SUPPLEMENTARY DATA

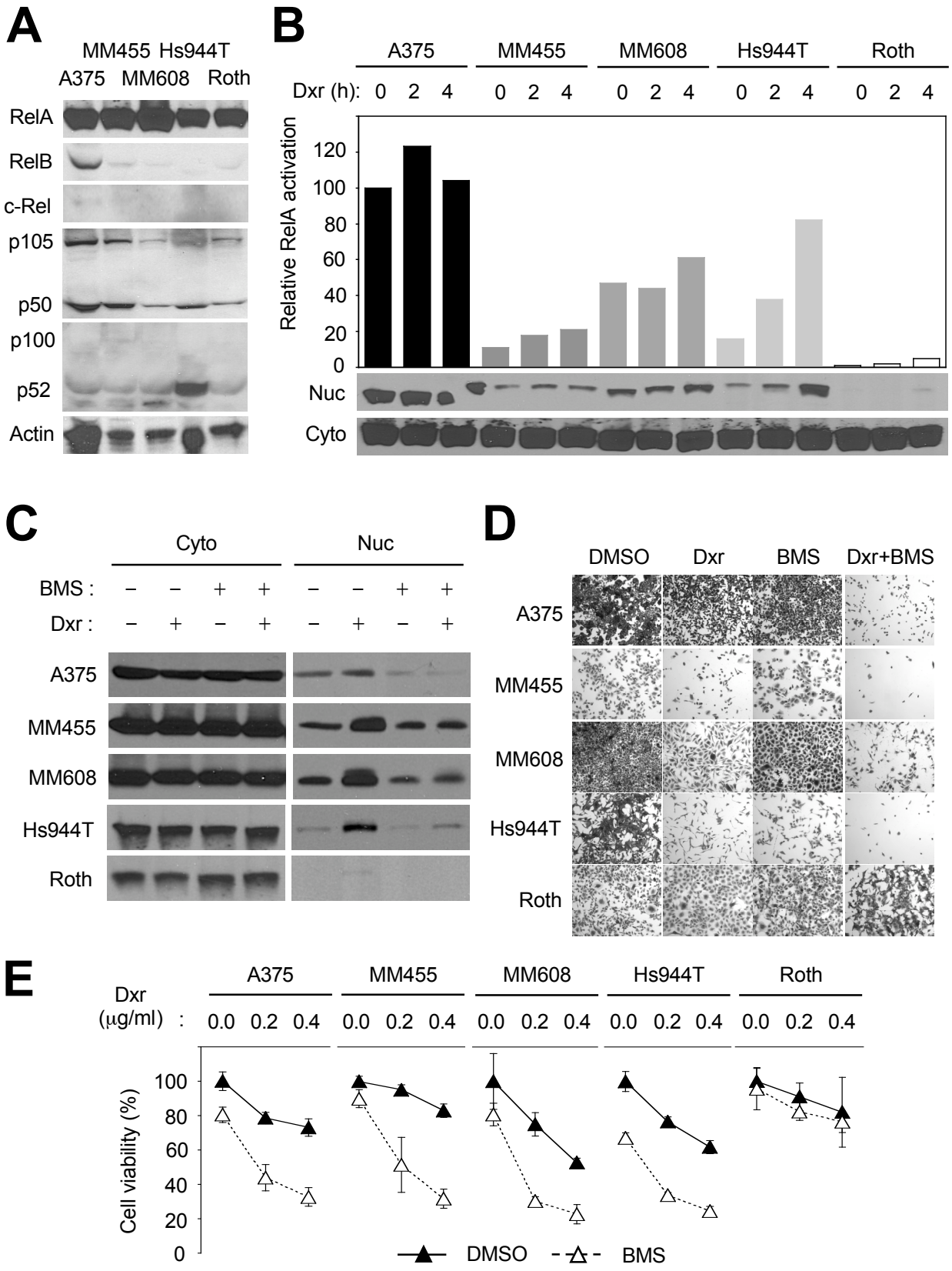

Figure S1. Doxorubicin-induced activation of NF-κB and NF-κB-mediated chemoresistance in various lines of human melanoma cells. A, Whole cell lysates from human melanoma cells were analyzed by immunoblotting with antibodies against the proteins indicated on the left. B, Cytoplasmic (Cyto) and nuclear (Nuc) extracts from human melanoma cells treated with doxorubicin (Dxr; 2 μg/ml) were prepared at the indicated time points and analyzed by immunoblotting with anti-RelA antibody. Values in the plot represent relative amounts of nuclear RelA. C, Human melanoma cells were treated with doxorubicin and BMS (75 μM; added 1 h prior to doxorubicin treatment) as indicated. Cyto and Nuc extracts were prepared 2 h after doxorubicin treatment and analyzed as in B. D, Human melanoma cells were treated with doxorubicin (0.5 μg/ml) and BMS (75 μM). Shown are viable adherent cells stained with crystal violet 24 h after doxorubicin treatment. (E) Human melanoma cells were treated with doxorubicin and BMS as in D. Cell viability was determined by MTT assay 24 h after doxorubicin treatment.

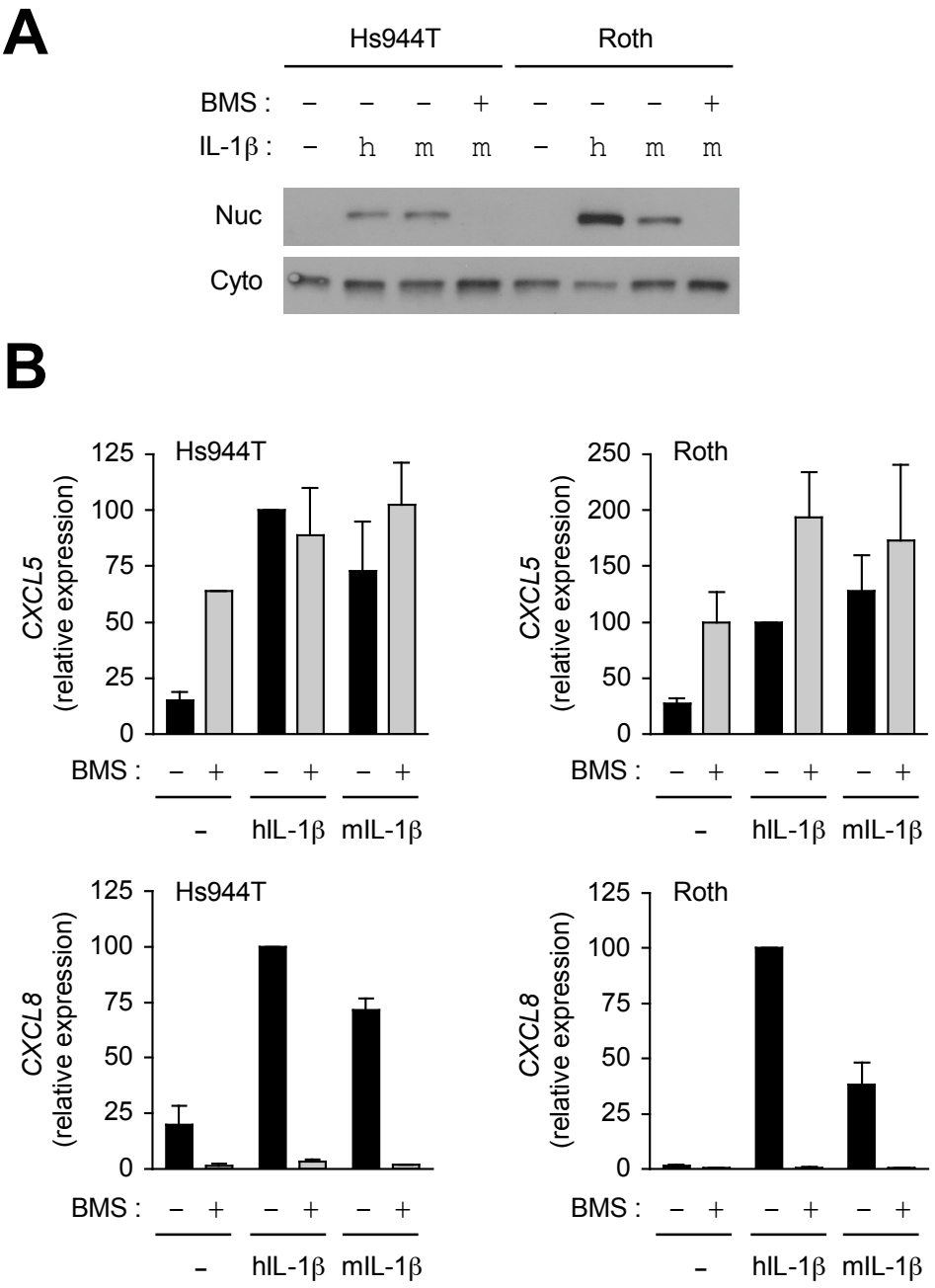

Figure S2. IL-1β-induced activation of NF-κB and chemokine gene expression in Hs944T and Roth cells. A, Hs944T and Roth cells were treated with human (h) or mouse (m) IL-1β (25 ng/ml) and BMS (75 μM; added 1 h prior to IL-1β treatment) as indicated. Cytoplasmic (Cyto) and nuclear (Nuc) extracts were prepared 15 min after IL-1β treatment and analyzed by immunoblotting with anti-RelA antibody. B, Hs944T and Roth cells were treated as in A. Total RNA was isolated 1 h after IL-1β treatment and analyzed by qPCR using primers specific to the genes indicated on the left. Expression is relative to that of *GAPDH*.

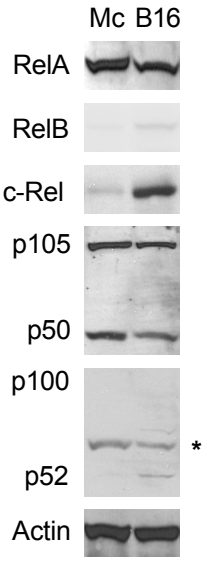

Figure S3. Expression of NF-κB proteins in mouse melanocytes and melanoma cells. Whole cell lysates from primary mouse melanocytes (Mc) and B16 mouse melanoma cells were analyzed by immunoblotting with antibodies against the proteins indicated on the left. Asterisk indicates a nonspecific signal.

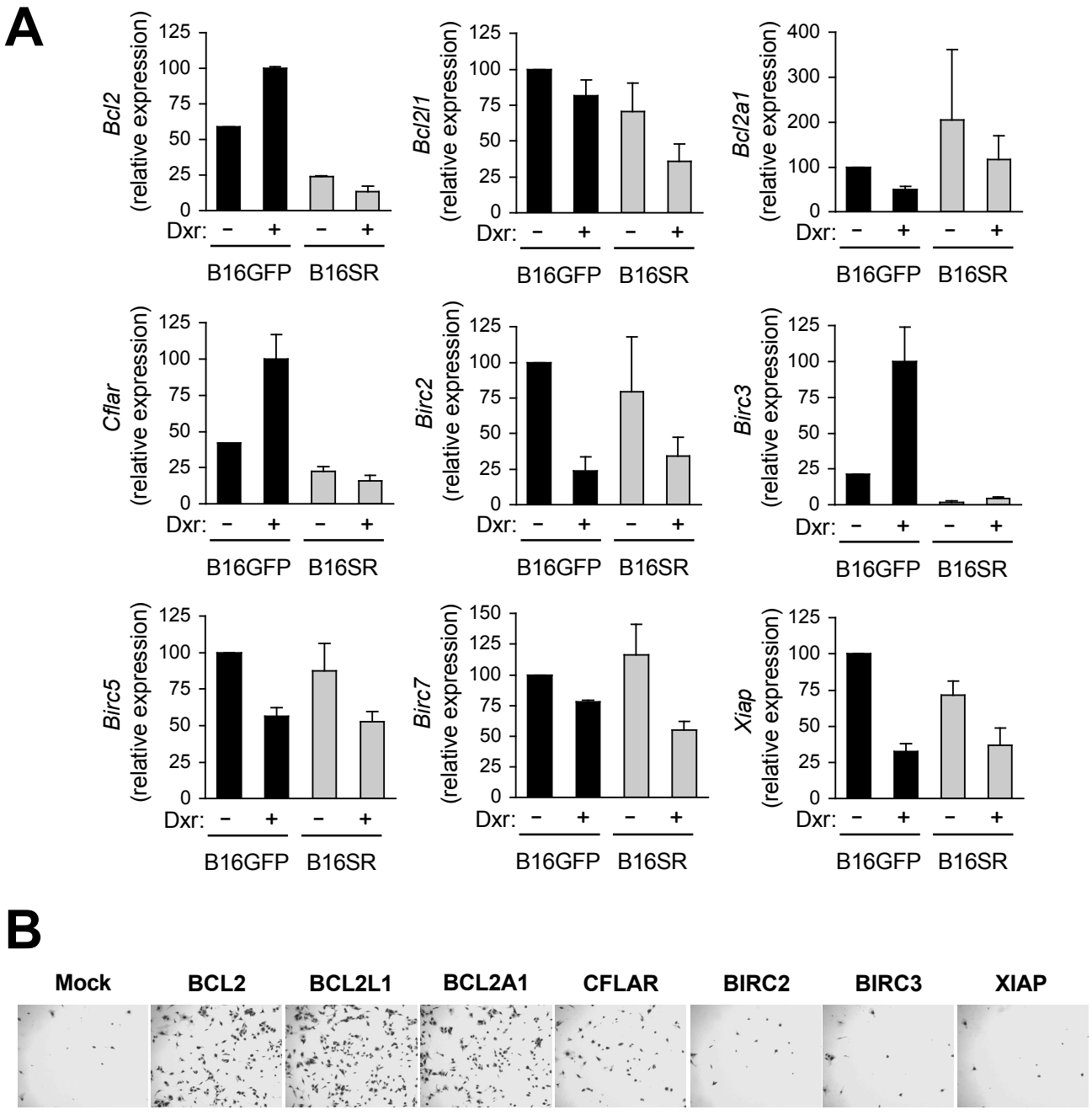

Figure S4. Antiapoptotic gene expression and function in Dxr-treated B16 cells. A, B16GFP and B16SR cells were treated with DMSO and doxorubicin (Dxr; 2 μg/ml) for 4 h. Total RNA was isolated and analyzed by qPCR using primers specific to the genes indicated on the left. Values represent percent mRNA relative to that in B16GFP cells. Expression is relative to that of *Ppia* (encoding cyclophilin). B, B16 cells were transfected with plasmid vectors expressing various antiapoptotic genes, left in culture for 48 h, and treated with doxorubicin (0.5 μg/ml). Shown are viable adherent cells stained with crystal violet 36 h after doxorubicin treatment.

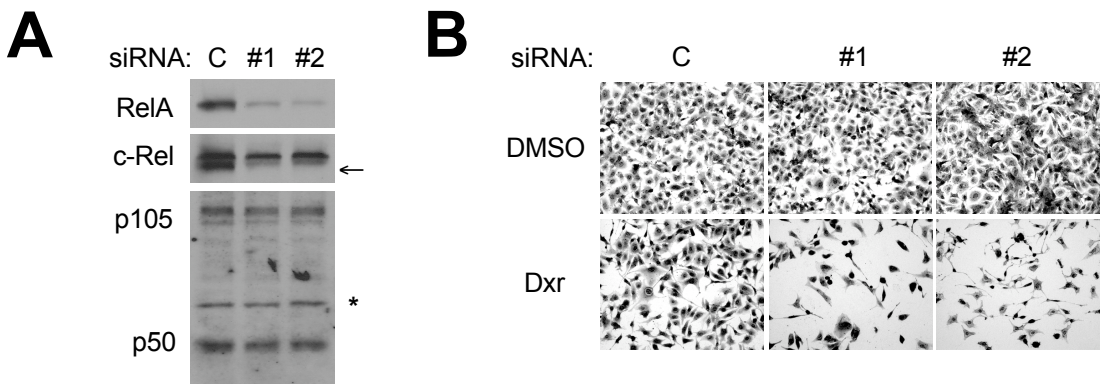

Figure S5. RelA-dependent chemoresistance in B16 cells. B16 cells were transfected with siRNA specific to mouse RelA mRNA (#1 and #2, targeting different sequences) and control siRNA (C), and left in culture for 48 h before analysis. A, Whole cell lysates were prepared analyzed by immunoblotting with antibodies against the proteins indicated on the left. Asterisk indicates a nonspecific signal. Arrow indicates a RelA-specific signal that originated from the previous round of immunoblotting. B, Cells were treated with DMSO and doxorubicin (Dxr; 0.5 μg/ml) as indicated. Shown are viable adherent cells stained with crystal violet 24 h after treatment.

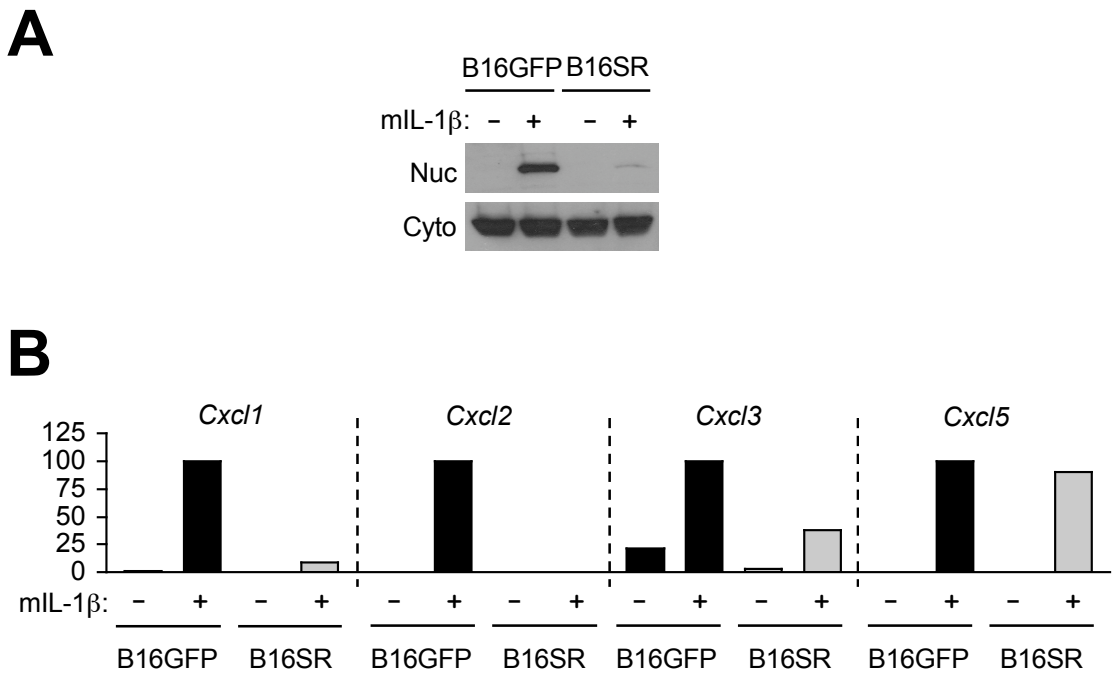

Figure S6. IL-1β-induced activation of NF-κB and chemokine gene expression in B16 cells. A, B16GFP and B16SR cells were treated with mouse IL-1β (25 ng/ml) as indicated. Cytoplasmic (Cyto) and nuclear (Nuc) extracts were prepared 15 min after IL-1β treatment and analyzed by immunoblotting with anti-RelA antibody. B, B16GFP and B16SR cells were treated as in A. Total RNA was isolated 1 h after IL-1β treatment and analyzed by qPCR using primers specific to the genes indicated on the left. Expression is relative to that of *Ppia*.

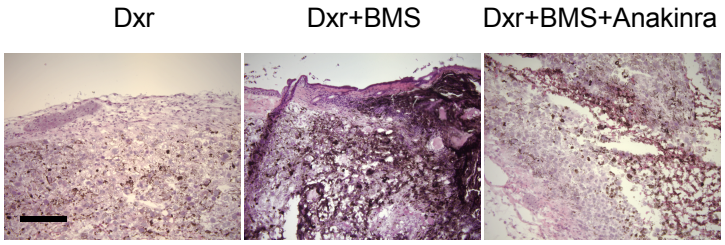

Figure S7. Effects of Dxr, BMS, and anakinra on B16 tumors in wild-type mice. C57BL/6 mice bearing B16 tumors were administered with doxorubicin (Dxr; 2 mg/kg), BMS (125 mg/kg), and anakinra (25 mg/kg) as indicated.Doxorubicin and BMS were administered on day 0, 2, 4, 6, and 8, and anakinra on day 3, 5, 7, 9, 11, and 13. Tumor sections were prepared on day 20 and analyzed by H&E staining. Scale bar, 100 μm.

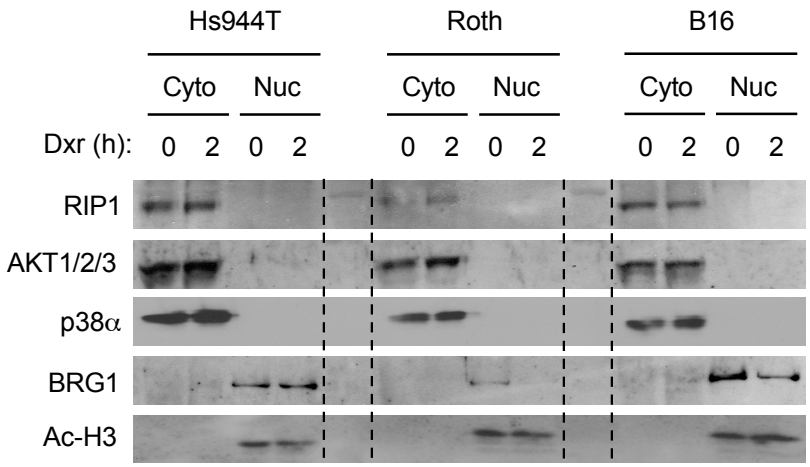

Figure S8. The purity of cytoplasmic and nuclear extracts of melanoma cells. Cytoplasmic (Cyto) and nuclear (Nuc) extracts prepared from melanoma cells, the same extracts used in the experiments of Fig. 1A and 3A, were analyzed by immunoblotting with antibodies against the following proteins: RIP1 (610458; BD Biosciences); AKT1/2/3 (sc-8312), p38α (sc-535), BRG1 (sc-10768; all from Santa Cruz Biotechnology); and acetylated histone H3 (Ac-H3; 06-599; Millipore). All samples were separated in the same gel, and transferred and subjected to immunoblotting on the same membrane. Dotted lines indicate space between groups of samples from different melanoma cell lines.