SUPPLEMENTARY METHODS AND MATERIALS

Fluorescence Polarization Binding Assay

The reaction buffer composition was 50 mM Tris, 200 mM NaCl, 2.0 mM DTT, and 0.005% Tween 20 (pH 8.0). Each sample was prepared in triplicate in a black 96-well plate (Greiner, Monroe, NC). Reactants were added to the plate in darkened conditions to minimize light exposure. The polarization values in millipolarization (mP) units were measured at an excitation wavelength of 488 nm and an emission wavelength of 535 nm with the following equation:

$$p = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + GI_{\perp}}$$

Where I_{II} is the intensity with polarizers parallel and I_L signifies the intensity with the polarizers perpendicular. G represents the instrumental error. Data was plotted as log concentration versus percent inhibition from the data and error bars calculated from standard deviation for each data point (Supplementary figures)

Immunofluorescence, PLK1 localization, and aberrant prometaphase/metaphase analysis

HeLa-H2B-GFP cells were plated at a density of 10,000 cells per coverslip 24 h prior to The cells were mounted and the coverslips examined with an Olympus IX81 microscope using a 100X objective, and the images were captured using a Hamamatsu OrcaR2 camera. For cells in metaphase, PLK1 localization was analyzed using MetaMorph software (Molecular Devices). Images were taken in z-stacks, overlayed and deconvoluted.

Analysis of apoptosis

At the indicated intervals, cells were collected by trypsinization, washed with PBS, fixed in 100% ethanol overnight, then incubated with propidium iodine (50 ug/mL) and RNase A (0.5 mg) for a minimum of 30 min. Staining with FITC-Annexin V was carried out as described by the manufacturer (BD Biosciences). The samples were analyzed using a Beckman-Coulter FC 500 flow cytometer. Data was quantified using ModFit LT software version 3.1 (Verity Software House). Experiments were performed in triplicate and the results averaged.

Statistical analysis

Calculations of the mean, SD, and standard error were performed using Microsoft Excel. Statistical analysis for comparison of each set of experimental means as well as statistical analysis of proportions was performed using Statistica 8 CS (StatSoft, Inc.).