SUPPLEMENTARY APPENDIX

# *MEIS1* regulates early erythroid and megakaryocytic cell fate

Sabrina Zeddies,[1] Sjoert B. G. Jansen,[1] Franca di Summa,[1] Dirk Geerts,[3] Jaap J. Zwaginga,[4] C. Ellen van der Schoot,[2] Marieke von Lindern,[1] and Daphne C. Thijssen-Timmer[1]

[1]Department of Hematopoiesis, Sanquin Research, Amsterdam; [2]Department of Experimental Immunohematology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center Amsterdam; [3]Department of Pediatric Oncology, Erasmus Medical Center Rotterdam; and [4]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center and the Jon J van Rood Center for Clinical Transfusion Research, Sanquin Blood Supply, Leiden, The Netherlands

 *doi:10.3324/haematol.2014.106567*
*Manuscript received on February 25, 2014. Manuscript accepted on August 1, 2014.*
*Correspondence: d.thijssen@sanquin.nl*

**Supplemental material and methods**

**DNA constructs**

*MEIS1* over expression vectors for both splice variants expressed in hematopoietic cells were generated by amplifying human *MEIS1* cDNA from splice variant1 (*MEIS1B*; NCBI NM_002398.2, 390 amino acid protein) or variant2 (*MEIS1D*; NCBI XM_005264322.1, 465 amino acid protein) devoid of the startcodon from a pcDNA3 vector by polymerase chain reaction (PCR) using the following primers for 30 cycles: *MEIS1*-Fwd: 5'-GAGATCAGATCTGCGCAAAGGTACGACGATC-3', *MEIS1*-Rev: 5'-GAGATCGTCGACTTACATGTAGTGCCACTGCC-3'.

The PCR-product and a GFP-carrying C1-vector (Agilent, Santa Clara, CA) were digested with *Sal*I and *Bgl*II and ligated. The GFP-*MEIS1* fusion was removed by *Nhe*I digestion. GFP was removed from the pRRL.PPT.SFFV.GFP.SIN lentiviral vector by *Cla*I digestion, blunting and *Sal*I digestion. The GFP-*MEIS1* insert and the lentiviral vector were ligated, resulting in lentiviral pRRL.PPT.SFFV.GFP-MEIS1.SIN vectors.

The lentiviral knock down vector SIN.PPT.CMV.GFP.U3*Nhe*1 was a kind gift from N.A Kootstra (Academic Medical Center, Amsterdam, The Netherlands). We cloned in a cassette containing a U6 promoter with the appropriate short hairpin RNA (shRNA) sequence. The shRNA sequence used against *MEIS1* exon 7 (sh72) was 5'-CCCTC TTGGAACAGAGATCAT-3', against the 3'UTR of *MEIS1* (sh68) was 5'-GCCGTGTGTTTAGAAGCCTAA-3' and as a non-targeting shRNA control (shc002) 5'-CAACAAGATGAAGAGCACCAA-3' was used. All vectors were verified by sequencing.

**Lentivirus production**

Lentiviral particles were produced in 293T cells using the third generation system[24]. In brief, constructs encoding *MEIS1* over expression or shRNA constructs were transfected together with a packaging (pMDLgp), reverse transcriptase (pRSV-REV) and envelope (pCMV-VSV-G) encoding plasmids using calcium chloride precipitation. Virus containing supernatant was harvested after transfection and concentrated by ultracentrifugation. Viruspellets were resuspended in CellGro medium (CellGenix, Frankfurt, Germany) and kept frozen until further use. The MOI (multiplicity of infection) was determined as virus particles per cell needed for efficient transduction of $CD34^+$ hematopoietic stem and progenitor cells (HSPCs). The amount of particles needed for 30% transduction or more (Poisson distribution) was taken as MOI =1.

**Human $CD34^+$ cells**

Adult bone marrow $CD34^+$ cells were isolated from bone marrow aspirates obtained after informed consent from patients undergoing cardiac surgery. Material was collected following the protocol for collecting bone marrow for research purposes approved by the medical ethical review board. Peripheral blood stem cells were provided by the Sanquin Laboratory for Cell Therapy and obtained from leukapheresis material of healthy donors treated with G-CSF (2x5 μg/kg/day subcutaneously, Filgastrim, Amgen, Thousand Oaks, CA) or from Hodgkin lymphoma, multiple myeloma or breast cancer patients in disease remission that had been treated with chemotherapy and G-CSF (5-10 μg/kg/day). All

donors and patients signed informed consent for the use of leftover material for research purposes.

**RNA isolation, cDNA synthesis and real-time PCR**

RNA was isolated from transduced $CD34^+$ cells 48 hours after transduction using the Qiagen RNeasy Micro Kit according to the manufacturer's specifications (Qiagen, Venlo, The Netherlands). For MEIS1 expression, cDNA was synthesized using the Superscript III First-Strand Synthesis Kit (Invitrogen, Bleiswijk, The Netherlands) according to the manufacturer's protocol followed by real-time PCR. Real-time PCR was conducted using specific primers for MEIS1B or MEIS1D and SYBR Green reagent (Applied Biosystems, Abingdon, UK). Gene expression was calculated relative to the expression of a housekeeping gene (beta-glucoronidase) using the deltaCT method.

*MEIS1*-specific primer forward: 5'-AGGAACACCTTATAATCATGATGGAC-3'

MEIS1B reverse: 5'-ATATTCATGCCCATTCCACTCATAG-3'

MEIS1D reverse: CTTTGCAGCCCTGGTGCT.

*GUS* (β-glucoronidase):

*GUS* primer forward: 5'-GAAAATATGT GGTTGGAGAGCTCATT-3'

*GUS* primer reverse: 5'-CCGAGTGAAGATCCCCTTTTTA-3'

**Microarray**

For microarray analysis on RNA samples from three independent experiments was conducted at ServiceXS (Leiden, The Netherlands). RNA was isolated using the Qiagen RNeasy Micro kit according to the manufacturers specifications. Samples were subjected

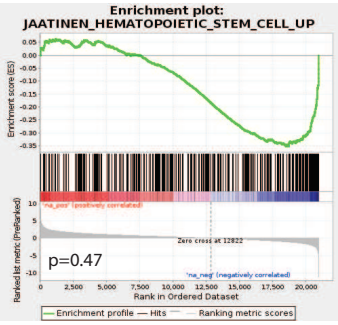
to NuGen labeling and hybridised to the Illumina Human HT-12 v4 expression beadarray (Illumina, San Diego, CA). The data discussed in this manuscript have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE 53263 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53263). **A reviewer link was created to access the dataset before publication: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=crkxmqyidncpxov&acc=GSE53263.** R version 2.15.3 (www.r-project.org) and Bioconductor version 2.11 (www.bioconductor.org) were used for quality control and VSN normalisation of the Illumina arrays. All calculations are run on an OpenSuSE 12.3 linux installation. The Bioconductor library "lumi" is used for loading the Illumina data into R. VSN normalization is applied on the data using the 'lumiN' function. All genes were included in further analysis. Limma library was used for the statistical analysis, with eBayes options used for estimating the 'average' variability over all genes and adjusting high variability genes down and simultanuously adjusts low variability genes up. Benjamini Hochberg FDR values are calculated using the 'multtest' library (function: 'mt.rawp2adjp'), q-values are calculated utilizing the 'qvalue' library. Bioconductor 'lumiHumanAll.db' and the 'GO.db' libraries are used to obtain the most recent annotations.

Gene Set Enrichment Analysis (GSEA) is a Java application provided by the Broad Institute. The t-values are used for the 'GSEAPreranked' test to determine which biological pathways are significant in the underlying microarray experiment. The biological pathways are obtained from the MSigDB database

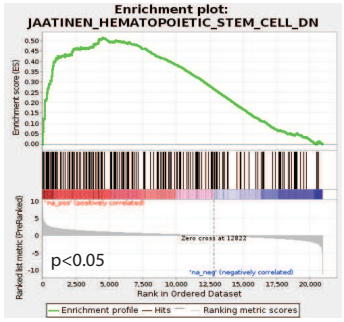
(http://www.broadinstitute.org/gsea/msigdb/index.jsp). Heatmaps are created using the function 'heatmap.2' in the 'gplots' library.

Supplemental figure

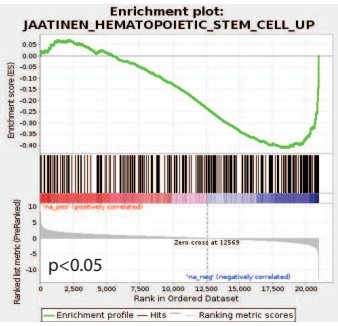
A MEIS1B




B MEIS1B




C MEIS1D




D MEIS1D

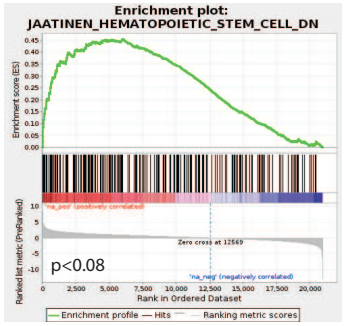


**Figure legends supplementary data**

**Supplemental Table S1. Complete gene expression profile of MPB CD34$^+$ cells after *MEIS1* overexpression.**

Gene expression is shown relative to empty vector after overexpression of either *MEIS1B* or *MEIS1D*.

**Supplemental Table S2. Differential expression of genes overlapping between *MEIS1* overexpression in CD34$^+$ cells and the Haematlas.**

**Supplemental Table S3. GSEA overview of genes found in microarray after MEIS1 overexpression in CD34$^+$ cells and in CD133$^+$ cells**

**Figure S1. GSEA gene enrichment plots comparing expression profiling after *MEIS1* overexpression in CD34$^+$ cells with a transcriptional profile of CB-derived CD133$^+$ cells.** (A) Transcriptional profile of *MEIS1B* overexpression compared to transcripts upregulated in CD133$^+$ cells and (B) downregulated in CD133$^+$ cells. (C) *MEIS1D*-related gene expression profile of CD34$^+$ cells compared to transcripts upregulated in CD133$^+$ cells and (D) downregulated in CD133$^+$ cells.