SUPPLEMENTARY METHODS

Confocal immunofluorescence. A Zeiss 510 META Laser Scanning Microscope was used for confocal immunofluorescence analysis. Cells were grown in 6-well plate format on glass cover slips. Glass slips were then treated with the immunostaining procedure described in methods, with the exception that antibodies were placed in complete media and incubated at 37°C to minimize cell lifting. After antibody incubations, cells were fixed in -20°C, 100% methanol for 10 minutes. Cells were counterstained with DAPI and mounted with fluorescent mounting medium (DakoCytomation, #S3023). Immunofluorescence staining of original tumor sections was performed by xylene-mediated depariffinization twice for 12 minutes, followed by 2x 100% EtOH washes for 5 minutes, 2x 95% EtOH for 5 minutes, 2x 90% EtOH for 5 minutes, 1x 70% EtOH for 5 minutes, 1x 50% EtOH for 5 minutes, and 1x H_2O wash for 5 minutes. Overnight blocking was then done in 1x DPBS with 2% BSA at 4°C. Anti-CD34 mAb (Novus Biologicals, #NB600-1071) was used at 1:50 dilution. Anti-p75 pcAb (Chemicon, #AB1554) was used at 1:150. Secondary anti-rabbit Alexa488 and anti-rat Alexa647 antibodies were both used at 1:1000 for indirect staining of p75 and CD34, respectively. DAPI was used as nuclear counterstain. All incubations and washes were the same as for the in vitro immunostaining protocol described in methods.

Immunostaining. Anti-mouse Epcam/CD326 (BD Pharmingen), Alexa647-conjugated anti-mouse/human SSEA-1, anti-mouse CD20, and anti-mouse CD202/Tie2 (eBioscience) were all used at 0.5μg/ml using the same immunostaining protocol described in methods

before analysis by flow cytometry. Anti-mouse S100 and Tyrp1 antibodies were used as previously described[23]. Alexa647-IgM isotype control for direct staining with anti-mouse/human Alexa647-SSEA-1 was used as negative control. Secondary antibody only was used for all other indirect antibody stains. All samples were treated independently and gates positioned where autofluorescence/non-specific positive signals were ≤0.01% as dictated by negative control samples.

PCR validation. Recombination of Cdkn2a, Pten, beta-catenin, and Braf were previously described[21-24].

Electron Microscopy. A JEOL JSM 6060 Scanning Electron Microscope (UVM Microscopy Imaging Center) was used for pictures taken of neoplastic pigmenting cells derived from tumor tissue sections for validation of melanosome production.

Cell cycle analysis. Melanoma subsets were sorted, spun down and resuspended in 500µl 1xDPBS. 500µl of 100% ice-cold ethanol was added dropwise while vortexing cell suspension and cell samples were fixed at -20°C for 30 minutes. Cells were then spun down and resuspended in 1xDPBS with 50 µg/ml propidium iodide and 100µg/ml RNase A in a final volume of 500µl and incubated at room temperature for 2 hours. Cells were quenched with 9.5ml 1xDPBS, spun down, and resuspended in 1ml 1xDPBS and data collected by flow cytometry using the BD LSRII™ cytometer. Data was analyzed by FlowJo 7.5.5 software. Doublet-discrimination was first performed and then G_1, S, and G_2 cell cycle phase percentages were discriminated by the Watson pragmatic curve-fit

mathematical model and manual adjustments made to constrain G_1 and G_2 peak width coefficient of variation (CV) values to within 1.0 of each other as suggested by the model.