```
Zebrafish      ---------MIERPDSAVSSVAQRNLEGYVGFANLPNQVYRKSVKRGFEFTLMVVGESGL
Human          MSVSARSAAAEERSVNSSTMGQQKNLEGYVGFANLPNQVYRKSVKRGFEFTLMVVGESGL
Mouse          MSVSARSAAAEERSVNCGTMAQPKNLEGYVGFANLPNQVYRKSVKRGFEFTLMVVGESGL
                          ** ..  :    :************************************

Zebrafish      GKSTLINSLFLTDLYSKDYPGPSQRIKKTVQVEQSKVLIKEGGVQLTLTIVDTPGFGDAV
Human          GKSTLINSLFLTDLYSPEYPGPSHRIKKTVQVEQSKVLIKEGGVQLLLTIVDTPGFGDAV
Mouse          GKSTLINSLFLTDLYSPEYPGPSHRIKKTVQVEQSKVLIKEGGVQLLLTIVDTPGFGDAV
               **************** :*****:********************* **************

Zebrafish      DNSNCWQPVINYIDSKFEDFLNAESRVNRRQMPDNRVHCCLYFIAPSGHGLKPLDIEFMK
Human          DNSNCWQPVIDYIDSKFEDYLNAESRVNRRQMPDNRVQCCLYFIAPSGHGLKPLDIEFMK
Mouse          DNSNCWQPVIDYIDSKFEDYLNAESRVNRRQMPDNRVQCCLYFIAPSGHGLKPLDIEFMK
               **********:********:******************:*********************

Zebrafish      RLHDKVNVIPLIAKADTLTPEECQLFKKQIMKEIQEHKIKIYEFPDTEDDEDSKLIRKIK
Human          RLHEKVNIIPLIAKADTLTPEECQQFKKQIMKEIQEHKIKIYEFPETDDEEENKLVKKIK
mouse          RLHEKVNIIPLIAKADTLTPEECQQFKKQIMKEIQEHKIKIYEFPETDDEEENKLVKKIK
               ***:***:**************** ********************:*:*:*:.**::***

Zebrafish      EKMPLAVVGSNVVIEVNGRKVRGRQYPWGVAEVENGEHCDFTVLRNMLIRTHMQDLKDVT
Human          DRLPLAVVGSNTIIEVNGKRVRGRQYPWGVAEVENGEHCDFTILRNMLIRTHMQDLKDVT
Mouse          DRLPLAVVGSNTIIEVNGKRVRGRQYPWGVAEVENGEHCDFTILRNMLIRTHMQDLKDVT
               :::********.:*****::**********************:*****************

Zebrafish      NNVHYENYRSKKLAAVTCNGVDATKNKGQLTKSPLAQMEEERREHVMKMKKMETEMEQVF
Human          NNVHYENYRSRKLAAVTYNGVDNNKNKGQLTKSPLAQMEEERREHVAKMKKMEMEMEQVF
Mouse          NNVHYENYRSRKLAAVTYNGVDNNKNKGQLTKSPLAQMEEERREHVAKMKKMEMEMEQVF
               **********:****** **** .********************** ****** ******

Zebrafish      EMKVKEKKQKLKDSEAELERRHEQMKKNLEAQYKELEEKRRQFEDEKANWEAQQRILEQQ
Human          EMKVKEKVQKLKDSEAELQRRHEQMKKNLEAQHKELEEKRRQFEDEKANWEAQQRILEQQ
Mouse          EMKVKEKVQKLKDSEAELQRRHEQMKKNLEAQHKELEEKRRQFEEEKANWEAQQRILEQQ
               ******* **********:*************:***********:***************

Zebrafish      KLDASKTMEKNKKKGKIF
Human          N--SSRTLEKNKKKGKIF
Mouse          N--SSRTLEKNKKKGKIF
               :  :*:*:**********
```

Fig. S1. Sequence alignment of septin 7 (Sept7b ENSDARG00000019649) proteins of zebrafish (*Danio rerio*), *Homo sapiens* (Sept 7-201 ENST00000350320, Chromosome-7), and *Mus musculus* (Sept7-201 ENSMUST00000060080 Chromosome-9). Septin 7 protein sequences were aligned with *ClustalW*. Zebrafish sept7b protein shares significant amino acid sequence similarity and identity with its mammalian counterparts.

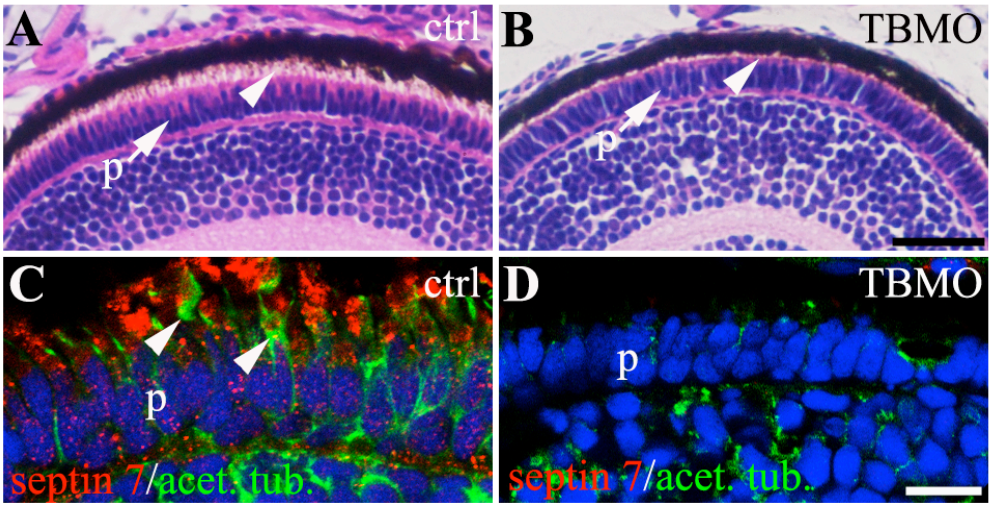

Fig. S2. *Sept7b* is required for the development of photoreceptor outer segment. (A) Hematoxylin-eosin stained histological section of 4 dpf zebrafish larva showing the photoreceptor cell layer (p). The photoreceptor outer segment, that comprises modified cilia, is marked with an arrowhead. **(B)** Histological section of 4 dpf *sept7b* translation blocking antisense morpholino oligonucleotide (TBMO)-treated zebrafish larva shows shortening of the photoreceptor (p) outer segment cilia (arrowhead). **(C-D)** Immunofluorescence staining of 4 dpf control (C) and *sept7b* TBMO-treated zebrafish (D) for septin (red) and acetylated tububulin (green). **(C)** Septin 7 localizes in a punctate fashion in the photoreceptor cells in the control, and the photoreceptor outer segment is visualized by acetylated tubulin (arrowheads). **(D)** The photoreceptor outer segment cilia are absent or clearly shorter in *sept7b* TBMO-treated zebrafish larva. Scale bar: 25 µm (A-B); 10µm (C-D).

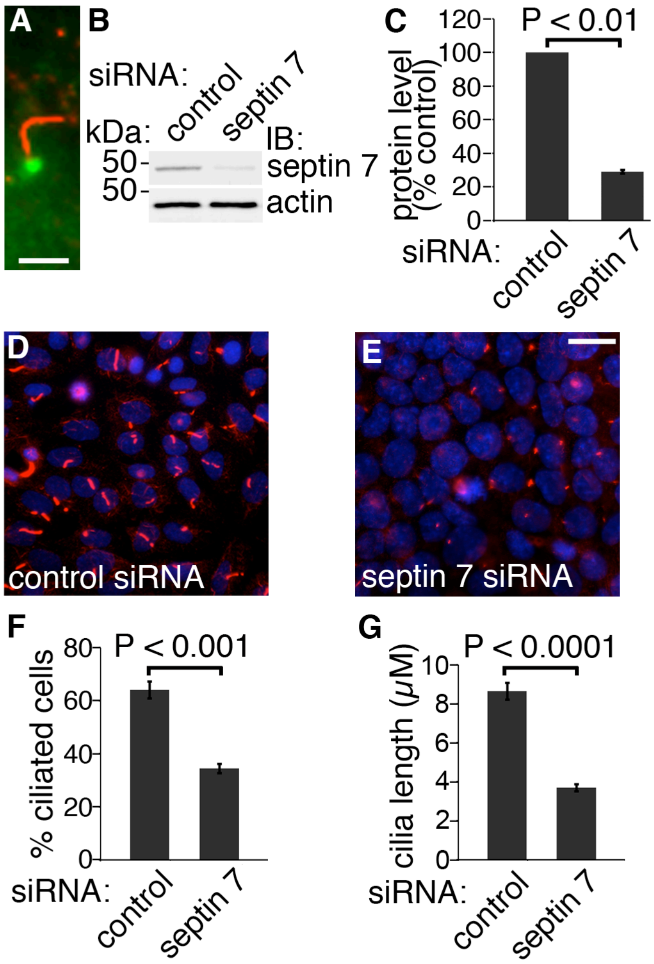

Fig. S3. Knockdown of septin 7 decreases the length of cilia in mIMCD3 cells. (**A**) In mIMCD3 cells, septin 7 (green) is expressed at the base of cilia, which are stained for acetylated tubulin (red). Scale bar: 2,5 µm. (**B**) mIMCD3 cells were transfected with 100 nmol ON-TARGET plus SMARTpool mouse Sept7 (L-042160-01-0005) or siCONTROL Non-Targeting Pool#2 (D-001206-14-05) siRNAs (Dharmacon, Lafayette, CO) using Lipofectamine 2000 (Invitrogen). Septin 7 siRNA leads to a 71% reduction in septin 7 expression in mIMCD3 cells. Actin was used as a loading control. (**C**) Quantification of protein levels of three replicate blots as in (B). (**D-E**) mIMCD3 cells treated with control siRNA (D) are decorated with cilia identified by staining for acetylated tubulin (red). In mIMCD3 cells transfected with septin 7 siRNA (E), the number and length of cilia are reduced. Nuclei are visualized with Hoechst stain (blue). Scale bar: 10 µm. (**F**) Quantification of 4000 cells indicates that 64% of cells transfected with the control siRNA had cilia whereas only 34.5% of cells transfected with septin 7 siRNA were ciliated. (**G**) Cilia length is reduced in septin 7 siRNA treated cells (3.69 ± 0.47 µm) compared to control siRNA transfected cells (8.86 ± 0.63 µm; n=200 for both). Graphs in C, F and G show the mean and error bars (STDEV) of three independent experiments, Student's t-test.

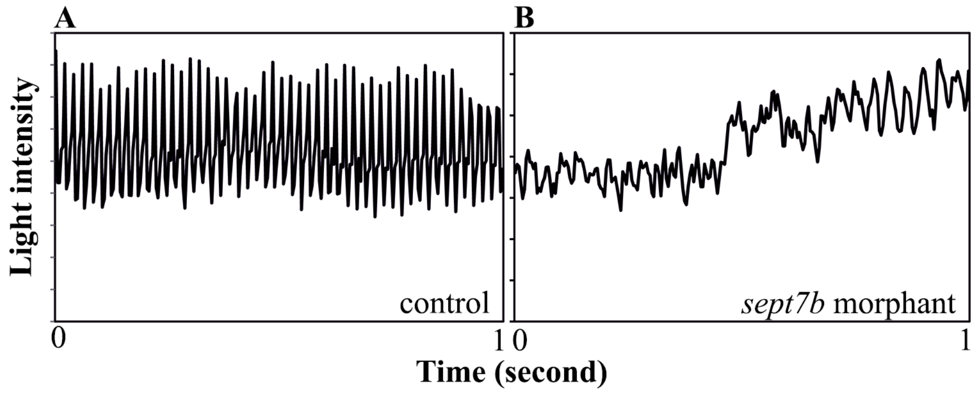

Fig. S4. Knockdown of *sept7b* leads to slow and irregular beating of the cilia. (A-B) The beating frequency of the pronephric cilia at 30 hpf control and *sept7b* TBMO-injected zebrafish was quantified based on the changes in the light intensity averaged over a region of interest placed on the path of the beating cilia. Each trace is 1 s in duration, and the curve shows the oscillations originating from the cilia. Beating of the cilia is regular in controls **(A)**, whereas the beating is slow and the beat pattern is irregular in *sept7b* morphants **(B)**.

Primers	
sept7b-1F	5´-TCTTCTGCTCTCGACCGCTT-3´
sept7b-1R	5´-ATCCCTCTGTGTGGTTTGAC-3´
sept7b-2F	5´-TCACGGTCCTGAGGAATATG-3´
sept7b-2R	5´-GATGAAGTTGCGTGTGGTTG-3´
sept7b-3F	5´-GTCACAGGACAATGATCGAG-3´
sept7b-3R	5´-CCACAGCCAGAGGCATCTTC-3´
sept7b-4F	5´-GTGTCCAGCGTTGCACAGAG-3´
sept7b-4R	5´-TCACCGACAACCATGAGCGT-3´
sept7b-5F	5´-GTTGCACAGAGGAATCTGGA-3´
sept7b-5R	5´-CCAGACTCACCGACAACCAT-3´
rps3-1F	5´-GCTCGCCGAGGATGGTTATT-3´
rps3-1R	5´-CGGTGTCGACGTAGTAGTTC-3´
rps3-2F	5´-CGTGTCACACCAACAAGA-3´
rps3-2R	5´-CAGCTTGTAGCGCAGAGA-3´
Morpholino antisense oligonucleotides	
TBMO	TCGGGTCTCTCGATCATTGTCCTGT
SBMO-2	AGAATTTAGCAACTTACCGACAACC
TBMO-MM	TCcGcTCTCTgGATgATTcTCCTGT
p53	GCGCCATTGCTTTGCAAGAATTG

Table S1. Primers and morpholino antisense oligonucleotides used in the study. TBMO targets the translation initiation site of *sept7b* and SBMO-2 targets the exon splice donor site of exon 2. The control MO, TBMO-MM, corresponds to the sequence of TBMO but harbours five mis-matching nucleotides marked by lowercase letters.

Movie S1. High-speed video microscopy of the pronephric cilia in 30 hpf control zebrafish. The movie shows the beating of the cilia (red arrow in a frame of the movie above). The movie was recorded at 250 frames/second.

Movie S2. High-speed video microscopy of the pronephric cilia in 30 hpf *sept7b* morphant zebrafish. The movie shows that the beating rate of the cilia is reduced and the beat pattern is irregular in *sept7b* TBMO-injected zebrafish (red arrow in a frame of the movie above). The movie was recorded at 250 frames/second.