Figure S2

A

![Diagram of Mad1 and Mad2 interaction sites](image)

B

![Western blot of Mad1 and Cdc2](image)

C

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Mad1-GFP</th>
<th>Plot1-mCherry</th>
<th>Mad1/Plot1</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C</td>
<td><img src="image" alt="Images of wild type, 306-676, 458-676, 564-676, 585-676" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D

![Graph showing kinetochore signal](image)

E

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Mad1-GFP</th>
<th>Mad2-mCherry</th>
<th>Mad1/Mad2</th>
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</thead>
<tbody>
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F

![Graph showing time in prometaphase](image)
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 ≥ 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**
