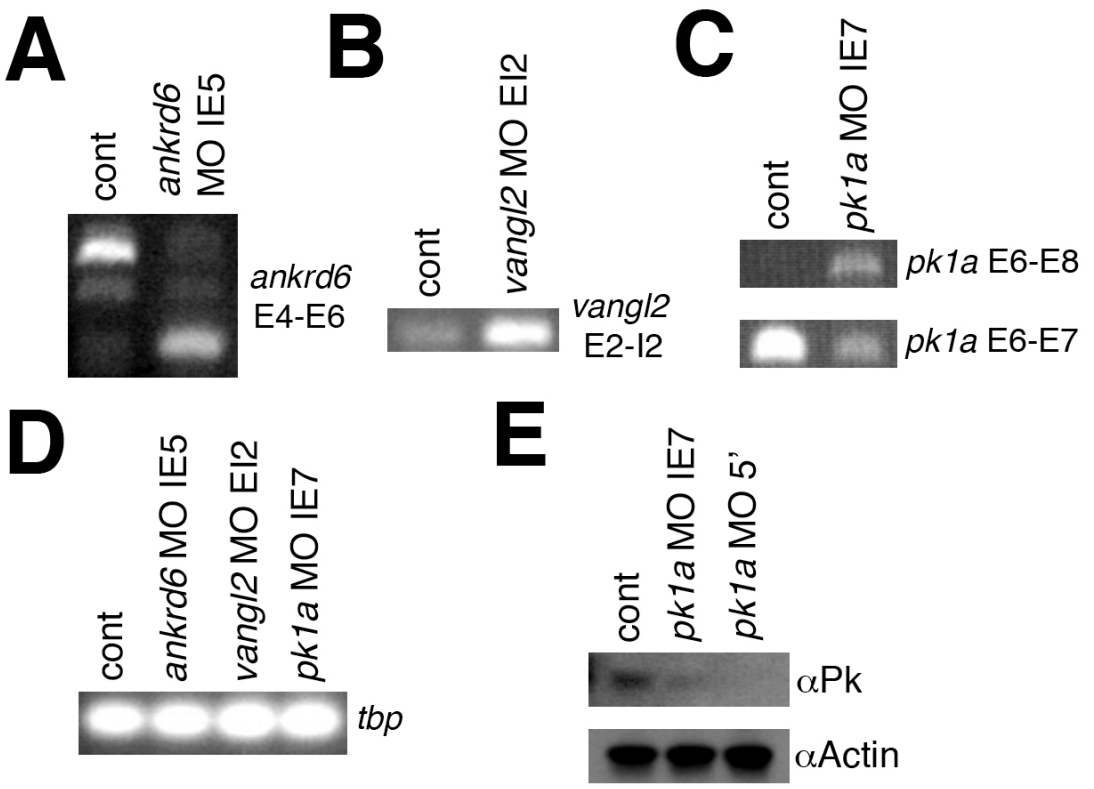

Figure S1. Documentation of morpholino knockdown. (A-D) PCR of 24 hpf embryos injected at the 1-cell stage with control and (A) *ankrd6* morpholino (MO), (B) *vangl2* MO, (C) *pk1a* MO, and each of the above (D), at the amounts used to elicit the phenotypes noted elsewhere (1.5 ng). (A) There is loss of a 375 bp *ankrd6* PCR product spanning exon 4 and exon 6 (E4-E6) in embryos injected with an *ankrd6* MO designed to inhibit splicing into exon 5. The lower part of the panel shows a 125 bp band using the same primers in the same embryos that results from loss of the ~250 bp exon 5. (B) A 202 bp *vangl2* PCR product spanning exon 2 (E2) and the intron between exons 2 and 3 (I2) in embryos injected with a *vangl2* MO designed to inhibit splicing out of I2, which introduces an in-frame stop codon from sequence within the second intron. (C) A novel 650 bp PCR product spanning *pk1a* exons 6 and 8 in embryos injected with *pk1a* MO designed to inhibit splicing into exon 7, as well as a decrease in a 200 bp PCR product spanning E6 and E7 in the same embryos. (D) Apparently similar amounts of a 100 bp *tbp* PCR product in embryos from each of the above knockdowns. The above results were confirmed using real-time quantitative PCR normalized against *hprt* or *cp* (ceruloplasmin). (E) Western blot analysis of 3 dpf larvae injected at 2 dpf with control (cont) or *pk1a* MOs. The upper panel shows a decrease in the 70 kDa Prickle band using anti-Prickle (αPk) antibody, while the lower shows constant Actin expression using αActin antibody.

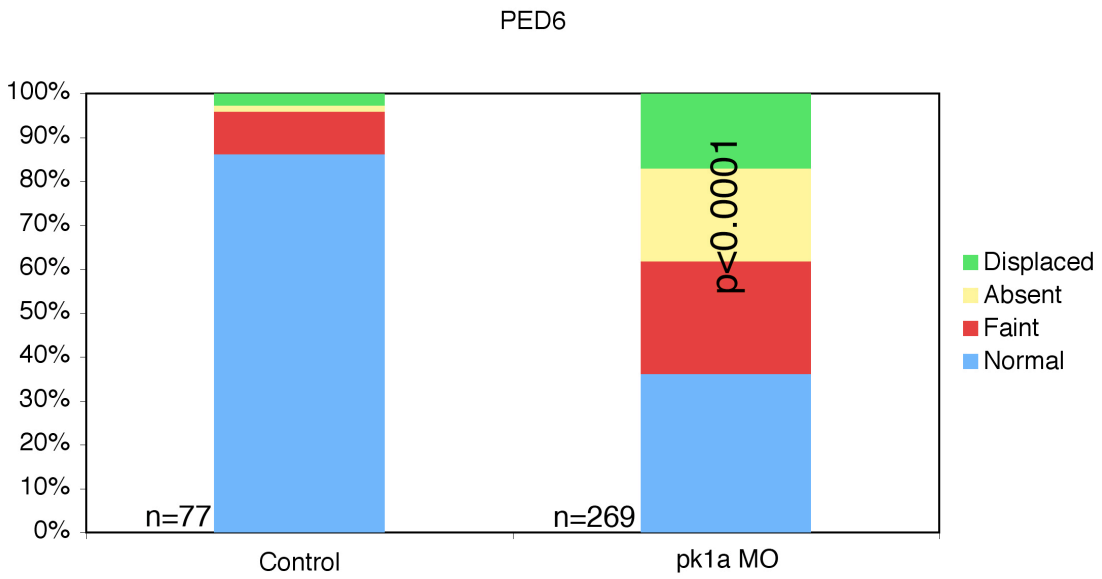

Figure S2. Abnormal gallbladder sidedness in *pk1a* morphants. PED6 uptake of control and *pk1a* MO-injected 5 dpf larvae, scored for normal right-sided placement, faint or absent intensity, or displacement to the left side. The total number of abnormal gallbladders is significantly increased in the *pk1a* morphants ($p<0.0001$, as depicted, by chi-square analysis), as is the number of displaced gallbladders ($p<0.0001$).

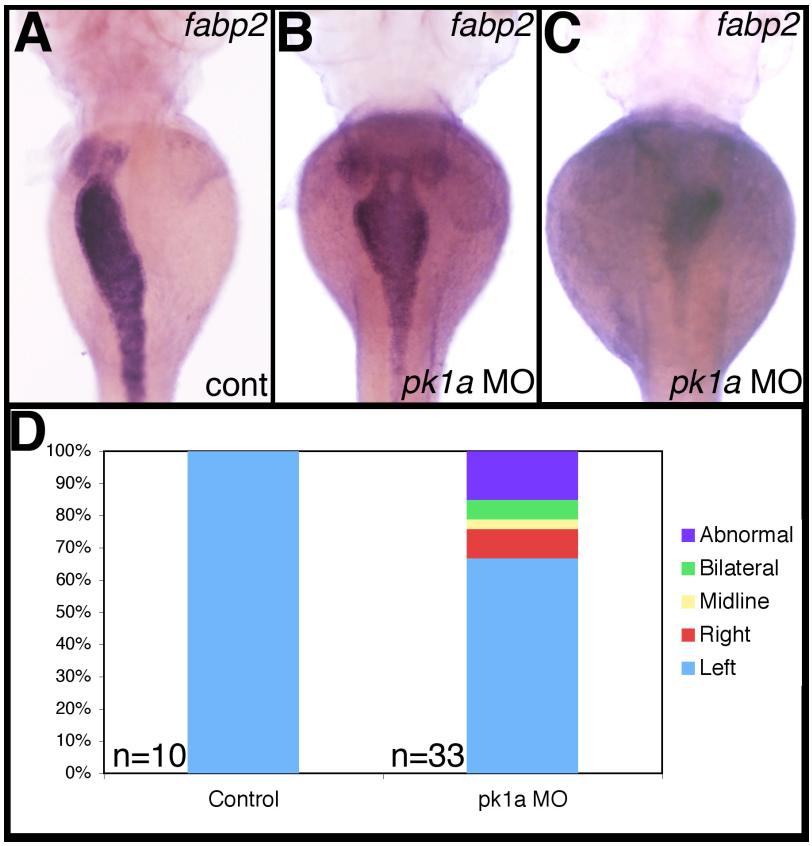

Figure S3. Abnormal intestine localization in *pk1a* morphants. Whole-mount in situ hybridization of fatty acid binding protein 2 (*fabp2*), an intestinal marker, in 3 dpf control (A) and *pk1a* morphants demonstrates abnormal intestine location, including "bilateral" (B), and "right" (C). (D) Graph depicting the scoring of *pk1a* morphants demonstrates a significant difference in the number of abnormally localized intestines (0% vs. 33%, $p<0.0001$ by chi-square test). Note that the *fabp2* probe also faintly stains the liver.

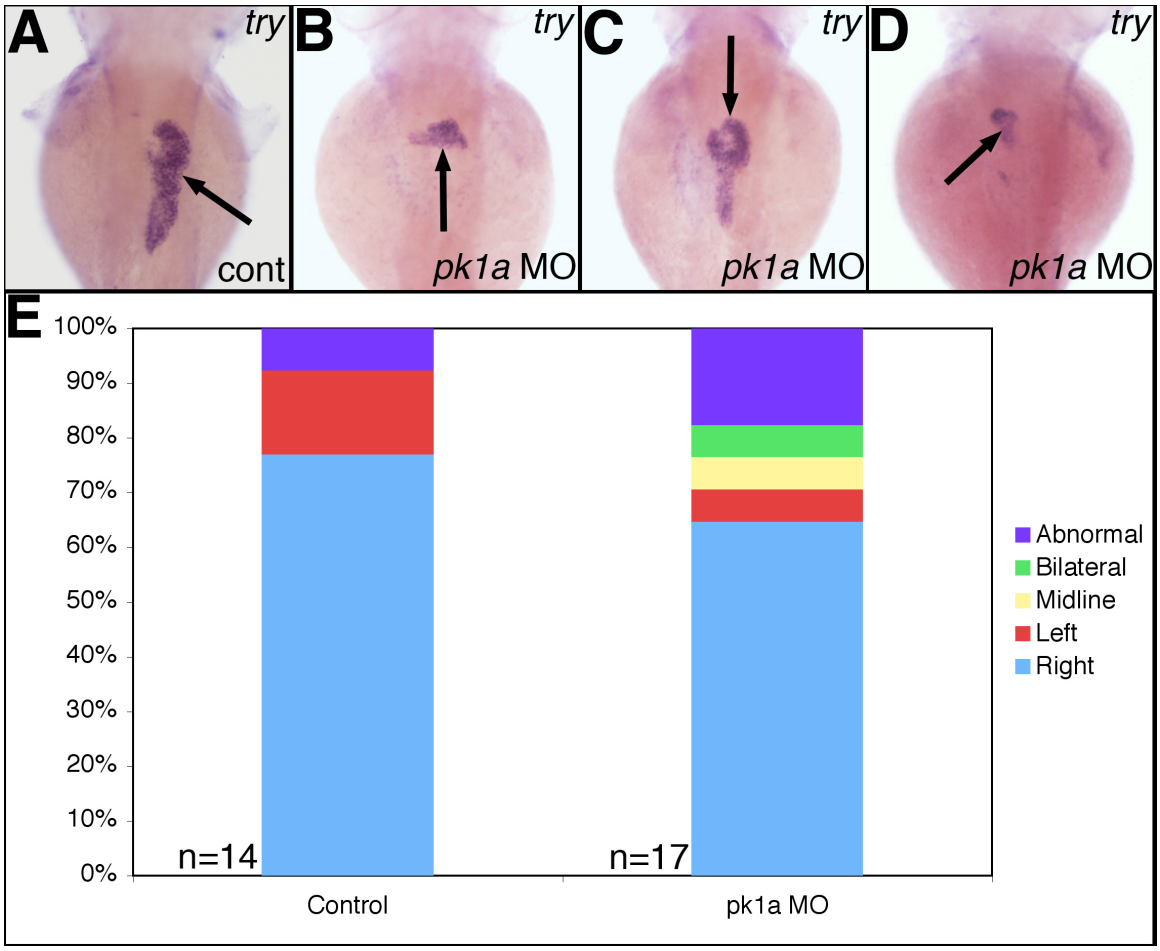

Figure S4. Marginally abnormal exocrine pancreas localization in *pk1a* morphants. Whole-mount in situ hybridization of *trypsin* (*try*), an exocrine pancreas marker, in 3 dpf control (A) and *pk1a* morphants demonstrates abnormal exocrine pancreas location, including "bilateral" (B), "midline" (C) and "left" (D). (E) Graph depicting the scoring of *pk1a* morphants demonstrates a slight difference in localization of the exocrine pancreas (p=NS by chi-square test).

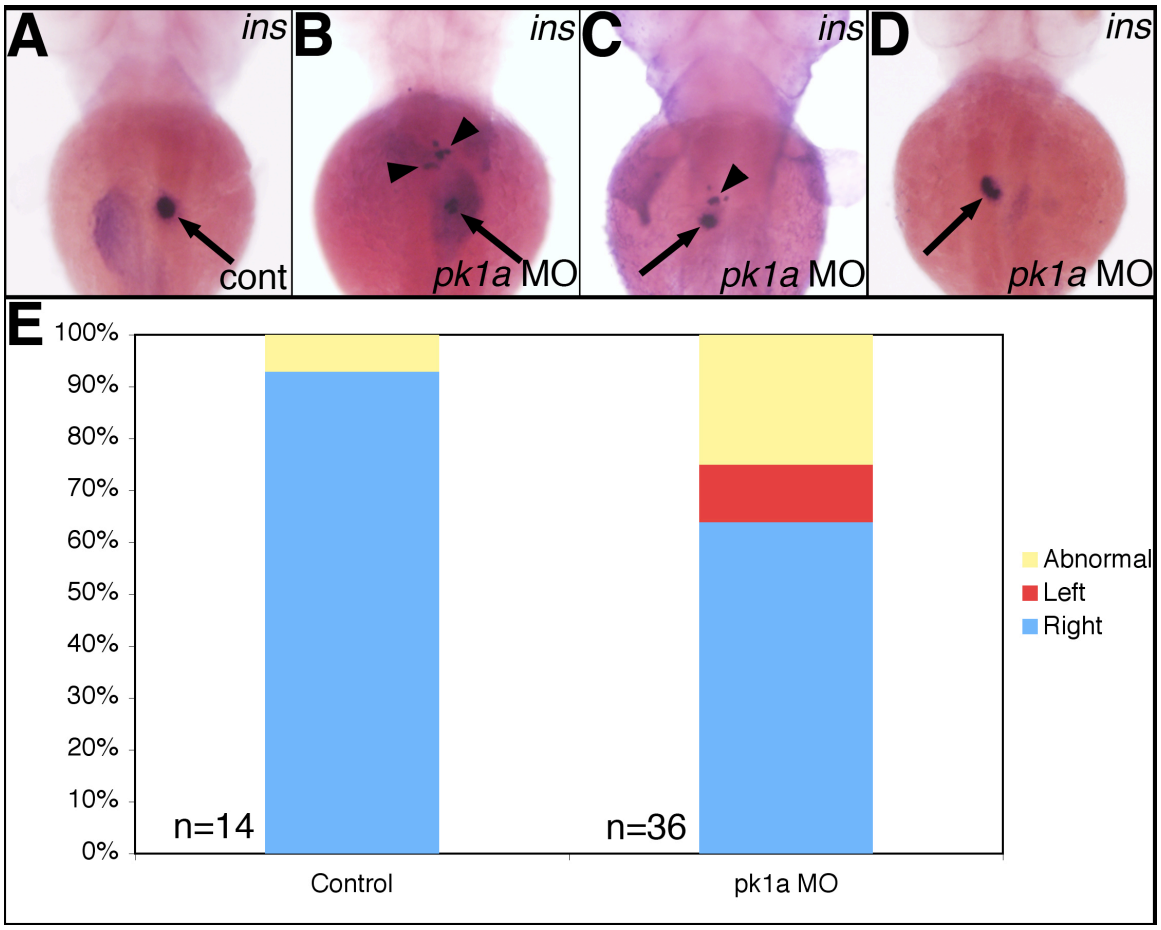

Figure S5. Abnormal endocrine pancreas localization in *pk1a* morphants. Whole-mount in situ hybridization of *insulin* (*ins*), an endocrine pancreas marker, in 3 dpf control (A) and *pk1a* morphants demonstrates abnormal exocrine pancreas location, including "abnormal" (B, C), and "left" (D). (E) Graph depicting the scoring of *pk1a* morphants demonstrates a significant difference in the localization of the endocrine pancreas ($p<0.0001$ by chi-square test). When combined with the localization of *try*, there is a significant difference in the localization of the pancreas in *pk1a* morphants ($p<0.0001$).

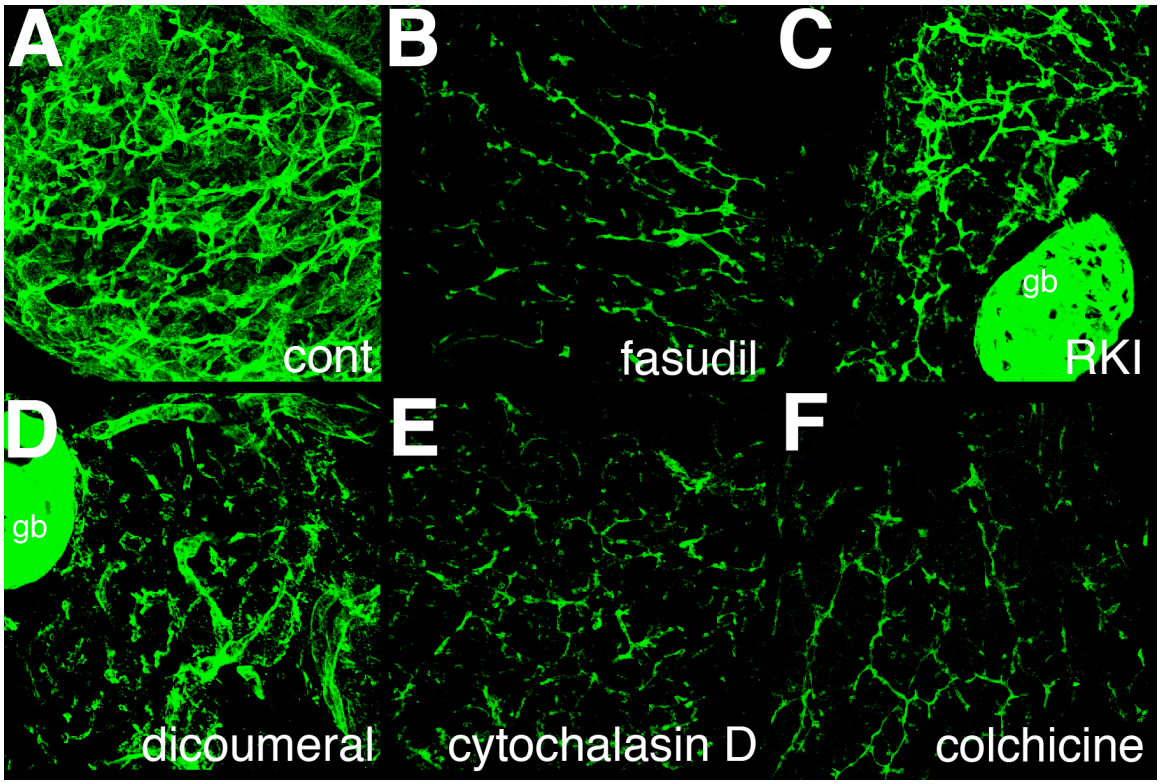

Figure S6. Additional examples of inhibition of Rho kinase, JNK, and cytoskeletal architecture negatively affecting biliary development. (A-F) Whole-mount projections of cytokeratin immunostaining of liver from a 5 dpf control larvae (A), compared to similar stainings from larvae treated with the Rho kinase inhibitors fasudil (B) and H-1152 (RKI, C), as well as the JNK inhibitor dicoumeral (D), the actin inhibitor cytochalasin D (E), and the microtubule and cytoskeleton inhibitor colchicine (F). gb, gallbladder.

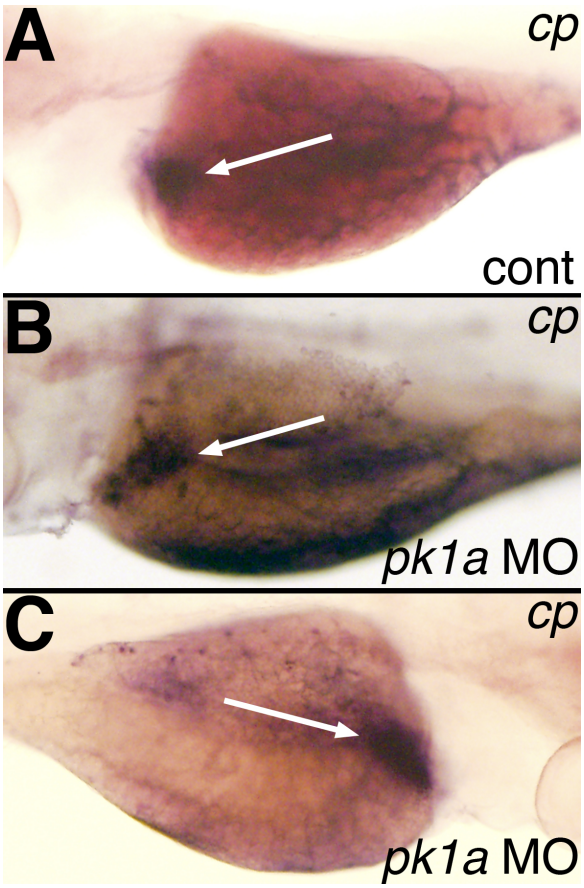

Figure S7. Ceruloplasmin (*cp*) staining of 4 dpf larvae injected with *pk1a* MO. (A) *In situ* hybridization of 4 dpf larva injected with control (cont) MO demonstrating liver staining (white arrow). (B-C) *In situ* hybridizations of 4 dpf larvae injected with *pk1a* MO demonstrating liver staining (white arrow) similar to (A). Views in A and B are left lateral, while that in C is right lateral, showing the liver on the right side, similar to other studies depicted here.

Supplementary Table 1. Sequences of primers and morpholino oligonucleotides.

Name	Sequence
Ankrd6 –F2	AGA TGC TGC CGA GAA AGT GT
Ankrd6-T3-B2	GCG CGA AAT TAA CCC TCA CTA AAG TGG CTC TGT GTC TCC TGA TG
Ankrd6-E4	ACG AGA ATA TCC GCA GCA GT
Ankrd6-E6	TCC TCA ATG TCC AGG TCA CA
Celsr1-F1	AAC AAT GGC ACT ACC GAA GG
Celsr1T3-B1	GCG CGA AAT TAA CCC TCA CTA AAG AGT CAC AGG GGA AAC AGG TG
Celsr2-F1	CCC CTG TGG TCA GCA TAA CT
Celsr2T3-B1	GCG CGA AAT TAA CCC TCA CTA AAG TCA CCA GGA ACA ACA CGG TA
Celsr3-F1	GTC AGA CTG CGA CTG GAT CA
Celsr3T3-B1	GCG CGA AAT TAA CCC TCA CTA AAG CAG CTC CAC AAA AGG AGG AG
Dsh2-F1	CAG ATG TGG TGG AGT GGT TG
Dsh2T3-B1	GGA TCC ATT AAC CCT CAC TAA AGG GAC AGA CCC TGT TGC GGT TAA T
Dsh3-F1	TGT GAT TTG TTG GCT GGT GT
Dsh3T3-B1	GGA TCC ATT AAC CCT CAC TAA AGG GAG CAA AGG AGC AAG AGT GTC C
Fabp2-F1	CAA CGT GAA GGA AGT CAG CA
Fabp2T3-B1	GAA TTC ATT AAC CCT CAC TAA AGG GAT CAC AGT GCA AAT GAC ACG A
Ins-F1	GCT CTG TTG GTC CTG TTG GT
InsT3-B1	GAA TTC ATT AAC CCT CAC TAA AGG GAG GAG AGC ATT AAG GCC TGT G
Prickle1a-F2	AAG TGC GGT ACT GCC AGT CT
Prickle1aT3-B2	GGA TCC ATT AAC CCT CAC TAA AGG GAG TTT AAA GCT CGC TCC ATG C
Prickle1a-E6	ATC ATG CAG CGT GTG AAC AT
Prickle1a-E7	GCA GTG CAC TTT CCC ATC AT
Prickle1a-E8	GTT TAA AGC TCG CTC CAT GC
Prickle2-F1	CCA GAG TGG GAA AGC TTC AG
Prickle2T3-B1	GCG CGA AAT TAA CCC TCA CTA AAG ATT GGC GTT GCT AAC CAA AC
Try-F1	GTC TCT GAA CAG CGG CTA CC
TryT3-B1	GAA TTC ATT AAC CCT CAC TAA AGG GAG AGC AGA CCT TGG CGT AAA C
Vangl2-E2	CAG AGA CAG CAG CAG CAG AG
Vangl2-I2	CCA GGA GCA CTC CTC AAA CT
Wnt11-F3	GAG CTC ATG CAC AGC ATT GT
Wnt11T3-B3	GAA TTC ATT AAC CCT CAC TAA AGG GAC AGT CTC TTC CCC TCA GTG C
Wnt11r-F3	CAC CAC AAA CCC ACC TCT CT
Wnt11Rt3-B3	GAA TTC ATT AAC CCT CAC TAA AGG GAT CCG TGT AAG GGT TGT AGC C
MO prickle-ATG	5’CACCGCGATTCTCCAGCTCCATCAC3’
MO prickle-IE7	5’CCACACTGCACACACAGCACATTAC3’
MO vangl2-ATG	5’GTACTGCGACTCGTTATCCATGTCG3’
MO vangl2-EI2	5’GTGTACTGACCCGATCATCTCCGCG3’
MO ankrd6-ATG	5’GAGGCATCGCGCTGGCTCATGAATC3’
MO ankrd6-IE2	5’CTCATGTCCTGAAGAGAATCCTGCA3’