Online supplementary material and methods

1. Expanded methods section
Animal procedures and cell cultures

Cardiac left ventricular fibroblasts were enzymatically isolated from hearts of male Wistar rats (Rattus norvegicus, 7-8 weeks old) as previously described\textsuperscript{19}. In brief, after heparine injection (625 units/100 g), deep anesthesia was induced with pentobarbital (50 mg/100 g body weight). The heart was removed and washed in sterile Joklik´s medium. Consequently, the aorta was cannulated and the heart was mounted on a Langendorff perfusion system. The hearts were perfused with Joklik´s medium for 5 minutes followed by Joklik´s medium containing 0.01% collagenase A (Roche, Germany) and 1% BSA during 30 minutes. The minced and filtered tissue suspension was settled for 15 minutes and the supernatant was centrifuged at 350 g for 10 minutes. The pellet was resuspended in DMEM (Gibco, Invitrogen, Belgium) supplemented with 10% fetal bovine serum (FB, Gibco, Invitrogen, Belgium), and 1% of penicillin/streptomycin solution and seeded in a 75 cm\textsuperscript{2} tissue culture flask. The cell cultures were then pre-incubated at 37°C in humidified air (humidity 95%) with 5% CO\textsubscript{2} for 6 hours. The medium with unattached cells was aspirated and fresh DMEM supplemented with 10% FB and penicillin/streptomycin was added. The medium was replaced every two days and cells were grown to confluence and then passaged with trypsin-EDTA. Under these conditions of isolation, smooth muscle and endothelial cells were rarely observed in the primary cultures. After second passage, Fb were seeded at a density of 2600 cells/cm\textsuperscript{2} and were treated 1) for 4 days with fresh DMEM medium with 10% FB containing either SD-208 (3 \textmu mol/L; Sigma-Aldrich, Belgium) which is a specific TGF-\textbeta receptor I kinase
inhibitor, 2) for 6 days with recombinant human TGF-β1 (400 pmol/L; PeproTech, USA) or 3) with a combination of SD-208 (3 µmol/L) and TGF-β1 (400 pmol/L). Spontaneous differentiation of Fb into proliferating myofibroblasts (p-MyoFb) was induced in normal culture medium for a period of 4 days. To investigate dedifferentiation of MyoFb, we added SD-208 (3 µmol/L) to p-MyoFb and TGF-β1 treated cultures during 4 days after washing of the cultured cells with standard medium. Reversal of TGF-β receptor I kinase inhibition was studied by applying TGF-β1 (400 pmol) to SD-208 + TGF-β1 pretreated Fb cultures. Afterwards cells were either prepared for immunostaining or trypsinated for proliferation assay, PCR, Western blotting and 3-DCM development. Furthermore, we also investigated the effect of Rho-kinase inhibitors by supplementing Y-27632 (10 µmol/L, CalBiochem 688001, Belgium) to DMEM culture medium for a period of 4 days. Afterwards, these cultures were trypsinated for PCR analysis. To evaluate proliferation capacity, 24 well culture plates (Costar, Corning Incorporated, USA) were seeded with 5000 cells per well and treated during 12 days with SD-208, TGF-β1 or with standard medium (control). After 12 days in culture, cells were trypsinated, stained with trypan blue and counted in a Bürker counting chamber.

**Unrestrained 3-Dimensional collagen matrices**

The ability of cardiac Fb and MyoFb to contract extracellular matrix was evaluated with disc-shaped unrestrained 3 dimensional collagen matrices and were prepared from rat tail collagen. A 15 ml collagen solution was prepared by mixing 5.50 ml collagen in 2.80 ml 100 mmol/l NaOH, 1.50 ml 10 × MEM Earle´s Salts (Gibco, Invitrogen, Belgium) and 5.20 ml of bidistilled water to obtain a final concentration of 1.50 mg collagen per ml.
The solution was kept on ice until addition of cells. 500 µl of this collagen solution was added to each BSA-treated well (24-well dish). Approximately 1x10^5 cells from second passaged cultures of p-MyoFb, SD-208 treated, TGF-β1 treated, Y-27632 treated cells were added in DMEM (Gibco, Invitrogen, Belgium) to this solution in the wells and incubated for 1 h at 37 °C for gel polymerization. Subsequently, 1 ml of DMEM was added to each well. The gels were detached spontaneously from the bottom of the wells and placed in a tissue culture incubator for 1–3 days. The volume of the collagen matrices was estimated by measurement of the 3H_2O distribution between the 3-DCM and medium. The gel volumes of the collagen matrices populated with cultured cells and of the collagen matrices without cells were compared. The influence of serum on the retraction of the collagen matrix was determined by calculating the reduction in matrix surface area between non-treated and serum-treated matrices. All experiments were performed in triplicate.

**Restrained 3-Dimensional collagen matrices**

Toroid-shaped restrained 3 dimensional collagen matrices were prepared by mixing fibroblastic cells with collagen type I and concentrated serum-containing culture medium (2x DMEM, 20% horse serum, 1% penicillin and streptomycin). pH was corrected to 7.4 by titration with NaOH (0.1N). The reconstitution mix was pipetted into circular casting molds and incubated for 30 to 45 minutes at 37°C and 5% CO2 to allow hardening of the reconstitution mixture. Thereafter, 6 ml serum-containing culture medium (DMEM, 20% horse serum, 1% penicillin and streptomycin) was added to each dish. Collagen rings
were cultured during 14 days. Culture media were changed 12 hours after casting and then every other 2 days while the culture was performed in casting molds.

**Immuno-staining of isolated cells**

Immunostaining of cultured cells was performed with Envision systems (Dako, Belgium) in slides (Becton-Dickinson, Belgium). The cells were fixed in methanol at –20°C for 5 minutes and treated with Triton X-100 in PBS followed by 1% H2O2 for 5 minutes. After blocking the non-specific binding with 1% BSA, cells were incubated at room temperature for 45 minutes with a primary antibody for α-SMA (Clone 1A4, Sigma, Belgium, dilution 1:400) or vinculin (H-300, Santa Cruz, Germany, dilution 1:50). The cells were then washed 3 x 2 minutes with Triton X-100 in PBS and consequently incubated for 30 minutes with anti-mouse IgG (Envision, Dako, Belgium, dilution 1:100). Secondary Ab was also added to cells without primary antibody to check for non-specific binding. Immunostaining was visualized by applying 3-amino-9-ethylcarbazole (AEC) for 10 minutes followed by a wash in 0.05M sodiumacetate (pH 4.9). Finally, nuclei were counterstained with Mayer’s haematoxylin and mounted in DPX. 150 cells from six randomly chosen fields were analyzed. Furthermore, the presence of stress fibers in cultured cells was evaluated by using Rhodamin-phalloidin actin staining (Invitrogen, Molecular Probes, Belgium, dilution 1:40). Cells were fixed during 30 minutes with 1% paraformaldehyde in PBS pH 7.4, permeabilized with 0.1% Triton X-100 and incubated with phalloidin-actin for 1 hour.
**Histology and immunohistochemistry**

Unrestrained 3-DCM were fixed in 1% paraformaldehyde in PBS (pH 7.4), permeabilized with 1% Triton X-100 and incubated with α-SMA antibody (Clone 1A4, Sigma, Belgium, dilution 1:400) for 1 hour. After washing, gels were incubated with a secondary labeled IgG antibody (Alexa 488, Invitrogen, Molecular Probes, Belgium). Consequently, the gels were double-stained with Rhodamin-Phalloidin actin for 45 minutes. Gels were washed with PBS, mounted on a glass slide by squeezing gently with the coverslip and analyzed with confocal microscopy. Restrained 3-DCM were fixed overnight in freshly prepared 4% paraformaldehyde in PBS pH 7.4 and were routinely embedded in paraffin. Serial sections of 7.5 µm were deparaffinized and antigen retrieval was performed in prewarmed citrate buffer for 20 minutes. After treatment with H2O2 diluted in methanol during 20 minutes, sections were washed and immunostained using α-SMA antibody (Clone 1A4, Sigma, Belgium, dilution 1:400) with overnight incubation. The following day, sections were incubated with biotin labeled secondary antibody followed by amplification with the Tyramide signal amplification system (Perkin Elmer, Belgium TSA Indirect NEL700001KT). Cells were analyzed for positivity in a 500 µm x 500 µm area of each section. Images were acquired using Carl Zeiss Axiovert 200M Inverted Microscope, numerical aperture (NA) lenses (Plan-Neofluar 10x/0.3, Plan-Neofluar 40x/1.3 Oil DIC), and a modern confocal scanhead (Zeiss LSM510). As excitation light sources, Argon (488 nm) and HeNe (543 nm) lasers were used. Image analysis was performed using Axiovision, LSM 510 and ImageJ (National Institutes of Health, http://rsb.info.nih.gov/ij/, USA) software. 3D reconstruction was made using ImageJ 3D viewer. Morphometric analyses were performed using a Zeiss...
Axioplan microscope with an Axiocam HrC camera and Axiovision morphometry software (Zeiss).

**Sircol collagen assay and cytokine array**

Fb were plated in T175 cm² culture flasks and differentiated into either Fb, p-MyoFb and non-p-MyoFb as described above. Standard culture medium was replaced by colorless DMEM medium (Gibco, Invitrogen, Belgium) containing 0.5% FBS. After 3 days in culture, cells were harvested, counted and lysed in 100 µl PBS + 0.5% Tx100. Conditioned medium was collected and used for cytokine analysis. All samples were were frozen in -80°C. Total intracellular collagen production was determined using the Sircol Collagen Assay kit (Biocolor, County Antrim, UK). Measurements were performed according to the manufacturer’s instructions. Samples were analyzed using a Bio-TEK EL808 (Winooski, USA) absorption reader at 540 nm. Collagen concentration was determined by a collagen standard series using bovine collagen (0.5 mg/ml) and normalized to cell number.

Cytokine secretion was measured in conditioned media using the Quantibody Rat Cytokine Array 2 (RayBiotech, Norcross, USA). Simultaneous quantification of 10 cytokine concentrations i.e. ICAM-1, IFNγ, IL-1β, IL-6, IL-10, Leptin, L-selectin, MCP-1, TIMP-1 and TNFα) was performed on a GenePix 4000B Microarray scanner and fluorescent signals were detected in the Cy3 channel. Data were acquired with the GenePix Pro 6.0 software. Raw data were further analyzed using a linear regression standard curve in the Q-Analyzer software (RayBiotech) and normalized to cell number.
Micro-array transcriptome analysis

RNA concentration and purity were determined spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies) and RNA integrity was assessed using a Bioanalyser 2100 (Agilent). Using the Ambion WT Expression Kit, per sample, an amount of 100 ng of total RNA spiked with bacterial poly-A RNA positive controls (Affymetrix) was converted to double stranded cDNA in a reverse transcription reaction. Next the sample was converted and amplified to antisense cRNA in an *in vitro* transcription reaction which was subsequently converted to single stranded sense cDNA. Finally, samples were fragmented and labeled with biotin in a terminal labeling reaction according to the Affymetrix WT Terminal Labeling Kit. A mixture of fragmented biotinylated cDNA and hybridisation controls (Affymetrix) was hybridised on Affymetrix GeneChip® Rat Gene 2.0 ST array followed by staining and washing in a GeneChip® fluidics station 450 (Affymetrix) according to the manufacturer’s procedures. To assess the raw probe signal intensities, chips were scanned using a GeneChip® scanner 3000 (Affymetrix). Biological pathway and network analysis were analyzed using Ingenuity Pathway Analysis software (IPA).

Statistical analysis

Values are expressed as mean ± SEM. All the data were obtained in cell cultures from primary isolation of 3 to 6 animals as indicated in the figure legends. All comparisons of fibroblast phenotypes were performed in matched cultures i.e. derived from the primary cell isolation; values in the figure legends refer to the number of animals from which primary cells were isolated. The statistical methods used are repeated measures analysis
of variance, Student’s two-tailed test for unpaired data, one-way analysis of variance with Bonferroni’s multiple comparison tests.

Intensity values from micro-array analysis were normalized using Robust Multi-array Average (RMA) and compared with limma (Bioconductor). Micro-array p-values were corrected for multiple testing with Benjamin-Hochberg and core analysis was performed with log ratio 1. A p value < 0.05 is considered significant.
2. Supplementary data

**SD-208 treated fibroblasts**

**Supplemental Figure 1: Configuration of stress fibers and $\alpha$-SMA incorporation in fibroblastic cells in unrestrained collagen matrices.**

A) Dendritic Fb in the center of the matrix and cultured without serum shows no stress fibers and only diffuse $\alpha$-SMA. B) Elongated proto-MyoFb at the periphery of the matrix shows stress fibers without $\alpha$-SMA incorporation (B, insert). C) Elongated p-MyoFb with co-localization of f-actin (D) and $\alpha$-SMA (E). (F) elongated non-p-MyoFb with co-localization of f-actin (G) and $\alpha$-SMA actin (H) in the absence of serum. Double immuno-staining shows stress fibers (red) and $\alpha$-SMA (green); scale bars represent 5 (A-H) and 20 (B insert, G) $\mu$m.
Supplemental Figure 2: 3-D reconstruction of fibroblastic cells in unrestrained 3-DCM. Cells are stained with Rhodamin-Phalloidin. A, B) Dendritic Fb; C, D) elongated p-MyoFb; E,F) elongated non p-MyoFb. All cells have a flattened phenotype in the YX and ZX plane of orientation. B, D) Higher magnification of cellular extensions indicated by arrows in (A) and (C). F) Higher magnification of the stress fiber network in the elongated non-p-MyoFb indicated by arrow in E. Scale bars represent 20 (A and E), 10 (C), 4 (B and F) and 2 (D) µm.
Supplemental Figure 3: Intercellular networks in the center of unrestrained collagen matrices. Cells are stained with Rhodamin-Phalloidin.

Fb (A, D), p-MyoFb (B, E) and non-p-MyoFb (C, F); A-C) 3-DCM are in the absence and D-F) presence of serum. G) 3-D reconstruction of the intercellular network showing cell contacts. Scale bars represent 40 (G) and 50 µm (A-F).
Supplemental Figure 4: Intercellular networks at the periphery of unrestrained collagen matrices.

Cells are stained with Rhodamin-Phalloidin. Fb (A, D), p-MyoFb (B, E) and non p-MyoFb (C, F) are cultured in 3-DCM in the absence (A-C) and presence (D-F) of serum. Scale bars represent 100 µm (A-F).
Supplemental Figure 5: Effect of p-MyoFb dedifferentiation on cell size. Total percentage of cells with α-SMA positive stress fibers arranged according to their cell-size in p-MyoFb and in SD-208 treated p-MyoFb cultures.

Supplemental video 1:
3-D reconstruction showing the flatness of a dendritic Fb in an unrestrained collagen matrix after 1 day in culture. Images were acquired using a laser-scanning confocal microscope (Carl Zeiss Axiovert 200M Inverted Microscope) and analyzed with Image J 3D software.

Supplemental video 2:
3-D reconstruction showing the flatness of a non-p-MyoFb network in an unrestrained collagen matrix after 1 day in culture. Images were acquired using a laser-scanning confocal microscope (Carl Zeiss Axiovert 200M Inverted Microscope) and analyzed with Image J 3D software.
Table 1: primers and reverse transcription conditions

|        | Primer 5' → 3'                                      |
|--------|-----------------------------------------------------|
| MRTF-A-F | 5'-GCCCGTCAGAACAGCACT-3'                           |
| MRTF-A-R | 5'-CCGCAACTTCAGCTCCTG-3'                           |
| MRTF-B-F | 5'-GCTGAGAAGGACCGCAAACCTT-3'                       |
| MRTF-B-R | 5'-TGGAGATGGGCTGGCTACAG-3'                          |
| GAPDH-F | 5'-TCATCATCTCCGCCCTTCCGC-3'                         |
| GAPDH-R | 5'-AGGCGGCAATGTCAGATCCACAACG-3'                     |
Table 2: top 30 of differentially expressed genes

2A: genes with higher expression in Fb vs p-MyoFb

| Gene description                                                                 | transcript_cluster_id | p-value     |
|---------------------------------------------------------------------------------|-----------------------|-------------|
| natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C) | 17745928              | 2.54E-06    |
| sphingomyelin phosphodiesterase 3, neutral membrane                             | 17733304              | 1.04E-05    |
| aldehyde dehydrogenase 3 family, member A1                                     | 17646585              | 1.70E-05    |
| G protein-coupled receptor 146                                                  | 17676317              | 1.83E-05    |
| interleukin 6 receptor                                                          | 17748948              | 1.89E-05    |
| adhesion molecule with Ig like domain 2                                          | 17840498              | 2.28E-05    |
| recombination signal binding protein for immunoglobulin kappa J region          | 17796762              | 2.66E-05    |
| arrestin, beta 1                                                                | 17618601              | 3.85E-05    |
| amiloride-sensitive cation channel 2, neuronal                                   | 17832236              | 3.85E-05    |
| TBC1 domain family, member 2B                                                   | 17854640              | 3.85E-05    |
| N-myc downstream regulated 1                                                     | 17837791              | 4.81E-05    |
| presenilin 2                                                                    | 17687143              | 5.14E-05    |
| ral guanine nucleotide dissociation stimulator,-like 1                           | 17685176              | 5.57E-05    |
| xanthine dehydrogenase                                                          | 17814063              | 6.32E-05    |
| adenosine A2a receptor                                                           | 17757925              | 6.95E-05    |
| serum/glucocorticoid regulated kinase 1                                          | 17627802              | 7.81E-05    |
| interferon regulatory factor 1                                                  | 17645837              | 7.81E-05    |
| epidermal growth factor receptor                                                | 17691651              | 8.10E-05    |
| ST3 beta-galactoside alpha-2,3-sialyltransferase 1                               | 17837809              | 9.71E-05    |
| RAS-like family 11 member A                                                     | 17675684              | 9.85E-05    |
| integrin, alpha 3                                                               | 17660303              | 0.00010499  |
| similar to hypothetical protein LOC340061                                       | 17725639              | 0.00010499  |
| selenium binding protein 1                                                      | 17749334              | 0.000107005 |
| poly (ADP-ribose) polymerase family, member 10                                  | 17838530              | 0.000154261 |
| interferon gamma inducible protein 47                                           | 17645314              | 0.000162164 |
| protein tyrosine phosphatase, receptor type, K, extracellular region            | 17627583              | 0.000171035 |
| selectin P ligand                                                               | 17674659              | 0.000191372 |
| RT1 class I b, locus S3                                                         | 17752507              | 0.000191372 |
| aldehyde oxidase 1                                                              | 17859335              | 0.000191372 |
| sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A | 17736974              | 0.000191913 |
2B: genes with lower expression in Fb vs p-MyoFb

| Gene Description                                                                 | transcript_cluster_id | p-value    |
|---------------------------------------------------------------------------------|-----------------------|------------|
| prostate transmembrane protein, androgen induced 1                              | 17779781              | 2.63E-07   |
| collagen, type XI, alpha 1                                                       | 17742074              | 1.34E-06   |
| similar to RIKEN cDNA 4931406P16                                                | 17631956              | 2.54E-06   |
| periostin, osteoblast specific factor                                            | 17738503              | 4.56E-06   |
| fibulin 2                                                                        | 17785473              | 4.56E-06   |
| WAP four-disulfide core domain 1                                                | 17730566              | 5.36E-06   |
| microfibrillar-associated protein 4                                              | 17646600              | 1.04E-05   |
| SERTA domain containing 4                                                        | 17687676              | 1.04E-05   |
| dihydropyrimidinase-like 3                                                      | 17726013              | 1.50E-05   |
| latent transforming growth factor beta binding protein 2                         | 17823226              | 1.50E-05   |
| zinc finger protein 521                                                          | 17724896              | 1.70E-05   |
| glutaminase                                                                     | 17859134              | 1.70E-05   |
| PDZ and LIM domain 3                                                             | 17711787              | 2.06E-05   |
| cytokine receptor-like factor 1                                                  | 17710939              | 2.13E-05   |
| 1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon) | 17712746              | 2.13E-05   |
| carbohydrate (chondroitin 4) sulfotransferase 11                                 | 17834711              | 3.74E-05   |
| gliomedin                                                                       | 17844208              | 3.74E-05   |
| similar to KIAA1357 protein                                                      | 17610635              | 3.97E-05   |
| kin of IRRE like 3 (Drosophila)                                                  | 17842978              | 4.81E-05   |
| runt-related transcription factor 1                                              | 17668012              | 5.19E-05   |
| integrin, beta 5                                                                | 17669524              | 5.63E-05   |
| chemokine-like receptor 1                                                        | 17674687              | 6.95E-05   |
| zinc finger protein 385D                                                         | 17701214              | 6.95E-05   |
| neural cell adhesion molecule 1                                                  | 17852225              | 6.95E-05   |
| PDZ domain containing RING finger 3                                              | 17793653              | 7.78E-05   |
| plexin domain containing 2                                                       | 17717308              | 9.36E-05   |
| integrin, alpha 11                                                               | 17844887              | 0.000112078|
| NADPH oxidase 4                                                                  | 17618091              | 0.000142524|
| serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 | 17658629              | 0.000146881|
| kinesin family member 26B                                                        | 17682462              | 0.000154261|
**2C: genes with higher expression in Fb vs non-p-MyoFb**

| Gene Description                                                   | transcript_cluster_id | p-value       |
|-------------------------------------------------------------------|-----------------------|---------------|
| selenium binding protein 1                                      | 17749334              | 1.62E-06      |
| sphingomyelin phosphodiesterase 3, neutral membrane               | 17733304              | 1.77E-06      |
| cyclin-dependent kinase inhibitor 1B                              | 17787711              | 2.36E-06      |
| aldehyde dehydrogenase 3 family, member A1                        | 17646585              | 2.42E-06      |
| arrestin, beta 1                                                   | 17618601              | 2.42E-06      |
| adenosine A2a receptor                                             | 17757925              | 2.63E-06      |
| recombination signal binding protein for immunoglobulin kappa J region | 17796762              | 2.70E-06      |
| natriuretic peptide receptor C/guanylate cyclase C (atrioatriuretic peptide receptor C) | 17745928              | 2.85E-06      |
| RAS-like family 11 member A                                       | 17675684              | 2.85E-06      |
| histone cluster 2, H2ab                                            | 17740971              | 2.86E-06      |
| prolyl 4-hydroxylase, alpha polypeptide II                         | 17645851              | 3.26E-06      |
| adhesion molecule with Ig like domain 2                            | 17840498              | 3.84E-06      |
| G protein-coupled receptor 146                                     | 17676317              | 3.88E-06      |
| interleukin 6 receptor                                            | 17748948              | 3.88E-06      |
| xanthine dehydrogenase                                            | 17814063              | 3.88E-06      |
| aldehyde dehydrogenase 3 family, member B1                        | 17639065              | 3.88E-06      |
| RAB3B, member RAS oncogene family                                 | 17800802              | 3.88E-06      |
| histone cluster 1, H4m                                             | 17715824              | 3.88E-06      |
| similar to histone 1, H2ai                                        | 17719495              | 3.88E-06      |
| protein tyrosine phosphatase, non-receptor type 1                 | 17769213              | 4.69E-06      |
| similar to Shb protein                                             | 17807027              | 4.80E-06      |
| aldehyde oxidase 1                                                 | 17859335              | 4.97E-06      |
| selectin P ligand                                                 | 17674659              | 5.80E-06      |
| ral guanine nucleotide dissociation stimulator,-like 1             | 17685176              | 6.49E-06      |
| serum/glucocorticoid regulated kinase 1                            | 17627802              | 7.68E-06      |
| integrin, alpha 3                                                  | 17660303              | 8.08E-06      |
| atonal homolog 8 (Drosophila)                                      | 17792236              | 8.08E-06      |
| toll-like receptor 5                                               | 17682865              | 8.95E-06      |
| epidermal growth factor receptor                                   | 17691651              | 1.06E-05      |
| autism susceptibility candidate 2-like                             | 17673265              | 1.06E-05      |
## 2D: genes with lower expression in Fb vs non-p-MyoFb

| Gene Description                                                                 | transcript_cluster_id | p-value   |
|---------------------------------------------------------------------------------|-----------------------|-----------|
| prostate transmembrane protein, androgen induced 1                              | 17779781              | 4.50E-08  |
| collagen, type XI, alpha 1                                                      | 17742074              | 8.09E-08  |
| similar to RIKEN cDNA 4931406P16                                                | 17631956              | 8.27E-08  |
| periostin, osteoblast specific factor                                            | 17738503              | 7.91E-07  |
| WAP four-disulfide core domain 1                                               | 17730566              | 7.91E-07  |
| fibulin 2                                                                       | 17785473              | 1.72E-06  |
| kin of IRRE like 3 (Drosophila)                                                | 17842978              | 1.72E-06  |
| microfibrillar-associated protein 4                                             | 17646600              | 1.77E-06  |
| SERTA domain containing 4                                                       | 17687676              | 1.77E-06  |
| latent transforming growth factor beta binding protein 2                        | 17823226              | 1.77E-06  |
| glutaminase                                                                     | 17859134              | 1.77E-06  |
| 1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon) | 17712746 | 1.77E-06 |
| zinc finger protein 521                                                         | 17724896              | 2.36E-06  |
| NADPH oxidase 4                                                                 | 17618091              | 2.36E-06  |
| dihydropyrimidinase-like 3                                                      | 17726013              | 2.38E-06  |
| platelet-derived growth factor alpha polypeptide                                | 17672282              | 2.38E-06  |
| pleckstrin homology domain containing, family G (with RhoGef domain) member 4 | 17729397              | 2.55E-06  |
| peroxidasin homolog (Drosophila)                                               | 17821373              | 2.70E-06  |
| semaphorin 7A, GPI membrane anchor                                              | 17844592              | 2.85E-06  |
| cAMP responsive element binding protein 3-like 2                               | 17790649              | 2.85E-06  |
| zinc finger protein 385D                                                        | 17701214              | 2.86E-06  |
| cytokine receptor-like factor 1                                                | 17710939              | 2.88E-06  |
| UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (GalNAc-T10) | 17646029 | 3.26E-06 |
| similar to Putative protein C21orf62 homolog                                   | 17667911              | 3.26E-06  |
| protease, serine, 35                                                           | 17846397              | 3.38E-06  |
| growth arrest and DNA-damage-inducible, beta                                  | 17833546              | 3.38E-06  |
| runt-related transcription factor 1                                             | 17668012              | 3.88E-06  |
| PDZ domain containing RING finger 3                                             | 17793653              | 3.88E-06  |
| complement component 1, q subcomponent-like 3                                  | 17720967              | 3.88E-06  |
| microtubule-associated protein 1B                                              | 17744691              | 3.88E-06  |
2E: genes with higher expression in non-p-MyoFb vs p-MyoFb

| Gene Description                                             | transcript_cluster_id | p-value      |
|--------------------------------------------------------------|-----------------------|--------------|
| pleckstrin homology domain containing, family G              | 17729397              | 0.000689309  |
| (with RhoGef domain) member 4                               |                       |              |
| semaphorin 7A, GPI membrane anchor                           | 17844592              | 0.000689309  |
| coiled-coil domain containing 3                             | 17720770              | 0.000689309  |
| complement component 1, q subcomponent-like 3               | 17720967              | 0.002030411  |
| CD34 molecule                                               | 17683339              | 0.002779918  |
| transmembrane protein with EGF-like and two                 | 17798789              | 0.007051762  |
| follistatin-like domains 1                                  |                       |              |
| v-ets erythroblastosis virus E26 oncogene homolog (avian)  | 17668088              | 0.009399225  |
| microRNA mir-181b-2                                         | 17761924              | 0.015048072  |
| cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)  | 17808573              | 0.019150091  |
| microRNA mir-181a-2                                         | 17761922              | 0.019150091  |
| matrilin 4                                                  | 17779191              | 0.019581473  |
| NADPH oxidase 4                                             | 17618091              | 0.022299972  |
| similar to Golgi phosphoprotein 2 (Golgi membrane protein GP73) | 17714096              | 0.022695042  |
| carbonic anhydrase 3                                        | 17746221              | 0.022695042  |
| beta-site APP-cleaving enzyme 2                             | 17664985              | 0.022695042  |
| potassium voltage gated channel, shaker related subfamily, | 17795064              | 0.028604028  |
| member 6                                                    |                       |              |
| ADAM metallopeptidase with thrombospondin type 1 motif, 12  | 17736515              | 0.030128347  |
| argininosuccinate synthase 1                               | 17761221              | 0.039107943  |
| fin bud initiation factor homolog (zebrafish)               | 17775237              | 0.03942209   |
| ankylosis, progressive homolog (mouse)                     | 17736813              | 0.04116751   |
| protease, serine, 35                                        | 17846397              | 0.042917123  |
| kelch repeat and BTB (POZ) domain containing 5             | 17856642              | 0.045664818  |
2F: genes with lower expression in non-p-MyoFb vs p-MyoFb

| Gene Description | transcript_cluster_id | p-value     |
|------------------|-----------------------|-------------|
| similar to histone 1, H2ai | 17719495 | 0.002779918 |
| histone cluster 1, H4m | 17715824 | 0.005772545 |
| Kruppel-like factor 4 (gut) | 17807351 | 0.007051762 |
| selenium binding protein 1 | 17749334 | 0.009399225 |
| histone cluster 2, H2ab | 17740971 | 0.009399225 |
| antigen identified by monoclonal antibody Ki-67 | 17637913 | 0.009399225 |
| cyclin A2 | 17746941 | 0.009399225 |
| topoisomerase (DNA) II alpha | 17660863 | 0.009862346 |
| ribonucleotide reductase M2 | 17815078 | 0.009862346 |
| cysteine-serine-rich nuclear protein 1 | 17856536 | 0.009862346 |
| stathmin 1 | 17802931 | 0.011015619 |
| shugoshin-like 2 (S. pombe) | 17859327 | 0.011015619 |
| DEP domain containing 1 | 17743717 | 0.016322314 |
| toll-like receptor 5 | 17682865 | 0.018374214 |
| cyclin-dependent kinase 1 | 17754704 | 0.01853393 |
| similar to histone 1, H2ai | 17719547 | 0.019101003 |
| minichromosome maintenance complex component 6 | 17683874 | 0.019150091 |
| histone cluster 1, H2ah | 17719497 | 0.0213261 |
| growth arrest-specific 2 like 3 | 17835023 | 0.0213261 |
| histone cluster 2, H4 | 17749618 | 0.0213261 |
| histone cluster 1, H2bf | 17715826 | 0.021945516 |
| prostaglandin reductase 1 | 17807657 | 0.02222536 |
| establishment of cohesion 1 homolog 2 (S. cerevisiae) | 17703831 | 0.022299972 |
| protein regulator of cytokinesis 1 | 17617801 | 0.022299972 |
| kinesin family member 4A | 17873058 | 0.022299972 |
| budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae) | 17777079 | 0.022299972 |
| TPX2, microtubule-associated, homolog (Xenopus laevis) | 17767437 | 0.022299972 |
| epithelial cell transforming sequence 2 oncogene | 17746602 | 0.022695042 |
| minichromosome maintenance complex component 7 | 17676520 | 0.022695042 |
| ASF1 anti-silencing function 1 homolog B (S. cerevisiae) | 17732679 | 0.022695042 |