Induction of Endothelial Cell from Fibroblast by 5 Defined Factors

Hyo-Soo Kim, MD, PhD, FAHA

Director, National Research Laboratory for Cardiovascular Stem Cell,

Seoul National University Hospital (SNUH),

Seoul, Republic of Korea

SNUH Cardiovascular Center

Presenter Disclosure

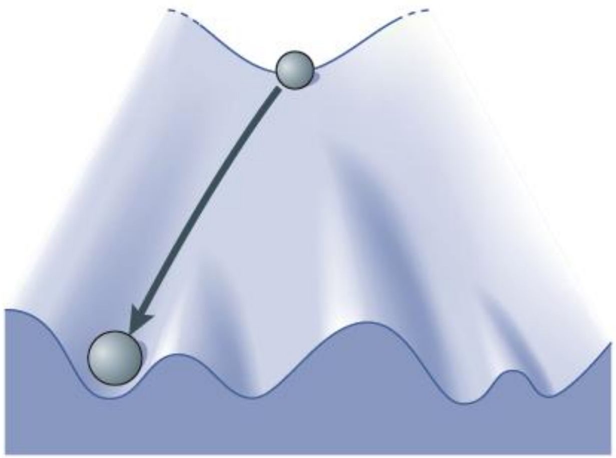
- Nothing to disclose

Background

- New vessel formation using endothelial cells holds great therapeutic promise.
- ESC- or iPSC-derived ECs
  - Ethical hurdles
  - No standardized protocol
  - Necessity for complex manipulation of EB
  - Low differentiation efficiency
  - Risk of contamination by feeder cells

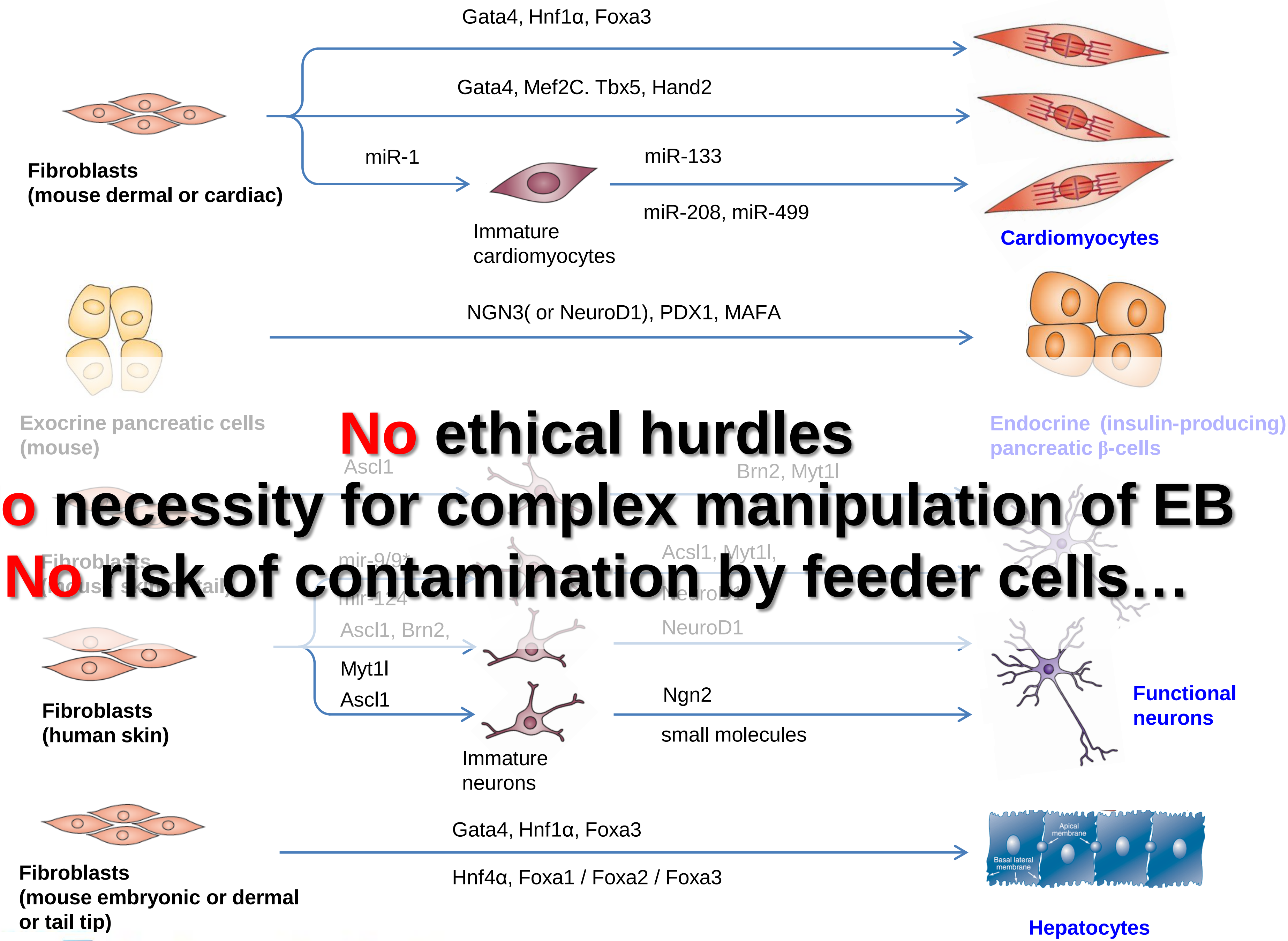
Waddington's Epigenetic Landscape

Conventional concept



SNUH Cardiovascular Center

Ladewig et al., *Nat. Rev. Mol. Cell Biol.* 2013



No ethical hurdles

No necessity for complex manipulation of EB

No risk of contamination by feeder cells…

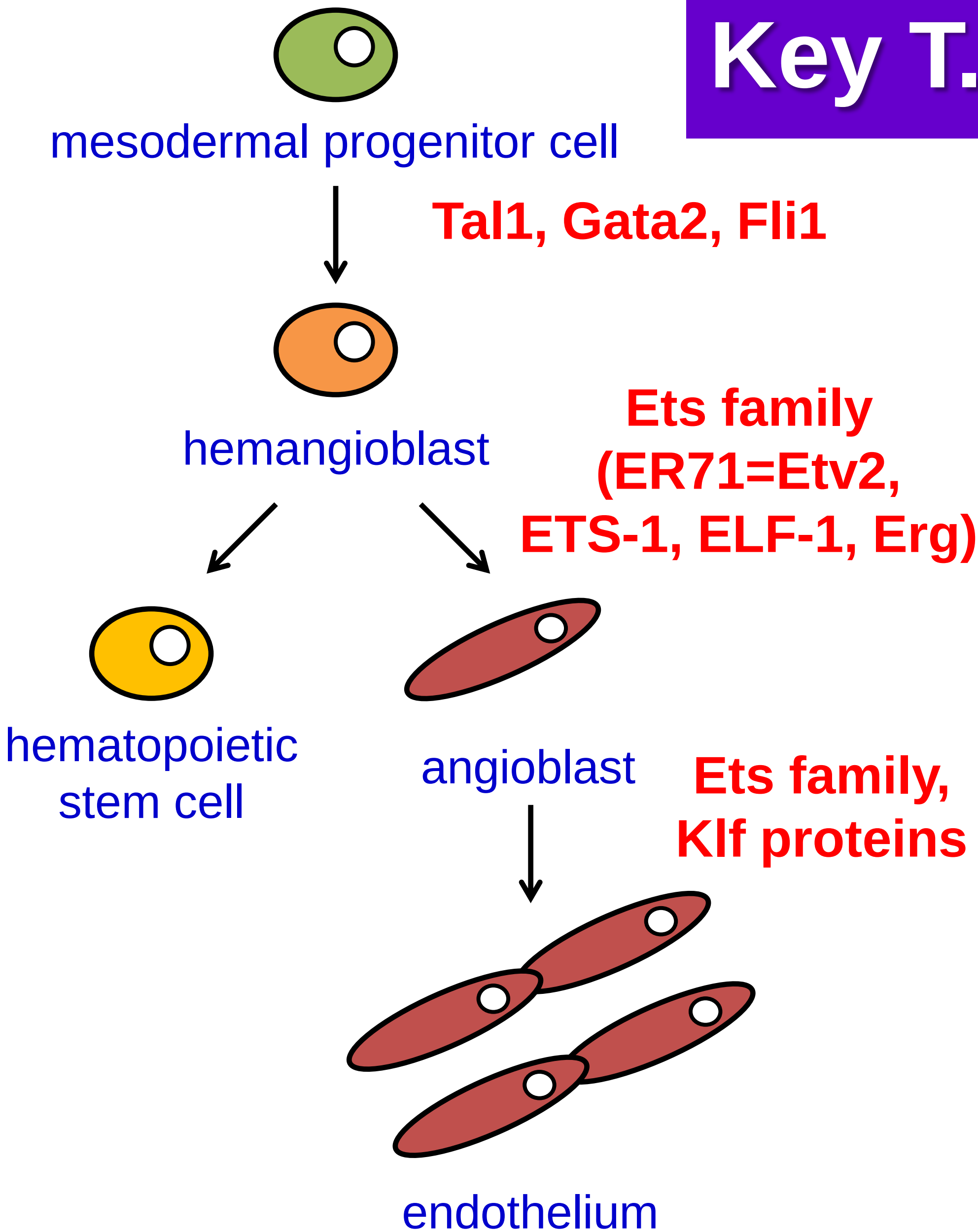
SNUH Cardiovascular Center

Adapted from Ladewig et al., *Nat. Rev. Mol. Cell Biol.* 2013

Aim:

- To develop a novel methodology providing **endothelial cells from fibroblasts** via **direct conversion.**

Key T.F.s in EC development



+ Co-workers of endothelial transcription
  – **FOXO1, FOXC, LMO2** (co-factor)
+ Regulator of endothelial functions
  – **Klf2**

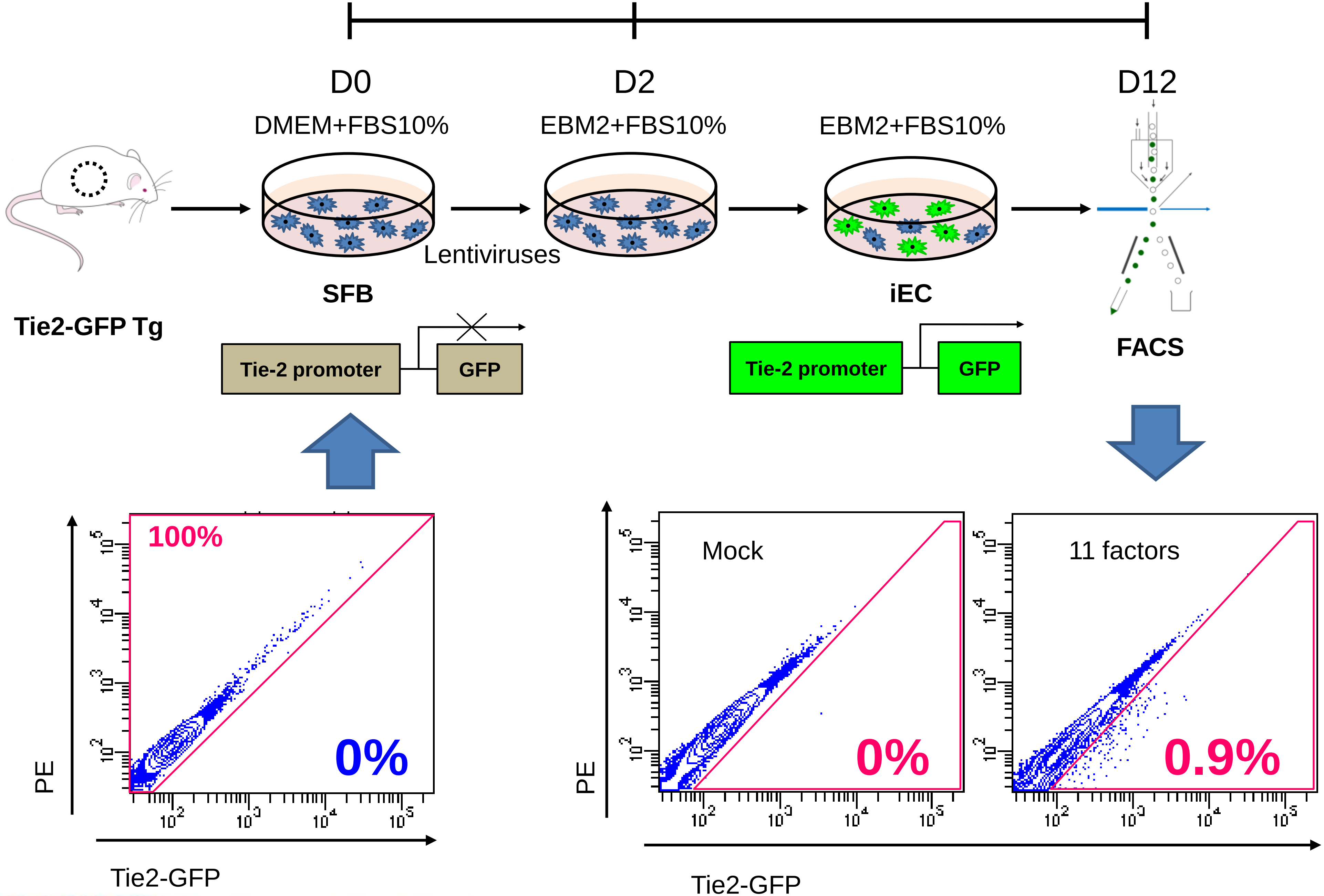
- **Target cells: adult fibroblast**
- **Species: mouse**
- **Read-out: tie2-GFP**

Adapted from De Val et al., *Cell* 2009

11 Candidate Factors

Gene symbol	Accession Number
Gata2	NM_008090
Foxc2	NM_013519
Elf1	NM_007920
Erg	NM_133659
Fli1	NM_008026
Tal1	NM_011527
Foxo1	NM_019739
Lmo2	BC057880
Ets1	BC010588
Er71(Etv2)	NM_007959
Klf2	NM_008452

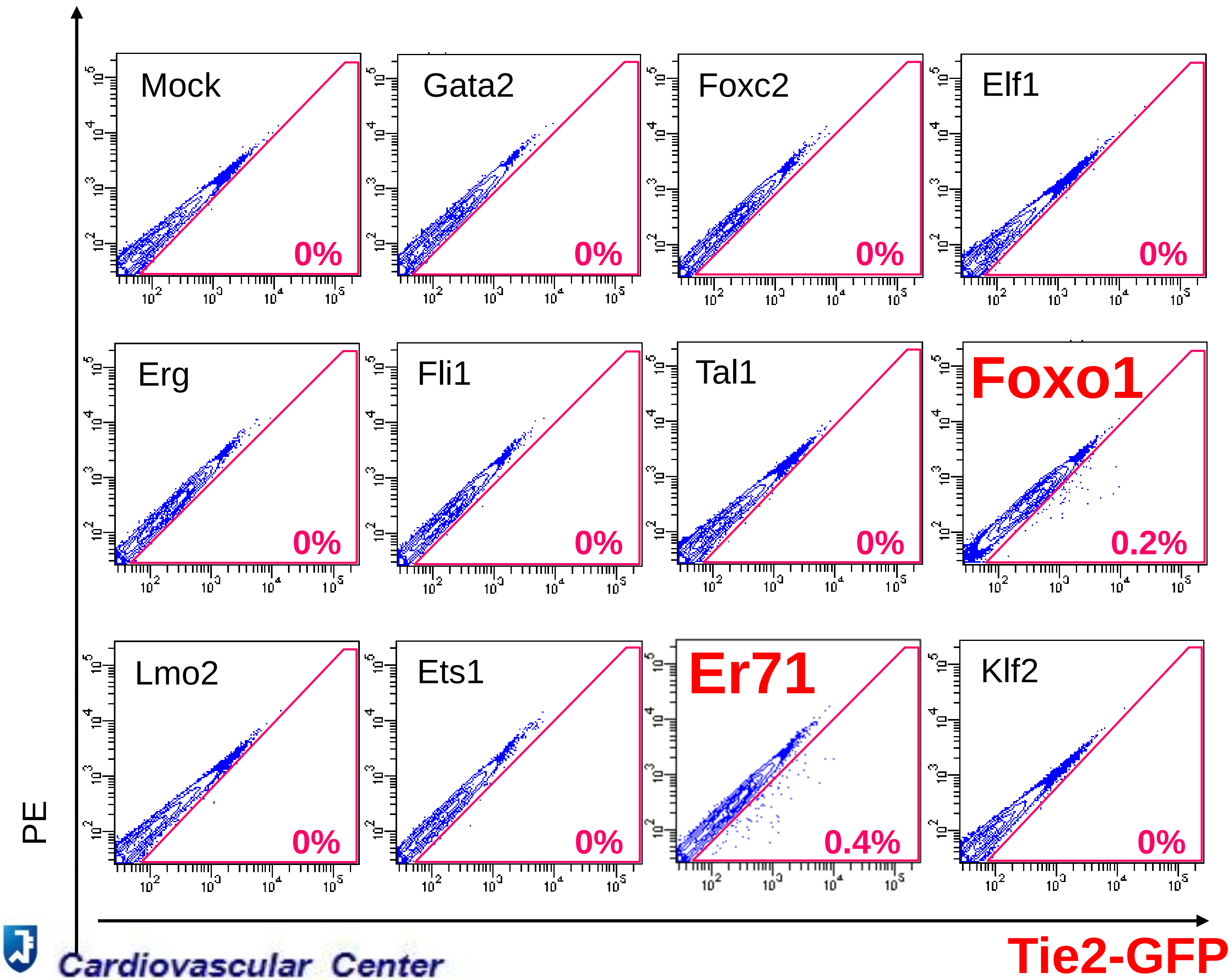
JK Han,,, HS Kim. Circulation 2014

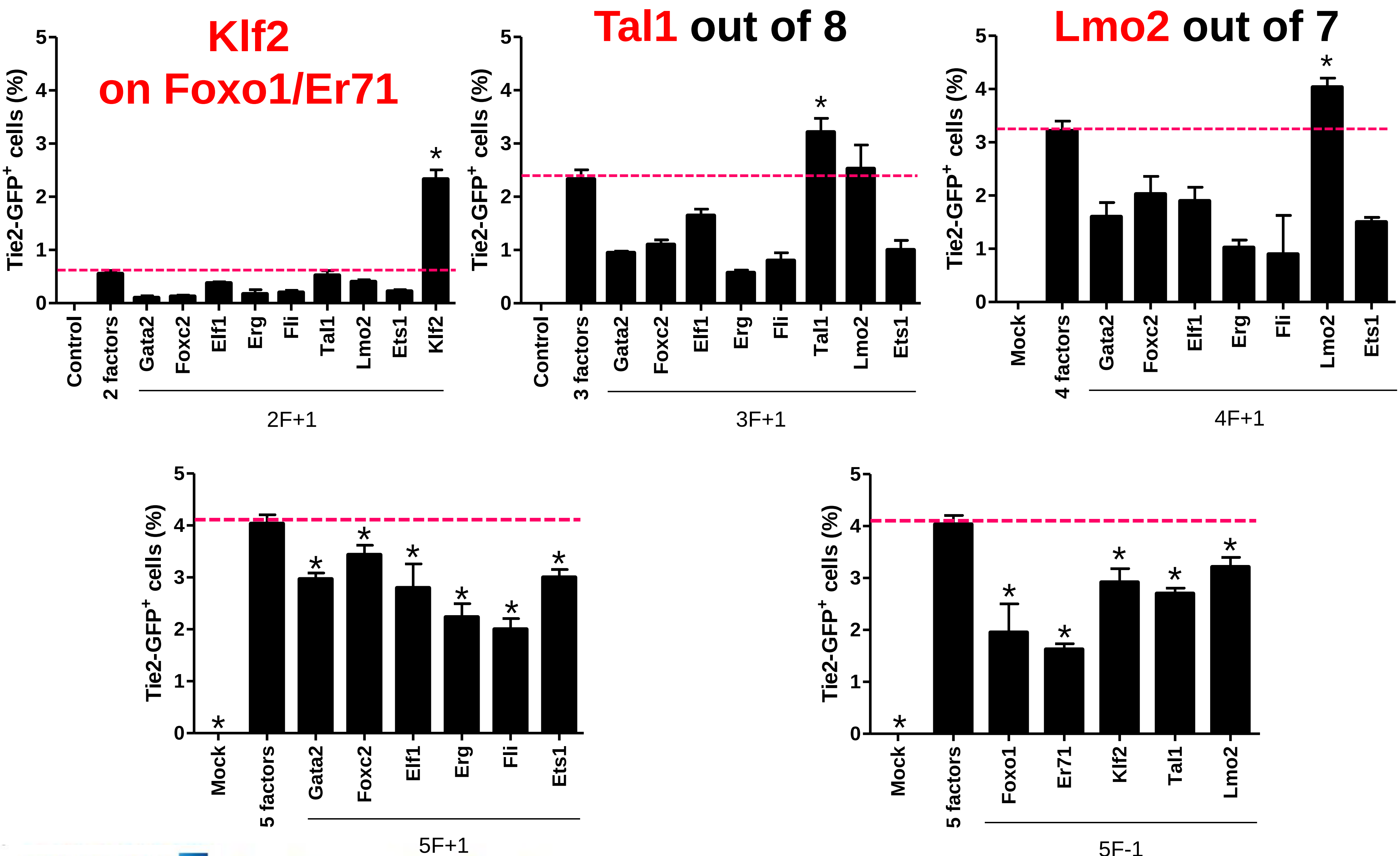


SNUH Cardiovascular Center

JK Han,,, HS Kim. Circulation 2014

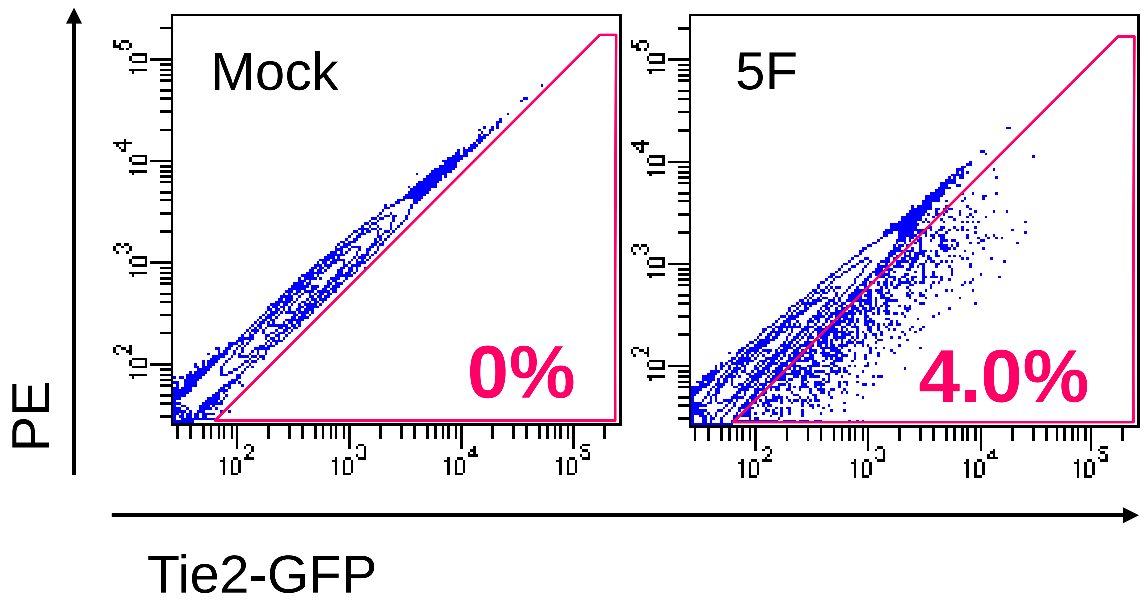
Series of 'Single Factor' Transduction Experiments

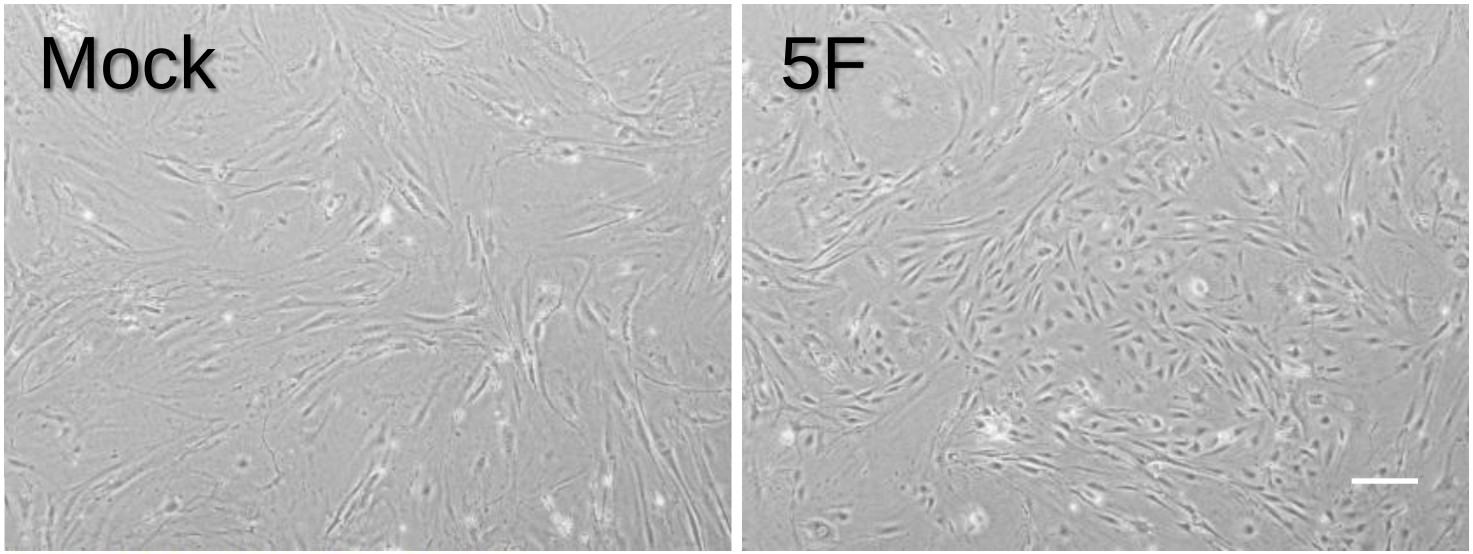




JK Han,,, HS Kim. Circulation 2014

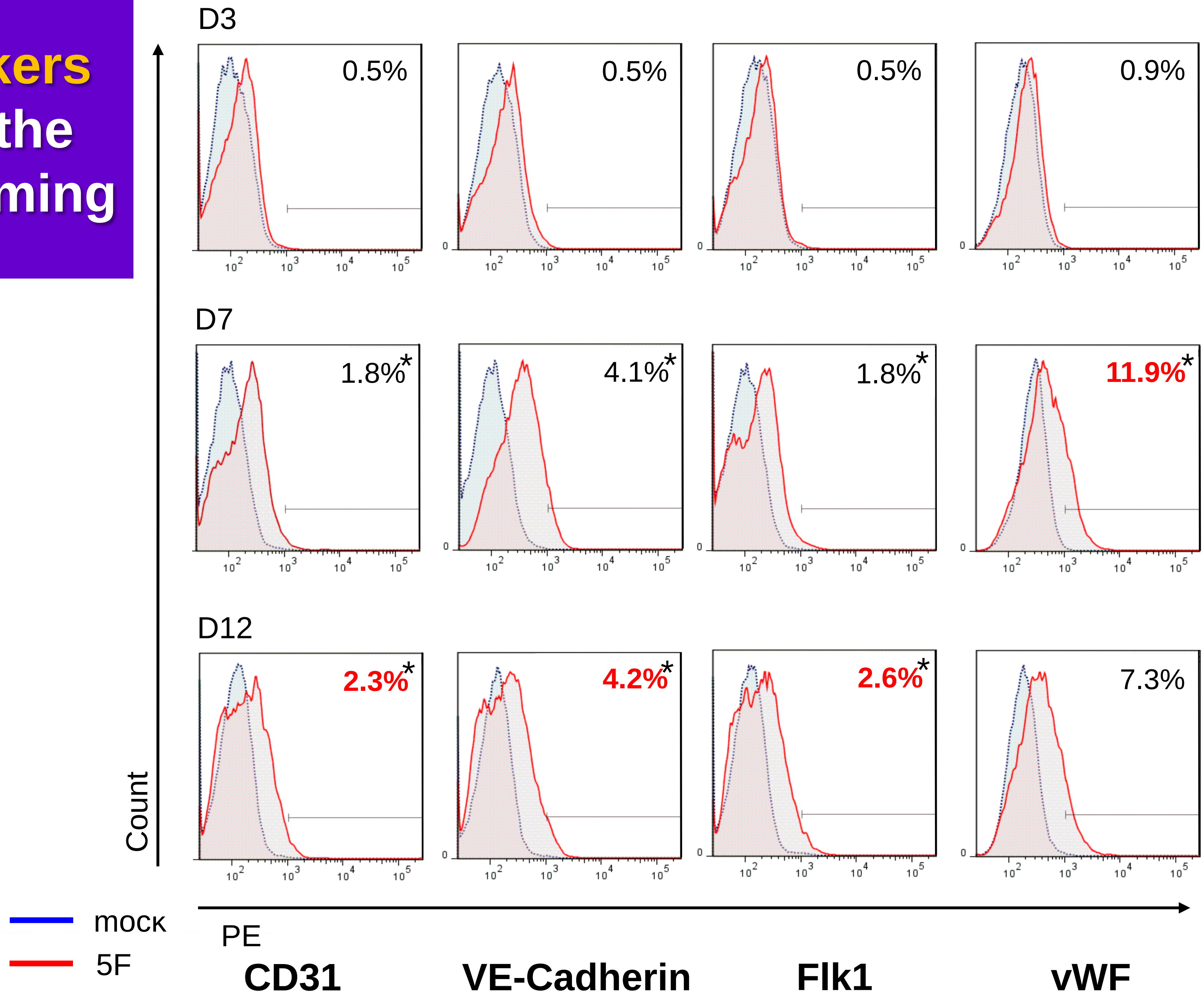
5 Key Factors for Endothelial Reprogramming





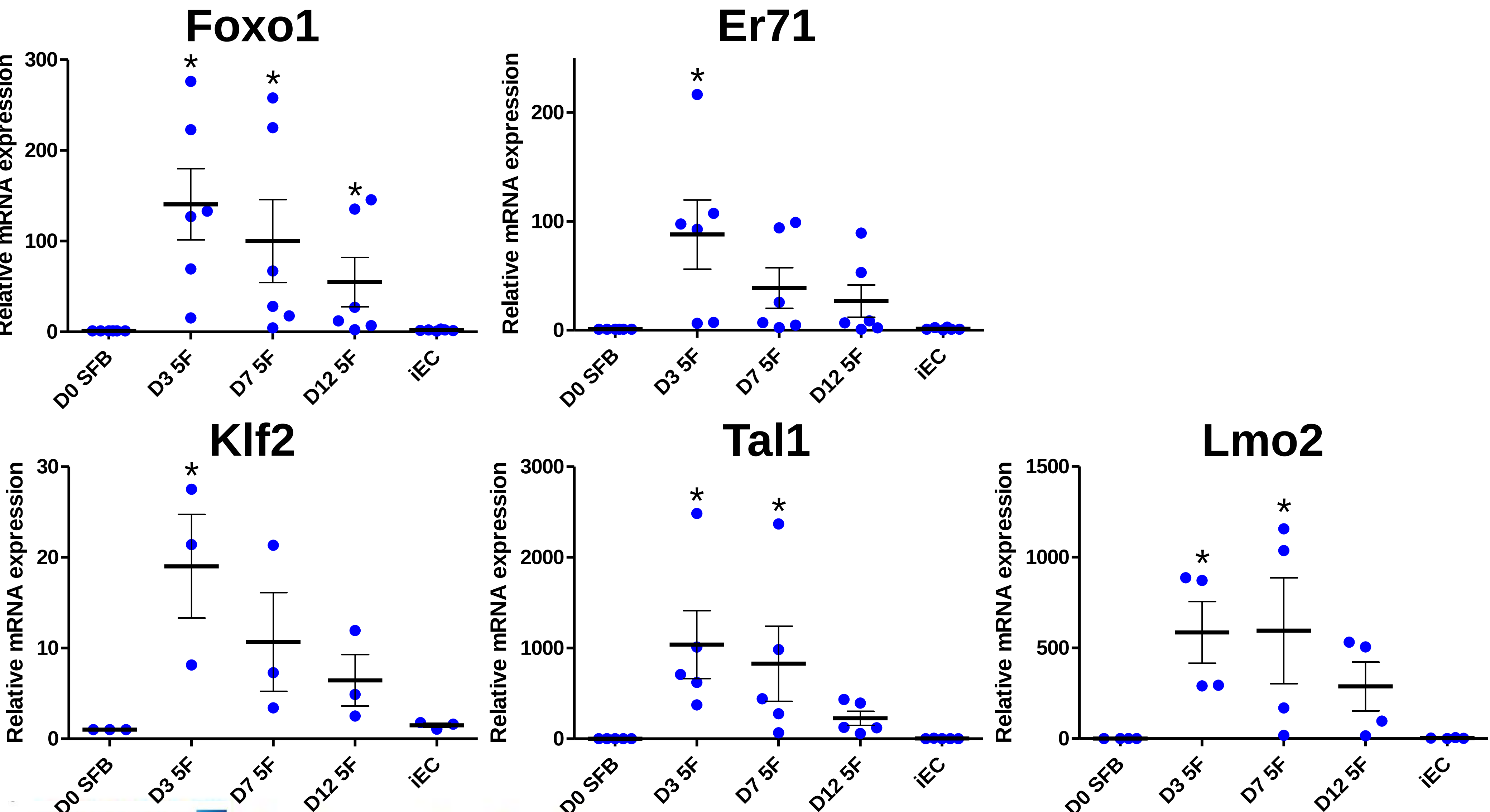
SNUH

EC markers during the Programming



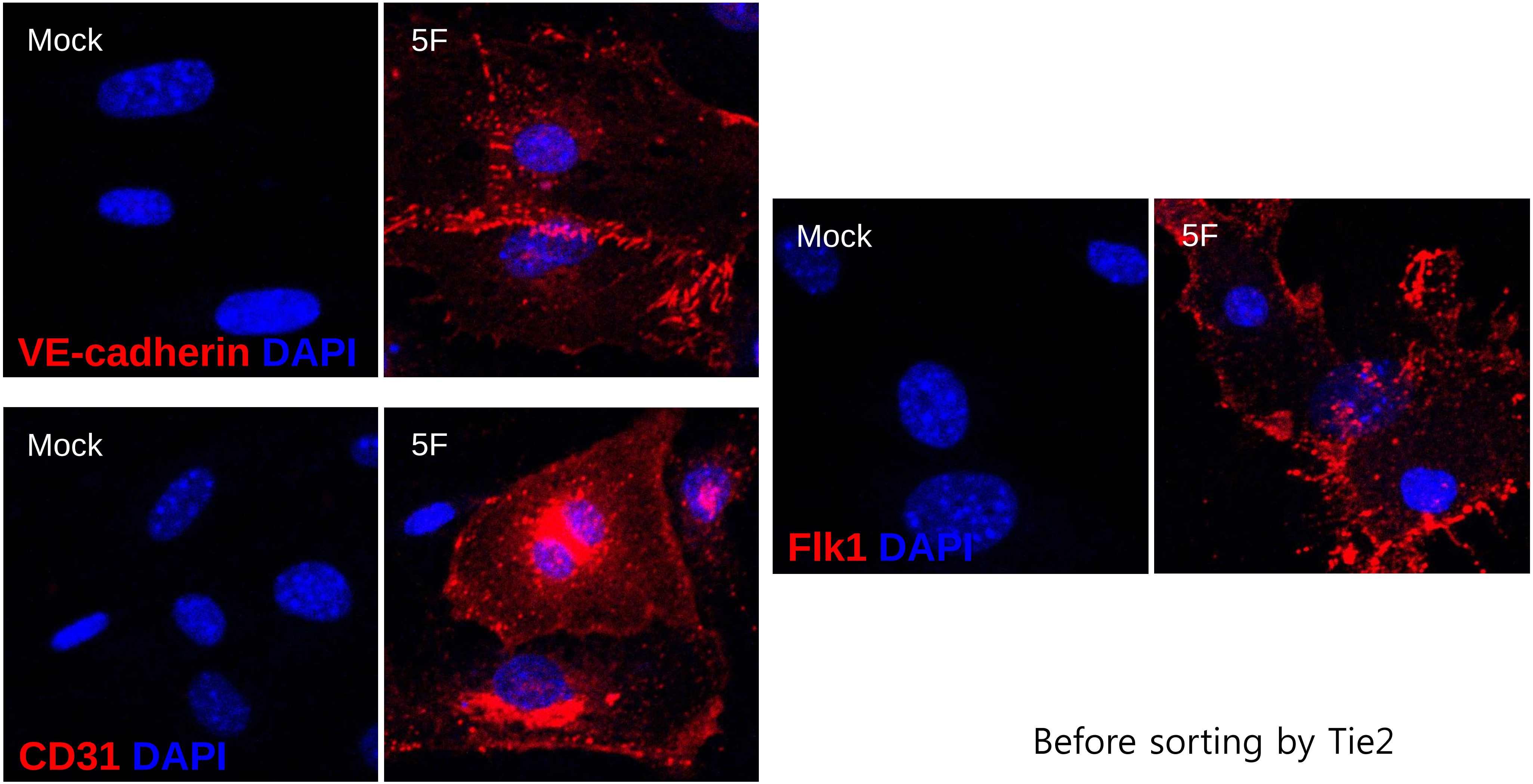
SNUH

Lentiviral Silencing



SNUH Cardiovascular Center

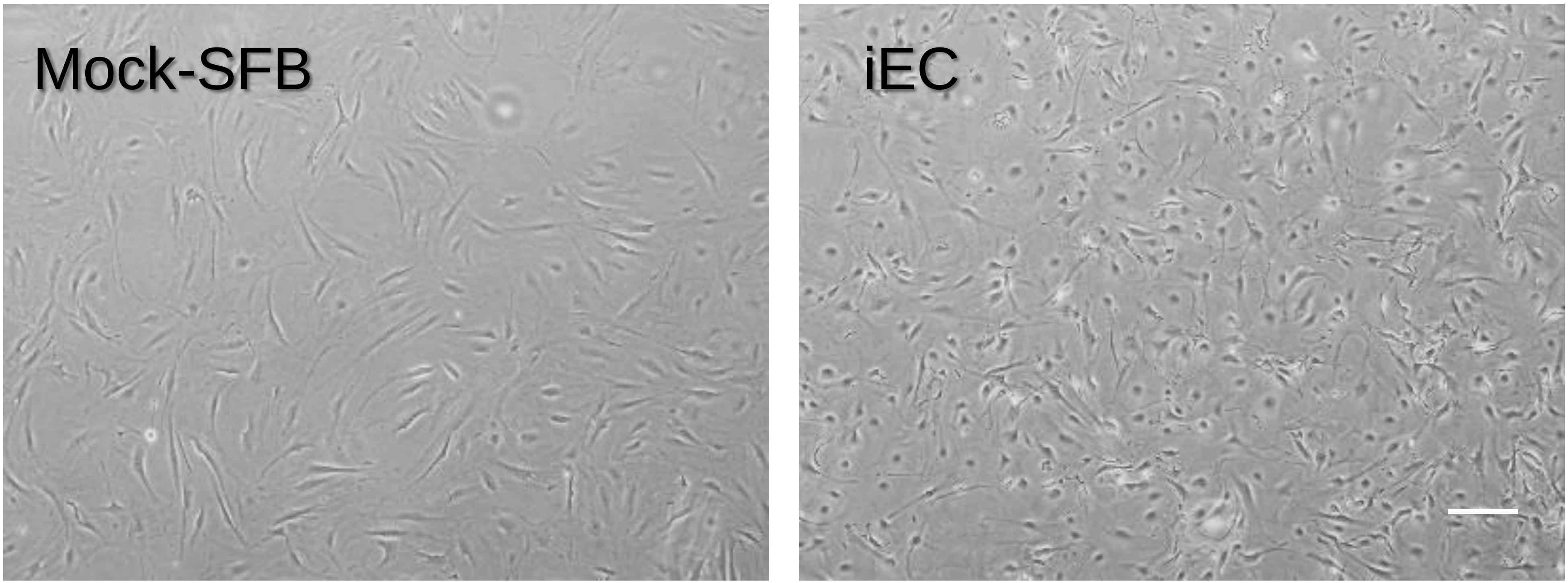
EC Markers after Endothelial Reprogramming



Before sorting by Tie2

JK Han,,, HS Kim. Circulation 2014

Induced Endothelial Cells:
after Tie2 sorting

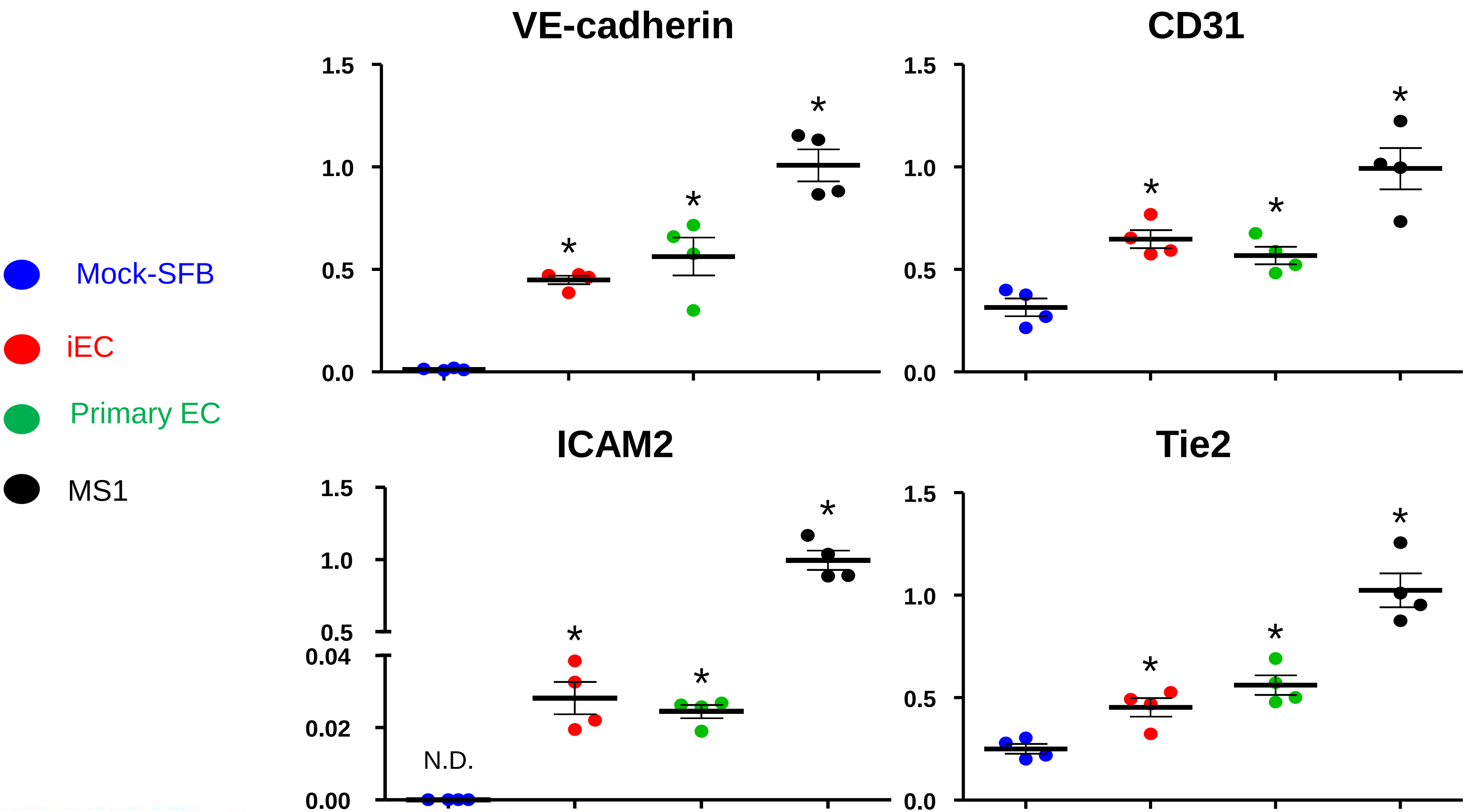


SNUH Cardiovascular Center

JK Han,,, HS Kim. Circulation 2014

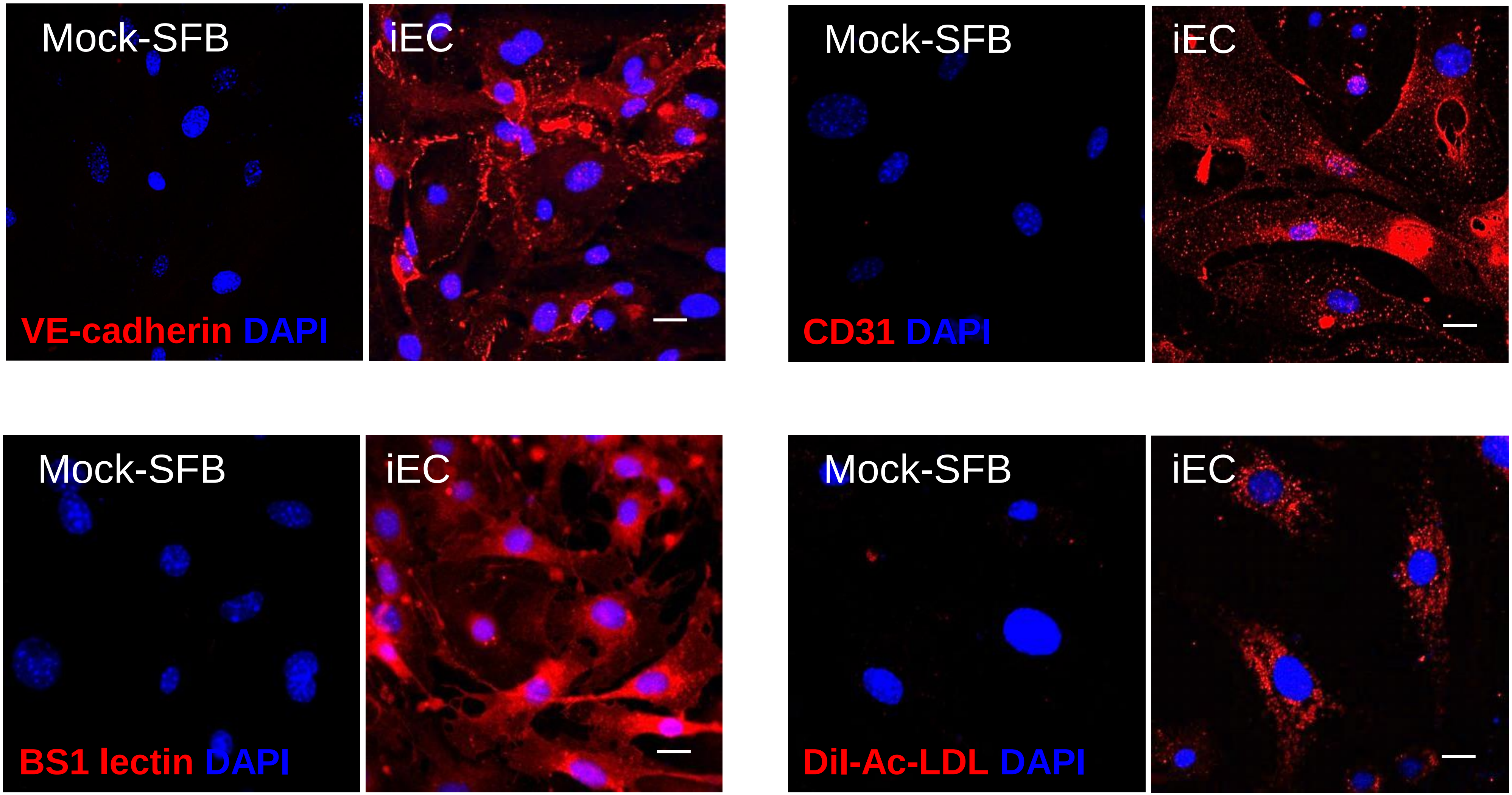
iEC Characterization: RT-PCR

Relative mRNA expression



JK Han,,, HS Kim. Circulation 2014

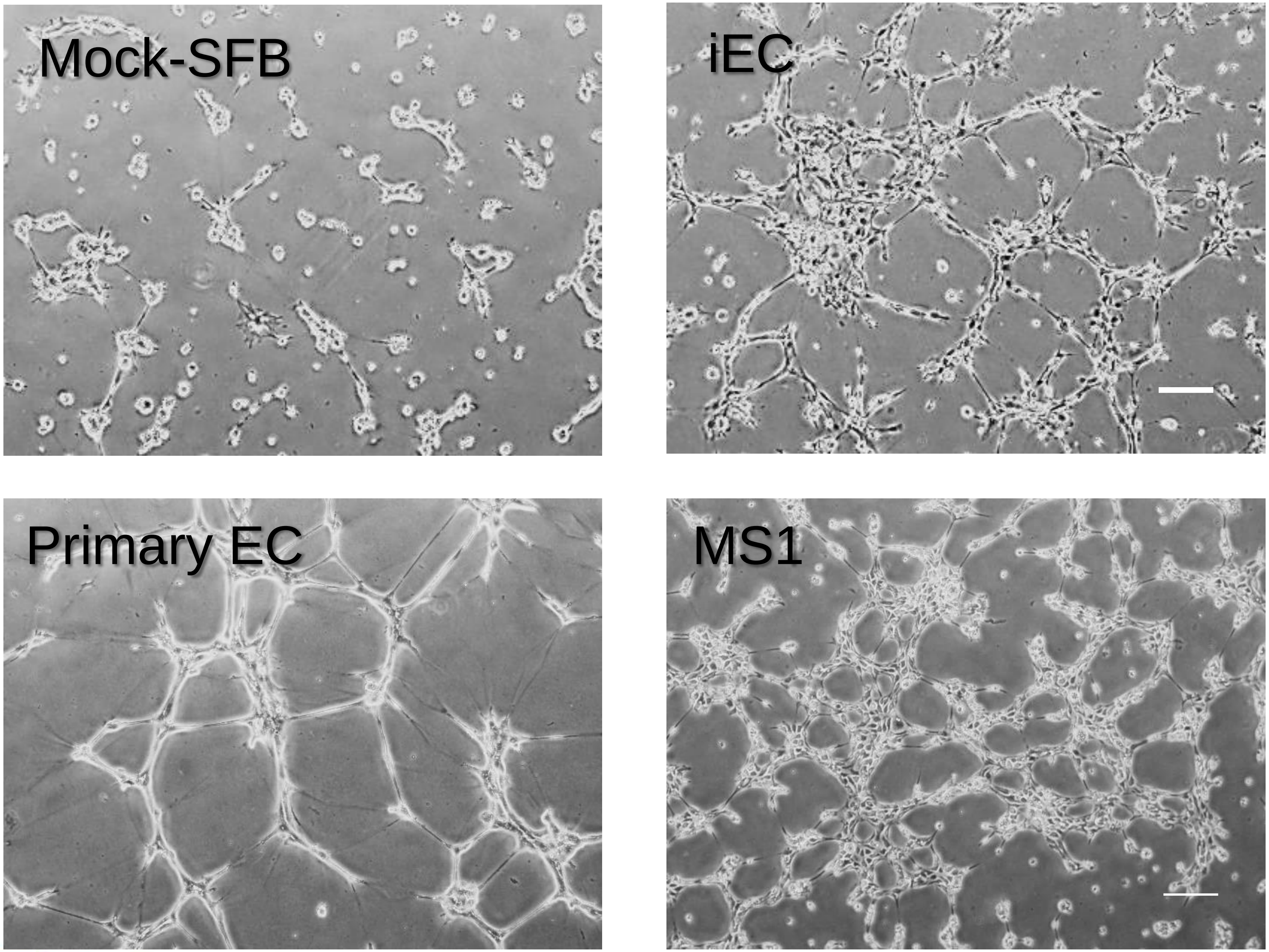
iEC Characterization: IF, EC Fx.



SNUH Cardiovascular Center

After sorting by Tie2

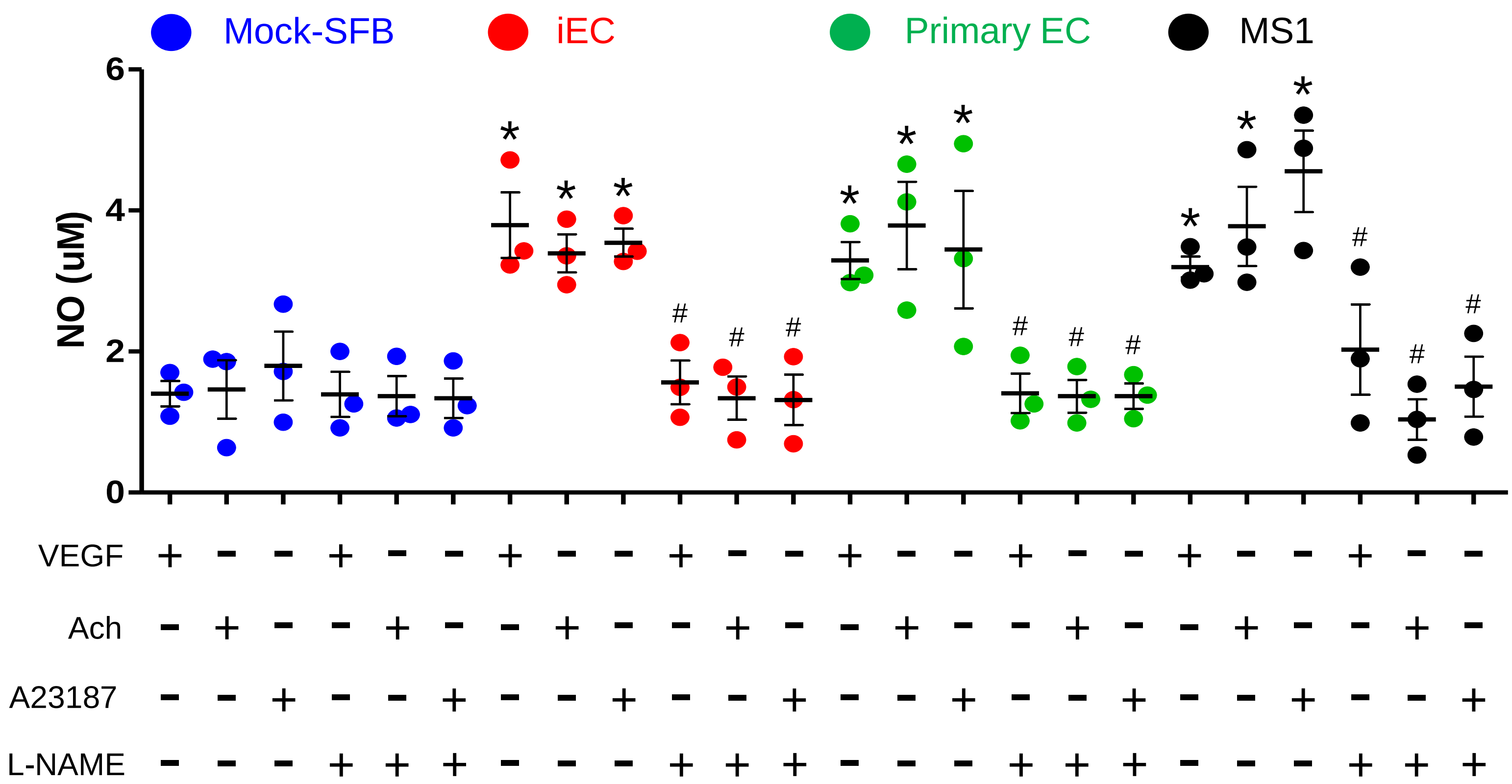
iEC Characterization: Matrigel Tube Formation



JK Han,,, HS Kim. Circulation 2014

iEC Characterization: NO Production

After overnight incubation in 2 ng/ml VEGF, culture supernatants were harvested, and NO was assayed using a NO Detection Kit.

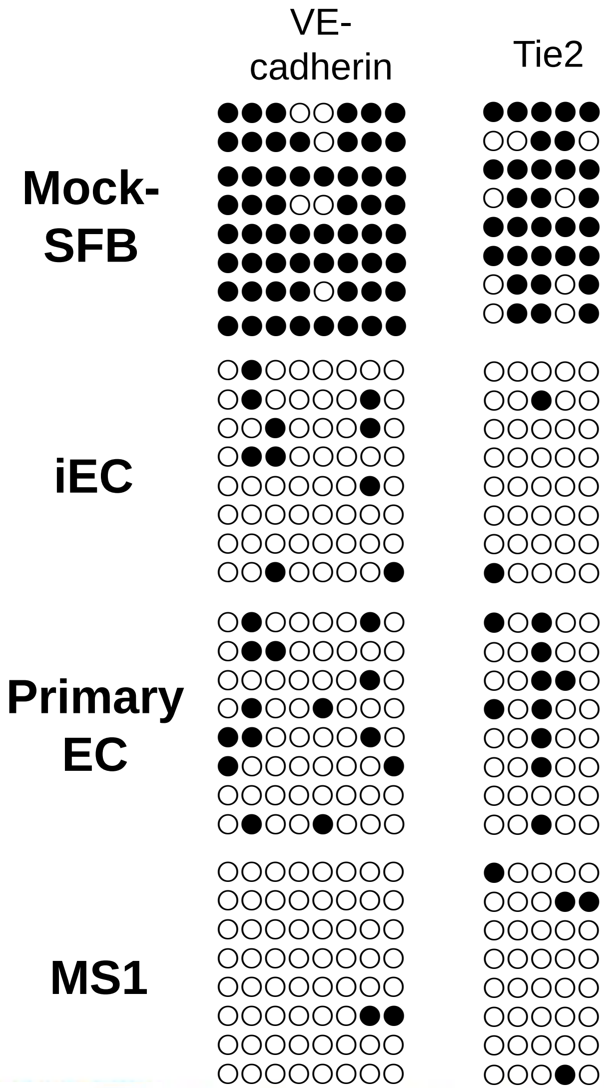


SNUH Cardiovascular Center

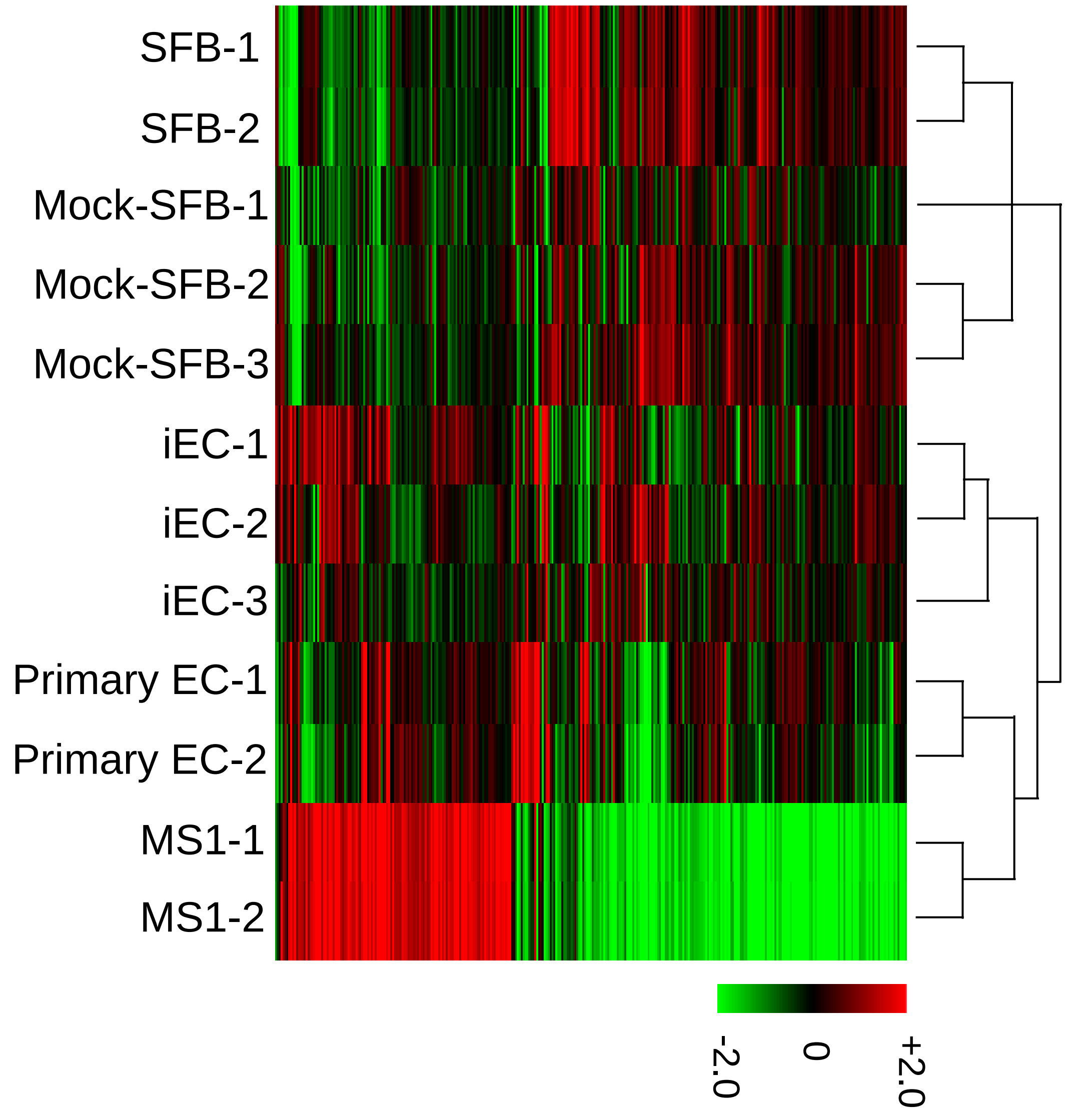
JK Han,,, HS Kim. Circulation 2014

Endothelial Epigenetics / Genetics

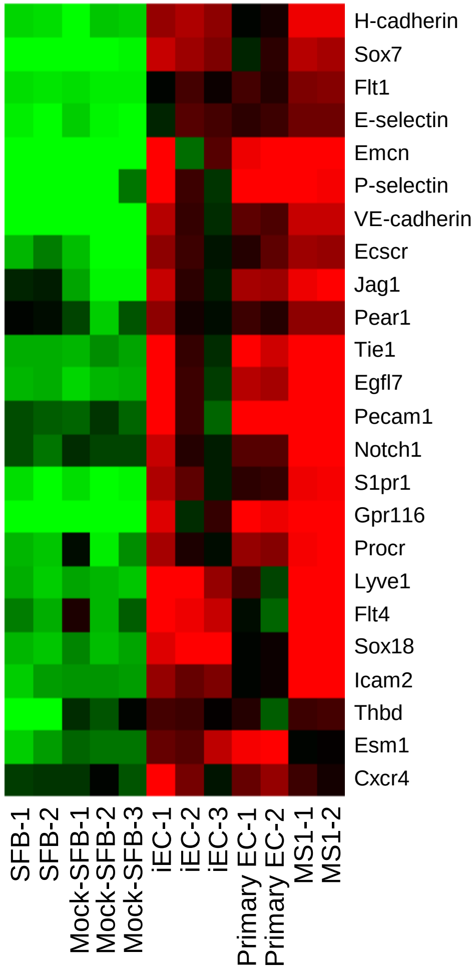
Bisulfite sequencing



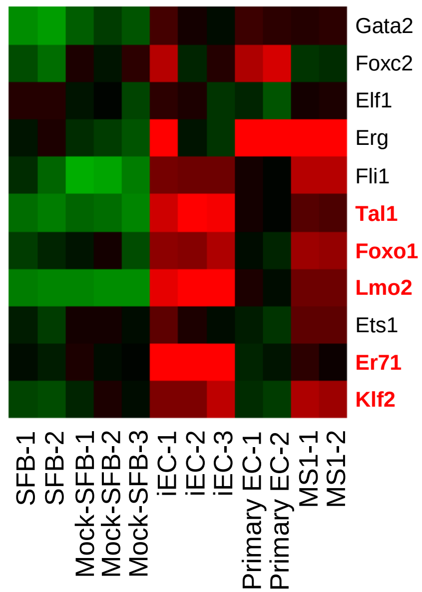
Affymetrix GeneChip Mouse Gene 1.0 ST Array



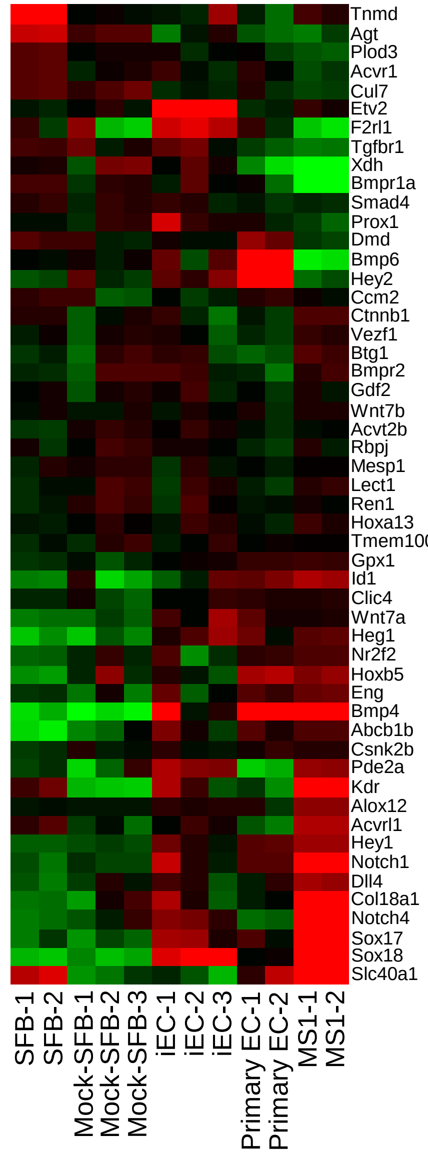
EC specific genes



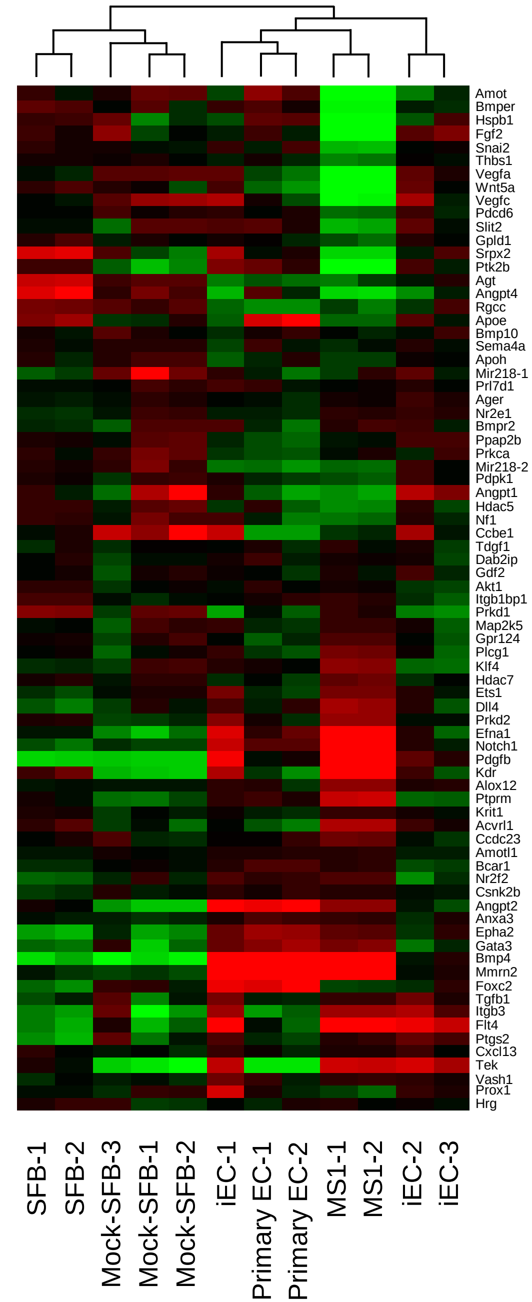
11 factors



Endothelium development (GO003158)



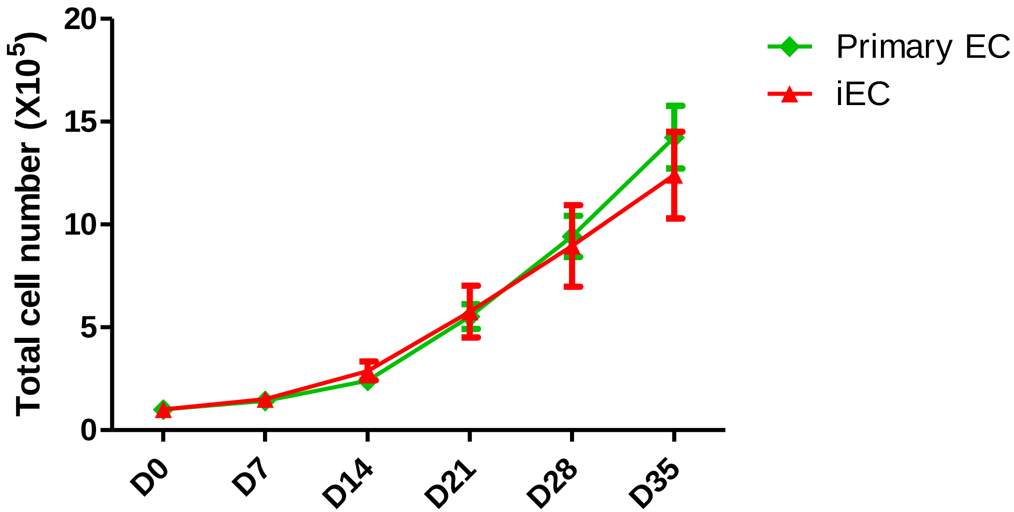
Regulation of EC migration (GO0010594)



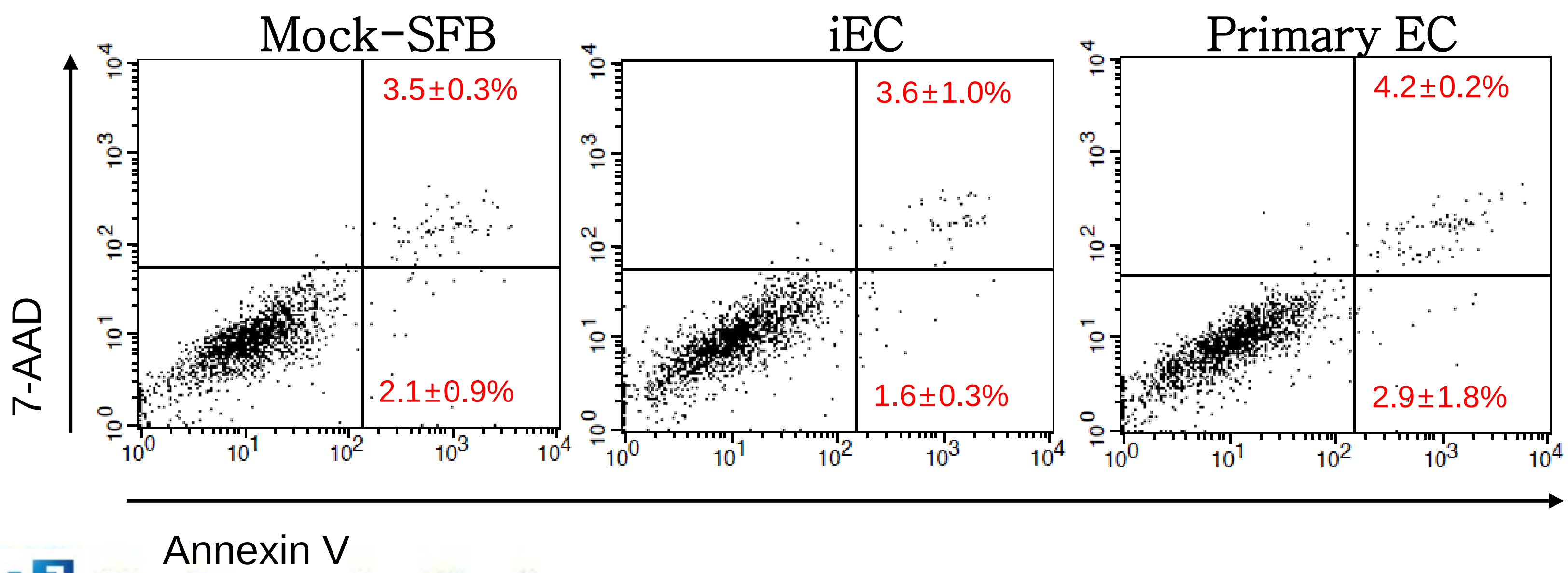
SNUH Cardiovascular Center

Proliferation & Apoptosis of iECs

Growth Curve



Serum Starvation

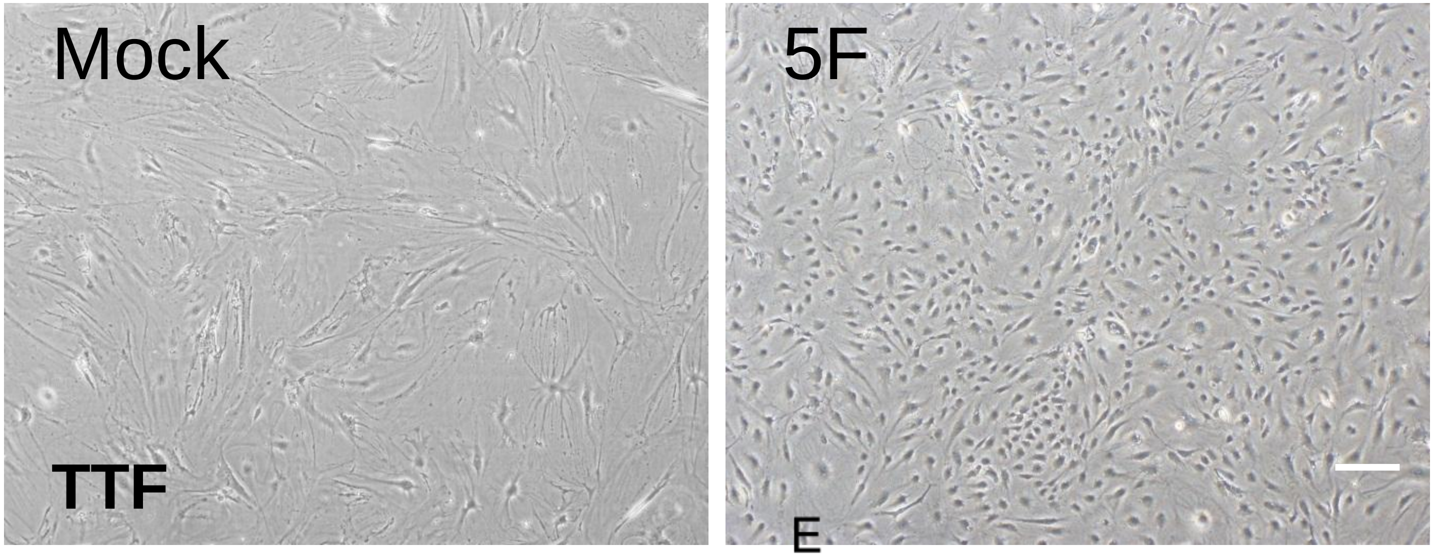


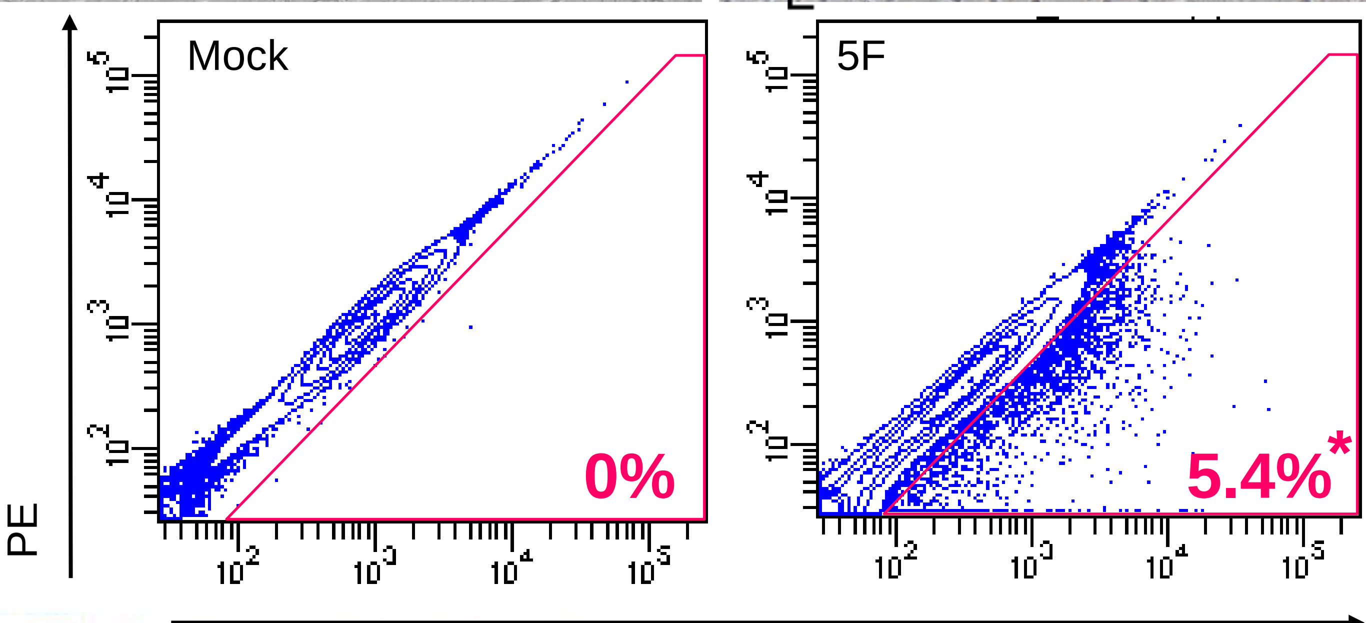
SNUH Cardiovascular Center

JK Han,,, HS Kim. Circulation 2014

Universal Effect of iEC-5 Factors: TTF

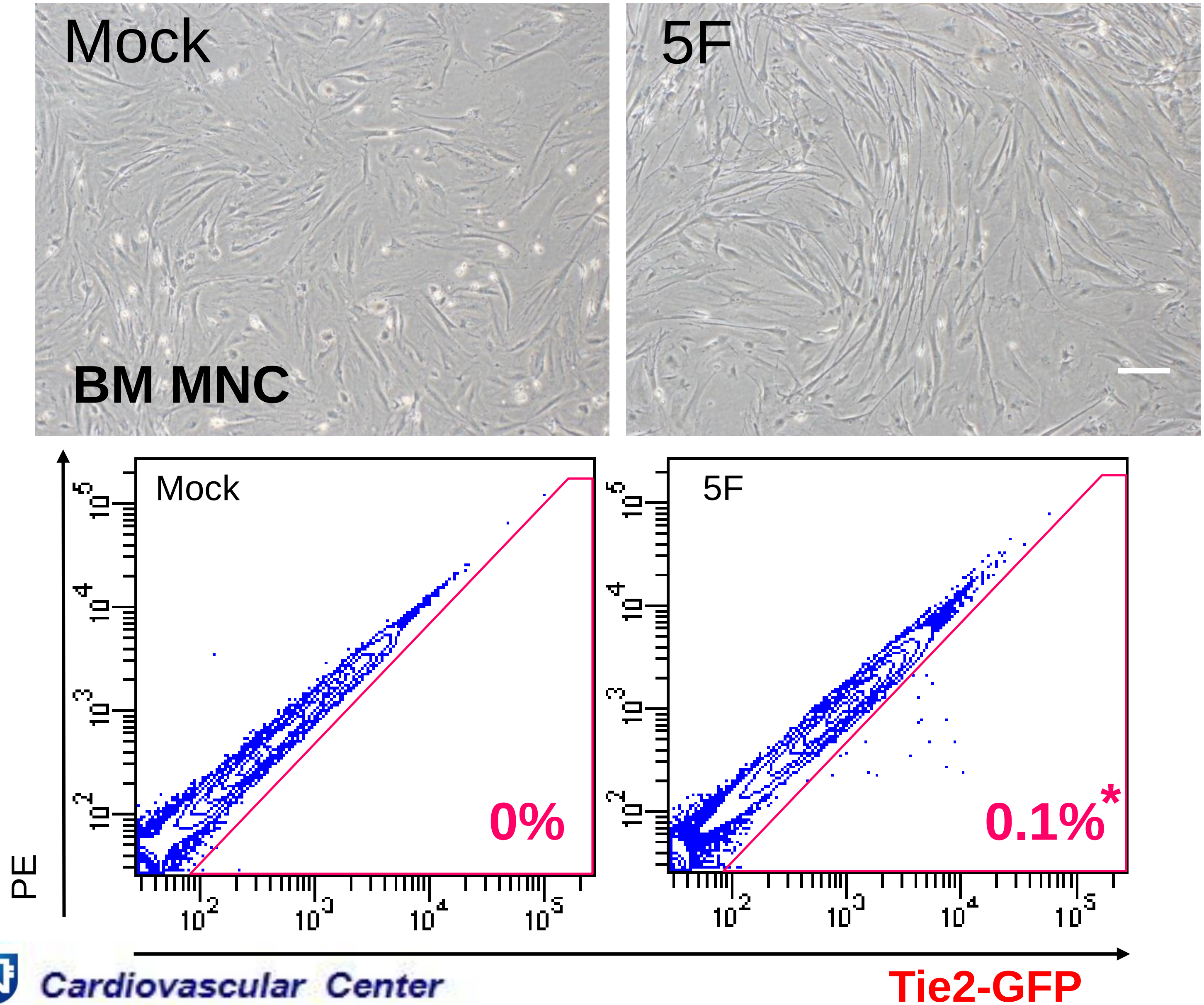
Tail-tip FB (TTF)





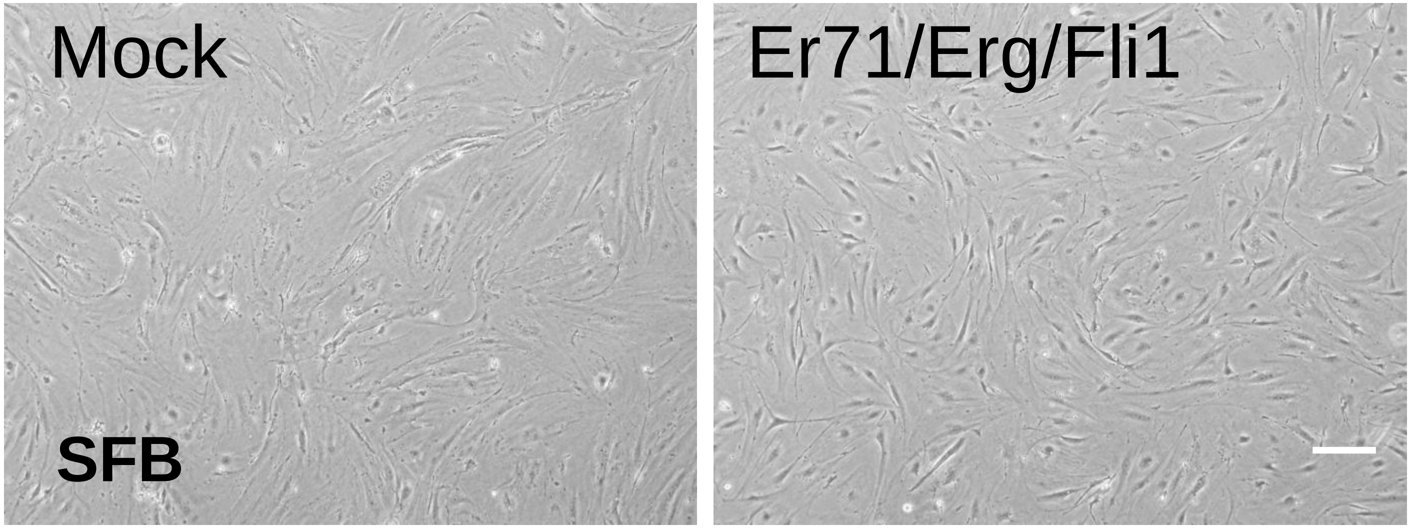
SNUH Cardiovascular Center

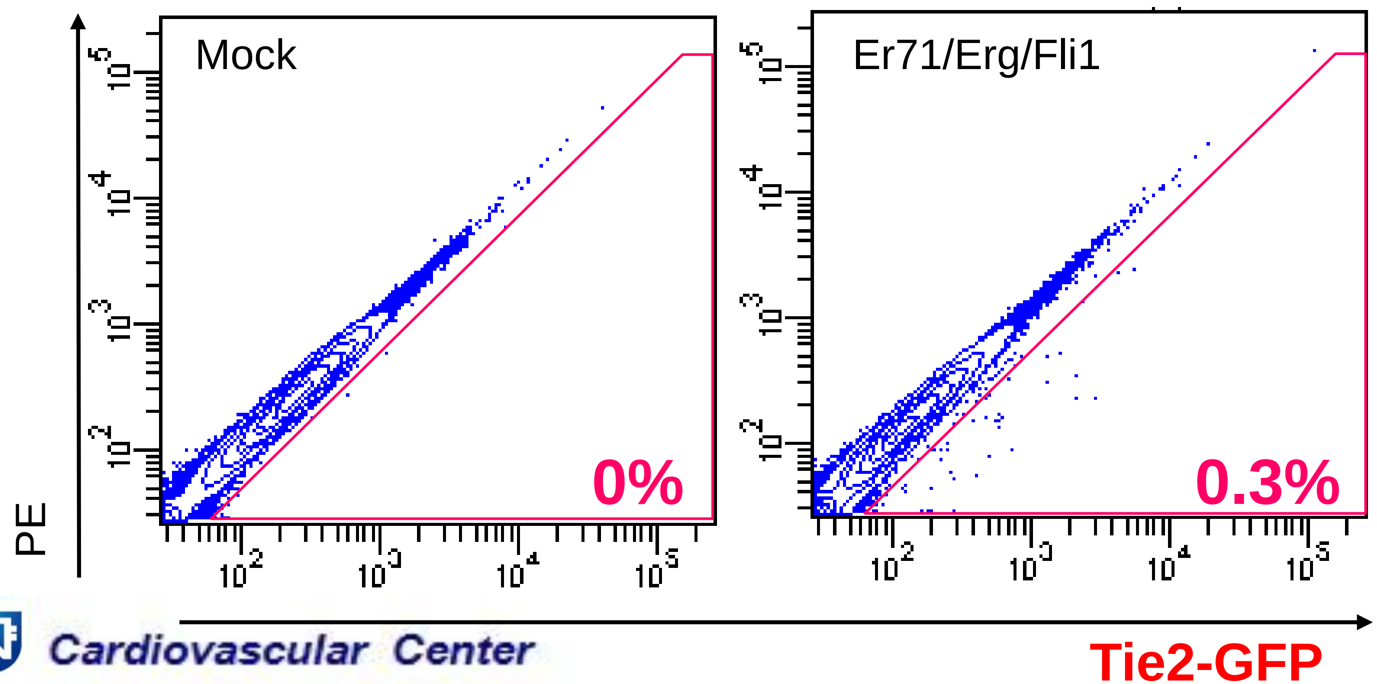
Effect of iEC-5 Factors on Monocytes



SNUH Cardiovascular Center

Unique Effect of iEC-5 Factors:
Compared with Rafii's 3 Factors

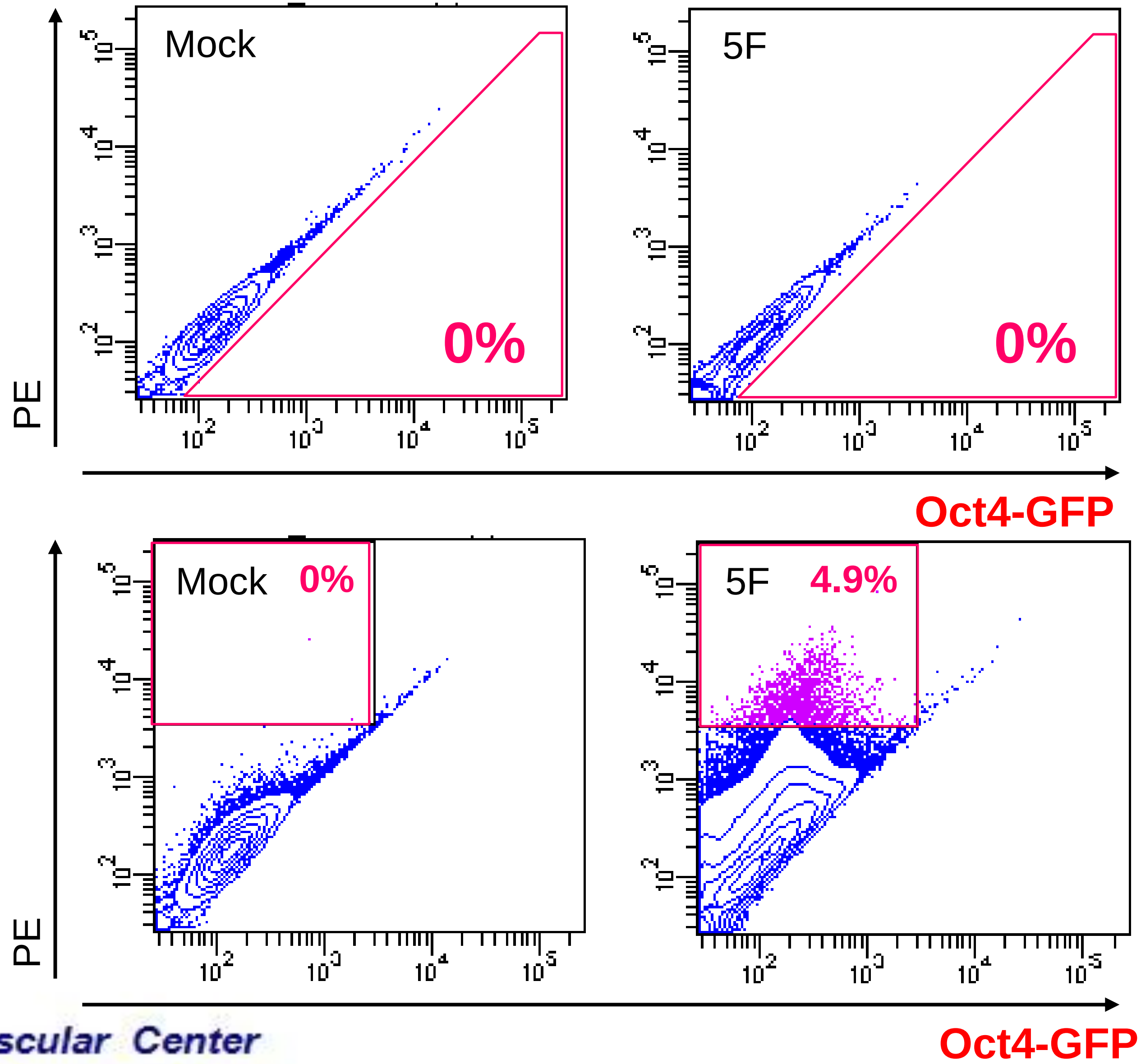




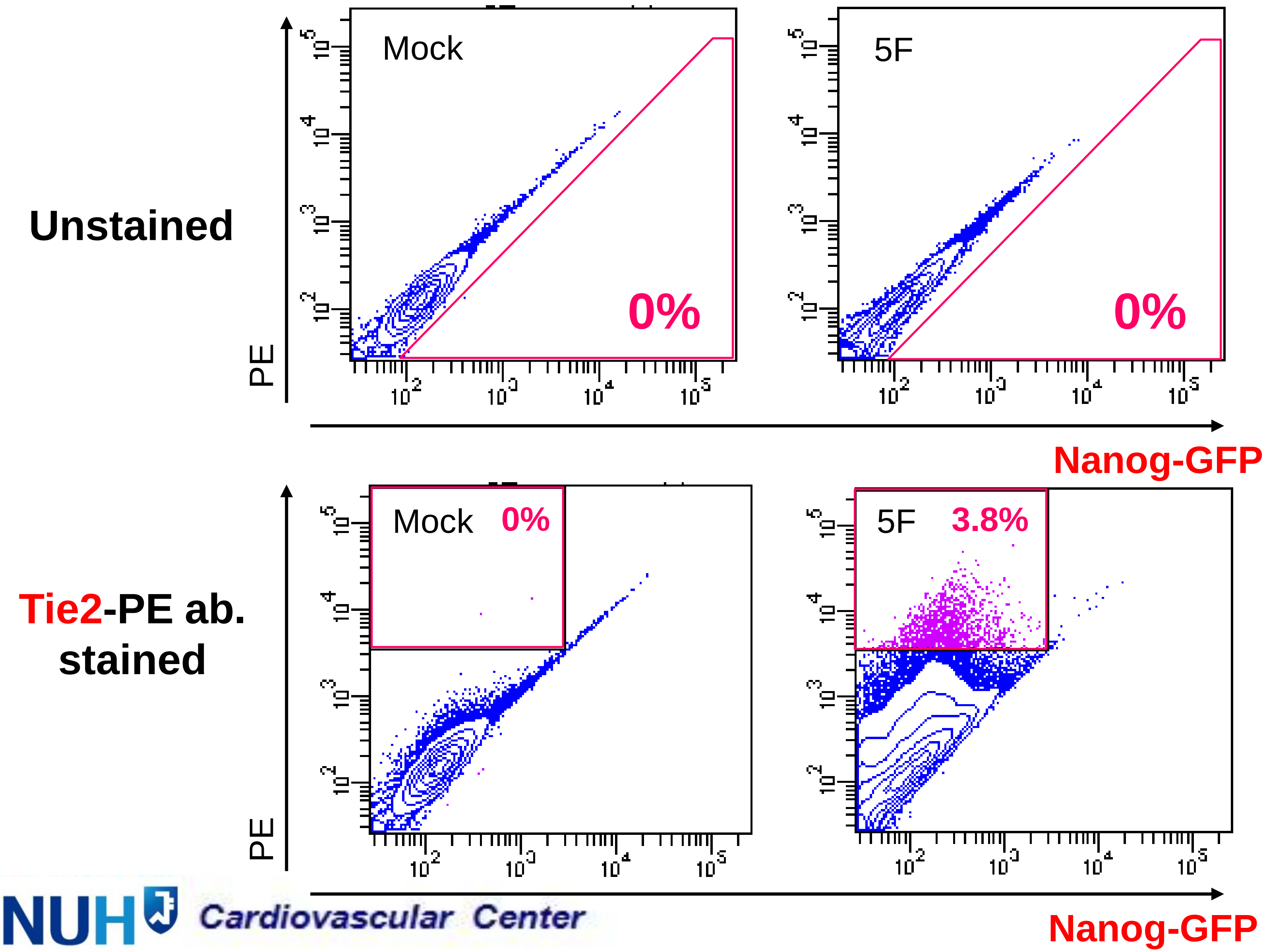
Not Through Pluripotency Induction: Oct4-GFP SFBs

Unstained

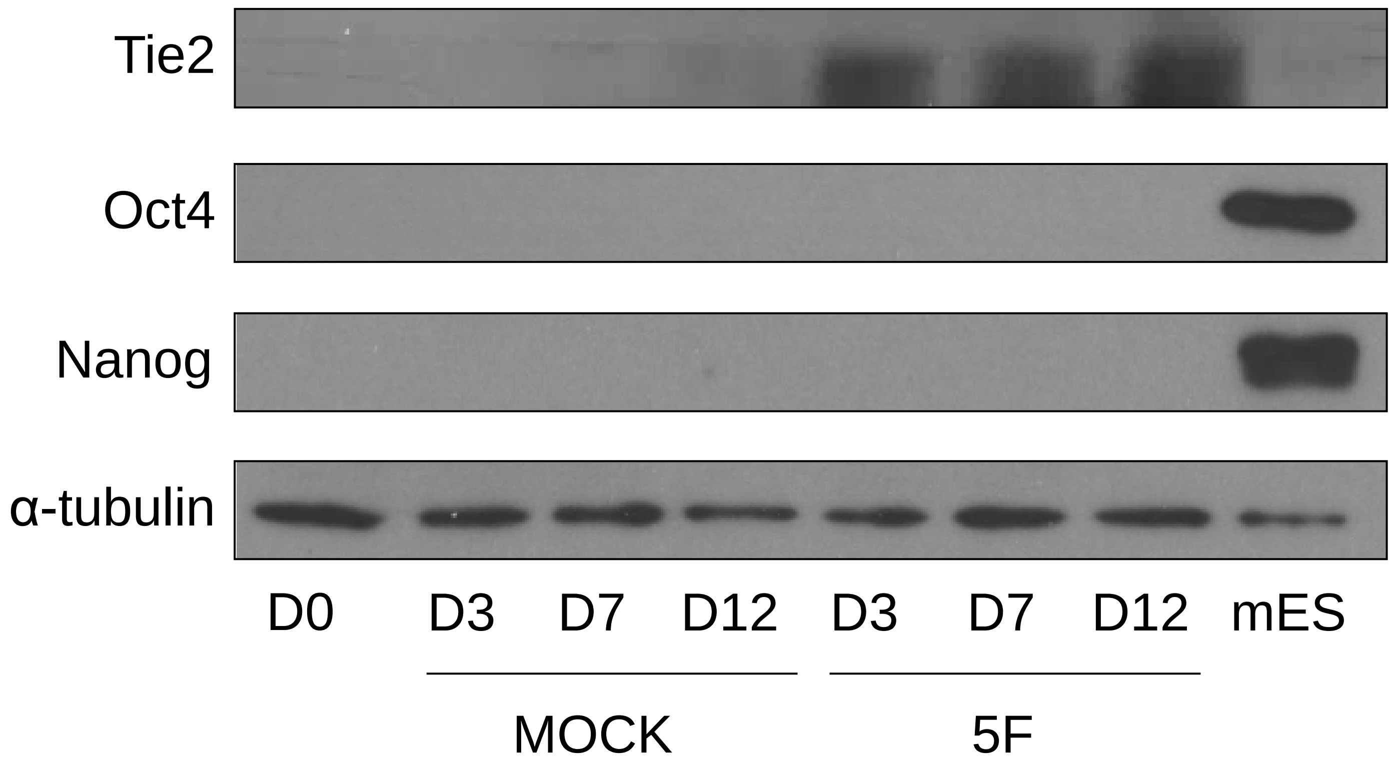
Tie2-PE ab. stained



Not Through Pluripotency Induction: Nanog-GFP SFBs



Not Through Pluripotency Induction: Oct4/Nanog stay silent during trans-differentiation



JK Han,,, HS Kim. Circulation 2014

In Vivo Functionality of iECs

Mouse Hindlimb
Ischemia Model: D14

Gross Pictures

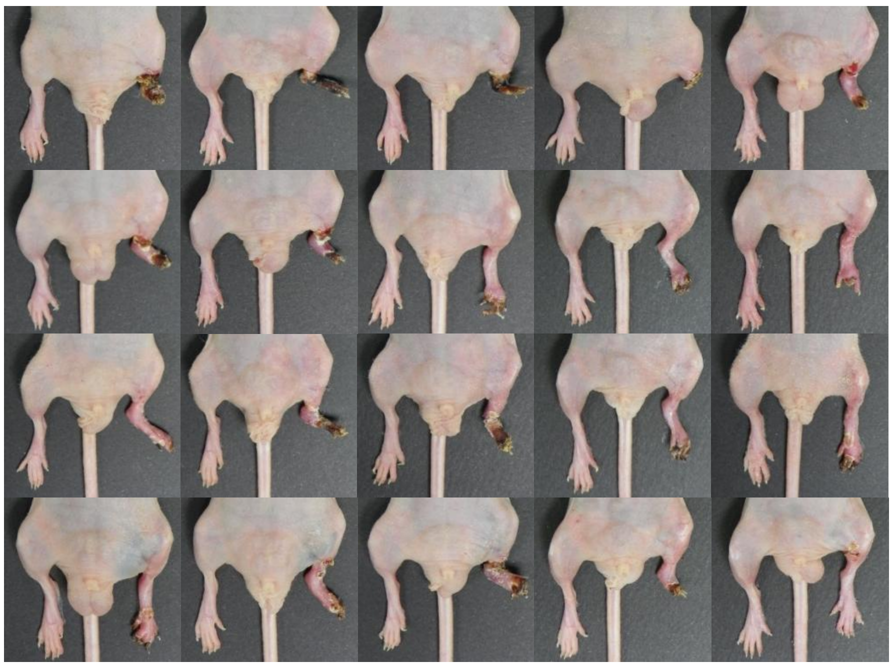
LDPI

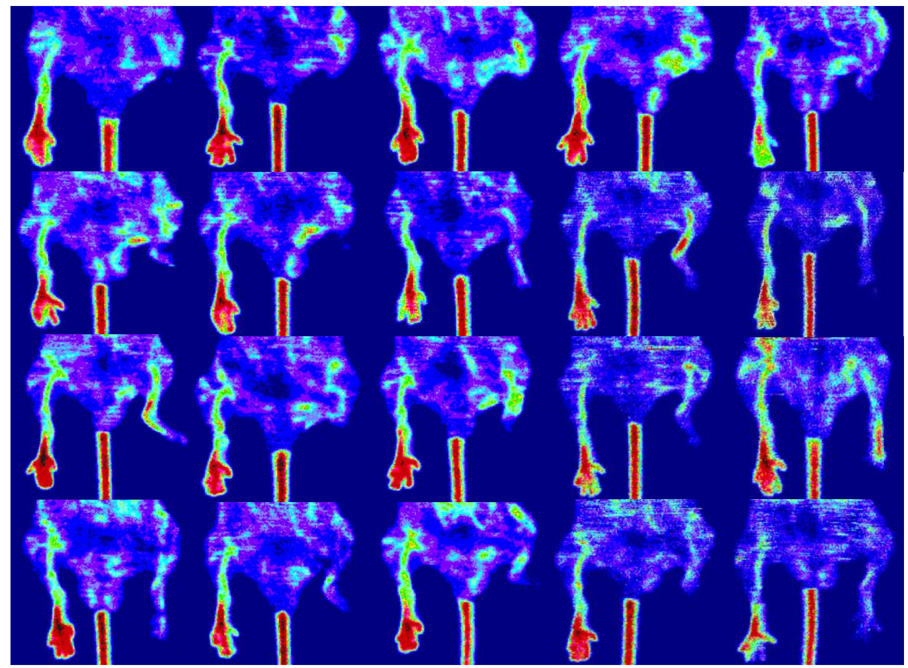
Mock-SFB

iEC

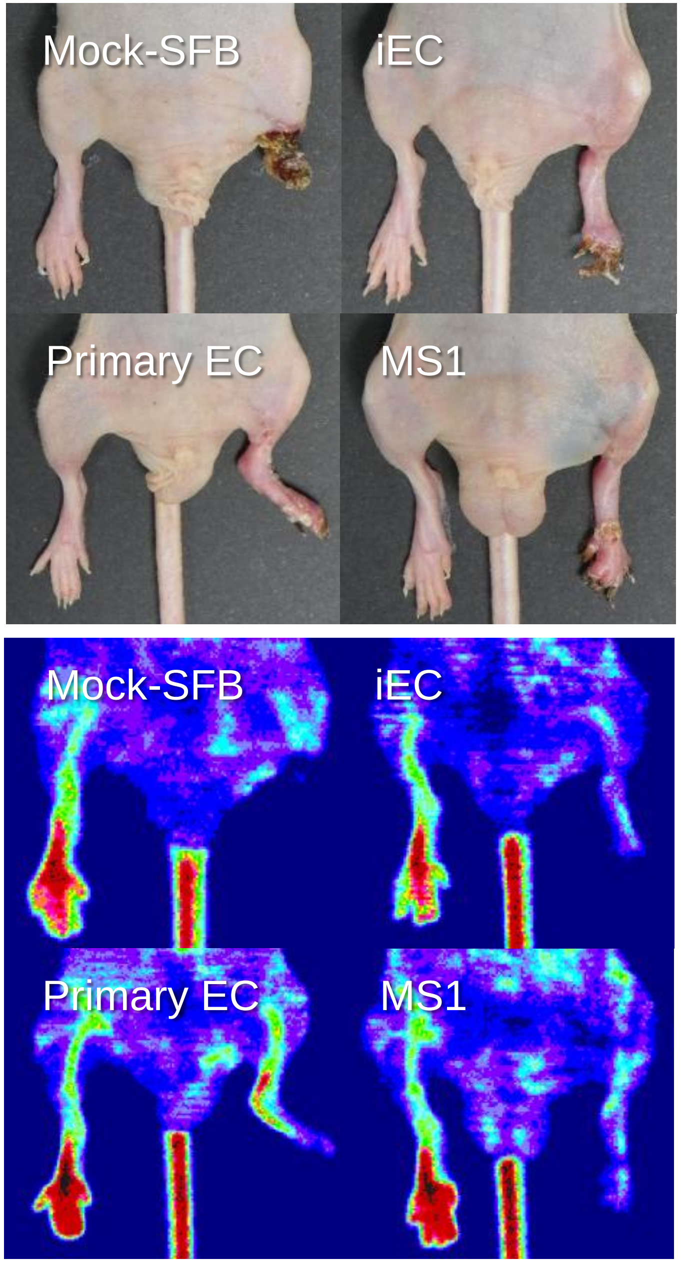
Primary EC

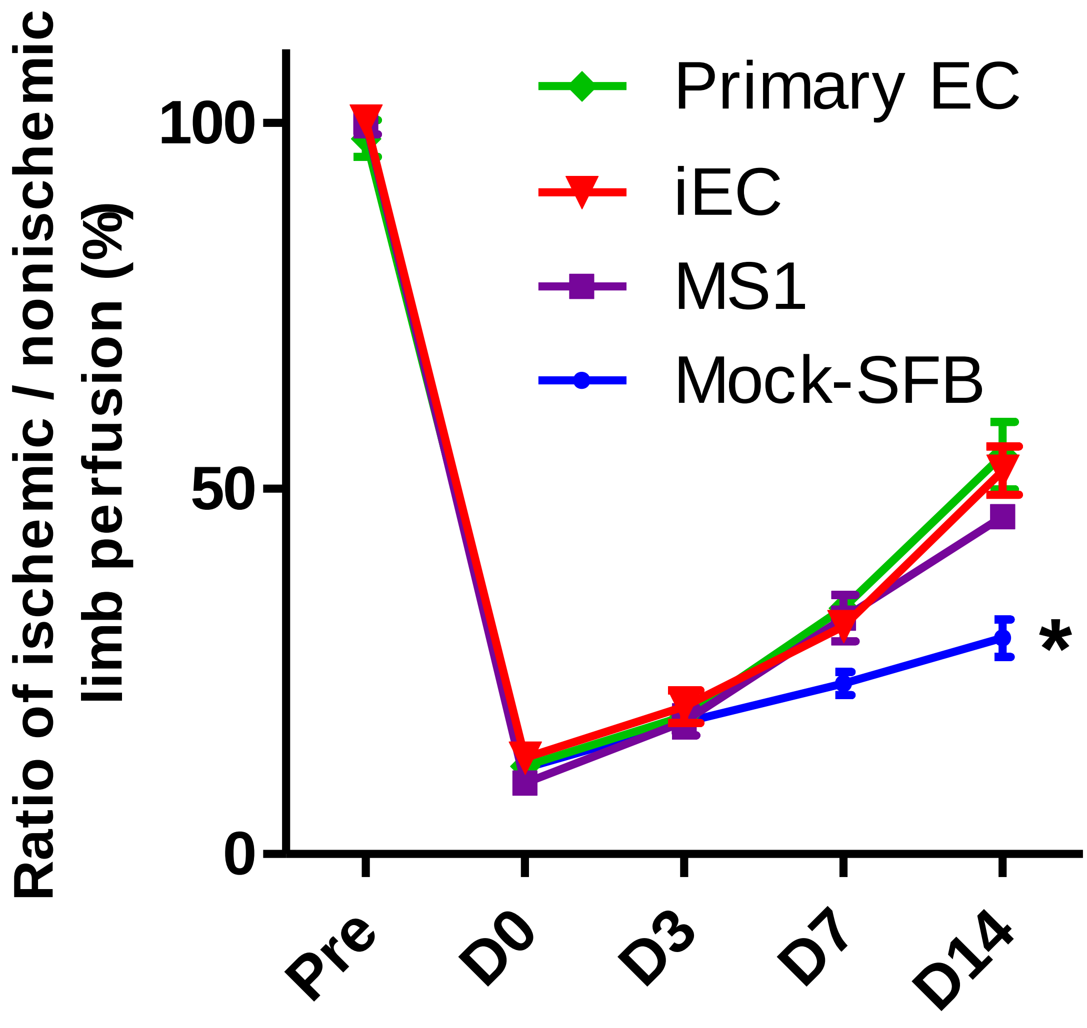
MS1





JK Han,,, HS Kim. Circulation 2014

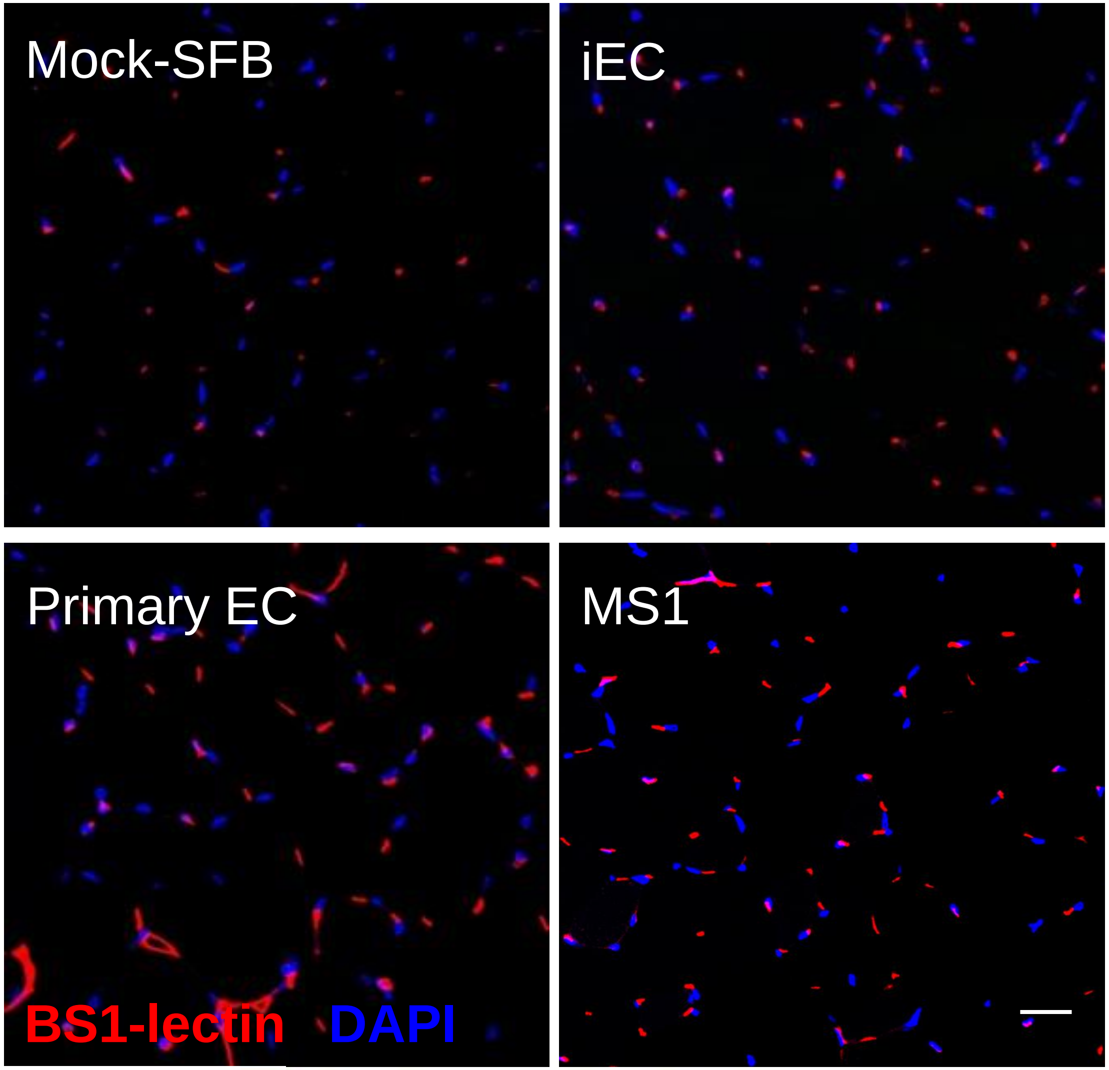


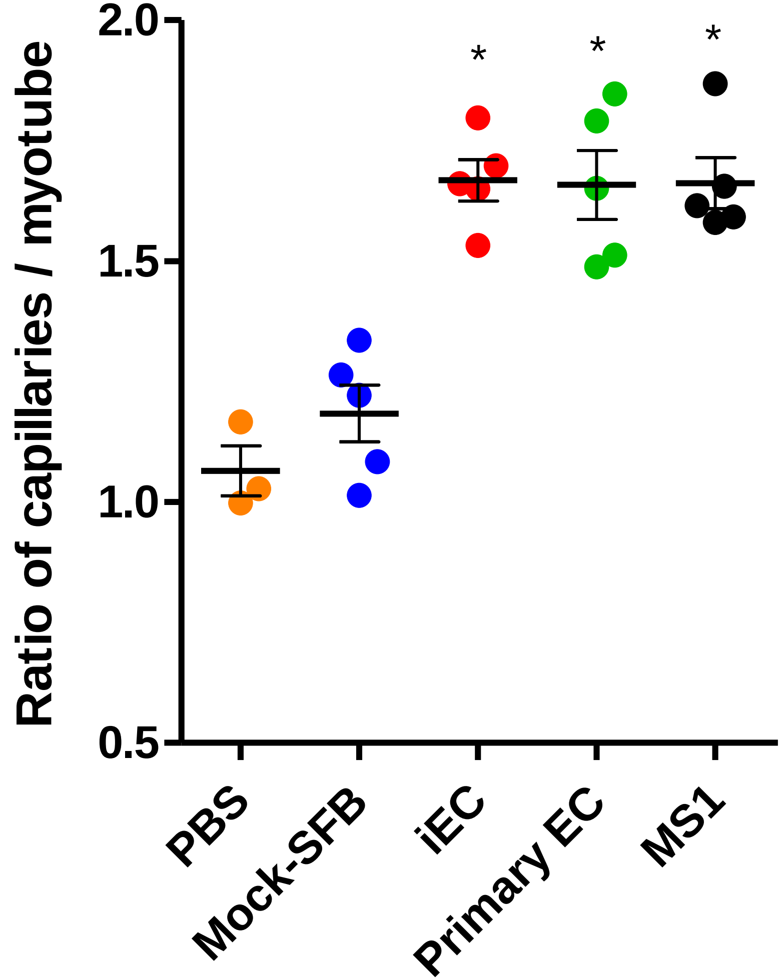


SNUH Cardiovascular Center

JK Han,,, HS Kim. Circulation 2014

Mouse Hindlimb Ischemia Model:
Capillary Density

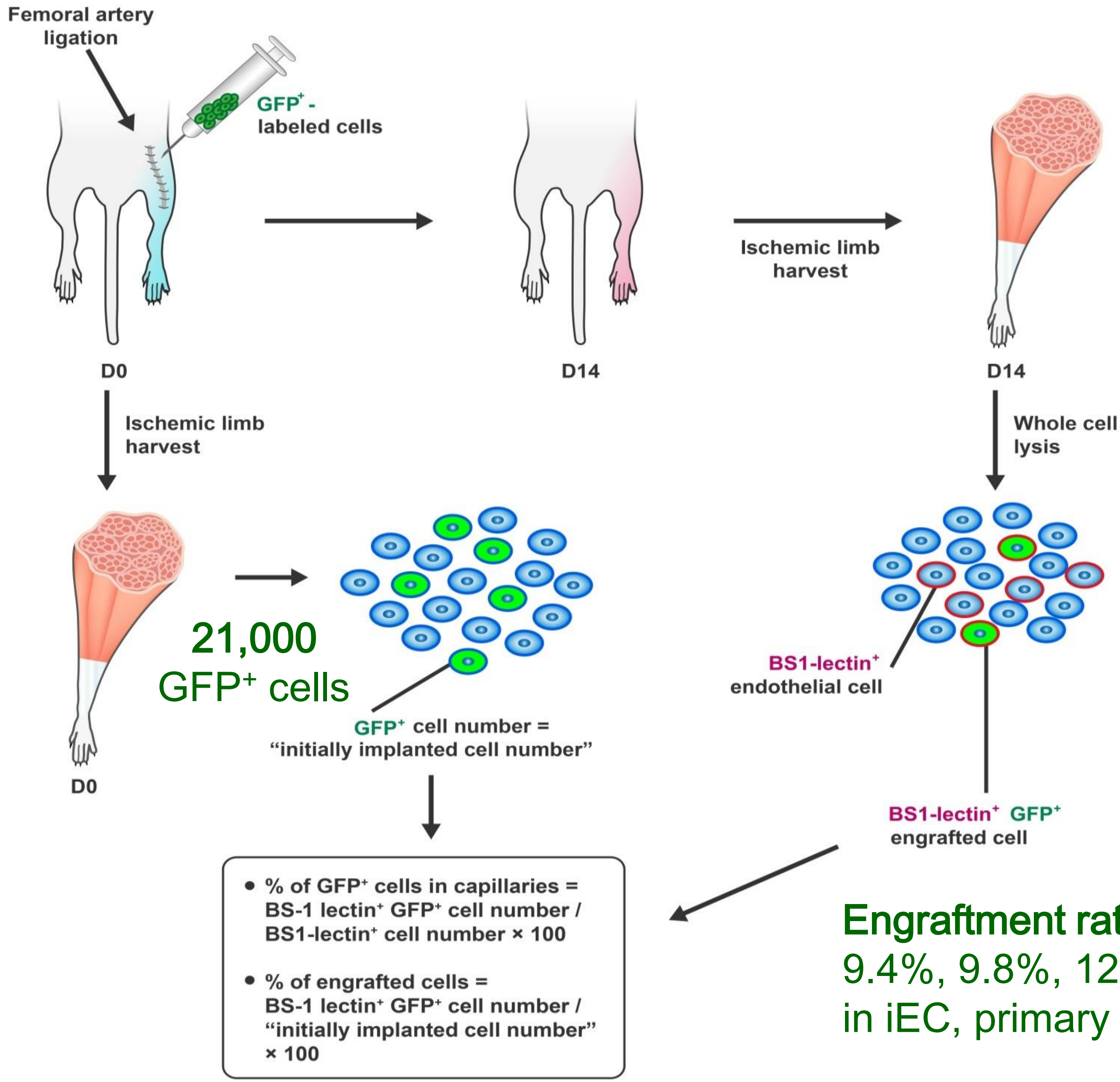




SNUH Cardiovascular Center

In Vivo Engraftment Ratio = *10%*

500,000
GFP+ cells

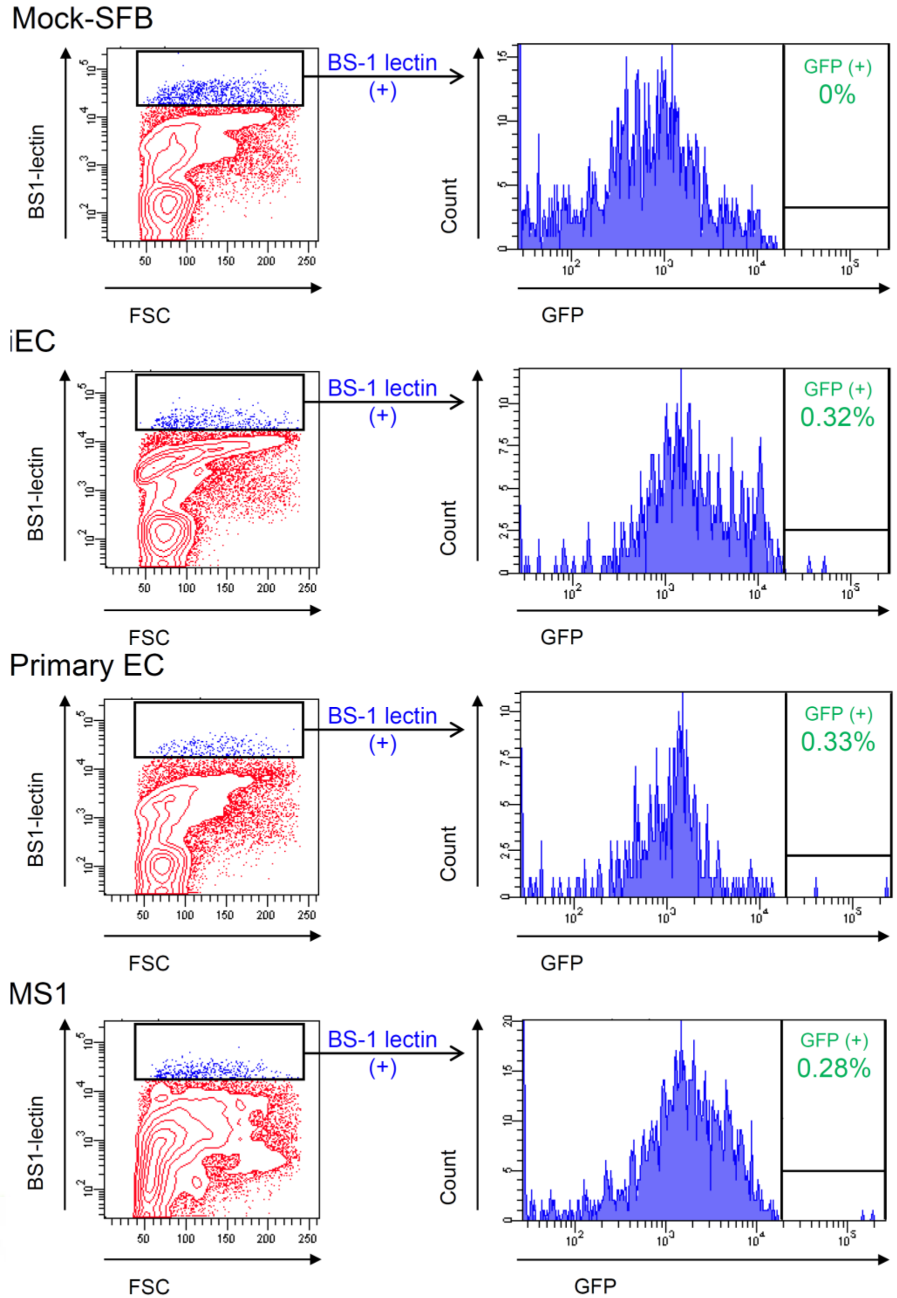


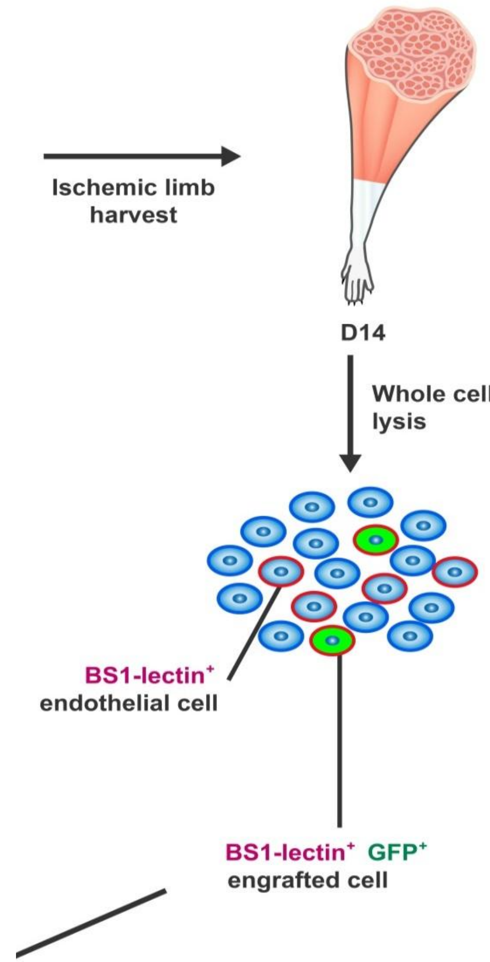
21,000
GFP⁺ cells

Engraftment ratio =
9.4%, 9.8%, 12.0%
in iEC, primary EC, and MS1

SNUH

In Vivo Participation as EC in Capillary: 0.3%

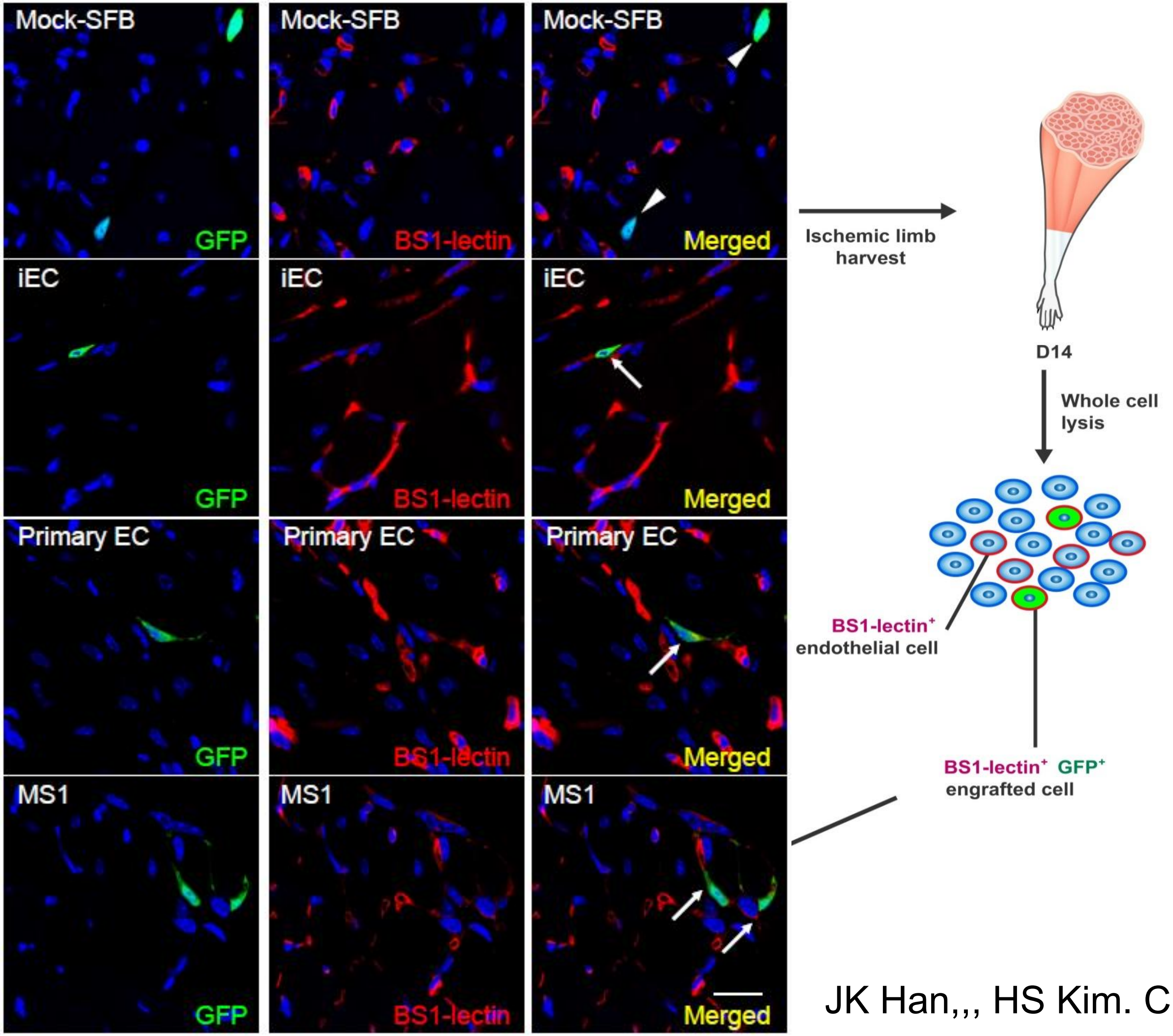




SNUH

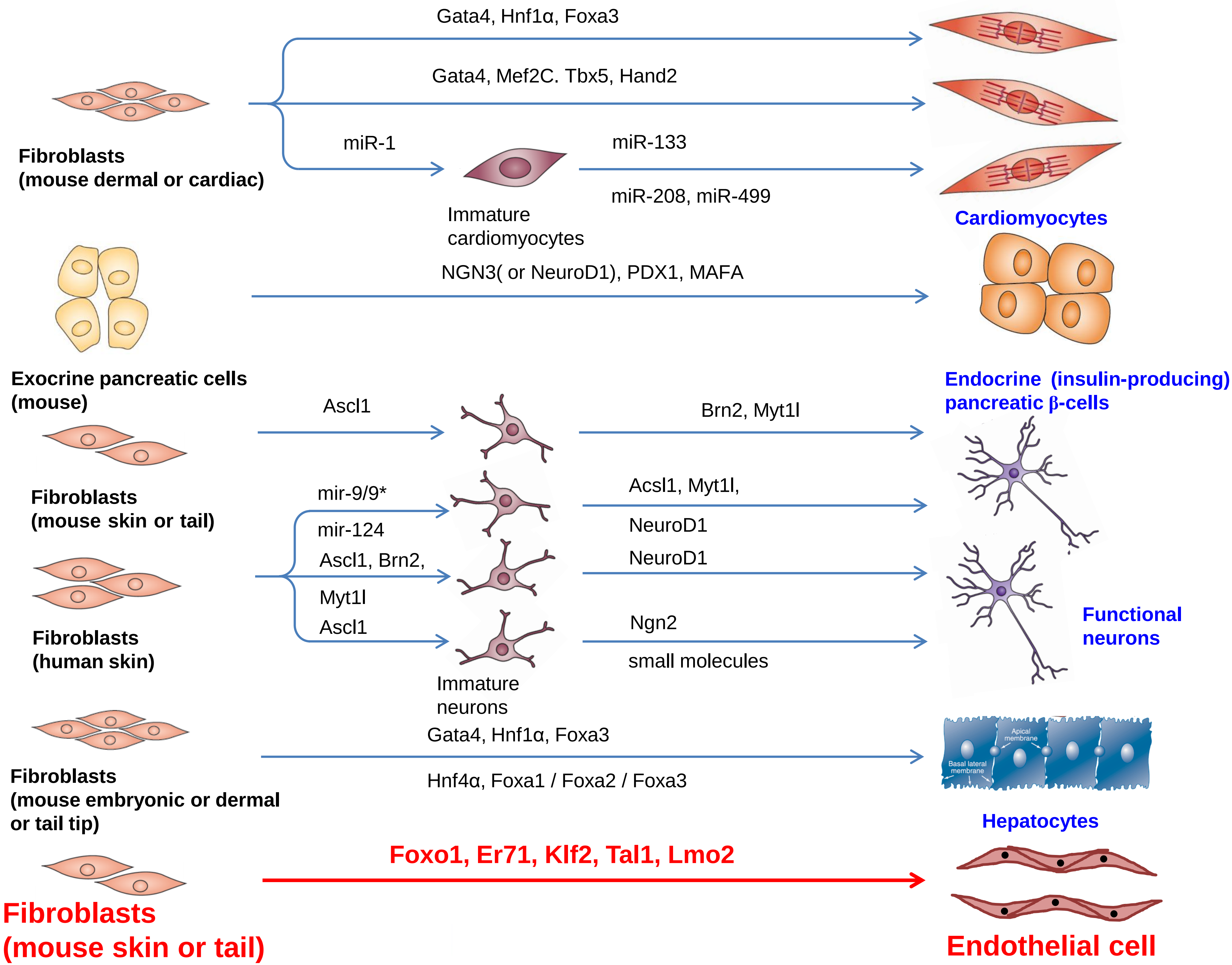
JK Han,,, HS Kim. Circulation 2014

In Vivo Participation as EC : 0.3%

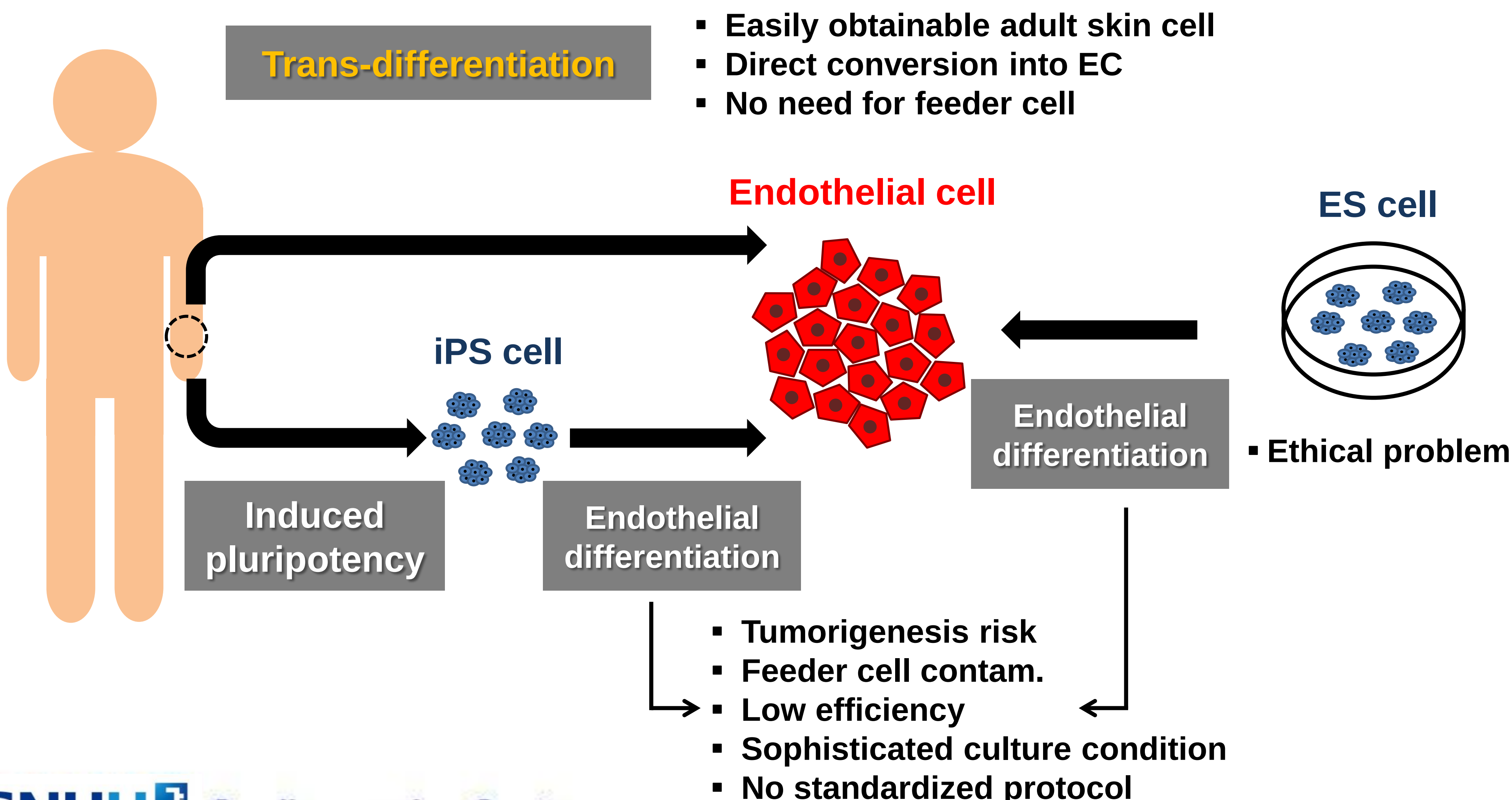


SNUH

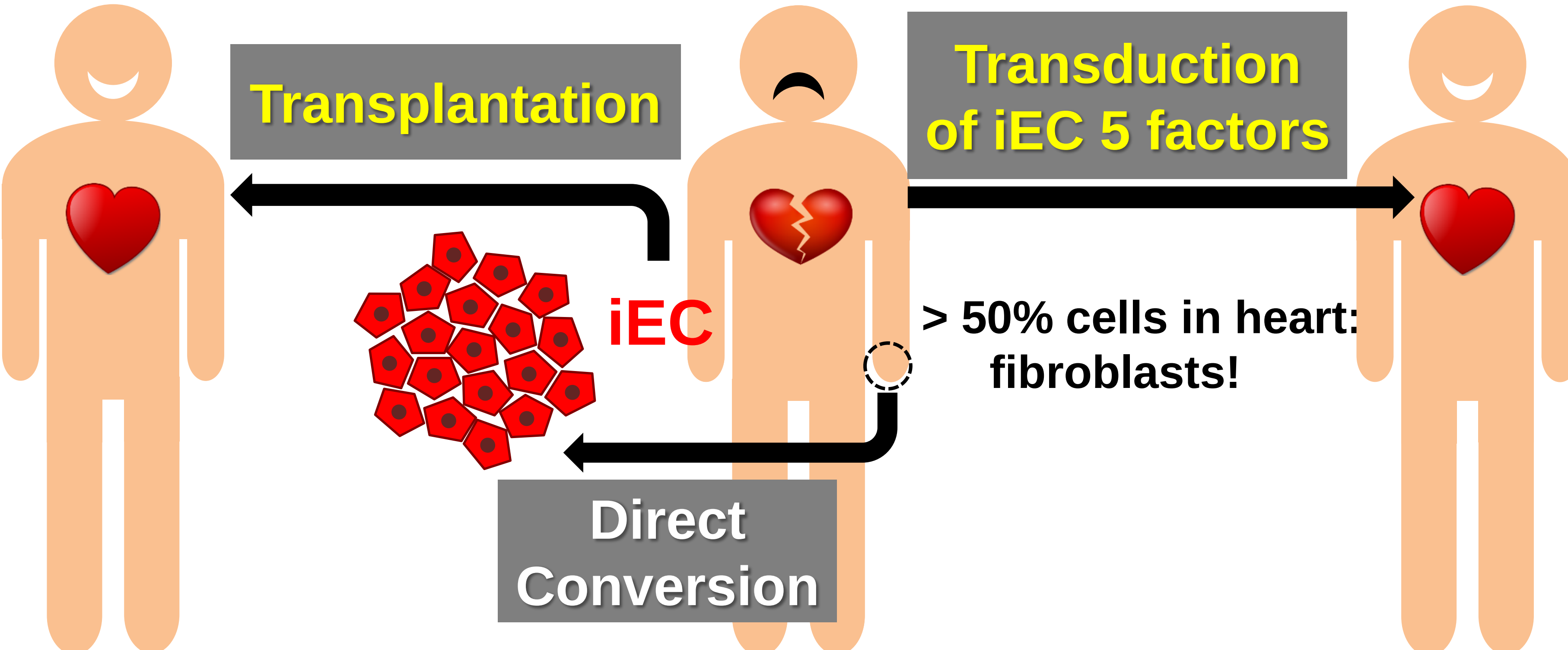
JK Han,,, HS Kim. Circulation 2014


Gata4, Hnf1α, Foxa3
Gata4, Mef2C. Tbx5, Hand2
miR-1
miR-133
miR-208, miR-499
Fibroblasts
(mouse dermal or cardiac)
Immature
cardiomyocytes
Cardiomyocytes
NGN3(or NeuroD1), PDX1, MAFA
Exocrine pancreatic cells
(mouse)
Endocrine (insulin-producing)
pancreatic β-cells
Ascl1
Brn2, Myt1l
Fibroblasts
(mouse skin or tail)
mir-9/9*
mir-124
Acsl1, Myt1l,
NeuroD1
Ascl1, Brn2,
Myt1l
NeuroD1
Ascl1
Ngn2
small molecules
Fibroblasts
(human skin)
Functional
neurons
Immature
neurons
Gata4, Hnf1α, Foxa3
Hnf4α, Foxa1 / Foxa2 / Foxa3
Apical
membrane
Basal lateral
membrane
Fibroblasts
(mouse embryonic or dermal
or tail tip)
Hepatocytes
Foxo1, Er71, Klf2, Tal1, Lmo2
Fibroblasts
(mouse skin or tail)
Endothelial cell

iEC vs. ESC/iPSC-derived ECs



Therapeutic Strategy



- ***Cell*, 2012**
- Human **amniotic** cells → ECs
- 3 ETS factors: ER71/ERG1/FLI1
- Only amniotic cells
  - Not readily available
  - Immunogenicity / allograft rejection
- Not terminally differentiated FBs

- ***Nat. Med*, 2012**
- ***PNAS*, 2012**
- Human FBs → ECs
- Y' iPS 4 factors: OCT4/SOX2/KLF4/MYC
- Via partial iPSC or progenitor cells status
  - Concerns over tumorigenic potential

Summary

- The first study demonstrating that adult fibroblasts can be directly converted to ECs by defined factors.
- These **iEC 5 factors** are Foxo1, Er71, Klf2, Tal1 and Lmo2.
- iECs exhibit endothelial features and functions *in vitro* and *in vivo.*

Conclusions

- Our study provides further evidence that **cell fate determination is not eternal, but plastic** by the formation of new transcriptional network.
- Our findings identify the **molecular background of endothelial differentiation and trans-differentiation**.
- This study makes significant progress towards **future clinical application**.

Direct Conversion of Adult Skin Fibroblasts to Endothelial Cells by Defined Factors

Jung-Kyu Han, MD*; Sung-Hwan Chang, BS*; Hyun-Ju Cho, PhD*; Saet-Byeol Choi, BS*; Hyo-Suk Ahn, MS; Jaewon Lee, BS; Heewon Jeong, BS; Seock-Won Youn, PhD; Ho-Jae Lee, BS; Yoo-Wook Kwon, PhD; Hyun-Jai Cho, MD; Byung-Hee Oh, MD; Peter Oettgen, MD; Young-Bae Park, MD; Hyo-Soo Kim, MD

Background—Cell-based therapies to augment endothelial cells (ECs) hold great therapeutic promise. Here, we report a novel approach to generate functional ECs directly from adult fibroblasts.

Methods and Results—Eleven candidate genes that are key regulators of endothelial development were selected. Green fluorescent protein (GFP)–negative skin fibroblasts were prepared from Tie2-GFP mice and infected with lentiviruses allowing simultaneous overexpression of all 11 factors. Tie2-GFP$^+$ cells (0.9%), representing Tie2 gene activation, were detected by flow cytometry. Serial stepwise screening revealed 5 key factors (Foxo1, Er71, Klf2, Tal1, and Lmo2) that were required for efficient reprogramming of skin fibroblasts into Tie2-GFP$^+$ cells (4%). This reprogramming strategy did not involve pluripotency induction because neither Oct4 nor Nanog was expressed after 5 key factor transduction. Tie2-GFP$^+$ cells were isolated using fluorescence-activated cell sorting and designated as induced ECs (iECs). iECs exhibited endothelium-like cobblestone morphology and expressed EC molecular markers. iECs possessed endothelial functions such as *Bandeiraea simplicifolia*-1 lectin binding, acetylated low-density lipoprotein uptake, capillary formation on Matrigel, and nitric oxide production. The epigenetic profile of iECs was similar to that of authentic ECs because the promoters of VE-cadherin and Tie2 genes were demethylated. mRNA profiling showed clustering of iECs with authentic ECs and highly enriched endothelial genes in iECs. In a murine model of hind-limb ischemia, iEC implantation increased capillary density and enhanced limb perfusion, demonstrating the in vivo viability and functionality of iECs.

Conclusions—We demonstrated the first direct conversion of adult fibroblasts to functional ECs. These results suggest a novel therapeutic modality for cell therapy in ischemic vascular disease. (*Circulation*. **2014;130:1168-1178.**)

Key Words: cell transdifferentiation ■ endothelial cells ■ fibroblasts

SNUH Cardiovascular Center

iEC Project

Jung-Kyu Han, MD
Sung-Hwan Chang,
Hyun-Ju Cho, PhD
Saet-Byeol Choi,
Hyo-Suk Ahn, MS
Jung-Soo Lee

Fund

- **Innovative Research Institute for Cell Therapy (IRICT), Korea**
- **National Research Foundation (NRF), Korea**