Appendix

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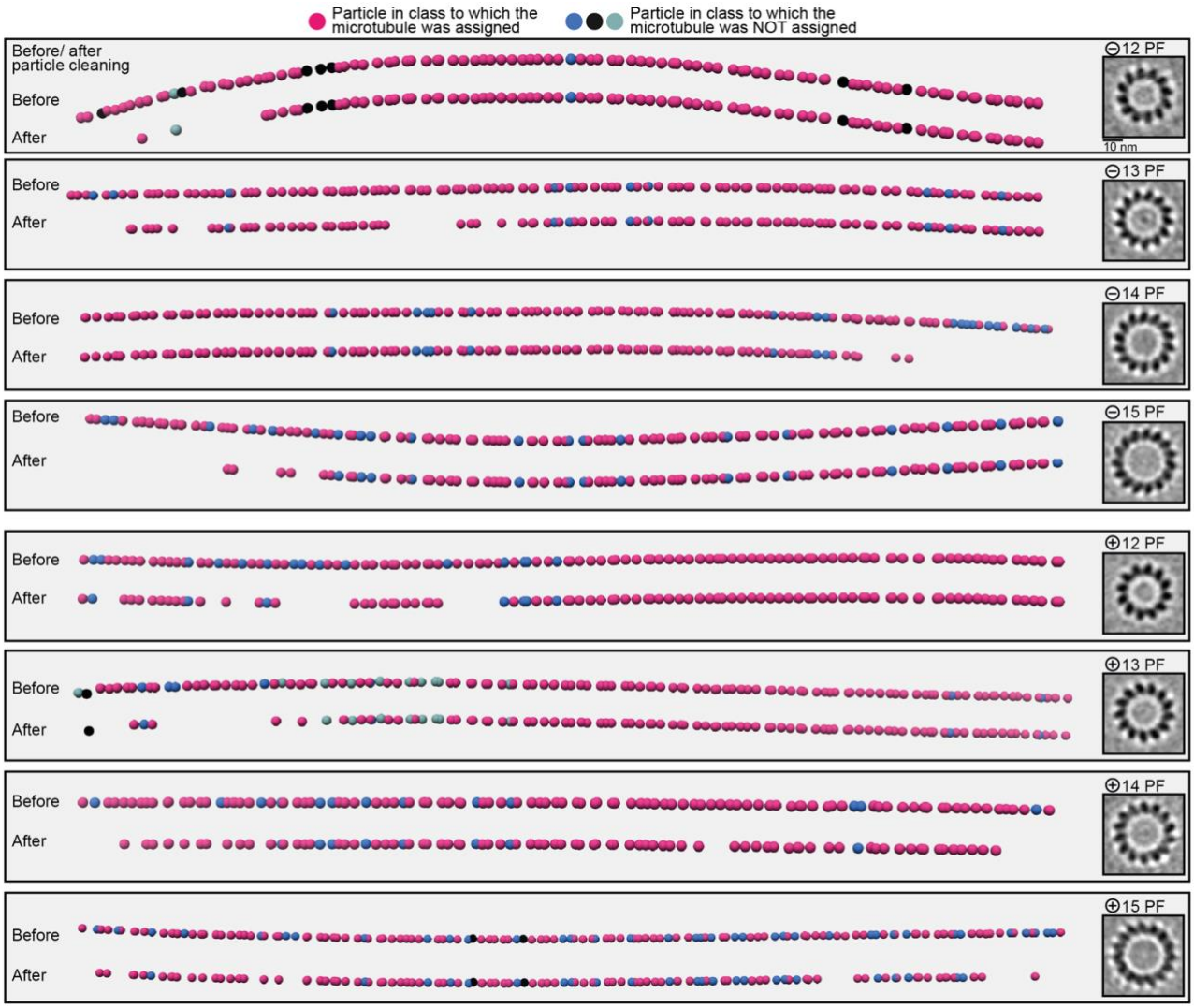
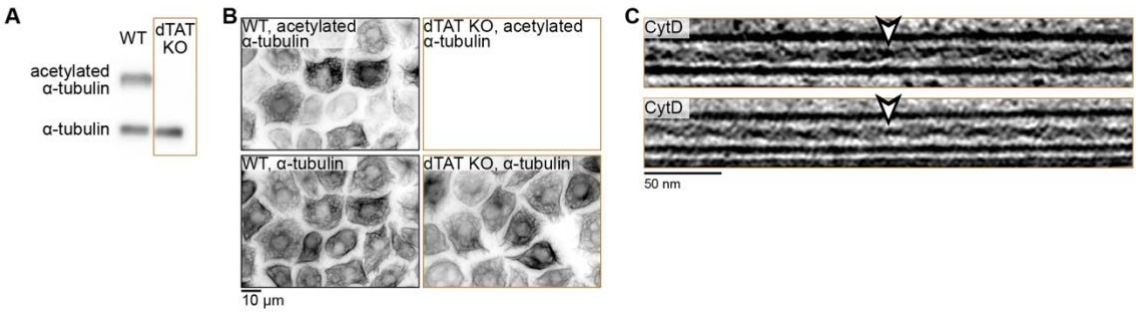


Appendix Figure S1. Validation of the microtubule orientation assignment. Microtubule particle positions and classes are shown for 8 representative microtubules with 12, 13, 14 or 15 protofilaments facing the minus and the plus ends. Pink spheres represent particles in the class, to which the microtubule was assigned. Blue, black and green spheres show particles in classes, to which the microtubule was not assigned. The particle class distribution before (top, 'Before') and after (bottom, 'After') removal of 20% of particles based on their cross-correlation scores ('cleaning') are shown for each microtubule. Projections of subtomogram averages of each of the microtubules before cleaning are shown on the right.



Appendix Figure S2. Validation of the *Drosophila* α-tubulin acetylase (dTAT) CRISPR knock-out. A) Western blot of wild-type (WT, left) or dTAT knock-out (KO, right) cells showing that dTAT KO cells lack acetylated α-tubulin (top). α-tubulin (bottom) was used as loading control. B) Immunofluorescence of WT (left) and dTAT KO (right) cells stained for acetylated α-tubulin (top) or α-tubulin (bottom) showing that dTAT KO cells lack acetylated α-tubulin. C) Tomogram slices of luminal filaments (white arrowheads) in dTAT KO cells showing that they have similar appearance to luminal filaments found in WT cells (Fig. 2A, B).

FAMILY OF ACTIN BINDING PROTEIN	EMD ENTRY NAME	EMD ID	HELICAL RISE [Å]	HELICAL TWIST [°]
ACTIN (BARE)	Helical ADP-F-actin	27114	28.1	-166.7
ACTININ	alpha-actinin CH1 bound to f-actin	5170	27.7	-166.8
BETA-SPECTRIN	F-actin with beta-III-spectrin actin-binding domain	8886	27.2	-166.9
CATENIN	alphaE-catenin - F-actin	21925	27.4	-166.9
CORONIN	coronin bound f-actin	6101	28.2	-166.3*
FILAMIN	FLNaABD-WT bound to phalloidin F-actin	7833	28.1	-166.8
PLASTIN	F-actin/Plastin2-ABD2 complex	21155	28.0	-166.5*
MYOSIN	cardiac myosin binding protein C C2 domain with f-actin	23497	27.3	-166.5
TROPOMYOSIN	F-actin-tropomyosin	6124	27.5	-166.4*
VINCULIN	vinculin-actin	6446	27.8	-166.8*
COFILIN	cofilactin	6844	27.6	-162.1
THIS STUDY	Subtomogram averaging structure of cofilactin filament inside microtubule lumen of Drosophila S2 cell protrusion	16877	28.0	-161.8

Appendix Table S1. EMDB entries of actin together with actin-binding proteins solved by helical reconstruction. Helical twist values marked with a star were deposited as positive values but are displayed with a negative sign because they describe left-handed helix symmetries. Helical parameters were rounded to one decimal.

MICROTUBULE ANALYSIS	**BIOLOGICAL REPLICATE 1**	**BIOLOGICAL REPLICATE 2**		**BIOLOGICAL REPLICATE 3**
GRID ID	**DZ4**	**DY2**	**DZ1**	**FB3/FB4**
DATASET	**dataset 1**	**dataset 2**	**dataset 3**	**dataset 4**
GENOTYPE	WT	WT		WT
SAMPLE CONDITIONS	4 h 2.5 µM CytD	4 h 2.5 µM CytD		4 h 5 µM CytD
INCREMENTS/ NUMBER OF TILTS	2°/ 61	2°/ 61	3°/ 41	3°/ 41
FRAMES PER TILT IMAGE	10	10	14	14
PIXEL SIZE [Å/PIXEL], DETECTOR	2.952, K2	2.952, K2		2.952, K2
TOTAL DOSE [$e^-/Å^2$]	123.44	121.68	123.57	122.25
PROGRAM USED FOR TILT ALIGNMENT	dautoalign and IMOD	IMOD		IMOD
ACQUIRED TILT SERIES	35	22	39	24
ANALYSED TOMOGRAMS	33	18	36	22
NUMBER OF ANALYZED PROTRUSIONS	34	56		22
NUMBER OF ANALYZED MICROTUBULES	109	299		166

Appendix Table S2. Acquisition and processing parameters for datasets 1 – 4 (EMPIAR-11450). These datasets were used for quantification and subtomogram averaging of microtubules in S2 cells.

CYTD & DMSO AND CYTD & TG	**BIOLOGICAL REPLICATE 1**		**BIOLOGICAL REPLICATE 2**		**BIOLOGICAL REPLICATE 3**		**DTAT KNOCK-OUT**
ACQUISITION DATE/ GRID ID	**17.06.2022**		**20.07.2022**		**21.07.2021**		**FE2/FE4**
DATASET	**dataset 5**		**dataset 6**		**dataset 7**		**dataset 8**
GENOTYPE	WT		WT		WT		dTAT KO
SAMPLE CONDITIONS	5 h 0.1% (v/v) DMSO, 4 h 2.5 µM CytD	5 h 2 µM TG, 4 h 2.5 µM CytD	5 h 0.1% (v/v) DMSO, 4 h 2.5 µM CytD	5 h 2 µM TG, 4 h 2.5 µM CytD	5 h 0.1% (v/v) DMSO, 4 h 2.5 µM CytD	5 h 2 µM TG, 4 h 2.5 µM	4 h 5 µM CytD
INCREMENTS/ NUMBER OF TILTS	3°/ 41		3°/ 41		3°/ 41		3°/ 41
FRAMES PER TILT IMAGE	14		14		14		14
PIXEL SIZE [Å/PIXEL], DETECTOR	2.952, K2		2.952, K2		2.659, K3		2.952, K2
TOTAL DOSE [e^- /Å^2]	118.08		122.19		121.54		122.25
PROGRAM USED FOR TILT ALIGNMENT	IMOD		AreTomo		AreTomo		IMOD
ACQUIRED TILT SERIES	12	14	23	18	25	18	65
ANALYSED TOMOGRAMS	12	12	19	14	20	13	62
NUMBER OF ANALYSED PROTRUSIONS CONTAINING MICROTUBULES	10	14	18	15	17	13	62
NUMBER OF ANALYZED MICROTUBULES	119		139		192		447

Appendix Table S3. Acquisition and processing parameters for datasets 5 – 7 (EMPIAR-11451) and dataset 8 (EMPIAR-11452). Datasets 5 – 7 were used for quantification of luminal filaments and had a final DMSO concentration of 0.125% (v/v). Datasets 5 – 8 were used for subtomogram averaging of luminal filaments. Dataset 8 was collected on the dTAT knock-out mutant.

CONTROL AND COFILIN KNOCK-DOWN	**BIOLOGICAL REPLICATE 1**		**BIOLOGICAL REPLICATE 2**		**BIOLOGICAL REPLICATE 3**		**BIOLOGICAL REPLICATE 4**	
ACQUISITION DATE/ GRID ID	**08.12.2022 (part 1) & 17.12.2022 (part 2)**		**16.12.2022**		**02.02.2023 (part 1) & 12.12.2022 (part 2)**		**03.02.2023**	
DATASET	**dataset 9**		**dataset 10**		**dataset 11**		**dataset 12**	
DAY 1 OF KNOCK-DOWN	01.12.2022		09.12.2022		24.11.2022		17.01.2023	
GENOTYPE	Control knock-down	Cofilin knock-down	Control knock-down	Cofilin knock-down	Control knock-down	Cofilin knock-down	Control knock-down	Cofilin knock-down
SAMPLE CONDITIONS	5 h 2 µM TG, 2 h 2.5 µM CytD		5 h 2 µM TG, 2 h 2.5 µM CytD		5 h 2 µM TG, 2 h 2.5 µM CytD		5 h 2 µM TG, 2 h 2.5 µM CytD	
INCREMENTS/ NUMBER OF TILTS	3°/ 41		3°/ 41		3°/ 41		3°/ 41	
FRAMES PER TILT IMAGE	14		14		14		14	
PIXEL SIZE [Å/PIXEL], DETECTOR	2.659, K3 (08.12.2022) & 2.952, K2 (17.12.2022)		2.952, K2		2.952, K2 (02.02.2023) & 2.659, K3 (12.12.2022)		2.952, K2	
TOTAL DOSE [$e^-/Å^2$]	113.74 (08.12.2022) & 111.61 (17.12.2022)		111.61		119.66 (part 1) & 111.61 (part 2)		120.91	
PROGRAM USED FOR TILT ALIGNMENT	IMOD & AreTomo		AreTomo		IMOD		IMOD	
ACQUIRED TILT SERIES	21	20	19	19	22	15	14	13
ANALYSED TOMOGRAMS	19	17	16	18	22	13	14	13
NUMBER OF ANALYSED PROTRUSIONS	20	17	18	18	22	14	14	13

Appendix Table S4. Acquisition and processing parameters for datasets 9 – 12 (EMPIAR-11453). These datasets were used for quantification of luminal filaments upon control or cofilin knock-down and treatment with CytD & TG (Fig. 3J – L). 8 tilt series from biological replicate 1 (dataset 11) and 3 tilt series from replicate 3 (dataset 13) were collected in different acquisition settings. All samples had a final DMSO concentration of 0.125% (v/v) DMSO.