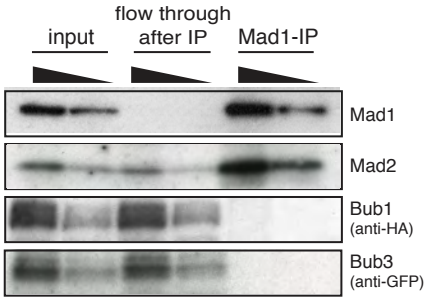
Figure S3

A

	Mad1-GFP IP *cdc25-22* release		Mad1-GFP IP *nda3-KM311* arrest		Bub1-GFP IP *nda3-KM311* arrest	
	pep.	seq.cov.	pep.	seq.cov.	pep.	seq.cov.
Mad1	111	81 %	94	82 %	0	0 %
Mad2	16	62 %	15	55 %	2	8 %
Bub1	0	0 %	0	0 %	75	62 %
Bub3	0	0 %	0	0 %	20	58 %
Spc7	0	0 %	0	0 %	15	15 %
Mis12	0	0 %	0	0 %	3	13 %
Mis13	0	0 %	0	0 %	6	20 %
Mis14	0	0 %	0	0 %	5	32 %
Nnf1	0	0 %	0	0 %	3	15 %
Ndc80	0	0 %	0	0 %	16	29 %
Nuf2	0	0 %	0	0 %	3	7 %
Spc24	0	0 %	0	0 %	3	21 %
Spc25	0	0 %	0	0 %	5	19 %
Nup211	45	28 %	2	1.1 %	0	0 %

B



C

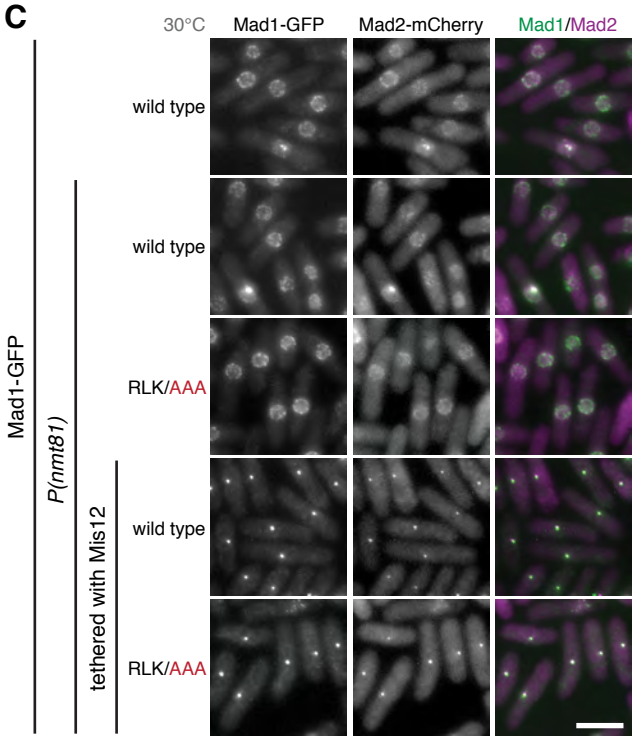


Figure S3 Absence of evidence for Bub1-Mad1 interaction and supplementary data for Mad1 kinetochore-tethering

A Mass spectrometry of immunoprecipitations of Mad1 and Bub1. Mad1-GFP was either immunoprecipitated from cells that were released from a *cdc25-22* arrest (at G2/M), were treated with the microtubule drug MBC and were harvested in mitosis (86 % mitotic cells) or from cells that were delayed in mitosis by the spindle assembly checkpoint due to the *nda3-KM311* tubulin mutant. Bub1-GFP was immunoprecipitated from cells that were delayed in mitosis by the spindle assembly checkpoint due to the *nda3-KM311* mutant. Samples were analysed by mass spectrometry. The table shows the number of identified peptides (pep.) and the amino acid sequence coverage (seq.cov.) reached for each protein, which are semi-quantitative measures for the abundance of the protein in the immunoprecipitate. Mad2 and Nup211 were recovered as interaction partners in the Mad1 immunoprecipitates, Bub3 and outer kinetochore proteins were recovered as interaction partners in the Bub1 immunoprecipitate.

B Anti-Mad1 immunoprecipitations from cells that were delayed in mitosis by the spindle assembly checkpoint due to the *nda3-KM311* tubulin mutant were analysed for the presence of Mad1, Mad2, Bub1 and Bub3 using anti-Mad1, anti-Mad2, anti-HA (Bub1) and anti-GFP (Bub3) antibodies. Input and flow through are shown on the left. A 1:2 dilution is additionally loaded for each sample.

C Mad2 colocalizes with Mad1-RLK/AAA

Representative images of cells expressing *mad2+-mCherry*, *nda3-KM311* and the indicated *mad1 wild type* or *RLK/AAA-GFP* fusions. Cells were grown at permissive temperature for the nda3-KM311 mutant (30 °C). *P(nmt81)* indicates expression of the construct from the *nmt81* promoter rather than from the endogenous *mad1* promoter. Scale bar: 10 μm.