An allosteric network in spastin couples multiple activities required for microtubule severing

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The AAA + ATPase spastin remodels microtubule arrays through severing and its mutation is the most common cause of hereditary spastic paraplegias (HSP). Polyglutamylation of the tubulin C-terminal tail recruits spastin to microtubules and modulates severing activity. Here, we present a ~3.2 Å resolution cryo-EM structure of the Drosophila melanogaster spastin hexamer with a polyglutamate peptide bound in its central pore. Two electrostatic loops arranged in a double-helical staircase coordinate the substrate sidechains. The structure reveals how concurrent nucleotide and substrate binding organizes the conserved spastin pore loops into an ordered network that is allosterically coupled to oligomerization, and suggests how tubulin tail engagement activates spastin for microtubule disassembly. This allosteric coupling may apply generally in organizing AAA + protein translocases into their active conformations. We show that this allosteric network is essential for severing and is a hotspot for HSP mutations.

Spastin is a microtubule-severing AAA ATPase (ATPases associated with diverse cellular activities) whose function is important in basic cell biological processes ranging from neurogenesis and axonal maintenance to nuclear envelope breakdown, vesicle trafficking, mitosis and cytokinesis. Spastin is recruited to endosomes and the midbody through its interaction with ESCRTIII and is thought to participate in the coordinated remodeling of membranes and microtubules. Spastin ATPase activity is stimulated by microtubules and microtubule severing activity requires adenosine triphosphate (ATP) hydrolysis. Spastin mutations are responsible for ~50% of hereditary spastic paraplegias (HSPs), a large and clinically diverse family of neurodegenerative disorders. In the pure form of the disease, HSP patients typically exhibit axonal degeneration in motor axons of the corticospinal tract, leading to progressive lower limb spasticity and weakness. Studies in model organisms and patient-derived induced pluripotent stem cells have shown that neurons are especially sensitive to spastin gene dosage. Cellular and biochemical studies have shown that many of the disease-associated mutations examined impair microtubule severing and ATP hydrolysis. However, the etiology of spastin induced HSP is still poorly understood.

Spastin has a modular structure comprising a three-helix bundle microtubule-interacting and trafficking (MIT) domain, a poorly conserved linker region and an AAA + ATPase domain. This domain architecture is shared among all three known microtubule severing enzymes: spastin, katanin and fidgetin. The ATPase domain is structurally homologous to that of other members of the AAA + protein family and consists of an α/β nucleotide binding domain (NBD) and a four-helix bundle domain (HBD). Both spastin and katanin differ from other AAA + ATPase enzymes in that they contain two additional helices within the NBD that are essential for severing. The AAA + ATPase domains hexamerize in a nucleotide-dependent manner and the tubulin substrate lowers the critical concentration for oligomerization. Consistent with this, high-affinity binding of spastin to microtubules is highly cooperative and requires ATP. The substrate-dependent oligomerization is likely important for the specificity and timing of action of these AAA + ATPases in the cell, as unregulated spastin and katanin microtubule severing activity is highly deleterious. The majority of disease-associated mutations of the SPAST gene are found in the ATPase domain of spastin.

Microtubule severing by spastin requires the β-tubulin C-terminal tail, an intrinsically disordered element that decorates the microtubule surface. While the α-tubulin tail contributes to binding, it is not required for severing activity. It was proposed that microtubule severing involves the hexamerization of spastin protomers around the ~20 residue negatively charged tubulin tail, and the subsequent pulling of the tubulin subunit out of the microtubule lattice using ATP-driven conformational changes in three pore loops that help translocate the substrate through the central pore of the hexameric ATPase. However, there has been no direct demonstration of the tubulin tail engagement by the spastin pore nor any atomistic structural information on the spastin hexamer or its interaction with the tubulin tail substrate. The interaction of spastin with the microtubule is strongly enhanced by the polyglutamylation of the β-tubulin tail, a post-translational modification that involves the addition of multiple glutamate chains of variable lengths; unmodified microtubules are not effectively severed at in vivo spastin concentrations. As many as 21 glutamates have been detected on tubulin tails in vivo. Polyglutamylation is highly abundant in neurons where spastin activity is required for neurite extension as well as axonal maintenance and regeneration.

Here, we present the cryo-EM structure of the spastin hexamer in complex with a polyglutamate peptide at ~3.2 Å resolution. The structure reveals an asymmetric hexamer with the AAA domains arranged in a split lock-washer conformation. Two conserved pore loops from the six protomers form a double-helical staircase gripping the odd and even glutamates of the substrate peptide, respectively. Residues in this double helix are positively charged and neutralize the electronegative polyglutamate peptide, consistent
with the regulation of microtubule severing by polyglutamylation of tubulin tails. The substrate binding pore loops are allosterically coupled to the ATP binding site as well as the oligomerization interfaces, providing a structural explanation for the tubulin substrate binding-induced spastin activation. The majority of the residues in this allosteric network are mutated in HSP patients, underscoring their importance to spastin function. Our comprehensive structural analysis of all reported HSP-associated spastin missense mutations in its AAA core provides a framework for understanding spastin molecular dysfunction.

**Results**

**Spastin forms a hexameric spiral.** Unlike many AAA+ ATPases, spastin exists as a monomer or dimer in the absence of nucleotide or bound to ADP, and only assembles into hexamers upon ATP binding. This hexamer is labile and falls apart at lower concentrations. To stabilize the hexameric state for structural studies by cryo-electron microscopy (cryo-EM) we used a commonly used mutation in the Walker B motif of the ATPase domain (E583Q), which retains ATP binding but prevents ATP hydrolysis. Analytical ultracentrifugation (AUC) shows that this construct assembles into hexamers in the presence of ATP at concentrations as low as 6 µM (Supplementary Fig. 1a). Initial structure determination yielded a reconstruction of the spastin hexamer limited to ~3.8 Å resolution showing cryo-EM density of adventitious peptide binding. As polyglutamylation enhances substrate binding and spastin has been shown to interact with polyglutamate peptides, we incubated our spastin preparation with polyglutamate (Methods). This increased the proportion of intact hexamers and allowed structural determination of the spastin-peptide complex to ~3.2 Å resolution (Table 1, Fig. 1 and Supplementary Figs. 2 and 3). Polyglutamate activates spastin ATPase with a maximal activation of approximately sixfold above the basal level (Supplementary Fig. 1b), comparable to the activation reported with microtubules. AUC with a fluorescently labeled polyglutamate peptide shows that it comigrates with the spastin hexamer (Supplementary Fig. 1c). The choice of polyglutamate also overcomes the experimental issue of placing enzymes in a specific register with respect to the tubulin tail sequence that is overrepresented in polyglutamates, but also contains other amino acid residues.

The overall architecture of the spastin hexamer resembles that of a split lock-washer, as previously observed for other homohexameric ATPases, including the related severing enzyme katanin (Fig. 1a). The individual protomers assemble into a right-handed spiral, with a ~6 Å rise for each protomer and a ~35 Å gap between the topmost and lowest protomers. The large (NBD) subdomain of the ATPase forms the central pore of the hexamer, with the HBD directed peripherally and in contact with the NBD of the neighboring protomer. The cryo-EM density corresponding to the ‘gating’ protomer complete the ATP binding site for all protomers except the gating protomer F. The Arg finger residue R641 is in a

| Table 1 | Cryo-EM data collection, refinement and validation statistics |
|-----------------|-----------------|
| **Spastin**<sup>E583Q</sup> | **EMD-20226, PDB 6P07** |
| **Data collection and processing** | |
| **Microscope** | Thermo Fischer Talos Arctica |
| **Camera** | Gatan K2 Summit DED |
| **Magnification (nominal)** | ≈36,000 |
| **Magnification (calibrated)** | ≈43,478.3 |
| **Voltage (kV)** | 200 |
| **Total electron exposure (e-/Å<sup>2</sup>)** | 52 |
| **Exposure rate (e-/pixel/s)** | 5.6 |
| **Defocus range (µm)** | −1.0 to −2.0 |
| **Pixel size (Å)** | 1.15 |
| **Micrographs collected (no.)** | 2,534 |
| **Micrographs used (no.)** | 2,534 |
| **Total extracted particles (no.)** | 2,736,865 |
| **Reﬁned particles (no.)** | 1,259,553 |
| **Final particles (no.)** | 488,385 |
| **Symmetry imposed** | C1 |
| **Resolution (global)** | 3.2 Å |
| **FSC 0.5 (unmasked/masked)** | 7.0/3.6 |
| **FSC 0.143 (unmasked/masked)** | 4.3/3.2 |
| **Resolution range (local)** | 3–5 |
| **Model composition** | |
| **Nonhydrogen atoms** | 14,089 |
| **Protein residues** | 1804 |
| **Ligands** | 12 |
| **Reﬁnement** | |
| **Initial model used (PDB code)** | 389P |
| **Average FSC** | 3.2 |
| **R<sub>b</sub> factors (Å<sup>2</sup>)** | 0.64 |
| **Protein residues** | 46.8 |
| **Ligands** | 50.5 |
| **R.m.s. deviations** | |
| **Bond lengths (Å)** | 0.01 |
| **Bond angles (°)** | 1.14 |
| **Validation** | |
| **MolProbity score** | 1.46 |
| **Clashscore** | 3.05 |
| **Poor rotamers (%)** | 0.0 |
| **C-beta deviations** | 0 |
| **Mean per-residue Ca r.m.s. deviation (Å)** | 0.64 |
| **Per-residue Ca r.m.s. deviation range (Å)** | 0.03–5.66 |
| **Ramachandran plot** | |
| **Favored (%)** | 94.65% |
| **Allowed (%)** | 5.35% |
| **Disallowed (%)** | 0.00% |
| **EMRinger score<sup>19</sup>** | 3.00 |
| **CaBLAM outliers** | 3.35% |
structure (Supplementary Fig. 5b) shows extensive conformational impair severing. The linker connecting helices 4 loop of the β-tide, establishing stabilizing interactions with the α4 helix11 of the neighboring protomer (Supplementary Fig. 6a). Interestingly, a recent study of the AAA ATPase VAP1 shows that it is able to translocate peptides in either direction11. It is possible that the polarity of the peptide through the spastin pore is not dictated by pore interactions alone and also results from the engagement of other interfaces with the microtubule substrate.

Consistent with the cryo-EM structures of other AAA+ ATPases, pore loop 1 (555–562), which is characterized as having a conserved aromatic residue important for substrate translocation, interacts directly with the bound substrate (Fig. 2b and Fig. 3a,b). Conserved Y556 intercalates between n + 2 residues of the substrate, sandwiching the glutamate sidechain and forming a hydrogen bond with one of the carboxyl oxygens (Fig. 3a,b). Consistent with its role in substrate engagement, its mutation abolishes severing while retaining full ATPase activity (Fig. 4). This intercalating organization between pore loops and substrate has been previously observed in other ATPases28,29,33 and likely serves as a general mode of substrate interaction for AAA+ ATPases.

Together with invariant K555, which forms a salt bridge with the glutamate of the n + 1 substrate residue, Y556 constitutes the first of two pore-loop spiral staircases that coordinate the substrate. The sidechain of pore loop 1 residue K555 is also likely stabilized by a hydrogen bond with conserved E462 in helix α1, the secondary structure element unique to severing enzymes34. Mutation of residues in α1 impair severing without a notable effect on ATPase activity35. Invariant H596 and R601 of pore loop 2 (594–601) interact directly with the bound substrate (Fig. 2b and Fig. 3a,b). Conserved Y556 intercalates between n + 2 residues of the substrate, sandwiching the glutamate sidechain and forming a hydrogen bond with one of the carboxyl oxygens (Fig. 3a,b). Consistent with its role in substrate engagement, its mutation abolishes severing while retaining full ATPase activity (Fig. 4). This intercalating organization between pore loops and substrate has been previously observed in other ATPases36,37,38 and likely serves as a general mode of substrate interaction for AAA+ ATPases.
of ClpA\textsuperscript{38}, where mutagenesis of certain residues within the loop inhibits substrate degradation but not substrate binding. Additionally, crystallographic studies of HSP104 show pore loop 2 interacting with an adjacent N’ domain molecule, acting as a ‘substrate mimic’\textsuperscript{39}.

**Fig. 2** | A double-helical staircase of pore loops surrounds the substrate and is coupled by a third pore loop spiral to the ATP binding site. \(\textbf{a}\), The three solvent exposed pore loops highlighted on the spastin hexamer structure. Pore loop 1 is colored in blue, pore loop 2 in gold and pore loop 3 in magenta. \(\textbf{b}\), Side (left) and top (right) views of the EM density of the triple spiral staircase generated by pore loops 1, 2 and 3. Pore loops 1 and pore loops 2 form a tight double spiral that engages with the peptide substrate (shown in light green). Pore loops 3 form a shallow spiral between pore loops 1–2 and the nucleotide. Ribbons colored as their corresponding pore loops are overlaid on the EM map to highlight the trajectory of the three pore loop spirals.

**Fig. 3** | A pore loop network couples nucleotide binding to substrate engagement and oligomerization. \(\textbf{a}\), Pore loops 1 and 2 interacting with the polyglutamate substrate. Substrate density is shown as a transparent surface with the polyglutamate model in light green. Pore loops are colored according to their assigned protomer, as in Fig. 1. Residues that interact directly with the substrate (K555, Y556, H596 and R601) are shown in stick representation. \(\textbf{b}\), Interactions between the substrate and conserved residues in pore loops 1 and 2. \(\textbf{c}\), A network of interactions between charged residues connects the peptide substrate to the nucleotide. Hydrogen bonds are shown as dashed lines. All interactions depicted have a measured distance of less than 4 Å.
Mutations in the three pore loops impair ATPase and microtubule severing. a, Microtubule severing assays of wild-type (WT) spastin and pore loop mutant R600A. Severing events are highlighted with blue arrows. Scale bar, 5 µm. b, Tukey plots of severing rates of spastin WT and mutants. n = 30 microtubules from 2, 4, 4, 4, 4 and 3 chambers for WT, Y556A, E561A, R600A, E629A, R630A and E633A mutants, respectively. c, Tukey plots of ATPase rates of spastin WT and mutants, n = 24, 16, 12, 8, 7 and 8 independent measurements for WT, Y556A, E561A, R600A, E629A, R630A and E633A mutants, respectively. For plots in panels b and c, plus sign denotes mean, box, 25th to 75th percentile, whiskers 1.5x interquartile (IQR) or the smallest (bottom) or largest (top) data point if less than 1.5x IQR, outliers shown as circles. ****P < 0.0001, determined by the Mann–Whitney test. ND, not detectable. Pore loop 1, 2 and 3 mutants shown in cyan, orange and magenta, respectively.

Fig. 4 | Effect of HSP mutations on spastin activity. There are over 200 HSP-associate spastin mutations reported to date and 30% of them are missense mutations. The majority of spastin missense mutations linked to HSP are located within the ATPase domain, and several missense disease mutations examined have been shown to inactivate or severely impair microtubule-severing activity in biochemical and cellular studies. Our structure provides a mechanistic understanding of how HSP mutations in the ATPase domain lead to loss of spastin function. Based on the high sequence identity and known structural conservation between Drosophila and human spastin, we generated a homology model of the human spastin ATPase domain (Supplementary Table 1). Thus, our structure sheds light on how to inactivate or severely impair microtubule-severing activity in biochemical and cellular studies. Our structure provides a mechanistic understanding of how HSP mutations in the ATPase domain lead to loss of spastin function. Based on the high sequence identity and known structural conservation between Drosophila and human spastin, we generated a homology model of the human spastin ATPase domain (Supplementary Table 1). Thus, our structure sheds light on how...
Fig. 5 | An allostERIC network mutated in HSP couples substrate binding to oligomerization and ATP hydrolysis. a, Interaction network centered on R591. Residues colored purple and cyan for protomers C and D, respectively. Residues mutated in HSP are indicated with an asterisk. All interactions depicted have a measured distance of less than 4 Å. b, W749 makes van der Walls contacts with several hydrophobic residues mutated in HSP and connects pore loop 3 to the C-terminal helix α11 involved in oligomerization. Residues are colored purple and cyan for protomers C and D, respectively. Residues mutated in HSP are indicated with an asterisk. All interactions depicted have a measured distance of less than 4 Å.

Fig. 6 | Structure-based insight into the mechanism of action of human spastin HSP disease mutations. a, Homology model of the human spastin hexamer depicting the location of missense mutations identified in HSP. Protomers are colored in alternating grey and white around the hexamer. Mutations are depicted as spheres at their Ca position and colored according to their proposed mechanism of action: interference with nucleotide binding (blue), defects in ATP hydrolysis and γ-phosphate sensing (green), disruption of interactions important for folding or conferring stability to the protomer (orange) and disruption of oligomerization interfaces (magenta). b, Close-up view of the nucleotide pocket, showing a large number of mutations that likely disrupt nucleotide binding or impair ATP hydrolysis and sensing. c, Close-up view of hydrophobic interfaces between α4, α5 and β2 highlighting potentially destabilizing HSP mutations. d, Close-up view of the NBD-HBD interaction interface between protomers showing a high density of HSP mutations.
(E42A or K, and R499C). E42 is part of the Walker B motif and participates in catalysis in all AAA ATPases, while R499 serves as the Arg finger. The Arg finger coordinates the γ-phosphate and electrostatically stabilizes the transition state after ATP hydrolysis. N487D (N629 in our Drosophila cryo-EM structure) is likely involved in both the sensing of the γ-phosphate and oligomerization interactions (Figs. 6b and 5a).

Other HSP mutations are likely deleterious because they disrupt the overall fold and stability of the AAA domain structure (Fig. 6c) (I406V, V423L, L426V, F427C, A428P, L447V, S458R and L461P among others). A large number of disease mutations map to protomer-protomer interfaces and likely interfere with oligomerization (Fig. 6d) (E356G, L360P, N361L/S, R364M, L367S, F368L/S, G370R, R372G, C448Y, A551P/Y, D555G/N, E536V, G559D, M561G, R562Q, D613H and T615I). Consistent with this, cellular studies have shown that the C448Y mutant is unable to associate with wild-type spastin and inhibit microtubule severing46. Thus, the preponderance of HSP mutations likely impact oligomerization interfaces or the overall stability of spastin and may lead to spastin haploinsufficiency. However, there is a large phenotypic variability in spastin-linked HSP and more recent work has shown that some spastin disease mutations can also have a toxic gain-of-function that is further exacerbated by spastin haploinsufficiency47. We note that spastin mutants that are deficient in oligomerization could still bind microtubules through a stretch of residues in the linker that connects the AAA and MIT62,63,64 domains and thus have a toxic gain-of-function on the microtubule cytoskeleton. Interestingly, mutations in the Arg finger R499 have been associated with early onset HSP15,16,17, possibly suggesting a dominant negative effect of this mutation. The Walker B residue E442 is the other strong candidate for a possible dynamin-like function to molecular mechanism. J. Biol. Chem. 217, 4057 (2018).

In summary, our structure of spastin elucidates the details of substrate engagement by a microtubule severing enzyme and reveals how three conserved pore loops in spastin establish a conduit that links substrate binding with oligomerization and ATP hydrolysis. Our comprehensive analysis of human HSP mutations reveals the extensive coverage of disease mutations on the spastin hexamer and how they would impact all major facets of hexamer function: ATP binding, hydrolysis and oligomerization.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41594-019-0257-3.

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Author contributions

C.R.S. froze EM grids, collected and processed EM data and built atomic models. A.S. purified all proteins, performed AUC and ATPhase assays. E.A.Z. performed severing assays. C.R.S., G.C.L. and A.R.M. interpreted structural models and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Methods
Protein expression and purification. Full-length Drosophila melanogaster spastin (sequence ID NP_001303437.1) with a Walker B mutation (E83Q) was expressed in Escherichia coli BL21DE3 cells as a C-terminal glutathione S-transferase (GST) fusion protein. For purification, GST tag was removed by PreScission protease cleavage of the GST-T. After cleavage with PreScission protease, spastin was further purified on a HiTrapQ chromatography column (subtractive step) followed by a MonoS column (GE Healthcare). Spastin eluted as two peaks that were collected separately. The purer spastin peak was concentrated to ~5 mg ml⁻¹ and injected on a Superdex 200 size exclusion column (GE Healthcare) in 20 mM HEPES pH 7.5, 300 mM KCl, 10 mM MgCl₂, 5 mM DTT, 5% glycerol. The sample was concentrated to 10 mg ml⁻¹, flash frozen in liquid nitrogen and stored at ~80 °C after supplementing the glycerol concentration to 15%. For ATPase and severing assays, the gel filtration step was omitted and the protein was concentrated to 5 mg ml⁻¹ and buffer exchanged into 20 mM HEPES pH 7.5, 300 mM KCl, 10 mM MgCl₂, 5 mM DTT, 5% glycerol. Single-use microscopes were flash frozen using liquid nitrogen and stored at ~80 °C. All structure-based point mutants were generated using Quickchange mutagenesis and subjected to the same purification protocol as the wild-type protein.

Analytical ultracentrifugation. AUC experiments were conducted in a ProteomeLab XL-I analytical ultracentrifuge (Beckman Coulter) using absorption optics. The spastin Walker B mutant was subjected to size exclusion chromatography in 20 mM HEPES, 300 mM KCl, 10 mM MgCl₂, and 1 mM tris(2-carboxyethyl)phosphine (TCEP). The eluted protein was used for the preparation of AUC samples in the same buffer as used for AUC and supplemented with 0.1 mM ATP. The sample was loaded into a 12 mm cell, placed in an AN-Ti rotor and equilibrated thermally in the ultracentrifuge. After thermal equilibrium was reached at 10 °C, the rotor was accelerated to 45,000 r.p.m. and intensity scans at 280 nm were started immediately and collected until no further sedimentation movement was observed. Data were analyzed in terms of continuous c(s) distributions using the SEADTT program. All accepted fits had r.m.s. deviations less than 0.008. Partial specific volume and buffer parameters were calculated using Sednterp (http://rasmb.org/sednterp/). Sedimentation coefficient distributions were corrected to standard conditions at 20 °C in water, s20w. For the detection of spastin and peptide co-sedimentation, a VGESEESEESEE peptide was synthesized and purified by biosynthesis and then its N terminus was labeled with Atto488 using Atto488-NHS ester (Sigma-Aldrich, no. A1698) according to the manufacturer’s protocol. After labeling, peptides were reverse phase HPLC purified. Experiments were performed for peptide (16.7 μM) alone and spastin (10 μM for the monomer or 1.67 μM for the hexamer) and excess peptide (16.7 μM). The samples were placed in the four-hole AN-Ti rotor and equilibrated thermally in the ultracentrifuge. After thermal equilibrium was reached at 10 °C, the rotor was accelerated to 50,000 r.p.m. and the intensity scans at 280 nm and 483 nm were started immediately and collected until no further sedimentation movement was observed. Data were analyzed in terms of continuous c(s) distributions using the SEDFIT program. All accepted fits had r.m.s. deviations less than 0.008. Partial specific volume and buffer parameters were calculated using Sednterp. Sedimentation coefficient distributions were corrected to standard conditions at 20 °C in water, s20,w. Modeling the 9.9 s peak as a monodisperse species gives statistically equivalent solutions, indicating that the 9.9 s peak is not a mixture of oligomers.

Sample preparation for electron microscopy. SpastinE583Q (1.2 mg ml⁻¹) was incubated on ice for ~20 min in 20 mM HEPES buffer (pH 7.5) containing 300 mM KCl, 10 mM MgCl₂, 5 mM DTT, 0.05% lauryl maltose neopentyl glycol (LMNG) detergent with and without 0.2 μM 1,500–5,500 molecular weight polyglutamate peptide (Sigma-Aldrich). Then 2.5 μl of sample was applied to a 400 mesh carbon-coated copper grid and negatively stained with 1% phosphotungstic acid (pH 6.8). Staining grids were manually blotted with filter paper (Whatman No.1) for ~4 s in a 4 °C cold room before plunge freezing in liquid ethane cooled by liquid nitrogen. Tenfold serial dilutions in 80 mM PIPES pH 6.8, 50 mM KCl, 1 mM EGTA, 2 mM MgCl₂ and 1 mM DTT for 50 mM spastin and point mutants. Initial rates were calculated from the linear portion of the reaction curve after addition of 2 mM ATP. Initial rates were calculated from the linear portion of the reaction curve after addition of 2 mM ATP. ATPase rates were corrected by subtraction of the measured release of phosphate in the absence of ATP. Polyglutamate stimulated ATP hydrolysis rates were measured at room temperature in 20 mM HEPES pH 7.5, 50 mM KCl, 10 mM MgCl₂, and 2 mM ATP at 290 nM spastin and polyglutamate concentrations from 0 to 100 μM. Stock solutions (100 mM) of polyglutamate (0.75–5.0 kDa) was made in water and adjusted to pH 7.5 with potassium hydroxide. Tenfold serial dilutions were made in water and added to the ATPase reaction, before the addition of ATP. Initial rates were calculated from the linear portion of the reaction curve.

ATP hydrolysis assays. Basal ATP hydrolysis rates were measured at room temperature using the EnzChek Phosphate Assay Kit (Thermo Fisher Scientific) in 80 mM PIPES pH 6.8, 50 mM KCl, 1 mM EGTA, 2 mM MgCl₂ and 1 mM DTT for 50 mM spastin and point mutants. Initial rates were calculated from the linear portion of the reaction curve after addition of 2 mM ATP. ATPase rates were corrected by subtraction of the measured release of phosphate in the absence of ATP. Polyglutamate stimulated ATP hydrolysis rates were measured at room temperature in 20 mM HEPES pH 7.5, 50 mM KCl, 10 mM MgCl₂, and 2 mM ATP at 290 nM spastin and polyglutamate concentrations from 0 to 100 μM. Stock solutions (100 mM) of polyglutamate (0.75–5.0 kDa) was made in water and adjusted to pH 7.5 with potassium hydroxide. Tenfold serial dilutions were made in water and added to the ATPase reaction, before the addition of ATP. Initial rates were calculated from the linear portion of the reaction curve.

Atomic model building and refinement. A crystal structure of the Drosophila spastin Walker B domain was used as a starting point (PDB 1Q39P) for modeling. Using Chimera, all loops and coils were deleted, and secondary structural elements were docked into the EM map. After one round of real-space refinement in Phenix, Coot was used to rebuild coils and improve main chain and side chains. The rebuilt model was then used as the input model for a multi-model generating pipeline, which allowed for accessing model quality. Overall, 200 models were generated in Rosetta using the refined map and model, and the top 10 scoring models were selected for further model refinement using Phenix with a per-residue Cα rms deviations. Regions with poor model convergence were remodeled and refined. The Molprobity server (http://molprobity.biochem.duke.edu/) and PDB validation service server (https://validate-rcsb-1.wwpdb.org/) were used to identify problems for subsequent correction in Coot. Residues 502–516 and 613–620 of protomer A are missing in the final model. The polyglutamate peptide was built de novo in Coot.
rates were corrected by subtraction of the measured release of phosphate in the absence of ATP and polyglutamate.

**Microtubule severing assays.** Microtubules were polymerized from 2 mg ml\(^{-1}\) bovine cytidylated brain tubulin (PurSolutions), 4% tetramethyl rhodamine labeled and 1% biotin-labeled porcine brain tubulin (Cytoskeleton). Microtubules were double-cycled with the slow-hydrolyzable GTP analog guanylyl (α,β)-methylene diphosphonate. The first polymerization was 1 h and the second polymerization double-cycled with the slow-hydrolyzable GTP analog guanylyl (α,β)-methylene diphosphonate. The microtubules were spun down at 126,000 g for 1 h and the second polymerization was subjected to a Mann–Whitney statistical test. N is reported for all experiments in figure legends. Data in Fig. 4b,c were subjected to a Mann–Whitney statistical test. Quantification and data analysis. Image analysis was carried out in Fiji. Prism (Graphpad Inc.) was used for graphing and statistical analysis.

**Homology modeling.** A human spastin hexamer homology model was generated using the online server SWISS-MODEL84 (https://swissmodel.expasy.org/). Coordinates for the homology model are available as Supplementary Data Set 1.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Electron microscopy map and the top scoring model of five atomic models obtained from an EM multi-model pipeline have been deposited at the Electron Microscopy Data Bank and Protein Data Bank under accession numbers EMD-20226 and PDB 6P07, respectively. All data used in this study are available from the corresponding authors upon reasonable request.

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