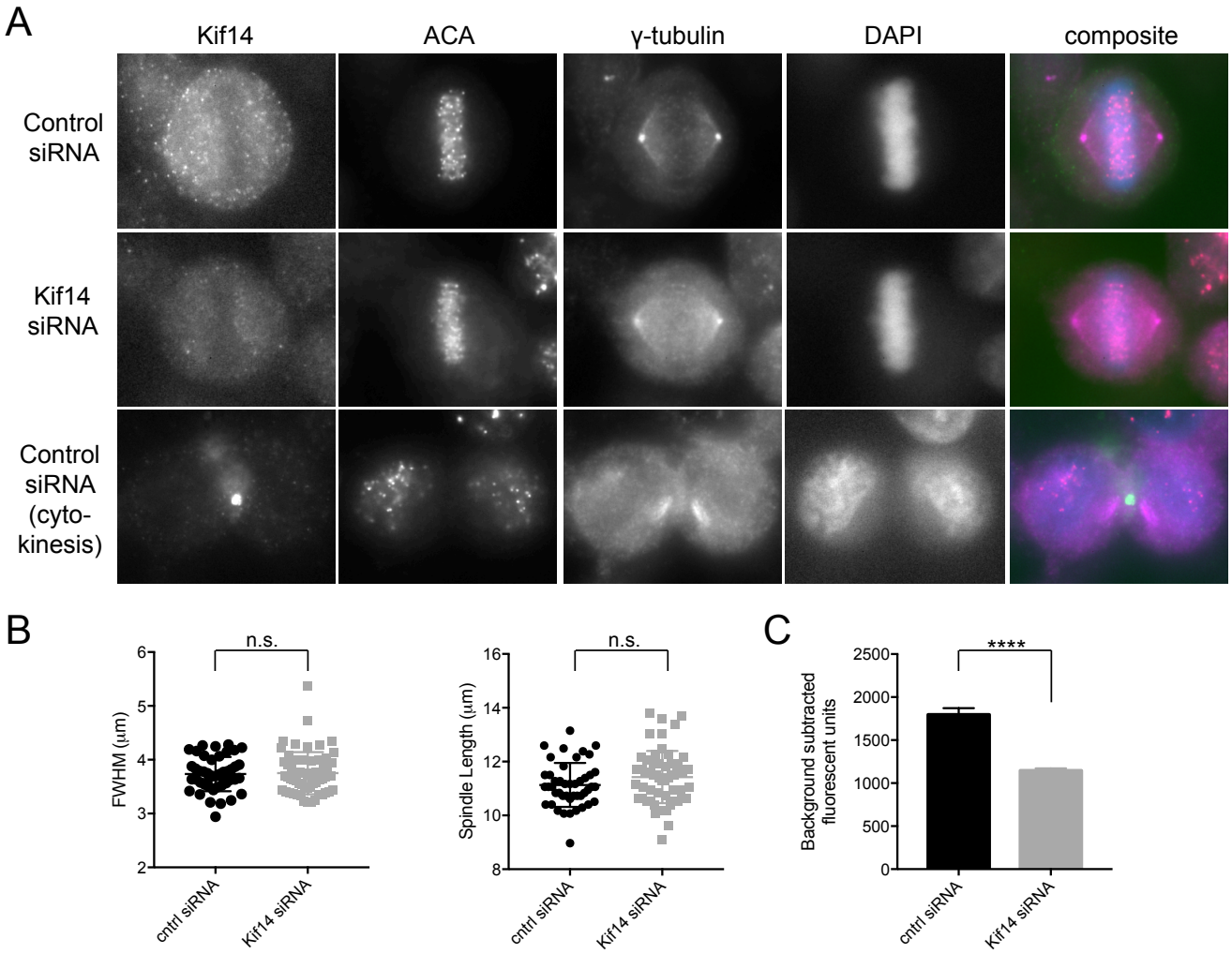


Figure S1. Cells deficient in Kif14 do not effect chromosome alignment or spindle length in metaphase. (**A**) Metaphase cells with endogenous Kif14 fluorescent antibody staining for control or Kif14 siRNA treatments. (**B**) Quantification of FWHM, spindle length, and background subtracted fluorescent intensity for both knockdown conditions. n.s., not significant; ****, $p < 0.0001$ by unpaired t test. Data obtained from two independent data sets with the following cell numbers: control siRNA (45), Kif14 siRNA (60).

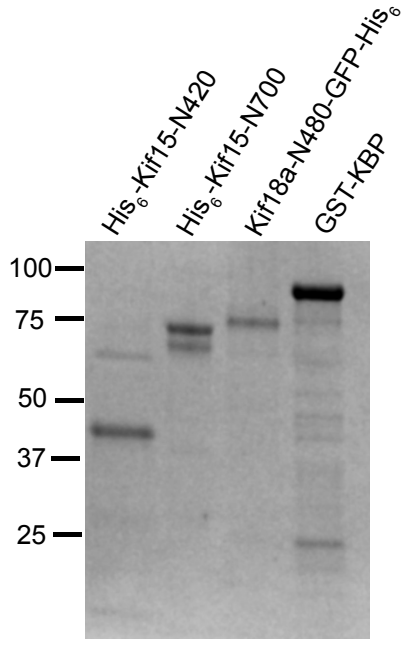
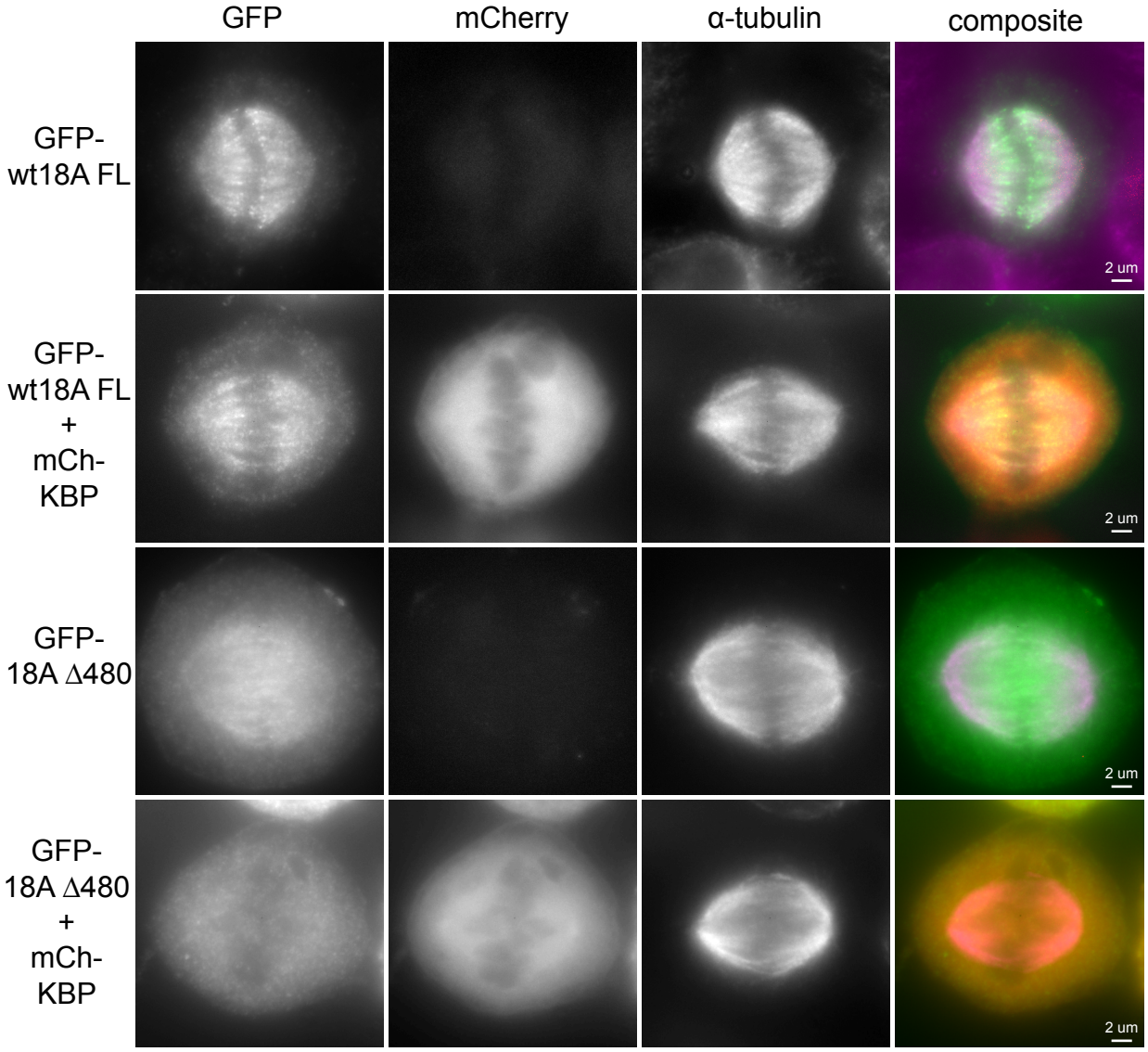


Figure S2. Purification of kinesin and KBP proteins. Coomassie stained gel of His_6-Kif15-N420 (MW = 45 kDa), His_6-Kif15-N700 (MW = 76 kDa), Kif18a-N480-GFP-His_6 (MW = 82 kDa) and GST-KBP (MW = 97 kDa). Molecular weight markers are indicated in kDa.

A


GFP
mCherry
α-tubulin
composite
GFP-
wt18A FL
GFP-
wt18A FL
+
mCh-
KBP
GFP-
18A Δ480
GFP-
18A Δ480
+
mCh-
KBP
2 um

B

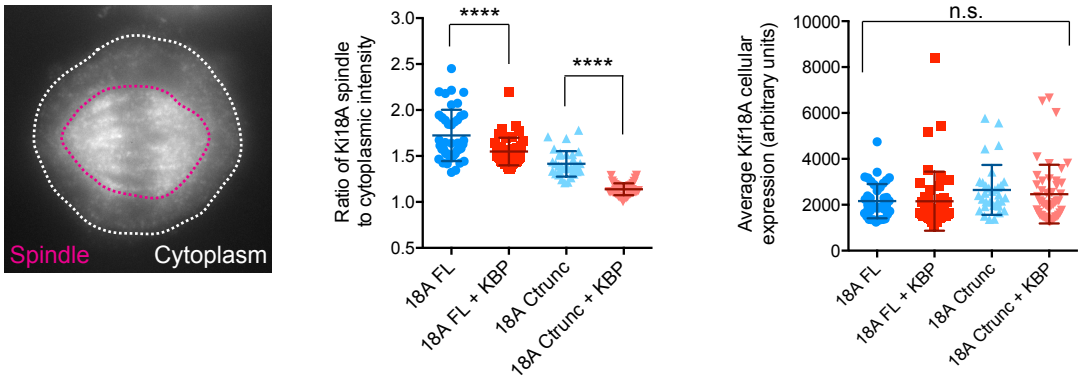

Spindle
Cytoplasm
Ratio of Ki18A spindle
to cytoplasmic intensity
3.0
2.5
2.0
1.5
1.0
0.5
****
****
18A FL
18A FL + KBP
18A Ctrunc
18A Ctrunc + KBP
Average Kif18A cellular
expression (arbitrary units)
10000
8000
6000
4000
2000
0
n.s.
18A FL
18A FL + KBP
18A Ctrunc
18A Ctrunc + KBP

Figure S3. Kif18A's C-terminal microtubule binding domain maintains spindle localization upon KBP overexpression. (**A**) Metaphase cells transfected with GFP-tagged Kif18A full length (FL) or C-terminally truncated GFP-tagged Kif18A (Δ480) with or without mCh-KBP. (**B**) (l*eft*) Pictoral definition of areas used to define spindle and cytoplasmic GFP Kif18A fluorescent signal intensity. (*middle*) Quantification of background subtracted spindle to cytoplasmic ratio determined for each cell measured in each condition. ****, adjusted $p < 0.0001$ with 95% confidence interval by one-way ANOVA with Tukey's multiple comparisons test. (*right*) Quantification of background subtracted average fluorescent intensity measured for each cell in each condition. n.s., not significant with 95% confidence interval by one-way ANOVA with Tukey's multiple comparisons test. Data obtained from three independent data sets with the following cell numbers: GFP Kif18A FL (41), GFP Kif18A FL + mCh KBP (47), GFP Kif18A Δ480 (32), GFP Kif18A Δ480 + mCh KBP (47).