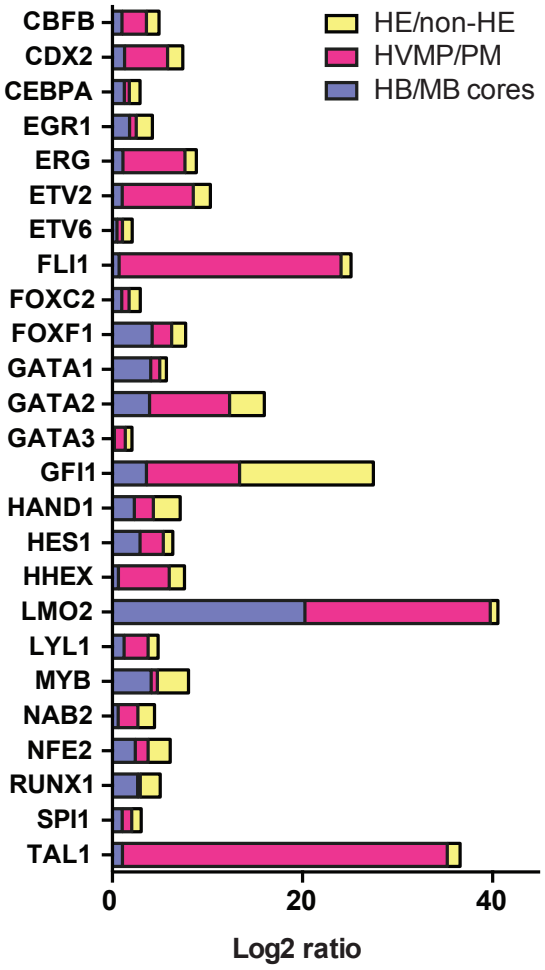
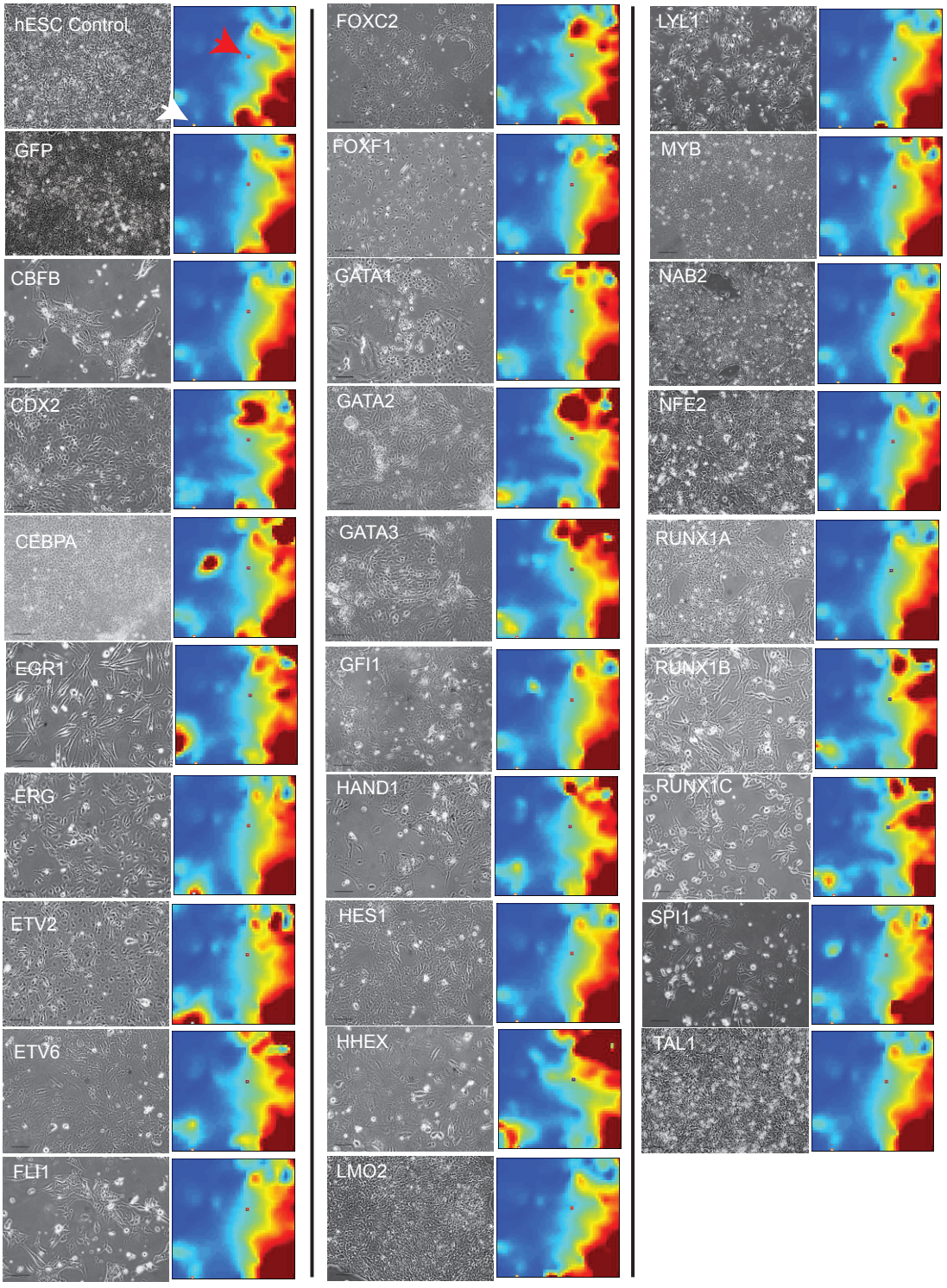
Supplementary Information Elcheva et al.

Supplementary Figure 1



Supplementary Figure 1. Prioritization of TFs for hematopoietic programming. TFs enriched in hESC-derived mesodermal and endothelial cells with hematopoietic activity [1,2]. Bars represent a ratio of TF expression from molecular profiling studies of indicated subpopulations obtained from hESCs differentiated in coculture with OP9. HE is VE-cadherin^{+}CD43^{-}CD73^{-} hemogenic endothelium; non-HE (VE-cadherin^{+}CD43^{-}CD73^{+} non-hemogenic endothelium); PM is apelin receptor positive primitive mesodermal cells with hemangioblast potential generated on day 3 hESC/OP9 coculture; HVMP is hematovascular mesodermal precursor highly enriched in cells forming hematoendothelial clusters on OP9 isolated on day 4 of differentiation; HB is endothelial intermediates (cores) with hematopoietic activity formed in hemangioblast clonogenic cultures; MB is endothelial intermediates (cores) without hematopoietic activity formed in mesenchymoangioblast clonogenic cultures.

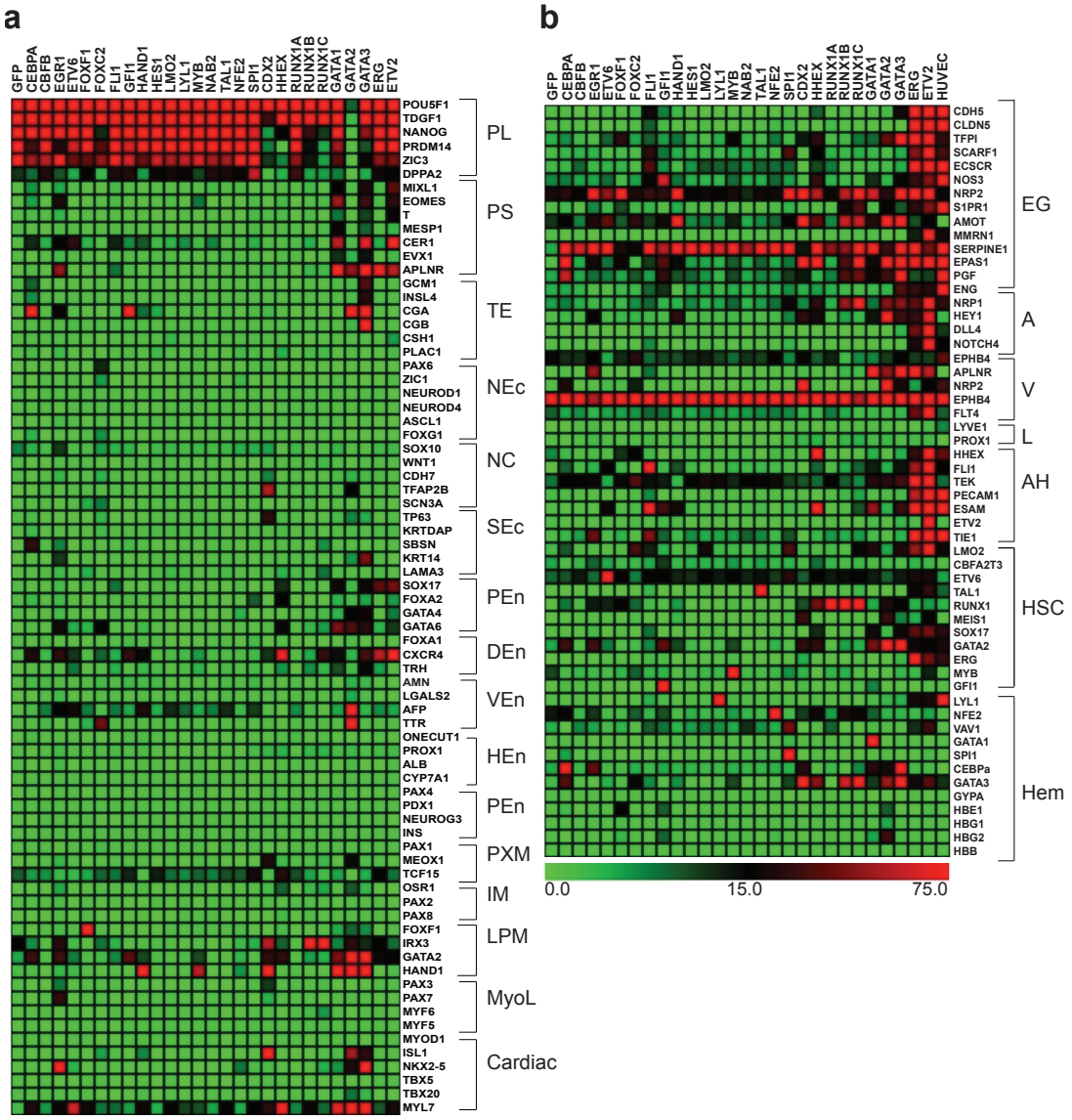
Supplementary Figure 2



Supplementary Figure 2. Morphologies and global gene expression profiles of hESCs differentiated by overexpression of single TF. Phase-contrast photographs of H1 hESCs

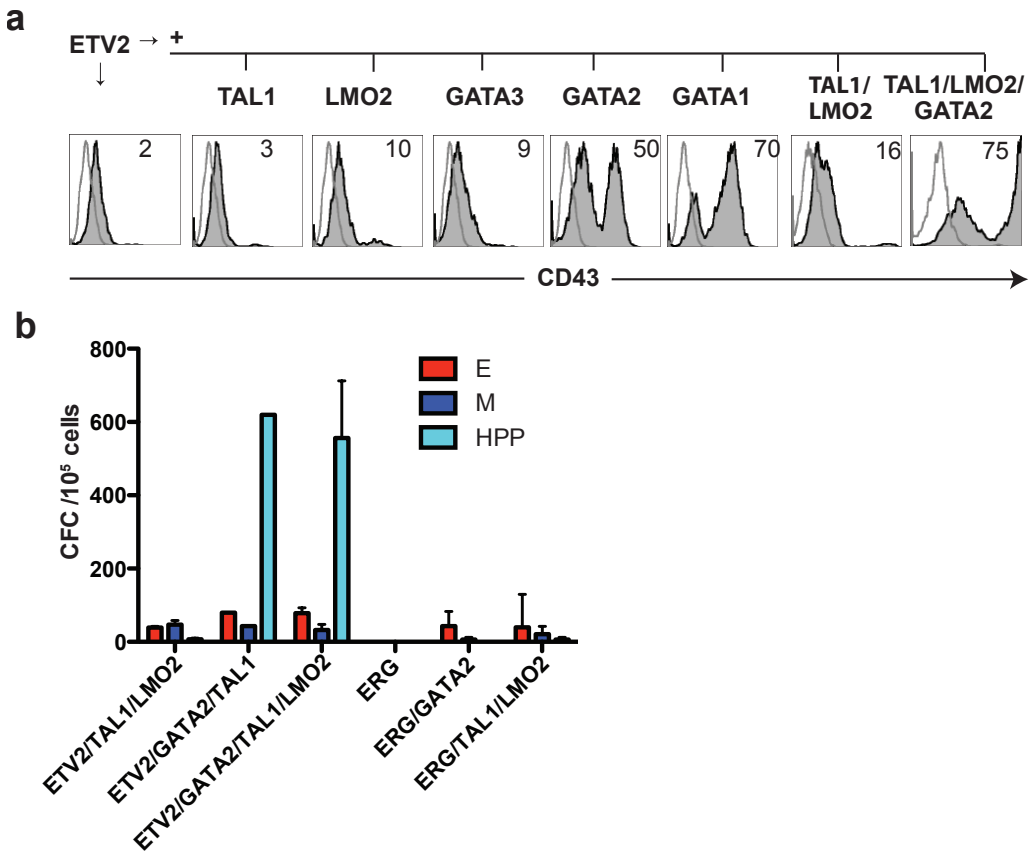
cultures transduced with GFP or indicated transcription factor. Scale bar, 100μm. Global analysis of changes in gene expression profiles of lentivirally transduced hESCs represented using gene expression dynamic investigator GEDI [3]. Plot gradients from blue to red represents lowly to highly expressed genes. The red arrow shows the position of *RUNX1* and the white arrow shows the position of *CDH5*. Genes inducing significant changes in transcriptome alter map configuration (e.g. compare control and *GATA2*), while genes with minimal effect on transcriptome show little changes in map configuration (e.g. compare control and *TAL1*). Representative experiment of 2-5 experiments is shown. Only ERG and ETV2 induce significant *CDH5* expression. HHEX weakly upregulates *RUNX1* expression.

Supplementary Figure 3



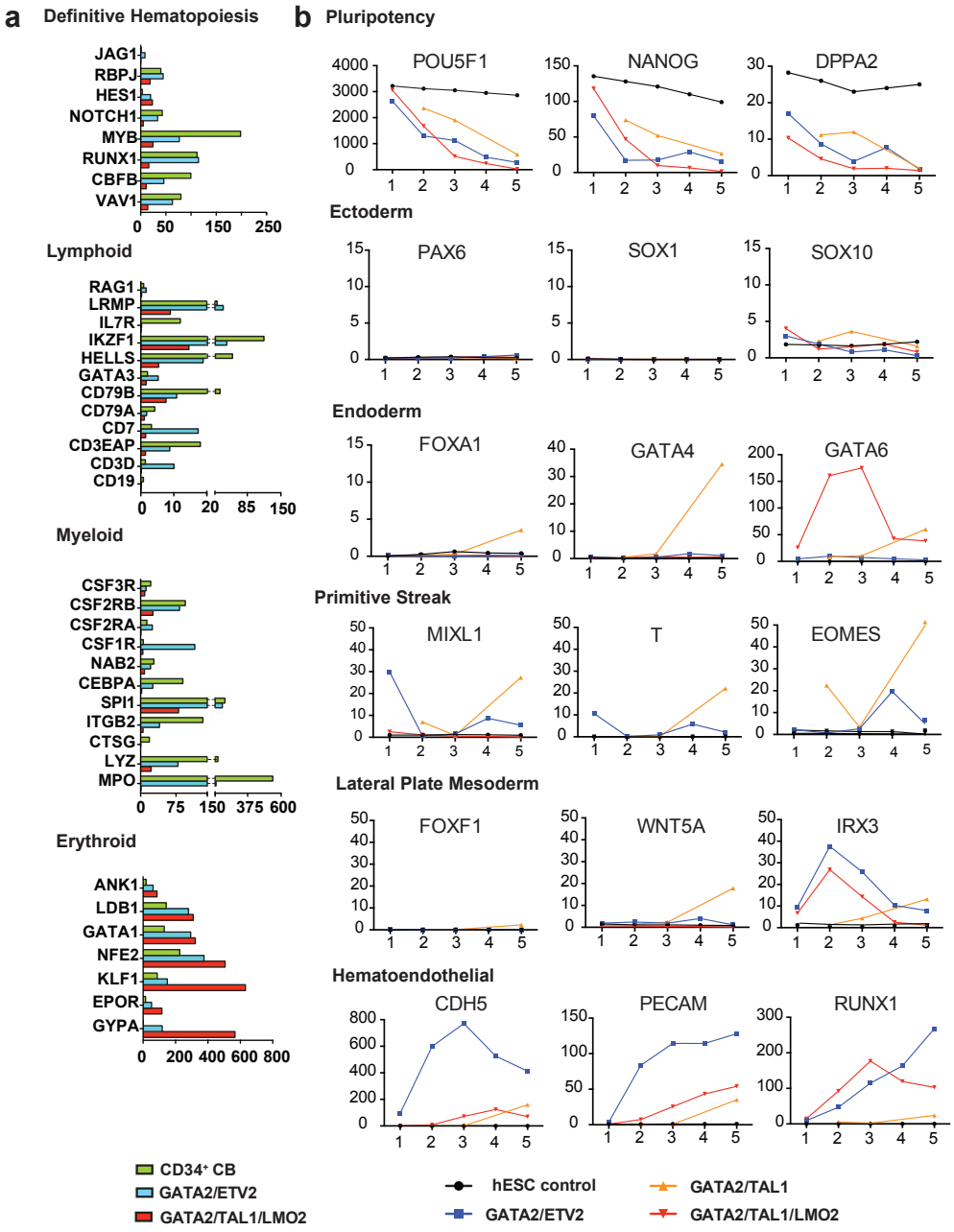
Supplementary Figure 3. Gene expression profiling of H1 hESCs differentiated by overexpression of single transcription factor. (a) Heat map of a selected set of genes associated with the development of germ layers and their derivatives. Pluripotency genes (PL); primitive streak (PS); trophectoderm (TE); neuroectoderm (NEc); neural crest (NC); surface ectoderm (Sec); primitive endoderm (Pen); definitive endoderm (Den); visceral endoderm (VEn); hepatic endoderm (HEn); pancreatic endoderm (PEn); paraxial mesoderm (PXM); intermediate mesoderm (IM); lateral plate mesoderm (LPM); myogenic lineage (MyoL). (b) Heat map of selected sets of genes associated with endothelial and hematopoietic differentiation. Typical endothelial genes (EG); arterial (A); venous (V); lymphatic (L); angiohematopoietic (AH); hematopoietic stem cells (HSC); typical hematopoietic genes (Hem). ERG and ETV2 selectively induce the endothelial program while GATA2 downregulates expression of pluripotency genes. Gene expression is estimated in tpm values.

Supplementary Figure 4



Supplementary Figure 4. Screening of different combinations of TFs based on co-expression with ETV2 and ERG. (a) CD43 expression by hESCs on day 7 after transduction with ETV2 plus indicated TFs. (b) CFC potential of hESCs transduced with indicated combinations of TFs. Results are mean for two to four independent experiments. Error bars shows s.e.m.

Supplementary Figure 5



Supplementary Figure 5. Analysis of gene expression in hESCs transduced with blood-inducing combinations of transcriptional regulators.
(a) The expression of genes associated with definitive hematopoiesis, lymphoid, myeloid, and erythroid specification in cells collected from hESCs cultures transduced with indicated combinations of factors. (b) Histograms comparing the kinetic of gene expression in control hESCs and hESCs transduced with indicated combinations of genes. Gene expression is estimated in tpm values

Supplementary Table 1. List of selected genes

	GENE	SEQUENCE
1	***CBFB*** *Core-binding factor subunit beta (CBF-beta)*	***NM_001755.2***
2	***CDX2*** *Caudal type homeobox 2*	***NM_001265.4***
3	***CEBAa*** *CCAAT/enhancer binding protein (C/EBP), alpha*	***BC160133.1***
4	***EGR1*** *Early growth response 1*	***NM_001964.2***
5	***ERG*** *v-ets erythroblastosis virus E26 oncogene homolog*	***NM_001243428.1***
6	***ETV2*** *ETS translocation variant 2*	***NM_014209.2***
7	***ETV6*** *ets variant 6*	***NM_001987.4***
8	***FOXF1*** *Forkhead box F1*	***NM_001451.2***
9	***FOXC2*** *Forkhead box C2 (MFH-1, mesenchyme forkhead 1)*	***BC113439.1***
10	***FLI1*** *Friend leukemia virus integration 1*	***NM_002017.3***
11	***GATA1*** *GATA binding protein 1*	***NM_002049.3***
12	***GATA2*** *GATA binding protein 2*	***BC051342.1***
13	***GATA3*** *GATA binding protein 3 trans-acting*	***BC003070.2***
14	***GFI1*** *Growth factor independent 1 transcription repressor*	***BC032751.1***
15	***HAND1*** *Heart and neural crest derivatives expressed 1*	***NM_004821.2***
16	***HES1*** *Hairy and enhancer of split 1*	***NM_005524.3***
17	***HHEX*** *Hematopoietically expressed homeobox*	***NM_002729.4***
18	***LMO2*** *LIM domain only 2 (rhombotin-like 1)*	***NM_001142315.1***
19	***LYL1*** *Lymphoblastic leukemia derived sequence 1*	***NM_005583.4***
20	***MYB*** *v-myb myeloblastosis viral oncogene*	***BC064955.1***
21	***NAB2*** *NGFI-A binding protein 2*	***BC065931.1***
22	***NFE2*** *Nuclear factor (erythroid-derived 2), 45kDa*	***BC005044.1***
23	***RUNX1 isoform RUNX1A*** *Runt-related transcription factor 1 (RUNX1) transcript variant 3 Acute myeloid leukemia 1 protein isoform a*	***NM_001122607.1***
24	***RUNX1 isoform RUNX1B*** *Runt-related transcription factor 1 (RUNX1) transcript variant 2 Acute myeloid leukemia 1 protein isoform b*	***NM_001001890.2***
25	***RUNX1 isoform RUNX1C*** *Runt-related transcription factor 1 (RUNX1) transcript variant 1 Acute myeloid leukemia 1 protein isoform c*	***NM_001754.4***
26	***SPI1*** *Spleen focus forming virus (SFFV) proviral integration oncogene spi1, PU-box binding protein (PU.1)*	***NM_003120.2***
27	***TAL1/SCL*** *T-cell acute lymphocytic leukemia 1*	***NM_003189.2***

Supplementary Table 2. Effect of individual transcription factors on cell morphology and phenotype

	Factor	Change morphology	Early Cell Death*	Markers by FACS**							
				APLNR	KDR	VEC	CD31	CD73	CD34	CD43	CD45
1	**CBFB**	No	No	–	–	–	–	–	–	–	–
2	**CDX2**	Yes	No	–	–	–	–	–	–	–	–
3	**CEBPA**	Yes	No	–	–	–	–	–	–	–	–
4	**EGR1**	Yes	No	–	–	–	–	–	–	–	–
5	**ERG**	Yes	No	–	++	++++	++++	+++	++	–	–
6	**ETV2**	Yes	No	+	+++	+++	+++	+++	+++	+	+
7	**ETV6**	Yes	No	–	–	–	–	–	–	–	–
8	**FOXF1**	Yes	Yes	–	–	–	–	++	+	–	–
9	**FOXC2**	Yes	No	–	–	–	–	–	–	–	–
10	**FLI1**	Yes	Yes	–	–	–	++	–	–	–	–
11	**GATA1**	Yes	No	+	+	–	–	+	++	+	–
12	**GATA2**	Yes	No	++	++	+	+	–	++	+	–
13	**GATA3**	Yes	No	+	+	–	–	–	++	–	–
14	**GFI1**	Yes	No	–	–	–	–	–	–	–	–
15	**HAND1**	Yes	No	–	–	+	+	++	–	–	–
16	**HES1**	No	No	–	–	–	–	–	–	–	–
17	**HHEX**	Yes	No	–	–	–	–	–	–	–	–
18	**LMO2**	No	No	–	–	–	–	–	–	–	–
19	**LYL1**	No	No	–	–	–	–	–	–	–	–
20	**MYB**	No	No	–	–	–	–	–	–	–	–
21	**NAB2**	No	Yes	–	–	–	–	–	–	–	–
22	**NFE2**	No	No	–	–	–	–	–	–	–	–
24	**RUNX1A**	No	Yes	–	–	+	–	+	–	–	–
25	**RUNX1B**	Yes	Yes	–	–	+	–	+	–	–	–
26	**RUNX1C**	Yes	Yes	–	–	+	–	+	–	–	–
23	**SPI1**	Yes	No	–	–	–	–	–	–	–	+
27	**TAL1**	No	No	–	–	–	–	–	–	–	–

* Cell death and morphologic changes were evaluated during 7 days of culture. Viable cell cultures collected on day 5 or 7 of culture. Cultures with cell death observed before 5 days of culture were collected and analyzed on 3rd day after transduction, i.e. before cell death was observed.

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Symbol	Expression Levels	Positive cells (%)
–	Negative	0-1
+	Low	2-5
++	Moderate	5- 20
+++	High	20-50
++++	Very high	> 50

Supplementary Table 3. The efficiency of CD43$^+$ blood cell generation following transduction of hPSC with ETV2/GATA2 and GATA2/TAL1/LMO2 combinations

Cell line	TFs Combination	Number of experiments (*n*)	Yield (10^6) of CD43+ cells*	Method of hematopoietic programming
H1 hESC	ETV2/GATA2	2	24.6	lentivirus
H9 hESC	ETV2/GATA2	2	7.3	lentivirus
DF-19-9-7T hiPSC	ETV2/GATA2	2	14.5	lentivirus
DF-4-3-7T hiPSC	ETV2/GATA2	1	2.7	lentivirus
H1 hESC	ETV2/GATA2	1	11.6	mmRNA
H1 hESC	GATA2/TAL1/LMO2	3	33.2	lentivirus
H9 hESC	GATA2/TAL1/LMO2	2	18.8	lentivirus
DF-19-9-7T hiPSC	GATA2/TAL1/LMO2	2	13.6	lentivirus
DF-4-3-7T hiPSC	GATA2/TAL1/LMO2	1	5.0	lentivirus

*Yield was calculated as a total number of CD43$^+$ cells obtained from 10^6 hPSCs after transduction with indicated combination of TFs and seven day of expansion in suspension cultures. To initiate blood formation, TFs were overexpressed in hPSCs using lentiviral vectors or mmRNA. After seven days, cells were collected and expanded for another seven days as described in materials and methods. Results show data from one experiment or mean of 2 or more experiments.

Supplementary Table 4. List of antibodies used in the study

Antigen	Label	Company	Cat. Number	Application	Dilution
Mouse IgG k Isotype control	FITC	BD Biosciences	554679	FACS	1/20
Mouse IgG k Isotype control	PE	BD Biosciences	554680	FACS	1/20
Mouse IgG k Isotype control	APC	BD Biosciences	554681	FACS	1/20
Anti-human CD29	FITC	Caltag-Invirogen	CD2901	FACS	1/50
Anti-human CD29	PE	Caltag-Invirogen	CD2904	FACS	1/50
Anti-human Tra-1-85	APC	R&D Systems	FAB3195A	FACS	1/10
Anti-human Tra-1-81	PE	Stemgent	09-0012	FACS	1/10
Anti-human Tra-1-60	PE	Stemgent	09-0009	FACS	1/10
Anti-human SSEA-3	PE	Stemgent	09-0044	FACS	1/10
Anti-human SSEA-4	PE	Stemgent	09-0003	FACS	1/10
IgG rabbit	None	EMD Millipore	NI01-100UG	IF	1 μg/ml
IgG mouse	None	EMD Millipore	NI03-100UG	IF	0.5 μg/ml
Anti-mouse/human REX1	None	Stemgent	09-0019	IF	1/100
Anti-mouse/human OCT4	None	Stemgent	09-0023	IF	1/100
Anti-mouse/human SOX2	None	Stemgent	09-0024	IF	1/100
Rabbit anti-human VEC	None	BenderMedSystem	BMS158	IF	1 μg/ml
Mouse anti-human CD43	None	BD Biosciences	551457	IF	0.5 μg/ml
Donkey Anti-mouse IgG	DyLight 594	JacksonResearch	715-516-150	IF	2 μg/ml
Donkey Anti-rabbit IgG	DyLight 488	JacksonResearch	715-486-152	IF	2 μg/ml
Anti-human APLNR (APJ)	APC	R&D Systems	FAB856A	FACS	1/30
Anti-human CD31	FITC	BD Biosciences	555445	FACS	1/20
Anti-human CD31	PE	BD Biosciences	555446	FACS	1/20
Anti-human CD32	PE	BD Biosciences	552884	FACS	1/20
Anti-human CD34	FITC	BD Biosciences	555821	FACS	1/20
Anti-human CD34	PE	BD Biosciences	348057	FACS	1/20
Anti-human CD41a	PE	BD Biosciences	555467	FACS	1/20
Anti-human CD41a	APC	BD Biosciences	559777	FACS	1/20
Anti-human CD43	FITC	BD Biosciences	55475	FACS	1/20
Anti-human CD43	PE	BD Biosciences	560199	FACS	1/20
Anti-human CD43	APC	BD Biosciences	560198	FACS	1/20
Anti-human CD45	APC	BD Biosciences	555485	FACS	1/20
Anti-human CD66b	FITC	Pelicluster	M1594	FACS	1/20
Anti-human CD73	APC	R&D Systems	FAB5795A	FACS	1/20
Anti-human CD144	FITC	BD Biosciences	560411	FACS	1/20
Anti-human CD163	PE	R&D Systems	FAB1607P	FACS	1/20
Anti-human CD226	PE	BD Biosciences	338305	FACS	1/20
Anti-human CD235a	PE	BD Biosciences	555570	FACS	1/50
Anti-human CD235a	APC	BD Biosciences	561775	FACS	1/50

Supplementary References

1 **Choi, K. D. *et al.* Identification of the hemogenic endothelial progenitor and its direct precursor in human pluripotent stem cell differentiation cultures. *Cell Rep* 2, (2012).**

2 **Vodyanik, M. A. *et al.* A mesoderm-derived precursor for mesenchymal stem and endothelial cells. *Cell Stem Cell* 7, 718-729, (2010).**

3 **Eichler, G. S., Huang, S. & Ingber, D. E. Gene Expression Dynamics Inspector (GEDI): for integrative analysis of expression profiles. *Bioinformatics* 19, 2321-2322 (2003).**