

Biology of perivascular progenitor cells

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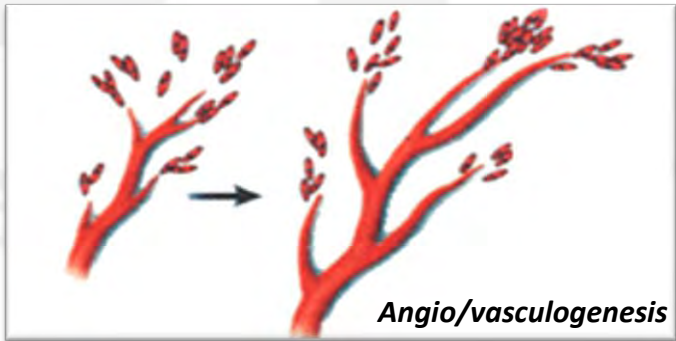
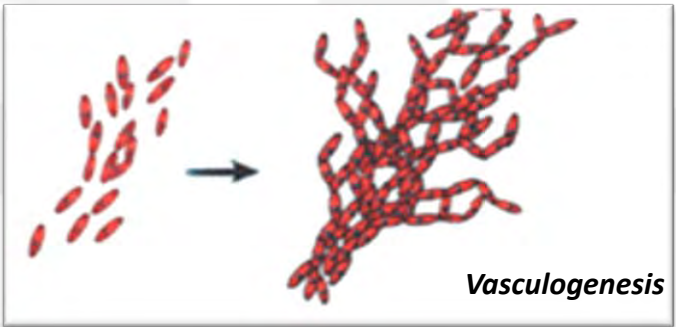
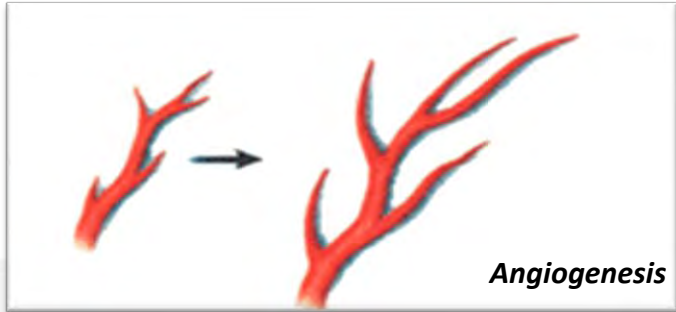
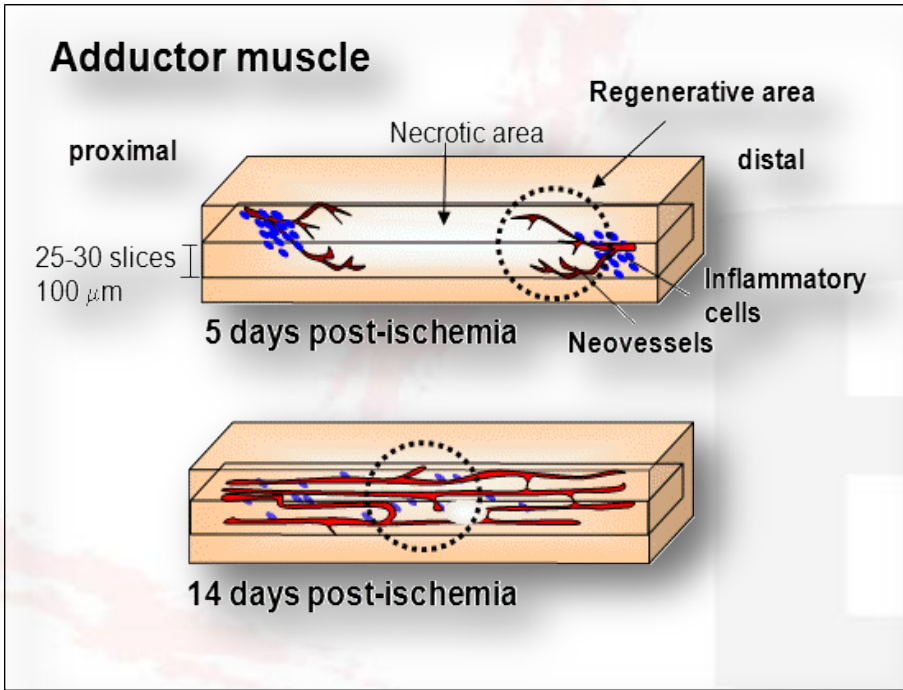
Monday 13th June 2011

CBCS Summer School on Cardiovascular Sciences

From Basic Mechanisms to Clinical Applications

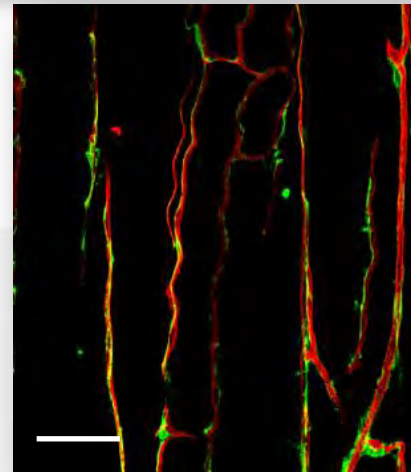
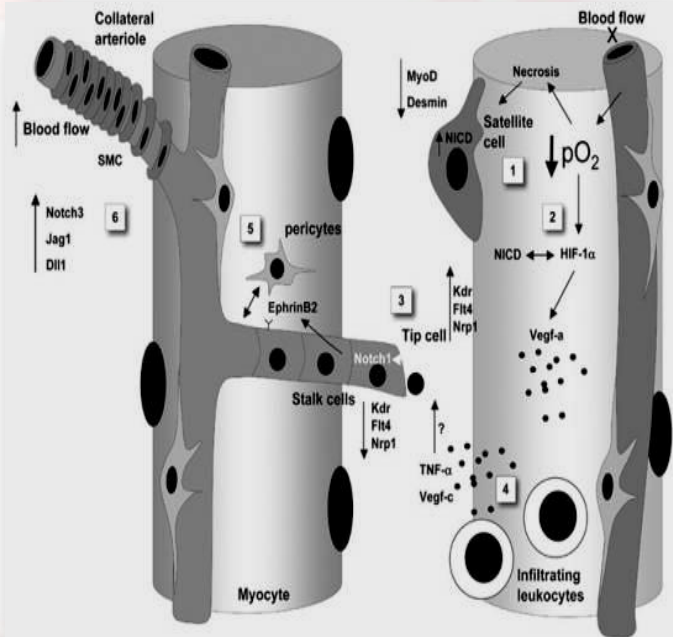
European Heart House 12/16 June 2011

Mechanisms of reparative vascularization

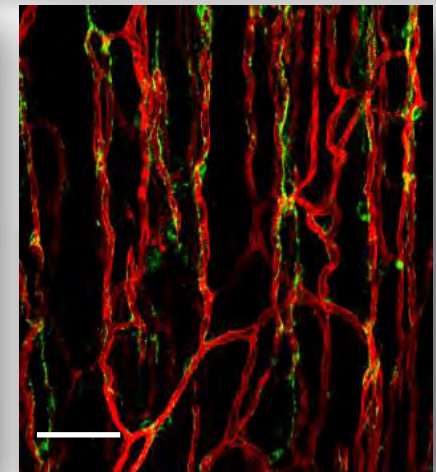


Pericyte/mural cell coverage is essential during vascular repair

Recruitment of NG2^{pos} pericytes around neovessels



Contralateral

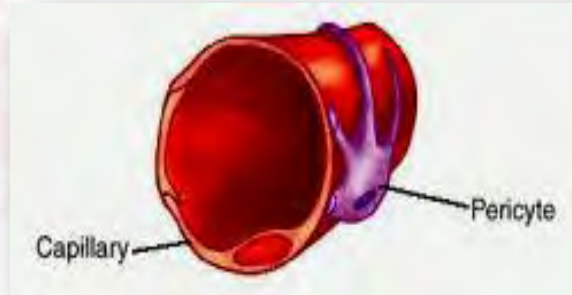


Ischemic

Isolectin/NG2

- **Risk factors and comorbidities impair pericyte recruitment, resulting in a fragile neovascularisation.**
- **Hence, a supply-side approach could be therapeutically helpful.**
- **However, difficulties in isolation and expansion have so far precluded clinical applications.**

General pericyte functions



Vascular stability by direct contacts and through matrix deposition



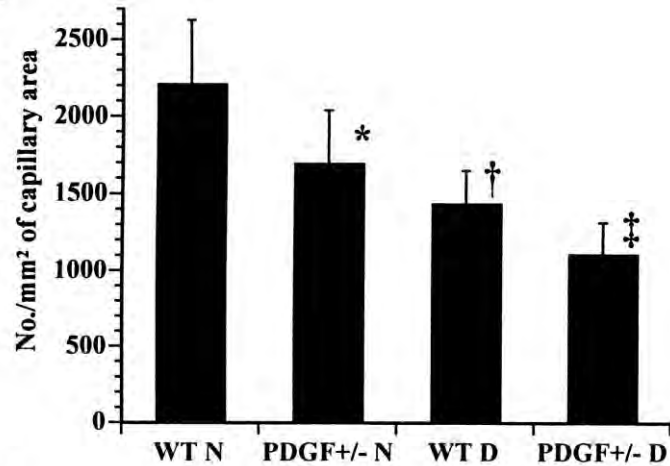
Presentation /activation of signals to induce quiescence/differentiation

Question 1: What is the status of pericytes in pathological conditions like diabetes

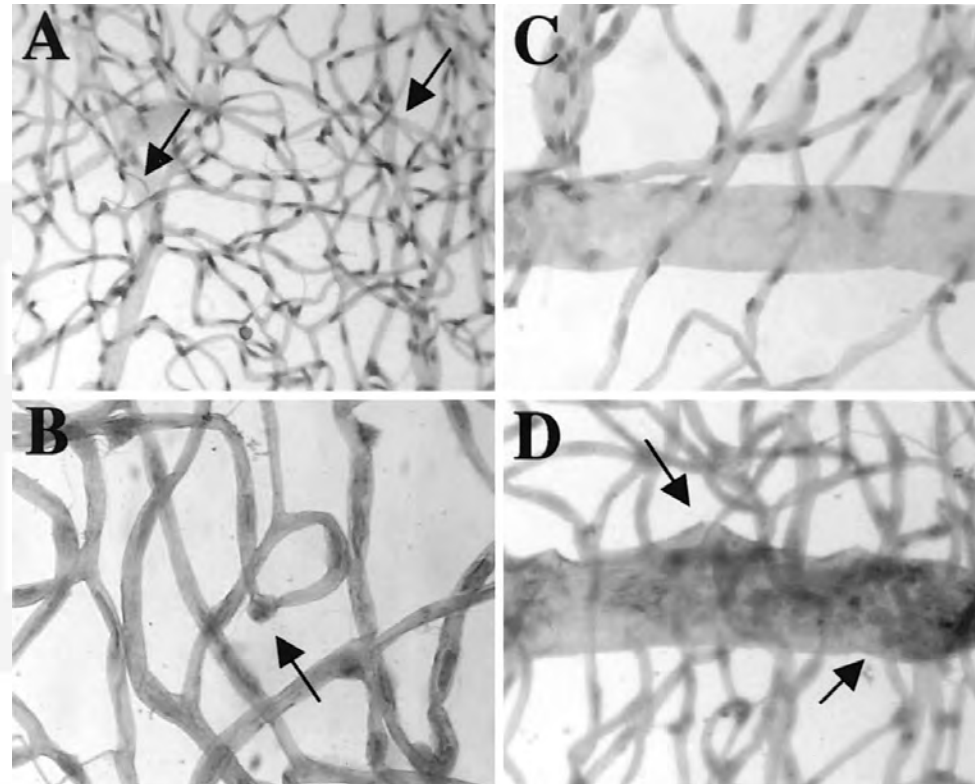
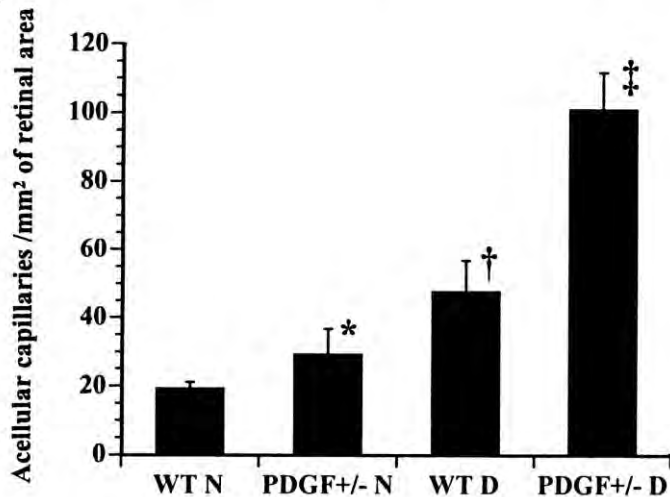
- 1. Increased in number*
- 2. More sticky*
- 3. Less abundant*
- 4. Non responsive to chemo-attractant*

Diabetes reduces pericyte number in the retina: role of PDGF

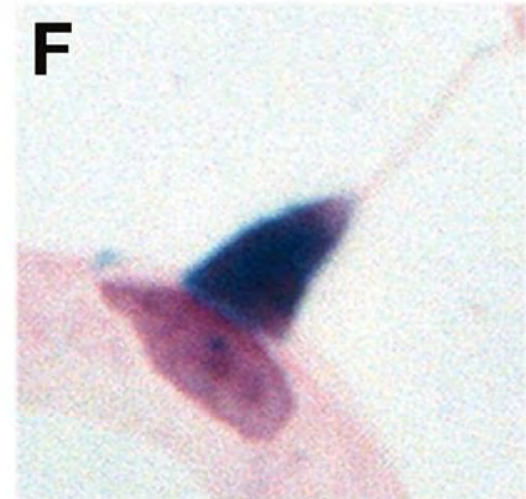
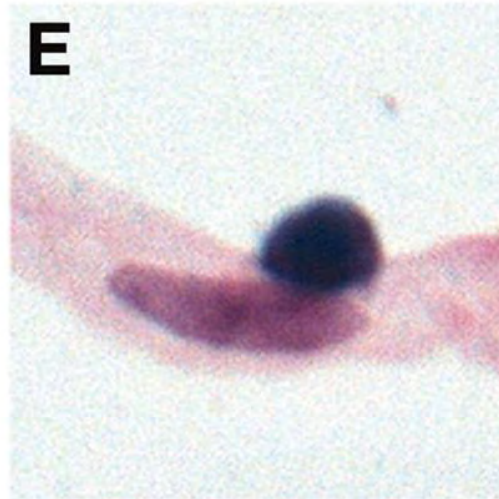
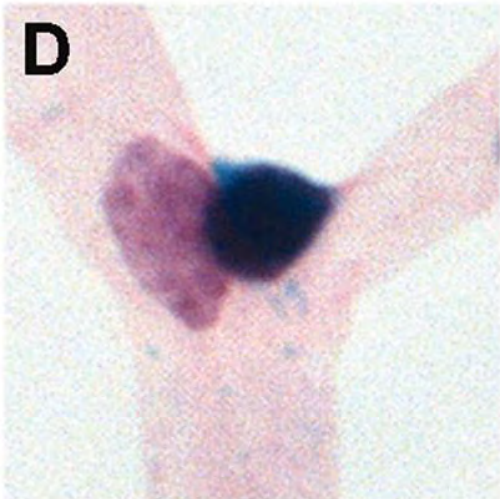
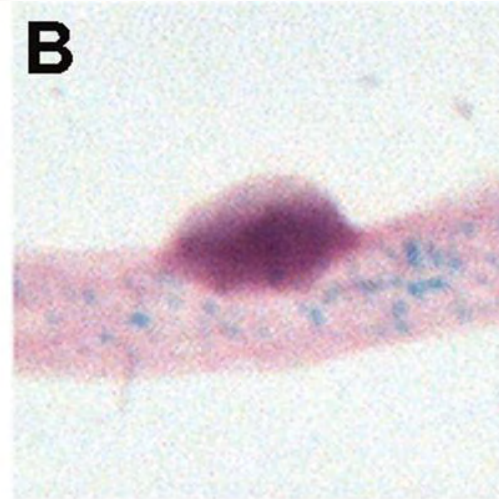
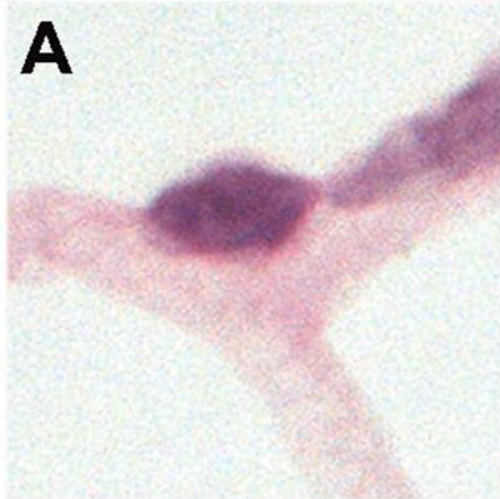
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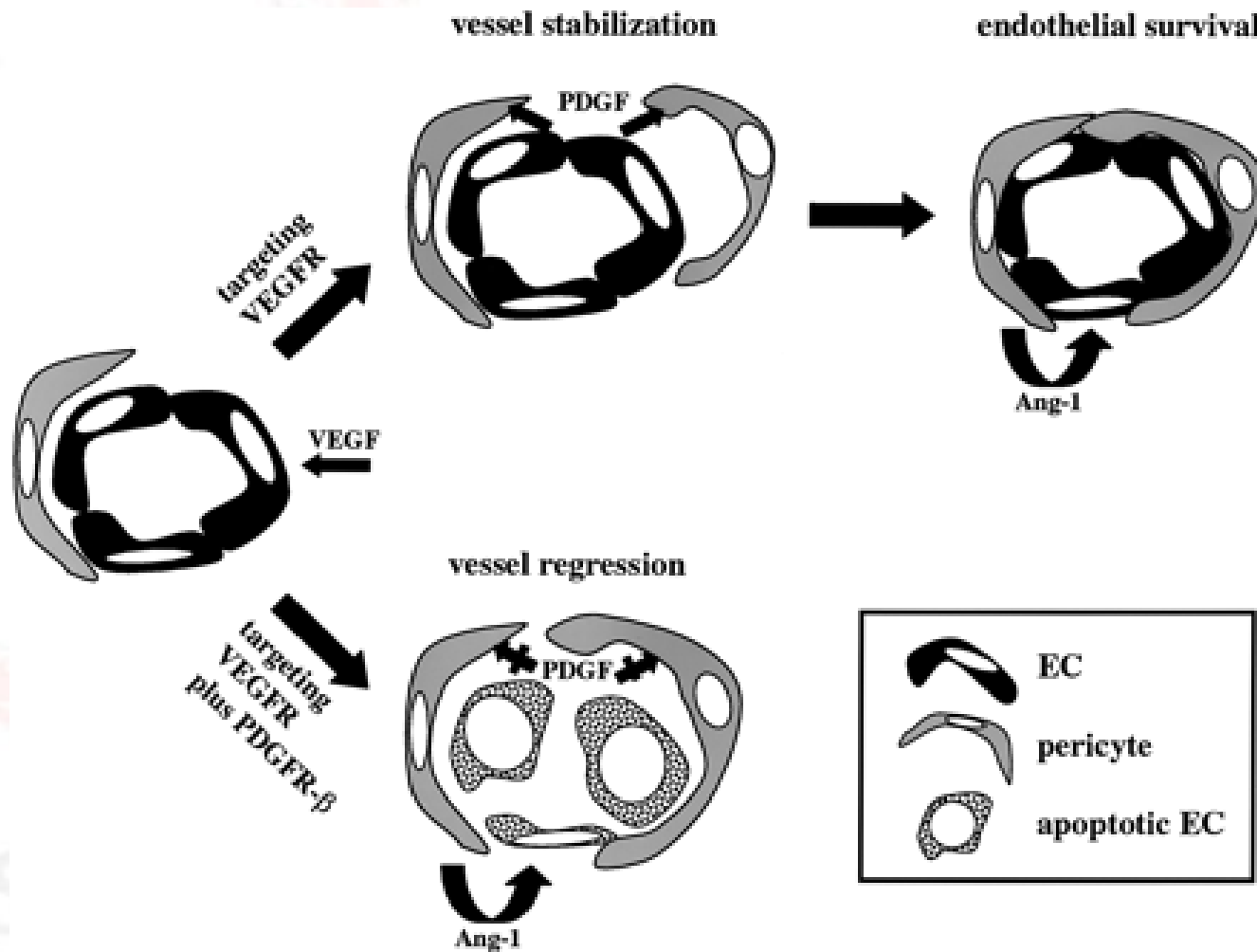
B



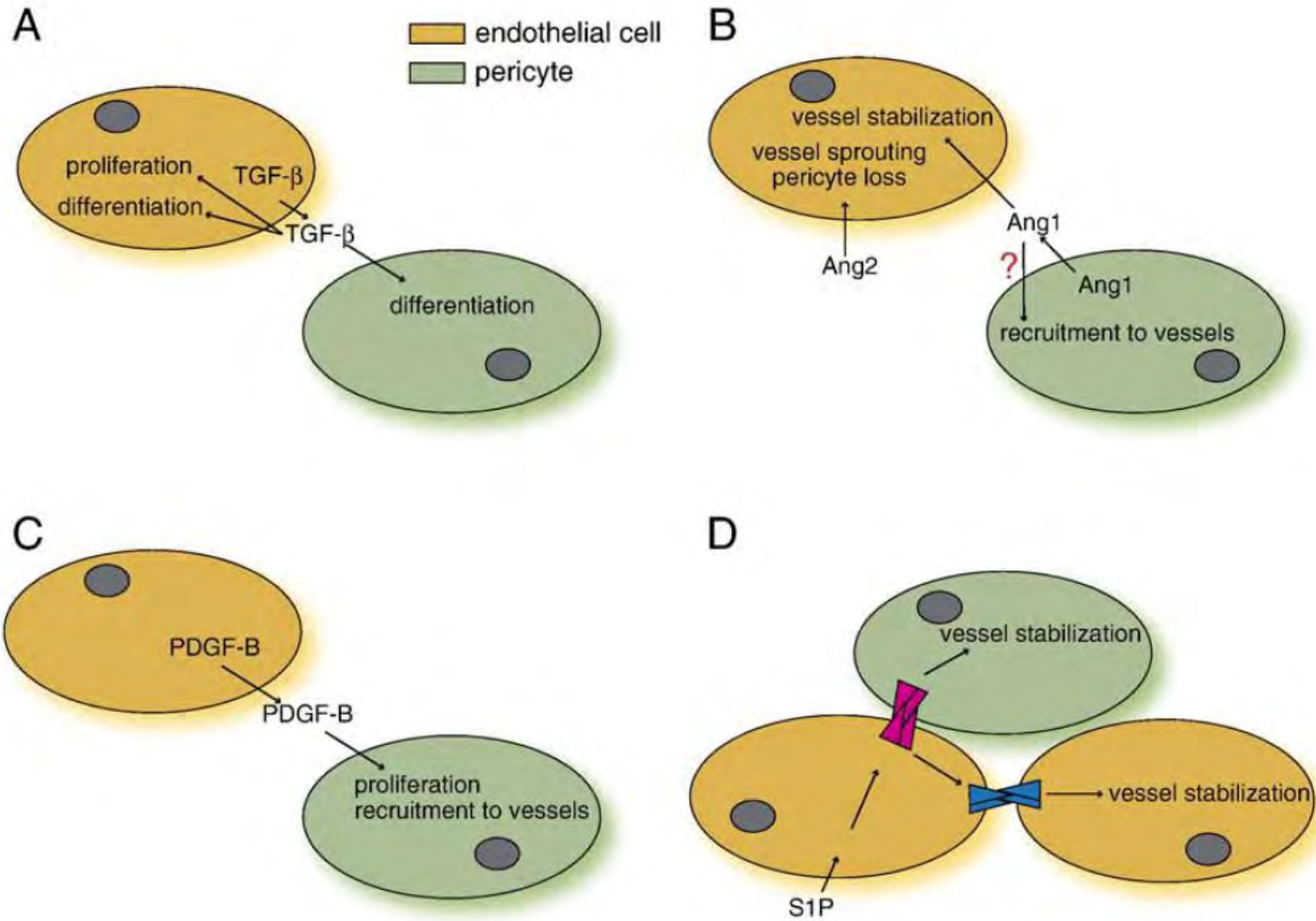
Diabetes reduces pericyte number in the retina: role of Ang-Tie2 in promotion of migration



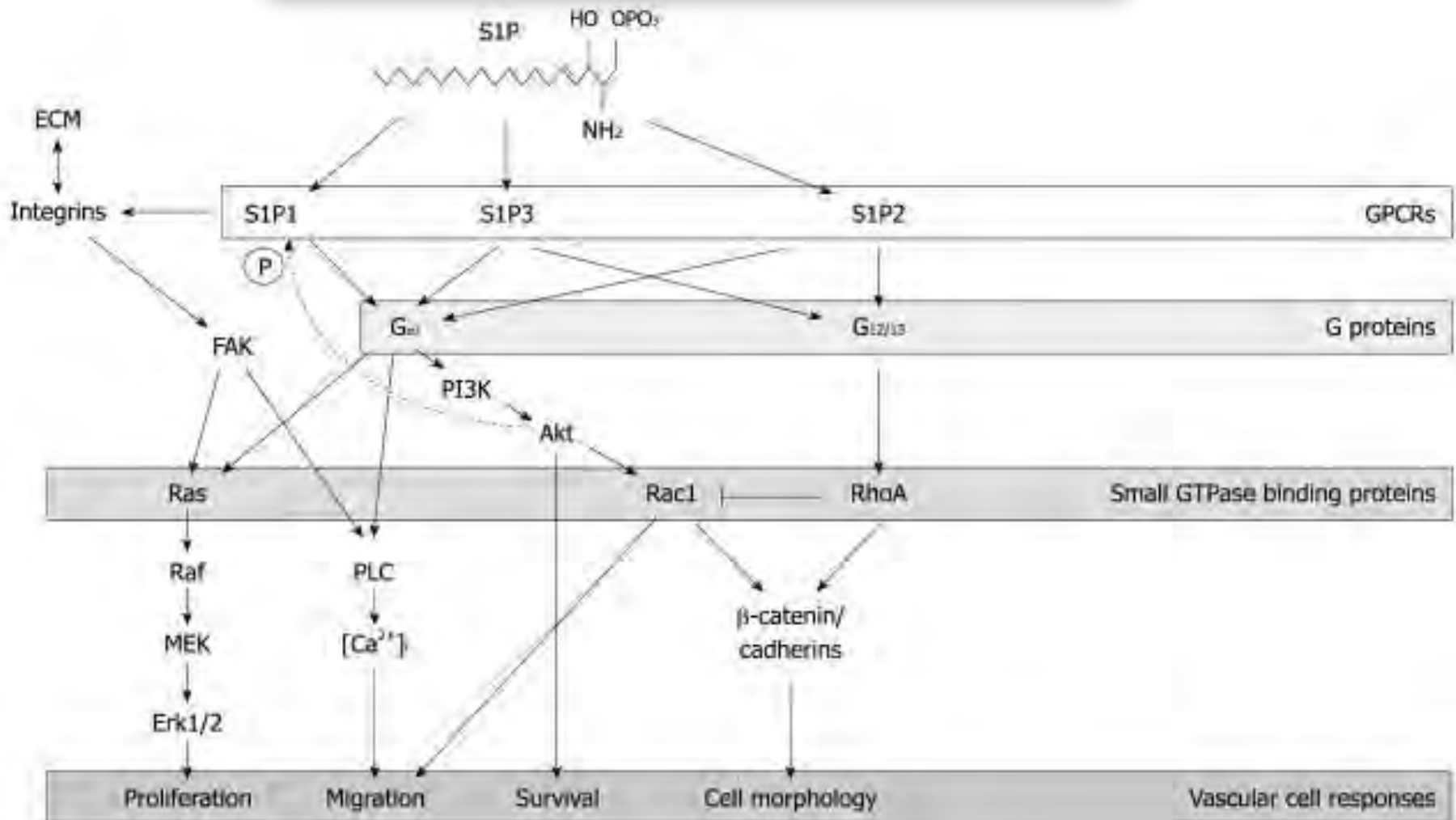
Anti-angiogenic therapy targeting VEGFR and PDGFR result in different effects because of pericytes



Signaling pathways involved in pericyte-EC interactions



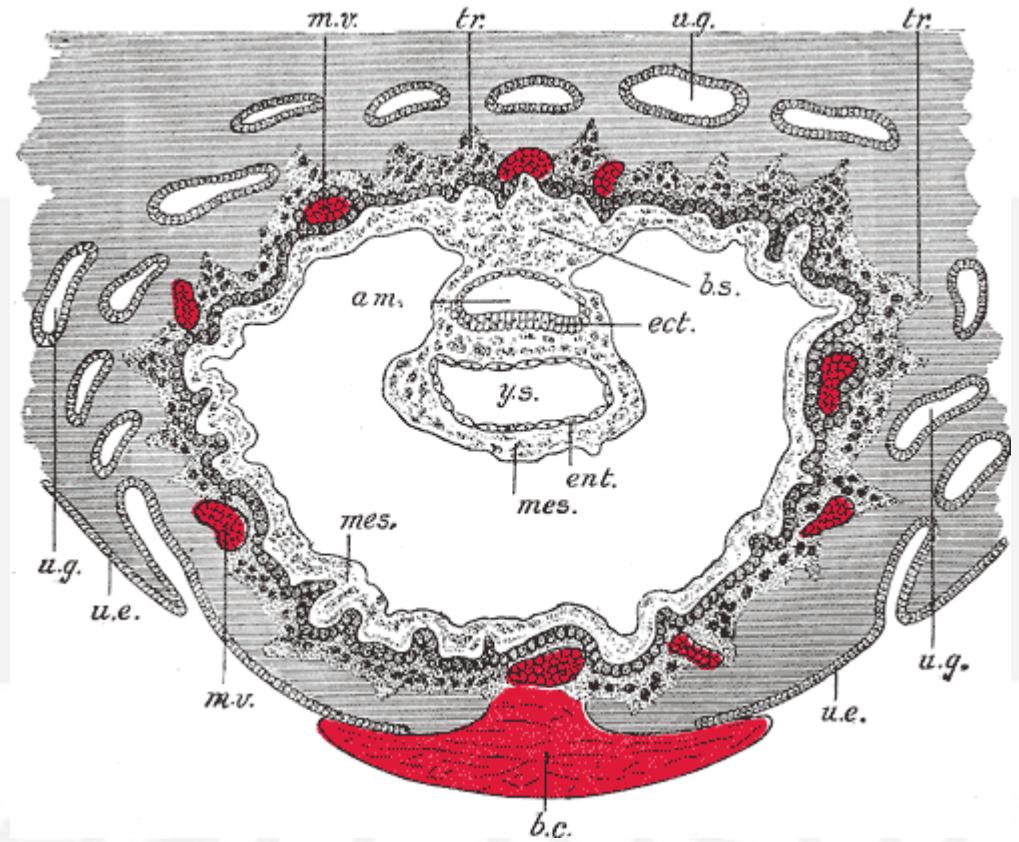
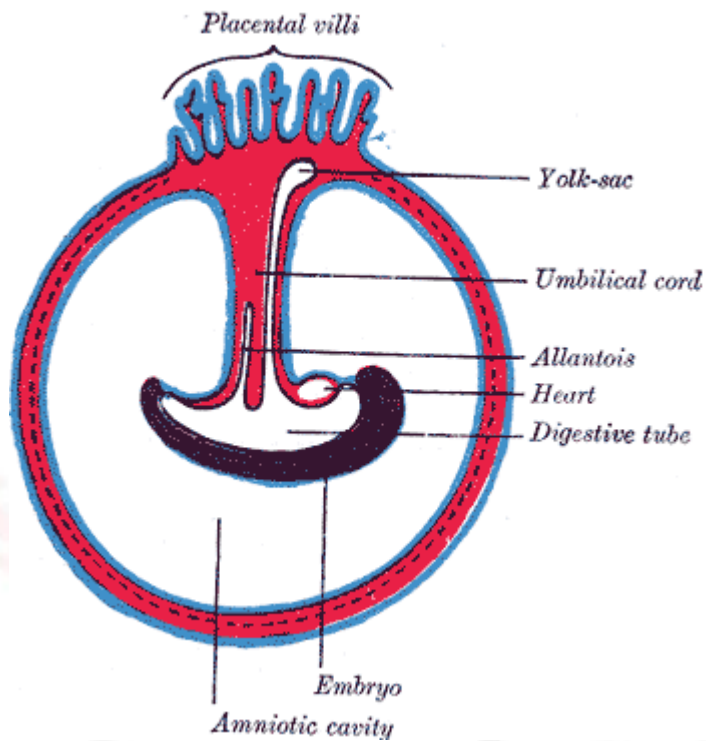
Signaling pathways involved in pericyte-EC interactions



Question 2: What is the origin of mural cells during development

- 1. Placenta*
- 2. Yolk sac*
- 3. Dorsal aorta*
- 4. Bone marrow*

The dorsal aorta and mesonephric region



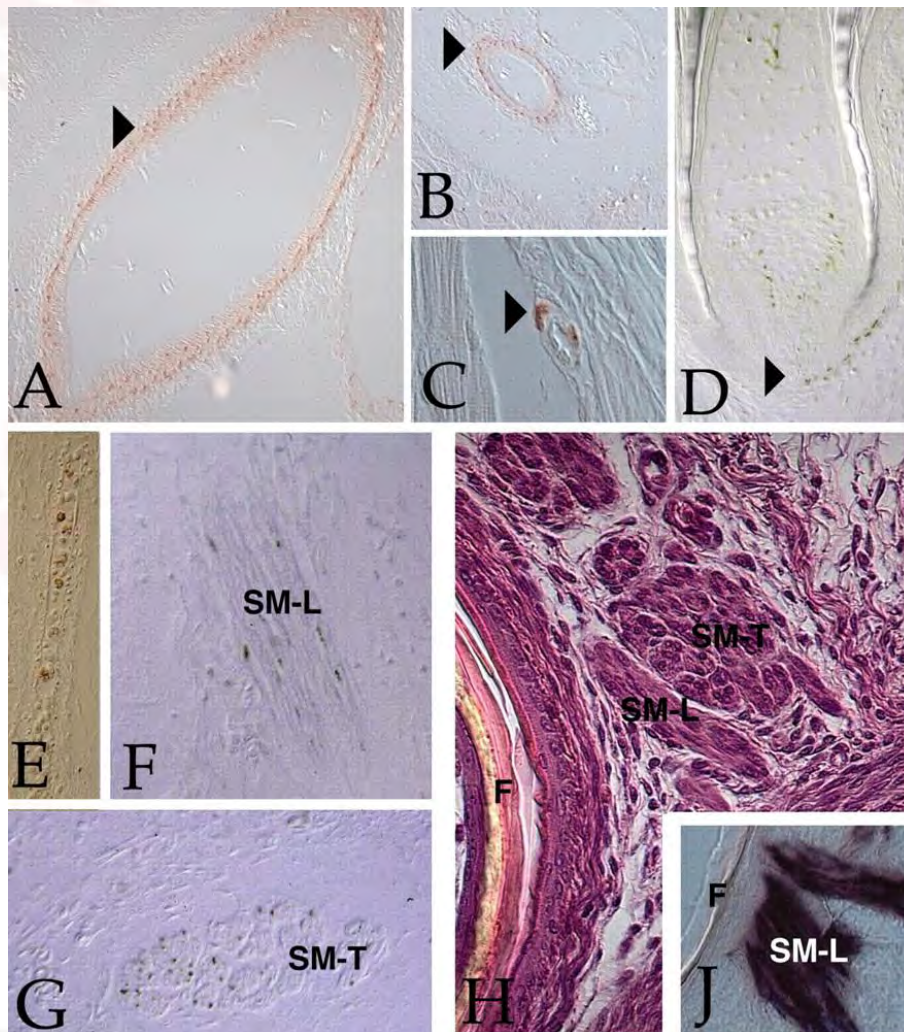
The meso-angioblast colonizes tissues forming peripheral mesenchymal niches

In 1999, Bianco and Cossu proposed that during tissue histogenesis, when vessels penetrate into developing tissues, vessel-associated progenitors, which possibly originate from a common ancestor (a 'meso-angioblast', rather than an hemo-angioblast) would leave the vessel and adopt the fate of the tissue where the vessel has entered.

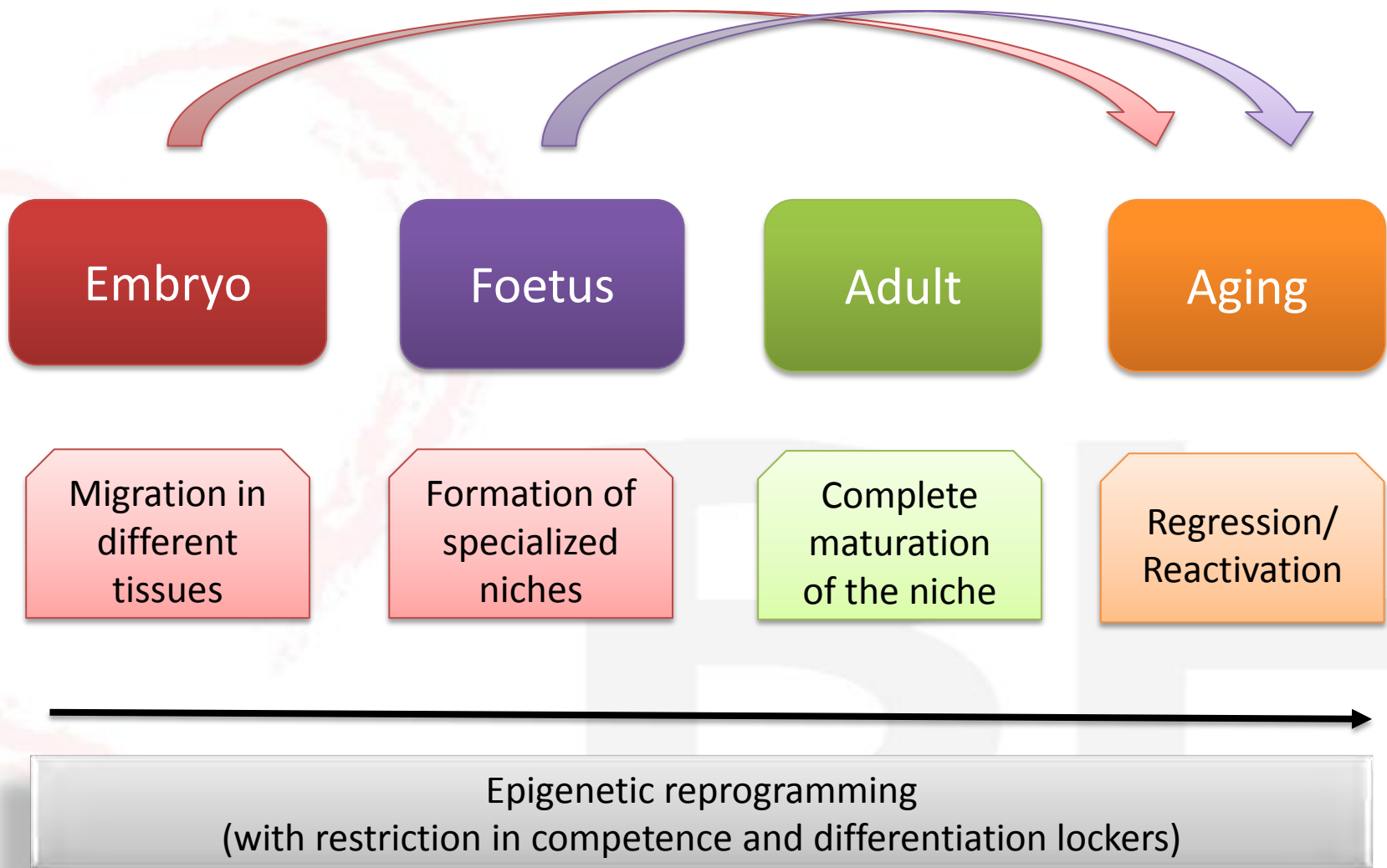
Next, they grafted quail or mouse embryonic aorta into host chick embryos. Donor cells, initially incorporated into the host vessels, were later integrated into mesodermal tissues, including blood, cartilage, bone, smooth, skeletal and cardiac muscle.

When expanded on a feeder layer of embryonic fibroblasts, the clonal progeny of a single cell from the mouse dorsal aorta acquired unlimited lifespan, expressed hemo-angioblastic markers (CD34, Flk1 and Kit) at both early and late passages, and maintained multipotency in culture or when transplanted into a chick embryo.

Immunoperoxidase staining with QCPN antibody of chick-quail chimeras sacrificed at E19-E20.



Minasi M G et al. *Development* 2002;129:2773-2783



	Mesenchymal									Pericytes					Endothelial					HMT		
	CD13	CD73	CD44	CD90	CD29	CD49	CD59	Desmin	aSMA	RGS5	NG2	ALP	CD105	PDGFR	Tie2	CD146	KDR	CD31	CD144	CD34	CD133	CD45
Mesangioblasts	+	+	+								+		+		+		-	-		-	-	-
Cord blood MSC											+	+				+				-		-
Humbelical cord perivascular cells			+	+				+		+										-		
Human foetal aorta VPC								+			+				+		+	+		+	+	-
Muscular and tissue pericytes	+	+	+	+					-	+	+	+	+	+		+		-	-	-	-	-
Vascular wall resident multipotent SC		+	+	+				+	+	+	+		+	-		-		-		-	-	-
SVP	+	+	+	+	+	+	+	+	-		+		+	+	+	-		-		+	-	-

Differentiation Potential of Human Postnatal Mesenchymal Stem Cells, Mesoangioblasts, and Multipotent Adult Progenitor Cells Reflected in Their Transcriptome and Partially Influenced by the Culture Conditions

VALERIE D. ROOBROUCK,^a CARLOS CLAVEL,^a SANDRA A. JACOBS,^b FERNANDO ULLOA-MONTOYA,^a STEFANIA CRIPPA,^c ABHISHEK SOHNI,^a SCOTT J. ROBERTS,^d FRANK P. LUYTEN,^d STEFAAN W. VAN GOOL,^b MAURILIO SAMPAOLESI,^c MICHEL DELFORGE,^e AERNOUT LUTTUN,^f CATHERINE M. VERFAILLIE^a

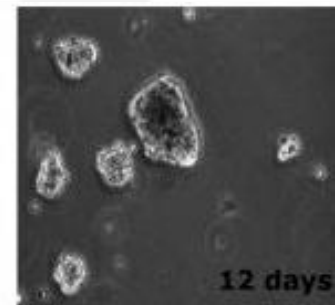
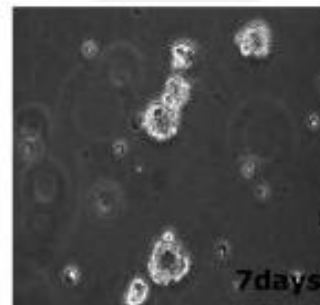
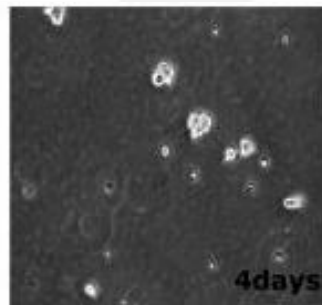
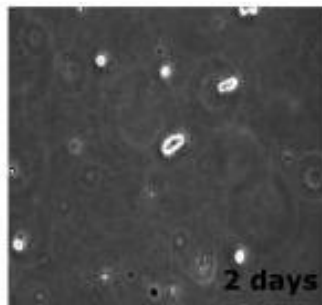
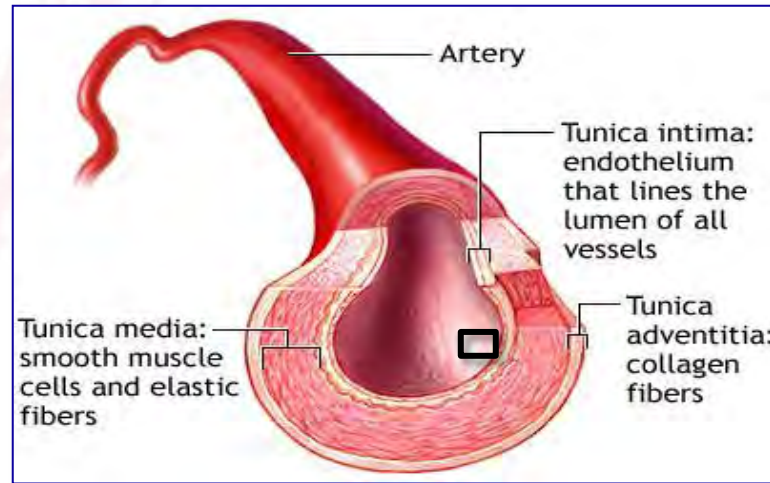
STEM CELLS 2011;29:871–882

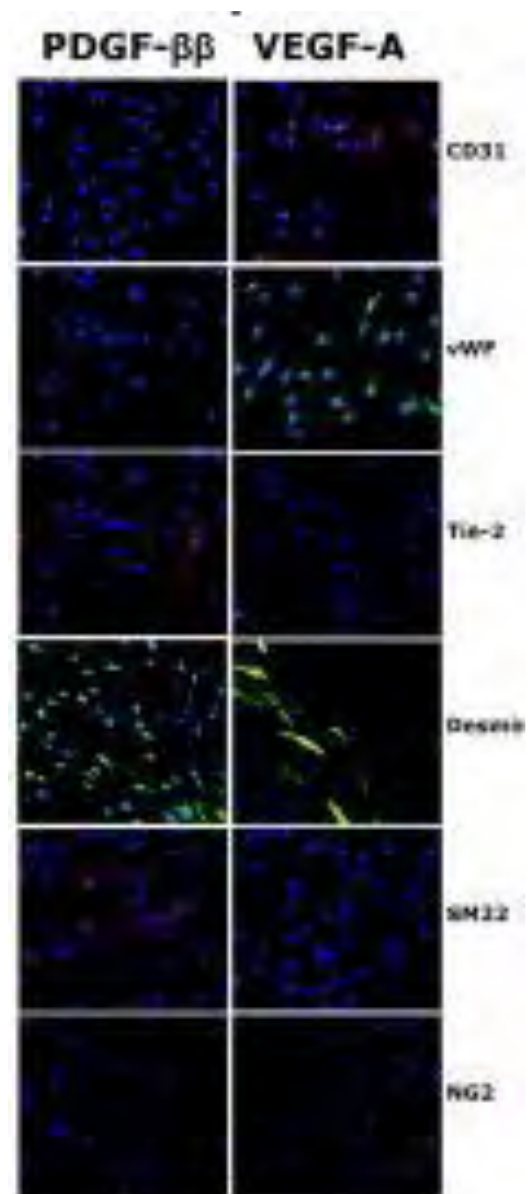
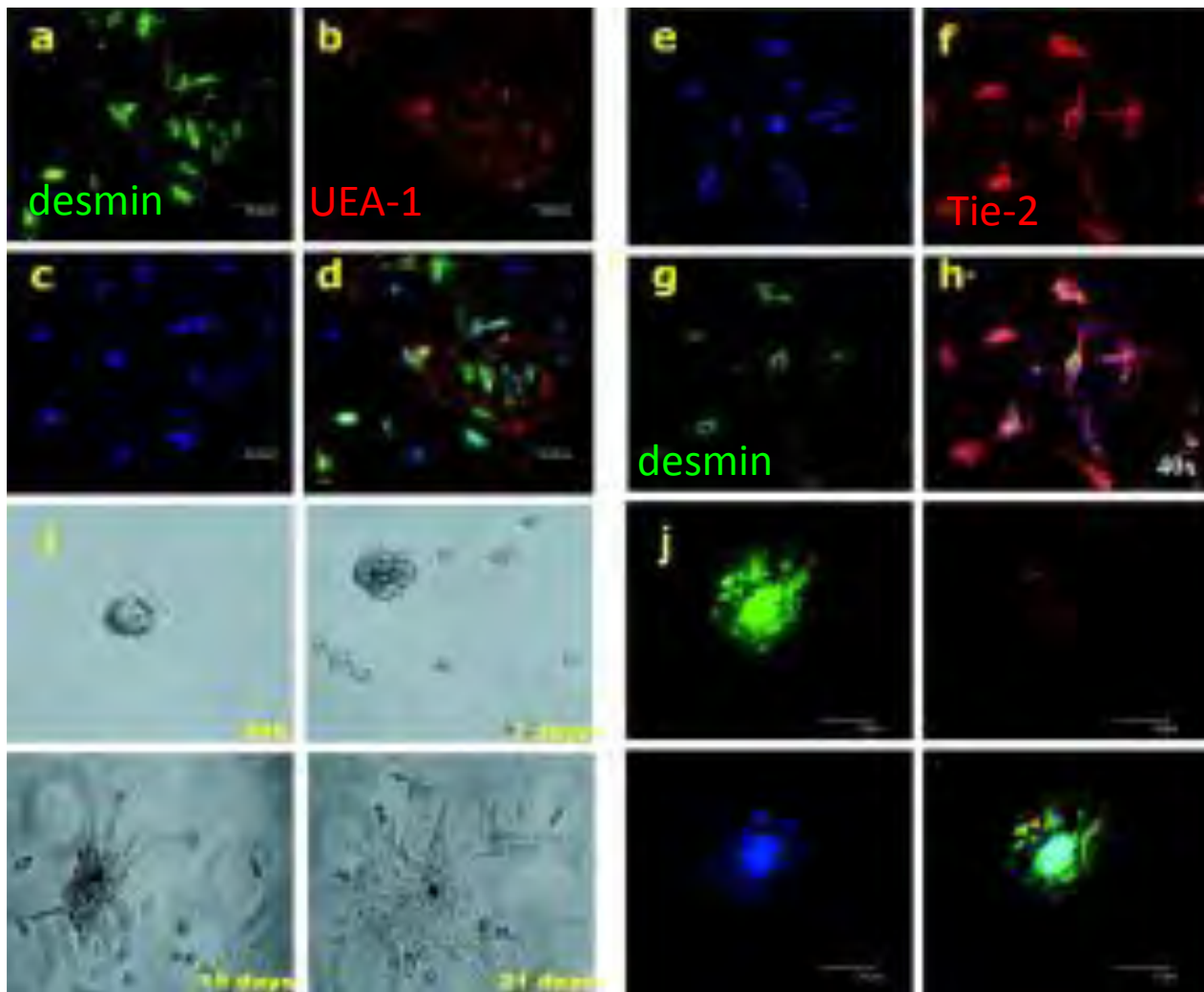
Bristol Heart Institute

Human Fetal Aorta Contains Vascular Progenitor Cells Capable of Inducing Vasculogenesis, Angiogenesis, and Myogenesis in Vitro and in a Murine Model of Peripheral Ischemia

Gloria Invernici, Costanza Emanuelli, Paolo Madeddu, Silvia Cristini, Sergio Gadau, Anna Benetti, Emilio Ciusani, Giorgio Stassi, Mauro Siragusa, Roberto Nicosia, Cesare Peschle, Umberto Fascio, Augusto Colombo, Tommaso Rizzuti, Eugenio Parati and Giulio Alessandri

American Journal of Pathology, The
[Volume 170, Issue 6, Pages 1879-1892](#) (June 2007)

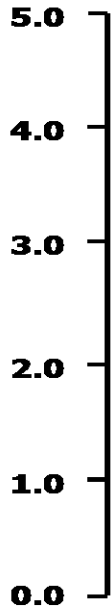




Tranplantation of hVPCs improves the healing of ischaemic limbs

Tranplantation of hVPCs stimulates arteriogenesis and myogenesis

n. of necrotic nails per ischemic foot (14 days)



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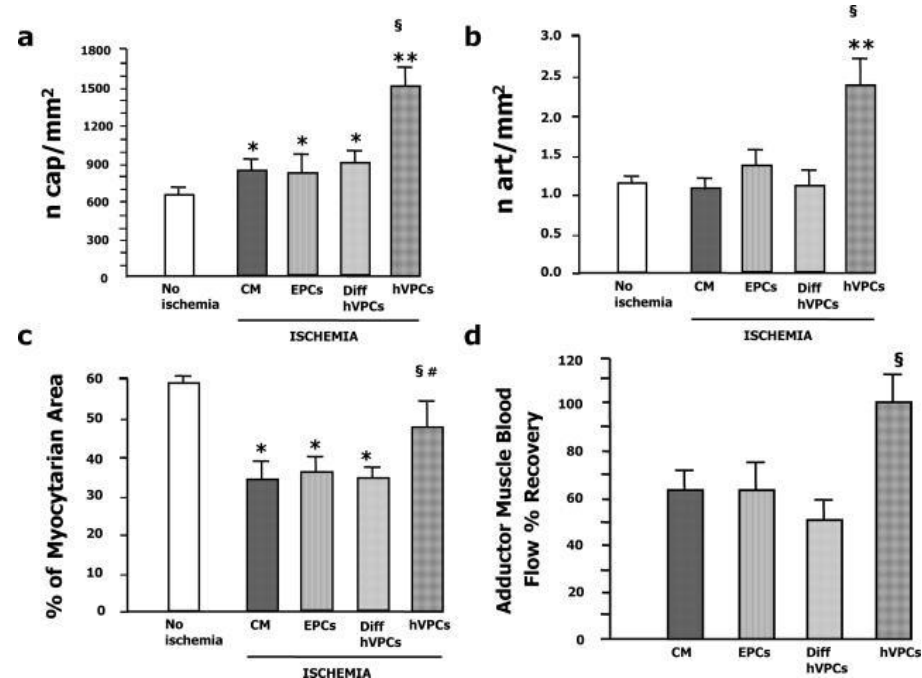
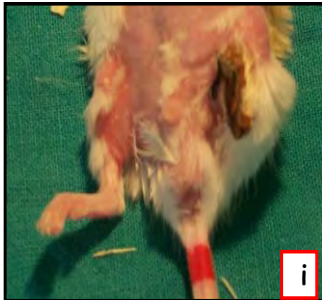
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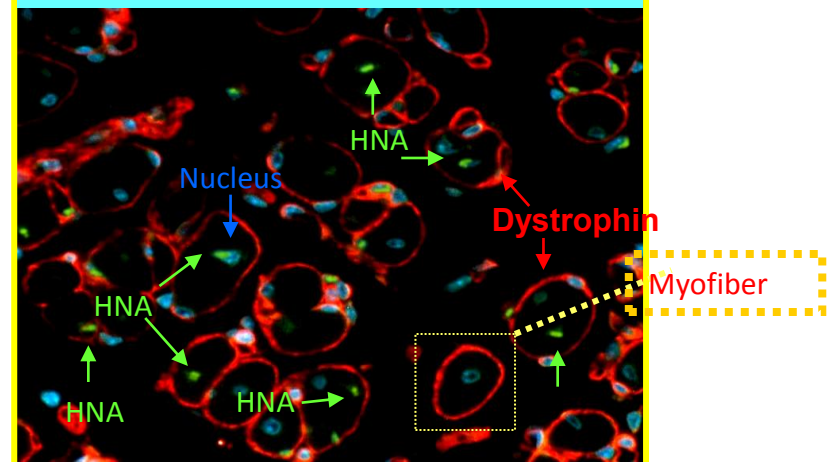
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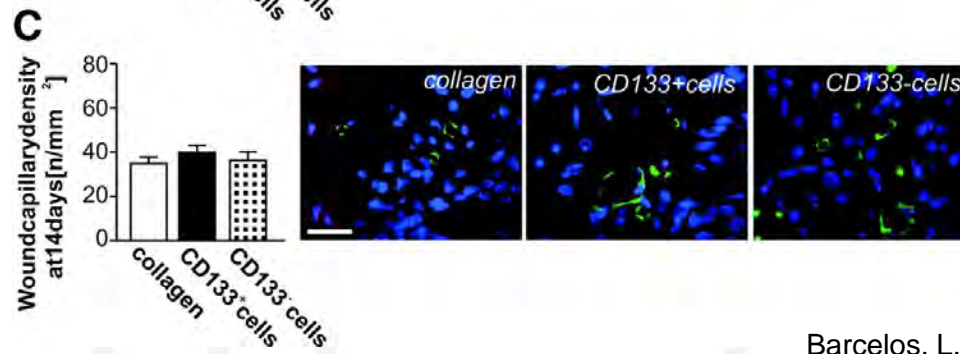
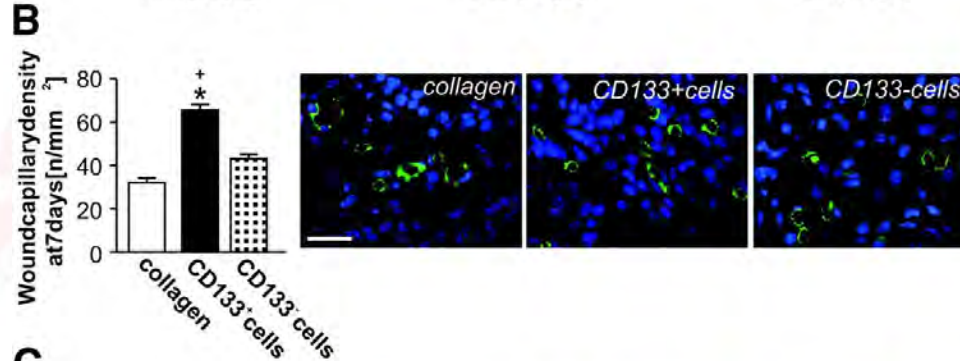
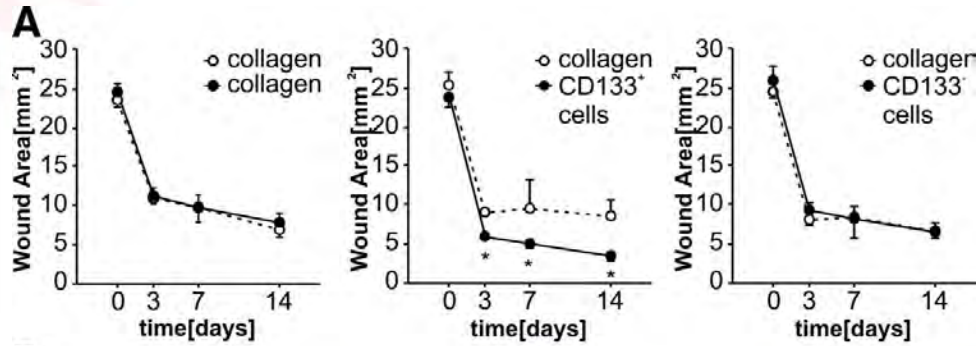
hVPCs



Human nuclear antigen (HNA) staining



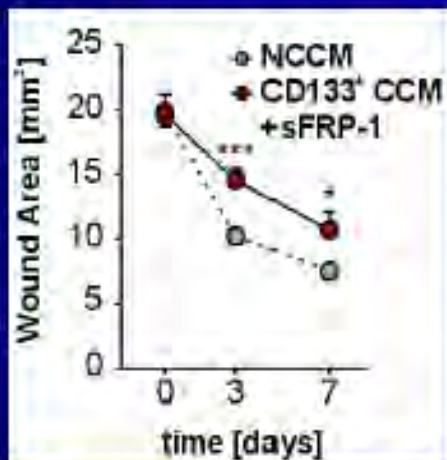
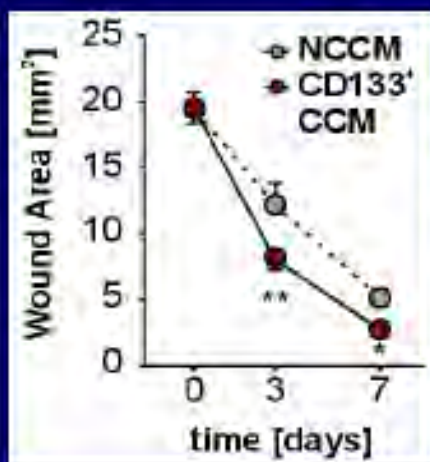
Accelerated wound closure in the presence of CD133+ cells (A, middle) is associated with higher temporary wound capillarization at day 7 (B)



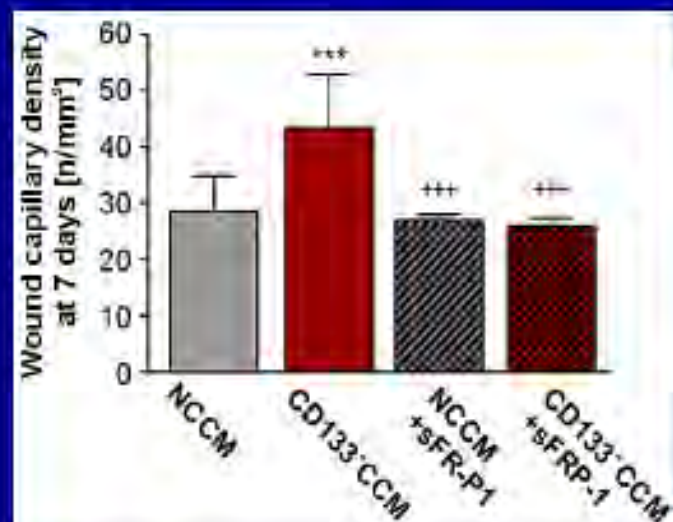
Barcelos, L. S. et al. Circ Res 2009

CD133+ cell conditioned medium activates a Wnt-dependent cicatrisation mechanism

Wound Closure



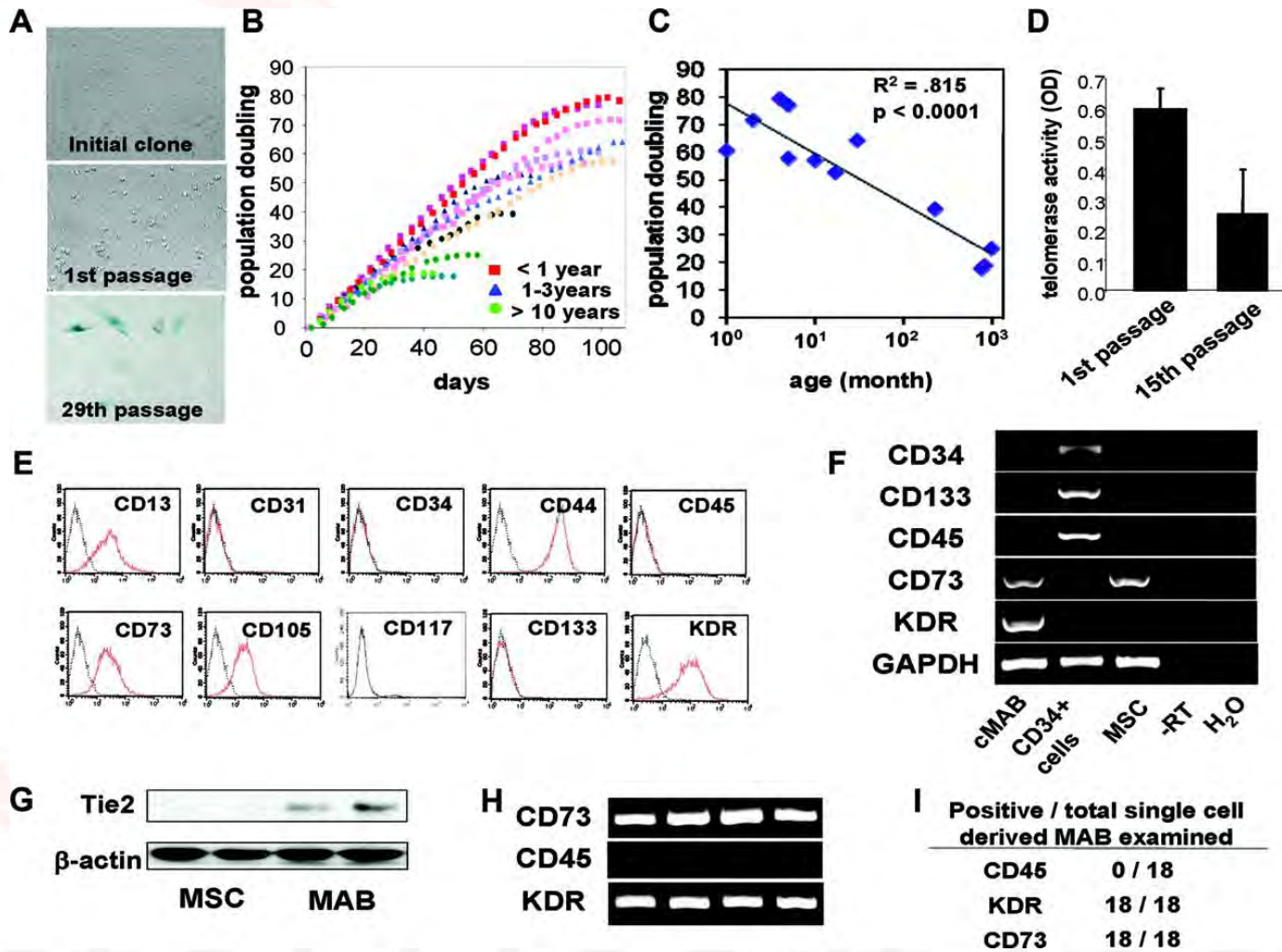
Wound Capillarization



Barcelos, L. S. et al. Circ Res 2009

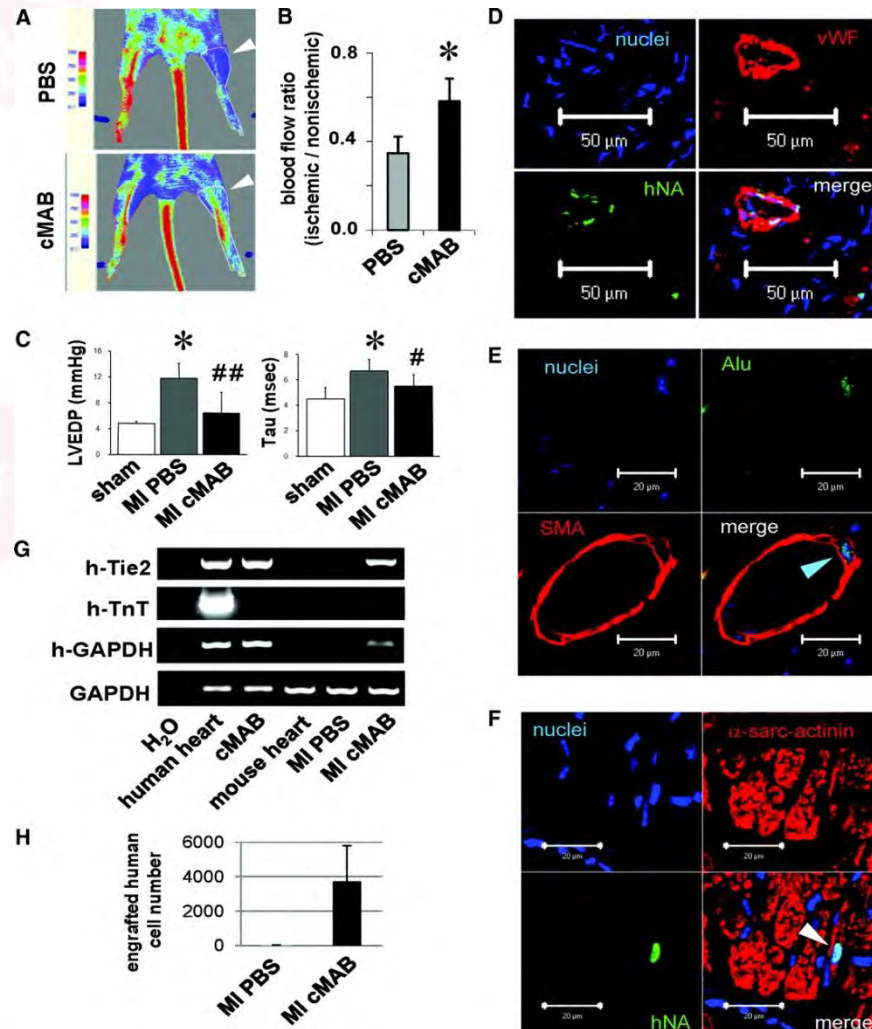
Sox2 Transduction Enhances Cardiovascular Repair Capacity of Blood-Derived Mesangioblasts

Characterization of children-derived circulating progenitor cells



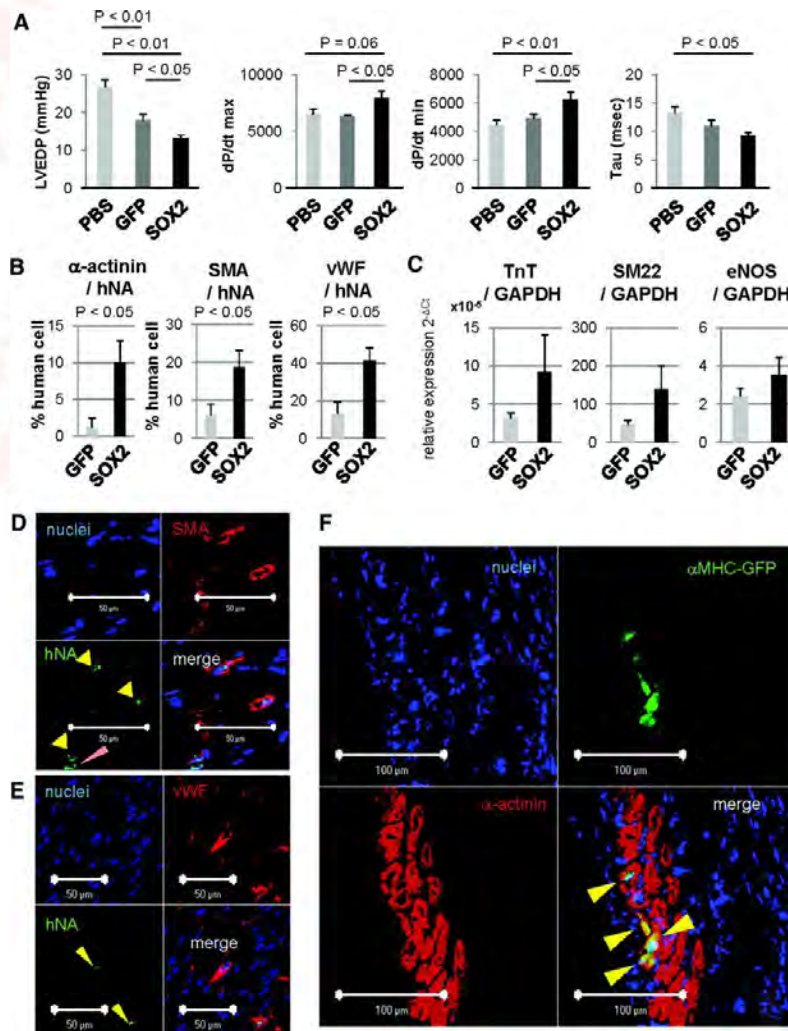
Koyanagi, M. et al. Circ Res 2010;106:1290-1302

Cell therapy with circulating MABs from children after induction of hind limb ischemia (A and B) or myocardial infarction (MI) (C through G)



Koyanagi, M. et al. Circ Res 2010;106:1290-1302

Sox2 increased the differentiation and therapeutic capacity of cMABs



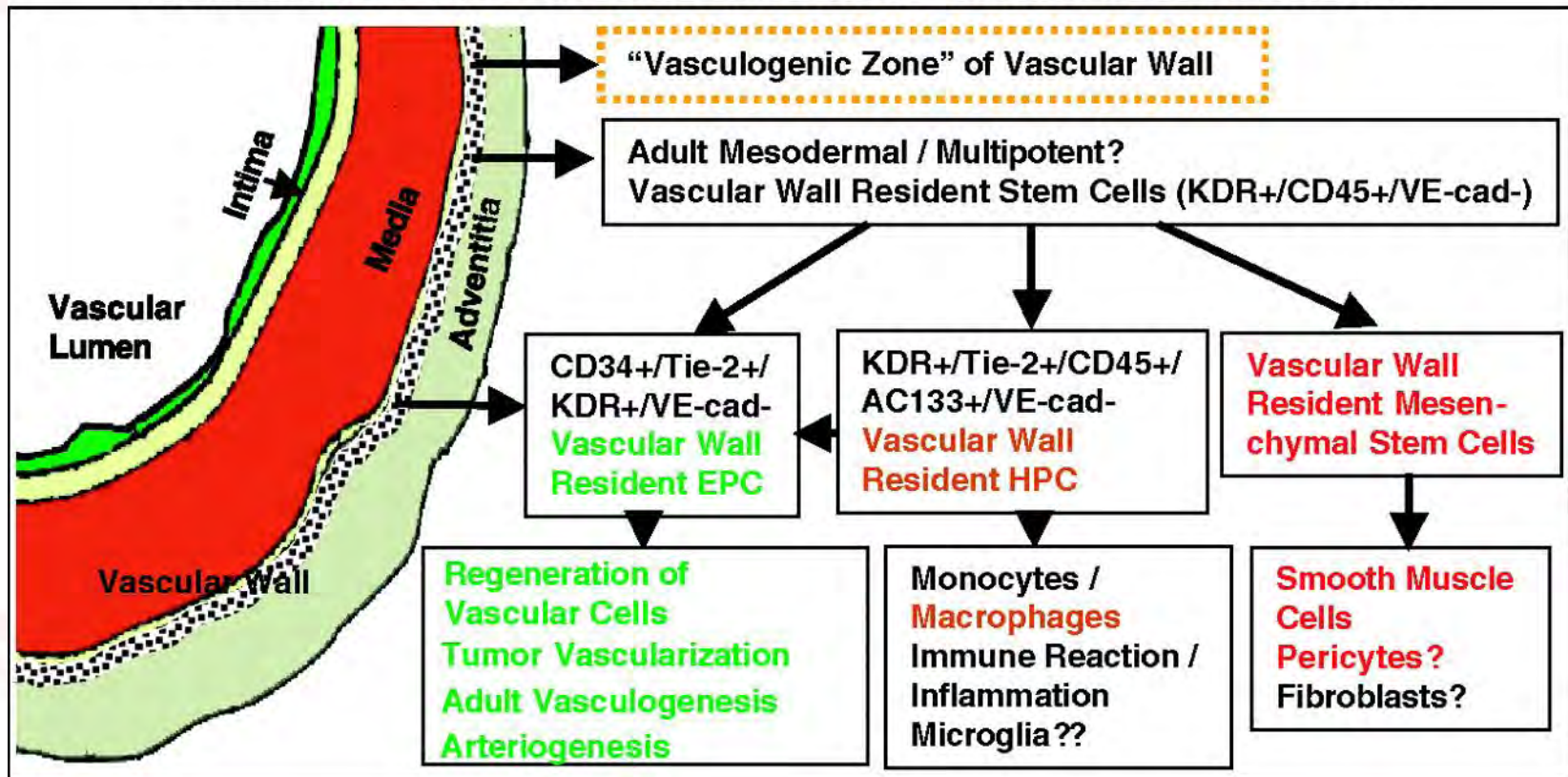
Koyanagi, M. et al. *Circ Res* 2010;106:1290-1302

Question 3: Which layer of the adult blood vessel contains progenitor cells

- 1. Intima*
- 2. Media*
- 3. Adventitia*
- 4. All the layers*

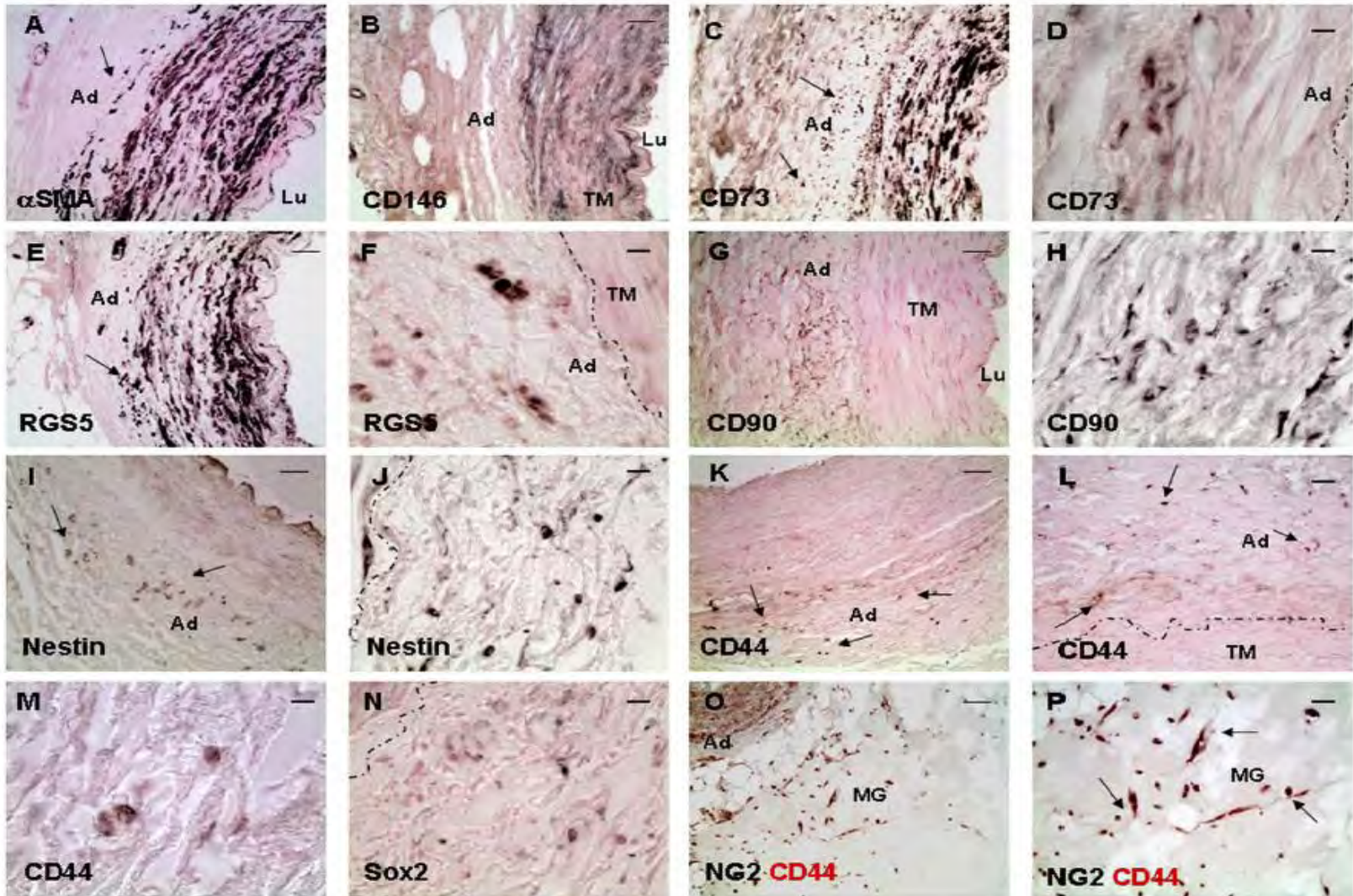
Hypothetical scheme of the 'vasculogenic zone'

Adult Vascular Wall Resident Stem Cells / Endothelial Precursor Cells

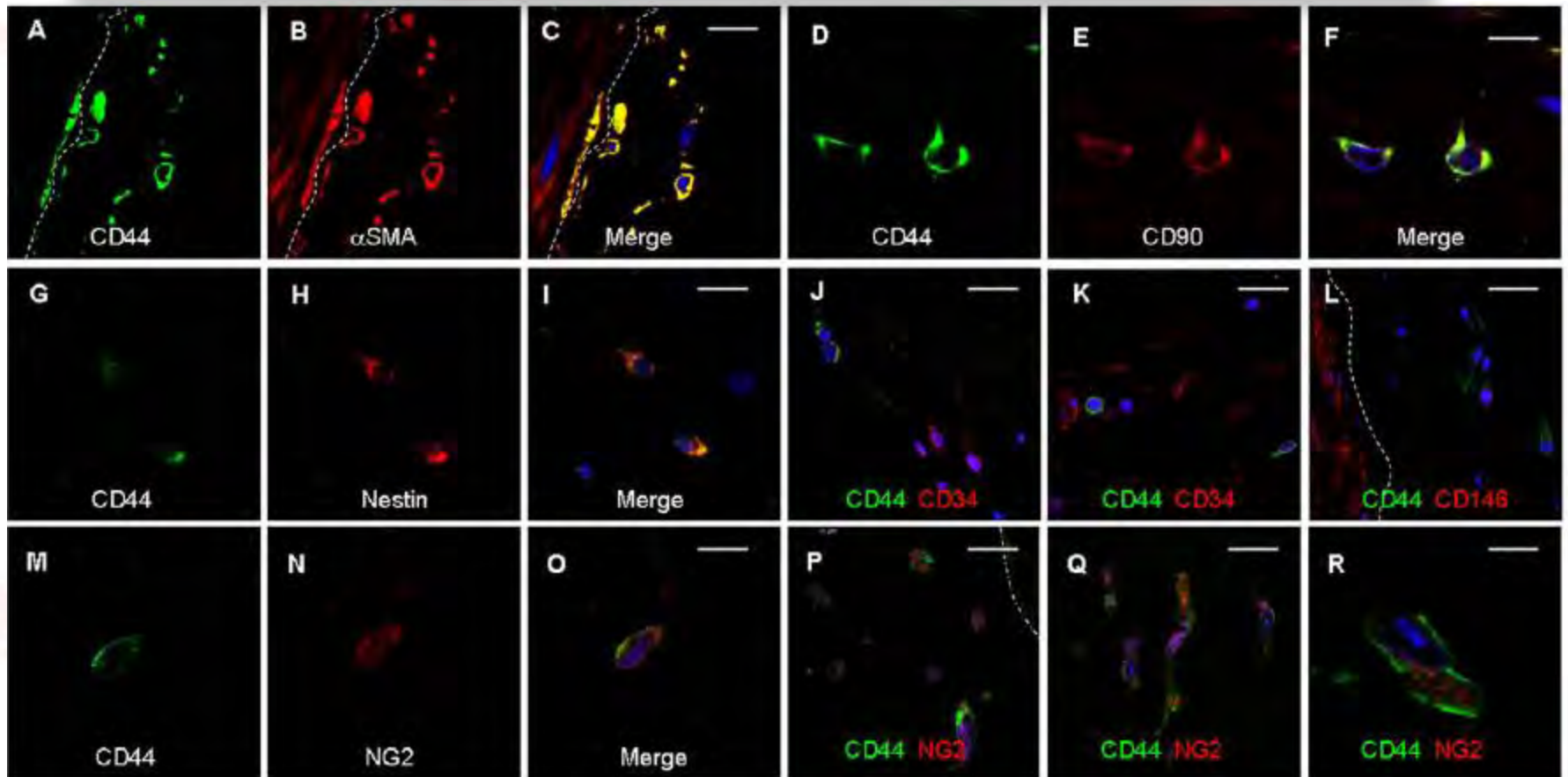


Zengin E et al. Development 2006;133:1543-1551

Immunostaining of cells of the 'vasculogenic zone'

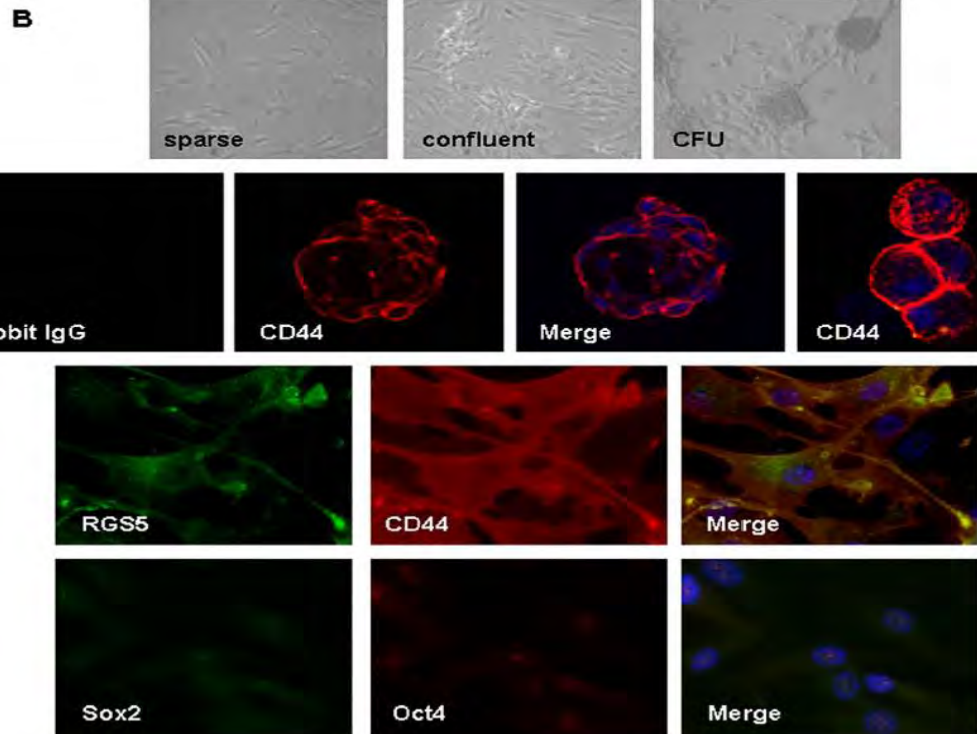
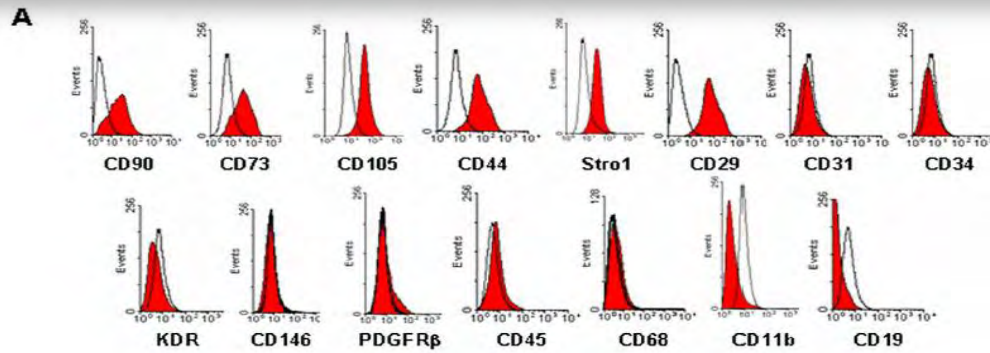


Double-immunostainings of hITA sections using antibodies against typical MSC maker proteins and CD44



← ring assay studies →

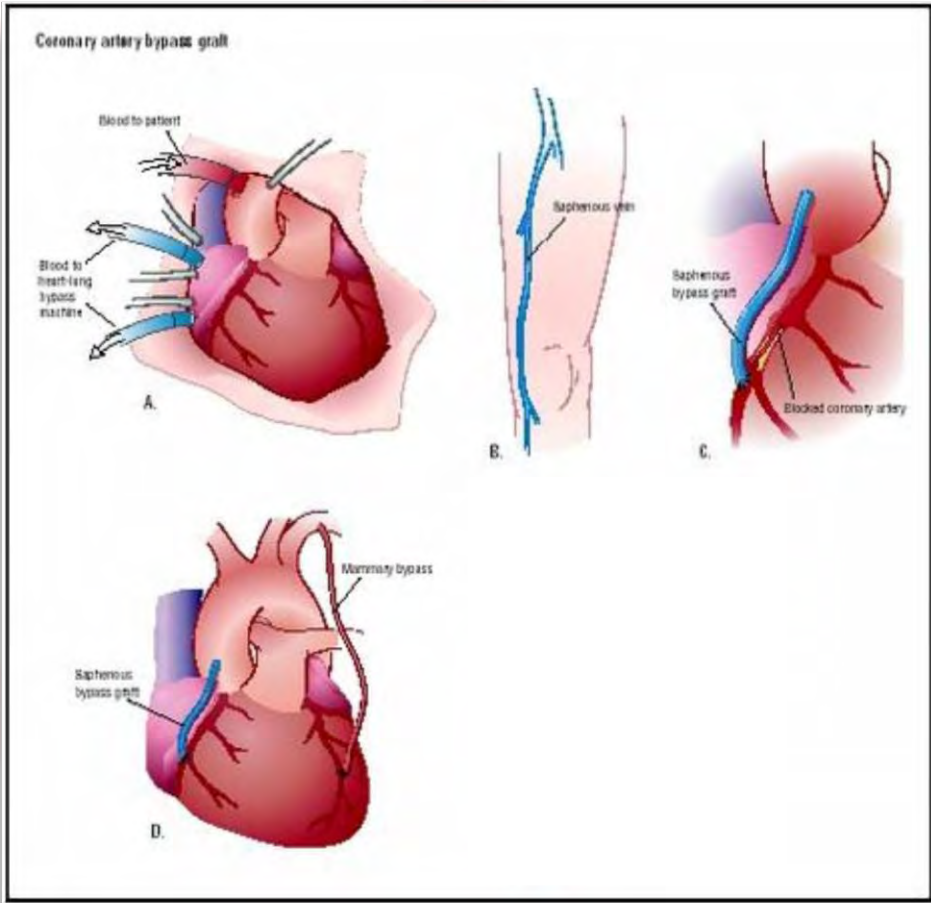
FACS analysis of CD44 sorted cells, CFU cultures and ICC of cultured cells



Question 4: Which vessel is most commonly use for bypass and is therefore a source of vscular progenitor cells

- 1. Mammary artery*
- 2. Saphenous vein*
- 3. Radial Artery*
- 4. Carotid artery*

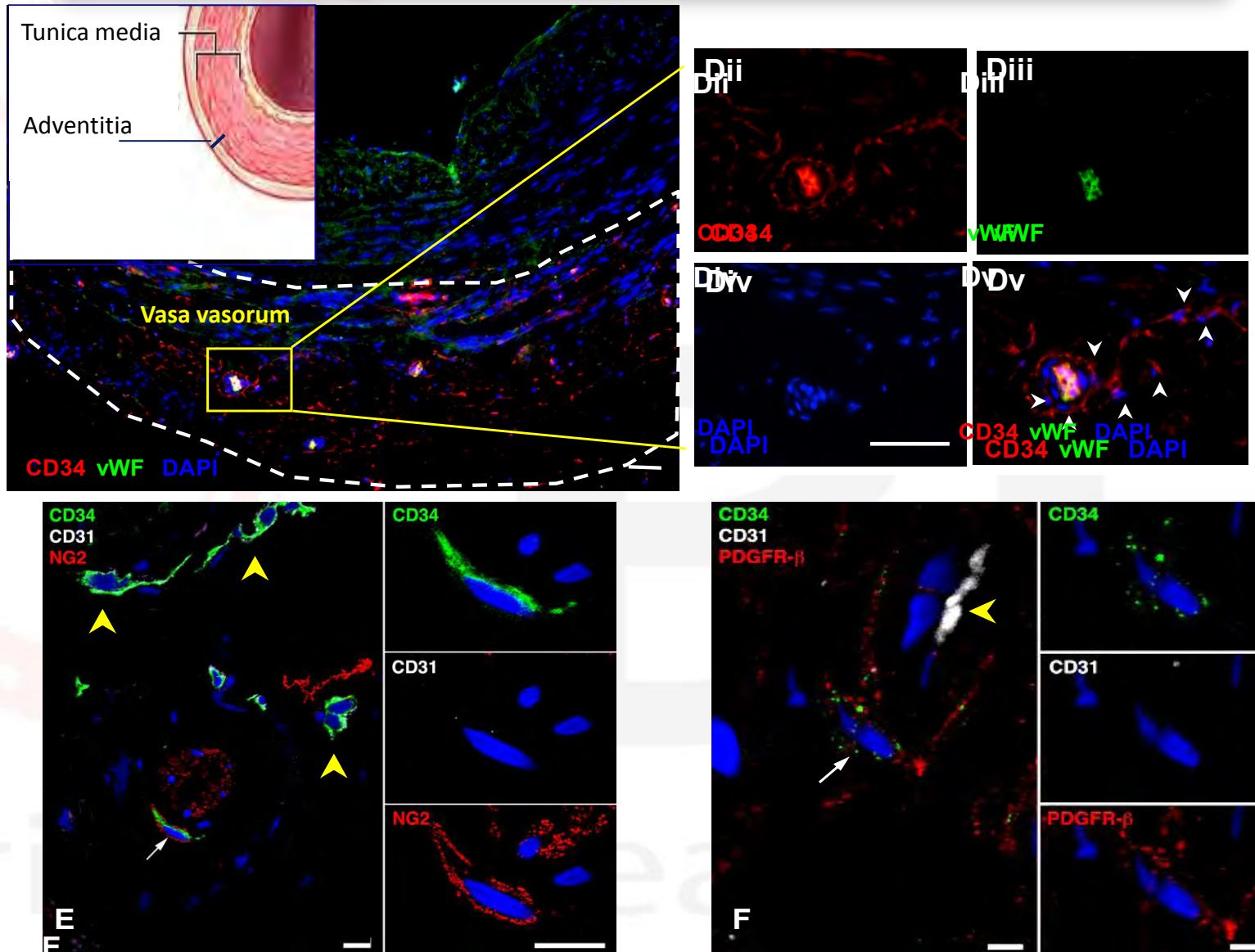
The saphenous vein: a convenient source of pericyte progenitor cells



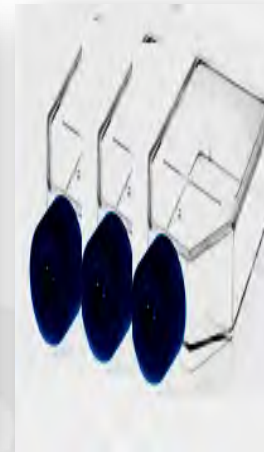
Vein leftover



Identification of pericytes in human saphenous vein vasa vasorum



Saphenous vein pericytes (SVP) Current protocol and roadmap to clinical development



SVP project progression

SVP cell prototype generation *1

Ex vivo expansion
GMP Manufacture

Molecular
Mechanisms

Validation
In mouse model
of ischemia

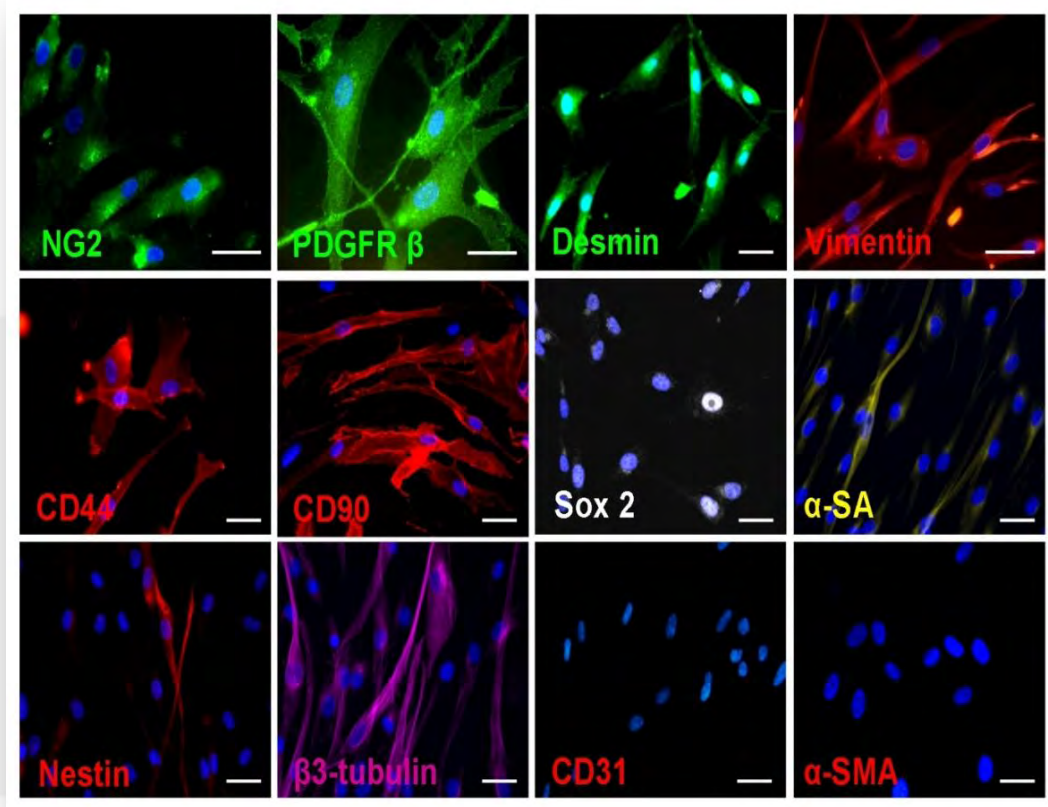
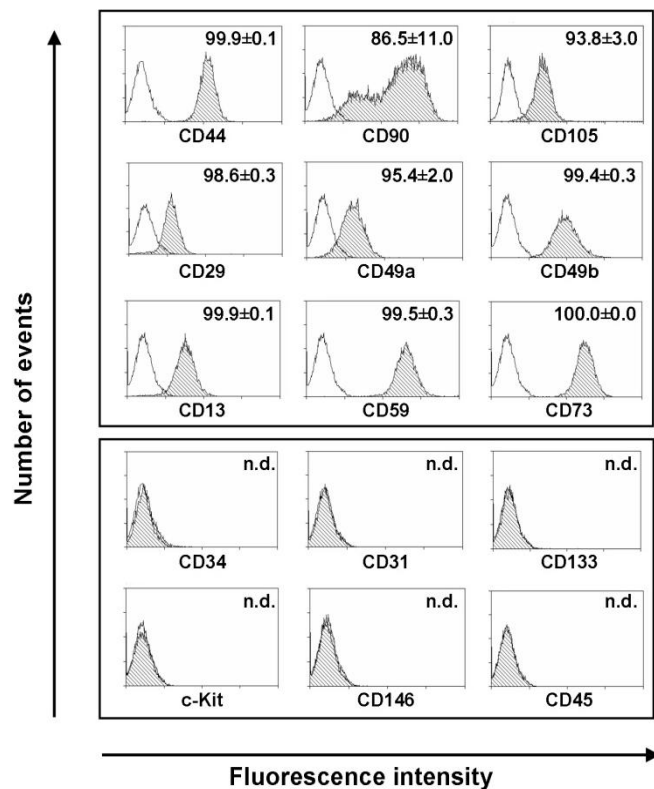
Combinatory
approaches using
cardiac stem cells

Validation in a
larger animal model

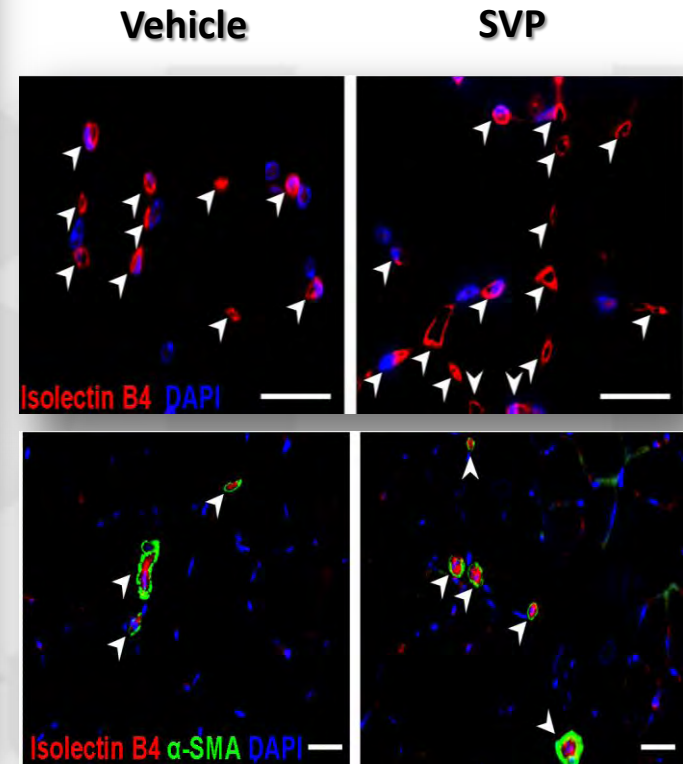
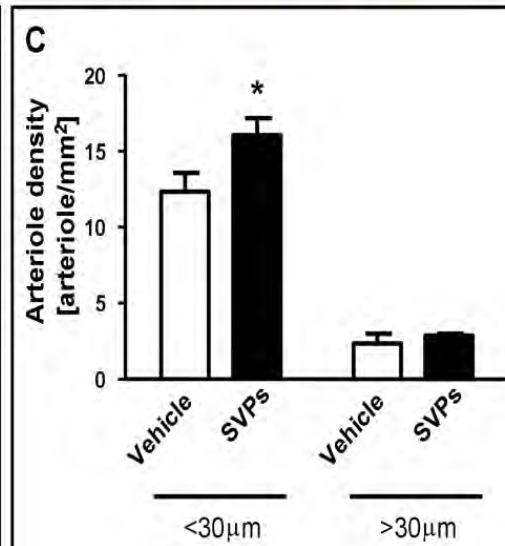
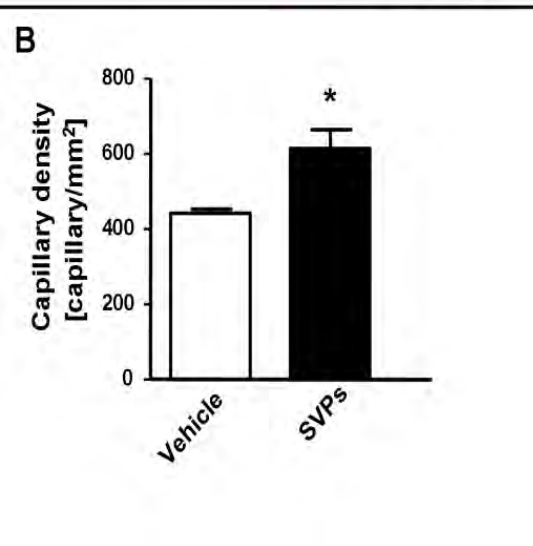
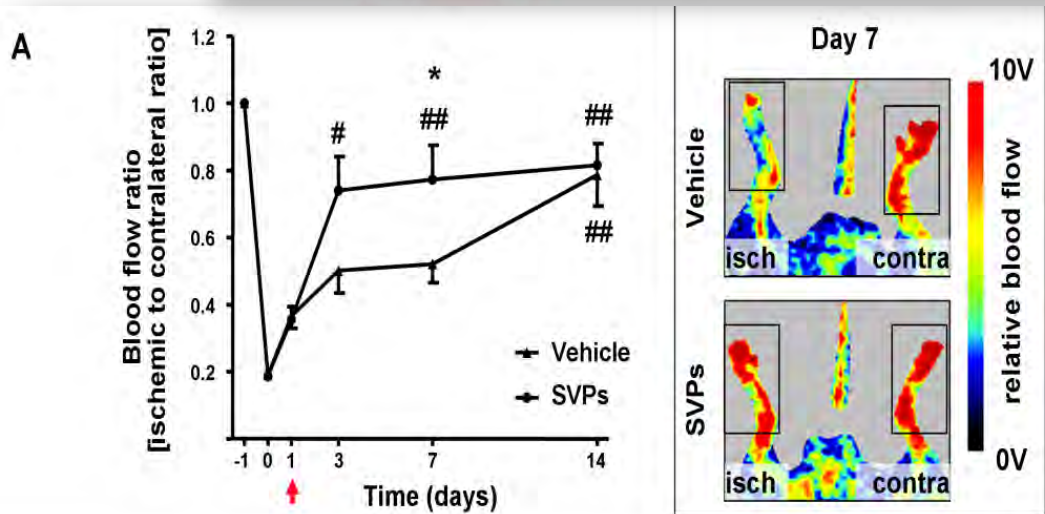
Phase I
Clinical Trial

*1: Campagnolo et al., 2010, *2: .

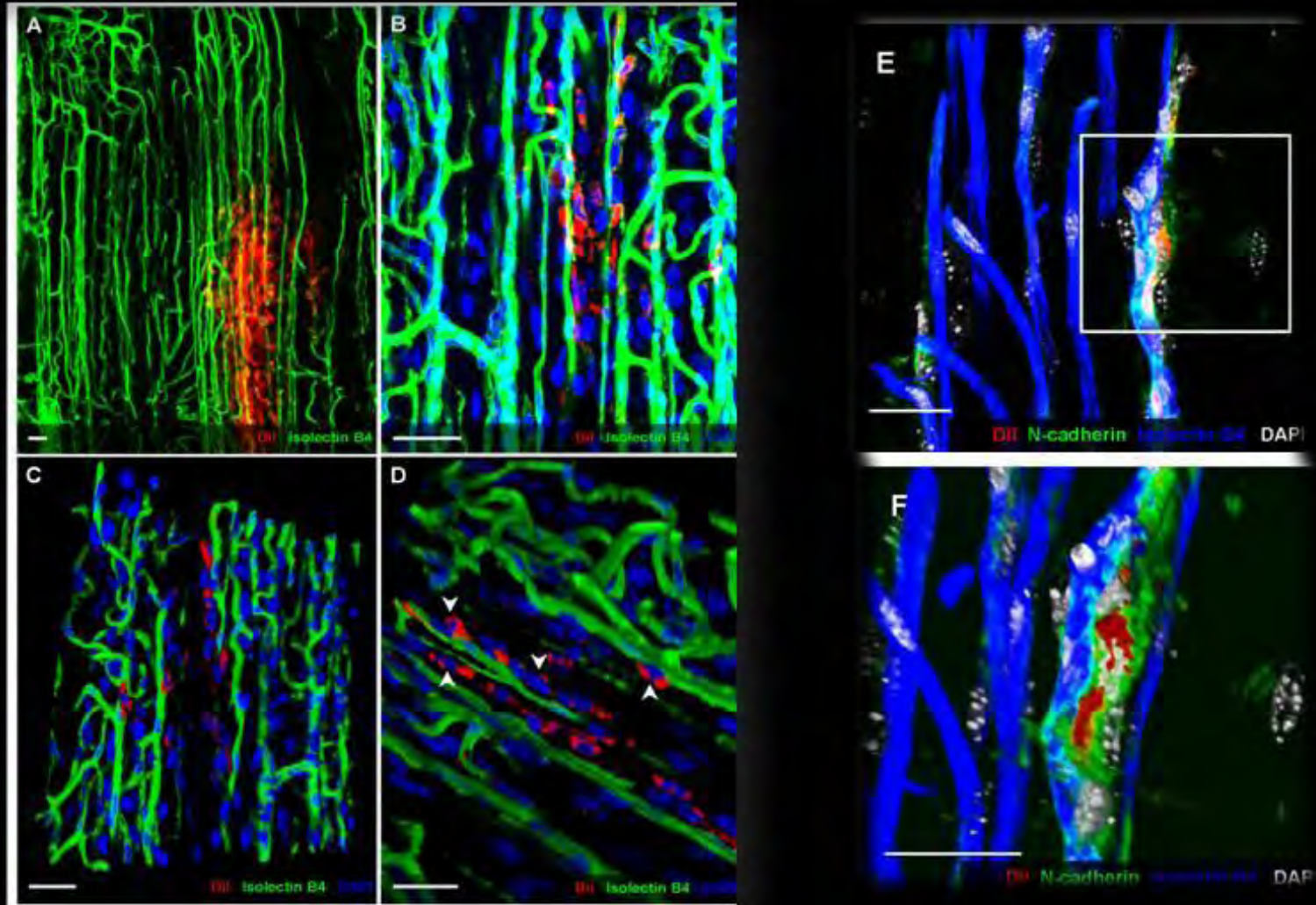
Expansion and characterization of SVP from polyclonal and monoclonal preparations



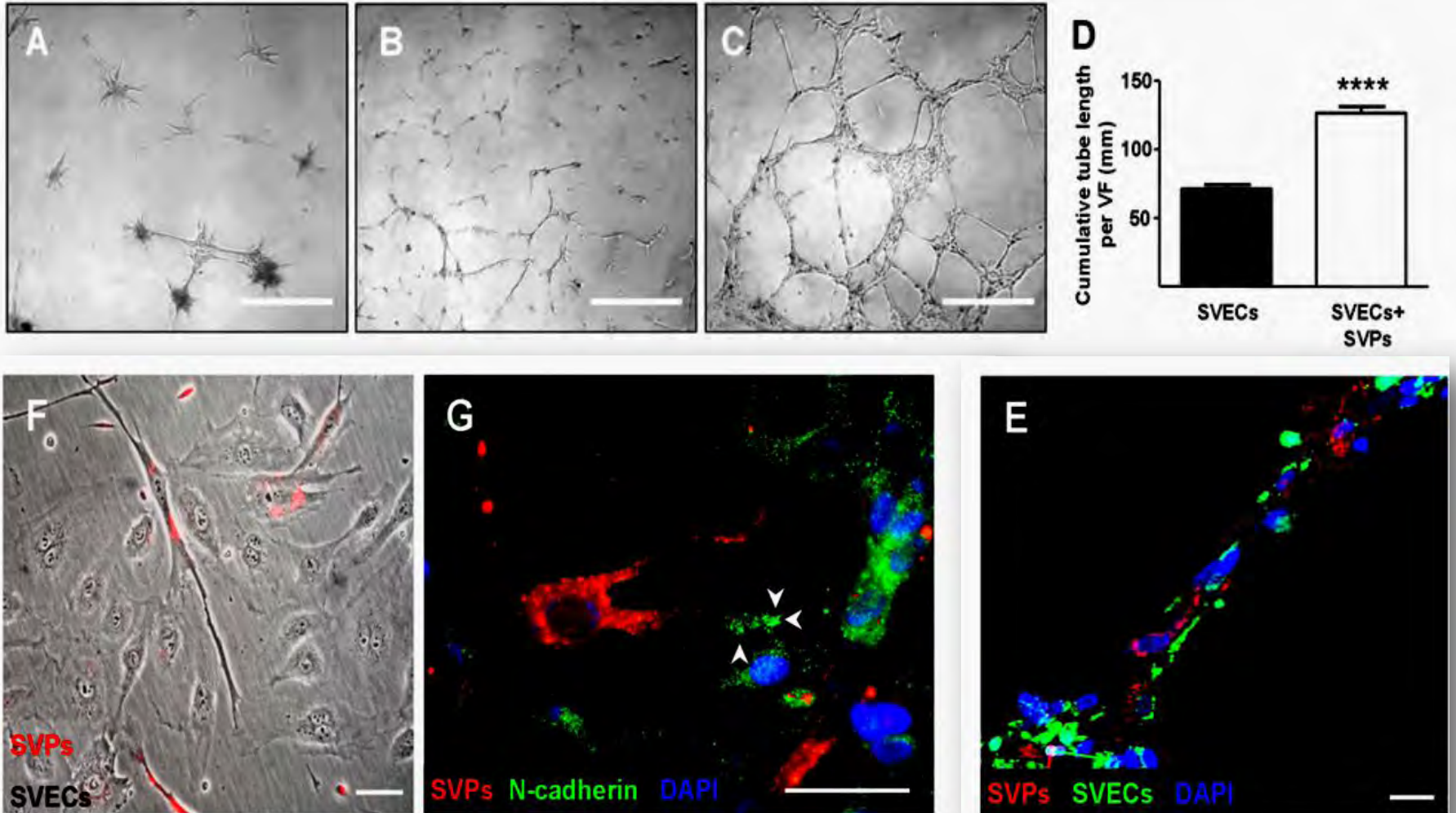
Human SVP improve post-ischemic recovery in a model of limb ischemia



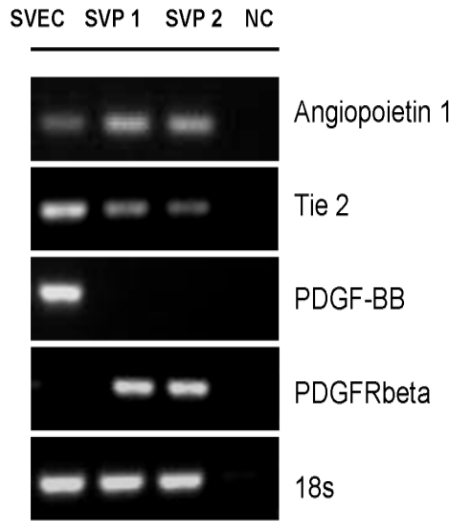
Human SVP establish physical contact with endothelial cells



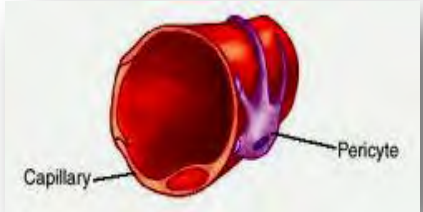
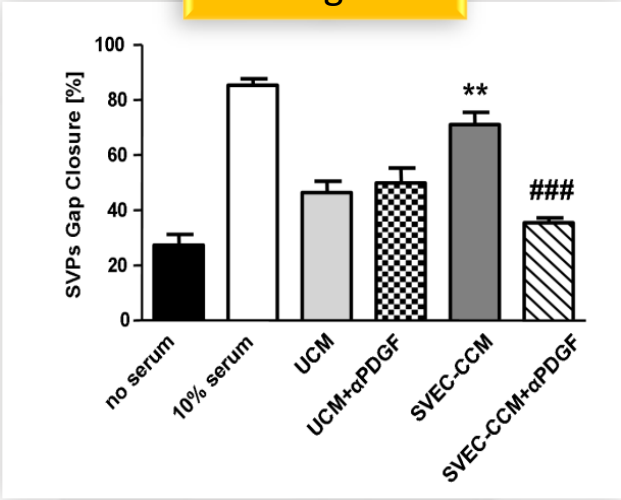
Physical interaction results in enhancement of vascular tube formation



Paracrine interactions with endothelial cells

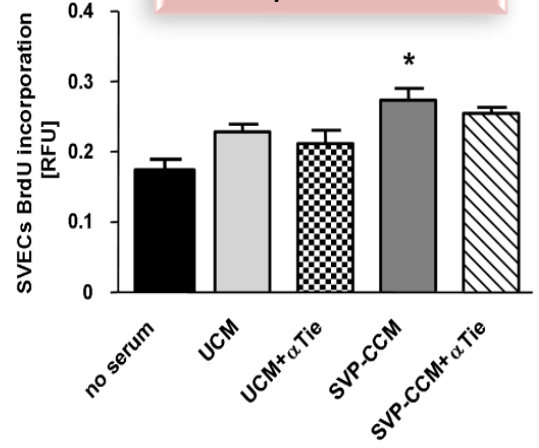


SVP migration

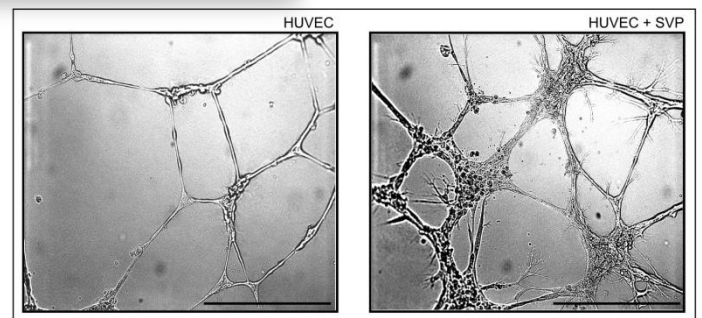
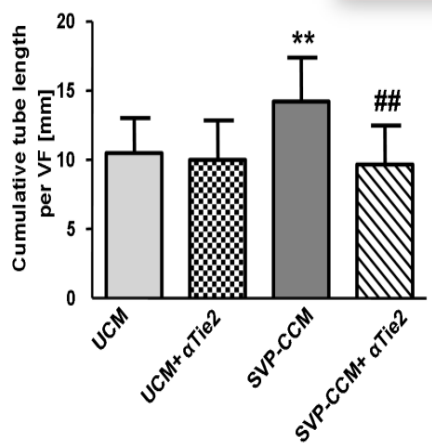


Born to be paracrine

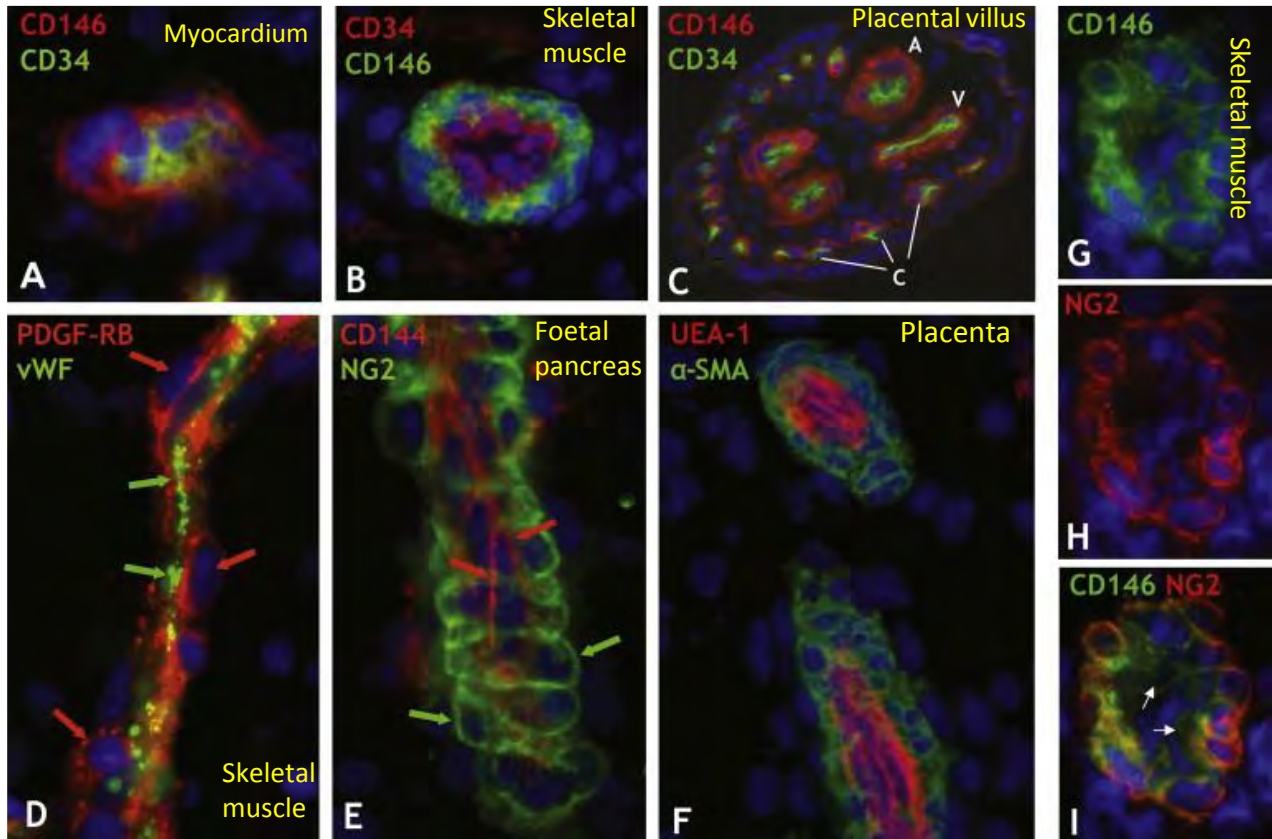
SVEC proliferation



SVEC tube formation



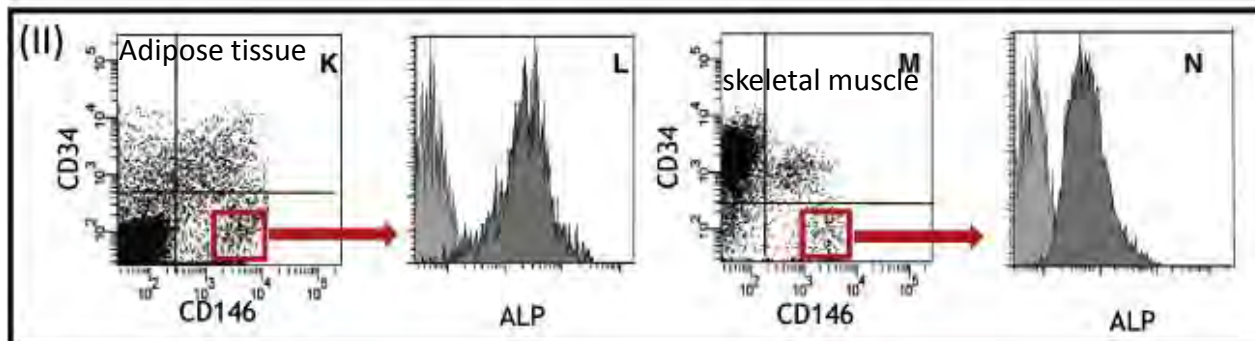
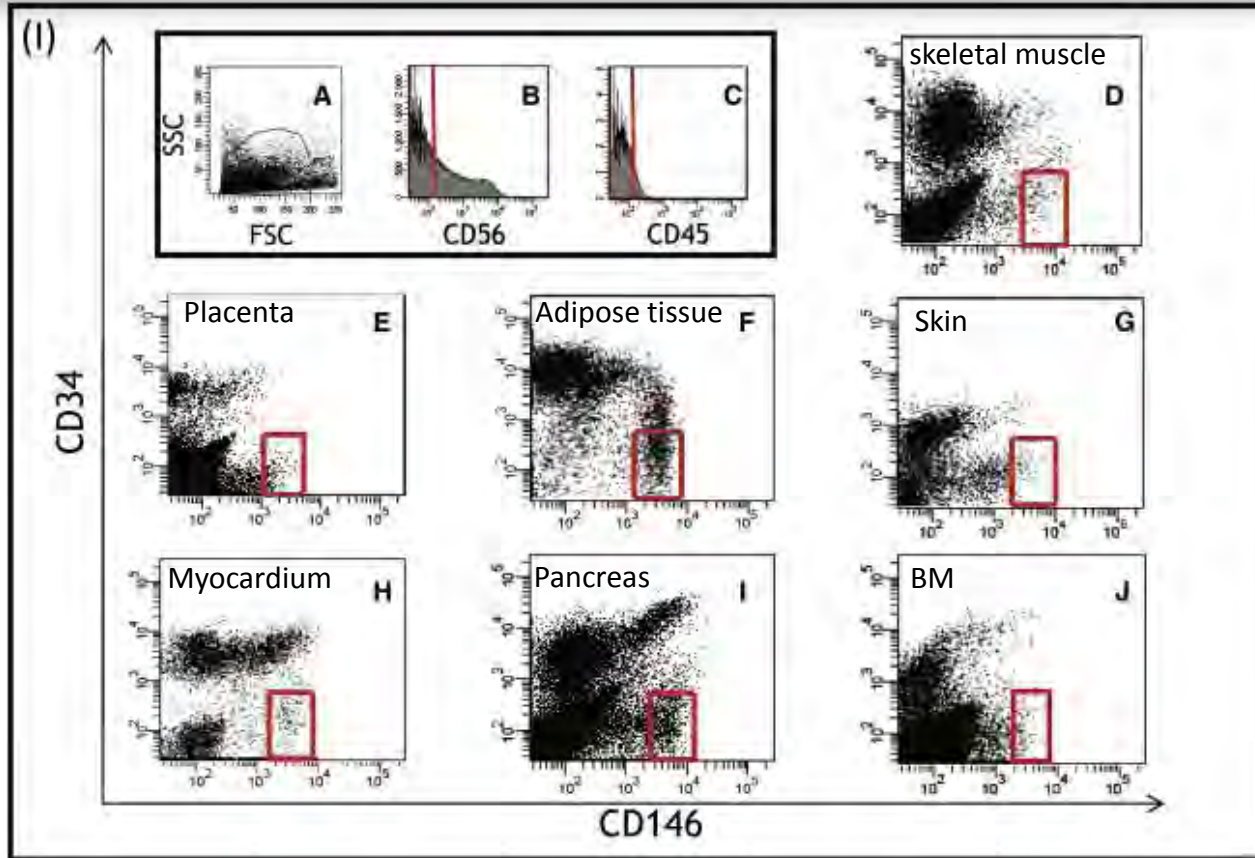
A Perivascular Origin for Mesenchymal Stem Cells in Multiple Human Organs



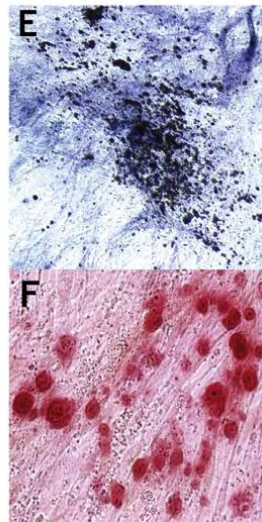
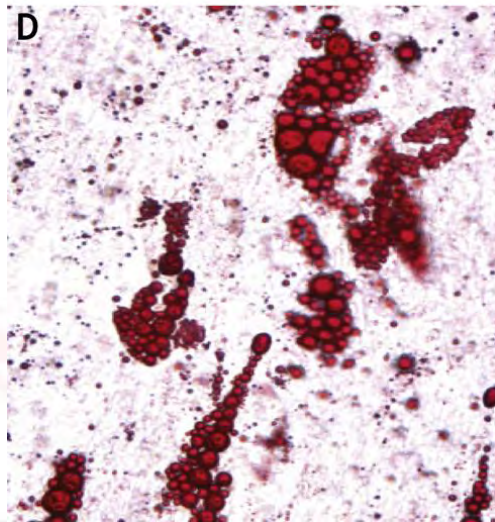
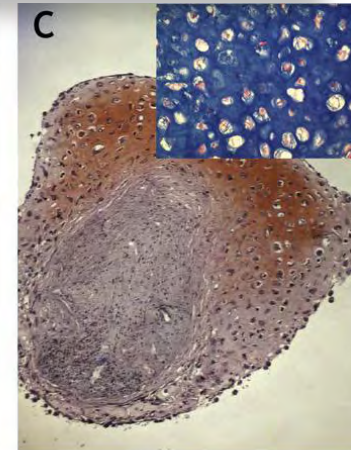
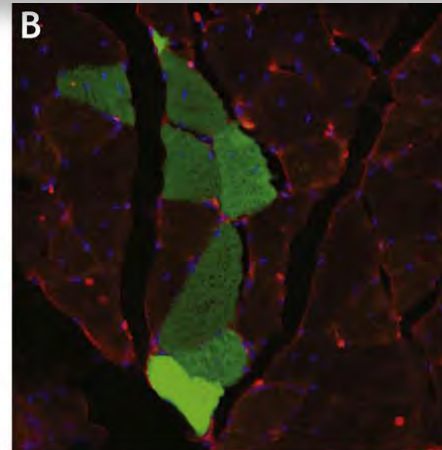
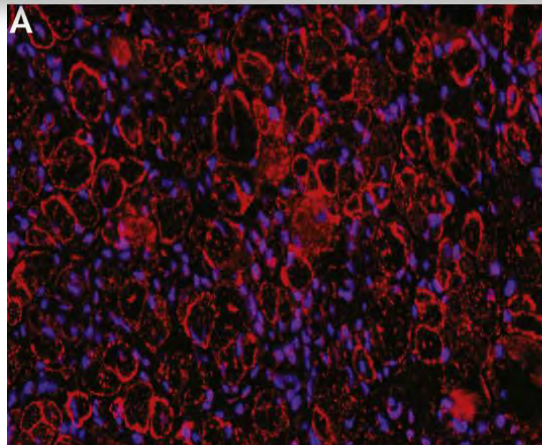
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Crisan et al Cell Stem Cell 2008

FACS Analysis of tissue perivascular cells from Multiple Human Organs



Multilineage Potential of Long-Term Cultured Perivascular Cells




Multilineage Potential of Cells From the Artery Wall

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Background—In diabetes or atherosclerosis, ectopic bone, fat, cartilage, and marrow often develop in arteries. However the mechanism is unknown. We have previously identified a subpopulation of vascular cells (calcifying vascular cells, CVC), derived by dilutional cloning of bovine aortic medial cells, and showed that they undergo osteoblastic differentiation and mineralization. We now show that CVC have the potential to differentiate along other mesenchymal lineages.

Methods and Results—To determine the multilineage potential of CVC, molecular and functional markers of multiple mesenchymal lineages were assessed. Chondrogenic potential of CVC was evidenced by expression of types II and IX collagen and cytochemical staining for Alcian blue. Leiomyogenic potential of CVC was evidenced by the expression of smooth muscle- α actin, calponin, caldesmon, and myosin heavy chain. Stromogenic potential of CVC was evidenced by the ability to support growth of colony-forming units of hematopoietic progenitor cells from human CD34⁺ umbilical cord blood cells for a period of 5 weeks. Adipogenic potential was not observed. CVC were immunopositive to antigens to CD29 and CD44 but not to CD14 or CD45, consistent with other mesenchymal stem cells. CVC retained multipotentiality despite passaging and expansion through more than 20 to 25 population doublings, indicating a capacity for self-renewal.


Conclusions—These results suggest that the artery wall contains cells that have the potential for multiple lineages similar to mesenchymal stem cells but with a unique differentiation repertoire. (*Circulation*. 2003;108:2505-2510.)



Abundant progenitor cells in the adventitia contribute to atherosclerosis of vein grafts in ApoE-deficient mice

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