## Supplemental Data

### Supplemental Methods

### Isolation and culture of primary BM-MSC

BM-MSC were isolated from BM of consented AML patients undergoing diagnostic BM aspiration and from healthy donors who were undergoing BM harvest for use in allogeneic BM transplantation. BM was subjected to centrifugation (700 *g* for 15 minutes at 4°C) over a Ficoll-Hypaque (Sigma-Aldrich) gradient to separate mononuclear cells. After centrifugation, the buffy coat layer was carefully extracted and resuspended in αMEM (Cellgro, Mediatech, Inc.) supplemented with 10% pooled human platelet lysate (pHPL, kindly provided by Dr. Dirk Strunk, Department of Hematology and Stem Cell Transplantation, Medical University of Graz, Austria), [1] supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin (Sigma-Aldrich). Detailed information on pHPL preparation method has been previously described. [1]Briefly, platelet rich plasma (PRP) from forty to fifty single blood donations units prepared by cytapheresis or derived from buffy coats are subjected to several freeze/thaw cycles to damage the platelet membranes and efficiently release growth factors into the plasma. Finally, the platelet fragments are removed by centrifugation to avoid extensive aggregate formation and deplete potential antigens and then the lysates are pooled.

The BM mononuclear cell content was analyzed by automated blood count (Beckman Coulter), and mononuclear cells were seeded at a density of $5 \times 10^4$ cells/cm$^2$ in tissue-culture flasks and cultured at 37°C in an atmosphere of 5% $CO_2$ at 95% humidity. The non-adherent cells were removed by completely changing the medium after 3 days, and the adherent cells were continuously cultured. The cultures were fed twice weekly by replacing 30% of the medium with fresh supplemented medium. The cells were harvested before reaching confluence by applying 0.25% trypsin and 1 mM EDTA (Life Technologies). BM-MSC aliquots were frozen after primary culture and stored in liquid nitrogen. For large-scale

expansion, the BM-MSC derived from primary culture were seeded in αMEM/10% pHPL at a density of 300 cells/cm$^2$ in four-layered cell factories. The culture medium was changed (i.e., 30% was replaced) twice weekly, and BM-MSC were harvested on days 11 through 15 by trypsinization. According to our previously published data these isolated BM-MSC are CD14-/CD31-/CD34-/CD45-/D44+/CD73+/CD90+/CD105+. [2]

**Isolation and Expansion of Endothelial Colony-forming Cells (ECFCs)**

Heparinized blood (6 mL) from a healthy donor was directly diluted in EGM/10% pHPL without additional cell separation and placed in a 75-cm$^2$ culture flask. Non-adherent cells were removed by washing with warm PBS after culturing for 24 hours. Cultures were maintained until the outgrowth of cobblestone-type colonies was observed, which were defined as ECFCs. The primary culture–derived ECFCs were then expanded in EGM/10% pHPL in two four-layered cell factories for 2 to 3 weeks.

**Lentiviral Constructs and Stably Transduced Cells.**

FLAG epitope-tagged wild-type IκBα or super-repressor (SR) IκBα (S32G/S36A) coding sequences [3] were subcloned into pCDH-CMV-MCS-EF1-copGFP lentiviral vector (System Biosciences, Cat#CD511B-1). A human codon optimized firefly luciferase sequence was excised from pGL4.51 (Promega) and cloned into pCDH-CMV-MCS-EF1-copGFP to generate pCDH-Luc-CopGFP. Lentiviral infections were carried out according to standard procedures. Briefly, 293T cells were co-transfected with pMD2.G and psPAX2 (Addgene, Inc.) along with pCDH-IκBα-SR, pCDH-Luc-CopGFP or pCDH empty vector using JetPrime transfection reagent (Polyplus-transfection, Inc.) according to the manufacturer's protocol. The transfection medium was replaced after 12 hours with fresh DMEM with 10% FBS, and 48 hours later the viral supernatants were collected and concentrated by using Centricon Plus-70 filter units (EMD Millipore). Normal BM-MSC were infected overnight with either pCDH empty vector (control) or

pCDH-IκBα-SR virus-containing supernatants supplemented with 8 μg/mL Polybrene (Sigma-Aldrich) to enhance lentiviral infection. NALM-6 cells were infected with pCDH-Luc-CopGFP viral supernatants. Two days after infection stably transduced BM-MSC and NALM-6 cells were sorted by FACS resulting in homogeneous populations of CopGFP-positive cells.

**Induction of MSC Differentiation**

MSCs were grown to 90% confluence in αMEM/10% pHPL and then moved to NH OsteoDiff medium or NH Adipo-Diff medium (Miltenyi Biotec, Inc.) to induce osteoblastic or adipogenic differentiation, respectively. Each differentiation medium was changed every 3 days. To confirm that BM-MSC differentiated into osteocytes and adipocytes, they were stained with alizarin red or alkaline phosphatase (to detect calcium deposits indicative of osteocytes) and oil red O (to detect lipids indicative of adipocytes).[4] Cells were photographed with a Hamamatsu-C4742-95 camera (Hamamatsu Photonics,) attached to an Olympus BX41 microscope (Olympus America, Inc.,).

**Co-culture Isolation and RNA Extraction**

Normal BM-MSC were plated at a density of ~ $3 \times 10^6$ cells/175-$cm^2$ flask. Approximately 24 hours later, the supernatant medium was replaced by 21 mL of RPMI with 10% FBS for BM-MSC alone or RPMI 10% FBS containing 5 to $7 \times 10^6$ REH cells for the REH−BM-MSC co-culture. REH/BM-MSC ratio was ~ 2 to 1 at the seeding time. After 48 hours of incubation, the supernatant medium was removed along with nonattached REH cells. Attached REH cells were collected by flushing the co-culture monolayer with 10 mL PBS. A second collection was done by incubating the co-culture monolayer with 5 mL of PBS with 5mM EDTA for 2 to 3 minutes. Both collections were pooled in one 50-mL tube. Monocultures of REH cells and MSC seeded at the same density as for the co-culture were collected as controls. After collection, the cells were stained for ~ 20 minutes with allophycocyanin (APC)-conjugated anti-human CD90 (Thy-1) antibody (BM-MSC marker) and phycoerythrin (PE)-conjugated anti-human CD45 antibody (leukemia cells marker) and then separated by FACS (FACSAria II; BD Biosciences).

After sorting, cells were pelleted and lysed with 0.7 mL of QIAzol lysis reagent for total RNA extraction. The same procedure was applied to co-cultures of BM-MSC with OCI-AML3 cells. In co-cultures experiments where ALL or AML patient samples where used, only $CD90^+$, $CD19^-$, $CD45^-$, BM-MSC were collected.

**mRNA Hybridization and Gene-expression Profiling.**

After confirmation of RNA quality using a Bioanalyzer 2100 instrument (Agilent Technologies, Inc.-), 300 ng of total RNA was amplified and biotin-labeled through an Eberwine procedure using an Illumina TotalPrep RNA Amplification kit (Life Technologies) and hybridized to Illumina HT12 version 4 human whole-genome arrays. Each of these arrays has an average of 15 beads for each of > 48,000 probes measuring > 25,000 annotated genes and additional transcripts. Bead-level data were processed by methods previously described. [5] In brief, outlier-filtered bead values underwent model-based background correction,[6] quantile normalization, filtering for probe quality,[7] and log2 transformation. Candidate differentially-expressed probes (DEPs) were then determined for each of 3 independent experiments, comparing co-cultured to monocultured MSC, by the Wilcoxon rank-sum test of processed bead values, with a significance threshold of p value < 0.01, false discovery rate q statistic < 0.1.[8] Final DEPs were those candidates found in all 3 experiments, and their fold-change was determined from the average of mean bead values for each experiment. Assessment of the possibility that DEPs were due to contamination of co-cultured and purified BM-MSC by REH cells was done as follows. For any probe, the intensity values from an experimental replicate were considered to be A for co-cultured and purified BM-MSC, B for the monocultured BM-MSC, and C for co-cultured and purified REH cells. A is the theoretical sum of A1, the unknown true value attributable to co-cultured BM-MSC, and A2, the calculated value attributable to contaminating REH cells. Based on 1.5% being the maximum measured frequency of contaminating REH cells, we calculated A2 as C*0.015. Setting a fold-change threshold of 1.5, a contaminating DEP must satisfy three conditions: 1) The DEP must be upregulated in co-cultured

BM-MSC samples, i.e., A must be > 1.5*B; 2) the contribution attributable to contaminating REH cells must be required for the fold-change threshold to be exceeded, (i.e., A-A2 must be < 1.5*B, or, using direct measurements, A-(C*0.015) must be < 1.5*B and 3) these conditions must be met in all 3 experiments.

Gene set enrichment analysis (GSEA)[9] was performed using gene sets from the Molecular Signatures Database (www.broadinstitute.org/gsea/msigdb/).

**Reverse Transcription-PCR Analysis**

Reverse transcription reactions were carried out using 1 μg of total RNA and the Super Script III First Strand cDNA synthesis kit according to the manufacturer's instructions (Life Technologies). All the cDNA samples were divided into aliquotes and stored at −70°C for further use. Real-time PCR reactions were performed on a 7900 Real-Time System (Applied Biosystems) using the SYBR Green qPCR kit (Applied Biosystems) and gene-specific primers. A list of primer sets used in this study is provided in Supplemental Table 3.

**Co-culture and BM-MSC−mediated Chemoresistance Experiments**

Normal BM-MSC were plated on day 0 at a density of ~ $2.5 \times 10^4$ cells/well in 24-well plates. Twenty four hours later, the supernatant medium was replaced with RPMI with 10% FCS containing ~ 1 x $10^5$ leukemia cells for co-culture purposes. The leukemic cells/BM-MSC ratio was ~ 4 to 1 at the seeding time. After incubation for 24 hours, all floating leukemia cells in the co-culture wells were removed by aspirating the supernatant medium and fresh medium (0.5 mL) was added to each well without disturbing the remaining attached leukemia cells. In order to determine the average number of attached leukemic cells per well in co-culture dishes and to establish the monoculture condition with same number of leukemia cells, the absolute number of adherent leukemia cells was determined in separate triplicate dishes destined for this purpose only. Once non-attached leukemia cells were removed from

these three wells by aspirating the supernatant medium, remaining attached leukemia cells were collected along with BM-MSC by mild trypsinization. After collection, leukemia cells were labeled with CD45 antibody and counted by flow cytometry using counting beads (CountBright Absolute Counting Beads, Life Technologies). Once the average absolute number of attached leukemia cells per well was determined, the same number of leukemia cells was plated in 0.5 mL of medium in each well of empty plates. Chemotherapy treatment started in both monoculture and co-culture plates immmediately at this point. Viability and absolute number of cells were determined after 48 to 72 hours of treatment by flow cytometry. Percentage of apoptosis was estimated by measuring phosphatidylserine externalization in cells using APC-conjugated annexin V (BD Biosciences) in combination with DAPI (Sigma-Aldrich) staining in $CD45^+$ cells. Percentage of viable cells was calculated by subtracting annexin $V^+$ and $DAPI^+$ cells from the total of $CD45^+$ cells. To determine the absolute number of viable cells a total of 10,000 beads was added to each test tube and the acquisition stop criterion was set at 1,000 beads. Absolute number of cells was calculated by multiplying number of events by ten.

**Immunohistochemical Analysis**

Fresh tissues collected from mice were fixed in 4% PFA and embedded in paraffin. The sections (5 μm) were stained with hematoxylin and eosin (Sigma-Aldrich) and analyzed by light microscopy. For immunohistochemical staining, the tissue sections were first incubated with sodium citrate buffer (pH 6.0) for antigen retrieval and then for 30 minutes in blocking solution (PBS, 0.5% Tween 20, 0.1% BSA and 10% FBS), followed an overnight incubation with the primary antibody or negative control antibody. The tissue sections were then sequentially incubated with a biotinylated antibody and peroxidase-labeled streptavidin (Dako North America, Inc.,). The staining was completed by a 5-minutes incubation with 3,3′-diaminobenzidine tetrahydrochloride/hydrogen peroxide, which yields a brown precipitate at the antigen site. Spectral images were obtained using a Cri attachment (CRi) on an Olympus IX81 DSU microscope equipped with disc-scanning unit confocal attachment using Nuance software (Nuance Communications, Inc.), and the images were analyzed using InForm software (InForm Software Corp.).

Five images per slide were quantified and averaged at three different focal depths within the tissue section.

BM biopsies from consented ALL patients were formalin-fixed and paraffin-embedded. Tissue sections (~ 5 μm thick) were backed in a 70°C oven for 30 minutes, deparaffinized in xylene, and then rehydrated through graded concentrations of alcohol. For the antigen retrieval, tissue sections were heated in Diva Decloaker (Biocare Medical, Concord, Calif) (Biocare Medical) for 30 minutes in a Decloaking Chamber (Biocare Medical). The tissue sections were incubated with 3% peroxidase blocking reagent for 10 minutes and subsequently with protein block Background Sniper (Biocare Medical) for 10 minutes. The tissue sections were then incubated with specific antibodies against human CD90 (1:50; Cat# 328101, Biolegend Inc.) and phospho-NF-κB p65 (Ser276) (1;100; Cat#3037, Cell Signaling Technologies) overnight at 4°C. Detection was achieved with Mach 2 dual stain kit #2 (Biocare Medical) using DAB (Dako) and Fast Red (Biocare Medical) as substrates for color development. All sections were counterstained with hematoxylin (Biocare Medical) for 2 minutes. The slides were then air dried and cover-slipped.

**Cell Fractionation and Western Blot Analysis**

Co-cultured cells were separated by extensive washing the BM-MSC monolayer with ice-cold PBS containing proteinase inhibitors cocktail (Roche) and phosphatases inhibitors. After separation cells were collected by centrifugation using a microcentrifuge at 1000 *g*. Cell pellets were resuspended in 5 pellet volumes (~ 150 μl) of CE buffer (10 mM HEPES, 60 mM KCl, 1 mM EDTA, 0.075% (v/v) NP40, 1mmol/L DTT and 1 mmol/L PMSF, adjusted to pH 7.6) and incubated on ice for 3 minutes. After centrifugation at 1000 *g* for 5 minutes, the cytoplasmic extract was collected and transferred to a clean tube without disturbing the nuclear pellet. Nuclei were gently washed four times with 300 μL of CE buffer without detergent and centrifuged 1000 *g* for 5 minutes. Nuclear pellets were resuspended in 50 μL of NE buffer (20 mM Tris-Cl, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mmol/L EDTA, 1 mM PMSF, adjusted

to pH 8.0) and incubated on ice for 10 minutes. Resuspended pellets were sonicated using microprobes three times for 5 seconds each on ice and the centrifugated at 12000 *g* for 5 minutes. The nuclear fractions were transferred to a clean tube and stored at −80°C until used.

Cell lysates were separated on 12% polyacrylamide gels, transferred to nitrocellulose membrane, immunoblotted with rabbit monoclonal anti–NF-κB p65 (1:500; Cat#8242, Cell Signaling Technologies), mouse monoclonal anti-PARP-1 (1:500; Cat#sc-8007, Santa Cruz Biotechnology, Inc.) and mouse monoclonal anti-GAPDH (1:5000; Cat#MAB374, EMD Millipore) followed by infrared secondary antibodies (LI-COR Biosciencesand then detected by the Odyssey imaging system (LI-COR Biosciences).

**Immunofluorescence Staining and Confocal Microscopy**

Monocultured or co-cultured BM-MSC were seeded on chamber slides at a density of $1 \times 10^4$ cells/slide. At the end of the experiment, cell cultures were washed with PBS twice and fixed with 4% PFA in PBS for 10 minutes at room temperature, then subjected to permeabilization with cold 0.5% Triton X-100 for 15 minutes. After extensive washing with PBS, fixed cells were incubated for 1 hour at 25°C in blocking buffer (PBS, 5% BSA) and then stained at 4°C overnight with anti–NF-κB p65 (1:100; Cat#8242, Cell Signaling Technologies). Slides were then washed with PBS and incubated for 60 minutes with Alexa Fluor 488 goat anti-rabbit IgG (1:1000) or Alexa Fluor 647 goat anti-rabbit IgG (1:1000) (Life Technologies) at 25°C according to the manufacturer's instructions. After further washing with PBS and counterstaining with DAPI, samples were mounted with Dako Fluorescent Mounting Medium and were visualized and photographed under a confocal laser-scanning microscope (Olympus IX81 DSU, (Olympus America, Inc.) featuring a water immersion 60 × 1.25 NA objective. Images were captured with an Orca II ER camera and processed with Slidebook 5.0 software (Intelligent Imaging Innovations).

***In Vivo* Extramedullary Bone Formation and Murine Leukemia Model**

NOD/SCID/IL-2rγ$^{null}$ mice were obtained from The Jackson Laboratory. All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee at The University of Texas MD Anderson Cancer Center and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Human BM-MSC ($1.5 \times 10^6$) transduced with empty vector (controls) or with IκBα-SR were mixed with human ECFCs ($1.5 \times 10^6$ cells) in 0.2 mL Matrigel (BD Biosciences) immediately before being subcutaneously injected into the flanks of NOD/SCID/IL-2rγ$^{null}$ mice. Each mouse was injected with control BM-MSC−ECFC−Matrigel in one flank and IκBα-SR−BM-MSC−ECFC−Matrigel in the other flank. Both the BM-MSC and ECFCs were obtained from the large-scale expansions described above, with low passages (i.e., passages 1 to 3). To monitor bone formation in mice, we utilized animal micro computed tomography weekly, starting on the fourth week after injection. At each time point, mice were anesthetized, and detailed three-dimensional images of the soft tissue and bone structure were obtained. When a positive signal was observed from the implants, the mice were injected with OsteoSense 750 and further scanned with a VisEn FMT 2500 imaging system to generate a tomographic database consisting of the bone structure and fluorescence signal.

The extramedullary bones were fully developed at ~ 8 weeks after implantation and the NALM6-luciferase-CopGFP leukemia cells were intravenously injected into each mouse. The leukemia burden was subsequently monitored by noninvasive imaging of isoflurane-anesthetized mice injected intraperitoneally with luciferin in the *in vivo* imaging system (IVIS system; Xenogen/Caliper Life Sciences). Ten days after injection of the leukemia cells, engraftment was confirmed and chemotherapy treatment started. The mice were injected i.p. with VCR (150 μg/kg) every 3 days for a total of 10 days. At the end of this chemotherapy regimen, the mice were imaged one more time and then sacrificed to remove extramedullary bones for *ex-vivo* imaging and immunohystochemical staining.

**Cell culture in VCAM-1–coated dishes**

Human recombinant VCAM-1 was purchased from R&D Systems, Inc. (cat# ADP5-050). Tissue culture dishes were coated with 0.25 mL of VCAM-1 solution (5 μg/mL in PBS) at 4 °C for 24 hours. After removing the VCAM-1 solution, dishes were incubated with 2% BSA in PBS for 30 minutes and then rinse twice with PBS before addition of the cell suspension.

**Supplemental References:**


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# Supplemental Tables and Figures

## Table S1: List of upregulated DEPs in co-culured BM-MSC

| ILMN_Gene | Probe ID | Mean_P | Mean_q | Mean_Fold_Change |
|---|---|---|---|---|
| ABCA1 | 4060358 | 1.94974E-07 | 4.19E-05 | 1.773097972 |
| ADAM15 | 3440452 | 0.002593522 | 0.025043 | 1.27824948 |
| ADAMTS1 | 6900086 | 0.000377196 | 0.006473 | 2.011960501 |
| ADSS | 5960097 | 0.004900036 | 0.038127 | 1.280485809 |
| AFAP1 | 2690187 | 0.000654003 | 0.010343 | 1.31177752 |
| ALDH1A3 | 4920148 | 2.92263E-05 | 0.00156 | 1.46045852 |
| ALDOC | 7330544 | 0.000635018 | 0.010135 | 1.249633565 |
| APBB3 | 4120279 | 3.45987E-05 | 0.001763 | 1.262693809 |
| ARPC1B | 130717 | 4.46352E-07 | 8.81E-05 | 2.959519524 |
| ASB1 | 1820347 | 0.001877551 | 0.012754 | 2.096819619 |
| ASNS | 1510296 | 7.49184E-05 | 0.002636 | 1.450951441 |
| BAX | 3520092 | 4.57368E-07 | 9.29E-05 | 2.001206552 |
| BHLHB2 | 2640735 | 0.002016317 | 0.018441 | 1.274387227 |
| BMS1 | 1190717 | 0.001110939 | 0.013185 | 1.302890382 |
| C19ORF10 | 1030458 | 1.94627E-05 | 0.001225 | 2.143667754 |
| CA12 | 150474 | 0.001761628 | 0.017394 | 1.452786704 |
| CALD1 | 4230328 | 1.26557E-06 | 0.00018 | 1.450299098 |
| CCDC16 | 5900112 | 0.002029948 | 0.025201 | 1.625541382 |
| CCL2 | 1030333 | 2.38791E-08 | 1.1E-05 | 3.037619312 |
| CCT7 | 7160719 | 0.000641461 | 0.011533 | 1.362747336 |
| CDC42EP4 | 1070047 | 0.001061204 | 0.01637 | 1.447680153 |
| CDK10 | 1850309 | 0.003516645 | 0.027728 | 1.478557567 |
| CDK6 | 670286 | 0.000243219 | 0.005302 | 1.530366597 |
| CDR2L | 3940482 | 0.000101549 | 0.002815 | 1.259702074 |
| CMTM7 | 2140239 | 3.88355E-06 | 0.000417 | 1.329387936 |
| CNTNAP1 | 4570358 | 0.004614007 | 0.038209 | 1.225674352 |
| COL6A2 | 2750356 | 7.97191E-06 | 0.000615 | 1.541904814 |
| COX4I1 | 3390022 | 0.001990145 | 0.019634 | 1.187638417 |
| CRIM1 | 3930040 | 5.69861E-05 | 0.002005 | 1.416028426 |
| CYR61 | 3930605 | 1.13023E-08 | 5.85E-06 | 1.764095259 |
| DCBLD2 | 60132 | 0.004105771 | 0.032772 | 1.292297 |
| DDOST | 6450605 | 0.000521999 | 0.00918 | 1.509965031 |
| DEXI | 2470386 | 0.001251681 | 0.017155 | 1.510976358 |

| | | | | |
|---|---|---|---|---|
| DKK3 | 4070133 | 0.001848961 | 0.01456 | 1.285937684 |
| DKK3 | 5390451 | 0.000312254 | 0.0067 | 1.238956164 |
| DRAM1 | 4280482 | 3.04768E-07 | 7.42E-05 | 1.644735281 |
| ECGF1 | 2350504 | 0.000260726 | 0.005509 | 1.738106113 |
| ECOP | 870692 | 0.00064891 | 0.009225 | 1.802510615 |
| EGLN2 | 7650653 | 0.002527153 | 0.02061 | 1.264537646 |
| EIF2S3 | 2850440 | 1.41994E-05 | 0.000571 | 1.32692448 |
| EIF4A1 | 3400403 | 0.005263818 | 0.044581 | 1.452442445 |
| EIF4H | 4540397 | 0.00018622 | 0.004604 | 1.536485782 |
| ERCC6 | 520168 | 0.002284312 | 0.023411 | 1.697202279 |
| FAM129B | 3180053 | 0.001179404 | 0.017001 | 1.281096963 |
| FER1L4 | 4640039 | 0.003660034 | 0.031476 | 1.245299219 |
| FKBP2 | 1010068 | 0.000382057 | 0.008808 | 1.723835885 |
| FLJ39632 | 1660341 | 0.000924017 | 0.012156 | 1.527887752 |
| FN1 | 4040592 | 0.00040257 | 0.005625 | 1.392973232 |
| GADD45A | 4880673 | 0.004363624 | 0.032533 | 1.303087946 |
| GAK | 7210767 | 0.001451926 | 0.019891 | 1.200249811 |
| GSTO2 | 2260730 | 0.001938643 | 0.021973 | 1.254404075 |
| HCLS1 | 1300408 | 0.001397915 | 0.019113 | 1.25112663 |
| HES4 | 5260070 | 0.000107777 | 0.002547 | 2.038181232 |
| HMGN1 | 4390195 | 0.001072403 | 0.014712 | 1.330689626 |
| IER3 | 1190367 | 6.02618E-07 | 9.79E-05 | 1.713170702 |
| IGF2R | 2810156 | 0.000682747 | 0.012666 | 1.265036593 |
| IL32 | 3440754 | 2.34684E-07 | 5.29E-05 | 2.431616936 |
| IL6 | 4040576 | 9.0641E-10 | 1.51E-06 | 2.092396402 |
| IL8 | 1980309 | 2.72556E-07 | 4.18E-05 | 35.6317444 |
| IRF1 | 6250064 | 1.04024E-05 | 0.000736 | 1.932757364 |
| KCNG1 | 1400427 | 7.53577E-05 | 0.00257 | 1.630269507 |
| KCTD11 | 2760008 | 0.002674098 | 0.025913 | 1.619102015 |
| KLF9 | 3390292 | 0.00144625 | 0.017978 | 1.280231716 |
| LDLR | 1440736 | 0.000926083 | 0.012196 | 1.54145786 |
| LEPREL1 | 4280524 | 0.006345432 | 0.047529 | 1.20726589 |
| LEPREL2 | 6760632 | 0.00189602 | 0.022684 | 1.494112325 |
| LOC100008589 | 6290142 | 1.08626E-08 | 8.02E-06 | 1.825911843 |
| LOC100128892 | 1770100 | 0.001225142 | 0.017718 | 1.488415814 |
| LOC100132717 | 650044 | 0.003140911 | 0.03098 | 1.440367826 |
| LOC100133372 | 2340703 | 0.002355015 | 0.017226 | 1.457644112 |
| LOC389168 | 3060148 | 0.002805297 | 0.025624 | 1.315391022 |
| LOC441089 | 6660086 | 0.003909958 | 0.03314 | 1.635250209 |
| LOC642755 | 5860242 | 0.003247375 | 0.032592 | 1.50258295 |
| LOC644063 | 1770470 | 0.000175414 | 0.004448 | 1.418317547 |

| | | | | |
|---|---|---|---|---|
| LOC645385 | 2490445 | 0.001714281 | 0.014283 | 1.472763361 |
| LOC647276 | 360653 | 0.000500737 | 0.010715 | 1.293970103 |
| LOC648210 | 6900048 | 0.000394137 | 0.005804 | 1.456050807 |
| LOC653888 | 4860093 | 2.44491E-07 | 4.89E-05 | 2.818566957 |
| LOC728059 | 4040519 | 0.000343508 | 0.006455 | 1.573304563 |
| LOC730417 | 7200612 | 0.003119008 | 0.026427 | 1.830145876 |
| LRRC42 | 5570524 | 0.001069391 | 0.011814 | 1.321066021 |
| LRRFIP2 | 4150438 | 1.95569E-06 | 0.000239 | 1.65071689 |
| MARCH6 | 780544 | 0.000382986 | 0.007844 | 1.627514576 |
| MICAL2 | 1430463 | 0.001957166 | 0.020678 | 1.708231477 |
| MLPH | 2120452 | 0.003340951 | 0.027821 | 1.362108752 |
| MRPL17 | 6660270 | 0.00303538 | 0.029961 | 1.269340172 |
| MSC | 7510377 | 0.000302247 | 0.006749 | 1.521234065 |
| MSRB2 | 2710646 | 0.00030646 | 0.006304 | 1.274865726 |
| MTP18 | 7400025 | 0.004655457 | 0.040678 | 1.259230322 |
| MX1 | 1690066 | 3.40706E-09 | 3.73E-06 | 2.570861001 |
| MYC | 6270646 | 0.000685148 | 0.00801 | 1.564085672 |
| NBPF10 | 1510681 | 0.004356735 | 0.035733 | 1.510912819 |
| NENF | 3120139 | 0.005804265 | 0.044775 | 1.262053843 |
| NFKB1 | 7400626 | 0.000740235 | 0.011394 | 1.375922077 |
| NFKBIA | 4280113 | 2.88467E-07 | 4.52E-05 | 2.012053708 |
| NOTCH3 | 2340692 | 5.42968E-05 | 0.001748 | 1.355252079 |
| PAM | 4210670 | 0.001944925 | 0.02427 | 1.239241139 |
| PAPPA | 730754 | 0.001239349 | 0.009847 | 1.421826274 |
| PCNX | 7050626 | 0.000843248 | 0.014804 | 1.159621711 |
| PENK | 6220019 | 0.002653865 | 0.02478 | 1.534531141 |
| PFKFB4 | 7400653 | 0.002271022 | 0.022063 | 1.254143601 |
| PIPSL | 4640070 | 0.002368947 | 0.022161 | 1.708645586 |
| PLSCR3 | 6940255 | 0.001772795 | 0.017956 | 1.254369737 |
| PPP3R1 | 6200768 | 0.002582297 | 0.021918 | 1.538764965 |
| PRIC285 | 5960343 | 0.005382949 | 0.046086 | 1.290265804 |
| PSAP | 6200086 | 0.000150563 | 0.003126 | 1.250088343 |
| PTPRF | 3060398 | 0.000851004 | 0.012776 | 1.275746259 |
| PTRF | 4850301 | 0.002876538 | 0.023371 | 1.272089655 |
| RAB2B | 670609 | 0.001491404 | 0.017866 | 1.208050657 |
| RCN3 | 2100431 | 0.004135131 | 0.038589 | 1.223992732 |
| RHBDF2 | 3420523 | 0.004754475 | 0.038619 | 1.455606745 |
| RNASET2 | 2850100 | 0.001224071 | 0.017993 | 1.250387667 |
| SAE1 | 7160753 | 0.001228878 | 0.018518 | 1.164416011 |
| SAT1 | 5490431 | 7.7087E-05 | 0.001735 | 1.379230902 |
| SBDSP | 5260717 | 0.00085691 | 0.01283 | 1.562675516 |

| | | | | |
|---|---|---|---|---|
| SCD | 2140128 | 2.62907E-06 | 0.000179 | 1.606536009 |
| SDF2L1 | 3120079 | 1.00646E-05 | 0.000753 | 1.734499398 |
| SDF4 | 3310167 | 0.003610239 | 0.032808 | 1.43170374 |
| SEPT5 | 6960022 | 0.001255626 | 0.013752 | 1.364371395 |
| SERPINB6 | 4220504 | 5.99051E-05 | 0.002659 | 1.25819048 |
| SFRS5 | 4730543 | 7.3385E-08 | 2.84E-05 | 1.807921499 |
| SFRS5 | 6380445 | 0.000570616 | 0.006193 | 1.560661307 |
| SIPA1 | 5810068 | 0.000272465 | 0.006463 | 1.521816907 |
| SIVA1 | 1450477 | 0.004410662 | 0.040676 | 1.320280185 |
| SLC25A28 | 2360392 | 0.002284892 | 0.014977 | 1.367274548 |
| SLC3A2 | 2450725 | 0.003691944 | 0.033775 | 1.590485068 |
| SOD2 | 3420373 | 5.05086E-05 | 0.002345 | 2.095530152 |
| SOD2 | 3890326 | 1.80093E-06 | 0.000247 | 3.857144773 |
| SQSTM1 | 4260044 | 0.001099174 | 0.015189 | 1.250007165 |
| SULF1 | 2570240 | 0.000643557 | 0.01041 | 1.320955122 |
| TAP1 | 7330392 | 3.77259E-07 | 8.29E-05 | 1.566465899 |
| TFPI | 1340039 | 1.55104E-05 | 0.000631 | 1.79542027 |
| TFPI | 4850731 | 1.09251E-05 | 0.000562 | 1.670571888 |
| TINF2 | 1740471 | 0.002426453 | 0.017208 | 1.240617189 |
| TKT | 6860202 | 0.001530007 | 0.017994 | 1.32290813 |
| TMBIM1 | 5820097 | 0.002849125 | 0.022812 | 1.669406308 |
| TMED3 | 5550408 | 2.03864E-06 | 0.000259 | 1.353798225 |
| TMED9 | 5390202 | 4.51066E-05 | 0.002136 | 1.657677932 |
| TMEM138 | 6290598 | 0.000954803 | 0.011576 | 1.320421768 |
| TMEM14C | 3840167 | 0.003383498 | 0.030195 | 1.188227835 |
| TMTC3 | 7000286 | 0.001571426 | 0.013905 | 1.746044751 |
| TNFAIP3 | 3360681 | 9.81875E-07 | 0.000146 | 2.983388209 |
| TNPO1 | 5080482 | 0.000411137 | 0.007379 | 2.166854013 |
| TNS3 | 5560561 | 2.78359E-05 | 0.001393 | 1.577449969 |
| TRIB3 | 1990630 | 0.001776982 | 0.019333 | 1.257730361 |
| TRPM4 | 870437 | 0.000135235 | 0.003675 | 1.406908078 |
| TUBB | 6580474 | 0.002273414 | 0.026985 | 1.145680987 |
| UPP1 | 7570673 | 0.000557313 | 0.008987 | 1.553847558 |
| VCAM1 | 1240519 | 0.000107605 | 0.002079 | 2.517183873 |
| VCAM1 | 2900390 | 8.65745E-10 | 1.62E-06 | 2.711085309 |
| VCAM1 | 4290390 | 6.66682E-05 | 0.002099 | 2.372113221 |
| VPS16 | 3830180 | 0.000528441 | 0.009271 | 1.238342447 |
| VPS37C | 6510528 | 0.002605891 | 0.022548 | 1.267716813 |
| WDR1 | 4860239 | 0.002026263 | 0.019563 | 1.606882241 |
| WDR45L | 2600646 | 0.000272882 | 0.006129 | 1.480419124 |
| WDR74 | 2190537 | 0.003538765 | 0.028579 | 1.542574566 |

| | | | | |
|---|---|---|---|---|
| YIF1A | 4590494 | 0.002601095 | 0.027045 | 1.22550939 |
| YRDC | 3440224 | 0.000322341 | 0.006817 | 1.324921502 |
| ZBTB43 | 2060037 | 0.000484724 | 0.01046 | 1.73562041 |
| ZFYVE21 | 380594 | 6.63238E-06 | 0.000575 | 2.209174276 |

**Supplemental Table 1. List of upregulated DEPs in co-culured BM-MSC.** Differentially-expressed probes (DEPs) were determined for each of 3 independent experiments, comparing co-cultured to monocultured MSC with a significance threshold of p value < 0.01, false discovery rate q statistic < 0.1. **ILMN_Gene:** Illumina gene nomenclature.

## Table S2: List of downregulated DEPs in co-culured BM-MSC

| ILMN_Gene | Probe ID | Mean_P | Mean_q | Mean_Fold_Change |
|---|---|---|---|---|
| ANGPTL4 | 4610433 | 5.26858E-06 | 0.000393912 | 0.716526063 |
| ARL4A | 1410113 | 3.1506E-05 | 0.001276343 | 0.629676042 |
| ARMCX6 | 4490215 | 0.002247328 | 0.024837408 | 0.599694544 |
| ATP5E | 3710725 | 0.001137393 | 0.013710578 | 0.725968044 |
| ATP5EP2 | 6900324 | 1.02826E-05 | 0.000781339 | 0.770178685 |
| ATP5J | 5860162 | 0.000131119 | 0.003983872 | 0.726632189 |
| ATP5J | 6560180 | 0.000613493 | 0.009710386 | 0.799816823 |
| ATP5L | 6370411 | 0.000593529 | 0.007819321 | 0.725251048 |
| ATP5O | 6110754 | 0.000291166 | 0.005537993 | 0.756586814 |
| ATP6V0E1 | 2320110 | 0.000378919 | 0.008580054 | 0.765110813 |
| ATP6V1D | 1660736 | 0.004171039 | 0.032210345 | 0.828137731 |
| BANF1 | 150767 | 0.001223371 | 0.015752948 | 0.795361703 |
| C11ORF1 | 610400 | 0.003884287 | 0.030752968 | 0.735561308 |
| C11ORF10 | 630445 | 0.001818016 | 0.021479648 | 0.847408956 |
| C11ORF51 | 6840100 | 0.001279444 | 0.018897623 | 0.525965949 |
| C14ORF156 | 5290025 | 1.28203E-06 | 0.000176514 | 0.704493976 |
| C1ORF166 | 6400097 | 0.001922271 | 0.023248964 | 0.628688906 |
| C20ORF117 | 290132 | 0.004974493 | 0.042819864 | 0.699797161 |
| C21ORF51 | 60041 | 0.001288365 | 0.016349919 | 0.688796161 |
| C22ORF25 | 630446 | 0.001642342 | 0.019838156 | 0.730544252 |
| C8ORF59 | 1510452 | 0.003207121 | 0.032004189 | 0.698284191 |
| CAT | 1770500 | 0.003108713 | 0.025368401 | 0.709289591 |
| CBX5 | 7150685 | 0.002955841 | 0.022204726 | 0.587068314 |
| CDC5L | 6760189 | 0.005687313 | 0.044314058 | 0.595799122 |
| CDK5RAP2 | 4260017 | 0.001163293 | 0.017292795 | 0.628467562 |
| CHMP5 | 6450326 | 0.002417858 | 0.026082008 | 0.813113788 |

| | | | | |
|---|---|---|---|---|
| CHST3 | 3930736 | 0.002360627 | 0.024224417 | 0.767728622 |
| CKLF | 5080367 | 0.000464377 | 0.007538655 | 0.710045202 |
| CNN1 | 4850630 | 0.001871341 | 0.020585672 | 0.67876572 |
| COPS4 | 4290400 | 0.003411182 | 0.027997838 | 0.773145169 |
| COX17 | 630735 | 5.65864E-05 | 0.001924115 | 0.725346632 |
| COX6B1 | 4250095 | 0.000112712 | 0.003890401 | 0.745480709 |
| COX7A1 | 5390138 | 0.00092959 | 0.013747331 | 0.819172462 |
| COX7A2 | 540491 | 0.001550036 | 0.016528998 | 0.797775865 |
| CPA4 | 520682 | 8.59212E-05 | 0.003293226 | 0.646035674 |
| CRIP1 | 1170047 | 0.003133502 | 0.026791921 | 0.748845719 |
| CRYAB | 6110079 | 0.003314881 | 0.026226935 | 0.70427908 |
| CSTB | 1430187 | 0.001106103 | 0.016174704 | 0.819984825 |
| DBI | 1010195 | 0.000593382 | 0.010500834 | 0.745107111 |
| DCLK2 | 2340129 | 0.003572337 | 0.029099377 | 0.594545661 |
| DCLK2 | 2470403 | 0.002888403 | 0.027146698 | 0.606348484 |
| DCP1A | 1450370 | 0.004344883 | 0.039311478 | 0.679938875 |
| DDIT3 | 830619 | 0.001082792 | 0.014044923 | 0.670073115 |
| DLX5 | 3370767 | 0.003299152 | 0.033268262 | 0.756391162 |
| DNAJB6 | 5860315 | 0.002431437 | 0.022569156 | 0.814236446 |
| DPY30 | 6960025 | 0.004456309 | 0.038706776 | 0.817471634 |
| DUSP3 | 6560156 | 8.39605E-05 | 0.003196195 | 0.704144061 |
| DYNLL1 | 6220086 | 0.000123242 | 0.004027128 | 0.653736553 |
| DYNLRB1 | 130603 | 0.001062051 | 0.013352971 | 0.755867477 |
| DYNLRB1 | 6580369 | 0.000106419 | 0.003240488 | 0.740117017 |
| DYNLRB1 | 7210224 | 0.001643103 | 0.014757821 | 0.750665707 |
| FAM96A | 6760202 | 0.002590665 | 0.028429579 | 0.574054948 |
| FLJ40504 | 4880333 | 0.001032193 | 0.011121764 | 0.671100438 |
| FLOT1 | 3780181 | 0.00316021 | 0.020879134 | 0.771031423 |
| FLYWCH2 | 4920162 | 0.00621369 | 0.049209027 | 0.718787591 |
| GDPD5 | 1010176 | 0.002761719 | 0.025002505 | 0.640981585 |
| GLRX | 4590228 | 0.00033358 | 0.00676316 | 0.694216764 |
| GOSR2 | 2600288 | 0.003640312 | 0.030471841 | 0.689606818 |
| GPC4 | 6330270 | 0.000175322 | 0.004976631 | 0.64951364 |
| GTF2A2 | 2450368 | 9.92136E-05 | 0.003470801 | 0.806999669 |
| HIST1H4C | 3890349 | 8.26847E-06 | 0.000624744 | 0.707623748 |
| HOXC4 | 6860717 | 0.00165459 | 0.020974998 | 0.629922162 |
| HS.553187 | 6620390 | 0.000944215 | 0.009881646 | 0.626949793 |
| HS.579631 | 3520168 | 0.002566409 | 0.021987776 | 0.730542178 |
| HSPB7 | 4560523 | 0.002820773 | 0.023342008 | 0.675102067 |
| HYOU1 | 5700041 | 0.000195314 | 0.005312529 | 0.578211386 |
| ID2 | 1260086 | 0.000877104 | 0.011129931 | 0.647510672 |

| | | | | |
|---|---|---|---|---|
| IGFBP4 | 7510414 | 0.001158264 | 0.012613064 | 0.760688762 |
| KIAA0367 | 3940392 | 0.000193975 | 0.004830939 | 0.629926738 |
| KRT81 | 430446 | 0.001214966 | 0.010290648 | 0.615428293 |
| LAGE3 | 1240482 | 0.002161026 | 0.016860618 | 0.783601255 |
| LBH | 2810246 | 0.000106253 | 0.002137329 | 0.596663749 |
| LOC100128731 | 5130142 | 0.001118804 | 0.014188175 | 0.79182526 |
| LOC100130516 | 6380037 | 0.000136288 | 0.003870431 | 0.666664218 |
| LOC100131801 | 5260360 | 5.71194E-05 | 0.001894105 | 0.668237014 |
| LOC100133477 | 3390072 | 0.000125634 | 0.004023846 | 0.736656857 |
| LOC100134537 | 2360082 | 0.00062077 | 0.011777942 | 0.700851876 |
| LOC134997 | 870537 | 0.000847774 | 0.008172289 | 0.661179919 |
| LOC389342 | 5090484 | 9.70036E-07 | 9.55909E-05 | 0.524001642 |
| LOC399988 | 4850136 | 0.001796967 | 0.02258815 | 0.752364481 |
| LOC400948 | 5860608 | 0.004289164 | 0.033110132 | 0.768173123 |
| LOC402175 | 520468 | 0.000200635 | 0.00466896 | 0.803001831 |
| LOC439953 | 2100112 | 0.002234664 | 0.019229024 | 0.802260639 |
| LOC440957 | 540240 | 0.000368966 | 0.006018455 | 0.707923875 |
| LOC644039 | 7000274 | 0.002447529 | 0.023040672 | 0.714238964 |
| LOC645058 | 1980600 | 0.001095148 | 0.015078799 | 0.749775745 |
| LOC646785 | 1170551 | 0.000392979 | 0.008017093 | 0.726406706 |
| LOC647886 | 4260440 | 0.000602793 | 0.01009495 | 0.67011336 |
| LOC650646 | 6960195 | 0.00147102 | 0.021038401 | 0.805127185 |
| LOC729236 | 2320709 | 2.60286E-05 | 0.001153251 | 0.6937029 |
| LOC730278 | 6770601 | 0.001433062 | 0.016218938 | 0.712450074 |
| LOC730833 | 580441 | 3.01864E-05 | 0.001456319 | 0.51971162 |
| MAP1A | 4920202 | 0.000253831 | 0.005627278 | 0.723903465 |
| MAPK7 | 1190300 | 0.00041501 | 0.008641566 | 0.626556704 |
| MEIS2 | 20358 | 4.99705E-06 | 0.000505847 | 0.683124941 |
| MGST1 | 5080131 | 0.000285519 | 0.006825836 | 0.777078957 |
| MGST3 | 7160400 | 0.000844036 | 0.012480832 | 0.792263879 |
| MRPL21 | 1300315 | 0.000740086 | 0.009728269 | 0.69400705 |
| MRPL21 | 6250576 | 0.002522671 | 0.019325008 | 0.79629083 |
| MRPL33 | 5570494 | 0.000886926 | 0.010958185 | 0.761299533 |
| MRPS18C | 1430639 | 0.000192583 | 0.004265842 | 0.723276562 |
| MRPS21 | 3830671 | 0.000119666 | 0.004017128 | 0.733915238 |
| MT1A | 6200402 | 0.001600002 | 0.018180527 | 0.807264781 |
| MT1E | 2070288 | 0.000892911 | 0.0118812 | 0.622530218 |
| MT1X | 6620528 | 3.68606E-07 | 6.39743E-05 | 0.625957936 |
| MT2A | 450615 | 0.000951932 | 0.010610786 | 0.739201597 |
| MYST3 | 3930195 | 0.003740799 | 0.037070445 | 0.60264629 |
| NDUFA2 | 6840189 | 0.001921535 | 0.019702953 | 0.74416866 |

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|---|---|---|---|---|
| NDUFA3 | 50240 | 0.00248142 | 0.024250167 | 0.806382759 |
| NDUFB3 | 1770102 | 0.001704368 | 0.015626356 | 0.800937512 |
| NDUFS5 | 6980398 | 0.000428667 | 0.008624778 | 0.775451172 |
| NEU1 | 4200692 | 0.001175552 | 0.017205074 | 0.646293799 |
| OBFC2B | 270196 | 0.002558842 | 0.026035066 | 0.558960621 |
| OLFML3 | 3180070 | 0.000845038 | 0.010664789 | 0.649013226 |
| PITX2 | 3990440 | 0.000268235 | 0.006927126 | 0.706718873 |
| PLIN2 | 460204 | 6.06819E-05 | 0.0022706 | 0.742037101 |
| PNMA1 | 6350608 | 0.004065148 | 0.031415261 | 0.719355538 |
| POLR2L | 6200017 | 0.000825359 | 0.011032208 | 0.518664315 |
| PPAP2B | 6220097 | 8.8928E-07 | 0.00013595 | 0.477428772 |
| PRICKLE4 | 730114 | 0.00285383 | 0.023027571 | 0.732524813 |
| PSG9 | 4120243 | 0.002873769 | 0.027985727 | 0.615587493 |
| RAB5C | 450164 | 0.001058337 | 0.016725464 | 0.767029663 |
| RBX1 | 2070746 | 0.002467085 | 0.026414148 | 0.745891369 |
| RDH10 | 7050433 | 0.000457524 | 0.010025094 | 0.606553397 |
| RHOB | 3400332 | 0.000106513 | 0.003500009 | 0.651326319 |
| RHOD | 4180270 | 0.004070359 | 0.035393304 | 0.728637104 |
| RNU6-1 | 3610279 | 0.000837876 | 0.012744314 | 0.77012134 |
| RPL14L | 2360102 | 0.007159898 | 0.054204167 | 0.803577587 |
| RPL24 | 1940546 | 0.001015995 | 0.011532046 | 0.825445407 |
| RPL26L1 | 6130390 | 0.001156601 | 0.017810546 | 0.810529797 |
| RPL35A | 6370504 | 0.002322074 | 0.023716831 | 0.812431761 |
| RPL36AL | 3170184 | 0.000449009 | 0.007791201 | 0.797810655 |
| RPL36AL | 5220161 | 0.000194257 | 0.005501862 | 0.720689893 |
| RPS15A | 7100717 | 0.000149295 | 0.003689508 | 0.770275108 |
| RPS21 | 2690338 | 0.000362133 | 0.006936411 | 0.814878574 |
| RPS26L | 4230121 | 0.003936607 | 0.03419686 | 0.747354127 |
| RPS26L | 5890730 | 0.000179262 | 0.004935114 | 0.690005129 |
| RTN4 | 2230161 | 0.003962981 | 0.030801505 | 0.888034163 |
| RXRA | 7000356 | 0.001533133 | 0.021096483 | 0.833341713 |
| SEC31A | 3840100 | 0.000756972 | 0.011123498 | 0.730166176 |
| SF3B14 | 2000500 | 0.00072264 | 0.011378352 | 0.736157846 |
| SFRS2 | 3940414 | 0.004362946 | 0.039534293 | 0.803944642 |
| SLC16A2 | 2650112 | 0.000221267 | 0.00576985 | 0.585892116 |
| SMG7 | 6270706 | 0.003193219 | 0.030167805 | 0.845194623 |
| SNHG5 | 1050475 | 0.004631248 | 0.034540312 | 0.80255382 |
| SRP14P1 | 1300072 | 0.002031228 | 0.023216198 | 0.703710326 |
| TBC1D24 | 3120161 | 0.003135765 | 0.025803169 | 0.634172414 |
| THEM2 | 1580427 | 0.000660585 | 0.009524499 | 0.715307305 |
| THYN1 | 2140176 | 0.000764193 | 0.010452478 | 0.647135073 |

| | | | | |
|---|---|---|---|---|
| TMEM119 | 3830762 | 0.003838014 | 0.034875244 | 0.782589748 |
| TMEM126A | 7560092 | 0.00100356 | 0.010675072 | 0.767123776 |
| TMEM189-UBE2V1 | 4390220 | 0.003501325 | 0.027466323 | 0.774653776 |
| TP53I3 | 1260020 | 0.002094459 | 0.021478238 | 0.823671011 |
| TRAPPC2P1 | 4590121 | 0.000594453 | 0.011025683 | 0.588438109 |
| TRAPPC4 | 1230639 | 0.00647702 | 0.049980142 | 0.812485026 |
| TRMT112 | 5420398 | 0.003447882 | 0.031325163 | 0.738424562 |
| TSC22D2 | 2450082 | 0.003298718 | 0.032740112 | 0.648745051 |
| TSC22D3 | 6350632 | 0.001771592 | 0.013287196 | 0.774360708 |
| TSPYL1 | 3780689 | 0.004461247 | 0.041245986 | 0.719109313 |
| TXN | 4290543 | 0.000198802 | 0.004433462 | 0.706048125 |
| UBE2A | 6960440 | 0.000416066 | 0.00876554 | 0.778115896 |
| UQCRB | 3830746 | 0.002192156 | 0.025528735 | 0.686763466 |
| UTP11L | 6280152 | 0.002853042 | 0.026744264 | 0.703124387 |
| VAMP5 | 2630195 | 0.002350105 | 0.015998328 | 0.710873955 |
| WNK4 | 7150411 | 0.000594928 | 0.010164536 | 0.6833168 |
| XKR8 | 7560435 | 0.004156889 | 0.03694921 | 0.699712499 |

**Supplemental Table 2: List of downregulated DEPs in co-culured BM-MSC.** Differentially-expressed probes (DEPs) were determined as described above (Supplemental Table 1). **ILMN_Gene:** Illumina gene nomenclature.

## Table S3: "Hinata_NFKB_Targets_Fibroblasts_Up" data set

| NAME | PROBE | DESCRIPTION<br>(from dataset) | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|---|---|---|---|---|---|---|
| row_0 | IL8 | 3576 | 0 | 5.011204243 | 0.23966059 | Yes |
| row_1 | SOD2 | 6648 | 1 | 1.790762305 | 0.3253037 | Yes |
| row_2 | TNFAIP3 | 7128 | 3 | 1.482197762 | 0.39610356 | Yes |
| row_3 | IL6 | 3569 | 10 | 1.085379481 | 0.44749472 | Yes |
| row_4 | NFKBIA | 4792 | 14 | 0.994617343 | 0.49480373 | Yes |
| row_5 | IER3 | 8870 | 34 | 0.759403825 | 0.5294848 | Yes |
| row_6 | MSC | 9242 | 65 | 0.625448227 | 0.5568115 | Yes |
| row_7 | RELB | 5971 | 107 | 0.53741473 | 0.5789801 | Yes |
| row_8 | SEC24A | 10802 | 168 | 0.483969897 | 0.5969553 | Yes |
| row_9 | GADD45A | 1647 | 273 | 0.420894474 | 0.6081221 | Yes |
| row_10 | RND3 | 390 | 276 | 0.418772727 | 0.62797755 | Yes |

| | | | | | | |
|---|---|---|---|---|---|---|
| row_11 | VEGFA | 7422 | 280 | 0.417971373 | 0.6477085 | Yes |
| row_12 | TNFAIP8 | 25816 | 306 | 0.407739908 | 0.6650542 | Yes |
| row_13 | NFKB1 | 4790 | 371 | 0.383867532 | 0.6778973 | Yes |
| row_14 | NFKB2 | 4791 | 537 | 0.333318114 | 0.67961895 | Yes |
| row_15 | BIRC2 | 329 | 540 | 0.333025903 | 0.6953736 | Yes |
| row_16 | GSTO1 | 9446 | 910 | 0.277104586 | 0.6768267 | No |
| row_17 | GABBR1 | 2550 | 1082 | 0.257824957 | 0.6744209 | No |
| row_18 | MAPK9 | 5601 | 1226 | 0.242013365 | 0.67367184 | No |
| row_19 | CDH2 | 1000 | 2064 | 0.177541375 | 0.61003244 | No |
| row_20 | ETS1 | 2113 | 2186 | 0.17010361 | 0.60774016 | No |
| row_21 | LITAF | 9516 | 2609 | 0.14549394 | 0.57833165 | No |
| row_22 | EGFR | 1956 | 2683 | 0.141073629 | 0.57878757 | No |
| row_23 | FGF1 | 2246 | 3997 | 0.080990486 | 0.4695103 | No |
| row_24 | MTSS1 | 9788 | 4174 | 0.072895199 | 0.45782933 | No |
| row_25 | FGF2 | 2247 | 4226 | 0.07118167 | 0.45683855 | No |
| row_26 | FN1 | 2335 | 5142 | 0.032139495 | 0.37952352 | No |
| row_27 | RAC1 | 5879 | 6555 | -0.028093996 | 0.25918493 | No |
| row_28 | CCNB1 | 891 | 7431 | -0.067342065 | 0.18700053 | No |
| row_29 | HDGF | 3068 | 7953 | -0.091711886 | 0.14648832 | No |
| row_30 | TAF15 | 8148 | 8109 | -0.100391373 | 0.13793208 | No |
| row_31 | VIM | 7431 | 8145 | -0.102516599 | 0.13981873 | No |
| row_32 | SOD1 | 6647 | 8616 | -0.127981603 | 0.10543617 | No |
| row_33 | ERCC1 | 2067 | 8801 | -0.138987169 | 0.096226625 | No |
| row_34 | EXOSC9 | 5393 | 9005 | -0.151375443 | 0.08597218 | No |
| row_35 | ARHGDIA | 396 | 9031 | -0.152561575 | 0.091114 | No |
| row_36 | ABCC1 | 4363 | 9293 | -0.170062497 | 0.07675499 | No |
| row_37 | FGF5 | 2250 | 9738 | -0.20191215 | 0.048148762 | No |
| row_38 | KRT7 | 3855 | 9795 | -0.206292331 | 0.05318876 | No |
| row_39 | MYBL1 | 4603 | 10218 | -0.240522489 | 0.028324967 | No |
| row_40 | RABEP1 | 9135 | 10340 | -0.252530605 | 0.029974792 | No |
| row_41 | CCNH | 902 | 10345 | - | 0.04173521 | No |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | 0.253113359 | 7 | |
| row_42 | AP2B1 | 163 | 10547 | -0.27699697 | 0.03766097 | No |
| row_43 | SERPINB2 | 5055 | 11077 | -0.359076947 | 0.009246081 | No |
| row_44 | CD9 | 928 | 11210 | -0.38908869 | 0.01647884 | No |
| row_45 | GREM1 | 26585 | 11395 | -0.444678873 | 0.021888986 | No |

**Supplemental Table 3: "Hinata_NFKB_Targets_Fibroblasts_Up" data set**. List of genes of corresponding to one of the Molecular Signatures Database that was significantly enriched in co-cultured samples (q value < 0.1). More information is available at http://www.broadinstitute.org/gsea/msigdb/search.jsp

## Table S4: "Kegg_Cytokine_Cytokine Receptor_Interaction" data set

| NAME | PROBE | DESCRIPTION<br>(from dataset) | RANK IN GENE LIST | RANK METRIC SCORE | RUNNING ES | CORE ENRICHMENT |
|---|---|---|---|---|---|---|
| row_0 | IL8 | 3576 | 0 | 5.011204243 | 0.23010653 | Yes |
| row_1 | CCL2 | 6347 | 2 | 1.608732104 | 0.30389053 | Yes |
| row_2 | IL6 | 3569 | 10 | 1.085379481 | 0.35312453 | Yes |
| row_3 | LIF | 3976 | 58 | 0.6397416 | 0.37843892 | Yes |
| row_4 | CCL5 | 6352 | 236 | 0.439167798 | 0.38330927 | Yes |
| row_5 | VEGFA | 7422 | 280 | 0.417971373 | 0.39878598 | Yes |
| row_6 | CCL3 | 6348 | 338 | 0.399056554 | 0.41218433 | Yes |
| row_7 | CX3CR1 | 1524 | 342 | 0.397123069 | 0.43016034 | Yes |
| row_8 | IL1RAP | 3556 | 344 | 0.39592424 | 0.44825414 | Yes |
| row_9 | IL9 | 3578 | 357 | 0.388776511 | 0.46506917 | Yes |
| row_10 | OSMR | 9180 | 402 | 0.372101545 | 0.4783532 | Yes |
| row_11 | CXCL2 | 2920 | 490 | 0.344279736 | 0.48664382 | Yes |
| row_12 | IL15 | 3600 | 591 | 0.324661046 | 0.4929102 | Yes |

| | | | | | | |
|---|---|---|---|---|---|---|
| row_13 | TNFRSF4 | 7293 | 612 | 0.321586132 | 0.5059486 | Yes |
| row_14 | LTBR | 4055 | 714 | 0.305877864 | 0.51126605 | Yes |
| row_15 | VEGFB | 7423 | 745 | 0.302081108 | 0.5225447 | Yes |
| row_16 | IFNGR2 | 3460 | 750 | 0.301187277 | 0.53602904 | Yes |
| row_17 | IL13RA1 | 3597 | 931 | 0.274996161 | 0.5331017 | Yes |
| row_18 | IL4R | 3566 | 1026 | 0.263670921 | 0.53708595 | Yes |
| row_19 | IL5RA | 3568 | 1166 | 0.248279899 | 0.5364748 | Yes |
| row_20 | TNFRSF14 | 8764 | 1195 | 0.245230988 | 0.5453158 | Yes |
| row_21 | IFNAR1 | 3454 | 1258 | 0.239603207 | 0.55096024 | Yes |
| row_22 | IL7R | 3575 | 1526 | 0.216726542 | 0.53783906 | No |
| row_23 | CSF1R | 1436 | 2193 | 0.169571489 | 0.48807278 | No |
| row_24 | CRLF2 | 64109 | 2200 | 0.169172108 | 0.4953224 | No |
| row_25 | EGFR | 1956 | 2683 | 0.141073629 | 0.460148 | No |
| row_26 | CXCL13 | 10563 | 2798 | 0.135534748 | 0.4565202 | No |
| row_27 | TNFRSF21 | 27242 | 3082 | 0.121778771 | 0.4376565 | No |
| row_28 | GHR | 2690 | 3202 | 0.116340019 | 0.4327152 | No |
| row_29 | IL24 | 11009 | 3294 | 0.112187698 | 0.43000287 | No |
| row_30 | IFNAR2 | 3455 | 3298 | 0.111881398 | 0.43488106 | No |
| row_31 | ACVR1B | 91 | 3300 | 0.111761793 | 0.43992656 | No |
| row_32 | ACVR1 | 90 | 3600 | 0.098425828 | 0.4186079 | No |
| row_33 | TNFSF4 | 7292 | 3816 | 0.088337667 | 0.4040849 | No |
| row_34 | TNFRSF10B | 8795 | 3992 | 0.081173472 | 0.39268953 | No |
| row_35 | VEGFC | 7424 | 4016 | 0.08010564 | 0.3943803 | No |
| row_36 | PDGFC | 56034 | 4435 | 0.061437551 | 0.36107972 | No |

| | | | | | | |
|---|---|---|---|---|---|---|
| row_37 | IL20RB | 53833 | 4739 | 0.048411239 | 0.3371188 | No |
| row_38 | ACVRL1 | 94 | 5542 | 0.014451066 | 0.26847717 | No |
| row_39 | TGFB2 | 7042 | 5646 | 0.010370494 | 0.26005256 | No |
| row_40 | TNFRSF12A | 51330 | 5704 | 0.008005543 | 0.25549448 | No |
| row_41 | BMPR1A | 657 | 5847 | 0.001971636 | 0.24331401 | No |
| row_42 | IL17RA | 23765 | 5868 | 0.001219181 | 0.24164169 | No |
| row_43 | TNFRSF25 | 8718 | 6437 | -0.022043359 | 0.19356988 | No |
| row_44 | TNFSF13B | 10673 | 6447 | -0.022935035 | 0.19384529 | No |
| row_45 | LEP | 3952 | 6479 | -0.024320684 | 0.19228317 | No |
| row_46 | IFNA21 | 3452 | 6590 | -0.029941706 | 0.18415235 | No |
| row_47 | IFNW1 | 3467 | 6656 | -0.032750066 | 0.18003917 | No |
| row_48 | CD40 | 958 | 6871 | -0.042174213 | 0.16348283 | No |
| row_49 | LEPR | 3953 | 6890 | -0.043082114 | 0.16390562 | No |
| row_50 | KITLG | 4254 | 7089 | -0.051394176 | 0.14915529 | No |
| row_51 | TGFBR2 | 7048 | 7468 | -0.069363609 | 0.1196753 | No |
| row_52 | BMPR2 | 659 | 7605 | -0.074760735 | 0.11135568 | No |
| row_53 | IL9R | 3581 | 7687 | -0.078446828 | 0.10795818 | No |
| row_54 | IL11RA | 3590 | 7800 | -0.08328530 | 0.10210398 | No |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | 9 | | |
| row_55 | TNFSF9 | 8744 | 8270 | -0.109697849 | 0.066612266 | No |
| row_56 | EDA2R | 60401 | 8322 | -0.112534843 | 0.0673725 | No |
| row_57 | MET | 4233 | 8388 | -0.116287924 | 0.06709525 | No |
| row_58 | PDGFRB | 5159 | 8455 | -0.12006937 | 0.06690522 | No |
| row_59 | KIT | 3815 | 8507 | -0.122138329 | 0.06810643 | No |
| row_60 | CXCL12 | 6387 | 8984 | -0.150400445 | 0.033878807 | No |
| row_61 | IL10RB | 3588 | 8995 | -0.150759012 | 0.03993727 | No |
| row_62 | IL25 | 64806 | 9004 | -0.151360288 | 0.046196166 | No |
| row_63 | CCL25 | 6370 | 9070 | -0.155226558 | 0.047706917 | No |
| row_64 | CLCF1 | 23529 | 9157 | -0.161198556 | 0.047677167 | No |
| row_65 | FAS | 355 | 9181 | -0.162685946 | 0.053159893 | No |
| row_66 | ACVR2A | 92 | 9190 | -0.163192108 | 0.05996209 | No |
| row_67 | TNFRSF19 | 55504 | 9228 | -0.166247696 | 0.06439855 | No |
| row_68 | TNFSF12 | 8742 | 9245 | -0.16746214 | 0.070705496 | No |
| row_69 | CCL3L1 | 6349 | 9473 | -0.183385059 | 0.05950993 | No |
| row_70 | TNFRSF1A | 7132 | 9890 | -0.214186564 | 0.033396192 | No |

| | | | | | | |
|---|---|---|---|---|---|---|
| row_71 | CCL26 | 10344 | 10555 | -0.278212786 | -0.011208601 | No |
| row_72 | TNFRSF11B | 4982 | 10654 | -0.29259932 | -0.006241624 | No |
| row_73 | TNFRSF6B | 8771 | 10929 | -0.331698775 | -0.014688387 | No |
| row_74 | PDGFRA | 5156 | 11131 | -0.372050077 | -0.014973951 | No |
| row_75 | TSLP | 85480 | 11155 | -0.376924068 | 3.46E-04 | No |
| row_76 | PPBP | 5473 | 11338 | -0.42049861 | 0.003927256 | No |
| row_77 | PLEKHO2 | 80301 | 11493 | -0.49788022 | 0.013481139 | No |

**Supplemental Table 4: "Kegg_Cytokine_Cytokine Receptor_Interaction" data set**. Another gene set of the Molecular Signatures Database that was significantly enriched in co-cultured samples (q value < 0.1). More information is available at http://www.broadinstitute.org/gsea/msigdb/search.jsp

## Table S5: Clinical data for leukemia patient samples

| Patient # | Source | Dx | % Blast | Status | Cytogenetics |
|---|---|---|---|---|---|
| 1 | PB | ALL | 90% | new dx | Diploid |
| 2 | PB | ALL | 87% | new dx | Hyperdiploid clone 49,XY,t(6;14;12)(q27;q31;p11.2),+8,+13,+18[3] |
| 3 | PB | ALL | 74% | relapse/refractory | Hyperdiploid metaphases 52~54,inv(1)(p36.2q31),+4,+6,add(7)(p21),+9,-10,+14,+17,+21,+1~4mar[cp10] |
| 4 | PB | ALL | 57% | new dx | Pseudodiploid |
| 5 | PB | ALL | 42% | new dx | Diploid |
| 6 | PB | AML | 79% | new dx | Pseudodiploid clone 46,XY,+13,17p+,-20[10] |
| 7 | PB | AML | 78% | relapse/refractory | Hypodiploid |
| 8 | PB | AML | 98% | relapse | Pseudodiploid clone 46,XX,der(16)del(16)(q22q22)inv(16)(p13q22),t(16;21)(p11.2;q22) |
| 9 | BM | AML | 51% | relapse/refractory | Pseudodiploid clone 46,XX,t(11;19)(q12;q13.3),add(12)(p13)[12] |
| 10 | PB | AML | 96% | primary refractory | Pseudodiploid clone 46,XY,t(9;11)(p22;q23) |
| 11 | PB | AML | 24% | new dx | Hypodiploid clones 45,XY,-7 |
| 12 | PB | AML | 67% | relapse | Hyperdiploid clone 47,XX,+8[20] |
| 13 | PB | AML | 67% | relapse | not data |
| 14 | PB | AML | 42% | relapse | not data |
| 15 | PB | AML | 82% | new dx | not data |

**Supplemental Table 5: ALL and AML Patients Clinical Data.** PB: peripheral blood. BM: bone marrow. Dx: Diagnosis. New dx: newly diagnosed.

## Table S6: Clinical data for AML-BM–MSC patient samples

| Patient # | source | %Blast | Cytogenetics | status | FAB | age | gender |
|---|---|---|---|---|---|---|---|
| 1 | BM | 45% | 47,XX,t(9;11)(p22;q23),+der(9)t(9;11)[17] | w/o maturation | AML-M1 | 21 | F |
| 2 | BM | 85 | Diploid female karyotype 46,XX[20] | w maturation | AML-M2 | 25 | F |
| 3 | BM | 93 | Diploid male karyotype 46,XY[20] | N/A | AML-M1 | 64 | M |
| 4 | BM | 58% | Pseudodiploid clone 46,XX,del(5)(q13q34)[20] | N/A | AML w/ MULTILINEAGE DYSPLASIA | 70 | F |
| 5 | BM | 61% | 48,XY,-7,+8,der(10)t(10;13)(p13;q14),+11,+15[19] | N/A | AML-M5a | 73 | M |
| 6 | BM | 63% | 46,XX,inv(3)(q21q26)[18] | N/A | N/A | 75 | F |
| 7 | BM | 95% | Diploid female karyotype 46,XX | N/A | AML-M4 | 63 | F |
| 8 | BM | 86% | Diploid female karyotype 46,XX | w/ mamturation | AML-M2 | 21 | F |
| 9 | BM | 35% | Diploid male karyotype 46,XY[19] | persistent w/multiple dysplacia | N/A | 80 | M |
| 10 | BM | 73% | Diploid male karyotype 46,XY | N/A | RAEB-T | 62 | M |
| 11 | BM | 55% | Pseudodiploid clones 46,XY,del(5)(q15q33),add(12)(p11.2) | N/A | AML-M4 | 49 | M |
| 12 | BM | 70% | Pseudodiploid clone 46,XX,t(8;21)(q22;q22)[10] | persistent/relapse | N/A | 57 | F |

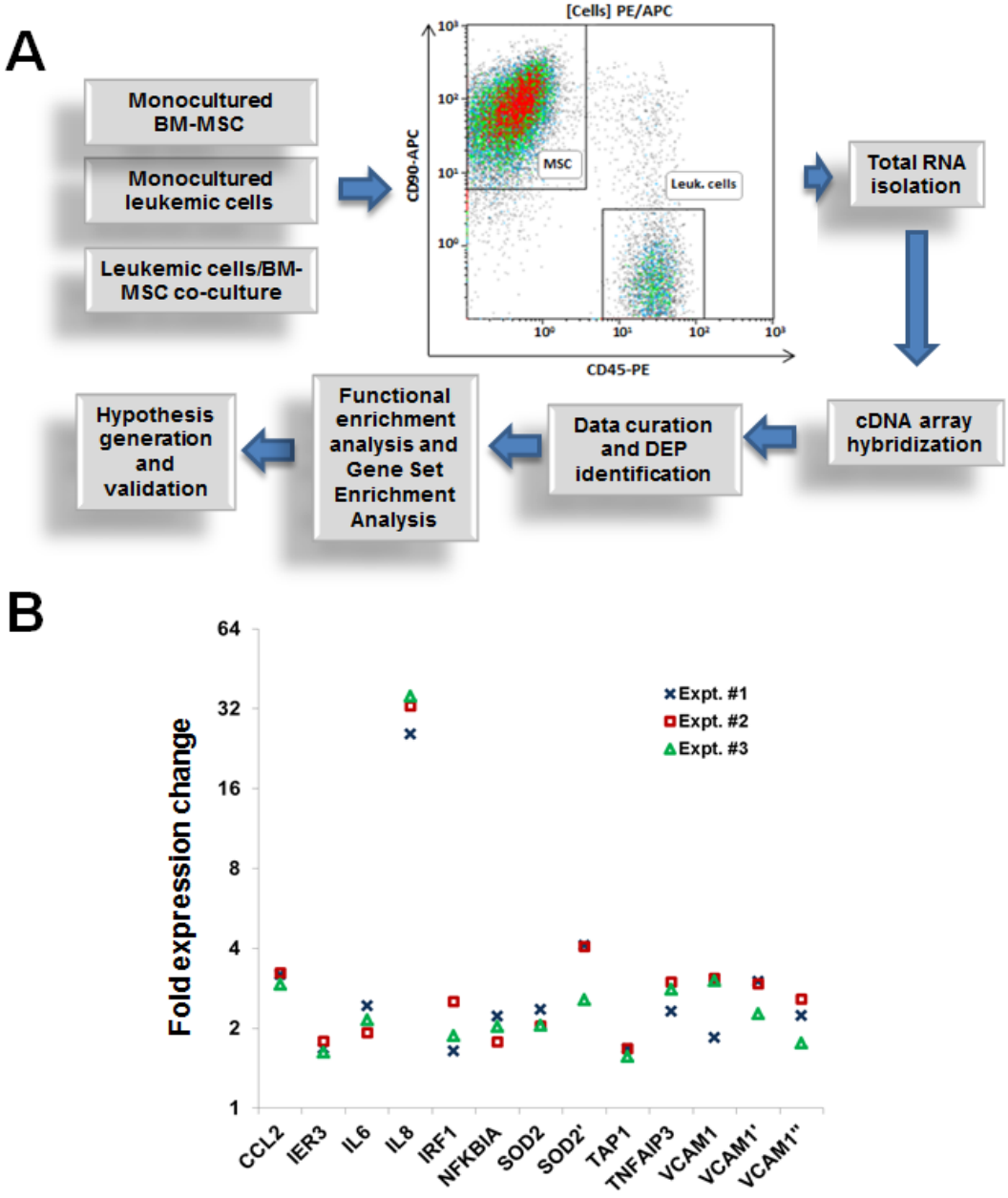
**Supplemental Table 6. Clinical data for AML-BM□MSC patient samples (n = 12).** FAB: French-American-British (FAB) classification system.

## Table S7: Primers used for SYBR Green qRT-PCR validation

| Gene name | Primer designation | lenght | sequence |
|---|---|---|---|
| VCAM-1 | VCAM-1-SyBR-FW | 24 | GAG GGG ACC ACA TCT ACG CTG ACA |
| | VCAM-1-SyBR-rev | 21 | ATC GGC TTC CCA GCC TCC AGA |
| TNFAIP3 | TNFAIP3-SyBR-FW | 20 | CCG GCT GCC CCT TCA CAC TG |
| | TNFAIP3-SyBR-rev | 20 | TCC TGG AGG CAG GCT TGG CA |
| IL8 | IL8-SyBR-FW | 23 | TGC AGC TCT GTG TGA AGG TGC AG |
| | IL8-SyBR-Rev | 21 | TGT GTT GGC GCA GTG TGG TCC |
| IL6 | IL6-SyBR-FW | 20 | TCC ACA AGC GCC TTC GGT CC |
| | IL6-SyBR-Rev | 21 | TGT CTG TGT GGG GCG GCT ACA |
| CCL2 | CCL2-SyBR-FW | 21 | TCG CAC TCT CGC CTC CAG CAT |
| | CCL2-SyBR-Rev | 22 | ACA GCA GGT GAC TGG GGC ATT G |
| IL-1β | IL1B-Sybr-rev | 21 | TTT TTG CTG TGA GTC CCG GAG |
| | IL1B-Sybr-FW | 22 | TTC GAC ACA TGG GAT AAC GAG G |
| IL-1α | IL1A-Sybr-Rev | 22 | ACT TTG ATT GAG GGC GTC ATT C |
| | IL1A-Sybr-FW | 21 | TGG TAG TAG CAA CCA ACG GGA |

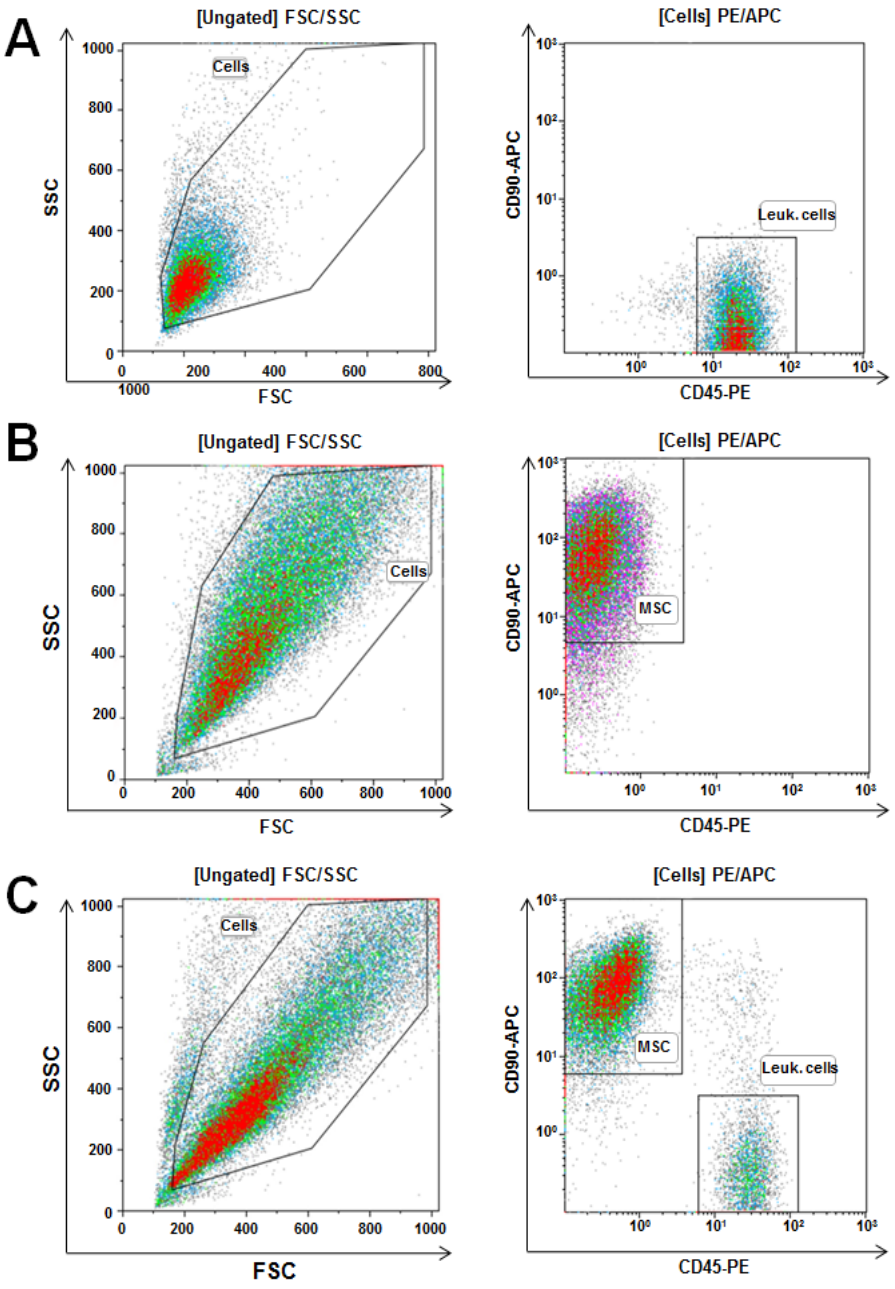
**Supplemental Table 7. List of primers used for qRT-PCR validation of NF-κB target genes.** Sets of primers were designed with Primer-Blast (NCBI) and specificity verified by PCR.

**Figure S1**



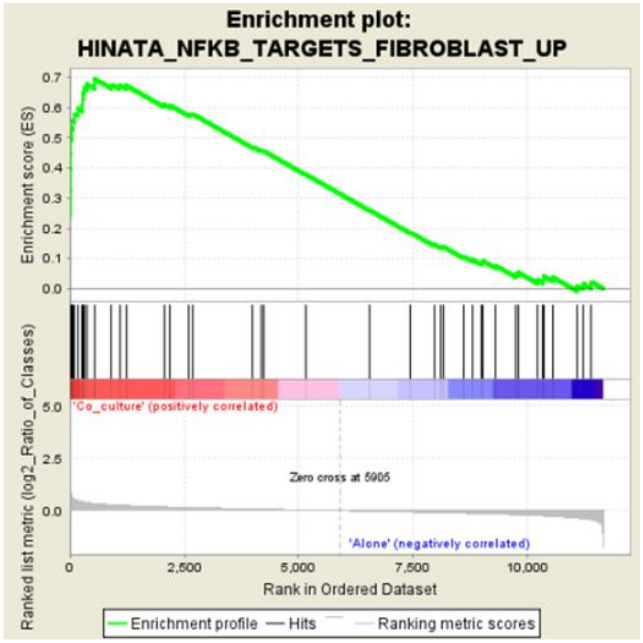

**Supplemental Figure 1. Gene expression changes in BM-MSC induced by co-culture with REH cells. A**, Experimental workflow of leukemia−BM-MSC co-culture experiments. Cells were co-cultured and separated by FACS based on their positive staining for CD90 (Thy-1) (BM-MSC marker) and CD45 (leukemia cells marker). Total RNA from either monocultured or co-cultured CD90 positive BM-MSC was amplified and hybridized to Illumina HT12 version 4 human whole-genome arrays. Data were processed and analyzed as described in Methods and differentially-expressed probes (DEP) were clustered and subjected to gene set enrichment analysis (GSEA). **B**, cDNA array fold expression change of a selected group of NF-κB transcriptional targets in co-cultured BM-MSC compared to the monocultured BM-MSC from three independent co-culture experiments.

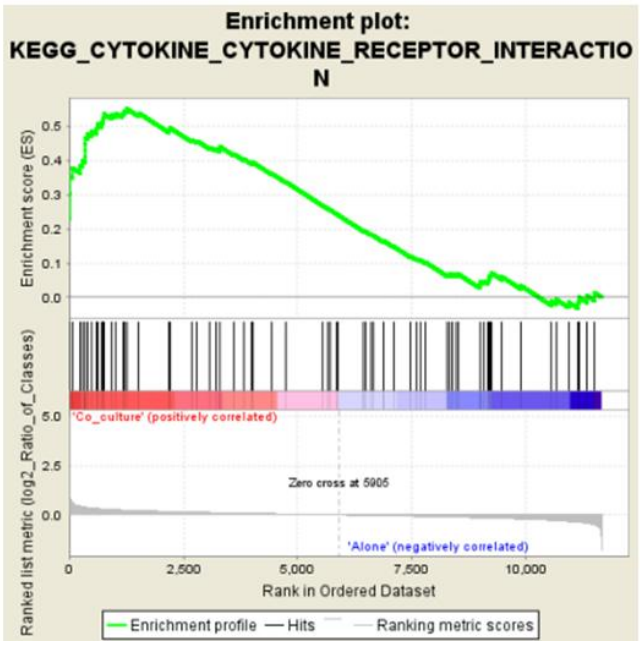
Figure S2




**Supplemental Figure 2. Representative example of Fluorescence Activated Cell Sorting (FACS) profiles of co-cultured BM-MSC/leukemic cells used for FACS-sorting of purified populations.** Monocultured leukemic cells (**A**) and BM-MSC (**B**) or co-cultures of leukemic cells and BM-MSC (**C**) were stained with Phycoerythrin (PE)-conjugated anti-human CD45 antibody (leukemic cells marker) and Allophycocyanin (APC)-conjugated anti-human CD90 (Thy-1) antibody (BM-MSC marker) and separated as indicated in Supplemental Methods.

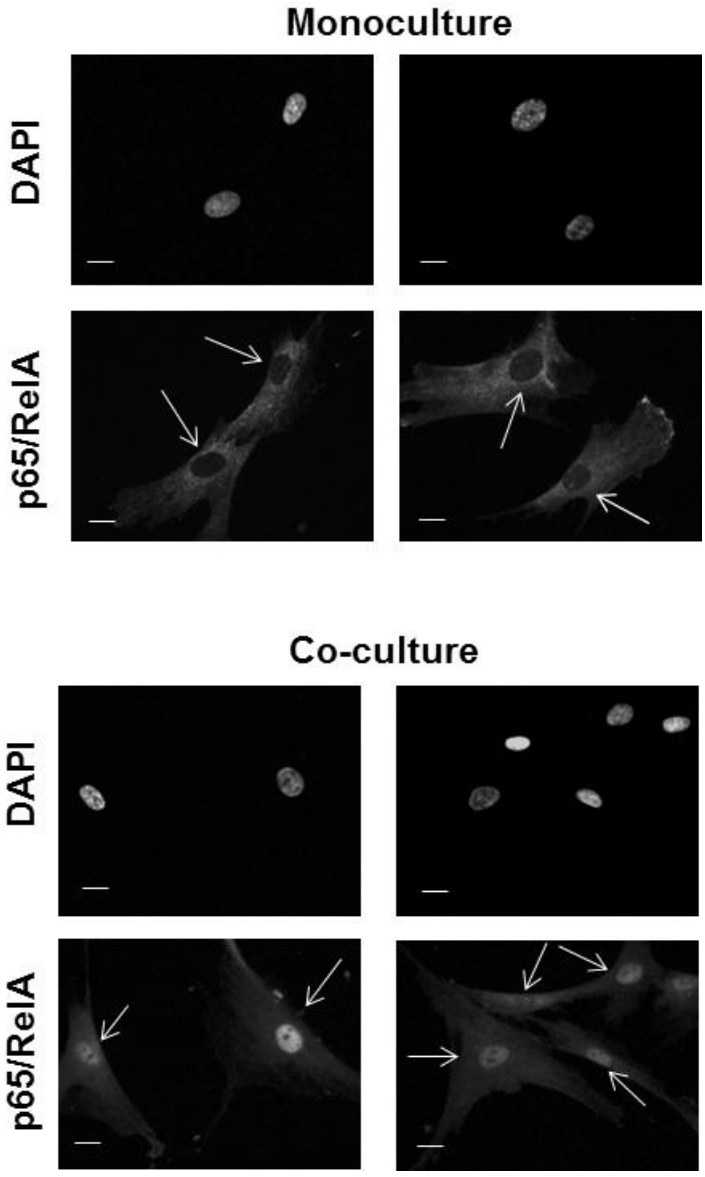
## Figure S3



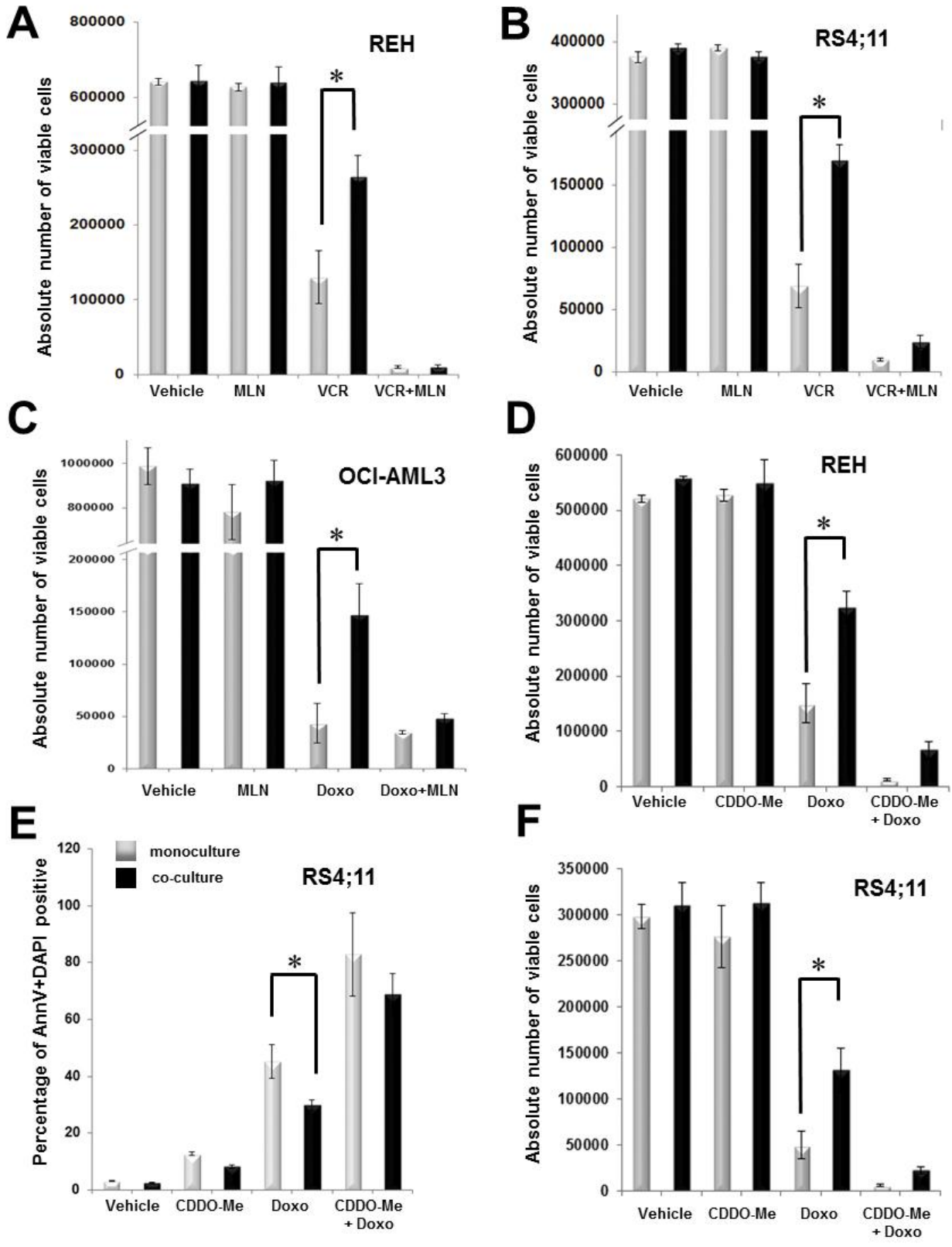

**Supplemental Figure 3. Examples of gene enrichment plots corresponding to BM-MSC in co-culture conditions.** The list of genes corresponding to each enrichment plot can be found in Supplemental Tables 3 and 4 respectively.

# Figure S4



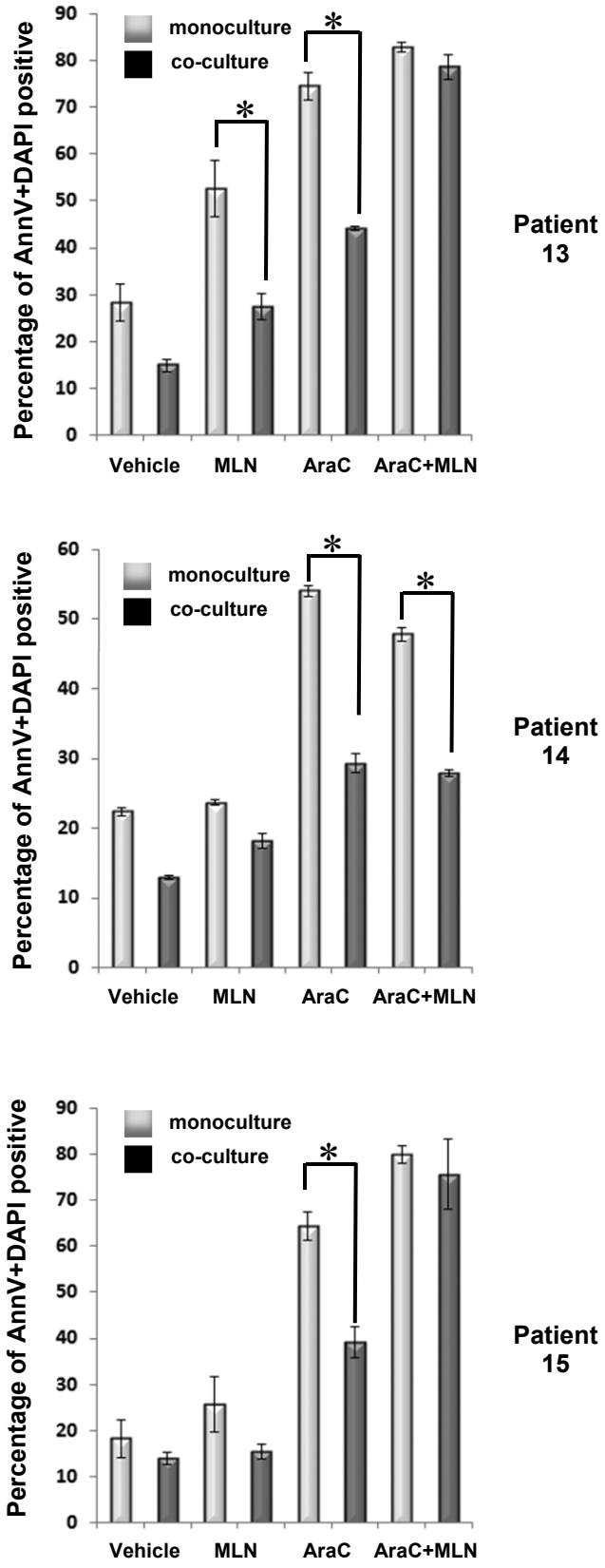

**Supplemental Figure 4. Co-culture with OCI-AML3 cells induces p65 (NF-κB) activation and nuclear translocation in BM-MSC**. BM-MSC were cultured alone (monoculture) or co-cultured with OCI-AML3 cells for 24 hours and then fixed with 4% PFA. Immunofluorescence staining for p65/RelA shows p65 translocation into BM-MSC nuclei upon interaction with OCI-AML3 cells in co-culture conditions. Nuclei were counterstained with DAPI. Scale bar: 10 µm. Arrows point at absence (monoculture panel) or presence (co-culture panel) of p65 in nuclei of BM-MSC.

# Figure S5


A
REH
Absolute number of viable cells
800000
600000
300000
200000
100000
0
*
Vehicle
MLN
VCR
VCR+MLN
B
RS4;11
Absolute number of viable cells
400000
300000
150000
100000
50000
0
*
Vehicle
MLN
VCR
VCR+MLN
C
OCI-AML3
Absolute number of viable cells
1000000
800000
200000
150000
100000
50000
0
*
Vehicle
MLN
Doxo
Doxo+MLN
D
REH
Absolute number of viable cells
600000
500000
400000
300000
200000
100000
0
*
Vehicle
CDDO-Me
Doxo
CDDO-Me + Doxo
E
monoculture
co-culture
RS4;11
Percentage of AnnV+DAPI positive
120
100
80
60
40
20
0
*
Vehicle
CDDO-Me
Doxo
CDDO-Me + Doxo
F
RS4;11
Absolute number of viable cells
350000
300000
250000
200000
150000
100000
50000
0
*
Vehicle
CDDO-Me
Doxo
CDDO-Me + Doxo

**Supplemental Figure 5**. **Blockade of NF-κB activation decreases viability in leukemic cells when combined with standard chemotherapy**. REH (**A** and **D**), RS4;11 (**B**, **E** and **F**) and OCI-AML3 (**C**) cells were cultured alone (monoculture) or co-cultured with BM-MSC as indicated in Methods. Monocultured and co-cultured cells were treated for 72 hours with either vincristine (VCR, **A** and **B**) or doxorubicin (Doxo, **C, D, E** and **F**) as monotherapy or in combination with one of the IKKβ inhibitors MLN120B (MLN, **A**, **B** and **C**) or CDDO-Me (**D, E** and **F**). The absolute number of viable cells (**A**, **B**, **C**, **D** and **F**) was and calculated by subtracting the absolute number of apoptotic cells (annexin$V^+$/$DAPI^+$) to the total number of cells determined by flow cytometry using annexin $V^+$/$DAPI^+$ staining and counting beads. The percentage of apoptotic cells (annexin$V^+$/$DAPI^+$) (**E**) was assessed by flow cytometry using annexin $V^+$/$DAPI^+$ staining and counting beads. Results are expressed as the mean of the absolute number of viable cells (± SEM) (**A**, **B**, **C**, **D** and **F**) and the mean of the percentage of annexin $V^+$/$DAPI^+$ (± SEM) (**E**) of three independent experiments. The symbol (*) indicates a statistically significant difference at $P \leq 0.05$. AnnV: annexinV.

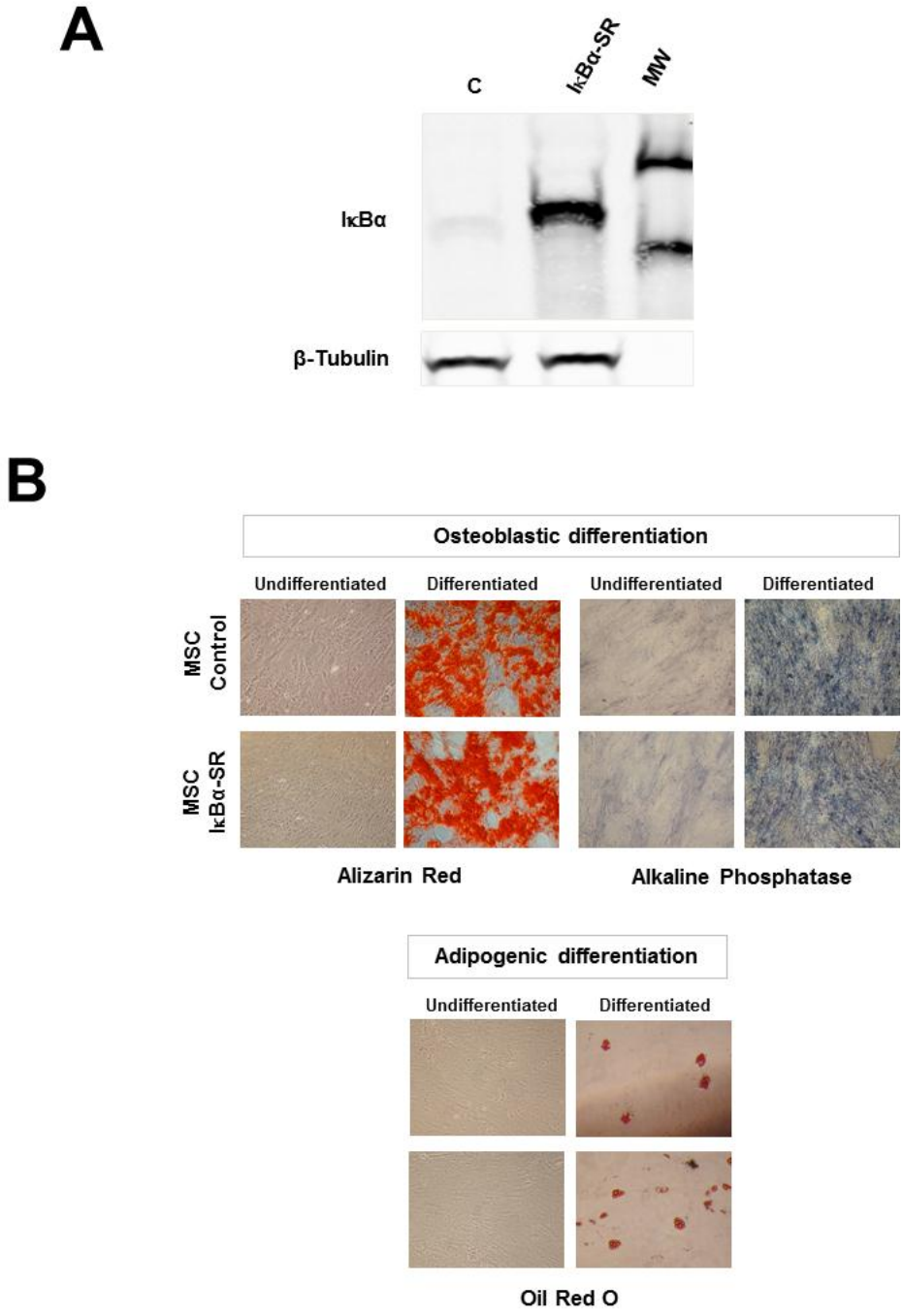
# Figure S6




**Supplemental Figure 6**. **Effect of NF-κB inhibition in combination with standard chemotherapy on primary leukemia samples**. Three AML primary samples were cultured alone (monoculture) or co-cultured with BM-MSC as indicated in Methods. Monocultured and co-cultured cells were treated for 48 hours with AraC (1 μmol/L) as monotherapy or in combination with MLN120B (MLN). The percentage

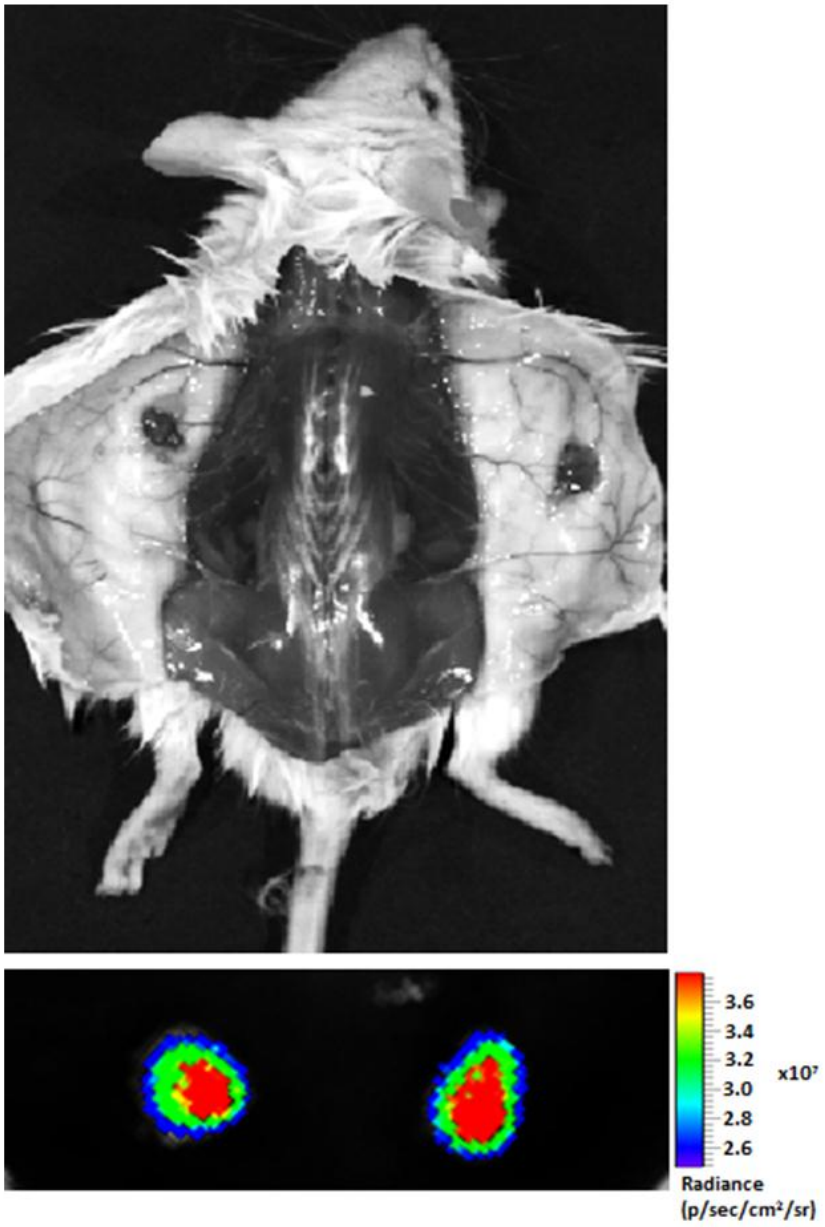
of apoptotic cells (annexin$V^+$/$DAPI^+$) was assessed by flow cytometry using annexin $V^+$/$DAPI^+$ staining and counting beads. Results are expressed as the mean of the percentage of annexin $V^+$/$DAPI^+$ (± SEM) of three independent experimental replicas. The symbol (*) indicates a statistically significant difference at $P \leq 0.05$. AnnV: annexinV.

Figure S7



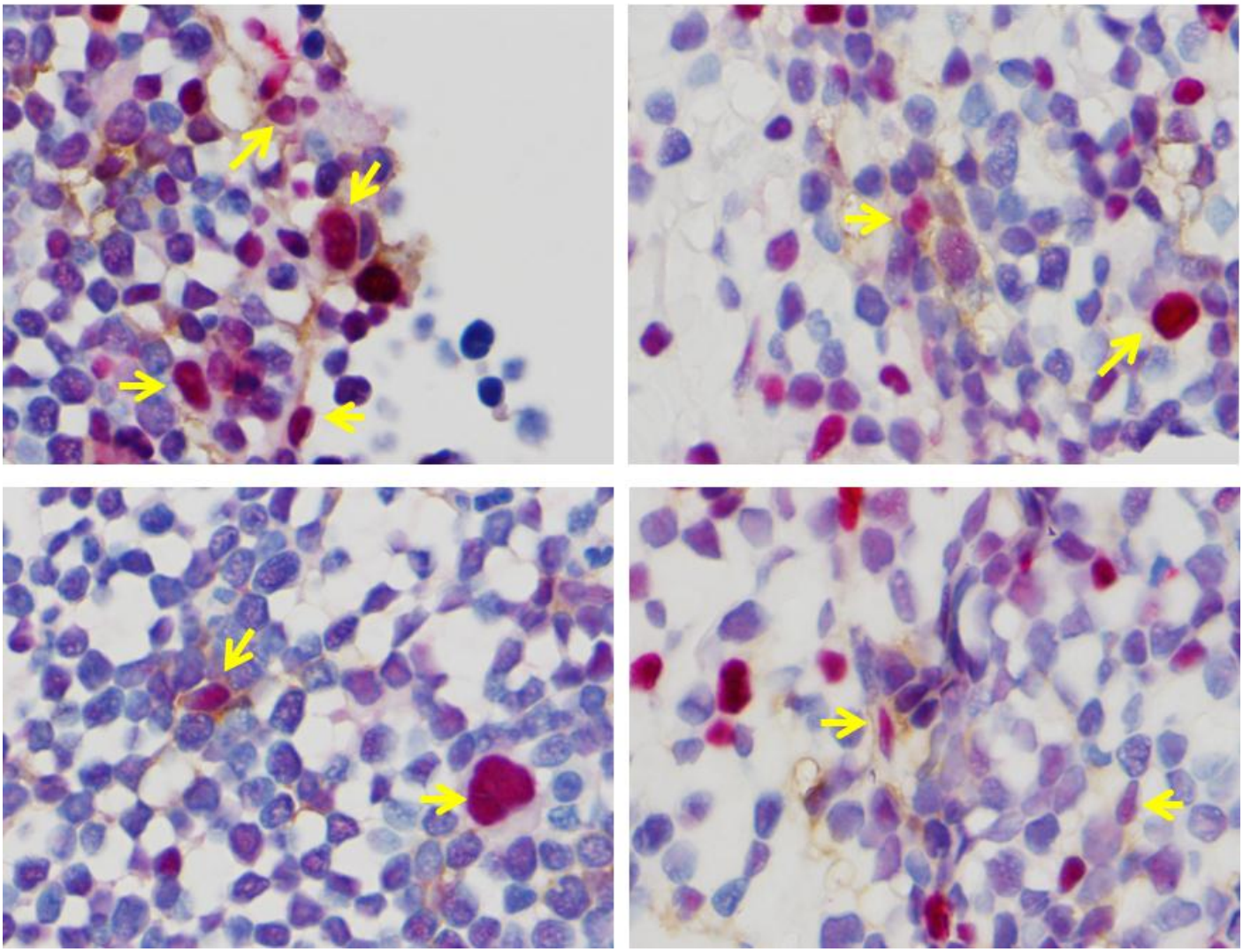

**Supplemental Figure 7**. **Characterization of BM-MSC expressing IκBα-SR**. **(A)** Western blot analysis of lysates from BM-MSC stably transduced with empty control or IκBα-SR lentivirus. β-tubulin was used as loading control. MW: Molecular weight marker. **B,** Osteoblastic and adipogenic differentiation in MSC-Control and MSC−IκBα-SR**.** The calcium deposits, indicative of osteocytes, were detected with alizarin red and alkaline phosphatase. Accumulation of lipids droplets was detected with oil red O. Photographs are representative of three independent wells.

## Figure S8



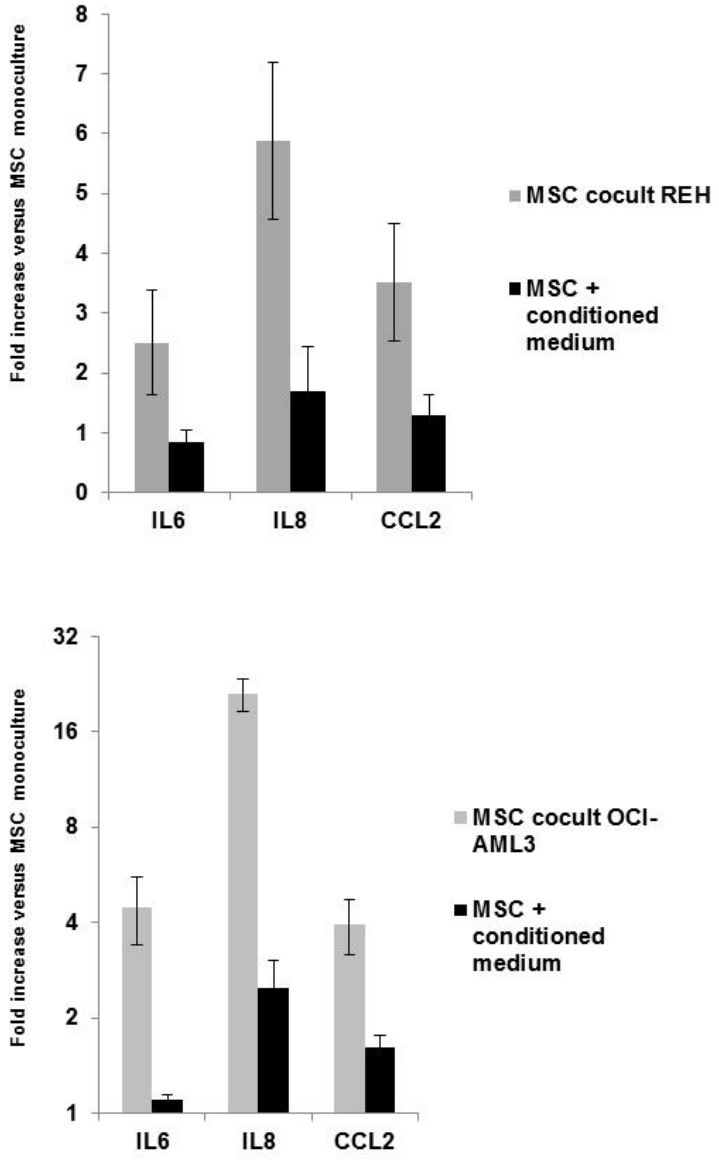

**Supplemental Figure 8. Imaging and measurement of osteoblastic activity in fully developed extramedullary bones.** Extramedullary bones with similar morphology were developed from control BM-MSC and IκBα-SR−transduced BM-MSC (upper panel; left and right, respectively). Osteoblastic activity in extramedullary bones was measured by injecting mice with OsteoSense 750 (lower panel)**.** Approximately 24 h after OsteoSense 750 injection, mice were sacrificed and extramedullary bones were removed and scanned with a VisEn FMT 2500 imaging system to detect the emerging fluorescence signal. Average radiance is expressed as photons per second per centimeter squared per steradian (p/s/cm2/sr).

# Figure S9



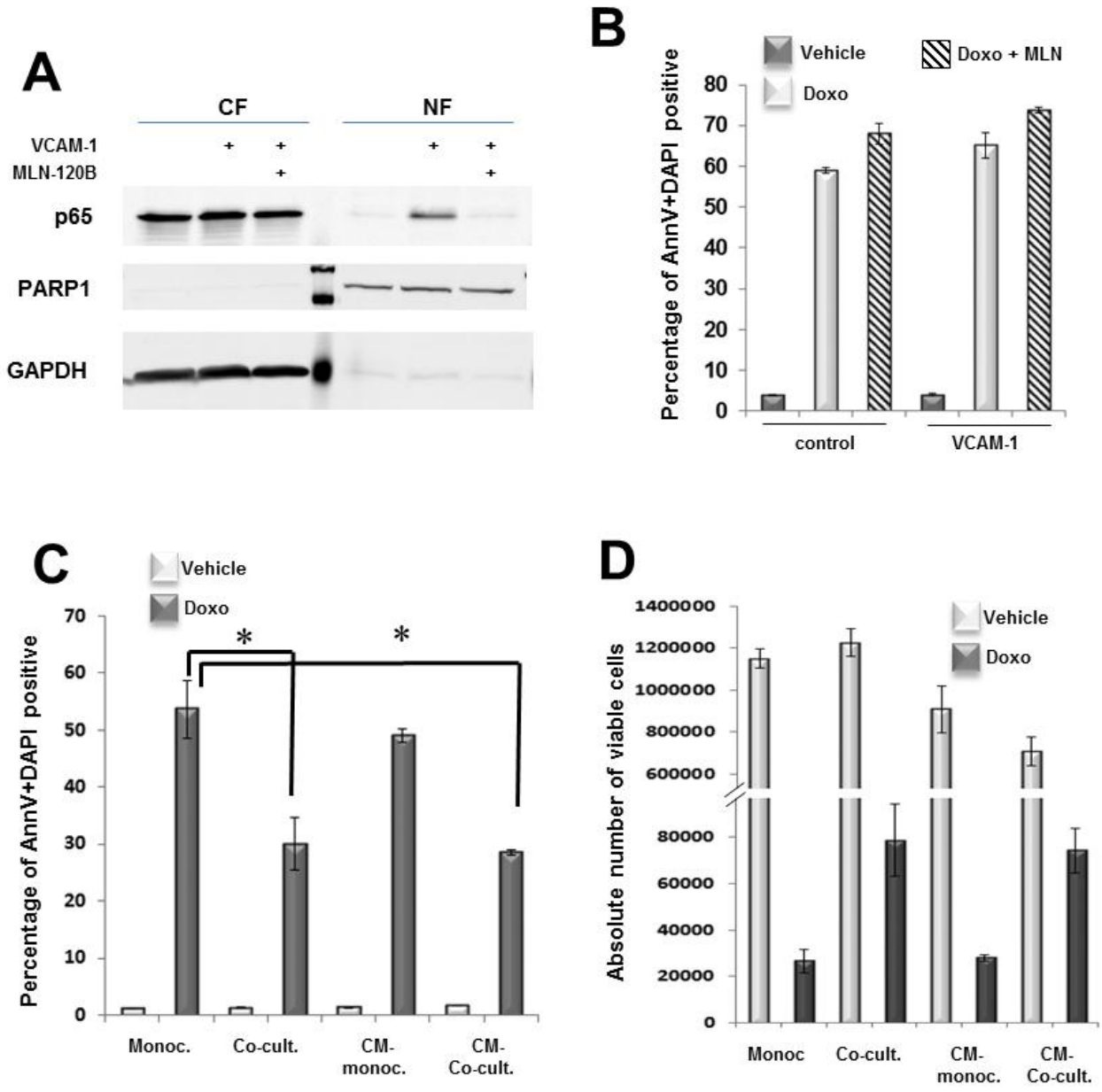
**Supplemental Figure 9. BM biopsy from a consented ALL patient.** The patient was a 33 year old Hispanic female, who presented to our institution on 01/03/2007 and was diagnosed with PH-negative, CD20-negative B lymphoblastic leukemia/lymphoma. The patient did not receive any therapy before the admission. The immunohistochemical studies were performed on diagnostic bone marrow biopsy core. Subsequently, the patient received hyper-CVAD therapy and achieved complete remission. The patient remained in complete remission at her last follow-up visit on 9/12/2013. Arrows indicate CD90-positive (brown staining) BM stroma cells showing nuclear localization of phospho-NF-κB p65 (Ser276) (red staining). Nuclear localization of phospho-NF-κB p65 is also observed in some CD90-negative ALL blasts.

# Figure S10




**Supplemental Figure 10. Direct leukemia–BM-MSC contact is needed to fully activate NF-κB transcriptional activity in BM-MSC.** BM-MSC were cultured alone (monoculture) or co-cultured with REH (upper panel) or OCI-AML3 (lower panel) cells for 24 hours. A second set of BM-MSC was incubated with the corresponding conditioned medium from leukemia cells that were previously cultured alone for 24 hours (i.e., the BM-MSC plus the conditioned medium). After separating leukemia cells from BM-MSC as indicated in Methods, total RNA from BM-MSC was extracted and qRT-PCR was carried out to detect the expression of a selected group of NF-κB target genes. Results of three independent experiments are expressed as the mean fold increase (± SEM) in different culture conditions over the expression levels in the monocultured BM-MSC in basal (not-conditioned) medium.

# Figure S11




**Supplemental Figure 11. The interaction between leukemic cells and BM-MSC is required to trigger the stroma-mediated chemoresistance. (A)** Western Blot analysis of cytosolic (CF) and nuclear (NF) fractions of lysates from OCI-AML3 cells cultured for 1 hour on VCAM-1 coated-dishes (VCAM-1 +) or regular culture plates with (+) or without MLN-120B. **(B)** OCI-AML3 cells were cultured on VCAM-1 coated-dishes (VCAM-1) or regular culture plates (control). After 24 hours, cells were treated with Doxo alone or in combination with MLN-120B for another 72 hours. **(C)** OCI-AML3 cells cultured alone (monoculture) or with BM-MSC (co-culture) in control medium. Conditioned mediums from 48 hours cultures of BM-MSC alone (CM-monoculture) or BM-MSC/OCI-AML3 co-cultures (CM-coculture) were clarified, filtered and used to culture two more sets of OCI-AML3 monocultures. Cells

were treated for 72 hours with control vehicle or Doxo. The percentage of apoptotic cells (annexinV+/DAPI+) and the absolute number of viable cells was assessed by flow cytometry as previously mentioned. Results are expressed as the mean of the percentage of annexin V+/DAPI+ (± SEM) **(B** and **C)** or mean absolute number of cells (± SEM) **(D)** of three independent replicas. The symbol (*) indicates a statistically significant difference at $P \leq 0.05$. AnnV: annexinV.