Expanded View Figures

Figure EV1. Morphology of embryoid bodies and differentiated cells.
EB formation and the morphology of cells during HSPC differentiation from ESCs. Scale bar = 100 μm.
Figure EV2. Gene expression analysis and validation.

A  PCA of mRNA microarray data.
B  Clustering analysis of mRNA expression array data.
C  qRT–PCR validation of several shRNA target genes with most increased or decreased expression in the LSK population compared with the LS and LK populations. Error bars represent standard deviation (SD), n=3. Student’s t-test shows no significance between the microarray and qRT-PCR data.
Figure EV3. Knockdown of Group XII, XI, IV, and X genes and analysis of their function in differentiation.

A qRT–PCR analysis showing the knockdown of Group XII genes Esrra, SI00a8, and Hcfcl2 in mESCs. Error bars represent standard deviation (SD) of technical triplicates. Student’s t-test, *P < 0.05.

B qRT–PCR analysis showing the knockdown of Group XI genes Atp5g3, Bub1b, Eci1, and Dis3l in mESCs. Error bars represent standard deviation (SD) of technical triplicates. Student’s t-test, *P < 0.05.

C qRT–PCR analysis showing the knockdown of Group IV genes Wbp5, Rbm26, Gdpd4, and Nrxn1 in mESCs. Error bars represent standard deviation (SD) of technical triplicates. Student’s t-test, *P < 0.05.

D Flow cytometric analysis of endoderm and mesoderm/endothelium cells in EBs on day 6 with knockdown of Wbp5, Rbm26, Gdpd4, and Nrxn1, respectively.

E Percentage of CXCR4+ cells in EBs on day 6 with knockdown of Wbp5, Rbm26, Gdpd4, and Nrxn1. Error bars represent standard deviation (SD), n = 2. Student’s t-test, *P < 0.05.

F Percentage of Flk1+ cells in EBs on day 6 with knockdown of Wbp5, Rbm26, Gdpd4, and Nrxn1. Error bars represent standard deviation (SD), n = 2. Student’s t-test shows no significance.

G qRT–PCR analysis showing the knockdown of Group X genes Ap2a1, Mett22, Lrsam1, and Hal in mESCs. Error bars represent standard deviation (SD) of technical triplicates. Student’s t-test, *P < 0.05.

H Flow cytometric analysis of endoderm and mesoderm/endothelium cells in EBs on day 6 with knockdown of Ap2a1, Mett22, Lrsam1, and Hal.

I Percentage of CXCR4+ cells in EBs on day 6 with knockdown of Ap2a1, Mett22, Lrsam1, and Hal. Error bars represent standard deviation (SD), n = 3. Student’s t-test shows no significance.

J Percentage of Flk1+ cells at day 6 with knockdown of Ap2a1, Mett22, Lrsam1, and Hal. Error bars represent standard deviation (SD), n = 3. Student’s t-test shows no significance.

K qRT–PCR analysis showing the knockdown AP2A1, METTL22, LRSAM1, and HAL in human CD34+ cells. Error bars represent standard deviation (SD) of technical triplicates. Student’s t-test, *P < 0.05.
A GO analysis of Groups IV (upper) and I (lower) target genes showing the pathways enriched during the endothelial–hematopoietic transition.

B Transcription factors enriched in Group I genes are related to cardiomyocyte development.

Figure EV 4. GO analysis (Groups IV and I genes) and heat map of Group I genes.
A GO analysis of Groups IV (upper) and I (lower) target genes showing the pathways enriched during the endothelial–hematopoietic transition.
B Transcription factors enriched in Group I genes are related to cardiomyocyte development.
Figure EV5. Identification of stage-specific gene networks that are critical to HSPC development. Stage-specific gene networks during HSPC development. The positioning of Stages I–III is shown in Fig 7. The top 20 genes shown in Fig 7 were used to build the interaction network.