

## Supplementary Material

**Table S1.** Proteins expressed only in osteoblasts. The table gives a list (corresponding gene name, protein name) of all proteins that could be quantified in osteoblasts but were not detected for any of the MSC donors.

Gene Name	Protein Name
<i>ACTC1</i>	Actin, alpha cardiac muscle 1
<i>ADA</i>	Adenosine deaminase
<i>SCIN</i>	Adseverin
<i>AIF1</i>	Allograft inflammatory factor 1
<i>ANGPTL3</i>	Angiopoietin-related protein 3
<i>ACE</i>	Angiotensin-converting enzyme;Angiotensin-converting enzyme, soluble form
<i>APOA1</i>	Apolipoprotein A-I;Proapolipoprotein A-I;Truncated apolipoprotein A-I
<i>APOE</i>	Apolipoprotein E
<i>ATXN3</i>	Ataxin-3
<i>NPR3</i>	Atrial natriuretic peptide receptor 3
<i>AURKA</i>	Aurora kinase A
<i>BCAM</i>	Basal cell adhesion molecule
<i>BCL7C</i>	B-cell CLL/lymphoma 7 protein family member C
<i>B3GALTL</i>	Beta-1,3-glucoyltransferase
<i>B4GALT5</i>	Beta-1,4-galactosyltransferase 5
<i>GUSB</i>	Beta-glucuronidase
<i>LACTB2</i>	Beta-lactamase-like protein 2
<i>BST2</i>	Bone marrow stromal antigen 2
<i>BRI3</i>	Brain protein I3
<i>BRD4</i>	Bromodomain-containing protein 4
<i>KCTD3</i>	BTB/POZ domain-containing protein KCTD3
<i>C4BPA</i>	C4b-binding protein alpha chain
<i>CAMSAP1</i>	Calmodulin-regulated spectrin-associated protein 1
<i>NTPCR</i>	Cancer-related nucleoside-triphosphatase
<i>CADM1</i>	Cell adhesion molecule 1
<i>CDCA2</i>	Cell division cycle-associated protein 2
<i>PDE5A</i>	cGMP-specific 3,5-cyclic phosphodiesterase
<i>CHMP1B</i>	Charged multivesicular body protein 1b
<i>CHMP2B</i>	Charged multivesicular body protein 2b
<i>CHI3L1</i>	Chitinase-3-like protein 1
<i>F9</i>	Coagulation factor IX
<i>COL5A3</i>	Collagen alpha-3(V) chain
<i>CRISPLD2</i>	Cysteine-rich secretory protein LCCL domain-containing 2
<i>DMBT1</i>	Deleted in malignant brain tumors 1 protein
<i>DAAM2</i>	Disheveled-associated activator of morphogenesis 2
<i>HECTD3</i>	E3 ubiquitin-protein ligase HECTD3
<i>RNF13</i>	E3 ubiquitin-protein ligase RNF13
<i>ENTPD1</i>	Ectonucleoside triphosphate diphosphohydrolase 1
<i>ENPP2</i>	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2
<i>EFHD1</i>	EF-hand domain-containing protein D1
<i>ELN</i>	Elastin
<i>EPHA4;EPHA5;EPHA3</i>	Ephrin type-A receptor 4;Ephrin type-A receptor 5;Ephrin type-A receptor 3
<i>EPHB4;EPHB3</i>	Ephrin type-B receptor 4;Ephrin type-B receptor 3
<i>FBN2</i>	Fibrillin-2
<i>FGG</i>	Fibrinogen gamma chain
<i>FBP2</i>	Fructose-1,6-bisphosphatase isozyme 2
<i>FUNDC2</i>	FUN14 domain-containing protein 2

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<i>GALT</i>	Galactose-1-phosphate uridylyltransferase
<i>LGALS9</i>	Galectin-9
<i>HEATR5A</i>	HEAT repeat-containing protein 5A
<i>HCLS1;PCMTD2;OTUD6A</i>	Hematopoietic lineage cell-specific protein
<i>HGFAC</i>	Hepatocyte growth factor activator
<i>HMG20A</i>	High mobility group protein 20A
<i>HNMT</i>	Histamine N-methyltransferase
<i>HDAC7</i>	Histone deacetylase 7
<i>HLA-A</i>	HLA class I histocompatibility antigen, A-23 alpha chain
<i>HLA-A</i>	HLA class I histocompatibility antigen, A-30 alpha chain
<i>HLA-A</i>	HLA class I histocompatibility antigen, A-36 alpha chain
<i>HLA-A</i>	HLA class I histocompatibility antigen, A-68 alpha chain
<i>HLA-F</i>	HLA class I histocompatibility antigen, alpha chain F
<i>HLA-B</i>	HLA class I histocompatibility antigen, B-45 alpha chain
<i>HLA-C</i>	HLA class I histocompatibility antigen, Cw-1 alpha chain
<i>HLA-C</i>	HLA class I histocompatibility antigen, Cw-18 alpha chain
<i>HLA-DRB4</i>	HLA class II histocompatibility antigen, DR beta 4 chain
<i>HLA-DRB1</i>	HLA class II histocompatibility antigen, DRB1-11 beta chain
<i>HLA-DRB1</i>	HLA class II histocompatibility antigen, DRB1-15 beta chain
<i>HLA-DRB1;HLA-DRB5</i>	HLA class II histocompatibility antigen, DRB1-16 beta chain
<i>IGHA1</i>	Ig alpha-1 chain C region
<i>IGKC</i>	Ig kappa chain C region
<i>IGFBP2</i>	Insulin-like growth factor-binding protein 2
<i>IFT140</i>	Intraflagellar transport protein 140 homolog
<i>KRT77</i>	Keratin, type II cytoskeletal 1b
<i>KRT80</i>	Keratin, type II cytoskeletal 80
<i>KYNU</i>	Kynureninase
<i>LPHN2</i>	Latrophilin-2
<i>LMOD1</i>	Leiomodin-1
<i>LRRC32</i>	Leucine-rich repeat-containing protein 32
<i>LRRC58</i>	Leucine-rich repeat-containing protein 58
<i>LIMS2</i>	LIM and senescent cell antigen-like-containing domain protein 2
<i>LCN1</i>	Lipocalin-1
<i>LYZ</i>	Lysozyme C
<i>MROH1</i>	Maestro heat-like repeat-containing protein family member 1
<i>NIPA1</i>	Magnesium transporter NIPA1
<i>MEST</i>	Mesoderm-specific transcript homolog protein
<i>STEAP4</i>	Metalloreductase STEAP4
<i>MFAP2</i>	Microfibrillar-associated protein 2
<i>MAP3K7</i>	Mitogen-activated protein kinase kinase kinase 7
<i>SLC16A5</i>	Monocarboxylate transporter 6
<i>CD14</i>	Monocyte differentiation antigen CD14
<i>MUC5B</i>	Mucin-5B
<i>NADK</i>	NAD kinase
<i>NTN4</i>	Netrin-4
<i>SPP1</i>	Osteopontin
<i>PALMD</i>	Palmdelphin
<i>PAPPA</i>	Pappalysin-1
<i>PALM</i>	Paralemmin-1
<i>SPG7</i>	Paraplegin
<i>PDDC1</i>	Parkinson disease 7 domain-containing protein 1
<i>PBLD</i>	Phenazine biosynthesis-like domain-containing protein
<i>FXYP1</i>	Phospholemman

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<i>PHYHD1</i>	Phytanoyl-CoA dioxygenase domain-containing protein 1
<i>PEAR1</i>	Platelet endothelial aggregation receptor 1
<i>CD36</i>	Platelet glycoprotein 4
<i>PODN</i>	Podocan
<i>PIGR</i>	Polymeric immunoglobulin receptor;Secretory component
<i>LMNA</i>	Prelamin-A/C;Lamin-A/C
<i>PSEN2</i>	Presenilin-2;Presenilin-2 NTF subunit;Presenilin-2 CTF subunit
<i>ATP9A</i>	Probable phospholipid-transporting ATPase IIA
<i>BICD1</i>	Protein bicaudal D homolog 1
<i>C12orf4</i>	Protein C12orf4
<i>FAM20A</i>	Protein FAM20A
<i>JAG1</i>	Protein jagged-1
<i>PRKCQ</i>	Protein kinase C theta type
<i>PRUNE2</i>	Protein prune homolog 2
<i>SAMD4A</i>	Protein Smaug homolog 1
<i>UNC13B</i>	Protein unc-13 homolog B
<i>TGM3</i>	Protein-glutamine gamma-glutamyltransferase E
<i>SLC46A1</i>	Proton-coupled folate transporter
<i>PIPSL</i>	Putative PIP5K1A and PSMD4-like protein
<i>DHX32</i>	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX32
<i>MRAS</i>	Ras-related protein M-Ras
<i>RAB27B</i>	Ras-related protein Rab-27B
<i>RGN</i>	Regucalcin
<i>RTN4</i>	Reticulon-4
<i>ARHGAP24</i>	Rho GTPase-activating protein 24
<i>RNF121</i>	RING finger protein 121
<i>SSPN</i>	Sarcospan
<i>CD163L1</i>	Scavenger receptor cysteine-rich type 1 protein M160
<i>DCLK1</i>	Serine/threonine-protein kinase DCLK1
<i>SLC17A5</i>	Sialin
<i>SIPA1L3</i>	Signal-induced proliferation-associated 1-like protein 3
<i>SLIT3</i>	Slit homolog 3 protein
<i>SNX21</i>	Sorting nexin-21
<i>STAB1</i>	Stabilin-1
<i>CXCL12</i>	Stromal cell-derived factor 1;SDF-1-beta(3-72);SDF-1-alpha(3-67)
<i>SYNPO2</i>	Synaptopodin-2
<i>TAPBPL</i>	Tapasin-related protein
<i>TBXAS1</i>	Thromboxane-A synthase
<i>TFPI2</i>	Tissue factor pathway inhibitor 2
<i>TRAF6</i>	TNF receptor-associated factor 6
<i>TANK</i>	TRAF family member-associated NF-kappa-B activator
<i>EBF3</i>	Transcription factor COE3
<i>TM4SF1</i>	Transmembrane 4 L6 family member 1
<i>TRIM47</i>	Tripartite motif-containing protein 47
<i>TINAGL1</i>	Tubulointerstitial nephritis antigen-like
<i>TNFAIP2</i>	Tumor necrosis factor alpha-induced protein 2
<i>TNFAIP8</i>	Tumor necrosis factor alpha-induced protein 8
<i>TNFRSF12A</i>	Tumor necrosis factor receptor superfamily member 12A
<i>TPD52L1</i>	Tumor protein D53
<i>FYN</i>	Tyrosine-protein kinase Fyn
<i>UPP1</i>	Uridine phosphorylase 1
<i>VEGFC</i>	Vascular endothelial growth factor C
<i>KCNAB2</i>	Voltage-gated potassium channel subunit beta-2

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WDR81  
WISP2  
ZNF281

WD repeat-containing protein 81  
WNT1-inducible-signaling pathway protein 2  
Zinc finger protein 281

**Table S2.** Proteins involved in differentiation and development of osteoblasts. The table shows a list of proteins that reached detectable levels only in osteoblasts, included in the GO terms GO:0045667 Regulation of osteoblastic differentiation, GO:22001044 Integrin-mediated signaling pathway, GO:0030509 BMP signaling pathway, GO:0030510 Regulation of BMP signaling pathway, GO:0031012 Extracellular matrix (Cellular compartment), GO:0030198 Extracellular matrix organization (biological process), GO:0001503 Ossification, GO:0030278 Regulation of ossification, GO:0016055 Wnt signaling pathway and GO0030111 Regulation of Wnt signaling pathway. The table shows the corresponding gene name and the protein name together with a brief description and keywords reflecting important characteristics of the proteins. The information is based on Uniprot (<https://www.uniprot.org/>, accessed on 12 March 2021), the gene card database (<https://www.genecards.org/>, accessed on 12 March 2021) and selected references from the databases.

PROTEIN	DESCRIPTION	KEY WORDS
ANGPTL3	<i>Angiopoietin-related protein 3.</i> This gene encodes a member of a family of secreted proteins that function in angiogenesis. Binds to endothelial cells via integrin alpha-V/beta-3 (ITGAV:ITGB3), activates FAK, MAPK and Akt signaling pathways and induces cell adhesion and cell migration (PMID 11877390). Secreted from podocytes, may modulate properties of glomerular endothelial cells involving integrin alpha-V/beta-3 and Akt signaling (PMID 18535744). May increase the motility of podocytes. May induce actin filament rearrangements in podocytes implicating integrin alpha-V/beta-3 and Rac1 activation. Binds to hematopoietic stem cells (HSC) and is involved in the regulation of HSC activity probably implicating down-regulation of IKZF1/IKAROS (By similarity).	Angiogenesis Integrin-mediated signaling pathways
APOA1	<i>Apolipoprotein A1.</i> This gene encodes the major protein component of high-density lipoprotein (HDL) in plasma. The encoded preproprotein is proteolytically processed to generate the mature protein, which promotes cholesterol efflux from tissues to the liver for excretion, and is a cofactor for lecithin cholesterolacyltransferase, an enzyme responsible for the formation of most plasma cholesteryl esters. Finally, APOA1 is important for induction of osteoblast differentiation (PMID 27359105).	Lipid metabolism Osteoblast differentiation
APOE	<i>Apolipoprotein E.</i> The protein is a major apoprotein of the chylomicron and is essential for triglyceride and cholesterol metabolism. The encoded protein is important for osteoblast differentiation and bone healing (PMID 31534056, 27074899).	Lipid metabolism Osteoblast differentiation
BCAM	<i>Basal cell adhesion molecule.</i> This glycoprotein is a member of the immunoglobulin superfamily and a receptor for the extracellular matrix protein laminin.	Extracellular matrix Laminin receptor
CHI3L1	<i>Chitinase 3 like 1.</i> Chitinases catalyze the hydrolysis of chitin, and this gene encodes a glycoprotein member of the glycosyl hydrolase 18 family. The protein is secreted and is thought to play a role in tissue remodeling. This secreted protein seems to be indirectly involved in the regulation of osteogenesis through effects on various cytokines and may also be important for osteoblastic differentiation though its effects on Notch signaling (PMID 28391634).	Glycosyl hydrolase Tissue remodeling Osteogenesis Osteoblast differentiation
COL5A3	<i>Collagen type V alpha 3 chain.</i> This fibrillar collagen appears to regulate the assembly of heterotypic fibers composed of both type I and type V collagen. Sp7/Osterix is an osteoblast-specific transcription factor that is important for osteoblast differentiation; this transcription factors increases Col5a3 transcription (PMID 20206127).	Extracellular matrix Osteoblast differentiation
CRISPLD2	<i>Cysteine rich secretory protein LCCL domain containing 2.</i> The protein seems to be important for craniofacial development (PMID 17616516, 22887593).	Craniofacial development
CXCL12	<i>C-X-C motif chemokine ligand 12.</i> This stromal cell-derived chemokine is the ligand for the G-protein coupled receptor CXCR4. The protein is important for maintaining musculoskeletal homeostasis (PMID 31336263), including osteogenic differentiation of MSCs (PMID 23935395). Disruption	Chemokine Osteoblast differentiation Osteoporosis

	of CXCL12/CXCR4 signaling may have a role in the development of osteoporosis (PMID 31336263, 32949783).	
DAAM2	<i>Dishevelled associated activator of morphogenesis 2</i> . WNT signals can be transduced to the RHOA signaling cascade through the Formin homology proteins DAAM1 and DAAM2 (PMID 16273260), and both WNT16 and DAAM2 are important for the predisposition to osteoporosis (PMID 30598549).	WNT RhoA Osteoporosis
DMBT1	<i>Deleted in malignant brain tumors 1</i> . The encoded protein precursor is a glycoprotein containing multiple scavenger receptor cysteine-rich (SRCR) domains separated by SRCR-interspersed domains (SID). Animal studies suggest that the protein has a proangiogenic effect and protects against steroid-induced osteonecrosis (PMID 32447063).	Proangiogenic Protect against osteonecrosis
ELN	<i>Elastin</i> . The encoded protein is one of the two components of elastic fibers that comprise part of the extracellular matrix. Degradation products of the encoded protein, known as elastin-derived peptides or elastokines, bind the elastin receptor complex. Elastokines can also contribute to cancer progression. Elastin is a marker of the osteogenic capacity of osteoblastic cells together with ALPL, COL1A2, DCN, ELN and RUNX2, and their effects are possibly mediated through regulation of TGF- $\beta$ 1 pathway signaling (PMID 27711115, 24984278).	Extracellular matrix Osteoblast Mineralization
	Elastin is involved in osteoblast mineralization of extracellular matrix (PMID 16817211).	
F9	<i>Coagulation factor IX</i> . This vitamin K-dependent coagulation factor circulates as an inactive zymogen. This factor is converted to an active form by factor XIa, which excises the activation peptide and thus generates a heavy chain and a light chain held together by one or more disulfide bonds. Clinical studies suggest excess of osteoporosis in patients with factor IX deficiency (hemophilia type B); direct effects of coagulation factors on osteoblasts/osteoblasts and/or indirect effects are possible mechanisms (PMID 29857920).	Coagulation Osteoporosis
FBN2	<i>Fibrillin-2</i> . The encoded protein is a component of connective tissue microfibrils and may be involved in elastic fiber assembly. Fibrillin-2 control bone formation by regulating osteoblast differentiation through the differential modulation of endogenous TGF $\beta$ and bone morphogenetic protein signals, and through these mechanisms they become important regulators of bone remodeling (PMID 20729550). The protein seems important for bone mineralization and development of osteoporosis (PMID 30690781), it is also important for TGF $\beta$ and BMP bioavailability during bone formation (PMID 20855508).	Extracellular matrix Mineralization Osteoporosis TGF- $\beta$ 1, BMP Osteoblast differentiation
FGG	<i>Fibrinogen gamma chain</i> . The encoded protein is the gamma component of the fibrinogen coagulation factor. The coagulation cascade may be important for regulation of bone mineralization, possibly through thrombin-mediated effects on osteoblasts (PMID 29857920).	Coagulation Mineralization
HDAC7	<i>Histone deacetylase 7</i> . Histones play a critical role in transcriptional regulation and cell cycle progression. HDAC7 suppresses osteoclastogenesis and attenuates $\beta$ -catenin function (PMID 23204328). Furthermore, BMP2 stimulated nuclear export of HDAC7, and BMP2 signaling also regulates Runx2 activity via inhibition of HDAC7 transcriptional repression (PMID 19029091).	Transcription Cell cycle Osteoclast BMP2 Runx2
IGFBP2	<i>Insulin like growth factor binding protein 2</i> . The protein encoded by this gene is one of six similar proteins that bind insulin-like growth factors (IGFs) I and II. The protein can be secreted into the bloodstream, where it binds IGF-I and IGF-II with high affinity, or it can remain intracellular, interacting with many different ligands. High expression levels of this protein promote the growth of several types of tumors and may be predictive of the chances of recovery of the patient. IGF is a main regulator of skeletal growth and	Skeletal growth Osteoporosis

	decreased levels during aging seem important for the risk of demineralization/osteoporosis (PMID 29626053).	
JAG1	<i>Jagged canonical Notch ligand 1</i> . The jagged 1 protein encoded by JAG1 is the ligand for the receptor notch 1; the latter is involved in signaling processes. Jag1 is important for osteoblast development and bone mineralization (PMID 29693255, 24491691).	Notch
LGALS9	<i>Galectin 9</i> . The galectins are a family of beta-galactoside-binding proteins implicated in modulating cell-cell and cell-matrix interactions. The protein encoded by this gene is an S-type lectin. Galectin-9 induces osteoblast differentiation through the CD44/Smad signaling pathway in the absence of BMPs.	Extracellular matrix Osteoblast differentiation CD44-Smad
LIMS2/PINCH2	<i>LIM zinc finger domain containing 2</i> . This gene encodes a member of a small family of focal adhesion proteins which interacts with ILK (integrin-linked kinase), a protein which effects protein-protein interactions with the extracellular matrix. The focal adhesion proteins Pinch1 and Pinch2/Lims2 regulate integrin activation and cell-extracellular matrix adhesion and migration; it is also important for bone homeostasis/mineralization (PMID 31723057).	Adhesion Integrin Mineralization
LRRC32	<i>Leucine rich repeat containing 32</i> . This gene encodes a type I membrane protein which contains 20 leucine-rich repeats. It is a TGF $\beta$ activating protein and may have growth enhancing and antiapoptotic effects in bone cells (PMID 33203838)	TGF $\beta$
MFAP2	<i>Microfibrillar-associated protein 2</i> . This protein is a major part of elastin-associated microfibrils. Microfibrillar-associated proteins (MFAPs) are extracellular matrix glycoproteins, which play a role in microfibril assembly, and MFAP2-deficient mice exhibit progressive osteopenia with elevated RANKL expression (PMID 32572962).	Extracellular matrix Osteogenesis RANKL
MUC5B	<i>Mucin 5B, oligomeric mucus/gel-forming</i> . This gene encodes a member of the mucin family of proteins, which are highly glycosylated macromolecular components. It can be expressed by chondrosarcoma (PMID 29138803).	Mucin
NTN4	Netrin 4. This gene encodes a member of the netrin family of proteins. Netrins are laminin-related proteins. The protein inhibits osteoclastic differentiation and prevents bone loss in an animal model of osteoporosis (PMID 24846137)	Laminin Mineralization Anti-osteoporosis Osteoclast
PSEN2	<i>Presenilin 2</i> . The encoded protein is possibly involved in the cleavage of the Notch receptor such that, they either directly regulate gamma-secretase activity, or themselves act as protease enzymes. The protein is induced during osteoblastic differentiation together with other key regulators of Wnt, TGF $\beta$ and Notch signaling (PMID 12203032, 23600707). The protein is also involved in the regulation of autophagy and mitochondrial functions (PMID 30412492).	Notch Osteoblast differentiation
PODN	<i>Podocan</i> . The protein is a member of the small leucine-rich repeat protein family and contains an amino terminal CX3CX7C cysteine-rich cluster followed by a leucine-rich repeat domain. The protein can bind collagen (PMID 30519093, 15063725)	Extracellular matrix Collagen
SPP1	<i>Secreted phosphoprotein 1/osteopontin</i> . The encoded protein is involved in the attachment of osteoclasts to the mineralized bone matrix. It is secreted and binds hydroxyapatite with high affinity. The osteoclast vitronectin receptor is found in the cell membrane and possibly important for this binding. This protein is also a cytokine that upregulates expression of interferon- $\gamma$ and IL12. SPP1 expression is regulated by Runx2 together with several other molecules involved in osteoblastic differentiation and functions. Runx2 is essential for osteoblast differentiation Its expression is upregulated in preosteoblasts, reaches the maximal level in immature osteoblasts, and is downregulated in mature osteoblasts. Runx2 enhances	Extracellular matrix Osteoclast Cytokine RUNX2

	osteoblast progenitor proliferation progenitors by regulating <i>Fgfr2</i> and <i>Fgfr3</i> and through direct regulation of hedgehog ( <i>Ihh</i> , <i>Gli1</i> , and <i>Ptch1/Lims2</i> ), Fgf ( <i>Fgfr2</i> and <i>Fgfr3</i> ), Wnt ( <i>Tcf7</i> , <i>Wnt10b</i> , and <i>Wnt1</i> ), and Pthlh ( <i>Pthr1</i> ) signaling pathway genes. Runx2 also induces the expression of major bone matrix protein genes, including <i>Spp1</i> together with <i>Col1a1</i> , <i>Ibsp</i> , <i>Bglap2</i> , and <i>Fn1</i> .	
TFPI2	<i>Tissue factor pathway inhibitor 2</i> . This serine proteinase inhibitor inhibits a variety of serine proteases including factor VIIa/tissue factor, factor Xa, plasmin, trypsin, chymotrypsin and plasma kallikrein. The coagulation system is involved in bone homeostasis; the TFPI2 protein may interact with TGF/BMP signaling (PMID 19450457).	Proteinase inhibitor Coagulation TGF/BMP
TINAGL1	<i>Tubulointerstitial nephritis antigen like 1</i> . The protein is a secreted glycoprotein. The encoded protein is regarded as a matrix protein, and animal models suggest that reduced function/level of Tinagl1 is associated with early craniofacial defects due to its Wnt-interacting effects (PMID 27243669).	Extracellular matrix Wnt
TRAF6	<ul style="list-style-type: none"> <li><i>TNF receptor-associated factor 6</i>. This gene encodes a member of the TNF receptor associated factor (TRAF) family of adaptor proteins that mediate signaling events from members of the TNF receptor and Toll/IL-1 receptor families to activate transcription factors such as NFκB and AP-1. Mice deficient in this protein exhibit osteopetrosis. This protein possesses ubiquitin ligase activity.</li> <li>Bone remodeling is a tightly coupled process consisting of repetitive cycles of bone resorption and formation. Both processes are governed by mechanical signals, including RANKL/RANK/OPG/TRAF6 signaling (PMID 17465692, 25368616).</li> </ul>	TNF NFκB Osteopetrosis Bone remodeling
VEGFC	<ul style="list-style-type: none"> <li><i>Vascular endothelial growth factor C</i>. The encoded protein promotes angiogenesis and endothelial cell growth, it can affect vessel permeability and can activate VEGFR-2 and VEGFR-3 receptors.</li> <li>An animal model of osteoblast differentiation suggests that expression of the VEGF receptors (VEGFR1, VEGFR2, VEGF165R/neuropilin) coincided with expression of their ligands, being maximally expressed during mineralization. The expression profile of VEGFs/VEGF receptors suggests that they contribute to the regulation of bone remodeling by attracting endothelial cells and osteoclasts and by stimulating osteoblast differentiation (PMID 10803575).</li> </ul>	Angiogenesis Osteoblast differentiation Bone remodelling

**Table S3.** Description of proteins expressed only in osteoblasts and included in the osteoblast-specific protein interaction network. The table gives a list (corresponding gene name, protein name) of those osteoblast-specific proteins that were included in the protein interaction networks presented in Figure 3. The keyword information is based on Uniprot (<https://www.uniprot.org/>), the gene card database (<https://www.genecards.org/>) and selected references from the databases.

PROTEIN	DESCRIPTION	KEY WORDS
APOA1	<i>Apolipoprotein A1</i> . This gene encodes the major protein component of high-density lipoprotein (HDL) in plasma. The encoded preproprotein is proteolytically processed to generate the mature protein, which promotes cholesterol efflux from tissues to the liver for excretion, and is a cofactor for lecithin cholesterol acyltransferase, an enzyme responsible for the formation of most plasma cholesteryl esters. Finally, APOA1 is important for induction of osteoblast differentiation (PMID 27359105).	Lipid metabolism
APOE	<i>Apolipoprotein E</i> . The protein is a major apoprotein of the chylomicron and is essential for triglyceride and cholesterol metabolism. The encoded protein is important for osteoblast differentiation and bone healing (PMID 31534056, 27074899).	Lipid metabolism
BRI3	<i>Brain protein I3</i> . Expressed in a wide range of organs. This molecular chaperon is a downstream target of Wnt signaling (PMID 20538055, 32555390).	Wnt Chaperon
BST2	<i>Bone marrow stromal cell antigen 2</i> . The protein is important for osteoblast differentiation, this effect is mediated via BMP2 (PMID 27359105). The protein may also play a role in pre-B-cell growth.	Osteoblast differentiation BMP2

B4GALT5	<i>Beta-1,4-galactosyltransferase 5</i> . This type II membrane-bound glycoprotein that appear to have exclusive specificity for UDP-galactose; it transfers galactose to similar acceptor sugars. It has an N-terminal hydrophobic signal sequence that directs the protein to the Golgi apparatus. Galactose administrations alters bone metabolism and leads to bone loss in animal models (PMID 32497865).	Galactose Golgi Metabolism Bone loss
CD14	<i>CD14 molecule</i> . The encoded protein is a surface antigen that cooperates with other proteins to inhibit severe inflammatory responses. CD14 is a coreceptor for Toll like receptor 4 (TLR4), and these receptors are involved in the regulation of osteoblast metabolism and bone mineral density as well as development of inflammation-associated osteoporosis (PMID 29867550).	Osteoblast TLR4 Mineralization
CD36	<i>CD36 molecule</i> . The encoded protein serves as a receptor for thrombospondin, widely distributed proteins involved in adhesive processes. CD36 binds to collagen, thrombospondin and phospholipids. It binds long chain fatty acids and may function in the transport and/or as a regulator of fatty acid transport.	Adhesion Thrombospondin Collagen Fatty acid
CHI3L1	<i>Chitinase 3 like 1</i> . Chitinases catalyze the hydrolysis of chitin, and this gene encodes a glycoprotein member of the glycosyl hydrolase 18 family. The protein is secreted and is thought to play a role in tissue remodeling. This chitinase is a marker of MSC differentiation, including differentiation in the direction of mature mesenchymal phenotypes, including osteocytes (PMID 23671604). It supports angiogenesis possibly through stimulation of proinflammatory and pro-angiogenic cytokines/chemokines (e.g. CCL2, CXCL2) (PMID 26733160).	Glycosyl hydrolase Tissue remodeling Angiogenesis Osteocyte differentiation Chemokine
CHMP1B	<i>Charged multivesicular body protein 1B</i> . CHMP1B belongs to the chromatin-modifying protein/charged multivesicular body protein (CHMP) family. These proteins are components of ESCRT-III (endosomal sorting complex required for transport III), a complex involved in degradation of surface receptor proteins and formation of endocytic multivesicular bodies. Some CHMPs have both nuclear and cytoplasmic/vesicular distributions, and CHMP1A is required for regulation of cell cycle progression. ESCRT-III associated proteins (e.g. CHMP1B) are important for intracellular transport and receptor trafficking, including BMP receptors (PMID 31587092).	Protein degradation Chromatin Cell cycle Receptor trafficking BMP receptors
CHMP2B	<i>Charged multivesicular body protein 2B</i> . This component of the heteromeric ESCRT-III complex (Endosomal Sorting Complex Required for Transport III) that functions in the recycling or degradation of cell surface receptors. ESCRT-III functions in the concentration and invagination of ubiquitinated endosomal cargos into intraluminal vesicles. ESCRT-III associated proteins are important for intracellular transport and receptor trafficking, including BMP receptors (PMID 31587092).	Ubiquitin Endosome Receptor trafficking
ELN	<i>Elastin</i> . The encoded protein is one of the two components of elastic fibers that comprise part of the extracellular matrix. Degradation products of the encoded protein, known as elastin-derived peptides or elastokines, bind the elastin receptor complex. Elastokines can also contribute to cancer progression. Elastin is a marker of the osteogenic capacity of osteoblastic cells together with ALPL, COL1A2, DCN, ELN and RUNX2, and their effects are possibly mediated through regulation of TGF- $\beta$ 1 pathway signaling (PMID 27711115, 24984278). Elastin is involved in osteoblast mineralization of extracellular matrix (PMID 16817211).	Extracellular matrix Osteogenesis TGF- $\beta$ 1
ENTPD1/C D39	<i>Ectonucleoside triphosphate diphosphohydrolase 1</i> . The protein encoded is a plasma membrane protein that hydrolyzes extracellular ATP and ADP to AMP. CD39/ENTPD1 drives the sequential hydrolysis of both adenosine triphosphate (ATP) and adenosine diphosphate (ADP) to adenosine monophosphate (AMP). AMP can be further degraded by CD73/ecto-5'-nucleotidase, to adenosine. (PMID 17502665).	Plasma membrane ATP/ADP hydrolysis

EPHA4	<p><i>EPH receptor A4</i>. This gene belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors have been implicated in mediating developmental events. The ephrin receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. Multiple transcript variants encoding different isoforms have been found for this gene.</p> <p>See EPHB4 for a detailed comment.</p>	Ephrine receptor
EPHB4	<p><i>EPH receptor B4</i>. Ephrin receptors and their ligands, the ephrins, mediate numerous developmental processes. Based on their structures and sequence relationships, ephrins are divided into the ephrin-A (EFNA) class, which are anchored to the membrane by a glycosylphosphatidylinositol linkage, and the ephrin-B (EFNB) class, which are transmembrane proteins. The Eph family of receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. Ephrin receptors make up the largest subgroup of the receptor tyrosine kinase (RTK) family. The protein encoded by this gene binds to ephrin-B2.</p> <p>EphA2 was expressed in osteoclast precursors and osteoblasts (PMID19299512). Bones cannot be properly formed or maintained without cell-cell interactions through ephrin ligands and Eph receptors; EphA2 is involved in the regulation of osteoblastic bone formation, mineralization and remodeling (PMID 22660185, 21724962, 25604255). Osteoblasts stimulated with ephrin-A2 in vitro significantly altered osteoblast gene expression including expression of RUNX2, ALPL and ALP (PMID 21724962). Furthermore, the bidirectional ephrin/receptor signaling then regulates bone remodeling at the initiation phase, and these effects of EphA2 are possibly mediated by RhoA (PMID 19299512). Finally, Local exosome-mediated communication between cells has an important role in bone homeostasis, and exosomes can specifically recognize osteoblasts through the interaction between ephrinA2 and EphA2 (PMID 27462462).</p>	Ephrine receptor Bone remodeling Osteoblast Mineralization RUNX2 RhoA Exosomes
FAM20A	<p><i>FAM20A Golgi associated secretory pathway pseudokinase</i>. This protein is likely secreted and may function in hematopoiesis.</p> <p>The family with sequence similarity 20 (Fam20) kinases phosphorylate extracellular substrates and play important roles in biomineralization (PMID 23754375). Fam20C is the Golgi casein kinase that phosphorylates secretory pathway proteins within Ser-x-Glu/pSer motifs. Mutations in Fam20C cause Raine syndrome, an osteosclerotic bone dysplasia (PMID 23754375), and FAM20A binds to and regulates FAM20C localization (PMID 27292199).</p>	Golgi Pseudokinase Mineralization Golgi casein kinase
FBN2	<p><i>Fibrillin-2</i>. The encoded protein is a component of connective tissue microfibrils and may be involved in elastic fiber assembly. Fibrillin-2 control bone formation by regulating osteoblast differentiation through the differential modulation of endogenous TGF<math>\beta</math> and bone morphogenetic protein signals, and through these mechanisms they become important regulators of bone remodeling (PMID 20729550). The protein seems important for bone mineralization and development of osteoporosis (PMID 30690781), it is also important for TGF<math>\beta</math> and BMP bioavailability during bone formation (PMID 20855508).</p>	Extracellular matrix Mineralization Osteoporosis TGF- $\beta$ 1, BMP Osteoblast differentiation
FGG	<p><i>Fibrinogen gamma chain</i>. The encoded protein is the gamma component of the fibrinogen coagulation factor.</p> <p>The coagulation cascade may be important for regulation of bone mineralization, possibly through thrombin-mediated effects on osteoblasts (PMID 29857920).</p>	Mineralization
FYN	<p><i>FYN proto-oncogene, Src family tyrosine kinase</i>. This gene encodes a membrane-associated tyrosine kinase that has been implicated in the control of cell growth. The protein associates with the p85 subunit of phosphatidylinositol 3-kinase and interacts with the fyn-binding protein.</p>	Osteoblast differentiation

	An experimental study suggests that Lyn, Fyn, and FGFR2 interacted with the ubiquitin ligase c-Cbl and ubiquitin in the regulation of osteoblast differentiation (PMID 15190072).	
GUSB	<i>Glucuronidase beta</i> . This gene encodes a hydrolase that degrades glycosaminoglycans, including heparan sulfate, dermatan sulfate, and chondroitin-4,6-sulfate. The enzyme forms a homotetramer that is localized to the lysosome.	Lysosome
HLA-A HLA-B HLA-C HLA-DRB1 HLA-F	HLA molecules are important in the regulation of both innate and adaptive immunity.	Immunoregulation Inflammation
KCNAB2	<i>Potassium voltage-gated channel subfamily A regulatory beta subunit 2</i> . Voltage-gated potassium channels represent the most complex class of voltage-gated ion channels. This gene encodes a member of the potassium channel, voltage-gated, shaker-related subfamily.	Potassium channel
KRT77	<i>Keratin 77</i> . Keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells and are subdivided into epithelial keratins and hair keratins. This gene encodes an epithelial keratin. Keratins are parts of the cytoskeleton (PMID 15495250, 21893596).	Keratin Cytoskeleton
KRT80	<i>Keratin 80</i> . This gene's expression profile shows that it encodes a type II epithelial keratin, although structurally the encoded protein is more like a type II hair keratin. This protein is involved in cell differentiation. Keratins are parts of the cytoskeleton (PMID 15495250, 21893596).	Keratin Cytoskeleton
LMNA	<i>Laminin A/C</i> . The nuclear lamina consists of a two-dimensional matrix of proteins located next to the inner nuclear membrane. The lamin family of proteins make up the matrix. Lamin proteins are thought to be involved in nuclear stability, chromatin structure and gene expression. Lamin A/C is fundamental for and differentiation of MSCs that are progenitors of osteoblasts; laminins thereby become important for bone homeostasis (PMID 32466483).	Nuclear envelope Transcription Differentiation Bone homeostasis
LYZ	<i>Lysozyme</i> . This gene encodes human lysozyme. The protein may be a modulator and adaptor of Mucin 5B (PMID 12568489).	Mucin 5B
MAP3K7	<i>Mitogen-activated protein kinase 7</i> . The protein is a member of the serine/threonine protein kinase family. This kinase mediates the signaling transduction induced by TGF $\beta$ and BMP and controls a variety of cell functions including transcription regulation and apoptosis. In response to IL-1, this protein forms a kinase complex including TRAF6, MAP3K7P1/TAB1 and MAP3K7P2/TAB2; this complex is required for the activation of NF $\kappa$ B. This kinase can also activate MAPK8/JNK, MAP2K4/MKK4. The protein is probably a modulator of osteoblastic differentiation (PMID 29753717).	Kinase Signal transduction TGF BMP TRAF6
MFAP2	<i>Microfibrillar-associated protein 2</i> . This protein is a major part of elastin-associated microfibrils. Microfibrillar-associated proteins (MFAPs) are extracellular matrix glycoproteins, which play a role in microfibril assembly, and MFAP2-deficient mice exhibit progressive osteopenia with elevated RANKL expression (PMID 32572962).	Extracellular matrix Osteogenesis RANKL
MUC5B	<i>Mucin 5B, oligomeric mucus/gel-forming</i> . This gene encodes a member of the mucin family of proteins, which are highly glycosylated macromolecular components. It can be expressed by chondrosarcoma (PMID 29138803).	Mucin Lysozyme
NTPCR	<i>Nucleoside-triphosphatase, cancer-related</i> . The protein is a non-specific nucleoside triphosphatase.	Nucleoside
PIGR	<i>Polymeric immunoglobulin receptor</i> . This gene is a member of the immunoglobulin superfamily. The encoded poly-Ig receptor binds polymeric immunoglobulin molecules and is secreted.	Protein secretion

PRKCQ	<p><i>Protein kinase C theta</i>. Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. Each member of the PKC family has a specific expression profile This protein is a calcium-independent and phospholipid-dependent protein kinase that is required for activation of NFκB and AP-1.</p> <p>A previous study suggests that PKC βII together with and PKC δ/θ mediate BMP-4-induced osteoblastic differentiation (PMID 20971075).</p>	NFκB AP-1 Osteoblast differentiation
PRUNE2	<p><i>Prune homolog 2 with BCH domain</i>. The encoded protein has several roles, including suppression of RhoA activity. A high molecular weight isoform colocalizes with Adaptor protein complex 2, beta-Adaptin and endodermal markers, suggesting an involvement in post-endocytic trafficking.</p> <p>Notch signaling seems to upregulate the transcription of PRUNE2 (PMID 28300657). Previous studies suggest that this molecule is involved in regulation of cell differentiation and apoptosis (PMID 22710163).</p>	RhoA Notch Differentiation Apoptosis
PSEN2	<p><i>Presenilin 2</i>. The encoded protein is possibly involved in the cleavage of the Notch receptor such that, they either directly regulate gamma-secretase activity, or themselves act are protease enzymes.</p> <p>The protein is induced during osteoblastic differentiation together with other key regulators of Wnt, TGFβ and Notch signaling (PMID 12203032, 23600707). The protein is also involved in the regulation of autophagy and mitochondrial functions (PMID 30412492).</p>	Notch Osteoblast differentiation
SPP1	<p><i>Secreted phosphoprotein 1/osteopontin</i>. The encoded protein is involved in the attachment of osteoclasts to the mineralized bone matrix. It is secreted and binds hydroxyapatite. The osteoclast vitronectin receptor may be involved in the binding to this protein. This protein is also a cytokine that upregulates expression of interferon-gamma and interleukin-12.</p> <p>RUNX2 induces the expression of this gene during osteoblastic differentiation (PMID 30987410).</p>	RUNX2 Extracellular release Osteoclast
TANK	<p><i>TRAF family member associated NFκB activator</i>. The TRAF (tumor necrosis factor receptor-associated factor) protease family associate with and transduce signals from members of the TNF superfamily. The protein encoded by this gene is found in the cytoplasm and can bind to TRAF1, TRAF2, or TRAF3, thereby inhibiting TRAF function by sequestering the TRAFs in a latent state in the cytoplasm and can inhibit NFκB activation.</p> <p>The protein is induced during osteoblast differentiation; it inhibits NFκB through inhibition of TRAF6 activation, is a negative regulator osteoclastogenesis and is thereby important for bone remodeling (PMID 22773835).</p>	Protease TRAF6 inhibition NFκB Bone remodeling
TRAF6	<ul style="list-style-type: none"> <li><i>TNF receptor-associated factor 6</i>. This gene encodes a member of the TNF receptor associated factor (TRAF) family of adaptor proteins that mediate signaling events from members of the TNF receptor and Toll/IL-1 receptor families to activate transcription factors such as NFκB and AP-1. Mice deficient in this protein exhibit osteopetrosis. This protein possesses ubiquitin ligase activity.</li> <li>Bone remodeling is a tightly coupled process consisting of repetitive cycles of bone resorption and formation. Both processes are governed by mechanical signals, including RANKL/RANK/OPG/TRAF6 signaling (PMID 17465692, 25368616).</li> </ul>	TNF NFκB Osteopetrosis Bone remodeling MAP3K7
VEGFC	<ul style="list-style-type: none"> <li><i>Vascular endothelial growth factor C</i>. The encoded protein promotes angiogenesis and endothelial cell growth, it can affect vessel permeability and can activate VEGFR-2 and VEGFR-3 receptors.</li> <li>An animal model of osteoblast differentiation suggests that expression of the VEGF receptors (VEGFR1, VEGFR2, VEGF165R/neuropilin) coincided with expression of their ligands, being maximally expressed during mineralization. The</li> </ul>	Angiogenesis Osteoblast differentiation Bone remodelling

expression profile of VEGFs/VEGF receptors suggests that they contribute to the regulation of bone remodeling by attracting endothelial cells and osteoclasts and by stimulating osteoblast differentiation (PMID 10803575).

**Table S4.** Proteins expressed only in MSCs. The table gives a list (corresponding gene name, protein name) of all proteins that could be quantified in MSCs but were not detected for any of the osteoblast donors.

<i>AASS</i>	Alpha-aminoadipic semialdehyde synthase, mitochondrial;Lysine ketoglutarate reductase;Saccharopine dehydrogenase
<i>ACTR6</i>	Actin-related protein 6
<i>ALAS1</i>	5-aminolevulinatase synthase, nonspecific, mitochondrial
<i>AMOTL1</i>	Angiomotin-like protein 1
<i>AP1B1</i>	AP-1 complex subunit beta-1
<i>ARID3A</i>	AT-rich interactive domain-containing protein 3A
<i>ATR</i>	Serine/threonine-protein kinase ATR
<i>BIN3</i>	Bridging integrator 3
<i>BRD9</i>	Bromodomain-containing protein 9
<i>BRMS1L</i>	Breast cancer metastasis-suppressor 1-like protein
<i>C19orf47</i>	Uncharacterized protein C19orf47
<i>C1QTNF3</i>	Complement C1q tumor necrosis factor-related protein 3
<i>C5orf30</i>	UNC119-binding protein C5orf30
<i>C7orf55;LUC7L2</i>	UPF0562 protein C7orf55;Putative RNA-binding protein Luc7-like 2
<i>CBX6;CBX8</i>	Chromobox protein homolog 6;Chromobox protein homolog 8
<i>CCDC115</i>	Coiled-coil domain-containing protein 115
<i>CDK17;CDK16</i>	Cyclin-dependent kinase 17;Cyclin-dependent kinase 16
<i>CENPV</i>	Centromere protein V
<i>CEP41</i>	Centrosomal protein of 41 kDa
<i>CEPT1</i>	Choline/ethanolaminephosphotransferase 1
<i>CHPT1</i>	Cholinephosphotransferase 1
<i>CLNS1A</i>	Methylosome subunit pICln
<i>COQ9</i>	Ubiquinone biosynthesis protein COQ9, mitochondrial
<i>CPNE7</i>	Copine-7
<i>CSNK1D</i>	Casein kinase I isoform delta
<i>CYB561D2</i>	Cytochrome b561 domain-containing protein 2
<i>DCAF7</i>	DDB1- and CUL4-associated factor 7
<i>EPB41L5</i>	Band 4.1-like protein 5
<i>FAM96A</i>	MIP18 family protein FAM96A
<i>FBLN2</i>	Fibulin-2
<i>FBXW9</i>	F-box/WD repeat-containing protein 9
<i>FLII</i>	Protein flightless-1 homolog
<i>FOXP2</i>	Forkhead box protein K2
<i>GDF15</i>	Growth/differentiation factor 15
<i>GTF2E2</i>	Transcription initiation factor IIE subunit beta
<i>HAUS1</i>	HAUS augmin-like complex subunit 1
<i>HCFC2</i>	Host cell factor 2
<i>HPDL</i>	4-hydroxyphenylpyruvate dioxygenase-like protein
<i>IER3IP1</i>	Immediate early response 3-interacting protein 1
<i>INO80C</i>	INO80 complex subunit C
<i>INTS5</i>	Integrator complex subunit 5
<i>KANK1</i>	KN motif and ankyrin repeat domain-containing protein 1
<i>KCTD21</i>	BTB/POZ domain-containing protein KCTD21

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<i>KLHDC4</i>	Kelch domain-containing protein 4
<i>KRT82</i>	Keratin, type II cuticular Hb2
<i>LCMT2</i>	tRNA wybutosine-synthesizing protein 4
<i>LRWD1</i>	Leucine-rich repeat and WD repeat-containing protein 1
<i>LYPLA2</i>	Acyl-protein thioesterase 2
<i>MAT2B</i>	Methionine adenosyltransferase 2 subunit beta
<i>METTL2A</i>	Methyltransferase-like protein 2A
<i>MEX3C</i>	RNA-binding E3 ubiquitin-protein ligase MEX3C
<i>MFSD5</i>	Molybdate-anion transporter
<i>MGAT5</i>	Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase A
<i>MIER1</i>	Mesoderm induction early response protein 1
<i>MKL2</i>	MKL/myocardin-like protein 2
<i>MLLT11</i>	Protein AF1q
<i>MORC2</i>	MORC family CW-type zinc finger protein 2
<i>MRPL53</i>	39S ribosomal protein L53, mitochondrial
<i>MTG1</i>	Mitochondrial ribosome-associated GTPase 1
<i>MT-ND5</i>	NADH-ubiquinone oxidoreductase chain 5
<i>MYO10</i>	Unconventional myosin-X
<i>MYO1B</i>	Unconventional myosin-Ib
<i>NACAP1</i>	Putative nascent polypeptide-associated complex subunit alpha-like protein
<i>NHSL1</i>	NHS-like protein 1
<i>NOP10</i>	H/ACA ribonucleoprotein complex subunit 3
<i>NRG1</i>	Pro-neuregulin-1, membrane-bound isoform;Neuregulin-1
<i>NRM</i>	Nurim
<i>NSMCE2</i>	E3 SUMO-protein ligase NSE2
<i>NT5C3B</i>	7-methylguanosine phosphate-specific 5-nucleotidase
<i>OSTM1</i>	Osteopetrosis-associated transmembrane protein 1
<i>PCDHGB5</i>	Protocadherin gamma-B5
<i>PDCD1LG2</i>	Programmed cell death 1 ligand 2
<i>PINX1</i>	PIN2/TERF1-interacting telomerase inhibitor 1
<i>PIP5K1A</i>	Phosphatidylinositol 4-phosphate 5-kinase type-1 alpha
<i>PMEPA1</i>	Protein TMEPAI
<i>POLR3D</i>	DNA-directed RNA polymerase III subunit RPC4
<i>PRIM2</i>	DNA primase large subunit
<i>PRPF18</i>	Pre-mRNA-splicing factor 18
<i>PTPMT1</i>	Phosphatidylglycerophosphatase and protein-tyrosine phosphatase 1
<i>RAB6B</i>	Ras-related protein Rab-6B
<i>RC3H1</i>	Roquin-1
<i>SAP130</i>	Histone deacetylase complex subunit SAP130
<i>SEPN1</i>	Selenoprotein N
<i>SESN2</i>	Sestrin-2
<i>SFXN4</i>	Sideroflexin-4
<i>SH3BP5L</i>	SH3 domain-binding protein 5-like
<i>SH3PXD2A</i>	SH3 and PX domain-containing protein 2A
<i>SHKBP1</i>	SH3KBP1-binding protein 1
<i>SLC16A6</i>	Monocarboxylate transporter 7
<i>SLC2A10</i>	Solute carrier family 2, facilitated glucose transporter member 10
<i>SLC6A9</i>	Sodium- and chloride-dependent glycine transporter 1
<i>SMAD1</i>	Mothers against decapentaplegic homolog 1

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SMARCD3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3
SMPDL3A	Acid sphingomyelinase-like phosphodiesterase 3a
SRRD	SRR1-like protein
SYNJ2BP	Synaptojanin-2-binding protein
TBX15	T-box transcription factor TBX15
TBX3	T-box transcription factor TBX3
TCF12	Transcription factor 12
TFB1M	Dimethyladenosine transferase 1, mitochondrial
TIMM17B	Mitochondrial import inner membrane translocase subunit Tim17-B
TMED8	Protein TMED8
TNFAIP8L3	Tumor necrosis factor alpha-induced protein 8-like protein 3
TOX2	TOX high mobility group box family member 2
UBE2T	Ubiquitin-conjugating enzyme E2 T
USP36	Ubiquitin carboxyl-terminal hydrolase 36
VPRBP	Protein VPRBP
WASF1	Wiskott-Aldrich syndrome protein family member 1
YAF2	YY1-associated factor 2
ZMAT2	Zinc finger matrin-type protein 2
ZNF280C	Zinc finger protein 280C

**Table S5.** Proteins involved in differentiation/development of osteoblasts and showing significant differences when comparing osteoblasts with MSCs. Only proteins with detectable levels both for osteoblasts and MSCs for at least three donors were included in the statistical comparison. Based on the QuickGO database 60 proteins involved in differentiation/development of osteoblasts have been identified, and 11 of these proteins could be quantified in our present study and showed significant differences between osteoblasts and MSCs. Three additional proteins showing significant differences were identified based on the Uniprot database when using a similar strategy. The 14 proteins are listed below (corresponding gene names) together with the protein name and a brief description of the protein together with keywords describing the most important/relevant biological functions. The data presented in the description/key word sections are based on information from the gene card database together with selected references from the gene database.

Protein	Description	Keywords
CEBPB	<i>CCAAT enhancer binding protein beta</i> . This gene encodes a transcription factor. The protein seems to be important for the regulation/balance of osteogenic versus adipogenic differentiation (PMID 28240601).	Transcription Osteoblast differentiation
CYR61 (CCN1)	<i>CCN family member 1/cellular communication network factor 1</i> . This secreted protein is growth factor-inducible and promotes adhesion. It interacts with several integrins and with sulfate proteoglycans. This protein also plays a role in cell proliferation, differentiation, angiogenesis, apoptosis and extracellular matrix formation. This matricellular protein serves regulatory rather than structural roles. It is found in mineralized tissues and influences bone healing <i>in vivo</i> and osteogenic differentiation <i>in vitro</i> . It exerts its effects on mature osteoblasts/osteocytes to modulate the bone mass and can alter the expression of the genes RANKL, VEGFA and SOST. The increased SOST expression is important for regulation of the bone mass through its effects on Wnt signaling; additional angiogenic effects are also important for CYR61 effects on bone mass (PMID 29351359). CYR61/CCN1 regulates the expression of PTH1R through interaction with the $\alpha$ V $\beta$ 3 and/or $\alpha$ V $\beta$ 5 integrins; the $\alpha$ V $\beta$ 3/ $\beta$ 5-	Osteoblast differentiation Extracellular matrix Angiogenic Adhesion Integrins Wnt PTH BMP2

	<p>binding domain of CCN1 is required for the PTH associated anabolic activity in bone cells (PMID 32634285). CYR61 also up-regulates BMP-2 protein expression, resulting in increased proliferation and osteoblastic differentiation through activation of <math>\alpha V\beta 3</math> integrin/integrin-linked kinase/ERK signaling (PMID 20675382)</p> <p>Gene expression profiling of Wnt3A stimulated MSCs showed increased expression of 220 genes, including CYR61 together with other members of the CNN family (CCN2/connective tissue growth factor, CCN5/WISP2). CCN1/Cyr61 was then upregulated at the early stage of Wnt3A stimulation. It is a direct target of canonical Wnt/<math>\beta</math>-catenin signaling and seems to have an important role in Wnt3A-induced osteoblast differentiation from MSCs (PMID 16581771).</p> <p>Finally, CYR61 is an inhibitor of osteoclast formation; given that CYR61 also stimulates osteoblasts it may therefore be a bifunctional local regulator of bone remodeling (PMID 17823253)</p>	
DDX21	<p><i>DExD-box helicase 21</i>. DEAD box proteins are putative RNA helicases. This protein unwinds double-stranded RNA, folds single-stranded RNA, and may play important roles in ribosomal RNA biogenesis, RNA editing, RNA transport, and general transcription.</p> <p>The protein has cell type specific effects; it is important in regulation of hematopoiesis, but it is not known whether the effect on hematopoiesis/bone marrow cells involve or influence osteoblasts or other mesenchymal cells (PMID 29364875).</p>	<p>RNA helicase Ribosome Transcription</p>
ENPP1	<p><i>Ectonucleotide pyrophosphatase/phosphodiesterase 1</i>. This gene is a member of the ecto-nucleotide pyrophosphatase-phosphodiesterase family. The encoded protein is a type II transmembrane glycoprotein, it has broad specificity and cleaves several substrates, including phosphodiester pyrophosphate bonds of nucleotides and nucleotide sugars.</p> <p>This protein is important for the regulation of osteoblastic functions and phosphate metabolism/transport, the formation of matrix vesicles and bone mineralization (PMID 29411103). It may also be involved in osteoblastic differentiation (PMID 30467547).</p>	<p>Membrane protein Nucleotide cleavage Phosphate and phosphodiester bonds Bone mineralization Differentiation</p>
EPHA2	<p><i>Ephrin type-A receptor 2</i>. This protein belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. Receptors in the EPH subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. The ephrin receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. This gene encodes a protein that binds ephrin-A ligands.</p> <p>EphA2 was expressed in osteoclast precursors and osteoblasts (PMID19299512). Bones cannot be properly formed or maintained without cell-cell interactions through ephrin ligands and Eph receptors; EphA2 is involved in the regulation of osteoblastic bone formation, mineralization and remodelling</p>	<p>Tyrosine kinase Transcription RhoA Exosome</p>

	<p>(PMID 22660185, 21724962, 25604255). Osteoblasts stimulated with ephrin-A2 in vitro significantly altered osteoblast gene expression including expression of RUNX2, ALPL and ALP (PMID 21724962).</p> <p>The bidirectional ephrin/receptor signaling then regulates bone remodeling at the initiation phase, and these effects of EphA2 are possibly mediated by RhoA (PMID 19299512). Local exosome-mediated communication between cells has an important role in bone homeostasis, and exosomes can specifically recognize osteoblasts through the interaction between ephrinA2 and EphA2 (PMID 27462462).</p>	
FASN	<p><i>Fatty acid synthase</i>. The enzyme is a multifunctional protein. Its main function is to catalyze the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain saturated fatty acids. Triglyceride metabolism and its regulation by this enzyme seem important in osteoblastic differentiation (PMID 23588618, 23588618).</p>	<p>Triglyceride Metabolism</p>
FBN1	<p><i>Fibrillin-1</i>. The encoded preproprotein is proteolytically processed to generate two proteins including the extracellular matrix component fibrillin-1 and the protein hormone asprosin. Fibrillin-1 is an extracellular matrix glycoprotein that serves as a structural component of calcium-binding microfibrils. These microfibrils provide force-bearing structural support in elastic and nonelastic connective tissue throughout the body. Asprosin regulates glucose homeostasis. FBN1 is a structural component of bone and is constitutively expressed by osteoblasts (PMID 10865210). It differentially regulates TGF-<math>\beta</math> and BMP bioavailability in bone and thereby bone formation (PMID 20855508); this seems to be at least partly mediated by altered Notch signaling in osteoblasts (PMID 21538048).</p>	<p>Extracellular matrix Bone Mineralization TGF<math>\beta</math> BMP Notch</p>
FHL2	<p><i>Four and a half lim domain 2</i>. This protein is a member of the four-and-a-half-LIM-only protein family. FHL2 interacts with many integrins and transcription factors and is important in osteoblast differentiation. Overexpression of FHL2 increases the accumulation of osteoblast differentiation markers and matrix mineralization. Integrin-matrix interaction plays a critical role in osteoblast function possibly through interactions with actin-binding proteins. FHL2 co-localizes with <math>\alpha</math>V<math>\beta</math>5 integrins at the focal adhesion sites. Osteoblasts overexpressing FHL2 exhibited increased adhesion to and migration on matrix proteins, and its stimulation of CREB activity is also dependent on integrins. Furthermore, the expression of osteoblast differentiation markers and Msx2 is upregulated and bone matrix mineralization is increased by FHL2 overexpression. Finally, However, FHL2 is also present in nuclei (PMID 16355270).</p> <p>Wnt signaling is an important pathway that controls the osteogenic differentiation of MSCs, and FHL2 controls MSC osteogenic differentiation as well as bone formation through its cooperation with Wnt/<math>\beta</math>-catenin signaling (PMID 23201222).</p>	<p>Integrin Mineralization Adhesion Migration Wnt</p>

GPNMB (osteactin)	<p><i>Glycoprotein Nmb</i>. The encoded protein is a type I transmembrane glycoprotein also referred to as osteoactivin. GPNMB RNA and protein expression increases during active bone regeneration post-fracture and high expression is seen especially during osteoblast differentiation (PMID 25899717, 24462663). It is expressed in several tissues but is most highly expressed in bone compared with non-osseous tissues (PMID 11746512). The highest osteoblast levels are observed during the later stages of bone matrix production/maturation and mineralization; the expression is correlated with expression of alkaline phosphatase and osteocalcin. Its expression in bone is regarded as osteoblast-specific (PMID 11746512), and MiR-150 is another regulator of GPNMB expression (PMID 32526406).</p>	Extracellular matrix Mineralization ALP
GTPBP4	<p><i>GTP binding protein 4</i>. The GTP-binding proteins are GTPases and function as molecular switches between the active GTP-bound and the GDP-bound state. When an extracellular ligand binds to a G-protein-linked receptor, the receptor changes its conformation to replace GDP with GTP. GTPBP4 can reorganized actin cytoskeleton through reduced RhoA activity (PMID 27720713); Rho GTPase and Wnt signaling have critical roles in bone cell physiology (PMID: 23856044).</p>	GTPase Actin RhoA Wnt
ITGA11	<p><i>Integrin subunit alpha 11</i>. This gene encodes an alpha integrin that binds collagen. Furthermore, Ostelectin/Clec11a is an osteogenic growth factor required to maintain the skeletal bone mass. It acts on osteogenic progenitors to promote their differentiation into osteoblasts. Integrin <math>\alpha 11</math> is an Ostelectin receptor, and ligation of integrin <math>\alpha 11\beta 1</math> activates the Wnt pathway (PMID 30632962).</p>	Integrin Collagen Ostelectin Clec11a Wnt
LOX	<p><i>Lysyl oxidase</i>. This protein is a member of the lysyl oxidase family. Alternative splicing results in multiple transcript variants; at least one of them encodes a preprotein that is proteolytically processed to generate a regulatory propeptide and the mature enzyme. Its copper-dependent amine oxidase activity functions in the crosslinking of collagens and elastin. Collagen cross-linking is regulated by lysyl oxidase. LOX can also influence bone remodeling through effects both on osteoblast and osteoclast activity (PMID 32165300) and is thereby important for maintenance of normal bone turnover (PMID 26627907). LOX-mediated organization of collagen fibers in the extracellular matrix seems to be an important regulator of osteoblastogenesis (PMID 26497171). Finally, LOX is important for mineral nodule formation and osteoblastic differentiation, i.e. expression of the osteoblastic markers type I collagen, bone sialoprotein and Runx2 (PMID 19458888).</p>	Extracellular matrix Collagen Elastin Bone turnover Osteoblast Osteoclast Runx2
TMEM119	<p><i>Transmembrane protein 119</i>. TNMEM119 expression is rapidly induced by PTH and it increases with time in MSCs committed to the osteoblast lineage by BMP-2. Overexpression of TMEM119 was associated with increased levels of Runx2, osteocalcin, alkaline phosphatase, and <math>\beta</math>-catenin. It is regarded as an important molecule in the pathway downstream of PTH</p>	PTH BMP2 Runx2 ALP Osteocalcin Wnt/catenin

and Smad3 signaling in osteoblasts (PMID 21239498, 24362451).

TPM4	<ul style="list-style-type: none"> <li><i>Tropomyosin 4</i>. This member of the tropomyosin family of actin-binding proteins modulates the cytoskeleton of non-muscle cells. It is a dimer of coiled-coil proteins that polymerize end-to-end along the major groove in most actin filaments. The protein provides stability to the filaments and regulates the access of other actin-binding proteins. The protein seems important for bone remodeling/mineralization (PMID 30739146).</li> </ul>	Cytoskeleton Actin Mineralization
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**Table S6.** Proteins showing a significant difference between male and female osteoblast donors. For each protein, the table presents the corresponding gene name, protein name, fold change from the comparison of males/females (log<sub>2</sub> transformed), the *p*-value and the *z*-score.

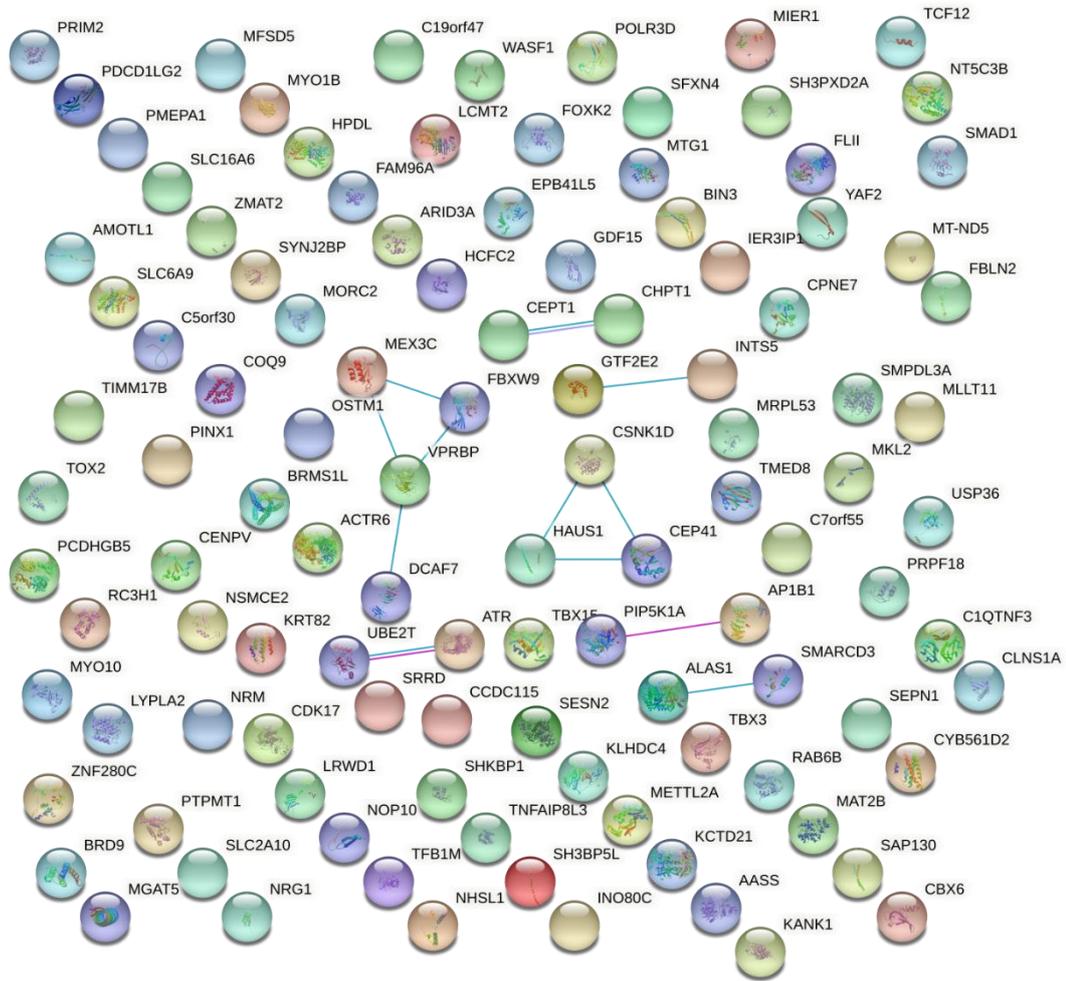
Gene Name	Protein Name	Fold Change Male/Female	<i>p</i> -value	<i>z</i> -score
<i>APOE</i>	Apolipoprotein E	-1,08	0.03467809	0,01676925
<i>ATP6V1F</i>	V-type proton ATPase subunit F	-1,44	0.0431466	0,00212157
<i>BPGM</i>	Bisphosphoglycerate mutase	0,99	0.02434383	0,00833979
<i>C1orf198</i>	Uncharacterized protein C1orf198	1,08	0.00930514	0,00488407
<i>C8orf82</i>	UPF0598 protein C8orf82	1,01	0.02221794	0.00756661
- <i>CHAMP1</i>	Chromosome alignment-maintaining phosphoprotein 1	-0.95	0.03732206	0.03120167
<i>CNN1</i>	Calponin-1	3.48	0.04878159	0
<i>COMMD9</i>	COMM domain-containing protein 9	-0.88	0.04879125	0.04173354
<i>CPNE2</i>	Copine-2	-1.33	0.00877175	0.00405265
<i>DCPS</i>	m7GpppX diphosphatase	-0.99	0.02901125	0.02516671
<i>DIAPH2</i>	Protein diaphanous homolog 2	1.23	0.03345499	0.00156539
<i>EIF2B1</i>	Translation initiation factor eIF-2B subunit alpha	0.88	0.01190378	0.01687002
<i>FAM129A</i>	Protein Niban	-1.31	0.02631683	0.00459538
<i>FAM195B</i>	Protein FAM195B	1.27	0.04580781	0.00117364
<i>FRG1</i>	Protein FRG1	1.01	0.0414877	0.00760812
<i>GPX1</i>	Glutathione peroxidase 1	-2.05	0.02552418	1.9318E-05
<i>IVNS1ABP</i>	Influenza virus NS1A-binding protein	-0.87	0.00559874	0.04442645
<i>LTBP1</i>	Latent-transforming growth factor beta-binding protein 1	-1.30	0.04820586	0.00487305
<i>MEST</i>	Mesoderm-specific transcript homolog protein	1.44	0.00437263	0.0002999
<i>METTL7A</i>	Methyltransferase-like protein 7A	1.31	0.02646207	0.00089613
<i>MIB1</i>	E3 ubiquitin-protein ligase MIB1	-1.57	0.01222727	0.00089299
<i>MRPS27</i>	28S ribosomal protein S27. mitochondrial	-0.99	0.00508971	0.02594273
<i>MRPS6</i>	28S ribosomal protein S6. mitochondrial	-0.97	0.04246351	0.0274775
<i>MYO1C</i>	Unconventional myosin-Ic	0.88	0.01406586	0.01647324
<i>NEGR1</i>	Neuronal growth regulator 1	-0.95	0.03486772	0.03033832
<i>NMT2</i>	Glycylpeptide N-tetradecanoyltransferase 2	-1.41	0.01500637	0.00257116
<i>NOL3</i>	Nucleolar protein 3	-1.16	0.02839415	0.01057664
<i>NTM</i>	Neurotrimin	-1.13	0.00862143	0.01234019
<i>NUMB</i>	Protein numb homolog	-0.90	0.00073514	0.03813309
<i>PAC3IN3</i>	Protein kinase C and casein kinase substrate in neurons protein 3	-0.89	0.0372482	0.04071722
<i>PAPOLA</i>	Poly(A) polymerase alpha	-1.39	0.00978221	0.00286055
<i>PCBP2</i>	Poly(rC)-binding protein 2	2.49	0.02675666	1.866E-09
<i>PDE12</i>	2.5-phosphodiesterase 12	-2.19	0.02395318	5.6127E-06

<i>PKN2</i>	Serine/threonine-protein kinase N2	-1.42	0.00967764	0.00234106
<i>PTP4A2;PTP4A1</i>	Protein tyrosine phosphatase type IVA 2;Protein tyrosine phosphatase type IVA 1	1.00	0.03041913	0.00819236
<i>RAB35</i>	Ras-related protein Rab-35	-0.98	0.02901174	0.02632575
<i>RPL22L1</i>	60S ribosomal protein L22-like 1	1.02	0.01187401	0.00719492
<i>RUNX2</i>	Runt-related transcription factor 2	-1.28	0.02650606	0.00547683
<i>SAR1A</i>	GTP-binding protein SAR1a	0.71	0.02283106	0.04095937
<i>SMG8</i>	Protein SMG8	-1.15	0.02471592	0.01160385
<i>TMEM192</i>	Transmembrane protein 192	0.69	0.02382637	0.04621599
<i>TNS3</i>	Tensin-3	1.04	0.00976532	0.00632694
<i>UBAC2</i>	Ubiquitin-associated domain-containing protein 2	1.23	0.04273789	0.00166153
<i>YIPF6</i>	Protein YIPF6	-0.99	0.03002568	0.02601649
<i>YY1;ZFP42</i>	Transcriptional repressor protein YY1;Zinc finger protein 42 homolog	-1.01	0.02415422	0.02322173

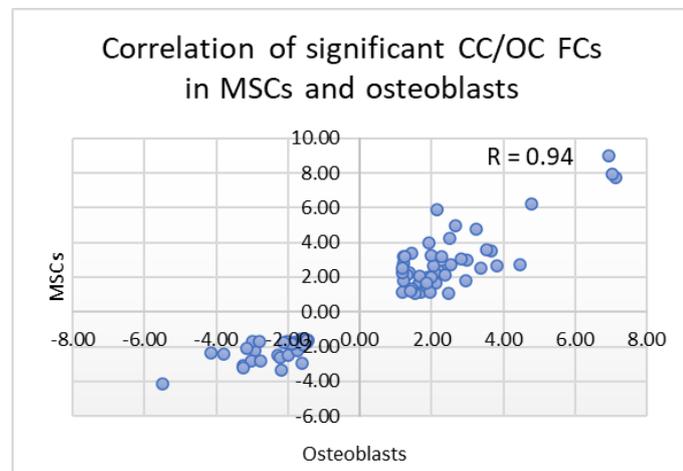
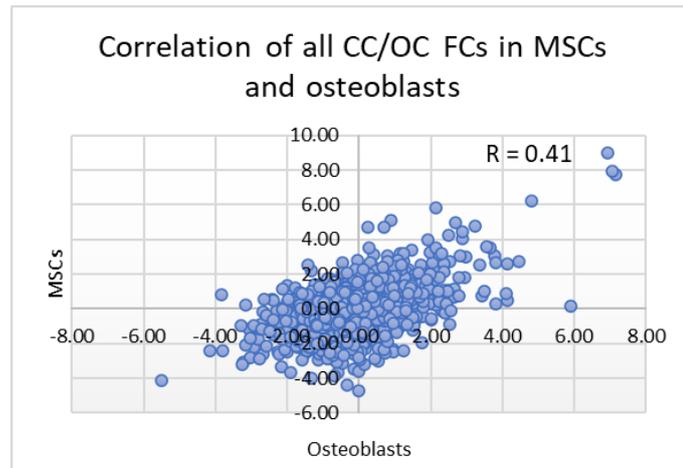
**Table S7.** Proteins showing a significant difference between osteoblast donors above and below 60 years of age. For each protein, the table presents the corresponding gene name, protein name, fold change from the comparison of elderly/younger donors ( $\log_2$ -transformed), the *p*-value and the z score.

Gene Name	Protein Name	Fold Change Elderly/Young	<i>p</i> -value	z-score
<i>ACO1</i>	Cytoplasmic aconitate hydratase	-0.92	0.0189653	0.01811366
<i>ALG1</i>	Chitobiosyldiphosphodolichol beta-mannosyltransferase	1.03	0.03518987	0.00296325
<i>ANKFY1</i>	Rabankyrin-5	-0.76	0.01206478	0.04843309
<i>AXL</i>	Tyrosine-protein kinase receptor UFO	-0.88	0.00683968	0.02407376
<i>BRX1</i>	Ribosome biogenesis protein BRX1 homolog	1.10	0.02451452	0.00177249
<i>CDC27</i>	Cell division cycle protein 27 homolog	-1.31	0.04136941	0.00092621
<i>COL12A1</i>	Collagen alpha-1(XII) chain	1.14	0.03423628	0.00130972
<i>COL1A1</i>	Collagen alpha-1(I) chain	2.01	0.03574206	1.7748E-07
<i>COMMD1</i>	COMM domain-containing protein 1	-0.87	0.02677572	0.02493297
<i>DERA</i>	Deoxyribose-phosphate aldolase	2.36	0.00180329	1.6698E-09
<i>DGKA</i>	Diacylglycerol kinase alpha	-0.84	0.01308061	0.03079946
<i>DNMBP</i>	Dynamin-binding protein	-0.80	0.02436019	0.03800298
<i>DPP4</i>	Dipeptidyl peptidase 4;Dipeptidyl peptidase 4 membrane form	-2.75	0.00556425	2.6305E-12
<i>EDIL3</i>	EGF-like repeat and discoidin I-like domain-containing protein 3	-1.29	0.0120985	0.00110111
<i>EPB41L1</i>	Band 4.1-like protein 1	-1.26	0.00596232	0.00142437
<i>GAR1</i>	H/ACA ribonucleoprotein complex subunit 1	-0.95	0.0294831	0.01517147
<i>GAS6</i>	Growth arrest-specific protein 6	-1.12	0.0119162	0.00442479
<i>GNE</i>	Bifunctional UDP-N-acetylglucosamine 2-epimerase	0.62	0.02649509	0.03857883
<i>GSTM2</i>	Glutathione S-transferase Mu 2	-1.47	0.0354085	0.00021246
<i>HLA-A</i>	HLA class I histocompatibility antigen. A-69 alpha chain	-1.21	0.01623657	0.00216293
<i>L3HYPDH</i>	Trans-3-hydroxy-L-proline dehydratase	-1.01	0.03641763	0.01005164
<i>METTL7A</i>	Methyltransferase-like protein 7A	-1.31	0.03613517	0.00095799
<i>MYCBP2</i>	E3 ubiquitin-protein ligase MYCBP2	-0.85	0.01300965	0.02860529
<i>MYO9B</i>	Unconventional myosin-IXb	-0.97	0.02360738	0.01279615
<i>NUP43</i>	Nucleoporin Nup43	1.01	0.01880347	0.00333926
<i>PPL</i>	Periplakin	-1.98	0.04990482	5.5718E-07
<i>PTPRF</i>	Receptor-type tyrosine-protein phosphatase F	0.63	0.00363919	0.03596013
<i>RPL38</i>	60S ribosomal protein L38	3.31	0.03381556	0
<i>SCRN1</i>	Secernin-1	-1.05	0.03716872	0.00754678

<i>SEC11A</i>	Signal peptidase complex catalytic subunit SEC11A	1.67	0.00994822	8.9979E-06
<i>SH2D4A</i>	SH2 domain-containing protein 4A	-1.21	0.02382157	0.00223084
<i>SLC8A1</i>	Sodium/calcium exchanger 1	1.53	0.03329056	4.0917E-05
<i>STRN</i>	Striatin	-0.79	0.03976304	0.03907754
<i>TIPRL</i>	TIP41-like protein	-0.86	0.0305352	0.02721873
<i>TRADD</i>	Tumor necrosis factor receptor type 1-associated DEATH domain protein	-0.84	0.00406154	0.02943871
<i>UAP1L1</i>	UDP-N-acetylhexosamine pyrophosphorylase-like protein 1	-0.84	0.00522373	0.03060842
<i>UROS</i>	Uroporphyrinogen-III synthase	-0.93	0.03501364	0.01668723
<i>VPS45</i>	Vacuolar protein sorting-associated protein 45	0.61	0.01243271	0.03915107
<i>WDR75</i>	WD repeat-containing protein 75	1.34	0.00861015	0.00024536



**Figure S1.** Network analysis of MSC-specific proteins, i.e. proteins only quantified for MSCs but not for any of the osteoblast population. The network was generated by the String database.



**Figure S2.** Scatter plot of the fold change (FC) for cells cultured in suboptimal serum-free IMDM medium (CC, cultured cells) vs the original cell (OC); a presentation of the fold changes (FCs) for osteoblasts and MSCs. The upper figure presents the results for 3677 proteins with fold change pairs in at least three donor samples for each cell type, and each dot represents a protein (Pearson  $R = 0.41$ ). The lower plot presents the results for the 87 proteins which were significantly altered by the suboptimal IMDM culture in both cell types (Pearson  $R = 0.94$ ).