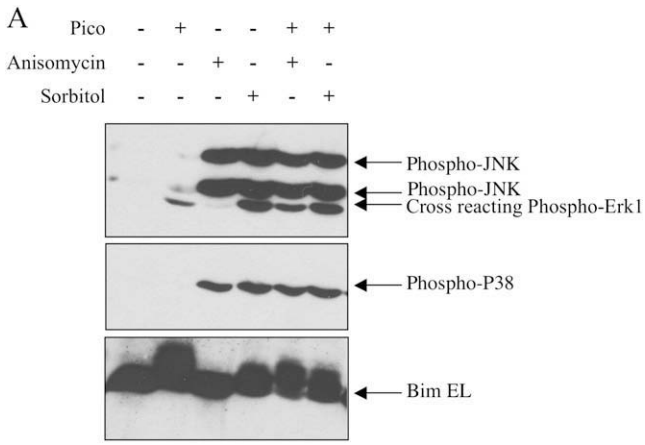
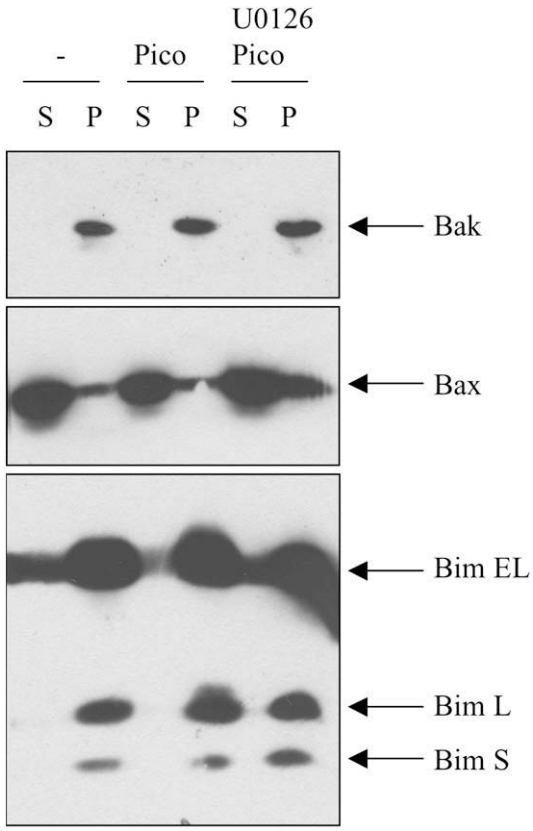
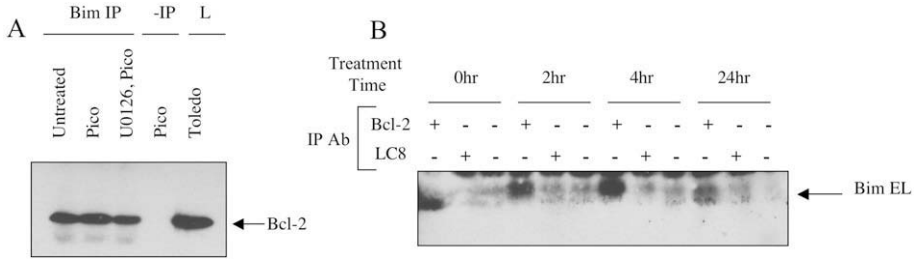
Appendix



Supplementary Figure S1. Lack of involvement of p38 and Jnk mitogen-activated protein (MAP) kinases in pico-induced Bim phosphorylation. (**A**) Effects of pico, anisomycin, and sorbitol, individually and in combinations, on Jnk, p38, and BimEL phosphorylation were evaluated in Toledo B-cell non-Hodgkins lymphoma (B-NHL) cells. (Note: the fastest migrating species reacting with the anti−phospho-Jnk antibody is a cross-reacting phospho-Erk1, according to the antibody supplier).



Supplementary Figure S2. Subcellular fractionation of Bak, Bax and Bim in Toledo B-cell non-Hodgkins lymphoma (B-NHL) cells after pico treatment alone or in combination with the Mek inhibitor U0126. Cells were either untreated, treated with pico for 24 hours, or treated with pico plus U0126 pretreatment. Cells were homogenized in hypotonic buffer and centrifuged at low speed to remove nuclei. Homogenates were then subjected to ultracentrifugation and thereby separated into P100 (P = particulate/membranes) and S100 (S = soluble) fractions [23]. Each fraction was then subjected to immunoblot analysis.



Supplementary Figure S3. Lack of effect of pico-treatment on Bim-Bcl-2 physical association. (**A**) Toledo B-cell non-Hodgkins lymphoma (B-NHL) cells were untreated, treated for 24 hours with 10 nM pico alone, or with pico plus U0126 pretreatment. Bim and Bcl-2 were immune-precipitated as described for Bax−Bak immune-precipitation. Immune-precipitates were then immunoblotted with an anti-Bcl-2 antibody. The specificity of the results was demonstrated by omitting the anti-Bim antibody. The identity of coprecipitated Bcl-2 was confirmed by comparison of the electrophoretic mobility with that of Bcl-2 in a cell lysate (L). (**B**) Toledo B-NHL cells were treated with pico for the indicated times and lysates were prepared as mentioned. Each lysate was divided into three portions, which were incubated with either anti−Bcl-2 antibody, anti-dynein light chain (LC8) antibody (unsuccessful positive control), or no antibody. After immune-precipitation, samples were immunoblotted with anti-Bim antibody.