SUPPLEMENTAL MATERIALS AND METHODS

Additional antibodies. Anti-A-RAF (Santa Cruz), anti-C-RAF and anti-$p27^{Kip1}$ (both BD Transduction labs) and $p57^{Kip2}$ antibodies (NeoMarkers) were utilized.

Lentiviral shRNA constructs. Control non-targeting and FOXD3 shRNAs were recombined into pLentineo/BLOCK-iT™-DEST, which was made by substituting the SV40/zeocin-resistance gene cassette for the human PGK promoter driving the neomycin-resistance gene. Lentivirus particles were packaged and used as above, with the exception of cells transduced with pLentineo/BLOCK-iT™-DEST-derived viruses, which were selected with 500 μg/ml G418 for two weeks.

Propidium iodide staining. Cells were trypsinized, washed and fixed in 70% ethanol for 4 hours. Cells were then permeabilized with PBS containing 0.1% Triton X100 and 200 μg/ml RNase A for 2 hours at 37°C and stained with 167 μg/ml propidium iodide for 30 min. DNA content was measured by flow cytometry on a BD FacsCanto™ FACSDiva software.

Supplemental Table 1: Oligonucleotide sequences for shRNA

Target	Oligonucleotide sequences
LacZ	5'-CACCAAATCGCTGATTTGTGTAGTCGGAGACGACTACACAAATCAGCGA-3' 5'-AAAATCGCTGATTTGTGTAGTCGTCTCCGACTACACAAATCAGCGATTT-3'
Non-targeting	5'-CACCGTAGCGACTAAACACATCAATTCAAGAGATTGATGTGTTTAGTCGCTA-3' 5'-AAAATAGCGACTAAACACATCAATCTCTTGAATTGATGTGTTTAGTCGCTAC-3'
B-RAF$^{\#1}$	5'-CACCACAGAGACCTCAAGAGTAATTCAAGAGATTACTCTTGAGGTCTCTG-3' 5'-AAAACAGAGACCTCAAGAGTAATCTCTTGAATTACTCTTGAGGTCTCTGT-3'
B-RAF$^{\#3}$	5'-CACCAGAATTGGATCTGGATCATTTCAAGAGAATGATCCAGATCCAATTCTTT-3' 5'-AAAAAAAGAATTGGATCTGGATCATTCTCTTGAAATGATCCAGATCCAATTCT-3'
B-RAF$^{\#7}$	5'-CACCGTATCACCATCTCCATATCATTTCAAGAGAATGATATGGAGATGGTGATAC-3' 5'- AAAAGTATCACCATCTCCATATCATTCTCTTGAAATGATATGGAGATGGTGATAC-3'
FOXD3$^{\#3}$	5'-CACCGCCGAGGACGTGGACATCGATTTCAAGAGAATCGATGTCCACGTCCTCGGC-3' 5'-AAAAGCCGAGGACGTGGACATCGATTCTCTTGAAATCGATGTCCACGTCCTCGGC-3'
FOXD3$^{\#4}$	5'-CACCGCCTAGTGAAGCCGCCTTACTTTCAAGAGAAGTAAGGCGGCTTCACTAGGC-3' 5'-AAAAGCCTAGTGAAGCCGCCTTACTTCTCTTGAAAGTAAGGCGGCTTCACTAGGC-3'
FOXD3$^{\#5}$	5'-CACCAGCATCGAGAACATCATAGGTTTCAAGAGAACCTATGATGTTCTCGATGC-3' 5'-AAAAGCATCGAGAACATCATAGGTTCTCTTGAAACCTATGATGTTCTCGATGCT-3'
FOXD3$^{\#7}$	5'-CACCGCCGTCGTTCAGCATCGAGAATTCAAGAGATTCTCGATGCTGAACGACGGC-3' 5'-AAAAGCCGTCGTTCAGCATCGAGAATCTCTTGAATTCTCGATGCTGAACGACGGC-3'

Supplemental Table 2: Primer sequences for qRT-PCR

Target	Primers	Product size (bp)
Actin	F: 5'-TACCTCATGAAGATCCTCACC-3' R: 5'-TTTCGTGGATGCCACAGGAC-3'	268
Cyclin A2	F: 5'-GAAGTACCAGACTACCATGAG-3' R: 5'-CTTCAAACTTTGAGGCTAACAG-3'	286
FOXD3	F: 5'-CATCCGCCACAACCTCTC-3' R: 5'-CATCATGAGCGCCGTCTG-3'	196
$p21^{Cip1}$	F: 5'-GCGATGGAACTTCGACTTTGT-3' R: 5'-GGGCTTCCTCTTGGAGAAGAT-3'	352

SUPPLEMENTAL FIGURES

Supplemental Figure 1. *A,* WM793TR Ctl shRNA and B-RAF shRNA cells were treated with doxycycline for 0, 1, 2, 3 and 4 days. Cell lysates were analyzed by Western blotting for B-RAF, C-RAF, phospho-ERK1/2 and total ERK1/2. *B*, WM793TR cells expressing B-RAF (hairpins #1, #3, and #7) shRNA were induced/non-induced for 6 days. Cells were lysed and samples analyzed by Western blotting for A-RAF and other targets as in A.

Supplemental Figure 2. Inducible expression of Foxd3 and LacZ in human melanoma cells. *A*, WM115TR, *B*, SK-MEL-28TR, and *C*, A375TR cells expressing doxycycline-inducible Foxd3 or LacZ were treated with or without 0.1 μg/ml doxycycline for 5 days. Lysates were Western blotted for the V5-epitope and ERK1/2.

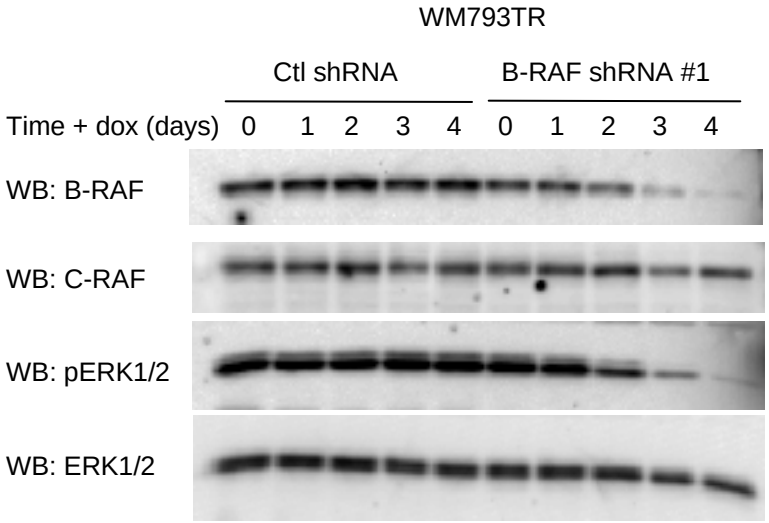
Supplemental Figure 3. *A*, WM793TR cells expressing LacZ or Foxd3 were induced with doxycycline for 5 days. Cells were fixed, permeabilized, treated with RNase A and stained with propidium iodide. DNA content was measured by flow cytometry. Bars indicate the percent of cells in G0/G1 (black), S phase (grey) or G2/M (white). Results represent the average and standard error of 4 independent experiments (* $p<0.05$). *B*, WM793 cells were treated with DMSO or AZD6244 (3.3 μM) for 24 hours. Cells were processed as in *A*. Results represent the average and standard error of 3 independent experiments. Significance between DMSO and AZD6244 treatment is indicated for G0/G1 (*** $p<0.001$), S (** $p<0.01$) and G2/M (* $p<0.05$) using two-tailed T-test.

Supplemental Figure 4. Regulation of CDK inhibitors by Foxd3 expression. Melanoma cells (WM793TR, WM115TR, and SK-MEL-28TR) were induced to express either β-galactosidase or Foxd3 for 5 days. Cell lysatses were analyzed by Western blotting for $p21^{Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$ and total ERK1/2 (loading control).

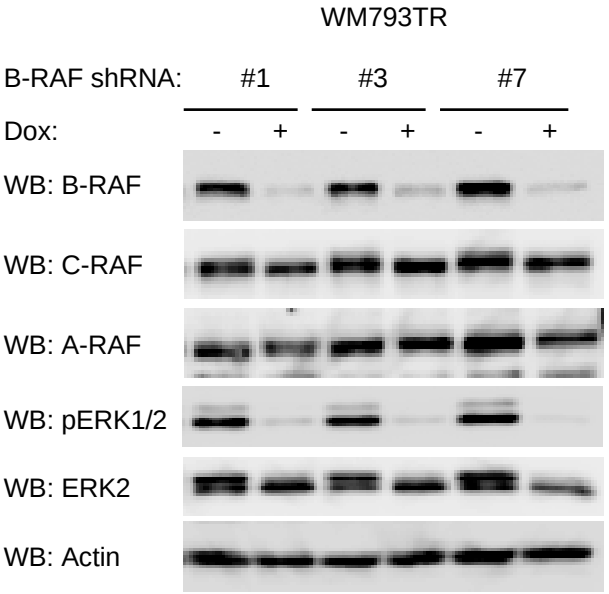
Supplemental Figure 5. FOXD3 knockdown is not sufficient to prevent AZD6244-induced growth arrest. *A*, WM793TR cells expressing a doxycycline-inducible non-targeting shRNA (Ctl) or shRNAs targeting FOXD3 were induced with doxycyline for 3 days. Next, cells were treated with 3.3 μM AZD6244 for 48 hours, lysed and Western blotted for FOXD3, phospho-ERK1/2 and total ERK1/2. *B*, Cells, as above, were incubated with 10 μM EdU for 8 hours, and analyzed for EdU incorporation by flow cytometry. Bar graphs represent the mean of three independent experiments and standard errors.

Abel & Aplin, 2009
Supplemental Figure 1

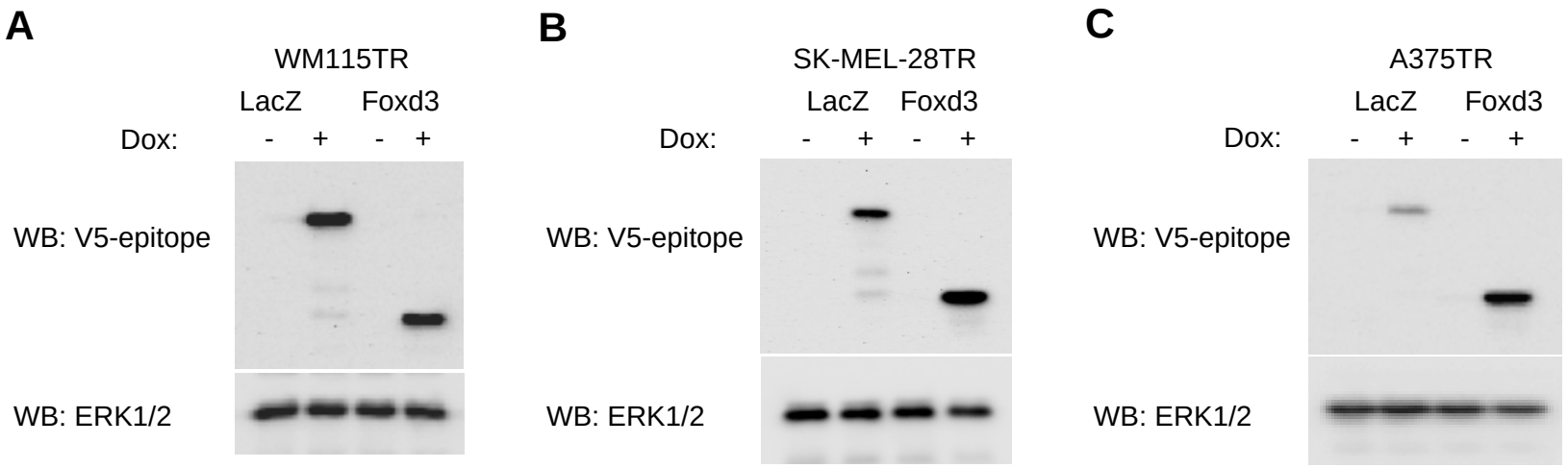
A



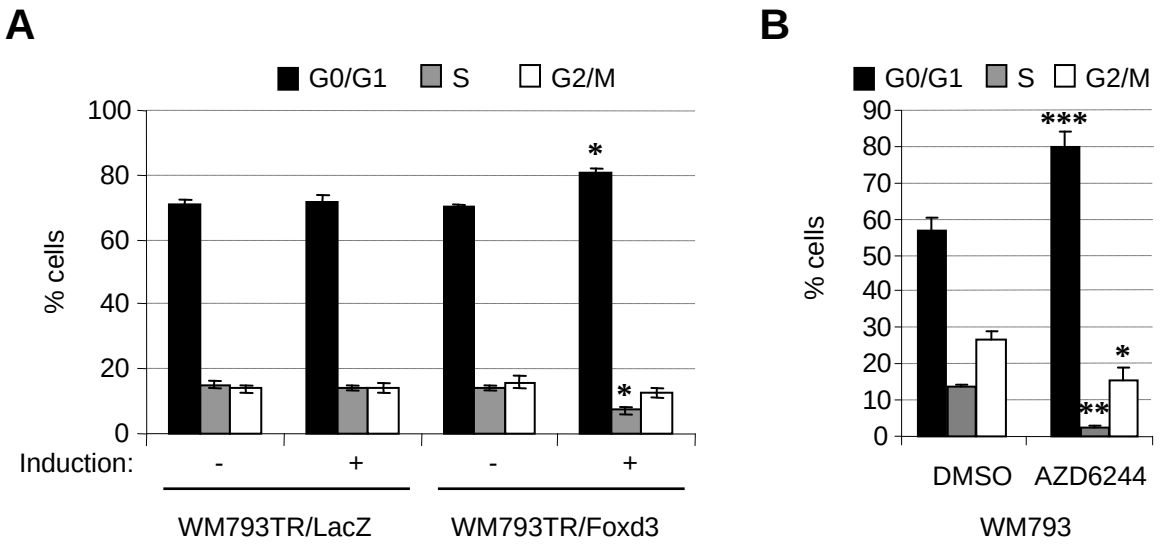
B



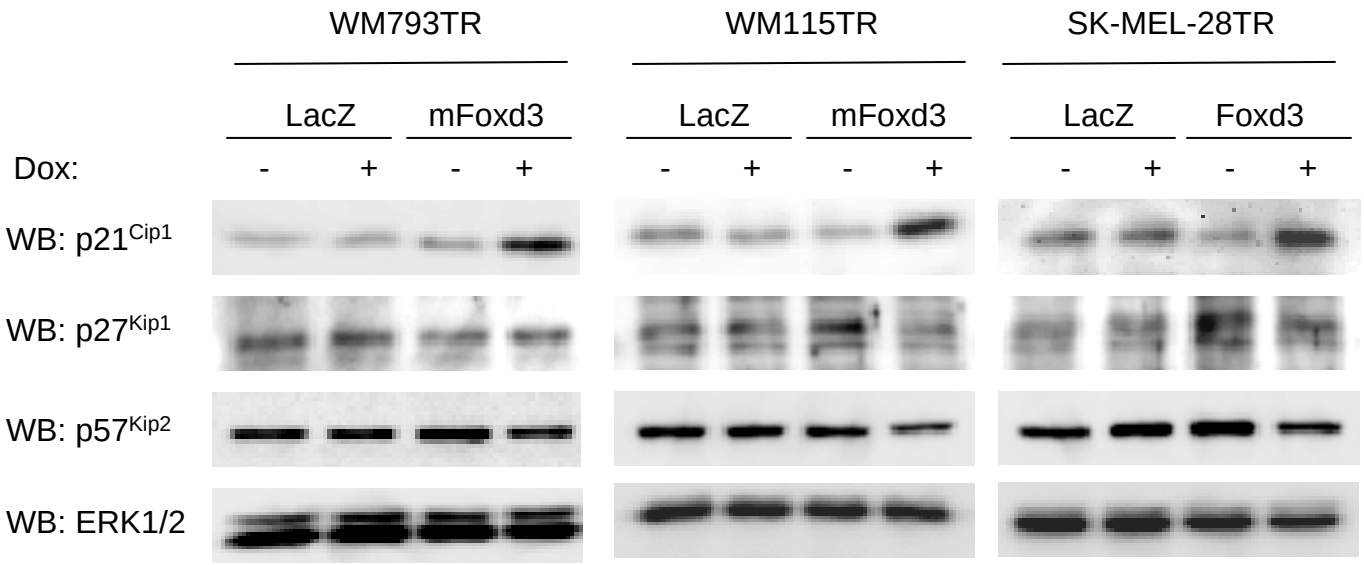
Abel & Aplin, 2009
Supplemental Figure 2



Abel & Aplin, 2009
Supplemental Figure 3



Abel & Aplin, 2009
Supplemental Figure 4



Abel & Aplin, 2009
Supplemental Figure 5