2* embryos at E15.5. (c) E15.5 *Inpp5e*$^{+/+}$*;CMVCre*, *Inpp5e*$^{-/-}$*;CMVCre*, *Inpp5e*$^{+/+}$*;CMVCre;SmoM2* and *Inpp5e*$^{-/-}$*;CMVCre;SmoM2* transverse neural tube section stained with hematoxylin and eosin. Arrow indicates neural tube. Bars, 250 µm.

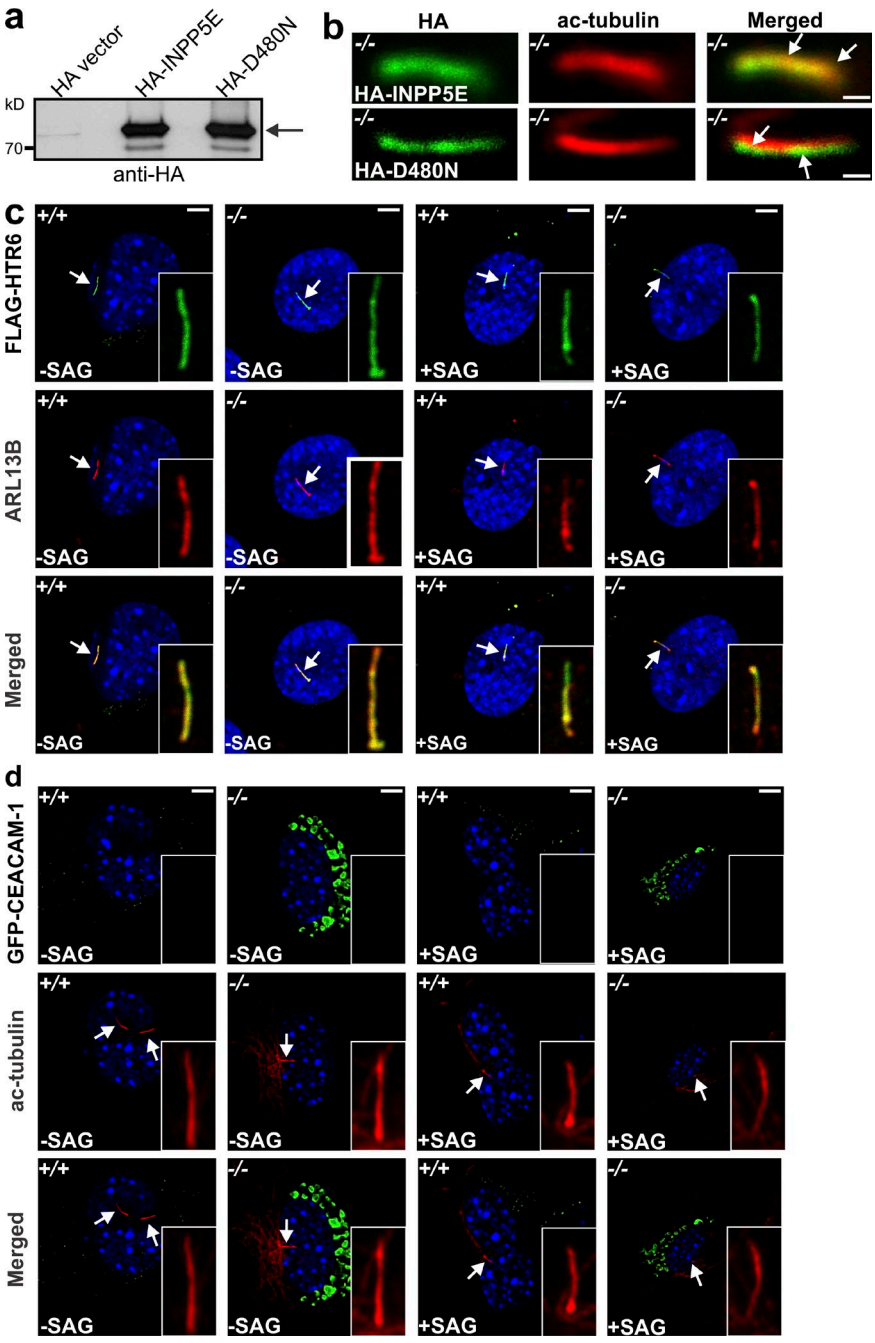

Figure S3. **INPP5E regulates the cilia accumulation/retention of cilia receptors after Hh pathway activation.** (a) *Inpp5e*$^{-/-}$ pMEFs transiently expressing HA vector, wild-type HA-INPP5E, or phosphatase-dead HA-INPP5E^{D480N} (HA-D480N) were harvested and lysates immunoblotted with HA antibodies. Arrow indicates HA-tagged recombinant proteins. Molecular weight markers are shown on the left. (b) *Inpp5e*$^{-/-}$ pMEFs transiently expressing wild-type HA-INPP5E or phosphatase-dead HA-INPP5E^{D480N} (HA-D480N) were fixed, permeabilized, and costained with HA and acetylated α-tubulin (ac-tubulin) antibodies. Arrow indicates colocalization. (c) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs transiently expressing FLAG-HTR6, either untreated (−SAG) or SAG treated (+SAG), were fixed, permeabilized, and costained with FLAG and ARL13B antibodies and DAPI and imaged using confocal microscopy. Arrow indicates cilia. Bars, 1 μm. Inset shows high magnification of cilia. (d) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs transiently expressing GFP–CEACAM-1, either untreated (−SAG) or SAG treated (+SAG), were fixed, permeabilized, and stained with ac-tubulin antibodies and imaged using confocal microscopy. Bars, 1 μm. Inset shows high magnification of cilia.

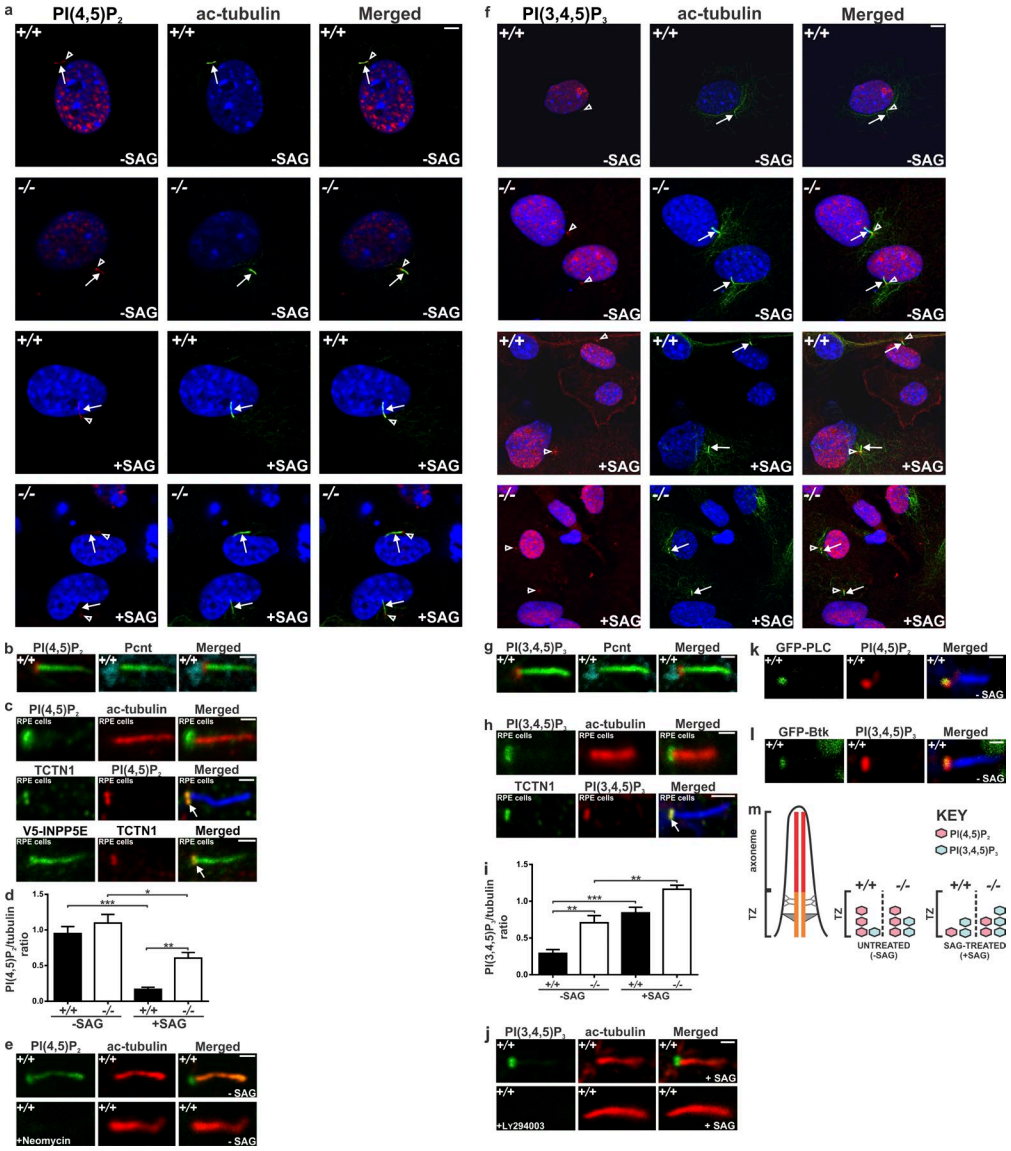

Figure S4. **PI(4,5)P_2 and PI(3,4,5)P_3 localize to the TZ and are regulated by INPP5E and Hh signaling.** (a) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs, either untreated (−SAG) or SAG treated (+SAG), were fixed, permeabilized, and costained with PI(4,5)P_2 and acetylated α-tubulin (ac-tubulin) antibodies and DAPI and imaged using confocal microscopy. Arrow indicates cilia axoneme, and arrowhead indicates PI(4,5)P_2 at the cilia base. Bar, 1 µm. (b) *Inpp5e*$^{+/+}$ (+/+) pMEFs were fixed, permeabilized, and costained with PI(4,5)P_2 (red), pericentrin (Pcnt; cyan), and ac-tubulin (green) antibodies and imaged using confocal microscopy. Bar, 500 nm. (c) RPE cells were serum starved for 24 h and costained with PI(4,5)P_2 and ac-tubulin antibodies (top) or TCTN1 (green), PI(4,5)P_2 (red), and ac-tubulin (blue) antibodies (middle). (bottom) Ciliated RPE cells transiently expressing V5-tagged wild-type INPP5E were costained with V5 (green) and TCTN1 (red) antibodies. Cells were imaged using confocal microscopy. The proximal and distal ends of the cilium are orientated left to right, respectively. Arrow indicates colocalization. Bars, 500 nm. (d) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs, either untreated (−SAG) or SAG treated (+SAG), were fixed, permeabilized, and costained with PI(4,5)P_2 and ac-tubulin antibodies and imaged using confocal microscopy as in panel a. The TZ PI(4,5)P_2 to ac-tubulin signal ratio was measured. Bars represent mean ± SEM. *n* = 3 pMEF lines per genotype, ∼100 cells per genotype. *, P < 0.05; **, P < 0.01; ***, P < 0.001. (e) *Inpp5e*$^{+/+}$ (+/+) pMEFs were fixed, treated with neomycin, permeabilized, and costained with PI(4,5)P_2 and ac-tubulin antibodies and imaged using confocal microscopy. Bar, 500 nm. (f) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs, either untreated (−SAG) or SAG treated (+SAG), were fixed, permeabilized, and costained with PI(3,4,5)P_3 and ac-tubulin antibodies and DAPI and imaged using confocal microscopy. Arrow indicates cilia axoneme, and arrowhead indicates PI(3,4,5)P_3 at cilia base. Bar, 1 µm. (g) *Inpp5e*$^{+/+}$ (+/+) pMEFs were fixed, permeabilized, and costained with PI(3,4,5)P_3 (red), pericentrin (Pcnt; cyan), and ac-tubulin (green) antibodies and imaged using confocal microscopy. Bar, 500 nm. (h) RPE cells were serum starved for 24 h and costained with PI(3,4,5)P_3 and ac-tubulin antibodies (top) or TCTN1 (green), PI(3,4,5)P_3 (red), and ac-tubulin (blue) antibodies (bottom) and imaged using confocal microscopy. The proximal and distal ends of the cilium are orientated left to right, respectively. Arrow indicates colocalization. Bars, 500 nm. (i) Untreated (−SAG) or SAG-treated (+SAG) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs were fixed, permeabilized, and costained with PI(3,4,5)P_3 and ac-tubulin antibodies and imaged using confocal microscopy. The TZ PI(3,4,5)P_3 to ac-tubulin signal ratio was measured. Bars represent mean ± SEM. *n* = 3 pMEF lines per genotype, ∼100 cells per genotype. **, P < 0.01; ***, P < 0.001. (j) SAG-stimulated (+SAG) *Inpp5e*$^{+/+}$ (+/+) pMEFs were treated with LY294003 (+LY294003); fixed, permeabilized, and costained with PI(3,4,5)P_3 and ac-tubulin antibodies; and imaged using confocal microscopy. Bar, 500 nm. (k and l) *Inpp5e*$^{+/+}$ (+/+) pMEFs expressing GFP-PLC (k) or GFP-Btk (l) were fixed, permeabilized, and coimmunostained with either PI(4,5)P_2 (k) or PI(3,4,5)P_3 (l) and ac-tubulin (blue) antibodies. Cells were imaged using confocal microscopy. Bar, 500 nm. (m) Schematic of a primary cilium to provide context for the transition zone (TZ). Schematic summarizing the relative levels of PI(4,5)P_2 (pink hexagon) and PI(3,4,5)P_3 (blue hexagon) at the TZ of either untreated (−SAG) or SAG-treated (+SAG) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs. A black dashed line divides the genotypes under each condition.

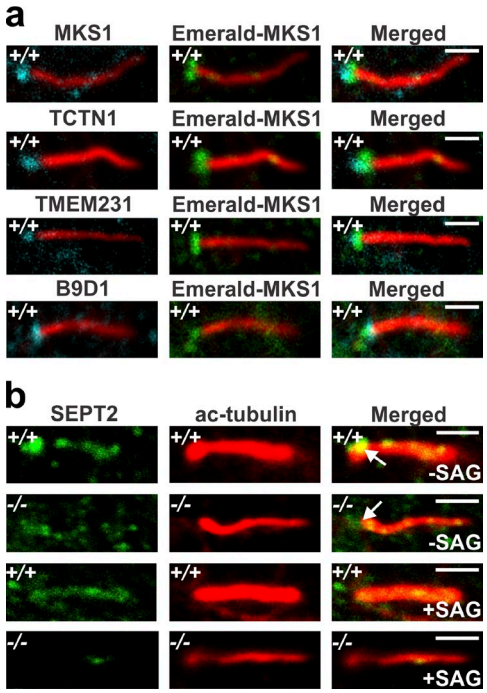

Figure S5. **INPP5E regulates the molecular organization of the cilia TZ.** (a) *Inpp5e*$^{+/+}$ (+/+) pMEFs transfected with Emerald-MKS1 were SAG treated (+SAG); fixed, permeabilized, and costained with acetylated α-tubulin (ac-tubulin) and either MKS1 (first panel), TCTN1 (second panel), TMEM231 (third panel), or B9D1 (bottom) antibodies; and imaged using confocal microscopy. The proximal and distal ends of the cilium are orientated left to right, respectively. Bars, 500 nm. (b) *Inpp5e*$^{+/+}$ (+/+) and *Inpp5e*$^{-/-}$ (−/−) pMEFs were either untreated (−SAG) or SAG treated (+SAG); fixed, permeabilized, and costained with SEPT2 and ac-tubulin antibodies; and imaged using confocal microscopy. The proximal and distal ends of the cilium are orientated left to right, respectively. Bars, 500 nm.

Table S1. **Penetrance of phenotypes in** *Inpp5e*$^{-/-}$*;CMVCre*, *Inpp5e*$^{+/+}$*;CMVCre;SmoM2* **and** *Inpp5e*$^{-/-}$*;CMVCre;SmoM2* **embryos at E15.5**

Phenotype	*Inpp5e−/−;CMVCre*	*Inpp5e+/+;CMVCre;SmoM2*	*Inpp5e−/−;CMVCre;SmoM2*
Exencephaly	66% (6/9)	0% (0/3)	0% (0/8)
Neural tube dysmorphology	0% (0/9)	100% (3/3)	0% (0/8)
Bilateral anophthalmos	100% (9/9)	100% (3/3)	0% (0/8)
Cleft palate	77% (7/9)	ND[a]	37.5% (3/8)
Edema	66% (6/9)	0% (0/3)	37.5% (3/8)
Hindlimb polydactyly	100% (9/9)	100% (3/3)	37.5% (3/8)
Forelimb polydactyly	0% (0/9)	100% (3/3)[b]	0% (0/8)
Syndactyly	0% (0/9)	100% (3/3)	0% (0/8)
Midline expansion	0% (0/9)	100% (3/3)	0% (0/8)
Number of embryos	9	3	8

Summary of *Inpp5e*$^{-/-}$*;CMVCre* (*n* = 9), *Inpp5e*$^{+/+}$*;CMVCre;SmoM2* (*n* = 3), and *Inpp5e*$^{-/-}$*;CMVCre;SmoM2* (*n* = 8) E15.5 phenotypes. Percentages indicate observed penetrance of the phenotype.
[a]For all *Inpp5e*$^{+/+}$*;CMVCre;SmoM2* embryos, a cleft palate phenotype could not accurately be determine (ND) because of the profound dysmorphology.
[b]One of the three *Inpp5e*$^{+/+}$*;CMVCre;SmoM2* embryos exhibited unilateral forelimb polydactyly.