

Figure S2. **Analysis of ciliary length and IFT in *dhc1b-2* and *bbs* mutants.** (A) Ciliary length of wild type and the four *bbs* mutant strains. (B) Western blot analysis of cilia isolated from wild type and *bbs4-1*. For quantification of relative protein amounts, values for band intensities were adjusted for IC2 loading and the ratios (left, parentheses) were then calculated. Velocity (C) and frequency (D) of anterograde and retrograde IFT for wild type and *dhc1b-2* and *bbs* mutants. Standard deviations are indicated in A, C, and D; *t* test probability data are shown in D.

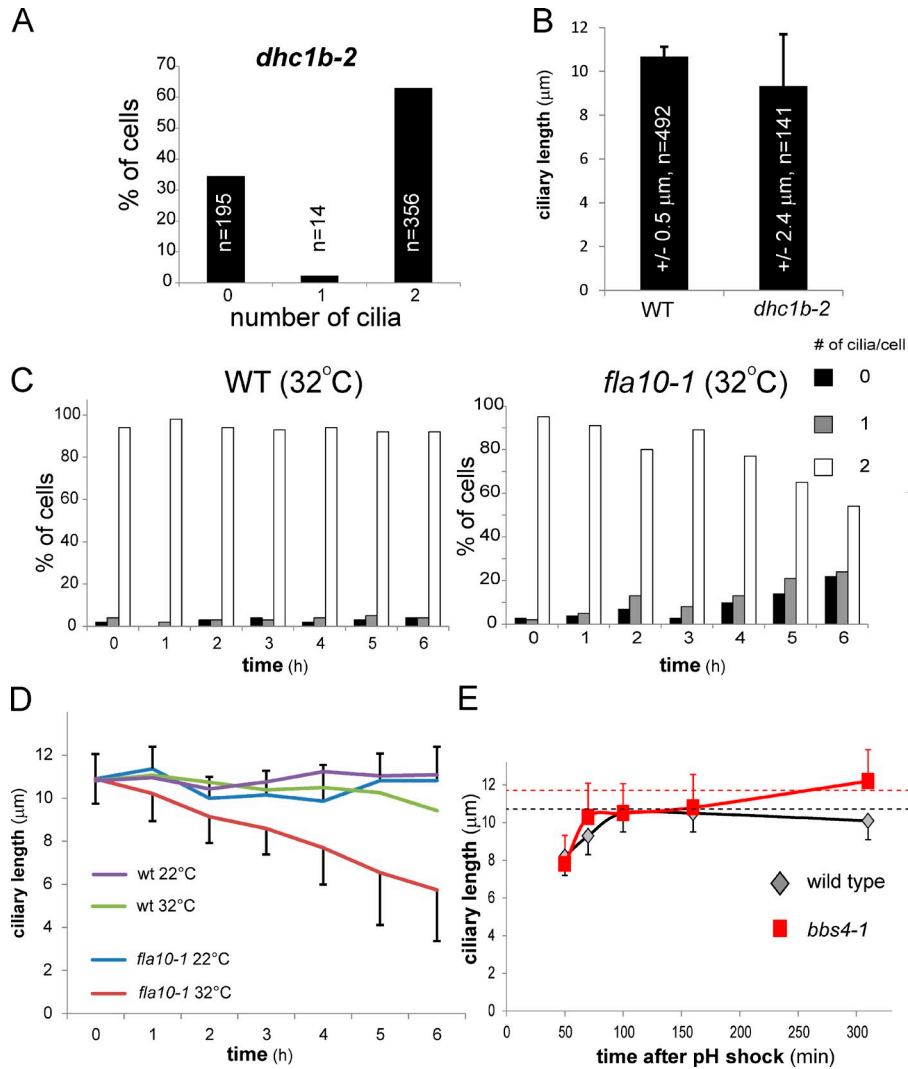


Figure S3. **Cilia length and number as a function of time and temperature in *dhc1b-2*, *fla10-1*, and *bbs4-1* mutants.** (A and B) Ciliary number and ciliary length, respectively, for the *dhc1b-2* mutant at the permissive temperature (21°C). (C) Ciliary number for wild-type and *fla10-1* cells before (0 h) and at various time points (1–6 h) after shifting the cells to 32°C. The data shown are from a single representative experiment out of two repeats. The number of cilia of wild-type or *fla10-1* cells maintained at 22°C remained at the 0-h level throughout the time course (not depicted). (D) Ciliary length of wild-type and *fla10-1* cells before (0 h) and after various times (1–6 h) of incubation at 22 or 32°C. (E) Ciliary length at various time points after deciliation by pH shock. Measurements are based on isolated cilia viewed by dark-field microscopy. Red, *bbs4-1*; gray, wild type; dashed lines, length of wild-type cilia (black, $10.8 \pm 0.91 \mu\text{m}$, $n = 29$) and *bbs4-1* cilia (red, $11.8 \pm 1.18 \mu\text{m}$, $n = 26$) before deciliation. At least 24 cilia from different cells were measured for each time point. Standard deviations are indicated in B, D, and E.

A

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M. musculus      -----MGRSSWRLVFAAGAGLALALEALPWLMRWLL-----AGRR-PR
H. sapiens      -----MGRLSWQVAAAAAVGLALTLEALPWLRLWLR-----SRRRRPR
D. rerio        MDVFKQMSFKELMKVLGLGTVAFLVGLVEWLNWLTRRLR-----DSRG-PL
C. reinhardtii  ---MGCASSKEEVALTPLSDVNAAKEVADLKAQVDQLKRQLASAGQSAAPAAAGAVKGGV
                :   :   .   .   :   *   *

M. musculus      REVLFFPSQVTCTEALLQAP--GLPPGPSGCPCLPHSESS-LSRLLRALLAARSSLELC
H. sapiens      REAPFFPSQVTCTEALLRAPGAELAELEPEGCPGLPHGESA-LSRLLRALLAARASLDLC
D. rerio        KEVLFPPSPQVCVEHLFTSHR-----SFPACPLPHGIQTSFSRLLLEHLLSARTSLEMC
C. reinhardtii  VETLFFPDEKLPCRNNRRPGG-----CKRQHCEYSHTPTS-LSRFLDYLGSAATRLDIC
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M. musculus      LFAFSSPQLGRAVQLLHQRGVVRVITDCDYMALNGSQIGLLRKAGIQVRHDQD-LGYMH
H. sapiens      LFAFSSPQLGHAVQLLHQRGVVRVITDCDYMALNGSQIGLLRKAGIQVRHDQD-PGYMH
D. rerio        IFSFSNMEMSRAILLLHKGVVVRVITDRDYMTITGSQIGALRKAGISVRHEMSSAVMHM
C. reinhardtii  VFTITNDDISDVVLELHNGVVRVRIISDNDQAHTQGSDDIDKFRQAGIAVRQDKT-AAHMH
                :*: : . : . :  ** : ** * : : *  *  * : : *  *  * : : *

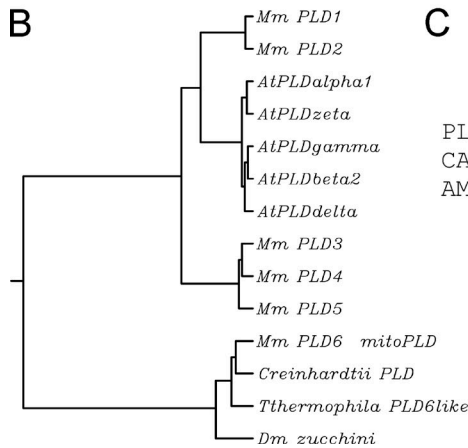
                #   ^   ^#   #   #

M. musculus      HKFAIVDKKVLITGSLNWTQAIQNNRENVLIMEDTEYVRLFLEEFERIWEFDPDKYSF
H. sapiens      HKFAIVDKRVLITGSLNWTQAIQNNRENVLITEDDEYVRLFLEEFERIWEQFNPTKYTF
D. rerio        HKFALVDGRKLSGSLNWTLTAVQSNKENVIITEEPELVRPFQQEFLKLWEASDPANHL
C. reinhardtii  HKFAIIDGRLLNGSFNWTRQAVTANNENVTVLSDPKLIASFQQQFDKLWDMFK-----
                **** : * : * : ** : ** *  *  * : * : * : * : * : * : *

M. musculus      FPQKRRGH-----
H. sapiens      FPPKSHSGSCAPPVSRAGRLLSWHRTCGTSSSQT
D. rerio        QSKNGQIKK-----
C. reinhardtii  -----

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B



C

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PLD      MGCASSKEEVALTPLSD...
CAH6     MCGGASVPQNGGGAPVT...
AMPK     MGACCSQPSEKYEYVQGG...

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Figure S4. **Alignment and phylogenetic analysis of PLDs.** (A) Alignment of *C. reinhardtii* and vertebrate PLDs. #, active site; ^, signature sites, #, both. (B) Rooted phylogenetic tree of murine PLDs (Mm_PLD1 [NCBI Protein accession no. NP_001157528.1], Mm_PLD2 [GenBank accession no. AAH68317], Mm_PLD3 [GenBank accession no. AAH76586], Mm_PLD4 [GenBank accession no. AAH58565], Mm_PLD5 [GenBank accession no. AAI00429], and Mm_PLD6 [GenBank accession no. CAI24298.1]), *Arabidopsis thaliana* PLDs (AtPLDalpha1 [GenBank accession no. AEE75720], AtPLDdelta [GenBank accession no. AEE86571], AtPLDgamma [GenBank accession no. AEE83056], AtPLDbeta2 [GenBank accession no. AEE81845], and AtPLDzeta [GenBank accession no. AED93432]), *Drosophila melanogaster* zucchini [GenBank accession no. AAM49862.1], *Tetrahymena thermophila* THERM_02188720 (NCBI Protein accession no. XP_001028688), and *C. reinhardtii* PLD (NCBI Protein accession no. XP_001693080). The tree is based on a CLUSTALW multiple sequence alignment using default settings. (C) N-Terminal sequences of PLD, CAH6, and AMPK. Residues predicted to be myristoylated are shown in red and residues predicted to be palmitoylated in green. Online tools (Myristylator, Myr Predictor, and CSS-Palm 2.0) were used for prediction of fatty acid modification.