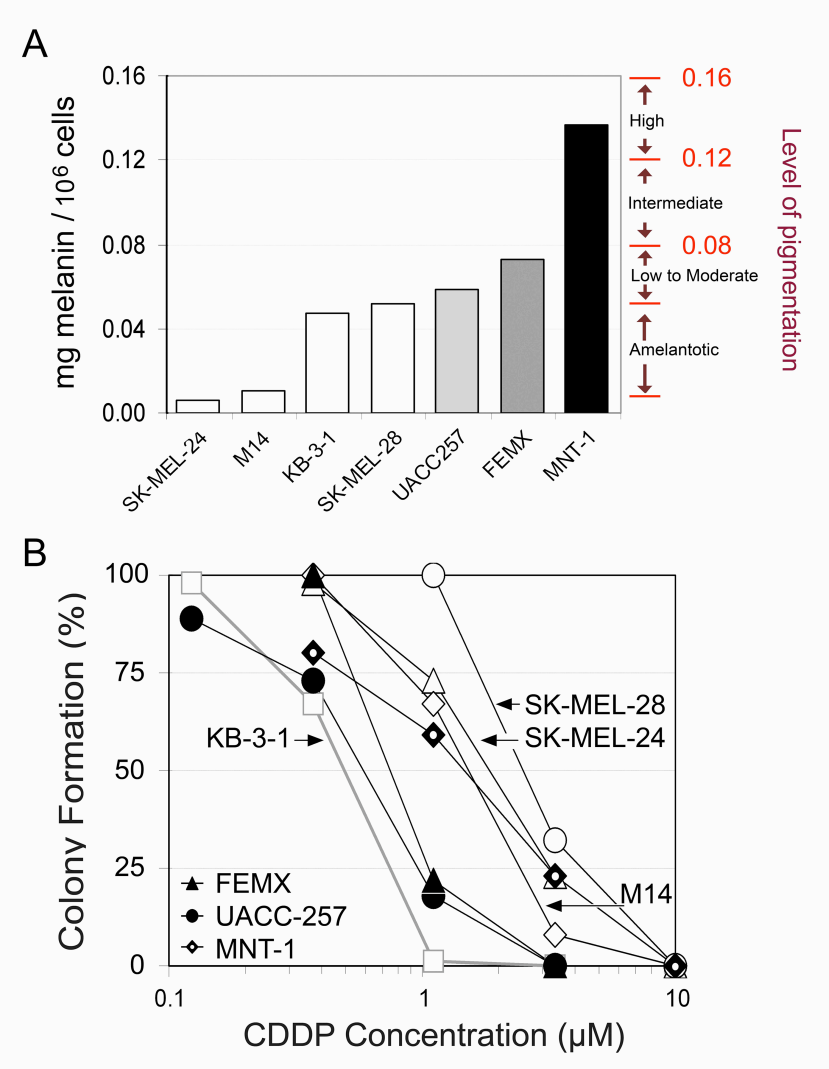
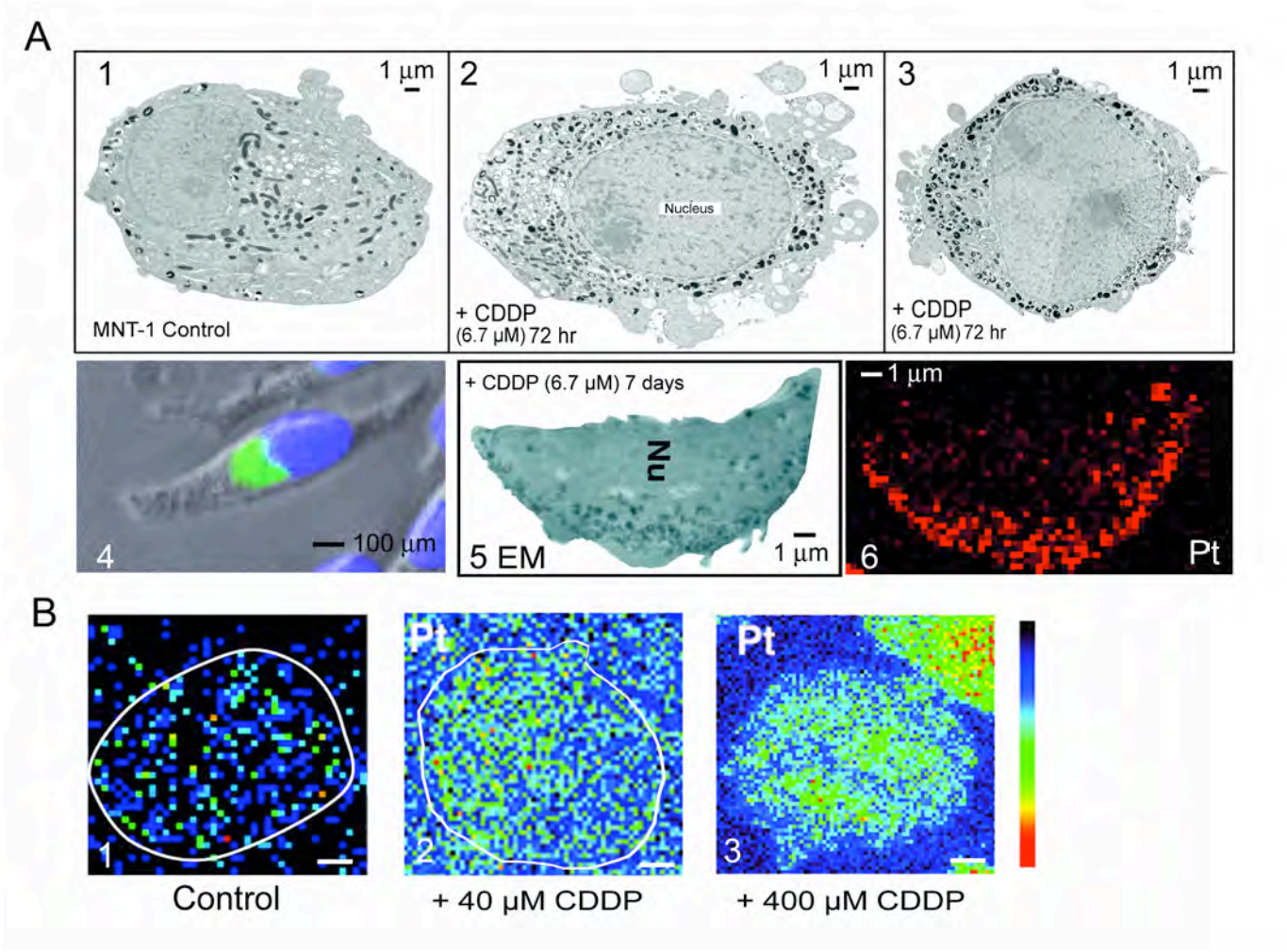
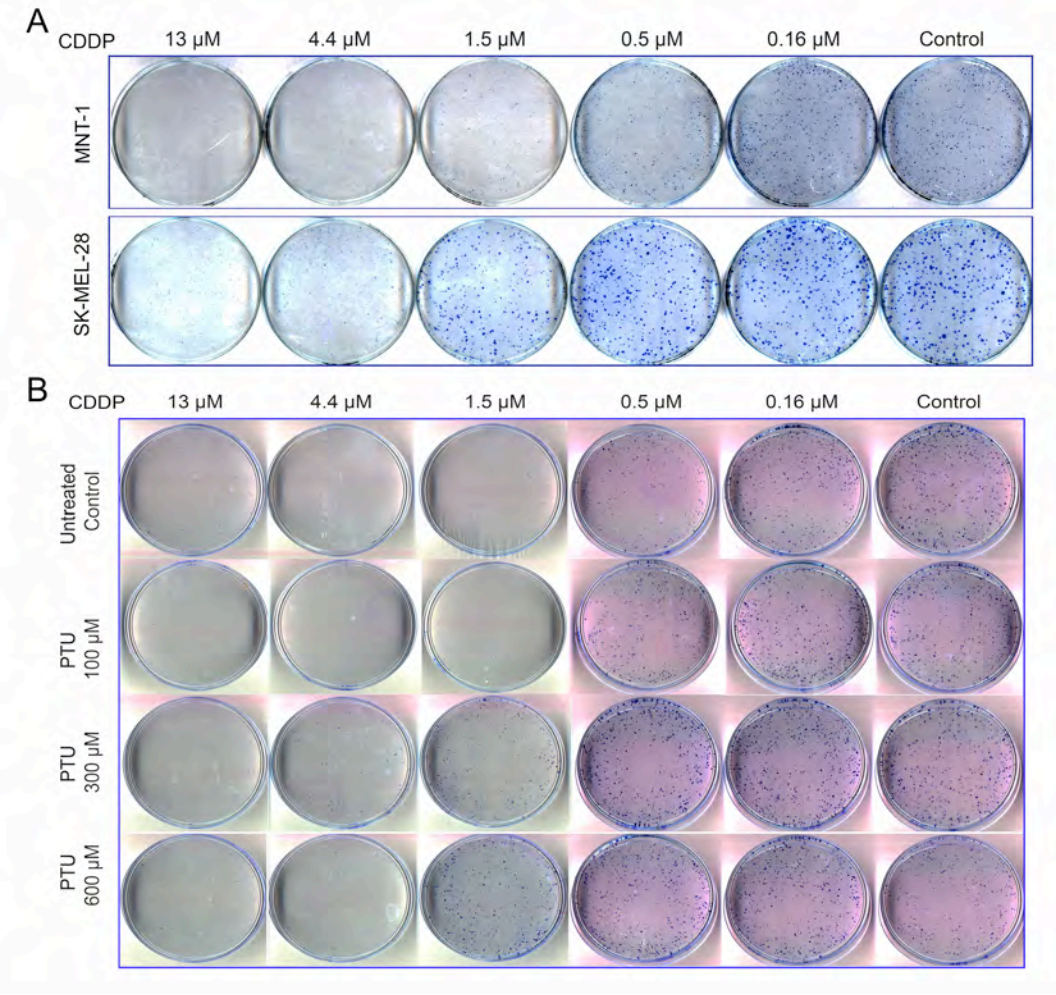


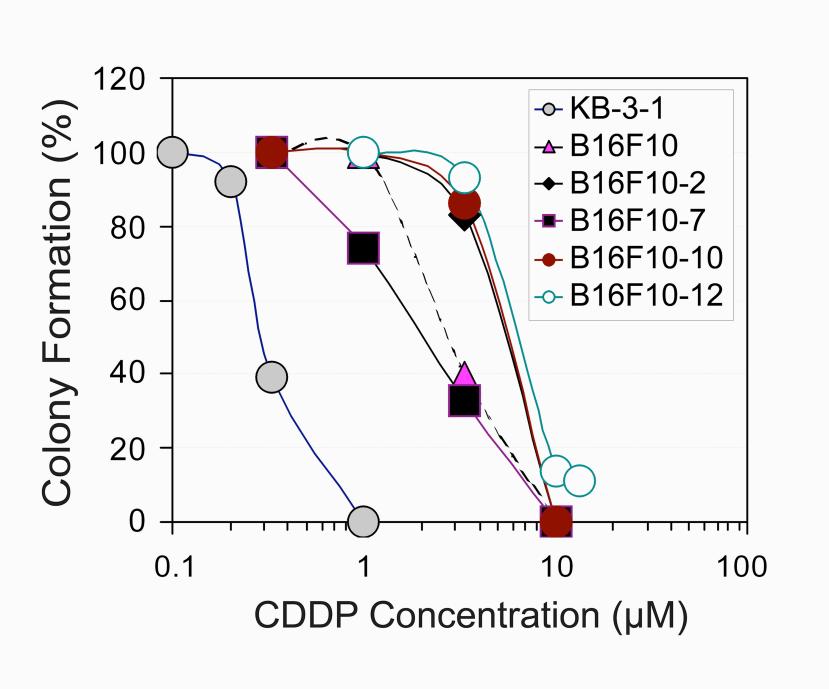
Supplementary Figure 1. The relationship between melanin content and drug resistance**. A**) Measurement of melanin content. The melanin content was measured in various melanoma cell line**s** (SK-MEL-24, M14, SK-MEL-28, UACC-257, FEMX, and MNT-1) and one non-melanoma cell line (KB-3-1, derived from HeLa cervical adenocarcinoma cells) and normalized to cell numbers. The cutoff points of the melanin content for defining the pigmentation status are shown; they classify melanoma cells as amelanotic, low to moderate, intermediate, and highly pigmented. **B**) Clonogenic assays of CDDP sensitivity. The assays were performed in three melanotic cell lines (MNT-1, FEMX, and UACC-257) and in three amelanotic cell lines (SK-MEL-28, SK-MEL-24, and M14). KB-3-1 cells were used as a non-melanoma control. Approximately 300 cells were seeded in 60-mm cell culture dishes. The cells were treated with CDDP for 10 days. Cytotoxic dose-response curves were determined as described in Materials and Methods. One of two similar experiments is shown.



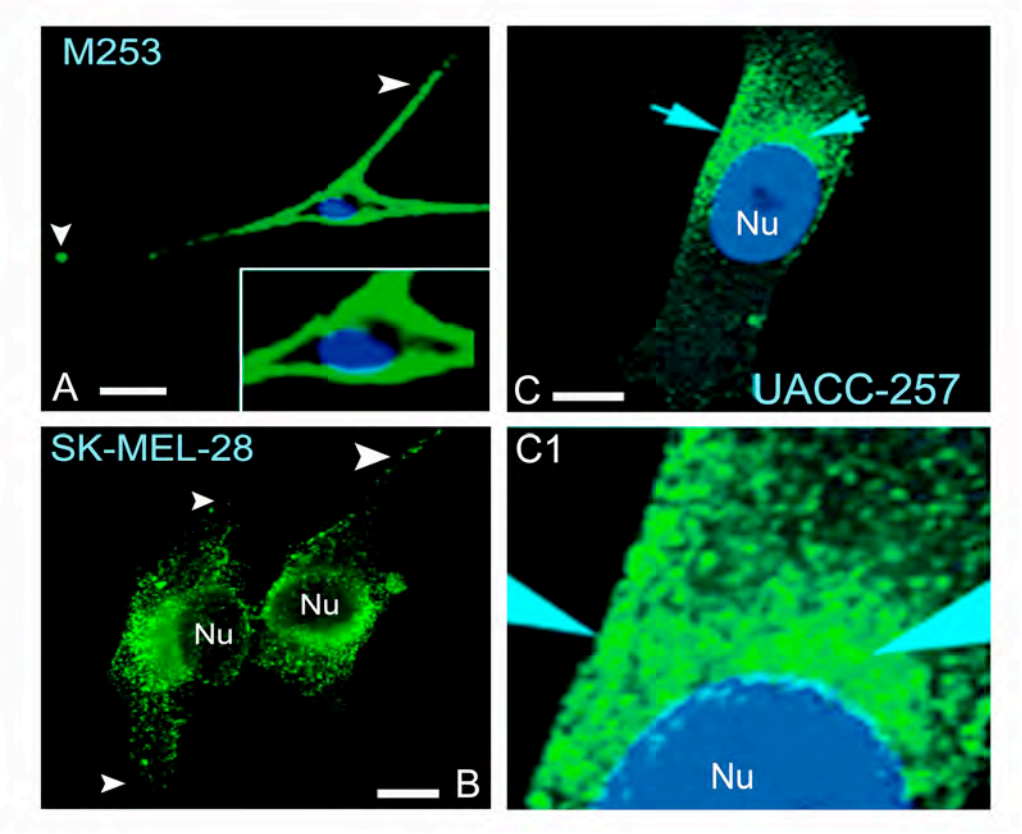
Supplementary Figure 2. Melanosome polarity and subcellular localization of CDDP. **A, 1**-**3**) Representative electron micrographs of MNT-1 cells treated with a sublethal dose (6.7 µM) of CDDP for 72 hr; **A4**) Untreated MNT-1 cells (grown in 6-well plates) were fixed with 4% paraformaldehyde, and stained with an antibody against the stage II melanosome marker HMB-45 (green) and DAPI (blue) and overlaid with a phase-contrast micrograph. Representative images were acquired with a 20X objective of the Zeiss Axiovert 200 microscope. **A5**) Unstained electron micrograph corresponding to the orientation of the X-ray micrograph in A6; **A6**) X-ray mapping of the platinum (Pt)-containing compound (CDDP) in MNT-1 cells treated with 6.7 µM CDDP for 7 days. **B**) X-ray mapping of CDDP (Platinum or Pt) in untreated MNT-1 cells (**B1**) or treated with 40 µM (**B2**) and 400 µM (**B3**) CDDP for 2.5 hr. Scale bars = 2 µm. Abbreviations: Nu, nucleus; EM, electron micrograph



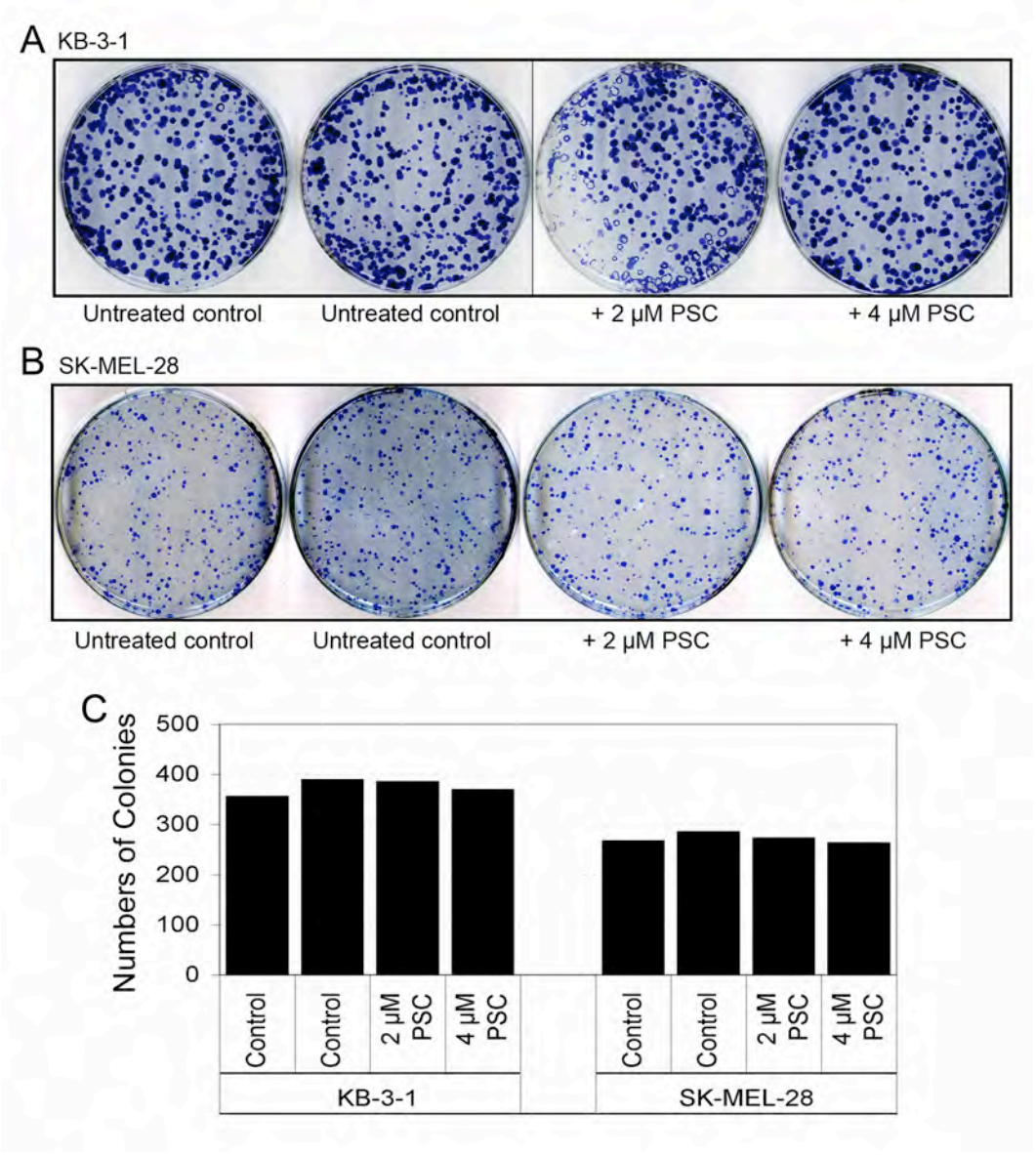
Supplementary Figure 3. Image presentation of clonogenic assays of CDDP sensitivity. Approximately 1,000 cells were seeded in 60-mm cell culture dishes, treated with indicated doses of CDDP for 3 days. The surviving colonies were stained with 0.5% methylene blue and counted at day 12 after removal of the drug-containing medium. Representatives of triplicate dishes of each treatment are shown. **A**) Clonogenic assays of CDDP sensitivity in SK-MEL-28 and MNT-1 cells. **B**) Clonogenic assays of CDDP sensitivity in MNT-1 cells in the presence of various 1-phenyl-2-thiourea (PTU) concentrations as indicated.



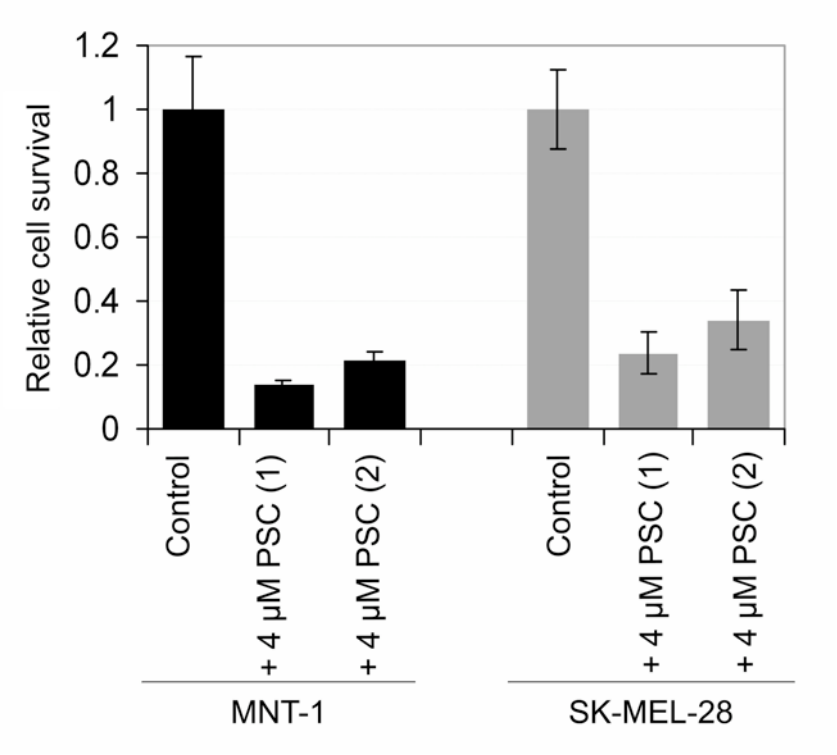
Supplementary Figure 4. Clonogenic assays of CDDP sensitivity in B16F10 subclones. Approximately 300 cells were seeded in 60-mm cell culture dishes. The cells were treated with CDDP for 10 days. Cytotoxic curves were determined as described in Materials and Methods. Representative cytotoxic dose-response curves from one experiment are shown. A comprehensive analysis of cytotoxic drug sensitivity and its association with melanin content is provided in Supplementary Table 1.



Supplementary Figure 5. Localization of the stage II melanosome marker HMB-45. Immunofluorescence analysis of HMB-45 expression was performed for M253 human melanocytes and in two melanoma cell lines (SK-MEL-28 and UACC-257). The cells were grown in two-chamber glass slides, fixed with 4% paraformaldehyde, and stained with the antibody against HMB-45 (green) and DAPI (blue) (in A, C, C1). **A**) HMB-45 expression in cultured and highly pigmented human melanocytes (M253 cells). Arrowheads point to dendrites of the cell. **B**) Cytoplasmic HMB-45 expression in amelanotic SK-MEL-28 cells. Arrowheads point to dendrite-like structures similar to melanocytes shown in A. **C**) HMB-45 expression in UACC-257 cells with an enlarged image shown in C1. Arrows (in both C and C1) indicate HMB-45 localizations at perinuclear and plasma membrane regions. Images were acquired with a Zeiss LSM 510 confocal microscope. Nu, nucleus; Scale bars = 100 μm.



Supplementary Figure 6. Clonogenic assays of PSC sensitivity. **A**) KB-3-1 cells. **B**) SK-MEL-28 cells. Approximately 1,000 cells were seeded in 60-mm cell culture dishes. The cells were treated on the third day by PSC for 3 days, as indicated. To accurately count the surviving colonies, we counted all dishes on day 12 after removal of the drug-containing medium. **C**) Histograms of colony numbers were determined by clonogenic assays (presented in both A and B).



Supplementary Figure 7. Cytotoxicity assays of PSC sensitivity in both MNT-1 and SK-MEL-28 cells cultured in high-density conditions. The exponentially growing cells were seeded in 6-well plates, grown near confluence, and treated with PSC for 3 days. The cells were then trypsinized, stained with Trypan Blue Stain (Invitrogen Inc., Carlsbad, CA), and counted with a hemocytometer. Two different batches of PSC stocks (labeled as 1 and 2) were used. Quantitative analysis of surviving cells is presented as histograms, with columns representing mean values of surviving cells in triplicate wells and error bars corresponding to 95% confidence intervals (CIs).