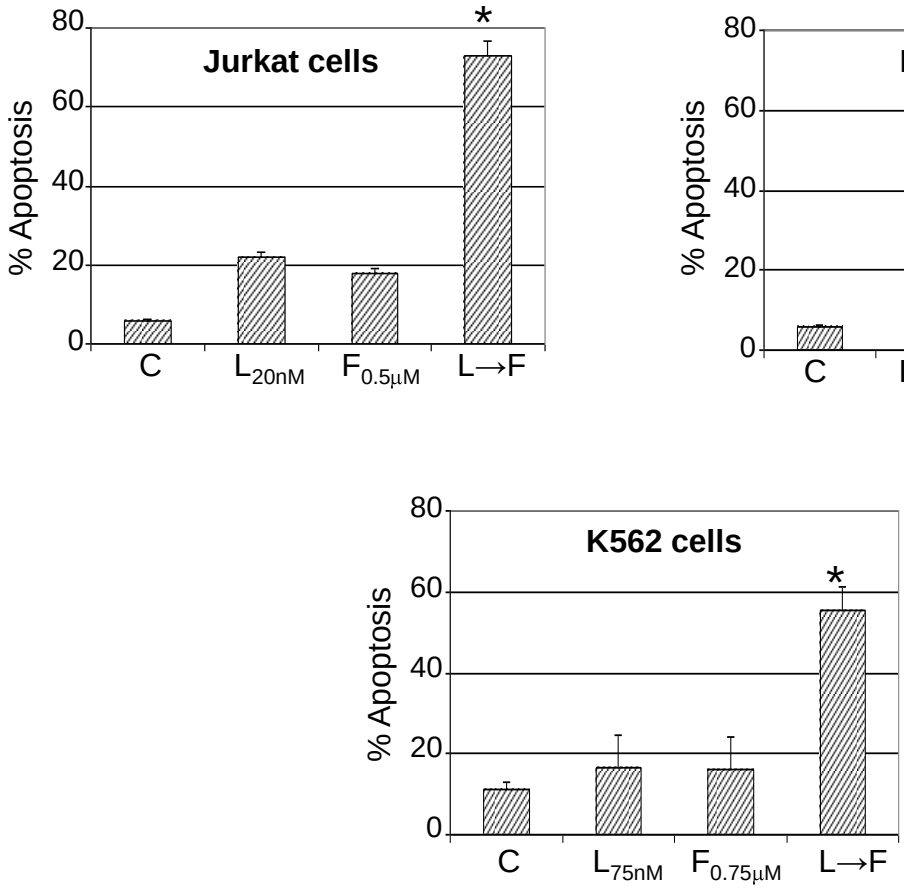
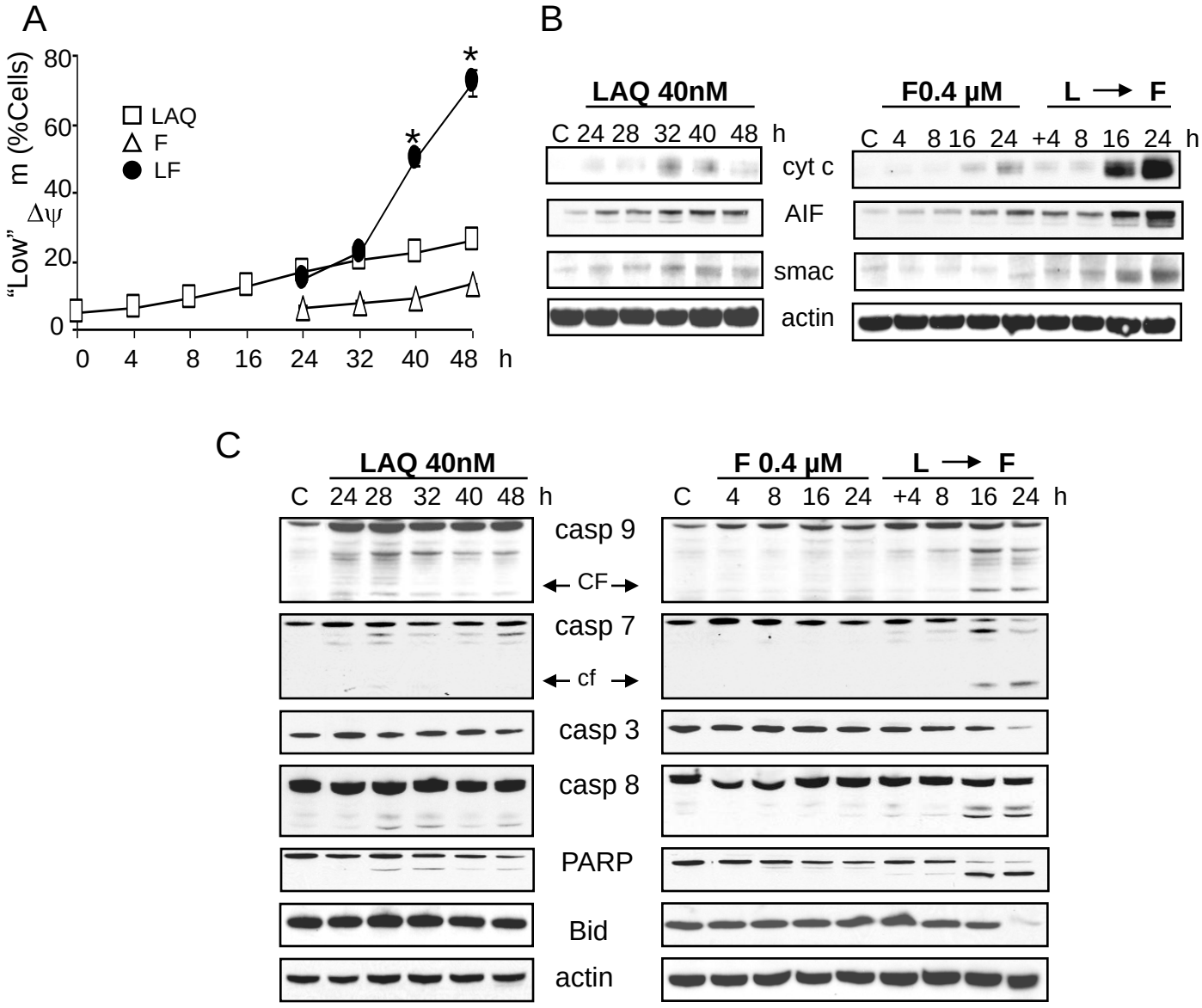
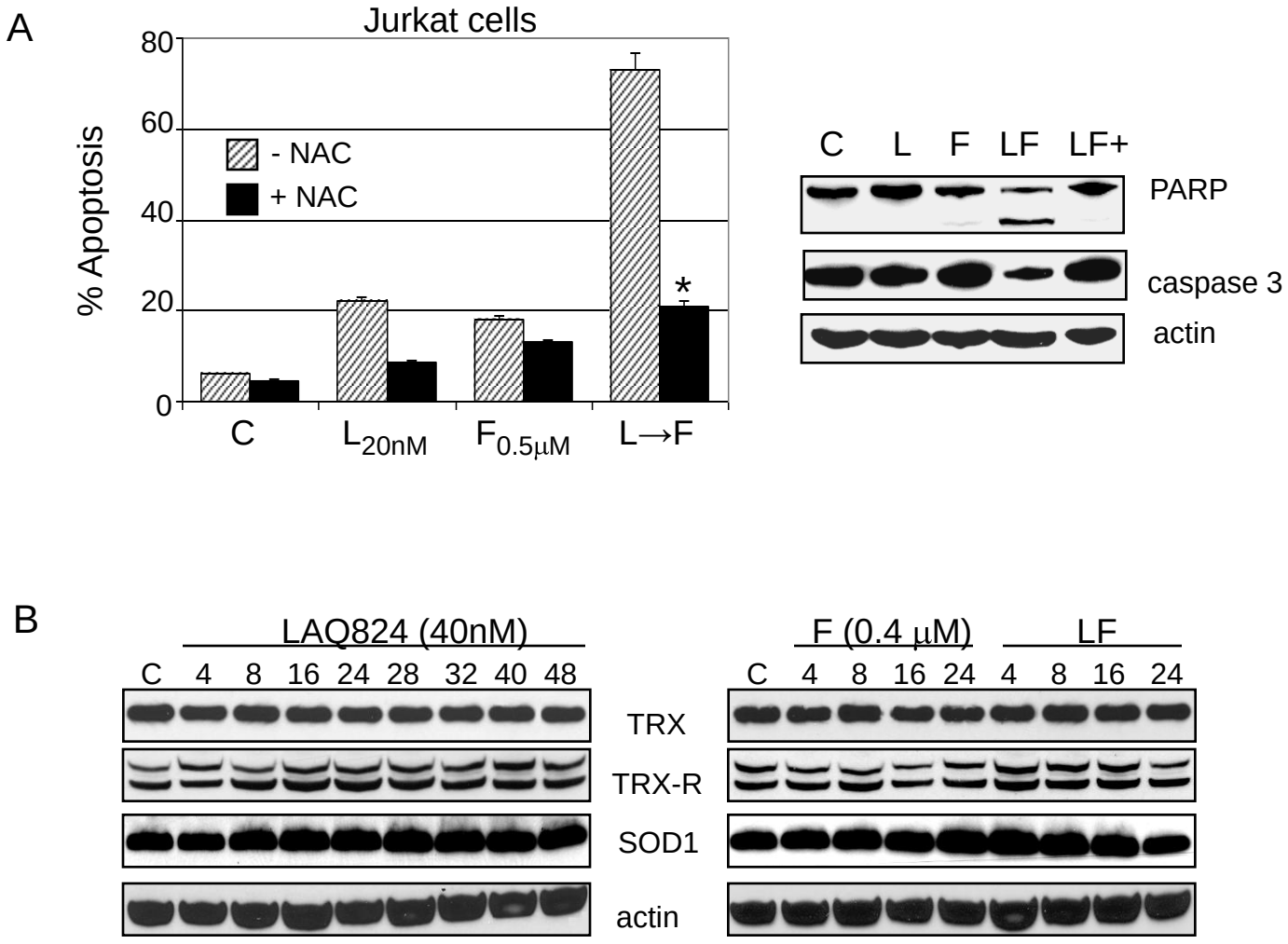


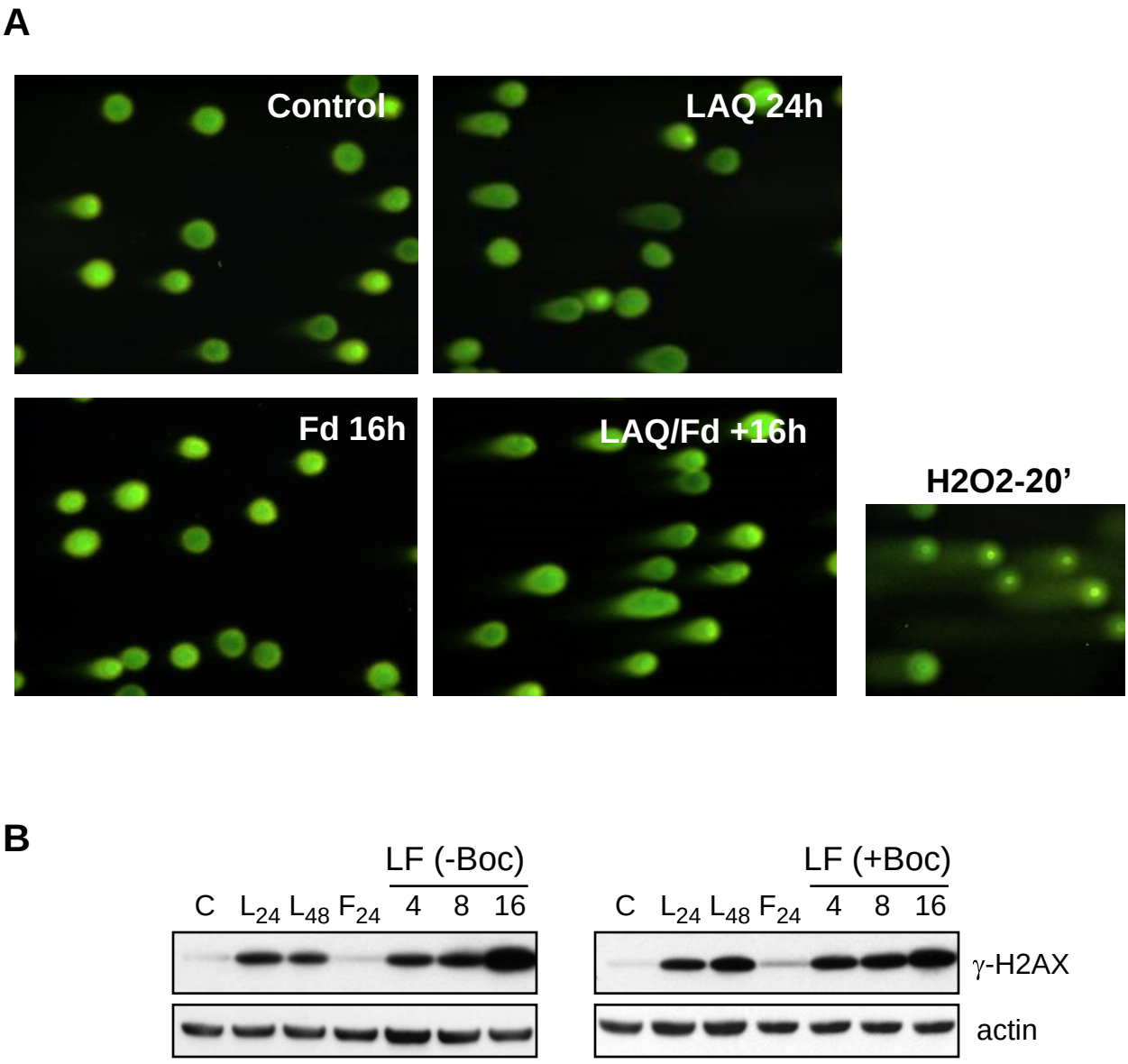
Supplementary Figure 1. Sequential administration of LAQ-824/fludarabine effectively induces apoptosis in various human leukemia cell types. Jurkat T-lymphoblastic leukemia cells, HL-60 promyelocytic leukemia cells, and K562 chronic myelogenous leukemia cells were sequentially exposed to LAQ-824 for 24 h followed by fludarabine for an additional 24 h at the indicated concentrations after which they were evaluated for apoptosis by flow cytometric analysis of Annnexin V/PI staining. Values represent the means ± SD for three separate experiments performed in triplicate. *: significantly greater than values for cells either untreated or exposed to the drugs alone; $p < 0.01$.



Supplementary Figure 2. Pre-exposure of U937 cells to LAQ-824 synergistically potentiates fludarabine-induced mitochondrial damage and apoptosis. *A*, U937 cells were sequentially exposed to 40 nM LAQ-824 (L) for 24 h after which they were either left untreated or exposed to fludarabine (F, 0.4 μM) without washing; cells were collected at the indicated intervals and the percentage of cells exhibiting a loss of $\Delta\psi$m was determined by flow cytometry as described in Methods. Values represent the means for three separate experiments ± S.E.M; *: significantly greater than values for cells exposed to either drug alone; $p < 0.01$. *B-C,* U937 cells were sequentially exposed to LAQ-824 and fludarabine as described in *A*. At the end of the treatment intervals, cells were pelleted, lysed, and protein extracted from either the cytosolic S-100 fraction (*B*), or whole cell lysates (*C*). In each case, 30 μg of protein were separated by SDS-PAGE, blotted and probed with the corresponding antibodies (*B*, cytochrome c, AIF, Smac; *C*, caspases 3, -7, -8 and -9, Bid, and PARP; actin was used as a loading control). The results of a representative study are shown; two additional experiments yielded similar results.

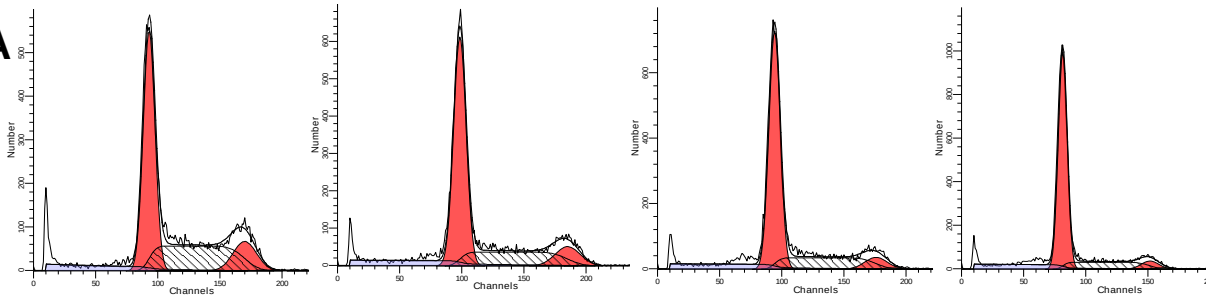


Supplementary Figure 3. *A*, Jurkat cells were sequentially exposed to the indicated concentrations of LAQ-824 (48 h), fludarabine (24 h) or the combination (LAQ-824_{24h}→$fludarabine_{24h}$) ± NAC (LF+; 15 mmol/L) added 2 h prior to the administration of LAQ-824. Cells were collected and analyzed for apoptosis (AnnexinV/PI) or by Western blot (right panel). *: significantly less than values for cells exposed sequentially to LAQ-824/F in the absence of NAC; $p < 0.01$; *B*, U937 cells were exposed to LAQ-824 (40 nM), fludarabine (0.4 μM) or the sequential combination for the indicated intervals after which cell lysates were analyzed by WB for expression of the indicated proteins. Lanes were loaded with 30 μg of protein; blots were subsequently stripped and reprobed with antibodies directed against actin to ensure equivalent loading and transfer. In all cases, the results of a representative study are shown; two additional experiments yielded similar results.



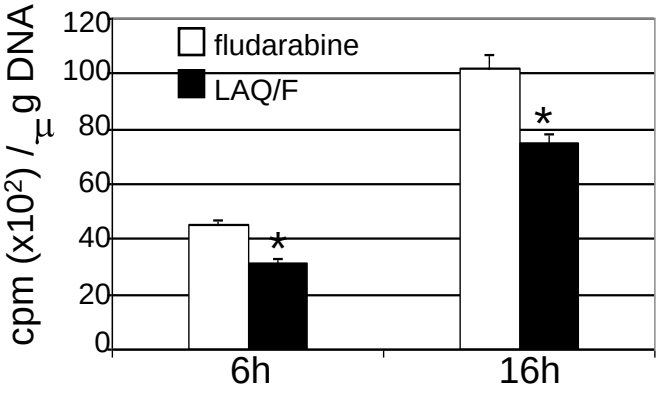
Supplementary Figure 4. *A*, analysis of DNA integrity by Comet assay. U937 cells were exposed to LAQ-824 (40 nM; 24 h), fludarabine (400 nM; 16 h) or sequentially to ($LAQ\text{-}824_{24h}$ → $fludarabine_{16h}$). After treatment, the assay was performed and the comets were visualized under a fluorescence microscope as described in Methods. Exposure to H_2O_2 (10 mM, 20 min) was used as a positive control. Representative photomicrographs are shown. *B,* U937 cells were treated with LAQ-824(40 nM) and fludarabine (400 nM) either administered alone or combined sequentially in the absence or presence of the pan-caspase inhibitor fmk-BOC (20 mM) for the indicated intervals (h), after which cell lysates were analyzed by WB. Lanes were loaded with 30 μg of protein; blots were subsequently stripped and reprobed with antibodies directed against actin to ensure equivalent loading and transfer. In all cases, the results of a representative study are shown; two additional experiments yielded similar results.

A



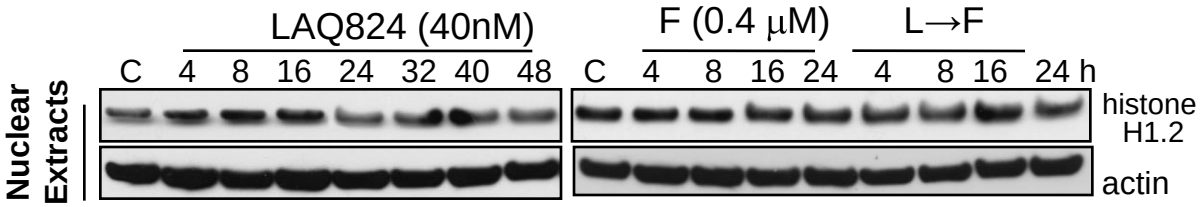
CONTROL	LAQ824- 24h	LAQ824- 32h	LAQ824 – 40h
G0-G1: 52.00 %	G0-G1: 62.96 %	G0-G1: 69.09 %	G0-G1: 75.20 %
G2-M: 12.73 %	G2-M: 10.61 %	G2-M: 6.87 %	G2-M: 5.69 %
S: 35.27 %	S: 26.43 %	S: 24.04 %	S: 19.10 %
G2/G1: 1.83	G2/G1: 1.88	G2/G1: 1.87	G2/G1: 1.87
Debris: 8.84 %	Debris: 11.70 %	Debris: 11.68 %	Debris: 13.30 %

B

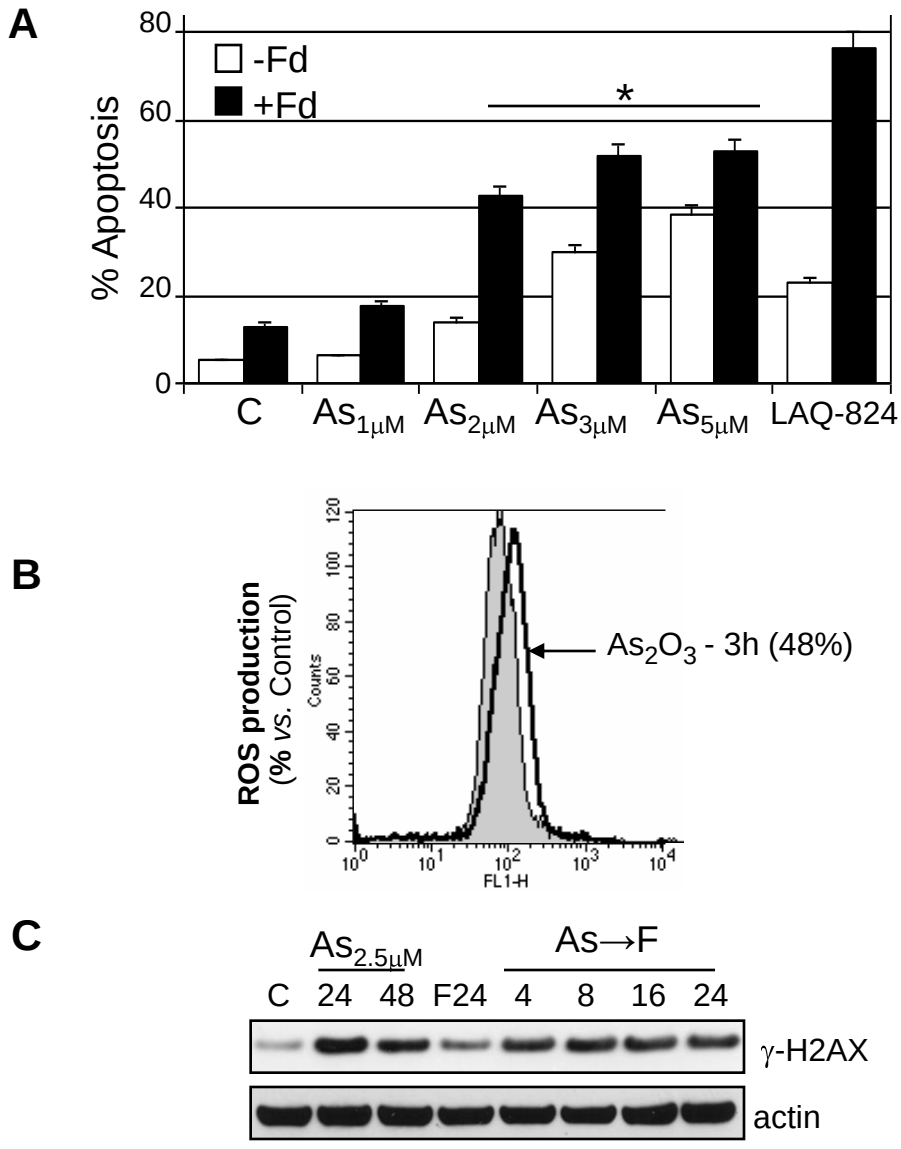


	ratio LF/F
6h	0.69
16h	0.73

C



Supplementary Figure 5. *A*, Cell cycle analysis, fludarabine DNA incorporation, and nuclear histone H1.2 expression in cells exposed to LAQ-824 ± fludarabine. *A*, following treatment with 40 nM LAQ-824, U937 cells were incubated in 70% ethanol, treated with PI, and subjected to cell cycle analysis using a Becton Dickinson FACScan flow cytometer and ModFit software as described in Materials and Methods. *B*, Incorporation of radioactive fludarabine into genomic DNA; U937 cells were incubated with fludarabine (0.4 µM) alone or in combination (LAQ-824$_{24h}$→fludarabine) with [3H]-F-ara-A after which DNA was extracted, and the amount of [3H]-F-ara-A incorporated was determined as described in Methods. Values, expressed as cpm [3H]-F-ara-A/µg DNA, represent the means for three experiments performed in triplicate; * significantly lower than values from cells exposed to fludarabine alone; $p<0.05$. Ratio: cpm [3H]-F-ara-A incorporated in LF-treated cells with respect to cpm [3H]-F-ara-A incorporated in F-treated cells. *C*, Western blot analysis of nuclear extracts from U937 cells collected after exposure to LAQ-824 (40 nM), fludarabine or the sequential combination for the indicated intervals. Lanes were loaded with 30 µg of protein; blots were subsequently stripped and reprobed with antibodies directed against actin to ensure equivalent loading and transfer. In all cases, the results of a representative study are shown; two additional experiments yielded similar results.



Supplementary Figure 6. *A*, U937 cells were sequentially exposed for 24 h to increasing concentrations of As_2O_3 (As) after which fludarabine (F, 0.4 μM) was added without washing; cells were collected 24h after addition of fludarabine (48h total) and the percentage of apoptotic cells was determined by flow cytometry as described in Methods. Values represent the means for three separate experiments ± S.E.M; *: significantly less than values for cells exposed to LAQ-824/F; $p < 0.05$. *B,* U937 cells were exposed to As_2O_3 (2.5μM) for 3 h, after which they were labeled with the oxidation-sensitive dye dihydroethidium (5 μM) and analyzed by flow cytometry to determine the percentage of cells displaying an increase in ROS production relative to untreated controls. Values represent the means for three separate experiments ± S.E.M. *C*, Western blot analysis of U937 cells treated sequentially as described in *A* with As2O3 (2.5μM) → fludarabine (F, 0.4 mM); at the end of the treatment intervals, cells were pelleted and lysed; 30 μg of protein were separated by SDS-PAGE, blotted and probed with an anti-γ-H2AX antibody; actin was used as a loading control. The results of a representative study are shown; two additional experiments yielded similar results.