Supplementary Figure 1. GSK461364A exhibits potent, time-dependent inhibition of Plk1.
Representative concentration-response plots for inhibition of full-length Plk1 by GSK461364A in the presence (open circles) and absence (closed circles) of a 60-minute enzyme-inhibitor preincubation. Plk1 activity was determined using the Z-lyte kit (Invitrogen, Carlsbad, CA) as described in Experimental Procedures. IC50 values were determined using two parameter fit (Hill coefficient and IC50) using GraFit software (Erithacus).

Supplementary Figure 2. Kinase selectivity of GSK461364A
The inhibition of 48 recombinant protein kinases was characterized using catalytic activity assays. pIC50 = -Log10(IC50).

Supplementary Figure 3: Activity of Plk1 inhibitor GSK461364A is specific for proliferating cells. Human Umbilical Vascular Endothelial Cells (HUVEC) were treated with GSK461364A at different concentrations in a 3-day assay and cell number determined by CTG assay. A) Cell were maintained with required supplements in exponentially growing phase (sub-confluent and proliferating HUVEC) or B) arrested in G0/G1 phase of the cell cycle by contact inhibition (confluent and non-proliferating HUVEC).

Supplementary Figure 4: Immunoblotting Analysis of GSK461364A treated tumor xenografts. Colo205 tumor xenografts harvested 24 and 48hrs post-dose1 after treatment with vehicle, 25mg/kg, 50mg/kg, or 100mg/kg GSK461364A. Data represents average & St Dev of n=3 tumors. Tumors were harvested and lysed, and lysates were immunoblotted for detection of phospho-Histone H3 (pHH3) and β-tubulin. Data represents the corrected (β-tubulin) and normalized average & St. Dev. of n=3 tumors. Results demonstrates a dose-dependent increase in mitotic marker pHH3. At Day1 post-dose, pHH3 levels for 100mg/kg treated mice are lower than for 50mg/kg, but increase by 48hrs post-dose.