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Trans-differentiation of Human Fibroblasts to Endothelial Cells: Role of Innate Immunity

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Houston, TX; Stanford, CA; Cincinnati, OH

Circulation, 2014

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Introduction

- **Shinya Yamanaka's Discovery:**
  - 2012 - Nobel Prize for Medicine
  - Forced reprogramming of somatic cells to induced
  - pluripotent stem cells (**iPSCs**)
  - By expression of the TFs: Oct4, Sox2, Klf4 and cMyc (**"OSKM")**

- **Disadvantage:**
  - Cells are transfected by viral vectors
  - Induce overexpression of TFs → cause global changes in expression and activity of epigenetic modifiers.
  - Only small percentage of cells transform (≈2–4 weeks)

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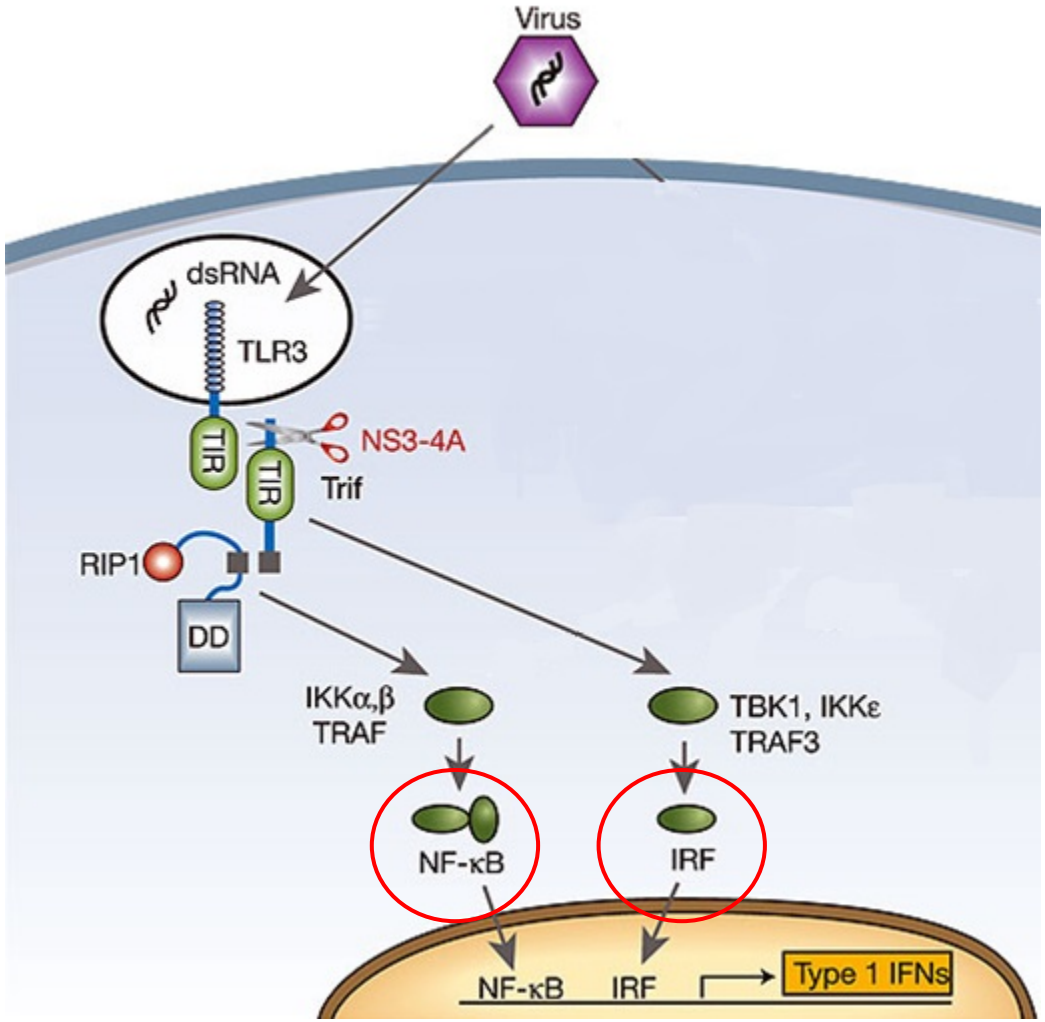
Introduction



Adapted from
Meylan et al., 2006

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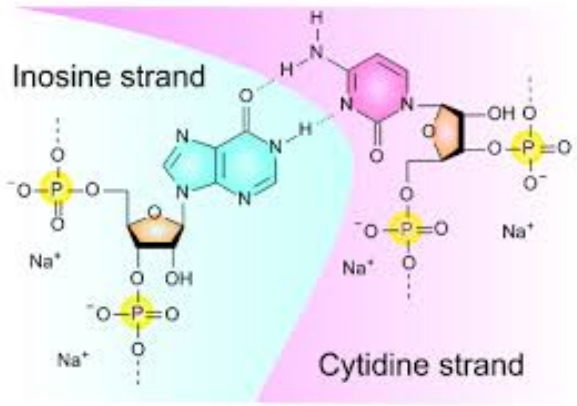
Aim

- **Ideal for Clinical Application:** Transdifferentiation of cells, but avoid genetic manipulation
- Generation of safe and functional induced ECs (“iECs”) from fibroblasts.
- Use small molecule to activate TLR3 and inductive growth cues, to induce trans-differentiation.

Polyinosinic-polycytidylic acid

- Poly(I:C) a synthetic analog of double stranded RNA (dsRNA)
- A molecular pattern associated with viral infection
- Poly(I:C) is recognized by Toll-like receptor 3 (TLR3)
- Activates TFs interferon regulatory factor 3 (IRF3), NF-κB and AP-14
- Triggers production of inflammatory cytokines and chemokines such as TNF-α, IL-6 and CXCL10



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Timeline

Direct reprogramming of fibroblasts to induced endothelial cells (iECs)

- BJ human new-born foreskin fibroblast cells

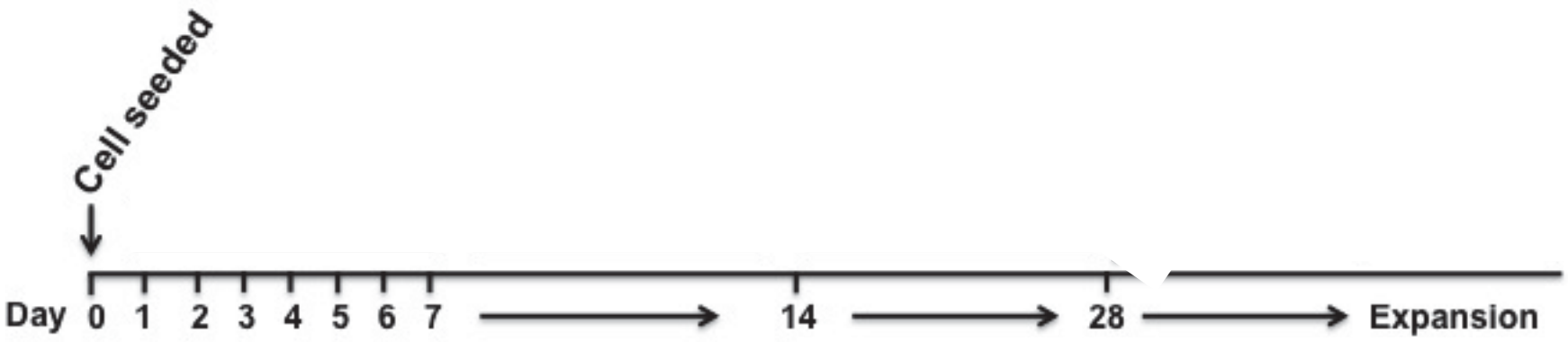

Figure 1A

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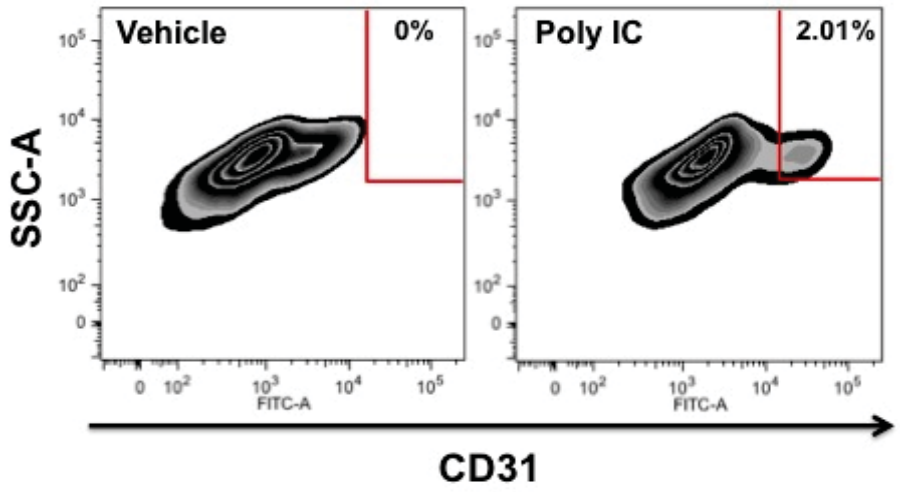
Detection of EC-Specific Markers After 28 Days of Differentiation

Human foreskin fibroblasts (BJ)



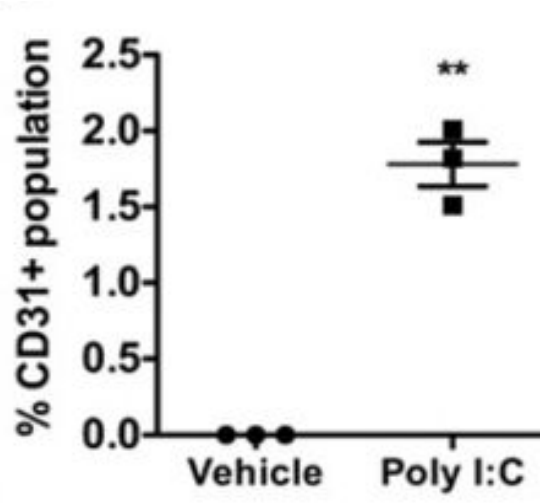


Figure 1B,C

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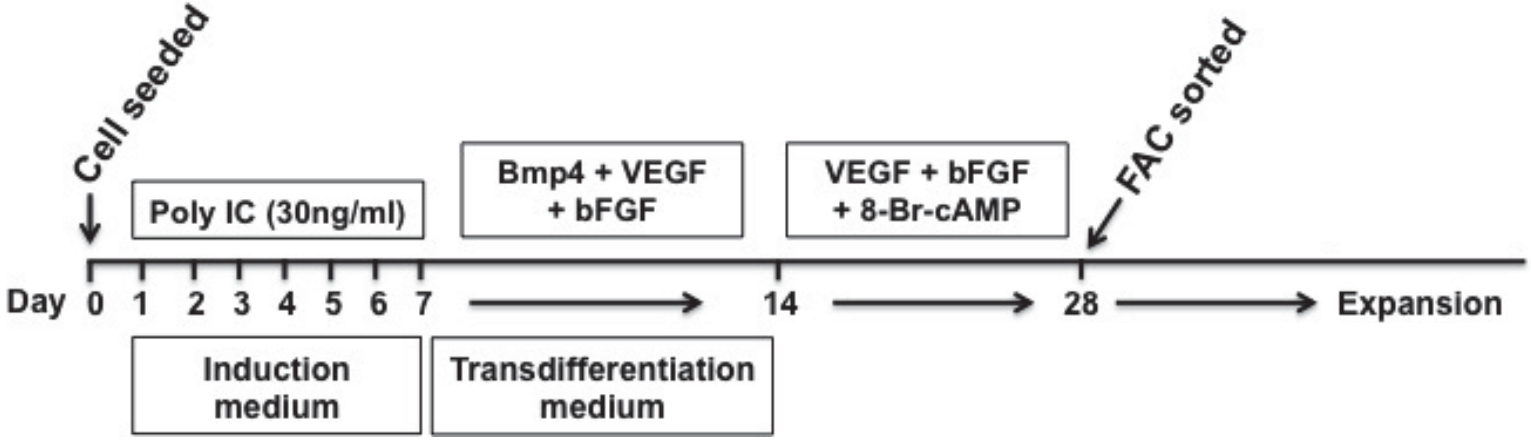
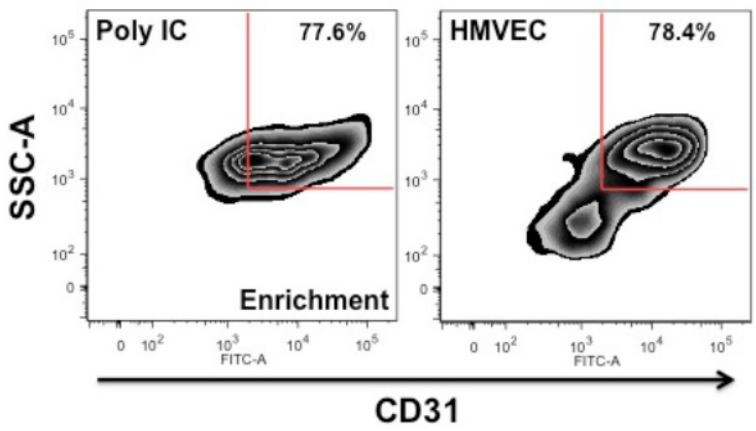
Expansion of Endothelial Cell Markers

Human foreskin fibroblasts





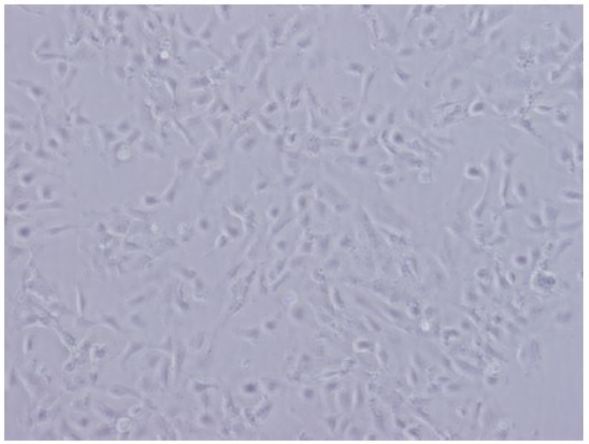


Figure S1A,B

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Expression of Endothelial Cell Markers

Human foreskin fibroblasts

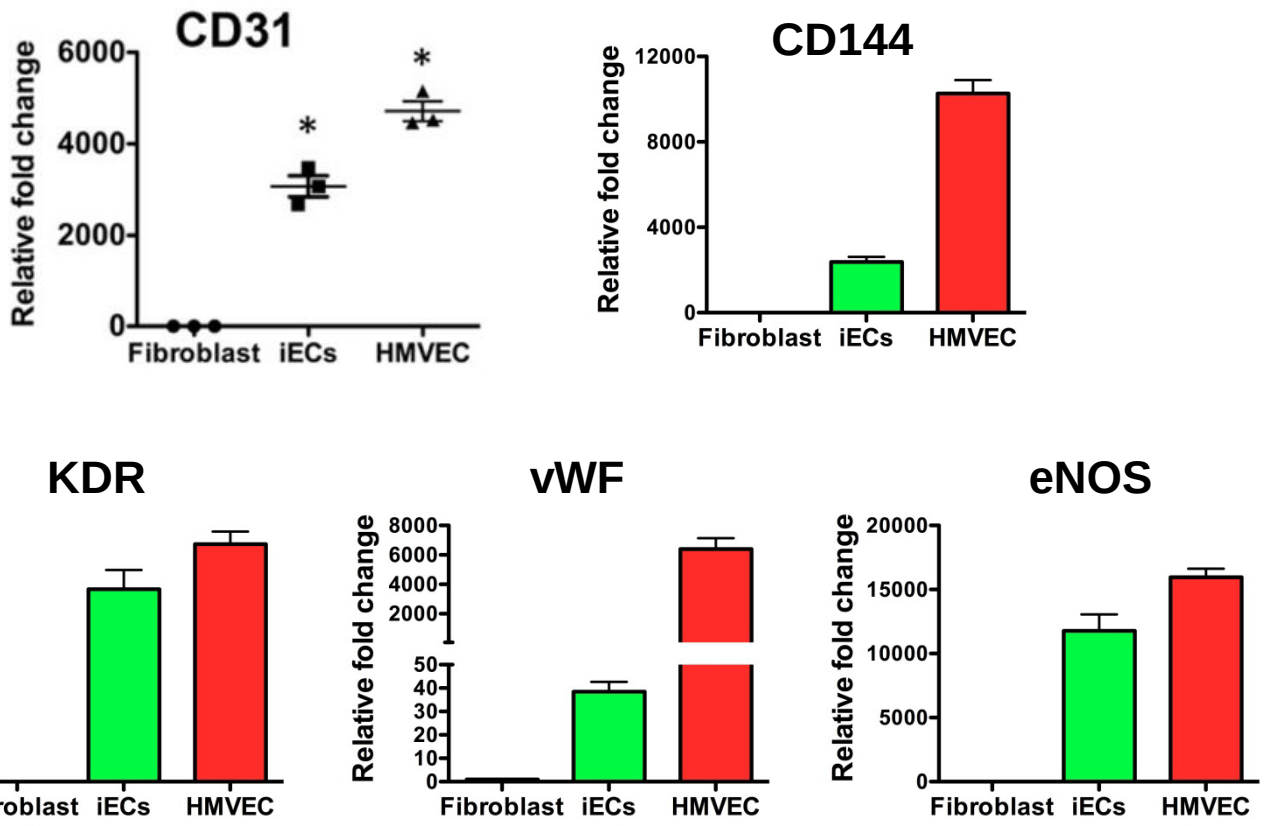

Figure 1D; S1C-F

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Expression of Endothelial Cell Markers

Human foreskin fibroblasts

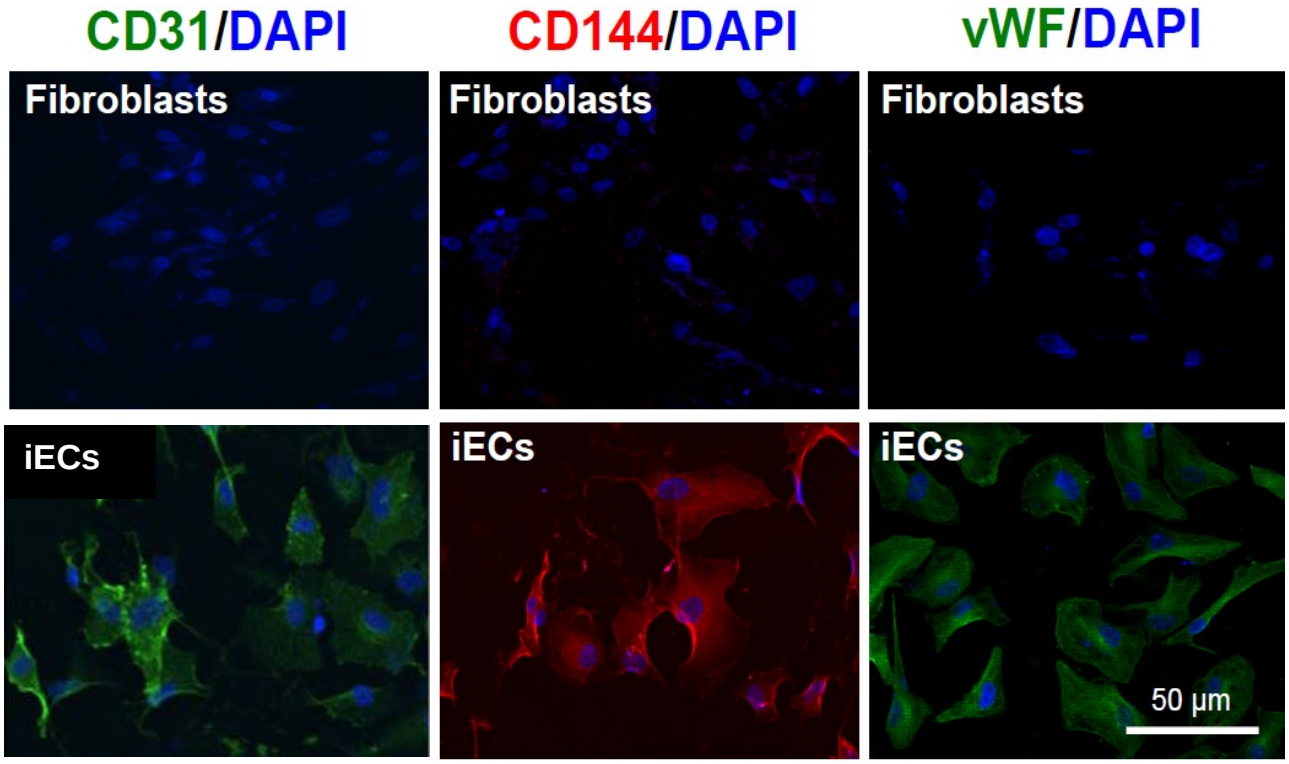

Figure 1E & S1G

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Uptake of Acetylated LDL & Tubular Network Formation

Human foreskin fibroblasts

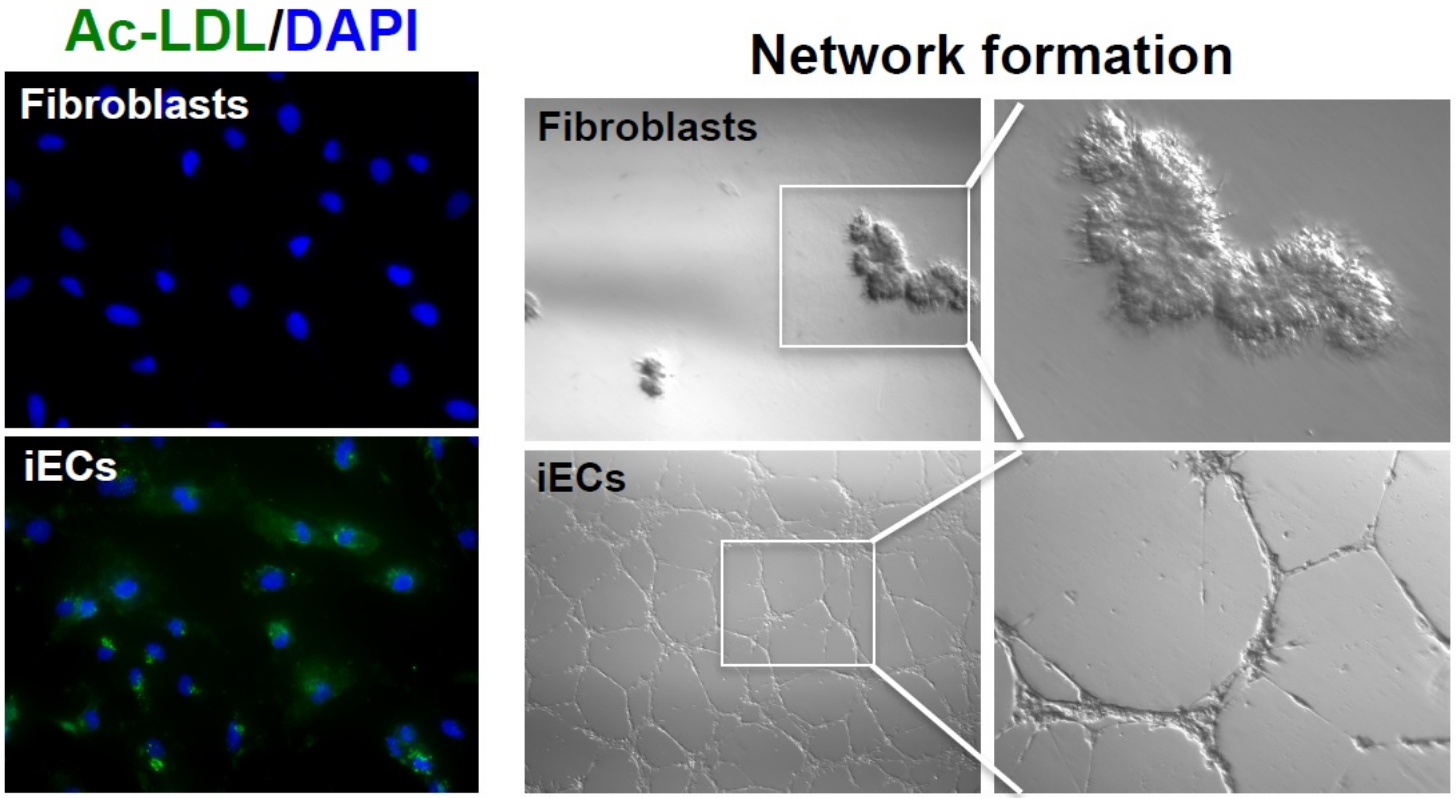

Fig.1F-G; S1H-I

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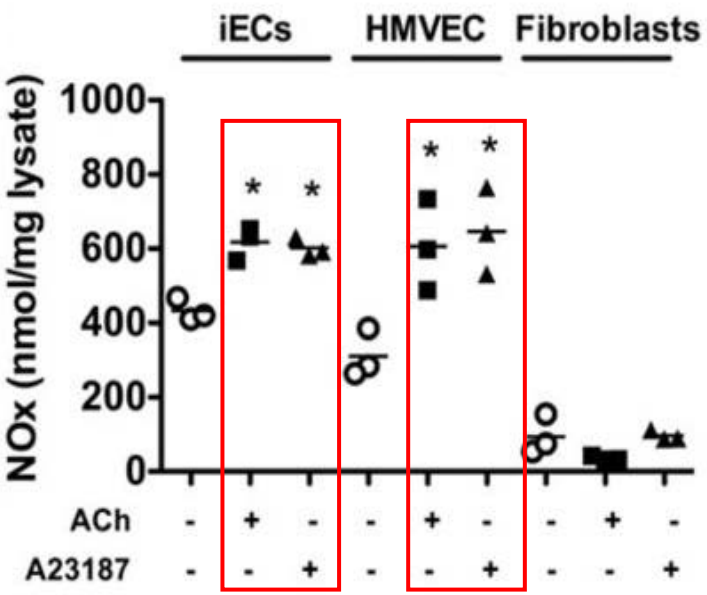
Nitric Oxide Production by iECs

Human foreskin fibroblasts



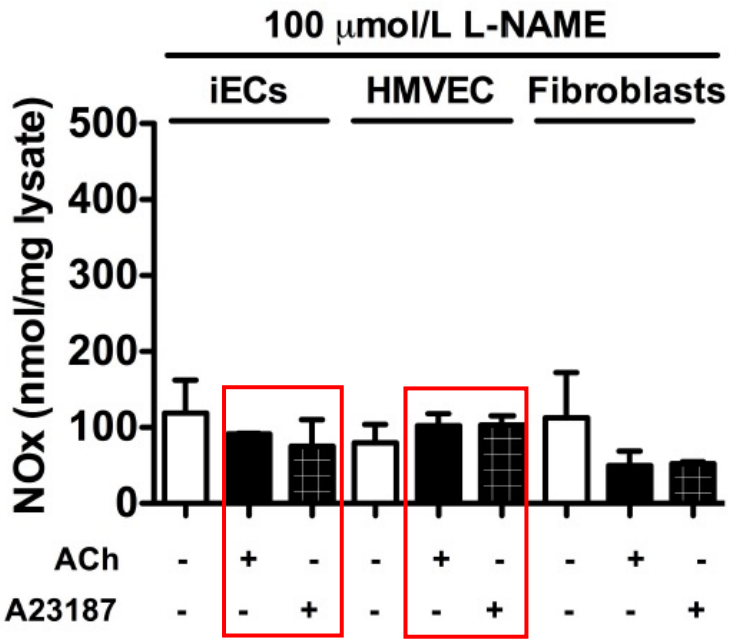


Figure 1H; S1J

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Expression of Angiogenic Cytokines

Human foreskin fibroblasts

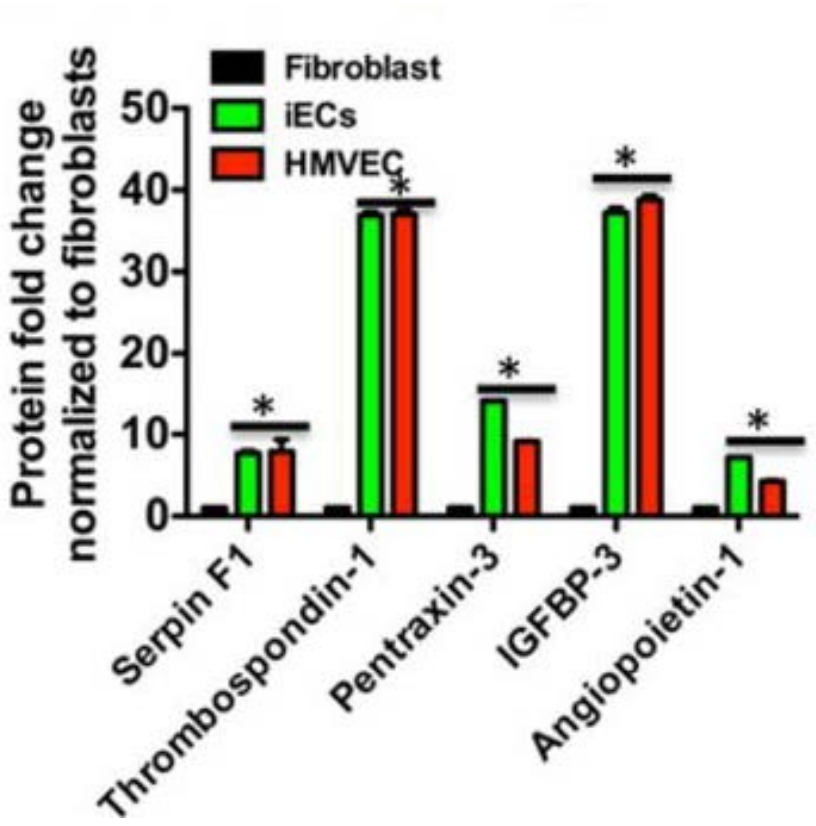

Figure 1I

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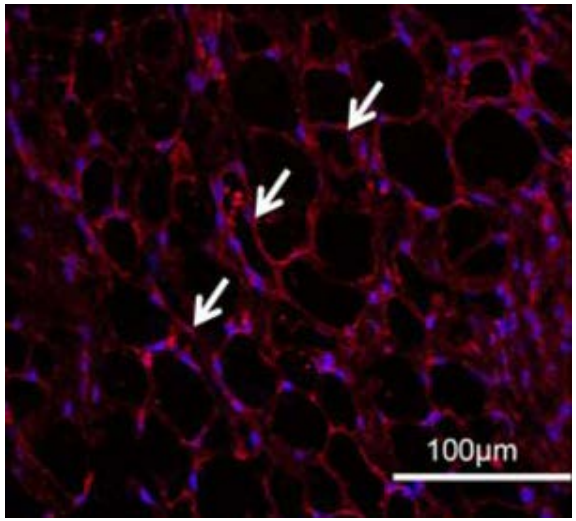
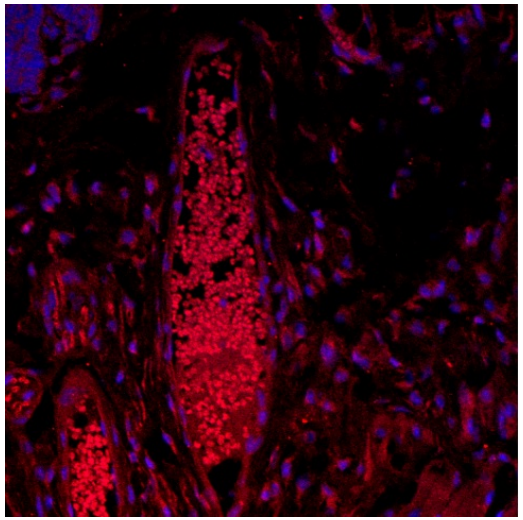
Capacity to Form Capillaries *InVivo*

Human foreskin fibroblasts





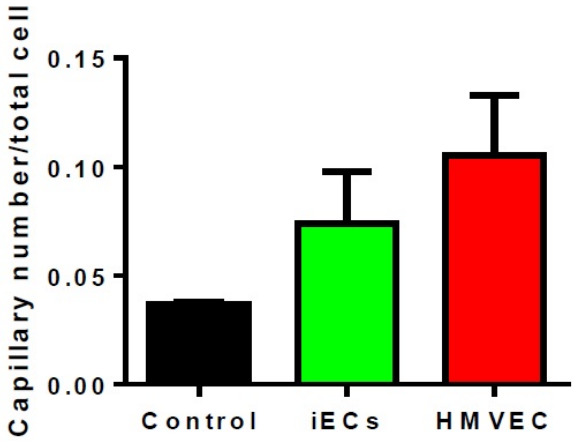


Figure 1J; S1K,L

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Sorting of GFP positive from GFP negative cells

Tie2GFP mice tail-tip fibroblasts

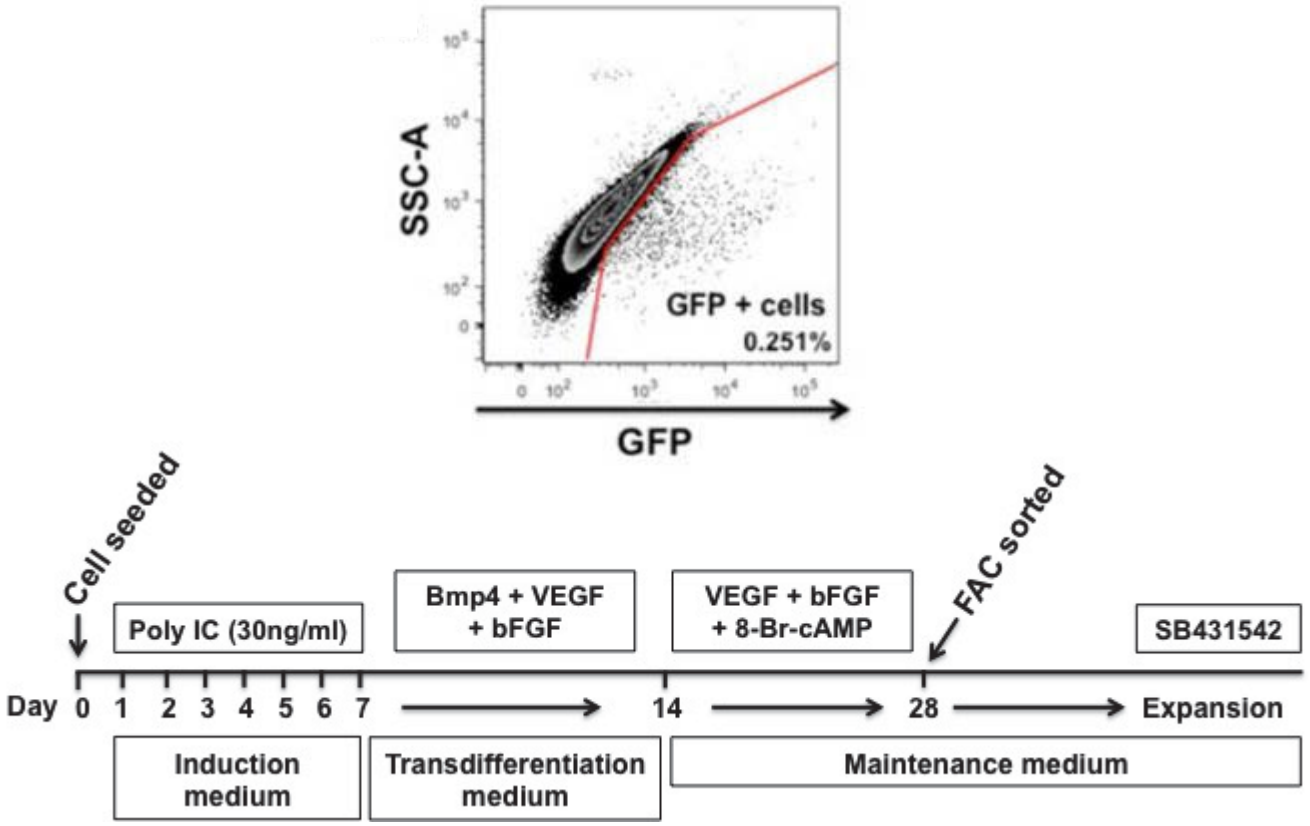

Figure 2A

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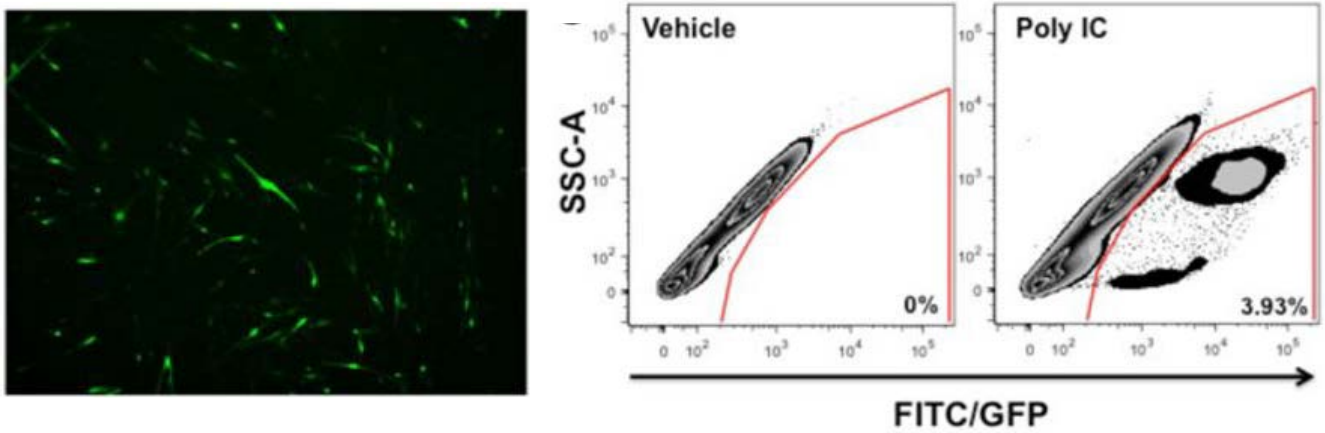
Detection of EC-Specific Markers After 28 Days of Differentiation

Tie2GFP mice tail-tip fibroblasts



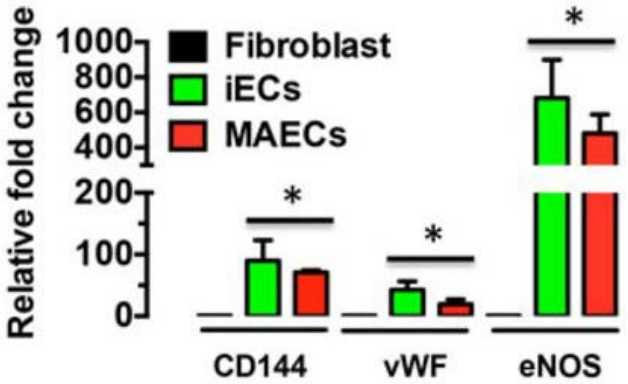


Figure 2B,C,D

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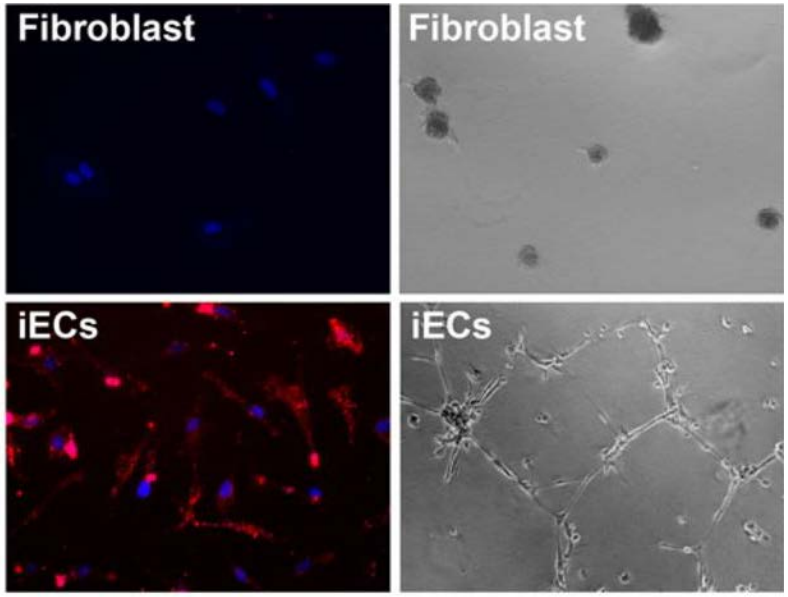
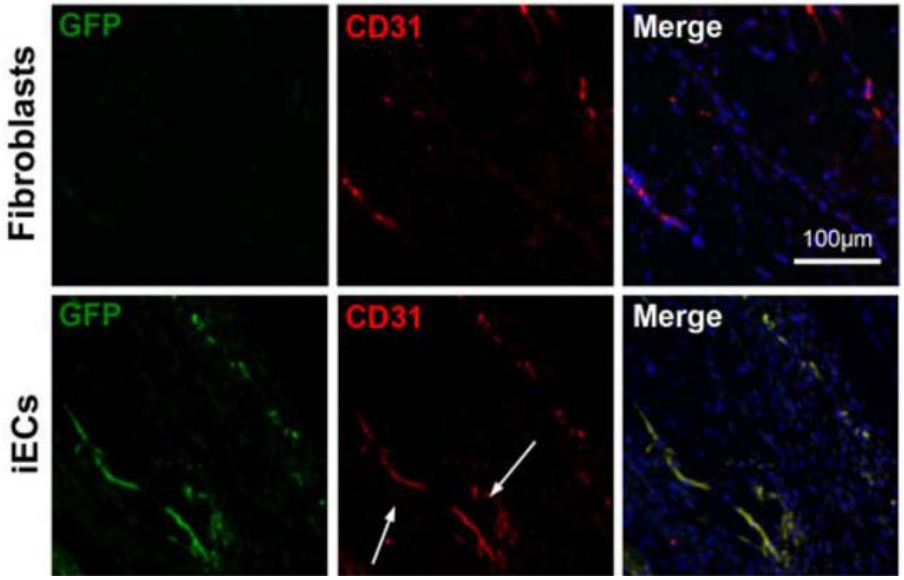
Uptake of Acetylated LDL & Tubular Network Formation

Tie2GFP mice tail-tip fibroblasts



In vitro



In vivo

Figure 2 E,F,G

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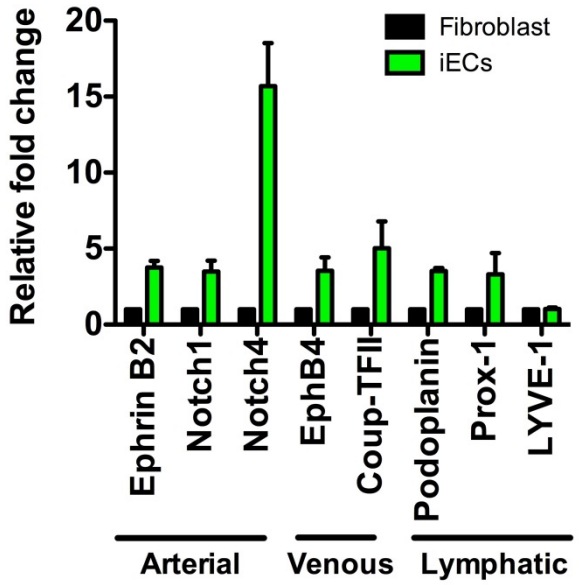
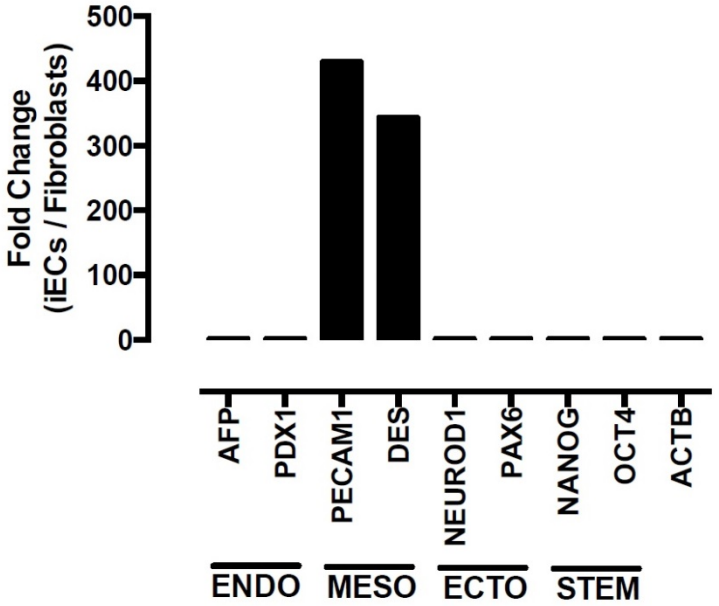
Arterial EC and Mesodermal Marker Expression Increased

Tie2GFP mice tail-tip fibroblasts



Arterial specific markers



Mesoderm specific markers

Figure S2A,B

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Histone modifications during direct reprogramming to iECs

Tie2GFP mice tail-tip fibroblasts

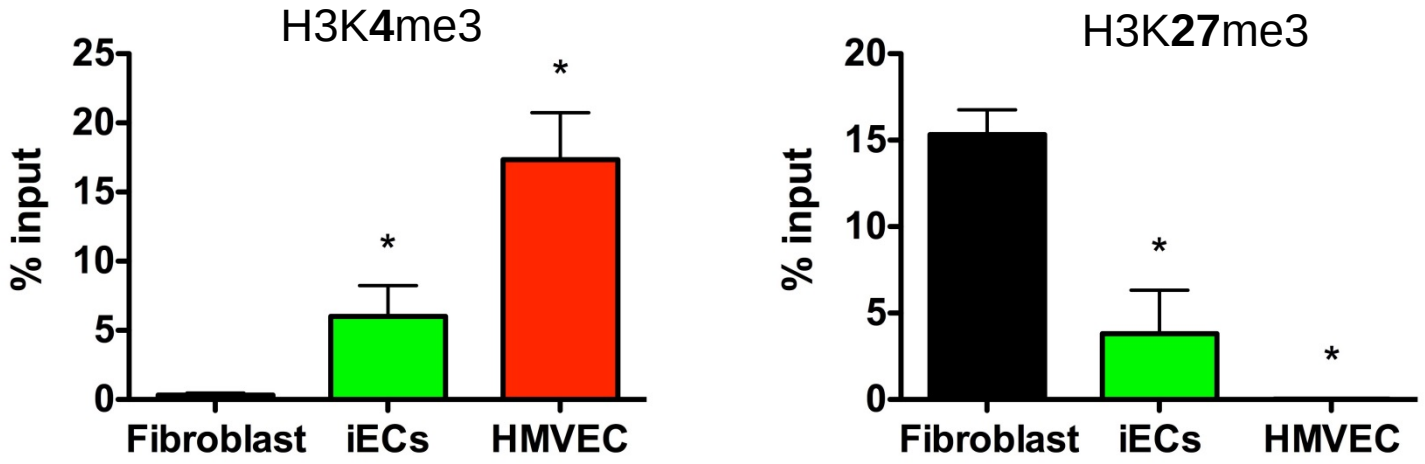

Figure S3A,B

Transcriptional profiling of induced-ECs

Tie2GFP mice tail-tip fibroblasts

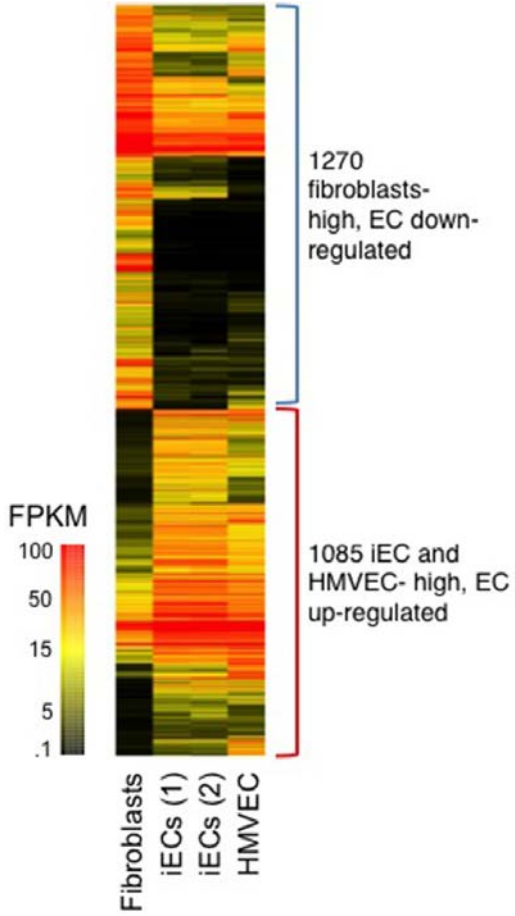

Figure 3A,B

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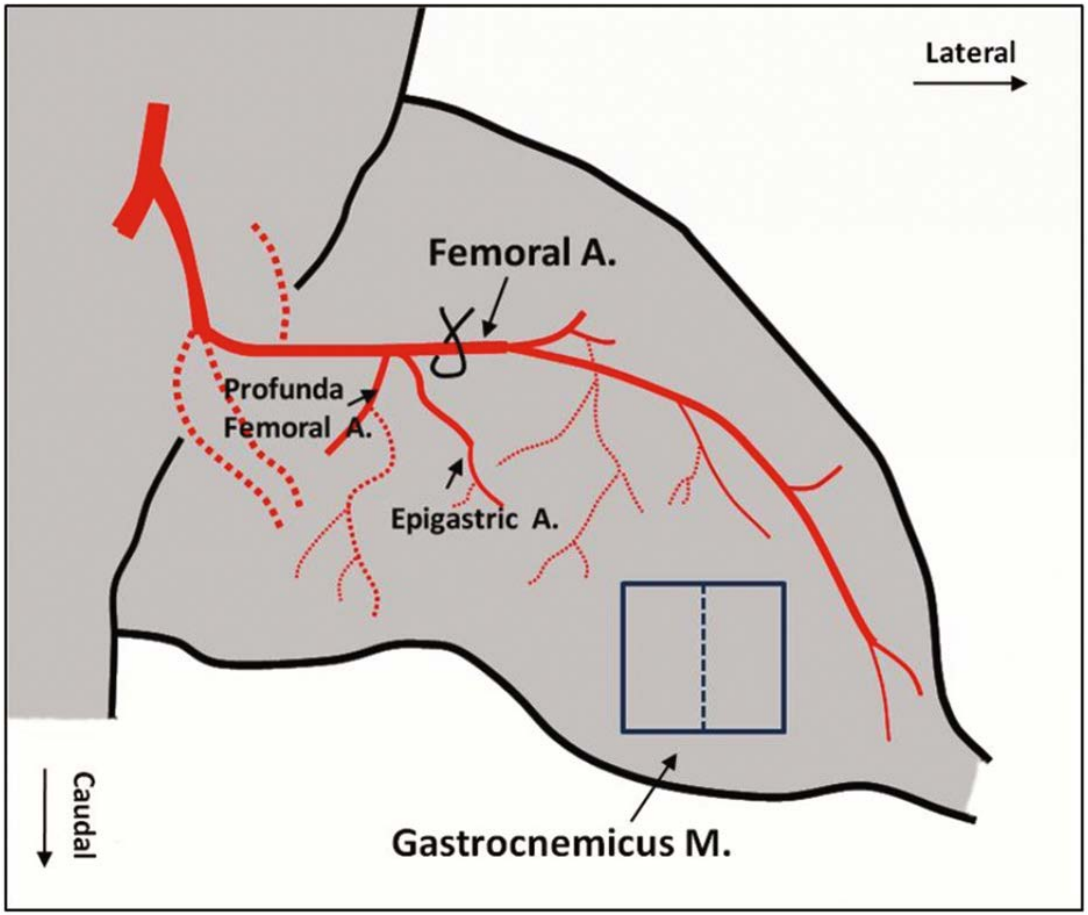
Therapeutic potential of iECs in a model of peripheral arterial disease



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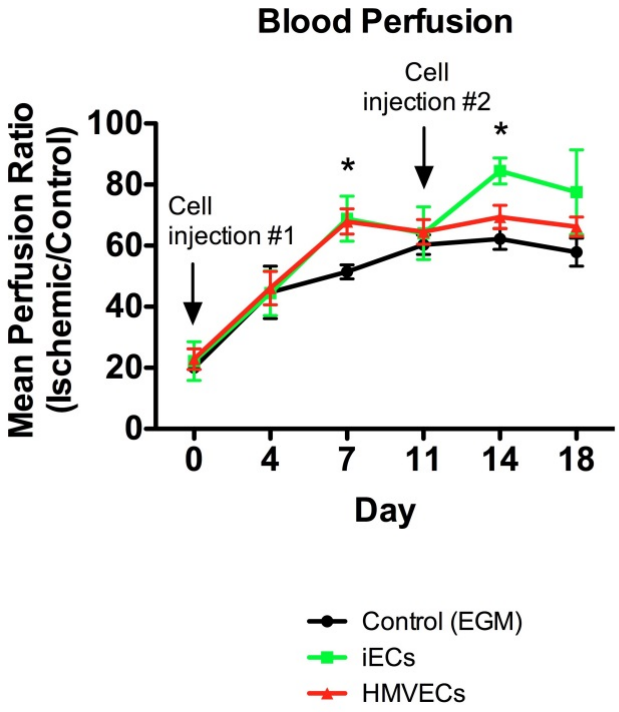
Therapeutic potential of iECs in a model of peripheral arterial disease

Tie2GFP mice tail-tip fibroblasts



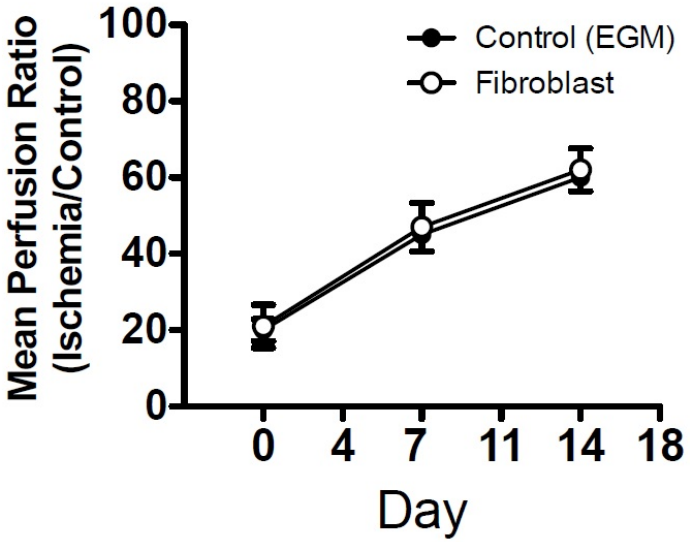


Figure S4A,B

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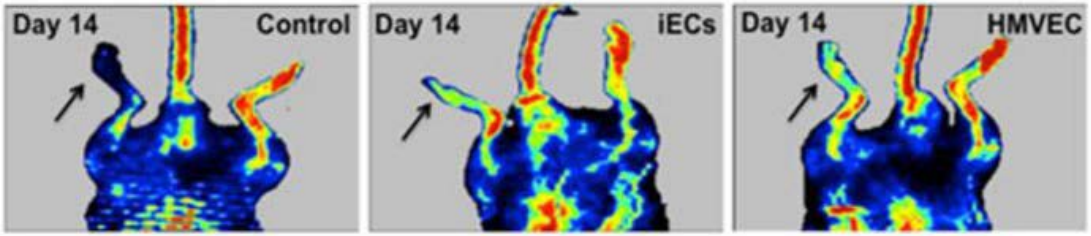
Therapeutic potential of iECs in a model of peripheral arterial disease

Tie2GFP mice tail-tip fibroblasts



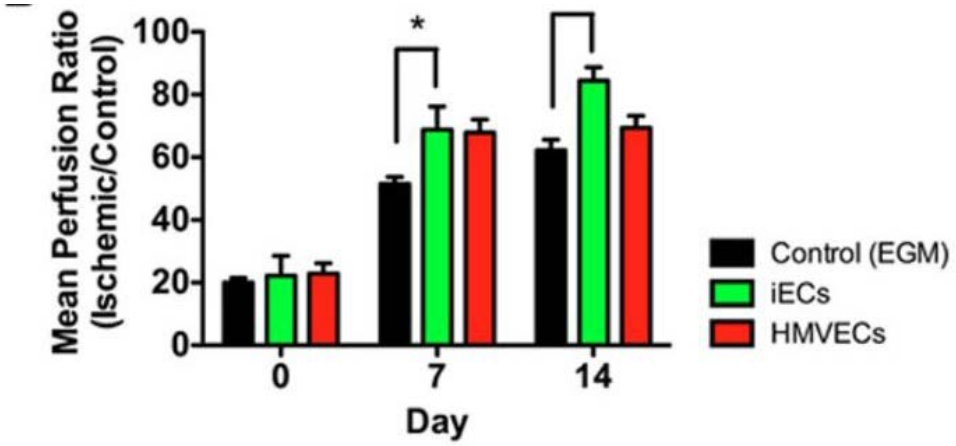


Figure 4A,B

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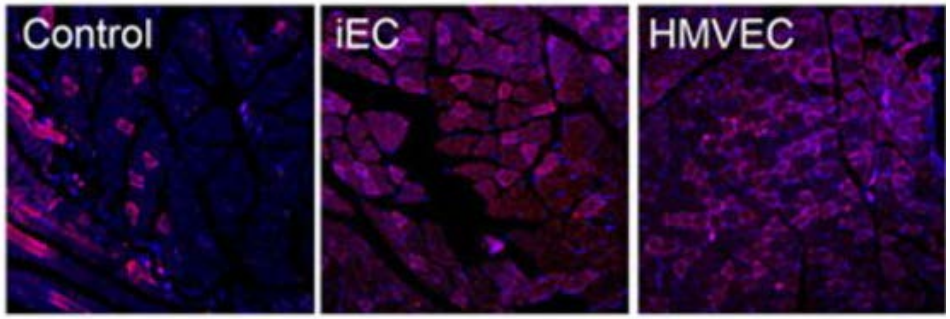
Therapeutic potential of iECs in a model of peripheral arterial disease

Tie2GFP mice tail-tip fibroblasts



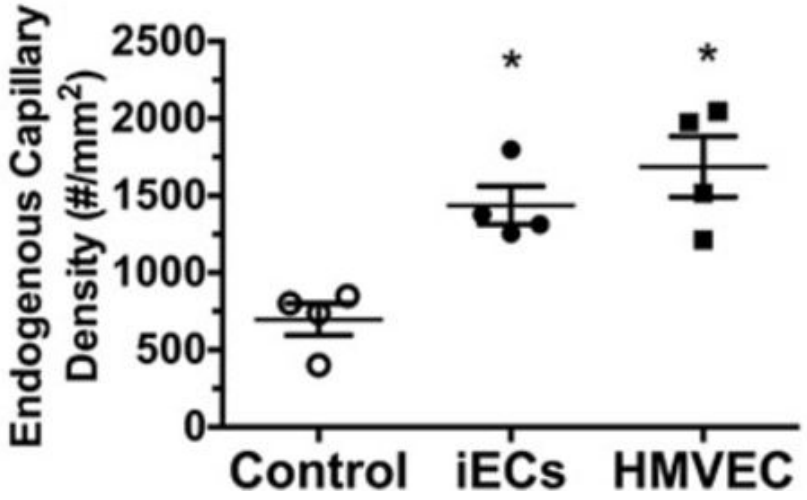


Figure 4C,D

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Therapeutic potential of iECs in a model of peripheral arterial disease

Tie2GFP mice tail-tip fibroblasts

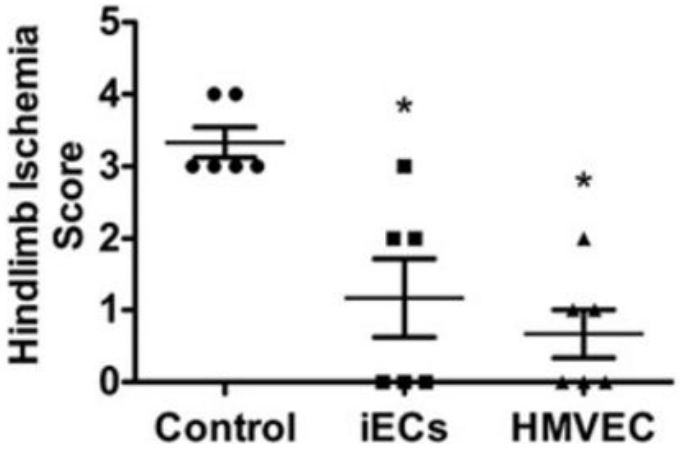

Figure 4E,F

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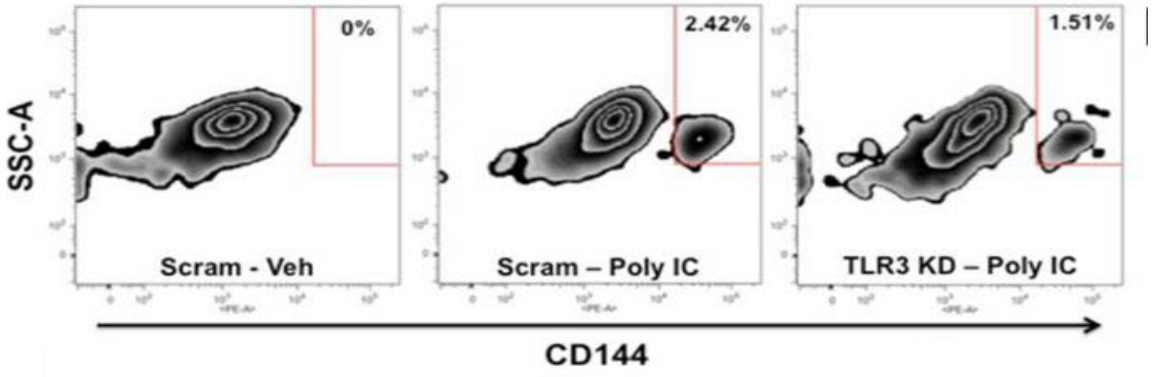
Innate immunity enables efficient transdifferentiation of fibroblasts to iECs

TLR3 knock-down mice tail-tip fibroblasts



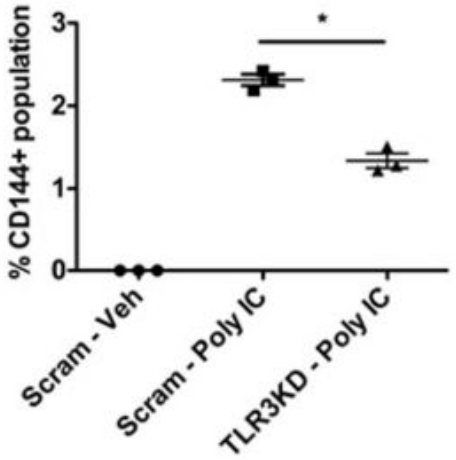


Figure 5A,B

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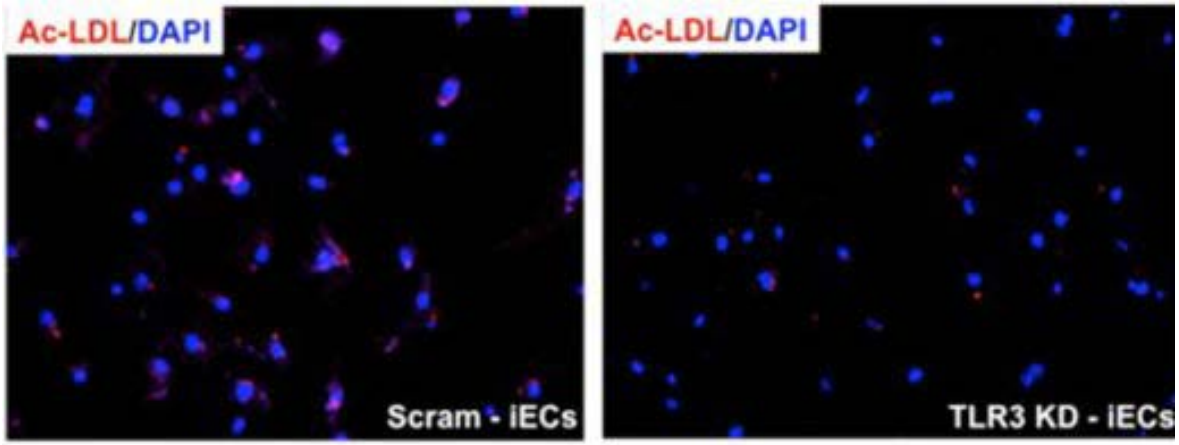
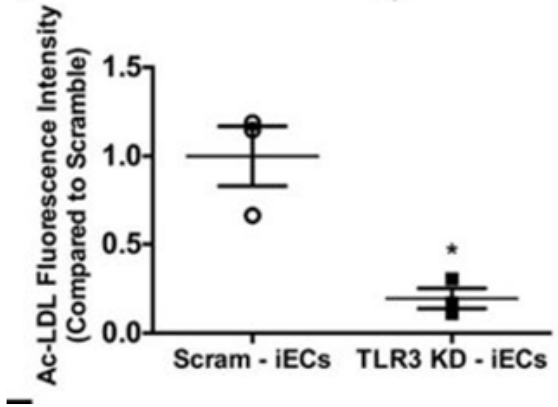
Reduced Capacity to Incorporate Ac-LDL

TLR3 knock-down mice tail-tip fibroblasts



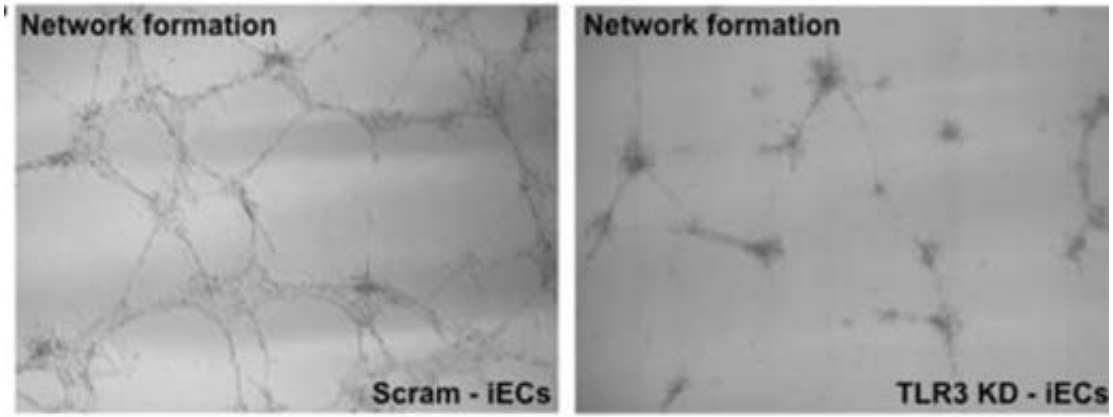


acetylated-LDL fluorescent intensity

Figure 5C,D

Failure to Form Capillary-like Networks

TLR3 knock-down mice tail-tip fibroblasts



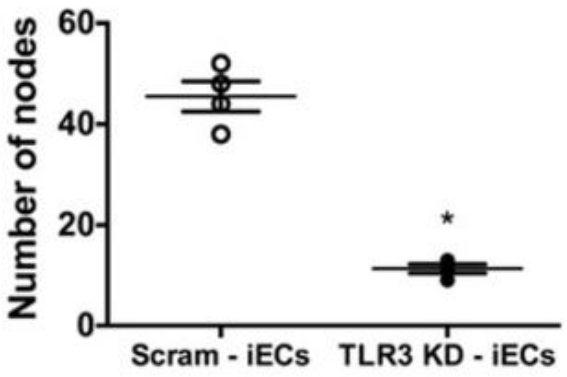


Figure 5E,F

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Changed Gene & Protein Expression

TLR3 knock-down mice tail-tip fibroblasts

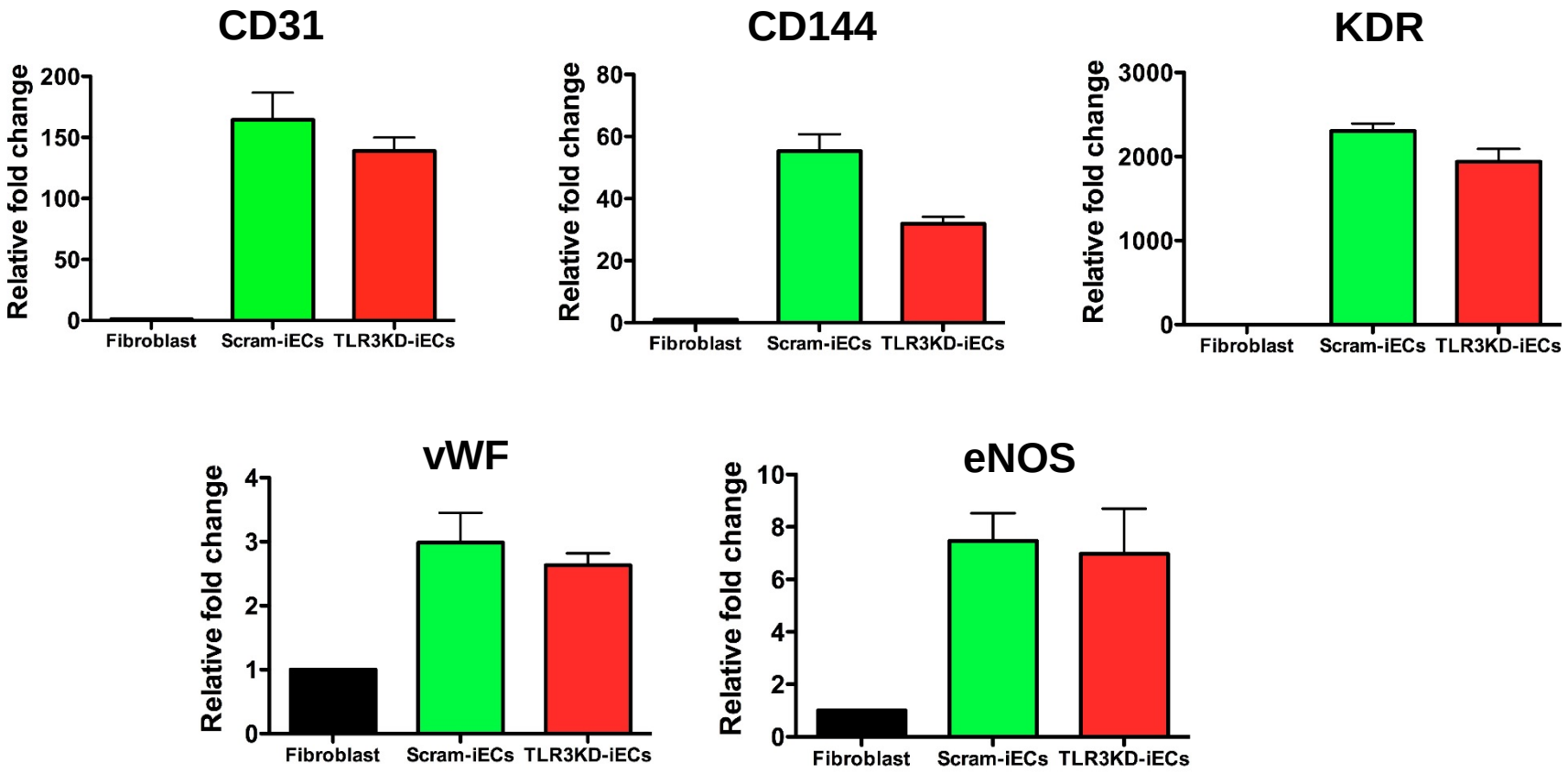

Figure S5A-E

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Changed Gene & Protein Expression

TLR3 knock-down mice tail-tip fibroblasts

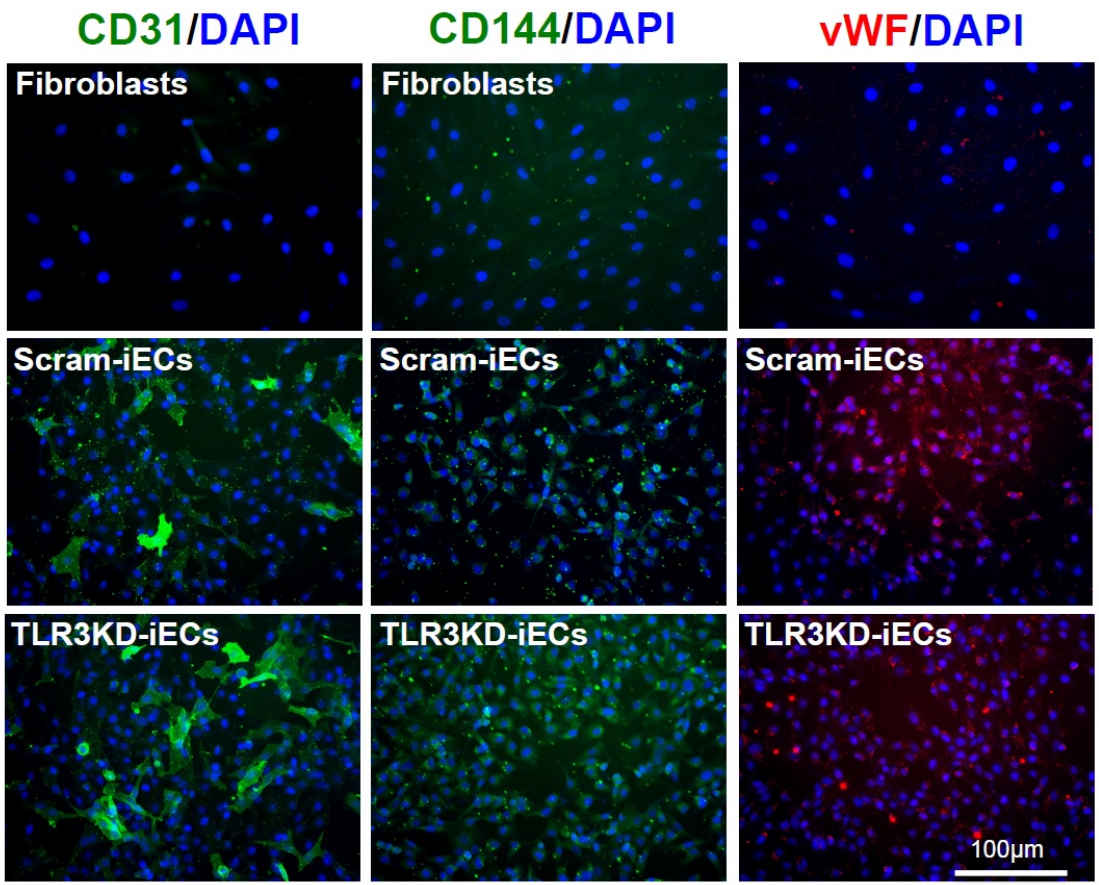

Figure S5F

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Heat Map of Genes Differential Expression in iECs

TLR3 knock-down mice tail-tip fibroblasts

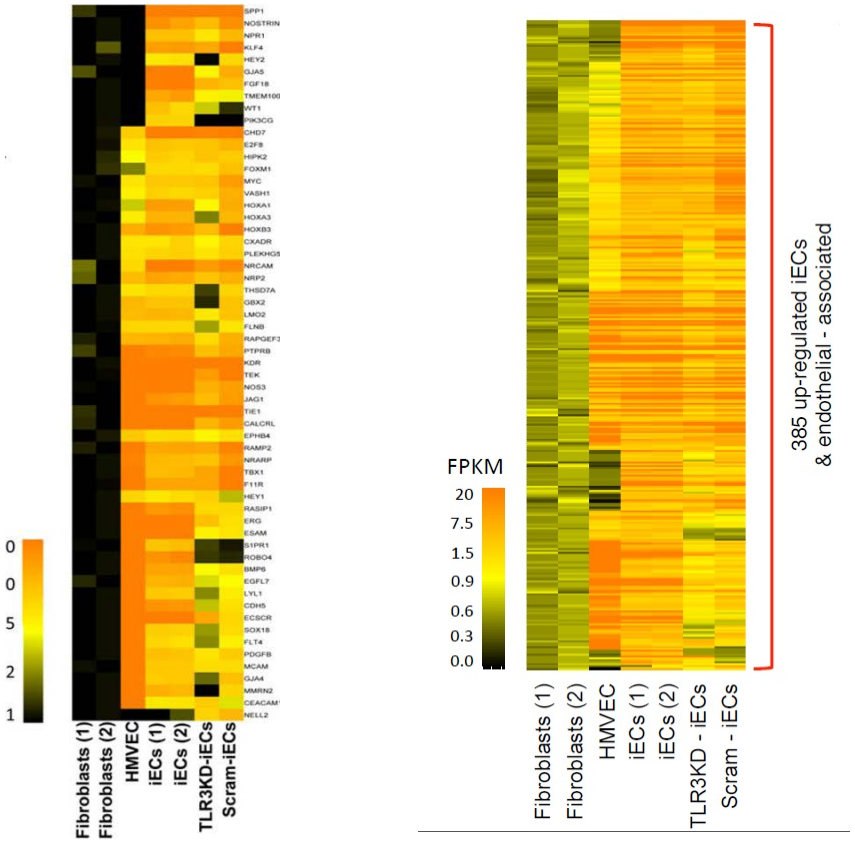

Figure 5G, S5G

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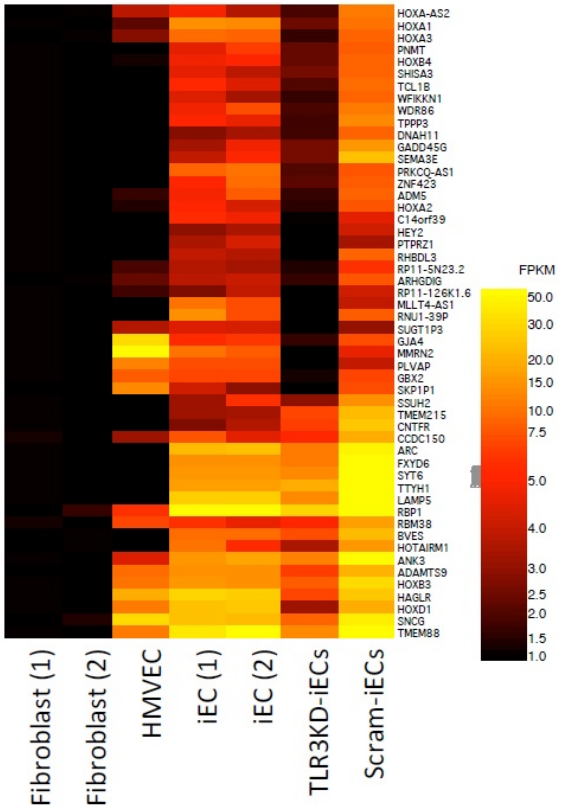
Heat Map of Genes Differential Expression in iECs

TLR3 knock-down mice tail-tip fibroblasts



Angiogenesis essential genes activated in iECs:

GJA4
HOXA1
HOXA3
HOXB3
HEY2
GBX2

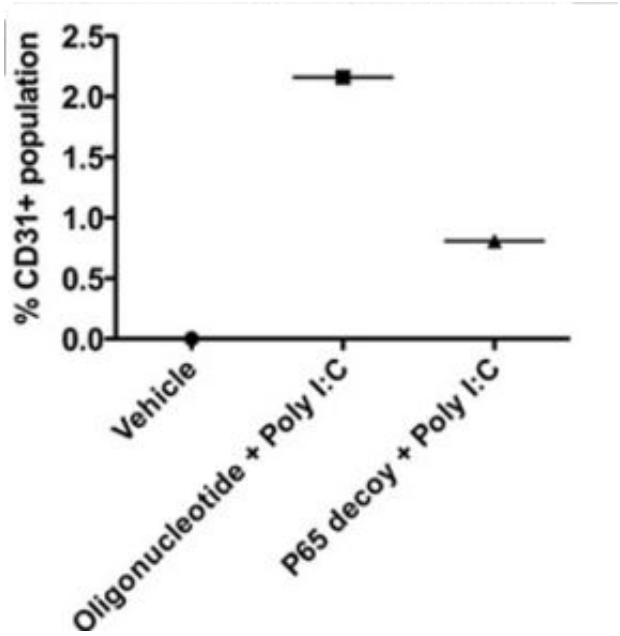


Figure S5H, 5H

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Reduced CD144+ Expression

TLR3 knock-out mice tail-tip fibroblasts

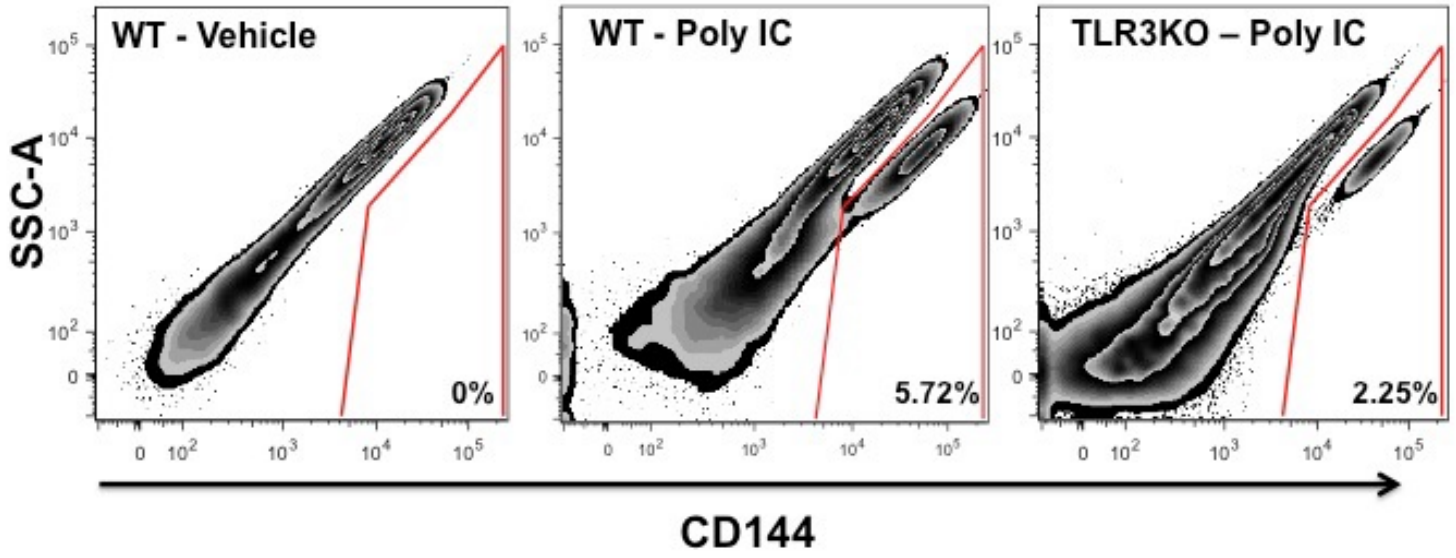

Figure S6

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Summary

- TLR3 agonist and endothelial growth factors is sufficient to transdifferentiate human fibroblasts to iECs
- Similarity to human microvascular endothelial cells:
  - Morphologically, immunohistochemically and transcriptional profile
- Administration of iECs improved perfusion and reduced tissue injury in ischemic hindlimb
- Absence of Poly I:C and ECF did not differentiate the fibroblasts into iECs
- TLR3 or NFKB knockdown each reduced the generation of iECs using this protocol

Thank you for your attention!

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

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Trans-differentiation of Human Fibroblasts to Endothelial Cells: Role of Innate Immunity

Nazish Sayed, Wing Tak Wong, Frank Ospino, Shu Meng, Jieun Lee, Arshi Jha, Philip Dexheimer, Bruce J. Aronow and John P. Cooke

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American Heart Association.