**Supplementary information**

**Supplementary Methods**

**Isolation of conditional lethal mutants with polarity defects**

Mutants that showed an altered morphology were selected visually by fluorescence microscopy after staining with Calcofluor white, as described previously (Hirata et al., 1998; Radcliffe et al., 1998). The isolated round mutants were further examined for sensitivity to the protein kinase inhibitor staurosporine (1.5 µg/ml). The temperature-sensitive (ts) pmo25 mutant was created by error-prone PCR mutagenesis as follows: The DNA fragments containing the pmo25\(^+\):GFP:kan\(^r\) gene amplified by PCR from the pmo25\(^+\):GFP:kan\(^r\) strain were integrated into the pmo25\(^+\) locus in wild-type cells; and from the resultant kan\(^r\) cells, the ts pmo25-35 mutant was isolated at 36°C.

**Immunochemical and kinase assays**

Preparation of cell extracts, immunoprecipitation, immunodetection, and kinase assays were performed as previously described (Matsusaka et al., 1995; Bähler and Nurse, 2001; Huang et al., 2003; Wiley et al., 2003). Immunoprecipitation was done by using anti-HA antibody (HA.11, BabCO), anti-GFP antibody (8362-1, Clontech), anti-Myc antibody (9E10, Calbiochem), and magnetizable beads conjugated to protein A or G (Dynabeads, DYNAL).
Microscopy techniques

For the observation of Pmo25-GFP, the cells expressing Pmo25-GFP were fixed with methanol (-20°C) for 10 min and washed three times with PEM buffer (Alfa et al. 1993). Fixed-cell images were collected with an Axiophot 2 MOT (ZEISS), the ApoTome sectioning system, and AxioCam MRm CCD camera; and the images were further processed with AxioVision software. For time-lapse microscopy, a 35 mm glass-bottomed culture dish (MatTek Corporation, P35G-1.5-10-C) was coated with 100 µg/ml concanavalin A. The culture of logarithmically growing cells (50 µl) was deposited in the well for a couple of minutes and then removed. The dish was filled with 3 ml of EMM medium and the cells that were attached to the bottom of the well were subjected to microscopic analysis. Live-cell images were collected with an IX70 (OLYMPUS) and DeltaVision sectioning system. Cytological techniques were performed according to Matsusaka et al. (1995).

In vitro binding assay

The expression plasmids for HA-Pmo25 or Myc-Nak1 proteins were generated as follow: An HA-tagged *pmo25* cDNA or Myc-tagged *nak1* cDNA was amplified from pACT2-HA-Pmo25 or pGBK7-Myc-Nak1, respectively, by PCR. The PCR products were inserted into the *Not*I and *Xho*I sites of the plasmid pTWIN1. The HA-Pmo25 and Myc-Nak1 proteins were synthesized using IMPACT-TWIN System (New England Biolabs). The HA-Pmo25 protein (10 µg/ml) was incubated with Myc-Nak1 protein (10
µg/ml) in Buffer A (Boudeau et al., 2004), which contained 50 mM Tris-HCl (pH7.5), 0.27 M Sucrose, 0.1 mM EDTA (pH8.0), 0.1%(v/v) mercaptoethanol and protease inhibitors, for 1 h at 4°C. The mixture was incubated in 1:1000 dilution of a mouse anti-HA antibody (HA.11, Babco) and magnetizable beads conjugated to protein A (Dynabeads, DYNAL) for 1.5 h at 4°C. The beads were washed three times with Buffer A, and the bound proteins were subjected to an immunoblot analysis.

**Supplementary References**

