Supporting Information

Liu and Lechtreck 10.1073/pnas.1713226115

SI Materials and Methods

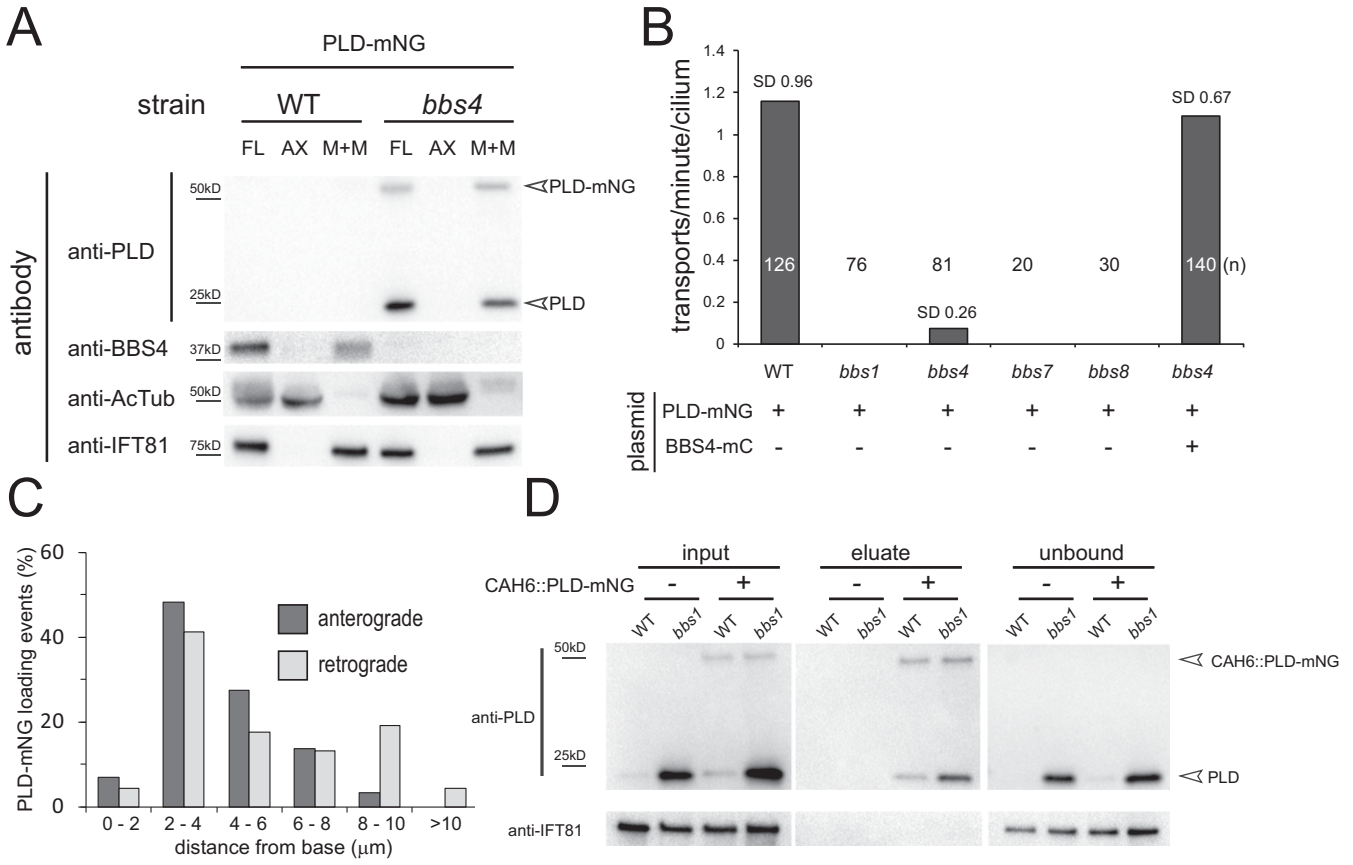


Fig. S1. Transport of PLD-mNG is diminished in *bbs* mutants. (*A*) Western blot comparing the distribution of PLD-mNG in isolated flagella of control (WT) and *bbs4*. Equivalent amounts of whole flagella (FL), axonemes (AX), and detergent fraction (membrane&matrix, M+M) were loaded. Antibodies to acetylated tubulin (AcTub) and IFT81 were used as loading controls and to show proper fractionation. (*B*) The frequencies of transports of PLD-mNG in *C. reinhardtii bbs* mutants. The SD and the number of cilia analyzed (n) are indicated. (*C*) Distribution of PLD-mNG loading events (i.e., transitions from diffusion to IFT-like transport) along the length of cilia (in percent). (*D*) Western blot analysis of a coimmunoprecipitation experiment using anti-mNG beads analyzing the ciliary M+M (input) of control (WT) and *bbs1* strains either untransformed or expressing CAH6::PLD-mNG. Shown are the input, flow-through (unbound), and eluate. Anti-IFT81 staining was used as a loading control.

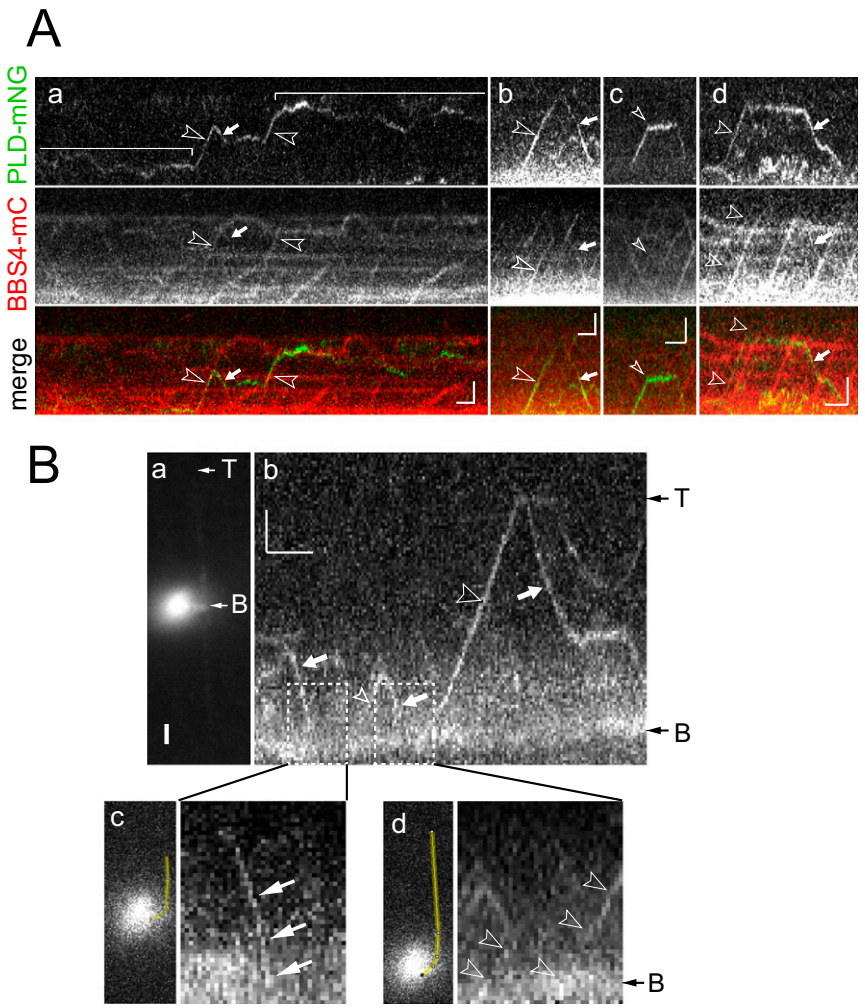


Fig. S2. Details of PLD-mNG transports. (*A*) Gallery of Kymograms from two-color imaging of PLD-mNG and BBS4-mC. Open arrowheads indicate anterograde transports; retrograde transports are marked by white arrows. Brackets in *a* indicate periods of PLD-mNG diffusion. (Scale bars: 2 s and 2 μm.) (*B*) Transport of PLD-mNG near the ciliary base. (*a*) Fifty-frame image average of cell; the ciliary base (B) and one tip (T) are marked. (Scale bar: 2 μm.) (*b*) Kymogram of the top cilium of the cell in *a*. (Scale bars: 2 s and 2 μm.) (*c* and *d*) Details of the boxed areas in *b*. Shown are still images with the lines used to prepare the kymograms and the corresponding kymograms. Due to minor movements of the cell, distinct lines were used for the kymograms shown in *b*–*d*. (*c*) Retrograde transport (white arrows) progressing to the ciliary base. (*d*) Anterograde transports (open arrowheads) of PLD-mNG entering the cilia.

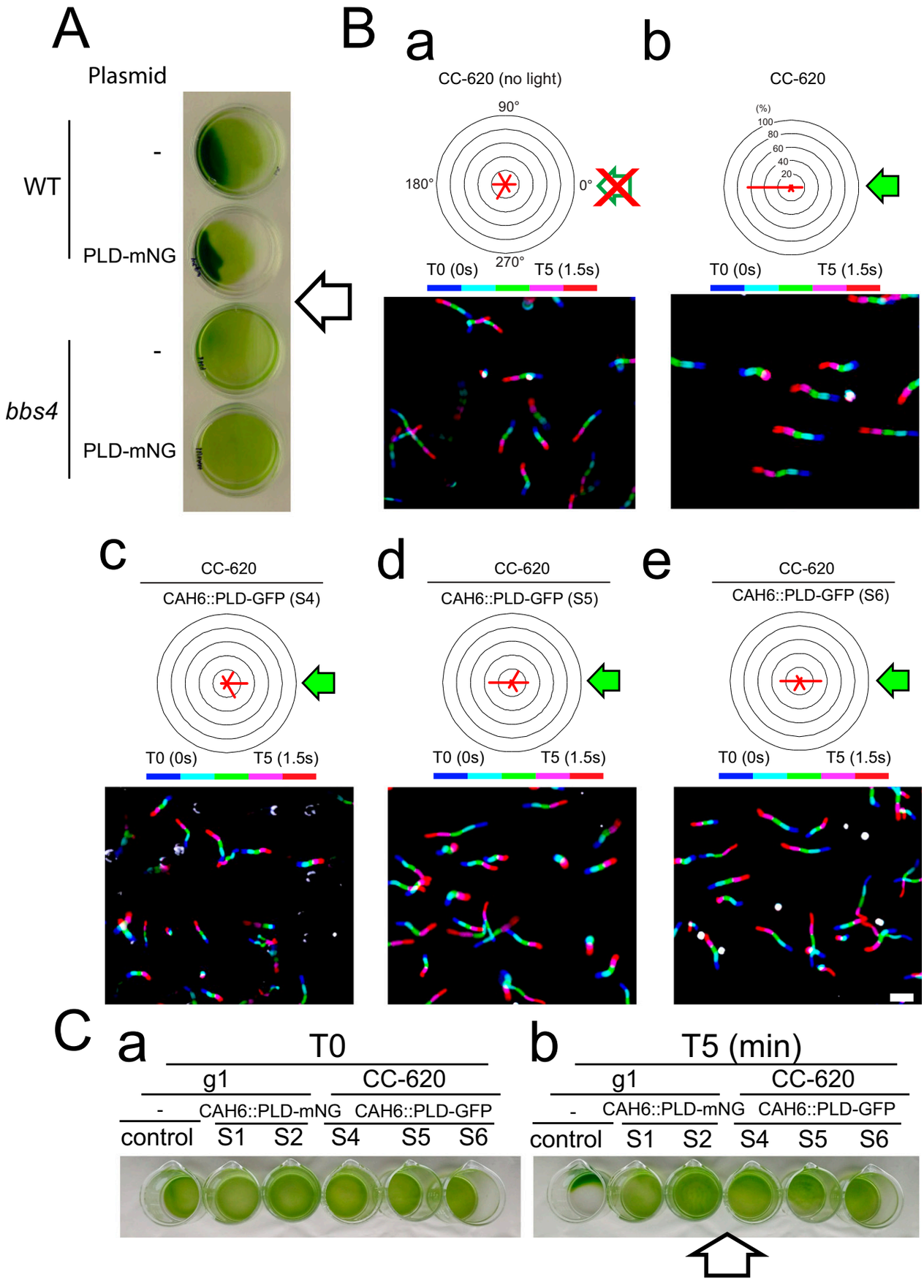


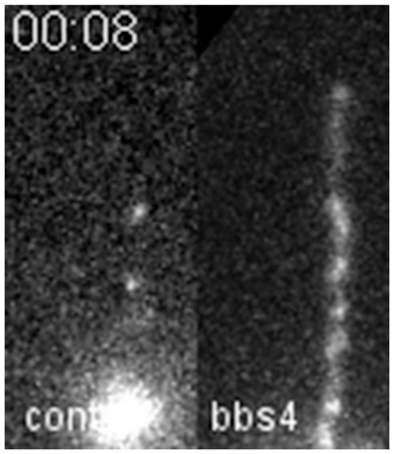
Fig. S3. Expression of CAH6::PLD-mNG diminishes phototaxis. (*A*) Population phototaxis assay of control (WT; CC-620) and *bbs4* cells and the strains expressing PLD-mNG. The direction of the light (arrow) is indicated. (*B*) Histograms and merged images from single cell phototaxis assays of WT control (CC-620; *a* and *b*) and of transformants (S4–S6) expressing CAH6::PLD-mNG/sfGFP (*c*–*e*). The control strain is shown in the dark (*a*) in addition to illumination with phototactic light (*b*). The direction of the light is indicated by the greens arrows. (Scale bar: 50 μm.) (*C*) Population phototaxis assay of control and five independent transformants expressing CAH6::PLD-mNG/sfGFP (S1, S2, S4–S6). Shown are images at T0 (*a*) and after 5 min of incubation in a light gradient (T5, *b*, arrow indicates direction of the light).

Table S1. List of strains

Name	Genotype	Source
CC-621 (WT)	*nit1, nit2, mt*$^{-}$	*Chlamydomonas* Genetics Center
CC-620 (WT)	*nit1, nit2, mt*$^{+}$	*Chlamydomonas* Genetics Center
g1 (WT)	*nit1, agg1, mt*$^{+}$	(21)
bbs1 (CC-4371)	*bbs1, mt*$^{-}$	(4)
bbs1 PLD-mNG	*bbs1, mt*$^{-}$	This study
bbs4 (CC-4377)	*agg1, mt*$^{+}$	(4)
bbs4 PLD-mNG	*agg1, mt*$^{+}$	This study
bbs4	*agg1, mt*$^{-}$	(4)
bbs4 PLD-mNG	*agg1, mt*$^{-}$	This study
bbs7 (CC-4381)	*agg1, nit1, mt*$^{+}$	(4)
bbs7 PLD-mNG	*agg1, nit1, mt*$^{+}$	This study
bbs8	*agg1, nit1, mt*$^{+}$	(5)
bbs8 PLD-mNG	*agg1, nit1, mt*$^{+}$	This study
Wild-type PLD-mNG	CC-620, *nit1, nit2, mt*$^{+}$	This study
bbs4 PLD-mNG BBS4-mC	*agg1, mt*$^{-}$	This study
Wild-type PLD$^{1\text{-}20}$-mNG	CC-620, *nit1, nit2, mt*$^{+}$	This study
bbs4 PLD$^{1\text{-}20}$-mNG	*agg1, mt*$^{+}$	This study
Wild-type PLD$^{1\text{-}58}$-mNG	CC-620, *nit1, nit2, mt*$^{+}$	This study
bbs4 PLD$^{1\text{-}58}$-mNG	*agg1, mt*$^{+}$	This study
Wild-type PLD$^{1\text{-}70}$-mNG	CC-620, *nit1, nit2, mt*$^{+}$	This study
bbs4 PLD$^{1\text{-}70}$-mNG	*agg1, mt*$^{+}$	This study
Wild-type PLD$^{1\text{-}95}$-mNG	CC-620, *nit1, nit2, mt*$^{+}$	This study
bbs4 PLD$^{1\text{-}95}$-mNG	*agg1, mt*$^{+}$	This study
bbs4 PLD^{MAA}-mNG	*agg1, mt*$^{+}$	This study
Wild-type PLD^{MAA}-mNG	g1, *nit1, agg1, mt*$^{+}$	This study
Wild-type CAH6$^{1\text{-}58}$PLD$^{59\text{-}223}$-GFP	620, *nit1, agg1, mt*$^{+}$	This study
Wild-type CAH6$^{1\text{-}58}$PLD$^{59\text{-}223}$-mNG	g1, *nit1, agg1, mt*$^{+}$	This study
bbs1 CAH6$^{1\text{-}58}$PLD$^{59\text{-}223}$-mNG	*bbs1, mt*$^{-}$	This study

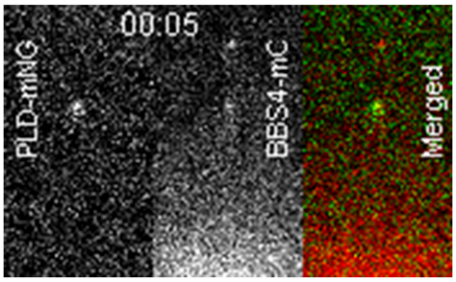
Table S2. List of primers

Primer no.	Sequence
1	CGCGTTAACATCTTAAAATGGCGGCCGCCAGCTCCAAGGAGGAG
2	CGCCTCGAGCTTGAACATGTCCC
3	CGCAAGCTTGCCTGCCGGGCGCGC
4	CGCCTCGAGGCGGGACAGGCTGGTAG
5	CGCCTCGAGGCAGGGGAGCTTCTCGTC
6	CGCCTCGAGCACAACACCGCCCTGCAA
7	CGCCTCGAGCTCGGCGCTGCTGCCGCC
8	CGCGTTAACATGGTGAGCGAAGACCGGCG
9	CGCAGATCTCTGCTCAGGCGATGCCCTAT
10	CGCAGATCTGAGACGTTGTTCTTCCCGG
11	CGCCTCGAGCTTGAACATGTCCCAGAG



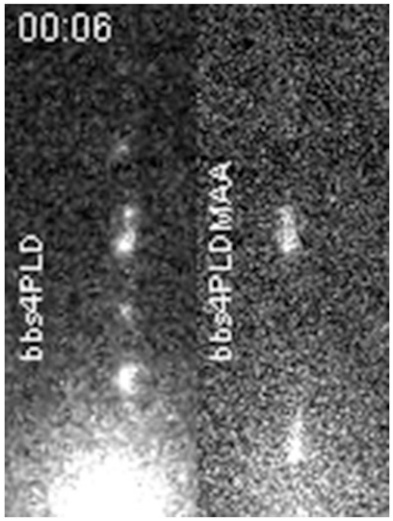
Movie S1. IFT of PLD-mNG in control and *bbs4* cilia. TIRF videos of a WT (*Left*) and a *bbs4* (*Right*) cell expressing PLD-mNG. The cilia tip points toward the top. Images were acquired at 10 fps, and playback is set at 20 fps (2× speed). The timer counts mm:ss.

Movie S1



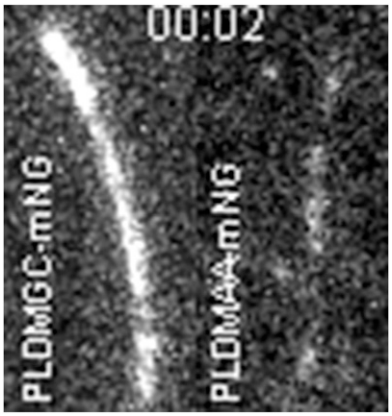
Movie S2. Cotransport of PLD-mNG and BBS4-mC. Two-color TIRF imaging of PLD-mNG (*Left*) and BBS4-mC (*Middle*) expressed in the *bbs4* BBS4-mC rescue strain; the merged video is shown on the *Right*. Note comigration of both proteins by retrograde IFT. The cilia tip points toward the top. Images were acquired at 10 fps, and playback is set at 10 fps (real time). The timer counts mm:ss. A kymogram of the recording is shown in Fig. 1*E*.

Movie S2



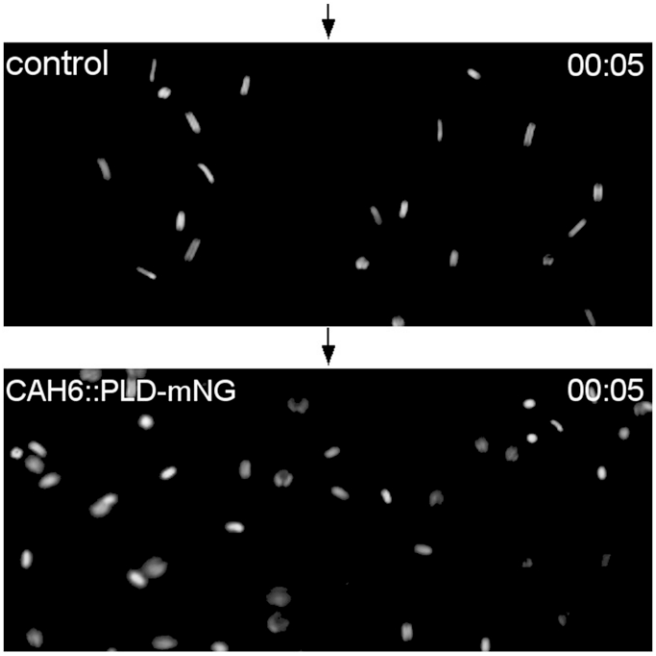
Movie S3. Diffusion of PLD and PLD^{MAA}-mNG in *bbs4* cilia. TIRF videos of PLD-mNG (*Left*) and PLD^{MAA}-mNG (*Right*) in *bbs4* cilia. The cilia tip points toward the top. Images were acquired at 10 fps, and playback is set at 20 fps (2× speed). The timer counts mm:ss.

Movie S3



Movie S4. PLD^{MAA}-mNG enters cilia faster than PLD-mNG. Photobleaching of mNG-tagged PLD (*Left*) and PLD^{MAA} (*Right*) in cilia of *bbs4* cells. Note lack of recovery for the PLD construct despite its significant higher concentration inside cilia before the bleach. The cilia tip points toward the top. Images were acquired at 2.5 fps, and playback is set at 10 fps (4× speed). The timer counts mm:ss.

Movie S4



Movie S5. Control cells expressing CAH6::PLD-mNG lack phototaxis. Single cell phototaxis assay of control cells (g1; *Top*) and control cells expressing CAH6::PLD-mNG (*Bottom*). The direction of the green phototactic light is indicated. The exposure time was 300 ms per frame, and the playback is set at ∼3.3 fps (real time). The timer counts mm:ss.

Movie S5