

Supplemental Table S1. Structure Determination Statistics

Supplemental Table S2. Pairwise comparison of meiotic clade AAA unfoldases. Percent sequence identities (<https://web.expasy.org/sim>) are shown for the sequence alignments of Figure 2E. Root mean square deviations between pairs of C α atoms (below) following overlaps of the five ATPase cassettes of subunits A-E treated as one rigid body. The number of residue pairs in the overlaps are indicated in parenthesis.

Supplemental Figure S1. Cryo-EM and 3D reconstruction. *A*, Representative electron cryomicrograph showing a homogeneous and monodisperse distribution of particles. *B*, The 19 2D classes used for *ab initio* model generation and 3D classification. Box size 256px (~242 Å) using a 160 Å circular mask. *C*, FSC of independent reconstructions using separate (odd:even particles) halves of the data. *D*, FSC between the refined model and the density map. *E*, Plot of angular distribution of particles used in final refinement. *F*, Color bar indicates local map resolution. *G*, Representative density and refined model for the β -sheet of subunit B (left) and the α -helix ($\alpha 5$) following the pore loop 2 residues of subunit D (right).

Supplemental Figure S2. Flow chart of 3D reconstruction. 2D classification filtered the 1,021,617 picked particles to 438,336 particles for 3D classification. Just under 30% (129,402) of those particles sorted into a class with good features, which was used to generate a 3D reconstruction at 4.3 Å resolution. Per particle CTF refinement and motion correction, and a final round of 3D classification, yielded a final reconstruction from 119,984 particles at 4.2 Å resolution after B-factor sharpening (-147 Å²).

Supplemental Figure S3. Subunit structure. The structure of a representative subunit (B) is shown with the secondary structural elements and sequence motifs labeled, as in Figure 2E.

Supplemental Figure S4. Katanin binds peptide in an identical fashion to spastin. Katanin spiral structure density (EMD-8794). Peptide and pore loop 1 residues were not modeled in the katanin structure determination (24). They are shown here for human spastin (colors) and *D. melanogaster* spastin (gray) following overlap on the large ATPase domains of subunits A-E. Human spastin subunit F, which is displaced from the spiral configuration, is not shown.

Supplemental Video S1. Refined model with cryo-EM map. Density is shown at 6.8 x sigma for the overall structure and the views of each individual subunit. For subunit F (magenta) the contour level is lowered to 4.4 x sigma.

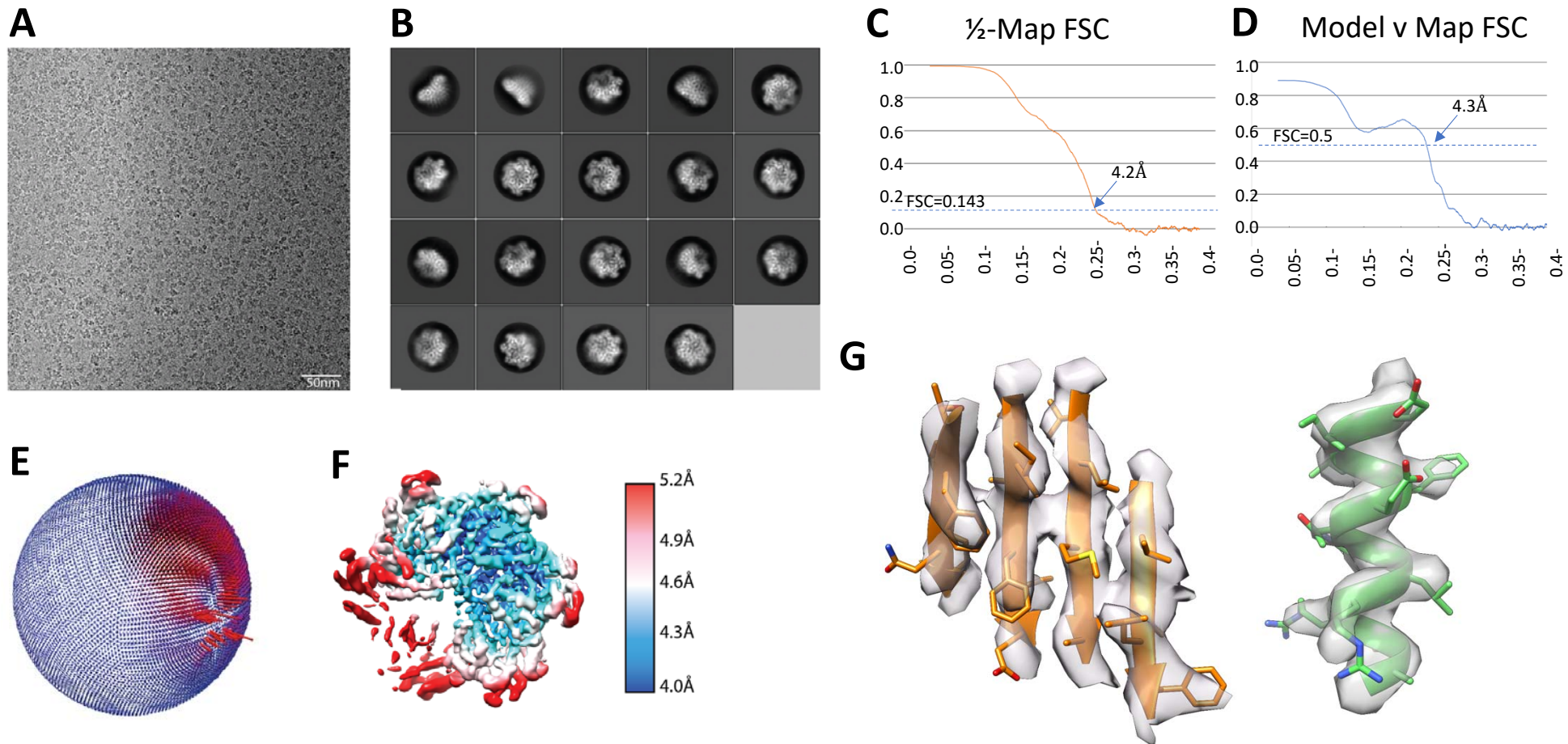
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Reconstruction	
Particle Images	119984
Resolution (unmasked, Å)	4.3
Resolution (masked, Å)	4.2
Map Sharpening B-factor (Å²)	-147
EM Databank Accession Number	EMD-20327
Refinement and Validation of Spastin Subunits A-E	
Resolution used for refinement (Å)	4.2
Number of Atoms	11106
RMSD bond length (Å)	0.003
RMSD bond angles (°)	0.427
Phi/psi favored (%)	99.21
Phi/psi allowed (%)	0.79
Phi/psi outlier (%)	0.00
Rotamer outliers (%)	0.00
Molprobity score / percentile (%)	1.21/100
Clashscore / percentile (%)	4.34/100
EMRinger score	0.82

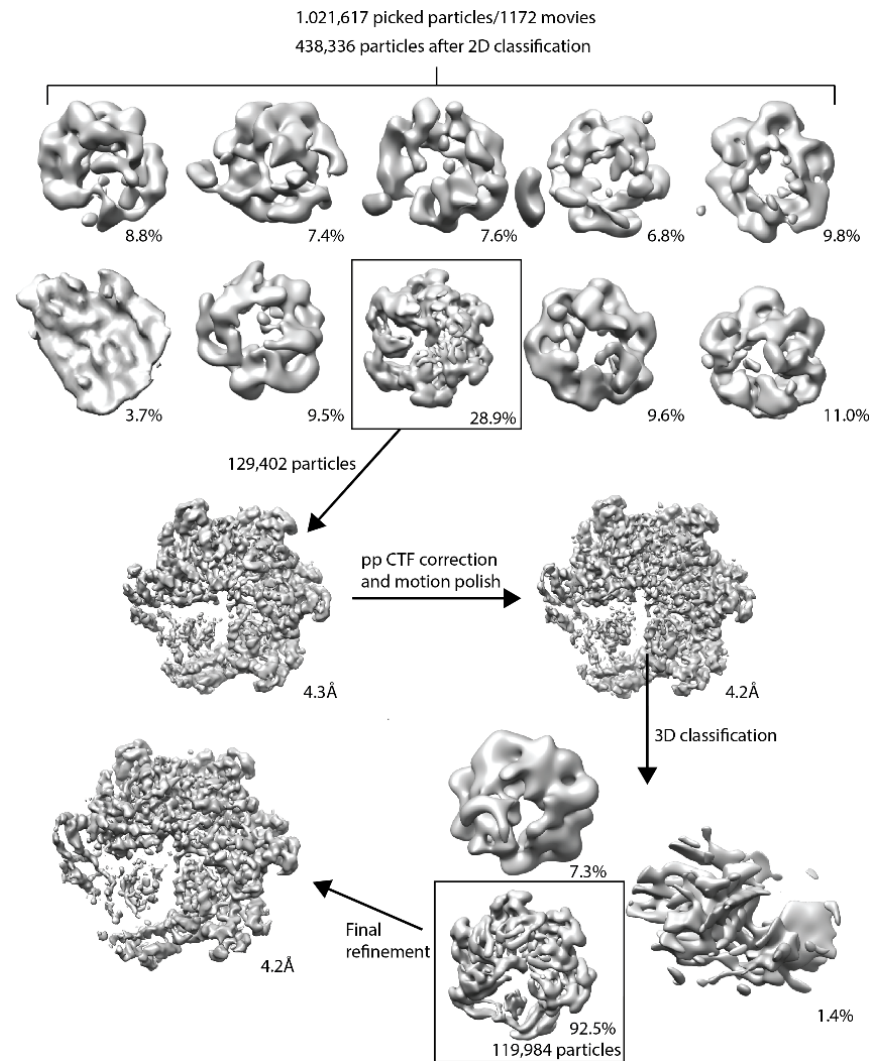
Supplemental Table S2

	<i>D.m.</i> Spastin	<i>C.e.</i> Katanin	<i>S.c.</i> Vps4
<i>H.s.</i> Spastin	76% 2.8 Å (1100)	46% 3.1 Å (1120)	50% 1.7 Å (1175)
<i>D.m.</i> Spastin		48% 2.9Å (1040)	48% 3.2Å (1100)
<i>C.e.</i> Katanin			50% 3.2Å (1120)

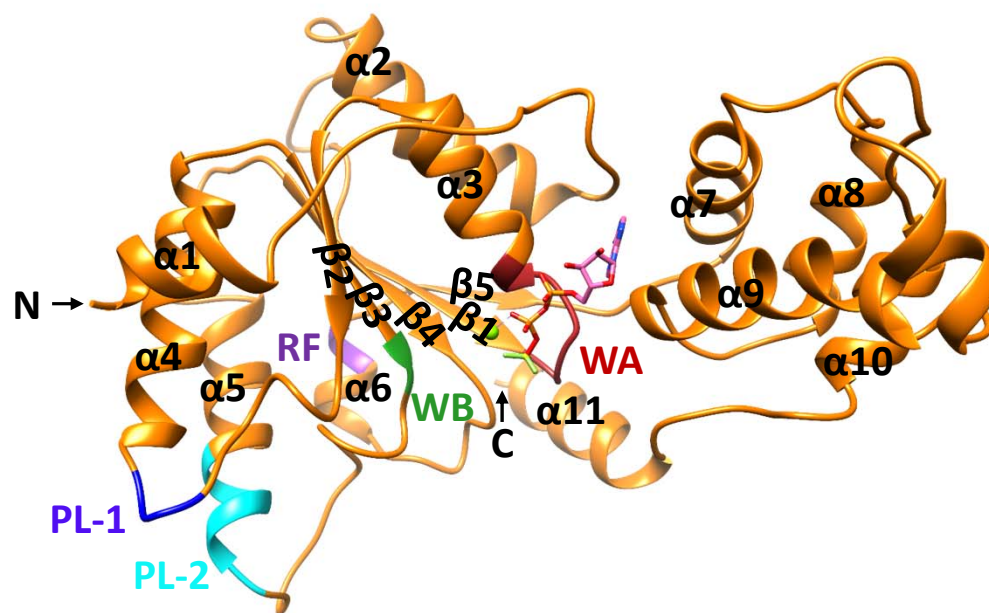
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4

