Chapter 6

The hypoxia target adrenomedullin is aberrantly expressed in multiple myeloma and promotes angiogenesis

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Leukemia 2013; 27(8): 1729–1737

ABSTRACT

In multiple myeloma (MM), angiogenesis is strongly correlated to disease progression and unfavorable outcome, and may be promoted by bone marrow hypoxia. Employing gene-expression profiling, we here identified the pro-angiogenic factor adrenomedullin (AM) as the most highly upregulated gene in MM cells exposed to hypoxia. Malignant plasma cells from the majority of MM patients, belonging to distinct genetic subgroups, aberrantly express AM. Already under normoxic conditions, a subset of MM highly expressed and secreted AM, which could not be further enhanced by hypoxia or cobalt chloride-induced stabilization of hypoxia-inducible factor (HIF)1α. In line with this, expression of AM did not correlate with expression of a panel of established hypoxia-/HIF1α-target genes in MM patients. We demonstrate that MM-driven promotion of endothelial cell proliferation and tube formation is augmented by inducible expression of AM and strongly repressed by inhibition of endogenous and hypoxia-induced AM activity. Together, our results demonstrate that MM cells, both in a hypoxia-dependent and independent fashion, aberrantly express and secrete AM, which can mediate MM-induced angiogenesis. Thus, AM secretion can be a major driving force for the angiogenic switch observed during MM evolution, which renders AM a putative target for MM therapy.

INTRODUCTION

Multiple myeloma (MM) is a neoplasm characterized by expansion of malignant plasma cells in the bone marrow. The transition of a normal plasma cell to a fully transformed, aggressive myeloma cell is a multistep process, which requires the acquisition of chromosomal translocations and mutations in multiple genes. Most of this evolution takes place in the bone marrow (BM), indicating that the interaction with the BM microenvironment has a critical role in the pathogenesis of MM.[1,2] In the MM-infiltrated BM, aberrant neovascularization (angiogenesis) is almost invariably present, and is associated with endothelial activation, increased capillary permeability and hyperperfusion.[3,4] Importantly, BM angiogenesis in MM parallels disease progression and is correlated with poor event-free and overall survival,[5,6] while after successful treatment, microvessel density returns to normal.[7,8]

The pathogenesis of MM-induced angiogenesis has not yet been fully elucidated. It is driven by genetic alterations in the MM cells resulting in an imbalance between the production of pro- and anti-angiogenic factors by myeloma cells and the microenvironment. This is reflected by elevated levels of pro-angiogenic factors, including VEGFA, bFGF, and HGF, in the BM plasma and peripheral blood of MM patients.[9] This imbalance results in an "angiogenic switch", which takes place on the verge of progression of monoclonal gammopathy of undetermined significance (MGUS) to active MM. As some of the important angiogenic factors secreted by myeloma cells, including VEGFA and bFGF, are equally expressed by tumor cells isolated from MGUS, smoldering MM and active MM,[10] it has been suggested that the angiogenic switch could also be the consequence of increasing tumor burden, rather than that of aberrant expression of pro-angiogenic factors alone. In line with this notion, a study by Hose *et al.*[11] revealed that even normal bone marrow plasma cells (BMPC) have significant pro-angiogenic properties.

On the other hand, chronic hypoxia may also have an important role in BM angiogenesis in MM.[12–14] This is suggested by studies demonstrating stabilization and nuclear localization of the HIF1α protein in malignant plasma cells.[12,13,15,16] Indeed, by employing gene-expression profiling, Colla *et al.*[13] demonstrated that hypoxia affects the transcriptional and angiogenic profiles of myeloma cells, leading to increased expression of VEGFA and IL-8 among other pro-angiogenic factors. Interestingly, HIF1α protein stabilization and activity in MM cells may also occur under normoxic conditions and can, in collaboration with c-MYC, induce VEGFA-mediated angiogenesis.[15] Together, these findings suggest a central role for the hypoxia-HIF1α axis in MM-related BM angiogenesis.

In the present study, we further explored this possibility by studying the transcriptional response of MM cells to hypoxia, using gene-expression microarrays. Interestingly, we identified the pro-angiogenic factor adrenomedullin (AM) as the most highly hypoxia-induced gene in MM cells. In primary myelomas, AM expression was found to be increased during disease progression from MGUS to MM. In addition, endogenous, ectopically expressed and hypoxia-triggered AM secretion by primary MMs and MM cell lines enhanced angiogenesis. Of note, several MM cell lines and primary MMs expressed high levels of AM under normoxic conditions, suggesting regulation independent of the hypoxia-HIF1α axis. Taken together, our results identify AM as a potential driver of the angiogenic switch and promising therapeutic target in MM.

MATERIALS AND METHODS

PREPARATION OF COMPLEMENTARY RNA, MICROARRAY HYBRIDIZATION AND GENE-EXPRESSION PROFILING ANALYSIS

RNA was extracted with the RNeasy Kit (Qiagen, Hilden, Germany) or the SV-total RNA extraction kit (Promega, Fitchburg, WI, USA) and Trizol (Invitrogen Life Technologies, Carslbad, CA, USA), in accordance with the manufacturer's instructions. Biotinylated complementary RNA was amplified with a double *in vitro* transcription, according to the Affymetrix small sample labeling protocol vII (Affymetrix, Santa Clara, CA, USA). The biotinylated complementary RNA was fragmented and hybridized to the HG-U133 Plus 2.0 GeneChip oligonucleotide arrays according to the manufacturer's instructions (Affymetrix). Fluorescence intensities were quantified and analyzed using the GCOS software (Affymetrix). Arrays were scaled to an average intensity of 100. Differentially expressed genes

were identified by a Student's *t*-test, and *P*-values were adjusted for multiple comparisons using the Benjamini and Hochberg correction. The threshold for significance was set to a *P*-value of ≤ 0.05. Among those genes, those with a fold change of ≥ 2 were retained. Among the genes with a fold change ≥ 2 in a given population, those with 100% absent call in this population were considered not to be biologically relevant and were removed. The call ("present" or "absent") is determined by Affymetrix GCOS software and indicates whether a gene is reliably expressed or not. For a global analysis of AM expression, gene-expression data publically available and deposited in the NIH Gene Expression Omnibus (GEO) National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/geo/ under accession number GSE2658, were used. These concerned the U133 Plus 2.0 Affymetrix oligonucleotide microarray data from 559 newly diagnosed MM patients included in total therapy 2/3(TT2, TT3), provided by the Donna D and Donald M Lambert Laboratory of Myeloma Genetics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.[17]

Primary MM samples used for additional studies were obtained during routine diagnostic procedures at the Academic Medical Center, Amsterdam, the Netherlands. Mononuclear cells were harvested by standard Ficoll/Paque density gradient centrifugation (Amersham Pharmacia Biosciences, Roozendaal, the Netherlands) and CD138+ cells were sorted by positive selection using anti-CD138 antibody (clone BB4, Instruchemie, Delfzijl, the Netherlands) and Dynabead-conjugated goat anti-mouse IgG (Dynal, Oslo, Norway).

NF-κB PROFILE

The MM NF-κB profile was determined by Annuziata *et al.*[18] Genes comprising the NF-κB profile in MM were those that were decreased in expression by > 40% in at least six of eight time points following treatment of L363 cells with IKKβ inhibitor (MLN120b) (Millennium Pharmaceuticals, Cambridge, MA, USA) for 8–24 h in three separate experiments (accession number GSE8487). Genes were chosen if they correlated in expression across the MM cell lines ($r > 0.5$). NF-κB profile genes, using Affymetrix U133 Plus 2.0 data, relied on the following probe sets: 210538_s_at (*BIRC3*), 202644_s_at (*TNFAIP3*), 207535_s_at (*NFKB2*), 204116_at (*IL2RG*), 203927_at (*NFKBIE*), 205205_at (*RELB*), 201502_s_at (*NFKBIA*), 209619_at (*CD74)*, 203471_s_at (*PLEK*), 210018_x_at (*MALT1)*, 223709_s_at (*WNT10A*).

CELL CULTURE

Human myeloma cell lines (HMCLs) L363, UM-1, OPM-1, NCI-H929 and RPMI8226 were cultured in RPMI medium 1640 (Invitrogen Life Technologies)

containing 10% clone I serum (HyClone, Waltham, MA, USA), 100 units per ml of penicillin, and 100 µg per ml of streptomycin. LME-1 cell line was cultured in IMDM medium (Invitrogen Life Technologies) supplemented with transferrin (20 µg/ml) and β-mercaptoethanol (50µM). An AM cDNA doxycycline-inducible NCI-H929 cell line (NCI-H929/TR/AM) was generated as described previously, using the T-REx System (Invitrogen Life technologies).[19] AM overexpression was obtained by incubating NCI-H929/TR/AM cells for 24 h with 0.2 µg/ml doxycycline. Conditioned medium was harvested from 48 h cultures initiated at a concentration of 10^6 cells/ml in RPMI 10% FCS medium. Control medium (RPMI 10% FCS) was pretreated for 48h in 37°C next to conditioned medium. NF-κB stimulation was performed by culturing MM cells (at a concentration of 10^6 cells/ml in RPMI medium containing 10% FCS) for 48h with PMA or TNFα (100ng/ml). The treatment with cobalt chloride ($CoCl_2$) was performed by culturing MM cells (at a concentration of 10^6 cells/ml in RPMI medium containing 10% FCS) for 24 h with the final concentration of $CoCl_2$ (100 uM).

HUVECs were prepared from human umbilical cord veins as described previously.[20] The adherent endothelial cells (culture flasks were coated with 1% gelatin) were maintained in RPMI medium (Invitrogen Life Technologies) containing 10% clone I serum (HyClone), 10% normal human serum, 3 µg/ml of basic fibroblast growth factor (bFGF), 100 units/ml of penicillin/streptomycin and 2mmol/l l-glutamine (complete medium), and incubated at 37°C in 5% CO2. At confluence, the cells were detached by trypsin and used in experiments before the sixth passage.

RT-PCR

Total RNA was isolated using Trizol according to the manufacturer's protocol (Invitrogen Life Technologies). The RNA was further purified using isopropanol precipitation and was concentrated using the RNeasy MinElute Cleanup kit (Qiagen). The quantity of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Five microgram of total RNA was used for cDNA synthesis as described previously.[21] The PCR mixture contained: 2 µl of cDNA, 1 x PCR Rxn buffer (Invitrogen Life Technologies), 0.2 mmol/l dNTP, 2 mmol/l $MgCl_2$, 0.2 µmol/l of each primer, and 1 U platinum Taq polymerase (Invitrogen Life Technologies). PCR conditions were: denaturing at 95°C for 5 min, followed by 30 cycles of 30 s at 95°C, 30s at 58°C and 30 s at 72°C. The reaction was completed for 10 min at 72°C. Primers used were: AM forward (5′-CTCTGAGTCGTGGGAAGAG G-3′); AM reverse (5′-CGTGTGCTTGTGGCTTAGAA-3′); PFKFB4 forward (5′-GGGATGGC GTCCCCACGGG-3′); PFKFB4 reverse (5′-CGCTCTCC

GTTCTCGGGTG-3′), AK3L forward (5′-TGCTGCCAGGCTAAGACAGTA CAA-3′); AK3L reverse (5′-TCTTCC TGGTTCTTCCATTGGGCA-3′) BNIP3 forward (5′-CACCTCGCTCGCAGACAC CAC-3′); BNIP3 reverse(5′-GAG AGCAGCAGAGATGGAAGGAAAAC-3′), VEGF forward (5′-CTCTACCT CCACCATGCCAAGT-3′); VEGF reverse (5′-ATCTGGTTC CCGAAAC CCTGAG-3′), CD74 forward (5′-TGACCAGCGCGACCTTATC-3′);CD74 reverse (5′-GAGCAGGTGCATCACATGGT-3′), RELB forward (5′-CATCG AGCTCCGGGATTGT-3′); RELB reverse (5′-CTTCAGGGACCCAGCGTT GTA-3′), calcitonin-receptor-like receptor (CRLR) forward (5′-TGCTCTGTG AAGGCATT TAC-3′); CRLR reverse (5′-CAGAATTGCTTGAACCTCTC-3′), RAMP2 forward (5′-GGACGGTGAAGAACTATGAG-3′); RAMP2 reverse (5′-ATCATGGCCAGGA GTACATC-3′), HPRT1 forward (5′-TGGCGTCG TGATTAGTGATG-3′); HPRT1 reverse (5′-TATCCAACACTTCGTGGGG T-3′). All primers were manufactured by Sigma-Aldrich (Haverhill, UK).

HUVEC PROLIFERATION AND TUBE FORMATION ASSAY

HUVECs were plated at a density of 5000 cells/well on pre-coated 96-well plates in 100 µl of RPMI 1640 medium containing 10% FCS and 10% NHS. The next day, the medium was removed and HUVECs were stimulated with MM conditioned medium (CM), harvested from 48 h cultures initiated at a concentration of 10^6cells/ml in RPMI with 10% FCS. The AM inhibitors (37133 and 16311) were obtained from the laboratory of Frank Cutitta and used at a final concentration of 1 µM.[22] bFGF was used at a final concentration of 3 µg/ml. After 72h, the number of living cells was determined based on a FSC/SSC dot plot. The results are presented as mean +/- s.d. of samples assayed in triplicate. All experiments were performed at least three times. Student's *t*-test was used for statistical data comparison. For the tube formation assay, HUVECs were seeded on matrigel in 15-well µ-slide angiogenesis plates, and were stimulated with MM conditioned medium. Cultures were then incubated for 6h at 37°C. At the end of the incubation period, from each well, three fields of view were photographed and the number of meshes per field was quantified. The data were expressed as mean ± s.d. of a representative experiment in triplicate.

ELISA AND WESTERN BLOT

The concentration of AM in MM supernatants was determined by ELISA (Phoenix Pharmaceutical, Burlingame, CA, USA). MM cell line conditioned media were obtained after 48 h culture at a concentration of 10^6cells/ml in RPMI 1% FCS medium. Control medium (RPMI 1% FCS) was pre-treated for 48 h at 37°C.

Protein for immunoblotting was harvested from MM cell lines, separated by 10% SDS-polyacrylamide gel electrophoresis and subsequently blotted. The following antibodies were used for immunoblotting: anti-HIF1α polyclonal antibody (NB100-449, Novus Biologicals, Littleton, CO, USA) and anti-β-actin monoclonal antibody (clone AC-15, Sigma-Aldrich, St. Louis, MO, USA). Primary antibodies were detected by HRP-conjugated secondary antibodies, followed by detection using Lumi-Light PLUS western blotting substrate (Roche, Penzberg, Germany).

STATISTICAL ANALYSIS

Nonparametric statistics were used with Prism 5.0 software (Graphpad Software, San Diego, CA, USA). Spearman rank correlation coefficients were used to determine correlations (in 559 newly diagnosed MM patients included in the Total Therapy 2/3(TT2, TT3) trials between (i) expression of the *AM* gene and the MM hypoxia target genes, (ii) expression of the *AM* gene and the NF-κB profile genes, (iii) *AM* gene and the *HOXB7* gene and (iv) expression of the *AM* gene and the *ING4* gene. The Kruskal-Wallis test was used to compare *AM* gene expression in plasma cells derived from 559 newly diagnosed MM patients treated in the TT2 and TT3 trials, 4 MGUS patients and 22 healthy donors BMPC. Two-tailed *t*-tests were employed to analyze the *in vitro* data using Prism 5.0 software (Graphpad Software, San Diego, CA, USA). A *P*-value of < 0.05 was considered to be statistically significant. The gene ontology (GO) biological pathways regulated by hypoxia were identified using DAVID software (Database for Annotations, Visualization and Integrated Discovery).[23]

RESULTS

TRANSCRIPTIONAL RESPONSE OF MULTIPLE MYELOMA CELLS TO HYPOXIA

To gain a global view of the transcriptional response of multiple myeloma cells to hypoxia, we compared the gene-expression profile of the HMCLs UM-1 and OPM-1 cultured under normoxic and hypoxic conditions for 16 h, using Affymetrix human genome U133 plus 2.0 arrays. HIF1α stabilization, a characteristic response to hypoxic stimulation, was confirmed by immunoblotting (Figure 1A). Hypoxia resulted in a consistent > 2-fold increase in gene expression of 311 genes in UM-1 cells and 290 genes in OPM-1 cells (Supplementary Table 1 and 2). Importantly, the hypoxic gene-expression signature (Table 1) contained many genes

that have previously been associated with hypoxia responses and/or represent established HIF1α target genes (underlined in Table 1). To identify if any GO classes were enriched in these two differentially expressed gene sets, GO analysis was performed using DAVID bioinformatics resource. The enriched GO "biological process" categories were found to be enriched with a *P*-value cutoff of $P < 0.05$ and a fold enrichment ≥ 2, and are shown in Supplementary Figures 1 and 2. Importantly, the "hypoxia" pathway, as well as pathways known to be regulated in response to hypoxia were found for both cell lines.

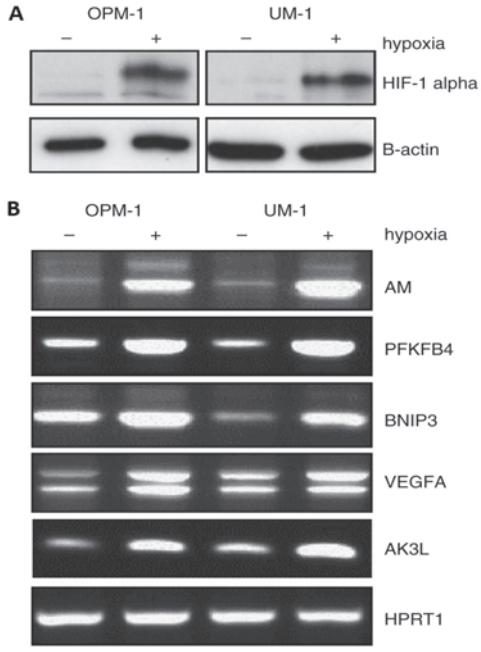


Figure 1. Transcriptional response of multiple myeloma cells to hypoxia

(A) HIF1α accumulation in the HMCLs OPM-1 and UM-1 in response to hypoxia. HMCLs were cultured under normoxic (-) or hypoxic (+) (1% O2) conditions for 16 h. HIF1α accumulation was detected using western blot. β-actin expression is shown as an input control. (B) Expression of hypoxia target genes in the HMCLs, OPM-1 and UM-1 MM cell lines, as determined by RT-PCR following 16 h exposure to normoxia (-) or hypoxia (+) (1% O2). HPRT1 expression is shown as an input control.

Those pathways contained many genes that have previously been associated with hypoxia responses and/or represent established HIF1α target genes (underlined in Table 1). It comprised genes involved in the metabolic response to hypoxia, including glucose import (*SLC2A6* and *SLC2A1*), glycolysis (*PFKFB4*, *PFKFB3*, *ALDOC*, *ENO1*, *HK2* and *HK1*), downregulation of oxidative phosphorylation

(*PDK1* and *MXI1*); genes involved in cell proliferation and apoptosis (*BNIP3*, *BNIP3L*, *PRF1*, *PLEKHF1* and *APLP1*), genes encoding transcription factors and signaling molecules (*SREBF1*, *JUN*, *MAF*, *WDR54*, *WDR5B*, *RAB20*, *RAP1* and *GAP*) and several pro-angiogenic genes (*VEGFA*, *AM* and *ANGPTL4*). For AM, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-4 (PFKFB4), BCL2/adenovirus E1B 19kDa interacting protein 3 (BNIP3), vascular endothelial growth factor (VEGF), and adenylate-kinase-3-like-1 (AK3L), hypoxia-mediated induction was confirmed by RT-PCR (Figure 1B). Among the hypoxia-repressed genes were a large group of genes linked to cell proliferation/DNA replication and protein synthesis, energy-costly processes that are typically repressed in response to hypoxic stress (Table 1).

Table 1. Common main genes induced by hypoxia in OPM-1 and UM-1 cell line

Main genes induced by hypoxia	**Main genes downregulated by hypoxia**
Extracellular glucose import *SLC2A6, SLC2A1*	Cell cycle and proliferation *CDC27, CDC23, CDC20, MYC, PLK1, CDK6, HDAC9*
Glycolytic breakdown of glucose *PFKFB4, PFKFB3, ALDOC, ENO1, HK2, HK1*	Regulation of DNA replication *RFC3, TOP1, CCDC88A*
Inhibition of mitochondrial activity *PDK1, MXI1*	Regulation of translation *EPRS, EIF2B3, FARSB, YARS2, EIF4G3*
Angiogenesis *VEGFA, AM, ANGPTL4*	— —
Apoptosis *BNIP3, BNIP3L, PRF1, PLEKHF1, APLP1*	— —
Transcription factors *SREBF1, JUN, MAF*	— —
Signal transduction *WDR54, WDR5B, RAB20,RAP1,GAP AK3L*	— —
Response to oxidative stress *IDH1*	— —
Chemokines and chemokine receptor *CXCR4, CCL28*	— —

Main genes induced by hypoxia, as revealed using DAVID Gene Functional Classification Tool. Confirmed HIF-1 alpha target genes are underlined.

Interestingly, in both OPM-1 and UM-1 cells, AM was identified as the top-regulated hypoxia target gene, displaying a 48- and 57-fold induction, respectively. The fact that AM has been reported to be a transcriptional target gene of HIF1α[24] and acts as a potent angiogenic factor in a number of solid tumors,[25–29] prompted

us to further investigate its expression, regulation and role in myeloma-induced angiogenesis.

EXPRESSION AND REGULATION OF AM IN HMCLS AND PRIMARY MULTIPLE MYELOMA CELLS

To explore the expression and regulation of AM in MM, we initially assessed the AM mRNA and protein expression in a panel of HMCLs cultured under normoxic conditions (Figures 2A–C). While high or moderate AM mRNA levels were found in L363, LME-1 and RPMI8226, AM expression in the other HMCLs was either low or undetectable (OPM-1, NCI-H929 and UM-1) (Figure 2A).

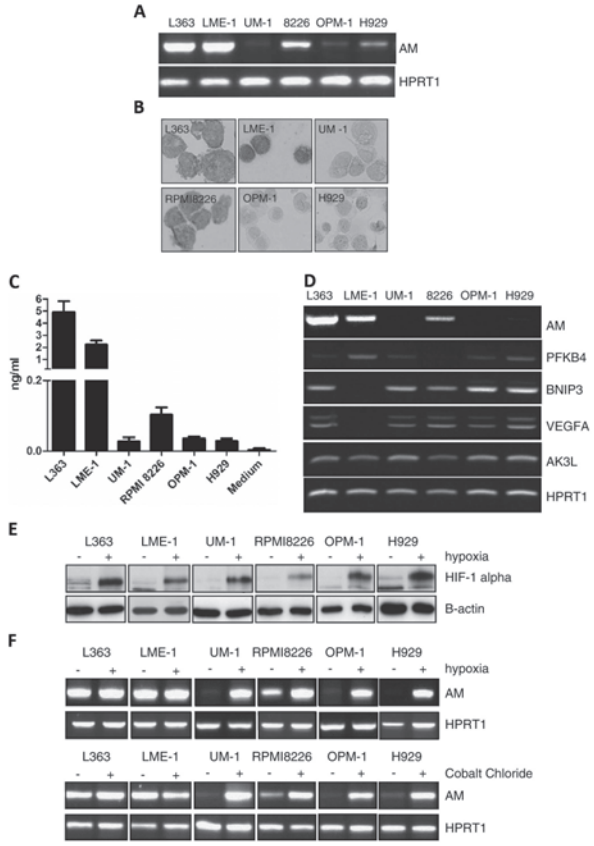


Figure 2. Expression and regulation of AM in MM cells

(A) Expression of AM mRNA in a panel of HMCLs. AM mRNA expression was analyzed by RT-PCR. HPRT1 was used as a loading control. (B) AM protein expression by HMCLs. Immunocytochemical staining of HMCLs with anti-AM antibody, showing intracytoplasmic expression (magnification: x 400). (C) AM protein secretion by HMCLs. AM concentration in the CM of HMCLs cells cultured for 48 h

was measured by ELISA ($n = 3$). (D) Expression of Am and selected hypoxia target genes in the panel of HMCLs. Hypoxia target genes expression was analyzed by RT-PCR. HPRT1 expression is shown as input control. (E) HIF1α accumulation in HMCLs in response to hypoxia. HMCLs were cultured under normoxic (-) or hypoxic (+) (1% O2) conditions for 16 h. HIF1α accumulation was determined by immunoblotting. β-actin expression is shown as an input control. (F) Upper panel: AM mRNA expression in HMCLs exposed to hypoxia. Cells were cultured for 16 h under normoxic (-) or hypoxic (+) conditions. HprT1 expression is shown as an input control. Lower panel: AM mRNA expression in HMCLs exposed to $CoCl_2$. AM mRNA expression in HMCLs cultured in the absence (-) or presence (+) of 100 μM $CoCl_2$ for 24 h.

Immunocytochemical AM protein detection matched these mRNA expression data (Figure 2B), showing strong cytoplasmic staining of L363, LME-1 and RPMI 8226, but no or very weak AM expression in the other cell lines. In addition, significant quantities of secreted AM protein could also be detected in the culture supernatants of L363 and LME-1 and, at lower levels, of RPMI8226 (Figure 2C). Hence, under normoxic culture conditions, HMCLs show highly variable levels of AM mRNA and protein expression.

As AM is a transcriptional target of HIF1α,[24] high expression of AM in HMCL under normoxic conditions could be a consequence of aberrant HIF1α pathway activation. If so, the AM-positive HMCLs L363, LME-1 and RPMI8226 would be predicted to be positive for an expression-signature comprising multiple HIF1α targets genes.[30] However, AM expression in the tested HMCLs did not correlate with that of other HIF1α/hypoxia target genes (Figure 2D). This suggests that the normoxic AM expression in L363, LME-1 and RPMI8226 cells is regulated by a HIF1α-independent mechanism.

Next, we subjected all HMCLs to hypoxia, which resulted in effective HIF1α stabilization (Figure 2E). Interestingly, AM expression was not only strongly induced in OPM-1 and UM-1 cells, but also in NCI-H929 and in RPMI8226 cells, with low and moderate normoxic AM gene expression, respectively. The already high normoxic AM expression in LME-1 and L363 cells was not further increased by hypoxia (Figure 2F upper panel). A similar induction of AM expression in HMCLs was obtained with $CoCl_2$ (Figure 2F lower panel). $CoCl_2$ stabilizes HIF1α by inhibiting prolyl hydroxylase domain-containing proteins (PHDs), which, in normoxic conditions, hydroxylate HIF1α. This hydroxylation is required for interaction of HIF1α with the VHL protein, which targets HIF1α for degradation by the 26S proteasome.[31] Thus, induction of AM expression by treatment with $CoCl_2$ confirms the role of HIF1α in hypoxia-driven upregulation of AM in MM cells (Figure 2F).

EXPRESSION OF AM IS RELATED TO MULTIPLE MYELOMA DISEASE PROGRESSION AND MOLECULAR SUBGROUP

Consistent with the results obtained in HMCLs, primary MMs isolated and processed under standard, that is, non-hypoxic, conditions, also showed highly variable levels of AM mRNA expression (Figure 3A). As shown in Figure 3B, hypoxic stimulation of these primary MM cells resulted in an increased AM mRNA levels in cells with low baseline AM expression.

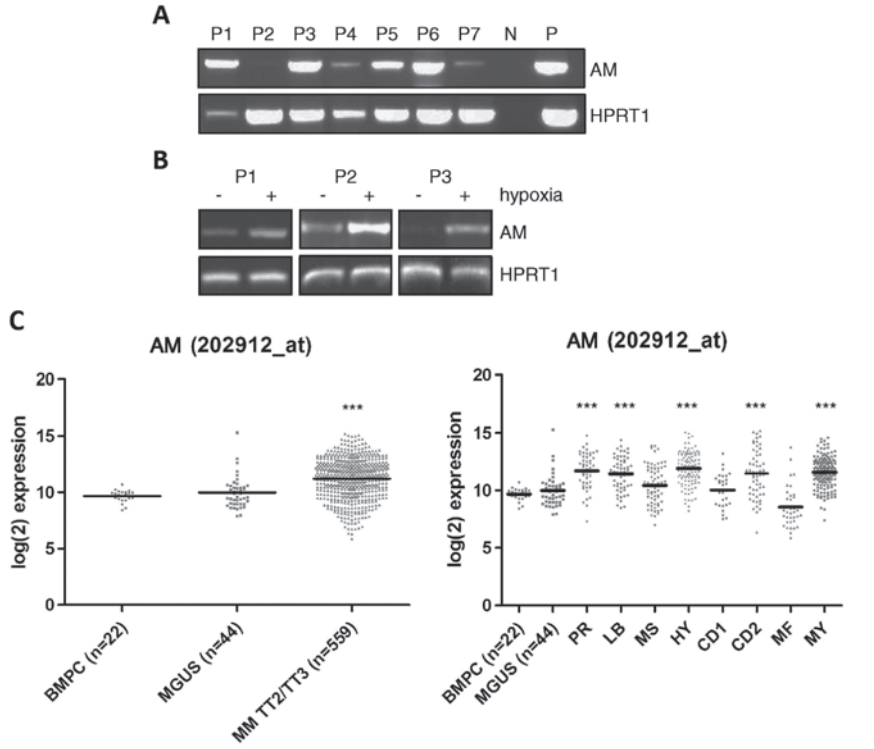


Figure 3. Expression of AM in primary MM samples

(A) Expression of AM mRNA in purified primary MM cells. Total RNA was isolated from primary MM cells and RT-PCR was performed. HPRT1 was used as a loading control. (B) Regulation of AM expression in primary MM cells by hypoxia. Primary MM cells were cultured for 48 h in normoxic (-) or hypoxic (+) (1% O2) conditions and RT-PCR was performed. HPRT1 expression is shown as an input control. (C) AM expression in MM is related to the disease progression and molecular subgroup. AM mRNA expression in 559 newly diagnosed MM patients from TT2/3, 44 MGUS patients and 22 healthy donors BMPC samples assessed by U133plus 2.0 Affymetrix oligonucleotide. Left panel: AM expression in BMPCs, MGUs, and MM. AM expression in MM cells was increased compared with BMPC ($P < 0.001$) and MGUS cells ($P < 0.001$). Right panel: AM expression in different genetic MM subgroups. Compared with BMPCs, AM expression was significantly increased in PR, LB, HY, CD2 and MY subgroups ($P < 0.001$).

To obtain a global view of AM expression in relation to disease progression and MM molecular subgroup, we analyzed Affymetrix oligonucleotide microarray data from a panel of 559 newly diagnosed MM patients included in total therapy 2/3 (TT2, TT3).[17] Interestingly, this analysis revealed that, compared with expression in normal BMPC, AM expression is already elevated in a small subset of MGUS patients and is markedly increased in the majority of primary MMs ($P < 0.001$) (Figure 3C, left panel). Further analysis of AM expression in specific MM molecular subgroups, as previously identified by gene-expression profiling (PR, LB, MF, HY, CD1, CD2, MS amd MY),[17] revealed significantly increased AM expression ($P < 0.05$) in the PR, LB, HY, CD2 and MY subgroups compared with BMPC and MGUS patients (Figure 3C, right panel). Hence, expression of AM by MM cells is related to disease progression and molecular subgroup.

ADRENOMEDULLIN EXPRESSION IN PRIMARY MMs IS NOT RELATED TO EXPRESSION OF OTHER HYPOXIA TARGET GENES OR NF-κB PROFILE GENES

The data presented above suggest that aberrant expression of AM by MM cells can be driven by hypoxia-dependent as well as hypoxia-independent mechanisms. To further strengthen this notion, we assessed the relation between expression of AM and a panel of 11 "MM hypoxia signature genes" (Supplementary Table 3). These genes were selected because they were (i) highly induced by hypoxia in both OPM-1 and UM-1 cells (Supplementary Table 1 and 2), and significantly expressed in at least 10% of primary MM patients; (ii) confirmed to be hypoxia target genes in multiple independent studies; (iii) contained functional hypoxia response elements (HREs) in their promoter.[30] Consistent with our observations in HMCLs, analysis of the TT2/TT3 data set[17] revealed that AM expression in primary MM is not correlated to most of the MM hypoxia signature genes. The only significant correlations found were between AM and PDK1 and HK2 (Supplementary Table 4); however, the correlation coefficients were low (< 0.2).

As the NF-κB pathway can drive angiogenesis[32–34] and expression of pro-inflammatory cytokines, including cytokines that can regulate AM,[35–37] we also assessed the relation between the NF-κB pathway and AM. As shown in Supplementary Table 4, analysis of the TT2/TT3 data set did not reveal an association between AM expression and expression of NF-κB profile genes, as defined by previous studies in MM.[18] Consistent with this finding, stimulation of the NF-κB pathway in MM cell lines (UM-1 and OPM-1) with PMA or TNFα, as confirmed by the upregulation of established NF-κB target genes, including *CD74* and *RELB*, did not enhance the (low) AM gene expression in these cells (Supplementary Figure 3).

Other candidate regulators of AM expression and MM-induced angiogenesis are the homeobox gene *HOXB7* and the tumor-suppressor gene inhibitor of growth family member 4 (*ING4*). HOXB7 can mediate tumor-induced angiogenesis,[38] and its expression in MM cells is correlated with that of several pro-angiogenic factors.[39] However, analysis of the TT2/3 primary MM patients' data set, did not reveal a correlation between *HOXB7* and *AM* gene expression (Supplementary Table 4). Similarly, expression of *ING4*, which represses angiogenesis in solid tumors[40] and is downregulated in MM cells compared with that in normal plasma cells,[41] was not correlated with AM expression (Supplementary Table 4).

ADRENOMEDULLIN CONTRIBUTES TO MM-INDUCED ANGIOGENESIS

MM cells generally express multiple angiogenic factors. Consequently, MM conditioned media (CM) show significant pro-angiogenic activity.[11] To assess whether AM can contribute to this activity, we generated NCI-H929 cells with inducible overexpression of AM, by stably transfecting a doxycycline-inducible AM cDNA (NCI-H929/TR/AM). Doxycycline treatment of these cells resulted in a clear induction of AM mRNA, resulting in a seven-fold increase in AM protein level, while doxycyclin treatment of control (NCI-H929/TR) cells did not affect AM expression levels (Figure 4A). Importantly, as shown in Figure 4B, CM derived from doxycycline-treated NCI-H929/TR/AM cells enhanced proliferation of human umbilical vein endothelial cells (HUVECs), which express the AM receptors CRLR and RAMP2-modifying protein (Supplementary Figure 4) by almost two-fold. Moreover, AM-enriched CM stimulated endothelial mesh formation by almost five-fold (Figure 4C). Both HUVEC proliferation and angiogenesis were AM-specific, as both were completely abrogated by 37133 and 16311, two highly specific small-molecule AM inhibitors that antagonize signaling by binding AM directly, thereby preventing receptor binding.[22] Of note, by the use of the CM from doxycycline-treated control NCI-H929/TR cells, non-specific effects of doxycycline were excluded and the AM-specificity of the small molecule inhibitors 37133 and 16311 was confirmed (Figure 4B right panel).

To address whether endogenously produced AM can also contribute to MM-induced angiogenesis, we next assessed the angiogenic properties of CM from L363 MM cells, which produce high levels of AM under normoxic conditions (Figure 2C). As shown in Figure 5, CM from L363 cells displayed potent angiogenic activity, promoting HUVEC proliferation, which was decreased two-fold by treatment with 16311 and 37133, respectively (Figure 5A) Furthermore,

blocking AM by 16311 and 37133 decreased L363 CM induced mesh formation approximately two-fold (Figure 5B). Taken together, these data demonstrate that overexpression of AM by malignant plasma cells can significantly contribute to their pro-angiogenic properties.

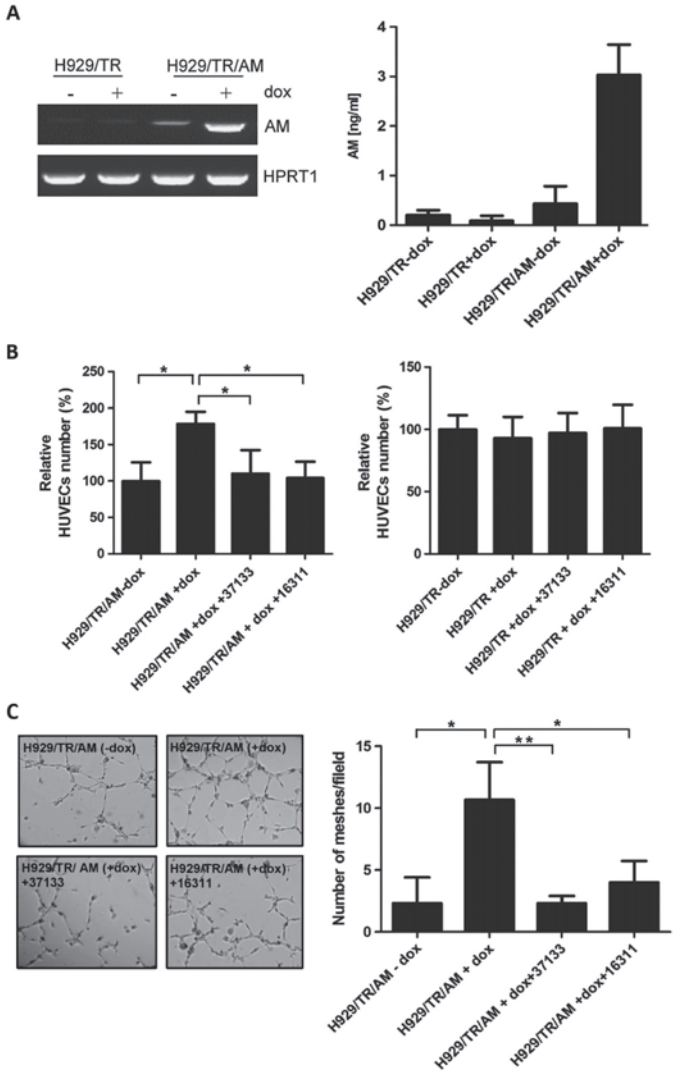


Figure 4. Overexpression of AM enhances the pro-angiogenic activity of MM cells

(A) Inducible expression of AM. NCI-H929 MM cells, containing either a doxycycline-inducible AM cDNA plus a TET repressor (NCI-H929/TR/AM) or a TET repressor only (NCI-H929/TR) were cultured without (-) or with (+) doxycycline for 24 h. AM expression was measured by RT-PCR (Left panel) and ELISA (Right panel). (B) AM overexpression enhances the pro-angiogenic activity of MM

supernatants Left panel. AM induction enhances HUVEC proliferation. HUVECs cells were cultured in supernatant of NCI-H929/TR/AM cells treated for 48 h without (- dox) or with (+ dox) doxycycline. This stimulating effect was fully blocked by 37133 and 16311, two small molecule inhibitors of AM (*P-value 0.05). The number of living cells in the control (supernatant from NCI-H929/TR/AM cells cultured for 48 h without doxycycline) was normalized to 100%. Right panel: specificity control: HUVECs cells were cultured in CM of NCI-H929/TR cells that had been treated for 48 h without (- dox) or with (+ dox) doxycycline. The number of living cells in the control (supernatant from NCI-H929/TR cells cultured for 48 h without doxycycline) was normalized to 100%. (C) AM promotes MM-induced angiogenesis. AM induction by doxycyclin strongly enhanced the formation of three-dimensional capillary-like tubular structures by HUVEC cells. HUVEC were cultured with CM from NCI-H929/TR/AM cell that had been cultured for 48 h without (- dox) or with (+ dox) doxycycline. The stimulating effect of the + dox CM was fully blocked by 37133 and 16311, two small molecule inhibitors of AM. Left panel: HUVEC cultures in matrigel showing endothelial mesh formation in cells cultured with CM from NCI-H929/TR/AM (- dox) or (+ dox) cells, in the presence or absence of the small molecule AM inhibitors 37133 and 16311. Right panel: quantification of endothelial mesh formation (*P-value < 0.05).

To determine whether hypoxia-driven AM secretion by primary MM cells can contribute directly to MM-induced angiogenesis, two independent primary MM cell samples were subjected to hypoxia and AM expression and angiogenic potential were investigated. Exposure to hypoxia resulted in a clear increase in AM mRNA levels in both primary MM cell samples (Figure 5C), as well as in an increased AM protein secretion (1.6- and 3.2-fold in MM1 and 2, respectively) (Figure 5D). Functionally, this hypoxia-driven AM secretion resulted in an approximately two-fold increase in HUVEC numbers, that was inhibited to normoxic or subnormoxic levels by blocking AM signaling with 16311. Importantly, 16311 did not influence bFGF-induced HUVEC proliferation, confirming specificity of the inhibition (Figure 5E). Taken together, these results demonstrate that AM is a key mediator of angiogenesis in MM, strongly upregulated under hypoxic conditions.

DISCUSSION

In this study, we explored the transcriptional response of MM cells to hypoxia, by comparing the gene expression profile of the HMCLs UM-1 and OPM-1 under normoxic and hypoxic conditions. The hypoxia-induced gene expression signature of these MM cells (Table 1, Supplementary Table 1 and 2, Figure 1) contained multiple genes that have previously been associated with hypoxia responses and/or represent established HIF1α target genes, including genes involved in the metabolic response to hypoxia, in cell proliferation and apoptosis. Furthermore, it comprised several pro-angiogenic genes, including *VEGFA*, *ANGPTL4* and *AM*, of which *AM* was identified as the top-regulated gene (40-50-fold induction).

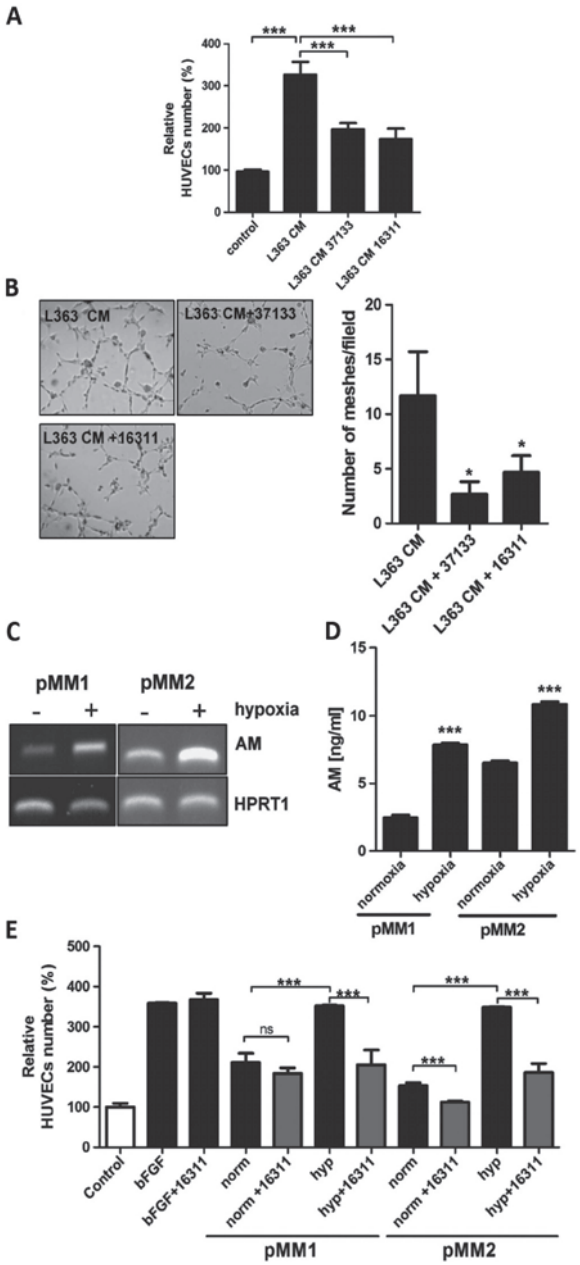


Figure 5. Endogenous AM expression contibutes to MM induced angiogenesis

(A) Contribution of endogenous AM to the mitogenic effect of CM from L363 cells. HUVEC proliferation was measured in CM in the presence or absence of the small molecule AM inhibitors 37133 and 16311. The mean ±s.d. of a representative experiment performed in triplicate is shown. (B) Contribution of endogenous AM to the angiogenic effect of CM from L363 cells. Endothelial mesh formation

was measured in L363 CM in the presence or absence of the small molecule AM inhibitors 37133 and 16311. The mean ±s.d. of a representative experiment performed in triplicate is shown. (**P*-value < 0.05; ****P*-value < 0.001). (C) Relative expression of AM mRNA in purified primary MM cells. Total RNA was isolated from primary MM (pMM) cells and RT-PCR was performed. AM expression was normalized to HPRT1 gene expression. (D) AM protein secretion by primary MM cells. AM concentration in the CM of primary MM cells cultured for 48 h in normoxic and/or hypoxic conditions was measured by ELISA. (E) Contribution of pMM-derived endogenous and hypoxia-induced AM to HUVECs proliferation, in the presence or absence of the small-molecule AM inhibitor 16311. bFGF was used as a specificity control for 16311. (****P*-value < 0.001).

Interestingly, however, although *AM* is a well-established HIF1α target gene containing HRE sites in its promoter, as major regulatory sequences,[42] we observed that several HMCLs and primary MMs also expressed high levels of AM under normoxic conditions (Figures 2A–C). Importantly, MM cells with normoxic AM expression did not show aberrant normoxic HIF1α stabilization and displayed no overexpression of other HIF1α/hypoxia target genes (Figures 2D–F), suggesting normoxic regulation of AM expression by HIF1α-independent mechanisms. In line with this notion, our analysis of a large MM gene-expression data set[17] revealed no consistent correlation between *AM* expression and expression of other hypoxia/HIF1α target genes. These findings imply that mechanisms other than hypoxia can contribute to AM expression in malignant plasma cells, and are consistent with a scenario in which both HIF1α-dependent and independent mechanisms contribute to the "angiogenic switch" in MM.

We observed that *AM* expression in malignant plasma cells of MM patients is significantly higher than in normal BMPC or plasma cells patients with MGUS (Figure 3C). This elevated *AM* expression was present in most molecular MM subgroups (PR, LB, HY, CD2 and MY) (Figure 3D). Hence, AM overexpression is related to disease progression, suggesting that AM may have an important functional contribution in angiogenesis, which is associated with progression of MGUS to clinically overt MM.[43] Our functional studies strongly support this scenario as they demonstrate that (i) endogenous, hypoxia-induced and ectopic expression of AM in MM cells strongly promotes angiogenic activity of MM cells, as shown by enhanced endothelial cell proliferation and mesh formation (Figures 4 and 5); (ii) blockage of AM strongly reduces the pro-angiogenic activity of MM cells (Figure 5).

Adrenomedullin is involved in blood vessel morphogenesis, vasculogenesis and tumor angiogenesis.[29,44] AM-null mice die *in utero* as a result of defective vasculogenesis,[45] while AM overexpression by tumors mediates tumor angiogenesis.[26,28,46] In addition, AM can directly promote tumor growth.[25,47] AM stimulates angiogenesis by binding the CRLR, which is widely expressed on normal and hypoxic endothelial cells.[48] Interestingly, binding of AM to the CRLR/RAMP2

can transactivate the VEGFR-2, which is responsible for most pro-angiogenic effects of VEGFA4[9], including the stimulation of endothelial cell differentiation, proliferation, migration and morphogenesis. This AM-induced VEGFR-2 transactivation does not require VEGFA, suggesting that AM can functionally mimic VEGFA, and thereby contribute to MM-induced angiogenesis.

Although it has recently been suggested that HIF1α is a major regulator of the pro-angiogenic profile of myeloma cells and of MM-induced angiogenesis, deregulation of other transcriptional pathways may potentially also contribute to the angiogenic switch and overexpression of *AM* in MM. Since pro-inflammatory cytokines have been shown to induce increased AM secretion,[35–37] we explored the role of NF-κB signaling in the regulation of AM. However, expression of NF-κB profile genes and *AM* in primary MM patients (Supplementary Table 4) were not correlated. Moreover, stimulation of the NF-κB pathway in MM cells *in vitro* did not influence expression of *AM* (Supplementary Figure 3), suggesting that the NF-κB pathway is not involved in the regulation of *AM* in malignant plasma cells. Other candidate regulators of AM expression and MM-induced angiogenesis are the homeobox gene *HOXB7* and the tumor-suppressor gene inhibitor of growth family member 4 (*ING4*). HOXB7 has been shown to mediate tumor-induced angiogenesis and tumor progression in solid cancers by regulating VEGF, IL-8, bFGF2 and Ang-2.[38] In MM cells, *HOXB7* expression is correlated with and can control overexpression of several pro-angiogenic factors.[39] However, our analysis of the TT2/3 primary MM patients' data set, did not reveal a correlation between *HOXB7* and *AM* gene expression (Supplementary Table 4). Similarly, expression of *ING4*, which acts as a repressor of angiogenesis in solid tumors[40] and shown reduced expression in MM cells compared with normal plasma cells,[41] was not correlated with AM expression (Supplementary Table 4). Hence, neither NF-κB, HOXB7 nor ING4, appear to have a role in AM deregulation in MM plasma cells, and further studies are needed to unravel the molecular mechanism(s) involved.

In conclusion, our results demonstrate that MM cells, both in a hypoxia-dependent and -independent fashion, aberrantly express and secrete AM, which can mediate MM-induced angiogenesis. This aberrant AM expression could be a major driving force for the angiogenic switch observed during MM progression, which renders AM a putative target for anti-angiogenic therapy in MM.

REFERENCES

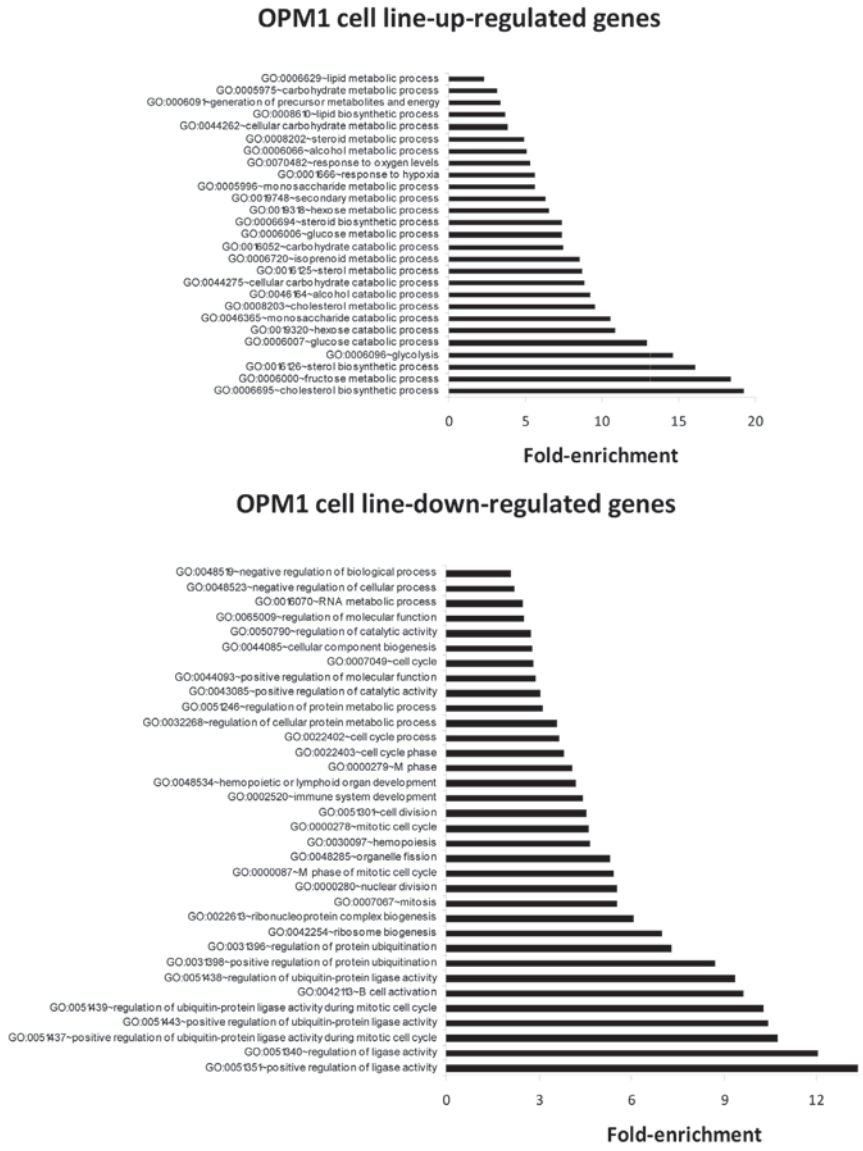
1. Kuehl WM, Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. Nat Rev Cancer 2002; 2: 175–187.
2. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 2007; 7: 585–598.
3. Vacca A, Ribatti D. Bone marrow angiogenesis in multiple myeloma. Leukemia 2006; 20: 193–199.
4. Giuliani N, Storti P, Bolzoni M, Palma BD, Bonomini S. Angiogenesis and multiple myeloma. Cancer Microenviron 2011; 4: 325–337.
5. Rana C, Sharma S, Agrawal V, Singh U. Bone marrow angiogenesis in multiple myeloma and its correlation with clinicopathological factors. Ann Hematol 2010; 89: 789–794.
6. Bhatti SS, Kumar L, Dinda AK, Dawar R. Prognostic value of bone marrow angiogenesis in multiple myeloma: use of light microscopy as well as computerized image analyzer in the assessment of microvessel density and total vascular area in multiple myeloma and its correlation with various clinical, histological, and laboratory parameters. Am J Hematol 2006; 81: 649–656.
7. Sezer O, Niemoller K, Kaufmann O, Eucker J, Jakob C, et al. Decrease of bone marrow angiogenesis in myeloma patients achieving a remission after chemotherapy. Eur J Haematol 2001; 66: 238–244.
8. Kumar S, Fonseca R, Dispenzieri A, Lacy MQ, Lust JA, et al. Bone marrow angiogenesis in multiple myeloma: effect of therapy. Br J Haematol 2002; 119: 665–671.
9. Ribatti D, Nico B, Vacca A. Importance of the bone marrow microenvironment in inducing the angiogenic response in multiple myeloma. Oncogene 2006; 25: 4257–4266.
10. Kumar S, Witzig TE, Timm M, Haug J, Wellik L, et al. Bone marrow angiogenic ability and expression of angiogenic cytokines in myeloma: evidence favoring loss of marrow angiogenesis inhibitory activity with disease progression. Blood 2004; 104: 1159–1165.
11. Hose D, Moreaux J, Meissner T, Seckinger A, Goldschmidt H, et al. Induction of angiogenesis by normal and malignant plasma cells. Blood 2009; 114: 128–143.
12. Martin SK, Diamond P, Gronthos S, Peet DJ, Zannettino AC. The emerging role of hypoxia, HIF-1 and HIF-2 in multiple myeloma. Leukemia 2011; 25: 1533–1542.
13. Colla S, Storti P, Donofrio G, Todoerti K, Bolzoni M, et al. Low bone marrow oxygen tension and hypoxia-inducible factor-1alpha overexpression characterize patients with multiple myeloma: role on the transcriptional and proangiogenic profiles of CD138 (+) cells. Leukemia 2010; 24: 1967–1970.
14. Asosingh K, De Raeve H, de Ridder M, Storme GA, Willems A, et al. Role of the hypoxic bone marrow microenvironment in 5T2MM murine myeloma tumor progression. Haematologica 2005; 90: 810–817.

15. Zhang J, Sattler M, Tonon G, Grabher C, Lababidi S, et al. Targeting angiogenesis via a c-Myc/hypoxia-inducible factor-1alpha-dependent pathway in multiple myeloma. Cancer Res 2009; 69: 5082–5090.
16. Giatromanolaki A, Bai M, Margaritis D, Bourantas KL, Koukourakis MI, et al. Hypoxia and activated VEGF/receptor pathway in multiple myeloma. Anticancer Res 2010; 30: 2831–2836.
17. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, et al. The molecular classification of multiple myeloma. Blood 2006; 108: 2020–2028.
18. Annunziata CM, Davis RE, Demchenko Y, Bellamy W, Gabrea A, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. Cancer Cell 2007; 12:115–130.
19. Reijmers RM, Groen RW, Rozemuller H, Kuil A, de Haan-Kramer A, et al. Targeting EXT1 reveals a crucial role for heparan sulfate in the growth of multiple myeloma. Blood 2010; 115: 601–604.
20. Baudin B, Bruneel A, Bosselut N, Vaubourdolle M. A protocol for isolation and culture of human umbilical vein endothelial cells. Nat Protoc 2007; 2: 481–485.
21. Derksen PW, Tjin E, Meijer HP, Klok MD, MacGillavry HD, et al. Illegitimate WNT signaling promotes proliferation of multiple myeloma cells. Proc Natl Acad Sci U S A 2004; 101: 6122–6127.
22. Martínez A, Julian M, Bregonzio C, Notari L, Moody TW, et al. Identification of vasoactive nonpeptidic positive and negative modulators of adrenomedullin using a neutralizing antibody-based screening strategy. Endocrinology 2004; 145: 3858–3865.
23. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009; 37: 1–13.
24. Takenaga K. Angiogenic signaling aberrantly induced by tumor hypoxia. Front Biosci 2011; 16: 31–48.
25. Ouafik L, Sauze S, Boudouresque F, Chinot O, Delfino C, et al. Neutralization of adrenomedullin inhibits the growth of human glioblastoma cell lines in vitro and suppresses tumor xenograft growth in vivo. Am J Pathol 2002; 160: 1279–1792.
26. Oehler MK, Hague S, Rees MC, Bicknell R. Adrenomedullin promotes formation of xenografted endometrial tumors by stimulation of autocrine growth and angiogenesis. Oncogene 2002; 21: 2815–2821.
27. Kaafarani I, Fernandez-Sauze S, Berenguer C, Chinot O, Delfino C, et al. Targeting adrenomedullin receptors with systemic delivery of neutralizing antibodies inhibits tumor angiogenesis and suppresses growth of human tumor xenografts in mice. FASEB J 2009; 23: 3424–3435.
28. Ishikawa T, Chen J, Wang J, Okada F, Sugiyama T, et al. Adrenomedullin antagonist suppresses in vivo growth of human pancreatic cancer cells in SCID mice by suppressing angiogenesis. Oncogene 2003; 22: 1238–1242.

29. Deville JL, Salas S, Figarella-Branger D, Ouafik L, Daniel L. Adrenomedullin as a therapeutic target in angiogenesis. Expert Opin Ther Targets 2010; 14:1059–1072.
30. Benita Y, Kikuchi H, Smith AD, Zhang MQ, Chung DC, et al. An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. Nucleic Acids Res 2009; 37: 4587–4602.
31. Yu F, White SB, Zhao Q, Lee FS. HIF-1alpha binding to VHL is regulated by stimulus-sensitive proline hydroxylation. Proc Natl Acad Sci U S A 2001; 98: 9630–9635.
32. Tabruyn SP, Griffioen AW, NF-kappa B. A new player in angiostatic therapy. Angiogenesis 2008; 11: 101–106.
33. Schmidt D, Textor B, Pein OT, Licht AH, Andrecht S, et al. Critical role for NF-kappaB-induced JunB in VEGF regulation and tumor angiogenesis. EMBOJ 2007; 26: 710–719.
34. Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene 2001; 20: 4188–4197.
35. Takahashi K, Nakayama M, Totsune K, Murakami O, Sone M, et al. Increased secretion of adrenomedullin from cultured human astrocytes by cytokines. J Neurochem 2000; 74: 99–103.
36. Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, et al. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. Biochem Biophys Res Commun 1994; 203: 719–726.
37. Horio T, Nishikimi T, Yoshihara F, Nagaya N, Matsuo H, et al. Production and secretion of adrenomedullin in cultured rat cardiac myocytes and non-myocytes: stimulation by interleukin-1beta and tumor necrosis factor-alpha. Endocrinology 1998; 139: 4576–4580.
38. Care A, Felicetti F, Meccia E, Bottero L, Parenza M, et al. HOXB7: a key factor for tumor-associated angiogenic switch. Cancer Res 2001; 61: 6532–6539.
39. Storti P, Donofrio G, Colla S, Airoldi I, Bolzoni M, et al. HOXB7 expression by myeloma cells regulates their pro-angiogenic properties in multiple myeloma patients. Leukemia 2011; 25: 527–537.
40. Garkavtsev I, Kozin SV, Chernova O, Xu L, Winkler F, et al. The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. Nature 2004; 428: 328–332.
41. Colla S, Tagliaferri S, Morandi F, Lunghi P, Donofrio G, et al. The new tumor-suppressor gene inhibitor of growth family member 4 (ING4) regulates the production of proangiogenic molecules by myeloma cells and suppresses hypoxia-inducible factor-1 alpha (HIF-1alpha) activity: involvement in myeloma-induced angiogenesis. Blood 2007; 110: 4464–4475.
42. Garayoa M, Martinez A, Lee S, Pio R, An WG, et al. Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during

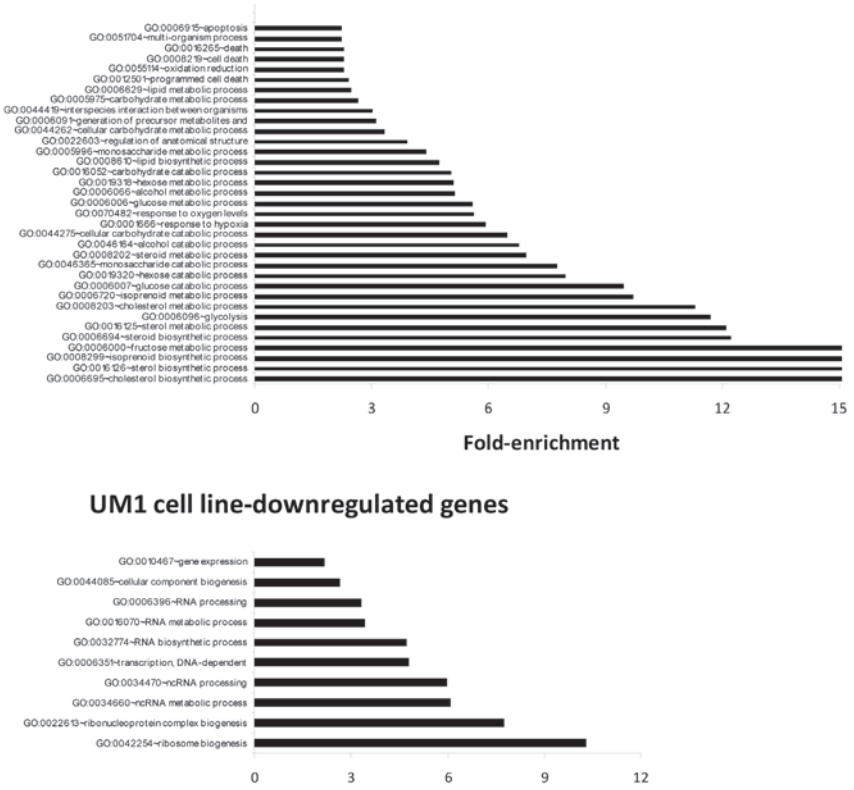
oxygen deprivation: a possible promotion mechanism of carcinogenesis. Mol Endocrinol 2000; 14: 848–862.
43. Jakob C, Sterz J, Zavrski I, Heider U, Kleeberg L, et al. Angiogenesis in multiple myeloma. Eur J Cancer 2006; 42: 1581–1590.
44. Kim W, Moon SO, Sung MJ, Kim SH, Lee S, et al. Angiogenic role of adrenomedullin through activation of Akt, mitogen-activated protein kinase, and focal adhesion kinase in endothelial cells. FASEB J 2003; 17: 1937–1939.
45. Caron KM, Smithies O. Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional Adrenomedullin gene. Proc Natl Acad Sci U S A 2001; 98: 615–619.
46. Martinez A, Vos M, Guedez L, Kaur G, Chen Z, et al. The effects of adrenomedullin overexpression in breast tumor cells. J Natl Cancer Inst 2002; 94: 1226–1237.
47. Ramachandran V, Arumugam T, Hwang RF, Greenson JK, Simeone DM, et al. Adrenomedullin is expressed in pancreatic cancer and stimulates cell proliferation and invasion in an autocrine manner via the adrenomedullin receptor, ADMR. Cancer Res 2007; 67: 2666–2675.
48. Fernandez-Sauze S, Delfino C, Mabrouk K, Dussert C, Chinot O, et al. Effects of adrenomedullin on endothelial cells in the multistep process of angiogenesis: involvement of CRLR/RAMP2 and CRLR/RAMP3 receptors. Int J Cancer 2004; 108: 797–804.
49. Guidolin D, Albertin G, Spinazzi R, Sorato E, Mascarin A, et al. Adrenomedullin stimulates angiogenic response in cultured human vascular endothelial cells: involvement of the vascular endothelial growth factor receptor 2. Peptides 2008; 29: 2013–2023.

SUPPLEMENTARY MATERIAL



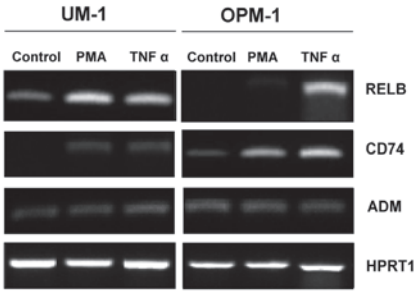
Supplementary Figure 1

Gene ontology groups with significant over/under-representation among genes differentially expressed in hypoxic conditions (fold change ≥ 2 or ≤ 0,5) in OPM-1 cells. Only the pathways having significant alteration ($p < 0.05$) are presented. For each GO pathway, the bar shows the x-fold enrichment of the pathway in the dataset.



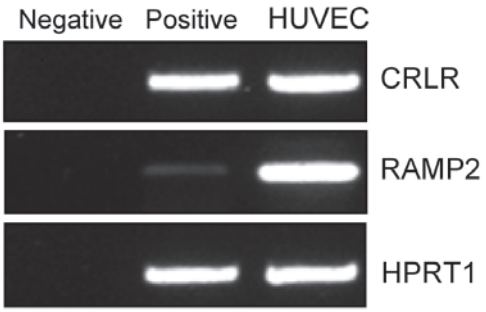
Supplementary Figure 2

Gene ontology groups with significant over/under-representation among genes differentially expressed in hypoxic conditions (fold change ≥ 2 or ≤ 0,5) in UM-1 cells. Only the pathways having significant alteration ($p < 0.05$) are presented. For each GO pathway, the bar shows the x-fold enrichment of the pathway in the dataset.



Supplementary Figure 3. Response of MM cell lines to NF-κB stimulation

RT-PCR analysis of NF-κB target genes and AM expression in OPM-1 and UM-1 MM cell lines following 48h exposure to PMA and/or TNF alpha (100ng/ml). HPRT1 expression is shown as an input control.



Supplementary Figure 4. Expression of AM receptors in HUVECs cells

Reverse transcriptase-PCR analysis for AM receptors: CRLR and RAMP2 modifying protein. HPRT1 expression is shown as an input control. As a positive control (positive) for RT-PCR the cDNA from kidney was used, as negative control (negative) water was used.

Supplementary Table 1. OPM-1 cell line

Probe Set ID	Score(d)	Gene Symbol	Gene Title
202912_at	48,48	ADM	adrenomedullin
203828_s_at	40,08	IL32	interleukin 32
223836_at	36,93	FGFBP2	fibroblast growth factor binding protein 2
228499_at	23,46	PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
214978_s_at	22,91	PPFIA4	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4
202887_s_at	10,39	DDIT4	DNA-damage-inducible transcript 4
204347_at	10,17	LOC645619 /// LOC731007	similar to Adenylate kinase isoenzyme 4, mitochondrial (ATP-AMP transphosphorylase) /// similar to Adenylate kinase isoenzyme 4, mitochondrial (Adenylate kinase 3-like 1) (ATP-AMP transphosphorylase)
202022_at	10,10	ALDOC	aldolase C, fructose-bisphosphate
1560112_at	9,29	WDFY2	WD repeat and FYVE domain containing 2
201170_s_at	9,04	BHLHB2	basic helix-loop-helix domain containing, class B, 2
225342_at	8,38	AK3L1	adenylate kinase 3-like 1
201212_at	7,73	LGMN	legumain
203438_at	6,93	STC2	stanniocalcin 2
207543_s_at	6,30	P4HA1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I
226985_at	6,23	FGD5	FYVE, RhoGEF and PH domain containing 5
201849_at	6,06	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
202718_at	5,51	IGFBP2	insulin-like growth factor binding protein 2, 36kDa

Probe Set ID	Score(d)	Gene Symbol	Gene Title
203027_s_at	4,98	MVD	mevalonate (diphospho) decarboxylase
221214_s_at	4,84	NELF	nasal embryonic LHRH factor
226682_at	4,82	LOC283666	hypothetical protein LOC283666
210512_s_at	4,75	VEGFA	vascular endothelial growth factor A
226549_at	4,68	SBK1	SH3-binding domain kinase 1
215616_s_at	4,62	JMJD2B	jumonji domain containing 2B
202934_at	4,62	HK2	hexokinase 2
202481_at	4,60	DHRS3	dehydrogenase/reductase (SDR family) member 3
226452_at	4,55	PDK1	pyruvate dehydrogenase kinase, isozyme 1
202497_x_at	4,42	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
227337_at	4,35	ANKRD37	ankyrin repeat domain 37
203282_at	4,22	GBE1	glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)
202464_s_at	4,16	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
235850_at	4,13	WDR5B	WD repeat domain 5B
205349_at	3,92	GNA15	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)
212689_s_at	3,82	JMJD1A	jumonji domain containing 1A
212813_at	3,76	JAM3	junctional adhesion molecule 3
219622_at	3,68	RAB20	RAB20, member RAS oncogene family
218205_s_at	3,67	MKNK2	MAP kinase interacting serine/threonine kinase 2
200697_at	3,67	HK1	hexokinase 1
223046_at	3,64	EGLN1	egl nine homolog 1 (C. elegans)
234312_s_at	3,57	ACSS2	acyl-CoA synthetase short-chain family member 2
221478_at	3,52	BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like
41386_i_at	3,47	JMJD3	jumonji domain containing 3
218274_s_at	3,36	ANKZF1	ankyrin repeat and zinc finger domain containing 1
225533_at	3,33	PHF19	PHD finger protein 19
222856_at	3,33	APLN	apelin, AGTRL1 ligand
226160_at	3,32	H6PD	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)
225249_at	3,30	SPPL2B	signal peptide peptidase-like 2B
225530_at	3,30	MOBKL2A	MOB1, Mps One Binder kinase activator-like 2A (yeast)
222446_s_at	3,26	BACE2	beta-site APP-cleaving enzyme 2

Probe Set ID	Score(d)	Gene Symbol	Gene Title
200831_s_at	3,24	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
211902_x_at	3,23	TRA@	T cell receptor alpha locus
227799_at	3,23	MYO1G	myosin IG
210130_s_at	3,14	TM7SF2	transmembrane 7 superfamily member 2
201389_at	3,09	ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
226971_at	3,09	CCDC136	coiled-coil domain containing 136
203823_at	3,08	RGS3	regulator of G-protein signalling 3
1569496_s_at	3,07	SPON2	Spondin 2, extracellular matrix protein
239004_at	3,07	SQSTM1	Sequestosome 1
240258_at	2,96	ENO1	enolase 1, (alpha)
202245_at	2,95	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
203911_at	2,94	RAP1GAP	RAP1 GTPase activating protein
219014_at	2,93	PLAC8	placenta-specific 8
235231_at	2,92	ZNF789	zinc finger protein 789
207030_s_at	2,91	CSRP2	cysteine and glycine-rich protein 2
200965_s_at	2,89	ABLIM1	actin binding LIM protein 1
235900_at	2,88	SPNS3	spinster homolog 3 (Drosophila)
209034_at	2,87	PNRC1	proline-rich nuclear receptor coactivator 1
207351_s_at	2,85	SH2D2A	SH2 domain protein 2A
215898_at	2,85	TTLL5	tubulin tyrosine ligase-like family, member 5
206655_s_at	2,83	GP1BB /// SEPT5	glycoprotein Ib (platelet), beta polypeptide /// septin 5
212518_at	2,83	PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma
221964_at	2,83	TULP3	tubby like protein 3
208308_s_at	2,80	GPI	glucose phosphate isomerase
228741_s_at	2,80	HCN3	hyperpolarization activated cyclic nucleotide-gated potassium channel 3
208154_at	2,79	LOC51336	mesenchymal stem cell protein DSCD28
202308_at	2,79	SREBF1	sterol regulatory element binding transcription factor 1
219862_s_at	2,79	NARF	nuclear prelamin A recognition factor
210132_at	2,78	EFNA3	ephrin-A3
218507_at	2,77	HIG2	hypoxia-inducible protein 2
226446_at	2,76	HES6	hairy and enhancer of split 6 (Drosophila)
208322_s_at	2,75	ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
200696_s_at	2,73	GSN	gelsolin (amyloidosis, Finnish type)

Probe Set ID	Score(d)	Gene Symbol	Gene Title
200632_s_at	2,72	NDRG1	N-myc downstream regulated gene 1
224473_x_at	2,70	LZTS2	leucine zipper, putative tumor suppressor 2
242361_at	2,70	IMMT	Inner membrane protein, mitochondrial (mitofilin)
220091_at	2,69	SLC2A6	solute carrier family 2 (facilitated glucose transporter), member 6
209462_at	2,68	APLP1	amyloid beta (A4) precursor-like protein 1
212218_s_at	2,67	FASN	fatty acid synthase
219752_at	2,66	RASAL1	RAS protein activator like 1 (GAP1 like)
221011_s_at	2,66	LBH	limb bud and heart development homolog (mouse)
223326_s_at	2,66	FLJ22795 /// LOC727751	hypothetical protein FLJ22795 /// similar to cis-Golgi matrix protein GM130
229902_at	2,65	FLT4	fms-related tyrosine kinase 4
1555842_at	2,65	LOC284356	hypothetical protein LOC284356
218625_at	2,64	NRN1	neuritin 1
203946_s_at	2,64	ARG2	arginase, type II
223621_at	2,63	PNMA3	paraneoplastic antigen MA3
225301_s_at	2,62	MYO5B	myosin VB
229055_at	2,62	GPR68	G protein-coupled receptor 68
221757_at	2,61	PIK3IP1	phosphoinositide-3-kinase interacting protein 1
219815_at	2,61	GAL3ST4	galactose-3-O-sulfotransferase 4
204981_at	2,60	SLC22A18	solute carrier family 22 (organic cation transporter), member 18
211037_s_at	2,60	LENG4	leukocyte receptor cluster (LRC) member 4
243363_at	2,60	LOC641518	hypothetical protein LOC641518
201313_at	2,58	ENO2	enolase 2 (gamma, neuronal)
204994_at	2,58	MX2	myxovirus (influenza virus) resistance 2 (mouse)
223640_at	2,56	HCST	hematopoietic cell signal transducer
1556599_s_at	2,55	ARPP-21	cyclic AMP-regulated phosphoprotein, 21 kD
225542_at	2,55	CENTB5	centaurin, beta 5
218697_at	2,55	NCKIPSD	NCK interacting protein with SH3 domain
201791_s_at	2,54	DHCR7	7-dehydrocholesterol reductase
210220_at	2,54	FZD2	frizzled homolog 2 (Drosophila)
204164_at	2,54	SIPA1	signal-induced proliferation-associated gene 1
204365_s_at	2,53	REEP1	receptor accessory protein 1
212496_s_at	2,51	JMJD2B	jumonji domain containing 2B
225262_at	2,49	FOSL2	FOS-like antigen 2
212430_at	2,48	RBM38	RNA binding motif protein 38
212045_at	2,48	GLG1	golgi apparatus protein 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
217838_s_at	2,48	EVL	Enah/Vasp-like
231720_s_at	2,48	JAM3	junctional adhesion molecule 3
241954_at	2,47	FDFT1	Farnesyl-diphosphate farnesyltransferase 1
214177_s_at	2,46	PBXIP1	pre-B-cell leukemia homeobox interacting protein 1
235226_at	2,46	CDC2L6	cell division cycle 2-like 6 (CDK8-like)
231124_x_at	2,45	LY9	lymphocyte antigen 9
236275_at	2,45	KRBA1	KRAB-A domain containing 1
227353_at	2,45	TMC8	Transmembrane channel-like 8
212561_at	2,44	RAB6IP1	RAB6 interacting protein 1
200737_at	2,44	PGK1	phosphoglycerate kinase 1
203894_at	2,43	TUBG2	tubulin, gamma 2
211924_s_at	2,43	PLAUR	plasminogen activator, urokinase receptor
1553611_s_at	2,42	FLJ33790	hypothetical protein FLJ33790
1562271_x_at	2,42	ARHGEF7	Rho guanine nucleotide exchange factor (GEF) 7
221778_at	2,42	JHDM1D	jumonji C domain-containing histone demethylase 1 homolog D (S. cerevisiae)
1565935_at	2,42	LOC91431	prematurely terminated mRNA decay factor-like
232946_s_at	2,42	NADSYN1	NAD synthetase 1
227383_at	2,42	LOC727820	hypothetical protein LOC727820
210711_at	2,40	MGC5457	hypothetical protein MGC5457
238740_at	2,39	AARSD1	alanyl-tRNA synthetase domain containing 1
208926_at	2,39	NEU1	sialidase 1 (lysosomal sialidase)
1554977_at	2,38	LOC198437	bA299N6.3
223216_x_at	2,37	FBXO16 /// ZNF395	zinc finger protein 395 /// F-box protein 16
203937_s_at	2,35	TAF1C	TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110kDa
209889_at	2,35	SEC31B	SEC31 homolog B (S. cerevisiae)
49679_s_at	2,35	MMP24	Matrix metallopeptidase 24 (membrane-inserted)
223727_at	2,34	KCNIP2	Kv channel interacting protein 2
224565_at	2,34	TncRNA	trophoblast-derived noncoding RNA
230619_at	2,34	ARNT	aryl hydrocarbon receptor nuclear translocator
36554_at	2,34	ASMTL	acetylserotonin O-methyltransferase-like
213270_at	2,34	MPP2	membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2)
226728_at	2,33	SLC27A1	solute carrier family 27 (fatty acid transporter), member 1
244385_at	2,32	JMJD2C	Jumonji domain containing 2C

Probe Set ID	Score(d)	Gene Symbol	Gene Title
239137_x_at	2,31	MGC45491	hypothetical protein MGC45491
226390_at	2,31	STARD4	START domain containing 4, sterol regulated
238551_at	2,30	FUT11	fucosyltransferase 11 (alpha (1,3) fucosyltransferase)
219236_at	2,30	PAQR6	progestin and adipoQ receptor family member VI
218019_s_at	2,30	PDXK	pyridoxal (pyridoxine, vitamin B6) kinase
214667_s_at	2,30	TP53I11	tumor protein p53 inducible protein 11
208964_s_at	2,30	FADS1	fatty acid desaturase 1
227281_at	2,30	SLC29A4	solute carrier family 29 (nucleoside transporters), member 4
229872_s_at	2,30	LOC642441 /// LOC730256 /// LOC730257	hypothetical LOC642441 /// hypothetical protein LOC730256 /// hypothetical protein LOC730257
217783_s_at	2,29	YPEL5	yippee-like 5 (Drosophila)
202769_at	2,29	CCNG2	cyclin G2
218756_s_at	2,28	MGC4172	short-chain dehydrogenase/reductase
228906_at	2,28	CXXC6	CXXC finger 6
225191_at	2,28	CIRBP	cold inducible RNA binding protein
222175_s_at	2,27	PCQAP	PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein
213060_s_at	2,26	CHI3L2	chitinase 3-like 2
209566_at	2,26	INSIG2	insulin induced gene 2
213011_s_at	2,26	TPI1	triosephosphate isomerase 1
201673_s_at	2,26	GYS1	glycogen synthase 1 (muscle)
203238_s_at	2,25	NOTCH3	Notch homolog 3 (Drosophila)
1566720_at	2,25	LOC376693	hypothetical LOC376693
209218_at	2,25	SQLE	squalene epoxidase
209348_s_at	2,25	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
201251_at	2,25	PKM2	pyruvate kinase, muscle
219499_at	2,25	SEC61A2	Sec61 alpha 2 subunit (S. cerevisiae)
228730_s_at	2,25	SCRN2	secernin 2
50965_at	2,24	RAB26	RAB26, member RAS oncogene family
201556_s_at	2,24	VAMP2	vesicle-associated membrane protein 2 (synaptobrevin 2)
1552455_at	2,23	PRUNE2	prune homolog 2 (Drosophila)
226068_at	2,23	SYK	Spleen tyrosine kinase
209640_at	2,23	PML	promyelocytic leukemia
209962_at	2,22	EPOR	erythropoietin receptor

Probe Set ID	Score(d)	Gene Symbol	Gene Title
203521_s_at	2,22	ZNF318	zinc finger protein 318
204118_at	2,22	CD48	CD48 molecule
218030_at	2,22	GIT1	G protein-coupled receptor kinase interactor 1
210010_s_at	2,22	SLC25A1	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1
1556058_s_at	2,22	SPEN	spen homolog, transcriptional regulator (Drosophila)
200808_s_at	2,21	ZYX	zyxin
222395_s_at	2,21	UBE2Z	ubiquitin-conjugating enzyme E2Z (putative)
228296_at	2,21	YPEL1	yippee-like 1 (Drosophila)
219566_at	2,20	PLEKHF1	pleckstrin homology domain containing, family F (with FYVE domain) member 1
206467_x_at	2,20	RTEL1 /// TNFRSF6B	tumor necrosis factor receptor superfamily, member 6b, decoy /// regulator of telomere elongation helicase 1
203317_at	2,20	PSD4	pleckstrin and Sec7 domain containing 4
203394_s_at	2,20	HES1	hairy and enhancer of split 1, (Drosophila)
36907_at	2,20	MVK	mevalonate kinase (mevalonic aciduria)
1553101_a_at	2,19	ALKBH5	alkB, alkylation repair homolog 5 (E. coli)
204379_s_at	2,19	FGFR3	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)
210854_x_at	2,19	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
235729_at	2,19	ZNF514	zinc finger protein 514
203950_s_at	2,19	CLCN6	chloride channel 6
1554077_a_at	2,19	TMEM53	transmembrane protein 53
233358_at	2,18	FLJ14311	hypothetical gene FLJ14311
1555843_at	2,18	HNRPM	Heterogeneous nuclear ribonucleoprotein M
214755_at	2,18	UAP1L1	UDP-N-acteylglucosamine pyrophosphorylase 1-like 1
223378_at	2,18	GLIS2	GLIS family zinc finger 2
208807_s_at	2,18	CHD3	chromodomain helicase DNA binding protein 3
212567_s_at	2,18	MAP4	microtubule-associated protein 4
218149_s_at	2,17	ZNF395	zinc finger protein 395
231823_s_at	2,16	SH3PXD2B	SH3 and PX domains 2B
224602_at	2,16	LOC401152	HCV F-transactivated protein 1
204044_at	2,16	QPRT	quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating))
222746_s_at	2,15	BSPRY	B-box and SPRY domain containing
41657_at	2,15	STK11	serine/threonine kinase 11

Probe Set ID	Score(d)	Gene Symbol	Gene Title
219188_s_at	2,15	LRP16	LRP16 protein
203366_at	2,14	POLG	polymerase (DNA directed), gamma
200827_at	2,14	PLOD1	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1
201749_at	2,14	ECE1	Endothelin converting enzyme 1
202328_s_at	2,13	PKD1	polycystic kidney disease 1 (autosomal dominant)
202068_s_at	2,13	LDLR	low density lipoprotein receptor (familial hypercholesterolemia)
200872_at	2,13	S100A10	S100 calcium binding protein A10
223666_at	2,12	SNX5	Sorting nexin 5
202624_s_at	2,12	CABIN1	calcineurin binding protein 1
35617_at	2,12	MAPK7	mitogen-activated protein kinase 7
229001_at	2,11	PPP1R3E	Protein phosphatase 1, regulatory (inhibitor) subunit 3E
225718_at	2,11	KIAA1715	KIAA1715
219825_at	2,11	CYP26B1	cytochrome P450, family 26, subfamily B, polypeptide 1
207196_s_at	2,11	TNIP1	TNFAIP3 interacting protein 1
238996_x_at	2,11	ALDOA	aldolase A, fructose-bisphosphate
206039_at	2,11	RAB33A	RAB33A, member RAS oncogene family
242956_at	2,11	IDH1	Isocitrate dehydrogenase 1 (NADP+), soluble
205633_s_at	2,11	ALAS1	aminolevulinate, delta-, synthase 1
202962_at	2,10	KIF13B	kinesin family member 13B
57082_at	2,10	LDLRAP1	low density lipoprotein receptor adaptor protein 1
219020_at	2,10	HS1BP3	HCLS1 binding protein 3
218759_at	2,10	DVL2	dishevelled, dsh homolog 2 (Drosophila)
218543_s_at	2,10	PARP12	poly (ADP-ribose) polymerase family, member 12
218068_s_at	2,09	ZNF672	zinc finger protein 672
203043_at	2,09	ZBED1	zinc finger, BED-type containing 1
48825_at	2,09	ING4	inhibitor of growth family, member 4
211529_x_at	2,09	HLA-G	HLA-G histocompatibility antigen, class I, G
201059_at	2,09	CTTN	cortactin
201537_s_at	2,08	DUSP3	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)
209051_s_at	2,08	RALGDS	ral guanine nucleotide dissociation stimulator
207100_s_at	2,08	VAMP1	vesicle-associated membrane protein 1 (synaptobrevin 1)
224824_at	2,08	FAM36A	family with sequence similarity 36, member A
242621_at	2,08	ZNF498	zinc finger protein 498

Probe Set ID	Score(d)	Gene Symbol	Gene Title
212669_at	2,08	CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma
208881_x_at	2,07	IDI1	isopentenyl-diphosphate delta isomerase 1
213787_s_at	2,07	EBP	emopamil binding protein (sterol isomerase)
211065_x_at	2,07	PFKL	phosphofructokinase, liver
228028_at	2,07	FAM59B	family with sequence similarity 59, member B
212793_at	2,06	DAAM2	dishevelled associated activator of morphogenesis 2
224821_at	2,06	ABHD14B	abhydrolase domain containing 14B
232463_at	2,06	CXYorf10	chromosome X and Y open reading frame 10
210070_s_at	2,06	CHKB /// CPT1B	choline kinase beta /// carnitine palmitoyltransferase 1B (muscle)
223392_s_at	2,06	TSHZ3	teashirt family zinc finger 3
204899_s_at	2,05	SAP30	Sin3A-associated protein, 30kDa
206724_at	2,05	CBX4	chromobox homolog 4 (Pc class homolog, Drosophila)
227392_at	2,05	NISCH	nischarin
37996_s_at	2,05	DMPK	dystrophia myotonica-protein kinase
209795_at	2,05	CD69	CD69 molecule
222795_s_at	2,04	PLCXD1	phosphatidylinositol-specific phospholipase C, X domain containing 1
208998_at	2,04	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
220310_at	2,03	TUBAL3	tubulin, alpha-like 3
32137_at	2,03	JAG2	jagged 2
224929_at	2,03	TMEM173	transmembrane protein 173
217584_at	2,03	NPC1	Niemann-Pick disease, type C1
37005_at	2,03	NBL1	neuroblastoma, suppression of tumorigenicity 1
221812_at	2,03	FBXO42	F-box protein 42
203402_at	2,02	KCNAB2	potassium voltage-gated channel, shaker-related subfamily, beta member 2
219894_at	2,02	MAGEL2	MAGE-like 2
207595_s_at	2,02	BMP1	bone morphogenetic protein 1
201625_s_at	2,02	INSIG1	insulin induced gene 1
212558_at	2,02	SPRY1	sprouty homolog 1, antagonist of FGF signaling (Drosophila)
205662_at	2,02	EPPB9	B9 protein
216953_s_at	2,02	WT1	Wilms tumor 1
218284_at	2,01	SMAD3	SMAD family member 3
214617_at	2,01	PRF1	perforin 1 (pore forming protein)

Probe Set ID	Score(d)	Gene Symbol	Gene Title
228000_at	2,01	ADC	arginine decarboxylase
231024_at	2,01	LOC572558	hypothetical locus LOC572558
202973_x_at	2,01	FAM13A1	family with sequence similarity 13, member A1
207163_s_at	2,01	AKT1	v-akt murine thymoma viral oncogene homolog 1
213827_at	2,01	SNX26	sorting nexin 26
214950_at	2,01	IL9R /// LOC729486	interleukin 9 receptor /// similar to Interleukin-9 receptor precursor (IL-9R) (CD129 antigen)
238590_x_at	2,01	TMEM107	transmembrane protein 107
202039_at	2,01	MYO18A /// TIAF1	TGFB1-induced anti-apoptotic factor 1 /// myosin XVIIIA
205536_at	2,01	VAV2	vav 2 oncogene
1567032_s_at	2,01	ZNF160	zinc finger protein 160
228149_at	2,00	FLJ31818	hypothetical protein FLJ31818
217937_s_at	2,00	HDAC7A	histone deacetylase 7A
1554240_a_at	2,00	ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
1556348_at	0,19	HEATR1	HEAT repeat containing 1
1556144_at	0,22	DHX30	DEAH (Asp-Glu-Ala-His) box polypeptide 30
201796_s_at	0,24	VARS	valyl-tRNA synthetase
242442_x_at	0,24	RG9MTD2	RNA (guanine-9-) methyltransferase domain containing 2
207793_s_at	0,25	EPB41	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)
206591_at	0,25	RAG1	recombination activating gene 1
218470_at	0,25	YARS2	tyrosyl-tRNA synthetase 2, mitochondrial
207057_at	0,27	SLC16A7	solute carrier family 16, member 7 (monocarboxylic acid transporter 2)
223527_s_at	0,28	CDADC1	cytidine and dCMP deaminase domain containing 1
1553749_at	0,29	FAM76B	family with sequence similarity 76, member B
201702_s_at	0,29	PPP1R10	protein phosphatase 1, regulatory (inhibitor) subunit 10
227361_at	0,30	HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1
238960_s_at	0,31	LARP4	La ribonucleoprotein domain family, member 4
204686_at	0,32	IRS1	insulin receptor substrate 1
201843_s_at	0,33	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
224188_s_at	0,33	XPNPEP3	X-prolyl aminopeptidase (aminopeptidase P) 3, putative
222808_at	0,33	ALG13	asparagine-linked glycosylation 13 homolog (S. cerevisiae)
201936_s_at	0,33	EIF4G3	eukaryotic translation initiation factor 4 gamma, 3
205072_s_at	0,33	XRCC4	X-ray repair complementing defective repair in Chinese hamster cells 4
239439_at	0,34	AFF4	AF4/FMR2 family, member 4
1554067_at	0,34	FLJ32549	hypothetical protein FLJ32549
235006_at	0,35	MGC13017	similar to RIKEN cDNA A430101B06 gene
244680_at	0,35	GLRB	glycine receptor, beta
240546_at	0,35	LOC389043	hypothetical gene supported by AK125982; BC042817
216550_x_at	0,35	ANKRD12	ankyrin repeat domain 12
221606_s_at	0,35	NSBP1	nucleosomal binding protein 1
222486_s_at	0,36	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1
220764_at	0,37	PPP4R2	protein phosphatase 4, regulatory subunit 2
205659_at	0,37	HDAC9	histone deacetylase 9
222849_s_at	0,37	SCRN3	secernin 3
1555829_at	0,38	FAM62B	family with sequence similarity 62 (C2 domain containing) member B
222011_s_at	0,38	TCP1	t-complex 1
1553810_a_at	0,38	KIAA1524	KIAA1524
235287_at	0,38	CDK6	cyclin-dependent kinase 6
219927_at	0,38	FCF1	FCF1 small subunit (SSU) processome component homolog (S. cerevisiae)
227338_at	0,38	LOC440983	hypothetical gene supported by BC066916
225028_at	0,39	LOC550643	hypothetical protein LOC550643
235338_s_at	0,39	SETDB2	SET domain, bifurcated 2
214056_at	0,39	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)
225158_at	0,39	GFM1	G elongation factor, mitochondrial 1
223465_at	0,40	COL4A3BP	collagen, type IV, alpha 3 (Goodpasture antigen) binding protein
219074_at	0,40	TMEM34	transmembrane protein 34
218859_s_at	0,40	ESF1	ESF1, nucleolar pre-rRNA processing protein, homolog (S. cerevisiae)
223758_s_at	0,40	GTF2H2	general transcription factor IIH, polypeptide 2, 44kDa
230352_at	0,40	PRPS2	Phosphoribosyl pyrophosphate synthetase 2

Probe Set ID	Score(d)	Gene Symbol	Gene Title
202362_at	0,40	RAP1A	RAP1A, member of RAS oncogene family
212824_at	0,40	FUBP3	far upstream element (FUSE) binding protein 3
1553645_at	0,40	FLJ39502	hypothetical protein FLJ39502
225484_at	0,40	TSGA14	testis specific, 14
213704_at	0,41	RABGGTB	Rab geranylgeranyltransferase, beta subunit
209388_at	0,41	PAPOLA	poly(A) polymerase alpha
238506_at	0,41	LRRC58	leucine rich repeat containing 58
214710_s_at	0,41	CCNB1	cyclin B1
231534_at	0,41	CDC2	Cell division cycle 2, G1 to S and G2 to M
214277_at	0,41	COX11 /// COX11P	COX11 homolog, cytochrome c oxidase assembly protein (yeast) /// COX11 homolog, cytochrome c oxidase assembly protein (yeast) pseudogene
215442_s_at	0,41	TSHR	thyroid stimulating hormone receptor
226019_at	0,42	OMA1	OMA1 homolog, zinc metallopeptidase (S. cerevisiae)
46947_at	0,42	GNL3L	guanine nucleotide binding protein-like 3 (nucleolar)-like
215081_at	0,42	KIAA1024	KIAA1024 protein
202124_s_at	0,42	TRAK2	trafficking protein, kinesin binding 2
1559343_at	0,42	SNRPN	Small nuclear ribonucleoprotein polypeptide N
1553801_a_at	0,42	C14orf126	chromosome 14 open reading frame 126
204120_s_at	0,42	ADK	adenosine kinase
214953_s_at	0,42	APP	amyloid beta (A4) precursor protein (peptidase nexin-II, Alzheimer disease)
235744_at	0,42	PPTC7	PTC7 protein phosphatase homolog (S. cerevisiae)
212249_at	0,42	PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)
236917_at	0,43	LRRC34	leucine rich repeat containing 34
205046_at	0,43	CENPE	centromere protein E, 312kDa
235388_at	0,43	CHD9	chromodomain helicase DNA binding protein 9
212570_at	0,43	ENDOD1	endonuclease domain containing 1
221079_s_at	0,43	METTL2A /// METTL2B	methyltransferase like 2B /// methyltransferase like 2A
235597_s_at	0,43	RGPD1 /// RGPD2 /// RGPD3	RANBP2-like and GRIP domain containing 1 /// RANBP2-like and GRIP domain containing 3 /// RANBP2-like and GRIP domain containing 2
220797_at	0,43	METT10D	methyltransferase 10 domain containing
210733_at	0,43	TRAM1	Translocation associated membrane protein 1
235003_at	0,43	UHMK1	U2AF homology motif (UHM) kinase 1
228530_at	0,43	RP11-11C5.2	Similar to RIKEN cDNA 2410129H14

Probe Set ID	Score(d)	Gene Symbol	Gene Title
225686_at	0,44	FAM33A	family with sequence similarity 33, member A
220060_s_at	0,44	C12orf48	chromosome 12 open reading frame 48
235737_at	0,44	TSLP	thymic stromal lymphopoietin
213320_at	0,44	PRMT3	protein arginine methyltransferase 3
215223_s_at	0,44	SOD2	superoxide dismutase 2, mitochondrial
213907_at	0,44	EEF1E1	Eukaryotic translation elongation factor 1 epsilon 1
235644_at	0,44	CCDC138	coiled-coil domain containing 138
221213_s_at	0,45	SUHW4	suppressor of hairy wing homolog 4 (Drosophila)
218647_s_at	0,45	YRDC	yrdC domain containing (E. coli)
208900_s_at	0,45	TOP1	topoisomerase (DNA) I
212615_at	0,45	CHD9	chromodomain helicase DNA binding protein 9
202240_at	0,45	PLK1	polo-like kinase 1 (Drosophila)
205369_x_at	0,45	DBT	dihydrolipoamide branched chain transacylase E2
205129_at	0,45	NPM3	nucleophosmin/nucleoplasmin, 3
202146_at	0,46	IFRD1	interferon-related developmental regulator 1
218535_s_at	0,46	RIOK2	RIO kinase 2 (yeast)
1569366_a_at	0,46	ZNF569	zinc finger protein 569
211406_at	0,46	IER3IP1	immediate early response 3 interacting protein 1
219036_at	0,46	CEP70	centrosomal protein 70kDa
216493_s_at	0,46	IGF2BP3 /// LOC645468 /// LOC651107	insulin-like growth factor 2 mRNA binding protein 3 /// similar to insulin-like growth factor 2 mRNA binding protein 3 /// similar to IGF-II mRNA-binding protein 3
221561_at	0,46	SOAT1	sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1
203276_at	0,46	LMNB1	lamin B1
212105_s_at	0,46	DHX9	DEAH (Asp-Glu-Ala-His) box polypeptide 9
202872_at	0,46	ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1
231820_x_at	0,46	ZNF587	zinc finger protein 587
239233_at	0,47	CCDC88A	coiled-coil domain containing 88A
211697_x_at	0,47	PNO1	partner of NOB1 homolog (S. cerevisiae)
225114_at	0,47	AGPS	alkylglycerone phosphate synthase
201151_s_at	0,47	MBNL1	muscleblind-like (Drosophila)
220346_at	0,47	MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like
214583_at	0,47	RSC1A1	regulatory solute carrier protein, family 1, member 1
219031_s_at	0,47	NIP7	nuclear import 7 homolog (S. cerevisiae)
221705_s_at	0,47	SIKE	suppressor of IKK epsilon

Probe Set ID	Score(d)	Gene Symbol	Gene Title
219493_at	0,47	SHCBP1	SHC SH2-domain binding protein 1
212575_at	0,47	C19orf6	chromosome 19 open reading frame 6
200841_s_at	0,47	EPRS	glutamyl-prolyl-tRNA synthetase
226794_at	0,47	STXBP5	syntaxin binding protein 5 (tomosyn)
223254_s_at	0,47	KIAA1333	KIAA1333
208042_at	0,47	AGGF1	angiogenic factor with G patch and FHA domains 1
217878_s_at	0,47	CDC27	cell division cycle 27 homolog (S. cerevisiae)
222850_s_at	0,47	DNAJB14	DnaJ (Hsp40) homolog, subfamily B, member 14
204807_at	0,47	TMEM5	transmembrane protein 5
213655_at	0,48	YWHAE	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide
205097_at	0,48	SLC26A2	solute carrier family 26 (sulfate transporter), member 2
227161_at	0,48	NOM1	nucleolar protein with MIF4G domain 1
223651_x_at	0,48	CDC23	cell division cycle 23 homolog (S. cerevisiae)
231784_s_at	0,48	WDSOF1	WD repeats and SOF1 domain containing
209257_s_at	0,48	SMC3	structural maintenance of chromosomes 3
225834_at	0,48	FAM72A /// LOC653820 /// LOC729533	family with sequence similarity 72, member A /// similar to family with sequence similarity 72, member A
209754_s_at	0,48	TMPO	thymopoietin
228204_at	0,48	PSMB4	Proteasome (prosome, macropain) subunit, beta type, 4
218712_at	0,48	C1orf109	chromosome 1 open reading frame 109
226762_at	0,48	PURB	purine-rich element binding protein B
231175_at	0,48	C6orf65	chromosome 6 open reading frame 65
230618_s_at	0,48	BAT2D1	BAT2 domain containing 1
210802_s_at	0,48	DIMT1L	DIM1 dimethyladenosine transferase 1-like (S. cerevisiae)
1557984_s_at	0,48	FLJ21908	hypothetical protein FLJ21908
205362_s_at	0,48	PFDN4	prefoldin subunit 4
235463_s_at	0,48	LASS6	LAG1 homolog, ceramide synthase 6 (S. cerevisiae)
222642_s_at	0,48	TMEM33	transmembrane protein 33
229128_s_at	0,48	ANP32E	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member E
209865_at	0,48	SLC35A3	solute carrier family 35 (UDP-N-acetylglucosamine (UDP-GlcNAc) transporter), member A3
1555638_a_at	0,48	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1
212952_at	0,48	CALR	Calreticulin

Probe Set ID	Score(d)	Gene Symbol	Gene Title
217759_at	0,48	TRIM44	tripartite motif-containing 44
226765_at	0,49	SPTBN1	Spectrin, beta, non-erythrocytic 1
213982_s_at	0,49	RABGAP1L	RAB GTPase activating protein 1-like
217547_x_at	0,49	ZNF675	zinc finger protein 675
236140_at	0,49	GCLM	glutamate-cysteine ligase, modifier subunit
220330_s_at	0,49	SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1
229194_at	0,49	PCGF5	polycomb group ring finger 5
222360_at	0,49	DPH5	DPH5 homolog (S. cerevisiae)
229125_at	0,49	ANKRD38	ankyrin repeat domain 38
203465_at	0,49	MRPL19	mitochondrial ribosomal protein L19
219918_s_at	0,49	ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)
204962_s_at	0,49	CENPA	centromere protein A
221683_s_at	0,49	CEP290	centrosomal protein 290kDa
236834_at	0,49	SCFD2	sec1 family domain containing 2
219546_at	0,49	BMP2K	BMP2 inducible kinase
213926_s_at	0,49	HRB	HIV-1 Rev binding protein
225300_at	0,49	C15orf23	chromosome 15 open reading frame 23
217620_s_at	0,49	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide
203790_s_at	0,49	HRSP12	heat-responsive protein 12
206989_s_at	0,49	SFRS2IP	splicing factor, arginine/serine-rich 2, interacting protein
1557132_at	0,50	WDR17	WD repeat domain 17
208653_s_at	0,50	CD164	CD164 molecule, sialomucin
237466_s_at	0,50	HHIP	hedgehog interacting protein
217299_s_at	0,50	NBN	nibrin
201996_s_at	0,50	SPEN	spen homolog, transcriptional regulator (Drosophila)
1554873_at	0,50	CSPP1	centrosome and spindle pole associated protein 1
224309_s_at	0,50	SUGT1	SGT1, suppressor of G2 allele of SKP1 (S. cerevisiae)
1552263_at	0,50	MAPK1	mitogen-activated protein kinase 1
209451_at	0,50	TANK	TRAF family member-associated NFKB activator
242648_at	0,50	KLHL8	kelch-like 8 (Drosophila)
227510_x_at	0,50	PRO1073	PRO1073 protein

Supplementary Table 2. UM-1 cell line

Probe Set ID	Score(d)	Gene Symbol	Gene Title
202912_at	57,63	ADM	adrenomedullin
228499_at	36,58	PFKFB4	6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 4
242064_at	24,83	SDK2	sidekick homolog 2 (chicken)
221009_s_at	13,21	ANGPTL4	angiopoietin-like 4
203438_at	12,97	STC2	stanniocalcin 2
214978_s_at	12,97	PPFIA4	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 4
227337_at	12,34	ANKRD37	ankyrin repeat domain 37
238551_at	11,21	FUT11	fucosyltransferase 11 (alpha (1,3) fucosyltransferase)
235850_at	10,99	WDR5B	WD repeat domain 5B
234312_s_at	10,88	ACSS2	acyl-CoA synthetase short-chain family member 2
204348_s_at	10,63	AK3L1	adenylate kinase 3-like 1
243435_at	10,59	KCNQ1OT1	KCNQ1 overlapping transcript 1
226682_at	10,47	LOC283666	hypothetical protein LOC283666
204347_at	9,57	LOC645619 /// LOC731007	similar to Adenylate kinase isoenzyme 4, mitochondrial (ATP-AMP transphosphorylase) /// similar to Adenylate kinase isoenzyme 4, mitochondrial (Adenylate kinase 3-like 1) (ATP-AMP transphosphorylase)
202022_at	9,51	ALDOC	aldolase C, fructose-bisphosphate
202497_x_at	9,47	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
227404_s_at	8,55	EGR1	Early growth response 1
202718_at	8,45	IGFBP2	insulin-like growth factor binding protein 2, 36kDa
201849_at	8,19	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
207543_s_at	8,08	P4HA1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I
215925_s_at	7,08	CD72	CD72 molecule
202934_at	6,91	HK2	hexokinase 2
202464_s_at	6,70	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
221478_at	6,63	BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like
226452_at	6,62	PDK1	pyruvate dehydrogenase kinase, isozyme 1
210512_s_at	6,58	VEGFA	vascular endothelial growth factor A

Probe Set ID	Score(d)	Gene Symbol	Gene Title
203282_at	6,00	GBE1	glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)
224797_at	5,93	ARRDC3	arrestin domain containing 3
210426_x_at	5,92	RORA	RAR-related orphan receptor A
203946_s_at	5,77	ARG2	arginase, type II
1558770_a_at	5,60	C17orf38	chromosome 17 open reading frame 38
206991_s_at	5,53	CCR5 /// LOC727797	chemokine (C-C motif) receptor 5 /// similar to C-C chemokine receptor type 5 (C-C CKR-5) (CC-CKR-5) (CCR-5) (CCR5) (HIV-1 fusion coreceptor) (CHEMR13) (CD195 antigen)
228483_s_at	5,46	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa
209566_at	5,39	INSIG2	insulin induced gene 2
219670_at	5,34	C1orf165	chromosome 1 open reading frame 165
223216_x_at	5,33	FBXO16 /// ZNF395	zinc finger protein 395 /// F-box protein 16
223046_at	5,04	EGLN1	egl nine homolog 1 (C. elegans)
218149_s_at	5,04	ZNF395	zinc finger protein 395
203027_s_at	5,01	MVD	mevalonate (diphospho) decarboxylase
206580_s_at	4,97	EFEMP2	EGF-containing fibulin-like extracellular matrix protein 2
225557_at	4,95	AXUD1	AXIN1 up-regulated 1
202218_s_at	4,92	FADS2	fatty acid desaturase 2
206686_at	4,89	PDK1	pyruvate dehydrogenase kinase, isozyme 1
207425_s_at	4,89	9-Sep	septin 9
218274_s_at	4,86	ANKZF1	ankyrin repeat and zinc finger domain containing 1
208513_at	4,83	FOXB1	forkhead box B1
205599_at	4,72	TRAF1	TNF receptor-associated factor 1
202149_at	4,69	NEDD9	neural precursor cell expressed, developmentally down-regulated 9
221757_at	4,68	PIK3IP1	phosphoinositide-3-kinase interacting protein 1
219622_at	4,55	RAB20	RAB20, member RAS oncogene family
202393_s_at	4,55	KLF10	Kruppel-like factor 10
209446_s_at	4,44	C7orf44	chromosome 7 open reading frame 44
201389_at	4,39	ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
212689_s_at	4,36	JMJD1A	jumonji domain containing 1A
201693_s_at	4,36	EGR1	early growth response 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
226985_at	4,33	FGD5	FYVE, RhoGEF and PH domain containing 5
217028_at	4,28	CXCR4	chemokine (C-X-C motif) receptor 4
224345_x_at	4,12	C3orf28	chromosome 3 open reading frame 28
209795_at	4,07	CD69	CD69 molecule
212281_s_at	4,07	TMEM97	transmembrane protein 97
244178_at	4,06	COMMD7	COMM domain containing 7
1569453_a_at	4,06	LOC692247	hypothetical locus LOC692247
201368_at	4,04	ZFP36L2	zinc finger protein 36, C3H type-like 2
202426_s_at	4,04	RXRA	retinoid X receptor, alpha
201790_s_at	3,93	DHCR7	7-dehydrocholesterol reductase
205484_at	3,93	SIT1	signaling threshold regulating transmembrane adaptor 1
226390_at	3,81	STARD4	START domain containing 4, sterol regulated
228891_at	3,72	C9orf164	chromosome 9 open reading frame 164
227868_at	3,68	LOC154761	hypothetical protein LOC154761
230398_at	3,63	TNS4	tensin 4
202973_x_at	3,58	FAM13A1	family with sequence similarity 13, member A1
211434_s_at	3,57	CCRL2 /// LOC727811	chemokine (C-C motif) receptor-like 2 /// similar to chemokine (C-C motif) receptor-like 2
240258_at	3,55	ENO1	enolase 1, (alpha)
201625_s_at	3,53	INSIG1	insulin induced gene 1
219014_at	3,53	PLAC8	placenta-specific 8
214593_at	3,53	PIAS2	protein inhibitor of activated STAT, 2
202887_s_at	3,51	DDIT4	DNA-damage-inducible transcript 4
212496_s_at	3,47	JMJD2B	jumonji domain containing 2B
202562_s_at	3,47	C14orf1	chromosome 14 open reading frame 1
210132_at	3,46	EFNA3	ephrin-A3
206039_at	3,44	RAB33A	RAB33A, member RAS oncogene family
218012_at	3,44	TSPYL2	TSPY-like 2
228749_at	3,43	ZDBF2	zinc finger, DBF-type containing 2
215649_s_at	3,43	MVK	mevalonate kinase (mevalonic aciduria)
208657_s_at	3,40	9-Sep	septin 9
226114_at	3,40	ZNF436	zinc finger protein 436
223553_s_at	3,35	DOK3	docking protein 3
238996_x_at	3,33	ALDOA	aldolase A, fructose-bisphosphate
202449_s_at	3,32	RXRA	retinoid X receptor, alpha
209034_at	3,28	PNRC1	proline-rich nuclear receptor coactivator 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
202245_at	3,27	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
224602_at	3,25	LOC401152	HCV F-transactivated protein 1
215734_at	3,24	C19orf36	chromosome 19 open reading frame 36
219236_at	3,21	PAQR6	progestin and adipoQ receptor family member VI
202996_at	3,19	POLD4	polymerase (DNA-directed), delta 4
209859_at	3,19	TRIM9	tripartite motif-containing 9
238965_at	3,16	C21orf2	Chromosome 21 open reading frame 2
209279_s_at	3,15	NSDHL	NAD(P) dependent steroid dehydrogenase-like
201673_s_at	3,13	GYS1	glycogen synthase 1 (muscle)
1567032_s_at	3,13	ZNF160	zinc finger protein 160
202644_s_at	3,12	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
222870_s_at	3,10	B3GNT2	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2
209822_s_at	3,09	VLDLR	very low density lipoprotein receptor
1555349_a_at	3,06	ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
242621_at	3,06	ZNF498	zinc finger protein 498
218638_s_at	3,05	SPON2	spondin 2, extracellular matrix protein
201464_x_at	3,04	JUN	jun oncogene
210130_s_at	3,03	TM7SF2	transmembrane 7 superfamily member 2
223522_at	2,99	C9orf45	chromosome 9 open reading frame 45
228098_s_at	2,96	MYLIP	myosin regulatory light chain interacting protein
237120_at	2,96	KRT77	keratin 77
205497_at	2,95	ZNF175	zinc finger protein 175
218368_s_at	2,90	TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A
233358_at	2,89	FLJ14311	hypothetical gene FLJ14311
207339_s_at	2,88	LTB	lymphotoxin beta (TNF superfamily, member 3)
201170_s_at	2,87	BHLHB2	basic helix-loop-helix domain containing, class B, 2
36711_at	2,85	MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
209608_s_at	2,84	ACAT2	acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)
208763_s_at	2,84	TSC22D3	TSC22 domain family, member 3
204243_at	2,84	RLF	rearranged L-myc fusion
202472_at	2,83	MPI	mannose phosphate isomerase
219183_s_at	2,83	PSCD4	pleckstrin homology, Sec7 and coiled-coil domains 4

Probe Set ID	Score(d)	Gene Symbol	Gene Title
1569835_at	2,81	LOC339352	Similar to ATP binding domain 3
202331_at	2,80	BCKDHA	branched chain keto acid dehydrogenase E1, alpha polypeptide
203950_s_at	2,79	CLCN6	chloride channel 6
218205_s_at	2,77	MKNK2	MAP kinase interacting serine/threonine kinase 2
1569909_at	2,77	KRT6L	keratin 6L
228738_at	2,76	D2HGDH	D-2-hydroxyglutarate dehydrogenase
224772_at	2,76	NAV1	neuron navigator 1
206907_at	2,75	TNFSF9	tumor necrosis factor (ligand) superfamily, member 9
219417_s_at	2,74	C17orf59	chromosome 17 open reading frame 59
209975_at	2,73	CYP2E1	cytochrome P450, family 2, subfamily E, polypeptide 1
206828_at	2,72	TXK	TXK tyrosine kinase
210105_s_at	2,72	FYN	FYN oncogene related to SRC, FGR, YES
206478_at	2,71	KIAA0125	KIAA0125
206348_s_at	2,70	PDK3	pyruvate dehydrogenase kinase, isozyme 3
1562403_a_at	2,69	SLC8A3	solute carrier family 8 (sodium-calcium exchanger), member 3
235948_at	2,69	FAM80A	family with sequence similarity 80, member A
241954_at	2,68	FDFT1	Farnesyl-diphosphate farnesyltransferase 1
205790_at	2,66	SKAP1	src kinase associated phosphoprotein 1
203317_at	2,65	PSD4	pleckstrin and Sec7 domain containing 4
201537_s_at	2,64	DUSP3	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)
1552343_s_at	2,64	PDE7A	phosphodiesterase 7A
226471_at	2,64	GGTL3	gamma-glutamyltransferase-like 3
219862_s_at	2,63	NARF	nuclear prelamin A recognition factor
1565611_at	2,62	DYNLRB1	Dynein, light chain, roadblock-type 1
206583_at	2,61	ZNF673	zinc finger protein 673
214847_s_at	2,60	GPSM3	G-protein signalling modulator 3 (AGS3-like, C. elegans)
201313_at	2,60	ENO2	enolase 2 (gamma, neuronal)
211423_s_at	2,59	SC5DL	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, S. cerevisiae)-like
204484_at	2,59	PIK3C2B	phosphoinositide-3-kinase, class 2, beta polypeptide
227134_at	2,57	SYTL1	synaptotagmin-like 1
219563_at	2,57	C14orf139	chromosome 14 open reading frame 139

Probe Set ID	Score(d)	Gene Symbol	Gene Title
200827_at	2,56	PLOD1	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1
212368_at	2,56	ZNF292	zinc finger protein 292
212190_at	2,56	SERPINE2	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
212958_x_at	2,55	PAM	peptidylglycine alpha-amidating monooxygenase
37796_at	2,53	LRCH4	leucine-rich repeats and calponin homology (CH) domain containing 4
224925_at	2,53	PREX1	phosphatidylinositol 3,4,5-trisphosphate-dependent RAC exchanger 1
207196_s_at	2,52	TNIP1	TNFAIP3 interacting protein 1
209146_at	2,49	SC4MOL	sterol-C4-methyl oxidase-like
202364_at	2,48	MXI1	MAX interactor 1
200697_at	2,47	HK1	hexokinase 1
213693_s_at	2,46	MUC1	mucin 1, cell surface associated
200832_s_at	2,46	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
205141_at	2,45	ANG	angiogenin, ribonuclease, RNase A family, 5
216086_at	2,44	SV2C	synaptic vesicle glycoprotein 2C
229063_s_at	2,44	CCDC107	coiled-coil domain containing 107
225530_at	2,43	MOBKL2A	MOB1, Mps One Binder kinase activator-like 2A (yeast)
241348_at	2,42	ZNF654	zinc finger protein 654
231056_at	2,42	LOC339352	similar to ATP binding domain 3
243296_at	2,42	PBEF1	Pre-B-cell colony enhancing factor 1
201251_at	2,41	PKM2	pyruvate kinase, muscle
222451_s_at	2,41	ZDHHC9	zinc finger, DHHC-type containing 9
225383_at	2,40	ZNF275	zinc finger protein 275
215189_at	2,40	KRT86	keratin 86
226470_at	2,39	GGTL3	gamma-glutamyltransferase-like 3
41386_i_at	2,39	JMJD3	jumonji domain containing 3
235231_at	2,39	ZNF789	zinc finger protein 789
34210_at	2,38	CD52	CD52 molecule
201309_x_at	2,36	C5orf13	chromosome 5 open reading frame 13
222906_at	2,36	FLVCR1	feline leukemia virus subgroup C cellular receptor 1
230086_at	2,35	FNBP1	formin binding protein 1
220091_at	2,34	SLC2A6	solute carrier family 2 (facilitated glucose transporter), member 6
218145_at	2,34	TRIB3	tribbles homolog 3 (Drosophila)
210561_s_at	2,33	WSB1	WD repeat and SOCS box-containing 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
205904_at	2,33	MICA	MHC class I polypeptide-related sequence A
223179_at	2,32	YPEL3	yippee-like 3 (Drosophila)
204899_s_at	2,32	SAP30	Sin3A-associated protein, 30kDa
202219_at	2,32	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
225262_at	2,31	FOSL2	FOS-like antigen 2
210337_s_at	2,31	ACLY	ATP citrate lyase
202803_s_at	2,31	ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
1553537_at	2,30	KRT73	keratin 73
221750_at	2,29	HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)
220310_at	2,29	TUBAL3	tubulin, alpha-like 3
232809_s_at	2,28	FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
212813_at	2,28	JAM3	junctional adhesion molecule 3
228149_at	2,27	FLJ31818	hypothetical protein FLJ31818
212552_at	2,27	HPCAL1	hippocalcin-like 1
242592_at	2,27	GPR137C	G protein-coupled receptor 137C
204669_s_at	2,26	RNF24	ring finger protein 24
222856_at	2,26	APLN	apelin, AGTRL1 ligand
1554860_at	2,26	PTPN7	protein tyrosine phosphatase, non-receptor type 7
218625_at	2,26	NRN1	neuritin 1
229086_at	2,25	C1orf213	chromosome 1 open reading frame 213
226442_at	2,24	ABTB1	ankyrin repeat and BTB (POZ) domain containing 1
201508_at	2,24	IGFBP4	insulin-like growth factor binding protein 4
225918_at	2,24	LOC146346	hypothetical protein LOC146346
214861_at	2,24	JMJD2C	jumonji domain containing 2C
212745_s_at	2,24	BBS4	Bardet-Biedl syndrome 4
225718_at	2,23	KIAA1715	KIAA1715
201275_at	2,23	FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)
1569212_at	2,22	LOC619207	scavenger receptor protein family member
202068_s_at	2,22	LDLR	low density lipoprotein receptor (familial hypercholesterolemia)
210362_x_at	2,22	PML	promyelocytic leukemia

Probe Set ID	Score(d)	Gene Symbol	Gene Title
201626_at	2,22	INSIG1	insulin induced gene 1
210203_at	2,21	CNOT4	CCR4-NOT transcription complex, subunit 4
205267_at	2,21	POU2AF1	POU domain, class 2, associating factor 1
204205_at	2,21	APOBEC3G	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
235213_at	2,21	ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B
226275_at	2,20	MXD1	MAX dimerization protein 1
202492_at	2,19	ATG9A	ATG9 autophagy related 9 homolog A (S. cerevisiae)
243264_s_at	2,18	C8orf44 /// SGK3	serum/glucocorticoid regulated kinase family, member 3 /// chromosome 8 open reading frame 44
217356_s_at	2,18	PGK1	phosphoglycerate kinase 1
226811_at	2,18	FAM46C	family with sequence similarity 46, member C
201810_s_at	2,17	SH3BP5	SH3-domain binding protein 5 (BTK-associated)
221011_s_at	2,16	LBH	limb bud and heart development homolog (mouse)
207590_s_at	2,16	CENPI	centromere protein I
209827_s_at	2,16	IL16	interleukin 16 (lymphocyte chemoattractant factor)
228906_at	2,16	CXXC6	CXXC finger 6
205839_s_at	2,16	BZRAP1	benzodiazapine receptor (peripheral) associated protein 1
238784_at	2,16	DPY19L2	dpy-19-like 2 (C. elegans)
220081_x_at	2,15	HSD17B7 /// HSD17B7P2 /// LOC730412	hydroxysteroid (17-beta) dehydrogenase 7 /// hydroxysteroid (17-beta) dehydrogenase 7 pseudogene 2 /// similar to hydroxysteroid (17-beta) dehydrogenase 7
200808_s_at	2,15	ZYX	zyxin
224027_at	2,15	CCL28	chemokine (C-C motif) ligand 28
215785_s_at	2,15	CYFIP2	cytoplasmic FMR1 interacting protein 2
210534_s_at	2,14	EPPB9	B9 protein
201102_s_at	2,14	PFKL	phosphofructokinase, liver
208881_x_at	2,14	IDI1	isopentenyl-diphosphate delta isomerase 1
217937_s_at	2,14	HDAC7A	histone deacetylase 7A
238540_at	2,14	LOC401320	hypothetical LOC401320
40020_at	2,13	CELSR3	cadherin, EGF LAG seven-pass G-type receptor 3 (flamingo homolog, Drosophila)
200872_at	2,13	S100A10	S100 calcium binding protein A10
213787_s_at	2,13	EBP	emopamil binding protein (sterol isomerase)
1554452_a_at	2,13	HIG2	hypoxia-inducible protein 2
203037_s_at	2,13	MTSS1	metastasis suppressor 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
221572_s_at	2,12	SLC26A6	solute carrier family 26, member 6
207908_at	2,12	KRT2	keratin 2 (epidermal ichthyosis bullosa of Siemens)
202260_s_at	2,11	STXBP1	syntaxin binding protein 1
207686_s_at	2,11	CASP8	caspase 8, apoptosis-related cysteine peptidase
227645_at	2,10	PIK3R5	phosphoinositide-3-kinase, regulatory subunit 5, p101
228667_at	2,10	AGPAT4	1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)
230012_at	2,10	C17orf44	chromosome 17 open reading frame 44
201250_s_at	2,10	SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1
204225_at	2,10	HDAC4	histone deacetylase 4
203208_s_at	2,10	MTFR1	mitochondrial fission regulator 1
205250_s_at	2,09	CEP290	centrosomal protein 290kDa
239137_x_at	2,09	MGC45491	hypothetical protein MGC45491
214730_s_at	2,09	GLG1	golgi apparatus protein 1
39966_at	2,09	CSPG5	chondroitin sulfate proteoglycan 5 (neuroglycan C)
202081_at	2,09	IER2	immediate early response 2
218498_s_at	2,09	ERO1L	ERO1-like (S. cerevisiae)
209882_at	2,09	RIT1	Ras-like without CAAX 1
212641_at	2,08	HIVEP2	human immunodeficiency virus type I enhancer binding protein 2
228194_s_at	2,08	SORCS1	sortilin-related VPS10 domain containing receptor 1
219125_s_at	2,08	RAG1AP1	recombination activating gene 1 activating protein 1
200921_s_at	2,08	BTG1	B-cell translocation gene 1, anti-proliferative
241905_at	2,07	PIK3C2A	Phosphoinositide-3-kinase, class 2, alpha polypeptide
213397_x_at	2,07	RNASE4	ribonuclease, RNase A family, 4
204106_at	2,07	TESK1	testis-specific kinase 1
218840_s_at	2,07	NADSYN1	NAD synthetase 1
203521_s_at	2,06	ZNF318	zinc finger protein 318
1558525_at	2,06	LOC283901	hypothetical protein LOC283901
221813_at	2,06	FBXO42	F-box protein 42
221828_s_at	2,06	FAM125B	family with sequence similarity 125, member B
210556_at	2,06	NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
202539_s_at	2,06	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
202769_at	2,05	CCNG2	cyclin G2

Probe Set ID	Score(d)	Gene Symbol	Gene Title
239761_at	2,05	GCNT1	glucosaminyl (N-acetyl) transferase 1, core 2 (beta-1,6-N-acetylglucosaminyltransferase)
205045_at	2,05	AKAP10	A kinase (PRKA) anchor protein 10
228550_at	2,05	RTN4R	reticulon 4 receptor
210031_at	2,05	CD247	CD247 molecule
223498_at	2,05	SPECC1	sperm antigen with calponin homology and coiled-coil domains 1
208964_s_at	2,05	FADS1	fatty acid desaturase 1
214500_at	2,04	H2AFY	H2A histone family, member Y
223392_s_at	2,04	TSHZ3	teashirt family zinc finger 3
203349_s_at	2,04	ETV5	ets variant gene 5 (ets-related molecule)
219629_at	2,04	FAM118A	family with sequence similarity 118, member A
233813_at	2,04	PPP1R16B	protein phosphatase 1, regulatory (inhibitor) subunit 16B
205107_s_at	2,03	EFNA4	ephrin-A4
228739_at	2,03	CYS1	cystin 1
225869_s_at	2,03	UNC93B1	unc-93 homolog B1 (C. elegans)
201637_s_at	2,03	FXR1	fragile X mental retardation, autosomal homolog 1
222790_s_at	2,03	RSBN1	round spermatid basic protein 1
225715_at	2,03	KIAA1303	raptor
212770_at	2,03	TLE3	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)
244556_at	2,02	LCP2	Lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
203929_s_at	2,02	MAPT	microtubule-associated protein tau
221214_s_at	2,02	NELF	nasal embryonic LHRH factor
203767_s_at	2,01	STS	steroid sulfatase (microsomal), arylsulfatase C, isozyme S
218630_at	2,01	MKS1	Meckel syndrome, type 1
59375_at	2,01	MYO15B	myosin XVB pseudogene
1554079_at	2,01	GALNTL4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 4
227412_at	2,01	PPP1R3E	protein phosphatase 1, regulatory (inhibitor) subunit 3E
234302_s_at	2,01	ALKBH5	alkB, alkylation repair homolog 5 (E. coli)
226925_at	2,01	ACPL2	acid phosphatase-like 2
217677_at	2,00	PLEKHA2	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 2
35617_at	2,00	MAPK7	mitogen-activated protein kinase 7

Probe Set ID	Score(d)	Gene Symbol	Gene Title
229437_at	2,00	BIC	BIC transcript
227383_at	2,00	LOC727820	hypothetical protein LOC727820
226037_s_at	0,13	LOC728198 /// TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa /// similar to transcription associated factor 9B
225779_at	0,22	SLC27A4	solute carrier family 27 (fatty acid transporter), member 4
219522_at	0,26	FJX1	four jointed box 1 (Drosophila)
225484_at	0,29	TSGA14	testis specific, 14
1557309_at	0,32	DENND1B	DENN/MADD domain containing 1B
224870_at	0,33	KIAA0114	KIAA0114
237466_s_at	0,34	HHIP	hedgehog interacting protein
226884_at	0,36	LRRN1	leucine rich repeat neuronal 1
235117_at	0,36	CHAC2	ChaC, cation transport regulator homolog 2 (E. coli)
219676_at	0,36	ZSCAN16	zinc finger and SCAN domain containing 16
209567_at	0,36	RRS1	RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)
222714_s_at	0,36	LACTB2	lactamase, beta 2
232155_at	0,38	KIAA1618	KIAA1618
220311_at	0,38	N6AMT1	N-6 adenine-specific DNA methyltransferase 1 (putative)
224632_at	0,38	GPATCH4	G patch domain containing 4
1554331_a_at	0,39	LRRC18	leucine rich repeat containing 18
1556348_at	0,39	HEATR1	HEAT repeat containing 1
212333_at	0,40	FAM98A	family with sequence similarity 98, member A
222162_s_at	0,40	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1
209771_x_at	0,40	CD24	CD24 molecule
1553111_a_at	0,40	KBTBD6	kelch repeat and BTB (POZ) domain containing 6
219050_s_at	0,41	ZNHIT2	zinc finger, HIT type 2
202870_s_at	0,41	CDC20	cell division cycle 20 homolog (S. cerevisiae)
225200_at	0,41	DPH3	DPH3, KTI11 homolog (S. cerevisiae)
205992_s_at	0,41	IL15	interleukin 15
228050_at	0,42	UTP15	UTP15, U3 small nucleolar ribonucleoprotein, homolog (S. cerevisiae)
232291_at	0,42	MIRH1	microRNA host gene (non-protein coding) 1
225748_at	0,42	LTV1	LTV1 homolog (S. cerevisiae)
204127_at	0,42	RFC3	replication factor C (activator 1) 3, 38kDa

Probe Set ID	Score(d)	Gene Symbol	Gene Title
221168_at	0,42	PRDM13	PR domain containing 13
1555950_a_at	0,43	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
228355_s_at	0,43	NDUFA12L	NDUFA12-like
222958_s_at	0,43	DEPDC1	DEP domain containing 1
223651_x_at	0,43	CDC23	cell division cycle 23 homolog (S. cerevisiae)
236302_at	0,43	PPM1E	protein phosphatase 1E (PP2C domain containing)
209595_at	0,43	GTF2F2	general transcription factor IIF, polypeptide 2, 30kDa
201656_at	0,43	ITGA6	integrin, alpha 6
207057_at	0,43	SLC16A7	solute carrier family 16, member 7 (monocarboxylic acid transporter 2)
231784_s_at	0,43	WDSOF1	WD repeats and SOF1 domain containing
228645_at	0,43	SNHG9	small nucleolar RNA host gene (non-protein coding) 9
231798_at	0,43	NOG	Noggin
218647_s_at	0,43	YRDC	yrdC domain containing (E. coli)
202715_at	0,44	CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
219031_s_at	0,44	NIP7	nuclear import 7 homolog (S. cerevisiae)
242260_at	0,44	MATR3	Matrin 3
224450_s_at	0,44	RIOK1	RIO kinase 1 (yeast)
223113_at	0,44	TMEM138	transmembrane protein 138
241933_at	0,44	QRSL1	Glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1
205215_at	0,44	RNF2	ring finger protein 2
213427_at	0,44	RPP40	ribonuclease P 40kDa subunit
230170_at	0,44	OSM	oncostatin M
203321_s_at	0,44	ZNF508	zinc finger protein 508
202345_s_at	0,44	FABP5 /// LOC728641 /// LOC729163 /// LOC731043 /// LOC732031	fatty acid binding protein 5 (psoriasis-associated) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP)
200848_at	0,44	AHCYL1	S-adenosylhomocysteine hydrolase-like 1
218590_at	0,44	PEO1	progressive external ophthalmoplegia 1
220083_x_at	0,44	UCHL5	ubiquitin carboxyl-terminal hydrolase L5
212510_at	0,45	GPD1L	glycerol-3-phosphate dehydrogenase 1-like
220295_x_at	0,45	DEPDC1 /// LOC730888	DEP domain containing 1 /// similar to DEP domain containing 1

Probe Set ID	Score(d)	Gene Symbol	Gene Title
215388_s_at	0,45	CFH /// CFHR1	complement factor H /// complement factor H-related 1
231102_at	0,45	CROT	carnitine O-octanoyltransferase
213320_at	0,45	PRMT3	protein arginine methyltransferase 3
1569190_at	0,45	SCLT1	sodium channel and clathrin linker 1
201023_at	0,45	TAF7	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa
203956_at	0,45	MORC2	MORC family CW-type zinc finger 2
225291_at	0,46	PNPT1	polyribonucleotide nucleotidyltransferase 1
231240_at	0,46	DIO2	deiodinase, iodothyronine, type II
204905_s_at	0,46	EEF1E1	eukaryotic translation elongation factor 1 epsilon 1
214277_at	0,46	COX11 /// COX11P	COX11 homolog, cytochrome c oxidase assembly protein (yeast) /// COX11 homolog, cytochrome c oxidase assembly protein (yeast) pseudogene
225682_s_at	0,46	POLR3H	polymerase (RNA) III (DNA directed) polypeptide H (22.9kD)
204033_at	0,46	TRIP13	thyroid hormone receptor interactor 13
203119_at	0,46	CCDC86	coiled-coil domain containing 86
226059_at	0,46	TOMM40L	translocase of outer mitochondrial membrane 40 homolog (yeast)-like
202903_at	0,46	LSM5	LSM5 homolog, U6 small nuclear RNA associated (S. cerevisiae)
221586_s_at	0,46	E2F5	E2F transcription factor 5, p130-binding
225768_at	0,47	NR1D2	nuclear receptor subfamily 1, group D, member 2
231863_at	0,47	ING3	inhibitor of growth family, member 3
204114_at	0,47	NID2	nidogen 2 (osteonidogen)
205929_at	0,47	GPA33	glycoprotein A33 (transmembrane)
222962_s_at	0,47	MCM10	minichromosome maintenance complex component 10
225158_at	0,47	GFM1	G elongation factor, mitochondrial 1
201516_at	0,47	SRM	spermidine synthase
205129_at	0,47	NPM3	nucleophosmin/nucleoplasmin, 3
203178_at	0,47	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
224441_s_at	0,47	USP45	ubiquitin specific peptidase 45
203196_at	0,47	ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
222771_s_at	0,47	MYEF2	myelin expression factor 2
205518_s_at	0,47	CMAH	cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminate monooxygenase)

Probe Set ID	Score(d)	Gene Symbol	Gene Title
235795_at	0,47	PAX6	paired box gene 6 (aniridia, keratitis)
219068_x_at	0,47	ATAD3A	ATPase family, AAA domain containing 3A
218670_at	0,47	PUS1	pseudouridylate synthase 1
209100_at	0,47	IFRD2	interferon-related developmental regulator 2
218488_at	0,48	EIF2B3	eukaryotic translation initiation factor 2B, subunit 3 gamma, 58kDa
221549_at	0,48	GRWD1	glutamate-rich WD repeat containing 1
203737_s_at	0,48	PPRC1	peroxisome proliferator-activated receptor gamma, coactivator-related 1
209205_s_at	0,48	LMO4	LIM domain only 4
230243_at	0,48	RG9MTD2	RNA (guanine-9-) methyltransferase domain containing 2
226882_x_at	0,48	WDR4	WD repeat domain 4
205046_at	0,48	CENPE	centromere protein E, 312kDa
228802_at	0,48	RBPMS2	RNA binding protein with multiple splicing 2
228381_at	0,48	ATF7IP2	Activating transcription factor 7 interacting protein 2
1554557_at	0,48	ATP11B	ATPase, Class VI, type 11B
235289_at	0,48	EIF5A2	eukaryotic translation initiation factor 5A2
227594_at	0,48	ZMYM6	zinc finger, MYM-type 6
219546_at	0,48	BMP2K	BMP2 inducible kinase
204769_s_at	0,48	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)
218719_s_at	0,48	GINS3	GINS complex subunit 3 (Psf3 homolog)
223035_s_at	0,48	FARSB	phenylalanyl-tRNA synthetase, beta subunit
224204_x_at	0,48	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2
223403_s_at	0,48	POLR1B	polymerase (RNA) I polypeptide B, 128kDa
238762_at	0,49	MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like
220688_s_at	0,49	MRTO4	mRNA turnover 4 homolog (S. cerevisiae)
218219_s_at	0,49	LANCL2	LanC lantibiotic synthetase component C-like 2 (bacterial)
226284_at	0,49	ZBTB2	zinc finger and BTB domain containing 2
204175_at	0,49	ZNF593	zinc finger protein 593
218680_x_at	0,49	HYPK	Huntingtin interacting protein K
228397_at	0,49	TUG1	taurine upregulated gene 1
219037_at	0,49	RRP15	ribosomal RNA processing 15 homolog (S. cerevisiae)
206235_at	0,49	LIG4	ligase IV, DNA, ATP-dependent

Probe Set ID	Score(d)	Gene Symbol	Gene Title
203708_at	0,49	PDE4B	phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)
224721_at	0,49	WDR75	WD repeat domain 75
224315_at	0,49	DDX20	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20
203465_at	0,49	MRPL19	mitochondrial ribosomal protein L19
218695_at	0,49	EXOSC4	exosome component 4
201644_at	0,50	TSTA3	tissue specific transplantation antigen P35B
202431_s_at	0,50	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
202069_s_at	0,50	IDH3A	isocitrate dehydrogenase 3 (NAD+) alpha
222765_x_at	0,50	ESF1	ESF1, nucleolar pre-rRNA processing protein, homolog (S. cerevisiae)
213189_at	0,50	MINA	MYC induced nuclear antigen
219131_at	0,50	UBIAD1	UbiA prenyltransferase domain containing 1
228043_at	0,50	UTP15	UTP15, U3 small nucleolar ribonucleoprotein, homolog (S. cerevisiae)
220643_s_at	0,50	FAIM	Fas apoptotic inhibitory molecule
225834_at	0,50	FAM72A /// LOC653820 /// LOC729533	family with sequence similarity 72, member A /// similar to family with sequence similarity 72, member A
1553535_a_at	0,50	RANGAP1	Ran GTPase activating protein 1
215011_at	0,50	SNHG3	small nucleolar RNA host gene (non-protein coding) 3
222500_at	0,50	PPIL1	peptidylprolyl isomerase (cyclophilin)-like 1
218470_at	0,50	YARS2	tyrosyl-tRNA synthetase 2, mitochondrial
218981_at	0,50	ACN9	ACN9 homolog (S. cerevisiae)
224654_at	0,50	DDX21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21

Supplementary Table 3. MM hypoxia-target genes

Gene ID	Probe set ID
ADM	202912_at
ALDOC	202022_at
AK3L1	225342_at
SLC2A3	202497_x_at
VEGFA	210512_s_at
BNIP3	201848_s_at
PDK1	226452_at
DDIT4	202887_s_at
P4HA1	207543_s_at
HK2	202934_at
ANKRD37	227337_at

Supplementary Table 4

Spearman's rank correlation coefficient matrix for AM in relation to HOXB7, ING4, NF-κB profile genes and hypoxia target genes in 559 primary MM patients, and in MM subgroups (PR, LB, HY, CD2, MY) with significantly elevated AM expression.

	MM patients (n = 559) AM	PR subgroup (n = 47) AM	LB subgroup (n = 58) AM	HY subgroup (n = 116) AM	CD2 subgroup (n = 60) AM	MY subgroup (n = 145) AM
ALDOC	ns	ns	ns	ns	ns	ns
AK3L1	ns	ns	ns	*-0.2	*-0.3	ns
SLC2A3	ns	ns	ns	ns	ns	ns
VEGFA	ns	ns	ns	*-0.2	ns	ns
BNIP3	ns	ns	ns	ns	ns	ns
PDK1	***0.1	ns	**0.3	*0.2	ns	ns
DDIT4	ns	ns	ns	ns	ns	ns
P4HA1	ns	ns	ns	ns	ns	ns
HK2	***0.2	ns	ns	***0.3	ns	**0.3
ANKRD37	ns	ns	ns	ns	ns	ns
HOXB7	ns	ns	ns	ns	ns	ns
ING4	ns	ns	ns	ns	ns	ns
NF-κB profile genes	ns	ns	ns	ns	ns	ns

ns-not significant, *** indicates p value < 0.001, **indicates p value < 0.01, *indicates p value < 0.05, NF-κB profile comprised 11 NF-κB MM signature genes determined by Annunziata et. al [19]