Supplemental figure legends

Figure S1. CDK1 expression is downregulated during EB- or RA-induced hESC differentiation. (**a**) mRNA levels of differentiation markers by qRT-PCR to confirm hESCs underwent effective EB-mediated differentiation. (**b**) hESC-derived EB images of which CDK1 was downregulated but did not affect proliferation during EB-induced differentiation. (**c**) Flow cytometry comparison of NANOG, OCT4, and CDK1 levels with EB-mediated differentiation (left panel) or RA-mediated differentiation of hESCs (right panel). (**d**) Flow cytometry comparison of NANOG, OCT4, and CDK1 levels at day 6 and 12 in NCCIT differentiation with EB formation together with addition of RA (left panel) or RA directly induced differentiation of NCCIT (right panel).

Figure S2. Downregulation of CDK1 induces differentiation. (**a**) Representative images of undifferentiated (upper left) or differentiated (upper right) and AP(+) (lower left) or AP(−) (lower right, arrows) hESC with or without RO3306 treatment. (**b**) Xenograft teratocarcinoma formation capacity by NCCIT cells that were pre-treated with vehicle (14 injections) or 0.5 μM JNJ-7706621 (13 injections). Statistical analysis was used to compare the percentage of formed tumor versus injection.

Figure S3. Downregulation of CDK1 in hESCs shows no significant effects on cell cycle arrest. (**a**) Quantification of the G_1, S, and G_2/M cell phases by BrdU- and PI-labeled DNA content in hESCs with shCDK1 knockdown and CDK1 inactivation by RO3306 (8 μM). The mean ± SD was from 2-3 independent experiments. (**b**) Cell phase distribution by BrdU in hESCs with knockdown of CDK1 by different oligos of shCDK1. (**c**) Immunoblot analysis of CDK1 by different oligos of shCDK1. (**d**) BrdU-labeled cell cycle of hESCs that were treated

with 8 μM RO3306 for 16 and 28 h. (**e**) Immunoblot analysis of CDK1 and cyclin B1 expression in hESCs that were treated with RO3306.

Figure S4. Correlation between differentiation and apoptosis in the CDK1 inactivation-mediated differentiation of hESCs. (**a**) Analysis of live, early apoptotic, and dead cell populations by MitoProbeDilC1(5) assay in hESCs that were transiently transfected with shControl or shCDK1 for 3 days or treated with low doses of RO3306 (2 μM) for 3 days. (**b**) Quantitation of live, early apoptotic, and dead cell populations in NCCIT cells that were treated with RO3306 (4 μM) for 4 days. (**c**) Percentage of TRA-1-60 negative and positive populations based on their proportion in each live, early apoptotic, and dead population in hESCs that were treated with or without RO3306 (8 μM) for 2 days. (**d**) Quantitation of the live, early apoptotic, and dead cell populations of (**c**). (**e**) Overall NANOG expression in in hESCs that were treated with RO3306 (8 μM) for 2 days.

Figure S5: Low dose of CDK1 inhibitor RO3306 does not directly suppress Akt activity. (**a**) Immunoblot analysis of the phosphorylation of Akt at Thr308 and Ser473 in hESCs that were treated with or without RO3306.

Figure S6: Downregulation of CDK1 induces a metabolic switch. (**a**) qRT-PCR of glycolytic genes, GLUT1, PFK1, HK2, and LDHA in CDK1 knockdown (by shRNA) or inactivation (by RO3306 treatment) hESCs. A statistical comparison was made between shCtrl and shCDK1 or DMSO (Ctrl) and RO3306. (**b**) hESCs were treated with or without RO3306 (6-8 μM), and the lactate concentration in the medium was quantitated at day 3. (**c**) qRT-PCR of glycolytic genes in RO3306 and MEK/ERK inhibitor UO126 treated hESCs. A statistical comparison was made between Ctrl, RO3306, and RO3306 + UO126. (**d**) qRT-PCR of glycolytic

genes in RO3306 and PS48 treated hESCs. A statistical comparison was made between Ctrl, RO3306, and RO3306 + PS48.

Figure S7: Role of Cyclin B1/CDK1 complex in iPSC formation. (**a**) AP(+) iPSC colonies that were reprogrammed from human fibroblast cells which were pre-transfected with vector and cyclin B1. (**b**) SSEA4, OCT4, NANOG, and TRA-1-60 expression by flow cytometry in nascent and replating iPSCs that were derived pre-transduced with vector or cyclin B1. (**c**) Liver cancer cells (97L) were pre-transduced with vector or cyclin B1 followed by iPSC generation. BrdU- and PI-labeled DNA content in nascent and replating iPSCs. (**d**) Phase contrast images of the formed cyclin B1-expressing iPSC colonies that were transfected with the reprogramming factors OSK and p53 shRNA but without LIN28 or L-MYC.