Supplemental Information for: '*HES6* knockdown in human hematopoietic precursor cells reduces their *in vivo* engraftment potential and their capacity to differentiate into erythroid, B, T 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 (BioRad, #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: burstforming 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 antimouse 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, #005523-00). 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 megakaryocyteerythroid 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)cultur e	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 Flt3L (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, #12000014) with 20% FCS	SCF and GMCSF (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 colonyforming 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 S

3. 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 S

4. 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)

Table S

* 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

5. 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 S . RNA-

6 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 S . RNA-

7 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 S . RNA-
8 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

Table S . RNA-

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

9 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

Table S

-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

10. 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

Table S . RNA-

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

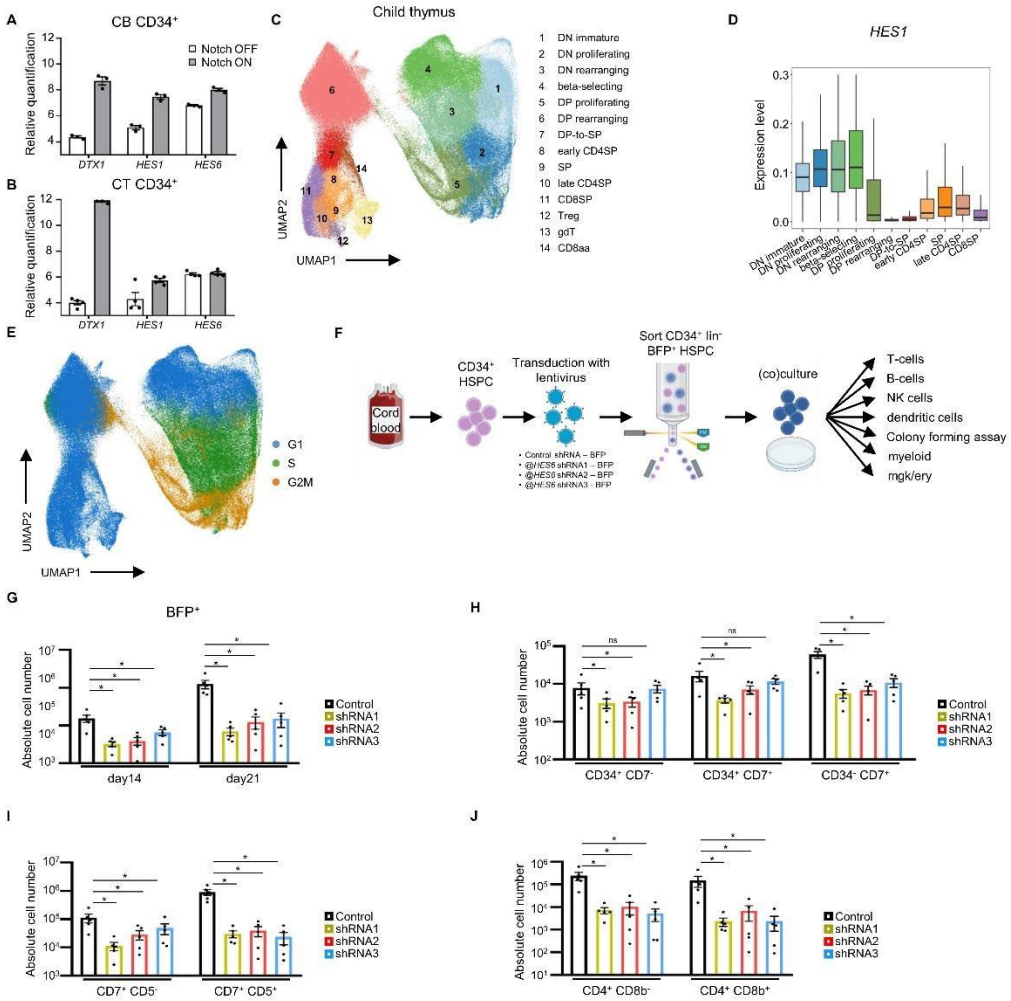
Table S13. RNA-seq results: down-regulated genes (HES6 knockdown vs control) in late erythroblasts

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

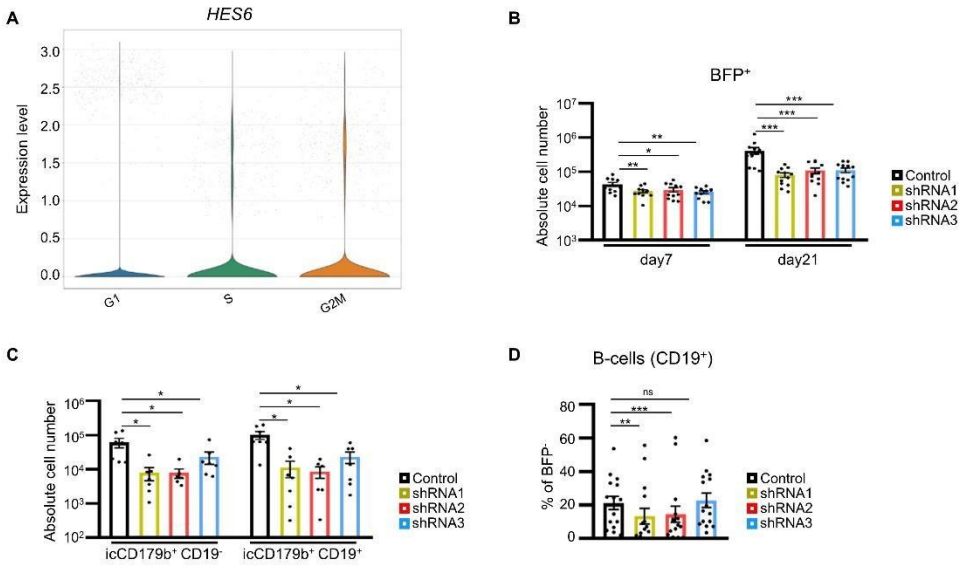
Supplemental figures

SUPPLEMENTAL FIGURE1



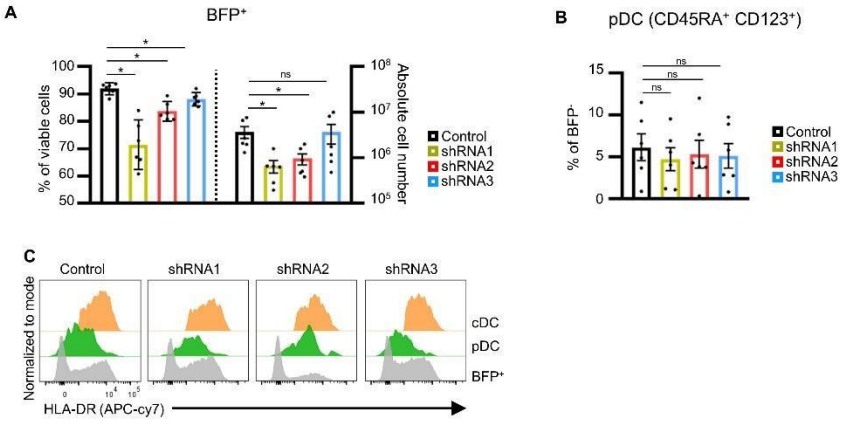
Supplemental Figure 1. (A-B) Bar graphs derived from micro-array data show log_2 normalized probe intensities for *DTX1*, *HES1* and *HES6* after culture of cord blood (CB) $CD34^+$ HSPCs for 72 hours (A) or $CD34^+$ thymocytes (CT) for 48 hours (B) on OP9-GFP (Notch OFF) or OP9-DLL1 (Notch ON) feeder [17,18]. Data is presented as the average expression of three (A) and four (B) replicates and error bars indicate standard error of the mean (SEM). (C-E) Results derived from scRNA-seq data of human post-natal thymocytes [19]. UMAP visualization with annotation of differentiation stadia (C) and cell-cycle stages (E) and boxplots (D) showing pseudo-bulk log-normalized imputed mRNA expression level of *HES1* across differentiation stadia of as annotated in C. DN: double negative ($CD4^-CD8^-$); DP: double positive ($CD4^+CD8^+$); SP: single positive. (F) Schematic overview illustrating the experimental workflow used to study the impact of *HES6* knockdown on human hematopoiesis. (G-J) Bar graphs of control shRNA and *HES6* shRNA-transduced $CD34^+Lin^-BFP^+$ HSPC cultured in T-lineage supporting conditions for a total of three weeks (N=5). Graphs showing absolute cell numbers of $CD45^+BFP^+$ cells at different time points (G) and of populations based on CD34 and CD7 expression at day 14 (H), CD7 and CD5 at day 21 (I) and CD4 and CD8b at day 21 (J). Data are presented as average of five replicates ± standard error of the mean (SEM) (Wilcoxon matched-pairs signed-rank test). *P<0.05; ns: not significant.

SUPPLEMENTAL FIGURE2



Supplemental Figure 2. (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-D) Bar graphs of control shRNA and *HES6* shRNA-transduced $CD34^+Lin^-BFP^+$ HSPC cultured in B-lineage supporting conditions for a total of three weeks, showing absolute cell numbers of $CD45^+BFP^+$ cells at day 7 (N=11) and day 21 (N=12; shRNA2: N=11) (B) and $icCD179b^+CD19^-$ and $icCD179b^+CD19^+$ cells at day 21 (N=7; shRNA2: N=6) (C). (D) Bar graph showing percentage of B-cells ($CD19^+$) within BFP^- population at day 21 (N=12; shRNA2: N=11). (B-D) Data are presented as average of all replicates ± SEM (Wilcoxon matchedpairs signed-rank test). *P<0.05, **P<0.01; ***P<0.001; ns: not significant.

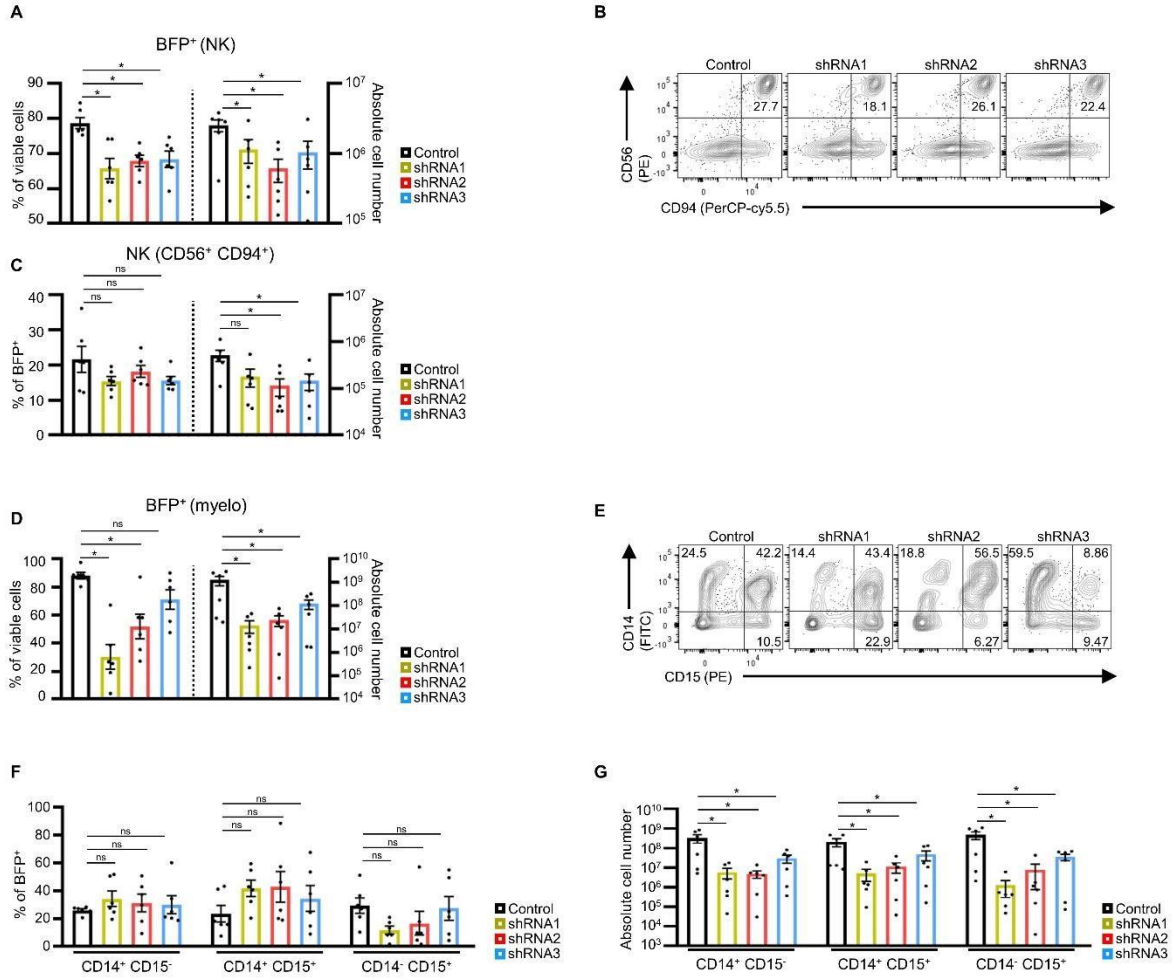
SUPPLEMENTAL FIGURE3



Supplemental Figure 3. 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

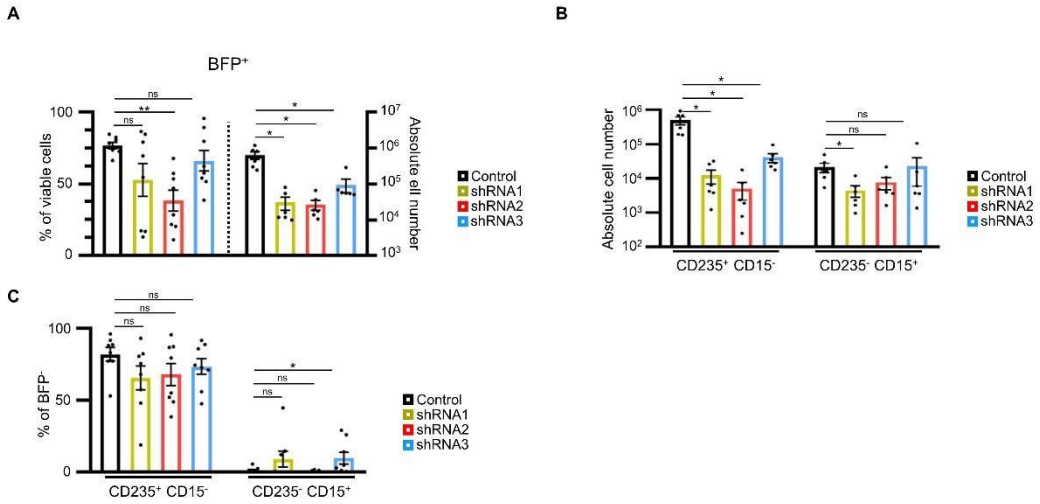
showing HLA-DR expression in all BFP$^+$ cells (grey), pDCs (green) and cDCs (CD4$^+$HLA-DR$^+$ non-pDCs) (orange) at day 14 for control or *HES6* knockdown. (A-B) Data are presented as average of six replicates ± SEM (Wilcoxon matched-pairs signed-rank test). *P<0.05; ns: not significant.

SUPPLEMENTAL FIGURE4



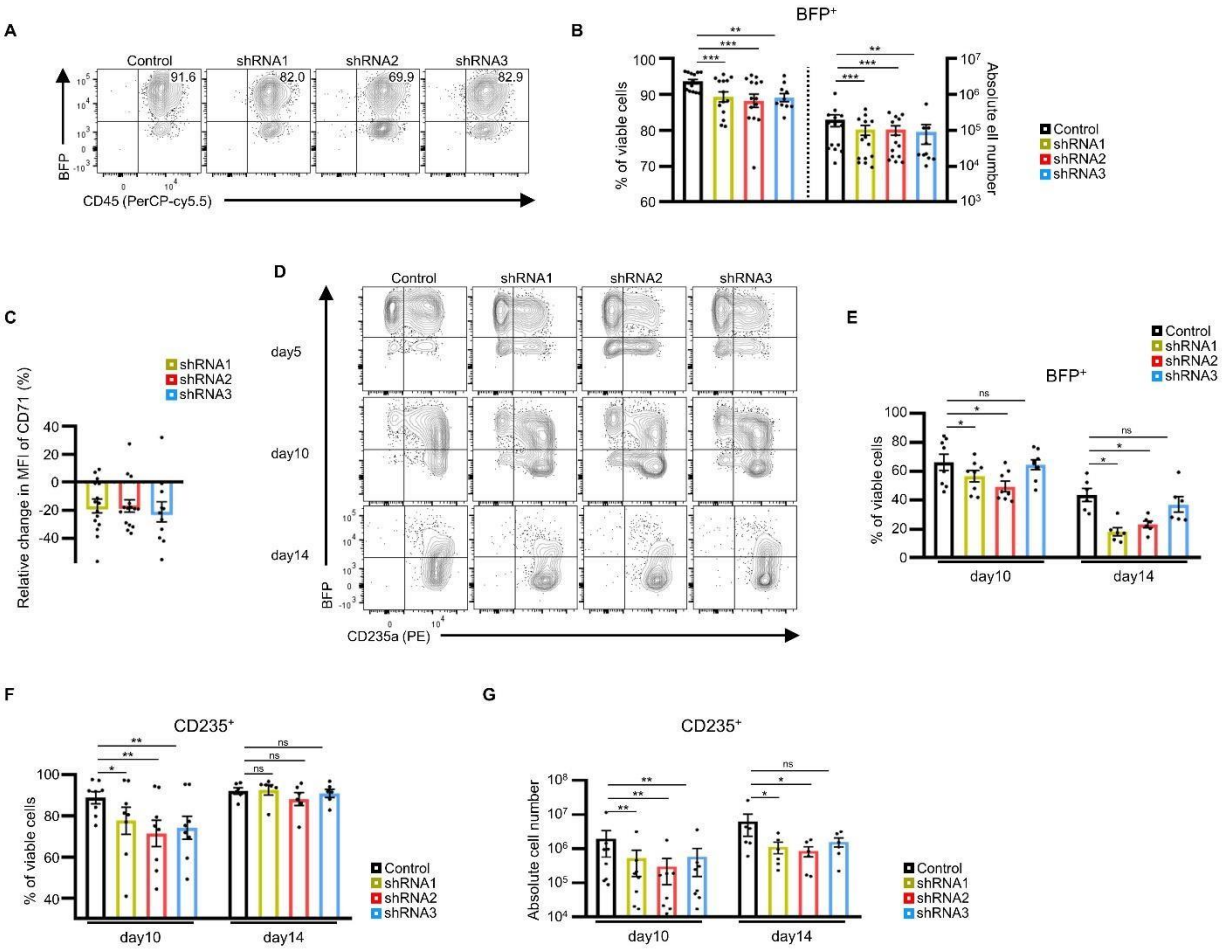
Supplemental Figure 4. (A-C) Flow cytometric analysis (B) and bar graphs (A, C) of control shRNA and *HES6* shRNA-transduced CD34$^+$Lin$^-$BFP$^+$ HSPC cultured in NK-lineage supporting conditions for a total of three weeks (N=6), showing frequencies and absolute cell numbers of CD45$^+$BFP$^+$ cells (A) and gating (B), frequencies and absolute cell numbers (C) of NK cells (CD56$^+$CD94$^+$) within BFP$^+$ population at day 21. (DG) 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 ± SEM (Wilcoxon matched-pairs signed-rank test). *P<0.05; ns: not significant.

SUPPLEMENTAL FIGURE5



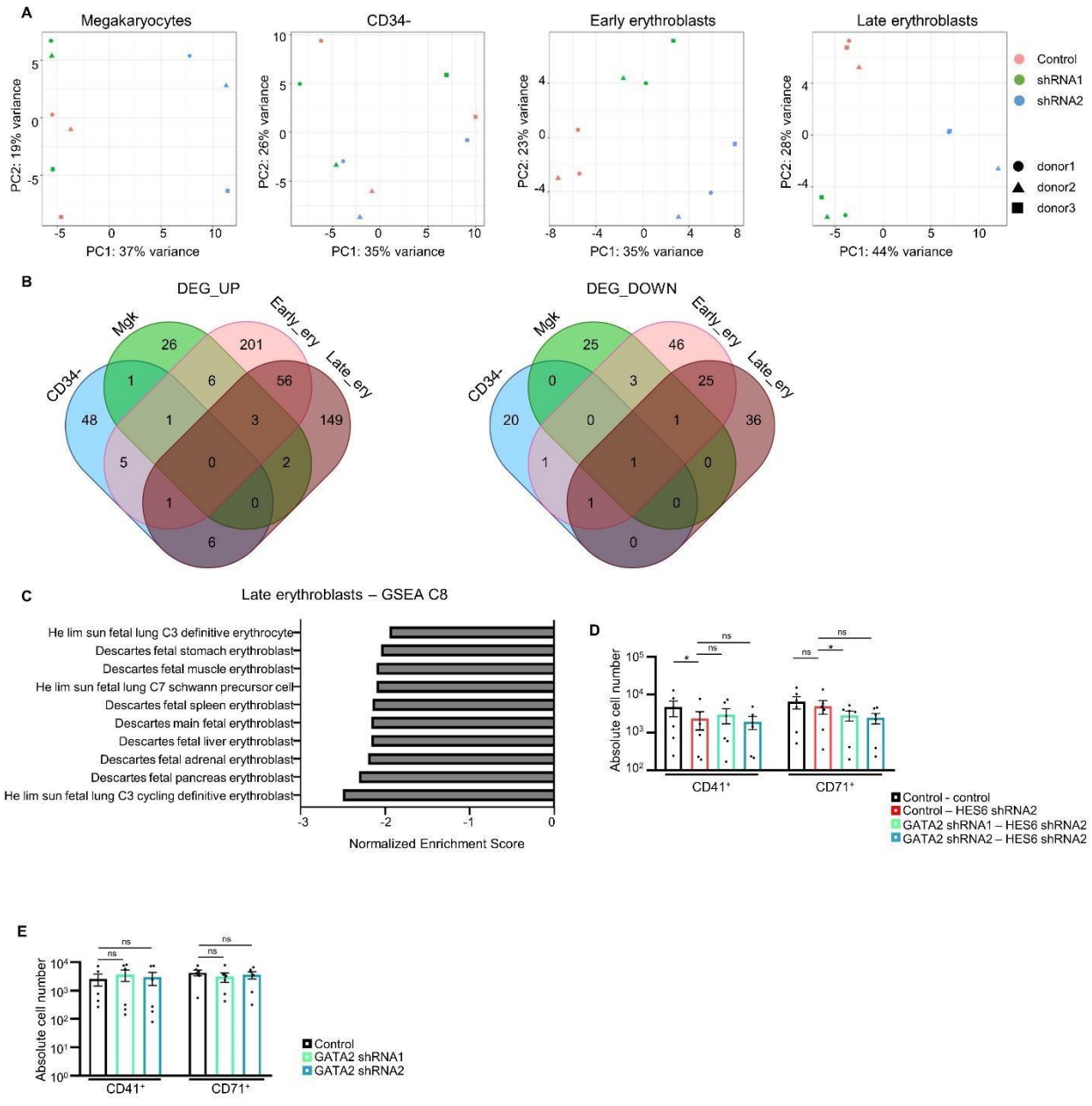
Supplemental Figure 5. (A-C) Bar graphs of control shRNA and *HES6* shRNA-transduced $CD34^{+}Lin^{-}BFP^{+}$ HSPC cultured in semi-solid colony-forming assay, Methocult, for a total of 10 days, showing frequencies (N=8) and absolute cell numbers (N=6) of $CD45^{+}BFP^{+}$ cells, gated on live cells (A), absolute cell numbers of erythroid ($CD235a^{+}CD15^{-}$) and myeloid ($CD235a^{-}CD15^{+}$) cells within BFP^{+} cells (N=6) (B) and frequencies of erythroid ($CD235a^{+}CD15^{-}$) and myeloid ($CD235a^{-}CD15^{+}$) cells within BFP^{-} cells (N=8) (C). (AC) Data are presented as average of all replicates ± SEM (Wilcoxon matched-pairs signed-rank test). *P<0.05, **P<0.01; ns: not significant.

SUPPLEMENTAL FIGURE6



Supplemental Figure 6. Flow cytometric analysis (A, D) and bar graph (B-C, E-G) 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) Gating (A) of CD45^{+}BFP^{+} population and the frequencies and total cell numbers (B) of this population at day5 (N=14). (C) Relative change in MFI of CD71 expression within the BFP^{+} early erythroblasts (gating as shown in Figure 4G-H) at day 5 for the three *HES6* shRNA conditions compared to the control. (D-E) Contour plots (D) showing BFP downregulation during erythroid (CD235a upregulation) development in all conditions, which explains extensive BFP downregulation over time (E). (F-G) Frequencies (F) and absolute cell numbers (G) of CD235^{+} erythroblasts (gating as shown in Figure 4G-H) within viable cell population at day 10 (N=8) and day 14 (N=6). (A, D) Contour plots shown are representative for one of the replicates. (B-C, E-G) Data are presented as average of the replicates ± SEM (Wilcoxon matched-pairs signed-rank test). *P<0.05, **P<0.01; ***P<0.001; ns: not significant.

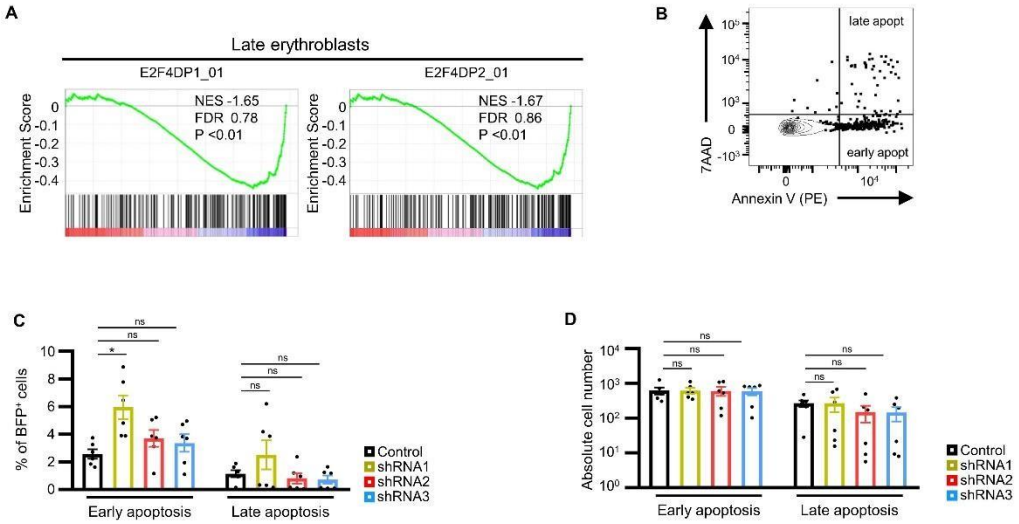
SUPPLEMENTAL FIGURE7



Supplemental Figure 7. (A-C) Results from data analysis of bulk RNA-seq of cell populations as discussed in Figure 6. (A) 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. (B) 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). (C) 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. (D-E) Results of *in vitro* experiments to study the impact of double *GATA2-HES6* knockdown (GFP^+BFP^+) (D) or single *GATA2* knockdown (GFP^+BFP^-) (E) on human megakaryo-erythropoiesis (N=6) as described in Figure

6E-H. Bar graphs show absolute cell numbers of indicated subpopulations within BFP^+ population in the different knockdown conditions. Data are presented as average of six replicates ± SEM (Wilcoxon matched-pairs signed-rank test). *P<0.05; ns: not significant.

SUPPLEMENTAL FIGURE8



Supplemental Figure 8. (A) Preranked GSEA results, from data analysis of bulk RNA-seq of cell populations as discussed in Figure 6, 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; NES: Normalized enrichment score; FDR: false detection rate. (B-D) Flow cytometry analysis (B) and bar graphs (C-D) of control shRNA and *HES6* shRNA-transduced $CD34^{+}Lin^{-}BFP^{+}$ HSPC cultured in megakaryocyte/erythroid-lineage supporting conditions for three days (N=6), showing gating (B), frequencies (C) and absolute cell numbers (D) of early and late apoptosis within BFP^{+} population. Contour plot shown is representative for all samples. Data are presented as average of six replicates ± SEM (Wilcoxon matched-pairs signed-rank test). *P<0.05, ns: not significant.

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