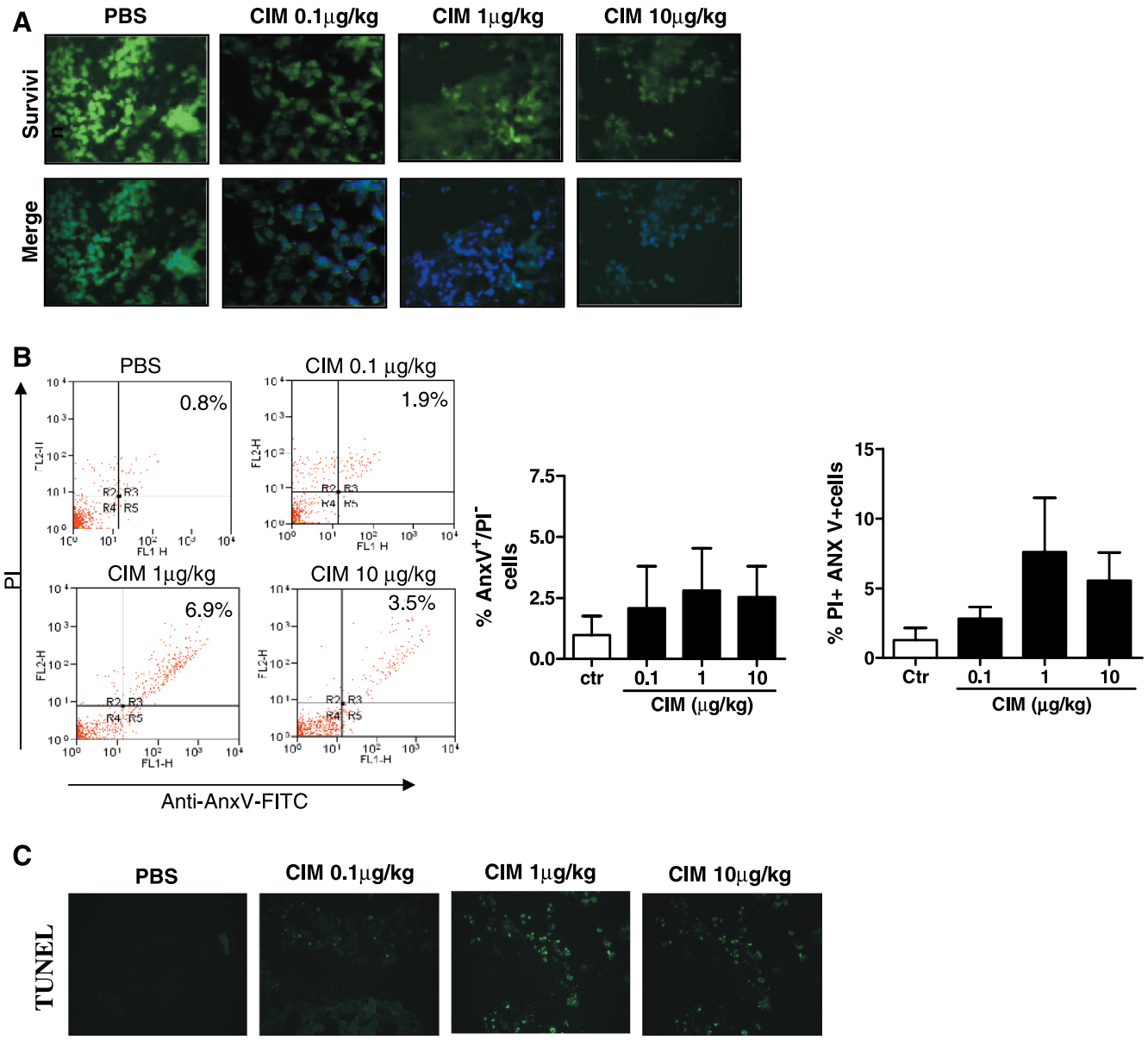

Figure W1. CIM treatment increases apoptosis in melanoma tissue. (A) Survivin expression detected by immunofluorescence analysis was reduced in CIM-treated mice compared with PBS. Data are representative of four independent experiments. Magnification, ×40. (B) The percentage of annexin V^+ cells (left graph) and annexin V and PI double-positive cells ($AnxV^+/PI^+$) (right graph) in mice treated with CIM (black bars) increased compared with PBS (open bar) (right panel). Representative dot plots are shown. Data are expressed as mean ± SEM (n = 7). (C) TUNEL+ staining (FITC) indicates apoptotic cells, which are increased in melanoma tissue harvested from mice treated with CIM compared with PBS. The pictures are representative of three experiments. Magnification, ×20.

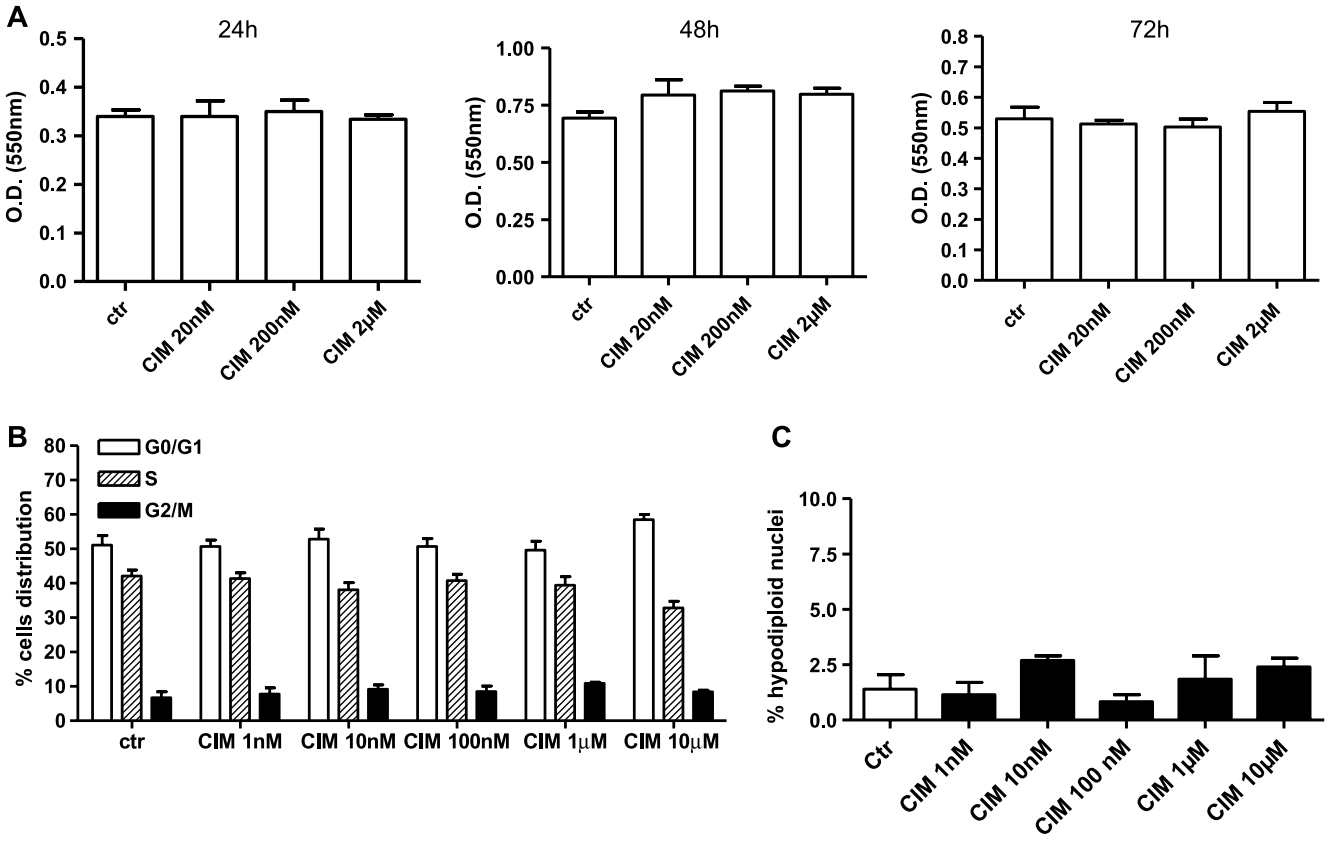

Figure W2. Effect of Cl-IB-MECA on B16-F10 cells. Cl-IB-MECA (20 nM to 2 μM) was added to the B16-F10 cells in culture and cell viability by MTT assay (A). Proliferation (B) and apoptosis (C) rates, by means of FACS analyses, were evaluated 24, 48, and 72 hours later. Shown are the results obtained from cell cycle and apoptosis analyses at 24 hours; similar results were obtained at 48 hours. Data are expressed as mean ± SEM (n = 6).

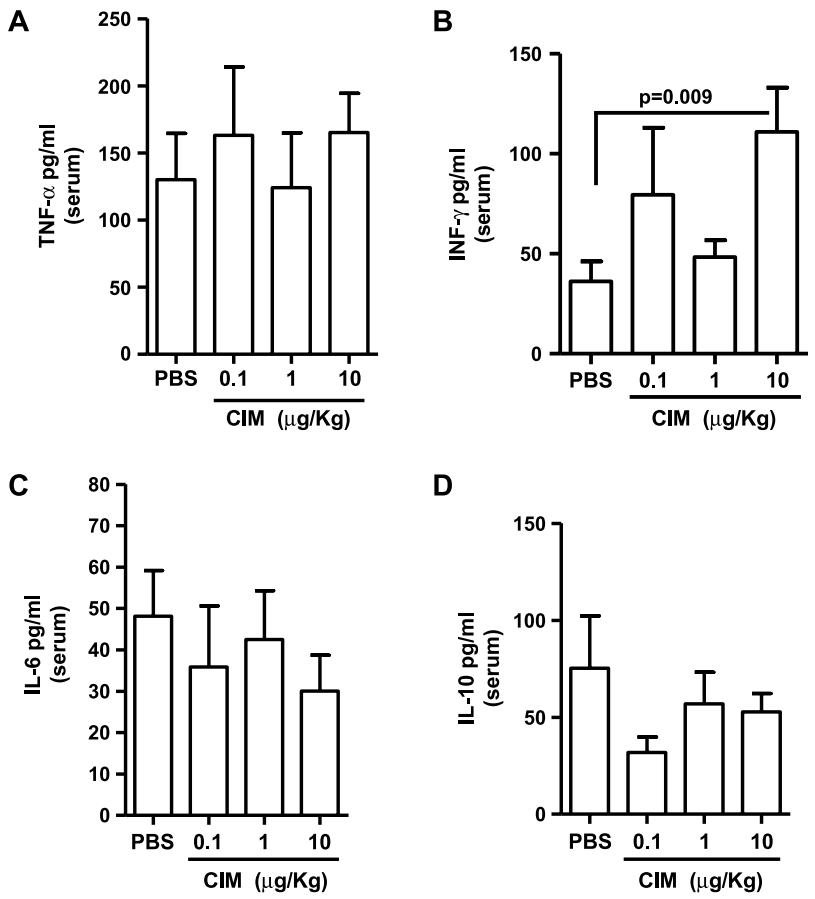

Figure W3. Serum levels of cytokines after CIM administration. Serum levels of TNF-α ($n = 10$; A), IL-6 ($n = 13$; C), and IL-10 ($n = 13$; D) did not change after CIM treatment whereas levels of IFN-γ significantly increased at the highest dose of CIM compared with PBS ($n = 8$) (B). Data are expressed as mean ± SEM. Statistical differences were determined by one-way ANOVA followed by Dunnett *post hoc* analysis.

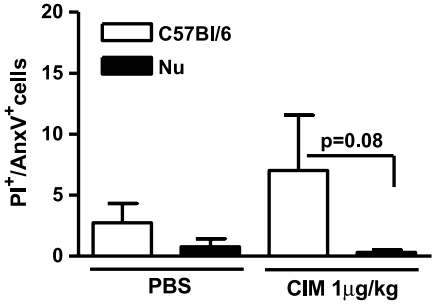

Figure W4. Apoptosis cells in nude melanoma-bearing mice after CIM treatment. Apoptosis as determined by the number of annexin V and propidium iodide (PI) double-positive ($AnxV^+PI^+$) cells was lower in Nu mice treated with CIM (black bar) than those obtained in C57Bl/6 mice (open bar). Data are expressed as mean ± SEM, $n = 10$.