SUPPLEMENTARY APPENDIX

Gene-expression and in vitro function of mesenchymal stromal cells are affected in juvenile myelomonocytic leukemia

Friso G.J. Calkoen,[1] Carly Vervat,[1] Else Eising,[2] Lisanne S. Vijfhuizen,[2] Peter-Bram A.C. 't Hoen,[2] Marry M. van den Heuvel-Eibrink,[3,4] R. Maarten Egeler,[1,5] Maarten J.D. van Tol,[1] and Lynne M. Ball[1]

[1]Department of Pediatrics, Immunology, Hematology/Oncology and Hematopoietic Stem Cell Transplantation, Leiden University Medical Center, the Netherlands; [2]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands; [3]Dutch Childhood Oncology Group (DCOG), The Hague, the Netherlands; [4]Princess Maxima Center for Pediatric Oncology, Utrecht, the Netherlands; and [5]Department of Hematology/Oncology and Hematopoietic Stem Cell Transplantation, Hospital for Sick Children, University of Toronto, ON, Canada

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Correspondence: f.g.j.calkoen@lumc.nl

Supplementary data:

Methods for online publication

Patients

Children referred to our center for HSCT were included in this study according to a protocol approved by the institutional review board (P08.001). Bone-marrow of 9 children with JMML was collected prior to treatment initiation. In addition, bone-marrow after HSCT was collected from 5 of these 9 children. The patients were classified following the criteria described by Loh *et al*.([1]) Bone-marrow samples were sent to the JMML-reference center in Freiburg, Germany for genetic analysis. Bone-marrow samples of healthy pediatric hematopoietic stem cell donors (n=10) were used as control group (HC). Informed consent was obtained from the children and/or their parents or guardians. This study was conducted according to the Declaration of Helsinki.([2])

MSC expansion and characterization

MSC were expanded and characterized as previously described.([3]) Briefly, bone-marrow mononuclear cells (MNC) obtained after Ficoll separation were cultured in DMEM (Invitrogen, Paisley, UK) containing 100 U/mL penicillin/100 µg/mL streptomycin (P/S; Invitrogen) and 10% (v/v) fetal bovine serum (FBS; VWR International, Bridgeport, NJ, USA). Non-adherent cells were removed by refreshing medium twice weekly. Upon reaching confluence MSC were harvested and passaged for further expansion. Phenotype (CD73, CD90, CD105 positive; CD3, CD31, CD34, CD45, CD86, HLA-DR negative) and differentiation capacity towards osteoblasts and adipocytes were investigated at passage 2-3 and 5-7, respectively. All but anti-CD105 (Ancell Corporation Bayport, MN) antibodies were derived from Becton Dickinson Biosciences (BD), San Diego, CA, USA.

To investigate common chromosome abnormalities in MSC and malignant cells, interphase fluorescence in situ hybridization (FISH) for chromosome 7 was performed on MSC from patients with known monosomy 7 using the Vysis LSI D7S486/CEP7 (Abbott Laboratories, Abbott Park, IL,USA) probe.([4]) Chimerism of MSC obtained after HSCT (donor or recipient origin) was studied by variable number of cytosine adenine (CA)-repeat analysis in MSC cultured from bone-marrow harvested after HSCT as previously described.([5]) MSC function was investigated using MSC obtained at passage 3-5 and MSC gene expression was analyzed using MSC obtained at passage 2-3.

Immunomodulatory assays

Effect of MSC on PHA-induced PBMC proliferation

The effect of MSC (30 Gy irradiated) on proliferation of peripheral blood mononuclear cells (PBMC) obtained from adult bloodbank donors (100.000 cells/well) after stimulation with phytohemagglutinin (PHA, PeproTech, London, UK, 2 μg/mL) was analyzed at MSC : PBMC ratios of 1:5 and 1:40. MSC and PBMC were co-cultured in RPMI P/S, 10% (v/v) fetal calf serum (FCS) for 5 days with the addition of ^{3}H-thymidine (1 μCi/well; Perkin Elmer, Wellesley, MA, USA) for the last 16 hours to measure proliferation using a β-counter (Perkin Elmer). Experiments were performed in triplicate.

Effect of MSC on NK-cell activation

The suppressive effect of MSC on NK-cell activation was determined using NK-cells isolated from PBMC of bloodbank donors with manual MACS cell separation technology and negative selection (Miltenyi Biotec, Bergisch Gladbach, Germany). NK-cell purity ($CD56^{+}CD3^{-}$ cells, using anti-CD3-PerCPC5.5 and anti-CD56-APC (Immunotech, Marseille, France)) was over 95%. NK-cells (0.1 x 10^{6}/well) were cultured with (0.02 x 10^{6}/well) or without MSC in the presence of IL-2 (30 IU/mL, Chiron Corporation, Emeryville, CA, USA) for 5 days. NK-cells were harvested and activation was measured by flow cytometry investigating the mean fluorescence intensity (MFI) of DNAM1 (BD), NKp30 (Immunotech) and NKp44 (Immunotech) expression using PE-labelled antibodies.

Effect of MSC on monocyte differentiation

To evaluate the effect of MSC on antigen presenting cells, monocytes were isolated from PBMC of blood bank donors using positive CD14 selection (Miltenyi Biotec) and cultured with IL-4 (40 ng/mL) and GM-CSF (800 IU/mL) (both from Tebu-Bio, Le Perray en Yvelines, France) for 5 days to differentiate towards immature dendritic cells (DC). Cells were harvested or cultured for 2 additional days with IL-4, GM-CSF, IFN-γ (500 U/mL, Boehringer, Mannheim, Germany) and CD40-ligand (0.25 μg/mL Beckman-Coulter, Marseille, France) to generate mature DC. Cells were phenotyped by flow cytometry for the expression of CD14 and CD1a (both antibodies from BD) on day 0, day 5 and day 7 after co-culturing of monocytes and MSC at MSC : monocyte ratios 1:5 or 1:40 or after culturing monocytes without MSC.

In vitro hematopoietic support of MSC

Short-term co-culture assays with MSC and hematopoietic progenitor cells (HPC) were performed to determine the supportive capacity of MSC for HPC maintenance and differentiation. Therefore, HPC were isolated from remaining material of G-CSF mobilized stem cell grafts from healthy transplant donors using CD34 positive selection (Miltenyi). After purification, >90% of selected cells expressed CD34. To investigate the effect of MSC on proliferation of $CD34^+$ cells, short-term cultures of 1000 CD34 selected cells/well without or with MSC (MSC : $CD34^+$ cell ratios 1:1 and 10:1) were performed in Stemspan medium (H3000, StemCell Technologies, Vancouver, Canada) with addition of 1% P/S, stem cell factor (SCF, 100 ng/mL, StemCell Technologies) and Flt3-ligand (Flt3-L, 100 ng/mL, StemCell Technologies), because SCF and Flt3-L are not produced by MSC. To investigate the effect of MSC on differentiation of $CD34^+$ cells, cultures were initiated with $10x10^3$ $CD34^+$ cells at a $CD34^+$ cell : MSC ratio of 1:5. Half of the culture medium was refreshed with the addition of growth factors on day 4, 7 and 11. Proliferation (day 7) and differentiation (day 7 and 14) were assessed using ^{3}H-thymidine during the last 16 hours or flow cytometry, respectively. Antibodies used for flow cytometry were anti-CD13-PE, anti-CD14-FITC, anti-CD33-APC, anti-CD34-PE, anti-CD45-FITC, anti-CD45-Percpc5.5, anti-CD163-PE and anti-CD235a-PE (all antibodies from BD).

To determine a direct effect of MSC on HPC differentiation into colony-forming units (CFU), MSC (30.000 per dish) were added to freshly purified HPC (500 cells/dish) in methylcellulose containing SCF, GM-CSF, IL-3 and EPO (H4434 StemCell Technologies), and cultured for 14 days (CFU-assay). Colonies were scored by two independent observers according to standard guidelines for the definition of CFU-GEMM, BFU-e, CFU-GM, CFU-G and CFU-M. Results are depicted as the average of duplicate dishes. After scoring of colonies in the CFU-assay, cells were harvested and phenotyped for the expression of CD45, CD14 and CD235a by flow cytometry as described above.

Gene expression

Total RNA was isolated from MSC obtained at passage 2-3 using a Qiagen RNeasy Minikit (Qiagen, Hilden, Germany). mRNA was profiled using Deep-SAGE sequencing using Illumina technology.([6]) CATG was added to the 5' end of the 17 base pair sequences obtained. Data were mapped against the UCSC hg19 reference genome using Bowtie for Illumina (version 1.1.2) with the permission of one mismatch and suppression of reads if more than one best match existed. Tags aligned to the same gene were summed for further

analysis. Gene information was added to the sequences with the biomaRt package in R (version 2.16.0).

Expression of genes of interest was validated using independent biological samples by RT-qPCR after generation of cDNA (cDNA synthesis kit, Roche, Basel, Switzerland) using the listed primers (Supplementary Table S1), as previously described.([6]) Expression levels were calculated relative to expression of the housekeeping genes *GAPDH* and *HPRT1*.

Statistical analysis

Graphpad 6 (Prism, La Jolla, CA) was used for data-analysis. Mann-Whitney and Wilcoxon matched-pairs signed rank tests were performed to compare different groups in functional assays. Validation of gene-expression amongst the different groups was compared using Mann Withney tests. Differential gene expression analysis was performed using the following data analysis packages in R (version 2.15.0): EdgeR (version 3.2.4) for data normalization, Globaltests (version 5.12.0) for gene-ontology, and Limma (version 3.16.7) for correction of multiple testing performed according to Benjamini and Hochberg.([7-10]) STRING version 9.1 software was used for analysis of protein interaction (string-db.org).([11]) Adjusted *p*-values <0.05 were considered statistically significant.

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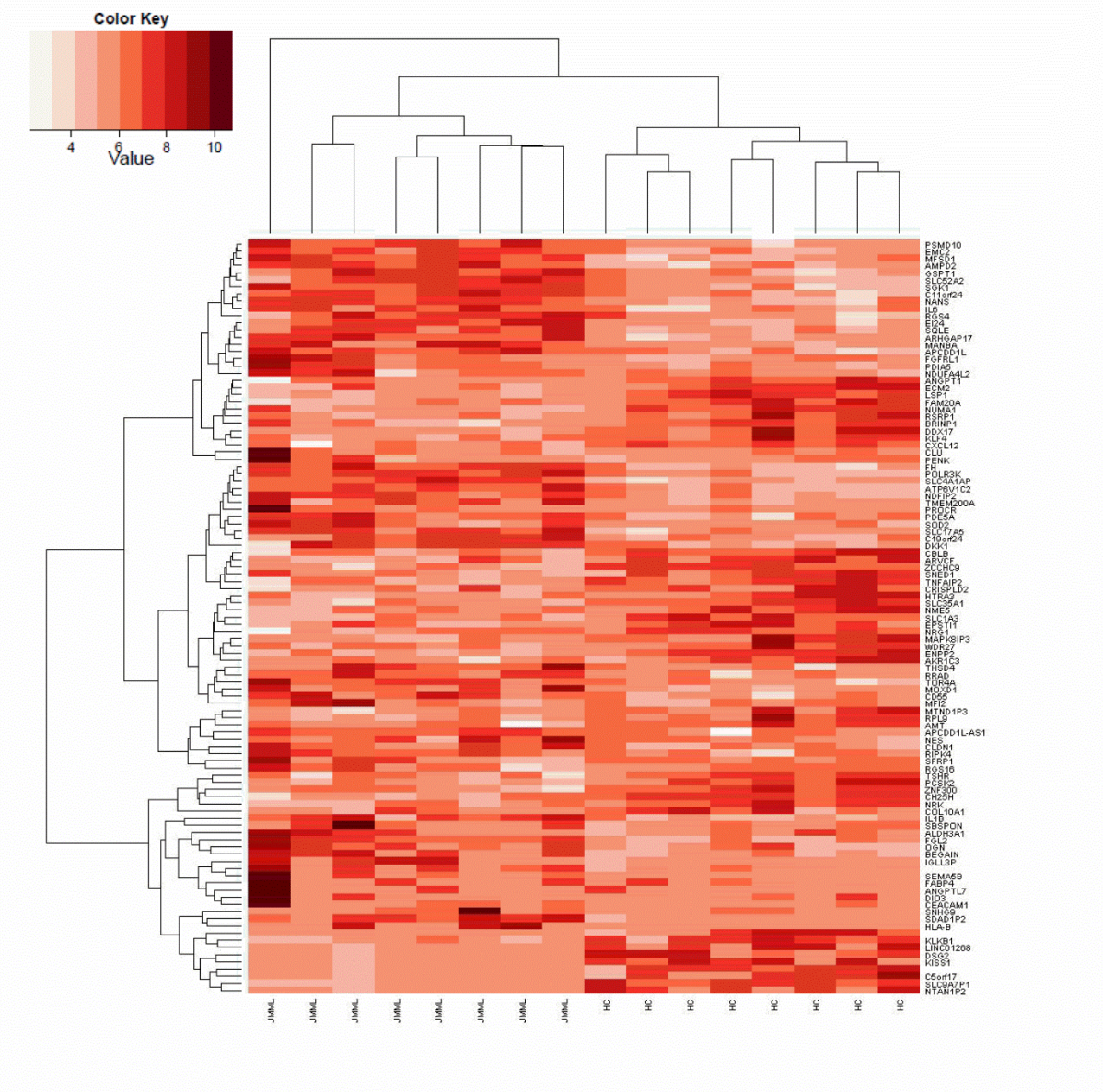
Supplementary Figure 1



Supplementary Figure 1. **JMML and healthy control (HC) MSCs have a different gene-expression profile.** In this heat-map the 100 differentially expressed genes detected by Deep-SAGE sequencing are depicted demonstrating clustering of healthy control (HC) derived bone-marrow (BM) MSC (n=8) and MSCs derived from BM of JMML patients at diagnosis (n=8). Darker colors correspond with increased expression.

Supplementary Table 1. Primer design for RT-PCR

ENPP2-forward	CAGCATCATCACCAGCTGTC
ENPP2-reverse	ATTGCAGCTCTCCTCGTTGT
DKK1-forward	TCCGAGGAGAAATTGAGGAA
DKK1-reverse	CCTGAGGCACAGTCTGATGA
MOXD1-forward	TGCTGAGTGGTCGATTCAAG
MOXD1-reverse	TGCAGGGAAGAGGAAGAAGA
DDX17-forward	TCACAGAGCTCTAGCCAGCA
DDX17-reverse	CAGTCTGCCCCATGTAACCT
APCDD1L-forward	GCAGCTCAGCTTTCCTGAGT
APCDD1L-reverse	CCCGGGAAAACTGGATTTAT
CXCR7-forward	GGCTATGACACGCACTGCTA
CXCR7-reverse	CTCATGCACGTGAGGAAGAA
CXCL12-forward	AGAGCCAACGTCAAGCATCT
CXCL12-reverse	CTTTAGCTTCGGGTCAATGC
IL-6-forward	GAAAGCAGCAAAGAGGCACT
IL-6-reverse	TTTCACCAGGCAAGTCTCCT
TNFAIP2-forward	CCTATTGCCGTGACAGGTTT
TNFAIP2-reverse	CTCCAGAAGGAGTGCAGGAC
HPRT1-forward	TGACACTGGCAAAACAATGCA
HPRT1-reverse	GGTCCTTTTCACCAGCAAGCT
GAPDH-forward	GGCCTCCAAGGAGTAAGACC
GAPDH-reverse	AGGGGAGATTCAGTGTGGTG

Supplementary Table 2. Differential expression between healthy control and JMML MSC

Ensembl gene id	Gene symbol	*p*-value	FDR	Average expression Healthy control	Average expression JMML	Analyzed[1]	Function[2]
ENSG00000130592	LSP1	4.67E-10	8.14E-06	1483	479	No available primer set	Adhesion
ENSG00000136960	ENPP2	5.65E-10	8.14E-06	365	168	Yes	Cell proliferation, chemotaxis
ENSG00000127951	FGL2	2.02E-09	1.94E-05	4	111	<100	Unknown
ENSG00000125851	PCSK2	1.91E-07	1.38E-03	206	7	<100	Protein processing
ENSG00000185215	TNFAIP2	3.52E-07	1.71E-03	479	154	Yes	Inflammation
ENSG00000138135	CH25H	3.57E-07	1.71E-03	66	6	<100	Cholesterol and lipid metabolism
ENSG00000010610	CD4	5.18E-07	2.13E-03	85	11	<100	Immunology
ENSG00000125538	IL1B	8.59E-07	3.09E-03	3	56	<100	Inflammation
ENSG00000230750	SDAD1P2	9.63E-07	3.09E-03	0	10	<100	Pseudogene
ENSG00000164764	SBSPON	1.88E-06	5.38E-03	1	89	<100	Polysaccharide binding
ENSG00000107984	DKK1	2.05E-06	5.38E-03	268	783	Yes	WNT inhibitor
ENSG00000079931	MOXD1	2.41E-06	5.79E-03	130	414	Yes	Copper binding, dopamine activity
ENSG00000065485	PDIA5	4.44E-06	9.31E-03	757	1729	No function	Unknown
ENSG00000226920		4.52E-06	9.31E-03	9	0	<100	Unknown
ENSG00000100201	DDX17	6.27E-06	1.20E-02	3361	1300	Yes	RNA processing
ENSG00000198768	APCDD1L	6.68E-06	1.20E-02	683	1265	Yes	Membrane protein
ENSG00000183092	BEGAIN	7.44E-06	1.26E-02	1	24	<100	Neuronal cell body
ENSG00000143882	ATP6V1C2	1.01E-05	1.62E-02	406	710	No	ATPase
ENSG00000123500	COL10A1	1.23E-05	1.79E-02	142	4	<100	Ossification
ENSG00000138735	PDE5A	1.24E-05	1.79E-02	279	667	No	Phosphodiesteras
ENSG00000108950	FAM20A	1.39E-05	1.91E-02	1248	461	No function	Unknown
ENSG00000082684	SEMA5B	1.73E-05	2.14E-02	0	14	<100	Axon growth
ENSG00000079215	SLC1A3	1.77E-05	2.14E-02	312	79	<100	Neurotransmission
ENSG00000181195	PENK	1.78E-05	2.14E-02	1547	18076	Based on one outlier	Neuropeptide activity
ENSG00000133106	EPSTI1	2.40E-05	2.76E-02	219	63	<100	Epithelial stroma interaction
ENSG00000239648	MTND1P3	2.69E-05	2.98E-02	127	24	<100	Pseudogene
ENSG00000206066	IGLL3P	3.00E-05	3.14E-02	0	9	<100	Pseudogene
ENSG00000234745	HLA-B	3.05E-05	3.14E-02	0	12	<100	Immunology
ENSG00000196139	AKR1C3	3.23E-05	3.21E-02	423	114	No	Aldo/keto reductase
ENSG00000118515	SGK1	3.55E-05	3.41E-02	1083	1929	No	Stress response
ENSG00000170323	FABP4	3.83E-05	3.49E-02	1	76	<100	Fatty acid metabolism
ENSG00000120885	CLU	4.15E-05	3.49E-02	2981	24019	Based on one outlier	Protein binding
ENSG00000163975	MFI2	4.17E-05	3.49E-02	138	522	No	Glycoprotein
ENSG00000114423	CBLB	4.37E-05	3.49E-02	535	244	No	Proto-oncogene
ENSG00000003137	CYP26B1	4.40E-05	3.49E-02	3	43	<100	Lipid metabolism

ENSG00000198113	TOR4A	4.52E-05	3.49E-02	63	186	<100	Protein folding
ENSG00000254366		4.58E-05	3.49E-02	11	0	<100	Unknown
ENSG00000170801	HTRA3	4.59E-05	3.49E-02	171	57	<100	Protease
ENSG00000164484	TMEM200A	5.36E-05	3.85E-02	359	671	No function	Unknown
ENSG00000227825	SLC9A7P1	5.36E-05	3.85E-02	5	0	<100	Pseudogene
ENSG00000255198	SNHG9	5.48E-05	3.85E-02	0	21	<100	RNA gene
ENSG00000196352	CD55	7.09E-05	4.78E-02	142	281	No	Complement activation
ENSG00000104332	SFRP1	7.12E-05	4.78E-02	105	795	No	WNT pathway
ENSG00000095380	NANS	8.43E-05	5.53E-02	1821	2588	No	
ENSG00000248874	C5orf17	8.97E-05	5.54E-02	8	0	No	
ENSG00000236318		9.17E-05	5.54E-02	0	12	No	
ENSG00000118855	MFSD1	9.37E-05	5.54E-02	1048	1467	No	
ENSG00000161980	POLR3K	9.42E-05	5.54E-02	477	703	No	
ENSG00000079385	CEACAM1	1.03E-04	5.89E-02	0	16	No	
ENSG00000046604	DSG2	1.04E-04	5.89E-02	6	0	No	
ENSG00000154188	ANGPT1	1.12E-04	5.95E-02	1424	668	No	
ENSG00000171819	ANGPTL7	1.14E-04	5.95E-02	1	46	No	
ENSG00000166592	RRAD	1.15E-04	5.95E-02	80	174	No	
ENSG00000101000	PROCR	1.15E-04	5.95E-02	379	1050	No	
ENSG00000106823	ECM2	1.16E-04	5.95E-02	1290	542	No	
ENSG00000078725	BRINP1	1.20E-04	6.05E-02	1544	510	No	
ENSG00000109323	MANBA	1.30E-04	6.47E-02	607	948	No	
ENSG00000091483	FH	1.59E-04	7.73E-02	539	762	No	
ENSG00000117152	RGS4	1.63E-04	7.73E-02	1369	3147	No	
ENSG00000104412	EMC2	1.67E-04	7.73E-02	1010	1378	No	
ENSG00000140750	ARHGAP17	1.67E-04	7.73E-02	649	918	No	
ENSG00000187720	THSD4	1.70E-04	7.73E-02	85	209	No	
ENSG00000106809	OGN	1.72E-04	7.73E-02	4	57	No	
ENSG00000010278	CD9	1.77E-04	7.78E-02	266	748	No	
ENSG00000145908	ZNF300	1.80E-04	7.78E-02	45	8	No	
ENSG00000119899	SLC17A5	1.86E-04	7.78E-02	246	370	No	
ENSG00000116337	AMPD2	1.87E-04	7.78E-02	919	1268	No	
ENSG00000185803	SLC52A2	1.87E-04	7.78E-02	1303	1840	No	
ENSG00000171067	C11orf24	1.89E-04	7.78E-02	1874	2677	No	
ENSG00000117616	RSRP1	1.97E-04	8.02E-02	1894	730	No	
ENSG00000131732	ZCCHC9	2.01E-04	8.04E-02	369	188	No	
ENSG00000143333	RGS16	2.07E-04	8.17E-02	50	311	No	
ENSG00000165409	TSHR	2.13E-04	8.29E-02	66	10	No	
ENSG00000197406	DIO3	2.21E-04	8.50E-02	1	37	No	

ENSG00000103342	GSPT1	2.35E-04	8.90E-02	1312	1835	No
ENSG00000138834	MAPK8IP3	2.42E-04	9.05E-02	325	137	No
ENSG00000228300	C19orf24	2.51E-04	9.20E-02	246	376	No
ENSG00000112096	SOD2	2.68E-04	9.66E-02	247	446	No
ENSG00000127418	FGFRL1	2.84E-04	9.98E-02	933	1639	No
ENSG00000137497	NUMA1	2.96E-04	9.98E-02	919	444	No
ENSG00000108602	ALDH3A1	3.01E-04	9.98E-02	3	53	No
ENSG00000250569	NTAN1P2	3.01E-04	9.98E-02	5	0	No
ENSG00000163798	SLC4A1AP	3.06E-04	9.98E-02	425	588	No
ENSG00000036672	USP2	3.08E-04	9.98E-02	21	63	No
ENSG00000164414	SLC35A1	3.15E-04	9.98E-02	110	45	No
ENSG00000145020	AMT	3.15E-04	9.98E-02	145	41	No
ENSG00000183421	RIPK4	3.15E-04	9.98E-02	17	68	No
ENSG00000184465	WDR27	3.15E-04	9.98E-02	303	114	No
ENSG00000104549	SQLE	3.31E-04	1.02E-01	710	1004	No
ENSG00000157168	NRG1	3.36E-04	1.02E-01	325	106	No
ENSG00000170498	KISS1	3.36E-04	1.02E-01	5	0	No
ENSG00000231290	APCDD1L-AS1	3.37E-04	1.02E-01	56	122	No
ENSG00000185633	NDUFA4L2	3.39E-04	1.02E-01	642	1629	No
ENSG00000163682	RPL9	3.55E-04	1.05E-01	56	13	No
ENSG00000164344	KLKB1	3.56E-04	1.05E-01	10	0	No
ENSG00000101843	PSMD10	3.64E-04	1.05E-01	1141	1499	No
ENSG00000136826	KLF4	3.65E-04	1.05E-01	2246	996	No
ENSG00000103196	CRISPLD2	3.67E-04	1.05E-01	749	223	No
ENSG00000149547	EI24	3.90E-04	1.10E-01	732	988	No
ENSG00000112981	NME5	4.01E-04	1.12E-01	169	62	No
ENSG00000102471	NDFIP2	4.07E-04	1.13E-01	357	515	No
ENSG00000099889	ARVCF	4.14E-04	1.14E-01	495	237	No
ENSG00000162804	SNED1	4.20E-04	1.14E-01	621	246	No
ENSG00000123572	NRK	4.28E-04	1.14E-01	31	3	No
ENSG00000227502	LINC01268	4.28E-04	1.14E-01	5	0	No
ENSG00000132688	NES	4.40E-04	1.16E-01	58	179	No
ENSG00000163347	CLDN1	4.44E-04	1.17E-01	22	77	No
ENSG00000184924	PTRHD1	4.54E-04	1.17E-01	1405	1880	No
ENSG00000198682	PAPSS2	4.57E-04	1.17E-01	2291	3753	No
ENSG00000164106	SCRG1	4.77E-04	1.20E-01	1223	2416	No
ENSG00000143248	RGS5	4.78E-04	1.20E-01	63	221	No
ENSG00000229729		4.88E-04	1.20E-01	5	0	No
ENSG00000148450	MSRB2	4.89E-04	1.20E-01	360	182	No

ENSG00000187514	PTMA	4.96E-04	1.21E-01	210	60	No
ENSG00000139874	SSTR1	5.04E-04	1.21E-01	5	35	No
ENSG00000147408	CSGALNACT1	5.05E-04	1.21E-01	53	238	No
ENSG00000184408	KCND2	5.38E-04	1.28E-01	7	0	No
ENSG00000114857	NKTR	5.65E-04	1.33E-01	623	302	No
ENSG00000170266	GLB1	5.76E-04	1.33E-01	1040	1386	No
ENSG00000105248	CCDC94	5.76E-04	1.33E-01	1	9	No
ENSG00000155363	MOV10	5.80E-04	1.33E-01	180	80	No
ENSG00000105426	PTPRS	5.92E-04	1.34E-01	401	210	No
ENSG00000196616	ADH1B	5.94E-04	1.34E-01	18	238	No
ENSG00000130600	H19	5.99E-04	1.34E-01	92	452	No
ENSG00000136244	IL6	6.14E-04	1.36E-01	1658	2495	Yes
ENSG00000125430	HS3ST3B1	6.21E-04	1.37E-01	122	49	No
ENSG00000110328	GALNT18	6.31E-04	1.38E-01	90	192	No
ENSG00000179091	CYC1	6.43E-04	1.39E-01	2716	3739	No
ENSG00000174109	C16orf91	6.45E-04	1.39E-01	220	310	No
ENSG00000129250	KIF1C	6.49E-04	1.39E-01	945	1228	No
ENSG00000254187		6.58E-04	1.39E-01	0	5	No
ENSG00000261857	MIA	6.65E-04	1.40E-01	8	62	No
ENSG00000179403	VWA1	6.78E-04	1.41E-01	227	96	No
ENSG00000251349	MSANTD3-TMEF	6.78E-04	1.41E-01	275	388	No
ENSG00000087494	PTHLH	6.92E-04	1.42E-01	17	62	No
ENSG00000185608	MRPL40	6.98E-04	1.43E-01	799	1038	No
ENSG00000258498	DIO3OS	7.05E-04	1.43E-01	4	72	No
ENSG00000171813	PWWP2B	7.41E-04	1.49E-01	159	69	No
ENSG00000150627	WDR17	7.72E-04	1.54E-01	1	15	No
ENSG00000102760	RGCC	7.75E-04	1.54E-01	56	127	No
ENSG00000231231	LINC01423	7.84E-04	1.55E-01	72	8	No
ENSG00000121039	RDH10	7.96E-04	1.56E-01	136	290	No
ENSG00000154174	TOMM70A	8.16E-04	1.59E-01	814	1047	No
ENSG00000184232	OAF	8.20E-04	1.59E-01	188	294	No
ENSG00000177542	SLC25A22	8.33E-04	1.59E-01	277	391	No
ENSG00000186469	GNG2	8.38E-04	1.59E-01	108	196	No
ENSG00000145337	PYURF	8.47E-04	1.59E-01	2593	3524	No
ENSG00000021762	OSBPL5	8.56E-04	1.59E-01	171	75	No
ENSG00000156042	CFAP70	8.59E-04	1.59E-01	87	12	No
ENSG00000153291	SLC25A27	8.60E-04	1.59E-01	175	59	No
ENSG00000197635	DPP4	8.62E-04	1.59E-01	179	397	No
ENSG00000151929	BAG3	8.90E-04	1.63E-01	658	870	No

ENSG00000145901	TNIP1	9.05E-04	1.65E-01	1059	1366	No
ENSG00000103449	SALL1	9.11E-04	1.65E-01	0	9	No
ENSG00000239382	ALKBH6	9.16E-04	1.65E-01	52	11	No
ENSG00000137965	IFI44	9.20E-04	1.65E-01	155	62	No
ENSG00000229563	LINC01204	9.53E-04	1.70E-01	5	0	No
ENSG00000113643	RARS	9.71E-04	1.71E-01	1951	2540	No
ENSG00000145476	CYP4V2	9.81E-04	1.71E-01	194	87	No
ENSG00000151376	ME3	9.84E-04	1.71E-01	118	45	No
ENSG00000144476	ACKR3	9.89E-04	1.71E-01	618	231	Yes = CXCR7
ENSG00000107159	CA9	9.97E-04	1.71E-01	3	23	No

p- value of the average of HC and JMML-MSC; average reads expressed as the number of reads per 10^6 reads analyzed

[1] Analyzed: this column indicates if the gene was further analyzed using RT-qPCR, based on FDR, expression threshold >100 copies and function.

[2] Function: a short discription, not intended to be exhaustive, of the function based on gene-ontology and www.genecards.org

HC: healthy control; JMML: juvenile myelomonocytic leukemia; FDR: false discovery rate with threshold $p<0.05$

Supplementary Table 3. GO-term analysis

GO-term	Holm's correction	alias	p-value	Covariates
GO:0002252	0.0001	immune effector process	8.65E-09	305
GO:0002253	0.0005	activation of immune response	3.27E-08	235
GO:0050778	0.0005	positive regulation of immune response	3.29E-08	283
GO:0007186	0.0006	G-protein coupled receptor signaling pathway	3.98E-08	520
GO:0043254	0.0011	regulation of protein complex assembly	6.57E-08	152
GO:0007005	0.0015	mitochondrion organization	9.59E-08	201
GO:0051091	0.0015	positive regulation of sequence-specific DNA binding transcription factor activity	9.66E-08	161
GO:0002250	0.0027	adaptive immune response	1.67E-07	148
GO:0002460	0.0028	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	1.77E-07	133
GO:0035270	0.0037	endocrine system development	2.33E-07	120
GO:0002449	0.0038	lymphocyte mediated immunity	2.42E-07	113
GO:0002443	0.0040	leukocyte mediated immunity	2.51E-07	148
GO:0016567	0.0041	protein ubiquitination	2.60E-07	513
GO:0031396	0.0043	regulation of protein ubiquitination	2.70E-07	160
GO:0051260	0.0061	protein homooligomerization	3.84E-07	205
GO:0031625	0.0083	ubiquitin protein ligase binding	5.30E-07	134
GO:0044389	0.0083	small conjugating protein ligase binding	5.30E-07	134
GO:0031398	0.0089	positive regulation of protein ubiquitination	5.64E-07	121

GO: gene-ontology