

Expanded View Figures

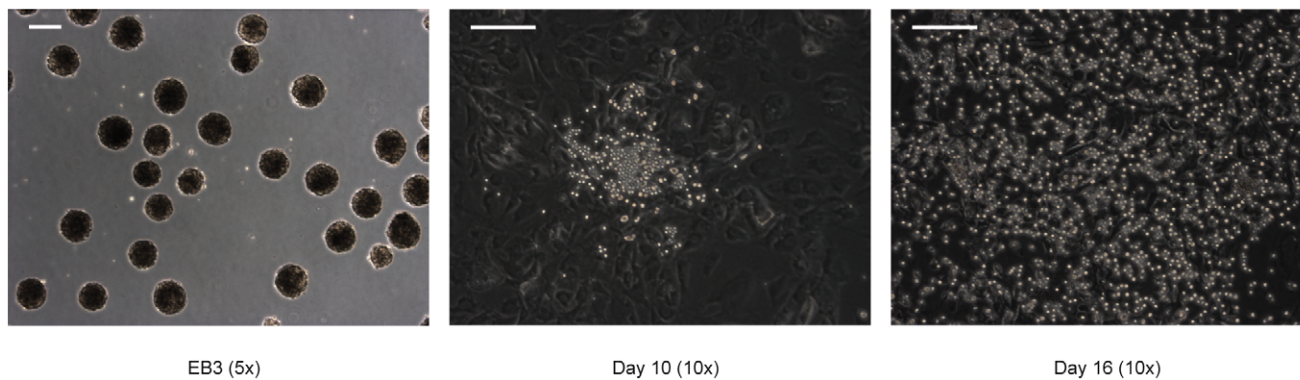


Figure EV1. Morphology of embryoid bodies and differentiated cells.

EB formation and the morphology of cells during HSPC differentiation from ESCs. Scale bar = 100 μ m.

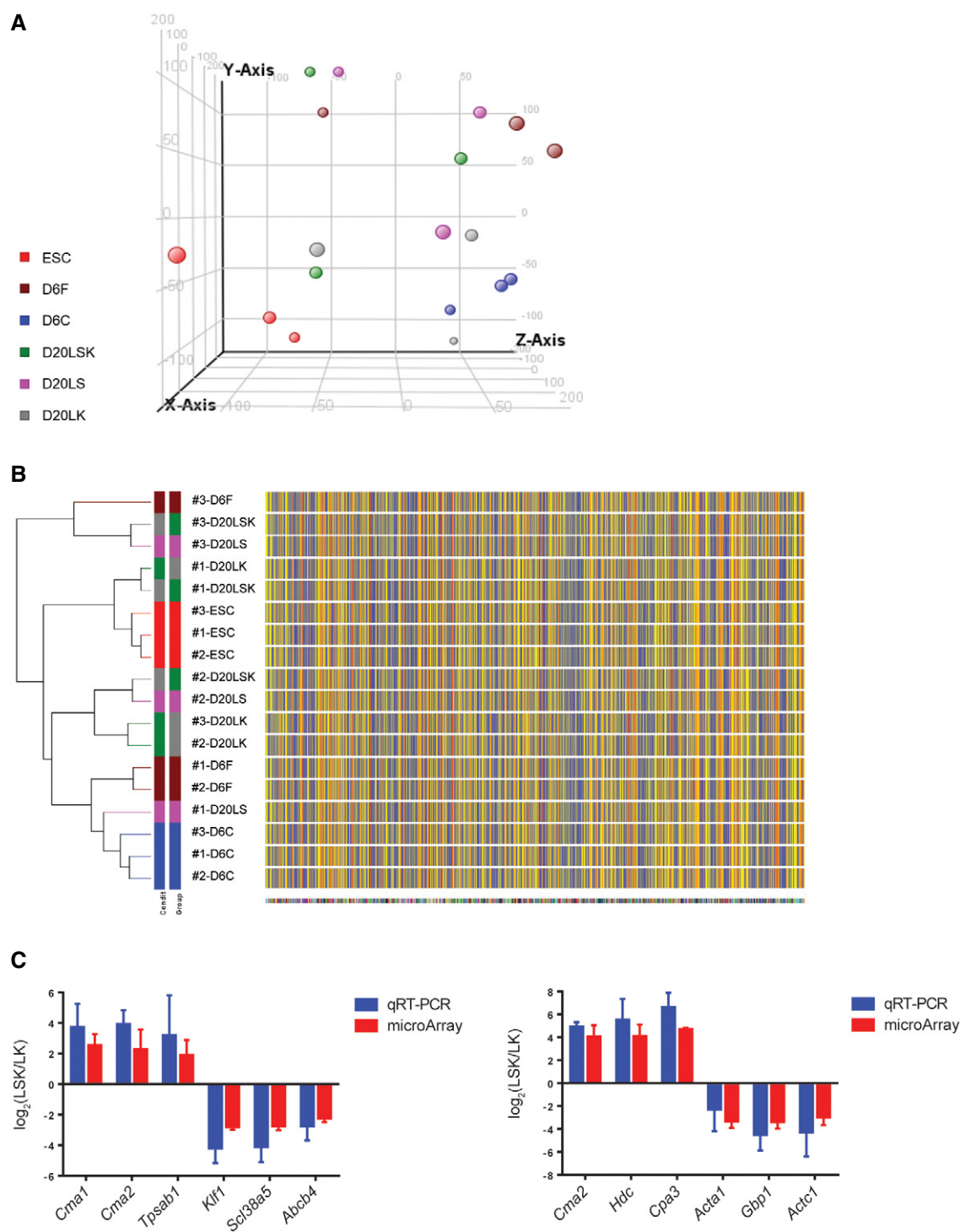


Figure EV2. Gene expression analysis and validation.

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.

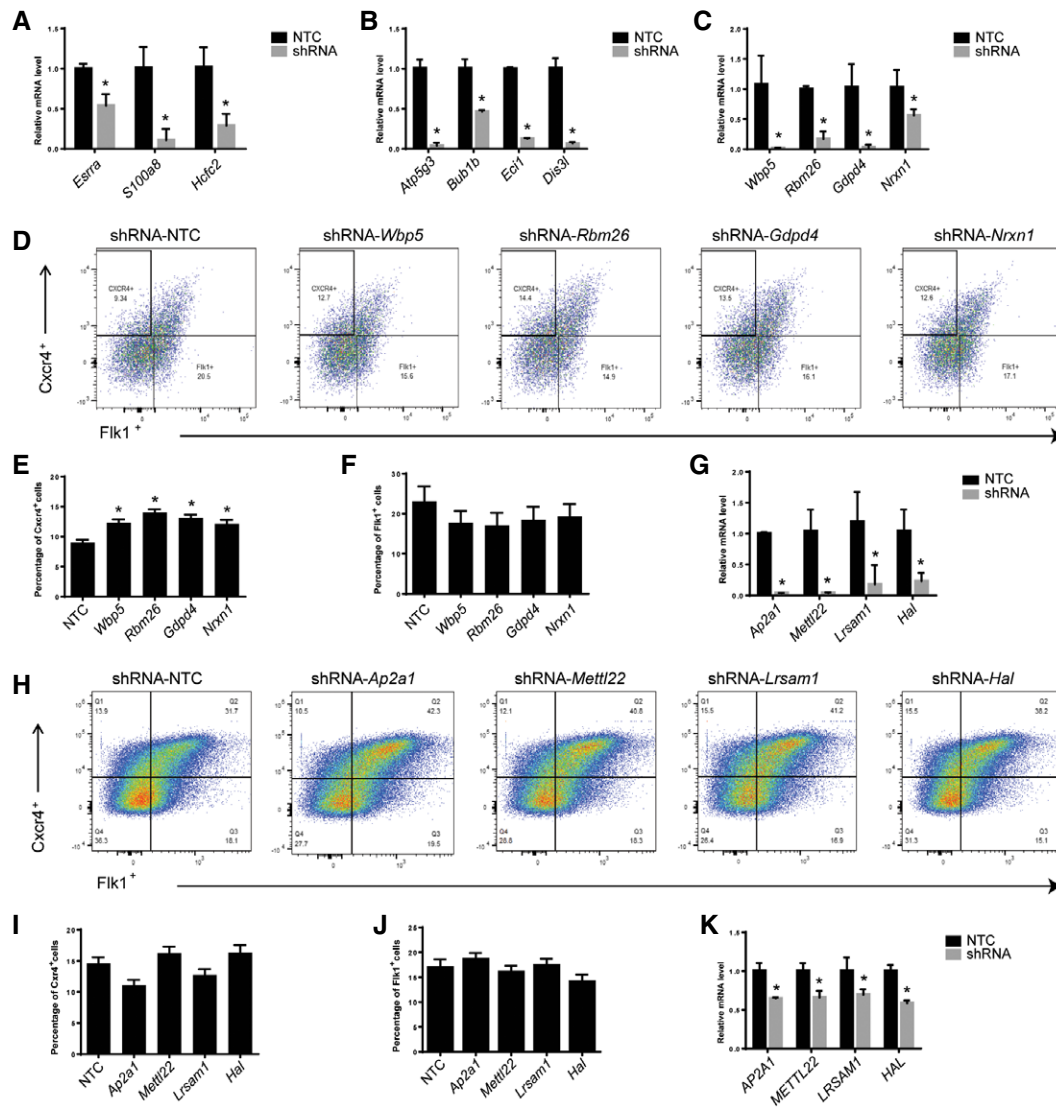


Figure EV3. Knockdown of Group XII, XI, IV, and X genes and analysis of their function in differentiation.

- A qRT-PCR analysis showing the knockdown of Group XII genes *Esrra*, *S100a8*, and *Hcfc2* 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*, *Mettl22*, *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*, *Mettl22*, *Lrsam1*, and *Hal*.
- I Percentage of CXCR4⁺ cells in EBs on day 6 with knockdown of *Ap2a1*, *Mettl22*, *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*, *Mettl22*, *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 of *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.

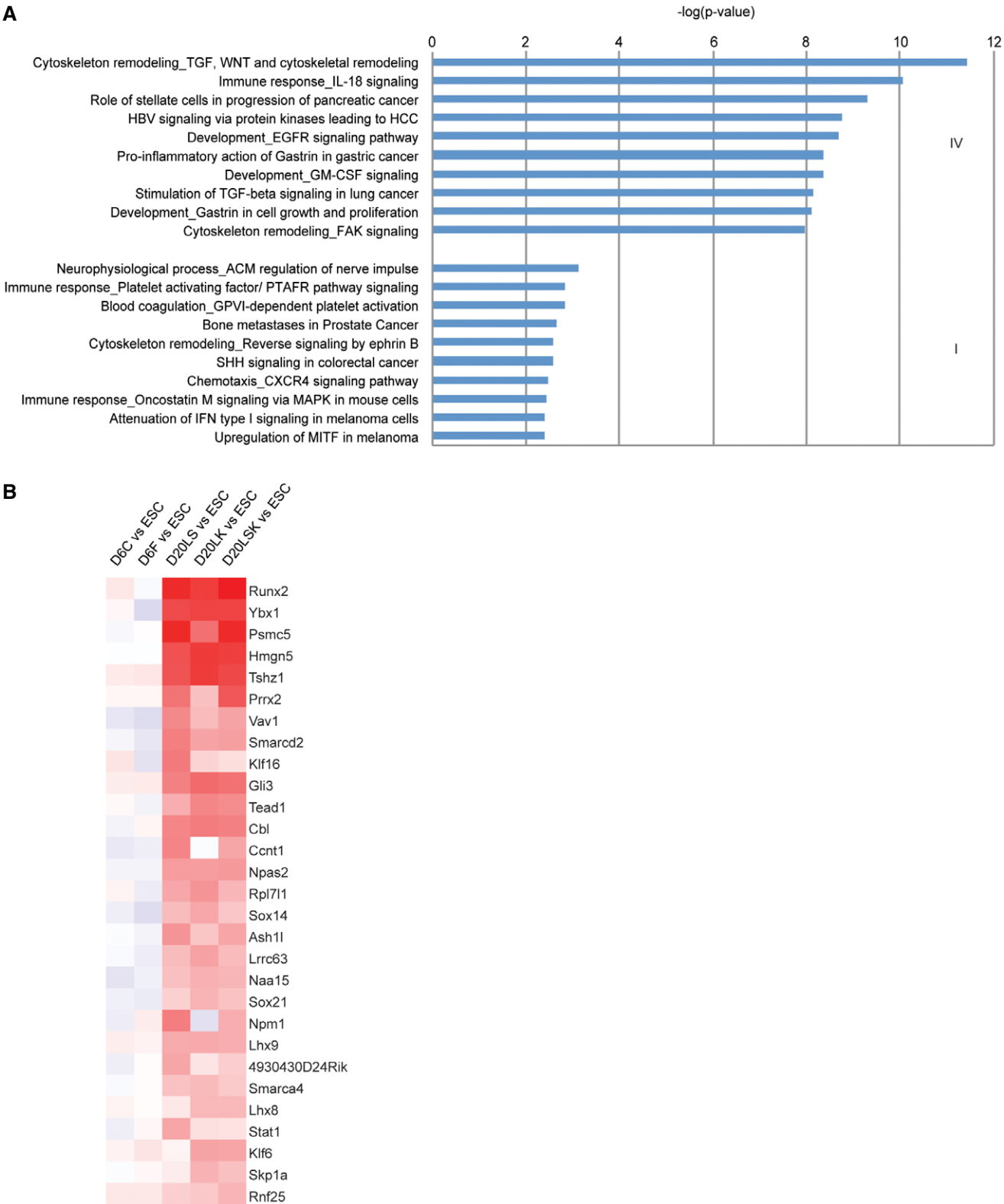


Figure EV4. GO analysis (Groups IV and I genes) and heat map of Group I genes.

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.

