Online methods

Animals. We obtained *Rag*$^{-/-}$ mice from the Mouse Models of Human Cancers Consortium Repository, US National Cancer Institute. *Igf1r*$^{fl/fl}$ and Osx1-GFP:Cre (The Jackson Laboratory) mouse lines have been described previously[22]. To generate Osx-Cre; *Igf1r*$^{fl/fl}$ (*Igf1r*$^{-/-}$) mice, we crossed hemizygous Osx-Cre transgenic mice with *Igf1r*$^{fl/fl}$ mice to generate heterozygous *Igf1r*-flox offspring with and without a Cre allele. We then intercrossed these offspring to generate the following offspring: Osx-Cre; *Igf1r*$^{fl/fl}$ (conditional knockout mice referred as *Igf1r*$^{-/-}$), Osx-Cre (referred as Igf*1r*$^{+/+}$), Osx-Cre; *Igf1r*$^{fl/+}$ (heterozygous conditional Knockout mice, referred as *Igf1r*$^{+/-}$), and mice without Osx-Cre. The liver-specific *Igf-1* deletion (LID) mice were created by crossing the mice expressing Cre-recombinase under the albumin enhancer/promoter (The Jackson Laboratory) with the mice floxed exon 4 of the *Igf-1* gene[38]. We performed genotyping using the genomic DNA isolated from tail biopsies. We purchased different aged male Sprague Dawley rats from the Harlan Laboratories, and used 20 months old rats in the local injection experiment. The average body weight of the old rats was 675 ± 25 g. All animals were maintained in the Animal Facility of the Johns Hopkins University School of Medicine. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University, Baltimore, MD, USA.

Isolation and culture of murine MSCs. We collected bone marrow cells from 6 weeks old male *Igf1r*$^{fl/fl}$ and wild-type mice euthanized by cervical dislocation, and cultured cells with Minimum Essential Medium [alpha] (α-MEM, Mediatech, Inc.) supplemented with penicillin (100 U ml^{-1}, Sigma-Aldrich), streptomycin sulfate (100 µg ml^{-1}, Sigma-Aldrich), and 20% lot-selected fetal bovine serum (FBS, Atlanta Biologicals) at 37 °C in a 5% CO_2 humidified incubator. After 72 hours of adhesion, we removed nonadherent cells and cultured adherent cells for an additional 7 days with a single media change. The adherent cells were then retrieved by trypsin digestion. We incubated cell aliquots for 20 minutes at 4 °C with phycoerythrin (PE)-,

fluorescein isothiocyanate (FITC)-, peridinin chlorophyll protein (Per CP)-, and allophycocyanin (APC)- conjugated antibodies against mouse Sca-1, CD29, CD45, and CD11b (Bio-legend). Acquisition was performed on a fluorescence-activated cell sorting (FACS) Aria model (BD Biosciences), and analysis was performed using a FACS DIVE software version 6.1.3 (BD Biosciences). The sorted $CD29^{+}Sca–1^{+}CD45^{-}CD11b^{-}$ cells were enriched by further culture.

Adenoviral infection. For the adenoviral (Ad) infections, we plated MSCs at a density of 5×10^5 cells in 100 mm plates. We added Ad-Cre-GFP viruses to the MSCs at a multiplicity of infection (MOI) of 100, suspended in PBS at 37 °C in 5% CO_2 atmosphere. Plates were swirled every 15 minutes and fresh α-MEM with 10% FBS was added after 1 hour of incubation. We exposed the cells to fresh α-MEM containing adenovirus for 48 hours. The control group Ad-GFP was treated with the same method. We obtained the adenovirus preparations from Vector Biolabs (Philadelphia, PA).

RNA interference. We transfected MSCs with siRNA targeting *Irs1* or scrambled control siRNA that has been tested without leading to the specific degradation of any known cellular mRNA (Santa Cruz) using Lipofectamine RNAiMAX in Opti-MEM (Invitrogen) according to the protocol recommended by the manufacturer (Invitrogen). Experiments were performed 48 hours following transfection. Scrambled siRNA and siRNA targeting *Irs1* had no significant effect on cellular morphology. We validated the successful knockdown of IRS1 using western blot analysis.

CFU-F and CFU-Ob assays. At the time of euthanasia, we collected bone marrow from femoral, tibial, and humeral medullary cavities, and determined cell numbers with Zapoglobin (Coulter Corp.) after removal of red blood cells. The numbers of CFU-Fs and CFU-Obs in murine bone marrow isolates and cocultures with irradiated guinea pig marrow cells were as reported[39]. We obtained marrow cells from the femurs and tibiae of 2 months old female Hartley guinea pigs by flushing with a 22

gauge needle and then resuspended. We γ-irradiated cells with a Co57 source for 50 minutes at 1.2 Grays min^{-1} as reported[40]. After rinsing by centrifugation, cells were resuspended in α-MEM medium with 20% FBS, counted and cultured at 2.5×10^6 per well of a six-well plate. For assay of CFU-F and CFU-Ob number, we plated 0.1, 0.5, or 1×10^5 murine marrow cells into six-well plates in 3 ml α-MEM supplemented with glutamine (2 mM), penicillin (100 U ml^{-1}), streptomycin sulfate (100 μg ml^{-1}), and 20% lot-selected FBS. We also established duplicate cultures. After 2 to 3 hours of adhesion, we removed unattached cells, and added 2.5×10^6 irradiated guinea pig feeder cells (provided by Dr. Brendan J. Canning) to cultivation medium of adherent cultures just after washing. On day 14, we fixed and stained the cultures with 0.5% Crystal Violet. We counted colonies that contained 50 or more cells. For CFU-Ob assay, we cultured the cells with osteogenic medium as described above for 21 days and analyzed with Alizarin Red staining. We determined the colony-forming efficiency by quantifying number of colonies per 10^5 marrow cells plated.

Bone marrow cavity transplantation. We used 2 months old male *Rag2*$^{-/-}$ mice with an immune-deficient background as recipients. We injected GFP-labeled cells in 10 μl of α-MEM media into the bone marrow cavity of the left femora, as previously described. The mice were euthanized 2 or 4 weeks after transplantation and processed the distal femora for staining. In some cases, we collected cells from bone marrows and compact bones by collagenase digestion, and assessed the total number of GFP positive cells by flow cytometry analysis. Bone surface GFP^+ cells were calculated as cells per millimeter of perimeter in sections (B.Pm), and bone matrix GFP^+ cells were calculated as cells per mm^2 of matrix area in sections (Bmx.Ar).

Subrenal capsule transplant. We utilized 2 months old male *Rag2*$^{-/-}$ mice with an immune-deficient background as recipients. 5×10^3 GFP-labeled cells in the matrigel (with 100 ng ml^{-1} IGF-1) were inoculated underneath the renal capsule of 2 months old *Rag2*$^{-/-}$ mice injected subcutaneously with rapamycin (3 mg kg^{-1} per day) or

vehicle for 4 weeks. We euthanized the mice 4 weeks after transplantation and processed the kidneys for frozen histology staining.

***In vitro* osteoblastic differentiation.** We plated MSCs cells at a density of 1,000 cells per well in the six-well plates and expanded in growth medium for 3 days. We induced osteoblastic differentiation in low serum conditions (2% FBS) in differentiation medium (αMEM supplemented with 50 μg ml^{-1} of ascorbic acid and 10 mM β-glycerol phosphate). We added mouse recombinant IGF-1(Abcam) to the differentiation medium at 100 ng ml^{-1} with or without PI3K inhibitor LY294002 (10 μM) or mTOR inhibitor rampamycin (20 nM). We changed the medium every 3 days and assayed cellular differentiation 20 days after induction through Alizarin Red staining.

Microcomputed tomography (μCT) analysis. We obtained long bone from mice and rats, dissected free of soft tissue, fixed overnight in 70% ethanol (10% PFA in Rats) and analyzed by a high resolution μCT (SkyScan1076 (1172 for rats) in-vivo CT, SKYSCAN Company). We used image reconstruction software: NRecon v1.6. Data analysis software: CTAn v1.9., three-dimensional model visualization software: μCTVol v2.0 to analyze parameters of the trabecular bone in the metaphysis. We set the scanner at a voltage of 89 kVp (50 kVp for rats), a current of 112 μA (200 μA for rats) and a resolution of 8.66596 μm $pixel^{-1}$ (12.1 μm $pixel^{-1}$ in rats). We established cross-sectional images of the proximal tibiae and femur to perform three-dimensional histomorphometric analysis of trabecular bone. The sample area selected for scanning was a 1.5 mm length of the metaphyseal secondary spongiosa, originating 1.0 mm below the epiphyseal growth plate and extending caudally. For aged rats, we used cross-sectional images of the distal femur to perform three-dimensional and two-dimensional morphometric analysis of the trabecular bone (400 cross-sections per specimen).

Histochemistry, immunohistochemistry, and histomorphometric analysis. At the time of euthanasia, we dissected the tibiae or femura and fixed the specimens in

10% buffered formalin for 48 hours, decalcified in 10% ethylenediamine tetraacetic acid (EDTA) (pH 7.0) for 14 days and embedded in paraffin. 4 μm thick longitudinally oriented sections of bone including the metaphysis and diaphysis were processed for TRAP staining (Sigma-Aldrich) and Goldner's Trichrome staining. For immunohistochemical staining, we incubated the sections with primary antibodies for overnight at 4 °C and used a horseradish peroxidase-streptavidin detection system (Dako) to detect immunoactivity followed by counterstaining with hematoxylin (Dako) or methyl green (Sigma-Aldrich). We used Isotype-matched negative control antibodies (R&D Systems) under the same conditions. We performed histomorphometric measurements of two-dimensional parameters of the trabecular bone in the secondary spongiosa in a 2 mm square, 1 mm distal to the lowest point of growth plate with OsteoMeasureXP Software (OsteoMetrics, Inc.). To label mineralization fronts, the mice were given subcutaneous injections of calcein (Sigma, 15 mg kg^{-1}) in 2% sodium bicarbonate solution 10 days and 3 days before death. We selected all histomorphometric parameters in four random visual fields per specimen, and measured five specimens per animal in each group.

Western blot analysis and ELISA. We prepared cell lysates in 2% sodium dodecyl sulfate, 2 M urea, 10% glycerol, 10 Mm Tris-HCl (pH 6.8), 10 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. We performed western blot analysis in conditional media or the protein of lysates from the individual groups. We centrifuged the lysates and separated the supernatants by SDS-PAGE, blotted onto a PVDF (Bio-Rad Laboratories) membrane, analyzed with specific antibodies, and visualized by enhanced chemiluminescence (ECL Kit; Amersham Biosciences). We used antibodies recognizing mouse IGF-1 (R&D Systems, Inc), IGF1R (Cell Signaling technology Inc.), and β-actin (Sigma-Aldrich) to examine the levels of IGF-1 in conditional media and IGF1R protein in lysates. We detected the amount of IGF-1 in the conditioned media with DuoSet ELISA Development Kit (R&D Systems, DY 791) according to the manufacturer's instructions.

In vitro **bone resorption assays.** For preparation of osteoclastic bone resorption-conditioned medium (BRCM), we isolated bone marrow macrophages (BMMs) from long bones of mice and cultured them in α-MEM containing 10% heat-inactivated fetal bovine serum in the presence of M-CSF. We cultured the cells for 2 days in the presence of 10 ng ml^{-1} recombinant M-CSF (R&D Systems Inc.), then plated the cells onto bone slices of bovine cortical bone in 24-well tissue culture plates ($1x10^5$ cells per well) and in the presence of 10 ng ml^{-1} recombinant M-CSF and 100 ng ml^{-1} of RANKL. Under these conditions, osteoclasts began to form and resorb bone at 6 to 7 days. The cultured cells were stained for TRAP activity at 8 to 10 days using a commercial kit (Sigma-Aldrich). We determined the resorption of the bovine cortical bone slices by scanning electronic microscopy. We harvested the conditioned media from the osteoclast-mediated resorption at 8 to 10 days with centrifugation. We added neutralizing antibodies for IGF-1, IGF-II, TGF-β1, PDGF (BD Biosciences) to the conditioned media or depleted the conditioned media (BRCM) of active IGF-1 by three cycles of immunoprecipitation using a monoclonal antibody specific for IGF-1 tagged to protein G-Sepharose.

Bone marrow cavity injection of IGF-1, IGFBP3 and IGF-1 with IGFBP3. We randomly divided forty rats into four groups with ten animals per group. We purchased recombinant IGF-1 and IGFBP3 from Abcam and R&D Systems. We delivered 8 μg of IGF-1, 42 μg IGFBP3, IGF-1 with IGFBP3 (8 μg of IGF-1 and 42 μg of IGFBP3, molar ratio 1:1) or vehicle (PBS) into bone marrow cavity once a week injected from the medial side of patellar tendon using 0.5 ml syringes with 27 gauge needles. We collected whole blood samples by cardiac puncture for biochemical assessments immediately after euthanasia by CO_2 asphyxiation after 4 weeks. We collected blood samples by centrifugation to obtain the sera, which were then stored at –80 °C until assayed.

Bone matrix extraction and bone marrow serum collection. We exposed bone marrow from freshly sacrificed animals at the growth plate and placed samples for

centrifugation with trabecular part of the bone at top. We removed bone marrow by centrifugation of the samples for 15 minutes at 5,000 rpm at 4 °C. We immersed the resected femurs in liquid nitrogen and mechanically crushed specimens into small fragments of several millimeters in diameter. The fragments were washed repeatedly in cold distilled water until they were free of blood and defatted in cold isopropylether. We grounded the defatted bone fragments into smaller particles by a biopulverizer (BioSpec Products, Inc., Bartlesville, OK, USA). We placed 40 mg to 50 mgs of the particles in a mini dialysis unit with a 3.5 Kd Molecular Weight Cut-Off (Termo). We added 0.5 ml of extraction solution (4 M Guanidine-HCl, 0.05 M EDTA, 30 mM Tris, 1 mg ml^{-1} bovine serum albumin, pH = 7.4) with protease inhibitors (1 mM phenylmethyl-sulfonyl fluoride, 5 mM benzamidine-HCl, 0.1 M E-aminocaproic-acid, 2 ug ml^{-1} leupeptin) into the mini dialysis unit to decalcify bone tissue and extract proteins from the bone matrix. The mini dialysis tube was in contact with the extraction solution in a beaker (500 ml for 15 mini dialysis units). We conducted the extraction procedure at 4 °C on a stir plate with a low-speed setting for 48 hours. We then re-dialyzed the bone sample against phosphate-buffered saline (PBS) solution for 72 hours at 4 °C. Finally, we stored the extracts at –80 °C until assayed for growth factor activity.

Systemic infusion by osmotic pumps. IGF-1(1.5 mg kg^{-1} per day), IGF-1 plus IGFBP3 (IGF-1: 1.5 mg kg^{-1} per day, IGFBP3: 7.8 mg kg^{-1} per day, molar ratio 1:1), or vehicle was infused into the circulation of four-weeks-old female liver-specific IGF-1 gene deletion (LID) mice with osmotic pumps for 4 weeks. Osmotic pumps (ALZET) were embedded subcutaneously.

Measurement of IGF-1 and IGFBP3 levels. We determined the concentration of IGF-1 by a mouse or rat IGF-1 Immunoassay kit (ELISA) (R & D Systems) according to the instructions provided. This assay was designed to eliminate interference by binding proteins. We diluted peripheral blood serum 1000 folds; bone marrow serum 500 folds and bone matrix protein 10 folds with the Calibrator Diluent RD5-38 before

detection. The assay sensitivity was 3.5 pg ml^{-1}; intra- and interassay coefficients of variation were 5.6% and 4.3%. We assessed Human IGF-1 levels by ELISA after an acid-ethanol extraction. The inter-assay variation was 5.3% and the intra-assay variation was 2.3%. We determined the concentration of IGFBP3 by a mouse or rat IGFBP3 Immunoassay kit (ELISA) (BioVendor, Candler) according to the instructions provided. The assay sensitivity was 18 pg ml^{-1}; intra- and interassay coefficients of variation were 4.6% and 8.4%.

Collection of human bone marrow samples. We collected bone marrow samples from individual subjects with osteoporosis, as defined by hip fractures and low bone mass (*n=12*), and individual subjects with osteoarthritis and normal bone mineral density of similar ages (*n=12*). The bone marrow collection was performed during total hip replacement with informed consent and approval by the Institutional Review Board at the University of Chile. Detailed information on the samples is shown in Supplementary Table 2.

Statistics. We presented data as the mean ± S.E.M. and analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's test or two-tailed *t-test*.

Primers used for quantitative real-time PCR

Primer	Sequence
*Gapdh*_Fw	5'-TGTGTCCGTCGTGGATCTGA-3'
*Gapdh*_Rv	5'-CCTGCTTCACCACCTTCTTGA-3'
*Alpl*_Fw	5'-CACAATATCAAGGATATCGACGTGA-3'
*Alpl*_Rv	5'-ACATCAGTTCTGTTCTTCGGGTACA-3'
*Runx2*_Fw	5'-TTACCTACACCCCGCCAGTC-3'
*Runx2*_Rv	5'-TGCTGGTCTGGAAGGGTCC-3'
*Sp7*_Fw	5'-ATGGCGTCCTCTCTGCTTGA-3'
Sp7 Rv	5'-GAAGGGTGGGTAGTCATTTG-3'
*Bglap*_Fw	5'-GGGCAATAAGGTAGTGAACAG-3'
*Bglap*_Rv	5'-GCAGCACAGGTCCTAAATAGT-3'
*Gpnmb*_Fw	5'-CCCCAAGCACAGACTTTTGAG-3'
*Gpnmb*_Rv	5'-GCTTTCTGCATCTCCAGCCT-3'
*Ogn*_Fw	5'-ACCATAACGACCTGGAATCTGT-3'
*Ogn*_Rv	5'-AACGAGTGTCATTAGCCTTGC-3'

Antibody list

Antigen	Clone	Company	Applications
Sca-1	D7	Biolegend	FCM
CD29	HMβ1-1	Biolegend	FCM
CD45	30-F11	Biolegend	FCM
CD11b	M1/70	Biolegend	FCM
GFP	4B10	Cell Signaling	IHC-P(1:800)
IGF1R	111A9	Cell Signaling	WB(1:1000)
IGF1R	polyclonal	Santa cruz	IHC-P(1:100)
p-IGF-1R	polyclonal	Cell Signaling	WB(1:400)
P-IGF-1R	polyclonal	Santa cruz	IHC-P(1:100)
Runx2	M-70	Santa cruz	IHC-P(1:100)
osterix	Polyclonal	Abcam	IHC-P(1:400)
osteocalcin	Polyclonal	Santa cruz	IHC-P(1:100)
osteocalcin	Polyclonal	Takara	IHC-P(1:100)
IGF-1	Polyclonal	R&D systems	WB(1:1000)
IRS-1	Polyclonal	Cell Signaling	WB(1:500)
Phosphotyrosine	4G10	Millpore	WB(1:500)
PI3 Kinase	Polyclonal	Cell Signaling	WB (1:400)
p-PI3 Kinase	polyclonal	Cell Signaling	WB(1:400)
AKT	40D4	Cell Signaling	WB(1:2000)
P-AKT	polyclonal	Cell Signaling	WB(1:500)
mTOR	polyclonal	Cell Signaling	WB(1:500)
P-mTOR	polyclonal	Cell Signaling	WB(1:400)

FCM: flow cytometry measurement, WB: western blot, IHC-P: immunohistochemical analysis

Reference list

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