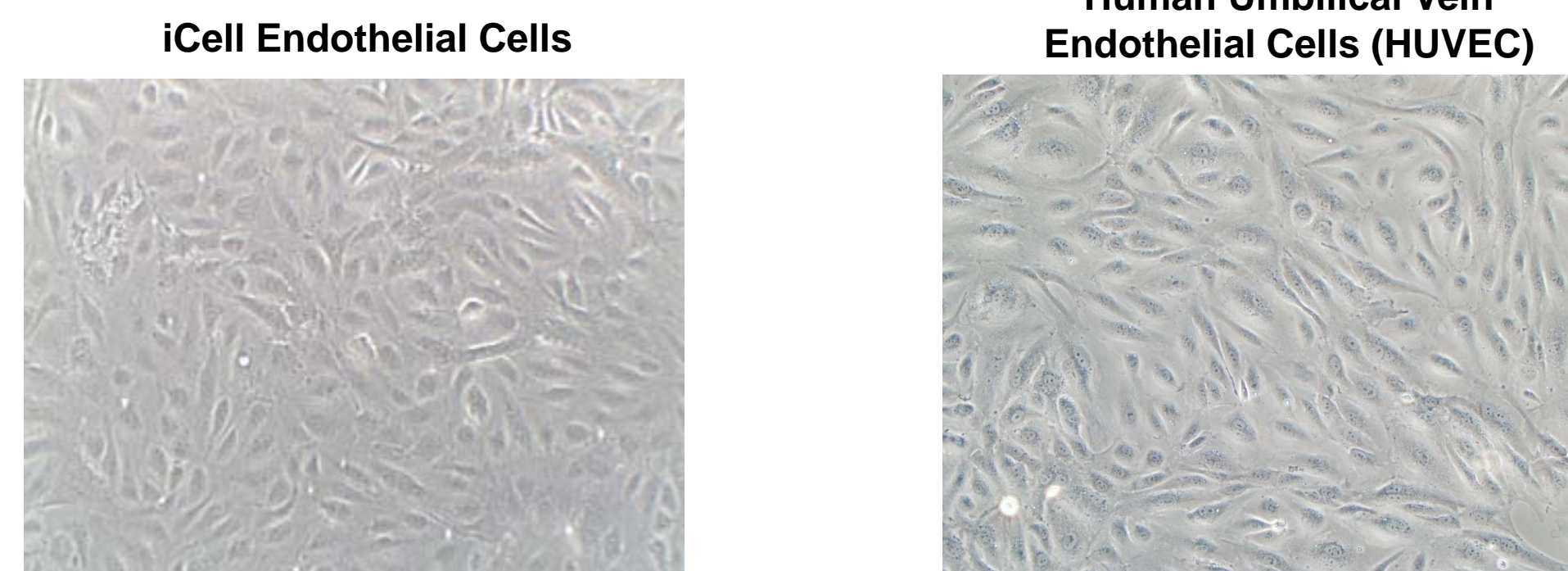


## Abstract

Endothelial cells play a key role in a diverse range of physiological, immunological and pathological processes including working as a selective barrier between the blood and the surrounding body tissues, controlling the inflammation of tissues, and regulating the angiogenesis process. They are metabolically active, producing physiologically active biochemical substances which play a significant role in health and disease. It is axiomatic that predictive translation of in vitro data requires a system capable of recapitulating appropriate in vivo biology. Of equal importance is the need for large quantities of high quality, high purity, and highly reproducible test system material. Human stem cell technology has addressed both of these needs and become an important focus for use in drug discovery, toxicity testing, and therapeutics. We have developed human iPSC-derived endothelial cells that are >95% pure and exhibit characteristic endothelial cell morphology, gene and protein expression, and functional properties. Data highlighting their generation, characterization, and expression of native behavior will be presented. The development of human iPSC-derived endothelial cells that recapitulate in vivo endothelial function will enable powerful new strategies for drug discovery and disease research.

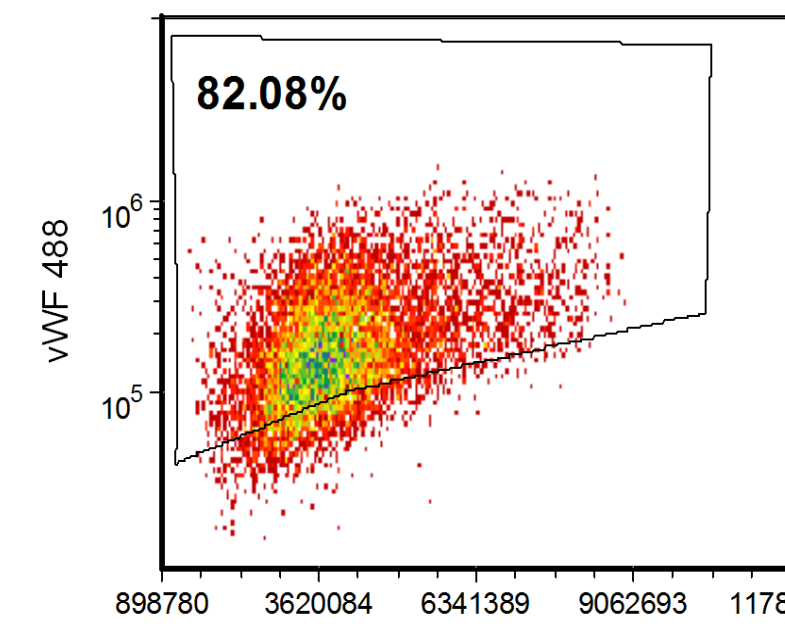
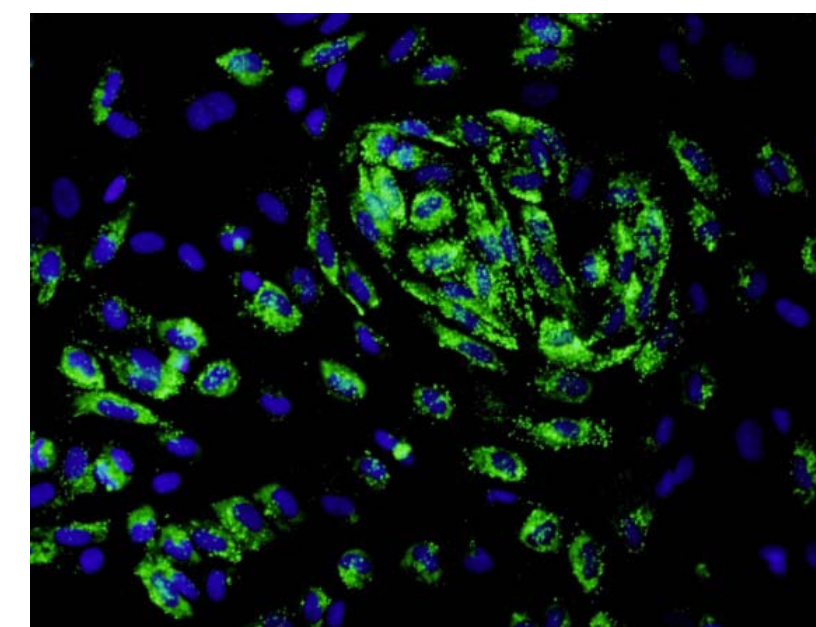
## iCell® Endothelial Cells: Morphology & Molecular Markers

### A. Morphology

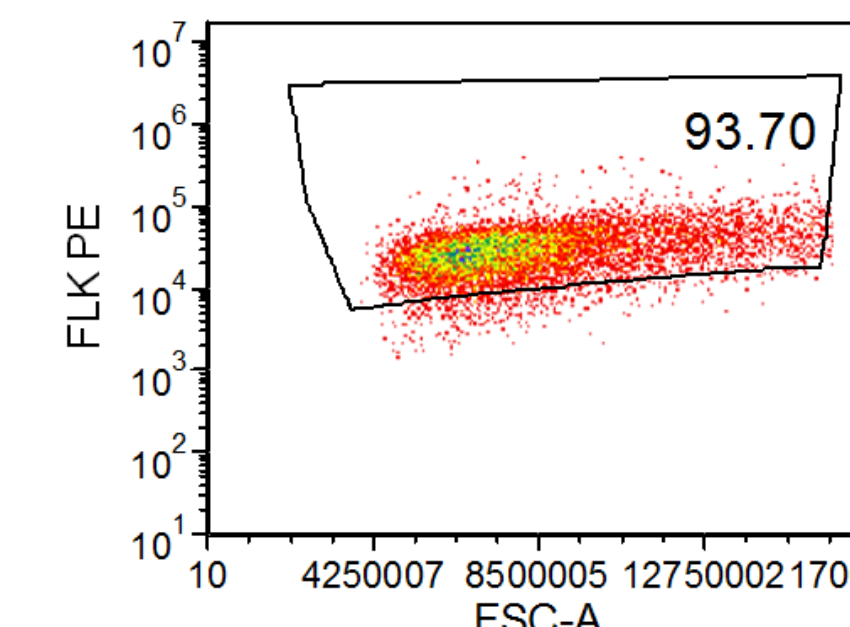


### B. Molecular Markers

#### I. von Willebrand Factor



#### II. Flk1



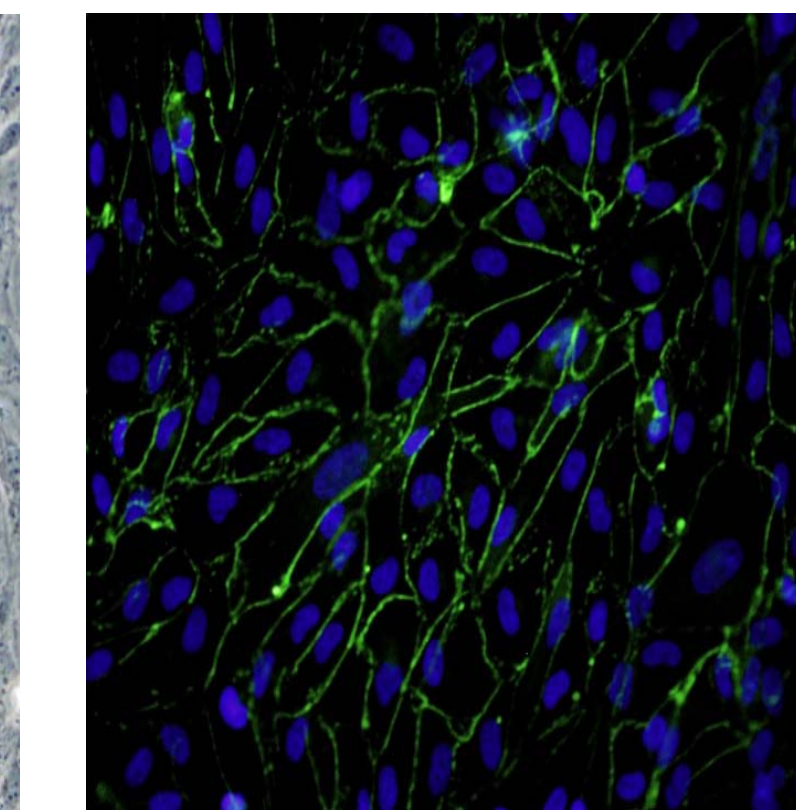
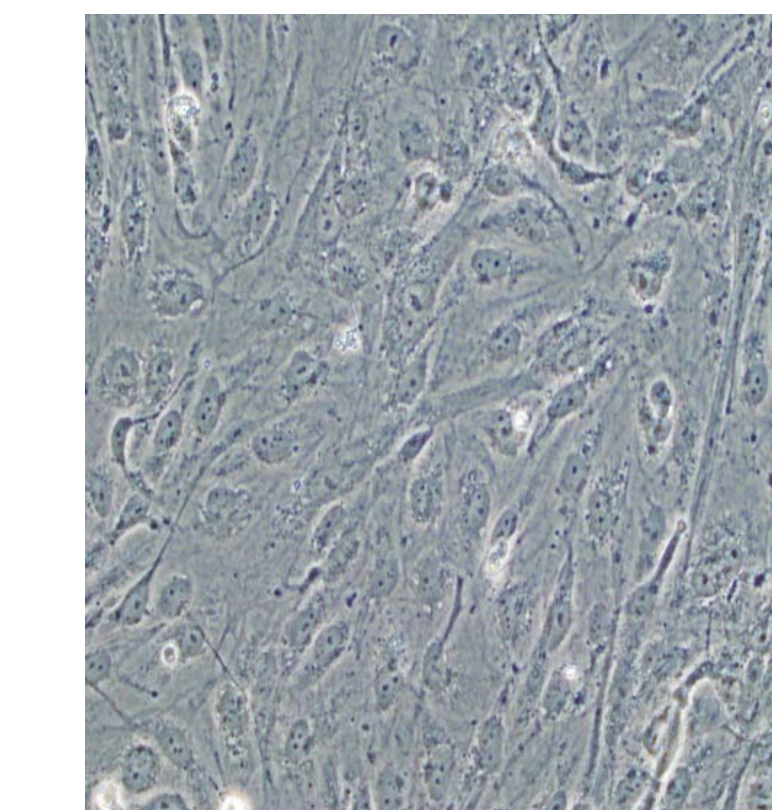
**A: iCell Endothelial Cells Morphology.** iCell Endothelial Cells and Human Umbilical Vein Endothelial Cells (HUVEC) were plated on fibronectin-coated plates. iCell Endothelial Cells form an adherent monolayer and exhibit a cobblestone morphology similar to HUVEC.

**B: iCell Endothelial Cells express endothelial proteins.** iCell Endothelial Cells express (I.) von Willebrand Factor as demonstrated by characteristic Wiebel-Palade body immunostaining (green) and FACS analysis, and (II.) Flk1 as determined by FACS analysis.

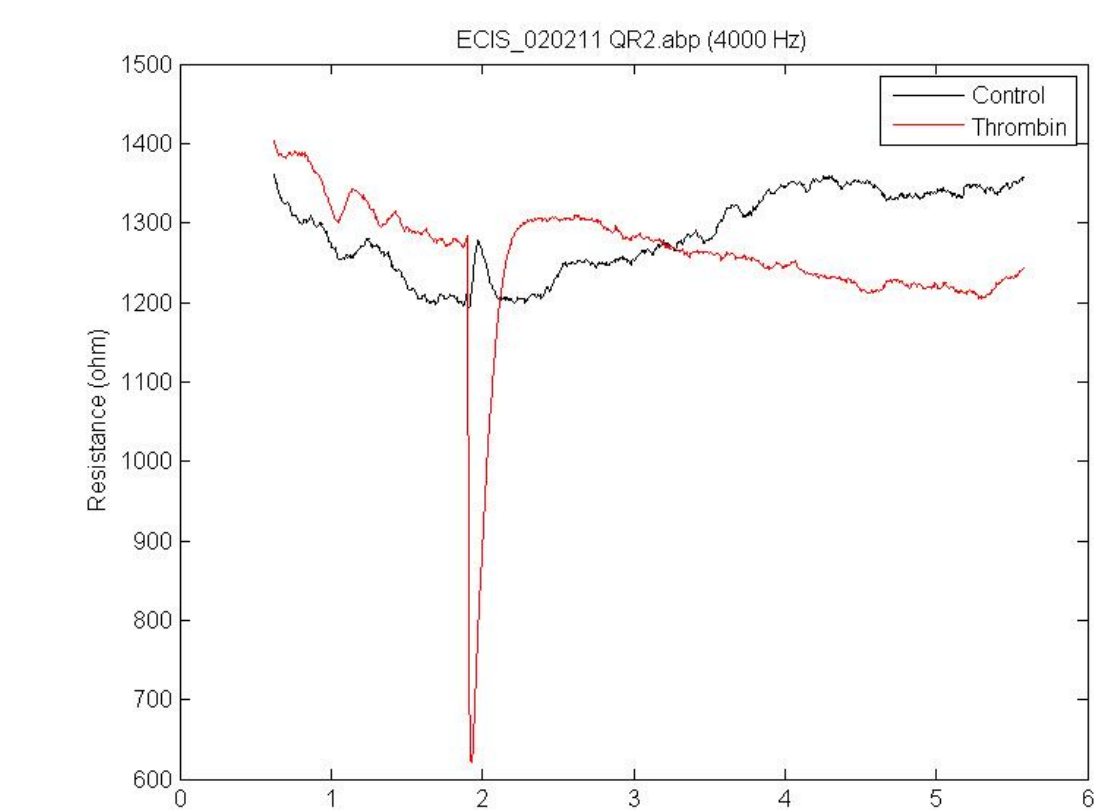
## iCell® Endothelial Cells: Functional Characterization

### A. Cell Barrier Protein Expression and Function

#### I. ZO-1 Protein Expression

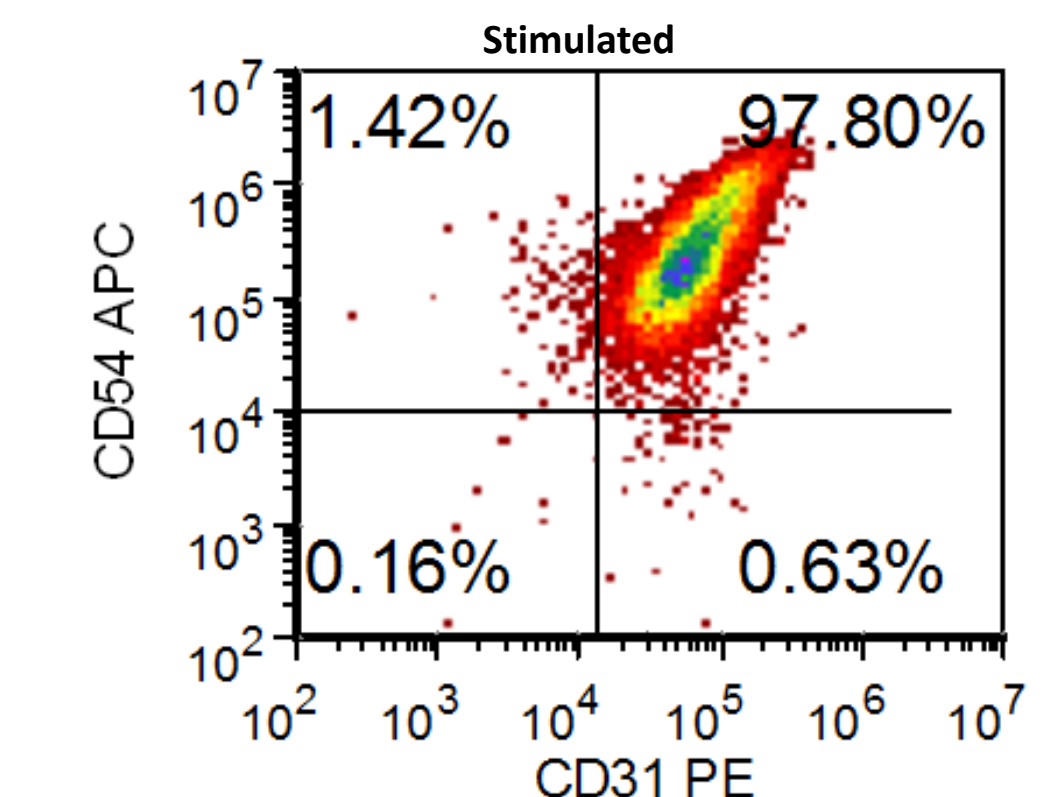
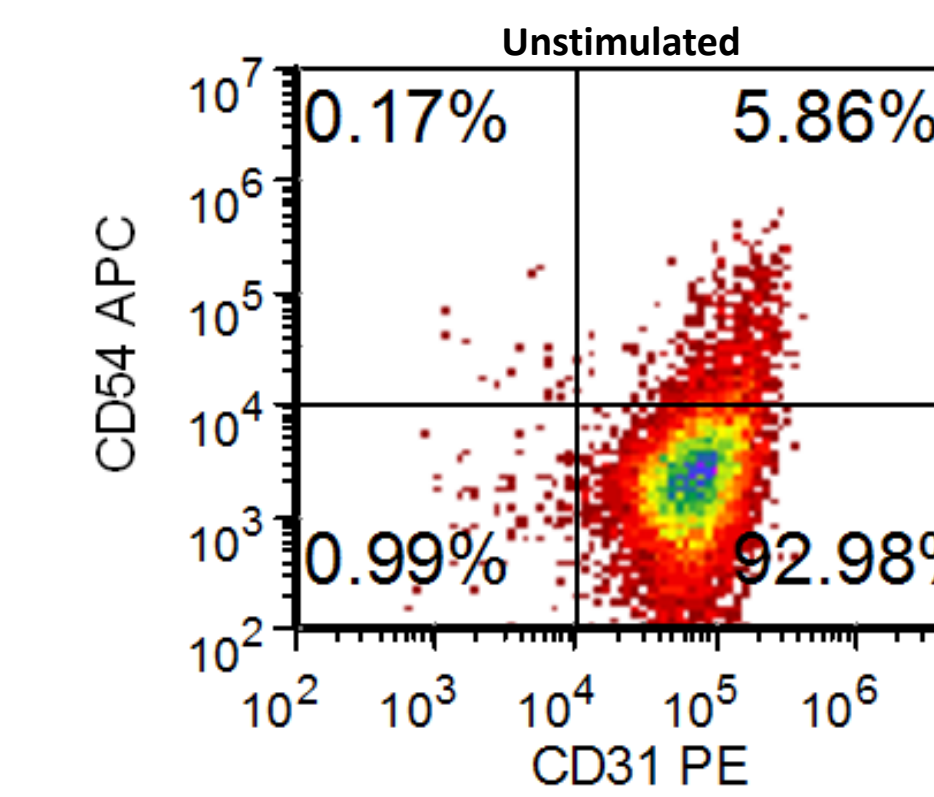


#### II. Electrical Resistance

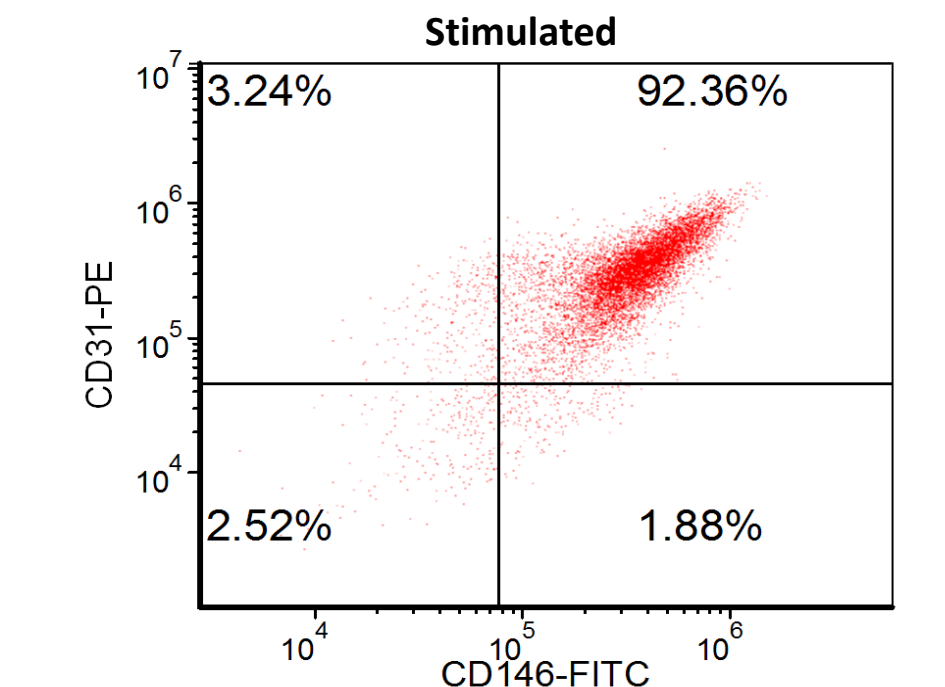
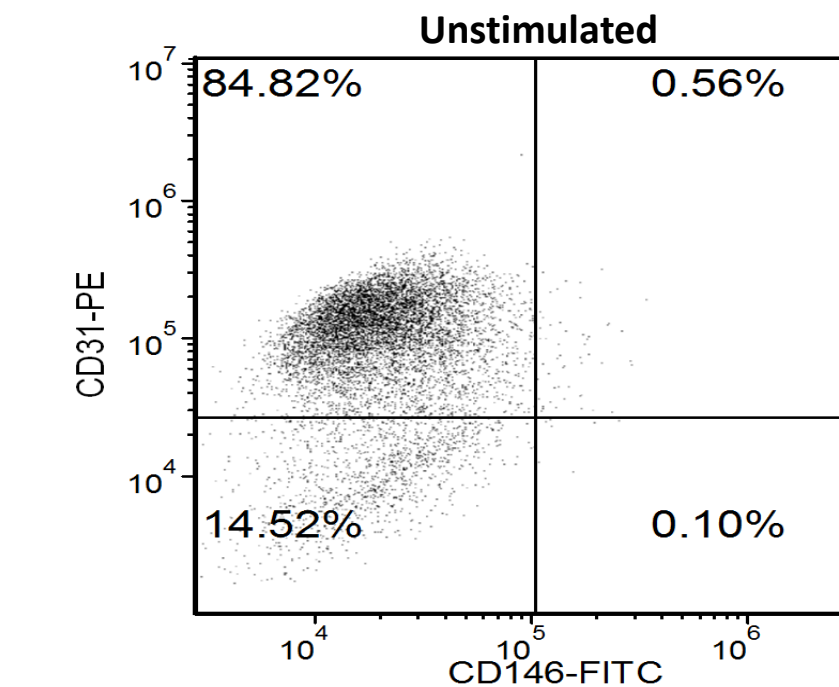


### B. Adhesion Molecule Stimulation

#### I. CD54 Stimulation



#### II. CD146 Stimulation



**A: iCell Endothelial Cells Demonstrate Endothelial Barrier Functions.** To assess the barrier function of iCell Endothelial Cells, cells were (I.) immunostained for the tight junction protein ZO-1 (green), and (II.) resistance levels were quantified by measuring the transcellular electrical resistance (TER). The baseline TER value for iCell Endothelial Cells is shown in black. The addition of Thrombin (red) disrupted the barrier function of the cells, thus decreasing the resistance, and cells were able to recover after a short period of time.

**B: iCell Endothelial Cells Exhibit Adhesion Molecule Stimulation.** (I.) iCell Endothelial Cells treated with 25 ng/ml TNF $\alpha$  for 24h showed increased stimulation of (I.) CD54, and (II.) CD146.

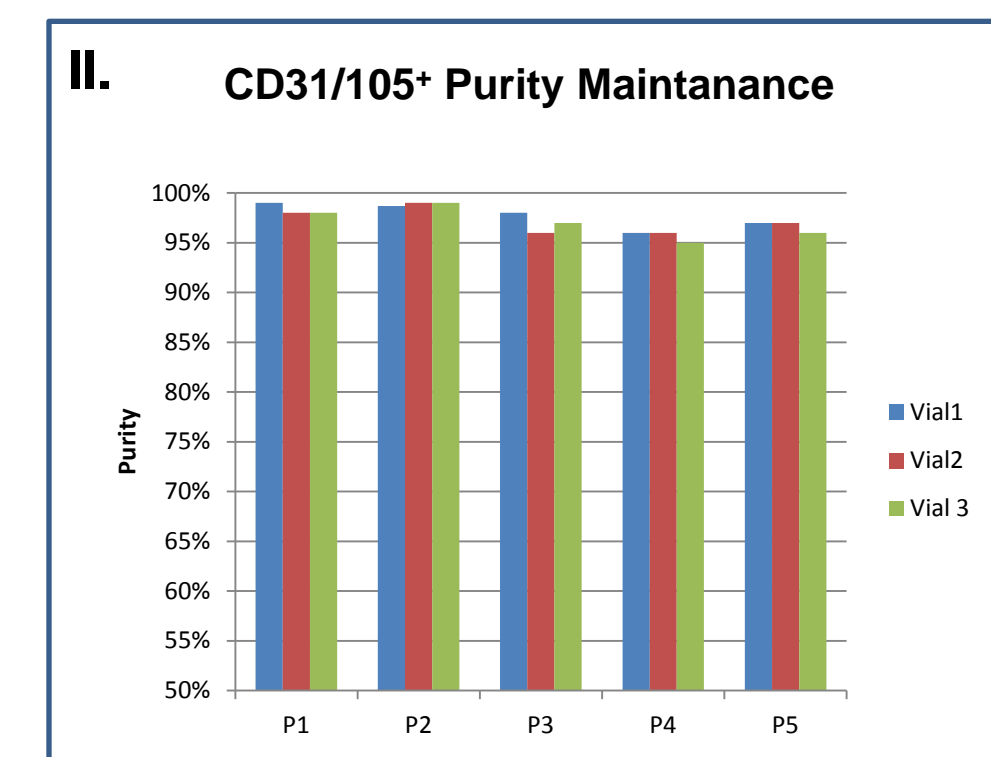
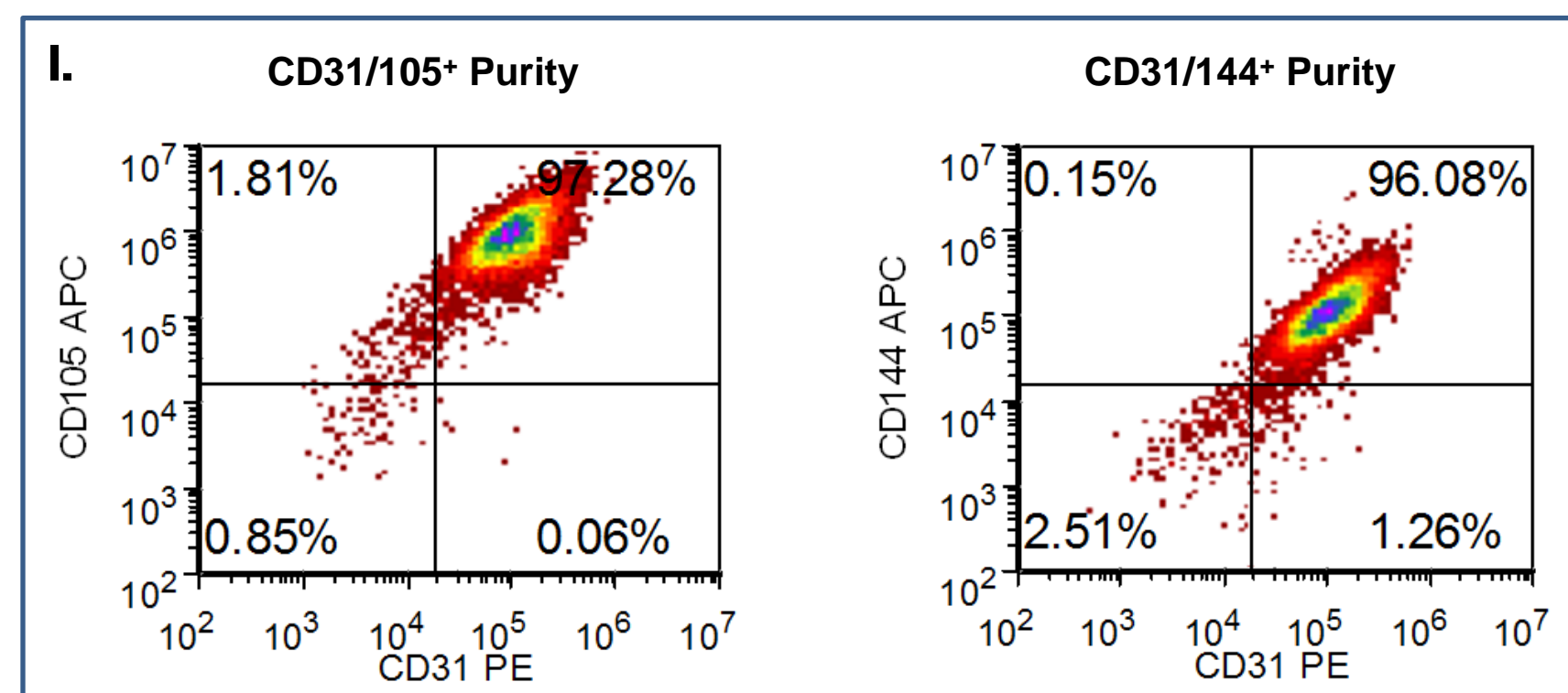
## iCell® Endothelial Cells

### Key Endothelial Cell Characteristics

<b>Morphology</b>	Cobblestone morphology
<b>Molecular Markers</b>	CD31 (PECAM), CD105 (Endoglin), CD144 (VE-cadherin), Flk1, ULEX, von Willebrand factor
<b>Functional Characteristics</b>	- Tubular structure formation - Barrier function – tight junction formation - Acetylated LDL uptake - Adhesion molecule stimulation - Wound healing enhancement

**Key Characteristics of an Endothelial Cell.** iCell Endothelial Cells exhibit the morphology and protein expression characteristic of an endothelial cell. In addition, they demonstrate barrier, angiogenesis, and inflammation functions.

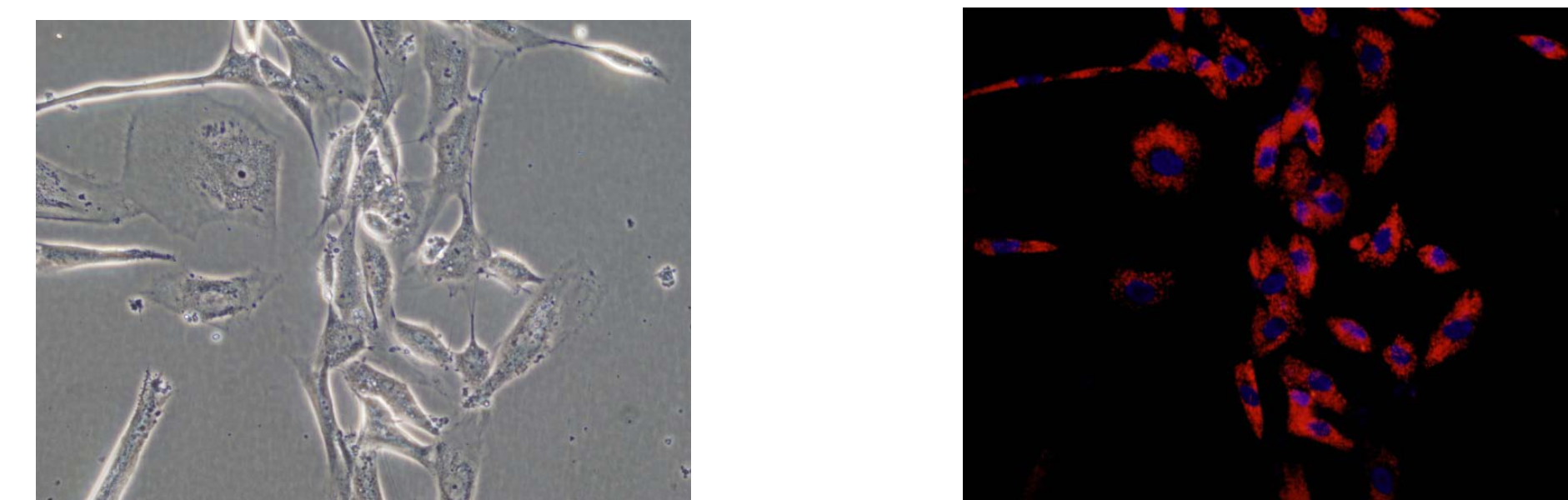
### A. iCell Endothelial Cells Purity



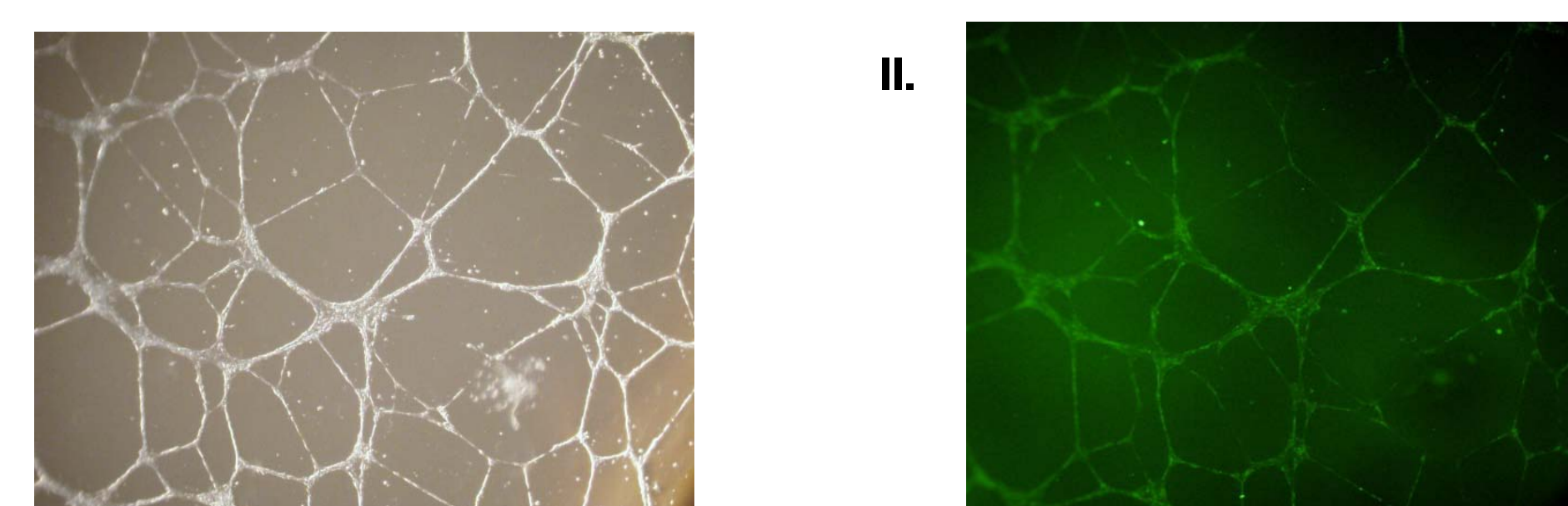
**Figure A: iCell Endothelial Purification.** Cellular Dynamics International's (CDI) proprietary differentiation technology and purification results in a highly pure endothelial population ( $\geq 90\%$ ) as determined by FACS analysis. (I.) iCell Endothelial Cells express CD31 (PECAM), CD105 (Endoglin), and CD144 (VE-cadherin). (II.) iCell Endothelial Cells maintain a >95% CD31/105+ purity over five passages.

## iCell® Endothelial Cells: Functional Characterization

### A. Acetylated LDL Uptake



### B. Tubular Structure Formation



**A: iCell Endothelial Cells Demonstrate Uptake of Acetylated LDL.** Cryopreserved iCell Endothelial Cells were thawed, grown for two passages and plated at 10,000 cells/cm $^2$ . The experiment was performed 48h later.

**B: iCell Endothelial Cells Display Typical Tubule Formation Properties.** (I.) iCell Endothelial Cells exhibit the capacity to form tubes in a thick layer of Matrigel. (II.) The same cells were also immunostained for ULEX (JEA-1) (green), which selectively binds to the surface glycoproteins of endothelial cells.

## Summary

### iCell® Endothelial Cells:

- Are generated from human iPSC cells.
- Exhibit typical endothelial cell morphology.
- Express characteristic endothelial cell molecular markers.
- Show characteristic tubular formation functions.
- Exhibit cell barrier functions and adhesion molecule stimulation.
- Will be commercially available late Q2 2011.