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Supplementary Material

MicroRNA-125b and chemokine CCL4 expression are associated with calcific aortic valve disease

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SUPPLEMENTARY METHODS

Patients

Aortic valves for this study were removed from 53 patients undergoing valve or aortic root surgery (Table 1) [1]. The study protocol was approved by the Research Ethics Committee of Oulu University Hospital, and it complied with the principles outlined in the Declaration of Helsinki. All operations were made following normal surgical procedures. After removal, valve cusps were placed immediately in liquid nitrogen and stored at -70°C until analyzed. For histological evaluation and mRNA and miRNA measurements, one leaflet of the valve was taken and divided into halves.

Based on clinical patient data and a macroscopic evaluation of the valvular tissue, the samples were divided into three groups. The control group contained aortic valves from patients undergoing surgery due to ascending aortic pathology (aneurysm or dissection) or aortic regurgitation. These valves were smooth, pliable and opalescent, and without macroscopically visible calcifications. The intermediate fibro(sclero)sis group consisted of valves that were macroscopically thickened, stiff and regurgitant. The patients had no significant transvalvular pressure gradient. In the AS group the patients had non-rheumatic, severe aortic valve stenosis with varying degrees of calcification. The peak transvalvular gradient in the AS group was 69.3 ± 21.4 mmHg ($n=28$) as assessed by Doppler echocardiography.

The patients' demographics are presented in Supplementary data Table 1 and 2. There were no significant differences in patients' gender, left ventricular ejection fraction or the proportion of bicuspid aortic valves. The average age of AS patients was significantly higher and they were more likely to have coronary artery disease or diabetes than the patients in the control or

fibro(sclero)sis groups. They were also more likely to use statins (Supplementary data Table 1). For miRNA-array and DNA microarray analyses, the samples were matched for all parameters including age (Supplementary data, Table 2).

Analysis of microRNA

The extraction of total microRNA from aortic valves was done with mirVana miRNATM Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. Briefly, the valves were homogenized using Magnet Lyser Instrument (Roche Applied Science, Penzberg, Upper Bavaria, Germany) followed by total RNA extraction without enrichment for small RNAs. The measurement of microRNAs was done using the miScript PCR system (Qiagen, Hilden, Germany). The quantification of real-time quantitative PCR results was done using the $\Delta\Delta CT$ method. Small nuclear RNA SNORA73 was used for normalization.

MicroRNA Array

Total RNA from three samples from both the control and the AS group (patient characteristics shown in Supplementary data Table 2) was extracted with the guanidine thiocyanate-CsCl method [2] and microRNA array analysis was done by using miRCURYTM LNA Array microRNA profiling services (Exiqon, Vedbaek, Denmark). Sample quality was analyzed using Bioanalyser 2100. Samples were labeled with miRCURYTM Hy3TM/Hy5TM power labeling kit and hybridized on the miRCURYTM LNA Array (v.10.0). Quantified signals were normalized using the global LOcally WEighted Scatterplot Smoothing (LOWESS) regression algorithm. miRNAs were defined as differentially expressed using two-tailed T-test calculations with a P value lower than 0.05. The heat map diagram shows the result of the two-way hierarchical clustering of miRNA and samples.

The clustering was performed on log₂(Hy5/Hy3) ratios which passed the filtering criteria using two-tailed t-test between the control and stenotic groups.

DNA microarray analysis

Total RNA (n=5 in each group; Supplementary data Table 2) was used as a template for the DNA microarray analysis done by using GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions as previously described [3]. Affymetrix CEL files were imported into GeneSpring 7.2 software (Agilent) and Robust Multichip Average (RMA) normalization was performed. Genes were defined as differentially expressed if the change was at least 2.0-fold and statistically significant (P<0.05, One-way ANOVA and Benjamini and Hochberg false discovery rate). The complete data sets are available from the NCBI's Gene Expression Omnibus (GEO) database and gene expression profiling data comply with the Minimum Information About a Microarray Experiment (MIAME) standard. The data can be obtained from the GEO database with the accession numbers GSE51472.

Differentially expressed genes were classified on the basis of biological function using a modified version of a previously established classification scheme [4] as previously described [5]. The Gene Ontology (www.geneontology.org) was used to make an initial assignment of functional classification. Because Gene Ontology output classifications did not necessarily address a specific pathophysiological condition or experimental treatment, the PubMed database (www.ncbi.nlm.nih.gov/sites/entrez?db=PubMed) was searched for each gene and the functional assignment was confirmed or adjusted under one of the following broad biological classifications: angiogenesis, apoptosis, biosynthesis and metabolism, cell adhesion, cell structure/motility, cell/organism defense, channel/transport proteins, inflammatory and immune response, protein

synthesis, posttranslational modifications and proteolysis, regulation of transcription, signal transduction, unclassified, unknown transcribed loci and expressed sequence tags (ESTs).

Pathway analysis

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) 9.05 database [6] was used to extract significant interactions within differentially expressed genes. The complete list of significantly altered genes between AS and control group (550 genes) was loaded into STRING 9.05 database, and directly interacting proteins were identified based on manually curated biological evidence of interaction with at least a high confidence score (defined by STRING to be 0.7000 or greater).

Analysis of RNA

Total RNA from aortic valve samples was isolated by the guanidine thiocyanate-CsCl method as previously described [2]. Human bone sialoprotein II, interleukin-8, osteopontin, S100 calcium binding protein A8 and chemokine (C-C motif) ligand (CCL) -3 and -4 as well as 18S mRNA levels were measured using TaqMan chemistry on a 7300 real-time quantitative PCR System (Applied Biosystems, Foster City, CA, USA) as previously described [1]. The sequences of the forward and reverse primers and the fluorogenic probes for RNA detection are provided in Supplementary Table 3. The results were normalized to 18S RNA quantified from the same samples.

Bioinformatic microRNA target prediction

To identify differentially expressed genes under miRNA regulation, the MicroCosm Targets web resource (<http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/>) was used to calculate a list of potential target genes for each significantly altered miRNA. The computational interactions

of miRNAs and target genes were predicted using the miRanda algorithm [7]. Each set of putative target genes was compared to the list of genes altered in the DNA microarray experiment to detect potentially functional interactions.

MicroRNA Transfection

Human monocytic leukemia cell line THP-1 was obtained from the American Type Culture Collection (Manassas, VA; cat. TIB-202) and cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 25 mM HEPES, 100 U/mL penicillin, and 100 µg/mL streptomycin (THP-1 medium). Monocyte-to-macrophage differentiation was initiated by supplementing the THP-1 medium with 50 nM phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich, St. Louis, MO) for 24 hours. The transfection mixtures were prepared according to the manufacturer's instructions containing 90 nM Pre-miRTM miRNA Precursors for miR -125b (mature miRNA sequence UCCCUGAGACCCUAACUUGUGA) mimics (by Ambion, Austin, USA). Transfection reagent was 1.4% Lipofectamin (Invitrogen) in Dulbecco's modification of Eagle's medium (DMEM). THP-1 cells (1.5×10^5 cells/well) were washed with PBS and incubated with the transfection reagents in THP-1 medium in the presence of 50 nM PMA for 24 hours, followed by a further 24 hour incubation with serum-free THP-1 medium. Successful uptake of microRNA mimics into cells was confirmed using a TaqMan® Cells-to-CtTM Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions and verified using qPCR (Supplementary Fig. 4). The cells and cell culture supernatants were collected for qPCR and ELISA analyses. In qPCR, the average miRNA-125b expression of cells transfected with miRNA-125b was 170-fold ($P < 0.001$) as compared to cells transfected with scrambled (negative) miRNA control, indicating successful transfection. The microRNAs were assayed using TaqMan® microRNA (Ambion, Austin, TX, USA) assays for miR-125b according to the manufacturer's instructions.

ELISA

CCL4 was detected from cell culture media with a commercial ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Histological, immunohistological and immunofluorescence analysis

The aortic valve samples were fixed in 10% buffered formalin solution and embedded in paraffin. For quantification of calcified area, 5- μ m sections were cut and stained with hematoxylin and eosin. When necessary, EDTA treatment was used to decalcify the sections. For area calculations (total valve area and calcified area), slides were photographed with Leica DFC420 camera. Digital images were saved on a standard PC running Windows XP and the measurements were performed with image analysis program ImageJ (<http://rsbweb.nih.gov/ij/>). Blood vessel density was determined on the basis of the number of vessels seen on the tissue divided by the area of the tissue sections. Factor VIII immunohistochemical staining was used to visualize density of blood vessels. A polyclonal rabbit anti-human antibody to factor VIII-related antigen Dako (Glostrup, Denmark) was used at a dilution of 1:50.

To assess localization of chemokines in aortic valve cusps, fresh cusps were cut in 5 μ m thick sections, dried and frozen. Specific primary antibodies for CCL3/MIP-1 α and CCL4/MIP-1 β (Sigma-Aldrich, St Louis, MO, USA) were used as primary antibodies in a dilution of 1:10 and 1:50, respectively. Macrophages were visualized by staining CD68 (clone PGM-1, DakoCytomation, Glostrup, Denmark) in a dilution of 1:100. Secondary antibodies for CCL3 and CCL4 were from Jackson ImmunoResearch, Baltimore Pike, West Grove, PA, USA and for CD68 from Life Technologies, Waltham, MA, USA. Before application of the primary antibodies, the sections of

valve samples were first fixated in -20°C acetone and dried shortly in room temperature. Then, the sections were incubated in 0.5% hydrogen peroxide in methanol for 10 minutes and washed twice in PBS Tween. Blocking serum (1% bovine serum albumin in PBS) was added and incubated for 10 minutes, sections were washed once in PBS Tween and primary antibody for CD68 was added and incubated for 1 hour. After washing twice with PBS Tween, secondary antibody (Alexa Fluor 594 goat anti-mouse IgG3 (red), 1:100) was incubated for 1 hour in room temperature and the samples were washed three times in PBS tween. Primary antibodies for CCL3 or CCL4 were incubated overnight in 4°C and washed three times in PBS tween. Secondary antibodies (Alexa Fluor 488 donkey anti-mouse IgG1 (green) for CCL3 and Alexa Fluor 488 goat anti-rabbit IgG (green) for CCL4) were added in dilution of 1:100 and incubated for 1 hour in room temperature. Slides were washed three times in PBS tween and once in distilled water and counterstained for 10 minutes with Hoechst stain (Sigma-Aldrich), dilution 1:1000. Negative control stainings were carried out by substituting nonimmune mouse or rabbit serum for the primary antibodies. Negative control stainings are presented in Supplementary Figure 6.

Localization of chemokines in aortic valve cusps was also confirmed by immunohistochemical staining. Specific antibodies for CCL3/MIP-1 α and CCL4/MIP-1 β (R&D Systems, Minneapolis, MN, USA) were used in a dilution of 1:20 and 1:10, respectively. Macrophages were visualized by staining CD68 (clone KP1, Dako, Glostrup, Denmark) in a dilution of 1:10,000. Before application of the primary antibodies, the 5- μ m thick sections of valve samples were heated in a microwave oven in 10 mM Tris-EDTA, pH 9.0 for 15 min (CCL3 and CD68) or in citrate buffer, pH 6.0 for 15 min (CCL4). The chromogen used was amino-9-ethylcarbazole (Life Technologies, Carlsbad, CA, USA) in chemokine stainings and 3,3'-diaminobenzidine (DAP) in other stainings. Negative control stainings were carried out by substituting nonimmune mouse or goat serum for the primary

antibodies. All histological analyses were made in a blinded manner by an experienced pathologist.

Statistical analysis

The patients' demographic data analysis between the control, fibrosis and AS groups was performed by analysis of variance (ANOVA), for continuous variables and by chi-square test or Fisher's exact test for categorical variables. Results are presented as mean \pm standard error of the mean (SEM). The data was first tested for normality by using the Kolmogorov-Smirnov test. For normally distributed variables the unpaired Student's t-test or one-way analysis of variance (ANOVA) followed by a least significant difference post hoc test for multiple comparisons was performed. For non-normally distributed groups Mann-Whitney's U-test was used. P values under 0.05 were considered statistically significant. Analyses were performed using IBM SPSS for Windows (version 19.0, IBM SPSS Inc., Chicago, IL, USA).

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Supplementary Table 1. Patients' characteristics

	Control	Fibro(sclero)sis	Aortic stenosis	P-value
Patients, n	13	12	28	
Male, n (%)	11 (85)	9 (75)	20 (71)	0.71
Age, year (range)	51 ± 16 (21-70)	60.6 ± 15.2 (21-70)	71.4 ± 8.8 (45-86)	0.002
Bicuspid valve, n (%)	2 (15)	8 (67)	10 (36)	0.53
LVEF, %	60.8 ± 9.5	50.9 ± 12.8	57 ± 11.3	0.64
DM, n (%)	0	0	8 (29)	0.015
Coronary disease, n (%)	4 (31)	5 (42)	21 (68)	0.061
ASO, n (%)	1 (8)	1 (8)	3 (11)	0.50
COPD, n (%)	0	0	0	
Statins, n (%)	4 (31)	5 (42)	21 (75)	0.014

Values are mean ± standard deviation (SD). The P-values are from ANOVA-model (class variables

are analyzed using Fisher's Exact Test).

LVEF, left ventricular ejection fraction; DM, diabetes mellitus; ASO, peripheral atherosclerosis;

COPD, chronic obstructive pulmonary disease.

Supplementary Table 2. Patients characteristics (DNA array and microRNA array analysis)

	Control	Fibro(sclerosis)	AS	<i>p</i> -value
N. of patients	5	5	5	
Male	5 (100%)	5 (100%)	5 (100%)	ns
Bicuspid valve	2 (40%)	1 (20%)	1 (20%)	ns.
Age (yrs)	43.8±18.4 (31-69)	57.8±12.9 (37-69)	56.8±9.9 (45-72)	ns.
LVEF (%)	56.6±6.5	59.8±10.5	57.8±14.9	ns.
DM	0	0	0	ns.
Coronary disease	0	0	1 (20%)	ns.
ASO	0	0	0	ns.
COPD	0	0	0	ns.
Statins	0	0	0	ns.

Values are mean ± standard deviation (SD). The P-values are from ANOVA-model (class variables are analysed using Fisher's Exact Test). LVEF, left ventricular ejection fraction; DM, diabetes mellitus; ASO, peripheral atherosclerosis; COPD, chronic obstructive pulmonary disease.

Supplementary Table 3. Probes and primers The forward (F) and reverse (R) primers for real-time quantitative RT-PCR.

Gene	Primers	Fluorogenic Probe
Bone sialoprotein II	(F) ACCAACAGCACAGAGGCAGA (R) CTTCCCTTCTCTCCATTGCT	AACGGCAACGGCAGCAGCG
Chemokine (C-C motif) ligand 3	(F) CAACCAAGTTCTGCATCACTTG (R) CGGGAGGGTAGCTGAAGCA	TGCTGACACGCCGACCGCC
Chemokine (C-C motif) ligand 4	(F) AGCGCTCTCAGCACCAATG (R) AGCTTCCTCGCAAGTGTAAAGAAA	CTCAGACCCCTCACCGCCTGC
Interleukin 8	(F) TGTGTGAAGGGTAGTTTGCCAAGG (R) GCCTTCCTGATTCTGCAGC	TGCACTGACATCTAAGTTCTTTAGCA
Osteopontin	(F) CATACAAGGCCATCCCCG (R) CTGTCCTTCCCACGGCTG	CCTGAACGGCCCTCTGATTGGG
Osteoprotegerin	(F) TGTACAGCAAAGTGGAAAGACCG (R) GGTGTGCCAGCTGTGTGT	TGCGCCCTTGCCCTGACC
S100 calcium binding protein A8	(F) TCATCGACGTTACCAAGTACTCCCTGA (R) AGCTGGAGAAAGCCCTTGAACCT	CGGCATGGAAATCCCT
18S	(F) TGGTTGCAAAGCTGAACCTAAAG (R) AGTCAAATTAAAGCCGAGGC	CCTGGTGGTGCCTCCGTCA

Supplementary Table 4. Comparison of DNA microarray results with real-time quantitative RT-PCR.

GenBank		Fold change*			
accession		DNA microarray		Quantitative-RT-PCR	
number	Description	Fibr	AS	Fibr	AS
M83248	Bone Sialoprotein II	1.8*	32.5***	8.5	15.7*
NM_000584	Interleukin-8	1.3	7.1*	1.3	6.6*
M83248	Osteopontin	3.2*	21.6***	13.0	53.8***
NM_002546	Osteoprotegerin	1.2	3.6	1.25	2.0
NM_002964	S100 calcium binding protein A8	2.2	12.6***	0.6	4.0*

*Results are expressed as fold change in gene expression between aortic stenosis (AS) group or fibro(sclero)sis group (Fibr) and control group (Ctrl). *P<0.05; ***P<0.001 vs. Ctrl group (n=5 in each group).

Supplementary Table 5. Differentially expressed genes between aortic stenosis group and control group.

Affymetrix probe set ID	Description	GenBank Accession number	Fold change*
Angiogenesis			
235086_at	Thrombospondin 1	AW956580	2.91
205609_at	Angiopoietin 1	NM_001146	2.16
206742_at	Vascular endothelial growth factor D	NM_004469	0.48
219700_at	Plexin domain containing 1	NM_020405	0.41
Apoptosis			
229147_at	Ras association (RalGDS/AF-6) domain family 6	AW070877	9.03
205681_at	BCL2-related protein A1	NM_004049	7.74
210164_at	Granzyme B	J03189	7.05
221601_s_at	Fas apoptotic inhibitory molecule 3	AI084226	4.08
1552280_at	T-cell immunoglobulin and mucin domain containing 4	NM_138379	3.65
1552553_a_at	NLR family, CARD domain containing 4	NM_021209	2.80
201859_at	Serglycin	NM_002727	2.59
210260_s_at	Tumor necrosis factor, alpha-induced protein 8	BC005352	2.45
211725_s_at	BH3 interacting domain death agonist	BC005884	2.26
206011_at	Caspase 1	AI719655	2.23
1552701_a_at	Caspase recruitment domain family, member 16	NM_052889	2.19
226223_at	PRKC, apoptosis, WT1, regulator	AI091432	2.11
Biosynthesis and metabolism			
205943_at	Tryptophan 2,3-dioxygenase	NM_005651	15.72
203397_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)	BF063271	7.70
213502_x_at	Glucuronidase, beta pseudogene 11	AA398569	6.32
205306_x_at	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	AI074145	5.02
206214_at	Phospholipase A2, group VII	NM_005084	4.69
217388_s_at	Kynureinase (L-kynureanine hydrolase)	D55639	4.11
242943_at	ST8 alpha-N-acetyl-neuraminate alpha-2,8-sialyltransferase 4	AA352113	3.74
207992_s_at	Adenosine monophosphate deaminase (isoform E)	NM_000480	3.32
219797_at	Mannosyl (alpha-1,3)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isoenzyme A	NM_012214	3.31
209696_at	Fructose-1,6-bisphosphatase 1	D26054	3.26
223405_at	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	AI042017	3.06
219403_s_at	Heparanase	NM_006665	3.01
224009_x_at	Dehydrogenase/reductase (SDR family) member 9	AF240697	2.90
205755_at	Inter-alpha-trypsin inhibitor heavy chain 3	NM_002217	2.80
205922_at	Vanin 2	NM_004665	2.63
205700_at	Hydroxysteroid (17-beta) dehydrogenase 6	NM_003725	2.38
224480_s_at	1-acylglycerol-3-phosphate O-acyltransferase 9	BC006236	2.26
201724_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)	NM_020474	2.19

219806_s_at	Chromosome 11 open reading frame 75	NM_020179	2.07
206210_s_at	Cholesteryl ester transfer protein, plasma	NM_000078	2.07
212449_s_at	Lysophospholipase I	BG288007	2.01
237292_at	Dihydropyrimidinase-like 3	BE669707	0.48
206024_at	4-hydroxyphenylpyruvate dioxygenase	NM_002150	0.43
226121_at	Dehydrogenase/reductase (SDR family) member 13	AI076793	0.42
205200_at	C-type lectin domain family 3, member B	NM_003278	0.34
230418_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1	AI097463	0.26
224400_s_at	Carbohydrate (N-acetylgalactosamine 4-O) sulfotransferase 9	AF332473	0.19
Cell adhesion			
209875_s_at	Recreted phosphoprotein 1 (osteopontin)	M83248	21.59
209933_s_at	CD300a molecule	AF020314	4.69
213416_at	Integrin, alpha 4	BG532690	4.32
202112_at	Von Willebrand factor	NM_000552	3.66
1554240_a_at	Integrin, alpha L	BC008777	3.61
215925_s_at	CD72 molecule	AF283777	3.59
203868_s_at	Vascular cell adhesion molecule 1	NM_001078	3.40
207224_s_at	Sialic acid binding Ig-like lectin 7	NM_016543	3.38
202803_s_at	Integrin, beta 2	NM_000211	3.27
223235_s_at	SPARC related modular calcium binding 2	AB014737	3.14
216250_s_at	Leupaxin	X77598	2.61
228094_at	Adhesion molecule, interacts with CXADR antigen 1	AL048542	2.52
206120_at	CD33 molecule	NM_001772	2.28
213241_at	Plexin C1	AF035307	2.26
233510_s_at	Parvin, gamma	AL355092	2.19
1552807_a_at	Sialic acid binding Ig-like lectin 10	AF301007	2.17
201578_at	Podocalyxin-like	NM_005397	0.48
230175_s_at	Discoidin, CUB and LCCL domain containing 2	AA805633	0.47
1552735_at	Protocadherin gamma subfamily A, 12	AL832028	0.45
225990_at	Boc homologue	W72626	0.44
223629_at	Protocadherin beta 5	BC001186	0.44
213993_at	Spondin 1, extracellular matrix protein	AI885290	0.41
208609_s_at	Tenascin XB	NM_019105	0.41
227297_at	Integrin, alpha 9	AI479176	0.41
231789_at	Protocadherin beta 15	AV722990	0.40
235494_at	Limbic system-associated membrane protein	AA702209	0.37
204713_s_at	Coagulation factor V (proaccelerin, labile factor)	AA910306	0.35
213948_x_at	Cell adhesion molecule 3	AI564838	0.32
219407_s_at	Laminin, gamma 3	NM_006059	0.31
204584_at	L1 cell adhesion molecule	AI653981	0.28
235118_at	Cell adhesion molecule 2	AV724769	0.18
207717_s_at	Plakophilin 2	NM_004572	0.15
230867_at	Collagen, type VI, alpha 6	AI742521	0.12
Cell structure/motility			
204320_at	Collagen, type XI, alpha 1	NM_001854	11.46
209758_s_at	Microfibrillar associated protein 5	U37283	3.20
209083_at	Coronin, actin binding protein, 1A	U34690	2.96
207705_s_at	Ninein-like	NM_025176	0.48
203637_s_at	Midline 1 (Opitz/BBB syndrome)	NM_000381	0.47
226029_at	Vang-like 2 (van gogh, Drosophila)	AB033041	0.45

201787_at	Fibulin 1	NM_001996	0.44
200965_s_at	Actin binding LIM protein 1	NM_006720	0.42
213075_at	Olfactomedin-like 2A	AL050002	0.41
209789_at	Coronin, actin binding protein, 2B	BF939649	0.39
239726_at	Ankyrin 3, node of Ranvier (ankyrin G)	AI743588	0.35
230104_s_at	Tubulin polymerization promoting protein	BG055052	0.35
229779_at	Collagen, type IV, alpha 4	BF476080	0.31
225817_at	Cingulin-like 1	AB051536	0.31
226003_at	Kinesin family member 21A	AB051495	0.30
222835_at	Thrombospondin, type I, domain containing 4	BG163478	0.30
228494_at	Protein phosphatase 1, regulatory subunit 9A	AI888150	0.27
214023_x_at	Tubulin, beta 2B class IIb	AL533838	0.25
222073_at	Collagen, type IV, alpha 3	AI694562	0.22
220161_s_at	Erythrocyte membrane protein band 4.1 like 4B	NM_019114	0.20
219747_at	Neuron-derived neurotrophic factor	NM_024574	0.19
Cell/Organism defense			
209795_at	CD69 molecule	L07555	8.69
204118_at	CD48 molecule	NM_001778	5.03
204122_at	TYRO protein tyrosine kinase binding protein	NM_003332	3.16
219079_at	Cytochrome b5 reductase 4	NM_016230	2.63
233085_s_at	Nucleic acid binding protein 1	AV734843	2.52
214617_at	Perforin 1	AI445650	2.41
1556213_a_at	BTG family, member 3	BC028229	0.50
214434_at	Heat shock 70kDa protein 12A	AB007877	0.48
223843_at	Scavenger receptor class A, member 3	AB007830	0.32
Channel/Transport proteins			
205568_at	Aquaporin 9	NM_020980	6.05
204430_s_at	Solute carrier family 2, member 5	NM_003039	5.36
218404_at	Sorting nexin 10	NM_013322	4.91
210423_s_at	Solute carrier family 11, member 1	L32185	3.99
222939_s_at	Solute carrier family 16, member 10	N30257	3.78
209267_s_at	Solute carrier family 39, member 8	AB040120	3.11
210119_at	Potassium inwardly-rectifying channel, subfamily J, member 15	U73191	3.03
202546_at	Vesicle-associated membrane protein 8	NM_003761	2.64
205542_at	Six transmembrane epithelial antigen of the prostate 1	NM_012449	2.37
228083_at	Calcium channel, voltage-dependent, alpha 2/delta subunit 4	AI433691	2.15
203922_s_at	Cytochrome b-245, beta polypeptide (chronic granulomatous disease)	AI308863	2.11
204204_at	Solute carrier family 31, member 2	NM_001860	2.04
1556116_s_at	Transportin	AI825808	0.46
229053_at	Synaptotagmin XVII	AI582818	0.45
225627_s_at	Cache domain containing 1	AK024256	0.45
214769_at	Chloride channel 4	AF052117	0.43
216452_at	Transient receptor potential cation channel, subfamily M, member 3	AB046836	0.42
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	NM_007168	0.37
224049_at	Potassium channel, subfamily K, member 17	AF339912	0.37
1566785_x_at	N-ethylmaleimide-sensitive	AK025172	0.37

203400_s_at	Transferrin	NM_001063	0.31
228504_at	Sodium channel, voltage-gated, type VII, alpha	AI828648	0.15
Inflammatory and immune response			
215121_x_at	Immunoglobulin lambda constant 1	AA680302	32.32
214974_x_at	Chemokine (C-X-C motif) ligand 5	AK026546	30.55
214146_s_at	Chemokine (C-X-C motif) ligand 7	R64130	17.25
214768_x_at	Immunoglobulin kappa constant	BG540628	16.25
202917_s_at	S100 calcium binding protein A8	NM_002964	12.62
205242_at	Chemokine (C-X-C motif) ligand 13	NM_006419	12.37
216510_x_at	Immunoglobulin heavy constant alpha 1	AB035175	11.40
217235_x_at	Immunoglobulin lambda-like polypeptide 5	D84140	11.15
216491_x_at	Immunoglobulin heavy constant mu	U80139	8.07
212657_s_at	Interleukin 1 receptor antagonist	U65590	7.52
205114_s_at	Chemokine (C-C motif) ligand 3	NM_002983	7.32
219434_at	Triggering receptor expressed on myeloid cells 1	NM_018643	7.29
202859_x_at	Interleukin 8	NM_000584	7.12
213674_x_at	Immunoglobulin heavy constant delta	AI858004	5.97
244313_at	Complement component 3b/4b receptor 1 (Knops blood group)	AI052659	5.92
214038_at	Chemokine (C-C motif) ligand 8	AI984980	5.17
226218_at	Interleukin 7 receptor	BE217880	5.06
216950_s_at	Fc fragment of IgG, high affinity Ia, receptor (CD64)	X14355	4.82
210895_s_at	CD86 molecule	L25259	4.76
210915_x_at	T cell receptor beta variable 19	M15564	4.66
229560_at	toll-like receptor 8	AW872374	4.62
210146_x_at	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	AF004231	4.59
211796_s_at	T cell receptor beta constant 1	AF043179	4.55
205859_at	Lymphocyte antigen 86	NM_004271	4.54
204007_at	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	J04162	4.42
205831_at	CD2 molecule	NM_001767	4.29
205098_at	Chemokine (C-C motif) receptor 1	AI421071	4.11
211339_s_at	IL2-inducible T-cell kinase	D13720	4.03
205269_at	Lymphocyte cytosolic protein 2	AI123251	4.02
201422_at	Interferon, gamma-inducible protein 30	NM_006332	3.83
204103_at	Chemokine (C-C motif) ligand 4	NM_002984	3.81
213095_x_at	Allograft inflammatory factor 1	AF299327	3.61
203535_at	S100 calcium binding protein A9	NM_002965	3.60
235735_at	Tumor necrosis factor (ligand) superfamily, member 8	AI936516	3.59
215806_x_at	T cell receptor gamma alternate reading frame protein	M13231	3.55
1554899_s_at	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	BC020763	3.52
212998_x_at	Major histocompatibility complex, class II, DQ beta 1	AI583173	3.51
206337_at	Chemokine (C-C motif) receptor 7	NM_001838	3.49
220088_at	Complement component 5 receptor 1 (C5a ligand)	NM_001736	3.40
211582_x_at	Leukocyte specific transcript 1	AF000424	3.31
219725_at	Triggering receptor expressed on myeloid cells 2	NM_018965	3.28
216920_s_at	T cell receptor gamma alternate reading frame protein/gamma constant 2	M27331	3.28
217147_s_at	T cell receptor associated transmembrane adaptor 1	AJ240085	3.14

213539_at	CD3d molecule	NM_000732	3.10
210031_at	CD247 molecule	J04132	3.08
237753_at	Interleukin 21 receptor	AW504569	3.06
223640_at	Hematopoietic cell signal transducer	AF285447	3.02
203561_at	Fc fragment of IgG, low affinity IIa, receptor (CD32)	NM_021642	3.01
210116_at	SH2 domain protein 1A	AF072930	2.92
226878_at	Major histocompatibility complex, class II, DO alpha	AL581873	2.92
205758_at	CD8a molecule	AW006735	2.87
206991_s_at	Chemokine (C-C motif) receptor 5	NM_000579	2.84
203645_s_at	CD163 molecule	NM_004244	2.79
204924_at	Toll-like receptor 2	NM_003264	2.78
214567_s_at	Chemokine (C motif) ligand 1	NM_003175	2.74
210548_at	Chemokine (C-C motif) ligand 23	U58913	2.62
207072_at	Interleukin 18 receptor accessory protein	NM_003853	2.58
210140_at	Cystatin F	AF031824	2.54
206420_at	Immunoglobulin superfamily, member 6	NM_005849	2.54
206366_x_at	Chemokine (C motif) ligand 2	U23772	2.52
209906_at	Complement component 3a receptor 1	U62027	2.52
209949_at	Neutrophil cytosolic factor 2, 65kDa	BC001606	2.52
202953_at	Complement component 1, q subcomponent, beta polypeptide	NM_000491	2.42
204787_at	V-set and immunoglobulin domain containing 4	NM_007268	2.41
205291_at	Interleukin 2 receptor, beta	NM_000878	2.39
210004_at	Oxidised low density lipoprotein (lectin-like) receptor 1	AF035776	2.36
223454_at	Chemokine (C-X-C motif) ligand 16	AF275260	2.34
204363_at	Coagulation factor III (thromboplastin, tissue factor)	NM_001993	2.32
209813_x_at	T cell receptor gamma alternate reading frame protein	M16768	2.32
205147_x_at	Neutrophil cytosolic factor 4, 40kDa	NM_000631	2.32
204470_at	Chemokine (C-X-C motif) ligand 1	NM_001511	2.31
207850_at	Chemokine (C-X-C motif) ligand 3	NM_002090	2.31
205789_at	CD1d molecule	NM_001766	2.26
206181_at	Signaling lymphocytic activation molecule family member 1	NM_003037	2.23
207339_s_at	Lymphotoxin beta (TNF superfamily, member 3)	NM_002341	2.14
211269_s_at	Interleukin 2 receptor, alpha	K03122	2.12
223583_at	Tumor necrosis factor, alpha-induced protein 8-like 2	AF271774	2.12
208075_s_at	Chemokine (C-C motif) ligand 7	NM_006273	2.04
218232_at	Complement component 1, q subcomponent, alpha polypeptide	NM_015991	2.02
230673_at	Polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	AV706971	0.22
Protein synthesis, posttranslational modifications and proteolysis			
204580_at	Matrix metallopeptidase 12	NM_002426	91.96
205624_at	Carboxypeptidase A3 (mast cell)	NM_001870	9.98
205488_at	Granzyme A	NM_006144	6.45
206666_at	Granzyme K	NM_002104	6.22
213566_at	Ribonuclease, RNase A family, k6	NM_005615	4.18
214533_at	Chymase 1, mast cell	NM_001836	4.05
205214_at	Serine/threonine kinase 17b	NM_004226	4.03

208146_s_at	Carboxypeptidase, vitellogenin-like	NM_031311	3.86
210084_x_at	Tryptase alpha/beta 1	AF206665	3.79
205653_at	Cathepsin G	NM_001911	3.77
202901_x_at	Cathepsin S	BC002642	3.52
219574_at	Membrane-associated ring finger (C3HC4) 1	NM_017923	3.44
225646_at	Cathepsin C	AI246687	3.34
203379_at	Ribosomal protein S6 kinase, 90kDa, polypeptide 1	NM_002953	3.26
208121_s_at	Protein tyrosine phosphatase, receptor type, O	NM_002848	3.09
228055_at	Mapsin B aspartic peptidase pseudogene	AI763426	2.63
206111_at	Ribonuclease, RNase A family, 2	NM_002934	2.45
205180_s_at	ADAM metallopeptidase domain 8	NM_001109	2.20
216905_s_at	Suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)	U20428	2.19
235177_at	Methyltransferase like 21A	AI625022	2.04
226944_at	HtrA serine peptidase 3	AW518728	0.49
219578_s_at	Cytoplasmic polyadenylation element binding protein 1	NM_030594	0.45
226360_at	Zinc and ring finger 3	AK022809	0.44
212915_at	PDZ domain containing RING finger 3	AL569804	0.43
229004_at	ADAM metallopeptidase with thrombospondin type 1 motif, 15	AI970797	0.35
209883_at	Glycosyltransferase 25 domain containing 2	AF288389	0.33
228523_at	Nanos homolog 1	AW970089	0.21
227725_at	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1	Y11339	0.19
243582_at	SH3 domain containing ring finger 2	AW082633	0.12
Regulation of transcription			
206715_at	Transcription factor EC	NM_012252	4.39
211597_s_at	Homeodomain-only protein	AB059408	4.07
204959_at	Myeloid cell nuclear differentiation antigen	NM_002432	4.00
204198_s_at	Runt-related transcription factor 3	AA541630	3.82
202672_s_at	Activating transcription factor 3	NM_001674	3.27
204057_at	Interferon regulatory factor 8	AI073984	2.80
1552634_a_at	Zinc finger protein 101	NM_033204	2.30
227935_s_at	Polycomb group ring finger 5	AA522681	2.08
206283_s_at	T-cell acute lymphocytic leukemia 1	NM_003189	0.50
214761_at	Zinc finger protein 423	AW149417	0.50
205931_s_at	cAMP responsive element binding protein 5	NM_004904	0.49
1558184_s_at	Zinc finger protein 17	AB075827	0.49
220184_at	Nanog homeobox	NM_024865	0.48
226676_at	Zinc finger protein 521	AK021452	0.48
212094_at	Paternally expressed 10	AL582836	0.47
244128_x_at	GLIS family zinc finger 1	AA872588	0.47
205619_s_at	Mesenchyme homeo box 1	NM_004527	0.46
236270_at	Nuclear factor of activated T-cells, cytoplasmic, calcineurin dependent 4	AI806528	0.46
209242_at	Paternally expressed 3	AL042588	0.46
224970_at	Nuclear factor I/A	AA419275	0.45
222771_s_at	Myelin expression factor 2	BF224052	0.44
207480_s_at	Meis homeobox 2	NM_020149	0.43
229273_at	Sal-like 1	AU152837	0.41

1560201_at	Zinc finger protein 713	AK097282	0.40
209505_at	Nuclear receptor subfamily 2, group F, member 1	AI951185	0.40
221234_s_at	BTB and CNC homology 1, basic leucine zipper transcription factor 2	NM_021813	0.40
213268_at	Calmodulin binding transcription activator 1	Z98884	0.39
202191_s_at	Growth arrest-specific 7	BE439987	0.38
228988_at	Zinc finger protein 6	AU157017	0.38
244521_at	Teashirt zinc finger homeobox 2	BG236742	0.36
205517_at	GATA binding protein 4	AV700724	0.34
228399_at	Odd-skipped related 1 (Drosophila)	AI569974	0.32
226913_s_at	SRY (sex determining region Y)-box 9	BF527050	0.31
243672_at	Sal-like 3	R42361	0.31
244043_at	Transcription factor Dp-2 (E2F dimerization partner 2)	AI049624	0.28
Signal transduction			
203000_at	Stathmin-like 2	BF967657	10.97
1555756_a_at	C-type lectin domain family 7, member A	AF400600	5.42
223809_at	Regulator of G-protein signalling 18	AF076642	5.31
230237_at	Adenylate cyclase activating polypeptide 1 (pituitary)	BE220888	4.77
223660_at	Adenosine A3 receptor	AF226731	4.63
205590_at	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	NM_005739	4.50
214467_at	G protein-coupled receptor 65	NM_003608	4.50
214470_at	Killer cell lectin-like receptor subfamily B, member 1	NM_002258	4.35
210258_at	Regulator of G-protein signalling 13	AF030107	4.20
203416_at	CD53 molecule	NM_000560	3.84
235740_at	Multiple C2-domains with two transmembrane regions 1	BG250585	3.72
204891_s_at	Lymphocyte-specific protein tyrosine kinase	NM_005356	3.65
218870_at	Rho GTPase activating protein 15	NM_018460	3.64
214770_at	Macrophage scavenger receptor 1	AI299239	3.62
223767_at	G protein-coupled receptor 84	AF237762	3.49
219279_at	Dedicator of cytokinesis 10	NM_017718	3.48
204220_at	Glia maturation factor, gamma	NM_004877	3.43
235046_at	Inositol polyphosphate-4-phosphatase, type II145kDa	AA456099	3.28
213603_s_at	Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	BE138888	3.25
204932_at	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	BF433902	3.23
210058_at	Mitogen-activated protein kinase 13	BC000433	3.17
204951_at	Ras homolog gene family, member H	NM_004310	3.11
205237_at	Ficolin (collagen/fibrinogen domain containing) 1	NM_002003	3.11
225166_at	Rho GTPase activating protein 18	AU158022	2.98
203471_s_at	Pleckstrin	NM_002664	2.94
228708_at	RAB20, member RAS oncogene family	BF438386	2.94
208018_s_at	Hemopoietic cell kinase	NM_002110	2.90
226068_at	Spleen tyrosine kinase	BF593625	2.85
230866_at	Cysteinyl leukotriene receptor	BE549540	2.79
224451_x_at	Rho GTPase activating protein 9	BC006107	2.73
210845_s_at	Plasminogen activator, urokinase receptor	U08839	2.69
1554741_s_at	Fibroblast growth factor 7 (keratinocyte growth	AF523265	2.64

	factor)		
230422_at	Formyl peptide receptor-like 2	AW026543	2.56
205479_s_at	Plasminogen activator, urokinase	NM_002658	2.46
228186_s_at	R-spondin 3	BF589322	2.42
209392_at	Ectonucleotide pyrophosphatase/phosphodiesterase 2	L35594	2.38
219358_s_at	ArfGAP with dual PH domains 2	NM_018404	2.35
228057_at	DNA-damage-inducible transcript 4-like	AA528140	2.32
205016_at	Transforming growth factor, alpha	NM_003236	2.32
204265_s_at	G-protein signalling modulator 3 (AGS3-like, <i>C. elegans</i>)	NM_022107	2.28
1552691_at	ADP-ribosylation factor-like 11	NM_138450	2.25
219412_at	RAB38, member RAS oncogene family	NM_022337	2.24
204829_s_at	Folate receptor 2	NM_000803	2.23
230925_at	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	AI093231	2.22
222218_s_at	Paired immunoglobulin-like type 2 receptor alpha	AJ400843	2.15
210340_s_at	Colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	BC002635	2.14
229625_at	Guanylate binding protein 5	BG545653	2.14
226847_at	Follistatin	BF438173	2.11
203332_s_at	Inositol polyphosphate-5-phosphatase, 145kDa	NM_005541	2.11
225685_at	CDC42 effector protein (Rho GTPase binding) 3	AI801777	2.10
223159_s_at	NIMA (never in mitosis gene a)-related kinase 6	BC000101	2.10
228258_at	TBC1 domain family, member 10C	AW008519	2.08
205349_at	Guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	NM_002068	2.04
204897_at	Prostaglandin E receptor 4 (subtype EP4)	AA897516	2.02
209909_s_at	Transforming growth factor beta 2	M19154	0.49
239218_at	Phosphodiesterase 1C, calmodulin-dependent 70kDa	AI800515	0.48
210473_s_at	G-protein-coupled receptor 125	M37712	0.47
206204_at	Growth factor receptor-bound protein 14	NM_004490	0.47
243792_x_at	Protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)	AI281371	0.47
226043_at	G-protein signalling modulator 1	AI242661	0.47
214721_x_at	CDC42 effector protein (Rho GTPase binding) 4	AL162074	0.45
227838_at	Unc-5 homolog C (<i>C. elegans</i>)	AW070250	0.45
225511_at	G protein-coupled receptor, family C, group 5, member B	AV725364	0.44
226197_at	Androgen receptor	AW173504	0.43
202920_at	Ankyrin 2, neuronal	BF726212	0.43
230144_at	Glutamate receptor, ionotropic, AMPA 3	AW294729	0.41
219764_at	Frizzled homolog 10 (<i>Drosophila</i>)	NM_007197	0.41
203071_at	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	NM_004636	0.41
209822_s_at	Very low density lipoprotein receptor	L22431	0.40
222696_at	Axin 2	BF684446	0.39
230708_at	Pickle homologue 1	AA206141	0.38
204442_x_at	Latent transforming growth factor beta binding protein 4	NM_003573	0.38
227997_at	Interleukin 17 receptor D	AW007080	0.38
221795_at	Neurotrophic tyrosine kinase, receptor, type 2	AI346341	0.37

215303_at	Doublecortin and CaM kinase-like 1	BE046461	0.37
204379_s_at	Fibroblast growth factor receptor 3	NM_000142	0.36
203680_at	Protein kinase, cAMP-dependent, regulatory, type II, beta	NM_002736	0.36
232267_at	G protein-coupled receptor 133	AL162032	0.35
217177_s_at	Protein tyrosine phosphatase, receptor type, B	AL080103	0.34
205111_s_at	Phospholipase C, epsilon 1	NM_016341	0.34
213436_at	Cannabinoid receptor 1	U73304	0.32
222317_at	Phosphodiesterase 3B, cGMP-inhibited	AA888858	0.31
241986_at	BMP binding endothelial regulator	AI423201	0.30
225016_at	Adenomatosis polyposis coli down-regulated 1	N48299	0.30
232282_at	WNK lysine deficient protein kinase 3	H06509	0.30
209763_at	Chordin-like 1	AL049176	0.23
229309_at	Adrenoceptor beta 1	AI625747	0.22
206423_at	Angiopoietin-like 7	NM_021146	0.16
222953_at	G protein-coupled receptor 83	BE670361	0.16
Unclassified			
215946_x_at	Immunoglobulin lamda-like polypeptide 3, pseudogene	AL022324	11.57
234994_at	Transmembrane protein 200A	AA088177	10.64
34210_at	CD52 molecule	N90866	8.98
230061_at	Transmembrane 4 L six family member 18	AW338625	6.61
220330_s_at	SAM domain, SH3 domain and nuclear localisation signals, 1	NM_022136	6.35
205569_at	Lysosomal-associated membrane protein 3	NM_014398	4.96
225687_at	Family with sequence similarity 83, member D	BC001068	4.89
219014_at	Placenta-specific 8	NM_016619	4.44
1559584_a_at	Chromosome 16 open reading frame 54	BC025741	4.21
236782_at	Sterile alpha motif domain containing 3	AI129628	3.94
1559065_a_at	C-type lectin superfamily 4, member G	BC039679	3.90
213888_s_at	TRAF3 interacting protein 3	AL022398	3.84
219386_s_at	SLAM family member 8	NM_020125	3.52
229390_at	Family with sequence similarity 26, member F	AV734646	3.45
219607_s_at	Membrane-spanning 4-domains, subfamily A, member 4	NM_024021	3.38
226459_at	Phosphoinositide-3-kinase adaptor protein 1	AW575754	3.37
203761_at	Src-like-adaptor	NM_006748	3.27
203021_at	Secretory leukocyte peptidase inhibitor	NM_003064	3.21
228532_at	Chromosome 1 open reading frame 162	AW662189	3.10
217916_s_at	Family with sequence similarity 49, member B	NM_016623	3.07
240572_s_at	C-type lectin domain family 2, member D pseudogene	BF436632	2.95
1553043_a_at	CD300 molecule-like family member F	NM_139018	2.88
241981_at	Family with sequence similarity 20, member A	AW291369	2.78
229070_at	Androgen-dependent TFPI regulating protein	AA470369	2.77
204923_at	SAM and SH3 domain containing 3	AL023653	2.67
210644_s_at	Leukocyte-associated Ig-like receptor 1	AF109683	2.61
212543_at	Absent in melanoma 1	U83115	2.55
233177_s_at	Paroxysmal nonkinesigenic dyskinesia	AB033010	2.53
204674_at	Lymphoid-restricted membrane protein	NM_006152	2.50
221081_s_at	DENN/MADD domain containing 2D	NM_024901	2.45
207571_x_at	Chromosome 1 open reading frame 38	NM_004848	2.45

219697_at	Heparan sulfate (glucosamine) 3-O-sulfotransferase 2	NM_006043	2.45
206513_at	Absent in melanoma 2	NM_004833	2.38
225105_at	Chromosome 12 open reading frame 12	BF969397	2.33
219666_at	Membrane-spanning 4-domains, subfamily A, member 6A	NM_022349	2.31
224779_s_at	Family with sequence similarity 96, member A	AI193090	2.27
221211_s_at	Chromosome 21 open reading frame 7	NM_020152	2.24
219812_at	Poliovirus receptor related immunoglobulin domain containing	NM_024070	2.24
209734_at	NCK-associated protein 1-like	BC001604	2.21
227353_at	Transmembrane channel-like 8	BE671663	2.20
219099_at	Chromosome 12 open reading frame 5	NM_020375	2.20
203414_at	Monocyte to macrophage differentiation-associated	NM_012329	2.08
225763_at	RCSD domain containing 1	AI659418	2.08
1556698_a_at	GPRIN family member 3	AI819722	2.06
220990_s_at	MIR21 micro RNA	NM_030938	2.04
243198_at	Testis expressed sequence 9	AA020920	2.04
231093_at	Fc receptor-like 3	BF514552	2.03
227341_at	BEN domain containing 7	AW195407	0.50
226884_at	Leucine rich repeat neuronal 1	N71874	0.50
235077_at	Maternally expressed 3 (non protein coding!!!)	BF956762	0.50
218888_s_at	Neuropilin (NRP) and tolloid (TLL)-like 2	NM_018092	0.50
242443_at	Echinoderm microtubule associated protein like 5	AW026978	0.49
213050_at	Cordon-bleu homolog	AA594937	0.49
213900_at	Family with sequence similarity 189, membrane A2	AA524029	0.49
230015_at	Progressive rod-cone degeneration	AV729651	0.49
239297_at	KIAA1456	BE932011	0.49
233520_s_at	Cardiomyopathy associated 5	AL359338	0.49
236325_at	KIAA1377 protein	BF057799	0.49
217551_at	Olfactory receptor, family 7, subfamily E, member 14, pseudogene	AA719797	0.49
215250_at	Transmembrane protein 111	AU147317	0.49
231024_at	PGM5 antisense RNA 1 (non-protein coding)	H12280	0.48
221272_s_at	Chromosome 1 open reading frame 21	NM_030806	0.47
228088_at	SEC14 and spectrin domains 1	AI092265	0.47
212976_at	Leucine rich repeat containing 8 family, member B	R41498	0.46
220751_s_at	Chromosome 5 open reading frame 4	NM_016348	0.46
228882_at	Tubby homolog	AL042088	0.44
222890_at	Coiled-coil domain containing 113	BG054922	0.44
231980_at	Docking protein 6	AL120332	0.44
218820_at	Chromosome 14 open reading frame 132	NM_020215	0.43
218974_at	Sine oculis binding protein homolog	NM_018013	0.43
230645_at	FERM domain containing 3	BF110588	0.43
209074_s_at	Family with sequence/uncharacterized	AL050264	0.43
1569256_a_at	Family with sequence similarity 43, member B	BC015675	0.42
231969_at	Storkhead box 2	AB037813	0.41
228790_at	Family with sequence similarity 110, member B	AW264082	0.41
225864_at	Family with sequence similarity 84, member B	AL039862	0.40
218824_at	paraneoplastic Ma antigen family-like 1	NM_018215	0.39
232412_at	F-box and leucine-rich repeat	AK024690	0.39
232166_at	KIAA1377 protein	AL045516	0.38

1559964_at	FLJ38717 protein	AK096036	0.38
228653_at	Sterile alpha motif containing 5	AI700341	0.38
226490_at	NHS-like 1	AA503387	0.38
209159_s_at	NDRG family member 4	AV724216	0.37
232887_at	Phosphoinositide-interacting regulation of transient receptor potential channels	AU144437	0.37
1559640_at	Ankyrin-repeat and fibronectin type III domain containing 1	BC037494	0.37
238865_at	Polyadenylate-binding protein, cytoplasmic 4-like	AI822134	0.36
240402_at	Kin of IRRE like 3	H05918	0.36
220518_at	ABI family, member 3 (NESH) binding protein	NM_024801	0.35
202421_at	Immunoglobulin superfamily, member 3	AB007935	0.34
229782_at	Non-coding RNA in rhabdomyosarcoma (RMS)	BE468066	0.32
228307_at	Elastin microfibril interfacer 3	AL137580	0.31
221886_at	DENN/MADD domain containing 2A	AL037701	0.30
223523_at	Transmembrane protein 108	BC000568	0.30
219791_s_at	Nbla00301	NM_024748	0.27
230087_at	Proline rich membrane anchor 1	AI823645	0.26
1564315_at	Chromosome 8 open reading frame 49	AK055534	0.25
209569_x_at	DNA segment on chromosome 4 (unique) 234 expressed sequence	NM_014392	0.23
232313_at	Transmembrane protein 132c	AL122107	0.21
228195_at	Chromosome 2 open reading frame 88	BE645119	0.21
229730_at	Smoothelin-like 2	AI193973	0.21
226415_at	Vesicle amine transport protein 1 homolog	AA156723	0.20
237802_at	X Kell blood group precursor-related family, member 4	R54212	0.17
Unknown transcribed loci and ESTs			
212592_at	Transcribed locus	AV733266	129.40
217022_s_at	Transcribed locus	S55735	50.72
215176_x_at	Transcribed locus	AW404894	47.52
211645_x_at	Transcribed locus	M85256	42.59
216576_x_at	Transcribed locus	AF103529	34.92
234764_x_at	Transcribed locus	U96394	31.87
216401_x_at	Transcribed locus	AJ408433	21.52
214777_at	Transcribed locus	BG482805	19.00
217258_x_at	Transcribed locus	AF043583	10.66
211798_x_at	Transcribed locus	AB001733	9.84
236280_at	Transcribed locus	AI225238	4.57
229635_at	Uncharacterized LOC100505702	AW043859	4.06
239294_at	Transcribed locus	AA810265	4.00
237625_s_at	Transcribed locus	BG548679	3.87
229937_x_at	Transcribed locus	AI681260	3.80
243366_s_at	Transcribed locus	AI936034	3.13
240232_at	Transcribed locus	AA503803	2.51
229629_at	Transcribed locus	AI923633	2.14
217384_x_at	Transcribed locus	AJ275374	2.13
230631_s_at	Uncharacterized LOC100288432	AI202642	2.09
230354_at	Transcribed locus	BG236273	2.05
217320_at	Transcribed locus	AJ275413	2.03
232182_at	LOC441444	AI142853	0.50
233984_at	Transcribed locus	AL133091	0.50

1557242_at	Transcribed locus	AI769729	0.50
243933_at	Transcribed locus	AI096634	0.49
232113_at	Transcribed locus	N90870	0.49
243184_at	Transcribed locus	AW173166	0.49
244420_at	Transcribed locus	AI128130	0.49
1564358_at	Transcribed locus	AK026284	0.48
237157_at	Transcribed locus	AW117547	0.48
239653_at	Transcribed locus	AI936859	0.48
233449_at	Transcribed locus	AU143940	0.47
1563217_at	Transcribed locus	BI598831	0.47
1562235_s_at	Transcribed locus	AL832146	0.47
230653_at	Transcribed locus	AI469960	0.47
239448_at	Transcribed locus	AI475033	0.47
240385_at	Transcribed locus	BF002339	0.47
241509_at	Transcribed locus	AI637733	0.47
215578_at	transcribed locus	AU145365	0.46
233546_at	Uncharacterized protein LOC283075	AK023065	0.46
242671_at	Transcribed locus	BF055144	0.46
242523_at	Transcribed locus	AW770320	0.45
236184_at	Transcribed locus	AI798959	0.45
235898_at	Transcribed locus	AW105010	0.44
240865_at	Transcribed locus	AA056086	0.44
1564699_at	Transcribed locus	BC017920	0.44
243791_at	Transcribed locus	AW979261	0.44
1569208_a_at	Transcribed locus	BC020895	0.44
232776_at	Transcribed locus	AU145289	0.44
234088_at	Transcribed locus	AU147125	0.43
213089_at	Uncharacterized LOC100272216	AU158490	0.43
241310_at	Transcribed locus	AI685841	0.43
1560049_at	Transcribed locus	AI125337	0.41
240245_at	Transcribed locus	AW237264	0.41
235123_at	Transcribed locus	AI951144	0.41
241459_at	Transcribed locus	AI494113	0.40
233214_at	Transcribed locus	AK021962	0.40
230785_at	Transcribed locus	AI634411	0.39
238827_at	Transcribed locus	BE843544	0.39
202350_s_at	Uncharacterized LOC/Matrilin 2	NM_002380	0.39
1558444_at	Transcribed locus	AW206560	0.38
1558714_at	Transcribed locus	BC043430	0.38
239301_at	Transcribed locus	BE551451	0.37
214295_at	Uncharacterized LOC57236	AW129056	0.37
219871_at	Uncharacterized FLJ13197	NM_024614	0.37
244112_x_at	Transcribed locus	N73550	0.37
207414_s_at	Uncharacterized/ Proprotein convertase subtilisin/kexin type 6	NM_002570	0.36
239999_at	Long intergenic non-protein coding RNA 478	N66393	0.36
1566163_at	Transcribed locus	AL832482	0.36
1563841_at	Transcribed locus	AL832549	0.35
240211_at	Uncharacterized LOC100130468	AI743177	0.34
239752_at	Transcribed locus	BE551781	0.33
232947_at	Transcribed locus	AU144382	0.31
232224_at	Uncharacterized LOC286272	AI274095	0.30

1561180_at	Transcribed locus	AK021807	0.30
239708_at	Transcribed locus	AA027105	0.29
233386_at	Transcribed locus	AK022037	0.28
242763_at	Transcribed locus	N39188	0.27
1559550_s_at	Transcribed locus	AL137360	0.24
222325_at	Transcribed sequence	AW974812	0.24
240558_at	Transcribed locus	AI023335	0.23
231181_at	Transcribed locus	AI683621	0.23
238178_at	Transcribed locus	BF110268	0.21
213904_at	Transcribed locus	AL390170	0.15
230319_at	Transcribed locus	AI222435	0.13
241560_at	Transcribed locus	AI927941	0.10

*Fold change represents statistically significant change ($P<0.05$) in gene expression between aortic stenosis group and control group.

Supplementary Table 6. Differentially expressed genes between aortic stenosis group and fibro(sclerotic) group.

Affymetrix probe set ID	Description	GenBank Accession number	Fold change*
Angiogenesis			
235086_at	Thrombospondin 1	AW956580	2.29
205609_at	Angiopoietin 1	NM_001146	2.01
219700_at	Plexin domain containing 1	NM_020405	0.41
Apoptosis			
205681_at	BCL2-related protein A1	NM_004049	5.61
1552280_at	T-cell immunoglobulin and mucin domain containing 4	NM_138379	4.91
210164_at	Granzyme B	J03189	4.80
229147_at	Ras association (RalGDS/AF-6) domain family 6	AW070877	4.75
221601_s_at	Fas apoptotic inhibitory molecule 3	AI084226	2.87
210260_s_at	Tumor necrosis factor, alpha-induced protein 8	BC005352	2.35
1552701_a_at	Caspase recruitment domain family, member 16	NM_052889	2.17
201859_at	Serglycin	NM_002727	2.13
202688_at	Tumor necrosis factor (ligand) superfamily, member 10	NM_003810	2.08
211725_s_at	BH3 interacting domain death agonist	BC005884	2.05
226223_at	PRKC, apoptosis, WT1, regulator	AI091432	2.03
209970_x_at	Caspase 1	M87507	2.02
Biosynthesis and metabolism			
205943_at	Tryptophan 2,3-dioxygenase	NM_005651	7.50
203397_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)	BF063271	3.69
205306_x_at	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	AI074145	3.63
217388_s_at	Kynureinase (L-kynurenine hydrolase)	D55639	3.58
213502_x_at	Glucuronidase, beta pseudogene 11	AA398569	3.40
219797_at	Mannosyl (alpha-1,3)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isoenzyme A	NM_012214	3.17
242943_at	ST8 alpha-N-acetyl-neuraminate alpha-2,8-sialyltransferase 4	AA352113	3.08
205700_at	Hydroxysteroid (17-beta) dehydrogenase 6	NM_003725	2.87
206214_at	Phospholipase A2, group VII	NM_005084	2.68
209696_at	Fructose-1,6-bisphosphatase 1	D26054	2.66
224009_x_at	Dehydrogenase/reductase (SDR family) member 9	AF240697	2.64
223405_at	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	AI042017	2.42
219403_s_at	Heparanase	NM_006665	2.33
205755_at	Inter-alpha-trypsin inhibitor heavy chain 3	NM_002217	2.25
207992_s_at	Adenosine monophosphate deaminase (isoform E)	NM_000480	2.16
204059_s_at	Malic enzyme 1, NADP(+) -dependent, cytosolic	NM_002395	2.16
203302_at	Deoxycytidine kinase	NM_000788	2.10
219099_at	Chromosome 12 open reading frame 5	NM_020375	2.03
205922_at	Vanin 2	NM_004665	2.02
238669_at	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	BE613133	0.49

208498_s_at	Amylase, alpha	NM_004038	0.47
205200_at	C-type lectin domain family 3, member B	NM_003278	0.43
223737_x_at	Carbohydrate (N-acetylgalactosamine 4-O) sulfotransferase 9	AF239821	0.39
230418_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1	AI097463	0.36
Cell adhesion			
209875_s_at	Secreted phosphoprotein 1 (osteopontin)	M83248	6.74
209933_s_at	CD300a molecule	AF020314	3.54
213416_at	Integrin, alpha 4	BG532690	2.93
203868_s_at	Vascular cell adhesion molecule 1	NM_001078	2.73
223235_s_at	SPARC related modular calcium binding 2	AB014737	2.69
207224_s_at	Sialic acid binding Ig-like lectin 7	NM_016543	2.68
1554240_a_at	Integrin, alpha L	BC008777	2.66
215925_s_at	CD72 molecule	AF283777	2.53
202803_s_at	Integrin, beta 2	NM_000211	2.53
206120_at	CD33 antigen	NM_001772	2.04
223629_at	Protocadherin beta 5	BC001186	0.49
235494_at	Limbic system-associated membrane protein	AA702209	0.49
225990_at	Boc homologue	W72626	0.48
213948_x_at	Cell adhesion molecule 3	AI564838	0.47
1552735_at	Protocadherin gamma subfamily A, 4	AL832028	0.46
208609_s_at	Tenascin XB	NM_019105	0.43
219407_s_at	Laminin, gamma 3	NM_006059	0.40
204584_at	L1 cell adhesion molecule	AI653981	0.37
235118_at	Cell adhesion molecule 2	AV724769	0.29
207717_s_at	Plakophilin 2	NM_004572	0.29
230867_at	Collagen, type VI, alpha 6	AI742521	0.23
Cell structure/motility			
37892_at	Collagen, type XI, alpha 1	J04177	8.11
209758_s_at	Microfibrillar associated protein 5	U37283	4.20
209083_at	Coronin, actin binding protein, 1A	U34690	2.26
213075_at	Olfactomedin-like 2A	AL050002	0.48
200965_s_at	Actin binding LIM protein 1	NM_006720	0.47
226029_at	Vang-like 2 (van gogh, Drosophila)	AB033041	0.46
214023_x_at	Tubulin, beta 2B class IIb	AL533838	0.44
209789_at	Coronin, actin binding protein, 2B	BF939649	0.44
222835_at	Thrombospondin, type I, domain containing 4	BG163478	0.40
228494_at	Protein phosphatase 1, regulatory subunit 9A	AI888150	0.39
225817_at	Cingulin-like 1	AB051536	0.39
239726_at	Ankyrin 3, node of Ranvier (Ankyrin G)	AI743588	0.38
219747_at	Neuron-derived neurotrophic factor	NM_024574	0.37
220161_s_at	Erythrocyte membrane protein band 4.1 like 4B	NM_019114	0.36
226003_at	Kinesin family member 21A	AB051495	0.36
222073_at	Collagen, type IV, alpha 3	AI694562	0.33
230104_s_at	Tubulin polymerization promoting protein	BG055052	0.33
Cell/Organism defense			
209795_at	CD69 molecule	L07555	5.82
210895_s_at	CD86 molecule	L25259	3.97
204118_at	CD48 molecule	NM_001778	3.78
219079_at	Cytochrome b5 reductase 4	NM_016230	2.56
204122_at	TYRO protein tyrosine kinase binding protein	NM_003332	2.49

210116_at	SH2 domain protein 1A	AF072930	2.46
222872_x_at	Nuclear acid binding protein 1	AU157541	2.01
223843_at	Scavenger receptor class A, member 3	AB007830	0.47
Channel/Transport proteins			
218404_at	Sorting nexin 10	NM_013322	4.19
205568_at	Aquaporin 9	NM_020980	3.58
204430_s_at	Solute carrier family 2, member 5	NM_003039	3.46
222939_s_at	Solute carrier family 16, member 10	N30257	3.44
209267_s_at	Solute carrier family 39, member 8	AB040120	2.60
202546_at	Vesicle-associated membrane protein 8 (endobrevin)	NM_003761	2.52
210423_s_at	Solute carrier family 11, member 1	L32185	2.46
203922_s_at	Cytochrome b-245, beta polypeptide (chronic granulomatous disease)	AI308863	2.05
223805_at	Oxysterol binding protein-like 6	AF323728	0.49
206306_at	Ryanodine receptor 3	NM_001036	0.47
204719_at	ATP-binding cassette, sub-family A (ABC1), member 8	NM_007168	0.46
1556116_s_at	Protein tyrosine phosphatase, receptor type, S	AI825808	0.45
224049_at	Potassium channel, subfamily K, member 17	AF339912	0.39
203400_s_at	Transferrin	NM_001063	0.31
228504_at	Sodium channel, voltage-gated, type VII, alpha	AI828648	0.20
Inflammatory and immune response			
212592_at	Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	AV733266	26.57
214974_x_at	Chemokine (C-X-C motif) ligand 5	AK026546	12.41
217022_s_at	Immunoglobulin heavy constant alpha 1	S55735	11.48
215214_at	Immunoglobulin lambda constant 1	H53689	10.12
215121_x_at	Immunoglobulin lambda light chain like	AA680302	8.72
209374_s_at	Immunoglobulin heavy constant mu	BC001872	8.52
214768_x_at	Immunoglobulin kappa constant	BG540628	8.09
205242_at	Chemokine (C-X-C motif) ligand 13	NM_006419	7.77
217235_x_at	Immunoglobulin lambda-like polypeptide 5	D84140	6.45
217258_x_at	Immunoglobulin lambda variable 1-44	AF043583	6.25
202917_s_at	S100 calcium binding protein A8	NM_002964	5.87
205114_s_at	Chemokine (C-C motif) ligand 3	NM_002983	5.66
202859_x_at	Interleukin 8	NM_000584	5.30
244313_at	Complement component (3b/4b) receptor 1, including Knops blood group system	AI052659	5.14
212657_s_at	interleukin 1 receptor antagonist	U65590	4.95
214146_s_at	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	R64130	4.82
213674_x_at	Immunoglobulin heavy constant delta	AI858004	4.56
216950_s_at	Fc fragment of IgG, high affinity Ia, receptor (CD64)	X14355	4.01
219434_at	Triggering receptor expressed on myeloid cells 1	NM_018643	3.82
229560_at	Toll-like receptor 8	AW872374	3.81
205831_at	CD2 molecule	NM_001767	3.63
205859_at	Lymphocyte antigen 86	NM_004271	3.32
204007_at	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	J04162	3.25
210915_x_at	T cell receptor beta constant 1	M15564	3.23
209901_x_at	Allograft inflammatory factor 1	U19713	3.22
211339_s_at	IL2-inducible T-cell kinase	D13720	3.22
214038_at	Chemokine (C-C motif) ligand 8	AI984980	3.20

226218_at	Interleukin 7 receptor	BE217880	3.08
204103_at	Chemokine (C-C motif) ligand 4	NM_002984	3.03
212998_x_at	Major histocompatibility complex, class II, DQ beta 1	AI583173	3.03
201422_at	Interferon, gamma-inducible protein 30	NM_006332	2.99
205098_at	Chemokine (C-C motif) receptor 1	AI421071	2.95
210146_x_at	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	AF004231	2.85
203535_at	S100 calcium binding protein A9	NM_002965	2.83
205269_at	Lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)	AI123251	2.76
1554899_s_at	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	BC020763	2.75
215806_x_at	T cell receptor gamma alternate reading frame protein	M13231	2.71
219725_at	Triggering receptor expressed on myeloid cells 2	NM_018965	2.69
206991_s_at	Chemokine (C-C motif) receptor 5	NM_000579	2.60
206337_at	Chemokine (C-C motif) receptor 7	NM_001838	2.57
235735_at	Tumor necrosis factor (ligand) superfamily, member 8	AI936516	2.52
223640_at	Hematopoietic cell signal transducer	AF285447	2.50
210629_x_at	Leukocyte specific transcript 1	AF000425	2.49
213539_at	CD3d molecule	NM_000732	2.44
206420_at	Immunoglobulin superfamily, member 6	NM_005849	2.40
210140_at	Cystatin F	AF031824	2.38
207850_at	Chemokine (C-X-C motif) ligand 3	NM_002090	2.36
210004_at	Oxidised low density lipoprotein (lectin-like) receptor 1	AF035776	2.36
214567_s_at	Chemokine (C motif) ligand 1	NM_003175	2.36
210644_s_at	Leukocyte-associated immunoglobulin-like receptor 1	AF109683	2.32
217147_s_at	T cell receptor associated transmembrane adaptor 1	AJ240085	2.30
226878_at	major histocompatibility complex, class II, DO alpha	AL581873	2.28
210031_at	CD247 molecule	J04132	2.28
220088_at	Complement component 5 receptor 1 (C5a ligand)	NM_001736	2.22
209906_at	Complement component 3a receptor 1	U62027	2.21
206366_x_at	Chemokine (C motif) ligand 2	U23772	2.19
205758_at	CD8a molecule	AW006735	2.17
204924_at	Toll-like receptor 2	NM_003264	2.16
209949_at	Neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	BC001606	2.12
202953_at	Complement component 1, q subcomponent, beta polypeptide	NM_000491	2.10
237753_at	Interleukin 21 receptor	AW504569	2.07
204787_at	V-set and immunoglobulin domain containing 4	NM_007268	2.05
218232_at	Complement component 1, q subcomponent, alpha polypeptide	NM_015991	2.02
232224_at	Mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	AI274095	0.40
230673_at	Polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	AV706971	0.25
Protein synthesis, posttranslational modifications and proteolysis			
204580_at	Matrix metallopeptidase 12	NM_002426	27.48

206666_at	Granzyme K	NM_002104	5.82
205488_at	Granzyme A	NM_006144	5.40
205624_at	Carboxypeptidase A3 (mast cell)	NM_001870	4.08
219574_at	Membrane-associated ring finger (C3HC4) 1	NM_017923	3.70
208146_s_at	Carboxypeptidase, vitellogenin-like	NM_031311	3.25
214533_at	Chymase 1, mast cell	NM_001836	3.21
202901_x_at	Cathepsin S	BC002642	2.87
205214_at	Serine/threonine kinase 17b	NM_004226	2.81
213566_at	Ribonuclease, RNase A family, k6	NM_005615	2.81
225646_at	Cathepsin C	AI246687	2.72
205653_at	Cathepsin G	NM_001911	2.43
235177_at	Methyltransferase like 21A	AI625022	2.26
203379_at	Ribosomal protein S6 kinase, 90kDa, polypeptide 1	NM_002953	2.26
228055_at	Napsin B aspartic peptidase pseudogene	AI763426	2.23
206111_at	Ribonuclease, RNase A family, 2	NM_002934	2.13
202693_s_at	Serine/threonine kinase 17a	AW194730	2.05
226435_at	Papilin, proteoglycan-like sulfated glycoprotein	AU145309	0.48
207414_s_at	Proprotein convertase subtilisin/kexin type 6	NM_002570	0.45
209883_at	Glycosyltransferase 25 domain containing 2	AF288389	0.42
229004_at	ADAM metallopeptidase with thrombospondin type 1 motif, 15	AI970797	0.40
228523_at	Nanos homolog 1 (<i>Drosophila</i>)	AW970089	0.37
227725_at	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1	Y11339	0.32
243582_at	SH3 domain containing ring finger 2	AW082633	0.26
Regulation of transcription			
211597_s_at	HOP homeobox	AB059408	3.99
206715_at	Transcription factor EC	NM_012252	3.64
204959_at	Myeloid cell nuclear differentiation antigen	NM_002432	3.38
202672_s_at	Activating transcription factor 3	NM_001674	2.34
204057_at	Interferon regulatory factor 8	AI073984	2.31
1552634_a_at	Zinc finger protein 101	NM_033204	2.18
228092_at	cAMP responsive element modulator	AL552470	2.13
204198_s_at	Runt-related transcription factor 3	AA541630	2.12
209780_at	Putative homeodomain transcription factor 2	AL136883	2.02
224013_s_at	SRY (sex determining region Y)-box 7	BC004299	0.49
202935_s_at	SRY (sex determining region Y)-box 9	AI382146	0.49
207480_s_at	Meis homeobox 2	NM_020149	0.49
228988_at	Zinc finger protein 711	AU157017	0.49
222357_at	Zinc finger and BTB domain containing 20	AW974823	0.46
229273_at	Sal-like 1	AU152837	0.45
222771_s_at	Myelin expression factor 2	BF224052	0.44
220184_at	Nanog homeobox	NM_024865	0.44
202191_s_at	Growth arrest-specific 7	BE439987	0.40
205517_at	GATA binding protein 4	AV700724	0.40
226913_s_at	SRY (sex determining region Y)-box 8	BF527050	0.40
244521_at	Teashirt zinc-finger homeobox 2	BG236742	0.39
243672_at	Sal-like 3	R42361	0.38
Signal transduction			
203001_s_at	Stathmin-like 2	NM_007029	4.70
223809_at	Regulator of G-protein signalling 18	AF076642	4.09

1555756_a_at	C-type lectin domain family 7, member A	AF400600	3.76
214467_at	G protein-coupled receptor 65	NM_003608	3.65
235740_at	Multiple C2-domains with two transmembrane regions 1	BG250585	3.61
208121_s_at	Protein tyrosine phosphatase, receptor type, O	NM_002848	3.58
210258_at	Regulator of G-protein signalling 13	AF030107	3.35
214470_at	Killer cell lectin-like receptor subfamily B, member 1	NM_002258	3.30
214770_at	Macrophage scavenger receptor 1	AI299239	3.19
205590_at	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	NM_005739	3.15
204891_s_at	Lymphocyte-specific protein tyrosine kinase	NM_005356	2.99
223660_at	Adenosine A3 receptor	AF226731	2.97
235046_at	Inositol polyphosphate-4-phosphatase, type II, 105 kDa	AA456099	2.86
204932_at	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	BF433902	2.79
225166_at	Rho GTPase activating protein 18	AU158022	2.77
203416_at	CD53 molecule	NM_000560	2.71
218870_at	Rho GTPase activating protein 15	NM_018460	2.68
230237_at	Adenylate cyclase activating polypeptide 1 (pituitary)	BE220888	2.67
204220_at	Glia maturation factor, gamma	NM_004877	2.64
210058_at	Mitogen-activated protein kinase 13	BC000433	2.56
203471_s_at	Pleckstrin	NM_002664	2.53
1554741_s_at	Fibroblast growth factor 7 (keratinocyte growth factor)	AF523265	2.45
223767_at	G protein-coupled receptor 84	AF237762	2.44
204951_at	Ras homolog gene family, member H	NM_004310	2.38
205016_at	Transforming growth factor, alpha	NM_003236	2.37
228708_at	RAB27B, member RAS oncogene family	BF438386	2.36
230866_at	Cysteinyl leukotriene receptor 1	BE549540	2.31
230422_at	Formyl peptide receptor-like 2	AW026543	2.27
1552691_at	ADP-ribosylation factor-like 11	NM_138450	2.27
208018_s_at	Hemopoietic cell kinase	NM_002110	2.21
205237_at	Ficolin (collagen/fibrinogen domain containing) 1	NM_002003	2.17
226068_at	Spleen tyrosine kinase	BF593625	2.16
213603_s_at	Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	BE138888	2.15
228186_s_at	R-spondin 3	BF589322	2.12
219358_s_at	ArfGAP with dual PH domains 2	NM_018404	2.10
204362_at	Src family associated phosphoprotein 2	NM_003930	2.03
226847_at	Follistatin	BF438173	2.02
219279_at	Dedicator of cytokinesis 10	NM_017718	2.02
227838_at	Unc-5 homolog C	AW070250	0.50
230144_at	Glutamate receptor, ionotropic, AMPA 3	AW294729	0.50
225511_at	G protein-coupled receptor, family C, group 5, member B	AV725364	0.49
205111_s_at	Phospholipase C, epsilon 1	NM_016341	0.49
215303_at	Doublecortin-like kinase 1	BE046461	0.49
226043_at	G-protein signalling modulator 1 (AGS3-like, C. elegans)	AI242661	0.49
226065_at	Prickle homolog 1	N98595	0.49
203071_at	Sema domain, immunoglobulin domain (Ig), short	NM_004636	0.46

	basic domain, secreted, (semaphorin) 3B		
232267_at	G protein-coupled receptor 133	AL162032	0.46
243792_x_at	Protein tyrosine phosphatase, non-receptor type 13	AI281371	0.45
204442_x_at	Latent transforming growth factor beta binding protein 4	NM_003573	0.43
221796_at	Neurotrophic tyrosine kinase, receptor, type 2	AA707199	0.43
203680_at	Protein kinase, cAMP-dependent, regulatory, type II, beta	NM_002736	0.42
213436_at	Cannabinoid receptor 1	U73304	0.42
241986_at	BMP binding endothelial regulator	AI423201	0.42
222317_at	Phosphodiesterase 3B, cGMP-inhibited	AA888858	0.41
217177_s_at	Protein tyrosine phosphatase, receptor type, B	AL080103	0.41
219764_at	Frizzled family receptor 10	NM_007197	0.40
209763_at	Chordin-like 1	AL049176	0.40
206423_at	Angiopoietin-like 7	NM_021146	0.39
204379_s_at	Fibroblast growth factor receptor 3	NM_000142	0.38
229309_at	Adrenoceptor beta 1	AI625747	0.36
225016_at	Adenomatosis polyposis coli down-regulated 1	N48299	0.35
222953_at	G protein-coupled receptor 83	BE670361	0.26
Unclassified			
234994_at	Transmembrane protein 200a	AA088177	5.32
205569_at	Lysosomal-associated membrane protein 3	NM_014398	4.47
220330_s_at	SAM domain, SH3 domain and nuclear localisation signals, 1	NM_022136	4.46
229390_at	Family with sequence similarity 26, member F	AV734646	3.98
225687_at	Family with sequence similarity 83, member D	BC001068	3.52
225105_at	Chromosome 12 open reading frame 75	BF969397	3.21
219014_at	Placenta-specific 8	NM_016619	3.20
34210_at	CD52 molecule	N90866	3.12
236782_at	Sterile alpha motif domain containing 3	AI129628	3.03
226459_at	Phosphoinositide-3-kinase adaptor protein 1	AW575754	2.97
221211_s_at	Chromosome 21 open reading frame 7	NM_020152	2.84
219607_s_at	Membrane-spanning 4-domains, subfamily A, member 4	NM_024021	2.80
1559065_a_at	C-type lectin superfamily 4, member G	BC039679	2.63
217916_s_at	Family with sequence similarity 49, member B	NM_016623	2.62
228489_at	Transmembrane 4 L six family member 18	AI721164	2.60
203021_at	Secretory leukocyte peptidase inhibitor	NM_003064	2.59
213888_s_at	TRAF3 interacting protein 3	AL022398	2.59
219386_s_at	SLAM family member 8	NM_020125	2.42
1559584_a_at	Chromosome 16 open reading frame 54	BC025741	2.34
228532_at	Chromosome 1 open reading frame 162	AW662189	2.31
204923_at	SAM and SH3 domain containing 3	AL023653	2.22
203761_at	Src-like-adaptor	NM_006748	2.22
205157_s_at	Junction plakophilin/keratin 17	NM_000422	2.22
229070_at	Androgen-dependent TFPI regulating protein	AA470369	2.18
227609_at	Epithelial stromal interaction 1	AA633203	2.12
1553043_a_at	CD300 antigen like family member F	NM_139018	2.10
221081_s_at	DENN/MADD domain containing 2D	NM_024901	2.10
206513_at	Absent in melanoma 2	NM_004833	2.09
243198_at	Testis expressed sequence 9	AA020920	2.02
231980_at	Docking protein 6	AL120332	0.50

225864_at	Family with sequence similarity 84, member B	AL039862	0.48
232166_at	KIAA1377	AL045516	0.48
228653_at	Sterile alpha motif domain containing 5	AI700341	0.48
231969_at	Storkhead box 2	AB037813	0.48
209074_s_at	Family with sequence similarity 107, member A	AL050264	0.48
218974_at	Sine oculis binding protein homolog	NM_018013	0.47
240402_at	Kin of IRRE like 3	H05918	0.47
202350_s_at	Matrilin 2	NM_002380	0.47
230015_at	Progressive rod-cone degeneration	AV729651	0.47
226344_at	Zinc finger, matrin type 1	AI741051	0.47
215250_at	Transmembrane protein 111	AU147317	0.47
218888_s_at	Neuropilin (NRP) and tolloid (TLL)-like 2	NM_018092	0.46
213050_at	Cordon-bleu homolog (mouse)	AA594937	0.46
231024_at	PGM5 antisense RNA 1 (non-protein coding)	H12280	0.45
202421_at	Immunoglobulin superfamily, member 3	AB007935	0.45
1559964_at	FLJ38717 protein	AK096036	0.45
228882_at	Tubby homolog (mouse)	AL042088	0.45
226490_at	NHS-like 1	AA503387	0.45
239999_at	Long intergenic non-protein condin RNA 478	N66393	0.41
209159_s_at	NDRG family member 4	AV724216	0.40
1564315_at	Chromosome 8 open reading frame 49	AK055534	0.40
226415_at	Vesicle amine transport protein 1 homologue	AA156723	0.40
228195_at	Chromosome 2 open reading frame 88	BE645119	0.39
228307_at	Elastin microfibril interfacer 3	AL137580	0.38
236141_at	Nbla00301	AA156933	0.38
230087_at	Proline rich membrane anchor 1	AI823645	0.38
237802_at	X Kell blood group precursor-related family, member 4	R54212	0.36
209569_x_at	DNA segment on chromosome 4 (unique) 234 expressed sequence	NM_014392	0.35
1559640_at	Ankyrin-repeat and fibronectin type III domain containing 1	BC037494	0.35
219791_s_at	FNbla00301	NM_024748	0.34
229730_at	Smoothelin-like 2	AI193973	0.34
221886_at	DENN/MADD domain containing 2A	AL037701	0.33
223523_at	Transmembrane protein 108	BC000568	0.31
229782_at	Rhabdomyosarcoma 2 associated protein (non-protein coding)	BE468066	0.30
232313_at	Transmembrane protein 132C	AL122107	0.29
Unknown transcribed loci and ESTs			
211645_x_at	Transcribed locus	M85256	15.08
217148_x_at	Transcribed locus	AJ249377	10.95
216401_x_at	Transcribed locus	AJ408433	10.05
214777_at	Transcribed locus	BG482805	8.72
216207_x_at	Transcribed locus	AW408194	8.36
211868_x_at	Transcribed locus	AJ225092	7.12
214836_x_at	Transcribed locus	BG536224	6.78
211798_x_at	Transcribed locus	AB001733	5.60
215946_x_at	Transcribed locus	AL022324	4.43
229635_at	Uncharacterized LOC100505702	AW043859	3.64
236280_at	Transcribed locus	AI225238	3.36
227827_at	Transcribed locus	AW138143	2.99

243366_s_at	Transcribed locus	AI936034	2.92
239294_at	Transcribed locus	AA810265	2.89
229937_x_at	Transcribed locus	AI681260	2.53
237625_s_at	Transcribed locus	BG548679	2.48
230631_s_at	Uncharacterized LOC100288432	AI202642	2.14
240232_at	Transcribed locus	AA503803	2.00
233607_at	Transcribed locus	AU145679	0.50
243933_at	Transcribed locus	AI096634	0.50
244420_at	Transcribed locus	AI128130	0.49
239908_at	Transcribed locus	AA496799	0.49
242671_at	Transcribed locus	BF055144	0.48
1561180_at	Transcribed locus	AK021807	0.48
1563217_at	Transcribed locus	BI598831	0.48
239653_at	Transcribed locus	AI936859	0.48
215578_at	Transcribed locus	AU145365	0.48
226587_at	Uncharacterized LOC100506965	BE783065	0.48
243462_s_at	Transcribed locus	BF590303	0.48
240385_at	Transcribed locus	BF002339	0.47
227576_at	Transcribed locus	AW003140	0.47
241459_at	Transcribed locus	AI494113	0.47
233449_at	Transcribed locus	AU143940	0.47
234088_at	Transcribed locus	AU147125	0.47
232182_at	Uncharacterized LOC286272	AI142853	0.46
233214_at	Transcribed locus	AK021962	0.46
1564358_at	Transcribed locus	AK026284	0.46
230653_at	Transcribed locus	AI469960	0.45
239708_at	Transcribed locus	AA027105	0.45
230785_at	Transcribed locus	AI634411	0.45
1566163_at	Transcribed locus	AL832482	0.45
243791_at	Transcribed locus	AW979261	0.44
1558444_at	Transcribed locus	AW206560	0.44
227663_at	Transcribed locus	BE674006	0.44
213089_at	Uncharacterized LOC153561	AU158490	0.44
240211_at	Uncharacterized LOC100130468	AI743177	0.44
235123_at	Transcribed locus	AI951144	0.43
219871_at	Uncharacterized FLJ13197	NM_024614	0.43
236184_at	Transcribed locus	AI798959	0.42
239301_at	Transcribed locus	BE551451	0.42
1563841_at	Transcribed locus	AL832549	0.42
239752_at	Transcribed locus	BE551781	0.41
240245_at	Transcribed locus	AW237264	0.39
1560049_at	Transcribed locus	AI125337	0.39
232947_at	Transcribed locus	AU144382	0.37
214295_at	Uncharacterized LOC57235	AW129056	0.36
1558714_at	Transcribed locus	BC043430	0.36
244112_x_at	Transcribed locus	N73550	0.34
242763_at	Transcribed locus	N39188	0.33
213904_at	Transcribed locus	AL390170	0.33
233386_at	Transcribed locus	AK022037	0.33

*Fold change represents statistically significant change ($P<0.05$) in gene expression between aortic stenosis group and fibro(sclerotic) group.

Supplementary Table 7. Differentially expressed genes between fibro(sclerotic) group and control group.

Affymetrix probe set ID	Description	GenBank Accession number	Fold change *
Biosynthesis and metabolism			
205943_at	Tryptophan 2,3-dioxygenase	NM_005651	2.10
203397_s_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)	BF063271	2.09
224400_s_at	Carbohydrate (N-acetylgalactosamine 4-O) sulfotransferase 9	AF332473	0.44
Cell adhesion			
209875_s_at	Secreted phosphoprotein 1 (osteopontin)	M83248	3.21
202112_at	Von Willebrand factor	NM_000552	2.18
Cell structure/motility			
37892_at	Collagen, type XI, alpha 1	J04177	2.54
Inflammatory and immune response			
212592_at	Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	AV733266	4.87
217022_s_at	Immunoglobulin heavy constant alpha 1	S55735	4.42
209138_x_at	Immunoglobulin lambda constant 1	M87790	4.06
209374_s_at	Immunoglobulin heavy constant mu	BC001872	3.84
214669_x_at	Immunoglobulin kappa constant	BG485135	3.72
214146_s_at	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	R64130	3.58
214974_x_at	Chemokine (C-X-C motif) ligand 5	AK026546	2.46
202917_s_at	S100 calcium binding protein A8	NM_002964	2.15
Protein synthesis, posttranslational modifications and proteolysis			
204580_at	Matrix metallopeptidase 12	NM_002426	3.35
205624_at	Carboxypeptidase A3 (mast cell)	NM_001870	2.44
216474_x_at	Tryptase alpha/beta 1	AF206667	2.01
243582_at	SH3 domain containing ring finger 2	AW082633	0.45
Regulation of transcription			
244043_at	Transcription factor Dp-2 (E2F dimerization partner 2)	AI049624	0.49
Signal transduction			
203000_at	Stathmin-like 2	BF967657	2.55
232282_at	WNK lysine deficient protein kinase 3	H06509	0.50
206423_at	Angiopoietin-like 7	NM_021146	0.42
Unclassified			
34210_at	CD52 molecule	N90866	2.88
230061_at	Transmembrane 4 L six family member 18	AW338625	2.59
234994_at	Transmembrane protein 200A	AA088177	2.00
237802_at	X Kell blood group precursor-related family, member 4	R54212	0.46
Unknown transcribed loci and ESTs			
215176_x_at	Transcribed locus	AW404894	3.41
211645_x_at	Transcribed locus	M85256	2.82
215946_x_at	Transcribed locus	AL022324	2.61
214777_at	Transcribed locus	BG482805	2.18
216401_x_at	Transcribed locus	AJ408433	2.14
241560_at	Transcribed locus	AI927941	0.46
213904_at	Homo sapiens cDNA clone IMAGE:4820330.	AL390170	0.45
240558_at	Transcribed locus	AI023335	0.28

* Fold change represents statistically significant change ($P<0.05$) in gene expression between fibro(sclerotic) group and control group.

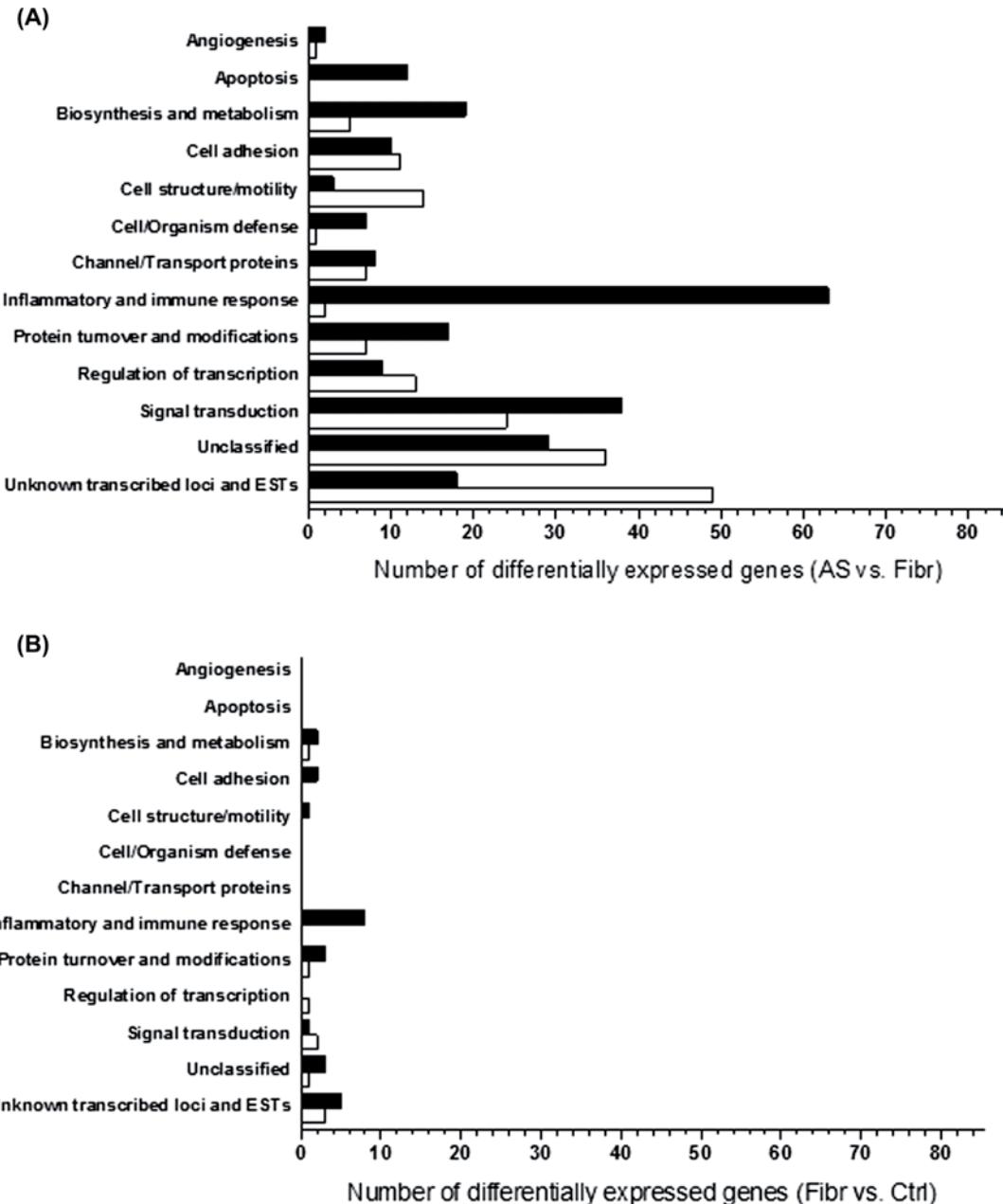
Supplementary Table 8. Abbreviations and full names of genes illustrated in Supplementary Fig. 2.

Abbreviation	Full name
ABLIM1	Actin binding LIM protein 1
ADAM8	ADAM metallopeptidase domain 8
ADCYAP1	Adenylate cyclase activating polypeptide 1 (pituitary)
ADRB1	Adrenergic beta-1-receptor
AMPD3	Adenosine monophosphate deaminase (isoform E)
ANGPT1	Angiopoietin 1
ANK2	Ankyrin 2, neuronal
APBB1IP	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
ARHGAP15	Rho GTPase activating protein 15
ARHGAP18	Rho GTPase activating protein 18
BID	BH3 interacting domain death agonist
CACNA2D4	Calcium channel, voltage-dependent, alpha 2/delta subunit 4
CASP1	Caspase 1
CCL7	Chemokine (C-C motif) ligand 7
CCL8	Chemokine (C-C motif) ligand 8
CCL23	Chemokine (C-C motif) ligand 23
CCR1	Chemokine (C-C motif) receptor 1
CCR5	Chemokine (C-C motif) receptor 5
CCR7	Chemokine (C-C motif) receptor 7
CD72	CD72 molecule
CD8A	CD8a molecule
CD247	CD247 molecule
CLEC7A	C-type lectin domain family 7, member A
COL4A3	Collagen, type IV, alpha 3
COL4A4	Collagen, type IV, alpha 4
COL6A6	Collagen, type VI, alpha 6
COL11A1	Collagen, type XI, alpha 1
CSF2RA	Colony stimulating factor 2 receptor, alpha, low-affinity
CTSG	Cathepsin G
CTSS	Cathepsin S
CXCL1	Chemokine (C-X-C motif) ligand 1
CXCL3	Chemokine (C-X-C motif) ligand 3
CXCL13	Chemokine (C-X-C motif) ligand 13
CXCL16	Chemokine (C-X-C motif) ligand 16
CYSLTR1	Cysteinyl leukotriene receptor 1
C3AR1	Complement component 3a receptor 1
C12orf5	Probable fructose-2,6-bisphosphatase
FBP1	Fructose-1,6-bisphosphatase 1
FIGF	Vascular endothelial growth factor D
FPR3	Formyl peptide receptor 3
F5	F5, Coagulation factor V
GALNT3	UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase 3
GALNTL1	UDP-N-acetyl-alpha-D-galactosamine-polypeptide N-acetylgalactosaminyltransferase-like 1

GNA15	Guanine nucleotide binding protein alpha
GPR65	G protein-coupled receptor 65
GRB14	Growth factor receptor-bound protein 14
GZMB	Granzyme B
HCK	Hemopoietic cell kinase
IRF8	Interferon regulatory factor 8
IFI30	Interferon, gamma-inducible protein 30
IL2RA	Interleukin 2 receptor, alpha
IL2RB	Interleukin 2 receptor, beta
IL8	Interleukin 8
IL18RAP	Interleukin 18 receptor accessory protein
INPP5D	Inositol polyphosphate-5-phosphatase, 145kDa
ITGA9	Integrin, alpha 9
ITGAL	Integrin, alpha L
ITK	IL2-inducible T-cell kinase
KMO	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)
KYNU	Kynureinase (L-kynurenine hydrolase)
LAMC3	Laminin, gamma 3
LCK	Lymphocyte-specific protein tyrosine kinase
LCP2	Lymphocyte cytosolic protein 2
LPXN	Leupaxin
L1CAM	L1 cell adhesion molecule
MAPK13	Mitogen-activated protein kinase 13
MMP12	Matrix metallopeptidase 12
NCF2	Neutrophil cytosolic factor 2
NCF4	Neutrophil cytosolic factor 4
NCKAP1L	NCK-associated protein 1-like
NFATC4	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
NLRC4	NLRC4, NLR family, CARD domain containing 4
NR2F1	Nuclear receptor subfamily 2, group F, member 1
PDE1C	Phosphodiesterase 1C, calmodulin-dependent 70kDa
PDE3B	Phosphodiesterase 3B, cGMP-inhibited
PIK3AP1	Phosphoinositide-3-kinase adaptor protein 1
PLAU	Plasminogen activator, urokinase
PLAUR	Plasminogen activator, urokinase receptor
PPBP	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)
PRICKLE1	Prickle homolog 1
PRF1	Perforin 1
PTGER4	Prostaglandin E receptor 4 (subtype EP4)
RAC2	Rho family, small GTP binding protein Rac2
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)
RGS18	Regulator of G-protein signaling 18
RHOH	Ras homolog gene family, member H
RORA	RAR-related orphan receptor A
RPS6KA1	Ribosomal protein S6 kinase, 90kDa, polypeptide 1
SPP1	Secreted phosphoprotein 1 (Osteopontin)
SRGN	Serglycin
ST6GALNAC1	ST6 (alpha-N-acetyl-neuraminy1-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1
SYK	Spleen tyrosine kinase
S100A8	S100 calcium binding protein A8

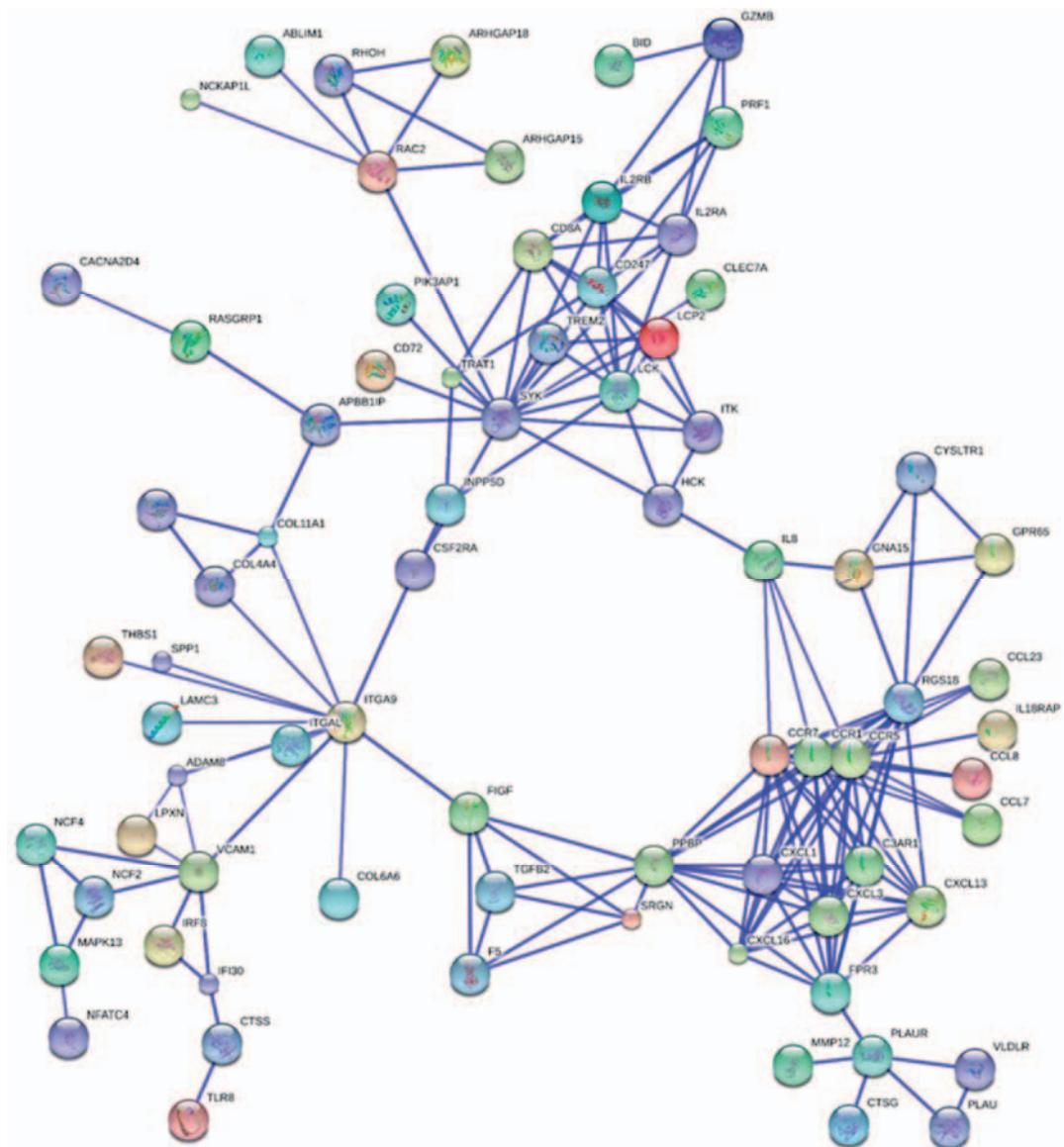
S100A9	S100 calcium binding protein A9
TDO2	Tryptophan 2,3-dioxygenase
TGFB2	Transforming growth factor, beta 2
THBS1	Thrombospondin 1
TLR8	Toll-like receptor 8
TRAT1	T cell receptor associated transmembrane adaptor 1
TREM2	Triggering receptor expressed on myeloid cells 2
VANGL2	Vang-like 2
VCAM1	Vascular cell adhesion molecule 1
VLDLR	Very low density lipoprotein receptor

Supplementary Figure 1. (A) Functional classification of genes differentially expressed in human aortic valve cusps in aortic stenosis group (AS) vs. fibro(sclero)sis group (Fibr) and (B) Fibr vs. control group (Ctrl).

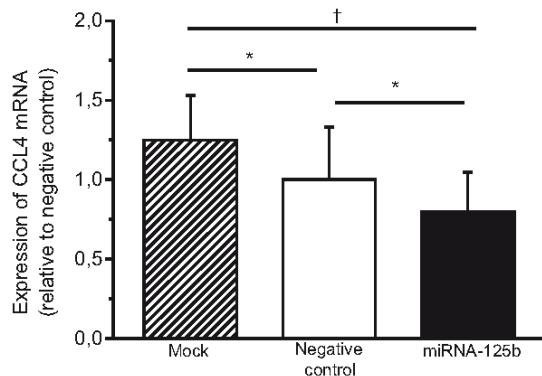


Supplementary Figure 2. Pathway analysis of differentially expressed genes in CAVD. Genes that were significantly changed in stenotic valves compared to control valves (Supplementary Table 5) were examined using the STRING database. The pathway shows potential biological connections between CAVD-associated genes. Lines represent regulatory connections between proteins. Protein nodes, network, and abbreviations are presented as they appear in STRING. ABLIM1, actin binding LIM protein 1; ADAM8, a Disintegrin and metalloproteinase domain-containing protein 8; APBB1IP, amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein; ARHGAP15, Rho GTPase activating protein 15; ARHGAP18, Rho GTPase activating protein 18; BID, BH3 interacting-domain death agonist; CACNA2D4, calcium channel, voltage-dependent, alpha 2/delta subunit 4; CCL7, chemokine (C-C motif) ligand 7; CCL8, chemokine (C-C motif) ligand 8; CCL23, chemokine (C-C motif) ligand 23; CCR1, C-C chemokine receptor type 1; CCR5, C-C chemokine receptor type 5; CCR7, C-C chemokine receptor type 7; CD8A, CD8a antigen; CD247, CD247 antigen; CD72, CD72 antigen; CLEC7A, C-type lectin domain family 7 member A; COL4A3, collagen, type IV, alpha 3; COL4A4, collagen, type IV, alpha 4; COL6A6, collagen, type VI, alpha 6; COL11A1, collagen, type XI, alpha 1; CSF2RA, colony stimulating factor 2 receptor, alpha; CTSG, cathepsin G; CTSS, cathepsin S; CXCL1, chemokine (C-X-C motif) ligand 1; CXCL3, chemokine (C-X-C motif) ligand 3; CXCL13, chemokine (C-X-C motif) ligand 13; CXCL16, chemokine (C-X-C motif) ligand 16; CYSLTR1, cysteinyl leukotriene receptor 1; C3AR1, complement component 3a receptor 1; FIGF, c-fos induced growth factor; FPR3, formyl peptide receptor 3; F5, coagulation factor V; GNA15, guanine nucleotide binding protein, alpha 15; GPR65, G protein-coupled receptor 65; GZMB, granzyme B; HCK, HCK proto-oncogene, Src family tyrosine kinase; IFI30, interferon, gamma-inducible protein 30; IL2RA, interleukin-2 receptor alpha; IL2RB, interleukin-2 receptor beta; IL8, interleukin 8; IL18RAP, interleukin 18 receptor accessory protein; INPP5D, inositol

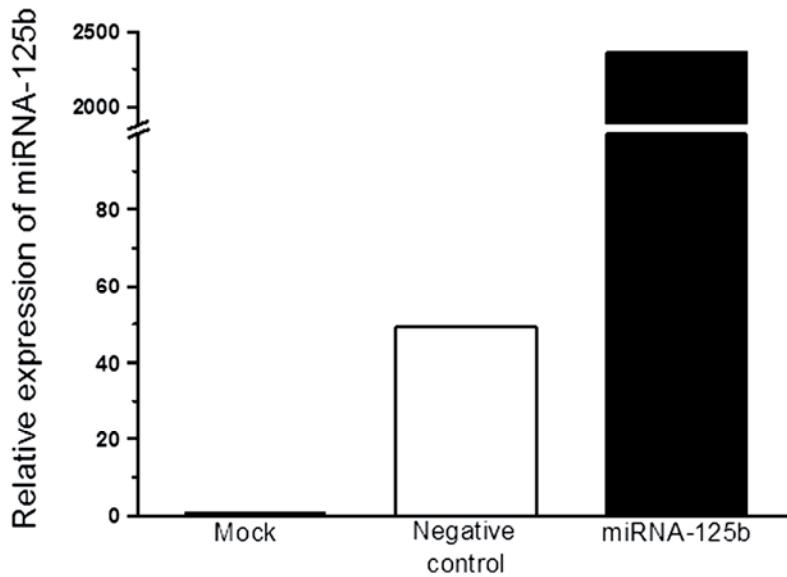
polyphosphate-5-phosphatase; IRF8, interferon regulatory factor 8; ITGAL, integrin alpha L chain; ITGA9, integrin alpha-9; ITK, IL2-inducible T-cell kinase; LAMC3, laminin subunit gamma-3; LCK, lymphocyte-specific protein tyrosine kinase; LPC2, lipocalin 2; LPXN, leupaxin; MAPK13, mitogen-activated protein kinase 13; MMP12, matrix metalloproteinase-12; NCF2, neutrophil cytosolic factor 2; NCF4, neutrophil cytosol factor 4; NCKAP1L, NCK-associated protein 1-like; NFATC4, nuclear factor of activated T-cells, cytoplasmic 4; PIK3AP1, phosphoinositide-3-kinase adaptor protein 1; PLAU, urokinase-type plasminogen activator; PLAUR urokinase-type plasminogen activator receptor; PPBP, pro-platelet basic protein (CXCL7); PRF1, perforin 1; RAC2, Ras-Related C3 Botulinum Toxin Substrate 2 (Rho Family, Small GTP Binding Protein Rac2); RASGRP1, RAS guanyl-releasing protein 1; RGS18, regulator of G-protein signaling 18; RHOH, ras homolog family member H; SPP1, osteopontin; SRGN, serglycin; SYK, spleen tyrosine kinase; TGFB2, transforming growth factor, beta 2; THBS1, thrombospondin 1; TLR8, toll-like receptor 8; TRAT1, T cell receptor associated transmembrane adaptor 1; TREM2, triggering receptor expressed on myeloid cells 2; VCAM1, vascular cell adhesion molecule 1; VLDLR, very-low-density-lipoprotein receptor.



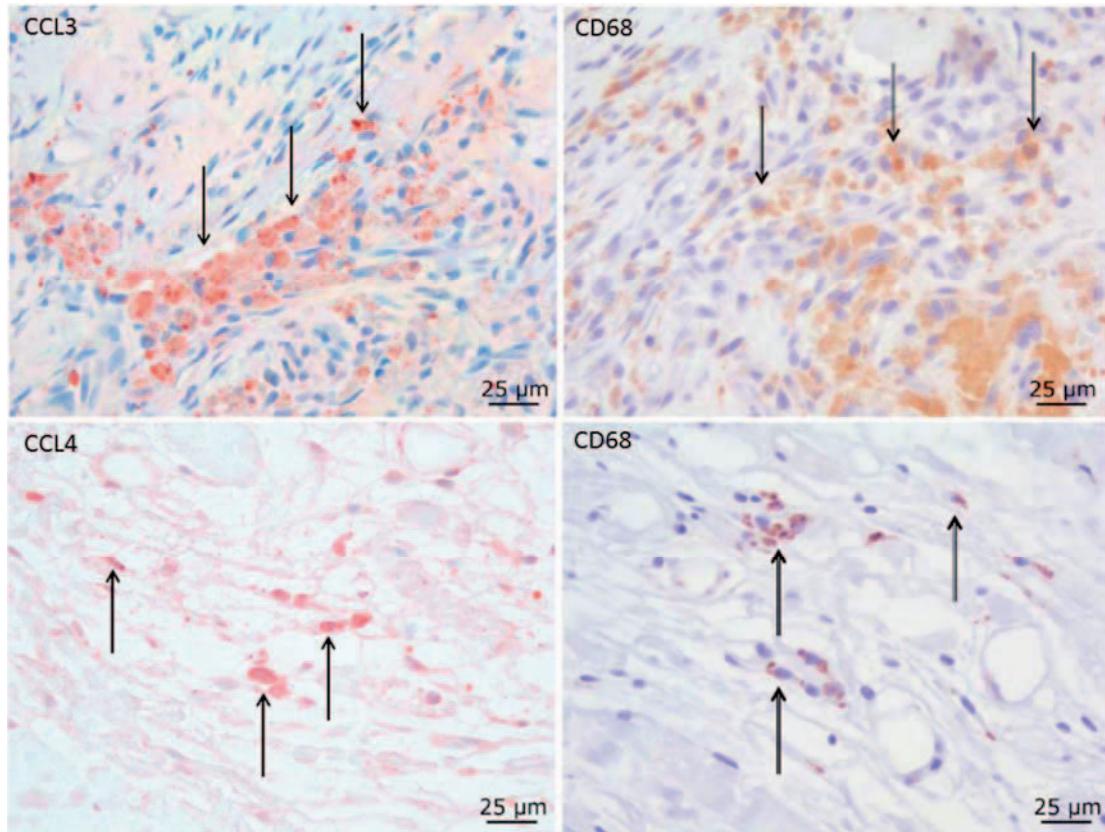
Supplementary Figure 3. Expression of CCL4 mRNA levels in THP-1 macrophages after transfection with transfection reagent only (mock), negative control miRNA and miRNA-125b. Results are expressed \pm SEM relative to negative control (* P< 0.05).



Supplementary Figure 4. Transfection of miRNA-125b was successful. The bars indicate the relative amount of miR-125b in human THP-1 macrophages after transfection with reagent only (mock), negative control and miRNA-125b.



Supplementary Figure 5. Representative immunohistochemical images demonstrating CCL3 and CCL4 expression in human aortic valve cusps. In the left-hand panels, CCL3 and CCL4 positivity is seen in cytoplasms as red granular staining (arrows). These cells are macrophages, as they are positive in CD68 staining of respective areas, shown on the right (brown cytoplasmic stain, double arrows).



Supplementary Figure 6. Negative control immunofluorescence stainings of calcific aortic valves.

