HES6 knockdown in human hematopoietic precursor cells reduces their *in vivo* engraftment potential and their capacity to differentiate into erythroid cells, B cells, T cells and plasmacytoid dendritic cells

Tamara De Vos,[1,2] Nicole Oatman,[1,2] Lena Boehme,[1] Tom Putteman,[1] Imke Velghe,[1,2] Yana Van Droogenbroeck,[1,2] Stijn De Munter,[1,2] Michaela Cesneková,[1,2] Filip Van Nieuwerburgh,[2,3] Bart Vandekerckhove,[1,2] Jan Philippé[1] and Tom Taghon[1,2]

[1]Department of Diagnostic Sciences, Ghent University; [2]Cancer Research Institute Ghent and [3]Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium

Correspondence: T. Taghon
tom.taghon@ugent.be

Received: April 27, 2023.
Accepted: March 21, 2024.
Early view: April 4, 2024.

https://doi.org/10.3324/haematol.2023.283432

Supplemental Information for: '*HES6* knockdown in human hematopoietic precursor cells reduces their *in vivo* engraftment potential and their capacity to differentiate into erythroid cells, B cells, T cells and plasmacytoid dendritic cells.'

Methods

HES6 and *GATA2* knockdown using lentiviral constructs

In the control SHC002 vector (MISSION pLKO.1, Sigma), the puromycin resistance gene was replaced by eBFP or eGFP as described[1,2] for *HES6* or *GATA2* knockdown, respectively. Three different shRNA sequences targeting *HES6*, of which two were previously validated[3] and two shRNA sequences targeting *GATA2* were selected (Table S2), were cloned in the pLKO.1 backbone and constructs were validated by sequencing. Lentivirus was produced by transfecting HEKT-cells with the transfer plasmid, envelope plasmid VSV-G and packaging plasmid P8.91 using the TransIT lenti Transfection reagent (Sopachem, #MIR6600).

In vitro HES6 knockdown (co)culture experiments

After transduction, $CD34^+lineage^-$ (lin^-: $CD3^-CD14^-CD19^-CD56^-$) BFP^+HSPCs were sorted (BD Biosciences, FACSAriaIII or FacsAria Fusion) with a purity of >95%. (Co)cultures were initiated with $2*10^4$ cells/ml in differentiation specific conditions (Table S1). For the Methocult™ (StemCell, #04445) colony-forming assays, 250 $CD34^+BFP^+$ HSPCs were plated per 35mm dish in duplicates.

RNA isolation, cDNA conversion and knockdown efficiency using qRT-PCR

Part of the $CD34^+lin^-BFP^+$ sorted cells, that were used in single *HES6* knockdown (co)cultures, was used for determining the knockdown efficiency of shRNAs targeting *HES6*. Part of the $CD34^+lin^-BFP^-GFP^+$ sorted cells, that were used in single *GATA2* knockdown cultures, was used for determining the knockdown efficiency of shRNAs targeting *GATA2*. After RNA extraction (Qiagen, #217084) and cDNA conversion (Bio-Rad, #1725038), qPCR was performed using SYBR Green (Bio-Rad, #172-5274) with primers for housekeeping genes[4] and genes of interest (Table S3).

Analysis of colony forming assay

After 10 days, three different colony types were counted based on their morphology (BFU-E/CFU-E: burst-forming unit-erythroid/colony-forming unit-erythroid, CFU-GM: colony-forming unit-granulocyte, macrophage, CFU-GEMM: colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte). After microscopic evaluation of the colonies, the complete culture was collected in a 15 ml tube for subsequent manual cell counting, flow cytometry and cytospin. For the analysis of colony counts, total cell counts and flow cytometry, we calculated the mean of duplicate samples. After counting of the various colonies up to $5*10^4$ cells of each duplicate culture condition were collected and pooled per condition. After washing with PBS, cells were resuspended in 300µl RPMI 1640 (Gibco, #11530586). Of this cell suspension, 5 drops were loaded in the cytospin chamber and spun down for 5 minutes (Tharmac, CellSpin

I). After air-drying, the cytospin slides were automatically stained in May-Grunwald-Giemsa solution and evaluated using the Leica DM 3000 LED microscope (original magnification 500x).

Analysis of previously published single cell RNA-seq

The thymic single cell data used in this study has previously been published and the analysis has been described in detail[5–8]. Markov affinity-based graph imputation of cells (MAGIC)[9] was used to denoise the data and impute dropout values. Cell cycle scoring was conducted using the G2/M and S phase marker genes provided in the Seurat package[10]. Two public bone marrow data sets (Setty et al.[11], https://data.humancellatlas.org/explore/projects/cc95ff89-2e68-4a08-a234-480eca21ce79, only adult peripheral blood and adult bone marrow data) were combined and preprocessed using Pegasus to remove cells will less than 500 or more than 6000 genes and cells with more than 10% mitochondrial reads. Data was batch corrected with bbknn and the UMAP visualization constructed with scanpy. An additional published bone marrow scRNA-seq data set[12] was used to extract developing B cells and annotations were transferred to the bone marrow data using celltypist[13]. Cell cycle scoring for bone marrow data was carried out using scanpy. Box plots, violin plots and dot plots for single cell data were generated with ggplot/Seurat or scanpy.

In vivo reconstitution

All animal experiments were performed with approval and in accordance with the guidelines of the Ethical Committee for Experimental Animals at the Faculty of Medicine and Health Sciences of Ghent University (ECD20-20). CB $CD34^+$ HSPC were isolated and pre-cultured for 1 to 3 days prior to transduction with lentivirus. Four hours after transduction, bulk HSPCs were intrahepatically injected in sublethally irradiated (100cGy) NOD SCID gamma (NSG) mice, aged one to three days old. 8-9 weeks post-injection, the mice were sacrificed by cervical dislocation and single-cell suspensions of liver, bone marrow and thymus were generated as previously described[14].

Flow cytometry

10^4-10^5 cells per sample were used for surface staining. Cells were blocked with anti-human and anti-mouse FcR (Miltenyi, #130-059-901 and #130-092-575) to avoid non-specific binding of antibodies. Subsequently, cells were stained with anti-human monoclonal antibodies, in different panels dependent on how many days cells were cultured and on culture conditions (Table S4). For intracellular staining of CD179b (clone HSL11, PE), up to 10^5 cells were fixated and permeabilized after surface staining using the Foxp3/Transcription Factor Staining Buffer Set according to manufacturer's instructions (eBioscience, #00-5523-00). Cell survival was investigated using the PE Annexin V apoptosis Detection kit I according to manufacturer's instructions (BD Pharmingen, #559763). Analysis of flow cytometric data was done using FlowJo (version 10.6.2).

RNA sequencing

RNA sequencing was performed on sorted control and *HES6* (shRNA1 and shRNA2) shRNA-transduced subpopulations for three donors after four days of culturing CB $CD34^+$ cells in the megakaryocyte-erythroid differentiation assay. Cells were stained with CD34-PerCP-cy5.5, CD71-APC-H7, CD235a-PE, CD41-PE-cy7, CD45-BV510. Within the BFP^+ population we sorted megakaryocytes ($CD41^+$ cells), early and late erythroblasts ($CD71^+$ $CD235^-$ $CD41^-$ and $CD71^+$ $CD235^+$ $CD41^-$). The fourth subset within the BFP^+

population was $CD71^-$ $CD235^-$ $CD41^-$ $CD34^-$. RNA from sorted cells was extracted as described above. The RNA sequencing libraries were prepared using the QuantSeq 3' mRNA-Seq Library Prep FWD kit (Lexogen) using different quantities of input RNA (Table S5) as described previously[15]. Differential gene expression was determined by R package DESeq2, adjusted p-values below 0.05 were considered significant. Heatmaps were generated with the R package "pheatmap". Volcano plots were made using the R package "ggplot2", adjusted p-values below 0.05 and absolute values of log2(foldchange) above 0.6 were considered significant. Enrichment analyses were performed with GSEA.

In vitro HES6-GATA2 double knockdown culture experiments

After transduction with a GFP lentivirus (control or *GATA2* knockdown) and BFP (control or *HES6* knockdown) lentivirus (33% cells in medium supplemented with 3x pre-culture cytokines, 33% GFP lentivirus and 33% BFP lentivirus), two sorting strategies were used (BD Biosciences, FACSAriaIII or FacsAria Fusion). For the control GFP – control BFP condition only $CD34^+lin^-$ BFP^+GFP^+ cells were sorted while for the *HES6* shRNA2 transduced conditions (with either control GFP, *GATA2* shRNA1 or *GATA2* shRNA2) both $CD34^+lin^-$ BFP^+GFP^+ as $CD34^+lin^-$ BFP^-GFP^+ cells were sorted, all with a purity of >95%. Cultures in megakaryocyte/erythroid-lineage supporting conditions (see Table S1) were initiated with maximum 2*10^4 cells/ml.

Cell cycle analysis

Cell cycle analysis was done using the Click-iT™ Plus EdU kit (Invitrogen, #C10646). EdU was added to the cultures for 45 minutes at 10µM (incubation at 37°), afterwards cells were collected and washed in PBS. Part of the cells was taken for intracellular cell cycle analysis according to the manufacturer's protocol, while part of the cells was used to determine BFP expression, without fixation or staining.

Statistical analysis

All statistical analysis was performed using Graphpad Prism (version 8.0.1). Bar graphs represent the mean and the error bar the standard error of the mean (SEM). For analysis of the *in vitro* co-cultures, comparison of percentages, colony numbers or absolute cell counts between control and a knockdown condition was done using non-parametric Wilcoxon signed-rank test, with a two-tailed p-value set at 0,05. For analysis of the *in vivo* experiment, comparison of control and *HES6* knockdown samples (combined shRNA1 and shRNA2) was done using non-parametric Mann Whitney U test, with a two-tailed p-value set at 0,05. Analysis of knockdown efficiencies using qPCR was done using non-parametric paired students t-test, with a two-tailed p-value set at 0,05.

Tables

Table S1. Pre-culture and (co)culture media according to application

(co)culture	Feeder	Pre-culture (before transduction)			(Co)culture		
		Cell culture medium*	**Cytokines****	**CO2 conditions**	**Cell culture medium***	**Cytokines****	**CO2 conditions**
B coculture	MS5	IMDM (Gibco, #12440053) with 10% FCS	SCF and Flt3-L (100ng/ml) TPO (20 ng/ml)	7%	IMDM (Gibco, #12440053) with 5% FCS, 10% hABserum	SCF and IL-7 (20ng/ml)	5%
NK coculture	MS5					IL-15 (10ng/ml) SCF, Flt3-L and IL-7 (5ng/ml)	
Myeloid coculture	MS5					SCF, Flt3-L and TPO (20ng/ml) GM-CSF and GCSF (10ng/ml)	
DC coculture	OP9-lie				Homemade MEMα (ThermoFisher, #12000-014) with 20% FCS	SCF and GM-CSF (20 ng/ml) Flt3-L (100 ng/ml) (Kirkling et al, 2018 [16])	7%
Mgk/ery culture	Feeder-free					TPO (50ng/ml) EPO and SCF (5ng/ml)	
T coculture	OP9-DLL4					SCF, Flt3-L and IL-7 (5ng/ml)	
Semi-solid colony-forming assay	Feeder-free	SFEM II (Stemcell, #09605)		5%	MethoCult™ H4435 Enriched (StemCell, # # 04445)	Supplemented in MethoCult™ medium	5%

** SCF (Peprotech, #300-07), Flt3L (Miltenyi, #130-096-479), TPO (Peprotech, #300-18), GMCSF (#), GCSF (#), EPO (#), IL-15 (#), IL-7 (#)

* supplemented with 100IU/ml penicillin, 100µg/ml streptomycin and 2mM L-glutamine

Table S2. shRNA constructs*

Target for knockdown	**Construct**	**Target sequence**	**Target region of *HES6***
HES6	shRNA1	AGCTTGAACTTGCCACTTCAG	3’ UTR
	shRNA2**	CAGCCTGACCACAGCCCAAAT	Exon 4
	shRNA3**	CGAGCTCCTGAACCATCTGCT	Exon 4
GATA2	shRNA1	CCGGCACCTGTTGTGCAAATT	Exon5
	shRNA2	GTGCAAATTGTCAGACGACAA	Exon5

*sequence for ligation into pLKO vector: 5’ CCGG—21bp sense—CTCGAG—21bp antisense—TTTTTG 3’

**validated by western blot by Xu et al[3]

Table S3. qPCR primers for housekeeping genes

	forward	**Reverse**
SDHA	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACAATGCAA
B2M	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT
ACTB	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAATTCATG
HES6	Ordered primer set from Bio-Rad, #10025636	
GATA2	CAGCAAGGCTCGTTCCTGTTCA	ATGAGTGGTCGGTTCTGCCCAT

Table S4. Flow cytometry staining panels per (co)culture condition

Staining panel	**Used antibodies ***
T	CD45 Percp-cy5.5 (clone HI30), CD34 FITC (clone 581), CD7 Alexa Fluor700 (clone MT701), CD5 PE-cy7 (clone UCHT2), CD4 BV510 (clone RPAT4), CD8β PE (clone 2ST8.5H7), HLA-DR APC-eFluor780 (clone LN3)
B - only surface	CD45 PerCP-cy5.5 (clone HI30), CD19 PE (cone HIB19), CD20 Alexa Fluor 700 (clone 2H7)
B – surface and intracellular	CD45 PerCP-cy5.5 (clone HI30), CD19 FITC (clone HIB19), CD34 APC (clone 581), intracellular CD179b PE (clone HSL11)
DC	CD45 Percp-cy5.5 (clone HI30), HLA-DR APC-eFluor780 (clone LN3), CD45RA APC (clone HI100), CD4 PE (clone RPAT4), CD123 FITC (clone AC145)
NK	CD45 FITC (clone 5B1), CD94 Percp-cy5.5 (clone DX22), CD56 PE (clone 5.1H11)
Methocult	CD45 BV510 (clone HI30), CD235a PE (clone KC16), CD15 PE (clone HI98)
Myeloid	CD45 PerCP-cy5.5 (clone HI30), CD14 FITC (clone 63D3), CD15 PE (clone HI98)
Mgk/ery	CD45 Percp-cy5.5 (clone HI30), CD42b APC (clone HIP1), CD71 APC-H7 (clone MA712), CD235a PE (clone KC16), CD41 PE-Cy7 (clone P2)
Myeloid**	hCD45 Percp-cy5.5 (clone HI30), CD33 APC (clone P67.6)
NK**	hCD45 Percp-cy5.5 (clone HI30), CD3 APC-cy7 (clone UCHT1), CD56 PE (clone 5.1H11)
Mgk/ery**	hCD45 Percp-cy5.5 (clone HI30), CD41 PE-Cy7 (clone P2), CD71 APC-H7 (clone MA712)
B (surface)**	hCD45 Percp-cy5.5 (clone HI30), CD19 PE (cloneHIB19), CD20 Alexa Fluor 700 (clone 2H7)
DC***	hCD45 Percp-cy5.5 (clone HI30), CD123 PE-cy7 (clone 6H6), CD45RA FITC (clone HI100), CD4 PE (clone RPAT4), HLA-DR APC-eFluor780 (clone LN3)

* in all panels Zombie Red™ Fixable Viability Kit (Biolegend, #423109) was used (1/1000 dilution) as a live/dead marker

** analysis of bone marrow samples of *in vivo* experiment

*** analysis of liver samples of *in vivo* experiment

Table S5. RNA input for RNA-seq per cell type

Cell type	**Input RNA (ng)**	**Exceptions (ng)**
$CD41^+$ (megakaryocytes)	9,35	
$CD34^-$ precursors	109,2	
$CD71^+CD235^-$ (early erythroblasts)	12,75	
$CD71^+CD235^+$ (late erythroblasts)	11,55	Donor 2 shRNA2: 4,4

Table S6. RNA-seq results: up-regulated genes (HES6 knockdown vs control) in $CD34^-$ precursors

log2FoldChange	pvalue	padj	symbol	Ensembl ID
1.409407	4.78E-14	2.07E-10	*PKIB*	ENSG00000135549
0.83337	2.09E-13	6.79E-10	*MS4A6A*	ENSG00000110077
1.163096	2.47E-12	6.41E-09	*CD1C*	ENSG00000158481
1.111889	5.67E-10	1.22E-06	*SLC7A11*	ENSG00000151012
0.634699	8.71E-10	1.61E-06	*ARPC3*	ENSG00000111229
1.209556	1.65E-09	2.68E-06	*CLEC5A*	ENSG00000258227
0.805721	2.02E-09	2.92E-06	*PLIN2*	ENSG00000147872
1.178945	7.67E-09	9.94E-06	*MRC1*	ENSG00000260314
0.957715	1.41E-08	1.66E-05	*SESN3*	ENSG00000149212
1.061511	1.85E-08	2E-05	*CLEC10A*	ENSG00000132514
2.067484	5.77E-08	5.75E-05	*PID1*	ENSG00000153823
1.256474	1.19E-07	0.00011	*IFI6*	ENSG00000126709
1.056071	2.23E-07	0.00017	*NCF2*	ENSG00000116701
0.994963	8.52E-07	0.000563	*RCBTB2*	ENSG00000136161
0.842862	1.3E-06	0.000803	*DSC2*	ENSG00000134755
1.332647	3.61E-06	0.00187	*HIC1*	ENSG00000177374
1.02294	4.28E-06	0.002131	*SVIL*	ENSG00000197321
1.934807	5.09E-06	0.002275	*TGM5*	ENSG00000104055
0.925298	7.02E-06	0.002937	*NDRG2*	ENSG00000165795
1.10785	7.31E-06	0.00296	*PDK4*	ENSG00000004799
0.775585	8.26E-06	0.003245	*LY86*	ENSG00000112799
0.986369	9.77E-06	0.003517	*SPRED1*	ENSG00000166068
1.430419	1.21E-05	0.004229	*PLA2G7*	ENSG00000146070
0.717067	1.31E-05	0.004353	*ASGR2*	ENSG00000161944
0.606664	1.47E-05	0.00465	*PPCS*	ENSG00000127125
0.946659	1.58E-05	0.00477	*MS4A7*	ENSG00000166927
0.845829	2.11E-05	0.005823	*SLC36A1*	ENSG00000123643
0.772088	2.79E-05	0.007368	*CTSD*	ENSG00000117984
2.249965	2.97E-05	0.007711	*TSHZ3*	ENSG00000121297
0.732279	3.34E-05	0.008492	*CPVL*	ENSG00000106066
1.54136	4.04E-05	0.010067	*SCN4B*	ENSG00000177098
0.746368	4.16E-05	0.010163	*IL6ST*	ENSG00000134352
1.202772	4.37E-05	0.010482	*RTN1*	ENSG00000139970
1.496493	5.09E-05	0.011586	*BCL2A1*	ENSG00000140379
0.661458	5.25E-05	0.011734	*THEMIS2*	ENSG00000130775
0.992436	5.51E-05	0.012111	*HERC5*	ENSG00000138646
0.950048	6.11E-05	0.013194	*SYT11*	ENSG00000132718
1.957835	8.03E-05	0.017073	*PROCR*	ENSG00000101000
0.697179	9.03E-05	0.01857	*TMEM170B*	ENSG00000205269
1.150539	0.000114	0.02144	*RGS1*	ENSG00000090104
1.521639	0.00012	0.02145	*PARM1*	ENSG00000169116
0.866426	0.000121	0.02145	*GPR82*	ENSG00000171657
1.628033	0.000118	0.02145	*PTGIR*	ENSG00000160013

2.550807	0.000136	0.023907	*VSIG4*	ENSG00000155659
2.854097	0.000139	0.024092	*MGAM*	ENSG00000257335
0.753344	0.00015	0.025345	*PTAFR*	ENSG00000169403
0.650268	0.000153	0.025345	*SLC30A1*	ENSG00000170385
1.238152	0.000151	0.025345	*CCL2*	ENSG00000108691
0.79658	0.000165	0.026371	*AFDN*	ENSG00000130396
1.875664	0.000205	0.030835	*FPR3*	ENSG00000187474
0.780869	0.000222	0.032291	*CPQ*	ENSG00000104324
2.369418	0.000232	0.03266	*IL31RA*	ENSG00000164509
1.926882	0.000232	0.03266	*GPNMB*	ENSG00000136235
1.153811	0.000234	0.03266	*CCNA1*	ENSG00000133101
0.878577	0.000239	0.032913	*MSMO1*	ENSG00000052802
5.16217	0.000293	0.037627	*C1QA*	ENSG00000173372
2.259803	0.000306	0.038526	*FAM171B*	ENSG00000144369
1.712009	0.000312	0.038878	*CMKLR1*	ENSG00000174600
0.636889	0.00037	0.045237	*TSEN34*	ENSG00000170892
0.658788	0.000396	0.0467	*CYRIA*	ENSG00000197872
3.307525	0.000396	0.0467	*OLFM1*	ENSG00000130558
0.814199	0.000409	0.047293	*ZMAT3*	ENSG00000172667

Table S7. RNA-seq results: down-regulated genes (HES6 knockdown vs control) in $CD34^-$ precursors

log2FoldChange	pvalue	padj	symbol	Ensembl ID
-1.12252	5.08E-16	6.59E-12	*TCF4*	ENSG00000196628
-1.88926	5.29E-15	3.43E-11	*MS4A2*	ENSG00000149534
-1.02793	1.4E-07	0.000121	*RUNX2*	ENSG00000124813
-0.68467	1.64E-07	0.000133	*CCDC50*	ENSG00000152492
-2.29414	2.48E-06	0.001458	*BLNK*	ENSG00000095585
-1.79242	3.42E-06	0.001849	*IGLL1*	ENSG00000128322
-0.61076	4.45E-06	0.002139	*TMEM109*	ENSG00000110108
-1.01678	6.31E-06	0.002728	*SLAMF7*	ENSG00000026751
-0.8301	1.25E-05	0.004254	*PRTN3*	ENSG00000196415
-1.07648	1.52E-05	0.004692	*KCNE5*	ENSG00000176076
-0.68315	1.76E-05	0.005174	*EIF4EBP2*	ENSG00000148730
-2.9281	2.07E-05	0.005823	*RUBCNL*	ENSG00000102445
-0.90605	2.46E-05	0.006645	*MYBL2*	ENSG00000101057
-0.72763	4.55E-05	0.010734	*CTSG*	ENSG00000100448
-0.64	5.06E-05	0.011586	*FADS1*	ENSG00000149485
-0.72988	8.66E-05	0.018112	*GUCD1*	ENSG00000138867
-0.93891	9.31E-05	0.018675	*COL24A1*	ENSG00000171502
-0.75543	0.000118	0.02145	*S100A10*	ENSG00000197747
-0.74822	0.000215	0.032094	*HMGN5*	ENSG00000198157
-0.68606	0.000242	0.033087	*PSME2*	ENSG00000100911
-0.65537	0.000263	0.035463	*IRF8*	ENSG00000140968
-0.67468	0.000272	0.036394	*PDLIM1*	ENSG00000107438
-1.33233	0.000395	0.0467	*IGHM*	ENSG00000211899
-0.80743	0.000401	0.046832		ENSG00000279602

Table S8. RNA-seq results: up-regulated genes (HES6 knockdown vs control) in megakaryocytes

log2FoldChange	pvalue	padj	symbol	Ensembl ID
1.663191	6.65E-13	5.93E-09	*CXCL8*	ENSG00000169429
2.48243	3.03E-10	8.44E-07	*SIGLEC6*	ENSG00000105492
1.08181	8.29E-09	1.92E-05	*CYP1B1*	ENSG00000138061
1.448119	1.61E-08	3.19E-05	*CCND2*	ENSG00000118971
0.8203	5.88E-08	0.000102	*COX7C*	ENSG00000127184
1.124102	7.18E-08	0.000111	*SLC22A17*	ENSG00000092096
2.232325	1.33E-07	0.000186	*SNHG19*	ENSG00000260260
1.722667	5.12E-07	0.000648	*C12orf76*	ENSG00000174456
0.811627	1.36E-06	0.001581	*ARPC3*	ENSG00000111229
4.148061	1.63E-06	0.001617	*INHBA*	ENSG00000122641
6.793755	2.75E-06	0.002558	*CRHBP*	ENSG00000145708
1.135222	3.18E-06	0.002606	*SYT11*	ENSG00000132718
1.068378	4.07E-06	0.003092	*KCNQ1OT1*	ENSG00000269821
0.814891	4.22E-06	0.003092	*MALAT1*	ENSG00000251562
6.812312	4.62E-06	0.003131	*HDC*	ENSG00000140287
1.787982	7.77E-06	0.003763	*SH2D2A*	ENSG00000027869
1.070072	7.81E-06	0.003763	*TUBA1A*	ENSG00000167552
1.499381	1.54E-05	0.006141	*ETS1*	ENSG00000134954
3.240239	1.93E-05	0.007482	*TRIM22*	ENSG00000132274
2.586995	2.29E-05	0.008248	*FOSL2*	ENSG00000075426
0.912391	3.01E-05	0.010468	*IRF2BPL*	ENSG00000119669
2.204223	3.98E-05	0.012885		ENSG00000234425
1.292893	4.89E-05	0.014637	*BTG2*	ENSG00000159388
0.771797	5.06E-05	0.014637	*CDK6*	ENSG00000105810
3.734066	5.07E-05	0.014637	*TNFRSF10B*	ENSG00000120889
1.203739	5.52E-05	0.015071	*KLHDC8B*	ENSG00000185909
0.697803	5.49E-05	0.015071	*NEAT1*	ENSG00000245532
1.029351	6.69E-05	0.017593	*IL1B*	ENSG00000125538
1.344376	0.000106	0.025961	*PAPPA*	ENSG00000182752
4.912364	0.000112	0.026865	*SLC7A8*	ENSG00000092068
0.718614	0.00012	0.02835	*SNHG3*	ENSG00000242125
5.898249	0.000123	0.028455	*NEB*	ENSG00000183091
0.662547	0.00014	0.03197	*HGD*	ENSG00000113924
2.311061	0.000145	0.032494	*ANXA1*	ENSG00000135046
1.150767	0.000153	0.03328	*VIM*	ENSG00000026025
0.970715	0.000158	0.033819	*CSF2RB*	ENSG00000100368
3.111682	0.000187	0.038909	*SGK1*	ENSG00000118515
1.711638	0.00019	0.038923	*LSR*	ENSG00000105699
5.841119	0.000225	0.044705	*ANKDD1B*	ENSG00000189045
1.336293	0.000237	0.045248	*BAZ2B*	ENSG00000123636
5.576799	0.000235	0.045248		ENSG00000225173

Table S9. RNA-seq results: down regulated genes (HES6 knockdown vs control) in megakaryocytes

log2FoldChange	pvalue	padj	symbol	Ensembl ID
-1.94036	8.51E-13	5.93E-09	*HEMGN*	ENSG00000136929
-1.88442	5.5E-11	2.56E-07	*HBB*	ENSG00000244734
-1.48478	1.94E-10	6.74E-07	*CD36*	ENSG00000135218
-1.37684	1.48E-06	0.001589	*CTNNAL1*	ENSG00000119326
-1.51214	3.16E-06	0.002606	*HBG1*	ENSG00000213934
-1.34752	4.72E-06	0.003131	*DGKI*	ENSG00000157680
-1.37067	4.96E-06	0.003141	*DYNLT5*	ENSG00000152760
-1.108	5.48E-06	0.00332	*DUSP7*	ENSG00000164086
-0.84544	6.67E-06	0.003572	*HMMR*	ENSG00000072571
-1.91464	6.62E-06	0.003572	*PAPSS2*	ENSG00000198682
-0.73172	6.28E-06	0.003572	*UBE2C*	ENSG00000175063
-4.95073	7.83E-06	0.003763	*FAM178B*	ENSG00000168754
-1.03695	8.9E-06	0.004134	*STK40*	ENSG00000196182
-0.83697	1.06E-05	0.004615	*ACER3*	ENSG00000078124
-1.97517	1.16E-05	0.004908	*LIPH*	ENSG00000163898
-0.69292	1.34E-05	0.005485	*MKI67*	ENSG00000148773
-0.98409	2.31E-05	0.008248	*HBD*	ENSG00000223609
-0.942	2.25E-05	0.008248	*SERPINH1*	ENSG00000149257
-1.07112	3.4E-05	0.011555	*EIF4EBP2*	ENSG00000148730
-0.83445	3.72E-05	0.012323	*CYB5R3*	ENSG00000100243
-0.89808	4.22E-05	0.013362	*DLK1*	ENSG00000185559
-1.65961	4.41E-05	0.013663	*ALKAL2*	ENSG00000189292
-0.89405	5.15E-05	0.014637	*CLEC1B*	ENSG00000165682
-0.61954	6.59E-05	0.017593	*UBE2J1*	ENSG00000198833
-1.24515	6.83E-05	0.017626	*RBPMS2*	ENSG00000166831
-1.16346	0.000101	0.025026	*CCN1*	ENSG00000142871
-0.8008	0.000152	0.03328	*RHAG*	ENSG00000112077
-4.20605	0.000176	0.037131	*LRRC17*	ENSG00000128606
-0.68183	0.000218	0.044055	*ARPC5*	ENSG00000162704
-0.91719	0.000232	0.045248	*PDE5A*	ENSG00000138735

Table S10. RNA-seq results: up-regulated genes (HES6 knockdown vs control) in early erythroblasts

log2FoldChange	pvalue	padj	symbol	Ensembl ID
2.097469	6.41E-40	8.71E-36	*MPO*	ENSG00000005381
2.751566	1.18E-25	5.37E-22	*MS4A3*	ENSG00000149516
0.975316	3.04E-22	1.03E-18	*CELF2*	ENSG00000048740
3.670641	1.45E-17	3.95E-14	*LINC00926*	ENSG00000247982
1.623884	5.22E-16	9.22E-13	*HDC*	ENSG00000140287
2.22121	1.16E-15	1.75E-12	*RNASE2*	ENSG00000169385
2.611048	1.2E-14	1.64E-11	*TSPOAP1*	ENSG00000005379
3.426254	6.31E-14	7.8E-11	*CSTA*	ENSG00000121552
1.329091	9.82E-13	1.11E-09	*HLA-DRA*	ENSG00000204287
1.783753	1.84E-11	1.78E-08	*HLA-DPB1*	ENSG00000223865
2.490429	2.79E-11	2.53E-08	*ATP8B4*	ENSG00000104043
1.617116	3.53E-11	3E-08	*TNFSF13B*	ENSG00000102524
2.316011	1.58E-10	1.26E-07	*ELANE*	ENSG00000197561
1.568268	2.04E-10	1.54E-07	*RFLNB*	ENSG00000183688
3.962926	5.94E-10	4.04E-07	*CD48*	ENSG00000117091
1.83306	7.09E-10	4.25E-07	*SORL1*	ENSG00000137642
2.864895	6.94E-10	4.25E-07	*CLEC12A*	ENSG00000172322
2.728413	7.59E-10	4.29E-07	*SERPINB10*	ENSG00000242550
2.059958	1.19E-09	6.45E-07	*KBTBD11*	ENSG00000176595
0.743683	1.78E-09	9.3E-07	*ACTG1*	ENSG00000184009
1.307296	3.07E-09	1.55E-06	*BASP1*	ENSG00000176788
1.358283	3.59E-09	1.74E-06	*HLA-DPA1*	ENSG00000231389
2.446985	4.86E-09	2.28E-06	*RAB32*	ENSG00000118508
1.527866	5.1E-09	2.31E-06	*PRKCB*	ENSG00000166501
1.220345	5.57E-09	2.44E-06	*HLA-A*	ENSG00000206503
0.943554	7.1E-09	3.01E-06	*LAPTM5*	ENSG00000162511
1.40235	7.89E-09	3.25E-06	*PKM*	ENSG00000067225
1.463545	1.27E-08	4.93E-06	*ATF7IP2*	ENSG00000166669
1.472023	4.43E-08	1.67E-05	*TENT5A*	ENSG00000112773
0.756416	5.01E-08	1.84E-05	*MLC1*	ENSG00000100427
1.412371	7.16E-08	2.46E-05	*SESN3*	ENSG00000149212
1.4018	8.13E-08	2.69E-05	*TUBA1A*	ENSG00000167552
0.688813	9.8E-08	3.17E-05	*ARPC2*	ENSG00000163466
1.841392	1.54E-07	4.87E-05	*MNDA*	ENSG00000163563
2.733461	1.97E-07	6.09E-05	*PRTN3*	ENSG00000196415
2.524546	2.37E-07	7E-05	*SYNE1*	ENSG00000131018
1.939355	2.75E-07	7.63E-05	*SRGN*	ENSG00000122862
2.829713	3.34E-07	8.9E-05	*CLEC5A*	ENSG00000258227
6.954929	3.62E-07	9.45E-05	*CD200*	ENSG00000091972
1.457034	5.54E-07	0.000134	*CAMK1D*	ENSG00000183049
2.09033	5.7E-07	0.000136	*CXCL8*	ENSG00000169429
1.442522	6.15E-07	0.000144	*FUT4*	ENSG00000196371
1.915807	7.01E-07	0.00016	*GFI1*	ENSG00000162676

1.072712	7.37E-07	0.000164	*COTL1*	ENSG00000103187
0.843122	1.01E-06	0.000221	*SERPINE2*	ENSG00000135919
6.801138	1.14E-06	0.000246	*PSMB9*	ENSG00000240065
3.263983	1.26E-06	0.000267	*BMERB1*	ENSG00000166780
0.827916	1.37E-06	0.000282	*GIHCG*	ENSG00000257698
0.768889	1.36E-06	0.000282		ENSG00000244879
1.802476	1.72E-06	0.000348	*SMYD3*	ENSG00000185420
0.994363	1.84E-06	0.000362	*APP*	ENSG00000142192
2.174151	2.04E-06	0.000386	*CYFIP2*	ENSG00000055163
1.153253	2.45E-06	0.000456	*FAM189B*	ENSG00000160767
2.943386	2.51E-06	0.000461	*TIMP2*	ENSG00000035862
6.124717	3.05E-06	0.000553	*ADA2*	ENSG00000093072
2.318926	3.26E-06	0.000582	*CLC*	ENSG00000105205
0.752138	3.59E-06	0.000633	*SNHG8*	ENSG00000269893
1.062842	6.23E-06	0.001019	*PNKD*	ENSG00000127838
2.101905	7.46E-06	0.001179	*TRIM22*	ENSG00000132274
2.416579	7.32E-06	0.001179	*SMIM24*	ENSG00000095932
5.039138	7.41E-06	0.001179	*GDF15*	ENSG00000130513
6.025457	8.15E-06	0.001258	*FAM171B*	ENSG00000144369
2.553982	8.46E-06	0.001276	*IL6R*	ENSG00000160712
0.723074	8.55E-06	0.001276	*HLA-B*	ENSG00000234745
3.496076	8.84E-06	0.001306	*SLC22A17*	ENSG00000092096
3.528654	9.15E-06	0.001322	*IL1RL1*	ENSG00000115602
0.738119	1.07E-05	0.001531	*GATA2*	ENSG00000179348
1.988767	1.1E-05	0.001558	*BEND4*	ENSG00000188848
1.346569	1.12E-05	0.001563	*NDRG1*	ENSG00000104419
1.04302	1.19E-05	0.00161	*DPYSL2*	ENSG00000092964
5.221764	1.22E-05	0.001646	*IL21R*	ENSG00000103522
5.444926	1.33E-05	0.001775	*IQCG*	ENSG00000114473
6.389607	1.38E-05	0.001815	*CEP83-DT*	ENSG00000278916
0.81915	1.4E-05	0.001823	*VAMP8*	ENSG00000118640
4.387756	1.41E-05	0.001823	*SCAT2*	ENSG00000257596
1.509764	1.46E-05	0.001871	*KCNAB2*	ENSG00000069424
2.996023	1.49E-05	0.001888	*SELL*	ENSG00000188404
0.949831	1.52E-05	0.001894	*CD74*	ENSG00000019582
0.769584	1.52E-05	0.001894	*SAMSN1*	ENSG00000155307
2.69743	1.62E-05	0.001985	*ANPEP*	ENSG00000166825
2.117503	1.96E-05	0.002294	*CREBRF*	ENSG00000164463
2.641709	1.92E-05	0.002294	*GSN*	ENSG00000148180
6.297301	1.99E-05	0.002311		ENSG00000270062
1.004938	2.05E-05	0.002355	*ID2*	ENSG00000115738
1.869451	2.07E-05	0.002358	*KCNQ1OT1*	ENSG00000269821
1.613004	2.12E-05	0.002383	*BGLT3*	ENSG00000260629
1.420124	2.2E-05	0.002452	*TPM4*	ENSG00000167460
1.076349	2.49E-05	0.002733	*EMB*	ENSG00000170571
1.326641	2.49E-05	0.002733	*PTGS1*	ENSG00000095303

2.71058	2.55E-05	0.00275	*KIF21B*	ENSG00000116852
4.159856	2.54E-05	0.00275	*FBXL15*	ENSG00000107872
2.294747	2.58E-05	0.002765	*CDC42SE1*	ENSG00000197622
2.830438	2.76E-05	0.002907	*NAP1L3*	ENSG00000186310
2.632759	2.75E-05	0.002907	*FAS*	ENSG00000026103
0.932314	3.19E-05	0.003256	*ARHGAP15*	ENSG00000075884
1.259231	3.15E-05	0.003256	*ANGPT1*	ENSG00000154188
6.148808	3.18E-05	0.003256		ENSG00000274922
0.70153	3.36E-05	0.00341	*APMAP*	ENSG00000101474
6.078896	3.55E-05	0.003563	*ARHGAP11B*	ENSG00000285077
5.555889	3.75E-05	0.003623	*CRYGD*	ENSG00000118231
0.809196	3.77E-05	0.003623	*CYTL1*	ENSG00000170891
2.069424	3.79E-05	0.003623	*NUDT11*	ENSG00000196368
3.623353	3.83E-05	0.003625	*HCST*	ENSG00000126264
6.704152	3.89E-05	0.003641	*CDC37L1-DT*	ENSG00000273061
0.896798	4.21E-05	0.003918	*ITPR1*	ENSG00000150995
6.407183	4.35E-05	0.00402		ENSG00000278376
1.842221	4.43E-05	0.004068	*MSRB3*	ENSG00000174099
1.242584	4.61E-05	0.004171	*FAM107B*	ENSG00000065809
4.357243	4.86E-05	0.004313	*KLHL13*	ENSG00000003096
1.270702	5.18E-05	0.004565	*RASGRP2*	ENSG00000068831
2.675919	5.28E-05	0.00463		ENSG00000271869
6.371641	5.89E-05	0.005064	*AKAP12*	ENSG00000131016
6.00349	6.76E-05	0.005634	*RAB7B*	ENSG00000276600
1.90678	6.7E-05	0.005634	*TFEC*	ENSG00000105967
2.187723	6.71E-05	0.005634	*PTPRE*	ENSG00000132334
5.832236	7.17E-05	0.005936	*SLC28A3*	ENSG00000197506
6.13082	7.47E-05	0.006076	*FBN2*	ENSG00000138829
6.117857	7.47E-05	0.006076		ENSG00000260588
0.750419	7.43E-05	0.006076	*RAC2*	ENSG00000128340
4.248149	7.63E-05	0.00617	*SH3BGRL3*	ENSG00000142669
3.452218	7.92E-05	0.006365	*VAV1*	ENSG00000141968
1.677858	8.22E-05	0.006528	*EIF3J-DT*	ENSG00000179523
3.40572	8.62E-05	0.006811	*MIR34AHG*	ENSG00000228526
6.279426	8.78E-05	0.006896	*S100P*	ENSG00000163993
1.427201	8.98E-05	0.007006	*PRAF2*	ENSG00000243279
0.806979	9.02E-05	0.007006	*GRAMD4*	ENSG00000075240
5.973596	9.19E-05	0.007092		ENSG00000267279
2.437881	9.39E-05	0.007168	*DCUN1D3*	ENSG00000188215
5.944113	9.55E-05	0.007244	*PARD3B*	ENSG00000116117
2.646527	0.000101	0.007517	*SYT11*	ENSG00000132718
5.995478	0.000103	0.00761	*CC2D2A*	ENSG00000048342
1.570489	0.000113	0.008329	*CTSG*	ENSG00000100448
2.377746	0.000118	0.008434	*FOSL2*	ENSG00000075426
1.095891	0.000118	0.008434	*IRAK3*	ENSG00000090376
1.009988	0.000116	0.008434	*MT2A*	ENSG00000125148

6.174995	0.000117	0.008434		ENSG00000274213
0.816303	0.000119	0.008454	*B4GALT5*	ENSG00000158470
1.465766	0.00012	0.008485	*ANKRD33B*	ENSG00000164236
4.550605	0.000122	0.008553	*ABCB1*	ENSG00000085563
0.799334	0.000122	0.008553	*DTD1*	ENSG00000125821
1.432472	0.000128	0.008929	*SPRED1*	ENSG00000166068
6.1109	0.000137	0.009399	*PSPN*	ENSG00000125650
4.893368	0.000143	0.009676	*SLC2A5*	ENSG00000142583
3.411536	0.000148	0.009963	*MRC2*	ENSG00000011028
1.189551	0.00015	0.009971	*WWC3*	ENSG00000047644
3.915008	0.000157	0.010337	*LDOC1*	ENSG00000182195
0.830028	0.00016	0.01051	*ST8SIA6*	ENSG00000148488
5.91576	0.000167	0.01074		ENSG00000285669
1.203523	0.000166	0.01074	*ASCC1*	ENSG00000138303
1.345524	0.000166	0.01074	*FRY*	ENSG00000073910
1.926857	0.000167	0.01074	*LYSMD2*	ENSG00000140280
1.643967	0.000168	0.01074	*SNHG20*	ENSG00000234912
2.809153	0.000178	0.011277	*FGL2*	ENSG00000127951
2.62229	0.000186	0.011673	*UBTD2*	ENSG00000168246
0.829506	0.000187	0.011679	*CBX6*	ENSG00000183741
6.118828	0.000191	0.011777	*NR5A2*	ENSG00000116833
2.752127	0.000192	0.011777	*ANXA6*	ENSG00000197043
3.598322	0.000191	0.011777	*PIMREG*	ENSG00000129195
1.397	0.000204	0.012379	*KCNK5*	ENSG00000164626
5.839532	0.000209	0.012606	*ADGRL1-AS1*	ENSG00000267169
2.315871	0.000213	0.012733	*ZNF462*	ENSG00000148143
1.286024	0.000214	0.012733	*PRXL2A*	ENSG00000122378
1.878279	0.000221	0.012989	*BST1*	ENSG00000109743
6.198964	0.000222	0.012989		ENSG00000277218
5.218952	0.000224	0.012989	*PTAFR*	ENSG00000169403
1.312936	0.000237	0.013588	*TUBB2B*	ENSG00000137285
0.7155	0.000235	0.013588	*VMP1*	ENSG00000062716
5.812117	0.000251	0.014287	*KCTD17*	ENSG00000100379
2.17904	0.000256	0.01448	*SHTN1*	ENSG00000187164
1.016408	0.000264	0.014835	*TPP1*	ENSG00000166340
5.99536	0.000274	0.01525		ENSG00000262089
6.29222	0.000277	0.015374	*RNVU1-6*	ENSG00000201558
5.834291	0.000294	0.016161	*SMOX*	ENSG00000088826
4.131385	0.000299	0.016324	*PTGER4*	ENSG00000171522
6.014024	0.000304	0.016541	*PPP1R3F*	ENSG00000049769
1.012591	0.000308	0.01666	*DRAM2*	ENSG00000156171
3.301227	0.00032	0.017253	*FGGY*	ENSG00000172456
2.188189	0.000322	0.017294	*ARHGAP30*	ENSG00000186517
3.540235	0.00033	0.017633	*TMEM259*	ENSG00000182087
5.710736	0.000331	0.017654		ENSG00000229666
0.719089	0.000349	0.018543	*ACTN1*	ENSG00000072110

3.936135	0.000355	0.018765	*MAP10*	ENSG00000212916
5.237301	0.000362	0.018996	*FAM43A*	ENSG00000185112
3.452211	0.000364	0.019003	*LOC554206*	ENSG00000262587
1.762562	0.000372	0.01936	*ERG*	ENSG00000157554
1.37313	0.000397	0.02056	*SMIM3*	ENSG00000256235
5.659547	0.000398	0.02056	*EDA2R*	ENSG00000131080
0.667954	0.000404	0.020699	*DAD1*	ENSG00000129562
5.673854	0.000403	0.020699	*FAM219B*	ENSG00000178761
5.249372	0.000409	0.020869	*CROCC*	ENSG00000058453
2.808229	0.000421	0.021406	*PXDN*	ENSG00000130508
3.889872	0.000429	0.02175	*HGF*	ENSG00000019991
3.183905	0.000433	0.021808	*GIMAP6*	ENSG00000133561
5.659714	0.000433	0.021808	*SPTAN1*	ENSG00000197694
0.622593	0.000439	0.022013	*AIF1*	ENSG00000204472
0.609775	0.000453	0.02248	*RNF168*	ENSG00000163961
2.045568	0.000453	0.02248	*FGFBP3*	ENSG00000174721
2.153347	0.000459	0.022612		ENSG00000275202
0.668579	0.000463	0.022686	*RGS10*	ENSG00000148908
0.828141	0.000471	0.022925	*TCEAL4*	ENSG00000133142
1.031466	0.000473	0.022925	*NEAT1*	ENSG00000245532
1.051138	0.000472	0.022925	*FAM30A*	ENSG00000226777
1.155501	0.00048	0.023207	*TRAPPC1*	ENSG00000170043
1.56524	0.000489	0.023527	*SLC22A15*	ENSG00000163393
5.960711	0.000492	0.023527	*LPAR4*	ENSG00000147145
5.548064	0.000492	0.023527		ENSG00000280129
1.26851	0.000506	0.023951	*UBE2W*	ENSG00000104343
5.599088	0.000529	0.024789	*BAALC*	ENSG00000164929
1.743959	0.000535	0.02495	*CXCR4*	ENSG00000121966
5.515986	0.000536	0.02495		ENSG00000285730
5.87233	0.000542	0.025117	*MS4A7*	ENSG00000166927
5.700245	0.000552	0.025436	*ZNF311*	ENSG00000197935
2.087712	0.000557	0.025578	*ETV5*	ENSG00000244405
3.287692	0.000561	0.025659	*PABIR3*	ENSG00000156500
1.828566	0.000571	0.026014	*MYO1F*	ENSG00000142347
5.698	0.000573	0.026043	*B3GNT7*	ENSG00000156966
1.343616	0.000576	0.026094	*RPS6KA1*	ENSG00000117676
0.636702	0.00059	0.026583	*ARHGDIB*	ENSG00000111348
5.601263	0.000595	0.026673		ENSG00000272663
2.060155	0.0006	0.026737	*TOX*	ENSG00000198846
4.109803	0.000608	0.027006	*INKA2*	ENSG00000197852
0.633279	0.000623	0.027412	*CCNY*	ENSG00000108100
3.711534	0.000624	0.027412		ENSG00000276900
1.442334	0.00065	0.028224	*RCSD1*	ENSG00000198771
4.559205	0.000666	0.02883		ENSG00000270871
5.55852	0.000673	0.029015	*PRUNE2*	ENSG00000106772
5.959459	0.00068	0.029224	*PLK2*	ENSG00000145632

5.052378	0.000686	0.029288		ENSG00000273837
1.150492	0.000697	0.029703	*NAP1L5*	ENSG00000177432
1.361988	0.000701	0.029732	*PSMB8*	ENSG00000204264
1.367787	0.000703	0.029732	*PI4KA*	ENSG00000241973
1.146168	0.000706	0.029797	*STK39*	ENSG00000198648
5.439349	0.000712	0.029848	*PRELID3A*	ENSG00000141391
5.644287	0.000711	0.029848	*C5AR1*	ENSG00000197405
1.161904	0.000724	0.030183	*DBNL*	ENSG00000136279
1.037603	0.00076	0.031178	*HLA-DRB1*	ENSG00000196126
3.796575	0.000766	0.031178	*GPM6B*	ENSG00000046653
0.949513	0.000757	0.031178	*C1GALT1C1*	ENSG00000171155
0.715562	0.000754	0.031178	*PDLIM1*	ENSG00000107438
2.040896	0.000772	0.03129	*CHMP4A*	ENSG00000254505
1.619419	0.000786	0.031671	*BEX1*	ENSG00000133169
1.529829	0.000791	0.03178	*CA5B*	ENSG00000169239
1.101381	0.000802	0.032146	*FCER1A*	ENSG00000179639
0.951539	0.000812	0.032428	*MARCKS*	ENSG00000277443
1.039366	0.000815	0.032488	*NQO2*	ENSG00000124588
2.645667	0.000822	0.032543	*DPF3*	ENSG00000205683
5.613345	0.000822	0.032543		ENSG00000277511
1.02485	0.000825	0.032588	*UTRN*	ENSG00000152818
2.097977	0.000858	0.033643	*TRERF1*	ENSG00000124496
1.892987	0.000859	0.033643	*DMXL2*	ENSG00000104093
4.620909	0.000873	0.033983	*HPSE*	ENSG00000173083
5.468499	0.000871	0.033983	*ZNF709*	ENSG00000242852
5.998278	0.000903	0.034857	*PHLDA3*	ENSG00000174307
0.75773	0.000943	0.036144	*ABI2*	ENSG00000138443
5.432641	0.000951	0.036201	*FRMD3*	ENSG00000172159
3.367231	0.000969	0.036577	*CXCL2*	ENSG00000081041
1.356049	0.00097	0.036577	*ZFP90*	ENSG00000184939
0.772262	0.000975	0.036594	*PXK*	ENSG00000168297
5.516707	0.001011	0.037732		ENSG00000277170
1.324954	0.001041	0.038635	*FXYD5*	ENSG00000089327
2.348588	0.00106	0.039251	*RASAL3*	ENSG00000105122
1.605766	0.001075	0.039686	*RNASE3*	ENSG00000169397
1.149921	0.001093	0.040064	*RHOQ*	ENSG00000119729
5.322563	0.001094	0.040064	*ANKRD18A*	ENSG00000180071
3.629447	0.001097	0.040067	*PPP1R16B*	ENSG00000101445
5.515987	0.001115	0.040297	*GABBR1*	ENSG00000204681
3.983901	0.001116	0.040297	*RBM26-AS1*	ENSG00000227354
3.356026	0.001108	0.040297	*PLXNB2*	ENSG00000196576
2.554845	0.001182	0.042035	*IRF5*	ENSG00000128604
1.205736	0.001191	0.042247	*LAMTOR4*	ENSG00000188186
5.734556	0.001195	0.042267	*LIN7A*	ENSG00000111052
4.426144	0.001202	0.042297	*STOX1*	ENSG00000165730
1.746907	0.0012	0.042297	*ATP2A3*	ENSG00000074370

2.070155	0.001216	0.042687	*MAP1A*	ENSG00000166963
1.338254	0.001249	0.043603	*XYLT1*	ENSG00000103489
3.171254	0.001287	0.044701	*RNVU1-19*	ENSG00000275538
0.826009	0.001293	0.044798	*TP53INP1*	ENSG00000164938
0.634542	0.001308	0.0452	*CCDC90B*	ENSG00000137500
5.612773	0.001337	0.045739		ENSG00000272432
5.319437	0.001334	0.045739	*HOMER3*	ENSG00000051128
1.791205	0.001333	0.045739		ENSG00000269688
3.870456	0.001367	0.046294	*CLDN10*	ENSG00000134873
0.659257	0.001361	0.046294	*PMP22*	ENSG00000109099
1.357256	0.001366	0.046294	*LAIR1*	ENSG00000167613
4.615352	0.00139	0.046844		ENSG00000224934
5.160194	0.0014	0.047079	*SIGLEC6*	ENSG00000105492
1.885338	0.001408	0.047106		ENSG00000273117
1.648771	0.001425	0.047463	*PAG1*	ENSG00000076641
5.698999	0.001425	0.047463		ENSG00000272186
1.930854	0.001437	0.047621	*GCNT2*	ENSG00000111846
1.903078	0.001436	0.047621	*C12orf57*	ENSG00000111678
1.085051	0.001444	0.04774	*SAC3D1*	ENSG00000168061
0.632958	0.001452	0.047863	*SNX12*	ENSG00000147164
2.408652	0.001477	0.048578	*ARL11*	ENSG00000152213
5.602542	0.001489	0.048873	*ARMH1*	ENSG00000198520
2.820799	0.001539	0.049882	*TRH*	ENSG00000170893
0.662158	0.001535	0.049882	*PRSS57*	ENSG00000185198
5.312195	0.001539	0.049882	*ADGRE5*	ENSG00000123146
2.975624	0.001549	0.04999	*GPR65*	ENSG00000140030

Table S11. RNA-seq results: down-regulated genes (HES6 knockdown vs control) in early erythroblasts

log2FoldChange	pvalue	padj	symbol	Ensembl ID
-2.70912	2.64E-33	1.79E-29	*EPCAM*	ENSG00000119888
-1.71198	1.84E-16	4.17E-13	*CA2*	ENSG00000104267
-0.76399	5.43E-16	9.22E-13	*TFRC*	ENSG00000072274
-0.73185	1.75E-11	1.78E-08	*FECH*	ENSG00000066926
-1.71152	4.99E-10	3.57E-07	*HES6*	ENSG00000144485
-2.22391	7.2E-10	4.25E-07	*HOOK1*	ENSG00000134709
-0.69878	9.09E-09	3.63E-06	*RHAG*	ENSG00000112077
-0.80274	6.46E-08	2.31E-05	*ZBTB7A*	ENSG00000178951
-0.90141	7.24E-08	2.46E-05	*UGCG*	ENSG00000148154
-0.74288	2.21E-07	6.68E-05	*NADK2*	ENSG00000152620
-0.72183	2.52E-07	7.22E-05	*MCM10*	ENSG00000065328
-0.74425	2.55E-07	7.22E-05	*OAT*	ENSG00000065154
-1.40369	2.91E-07	7.9E-05	*TLCD4*	ENSG00000152078
-1.20704	3.85E-07	9.88E-05	*CDH1*	ENSG00000039068
-0.6049	5.06E-07	0.000125	*RPL22L1*	ENSG00000163584
-1.34558	7.07E-07	0.00016	*HBB*	ENSG00000244734
-0.70158	1.78E-06	0.000355	*FAM210B*	ENSG00000124098
-1.06392	1.92E-06	0.000372	*SLC29A1*	ENSG00000112759
-0.85293	1.95E-06	0.000373	*HMGN5*	ENSG00000198157
-0.84831	5.18E-06	0.00089	*METTL13*	ENSG00000010165
-0.91649	5.3E-06	0.0009	*TGM2*	ENSG00000198959
-1.75659	5.49E-06	0.000921	*FAM178B*	ENSG00000168754
-1.40211	5.84E-06	0.000967	*GAL*	ENSG00000069482
-0.60252	8.1E-06	0.001258	*NUP210*	ENSG00000132182
-0.98365	9E-06	0.001314	*XK*	ENSG00000047597
-1.80935	1.18E-05	0.00161	*COCH*	ENSG00000100473
-0.77328	1.53E-05	0.001894	*EIF4EBP2*	ENSG00000148730
-0.88294	1.94E-05	0.002294	*STRADB*	ENSG00000082146
-0.65998	1.94E-05	0.002294	*PM20D2*	ENSG00000146281
-1.03803	3.08E-05	0.003221	*B3GALNT1*	ENSG00000169255
-1.8015	3.61E-05	0.003563	*SPTB*	ENSG00000070182
-0.77764	3.58E-05	0.003563	*TUBB6*	ENSG00000176014
-1.17577	3.84E-05	0.003625	*PI4K2B*	ENSG00000038210
-0.76976	4.78E-05	0.004274	*RIDA*	ENSG00000132541
-1.89594	5.5E-05	0.00479	*LINC01133*	ENSG00000224259
-0.6596	5.61E-05	0.004857	*E2F4*	ENSG00000205250
-0.79287	6.51E-05	0.005565	*CCDC71L*	ENSG00000253276
-0.66026	6.72E-05	0.005634	*APOC1*	ENSG00000130208
-1.50813	9.77E-05	0.007373	*DNAJA4*	ENSG00000140403
-2.11	9.93E-05	0.007456	*LGALS3*	ENSG00000131981
-0.6106	0.000117	0.008434	*TRIB2*	ENSG00000071575
-0.8128	0.000115	0.008434	*GUCD1*	ENSG00000138867
-0.80379	0.000131	0.009111	*HLTF*	ENSG00000071794

-4.43441	0.000141	0.009651	*CTSE*	ENSG00000196188
-1.6623	0.000149	0.009963	*IL15RA*	ENSG00000134470
-0.71041	0.000152	0.01005	*CA8*	ENSG00000178538
-4.45084	0.000169	0.010773	*HBA2*	ENSG00000188536
-3.52608	0.000178	0.011277	*EMP2*	ENSG00000213853
-0.71923	0.000188	0.011689	*JAK2*	ENSG00000096968
-0.65898	0.000192	0.011777	*ALDH5A1*	ENSG00000112294
-0.61876	0.000195	0.011908	*CPNE3*	ENSG00000085719
-0.63566	0.000214	0.012733	*KRR1*	ENSG00000111615
-0.87732	0.000224	0.012989	*LEPR*	ENSG00000116678
-0.67462	0.000237	0.013588	*ALAD*	ENSG00000148218
-1.60605	0.000262	0.014762	*SLC2A14*	ENSG00000173262
-0.60969	0.000285	0.015754	*LRRCC1*	ENSG00000133739
-0.67964	0.000356	0.018765	*NME4*	ENSG00000103202
-1.09089	0.000443	0.022128	*DUSP2*	ENSG00000158050
-1.00857	0.000457	0.022595	*MEX3D*	ENSG00000181588
-0.74091	0.000494	0.023566	*RFESD*	ENSG00000175449
-0.7148	0.000497	0.023611	*RAD17*	ENSG00000152942
-0.6939	0.000526	0.024775	*PIK3R4*	ENSG00000196455
-0.67712	0.000591	0.026583	*BLVRB*	ENSG00000090013
-0.67616	0.000597	0.026691	*JMJD1C*	ENSG00000171988
-0.7057	0.000612	0.027065	*MXD1*	ENSG00000059728
-6.15031	0.000685	0.029288	*ESYT3*	ENSG00000158220
-0.65472	0.000744	0.030914	*BAG2*	ENSG00000112208
-0.72152	0.000763	0.031178	*ZFP36L1*	ENSG00000185650
-0.77256	0.000774	0.031292	*FOXJ2*	ENSG00000065970
-0.82454	0.000848	0.033407	*TRMT11*	ENSG00000066651
-1.27746	0.000947	0.036145	*SLC39A4*	ENSG00000147804
-0.62617	0.000978	0.036613	*ADD2*	ENSG00000075340
-4.33378	0.001118	0.040297	*SLC6A8*	ENSG00000130821
-0.66747	0.001163	0.041685	*CD44*	ENSG00000026508
-0.8938	0.00118	0.042035	*CAPRIN2*	ENSG00000110888
-0.69477	0.00132	0.045506	*HBG1*	ENSG00000213934
-0.91225	0.001386	0.046837	*TOB1*	ENSG00000141232
-0.86797	0.001542	0.049882	*LIN9*	ENSG00000183814

Table S12. RNA-seq results: up-regulated genes (HES6 knockdown vs control) in late erythroblasts

log2FoldChange	pvalue	padj	symbol	Ensembl ID
2.856788	4.11E-22	2.6E-18	*CELF2*	ENSG00000048740
2.116957	2.71E-19	5.71E-16	*ID2*	ENSG00000115738
1.54921	4.35E-16	7.86E-13	*NEAT1*	ENSG00000245532
1.76249	4.8E-13	6.76E-10	*EGR1*	ENSG00000120738
2.362142	1.14E-11	1.2E-08	*GATA2*	ENSG00000179348
1.630967	1.61E-10	1.46E-07	*ATF7IP2*	ENSG00000166669
2.907682	6.69E-10	5.65E-07	*PTGS1*	ENSG00000095303
1.959083	4.2E-09	3.13E-06	*PRAF2*	ENSG00000243279
3.486418	4.86E-09	3.42E-06	*HLA-DRB1*	ENSG00000196126
1.014799	6.38E-09	4.23E-06	*ACTG1*	ENSG00000184009
8.237323	7.9E-09	4.76E-06	*MIR34AHG*	ENSG00000228526
2.121954	1.91E-08	1.05E-05	*TP53INP1*	ENSG00000164938
5.29072	2.04E-08	1.05E-05	*GDF15*	ENSG00000130513
4.451133	2.29E-08	1.07E-05	*ITGA2B*	ENSG00000005961
3.219238	2.93E-08	1.32E-05	*ZNF462*	ENSG00000148143
1.762966	4.34E-08	1.83E-05	*LAPTM5*	ENSG00000162511
1.989766	4.26E-08	1.83E-05	*FYN*	ENSG00000010810
2.665699	5.79E-08	2.36E-05	*TIMP1*	ENSG00000102265
3.126627	8.04E-08	3.08E-05	*CFAP210*	ENSG00000154479
2.274598	9.63E-08	3.59E-05	*EIF3J-DT*	ENSG00000179523
2.134195	1.65E-07	5.98E-05	*HLA-DPA1*	ENSG00000231389
1.061558	1.76E-07	6.18E-05	*STAT3*	ENSG00000168610
2.549163	2.37E-07	8.12E-05	*GCSAML*	ENSG00000169224
7.398936	3.52E-07	0.000111	*TMEM233*	ENSG00000224982
1.493107	3.88E-07	0.00012	*FLNA*	ENSG00000196924
0.999584	4.4E-07	0.000133	*CBX6*	ENSG00000183741
1.444342	4.93E-07	0.000142	*FAM189B*	ENSG00000160767
1.585084	4.84E-07	0.000142	*MAGEF1*	ENSG00000177383
2.162977	6.64E-07	0.000187	*HLA-DRA*	ENSG00000204287
2.353936	8.84E-07	0.000243	*SESN3*	ENSG00000149212
5.222117	1.06E-06	0.000286	*CYFIP2*	ENSG00000055163
4.508767	1.47E-06	0.000387	*PINLYP*	ENSG00000234465
1.483148	1.55E-06	0.0004	*CYTOR*	ENSG00000222041
3.287503	1.65E-06	0.000417	*PRKCB*	ENSG00000166501
1.487929	1.75E-06	0.000434	*H1-10*	ENSG00000184897
7.315048	1.93E-06	0.000471	*PLXNB2*	ENSG00000196576
1.60183	2.01E-06	0.000478	*PDLIM1*	ENSG00000107438
7.127615	2.15E-06	0.000486		ENSG00000272843
4.186563	2.15E-06	0.000486	*SND1-IT1*	ENSG00000279078
1.157974	2.91E-06	0.000635	*ULK3*	ENSG00000140474
1.188546	3.06E-06	0.000656	*ABI2*	ENSG00000138443
2.29248	3.16E-06	0.000667	*GDF11*	ENSG00000135414
1.874801	3.28E-06	0.00068	*KAZALD1*	ENSG00000107821

0.860729	3.71E-06	0.000758	*RPS18*	ENSG00000231500
0.926889	3.88E-06	0.00078		ENSG00000244879
1.067011	4.42E-06	0.000861	*ST3GAL2*	ENSG00000157350
4.103259	4.84E-06	0.000917	*DYNLT2*	ENSG00000184786
6.732813	5.56E-06	0.001035	*AKAP12*	ENSG00000131016
1.299791	5.75E-06	0.001055	*ZNF271P*	ENSG00000257267
5.377805	6.16E-06	0.001114	*LINC02987*	ENSG00000267575
2.686271	6.4E-06	0.001141	*NT5M*	ENSG00000205309
6.040577	7.08E-06	0.001245	*FIZ1*	ENSG00000179943
6.977438	7.18E-06	0.001245	*CPB2-AS1*	ENSG00000235903
6.613806	9.33E-06	0.001541	*AMHR2*	ENSG00000135409
1.54518	9.37E-06	0.001541	*CBFA2T3*	ENSG00000129993
2.430026	9.15E-06	0.001541		ENSG00000267002
2.024891	1.09E-05	0.001751	*HLA-A*	ENSG00000206503
2.693389	1.09E-05	0.001751	*CSKMT*	ENSG00000214756
1.488943	1.24E-05	0.001936	*ATP6V1F*	ENSG00000128524
3.687718	1.23E-05	0.001936	*MPO*	ENSG00000005381
2.075687	1.38E-05	0.002125	*ZSCAN16-AS1*	ENSG00000269293
1.084766	1.42E-05	0.00217	*MLC1*	ENSG00000100427
1.300774	1.49E-05	0.002225	*ACTN1*	ENSG00000072110
0.975996	1.56E-05	0.002292	*PIM2*	ENSG00000102096
0.791035	1.93E-05	0.00278	*TRIAP1*	ENSG00000170855
1.788161	2.29E-05	0.003187	*CD74*	ENSG00000019582
7.061471	2.46E-05	0.003346		ENSG00000272795
3.45034	2.86E-05	0.003858	*PIK3R3*	ENSG00000117461
6.437087	3.1E-05	0.004133	*TRERF1*	ENSG00000124496
6.345263	3.23E-05	0.004254	*LINC00562*	ENSG00000260388
6.33226	3.33E-05	0.004349	*DOCK2*	ENSG00000134516
4.13847	3.9E-05	0.004993	*H2BC8*	ENSG00000273802
0.606298	4.02E-05	0.005087	*CD63*	ENSG00000135404
2.11049	4.12E-05	0.00517	*KCTD10*	ENSG00000110906
6.552066	4.45E-05	0.005425	*NRM*	ENSG00000137404
2.218904	4.39E-05	0.005425	*MS4A6A*	ENSG00000110077
1.790575	4.46E-05	0.005425	*ARRB2*	ENSG00000141480
3.641854	4.57E-05	0.005512	*MARCHF2*	ENSG00000099785
2.203473	5.12E-05	0.006008	*FDXR*	ENSG00000161513
1.700942	5.33E-05	0.006191	*WWC3*	ENSG00000047644
6.880276	6.14E-05	0.007073		ENSG00000283341
5.974731	6.23E-05	0.007103	*ARL10*	ENSG00000175414
3.874786	6.62E-05	0.007483	*PTENP1*	ENSG00000237984
6.542763	6.88E-05	0.007705	*PGM1*	ENSG00000079739
1.576905	7.25E-05	0.008056	*DPYSL2*	ENSG00000092964
1.875023	7.49E-05	0.008184	*TUBB2B*	ENSG00000137285
0.971904	8.14E-05	0.008658	*HDGFL3*	ENSG00000166503
2.115118	8.08E-05	0.008658	*SELENOW*	ENSG00000178980
6.33289	8.32E-05	0.008777	*ARHGAP30*	ENSG00000186517

4.531312	8.43E-05	0.008824	*CERK*	ENSG00000100422
3.451001	8.56E-05	0.008879		ENSG00000260793
6.635021	8.78E-05	0.009034		ENSG00000286138
1.334002	0.000103	0.010121	*CCND3*	ENSG00000112576
1.338286	0.000103	0.010121	*AKR1B1*	ENSG00000085662
6.077029	0.000105	0.010171		ENSG00000230177
4.6194	0.000107	0.010303	*CD1C*	ENSG00000158481
6.566592	0.000111	0.010534	*PHLDA3*	ENSG00000174307
2.406939	0.000127	0.011836	*SPEN-AS1*	ENSG00000179743
0.906373	0.000127	0.011836	*CPEB4*	ENSG00000113742
6.29021	0.000129	0.011906		ENSG00000226853
1.990765	0.00013	0.011906	*HLA-DPB1*	ENSG00000223865
3.344786	0.000132	0.011983	*PSMB8*	ENSG00000204264
0.64902	0.000143	0.012804	*CAPZB*	ENSG00000077549
2.758007	0.000145	0.012929		ENSG00000278774
3.116223	0.000154	0.013493	*ATXN1*	ENSG00000124788
6.139386	0.000153	0.013493	*XAGE2*	ENSG00000155622
0.922376	0.000163	0.013956	*TIMP3*	ENSG00000100234
2.980254	0.000167	0.014228	*KCNK6*	ENSG00000099337
5.569186	0.000173	0.014572		ENSG00000205464
1.384977	0.000177	0.014718	*IMPA1*	ENSG00000133731
6.140039	0.000176	0.014718		ENSG00000232470
3.707969	0.000178	0.014736	*ATP6V0E2*	ENSG00000171130
1.641017	0.000182	0.014962	*TUBA1A*	ENSG00000167552
1.213687	0.000183	0.014962	*TST*	ENSG00000128311
1.537467	0.000189	0.015253	*MOSMO*	ENSG00000185716
6.413102	0.000191	0.015326		ENSG00000272186
6.001609	0.000199	0.015526	*CDKN1A*	ENSG00000124762
1.105393	0.0002	0.015526	*BRI3*	ENSG00000164713
2.559531	0.000195	0.015526	*DAPK1*	ENSG00000196730
3.063004	0.000198	0.015526	*PKM*	ENSG00000067225
6.079845	0.000211	0.016269	*PDK4*	ENSG00000004799
0.692278	0.000213	0.016274	*ARPC2*	ENSG00000163466
6.380642	0.000213	0.016274	*TTI2*	ENSG00000129696
1.407132	0.000216	0.016277	*KLHDC8B*	ENSG00000185909
1.717993	0.000215	0.016277	*GTF3C5*	ENSG00000148308
1.633014	0.000219	0.016288	*SSR4*	ENSG00000180879
6.297715	0.000218	0.016288		ENSG00000225511
0.851441	0.000221	0.016366	*BTG1*	ENSG00000133639
1.70452	0.000222	0.016366	*CALM3*	ENSG00000160014
1.733382	0.000227	0.016628		ENSG00000260708
1.35076	0.00023	0.016759	*GNA15*	ENSG00000060558
1.035924	0.000236	0.017069	*IRF2BPL*	ENSG00000119669
2.914084	0.000238	0.017109	*LEF1*	ENSG00000138795
1.449417	0.000241	0.017225	*PEA15*	ENSG00000162734
1.322418	0.000245	0.017439	*SERF2*	ENSG00000140264

0.926422	0.000247	0.017439	*AGPAT3*	ENSG00000160216
1.759741	0.000251	0.017666	*TYMSOS*	ENSG00000176912
3.571767	0.000253	0.01768	*CXCL3*	ENSG00000163734
1.889473	0.000264	0.018362		ENSG00000272716
6.654569	0.000289	0.019464		ENSG00000272662
5.934963	0.000288	0.019464	*PRDM1*	ENSG00000057657
1.013295	0.000289	0.019464	*RAC2*	ENSG00000128340
2.662637	0.000295	0.019735	*HLA-E*	ENSG00000204592
3.134382	0.000319	0.021011	*DMAP1*	ENSG00000178028
3.632636	0.000326	0.021398	*SYNE1*	ENSG00000131018
1.187807	0.000337	0.021973	*SERPINE2*	ENSG00000135919
1.076273	0.00034	0.022054	*PMP22*	ENSG00000109099
1.677428	0.000343	0.022144	*ITPR1*	ENSG00000150995
2.849946	0.000354	0.022774	*PTK2B*	ENSG00000120899
0.699995	0.000364	0.023257	*MTHFR*	ENSG00000177000
6.113946	0.000371	0.023269	*CD244*	ENSG00000122223
3.442952	0.000367	0.023269	*NEURL1B*	ENSG00000214357
1.355781	0.000371	0.023269	*HSD17B10*	ENSG00000072506
7.537513	0.00037	0.023269	*EDA2R*	ENSG00000131080
4.808107	0.000376	0.023371	*TRMT61A*	ENSG00000166166
2.206183	0.000385	0.023784	*SHTN1*	ENSG00000187164
3.171261	0.00041	0.025173	*OAS3*	ENSG00000111331
0.839137	0.000412	0.025222	*MTSS1*	ENSG00000170873
3.020208	0.000424	0.025814		ENSG00000272086
5.109329	0.000429	0.02587	*CD70*	ENSG00000125726
6.195852	0.000433	0.02587	*MTMR11*	ENSG00000014914
1.518515	0.000431	0.02587	*LAMTOR4*	ENSG00000188186
2.095145	0.000441	0.025891	*IFI6*	ENSG00000126709
5.876827	0.000445	0.025891	*RNVU1-19*	ENSG00000275538
6.227324	0.000447	0.025891	*TBCE*	ENSG00000285053
2.647214	0.000448	0.025891	*CBLL1-AS1*	ENSG00000241764
0.669552	0.000437	0.025891	*STOM*	ENSG00000148175
3.262193	0.00044	0.025891	*SMPD1*	ENSG00000166311
1.638861	0.000458	0.026264	*MANF*	ENSG00000145050
2.341182	0.000469	0.026623		ENSG00000201674
5.989042	0.000467	0.026623		ENSG00000273619
4.343128	0.000476	0.026881	*JAKMIP2*	ENSG00000176049
2.994618	0.000492	0.027434	*ILRUN-AS1*	ENSG00000272288
5.431713	0.000491	0.027434	*TRO*	ENSG00000067445
3.984692	0.000501	0.027739		ENSG00000248124
5.715525	0.000502	0.027739	*LOC105371414*	ENSG00000260279
5.042785	0.000505	0.027801		ENSG00000273148
1.14386	0.000508	0.027872	*ACSM3*	ENSG00000005187
5.235046	0.000532	0.028339	*TMEM158*	ENSG00000249992
5.82877	0.000528	0.028339	*ZDHHC11*	ENSG00000188818
1.802991	0.000532	0.028339	*SRA1*	ENSG00000213523

5.902059	0.000523	0.028339		ENSG00000271888
4.846929	0.000528	0.028339	*SNHG11*	ENSG00000174365
5.402242	0.000548	0.028989	*STAG3*	ENSG00000066923
5.629016	0.000567	0.029655	*BEND4*	ENSG00000188848
4.359445	0.000571	0.02974	*CLDND2*	ENSG00000160318
1.330824	0.000586	0.03038	*AGO1*	ENSG00000092847
1.227258	0.000588	0.03038	*VPS4A*	ENSG00000132612
1.899023	0.000598	0.030801	*PIEZO1*	ENSG00000103335
2.00697	0.000618	0.031435	*WAS*	ENSG00000015285
5.176286	0.000633	0.031819		ENSG00000243193
0.671543	0.000647	0.03224	*EID1*	ENSG00000255302
3.788006	0.00071	0.033947		ENSG00000273437
5.575865	0.00071	0.033947	*CLU*	ENSG00000120885
4.670718	0.000695	0.033947	*PYGB*	ENSG00000100994
0.695155	0.000749	0.035265	*MAN1A2*	ENSG00000198162
6.112771	0.000747	0.035265	*FRMD6-AS1*	ENSG00000273888
1.53441	0.000759	0.035532	*ANXA4*	ENSG00000196975
1.339947	0.00076	0.035532	*REC8*	ENSG00000100918
2.582403	0.000765	0.035626	*HLA-F-AS1*	ENSG00000214922
5.892826	0.000769	0.035649	*RTN1*	ENSG00000139970
1.429017	0.000778	0.035952	*KCNK5*	ENSG00000164626
2.212922	0.000818	0.037134	*AIF1*	ENSG00000204472
3.104509	0.000812	0.037134	*ABLIM1*	ENSG00000099204
0.764653	0.000813	0.037134	*TM7SF3*	ENSG00000064115
0.667198	0.000817	0.037134	*RDH11*	ENSG00000072042
3.114948	0.000824	0.037283	*MLLT11*	ENSG00000213190
1.728171	0.000839	0.037798	*MEX3A*	ENSG00000254726
1.281183	0.000873	0.039218	*STK39*	ENSG00000198648
7.508341	0.000889	0.039632	*NUDT11*	ENSG00000196368
5.629854	0.000937	0.041343	*RHOC*	ENSG00000155366
0.697894	0.000965	0.04198	*EIF4E2*	ENSG00000135930
0.622706	0.00096	0.04198	*JPT1*	ENSG00000189159
5.962074	0.000996	0.042287		ENSG00000270742
2.840021	0.000982	0.042287	*FAXDC2*	ENSG00000170271
0.709682	0.000998	0.042287	*TMEM258*	ENSG00000134825
2.336821	0.000999	0.042287	*ST8SIA1*	ENSG00000111728
3.76995	0.000986	0.042287	*TFIP11-DT*	ENSG00000261188
0.791471	0.001005	0.042406	*NPM3*	ENSG00000107833
2.86848	0.001021	0.042796	*MAP1A*	ENSG00000166963
6.04887	0.001018	0.042796	*RAI1*	ENSG00000108557
3.906269	0.001026	0.042864	*CAVIN1*	ENSG00000177469
5.226404	0.001032	0.04298		ENSG00000274428
4.22161	0.00104	0.043194	*SPINDOC*	ENSG00000168005
5.699726	0.00105	0.043458	*SLCO2B1*	ENSG00000137491
1.873032	0.001058	0.043623	*IRAK3*	ENSG00000090376
3.074392	0.001064	0.043626	*KCNQ1OT1*	ENSG00000269821

5.127526	0.001078	0.043873	*DMTF1-AS1*	ENSG00000224046
1.060246	0.001093	0.044347	*SEC14L1*	ENSG00000129657
4.248693	0.00111	0.04482		ENSG00000263934
6.042369	0.00115	0.046081	*MIR762HG*	ENSG00000260083
0.60944	0.001161	0.046388	*NDUFS6*	ENSG00000145494
4.718078	0.001182	0.046787	*CCNG2*	ENSG00000138764
1.564262	0.001177	0.046787	*TENT5A*	ENSG00000112773
1.321602	0.00118	0.046787	*MAGEH1*	ENSG00000187601
5.757167	0.001194	0.047102	*LIPE-AS1*	ENSG00000213904
4.263923	0.001203	0.047262		ENSG00000273382
1.845635	0.001206	0.047262	*STON2*	ENSG00000140022
5.728386	0.00122	0.04752	*LINC00173*	ENSG00000196668
0.788269	0.001239	0.048135	*SOCS7*	ENSG00000274211
2.742942	0.001273	0.048838	*MRC1*	ENSG00000260314
1.916529	0.001285	0.049174		ENSG00000271869
5.126631	0.00131	0.049227	*HHLA3*	ENSG00000197568
2.124147	0.001297	0.049227	*CST3*	ENSG00000101439
3.554307	0.001301	0.049227	*ZNF554*	ENSG00000172006
3.778147	0.001338	0.049969	*GDI1*	ENSG00000203879

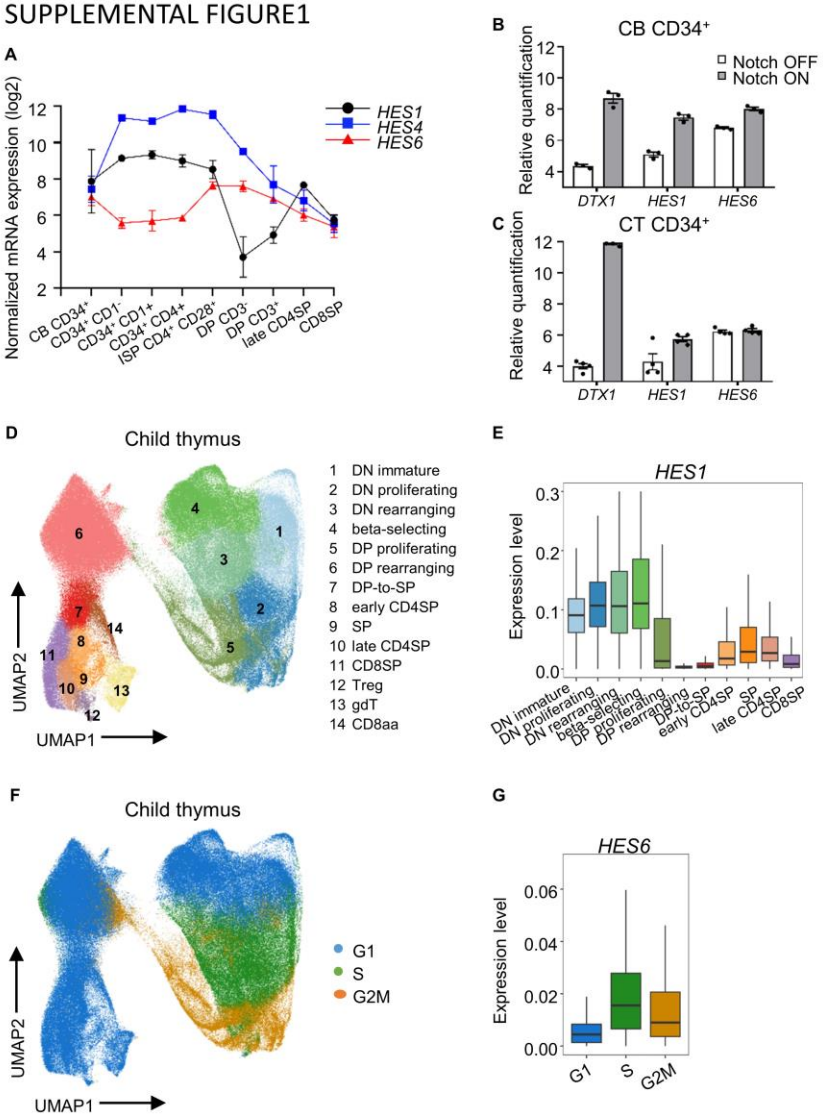
Table S13. RNA-seq results: down-regulated genes (HES6 knockdown vs control) in late erythroblasts

log2FoldChange	pvalue	padj	symbol	Ensembl ID
-2.08861	3.03E-25	3.83E-21	*EPCAM*	ENSG00000119888
-0.9478	2.07E-20	6.54E-17	*TFRC*	ENSG00000072274
-2.06663	1.83E-20	6.54E-17	*COCH*	ENSG00000100473
-1.03261	5.53E-20	1.4E-16	*HBB*	ENSG00000244734
-1.57737	6.23E-15	9.86E-12	*HES6*	ENSG00000144485
-1.19767	8.63E-13	1.09E-09	*RPL22L1*	ENSG00000163584
-1.8797	4.11E-12	4.74E-09	*LGALS3*	ENSG00000131981
-0.94644	1.26E-10	1.23E-07	*NME4*	ENSG00000103202
-1.79417	8.33E-10	6.59E-07	*HOOK1*	ENSG00000134709
-1.00777	6.68E-09	4.23E-06	*CD44*	ENSG00000026508
-1.14908	1.51E-08	8.7E-06	*PM20D2*	ENSG00000146281
-1.00226	2.08E-08	1.05E-05	*CA2*	ENSG00000104267
-1.92134	2.23E-08	1.07E-05	*CPVL*	ENSG00000106066
-2.19529	8.02E-08	3.08E-05	*SQOR*	ENSG00000137767
-1.9092	3.01E-07	9.79E-05	*PTGR1*	ENSG00000106853
-0.81286	2.99E-07	9.79E-05	*DLGAP5*	ENSG00000126787
-0.82502	2.04E-06	0.000478	*CAPRIN2*	ENSG00000110888
-0.89996	2.44E-06	0.000541	*ATAD5*	ENSG00000176208
-1.01804	4.01E-06	0.000793	*RHNO1*	ENSG00000171792
-2.43469	4.85E-06	0.000917	*HBM*	ENSG00000206177
-0.92909	1.48E-05	0.002225	*STYX*	ENSG00000198252
-0.70204	1.86E-05	0.002702	*ZFP36L1*	ENSG00000185650
-0.72307	2.01E-05	0.002866	*HMGN5*	ENSG00000198157
-2.28525	2.23E-05	0.003134	*GLIPR2*	ENSG00000122694
-0.7603	2.44E-05	0.003346	*NMI*	ENSG00000123609
-0.95144	3.67E-05	0.004736	*SLC31A1*	ENSG00000136868
-0.94954	4.71E-05	0.005621	*GABPB2*	ENSG00000143458
-0.84127	8E-05	0.008655	*EIF4EBP2*	ENSG00000148730
-0.73325	9.3E-05	0.009417	*SMARCAD1*	ENSG00000163104
-4.02644	9.27E-05	0.009417	*DSG2*	ENSG00000046604
-0.89204	9.44E-05	0.009487	*B3GALNT1*	ENSG00000169255
-0.7245	0.000103	0.010121	*NUP210*	ENSG00000132182
-1.66262	0.000135	0.012212	*SEC14L4*	ENSG00000133488
-0.69639	0.000156	0.013502	*KIF23*	ENSG00000137807
-0.85458	0.00016	0.013796	*STRADB*	ENSG00000082146
-0.68232	0.000185	0.015013	*IPO11*	ENSG00000086200
-1.59052	0.000197	0.015526	*FSBP*	ENSG00000265817
-3.55975	0.000274	0.018965	*WIPF3*	ENSG00000122574
-1.47081	0.000286	0.019464	*CTSZ*	ENSG00000101160
-0.73338	0.000296	0.019735	*E2F4*	ENSG00000205250
-1.02243	0.000377	0.023371	*VANGL1*	ENSG00000173218
-0.87567	0.000429	0.02587	*ICA1*	ENSG00000003147
-0.6835	0.000444	0.025891	*BAG2*	ENSG00000112208

-0.60567	0.000489	0.027434	*TUBB6*	ENSG00000176014
-0.65062	0.000549	0.028989	*ALAD*	ENSG00000148218
-1.09519	0.000564	0.029621	*ADI1*	ENSG00000182551
-0.71909	0.000607	0.031104	*METTL13*	ENSG00000010165
-0.91825	0.000674	0.033451	*RGCC*	ENSG00000102760
-0.74214	0.000679	0.033583	*CYBRD1*	ENSG00000071967
-0.60999	0.000709	0.033947	*ATP1A1*	ENSG00000163399
-1.43704	0.000693	0.033947	*CMAHP*	ENSG00000168405
-0.83017	0.000698	0.033947	*CA8*	ENSG00000178538
-0.77524	0.00072	0.034257	*KLF13*	ENSG00000169926
-0.99073	0.000737	0.034932	*LIN9*	ENSG00000183814
-0.75437	0.000894	0.039736	*WASHC5*	ENSG00000164961
-0.79023	0.000902	0.039918	*PSAT1*	ENSG00000135069
-0.91801	0.000998	0.042287	*METTL9*	ENSG00000197006
-0.82103	0.000978	0.042287	*ENOSF1*	ENSG00000132199
-0.90647	0.001065	0.043626	*FBXO34*	ENSG00000178974
-1.9091	0.001248	0.048324	*SLC22A16*	ENSG00000004809
-0.89517	0.001254	0.048428	*TBC1D24*	ENSG00000162065
-0.63655	0.001265	0.04868	*XK*	ENSG00000047597
-0.90135	0.0013	0.049227	*E2F8*	ENSG00000129173
-0.81084	0.001329	0.049768	*DNAJA4*	ENSG00000140403

Supplemental figures

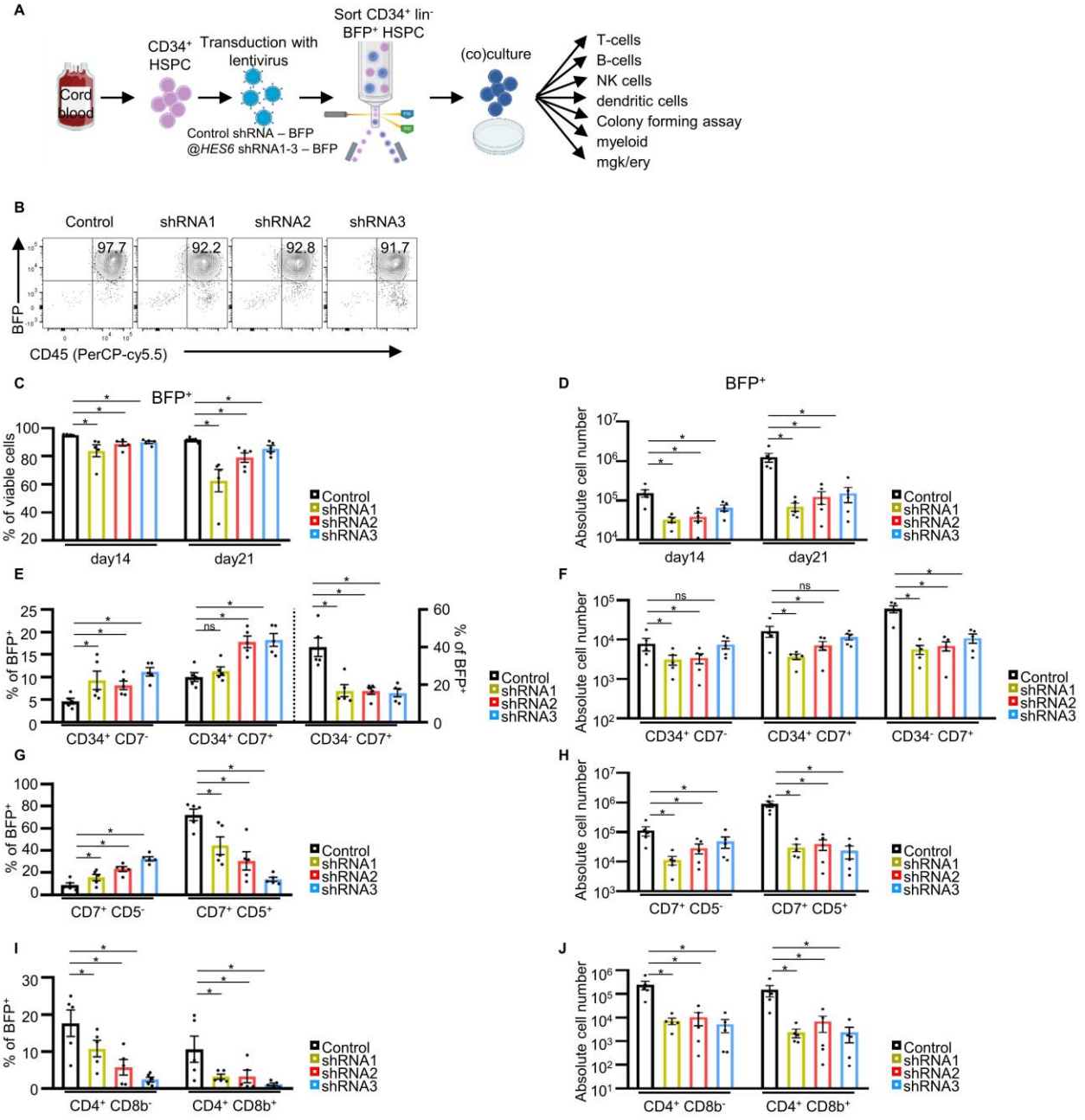
SUPPLEMENTAL FIGURE1



Supplemental Figure 1. *HES6* expression in human post-natal thymocytes. (A) Line plots showing log_2 normalized mRNA expression of *HES1* (black), *HES4* (blue) and *HES6* (red) based on bulk RNA-seq on sorted subsets of human post-natal thymocytes[17]. Data is presented as the average expression of two independent experiments from two donors and error bars indicate the standard error of the mean (SEM). CB: cord blood; ISP: immature single positive ($CD4^+$); DP: double positive ($CD4^+CD8^+$); SP: single positive. (B-C) Bar graphs derived from micro-array data show log_2 normalized probe intensities for *DTX1*, *HES1* and *HES6* after culture of cord blood (CB) $CD34^+$ HSPCs for 72 hours (B) or $CD34^+$ thymocytes (CT) for 48 hours (C) on OP9-GFP (Notch OFF) or OP9-DLL1 (Notch ON) feeder [18,19]. Data is presented as the average expression of three (B) and four (C) replicates and error bars indicate SEM. (D-G) Results derived from scRNA-seq data of human post-natal thymocytes [20]. UMAP visualization with annotation of differentiation stadia (D) and cell-cycle stages (F) and boxplots (E,G) showing pseudo-bulk log-normalized imputed mRNA expression level of *HES1* across differentiation stadia (E) and showing pseudo-bulk log-normalized imputed mRNA expression level of *HES6* across cell-cycle stadia (G). DN:

double negative ($CD4^-CD8^-$); DP: double positive ($CD4^+CD8^+$); SP: single positive. Boxplots show median and first and third quartile.

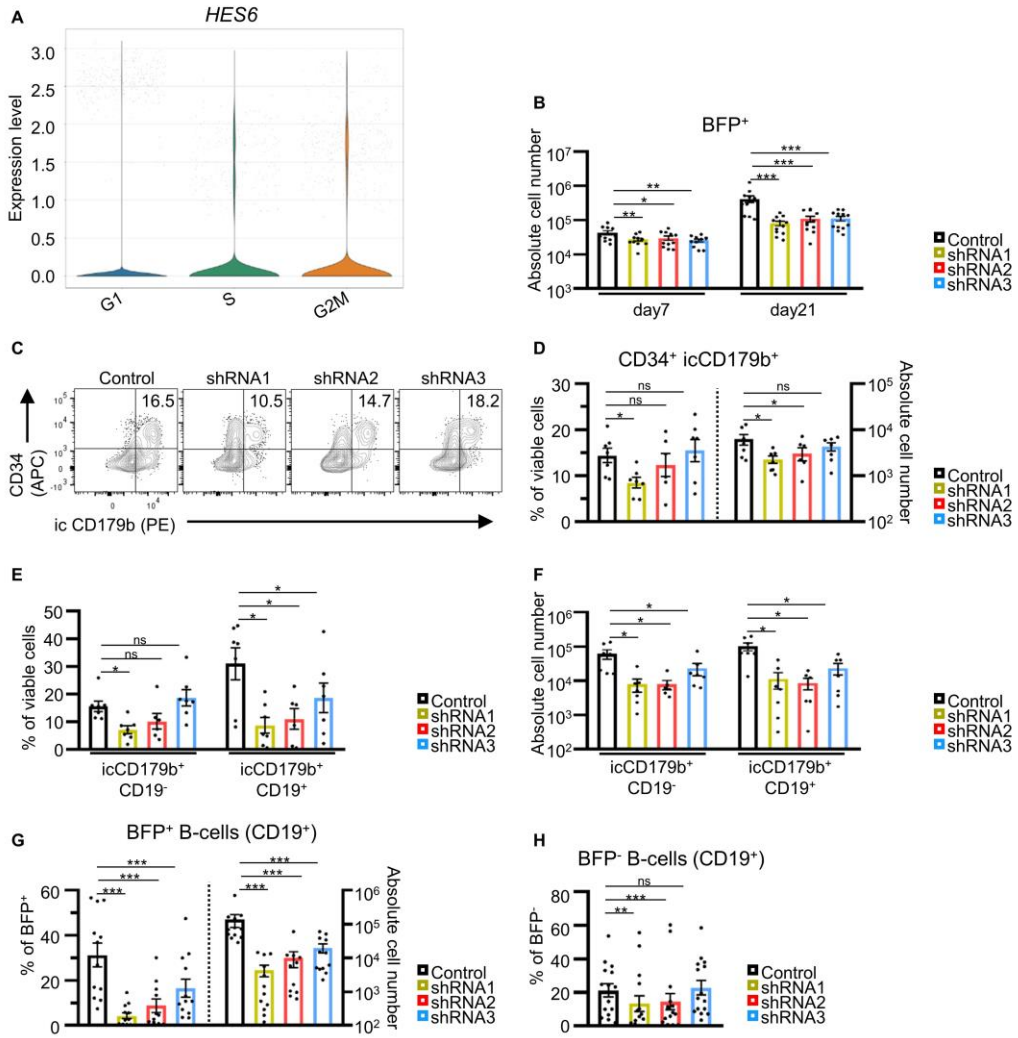
SUPPLEMENTAL FIGURE2



Supplemental Figure 2. *HES6* is important for early *in vitro* T-cell development. (A) Schematic overview illustrating the experimental workflow used to study the impact of *HES6* knockdown on human hematopoiesis. (B-J) Flow cytometric analysis (B) and bar graphs (C-J) of control shRNA and *HES6* shRNA-transduced CD34$^+$Lin$^-$BFP$^+$ HSPC cultured *in vitro* in T-lineage supporting conditions for a total of three weeks (N=5). Flow cytometric analysis of CD45 and BFP after 14 days (B) and bar graphs showing CD45$^+$BFP$^+$ frequencies within live cells (C) and absolute cell numbers of CD45$^+$BFP$^+$ cells (D) at different time points. Bar graphs showing frequencies (E) and absolute cell numbers (F) of populations based on CD34 and CD7 expression within BFP$^+$ population at day 14. Bar graphs showing frequencies (G) and absolute cell numbers (H) of populations based on CD7 and CD5 expression within BFP$^+$ population at

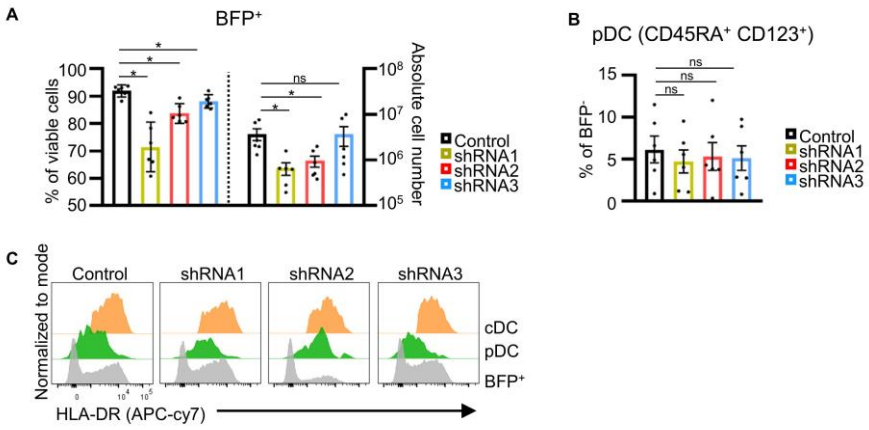
day 21. Bar graphs showing frequencies (I) and absolute cell numbers (J) of populations based on CD4 and CD8b expression within BFP^+ population at day 21. (C-J) Data are presented as average of five replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05; ns: not significant). Contour plots shown are representative for five replicates.

SUPPLEMENTAL FIGURE3



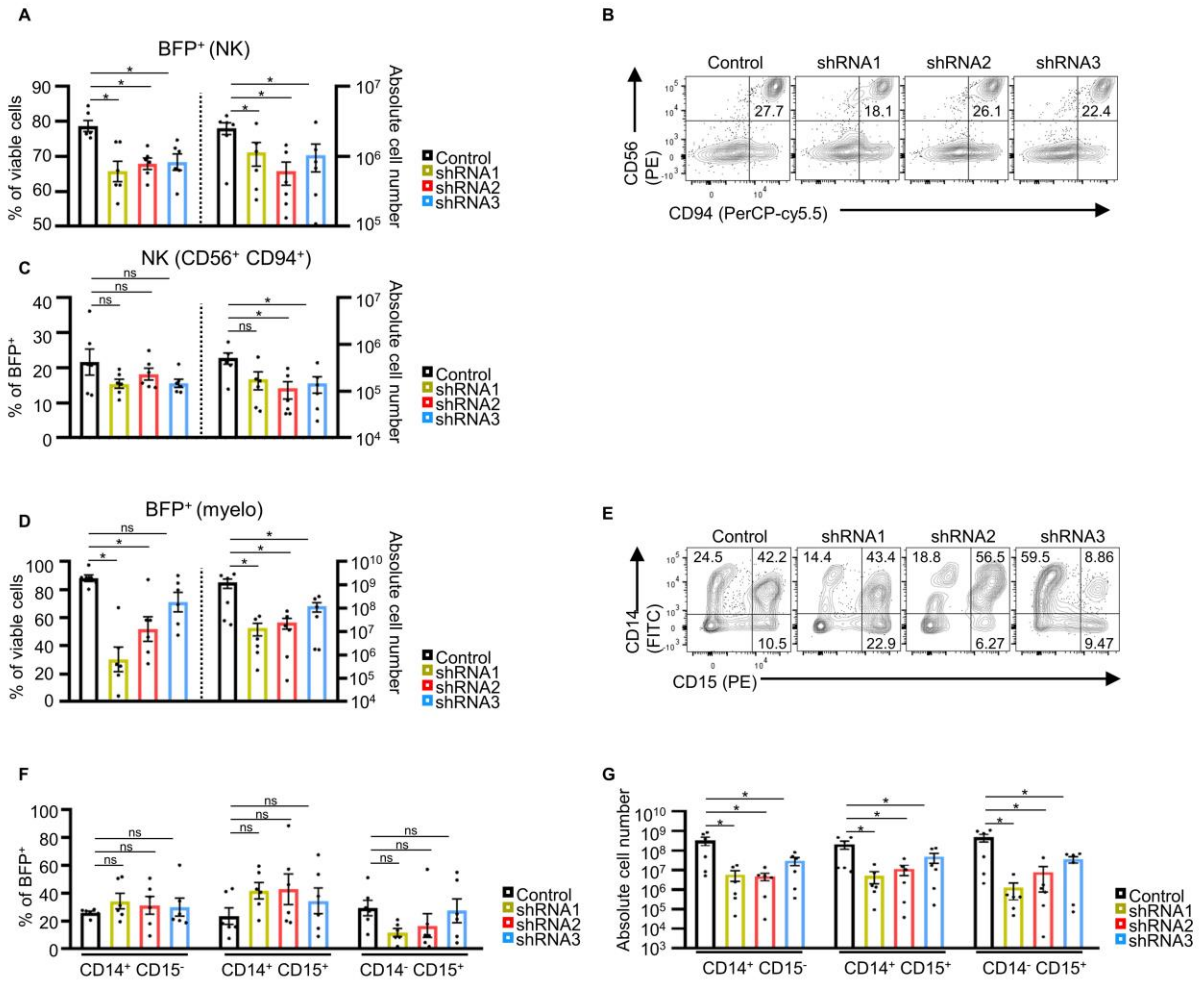
Supplemental Figure 3. *HES6* is important for early *in vitro* B-cell development. (A) Violin plot showing pseudo-bulk log-normalized expression level of *HES6* in different cell-cycle stages within B cells from human bone marrow (Figure 2B). (B-H) Flow cytometric analysis (C) and bar graphs (B, D-H) of control shRNA and *HES6* shRNA-transduced $CD34^+Lin^-BFP^+$ HSPC cultured *in vitro* in B-lineage supporting conditions for a total of three weeks. (B) Bar graphs showing absolute cell numbers of $CD45^+BFP^+$ cells at day 7 (N=11) and day 21 (N=12; shRNA2: N=11). Flow cytometric analysis (C) showing gating and bar graphs (D) showing frequencies and absolute cell numbers of $CD34^+$ and intracellular (ic) $CD179b^+$ population within live cells at day 7 (N=7; shRNA2: N=6). Contour plots (C) with frequencies shown are from a representative replicate. Bar graphs showing frequencies (E) and absolute cell numbers (F) of $CD19^+$ $icCD179b^+$ population within live cells at day 21 (N=7; shRNA2: N=6), and frequencies and absolute cell numbers of B-cells ($CD19^+$) within $CD45^+BFP^+$ cells (G) or frequencies of B-cells ($CD19^+$) within $CD45^+BFP^-$ cells (H) at day 21 (N=12; shRNA2: N=11). Data are presented as average of all replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05, **P<0.01; ***P<0.001; ns: not significant).

SUPPLEMENTAL FIGURE4



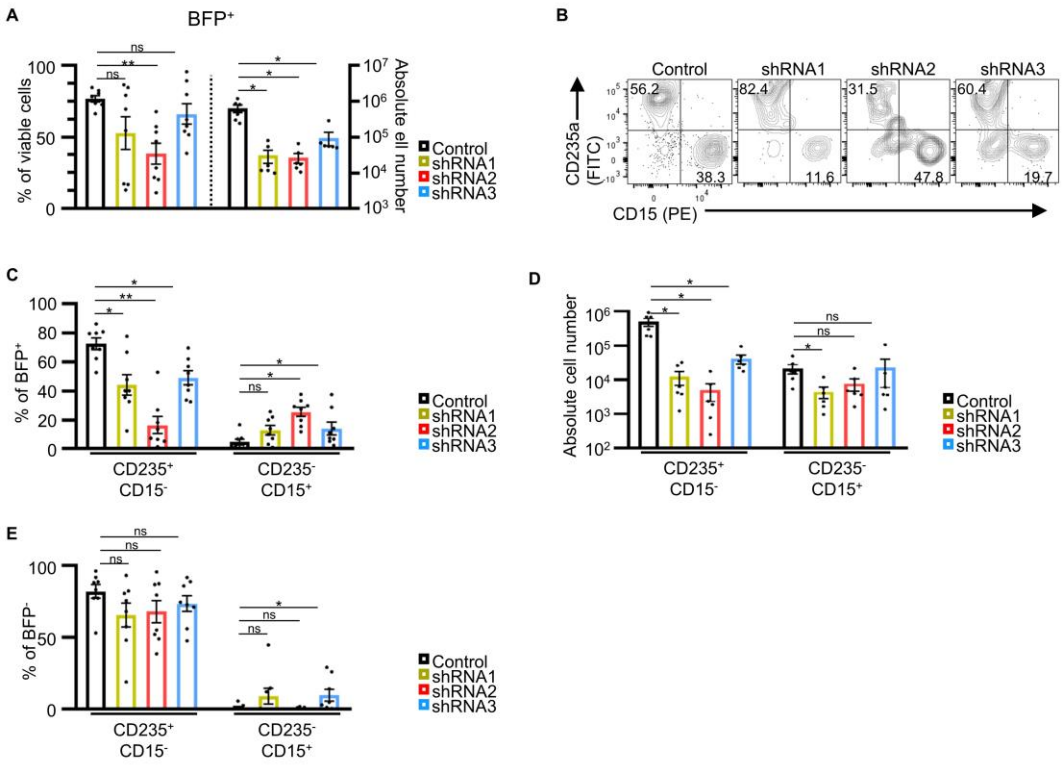
Supplemental Figure 4. *HES6* knockdown impairs *in vitro* pDC development. Bar graphs (A-B) and flow cytometric analysis (C) of control shRNA and *HES6* shRNA-transduced $CD34^+Lin^-BFP^+$ HSPC cultured in DC-lineage supporting conditions for a total of two weeks (N=6), showing frequencies and absolute cell numbers of $CD45^+$ BFP^+ cells, gated on live cells (A) and frequencies of pDCs ($CD45RA^+CD123^+$) within the BFP^- population (B) at day 14. (C) Histogram showing HLA-DR expression in all BFP^+ cells (grey), pDCs (green) and cDCs ($CD4^+$HLA-DR^+ non-pDCs) (orange) at day 14 for control or *HES6* knockdown. (A-B) Data are presented as average of six replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05; ns: not significant).

SUPPLEMENTAL FIGURE5



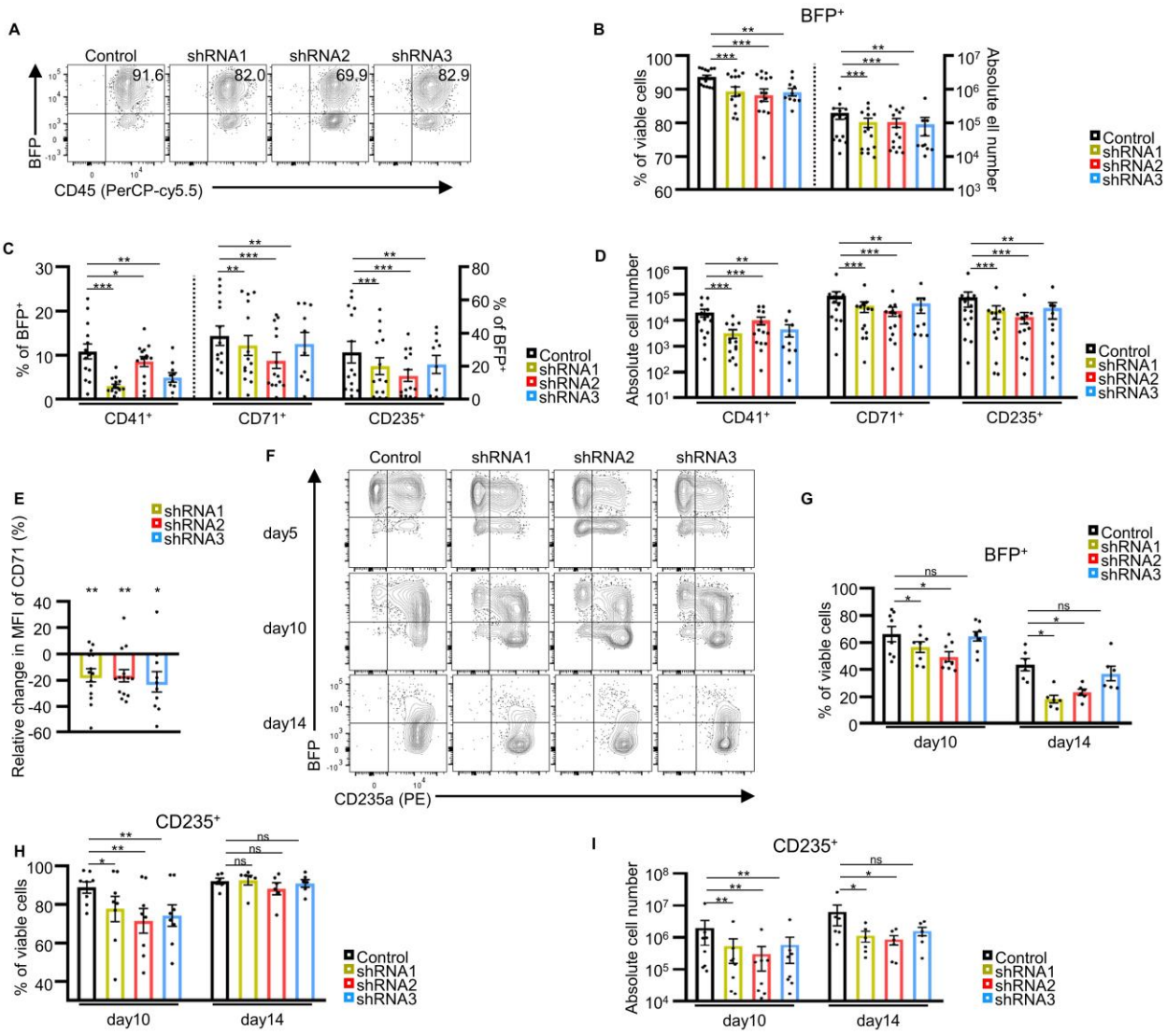
Supplemental Figure 5. *HES6* knockdown doesn't affect *in vitro* NK and myeloid development. (A-C) Flow cytometric analysis (B) and bar graphs (A, C) of control shRNA and *HES6* shRNA-transduced $CD34^+Lin^-BFP^+$ HSPC cultured in NK-lineage supporting conditions for a total of three weeks (N=6), showing frequencies and absolute cell numbers of $CD45^+BFP^+$ cells (A) and gating (B), frequencies and absolute cell numbers (C) of NK cells ($CD56^+CD94^+$) within BFP^+ population at day 21. (D-G) Bar graphs (D, F-G) and flow cytometric analysis (E) of control shRNA and *HES6* shRNA-transduced $CD34^+Lin^-BFP^+$ HSPC cultured in myeloid-lineage supporting conditions for a total of four weeks (N=6), showing frequencies and absolute cell numbers of $CD45^+BFP^+$ cells (D) and frequencies and absolute cell numbers of cell populations based on CD14 and CD15 expression within BFP^+ cells (E-G) at day 28. (B,E) Contour plots shown are representative for six replicates. (A-G) Data are presented as average of six replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05; ns: not significant).

SUPPLEMENTAL FIGURE6



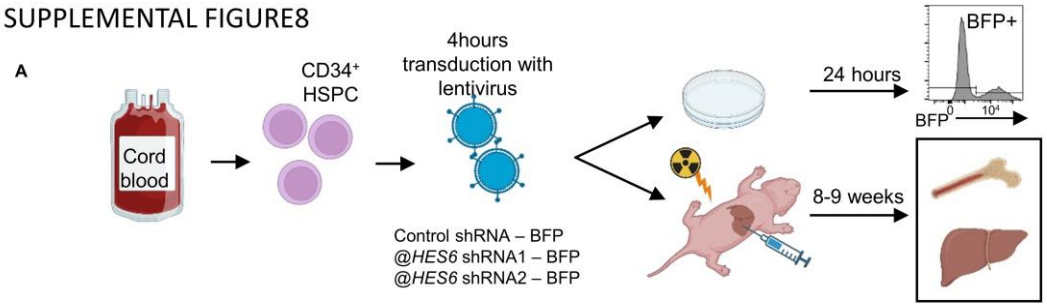
Supplemental Figure 6. *HES6* knockdown impairs colony forming capacity of HSPCs. (A-E) Flow cytometric analysis (B) and bar graphs (A, C-E) of control shRNA and *HES6* shRNA-transduced $CD34^{+}Lin^{-}BFP^{+}$ HSPC cultured in semi-solid colony-forming assay, Methocult, for a total of 10 days. (A) Bar graphs showing frequencies (N=8) and absolute cell numbers (N=6) of $CD45^{+}BFP^{+}$ cells, gated on live cells. (B-D) Contour plots (B) showing CD235a and CD15 expression within $CD45^{+}BFP^{+}$ cells and bar graphs showing frequencies (C) and absolute cell numbers (D) of erythroid ($CD235a^{+}CD15^{-}$) and myeloid ($CD235a^{-}CD15^{+}$) cells within BFP^{+} cells (N=6). Contour plots shown are representative for eight replicates. (E) Bar graphs showing frequencies of erythroid ($CD235a^{+}CD15^{-}$) and myeloid ($CD235a^{-}CD15^{+}$) cells within BFP^{-} cells (N=8). Data are presented as average of all replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05, **P<0.01; ns: not significant).

SUPPLEMENTAL FIGURE7



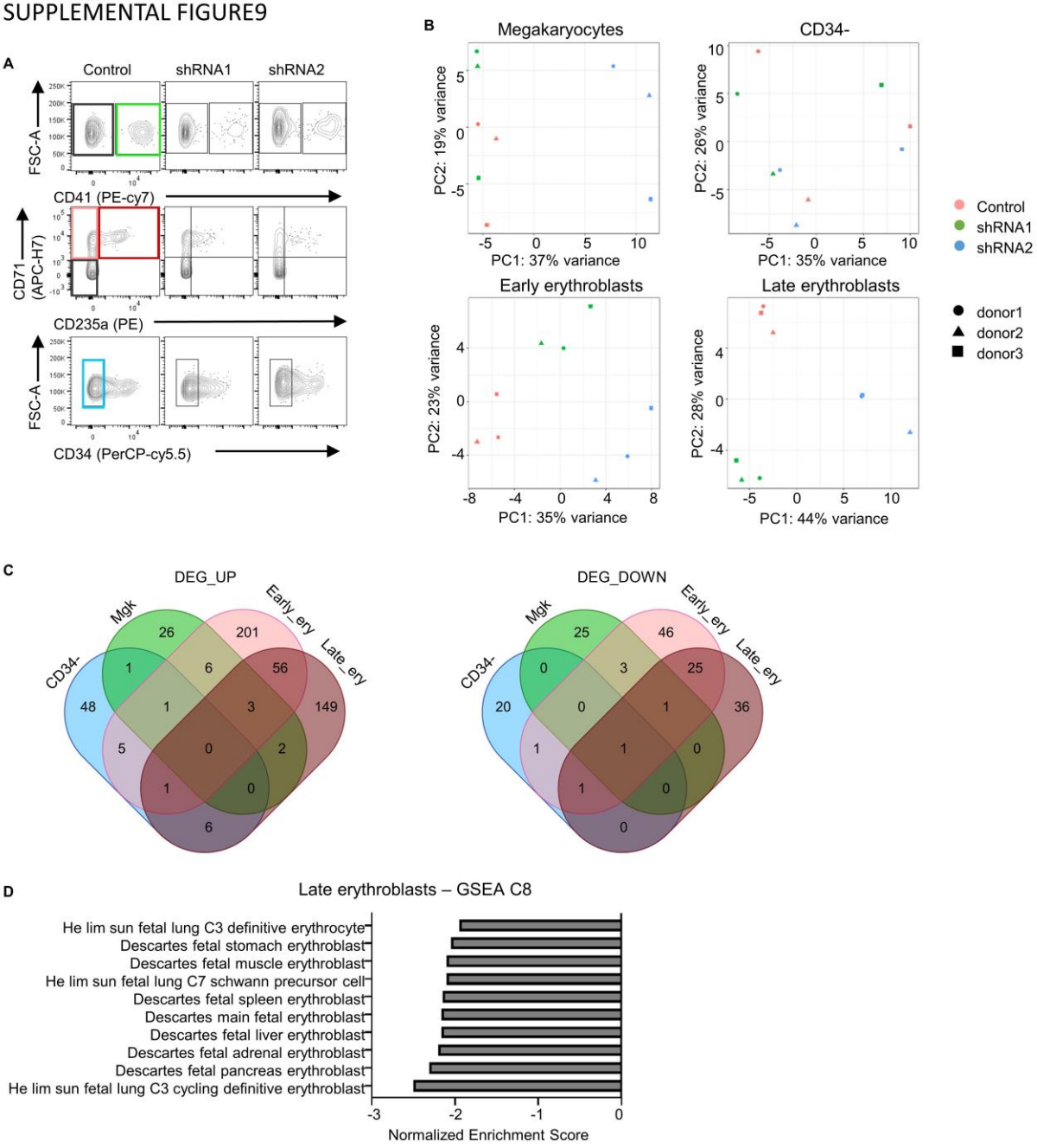
Supplemental Figure 7. *HES6* knockdown reduces *in vitro* megakaryocyte and erythroid development. Flow cytometric analysis (A, F) and bar graph (B-E, G-I) of control shRNA and *HES6* shRNA-transduced CD34$^+$Lin$^-$BFP$^+$ HSPC cultured in megakaryocyte/erythroid-lineage supporting conditions for a total of two weeks. (A-B) Contour plots (A) showing gating of CD45$^+$BFP$^+$ population and the frequencies and total cell numbers (B) of this population within live cells at day five (N=14, shRNA3: N=11). (B-D) Bar graphs showing frequencies (C) and absolute cell numbers (D) of megakaryocytes (CD41$^+$) and CD71$^+$ or CD235a$^+$ erythroblasts, gated as shown in Figure 4E-F), within CD45$^+$BFP$^+$ population at day five (N=14, shRNA3: N=11). (E) Relative change in MFI of CD71 expression within the BFP$^+$ CD71$^+$ erythroblasts (gating as shown in Figure 4E-F) at day five for the three *HES6* shRNA conditions compared to the control (N=14, shRNA3: N=11). Data are presented as average of the replicates ± standard error of the mean (SEM) (one sample Wilcoxon test). (F-G) Contour plots (F) and bar graphs (G) showing BFP downregulation during erythroid (CD235a upregulation) development in all conditions, which explains

extensive BFP downregulation over time (day10: N=8, day14: N=6). (H-I) Bar graphs showing frequencies (H) and absolute cell numbers (I) of $CD235^{+}$ erythroblasts (gating as shown in Figure 4E-F) within viable cell population at day 10 (N=8) and day 14 (N=6). (A, F) Contour plots shown are representative for one of the replicates. (B-D, G-I) Data are presented as average of the replicates ± (Wilcoxon matched-pairs signed-rank test, *P<0.05, **P<0.01; ***P<0.001; ns: not significant).



Supplemental Figure 8. *HES6* knockdown *in vivo* experimental set-up. (A) Schematic overview showing preculture, transduction and intrahepatic injection of cord blood $CD34^+$ enriched HSPC (BFP^+ and BFP^-) in NSG mice, which were sublethally irradiated and analyzed after 8-9 weeks (results are shown in Figure 5).

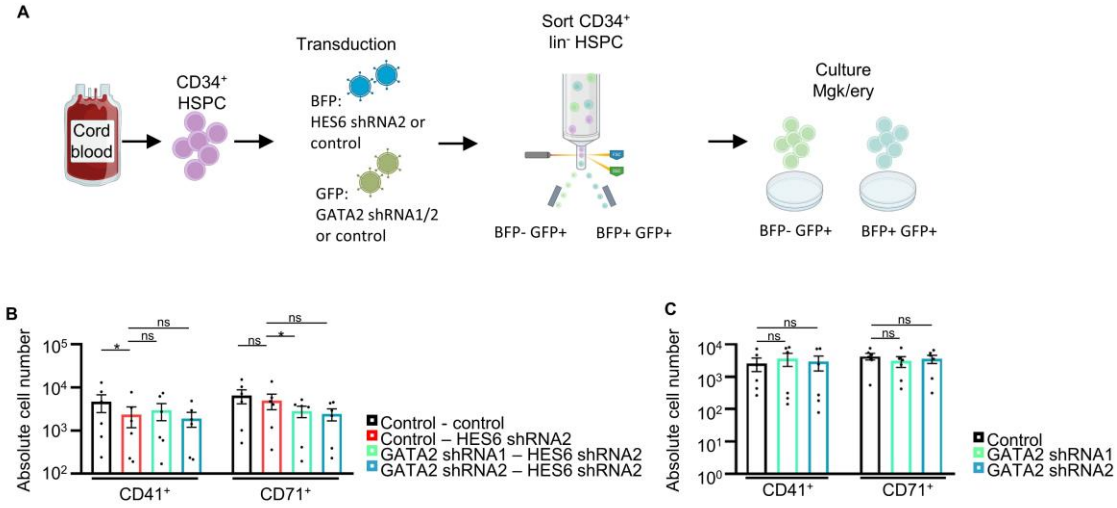
SUPPLEMENTAL FIGURE9



Supplemental Figure 9. RNA-seq analysis reveals molecular pathways downstream of *HES6* knockdown during erythroid development. (A) Flow cytometry data of sorted subpopulations of control shRNA and *HES6* shRNA-transduced (shRNA1 and shRNA2 were used) $CD34^{+}Lin^{-}BFP^{+}$ HSPC cultured for four days in megakaryocyte/erythroid-lineage supporting conditions. Bulk RNA-seq was performed on these four subpopulations. Sorted megakaryocytes ($CD41^{+}$, green) within BFP^{+} population, erythroblasts (early erythroblasts, pink, $CD71^{+}CD235^{-}$ and late erythroblasts, red, $CD71^{+}CD235^{+}$) are gated within $BFP^{+}CD41^{-}$ population and $CD34^{-}$ precursors are gated within $BFP^{+}CD41^{-}CD71^{-}CD235^{-}$ population (light blue, $CD34^{-}$). Contour plots shown are representative for three independent donors. (B-D) Results from data analysis of bulk RNA-seq of cell populations as discussed in Figure 6. (B) PCA plots from three

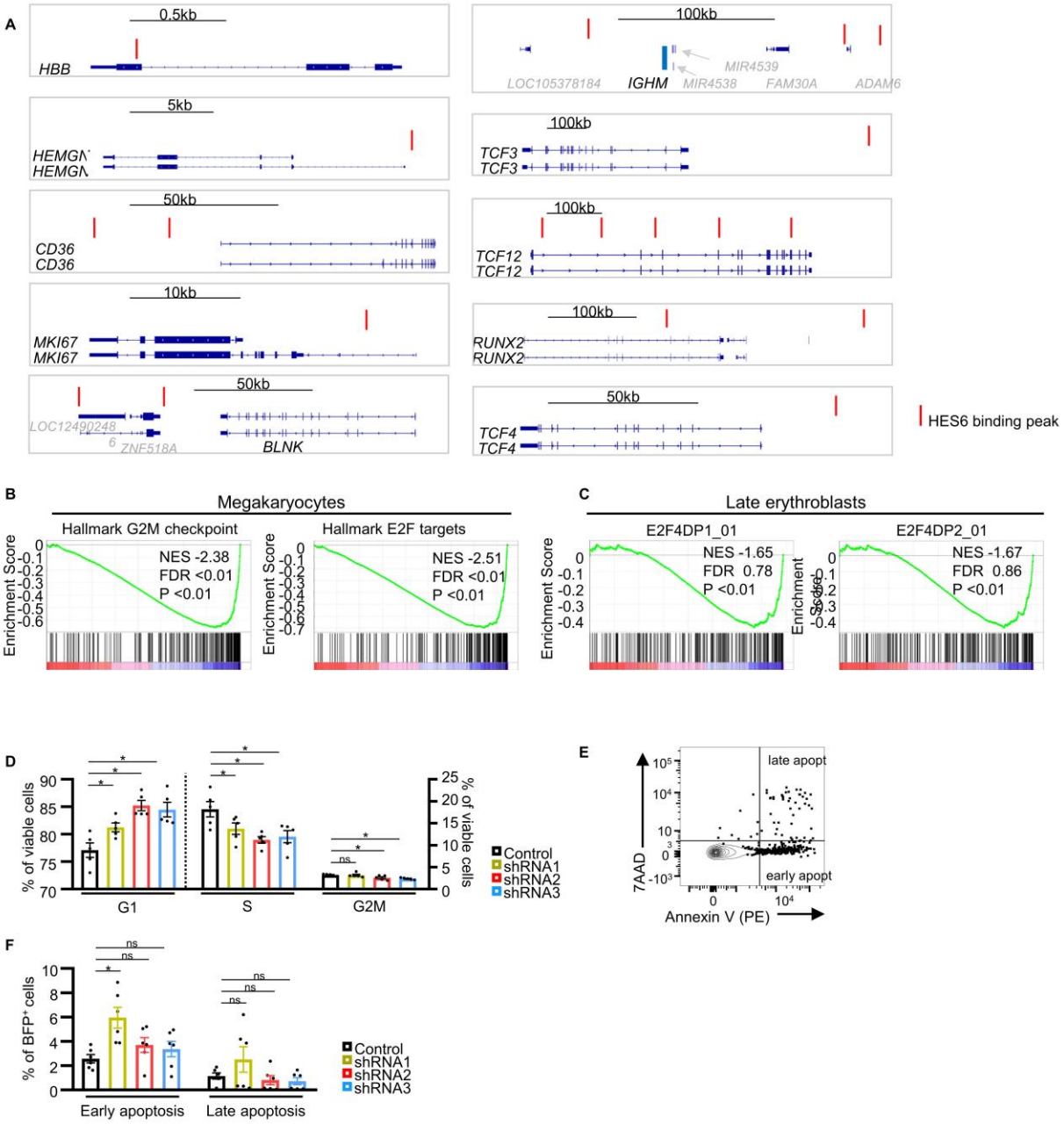
independent donors with two *HES6* knockdown conditions and control condition for megakaryocytes ($CD41^+$), $CD34^-$ precursor cells and early ($CD71^+CD235^-$) and late ($CD71^+CD235^+$) erythroblasts. (C) Venn diagram showing overlap between differentially up- and downregulated genes (DEG_UP and DEG_down respectively) in different cell populations between control and HES6 knockdown (Table S6-13). (D) GSEA results showing normalized enrichment score for top 10 of gene sets (FDR <25% and p<0.01) enriched in control sample within late erythroblasts.

SUPPLEMENTAL FIGURE10



Supplemental Figure 10. *GATA2* knockdown fails to rescue impaired *in vitro* megakaryo-erythroid development upon *HES6* knockdown. (A) Schematic overview illustrating the experimental workflow used to study the impact of double *GATA2-HES6* knockdown (GFP^+BFP^+) or single *GATA2* knockdown (GFP^+BFP^-) on human megakaryo-erythropoiesis. (B-C) Bar graphs showing results of *in vitro* experiments to study the impact of double *GATA2-HES6* knockdown (GFP^+BFP^+) (B) or single *GATA2* knockdown (GFP^+BFP^-) (C) on human megakaryo-erythropoiesis (N=6) as described in Figure 7. Bar graphs showing absolute cell numbers of indicated subpopulations within GFP^+BFP^+ population (B) or GFP^+BFP^- population (C) in the different knockdown conditions. Data are presented as average of six replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05; ns: not significant).

SUPPLEMENTAL FIGURE11



Supplemental Figure 11. *HES6* knockdown impacts cell cycle progression. (A) Analysis of recently published ChIP-seq data of *HES6* in erythroid cells[21] and significant *HES6* binding peaks are indicated in red. Genes shown are important for erythroid (*HBB*), megakaryocyte (*HEMGN*, *CD36*, *MKI67*), T- and B-cell (*BLNK*, *IGHM*, *TCF3*, *TCF12*) and pDC (*RUNX2*, *TCF4*) development. (B-C) Results from data analysis of bulk RNA-seq of sorted cell populations as discussed in Figure 6. Preranked GSEA results showing enrichment of cell-cycle related gene sets in control sample within megakaryocytes (B) and showing enrichment, though with a high FDR, of genes with *E2F4* motif regions within region around transcription start site in control sample within late erythroblasts (C). NES: Normalized enrichment score; FDR: false detection rate. (D-F) Flow cytometry analysis (E) and bar graphs (D, F) of control shRNA

and *HES6* shRNA-transduced $CD34^{+}Lin^{-}BFP^{+}$ HSPC cultured in megakaryocyte/erythroid-lineage supporting conditions for three days. (D) Bar graphs showing frequencies of different cell cycle stages, gating as shown in Figure 8C, within live population, at day3 (N=5). (E-F) Contour plot showing gating (E) and bar graphs showing frequencies (F) of early and late apoptosis within BFP^{+} population at day3 (N=6). Contour plot shown is representative for six replicates. Data are presented as average of all replicates ± standard error of the mean (Wilcoxon matched-pairs signed-rank test, *P<0.05, ns: not significant).

References

1. Van De Walle I, Dolens AC, Durinck K, et al. GATA3 induces human T-cell commitment by restraining Notch activity and repressing NK-cell fate. Nat Commun. 2016;7.

2. Dolens A, Durinck K, Lavaert M, et al. Distinct Notch1 and BCL11B requirements mediate human γδ/αβ T cell development. EMBO Rep. 2020;21(5).

3. Xu Y, Liu X, Zhang H, et al. Overexpression of HES6 has prognostic value and promotes metastasis via the Wnt/ß-catenin signaling pathway in colorectal cancer. Oncol Rep. 2018;40(3):1261-1274.

4. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7):research0034.1. doi:10.1186/gb-2002-3-7-research0034

5. Roels J, Van Hulle J, Lavaert M, et al. Transcriptional dynamics and epigenetic regulation of E and ID protein encoding genes during human T cell development. Front Immunol. 2022;13(July):1-24.

6. Lavaert M, Liang KL, Vandamme N, et al. Integrated scRNA-Seq Identifies Human Postnatal Thymus Seeding Progenitors and Regulatory Dynamics of Differentiating Immature Thymocytes. Immunity. 2020;52(6):1088-1104.e6. doi:10.1016/j.immuni.2020.03.019

7. Park JE, Botting RA, Domínguez Conde C, et al. A cell atlas of human thymic development defines T cell repertoire formation. Science (1979). 2020;367(6480):eaay3224.

8. Le J, Park JE, Ha VL, et al. Single-Cell RNA-Seq Mapping of Human Thymopoiesis Reveals Lineage Specification Trajectories and a Commitment Spectrum in T Cell Development. Immunity. 2020;52(6):1105-1118.e9.

9. van Dijk D, Sharma R, Nainys J, et al. Recovering Gene Interactions from Single-Cell Data Using Data Diffusion. Cell. 2018;174(3):716-729.e27.

10. Stuart T, Butler A, Hoffman P, et al. Comprehensive Integration of Single-Cell Data. Cell. 2019;177(7):1888-1902.e21.

11. Setty M, Kiseliovas V, Levine J, Gayoso A, Mazutis L, Pe'er D. Characterization of cell fate probabilities in single-cell data with Palantir. Nat Biotechnol. 2019;37(4):451-460.

12. Suo C, Dann E, Goh I, et al. Mapping the developing human immune system across organs. Science (1979). 2022;376(6597).

13. Domínguez Conde C, Xu C, Jarvis LB, et al. Cross-tissue immune cell analysis reveals tissue-specific features in humans. Science (1979). 2023;376(6594):eabl5197.

14. Filtjens J, Taveirne S, Van Acker A, et al. Abundant stage-dependent Ly49E expression by liver NK cells is not essential for their differentiation and function. J Leukoc Biol. 2013;93(5):699-711.

15. De Decker M, Lavaert M, Roels J, et al. HES1 and HES4 have non-redundant roles downstream of Notch during early human T-cell development. Haematologica. 2020;106(1):130-141.

16. Kirkling ME, Cytlak U, Lau CM, et al. Notch Signaling Facilitates In Vitro Generation of Cross-Presenting Classical Dendritic Cells. Cell Rep. 2018;23(12):3658-3672.e6.

17. Roels J, Kuchmiy A, De Decker M, et al. Distinct and temporary-restricted epigenetic mechanisms regulate human αβ and γδ T cell development. Nat Immunol. 2020;21(10):1280-1292.

18. Durinck K, Wallaert A, Van de Walle I, et al. The notch driven long non-coding RNA repertoire in T-cell acute lymphoblastic leukemia. Haematologica. 2014;99(12):1808-1816.

19. Loontiens S, Dolens AC, Strubbe S, et al. PHF6 Expression Levels Impact Human Hematopoietic Stem Cell Differentiation. Front Cell Dev Biol. 2020;8.

20. Roels J, Van Hulle J, Lavaert M, et al. Transcriptional dynamics and epigenetic regulation of E and ID protein encoding genes during human T cell development. Front Immunol. 2022;13(July):1-24.

21. Wang Z, Wang P, Zhang J, et al. The novel GATA1-interacting protein HES6 is an essential transcriptional cofactor for human erythropoiesis. Nucleic Acids Res. Published online March 17, 2023.