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Supplemental information

Feeder-free generation and characterization of endocardial and cardiac valve cells from human pluripotent stem cells

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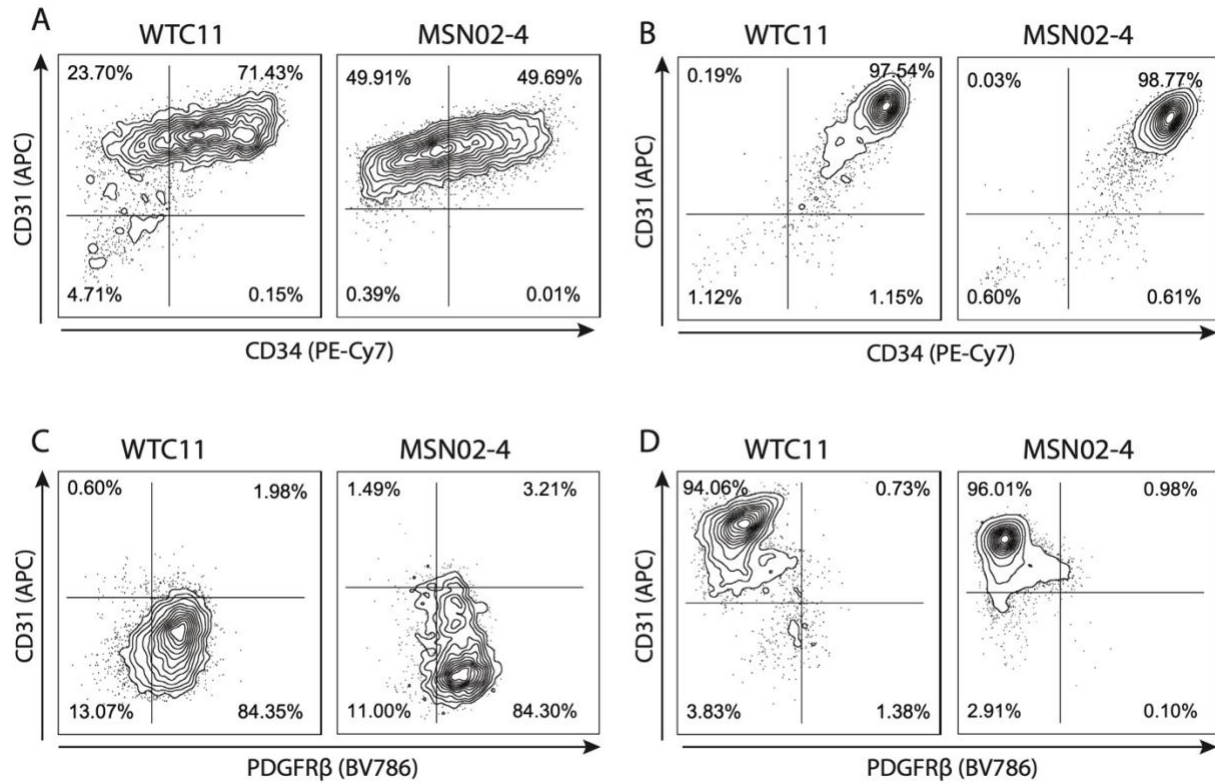


Figure S1: Validation of sort efficiencies following MACS, related to Figures 1 and 3. (A)

Sort efficiency following CD31 MACS on Day 14 endocardial cells. **(B)** Sort efficiency

following CD34 MACS on Day 14 endothelial cells. **(C)** Sort efficiency following negative

selection with CD31 MACS for post-EndMT VICs. **(D)** Sort efficiency following positive

selection with CD31 MACS for post-EndMT VECs.

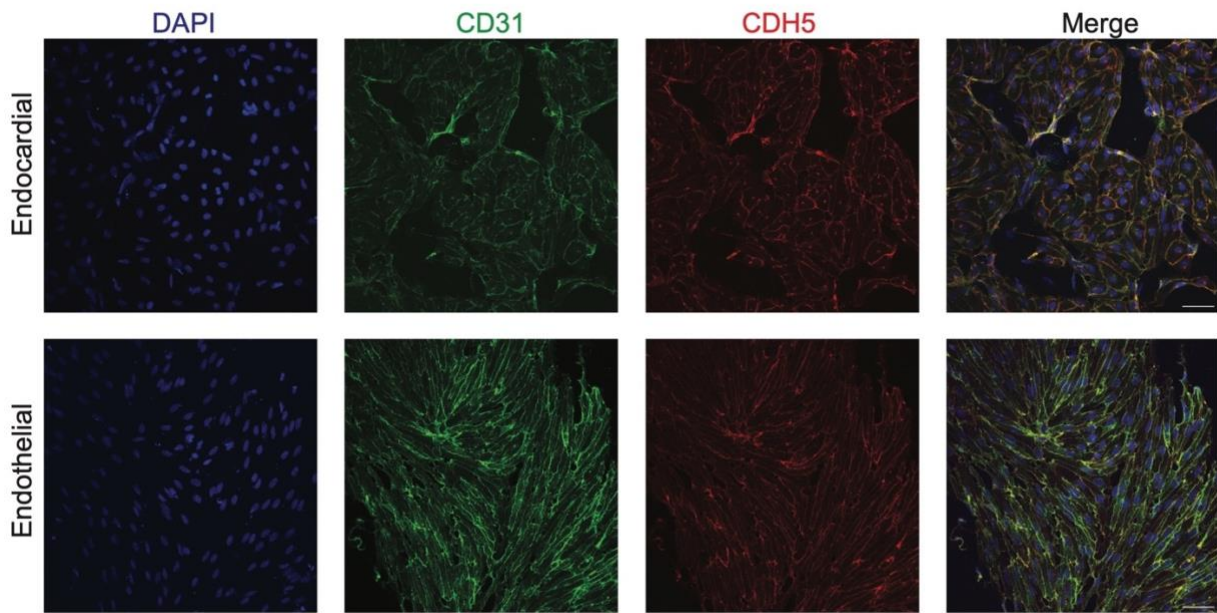


Figure S2: Immunofluorescence analysis of CD31 and CDH5 of endocardial and endothelial cells, related to Figure 2. Representative confocal microscopy of MSN02-4 endocardial and endothelial cells stained for CD31 (GFP) and CDH5 (RFP).

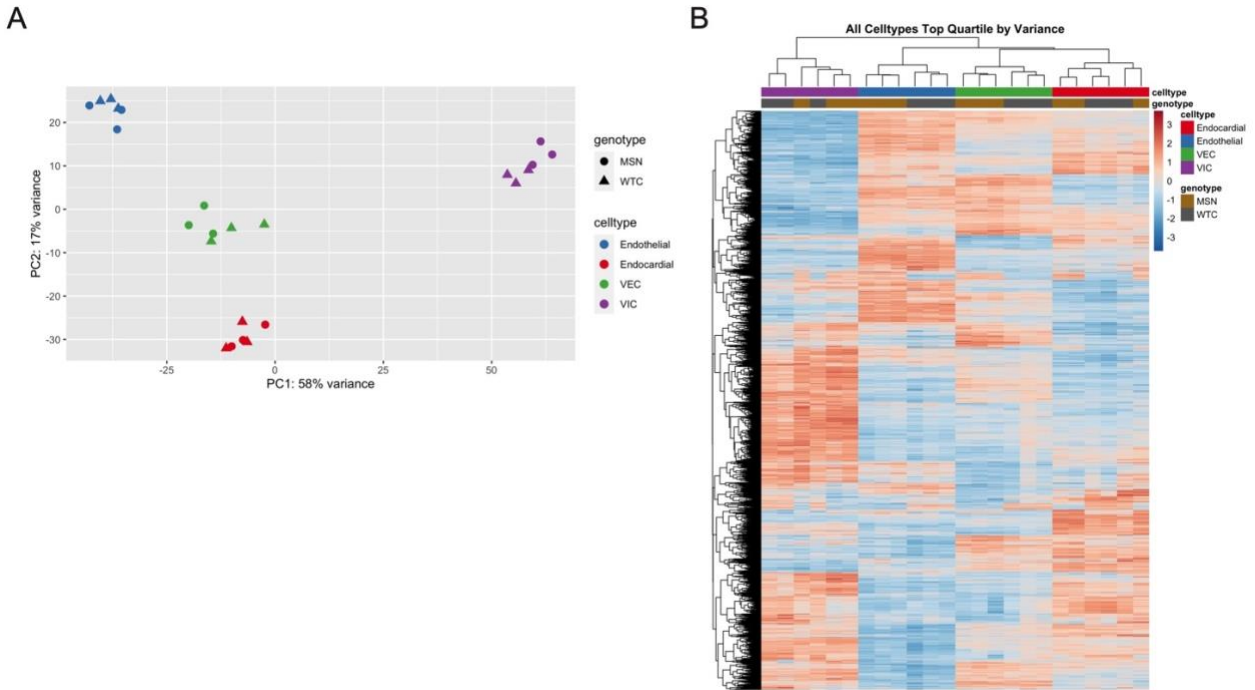


Figure S3: Bulk-RNaseq analysis on all cell populations, related to Figure 5. (A) PCA plot of each of the cell populations from both WTC11 and MSN02-4 iPSC lines. **(B)** Heatmap with unsupervised clustering on the top quartile of genes ranked by variance for all cell types.

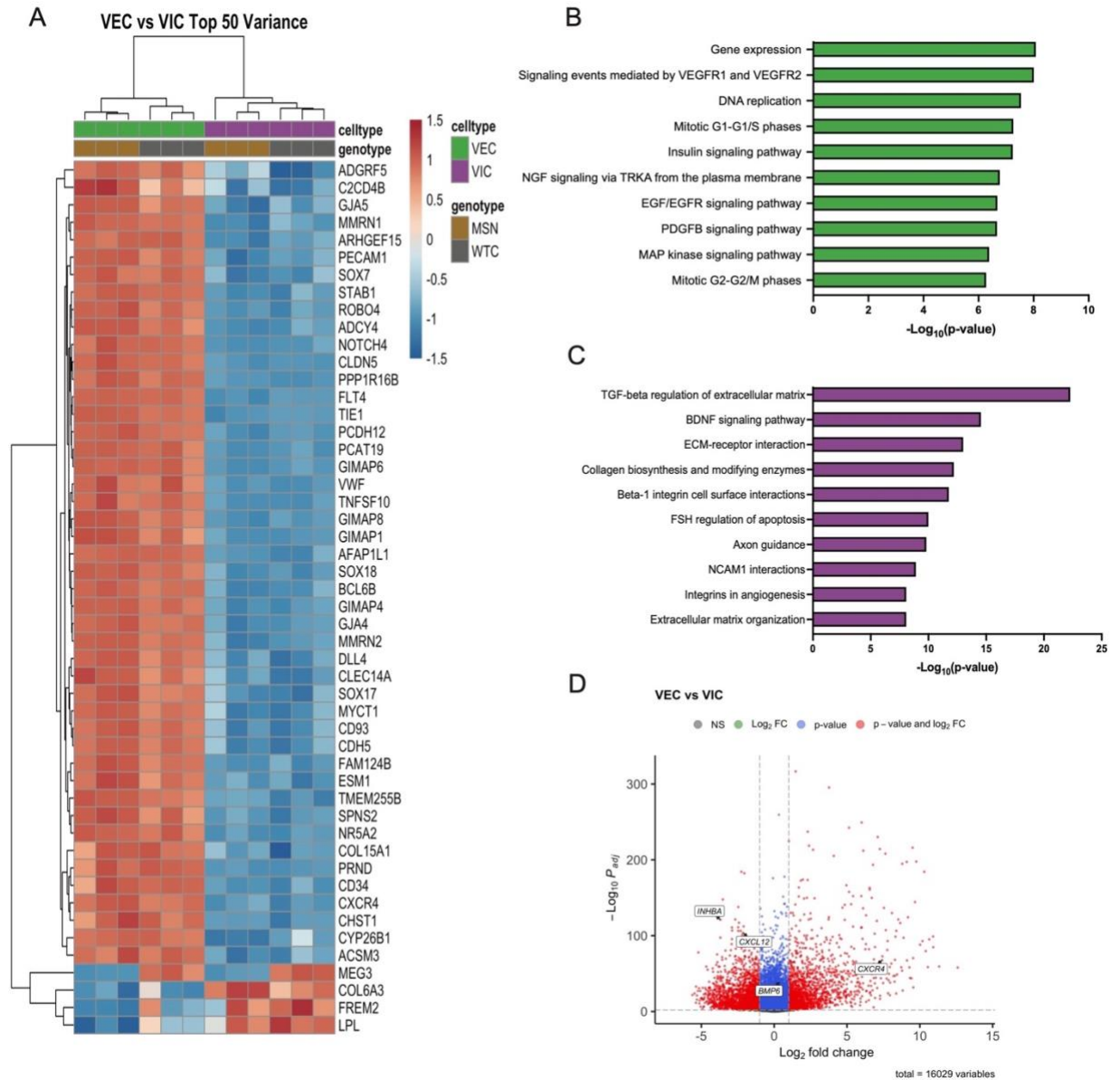


Figure S4: Bulk-RNAseq characterization of VECs vs VICs, related to Figure 6. (A)

Heatmap of the top 50 DEGs by variance between VECs and VICs. **(B)** Top 10 BioPlanet GO terms to assess signaling pathway changes for DEGs upregulated in VECs relative to VICs. **(C)** Top 10 BioPlanet GO terms to assess signaling pathway changes for DEGs upregulated in VICs relative to VECs. **(D)** Volcano plot to assess relative expression of *CXCL12*, *INHBA*, *BMP6*, and *CXCR4* between VECs and VICs. Cutoff for \log_2 fold change was set to 1 and -1; $p_{adj} < 10^{-3}$.

Table S1- List of Primers, Related to STAR Methods

Gene Name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
CAD11	CCAACAGCCCGATAAGGTATT	TTGGGAGCATTATCGTTGACA
CD31	GACGTGCAGTACACGGAAGT	GGAGCCTTCCGTTCTAGAGTAT
CDH5	GGCAGTCCAACGGAACAGA	CTCTCTTTTGGCGCCCGT
COL1A1	CAATGCTGCCCTTTCTGCTCCTTT	CACTTGGGTGTTTGTGAGCATTGCCT
COL3A1	GAATTTGGTGTGGACGTTGG	CTTGCACTGGTTGACAAGATTAG
GATA4	CGAATGACGGCATCTGTTTGCCAT	ATTTGGTATTAGGGATGCAGGGCG
GATA5	CTCGTACTCCGCCACCTACC	AGTTGACACACTCACGACCC
HAPLN1	AGCCACCTGATTAAGAAACCA	TGACAGAGTTTGACACAAAGAAAA
ISL1	TCCCTATGTGTTGGTTGCGG	TCGTTCTTGCTGAAGCCGAT
MEIS2	AGCGCAAGACACAGGACTTA	AAACCTGCTCGATTTGACTGG
N-CAD	ATGTGCATGAAGGACAGCCTCT	GTGGAAAGCTTCTCACGGCA
NFATc1	GCATCACAGGGAAGACCGTGTC	GAAGTTCAATGTCCGAGTTTCTGAG
NKX2.5	TTTGCATTCCTCCTGCGGAGACCTA	ACTCATTGCACGCTGCATAATCGC
NPR3	CGGGAAGATTCCATCAGATCC	GTCTTTCAACCCGTCTATCTCC
NRG1	CCGAAAGCCACTCTGTAATC	CCTGAGGAAGCTGTTACATTC
POSTN	TGCCCAGCAGTTTTGCCCAT	CGTTGCTCTCCAAACCTCTA
SOX9	ACACTCCTCCTCCGGCATGAG	GCTTCAGGTCAGCCTTGCC
TBP	TGAGTTGCTCATACCGTGCTGCTA	CCCTCAAACCAACTTGTCAACAGC
TGFB2	TCTGTGGGTACCTTGATGCC	GCGCTGGGTGGAGATGTTA
TMEM100	TGCTCCTTACTGCATTGGCTT	GCTTGGTAAGAGGAGCATGGA
VIM	GGACCAGCTAACCAACGACA	AAGGTCAAGACGTGCCAGAG