Supplementary Figure S1. p15, p16, and Ki-67 expression in a benign BRAF(V600E) nevus. Immunohistochemistry staining for p15, p16, Ki-67, and BRAF(V600E) expression in a representative benign intradermal nevus. Photomicrographs taken from 10-μm-thick sections from a FFPE tissue block. Scale bars = 100μm.

Supplementary Figure S2. Active CDK4 reverses BRAF(V600E)-mediated melanocyte growth arrest. A) Ki-67 expression (green) in nevus MC (left) and nevus MCs transduced with *CDK4*(R24C) (right). Cells were counterstained with Mel-A (red) and DAPI (blue). **B**) Percent Ki-67 positive cells in normal MCs, nevus MCs, and nevus MCs expressing CDK4(R24C). n = 3 biological replicates per experiment. *P<0.05. Scale bars, 10μm. **C**, Relative proliferation of diBRAF(V600E) MCs transduced with either a luciferase control (Luci) or activated CDK4 (CDK4(R24C)), and treated with or without 0.25μg/mL doxycycline. Values reflect the average ± SEM of n = 3 biological replicates.

Supplementary Figure S3. BRAF(V600E), p15, and p16 expression in normal and nevus MCs. Immunoblot of total cell extracts of normal and nevus-derived melanocytes for BRAF(V600E), p16, and p15. β–Actin blot is shown as loading control.

Supplementary Figure S4. Engineered p15 and p16 expression in melanocytes **A**) Immunoblot of total cell extracts from melanocytes transduced with or without lentiviral p15 (left) or p16 (right). β–Actin is shown as loading control. **B**) Relative viral titers of luciferase control, p15, and p16 lentiviruses transduced into human melanocytes. **C**) Relative luciferase, p15, and p16 mRNA expression following transduction of each virus into human melanocytes. **D**) Rb and phospho-Rb immunoblot of total cell extracts from melanocytes transduced with luciferase control, p15, p16, or both p15 and p16 lentivirus. β–Actin is shown as loading control. **E**) Growth curve of human melanocytes transduced with luciferase control, p15, p16, or both p15 and p16 lentivirus. Viral titers, relative mRNA expression, and growth curves represent the mean ± SEM for each group. n = 3 biological replicates for each group.

Supplementary Figure S5. Effects of p15 shRNA rescue and lentiCRISPR-CAS9 mediated p15 loss in diBRAF(V600E) MCs. A) Immunoblot of total cell extracts from melanocytes transduced with diBRAF(V600E) and either non-silencing shRNA (NSi), p15 shRNA (p15i), or p15 shRNA with lentiviral p15 (p15i + p15). β–Actin blot is shown as loading control. **B**) Relative proliferation of doxycycline-treated diBRAF(V600E) MCs transduced with either non-silencing shRNA (NSi), p15 shRNA (p15i), or p15 shRNA with lentiviral p15 (p15i + p15). Proliferation is relative to the initial $2.5x10^5$ cells plated for each group. n = 3 biological replicates for each group. **C**) Immunoblot of total cell extracts from diBRAF(V600E) melanocytes transduced with a control lentiviral CRISPR Cas9 and guide RNAs targeting GFP or p15 and induced with doxycycline. β–Actin is

shown as loading control. **D**) Growth curve representing relative growth of diBRAF(V600E) melanocytes transduced with either a lentiviral CRISPR-Cas9 and guide RNAs targeting GFP or lentiviral CRISPR-Cas9 and guide RNAs targeting p15, in the absence or presence of 0.25μg/mL doxycycline. Data represent the mean ± SEM for each group. n = 3 biological replicates for each group.

Supplementary Figure S6. Loss of CDKN2A and CDKN2B promotes proliferation in BRAF(V600E) Melanocytes. Relative proliferation after 6 days in culture, comparing diBRAF(V600E) MCs expressing shRNAs targeting scrambled control (NSi), p15 (p15i), p16 (p16i), p16 with simultaneous expression of dominant negative p53 (R248W), or a combination of p15 and p16 with dnp53 (p15i + p16i) in the presence or absence of 0.25ug/mL doxycycline. Data represent the mean ± SEM for each group. n = 3 biological replicates for each group.

Supplementary Figure S7. p15 and p16 Expression in Benign Nevi and Melanoma. A) Hematoxylin and eosin staining, and Immunohistochemistry (IHC) staining for Mart-1 (red) / Ki-67 (brown), p15 (brown), and p16 (red) in representative benign intradermal nevus and malignant melanoma specimens. Photomicrographs of 10-μm-thick sections are representative of 15 unique nevus cases and 12 unique malignant melanoma cases. Scale bars = 100μm. **B**) Quantification of p15 staining in 15 benign intradermal nevi and 12 malignant melanoma cases. Black bars represent the median quantified expression of p15 in the set of nevus (blue) or melanoma (red) cases. **C**) Quantification of p16 staining in n = 15 benign intradermal nevi and n = 12 malignant melanoma cases. Black bars represent the median quantified expression of p16 in either benign intradermal nevi (left side) or malignant melanoma (right side) of cases.

Supplementary Figure S8. p15 and p16 Expression in a Benign Nevus with Adjacent Melanoma. IHC staining profile Mart-1 (red) / Ki-67 (brown), p15 (brown), and p16 (red) in a malignant melanoma case (white arrow) arising in continuity with a benign intradermal nevus (black arrow). Photomicrographs of 10-μm-thick sections. Scale bars = 100μm.

Supplementary Figure S9. Erk 1/2 Target EGR-1 is Upregulated in BRAFV600E Nevi. A) Relative EGR-1 mRNA levels in normal and nevus melanocytes. Data represent the mean ± SEM for n = 3 biological replicates. *P<0.05. **B**) Relative EGR-1 mRNA levels in diBRAF(V600E) melanocytes +/- doxycycline. Data represent the mean ± SEM n = 3 biological replicates. *P<0.05. **C**) Immunoblots of total cell extracts isolated from doxycycline-inducible BRAF(V600E) (diBRAFV600E) melanocytes +/- doxycycline and +/- the MEK inhibitor UO126 showing the effects of ERK 1/2 inhibition p15 expression. Total cell extracts were blotted for BRAF(V600E), phospho-ERK 1/2, p16, and p15. β−Actin blot is shown as loading control.

Supplementary Figure S10. BRAF(V600E) inhibitor PLX4720 prevents induction of p15- mediated growth arrest but is insufficient to reverse previously-established growth arrest. **A**) Relative proliferation of diBRAF(V600E) MCs treated +/- doxycycline and +/- PLX4720. Pre-dox PLX indicates pre-treatment with 0.25ug/mL doxycycline followed by treatment with PLX4720. Dox 1uM PLX indicates simultaneous treatment with PLX4720 and 0.25ug/mL doxycycline. **B**) Relative proliferation of BRAF(V600E) positive nevus MCs treated with or without PLX4720. **C**) Immunoblot of p15 expression in total cell extracts from diBRAF(V600E) MC populations treated with or without 0.25ug/mL doxycycline and subsequently treated with 1uM PLX4720, and diBRAF(V600E) MCs treated with or without 0.25ug/mL doxycycline and 1uM PLX4720 simultaneously. **D**) Relative proliferation of diBRAF(V600E) MCs +/- 0.25ug/mL doxycycline in which the doxycycline treatment remains constant for the duration of the proliferation assay, or the doxycycline is treatment is stopped after 3 days. Values represent the mean ± SEM for n = 3 biological replicates in **A**, **B**, and **D**.

Supplementary Figure S11. BRAF(V600E) Induces Loss of Repressive Chromatin Marks at the CDKN2A/B Locus. A) relative H3K27me^3 localization (left), and H3K9me^3 localization (right) at the p15*INK4B* promoter in diBRafV600E MC +/- doxycycline. **B**) relative H3K27me^3 localization (left), and H3K9me^3 localization (right) at the p16*INK4A* promoter in diBRafV600E MC +/- doxycycline. Values represent the mean ± SEM for n = 3 biological replicates in **A** and **B**.

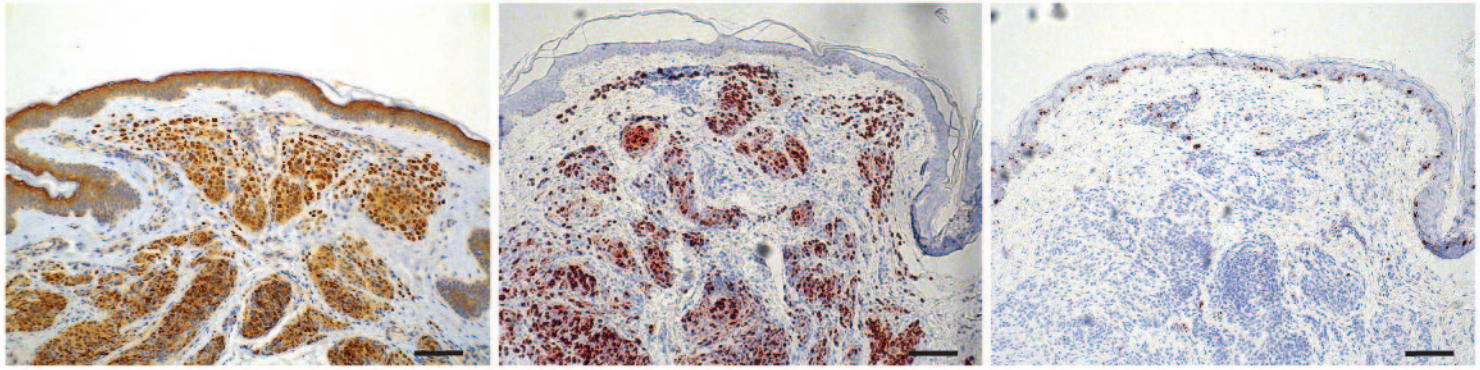
Supplementary Figure S12. Effects of hTERT on p15 in BRAF(V600E) Melanocytes. **A**) Relative telomerase activity in normal MCs and MCs overexpressing hTERT as compared to positive control cells. Values represent the mean ± SEM for n = 3 biological replicates. **B**) Immunoblot of total cell extracts for BRAF(V600E) and p15 expression in diBRAF(V600E) MCs transduced with control luciferase or hTERT lentivirus and treated with or without 0.25ug/mL doxycycline. β–Actin is shown as loading control.

Supplementary Figure S13. Senescence Marker Expression in human xenografts derived from Nevus-MCs. A) p16 immunohistochemistry (red) in the human xenografts derived from Nevus-MCs used in Main Text Fig. 6. **B**) TUNEL staining (green) with DAPI staining (blue) on the same xenografts derived from Nevus-MCs. UVB treated mouse ear skin served as the positive TUNEL control. Photomicrographs are of 10-μm-thick sections. Scale bars = 100μm.

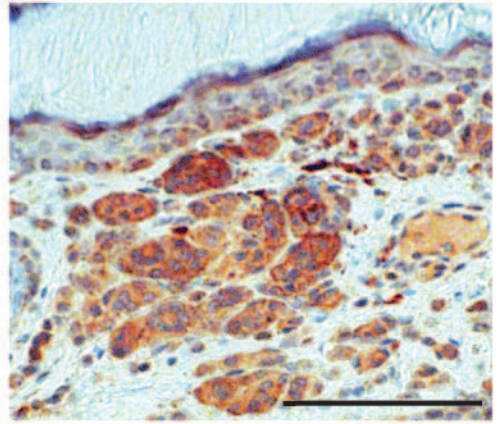
Supplementary Figure S14. Effects of p53(R248W) Transduction on Melanocytes. Relative p21 mRNA levels in nevus MCs transduced with or without lentiviral p53(R248W). Values represent the mean ± SEM for n = 3 biological replicates.

Supplementary Figure S15. p16 Loss Results in Partial Proliferation Rescue in BRAFV600E Nevus Melanocytes. **A**, Immunohistochemistry (IHC) staining for melanocyte marker, Mart-1 (burgundy), and proliferation marker, Ki-67, expression on representative tissue sections of 3-dimensional skin reconstruct xenografts harvested from SCID mice 60 days post-grafting. Xenografts were representative of n = 3 biological replicates and comprised normal human keratinocytes and nevus-derived human melanocytes transduced with lentiviral p53(R248W), hTERT, and either pLKO anti-NS (NSi), pLKO anti-p15i, or pLKO anti-p16 (p16i). Photomicrographs of 10-μm-thick sections. Scale bars = 100μm. **B**, Number of melanocytes per millimeter (mm) of tissue in Nevus anti-NS, Nevus anti-p15, and Nevus anti-p16 xenografts from **A**. Values are an average of n = 3 biological replicates.

Supplementary Fig. S1. p15, p16, and Ki-67 Expression in a Benign BRAF(V600E) Nevus

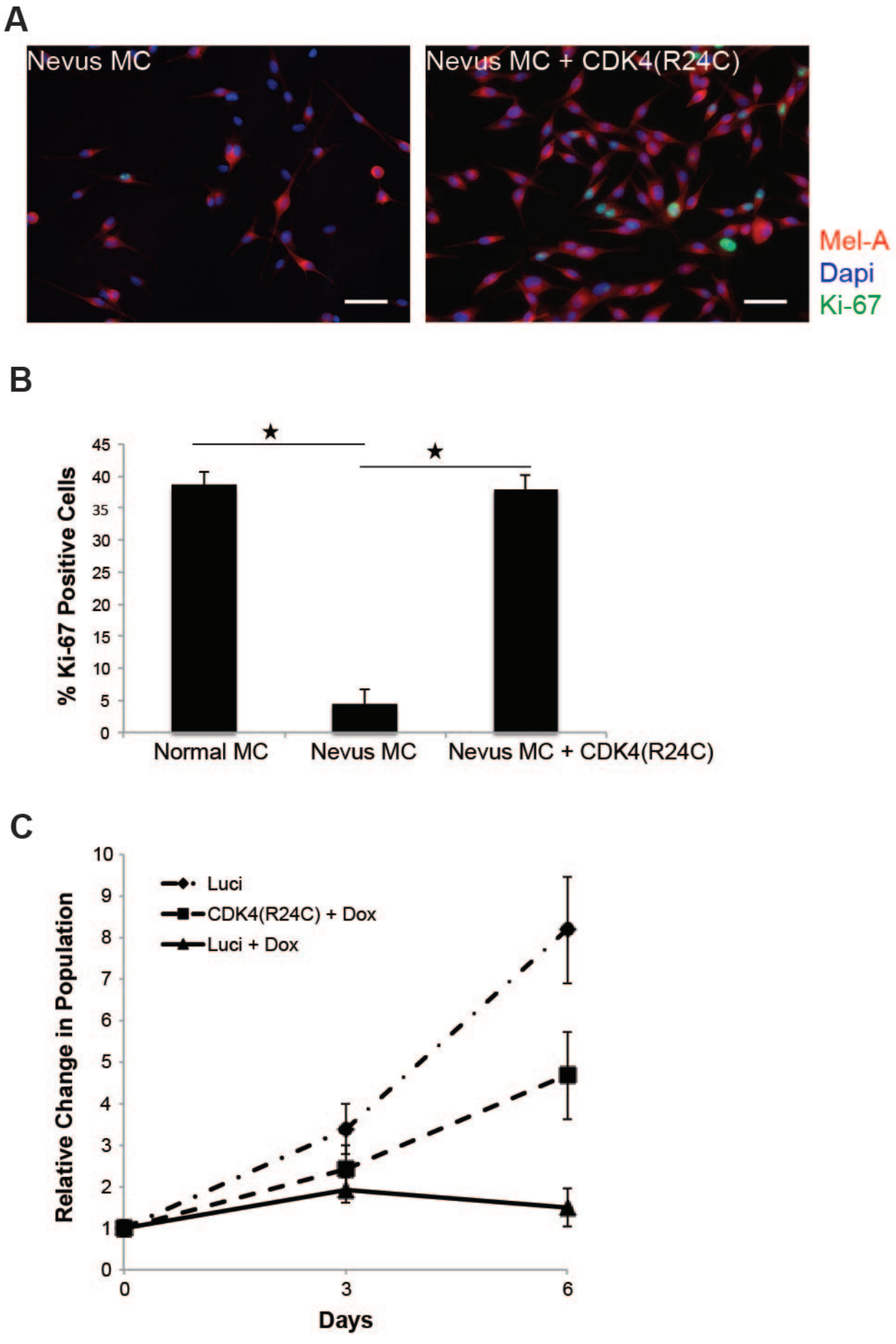


p15 p16 Ki-67

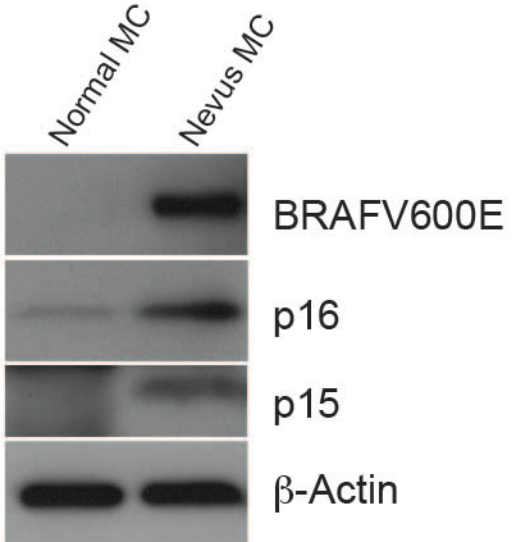


BRAF(V600E) mutant specific antibody

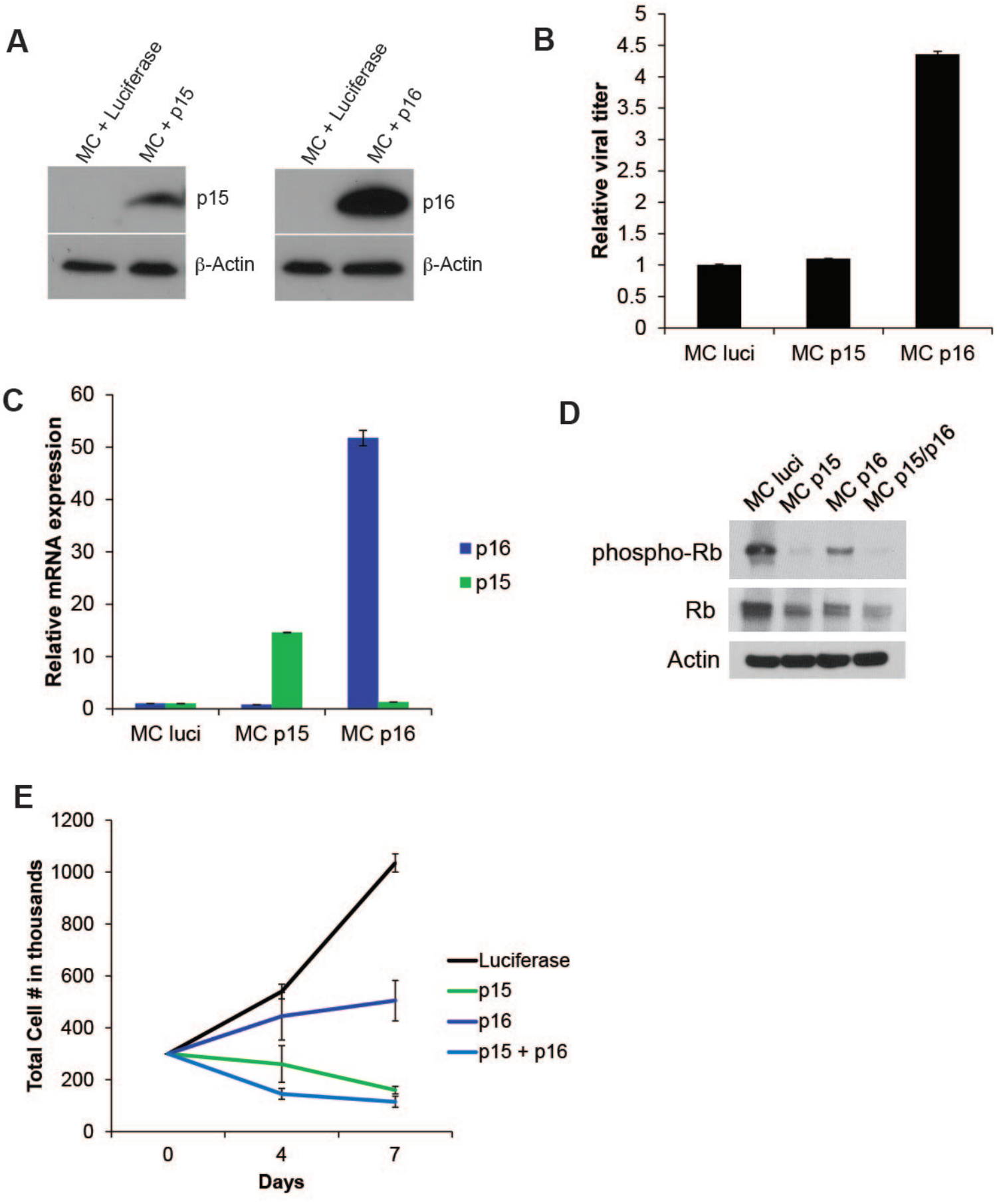
Supplementary Fig. S2. Active CDK4 Reverses BRAF(V600E)-mediated Melanocyte Growth Arrest



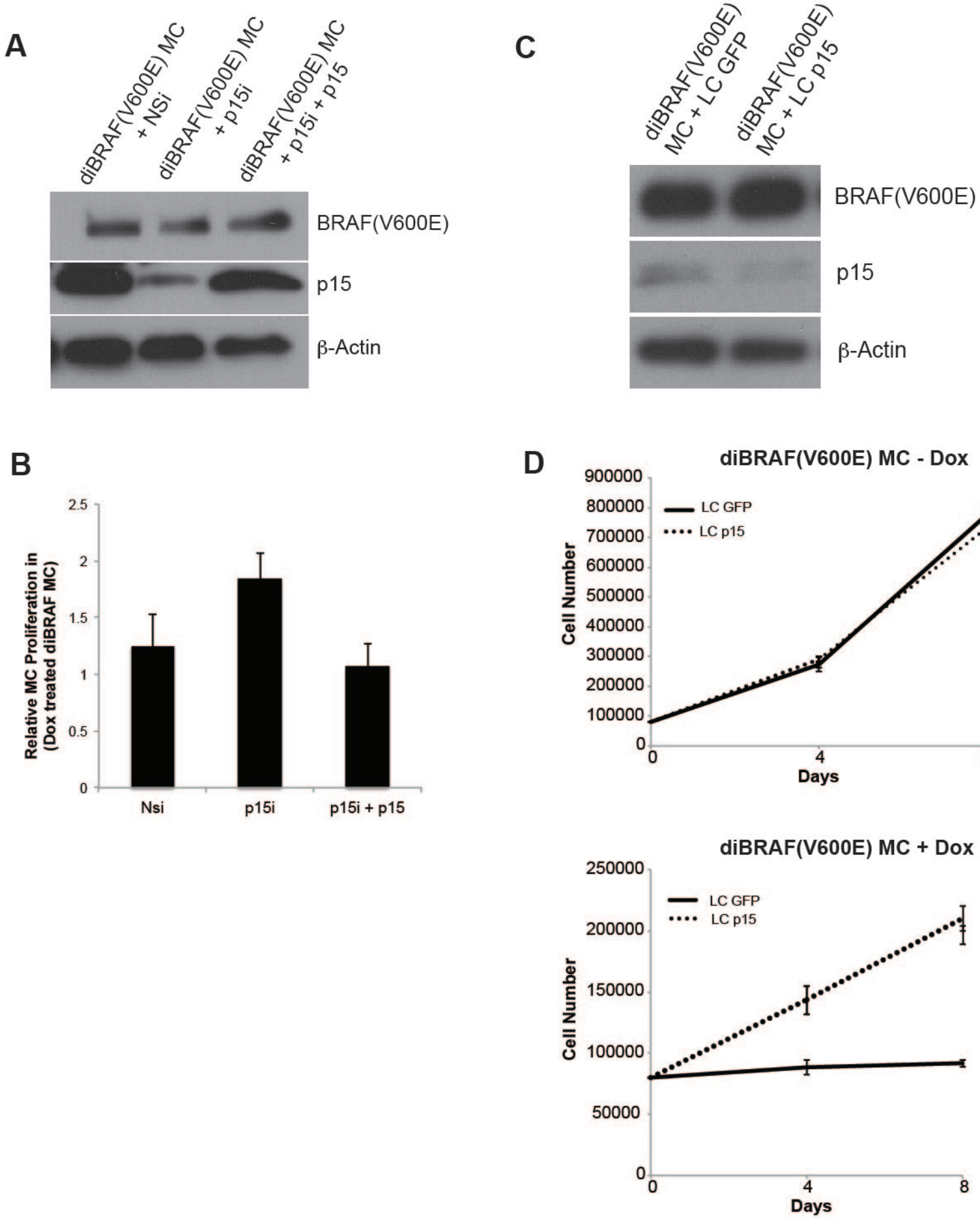
Supplementary Fig. S3. BRAF(V600E), p15, and p16 Expression in Normal and Nevus MCs



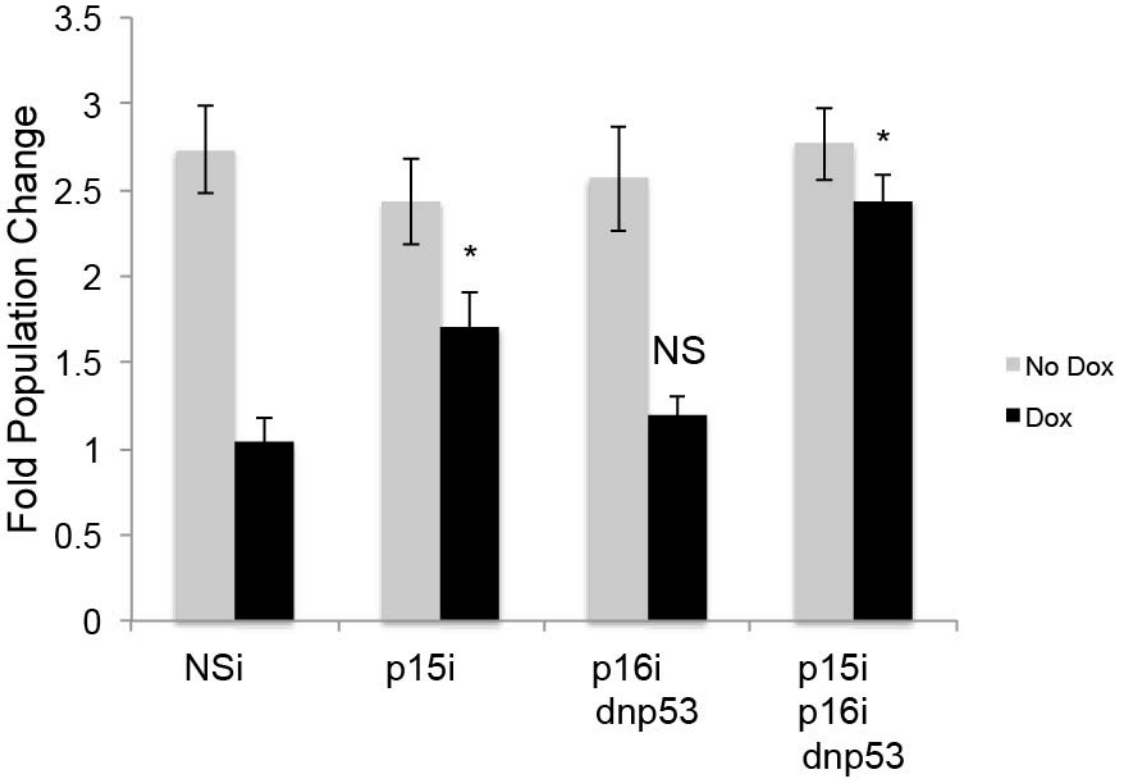
Supplementary Fig. S4. Engineered p15 and p16 Expression in Melanocytes



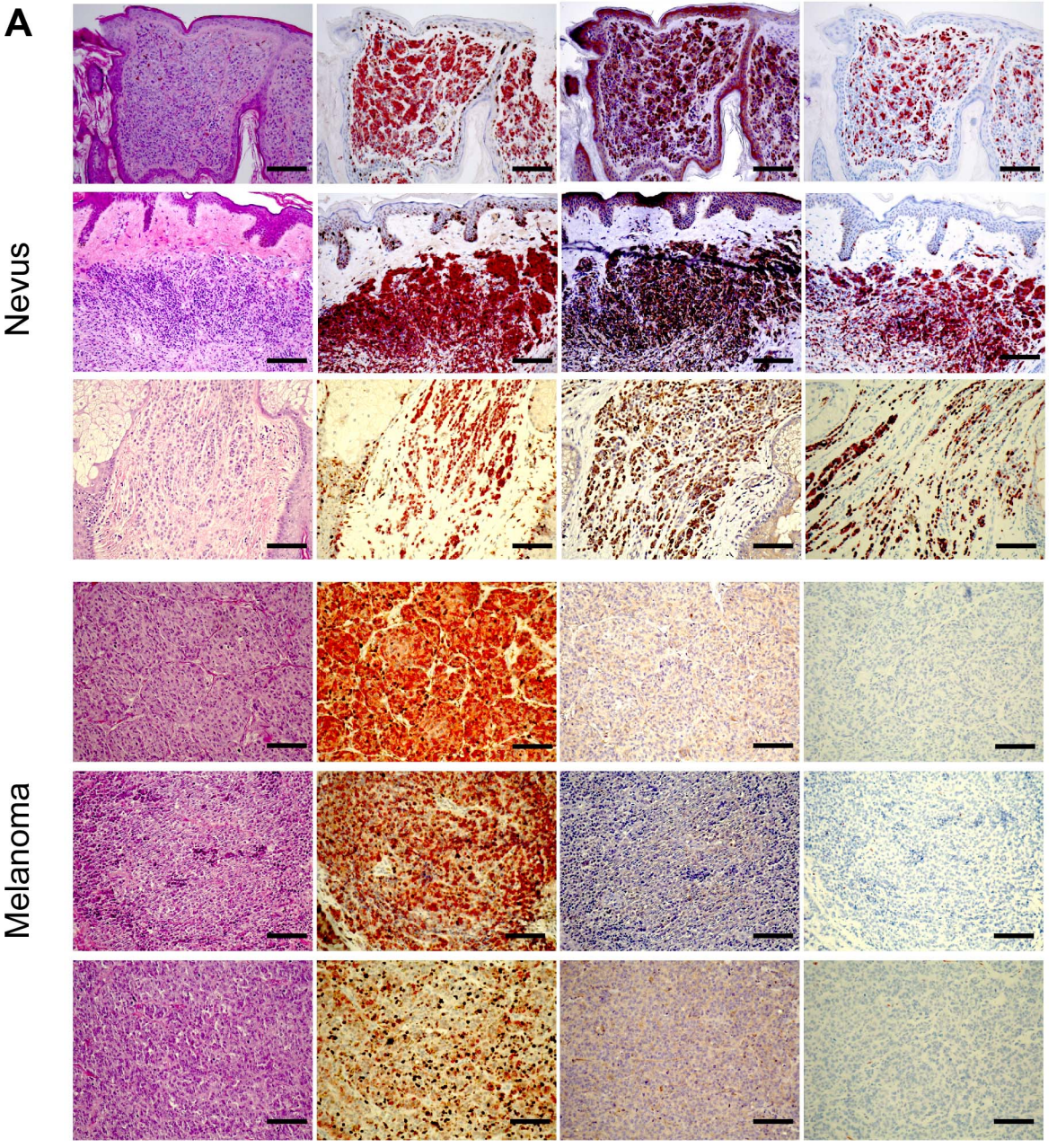
Supplementary Fig. S5. Effects of p15 shRNA Rescue and lentiCRISPR-Cas9 Mediated p15 Loss in diBRAF(V600E) MCs

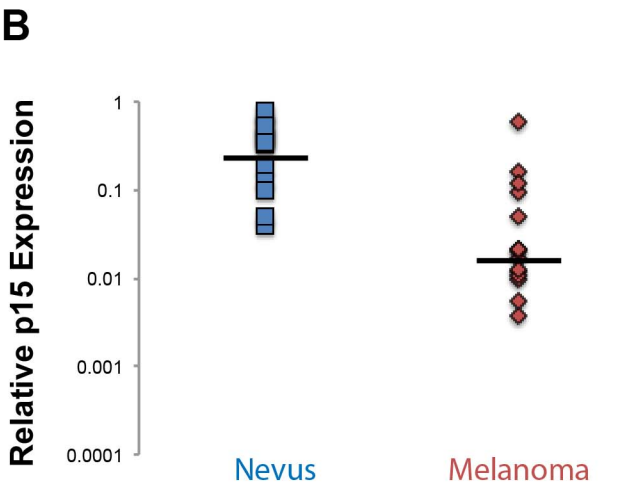


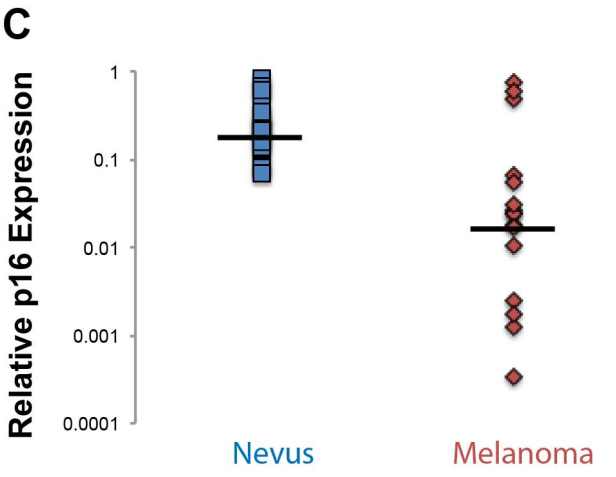
Supplementary Fig. S6. Loss of CDKN2A and CDKN2B Promotes Proliferation in BRAF(V600E) MCs.



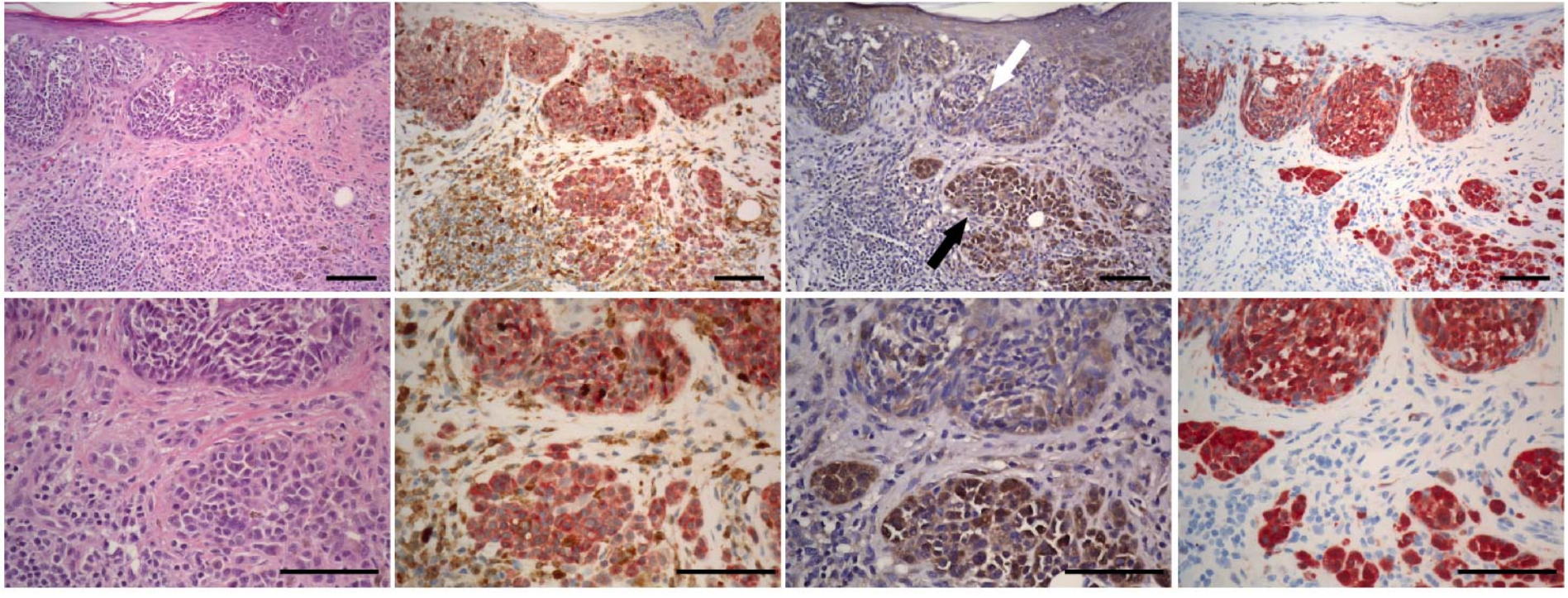
Supplementary Fig. S7. p15 and p16 Expression in Benign Nevi and Melanoma



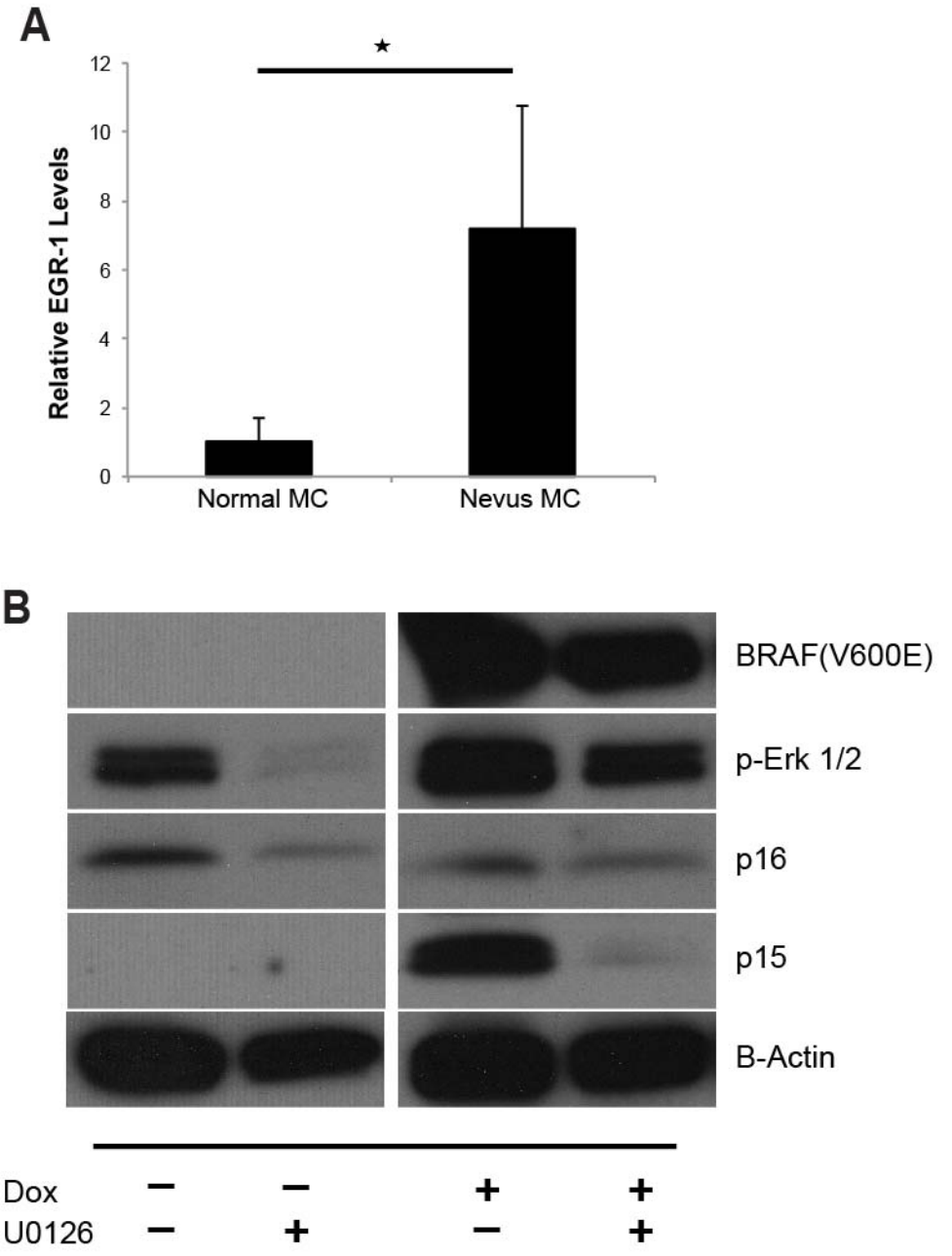




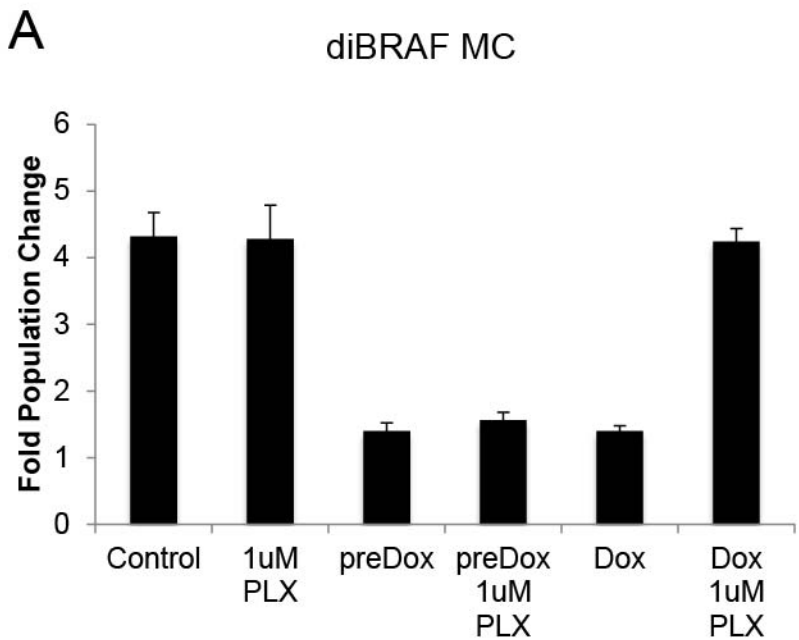
Supplementary Fig. S8. p15 and p16 Expression in Benign Nevus with Adjacent Melanoma

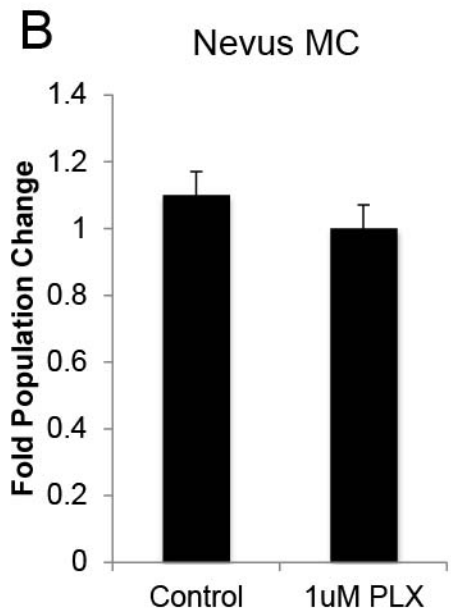


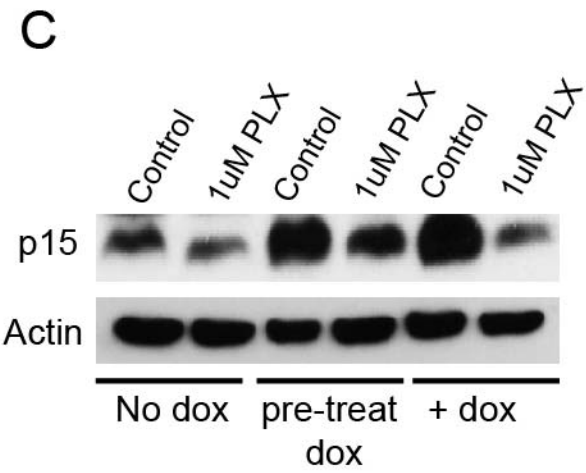
Supplementary Fig. S9. Erk 1/2 Target EGR-1 is Upregulated in BRAFV600E Nevi

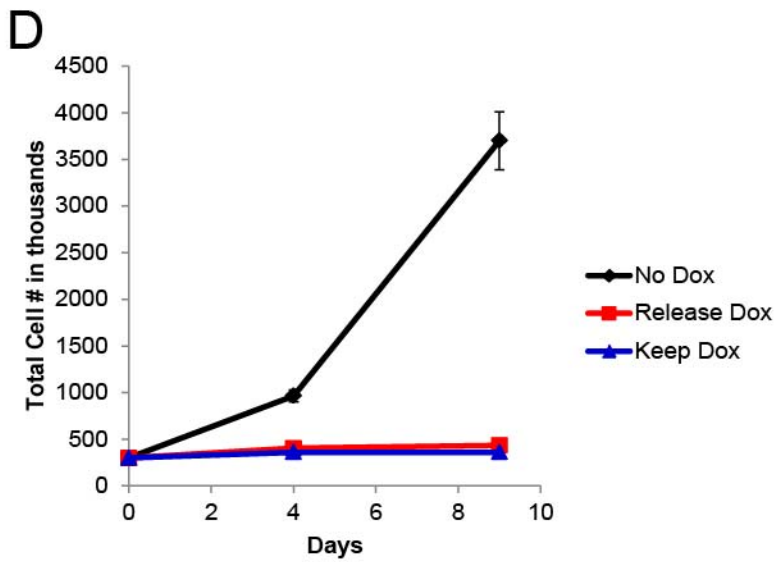


Supplementary Fig. S10. BRAF(V600E) Inhibitor PLX4720 Prevents Induction of p15-Mediated Growth Arrest but is Insufficient to Reverse Established Growth Arrest Phenotype.

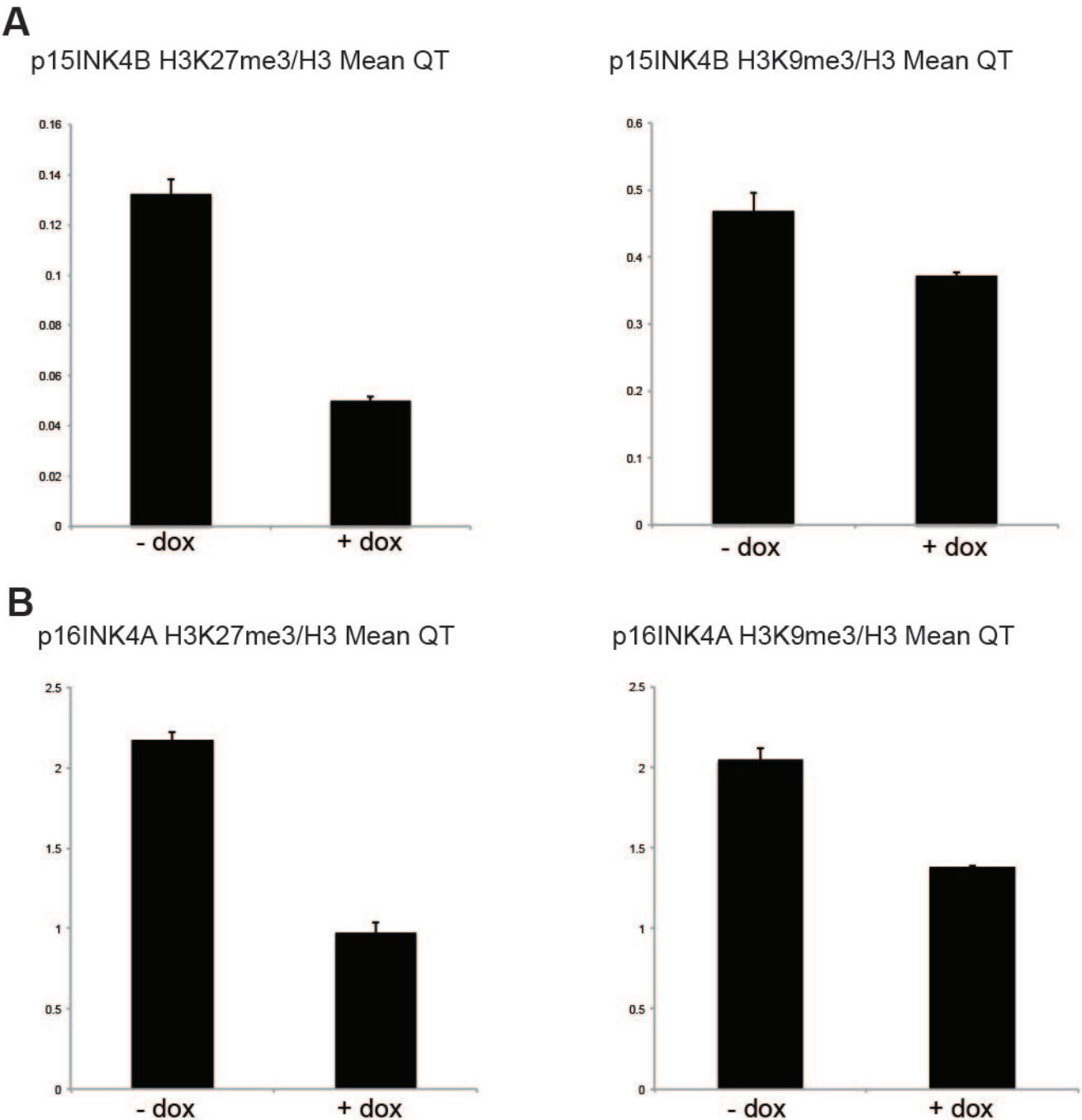




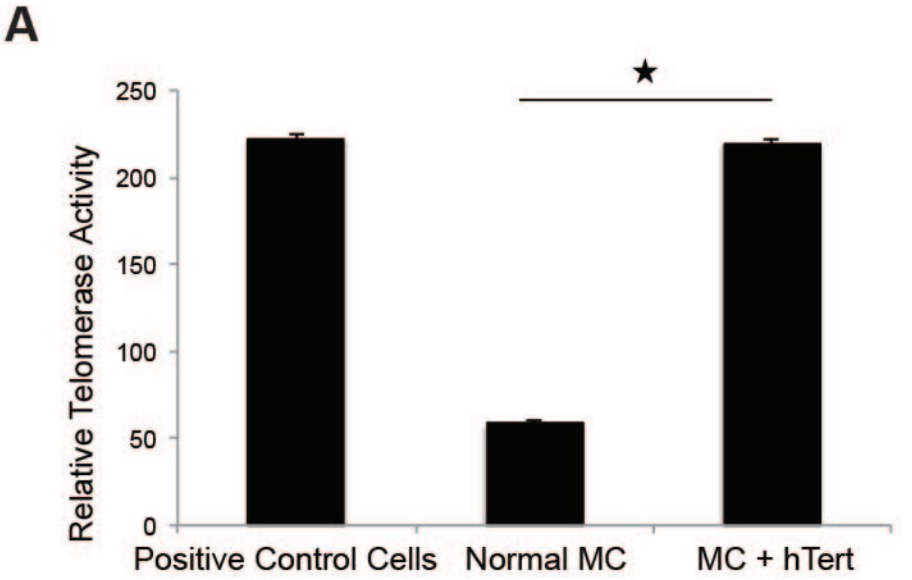


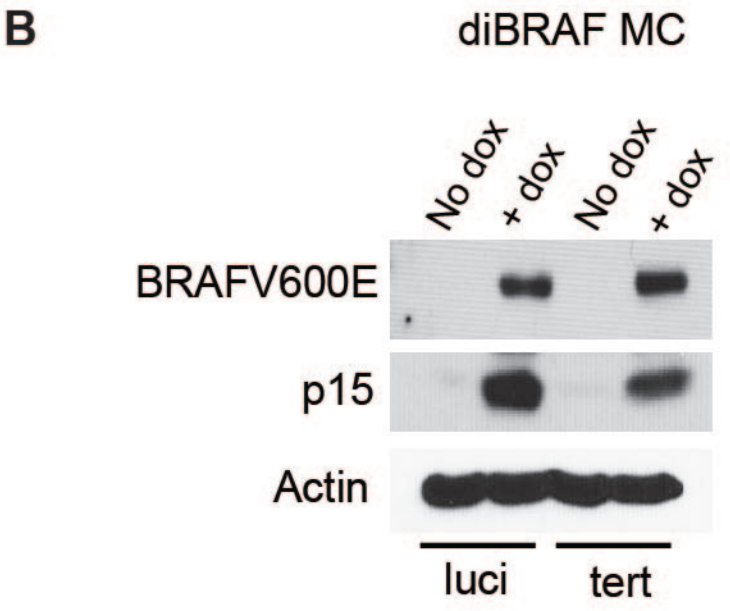


Supplementary Fig. S11. BRAF(V600E) Induces Loss of Repressive Chromatin Marks at the *CDKN2A/B* Locus



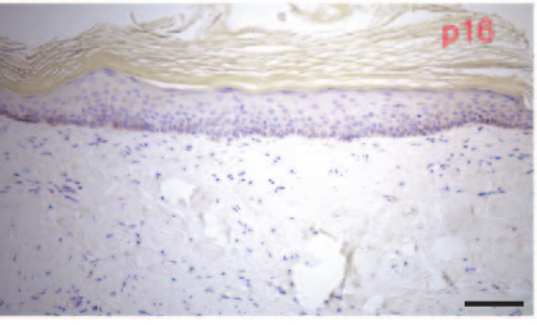
Supplementary Fig. S12. Effects of hTERT Transduction on Melanocytes



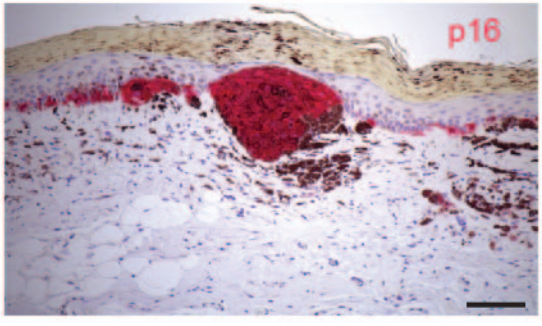


Supplementary Fig. S13. Senescence and Apoptosis Marker Expression in BRAF(V600E) Nevus- derived Skin Grafts.

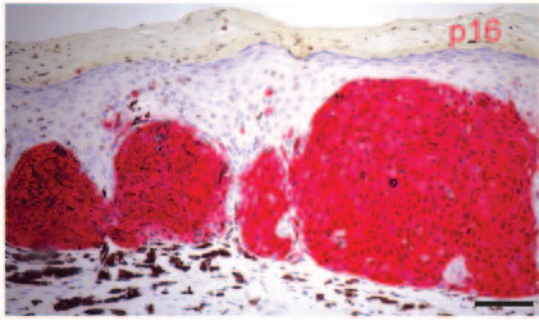
A



Nevus MC +
CDK4(R24C)

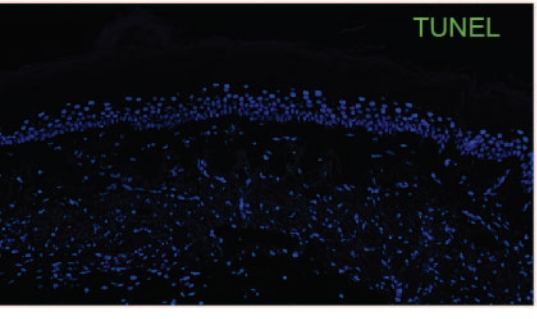


Nevus MC +
CDK4(R24C) + hTert



Nevus MC +
CDK4(R24C) + hTert +
p53(R248W)

B



Nevus MC +
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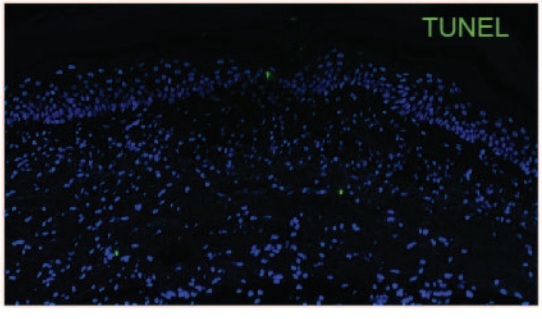

Nevus MC +
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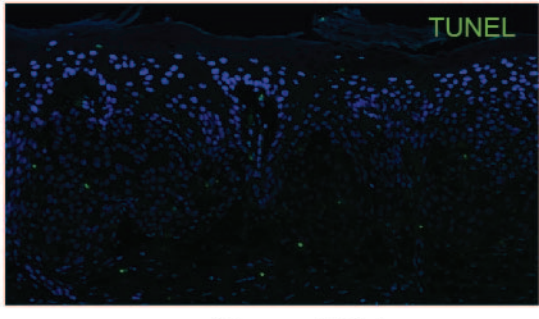
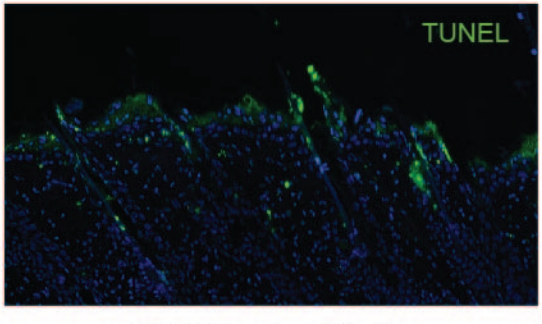
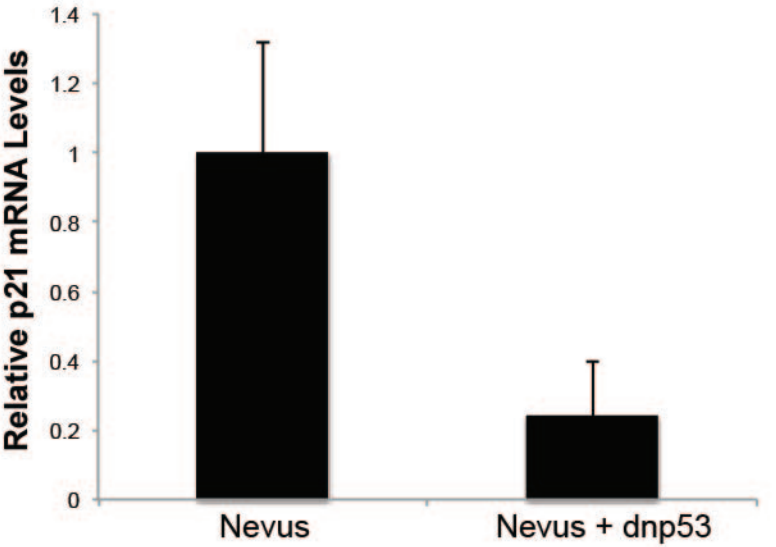


Nevus MC +
CDK4(R24C) + hTert +
p53(R248W)



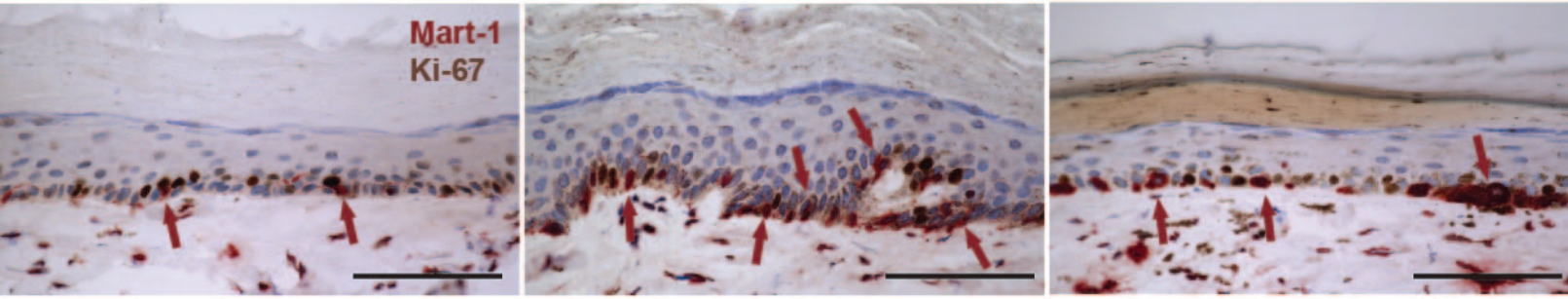
UVB Treated Murine
Skin

Supplementary Fig. S14. Effects p53(R248W) Transduction on Melanocytes



Supplementary Fig. S15. p16 Loss Results in Partial Proliferation Rescue in BRAF(V600E) Nevus Melanocytes.

A

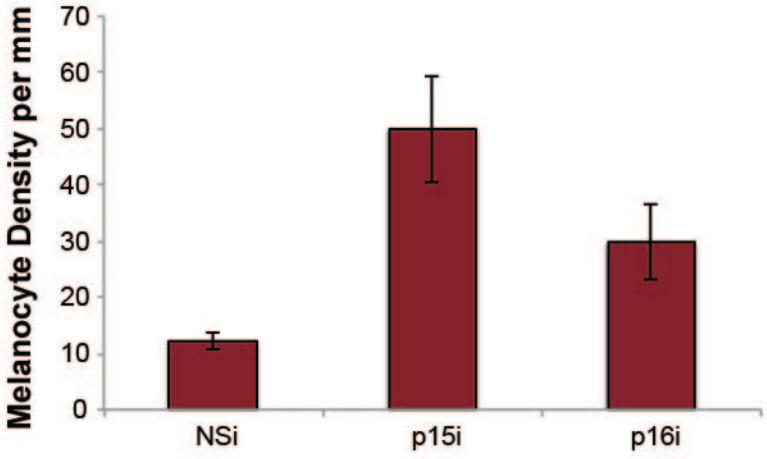


Nevus MC NSi + p53(R248W) + hTert

Nevus MC p15i + p53(R248W) + hTert

Nevus MC p16i + p53(R248W) + hTert

B



Supplemental Methods

Isolation of Melanocytes from Human Nevi

Benign human melanocytic nevi were excised from patient donors seen in the Dermatology clinic at the Hospital of the University of Pennsylvania according to an IRB approved protocol. A portion of each specimen was processed for routine histological examination to confirm the clinical diagnosis of benign nevus. The tissue samples were microdissected to isolate nevus from surrounding normal tissue. Nevus tissue was then mechanically separated into fine pieces, and enzymatically dissociated in a mixture of dispase and collagenase for 2 hours. The resulting supernatant was spun at 218*g* for 5 minutes to pellet the extracted cells, which were subsequently resuspended in melanocyte Medium 254 (Invitrogen). The cells were plated onto 6-cm collagen-coated tissue culture plates (BD Falcon). Melanocytes were further isolated from contaminating fibroblasts by selective trypsinization and 4 day exposure to 100 μg/mL G418.

Melanocyte Culture

Primary melanocytes were extracted from fresh discarded surgical human foreskin specimens as described previously (28) with some modifications detailed as follows. After overnight incubation in Dispase, the epidermis was separated from the dermis and treated with trypsin for 10 minutes. Cells were pelleted and plated on selective MC Medium 254 (Invitrogen) with Human Melanocyte Growth Supplement, and 1% penicillin and streptomycin. TGFb-1 was purchased from R&D Systems and used at a concentration of 100 pM. Doxycycline (hyclate) hydrochloride was purchased from Sigma, dissolved in sterile, deionized water, and used at a concentration of 0.25μg/mL. SB-431542 was purchased from EMD Millipore, dissolved in DMSO, and used at a concentration of 50μM.

Immunoblot Analyses and Antibodies

Adherent cells were washed once with DPBS and lysed with 1% NP-40 buffer (150 mM NaCl, 50 mM Tris, pH 7.5, 1 mM EDTA, and 1% NP-40) containing 1X protease inhibitors (Roche) and 1X phosphatase inhibitors (Roche). Lysates were quantified (Bradford assay), normalized, reduced, denatured (95 ˚C) and resolved by SDS gel electrophoresis on 4-15% Tris/Glycine gels (Bio-Rad). Resolved protein was transferred to PVDF membranes (Millipore) using a Semi-Dry Transfer Cell (Bio-Rad), blocked in 5% dry milk in TBS-T and probed with primary antibodies recognizing BRAF (Abcam, ab33899, 1:500), BRAF-V600E (Spring Bioscience, VE1, 1:500), p15 (Abcam, ab 53034, 1:500), p16 (BD Pharmingen, #551153, 1:500), and β-Actin (Cell Signaling Technology, #3700, 1:4000). After incubation with the appropriate secondary antibody, proteins were detected using either Luminata Crescendo Western HRP Substrate (Millipore) or ECL Western Blotting Analysis System (GE Healthcare).

Immunohistochemistry

Formalin fixed paraffin embedded (FFPE) human skin tissue sections from patients with benign nevus and melanoma, or human xenograft tissues were screened for p15 protein expression using peroxidase/DAB (diaminobenzidine)-complex method (EXPOSE biotin free detection kit, Abcam, Cambridge, MA). Tissue sections were deparaffinized and rehydrated. To retrieve the antigens, tissue sections were microwaved in 10mM Citrate buffer pH 6.0. To quench endogenous peroxidase, tissues were incubated in 3% H_2O_2 . Tissues were then incubated with primary antibody to p15 (1:200; Abcam, Cambridge, MA) for three hours at room temperature. The secondary antibody, goat anti-rabbit HRP conjugated, was supplied with the kit and incubated 18 min at room temperature. Following multiple washes in TBS, the antigen-antibody

complex was visualized with 3.3% DAB-solution. The tissues were counterstained with hematoxilin, dehydrated and a coverslip was applied.

TUNEL Staining

To identify apoptotic cells we used a terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay. Briefly, FFPE tissue sections were deparaffinized and rehydrated as described in the IHC section. The tissues were permeabilized using 10mM Citrate Buffer, pH 6.0 in microwave at 350W. Following washing in TBS-T, sections were outlined by creating a hydrophobic border with a PAP pen (Cancer Diagnostics, Durham, North Carolina). TUNEL labeling was performed with an In Situ Cell Death Detection Kit, Fluorescein (Roche, Manheim, Germany). The sections were covered with parafilm, incubated for 60 minutes at 37°C, and washed with PBS. Coverslips were mounted with Vectashield/DAPI (Vector Labs, Burlingame, California).

Quantification of Immunohistochemistry Staining

Tissue sections from human nevi, melanomas, and melanomas arising in continuity with existing nevi were stained for p15 or p16 expression using methods described in the previous immunohistochemistry section. 10X photomicrograph images of representative tissue sections were taken using the Zeiss microscope. Tiff files of the images were saved and transferred to Adobe Photoshop where pixels corresponding to p15 or p16 IHC staining were selected using the color selection tool. Images corresponding to the single specific color were then analyzed using FIJI (Image J) to determine the number of pixels in each sample. The number of pixels representing the total staining, including the DAPI counterstain, was also obtained so that the

levels of p15 and p16 expression could be normalized to the total amount of label in each section. Final ratios of p15 and p16 expression were calculated by dividing the number of p15 or p16 pixels by the total pixels in each section.

Generation of Lentiviral Vectors

The following lentiviral plasmids were used to express the corresponding human genes: diBRAF, p15, p16, CDK4(R24C), dominant-negative p53(R248W), hTERT, and luciferase. The human BRAF (V600E) gene was inserted immediately after the TetO operator in a modified version of the doxycycline-inducible lentiviral pTRIPZ vector (Thermo Scientific), in which the shRNA hairpin sequences were deleted. Human p15 (Addgene 16454), CDK4(R24C), p53(R248W), and hTERT (28) were cloned into the pRRL lentivector (40). Human p16 was expressed using Addgene plasmid #22263 (Eric Campeau). shRNAs were expressed from pLKO.1 and are available through OpenBiosystems. The nucleotide sequences of the specific hairpin sequences used are available upon request.

Quantitative RT/PCR

mRNA was extracted from melanocytes according to the RNeasy Mini Kit protocol (Qiagen), and reverse transcribed to cDNA using the High Capacity RNA-to cDNA kit (Applied Biosystems). Quantitative PCR of the resulting cDNA was carried out using Power SYBR Green Master Mix (Applied Biosystems) and gene-specific primers, in triplicate, on a ViiA 7 Real-Time PCR System (Life Technologies). The following primers were used for detection; CDK2 forward: 5'-CAG GAT GTG ACC AAG CCA GT-3'; CDK2 reverse: 5'-GGC TGG CCA AGA CTA GAA GG-3'; CDK4 forward: 5'- GTG CAG TCG GTG GTA CCT GA-3'; CDK4

reverse: 5'- AAG GCA AAG ATT GCC CTC TCA-3'; CDK6 forward: 5' – ACA GAG CAC CCG AAG TCT TG – 3'; CDK6 reverse: 5'-CTG GGA GTC CAA TCA CGT CC-3'; p14ARF forward: 5'- ACG GGT CGG GTG AGA GTG -3'; p14ARF reverse: 5'- GTG GCC CTC GTG CTG ATG -3'; p15 forward: 5'- CAC CCC CAC CCA CCT AAT TC-3'; p15 reverse: 5'-TGA GTG TCG AGG GCC AGA TA-3'; p16 forward: 5'-GCC CAA CGC ACC GAA TAG-3'; p16 reverse: 5'- ACG GGT CGG GTG AGA GTG -3'; p18 forward: 5'- GTG GGG CAT CGG AAC CAT AA-3'; p18 reverse: 5'- AAA GTA GAG GCA ACG TGG GG-3'; p21 forward: 5'- GAT GTA GAG CGG GCC TTT GA-3'; p18 reverse: 5'- AAA GTA GAG GCA ACG TGG GG -3'. Relative expression was determined using the 2-[delta][delta] Ct method followed by normalization to the wild-type melanocyte transcript levels.

Growth Curves

Cells were seeded into 6-well plates at a density of 2.0×10^5 or 2.5×10^5 cells per well. At regular intervals, cells were trypsinized, resuspended, and manually counted on Kova Glasstic Slide 10 (Hycor). Cells were subsequently spun down and re-seeded into 6-well plates at equal densities.

Sanger Sequencing

mRNA was isolated from nevus melanocytes using the RNeasy kit and reverse transcribed to cDNA. Sanger sequencing was conducted by the University of Pennsylvania DNA Sequencing facility. Samples submitted contained 60 ng DNA, and 3.2 pmoles each of forward and reverse primers in a volume of 9 μL. The sequencing primers used were ordered from Integrated DNA

Technologies and are as follows; BRAF forward: 5'- GCA CGA CAG ACT GCA CAG GG -3'; BRAF reverse: 5'- AGC GGG CCA GCA GCT CAA TAG -3'.

Preparation of 3-D Organotypic Skin Cultures

Organotypic skin grafts containing MCs were established using modifications to previously detailed methods (10, 28). The Keratinocyte Growth Media (KGM) used for keratinocyte-only skin grafts was replaced with Melanocyte Xenograft Seeding Media (MXSM). MXSM is a 1:1 mixture of KGM and Keratinocyte Media 50/50 (Gibco) containing 2% FBS, 1.2 mM calcium chloride, 100 nM Et-3 (endothelin 3), 10 ng/mL rhSCF (recombinant human stem cell factor), and 4.5 ng/mL r-basic FGF (recombinant basic fibroblast growth factor). 1.5×10^5 melanocytes and 5.0×10^5 keratinocytes were suspended in 80 μL MXSM, seeded onto the dermis, and incubated at 37 ˚C for 4 days at the air-liquid interface.

Mouse Xenografting

Organotypic skin tisssues were grafted onto 5-7 week old female ICR Scid mice (Taconic) according to an IACUC-approved protocol at the University of Pennsylvania. Mice were anesthetized in an isoflurane chamber. Murine skin was removed from the upper dorsal region of the mouse. Reconstituted human skin was reduced to a uniform 11 x 11 mm square and grafted onto the back of the mouse with individual interrupted 6-0 nylon sutures. Mice were dressed with Bactroban ointment, Adaptic, Telfa pad and Coban wrap. Dressings were removed 2 weeks after grafting.

Chromatin Immunoprecipitation Followed by Quantitative PCR (ChIP-qPCR)

Cells in $10cm^2$ dishes were fixed in 1% formaldehyde for 5 minutes and fixation was quenched with addition of glycine to 125 mM for an additional 5 minutes. Cells were harvested by scraping from plates, and washed twice in 1x PBS before storage at -80°C. ChIP was performed as previously described (41) except that extracts were sonicated six times for 7.5 minutes each round (30 seconds sonication with intermediate incubation of 30 seconds per round) using a Bioruptor (Diagenode). All ChIPs were performed using 150 µg of extract and 2 µg of antibody per sample. 30 µl of Protein G Dynabeads (Invitrogen 100.02D) were used per ChIP. Antibodies included Anti-Histone H3 (Abcam ab1791), Anti-Histone H3 (tri methyl K9) (Abcam ab8898), Anti-Histone H3 (tri methyl K4) (Abcam ab8580), Anti-Histone H3 (tri methyl K27) (Abcam ab6002). Following elution, ChIP DNA was analyzed by standard qPCR methods on a 7900HT Fast-Real-Time PCR (ABI). Primer sequences are available upon request.

Viral Transfection and Transduction

Viral transductions were performed as described previously (10, 27, 28). HEK293T cells were cultured to 40% confluency and incubated in DMEM supplemented with 5% FBS and 1% antibiotic and antimycotic in 6-well plates. For each well, 1.22 µg lentiviral vector was mixed with viral packaging plasmids pCMVΔR8.91 (0.915 µg) and pUC-MDG (0.305 µg). This plasmid solution and 7.2 µl of Fugene 6 Transfection Reagent (Promega) were added to 96 µl of supplement-free DMEM. The resulting mixture was incubated at room temperature for 15 minutes and then added into the HEK293T culture media. At 16 hours post-transfection, 10 mM sodium butyrate was added onto the cells. At 24 hours, the culture media was replaced and the cells were incubated at 32°C. At 45 hours, viral supernatant was collected by filtering the media through a .45 µm syringe filter (Argos). Melanocytes seeded at 2.5×10^6 cells per well were

incubated in viral supernatant in the presence of 5 μg mL^{-1} polybrene and centrifuged at 300 x g for 60 minutes at room temperature. After incubating the transduced melanocytes for 15 minutes at 37ºC, viral supernatant was removed and replaced with growth media.

CRISPR-Cas9 Cloning and Transduction

Guide RNAs were designed using software tools developed by the Zhang Lab and provided on the website http://www.genome-engineering.org/ (Hsu et al., 2013). Guide RNAs were subsequently cloned into lentiCRISPRv2 (Addgene # 52961) according to the accompanying protocol (Sanjana et al., 2014 and Sanjana et al., 2014). Guide RNA sequences are as follows: lentiCRISPR GFP 5' GAA GTT CGA GGG CGA CAC CC 3'; lentiCRISPR p15.2 5' CCC GAA ACG GTT GAC TCC GT 3'. LentiCRSIPR transductions in human melanocytes were conducted as previously described for the other lentiviral constructs used in this study.

Statistics

Mean values were compared using an unpaired Student's two-tailed T-Test.