Supplemental Materials

Molecular Biology of the Cell

Pathak et al.

Figure S1

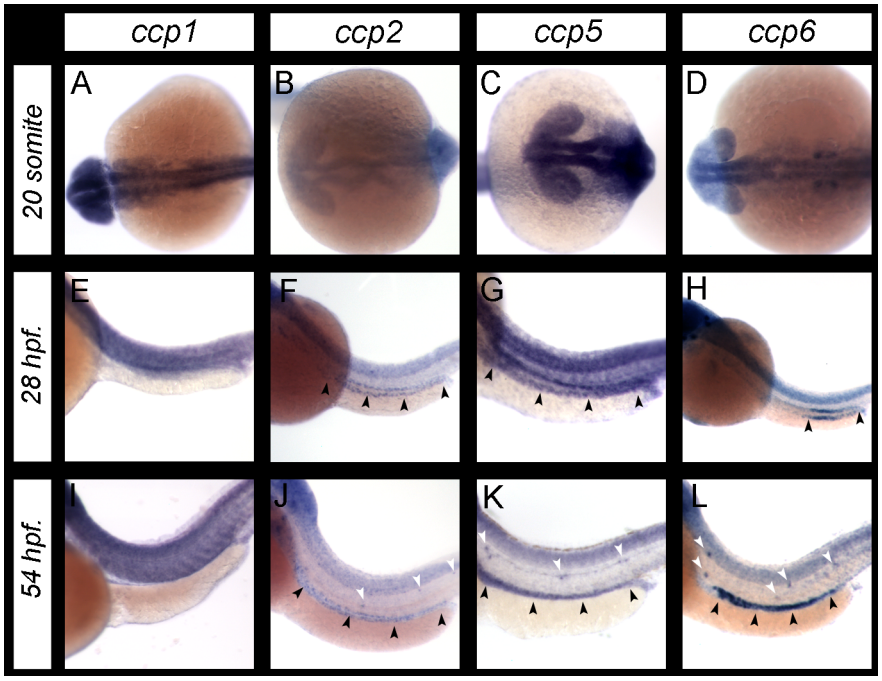


Figure S1. Persistent expression of *ccp* deglutamylase genes during zebrafish larval development.
(A-D) Dorsal views of 20 somite embryos showing that (A) *ccp1*, (B) *ccp2*, (C), *ccp5* and (D) *ccp6* are widely expressed in the developing CNS. (E-H) Lateral views of the zebrafish trunk at 28 hpf showing (E) diffuse *ccp1* expression in the somitic mesoderm and prominent expression of (F) *ccp2*, (G*) ccp5* and (H) *ccp6* in the bilateral pronephric ducts (black arrowheads) and medially in neural tube. In (H), note distinct enrichment of *ccp6* expression in the posterior pronephric segment. (I-L) Lateral views of the trunk in 54hpf larvae showing that expression of (I) *ccp1* persists in the somites and that of (J) *ccp2*, (K) *ccp5* and (L) *ccp6* persists in the pronephros (black arrowheads), spinal canal and also emerges in lateral line organs (white arrowheads).

Figure S2

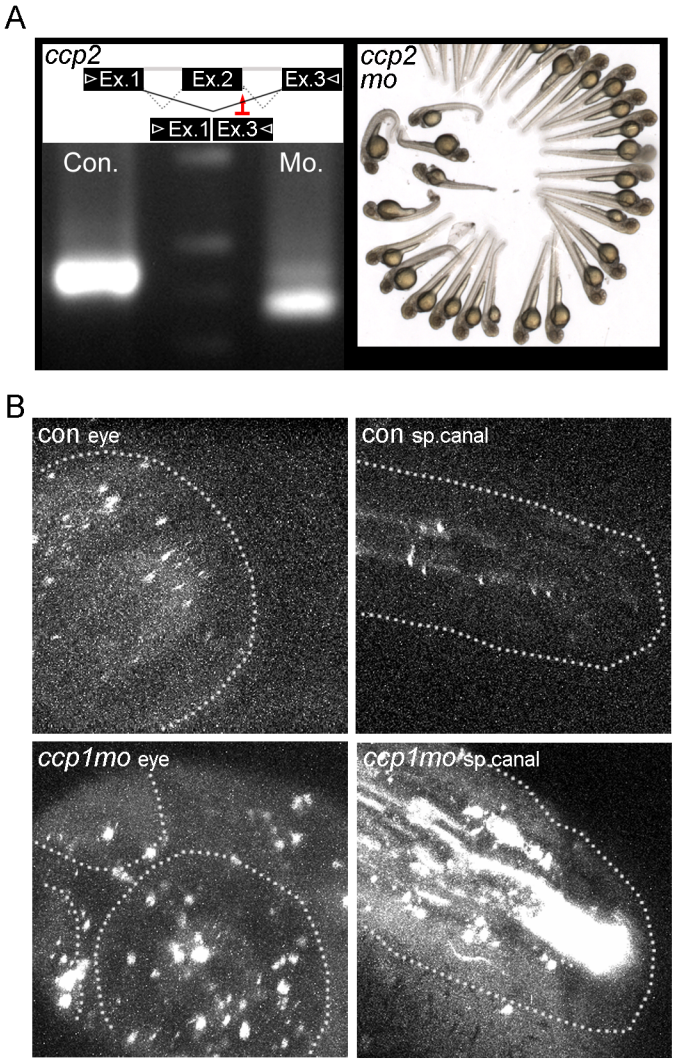
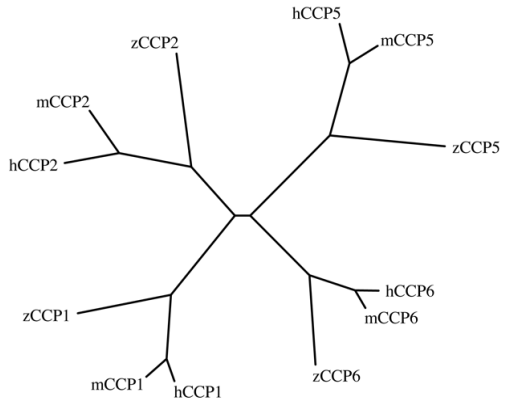


Figure S2. Additional phenotypes in *ccp1* and *ccp2* morphants.
A. Molecular and phenotypic defects induced by knockdown of *ccp2*. Antisense morpholinos designed to block the splice donor site at *ccp2* exon 2. Although agarose gel analysis showing smaller size of *ccp2* RT PCR amplicons in morphants relative to control indicated deletion of exon2, a surprisingly small fraction of morpholino injected larvae exhibit curved body axis but no pronephric cysts.
B. Annexin-GFP labeling indicating normal apoptosis in the eye and spinal canal of control zebrafish larvae at 48 hpf, and severely enhanced apoptosis in the eye and accumulation of apoptotic cells in the spinal canal of *ccp1* morphants.

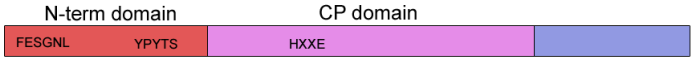
Figure S3.

A

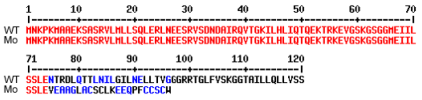


Zebrafish deglutamylase	Mouse deglutamylase	Human deglutamylase
zCcp1 (gi|66773052)	mCCP1 (gi|114158695)	hCCP1 (gi|55787673)
zCCP2 (gi|149773464)	mCCP2 (gi|160420289)	hCCP2 (gi|31657119)
zCCP5 (gi|51871616)	mCCP5 (gi|114150571)	hCCP5 (gi|118421091)
zCCP6 (gi|62955481)	mCCP6 (gi|114150581)	hCCP6 (gi|190194403)

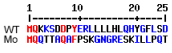
B Ccp (conserved regions)



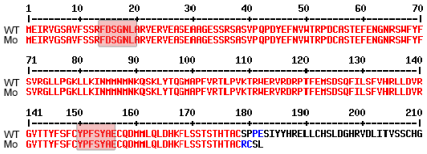
C Ccp1 Mo



D Ccp2 Mo



E Ccp5 Mo



F Ccp6 Mo

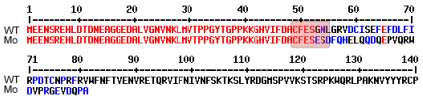


Figure S3. Comparisons of zebrafish Ccp deglutamylases and their morpholino induced truncations

(A) Phylogeny of zebrafish Ccp deglutamylases obtained by alignment of zebrafish, mouse and human polypeptide sequences indicated by abbreviated protein names in accompanying table. (B) Schematic diagram showing key conserved amino acid motifs in the N-terminal and carboxy peptidase domain of all functional mouse CCP deglutamylases. (C-F) N terminal truncations deduced by amino acid alignments of morphant and wildtype polypeptides of zebrafish Ccp deglutamylases (C) Ccp1, (D) Ccp2, (E) Ccp5 and (F) Ccp6 .

Figure S4.

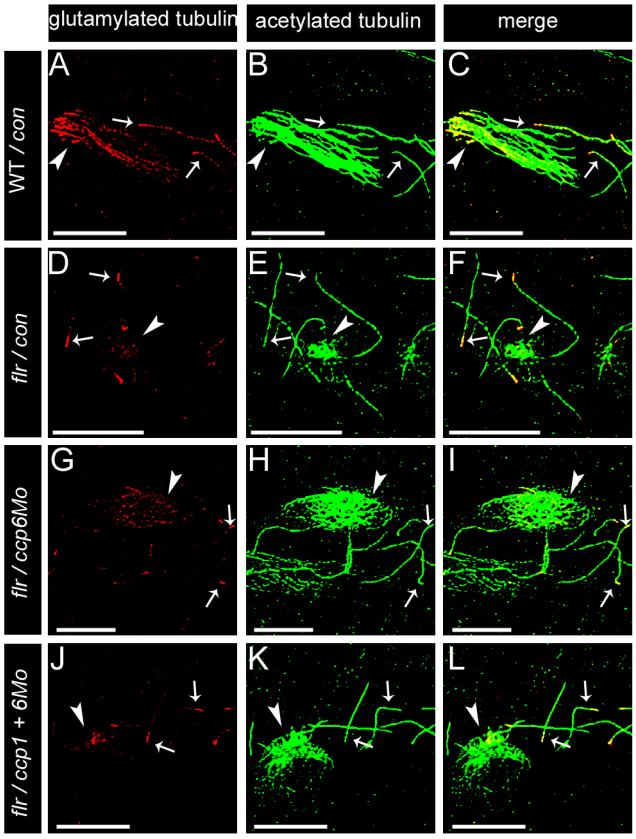


Figure S4. Inactivation of *ccp6* alone or double knockdown of *ccp1/ccp6* is not sufficient to restore tubulin glutamylation in defective cilia of the *fleer* mutant.
(A-L) Representative images of pronephric single cilia (white arrows) and multicilia (white arrowheads) of 2.5 day zebrafish larvae, double immunolabeled with glutamylated tubulin specific mAb GT335 (red) and acetylated tubulin specific mAb 6-11B-1 (green). Scale bars = 10μm. (A-C) Pronephric cilia in control larva showing (A) glutamylated tubulin levels gradually decrease from the base to tip of axonemes, (B) acetylated tubulin is uniformly distributed along the entire length of axonemes and (C) their merge. (D-F) Pronephric cilia of *fleer* mutant injected with control morpholino showing (D) glutamylated tubulin restricted near the base of single cilia and absent in multicilia, (E) acetylated tubulin in axonemes of single cilia and accumulated in cytoplasm of multiciliated cells and (F) their merge. (G-I) Pronephric cilia of *fleer* mutants injected with *ccp6x3* morpholino alone or (J-L) a combination of *ccp1x5mo* and *ccp6x3mo* showing (G, J) glutamylated tubulin is not enhanced in single or multicilia and (H, K) acetylated tubulin persistently accumulated in cytoplasm of multiciliated cells (arrowheads).

Figure S5.

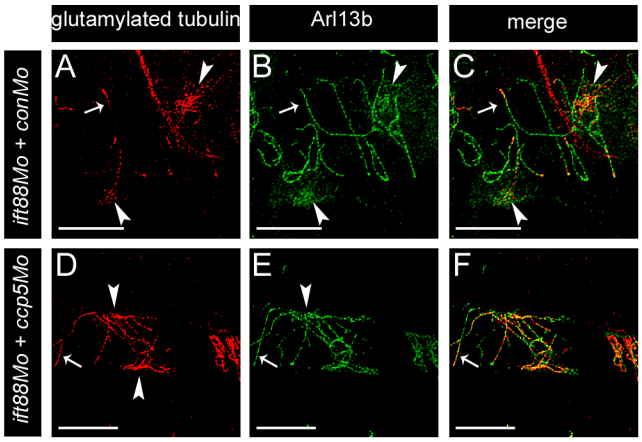


Figure S5. Ccp5 knockdown in Ift88 deficient zebrafish promotes assembly of pronephric multicilia visualized by Arl13b.

A-F) Representative images of pronephric single cilia (white arrows) and multicilia (white arrowheads) of 2.5 day zebrafish larvae, double immunolabeled with glutamylated tubulin specific mAb GT335 (red) and a polyclonal antibody to Arl13b (green). Scale bars = 10μm. (A-C) Pronephric cilia of *ift88* morphants showing (A) glutamylated tubulin gradually decreasing along single cilia and abnormally accumulated at the base of multicilia, (B) Arl13b present along single cilia and accumulated at the base of multiciliated cells and (C) their merge. (D-F) Pronephric cilia in double morphant of *ift88* and *ccp5* showing (D) glutamylated tubulin at elevated levels in axonemes of both single and restored multicilia (note reduced cytoplasmic accumulation of glutamylated tubulin in multiciliated cells), (E) Arl13b in axonemes of both single and restored multicilia and (F) their merge.

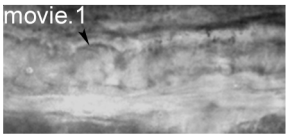
Supplemental table 1

Gene	Exon	Forward Primer (5'-3")	Exon	Reverse primer (5'-3')
ccp1	3(O)	CAGGGAAAATCCTTCACCTCATC	7(O)	TTTCAAACAGGATCTCCAAAGCA
	4 (I)	TGGGATGGAGATCATTCTGTCAT	7 (I)	ATAGACCCGAAGAACCTGCAGAC
ccp2	1(O)	ATGACTTCATGACTTGCGTGCAG	5(O)	CATTGCCTGTTGGCTGATAGAAC
	1(I)	TCGCTCTTACGGTTACAGGAATG	4(I)	GTCCCATTCGATATGCTGGATTT
ccp5	4(O)	GACAGTCAGTTCATCCTGTCGTTT	9(O)	GGTTGCACTCTGCCATGTCC
	4(I)	GGCGTCACCACCTACTTCTCCTT	6(I)	AAACCATTGAACACGAAGCTGGA
	7(I)	TCCAGAGAGAGGCGGAGCAT	9(I)	GCCCACCTGCTCGTACACCT
ccp6	1(O)	TAATGAAGCAGGAGGAGAGGATG	5(O)	TAATGTTGCAGACGCGAATAGGT
	2(I)	ACAAACTGATGGTCACTCCTCCA	5(I)	TAAACATCATCCTCTCGGTCGAA

Nucleotide sequence of nested primers used for generating RT PCR amplicons of zebrafish *ccp1*, *ccp2*, *ccp5* and *ccp6* genes. The letter O in front of the exon position denotes outside and the letter I denotes inside.

Legends for Supplemental Quicktime movies

Highspeed microvideo images were acquired at 235-245 frames per second (fps). Except movie 2 which is 6 seconds long all final movies are 3 seconds long. All movie frame rates were slowed down 20-fold to represent an equivalent of 200 milliseconds of real time in a 3 or 6 second long movie. All pronephric tubules are oriented with the normal direction of fluid flow (to the cloaca) to the right.



Movie 1. Motile pronephric cilia in 2.5dpf zebrafish larva injected with control morpholino. Synchronously beating bundles of numerous multicilia beating are seen to create a unidirectional fluid flow in the pronephric duct of the zebrafish larva injected with control morpholino. The anterior end of the pronephros is to the left and the posterior is to the right. For clear visualization of individual cilia bundles, distension of the pronephric lumens was induced by mechanical obstruction near the cloaca. A black arrow points towards an optimally focused multicilia bundle.

Movie 2. Aberrant motility of pronephric cilia in 2.5dpf zebrafish injected with *ccp5ex5* morpholino. This is the representative movie used to depict line scans in the analysis of cilia beat amplitude in figure 4 A, B. Multiple bundles of pronephric multicilia cilia appear to move in an asynchronous manner with varying beat amplitude in this movie. Arrowheads numbered i through v identify cilia bundles whose beat amplitudes were measured. Note that cilia bundles i and ii are barely moving and that of v are rigid and twitching due to fluid flow. Anterior end of the pronephros is to the left and posterior is to the right.