Figure S1

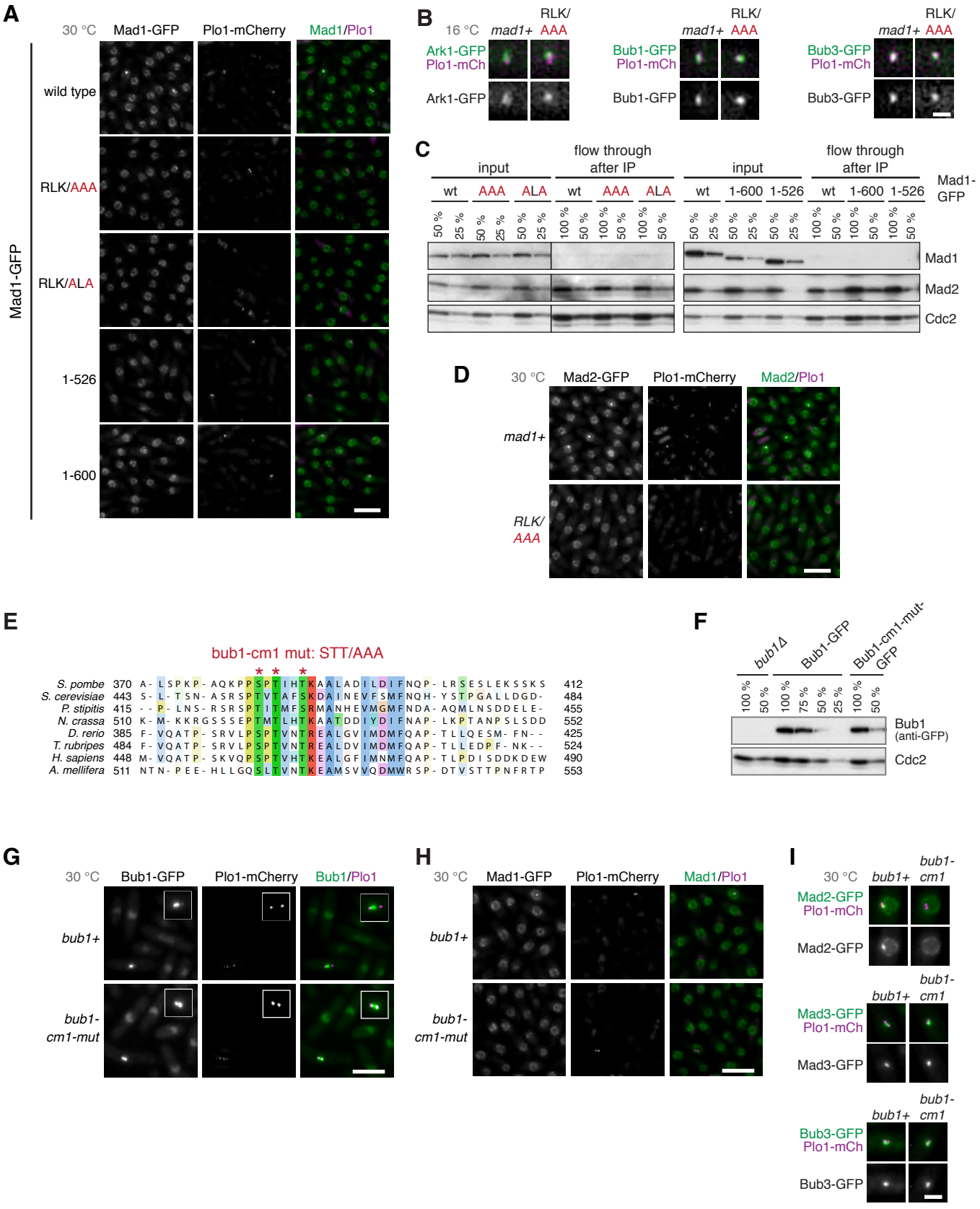
A
30 °C
Mad1-GFP
Plo1-mCherry
Mad1/Plo1
Mad1-GFP
wild type
RLK/AAA
RLK/ALA
1-526
1-600
B
16 °C
mad1+
RLK/AAA
Ark1-GFP Plo1-mCh
Ark1-GFP
Bub1-GFP Plo1-mCh
Bub1-GFP
Bub3-GFP Plo1-mCh
Bub3-GFP
C
input
flow through after IP
wt
AAA
ALA
1-600
1-526
Mad1-GFP
Mad1
Mad2
Cdc2
D
30 °C
Mad2-GFP
Plo1-mCherry
Mad2/Plo1
mad1+
RLK/AAA
E
bub1-cm1 mut: STT/AAA
S. pombe 370 A-LSPKP-AQKPPSPTIHTKAALADILDIFNQP-LRSESLEKSSKS 412
S. cerevisiae 443 S-L-TSN-ASRSPTVTAFSKDAINEVFSMFNQH-YSTPGALLDGD- 484
P. stipitis 415 --P-LNS-RSRSPTITMFSRMANHEVMGMFNDA-AQMLNSDDELE- 455
N. crassa 510 K-M-KKRGSSSEPTMTLHTKAATDDIYDIFNAP-LKPTANPSLSDD 552
D. rerio 385 F-VQATP-SRVLPSPTVNTREALGVIMDMFQAP-TLLQESM-FN-- 425
T. rubripes 484 F-VQATP-SRVLPSPTVNTREALDVIMDMFQAP-TLLEDPF-NK-- 524
H. sapiens 448 M-VQATP-SKVQPSPTVHTKEALGFIMNMFQAP-TLPDISDDKDEW 490
A. mellifera 511 NTN-PEE-HLLGQSLTVNTKEAMSVVQDMWRSP-DTVSTTPNFRTP 553
F
bub1Δ
Bub1-GFP
Bub1-cm1-mut-GFP
Bub1 (anti-GFP)
Cdc2
G
30 °C
Bub1-GFP
Plo1-mCherry
Bub1/Plo1
bub1+
bub1-cm1-mut
H
30 °C
Mad1-GFP
Plo1-mCherry
Mad1/Plo1
bub1+
bub1-cm1-mut
I
30 °C
bub1+
bub1-cm1
Mad2-GFP Plo1-mCh
Mad2-GFP
Mad3-GFP Plo1-mCh
Mad3-GFP
Bub3-GFP Plo1-mCh
Bub3-GFP

Figure S1 Additional analysis of Mad1-RLK and Bub1-cm1 mutants

A Mad1-RLK mutants and C-terminal truncations localize to the nuclear rim

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). A localized Plo1-mCherry signal indicates that cells are in mitosis (scale bar: 10 *μ*m).

B Ark1, Bub1 and Bub3 localize to kinetochores in the *mad1-RLK/AAA* mutant

Cells expressing *plo1+-mCherry*, *nda3-KM311* and the indicated GFP fusion proteins were analysed at the restrictive temperature for *nda3-KM311* (16 °C) as in Fig 1E. Representative nuclei of mitotic cells are shown (scale bar: 2 *μ*m).

C Mad1 is efficiently depleted by immunoprecipitation

Input and flow through of the anti-Mad1 immunoprecipitation shown in Fig 1H. To detect the Mad1-RLK mutants, anti-GFP antibody was used; to detect the Mad1 truncations, anti-Mad1 antibody was used. Cdc2 serves as loading control. The C-terminal truncations Mad1-1-600 and Mad1-1-526 contain more Mad2 in the flow through, in agreement with inefficient binding of Mad2 to Mad1 (Fig 1H).

D Mad2 localisation to kinetochores is strongly reduced in the *mad1-RLK/AAA* mutant

Representative images of cells expressing *mad1+* or *mad1-RLK/AAA*, as well as *mad2+-GFP*, *plo1+-mCherry* and *nda3-KM311*. Cells were grown at permissive temperature for *nda3-KM311* (30 °C). Mad2-GFP localisation to the nuclear rim in interphase is similar between wild type and *mad1-RLK/AAA* cells, but localisation to the kinetochore in mitosis is impaired in the *mad1-RLK/AAA* mutant (scale bar: 10 *μ*m).

E Alignment of the Bub1 region containing the conserved motif 1 (cm1).

Sequences from OrthoMCL group OG5_130700 were aligned using M-Coffee. Selected sequences are shown with Clustal X colouring scheme. Red asterisks indicate the amino acids mutated in the *bub1-cm1* mutant (S381A, T383A and T386A).

F Bub1-cm1 mutant is present at similar levels as wild type Bub1

Extracts were analysed by immunoblotting using anti-GFP (to detect Bub1-GFP or Bub1-cm1-mut-GFP) and anti-Cdc2 (loading control) antibodies. Percentages on top indicate how much of the extract was loaded.

G Bub1-cm1 mutant localisation resembles Bub1 wt localisation

Representative images of cells expressing *bub1-GFP* (wild type or cm1 mutant), *plo1+-mCherry* and *nda3-KM311*. Cells were grown at permissive temperature for *nda3-KM311* (30 °C). Both wild type Bub1 and Bub1-cm1 enrich in the nucleus in interphase. Inset: Plo1-mCherry marks mitotic spindle pole bodies, and both Bub1 and Bub1-cm1 localize to the region of the mitotic spindle (most likely by localizing to unattached kinetochores). Scale bar: 10 *μ*m. Insets are additionally magnified 1.87-fold.

H Mad1-GFP localisation to the nuclear rim is not impaired by the *bub1-cm1* mutation

Representative images of cells expressing *bub1* (wild type or cm1 mutant), *mad1+-GFP, plo1+-mCherry* and *nda3-KM311*. Cells were grown at permissive temperature for *nda3-KM311* (30 °C). Nuclei of mitotic cells with Mad1-GFP signal from these panels are shown in Fig 1J. Scale bar: 10 *μ*m.

I Bub1-cm1 mutation affects kinetochore localisation of Mad2 but not Mad3 and Bub3

Representative nuclei of mitotic cells expressing *bub1* (wild type or cm1 mutant), the indicated GFP fusions, *plo1+-mCherry* and *nda3-KM311*. Cells were grown at permissive temperature for *nda3-KM311* (30 °C). Scale bar: 2 *μ*m