Supplementary Materials

Molecular Biology of the Cell

Wolff *et al*.

Supplemental Materials

Acentrosomal spindle assembly and maintenance in *C. elegans* oocytes requires a kinesin-12 non-motor microtubule interaction domain

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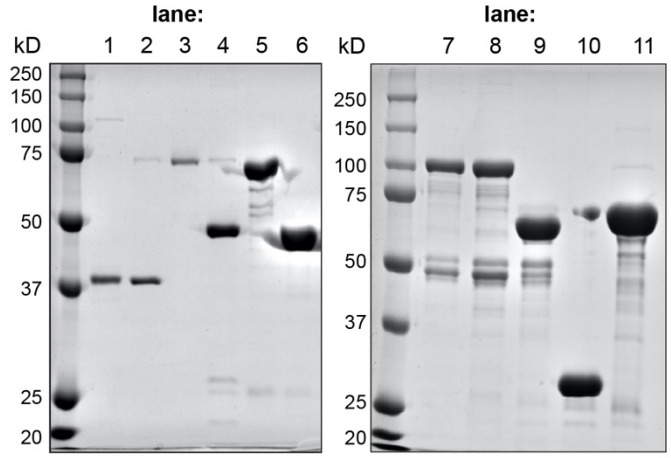
3 Supplemental Figures and Legends

2 Supplemental Video Legends

Figure S1

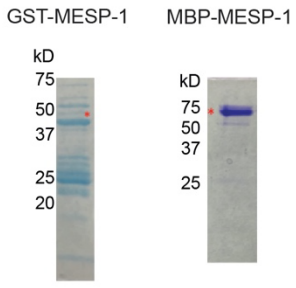
A



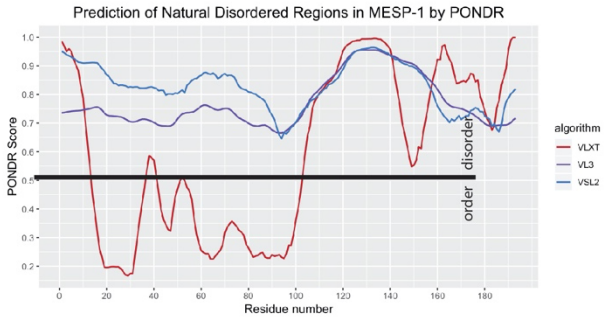
lane	protein	residues	tag	M.W.	plasmid	exp time / temp
1	N-stalk	329-634	C-term 6xHis	35kD	pIW9	16°C, overnight
2	C-stalk	635-932	C-term 6xHis	35kD	pIW10	16°C, overnight
3	stalk	329-932	C-term 6xHis	70kD	pIW1	30°C, 4 hours
4	Δhinge	329-559, 770-932	C-term 6xHis	47kD	pIW8	16°C, overnight
5*	MBP-MESP-1	full length	N-term 6xHis-MBP	64kD	pIW11	18°C, overnight
6	MBP	full length	N-term 6xHis	42kD	pET MBP	18°C, overnight
7	GFP-stalk	329-932	N-term 6xHis-GFP	98kD	pIW6	16°C, overnight
8	GFP-*klp-18ts*	329-932 (V854M, G876S)	N-term 6xHis-GFP	98kD	pIW12	16°C, overnight
9	GFP-ΔCstalk	329-634	N-term 6xHis-GFP	63kD	pIW13	16°C, overnight
10	GFP	full length	-	28kD	addgene:#29663	16°C, overnight
11**	MBP-MESP-1	full length	N-term 6xHis-MBP	64kD	pIW11	18°C, overnight

* purification used in experiments without GFP
** purification used in experiments with GFP (Figure 3D-E)

B



C



D

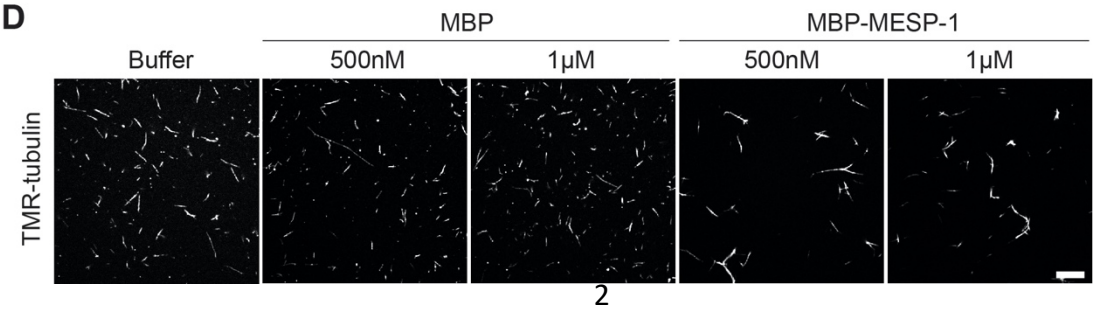


Figure S1: Protein expression and purification details.

a) SDS-PAGE gels stained with Coomassie showing purifications of all proteins used. For purification details, see Materials and Methods. Protein of interest is major band in each lane. Each purification was confirmed by Western Blot probed with an antibody against protein of interest. The corresponding residues, affinity tag(s), molecular weight (M.W.), plasmid name, and expression time / temperature (after 0.1mM IPTG induction) for each protein are shown in the chart. The purification of MBP-MESP-1 shown in lane 5 was used in Figures S1D, 3A, 3B, and the purification shown in lane 11 was used in the GFP experiments in Figure 3D and 3E; the conditions for these purifications were the same but they were performed on different days.

b) Representative purifications shown for GST- and MBP-tagged MESP-1. GST-MESP-1 and MBP-MESP-1 band marked by red asterisk. GST-MESP-1 shows more degradation during purification than MBP-MESP-1.

c) Prediction of disordered regions within MESP-1 using PONDR (Xue *et al.*, 2010). PONDR score for three algorithms (VXLT (red), VL3 (purple), and VSL2 (blue)) shown for each residue. Residues with PONDR scores above the black line (> 0.5) are predicted to be disordered.

d) MBP-MESP-1 microtubule binding activity tested by microtubule bundling assay. Representative images of TMR-microtubules incubated with buffer alone, MBP, and MBP-MESP-1. Scale bar = 10μm.

Figure S2

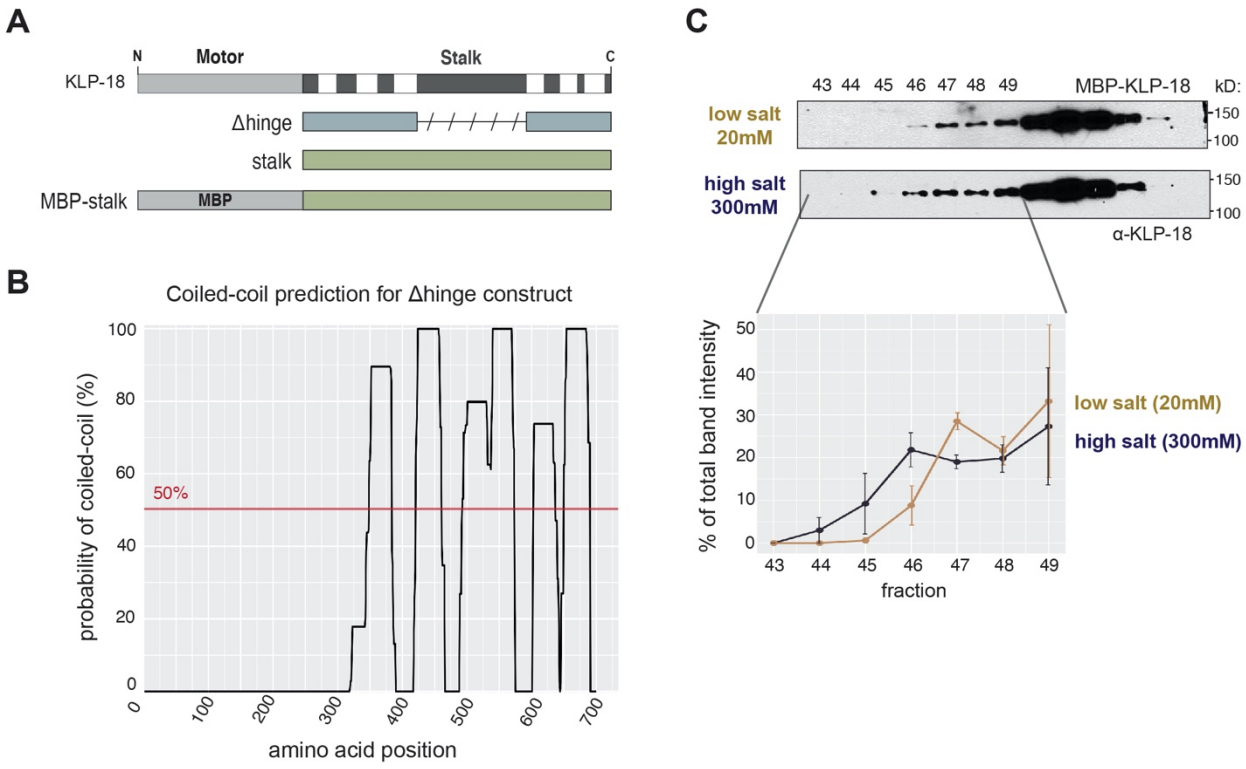


Figure S2: KLP-18 stalk contains a putative hinge region.

a) Schematics of Δhinge, full length stalk, and MBP-stalk constructs. Slanted dashed lines show area deleted in Δhinge.

b) Paircoil2 prediction for Δhinge construct with putative hinge deleted. Δhinge is predicted to be completely coiled-coil and therefore presumably rigid (compare to full-length stalk in Figure 1A).

c) KLP-18 stalk flexibility tested in size exclusion chromatography experiment. MBP-stalk applied to a size exclusion column in high salt (300mM, blue) and low salt (20mM, gold) buffer. Indicated fractions probed for KLP-18 in a Western blot then quantified. Mean +/- sd of percent of total band intensity is shown. High salt: n = 3 over 2 purifications, low salt: n = 2 over 1 purification.

Figure S3

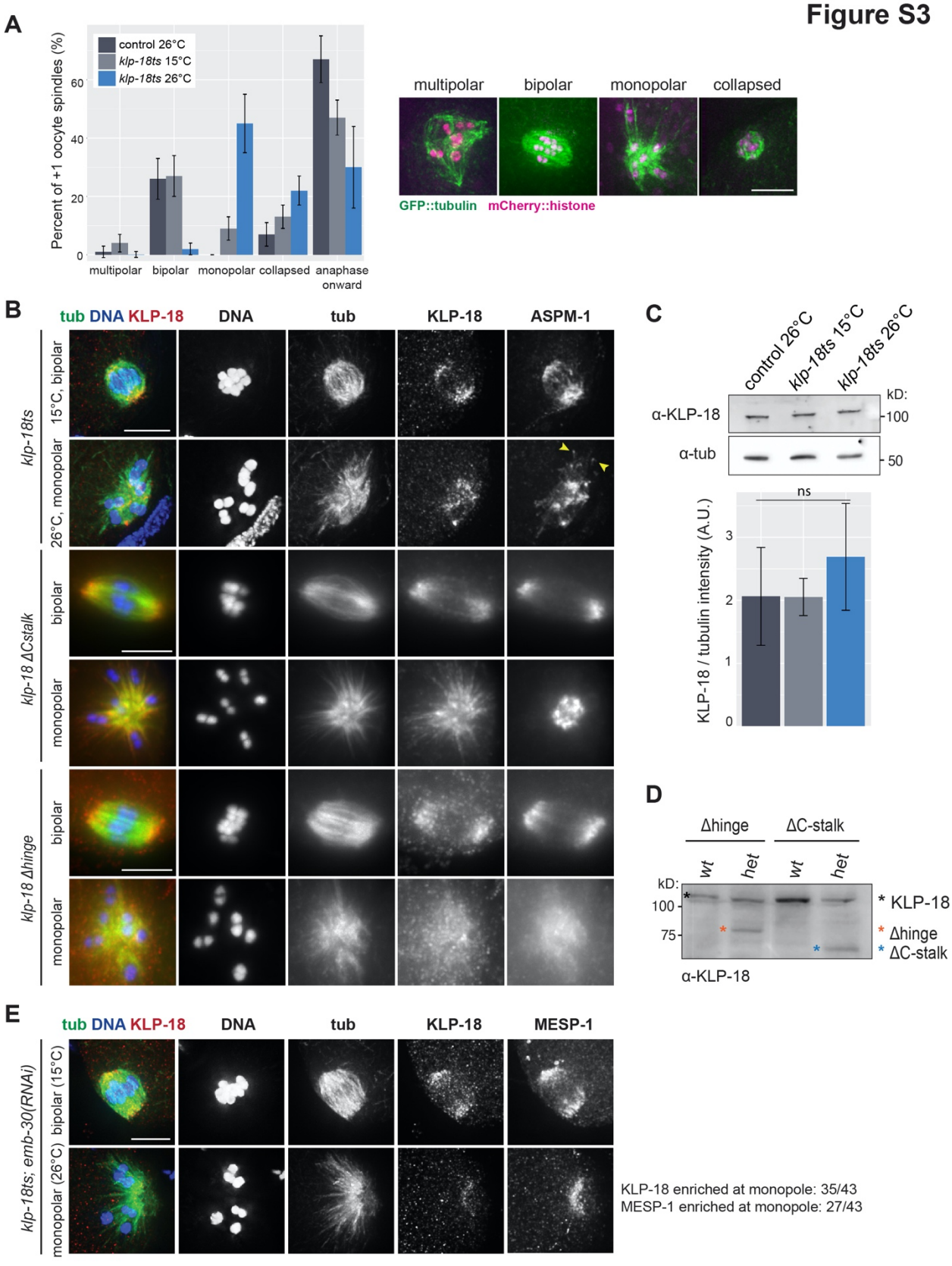

A
control 26°C
klp-18ts 15°C
klp-18ts 26°C
Percent of +1 oocyte spindles (%)
multipolar
bipolar
monopolar
collapsed
anaphase onward
multipolar
bipolar
monopolar
collapsed
GFP::tubulin
mCherry::histone
B
tub DNA KLP-18
DNA
tub
KLP-18
ASPM-1
klp-18ts
15°C, bipolar
26°C, monopolar
klp-18 ΔCstalk
bipolar
monopolar
klp-18 Δhinge
bipolar
monopolar
C
control 26°C
klp-18ts 15°C
klp-18ts 26°C
kD:
α-KLP-18
α-tub
100
50
ns
KLP-18 / tubulin intensity (A.U.)
D
Δhinge
ΔC-stalk
wt
het
wt
het
kD:
100
75
* KLP-18
* Δhinge
* ΔC-stalk
α-KLP-18
E
tub DNA KLP-18
DNA
tub
KLP-18
MESP-1
klp-18ts; emb-30(RNAi)
bipolar (15°C)
monopolar (26°C)
KLP-18 enriched at monopole: 35/43
MESP-1 enriched at monopole: 27/43

Figure S3: KLP-18 mutant strains express truncated protein and result in monopolar spindles.

a) Oocyte spindle morphology in the most recently fertilized (+1) embryo of control (dark gray) and *klp-18ts* worms expressing GFP::tubulin and mCherry::histone. During normal spindle assembly, minus ends are sorted away from chromosomes, to form multiple poles ("multipolar") that coalesce to form a bipolar spindle ("bipolar") (Wolff *et al.*, 2016). If minus ends are not sorted outwards, a monopolar spindle forms with chromosomes arranged in a rosette ("monopolar") (Segbert *et al.*, 2003; Wignall and Villeneuve, 2009; Connolly *et al.*, 2014; Wolff *et al.*, 2016); these chromosomes then move inwards in anaphase ("collapsed") (Muscat *et al.*, 2015). Spindle morphology in *klp-18ts* was quantified at permissive (15°C, light gray) and restrictive (26°C, blue) temperatures; spindles that had entered anaphase (with two sets of separated chromosomes) and Meiosis II spindles were grouped together as "anaphase onwards". Note that this data is from the same quantification depicted in Figure 2B, but in this case all categories are represented, including spindles that had progressed to anaphase (in Figure 2B the "anaphase onwards" category and the "collapsed" category, representing monopolar anaphase, were excluded to emphasize the spindle assembly phenotype). Bars represent mean percentage +/- sd. For *klp-18ts* conditions, n = 4 experiments; for control, n = 3 experiments.

b) DNA (blue), tubulin (green), KLP-18 (red), and ASPM-1 (not in merge) localization in *klp-18(or447ts)*, *klp-18ΔC-stalk*, and *klp-18Δhinge* mutant worms. Representative images of wild type bipolar and mutant monopolar spindles shown for each strain. In the *klp-18ts* mutant, there are some ASPM-1 foci on the outside of the monopolar spindle (arrowheads), suggesting that there is weak microtubule sorting activity in this mutant that allows some minus ends to be pushed outwards. Images for *klp-18ts* are deconvolved to better show ASPM-1 foci on the outside of the aster; other images are not deconvolved. Scale bars = 5μm.

c) Western blot of control (dark gray) and *klp-18ts* worms at permissive (15°C, light gray) and restrictive (26°C, blue) temperature probed with an anti-KLP-18 antibody. Representative blot (top) with quantification of normalized KLP-18 band intensity (below, mean +/- sd). Quantified KLP-18/tubulin intensity was not significantly different between any of the conditions over n = 3 experiments ($p > 0.05$, paired one-tail Student's t-Test).

d) Western blot of progeny from homozygous wild type ('wt') and heterozygous mutant ('het') *klp-18Δhinge* or *klp-18ΔC-stalk* parents probed with an anti-KLP-18 antibody. Wild type (black), *Δhinge* (orange), and *ΔC-stalk* (blue) bands indicated by asterisks.

e) DNA (blue), tubulin (green), KLP-18 (red), and MESP-1 (not in merge) localization in *klp-18ts* worms at 15°C and 26°C; *emb-30(RNAi)* was used to induce metaphase I arrest. These images are deconvolved. Quantification shown to the right of the images; for details on how enrichment was defined see “Figure Quantification” section of Materials and Methods. Scale bar = 5μm.

SUPPLEMENTAL VIDEO LEGENDS

Video S1. Metaphase I-arrested oocyte spindle shortens and then forms a monopole upon temperature shift in the *klp-18(or447ts)* mutant.

Representative movie of spindle reorganization in the *klp-18 (or447ts)* mutant upon temperature shift; corresponds to Figure 4B. Chromosomes (mCherry::histone, magenta) and microtubules (GFP::tubulin, green) visualized in an *emb-30(RNAi) klp-18(or447ts)* oocyte dissected then shifted to the restrictive temperature. Scale bar = 10μm.

Video S2. Control Metaphase I-arrested oocyte spindle maintains bipolarity.

Representative movie of a Metaphase I-arrested control spindle; corresponds to Figure 4B. Chromosomes (mCherry::histone, magenta) and microtubules (GFP::tubulin, green) visualized in an *emb-30(RNAi)* oocyte dissected then shifted to the restrictive temperature. Scale bar = 10μm.

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