Supporting Materials and Methods

Plasmids and Cloning. The pHF7 plasmid expressing GFP from the SV40 early promoter has been described (1). The pTTKII plasmid (2) was the generous gift of G. Mills (M. D. Anderson Cancer Center, Houston). The hMps1 ORF from pTTKII was amplified by PCR and cloned into pHF7 to create the GFP-hMps1 expression plasmid pHF36. An 1,139-bp *Bsp*EI–*Nde*I fragment containing the D664A mutation was generated by PCR and cloned into the *Bsp*EI and *Nde*I sites of pHF36 to create the GFP-hMps1KD expression plasmid pHF56. To create the GST-hMps1-ha expression plasmid pGEX-hMps1-ha, the hMps1 ORF was tagged with the hemagglutinin (HA) epitope by PCR and cloned into the pGEX-6P-1 plasmid (Amersham Biosciences). The 1,139-bp D664A containing *Bsp*EI–*Nde*I fragment from pHF56 was cloned into the *Bsp*EI and *Nde*I sites of pGEX-hMps1-ha to create pGEX-hMps1KD-ha. Human Centrin2 was amplified by PCR and cloned into pHF7 to create the GFP-centrin expression plasmid pHF80. All PCR-generated regions of pHF36, pHF56, pGEX-hMps1-ha, and pHF80 were sequenced to verify the absence of unintended PCR induced errors.

Purification of hMps1 Fusion Proteins. The GST-hMps1400-507 fusion protein has been described (3). The GST-hMps1-ha and GST-hMps1KD-ha fusion proteins were expressed in the *Escherichia coli* strain BL21(DE3)pLysS (Invitrogen) from the pGEX-hMps1-ha and pGEX-hMps1KD-ha plasmids. Bacteria were lysed in PBS containing 50 mM NaCl, 5 mM $MgCl_2$, 10% glycerol (vol/vol), 0.2% Triton X-100 (vol/vol), 0.1% 2-mercaptoethanol (vol/vol), and 125 μg/ml egg white lysozyme. Fusion proteins from clarified lysates were bound to glutathione-Sepharose (Amersham Biosciences), columns were washed extensively, and fusion proteins were eluted in 50 mM Tris•HCl (pH 8.0) containing 10% glycerol (vol/vol), 0.1% 2-mercaptoethanol (vol/vol), and 10 mM reduced glutathione. Protein was determined by Bradford assay, and peak fractions were pooled and frozen.

Generation of siRNA. The following primer sets were used to generate Lamin A/C and hMps1 PCR products flanked by T7 promoter sequences (lowercase represents T7,

uppercase represents gene-specific sequence); Lamin A/C 153-719, 5'-taatacgactcactatagggagaGCTGGAAACGGAGAACGCAGG-3' and 5'-taatacgactcactatagggagaCGGCTCTCAAACTCACGCTGC-3'; hMps1 14-547, 5'-taatacgactcactatagggagaATTTAAGTGGCAGAGAATTGAC-3' and 5'-taatacgactcactatagggagaCAATTTCCAGCATTTCTAGTGG-3'; hMps1 997-1543, 5'-taatacgactcactatagggagaAGTCATTTCAAGGAACCTCTGG-3' and 5'-taatacgactcactatagggagaTTGCTGAAGAAGATGCTAAAAC-3'.

1. Fisk, H. A. & Winey, M. (2001) *Cell* **106,** 95–104.
2. Mills, G. B., Schmandt, R., McGill, M., Amendola, A., Hill, M., Jacobs, K., May, C., Rodricks, A., Campbell, S. & Hogg, D. (1992) *J. Biol. Chem.* **267,** 16000–16006.
3. Liu, S. T., Chan, G. K., Hittle, J. C., Fujii, G., Lees, E. & Yen, T. J. (2003) *Mol. Biol. Cell* **14,** 1638–1651.