

## Supplementary Information

### **The osteogenic commitment of CD271+CD56+ bone marrow stromal cells (BMSCs) in osteoarthritic femoral head bone**

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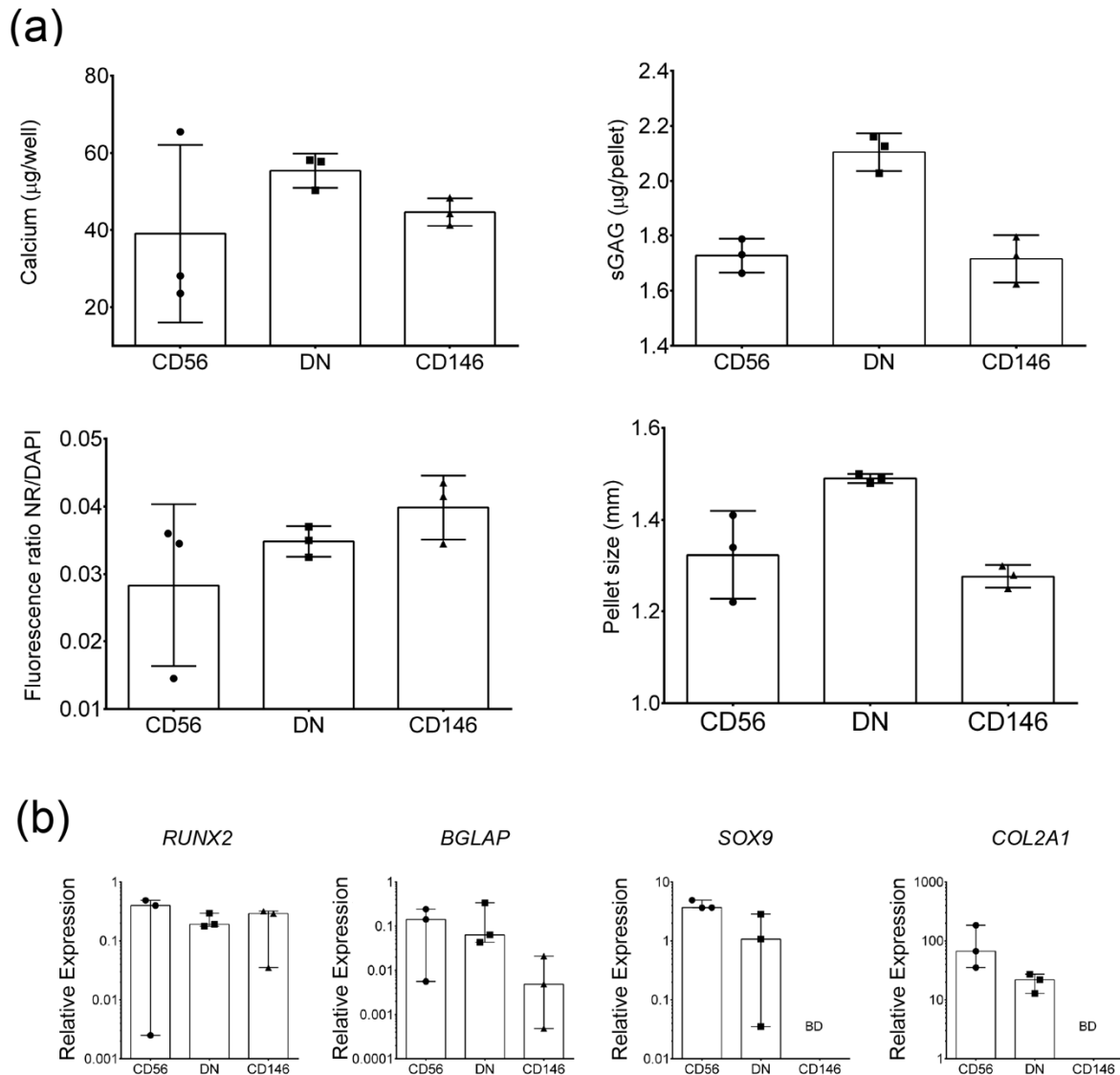
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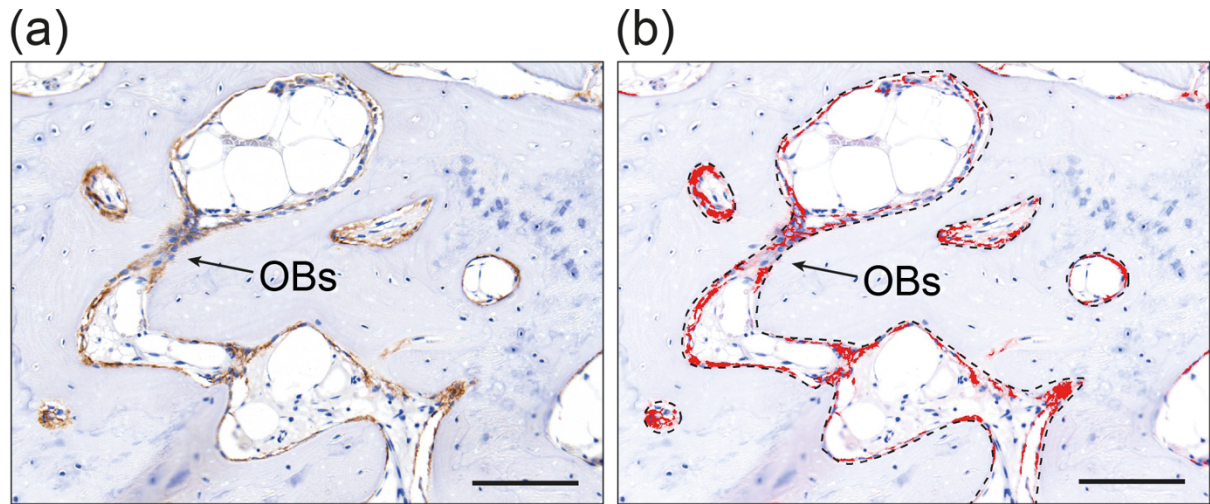
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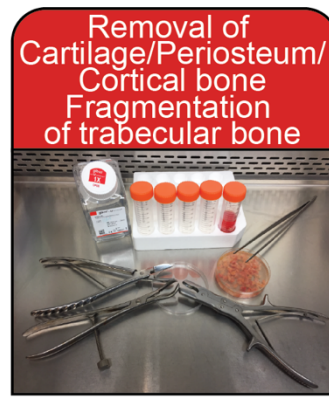
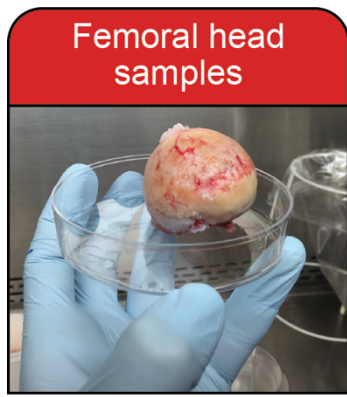
**Supplementary Figure 1. Quantitative assessment of tri-lineage multipotentiality of CD271+ BMSCs subsets following minimal culture-expansion.**

(a): Functional data. Calcium production of sorted, culture-expanded and osteogenically-induced cells following 21 days of induction (top left). Adipogenesis quantification by uptake of Nile red normalised to DAPI following 21 days of adipogenic induction (bottom left). Quantification of GAG in chondrogenic pellets (top right) and measurement of pellet sizes (bottom right). Functional assays data is presented as dot plots with bars indicating mean values and whiskers representing standard deviation, n=3 donors. (b) Gene expression data. Transcriptional analysis was performed for osteogenic transcripts *RUNX2* and *BGLAP* and chondrogenic transcripts *SOX9* and *COL2A1* on day 21 post-induction and data are presented as dot plots with bars indicating median values and whiskers representing interquartile ranges; n=3 donors. BD: below detection levels.



### Supplementary Figure 2. Quantification of CD56+ cells in OA bone.

Representative photomicrographs showing IHC of CD56 positive cells in OA femoral heads sclerotic areas (a). The areas were selected for quantification using Nuance software by tracing a dotted line around the bone cavity areas (b). CD56 area quantification was performed based on DAB+ staining (brown), within the selected bone cavity areas (dashed lines) above negative control threshold (indicated by red colour). OBs: osteoblasts; Scale bar: 100  $\mu$ m.



**IHC**  
CD271, CD56 and E11

**Picrosirius Red**

**IF**  
CD271, CD56 and DAPI

**FACS**

- CD271+ MSCs
  - CD56+
  - CD146+
  - DN
- CD45+ HLCs

**Minimal culture expansion**

**qPCR**  
96a TLDA

**Holomonitor**

**Functional assays**

**Supplementary Figure 3. Experimental design for CD271+ BMSCs subsets isolation and characterisation from OA femoral head trabecular bone samples.**

For histological analysis, femoral heads from n=4 OA donors were slowly decalcified in EDTA solution, bisected using a standard pathology knife and tissue sections were used for IHC, IF or Picrosirius Red staining. To obtain trabecular bone-resident live cells, soft tissue (ligament, periosteum) and cartilage were first removed from femoral heads using a scalpel then cortical bone was taken out using rongeur. The remaining trabecular bone was then mechanically broken down into small fragments using rongeur and enzymatically digested. From the obtained cell suspension, the BMSCs were isolated by FACS and used for subsequent gene expression analysis and functional in vitro assays. For gene expression analysis a total of n=6 OA donors were used for analysis by qPCR using a 96a gene panel TaqMan low-density array card. Each of the three subsets (CD56+, CD146+ and DN) isolated by FACS from n=3 OA donors were induced towards osteogenic, adipogenic and chondrogenic lineages and assessed by standard in-vitro quantitative and qualitative assays. The three subsets from different n=3 OA donors were used to study cell motility using the holographic imaging (Holomonitor)

**Supplementary Table 1. List of TaqMan assays ID and the corresponding gene names used for gene expression analysis.**

Assay ID	Gene Symbol	Synonyms	References
Hs00153936_m1	<i>ACAN</i>	Aggrecan	67
Hs00909449_m1	<i>ACTA2</i>	actin, alpha 2, smooth muscle, aorta	26,67,68
Hs00244715_m1	<i>ACVR1B</i>	activin A receptor, type IB	26,67,68
Hs00155658_m1	<i>ACVR2A</i>	activin A receptor, type IIA	26,67,68
Hs00758162_m1	<i>ALPL</i>	alkaline phosphatase, liver/bone/kidney	17,29,67,69
Hs00181613_m1	<i>ANGPT1</i>	angiopoietin 1	29,67
Hs01101127_m1	<i>ANGPTL4</i>	angiopoietin-like 4	28,67
Hs03044164_m1	<i>BAMBI</i>	BMP and activin membrane-bound inhibitor homolog	26,67,68
Hs00609452_g1	<i>BGLAP</i>	bone gamma-carboxyglutamate protein (osteocalcin)	26,67–69
Hs00154192_m1	<i>BMP2</i>	bone morphogenetic protein 2	26,67,68
Hs00370078_m1	<i>BMP4</i>	bone morphogenetic protein 4	29,67
Hs00234930_m1	<i>BMP5</i>	bone morphogenetic protein 5	29,67
Hs00403062_m1	<i>BMPER</i>	BMP binding endothelial regulator	26,67,68
Hs00831730_s1	<i>BMPRIA</i>	bone morphogenetic protein receptor, type IA	26,67,68
Hs00156438_m1	<i>CDH11</i>	cadherin 11, type 2, OB-cadherin	29,67,69
Hs00901463_m1	<i>CDH5</i>	cadherin 5, type 2, VE-cadherin	26,67,68
Hs00269972_s1	<i>CEBPA</i>	CCAAT/enhancer binding protein (C/EBP), alpha	17,29,67
Hs00154382_m1	<i>CHAD</i>	Chondroadherin	67
Hs00253550_m1	<i>CLEC4M</i>	CD209 molecule,C-type lectin domain family 4, member M	26,67,68
Hs00166657_m1	<i>COL10A1</i>	collagen, type X, alpha 1	67
Hs01076777_m1	<i>COL1A1</i>	collagen, type I, alpha 1	28,67
Hs01028971_m1	<i>COL1A2</i>	collagen, type I, alpha 2	28,67
Hs00264051_m1	<i>COL2A1</i>	collagen, type II, alpha 1	67
Hs00164359_m1	COMP	Cartilage Oligomeric Matrix Protein	28,67

Hs00426981_m1	<i>CSPG4</i>	chondroitin sulfate proteoglycan 4	67
Hs00171022_m1	<i>CXCL12</i>	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	29,67
Hs00601975_m1	<i>CXCL2</i>	C-X-C Motif Chemokine Ligand 2	26,67,68
Hs00322497_m1	<i>DAAM2</i>	dishevelled associated activator of morphogenesis 2	26,67,68
Hs00178815_m1	<i>DDR2</i>	discoidin domain receptor tyrosine kinase 2	26,67,68
Hs00182901_m1	<i>DVL2</i>	dishevelled, dsh homolog 2	26,67,68
Hs00358886_m1	<i>EFNA1</i>	ephrin-A1	26,67,68
Hs00174752_m1	<i>EPHB4</i>	EPH receptor B4	29,67
Hs00191912_m1	<i>EPYC</i>	epiphycan	67
Hs00609791_m1	<i>FABP4</i>	fatty acid binding protein 4, adipocyte	29,67
Hs00170454_m1	<i>FGF5</i>	fibroblast growth factor 5	26,67,68
Hs00241111_m1	<i>FGFR1</i>	fibroblast growth factor receptor 1	26,67,68
Hs00179829_m1	<i>FGFR3</i>	fibroblast growth factor receptor 3	26,67,68
Hs00173503_m1	<i>FRZB</i>	frizzled-related protein	28,29,67
Hs00268943_s1	<i>FZD1</i>	frizzled homolog 1	29,67
Hs00201853_m1	<i>FZD4</i>	frizzled homolog 4	29,67
Hs00275833_s1	<i>FZD7</i>	frizzled homolog 7	29,67
Hs00259040_s1	<i>FZD8</i>	frizzled homolog 8	26,67,68
Hs00268954_s1	<i>FZD9</i>	frizzled homolog 9	29,67
Hs99999905_m1	<i>GAPDH</i>	glyceraldehyde-3-phosphate dehydrogenase	29,67
Hs00231119_m1	<i>GATA2</i>	GATA binding protein 2	29,67
Hs00167060_m1	<i>GDF5</i>	Growth Differentiation Factor 5	67
Hs01075601_m1	<i>GHR</i>	growth hormone receptor	29,67
Hs00748445_s1	<i>GJAI</i>	gap junction protein, alpha 1, 43kDa	26,67,68
Hs00157103_m1	<i>HAPLN1</i>	Hyaluronan And Proteoglycan Link Protein 1	67
Hs99999909_m1	<i>HPRT1</i>	hypoxanthine phosphoribosyltransferase 1	26,67,68
Hs99999041_m1	<i>IFNG</i>	interferon, gamma,	26,67,68
Hs00181211_m1	<i>IGFBP3</i>	insulin-like growth factor binding protein 3	29,67

Hs00961622_m1	<i>IL10</i>	interleukin 10	26,67,68
Hs00174202_m1	<i>IL7</i>	interleukin 7	29,67
Hs01070036_m1	<i>JAG1</i>	jagged 1	29,67
Hs00174492_m1	<i>LEPR</i>	leptin receptor	29,67
Hs01012571_m1	<i>LPL</i>	lipoprotein lipase	28,29,67
Hs00174838_m1	<i>MCAM</i>	melanoma cell adhesion molecule	17,28,67
Hs00427183_m1	<i>MSX1</i>	msh homeobox 1	26,67,68
Hs02379661_g1	<i>MT2A</i>	metallothionein 2A	26,67,68
Hs00159522_m1	<i>MYH9</i>	myosin, heavy chain 9, non-muscle	26,67,68
Hs02387400_g1	<i>NANOG</i>	Nanog homeobox	17,67
Hs00707120_s1	<i>NES</i>	nestin	29,67
Hs00182120_m1	<i>NGFR</i>	nerve growth factor receptor (TNFR superfamily, member 16)	17,28,67
Hs00271352_s1	<i>NOG</i>	noggin	26,67,68
Hs00192325_m1	<i>OMD</i>	osteomodulin	26,67-69
Hs00190682_m1	<i>PAPSS2</i>	3'-Phosphoadenosine 5'-Phosphosulfate Synthase 2	67
Hs00170179_m1	<i>PCOLCE</i>	procollagen C-endopeptidase enhancer	26,67,68
Hs00998018_m1	<i>PDGFRA</i>	platelet-derived growth factor receptor, alpha polypeptide	26,67,68
Hs00185122_m1	<i>PDGFRL</i>	platelet-derived growth factor receptor-like	26,67,68
Hs01370291_g1	<i>PHOSPHO1</i>	phosphatase, orphan 1	26,67,68
Hs00999632_g1	<i>POU5F1</i>	POU class 5 homeobox 1	17,67
Hs01115513_m1	<i>PPARG</i>	peroxisome proliferator-activated receptor gamma	29,67
Hs00160431_m1	<i>PRELP</i>	Proline and Arginine Rich End Leucine Rich Repeat Protein	28,67
Hs00231079_m1	<i>RUNX1</i>	runt-related transcription factor 1	26,67,68
Hs00231692_m1	<i>RUNX2</i>	runt-related transcription factor 2	29,67,69
Hs00173499_m1	<i>SIPRI</i>	Sphingosine-1-Phosphate Receptor 1	26,67,68
Hs00610060_m1	<i>SFRP1</i>	secreted frizzled-related protein 1	29,67
Hs00180066_m1	<i>SFRP4</i>	secreted frizzled-related protein 4	26,67,68



Hs00361747_m1	<i>SORT1</i>	sortilin 1	26,67,68
Hs01053049_s1	<i>SOX2</i>	SRY (sex determining region Y)-box 2	17,67
Hs00165814_m1	<i>SOX9</i>	SRY (sex determining region Y)-box 9	29,67
Hs00541729_m1	<i>SP7</i>	Sp7 transcription factor	26,67,68
Hs00277762_m1	<i>SPARC</i>	secreted protein, acidic, cysteine-rich (osteonectin)	29,67,69
Hs00959010_m1	<i>SPP1</i>	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I)	29,67,69
Hs00559661_m1	<i>TGFBR2</i>	transforming growth factor, beta receptor II (70/80kDa)	26,67,68
Hs00234257_m1	<i>TGFBR3</i>	transforming growth factor, beta receptor III	29,67
Hs01113602_m1	<i>TNFAIP6</i>	TNF Alpha Induced Protein 6	67
Hs00900360_m1	<i>TNFRSF11B</i>	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	26,67,68
Hs00361186_m1	<i>TWIST1</i>	twist homolog 1	26,67,68
Hs00382379_m1	<i>TWIST2</i>	twist homolog 2	26,67,68
Hs01097550_m1	<i>UGDH</i>	UDP-Glucose Dehydrogenase	67
Hs00900058_m1	<i>VEGFA</i>	vascular endothelial growth factor A	26,67,68
Hs01099206_m1	<i>VEGFC</i>	vascular endothelial growth factor C	29,67
Hs00183662_m1	<i>WIF1</i>	WNT inhibitory factor 1	26,67,68