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Transcriptome profiling of human papillary and reticular fibroblasts from adult interfollicular dermis pinpoints the 'tissue skeleton' gene network as a component of skin chrono-ageing

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A B S T R A C T

Interactions between extracellular matrix (ECM) and fibroblasts are essential for maintaining dermis integrity, and are subject to ageing. The ötissue skeleton' network connects ECM to the nucleus and DNA, impacting nuclear shape and gene expression. In a previous *Mech Ageing Dev* publication, we have presented a transcriptomic study of papillary (Fp) and reticular (Fr) fibroblasts, with a main focus on Fp ageing. As shown here, ageing affects ötissue skeleton' transcripts, even more clearly in Fr than in Fp. Accordingly, using circular index measurement, we show that nuclear shape is affected by ageing in both cell fractions.

In paucicellular tissues such as the human interfollicular dermis, the relationship between fibroblasts and extracellular matrix (ECM) has a major functional importance (Vedrenne et al., 2012; Cole et al., 2018). This relay initiates the perception of intra-tissue variations of biological parameters (growth factor and hormone levels, toxins…) and mechanical changes. Although the roles of biological effectors have been studied for years, mechanical stimulations are studied only since recently (Caberlotto et al., 2017). Some mechano-sensible transcription factors are involved in rapid (minutes) responses to mechanical stimulations (Dupont et al., 2011), while others follow longer (days) kinetics (Dingal et al., 2015). In addition, mechanical solicitations impact cellular physiology via epigenetic mechanisms. For example, substrate rigidity can modify nuclei volume and polarization, and influence subnuclear localization of acetylated histones (Kim and Wirtz, 2015 and Kim et al., 2017). This control is ensured by a protein network that bridges ECM fibrillar proteins and nuclear DNA. ECM proteins connect to the intracellular cytoskeleton via focal adhesion points, which itself connects to the nucleoskeleton via complexes classified as linkers of nucleoskeleton and cytoskeleton (LINCs) (for reviews, see Zhong et al., 2010; Dahl and Kalinowski, 2011). At the end of this chain, the nucleoskeleton is connected with genomic DNA. A scheme of the 'tissue skeleton' network is presented in Fig. 1A.

The genomic profiles of human fibroblasts are highly heterogeneous with regards to their different anatomical origins (Chang et al., 2002), which has been documented using a list of 267 transcripts that discriminated human fibroblast anatomic demarcations (Rinn et al., 2006).

Notably, 29.2% of these transcripts are differentially expressed, comparing Fp fibroblasts (superficial localization, between the epidermis and the rete subpapillae plexus) and Fr fibroblasts (of a deeper localization, 700 µm below the skin surface) (Table 1). The molecular identity of the different human dermal fibroblast populations still needs further investigation. A comparative review of recently published molecular signatures is presented in Table 2.

In previous *Mech Ageing Dev* publications, we have reviewed the evolutions of human dermis ECM and fibroblast characteristics, as a function of developmental stage and ageing (Haydont et al., 2018a) and presented a genome-wide transcriptome comparison of 'young' and 'old' dermal fibroblasts (Haydont et al., 2018b). A finding that emerged from the comparison of 'young' and 'old' Fp was the modulation of 186 transcripts related to the 'tissue skeleton' concept (Fig. 1B, purple bars). Interestingly, this phenomenon was even more marked in Fr, with a modulation of 295 'tissue skeleton' transcripts (Fig. 1B, green bars). Modulated Fr transcripts included 54 ECM genes, 61 focal adhesion point genes, 46 cytoskeleton genes, 2 LINC complex genes, and 7 nucleoskeleton genes. Signatures of transcripts involved in 'tissue

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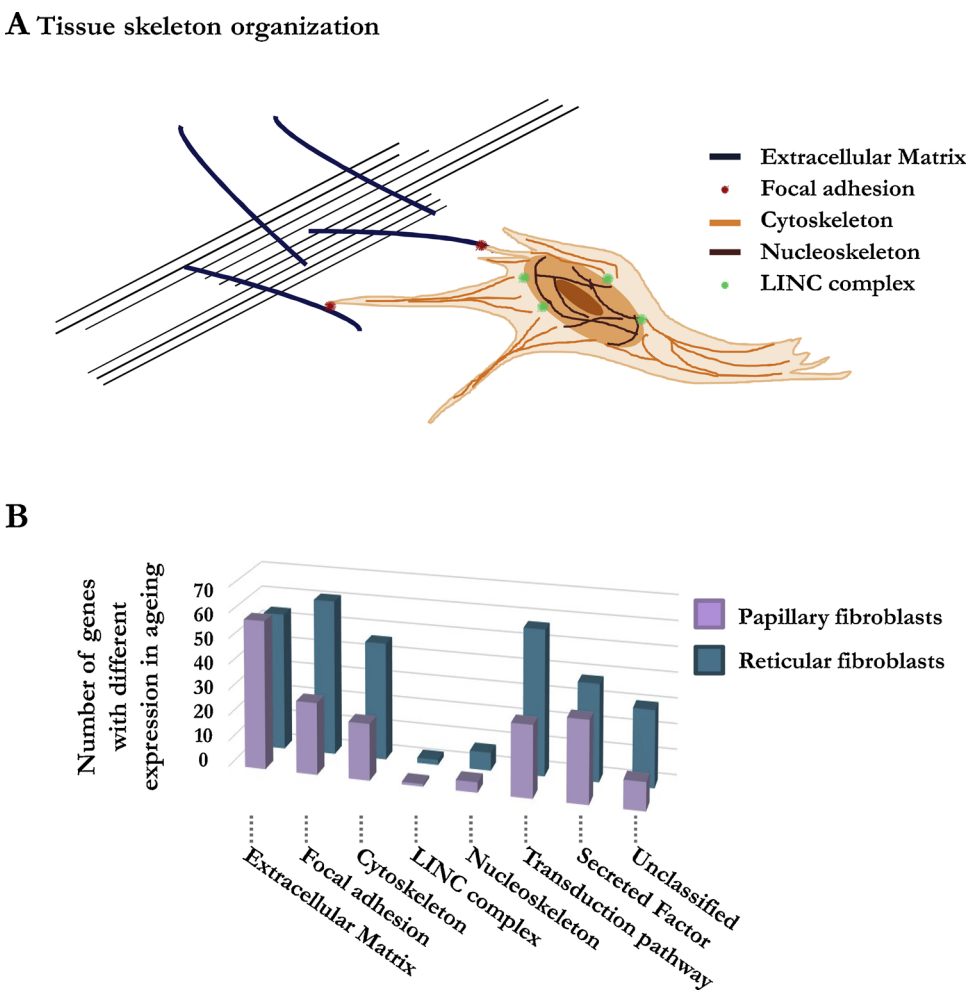

Fig. 1. Concept of 'tissue skeleton'. **A** – Schematic representation of the tissue skeleton network that connects ECM and genomic DNA via successive molecular relays: focal adhesion points, cytoskeleton, LINC complexes, and nucleoskeleton. This network participates to the transmission of mechanical stimulations to the nucleus and transformation into genetic and epigenetic responses (gene expression, DNA compaction, histone acetylation, spatial organization of DNA). **B** – Modulation with chrono-ageing of transcripts related to the tissue skeleton biology in papillary fibroblasts (Fp) [histogram purple bars] and reticular fibroblasts (Fr) [histogram green bars] from human adult dermis. The presented data were extracted from our previously published transcriptome comparative analysis of Fp and Fr cells (full details on the biological material, approach, and procedures, are provided in Haydont et al., 2018b). Briefly, Fp and Fr were extracted from adult human breast skin biopsies. We could not completely exclude that sample have been occasionally exposed to solar radiation. Three 'younger' (22, 25 and 28 years old) and 3 'older' (55, 61 and 65 years old) donors were selected for micro-array profiling. (full-genome Affymetrix GeneChip HG-U133 Plus 2.0). A 1.5 fold-change threshold together with a p-value of 0.05 were used. In Fp, age-related transcript modulation concerned 113 genes directly involved in the tissue skeleton structure (focal adhesion points, cytoskeleton, LINC complexes, nucleoskeleton), and 73 genes involved in the regulation of its biology (signal transduction, secreted factors, unclassified). In Fr, transcriptional modulation concerned 170 genes directly involved in the tissue skeleton structure, and 125 genes that regulate its biology.

skeleton' biology and modulated with ageing in Fp and/or Fr are detailed in Table 3.

Remarkably, the detected transcript signatures suggested changes in ECM organization and quality during Fr chrono-ageing. Modulations (Log2 fold-change) concern genes related to collagen fibrils and bundles. Age-related transcript modulations in Fr also affect genes of the proteoglycan network. Interestingly, some transcripts found up-modulated in aged skin fibroblasts (*ACAN*, *ASPN* and *OGN*), are expressed in tendon and cartilage (Kiani et al., 2002; Tanaka et al., 2012; Nakajima et al., 2007), which stiffness is much higher than that of the dermis. Varani et al. have shown that, in human skin, the contact surface between Fp and ECM reduces with ageing (Varani et al., 2006) and here, other up-modulated proteoglycans (*EMCN* and *TNXB*) are known to reduce cell adhesion to ECM (Kinoshita et al., 2001; Fujie et al., 2009). Our results suggest that chrono-ageing also affects the expression of genes related to the actomyosin-microtubule cytoskeleton. Linker protein genes were found up-modulated in aged Fr cells: *PLEC1* (+1.51) and *TNNC1* (+1.53). Moreover, up-modulation of genes encoding F-actin capping proteins such as *GSN* (+1.71) and *ARPC4* (+1.75), together with up-modulation of the *TMSB15A* gene (+2.32), which encodes a G-actin trapping protein, suggested a decrease of the actin polymerization-depolymerization dynamics. The microtubule network was also concerned by transcriptional changes in Fr cells from 'old' donors. Dynamin transcripts, which are involved in mobilization of microtubules during endocytosis (Ferguson and Dynamin, 2012), were found up-modulated: *DNM1* (+1.58) and *DNM3* (+2.10). Transcript up-modulation in aged Fr also concerned genes involved in chromosome segregation by the microtubule network (Ducat and Zheng, 2004; Cross and McAinsh, 2014; Petry, 2016). Transcript signatures related to ECM and cytoplasmic functions, with predominant expression in 'young' or 'old' Fr cells are schematized in Fig. 2A and B. Furthermore, transcriptional changes detected in Fr cells from 'old' donors concerned genes encoding nuclear components, including 2 LINC complex genes and 7 nucleoskeleton genes. Microarray screen validation data are summarized in Table 4. Interestingly, 27.7% of the 267 transcripts analyzed for anatomical modulations (Rinn et al., 2006) were also detected here as modulated with ageing. Moreover, 48.6% of these transcripts are involved in the 'tissue skeleton' (Table 5). Finally, the 'tissue skeleton' transcript signatures identified here as modulated with ageing in Fp and/or Fr fibroblasts were compared to available data from the literature (Table 6). The moderate convergence of independent studies highlighted the complexity of tissue ageing biology.

Table 1
Rinn et al., have described a list of 337 probesets (267 genes) that can be used to discriminate fibroblasts according to their anatomical origins (Rinn et al., 2006). Cross-analysis of these data together with data from the present study was performed, and revealed that 29.2% of these 267 genes exhibit differential expression when comparing dermis Fp and Fr fibroblasts. The corresponding probeset list and their fold-changes in Fr versus Fp are provided.

Papillary Fibroblasts			Reticular Fibroblasts		
Gene Symbol	Gene Title	Fold Change	Gene Symbol	Gene Title	Fold Change
APCDD1	adenomatosis polyposis coli down-regulated 1	29.49	COL11 A1	collagen, type XI, alpha 1	51.70
PLAC8	placenta-specific 8	5.80	COL11 A1	collagen, type XI, alpha 1	38.65
GPR126	G protein-coupled receptor 126	4.05	ZFPM2	zinc finger protein, multitype 2	38.54
TFAP2C	transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	3.59	APBB1IP	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	28.37
COLEC12	collectin sub-family member 12	3.41	COL11 A1	collagen, type XI, alpha 1	18.74
RAB11FIP1	RAB11 family interacting protein 1 (class I)	3.34	RGS4	regulator of G-protein signaling 4	11.94
FOXF1	forkhead box F1	3.13	RGS4	regulator of G-protein signaling 4	9.68
CRIP1	cysteine-rich protein 1 (intestinal)	2.88	TM4SF1	transmembrane 4 L six family member 1	9.47
RAB11FIP1	RAB11 family interacting protein 1 (class I)	2.83	TM4SF1	transmembrane 4 L six family member 1	8.66
VIT	vitrin	2.80	TM4SF1	transmembrane 4 L six family member 1	8.22
COL10A1	collagen, type X, alpha 1	2.63	RGS4	regulator of G-protein signaling 4	8.18
COL10A1	collagen, type X, alpha 1	2.59	DSP	desmoplakin	7.51
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5	2.58	NTN4	netrin 4	6.78
PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	2.44	PTGER3	prostaglandin E receptor 3 (subtype EP3)	5.57
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5	2.44	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	5.02
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	2.40	LRRC17	leucine rich repeat containing 17	4.93
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5	2.37	CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	4.41
NFE2L3	nuclear factor (erythroid-derived 2)-like 3	2.33	NCALD	neurocalcin delta	4.03
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif, 5	2.25	CD9	CD9 molecule	3.70
RARRES2	retinoic acid receptor responder (tazarotene induced) 2	2.25	NR2F2	nuclear receptor subfamily 2, group F, member 2	2.99
SLC1 A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	2.24	WISP2	WNT1 inducible signaling pathway protein 2	2.94
PLN	phospholamban	1.96	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	2.91
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	1.94	NR2F2	nuclear receptor subfamily 2, group F, member 2	2.70
ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	1.93	NR2F2	nuclear receptor subfamily 2, group F, member 2	2.66
AMPH	amphiphysin	1.92	KLHL13	kelch-like 13 (Drosophila)	2.53
MAP3K1	mitogen-activated protein kinase kinase kinase 1	1.85	NR2F2	nuclear receptor subfamily 2, group F, member 2	2.47
PDE5 A	phosphodiesterase 5 A, cGMP-specific	1.85	MSX1	msh homeobox 1	2.41
BHMT2	betaine-homocysteine methyltransferase 2	1.78	MARVELD2	MARVEL domain containing 2	2.36
FBXO32	F-box protein 32	1.76	SERPINB7	serpin peptidase inhibitor, clade B (ovalbumin), member 7	2.30
ADM	adrenomedullin	1.76	MYO10	myosin X	2.27
PSG1	pregnancy specific beta-1-glycoprotein 1	1.74	MYO10	myosin X	2.18
CTSZ	cathepsin Z	1.74	PHLDB2	pleckstrin homology-like domain, family B, member 2	2.17
MAP3K5	mitogen-activated protein kinase kinase kinase 5	1.72	NR2F2	nuclear receptor subfamily 2, group F, member 2	2.16
SOCS3	suppressor of cytokine signaling 3	1.70	MITF	microphthalmia-associated transcription factor	1.98
MAP3K5	mitogen-activated protein kinase kinase kinase 5	1.67	DKK2	dickkopf homolog 2 (Xenopus laevis)	1.98
HOXA13	homeobox A13	1.65	FN1	fibronectin 1	1.97
COL8A2	collagen, type VIII, alpha 2	1.63	FN1	fibronectin 1	1.96
NRN1	neuritin 1	1.63	MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	1.92
RASSF4	Ras association (RalGDS/AF-6) domain family member 4	1.58	LOX	lysyl oxidase	1.89
WNT5B	wingless-type MMTV integration site family, member 5B	1.58	HOXA5	homeobox A5	1.88
PSG1	pregnancy specific beta-1-glycoprotein 1	1.57	IGF2	insulin-like growth factor 2 (somatomedin A)	1.85
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein	1.56	HSPB2	heat shock 27 kDa protein 2	1.84
COL8A2	collagen, type VIII, alpha 2	1.53	GREM2	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)	1.83
ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	1.51	MYO10	myosin X	1.83
PSG1	pregnancy specific beta-1-glycoprotein 1	1.51	MITF	microphthalmia-associated transcription factor	1.78

(*continued on next page*)

Table 1 (*continued*)

Papillary Fibroblasts			Reticular Fibroblasts		
Gene Symbol	Gene Title	Fold Change	Gene Symbol	Gene Title	Fold Change
ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.50	GREM2	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)	1.77
WNT5B	wingless-type MMTV integration site family, member 5B	1.50	NR2F1	Nuclear receptor subfamily 2, group F, member 1	1.74
			GLRX	glutaredoxin (thioltransferase)	1.74
			PHACTR2	phosphatase and actin regulator 2	1.72
			DHRS3	dehydrogenase/reductase (SDR family) member 3	1.69
			F2R	coagulation factor II (thrombin) receptor	1.68
			MYO10	myosin X	1.66
			GREM2	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)	1.66
			GLRX	glutaredoxin (thioltransferase)	1.65
			LOX	lysyl oxidase	1.63
			SLC6A15	solute carrier family 6 (neutral amino acid transporter), member 15	1.63
			ITGBL1	Integrin, beta-like 1 (with EGF-like repeat domains)	1.62
			PHACTR2	phosphatase and actin regulator 2	1.61
			FOXP1	forkhead box P1	1.59
			NR2F1	nuclear receptor subfamily 2, group F, member 1	1.59
			MEG3	maternally expressed 3 (non-protein coding)	1.59
			ITPR1	inositol 1,4,5-triphosphate receptor, type 1	1.58
			PHACTR2	phosphatase and actin regulator 2	1.57
			MSX1	Msh homeobox 1	1.57
			HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	1.56
			MYO10	myosin X	1.56
			HIPK2	homeodomain interacting protein kinase 2	1.54
			C7orf10	chromosome 7 open reading frame 10	1.53
			FN1	fibronectin 1	1.52
			PHACTR2	phosphatase and actin regulator 2	1.51
			MEG3	maternally expressed 3 (non-protein coding)	1.51
			PTGER3	prostaglandin E receptor 3 (subtype EP3)	1.51
			HIPK2	homeodomain interacting protein kinase 2	1.50

Mechanical variations due to changes in ECM composition have been reported to affect nuclear shape, chromatin organization, and histone deacetylation activity (Jagielska et al., 2017). Geometrical modifications in the nucleus can thus influence chromatin dynamics, transcription, and genomic integrity (Makhija et al., 2016; Oshidari et al., 2018). Accordingly, analysis of nucleus shape modification may bridge to two of the nine ageing hallmarks (epigenetic alterations and genomic instability) (López-Otín et al., 2013). Modifications of nucleus shape and size have been reported in premature ageing disorders such as the Hutchinson-Gilford progeria syndrome (Cau et al., 2014), and during physiological chrono-ageing (Jeanny and Gontcharoff, 1981; Pienta et al., 1992). To explore these characteristics in normal dermal fibroblasts, we performed measurements of Fp and Fr nuclei surface and circularity in bidimensional cultures, as a function of donor's age. Comparative image analysis of Fp and Fr nuclei indicated that mean nucleus surface was significantly higher in Fr nuclei ($p < 0.001$) (Fig. 3A), but chrono-ageing had no impact on this parameter. In contrast, we found that circularity (isoperimetric quotient), a parameter that is linked to the regularity of nuclei shape, decreased with ageing in both Fp and Fr fibroblasts ($p < 0.001$) (Fig. 3B).

The present study contributes to increase the knowledge on Fp and Fr dermal fibroblasts (Haydont et al., 2018b; Nauroy et al., 2017; Driskell et al., 2013; Mine et al., 2008), and highlights the possible involvement of the 'tissue skeleton' gene network in the tissue and cellular evolutions that occur during chrono-ageing. This cartography certainly provides a new source of potential regulators of human skin ageing. Its understanding will require functional explorations at the genomic and epigenomic levels to address the critical question of distinguishing causal factors and consequential responses. The data shown here concern protein-coding transcripts. Further transcriptomic work using next-generation sequencing would open perspectives for characterization of epigenetic factors involved, such as non-coding RNAs (ncRNAs). Moreover, protein maturation constitutes an important complementary field that needs further exploration.

Competing interests

VH, VN, DA are L'Oréal employees. NF is a CEA employee and acts as L'Oréal scientific consultant, free of charge. VH and DA are inventors on the filed patent application numbered 1759023 (September 28th 2017) entitled "Signatures moléculaires du vieillissement de fibroblastes dermiques et équivalent de derme comprenant ces fibroblastes âgés".

Table 2

The distinction of Fp and Fr fibroblasts originates from their dermis localization (between the epidermis and superficial plexus for Fp, and between the superficial plexus and deep plexus for Fr). Fp and Fr were originally shown to differ in their proliferative capacity (Harper and Grove, 1979) contractility (Schafer et al., 1989), and secretory profile (Schönherr et al., 1993; Sorrell et al., 2004). Since then, a search for Fp and Fr biomarkers has been conducted by different groups (Janson et al., 2012; Nauroy et al., 2017; Philippeos et al., 2018; Korosec et al., 2018). Selections of markers identified independently for human cells are listed. Convergences are highlighted in green color, and divergences in red color. Procedures used for cell isolation are indicated for each study.

		Non-cultivated cells										Cells expanded in culture					
		Risk of contamination by other cell types +++ Risk of phenotype modifications induced by culture ---										Risk of contamination by other cell types +/- Risk of phenotype modifications induced by culture +/-					
		Philippeos et al.						Korosec et al.				Nauroy et al.		Janson et al.		Present paper	
		Microdissection		Single-cell RNA-seq				Sorting FAP / CD90 or dermatome				Dermatome		Dermatome		Dermatome	
		Enriched in Papillary fibroblasts	Enriched in pre-adipocytes	Group 1 Undefined	Group 2 Pericytes (and others)	Group 3 Probably papillary	Group 4 Probably reticular	Group 5 Macrophages dendritic cells, pre-adipocytes	Papillary dermis Enriched in FAP+ CD90-	Upper reticular dermis Enriched in FAP+ CD90+	Lower reticular dermis Enriched in FAP- CD90+	Enriched in Papillary fibroblasts	Enriched in Reticular fibroblasts	Enriched in Papillary fibroblasts	Enriched in Reticular fibroblasts	Enriched in Papillary fibroblasts	Enriched in Reticular fibroblasts
Tissue localization		Dermis depth: from 0 to 100µm	Dermis depth: from 200µm to 500µm						Dermis depth: from 0 to 200µm	Dermis depth: from 200µm to 1000µm	Dermis depth: below 1000µm	Dermis depth: from 0 to 200µm	Dermis depth: below 600µm	Dermis depth: from 0 to 200µm	Dermis depth: below 600µm	Dermis depth: from 0 to 200µm	Dermis depth: below 600µm
Philippeos et al.	Enriched in Papillary Fibroblasts	APCDD1 AXIN2 C8orf22 CCL14 CCL15 CCL5 CD3D CD3E CD3G CD39 CD52 CLEC10A CLEC2A CLEC7A COL18A1 COL23A1 COL6A5 CTSW DIRAS3 ESRG FCER1A FREM1 HIGD1B HSPB3 IFNG IGJ IGLL5 LAMC3 LINC01091 LYZ YL3 NPTX2 CED1B-AS1 PTGDS PTGS1 PTK7 RNASE6 ROBO2 RSP01 SGCA SGCG SPON1 TRAT1 UBXN11 WIF1 XCL1			COL6A5 COL23A1 HSPB5				CD39			COL18A1				APCDD1 AXIN2 COL18A1 COL23A1 HSPB3 NPTX2 PTGDS ROBO2	DIRAS3 SPON1
	Enriched in Pre-Adipocytes	AQP5 AZGP1 CA6 CD36 CEACAM5 CEACAM6 CLDN10 CLDN10-AS1 CLDN7 CRISP3 DCD DNER ELF3 FABP9 GABRP GRB14 KRT25 KRT27 KRT28 KRT35 KRT7 KRT71 KRT8 KRTAP11-1 LOC102723505 LOC102723517 MUCL1 OBP2A OBP2B PART1 PIP PRR9 ROPN1B S100A1 SCGB1B2P SCGB1D2 SCGB2A1 SCGB2A2 SCL6A14 SCLC12A2 SLC13A2 SMIM22 STAC2 TCHH ZG16B									CD36					DNER GRB14

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Table 2 (*continued*)

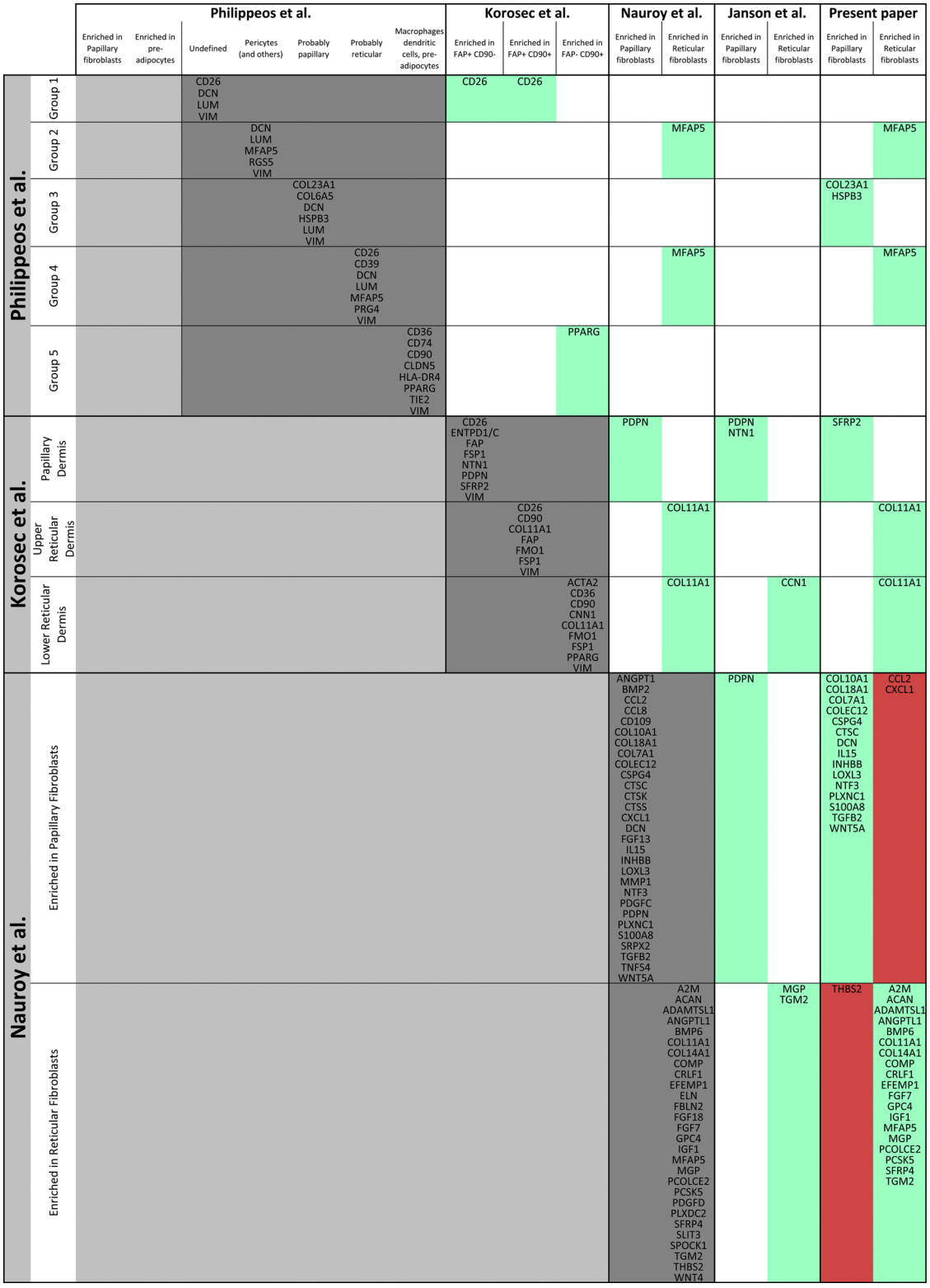

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Table 2 (*continued*)

		Philippeos et al.							Korosec et al.			Nauroy et al.		Janson et al.		Present paper	
		Enriched in Papillary fibroblasts	Enriched in pre-adipocytes	Undefined	Pericytes (and others)	Probably papillary	Probably reticular	Macrophages dendritic cells, pre-adipocytes	Enriched in FAP+ CD90-	Enriched in FAP+ CD90+	Enriched in FAP- CD90+	Enriched in Papillary fibroblasts	Enriched in Reticular fibroblasts	Enriched in Papillary fibroblasts	Enriched in Reticular fibroblasts	Enriched in Papillary fibroblasts	Enriched in Reticular fibroblasts
Janson et al.	Enriched in Papillary Fibroblasts													CCRL1 GPER ITMC2 NTN1 PDPN STEAP1 TNFRSF25		TNFRSF19	GPER STEAP1
	Enriched in Reticular Fibroblasts														CDH2 CCN1 MAP1B MGP NEXLN PPP1R14A TAGLN TGM2 TMEM200		CDH2 MAP1B MGP PPP1R14A TAGLN TGM2 TMEM200
Present paper	Enriched in Papillary Fibroblasts															APCDD1 AXIN2 COL10A1 COL18A1 COL23A1 COL7A1 COLEC12 CSPG4 CTSC DCN HSPB3 IL15 INHBB LOXL3 NPTX2 NTF3 PLXNC1 PTGDS ROBO2 S100A8 SFRP2 TGFB2 THBS2 TNFRSF19 WNT5A	
	Enriched in Reticular Fibroblasts																A2M ACAN ADAMTSL1 ANGPTL1 BMP6 CCL2 CDH2 COL11A1 COL14A1 COMP CRLF1 CXCL1 DIRAS3 DNER EFEMP1 FGF7 GPC4 GPER GRB14 IGF1 MAP1B MFAP5 MGP PCOLCE2 PCSK5 PPP1R14A SFRP4 SPON1 STEAP1 TAGLN TGM2 TMEM200A

Table 3

Tissue skeleton-related transcripts modulated during chrono-ageing in Fp and Fr fibroblasts. Data were extracted from our previously published micro-array genome-wide transcriptome analysis (Haydont et al., 2018b). Transcriptional changes that were detected in Fp and Fr cells concerned respectively 113 and 170 genes directly involved in tissue skeleton structure, comprising 58 and 54 ECM genes, 28 and 61 focal adhesion point genes, 22 and 46 cytoskeleton genes, 1 and 2 LINC complex genes, and 4 and 7 nucleoskeleton gene. In addition, transcript modulation in Fp and Fr cells concerned respectively 73 and 125 genes with possible regulatory roles in tissue skeleton biology, comprising 28 and 57 signal transduction genes, 34and 38 soluble factor genes, as well as 11 and 30 unclassified genes. Genes highlighted in green were detected as over-expressed in young Fp and Fr, thus representing common signatures of the younger adult age group in both Fp and Fr. Genes highlighted in purple were detected as over-expressed in old Fp and Fr, thus representing common signatures of the older adult age group in both Fp and Fr.

		Number of modulated transcripts	Up modulated in Young			Up modulated in old		
			Gene Symbol	Gene Title	Fold Change	Gene Symbol	Gene Title	Fold Change
Papillary Fibroblasts	Extracellular matrix	58	CLEC12B	C-type lectin domain family 12, member B	1,58	ACAN	aggrecan	3,04
			COL12A1	collagen, type XII, alpha 1	1,58	ADAM23	ADAM metallopeptidase domain 23	1,62
			COL15A1	collagen, type XV, alpha 1	1,65	CLEC2B	C-type lectin domain family 2, member B	1,5
			COL3A1	Collagen, type III, alpha 1	2,29	COL11A1	collagen, type XI, alpha 1	3,21
			COL8A1	collagen, type VIII, alpha 1	1,57	COL6A1	collagen, type VI, alpha 1	1,54
			COLEC12	collectin sub-family member 12	2,08	CSTA	cystatin A (stefin A)	3,7
			CTSC	cathepsin C	1,54	DPP4	dipeptidyl-peptidase 4	2,16
			CTSK	cathepsin K	1,51	ELN	elastin	1,78
			EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	2,21	EMILIN2	elastin microfibril interfacer 2	1,81
			FBLN7	fibulin 7	1,61	ESM1	endothelial cell-specific molecule 1	1,57
			FBN2	fibrillin 2	6,14	HSPG2	heparan sulfate proteoglycan 2	1,62
			GPC4	glypican 4	2	LGALS8	lectin, galactoside-binding, soluble, 8	1,79
			HAPLN1	hyaluronan and proteoglycan link protein 1	2,97	LOX	lysyl oxidase	3,18
			HMCN1	hemicentin 1	1,63	LOXL2	lysyl oxidase-like 2	1,68
			HTRA3	HtrA serine peptidase 3	1,72	LTBP1	latent transforming growth factor beta binding protein 1	2,14
			IGFBP2	insulin-like growth factor binding protein 2, 36kDa	1,57	MASP1	mannan-binding lectin serine peptidase 1	1,55
			ITIH5	inter-alpha (globulin) inhibitor H5	1,54	MFAP5	microfibrillar associated protein 5	1,69
			LOXL4	lysyl oxidase-like 4	1,92	MMP12	matrix metallopeptidase 12 (macrophage elastase)	4,27
			MATN3	matrilin 3	1,61	MMP14	matrix metallopeptidase 14 (membrane-inserted)	1,69
			MUC1	mucin 1, cell surface associated	1,91	MMP16	matrix metallopeptidase 16 (membrane-inserted)	1,76
			PRELP	proline/arginine-rich end leucine-rich repeat protein	2,23	MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)	1,57
			SULF2	sulfatase 2	2,73	NID1	nidogen 1	1,56
			THBS2	thrombospondin 2	2,04	PAPPA	pregnancy-associated plasma protein A, pappalysin 1	1,58
			THBS3	thrombospondin 3	1,53	PCOLCE2	procollagen C-endopeptidase enhancer 2	2,96
			THBS5/COMP	cartilage oligomeric matrix protein	2,44	PCSK5	proprotein convertase subtilisin/kexin type 5	2,15
						PI3	peptidase inhibitor 3, skin-derived	1,61
						SERPIN B2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	2,99
						SERPIN B6	serpin peptidase inhibitor, clade B (ovalbumin), member 6	1,51
						SERPIN B7	serpin peptidase inhibitor, clade B (ovalbumin), member 7	1,57
						SERPIN G1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	1,63
						SERPINE1	serpin peptidase inhibitor, clade E member 1	1,97
						THBS1	thrombospondin 1	1,71
						TIMP3	TIMP metallopeptidase inhibitor 3	2,66
	Focal adhesoin point	28	ACVR2	activin A receptor, type IIA	1,53	ADD2	adducin 2 (beta)	2,11
			CELSR1	cadherin, EGF LAG seven-pass G-type receptor 1	2,13	ANKRD12	Ankyrin repeat domain 12	1,75
			CRLF1	cytokine receptor-like factor 1	2,51	ANKRD36	ankyrin repeat domain 36B	1,53
			EFNB3	ephrin-B3	1,88	APBB1IP	amyloid beta (A4) precursor protein-binding, B1 interacting protein	1,76
			FGFR2	fibroblast growth factor receptor 2	1,68	BMPR1B	bone morphogenetic protein receptor, type IB	1,56
			GAS1	growth arrest-specific 1	1,64	FLOT1	flotillin 1	2,29
			GPR126	G protein-coupled receptor 126	1,58	GPR133	G protein-coupled receptor 133	3,19
			GPR37	G protein-coupled receptor 37 (endothelin receptor type B-like)	1,71	GPRC5B	G protein-coupled receptor, family C, group 5, member B	1,78
			GPR56	G protein-coupled receptor 56	1,74	IL15RA	interleukin 15 receptor, alpha	1,65
			ITGA8	integrin, alpha 8	1,57	ITGA3	integrin, alpha 3 (antigen CD49C)	1,72
			MCAM	melanoma cell adhesion molecule	1,55	ITGA6	integrin, alpha 6	1,71
			TGFBR1	transforming growth factor, beta receptor 1	1,62	ITGA9	integrin, alpha 9	1,55
			TNFSF4	tumor necrosis factor (ligand) superfamily, member 4	2,79	MFGE8	milk fat globule-EGF factor 8 protein	1,57
						NFASC1	neurofascin homolog (chicken)	1,51
						PLAUR	plasminogen activator, urokinase receptor	1,72
	Cytoskeleton	22	DMD	dystrophin	1,61	ABLIM1	actin binding LIM protein 1	1,64
			DNALI1	dynein, axonemal, light intermediate chain 1	1,72	FBLIM1	filamin binding LIM protein 1	1,68
			MYLIP	myosin regulatory light chain interacting protein	1,85	KIF22	kinesin family member 22	1,72
			NEFH	neurofilament, heavy polypeptide	1,87	KIF26A	kinesin family member 26A	1,55
			PHACTR3	phosphatase and actin regulator 3	3,64	MAP1B	microtubule-associated protein 1B	1,53
			PPP1CB	protein phosphatase 1, catalytic subunit, beta isoform	1,5	MYO10	myosin X	2,19
			S100A10	S100 calcium binding protein A10	1,56	NDNF	Neuron-Derived Neurotrophic Factor	1,6
			TUBB2B	tubulin, beta 2B	2,24	NEFL	neurofilament, light polypeptide	1,63
						NTNG1	netrin G1	4,28
						PHACTR2	phosphatase and actin regulator 2	1,51
						PPP1R15A	protein phosphatase 1, regulatory (inhibitor) subunit 15A	1,50
						S100A7	S100 calcium binding protein A7	3,27
						S100A8	S100 calcium binding protein A8	3,28
						TNS1	tensin 1	1,59
	LINC Complexes	1	UNC84A	unc-84 homolog A (C. elegans)	1,66			
	Nucleoskeleton	4	H1F0	H1 histone family, member 0	1,8	ARPC4	actin related protein 2/3 complex, subunit 4, 20kDa	1,58
			SMARCA1	NF related, matrix associated, actin dependent regulator of chroma	1,65	HIST1H2BE	histone cluster 1, H2be	1,51
	Transduction pathways	28	AXIN2	axin 2	1,59	AKAP12	A kinase (PRKA) anchor protein 12	1,76
			bCATENIN	catenin (cadherin-associated protein), beta 1, 88kDa	1,68	ARF1	ADP-ribosylation factor 1	1,86
			GNG7	guanine nucleotide binding protein (G protein), gamma 7	1,59	ARFRP1	ADP-ribosylation factor related protein 1	1,77
			ITGB1BP1	integrin beta 1 binding protein 1	1,88	ARHGDIA	Rho GDP dissociation inhibitor (GDI) alpha	1,66
			LDB3	LIM domain binding 3	4,12	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1,5
			MAP2K6	mitogen-activated protein kinase kinase 6	1,59	GNG12	guanine nucleotide binding protein (G protein), gamma 12	1,58
			MAP3K1	mitogen-activated protein kinase kinase kinase 1	1,59	ID4	inhibitor of DNA binding 4	1,61
			MAPK13	mitogen-activated protein kinase 13 (p38 delta)	1,55	KANK4	KN motif and ankyrin repeat domains 4	3,4
			RAB30	RAB30, member RAS oncogene family	1,78	MAP2K2	mitogen-activated protein kinase kinase 2	2,1
			RAB3D	RAB3D, member RAS oncogene family	1,64	NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	1,77
			RAB8B	RAB8B, member RAS oncogene family	1,67	RAB3B	RAB3B, member RAS oncogene family	3,31
			RASGEF1A	RasGEF domain family, member 1A	1,66	RAB5C	RAB5C, member RAS oncogene family	1,57
			RGS4	regulator of G-protein signaling 4	1,74	RAC2	ras-related C3 botulinum toxin substrate 2	2,42
						RANGAP1	Ran GTPase activating protein 1	2,31
						SMAD5	SMAD family member 5	1,53
	Secreted factors	34	EDIL3	EGF-like repeats and discoidin I-like domains 3	2,88	ANGPTL4	angiopoietin-like 4	2,55
			FGF1	fibroblast growth factor 1 (acidic)	1,51	CCL5	chemokine (C-C motif) ligand 5	2,69
			INHBB	inhibin, beta B	2,05	CXCL1	chemokine (C-X-C motif) ligand 1	3,18
			PDGFC	platelet derived growth factor C	1,52	CXCL14	chemokine (C-X-C motif) ligand 14	2,35
			POSTN	periostin, osteoblast specific factor	2,54	CXCL5	chemokine (C-X-C motif) ligand 5	6,91
			SEMA3B	sema domain, short basic domain, secreted, (semaphorin) 3B	2,04	CXCL6	chemokine (C-X-C motif) ligand 6	7
			SEMA5A	ain, seven thrombospondin repeats (type 1 and type 1-like), (sema	1,52	GDF15	growth differentiation factor 15	1,55
			SEMA6D	ain, transmembrane domain (TM), and cytoplasmic domain, sema	2,09	IL15	interleukin 15	1,86
			SFRP2	secreted frizzled-related protein 2	4,85	IL32	interleukin 32	4,05
			TGFB2	transforming growth factor, beta 2	1,62	IL7	interleukin 7	1,65
			WISP1	WNT1 inducible signaling pathway protein 1	1,6	IL8	interleukin 8	3,77
						MDK	midkine (neurite growth-promoting factor 2)	1,88
						NRG1	neuregulin 1	1,95
						PF4V1	platelet factor 4 variant 1	1,67
						PGF	placental growth factor	1,93
						PLXNC1	plexin C1	1,72
						RSPO3	R-spondin 3 homolog (Xenopus laevis)	2,15
						SEMA7A	semaphorin 7A, GPI membrane anchor	1,59
						SFRP1	secreted frizzled-related protein 1	3,74
						TGFA	transforming growth factor, alpha	1,89
						VEGFA	vascular endothelial growth factor A	1,56
						WISP2	WNT1 inducible signaling pathway protein 2	1,99
						WNT5B	wingless-type MMTV integration site family, member 5B	1,62
	Unclassified	11	CPAMD8	C3 and PZP-like, alpha-2-macroglobulin domain containing 8	1,85	C1QTNF2	C1q and tumor necrosis factor related protein 2	1,52
			CSRP2	cysteine and glycine-rich protein 2	1,99	CRELD1	cysteine-rich with EGF-like domains 1	1,64
			LMAN1	lectin, mannose-binding, 1	1,53	CST6	cystatin E/M	1,9
			POMZP3	POM (POM121 homolog, rat) and ZP3 fusion	1,99	FHL1	four and a half LIM domains 1	1,54
			VWA5A	von Willebrand factor A domain containing 5A	1,64	MDC1	mediator of DNA damage checkpoint 1	1,54
						OGFOD1	2-oxoglutarate and iron-dependent oxygenase domain containing 1	1,52

(*continued on next page*)

Table 3 (*continued*)

		Number of modulated transcripts	Up modulated in Young			Up modulated in old		
			Gene Symbol	Gene Title	Fold Change	Gene Symbol	Gene Title	Fold Change
Reticular Fibroblasts	**Extracellular matrix**	54	ADAMTSL1	ADAMTS-like 1	1,75	ACAN	aggrecan	3,5
			CLEC3B	C-type lectin domain family 3, member B	1,74	ADAM23	ADAM metallopeptidase domain 23	2,45
			COL12A1	collagen, type XII, alpha 1	2,85	ASPN	asporin	1,67
			COL3A1	Collagen, type III, alpha 1	1,74	CCBE1	collagen and calcium binding EGF domains 1	1,84
			COL4A5	collagen, type IV, alpha 5	1,57	CLEC2B	C-type lectin domain family 2, member B	1,75
			CSTA	cystatin A (stefin A)	1,75	COL14A1	collagen, type XIV, alpha 1	2,1
			EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	1,58	COL4A1	collagen, type IV, alpha 1	1,53
			FBN2	fibrillin 2	6,52	COL4A2	collagen, type IV, alpha 2	2,03
			FN1	fibronectin 1	1,71	DCN	decorin	1,71
			GPC4	glypican 4	1,71	DPT	dermatopontin	2,88
			HAPLN1	hyaluronan and proteoglycan link protein 1	1,72	EMCN	endomucin	2,81
			IGFBP5	insulin-like growth factor binding protein 5	2,69	FBLN1	fibulin 1	2,07
			IGFBP7	insulin-like growth factor binding protein 7	1,51	IBSP	integrin-binding sialoprotein	1,86
			ITIH5	inter-alpha (globulin) inhibitor H5	1,53	IGF2BP3	insulin-like growth factor 2 mRNA binding protein 3	1,84
			LOXL3	lysyl oxidase-like 3	1,61	IGFBP2	insulin-like growth factor binding protein 2, 36kDa	2,35
			MUC20	Mucin 20, cell surface associated	2,12	LGALS8	lectin, galactoside-binding, soluble, 8	1,59
			PAPPA	pregnancy-associated plasma protein A, pappalysin 1	1,52	LOX	lysyl oxidase	2,73
			PLAU	plasminogen activator, urokinase	1,53	LTBP1	latent transforming growth factor beta binding protein 1	1,51
			SPOCK1	rc/osteonectin, cwcv and kazal-like domains proteoglycan (testicar	1,9	MASP1	mannan-binding lectin serine peptidase 1	1,76
			SULF1	sulfatase 1	1,66	MIIP	migration and invasion inhibitory protein	1,56
			SULF2	sulfatase 2	2,93	MMP12	matrix metallopeptidase 12 (macrophage elastase)	3,85
			TGM2	transglutaminase 2	2,2	MMP14	matrix metallopeptidase 14 (membrane-inserted)	1,88
			THBS2	thrombospondin 2	1,7	MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)	1,62
			THSB5/COMP	cartilage oligomeric matrix protein	3,45	OGN	osteoglycin	2,23
			THSD4	thrombospondin, type I, domain containing 4	1,73	PCOLCE2	procollagen C-endopeptidase enhancer 2	1,56
						SERPINB2	serpin peptidase inhibitor, clade B, member 2	2,19
						SERPINF1	serpin peptidase inhibitor, clade F, member 1	2,07
						SPON1	spondin 1, extracellular matrix protein	2,83
						TNXB	tenascin XB	2,08
	Focal adhesoin points	61	ACVR2A	activin A receptor, type IIA	1,59	ADD2	adducin 2 (beta)	2,66
			ANKRD10	ankyrin repeat domain 10	1,62	AGTR1	angiotensin II receptor, type 1	2,26
			ANKRD57	ankyrin repeat domain 57	1,87	AGTRAP	angiotensin II receptor-associated protein	1,54
			BMPR2	ne morphogenetic protein receptor, type II (serine/threonine kinas	1,97	BMPR1B	bone morphogenetic protein receptor, type IB	1,65
			CADM1	cell adhesion molecule 1	1,74	CCRL1	chemokine (C-C motif) receptor-like 1	2,75
			CLDN1	claudin 1	1,5	CLDN11	claudin 11	1,62
			CRLF1	cytokine receptor-like factor 1	2,18	DSG2	desmoglein 2	2,12
			CXCR7	Chemokine (C-X-C motif) receptor 7	1,58	F2RL1	coagulation factor II (thrombin) receptor-like 1	1,74
			EFNB2	ephrin-B2	2,14	GLDN	gliomedin	1,53
			F2R	coagulation factor II (thrombin) receptor	1,85	GPR161	G protein-coupled receptor 161	2,06
			F2RL2	coagulation factor II (thrombin) receptor-like 2	2,9	GPR176	G protein-coupled receptor 176	1,57
			FGFR2	fibroblast growth factor receptor 2	1,52	GPR177	G protein-coupled receptor 177	2,13
			FLRT2	Fibronectin leucine rich transmembrane protein 2	1,71	GPRC5A	G protein-coupled receptor, family C, group 5, member A	2,03
			GAS1	growth arrest-specific 1	1,64	GPRC5B	G protein-coupled receptor, family C, group 5, member B	3,6
			GPR116	G protein-coupled receptor 116	3,97	IL13RA2	interleukin 13 receptor, alpha 2	2,42
			IL6R	interleukin 6 receptor	1,85	IL21R	interleukin 21 receptor	1,5
			IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	1,61	ITGA6	integrin, alpha 6	1,55
			ITGB2	integrin, beta 2	1,6	ITGA8	integrin, alpha 8	3,21
			ITGBL1	integrin, beta-like 1 (with EGF-like repeat domains)	1,55	ITGA9	integrin, alpha 9	3,56
			LEPR	leptin receptor	1,53	ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	2,03
			PCDH19	protocadherin 19	3,03	ITGB5	Integrin, beta 5	1,83
			PCDH7	protocadherin 7	1,85	LAMP1	lysosomal-associated membrane protein 1	1,84
			PCDH9	protocadherin 9	2,14	NCAM1	neural cell adhesion molecule 1	1,7
			PCDHB10	protocadherin beta 10	1,69	RARB	retinoic acid receptor, beta	1,84
			PCDHB14	protocadherin beta 14	1,66	THBD	thrombomodulin	2,17
			PDGFRB	platelet-derived growth factor receptor, beta polypeptide	1,53	TSPAN4	tetraspanin 4	1,69
			PDGFRL	platelet-derived growth factor receptor-like	1,85	TSPAN8	tetraspanin 8	2,02
			PPFIA4	tyrosine phosphatase, receptor type, PTPRF, interacting protein, a	1,94			
			TMEM106B	transmembrane protein 106B	1,7			
			TNFRSF10A	tumor necrosis factor receptor superfamily, member 10a	1,5			
			TNFRSF21	tumor necrosis factor receptor superfamily, member 21	1,54			
			TNFSF4	tumor necrosis factor (ligand) superfamily, member 4	3,84			
			TSPAN14	tetraspanin 14	1,6			
			VCAM1	vascular cell adhesion molecule 1	3,32			
	Cytoskeleton	46	ACTA2	actin, alpha 2, smooth muscle, aorta	1,55	ABLIM1	actin binding LIM protein 1	1,58
			CALD1	caldesmon 1	2,84	AURKA	aurora kinase A	1,65
			CORO2B	coronin, actin binding protein, 2B	2,02	AURKB	aurora kinase B	1,67
			EZR	ezrin	1,63	FIBCD1	fibrinogen C domain containing 1	2,17
			FAM20A	family with sequence similarity 20, member A	1,7	GSN	gelsolin (amyloidosis, Finnish type)	1,71
			FLG	filaggrin	1,88	KIF14	kinesin family member 14	1,63
			GOLGA8A	golgi autoantigen, golgin subfamily a, 8A	2,48	KIF22	kinesin family member 22	2,36
			GOLGA8B	golgi autoantigen, golgin subfamily a, 8B	1,71	KIF2C	kinesin family member 2C	1,53
			GOLIM4	golgi integral membrane protein 4	1,92	KRT18	keratin 18	1,61
			GOLSYN	Golgi-localized protein	1,81	KRT33A	keratin 33A	1,87
			IQGAP1	IQ motif containing GTPase activating protein 1	1,91	KRT7	keratin 7	1,71
			MYH10	myosin, heavy chain 10, non-muscle	2,11	KRTAP1-1	keratin associated protein 1-1	1,56
			MYO6	myosin VI	2,06	KRTAP1-3	keratin associated protein 1-3	1,51
			NEFH	neurofilament, heavy polypeptide	1,85	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog	1,69
			TMEM43	Transmembrane protein 43	1,52	MAP2	microtubule-associated protein 2	1,68
			TPM1	Tropomyosin 1 (alpha)	1,5	MYH1	myosin, heavy chain 1, skeletal muscle, adult	2,02
						MYH2	myosin, heavy chain 2, skeletal muscle, adult	2,13
						MYO10	myosin X	1,73
						NEFL	neurofilament, light polypeptide	1,98
						NEFM	neurofilament, medium polypeptide	2,49
						NES	nestin	1,7
						PLEC1	plectin 1, intermediate filament binding protein 500kDa	1,51
						TMED2	transmembrane emp24 domain trafficking protein 2	1,56
						TMSB15A	thymosin beta 15a	2,32
						TNNC1	troponin C type 1 (slow)	1,53
						TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	1,68
						TRAPPC3	trafficking protein particle complex 3	1,5
						TUBB2A	tubulin, beta 2A	1,99
						TUBB2B	tubulin, beta 2B	1,99
						TUBD1	tubulin, delta 1	1,53
	LINC Complexes	2	SPAG4	sperm associated antigen 4	1,56	SYNE2	spectrin repeat containing, nuclear envelope 2	1,62
	Nucleoskeleton	7	LAMA1	laminin, alpha 1	1,82	LAMA2	laminin, alpha 2	2,07
			LAMA4	laminin, alpha 4	1,58	ARPC4	actin related protein 2/3 complex, subunit 4, 20kDa	1,75
						ACTG2	actin, gamma 2, smooth muscle, enteric	1,67
						SMARCA4	SWI/SNF related, actin dependent regulator of chromatin, a4	1,58
						H2AFX	H2A histone family, member X	2,07

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Table 3 (*continued*)

		Number of modulated transcripts	Up modulated in Young			Up modulated in old		
			Gene Symbol	Gene Title	Fold Change	Gene Symbol	Gene Title	Fold Change
Reticular Fibroblasts	**Transduction pathways**	57	AKT3	v-akt murine thymoma viral oncogene homolog 3	1,69	DIRAS3	DIRAS family, GTP-binding RAS-like 3	2,18
			ARHGAP21	Rho GTPase activating protein 21	1,79	DNM1	dynamin 1	1,58
			ARHGAP5	Rho GTPase activating protein 5	1,52	DNM3	dynamin 3	2,1
			ARHGEF19	Rho guanine nucleotide exchange factor (GEF) 19	1,54	HHIP	hedgehog interacting protein	1,64
			ARHGEF4	Rho guanine nucleotide exchange factor (GEF) 4	1,78	JAK1	Janus kinase 1	1,5
			ARHGEF7	Rho guanine nucleotide exchange factor (GEF) 7	1,65	KANK4	KN motif and ankyrin repeat domains 4	2,5
			ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1	1,85	MAP2K2	mitogen-activated protein kinase kinase 2	2,57
			BMP2K	BMP2 inducible kinase	1,58	MAP3K5	mitogen-activated protein kinase kinase kinase 5	1,73
			CDC42EP2	CDC42 effector protein (Rho GTPase binding) 2	1,53	MAPK13	mitogen-activated protein kinase 13	1,55
			CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	1,63	PKIA	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	2,52
			CNKSR2	connector enhancer of kinase suppressor of Ras 2	1,51	PRKAB1	protein kinase, AMP-activated, beta 1 non-catalytic subunit	1,66
			CREB5	cAMP responsive element binding protein 5	1,65	PRKCDBP	protein kinase C, delta binding protein	1,66
			DAB2	disabled homolog 2, mitogen-responsive phosphoprotein	1,67	RAB3B	RAB3B, member RAS oncogene family	2,78
			G3BP1	GTPase activating protein (SH3 domain) binding protein 1	1,98	RAB5C	RAB5C, member RAS oncogene family	1,61
			ITGB1BP1	integrin beta 1 binding protein 1	1,85	RAC2	ras-related C3 botulinum toxin substrate 2	2,88
			LDB3	LIM domain binding 3	4,7	RANGAP1	Ran GTPase activating protein 1	3,26
			LHX8	LIM homeobox 8	2,56	RASA4	RAS p21 protein activator 4	1,73
			LHX9	LIM homeobox 9	3,03	RASD1	RAS, dexamethasone-induced 1	1,52
			LIMCH1	LIM and calponin homology domains 1	1,64	RASD2	RASD family, member 2	2,57
			LIMS3	LIM and senescent cell antigen-like domains 3	1,76	RHEB	Ras homolog enriched in brain	1,5
			LMCD1	LIM and cysteine-rich domains 1	2,59			
			LMO7	LIM domain 7	1,51			
			NFAT5	nuclear factor of activated T-cells 5, tonicity-responsive	1,67			
			PIK3C2A	Phosphoinositide-3-kinase, class 2, alpha polypeptide	1,75			
			PLEKHA2	pleckstrin homology domain containing, A 2	2,77			
			PPP1R10	protein phosphatase 1, regulatory (inhibitor) subunit 10	1,53			
			RAB11FIP1	RAB11 family interacting protein 1 (class I)	1,95			
			RAB30	RAB30, member RAS oncogene family	1,52			
			RANBP17	RAN binding protein 17	2,41			
			RANBP2	RAN binding protein 2	1,51			
			RASSF2	Ras association (RalGDS/AF-6) domain family member 2	1,59			
			RASSF8	Ras association (RalGDS/AF-6) domain family (N-terminal) 8	1,68			
			RGS4	regulator of G-protein signaling 4	2,34			
			RHOJ	ras homolog gene family, member J	2,57			
			STAT1	signal transducer and activator of transcription 1, 91kDa	1,6			
			STAT4	signal transducer and activator of transcription 4	1,78			
			STK32B	serine/threonine kinase 32B	1,91			
	Secreted factors	38	ANGPT1	angiopoietin 1	1,86	ANGPTL4	angiopoietin-like 4	1,66
			ANGPTL1	angiopoietin-like 1	1,65	BMP2	bone morphogenetic protein 2	1,63
			CSF2RB	colony stimulating factor 2 receptor, beta, low-affinity	1,68	CCL5	chemokine (C-C motif) ligand 5	1,85
			FGF9	fibroblast growth factor 9 (glia-activating factor)	3,28	CXCL1	chemokine (C-X-C motif) ligand 1	2,16
			GDNF	glial cell derived neurotrophic factor	1,96	CXCL2	chemokine (C-X-C motif) ligand 2	2,04
			INHBA	inhibin, beta A	2,29	CXCL3	chemokine (C-X-C motif) ligand 3	2,1
			INHBB	inhibin, beta B	1,91	CXCL5	chemokine (C-X-C motif) ligand 5	6,75
			NTF3	neurotrophin 3	2,23	CXCL6	chemokine (C-X-C motif) ligand 6	4,99
			PLXNA2	plexin A2	1,66	IGF2	insulin-like growth factor 2 (somatomedin A)	2,37
			POSTN	periostin, osteoblast specific factor	5,83	IL15	interleukin 15	1,62
			SCUBE3	signal peptide, CUB domain, EGF-like 3	1,99	IL32	interleukin 32	2,26
			SEMA3B	sema domain, immunoglobulin domain, short basic domain, 3B	1,53	IL8	interleukin 8	5,8
			SEMA5A	sema domain, seven thrombospondin repeats, (semaphorin) 5A	2,38	MDK	midkine (neurite growth-promoting factor 2)	2,06
			SFRP2	secreted frizzled-related protein 2	2,22	NOV	nephroblastoma overexpressed gene	1,67
			SFRP4	secreted frizzled-related protein 4	2,14	PF4	platelet factor 4	1,5
			WNT5A	wingless-type MMTV integration site family, member 5A	2,04	PF4V1	platelet factor 4 variant 1	2,19
						PGF	placental growth factor	1,74
						PLXNC1	plexin C1	1,54
						PTN	pleiotrophin	1,83
						VEGFA	vascular endothelial growth factor A	1,66
						WISP2	WNT1 inducible signaling pathway protein 2	1,75
						WNT5B	wingless-type MMTV integration site family, member 5B	1,79
	Unclassified	30	AMIGO2	adhesion molecule with Ig-like domain 2	1,52	C1QTNF2	C1q and tumor necrosis factor related protein 2	1,52
			CLIP1	CAP-GLY domain containing linker protein 1	2,27	CRELD1	cysteine-rich with EGF-like domains 1	1,52
			CYTL1	cytokine-like 1	1,73	F10	coagulation factor X	1,92
			FNDC3B	fibronectin type III domain containing 3B	1,63	FHL1	four and a half LIM domains 1	1,62
			MEGF8	multiple EGF-like-domains 8	1,51	FIBCD1	fibrinogen C domain containing 1	2,17
			POMZP3	POM and ZP3 fusion/zona pellucida glycoprotein 3	2,28	HLA-B	major histocompatibility complex, class I, B	1,51
			PRKAB2	protein kinase, AMP-activated, beta 2 non-catalytic subunit	1,72	HLA-DMA	major histocompatibility complex, class II, DM alpha	1,61
						LAPTM4B	lysosomal protein transmembrane 4 beta	2,12
						LEPREL1	leprecan-like 1	2,13
						OGFOD1	2-oxoglutarate and iron-dependent oxygenase domain containing 1	1,57
						OLFM1	olfactomedin 1	1,65
						OLFML2B	olfactomedin-like 2B	1,84
						PRSS12	protease, serine, 12 (neurotrypsin, motopsin)	2,36
						S100P	S100 calcium binding protein P	3,1
						TM4SF1	transmembrane 4 L six family member 1	5,37
						TMCC2	transmembrane and coiled-coil domain family 2	1,56
						TMCO6	transmembrane and coiled-coil domains 6	1,61
						TMEM155	transmembrane protein 155	1,95
						TMEM35	transmembrane protein 35	1,52
						TMEM55B	transmembrane protein 55B	1,5
						TMSB15A	thymosin beta 15a	2,32
						TMTC4	transmembrane and tetratricopeptide repeat containing 4	1,55
						TOR3A	torsin family 3, member A	2,06

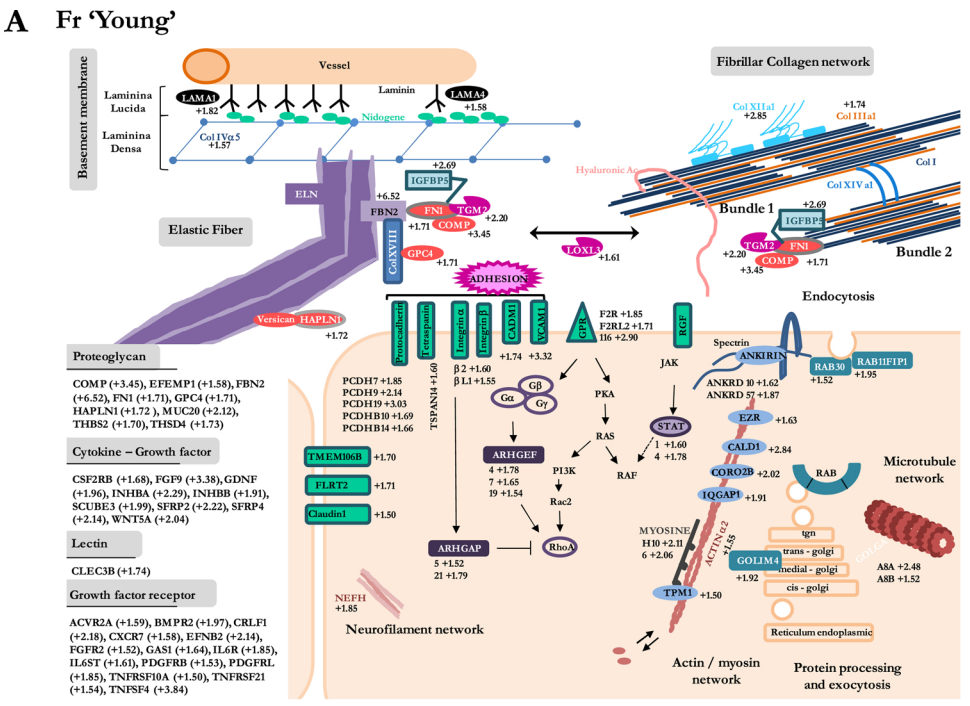

Fig. 2. Functional classification of transcripts modulated during chrono-ageing in Fr fibroblasts. The global transcriptome analysis identified a total of 779 probe sets up-modulated in Fr from the 'younger' donor group, and 759 probe sets up-modulated in Fr from the 'older'donor group (fold-change cut-off at 1.5, $p < 0.05$) (Haydont et al., 2018b). The transcripts that are indicated in the present figure were selected according to direct involvement in tissue skeleton structure: 81 transcripts predominantly expressed in 'young' Fr (**A**), and 99 transcripts predominantly expressed in 'old' Fr (**B**). Notably, evolution of Fr transcriptome with ageing included modulation of transcripts related to the structuration and organization of collagen bundles and fibrils, proteoglycan biology, and cytoskeleton polymerization.

Table 4
Validation of the micro-array data. qRT-PCR analyses of transcript levels were performed as described in Haydont et al., 2018b using expanded fibroblasts at 7 to 10 population doublings. RNA extractions were systematically performed at 80% culture confluency, and 24 h after medium renewal. Cell samples from 8 donors were used for qRT-PCR validations: 5 'younger' (20, 22, 25, 28, and 31 years old) and 4 'older' (55, 61, 65, and 65 years old). Gene expression was normalized according to *GAPDH* (Reference QT01192646) and *TBP* (Reference QT00000721) transcript levels. Primer source: QIAgen, Courtaboeuf, France. Signals analysis: iQ™ 5 software (Biorad, Marnes-la-Coquette, France). For validation of the screen at the protein level, skin sections from 15 donors were used: 7 'younger' (18, 20, 22, 23, 25, 28, and 31 years old) and 8 'older' (53, 55, 57, 58, 59, 61, 64, and 65 years old). Comparative immunofluorescence detection of ACAN, Col XI α1 and KANK4 proteins was performed as described in Haydont et al., 2018b. Picture acquisition: Leica microscope coupled with a QIMAGINE RETIGA 2000R Fast 1394 camera. Signal quantification: using the Histolab© software.

	Gene symbol	Gene Title	Fold Change By Probe Set on U133 + 2.0	RT-qPCR validations		Validations at the protein level (IHC in skin sections) (Haydont et al., 2018b)	
				Fold Change (vs Young)	Primers Supplier References	Fold Change (vs Young)	Antibody Supplier References
Young Fp	CADM1	cell adhesion molecule 1	3.87 / 2.19	2.05 ± 0.3	QT00050001		
vs. Old Fp	HAPLN1	hyaluronan and proteoglycan link protein 1	3.55 / 3 / 2.97 / 2.87	2.5 ± 0.8	QT00001694		
	WISP1	WNT1 inducible signaling pathway protein 1	1.6	1.44 ± 0.14	QT00079492		
	SFRP2	secreted frizzled-related protein 2	4.85 / 3.78	4.7 ± 2.9	QT00073220		
	TGF β2	transforming growth factor, beta 2	1.62 / 1.53	1.41 ± 0.3	QT02290316		
	INHBB	inhibin, beta B	2.05	1.86 ± 0.42	QT00199878		
Old Fp	LOX	lysyl oxidase	3.18	1.5 ± 0.05	QT00017311		
vs. Young Fp	ACAN	aggrecan	3.04 / 2.87 / 2.85	1.37 ± 0.3	QT00001365	3.21 ± 1.20	ab3778 Abcam (Paris - France)
	COLXIA1	collagen, type XI, alpha 1	3.21 / 2.67	1.6 ± 0.4	QT00088711	4.28 ± 2.28	SAB4500393 Sigma (Saint-Quentin Falaviers - France)
	WISP2	WNT1 inducible signaling pathway protein 2	1.99	1.40 ± 0.56	QT00211792		
	SFRP1	secreted frizzled-related protein 1	3.74 / 2.60	1.71 ± 0.63	QT00031927		
	BMPR1B	bone morphogenetic protein receptor, type IB	1.56	1.41 ± 0.33	QT02288328		
	LTBP1	latent transforming growth factor beta binding protein 1	2.14 / 1.61	1.45 ± 0.13	QT00010206		
	KANK4	KN motif and ankyrin repeat domains 4	3.4	2.4 ± 0.8	QT00030905	4.1 ± 1.14	ab121410 Abcam (Paris - France)
Young Fr	FGF9	fibroblast growth factor 9 (glia-activating factor)	3.38 / 2.34	2.6 ± 1.2	QT00000091		
vs. Old Fr	RHO J	ras homolog gene family, member J	2.57	1.9 ± 0.5	QT00092078		
	VCAM1	vascular cell adhesion molecule 1	3.32	2.6 ± 1.1	QT00018347		
	HAPLN1	hyaluronan and proteoglycan link protein 1	1.72 / 1.52 / 1.50	1.49 ± 0.75	QT00001694		
	SFRP2	secreted frizzled-related protein 2	2.22	1.76 ± 0.27	QT00073220		
Old Fr	ACAN	aggrecan	3.5 / 3.41	3.0 ± 1.0	QT00001365	4.82 ± 1.54	ab3778 Abcam (Paris - France)
vs. Young Fr	EMCN	endomucin	2.81 / 2.78 / 2.61	1.7 ± 0.2	QT00025158		
	BMPR1B	bone morphogenetic protein receptor, type IB	1.65	1.75 ± 0.56	QT02288328		

Table 5

The probset list described by Rinn et al., (Rinn et al., 2006) [transcript signatures associated with specific anatomic sites] was cross-analyzed together our lists that distinguished young versus old Fp and Fr. Convergence between the two studies is shown. Fold-changes between old and young donor ages are indicated, as well as involvement in 'tissue skeleton', highlighted in green color.

	Up modulated in Young			Up modulated in old		
	Gene Symbol	Gene Title	Fold Change	Gene Symbol	Gene Title	Fold Change
Papillary Fibroblasts	ABCC4	ATP-binding cassette, sub-family C, member 4	1,67	AGTR1	angiotensin II receptor, type 1	2,79
	AMPH	amphiphysin	1,75	AGTR1	angiotensin II receptor, type 1	2,04
	AQP1	aquaporin 1 (Colton blood group)	1,74	APBB1IP	beta (A4) precursor protein-binding, family B, member 1 interactin	1,76
	AQP1	aquaporin 1 (Colton blood group)	2,27	B4GALT1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	1,98
	C10orf10	chromosome 10 open reading frame 10	1,63	COL11A1	collagen, type XI, alpha 1	2,67
	CALD1	caldesmon 1	1,62	COL11A1	collagen, type XI, alpha 1	3,21
	COLEC12	collectin sub-family member 12	2,08	CST6	cystatin E/M	1,90
	F2RL2	coagulation factor II (thrombin) receptor-like 2	1,81	CXCL1	chemokine (C-X-C motif) ligand 1	3,18
	GAS1	growth arrest-specific 1	1,64	DHRS3	dehydrogenase/reductase (SDR family) member 3	1,74
	GPR126	G protein-coupled receptor 126	1,58	DSP	desmoplakin	1,66
	IGF2	insulin-like growth factor 2	1,51	EMILIN2	elastin microfibril interfacer 2	1,81
	MAP3K1	mitogen-activated protein kinase kinase kinase 1	1,59	GADD45B	growth arrest and DNA-damage-inducible, beta	1,70
	MOXD1	monooxygenase, DBH-like 1	1,59	GREM2	gremlin 2, cysteine knot superfamily	1,53
	MOXD1	monooxygenase, DBH-like 1	1,64	HIPK2	homeodomain interacting protein kinase 2	1,89
	NFE2L3	nuclear factor (erythroid-derived 2)-like 3	1,53	HIPK2	homeodomain interacting protein kinase 2	1,70
	NFIB	nuclear factor I/B	1,82	HIPK2	homeodomain interacting protein kinase 2	1,94
	NFIB	nuclear factor I/B	1,86	HIPK2	homeodomain interacting protein kinase 2	1,83
	NFIB	nuclear factor I/B	1,97	LOX	lysyl oxidase	3,18
	NFIB	nuclear factor I/B	1,77	MASP1	mannan-binding lectin serine peptidase 1	1,55
	PLAC8	placenta-specific 8	2,34	MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)	1,57
	PLN	phospholamban	1,59	MRPL38	mitochondrial ribosomal protein L38	1,61
	QPCT	glutaminyl-peptide cyclotransferase	1,55	MX1	myxovirus resistance 1, interferon-inducible protein p78	2,78
	RARRES2	retinoic acid receptor responder 2	2,37	MYO10	myosin X	2,19
	RASSF4	Ras association domain family member 4	2,53	MYO10	myosin X	1,53
	RGS4	regulator of G-protein signaling 4	1,57	MYO10	myosin X	1,60
	RGS4	regulator of G-protein signaling 4	1,74	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	1,57
	SLC16A4	solute carrier family 16, member 4	1,57	PAPPA	pregnancy-associated plasma protein A, pappalysin 1	1,58
	UACA	uveal autoantigen with coiled-coil domains and ankyrin repeats	1,67	PHACTR2	phosphatase and actin regulator 2	1,51
				PSG1	pregnancy specific beta-1-glycoprotein 1	9,80
				PSG1	pregnancy specific beta-1-glycoprotein 1	10,63
				PSG3	pregnancy specific beta-1-glycoprotein 3	1,91
				PTGER3	prostaglandin E receptor 3 (subtype EP3)	2,06
				SERPINB7	serpin peptidase inhibitor, clade B (ovalbumin), member 7	1,57
				SLC7A1	solute carrier family 7 , member 1	1,51
				TBX2	T-box 2	1,51
				THBS1	thrombospondin 1	1,71
				TM4SF1	transmembrane 4 L six family member 1	3,31
				TM4SF1	transmembrane 4 L six family member 1	3,21
				TM4SF1	transmembrane 4 L six family member 1	3,36
				WISP2	WNT1 inducible signaling pathway protein 2	1,99
				WNT5B	wingless-type MMTV integration site family, member 5B	1,62
				ZFPM2	zinc finger protein, multitype 2	3,22
Reticular Fibroblasts	CALD1	caldesmon 1	1,58	AGTR1	angiotensin II receptor, type 1	1,93
	CALD1	caldesmon 1	1,65	AGTR1	angiotensin II receptor, type 1	2,26
	CALD1	caldesmon 1	1,94	AQP1	aquaporin 1 (Colton blood group)	1,59
	CALD1	caldesmon 1	2,10	B4GALT1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	2,42
	CALD1	caldesmon 1	2,84	CCRL1	chemokine (C-C motif) receptor-like 1	2,75
	COL11A1	collagen, type XI, alpha 1	1,50	COL4A2	collagen, type IV, alpha 2	2,03
	COL11A1	collagen, type XI, alpha 1	1,56	CXCL1	chemokine (C-X-C motif) ligand 1	2,16
	COL4A5	collagen, type IV, alpha 5	1,57	DHRS3	dehydrogenase/reductase (SDR family) member 3	1,50
	DKK2	dickkopf homolog 2 (Xenopus laevis)	1,86	FBXO32	F-box protein 32	1,80
	F2R	coagulation factor II (thrombin) receptor	1,85	GADD45B	Growth arrest and DNA-damage-inducible, beta	1,61
	F2RL2	coagulation factor II (thrombin) receptor-like 2	2,90	GADD45B	growth arrest and DNA-damage-inducible, beta	1,66
	FN1	fibronectin 1	1,71	HIPK2	homeodomain interacting protein kinase 2	1,53
	GAS1	growth arrest-specific 1	1,64	LOX	lysyl oxidase	2,73
	IGFBP5	insulin-like growth factor binding protein 5	1,59	LRRC17	leucine rich repeat containing 17	2,43
	IGFBP5	insulin-like growth factor binding protein 5	1,69	MAP3K5	mitogen-activated protein kinase kinase kinase 5	1,54
	IGFBP5	insulin-like growth factor binding protein 5	2,23	MAP3K5	mitogen-activated protein kinase kinase kinase 5	1,73
	IGFBP5	insulin-like growth factor binding protein 5	2,39	MASP1	mannan-binding lectin serine peptidase 1	1,75
	IGFBP5	insulin-like growth factor binding protein 5	2,69	MASP1	mannan-binding lectin serine peptidase 1	1,76
	MEG3	maternally expressed 3 (non-protein coding)	1,58	MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)	1,62
	MEG3	maternally expressed 3 (non-protein coding)	1,85	MSX1	Msh homeobox 1	1,98
	MX1	myxovirus resistance 1, interferon-inducible protein p78	1,61	MYO10	myosin X	1,71
	NR2F2	nuclear receptor subfamily 2, group F, member 2	1,65	MYO10	myosin X	1,73
	NR2F2	nuclear receptor subfamily 2, group F, member 2	1,66	NCALD	neurocalcin delta	1,52
	OSBPL10	oxysterol binding protein-like 10	1,82	NFIB	nuclear factor I/B	1,68
	PAPPA	pregnancy-associated plasma protein A, pappalysin 1	1,52	NFIB	nuclear factor I/B	1,76
	PHLDB2	pleckstrin homology-like domain, family B, member 2	2,06	NFIB	nuclear factor I/B	1,79
	PLAC8	placenta-specific 8	1,93	NFIB	nuclear factor I/B	1,89
	PLAU	plasminogen activator, urokinase	1,53	PSG1	pregnancy specific beta-1-glycoprotein 1	3,31
	QPCT	glutaminyl-peptide cyclotransferase	1,57	PSG1	pregnancy specific beta-1-glycoprotein 1	3,33
	RAB11FIP1	RAB11 family interacting protein 1 (class I)	1,70	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9	1,51
	RAB11FIP1	RAB11 family interacting protein 1 (class I)	1,95	PTGER3	prostaglandin E receptor 3 (subtype EP3)	1,72
	RGS4	regulator of G-protein signaling 4	1,91	PTGER3	prostaglandin E receptor 3 (subtype EP3)	1,78
	RGS4	regulator of G-protein signaling 4	2,34	PTGER3	prostaglandin E receptor 3 (subtype EP3)	1,83
	SLC16A4	solute carrier family 16, member 4	1,52	PTGER3	prostaglandin E receptor 3 (subtype EP3)	1,86
	TRPS1	trichorhinophalangeal syndrome I	1,58	PTGER3	prostaglandin E receptor 3 (subtype EP3)	2,14
	UACA	uveal autoantigen with coiled-coil domains and ankyrin repeats	1,57	PTGER3	prostaglandin E receptor 3 (subtype EP3)	5,58
	UACA	uveal autoantigen with coiled-coil domains and ankyrin repeats	1,96	TM4SF1	transmembrane 4 L six family member 1	4,00
				TM4SF1	transmembrane 4 L six family member 1	4,94
				TM4SF1	transmembrane 4 L six family member 1	5,37
				WISP2	WNT1 inducible signaling pathway protein 2	1,75
				WNT5B	wingless-type MMTV integration site family, member 5B	1,79

Table 6

The search of molecular signatures of ageing has been conducted using unfractionated fibroblast populations (Coppé et al., 2008; Waldera Lupa et al., 2015; Kaisers et al., 2017; Kaur et al., 2018; Sun et al., 2018). The different signatures were examined and compared. Comparisons were also performed together with the 'tissue skeleton' transcript list identified in the present study. Convergences are highlighted in green color, and divergences in red color. The moderate convergence of the independently identified signatures highlighted the importance of considering the biological specificities of different ageing cellular models.

		Kaur et al. Age-related changes in secretome, cultured dermal fibroblasts, proteomics analysis		**Waldera Lupa et al.** Age-related changes in secretome, cultured dermal fibroblasts, proteomics analysis,		**Kaisers et al.** Age-related changes in transcriptome, cultured dermal fibroblasts, RNA-seq analysis		**Coope et al. and Sun et al.**	**Present paper** Age-related changes in transcriptome, cultured dermal fibroblasts, mico-array profiling	
		UP with Ageing	DOWN with Ageing	UP with Ageing	DOWN with Ageing	UP with Ageing	DOWN with Ageing	SASP markers	UP with Ageing	DOWN with Ageing
Kaur et al.	**UP with Ageing**	ADA ADH1A ADH1B AGT AOC1 ABP1 APLP2 ASAH1 BASP1 CDC42 CLU COA6 COL15A1 COX5B CSTA CXCL1 CXCL2 CXCL3 DHX9 DPP4 DSC1 DSG1 DSP FAP FBLN1 FUS GAA HDLBP IGF2R IGFBP2 KHDRBS1 KPRP LAMB2 MARCKS MYH10 PCBP2 PCNP PDLIM5 PI16 PRKDC PSG4 RARRES2 S100A10 SFRP2 SYCP1 TCEAL3 TCEAL5 TCEAL6 TNXB WISP2 YBX3						CXCL1 CXCL2 CXCL3 IGFBP2 SFRP2	CSTA CXCL1 CXCL2 CXCL3 DPP4 FBLN1 IGFBP2 MYH10 TNXB WISP2	COL15A1 COL4A5 S100A1 SFRP2
	DOWN with Ageing		ACLY AGRN ANXA2 ANXA2P2 BTD COL4A1 COL4A5 COL6A3 DKFZp686K04147 COTL1 CPPED1 CPXM2 CTHRC1 DDAH2 DKK1 DKK2 DKK3 ENPP1 DKFZp686P13218 FBN2 FDPS GAS6 DKFZp666G247 GDI2 GPC4 GPC6 HAPLN1 HMCN1 LGALS3BP LOXL2 MDK MFAP2 MFGE8 MMP1 MMP10 MMP3 PCOLCE PFN2 PLAUR PLOD2 POSTN PRDX6 PTGDS PTN RGMB SERPINB7 DKFZp686D06190 SERPINF1 SOD3 SSC5D STC1 UCHL1 VPS26A	MMP3 MMP10	FBN2			MMP10 MMP3	COL4A1 LOXL2 MDK MFGE8 MMP3 PLAUR PTN SERPINF1	FBN2 GPC4 HAPLN1 HMCN1 POSTN

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Table 6 (*continued*)

		Kaur et al. Age-related changes in secretome, cultured dermal fibroblasts, proteomics analysis		Waldera Lupa et al. Age-related changes in secretome, cultured dermal fibroblasts, proteomics analysis,		Kaisers et al. Age-related changes in transcriptome, cultured dermal fibroblasts, RNA-seq analysis		Coope et al. and Sun et al.	Present paper Age-related changes in transcriptome, cultured dermal fibroblasts, mico-array profiling	
		UP with Ageing	DOWN with Ageing	UP with Ageing	DOWN with Ageing	UP with Ageing	DOWN with Ageing	SASP markers	UP with Ageing	DOWN with Ageing
Waldera Lupa et al.	UP with Ageing			CD14 CDH11 CHI3L2 CXCL10 FBLN2 FBLN5 FKBP7 FKBP9 GBA IFNG IL15 IL1B IL1RN IL4 IL8 LGALS3 MAN1C1 MMP1 MMP10 MMP14 MMP3 MXRA5 NEU1 NID2 OLFM1 POFUT1 PRELP PRSS23 RNH1 SERPINE2 SIRPA STC1 THBS1 TNF TPST1		STC1		IL15 IL1B IL8 MMP1 MMP10 MMP3	IL15 IL8 MMP14 MMP3 OLFM1 THBS1	PRELP
	DOWN with Ageing				ADAMTSL1 ATP6AP1 CD248 CDH2 COL1A1 CPEPE ENO2 F2 FBN2 FLNC FST FSTL1 GGCT GLT8D1 GSN HSPA13 IGFBP6 ISLR ITGBL1 LMAN2 LOXL1 MFAP5 MYL12A NT5E NTNG1 NUCB2 OAF PRNP PSAP SLC3A2 SUMF1 TFPI TWSG1 TXNDC5 VIT			IGFBP6	GSN MFAP5 NTNG1	ADAMTSL1 FBN2 ITGBL1
Kaisers et al.	UP with Ageing					ADGRL4 BACE2 CPZ FGF13 GJA1 KIAA1324L PENK ROBO1 STC1 USP41 ZNF385D				
	DOWN with Ageing						ACSS3 ARHGAP23P1 ATOH8 CKB CNN1 CRISPLD2 DDR1 EHD1 ENC1 ERRFI1 EVA1A FAM83G FGFRL1 FILIP1L HSPB7 ID1 ID3 KCNC4 MEG3 PODXL PPP1R3C PRPS1 PRRX2 RP11-309L24.6 SEPT5 SERTAD1 SH2D4A SMAD7 SNAI1 SPHK1 TRNP1			

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Table 6 (*continued*)

		Kaur et al. Age-related changes in secretome, cultured dermal fibroblasts, proteomics analysis		Waldera Lupa et al. Age-related changes in secretome, cultured dermal fibroblasts, proteomics analysis,		Kaisers et al. Age-related changes in transcriptome, cultured dermal fibroblasts, RNA-seq analysis		Coope et al. and Sun et al.	Present paper Age-related changes in transcriptome, cultured dermal fibroblasts, mico-array profiling	
		UP with Ageing	DOWN with Ageing	UP with Ageing	DOWN with Ageing	UP with Ageing	DOWN with Ageing	SASP markers	UP with Ageing	DOWN with Ageing
Coope et al. and Sun et al.	UP in SASP							ANG ANGPTL4 AREG ATM AXL FGF2 cGAS CXCL1 CXCL2 CXCL3 CXCR2 CCL24 EPHA2 EREG NPRSF6 FGF7 GATA4 GM-CSF GROA GROB GROG HCC4 HGF HSP90 ICAM1 ICAM3 IGFBP1 IGFBP2 IGFBP4 IGFBP6 IGFBP7 IKK IL11 IL13 IL15 IL1B IL6 IL7 IL8 IRF3 JAK2 LEP macroH2A1 MAPK11 MAPK12 MAPK13 MAPK14 MCP1 MCP2 MCP4 MIF MIP1A MIP3A MMP1 MMP10 MMP12 MMP3 mTOR NOTCH1 OSM PAI1 PIGF PTP1B phosphatase SCF SFRP2 SGP130 STAT3 STING sTNFRI sTNFRII TBK1 TIMP2 TNFRSF11B TRAF3IP2 TRAILR3 uPAR VEGF WNT16B	ANGPTL4 CXCL1 CXCL2 CXCL3 IL15 IL7 IL8 MMP12 MMP3	IGFBP7 SFRP2 VEGF
Present paper	UP with Ageing								ANGPTL4 COL4A1 CSTA CXCL1 CXCL2 CXCL3 DPP4 FBLN1 GSN IGFBP2 IL15 IL7 IL8 LOXL2 MDK MFAP5 MFGE8 MMP12 MMP14 MMP3 MYH10 NTNG1 OLFM1 PLAUR PTN SERPINF1 THBS1 TNXB WISP2	
	DOWN with Ageing									ADAMTSL1 COL15A1 COL4A5 FBN2 GPC4 HAPLN1 HMCN1 ITGBL1 IGFBP7 POSTN PRELP S100A1 SFRP2 VEGF

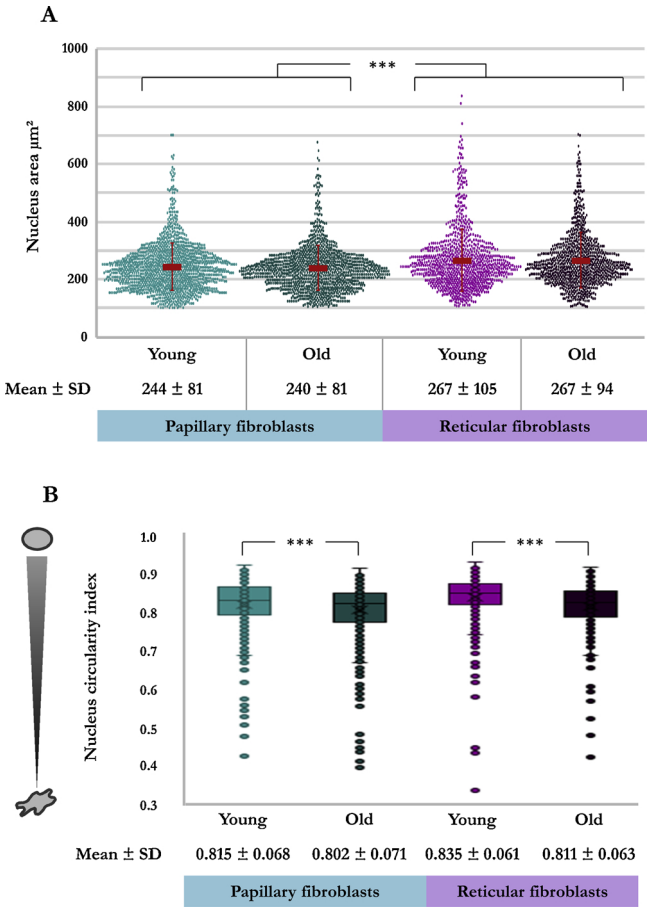

Fig. 3. Impact of ageing on Fp and Fr fibroblast nuclear shape. Bidimensional cultures of Fp and Fr dermal fibroblasts (from mammary skin biopsies) were used to analyze nucleus surface and circularity, as parameters of shape. Samples included cells from 5 'younger' donors (20, 22, 25, 28, and 31 years old) and 4 'older' donors (55, 61, 65, and 65 years old). Cell cultures were fixed with 4% paraformaldehyde and permeabilized in 0.1% SDS. Then, nuclei were stained with DAPI, and samples were mounted in ProLong Gold medium (Invitrogen - Molecular Probes, France). Fluorescence pictures were acquired using a Leica microscope (x200 magnification) coupled with a QIMAGINE RETIGA 2000R Fast 1394 camera (QImaging, Canada). The Image J software was used to determine the surface in μm^2 (**A**) and circularity index* (**B**) of individual nuclei. Presented data correspond to 1240 and 906 analyzed nuclei for 'young' Fp and Fr, respectively (pooled analysis from the 5 donors), and 1183 and 851 analyzed nuclei for 'old' Fp and Fr, respectively (pooled analysis from the 4 donors). Error bars represent SD. The Student's *t*-test was applied to determine p-values [***$p < 0.001$].

*Circularity index (x) was calculated according to the formula $x = 4\pi\frac{Area}{perimeter^2}$.

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