



Supplementary information, Figure S3 FACS analyses of KDR expression in the

differentiated hESCs into endothelial lineage cells.

(A) Expression of KDR and TRA-1-60 on day 0 and 3. The undifferentiated hESCs (day 0) were positive for TRA-1-60 and showed partial and weak expression of KDR, whereas the differentiated hESCs (day 3) were negative for TRA-1-60 and showed partial but strong expression of KDR. **(B)** Time course of expression of KDR and VEC in the endothelial differentiation induced with BMP4 and VEGF-A. During mesodermal specification (day 0 to 4), the KDR⁺ cell ratio was decreased once and then increased with stronger intensity. VEC⁺ cells did not appear by day 4 but was induced with VEGF-A treatment on day 5–6. **(C)** Comparison of the KDR⁺ cell ratios on day 4 induced with each treatment: medium alone, BMP4, and BMP4 plus GSK-3 β I. **(D)** The EC (VEC⁺CD31⁺) induction ratios derived from KDR⁺ and KDR⁻ cell populations sorted on day 4. The sorted cells were then treated with VEGF-A and analyzed by FACS on day 8. **(E)** Positive correlation between the KDR⁺ cell ratio on day 4 and the VEC⁺ cell ratio on day 6 in the endothelial differentiation.