

Supplemental information

BCL6B-dependent Suppression of ETV2 Hampers Endothelial Cell Differentiation

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Figure S1. Generation and characterization of hiPSC-derived ECs

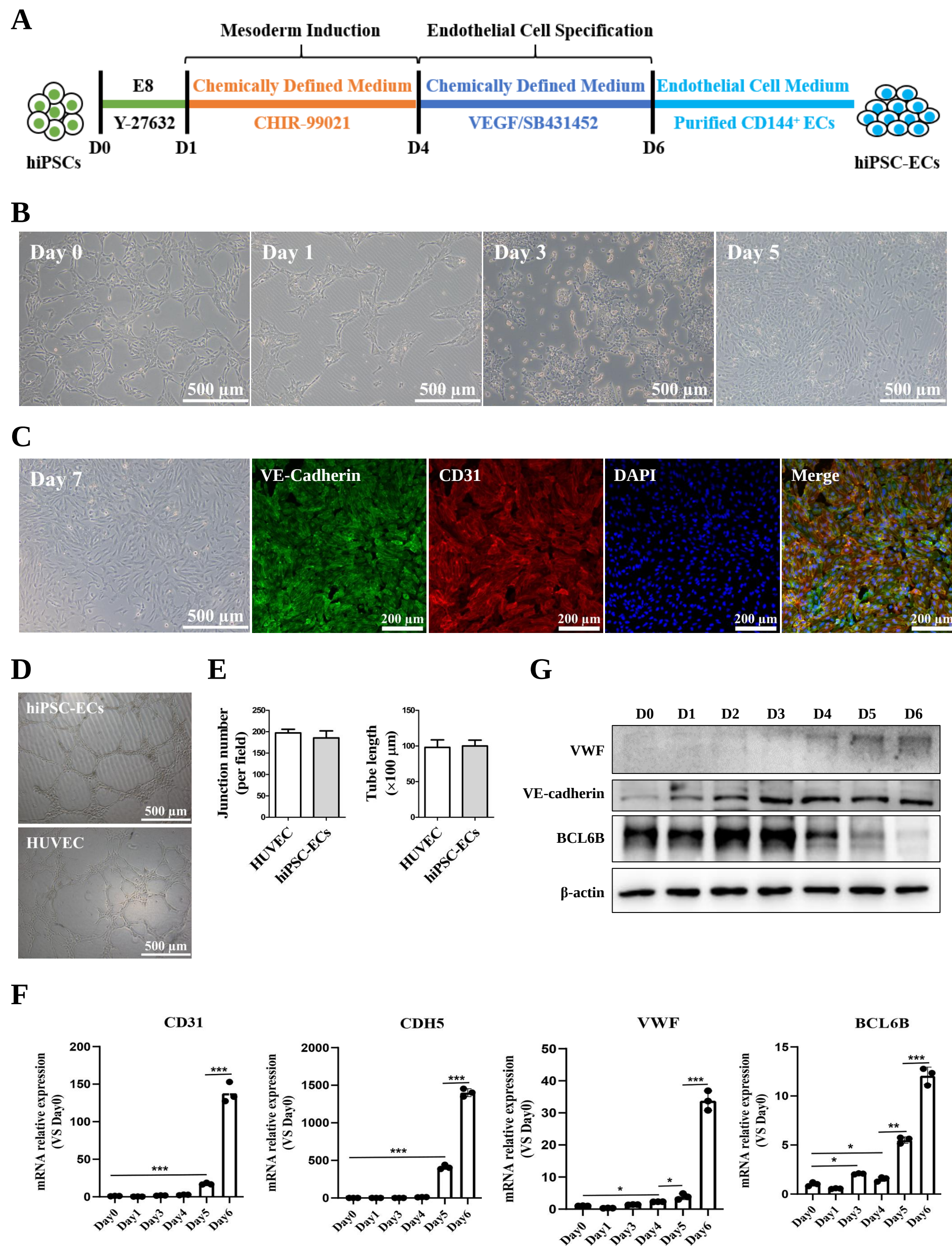


Figure S1. Generation and characterization of hiPSC-derived ECs

(A) Schematic illustration outlining the strategy for EC differentiation from hiPSCs. The differentiation of hiPSCs into ECs involved two stages: mesoderm induction and EC specification, with the entire process typically taking 6 days.

(B) Brightfield images depicting the cell morphology at different time points during EC differentiation.

(C) Brightfield image showing purified ECs at D7, along with confocal immunofluorescence images highlighting EC markers VE-Cadherin (green) and CD31 (red) with DAPI (blue).

(D) Brightfield images displaying tube-like structures formed by hiPSC-ECs and HUVEC.

(E) Quantitative analysis of junction number and tube length.

(F) mRNA expression of EC markers (*VWF*, *CDH5*, *CD31*) and *BCL6B* during EC differentiation.

(G) Protein levels of VWF, VE-Cadherin and BCL6B throughout EC differentiation from D0 to D6, as determined by western blot.

Figure S2. Generation and characterization of blood VOs

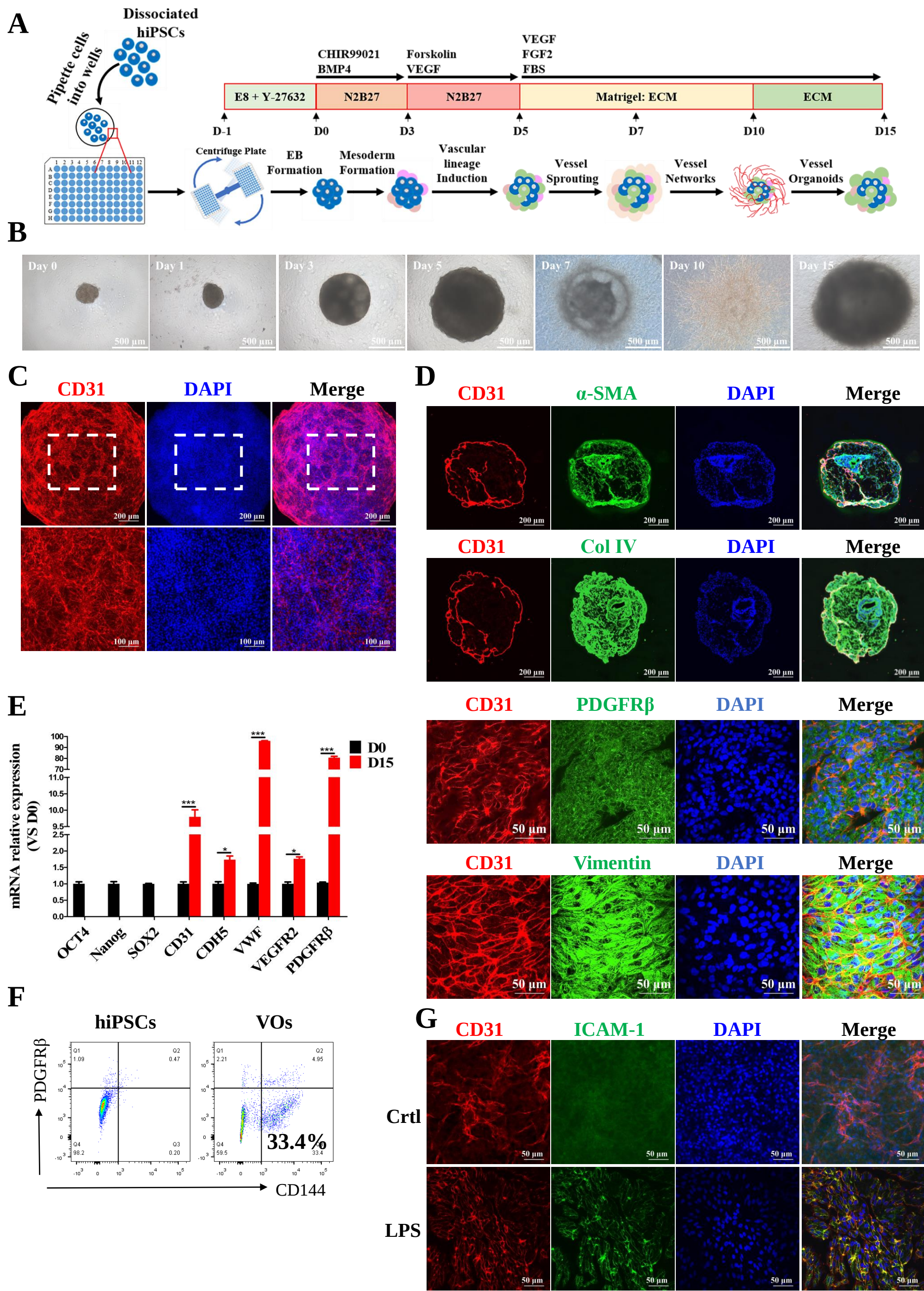


Figure S2. Generation and characterization of blood VOs

(A) A schematic diagram illustrating the protocol used to generate VOs from hiPSCs.

(B) Brightfield images depicting the development of VOs over a 15-day differentiation period.

(C) Confocal imaging of CD31 in D15 VOs.

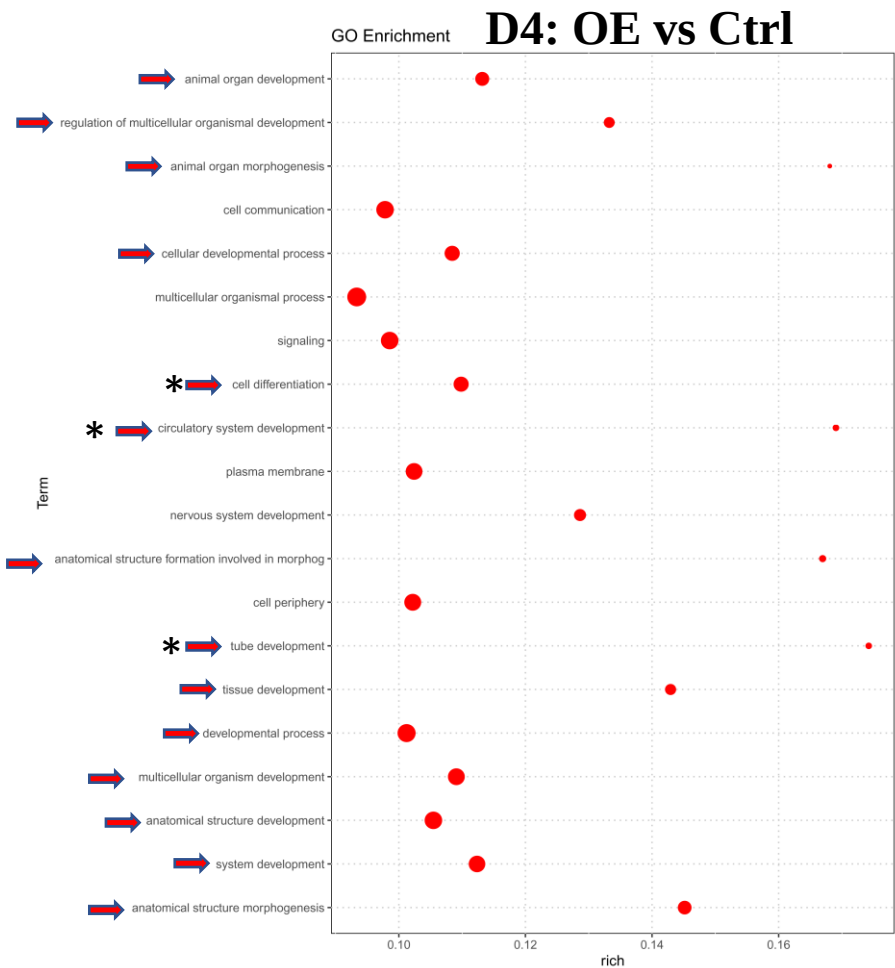
(D) Immunostaining of α -SMA, Col IV and CD31 in D15 VOs. α -SMA, alpha- smooth muscle cell actin; Col IV, Collagen IV.

(E) mRNA expression of stem markers (*Nanog*, *OCT4*, *SOX2*) and vessel markers (*VWF*, *CDH5*, *CD31*, *VEGFR2* and *PDGFR β*) in D15 VOs (n=10) compared to D0. Data normalized to *GAPDH* expression.

(F) Flow cytometry analysis of PDGFR β^+ and CD144 $^+$ cells in D15 VOs.

(G) Immunostaining of ICAM-1 expression in D15 VOs after treatment of LPS (2 μ g/mL) for 24 hours.

E



F

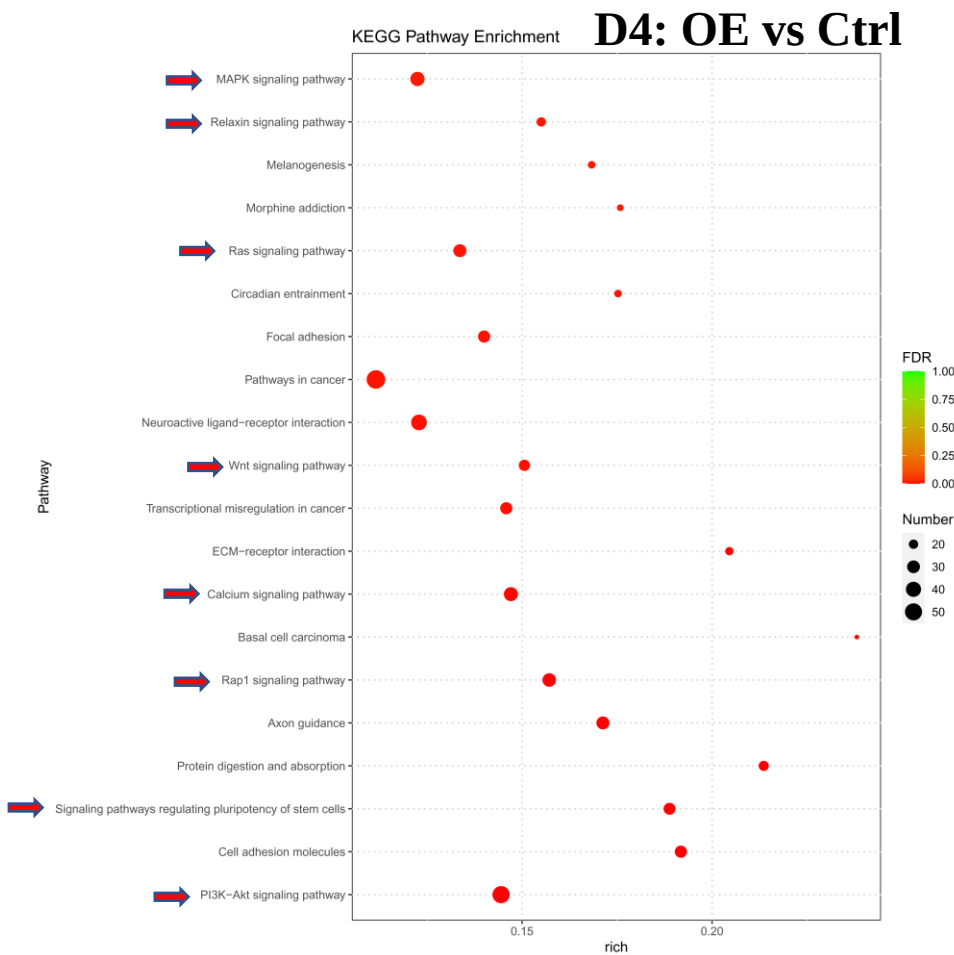


Figure S3. RNA-Seq analysis showing GO enrichment and pathway changed during EC differentiation with or without BCL6B overexpression

(A) GO enrichment analysis of differently expressed genes (DEGs) between day 4 and day 2 differentiating hiPSCs.

(B) KEGG pathways of DEGs between day 4 and day 2 differentiating hiPSCs.

(C) GO enrichment analysis of DEGs at day 2 of differentiating hiPSCs between control (Ctrl) and BCL6B overexpression (OE).

(D) KEGG pathways of DEGs at day 2 of differentiating hiPSCs between control (Ctrl) and BCL6B overexpression (OE).

(E) GO enrichment analysis of DEGs at day 4 of differentiating hiPSCs between control (Ctrl) and BCL6B overexpression (OE).

(F) KEGG pathways of DEGs at day 4 of differentiating hiPSCs between Ctrl and OE.

Figure S4. BCL6B inhibited ETV2 expression

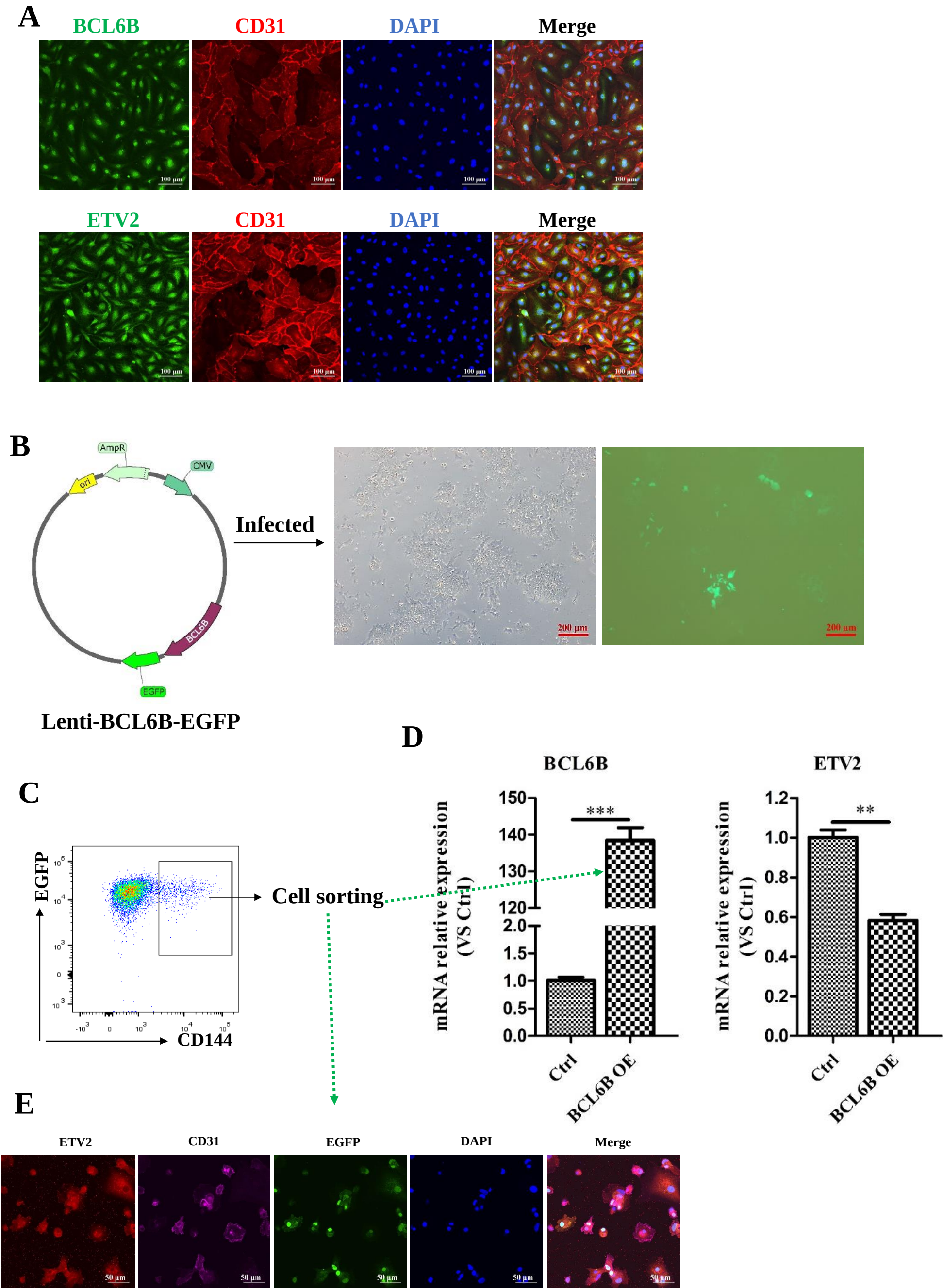


Figure S4. BCL6B inhibited ETV2 expression

(A) Immunofluorescence staining of BCL6B and ETV2 in HUVEC.

(B) Schematic illustration of BCL6B with EGFP overexpression vector and transfected into hiPSCs.

(C) Cell sorting of EGFP⁺ differentiated ECs at day 6.

(D) mRNA expression of BCL6B and ETV2 expression in control and sorted ECs (EGFP⁺/CD144⁺).

(E) Immunofluorescence staining of CD31 and ETV2 in sorted ECs (EGFP⁺/CD144⁺).