Supplemental Methods

Patient and control samples

Peripheral blood samples were collected from pediatric patients with histologically confirmed, newly diagnosed AML treated at Texas Children's Hospital, Houston TX between 2009 and 2013. Informed consent was obtained from patients or their guardian in accordance with the US National Cancer Institute (NCI), in accordance with the Declaration of Helsinki, and following all institutional policies before entry onto this study. Samples were collected in Cell-Save preservation tubes from patients with an absolute blast count of at least 1000 blasts/μL. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood using sucrose centrifugation. Magnetic bead separation (Miltenyi Biotech, Germany) was used to enrich for AML cells using Anti-CD3 and anti-CD19 depletion. Tumor cells were frozen as pellets for immunoblotting and lysates made for immunoblot analyses as previously described (30). Negative controls were obtained from peripheral blood collected from healthy siblings of bone marrow transplant recipients and from healthy adult volunteers. Patients were treated using either standard therapy (cytarabine-daunorubicin-etoposide) or protocol-specific drugs as noted in Supplemental Table 1.

Protein detection

Capillary electrophoresis immunoblotting was performed using ProteinSimple WES (ProteinSimple, San Jose, CA, USA). Minimum concentration of samples loaded for detection was optimized at 1mg/ml. Antibodies were used at the following concentrations:

Grp78 (BD Pharmingen, Franklin Lakes, NJ, USA; 610978; 1:500), Actin (Sigma Aldrich, St. Louis, MO, USA; A5441; 1:250), phospho-eIF2α (Cell Signaling 9721; 1:50), total eIF (Cell Signaling, Danvers, MA, USA; 9722; 1:50) and IRE1 (Cell Signaling 3294; 1:50). UPR protein expression was quantified using the area under the curve (AUC) for the peak on the electropherogram for the protein of interest (ProteinSimple Compass software v2.7). The AUC for the UPR protein was normalized to the AUC for endogenous control actin for the same patient sample. UPR induction post chemotherapy was defined as more than 2-fold increase from baseline expression of the UPR protein.

RNA isolation and qRT-PCR-

MSCs, OPCs, and KSL cells were sorted directly into 350μL of Buffer RLT (Qiagen, Germany). RNA was extracted using the manufacturers protocol from the RNeasy kit (Qiagen) and quantified using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). RNA was converted into complementary DNA using the SuperScript III First-Strand Synthesis kit (Invitrogen) with oligo(dT) priming, followed by RT-PCR analysis using TaqMan. Sequences of the primers used are listed in **Supplemental Table 3**. Relative quantification was calculated using the ΔΔCT algorithm with *Gapdh*/*GAPDH* as the endogenous control when appropriate.

Cell culture and EV preparation

Molm-14, HL60, and U937 cells were obtained from the laboratory of J. Tyner and were cultured in RPMI (Invitrogen/Gibco, Waltham, MA, USA) with 10% fetal bovine serum (FBS) (Gemini, West Sacramento, CA, USA) and 1X penicillin/streptomycin (Invitrogen/Gibco) at 37°C, 5% CO_2, and >95% humidity. Cell lines are routinely checked for *Mycoplasma* contamination using the MycoAlert Plus assay (Lonza, Switzerland). For

imaging experiments, AML cells were transduced with lentivirus harboring the mGFP transgene (Addgene Plasmid #17481) and then purified using flow cytometry. Human $CD34^+$ bone marrow progenitors were purchased from ATCC and cultured in X-Vivo medium (Lonza) supplemented with BIT 9500 Serum Substitute (STEMCELL Technologies, Vancouver, BC, Canada), FMS-like tyrosine kinase 3 ligand (50 ng/ml), G-CSF (10 ng/ml), IL-3 (10 ng/ml), IL-6 (10 ng/ml), stem cell factor (SCF) (50 ng/ml), and thrombopoietin (25 ng/ml) (Peprotech, Rocky Hill, NJ, USA). EVs were isolated by differential centrifugation as described previously (30). Briefly, cells were cultured for 48 hours, the culture medium was spun at 300*g* for 10 min to remove cells, and then the supernatant was spun at 2000*g* for 20 min and at 10,000*g* for 20 min to remove cellular debris. The supernatant was centrifuged at 100,000*g* for 2 hours. EVs were either resuspended in phosphate-buffered saline (PBS) or media, depending on the experiment.

Live cell microscopy of cells sorted from Xenografts-

MSCs and OPCs were sorted into 2 ml Eppendorf tubes containing 1 ml of MSC media (as described above). Freshly sorted cells were centrifuged at 1000xg for 10 minutes at 4C and resuspended in fresh phenol-free media, before being replated onto Matrigel (growth factor reduced/phenol free) diluted 1:9 in 35mm live cell culture chambers with 4 well inserts and #1.5 polymer coverslip bottom (Ibidi, Germany). Cells were then incubated at 37°C 5% CO2 for 45 minutes to allow for attachment to Matrigel coated chamber. Cells were then stained with Hoechst (250ng/ml; ThermoFisher) and Cellmask Deep Red (1ul/ml; ThermoFisher) for 15 minutes at 37°C and gently washed twice with warmed

phosphate buffered saline (1X) before adding fresh media. Cells were then imaged using a Deltavision CoreDV/Olympus IX71 microscope, equipped with 60X Plan Apo N 1.49 objective, 7-color solid state LED illumination, motorized stage, Nikon Coolpix HQ CCD camera, and live cell chamber supplying 37°C/5% CO_2. 3D Z-stacks were acquired in 3 channels using a 200nm Z-step through the entire cell volume to maximize capture speed while maintaining adequate axial resolution. Exposure times and laser intensity were held constant between conditions. To identify non-specific background for the 488 channel (GFP), z-stacks of MSCs and OPCs from non-xenografted NSG mice were captured to determine thresholding value. Images were deconvolved using SoftworX and analyzed using Imaris Bitplane.

Live cell microscopy in vitro UPR stress experiments-

FACS sorted MSCs and OPCs were expanded for one week in culture on Matrigel (1:11 dilution) coated Ibidi gridded culture slides with #1.5 coverglass. Cells were then treated with either 1ng/ml Thapsigargin, $1x10^8$ Molm-14 mGFP EVs or vehicle at 0 and again at 24 hours. At hour 36, cells were washed in 37°C phosphate buffered saline, stained with ER-Tracker (500nM in Hank's Balanced Salt Solution) for 1 hour, rinsed and stained with Cell Mask and Hoechst (as described above). Cells were then imaged under live cell conditions using a Nikon TiE microscope equipped with Yokogawa CSU-W1 Spinning Disk confocal, 100X Plan Apo TIRF 1.49 objective, 4-channel laser excitation, motorized stage, Nikon CCD camera, and live cell chamber supplying 37C/5% CO2. Z-stacks were acquired at 100nm Z-step using 50nm pinhole through the total volume of the cell with exposure times and laser power held constant across conditions. Images were analyzed using imaris bitplane. Cellmask staining demarcating the plasma membrane was used to determine the outer limit of the cells and determine mGFP foci internal to the plasma

membrane, while masking those found external to the cells. To measure mGFP foci and their association with the ER territory, Imaris functions *Spots* function was used to identify, count and differentially pseudo-color internal mGFP foci based on association with ER surface volume as identified using Surface function. If mGFP foci were found colocalized or directly touching the ER surface GFP+ foci were defined as ER-associated.

EV imaging and concentration determination-

For vesical imaging, EVs were mounted in solid capture by embedding Molm-14 mGFP EVs into a solid hydrogel to prevent Brownian motion. To embed vesicles, EVs were resuspended up to 100ul in phenol-free media, shaken over night at 4C, then added on ice to Matrigel in a 1:6 v/v ratio with pre-cooled pipet tips. The ice-cold EV/Matrigel mixture is vortexed and immediately pipetted with pre-cooled pipet tips into a well of an 96-well imaging plate with #1.5 bottom (Ibidi) and incubated at 37C for 4 hours. Following incubation, solid gels containing mGFP-labled EVs are imaged by using a Nikon Yokogawa CSU-W1 Spinning Disk confocal as described above, and counted using Imaris Bitplane. Concentrations are determined by acquiring 3D Z-stacks with a fixed volume (100µm x 100µm x 10µm = $1x10^{-7}$ ml) using 488 laser excitation. Images were then thresholded to remove non-specific background which was calculated by imagining embedded non-fluorescently labeled Molm-14 EVs. Individual mGFP foci within a fixed volume in 5 different fields were counted using Imaris Surfaces function. The average value of 5 fields was then used to compare concentration across conditions in fold-change. To validate resolution of sub-diffraction level structure size, 34nm, 100nm and 180nm reference beads (ThermoFisher) were utilized to determine detection limit and relative signal point-spread estimation. Using a 100X Plan Apo 1.49 TIRF objective and 50nm pinhole, signals from all three beads were detectable with consistent size and intensity

without observable aggregation, with a point-spread roughly 4-fold larger than the actual size of the respective reference bead. This ensured that detection of EVs in the nanoscale is possible using our microscopy system and approach.

CFU-F assay

One thousand MSCs and OPCs from NSG mice were sorted directly into 6-well plates containing MSC media (MEMα, 15% FBS, 1X penicillin/streptomycin) and cultured at 37°C, 5% CO_2, and >95% humidity for 14 days, replacing half of the media every three days. On day 15, cells were gently washed in PBS and then fixed in methanol. Cells were then incubated with Giemsa (EMD Millipore, Burlington, MA, USA) for one hour. Stain was removed and cells washed with water. CFU-Fs were counted and only colonies containing >50 cells were scored.

Osteo- and adipogenic differentiation assays

Sorted MSCs were allowed to reach confluency in culture (2-3 weeks) in MSC media prior to induction. For osteogenic differentiation, cells were cultured in MSC media containing 100 nmol/L dexamethasone, 10 mmol/L beta-glycerophosphate, and 0.05 mmol/L L-ascorbic acid-2-phosphate for 3 weeks. Cells were fixed in 4% PFA and calcium mineralization of osteoblasts was detected by alizarin red staining and staining intensity was quantified by colorimetric detection of re-extracted alizarin red at 405nm using a plate reader. For adipogenic differentiation, cells were incubated in MSC media containing 1µM dexamethasone, 1µM indomethacin, 500 µM 3-isobutyl-1-methylxantine (IBMX) and 10 µg/ml human recombinant insulin for 3 weeks. Cells were fixed in 4% PFA and lipid

vacuoles were detected using Oil red-O staining and staining intensity was quantified by colorimetric detection of re-extracted stain at 488nm using a plate reader.

ELISA

EVs were generated as previously described, and re-suspended in manufacturer's diluent solution containing 1% Triton-X. Supernatant (SN) from the EV prep was used to detect vesicle free BMP2. Experiment was conducted per manufacturer's instructions (AbCam, United Kingdom; BMP2 detection kit, ab119581). Samples and standards were read using a SyngeryH1 plate reader (BioTek, Winooski, VT, USA). BMP2 concentrations were generated using a standard curve.

Supplemental References

1. Horton TM, Pati D, Plon SE, Thompson PA, Bomgaars LR, Adamson PC, et al. A phase 1 study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. Clin Cancer Res. 2007;13(5):1516-22.