Supplementary material

Functional Characterization of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells

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Supplementary Table S1. List of genes, RefSeq numbers and primers for qPCR.

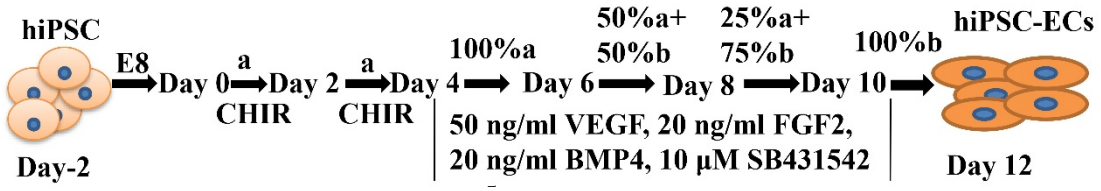
Gene symbol	**RefSeq No.**	**Company**
ABCC8 (KATP, beta-subunit SUR1)	NM_000352	Qiagen
ADRA1A	NM_033303	Qiagen
ADRA2A	NM_000681	Qiagen
ADRB1	NM_000684	Qiagen
ADRB2	NM_000024	Qiagen
CHRM2	NM_000739	Qiagen
CHRM3	NM_000740	Qiagen
HCN2	NM_001194	Qiagen
HCN4	NM_005477	Qiagen
KCNN2 (SK2)	NM_021614	Qiagen
KCNN4	NM_002250	Qiagen
KCNQ1 (I_{Ks}, Kv7.1)	NM_000218	Qiagen
SLC8A1 (NCX1)	NM_021097	Qiagen
TRPV2	NM_016113	Qiagen
KCNJ2	NM_000891	Qiagen
KCNMA1	NM_002247	Qiagen
ADRA1A	NM_033303	Qiagen
ADRA2A	NM_000681	Qiagen
ADRB1	NM_000684	Qiagen

ADRB2	NM_000024	Qiagen
CHRM2	NM_000739	Qiagen
CHRM3	NM_000740	Qiagen
DRD1	NM_000794	Qiagen
DRD2	NM_000795	Qiagen
DRD3	NM_033660	Qiagen
DRD4	NM_000797	Qiagen
DRD5	NM_000798	Qiagen
GNAS	HP101598	Sino Biological

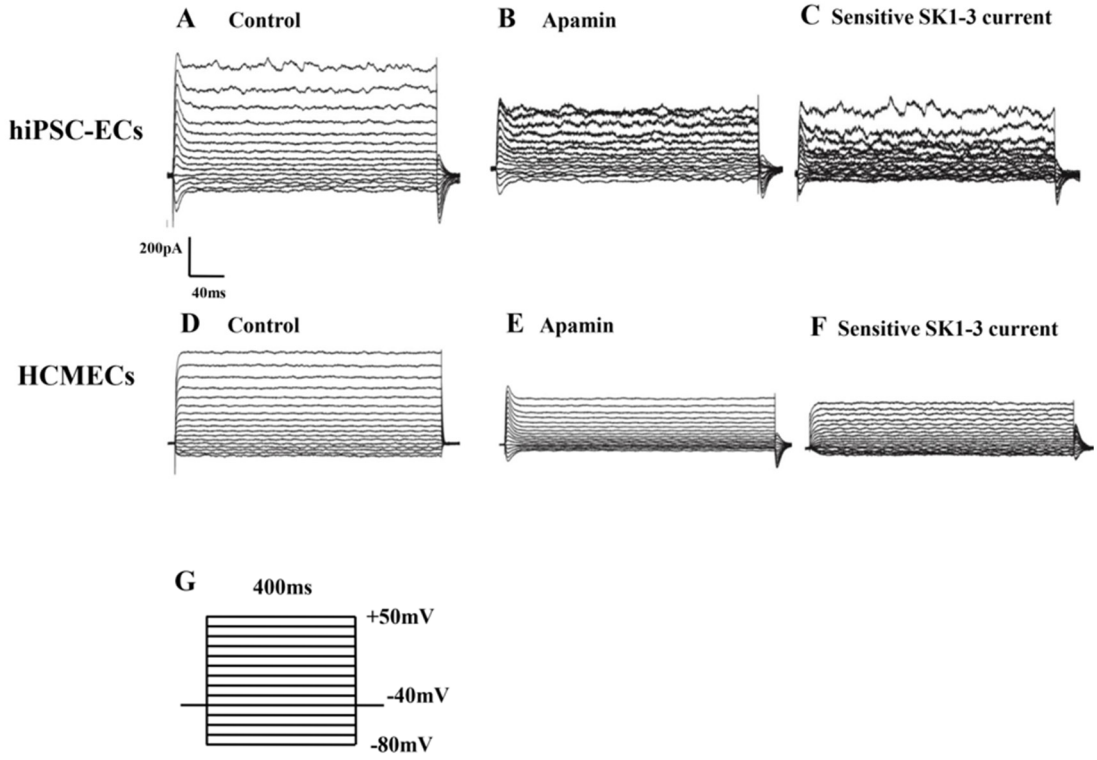
Supplementary Table S2. Primer sequences used

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
KCNN1	CAGCATCTCCTCCTGGATCAT	GCTGGTCACTTCCTGCTTGTC
KCNN3	GCCAACACTCTGGTGGACCT	GTTGAAGCTGGCGGTGAGAT
AT1 receptor	GCCCTTTGGCAATTACCTATGT	CGTGAGTAGAAACACACTAGCGT
AT2 receptor	CCGCATTTAACTGCTCACACA	ATCATGTAGTAGAGAACAGGAATT G CTT
GNA11	GATCCTCTACAAGTACGAGCAG AAC	ACTGATGCTCGAAGGTGGTC
Gai2	CTTGTCTGAGATGCTGGTAATG G	CTCCCTGTAAACATTTGGACTTG
GNAQ	GACTACTTCCCAGAATATGATG GAC	GGTTCAGGTCCACGAACATC
PECAM1	ATTGCAGTGGTTATCATCGGAG TG	CTCGTTGTTGGAGTTCAGAAGTGG
CDH5	AGACACCCCCAACATGCTAC	GCAAACTCTCCTTGGAGCAC
VWF	GGGGTCATCTCTGGATTCAAG	TCTGTCCTCCTCTTAGCTGAA

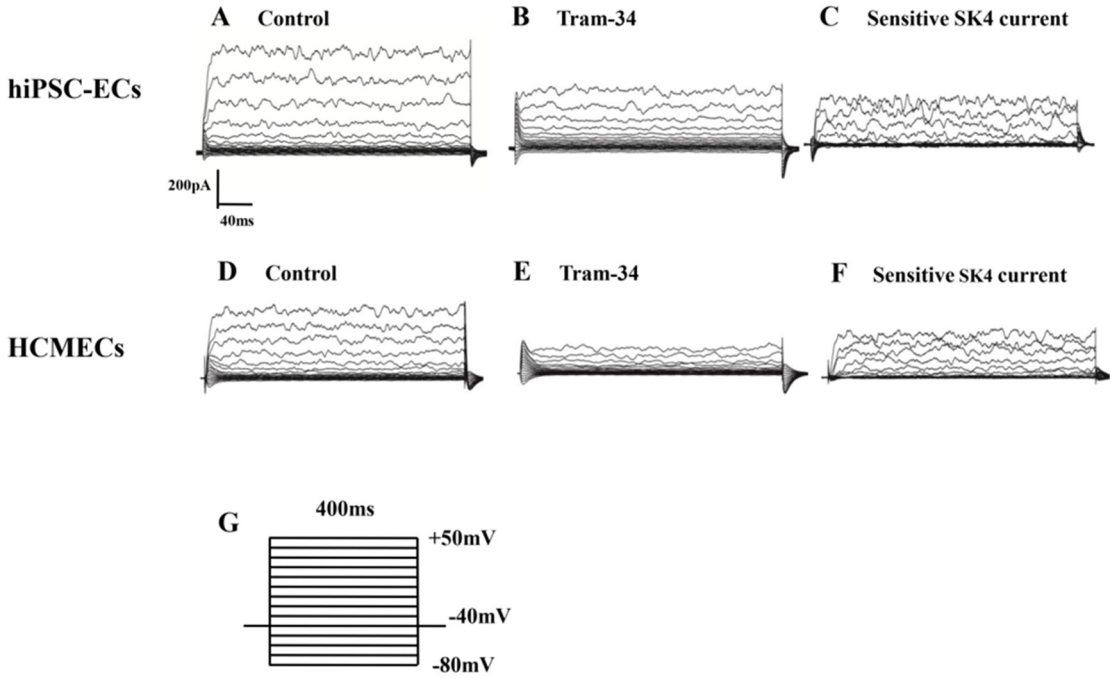
Figure legends



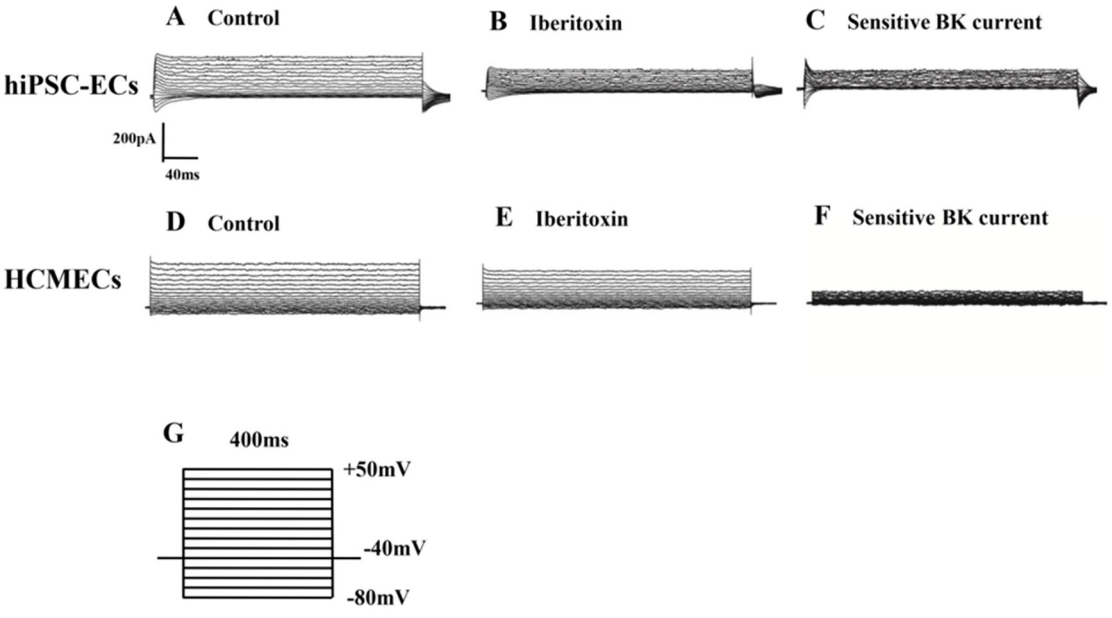
Supplementary Figure S1: Schematic of hiPSC-ECs differentiation. hHiPSCs were seeded and maintained in E8 medium to establish single colonies (day -2 to day 0). hHiPSCs were exposed for four days to E8 medium supplemented with different concentration of CHIR (day 0 to day 4). hHiPSCs were then exposed for eight days to different ratio of differentiation medium and EMV2 supplemented with different combinations of cytokines as indicated (day 4 to day 12) and subsequently analyzed.



Supplementary Figure S2. SK1-3 currents in hiPSC-ECs and HCMECs. Membrane currents were recorded using the protocol shown in G. Apamin (100 nM), a specific blocker of SK1-3, was used to separate I_{SK1-3} from other currents. (A) Representative current traces in the absence of apamin (control) in hiPSC-ECs. (B) Representative current traces after application of apamin in hiPSC-ECs. (C) Apamin sensitive currents (I_{SK1-3}) in hiPSC-ECs. (D) Representative current traces in the absence of apamin (control) in HCMECs. (E) Representative current traces after application of apamin in HCMECs. (F) Apamin sensitive currents (I_{SK1-3}) in HCMECs. (G) The protocol for recording currents. The holding potential was -40 mV, test potentials ranged from -80 mV to +50 mV for 400 ms.

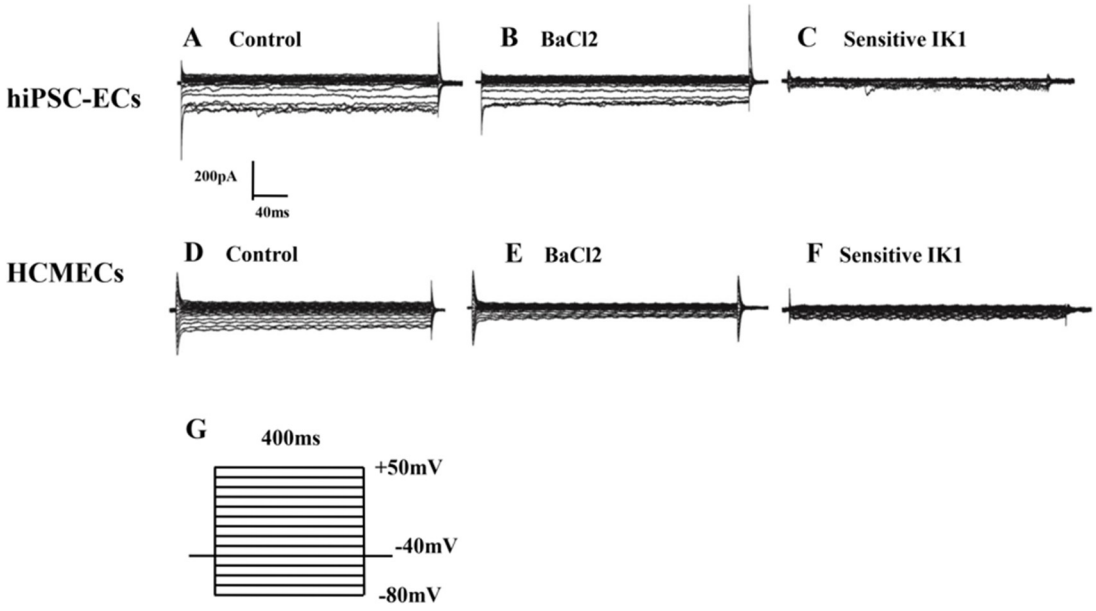


Supplementary Figure S3. SK4 currents in hiPSC-ECs and HCMECs. Membrane currents were recorded using the protocol shown in G. Tram-34 (1 μM), a specific blocker of SK4, was used to separate I_{SK4} from other currents. (A) Representative current traces in the absence of Tram-34 (control) in hiPSC-ECs. (B) Representative current traces after application of Tram-34 in hiPSC-ECs. (C) Tram-34 sensitive currents (I_{SK4}) in hiPSC-ECs. (D) Representative current traces in the absence of Tram-34 (control) in HCMECs. (E) Representative current traces after application of Tram-34 in HCMECs. (F) Tram-34 sensitive currents (I_{SK4}) in HCMECs. (G) The protocol for recording currents. The holding potential was -40 mV, test potentials ranged from -80 mV to +50 mV for 400 ms.

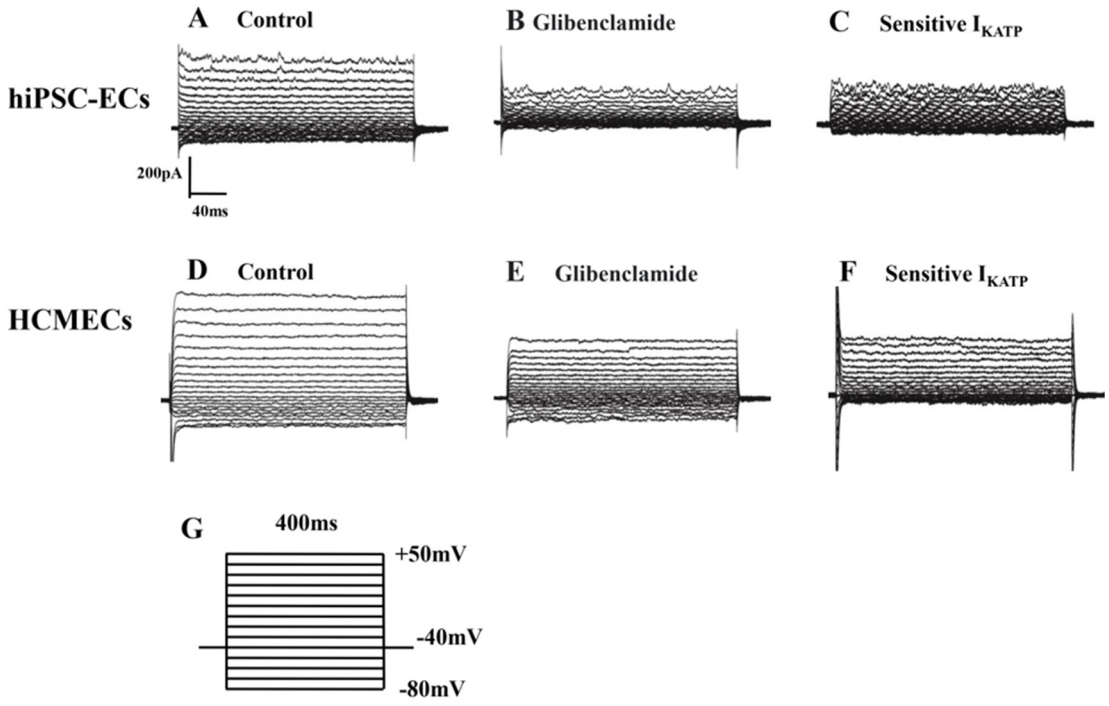


Supplementary Figure S4. BK currents in hiPSC-ECs and HCMECs. Membrane currents were

recorded using the protocol shown in G. Iberitoxin (100 nM), a specific blocker of BK current (I_{BK}), was used to separate I_{BK} from other currents. (A) Representative current traces in the absence of iberitoxin (control) in hiPSC-ECs. (B) Representative current traces after application of iberitoxin in hiPSC-ECs. (C) Iberitoxin sensitive currents (I_{BK}) in hiPSC-ECs. (D) Representative current traces in the absence of iberitoxin (control) in HCMECs. (E) Representative current traces after application of iberitoxin in HCMECs. (F) Iberitoxin sensitive currents (I_{BK}) in HCMECs. (G) The protocol for recording currents. The holding potential was -40 mV, test potentials ranged from -80 mV to +50 mV for 400 ms.



Supplementary Figure S5. I_{K1} in hiPSC-ECs and HCMECs. Membrane currents were recorded using the protocol shown in G. $BaCl_2$ (100 μM), a blocker of I_{K1} current, was used to separate I_{K1} from other currents. (A) Representative current traces in the absence of $BaCl_2$ (control) in hiPSC-ECs. (B) Representative current traces after application of $BaCl_2$ in hiPSC-ECs. (C) $BaCl_2$ sensitive currents (I_{K1}) in hiPSC-ECs. (D) Representative current traces in the absence of $BaCl_2$ (control) in HCMECs. (E) Representative current traces after application of $BaCl_2$ in HCMECs. (F) $BaCl_2$ sensitive currents (I_{K1}) in HCMECs. (G). The protocol for current recording, with a holding potential of -40 mV and test potentials ranging from -120 mV to +50 mV for 400 ms.



Supplementary Figure S6. I_{KATP} in hiPSC-ECs and HCMECs. Membrane currents were recorded using the protocol shown in G. Glibenclamide (10 μM), a blocker of I_{KATP} current, was used to separate I_{KATP} from other currents. (A) Representative current traces in the absence of glibenclamide (control) in hiPSC-ECs. (B) Representative current traces after application of glibenclamide in hiPSC-ECs. (C) Glibenclamide sensitive currents (I_{KATP}) in hiPSC-ECs. (D) Representative current traces in the absence of glibenclamide (control) in HCMECs. (E) Representative current traces after application of glibenclamide in HCMECs. (F) Glibenclamide sensitive currents (I_{KATP}) in HCMECs. (G). The protocol for current recording, with a holding potential of -40 mV and test potentials ranging from -120 mV to +50 mV for 400 ms.