

Supplementary information

Full Title: Transient existence of circulating multipotential stromal cells in the Deep Veins in Man following long bone intramedullary reaming.

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CONTENTS

Supplementary Table 1. Surgical patient information

Supplementary Table 2. Rheumatoid arthritis patient information

Supplementary Table 3. Taqman assays used

Supplementary Figure 1A (*ACTA1-GATA2*) Relative gene expression in MSCs of different origin

Supplementary Figure 1B (*GHR-WIF1*) Relative gene expression in MSCs of different origin

Supplementary Figure 2 Genes with no overlapping gene expression between PVB- and FVB-MSCs

Supplementary Table 1. Surgical patient information

Surgical Group	Donor number	Gender	Age	Diagnosis
Concomitant with reaming	1	Male	40	IM nailing of closed tibial shaft fracture
Concomitant with reaming	2	Male	28	IM nailing of closed tibial shaft fracture
Concomitant with reaming	3	Female	46	IM nailing of closed tibial shaft fracture
Concomitant with reaming	4	Male	30	IM nailing of closed tibial shaft fracture
Concomitant with reaming	5	Female	53	IM nailing of closed tibial shaft fracture
Concomitant with reaming	6	Male	18	IM nailing of closed tibial shaft fracture
Concomitant with reaming	7	Male	21	IM nailing of closed tibial shaft fracture
Concomitant with reaming	8	Female	53	IM nailing of closed tibial shaft fracture
Concomitant with reaming	9	Male	73	IM nailing of closed tibial shaft fracture
Post-reaming	1	Male	19	Nonunion femur
Post-reaming	2	Female	50	Nonunion femur
Post-reaming	3	Male	22	Nonunion femur
Post-reaming	4	Female	64	Nonunion tibia
Post-reaming	5	Male	33	Nonunion femur
Post-reaming	6	Male	43	Nonunion femur
Post-reaming	7	Male	62	Nonunion tibia
Post-reaming	8	Male	63	Nonunion femur
Post-reaming	9	Female	58	Nonunion tibia
Post-reaming	10	Female	44	Nonunion tibia
Post-reaming	11	Male	38	Nonunion tibia
Post-reaming	12	Female	24	Nonunion tibia
Post-reaming	13	Female	50	Non union tibia
Post-reaming	14	Female	45	Non union tibia
Post-reaming	15	Male	66	Non union radius
Prior surgery	1	Female	39	Sacroilitis for sacroiliac screws
Prior surgery	2	Male	67	Removal of metal work ankle
Prior surgery	3	Male	62	Removal of metal work tibia
Prior surgery	4	Female	42	Sacroilitis for sacroiliac screws
Prior surgery	5	Male	21	Removal of metal work tibia
Prior surgery	6	Male	18	Osteomyelitis tibia for debridement
Prior surgery	7	Female	46	Coccyx dinea for local injection
Prior surgery	8	Male	18	Osteomyelitis tibia for debridement
Prior surgery	9	Male	42	Sacroilitis for sacroiliac screws
Prior surgery	10	Male	41	Meniscal tear of knee for arthroscopy
Prior surgery	11	Female	31	Sacroilitis for sacroiliac screws
Prior surgery	12	Male	38	Osteomyelitis tibia for debridement

Supplementary Table 2. Rheumatoid arthritis patient information

RA category	Donor number	Sex	Age	CRP (mg/l)	ESR (mm/h)	Plasma viscosity (mPa.s)	RF (Iu/ml)	AntiCCP (U/ml)
Early	1	Female	77	<5.0	11	1.53	66	1.5
Early	2	Female	37	27	39	na	<15	0.4
Early	3	Female	41	<5.0	12	na	41	7.7
Early	4	Female	59	34	42	na	63	1.1
Early	5	Male	70	<5.0	10	na	210	101
Early	6	Male	74	28	na	1.73	65	>300.0
Early	7	Female	51	<5.0	na	1.74	<15	2.10
Early	8	Male	49	56	na	2.03	<15	1.70
Early	9	Female	73	54	9	na	22	16
Early	10	Male	73	<5.0	9	na	<15	2.6
Early	11	Female	49	<5.0	9	na	115	7.3
Early	12	Female	23	46	7	na	31	1.0
Early	13	Female	52	23	na	na	425	24.4
Early	14	Female	48	<5	na	na	624	77.30
Early	15	Female	51	17.9	56	1.81	71	>300.0
Established	1	Female	45	10.1	59	na	<15	>300.0
Established	2	Female	41	<5	9	1.54	15	0.50
Established	3	Male	56	<5	3	na	60	>300.00
Established	4	Female	47	<5	24	1.74	<15	<0.50
Established	5	Male	66	<5	8	na	119	>300.0
Established	*6	Female	34	5.9	26	na	51	1.6
Established	7	Female	42	<5	na	na	52	10.9
Established	8	Male	48	<5	na	na	234	>300.0
Established	9	Female	54	<5	50	na	98	253.5
Established	10	Male	54	<5	5	na	16	>300.0
Established	11	Female	32	<5	na	1.45	<15	111.2

Early RA is defined by < 1 year disease duration. CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, RF = rheumatoid factor, CCP = cyclic citrullinated peptide. na = not available. * yielded 1 colony

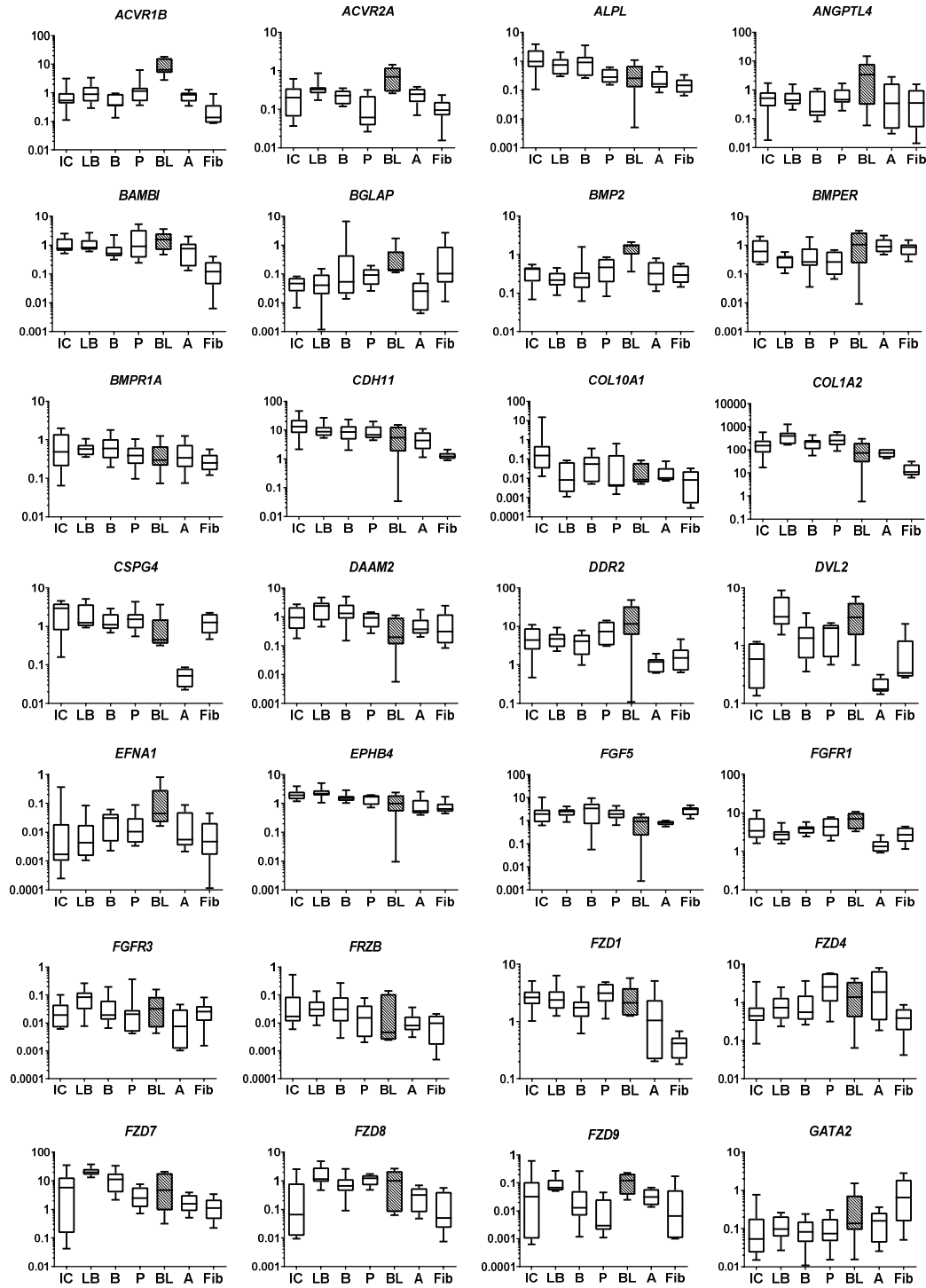
Supplementary Table 3. Taqman assays used

Taqman assay identification	Gene Symbol	Description
Hs00909449_m1	ACTA2	actin, alpha 2, smooth muscle, aorta
Hs00244715_m1	ACVR1B	activin A receptor, type IB
Hs00155658_m1	ACVR2A	activin A receptor, type IIA
Hs00758162_m1	ALPL	alkaline phosphatase
Hs00181613_m1	ANGPT1	angiopoietin 1
Hs01101127_m1	ANGPTL4	angiopoietin-like 4
Hs03044164_m1	BAMBI	BMP and activin membrane-bound inhibitor homolog
Hs00609452_g1	BGLAP	bone gamma-carboxyglutamate (gla) protein/ osteocalcin
Hs00154192_m1	BMP2	bone morphogenetic protein 2
Hs00234930_m1	BMP5	bone morphogenetic protein 5
Hs00403062_m1	BMPER	BMP binding endothelial regulator
Hs00831730_s1	BMPR1A	bone morphogenetic protein receptor, type IA
Hs00156438_m1	CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)
Hs00269972_s1	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
Hs00166657_m1	COL10A1	collagen, type X, alpha 1
Hs01076777_m1	COL1A1	collagen, type I, alpha 1
Hs01028971_m1	COL1A2	collagen, type I, alpha 2
Hs00264051_m1	COL2A1	collagen, type II, alpha 1
Hs00426981_m1	CSPG4	chondroitin sulfate proteoglycan 4/ NG2
Hs00171022_m1	CXCL12	chemokine (C-X-C motif) ligand 12/ stromal cell-derived factor 1
Hs00322497_m1	DAAM2	dishevelled associated activator of morphogenesis 2
Hs00178815_m1	DDR2	discoidin domain receptor tyrosine kinase 2
Hs00182901_m1	DVL2	dishevelled, dsh homolog 2
Hs00358886_m1	EFNA1	ephrin-A1
Hs00174752_m1	EPHB4	EPH receptor B4
Hs00191912_m1	EPYC	epiphygan
Hs00609791_m1	FABP4	fatty acid binding protein 4, adipocyte
Hs00170454_m1	FGF5	fibroblast growth factor 5
Hs00241111_m1	FGFR1	fibroblast growth factor receptor 1
Hs00179829_m1	FGFR3	fibroblast growth factor receptor 3
Hs00173503_m1	FRZB	frizzled-related protein/ secreted frizzled-related protein 3
Hs00268943_s1	FZD1	frizzled homolog 1
Hs00201853_m1	FZD4	frizzled homolog 4
Hs00275833_s1	FZD7	frizzled homolog 7
Hs00259040_s1	FZD8	frizzled homolog 8
Hs00268954_s1	FZD9	frizzled homolog 9
Hs99999905_m1	GAPDH	glyceraldehyde-3-phosphate dehydrogenase
Hs00231119_m1	GATA2	GATA binding protein 2
Hs01075601_m1	GHR	growth hormone receptor
Hs00748445_s1	GJA1	gap junction protein, alpha 1, 43kDa/ Connexin 43
Hs99999909_m1	HPRT1	hypoxanthine phosphoribosyltransferase 1
Hs01005963_m1	IGF2	insulin-like growth factor 2 (somatomedin A),INS-IGF2
Hs00181211_m1	IGFBP3	insulin-like growth factor binding protein 3
Hs00961622_m1	IL10	interleukin 10
Hs00174202_m1	IL7	interleukin 7

Hs01070036_m1	JAG1	jagged 1
Hs00174492_m1	LEPR	leptin receptor
Hs01012571_m1	LPL	lipoprotein lipase
Hs00174838_m1	MCAM	melanoma cell adhesion molecule/ CD146
Hs00427183_m1	MSX1	msh homeobox 1
Hs02379661_g1	MT2A	metallothionein 2A
Hs00159522_m1	MYH9	myosin, heavy chain 9, non-muscle
Hs02387400_g1	NANOG	Nanog homeobox
Hs00707120_s1	NES	nestin
Hs00182120_m1	NGFR	nerve growth factor receptor (TNFR superfamily, member 16)/ CD271
Hs00918411_s1	NGFRAP1	nerve growth factor receptor (TNFRSF16) associated protein 1
Hs00271352_s1	NOG	noggin
Hs00192325_m1	OMD	osteomodulin
Hs00170179_m1	PCOLCE	procollagen C-endopeptidase enhancer
Hs00998018_m1	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide
Hs00185122_m1	PDGFRL	platelet-derived growth factor receptor-like
Hs01370291_g1	PHOSPHO1	phosphatase, orphan 1
Hs00999632_g1	POU5F1	POU class 5 homeobox 1 (Oct 4)
Hs01115513_m1	PPARG	peroxisome proliferator-activated receptor gamma
Hs00231079_m1	RUNX1	runt-related transcription factor 1
Hs00231692_m1	RUNX2	runt-related transcription factor 2
Hs00173499_m1	S1PR1	sphingosine-1-phosphate receptor 1
Hs00610060_m1	SFRP1	secreted frizzled-related protein 1
Hs00180066_m1	SFRP4	secreted frizzled-related protein 4
Hs00361747_m1	SORT1	sortilin 1
Hs01053049_s1	SOX2	SRY (sex determining region Y)-box 2
Hs00165814_m1	SOX9	SRY (sex determining region Y)-box 9
Hs00277762_m1	SPARC	secreted protein, acidic, cysteine-rich/ osteonectin
Hs00959010_m1	SPP1	osteopontin/ secreted phosphoprotein 1/ bone sialoprotein I
Hs00559661_m1	TGFBR2	transforming growth factor, beta receptor II (70/80kDa)
Hs00234257_m1	TGFBR3	transforming growth factor, beta receptor III
Hs00900360_m1	TNFRSF11B	tumor necrosis factor receptor superfamily, member 11b/ osteoprotegerin
Hs00361186_m1	TWIST1	twist homolog 1
Hs00382379_m1	TWIST2	twist homolog 2
Hs00900058_m1	VEGFA	vascular endothelial growth factor A
Hs01099206_m1	VEGFC	vascular endothelial growth factor C
Hs00183662_m1	WIF1	WNT inhibitory factor 1

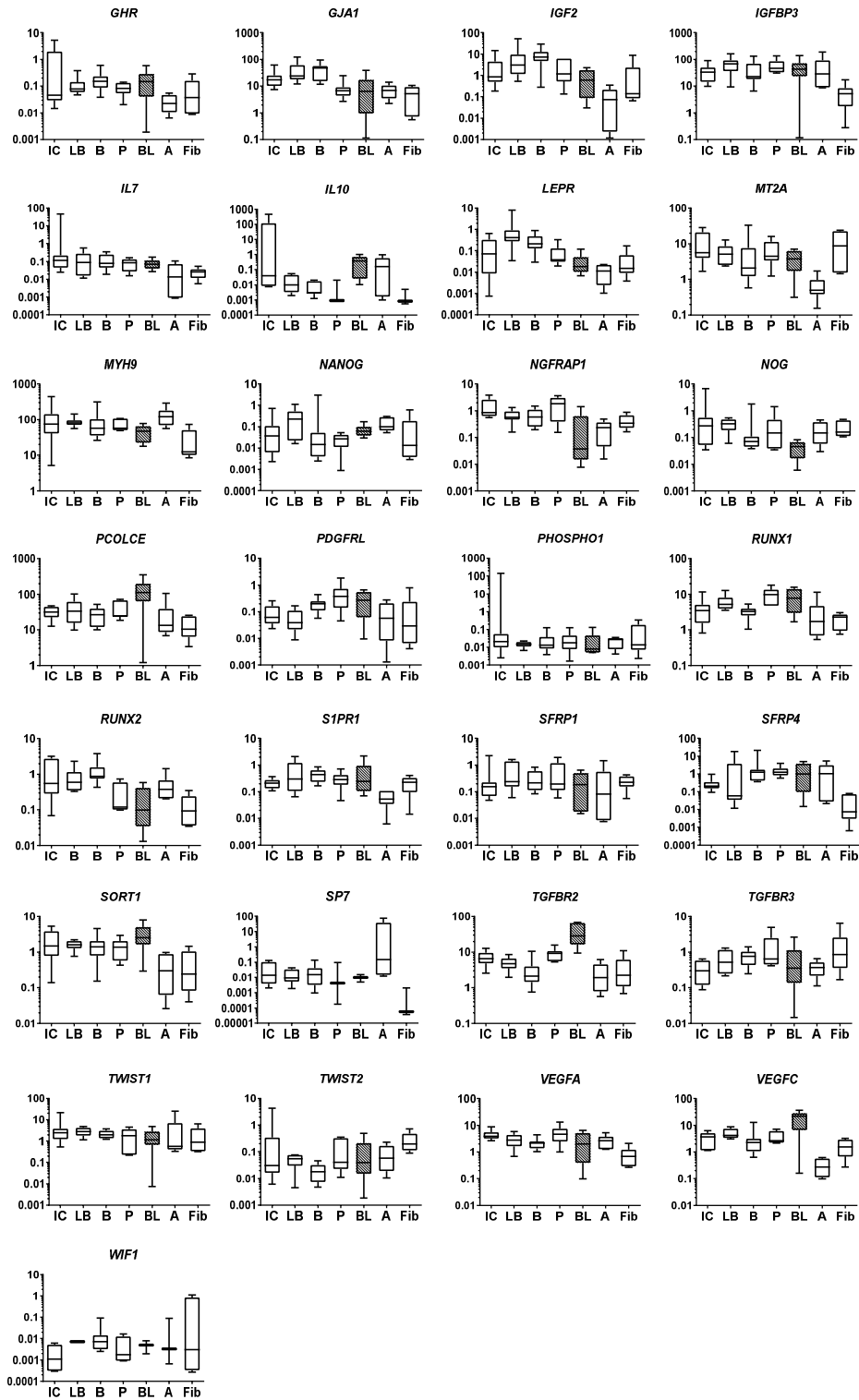
HPRT was used as the reference gene; *GAPDH* was used as a technical internal control only

Supplementary Figure 1A (*ACTA1-GATA2*) Relative gene expression in MSCs of different origin



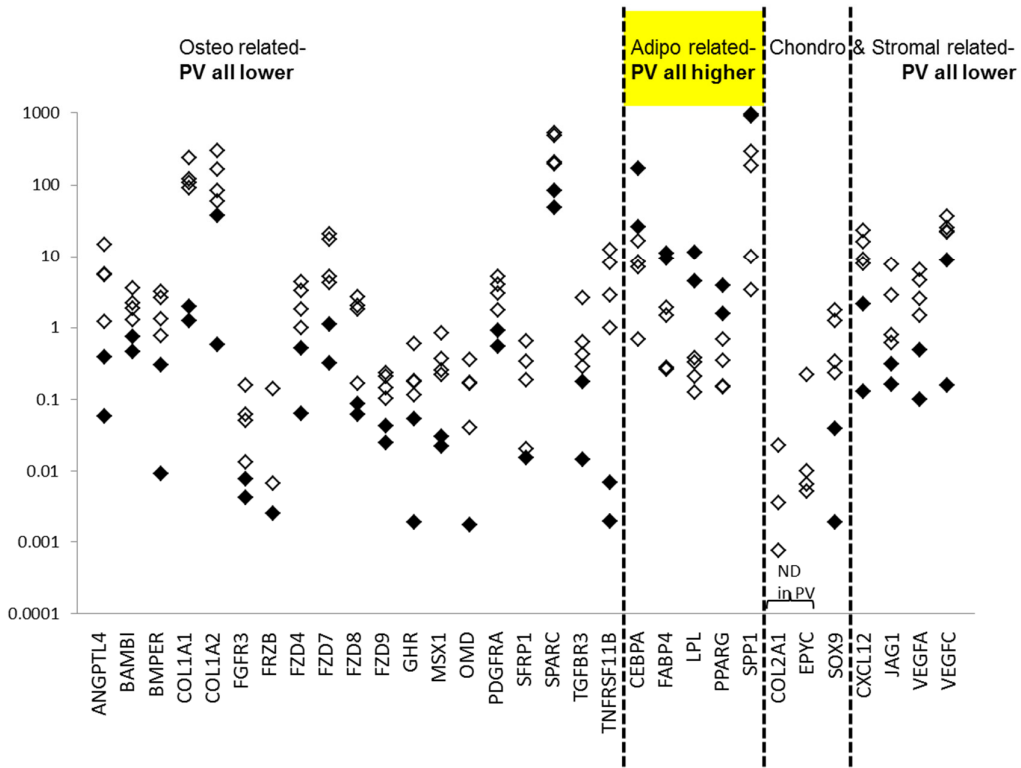
Gene expression normalised to *HPRT* using $2^{-\Delta C_t}$. IC=iliac crest marrow, LB=long-bone marrow, B=control bone, P=periosteum, BL=blood, A=adipose, Fib=fibroblast

Supplementary Figure 1B (*GHR-WIF1*) Relative gene expression in MSCs of different origin



Gene expression normalised to *HPRT* using $2^{-\Delta C_t}$. IC=iliac crest marrow, LB=long-bone marrow, B=control bone, P=periosteum, BL=blood, A=adipose, Fib=fibroblast

Supplementary Figure 2 Thirty-one genes with no overlapping gene expression between PVB- and FVB-MSCs



Black diamonds = PVB-MSCs, open diamonds FVB-MSCs. ND = not detected