Figure S1. Microtubule protein polymerization in the presence of plinabulin and colchicine. Cell-free microtubule-associated protein rich microtubule protein polymerized for 60 min in the presence of indicated drugs or 1% dimethyl sulfoxide (control). At least 100 microtubules per condition were counted. Error bars, standard deviation.

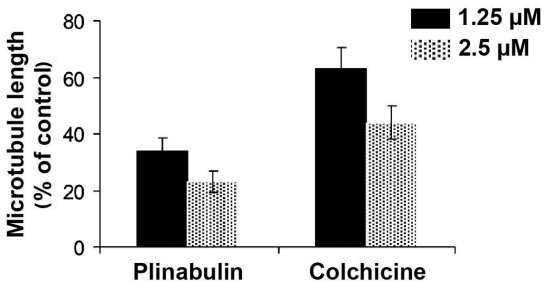

Figure S2. Plinabulin inhibits mitosis during prometaphase. MCF7 cells were treated with 10 nM plinabulin or 1% DMSO for 20 h prior to staining of α- and β-tubulin utilizing monoclonal mouse antibodies, or of DNA using 4',6-DAPI, diamidino-2-phenylindole. Narrow arrows indicate mitotic spindle polymer and mitotic chromosomes and thicker arrows indicate interphase arrays and nuclei. DMSO, dimethyl sulfoxide.

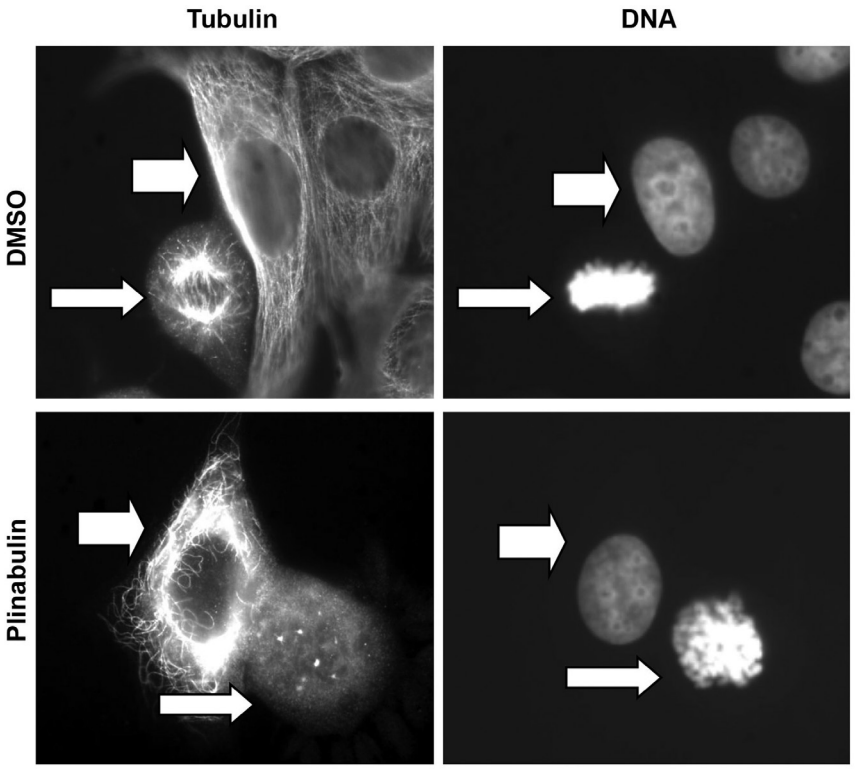

Figure S3. Co-treatment with plinabulin decreases tumor growth in murine xenotransplantation models. (A) HCT-15 colon cancer and (B) A549 non-small cell lung cancer. Tumor weights were determined upon microdissection following 28 days in N=5-10 mice pertreatment group. Two-sided t-test was performed to compare tumor weights. *P<0.05 and **P<0.01. n.s., not significant; iri, irinotecan; plin, plinabulin; doc, docetaxel.

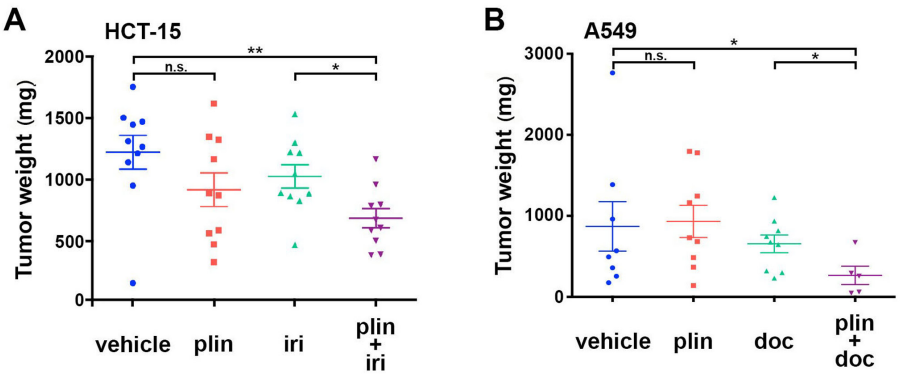

Figure S4. Distribution of plinabulin into central nervous system regions. ^{14}C-Plinabulin (5 mg/kg) was administered intravenously to male Sprague-Dawley rats. At predetermined time-points, blood was drawn, and animals were euthanized (N=3 per timepoint) and deep-frozen in a mixture of hexane and dry ice for 20 min and 30 μm sections were collected for quantitative autoradioautoluminography. Each section was exposed to an imaging plate for 4 days, in a lead box and refrigerated at ~4˚C to minimize background radiation artifacts. Following exposure, the imaging plate was read by the BAS-2500 scanner and its Image Reader software version 1.1 (both from Fuji, Tokyo, Japan). From the autoradioluminograms obtained, the amount of radioactivity in the specified tissues was quantified from each animal with reference to calibration curves generated by known ^{14}C-glucose blood standard solution radioactivity concentrations.