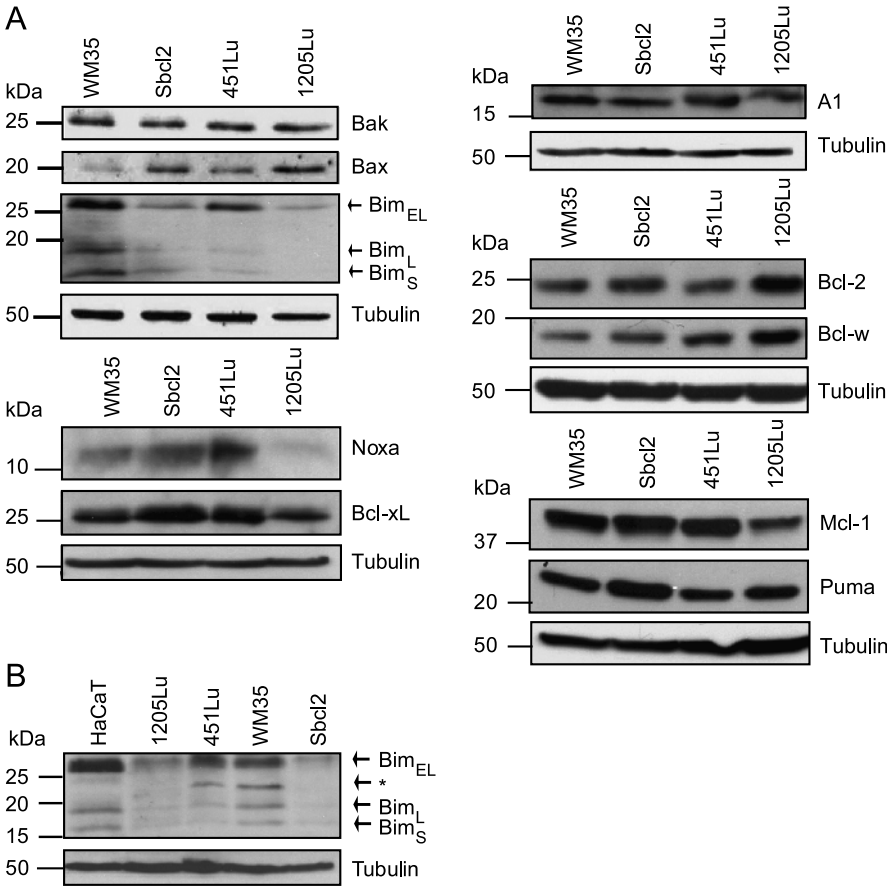

Figure W1. Expression profile of proapoptotic and antiapoptotic proteins in melanoma cell lines. (A) Whole-cell lysates were prepared from 1205Lu, 451Lu, WM35, and Sbcl2 cells, and equal amounts of protein were loaded and run on 12.5% SDS-PAA gels. Tubulin served as a loading control. All Western blots are representatives of three independent experiments. (B) Western blot analysis of whole-cell lysates showing the levels of Bim expression in HaCaT keratinocytes in comparison to melanoma cells. *Protein of unknown origin.

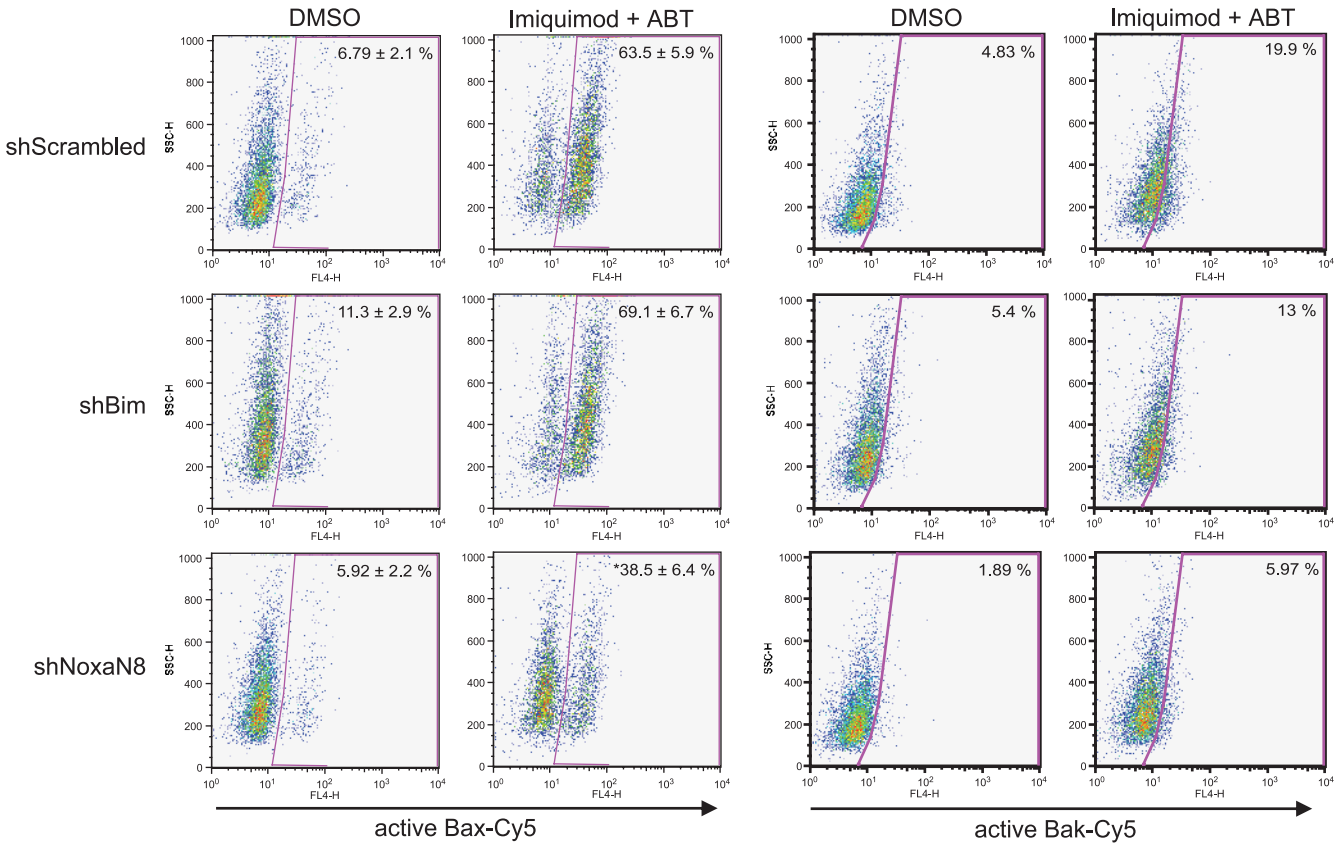

Figure W2. Knockdown of Noxa protects from imiquimod-induced activation of Bax and Bak in 451Lu melanoma cells. Cells were treated with DMSO (0.7%) or 50 μg/ml imiquimod plus ABT-737 (ABT; 1 μM) for 24 hours. Staining of active Bax and Bak in 451Lu scrambled, Bim, and NoxaN8 RNAi knockdown cells was performed as described in Figure W3. The percentages of active Bax represent means ± SEM of three experiments. *$P < .05$ compared with shScrambled. Staining for active Bak was performed once.

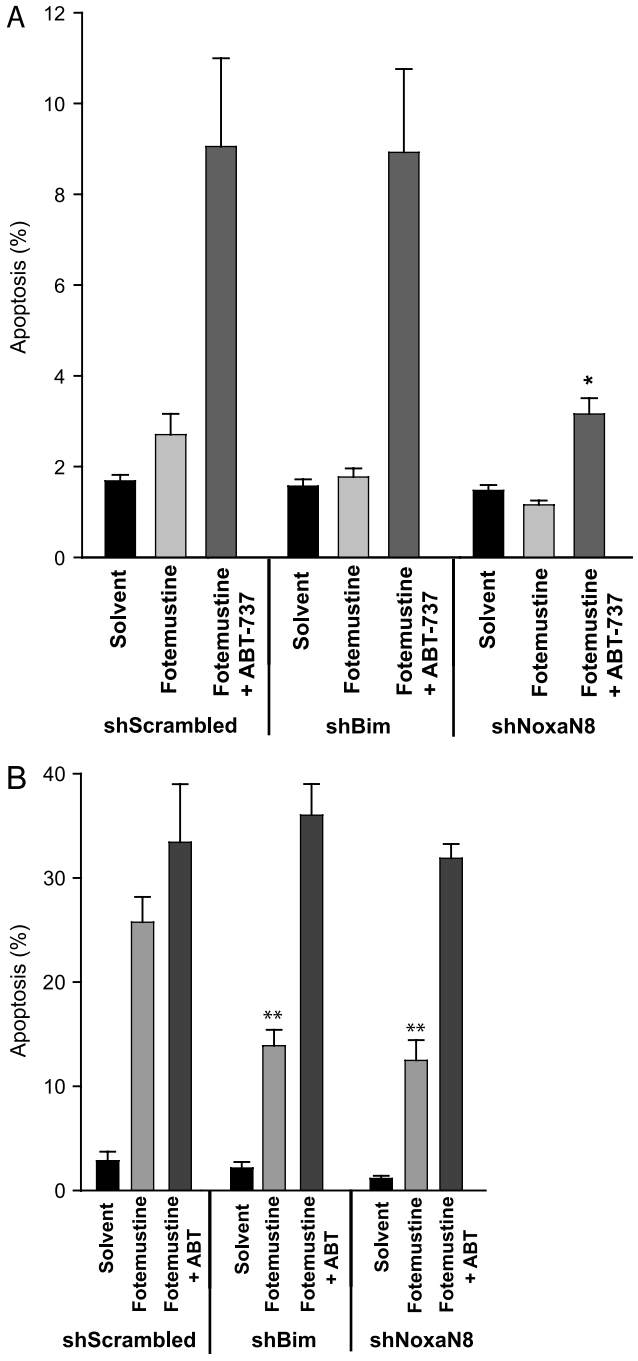

Figure W3. Requirement for Bim or Noxa in 1205Lu melanoma during apoptosis induced by fotemustine and in combination with ABT-737. Cells were treated for 6 (A) or 24 hours (B) with fotemustine (50 μg/ml) alone or in combination with ABT-737 (1 μM), and cell death was assessed by staining for active caspase 3 (A, B), followed by flow cytometric analysis. Data represent means $\pm$ SEM of at least four experiments. *$P < .05$; ** $P < .005$ compared with scrambled control.

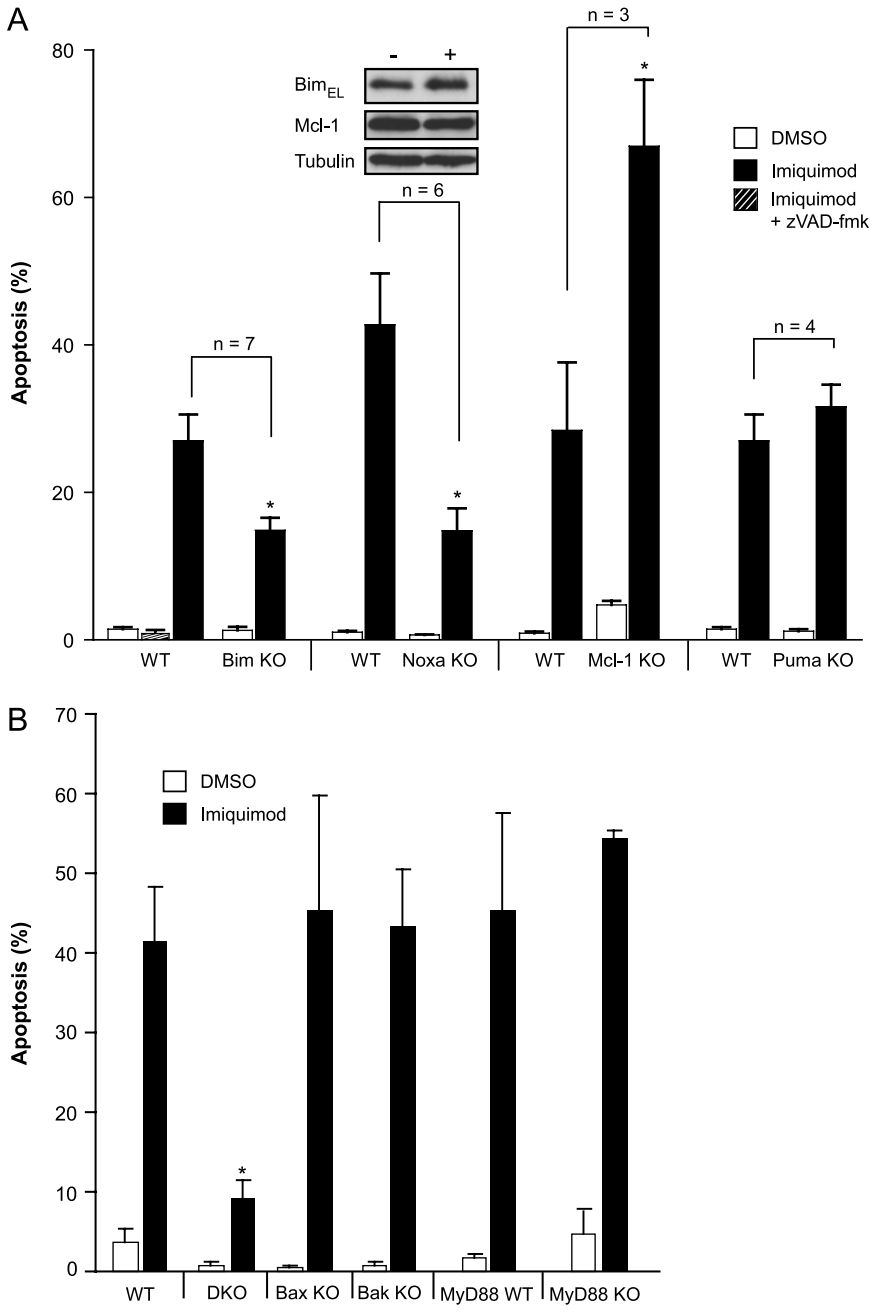

Figure W4. Loss of Bim or Noxa affords protection against imiquimod-induced killing of MEF cells. (A) Mouse embryonic fibroblast cells from Bim, Noxa, Mcl-1, and Puma knockout (KO) mice and their corresponding wild type cells (WT) were treated for 19 hours with DMSO (1%) or 100 μg/ml imiquimod, and apoptosis was assessed by staining for active caspase 3, followed by flow cytometric analysis. Where indicated, 50 μM zVAD-fmk was added before stimulation. Data represent means ± SEM of at least three independent experiments as indicated (*n*). *$P < .04$ significantly less or more apoptosis compared with the equivalent wild type (WT). (inset) Whole-cell lysates of MEF NoxaWT cells 18 hours after stimulation with 100 μg/ml imiquimod (+) or with 1% DMSO were tested by Western blot analysis for Bim and Mcl-1 expression. Tubulin served as a loading control. Similar results were obtained in two separate experiments. (B) Bax and Bak can both mediate imiquimod-induced killing in MEF cells, but MyD88 is not required. $Bax^{+/-}$ $Bak^{+/-}$ (labeled WT), $Bax^{-/-}$ $Bak^{-/-}$ (DKO), $Bax^{-/-}$ $Bak^{+/-}$ (Bax KO), $Bax^{+/-}$ $Bak^{-/-}$ (Bak KO), MyD88 wild type, and knockout MEFs were incubated with 75 μg/ml imiquimod or DMSO (0.75% alone) alongside the respective wild type cells. At 40 hours after stimulation, cells were collected and stained with active caspase 3 followed by flow cytometric analysis. Data represent means ± SEM of at least three experiments. *$P < .05$, significantly less apoptosis compared with WT MEFs incubated with imiquimod.

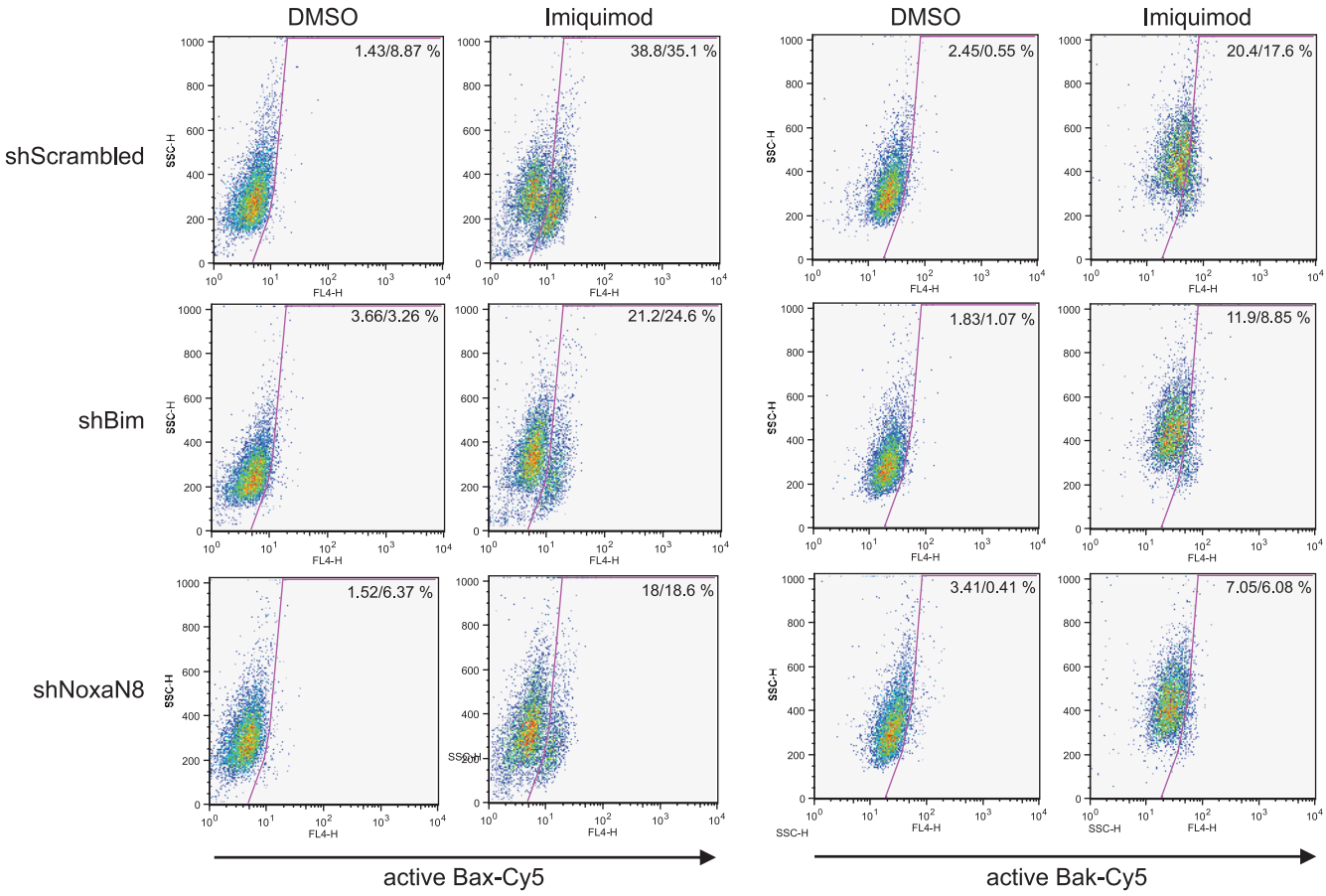

Figure W5. Knockdown of Bim or Noxa protects HaCaT keratinocytes from imiquimod-induced activation of Bax and Bak. Cells were treated with DMSO alone (solvent control, 1%) or with 100 μg/ml imiquimod for 24 hours, and Bax activation was monitored by flow cytometry after staining with anti–conformationally changed Bax (Clone3)/Cy5–conjugated goat–antimouse IgG. In parallel, Bak activation was detected using flow cytometry after staining with anti–conformationally changed Bak (AB-1)/Cy5–conjugated goat–antimouse IgG. Results are given for two separate experiments and one representative dot blot.