

## **IL21R expressing CD14<sup>+</sup>CD16<sup>+</sup> monocytes expand in multiple myeloma patients leading to increased osteoclasts**

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## SUPPLEMENTAL METHODS

### *Immunophenotype of BM CD14<sup>+</sup> in patients with monoclonal gammopathies.*

Briefly, 100  $\mu$ l of total BM aspirate was incubated in the dark with anti-human HLA-DR-PE (clone L243; BD), anti-human CD14-PerCP-Cy 5.5, anti-human CD16-PE-Cy7 (clone B73.1; BD) and anti-human CD45-APC-H 7 (clone 2D1; BD) for 20 min. Flow cytometry analysis was performed as 6-color experiment by using BD FACS Canto II with Diva software. First, a gate was set around monocytes based on their light-scatter properties. Then, two monocyte populations were defined in that gate: the classical CD14<sup>bright</sup>CD16<sup>-</sup> monocytes and CD14<sup>dim</sup>CD16<sup>bright</sup> monocytes. In some experiments, 10<sup>6</sup> mononuclear cells (MNCs), obtained from BM aspirates by density gradient centrifugation (Lympholite-H; Burlington, NC), were stained with anti-human CD14-PerCP-Cy 5.5, anti-human CD16-PE-Cy7 and anti-human CD360/IL-21R APC (Clone 17A12; BD), using the gating strategy described above.

### *Fluorescence in situ hybridization (FISH) analysis.*

FISH analysis has been performed on purified CD138<sup>+</sup> cells to test the presence of: hyperdiploidy (ON9RED/15GREEN; Kreatech Diagnostics; Durham, NC); del(13q) (D13 S319SO/CEP 12SG; Metasystems; Altlussheim, Germany); del(17p) (LSI ATM SG/p53SO; Metasystems); chromosome 14 translocation (14 BREAK-APART; Metasystems). If samples were positive for chromosome 14 translocation, the presence of t(4;14) (FGFR3SO/IGHSG; Abbott Laboratories; Abbott Park, IL), t(11;14) (LSI IGH/CCND1XT; Abbott Laboratories) and t(14;16) (IGH/MAF; Abbott Laboratories) were checked.

### *Microarrays analysis*

Total RNA was extracted from BM CD14<sup>+</sup> cells using the RNeasy total RNA isolation kit (Qiagen; Hilden, Germany). Gene expression profiles of BM CD14<sup>+</sup> cells previously isolated were generated on GeneChip<sup>®</sup> HG-U133 Plus 2.0 arrays (Affymetrix; Santa Clara, CA). The biotin-labeled cRNA was prepared according to the Affymetrix GeneChip Expression Analysis Technical Manual

protocol. Log<sub>2</sub>-transformed expression values were extracted from CEL files and normalized using RMA procedure in the *affy* package for Bioconductor and the annotations included in the cdf definition files version 20 available at the Brainarray portal (<http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/20.0.0/version.html>).

Unsupervised and supervised analyses of gene expression data were carried out using the Significant Analysis of Microarrays (SAM) software version 4.00, as previously reported.<sup>13</sup>

Data were deposited in NCBI's Gene Expression Omnibus and are accessible through GEO series accession number GSE70345.

Correlation between *IL21R* expression and that of *CCR5*, *CD40* and *SLAMF7* by BM monocytes arisen from gene expression analysis were evaluated by Spearman *rho* correlation test.

#### ***Real Time PCR.***

RNA (0,5 µg) was reverse-transcribed with 400 U Moloney murine leukemia reverse transcriptase (Applied Biosystems, Life Technologies) in accordance with the manufacturer's protocol. Real Time PCR was performed by adding complementary DNA to a universal master Mix primers and TaqMan probes (Applied Biosystems) for the following genes: *IL21R*: Hs00222310\_m1, *SLAMF7*: Hs00221793\_m1 and *ABL*: Hs01104728\_m1. The Real Time ready assays for *CCR5* (Assay ID: 104069) and *CD40* (Assay ID: 100254) genes were purchased from Roche Diagnostics (Mannheim, Germany). The expression of selected genes was checked by Real Time PCR by Light Cycler 480 (Roche Diagnostics). To normalize the differences in RNA quality and reverse transcription efficiency, we applied the comparative Ct method using the endogenous reference gene *ABL*. In the experiments where treated cells were compared to untreated controls, the fold change in mRNA expression (n-fold) of each analyzed gene was calculated as  $2^{-\Delta\Delta C_t}$ , as previously described.<sup>30</sup> However, when the aim of the analysis was to check the differences between groups of patients, mRNA expression levels were expressed as  $-\Delta C_t$ .

#### ***STAT3 activity assay.***

STAT3 activity was evaluated by means of an ELISA-based commercially available kit (Trans AM STAT Family, Active Motif, Vinci Biochem; Vinci, Florence, Italy), used in accordance with the manufacturers' procedures. We tested duplicates of nuclear extracts (each containing 5 µg of proteins) of CD14<sup>+</sup> cells purified from 3 MGUS, 3 SMM and 3 MM patients and nuclear extracts of HepG2 treated with rhIL-6 (100 ng/ml) were used as positive controls, as suggested in the manufacturers' procedures. Cell nuclear extracts were obtained and total amount of proteins quantified as previously described.<sup>30</sup>

***BM IL-21 levels in patients with monoclonal gammopathies.***

The levels of IL-21 in BM plasma were measured in a total cohort of 76 newly diagnosed MM, 42 SMM and 41 MGUS patients. Plasma (5 ml) was obtained after centrifugation from BM aspirates, aliquoted and stored at -20°C until analysis. BM IL-21 plasma levels were detected by Human Interleukin-21 ELISA kit (BioVendor; Brno, Czech Republic), following the manufacturers' protocol.

## SUPPLEMENTAL TABLES

**Supplemental Table 1:** mRNA expression levels of *CD14* (929\_at) and *SDCI* (6382\_at).

Monocyte samples reported in italic style were excluded from gene expression analysis due to high PC contamination according to 2-groups stratification based on K-means clustering of *SDCI* expression.

<b>Monocyte sample</b>	<b><i>CD14</i></b>	<b><i>SDCI</i></b>
MGUS1	8789.818	40.304
MGUS2	9006.552	38.80391
MGUS3	6403.874	35.66097
MGUS4	9964.512	43.99736
MGUS5	8146.314	59.22493
MGUS6	8589.581	57.34123
MGUS7	7803.366	44.29881
MGUS8	9120.798	35.85622
MGUS9	8895.179	32.07205
SMM1	8114.306	37.218
SMM2	8029.939	39.68942
SMM3	7796.826	28.66408
SMM4	9010.986	43.27907
SMM5	8058.92	43.83396
SMM6	9088.744	52.35158
SMM7	7662.433	43.14486
SMM8	5344.555	46.07221
SMM9	8406.226	36.69782
SMM10	11324.39	60.34423
SMM11	9027.734	48.58276
SMM12	7547.065	37.88982
SMM13	7526.294	65.80806
SMM14	8964.173	28.70343
SMM15	8211.26	45.07396
MM1	7492.444	32.61221
MM2	6873.478	163.3324
MM3	6079.989	42.57115
MM4	8406.226	70.01866
MM5	6458.982	49.30689
MM6	5512.166	55.35385
MM7	10195.97	37.92994
MM8	8460.027	33.74889
MM9	7921.082	80.19244
MM10	7397.48	47.13206
MM11	11284.27	30.44155
MM12	7538.09	64.00715

MM13	8060.529	100.2527
MM14	9567.787	37.23853
MM15	8702.203	166.6147
MM16	8484.997	99.10607
MM17	8436.607	35.66015
MM18	8612.851	35.29721
MM19	5049.836	56.44912
MM20	8848.109	74.04942
MM21	7571.708	61.97922
MM22	9838.354	104.0483
MM23	9886.636	45.53263
<i>MM24</i>	<i>4579.45</i>	<i>655.08</i>
<i>MM25</i>	<i>5069.29</i>	<i>1464.04</i>
<i>MM26</i>	<i>7199.53</i>	<i>1209.62</i>
<i>MM27</i>	<i>1372.92</i>	<i>7674.54</i>
<i>MM28</i>	<i>7325.70</i>	<i>702.92</i>
<i>MM29</i>	<i>8592.04</i>	<i>1117.68</i>
<i>MM30</i>	<i>7947.35</i>	<i>2153.92</i>
<i>MM31</i>	<i>5844.00</i>	<i>555.92</i>
<i>MM32</i>	<i>5309.73</i>	<i>2219.51</i>

Abbreviations: MGUS, Monoclonal Gammopathy of Undetermined Significance; SMM, Smoldering Multiple Myeloma; MM, Multiple Myeloma.

**Supplemental Table 2:** List of genes significantly modulated in BM monocytes across the three groups of patients (MM, SMM and MGUS) by multiclass analysis of gene expression data.

Gene ID	Gene Name	SAM Score(d)*
FCER1A	Fc fragment of IgE receptor Ia	1.49
SLCO4C1	solute carrier organic anion transporter family member 4C1	1.46
PTPN22	protein tyrosine phosphatase, non-receptor type 22	1.45
PADI4	peptidyl arginine deiminase 4	1.45
SLAMF7	SLAM family member 7	1.44
PGM1	phosphoglucomutase 1	1.38
RAB37	RAB37, member RAS oncogene family	1.34
RPS17	ribosomal protein S17	1.33
FLVCR2	feline leukemia virus subgroup C cellular receptor family member 2	1.33
ANXA6	annexin A6	1.32
SERPINB10	serpin family B member 10	1.32
WLS	wntless Wnt ligand secretion mediator	1.31
CLTCL1	clathrin heavy chain like 1	1.29
TMEM55A	transmembrane protein 55A	1.26
SSBP4	single stranded DNA binding protein 4	1.25
GNMT2	G protein subunit gamma transducin 2	1.22
LOC100506844	uncharacterized LOC100506844	1.22
CPNE2	copine 2	1.22
CCR5	C-C motif chemokine receptor 5	1.21
UBR4	ubiquitin protein ligase E3 component n-recognin 4	1.21
SQLE	squalene epoxidase	1.20
CTSL	cathepsin L	1.20
SLC9A3R1	SLC9A3 regulator 1	1.20
PNMA1	paraneoplastic Ma antigen 1	1.19
LGALS12	galectin 12	1.19
CHST7	carbohydrate sulfotransferase 7	1.19
LHFPL2	lipoma HMGIC fusion partner-like 2	1.19
MYB	MYB proto-oncogene, transcription factor	1.19
ADA	adenosine deaminase	1.18
SRPK1	SRSF protein kinase 1	1.17
CENPK	centromere protein K	1.17
RUNX3	runt related transcription factor 3	1.17
VAMP5	vesicle associated membrane protein 5	1.16
IL21R	interleukin 21 receptor	1.16
HADHA	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase , alpha subunit	1.15
TMEM170B	transmembrane protein 170B	1.14
FBN2	fibrillin 2	1.14
NCAPD2	non-SMC condensin I complex subunit D2	1.14

MTSS1	MTSS1, I-BAR domain containing	1.13
CCL18	C-C motif chemokine ligand 18	1.13
EIF2D	eukaryotic translation initiation factor 2D	1.13
TCN2	transcobalamin 2	1.12
DGKZ	diacylglycerol kinase zeta	1.12
AZU1	azurocidin 1	1.12
SLC2A9	solute carrier family 2 member 9	1.11
CDCA7	cell division cycle associated 7	1.11
TUBB	tubulin beta class I	1.11
SPNS3	sphingolipid transporter 3	1.10
TMEM51	transmembrane protein 51	1.10
KBTBD11	kelch repeat and BTB domain containing 11	1.10
IPCEF1	interaction protein for cytohesin exchange factors 1	1.10
ECRP	ribonuclease A family member 2 pseudogene	1.10
SCCPDH	saccharopine dehydrogenase	1.09
ORM1	orosomucoid 1	1.08
GAS2L1	growth arrest specific 2 like 1	1.08
ANP32E	acidic nuclear phosphoprotein 32 family member E	1.08
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif 5	1.08
GOT2	glutamic-oxaloacetic transaminase 2	1.08
MIR205	microRNA 205	1.07
NECTIN2	nectin cell adhesion molecule 2	1.07
KIF11	kinesin family member 11	1.07
NRM	nurim	1.07
CTSV	cathepsin V	1.07
SIPA1L2	signal induced proliferation associated 1 like 2	1.07
SLC26A8	solute carrier family 26 member 8	1.07
EMB	embigin	1.06
FAM101B	refilin B	1.06
MKI67	marker of proliferation Ki-67	1.06
CDCA7L	cell division cycle associated 7 like	1.06
HEG1	heart development protein with EGF like domains 1	1.06
B4GALT6	beta-1,4-galactosyltransferase 6	1.05
IGF1R	insulin like growth factor 1 receptor	1.05
DNASE2	deoxyribonuclease 2, lysosomal	1.05
IFNGR2	interferon gamma receptor 2	1.05
SELM	selenoprotein M	1.05
EIF3L	eukaryotic translation initiation factor 3 subunit L	1.05
TRAF5	TNF receptor associated factor 5	1.05
TMC8	transmembrane channel like 8	1.05
E2F8	E2F transcription factor 8	1.05
PRRG2	proline rich and Gla domain 2	1.05
CIT	citron rho-interacting serine/threonine kinase	1.04
PTCH1	patched 1	1.04
PSRC1	proline and serine rich coiled-coil 1	1.04
INSIG1	insulin induced gene 1	1.04
MRPL37	mitochondrial ribosomal protein L37	1.04



RRAS	related RAS viral oncogene homolog	1.04
MAP4K4	mitogen-activated protein kinase kinase kinase 4	1.04
PIM1	Pim-1 proto-oncogene, serine/threonine kinase	1.03
IFITM1	interferon induced transmembrane protein 1	1.03
HIP1	huntingtin interacting protein 1	1.03
ANLN	anillin actin binding protein	1.03
PGD	phosphogluconate dehydrogenase	1.03
SPOCK1	SPARC/osteonectin, cwcv and kazal like domains proteoglycan 1	1.03
ISG20	interferon stimulated exonuclease gene 20	1.03
LOC152225	uncharacterized LOC152225	1.03
BPI	bactericidal/permeability-increasing protein	1.03
CKAP4	cytoskeleton associated protein 4	1.03
ZBED3	zinc finger BED-type containing 3	1.02
PKM	pyruvate kinase, muscle	1.02

\*Linear discriminant score defined by the Significance Analysis of Microarrays (SAM) analysis.

**Supplemental Table 3:** List of genes differentially expressed by BM monocytes obtained from supervised analysis of MM patients as compared to SMM.

**DOWN**

Gene ID	Gene Name	SAM score(d)*	Fold Change
PTPN22	protein tyrosine phosphatase, non-receptor type 22	-4.76	0.59
PADI4	peptidyl arginine deiminase 4	-4.65	0.44
FCER1A	Fc fragment of IgE receptor 1a	-4.62	0.42
SLCO4C1	solute carrier organic anion transporter family member 4C1	-4.57	0.45
RAB37	RAB37, member RAS oncogene family	-4.54	0.58
ECRP	ribonuclease A family member 2 pseudogene	-4.46	0.47
SERPINB10	serpin family B member 10	-4.38	0.29
PGM1	phosphoglucomutase 1	-4.29	0.64
RPS17	ribosomal protein S17	-4.29	0.79
FBN2	fibrillin 2	-4.18	0.54
LGALS12	galectin 12	-4.16	0.60
ANXA6	annexin A6	-4.08	0.65
MIR205	microRNA 205	-4.02	0.76
SPNS3	sphingolipid transporter 3	-3.99	0.64
TMEM55A	transmembrane protein 55A	-3.98	0.67
SCCPDH	saccharopine dehydrogenase	-3.97	0.72
ADAMTS5	ADAM metalloproteinase with thrombospondin type 1 motif 5	-3.97	0.73
SLC2A9	solute carrier family 2 member 9	-3.96	0.74
WLS	wntless Wnt ligand secretion mediator	-3.92	0.64
KBTBD11	kelch repeat and BTB domain containing 11	-3.88	0.66
SRPK1	SRSF protein kinase 1	-3.86	0.69
PGD	phosphogluconate dehydrogenase	-3.85	0.72
SLC26A8	solute carrier family 26 member 8	-3.85	0.66
UBR4	ubiquitin protein ligase E3 component n-recognin 4	-3.82	0.74
LOC100506844	uncharacterized LOC100506844	-3.78	0.62
SLC9A3R1	SLC9A3 regulator 1	-3.78	0.70
CLTCL1	clathrin heavy chain like 1	-3.76	0.70
CENPK	centromere protein K	-3.73	0.43
MYB	MYB proto-oncogene, transcription factor	-3.71	0.51
EMB	embigin	-3.70	0.55
TMC8	transmembrane channel like 8	-3.68	0.74
EIF2D	eukaryotic translation initiation factor 2D	-3.61	0.78
MTMR11	myotubularin related protein 11	-3.60	0.63
BPI	bactericidal/permeability-increasing protein	-3.55	0.42
MRPL33	mitochondrial ribosomal protein L33	-3.53	0.81
NRM	nurim	-3.52	0.79
FMO5	flavin containing monooxygenase 5	-3.52	0.72

HIP1	huntingtin interacting protein 1	-3.52	0.75
KIF21B	kinesin family member 21B	-3.51	0.78
B4GALT6	beta-1,4-galactosyltransferase 6	-3.49	0.77
DGKZ	diacylglycerol kinase zeta	-3.49	0.74
CDA	cytidine deaminase	-3.48	0.65
HDDC2	HD domain containing 2	-3.46	0.78
MARC1	mitochondrial amidoxime reducing component 1	-3.46	0.61
MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	-3.46	0.79
TMEM170B	transmembrane protein 170B	-3.43	0.62
CPNE2	copine 2	-3.43	0.73
ADGRA2	adhesion G protein-coupled receptor A2	-3.43	0.70
IPCEF1	interaction protein for cytohesin exchange factors 1	-3.42	0.61
CACNA2D3	calcium voltage-gated channel auxiliary subunit alpha2delta 3	-3.41	0.60
MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	-3.41	0.74
CDCA7	cell division cycle associated 7	-3.41	0.50
PKM	pyruvate kinase, muscle	-3.40	0.69
RNASE2	ribonuclease A family member 2	-3.40	0.58
PSRC1	proline and serine rich coiled-coil 1	-3.40	0.73
CKAP4	cytoskeleton associated protein 4	-3.39	0.65
FAM101B	refilin B	-3.39	0.62
SPOCK1	SPARC/osteonectin, cwcv and kazal like domains proteoglycan 1	-3.38	0.65
NME8	NME/NM23 family member 8	-3.38	0.74
CYTL1	cytokine like 1	-3.37	0.62

### *UP*

<b>Gene ID</b>	<b>Gene Name</b>	<b>SAM score(d)*</b>	<b>Fold Change</b>
SLAMF7	SLAM family member 7	4.74	2.44
CCR5	C-C motif chemokine receptor 5	4.55	2.26
CTSL	cathepsin L	4.46	2.13
CHST7	carbohydrate sulfotransferase 7	4.33	1.87
LHFPL2	lipoma HMGIC fusion partner-like 2	4.15	2.20
PNMA1	paraneoplastic Ma antigen 1	4.07	1.55
SSBP4	single stranded DNA binding protein 4	4.06	1.28
PIM1	Pim-1 proto-oncogene, serine/threonine kinase	4.03	1.63
GAS2L1	growth arrest specific 2 like 1	3.98	1.37
GNGT2	G protein subunit gamma transducin 2	3.97	1.40
TCN2	transcobalamin 2	3.91	1.50
FLVCR2	feline leukemia virus subgroup C cellular receptor family member 2	3.90	1.51
YES1	YES proto-oncogene 1, Src family tyrosine kinase	3.87	1.22
SQLE	squalene epoxidase	3.86	1.84
VAMP5	vesicle associated membrane protein 5	3.85	1.62
ADA	adenosine deaminase	3.83	1.79
CTSV	cathepsin V	3.81	1.43

IFNGR2	interferon gamma receptor 2	3.75	1.38
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\*Ratio of change in gene expression to standard deviation.

**Supplemental Table 4:** List of differentially expressed genes by BM monocytes arisen from supervised analysis of MM *versus* (MGUS plus SMM) patients.

**DOWN**

Gene ID	Gene Name	SAM Score(d)*	Fold Change
FCER1A	Fc fragment of IgE receptor Ia	-5.70	0.40
SLCO4C1	solute carrier organic anion transporter family member 4C1	-5.65	0.45
PADI4	peptidyl arginine deiminase 4	-5.58	0.46
PTPN22	protein tyrosine phosphatase, non-receptor type 22	-5.57	0.60
PGM1	phosphoglucomutase 1	-5.35	0.64
RPS17	ribosomal protein S17	-5.15	0.79
ANXA6	annexin A6	-5.12	0.64
RAB37	RAB37, member RAS oncogene family	-5.12	0.60
WLS	wntless Wnt ligand secretion mediator	-5.03	0.63
SERPINB10	serpin family B member 10	-5.01	0.32
CLTCL1	clathrin heavy chain like 1	-4.92	0.68
TMEM55A	transmembrane protein 55A	-4.89	0.67
LOC100506844	uncharacterized LOC100506844	-4.71	0.62
UBR4	ubiquitin protein ligase E3 component n-recogin 4	-4.66	0.74
SLC9A3R1	SLC9A3 regulator 1	-4.63	0.70
CPNE2	copine 2	-4.59	0.71
MYB	MYB proto-oncogene, transcription factor	-4.58	0.51
CENPK	centromere protein K	-4.54	0.43
SRPK1	SRSF protein kinase 1	-4.53	0.70
LGALS12	galectin 12	-4.52	0.63
TMEM170B	transmembrane protein 170B	-4.40	0.61
HADHA	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase , alpha subunit	-4.38	0.83
EIF2D	eukaryotic translation initiation factor 2D	-4.37	0.78
DGKZ	diacylglycerol kinase zeta	-4.33	0.74
CDCA7	cell division cycle associated 7	-4.29	0.50
FBN2	fibrillin 2	-4.26	0.58
AZU1	azurocidin 1	-4.25	0.45
IPCEF1	interaction protein for cytohesin exchange factors 1	-4.25	0.61
TUBB	tubulin beta class I	-4.23	0.68
SPNS3	sphingolipid transporter 3	-4.23	0.65
NCAPD2	non-SMC condensin I complex subunit D2	-4.22	0.59
ANP32E	acidic nuclear phosphoprotein 32 family member E	-4.17	0.72
ADAMTS5	ADAM metalloproteinase with thrombospondin type 1 motif 5	-4.17	0.72
ORM1	orosomucoid 1	-4.17	0.49
KIF11	kinesin family member 11	-4.14	0.40
NRM	nurim	-4.14	0.79

KBTBD11	kelch repeat and BTB domain containing 11	-4.13	0.69
GOT2	glutamic-oxaloacetic transaminase 2	-4.13	0.75
SLC2A9	solute carrier family 2 member 9	-4.12	0.76
FAM101B	refilin B	-4.11	0.63
EMB	embigin	-4.10	0.56
CDCA7L	cell division cycle associated 7 like	-4.08	0.54
SLC26A8	solute carrier family 26 member 8	-4.07	0.68
B4GALT6	beta-1,4-galactosyltransferase 6	-4.07	0.77
E2F8	E2F transcription factor 8	-4.05	0.44
MKI67	marker of proliferation Ki-67	-4.04	0.54
EIF3L	eukaryotic translation initiation factor 3 subunit L	-4.04	0.81
PSRC1	proline and serine rich coiled-coil 1	-4.00	0.74
MRPL37	mitochondrial ribosomal protein L37	-3.99	0.84
ANLN	anillin actin binding protein	-3.99	0.44
SPOCK1	SPARC/osteonectin, cwcv and kazal like domains proteoglycan 1	-3.97	0.66
MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	-3.96	0.80
ZBED3	zinc finger BED-type containing 3	-3.95	0.70
HIP1	huntingtin interacting protein 1	-3.94	0.76
CKAP4	cytoskeleton associated protein 4	-3.93	0.67
CIT	citron rho-interacting serine/threonine kinase	-3.92	0.65
PKM	pyruvate kinase, muscle	-3.92	0.71
RAB27A	RAB27A, member RAS oncogene family	-3.90	0.71
ZWINT	ZW10 interacting kinetochore protein	-3.90	0.49
FOXM1	forkhead box M1	-3.89	0.61
S100A12	S100 calcium binding protein A12	-3.89	0.69
CENPH	centromere protein H	-3.87	0.59
MS4A3	membrane spanning 4-domains A3	-3.87	0.43
PECR	peroxisomal trans-2-enoyl-CoA reductase	-3.86	0.67
TOP2A	topoisomerase II alpha	-3.86	0.40
CDA	cytidine deaminase	-3.84	0.67
CENPF	centromere protein F	-3.83	0.55
LOC100288637	OTU deubiquitinase 7A pseudogene	-3.82	0.59
BPI	bactericidal/permeability-increasing protein	-3.82	0.46
SCCPDH	saccharopine dehydrogenase	-3.82	0.76
GPSM2	G-protein signaling modulator 2	-3.82	0.63
TMC8	transmembrane channel like 8	-3.81	0.77
ZNF281	zinc finger protein 281	-3.81	0.73
MRPL33	mitochondrial ribosomal protein L33	-3.80	0.81
MIR205	microRNA 205	-3.80	0.79
MARC1	mitochondrial amidoxime reducing component 1	-3.79	0.65
HIPK2	homeodomain interacting protein kinase 2	-3.79	0.75
KIF18B	kinesin family member 18B	-3.78	0.50
NCAPG	non-SMC condensin I complex subunit G	-3.78	0.47
RETN	resistin	-3.78	0.58
TPCN1	two pore segment channel 1	-3.77	0.81
TYMS	thymidylate synthetase	-3.77	0.44

RACGAP1	Rac GTPase activating protein 1	-3.77	0.56
SHCBP1	SHC binding and spindle associated 1	-3.77	0.56
GINS1	GINS complex subunit 1	-3.76	0.51
CACNA2D3	calcium voltage-gated channel auxiliary subunit alpha2delta 3	-3.76	0.63
PTDSS1	phosphatidylserine synthase 1	-3.76	0.82
ECRP	ribonuclease A family member 2 pseudogene	-3.75	0.54
PGD	phosphogluconate dehydrogenase	-3.75	0.76
KIAA0101	KIAA0101	-3.74	0.46
MSL1	male specific lethal 1 homolog	-3.74	0.81
TPX2	TPX2, microtubule nucleation factor	-3.73	0.48
TK1	thymidine kinase 1	-3.73	0.66
FAM217B	family with sequence similarity 217 member B	-3.73	0.67
NUSAP1	nucleolar and spindle associated protein 1	-3.73	0.51
NCAPG2	non-SMC condensin II complex subunit G2	-3.72	0.62
KIF20A	kinesin family member 20A	-3.72	0.47
BMX	BMX non-receptor tyrosine kinase	-3.71	0.72
KIF15	kinesin family member 15	-3.70	0.50
HMGB2	high mobility group box 2	-3.69	0.78
IGF1R	insulin like growth factor 1 receptor	-3.69	0.73
NME8	NME/NM23 family member 8	-3.68	0.76
KIFC1	kinesin family member C1	-3.68	0.72
MEGF9	multiple EGF like domains 9	-3.68	0.62
OIP5	Opa interacting protein 5	-3.68	0.54
MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	-3.67	0.76
ADGRA2	adhesion G protein-coupled receptor A2	-3.67	0.71
CEP55	centrosomal protein 55	-3.67	0.47
TACC3	transforming acidic coiled-coil containing protein 3	-3.65	0.75
NUF2	NUF2, NDC80 kinetochore complex component	-3.64	0.50
RASSF1	Ras association domain family member 1	-3.64	0.82
GPI	glucose-6-phosphate isomerase	-3.64	0.79
CENPM	centromere protein M	-3.63	0.62
ZNF367	zinc finger protein 367	-3.62	0.51
EFCAB2	EF-hand calcium binding domain 2	-3.61	0.67
CTH	cystathionine gamma-lyase	-3.61	0.53
KIF21B	kinesin family member 21B	-3.60	0.80
HIST1H4C	histone cluster 1 H4 family member c	-3.60	0.57
PCNA	proliferating cell nuclear antigen	-3.59	0.62
MTMR11	myotubularin related protein 11	-3.59	0.68
TMC4	transmembrane channel like 4	-3.58	0.72
C20orf27	chromosome 20 open reading frame 27	-3.58	0.78
PLD1	phospholipase D1	-3.58	0.81
UBE2C	ubiquitin conjugating enzyme E2 C	-3.57	0.54
TTK	TTK protein kinase	-3.57	0.50
FAM110B	family with sequence similarity 110 member B	-3.56	0.76
CBX4	chromobox 4	-3.56	0.78
FARSB	phenylalanyl-tRNA synthetase beta subunit	-3.55	0.80

MAD2L1	MAD2 mitotic arrest deficient-like 1	-3.54	0.54
FMO5	flavin containing monooxygenase 5	-3.54	0.76
ERG	ERG, ETS transcription factor	-3.53	0.85
ANAPC15	anaphase promoting complex subunit 15	-3.53	0.82
FAM64A	family with sequence similarity 64 member A	-3.52	0.70
KIF14	kinesin family member 14	-3.51	0.55
SLC25A40	solute carrier family 25 member 40	-3.51	0.75
THBS4	thrombospondin 4	-3.50	0.79
SSR1	signal sequence receptor subunit 1	-3.50	0.85
POLR3B	RNA polymerase III subunit B	-3.50	0.79
PCMT1	protein-L-isoaspartate O-methyltransferase	-3.49	0.78
SIDT1	SID1 transmembrane family member 1	-3.48	0.71
ITGAM	integrin subunit alpha M	-3.47	0.76
CAT	catalase	-3.47	0.72
NCL	nucleolin	-3.46	0.87
LPGAT1	lysophosphatidylglycerol acyltransferase 1	-3.46	0.76
CCNE2	cyclin E2	-3.45	0.62
HAL	histidine ammonia-lyase	-3.45	0.73
MCM7	minichromosome maintenance complex component 7	-3.45	0.72
STMN1	stathmin 1	-3.44	0.79
BIRC5	baculoviral IAP repeat containing 5	-3.44	0.64
SDHD	succinate dehydrogenase complex subunit D	-3.44	0.82
CDC20	cell division cycle 20	-3.43	0.50
PHKA2	phosphorylase kinase regulatory subunit alpha 2	-3.43	0.83
MPO	myeloperoxidase	-3.43	0.49
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	-3.43	0.55
DTL	denticleless E3 ubiquitin protein ligase homolog	-3.43	0.51
RRM2	ribonucleotide reductase regulatory subunit M2	-3.43	0.46
TFF3	trefoil factor 3	-3.43	0.80
VRK1	vaccinia related kinase 1	-3.42	0.74
PTTG1	pituitary tumor-transforming 1	-3.42	0.64
TSPAN2	tetraspanin 2	-3.42	0.69
SLC40A1	solute carrier family 40 member 1	-3.42	0.45
GYS1	glycogen synthase 1	-3.42	0.79
KIF4A	kinesin family member 4A	-3.42	0.56
NPIP15	nuclear pore complex interacting protein family member B15	-3.41	0.73
PLCL2	phospholipase C like 2	-3.41	0.77
TOP1MT	topoisomerase I, mitochondrial	-3.40	0.75
TCF19	transcription factor 19	-3.40	0.67
SFXN5	sideroflexin 5	-3.40	0.87
MELK	maternal embryonic leucine zipper kinase	-3.39	0.56
CYTL1	cytokine like 1	-3.39	0.65
CSF3R	colony stimulating factor 3 receptor	-3.38	0.82
MCM4	minichromosome maintenance complex component 4	-3.36	0.73
USP48	ubiquitin specific peptidase 48	-3.36	0.78
CDK1	cyclin dependent kinase 1	-3.35	0.54



CIDEB	cell death-inducing DFFA-like effector b	-3.35	0.76
CARS2	cysteinyl-tRNA synthetase 2, mitochondrial	-3.35	0.80
IVNS1ABP	influenza virus NS1A binding protein	-3.34	0.74
DLGAP5	DLG associated protein 5	-3.34	0.43
TMEM106C	transmembrane protein 106C	-3.34	0.71
RCC1	regulator of chromosome condensation 1	-3.33	0.85
ASPM	abnormal spindle microtubule assembly	-3.33	0.54
GTSE1	G2 and S-phase expressed 1	-3.32	0.73
BZW2	basic leucine zipper and W2 domains 2	-3.32	0.83
RNASE2	ribonuclease A family member 2	-3.32	0.63
EP300-AS1	EP300 antisense RNA 1	-3.32	0.85
SMC2	structural maintenance of chromosomes 2	-3.31	0.65
RALGAPA2	Ral GTPase activating protein catalytic alpha subunit 2	-3.31	0.84
CCNA2	cyclin A2	-3.31	0.55
VILL	villin like	-3.30	0.83
SSRP1	structure specific recognition protein 1	-3.30	0.82
GGH	gamma-glutamyl hydrolase	-3.30	0.65
SMYD2	SET and MYND domain containing 2	-3.30	0.81

*UP*

<b>Gene ID</b>	<b>Gene Name</b>	<b>SAM Score(d)*</b>	<b>Fold Change</b>
SLAMF7	SLAM family member 7	5.56	2.35
FLVCR2	feline leukemia virus subgroup C cellular receptor family member 2	5.01	1.58
SSBP4	single stranded DNA binding protein 4	4.81	1.29
GNGT2	G protein subunit gamma transducin 2	4.71	1.41
SQLE	squalene epoxidase	4.64	1.81
PNMA1	paraneoplastic Ma antigen 1	4.58	1.52
ADA	adenosine deaminase	4.55	1.75
VAMP5	vesicle associated membrane protein 5	4.49	1.61
IL21R	interleukin 21 receptor	4.48	1.49
RUNX3	runt related transcription factor 3	4.47	1.62
LHFPL2	lipoma HMGIC fusion partner-like 2	4.44	2.03
MTSS1	MTSS1, I-BAR domain containing	4.38	1.43
TCN2	transcobalamin 2	4.25	1.45
CTSL	cathepsin L	4.22	1.87
TMEM51	transmembrane protein 51	4.22	1.71
CHST7	carbohydrate sulfotransferase 7	4.20	1.68
CCR5	C-C motif chemokine receptor 5	4.20	1.95
NECTIN2	nectin cell adhesion molecule 2	4.13	1.25
HEG1	heart development protein with EGF like domains 1	4.08	1.51
SIPA1L2	signal induced proliferation associated 1 like 2	4.07	1.34
TRAF5	TNF receptor associated factor 5	4.05	1.36
DNASE2	deoxyribonuclease 2, lysosomal	4.05	1.60
INSIG1	insulin induced gene 1	4.01	1.69

RRAS	related RAS viral oncogene homolog	4.01	1.48
IFITM1	interferon induced transmembrane protein 1	4.00	2.52
PTCH1	patched 1	3.99	1.12
ISG20	interferon stimulated exonuclease gene 20	3.98	1.68
LOC152225	uncharacterized LOC152225	3.97	1.43
PIM1	Pim-1 proto-oncogene, serine/threonine kinase	3.96	1.58
IFNGR2	interferon gamma receptor 2	3.95	1.34
CD9	CD9 molecule	3.94	2.26
PRRG2	proline rich and Gla domain 2	3.94	1.20
LFNG	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	3.92	1.37
GAS2L1	growth arrest specific 2 like 1	3.91	1.31
DHRS9	dehydrogenase/reductase 9	3.91	1.77
PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	3.91	1.49
CTSV	cathepsin V	3.88	1.36
MAP3K7CL	MAP3K7 C-terminal like	3.87	1.94
EPSTI1	epithelial stromal interaction 1	3.87	2.05
STAT4	signal transducer and activator of transcription 4	3.83	1.40
AMPD1	adenosine monophosphate deaminase 1	3.82	1.72
CASP5	caspase 5	3.79	1.64
MARCO	macrophage receptor with collagenous structure	3.79	1.47
CD40	CD40 molecule	3.78	1.29
SELM	selenoprotein M	3.78	1.55
CACNA1A	calcium voltage-gated channel subunit alpha 1 A	3.77	1.25
ZDHHC23	zinc finger DHHC-type containing 23	3.76	1.24
HIVEP2	human immunodeficiency virus type I enhancer binding protein 2	3.75	1.34
LINC00847	long intergenic non-protein coding RNA 847	3.74	1.42
IDI1	isopentenyl-diphosphate delta isomerase 1	3.72	1.27
PRORS1P	prolyl-tRNA synthetase associated domain containing 1, pseudogene	3.72	1.22
GJA3	gap junction protein alpha 3	3.71	1.16
ADGRE2	adhesion G protein-coupled receptor E2	3.71	1.50
TESK1	testis-specific kinase 1	3.70	1.21
LAP3	leucine aminopeptidase 3	3.69	1.44
MLLT11	myeloid/lymphoid or mixed-lineage leukemia; translocated to, 11	3.68	1.31
SLC16A6	solute carrier family 16 member 6	3.67	1.50
EIF5A2	eukaryotic translation initiation factor 5A2	3.66	1.29
PIK3IP1	phosphoinositide-3-kinase interacting protein 1	3.64	1.21
IFI27	interferon alpha inducible protein 27	3.62	3.13
ERGIC1	endoplasmic reticulum-golgi intermediate compartment 1	3.60	1.25
CXCL11	C-X-C motif chemokine ligand 11	3.60	2.01

\*Ratio of change in gene expression to standard deviation.

**Supplemental Table 5:** IL-21 expression by BM microenvironment cells.

	<b>-ΔCt</b>
BM MSCs	ND
CD14 <sup>+</sup> cells	ND
CD138 <sup>+</sup> cells	ND
CD3 <sup>+</sup> cells	-8,3
Activated CD3 <sup>+</sup> cells	-1
CD8 <sup>+</sup> cells	ND
CD4 <sup>+</sup> cells	-8,6

Abbreviation: ND=not detectable

## **LEGENDS OF SUPPLEMENTAL FIGURES**

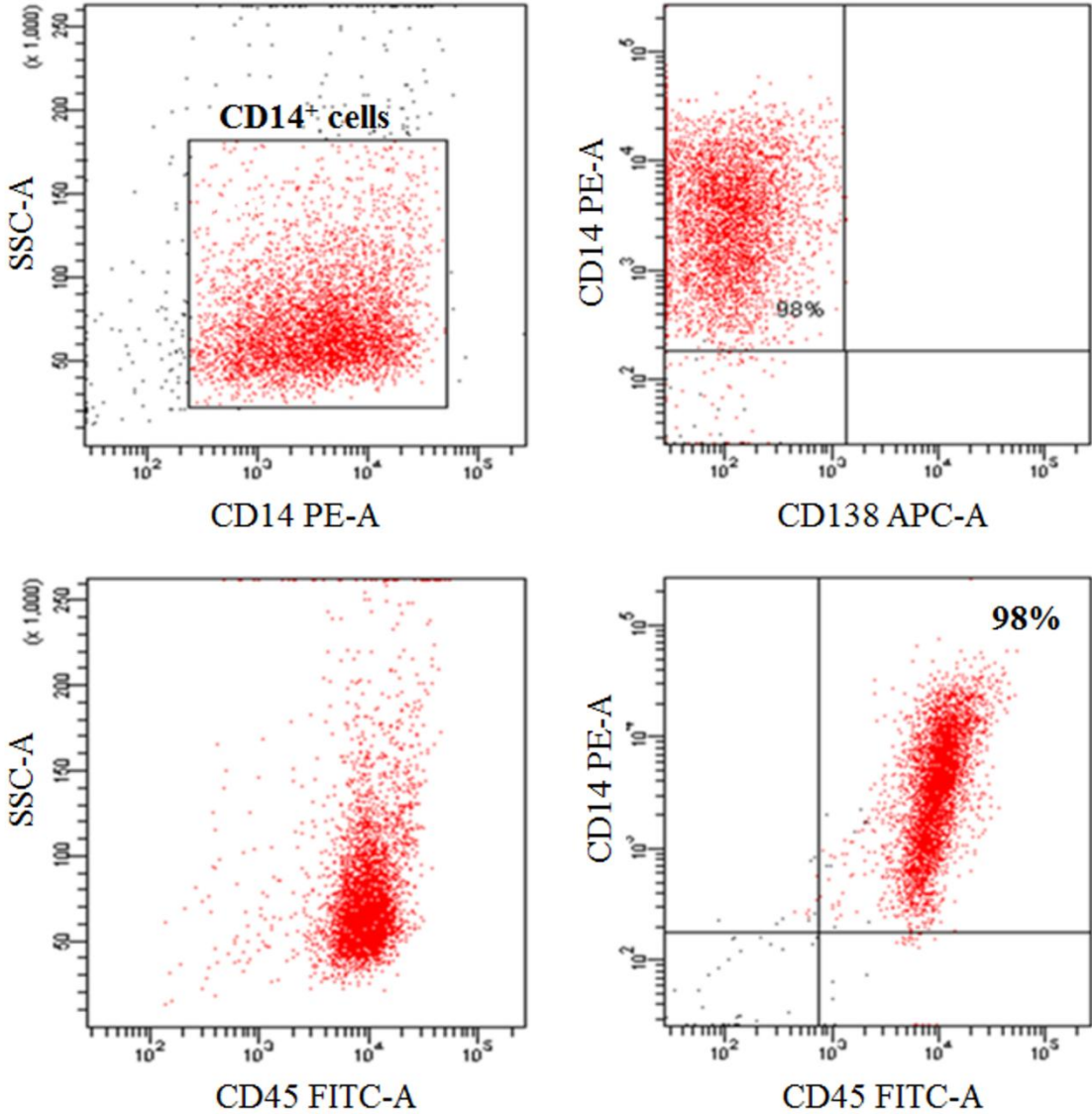
**Supplemental Figure 1: BM monocytes purity.** Representative flow cytometry plots to assess the purity of BM monocytes after purification with anti-CD14 Ab conjugated microbeads. Monocytes were identified as CD14<sup>+</sup>CD45<sup>+</sup>CD138<sup>-</sup> cells.

**Supplemental Figure 2: Monocyte sub-population sorting.** Example of flow cytometry analysis with anti-human CD16 and anti-human CD14, showing the two sub-population of monocytes before (A) and after (B, C) sorting. Purity of CD14<sup>+</sup>CD16<sup>+</sup> (B) and CD14<sup>+</sup>CD16<sup>-</sup> (C) cells.

**Supplemental Figure 3: Gene expression profiling of purified BM CD14<sup>+</sup> cells from patients with different monoclonal gammopathies.** (A) Heatmap of the differentially expressed genes resulted from the multiclass Significant Analysis of Microarrays (SAM) of the monocytes samples clustered according to the diagnosis of the patients. Heatmaps of the differentially expressed genes arisen from the supervised SAM analyses comparing BM CD14<sup>+</sup> cells from MM *versus* SMM (B) or active MM *versus* asymptomatic forms (MGUS plus SMM) (C).

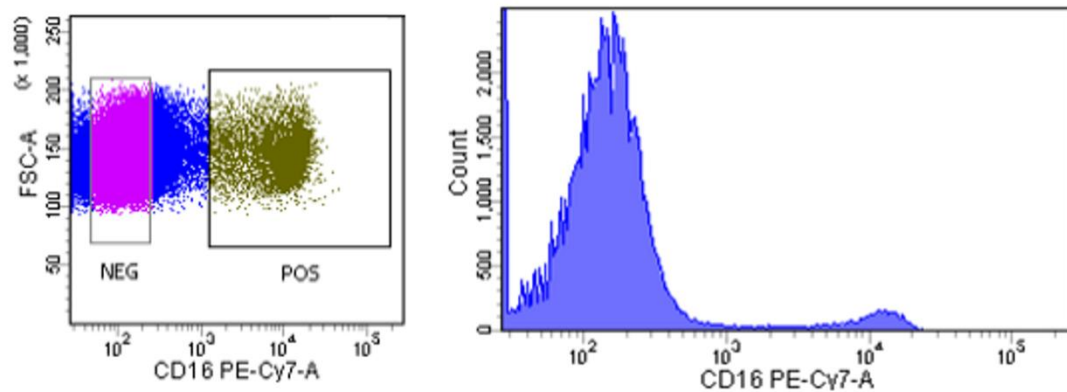
SUPPLEMENTAL FIGURES

Supplemental Figure 1

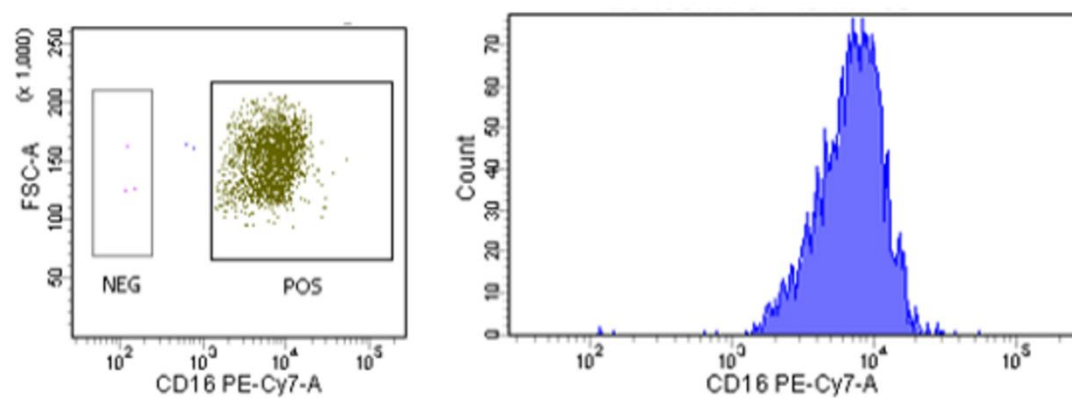


## Supplemental Figure 2

**A**



**B**



**C**

