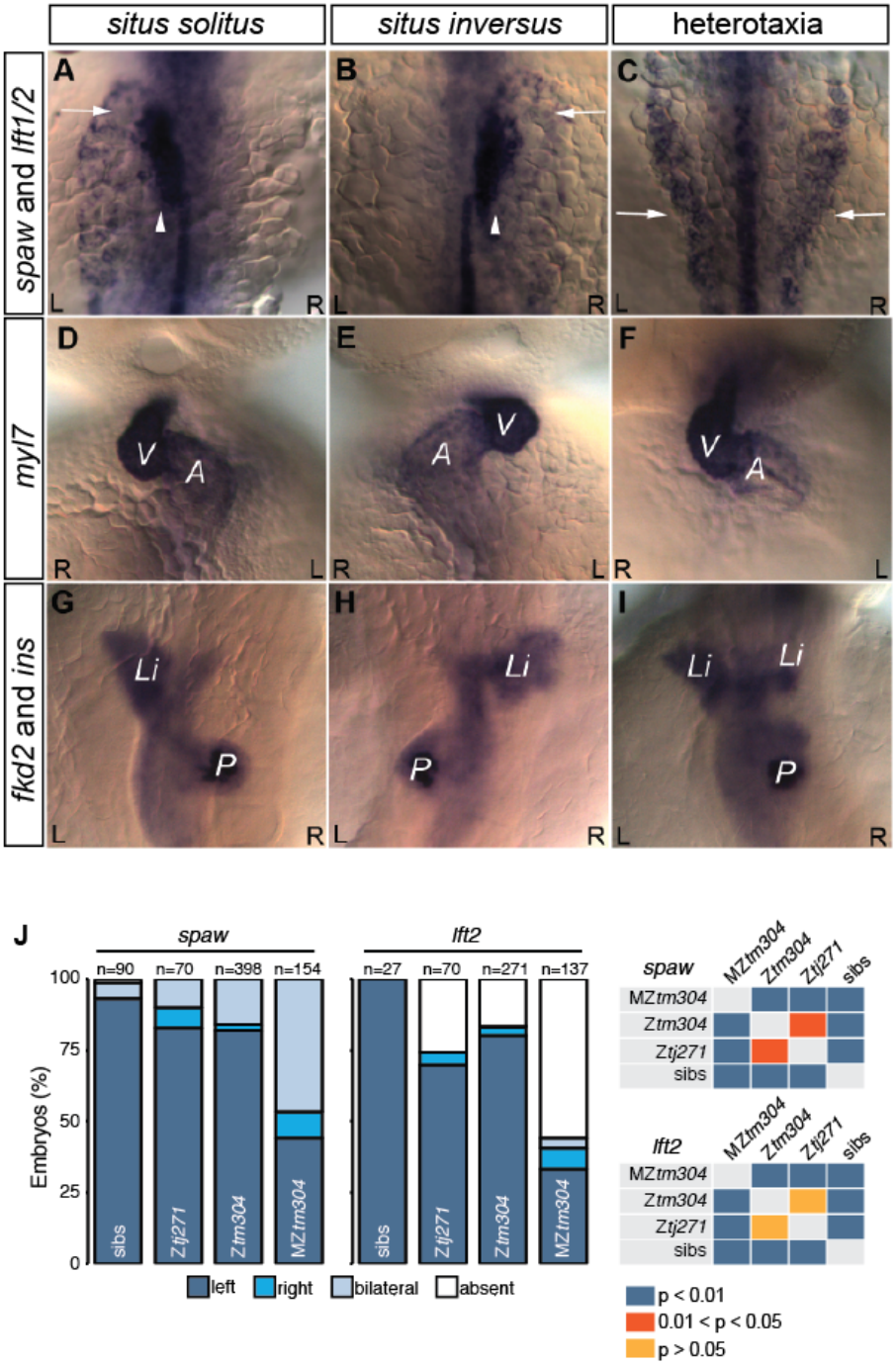
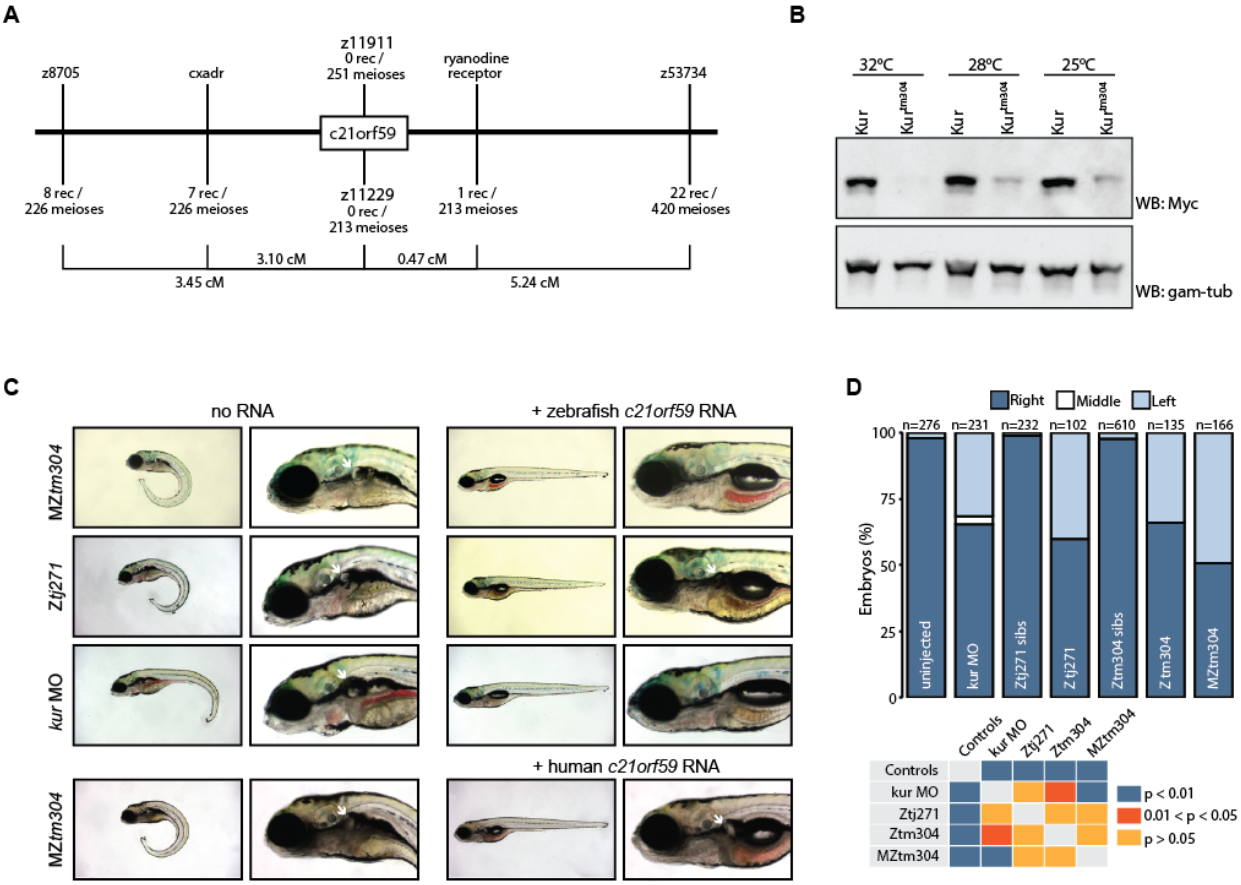
Supplemental Figures and Legends

Figure S1, Related to Figure 1. Laterality Defects in *kur* Mutants



(A-I) WISH for *spaw*, *lft1/2* as well as makers of the heart (*myl7*), liver (*fkd2*) and pancreas (*ins*) showing examples laterality defects observed in mutants. (J) Quantitation of *spaw* and *lft2* expression patterns observed in mutants. Statistical comparison of different genotypes was performed using chi-square analysis with $p < 0.05$ being considered to report significant differences.

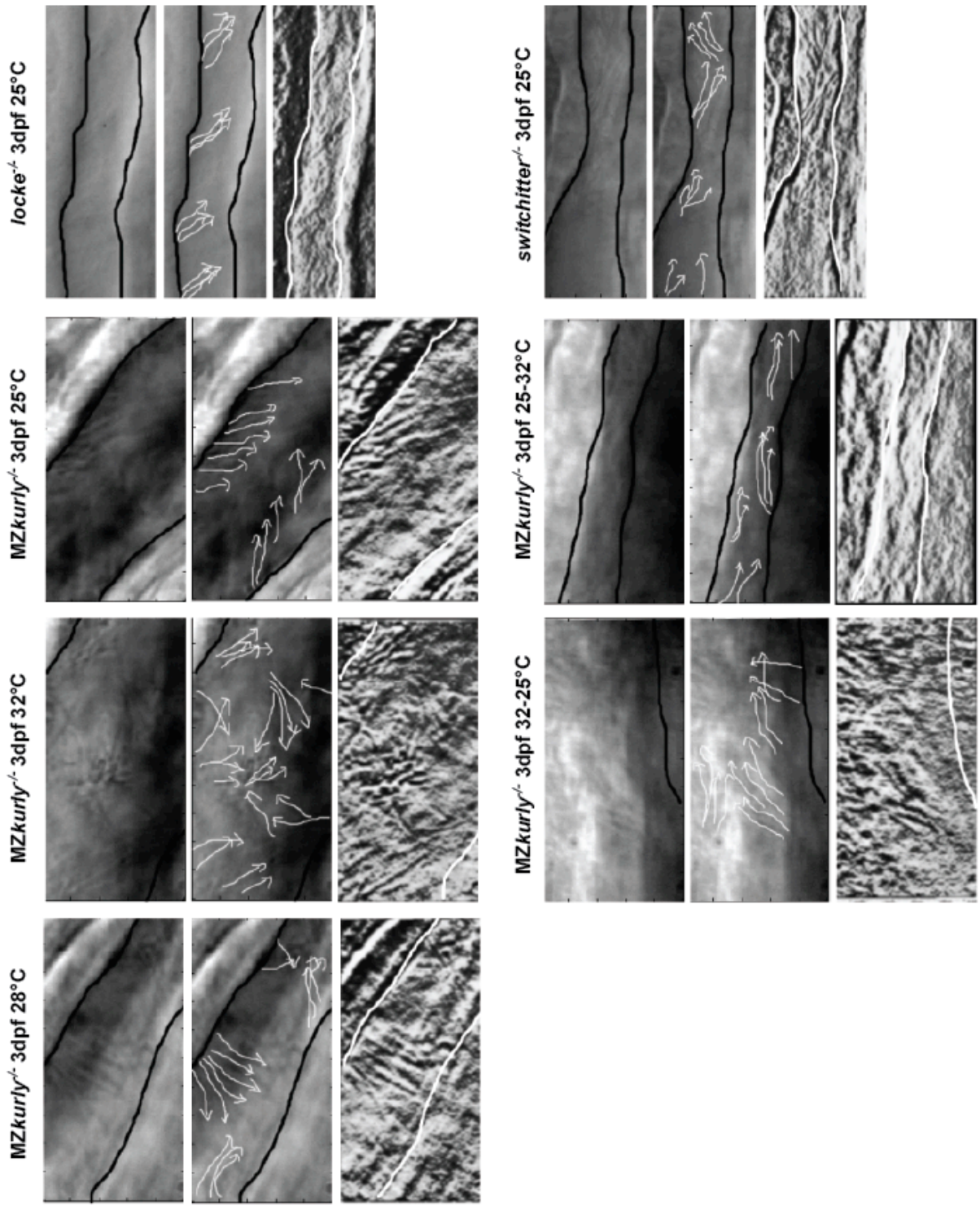
Figure S2, Related to Figure 1. *kur* Alleles Carry *c21orf59* Mutations



(A) Schematic diagram of the region *kur* was mapped to, including *c21orf59*. (B) Western blot of Myc-tagged Kur after expression in zebrafish embryos which were subsequently raised at the given temperatures. At higher temperatures, wild-type Kur is present as a strong band but Kur^{tm304} protein has either not been generated or has decayed. Gamma-tubulin was used as a

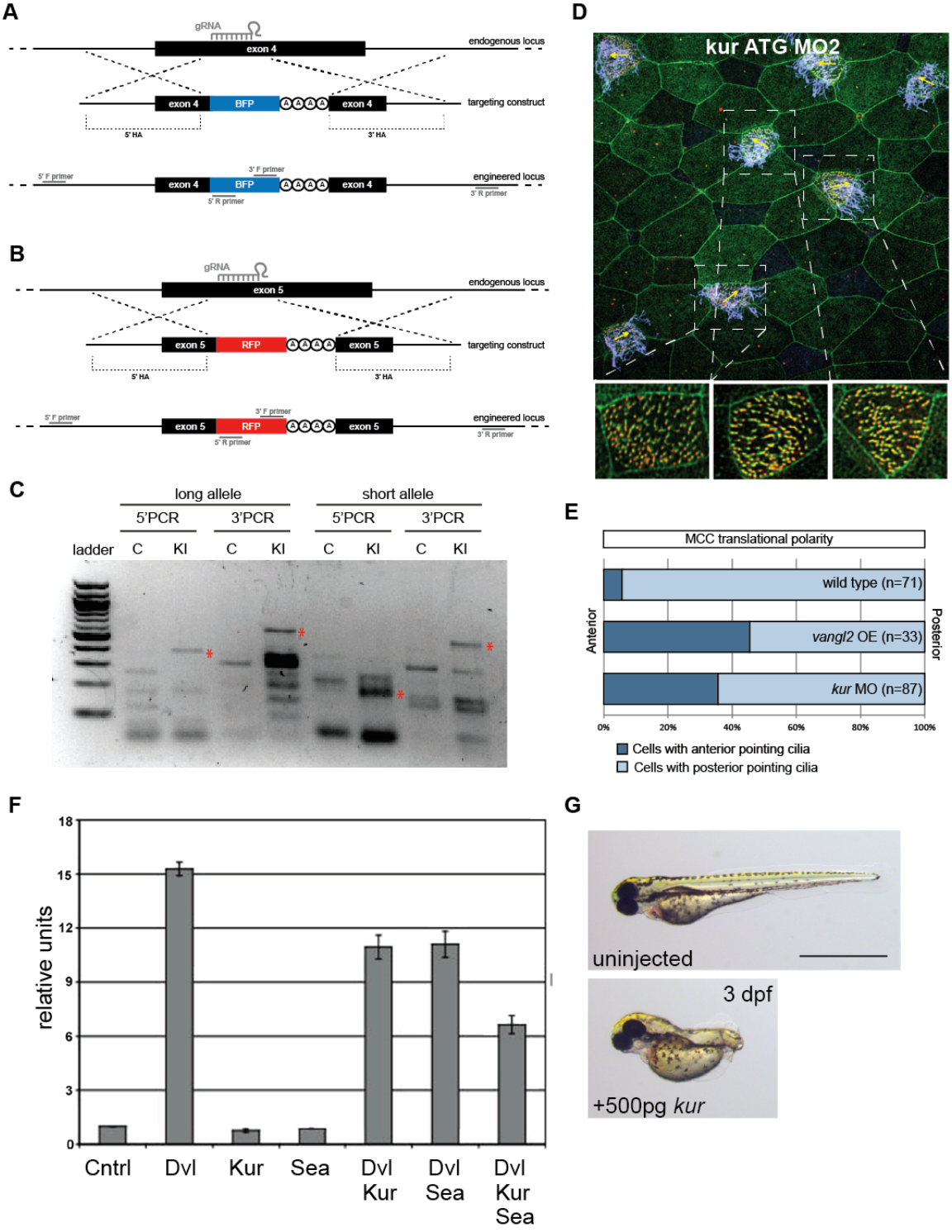
loading control. (C) *kur* mutants and morphants were rescued (body axis defect was completely rescued, whereas kidney cysts (white arrows) were partially rescued) by injection at the 1-cell stage of either zebrafish or human *c21orf59* RNA. (D) Heart looping scored by *myl7* ISH at 48 hpf; mutants and morphants exhibited laterality defects that were not present in uninjected or sibling controls. Statistical comparison of different genotypes was performed using chi-square analysis with $P < 0.05$ being considered to report significant differences.

Figure S3, Related to Figure 3. Kidney Cilia Polarization is Altered in *kur* Mutants



Stills of live imaging of mutant kidneys at 3 dpf. Black and white lines mark the edges of the pronephros whilst white lines show the direction of cilia.

Figure S4, Related to Figure 3. Generation of *Xenopus kur* Mutants, Tissue-Level Polarity Defects, and Repression of Canonical Wnt Signaling by Kur



(A-C) Schematic diagram of CRISPR/Cas9-induced homologous recombination (HR) knock-in (KI) strategy. A single guide RNA (gRNA) cuts both the long and short *kur* alleles, then targeting constructs with allele-specific homology arms (HA) are used to integrate BFP (A) and RFP (B), respectively, into the endogenous locus, followed by termination codons and a polyA tail. PCR using a BFP- or RFP-specific primer and a primer outside the region of homology (C) indicated successful KI in F0 mosaic embryos. (D-E) Tissue level polarity is perturbed when *kur* is depleted. Representative image from a *kur* MO-treated embryo (ATG MO 2) expressing the rootlet marker GFP-CLAMP (green) and the basal body marker Centrin-RFP (red) stained with anti-acetylated tubulin antibody (purple) (D). Yellow arrows indicate average cilia orientation of individual ciliated cells. Three cells are enlarged without acetylated tubulin staining showing cilia orientation. Quantitation of ciliated cell polarity in control embryos, embryos overexpressing the PCP component *vangl2*, and *kur* morphant embryos (E). (F) Luciferase readouts of the Super8XTopFlash reporter. Expression of Dvl alone activates the reporter whereas co-expression of Dvl and Kur or Dvl and Sea reduces activity, which is further reduced by expression of Kur and Sea together. Error bars represent standard deviation. (G) Overexpression of *kur* by RNA injection at the 1-cell-stage induced moderate dorsalization.

Movie S1, Related to Figure 2

Pronephric cilia in an MZ*kur*^{tm304} sibling raised at 32^{o}C with posterior towards the top right at 2 dpf. Bundled and beating cilia can be seen in a non-dilated tubule. At the start of the movie, the edges of the tubules are lined with black dots and cilia are indicated by white arrows.

Movie S2, Related to Figure 2

Pronephric cilia in an MZ*kur*$^{tm304/tm304}$ mutant raised at 25^{o}C with posterior towards the top right at 2 dpf. Cilia are pointed posteriorly and are motile, whilst the tubule is slightly dilated. At the start of the movie, the edges of the tubules are lined with black dots and cilia are indicated by white arrows.

Movie S3, Related to Figure 2

Pronephric cilia in an MZ*kur*$^{tm304/tm304}$ mutant raised at 32^{o}C with posterior towards the top right at 2 dpf. Cilia are pointed in all directions and have mostly failed to bundle and are immotile. The tubule is very dilated, with edges just noticeable at the upper left and bottom right. At the start of the movie, the edges of the tubules are lined with black dots and cilia are indicated by white arrows.

Movie S4, Related to Figure 2

Pronephric cilia in an MZ*kur*$^{tm304/tm304}$ mutant raised at 32^{o}C for the first 48 hours then shifted to 25^{o}C for 12 hours prior to imaging. The posterior is towards the top right. The tubule is dilated but motility has returned to many of the cilia. At the start of the movie, the edges of the tubules are lined with black dots and cilia are indicated by white arrows. Due to the extensive dilation of the tubule in this embryo, only one edge of the tubule is visible in the movie.

Supplemental Experimental Procedures

Mapping, cloning and genotyping *kur*

Mapping of the *kur* locus [S1] was performed using *kur*^{tm304} embryos and single sequence length polymorphism (SSLP) markers. Bulk segregant analysis placed *kur* on chromosome 10 and the genomic interval was subsequently narrowed to between SSLP markers z8705 and z53734. Single nucleotide polymorphism (SNP) analysis was used to further narrow the region to between z11911 and z11229. Sequencing of candidate open reading frames (ORFs) revealed mutations in *c21orf59* in both *kur*^{tm304} and *kur*^{tj271}. The zebrafish *c21orf59* clone was obtained from American Type Culture Collection (ATCC) in the pSPORT6.1 vector and was used to generate RNA and *in situ* probes (see below). The coding region was cloned into the pGBKT7 vector which tagged the *c21orf59* ORF with N-terminal myc.

Genotyping was performed from fin clip DNA isolated by treatment with Proteinase K overnight. The *kur*^{tm304} mutation was genotyped by PCR using Forward (CCCCATGGGCAGATTATTTAGC) and Reverse (CCTGACACAACTGTTAAACC) primers to generate an 800 bp product. Wild-type sequence was cleaved by *HinfI* to produce 560, 140, and 100 bp bands whereas one *HinfI* site is eliminated by the *kur*^{tm304} mutation such that mutant DNA is cleaved once to produce 560 and 240 bp bands. The *kur*^{tj271} mutation was genotyped by PCR using Forward (GCAAGTATGTAAACAATGGCG) and Reverse (CAGAATTATCGAGTGGTC) primers to generate a 670 bp product. Wild-type sequence was cleaved by *BstNI* digestion to generate 495 and 265 bp bands whereas the mutant band is not cut by *BstNI*.

In situ hybridization

DIG-labeled RNA probes were transcribed from linearized DNA template and used for whole mount in situ hybridization by standard protocols. Embryos were mounted in modified GMM (100 ml Canada Balsam (Sigma), 10 ml methylsalicylate (Sigma)), visualized using a Leica

DMRA microscope at 10x magnification and photographed with a ProgressC14 Digital Camero (Jenoptik).

Live imaging of cilia

Live imagining of cilia motility in the pronephric tubules was performed as described previously [S2]. Movies were captured at 100 frames per second.

RNA and morpholino injections

The *c21orf59* clone in the pSPORT6.1 vector was linearized with *XhoI* and *c21orf59* RNA, including its endogenous 5' and 3'UTRs, was *in vitro* transcribed with the Ambion mMessage mMachine SP6 kit. 1 ng of *c21orf59* RNA was injected at the 1-cell stage to rescue the phenotype of *kur* mutants and *c21orf59* morphants. pGBKT7-*c21orf59* was linearized with *NotI* and RNA made using the Ambion mMessage mMachine T7 kit. This myc-c21orf59 RNA was also able to rescue *kur* mutants at concentrations of 700 pg – 1 ng when injected at the 1-cell stage. For immunoprecipitation experiments, 1-2 ng of tagged *c21orf59* RNA was used. Human *c21orf59* RNA could rescue *kur* mutants when injected at 500 pg – 1 ng. An independently cloned c21orf59-LAP (Localization and Affinity Purification) and untagged c21orf59 without endogenous 5' or 3' UTRs overexpressed at 500 pg induced dorsalization (**Figure S4B**).

Morpholino antisense oligonucleotides (MO) (Gene Tools LLC) were maintained in 50 µg/µl stock solutions in water. MO were mixed with 5 mg/ml phenol red and micro-injected into the yolk at the 1-4-cell stage. The *c21ord59* MO (CACGTAAAGATCAGTCATACCAGTG) is a splice site MO that overlaps the exon 2-intron 2 junction. 6-9 ng of MO was sufficient to accurately phenocopy *kur* mutants.

Fertilized *Xenopus laevis* tadpoles were obtained using standard protocols. The following *c21orf59* specific morpholino oligonucleotides (Gene Tools LLC) were used: SPL MO: TGGTGCTGCAAATAAACACACTCAC; ATGMO1: TGTAAGCGCACCATTTTTCCAATCA; ATGMO2: CAATCACTTGTGTGAAACGAATGCA. Morpholinos were injected at the 4-cell stage

into all 4 blastomeres. Synthesized mRNA encoding Prickle2-GFP, mem-RFP, GFP-CLAMP and Centrin4-RFP were variously co-injected as polarity markers as has been described previously [S3, S4, S5]. After *in vitro* transcription, mRNAs were purified and injected into all blastomeres of 2- or 4-cell stage embryos. MOs and mRNA were injected sequentially into the same blastomere. Embryos were allowed to develop in 0.1% Marc's Modified Ringers solution with gentamycin at 16^{o}C until they reached stage 29.

Xenopus laevis **microcopy and quantification.**

Displacement of dyed polystyrene 10 µm microspheres (Ploysciences Inc.) was visualized using a DCM130 digital camera mounted on a dissecting scope. Images were acquired using ScopePhoto software and flow velocity was quantified using Nikon Elements software. At least 10 flow lines for at least 5 embryos from 2 independent experiments were quantified.

For kurly morphants the Prickle2-GFP asymmetric localization was quantified relative to memRFP using fluorescent intensities taken from images of live embryos and measured with Nikon Elements software. The log 2 was calculated after dividing the ratio of posterior intensity of Prickle2-GFP/memRFP by the ratio of anterior intensity of Prickle2-GFP/mem-RFP [S5]. For the CRISPR/Cas knock-in experiments, the log2 was calculated after dividing the posterior Prickle2-GFP intensity by the anterior intensity.

For the cilia polarity assay embryos injected with GFP-CLAMP and Centrin4-RFP where fixed in 3% paraformaldehyde (PFA) and stained with mouse anti-acetylated alpha-tubulin primary antibody (Sigma T7451) and Cy-5 conjugated secondary antibody (Jackson Immuno.) with standard procedures. Ciliated cells were imaged using a Nikon A1R confocal microscope using a 60X plan-Apo objective. Cilia orientation was scored using Nikon Elements software.

Immunoprecipitation and immunofluorescence

For protein collection, zebrafish embryos were manually dechorionated and placed into 2 ml epitubes on ice for 1 minute. Water was removed and embryos were rinsed three times with 500

µl of E3 buffer (5.m mM NaCl, 0.2 mM KCl, 0.3 mM $CaCl_2$, 0.3 mM $MgSO_4$, 0.2 % v/v Methylene Blue, pH 6.5). Embryos were then disrupted by vigorous pipetting after which 1 ml of ½ Ringer's Solution (55 mM NaCl, 1.8 mM KCl, 1.25 mM $NaHCO_3$; Ginzburg Fish Ringer, no Ca^{2+}) was added. Samples were vortexed for 30 seconds then placed on ice for 30 seconds, and this procedure was repeated 5 times. Subsequently, samples were centrifuged for 5 minutes at 4^{o}C at 20,000g, supernatant was removed and samples were placed in buffer (20 mM Tris/HCl pH 7.5, 0.32 M sucrose, 2 mM EDTA) plus 1/3 v/v Laemmli Buffer. Samples were heated to 70^{o}C for 20 minutes and approximately 15 µg of protein was run per lane on 12% MOPS Nu-PAGE gel. Transfer to nitrocellulose membranes was performed for 3 hours at 0.18 A at 4^{o}C. Membranes were blocked for 1 hour in 5% milk/TBST solution, incubated in primary antibody in milk/TBST for 2 hours, washed three times in TBST, incubated for 1 hour in secondary antibody in milk/TBST, followed by six 5 minute washes in TBST. Amersham ECL Western Blotting Detection Reagents (GE Healthcare) were used for detection.

For kidney immunofluorescence, embryos were fixed in Dent's fixative at 4^{o}C overnight, gradually rehydrated into PBT/1% DMSO (PBDT), then blocked for 2 hours in 10% goat serum/0.1% PBDT solution. Immunostaining was performed as described previously [S6]. Samples were mounted in Aqua PolyMount (Polysciences Inc.) and visualized using a Zeiss LSM 510 microscope. For KV immunofluorescence, embryos were harvested at the 8 somite stage and fixed in 4% PFA overnight at 4°C, then washed in 1X PBST, transitioned to 100% MeOH, and placed at -20°C overnight. Five minute incubations were subsequently performed in 75% MeOH: 25% PBST, 50% MeOH: 50% PBST, 25% MeOH: 75% PBST, followed by another four washes (five minutes each) in PBST. The embryos were then washed in H_2O for five minutes, incubated in ice-cold acetone for seven minutes, washed in H_2O for five minutes, and washed for five minutes in PBDT. The embryos were blocked for two hours in 1X PBDT containing 10% normal sheep serum (NSS) and rocked gently at room temperature. The embryos were incubated overnight at 4°C with 1:400 anti-acetylated tubulin (T6793, Sigma) and

1:400 anti-gamma-tubulin (T5192, Sigma) in 1X PBDT containing 10% NSS. The following day, the embryos were washed in 1X PBDT containing 1% NSS and 0.1 M NaCl on a rocker at room temperature. The first wash was for 1 min and the five subsequent washes were for 30 min each, followed by a 30 min wash in 1X PBDT containing 1% NSS. The embryos were incubated with 1:400 TRITC conjugated anti-mouse IgG_{2b} (1090-03, SouthernBiotech), 1:400 Alexa Fluor(R) 647 conjugated anti-rabbit IgG (A21246, life technologies), 1:125 Hoechst stain, and 1X PBDT containing 1% NSS. The incubation was performed overnight at 4°C. The following day, the embryos were washed in 1X PBDT containing 1% NSS and 0.1 M NaCl on a rocker at room temperature. The first wash was for 1 min and the five subsequent washes were for 30 min each, followed by a 30 min wash in 1X PBDST containing 1% NSS. The final wash was performed for 30 min in PBDT. The embryos were then flat mounted and imaged using a 40X objective on a Nikon A1 confocal microscope. The images were analyzed using IMARIS software.

TopFlash assay

The luciferase reporter construct used was SUPER 8XTOPFLASH as described [S7]. The Renilla construct used was a gift from K. Horvath. By removing the promoter of the Renilla construct to create a null construct, extremely high background readings have been eliminated. Briefly, NIH-3T3 cell transfection experiments were performed with 1100 ng of DNA total per well transfected, 100 ng of each reporter and 300 ng of each construct. Those wells that did not contain equal amounts of experimental constructs were transfected with appropriate amounts of empty vector. Cells were transfected using the Lipofectamine™ 2000 system (Invitrogen, Carlsbad, CA). Cells were lysed according to manufacturer's protocol using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI). Assays were performed using a Turner 20/20n luminometer (Turner Biosystems, Sunnyvale, CA).

Supplemental References

S1. Haffter P, Nusslein-Volhard C (1996) Large scale genetics in a small vertebrate, the zebrafish. Int J Dev Biol 40: 221-227.

S2. Sullivan-Brown J, Schottenfeld J, Okabe N, Hostetter CL, Serluca FC, et al. (2008) Zebrafish mutations affecting cilia motility share similar cystic phenotypes and suggest a mechanism of cyst formation that differs from pkd2 morphants. Dev Biol 314: 261-275.

S3. Mitchell B, Stubbs JL, Huisman F, Taborek P, Yu C, et al. (2009) The PCP pathway instructs the planar orientation of ciliated cells in the Xenopus larval skin. Curr Biol 19: 924-929.

S4. Park TJ, Mitchell BJ, Abitua PB, Kintner C, Wallingford JB (2008) Dishevelled controls apical docking and planar polarization of basal bodies in ciliated epithelial cells. Nat Genet 40: 871-879.

S5. Butler MT, Wallingford JB (2015) Control of vertebrate core planar cell polarity localization and dynamics by Prickle 2. Development 142: 3429-3439.

S6. Schottenfeld J, Sullivan-Brown J, Burdine RD (2007) Zebrafish curly up encodes a Pkd2 ortholog that restricts left-side-specific expression of southpaw. Development 134: 1605-1615.

S7. Veeman MT, Slusarski DC, Kaykas A, Louie SH, Moon RT (2003) Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. Curr Biol 13: 680-685.