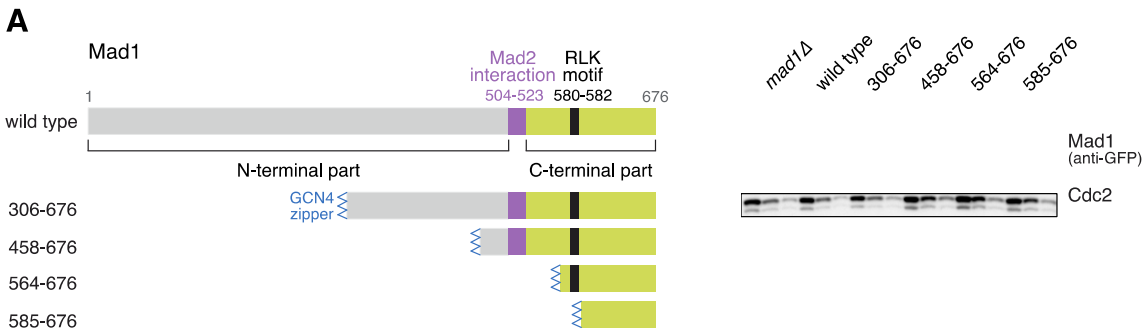
Figure S2



some

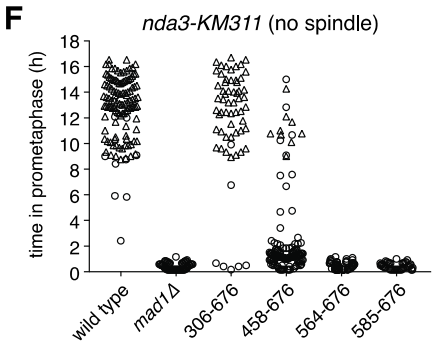


Figure S2 The Mad1 N-terminal part is not required for kinetochore localisation, the C-terminal part is not sufficient

A Domain structure of the Mad1 protein and N-terminal truncations

A fragment of *S. cerevisiae* Gcn4p (aa250-277; GCN4 zipper) was used to aid coiled-coil formation of the remaining alpha-helical parts and dimerization [1, 2].

B N-terminal truncation mutants of Mad1 are expressed, but to different levels

Immunoblotting of cell extracts using anti-GFP and anti-Cdc2 (loading control) antibodies. A dilution series was loaded for each strain to compare intensities. The N-terminal Mad1 truncations were expressed, but not all to the same level as wild type Mad1-GFP (also see (C)).

C Truncation of the Mad1 N-terminus abolishes nuclear rim localisation

Representative images of cells expressing *plo1+-mCherry*, *nda3-KM311* and the indicated *mad1-GFP* fusions. Cells were grown at permissive temperature for *nda3-KM311* (30 °C). Scale bar: 10 µm; scale bar in inset: 2 µm. Nuclear rim localisation was lost in all N-terminal truncations, whereas kinetochore localisation was at least partly preserved in mutants that retained parts of the N-terminal coiled-coil. The C-terminal part of Mad1 was not sufficient for kinetochore localisation.

D Only the shortest N-terminal Mad1 truncation (Mad1-306-676) preserves kinetochore localisation of Mad1-GFP at the restrictive temperature for *nda3-KM311*

The same strains as in (C) were shifted to the restrictive temperature for *nda3-KM311* (16 °C) and imaged as in Fig 1C. Mad1-GFP signals were quantified at the kinetochore as cells entered mitosis (a.u. = arbitrary units; error bars = s.d.; $n \geq 20$ cells). The kinetochore localisation of Mad1-458-676 was almost undetectable in live cell imaging (left panel), but was visible at 16 °C when the same image acquisition settings as in (C) were used (right panel; representative nuclei of mitotic cells). Mad1-458-676 localisation seems weaker at 16 °C than at 30 °C. The schematic depicts the situation in the example pictures and shows a nucleus with unclustered chromosomes (light blue). Unclustering occurs in the absence of microtubules when cells delay in mitosis.

E Mad2-mCherry shows the same localisation pattern as Mad1-GFP in the truncation mutants

Cells expressing *mad2+-mCherry*, *nda3-KM311* and the indicated *mad1-GFP* fusions were imaged at 30 °C. Representative nuclei of cells in mitosis are shown. Scale bar: 2 µm. Mad1-306-676 and Mad1-458-676 co-recruit Mad2 to the kinetochore, indicating that the interaction with Mad2 is preserved.

F The shorter N-terminal Mad1 truncation (Mad1-306-676) largely preserves checkpoint activity.

Checkpoint function in the indicated strains was analysed as in Fig 1F. Checkpoint activity in Mad1-306-676 was largely preserved (although the abundance seemed lower than wild type Mad1 (B,C)). Checkpoint activity in Mad1-458-676 was impaired, which coincided with an impairment of localisation to the kinetochore that was more pronounced at 16 °C (C,D,E). The two shortest Mad1 fragments (Mad1-564-676 and 585-676) were checkpoint-deficient, which was expected from the lack of the Mad2-interaction motif.

Supplementary References

1. Kammerer RA, Schulthess T, Landwehr R, Lustig A, Engel J, Aebi U, Steinmetz MO (1998) An autonomous folding unit mediates the assembly of two-stranded coiled coils. *Proc Natl Acad Sci U S A* **95:** 13419-13424

2. O'Shea EK, Klemm JD, Kim PS, Alber T (1991) X-ray structure of the GCN4 leucine zipper, a two-stranded, parallel coiled coil. *Science* **254:** 539-544