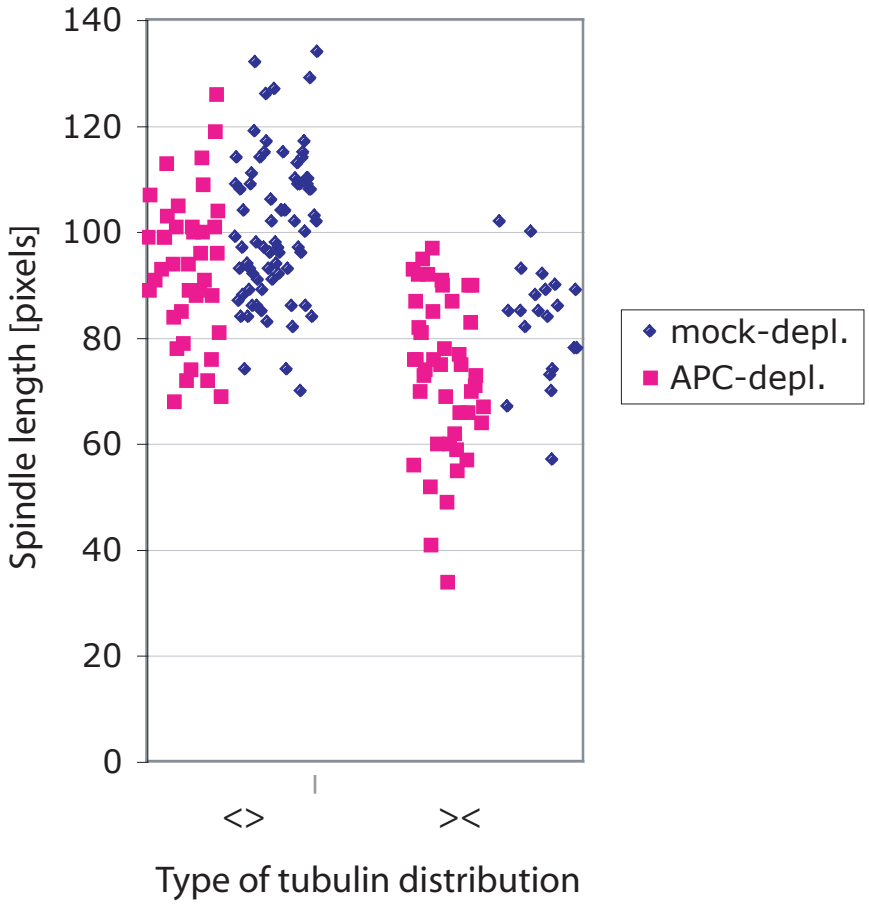
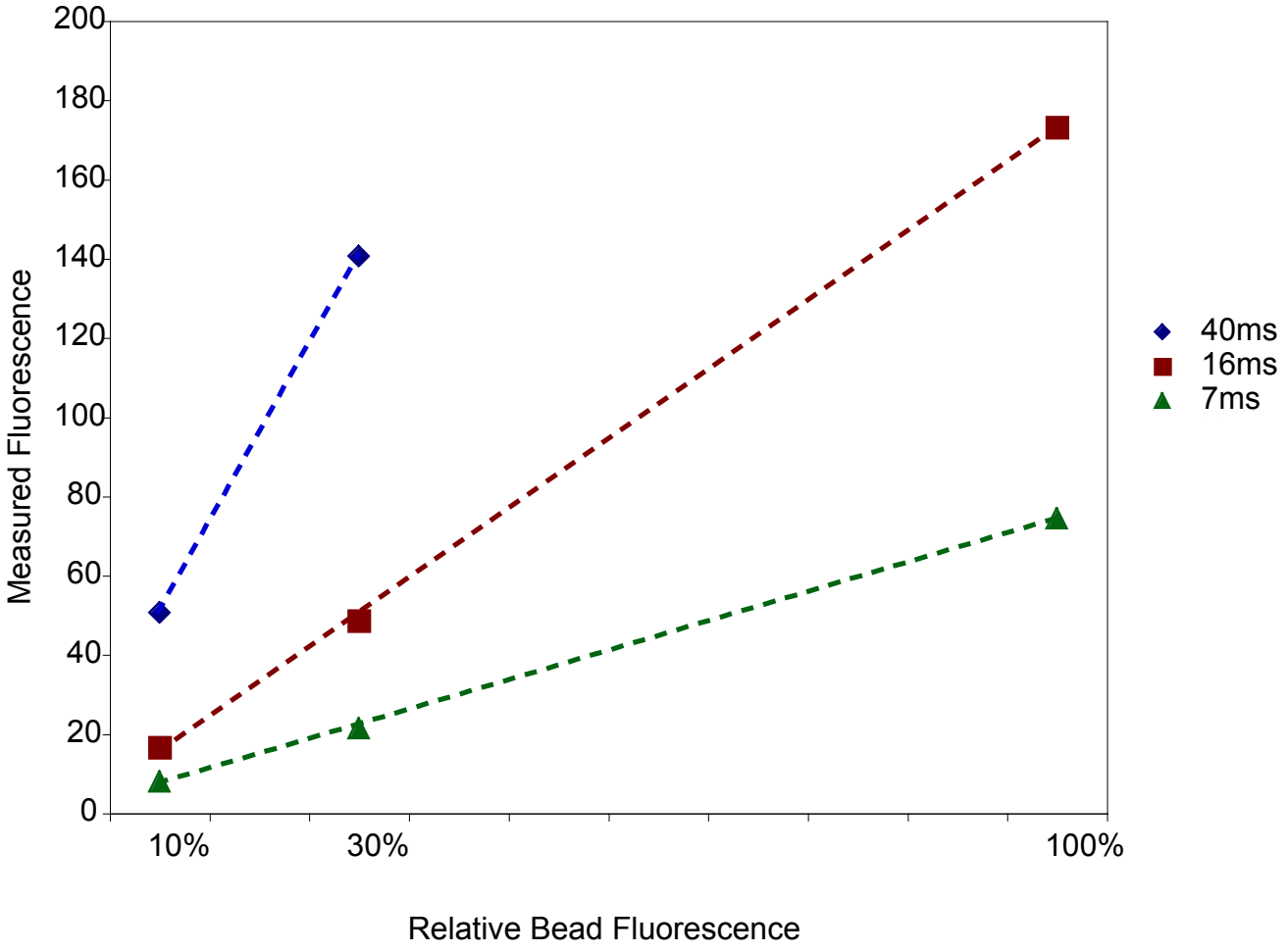
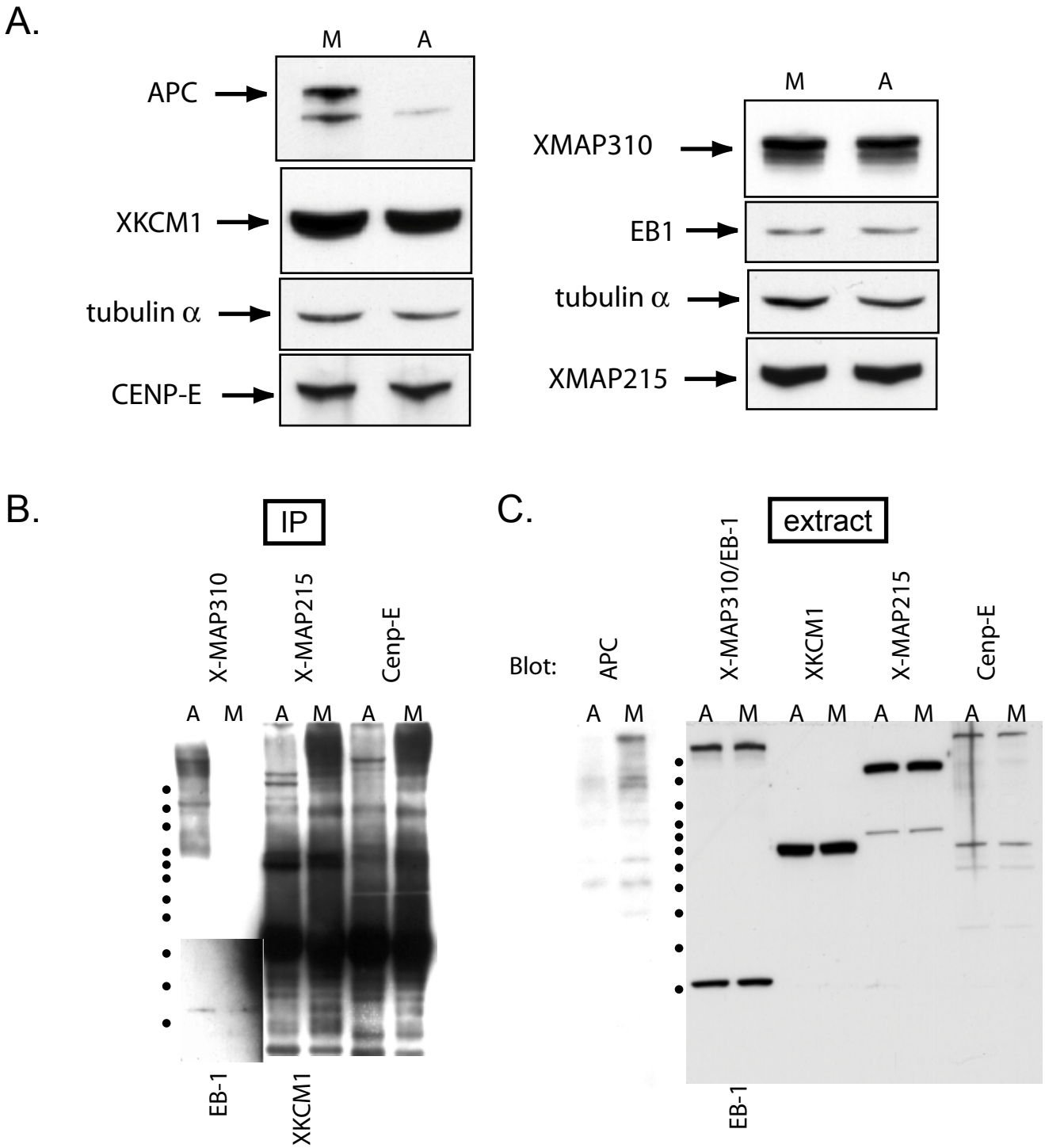
Correlation between microtubule mass distribution and spindle length



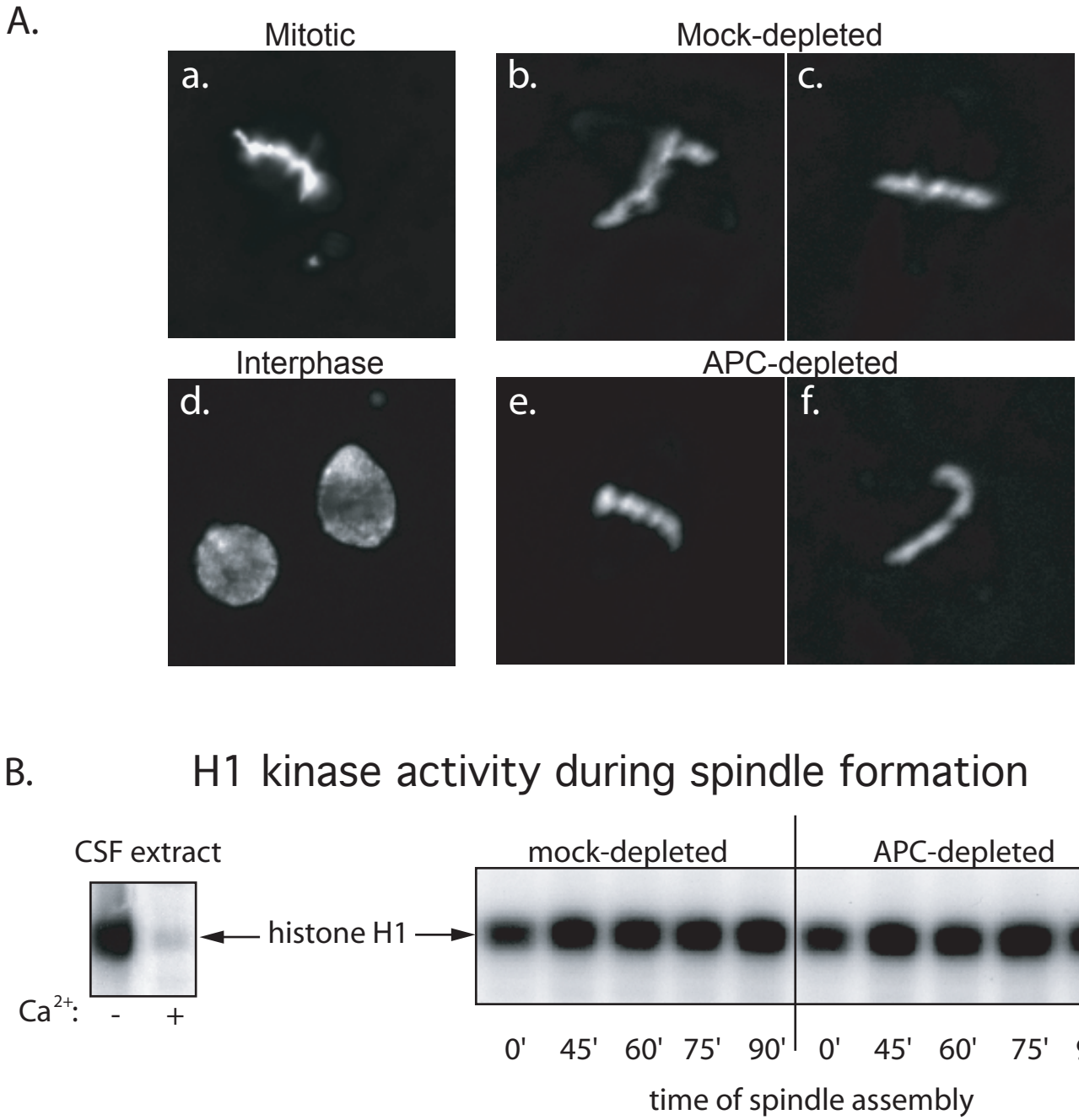
Supplemental Figure 1:
Spindles formed in mock- and APC-depleted extracts supplemented with rhodamine-labelled tubulin were imaged and analysed as described in Figure 3c. Spindles were classified into two groups based on the tubulin distribution along the pole-to-pole axis: one group had more tubulin in the central region (<>) whereas the other type contained more tubulin at the poles (><). These categories correspond to the typical distribution of tubulin in strong and weak spindles respectively. The pole-to-pole distance (spindle length) was then plotted for spindles in each groups for mock- and APC-depleted spindles.
Note that spindles with the tubulin distribution that is associated with weak spindles tended to be shorter and these spindles were more abundant in APC-depleted extracts. This establishes a correlation between two parameters used to describe the spindle phenotype: length and distribution.



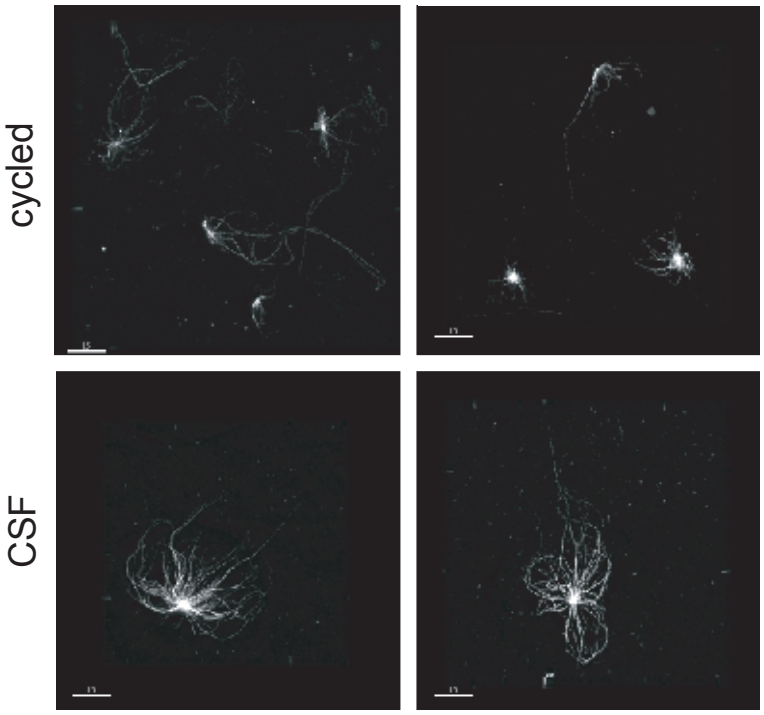
Supplemental Figure 2:
Beads with known fluorscence content were imaged and processed identically to spindles as described in the methods. The calculated average intensities were plotted against the true relative fluorscence of the beads for three different exposure times.
The data show that below saturation of the camera (which is reached for 100% beads at 40ms exposure time), there is a linear relationship between the measured and true fluorescence. This experiment confirmed that manipulations and transfers of spindle images did not affect the relative intensity values we recorded.



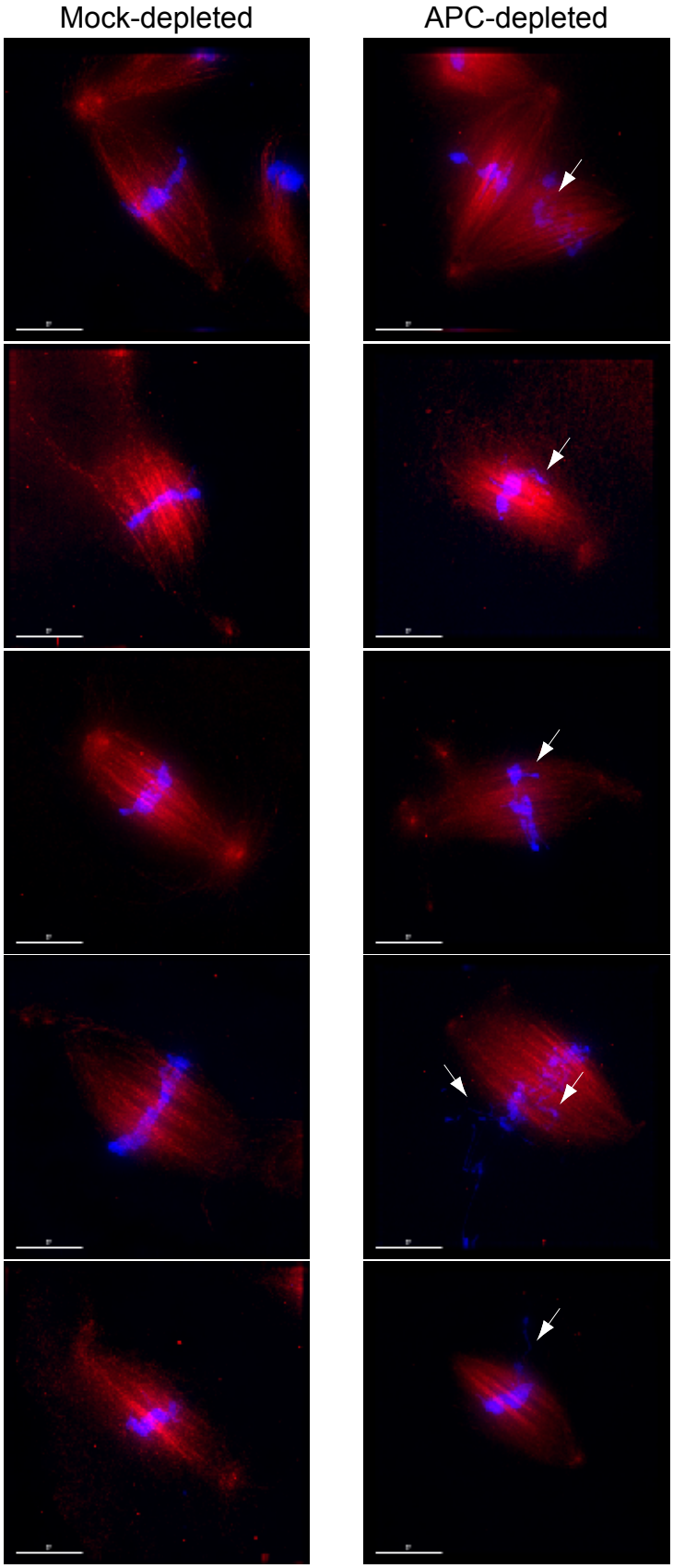
Supplemental Figure 3:
A. APC- (A) and mock-depleted (M) extracts were separated on 4-12 % gels and probed with the indicated antibodies after transfer to Nitrocellulose membranes to show that depleting APC did not co-deplete other proteins important in spindle assembly.
B. Material that co-immunoprecipiated with the APC antibody (A) or rabbit immunoglobulin (M) was probed with the same panel of antibodies to reveal that minute quantities of XMAP310, XMAP215, XKCM1, and CenpE were immunoprecipitated with APC, however, the total amount of these proteins was not changed after APC depletion as shown in C. which shows the corresponding extracts after APC- or mock-depletion.



Supplemental Figure 4.
A. Images of chromatin in control (a), mock- (b, c) or APC-depleted (e, f) mitotic or interphase (d) CSF extracts to show that the gross morphology of chromatin was not altered after APC depletion. B. Samples of spindle assembly reactions in mock- or APC-depleted CSF extracts were collected at indicated time points and assayed for the activity of mitotic kinase toward histone H1. Phosphorylation of histone H1 is shown in the autoradiograph. There was no detectable difference between the two types of extracts confirming that altered spindle morphology upon APC depletion could not be attributed to premature exit from mitosis. The control on the left shows the relative levels of H1 kinase activity in mitotic extract and the same extract 40 minutes after exit from mitosis was induced by the addition of Calcium.



Supplemental Figure 5:
Centrosomes were added to cycled and CSF extracts supplemented with rhodamine tubulin and asters collected after 10 minutes by centrifugation through a glycerol cushion onto coverslips. Asters in cycled extracts were significantly smaller and less abundant than in CSF extracts. Bars represent 15μm.



Supplemental Figure 6:
Examples of spindles assembled in mock- and APC-depleted cycled extracts show that chromosomes are not as well aligned in spindles formed in the absence of APC. In most mitotic spindles in APC-depleted extracts some portion of the chromsomes is not aligned with the rest, however, the bulk of the DNA forms a typical metaphase plate.