Supplemental Materials

Molecular Biology of the Cell

Fu et al.

Supplemental Information for

The I1 dynein-associated tether and tether head complex is a conserved regulator of ciliary motility

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TABLE S1. LC-MS/MS analyses of axonemal proteins from wild-type (WT) and mutant cells from *T. thermophila* and *C. reinhardtii*[#]

	T. thermophila		***C. reinhardtii***						
Proteins	WT	*fap43*	WT	*fap44*	*fap43*	*fap244*	*pf9-3*	*fap43 / WT**	*fap244 / WT**
Representative I1 dynein proteins									
I1α-DHC	258	258	253	218	232	245	**9**	1.03	0.96
I1β-DHC	267	267	260	235	244	262	**8**	0.99	1.15
IC97	55	49	41	42	35	40	**0**	0.7	1.16
IC138	55	42	58	51	57	56	**0**	0.85	0.91
IC140	45	36	51	44	50	48	**0**	1.08	1
T/TH complex proteins									
FAP43	123	**0**	86	**0**	**0**	88	87	0	1.28
FAP44	151	**0**	112	**0**	72	101	107	0.65	0.87
FAP244	N/A**	N/A**	55	**0**	67	**0**	56	2.21	0

Number of unique peptides identified for different I1 dynein and T/TH complex proteins in wild-type and mutant axonemes. For the *C. reinhardtii* mutants, each LC-MS/MS experiment was performed independently (each with WT control) and the number of unique peptides was normalized to the average value of the WT samples. Findings of zero or greatly reduced unique peptides are shown in bold font.

* Ratio of the number of unique peptides from each tested protein from the *fap43 mutant* to that from WT, and from the *fap244 mutant* to that of the WT.

** No homologue protein of *C. reinhardtii* FAP244 was identified in the proteome of *T. thermophila*.

TABLE S2. Summary of the strains used in this study and image processing information

Strains	No. of tomograms	Averaged repeats	Resolution (nm)
T. thermophila WT	12	1622	3.3
T. thermophila fap43	10	1150	3.9
T. thermophila fap43bccp	27	3250	3.4
T. thermophila fap43bccp control	18	2200	3.6
T. thermophila fap44bccp	20	2451	3.5
T. thermophila fap44bccp control	24	3174	3.4
C. reinhardtii WT	25	3736	3.0
C. reinhardtii WT*	19	2381	2.7
C. reinhardtii fap43	13	1711	3.7
C. reinhardtii fap44	15	1885	4.0
*C. reinhardtii fap244**	13	1601	2.5

* data recorded using Titan Krios TEM with K2 and Volta-Phase-Plate (compared to remaining data recorded using F30 with CCD)

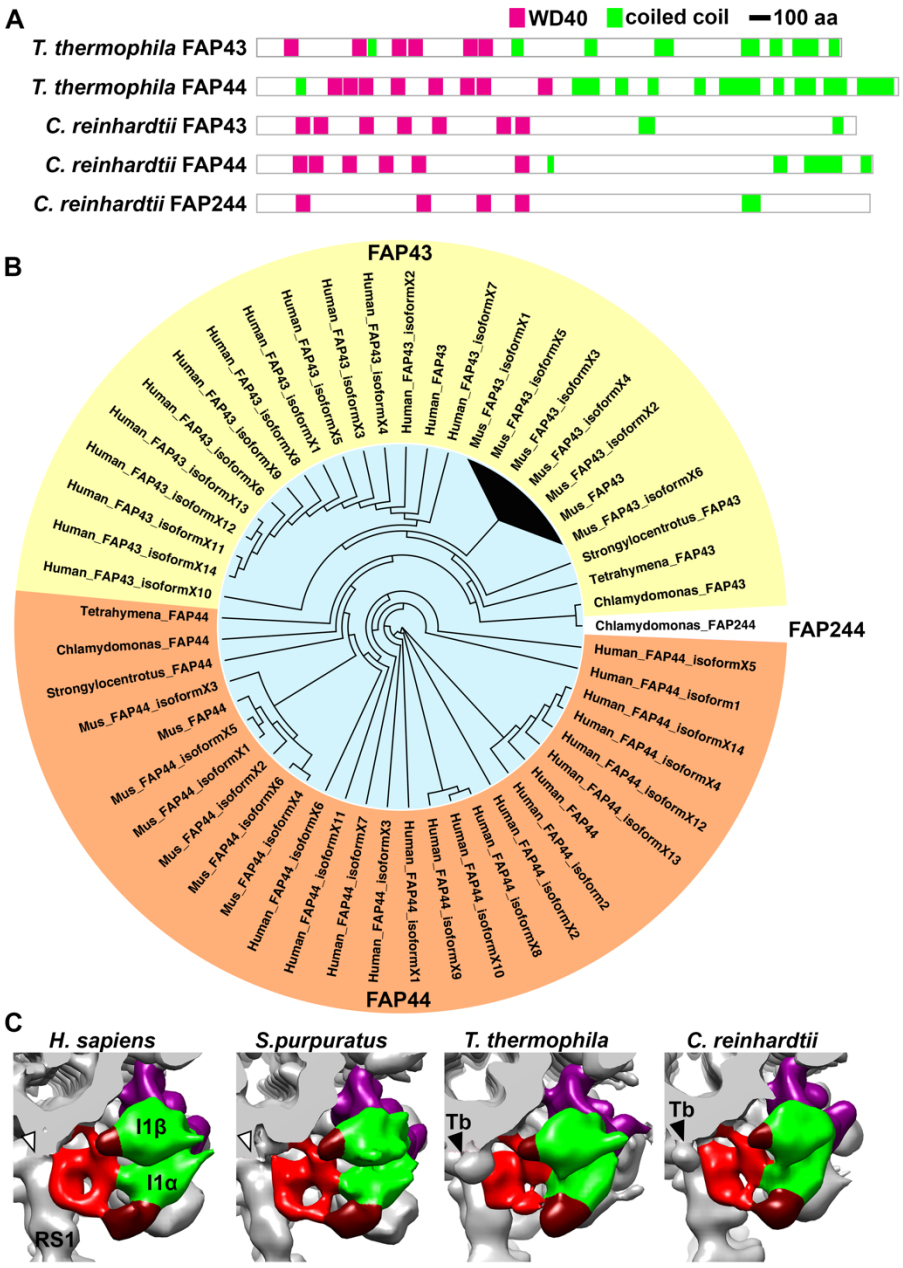

FIGURE S1: The protein components and structural morphology of the T/TH complex are conserved among eukaryotes. (A) Domain organization of *T. thermophila* FAP43 and FAP44 proteins, and of *C. reinhardtii* FAP43, FAP44 and FAP244 proteins, show all of them to be enriched with WD40 domains (red) in their N-terminal regions, and with coiled-coil domains (green) in their C-terminal regions. Length of 100 amino acids is indicated. (B) A maximum

likelihood phylogenetic tree constructed from 53 T/TH protein sequences in evolutionarily diverse organisms, i.e. human, mouse, sea urchin, *T. thermophila* and *C. reinhardtii*. The tree shows the presence of two clades, corresponding to the FAP43 (yellow) and FAP44 (orange) proteins. The algae-specific FAP244 best fits into the FAP43 clade. (C) 3D isosurface renderings of the structurally conserved T/TH complexes and the I1 dynein in cross-sectional view of the averaged axonemal repeat from human airway cilia (*H. sapiens*), sea urchin sperm flagella (*S. purpuratus*), *Tetrahymena* cilia and *Chlamydomonas* flagella. Note that a tether-associated base (Tb) is only observed in *Tetrahymena* and *Chlamydomonas* (black arrowheads), but not in higher organisms (white arrowheads). Coloring of the 3D isosurface renderings: green, I1α and I1β motor domains; red, tether; dark red, tether head; purple, intermediate and light chain of I1 dynein complex; RS, radial spoke. [Data of human airway cilia and sea urchin sperm were adapted from Lin et al., 2014].

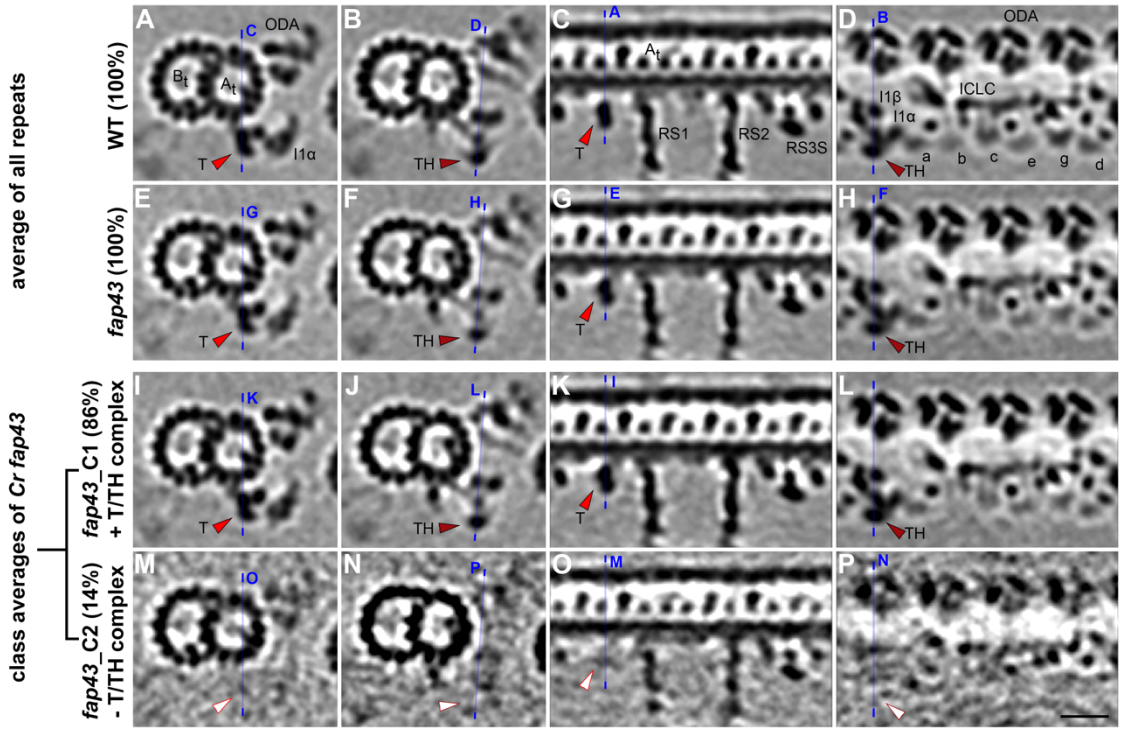

FIGURE S2: Classification analysis of the *C. reinhardtii fap43* mutant reveals that only a small percentage of the axonemal repeats lack the T/TH complex. (A–H) Tomographic slices of the averages from all axonemal repeats (100%) from *Chlamydomonas* wild-type (WT, A–D) and *fap43* (E–H) in cross-sectional (A, B, E and F) and longitudinal (C, D, G and H) orientations. Blue lines indicate the locations of the slices in the respective panels. Electron densities representing tether (T, red arrowheads) and tether head (TH, dark red arrowheads) are indicated. (I–P) Classification analysis of the axonemal repeats from *fap43* mutant focused on the T/TH complex resulted in two class averages: class 1, with T/TH present (I–L) and class 2 without T/TH (M–P); the percentages of repeats included in each class are indicated. Other labels: A_t and B_t, A- and B-tubule; a-e & g, inner dynein arm isoforms; ICLC, intermediate and light chain complex; ODA, outer dynein arm; RS/RS3S, radial spoke/radial spoke 3 stand-in. Scale bar: 20 nm (valid for A–P).

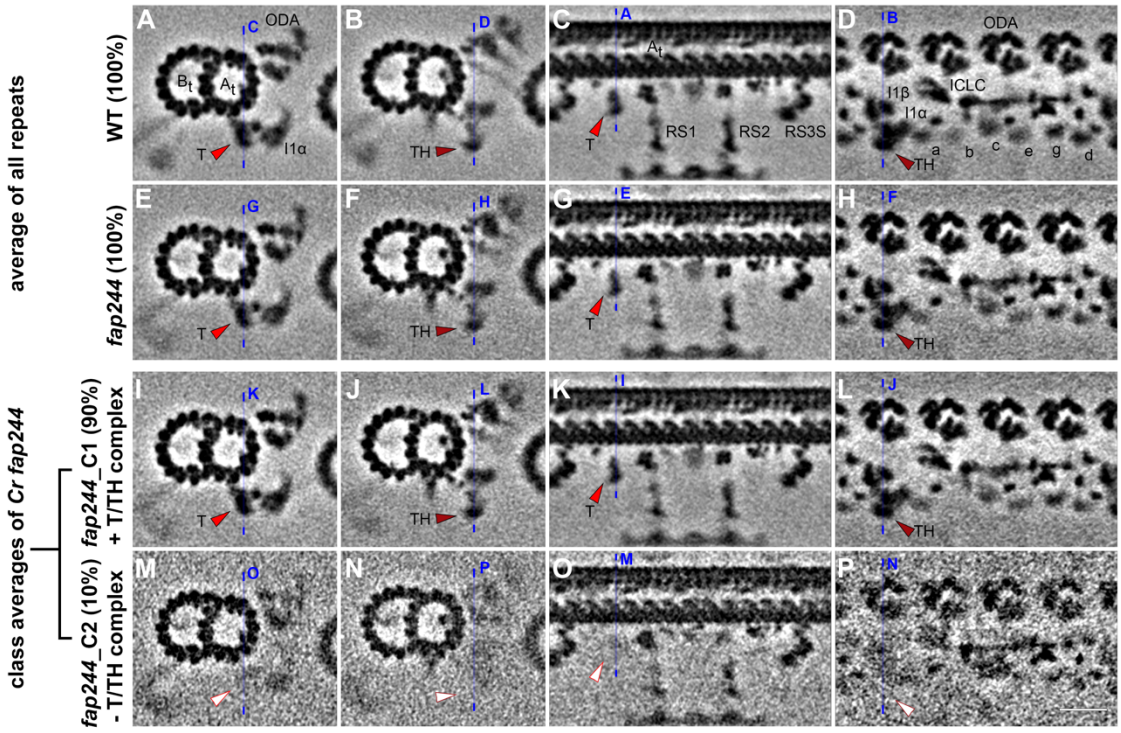

FIGURE S3: Classification analysis of the *C. reinhardtii fap244* mutant reveals that only a small percentage of the axonemal repeats lack the T/TH complex. (A–H) Tomographic slices of the averages from all axonemal repeats (100%) from *Chlamydomonas* wild-type (WT, A–D) and *fap244* (E–H) in cross-sectional (A, B, E and F) and longitudinal (C, D, G and H) orientations. Blue lines indicate the locations of the slices in the respective panels. Electron densities representing tether (T, red arrowheads) and tether head (TH, dark red arrowheads) are indicated. Note that for both averages, wild-type and *fap244*, the data were recorded on a Titan Krios TEM with K2 and Volta-Phase-Plate, resulting in higher resolution (see also Table S2 and *Materials and methods*). (I–P) Classification analysis of the axonemal repeats from *fap244* mutant focused on the T/TH complex resulted in two class averages: class 1, with T/TH present (I–L) and class 2 without T/TH (M–P); the percentages of repeats included in each class are indicated. Other labels: A_t and B_t, A- and B-tubule; a-e & g, inner dynein arm isoforms; ICLC, intermediate and light chain

complex; ODA, outer dynein arm; RS/RS3S, radial spoke/radial spoke 3 stand-in. Scale bar: 20 nm (valid for A–P).

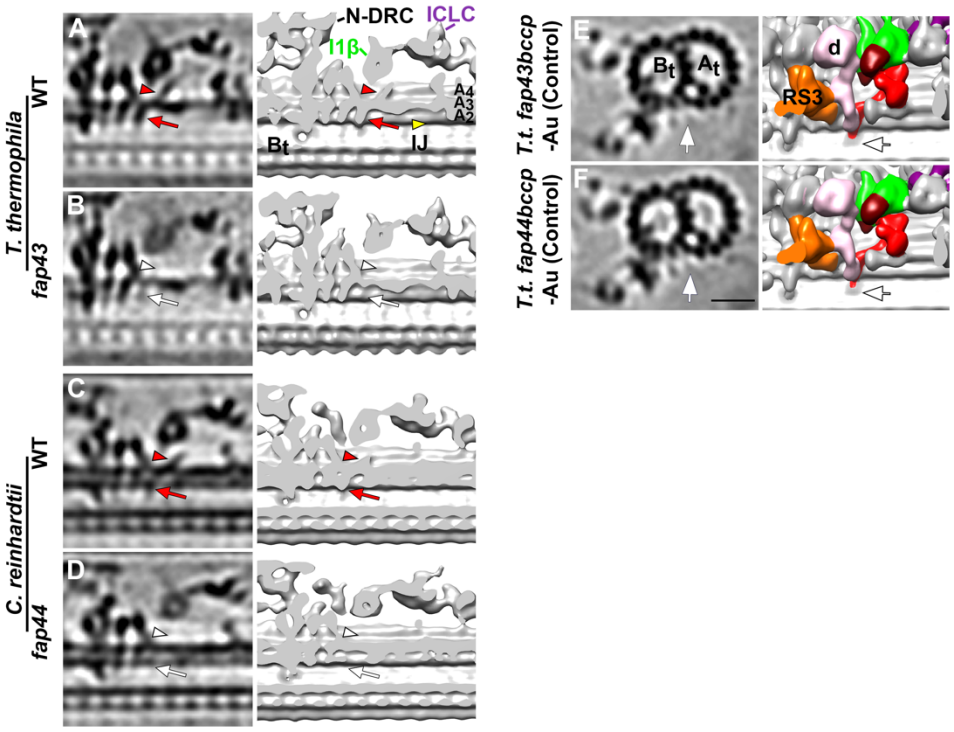

FIGURE S4: Structural details of the tether ridge structure and control samples for the BCCP-gold-labeling experiments. (A–D) Tomographic slices (left) and 3D isosurface renderings (right) of the averaged axonemal repeats from wild-type (WT) *T. thermophila* and *C. reinhardtii*, and the *T. thermophila fap43* and *C. reinhardtii fap44* mutants show the ridge structure in bottom view (as indicated by the blue plane in Figure 3A); in (A and C) red arrowheads indicate the tether region attached to A-tubule protofilaments A4-A3, and arrows indicate regions anchored to A3-A2 near the inner junction (IJ) between the A- and B-tubule (A_t and B_t). The ridge was not observed in *T. thermophila fap43* and *C. reinhardtii fap44* mutant (white arrowheads and arrows in B and D). (E and F) Tomographic slices (left, in cross-sectional direction, viewed from the ciliary tip towards the cell body as in Figure 3) and 3D isosurface renderings (right, in bottom view) of the averaged axonemal repeats from *T. thermophila fap43bccp* (E) and *fap44bccp* (F) without adding streptavidin-gold (control samples). Note the absence (white arrows) of a label-density when

compared to Figure 3, H and I from the tether (red) and tether head (dark red) complex. Labels: d (rose), inner dynein arm d; I1β (green), I1β motor domain; ICLC (purple), intermediate and light chain complex; N-DRC, nexin dynein regulatory complex; ODA, outer dynein arm; RS (orange), radial spoke. Scale bar: 20 nm (valid for EM images in A–F).

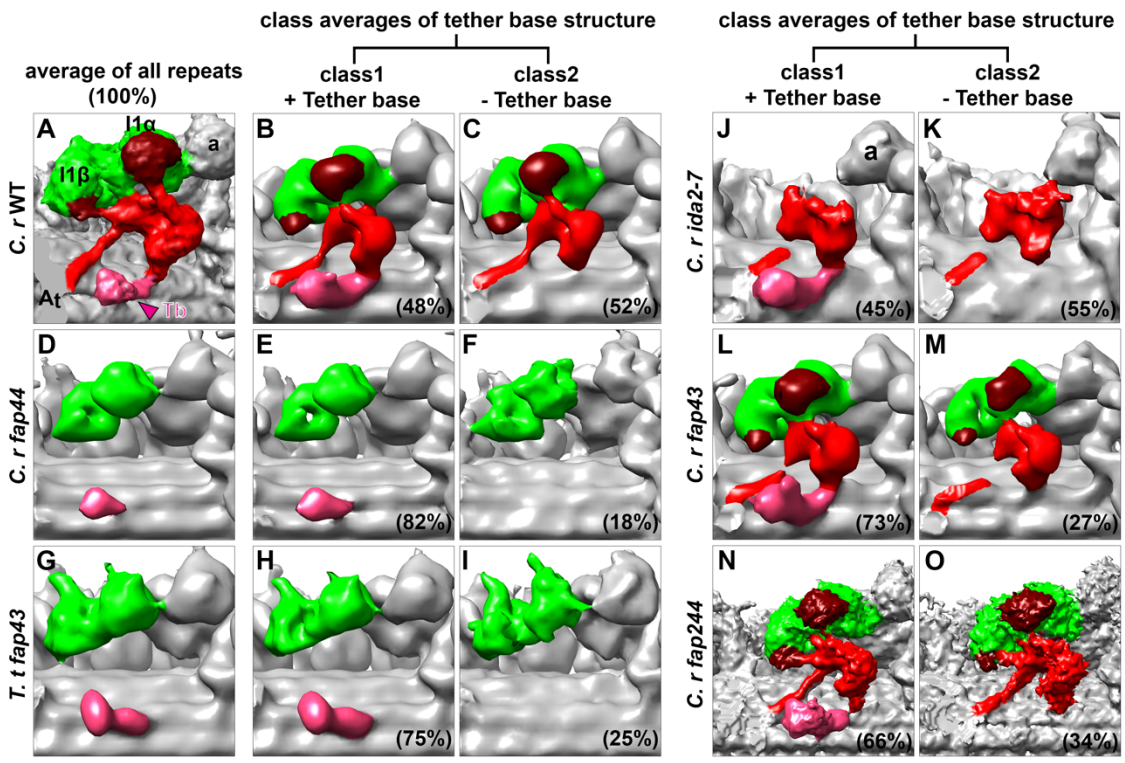

FIGURE S5: Classification analyses focused on the tether-associated base (Tb) structure reveals species-specific differences in wild-type (WT) and T/TH mutants. All panels show 3D isosurface renderings of averaged axonemal repeats in bottom view. (A, D and G) Average of all axonemal repeats from *C. reinhardtii* wild-type (WT) (A) and *fap44* (D), as well as *T. thermophila fap43* (G) showing the Tb (pink, arrowhead in *A*), even when the tether (red) and tether head (dark red) complex is missing (D-I). (B-O) Classification focused on the Tb resulted in two classes for each studied strain: class 1 with the Tb present and class 2 without the Tb; strains studied from *C. reinhardtii*: wild-type (B and C), *fap44* (*E* and *F*), the I1 dynein-missing mutant *ida2-7* (J and K), *fap43* (L and M), and *fap244* (N and O); from *T. thermophila fap43* (H and I). The percentages of repeats included in each class are indicated. Additional colors: green, I1α and I1β motor domains. Please note that the isosurface renderings in (A, N and O) appear "bumpy" because of the higher

resolution achieved by collecting the data using a Titan Krios TEM with K2 and Volta-Phase-Plate (see also Supplemental Figure S3, Table S2 and *Materials and methods*).