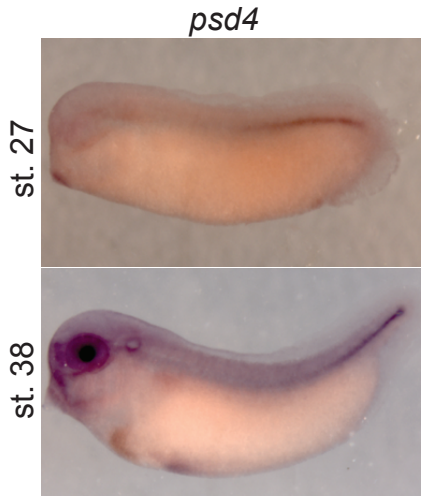
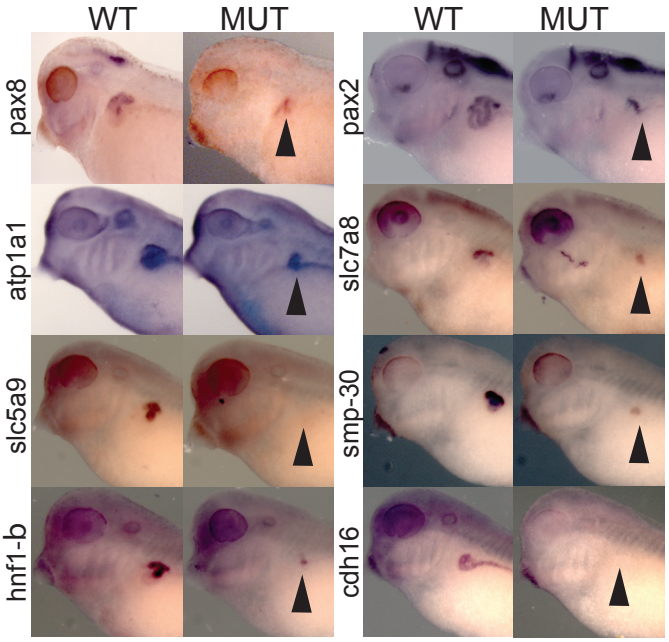
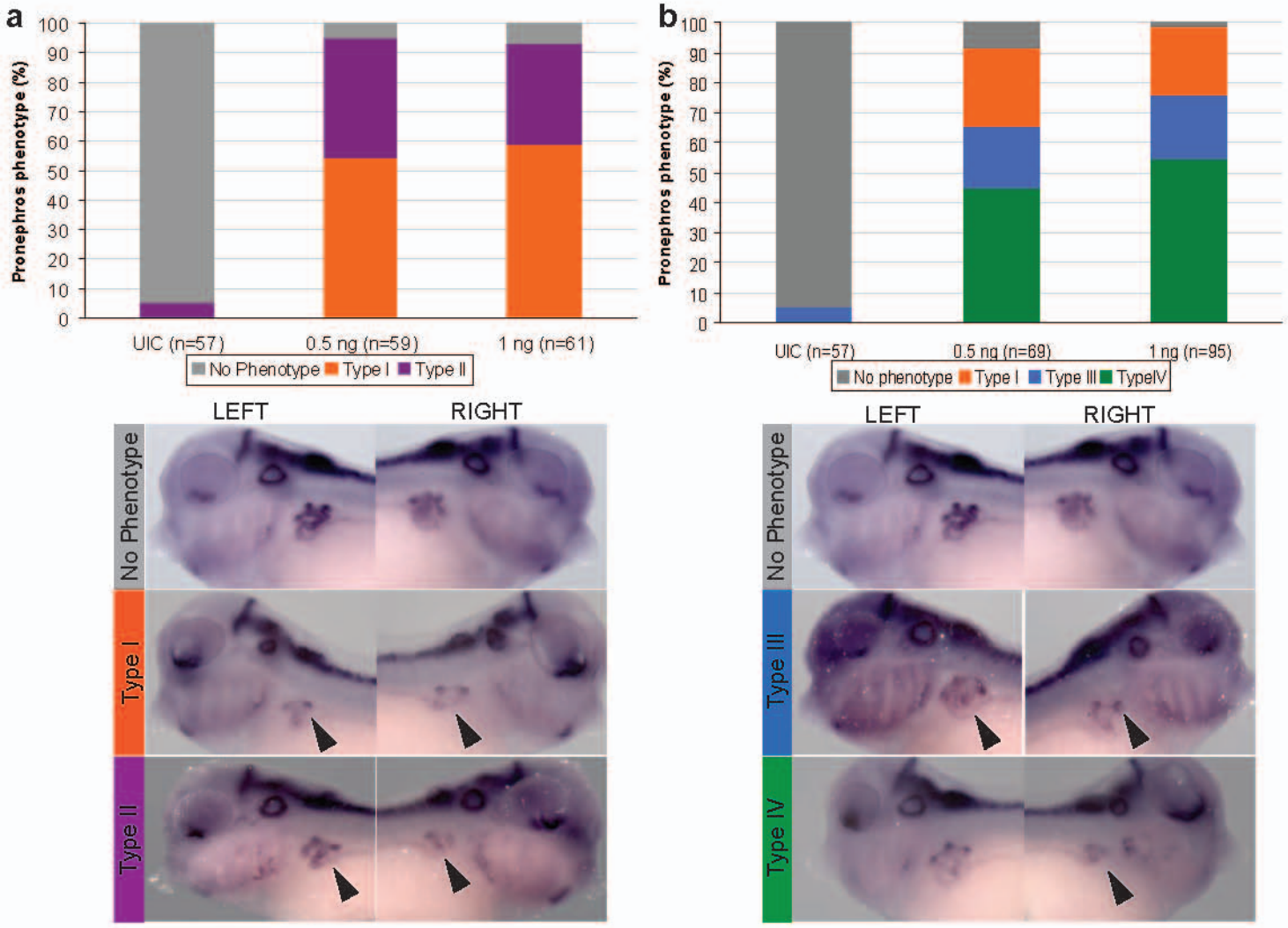


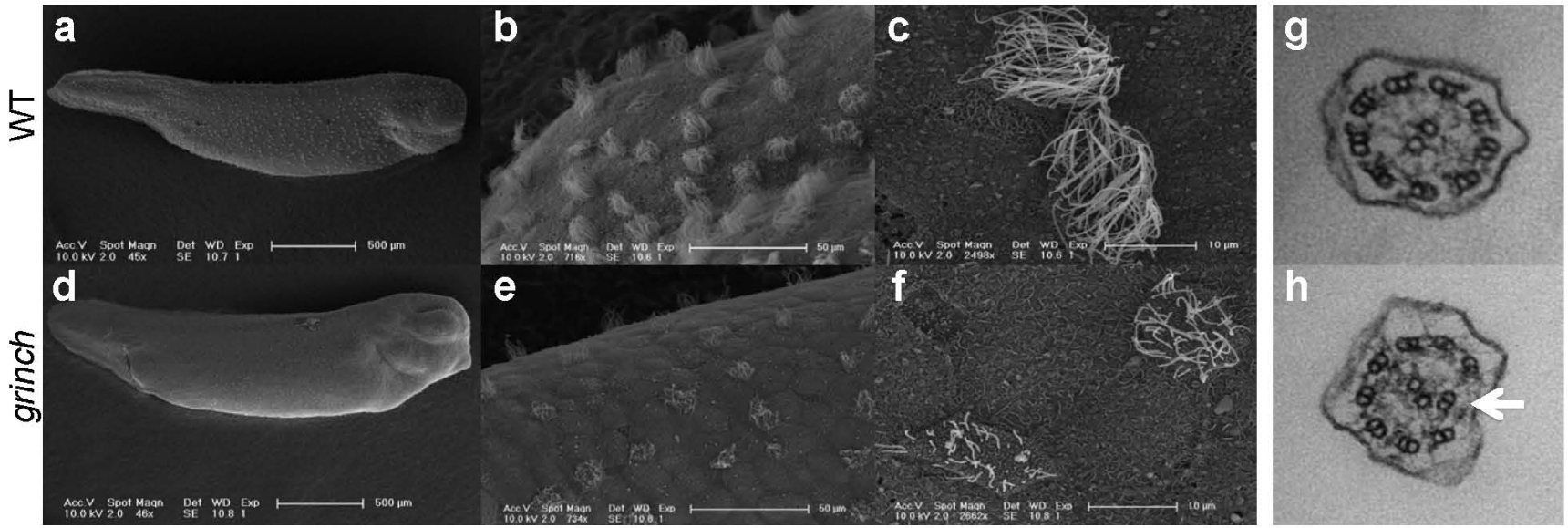
Supplementary Figure 1: *psd4* expression by WMISH at stages before pronephros are functional (st. 27) and when they are starting to function (st. 38).



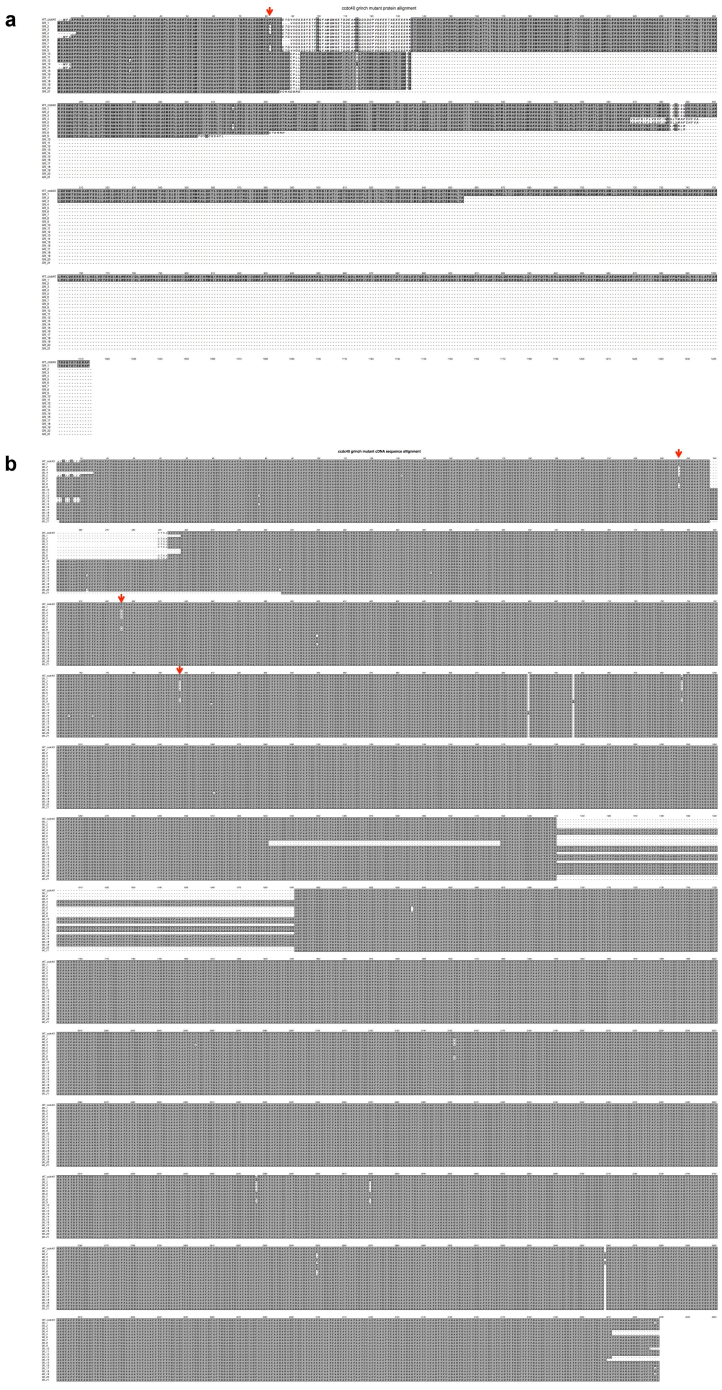
Supplementary Figure 2: Expression of pronephric markers in wt and ruby mutant embryos. Embryos from a heterozygous carrier cross were fixed at 36-37 stage and the expression of pronephric markers: *pax8* (*paired box 8*), *pax2* (*paired box 2*), *atp1a1* (*ATPase, Na+/K+ transporting, alpha 1 polypeptide*) , *slc7a8* (*solute carrier family 7 (amino acid transporter light chain, L system)*, *member 8*), *slc5a9* (*solute carrier family 5 (sodium/glucose cotransporter), member 9*), *smp-30* (*senescence marker protein-30*), *hnf1*-b (*HNF1 homeobox* b) and *cdh16* (*cadherin 16*), were studied by WMISH. Embryos were genotyped to confirm the mutation after imaging. Arrowheads indicate abnormal pronephros.



Supplementary Figure 3: Phenocopy of *ruby* mutant by *pax8* MO injection. *Pax8* morpholino was injected at one **(a)** or two cell stage (single cell injected) **(b)**. Embryos were fixed at st 36-37 and pronephric development was assessed by *pax2* expression. Abnormalities in pronephros development were classified: No phenotype; pronephros similar to uninjected controls (UIC); Type I: disrupted and abnormal pronephros similar to *ruby* mutants on both sides of the embryos. Type II: abnormal pronephros on one side and moderate abnormal pronephros on the other side, Type III: disrupted and abnormal pronephros on the injected side and moderate abnormal phenotype on the uninjected side. Type IV: disrupted and abnormal on the injected side and normal pronephros on the uninjected side. Images of *Pax2* WMISH showing each type of phenotype are shown. For Type III and IV, embryos shown were injected on the right side. Arrowheads indicate disrupted and abnormal pronephros.



Supplementary Figure 4: SEM and TEM of WT and *grinch* embryos. **(a-f)** SEM; **(g,h)** TEM; **(a-c)** WT embryos; **(d-f)** *grinch* mutant embryos. **(f)** *grinch* mutants have fewer, shorter and abnormal cilia per cell. **(g)** WT cilia showing the 9+2 microtubule structure. **(h)** mutant cilia showing a displaced outer ring microtubule doublet (arrow). Embryos in **(a,d)** are lateral view with dorsal on top and anterior to the right.



Supplementary Figure 5:
(a) Protein sequence alignment of the 21 *grinch* mutant clones. **(b)** cDNA sequence of the 21 mutant clones. Heterozygosity at the mutant locus (red arrows).

Supplementary Table 1: Genotyping primers used for fine mapping in *ruby* and *grinch*. n/a: not applicable.

Mutant	Marker	Genotyping Primers	Enzyme
ruby	1790	5' AATAAAGGTCCTTGCCTTGC 3' 5' CAATGTCAAGATGGCTTTGG 3'	StyI
	1211	5' CTGAGAGCCAGAATGAGAGG 3' 5' ATCATGCCTACACCAAGTGC 3'	PstI
	735	5' GCAGTCCTTCGACAATCAGC 3' 5' TTTGTATGGGCACTGGAACC 3'	PvuII
	1364	5' ATGAGGGAGAAATTCAGAGG 3' 5' GGTACTGGTGTGCGAATGG 3'	PstI
	1571	5' CTTCACCACAGTTCATCATGG 3' 5' CAGGAGACGCAAATAATCTTCC 3'	PstI
	1627	5' TCCGGTTCAATCTTCTCTGC 3' 5' CTCTAACGTTTCAGGGTTCC 3'	BamHI
	1789	5' GAGCCTTCTTAGTGGGATGG 3' 5' CAGTGCCATGAACAATCTCC 3'	PvuII
	1791	5' TGCTGATTCCCTGCACATAC 3' 5' GCAGCTCGAAGTGAAGATCC 3'	NspI
	1862	5' GGTGGCCACAAACGTTACC 3' 5' CGTTACCTCTGCAATCAAACC 3'	StyI
	2011	5' CCCATTCGAGAACTGGAAAA 3' 5' GGTTGTATTGGGAGCAATGG 3'	PsiI
	2043	5' CCACCCCTTTAAATGGAACC 3' 5' TTTGGCTGCCAGATACTCCT 3'	DraI
	2056	5' GCAAGCTGGGAAGGATGC 3' 5' GTTGGAAGTTGATGTTCTGC 3'	PstI
	2380	5' TGTGAAACAGGGAATGTAGG 3' 5' GGTAATGCCGAATGTCATGG 3'	PvuII
	6051	5' GCAAGCAGGAGCCTATAACG 3' 5' ATGACTGGCAAGCTGTTGG 3'	BamHI
grinch	008C01	5' AGACCCTGCCAAGAAAAGAC 3' 5' AGTCCACTCAGCCCTGTTC 3'	n/a
	016B06	5' AAAGGCAACAATAATGGCAG 3' 5' GCCAAGTCAGCATCTAATGG 3'	n/a
	100A10	5' ATTCTATCCCCTGATCCACC 3' 5' TGCAACTATGGGGCTTATTG 3'	n/a
	148E4	5' TCCCAGTCACGACGTATATTGAGGTGCCAGGATGC 3' 5' CACAAACAAGGCCGAAACTT 3'	n/a
	148M1	5' TCCCAGTCACGACGTGCATGGAACAAAACGTTGG 3' 5' ACCTTGGGTCAAAACATGGA 3'	n/a
	s148-783K	5' CATGTCATATTTGCCGTTGG 3' 5' AGCAACATTCAAGGGGTTGG 3'	SsiI
	s148-146K	5' CAGCAAGCTGGTTGTAGTGG 3' 5' GCCAGAGCTCTCAAGTAGAGG 3'	PstI
	s148-20K	5' CTTTTCAACAGCCTCTGATGG 3' 5' ATGCCAGAGTCCCATAAAGC 3'	AvaII
	s304-1.3M	5' TAGCTGCCCCTATCACCATC 3' 5' TACATGTCCCGGGCTTCTAT 3'	AlwNI
	s304-1M	5' CAGGGGTTGGACATGTAAGC 3' 5' AGGCAGATCCAGAGCTTTCC 3'	AvaII
	s304-875K	5' TCCCAGTCACGACGTCTCGAGCAATGAGTGACAGC 3' 5' TGGCACCAATAGGATTGACA 3'	n/a

	s304-219K	5’ TCCCAGTCACGACGTAGAGCTGGCAGATGGCTAGA 3’ 5’ TGTGCCCATTTACCTGTTTG 3’	n/a
	560E1	5’ TCCCAGTCACGACGTGGCATGGAGATGTTGCTCTT 3’ 5’ ATGGCCCATGACTCTCTGTT 3’	n/a
	560B2	5’ TCCCAGTCACGACGTCATCCCAGTGCTTTGCTTTA 3’ 5’ GGCACTCACAAGGCTTTTATG 3’	n/a
	029C01	5’ CGAGTTCGGCTATGACAGAC 3’ 5’ ATCCCTTTTGGGTTGGTTAC 3’	n/a
	045F05	5’ TGCATTTTGTAATGGGGTG 3’ 5’ TTTGTCAGGCACAACAAGTC 3’	n/a
	N3_34	5’ AGGAGATGCAATTTGGTTGG 3’ 5’ AGAGGCACACACAGGAAAGC 3’	n/a
	044G07	5’ ACTGTGACCTTCCTACACGC 3’ 5’ ATTATCTTGTTGGGGACACG 3’	n/a
	006G10	5’ TGCTTCAGGGGTCTTCATAC 3’ 5’ CGAGATAGCCAGATAGCCAG 3’	n/a

Supplementary Table 2: RT-PCR primers used for *grinch* transcript amplification and sequencing.

Name	Primer
UTR5-1F	5' AGCGTCGCTTAGCAACAGTTAT 3'
UTR3-1R	5' CCCAGCTACACAGCAGCTTATT 3'
UTR3-2R	5' TCAATAGAGATTTACTAGCCAGTG 3'C
UTR3-3R	5' CATTTCTTTTGTTTGTCAGTGTTTAG 3'
RT_full-F	5' ATGTTCCTTAGCCCTAGATCTGG 3'
RT_full-R2	5' TCAAGGTGCCCTCTCGGATGTA 3'
E2tE3-F	5' AACAACCTCTTTCACCAAAAGC 3'
E3tE4-R	5' ATAGGTCTTGAGAGCCGTCTGG 3'
E6tE7-F	5' GAGAATATGAATCGGGATGTGC 3'
RT-3F	5' TTCAGGAGGCTTTTAGTCTTGC 3'
RT-3R	5' CCCTTTGCTTGGTTAGATTCAC 3'
RT-4F	5' TACACTGGCAATTGAGAGGAAA 3'
RT-4R	5' TCACTGACGGTTAACTGCTTCT 3'
RT-5F	5' TCAGATCATGTTGTGGGAAAAG 3'
(Fl.)-m13-F	5' TCCCAGTCACGACGT 3'