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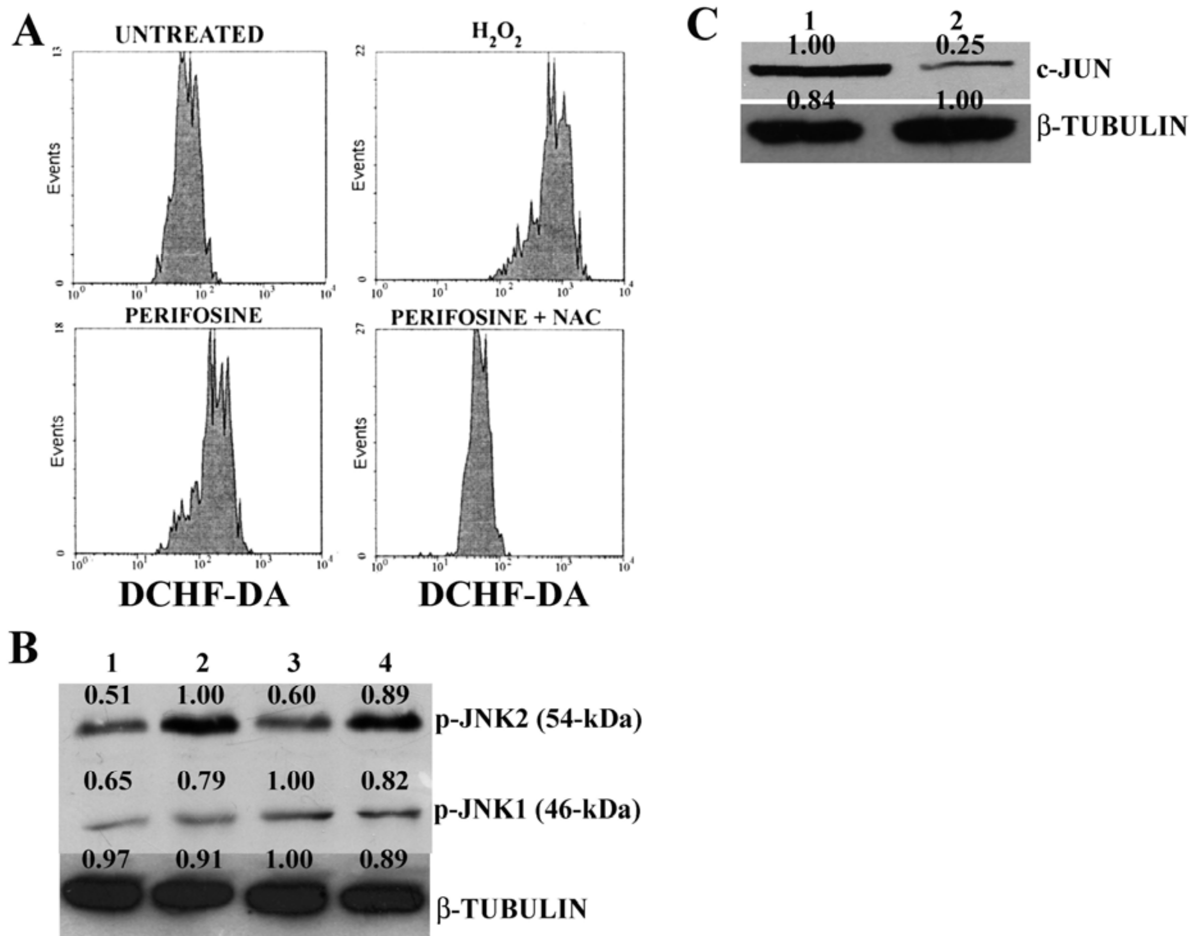

Supplementary Figure 1. Perifosine upregulates ROS, and increases p-JNK 2 levels in THP-1 cells. A: flow cytometric analysis demonstration of ROS generation. Cells were treated with perifosine (2 μM) for 16 hr, then loaded with the ROS selective probe DCHF-DA (5 μM for 1 h). Hydrogen peroxide (H_2O_2) served as a positive control for ROS generation (200 μM for 1 hr). The ROS scavenger NAC was employed at 15 mM. One representative of three different experiments is shown. B: western blot analysis for p-JNK 1/2. Lane 1: untreated cells; Lane 2: cells treated with perifosine (2 μM for 16 hr); Lane 3: perifosine-treated cells that had PKCα downregulated by siRNA; Lane 4: perifosine-treated cells exposed to scrambled siRNA. C: western blot analysis for c-Jun in cells treated with siRNA specific for c-Jun. Lane 1: untreated cells; Lane 2: cells treated with siRNA specific for c-Jun. Cells were analyzed 48 hr after transfection. Forty μg protein was loaded in each lane and β-tubulin served as loading control.

Type of file: figure
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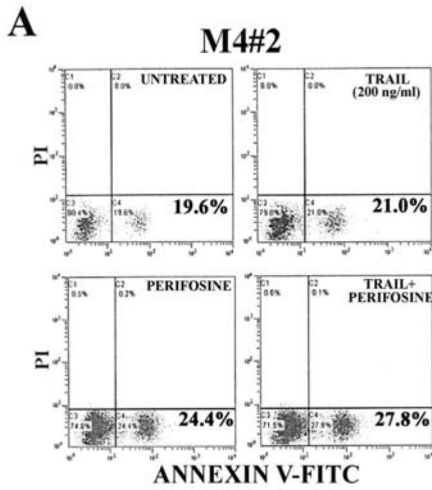
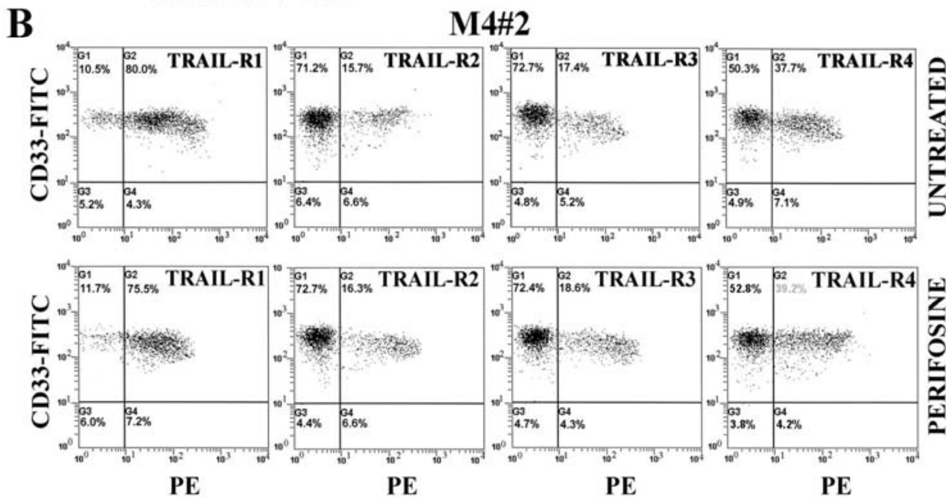
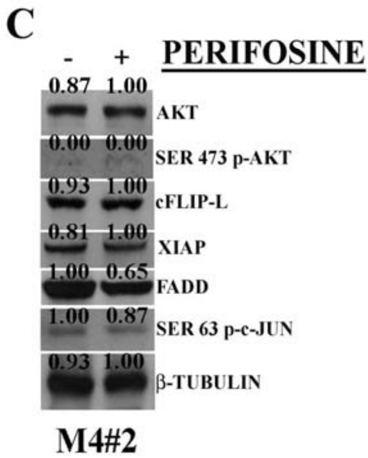
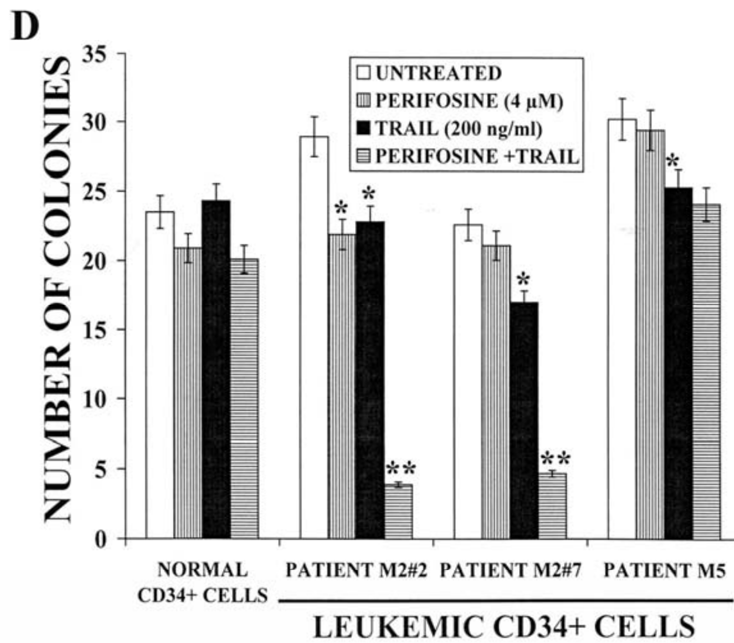
Filename: Supplementary Figure 2.pdf









SUPPLEMENTARY FIGURE 2

Supplementary Figure 2. Perifosine + TRAIL combination is not synergistic in AML primary cells without activated Akt, but downregulates clonogenic activity of AML CD34$^+$ cells with activated Akt. A: Annexin V-FITC/PI staining analysis of cells from patient M4#2 treated with either perifosine or TRAIL alone and with two drugs together for 48 hr. The numbers in the lower right quadrants correspond to the percentage of cells which are Annexin V-positive and PI-negative (early apoptotic cells). B: flow cytometric analysis showing surface expression of TRAIL receptors and CD33 in cells from patient M4#2, either untreated or treated with 4.0 μM perifosine for 24 hr. Anti-TRAIL receptors antibodies were conjugated to PE, while anti-CD33 antibody was FITC-conjugated. C: western blot analysis for Akt, Ser 473 p-Akt, cFLIP-L, XIAP, FADD, and Ser 63 p-c-Jun in extracts from AML patient M4#2 primary cells. Perifosine treatment was for 24 hr at 4.0 μM concentration. D: colony forming assay of CD34$^+$ cells isolated from cord blood and patients with or without Akt activation. Cells were seeded in a semisolid methylcellulose medium containing cytokines according to standard procedures. The X axis indicates the number of colonies per view (observation at 25x under an Olympus light microscope). One asterisk: $p<0.05$. Two asterisks: $p<0.01$.