

Angio-vasculogenic potential of CD31⁺ cells

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Stem cells for the treatment of various ischemic organs

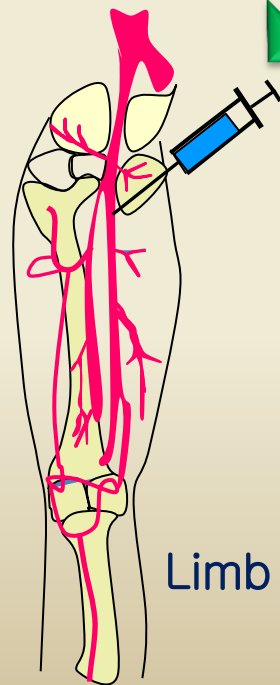
Stem or progenitor cells



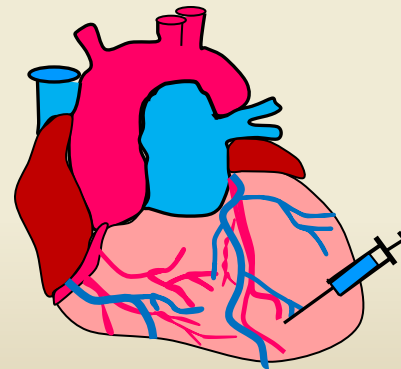
Cell therapy



Ischemic organ

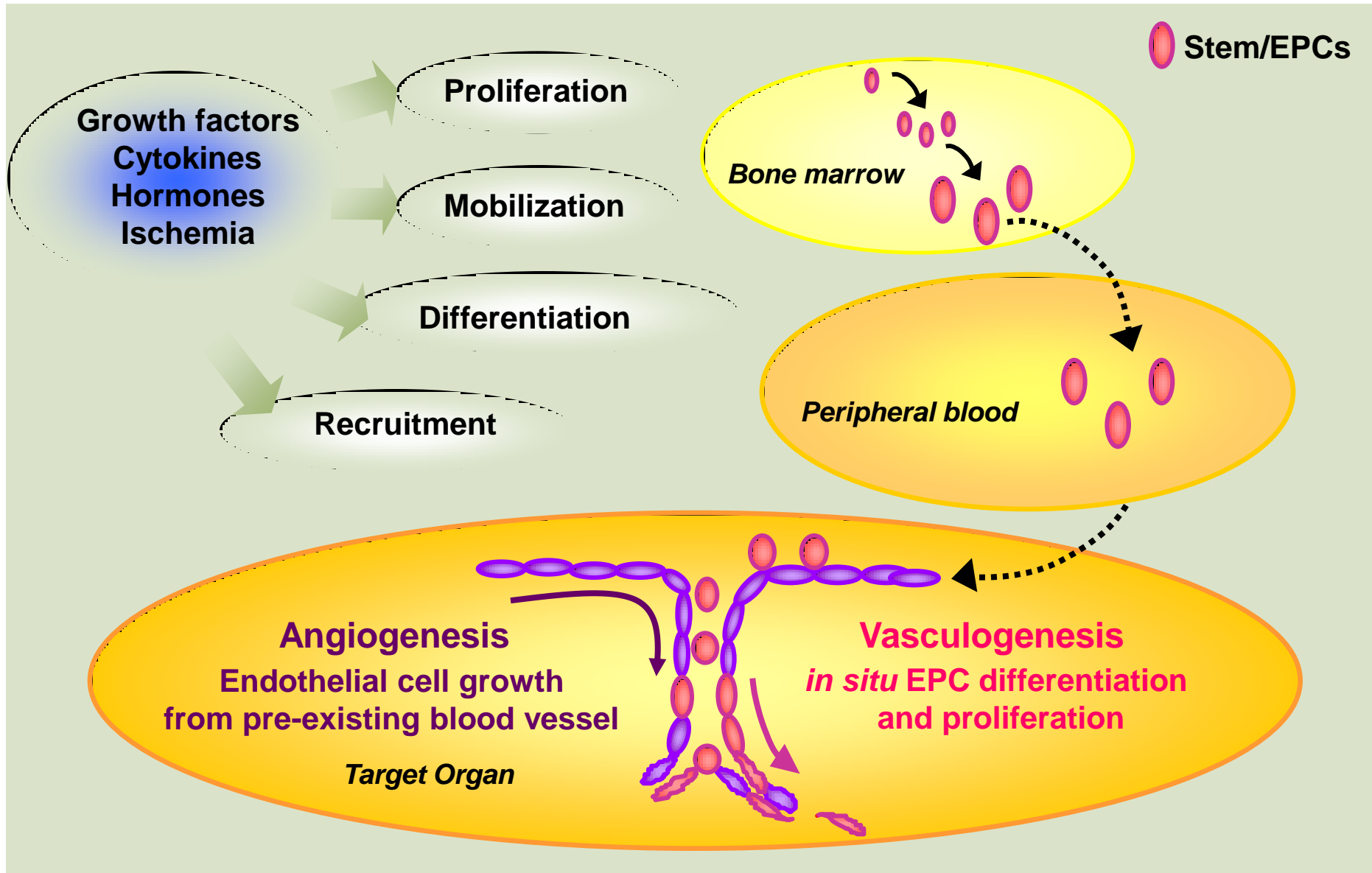


Limb



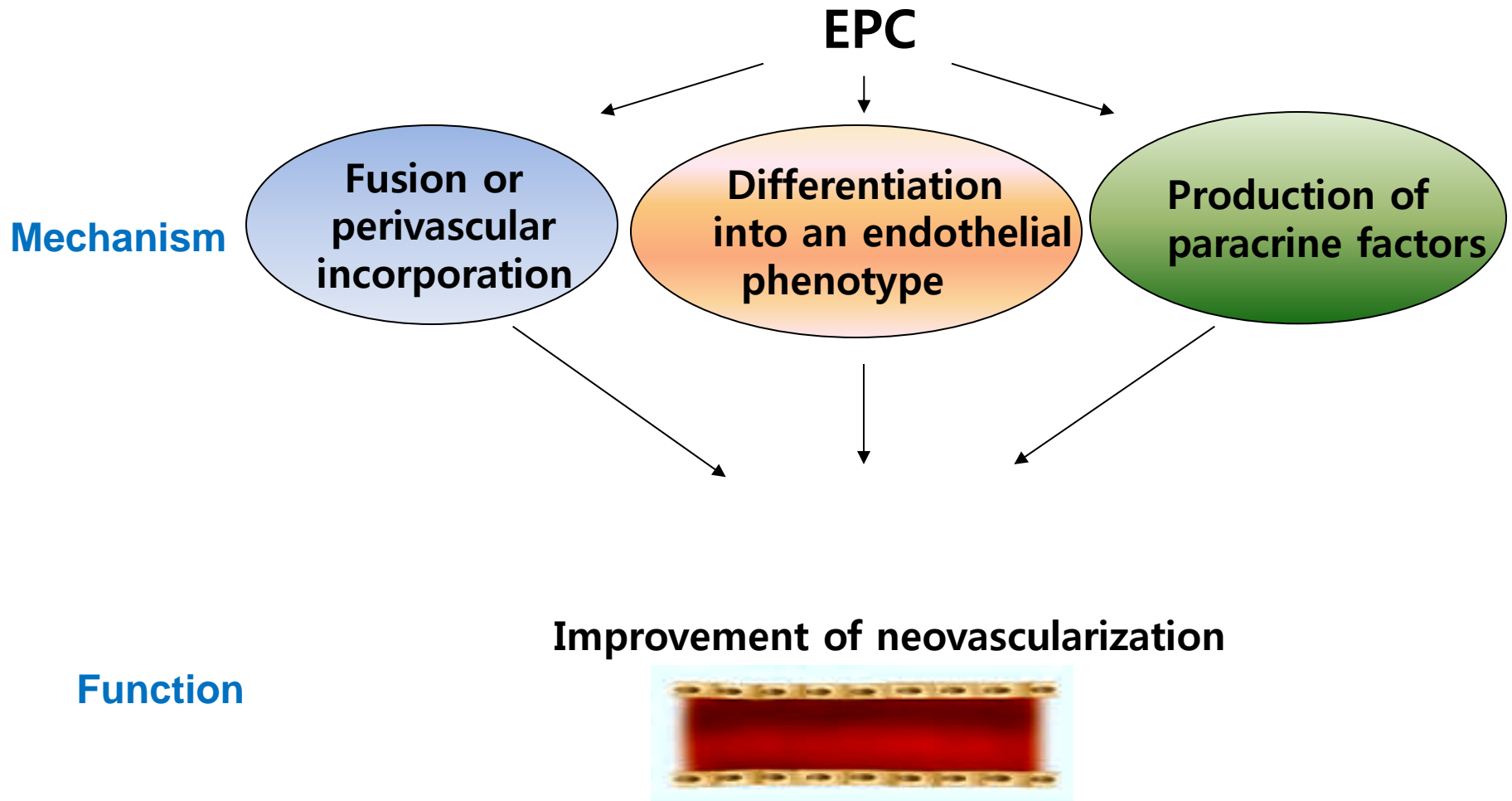
Myocardium

Neovascularization in Adult Mammals



(Asahara, Science 1997, Circ Res 1999)

How these EPC enhance neovascularization?



Ongoing Issues with BM Cells

1. Severe controversy exists regarding the **transdifferentiation of BM-derived cells**
: does **vasculogenesis** really occur in vivo?

Ziegelhoff *et al.* Circ 2004

2. Therapeutic effects of EPCs or other BM cells are predominantly attributed to **paracrine mechanism** than vasculogenic effects

Rehman *et al.* Circ 2003

Gnecchi *et al.* Nature Med 2005

Kinnaird *et al.* Cir Res 2005

Cho *et al.* JEM 2007

3. Lack of circulating stem/progenitors (CD34+, CD133+, KDR+, c-kit +) in PB or BM
Definition of 'true EPCs' is now controversial.

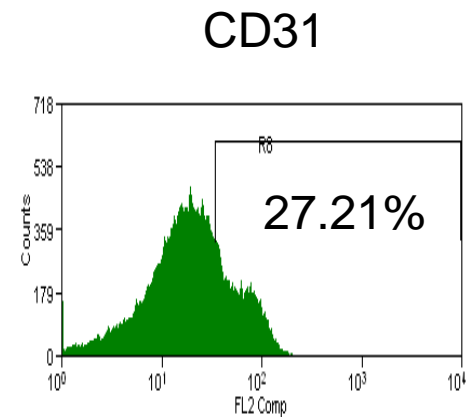
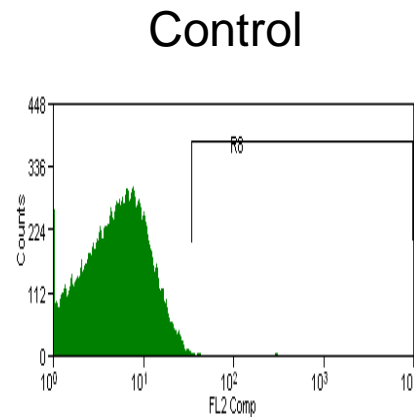
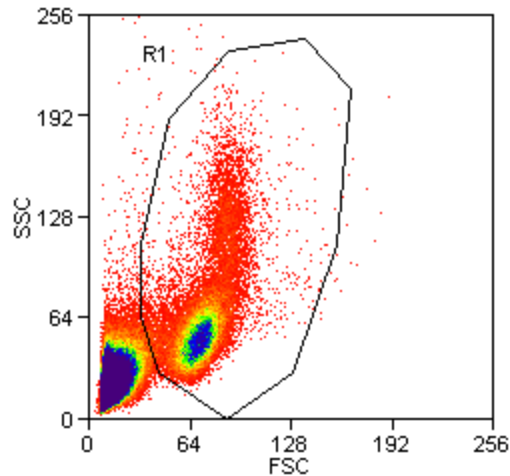
Ingram *et al.* Blood 2005

There is no specific marker(s) to prospectively isolate genuine EPCs

- : Is there any surface marker that can encompass cells having high angiogenic and vasculogenic activities?

CD31 expression in human PB cells

: About **30%** of total **murine BM** and **human PB, BM-MNC** express CD31

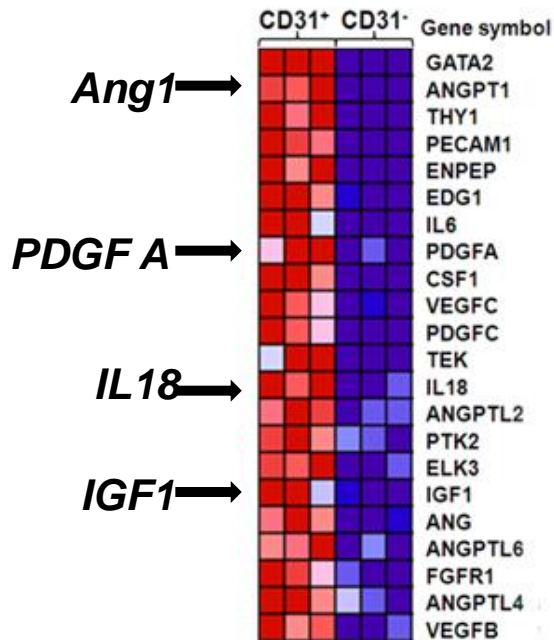


Human peripheral blood (PB) mononuclear cells

CD31 and Angiogenic Potential

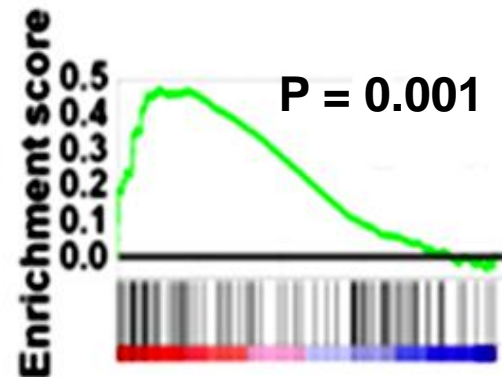
- Murine BM-CD31+ cells showed higher angiogenic properties than the CD31- cells

[Proangiogenic Gene Set]



Gene Set Enrichment Analysis (GSEA)

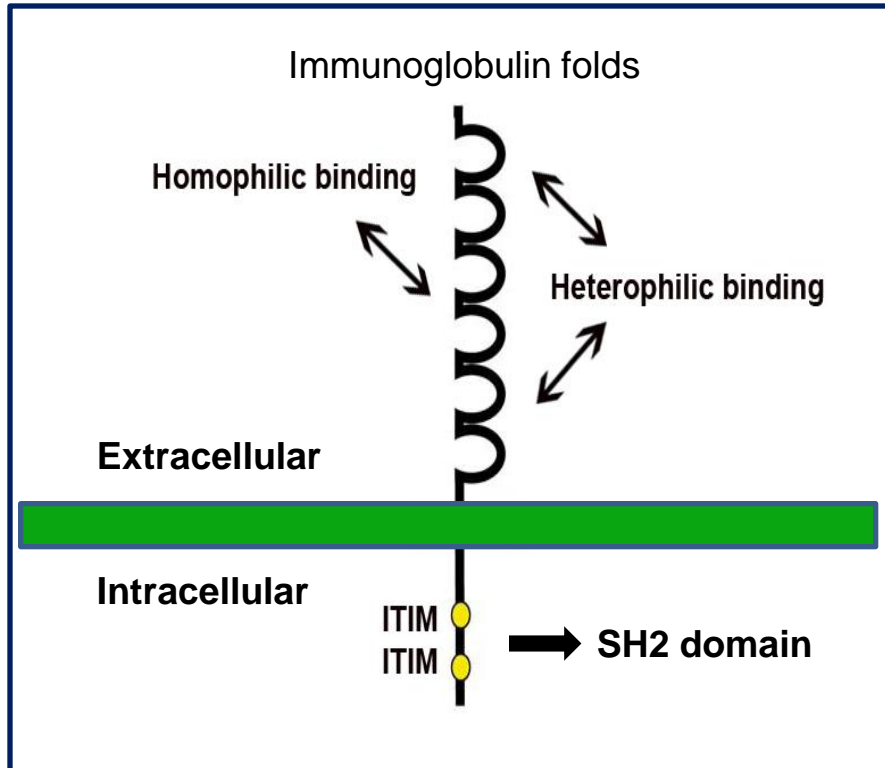
CD31+ cells vs. CD31- cell



Proangiogenic Gene Set
; 113 genes

Structure and Function of CD31

; CD31(PECAM-1, Platelet-endothelial cell adhesion molecule-1)



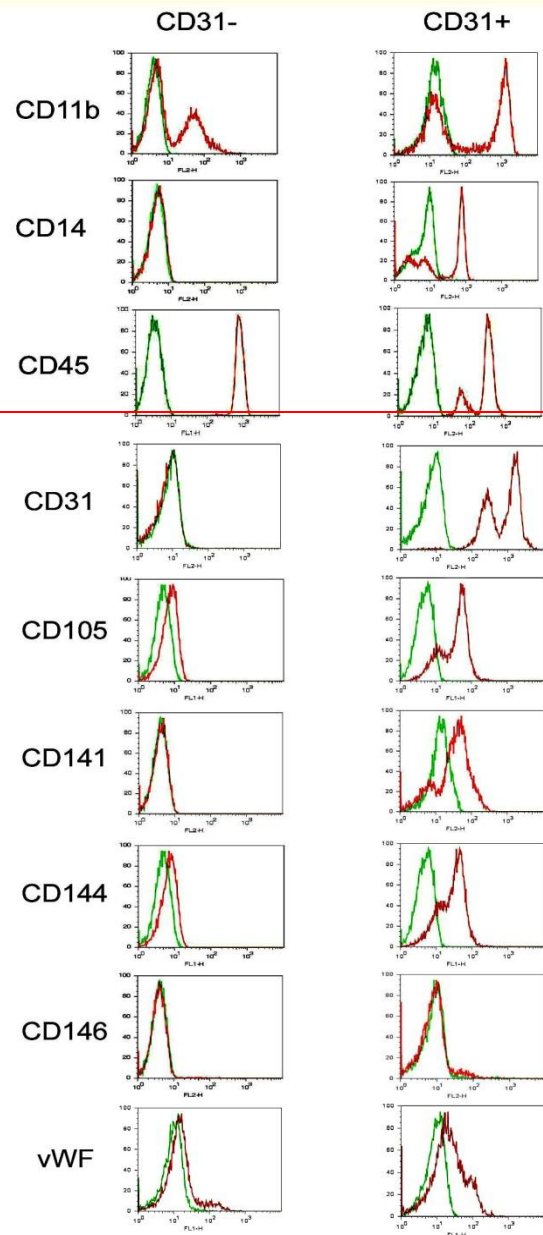
[CD31 structure]

- A member of Ig gene superfamily, 130 kDa type I transmembrane glycoprotein
- [Expression] Express in endothelial cells, naïve T, B cells, monocytes megakaryocytes, NK cells and platelet
- [Function] Regulation of platelet, leukocyte migration, integrin activation, cell survival, angiogenesis etc.

Study Aim

- **To investigate whether hPB-CD31⁺ cells have angio-vasculogenic property**

CD31⁺ cells highly express endothelial specific markers



**Endothelial cell
marker**

Green: isotype control

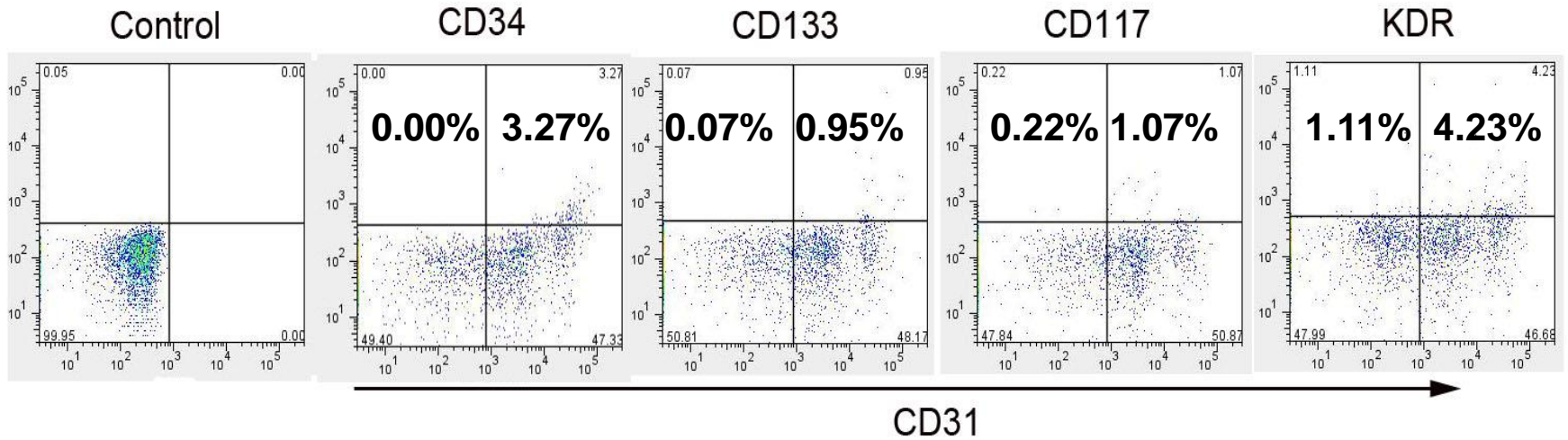
CD31+ cells significantly express monocyte, endothelial markers

	CD31+ cells	CD31- cells
CD3	42.2±6.6	56.5±8.9
CD11b	53.4±3.5	39.7±8.1
CD14	32.0±5.2**	0.2±0.1
CD19	14.1±4.9	7.0±1.9
CD45	99.4±0.4	99.5±0.4
CD31	94.5±3.4**	4.1±1.6
CD105	55.6±8.3**	6.6±2.3
CD141	29.6±4.7**	0.6±0.1
CD144	54.6±11.1*	6.2±3.6
CD146	0.5±0.3	0.8±0.5
vWF	36.3±12.1*	9.1±4.4

Endothelial cell
marker

(n=3 each, *P<0.05, **P<0.01)

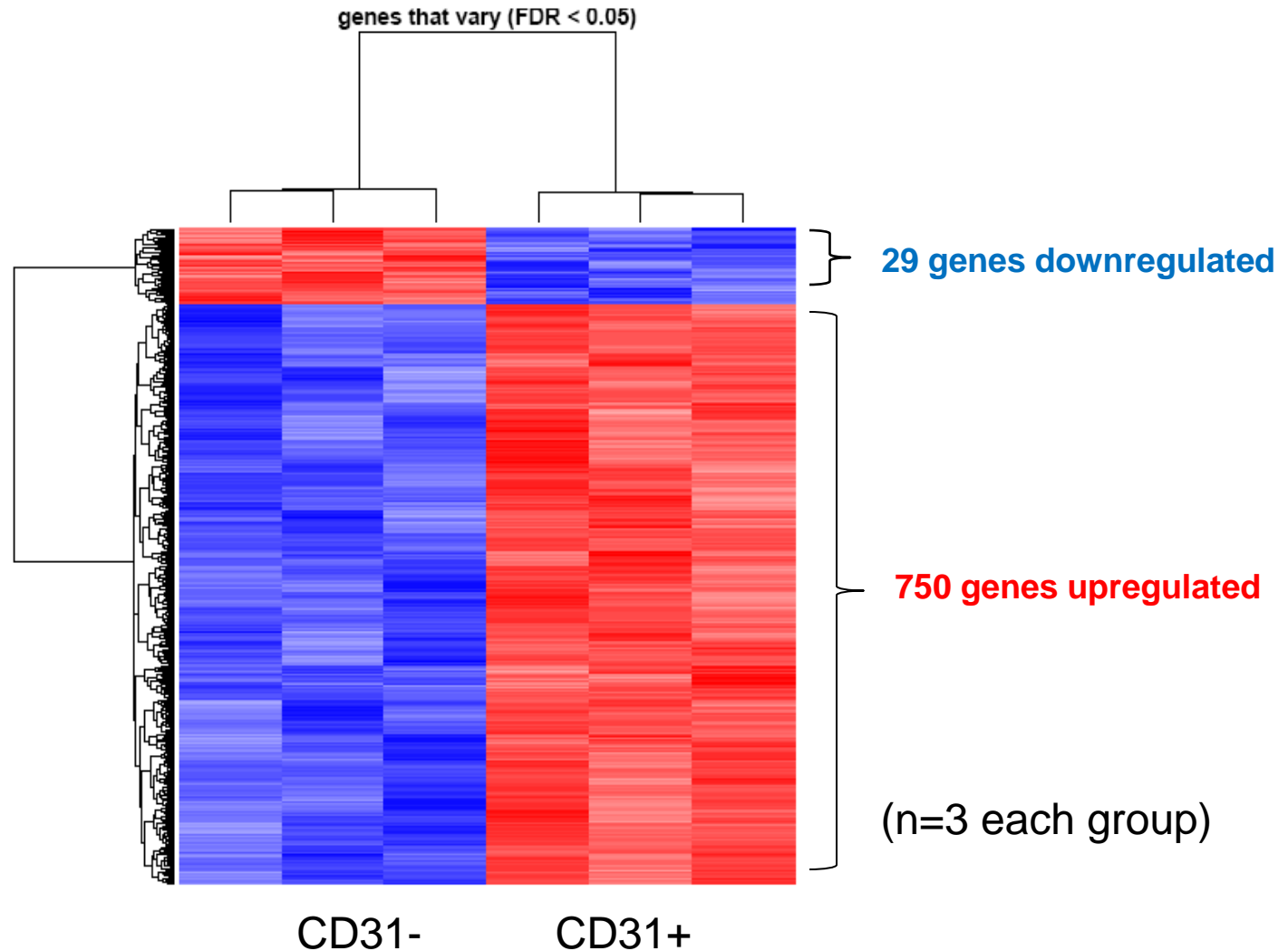
CD31⁺ cells significantly express stem cells markers



	CD31 ⁺ cells	CD31 ⁻ cells
CD34	3.3±0.2^{**}	0.2±0.05
CD133	2.4±0.4[*]	0.7±0.2
CD117	3.1±1.2[*]	1.2±0.5
KDR	7.4±3.1[*]	3.9±2.7

(n=4 each, *P<0.05, **P<0.01)

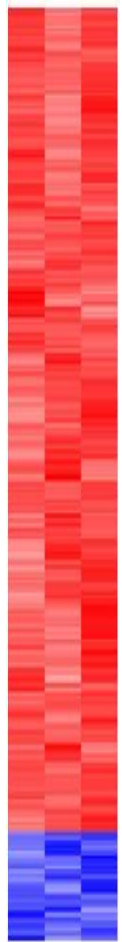
Microarray analysis of human PB CD31⁺ and CD31⁻ cells



Human gene 1.0 ST array: 33,265 genes

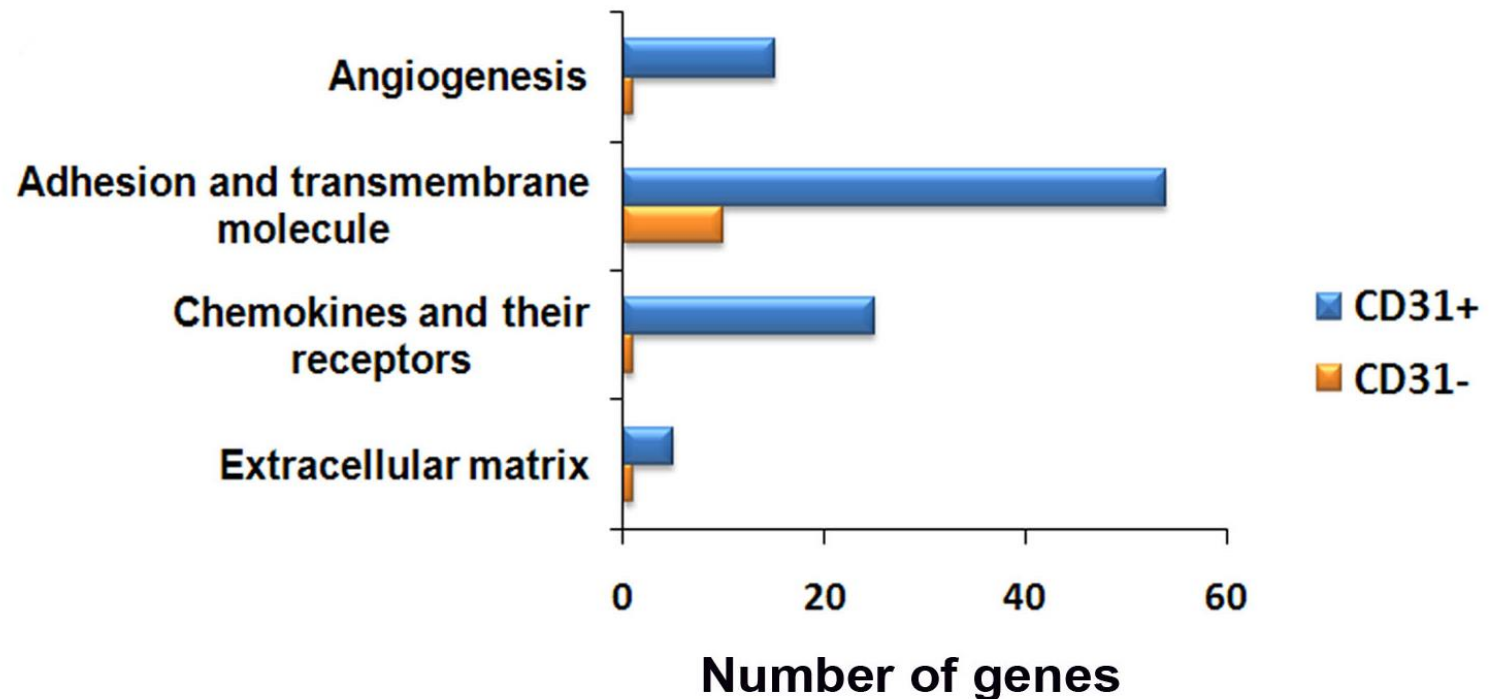
Kim et al. JACC 2010

CD31⁺ cells significantly express angiogenic genes



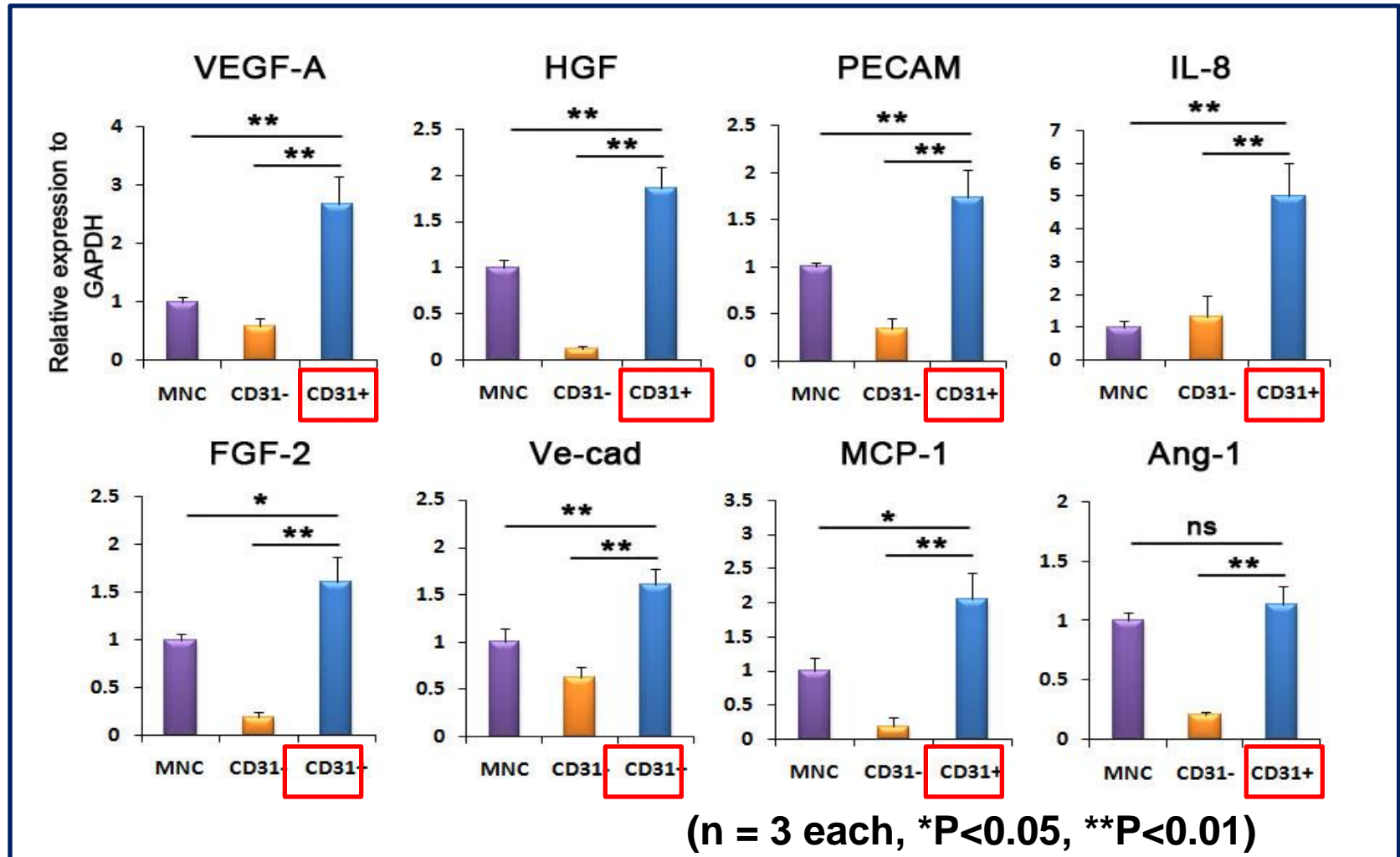
Gene Symbol	mRNA - Description	Fold change
NRP1	Neuropilin 1 (NRP1), transcript variant 1, mRNA.	11.8
ANPEP	Alanyl (membrane) aminopeptidase (ANPEP), mRNA.	6.6
IL8	Interleukin 8 (IL8), mRNA.	6.5
HGF	Hepatocyte growth factor (HGF), transcript variant 1, mRNA.	5.8
PDGFC	Platelet derived growth factor C (PDGFC), mRNA.	5.2
VEGFA	Vascular endothelial growth factor A (VEGFA), transcript variant 1, mRNA.	4.7
EDG3	Endothelial differentiation, sphingolipid G-protein-coupled receptor 3, mRNA.	4.2
PECAM1	Platelet/endothelial cell adhesion molecule (CD31 antigen) (PECAM1), mRNA.	3.9
FLT3	Fms-related tyrosine kinase 3 (FLT3), mRNA.	3.5
ANG	Angiogenin, ribonuclease, RNase A family, 5 (ANG), transcript variant 1, mRNA.	3.2
KLF5	Kruppel-like factor 5 (intestinal) (KLF5), mRNA.	3
ANGPT1	Angiopoietin 1 (ANGPT1), mRNA.	2.2
JAG1	Jagged 1 (Alagille syndrome) (JAG1), mRNA.	2.1
PDGFA	Platelet-derived growth factor alpha polypeptide (PDGFA), transcript variant 2, mRNA.	2
ENG	Endoglin (Osler-Rendu-Weber syndrome 1) (ENG), mRNA.	1.8
PDGFRB	Platelet-derived growth factor receptor, beta polypeptide (PDGFRB), mRNA.	-1.5
PDGFD	Platelet derived growth factor D (PDGFD), transcript variant 1, mRNA.	-2
FGFBP2	Fibroblast growth factor binding protein 2 (FGFBP2), mRNA.	-2.2

Microarray analysis of human PB CD31⁺ and CD31⁻ cells

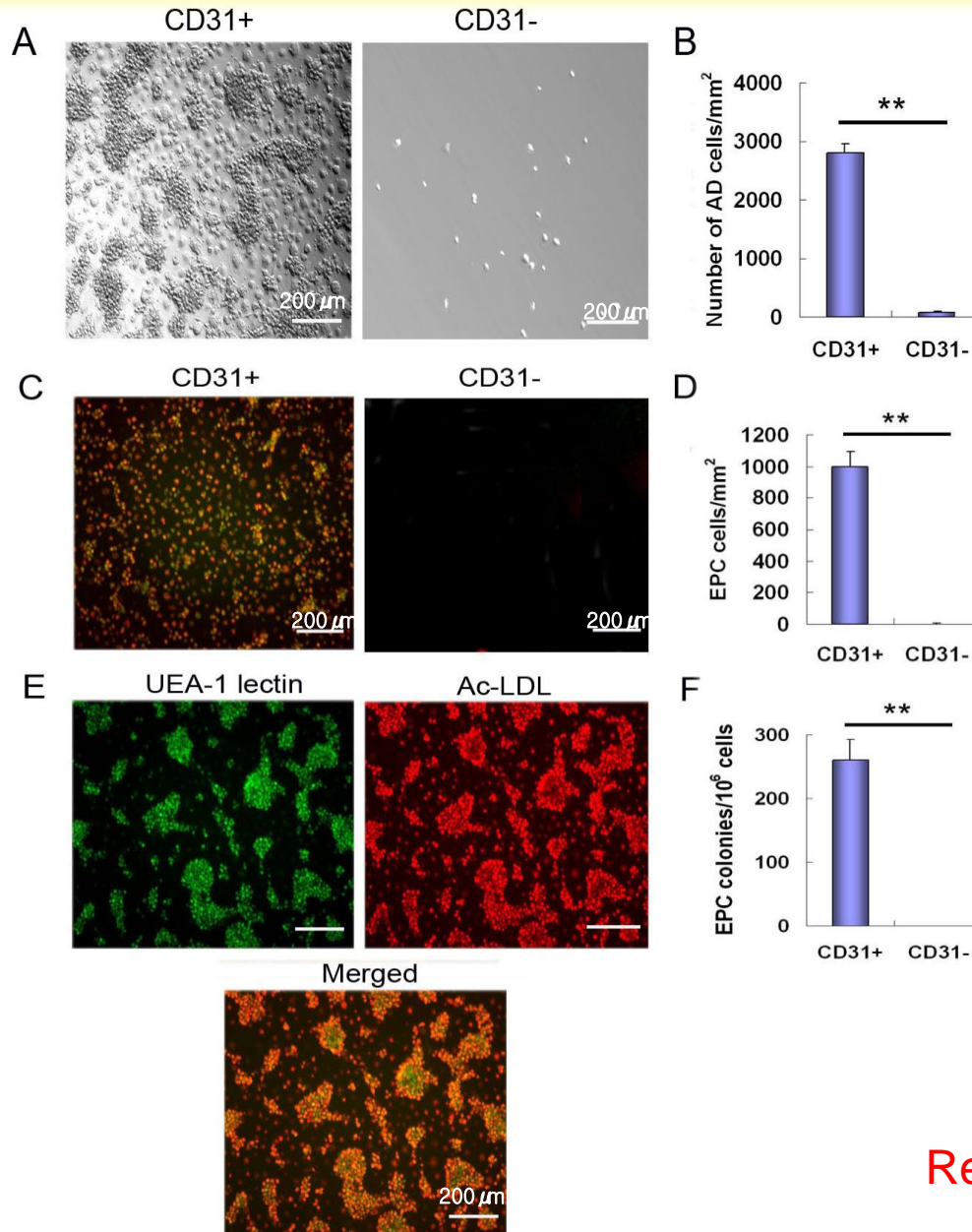


CD31⁺ cells significantly express multiple angiogenic genes

[qRT PCR]



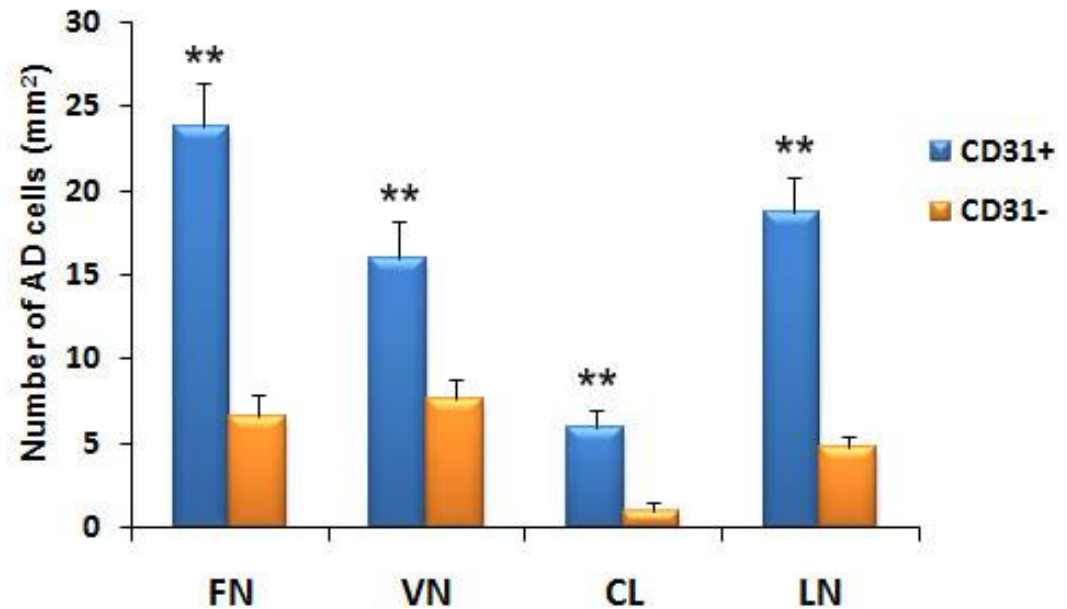
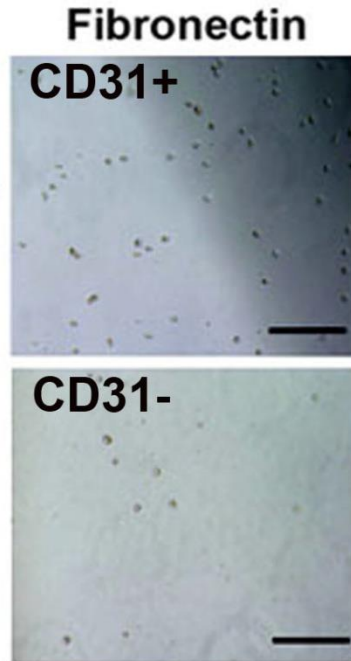
CD31⁺ cells gave rise to EPC



(n=5 each, **P<0.01)

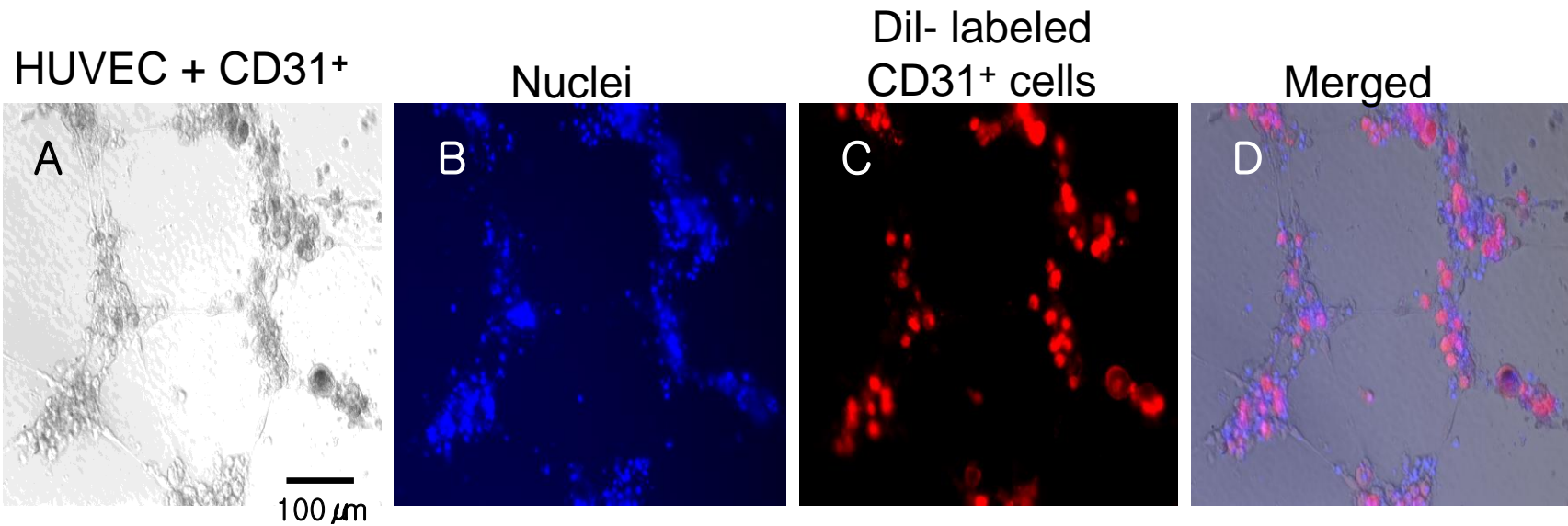
Red: acLDL, Green: UEA-lectin

CD31⁺ cells are significantly adherent

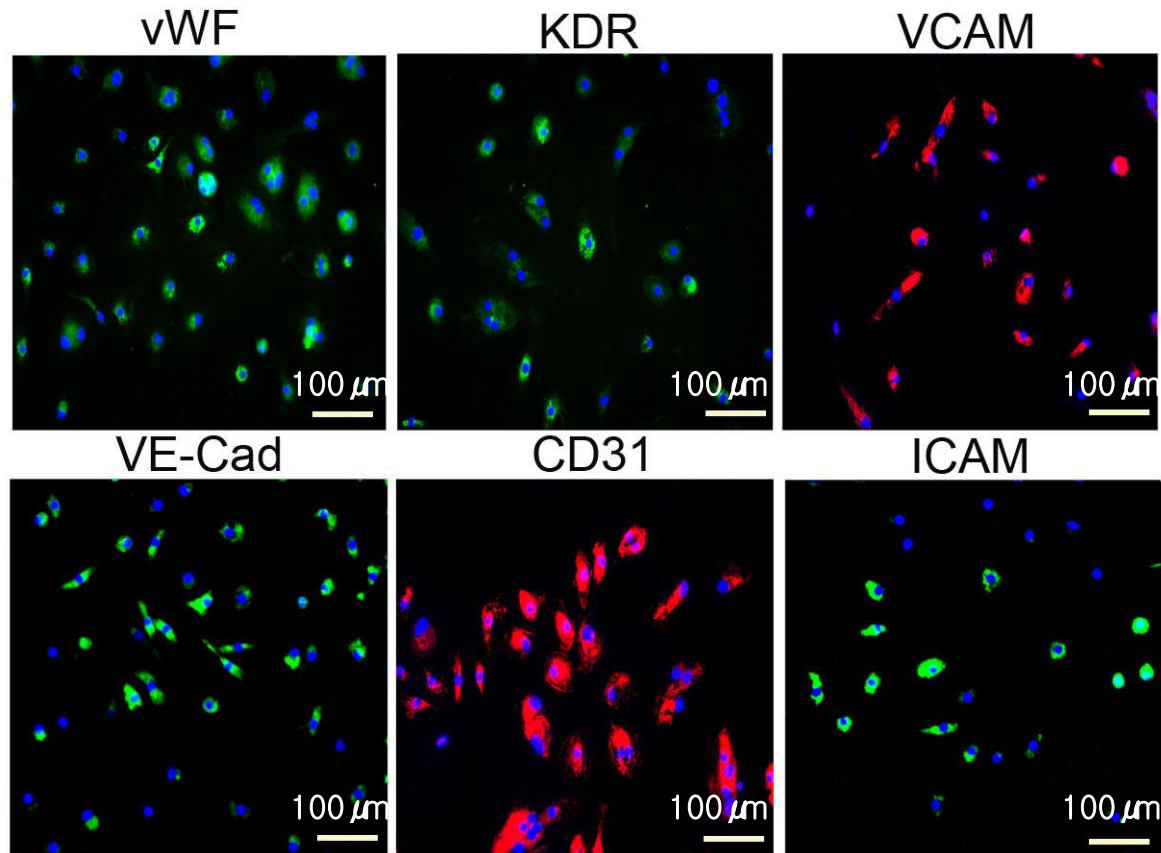


(n=3 each, **P<0.01)

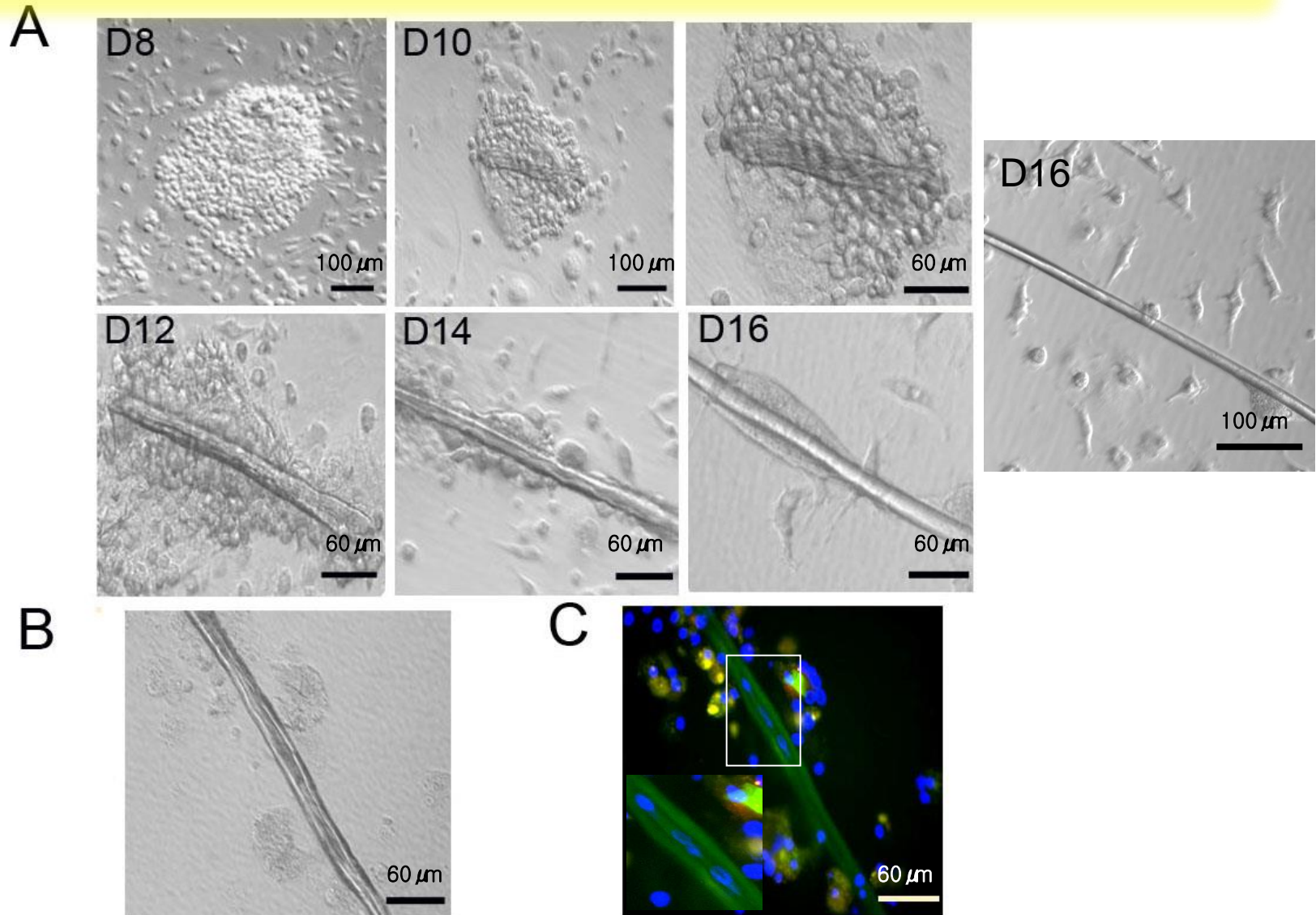
Incorporation of Dil-labeled CD31⁺ cells into the non-labeled HUVEC network (in vitro capillary network formation)



In vitro differentiation of CD31⁺ cells into endothelial lineages

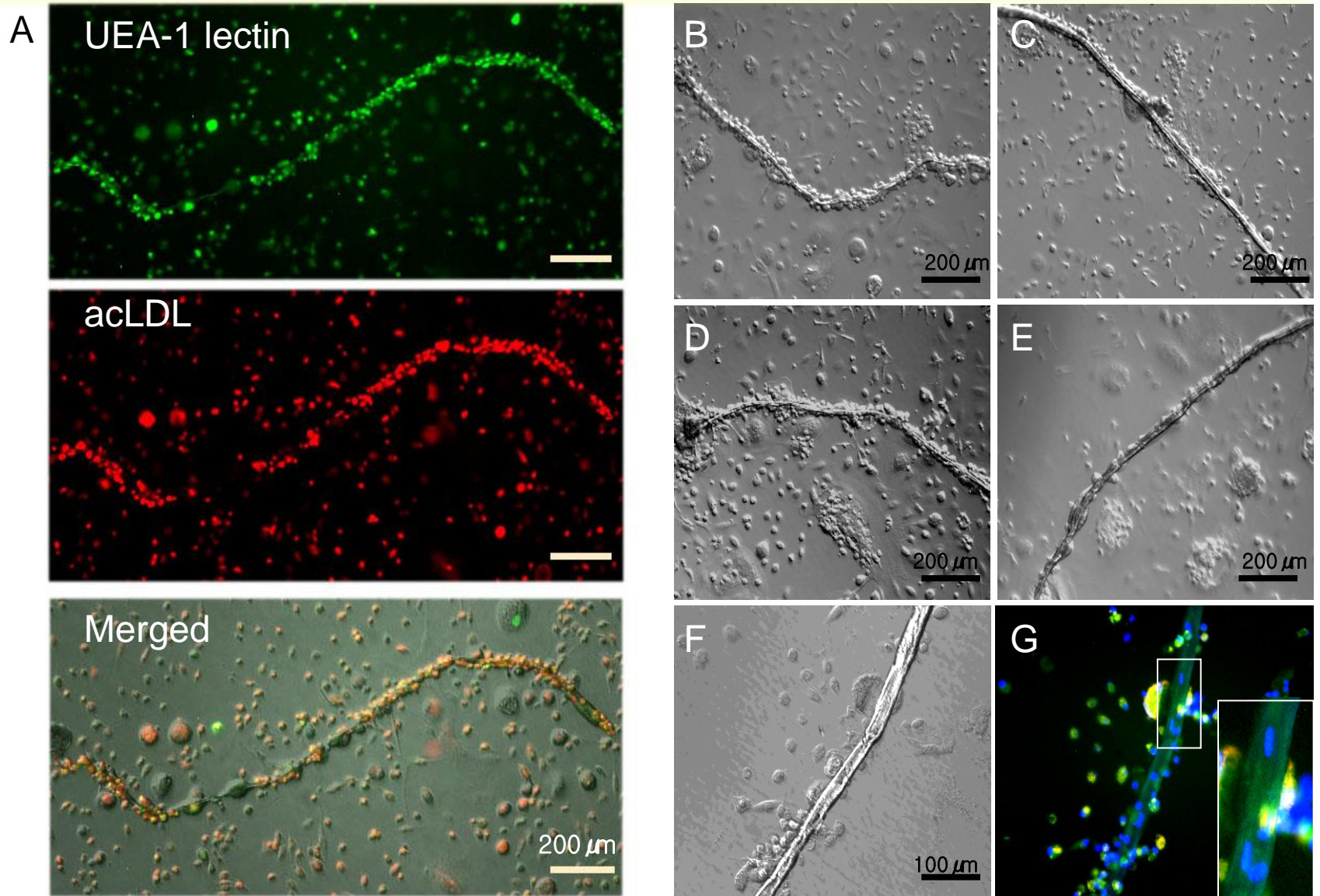


In vitro differentiation of CD31⁺ cells into vascular-like tube (EPC culture condition)

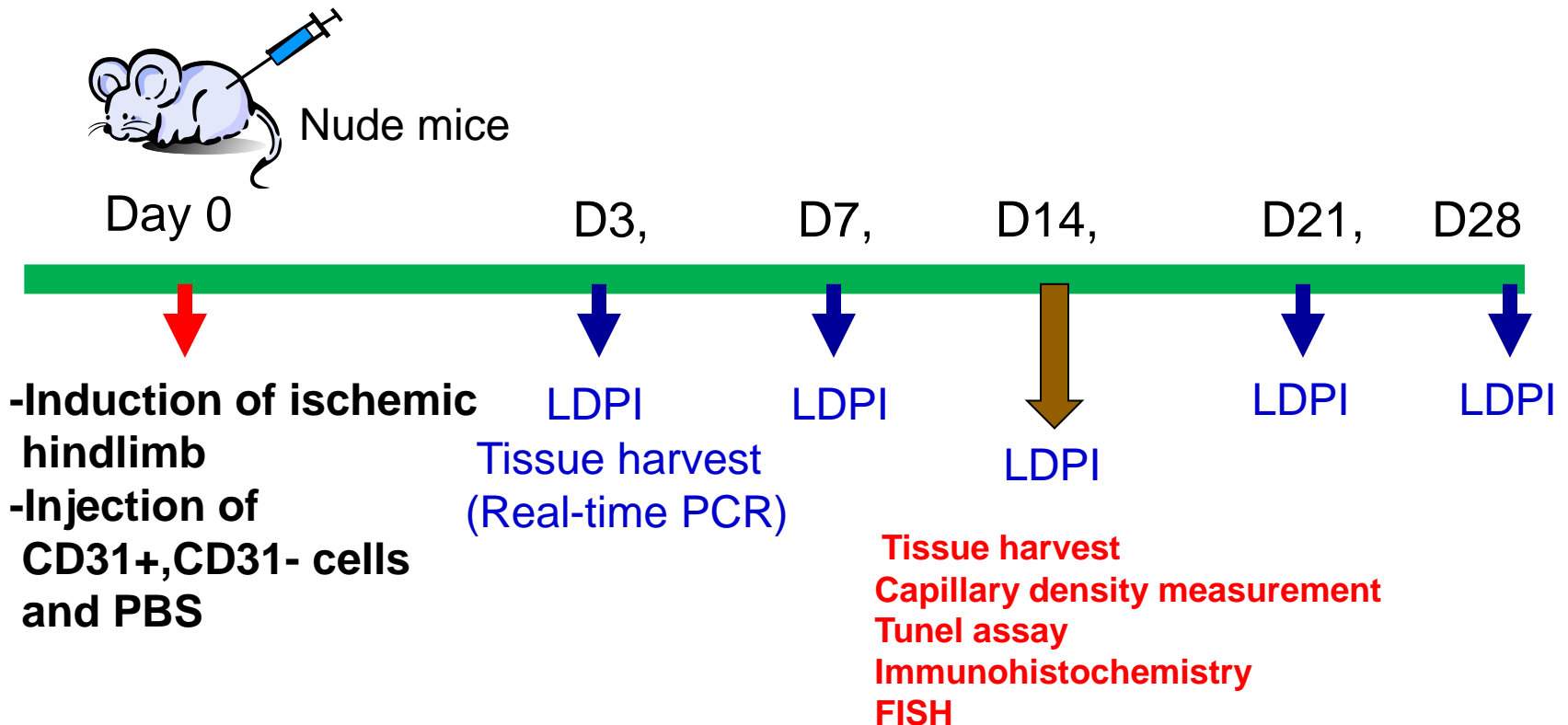
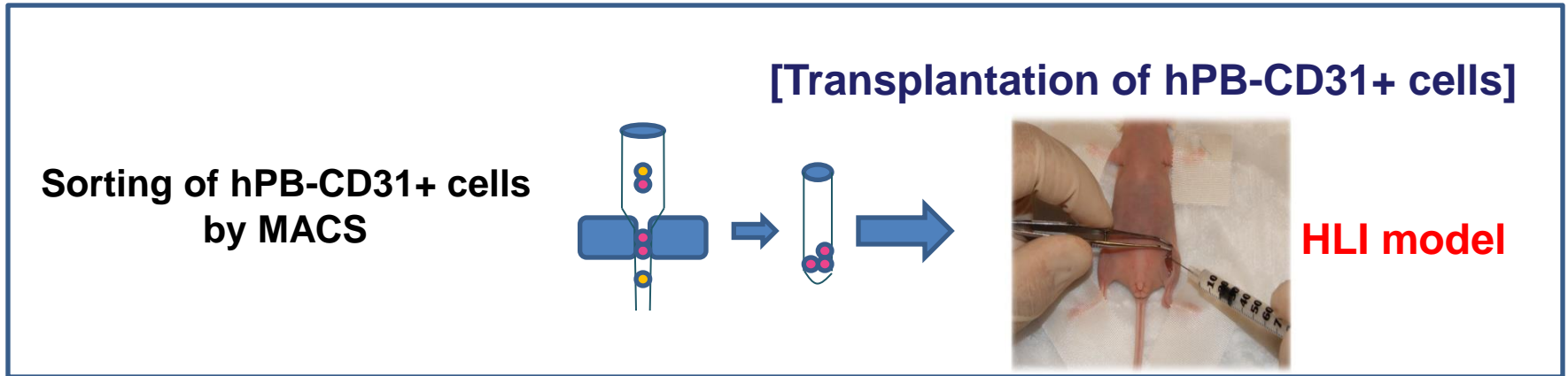


Red: acLDL, Green: UEA-lectin, Blue: DAPI

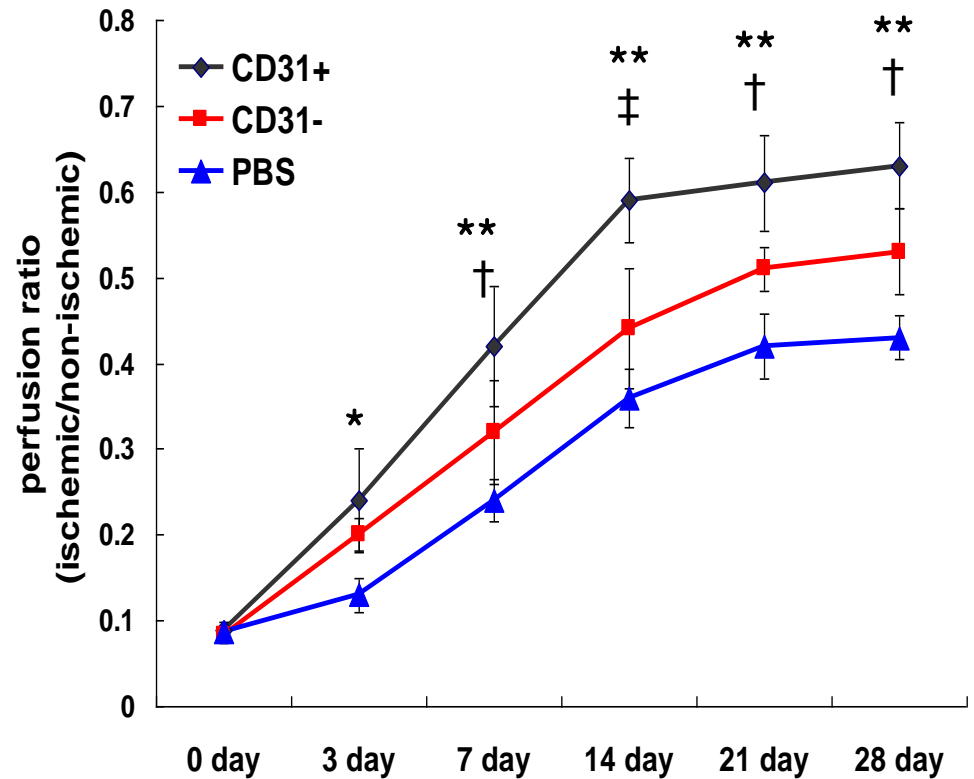
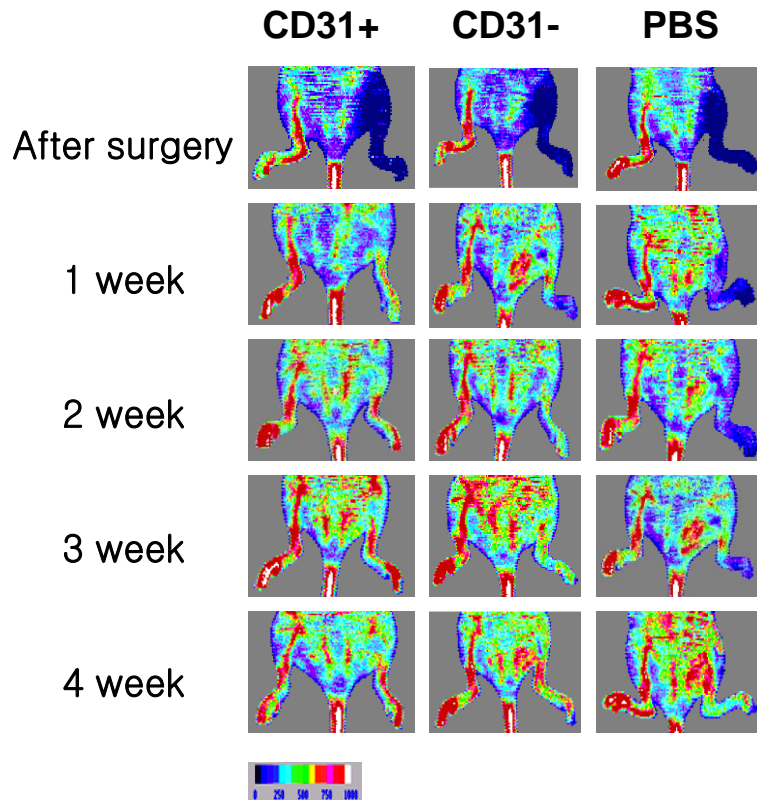
In vitro differentiation of CD31⁺ cells into vascular-like tube



Study design (in vivo experiment)



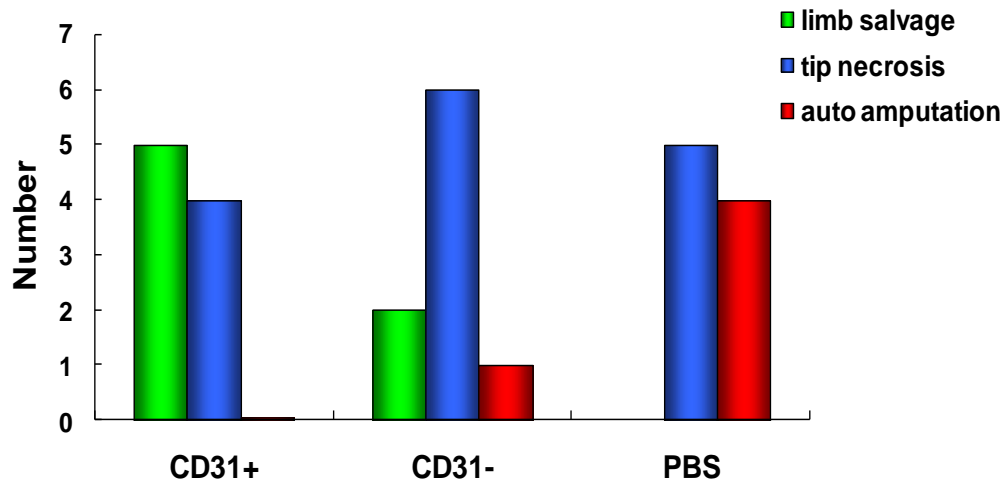
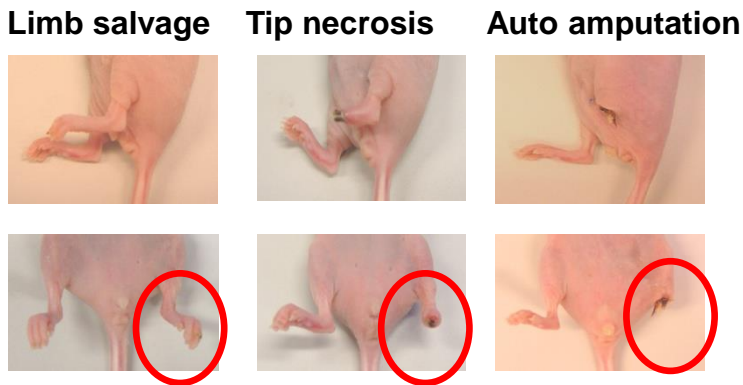
CD31⁺ cells transplantation increased blood perfusion



(n=9 each group)

*P<0.05, **P<0.01 vs PBS; † P<0.05, ‡ P<0.01 vs CD31-

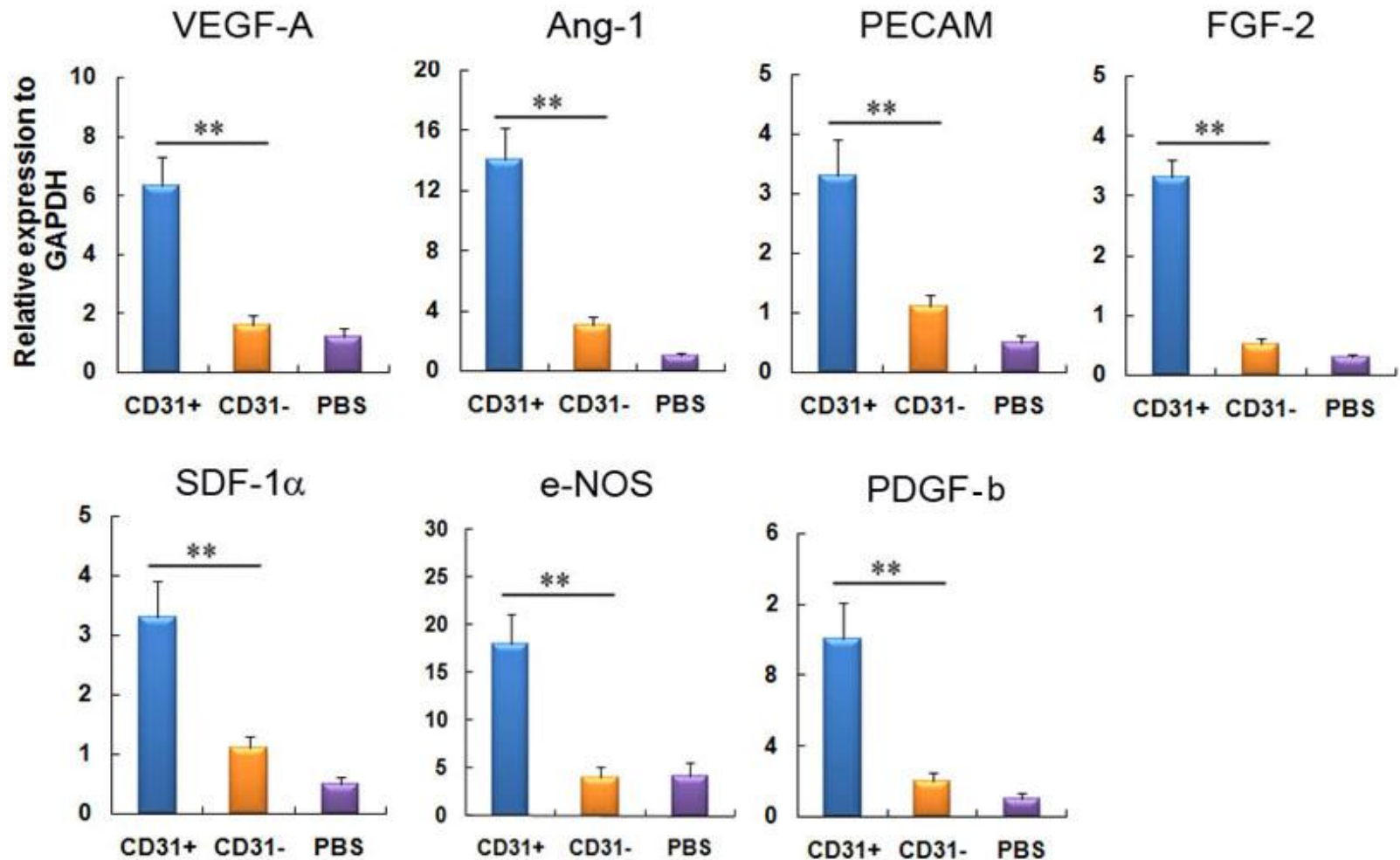
CD31⁺ cells transplantation increased limb salvage



(n=9 each group)

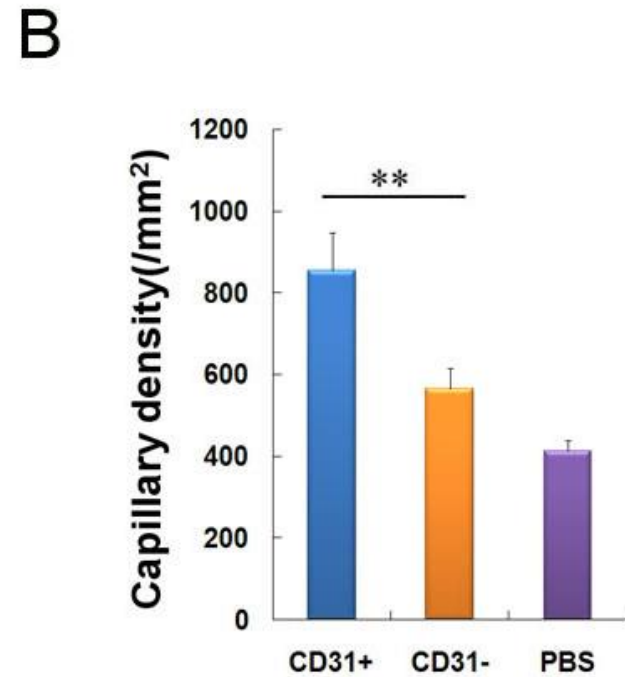
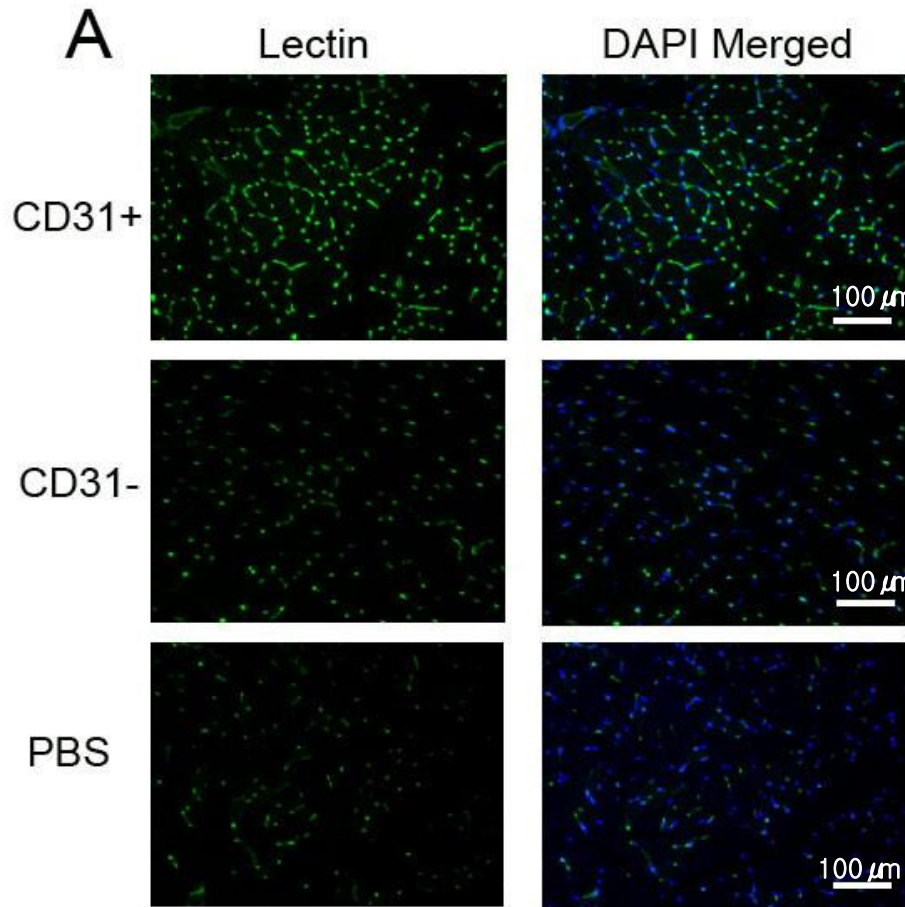
CD31⁺ cells transplantation increased angiogenic factors in ischemic hindlimb

(In vivo angiogenic gene expression)



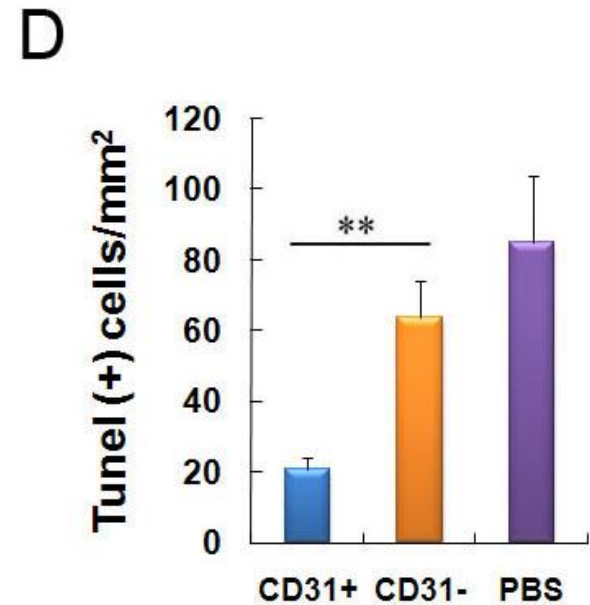
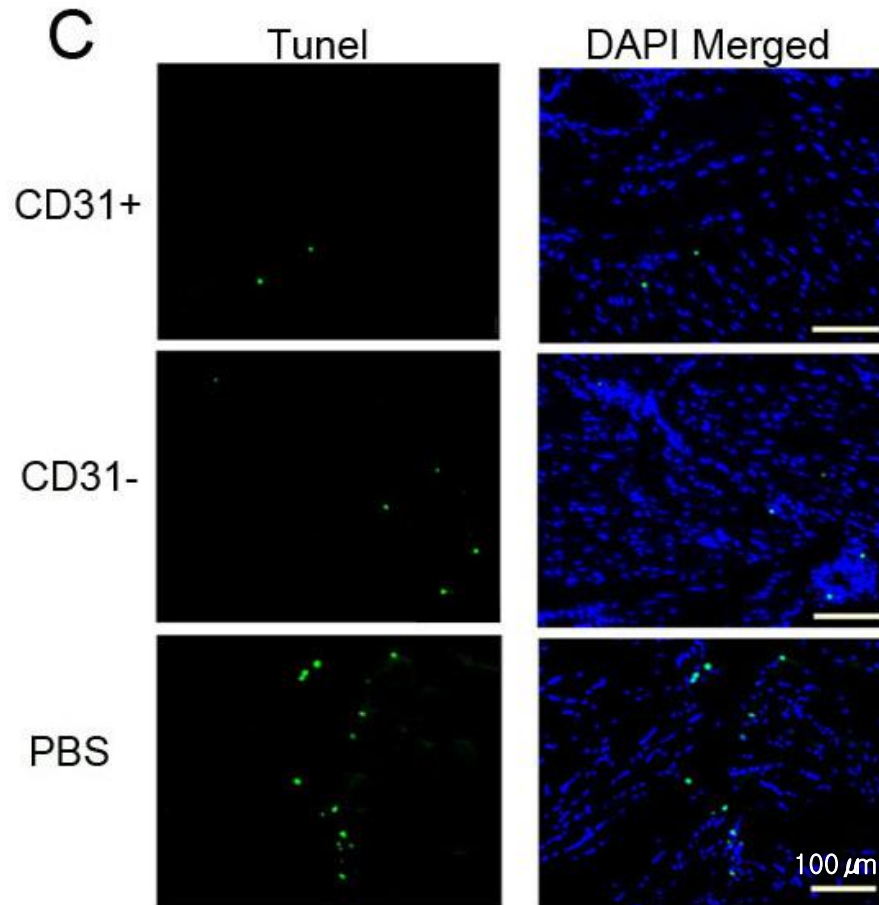
(n=3 each, *P<0.05, **P<0.01)

CD31⁺ cells transplantation increased capillary density



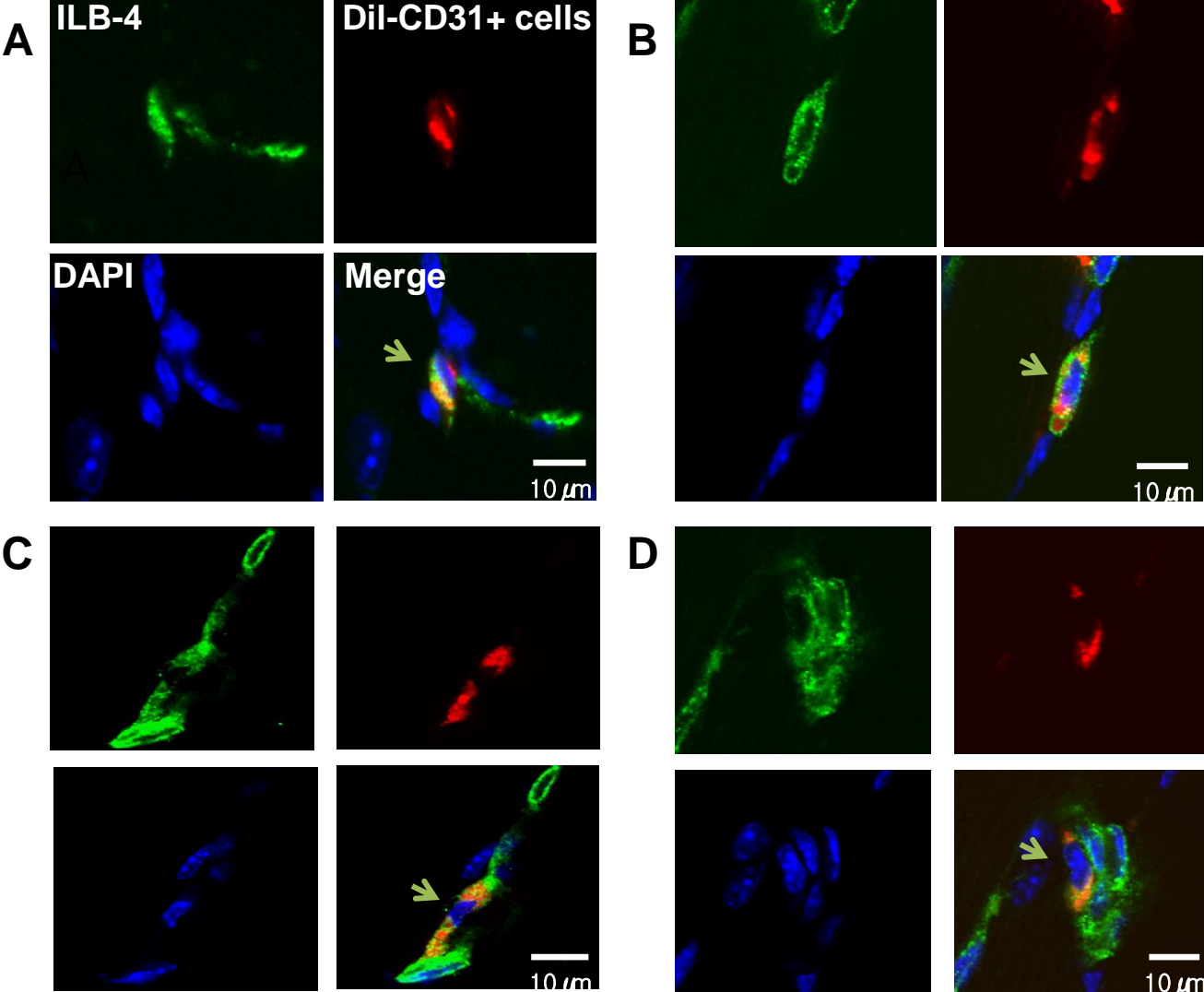
(n=5 each group, **P<0.01)

CD31⁺ cells transplantation decreased apoptosis

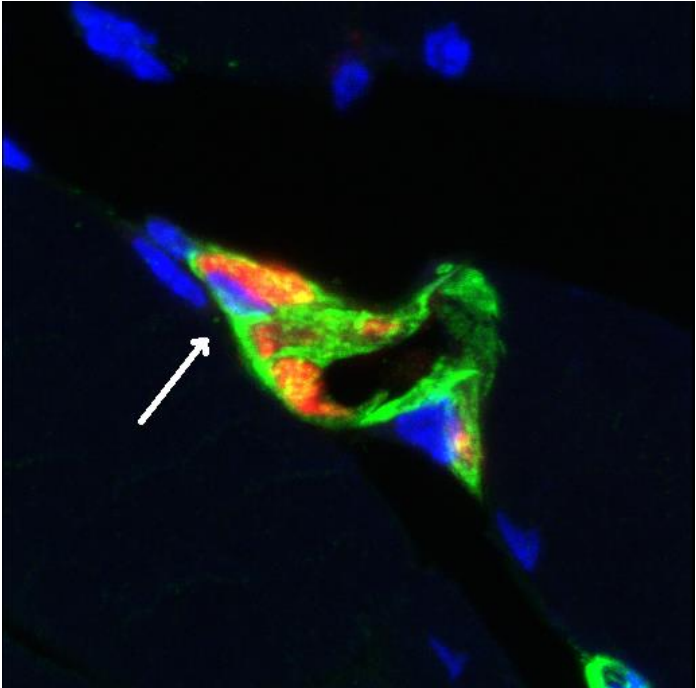
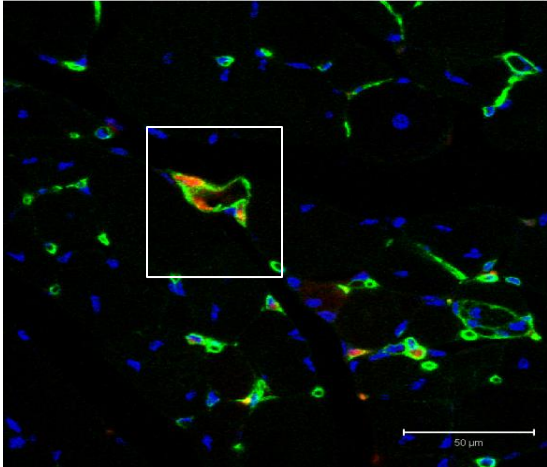
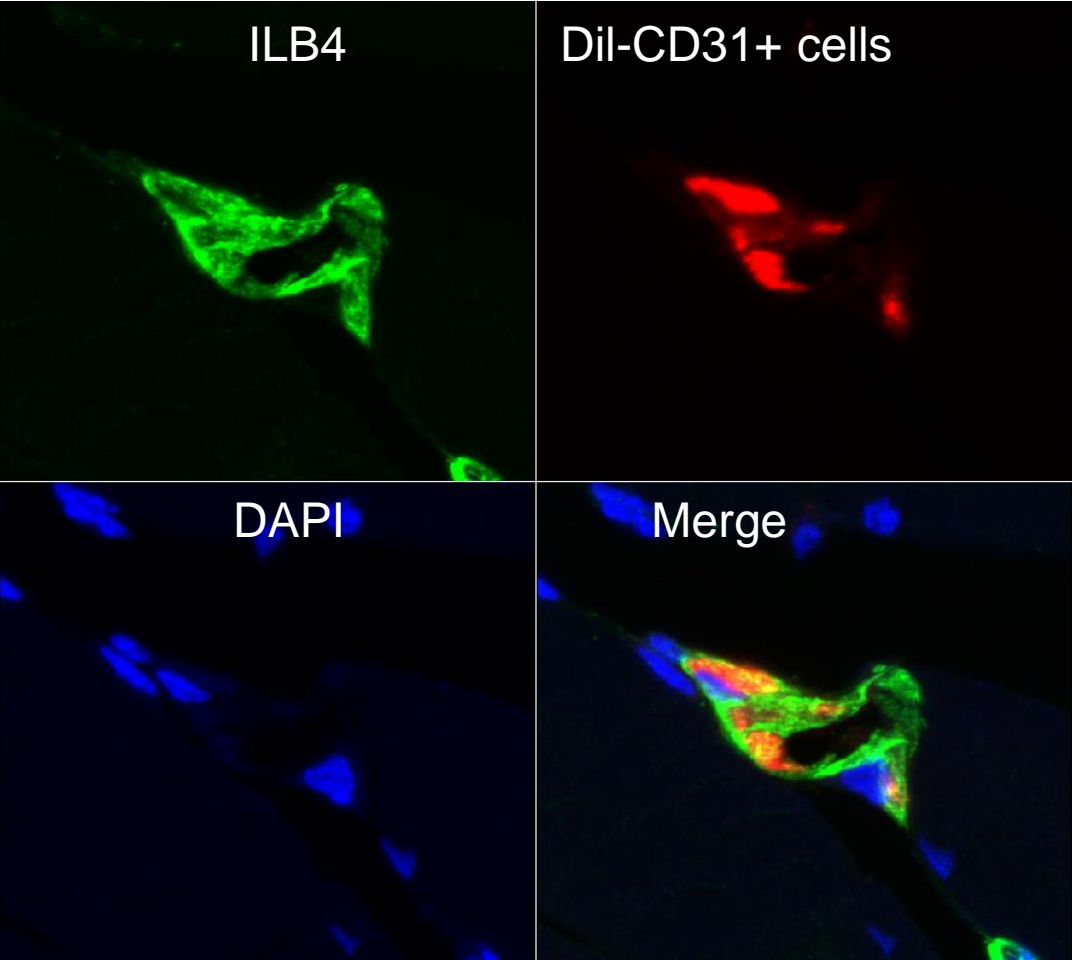


(n=5 each group, **P<0.01 vs CD31⁻)

Transdifferentiation of CD31+ cells into endothelial cells in vivo

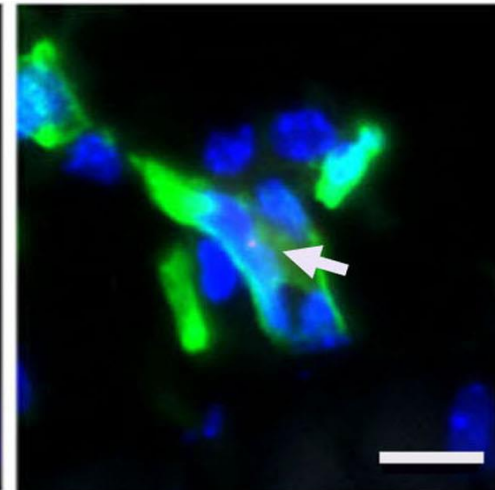
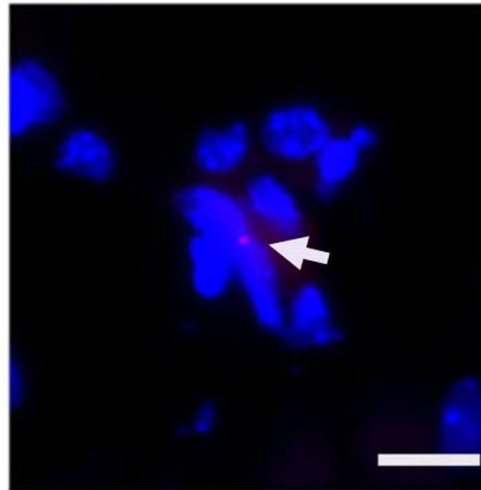
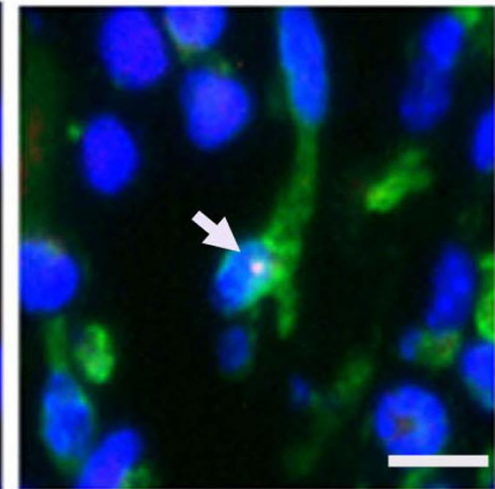
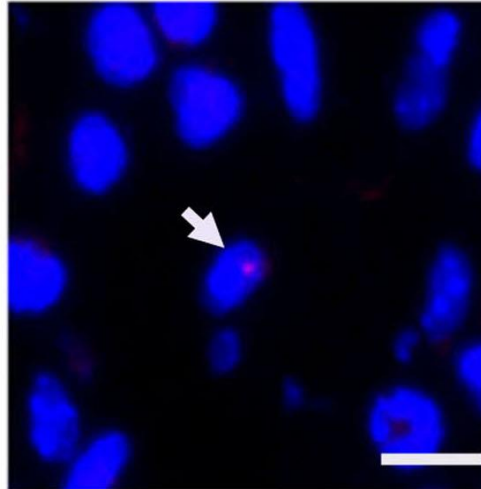
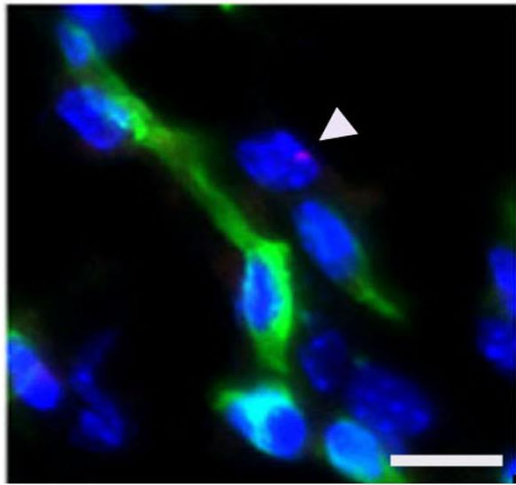


Transdifferentiation of CD31+ cells into endothelial cells in vivo



Transdifferentiation of CD31⁺ cells into endothelial cells in vivo

FISH analysis

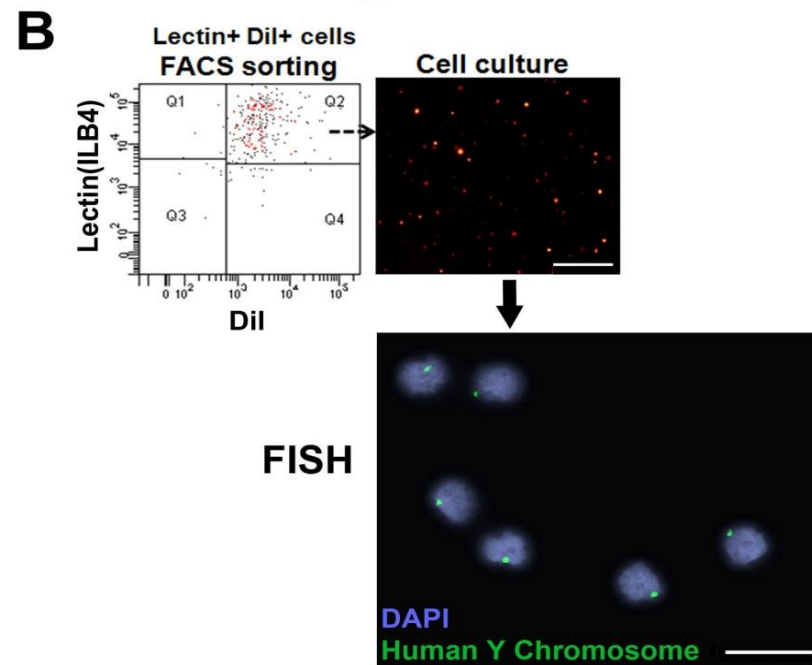
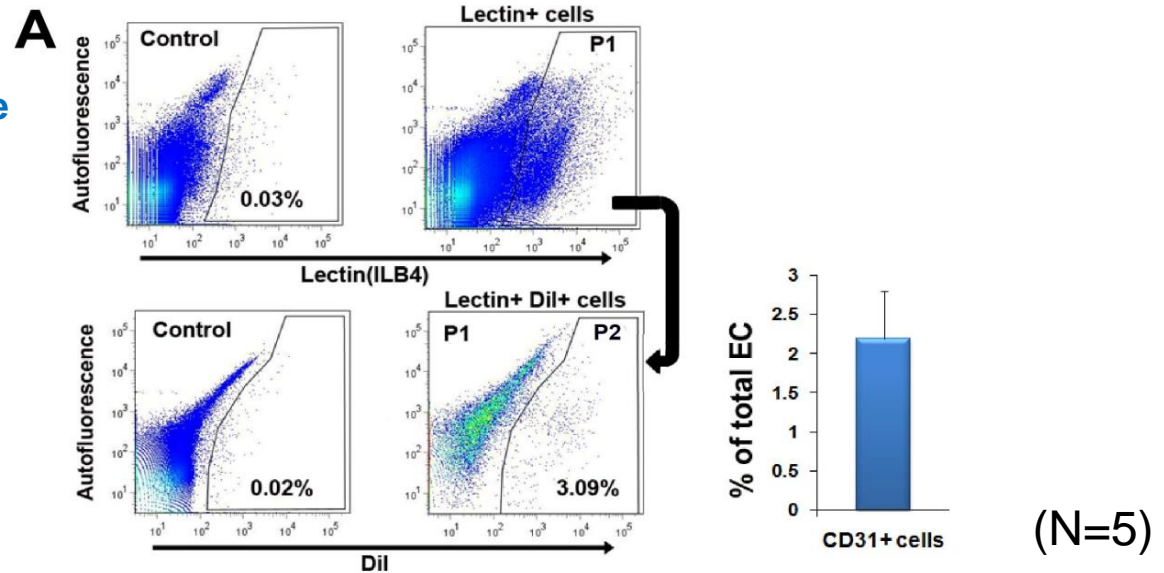


Arrow head: pericytic area

Green: ILB-4, Red: Human Y chromosome
Blue: DAPI

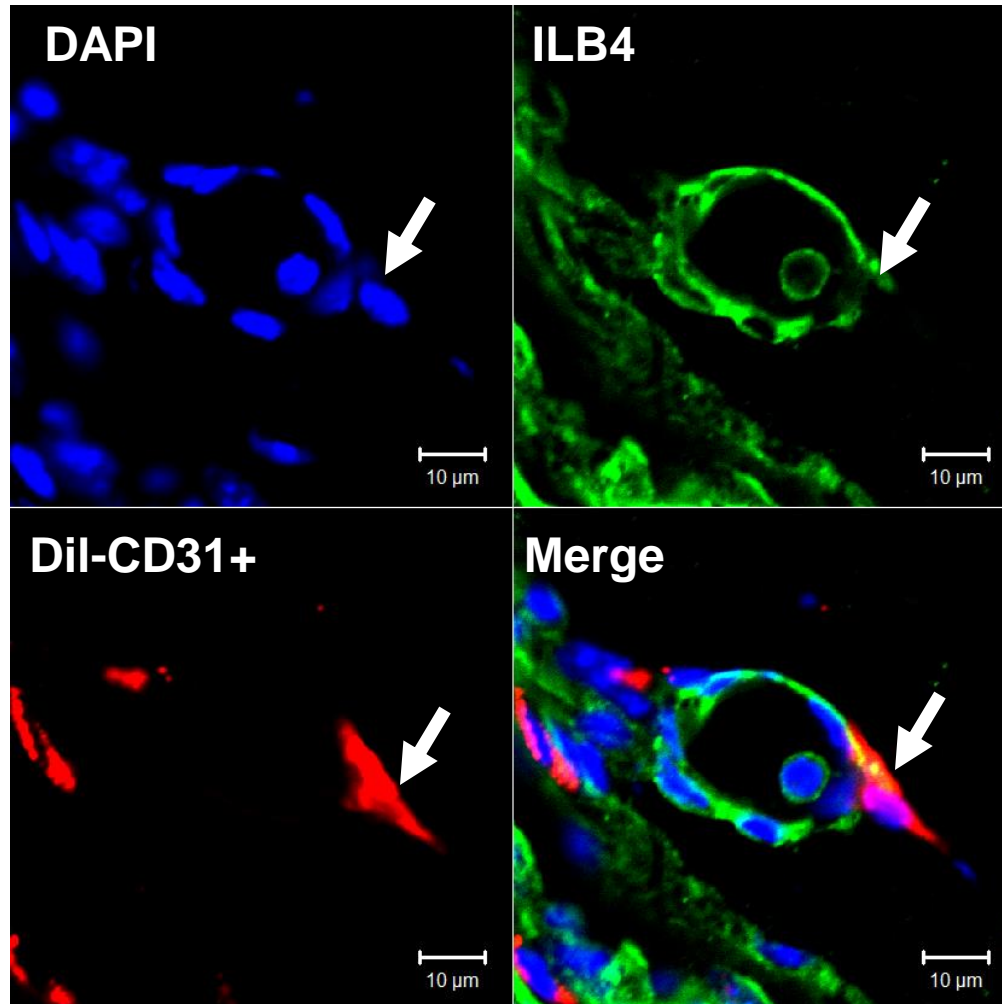
Quantification of Endothelial Cell Transdifferentiated CD31+ Cells in vivo

1. Lectin perfusion into nude mice
2. Collect hindlimb tissue
3. Enzyme digestion of hindlimb tissues
4. FACS analysis



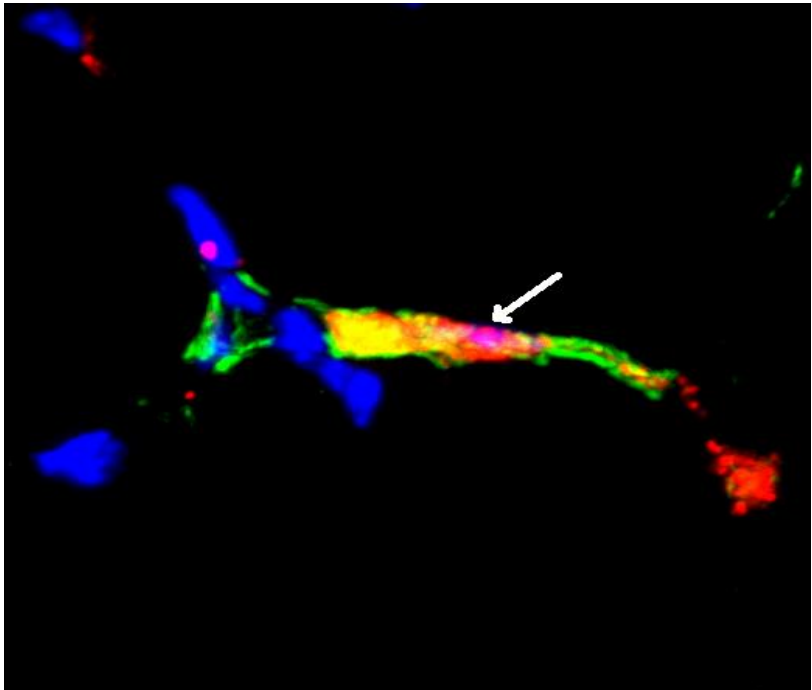
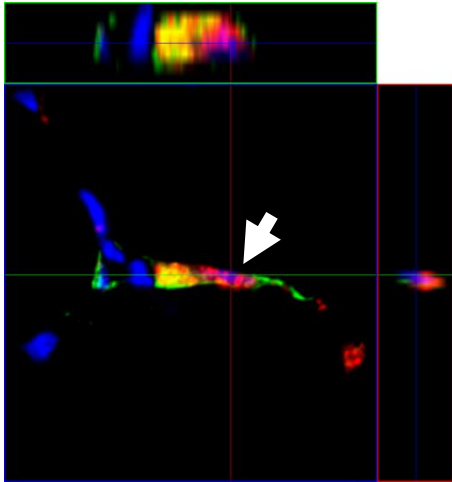
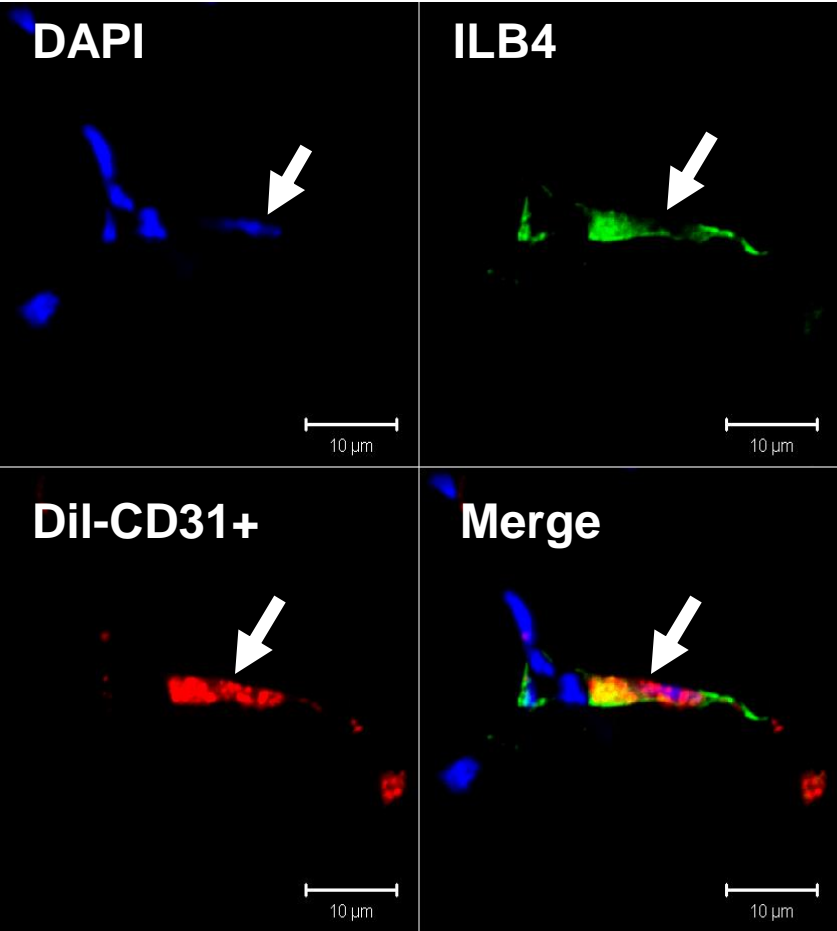
Perivascular Incorporation of CD31+ Cells

[Confocal images at **10 months** after cell transplantation]



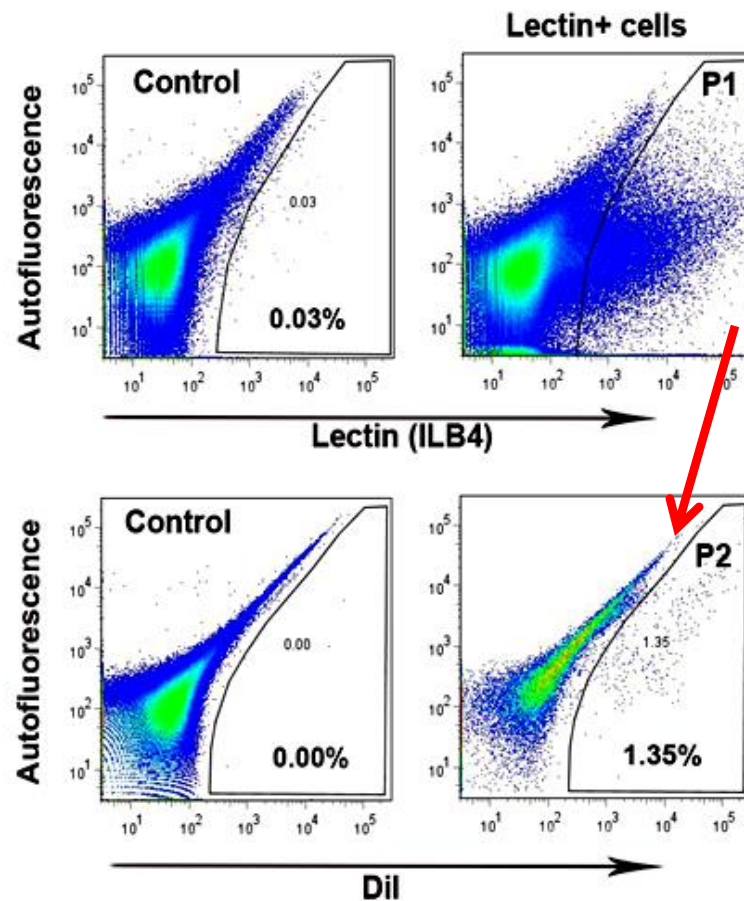
Endothelial Cells Transdifferentiation of CD31+ Cells

[Confocal images at 10 months after cell transplantation]



Durable Endothelial Cell Transdifferentiation of CD31⁺ Cells

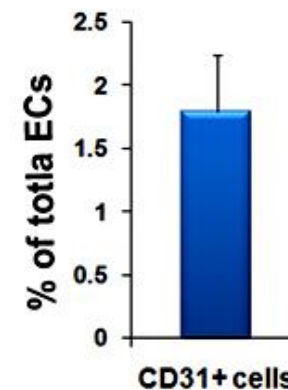
[FACS analysis of hindlimb tissues]
; **10 months** after CD31⁺ cell transplantation



Quantification of endothelial cell transdifferentiated cells

P1: Lectin+ cells

P2: Lectin+Dil+ cells



(n = 6)

Summary

- **CD31⁺ cells exhibited higher angiogenic gene expressions.**
- **CD31⁺ cells showed high blood perfusion rate and limb salvage in ischemic hindlimb model.**
- **CD31⁺ cells showed long-term engraftment and transdifferentiated into endothelial cells.**

Conclusion

- **These data suggested that CD31 might be alternative marker for the isolation of angio-vasculogenic cells for treating ischemic cardiovascular diseases.**

Acknowledgement

- **Emory Univ. USA**
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- **Seoul National Univ. Hospital**
Hyun-Jai Cho, MD,PhD

Thank you!