

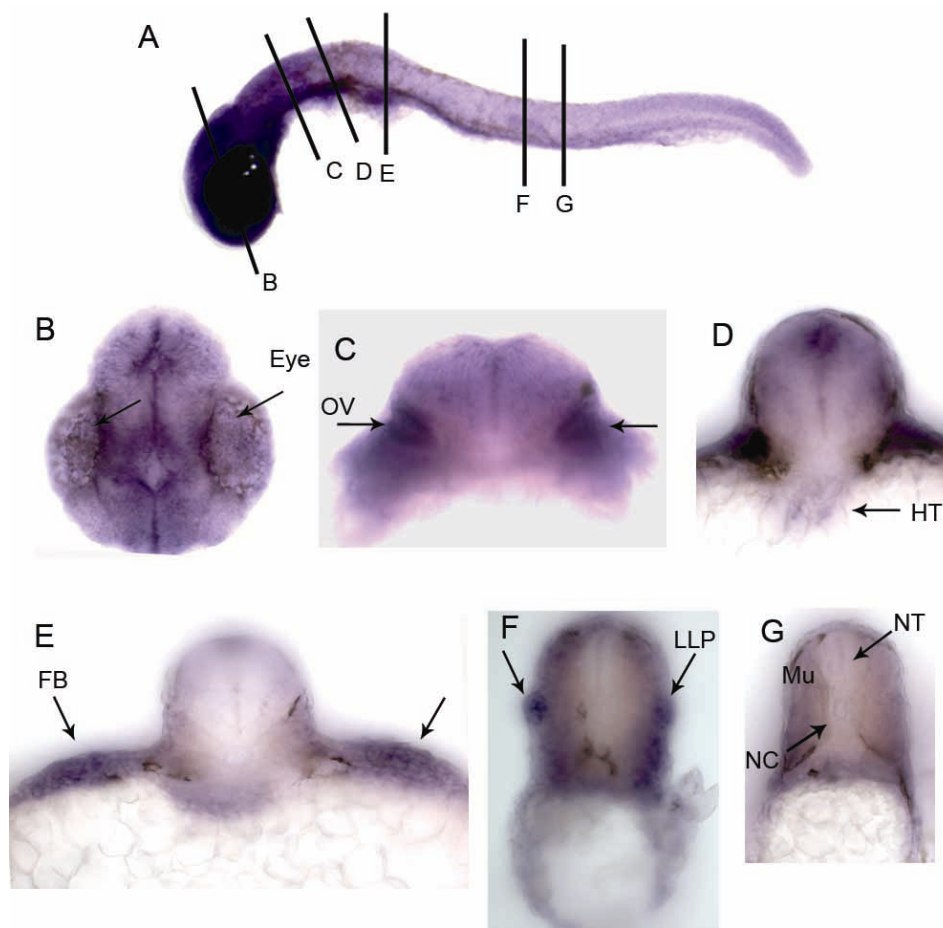
SUPPLEMENTARY FILE

HMG-2008-D-00705

Convergent extension movements and ciliary function are mediated by *ofd1*, a zebrafish orthologue of the human oral-facial-digital type 1 syndrome gene.

Maria I. Ferrante, Leila Romio *et al.*

Supplementary Figure 1. Sections of whole-mount ISH probed with *ofd1*.

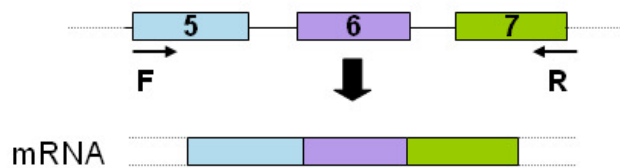


(A) Lateral view of a 28 hpf embryo stained with the *ofd1* antisense probe, with planes of sections in following panels as indicated. (B) Section through the head, with eyes indicated by arrows. (C) Section through the hindbrain, with arrows indicating otic vesicles. (D) Caudal part of the hindbrain and the heart tube (HT, arrow). (E) Section at level of the fin buds (FB), indicated by arrows. (F) Sections through lateral line organ primordia (LLP). (G) Section through the trunk, NT neural tube, Mu, trunk musculature, NC, notochord.

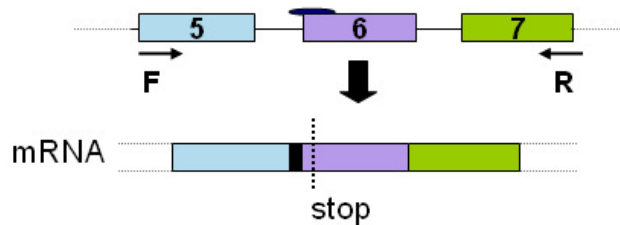
Supplementary Figure 2. Effect of SPL6 MO on *ofd1* RNA splicing.

A

- Normal *ofd1* splicing

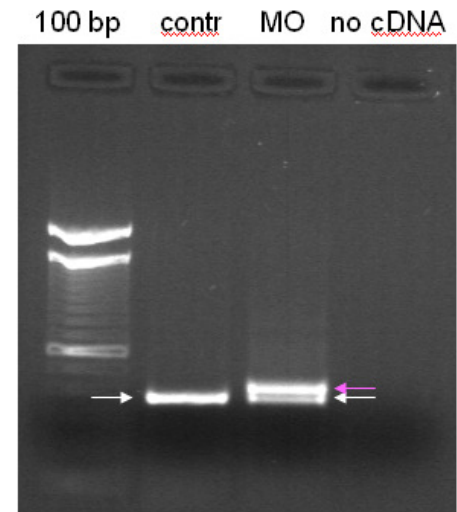


- disruption produced by *ofd1* spl6 MO



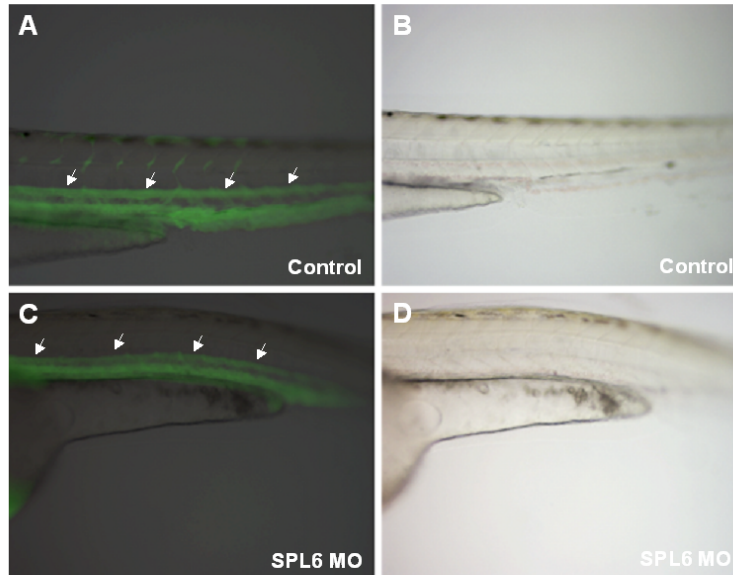
B

RT-PCR



SPL6 MO targets the donor splice site of intron 5/exon 6 activating a cryptic splice site in intron 5. By sequencing the PCR product amplified from the aberrant mRNA we found that 43 bp from intron 5 had been inserted in the transcript, causing a frameshift resulting in premature termination of translation a few base pairs downstream of the insertion (represented in A). Injection of 4 ng of SPL6 MO did not completely disrupt normal splicing: amplification of cDNA with primers positioned in exons 5 and 7 produced a band of the normal size (white arrow in B) in addition to the aberrant product of higher molecular weight (pink arrow in B).

Supplementary Figure 3. Visualisation of FITC-dextran within the embryonic vasculature after injection into the pericardial space.



FITC-dextran (10,000 MW) was injected into the pericardial space of 60 hpf zebrafish embryos. Within minutes, fluorescence was detected in the dorsal aorta (arrowheads) and the posterior cardinal vein (the thick green line below the aorta) in both wild-type control (A and B) and *ofdl* MO injected (C and D) embryos.

Supplementary Movie 1.

Movie of beads injected in KV of a control embryo. Recorded at 5 frames/second and subsequently speeded up at 10 frames/second.

Supplementary Movie 2.

Movie of beads injected in KV of an *ofdl* MO injected embryo. Recorded at 5 frames/second and subsequently speeded up at 10 frames/second.

Supplementary Table 1. Full list of genes up- and downregulated in *ofdl* ATG MO-injected embryos at shield stage

Probe ID, Fold Change, Log2 of Fold Change, Adjusted P value, the number of times the probe appears on the array, Ensembl ID and description, EMBL IDs and ZFIN ID for the genes that are up- or downregulated in *ofdl* MO-injected embryos are given in the columns. Genes are sorted by descending Fold Change. Data were Loess normalised and analysed using the bioconductor (<http://www.bioconductor.org/>) limma package and P-value adjusted as described in Methods.