**Supplementary Methods**

*Cell viability assays:*

# For viability assays, cells were plated in tissue culture-treated multiwell plates (TPP, Trasadingen, Switzerland), left to adhere for 16-24h, treated with serial dilutions of docetaxel, and analyzed at indicated time point for viability by MTT as previously described (1). Optical density for 570 and 630 was read with either a SpectraMax M5e plate reader (Molecular Devices, Sunnyvale, CA) or a Synergy HT plate reader (BioTek/Agilent), and the OD570-630 of each technical replicate was divided by the average of untreated samples of the same line to obtain % viability. Where indicated, logIC50 was statistically determined using nonlinear fit by least squares regression (four parameter, variable slope) in Prism 8.

*Murine bone marrow stromal cell co-culture*

# For co-culture assays, murine primary bone marrow stromal cells (BMSCs) were harvested from pelvic or leg bones and cultured as previously described (2). Breast cancer cells (0.5-1x104/well) were parachuted onto confluent BMSC in 96-well tissue culture-treated plates (TPP) and left to adhere overnight. Co-cultures were then treated with serial dilution of docetaxel (0.8 nM to 1μM in media) and analyzed by MTT after 72h. BMSC alone showed no reduction of formazan fixation at the doses in use, so OD750-630 was normalized by cell line as described.

*Galuminox imaging of radical oxygen species*

Live cell fluorescence imaging studies were performed at Washington University Center for Cellular Imaging (WUCCI). For imaging studies, β3WT and β3KO 4T1 cells were plated onto borosilicate 8-well chambered coverglass (Labtek), allowed to grow to approximately 50% confluence at 37°C under 5% CO2 atmosphere in culture media (200μL), and treated with DTX (10nM) for 24h. Following DTX treatments, all wells were rinsed with fresh media. For evaluating impact of ROS, cells were incubated either with Galuminox (20μM), a mitochondrial ROS sensitive metalloprobe (3), or media alone at 37°C for 1h under continuous influx of 5% CO2. After 1h, cellular accumulation studies were performed with an inverted Nikon A1Rsi laser scanning confocal microscope using a 60x oil objective lens (Nikon Instruments Inc., NY, USA). 405 nm lasers were used for the detection of Galuminox. Throughout the data acquisition process, cells were maintained at 37 °C with 5% CO2, controlled by a Tokai Hit stage-top incubation system (Shizuoka, Japan). Acquisition was performed using Nikon NIS-Elements software (Nikon Instruments Inc., NY, USA.). Images were processed and analyzed using the ImageJ software package (NIH, Bethesda, Maryland; RRID:SCR\_003070). Regions of interest were manually drawn around cells, the uptake of Galuminox was quantified (wherein corrected total cellular fluorescence (CTCF) = integrated density–(area of selected cell × mean fluorescence of background readings)) using protocols described elsewhere (4, 5).

*Bioluminescence imaging and radiography*

*In vivo* bioluminescence imaging was performed on the days indicated using an IVIS Lumina (PerkinElmer, Waltham, MA; Living Image 4.2), 5min to 1sec exposure, bin2-8, FOV12.5cm, f/stop1, open filter). Mice were injected intraperitoneally with D-luciferin (150mg/kg in PBS; Gold Biotechnology, St. Louis, MO) and imaged using isoflurane anesthesia (2% vaporized in O2). Mice were euthanized immediately after *in vivo* confirmation of successful intraperitoneal administration of D-luciferin. Organs of interest were then dissected out and imaged separately. Total photon flux (photons/sec) was measured from fixed regions of interest (ROIs) using Living Image 2.6 (RRID:SCR\_014247). Investigators were blinded to treatment groups during BLI analyses.

Osteolytic lesions were imaged by X-Ray imaging system (Faxitron). Tibiofemoral lesion area was quantified using ImageJ (NIH, Bethesda, Maryland; RRID:SCR\_003070) with investigators blinded to treatment group.

*Drug preparation for in vivo studies*

Docetaxel (LC Laboratories) was initially solubilized in 100% ethanol and stored at -20°C. A 10mg/mL working solution was freshly prepared on the day of injection by dilution in a Tween 80/PBS solution (final Tween 80 : ethanol : PBS ratio of 20:13:67) to prevent precipitation. Finally, working solution was further diluted to 0.9-1mg/mL in PBS. Vehicle control was prepared and diluted in a similar manner using 100% ethanol without docetaxel.

Rapamycin (Sigma R0395) was solubilized in 100% ethanol, diluted to a 1mg/mL working solution in 5% PEG400, 5% Tween 80, then aliquoted and stored at -20°C. 100% ethanol without rapamycin was prepared and stored in a similar way for mice receiving vehicle control. Aliquots of working solution or equivalent vehicle were freshly thawed on the day of injection and administered 2mg/kg by i.p. injection. An equimolar equivalent of nanoparticle encapsulated rapamycin or cargo-free nanoparticle control was administered by tail vein injection for nanoparticle experiments.

*Bone sample preparation for transmission electron microscopy*

At Day 15 experimental endpoint, mice were euthanized and perfused with heparin-supplemented PBS, followed with a 2.5% glutaraldehyde, 2% paraformaldehyde fixative solution buffered at pH 7.4 by 0.15M cacodylate with 2mM CaCl2 (TEM fixative). Hindlimb bones were dissected out and submerged in TEM fixative overnight at 4°C. Samples were then decalcified in 14% M EDTA (pH 7.2) at 4°C for 14 days with mild agitation and periodic switch into fresh EDTA. Afterwards, samples were rinsed in cacodylate buffer 3 times for 10 minutes each, and subjected to a secondary fixation in 1% osmium tetroxide/1.5% potassium ferrocyanide in cacodylate buffer for one hour, rinsed in ultrapure water 4 times for 10 minutes each, and stained in an aqueous solution of 2% uranyl acetate for one hour. The samples were washed again in ultrapure water 4 times for 10 minutes each and dehydrated in a graded acetone series (10%, 20%, 30%, 50%, 70%, 90%, 100% x4) for 15 minutes in each step. Tissues were then infiltrated with microwave assistance (Pelco BioWave Pro, Redding, CA) into Spurr’s resin and cured in an oven at 60˚C for 80 hours.

*Flow cytometric analysis*

*In vitro* tumor cells were lifted with 1x Versene (Gibco: 15040066) unless otherwise indicated. For *ex vivo* analysis of murine bone metastases, hindlimb bones were dissected and the tibiofemoral joint isolated and finely crushed with surgical scissors. Manually processed samples were further digested in collagenase A (Roche) and DNase I (Sigma-Aldrich) at 37°C for 1 hour with agitation. Digested samples were strained through 70μm Falcon nylon filters (Corning: 352350) and prepared as single cell suspensions in 5% FBS PBS with 1mM EDTA (Corning). Cells were stained with either PE- or AlexaFluor-647-conjugated anti-mouse integrin β3 (1:200, clone: 2C9.G2, BD Pharmingen; PE RRID:AB\_394800; AF647 RRID:AB\_2738255), CD45.2, (1:200, clone: 104, BioLegend, RRID:AB\_492872) and DAPI (Sigma: D9542) and acquired on the LSRFortessa (BD Biosciences). FlowJo (TreeStar, RRID:SCR\_008520) was used for data analysis and representative flow plot generation.

Protein synthesis assays were performed by incubation of cells with the synthetic methionine analog Click-iT® HPG (L-homopropargylglycine) in methionine-free Dulbecco’s Modified Eagle Medium (DMEM, Gibco: 21013) supplemented with 200uM L-cyteine (Sigma: 1.02452), 2mM glutamate (Agilent: 103579-100), and 1mM pyruvate (Agilent: 103578-100) for 30 minutes. Cells were lifted with 0.25% trypsin (Gibco: 25200056), and incorporated Click-iT® HPG was further processed for fluorescent readout by flow cytometry using the Invitrogen Click-iT® HPG Alexa Fluor 594 Protein Synthesis Assay Kit (ThermoFisher: C10428) according to manufacturer’s recommendations.

For cell proliferation assays using BrdU incorporation, S-phase entry of proliferating cells was assessed by flow cytometry analysis of 5-bromo-2’-deoxyuridine (BrdU) incorporation overnight using the eBioscience BrdU Staining Kit for Flow Cytometry FITC (ThermoFisher: 8811-6600-42), according to manufacturer’s recommendations.

*RNA sequencing and analysis*

For transcriptomic profiles of β3KO1-BO1 cells, the pMx, Δβ3, and hβ3 lines were cultured in biological triplicate on tissue culture-treated 6-well plates for 24h in the presence of DMSO or 10nM DTX, followed by lysis and RNA extraction using the RNeasy Plus Mini Kit (QIAGEN: 74134). Total RNA integrity was determined using Agilent Bioanalyzer. Library preparation was performed with 1ug of total RNA. Ribosomal RNA was removed by a hybridization method using Ribo-ZERO kits (Illumina-EpiCentre). mRNA was then fragmented and reverse transcribed to yield cDNA using SuperScript III RT enzyme (Life Technologies, per manufacturer’s instructions) and random hexamers. A second strand reaction was performed to yield ds-cDNA. cDNA was blunt ended, had an A base added to the 3’ ends, and then had Illumina sequencing adapters ligated to the ends. Ligated fragments were then amplified for 13 cycles using primers incorporating unique index tags. Fragments were sequenced on an Illumina HiSeq3000 using single end reads extending 50 bases. Sequencing reads were checked for quality using FastQC (RRID:SCR\_014583) (7) and aligned to the mouse reference genome (mm10) using the splice-aware alignment tool HISAT2 (RRID:SCR\_015530) (8) guided by the transcript annotation downloaded from the UCSC genome browser (RRID:SCR\_005780) (9). Subsequently, featureCounts (RRID:SCR\_012919) (10) was used to quantify the raw count of reads mapped to the transcripts.

For transcriptomic profiles of 4T1 lines, β3WT and β3KO were cultured in biological triplicate on poly-L-lysine-coated 6-well petri dishes for 24h in the presence of DMSO or 10nM DTX. After a 48h drug-free recovery period, cells were lysed and RNA extracted using the RNeasy Plus Mini Kit (QIAGEN: 74134). Total RNA integrity was determined using Agilent 4200 Tapestation. Library preparation was performed with 1ug of total RNA. Ribosomal RNA was removed by an RNase-H method using RiboErase kits (Kapa Biosystems). mRNA was then fragmented in reverse transcriptase buffer and heated to 94 degrees for 8 minutes. mRNA was reverse transcribed to yield cDNA using SuperScript III RT enzyme (Life Technologies, per manufacturer’s instructions) and random hexamers. A second strand reaction was performed to yield ds-cDNA. cDNA was blunt ended, had an A base added to the 3’ ends, and then had Illumina sequencing adapters ligated to the ends. Ligated fragments were then amplified for 12 cycles using primers incorporating unique dual index tags. Fragments were sequenced on an Illumina NovaSeq-6000 using paired end reads extending 150 bases. Basecalls and demultiplexing were performed with Illumina’s bcl2fastq software (RRID:SCR\_015058) and a custom python demultiplexing program with a maximum of one mismatch in the indexing read. RNA-Seq reads were then aligned to the Ensembl release 76 primary assembly with STAR version 2.5.1a (RRID:SCR\_015899) (11). Gene counts were derived from the number of uniquely aligned unambiguous reads by Subread:featureCount version 1.4.6-p5 RRID:SCR\_012919) (10). Isoform expression of known Ensembl transcripts was estimated with Salmon version 0.8.2 (RRID:SCR\_017036) (12). Sequencing performance was assessed for the total number of aligned reads, total number of uniquely aligned reads, and features detected. The ribosomal fraction, known junction saturation, and read distribution over known gene models were quantified with RSeQC version 2.6.2 (RRID:SCR\_005275) (13).

For both projects, gene counts were used for expression normalization and differential expression analysis using edgeR (RRID:SCR\_012802) (14). Ranked lists of normalized expression values were then imported into GSEA v4.0.1 (Broad Institute, RRID:SCR\_003199) (15, 16) for hallmark gene set enrichment analysis (17).

*Integrin αvβ3 antagonist homing ligand*

The vitronectin antagonist specific for activated integrin αvβ3 was a quinalone nonpeptide, developed by Bristol-Myers Squibb Medical Imaging (US patent 6,511,648 and related patents) and coupled to phosphatidylethanolamine-polyethylene glycol 2000 (αvβ3-PEG2000-PE, **Supplementary Fig. S7A**). The antagonist was initially characterized as the 111In-DOTA conjugate RP478 and cyan 5.5 homologue TA145 (18–20). The ανβ3 peptidomimetic had a 15-fold preference for the Mn2+-activated receptor (21, 22) and an IC50 for ανβ5, α5β1 and Gp-IIbIIIa of >10 μM (Bristol-Myers Squibb Medical Imaging, unpublished data). Integrin αvβ3-targeted nanoparticles have an IC50 of 50pM for the Mn22+ activated integrin αvβ3 receptor (Kereos, Inc., unpublished data). Homing specificity to neovascular sprouts was previously demonstrated in a well-defined Matrigel® plug study using the Rag1tm1Mom Tg(TIE-2-lacZ)182-Sato mouse (Jax, RRID:IMSR\_JAX:005707) (23).

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