Supplementary information

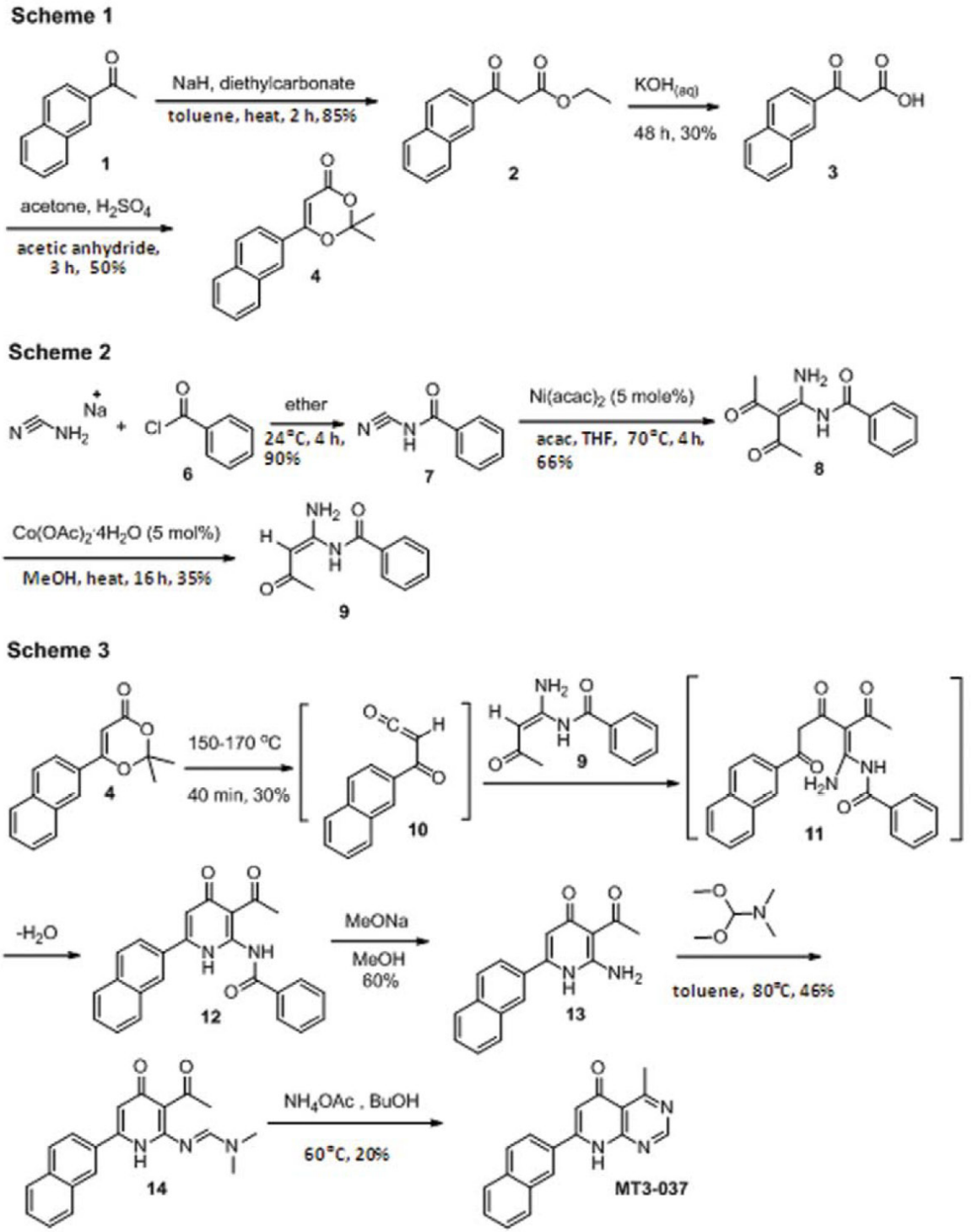
Materials and methods

Quantitative reverse transcription PCR (qRT-PCR)

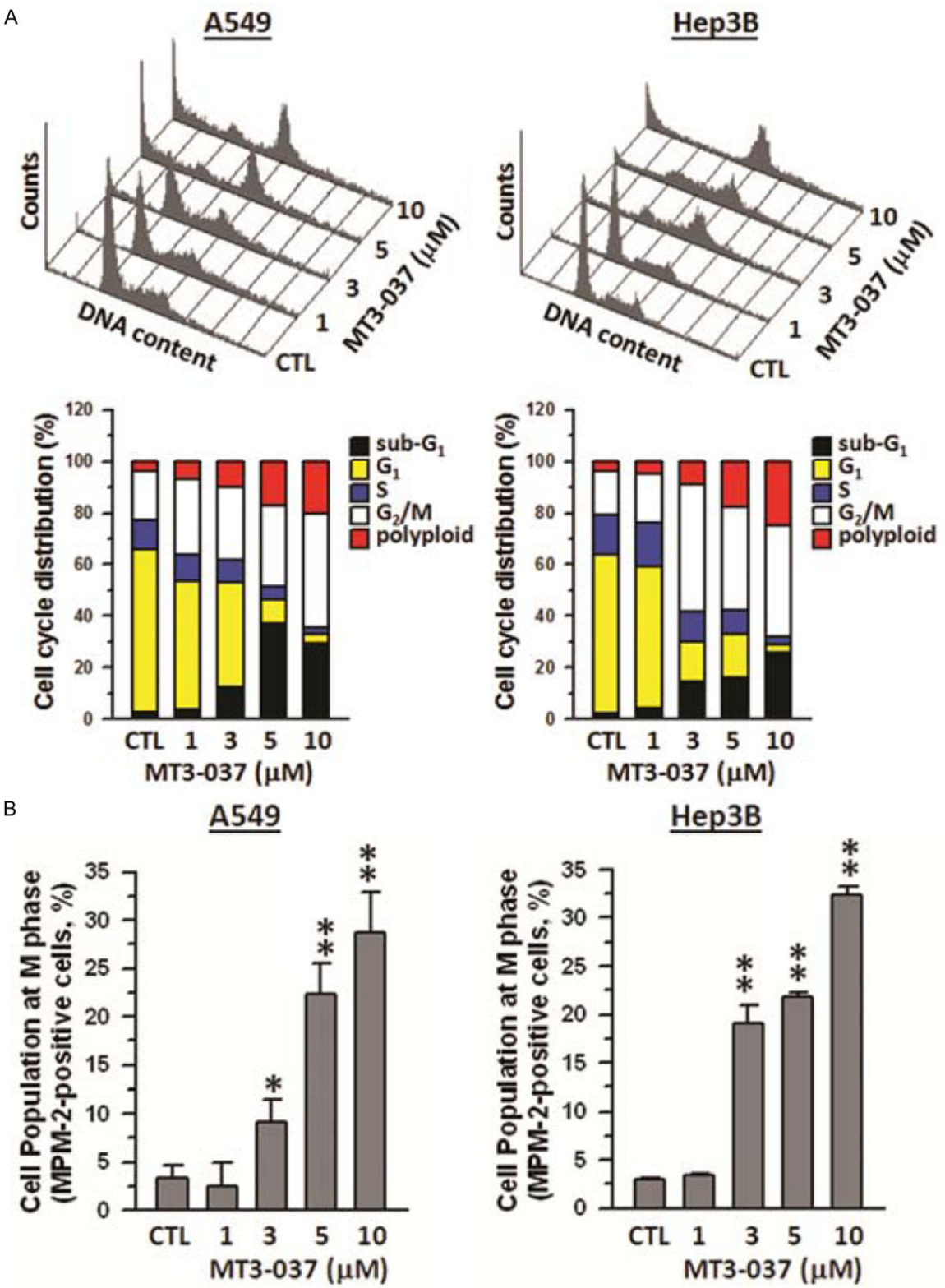
Using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA, USA), the total RNA was isolated from cells that had been treated with MT3-037. cDNA was reverse transcribed with an oligo dT(15) primer and M-MLV reverse transcriptase (Invitrogen). Quantitative PCR analysis was performed with a LightCycler 480 II RTPCR system (Roche Applied Sciences, Manheim, Germany) using the Fast Start DNA Master Plus SYBR Green I kit (Roche Applied Sciences). PCR primers were as follows: human *CDK1* (XM_006718082.1) 5'-TTTTCAGAGCTTTGGGCACT-3' (forward), 5'-AAATCCAAGCCATTTTCATCC-3' (reverse); human *cyclin B1* (NM_031966.3), 5'-CGGGAAGTCACTGGAAACAT-3' (forward), 5'-AAACATGGCAGTGACACCAA-3' (reverse); and human *GAPDH* (NM_002046.3) 5'-AGCCACATCGCTCAGACAC-3' (forward); 5'-GCCCAATACGACCAAATC-3' (reverse). The mRNA expression levels were normalized to the level of *GAPDH* mRNA in the same sample.

Immunoprecipitation

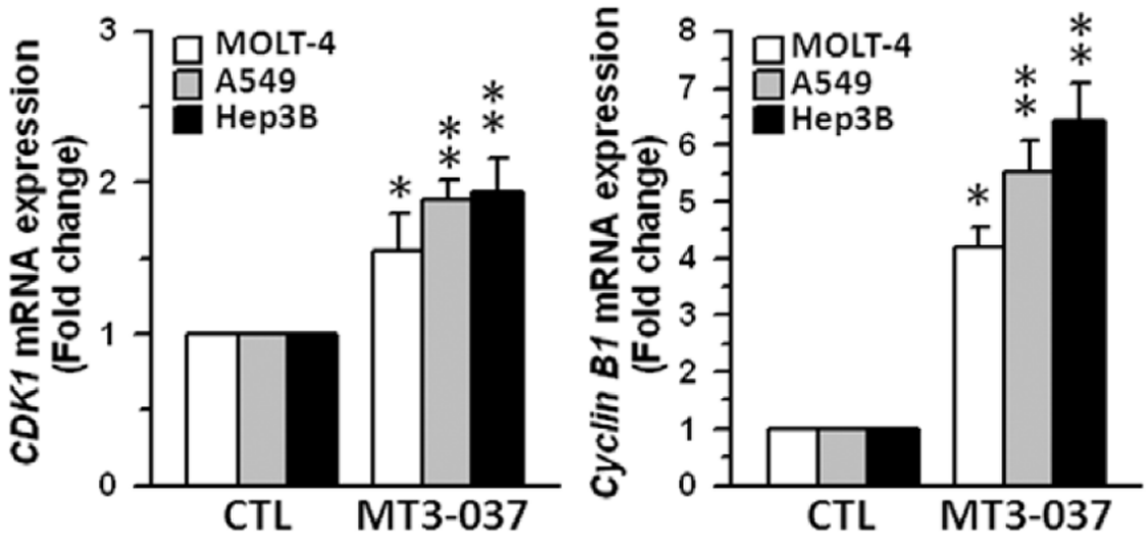
Cells were lysed with buffer containing 50 mM Tris-Cl (pH 7.5), 1% (v/v) Igepal CA-630, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 5 mM NaF, 1 mM PMSF, and 5 μg/ml each of leupeptin, aprotinin, and pepstatin A, and then sonicated. Cell lysates were incubated at 4°C overnight with specific primary antibodies and protein A-Sepharose beads (GE Healthcare Life Sciences, Pittsburgh, PA, USA). After washing with the same buffer, precipitated proteins were boiled in 1 × Laemmli sample buffer and then separated by SDS-PAGE.



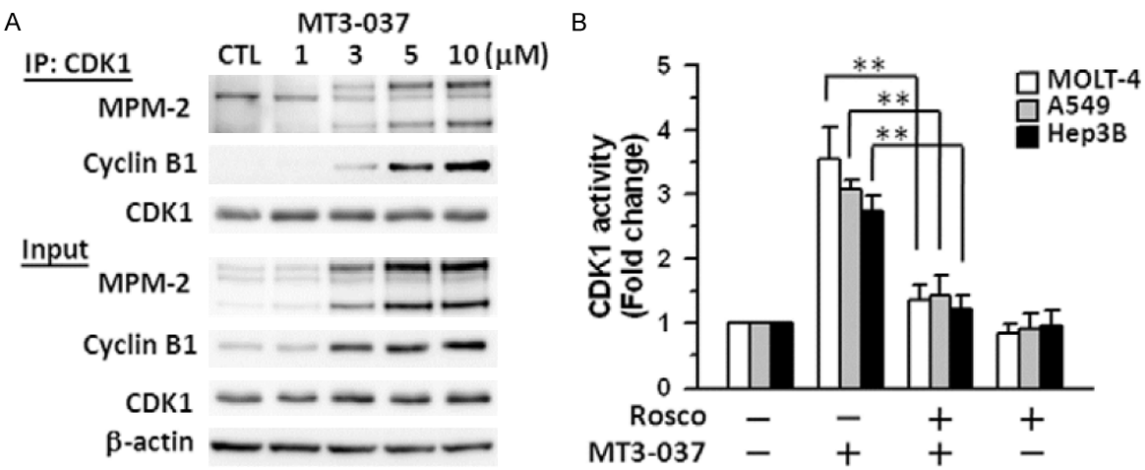
Supplementary Figure 1. Synthesis steps for MT3-037. Compound 4 was readily prepared from commercially available 2'-acetonaphthone (Sigma-Aldrich) by synthetic Scheme 1. Carboethoxylation of compound 1 followed by hydrolysis with KOH(aq) gave acid 3, which was subjected to acetal formation to produce dioxinone 4. Compound 9 was prepared via three-step sequence outlined in Scheme 2. For the synthesis of MT3-037 shown in Scheme 3, dioxinone 4 was reacted with compound 9 to give pyridone 12. Hydrolysis of pyridine 12 by sodium methoxide gave compound 13, which was in turn heated with N, N-dimethylformamide dimethylacetal to generate compound 14. Finally, cyclization of compound 14 gave MT3-037.



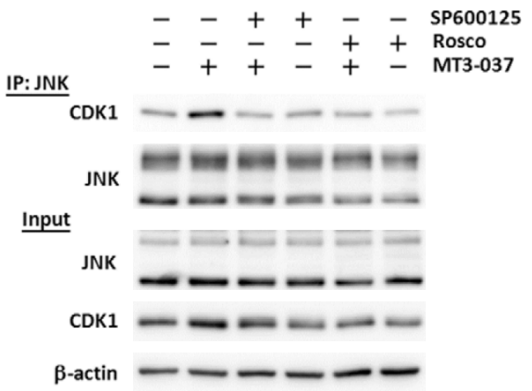
Supplementary Figure 2. MT3-037 causes the cell cycle arrest. A. A549 and Hep3B cells were treated with vehicle (DMSO, CTL) or different concentrations of MT3-037 for 24 h, and the DNA content/cell cycle distribution was assessed by flow cytometry. B. Expression of mitotic proteins in MT3-037-treated MOLT-4 cells. Cells were harvested after 24 h incubation with the indicated concentrations of MT3-037. MPM-2- and propidium iodide-stained cells were examined by flow cytometry. $*P < 0.05$; $**P < 0.01$, compared with control (untreated) values for the corresponding incubation period.



Supplementary Figure 3. MT3-037 increases the *CDK1* and *cyclin B1* gene expression. Cells were treated with 5 μM MT3-037 for 12 h and then collected for detection of *CDK1* and *cyclin B1* mRNA with qRT-PCR. **P* < 0.05; ***P* < 0.01, compared with control (untreated).



Supplementary Figure 4. MT3-037 increases the association of MPM-2, cyclin B1, and CDK1. A. MOLT-4 cells were pretreated with 30 μM SP600125 or 20 μM roscovitine and then treated with 5 μM MT3-037 for 18 h. Cells were harvested and lysed for immunoprecipitation (IP) with anti-CDK1. B. Roscovitine inhibits the MT3-037-induced CDK1 kinase activity. Cells were pretreated with 20 μM MT3-037 for 18 h. Cells were collected for measurement of CDK1 kinase activity using CDK1 Kinase Assay Kit (MBL International). ***P* < 0.01.



Supplementary Figure 5. MT3-037 increases the interaction of JNK and CDK1. MOLT-4 cells were pretreated with 30 μM SP600125 or 20 μM roscovitine and then treated with 5 μM MT3-037 for 18 h. Cells were harvested and lysed for immunoprecipitation (IP) with anti-JNK.