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Supplemental Data

Mutations in Radial Spoke Head Protein Genes *RSPH9* and *RSPH4A* Cause Primary Ciliary Dyskinesia with Central Microtubular Pair Abnormalities

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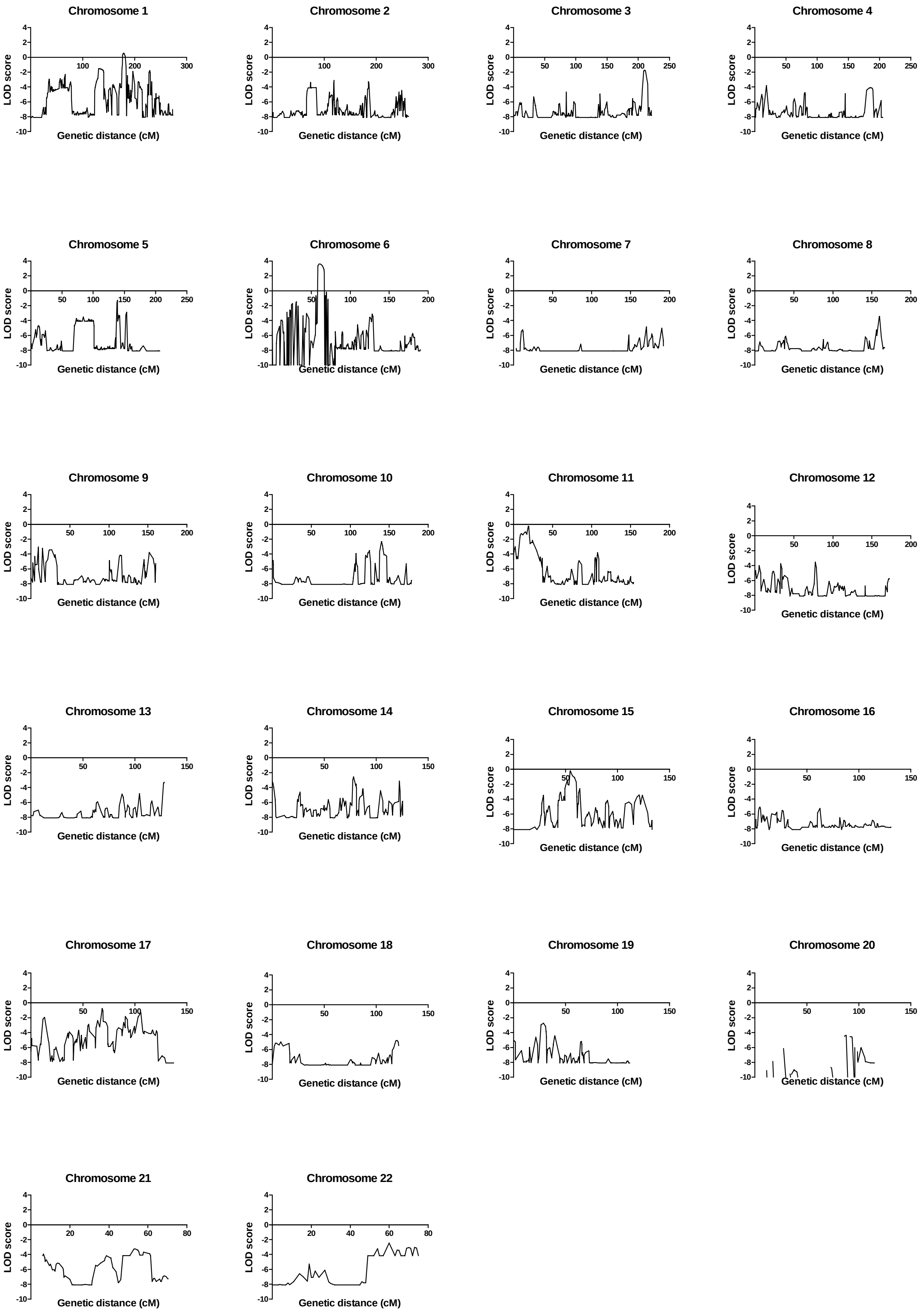
A.


Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
Chromosome 10
Chromosome 11
Chromosome 12
Chromosome 13
Chromosome 14
Chromosome 15
Chromosome 16
Chromosome 17
Chromosome 18
Chromosome 19
Chromosome 20
Chromosome 21
Chromosome 22
LOD score
Genetic distance (cM)
4
2
0
-2
-4
-6
-8
-10
50
100
150
200
250
300
20
40
60
80

B.

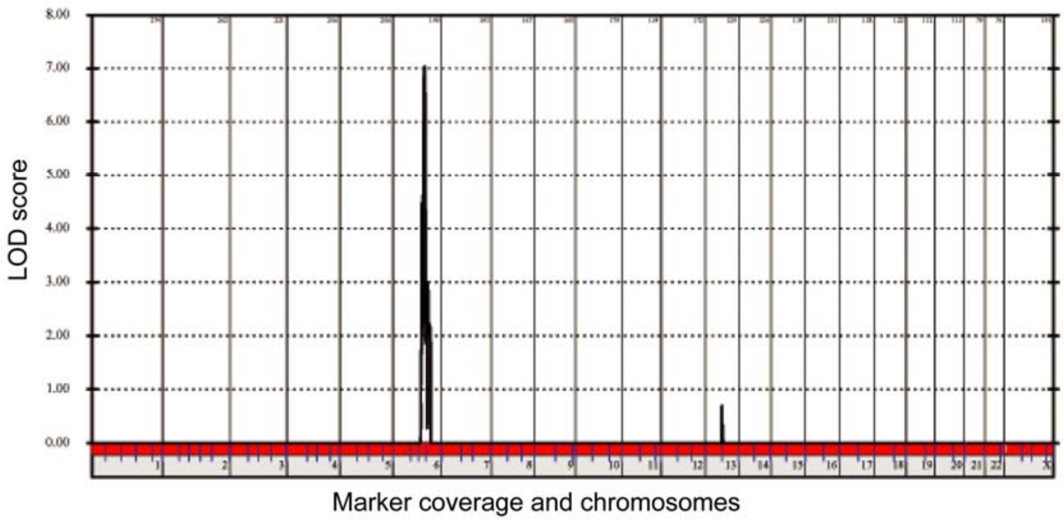


Figure S1. Whole Genome *RSPH9* and *RSPH4A* Linkage Scan Results

(A) Genome scan results for Family UCL152 showing results for all chromosomes. Family UCL146 was subsequently genotyped across the UCL152 chromosome 6 *RSPH9* locus to generate the joint lodscore for both families shown in Figure 1. Analysis was performed using GENEHUNTER 2.1r5.

(B) Genome scan results for the five transposition defect families linked to *RSPH4A*: UCL105, UCL131, UCL132, UCL138 and UCL170, showing results for all chromosomes. Analysis was performed using MERLIN 1.01 within *easyLINKAGE*.

A RSPH9

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                                                       p.Lys268del
Human                                 LTFYHAPRTKNYGYVYVGTGEKNMDLPFML 276
Canis familiaris                      LTFFHAPRTKNYGYIYMGTGEKNIDLPFML 246
Bos taurus                            LTFYHAPRTKNYGYIYVGTGEKNLDLPFML 276
Sus scrofa                            LTFYHAPRTKNCGYIYVGTGEKNMDLPFML 276
Monodelphis domestica                 LTFFHAPRTKNHGYIYVGTGEKNIDLPFML 276
Ornithorhynchus anatinus              LTFFHVPQTKNYGYFYVGTGEKNMDLPFML 276
Mus musculus                          LTFYHAPRTKNYGYIYVGTGEKNMDLPFML 276
Gallus gallus                         LTFYHVPMTKQYGYIYCGTGEKNLDLPFML 275
Danio rerio                           FTFFHVPQTPQHGYIYMGDGLMNLDLPFML 277
Apis mellifera                        MMFYHKLNSPHYGFLYFGHGKKNMDIVFMV 279
Drosophila melanogaster               MTFFHKINSHKHGFLYLGDGKKNFDLLFMY 281
Nematostella vectensis                FTFFHVPETRKYGMVYIGTGNKNLDLPFML 246
Xenopus tropicalis                    MTFYHVPLTPLHGHLYIGTGERNLDLPFMI 277
Chlamydomonas reinhardtii             YFFYYAANELTWGSLYVGDGLRNNDLIFML 269
Tetrahymena thermophila               FVGYHRANSQIFGYAYIGNGIKNSDLPFLL 296
Trypanosoma brucei                    SLFWYRPGSKDCGQVYCGSGERDFEVCFLL 297
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NCBI ID numbers:
Human NP_689945; C.familiaris XP_851950; B.taurus XP_612744; S.scrofa XP_001927232; M.domestica XP_001362778; O.anatinus XP_001506544; M.musculus NP_083614; D.rerio NP_001025284; G.gallus XP_419496; A.mellifera XP_001121220; D.melangaster NP_724049; N.vectensis XP_001619872; X.tropicalis NP_001005021; C.reinhardtii ABC02020; T.thermophila ABB03904; T.brucei XP_828524.

B RSPH4A

CLUSTAL 2.0.8 multiple sequence alignment.
Human RSPH4A (NP_001010892)
against *Chlamydomonas* RSP4 (XP_001700728) and RSP6 (XP_001700729):

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RSP4            ------------------------------------------------------------
RSP6            ------------------------------------------------------------
RSPH4A          MEDSTSPKQEKENQEELGETRRPWEGKTAASPQYSEPESSEPLEAKQGPETGRQSRSSRP 60

                                      p.Pro87Ser            p.Gln109X
RSP4            ------------------------------------------------------------
RSP6            ------------------------------------------------------------
RSPH4A          WSPQSRAKTPLGGPAGPETSSPAPVSPREPSSSPSPLAPARQDLAAPPQSDRTTSVIPEA 120

                                              p.Gln154X
RSP4            ------------------------------------------------------------
RSP6            ------------------------------------------------------------
RSPH4A          GTPYPDPLEQSSDKRESTPHHTSQSEGNTFQQSQQPKPHLCGRRDVSYNNAKQKELRFDV 180


RSP4            ------------------------MAAVDSVAQALAYLQVHSPQDGTSMYDHLVKLVSKV 36
RSP6            --------------------------MAADVGQALAFLQQVKTTQGASIYEGLKAALAKV 34
RSPH4A          FQEEDSNSDYDLQQPAPGGSEVAPSMLEITIQNAKAYLLKTSSNSGFNLYDHLSNMLTKI 240

RSP4            LEDQPKNAVDLLETSLLVKKSTFDPKESSPLVPIPVAPDATQTQAAVSIFGDPELPINPA 96
RSP6            LEDRPVNAVEALETSVLSTPPAAN--LSVPLVPAASAAAAAAAVAKASLFGDPEPVLDPE 92
RSPH4A          LNERPENAVDIFEN---ISQDVKMAHFSKKFDALQNENELLPTYEIAEKQKALFLQGHLE 297

RSP4            TGEPVPADPPNEFEAENMLGAAAVLDCLGVGLGRELGVNIALAAKRIGEDPKLAVRSVRF 156
RSP6            SGEPIDPDAPNEFECEDVEGDGDLLDGLGVGLGRQEMYAAMLAVKRLGEDAKRGVSTVRF 152
RSPH4A          GVDQELEDEIAENALPNVMESAFYFEQAGVGLGTDETYRIFLALKQLTDT--HPIQRCRF 355

RSP4            FGKFLGLYSDYFVFEVAFKKEAAKEAAPAAPAPERVEGEAASSS--------------AP 202
RSP6            FGKFFGTQADYYVFETTLQSNPDMPEAPEG------------------------------ 182
RSPH4A          WGKILGLEMNYIVAEVEFREGEDEEEVEEEDVAEERDNGESEAHEDEEDELPKSFYKAPQ 415

RSP4            EVPVEEPGKGANKFTYLVCSSLGGPLTRLPDVTPAQVKASRRIKKLLTGRLTSHVSTYPA 262
RSP6            TIPLEPYGEGVNAYIYFVSNTLGGPLQQLPYVTPEQIKASRLLRRYLTGRLDAPVSAFPA 242
RSPH4A          AIPKEESRTGANKYVYFVCNEPGRPWVKLPPVIPAQIVIARKIKKFFTGRLDAPIISYPP 475
                          p.Arg490X
RSP4            FPGNEANYLRALIARISAATVVAPSDLFSLNDETGELERAEDWEPPAGRE---------- 312
RSP6            FPGNEANYLRALIARISAATVCCPRGFFTADDDSAELSANDEWVPLKGRE---------- 292
RSPH4A          FPGNESNYLRAQIARISAGTHVSPLGFYQFGEEEGEEEEEAEGGRNSFEENPDFEGIQVI 535

RSP4            --MAAPTAWVHVRPHLKSQGRC----EVHKRELPEDADEDE--FYNEDELEEGPDLLAAL 364
RSP6            --MALPVNWSHRYAHLKGQGRT----VTHKRDPPDEEEEPEKNFWTAEEMEAGPPPLATL 346
RSPH4A          DLVESLSNWVHHVQHILSQGRCNWFNSIQKNEEEEEEEDEEKDDSDYIEQEVGLPLLTPI 595

RSP4            EEDAQLPGEQ------AAWTPIYSSASEAVKTQAGGLRSLVWPGAVCGGRGSEWTCVYVG 418
RSP6            DTDAPLPAATGDKVPPPAWSPVFASASVTTRNQVAGVRSNRWPGAVCACAGRHFTSMYVG 406
RSPH4A          SEDLEIQNIP-------PWTTRLSSN-LIPQYAIAVLQSNLWPGAYAFSNGKKFENFYIG 647

RSP4            WGVKN--APFVPLPPPPVAQEF----------------AWGEVETQELELKP--APPPPE 458
RSP6            WGIKAG-GEWSPCPPPPPVPQWGA-------------PAAGVEGGQQLLLECNDLPPKPA 452
RSPH4A          WGHKYSPDNYTPPVPPPVYQEYPSGPEITEMDDPSVEEEQAFRAAQEAVLLAAENEESEE 707

RSP4            EEAEADE-- 465
RSP6            PPEEEDE-- 459
RSPH4A          DEDEEDDYD 716
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Figure S2. Evolutionary Conservation of Amino Acid Residue Changes in RSPH9 and Homology of RSPH4A

(A) Conservation of RSPH9 amino acid Lys268 across multiple species and phyla (red).
(B) Alignment of human RSPH4A and the two *Chlamydomonas* homologs, RSP4 and RSP6 which are 31% and 30% percent identical to human respectively. The *Chlamydomonas* proteins are shorter and align to the human protein C-terminus starting from position Ile211. The radial spoke domain in RSPH4A (Pfam PF04712) is located at positions c.207-697 of NP_001010892. Human mutations identified in PCD patients are highlighted in red. Alignment of 16 species (not shown) shows the RSPH4A Pro87, Gln109 and Gln154 residues are located in a mammalian specific N-terminal region not present in ciliate homologs. However, the C-terminal Arg490 is highly conserved between mammals and ciliates.

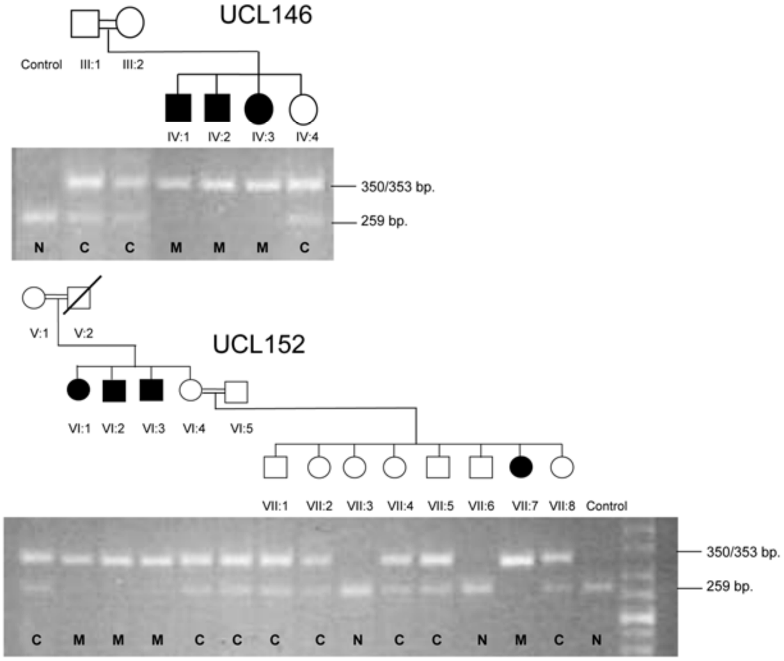

Figure S3. Representative Example of Confirmation of Mutation Inheritance

MboII restriction digest of exon 5 PCR product confirming the *RSPH9* c.804_806delGAA mutation co-segregates with the disease status in both extended Bedouin kindreds, in accordance with haplotype data. N, wild-type allele homozygote; C, carrier; M, homozygous for mutation.

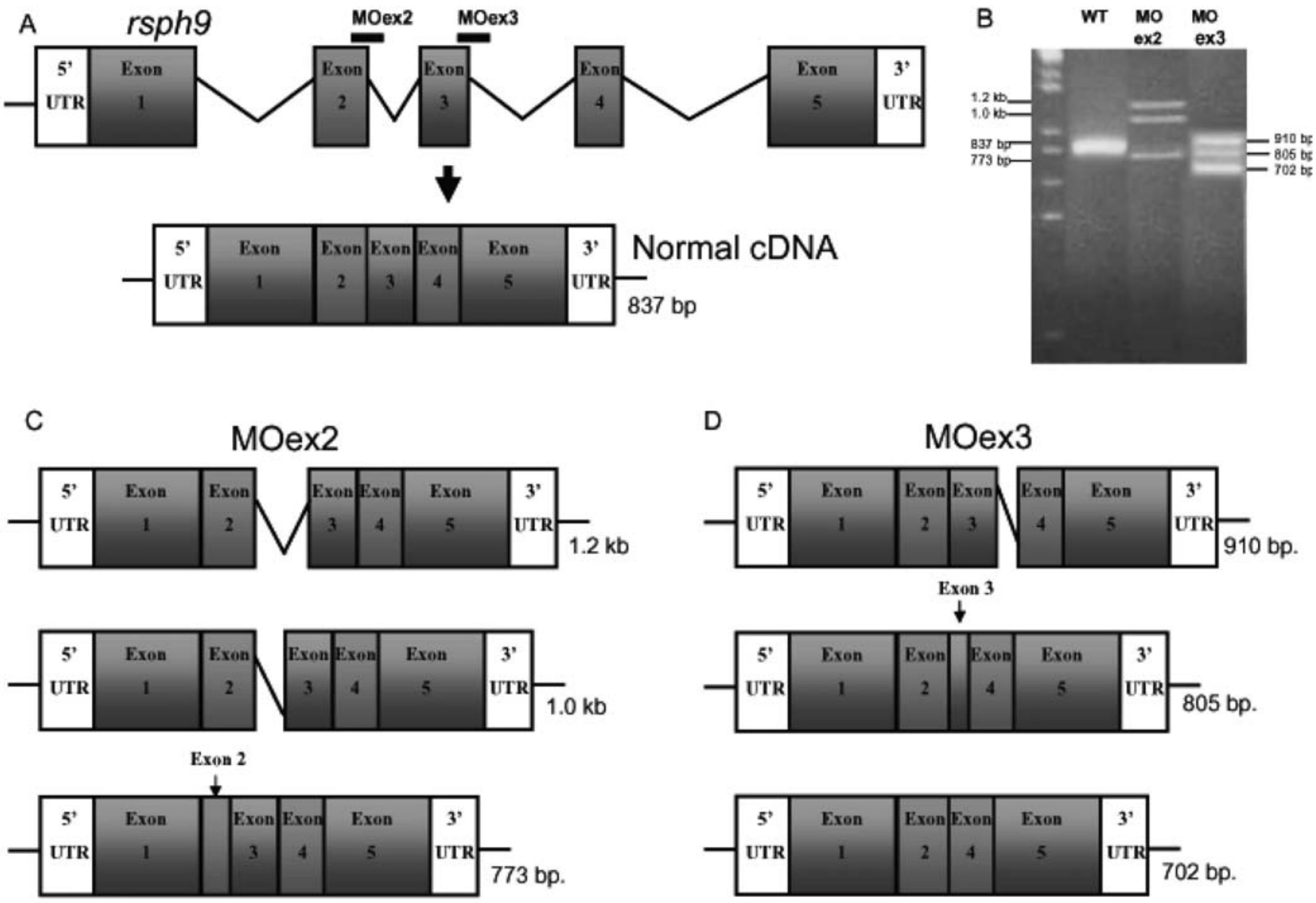

Figure S4. Disruption of Zebrafish *rsph9* mRNA Splicing by Morpholino Injection

(A) Genomic (7.2 kb) and cDNA (827 bp) structure of zebrafish *rsph9* indicating exon 2 (MOex2) and exon 3 (MOex3) MO location.
(B) RT-PCR of 72 hpf control or morpholino-injected embryos shows MO effectiveness indicating suppression of correct mRNA splicing persists for at least 72 hpf. 24 hours hpf showed the same results (not shown). An 837 bp fragment is generated in control embryos, but no wild-type mRNA was expressed after either MOex2 or MOex3 injection. Both only produced aberrant transcripts.
(C) Schematic of RT-PCR amplicons generated in MOex2-injected embryos. Three products were produced due to disrupted intron 2 splicing: either non-splicing (1.2 kb product), partial splicing of intron 2 (1.0 kb product), or deletion of 64 bp from the 3' end of exon 2 (773 bp product) presumably due to use of a cryptic splice donor in exon 2.
(D) Schematic of RT-PCR amplicons generated in MOex3-injected embryos. Three products were produced due to disrupted intron 3 splicing: either partial splicing of intron 3 (910 bp product) or a 28 bp deletion at the 3' end of exon 3 (805 bp product), presumably due to cryptic splice sites, or complete deletion of exon 3 (702 bp product). The MOex2 and MOex3 aberrant transcripts all predict premature truncation of the 277 residue protein due to frameshifts. This occurs for MOex2 after 155 amino acids in the 1.2 and 1.0 kb products, and after 111 amino acids in the 773 bp product; and for MOex3 after 205, 187 or 132 amino acids for the 910, 805 and 702 bp products, respectively.

Table S1. Genomic Sequencing Primers

Exon	**Primer 1**	**Primer2**
RSPH9		
Exon 1	5’-CCCAAACTCTGGAAAGGATG-3’	5’-CCATTCCACTCATCTCAGATCC-3’
Exon 2	5’-GCCCAACCCACTAGACAGAA-3’	5’-CCAGCCTGTTGCCATAATTT-3’
Exon 3	5’-AGCGTGATCTGTGTGGCTCT-3’	5’-CTGCTGGACTCCCAGACATA-3’
Exon 4	5’-GGTCCAGTGCAGACAGAAA-3’	5’-GTGAACCACCAATTCCCACT-3’
Exon 5	5’-CCAGTGGAACCATAGCACCT-3’	5’-ACCAGACGGAGGTGGAGTTA-3’
RSPH4A		
Exon1.1	5’-AGGGGTTGTACCGAATCACA-3’	5’-TCAGGAATCACACTCGTGGT-3’
Exon1.2	5’-GGACCAGAAACATCATCACC-3’	5’-CAGAGATACTCACACACCCT-3’
Exon 2	5’-CTAATGACCCCAGAAATGTC-3’	5’-GATCACGCCATTGTACTCCA-3’
Exon 3.1	5’-TCACCATGTTGGCCAGTCTT-3’	5’-ATGAACCAGGAAGACCATGG-3’
Exon 3.2	5’-GATGTAGCTGAAGAGAGGGA-3’	5’-ATCCCTGGGACCTTCAGTTA-3’
Exon 4	5’-AGCACGGTCCTTTCCTTGAA-3’	5’-ATGGGAAGAGGATGAGACCT-3’
Exon 5	5’-TCATGAAGTACCCTCCTGAT-3’	5’-CAGAGCAAGACTCTGTCTCA-3’
Exon 6	5’-TGCTTGTGGTCTAGGTGTAG-3’	5’-ACACACCCCTTATATGGGAC-3’