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Proteome analysis of human mesenchymal stem cells undergoing chondrogenesis when exposed to the products of various magnesium-based materials degradation

Adela Helvia Martínez Sánchez[a,1], Maryam Omidi[b,1], Marcus Wurlitzer[b], Marceline Manka Fuh[b], Frank Feyerabend[a], Hartmut Schlüter[b], Regine Willumeit-Römer[a], Bérengère J.C. Luthringer[a,*]

[a] Division of Metallic Biomaterials, Institute of Material Research, Helmholtz-Zentrum Geesthacht, Max Planck Strasse 1, 21502, Geesthacht, Germany
[b] Institute of Clinical Chemistry and Laboratory Medicine, Mass Spectrometric Proteomics, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246, Hamburg, Germany

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ABSTRACT

Treatment of physeal fractures (15%–30% of all paediatric fractures) remains a challenge as in approximately 10% of the cases, significant growth disturbance may occur. Bioresorbable Magnesium-based implants represent a strategy to minimize damage (*i.e.*, load support until bone healing without second surgery). Nevertheless, the absence of harmful effects of magnesium-implants and their degradation products on the growth plate should be confirmed. Here, the proteome of human mesenchymal stem cells undergoing chondrogenesis was evaluated when exposed to the products of various Magnesium-based materials degradation. The results of this study indicate that the materials induced regulation of proteins associated with cell chondrogenesis and cartilage formation, which should be beneficial for cartilage regeneration.

1. Introduction

Biodegradable magnesium (Mg)-based materials are promising candidates for substituting permanent implants for orthopaedic application as a second surgery and chronic inflammation will be avoided [1]. Furthermore, as an element, Mg is essential to the human body and, as metal, has mechanical properties close to the ones of bone. Special emphasis should be given to children, a sector of the population with a high incidence of bone damage [2]. Bone repair in children is a fast process, and the main requirement from an implant for appropriate healing is to reduce bone load bearing. Therefore, an additional and undesirable immobilisation of the patients will always be necessary to remove a permanent implant. Growing long bones have specific cartilaginous discs at both ends, growth plates, responsible for endochondral ossification and bone formation until adult stage. Any damage due to the implant application or removal, as well as due to the degradation products, could generate irreversible malformations [3,4].

Foetal bone development starts with stem cells condensation, chondrocyte differentiation, proliferation, maturation and ossification. Every step is characterised by changes in cell morphology, proliferation and extracellular matrix (ECM) production, and by a complex molecular regulation [5]. After stem cell condensation, most of the cells become chondrocytes with a rounded morphology and express specific genes such as SRY (sex determining region Y)-box9 (*SOX9*), aggrecan (*ACAN*; a proteoglycan) and collagen, type II *COL2*. The ECM is rich in COL2 and glycosaminoglycans (GAG). Then chondrocytes proliferate and synthesize more ECM, enlarging cartilage [6–9]. Chondrocytes undergo hypertrophy (or maturation), showing a notably enlarged size, a high expression of collagen, type-X gene (*COL10*) [10,11], and regulating mineralisation of surrounding matrix by expressing other gene markers which are also bone markers, such as osteopontin (*OPN*) and collagen, type I alpha 1 (*COL1A1*) [5]. At the final stage of maturation, the chondrocytes can undergo apoptosis.

Previous proteomic studies of mesenchymal stem cell (MSC) chondrogenesis [12–17] have shown that the majority of regulated proteins are related to cell metabolism (*e.g.*, adenosine triphosphate (ATP) synthase subunits alpha (α) and beta (β), carbonyl reductase, aldose reductase, α-enolase, dihydropyrimidinase-like 2, glyceraldehyde-3-phosphate dehydrogenase, and glycogen phosphorylase), ECM and cytoskeleton (*e.g.*, annexins, actin-related proteins, biglycan,

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* Corresponding author.
E-mail address: berengere.luthringer@hzg.de (B.J.C. Luthringer).
[1] Those authors contributed equally to this work.

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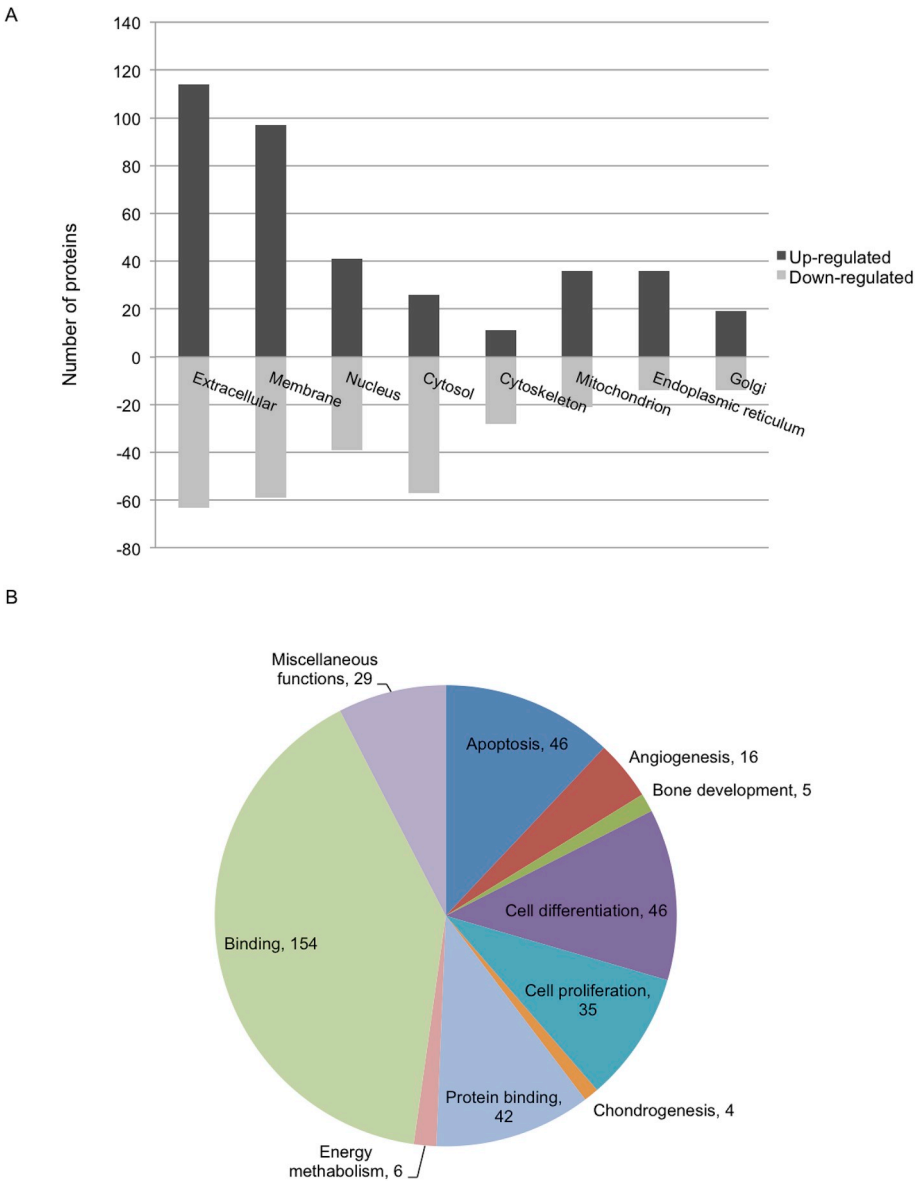

Fig. 1. The number of regulated proteins with more than two-fold change in at least one of the Mg-alloys sorted according to (a) their location in the cells and (b) their involvement in physiological processes.

chondroadherin, collagen α-2(VI) chain, collagen α-3(VI) chain, fibronectin, vimentin, gelsolin, procollagen-lysine, and transforming growth factor-beta-induced protein ig-h3 precursor), and response to stress (*e.g.*, 78 kDa glucose-regulated protein, endoplasmin, peroxiredoxin-6, peptidyl-prolyl cis-trans isomerise A, superoxide dismutase, heat shock protein beta-1, and stress-induced phosphoprotein 1). Human umbilical cord perivascular (HUCPV) cells are mesenchymal stem cells (MSC), isolated from the vessels surface of umbilical cords, with a high proliferation rate and strong potential for differentiation into the skeletal lineages (both bone and cartilage) [18–20].

In order to prove the potential of Mg-based materials for application in cartlage treatment, a better understanding of the chondrogenic mechanisms influenced by Mg-based materials degradation is still necessary. This study aimed at (I) evaluating the differently expressed proteins during chondrogenesis under the influence of Mg-materials degradation products, contained in the degradation medium (extracts), and (II) determining which of those proteins are directly involved in the chondrogenic differentiation. For this purpose, differential proteomics *via* label-free quantification of HUCPV cells driven toward chondrogenesis under the influence of three materials (Pure-Mg, Mg-2Ag and Mg-10Gd) was performed. Silver (Ag) was selected as alloying elements due to its antibacterial properties and gadolinium (Gd) to improve material mechanical properties. Cyto- and biocompatibility of these two alloys have been earlier tested and demonstrated ([21,22,23,24] for Ag and Gd, respectively).

2. Experimental procedures

2.1. Extract preparation and characterization

Extracts of Pure Mg (Pure-Mg, 99.95%), Mg with 2 wt% silver (Mg-2Ag) and Mg with 10 wt% gadolinium (Mg-10Gd) materials were prepared according to EN ISO standards 10993:5 [25] and 10993:12 [26]. Pure elutes were characterised (composition and pH) and diluted in differentiation medium to obtain a common concentration of Mg (*i.e.*, 6.08 mM).

2.2. Induction of micropellets formation and chondrogenic differentiation

Ethical approval for the isolation of HUCPV was obtained from the

Ethik-Kommission der Ärztekammer Hamburg. Umbilical cord samples were provided by Asklepios Klinik Altona immediately after caesarean sections of consenting donors. Cell micromasses were obtained from HUCPV cell (passage 2) pellets after 3 days. Chondrogenesis was then chemically induced for up to 11 days with or without Mg-extracts, followed by proteome analysis. For each group (*i.e.*, control, Pure-Mg, Mg-2Ag, and Mg-10Gd) 3 biological replicates were established. Furthermore, 3 technical replicates (*i.e*, LC MSMS injections) were performed. Control group refers to micropellets driven toward chondrogenesis without any Mg extract (only differentiation medium). More details can be found in Supplemental experimental procedures.

2.3. Proteomic analysis

Proteins from the pellets were extracted using TissueLyzer II (QIAGEN), tryptic in-solution digested and then desalted.

For liquid chromatography–mass spectrometry (LC-MSMS) measurements, all the tryptic digested peptides were subjected to a nano-flow UPLC-column (DionexUltiMate 3000 RSLCnano, Thermo Scientific, Bremen, Germany) coupled *via* electrospray ionization (ESI) to an Orbitrap mass spectrometer (Orbitrap-Fusion, Thermo Fisher Scientific).

To compare the relative protein abundance, raw data files obtained from the LC-MSMS were processed by MaxQuant 1.5.2.8 [27]. These parameters were used for identification and label-free quantification: identification of the peptides against SwissProt database downloaded from UniProt in July 2015 (with internal contaminants database of MaxQuant); trypsin was used as an enzyme with one missed cleavage; carbamidomethylation on cysteine was set as fixed modification and oxidation of methionine as variable modifications; precursor mass of 20 ppm and fragment mass tolerance of 0.5 Da; and minimum peptide length of 6 amino acids for identification and match between runs.

Peptide spectrum match (PSM) and protein false discovery rate (FDR) were 0.01; and at least 2 ratio count for LFQ was used.

Perseus 1.5.2.6 [28] and Wolfram Mathematica 10.0 (Wolfram Research Europe Ltd., Oxfordshire, United Kingdom) were used for bioinformatics analysis.

Heat maps (Figs. 1–5; Fig. A1), based on two-sided student's T test, prepared in Perseus, indicates the fold change and significance of each protein of HUCPV cells incubated for 11 days with Mg-alloys (Mg-10Gd, Mg-2Ag, and Pure-Mg) compared to control cells after 11 days incubation without Mg-alloys (permutation-based FDR of 0.01, s0 = 0.1).

Other and more detailed experimental procedures are described in Supplemental experimental procedures.

3. Results

3.1. Composition of the extracts

As it can be observed in Table 1, Mg contents increased strongly in the extracts compared to the extraction media (α-MEM supplemented with 10% foetal bovine serum for mesenchymal stem cells (SC-FBS; Stem Cell Technologies, Vancouver, Canada) and 1% antibiotics Penicillin/Streptomycin (Pen strep; Invitrogen, Bremen, Germany)) while Ca and P ones decreased. To avoid osmotic choc and in order to study the effect of alloying element independently of Mg content, extracts were diluted with differentiation medium to obtain a common Mg concentration of about 6.08 mM.

3.2. Effects of the Mg-alloys degradation products on chondrogenic-differentiated HUCPV proteome

In order to determine the influence of Pure-Mg, Mg-10Gd, and Mg-2Ag extracts on HUCPV cells driven toward chondrogenesis, proteins from each condition were analysed with differential bottom-up proteomics using label-free quantification (LFQ).

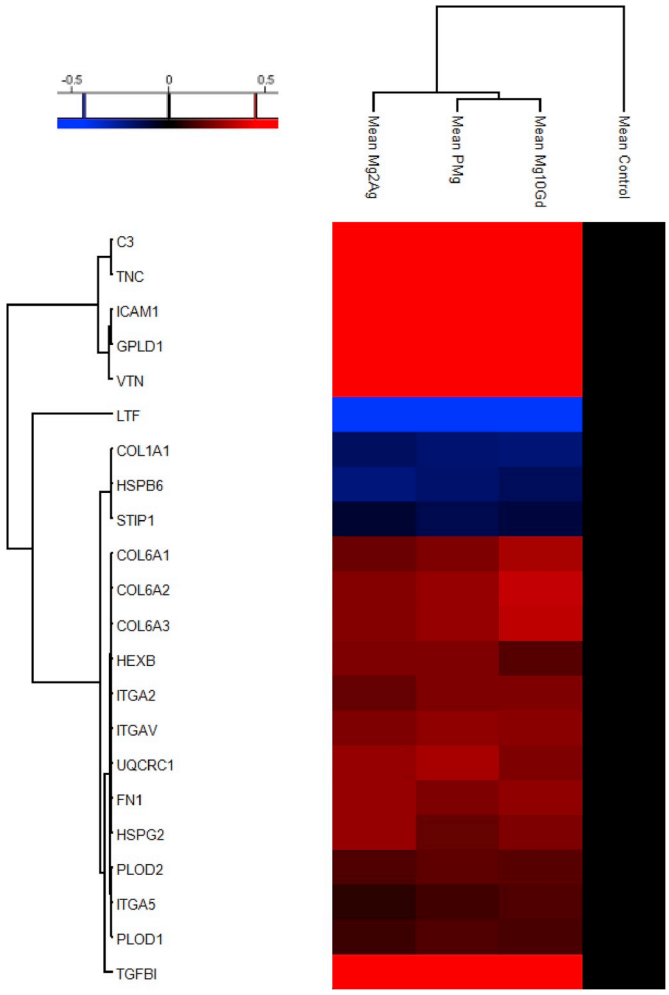

Fig. 2. Heat-map and hierarchical clustering of the up- and down-regulated proteins involved in chondrogenesis and cartilage development (P-value = 0.05; min. fold-change of 2) in all Mg-alloys compared based on the mean values of the biological replicates (normalized to Control).

246 significantly regulated proteins (Table A1) were found under the influence of the extracts and clustered in a heat map (Fig. A1). 136 proteins were upregulated in the presence of Mg-10Gd, Mg-2Ag and Pure-Mg (from which 134 proteins were common for the three extracts) while 110 proteins were downregulated. A Gene Ontology (GO) annotation downloaded from UniProt was performed for each protein of the list of regulated proteins. Clustering these proteins regarding their localisation in the cells (cellular compartment; Fig. 1a) indicated that the number of upregulated extracellular proteins and membrane proteins (involved not only in ECM composition) was considerably higher than downregulated ones in the presence of Mg alloys. Additionally, the number of regulated cytosol proteins was high. Cytosolic and cytoskeletal proteins were mostly downregulated. Fig. 1b shows the most affected biological processes. The highest number of regulated proteins were involved in cell binding, differentiation, apoptosis, and cell proliferation. To a lesser extent, proteins involved in angiogenesis, energy metabolism, bone development, and chondrogenesis were influenced by the extracts. Proteins involved in chondrogenesis and cartilage formation are depicted in Fig. 2. Heat maps in Figs. 3–5 illustrate the regulated proteins clustered according to their involvement in apoptosis, response to cell toxicity and angiogenesis, respectively.

In a second step, Mg-alloy specific proteins will be discussed.

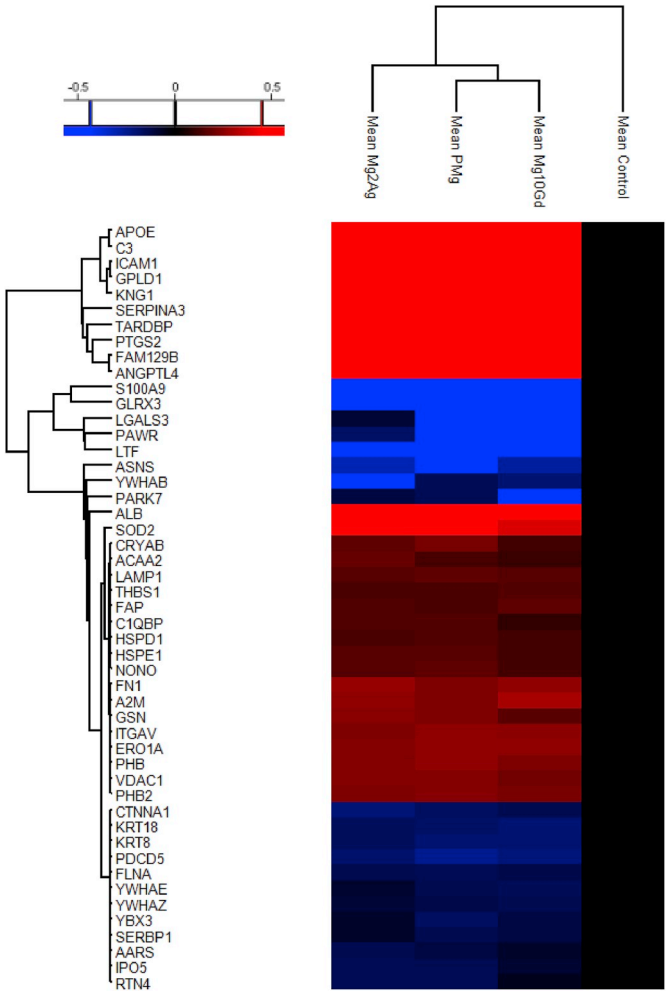

Fig. 3. Heat-map and hierarchical clustering of the up- and down-regulated proteins involved in apoptosis (P-value = 0.05; min. fold-change of 2) in all Mg-alloys compared based on the mean values of the biological replicates (normalized to Control).

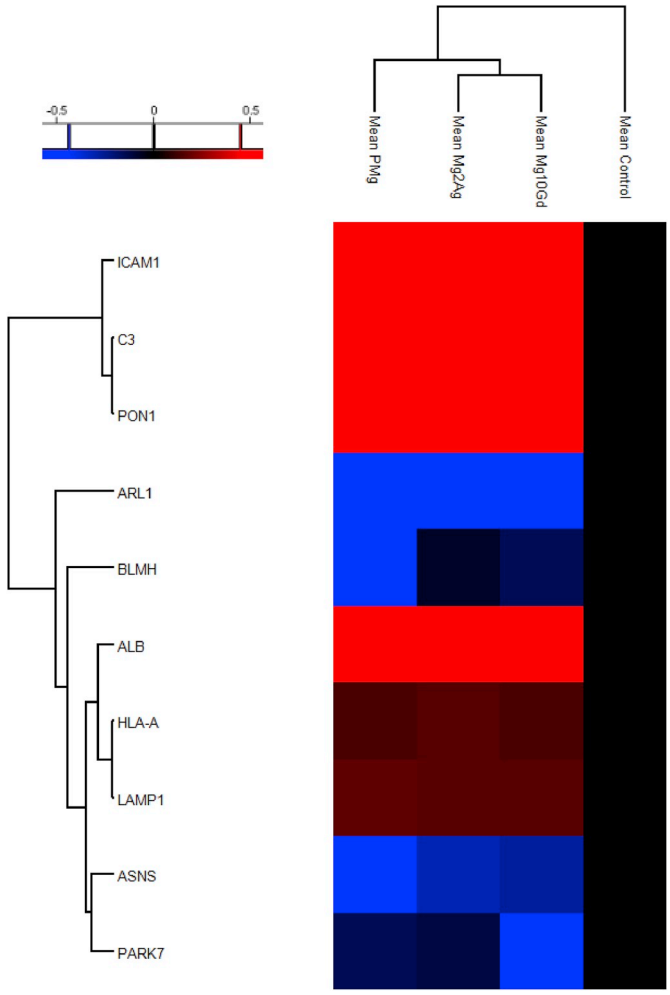

Fig. 4. Heat-map and hierarchical clustering of the up- and down-regulated proteins involved in cellular response to toxicity (P-value = 0.05; min. fold-change of 2) in all Mg-alloys compared based on the mean values of the biological replicates (normalized to Control).

3.2.1. Regulated proteins involved in chondrogenesis and cartilage formation

Fig. 2 illustrates the regulated chondrogenesis-related proteins. Four chondrogenesis-related proteins were upregulated by the extracts: glycosylphosphatidylinositol specific phospholipase D1 (GPLD1) was upregulated in the presence of Mg-alloys, while hexosaminidase B (beta polypeptide) (HEXB) and transforming growth factor, beta-induced, 68 kDa (TGFBI) were upregulated compared to the control. In addition, proteins involved in cartilage ECM formation and organisation (fibronectin 1 (FN1), collagen, type VI (COL6), tenascin C (TNC), intercellular adhesion molecule 1 (ICAM1), vitronectin (VTN), heparan sulfate proteoglycan 2 (HSPG2), procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 and 2 (PLOD1 (significantly in the presence of pure-Mg and Mg10Gd) and PLOD2) and integrins α-2 (ITGA2), α-5 (ITGA5)) were upregulated in the presence of Mg. COL1A1 was downregulated by all the extracts. ITGA5 was significantly upregulated in the presence of Mg-10Gd but not with the other extracts.

3.2.2. Regulated proteins involved in apoptosis

Regulated proteins involved in apoptosis were clustered in the heat map shown in Fig. 3. From the total 49 proteins identified, 29 were upregulated, while 20 of them were downregulated in the presence of Mg-alloys. S100 calcium binding protein A9 (S100A9) was completely absent in the presence of Mg-alloys. Galectin 3 (LGALS3) was downregulated in the presence of Mg-2Ag (in less than 2 fold) and absent in any biological replicates in the presence of the other Mg-alloys. On the other hand, 10 apoptotic-related regulated proteins in at least two biological replicates of each condition were only present after incubation of HUCPV with Mg-alloys. From those proteins, 6 were stimulators of apoptosis: C3, Kininogen-1 (KNG1), prostaglandin-endoperoxide synthase 2 (PTGS2), TAR DNA-binding protein (TARDBP), SERPINA3 and GPLD1 while 4 were inhibitors: apolipoprotein E (APOE), ICAM1, niban-like protein 1 (FAM129B) and angiopoietin-related protein 4 (ANGPTL4). Regarding the downregulated proteins in the presence of the extracts, programmed cell death protein 5 (PDCD5) and PRKC apoptosis WT1 regulator protein (PAWR) were positive regulators of apoptosis. The regulation of the other apoptotic-related proteins is significant in all Mg-alloys.

3.2.3. Regulated proteins involved in the cellular response to toxicity

The heat map in Fig. 4 indicates the regulated proteins involved in cellular response to toxic substances in the presence of Mg-alloys. 6 of those 10 proteins involved in the cellular response to toxicity were upregulated while 4 were downregulated. ICAM1, C3, and paraoxonase 1 (PON1) were only present in the HUCPV cells incubated with Mg-alloys.

3.2.4. Regulated proteins involved in angiogenesis

18 of angiogenesis-related proteins were regulated in the presence of Mg-alloys, 17 of those were significantly increased in the presence of

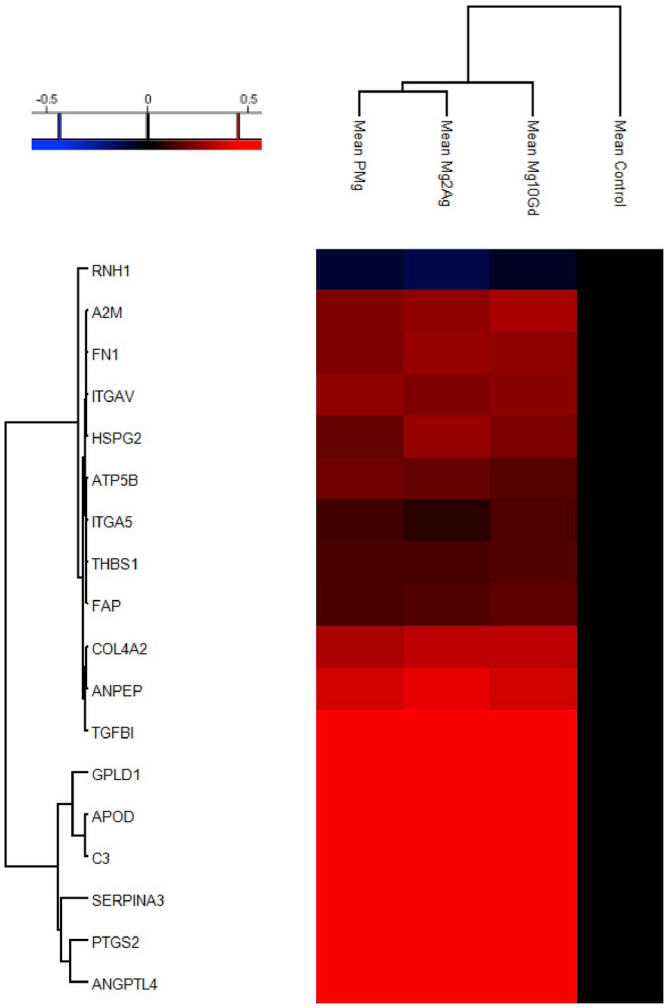

Fig. 5. Heat-map and hierarchical clustering of the up- and down-regulated proteins involved in angiogenesis and bone formation (P-value = 0.05; min. fold-change of 2) in all Mg-alloys compared based on the mean values of the biological replicates (normalized to Control).

at least one of the Mg-alloys. Apolipoprotein D (APOD), Complement Component 3 (C3), PTGS2, GPLD1, Alpha-1-antichymotrypsin (SERPINA3) and angiopoietin-like 4 (ANGPTL4) were present in at least two biological replicates of the HUCPV cells incubated with Mg-alloys, and not present in the control (Fig. 5). Ribonuclease/angiogenin inhibitor 1 (RNH1) was downregulated in the presence of all Mg-alloys. However, downregulation of this protein is significant only in the presence of Mg-2Ag. Moreover, ITGA5 was significantly upregulated in the presence of Mg-10Gd, while there was no significant change in the presence of the other Mg-alloys. Thrombospondin 1 (THBS1) was up-regulated with all the extracts. The upregulation of the other angiogenesis-related proteins was significant in all Mg-alloys.

3.2.5. Regulated proteins in all biological replicates in the presence of Mg-10Gd & Mg-2Ag

The significantly regulated proteins in the presence of Mg-10Gd and Mg-2Ag (5 proteins) are listed in Table 2. Charged multivesicular body protein 4B (CHMP4B) was upregulated (while downregulated with Pure-Mg) and Asparagine synthetase (NARS) was downregulated by the three extracts (being also significantly decreased with Mg-10Gd and Mg-2Ag compared to Pure-Mg. Both proteins are involved in cell apoptosis and NARS also in response to toxic substrates. Thioredoxin reductase 1 (TXNRD1) was downregulated only with Mg-2Ag and Mg-10Gd. Keratin 19 (KRT19) was downregulated with the three extracts (but the downregulation was significantly lower than with Pure-Mg (Table A1). SERPINE2, an ECM protein, was present only in the incubated cells with the three Mg-alloys, exhibiting higher expression in Mg-10Gd and Mg-2Ag (in all of the biological replicates of Mg-10Gd and Mg-2Ag) than in Pure-Mg (Table A1).

3.2.6. Regulated proteins in the presence of Mg-10Gd & Pure-Mg

Regulated proteins (22 proteins) in all of the biological replicates in HUCPV cells incubated with Mg-10Gd and Pure-Mg are listed in Table 2. Among them, 6 and 9 proteins expression was increased and decreased, respectively, compared to control cells. Three proteins were identified only in the presence of Mg-10Gd and Pure-Mg, whereas 3 proteins were absent in the presence of both extracts. Four proteins involved in the apoptotic process were regulated: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide (YWHAE) and LGALS3 were downregulated, the latter being absent in cells incubated with those extracts. PTGS2 was remarkably upregulated with the three extracts, but significantly higher with Mg-10Gd and Pure-Mg than with Mg-2Ag. Among the proteins involved in transport, sideroflexin 3 (SFXN3) involved in iron homeostasis was absent in control cells and orosomucoid 2 (ORM2) was missing in the presence of Mg-alloys. Four downregulated proteins with a role in cell differentiation, four and a half LIM domains protein 1 (FHL1), PLS3, LGALS3, and calpain 1 (CAPN1) are listed in Table 2. Additionally, downregulation of calmodulin 1 (CALM1) was observed with the three extracts (although not significantly), being more notable with Mg-10Gd and Mg-Pure than with Mg-2Ag.

3.2.7. Regulated proteins in the presence of Mg-2Ag & Pure-Mg

In the presence of Mg-2Ag and pure-Mg, 29 proteins were significantly regulated in all of the biological replicates of these two conditions (Table 2). Eight of these proteins were up- and 13 of them were down-regulated. Six proteins were only present in all biological replicates of the incubated cells with Mg-2Ag and pure-Mg. Furthermore, there are two proteins which were presented only in the cells without Mg-alloys. Regulated proteins involved in apoptosis: Reticulon 4 (RTN4), Filamin-A (FLNA), Glutaredoxin-3 (GLRX3) and Importin-5 (IPO5) were down-regulated in the presence of Mg-2Ag and pure-Mg. 10 kDa heat shock protein (HSPE1) was up-regulated while 60 kDa heat

Table 1
Elemental characterisation of the extraction medium (growth medium) initial extracts (pure) and after dilution to a Mg concentration of 6.08 mM (diluted) measured *via* ICP-MS. All concentrations are in millimolar (mM).

			Mg (mM)	Ca (mM)	P (mM)	Gd (mM)	Ag (mM)	pH
Extract	Pure	Pure-Mg	51.43	0.70	0.30	n.d.	n.d.	8.68
		Mg-10Gd	80.64	0.32	0.32	2.16×10^{-3}	n.d.	8.51
		Mg-2Ag	50.60	0.54	0.54	n.d.	71×10^{-3}	8.68
	Diluted	Pure-Mg	6.08	1.79	1.26	n.d.	n.d.	8.15
		Mg-10Gd	6.08	1.77	1.26	1.63×10^{-4}	n.d.	8.15
		Mg-2Ag	6.08	1.72	1.16	n.d.	11×10^{-3}	8.25
Growth or expansion medium			0.81	1.80	1.01	n.d.	n.d.	7.34
Differentiation or chondrogenic medium			0.82	1.89	1.37	n.d.	n.d.	7.40

Table 2
Significantly regulated proteins (⇧ upregulation - ⇩ downregulation) under different conditions. Function based on UniProt database search. Protein names and symbol are according to Hugo Gene Nomenclature Committee – synonyms/previous names are italicised.

	Protein name (gene name) *synonym*	Function	Fold change in Pure-Mg	Fold change in Mg-10Gd	Fold change in Mg-2Ag
Significantly regulated proteins in Mg-10Gd and Mg-2Ag (not in Pure-Mg).	Charged multivesicular body protein 4B (CHMP4B) *Chromatin modifying protein 4B*	negative regulation of cell death	/	⇧ 2.29 ±0.199	⇧ 2.57 ±0.068
	Eukaryotic translation initiation factor 3 subunit C (EIF3C)	translation initiation factor activity	/	⇩ 2.04 ±0.0.97	⇩ 2.34 ±0.056
	Thioredoxin reductase 1 (TXNRD1)	cell proliferation, response to reactive oxygen species, thioredoxin-disulfide reductase activity	/	⇩ 2.14 ±0.02	⇩ 2.24 ±0.018
	Keratin 19 (KRT19)	cell differentiation, involved in embryonic placenta development, structural constituent of cytoskeleton	/	⇩ 16.22 ±0.319	⇩17.78 ±0.068
	Asparagine-tRNA ligase (NARS)	negative regulation of apoptotic process, response to toxic substance	/	⇩ 4.17 ±0.083	⇩ 5.13 ±0.069
			Fold change in Pure-Mg	Fold change in Mg-10Gd	Fold change in Mg-2Ag
Significantly regulated proteins in Mg-10Gd and Pure-Mg (not in Mg-2Ag)	Transmembrane p24 trafficking protein 7 (TMED7) *Transmembrane emp24 domain-containing protein 7*	protein transport	⇧ 3.39 ±0.077	⇧ 2.82±0.0056	/
	Sideroflexin 3 (SFXN3)	transporter activity	Not present in control	Not present in control	/
	IKBKB interacting protein (IKBIP) *Inhibitor of nuclear factor kappa-B kinase-interacting protein*	response to X-ray	⇧ 2.09 ±0.036	⇧ 2.29 ±0.072	/
	Pyrophosphatase (inorganic) 1 (PPA1) *PP*	magnesium ion binding; phosphate-containing compound metabolic process	⇩ 2.45 ±0.022	⇩ 2.00 ±0.060	/
	Bleomycin hydrolase (BLMH)	aminopeptidase activity, proteolysis, response to toxic substance	Not present in Pure-Mg	⇩ 2.19 ±0.056	/
	Four and a half LIM domains protein 1 (FHL1) *LIM protein SLIMMER (SLIM1)*	cell differentiation, positive regulation of potassium ion transport, zinc ion binding	⇩ 3.31 ±0.194	⇩ 2.69 ±0.065	/
	Nexilin F-actin binding protein (NEXN) *NELIN, nexilin*	regulation of cytoskeleton organisation	Not present in Pure-Mg	Not present in Mg-10Gd	/
	AHNAK nucleoprotein (AHNAK) *neuroblast differentiation-associated protein, desmoyokin*	regulation of voltage-gated calcium channel activity	⇩ 2.57 ±0.098	⇩ 2.69 ±0.24	/
	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 (PLOD1) *lysyl hydroxlase 1, LH1*	oxidation-reduction process, procollagen-lysine 5-dioxygenase activity	⇧ 2.09 ±0.020	⇧ 2.00 ±0.133	/
	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon (YWHAE) *14-3-3 protein epsilon*	negative regulation of cysteine-type endopeptidase activity involved in apoptotic process, positive regulation of protein insertion into mitochondrial membrane, involved in apoptotic signalling pathway	⇩ 2.09 ±0.117	⇩ 2.24 ±0.178	/
	Prostaglandin-endoperoxide synthase 2 (PTGS2) *prostaglandin G/H synthase 2, cyclooxygenase 2, COX2*	positive regulation of apoptotic process, angiogenesis, involved in sprouting angiogenesis, bone mineralisation	Not present in control	Not present in control	/
	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) *protein kinase C inhibitor protein 1*	positive regulation of protein insertion into mitochondrial membrane involved in	⇩ 2.24 ±0.067	⇩ 2.75 ±0.078	/

(*continued on next page*)

Table 2 (*continued*)

	Protein name (gene name) *synonym*	Function	Fold change in Pure-Mg	Fold change in Mg-10Gd	Fold change in Mg-2Ag
		apoptotic signalling pathway			
	ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1(ATP5F1)	ATP biosynthetic process, transporter activity	⇧ 2.19 ±0.061	⇧ 2.63 ±0.132	/
	Galectin 3 (LGALS3) *lectin, galactoside-binding, soluble, 3*	epithelial cell differentiation, regulation of extrinsic apoptotic signalling pathway via death domain receptors, regulation of T cell apoptotic process, regulation of T cell proliferation	Not present in Pure-Mg	Not present in Mg-10Gd	/
	Plastin 3 (PLS3) *T-plastin*	auditory receptor cell differentiation, bone development, calcium ion binding	⇩ 2.04 ±0.071	⇩ 2.09 ±0.068	/
	Cytochrome c oxidase subunit 4 isoform 1 (COX4I1) *cytochrome c oxidase subunit IV, COX4*	generation of precursor metabolites and energy, response to nutrient	⇧ 2.19 ±0.066	⇧ 2.04 ±0.041	/
	Calpain 1 (CAPN1) *calpain 1, (mu/I) large subunit*	extracellular matrix disassembly, positive regulation of cell proliferation, proteolysis	⇩ 3.47 ±0.083	⇩ 3.16 ±0.061	/
	Lecithin-cholesterol acyltransferase (LCAT) *phosphatidylcholine-sterol acyltransferase*	lipoprotein biosynthetic process, response to copper ion	Not present in control	Not present in control	/
	H2A histone family, member (H2AFY) *Core histone macro-H2A.1, MACROH2A1*	SH3/SH2 adaptor activity	⇧ 2.04 ±0.099	⇧ 2.29 ±0.108	/
	Actinin alpha 4 (ACTN4)	BAT3 complex binding, positive regulation of ER-associated ubiquitin-dependent protein catabolic process	⇩ 2.09 ±0.035	⇩ 2.19 ±0.027	/
	Orosomucoid 1 (ORM1) *alpha-1-acid glycoprotein 1, OMD, ORM*	metal ion binding, SMAD protein signal transduction, transport	Not present in Pure-Mg	Not present in Mg-10Gd	/
	Alpha fetoprotein (HPAFP)	oxygen transporter activity, heme binding	⇩ 2.51 ±0.0137	⇩ 2.88 ±0.202	/
			Fold change in Pure-Mg	Fold change in Mg-10Gd	Fold change in Mg-2Ag
Significantly regulated proteins in Mg-2Ag and Pure-Mg (not in Mg-10Gd)	REX2, RNA exonuclease 2 homolog (S. cerevisiae) (REXO2), *Oligoribonuclease, mitochondrial precursor*	3′-5′ exonuclease activity, focal adhesion, nucleotide metabolic process	Not present in control	/	Not present in control
	LIM domain and actin-binding protein 1 (LIMA1), *Epithelial protein lost in neoplasm, EPLIN, FLJ38853*	focal adhesion, negative regulation of actin filament depolymerisation, stress fiber	⇩ 4.07 ±0.036	/	⇩ 2.51 ±0.112
	Reticulon-4 (RTN4), *ASY, Foocen, KIAA0886, My043, Nbla00271*	negative regulation of cell growth, of apoptotic process, regulation of cell migration	⇩ 2.19 ±0.160	/	⇩ 2.14 ±0.14
	Histone cluster 1, H2bl (HIST1H2BL) *Histone H2B type 1-L, H2BFC, Histone H2B.c*	nucleosome assembly	⇧ 2.24 ±0.044	/	⇧ 2.75 ±0.035
	Kinectin (KTN1), *CG1, CG-1 antigen, kinesin receptor*	microtubule-based movemen	Not present in Pure-Mg	/	Not present in Mg2Ag
	non-POU domain containing, octamer-binding (NONO), *NonO protein, Non-POU domain-containing octamer-binding protein*	DNA recombination, DNA repair, negative regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway [	⇧ 2.45 ±0.097	/	⇧ 2.24 ±0.040
	LIM and SH3 domain protein 1 (LASP1), *Metastatic lymph node gene 50 protein,Lasp-1*	focal adhesion;ion transmembrane transporter activity,	⇩ 4.07 ±0.089	/	⇩ 2.88 ±0.23

(*continued on next page*)

Table 2 (*continued*)

Protein name (gene name) *synonym*	Function	Fold change in Pure-Mg	Fold change in Mg-10Gd	Fold change in Mg-2Ag
	ion transport, zinc ion binding			
Major vault protein (MVP), *LRP, Lung resistance-related protein, Major vault protein*	ERBB signaling pathway, protein transport	⇧ 2.29 ±0.115	/	⇧ 2.04 ±0.006
Caldesmon 1 (CALD1), *CDM, L-caldesmon, Non-muscle caldesmon*	movement of cell or subcellular component, muscle contraction	⇩ 3.09 ±0.076	/	⇩ 2.04 ±0.090
Nucleobindin 1 (NUCB1), *CALNUC, DKFZp686A15286*	regulation of protein targeting	⇧ 2.34 ±0.108	/	⇧ 2.40 ±0.008
heat shock 10 kDa protein 1 (chaperonin 10), (HSPE1), 10 kDa *heat shock protein,* 10 kDa *chaperonin, CPN10*	activation of cysteine-type endopeptidase activity involved in apoptotic process, osteoblast differentiation	⇧ 2.24 ±0.107	/	⇧ 2.24 ±0.029
Branched-chain-amino-acid aminotransferase 1, cytosolic (BCAT1), *BCAT(c), BCATC, BCT1, Branched-chain-amino-acid aminotransferase, cytosolic*	aspartate biosynthetic process, cell proliferation	⇩ 2.24 ±0.034	/	⇩ 2.14 ±0.103
Tu translation elongation factor, mitochondrial (TUFM), *Elongation factor Tu, mitochondrial, P43, COXPD4*	GTP binding, GTPase activity, mitochondrial translational elongation [	⇩ 2.29 ±0.041	/	⇩ 2.29 ±0.105
Serum amyloid A4, constitutive (SAA4), *Serum amyloid A-4 protein, CSAA, C-SAA*	acute-phase response, cell chemotaxis, chemoattractant activity	Not present in control	/	Not present in control
Phosphatidylethanolamine-binding protein 1 (PEBP1), *HCNP, HCNPpp, Neuropolypeptide h3, PBP*	ATP binding, serine-type endopeptidase inhibitor activity	⇩ 3.89 ±0.110	/	⇩ 2.88 ±0.117
Filamin-A, akoga (FLNA), *ABP-280, ABPX, Actin-binding protein 280, Alpha-filamin*	cell junction assembly, focal adhesion, negative regulation of apoptotic process, wound healing	⇩ 2.14 ±0.062	/	⇩ 2.09 ±0.020
Spermidine synthase (SRM), *PAPT, Putrescine aminopropyltransferase, SPDSY*	polyamine metabolic process, protein homodimerization activity, spermidine biosynthetic process	⇩ 2.29 ±0.152	/	⇩ 2.40 ±0.067
Ezrin (EZR), *CVIL, CVL, Cytovillin, DKFZp762H157, Ezrin*	focal adhesion, regulation of cell shape	⇩2.19 ±0.116	/	⇩ 2.29 ±0.017
Heat shock 60 kDa protein 1 (chaperonin) (HSP60), 60 kDa *heat shock protein,Chaperonin 60, Chaperonin 60, CPN60, GROE*	negative regulation of apoptotic process, positive regulation of interleukin-6,10,12	⇧ 2.14 ±0.099	/	⇧ 2.00 ±0.045
L-lactate dehydrogenase B (LDHB), *L-lactate dehydrogenase B chain, LHD heart subunit, LHD-B*	L-lactate dehydrogenase activity, pyruvate metabolic process	⇩ 2.04 ±0.034	/	⇩ 2.09 ±0.049
Apolipoprotein A-II (APOA2), *apoAII, ApoA-II, Apo-AII, Apolipoprotein A2*	acute inflammatory response, protein oxidation	Not present in control	/	Not present in control
Crystallin alpha B (CRYAB), *Alpha-crystallin B chain, Alpha (B)-crystalline, HspB5, HSPB5*	negative regulation of extrinsic apoptotic signaling pathway, positive regulation of cell aging, positive regulation of osteoblast differentiation, negative regulation of adipose tissue development,	⇧ 3.02 ±0.084	/	⇧ 2.45 ±0.048
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1), *A1A, A1AT, Alpha-1-antiproteinase, alpha-1-antitrypsin*	acute-phase response, inflammatory response, serine-type endopeptidase inhibitor activity	Not present in control	/	Not present in control
Serpin peptidase inhibitor, clade C (antithrombin), member 1 (SERPINC1) *Antithrombin-III, AT3, ATIII, Serpin C1*	acute-phase response, serine-type	Not present in control	/	Not present in control

(*continued on next page*)

Table 2 (*continued*)

Protein name (gene name) *synonym*	Function	Fold change in Pure-Mg	Fold change in Mg-10Gd	Fold change in Mg-2Ag
Haptoglobin-related protein (HPR), *A-259H10.2, Haptoglobin-related protein, HP*	endopeptidase inhibitor activity extracellular matrix disassembly, negative regulation of cell proliferation, negative regulation of cell-cell adhesion mediated by cadherin, positive regulation of fibrinolysis, serine-type endopeptidase activity	Not present in control	/	Not present in control
Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2) (GOT2), *Aspartate aminotransferase, mitochondrial, FABP-1, FABPpm*	canonical glycolysis,epithelial cell differentiation	⇧ 2.63 ±0.088	/	⇧ 2.51 ±0.48
Glutaredoxin 3 (GLRX3), *Glutaredoxin-3, GRX3, GRX4, PICOT, PKC-interacting cousin of thioredoxin*	osteoblast differentiation, regulation of apoptotic process	Not present in P-Mg	/	Not present in Mg-2Ag
Programmed cell death protein 5 (PDCD5), *Protein TFAR19, TF-1 cell apoptosis-related protein 19, TFAR19*	chloride transmembrane transport, lipid transport	⇩ 3.63 ±0.067	/	⇩ 2.63 ±0.196
Importin5 (IPO5), *Imp5, Importin-5, Importin subunit beta-3*	positive regulation of apoptotic process, positive regulation of intrinsic apoptotic signaling pathway	⇩2.14 ±0.039	/	⇩ 2.19 ±0.008

shock protein (HSPE1) and Alpha-crystallin B chain (CRYAB) were up-regulated. Regarding proteins involved in cell differentiation, Aspartate aminotransferase (GOT2) was up-regulated and Glutaredoxin-3 (GLRX3) was down-regulated in cells cultured with Mg-2Ag and pure Mg. Moreover, the proteins involved in transportation such as LIM and SH3 domain protein 1 (LASP1), Major vault protein (MVP), Ezrin (EZR), Programmed cell death protein 5 (PDCD5), were down- or up-regulated in the presence of Mg-2Ag and pure-Mg. Among the proteins observed in the presence of Mg-2Ag and pure-Mg, which were absent in control cells, APOA2, Antithrombin-III (SERPINA3), and Alpha-1-anti-trypsin (SERPINA1) are involved in the acute-phase response. Non-POU domain-containing octamer-binding protein (NONO) was upregulated in the presence of Mg-2Ag and pure-Mg.

4. Discussion

According to the overall results, it is obvious that the increased concentration of Mg^{2+} ions is responsible for the main effects observed in this study. In comparison to the effect of Mg^{2+} ions, Ag^{+}-ions and Gd^{3+}-ions have minor effects. Additionally, increased extracts pH probably have an influence on chondrogenesis. Indeed, lower pH (as observed in diabetes and aging) negatively influence bone homeostasis (altered bone structure and density). Furthermore, an alkaline pH (about 8) is optimal for alkaline phosphatase activity and hydroxyapatite precipitation while switching off osteoclast resorption [29,30]. Moreover, Moghadam et al. demonstrated that chondrogenesis was more efficient after short-term culture in alkaline medium [31]. *In vitro* and even *in vivo* magnesium-based material degradation is a complex mechanism accompanied by increased pH, ion released (increased osmolality) and other phenomenon. Therefore, the already observed positive effects of these biomaterials on bone healing are probably multifactorial and due to the synergistic effects of magnesium-based degradation. Furthermore, pH of the different extracts are similar thus, the proteomics variation measured between the different extracts are probably due to the material compositions themselves.

Mg^{2+} is an endogenous element in living organisms and its doubly charged ion involved in a multitude of physiological processes, in many cases enabling defined functions of proteins as their ligands. Living organisms are equipped with a fine-tuned system guaranteeing constant levels of Mg ions in the intra- and extracellular space. Thus, it is not surprising that the increase of Mg ions in the culture medium, will lead to an active cell reaction (*e.g.*, regulation of 246 proteins). Extracellular proteins and cytosolic/cytoskeletal proteins were mostly upregulated and downregulated, respectively. Among the main cell functions of the proteins influenced by the extracts, cell attachment, growth, differentiation and survival or apoptosis were identified. Such functions are involved also in the interactions between chondrocytes and ECM and are important for cartilage homeostasis and cartilage repair [32]. Mg has a key role in cellular energy metabolism and Mg^{2+} ions are known to enhance the activity of adenosine triphosphate (ATP) synthase [33]. This enzyme consists of two main regions, F_0 and F_1 themselves composed of subunits. Here, accordingly, several subunits were upregulated (Fig. A1): from the F_0 complex: ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit B1 (ATP5F1) and from F_1: ATP synthase, H^+ transporting, mitochondrial F1 complex, alpha subunit 1 (ATP5A1), gamma polypeptide 1 (ATP5C1), beta polypeptide (ATP5B) and O subunit (ATP5O). Similarly, upregulation of voltage-dependent anion channel 1 (VDAC1) was induced by the extracts. This protein interacts with hexokinase and creatine kinase to convert newly generated ATP into high-energy storage molecules. Therefore the increased synthesis of VDAC1 is also associated with high metabolically active and energy-demanding cells [34]. A possible explanation may be that the increased Mg^{2+}-concentration induces the increased synthesis of energy-rich (phosphate-rich) metabolites for binding free Mg^{2+}- ions for maintaining Mg^{2+} homeostasis. Increased of energy-rich metabolites may increase biosynthesis by which the energy-rich metabolites are consumed [35].

Proteins involved in cholesterol metabolism were strongly

upregulated by the extracts. Lecithin-cholesterol acyltransferase (LCAT) showed 2-fold higher expression with Mg-10Gd and Pure-Mg than with Mg-2Ag. This protein is the central enzyme involved in the extracellular metabolism of lipoproteins. Apolipoprotein A-I (APOA1) is the most potent phosphatidylcholine-sterol acyltransferase activator in plasma (although it can also be activated by APOE, APOC1 and APOA4). All those apolipoproteins involved in cholesterol efflux (as well as additional ones as APOD) were strongly upregulated with the three extracts (Appendix A). Both LCAT and APOAI lack or deficiency give rise to cartilage degeneration and the development of osteoarthritis (OA) [36,37]. Thus, the possible inhibitory effect of those extracts on OA (*i.e.*, through the upregulation of the aforementioned proteins), is an interesting subject for future investigations.

The three extracts induced the upregulation of proteins involved in cartilage development (both ECM integrity and ECM-cell adhesion) (Appendix A). Among them, TNC is notably upregulated during cartilage development, and it is involved in ECM remodelling and cell differentiation [38]. HSPG2 is involved in the metabolism (synthesis and catabolism) of GAG, one of the main components of cartilage ECM. It is required for cartilage development, where it plays a role in ECM organization. ICAM1 (whose expression was not detected in control cells) has multiple functions, being relevant its role in cell adhesion (specifically integrin-mediated adhesion). Its expression in human chondrocytes can be induced by exogenous interleukin 1 α (IL1α), which was added to the culture medium in the study of Davies et al. in order to induce chondrogenesis [39]. Those results suggest a synergistic effect of IL1α in the presence of Mg-extracts or a direct effect of the Mg ions on ICAM1 expression.

Another group of proteins, upregulated by the three extracts, was the integrin family. Integrins are responsible for primary adhesion of cells to orthopaedic or dental implants, therefore addition of Mg ions to the surface of biomaterials enhance cell-material interaction, reducing the possibilities of implant rejection by the body [40]. Furthermore, the integrin family plays a major role in mediating cell-matrix interactions that are important in regulating cartilage development and repair. Integrins and cell-matrix interactions have been shown to be involved in chondrogenesis of MSC [38] and enhance MSC attachment to endochondral defects (enhancing its repair). Additionally, integrins are involved in the negative regulation of apoptosis. Upregulation of integrins in response to Mg extracts seems therefore beneficial, not only for enhancing chondrogenesis of HUCPV cells, but also for generating a good quality cartilaginous matrix. In native cartilage, chondrocytes express several members of the integrin family, which can serve as receptors for relevant proteins in the structure of the ECM (which also were upregulated under the influence of the extracts): ITGA5 is a receptor for FN1, ITGAV for VTN and ITGA2 for COL6. Since divalent cations, including Mg^{2+}, are ligands for integrins and activate them, an increased cell adhesion of HUCPV cells under the influence of the three extracts is expected. The integrin-signalling proteins are important components of the cartilage ECM. VTN interacts with glycosaminoglycans and proteoglycans and serves as a cell-to-substrate adhesion molecule. Furthermore, it inhibits the membrane-damaging effect of the terminal cytolytic effect of the complement pathway. FN1 is involved in early chondrocyte differentiation events after birth. Its upregulation takes place during the condensation of stem cells [41]. COL4 is found in connective tissue. A notable upregulation of this protein has been reported during early stages of human MSC chondrogenesis (after 10 days), possibly due to the influence of this protein on Sox9 [42]. Two other chondrogenic-related proteins were upregulated by the three extracts: GPLD1, which stimulates chondrocyte differentiation and HEXB, which has a role in the catabolic process of chondroitin sulphate.

The presence of Mg-extracts on HUCPV cells during chondrogenesis also induced the upregulation of TGFBI, a protein involved in the cell-collagen interaction, and important for ECM remodelling during chondrocyte differentiation [43]. TGFBI overexpression positively enhances the proliferation and chondrogenic potential of human synovium-derived MSC [44]. Furthermore, TGFBI induces upregulation of integrins [45]. Therefore, Mg extracts may induce an enhancement of TGFBI, which will in turn upregulate integrin production, and subsequently, integrin-mediated cell adhesion and chondrogenesis. In addition, TGFBI induces expression of PLOD1 and PLOD2, proteins upregulated in regenerated cartilage *in vivo* (regarding the natural cartilage).

Angiogenesis is a fundamental component of bone repair due to the development of blood vessels in the fracture callus [46] and a vital part of bone formation [46,47]. Hypertrophic cartilage produces angiogenic stimulators [48–50], unlike angiogenesis inhibitors, which are secreted by immature chondrocytes [51,52]. The 3 processes, chondrogenesis, angiogenesis and bone formation are closely related. Hence, some of the regulated proteins are involved in all of them (*e.g.*, ITGA5 and COL1A1). Upregulation of 15 of the 16 regulated proteins involved in angiogenesis shows a hypertrophic stage of chondrocytes. Furthermore, THBS1 upregulation could be of interest since it has been shown that this protein inhibits vascular endothelial growth factor (VEGF)-induced migration in human microvascular cells [53].

COL1A1 was downregulated under the influence of the three extract. COL1A1 is involved in bone trabecula formation and final stage of cartilage development. Nevertheless, the downregulation of this protein versus the lack of effect on COL2 production is indicating a reduction in the ratio COL2/COL1 characteristic in cartilage tissue. PLS3 is involved in bone development and its downregulation in the presence of Mg-alloys may avoid cartilage mineralisation. Consequently, the downregulation of those two proteins is beneficial in order to keep the chondrogenic phenotype of the differentiated HUCPV cells. Furthermore, two proteins PTGS2 and GPLD1 were only detected in the presence of extracts. PTGS2 (or cyclooxygenase 2, COX 2) is responsible for production of inflammatory prostaglandins. Furthermore, PTGS2 is also associated with increased cell adhesion, phenotypic changes and resistance to apoptosis. PTGS2 is a target of nonsteroidal anti-inflammatory drugs (NSAID) including acetylsalicylic acid (“aspirin”) and isobutylphenylpropionic acid (“ibuprofen”). NSAID have been reported to have (controversial/negative) influence osteogenesis during bone fracture healing [54]. Welting et al. demonstrate the role of PTGS2 in chondrocyte maturation-hypertrophy [55]. GPLD1 hydrolyses the inositol phosphate linkage in proteins anchored by glycosylphosphatidylinositol (GPI) to the outer leaflet of the plasma membrane, thereby releasing the attached protein. Over 250 GPI-proteins are known, among them heparan sulfate proteoglycans (HSPG) [56], ephrin A ligands (for Eph receptors - the largest known subfamily of receptor protein-tyrosine kinases), putative adhesion/signalling molecules of the Ly6 family, and enzymes like alkaline phosphatase [57]. GPI-anchored proteins are believed to have a role in cell adhesion events involved in tissue patterning and cell signalling. Indeed, Ahrens et al. demonstrated that GPI-anchored proteins are necessary to the columnar tissue arrangement and the proper development of the growth plate [57].

Apoptosis is a tightly regulated process, inevitable and essential during development, particularly during formation of articular cartilage and endochondral ossification of growth plate [58]. Increased apoptosis in native cartilage is associated with matrix degradation. Induction of MSC chondrogenesis *in vitro* using micromasses formation models increases the possibility of apoptosis due to the severe hypoxic conditions that cells suffer in the centre of the spheres. Nevertheless, some differences in protein expression (involved in both positive and negative regulation of apoptosis) were found due to the action of the extracts (Fig. 4). On the one hand, 9 apoptosis-related proteins were found only in presence of extracts: 5 were stimulators of apoptosis (C3, KNG1, PTGS2, TARDBP, and GPLD1) and 4 inhibitors (APOE, ICAM1,

FAM129B, and ANGPTL4). On the other hand, 2 proteins stimulating apoptosis were downregulated, S100A9 and LGALS3. S100A9 was completely absent in the presence of Mg-alloys. This protein also has a role in actin cytoskeleton reorganization, proinflammatory response and oxidant-scavenging. LGALS3 was downregulated in the presence of Mg-2Ag, and absent in the presence of Pure-Mg and Mg-10Gd, which may indicate a toxic action of Ag. Galectin-3 (LGALS3) is also found to be a potent inhibitor for osteoclastogenesis *in vitro* [59]. Those results show a clear influence on apoptosis and suggest a reduction of cell death by the extracts.

Since Mg-alloy degradation products might have undesired side effects like toxicity to the cells, regulated proteins involved in the response to toxic effect were evaluated. Six from the 10 regulated proteins known to be associated with stress response showed increased expression in the presence of extracts. From those six, four proteins were not found in the absence of the extracts, which might suggest a response of the cells toward toxicity; however, these proteins have also other roles in the cells. For instance, C3 is one of the stimulators for angiogenesis and ICAM1 is involved in cell migration and adhesion, therefore reinforcing cartilage repair.

Some proteins were regulated only by 2 extracts, or showed significant differences in the expression (always normalised to control) among the 3 extracts. In principle, proteins up- or downregulated only by Mg-2Ag or Mg-10Gd extracts should give information about the effects of the alloying elements (Ag and Gd) on HUCPV chondrogenesis, as Mg concentration was constant. CHMP4B, having a role apoptosis suppression, was upregulated with those extracts, could be beneficial for cell viability. However, NARS with the same function was downregulated. Regarding the other function of NARS in the cellular response to toxic substrate, its downregulation supports suggests a lack of toxic effect of Mg-2Ag and Mg-10Gd. Interestingly, serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2 (SERPINE2), was only expressed in the presence of the extracts, and its expression was significantly higher with Mg-10Gd and Mg-2Ag than with Pure-Mg. It has been shown that SERPINE2 expression in human chondrocytes might prevent cartilage catabolism by inhibiting the expression of matrix metallopeptidase 13 (MMP13), one of the most relevant collagenases, involved in cartilage breakdown in OA [60]. Therefore, alloying elements could have a positive effect on the maintenance of cartilage integrity. TXNRD1, protein involved in cell proliferation, was downregulated only with Mg-10Gd and Mg-2Ag. In correlation with this, a strong downregulation of the microtubule-associated protein 9 (MAP9) with Mg-10Gd extract was observed, while a slight upregulation was detected with Pure-Mg and Mg-2Ag. MAP9 is required for mitosis progression and cytokinesis (UniProt). Therefore, its downregulation could decrease cell cycle progression and cell division. The proteins commonly regulated only by Pure-Mg and Mg-2Ag as well as Pure-Mg and Mg-10Gd were mainly involved in apoptosis, indicating that Mg itself has a significant influence on this cellular process.

Mg-2Ag and Pure-Mg showed a beneficial effect on HUCPV viability by downregulating proteins positively involved in apoptosis (RTN4,FLNA, GLRX3, and IPO5) and upregulating two proteins involved in negative regulation of apoptosis (60 kDa heat shock protein and α-crystallin B chain). However, FLNA, having a role in protecting cells from apoptosis was also downregulated. An interesting upregulated protein in the presence of Mg-2Ag and Pure-Mg is non-POU domain containing, octamer-binding protein (NONO). This protein has a protective role in the regulation of oxidative stress-induced neuron intrinsic apoptotic signalling pathway, Furthermore, NONO promotes chondrogenesis by interacting with SOX9 thus allowing/promoting transcription of SOX9 target genes such *COL2A1* [61]. Both functions suggest that NONO upregulation is beneficial for cartilage development and bone healing. Some proteins involved in acute-phase or response to inflammation (Apolipoprotein A-II2, ATIII and SERPINA1) were only observed under the influence of Mg-2Ag and Pure-Mg, making them interesting for further research.

Mg-10Gd and Pure-Mg showed beneficial effects on chondrogenesis and maintenance of cartilage integrity. First, LGALS3, which has a role in the regulation of extrinsic apoptotic signalling pathway *via* death domain receptors, was absent. PTGS2 (having roles previously described in apoptosis, angiogenesis and bone formation) was remarkably upregulated with the three extracts, but significantly lower with Pure-Mg and Mg-2Ag than with Mg-10Gd. In the second place, the downregulation of calpain 1 (CAPN1), supported by the decreased expression of calmodulin 1 (phosphorylase kinase, delta) (CALM1) (Appendix A), which expression has been reported to diminish during chondrogenic differentiation of stem cells [62], indicate that Mg-10d and Pure-Mg extracts could enhance cell chondrogenesis. This idea is also supported by the absence of CAPN1 protein in the presence of Mg-10Gd and Pure-Mg. The serum concentration of CAPN1 raises several folds during an acute phase response (the systemic answer to a local inflammatory stimulus). Therefore its absence suggests a lack or decrease of immunological reactions against the degradation products of the material [63].

To conclude, various regulated proteins were identified in response to Mg-alloy degradation products. Regulation of specific proteins indicate a positive effect on chondrogenesis (*i.e.*, integrins, TGFBI, FN1, VTN, CALM1, NONO) and cell viability (apoptotic-related proteins), as well as possible influence on reducing or inhibiting OA (cholesterol metabolism-related proteins and SERPINE2) and acute-phase response (APOA2, ATIII and SERPINA1). These results show that the Mg-based materials have potential to stimulate cartilage *in vitro*. Further investigation *in vivo* will be pursuit to validate these results.

Author Contributions

A.M.S. and F.F. designed the study; A.M.S, M.O., M.W., M.M., and B.L. conducted cell cultures, processing, and data analysis; H.S., R.W.R. and B.L. provided intellectual contributions; A.M.S. and M.O. cowrote the paper.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bioactmat.2019.04.001.

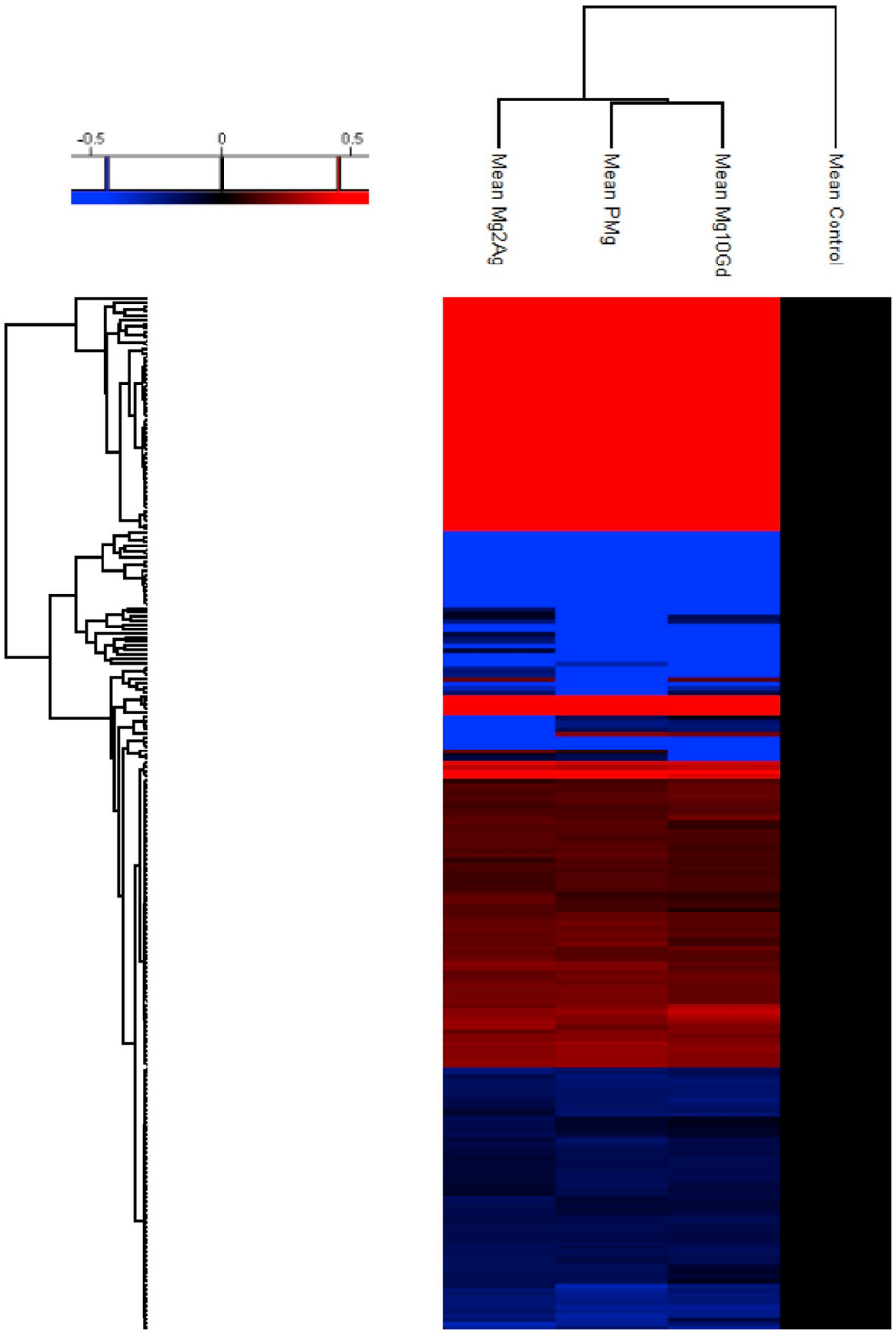

Fig. A1. Heat-map and hierarchical clustering of all significantly up- and down-regulated proteins (P-value = 0.05; min. fold-change of 2) in all Mg-alloys compared based on the mean values of the biological replicates (normalized to Control).

Table A1

Table showing the significantly (P-value = 0.05; min. fold-change of 2) regulated proteins under the influence of the Mg extracts (Mg-10Gd, Mg-2Ag and Pure-Mg). Data express the fold change of expression (as log10) based on the mean values of the biological replicates normalised to the control. For proteins that were not found in either group, no fold change could be determined. A log10-fold change of > 1 or < -1 was assumed in that case, reflecting an increase or decrease in protein intensity of more than 10-fold. Official protein names and symbols (Uniprot-HGNC) as well as synonyms (in grey) are listed.

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
Apolipoprotein D ApoD, Apo-D, Apolipoprotein D	APOD	> 1	> 1	> 1
Plasminogen	PLG	> 1	> 1	> 1
Complement component 4 binding protein, alpha C4b-binding protein alpha chain, C4bp, C4BP, Proline-rich protein, PRP	C4BPA	> 1	> 1	> 1

(*continued on next page*)

Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
Immunoglobulin heavy constant gamma 1 (G1m marker)	IGHG1	> 1	> 1	> 1
Immunoglobulin heavy constant gamma 1 (G1m marker)				
Immunoglobulin heavy constant alpha 1	IGHA1	> 1	> 1	> 1
FLJ14473, FLJ35065, FLJ35500, FLJ36402, FLJ39698, FLJ40001, FLJ41548, FLJ41552, FLJ41789, FLJ43248, FLJ43594, FLJ44293, FLJ46028, FLJ46621, FLJ46724, FLJ46811, FLJ46824, FLJ90170, IgA1, Ig alpha-1 chain C region, MGC102857				
Ceruloplasmin (Ferroxidase)	CP	> 1	> 1	> 1
Ceruloplasmin, ferroxidase				
Immunoglobulin kappa constant	IGKC	> 1	> 1	> 1
HCAK1, Ig kappa chain C region, Ig kappa chain V-I region HK101, Ig kappa chain V-I region Walker, Km, MGC111575, MGC62011, MGC72072, MGC88770, MGC88771, MGC88809				
GC, vitamin D binding protein	GC	> 1	> 1	> 1
Vitamin D-binding protein (DBP) (VDB) (Gc protein-derived macrophage activating factor) (Gc-MAF) (GcMAF) (Gc-globulin) (Group-specific component) (Gc) (Vitamin D-binding protein-macrophage activating factor) (DBP-maf)				
Complement C3	C3	> 1	> 1	> 1
(C3 and PZP-like alpha-2-macroglobulin domain-containing protein 1) [Cleaved into: Complement C3 beta chain;C3-beta-c (C3bc);Complement C3 alpha chain; C3a anaphylatoxin; Acylation stimulating protein (ASP) (C3adesArg); Complement C3b alpha' chain; Complement C3c alpha' chain fragment 1; Complement C3dg fragment; Complement C3g fragment; Complement C3d fragment; Complement C3f fragment; Complement C3c alpha' chain fragment 2], CPAMD1				
immunoglobulin heavy constant mu	IGHM	> 1	> 1	> 1
AGM1, DKFZp686I15196, DKFZp686I15212, FLJ00385, Ig mu chain C region, MGC104996, MGC52291, MU, VH				
Paraoxonase 1	PON1	> 1	> 1	> 1
Serum paraoxonase/arylesterase 1 (PON 1) (EC 3.1.1.2) (EC 3.1.1.81) (EC 3.1.8.1) (Aromatic esterase 1) (A-esterase 1) (K-45) (Serum aryldialkylphosphatase 1), PON				
Apolipoprotein A4	APOA4	> 1	> 1	> 1
ApoA-IV, Apo-AIV, Apolipoprotein A4, Apolipoprotein A-IV, MGC142154, MGC142156				
Tenascin C	TNC	> 1	> 1	> 1
150-225, Cytotactin, Glioma-associated-extracellular matrix antigen, GMEM, GP, GP 150–225, Hexabrachion, HXB, JI, MGC167029, Myotendinous antigen, Neuronectin, Tenascin, Tenascin-C, TN, TN-C				
Apolipoprotein E	APOE	> 1	> 1	> 1
APOE				
serum amyloid A4, constitutive	SAA4	> 1	> 1	> 1
Constitutively expressed serum amyloid A protein, CSAA, C-SAA, Serum amyloid A-4 protein				
Apolipoprotein M	APOM	> 1	> 1	> 1
Apolipoprotein M, ApoM, Apo-M, G3a, G3A, HSPC336, MGC22400, NG20, Protein G3a				
Transthyretin	TTR	> 1	> 1	> 1
ATTR, CTS, CTS1, HsT2651, PALB, Prealbumin, TBPA, Transthyretin				
Apolipoprotein A2	APOA2	> 1	> 1	> 1
apoAII, ApoA-II, Apo-AII, Apolipoprotein A2, Apolipoprotein A-II				
Hemopexin	HPX	> 1	> 1	> 1
Beta-1B-glycoprotein, FLJ56652, Hemopexin, HX				
Immunoglobulin heavy constant gamma 2 (G2m marker)	IGHG2	> 1	> 1	> 1
Ig gamma-2 chain C region				
Haptoglobin-related protein	HPR	> 1	> 1	> 1
A-259H10.2, Haptoglobin-related protein, HP				
Apolipoprotein C3	APOC3	> 1	> 1	> 1
APOCIII, ApoC-III, Apo-CIII, Apolipoprotein C3, Apolipoprotein C-III, MGC150353				
Nidogen 2	NID2	> 1	> 1	> 1
NID-2, Nidogen-2, Osteonidogen				
Alpha-1-B glycoprotein	A1BG	> 1	> 1	> 1
A1B, ABG, Alpha-1B-glycoprotein, Alpha-1-B glycoprotein, DKFZp686F0970, GAB, HYST2477				
Inter-alpha-trypsin inhibitor heavy chain1	ITIH1	> 1	> 1	> 1
H1P, IATIH, IGHEP1, Inter-alpha-inhibitor heavy chain 1, Inter-alpha-trypsin inhibitor complex component III, Inter-alpha-trypsin inhibitor heavy chain H1, ITIH, ITI-HC1, ITI heavy chain H1, MGC126415, Serum-derived hyaluronan-associated protein, SHAP				
Kininogen 1	KNG1	> 1	> 1	> 1
Alpha-2-thiol proteinase inhibitor, BDK, Fitzgerald factor, High molecular weight kininogen, HMWK, Kininogen-1, KNG, Williams-Fitzgerald-Flaujeac factor				
AE binding protein 1	AEBP1	> 1	> 1	> 1
ACLP, Adipocyte enhancer-binding protein 1, AE-binding protein 1, Aortic carboxypeptidase-like protein, FLJ33612				
Perilipin 2	PLIN2	> 1	> 1	> 1
adipose differentiation-related protein, ADFP				
Apolipoprotein L1	APOL1	> 1	> 1	> 1
ApoL, APOL, Apo-L, APO-L, ApoL-I, APOL-I, Apolipoprotein L, Apolipoprotein L1, Apolipoprotein L-I, FSGS4				
Ig kappa chain V-III region GOL (Rheumatoid factor)		> 1	> 1	> 1
Tubulointerstitial nephritis antigen like 1	TINAGL1	> 1	> 1	> 1
ARG1, GIS5, Glucocorticoid-inducible protein 5, LCN7, LIECG3, OLRG2, OLRG-2, Oxidized LDL-responsive gene 2 protein, P3ECSL, PP6614, PSEC0088, TINAGL, TIN Ag-related protein, TINAGRP, TIN-Ag-RP, Tubulointerstitial nephritis antigen-like, Tubulointerstitial nephritis antigen-related protein, UNQ204/PRO230				
Glutathione peroxidase 3	GPX3	> 1	> 1	> 1
Extracellular glutathione peroxidase, Glutathione peroxidase 3, GPx-3, GPXP, GPx-P, GSHPx-3, GSHPx-P, Plasma glutathione peroxidase				
Complement C1q C chain	C1QC	> 1	> 1	> 1
Complement component 1, q subcomponent, C chain, C1Q-C, C1QG, Complement C1q subcomponent subunit C, FLJ27103				
Orosomucoid 2	ORM2	> 1	> 1	> 1
AGP2, AGP 2, AGP-B, AGP-B′, Alpha-1-acid glycoprotein 2, OMD 2, Orosomucoid-2				
Apolipoprotein C-I	APOC1	> 1	> 1	> 1
APOC1				
Coagulation factor XII	F12	> 1	> 1	> 1
Coagulation factor XII, HAE3, HAEX, HAF, Hageman factor				
serpin family C member 1	SERPINC1	> 1	> 1	> 1

(*continued on next page*)

Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
Serpin peptidase inhibitor, clade C (antithrombin), member 1, Antithrombin-III, AT3, ATIII, MGC22579, PRO0309, Serpin C1				
HtrA serine peptidase 1	HTRA1	> 1	> 1	> 1
ARMD7, HtrA, HTRA, IGFBP5-protease, L56, ORF480, PRSS11, Serine protease 11, Serine protease HTRA1				
Amyloid P component, serum	APCS	> 1	> 1	> 1
9.5S alpha-1-glycoprotein, MGC88159, PTX2, SAP, Serum amyloid P-component				
Intercellular adhesion molecule 1	ICAM1	> 1	> 1	> 1
BB2, CD54, ICAM-1, Intercellular adhesion molecule 1, Major group rhinovirus receptor, P3.58				
Serpin family A member 3	SERPINA3	> 1	> 1	> 1
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3, AACT, ACT, alpha-1-antichymotrypsin, Alpha-1-antichymotrypsin, Cell growth-inhibiting gene 24/25 protein, GIG24, GIG25, MGC88254, Serpin A3				
Serpin family E member 2	SERPINE2	> 1	> 1	> 1
Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2, DKFZp686A13110, GDN, Glia-derived nexin, nexin, Peptidase inhibitor 7, PI7, PI-7, PN1, PN-1, PNI, Protease nexin 1, Protease nexin I, Serpin E2				
RNA exonuclease 2	REXO2	> 1	> 1	> 1
REX2, RNA exonuclease 2 homolog (*S. cerevisiae*), CGI-114, DKFZp566E144, DKFZP566E144, MGC111570, Oligoribonuclease, mitochondrial, REX2, RFN, RNA exonuclease 2 homolog, SFN, Small fragment nuclease, SMFN				
Malectin	MLEC	> 1	> 1	> 1
Oligosaccharyltransferase complex subunit (non-catalytic), KIAA0152				
Glycosylphosphatidylinositol specific phospholipase D1	GPLD1	> 1	> 1	> 1
Glycoprotein phospholipase D, Glycosyl-phosphatidylinositol-specific phospholipase D, GPIPLD, GPI-PLD, GPIPLDM, GPI-specific phospholipase D, MGC22590, Phosphatidylinositol-glycan-specific phospholipase D, PIGPLD, PI-G PLD, PIGPLD1				
Progesterone receptor membrane component 1	PGRMC1	> 1	> 1	> 1
HPR6.6, Membrane-associated progesterone receptor component 1, mPR, MPR, PGRMC				
Vitronectin	VTN	> 1	> 1	> 1
Serum-spreading factor, S-protein, V75, Vitronectin, VN, VNT				
Serpin family A member 1	SERPINA1	> 1	> 1	> 1
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1, A1A, A1AT, AAT, Alpha-1-antiproteinase, alpha-1-antitrypsin, Alpha-1-antitrypsin, alpha1AT, Alpha-1 protease inhibitor, MGC23330, MGC9222, PI, PI1, PRO0684, PRO2209, PRO2275, Serpin A1				
Family with sequence similarity 129, member B	FAM129B	> 1	> 1	> 1
bA356B19.6, C9orf88, DKFZP434H0820, FLJ13518, FLJ22151, FLJ22298, Meg-3, MEG-3, MINERVA, Niban-like protein 1, OC58, Protein FAM129B				
Angiopoietin- like 4	ANGPTL4	> 1	> 1	> 1
Angiopoietin-like protein 4, Angiopoietin-related protein 4, ANGPTL2, ARP4, FIAF, Hepatic fibrinogen/angiopoietin-related protein, HFARP, NL2, PGAR, pp1158, PP1158, PSEC0166, UNQ171/PRO197				
TAR DNA-binding protein	TARDBP	> 1	> 1	> 1
ALS10, TAR DNA-binding protein 43, TDP43, TDP-43				
Fibrillin1	FBN1	> 1	> 1	> 1
FBN, Fibrillin-1, MASS, MFS1, OCTD, SGS, SSKS, WMS				
Prostaglandin-endoperoxide synthase 2	PTGS2	> 1	> 1	> 1
COX2, COX-2, Cyclooxygenase-2, GRIPGHS, hCox-2, PGG/HS, PGHS-2, PGH synthase 2, PHS-2, PHS II, Prostaglandin-endoperoxide synthase 2, Prostaglandin G/H synthase 2, Prostaglandin H2 synthase 2				
Lecithin-cholesterol acyltransferase	LCAT	> 1	> 1	> 1
Lecithin-cholesterol acyltransferase, Phosphatidylcholine-sterol acyltransferase, Phospholipid-cholesterol acyltransferase				
Apolipoprotein B	APOB	> 1	> 1	> 1
Apo B-100, Apolipoprotein B-100, FLDB, LDLCQ4				
Glutaminyl-tRNA synthetase	QARS	> 1	> 1	> 1
GlnRS, GLNRS, Glutamine–tRNA ligase, Glutaminyl-tRNA synthetase, PRO2195				
Sideroflexin 3	SFXN3	> 1	> 1	> 1
BA108L7.2, SFX3, Sideroflexin-3				
Apolipoprotein A1	APOA1	1.872	1.892	1.839
ApoA-I, Apo-AI, Apolipoprotein A-I				
Albumin	ALB	1.652	1.814	1.788
DKFZp779N1935, GIG20, GIG42, PRO0883, PRO0903, PRO1341, PRO1708, PRO2044, PRO2619, PRO2675, Serum albumin, UNQ696/PRO1341				
Haptoglobin	HP	1.578	1.698	1.693
BP, HP2ALPHA2, HPA1S, MGC111141				
Transferrin	TF	1.208	1.482	1.372
Beta-1 metal-binding globulin, DKFZp781D0156, PRO1400, PRO1557, PRO2086, Serotransferrin, Siderophilin, Transferrin				
Transforming growth factor beta induced	TGFBI	1.050	1.080	1.076
Beta ig-h3, BIGH3, CDB1, CDG2, CDGG1, CSD, CSD1, CSD2, CSD3, EBMD, Kerato-epithelin, LCD1, RGD-CAP, RGD-containing collagen-associated protein, Transforming growth factor-beta-induced protein ig-h3				
Superoxide dismutase 2	SOD2	0.870	1.031	1.012
IPOB, MNSOD, MVCD6				
Alanyl aminopeptidase, membrane	ANPEP	0.835	0.921	0.831
Alanyl aminopeptidase, Aminopeptidase M, Aminopeptidase N, AP-M, APN, AP-N, CD13, gp150, GP150, hAPN, LAP1, Microsomal aminopeptidase, Myeloid plasma membrane glycoprotein CD13, p150, P150, PEPN				
Collagen type IV alpha 2 chain	COL4A2	0.764	0.770	0.691
Collagen alpha-2(IV) chain, DKFZp686I14213, FLJ22259				
Fibronectin 1	FN1	0.577	0.616	0.503
CIG, Cold-insoluble globulin, DKFZp686F10164, DKFZp686H0342, DKFZp686I1370, DKFZp686O13149, ED-B, Fibronectin, FINC, FN, FNZ, GFND, GFND2, LETS, MS				
Heparan sulfate proteoglycan 2	HSPG2	0.503	0.613	0.415
Basement membrane-specific heparan sulfate proteoglycan core protein, HSPG, perlecan, Perlecan, PLC, PRCAN, SJA, SJS, SJS1				
Ubiquinol-cytochrome c reductase core protein I	UQCRC1	0.523	0.594	0.656
Complex III subunit 1, Core protein I, Cytochrome b-c1 complex subunit 1, mitochondrial, D3S3191, QCR1, Ubiquinol-cytochrome-c reductase complex core protein 1, UQCR1				
Ubiquinol-cytochrome c reductase core protein II	UQCRC2	0.540	0.581	0.584

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Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
Complex III subunit 2, Core protein II, Cytochrome b-c1 complex subunit 2, mitochondrial, QCR2, Ubiquinol-cytochrome-c reductase complex core protein 2, UQCR2,				
Alpha-2-macroglobulin	A2M	0.673	0.572	0.506
Alpha-2-M, Alpha-2-macroglobulin, C3 and PZP-like alpha-2-macroglobulin domain-containing protein 5, CPAMD5, DKFZp779B086, FWP007, S863-7				
Stomatin like 2	STOML2	0.512	0.560	0.613
Stomatin (EPB72)-like 2, EPB72-like protein 2, HSPC108, SLP2, SLP-2, Stomatin-like protein 2				
Gelsolin	GSN	0.375	0.548	0.514
Actin-depolymerizing factor, ADF, AGEL, Brevin, DKFZp313L0718, Gelsolin				
Collagen type VI alpha 3 chain	COL6A3	0.741	0.543	0.594
Collagen alpha-3(VI) chain, DKFZp686D23123, DKFZp686K04147, DKFZp686N0262, FLJ34702, FLJ98399				
Endoplasmic reticulum oxidoreductase 1 alpha	ERO1A	0.571	0.539	0.587
Endoplasmic oxidoreductin-1-like protein, ERO1A, ERO1-alpha, ERO1-L, ERO1-L-alpha, ERO1-like protein alpha, Oxidoreductin-1-L-alpha, UNQ434/PRO865				
Collagen type VI alpha 2 chain	COL6A2	0.778	0.530	0.590
Collagen alpha-2(VI) chain, DKFZp586E1322, FLJ46862, PP3610				
Voltage dependent anion channel 1	VDAC1	0.454	0.529	0.530
hVDAC1, MGC111064, Outer mitochondrial membrane protein porin 1, Plasmalemmal porin, PORIN, Porin 31HL, Porin 31HM, VDAC, VDAC-1, Voltage-dependent anion-selective channel protein 1				
Prohibitin	PHB	0.498	0.525	0.588
PHB1				
Integrin subunit alpha V	ITGAV	0.562	0.512	0.573
integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51, CD51, DKFZp686A08142, Integrin alpha-V, MSK8, Vitronectin receptor subunit alpha, VNRA				
Prohibitin 2	PHB2	0.489	0.504	0.542
BAP, Bap37, BCAP37, B-cell receptor-associated protein BAP37, D-prohibitin, MGC117268, p22, PNAS-141, Prohibitin-2, REA, Repressor of estrogen receptor activity				
Hexosaminidase subunit beta	HEXB	0.348	0.503	0.504
Beta-hexosaminidase subunit beta, Beta-N-acetylhexosaminidase subunit beta, Cervical cancer proto-oncogene 7 protein, HCC7, HCC-7, Hexosaminidase subunit B, N-acetyl-beta-glucosaminidase subunit beta				
DnaJ heat shock protein family (Hsp40) member B11	DNAJB11	0.406	0.482	0.478
ABBP2, ABBP-2, APOBEC1-binding protein 2, DJ9, DnaJ homolog subfamily B member 11, DnaJ protein homolog 9, EDJ, ER-associated DNAJ, ER-associated dnaJ protein 3, ER-associated Hsp40 co-chaperone, ERdj3, ERj3, ERJ3, ERj3p, hDj9, HDJ9, hDj-9, HEDJ, Human DnaJ protein 9, PRO1080, PSEC0121, PWP1-interacting protein 4, UNQ537, UNQ537/PRO1080				
Fumarate hydratase	FH	0.390	0.475	0.460
Fumarase, Fumarase, Fumarate hydratase, mitochondrial, HLRCC, LRCC, MCL, MCUL1				
ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	ATP5C1	0.405	0.468	0.485
ATP5C, ATP5CL1, ATP synthase subunit gamma, mitochondrial, F-ATPase gamma subunit				
Enoyl CoA hydratase 1	ECH1	0.397	0.456	0.468
Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial, HPXEL				
dihydrolipoamide S-succinyltransferase	DLST	0.403	0.455	0.488
2-oxoglutarate dehydrogenase complex component E2, Dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex, Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial, DLTS, E2K, OGDC-E2				
ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	ATP5O	0.418	0.453	0.435
ATPO, ATP synthase subunit O, mitochondrial, Oligomycin sensitivity conferral protein, OSCP				
Histone cluster 1 H2B family member l	HIST1H2BL	0.337	0.436	0.354
dJ97D16.4, H2B/c, H2BFC, Histone H2B.c, Histone H2B type 1-L				
Collagen type VI alpha 1 chain	COL6A1	0.676	0.433	0.510
Collagen alpha-1(VI) chain				
Acetyl-CoA acyltransferase 2	ACAA2	0.243	0.428	0.295
3-ketoacyl-CoA thiolase, mitochondrial, Acetyl-CoA acyltransferase, Beta-ketothiolase, DSAEC, FLJ35992, FLJ95265, Mitochondrial 3-oxoacyl-CoA thiolase, T1				
Malate dehydrogenase 2	MDH2	0.332	0.426	0.422
Malate dehydrogenase, mitochondrial, MDH, MGC:3559, M-MDH, MOR1, malate dehydrogenase 2, NAD (mitochondrial)				
ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	ATP5B	0.360	0.425	0.456
ATPMB, ATPSB, ATP synthase subunit beta, mitochondrial, MGC5231				
Histone cluster 1 H4 family member a	HIST1H4A	0.413	0.419	0.345
Histone cluster 1, H4a, H4/a, H4FA, HIST1H4B, HIST1H4C, HIST1H4D, HIST1H4E, HIST1H4F, HIST1H4H, HIST1H4I, HIST1H4J, HIST1H4K, HIST1H4L, HIST2H4A, HIST2H4B, HIST4H4				
Charged multivesicular body protein 4B	CHMP4B	0.359	0.414	< −1
Chromatin modifying protein 4B, C20orf178, Charged multivesicular body protein 4b, CHMP4A, CHMP4b, Chromatin-modifying protein 4b, CTPP3, dJ553F4.4, hSnf7-2, hVps32-2, Shax1, SHAX1, SNF7, SNF7-2, SNF7 homolog associated with Alix 1, Vacuolar protein sorting-associated protein 32-2, Vps32-2, VPS32B				
Histone cluster 2 H2B family member e	HIST2H2BE	0.366	0.407	0.315
Histone cluster 2, H2be, GL105, H2B, H2B/q, H2B.1, H2BFQ, H2BGL105, H2BQ, Histone H2B.q, Histone H2B-GL105, Histone H2B type 2-E, MGC119802, MGC119804, MGC129733, MGC129734				
Integrin subunit alpha 2	ITGA2	0.520	0.404	0.511
Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor), BR, CD49 antigen-like family member B, CD49b, CD49B, Collagen receptor, GPIa, Integrin alpha-2, Platelet membrane glycoprotein Ia, VLA-2, VLA-2 subunit alpha, VLAA2				
5′-nucleotidase ecto	NT5E	0.407	0.400	0.431
5′-NT, 5′-nucleotidase, CD73, E5NT, Ecto-5′-nucleotidase, eN, eNT, NT, NT5, NTE				
Brain abundant membrane attached signal protein 1	BASP1	0.305	0.399	0.358
22 kDa neuronal tissue-enriched acidic protein, Brain acid soluble protein 1, CAP23, CAP-23, MGC8555, NAP22, NAP-22, Neuronal axonal membrane protein NAP-22				
Glutamic-oxaloacetic transaminase 2	GOT2	0.267	0.399	0.418
Aspartate aminotransferase, mitochondrial, FABP-1, FABPpm, Fatty acid-binding protein, FLJ40994, Glutamate oxaloacetate transaminase 2, KAT4, KATIV, mAspAT, mitAAT, Plasma membrane-associated fatty acid-binding protein, Transaminase A				
Glutamate dehydrogenase 1	GLUD1	0.338	0.397	0.420
GDH, GDH1, GDH 1, GLUD, Glutamate dehydrogenase 1, mitochondrial, MGC132003				
Keratin 9	KRT9	0.196	0.393	0.241
CK-9, Cytokeratin-9, EPPK, K9, Keratin, type I cytoskeletal 9, Keratin-9				

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Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
Microtubule associated protein 9	MAP9	< −1	0.392	0.172
ASAP, Aster-associated protein, FLJ21159, Microtubule-associated protein 9				
Crystallin alpha B	CRYAB	0.270	0.386	0.476
Alpha(B)-crystallin, Alpha-crystallin B chain, CRYA2, CTPP2, Heat shock protein beta-5, HspB5, HSPB5, Renal carcinoma antigen NY-REN-27, Rosenthal fiber component				
Transmembrane p24 trafficking protein 9	TMED9	0.432	0.385	0.455
Glycoprotein 25L2, GP25L2, HSGP25L2G, Transmembrane emp24 domain-containing protein 9				
Nucleobindin 1	NUCB1	0.231	0.382	0.373
CALNUC, DKFZp686A15286, FLJ40471, NUC, Nucleobindin-1				
Signal sequence receptor subunit 4	SSR4	0.362	0.378	0.437
Signal sequence receptor, delta (translocon-associated protein delta), Signal sequence receptor subunit delta, SSR-delta, Translocon-associated protein subunit delta, TRAPD, TRAP-delta				
histone cluster 1 H2A family member j	HIST1H2AJ	0.466	0.365	0.310
Histone cluster 1, H2aj, dJ160A22.4, H2A/E, H2AFE, Histone H2A/e, Histone H2A type 1-J				
Lysosome associated membrane protein 1	LAMP1	0.352	0.364	0.400
CD107a, CD107 antigen-like family member A, LAMP-1, LAMPA, LGP120, Lysosome-associated membrane glycoprotein 1, Lysosome-associated membrane protein 1				
Phosphoglycerate kinase 1	PGK1	0.260	0.355	0.273
Cell migration-inducing gene 10 protein, MGC117307, MGC142128, MGC8947, MIG10, OK/SW-cl.110, PGKA, Phosphoglycerate kinase 1, Primer recognition protein 2, PRP 2				
Major histocompatibility complex, class I, A	HLA-A	0.305	0.353	0.305
HLA class I histocompatibility antigen, A-24 alpha chain (Aw-24) (HLA class I histocompatibility antigen, A-9 alpha chain) (MHC class I antigen A*24)				
Heat shock protein family E (Hsp10) member 1	HSPE1	0.281	0.351	0.346
10 kDa chaperonin, 10 kDa heat shock protein, mitochondrial, Chaperonin 10, CPN10, Early-pregnancy factor, EPF, GROES, Hsp10, HSP10				
Pyrroline-5-carboxylate reductase 1	PYCR1	0.307	0.350	0.347
ARCL2B, P5C, P5CR, P5CR 1, P5C reductase 1, PIG45, PP222, PRO3, PYCR, Pyrroline-5-carboxylate reductase 1, mitochondrial				
Proteasome 26S subunit, non-ATPase 14	PSMD14	0.308	0.348	0.323
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 14, 26S proteasome-associated PAD1 homolog 1, 26S proteasome non-ATPase regulatory subunit 14, 26S proteasome regulatory subunit RPN11, pad1, PAD1, POH1, Rpn11, RPN11				
Non-POU domain containing octamer binding	NONO	0.287	0.347	0.386
54 kDa nuclear RNA-and DNA-binding protein, 55 kDa nuclear protein, DNA-binding p52/p100 complex, 52 kDa subunit, NMT55, NonO protein, Non-POU domain-containing octamer-binding protein, NRB54, P54, p54(nrb), p54nrb, P54NRB				
High mobility group AT-hook 1	HMGA1	0.192	0.345	0.203
High mobility group AT-hook protein 1, High mobility group protein A1, High mobility group protein HMG-I/HMG-Y, High mobility group protein R, HMGA1A, HMG-I(Y), HMGIY, HMG-R, MGC12816, MGC4242, MGC4854				
Transmembrane p24 trafficking protein 10	TMED10	0.410	0.342	0.329
21 kDa transmembrane-trafficking protein, p23, P24(DELTA), p24delta, S31I125, S31III125, TMP21, Tmp-21-I, Transmembrane emp24 domain-containing protein 10, Transmembrane protein Tmp21				
Fibroblast activation protein alpha	FAP	0.384	0.336	0.307
170 kDa melanoma membrane-bound gelatinase, DKFZp686G13158, DPPIV, FAPA, Fibroblast activation protein alpha, Integral membrane serine protease, Seprase				
Prolyl 4-hydroxylase subunit alpha 1	P4HA1	0.329	0.334	0.352
4-PH alpha-1, C-P4Halpha(I), P4HA, Procollagen-proline,2-oxoglutarate-4-dioxygenase subunit alpha-1, Prolyl 4-hydroxylase subunit alpha-1, prolyl 4-hydroxylase, alpha polypeptide I				
Complement C1q binding protein	C1QBP	0.201	0.331	0.337
Complement component 1, q subcomponent binding protein, C1qBP, Complement component 1 Q subcomponent-binding protein, mitochondrial, GC1QBP, gC1qR, gC1Q-R, GC1q-R protein, Glycoprotein gC1qBP, HABP1, Hyaluronan-binding protein 1, Mitochondrial matrix protein p32, p32, p33, SF2p32, SF2P32				
ER lipid raft associated 2	ERLIN2	0.339	0.330	0.403
C8orf2, Endoplasmic reticulum lipid raft-associated protein 2, Erlin-2, MGC87072, NET32, SPFH2, SPFH domain-containing protein 2, Stomatin-prohibitin-flotillin-HflC/K domain-containing protein 2, UNQ2441/PRO5003/PRO9924				
Signal sequence receptor subunit 1	SSR1	0.416	0.327	0.369
Signal sequence receptor, alpha, DKFZp781N23103, FLJ14232, FLJ22100, FLJ23034, FLJ78242, FLJ93042, PSEC0262, Signal sequence receptor subunit alpha, SSR-alpha, Translocon-associated protein subunit alpha, TRAPA, TRAP-alpha				
Tropomodulin 3	TMOD3	0.136	0.320	0.281
Tropomodulin-3, Ubiquitous tropomodulin, UTMOD, U-Tmod				
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	PLOD2	0.372	0.320	0.375
LH2, Lysyl hydroxylase 2, Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2, TLH				
Major vault protein	MVP	0.273	0.313	0.361
LRP, Lung resistance-related protein, Major vault protein, VAULT1				
Thrombospondin 1	THBS1	0.342	0.305	0.302
THBS, THBS-1, Thrombospondin-1, TSP, TSP1, TSP-1				
Heat shock protein family D (Hsp60) member 1	HSPD1	0.281	0.301	0.334
60 kDa chaperonin, 60 kDa heat shock protein, mitochondrial, Chaperonin 60, CPN60, GROEL, Heat shock protein 60, HLD4, Hsp60, HSP60, HSP-60, HSP65, HuCHA60, Mitochondrial matrix protein P1, P60 lymphocyte protein, SPG13				
IKBKB interacting protein	IKBIP	0.363	0.299	0.321
Inhibitor of nuclear factor kappa-B kinase-interacting protein (I kappa-B kinase-interacting protein) (IKBKB-interacting protein) (IKK-interacting protein)				
ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	ATP5A1	0.290	0.290	0.312
ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle, ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 2, non-cardiac muscle-like 2, ATP5AL2, ATPM				
ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1	ATP5F1	0.422	0.287	0.337
ATP synthase F(0) complex subunit B1, mitochondrial (ATP synthase proton-transporting mitochondrial F(0) complex subunit B1) (ATP synthase subunit b) (ATPase subunit b)				
Atlastin GTPase 3	ATL3	0.260	0.272	0.321
Atlastin 3, DKFZP564J0863				
Basigin (Ok blood group)	BSG	0.286	0.266	0.302
5F7, Basigin, CD147, Collagenase stimulatory factor, EMMPRIN, Extracellular matrix metalloproteinase inducer, Leukocyte activation antigen M6, M6, OK, OK blood group antigen, TCSF, Tumor cell-derived collagenase stimulatory factor, UNQ6505/PRO21383				
Adipocyte plasma membrane-associated protein	APMAP	0.293	0.264	0.333

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Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
Chromosome 20 open reading frame 3, C20orf3, BSCv, Protein BSCv, UNQ1869/PRO4305				
H2A histone family member Y	H2AFY	0.363	0.263	0.307
Core histone macro-H2A.1, H2A/y, H2A.y, H2AF12M, H2AFJ, Histone H2A.y, Histone macroH2A1, MACROH2A1, MACROH2A1.1, macroH2A1.2, Medulloblastoma antigen MU-MB-50.205, mH2A1				
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	PLOD1	0.304	0.260	0.324
FLJ42041, LH, LH1, LLH, Lysyl hydroxylase 1, PLOD, Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1				
Cytochrome c oxidase subunit 4I1	COX4I1	0.309	0.246	0.339
COX4, COX4-1, COXIV, COX IV-1, Cytochrome c oxidase polypeptide IV, Cytochrome c oxidase subunit 4 isoform 1, mitochondrial, Cytochrome c oxidase subunit IV isoform 1, FLJ23483, MGC72016				
Aspartyl-tRNA synthetase	DARS	0.261	0.205	0.329
Aspartate–tRNA ligase, Aspartyl-tRNA synthetase, cytoplasmic, AspRS, Cell proliferation-inducing gene 40 protein, DKFZp781B11202, MGC111579, PIG40				
Integrin subunit alpha 5	ITGA5	0.316	0.183	0.268
CD49 antigen-like family member E, CD49e, Fibronectin receptor subunit alpha, FNRA, Integrin alpha-5, Integrin alpha-F, VLA-5, VLA5A				
Eukaryotic translation initiation factor 2 subunit beta	EIF2S2	< −1	−0.119	< −1
DKFZp686L18198, EIF2, EIF2B, EIF2beta, eIF-2-beta, Eukaryotic translation initiation factor 2 subunit 2, Eukaryotic translation initiation factor 2 subunit beta, MGC8508				
Bleomycin hydrolase	BLMH	−0.338	−0.155	< −1
BH, Bleomycin hydrolase, BLM hydrolase, BMH				
FK506 binding protein 1A	FKBP1A	< −1	−0.162	−0.324
12 kDa FK506-binding protein, 12 kDa FKBP, FK506-binding protein 1A, FKBP1, FKBP12, FKBP-12, FKBP12C, FKBP-1A, Immunophilin FKBP12, Peptidyl-prolyl cis-trans isomerase FKBP1A, PKC12, PKCI2, PPIASE, PPIase FKBP1A, Rotamase				
SERPINE1 mRNA binding protein 1	SERBP1	−0.264	−0.170	−0.324
CGI-55, CHD3IP, DKFZp564M2423, DKFZP564M2423, FLJ90489, HABP4L, PAI1 RNA-binding protein 1, PAIRBP1, PAI-RBP1, Plasminogen activator inhibitor 1 RNA-binding protein, SERPINE1 mRNA-binding protein 1				
Y-box binding protein 3	YBX3	−0.264	−0.174	−0.385
Cold-shock domain containing A1, CSDA1, dbpA, ZONAB				
A-kinase anchoring protein 12	AKAP12	−0.386	−0.186	−0.426
A kinase (PRKA) anchor protein 12, AKAP-12, AKAP250, AKAP 250, A-kinase anchor protein 12, A-kinase anchor protein 250 kDa, DKFZp686M0430, DKFZp686O0331, FLJ20945, FLJ97621, Gravin, Myasthenia gravis autoantigen				
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon	YWHAE	−0.346	−0.191	−0.321
14-3-3E, 14-3-3 protein epsilon, FLJ45465, FLJ53559, KCIP-1, MDCR, MDS				
Stress induced phosphoprotein 1	STIP1	−0.247	−0.196	−0.307
Hop, HOP, Hsc70/Hsp90-organizing protein, IEF-SSP-3521, P60, Renal carcinoma antigen NY-REN-11, STI1, STI1L, Stress-induced-phosphoprotein 1, Transformation-sensitive protein IEF SSP 3521				
Zyxin	ZYX	−0.285	−0.206	−0.339
ESP-2, HED-2, Zyxin, Zyxin-2				
Diazepam binding inhibitor, acyl-CoA binding protein	DBI	−0.483	−0.212	−0.445
Diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein), ACBD1, ACBP, Acyl-CoA-binding protein, CCK-RP, Diazepam-binding inhibitor, Endozepine, EP, MGC70414				
Myosin light chain 9	MYL9	−0.304	−0.216	−0.333
20 kDa myosin light chain, LC20, MGC3505, MLC2, MLC-2C, MRLC1, Myosin regulatory light chain 2, smooth muscle isoform, Myosin regulatory light chain 9, Myosin regulatory light chain MRLC1, Myosin regulatory light polypeptide 9, Myosin RLC, MYRL2				
Keratin 7	KRT7	−0.305	−0.217	−0.307
CK7, CK-7, Cytokeratin-7, K2C7, K7, Keratin, type II cytoskeletal 7, Keratin-7, MGC129731, MGC3625, Sarcolectin, SCL, Type-II keratin Kb7				
Galectin 3	LGALS3	< −1	−0.218	< −1
Lectin, galactoside-binding, soluble, 3, 35 kDa lectin, Carbohydrate-binding protein 35, CBP35, CBP 35, GAL3, Gal-3, Galactose-specific lectin 3, Galactoside-binding protein, GALBP, Galectin-3, GALIG, IgE-binding protein, L31, L-31, Laminin-binding protein, Lectin L-29, LGALS2, MAC2, MAC-2, Mac-2 antigen				
Calmodulin 1	CALM1	−0.321	−0.223	−0.342
Calmodulin 1 (phosphorylase kinase, delta), CALM2, CALM3, CALML2, caM, CAMI, DD132, PHKD				
Myotrophin	MTPN	< −1	−0.226	< −1
FLJ31098, FLJ99857, GCDP, Myotrophin, MYOTROPHIN, Protein V-1, V-1				
Tropomyosin 4	TPM4	−0.329	−0.232	−0.312
TM30p1, Tropomyosin-4, Tropomyosin alpha-4 chain				
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	YWHAZ	−0.326	−0.235	−0.286
14-3-3 protein zeta/delta, 14-3-3-zeta, KCIP-1, MGC111427, MGC126532, MGC138156, Protein kinase C inhibitor protein 1, YWHAD				
Malate dehydrogenase1	MDH1	−0.258	−0.244	−0.320
Malate dehydrogenase 1, NAD (soluble), Cytosolic malate dehydrogenase, Malate dehydrogenase, cytoplasmic, MDHA, MDH-s, MGC:1375, MOR2				
Microtubule associated protein 4	MAP4	−0.290	−0.247	−0.418
DKFZp779A1753, MAP-4, MGC8617, Microtubule-associated protein 4				
Parkinsonism associated deglycase	PARK7	< −1	−0.260	−0.339
Parkinson disease (autosomal recessive, early onset) 7, DJ1, DJ-1, FLJ27376, FLJ34360, FLJ92274, Oncogene DJ1, Parkinson disease protein 7, Protein DJ-1				
Cysteine and glycine rich protein 1	CSRP1	−0.297	−0.263	−0.319
CRP, CRP1, CSRP, CYRP, Cysteine and glycine-rich protein 1, Cysteine-rich protein 1, D1S181E, DKFZp686M148				
Actinin alpha 4	ACTN4	−0.344	−0.280	−0.321
ACTININ-4, Alpha-actinin-4, DKFZp686K23158, F-actin cross-linking protein, FSGS, FSGS1, Non-muscle alpha-actinin 4				
Pyrophosphatase (inorganic) 1	PPA1	−0.304	−0.290	−0.388
Inorganic pyrophosphatase, IOPPP, MGC111556, PP, PP1, Ppase, PPase, Pyrophosphate phospho-hydrolase, SID6-8061				
Alpha fetoprotein	AFP	−0.457	−0.293	−0.395
Alpha-1-fetoprotein, Alpha-fetoglobulin, Alpha-fetoprotein, FETA, HPAFP				
AHNAK nucleoprotein	AHNAK	−0.426	−0.299	−0.411
AHNAKRS, Desmoyokin, MGC5395, Neuroblast differentiation-associated protein AHNAK, PM227				
Plastin 3	PLS3	−0.319	−0.300	−0.315
Plastin-3, T-plastin				
Glycine-tRNA synthetase	GARS	−0.261	−0.301	−0.229

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Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
AP-4-A synthetase, CMT2D, Diadenosine tetraphosphate synthetase, DSMAV, Glycine–tRNA ligase, Glycyl-tRNA synthetase, GlyRS, HMN5, SMAD1				
Filamin C	FLNC	−0.269	−0.301	−0.298
ABP-280, ABP280A, ABP-280-like protein, ABPA, ABPL, ABP-L, Actin-binding-like protein, Filamin-2, Filamin-C, FLJ10186, FLN2, FLNc, FLN-C, Gamma-filamin, filamin C, gamma				
Ribonuclease/angiogenin inhibitor 1	RNH1	−0.142	−0.304	−0.204
MGC18200, MGC4569, MGC54054, Placental ribonuclease inhibitor, Placental RNase inhibitor, PRI, RAI, Ribonuclease/angiogenin inhibitor 1, Ribonuclease inhibitor, RNH				
Calpain 2	CAPN2	−0.183	−0.304	−0.183
Calcium-activated neutral proteinase 2, Calpain-2 catalytic subunit, Calpain-2 large subunit, Calpain large polypeptide L2, Calpain M-type, CANP2, CANP 2, CANPL2, CANPml, FLJ39928, M-calpain, mCANP, Millimolar-calpain				
PDZ and LIM domain 3	PDLIM3	−0.497	−0.305	−0.712
Actinin-associated LIM protein, ALP, Alpha-actinin-2-associated LIM protein, DKFZp686L0362, FLJ26096, PDZ and LIM domain protein 3				
Ribosomal protein L10a	RPL10A	−0.224	−0.307	−0.247
60S ribosomal protein L10a, Csa-19, CSA-19, L10A, NEDD6, NEDD-6, Neural precursor cell expressed developmentally down-regulated protein 6				
Solute carrier family 3 member 2	SLC3A2	−0.119	−0.312	−0.191
Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2, 4F2, 4F2 cell-surface antigen heavy chain, 4F2hc, 4F2HC, 4F2 heavy chain antigen, 4T2HC, CD98, CD98HC, Lymphocyte activation antigen 4F2 large subunit, MDU1, NACAE				
Caldesmon 1	CALD1	−0.375	−0.313	−0.488
CAD, Caldesmon, CDM, HCAD, H-CAD, LCAD, L-CAD, MGC21352, NAG22				
GDP dissociation inhibitor 2	GDI2	−0.486	−0.315	−0.396
FLJ16452, FLJ37352, GDI-2, Guanosine diphosphate dissociation inhibitor 2, RABGDIB, Rab GDI beta, Rab GDP dissociation inhibitor beta				
Lactate dehydrogenase B	LDHB	−0.270	−0.316	−0.308
LDH-B, LDH-H, LDH heart subunit, L-lactate dehydrogenase B chain, Renal carcinoma antigen NY-REN-46, TRG-5				
Filamin A	FLNA	−0.300	−0.318	−0.333
ABP-280, ABPX, Actin-binding protein 280, Alpha-filamin, CVD1, DKFZp434P031, Endothelial actin-binding protein, Filamin-1, Filamin-A, FLN, FLN1, FLN-A, FMD, MNS, NHBP, Non-muscle filamin, OPD, OPD1, OPD2, XLVD, XMVD				
Branched chain amino acid transaminase 1	BCAT1	−0.232	−0.325	−0.350
BCAT(c), BCATC, BCT1, Branched-chain-amino-acid aminotransferase, cytosolic, DKFZp686E12175, ECA39, MECA39, PNAS121, PP18, Protein ECA39				
Alanine-tRNA synthetase	AARS	−0.173	−0.326	−0.255
Alanine–tRNA ligase, Alanyl-tRNA synthetase, cytoplasmic, AlaRS, Renal carcinoma antigen NY-REN-42				
Phosphoglucomutase 1	PGM1	−0.282	−0.329	−0.299
Glucose phosphomutase 1, PGM 1, Phosphoglucomutase-1				
Citrate synthase	CS	−0.099	−0.334	−0.181
Citrate synthase, mitochondrial				
Reticulon 4	RTN4	−0.120	−0.335	−0.335
ASY, Foocen, KIAA0886, My043, Nbla00271, Nbla10545, Neurite outgrowth inhibitor, Neuroendocrine-specific protein, Neuroendocrine-specific protein C homolog, NI220/250, NOGO, NOGO-A, Nogo-B, NOGOC, Nogo-C, Nogo protein, NSP, NSP-CL, Reticulon-4, Reticulon-5, RTN4-A, RTN4-B1, RTN4-B2, RTN4-C, RTN-x, RTN-X, SP1507				
Eukaryotic translation initiation factor 3 subunit D	EIF3D	−0.221	−0.337	−0.218
eIF3d, eIF3 p66, eIF3-p66, EIF3S7, eIF3-zeta, eIF-3-zeta, Eukaryotic translation initiation factor 3 subunit 7, Eukaryotic translation initiation factor 3 subunit D, MGC126526, MGC17258				
Importin 5	IPO5	−0.199	−0.337	−0.332
DKFZp686O1576, FLJ43041, IMB3, Imp5, Importin-5, Importin subunit beta-3, Karyopherin beta-3, KPNB3, MGC2068, Ran-binding protein 5, RanBP5, RANBP5				
Fermitin family member 2	FERMT2	−0.278	−0.338	−0.253
DKFZp686G11125, Fermitin family homolog 2, FLJ34213, FLJ44462, KIND2, Kindlin-2, MIG2, mig-2, MIG-2, Mitogen-inducible gene 2 protein, PH domain-containing family C member 1, Pleckstrin homology domain-containing family C member 1, PLEKHC1, UNC112, UNC112B				
Peroxiredoxin 6	PRDX6	−0.359	−0.343	−0.346
1-Cys, 1-Cys peroxiredoxin, 1-Cys PRX, 24 kDa protein, Acidic calcium-independent phospholipase A2, aiPLA2, Antioxidant protein 2, AOP2, KIAA0106, Liver 2D page spot 40, MGC46173, Non-selenium glutathione peroxidase, NSGPx, p29, Peroxiredoxin-6, PRX, Red blood cells page spot 12				
Asparagine-tRNA synthetase	NARS	−0.353	−0.343	−0.309
AsnRS, ASNRS, Asparagine–tRNA ligase, Asparaginyl-tRNA synthetase, cytoplasmic, NARS1				
Thioredoxin reductase 1	TXNRD1	−0.329	−0.355	−0.288
Gene associated with retinoic and IFN-induced mortality 12 protein, Gene associated with retinoic and interferon-induced mortality 12 protein, GRIM12, GRIM-12, KDRF, KM-102-derived reductase-like factor, MGC9145, Thioredoxin reductase 1, cytoplasmic, Thioredoxin reductase TR1, TR, TR1, Trxr1, TRXR1, TXNR				
Ezrin	EZR	−0.300	−0.356	−0.344
CVIL, CVL, Cytovillin, DKFZp762H157, Ezrin, FLJ26216, MGC1584, p81, VIL2, Villin-2				
Thioredoxin	TXN	−0.413	−0.364	−0.407
ADF, ATL-derived factor, DKFZp686B1993, MGC61975, SASP, Surface-associated sulphydryl protein, Thioredoxin, TRDX, Trx, TRX, TRX1				
Tu translation elongation factor, mitochondrial	TUFM	−0.181	−0.364	−0.357
COXPD4, EFTu, EFTU, EF-Tu, EF-TuMT, Elongation factor Tu, mitochondrial, P43				
Cell division cycle 37	CDC37	< −1	−0.365	< −1
CDC37A, Hsp90 chaperone protein kinase-targeting subunit, Hsp90 co-chaperone Cdc37, p50Cdc37, P50CDC37				
Keratin 8	KRT8	−0.434	−0.365	−0.444
CARD2, CK8, CK-8, CYK8, Cytokeratin-8, K2C8, K8, Keratin, type II cytoskeletal 8, Keratin-8, KO, Type-II keratin Kb8				
Keratin 18	KRT18	−0.437	−0.367	−0.399
Cell proliferation-inducing gene 46 protein, CK-18, CYK18, Cytokeratin-18, K18, Keratin, type I cytoskeletal 18, Keratin-18, PIG46				
Eukaryotic translation initiation factor 3 subunit C	EIF3C	−0.314	−0.374	−0.280
eIF3c, EIF3CL, eIF3-p110, EIF3S8, FLJ53378, FLJ54400, FLJ54404, FLJ55450, FLJ55750, FLJ78287, MGC189737, MGC189744				
Spermidine synthase	SRM	−0.236	−0.375	−0.356
PAPT, Putrescine aminopropyltransferase, SPDSY, Spermidine synthase, SPS1, SRML1				
Pentraxin 3	PTX3	−0.284	−0.379	−0.224
Pentaxin-related protein PTX3, Pentraxin-related protein PTX3, TNFAIP5, TNF alpha-induced protein 5, TSG14, TSG-14, Tumor necrosis factor alpha-induced protein 5, Tumor necrosis factor-inducible gene 14 protein				
Collagen type I alpha 1 chain	COL1A1	−0.468	−0.387	−0.441
Alpha-1 type I collagen, Collagen alpha-1(I) chain, OI4, collagen, type I, alpha 1				
Pro-apoptotic WT1 regulator	PAWR	< −1	−0.400	< −1

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Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
PAR4, par-4, Par-4, PRKC apoptosis WT1 regulator protein, Prostate apoptosis response 4 protein				
LIM domain and actin binding 1	LIMA1	−0.266	−0.400	−0.610
Epithelial protein lost in neoplasm, EPLIN, FLJ38853, LIM domain and actin-binding protein 1, MGC131726, PP624, SREBP3				
Transgelin	TAGLN	−0.561	−0.408	−0.553
22 kDa actin-binding protein, DKFZp686B01212, DKFZp686P11128, Protein WS3-10, SM22, SM22-alpha, SMCC, Smooth muscle protein 22-alpha, TAGLN1, Transgelin, WS3-10				
Programmed cell death 5	PDCD5	−0.479	−0.415	−0.564
FLJ42784, MGC9294, Programmed cell death protein 5, Protein TFAR19, TF-1 cell apoptosis-related protein 19, TFAR19				
EH domain containing 2	EHD2	−0.283	−0.422	< −1
EH-domain-containing protein 2, FLJ96617, PAST2, PAST homolog 2				
Histidyl-tRNA synthetase	HARS	< −1	−0.444	< −1
FLJ20491, HisRS, Histidine–tRNA ligase, Histidyl-tRNA synthetase, cytoplasmic, HRS				
Phosphatidylethanolamine binding protein 1	PEBP1	−0.574	−0.457	−0.595
HCNP, HCNPpp, Neuropolypeptide h3, PBP, PEBP, PEBP-1, Phosphatidylethanolamine-binding protein 1, Prostatic-binding protein, Raf kinase inhibitor protein, RKIP				
LIM and SH3 protein 1	LASP1	−0.435	−0.460	−0.606
Lasp-1, LASP-1, LIM and SH3 domain protein 1, Metastatic lymph node gene 50 protein, MLN50, MLN 50				
PDZ and LIM domain 5	PDLIM5	−0.481	−0.460	−0.520
Enh, ENH, ENH1, Enigma homolog, Enigma-like PDZ and LIM domains protein, L9, LIM, PDZ and LIM domain protein 5				
Catenin alpha 1	CTNNA1	−0.325	−0.470	−0.380
Catenin (cadherin-associated protein), alpha 1, 102 kDa, Alpha E-catenin, Cadherin-associated protein, CAP102, Catenin alpha-1, FLJ36832, FLJ52416, Renal carcinoma antigen NY-REN-13				
Tumor protein D54 like 2	TPD52L2	−0.469	−0.481	−0.542
D54, DKFZp686A1765, hD54, Tumor protein D52-like 2, Tumor protein D54				
Heat shock protein family B (small) member 6	HSPB6	−0.356	−0.486	−0.415
Heat shock protein, alpha-crystallin-related, B6, FLJ32389, Heat shock 20 kDa-like protein p20, Heat shock protein beta-6, Hsp20, HspB6				
Eukaryotic translation initiation factor 4 gamma 1	EIF4G1	−0.411	−0.487	< −1
DKFZp686A1451, EIF4F, EIF4G, eIF-4G1, EIF-4G1, eIF-4G 1, eIF-4-gamma 1, EIF4GI, Eukaryotic translation initiation factor 4 gamma 1, p220, P220				
LIM domain 7	LMO7	< −1	−0.494	< −1
F-box only protein 20, FBX20, FBXO20, KIAA0858, LIM domain only protein 7, LMO-7, LOMP				
Ribosome binding protein 1	RRBP1	−0.532	−0.498	−0.646
Ribosome binding protein 1 homolog 180 kDa (dog), 180 kDa ribosome receptor homolog, DKFZp586A1420, ES/130, ES/130-related protein, ES130, FLJ36146, hES, KIAA1398, MGC157720, MGC157721, Ribosome-binding protein 1, Ribosome receptor protein, RRp				
Thioredoxin domain containing 17	TXNDC17	< −1	−0.521	< −1
14 kDa thioredoxin-related protein, MGC14353, Protein 42-9-9, Thioredoxin domain-containing protein 17, Thioredoxin-like protein 5, TRP14, TXNL5				
Tubulin folding cofactor A	TBCA	< −1	−0.547	< −1
CFA, TCP1-chaperonin cofactor A, Tubulin-folding cofactor A, Tubulin-specific chaperone A				
Phosphoserine aminotransferase 1	PSAT1	−0.625	−0.641	−0.430
EPIP, MGC1460, Phosphohydroxythreonine aminotransferase, Phosphoserine aminotransferase, PSA, PSAT				
Asparagine synthetase (glutamine-hydrolyzing)	ASNS	−0.622	−0.707	< −1
Cell cycle control protein TS11, Glutamine-dependent asparagine synthetase, TS11				
Glutaminase	GLS	−0.434	−0.722	−0.587
AAD20, DKFZp686O15119, FLJ10358, GLS1, Glutaminase kidney isoform, mitochondrial, K-glutaminase, KIAA0838, L-glutamine amidohydrolase				
Calponin 1	CNN1	−1.106	−0.982	−1.022
Calponin 1, basic, smooth muscle, Basic calponin, Calponin-1, Calponin H1, smooth muscle, Sm-Calp, SMCC				
Apolipoprotein A2	APOA2	−1.336	−1.176	−1.202
ApoAII, ApoA-II, Apo-AII, Apolipoprotein A2, Apolipoprotein A-II				
Keratin 19	KRT19	−1.214	−1.246	< −1
CK19, CK-19, Cytokeratin-19, K19, K1CS, Keratin, type I cytoskeletal 19, Keratin-19, MGC15366				
Hemoglobin subunit alpha 1	HBA1	−1.225	−1.325	−1.284
CD31, MGC126895, MGC126897				
Transmembrane p24 trafficking protein 7	TMED7	0.451	< −1	0.533
CGI-109, FLJ57776, FLJ90481, Transmembrane emp24 domain-containing protein 7				
GDP dissociation inhibitor 1	GDI1	< −1	< −1	< −1
FLJ41411, GDI-1, GDIL, Guanosine diphosphate dissociation inhibitor 1, MRX41, MRX48, Oligophrenin-2, OPHN2, Protein XAP-4, RABGD1A, RABGDIA, Rab GDI alpha, Rab GDP dissociation inhibitor alpha, XAP4, XAP-4				
Aldehyde dehydrogenase 18 family member A1	ALDH18A1	−0.071	< −1	−0.350
Aldehyde dehydrogenase family 18 member A1, Delta-1-pyrroline-5-carboxylate synthase, GSAS, MGC117316, P5CS, PYCS				
Cystatin B	CSTB	< −1	< −1	< −1
CPI-B, CST6, Cystatin-B, EPM1, Liver thiol proteinase inhibitor, PME, Stefin-B, STFB				
Aldehyde dehydrogenase 1 family member L2	ALDH1L2	< −1	< −1	< −1
Aldehyde dehydrogenase family 1 member L2, DKFZp686A16126, DKFZp686M064, DKFZp686P14145, FLJ36769, FLJ38508, MGC119536, MGC119537, mtFDH				
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta	YWHAB	−0.441	< −1	−0.346
14-3-3 protein beta/alpha, GW128, HS1, KCIP-1, Protein 1054, Protein kinase C inhibitor protein 1, YWHAA				
Lactotransferrin	LTF	< −1	< −1	< −1
GIG12, HLF2, Lactoferrin, Lactotransferrin, LF, Talalactoferrin				
Calpain 1	CAPN1	−0.495	< −1	−0.537
Calcium-activated neutral proteinase 1, Calpain-1 catalytic subunit, Calpain-1 large subunit, Calpain mu-type, CANP, CANP1, CANP 1, CANPL1, Cell proliferation-inducing gene 30 protein, Micromolar-calpain, muCANP, muCL, PIG30				
Latexin	LXN	< −1	< −1	−0.706
ECI, Endogenous carboxypeptidase inhibitor, Latexin, Protein MUM, TCI, Tissue carboxypeptidase inhibitor				
Four and a half LIM domains 1	FHL1	−0.432	< −1	−0.519
bA535K18.1, FHL-1, FHL1A, FHL1B, FLH1A, Four and a half LIM domains protein 1, KYOT, KYO-T, MGC111107, Skeletal muscle LIM-protein 1, SLIM, SLIM1, SLIM-1, SLIMMER, XMPMA				
Nexilin F-actin binding protein	NEXN	< −1	< −1	< −1

(*continued on next page*)

Table A1 (*continued*)

Protein name	Gene symbol	Log10 fold change compared to control		
		Mg-10Gd	Mg-2Ag	Pure-Mg
F-actin-binding protein, MGC104234, MGC138865, MGC138866, Nelin, NELIN, nexilin, Nexilin				
Orosomucoid 1	ORM1	< −1	< −1	< −1
AGP1, AGP 1, AGP-A, Alpha-1-acid glycoprotein 1, OMD 1, ORM, Orosomucoid-1				
Serine and arginine rich splicing factor 6	SRSF6	< −1	< −1	< −1
Serine/arginine-rich splicing factor 6, SFRS6, splicing factor, arginine/serine-rich 6, B52, pre-mRNA splicing factor SRP55, SR splicing factor 6, SRP55				
Kinectin 1	KTN1	< −1	< −1	< −1
CG1, CG-1 antigen, KIAA0004, Kinectin, Kinesin receptor, KNT, MGC133337, MU-RMS-40.19				
6-phosphogluconolactonase	PGLS	< −1	< −1	< −1
6PGL				
ADP ribosylation factor like GTPase 1	ARL1	< −1	< −1	< −1
ADP-ribosylation factor-like 1, ADP-ribosylation factor-like protein 1, ARFL1				
Protein phosphatase 1 regulatory subunit 12A	PPP1R12A	< −1	< −1	< −1
Protein phosphatase 1, regulatory (inhibitor) subunit 12A, MBS, MGC133042, Myosin phosphatase-targeting subunit 1, Myosin phosphatase target subunit 1, MYPT1, Protein phosphatase 1 regulatory subunit 12A, Protein phosphatase myosin-binding subunit				
Synaptopodin 2	SYNPO2	< −1	< −1	< −1
Genethonin-2, Myopodin, MYOPODIN, Synaptopodin-2				
Glutaredoxin 3	GLRX3	< −1	< −1	< −1
bA500G10.4, FLJ11864, GLRX4, Glutaredoxin-3, GRX3, GRX4, HUSSY-22, PICOT, PKC-interacting cousin of thioredoxin, PKCq-interacting protein, PKC-theta-interacting protein, Thioredoxin-like protein 2, TXNL2, TXNL3				
NSFL1 cofactor	NSFL1C	< −1	< −1	< −1
NSFL1 (p97) cofactor (p47), dJ776F14.1, FLJ46889, MGC3347, NSFL1 cofactor p47, p47, P47, p97 cofactor p47, UBX1, UBXD10, UBX domain-containing protein 2C, UBXN2C				
Cold inducible RNA binding protein	CIRBP	< −1	< −1	< −1
A18HNRNP, A18 hnRNP, CIRP, Cold-inducible RNA-binding protein, Glycine-rich RNA-binding protein CIRP				
Heterogeneous nuclear ribonucleoprotein D like	HNRNPDL	< −1	< −1	< −1
Heterogeneous nuclear ribonucleoprotein D-like (hnRNP D-like) (hnRNP DL) (AU-rich element RNA-binding factor) (JKT41-binding protein) (Protein laAUF1), HNRPDL, JKTBP				
Phosphoenolpyruvate carboxykinase 2, mitochondrial	PCK2	< −1	< −1	< −1
PEPCK, PEPCK2, PEPCK-M, Phosphoenolpyruvate carboxylase				
Desmin	DES	< −1	< −1	< −1
CMD1I, CSM1, CSM2, FLJ12025, FLJ39719, FLJ41013, FLJ41793				
Alpha fetoprotein	AFP	< −1	< −1	< −1
Alpha-1-fetoprotein, Alpha-fetoglobulin, Alpha-fetoprotein, FETA, HPAFP				
Spectrin repeat containing nuclear envelope protein 1	SYNE1	< −1	< −1	< −1
8B, ARCA1, C6orf98, CPG2, dJ45H2.2, DKFZp781J13156, EDMD4, enaptin, Enaptin, FLJ30878, FLJ41140, KIAA0796, KIAA1262, KIAA1756, MYNE1, Myne-1, Myocyte nuclear envelope protein 1, nesprin-1, Nesprin-1, Nuclear envelope spectrin repeat protein 1, SCAR8, Synaptic nuclear envelope protein 1, Syne-1, SYNE-1B				
S100 calcium binding protein A9	S100A9	< −1	< −1	< −1
60B8AG, CAGB, Calgranulin-B, Calprotectin L1H subunit, CFAG, CGLB, L1AG, Leukocyte L1 complex heavy chain, LIAG, MAC387, MIF, Migration inhibitory factor-related protein 14, MRP14, MRP-14, NIF, p14, P14, Protein S100-A9, S100 calcium-binding protein A9				
Ubiquitin conjugating enzyme E2 N	UBE2N	< −1	< −1	< −1
Bendless-like ubiquitin-conjugating enzyme, BLU, MGC131857, MGC8489, Ubc13, UBC13, UbcH-ben, Ubiquitin carrier protein N, Ubiquitin-conjugating enzyme E2 N, Ubiquitin-protein ligase N				

Table B1
Medium composition of the different conditions.

Condition	Composition
Growth medium (expansion media)	MEM + 10% SC-FBS + 1% antibiotics penicillin/streptomycin
Differentiation medium (control)	αMEM + 10% SC-FBS + 1% antibiotics penicillin/streptomycin +0.28 nM L-Ascorbic acid 2-phosphate + 1 mM L-Cystein + 100 ng/mL IGF1 + 20 ng/mL TGFB1 + 10 ng/mL IL4
Pure-Mg extract	Differentiation medium + pure extract
Mg-10Gd extract	Differentiation medium + pure extract
Mg-2Ag extract	Differentiation medium + pure extract

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