Differentiation of cytoplasmic and meiotic spindle assembly MCAK functions by Aurora B-dependent phosphorylation

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SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Immunoprecipitations. Mock-depleted, MCAK-depleted, and MCAK-depleted extracts supplemented with wild type (wt) XMCAK or XMCAK-4A were prepared as described in the main text. For each immunoprecipitation, 5 µg rabbit IgG (Jackson ImmunoResearch Laboratories) or anti-ICIS antibodies (Ohi *et al.*, 2003) were bound to 5 µl Affi-Prep protein A (BioRad) in TBST for 1 hour at 4°C. Antibody-bound beads were washed once with TBST, three times with CSF-XB, and incubated in 50 µl of the appropriate extract for 1 hour at 4°C. Beads were washed twice with CSF-XB, six times with NP-40 buffer (10 mM Na-phosphate (pH=7.2), 150 mM NaCl, 2 mM EDTA, 1% IGEPAL CA-630), boiled in sample buffer, and analyzed by immunoblotting. Anti-ICIS antibodies were used at 0.5 µg/ml in 5% w/v skim milk in TBST.

FIGURE LEGEND

Figure S1. XMCAK-4A associates with ICIS in *Xenopus* extract. IgG and anti-ICIS immunoprecipitates from mock-depleted, MCAK-depleted (ΔMCAK), and MCAK-depleted extracts supplemented with either wt XMCAK or XMCAK-4A

were analyzed by immunoblotting using either anti-ICIS or anti-XMCAK antibodies.

SUPPLEMENTARY REFERENCE

Ohi, R., Coughlin, M.L., Lane, W.S., and Mitchison, T.J. (2003). An Inner Centromere Protein that Stimulates the Microtubule Depolymerizing Activity of a KinI Kinesin. Dev Cell *5*, 309-321.