SUPPLEMENTAL MATERIALS AND METHODS

Generation of stable cell lines by lentiviral infection.

Lentiviruses were produced by transfection with Effectene reagent of 10μg vector plasmid (pRRLSIN.cPPT.PGK-GFP.WPRE or PLKO) together with 15μg packaging (pCMVdR8.74) and 6μg envelope (pMD2.G) plasmids in 293T cells, according to Trono's lab protocol (http://tronolab.epfl.ch). Supernatant was harvested 48h post-transfection, filtered with 0.22μm filters, diluted 1:2 with fresh media and used to infect 2×10⁵ cells in 6-well plates, in presence of 8μg/ml Polybrene (Sigma-Aldrich).

Analysis of cell circularity, proliferation, migration and adhesion.

Cell circularity was quantified using the Shape Factor \[4\pi(\text{area})/(\text{perimeter})^2\], where a value of 1 indicates a perfect circular perimeter and decreasing values indicate more elongated perimeters.

For proliferation assays, 2×10⁴ cells/well were plated in 12-well plates in complete medium and analyzed at days 0,1, 2, 3,4 and 5 by quantification of crystal violet staining at 590nm wavelength.

For the wound healing assay, 10⁵ cells were seeded in 24-well plates containing silicone inserts that create a 500μm thick wall between two chambers (Culture-Insert 24, Ibidi). The cells were grown to confluency, serum starved for 24h, and the inserts removed. The cells were rinsed with PBS, placed in complete media and photographed at the indicated times. The area covered by migrated cells was calculated using ImageJ software. For transwell migration assay, 2×10⁴ cells were seeded in serum-free media in the upper chamber of transwells with 8.0μm pore size membrane (24-well format, Costar, Corning)
Incorporated) and complete media in the lower chamber. Cells migrating to the lower side of the membrane were fixed and quantified by crystal violet staining. For transendothelial migration assays, $10^5$ HUVEC cells were seeded in complete medium in the upper chamber of transwell inserts with 5.0μm pore size membrane (24-well format, Costar, Corning Incorporated) coated by fibronectin at 5μg/cm², and grown till confluency. $10^5$ melanoma cells were then seeded on top of the HUVEC monolayer in HUVEC's complete medium. Cells reaching the lower side of the membrane were fixed and quantified by crystal violet staining at 24h.

For gelatin zymography, equal number of cells were seeded, grown to 80% confluence and serum starved for 16h. The media was collected and at the moment of media collection the cells were counted to confirm an equal number. The conditioned media was centrifuged to remove cellular debris, normalized to the cell number, and run on 10% acrylamide/bisacrylamide gel containing 0.1% (w/v) gelatin. The gel was then incubated in 2.5% Triton X-100 for 30 minutes at room temperature (RT), rinsed in distilled water and incubated at 37°C in 50mM Tris pH 7.8, 200mM NaCl, 5mM CaCl₂ for 16 hours before staining with Coomassie blue R-250 to evaluate gelatinase activity.

To assay adhesion, $5 \times 10^4$ cells/well were seeded onto 5μg/ml collagen I or 10μg/ml fibronectin (both from Sigma-Aldrich) pre-coated 96-well plates, for 15 and 30 minutes at 37°C. These were washed with PBS, and the adherent cells quantified by crystal violet staining.

**Analysis of cell survival and drug treatment response.**

Cells were plated at equal density on plates that were uncoated (for starvation analyses) or pre-coated with 12mg/ml Poly(2-hydroxyethyl methacrylate) (Sigma Aldrich) in 95%
ethanol (for anoikis assays) and maintained in serum-free medium for 72h. The cells were assayed by western blot analysis for cleaved PARP or trypsinized and stained with AnnexinV-APC (BD Biosciences) for FACS analysis. Total apoptotic cells were measured by gating for AnnexinV-positive cells using a FACSCalibur system.

For drug treatment assays, PLX4720 (Selleck) was dissolved in DMSO and used at the indicated concentrations. For growth inhibition experiments, 3x10^4 cells were plated in 6-well plates and treated with PLX4720 for 18 days, media with fresh drug was replaced every other day. Resistant populations were derived exposing MA2 cell variants (CTL and BMI1) to different concentration of PLX4720 (0.5-2-3 and 5μM). Drug was replaced every other day and cells were considered resistant after two months. For low seeding assays, 500 cells were plated in 10cm plates and cultured for 10 days in complete medium. In both cases, the surviving cells were quantified by crystal violet staining.

**Real time PCR.**

RNA was isolated and purified using TRIzol® Reagent and RNAeasy, and reverse-transcribed using SuperScript III reverse transcriptase (Invitrogen). Real-time PCR reactions were performed with SYBR Green (Applied Biosystems) on the StepOnePlus™ Real-Time PCR System (Applied Biosystems) using primers listed in Table 3. At least three independent biological samples were analyzed. Relative mRNA levels were normalized to 18S rRNA expression.

**Preparation and western blot analysis of cytoplasmic and nuclear extracts.**

Total cell lysates were prepared in RIPA buffer (0.1% SDS, 1% Sodium Deoxycholate, 1% IGEPAL, 25mM TrisHCl at pH 7.6, 150mM Sodium Chloride, 5% 2-mercaptoethanol, bromophenol blue). Nuclear and cytoplasmic fractions were prepared
by suspending harvested cells in hypotonic buffer (pH 7.9, 10mM HEPES, 0.2mM
EDTA, 0.2mM EGTA, 1mM DTT, plus protease inhibitor) on ice for 10 min. Triton-
X100 was added to the final concentration of 0.1%, cells were vortexed 15 seconds and
centrifuged at 4000 rpm at 4°C for 5 minutes. The supernatants, containing the
cytoplasmic fractions, were removed and the pellets resuspended in RIPA buffer with 1%
SDS. These samples were boiled for 10 minutes, sonicated and centrifuged at 12000 rpm
for 10 minutes and the supernatants, containing the nuclear fractions, recovered. The
whole cell, nuclear and cytoplasmic cell extracts were quantified by BCA Protein Assay
(Thermo Scientific) and equal amounts separated by SDS-PAGE and transferred to
nitrocellulose membranes. The membranes were blocked in 5% BSA Tris-buffered saline
(TBS)–Tween buffer (137mM NaCl, 20mM Tris/HCl, pH 7.6, 0.3% Tween-20) for 1 h at
RT, and incubated with primary (overnight at 4°C) then secondary (1 h at RT) antibodies
in 1% BSA TBS–Tween buffer, washed and the signals visualized by enhanced
chemiluminescence (Pierce ECL Western Blotting Substrate, Thermo Scientific). Primary
antibodies were used from: Millipore to detect BMI1 (05-637); BD Bioscences to detect
β-Catenin (610154), Fibronectin (610077) and HSP90 (610418); Life Technologies to
detect GAPDH (AM4300), Roche to detect GFP (11814460001) and Cell Signaling to
detect: TGFβR1 (3712), PDGFRα (3174), PDGFRβ (3169), Phospho-PKCα/β (9375),
PKCα (2056), PKCδ (2058), PKCζ (9368), PKCµ (2052), AKT (4691), AKT1 (2938),
AKT2 (3063), Phospho AKT (4058), Phospho-GSK-3β (9323), GSK-3β (12456), PTEN
(9559), EGFR (2232), Phospho-c-Jun (9261), c-Jun (9165), Active β-Catenin (8814),
PARP (9542), Cleaved PARP(Asp214) (mouse specific 9544, human specific 9541),
Phospho ERK1/2 (4370), Phospho MEK (9154), SMAD2 (5339), SMAD3 (9523), SNAIL (3879), Vinculin (4650) and Lamin A/C (2032).

**Immunohistochemistry.**

Ki67 and S100 immunohistochemistry was performed with a citric acid unmasking protocol as described (Hilgendorf et al. 2013). Slides were blocked for 1 h at RT in 2% normal horse serum (Ki67) or 10% goat serum (S100) in PBS. Ki67 (1:50; BD Biosciences, 550609) and S100 (1:400; Thermo Scientific RB-044-A0) antibodies were used in 0.15% Triton/PBS overnight at 4°C. Secondary antibodies (Vector Laboratories) were used at 1:200 in the relevant blocking buffer, and detected using a DAB substrate kit (Vector Laboratories), with haematoxylin counterstaining. When needed a melanin bleaching step was performed after antigen retrieval (Momose et al. 2011).

**Supplemental References**

Hilgendorf, K.I., Leshchiner, E.S., Nedelcu, S., Maynard, M.A., Calo, E., Ianari, A., Walensky, L.D., and Lees, J.A. 2013. The retinoblastoma protein induces apoptosis directly at the mitochondria. *Genes Dev* **27**(9): 1003-1015.

Momose, M., Ota, H., and Hayama, M. 2011. Re-evaluation of melanin bleaching using warm diluted hydrogen peroxide for histopathological analysis. *Pathol Int* **61**(6): 345-350.