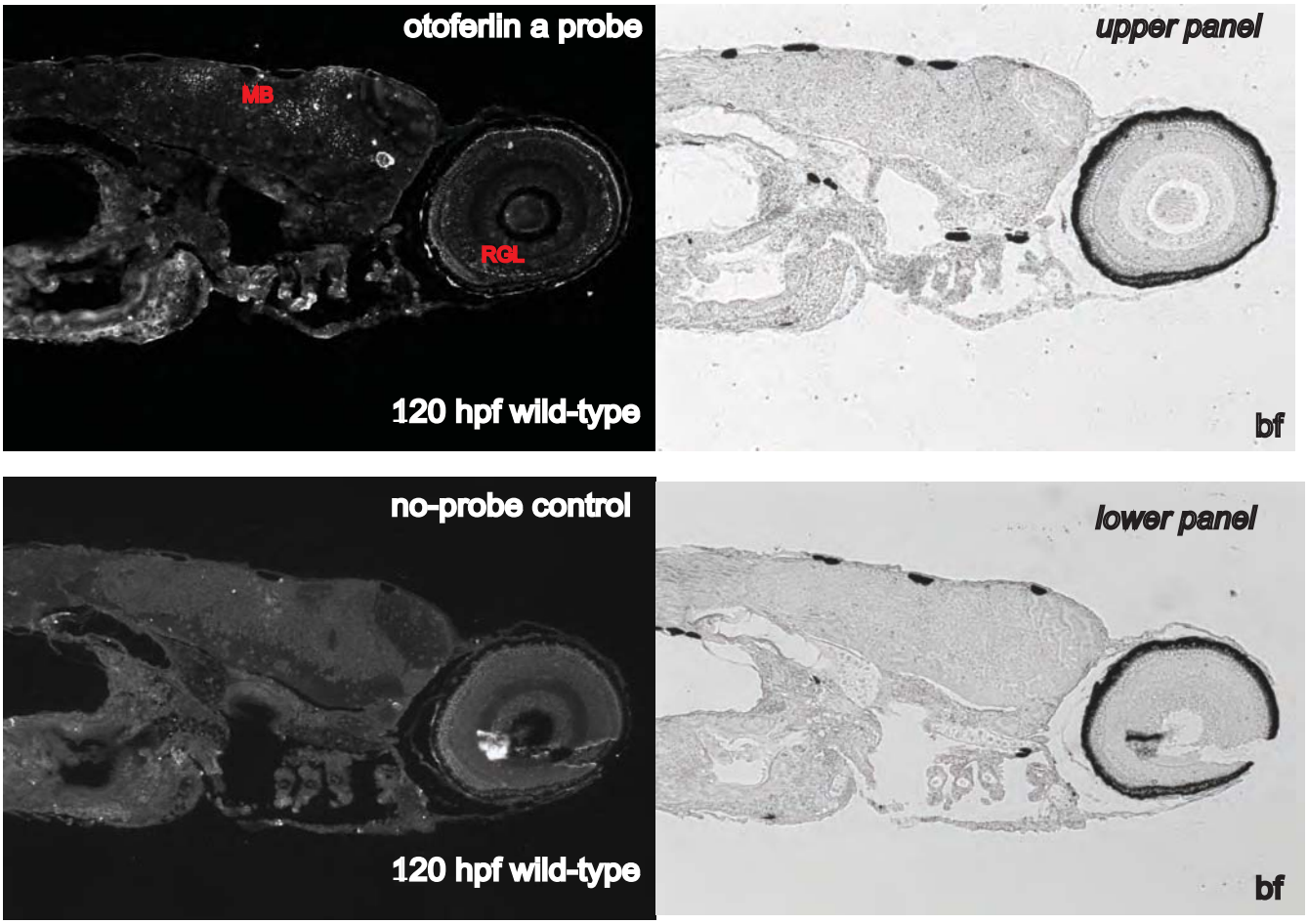
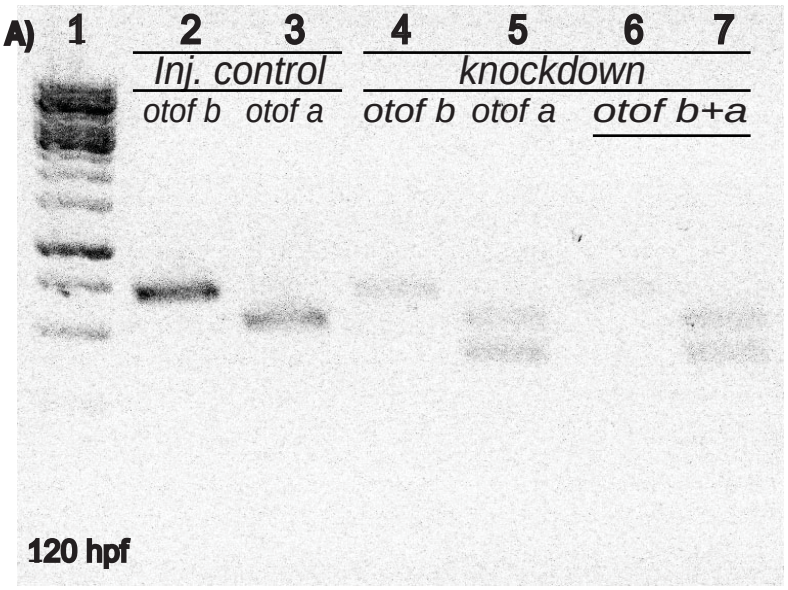
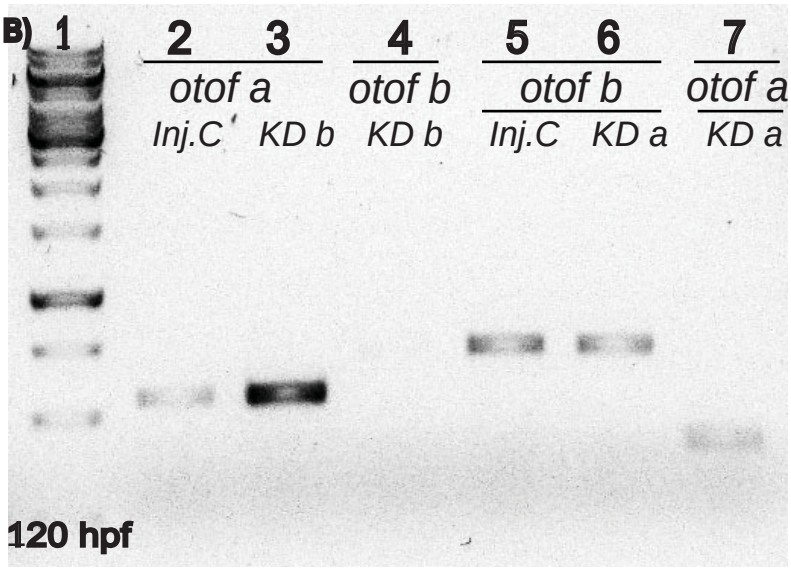


Supplementary Figure 1 - *In situ* hybridization images of 120 hpf wild-type larval zebrafish paraffin sections showing expression of otoferlin a in the mid-brain (MB) and retinal ganglion cell layer (RGL) .
upper panel - otoferlin a probe and corresponding bright-field (bf) images. lower panel - no-probe control and corresponding bright-field (bf) images.

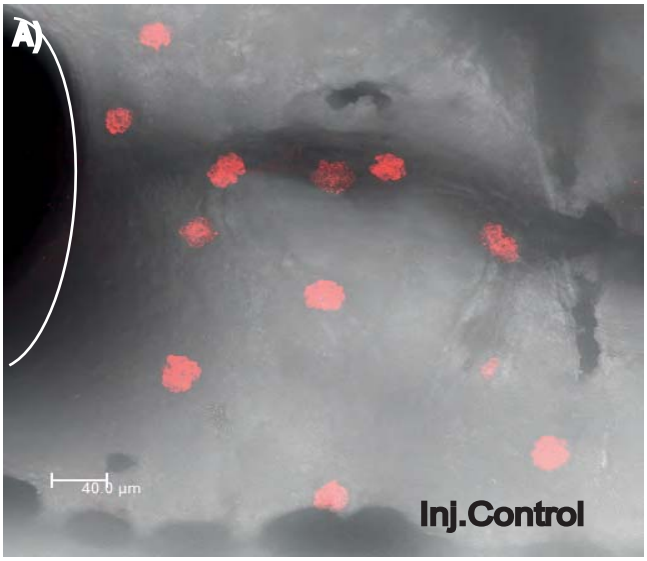


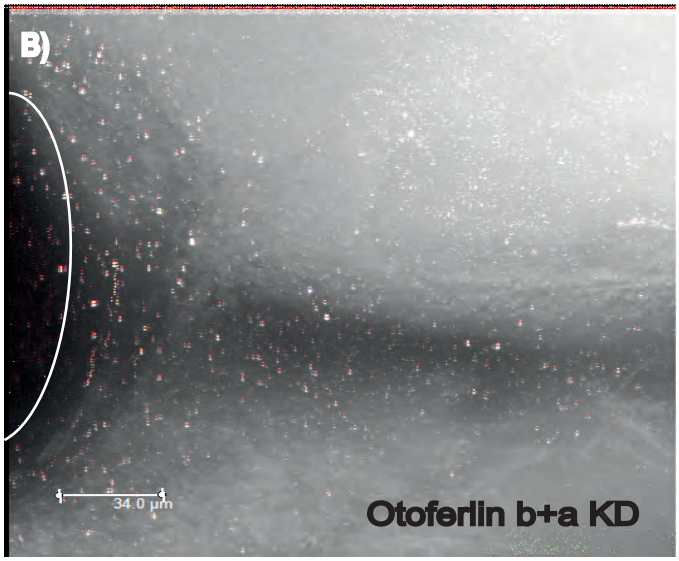
morpholino knockdown



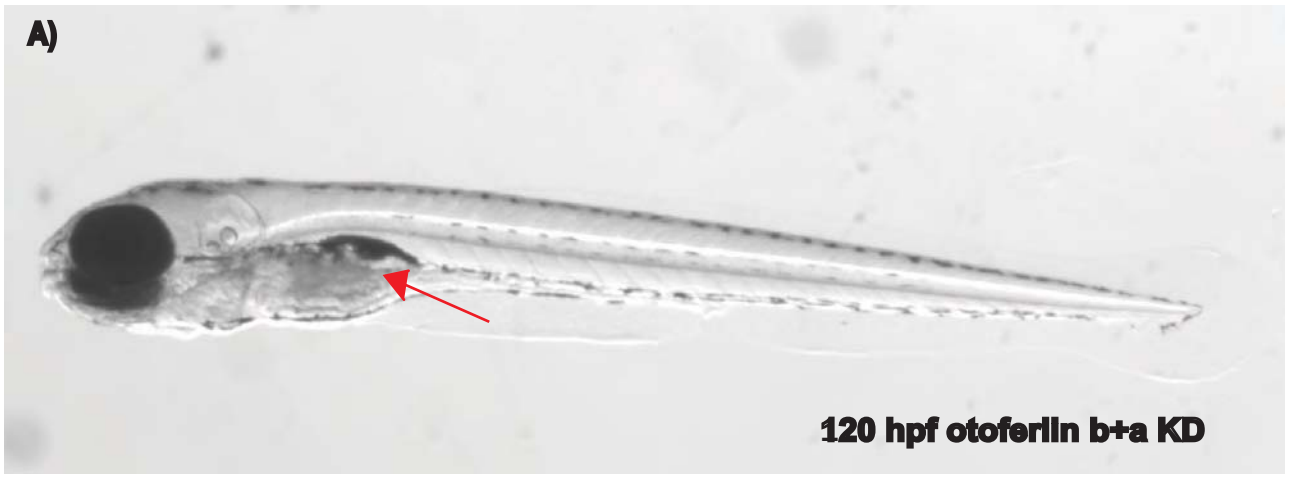
cross-expression

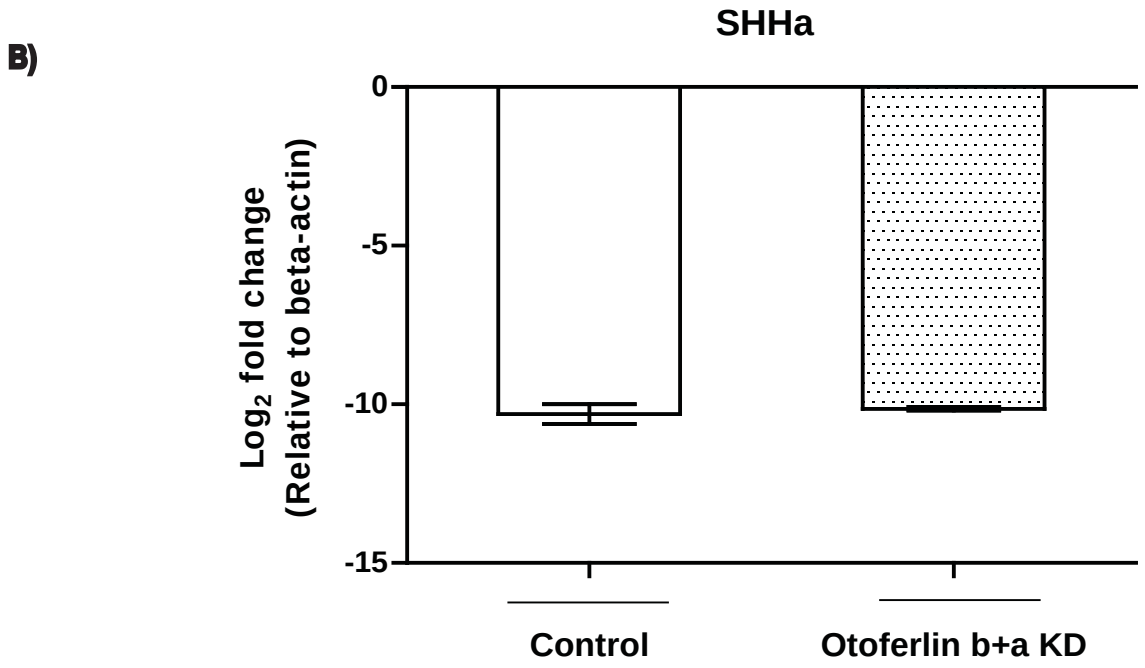
Supplementary Figure 2 - Morpholino knockdown of otoferlin in zebrafish larvae. **A)** RT-PCR gel image of otoferlin KD zebrafish larvae at 120 hpf, (lane 1) = molecular weight marker ; (lane 2) = negative control tested for otoferlin b; (lane 3) = negative control injected tested for otoferlin a; (lane 4)= otoferlin b KD tested for otoferlin b; (lane 5) = otoferlin a KD tested for otoferlin a; (lane 6) = otoferlin b+a double KD tested for otoferlin b; (lane 7) = otoferlin b+a double KD tested for otoferlin a. **B)** Cross expression studies with 120 hpf zebrafish larvae. RT-PCR gel image shows expression of: (lane 1)= molecular weight marker; (lane 2) = otoferlin a in control; (lane 3)= otoferlin a in otoferlin b KD; (lane 4) = otoferlin b in otoferlin b KD; (lane 5) = otoferlin b in control; (lane 6)= otoferlin b in otoferlin a KD; (lane 7) = otoferlin a in otoferlin a KD. (Inj. C – injected control, KD – Knockdown, otof a – otoferlin a, otof b – otoferlin b, otof b+a – otoferlin b+a)





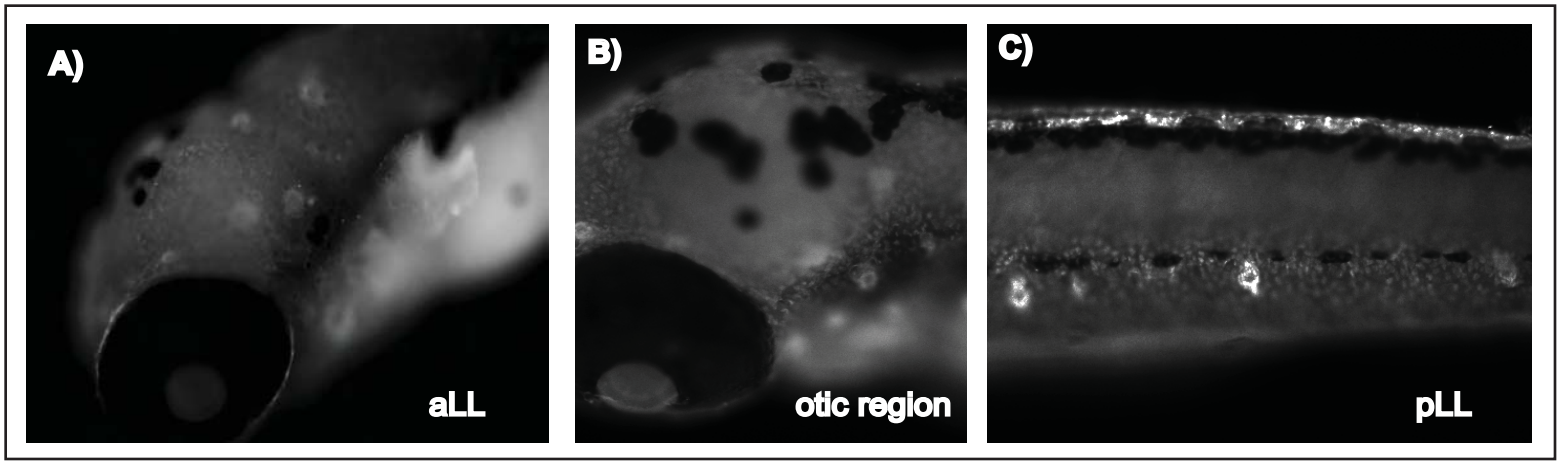
Supplementary Figure 3 - Compressed z-stack through the ear region showing otoferlin expression in 120 hpf injected control **(A)** larval zebrafish, and, otoferlin expression in double knockdown **(B)** 120 hpf larval fish (white hemicircles denotes the eye). Red clusters in Figure are otoferlin positively stained hair cell neuromasts.

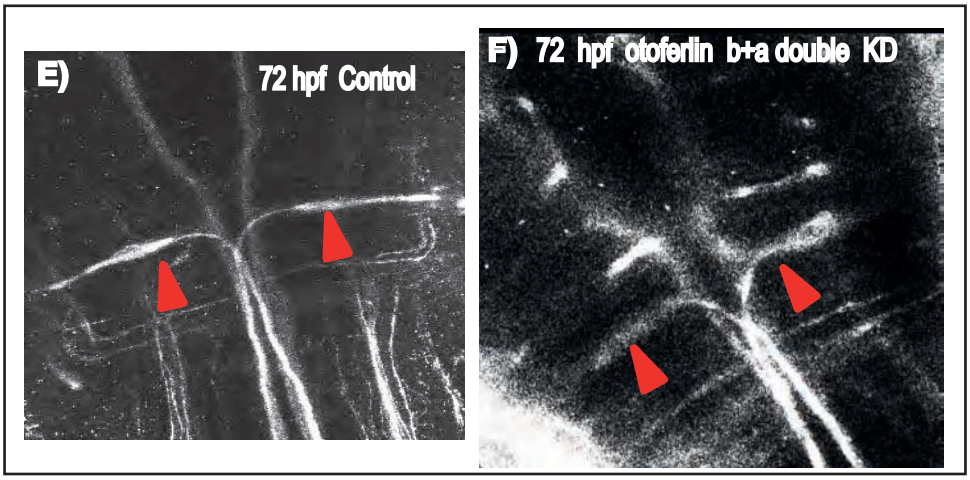




Supplementary Figure 4 - **A)** Observable phenotypes associated with the otoferlin b+a KD in120 hpf larval zebrafish with second set of morpholinos (e11i11 - otoferlin a and e2i2- otoferlin b). Arrow indicates swim bladder.
B) qPCR showing relative expression of shha in 72 hpf otoferlin control and double morphants normalized to beta-actin expression. Data shows no statistically significant differences in the expression of shha in control and double morphants.
The statistical significance is calculated through Mann-Whitney test.

96 hpf otoferlin b+a KD co-injected with mouse-FL otoferlin construct

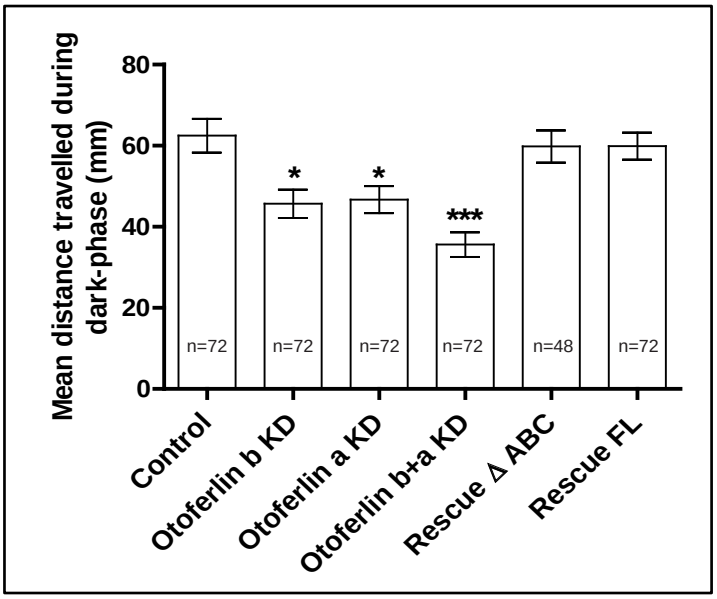




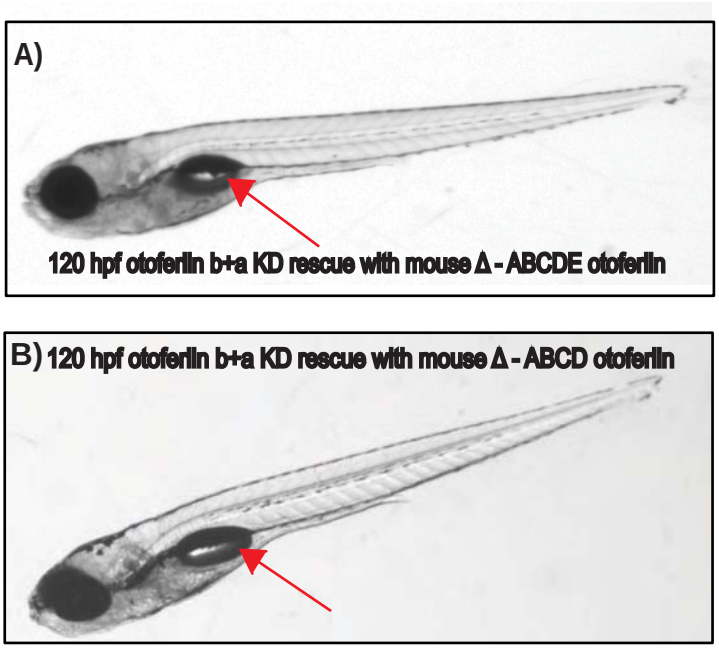
G)

Groups	Number of fish	Mean distance moved	sem
Control	23	63.94	5.69
Otoferlin a KD	18	66.03	5.73
Otoferlin b KD	17	66.79	7.53
Otoferlin b+a KD	16	22.92	2.65

Supplementary Figure 5 - **(A-C)** Whole-mount *in situ* hybridization on otoferlin double morphants co-injected with the full-length mouse otoferlin construct under the hair cell specific promoter at 96hpf. **A, B,** and **C** shows mRNA expression in the anterior lateral line (aLL) , posterior lateral line (pLL), and otic vesicle. **(E-F)** Confocal images of whole mount immunohistochemistry of 72 hpf larval zebrafish showing mauthner cells. **E)** Injected control, **F)** Otoferlin b+a double morphants. Arrowhead indicates the mauthner cells.**G)** Summary statistics of the startle between otoferlin morphants and control groups, sem - Std err of mean.



Supplementary Figure 6 - Dark-light behavioral assay : Distances travelled (in mm) during the dark-phase by larvae are shown. Mean distances moved in mm: Injected control (n=72) = 62.73, otoferlin b KD (n=72) = 45.67, otoferlin a KD (n=72) = 46.68, otoferlin b+a KD (n=72) = 35.61, Rescue FL (n=72) = 59.89, Rescue ΔABC (n=48) = 59.81. Dunn's multiple comparison test with standard 5% significance level shows significant difference between control and KD groups and no significant difference between control and rescue groups. Error bars indicate 95% confidence interval of the sample mean.



Supplementary Figure 7 - Rescue of zebrafish otoferlin KD with mouse otoferlin constructs. Rescue of swim bladder defect in 120 hpf otoferlin b+a KDs with mouse otoferlin, **A)** del-ABCDE, **B)** del-ABCD constructs. Arrow indicates inflated swim bladder.