

Supplementary Material

1 Supplementary Tables

1.1 Supplementary Table 1

Supplementary Table 1. The Taqman probes used to assess the gene expression

Gene symbol	Gene name	Assay number
ACAN	aggrecan	HS00153936-m1
ADAMTS4	a disintegrin and metalloproteinase with thrombospondin motifs 4	HS00192708-m1
ADAMTS5	a disintegrin and metalloproteinase with thrombospondin motifs 5	HS01095524-m1
ANKH	human homolog of the murine progressive ankylosis gene	HS01064613-m1
ARNTL	aryl hydrocarbon receptor nuclear translocator-like protein 1	HS00154147-m1
ASPN	asporin	HS01558901-m1
BCL2	BCL2, apoptosis regulator	HS00608023-m1
BGLAP	Bone Gamma-Carboxyglutamate Protein	HS01587814-g1
BMPR1B	Bone Morphogenetic Protein Receptor Type 1B	HS01010965-m1
CCL19	C-C Motif Chemokine Ligand 19	HS00171149-m1
CCL2	C-C Motif Chemokine Ligand 2	HS00234140-m1
CCL20	C-C Motif Chemokine Ligand 20	HS01011368-m1
CCL5	C-C Motif Chemokine Ligand 5	HS00982282-m1
CCR1	C-C Motif Chemokine Receptor 1	HS00174298-m1
CCR10	C-C Motif Chemokine Receptor 10	HS00706455-s1
CCR2	C-C Motif Chemokine Receptor 2	HS00356601-m1
CCR3	C-C Motif Chemokine Receptor 3	HS99999027-s1
CCR5	C-C motif chemokine receptor 5	HS00152917-m1
CCR6	C-C Motif Chemokine Receptor 6	HS00171121-m1
CCR7	C-C Motif Chemokine Receptor 7	HS04398702-m1

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COL10A1	Collagen Type X Alpha 1 Chain	HS00166657-m1
COL1A1	Collagen Type I Alpha 1 Chain	HS01076777-m1
COL1A2	Collagen Type I Alpha 2 Chain	HS01028971-m1
COL2A1	Collagen Type II Alpha 1 Chain	HS00264051-m1
COMP	Cartilage Oligomeric Matrix Protein	HS00164359-m1
CTGF	Connective Tissue Growth Factor	HS00170014-m1
CXCL12	C-X-C Motif Chemokine Ligand 12	HS00171022-m1
CXCR1	C-X-C Motif Chemokine Receptor 1	HS01921207-m1
CXCR4	C-X-C Motif Chemokine Receptor 4	HS00607978_S1
CYR61	Cysteine Rich Angiogenic Inducer 61	HS00155479-m1
DDR2	Discoidin Domain Receptor Tyrosine Kinase 2	HS01025956-m1
DIO2	Iodothyronine Deiodinase 2	HS00255341-m1
DIRAS2	DIRAS Family GTPase 2	HS01107862-m1
FABP4	Fatty Acid Binding Protein 4	HS00609791-m1
GDF5	Growth Differentiation Factor 5	HS00167060-m1
GDF6	Growth Differentiation Factor 6	HS01377663-m1
GREM1	Gremlin 1, DAN Family BMP Antagonist	HS00171951-m1
HGF	Hepatocyte Growth Factor	HS00300159-m1
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	HS99999909-m1
IBSP	integrin binding sialoprotein	HS00173720-m1
IGF1	Insulin Like Growth Factor 1	HS03986524-m1
IGF1R	Insulin Like Growth Factor 1 Receptor	HS00609566-m1
IGF2	Insulin Like Growth Factor 2	HS00171254-m1
IGFBP3	Insulin Like Growth Factor Binding Protein 3	HS00426289-m1
IL10	Interleukin 10	HS00961622-m1
IL1B	Interleukin 1 Beta	HS01555413-m1

IL6	Interleukin 6	HS00174131-m1
LCN2	Lipocalin 2	HS01008571-m1
LEPR	Leptin Receptor	HS00174492-m1
MMP1	Matrix Metalloproteinase 1	HS00899658-m1
MMP13	Matrix Metalloproteinase 13	HS00942589-m1
MMP14	Matrix Metalloproteinase 14	HS00237119-m1
MMP2	Matrix Metalloproteinase 2	HS01548728-m1
MMP3	Matrix Metalloproteinase 3	HS00968308-m1
MMP9	Matrix Metalloproteinase 9	HS00957562-m1
NGF	Nerve Growth Factor	HS00171458-m1
NGFR	Nerve Growth Factor Receptor	HS00182120-m1
NOS2	Nitric Oxide Synthase 2	HS01075529-m1
NOTCH1	Notch 1	HS01062014_m1
POSTN	Periostin	HS01566750-m1
PPARd	Peroxisome Proliferator Activated Receptor Delta	HS00602622-m1
PPARg	Peroxisome Proliferator Activated Receptor Gamma	HS01115513-M1
PSIP1	PC4 And SFRS1 Interacting Protein 1	HS01045714-g1
PTGS2	Prostaglandin-Endoperoxide Synthase 2	HS00153133-m1
PTH1R	Parathyroid Hormone 1 Receptor	HS00896824-m1
PTH1H	Parathyroid Hormone Like Hormone	HS00174969-m1
ROR2	Receptor Tyrosine Kinase Like Orphan Receptor 2	HS00171695-m1
RUNX2	Runt Related Transcription Factor 2	HS00234692-m1
S1PR1	Sphingosine-1-Phosphate Receptor 1	HS00173499-m1
SERPINE1	Serpin Family E Member 1	HS00167155-m1
SFRP1	Secreted Frizzled Related Protein 1	HS00610060-m1
SFRP4	Secreted Frizzled Related Protein 4	HS00180066-m1
SOX9	SRY-Box 9	HS00165814-m1

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SP7	Sp7 Transcription Factor	HS00541729-m1
SPARC	Secreted Protein Acidic And Cysteine Rich	HS00277762-m1
SPHK1	Sphingosine Kinase 1	HS00184211-m1
SPP1	Secreted Phosphoprotein 1	HS00959010-m1
STMN2	Stathmin 2	HS00975800-m1
TGFB1	transforming growth factor beta 1	HS00998133-m1
TGFB2	transforming growth factor beta 2	HS00234244-m1
TGFB3	Transforming Growth Factor Beta 3	HS01085997-m1
TGFBR1	Transforming Growth Factor Beta Receptor 1	HS00610319-m1
TGFBR2	transforming growth factor beta receptor 2	HS00559661-m1
TGFBR3	Transforming Growth Factor Beta Receptor 3	HS00234257-m1
THBS4	Thrombospondin 4	HS00170261-m1
TIMP1	Tissue inhibitor of metalloproteinase- 1	HS00171558-m1
TIMP2	Tissue inhibitor of metalloproteinase- 2	HS01091319-m1
TIMP3	Tissue inhibitor of metalloproteinase- 3	HS00927214-m1
TNFa	Tumor Necrosis Factor	HS99999043-m1
TNFRSF11B	TNF Receptor Superfamily Member 11b	HS00900360-m1
TNFSF11	TNF Superfamily Member 11	HS01092186-m1
VEGFA	Vascular Endothelial Growth Factor A	HS00900058-m1
VEGFC	Vascular Endothelial Growth Factor C	HS01099206-m1
WISP1	WNT1 Inducible Signaling Pathway Protein 1	HS04234730-m1
WNT10b	Wnt Family Member 10B	HS00559664-m1

1.2 Supplementary Table 2

Supplementary Table 2. Genes not differentially expressed between SF and SB MSCs.

Similar expression in SF and SB MSCs	
Gene symbol	Gene name
ARNTL	Aryl hydrocarbon receptor nuclear translocator-like protein 1
ADAMTS4	A disintegrin and metalloproteinase with thrombospondin motifs 4
ANKH	Human homolog of the murine progressive ankylosis gene
BCL2	BCL2, apoptosis regulator
COL1A1	Collagen Type I Alpha 1 Chain
COL1A2	Collagen Type I Alpha 2 Chain
CYR61	Cysteine Rich Angiogenic Inducer 61
DIO2	Iodothyronine Deiodinase 2
FABP4	Fatty Acid Binding Protein 4
GDF5	Growth Differentiation Factor 5
GDF6	Growth Differentiation Factor 6
HGF	Hepatocyte Growth Factor
IGF1R	Insulin Like Growth Factor 1 Receptor
MMP13	Matrix Metalloproteinase 13
MMP14	Matrix Metalloproteinase 14
Notch1	Notch 1
PPARG	Peroxisome Proliferator Activated Receptor Gamma
PSIP1	PC4 And SFRS1 Interacting Protein 1
ROR2	Receptor Tyrosine Kinase Like Orphan Receptor 2
S1PR1	Sphingosine-1-Phosphate Receptor 1
SFRP1	Secreted Frizzled Related Protein 1
SFRP4	Secreted Frizzled Related Protein 4
SOX9	SRY-Box 9
SPARC	Secreted Protein Acidic And Cysteine Rich
SPHK1	Sphingosine Kinase 1

SPP1	Secreted Phosphoprotein 1
TGFB1	Transforming growth factor beta 1
TGFB2	Transforming growth factor beta 2
TGFB3	Transforming Growth Factor Beta 3
TGFR1	Transforming Growth Factor Beta Receptor 1
TIMP1	Tissue inhibitor of metalloproteinase- 1
TNFRSF11B	TNF Receptor Superfamily Member 11b
TNFSF11	TNF Superfamily Member 11
VEGFA	Vascular Endothelial Growth Factor A
WISP1	WNT1 Inducible Signaling Pathway Protein 1
Expression below detection in both SF and SB MSCs	
Gene symbol	Gene name
ASPN	Asporin
BGLAP	Bone Gamma-Carboxyglutamate Protein
BMPR1B	Bone Morphogenetic Protein Receptor Type 1B
CCL19	C-C Motif Chemokine Ligand 19
CCL20	C-C Motif Chemokine Ligand 20
CCR10	C-C Motif Chemokine Receptor 10
CCR2	C-C Motif Chemokine Receptor 2
CCR3	C-C Motif Chemokine Receptor 3
CCR5	C-C motif chemokine receptor 5
CCR6	C-C Motif Chemokine Receptor 6
CCR7	C-C Motif Chemokine Receptor 7
COL10A1	Collagen Type X Alpha 1 Chain
COL2A1	Collagen Type II Alpha 1 Chain
COMP	Cartilage Oligomeric Matrix Protein
CXCR1	C-X-C Motif Chemokine Receptor 1
CXCR4	C-X-C Motif Chemokine Receptor 4
DIRAS2	DIRAS Family GTPase 2
IGF1	Insulin Like Growth Factor 1
IL10	Interleukin 10
IL1B	Interleukin 1 Beta
IL6	Interleukin 6
LCN2	Lipocalin 2
NGFR	Nerve Growth Factor Receptor
NOS2	Nitric Oxide Synthase 2

TIMP2	Tissue inhibitor of metalloproteinase- 2
TNFa	Tumor Necrosis Factor
Wnt10b	Wnt Family Member 10B

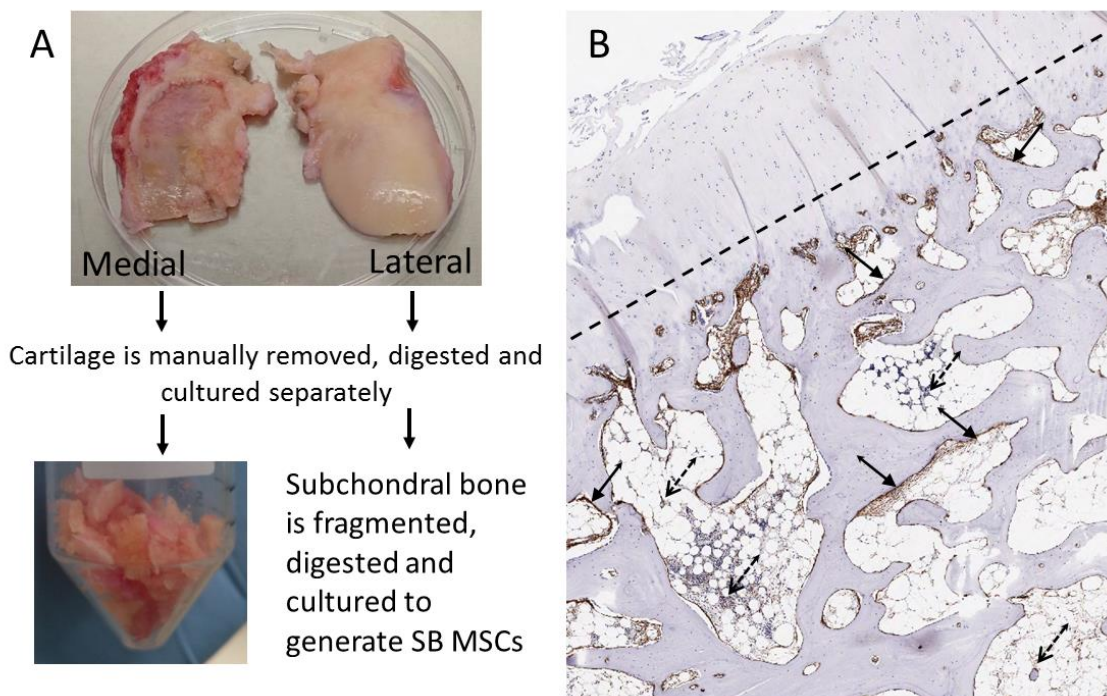
2 Supplementary Materials and methods

2.1. Immunohistochemistry

As described before (Sanjurjo-Rodriguez, et al. 2019), whole condyles were fixed for 1 week in 3.7% formaldehyde (ThermoFisher Scientific, UK), decalcified for at least 6 months using 0.5M ethylenediaminetetraacetic acid (EDTA; Sigma, USA), re-fixed for further 2 days in formaldehyde before embedding in paraffin blocks. Immunohistochemical staining for CD271 was performed using monoclonal mouse anti-human antibody (clone ME20.4; Invitrogen, USA) at 1:200 dilution and EnVision+ Dual Link System-HRP (DAB+) (Dako, Agilent, USA). Sections were counterstained with hematoxylin and the slides scanned using Leica Aperio AT2.

3 Supplementary Figures

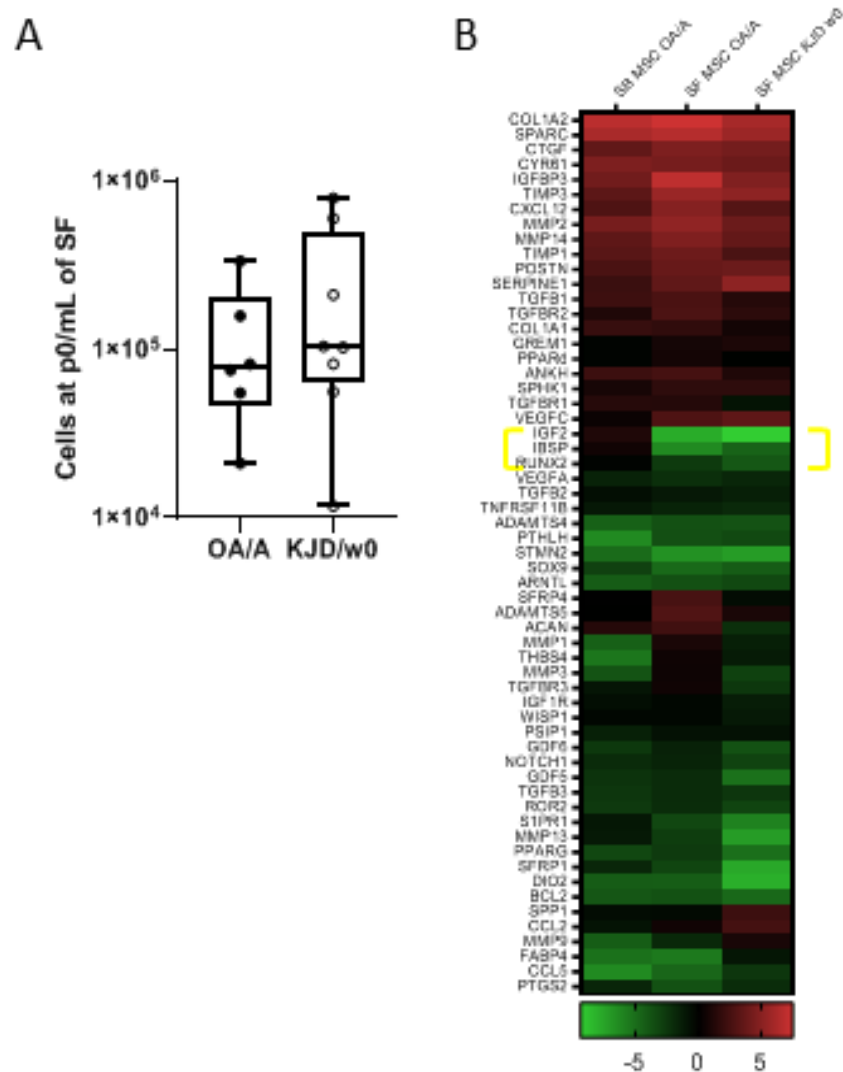
3.1. Supplementary Figure 1



Supplementary Figure 1: Tissue processing (A) and the immunohistochemical staining of an example osteochondral specimen (B) showing an absence of intact cartilage superficial layer and the presence of CD271+ MSCs in marrow cavities (dotted arrows) and in bone-lining locations (black arrows). Dotted line represents an approximate position of slicing-off the cartilage using a scalpel.

The method for processing and immunohistochemical staining of OA knee osteochondral specimens can be found in “Material and methods” and “Supplementary Materials and methods”.

3.2. Supplementary Figure 2.



Supplementary Figure 2: Comparison between SF MSCs from OA arthroplasty (OA/A) cohort and SF MSCs from KJD cohort at baseline (week 0). Similar growth potentials were observed (A) (lines represent medians, each symbol represents an individual donor). Gene expression heatmap of SF MSCs from both cohorts: OA/A (n=6), KJD (n=8), and SB MSCs from OA/A cohort (n=11) used as comparator (B). Heatmap is generated using Graphpad Prism version 8.4.3. Log₂ transformation and data filtering (filter = 67% present) were performed on the data and colour coding represents medians. Osteogenic genes differentially expressed between SB MSCs and both groups of SF MSCs are shown in yellow brackets.