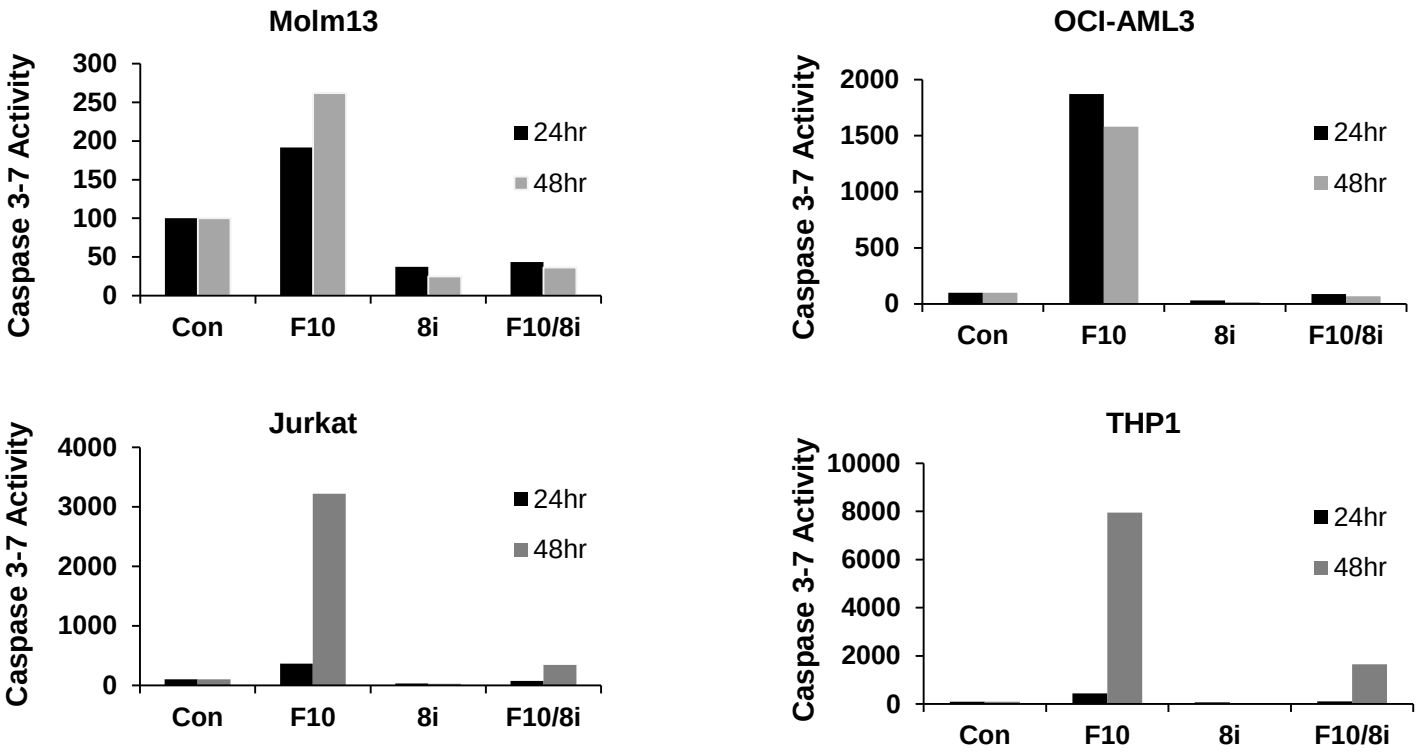
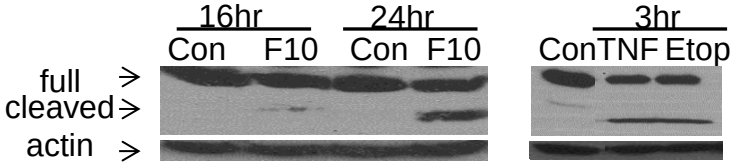
Supplementary for “Thymineless death in AML cells via extrinsic pathway”

Leukemia Research

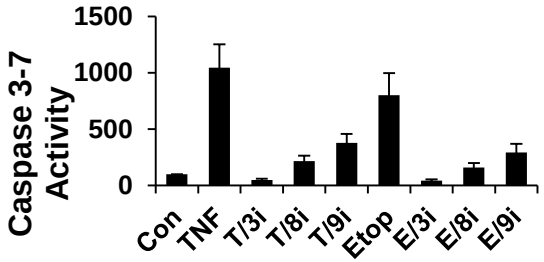


Supplementary Figure S1: Caspase-glo 3/7 assay results for Molm13, OCI-AML3, Jurkat, and THP1 cells treated with F10 for either 24 or 48 h. For all cell lines co-treatment with the caspase 8 inhibitory peptide IETD decreased caspase 3/7 activity to control levels consistent with the extrinsic pathway being essential for apoptosis initiation.

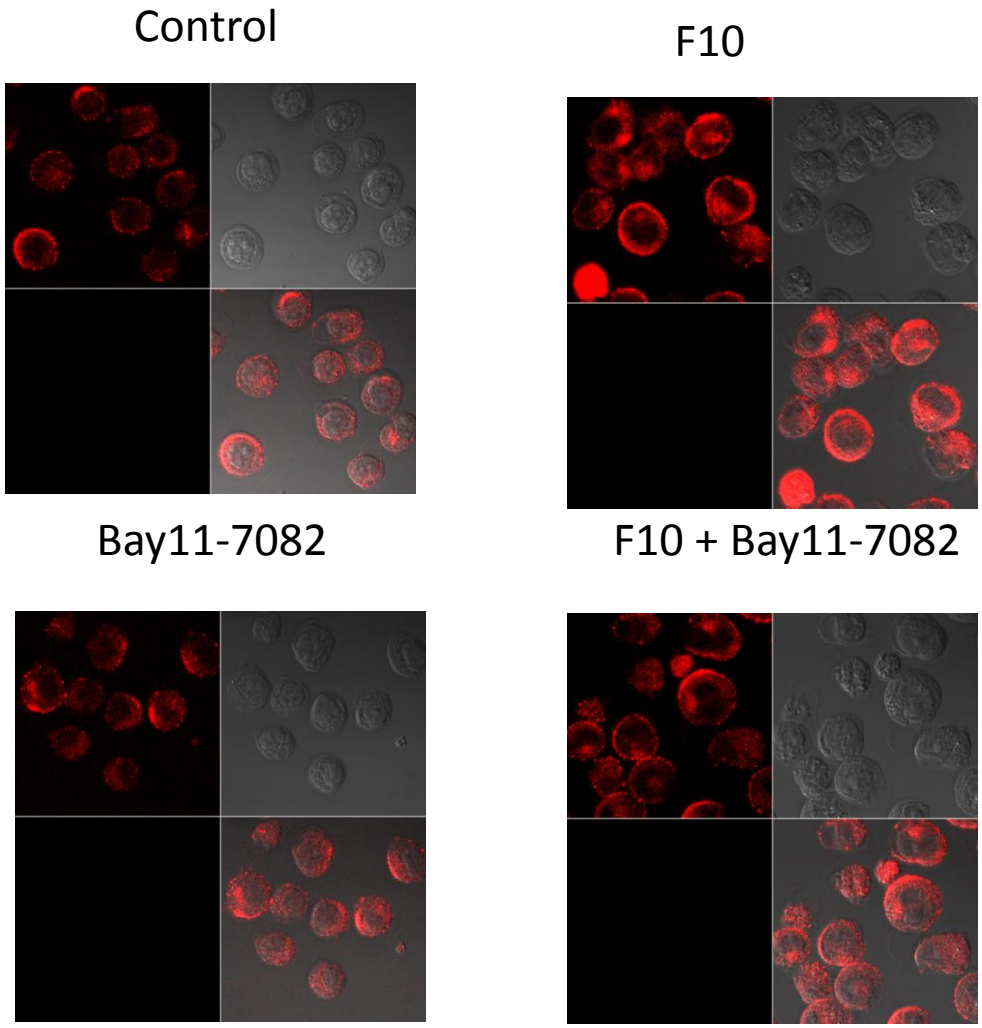
A



B



Supplementary Figure S2: F10 treatment causes HL-60 cells to undergo a type 2 apoptotic process in which the effects of extrinsic pathway activation are amplified by Bid cleavage leading to caspase 9 activation. (A) Western blot showing cleaved Bid in F10-treated cells and also in HL-60 cells treated with TNFα + cycloheximide (0.3 nM/100 μM; 3 h – TNF or T)or etoposide (100 μM; 3 h – Etop or E). (B) Caspase glo 3/7 assay showing execution caspase activity is decreased by inhibiting either caspase 8 and caspase 9 in cells treated with TNFα (T) or etoposide which is consistent with a type 2 apoptotic process.



Supplementary Figure S3: Confocal Microcopy Images of Fas in HL-60 cells with the indicated treatments. NF-kB inhibition had no apparent effect on Fas localization. Similar results were obtained with FasL (data not shown)

Supplementary Figure S4: Results of (left panels) cell-titer glo cell viability assays and caspase 3/7 glo apoptosis assays (right panels) evaluating F10 and simvastatin and combinations of these agents at the indicated concentrations in Molm13, OCI-AML3, and KG1a AML cells