Sex-dependent VEGF expression underlies variations in human pluripotent stem cell to endothelial progenitor differentiation

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Supplementary Table 1: Antibodies used in immunostaining analysis.

Antibody	Source	Application
CD144-FITC	Miltenyi Biotec, recombinant human IgG1, Clone: REA199, Cat#: 130-100-742	1:25 (FC)
VE-cadherin	Santa Cruz, mouse igG1, Clone: F-8, Cat#: sc-9989	1:1000 (WB)
β-actin-HRP	Cell Signaling Technology, rabbit, Clone: 13E5, Cat#: 5125S	1:20000 (WB)
Secondary Antibody	Alexa 488 Goat anti Mouse IgG1, Cat#: A-21121	1:1000 (IF)
Secondary Antibody	Anti-mouse IgG HRP-linked, Cell Signaling Technology, Cat#: 7076S	1:1000 (WB)

Supplementary Table 2: Primers used in qPCR experiments.

Primer	Sequence	Size (bp) / T_m (°C) / No. of Cycles
GAPDH_FWD	5'-GTGGACCTGACCTGCCGTCT-3'	152 / 55 / 40
GAPDH_REV	5'-GGAGGAGTGGGTGTCGCTGT-3'	
VEGF_FWD	5'-GGCAAAGTGAGTGACCTGCT-3'	106 / 55 / 40
VEGF_REV	5'-CTGTCTGTCTGTCCGTCAGC-3'	
SOX2_FWD	5'-CAAGATGCACAACTCGGAGA-3'	300 / 55 / 40
SOX2_REV	5'-GTTCATGTGCGCGTAACTGT-3'	
CD34_FWD	5'-CCTAAGTGACATCAAGGCAGAA-3'	201 / 55 / 40
CD34_REV	5'-GCAAGGAGCAGGGAGCATA-3'	

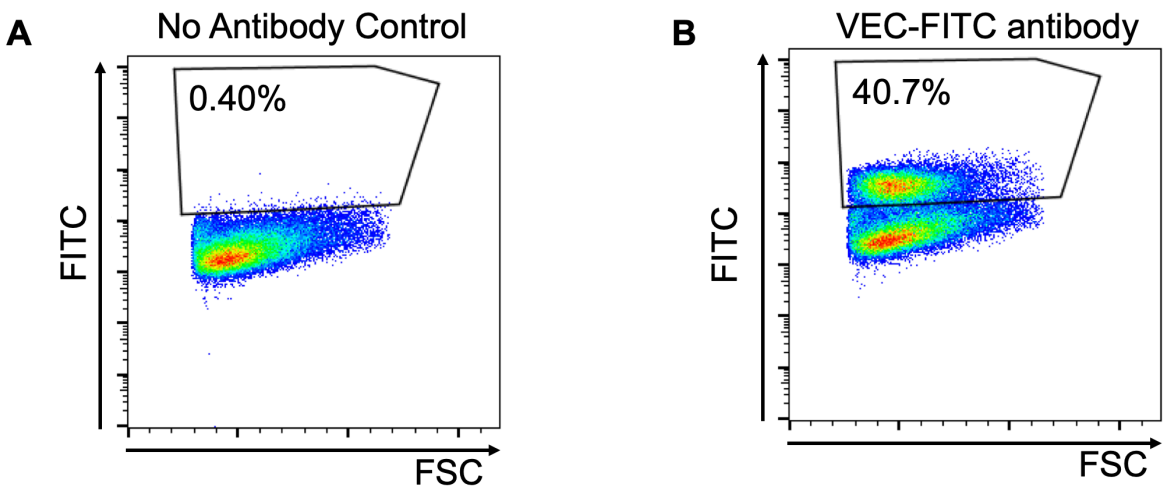

Figure S1: Flow cytometry analysis for D5 endothelial progenitor cells derived from WT H9 cells. H9 cells were differentiated using a GSK3 inhibitor based endothelial progenitor differentiation protocol (Gi protocol). On day 5 of differentiation, cells were analyzed for VEC expression by flow cytometry. (A) No antibody control (B) VEC-FITC antibody.

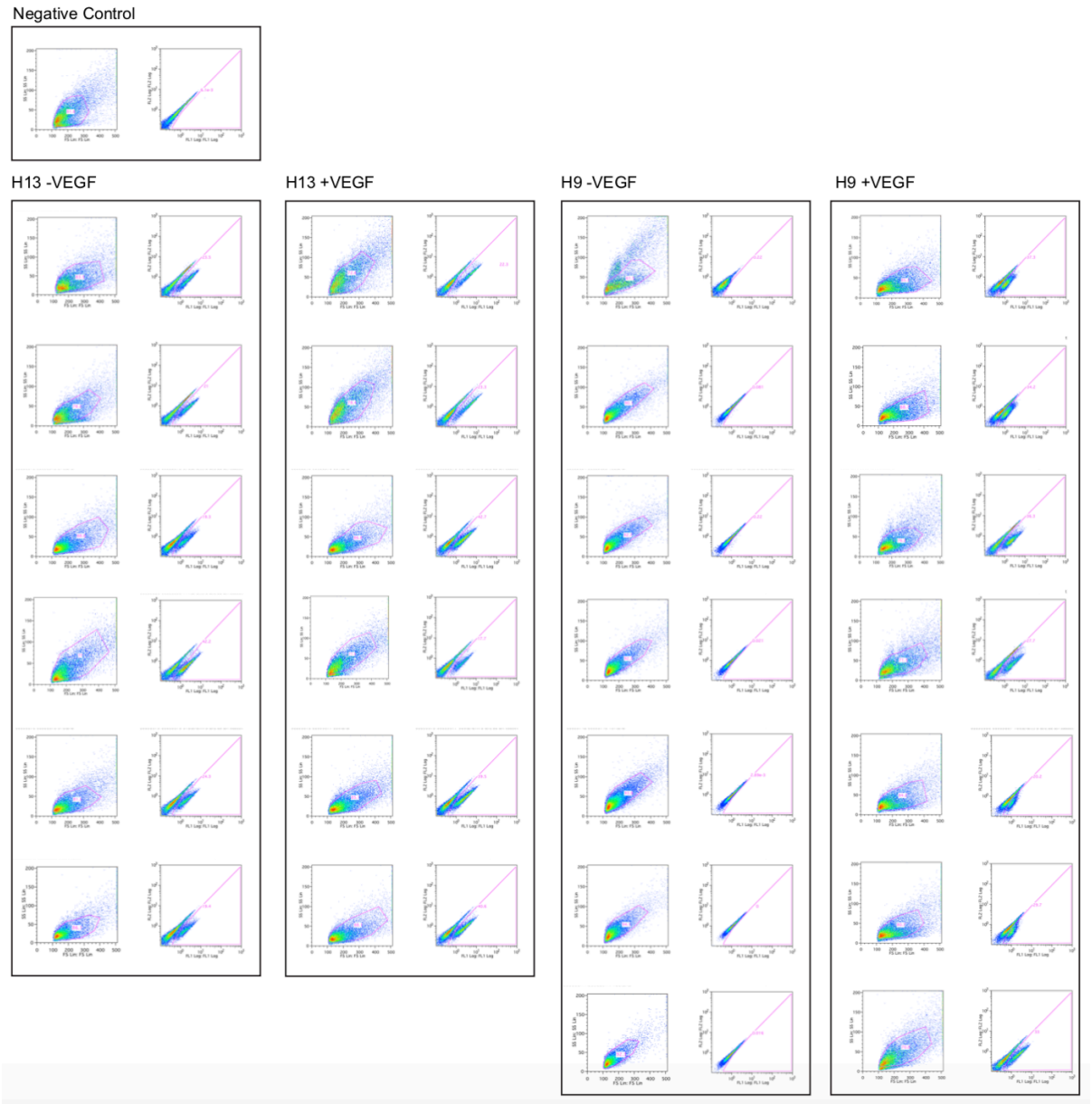

Figure S2: Gating for all flow plots included in data set. H9 and H13 VEC-GFP KI cell lines were differentiated using Gi protocol. On day 5 of differentiation, GFP expression was analyzed via flow cytometry.

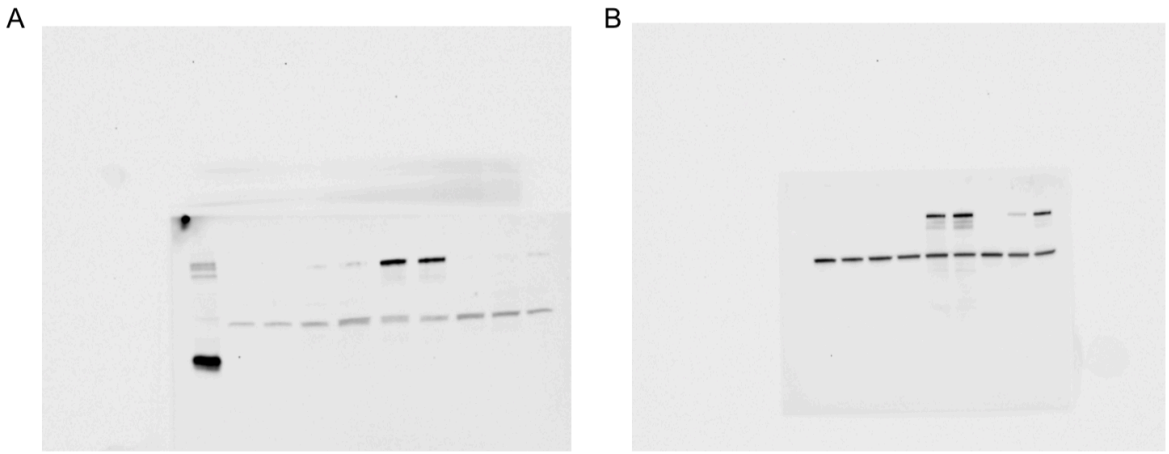

Figure S3: Full blots for Western blot data. A) H9 cells B) H13 cells were differentiated with Gi protocol. VEC and beta-actin expression was analyzed via western blot.

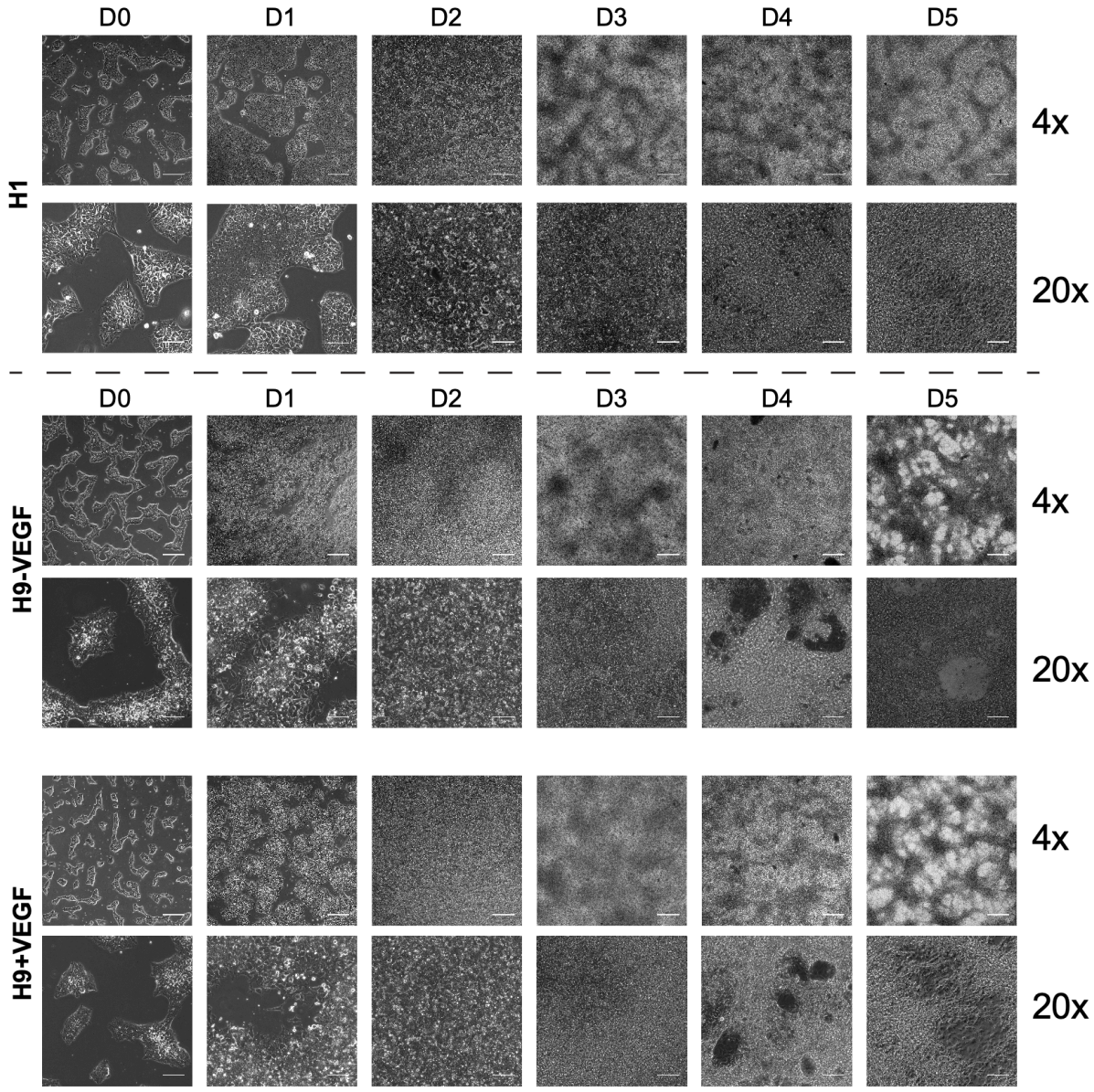

Figure S4: Representative bright field images of differentiation. H1 and H9 cells were differentiated with Gi protocol. Bright field images were taken daily during differentiation. Scale bars for 4x images are 500 μm. Scale bars for 20x images are 100 μM.