Supplemental Materials

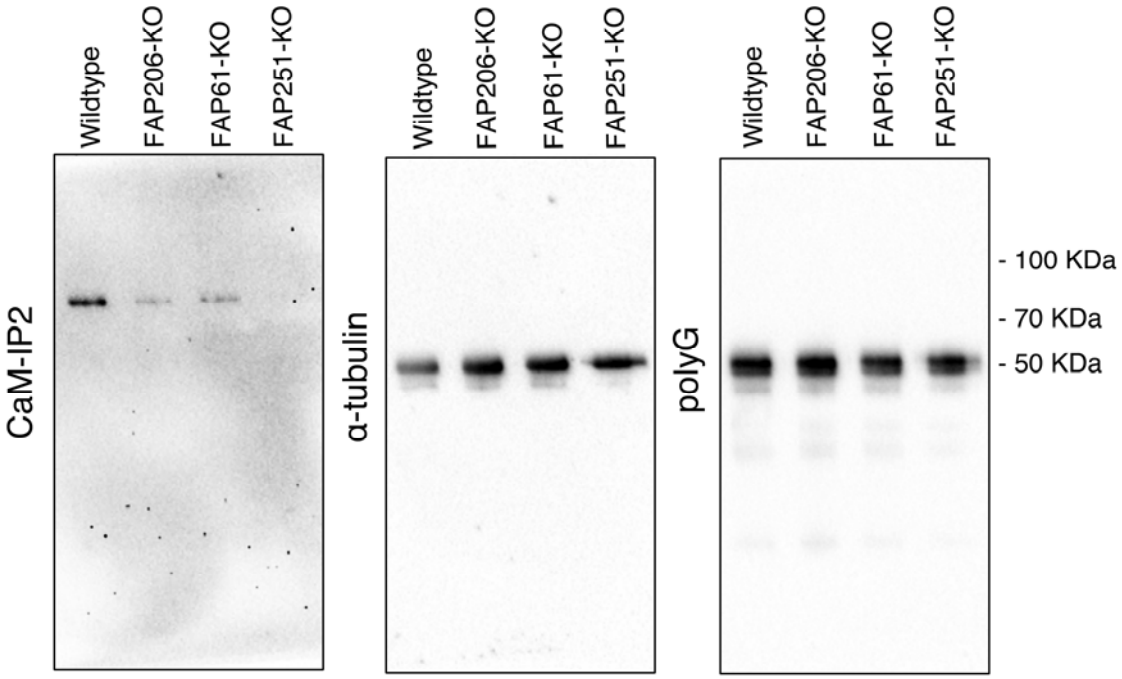
Molecular Biology of the Cell

Vasudevan et al.

Supplemental Data

Supplemental Figure

Supplemental Figure S1. Western blotsofciliaisolatedfromwildtypeandseveral knockout strainsusingthe FAP91/Cam-IP2 antibodies, 12G10 anti-α-tubulinandpolyG, polyglycylatedtubulinantibodies. Fragmentsoftheblotsshownontheleftand in themiddle are used in Figure 7A.



SupplementalMovies

SupplementalMovie 1. TIRF imaging of live cells that express FAP206-GFP under the native promoter.

SupplementalMovie2. A wild-type cell recorded at 500 fps. The video is played at 30 fps.

SupplementalMovie3. A FAP206-KO cell recorded at 500 fps. The video is played at 30 fps. Note that the mutant cilia tend to be more straight, indicating a decreased bend amplitude (compare to Supplemental Movie 2).

SupplementalMovie4. 3D visualization of the averaged 3D structure of the wildtype 96 nm axonemalrepeat.

SupplementalMovie5. 3D visualization of the averaged 3D structure of the FAP206-KO 96 nm axonemalrepeat.

SupplementalMovie6. An overlay comparison of the RS2 structure between wildtype and FAP206-KO based on subtomogram averages of all axonemal repeats. The meshed area shows the wildtype, while the solid area shows the remaining structures in the FAP206-KO mutant, including the side prong (light blue).

SupplementalMovie7. A comparison of the averaged RS2 structure between wildtype (left) and FAP206-KO after separation into two classes that either assemble a partial RS2 (+RS2) (middle) or lack RS2 entirely (-RS2) (right). Note that even when RS2 is assembled in FAP206-KO(+RS2), the RS2 base lacks the front prong (red) and the back prong density (yellow) is reduced.#

Supplemental Tables.

Table S1. Primers used for amplification of fragments of *FAP206* that were cloned on sides of the neo4 marker to construct a targeting fragment for the gene knockout.

Primer sequence	Size of amplified fragment (Kb)
Forward 5'-ATAAGGGCCCTCGTAATGCAAGCTGAGAAT-3' Reverse 5'-AATTCCCGGGCTATATATCTATTTATAATTTGCT-3'	1.21
Forward: 5'-AATTCTGCAGCTTGGACCTTTTGTTTCTCT-3' Reverse 5'-TTATCCGCGGTGACCACAAGCAAAATCCTA-3'	1.12

Table S2. Diagnostic primers used for verification of *FAP206* gene disruption (amplify the region that was intended to be deleted).

Primer sequence	Size of amplified fragment (Kb)
Forward 5'-ATACCCATTTTAGATTCTAACA-3' Reverse 5'-CACTGTCATAAGCTATATTTC-3'	0.959

Table S3. Primers used for amplification of fragments of genomic DNA of *Tetrahymena*for making a plasmid that targeted GFP to the native locus of *FAP206*. In the targeting fragment the 1.24 kb fragment was followed by GFP, TGA stop codon, *BTU1* transcription terminator, and the 1.18 fragment.

Primer sequence	Size of amplified fragment (Kb)
Forward 5'-AATACCGCGGATCTAATGAGGAATAAGTAGAA-3' Reverse 5'-TATTACGCGTGCATTAGTGTCTTTGTCTCTTAAT-3'	1.24
Forward 5'-TATAATCGATAGTACTAATTAATATTTATTTTGC-3' Reverse 5'-TATTGAGCTCTAATACAAAACAGTACTCAGAT-3'	1.18

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