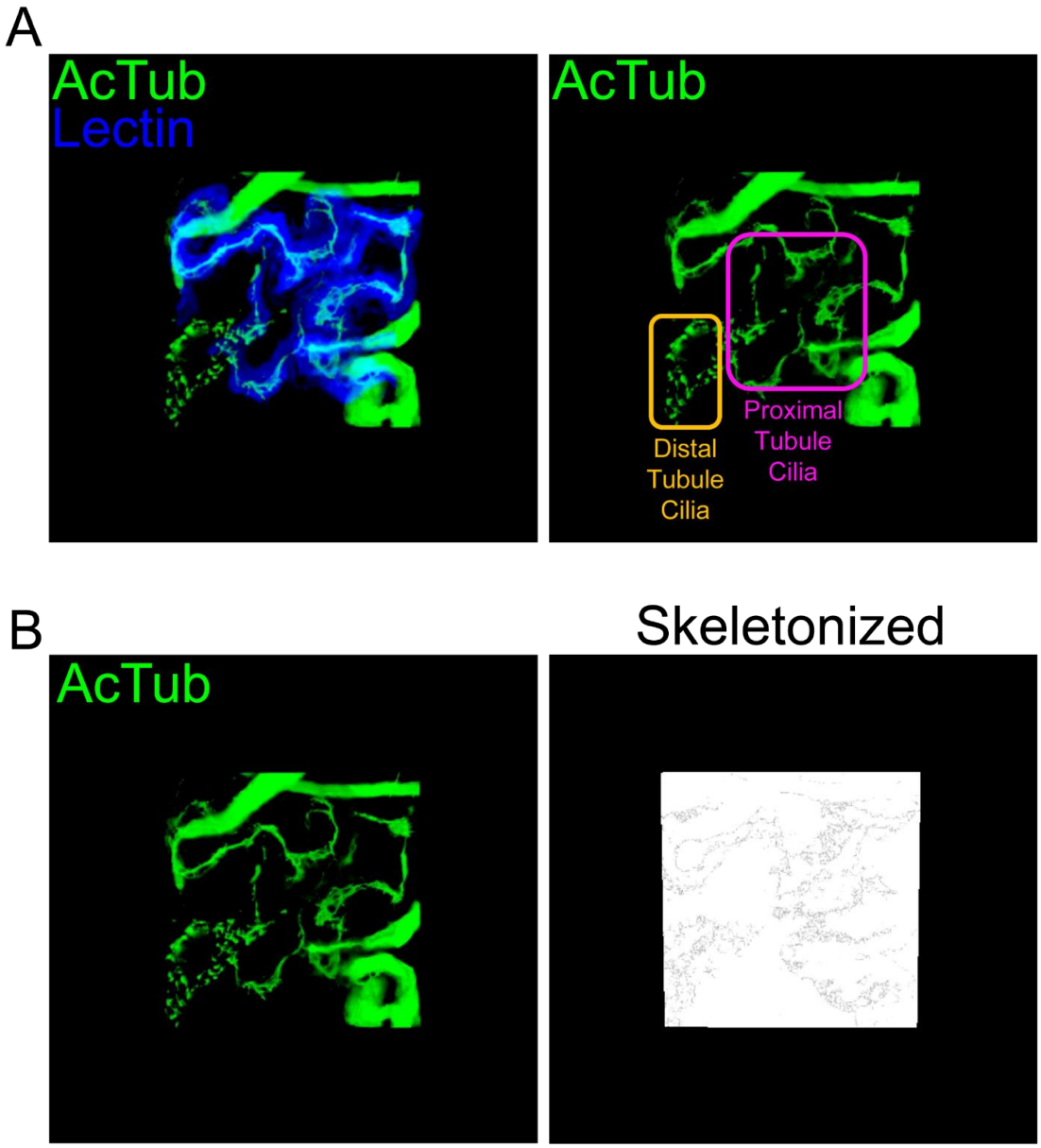
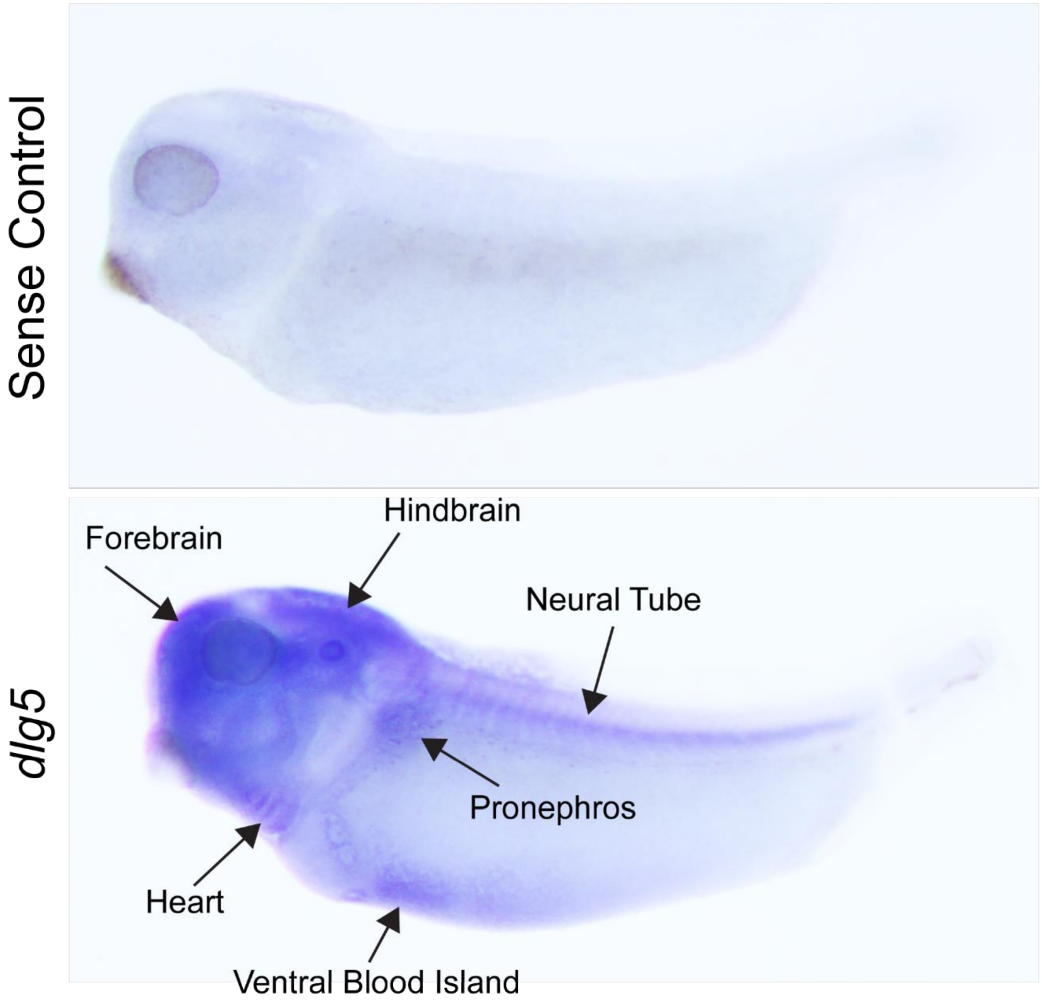
Fluorescence Reagent	Vendor	Catalog	Dilution	Species Origin
A594 Conjugated Phalloidin	Thermo Fisher Scientific	A12381	1:30	*Amanita phalloides*
A647 Conjugated Anti-Acetylated α-Tubulin	Santa Cruz Botechnology	sc-23950	1:1000	*Mus musculus*
Fluorescein Conjugated Lectin	Vector Laboratories	FL-1141	1:1000	*Erythrina cristagalli*

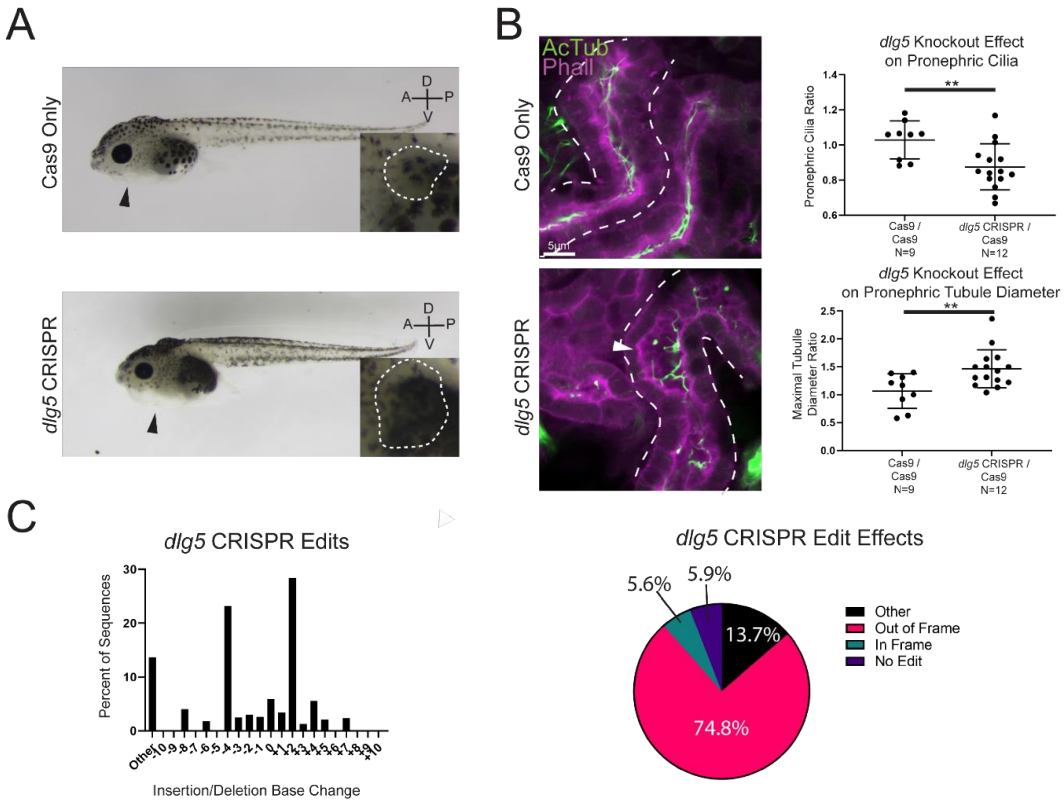
Supplemental Table 1 Information for fluorescence labeling reagents used in this study.



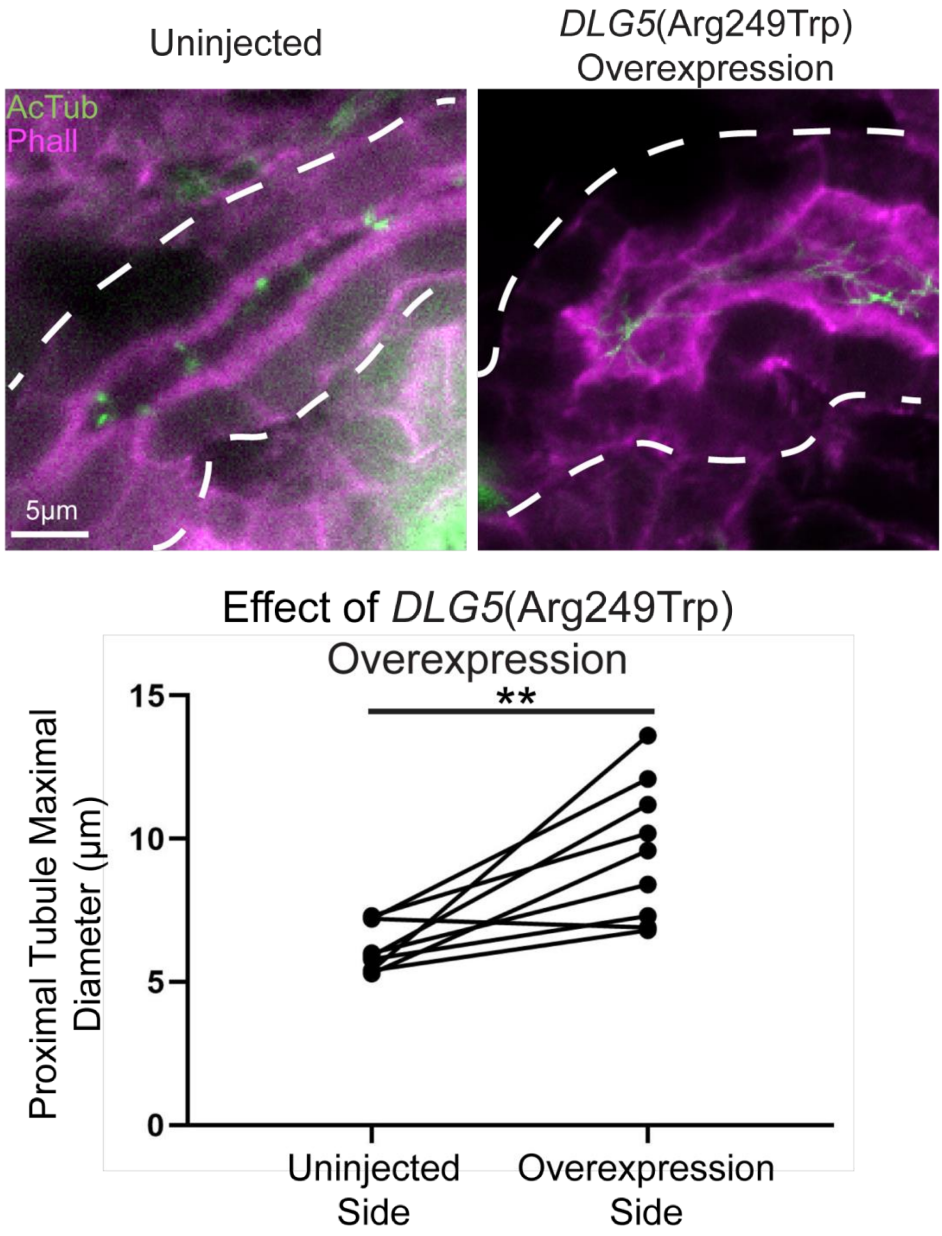
Supplemental Figure 1 Example of methodological process used to assess tubule phenotypes. (A) Representative 3D projection of images acquired for fluoro-lectin and α-acetylated tubulin to determine proximal tubule tissue and its cilia. (B) Representative 3D projection of images acquired for α-acetylated tubulin and its skeletonization used to then count cilia in portions of the proximal pronephros.



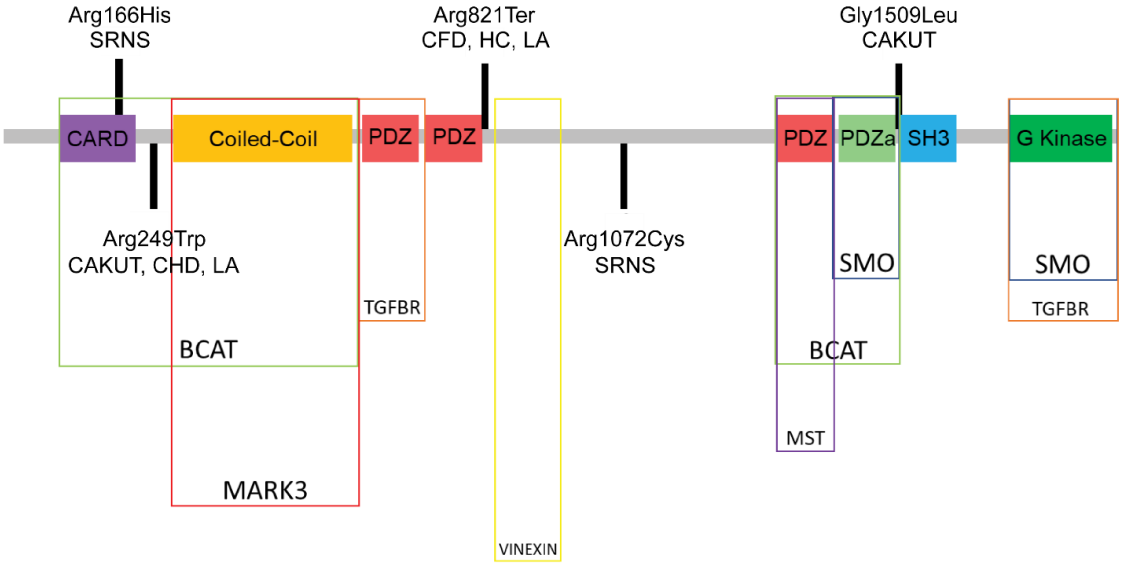
Supplemental Figure 2 WISH in untreated stage 36 embryos with either sense or antisense probe against a region of *dlg5* mRNA demonstrates the expression of *dlg5* in the ventral blood island, brain, heart, neural tube, and proximal pronephros.



Supplemental Figure 3 F0 mosaic CRISPR knockout of *dlg5* recapitulates the phenotypic abnormalities observed with MO based knockdown. (A) Representative images of stage 45 embryos injected with either Cas9 alone or Cas9 with a guide RNA targeting *dlg5* show the edema and kidney dysplasia resulting from mosaic *dlg5* knockout. (B) Representative images of injected sides for Cas9 alone and Cas9 with s *dlg5* guide RNA of stage 45 embryos along with quantification reveal a loss of cilia and increased proximal tubule diameter in the pronephroi. (C) Example TIDE analysis of insertion and deletion sizes along with their predicted effects in a pool of 5 embryos. “Other” indicates changes that could not be analyzed via TIDE due to large size of indel. Statistical tests carried out as unpaired T-tests with ** designating $p < 0.01$. Bars indicate mean and SD of replicate means.



Supplemental Figure 4 Overexpression of *DLG5* (Arg249Trp) in *Xenopus* embryos causes kidney dysmorphology. (B) Representative images of uninjected and *DLG5* (Arg249Trp) mRNA injected sides of stage 45 embryos along with quantitation reveal increased proximal tubule diameter in the pronephroi. Each data point is the maximal diameter of the pronephric tubule in either the paired uninjected or injected side of embryos. Statistical test carried out a paired T-test with ** designating $p < 0.005$.



Supplemental Figure 5 Schematic diagram of previously identified Dlg5 interactions mapped onto the structure of Dlg5. Boxes depict the minimal regions of Dlg5 that were previously shown to interact with β-catenin, Mark3, TGFβ receptor, Vinexin, Mst, and Smo.