Supplemental Figure 1. U937 cells (5 x 10^5 cells/ml) were treated with EX and 2-DG for 6 h and oxygen consumption (for EX only) and ATP levels were quantitated as described in the Methods. * = *P<0.01* from control

Supplemental Figure 2. OCI-AML3 (5 x 10^5 cells/ml) and a primary sample (AML6; 1 x 10^6 cells/ml) were treated with ranolazine (RAN; 500 μmol/L) for 6 h and ATP levels and oxygen consumption were quantitated as described in the methods. * = *P<0.001* from control.

Supplemental Figure 3. The increased glycolytic activity induced by FAO inhibitors may be a compensatory mechanism to sustain ATP production in the face of decreased PDH activity.

Supplemental Figure 4. OCI-AML3 (5 x 10^5 cells/ml) and a primary sample (AML6; 1 x 10^6 cells/ml) were treated with ranolazine (RAN; 250 μmol/L) or EX (100 μmol/L), respectively, and after 48 h exposure, lactate levels in the medium were quantitated and normalized to viable cell numbers as described in the Methods. * = *P<0.01* from control.

Supplemental Figure 5. OCI-AML3 cells were cultured alone or on MSC feeder layers in the presence of 0.2 mM oleate and 30 μCi/l [9,10-^{3}H] oleate. The rates of oleate oxidation were determined by monitoring the release of ^3H_2O in the culture medium. Results are expressed as fmol/cell after counting total viable cells by trypan blue exclusion in a hemocytometer.

Supplemental Figure 6. U937 cells (5 x 10^5 cells/ml) were treated with 100 μmol/L EX and 250 μmol/L ranolazine (RAN) for 24 h and the percentages of viable and apoptotic cells were determined by flow cytometry as described in the Methods. * = *P<0.05* from control.

Supplemental Figure 7. MOLM13 cells were treated with 200 μmole/L EX for 24 h alone or in the presence of the pan-caspase inhibitor z-VAD-fmk (50 μmole/L). Apoptotic cells were quantitated by flow cytometry.

Supplemental Figure 8. OCI-AML3 and MOLM13 cells were treated with 100 μmole/L EX for 1, 3, and 24 h. Replicates of untreated cells were exposed to 1 μmole/L rotenone for 1 h as a positive control for superoxide generation. 1 h before sample collection, cells were stained with dihydroethidine (50 nmole/L; Invitrogen). Cells were then washed several times in PBS and analyzed by flow cytometry. Ethidium/DNA adducts were detected in FL2.

Supplemental Figure 9. OCI-AML3 cells were treated with 100 μmole/L EX for 20 h, and intracellular long chain fatty acyl-CoAs (LCACoA) contents were determined by HPLC as described in the Supplemental Methods. The species analyzed were lauroyl-CoA (12:0), myristoyl-CoA (14:0), palmitoleoyl-CoA (16:1), linoleoyl-CoA (18:2), palmitoyl-CoA (16:0), oleoyl-CoA (18:1), and stearoyl-CoA (18:0). Results shown are the average of two independent experiments.

Supplemental Figure 10. OCI-AML3 (5 x 10^5 cells/ml) were treated with ranolazine (RAN; 250 □mol/L) alone or in combination with 2 μmol/L ABT-737 for 24 h and Annexin V positive cells were quantitated by flow cytometry as described in the Methods. * = *P<0.001* from control.

Supplemental Figure 11. OCI-AML3 cells were treated with 0, 50, or 100 μmole/L EX for 20 h,

and ceramide levels were determined by HPLC as described in the Supplemental Methods. Results shown are the average of two independent experiments.

Supplemental Figure 12. Representative carboxyfluorescein diacetate succinimidyl ester (CFSE) histograms gating on viable CD34+ leukemia progenitors in AML samples.

Supplemental Figure 13. Representative carboxyfluorescein diacetate succinimidyl ester (CFSE) histogram gating on viable cells in CML sample L. Red arrows indicate the location of CFSE high as determined by colcemid control.

Supplemental Figure 14. Two normal bone marrow samples, and one normal pheresis sample were loaded with the cell tracing dye CFSE as described in the Methods and exposed to increasing concentrations of EX for 5 days. Cells were then collected and stained with CD34-APC and 7-AAD, and viable CFSE high/CD34+ cells were quantitated by flow cytometry as described in the Methods. Samples were collected during routine diagnostic procedures after informed consent was obtained in accordance with regulations and protocols approved by the Human Subjects Committee of the University of Texas M.D. Anderson Cancer Center (Houston, TX). Mononuclear cells were separated by Ficoll-Hypaque (Sigma Aldrich) density gradient centrifugation.

Supplemental Methods

High performance liquid chromatography quantification of ceramide. Total lipids were extracted from cell pellets according to the method of Bligh and Dyer (S1). Ceramide content was determined by HPLC according to the method of Previati et al. (S2) with slight modifications. Briefly, lipid standards or samples were dissolved in 100 µl of anhydrous chloroform (amylenes stabilized) and 10 µl 100 mM naproxen, 10 µl 100 mM *N,N'*-Dicyclohexylcarbodiimide, 10 µl 100 mM 4-(Dimethylamino)pyridine, all dissolved in anhydrous chloroform, were added. The samples were mixed and incubated at -20°C for 5 h. The derivatization was stopped by rotoevaporation. The samples were then resuspended in 15 µl of anhydrous chloroform, and 2 ml of hexane were added before vortexing and centrifuging for 10 min at 14,000g. The supernatant fraction was extracted twice with 5 ml of methanol:H2O (4:1). Chromatography was carried out on a LC-10ATVP instrument (Shimadzu Scientific Instruments) equipped with a Econosphere CN 5U, 250 x 4.6-mm column (Grace) and a Opti-Guard® 1 mm guard column (Optimize Technologies). UV absorption was determined at 230 nm. The mobile phase was delivered at the flow rate of 1ml/min. The solvents used for separation were (A) hexane and (B) isopropanol. After equilibration of the column with 100% A, 0% B, the separation was accomplished with a linear gradient to 6 min to 95% A, 5% B, followed by isocratic conditions to 16 min. Peak area was used to estimate the amount of material based on a standard curve realized with authentic bovine brain ceramides. Within and between assay variation was 1.89% and 4.80%, respectively.

Quantitative and qualitative analysis of cardiac long-chain acyl-CoA species. Long-chain acyl-CoA thioesters (LCACoAs) were extracted and quantified according to the method developed by Mangino et al. (S3). The cell pellets were homogenized in presence of 1 µg of n-heptadecanoyl-CoA used as an internal standard, and LCACoAs were recovered with two successive chloroform:methanol (1:2) extractions. Separation was carried out on a Ultrasphere C18 ODS, 250 x 4.6-mm column (Beckman Coulter). UV absorption was determined at 254 nm.

The following solvents were delivered at a flow-rate of 1 ml/min: (A) acetonitrile and (B) 10 mM KH_2PO_4, pH 5.3. The program was started with 24% A, 76% B with linear gradient to 4 min to 34% A, 66% B; a linear gradient to 14 min to 45% A, 55% B; a linear gradient to 23 min to 57% A, 43% B; and a isocratic run to 70% A, 30% B. After 25 min, the system was re-equilibrated to the initial time zero solvent composition for 10 min before injecting another sample. The ratio of the peak areas of LCACoA species to the area obtained from the internal standard was used for quantification based on curves realized with authentic standards.

Determination of oleate oxidation. Cells were incubated in RPMI medium (as described above) supplemented with 0.2 mM sodium oleate and 30 μCi/l [9,10-^{3}H]oleate (Sigma Aldrich) bound to 1% bovine serum albumin (Probumin™, fatty acid free, Millipore, Billerica, MA). Oleate oxidation was determined by measuring the release of ^3H_2O(51). Briefly, a 0.5 ml medium aliquot was added to a 1.5 ml column of a strong anion exchange resin (AG□1-X8-hydroxide form (100-200 mesh), BioRad Laboratories). The ^3H_2O was eluted with two washes of 2 ml double distilled water, mixed with 10 ml of
liquid scintillation cocktail, and radioactivity was determined in a liquid scintillation counter.

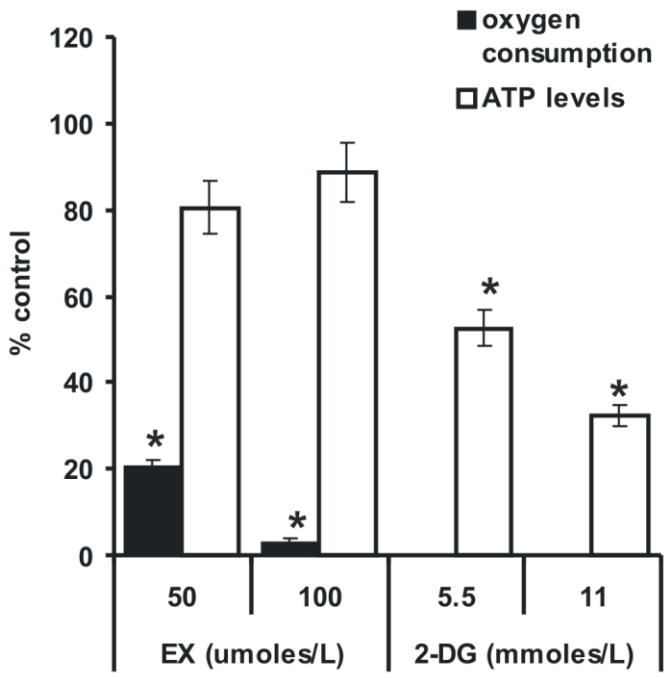
Supplemental references

S1. Bligh EG, Dyer, W.J.: A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37:911-917, 1959

S2. Previati M, Bertolaso L, Tramarin M, Bertagnolo V, Capitani S: Low nanogram range quantitation of diglycerides and ceramide by high-performance liquid chromatography. *Anal Biochem* 233:108-114, 1996

S3. Mangino MJ, Zografakis J, Murphy MK, Anderson CB: Improved and simplified tissue extraction method for quantitating long-chain acyl-coenzyme A thioesters with picomolar detection using high-performance liquid chromatography. *J Chromatogr* 577:157-162, 1992

Supplemental Figure 1



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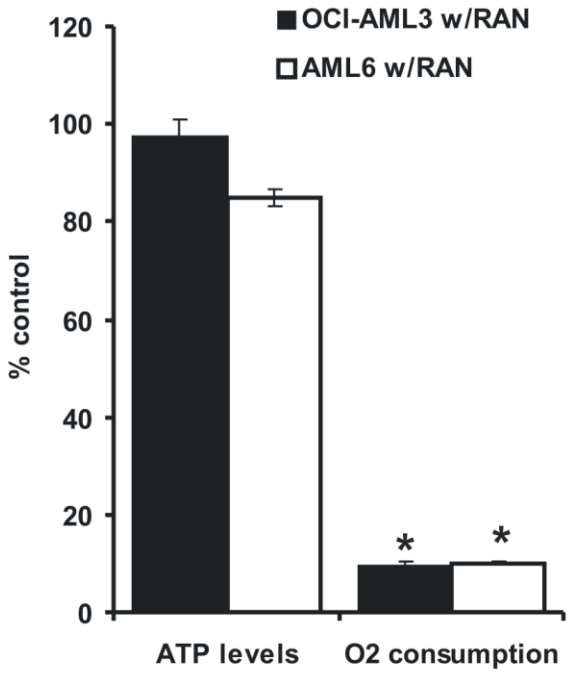

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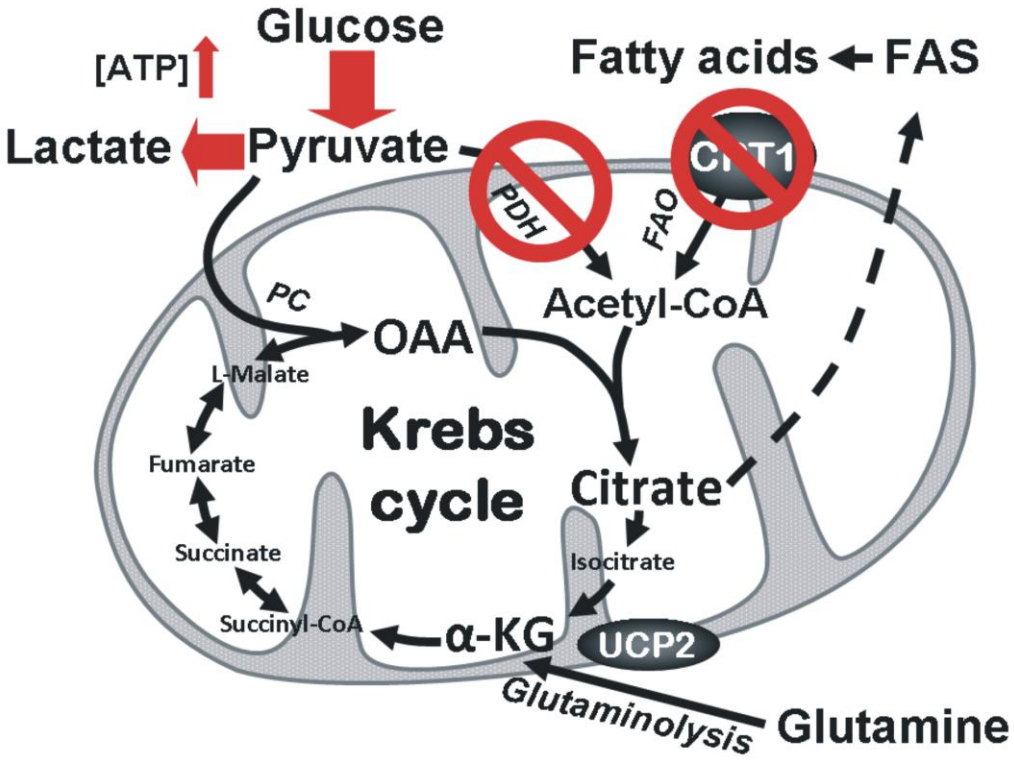

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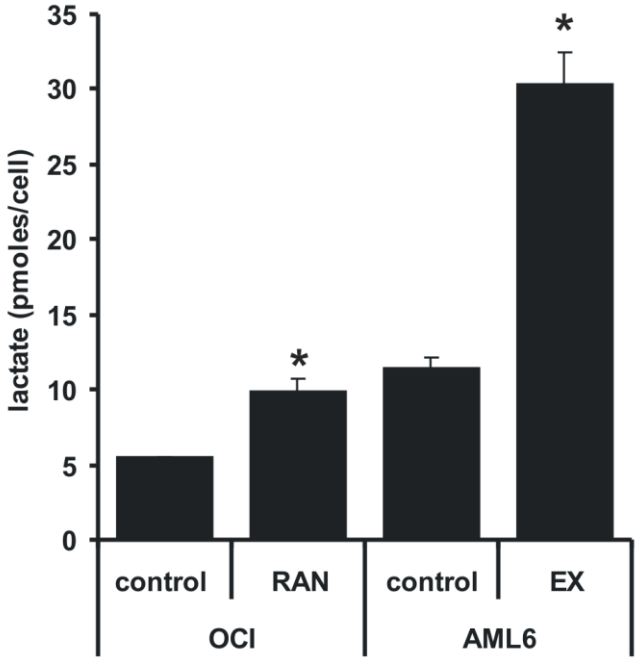

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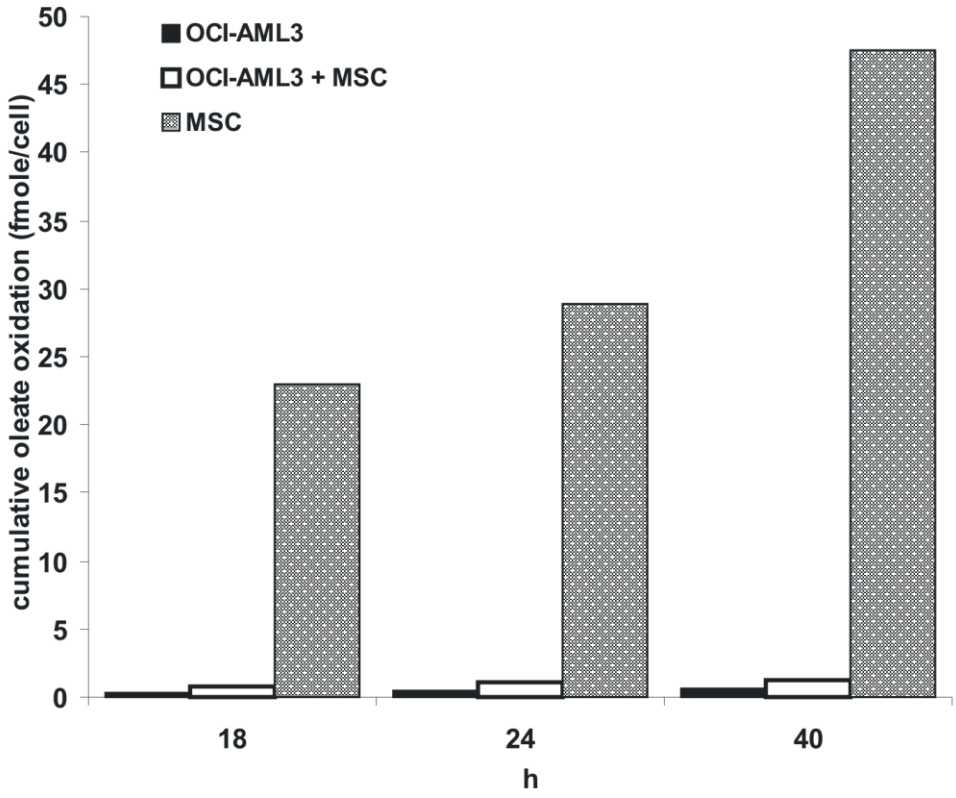

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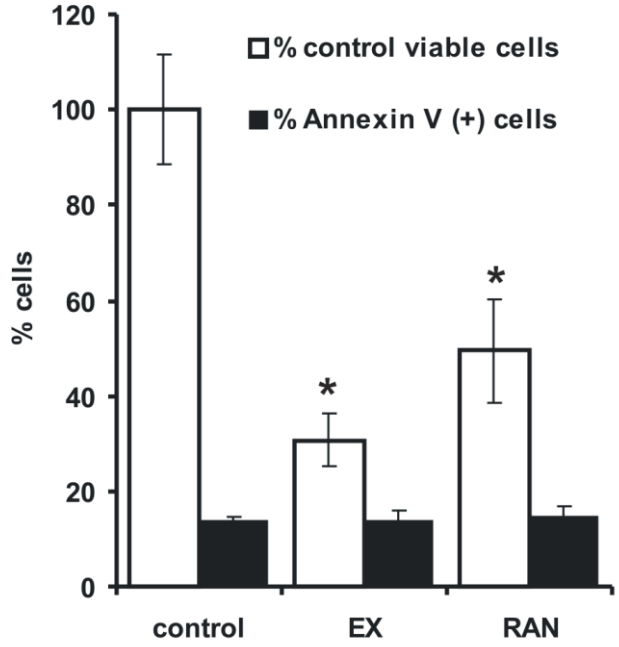

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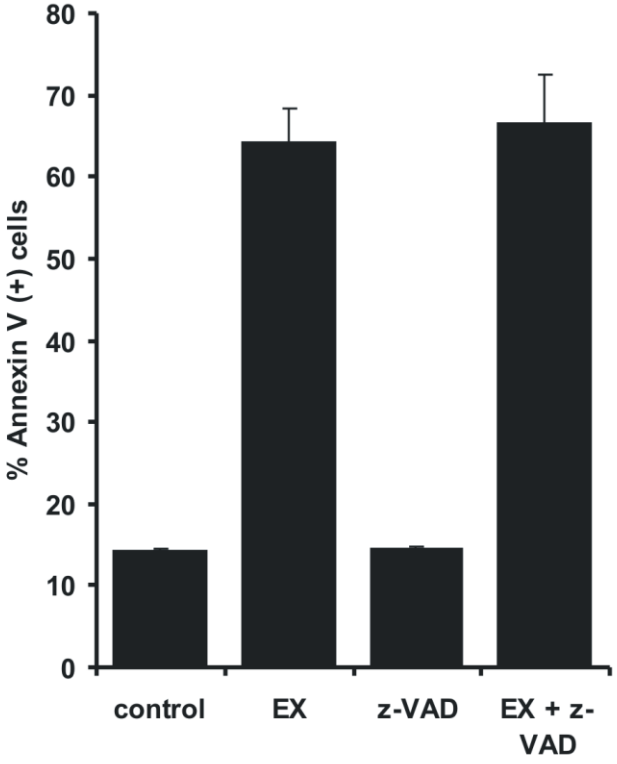

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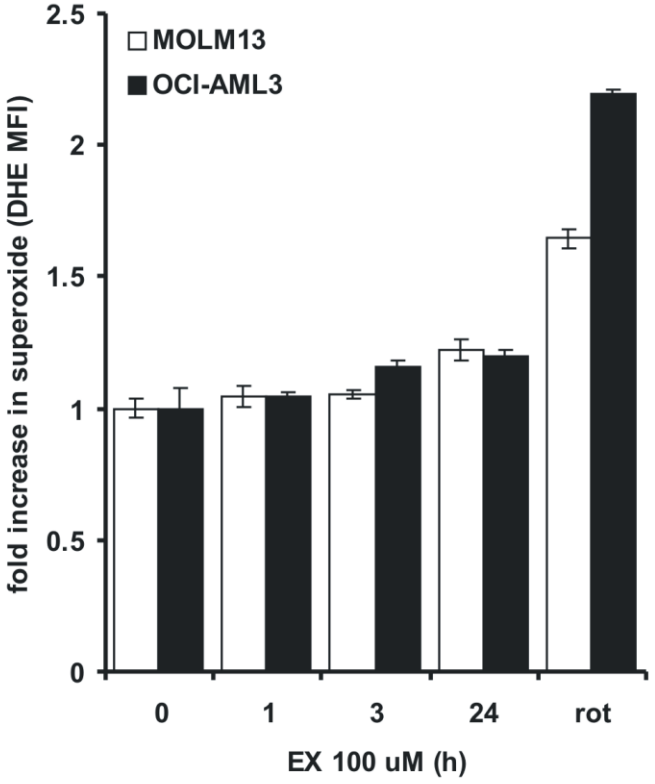

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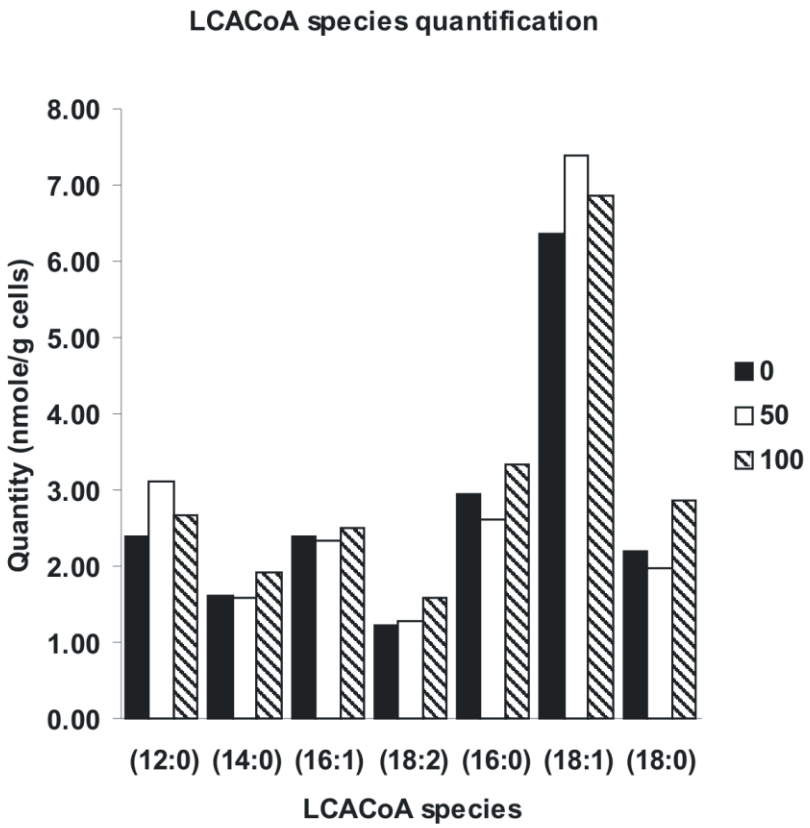

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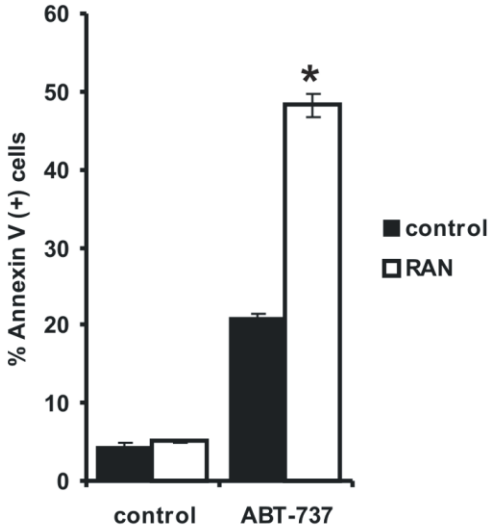

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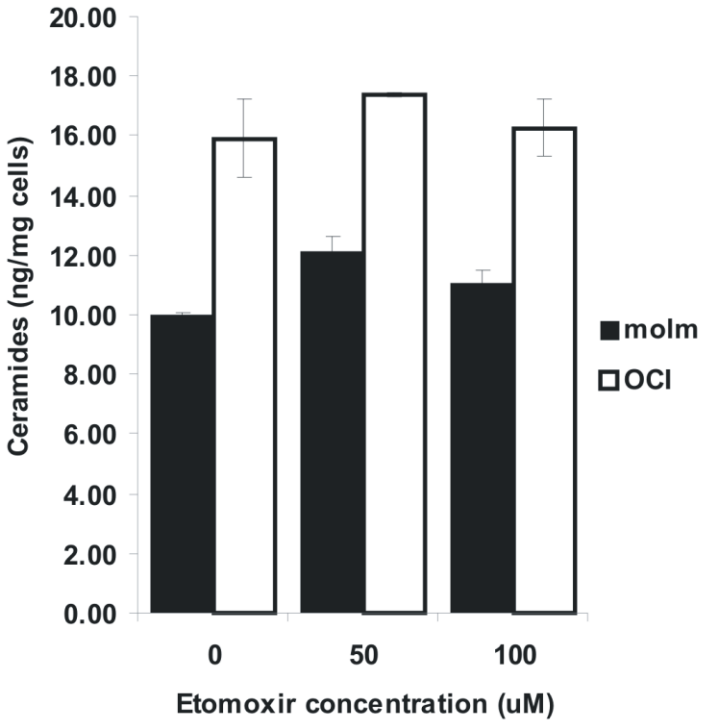

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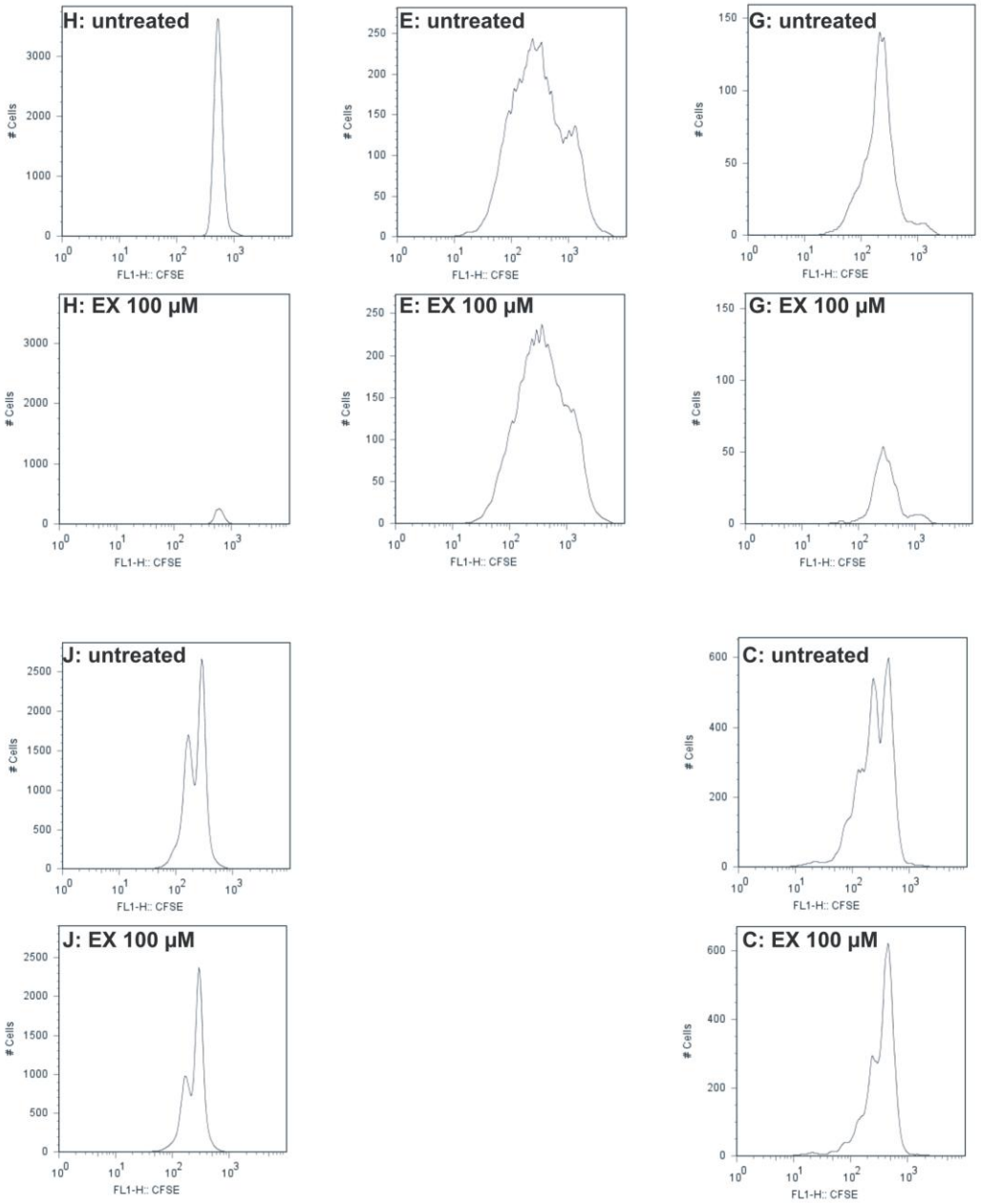

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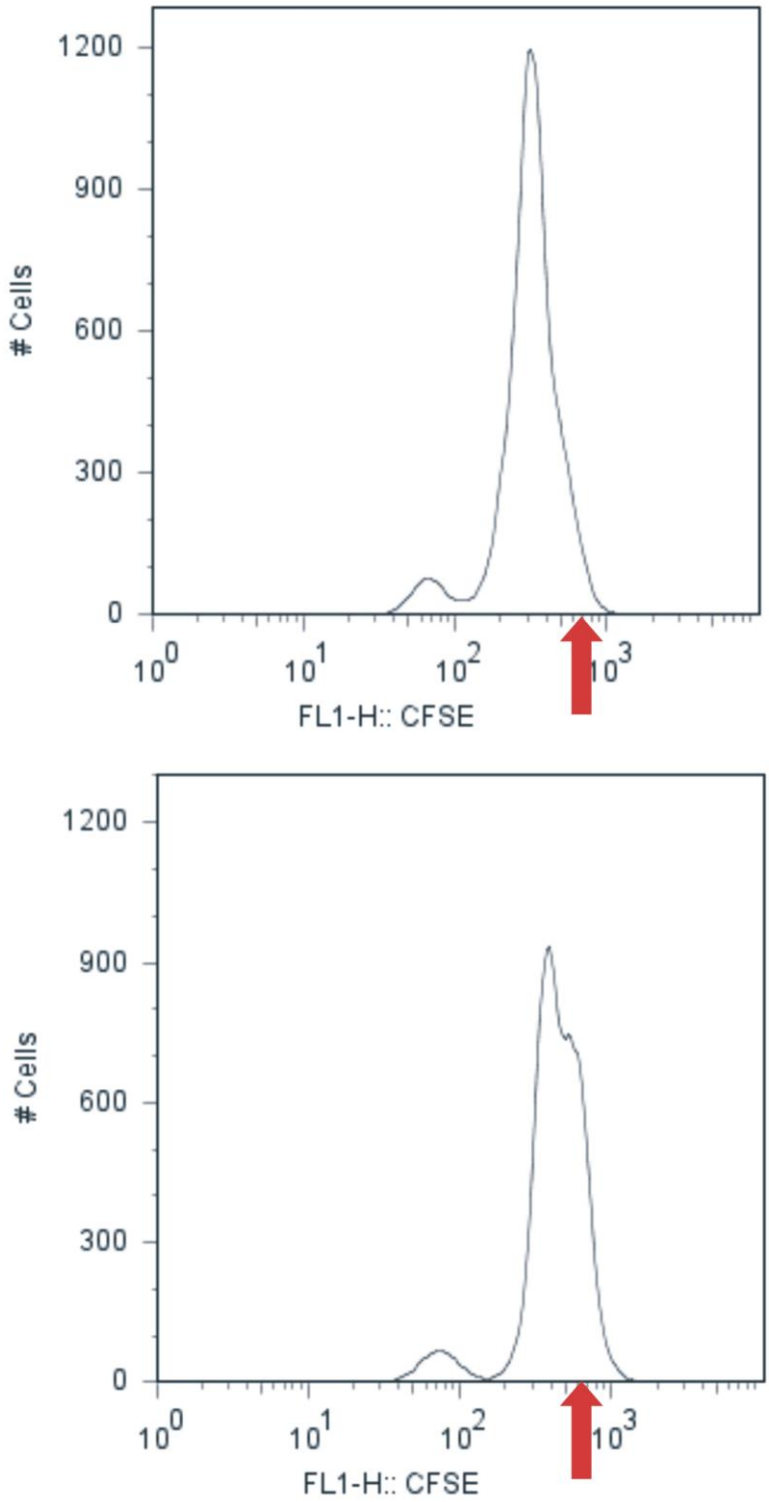

Supplemental Figure 14