Efficient Differentiation of Human Pluripotent Stem Cells to Endothelial Progenitors via Small-Molecule Activation of WNT Signaling

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**Figure S1.** Differentiation of H1 human embryonic stem cells to endothelial progenitors, related to Figure 1. H1 Human embryonic stem cells were cultured on Matrigel in LaSR basal medium containing 6 μM CH for 2 days followed by another 3 days in Stem-Pro 34 medium. Flow cytometry analysis of CD34 and CD31, CD31 and CD144, CD31 and CD45 expression was performed after 5 days of differentiation.
Figure S2. Endothelial differentiation requires β-catenin expression, related to Figure 2. 19-9-11 ishcats iPSCs were cultured in LaSR basal medium containing 6 μM CH and/or 2 μg/ml doxycycline for 2 days. Flow cytometry analysis of brachyury expression was performed after 2 days of differentiation.
**Figure S3.** Differentiation of multiple hESC and iPSC lines to endothelial progenitors, related to Figure 5. Purified CD34+ cells were generated as described in Fig. 5A from different hPSC lines: (A) hESC H13, (B) hESC H14, (C) iPSC 19-9-7 and (D) iPSC 6-9-9. Flow cytometry analysis of CD34 expression was performed before (left) and after (right) EasySep™ Human CD34 Positive Selection Kit. Single CD34+ cells were cultured in a combined medium for 10 days (E, F). Immunostaining of (E) calponin and (F) VE-cadherin/vWF/SMA were performed.
Figure S4. Analysis of endothelial progenitor marker expression, related to Figure 6. Flow cytometry analysis of developmental markers (A) Tie-2, (B) CD117, (C) KDR, and (D) I-CAM1 in day 5 unsorted CD34+ endothelial progenitors were performed.
**Figure S5.** Analysis of endothelial marker expression in day 60 endothelial cells, related to Figure 7.

Flow cytometry analysis of VE-cadherin (A & B) and CD31 (C & D) expression in day 60 endothelial cells was performed. (A) and (C) are corresponding isotype controls.
## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Primers for Q-PCR

| Genes | Sequences (5' - 3') | Size (bp)/Tm (°C) |
|-------|---------------------|-------------------|
| OCT4  | F: CAGTGCCCCAAACCCACAC  
        | R: GGAGACCCAGCAGCCTCAAA | 161/58 |
| SOX2  | F: CAAGATGCAACAATCGGAGA  
        | R: GTTCATGTGCAGCGTAACTGT | 300/58 |
| MIXL1 | F: CAGAGTGGGAATCTTTCCA  
        | R: TGAGTCCAGCTTTGAAACCAA | 231/58 |
| CD31  | F: GCTGACCCTTCTGCTCTGT  
        | R: TGAGAGGTGGGTGCTGACATC | 238/55 |
| CD34  | F: CCTAAGTGACATCAAGGCAGAA  
        | R: GCAAGGAACAGGGAGCATA | 201/55 |
| ACTB  | F: CCTGAACCCTAAGGCCAACCG  
        | R: GCTCATAGCTCTTTCTCCAGGG | 400/58 |
| GAPDH | F: GTGGACCTGACCTGCCCCAGTCT  
        | R: GGAGGAGTGTTGCTGCTGT | 152/58 |
| T     | F: AAGAAGGAATGCGACCTCA  
        | R: TACTGCAGGTGTGAGCAAGG | 101/58 |
| CTNNB1| F: CCCACTAATGTCCAGCGTTT  
        | R: AACGCATGATAGCGTGCTGT | 217/58 |
## Antibodies for immunostaining (IS), western blotting (WB) and flow cytometry (FC)

| Antibody       | Application                                      |
|----------------|--------------------------------------------------|
| CD31-APC       | Miltenyi Biotec, mouse IgG1, Clone: AC128        |
|                | Cat. no: 130-092-652                             |
| CD34-FITC      | Miltenyi Biotec, mouse IgG2a, Clone: AC136       |
|                | Cat. no: 130-081-001                             |
| Brachyury      | R&D Polyclonal Ab, GoatIgG Clone: AF2085         |
|                | 1:100 (FC)                                       |
| VE-cadherin    | Santa Cruz, mouse IgG1, Clone: F-8 sc9989        |
|                | 1:100 (IS)                                       |
|                | 1:1000 (WB)                                      |
| OCT-3/4        | Santa Cruz, Mouse IgG2b Clone: C-10 sc-5279      |
|                | 1:100 (IS)                                       |
|                | 1:1000 (WB)                                      |
| SMMHC          | Abcam 82541, RabbitIgG, Cat. no: 82541           |
|                | 1:800 (IS)                                       |
| Actin, smooth muscle | Thermo Scientific, mouse IgG2a/κ Clone: 1A4 MS-113-P |
|                | 1:100 (IS)                                       |
| CD117 (c-kit)  | BD Pharmingen, mouse IgG1/κ Clone: YB5.B8        |
|                | Cat. no:555713                                   |
| TIE-2          | Santa Cruz, rabbit IgG, Clone: H-176 sc9026      |
|                | 1:200 (IS)                                       |
| KDR            | Santa Cruz, mouse IgG1, Clone: A-3 sc6251        |
|                | 1:200 (IS)                                       |
| vWF            | Dako, rabbit IgG, Cat. no: A008202-5             |
|                | 1:500 (IS)                                       |
| ICAM-1 (CD54)  | DSHB, mouse IgG1, P2A4                          |
|                | 1:30 (IS)                                        |
| Calponin       | Abcam, Mouse IgG1, Clone: CALP Cat. no: ab700    |
|                | 1:200 (IS)                                       |
| β-Actin        | Cell Signaling Technology, Rabbit mAb (HRP Conjugate), 13E5,5125S goat anti-mouse IgG-HRP Santa Cruz, sc-2005 |
|                | 1:5000 (WB)                                      |
|                | 1:3000 (WB)                                      |