**Supplemental Information**

## METHODS

### A-PAC Extraction, Chemicals and Reagents

The cranberry powder CystiCran-40 (CYS), consisting of 40% A-PACS, was provided by NATUREX-DBS LLC, (Sagamore, MA). A-PACs were isolated from this powder similar to the methods of Foo et al., 2000[6], with minor modifications. Briefly, the CYS powder was mixed in water, defatted with hexane (Sigma-Aldrich; St. Louis, MO), and then the aqueous layer was extracted with ethyl acetate (Sigma-Aldrich; St. Louis, MO). This extract was concentrated before it was added to a Sephadex LH-20 column (GE Healthcare Co.; Buckinghamshire, UK) and eluted with 100% ethanol (Koptec; King of Prussia, PA), followed by 70% acetone (Sigma-Aldrich; St. Louis, MO). After elution with 70% acetone, the A-PAC fraction was lyophilized and then evaluated using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as previously done at the University of Massachusetts (Amherst Mass Spectroscopic Facility with Dr. Stephen Eyles).[7] The degree of polymerization of the A-PACs was found to range from 2 to 6.

### Primary Samples and Cell lines

Primary AML cells were obtained with informed consent and IRB approval from Weill Cornell Medical College-New York Presbyterian Hospital or University of Rochester. Primary AML samples were thawed and cultured as previously described.[15] Normal bone marrow from volunteer donors was treated in the same fashion. Cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA; Deutsche Sammlung von Mikroorganismen (DSMZ; Braunschweig, Germany). All cell lines were authenticated (Bio-Synthesis; Lewisville, TX) and tested for mycoplasma. All cell lines were cultured in Iscove's Modified Dulbecco's Medium (Life technologies; Gaithersburg, MD) supplemented with either 10 or 20% fetal bovine serum (Life technologies, Gaithersburg, MD) according to supplier's instructions and 1% penicillin/streptomycin (Life Technologies; Gaithersburg, MD).

### Cell Viability Assays

Cell viability was determined using annexin V (BD Biosciences, San Jose, CA) or YO-PRO™ dye (ThermoFisher Scientific, Grand Island, NY) and/or 7-aminoactinomycin (7-

AAD, ThermoFisher Scientific; Grand Island, NY). In addition, cells were also stained with antibodies against phenotypic markers CD45, CD34, and CD38 (BD Biosciences; San Diego, CA) to assess viability in phenotypically defined AML subpopulations as previously described.[15] At least 50,000 events were recorded per condition on an BD-LSR II flow cytometer. Cells that are negative for annexin-V or YO-PRO and/or 7-AAD are considered viable. Data analysis was conducted using FlowJo software for Mac OS X (TreeStar; Ashland, OR).

**Measurement of NF-κB (p65 subunit) activity**

The DNA-binding capacity of NF-κB (p65 subunit) was measured from whole cell extracts using the Nuclear Extract and TransAM™ NF-κB Kits (Active Motif; Carlsbad, CA) according to the manufacturer's instructions.

**Cytokine Assay**

Supernatants from MOLM13 cell cultures, treated with and without A-PACs or vehicle (0.3% DMSO), were assessed for various human proinflammatory chemokine analytes using the LEGENDplex multi-analyte flow assay kit (BioLegend; Sand Diego, CA) following the manufacturer's instructions. Specifically, IL-8, IP-10, TARC, ENA-78, I-TAC MIP-1b were assessed.

**Immunoblot**

We followed a modified version of the western blot methods previously described.[15] Cleaved PARP was evaluated in MOLM-13 cells after 8 hours of treatment with cytarabine (Selleck Chemicals; Houston, TX) (ARA-C; 1μM), CYS (500 μg/mL), A-PAC (250 μg/mL), or the untreated cells with the vehicle (DMSO: Sigma-Aldrich; St. Louis, MO). Membranes were probed with the following primary antibodies: Cleaved PARP (Asp214)(19F4) Mouse mAb (Human Specific) #9546 (Cell Signaling Technology; Danvers, MA) at 1:1000 with blocking buffer and Monoclonal anti-β-Actin antibody produced in mouse #A5316 (Sigma-Aldrich; St. Louis, MO) at 1:5000 with blocking buffer. The secondary antibody was Ab - LI-COR Goat Anti-Mouse IgG Polyclonal Antibody (IRDye® 800CW), LI-COR 926-32210 (BD Biosciences; San Diego, CA) at 1:10,000 with blocking buffer.

**RNA extraction/sequencing data**

Cell lines (MV4-11, K562, REH, MOLM-13, SKNO-1, and Ramos) were treated with A-PACs (62.5 µg/ml;31.25 µg/mL for Ramos) for 4 hours. RNA was extracted using the RNeasy plus mini kit (Qiagen; Hilden, Germany) and assessed as previously described.[16] We also performed gene set enrichment analysis (GSEA) on the RNA sequencing data for Ramos cell lines treated with A-PAC and the control.

**Colony forming assays.**

Colony forming assays were performed as previously described.[15] Briefly, primary AML or cord blood cells were cultured in the presence or absence of A-PACs. Cells were plated in MethoCult H4434 (Stem Cell Technologies; Vancouver, Canada). Colonies were scored after 14 days of culture using the StemVision (Stem Cell Technologies; Vancouver, Canada).

**Xenotransplants**

Experiments were carried out under an Institutional Animal Care and Use Committee (IACUC)-approved protocol, and institutional guidelines for the proper and humane use of animals in research were followed. Xenotransplant assays were performed as described previously.[15] In short, nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice Jackson Laboratories (JAX) were sub-lethally irradiated with 2.5 Gy (250 Rads) using a RadSource X-Ray irradiator before transplantation. For functional assays: cells were treated for 18 hours and then 2.5 x $10^6$ AML or CB cells were injected via the tail vein. At 6-8 weeks post-injections, animals were sacrificed and BM was analyzed for the presence of human cells by flow cytometry. The BM cells were stained with mouse CD45-PE-Cy5 (mCD45), human CD45-APC-H71 (hCD45), human CD33-PE (hCD33), human CD19-Alexa Fluor 700 (hCD19), APC conjugated human CD34 (hCD34) and PE-Cy7 conjugated human CD38 (hCD38) and DAPI (BD Biosciences; San Diego, CA). For in vivo treatment: patient-derived xenografts (PDX) were established by injecting 1 × $10^6$ human primary AML cells into sub-lethally irradiated immunodeficient mice.[15] PDX mice were treated with either PBS or 25 mg/kg A-PACs for 2 times a week for 3 weeks, or with 60 mg/kg of ara-C every day for 5 days.

**Statistical Analyses**

Statistical analyses were performed by using GraphPad Prism (GraphPad Software, La Jolla, CA). For the in vivo mouse xenograft experiment, statistical significance was calculated by using 1-way ANOVA with post hoc Tukey test. Otherwise, a 2-tailed Student *t* test was performed to evaluate statistical significance. The optim() function in R (www.R-project.org) was used to fit logistic functions predicting viability (% surviving) from log-transformed ($log_{10}(x)$) concentration values for each cell line. We calculated LD50 estimates for the model fits. Dotted lines in the plots represent models using parameters based on the upper and lower 95% confidence intervals of each parameter for each of the three groups A, B, C.

## TABLES

### Supplemental Table 1. Cell line information

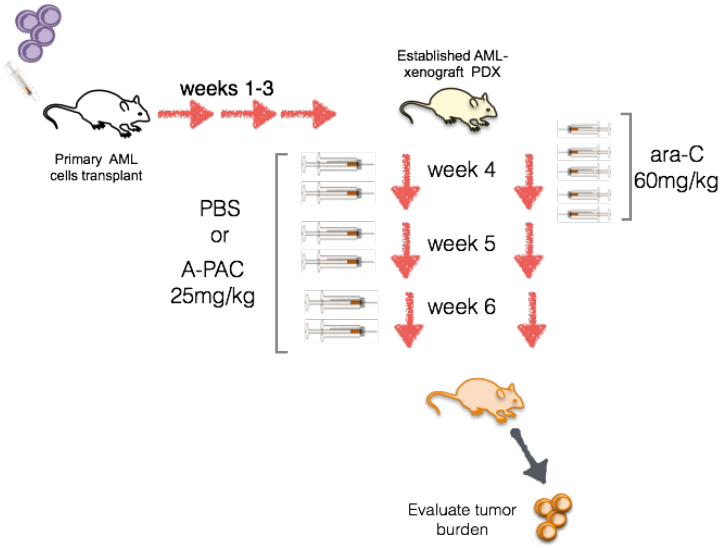
| **Cell Lines** | **Species** | **Morphology** | **Type** | **Karyotype and/or mutation(s)** | **LD50** μg/mL |
|---|---|---|---|---|---|
| U-937 | Human | monocytic | AML, M5 | CALM-AF10, Homozygous for TP53 c.559+1G>A; splice donor mutation | 140.2 |
| TUR | Human | monocytic | AML, M5 | TPA-U937-Resistant, Homozygous for TP53 c.559+1G>A; splice donor mutation | 134.4 |
| THP-1 | Human | monocytic | AML, M5 | MLL-AF9, Heterozygous for TP53 p.Arg174fs*3 (c.520_545del26) | 204.6 |
| SUDHL6 | Human | lymphoblast-like | B lymphocyte | t(14;18)(q32;q21), Heterozygous for TP53 c.673-2A>C; splice acceptor mutation and p.Tyr234Cys (c.701A>G) | 22.72 |
| SKNO-1 | Human | myeloblastic | AML, M2 | AML1-ETO, N822K c-kit, Homozygous for TP53 p.Arg248Gln (c.743G>A) and p.His368Arg (c.1103G>A) | 43.06 |
| SET-2 | Human | megakaryoblastic | essential thrombocythemia | JAK2V617F, Heterozygous for TP53 p.Arg248Trp (c.742C>T) and (c.559+2T>A) | 54.74 |
| REH | Human | lymphoblast | ALL (Non-T, Non-B) | CDKN2Adel, CDKN2Bdel, Heterozygous for TP53 p.Arg181Cys (c.541C>T) | 49.54 |
| Ramos Blue (Ramos B) | Human | lymphoblast | B lymphocyte | NF-kB/AP-1-inducible SEAP, Homozygous for TP53 p.Ile254Asp (c.760_761AT>GA) | 23.32 |
| Ramos | Human | lymphoblast | B lymphocyte | Homozygous for TP53 p.Ile254Asp (c.760_761AT>GA) | 23.48 |
| MV4-11 | Human | monocytic | AML, M5 | FLT3-ITD, MLL-AF4 | 236.8 |
| MOLM-13 | Human | monocytic | AML, M5 | FLT3-ITD, MLL-AF9, CDKN2Adel, CDKN2Bdel | 47.51 |
| KG-1 | Human | myeloblastic | AML | 2n = 46, diploid, | 189.8 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | Homozygous for TP53 c.672+1G>A; splice donor mutation | |
| Kasumi-4 | Human | myeloblastic | CML | BCR-ABL | 134.7 |
| Kasumi-1 | Human | myelomonocytic | AML, M2 | AML1-ETO, N822K c-kit, T Homozygous for TP53 p.Arg248Gln (c.743G>A) | 127.8 |
| K562 | Human | lymphoblast | CML | BCR-ABL CDKN2Adel, CDKN2Bdel, Homozygous for TP53 p.Gln136fs*13 (c.406_407insC) | 90.46 |
| HL-60 | Human | promyelocitic | AML | c-myc+ | 87.13 |
| HEL | Human | erythroleukemia | AML | JAK2V617F, CDKN2Adel, CDKN2Bdel, Homozygous for TP53 p.Met133Lys (c.398T>A) | 24.57 |

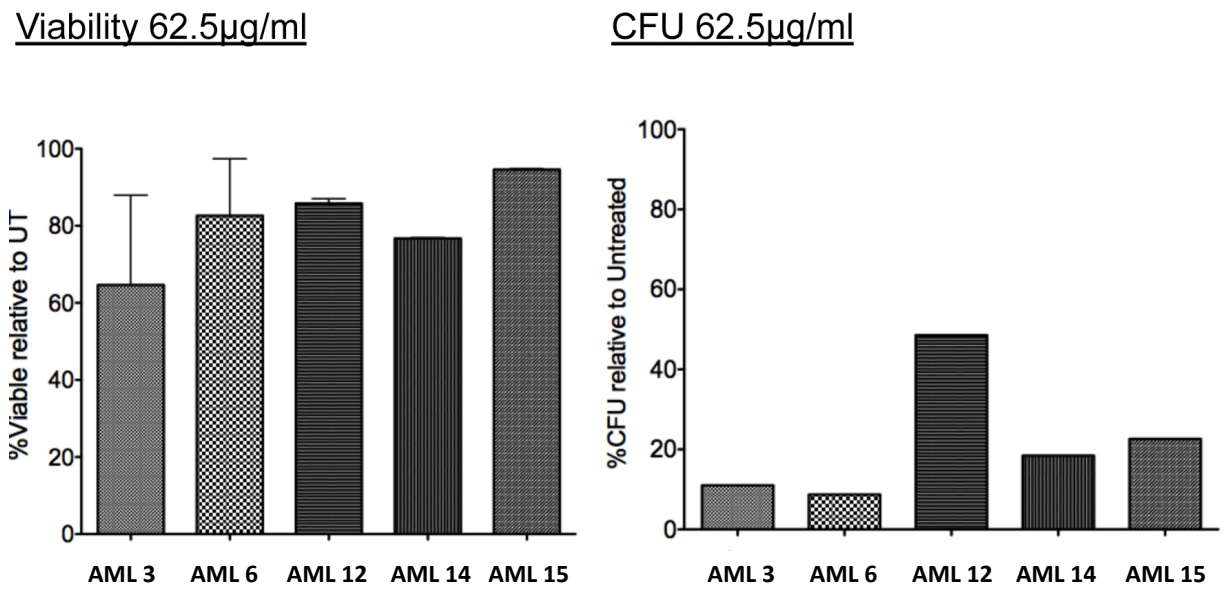
**Supplemental Table 2. Primary sample information**

| **ID** | **Sample information** | **FLT3-ITD** | **LD50** μg/mL |
|---|---|---|---|
| AML1 | monosomy 7;NPM1 wt; de novo | positive | ND |
| AML2 | M4; Normal cytogenetics; Relapsed | positive | ND |
| AML3 | N/A | positive | ND |
| AML4 | De Novo; M2; Normal cytogenetics | positive | ND |
| AML5 | MDS progression to AML | positive | 336.3 |
| AML6 | N/A | negative | 80.62 |
| AML7 | N/A | negative | 161.2 |
| AML8 | N/A | negative | 285.7 |
| AML9 | N/A | negative | 40.7 |
| AML10 | del(9)(q13)[1] ; De novo | positive | 30.20 |
| AML11 | De Novo, Normal cytogenetics | negative | 190.4 |
| AML12 | De Novo, Normal cytogenetics | positive | 177.8 |
| AML13 | MDS progression to AML; cytogenetics | negative | 113.5 |
| AML14 | MDS progression to AML | negative | 91.37 |
| AML15 | Refractory; 47,XY,+8[3] | positive | 216.8 |

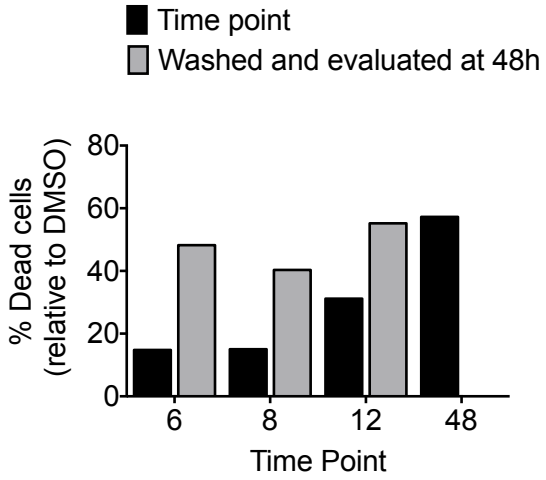
**FIGURES**



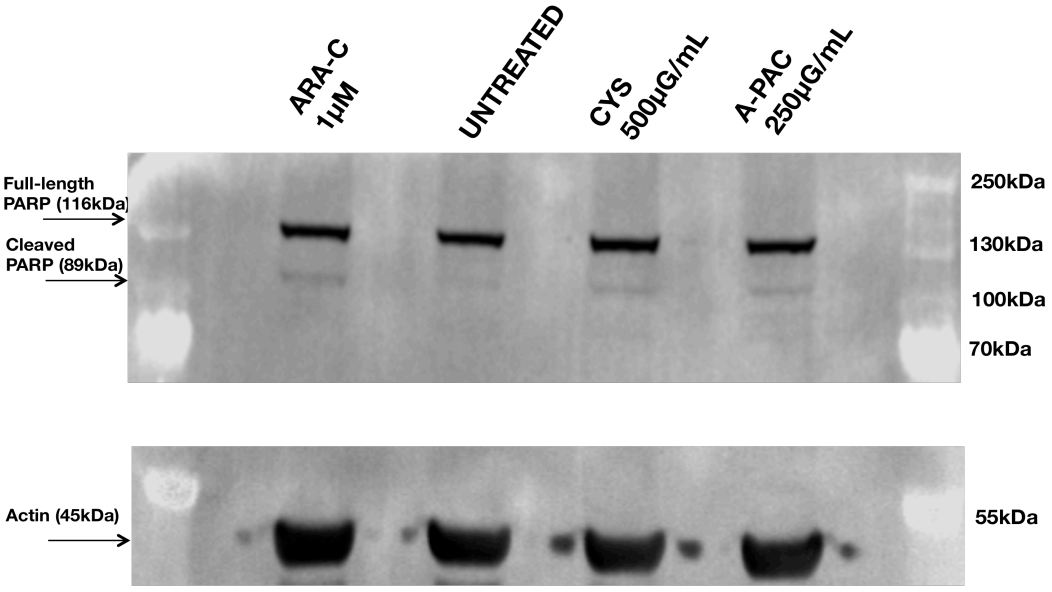

**Supplemental Figure 1.** Schematic representation of the experimental design. Mice were treated i.p. with 25mg/kg of A-PACs or PBS 2 times/week for 3 weeks or with 60 mg/kg ara-C daily for 5 days.



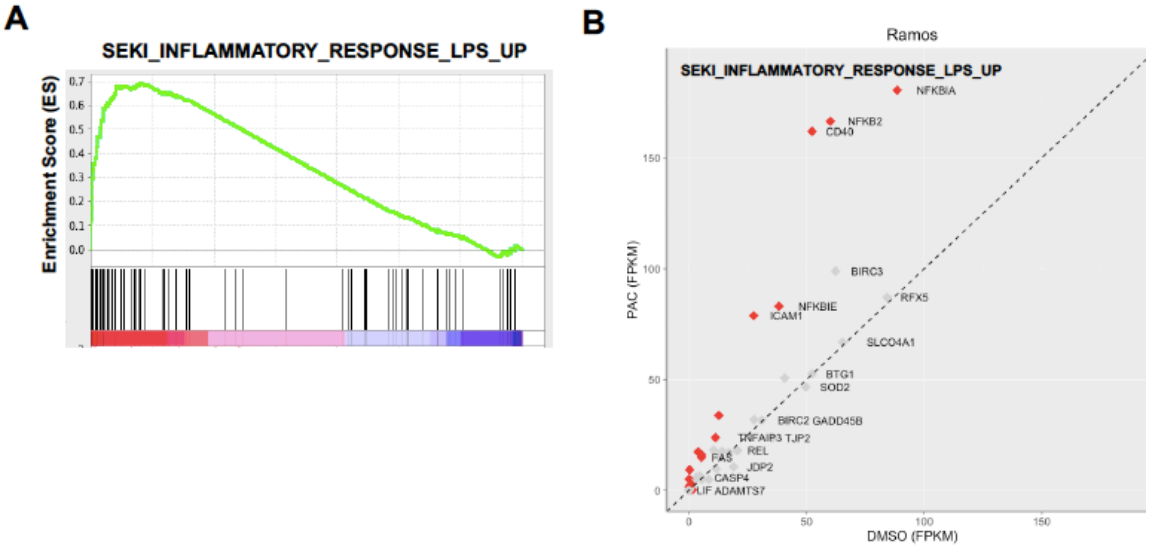

**Supplemental Figure 2**. Left panel: Percent viability of CD34+CD38- AML cells after 18 hours of treatment with A-PAC. Right panel: Percent colony forming units (CFU) relative to vehicle for the same samples shown on the left panel after 48 hours.



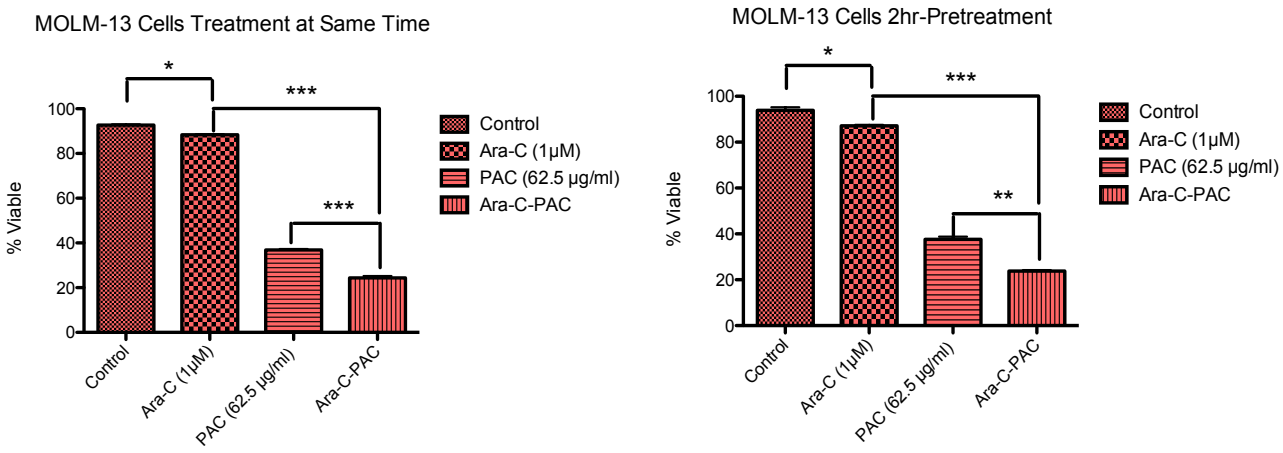

**Supplemental Figure 3**. Cell death at wash time points following 250 µg/mL A-PAC treatment (black bars) and 48 hours later (gray bars).



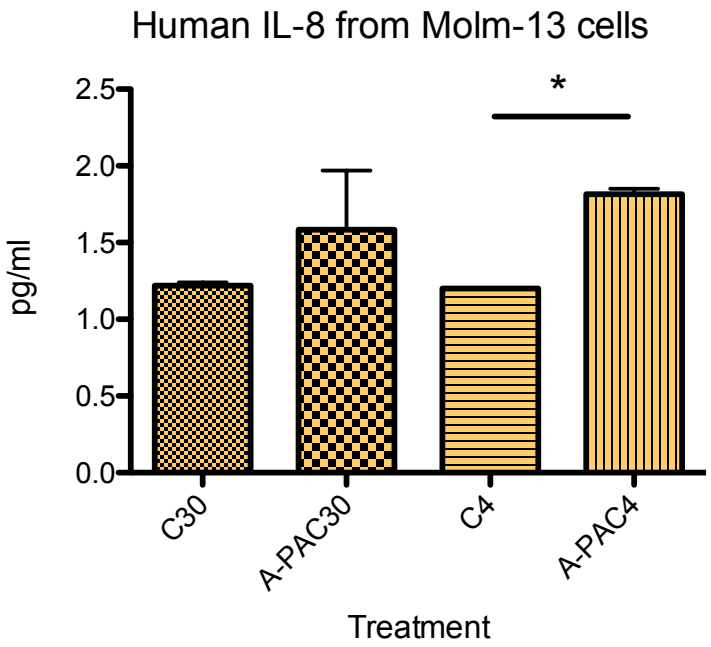

**Supplemental Figure 4**. Western blot analysis of MOLM-13 cells treated with A-PAC, CYS (cysticran), or ARA-C (cytarabine) after 8 hours indicated increased expression of cleaved PARP in comparison to untreated cells with vehicle (DMSO).




**Supplemental Figure 5**. GSEA Analysis: inflammatory response for ramos cells (A) Enrichment scores (B) A-PAC treatment vs. control (DMSO).




**Supplemental Figure 6**. Cytarabine (ara-C) treated in combination with A-PAC either at same time or 2 hours after treatment with A-PACs significantly reduced the percent viable cells. The control were cells treated with the vehicle (0.3% DMSO in media). Data mean ± SEM were significant if *P<0.05, **P<0.01, ***P<0.001 (One-way ANOVA; post test: Tukey's multiple comparison).




**Supplemental Figure 7**. IL-8 was the only inflammatory chemokine increased in cell culture supernatants as early as 30 minutes and significantly at 4 hours post-treatment with 62.5 μg/mL of A-PACs in an AML cell line (MOLM13). C30 and C4 represent vehicle treatment (PBS) at 30 minutes and 4 hours, respectively. A-PAC30 and A-PAC4 represent treatment after 30 minutes and 4 hours, respectively. Data mean ± SEM were significant if *P<0.05, **P<0.01, ***P<0.001 (student's t-test).