## Online Supplemental Material

## Lindon et al. http://www.jcb.org/cgi/doi/10.1083/jcb.200309035

## Materials and methods

## Immunofluorescence microscopy

Cells were fixed and permeabilized in ice-cold methanol/acetone, then preincubated for 15 min in PBS containing 3% BSA and 0.2% Tween 20. Primary (anti-Plk1 NH<sub>2</sub>-terminus; Upstate Biotechnology) and secondary (Alexa® Fluor 568–conjugated goat anti–rabbit; Molecular Probes, Inc.) antibody incubations (1 h each) were performed using this buffer, whereas PBS/0.2% Tween 20 was used to wash cells extensively between incubations. Finally, cells were mounted in Mowiol (Calbiochem) and viewed on a confocal microscope (Radiance 2000; Bio-Rad Laboratories) using sequential excitation at 488 and 543 nm.