Morphological restriction of human coronary artery endothelial cells substantially impacts global gene expression patterns

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Introduction

Regulation of the vascular system is essential for tissue growth and homeostasis, and aberrant vascular signalling has been implicated in a vast number of diseases, such as cancer, diabetes, arthritis, macular degeneration and cardiovascular disorders [1]. The majority of research examining endothelial function has focused on the effects of secreted growth factors and cytokines such as vascular endothelial growth factor, fibroblast growth factor, transforming growth factor (TGF)β and a host of other molecules on endothelial cell signalling and physiology. Although these factors undoubtedly play a critical role in regulating cardiovascular development, function and disease, a growing number of studies indicate that Alterations in cell shape have been shown to modulate chromatin condensation and cell lineage specification; however, the mechanisms controlling these processes are largely unknown. Because endothelial cells experience cyclic mechanical changes from blood flow during normal physiological processes and disrupted mechanical changes as a result of abnormal blood flow, cell shape deformation and loss of polarization during coronary artery disease, we aimed to determine how morphological restriction affects global gene expression patterns. Human coronary artery endothelial cells (HCAECs) were cultured on spatially defined adhesive micropatterns, forcing them to conform to unique cellular morphologies differing in cellular polarization and angularity. We utilized pattern recognition algorithms and statistical analysis to validate the cytoskeletal pattern reproducibility and uniqueness of each micropattern, and performed microarray analysis on normal-shaped and micropatterned HCAECs to determine how constrained cellular morphology affects gene expression patterns. Analysis of the data revealed that forcing HCAECs to conform to geometrically-defined shapes significantly affects their global transcription patterns compared to non-restricted shapes. Interestingly, gene expression patterns were altered in response to morphological restriction in general, although they were consistent regardless of the particular shape the cells conformed to. These data suggest that the ability of HCAECs to spread, although not necessarily their particular morphology, dictates their genomics patterns.

Abbreviations
DAPI, 4′,6-diamidino-2-phenylindole; HCAEC, human coronary artery endothelial cell; KS, Kolmogorov–Smirnov; TGF, transforming growth factor; Wnt, wingless-type.
endothelial physiology, as well as that of many other cell types, is directed by an intimate combination of physical, chemical and biological cues present in the tissue microenvironment [2,3]. Over a century ago, physical cues were hypothesized to play important roles in tissue development and there are no better examples in the human body than the deleterious effects of microgravity on bone structure [4] and hypertension on cardiovascular function [5]. However, almost all organisms have evolved specific structures that are tailored to respond to nano- and macroscale physical forces whereby cells are able to detect and respond to external forces through mechanically induced conformational or organizational changes in cellular molecules, such as stretch-sensitive ion channels, G protein coupled receptors, tyrosine kinase receptors, cadherins and integrins located on the plasma membrane and in cell-to-cell and cell-to-extracellular matrix junctions [6].

Over the past decade, a large number of studies have manipulated endothelial tension, compression and shear stress aiming to determine how mimicking blood flow affects endothelial function [7]. Despite the progress made in this area, many of the mechanisms regulating how extrinsic mechanical stresses affect endothelial physiology remain unknown, and the implications of such studies are primarily limited to extrapolations of how luminal blood flow from normal, hypertensive and sclerotic conditions affects endothelial cells. A wealth of primarily qualitative evidence suggests that cell morphology-specific regulation of mechanotransduction is essential for cellular fate decisions such as proliferation, apoptosis, differentiation and quiescence [8–12]. For example, restriction of endothelial cell spreading using micropatterned substrates induces cell cycle arrest and apoptosis [8]. Alternatively, cell proliferation increases when cell spreading is allowed, whereas cells preferentially undergo differentiation in a moderately spread state. Endothelial migration is significantly more guided and regulated on narrower adhesive surfaces than on larger ones and geometric cues have been shown to modulate endothelial differentiation [13]. Other cell types may show distinct phenotypes solely on morphological alterations. For example, it has been reported that human stem cells can be directed to osteogenic or adipogenic developmental lineages by simply manipulating cell shape and thereby altering cellular mechanics [14], although more recent follow-up studies conducted in a separate laboratory suggest that adipogenic potential is not dependent on cell geometry [15]. Previously reported data obtained in our laboratory and others indicate that alterations in cell shape and cytoskeletal dynamics are capable of markedly overriding external mitogenic signalling [12,16]. This suggests that, as opposed to a model in which cell proliferation, death and differentiation are largely independent of cell shape, these processes are coordinately regulated and modulated by cellular mechanics. Thus, the local differentials in growth factors, biochemistry and internal and external mechanical stress synergize to modulate the specificity that drives tissue heterogeneity during development, normal function and disease.

Cell shape changes have been associated with nuclear shape remodelling [11,17–19]. It has been hypothesized that the transduction of mechanical information through cytoskeletal/nuclear coupling results in alterations that modulate chromosomal architecture and subsequent accession of transcription factors to their target genes [20–24]. Indeed, recent work has demonstrated that large-scale changes in cell shape induce alterations in chromosome condensation leading to marked effects on cell proliferation [25]. Thus, distinct cellular morphologies may drive the patterning of unique cytoskeletal architectures that govern global gene expression [26]. Despite these findings, it is not known how cell shape and its effects on cytoskeletal structures modulate global transcriptional patterns.

Although the normal surface of arteries is smooth, atherosclerotic arteries are characterized by irregular arrangement of endothelial cells, compromised monolayer integrity, irregular protrusions in the shape of scales or plates, and altered endothelial cell geometry [27,28]. Thus an understanding of how endothelial cell shape changes affect cellular function may shed light on the deregulation of endothelial cells during aberrant states such as hypertension, arteriosclerosis and coronary artery disease. In the present study, we examined the global gene expression changes that occur when human coronary artery endothelial cells (HCAECs) are shape and spread restricted by micropatterning into reproducibly unique cellular morphologies that are distinctive in polarization, morphological angularity and actin cytoskeleton patterning. Given the wealth of data suggesting that cell shape and cytoskeletal patterning can alter cellular physiology across a large number of cell types, we specifically investigated whether unique alterations in these cellular properties are capable of modulating global gene expression changes in endothelial cells. Our data demonstrate that geometric restriction induces dramatic alterations in the HCAEC transcriptome, although these changes are independent of the exact cell shape and/or actin orientation assumed by the cell.
Results

Quantitative analysis of cell shape-induced cytoskeletal and nuclear changes in HCAECs

To determine how cell shape alterations regulate the endothelial transcriptome, we must first utilize a system that manipulates cellular morphology at the same time as consistently maintaining all other growth variables. Accordingly, we seeded HCAECs on collagen I-coated spatially defined micropatterns, allowing cells to adapt to reproducible large (1600 μm²) geometric patterns, including a disc, crossbow, H, Y and L (Fig. 1A). We specifically utilized this cell type because endothelial cells of the coronary artery are constantly exposed to cyclic mechanical changes from blood flow during normal physiological processes and disrupted mechanical changes as a result of abnormal blood flow, cell shape deformation and loss of polarization during coronary artery disease. The size of the micropattern was specifically chosen because we tested micropatterns restricting the cells to either 700 or 1100 μm²; however, at these sizes, the cells failed to reproducibly conform to the intended shape (data not shown). Moreover, larger micropatterns would allow multiple cells to attach to one micropattern, thus dramatically affecting reproducibility of cell shape. As a control for nonrestricted morphology, cells were also plated at subconfluent levels on the chip in an area coated in collagen I. These patterns were specifically chosen for their ability to alter cell polarization (because this affects stress fibre architecture and nuclear orientation) [25] and the angularity of the cells’ morphologies. Disk-shaped cells adopted a round morphology with obtuse cellular edges and random polarization. Crossbow and H-shaped cells exhibit a combination of obtuse and acute edges and become strongly and moderately polarized, respectively. Y- and L-shaped cells were dominated by acute angles, with strong polarization in the Y-shaped cells and no polarization in the L-shaped cells.

To quantify how endothelial cell shape drives actin cytoskeleton patterning, we performed immunofluorescent confocal imaging of normally-shaped and micropatterned HCAECs labelled with rhodamine-conjugated phalloidin (which stains the actin cytoskeleton), phosphorylated focal adhesion kinase (which stains cellular attachments to the extracellular matrix) and 4’,6-diamidino-2-phenylindole (DAPI) (which highlights the nucleus) (Fig. 1B). For a full understanding of the quantitative differences in the actin cytoskeletal orientation of each immunofluorescent image, we implemented algorithms to separate the structures of interest from the remainder of the image, thus allowing us to describe the image quantitatively rather than using standard qualitative methods. Accordingly, we employed techniques for linear feature extraction to segment and obtain orientation and length of the actin fibres from each image. The techniques included preprocessing the images to enhance foreground elements, actin fibre detection using...
FIBERSCORE [29] and filtering including thresholding and mathematical morphology (Fig. 2). Figure 2B provides a more suitable input image to FIBERSCORE for detection because the actin fibres are brighter and display higher contrast. Figure 2C,D shows the correlation and orientation outputs of FIBERSCORE and is used for further analysis of length and orientation, respectively.

We first statistically analyzed the actin fibre orientations using images similar to those shown in Fig. 2D to quantitatively illustrate that HCAECs conforming to one micropatterned shape are indeed unique in cytoskeletal organization compared to those of another micropatterned shape. Immunofluorescent actin images from each shape were tiled into grid regions and the two-sample Kolmogorov-Smirnov (KS) test [30] was utilized to determine whether fibre orientation between cell shapes is truly unique and reproducible in structure (Table 1). High scores (closer to 1.0) occur when actin fibre orientations are largely dissimilar between cells and were observed across shape to shape comparisons. With the exception of normal-shaped cells (which demonstrated high actin orientation variability), we find relatively low rejection scores when comparing all the individual cells with their underlying cumulative tiling, meaning that cells of the same shape have fibre orientations more similar to each other than to other shapes. Note that these comparisons are not symmetric (e.g. comparing X-bow to disk yields slightly different scores than disk to X-bow). This asymmetry is a result of the fact that the orientation of individual images is being compared to the cumulative histogram of a specific shape; we are thus comparing individual disk image fibre orientations with the

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**Fig. 2.** Cytoskeletal image processing. Actin cytoskeleton images were processed as described in the Materials and methods. The processed images for a X-bow-shaped cell are shown. (A) Original immunofluorescence image in greyscale. (B) Preprocessing: contrast-limited adaptive histogram equalization. (C) Correlation image result from detection with FIBERSCORE. (D) Orientation image result from detection with FIBERSCORE. (E) Postprocessing: threshold. (F) Postprocessing: skeleton.
cumulative X-bow orientations and vice versa. These comparisons will yield similar but not identical results. These findings strongly validate the idea that the cellular morphologies induced by the micropatterned substrates result in reproducibly unique actin orientations between cell shapes. The analysis of shapes using the tiled grid regions, however, shows similarities in certain regions of a cell between shapes. Detailed analysis of the dominant and second dominant angles in actin orientation between cell shapes revealed that (a) crossbow-shaped cells have more contribution from actin angles close to 0° along the horizontal projection and angles oriented in opposite directions when comparing the widest regions of the crossbow with the narrowest regions; (b) disk-shaped cells have a more uniform distribution of actin angle orientations; (c) H-shaped cells have more contributions from angles close to 0° along the vertical centre; and (d) Y- and L-shaped cells display non-uniform orientation distributions each with a different dominant angle (Fig. 3A–C). This 3 × 3 tiling is applied in the same manner to all images; the consistency in the KS test results indicate the robustness of the results with respect to this choice of tiling. Note that in regions where it appears that there are no fibres and thus no orientation information (e.g. Y-shape left upper and lower corners) as a result of image variation, we do obtain a small amount of orientation information, as shown in Fig. 3C. We then analyzed the median fibre length using images similar to Fig. 3C between normal and micropatterned HCAECs using the previously described modified FIBERSCORE analysis. As indicated in Fig. 3D, the median fibre length (±SEM) for normal-shaped HCAECs was significantly greater (6.84 ± 0.9 µm) than for crossbow- (2.9 ± 0.1 µm), disk- (3.3 ± 0.2 µm), H- (2.6 ± 0.2 µm), Y- (2.9 ± 0.3 µm) and L- (4.3 ± 0.4 µm) shaped cells. Thus, these data strongly indicate that actin orientation and length are truly unique between each cell shape and, if genomic alterations are truly shape and actin confirmation dependent, this model system is sufficient in both design and reproducibility to identify those changes.

Using shape-engineered endothelial cells on circular, square and various rectangular adhesive micropatterns mimicking elongated bipolar shapes, Versaevel et al. [25] indicated that cell elongation and spreading is a key parameter of nuclear deformation and this process is absolutely dependent on lateral compressive forces generated by an actomyosin-mediated mechanism. It was further demonstrated that cell elongation leads to successive changes in the level of chromatin condensation as the nuclear shape index is decreased. To test whether changes in cell shape in general (as opposed to solely cell elongation, as shown previously) [25] induce nuclear deformation, we analyzed top and side images of the nuclei from normal and micropatterned HCAECs using confocal microscopy (at least 40 nuclei per condition). The prototypical HCAEC nucleus is ~15–18 µm long by 5–8 µm high and maintains a distinctive oval appearance (Fig. 4A, left), whereas deformed nuclei show variability from this norm, as shown in Fig. 4A (middle and right). Although irregularity in nuclear shape occurred relatively infrequently in normal-shaped cells (~6% of the cells exhibited nonprototypical nuclei), the percentages were significantly higher in the micropatterned HCAECs, ranging from just over 20% of the L- and Y shaped cells to approximately three-quarters of the population in disc shaped cells (Fig. 4B).

**Morphological restriction in HCAECs results in large-scale changes in endothelial global gene transcription independent of the unique shape adopted**

Distinct micropattern-mediated alterations in cell shape have been shown to affect lineage specification in mesenchymal progenitor cells [14], although less is known regarding how changes in cell morphology affect terminally differentiated cell types (such as an endothelial cells). Thus, we sought to address two questions: (a) does morphological restriction affect endothelial global transcription and (b) does a distinct cellular morphology uniquely affect endothelial global transcription. Using the reproducible micropatterning system described above, we can effectively address both questions.

We performed whole genome microarray analysis on total RNA collected from nonrestricted and micropatterned HCAECs cultured on 96-well collagen I-coated micropatterned plates and grown in standard growth media. The nonrestricted cells were grown at low

|                | Crossbow | Disk | H-cell | Y-cell | L-cell | Normal cell |
|----------------|----------|------|--------|--------|--------|-------------|
| Crossbow       | 0.54     | 0.93 | 0.94   | 0.68   | 0.96   | 0.95        |
| Disk           | 0.96     | 0.78 | 0.95   | 0.99   | 0.91   | 0.99        |
| H-cell         | 0.93     | 0.91 | 0.6    | 0.93   | 0.75   | 0.95        |
| Y-cell         | 0.71     | 0.99 | 0.94   | 0.5    | 0.95   | 0.96        |
| L-cell         | 0.99     | 0.95 | 0.88   | 0.97   | 0.39   | 0.93        |
| Normal cell    | 0.95     | 1    | 0.89   | 0.95   | 0.88   | 0.86        |

**Table 1.** Correlation of actin fibre orientation between each shape. The data presented are the mean scores of the output via a two-sample KS test (scale of 0 to 1 where 1 completely rejects the null hypothesis of the test).
confluence to minimize cell-to-cell contacts. Our data revealed large-scale alterations in gene expression as a result of HCAEC morphological restriction. As shown in Fig. 5A,B and Table 2, 361 statistically relevant gene expression changes were equal or greater than two-fold in magnitude ($P < 0.05$) in at least one of the cell shapes compared to normally-shaped HCAECs cultured on the same micropatterned plate. The complete data set is publically available via the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) (accession number GSE43349). These results provide strong evidence that restricting cell shape induces changes in the global transcriptional patterns of endothelial cells.

Although seeding density was controlled in these experiments to minimize cell-to-cell contact (particularly in the control samples where cellular interactions are possible), it is probable that the use of rich growth media encourages the proliferation of the nonrestricted cells but, because shape restriction has been shown to inhibit proliferation [8], is unable to do so in the shape-restricted cells. This could potentially induce bias in the interpretation of the data from the unrestricted HCAECs as a result of differences in cell cycle progression or cell-to-cell contacts that arise between the mother and daughter cells following mitosis. To address this potential concern, we performed the same micropatterning experiment as described above, except the cells in both the nonrestricted and shape-restricted conditions were serum starved for 48 h before RNA collection to block cell proliferation, thus eliminating variables such as cell-to-cell contact, cell cycle progression, and proliferation in the restricted samples.

Fig. 3. Quantification of actin fibre orientation and length in normal and micropatterned HCAECs. (A) FIBERSCORE: orientation heatmaps depicting actin orientation for normal and micropatterned HCAECs in relation to their cellular axis. An angle starting at 0° is coincident with the x-axis and increases in a counter clockwise direction to 180°. (B) Representative 3 × 3 tiling of a crossbow-shaped HCAEC orientation heatmap. (C) Dominant and second dominant fibre angles in each grid block for each shape. The dominant and second dominant angles are calculated by combining angle information in all images of the particular shape tile. Angles are detected using the restricted angle resolution of FIBERSCORE. (D) FIBERSCORE: correlation quantification of the median fibre length of the actin fibres from normal and micropatterned HCAECs. At least 11–14 images were analyzed from each condition.
differences, etc. A comparison of the profile plots of nonrestricted versus shape-restricted HCAECs grown in standard growth conditions or subsequent to serum starvation yielded similar results, indicating that, regardless of growth conditions, proliferation or cell-to-cell contact, morphological restriction induced significant changes in the global gene expression profiles (Fig. 5C). The complete microarray data set for the serum starvation experiment is publically available via the Gene Expression Omnibus (accession number GSE44168).

Previous data collected from mesenchymal progenitor cells conforming to micropatterns that induced the cells to form obtuse versus acute morphological angles suggested that cell morphology controls lineage specification. Although endothelial cells are terminally differentiated, we aimed to determine whether such distinct morphological as well as polarity changes might influence the endothelial transcriptome. By excluding the nonrestricted conditions from the analysis, we compared the gene expression changes between only the micropatterned endothelial cells adhering to crossbow, disc, H, L and Y shapes to examine whether distinct cellular morphology can affect endothelial gene expression patterns. As shown in Fig. 5B,C and Table 2, gene expression changes did not significantly vary based on the particular shape, actin patterning or polarity to which the cells conformed. Indeed, statistical analysis of the genomic data set failed to reveal a single two-fold or greater ($P < 0.05$) alteration in gene expression between any of the cell shapes. These data suggest that, unlike mesenchymal stem cells whose phenotype can be modulated by cellular angularity, endothelial cells grown under these unique geometric constraints do not differ in their global gene expression patterns. Cumulatively, our data indicates that morphological constraint, rather than cellular angularity and polarity, alter the global transcriptome under these conditions.

Pathway analysis of the morphology induced transcriptome changes

We next implemented a systems level approach to understand how geometric constraint may affect the overall cellular phenotype. Our initial analysis reported above included two-fold or greater changes in gene expression, yet, for this network analysis, we broadened our microarray data set (from the standard growth condition experiment) to include the 1.4-fold or greater statistically relevant ($P < 0.05$) changes in gene expression. This cut-off was selected not only to refrain from limiting our network analysis to solely the highest expression changes, but also to take into account transcriptional changes that were less pronounced but still relevant with regard to modulating cellular physiology. This resulted in ~8% of the human genome experiencing changes in gene expression (642 up-regulated genes and 1218 down-regulated genes). We then performed Metacore pathway analysis of these gene expression changes to predict significant alterations in major cellular processes, including cell cycle regulation ($P < 3.3 \times 10^{-8}$) (Table 3), cytoskeletal dynamics and cell adhesion ($P < 4.2 \times 10^{-5}$) (Table 4), glycolysis/gluconeogenesis ($P < 2.7 \times 10^{-4}$) (Table 5), TGFβ signalling ($P < 1.6 \times 10^{-5}$) (Table 6) and wingless-type (Wnt) signalling ($P < 1.6 \times 10^{-5}$) (Table 7). Because TGFβ signalling has been shown to play a major role in arteriosclerotic disease progression, we confirmed our microarray data utilizing quantitative PCR to detect the shape-induced alterations in mRNA expression levels of the TGFβ signalling genes SMAD6, SMAD7 and TGFB2, as well as several genes...
reportedly involved in the atherosclerotic process, including LPL, MMP1, KDR, ITGA2, ACE, BIRC3, IL1R1, ICAM1, Hey1, BCL2, CSF2, APOE, PDGFB, BCL2A1, CCL2 and LDLR (Fig. 6).

**Discussion**

The interplay between the physical, chemical and biological cues to which cells are constantly exposed modulates processes ranging from those as broad as cellular lineage determination to those as subtle as the functional nuances between two adjacent cells. Despite the number of studies addressing this area of research, the molecular mechanisms by which these cues synergize is largely unknown. It has been reported that cellular morphology and cytoskeletal angularity greatly influence progenitor lineage specification [14] and that changes in cell shape influence chromatin condensation via nuclear deformation [25]. In the present study, we aimed to determine whether morphological changes in coronary artery endothelial cells could affect the global patterns of gene expression. Understanding how cell shape change affects the coronary artery endothelial cell transcriptome may allow us to better understand the molecular aberrations that underlie coronary artery disease. The present study made use of micropatterned growth substrates that force cells to conform to precise geometric shapes. Although micropatterned cell growth has been utilized in a limited number of studies, there is little evidence that such techniques consistently lead to morphological and cytoskeletal patterns that are highly reproducible and truly unique between different micropatterns. We utilized pattern recognition algorithms and statistical analysis to confirm that cells conforming to the crossbow, disk, H, L or Y shapes had truly reproducible cellular morphology and cytoskeletal architecture unique for each cell shape adopted. Given that most analysis of cytoskeletal organization in the available literature is qualitative in nature, this algorithm can be extensively used in the future to provide quantitative interpretations of the differences in both static (as we have analyzed) and dynamic cytoskeletal structures between two or more treatment groups.
Table 2. Two-fold or greater alterations in gene expression compared to normal-shaped coronary artery endothelial cells (standard growth media).

| Gene symbol | Gene name | Accession number | X-bow | Disc | H | L | Y |
|-------------|-----------|------------------|-------|------|---|---|---|
| TMEM100     | Transmembrane protein 100, TV2 | NM_018286.2 | 6.4   | 6.3  | 6.4 | 7  |
| PTGS2       | Prostaglandin-endoperoxide synthase 2 | NM_000963.1 | 4.3   | 3.7  | 4   | 3.9| 4.1|
| IRF6        | Interferon regulatory factor 6 | NM_006147.2 | 3.4   | 2.9  | 2.8 | 3  | 3.5|
| ALPL        | Alkaline phosphatase, liver/bone/kidney, TV1 | NM_000478.3 | 3.3   | 3.5  | 3.3 | 3.4| 3.1|
| C8orf4      | Chromosome 8 ORF 4 | NM_020130.3 | 3.2   | 2.6  | 3   | 2.1| 2.7|
| HEY1        | Hairy/enhancer-of-split related with YRPW motif 1, TV2 | NM_001040708.1 | 3.1   | 2.7  | 2.7 | 2.7| 3.1|
| BMF         | Bcl2 modifying factor, TV2 | NM_033503.3 | 3     | 3.3  | 2.9 | 2.9| 3  |
| LOC730525   | Hypothetical protein | NM_001126202.1 | 3     | 2.8  | 2.7 | 3  | 4  |
| SEMA3G      | Semaphorin 3G | NM_020163.1 | 2.9   | 3.2  | 2.6 | 2.9| 2.6|
| HSD17B11    | Hydroxysteroid (17β) dehydrogenase 11 | NM_016245.2 | 2.8   | 2.2  | 2.6 | 2.5| 3.3|
| F2RL3       | Coagulation factor II (thrombin) receptor-like 3 | NM_003950.2 | 2.8   | 2.8  | 2.7 | 2.9| 2.6|
| TOX2        | TOX high mobility group box family member 2, TV4 | NM_001098796.1 | 2.8   | 3.1  | 2.7 | 2.4| 2.6|
| C20orf100   | TOX high mobility group box family member 2, TV1 | NM_001098797.1 | 2.8   | 2.8  | 2.6 | 2.3| 2.9|
| SPRY1       | Sprouty homologue 1, antagonist of FGF signalling (Drosophila), TV1 | NM_005844.1 | 2.7   | 2.5  | 2.7 | 2.6| 3.3|
| SEV34       | Zinc finger and BTB domain containing 16, TV2 | NM_001018011.1 | 2.6   | 2.6  | 2.6 | 2.7| 2.7|
| TMEM140     | Transmembrane protein 140 | NM_018295.2 | 2.6   | 2.2  | 2.3 | 2  | 2.5|
| NPTX1       | Neuronal pentraxin I | NM_002522.2 | 2.6   | 2.7  | 2.2 | 2.1| 2.6|
| SMAD7       | SMAD family member 7 | NM_005904.2 | 2.6   | 2.4  | 2.5 | 2.7| 2.6|
| ANKRD1      | Ankyrin repeat domain 1 (cardiac muscle) | NM_014391.2 | 2.5   | 2.8  | 2.7 | 2.7| 3.1|
| CXC4        | Chemokine (C-X-C motif) receptor 4, TV1 | NM_001008540.1 | 2.4   | 2.5  | 2.3 | 2.1| 2.5|
| SYN1        | Synemin, intermediate filament protein, TVB | NM_015298.5 | 2.4   | 2.1  | 2  | 2.2| 2.4|
| HLX         | H2.0-like homeobox | NM_021958.2 | 2.4   | 2.7  | 2.2 | 2  | 2  |
| EFNB2       | Ephrin-B2 | NM_004932.2 | 2.3   | 2.2  | 2.1 | 2  | 2.2|
| TNFAIP8L3   | Tumour necrosis factor, α-induced protein 8-like 3 | NM_0207381.2 | 2.2   | 2.3  | 1.9 | 2  | 2.2|
| NEDD9       | Neural precursor cell expressed, develop. down-regulated 9, TV2 | NM_182966.2 | 2.2   | 1.7  | 1.9 | 1.9| 2.6|
| GDF15       | Growth differentiation factor 15 | NM_004864.1 | 2.1   | 2.2  | 2  | 1.9| 2.1|
| CALCR       | Calcitonin receptor-like | NM_005795.4 | 2.1   | 1.8  | 2  | 1.7| 2  |
| RDX         | Radixin, TV3 | NM_002906.3 | 2.1   | 1.9  | 1.9 | 1.6| 2.1|
| MMP10       | Matrix metalloproteinase 10 (stromelysin 2) | NM_002425.1 | 2  | 2  | 1.6 | 1.4| 1.7|
| CMTM8       | CKLF-like MARVEL transmembrane domain containing 8 | NM_178868.3 | 2  | 2  | 1.8 | 1.8| 2  |
| C13orf15    | Regulator of cell cycle | NM_014059.2 | 2  | 2  | 1.8 | 1.7| 2.1|
| NDRG4       | NDRG family member 4 | NM_022910.1 | 2  | 2.1  | 1.9 | 1.7| 1.9|
| LOC100132564| Hypothetical protein | NM_001713808.1 | 2  | 2.3  | 1.4 | 1.4| 2.1|
| CRYAB       | Crystallin, alpha B | NM_001885.1 | 1.9  | 1.9  | 1.7 | 1.9| 2.5|
| RAGD        | Ras-related GTP binding D | NM_021244.3 | 1.9  | 1.6  | 1.8 | 1.7| 2  |
| IL10        | Interleukin 10 | NM_000572.2 | 1.9  | 1.6  | 1.4 | 1.5| 2.6|
| LOC100129211| Hypothetical protein | NM_001719861.1 | 1.8  | 1.8  | 1.5 | 1.6| 2  |
| GRAP        | GRB2-related adaptor protein | NM_006613.3 | 1.8  | 1.8  | 1.6 | 1.6| 2  |
| C8orf45     | Chromosome 8 open reading frame 45 | NM_173518.2 | 1.8  | 1.7  | 1.5 | 1.6| 2.1|
| PDGFβ       | Platelet-derived growth factor | NM_002608.1 | 1.8  | 2.2  | 1.8 | 1.8| 2  |
| LOC100190986| Nuclear pore complex interacting protein pseudogene | NR_024456.1 | 1.8  | 1.7  | 1.8 | 1.6| 2.5|
| PGF         | Placental growth factor | NM_002632.4 | 1.8  | 2  | 1.6 | 1.5| 1.6|
| LOC100132247| Nuclear pore complex interacting protein related gene | NM_001135865.1 | 1.7  | 1.4  | 2.2 | 1.7| 1.9|
| Gene symbol | Gene name                          | Accession number | X-bow | Disc | H | L | Y |
|-------------|------------------------------------|------------------|-------|------|---|---|---|
| FAM175A     | Family with sequence similarity 175, member A | NM_139076.2     | 1.7   | 1.4  | 1.4 | 1.4 | 2.1 |
| PDGF8       | Platelet-derived growth factor beta (oncogene homolog), TV2 | NM_033016.1     | 1.7   | 2.1  | 2.1 | 1.7 | 1.7 |
| LOC440353   | Nuclear pore complex interacting protein pseudogene | NR_002603.1     | 1.7   | 1.5  | 2.1 | 1.7 | 2.1 |
| KIAA1751    | KIAA1751                           | NM_001080484.1  | 1.6   | 1.7  | 1.5 | 1.5 | 2.5 |
| LOC613037   | Nuclear pore complex interacting protein pseudogene | NR_002555.2     | 1.6   | 1.4  | 2   | 1.5 | 2.1 |
| MAGT1       | Magnesium transporter 1            | NM_032121.4     | 1.6   | 1.7  | 1.7 | 1.5 | 2   |
| ZNF738      | Misc_RNA, partial miscRNA          | XR_040185.1     | 1.6   | 1.5  | 1.4 | 1.4 | 2   |
| DMC1        | DMC1 dosage suppressor of mck1 homolog | NM_007068.2     | 1.6   | 1.7  | 1.4 | 1.4 | 2.4 |
| LOC729978   | Similar to LOC339047 protein, TV2  | XM_001723016.1  | 1.6   | 1.5  | 1.5 | 1.3 | 2   |
| LOC23117    | KIAA0220-like protein, TV16        | XM_933634.2     | 1.6   | 1.5  | 1.6 | 1.6 | 2.1 |
| LOC100132568| Hypothetical protein               | XM_001722111.1  | 1.6   | 1.5  | 1.5 | 1.3 | 2.2 |
| LOC440348   | Nuclear pore complex interacting protein-like 2 | NM_00108059.2  | 1.6   | 1.7  | 1.9 | 1.7 | 2.1 |
| LOC440345   | Hypothetical protein, TV6          | XM_933717.1     | 1.6   | 1.5  | 2.1 | 1.7 | 2.5 |
| LOC728809   | Hypothetical LOC728809             | XM_001719546.1  | 1.6   | 1.4  | 1.3 | 1.2 | 2   |
| TRIM13      | Tripartite motif containing 13, TV4| NM_001007278.1  | 1.6   | 1.5  | 1.6 | 1.5 | 2.1 |
| IMAGE:2760913| NCI_CGP_Lu29 Homo sapiens cDNA    | AVU276479       | 1.6   | 1.4  | 1.6 | 1.5 | 2.5 |
| CATSPer2    | Cation channel, sperm associated 2, TV4| NM_172097.1     | 1.5   | 1.3  | 1.4 | 1.4 | 2.1 |
| MCART1      | Mitochondrial carrier triple repeat 1| NM_033412.1     | 1.5   | 1.7  | 1.3 | 1.2 | 2.3 |
| NLRR8       | NLR family, pyrin domain containing 8| NM_170811.2     | 1.5   | 1.5  | 1.3 | 1.4 | 2.1 |
| LOC255167   | Uncharacterized LOC255167          | NR_024424.1     | 1.5   | 1.6  | 1.3 | 1.3 | 2.2 |
| DDX51       | DEAD (Asp-Glu-Ala-Asp) box polypeptide 51 | NM_175066.2     | 1.4   | 1.3  | 1.3 | 1.4 | 2.2 |
| C21orf55    | Chromosome 21 ORF 55               | NM_017633.2     | 1.4   | 1.4  | 1.2 | 1.3 | 2.1 |
| LOC95986    | Amine oxidase, copper containing 3 pseudogene | NR_002773.1     | 1.4   | 1.6  | 1.4 | 1.5 | 2.2 |
| LOC100130168| Hypothetical protein               | XM_001719127.1  | 1.4   | 1.4  | 1.2 | 1.2 | 2   |
| MAPK8F3     | Mitogen-activated protein kinase 8 interacting protein 3, TV2 | NM_001040439.1  | 1.4   | 2    | 1.6 | 1.6 | 1.7 |
| ZNF682      | Zinc finger protein 682, TV1       | NM_033196.2     | 1.4   | 1.4  | 1.3 | 1.3 | 2.2 |
| ZNF486      | Zinc finger protein 486            | XM_371152.3     | 1.3   | 1.4  | 1  | 1.2 | 2.1 |
| SULT1A1     | Sulfotransferase family, cytosolic, 1A, phenol-prefering, member 1, TV3 | NM_177530.1     | 1.3   | 1.4  | 1.4 | 1.3 | 2   |
| LOC100128510| Hypothetical protein               | XM_001716759.1  | 1.3   | 1.5  | 1.4 | 1.5 | 2   |
| LOC653994   | Similar to eukaryotic translation initiation factor 4H, TV2 | XM_944429.1     | -1.3  | -2.2 | -1.3 | -1.5 | 1 |
| LOC648924   | Similar to eukaryotic translation initiation factor 4A, TV1 | XR_018316.1     | -1.3  | -1.8 | -1.6 | -2.1 | -1.3 |
| NDUFA8      | NADH dehydrogenase (ubiquinone)    | NM_014222.2     | -1.4  | -1.3 | -1.7 | -2.0 | -1.4 |
| TNP01       | Transportin 1, TV2                 | NM_153882.2     | -1.4  | -2.3 | -1.5 | -1.7 | -1.3 |
| SNAP23      | Synaptosomal-associated protein, 23 kDa, TV1 | NM_003825.2     | -1.4  | -2.0 | -1.4 | -1.6 | -1.3 |
| TCEA1       | Transcription elongation factor A (SII), TV2 | NM_201437.1     | -1.4  | -2.0 | -1.6 | -1.8 | -1.4 |
| ALCAM       | Activated leukocyte cell adhesion molecule | NM_001627.2     | -1.4  | -1.4 | -1.6 | -2.0 | -1.8 |
| TCEAL8      | Transcription elongation factor A (SII)-like 8, TV2 | NM_001066684.1  | -1.4  | -1.5 | -1.7 | -2.0 | -1.4 |
| TEME189-UBE2V1| TMEM189-UBE2V1 readthrough transcript, TV2 | NM_003349.4     | -1.4  | -2.0 | -1.5 | -1.7 | -1.3 |
| LOC730052   | Misc_RNA (LOC730052)               | XR_016054.2     | -1.4  | -2.0 | -1.4 | -1.7 | -1.3 |
| TXNDC5      | Thioredoxin domain containing 5 (endoplasmic reticulum), TV1 | NM_030810.2     | -1.4  | -2.0 | -1.4 | -1.5 | -1.6 |
| BCLL21      | BCL2-like 1, nuclear gene encoding mitochondrial protein, TV1 | NM_138578.1     | -1.5  | -2.0 | -1.3 | -1.6 | -1.3 |
| EIF4F2      | Eukaryotic translation initiation factor 4y, 2, TV1 | NM_001418.3     | -1.5  | -2.2 | -1.5 | -2.0 | -1.5 |
| TCP1        | T-complex 1, TV1                   | T_030752.2      | -1.5  | -2.1 | -1.4 | -1.8 | -1.4 |
| CCT6A       | Chaperonin containing TCP1, subunit 6A (zeta 1), TV1 | NM_001762.3     | -1.5  | -2.1 | -1.5 | -1.9 | -1.4 |
Table 2. (Continued).

| Gene symbol | Gene name                                      | Accession number | X-bow | Disc | H   | L   | Y   |
|-------------|------------------------------------------------|------------------|-------|------|-----|-----|-----|
| LOC644063   | Similar to heterogeneous nuclear ribonucleoprotein K | XR_016547.1      | −1.5  | −2.2 | −1.6 | −2.0 | −1.3 |
| LSM5        | LSM5 homologue, U6 small nuclear RNA associated (Saccharomyces cerevisiae) | NM_012322.1      | −1.5  | −1.6 | −2.0 | −1.9 | −1.8 |
| FEZ2        | Fasciculation and elongation protein zeta 2 (zygin II), TV1 | NM_005102.2      | −1.5  | −1.7 | −1.8 | −2.1 | −1.6 |
| C14orf1149  | Chromosome 14 ORF 149                            | NM_144581.1      | −1.5  | −1.6 | −1.8 | −2.0 | −1.9 |
| LOC728059   | Misc_RNA                                        | XR_015606.1      | −1.5  | −2.4 | −1.6 | −2.3 | −1.7 |
| THOC4       | THO complex 4                                    | XM_001134346.1   | −1.5  | −1.8 | −1.6 | −2.0 | −1.5 |
| LYPLA1      | Lysocephospholipase I                            | NM_006330.2      | −1.5  | −1.9 | −1.8 | −2.1 | −1.5 |
| EDG1        | Endothelial differentiation, sphingolipid G-protein-coupled receptor, 1 | NM_001400.3      | −1.5  | −1.5 | −1.5 | −2.2 | −1.6 |
| LOC648695   | Similar to retinoblastoma binding protein 4, TV5 | XM_944246.2      | −1.5  | −2.2 | −1.8 | −2.2 | −1.7 |
| MALL        | Mal, T-cell differentiation protein-like          | NM_005434.3      | −1.5  | −1.3 | −1.7 | −2.0 | −1.7 |
| ZFAND6      | Zinc finger, AN1-type domain 6                   | NM_019006.2      | −1.5  | −2.2 | −1.6 | −1.8 | −1.6 |
| ADK         | Adenosine kinase, transcript variant ADK-short   | NM_001123.2      | −1.5  | −1.6 | −1.9 | −2.0 | −1.5 |
| ZYX         | Zyxin, TV1                                       | NM_003461.4      | −1.5  | −1.4 | −2.0 | −1.7 | −1.7 |
| PAPSS2      | 3′-phosphoadenosine                              | NM_004670.3      | −1.5  | −1.5 | −1.7 | −2.1 | −1.5 |
| G3BP2       | GTPase activating protein (SH3 domain) binding protein 2, TV3 | NM_203504.1    | −1.5  | −1.6 | −1.6 | −2.1 | −1.6 |
| LOC100130661| Similar to high-mobility group protein 1-10, TV2 | XM_001723189.1   | −1.5  | −2.1 | −1.6 | −1.9 | −1.5 |
| HIGD1A      | HIG1 hypoxia inducible domain family, member 1A, TV1 | XM_001099668.1  | −1.6  | −2.0 | −1.8 | −2.1 | −1.7 |
| EPB41L3     | Erythrocyte membrane protein band 4.1-like 3     | NM_012307.2      | −1.6  | −1.7 | −1.6 | −2.0 | −1.8 |
| IARS        | Isoleucyl-tRNA synthetase, TV short              | NM_002161.3      | −1.6  | −1.5 | −1.7 | −2.0 | −1.5 |
| RRAS2       | Related RAS viral (r-ras) oncogene homologue 2   | NM_012250.3      | −1.6  | −1.9 | −1.8 | −2.0 | −1.3 |
| RANBP1      | RAN binding protein 1                            | NM_002882.2      | −1.6  | −1.6 | −2.1 | −2.0 | −1.7 |
| NOL6        | Nucleolar protein family 6 (RNA-associated), TV γ | NM_139235.3      | −1.6  | −1.3 | −2.0 | −1.7 | −1.5 |
| C18orf55    | Chromosome 18 ORF 55                             | NM_014177.1      | −1.6  | −1.7 | −1.7 | −2.1 | −1.6 |
| CSE1L       | CSE1 chromosome degradation 1-like (yeast)       | NM_001316.2      | −1.6  | −1.6 | −1.8 | −2.0 | −1.6 |
| TIMM23      | Translocase of inner mitochondrial membrane 23 homologue | NM_006327.2    | −1.6  | −1.7 | −1.8 | −2.1 | −1.6 |
| FHL2        | Four and a half LIM domains 2, TV4               | NM_201557.2      | −1.6  | −1.6 | −1.8 | −2.0 | −1.6 |
| AP15S       | Adaptor-related protein complex 1, sigma 1 subunit, TV4 | NM_057089.2    | −1.6  | −1.5 | −1.9 | −2.1 | −1.4 |
| HNRPA2B1    | Heterogeneous nuclear ribonucleoprotein A2/B1, TV B1 | NM_031243.1      | −1.6  | −1.5 | −1.8 | −2.0 | −1.8 |
| CCNC        | Cycin C, TV2                                     | NM_001013399.1   | −1.6  | −2.0 | −1.7 | −1.9 | −1.5 |
| PTPLAD1     | Protein tyrosine phosphatase-like A domain containing 1 | NM_016395.2    | −1.6  | −1.7 | −1.8 | −2.1 | −1.5 |
| HNRNPK      | Heterogeneous nuclear ribonucleoprotein K, TV2   | NM_031263.2      | −1.6  | −1.8 | −1.5 | −2.0 | −1.5 |
| HAT1        | Histone acetyltransferase 1, TV1                | NM_003642.2      | −1.6  | −1.7 | −2.0 | −1.9 | −1.7 |
| PSME3       | Proteasome (prosome, macropain) activator subunit 3, TV1 | NM_005789.2   | −1.6  | −1.3 | −2.0 | −2.0 | −1.6 |
| HIGD1A      | HIG1 hypoxia inducible domain family, member 1A, TV1 | NM_001099668.1  | −1.6  | −1.7 | −1.8 | −2.1 | −1.6 |
| Gene symbol | Gene name | Accession number | X-bow | Disc | H | L | Y |
|-------------|-----------|------------------|-------|------|---|---|---|
| ARM1C3      | Armadillo repeat containing, X-linked 3, TV2 | NM_177947.2 | −1.6  | −2.0 | −1.5 | −2.0 | −1.6 |
| LOC100128266| PREDICTED: Misc_RNA | XR_038984.1 | −1.6  | −2.1 | −1.8 | −2.1 | −1.7 |
| DCCB2       | Dicoidin, CUB and LCCL domain containing 2 | NM_080927.3 | −1.6  | −1.6 | −2.1 | −1.9 | −1.8 |
| SMS         | Spermine synthase | NM_004595.2 | −1.6  | −1.9 | −1.8 | −2.0 | −1.7 |
| TPM3        | Tropomyosin 3, TV1 | NM_152263.2 | −1.6  | −1.9 | −1.8 | −2.1 | −1.4 |
| LOC653884   | Similar to FUS interacting protein (serine-arginine rich) 1 | XM_936240.1 | −1.6  | −1.9 | −1.6 | −2.0 | −1.5 |
| ATP5G1      | ATP synthase, mitochondrial Fo complex, subunit C1, TV2 | NM_001002027.1 | −1.6  | −1.5 | −1.9 | −2.0 | −1.7 |
| SDCBP       | Syndecan binding protein (syntenin), TV2 | NM_001007067.1 | −1.6  | −2.0 | −1.6 | −2.1 | −1.7 |
| MCM6        | Minichromosome maintenance complex component 6 | NM_005915.4 | −1.6  | −1.7 | −2.0 | −2.0 | −1.7 |
| BRI1X       | BR1X, biogenesis of ribosomes, homologue (S. cerevisiae) | NM_018321.3 | −1.6  | −1.7 | −1.7 | −2.0 | −1.7 |
| RPL29       | Ribosomal protein L29 | NM_000992.2 | −1.6  | −2.1 | −2.0 | −2.2 | −1.6 |
| LOC684330   | Similar to tropomyosin 3 isoform 2 | XR_017492.1 | −1.6  | −2.0 | −1.8 | −1.9 | −1.6 |
| LPXN        | Leupaxin | NM_004511.1 | −1.6  | −1.6 | −1.9 | −2.0 | −1.8 |
| LOC100130506| Hypothetical protein | XM_001724500.1 | −1.6  | −1.7 | −2.0 | −2.0 | −1.7 |
| DD2X1       | DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 | NM_004728.2 | −1.6  | −1.6 | −1.7 | −2.0 | −1.5 |
| LDHA        | Lactate dehydrogenase A | NM_005566.1 | −1.6  | −1.6 | −1.9 | −2.0 | −1.8 |
| LOC684590   | Misc_RNA | XR_016251.2 | −1.6  | −1.7 | −2.0 | −1.9 | −1.7 |
| FKBPL1      | FK506 binding protein 14, 22 kDa | NM_017948.2 | −1.6  | −1.7 | −1.9 | −2.1 | −1.6 |
| NME1        | NME/NM23 nucleoside diphosphate kinase 1, TV2 | NM_006269.2 | −1.6  | −1.8 | −2.1 | −2.1 | −1.7 |
| AHNAK       | AHNAK nucleoprotein, TV1 | NM_001620.1 | −1.6  | −1.6 | −2.0 | −1.8 | −1.4 |
| CKS2        | CDC28 protein kinase regulatory subunit 2 | NM_001827.1 | −1.6  | −1.5 | −2.0 | −2.1 | −1.7 |
| CYCCL1      | Cytochrome c, somatic-like 1 on chromosome 6 | NR_001561.1 | −1.6  | −2.0 | −1.9 | −2.1 | −1.7 |
| LOC684547   | Misc_RNA | XR_017680.1 | −1.7  | −1.9 | −1.9 | −2.1 | −1.8 |
| WDR4        | WD repeat domain 4, TV2 | NM_032661.3 | −1.7  | −1.5 | −1.9 | −2.2 | −1.6 |
| ALDH1A3     | Aldehyde dehydrogenase 1 family, member A3 | NM_006993.1 | −1.7  | −1.7 | −2.0 | −2.4 | −1.9 |
| CLINT1      | Clathrin interactor 1 | NM_014662.2 | −1.7  | −1.6 | −1.8 | −2.0 | −1.6 |
| GNG12       | Guanine nucleotide binding protein (G protein), γ 12 | NM_018841.4 | −1.7  | −2.5 | −1.6 | −2.1 | −1.6 |
| TOMM5       | Translocase of outer mitochondrial membrane 5 homologue, TV1 | NM_001001750.2 | −1.7  | −1.7 | −2.0 | −1.9 | −1.9 |
| MPZL2       | Myelin protein zero-like 2, TV1 | NM_005797.2 | −1.7  | −1.8 | −1.9 | −2.2 | −1.7 |
| DUSP14      | Dual specificity phosphatase 14 | NM_007026.2 | −1.7  | −1.6 | −1.9 | −2.1 | −1.9 |
| IDH1        | Isocitrate dehydrogenase 1 (NADP+), soluble | NM_005896.2 | −1.7  | −2.0 | −1.7 | −1.9 | −1.8 |
| CYT1        | Cytokine-like 1 | NM_018659.2 | −1.7  | −1.8 | −2.0 | −2.1 | −1.6 |
| MLK1        | Mixed lineage kinase domain-like | NM_152649.1 | −1.7  | −1.6 | −1.8 | −2.0 | −1.6 |
| CTHRC1      | Collagen triple helix repeat containing 1 | NM_138455.2 | −1.7  | −1.8 | −1.8 | −2.0 | −1.6 |
| C6orf173    | Chromosome 6 ORF 173 | NM_001012507.1 | −1.7  | −1.6 | −2.1 | −1.8 | −1.8 |
| MGSC4089    | Hypothetical protein | XR_016048.1 | −1.7  | −1.8 | −1.7 | −2.0 | −1.7 |
| KDEL3       | KDEL endoplasmic reticulum protein retention receptor 3, TV1 | NM_006855.2 | −1.7  | −1.6 | −1.8 | −1.8 | −2.0 |
| TNFSF4      | Tumour necrosis factor (ligand) superfamily, member 4 | NM_003326.2 | −1.7  | −1.7 | −1.7 | −2.1 | −1.9 |
| AURKA       | Aurora kinase A, TV5 | NM_198436.1 | −1.7  | −1.7 | −2.1 | −1.9 | −1.9 |
| SMS         | Spermine synthase | NM_004595.2 | −1.7  | −2.0 | −1.8 | −2.0 | −1.7 |
| RND3        | Rho family GTPase 3 | NM_005168.3 | −1.7  | −1.6 | −2.0 | −1.9 | −1.7 |
| CLDN5       | Claudin 5 (transmembrane protein deleted in velocardiofacial syndrome) | NM_003277.2 | −1.7  | −1.4 | −1.8 | −2.1 | −1.9 |
| EDN1        | Endothelin 1 | NM_001955.2 | −1.7  | −1.7 | −2.0 | −1.7 | −1.7 |
| PVRL3       | Poliovirus receptor-related 3 | NM_015480.1 | −1.7  | −1.6 | −2.0 | −2.1 | −1.8 |
| LOX         | Lysyl oxidase | NM_002317.3 | −1.7  | −1.9 | −1.9 | −2.0 | −1.6 |
| ICMT        | Isoprenylcysteine carboxyl methyltransferase | NM_012405.3 | −1.7  | −1.6 | −2.0 | −1.8 | −1.7 |
| PRDX3       | Peroxiredoxin 3, nuclear gene encoding mitochondrial protein, TV1 | NM_006793.2 | −1.7  | −1.9 | −1.8 | −2.1 | −1.6 |
### Table 2. (Continued).

| Gene symbol | Gene name | Accession number | X-bow | Disc | H | L | Y |
|-------------|-----------|------------------|-------|------|---|---|---|
| TUBB6       | Tubulin, β class V | NM_032525.1 | −1.7 | −2.2 | −1.6 | −1.8 | −1.6 |
| VAMP5       | Vesicle-associated membrane protein 5 (myoovrin) | NM_006634.2 | −1.7 | −1.9 | −1.9 | −2.1 | −1.6 |
| MORF4L2     | Mortality factor 4-like 2 | NM_012296.1 | −1.7 | −1.8 | −1.9 | −2.2 | −1.7 |
| NOP56       | NOP56 ribonucleoprotein homologue (yeast), TV1 | NM_006392.2 | −1.7 | −1.8 | −1.9 | −2.1 | −1.7 |
| HNRPK       | Heterogeneous nuclear ribonucleoprotein K, TV3 | NM_031263.1 | −1.7 | −1.8 | −1.8 | −2.0 | −1.9 |
| RNF121      | Ring finger protein 121, TV1 | NM_018320.3 | −1.7 | −1.4 | −2.0 | −2.0 | −1.8 |
| KDELRC2     | KDEL (Lys-Asp-Glu-Leu) containing 2 | NM_153705.4 | −1.7 | −1.7 | −1.9 | −2.2 | −1.8 |
| FJX1        | Four jointed box 1 (Drosophila) | NM_014344.2 | −1.7 | −1.6 | −2.0 | −2.1 | −1.9 |
| DNMT1       | DNA (cytosine-5-methyltransferase 1 | NM_001379.1 | −1.7 | −1.4 | −1.8 | −2.0 | −1.7 |
| LOC729779   | MiscRNA (LOC729779) | XR_019592.2 | −1.7 | −2.0 | −1.8 | −1.6 | −1.7 |
| FABP5       | Fatty acid binding protein 5 (psoriasis-associated) | NM_001444.1 | −1.7 | −1.6 | −1.9 | −2.0 | −1.7 |
| ZDHHC6      | Zinc finger, DHHC-type containing 6 | NM_022494.1 | −1.7 | −1.8 | −1.9 | −2.2 | −1.7 |
| IL1RL1      | Interleukin 1 receptor-like 1 (IL1RL1), TV2 | NM_003856.2 | −1.7 | −1.9 | −1.8 | −2.0 | −1.7 |
| EBN18P2     | EBN1 binding protein 2 | NM_006824.1 | −1.7 | −1.8 | −2.1 | −2.1 | −1.6 |
| TFDP1       | Transcription factor Dp-1 | NM_007111.3 | −1.7 | −1.6 | −1.8 | −2.1 | −1.7 |
| PAICS       | Phosphobovinaminomidaso | NM_006452.3 | −1.7 | −1.7 | −2.0 | −2.2 | −1.6 |
| CSD1        | CDGSH iron sulfur domain 1 | NM_018464.2 | −1.7 | −1.7 | −2.2 | −2.1 | −1.7 |
| LOC100129086 | Similar to HIG1 domain family, member 1A | XM_001725669.1 | −1.7 | −2.1 | −2.0 | −2.1 | −1.7 |
| POL2E4      | Polymerase (DNA-directed), α4, accessory subunit | NM_019896.2 | −1.8 | −1.8 | −2.0 | −2.0 | −1.6 |
| FER1L3      | Fer-1-like 3, myoferlin (Caenorhabditis elegans), TV2 | NM_133337.1 | −1.8 | −1.5 | −1.9 | −2.0 | −1.9 |
| PVR3        | Poliovirus receptor-related 3 | NM_015480.1 | −1.8 | −1.9 | −2.2 | −2.2 | −1.9 |
| RANBP1      | RAN binding protein 1 | NM_002882.2 | −1.7 | −2.1 | −2.2 | −2.3 | −1.8 |
| RAB11A      | RAB11A, member RAS oncogene family | NM_004663.3 | −1.8 | −1.5 | −1.8 | −2.0 | −1.7 |
| SLC38A1     | Solute carrier family 38, member 1, TV1 | NM_030674.3 | −1.8 | −1.7 | −2.1 | −2.0 | −2.0 |
| IL8         | Interleukin 8 | NM_005894.2 | −1.8 | −1.9 | −2.1 | −2.0 | −1.7 |
| LOC100132715 | MiscRNA | XR_039129.1 | −1.8 | −1.5 | −1.9 | −2.0 | −1.6 |
| LOC644330   | Similar to tropomyosin 3 isoform 2 | XR_017492.1 | −1.8 | −2.4 | −2.0 | −2.1 | −1.7 |
| ZNF185      | Zinc finger protein 185 (LM domain) | NM_007150.2 | −1.8 | −1.6 | −1.9 | −2.0 | −1.7 |
| COL13A1     | Collagen, type XIII, x1 | NM_080915.2 | −1.8 | −1.6 | −2.1 | −2.0 | −1.8 |
| PKD2        | Polycystic kidney disease 2 (autosomal dominant) | NM_002972.1 | −1.8 | −1.6 | −1.9 | −2.0 | −2.0 |
| MAGE1D      | Melanoma antigen family D, 1, TV2 | NM_006996.3 | −1.8 | −1.7 | −1.9 | −1.9 | −2.3 |
| POL3        | Polymerase (DNA directed), ε3 (p17 subunit) | NM_017443.3 | −1.8 | −1.6 | −2.0 | −2.1 | −1.7 |
| CORO1C      | Coronin, actin binding protein, 1C, TV1 | NM_014325.2 | −1.8 | −1.5 | −1.8 | −2.0 | −1.8 |
| LOC652481   | Similar to mitochondrial import inner membrane translocase subunit Tim23 | XM_941942.1 | −1.8 | −2.2 | −1.9 | −1.9 | −1.7 |
| SLFN11      | Schlafen family member 11 | NM_152270.2 | −1.8 | −1.4 | −2.0 | −1.9 | −1.8 |
| PRNP        | Prion protein (PRNP), TV3 | NM_001080121.1 | −1.8 | −1.3 | −1.9 | −2.0 | −2.2 |
| FRMD6       | FERM domain containing 6 | NM_152330.2 | −1.8 | −1.8 | −2.1 | −2.2 | −1.9 |
| PTS         | 6-pyruvoyl-tetrahydropterin synthase | NM_000317.1 | −1.8 | −1.8 | −1.9 | −2.0 | −1.5 |
| PECI        | Enoyl-CoA δ isomerase 2 (ECII2), TV1 | NM_006117.2 | −1.8 | −2.4 | −2.2 | −2.5 | −1.9 |
| MGAT2       | Mannosyl-glycoprotein-acetylglucosaminyltransferase, TV2 | NM_001015883.1 | −1.8 | −2.1 | −1.6 | −2.1 | −1.6 |
| ATP6V0E2    | ATPase, H+ transporting V0 subunit e2, TV1 | NM_145230.2 | −1.8 | −1.5 | −2.0 | −1.9 | −1.8 |
| RPL6        | Ribosomal protein L6, TV1 | NM_001024662.1 | −1.8 | −2.0 | −1.9 | −2.2 | −1.8 |
| CGNL1       | Cingulin 1 | NM_032866.3 | −1.8 | −1.8 | −2.2 | −2.3 | −2.0 |
| LDHA        | Lactate dehydrogenase A, TV2 | NM_001135239.1 | −1.8 | −1.8 | −2.0 | −2.1 | −1.9 |
| PGK1        | Phosphoglycerate kinase 1 | NM_000291.2 | −1.8 | −1.9 | −1.9 | −2.2 | −1.8 |
| CCND3       | Cyclin D3 | NM_007160.2 | −1.8 | −1.6 | −2.0 | −2.0 | −2.0 |
| SFRS2       | Serine/arginine-rich splicing factor 2 | NM_0030163 | −1.8 | −1.7 | −2.3 | −2.2 | −1.9 |
| F2RL1       | Coagulation factor II (thrombin) receptor-like 1 | NM_005242.3 | −1.8 | −1.8 | −1.8 | −2.2 | −1.8 |
| PLSCR4      | Phospholipid scramblase 4 | NM_020353.1 | −1.8 | −1.7 | −1.8 | −2.1 | −1.6 |
| KDELRC3     | KDEL endoplasmic reticulum protein retention receptor 3, TV2 | NM_016657.1 | −1.8 | −2.0 | −2.0 | −2.2 | −1.9 |
Table 2. (Continued).

| Gene symbol   | Gene name                                                                 | Accession number | X-bow | Disc H | L   | Y   |
|---------------|---------------------------------------------------------------------------|------------------|--------|--------|-----|-----|
| LOC653226     | Similar to signal recognition particle 9 kDa protein (SRP9)               | XM_927451.2      | -1.8   | -2.2   | -1.8| -2.0| -1.5|
| LOC387882     | Hypothetical protein                                                      | NM_207376.1      | -1.8   | -1.8   | -2.1| -2.1| -1.7|
| PPM1F         | Protein phosphatase, Mg2+/Mn2+ dependent, 1F                              | NM_014634.2      | -1.8   | -1.4   | -1.9| -2.1| -1.7|
| PRICKLE1      | Prickle homologue 1 (Drosophila)                                         | NM_153026.1      | -1.8   | -1.4   | -2.0| -1.7| -1.8|
| TSPAN5        | Tetraspanin 5                                                            | NM_005732.3      | -1.8   | -1.7   | -2.0| -2.2| -1.6|
| PDCD6IP       | Programmed cell death 6 interacting protein                              | NM_013374.3      | -1.8   | -1.7   | -1.8| -2.2| -1.9|
| EFEMP1        | EGF-containing fibulin-like extracellular matrix protein 1               | NM_004105.3      | -1.8   | -3.0   | -1.8| -2.2| -1.7|
| CDC20         | Cell division cycle 20 homologue (S. cerevisiae)                         | NM_001255.2      | -1.8   | -1.9   | -2.1| -1.8| -2.0|
| LOC642590     | Misc_RNA                                                                 | XR_037021.1      | -1.8   | -1.8   | -1.8| -2.2| -1.7|
| PRKAG2        | Protein kinase, AMP-activated, γ2 noncatalytic subunit, Tvβ                | NM_024429.1      | -1.9   | -1.9   | -2.0| -2.1| -2.0|
| MRPL39        | Mitochondrial ribosomal protein L39, Tv1                                 | NM_017446.3      | -1.9   | -1.9   | -1.9| -2.2| -1.7|
| TRAM2         | Translocation associated membrane protein 2                              | NM_012288.3      | -1.9   | -1.6   | -2.0| -2.1| -1.8|
| B4GALTL5      | UDP-GalβGlcNAc β1,4-galactosyltransferase, polypeptide 5                  | NM_004776.2      | -1.9   | -1.8   | -2.2| -2.4| -2.3|
| TUBA1A        | Tubulin, α1β                                                           | NM_006009.2      | -1.9   | -2.0   | -1.8| -2.3| -1.9|
| KPNA2         | Karyopherin α2 (RAG cohort 1, importin α1)                               | NM_002766.2      | -1.9   | -2.3   | -2.0| -2.2| -1.9|
| FERL13        | Fer-like 3, myoferlin (C. elegans) (FERL13), Tv1                          | NM_013451.2      | -1.9   | -1.9   | -1.8| -2.0| -2.0|
| NLGN1         | Neuroligin 1                                                             | NM_014932.2      | -1.9   | -1.9   | -2.3| -2.5| -2.0|
| ALDH3A2       | Aldehyde dehydrogenase 3 family, member A2, Tv2                         | NM_003362.2      | -1.9   | -1.9   | -1.8| -2.1| -1.8|
| LOC732007     | Similar to phosphoglycerate mutase 1                                    | XR_015684.1      | -1.9   | -1.9   | -1.8| -2.2| -2.0|
| C2orf63       | Family with sequence similarity 176, member C                            | NM_058187.3      | -1.9   | -1.7   | -2.2| -1.9| -1.7|
| MSRB3         | Methionine sulfoxide reductase B3, Tv1                                   | NM_198080.2      | -1.9   | -2.1   | -1.7| -2.0| -1.7|
| PLXNA2        | Plexin A2                                                                | NM_025179.3      | -1.9   | -1.5   | -2.1| -1.9| -1.9|
| UCHL3         | Ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)       | NM_006002.3      | -1.9   | -2.0   | -2.3| -2.3| -2.0|
| MT1G          | Metallothionein 1G                                                        | NM_005950.1      | -1.9   | -1.4   | -2.0| -1.8| -1.8|
| NEXN          | Nexilin (F actin binding protein), Tv1                                   | NM_144573.3      | -1.9   | -2.2   | -2.1| -2.1| -1.9|
| CRIM1         | Cysteine rich transmembrane BMP regulator 1 (chordin-like)               | NM_016441.1      | -1.9   | -2.5   | -2.0| -2.4| -1.9|
| LOC644774     | Similar to phosphoglycerate kinase 1                                     | XM_927868.1      | -1.9   | -2.3   | -2.1| -2.4| -2.0|
| UBE2T         | Ubiquitin-conjugating enzyme E2T (putative)                              | NM_014176.2      | -1.9   | -1.9   | -2.0| -2.0| -1.8|
| LOC441019     | Hypothetical LOC441019                                                   | XM_498969.2      | -1.9   | -1.5   | -2.1| -2.1| -2.1|
| PGAM1         | Phosphoglycerate mutase 1 (brain)                                        | NM_002629.2      | -1.9   | -2.4   | -1.8| -1.9| -1.6|
| LPHN2         | Latrophilin 2                                                            | NM_012302.2      | -1.9   | -1.6   | -2.1| -2.3| -1.9|
| EHD4          | EHD-domain containing 4                                                  | NM_139665.2      | -1.9   | -1.6   | -1.9| -2.0| -1.7|
| MYOF          | Myoferlin, Tv1                                                           | NM_013451.3      | -1.9   | -1.8   | -1.9| -2.2| -1.9|
| PTG1          | Pituitary tumour-transforming 1                                           | NM_004219.2      | -1.9   | -2.2   | -2.1| -2.1| -1.8|
| TUBA1C        | Tubulin, α1c                                                             | NM_032704.2      | -1.9   | -2.0   | -1.9| -2.2| -1.9|
| ANXA2         | Annexin A2, Tv2                                                          | NM_001002857.1    | -1.9   | -2.8   | -2.2| -2.5| -1.8|
| FILIP1L       | Filamin A interacting protein 1-like, Tv3                                | NM_001042459.1    | -1.9   | -1.8   | -2.1| -1.9| -2.0|
| TRIP6         | Thyroid hormone receptor interactor 6                                    | NM_003302.2      | -1.9   | -1.8   | -2.1| -1.9| -1.9|
| GIMAP7        | GTPase, IMAP family member 7                                             | NM_153236.3      | -1.9   | -1.9   | -2.1| -2.6| -2.0|
| PECI          | Enoyl-CoA isomerase 2, Tv1                                                | NM_006172.2      | -1.9   | -1.9   | -2.1| -2.1| -1.9|
| TMEM14A       | Transmembrane protein 14A                                                | NM_014051.3      | -1.9   | -2.1   | -2.1| -2.3| -2.3|
| CALD1         | Caldesmon 1 (CALD1), Tv5                                                 | NM_033140.2      | -2.0   | -2.0   | -2.3| -2.1| -2.0|
| LOC402221     | Similar to actin x1 skeletal muscle protein                              | XM_938988.1      | -2.0   | -2.0   | -1.8| -2.2| -2.2|
| CCND2         | Cyclin D2                                                                | NM_017592.2      | -2.0   | -1.7   | -1.9| -2.1| -2.1|
| PRNP          | Prion protein (PRNP), Tv2                                                | NM_183079.2      | -2.0   | -2.0   | -2.2| -2.2| -2.4|
| FRMD6         | FERM domain containing 6, Tv2                                             | NM_152330.3      | -2.0   | -2.0   | -2.0| -2.1| -1.9|
| EFHD2         | EF-hand domain family, member D2                                         | NM_024329.4      | -2.0   | -1.7   | -2.2| -2.2| -2.0|
| AADACL1       | Arylacetamide deacetylase-like 1                                          | NM_020792.3      | -2.0   | -2.3   | -2.2| -2.4| -2.2|
| Gene symbol | Gene name | Accession number | X-bow | Disc | H | L | Y |
|-------------|-----------|-----------------|-------|------|---|---|---|
| TGM2 | Transglutaminase 2, TV1 | NM_004613.2 | -2.0 | -1.8 | -2.3 | -2.1 | -1.9 |
| CAV2 | Caveolin 2 (CAV2), TV1 | NM_001233.3 | -2.0 | -2.7 | -2.3 | -2.6 | -2.0 |
| NNMT | Nicotinamide N-methyltransferase | NM_006169.2 | -2.0 | -2.1 | -2.2 | -2.2 | -2.0 |
| UAP1 | UDP-N-acetylglucosamine pyrophosphorylase 1 | NM_003115.3 | -2.0 | -1.6 | -2.2 | -2.0 | -1.9 |
| TJP2 | Tight junction protein 2 (zona occludens 2), TV2 | NM_201629.1 | -2.0 | -1.8 | -2.2 | -2.0 | -2.0 |
| AURKA | Aurora kinase A, TV3 | NM_198434.1 | -2.0 | -1.9 | -2.2 | -2.1 | -2.1 |
| CSTF3 | Cleavage stimulation factor, 3’ pre-RNA, subunit 3, 77 kDa, TV2 | NM_001033506.1 | -2.0 | -2.2 | -2.1 | -2.3 | -1.9 |
| PTPLA | Protein tyrosine phosphatase-like, member A | NM_014241.3 | -2.0 | -1.9 | -2.1 | -2.3 | -2.0 |
| CAV1 | Caveolin 1, caveolea protein, 22 kDa | NM_001753.3 | -2.0 | -2.0 | -2.3 | -2.3 | -1.9 |
| EXT1 | Exostosin 1 | NM_000127.2 | -2.0 | -1.7 | -2.0 | -2.4 | -2.2 |
| CEN2A | Cyclin A2 | NM_001237.2 | -2.0 | -1.9 | -2.1 | -1.9 | -1.9 |
| CD59 | CD59 molecule, complement regulatory protein, TV2 | NM_006111.4 | -2.0 | -1.5 | -2.0 | -2.1 | -2.1 |
| TUBB2C | Tubulin, β4B class Ib | NM_006088.5 | -2.0 | -1.9 | -2.2 | -2.4 | -2.4 |
| SFRA5 | Splicing factor, arginine/serine-rich 3 | NM_003017.3 | -2.0 | -2.0 | -2.1 | -2.2 | -2.0 |
| RAN | RAN, member RAS oncogene family | NM_006325.2 | -2.0 | -2.2 | -2.3 | -2.4 | -2.4 |
| ADAM9 | ADAM metallopeptidase domain 9, TV1 | NM_003816.2 | -2.0 | -2.8 | -2.0 | -2.3 | -1.9 |
| LRP8 | Low density lipoprotein receptor-related protein 8, TV3 | NM_017522.3 | -2.0 | -1.9 | -2.2 | -2.2 | -2.2 |
| MELK | Maternal embryonic leucine zipper kinase | NM_014791.2 | -2.0 | -2.0 | -2.1 | -2.3 | -2.0 |
| GALNT10 | Polypeptide N-acetylgalactosaminyltransferase 10, TV2 | NM_017540.3 | -2.0 | -1.9 | -1.9 | -2.0 | -1.9 |
| CBX6 | Chromobox homologue 6 | NM_014292.3 | -2.0 | -1.6 | -2.3 | -2.5 | -2.0 |
| CALM1 | Calmodulin 1 (phosphorylase kinase, β) | NM_006888.3 | -2.0 | -1.7 | -2.3 | -2.4 | -2.1 |
| PTTG1 | Pituitary tumour-transforming 1 | NM_004219.7 | -2.1 | -1.9 | -2.1 | -2.2 | -1.8 |
| IL8 | Interleukin 8 | NM_000584.2 | -2.3 | -2.9 | -3.0 | -3.2 | -2.5 |
| IL1RL1 | Interleukin 1 receptor-like 1, TV2 | NM_003856.2 | -2.1 | -2.0 | -2.5 | -2.5 | -1.9 |
| FZD4 | Frizzled homologue 4 (Drosophila) | NM_012193.2 | -2.1 | -1.6 | -2.0 | -2.1 | -2.1 |
| GLCE | Glucuronic acid epimerase | NM_015554.1 | -2.1 | -2.0 | -2.5 | -2.7 | -2.4 |
| UBE2C | Ubiquitin-conjugating enzyme E2C, TV6 | NM_181802.1 | -2.1 | -2.1 | -2.0 | -2.1 | -1.9 |
| FAM176A | Family with sequence similarity 176, member A, TV1 | NM_001135032.1 | -2.1 | -2.1 | -2.1 | -2.2 | -2.2 |
| ICAM2 | Inter cellular adhesion molecule 2, TV1 | NM_00109786.1 | -2.1 | -2.1 | -2.4 | -2.8 | -2.1 |
| TGM2 | Transglutaminase 2, TV2 | NM_198951.1 | -2.1 | -2.0 | -2.5 | -2.4 | -2.0 |
| EPH1A2 | EPH receptor A2 | NM_004431.2 | -2.1 | -1.7 | -2.0 | -2.1 | -2.0 |
| FEN1 | Flap structure-specific endonuclease 1 | NM_004111.4 | -2.1 | -2.2 | -2.6 | -2.5 | -2.2 |
| ATP1B1 | ATPase, Na^+/-K^+ transporting, β1 polypeptide | NM_001677.3 | -2.1 | -2.0 | -2.1 | -2.6 | -2.1 |
| ODZ3 | Ozd, odd Oz-ten-m homologue 3 (Drosophila) | NM_01080477.1 | -2.1 | -1.9 | -2.3 | -2.5 | -2.1 |
| FILIP1L | Filamin A interacting protein 1-like, TV1 | NM_182909.2 | -2.1 | -1.8 | -2.1 | -2.1 | -1.9 |
| NMT2 | N-methyltransferase 2 | NM_004808.1 | -2.1 | -2.1 | -2.4 | -2.5 | -2.3 |
| PHACTR2 | Phosphatase and actin regulator 2, TV1 | NM_001100164.1 | -2.1 | -1.9 | -2.2 | -2.5 | -1.9 |
| TUBA1B | Tubulin, α1b | NM_006082.2 | -2.1 | -1.9 | -2.4 | -2.1 | -2.3 |
| C20orf127 | Chromosome 20 ORF 127 | NM_080757.1 | -2.1 | -1.8 | -2.7 | -2.6 | -2.0 |
| NPFPR2 | Neuropeptide FF receptor 2, TV1 | NM_004885.1 | -2.1 | -2.1 | -2.3 | -2.2 | -2.3 |
| LIM1 | LIM domain and actin binding 1 | NM_016357.3 | -2.2 | -2.1 | -2.2 | -2.2 | -1.9 |
| BASP1 | Brain abundant, membrane attached signal protein 1 | NM_006317.3 | -2.2 | -2.0 | -2.4 | -2.5 | -2.2 |
| TNFRSF12A | Tumour necrosis factor receptor superfamily, member 12A | NM_016639.1 | -2.2 | -1.9 | -2.6 | -2.2 | -2.1 |
| KRT7 | Keratin 7 | NM_005556.3 | -2.2 | -1.8 | -2.2 | -2.2 | -2.0 |
| NCPG | Non-SMC condensin I complex, subunit G | NM_022346.3 | -2.2 | -2.1 | -2.2 | -2.3 | -2.4 |
| CCNA1 | Cyclin A1 | NM_003914.2 | -2.2 | -2.4 | -2.4 | -2.5 | -2.5 |
| DIO2 | Deiodinase, iodothyronine, type II, TV3 | NM_001007023.2 | -2.2 | -2.0 | -2.1 | -1.9 | -2.2 |
| DDAH1 | Dimethylarginine dimethylaminohydrolase 1 | NM_012137.2 | -2.2 | -2.1 | -2.8 | -2.6 | -2.4 |
| CAV1 | Caveolin 1, caveolea protein, 22 kDa | NM_001753.3 | -2.2 | -2.4 | -2.4 | -2.7 | -2.4 |
| TYMS | Thymidylate synthetase | NM_001071.1 | -2.2 | -2.3 | -2.4 | -2.2 | -2.0 |
| GRB14 | Growth factor receptor-bound protein 14 | NM_004490.2 | -2.2 | -2.1 | -2.5 | -2.4 | -2.1 |
| CAV2 | Caveolin 2, TV1 | NM_001233.3 | -2.2 | -2.5 | -2.3 | -2.6 | -2.2 |
| Gene symbol | Gene name                                                                 | Accession number | X-bow | Disc | H   | L   | Y   |
|-------------|---------------------------------------------------------------------------|------------------|-------|------|-----|-----|-----|
| MGLL        | Monoglyceride lipase (MGLL), TV1                                         | NM_007283.5      | −2.2  | −1.8 | −2.1| −2.2| −2.1|
| FILIP1L     | Filamin A interacting protein 1-like, TV2                                | NM_014890.2      | −2.2  | −1.8 | −2.6| −2.5| −2.1|
| CEP55       | Centrosomal protein 55 kDa                                               | NM_018131.3      | −2.3  | −2.2 | −2.2| −2.4| −2.4|
| CALD1       | Cafesmon 1, TV3                                                           | NM_033157.2      | −2.3  | −2.8 | −2.5| −2.3| −1.9|
| UBE2C       | Ubiquitin-conjugating enzyme E2C, TV3                                     | NM_181800.1      | −2.3  | −2.4 | −2.4| −2.7| −2.2|
| MTE         | Metallothionein E                                                         | NM_175621.2      | −2.3  | −2.0 | −3.2| −2.4| −2.5|
| MCM4        | Minichromosome maintenance complex component 4, TV1                      | NM_005914.2      | −2.3  | −2.2 | −2.5| −2.6| −2.3|
| FABP4       | Fatty acid binding protein 4, adipocyte                                  | NM_001442.1      | −2.3  | −2.1 | −2.2| −2.3| −2.5|
| PLOD2       | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, TV2                  | NM_00935.2       | −2.3  | −2.2 | −2.6| −2.8| −2.5|
| TXNRD2      | Thioredoxin reductase 2, nuclear gene encoding mitochondrial protein      | NM_006440.3      | −2.4  | −2.2 | −2.9| −2.8| −2.4|
| LDLR        | Low-density lipoprotein receptor (familial hypercholesterolaemia)         | NM_000527.2      | −2.4  | −2.2 | −2.7| −2.6| −2.5|
| GIMAP4      | GTPase, MAP family member 4                                              | NM_018326.1      | −2.5  | −2.2 | −2.6| −2.9| −2.8|
| PRC1        | Protein regulator of cytokinesis 1, TV2                                   | NM_199413.1      | −2.5  | −2.1 | −2.2| −2.3| −2.2|
| MGLL        | Monoglyceride lipase, TV1                                                 | NM_007283.5      | −2.5  | −2.8 | −2.8| −2.4| −2.4|
| FKG30       | Actin-like protein                                                        | NM_001017421.1   | −2.5  | −2.4 | −2.3| −2.6| −2.2|
| ALDH1A3     | Aldehyde dehydrogenase 1 family, member A3                               | NM_000693.2      | −2.5  | −2.6 | −2.6| −2.9| −2.6|
| CYR61       | Cysteine-rich, angiogenic inducer, 61                                     | NM_001554.3      | −2.5  | −2.1 | −2.5| −2.3| −2.7|
| MAD2L1      | MAD2 mitotic arrest deficient-like 1 (yeast)                              | NM_002358.2      | −2.5  | −2.7 | −2.7| −2.6| −2.6|
| CCL15       | Chemokine (C-C motif) ligand 15, TV1                                      | NM_032964.2      | −2.5  | −2.2 | −2.5| −2.3| −2.3|
| S1PR3       | Sphingosine-1-phosphate receptor 3                                        | NM_000226.2      | −2.5  | −2.0 | −2.5| −2.4| −2.5|
| C6orf105    | Chromosome 6 ORF 105                                                      | NM_002744.1      | −2.5  | −3.1 | −2.7| −2.9| −2.6|
| TACSTD2     | Tumour-associated calcium signal transducer 2                            | NM_002353.1      | −2.6  | −2.2 | −2.6| −3.1| −2.7|
| MT1E        | Metallothionein 1E                                                         | NM_175617.3      | −2.7  | −2.2 | −3.1| −2.7| −2.2|
| PLOD2       | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, TV1                  | NM_182943.2      | −2.7  | −3.1 | −2.9| −3.4| −2.7|
| STC2        | Stanniocalcin 2                                                           | NM_003714.2      | −2.7  | −2.2 | −3.3| −3.0| −2.9|
| SDPR        | Serum deprivation response (phosphatidylserine binding protein)           | NM_004657.4      | −2.8  | −3.1 | −3.2| −3.7| −3.0|
| PLOD2       | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, TV2                  | NM_000935.2      | −2.8  | −2.4 | −2.9| −3.0| −2.9|
| LOC399942   | Similar to tubulin x-2 chain (x-tubulin 2), TV5                          | NM_034471.1      | −3.0  | −3.3 | −2.9| −3.0| −2.8|
| CXCL1       | Chemokine (C-X-C motif) ligand 1                                          | NM_001511.1      | −3.1  | −3.0 | −3.3| −3.2| −2.9|
| UHRF1       | Ubiquitin-like with PHD and ring finger domains, 1, TV1                   | NM_001048201.1   | −3.2  | −2.7 | −3.4| −3.1| −3.5|
| PTGER4      | Prostaglandin E receptor 4 (subtype EP4)                                   | NM_000958.2      | −3.3  | −2.5 | −3.4| −3.9| −3.4|
| MGC87042    | Similar to six transmembrane epithelial antigen of prostate               | NM_001128032.1   | −3.4  | −2.9 | −3.7| −3.8| −3.4|
| TOP2A       | Topoisomerase (DNA) II α 170 kDa                                        | NM_001067.2      | −3.5  | −3.3 | −3.4| −3.5| −3.4|
| LOC399959   | Mtr-100-let-7a-2 cluster host gene (nonprotein coding)                    | NR_024430.1      | −3.6  | −3.0 | −4.2| −4.0| −3.9|
| STEAP1      | Six transmembrane epithelial antigen of the prostate                      | NM_012449.2      | −3.6  | −3.5 | −3.6| −4.0| −3.7|
| BMP4        | Bone morphogenetic protein 4, TV3                                        | NM_130851.1      | −3.6  | −2.8 | −4.3| −3.8| −3.8|
| LOC158376   | Hypothetical protein                                                      | NM_001129749.1   | −3.9  | −3.3 | −3.1| −3.6| −3.6|
| DKK1        | Dickkopf 1 homologue (Xenopus laevis)                                     | NM_012242.2      | −5.2  | −4.0 | −6.2| −6.0| −5.5|
| RGS4        | Regulator of G-protein signalling 4                                       | NM_005613.3      | −7.6  | −6.5 | −9.9| −10.2| −9.0|
Gene X-bow Disc H L Y
---
H1F0 1.6 1.4 1.5 1.5 1.8
TUBB6 −1.3 −1.4 −1.4 −1.6 −1.4
CCT2 −1.4 −1.3 −1.5 −1.6 −1.4
TUBA1C −1.4 −1.3 −1.7 −1.5 −1.5
DYNNL1 −1.4 −1.4 −1.6 −1.7 −1.3
H3F3B −1.4 −1.4 −1.8 −1.7 −1.5
TUBA1A −1.5 −1.4 −1.6 −1.6 −1.7
RBX1 −1.5 −1.4 −1.5 −1.8 −1.5
PCNA −1.5 −1.6 −1.8 −1.8 −1.5
TCP1 −1.5 −2.1 −1.4 −1.8 −1.4
CCTB −1.5 −2.1 −1.5 −1.9 −1.4
BUB3 −1.5 −1.4 −1.5 −1.5 −1.4
CSE1L −1.5 −1.2 −1.5 −1.7 −1.4
TUBB2A −1.5 −1.5 −1.7 −1.5 −1.6
CDK6 −1.6 −1.3 −1.5 −1.5 −1.6
PPP2CA −1.6 −1.6 −1.8 −1.9 −1.7
CSK1B −1.6 −1.6 −1.7 −1.8 −1.6
AURKA −1.7 −1.7 −2.1 −1.9 −1.9
TPD51 −1.7 −1.6 −1.7 −1.9 −1.7
CCND2 −1.7 −1.7 −2.0 −2.2 −2.0
CCND3 −1.8 −1.6 −2.0 −2.0 −2.0
CDC20 −1.8 −1.9 −2.1 −1.8 −2.0
CCNB2 −1.9 −1.8 −1.8 −1.7 −1.9
PTTG1 −1.9 −2.2 −2.1 −2.1 −1.8
CCNA2 −2.0 −1.9 −2.1 −1.9 −1.9
RAN −2.0 −2.2 −2.3 −2.4 −2.0
TUBA1B −2.1 −1.9 −2.4 −2.1 −2.3
NAPG −2.2 −2.1 −2.2 −2.3 −2.4
CCNA1 −2.2 −2.4 −2.4 −2.5 −2.5
MAD2L1 −2.5 −2.7 −2.7 −2.6 −2.6
TOP2A −3.5 −3.3 −3.4 −3.5 −3.4

Table 3. Fold changes in mRNA expression levels of genes involved in cell cycle progression.

Gene X-bow Disc H L Y
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SYNM 2.4 2.1 2.0 2.2 2.4
MMP10 2.0 2.0 1.6 1.4 1.7
MMP1 1.7 1.9 1.3 1.4 1.3
ITGB4 1.7 1.8 1.7 1.7 1.6
JUN 1.6 1.5 1.4 1.4 1.5
RPS6KA5 1.6 1.4 1.5 1.4 1.8
AXIN2 1.5 1.5 1.4 1.4 1.5
MYLK 1.5 1.3 1.5 1.3 1.4
CSNK2A2 1.5 1.5 1.3 1.2 1.4
TUBB6 −1.3 −1.4 −1.4 −1.6 −1.4
CD44 −1.4 −1.6 −1.3 −1.7 −1.4
TUBA1C −1.4 −1.3 −1.7 −1.5 −1.5
FLOT2 −1.4 −1.3 −1.6 −1.7 −1.5
MYL9 −1.4 −1.7 −1.5 −1.6 −1.3
TUBA1A −1.5 −1.4 −1.6 −1.6 −1.7
EIF4G2 −1.5 −2.2 −1.5 −2.0 −1.5
SHC1 −1.5 −1.4 −1.1 −1.3 −1.4
ROCK2 −1.5 −1.2 −1.4 −1.6 −1.5
VCL −1.5 −1.6 −1.4 −1.7 −1.6
ZYX −1.5 −1.4 −2.0 −1.7 −1.7
TUBB2A −1.5 −1.5 −1.7 −1.6 −1.6
ACTN4 −1.6 −1.3 −1.8 −1.7 −1.6
CAV2 −1.6 −1.5 −1.8 −1.8 −1.7
NES −1.7 −1.5 −1.7 −1.8 −1.5
ACTR2 −1.7 −1.5 −1.6 −1.8 −1.5
GNL2 −1.7 −2.5 −1.6 −2.1 −1.6
TUBB6 −1.7 −2.2 −1.6 −1.8 −1.6
TUBG1 −1.7 −1.4 −1.6 −1.9 −1.6
IL8 −1.8 −1.9 −2.1 −2.0 −1.7
TUBA1A −1.9 −2.0 −1.8 −2.3 −1.9
TJP2 −2.0 −1.7 −1.6 −1.7 −1.7
TUBA1B −2.1 −1.9 −2.4 −2.1 −2.3
CAV1 −2.2 −2.4 −2.4 −2.7 −2.4
CXCL1 −3.1 −3.0 −3.3 −3.2 −2.9

Table 4. Fold changes in mRNA expression levels of genes involved in cytoskeletal dynamics and cell adhesion.

Upon demonstrating the reproducibility and applicability of micropatterns to control cellular morphology, we utilized microarray technology to analyze how morphological restriction and unique cellular morphologies affect the HCAEC transcriptome. Our data indicate that morphological restriction (i.e. ability of the cell to spread) is a major regulator of endothelial gene expression patterns, as demonstrated by large-scale changes in gene expression after morphological restriction of HCAECs. Our data indicate that morphological restriction via micropattern adherence greatly increases the incidence of nuclear deformation in HCAECs. Given that large-scale cell shape changes results in a drastic condensation of chromatin as a result of lateral compressive force-induced nuclear orientation shifts and deformation [25], it is possible that restricting cell spreading affects the dynamic genome architecture in the nuclear space, thus regulating gene expression by modulating the geometric constraints that regulate dynamic chromatin positioning. We suspect that shape-induced gene expression changes are more complex than simply a consequence of nuclear deformation given that the transcriptome between each of the micropatterned shapes was remarkably similar, whereas the level of nuclear deformation varied drastically between the individual micropatterns. Indeed, although distinct cell shapes and cytoskeletal patterning have been reported to regulate mesenchymal progenitor lineage differentiation and endothelial cell chromatin condensation [14,25], we were very surprised to discover that shape induced gene expression patterns were remarkably constant across all altered cellular morphologies tested relative to each other. Moreover, considering a recent study suggesting that cell geometry does not regulate the adipogenic differentiation of mesenchymal stem cells [15], further follow-up studies are needed to determine how cellular geometry affects the phenotype of different cell types. Our data do not necessarily...
contradict the report of shape-induced differentiation in mesenchymal progenitor cells [14] but, instead, suggest that there are varying levels of responsiveness to morphology driven cellular outputs between different cell types (mesenchymal progenitor versus coronary artery endothelial cells). Cumulatively, our data suggest that the ability of HCAECs to spread (but not necessarily their particular morphology) dictates their genomics patterns. These data build on and corroborate the findings reported in earlier work indicating that endothelial spreading regulates cell fate decisions between proliferation and death [8,11].

Bioinformatics analysis of the microarray data revealed that the largest functional groupings of genes whose expression was altered upon morphological restriction were those involved in cell cycle regulation (30 genes) and cytoskeletal dynamics/cell adhesion (34 genes). Within the identified cell cycle regulators, a number of genes were strongly involved in spindle assembly, cell cycle phase transition, nucleocytoplasmic transport of cyclins and cyclin-dependent kinases, and chromosome condensation. With the exception of one gene (H1F0, which encodes for a histone protein), the expression the identified cell cycle-related genes was down-regulated, including the major cell cycle promoters CDK6, CCNA1, CCNB2, CCND2 and CCND3. Considering the previously proposed impact of cell shape on chromosome condensation, we were intrigued at the down-regulation of genes involved in DNA accessibility, including condensin (NCAPG), topoisomerase II α (TOP2A), histone H3 (H3F3B) and histone H1 (H1F0). These particular changes could have a role in modulating global gene expression, lineage specification and the cellular physiology of endothelial cells and their progenitors. In mesenchymal progenitor cells, it has been reported that shape-induced contraction enhances c-Jun N-terminal kinase and extracellular-related kinase 1/2 activity in conjunction with wingless-type signalling [14]. Pathway analysis of the microarray data from the shape confirmed that HCAECs revealed shape-induced alterations in the expression of genes involved in Wnt signalling (up-regulation of TCF4 and down-regulation of RUVBL2, SNAI2, FZD4 and DKK1) and an up-regulation in JUN expression, indicating that similar changes in these signalling pathways likely occur when the endothelial cell morphology is altered. Additionally, the expression of several genes encoding members of the TGFB signalling cascades was altered upon changes in HCAEC shape, including the ligands BMP2, BMP4 and TFGB2, the type II receptor BMPR2, and the signalling effectors SMAD6 and SMAD7. Given that aberrant TGFB signalling is critically implicated in the progression of coronary artery disease and arteriosclerosis [31], it is possible that endothelial cell shape changes could initiate and/or exacerbate disease progression via alterations in the expression of key genes involved in these processes.

Materials and methods

Cell culture and treatments

Primary cultures of human coronary artery endothelial cells (HCAECs; < 5 passages; #PCS-100-020; ATCC, Manassas, VA, USA) were cultured in vascular cell basal media...
media (PCS-100-030; ATCC) supplemented with 0.2% bovine brain extract, 5 ng/mL human epidermal growth factor, 10 mM L-glutamine, 0.75 units/mL heparin sulfate, 1 μg/mL hydrocortisone, 50 μg/mL ascorbic acid, 2% fetal bovine serum and pen/strep. For serum starvation experiments, HCAECs were cultured in vascular cell basal media supplemented with 10 mM L-glutamine, 0.75 units/mL heparin sulfate, 1 μg/mL hydrocortisone, 50 μg/mL ascorbic acid and pen/strep for 48 h before RNA collection. For cell shape patterning, collagen I-coated coverslips and 96-well plates with micropatterns were seeded with ~5000 or 50,000 HCAECs per well and coverslip, respectively, in accordance with the manufacturer’s instructions. For the control, cells were seeded at low density approximately equal to that seen in the micropatterned conditions (to minimize cell-to-cell contacts) on collagen I-coated coverslips and 96-well plates. For all patterned conditions (to minimize cell-to-cell contacts), the orientation of the micropattern for all analyses. For automatic detection of actin fibres, we utilized the FIBERSCORE algorithm reported by Lichtenstein et al. [29], which bases the segmentation of fibres on the probability that a pixel neighbourhood belongs to a fibre. The output of the FIBERSCORE algorithm comprises a correlation image (Fig. 2C), which indicates pixels with higher probability of belonging to a fibre, and an orientation image (Fig. 2D), which indicates the orientation of the fibre at each pixel location. To remove fibres from the resulting FIBERSCORE output that are less correlated than other image regions, we performed a two-step post processing method: (a) remove pixels with correlation values below a predetermined threshold (Fig. 2E) and (b) skeletonize the fibre structures with combinations of the basic morphological operations erosion and opening [32] (Fig. 2F). The skeletonization process removes repetitive information within each detected fibre. Individual and median fibre lengths were obtained by measuring the processed fibre length in the skeletonized images.

Immunofluorescence

Micropatterned coverslips (Cytoo Inc.) were fixed in fresh 4% paraformaldehyde, blocked in 0.5% Tween-20, and incubated with 1 : 200 phospho-FAK (#3283; Cell Signaling, Danvers, MA, USA) antibody, 1 : 350 rhodamine-conjugated phalloidin (Cytoskeleton Inc., Denver, CO, USA) and 1 : 1000 DAPI. Anti-phospho-FAK was labelled with a FITC-conjugated secondary antibody and immunofluorescent images were captured in 0.1-μm Z-stacks using a C2SI scanning laser confocal microscope (Nikon, Tokyo, Japan). Images were equivalently processed in NIKON ELEMENTS 3.2, surface rendering images were obtained using IMARIS, version 6.0 (Bitplane AG, Zurich, Switzerland) and three-dimensional deconvolution was performed using Autoquant X3 (Media Cybernetics, Inc., Bethesda, MD, USA).

Quantification of actin fibre length

For each analysis, 11–14 images of each shape from the actin immunofluorescent images were utilized. Images were initially preprocessed by implementing contrast-limited adaptive histogram equalization, which enhances the contrast of the image in small regions rather than as a whole [32] (Fig. 2B). Images are rotated to have consistent orientation of the micropattern for all analyses. For automatic detection of actin fibres, we utilized the FIBERSCORE algorithm reported by Lichtenstein et al. [29], which bases the segmentation of fibres on the probability that a pixel neighbourhood belongs to a fibre. The output of the FIBERSCORE algorithm comprises a correlation image (Fig. 2C), which indicates pixels with higher probability of belonging to a fibre, and an orientation image (Fig. 2D), which indicates the orientation of the fibre at each pixel location. To remove fibres from the resulting FIBERSCORE output that are less correlated than other image regions, we performed a two-step post processing method: (a) remove pixels with correlation values below a predetermined threshold (Fig. 2E) and (b) skeletonize the fibre structures with combinations of the basic morphological operations erosion and opening [32] (Fig. 2F). The skeletonization process removes repetitive information within each detected fibre. Individual and median fibre lengths were obtained by measuring the processed fibre length in the skeletonized images.
difference between individual cell distributions and the cumulative distributions. This tile-by-tile comparison is used to pinpoint similar regions between cell shapes that can be result in less uniqueness in global shape comparisons. Both methods count the number of null hypothesis rejections (at a significance level of 0.05) and normalize according to the number of KS tests.

**Gene expression analysis**

For each shape tested, as well as the nonrestricted controls, ~5000 HCAECs were grown in each well of a 96-well micropatterned plate. This was replicated in 16 independent wells per plate to minimize experimental error. Total RNA for each shape was isolated using the Purelink RNA Micro kit (Invitrogen, Grand Island, NY, USA) after 24 h of the cells adhering to the substrate. The isolated RNA from the replicates (5000 cells per shape multiplied by 16 independent replicates) were pooled, amplified and biotin-labelled using an Illumina TotalPrep RNA Amplification Kit (Illumina, San Diego, CA, USA). Some 750 ng of biotinylated aRNA was then briefly heat-denatured and loaded onto expression arrays to hybridize overnight. Following hybridization, arrays were labelled with Cy3-streptavidin and imaged using the Illumina ISCAN. Intensity values were transferred to Gene Expression Omnibus (standard growth conditions = accession number GSE43349; Serum starvation conditions = accession number GSE44168). For confirmation of microarray results, RNA from normal- and cross-bow-shaped cells was converted to cDNA using the Verso cDNA kit (Thermo-Scientific, Waltham, MA, USA) and quantitative PCR was performed using SYBR Green probes (Invitrogen) with an ABI7900HT real-time PCR instrument (Invitrogen).

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