

Figure S1. Cell proliferation in 293T cells treated with multiple concentrations (0, 1, 2.5, 5, 10 and 25  $\mu$ M) of 7-DHC or ethanol as a control for 48 h. 7-DHC, 7-dehydrocholesterol.

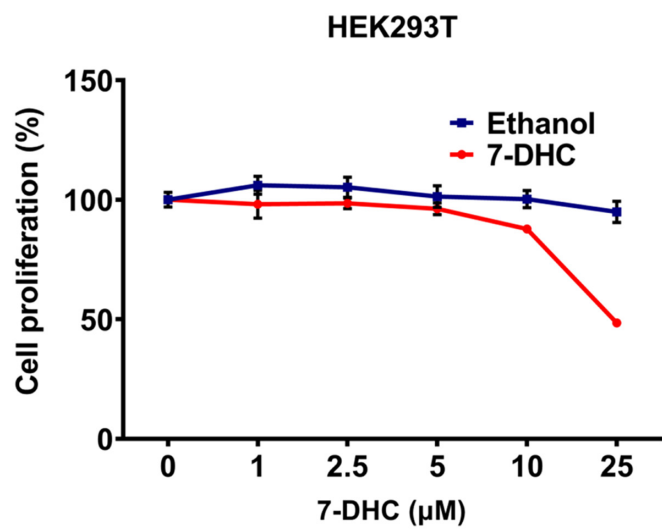


Figure S2. 7-DHC increases melanoma cell apoptosis and inhibits melanoma cell migration. (A) Hoechst-PI double staining of A375 and A2058 melanoma cells treated with 7-DHC (eye lens x objective lens, 10x10). (B) Migration rates of A375 melanoma cells treated with 7-DHC (0, 1, 5, 10 and 25  $\mu\text{M}$ ) were measured via wound-healing assay (eye lens x objective lens, 10x10). 7-DHC, 7-dehydrocholesterol.

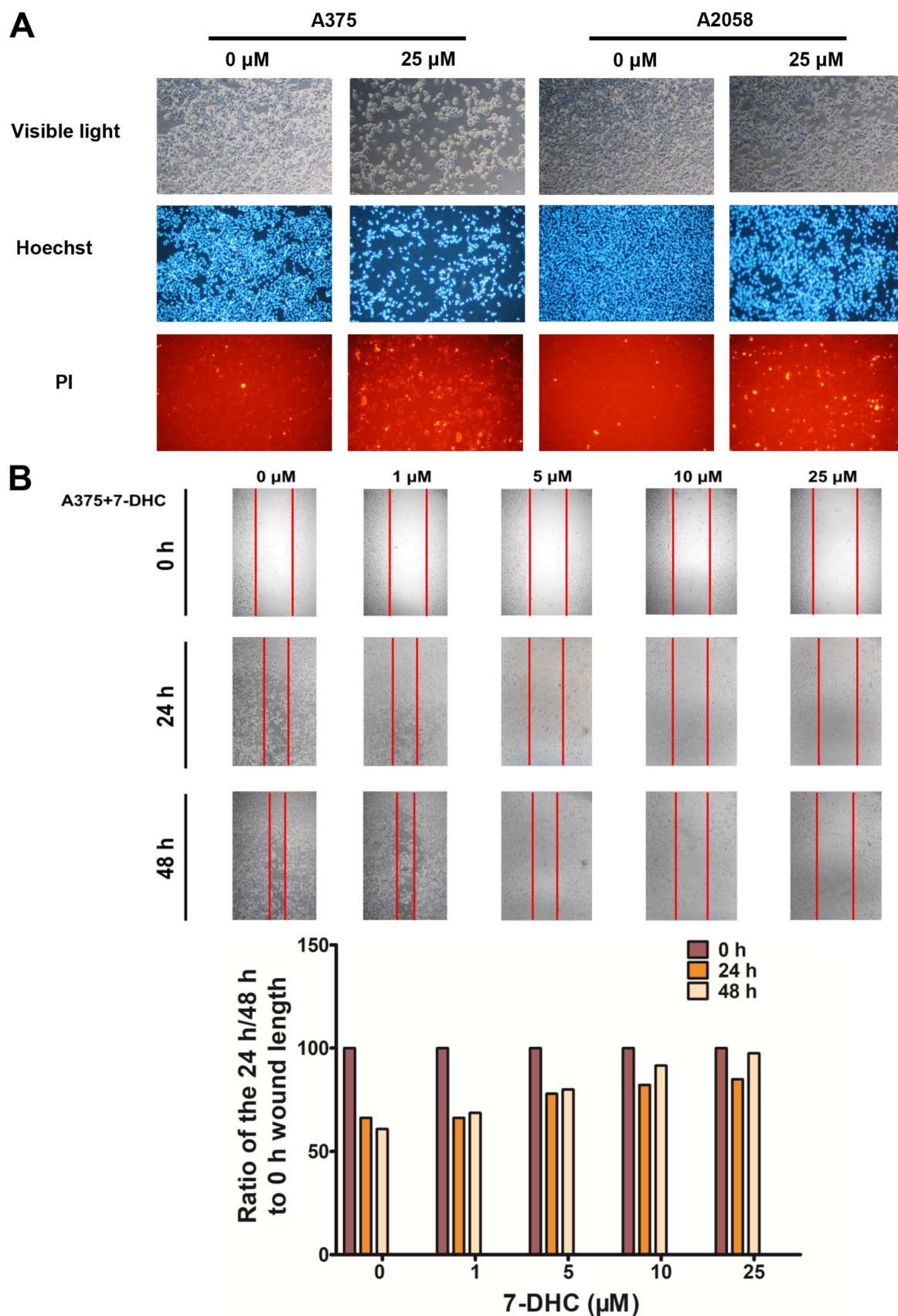


Figure S3. 7-DHC induces cell cycle arrest of melanoma cells at S phase. Results of flow cytometry detecting the cell cycle profile of (A) A375 and (B) A2058 melanoma cells treated with different concentration of 7-DHC. Cell cycle analysis of (C) A375 and (D) A2058 melanoma cells treated with various concentrations (0, 1, 2.5, 5, 10 and 25  $\mu$ M) of 7-DHC. 7-DHC, 7-dehydrocholesterol.

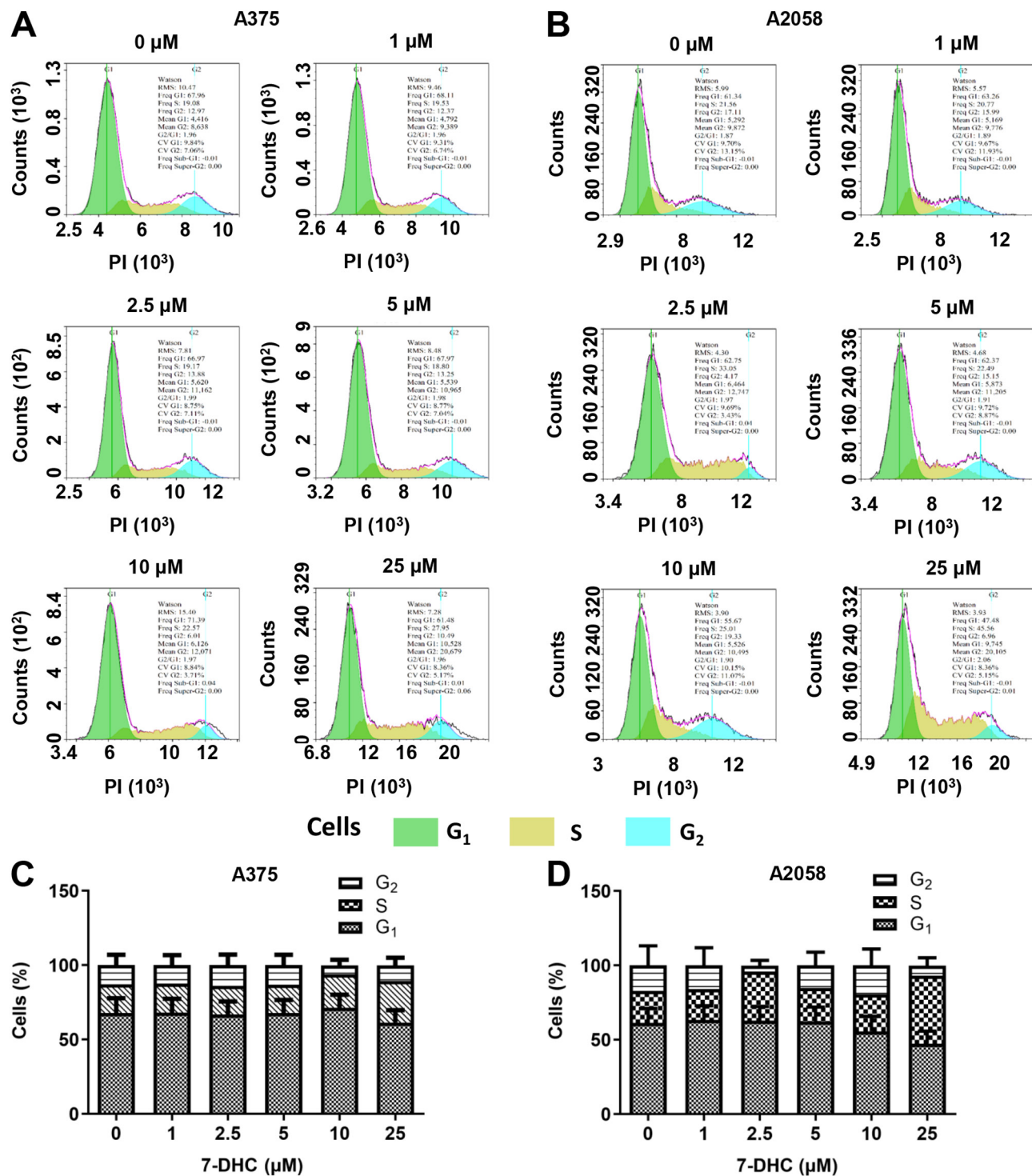


Figure S4. 7-DHC inhibits melanoma via the Akt1/NF- $\kappa$ B signaling pathway. (A) Western blot analysis of A2058 cells treated with several concentrations (0, 1, 2.5, 5, 10 and 25  $\mu$ M) of 7-DHC were compared with ethanol as the NC for the analysis of phosphorylated and non-phosphorylated levels of Akt1-Ser473 normalized to GAPDH. (B) Western blot analysis of A2058 cells treated with several concentrations (0, 1 and 2.5  $\mu$ M) of 7-DHC were compared with ethanol as the NC to analyze the entry of RELA into the nucleus. (C) Western blot analysis of A375 cells treated with several concentrations (1, 5 and 10  $\mu$ M) of 7-DHC and the Akt1 activator IGF1 (1  $\mu$ M) were compared with ethanol as the NC for the analysis of phosphorylated and non-phosphorylated levels of Akt1-Ser473 normalized to GAPDH. Relative expression of reported RELA target genes, AMACR, HMOX1, IL11, LCN2 and PTGS2, in (D) A375 and (E) A2058 cells treated with the Akt1 inhibitors, MK-2206 (1  $\mu$ M) and AKT inhibitor III (1  $\mu$ M). \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001. 7-DHC, 7-dehydrocholesterol; RELA, RELA proto-oncogene NF- $\kappa$ B subunit; IGF, insulin-like growth factor 1; p, phosphorylated; NC, negative control; C, cytoplasmic; N, nuclear; AMACR,  $\alpha$ -methylacyl-CoA racemase; HMOX1, heme oxygenase 1; LCN2, lipocalin 2; PTGS2, prostaglandin-endoperoxide synthase 2.

