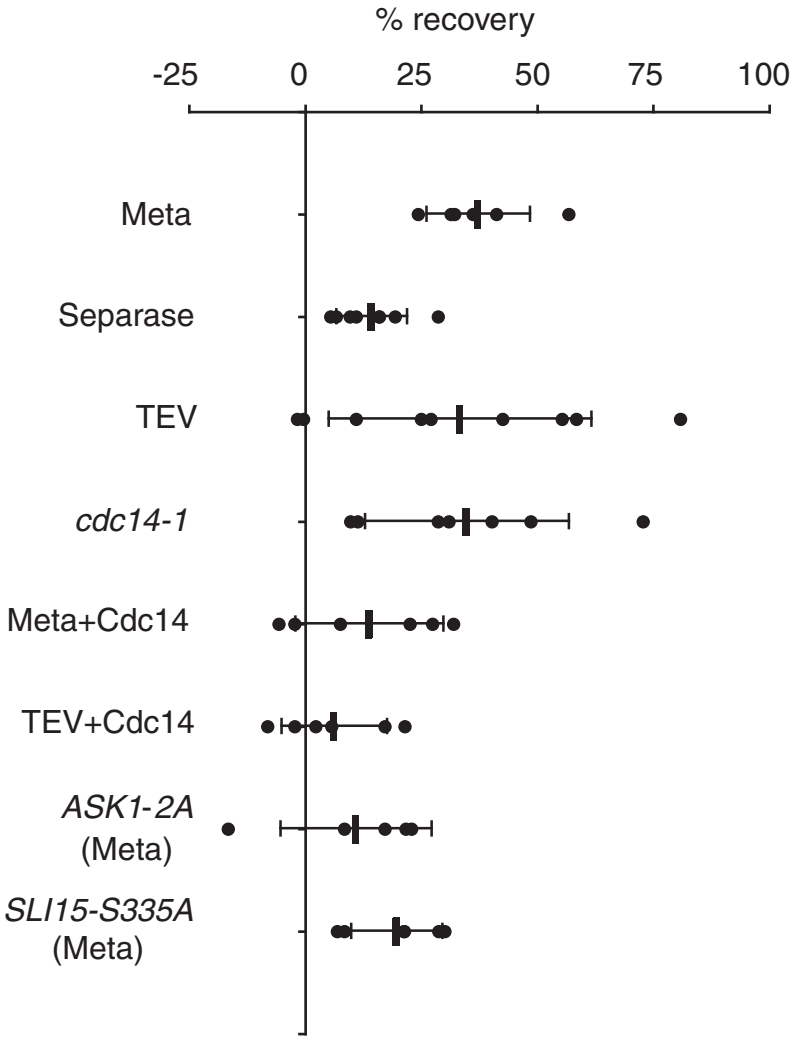
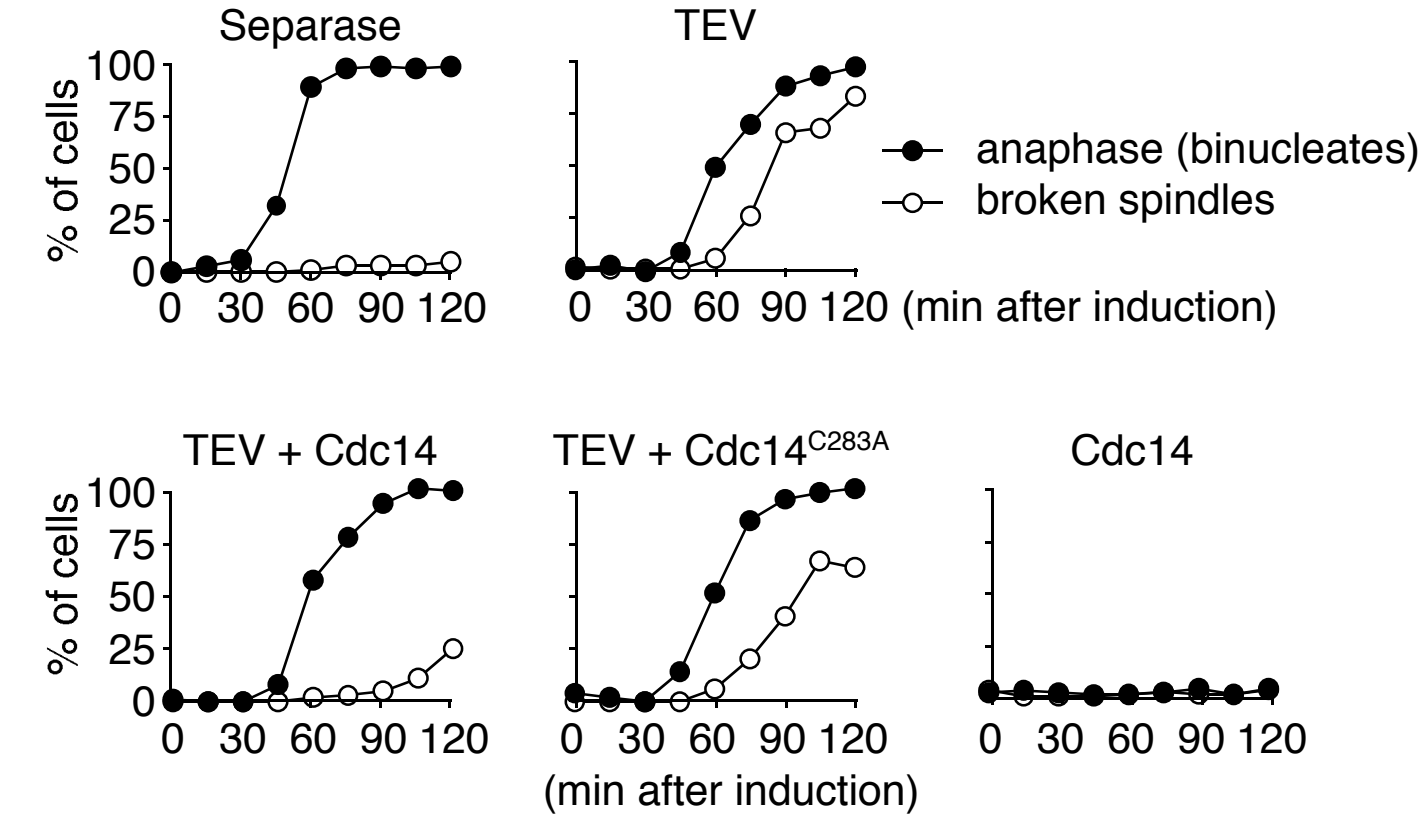
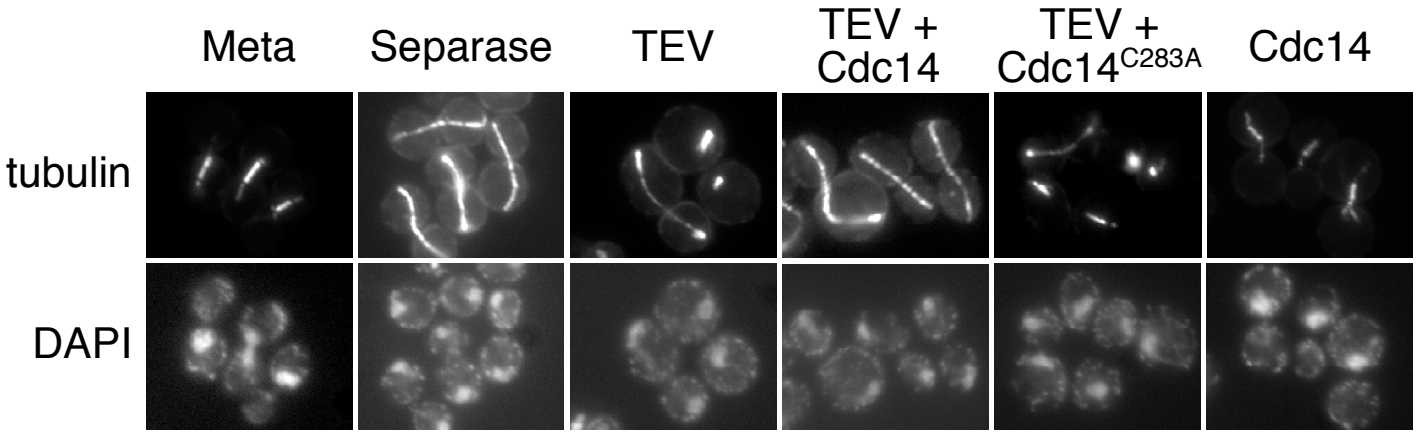


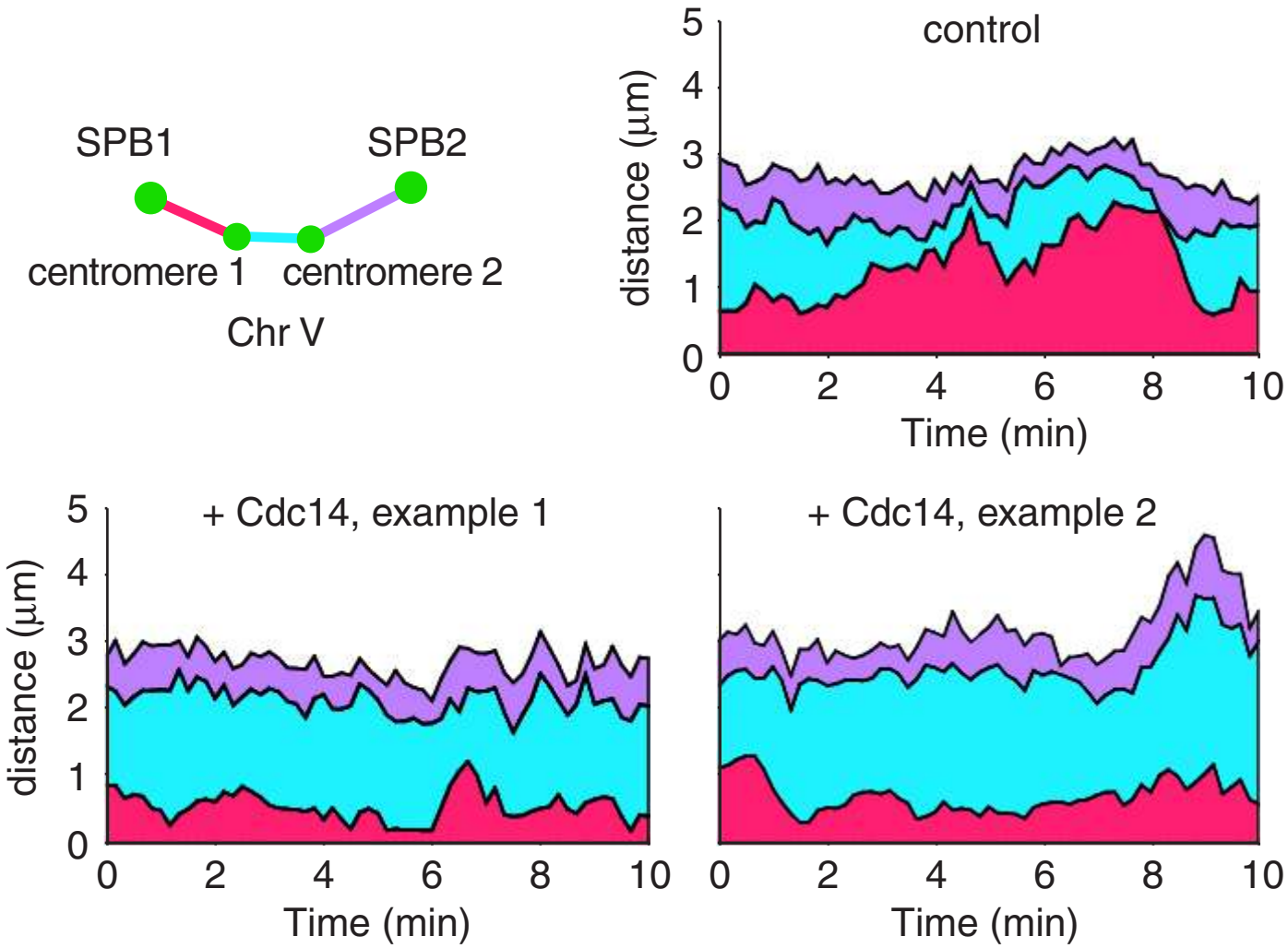

| | Average ± s. d. | p-value (*t*-test) vs. Meta | vs. Separase |
|---|---|---|---|
| Meta | 37.0 ± 11.2 | N/A | 0.002 |
| Separase | 14.1 ± 7.6 | 0.002 | N/A |
| TEV | 33.1 ± 28.3 | 0.719 | 0.083 |
| *cdc14-1* | 34.6 ± 22.0 | 0.806 | 0.049 |
| Meta+Cdc14 | 13.6 ± 15.9 | 0.016 | 0.945 |
| TEV+Cdc14 | 6.0 ± 11.4 | 0.001 | 0.170 |
| *ASK1-2A* (Meta) | 10.7 ± 16.3 | 0.019 | 0.683 |
| *SLI15-S335A* (Meta) | 19.4 ± 9.8 | 0.016 | 0.295 |

**Supplementary Figure S1** Analysis of microtubule dynamics by FRAP of GFP-tubulin labelled spindles. Summary of results, examples of which are presented in Figures 1, 2, and 5. Recovery was determined as follows: fluorescence intensity of an area within the bleached region was measured before bleach. The intensity after bleach in the same area was taken as baseline (0% recovery), from which recovery towards the prebleach value was calculated. To compensate for fluctuations, recovery was measured as the average fluorescence intensity within the 70-100 seconds time window after bleach. Fluorescence within control regions did not normally decrease more that 10% during the course of the experiment, and is therefore not corrected for (in some experiments after TEV protease-triggered anaphase, fluorescence in the control region decreased to a larger extend, which is likely because of a change in microtubule density in this region caused by dynamic instability, see e.g. Figure 1).
Six or more spindles were analysed under each condition. Average recovery and standard deviation are indicated by the bar and whisker lines, and the numerical values are given to the right. A two-sided Student's *t*-test, allowing unequal variance between samples, was performed to evaluate whether recovery significantly differed under the conditions tested. Recovery was compared to spindles in metaphase (Meta), and to spindles in anaphase triggered by separase expression (Separase).
These results show that microtubule turnover during anaphase triggered by TEV protease expression, and in *cdc14-1* mutant cells, is not significantly different from that in metaphase. After expression of separase or Cdc14, or in cells containing either the *SLI15-S335A* or *ASK1-2A* alleles, microtubule dynamics are significantly reduced ($p<0.02$ in these cases).



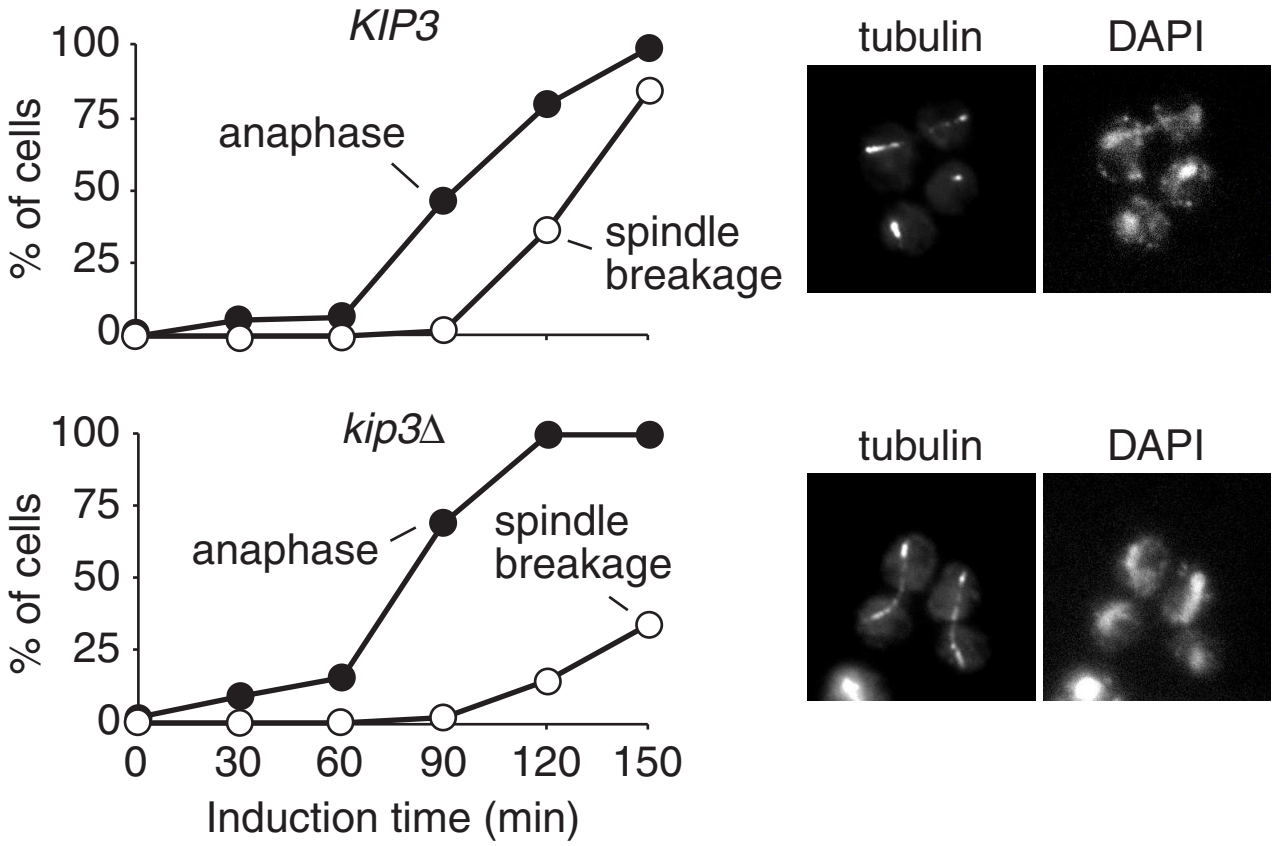




**Supplementary Figure S2** Cdc14 rescues stability of the breaking spindle during TEV protease-induced anaphase. As control, after anaphase is triggered in metaphase arrested cells by expression of separase, in strain Y393 (*Mata MET-CDC20 GAL-ESP1*), the anaphase spindle is stable. In contrast, the anaphase spindle after chromosome segregation triggered by expression of TEV protease, in strain Y353 (*Matα MET-CDC20 GAL-TEV SCC1$^{TEV}$*), is unstable and breaks down. Coexpression with TEV protease of Cdc14, but not phosphatase inactive Cdc14$^{C283A}$, rescues the stability of anaphase spindles. Strains Y1239 (as Y353, plus *GAL-CDC14*), and Y1294 (as Y353, plus *GAL-cdc14(C283A)*). Similar expression levels of Cdc14 and Cdc14$^{C283A}$ were confirmed by Western blotting (not shown). The effect of Cdc14 on spindle stability was not due to indirect activation of separase, because expression of Cdc14 alone did not initiate anaphase nor could we detect evidence of Scc1 cleavage by Western blotting (strain Y1304 (*Mata MET-CDC20 GAL-CDC14*)). Similar expression levels of Cdc14 and Cdc14$^{C283A}$ in all cases were confirmed by Western blotting.




**Supplementary Figure S3** Ectopic Cdc14 in metaphase reduces centromere oscillations. Strain Y2182 (*MATα MET-CDC20 cenV-GFP SPC42-GFP*) and Y2184 (as Y2182, but *GAL-CDC14-CFP*) were arrested in metaphase, and galactose was added to start expression of Cdc14 in strain Y2184. Movement of the centromeres relative to the spindle poles and relative to each other was tracked over time. z-stacks with 16 frames in 0.2 μm distance were recorded every 10 seconds. The distances were determined in three dimensions according to the scheme on the top left, and plotted over time. The colours in the diagrams follow the colour code in the scheme. Note that changes in the sum of the three recorded distances do not necessarily reflect changes in the spindle pole-to-pole distance that remained constant.

In control metaphase cells, centromere oscillations are seen with the sister centromeres separating and rejoining, indicative of dynamic kinetochore microtubules, as has been previously described (Ref. 11). After Cdc14 expression, the amplitude of centromere movement decreased, and sister centromeres remained separated from each other in the vicinity of opposite spindle poles (4 out of 4 cells observed). This suggests that kinetochore microtubules have shortened and become less dynamic.




**Supplementary Figure S4** Rescue of spindle stability in the absence of Cdc14 by deletion of Kip3. Anaphase was induced by expression of TEV protease in strains Y1715 (*MAT*a *MET-CDC20 GAL-TEV SCC1*$^{TEV}$), and Y1845 (as 1715, but *kip3*Δ). Anaphase (spindle elongation and binucleate formation) and breakage of the anaphase spindle were scored after processing cells for immunofluorescence. Examples of the cells are shown at 120 min.