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Supplemental Material to:

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CDK1 stabilizes HIF-1α via direct phosphorylation of Ser668 to promote tumor growth

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Supplemental Figures

Supplemental Figure 1. Ro-3306 inhibits CDK1 activity and causes G2/M phase arrest after prolonged treatment. A) HCT116 cells were treated with DMSO or Ro-3306 (5 μM) for the indicated times. The cells then stained with PI and flow cytometry was used to assess the cell cycle profiles (number in the top right corner of the histograms represents the % of cells in G2/M phase). B) *In vitro* kinase assays were performed to determine the IC50 of Ro-3306 against CDK1/cyclin B1, using Histone H1 as a substrate (n=3).

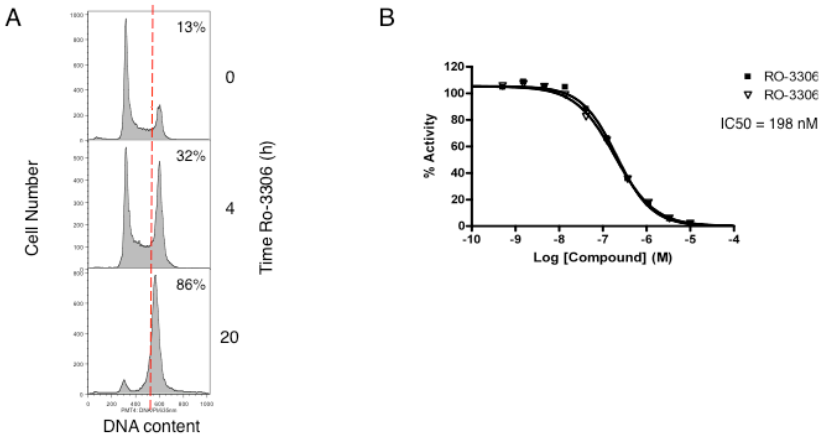
Supplemental Figure 2. Synchronization and release of HCT116 cells. Representative histograms of HCT116 cells after synchronization in S-phase (double thymidine block) and release in DMEM medium containing 10% FBS. A = asynchronous population.

Supplemental Figure 3. Characterization of RKO cells stably expressing HIF-1α constructs. A) RKO cells stably expressing the indicated HIF-1α constructs were cultured in normoxia or hypoxia for 6 h, and western blotting and RT-PCR was used to monitor the expression of endogenous and exogenous HIF-1α protein and B) mRNA . C) One thousand cells from each of the indicated stable cell lines were plated in a 96 well plate. On 5 consecutive days, CellTiter-Glo was added to three wells containing each cell line, and bioluminescence was used to measure the number of viable cells per well. D) RKO cells stably expressing the indicated HIF-1α constructs were cultured in normoxia or hypoxia and mRNA was isolated after 6 h. VEGF mRNA expression was measure by RT-PCR, and relative levels VEGF were calculated using densitometry.

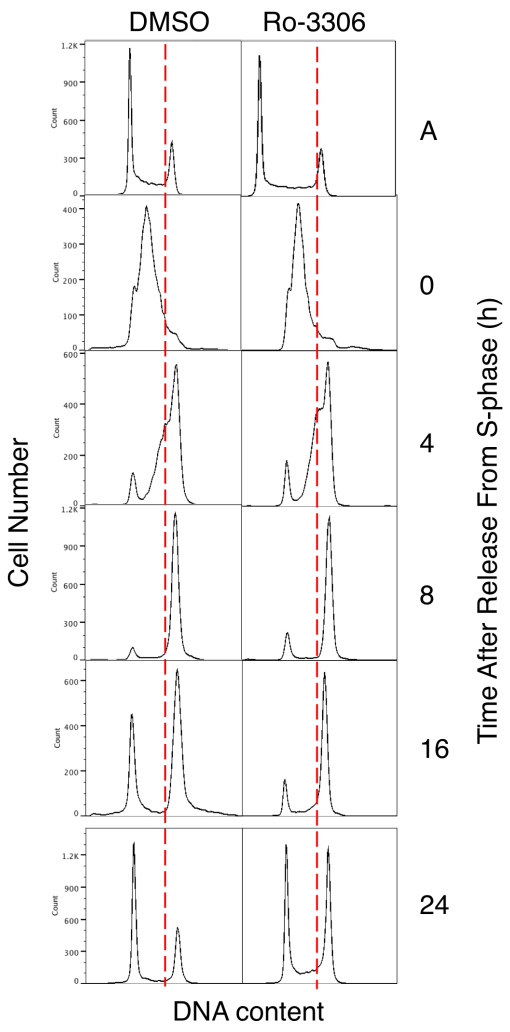
Supplemental Figure 4. Characterization of HCT116 cells stably expressing HIF-1α constructs. A) HCT116 cells stably expressing the indicated HIF-1α constructs were cultured in normoxia or hypoxia for 6 h, and western blotting and RT-PCR was used to monitor the expression of endogenous and exogenous HIF-1α protein and B) mRNA . C) One thousand cells from each of the indicated stable cell

lines were plated in a 96 well plate. On 5 consecutive days, CellTiter-Glo was added to three wells containing each cell line, and bioluminescence was used to measure the number of viable cells per well.

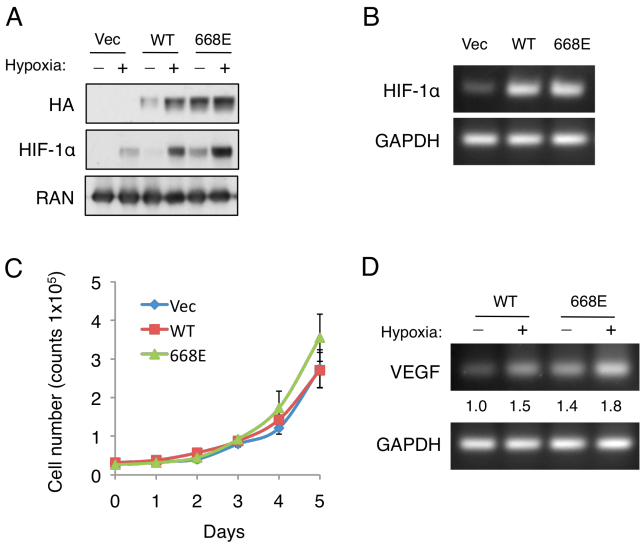
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4