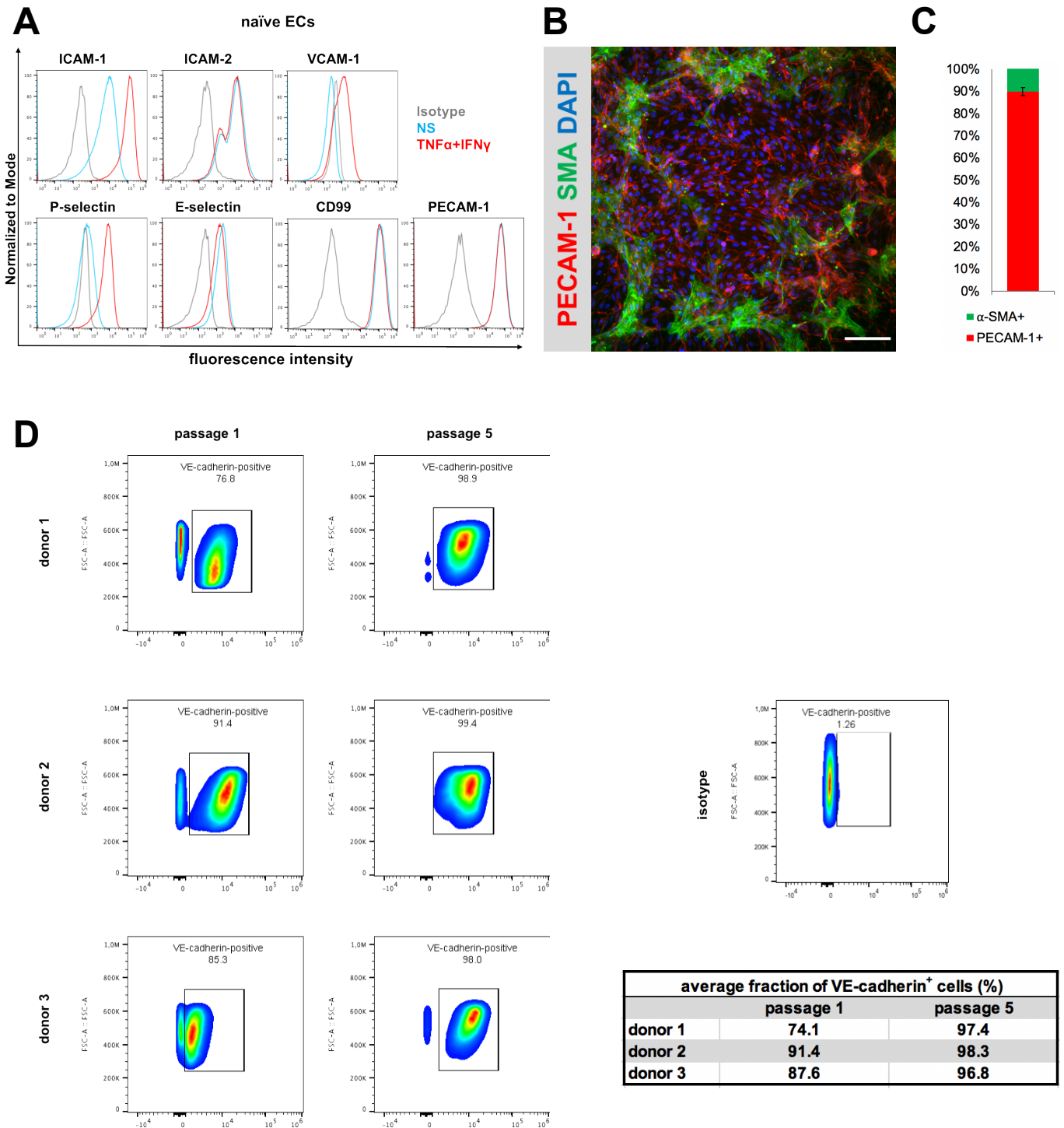


Supplementary Figure 2



Supplementary Figure 2. Adhesion molecule phenotype and morphology of naïve ECs from additional hiPSC clone, and purity of VE-cadherin⁺ cells at passage 1 and passage 5.

(A) Cell surface staining of naïve ECs for the adhesion molecules ICAM-1, ICAM-2, VCAM-1, P-selectin, E-selectin, CD99, and PECAM-1 was analyzed by flow cytometry. Isotype control, non-stimulated (NS) and 16 h pro-inflammatory cytokine-stimulated condition (1 ng/mL TNF- α + 20 IU/mL IFN- γ) are represented respectively in grey, blue, and red lines in histogram overlays. Representative data from donor 1 is shown. (B) Immunofluorescence staining of naïve ECs grown on 0.4 μ m pore Transwell filter for PECAM-1 (red) and smooth muscle actin (green). Nuclei were stained with DAPI (blue). Scale bar = 100 μ m. (C) Quantification of PECAM-1⁺ and α -smooth muscle actin⁺ cells in immunofluorescence staining images. Bar shows mean \pm SD of 4 differentiations using IMR90-4 line. (D) Staining of ECs for the adherens junction molecule VE-cadherin was analyzed by flow cytometry. Isotype control, passage 1 and passage 5 ECs are reported. Representative data are shown. Three different hiPSC clones derived from 3 individuals (donor 1, 2, 3) were evaluated in this assay.