Supplementary figure legends

Figure S1. Generation of cyclin A2 conditional knockout mice. The genomic locus of cyclin A2 (*I*) was modified in ES cells using the targeting vector (*II*). LoxP recombination sites (red triangles) along with an FRT (green rectangles)-flanked neomycin-selection cassette were introduced flanking exons 3 and 5. Expression of FLP recombinase results in removal of the neomycin cassette (*III*). Expression of Cre recombinase results in excision of exons 3-5 (I*V*) and a frame shift, generating a null allele (A). Following StuI digestion, 5′ and 3′ probes located outside of the targeting vector (Chr3: 20802167-20802566 bp; PKB997 and Chr3: 20812767-20813766 bp; PKB998) were used for Southern blot analysis, resulting in 12.7 kb (5′) for wild type or 7.4 kb (5') for the floxed locus (B). Cyclin $A2^{flox}$ mice were crossed to the Rosa26-CreERT2 strain and cyclin A2 loss in fibroblasts derived from cyclin $A2^{WT/flox}$Rosa26-$CreERT2^{Tg/Tg}$ or cyclin $A2^{flox/flox}$Rosa26-$CreERT2^{Tg/Tg}$ embryos was induced by addition of 4-OHT. PCR genotyping with primers P1 (5'-CATCTGCTCATCTCTGTGGAGG-3'; PKO1680), P2 (5'-GTGGTGATTCTGTGTGTGTGAG-3'; PKO1681) and P3 (5'-CTGAACCTCTCAGCCCCTATCC-3'; PKO1682) demonstrates complete loss of the cyclin A2 floxed band (C). RNA and protein samples were collected at different time points after addition of 4-OHT and analyzed by RT-PCR and Western blot to monitor cyclin A2 loss (D). Cyclin $A2^{flox}$ mice were crossed with Albumin-Cre transgenic mice and sections from wild type and cyclin A2 knockout liver were stained with Feulgen to visualize nuclei (E). Protein extracts were prepared from individual wild type and cyclin $A2^{Liv-/-}$ liver tumors (isolated at the indicated months) and subjected to SDS-PAGE followed by Western blots with the indicated antibodies (F).

Figure S2. Tumorigenesis using transformed MEFs. Cyclin $A2^{flox}$ and $Cdk2^{null}$cyclin $A2^{flox}$ MEFs were transformed with activated Ras/$p53^{DN}$ and treated with 4-OHT to induce cyclin A2 knockout. Protein extracts were subjected to SDS-PAGE and Western blotting with the indicated antibodies. Activated Ras signaling was confirmed by detection of phospho-Erk expression which was increased in transformed MEFs (A). Inactivation of p53 was confirmed by treating MEFs with 1 μM Adriamycin for 24 hours, followed by Western blotting of protein extracts with indicated antibodies. Increased p53 expression upon adriamycin treatment was detected in non-

transformed MEFs only. Adriamycin treatment also resulted in decreased expression of cyclin A2 (B). Cyclin $A2^{flox}$ and $Cdk2^{null}$cyclin $A2^{flox}$ MEFs were oncogenically transformed with activated Ras/c-Myc and treated with 4-OHT to induce cyclin A2 knockout. Endogenous c-Myc expression was undetectable in non-transformed MEFs. Activated Ras signaling was confirmed by detection of phospho-Erk expression (C). Transformed MEFs were assessed for colony formation (D). $Cdk2^{null}$cyclin $A2^{null}$ MEFs were found to be resistant to transformation by Ras/c-Myc. Absence of cyclin A2 in tumors generated from transformed cyclin $A2^{null}$ and DKO MEFs was confirmed by Western blot (E) and immunohistochemistry (F). Immunohistochemistry images are representative of 4 tumors analyzed per genotype.

Figure S3. Deletion of cyclin A2 in growing tumors. Nude mice with growing tumors from cyclin $A2^{WT}$Rosa26-CreERT2 or cyclin $A2^{flox}$Rosa26-CreERT2 MEFs transformed with activated Ras/$p53^{DN}$ were implanted with Tamoxifen pellets (A-B). Cyclin $A2^{flox}$Rosa26-CreERT2 tumors grew at decreased rate in the presence of Tamoxifen. Mice in (A-B) were photographed 4 weeks after MEF allograft. Data is representative of 2 independent MEF clones and 6-8 nude mice were used per genotype. Tumors were genotyped for cyclin A2 and Cre-mediated excision of cyclin A2 was confirmed by the presence of "Null" band (C).

Figure S4. Premature senescence in $Cdk2^{null}$cyclin $A2^{null}$ MEFs. Cyclin A2 loss was induced in cyclin $A2^{flox}$ and $Cdk2^{null}$cyclin $A2^{flox}$ MEFs by addition of 4-OHT. Acidic β-galactosidase staining at various passages reveals premature senescence in $Cdk2^{null}$cyclin $A2^{null}$ MEFs .

Figure S5. FACS analysis of primary MEFs. MEFs were synchronized at G0/G1 by serum starvation for 72 hours and released into S phase by serum addition. Cyclin A2 loss was induced by addition of 4-OHT during serum starvation. Following BrdU pulse labeling, cells were stained with propidium iodide (PI) and analyzed by FACS to determine the percentage of cells in S, G1 phase, and cells with 4n DNA content. $Cdk2^{null}$cyclin $A2^{null}$ MEFs displayed a small increase in

cells with 4n DNA content, defective S phase entry (as seen in Cdk2^{null} MEFs) and delayed exit from S phase (as seen in cyclin A2^{null} MEFs).

Figure S6. Defective and error-prone recovery from quiescence in late passage Cdk2^{null}cyclin A2^{null} cells. Proliferative potential of passage 30 3T3 MEFs was measured by alamarBlue proliferation assays in normal growth medium containing 10% serum (A) and in growth medium containing 1% serum (B). Cdk2^{null}cyclin A2^{null} MEFs were unable to grow in 1% serum. Comparative cell cycle profile analysis of primary and passage 30 Cdk2^{null}cyclin A2^{null} MEFs is shown in (C). MEFs were synchronized at G0/G1 by serum starvation for 72 hours and released into S phase in the presence of 10% serum. Following BrdU pulse labeling, cells were stained with propidium iodide (PI) and analyzed by FACS to determine the percentage of cells in S, G1 phase, and cells with 4n DNA content. The FACS profile shown for "released" MEFs (bottom panel) is at 72 hours after release from serum starvation. Extensive apoptosis observed in passage 30 MEFs is absent in primary MEFs (C).

Figure S7. FACS analysis after induction of cyclin A2 loss in immortalized MEFs. Immortalized passage 30 cyclin A2^{flox} and Cdk2^{null}cyclin A2^{flox} MEFs were synchronized in G0/G1 phase by serum starvation for 72 hours and simultaneously treated with 4-OHT to induce cyclin A2 knockout. Immortalized MEFs were released into S phase by serum addition and were collected at the indicated time points. Following BrdU pulse labeling, cells were stained with propidium iodide (PI) and analyzed by FACS to determine the percentage of cells in S, G2/M, and G1 phase. Entry into the cell cycle is associated with extensive apoptosis and re-replication upon loss of Cdk2 and cyclin A2.

Figure S8. Biochemical analysis of primary MEFs and primary liver tumor cells. Primary MEFs were synchronized in G0/G1 phase by serum starvation for 72 hours and simultaneously treated with 4-OHT to induce cyclin A2 knockout. MEFs were released into S phase by serum addition and were collected at the indicated time points. Protein extracts were prepared and subjected to SDS-PAGE followed by Western blotting with the indicated antibodies (A). Quantitative analysis of Cdk1-associated activity of primary MEFs in Fig. 5A is presented in (B). *Student's t-test indicating statistically significant increase in Cdk1 activity in cyclin A2^{null} MEFs

when compared to cyclin $A2^{flox}$ MEFs at the corresponding time point, using three independent assays performed with three different MEF clones; $p<0.05$ (B). Protein extracts from primary MEFs were subjected to co-immunoprecipitation with antibodies against Cdk2, followed by SDS-PAGE and Western blotting with the indicated antibodies (C). Cyclin A2 knockout in unsynchronized primary liver tumor cells was achieved by addition of 4-OHT, whereas Cdk2 was silenced by retroviral shRNA transduction. Protein extracts were subjected to Western blotting with the indicated antibodies (D). Cdk1-associated kinase activity in protein extracts of unsynchronized primary liver tumor cells was measured using radiolabeled ATP and histone H1 as substrates. *Student's t-test performed on three independent kinase assays using two different tumor cell lines indicating statistically significant increase in Cdk1 activity in cyclin $A2^{null}$ cells when compared to cyclin $A2^{flox}$ cells; $p<0.05$ (E). Data in (D-E) is representative of two tumor cell lines established from two mice. NPIU: normalized phosphoimager units.