The effect of the cyclin-dependent kinase inhibitor flavopiridol on anaplastic large cell lymphoma cells and relationship with NPM-ALK kinase expression and activity

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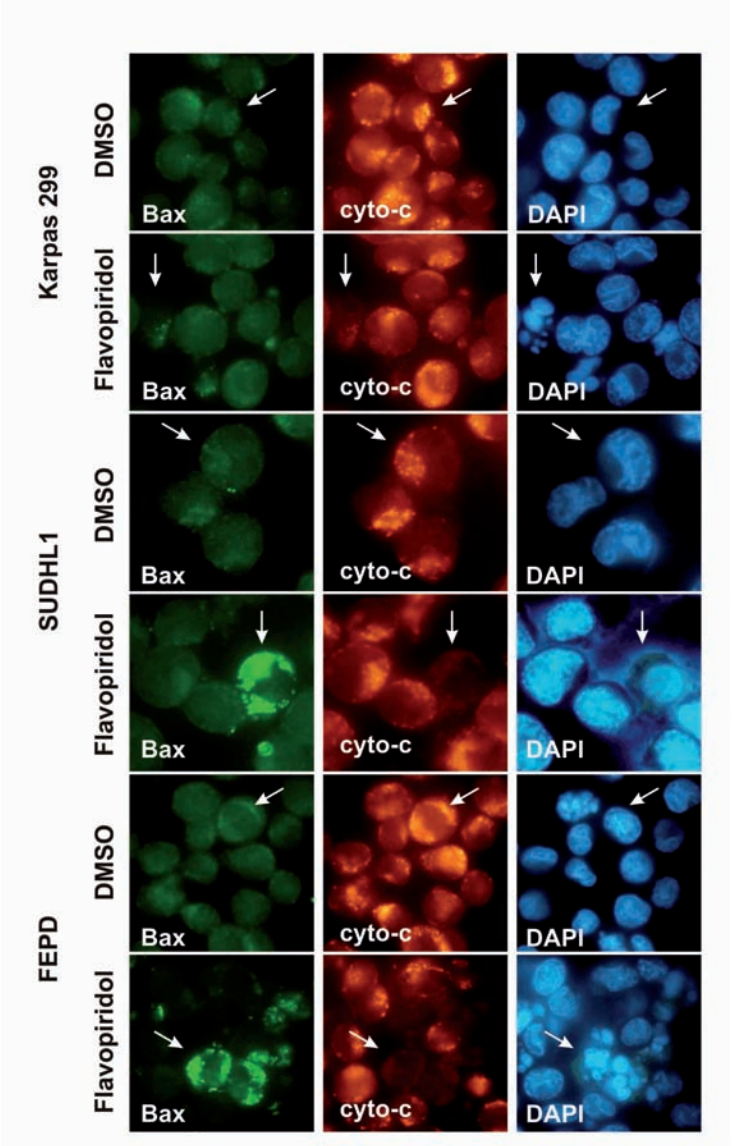
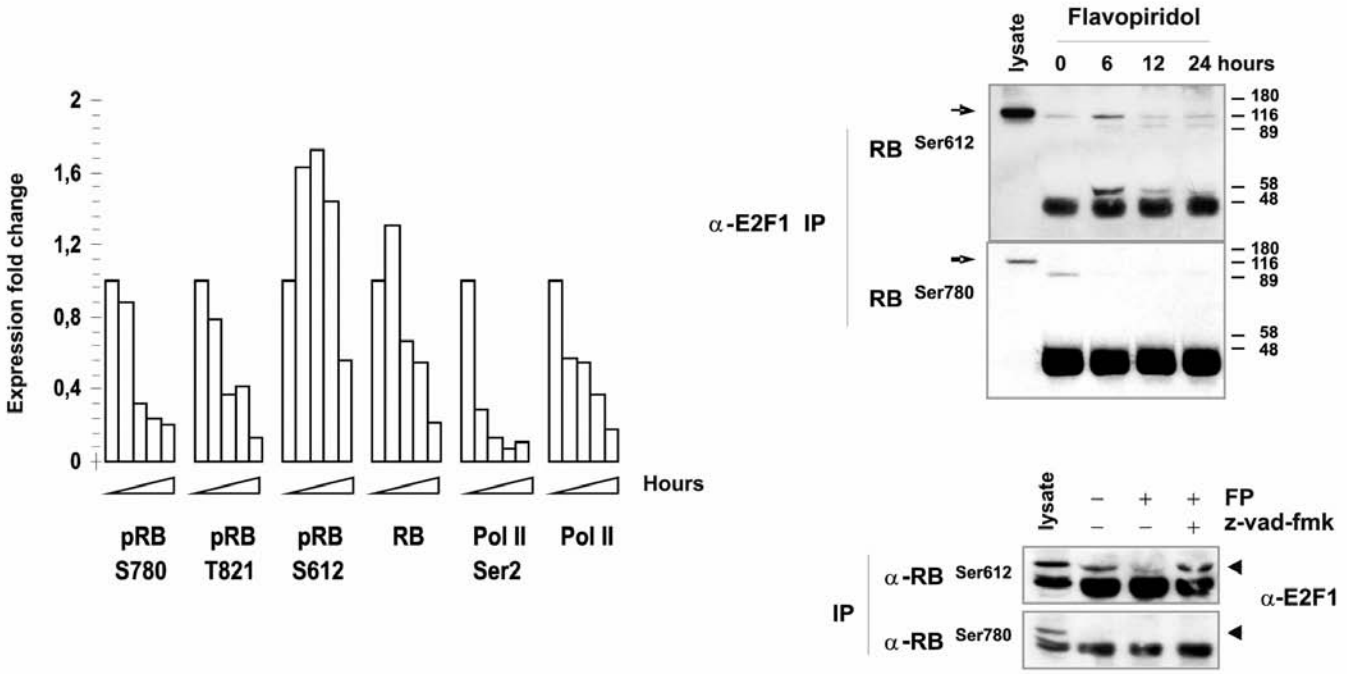


Online Supplementary Figure S1. Subcellular localization of Bax and cytochrome c in flavopiridol-treated (FP) and –untreated (DMSO) cells. ALCL cells, Karpas 299, SUDHL1 and FEPD cells were treated with 200 nM flavopiridol for 6 h. After treatment the cells were fixed in 3.7% paraformaldehyde, permeabilized as described in the Design and Methods section, and stained with the indicated antibodies (Bax; cyto-c), or with DNA intercalating dye DAPI. Bright perinuclear fluorescence in DMSO-treated cells reflects mitochondrial localization of cytochrome-c, whereas in flavopiridol-treated cells it indicates activated Bax in damaged mitochondria (arrowheads).



Online Supplementary Figure S2. Changes in the levels of expression and degree of phosphorylation of RB and RNA polymerase II were investigated by western blotting in Karpas299 cells exposed to flavopiridol (FP) for 3, 6, 12 and 24 h. Phosphorylation status of both proteins was measured by using site-specific antibodies, as indicated in the figure, and protein band densities are expressed in the graph as fold changes of controls at time 0. To detect complex formation between RB and E2F1 in Karpas299 cells, flavopiridol (200 nM) was added for the indicated periods. Cell lysates (500 μg) were immunoprecipitated with anti-E2F1 antibody, and the immunocomplexes were fractionated by SDS-PAGE and analyzed by western blotting using phospho-specific antibodies. Reverse immunoprecipitation was also performed using anti-phospho-Ser612 and anti-phospho-Ser780 antibodies, in the presence or absence of z-vad-fmk caspase inhibitor. Thirty μg of total cell lysates were included.