Supplementary Material – Portions of Materials and Methods and Supplementary Figure Legends

**Materials and Methods**
**Materials**
Antibody against phospho-Ser/Thr-Pro MPM-2 (MPM-2) (05-368) was purchased from Millipore (Billerica, MA) and antibodies against GAPDH (2118) and poly(ADP-ribose) polymerase (PARP) (9532) were purchased from Cell Signaling (Beverly, MA). Vincristine sulfate (sc-201434) was from Santa Cruz Biotechnology (Santa Cruz, CA); Taxol (T7191), antibody against α-tubulin (T9026), cholesterol (C3045), amphotericin (A2942), human apo-transferrin (T1147) and vinblastine sulfate (V1377) were purchased from Sigma-Aldrich (St. Louis, MO); human serum albumin (HSA) (1006.HSA.CCPL) was purchased from Nova Biologics (Oceanside, CA); insulin (128-100) was purchased from Cell Applications (San Diego, CA); docetaxel (A3494) was from ApexBio (Houston, TX); and MLN8237 (S1133) was from Selleckchem (Houston, TX). ALL cell cultures were provided by Dr. Fred Falkenburg (University of Leiden, Leiden, The Netherlands).

**Cell extraction and immunoblotting**

Cells were collected by centrifugation at 2,000 x g for 10 min, washed in PBS, and resuspended in lysis buffer which consisted of 40 mM HEPES (pH 7.5), 0.12 M NaCl, 1% Triton X100, 1 mM EDTA, plus EDTA-free complete protease inhibitor tablets [Roche] supplemented with 20 µg/mL aprotinin, 50 µg/mL leupeptin, 10 µM pepstatin, and 1 mM phenylmethylsulfonyl fluoride. After 45 min at 4°C, the suspension was centrifuged at 16,300 x g for 15 min, the protein concentration of the supernatant was determined, and extracts containing 30-50 µg of protein were subjected to SDS-PAGE and immunoblotting. Apparent molecular weights of the proteins examined are as follows: GAPDH, 37 kDa; mitotic phosphoproteins reactive with MPM-2 antibody, 60 kDa and above; PARP, 116 and 85 kDa for uncleaved and cleaved, respectively.

**Cell cycle analysis**

Cells were harvested and resuspended in PBS at a concentration of 106 cells/mL. Cell cycle analysis was performed after fixation using propidium iodide staining and flow cytometry according to the manufacturer’s instructions (BD Pharmingen) by the UAMS Flow Cytometry Core Facility using a FACSCalibur (Becton Dickinson, Mountain View, CA). The data presented were derived from 95-97% of recorded events for each experiment, and events originating from cellular debris and aggregates, representing 3-5% of the total, were excluded. The data were analyzed using the FACS Diva (BD) software.

**Analysis of microtubule content**

To determine the relative amounts of polymerized versus depolymerized tubulin in control and MTA-treated ALL cells, a procedure described in the microtubules/tubulin assay kit (Cytoskeleton, Denver, CO) was followed, with all steps conducted at 37°C unless otherwise stated. Briefly, 106 cells were suspended in 100 µL lysis buffer (100 mM PIPES, pH 6.9, 5 mM MgCl2,1 mM EGTA, 30% glycerol, 0.1% Nonidet P40, 0.1% Triton X-100, 0.1% Tween 20, 0.1% 2-mercaptoethanol, 0.1 mM GTP, 1 mM ATP, and a mixture of protease inhibitors, as indicated above). Cells were homogenized by passing the suspension through a 200 uL pipette tip eight times, centrifuged at 2,000 x g for 5 min, and the supernatant centrifuged at 100,000 x g for 30 min. Supernatants containing soluble tubulin were removed and stored on ice. Pellets containing polymerized tubulin were suspended, with occasional passage through a 200 µL pipette tip, in 100 µL of 2 mM CaCl2 for 15 min at room temperature to depolymerize the microtubules. Equivalent volumes of each fraction were subjected to immunoblotting for α-tubulin, with quantitation performed using ImageJ software.

**Statistical analysis**

Data were analyzed using Student’s t-test with p value of ≤ 0.05 considered significantly different. Assays were conducted with replicates of at least three and all experiments were repeated at least once with essentially identical results.

**Supplementary Figure Legends**

**Fig. S1. Cellular sensitivity to vincristine.** Cell viability was assessed by MTT assay as described in Materials and Methods. Cells were treated with vehicle (100% viability) or increasing concentrations of vincristine for twice the doubling time (KB3, 48 h; RS4;11, 72 h; ALL-2, 120 h; ALL-5, 134 h). Results are given as mean ± S.D. (n = 6).

**Fig. S2. Docetaxel and vinblastine induce cell death without substantial mitotic accumulation.** ALL- 5 cells were treated with 100 nM docetaxel (DTx) or 100 nM vinblastine (VBL) for the times indicated, stained with PI, and subjected to flow cytometry. The proportion of cells with either <2N (left panel) or 4N (right panel) DNA content are shown (mean ± S.D., n = 3). \*p ≤ 0.05 (Student’s t test).

**Fig. S3. The Aurora A kinase inhibitor MLN8237 induces M phase arrest.** ALL-2 cells were treated with 0.5 µM MLN8237 for the times indicated, stained with PI, and subjected to flow cytometry. The proportion of cells with 4N DNA content is indicated. Histograms are representative of three independent experiments.

**Fig. S4. Correlation of DNA content and cell size.** Flow cytometric analysis of primary ALL cultures is shown after staining with PI (x-axis), color coded according to the key on the right. Cell size, determined by side-scatter, is shown on the y-axis. Each dot-plot is representative of 3 independent experiments.

**Fig. S5. Separation of ALL cells into distinct cell cycle phases by centrifugal elutriation.** ALL-2 cells were subjected to centrifugal elutriation, as described in Materials and Methods, and pools of select fractions subjected to propidium iodide (PI) staining and flow cytometry. **A**, early fractions 5-8 (early G1 phase, 23% of total cells); **B**, intermediate fractions 13-16 (late G1 and S phase, 25% of total cells); **C**, late fractions 19-20 (S, G2, and M phases, 16% of total cells). Note that the data shown are from a select experiment for illustrative purposes, and there was experimental variability in the pools of fractions needed to enrich for cells in specific cell cycle phases.

**Fig. S6. Cell cycle distribution of elutriated ALL cells before and after vincristine treatment.** ALL-5 cells were separated by centrifugal elutriation into distinct cell cycle phases. **A**. G1-enriched cells (2N DNA) or, **B**. G2/M-enriched cells (4N DNA), were treated with 0.1% DMSO or 100 nM vincristine (VCR) for the times indicated. Cells were stained with propidium iodide and analyzed by flow cytometry. Cell cycle distributions for ALL-5 cells are shown in graphical form, according to the color key. The graphs are representative of 3 independent experiments.

**Fig. S7. Immunoblotting for PARP and MPM2 in vehicle-treated elutriated cells.** ALL-2 (**A**) or ALL-5 (**B**) cells in G1 phase (2N) or G2/M phase (4N) were treated with 0.1 % DMSO for the times indicated and extracts subjected to immunoblotting for PARP or MPM2. Migration positions of intact and cleaved species of PARP are indicated. Vehicle or 100 nM VCR-treated KB3 cells (left two lanes) served as positive control. GAPDH was used as a loading control.

**Fig. S8. Primary ALL-2 cells in G1 phase undergo vincristine-induced death without transit to S phase**. ALL-2 cells in G1 phase obtained by centrifugal elutriation were incubated with EdU and either 0.1% DMSO or 100 nM vincristine (VCR) for the times indicated. Cells were harvested, fixed and stained for EdU incorporation and with PI and analyzed by flow cytometry. The graphs show distribution between EdU-negative and EdU-positive for (**A**) live cells (>2N DNA) or (**B**) dead cells (<2N DNA), expressed as percentage of total cells. Results are expressed as mean ± S.D. (n = 3).

**Fig. S9. Primary ALL cultures lack a quiescent subpopulation.**  ALL-5 cells were labeled with cell proliferation dye eFluor® 450 and harvested at day-zero and at daily intervals thereafter and subjected to flow cytometry, as described in Materials and Methods. The lighter black line marks the raw fluorescence data, and the colored peaks represent the data after deconvolution. Histograms shown are representative of three independent experiments.