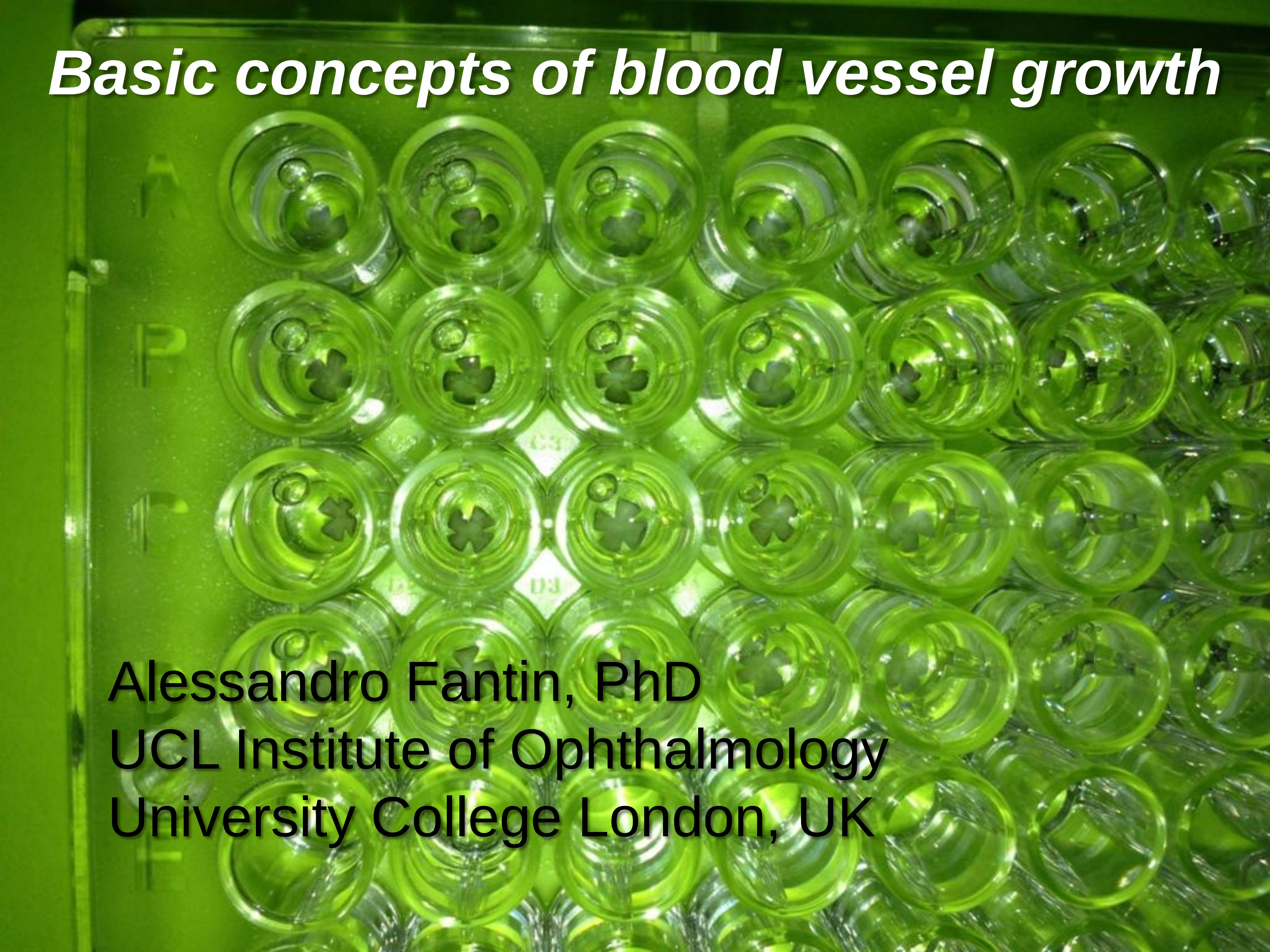


Basic concepts of blood vessel growth

Alessandro Fantin, PhD
UCL Institute of Ophthalmology
University College London, UK

Blood vessel formation

4 main processes:

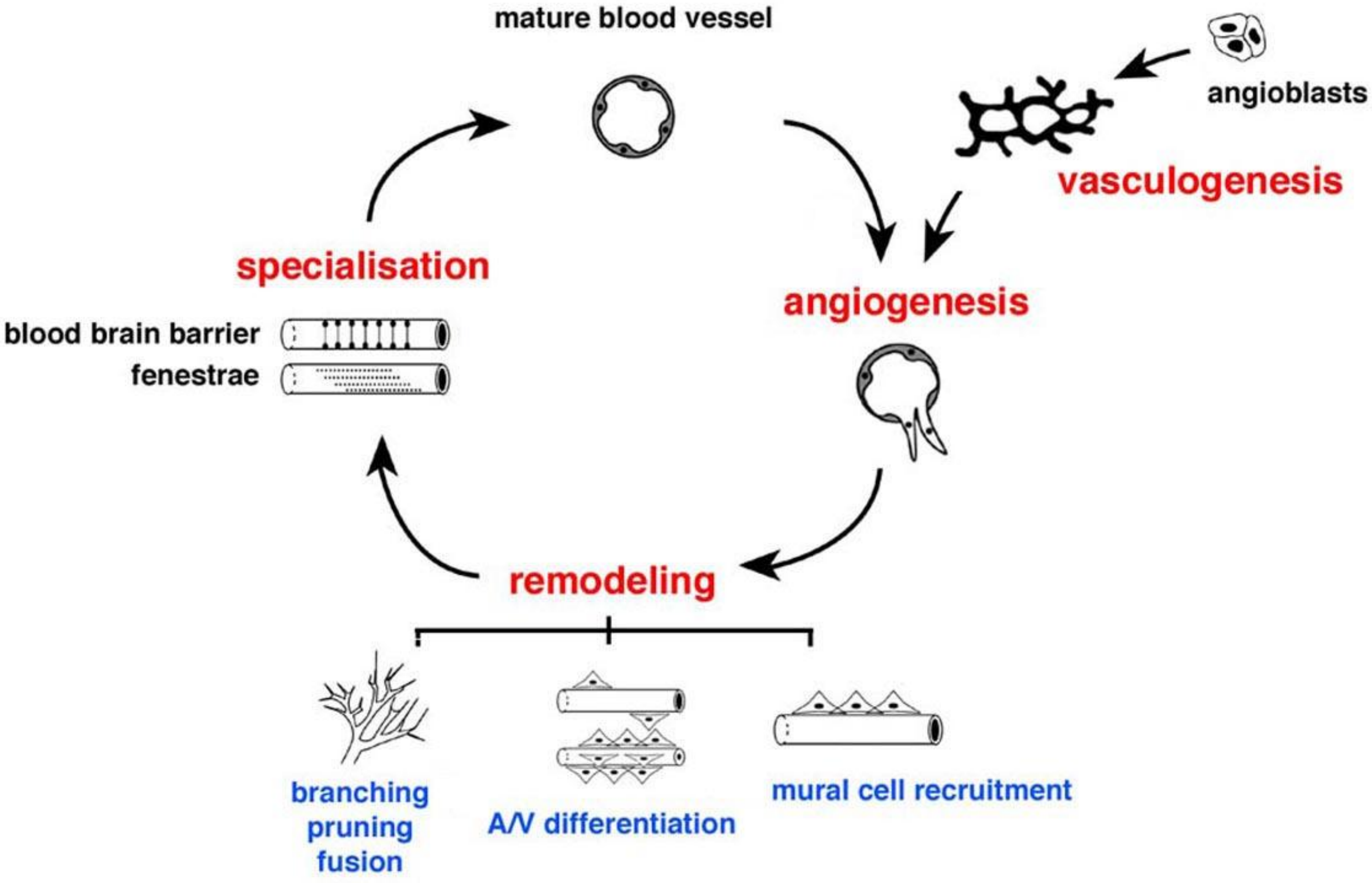
1) vasculogenesis
2) angiogenesis

➢ establish vascular network

3) vascular remodelling
4) endothelial specialisation

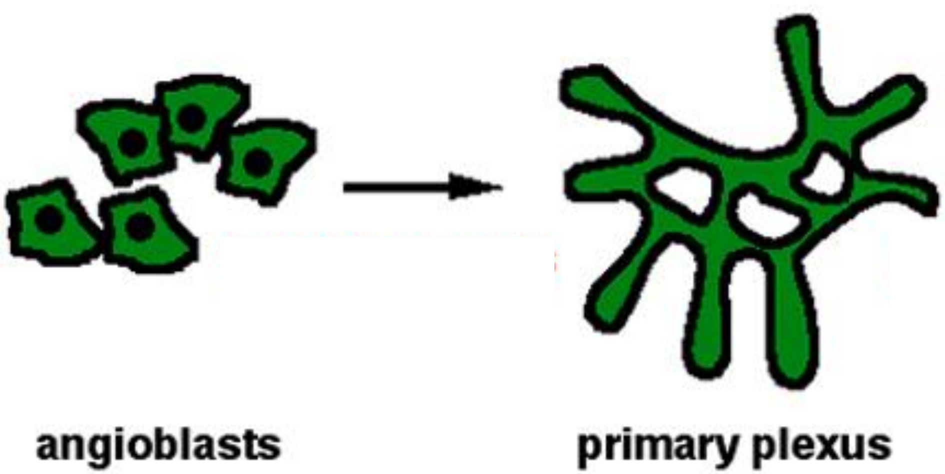
➢ modify the network to physiological needs

the "blood vessel cycle"



1. Vasculogenesis

vasculum - duct, vessel; *genesis* - creation

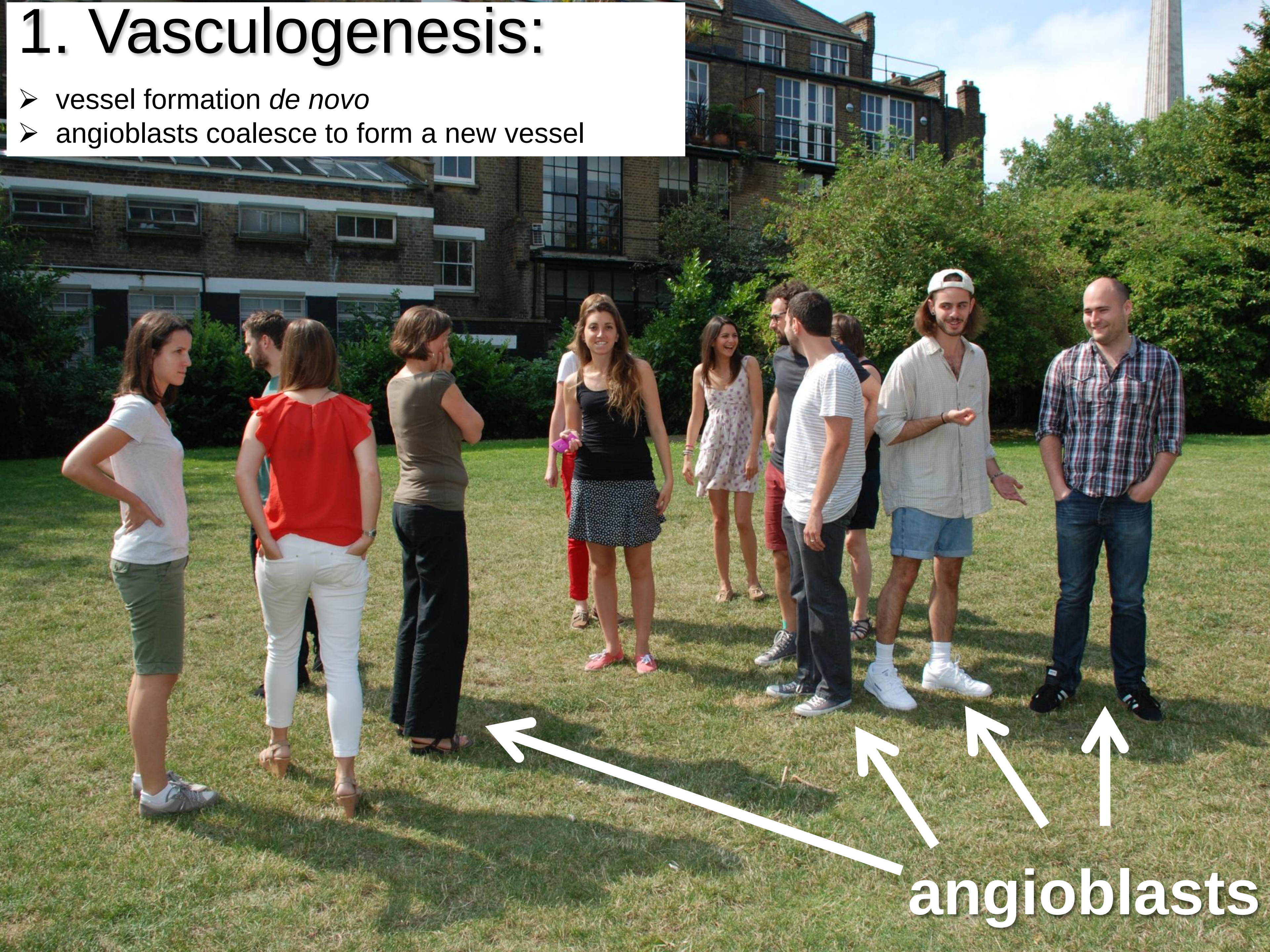


vessel formation *de novo*

Ruhrberg et al., *Bioassays* 2003

1. Vasculogenesis:

- vessel formation *de novo*
- angioblasts coalesce to form a new vessel



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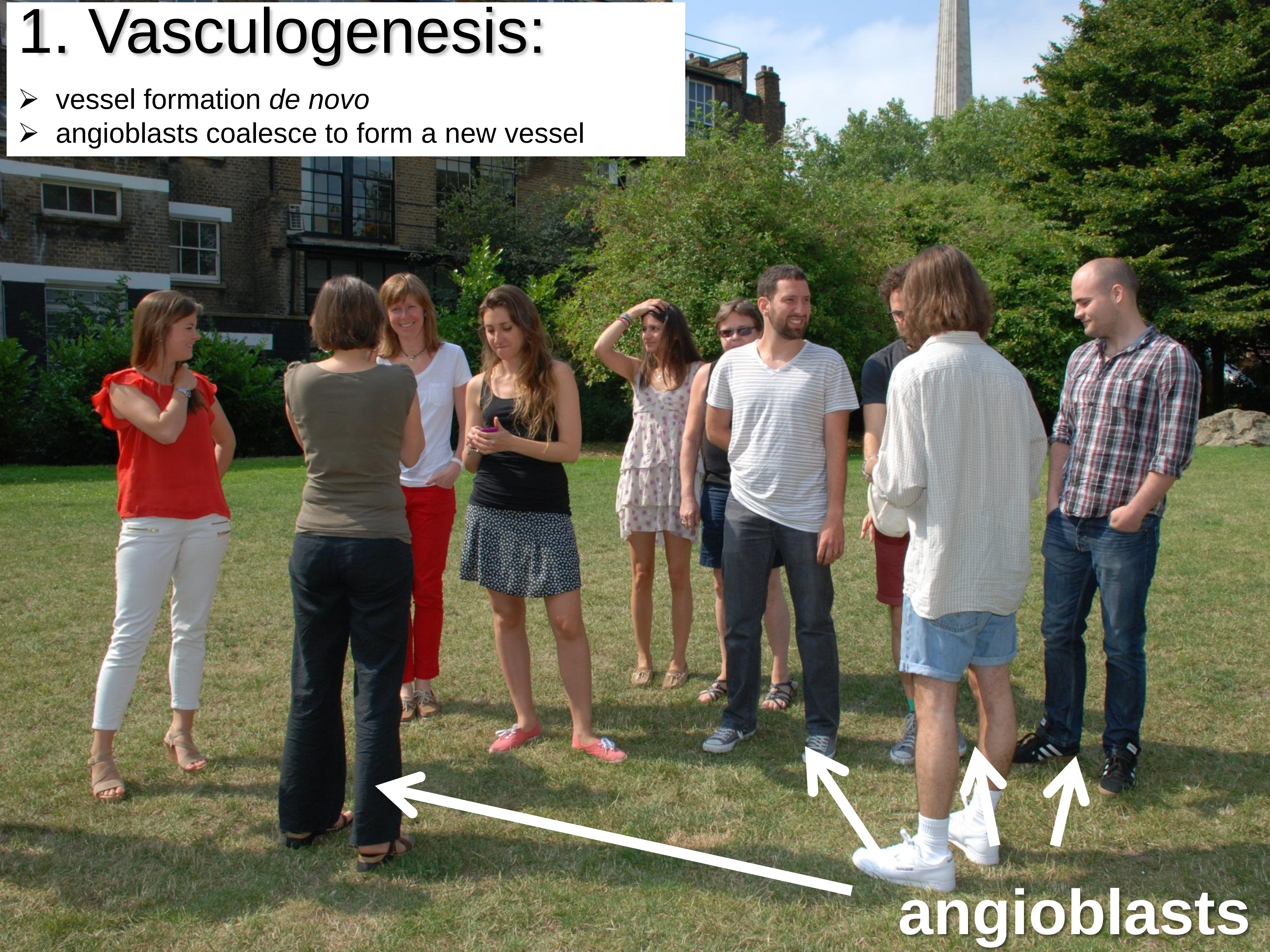

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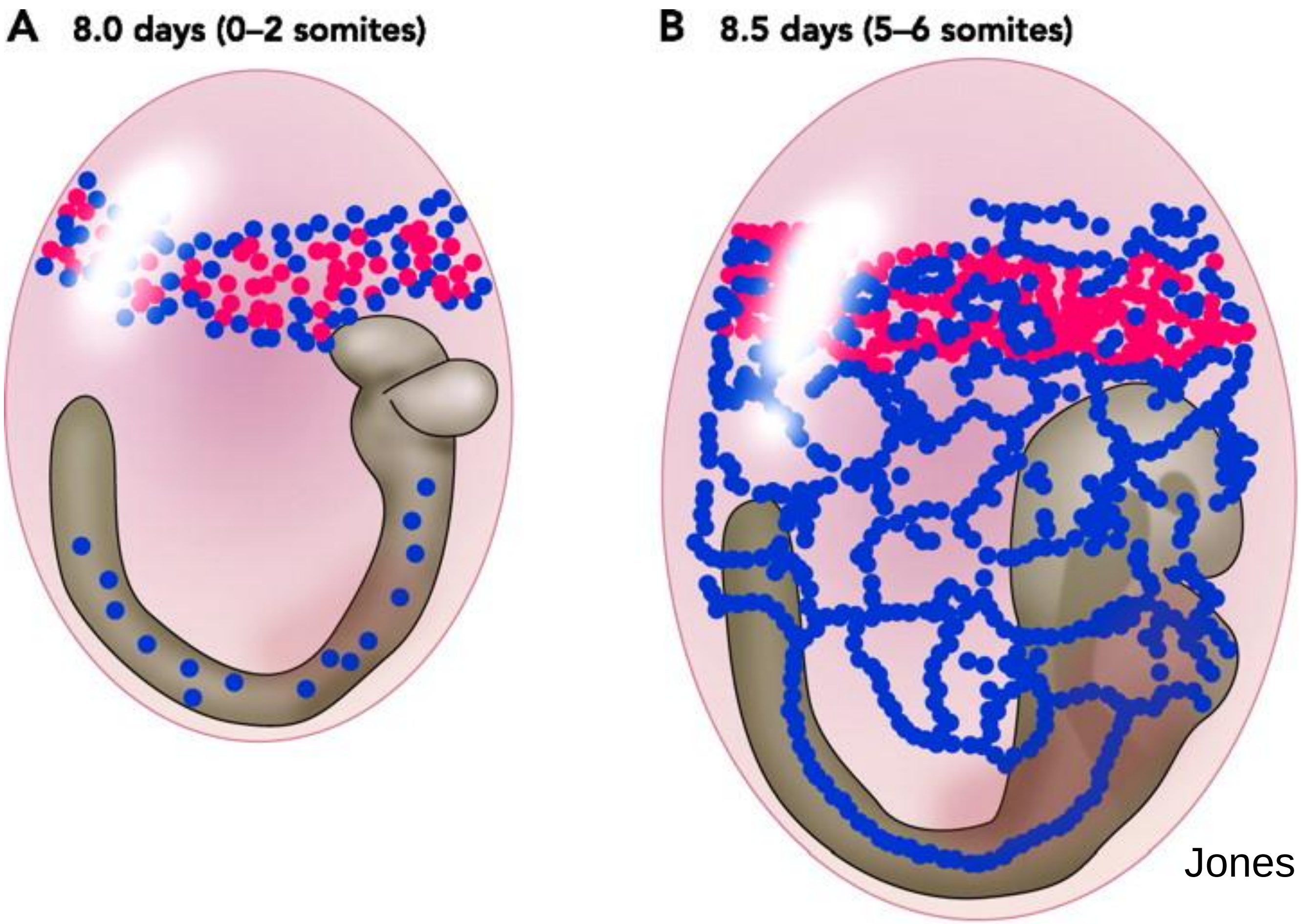


Jones et al., 2006

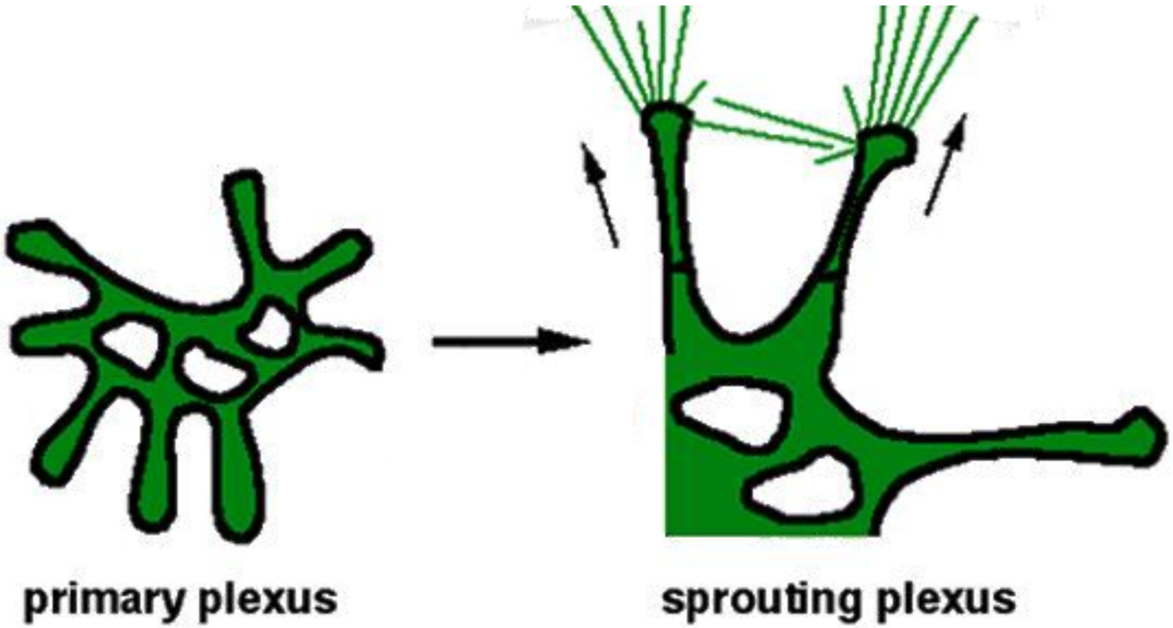
Yolk sac: blood islands

Embryo proper: paired dorsal aortas (& cardinal veins)

2. Angiogenesis

angeion - container, vessel; *genesis* - creation

1787: British surgeon Dr. John Hunter first uses the term "angiogenesis“ to describe blood vessels growing in the reindeer antler.



vessel growth by sprouting (or intussusception) from pre-existing vessels

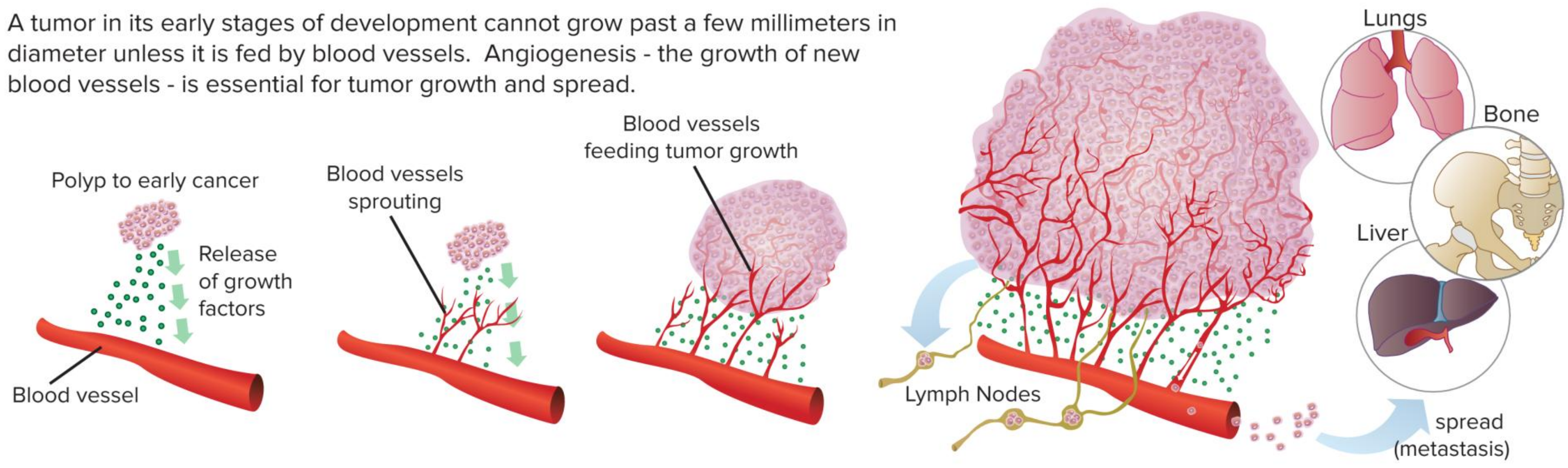
Ruhrberg et al., *Bioassays* 2003

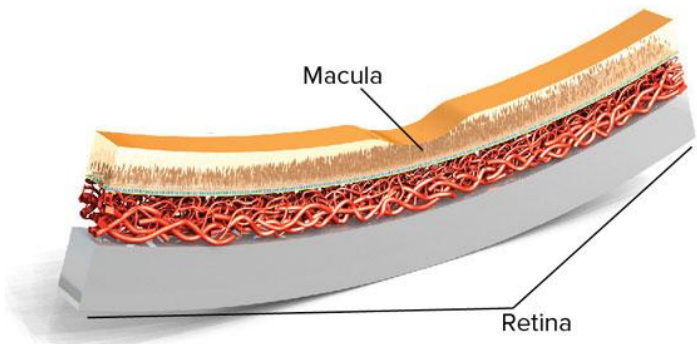
2. Angiogenesis

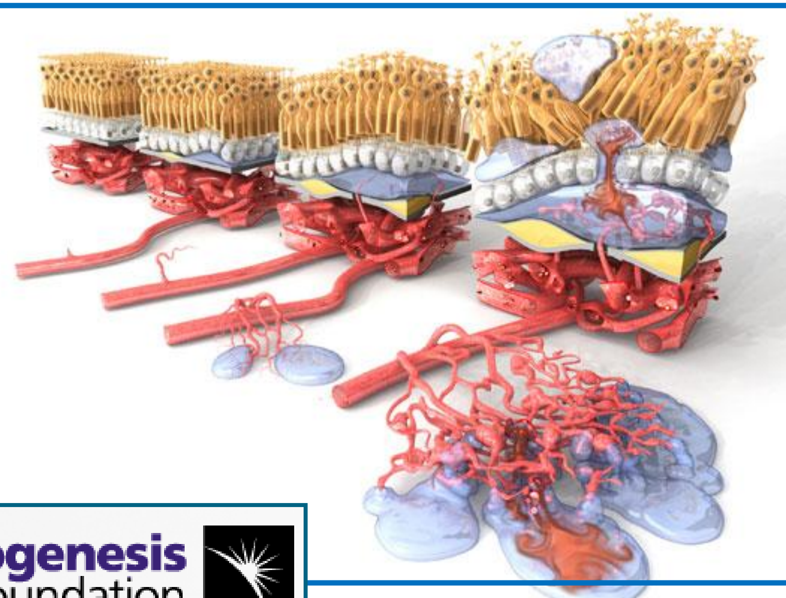
1) vascular expansion from pre-existing vessels
2) sprouting toward avascular areas

Why study angiogenesis?

Abnormal blood vessel growth, either excessive or insufficient, is a “common denominator” underlying many deadly and debilitating conditions (eg **cancer**, **age-related blindness (wet AMD)**, cardiovascular disease, stroke)







The Angiogenesis Foundation

Angiogenesis HISTORY in the clinic

1971: Surgeon Judah Folkman hypothesizes that tumour growth is dependent upon angiogenesis. His theory, published in the *New England Journal of Medicine*, is initially regarded as heresy by leading physicians and scientists.

1989: One of the most important angiogenic factors, vascular endothelial growth factor (VEGF), is discovered Dr. Napoleone Ferrara. It turns out to be identical to a molecule called Vascular Permeability Factor (VPF) discovered in 1983 by Dr. Harold Dvorak.

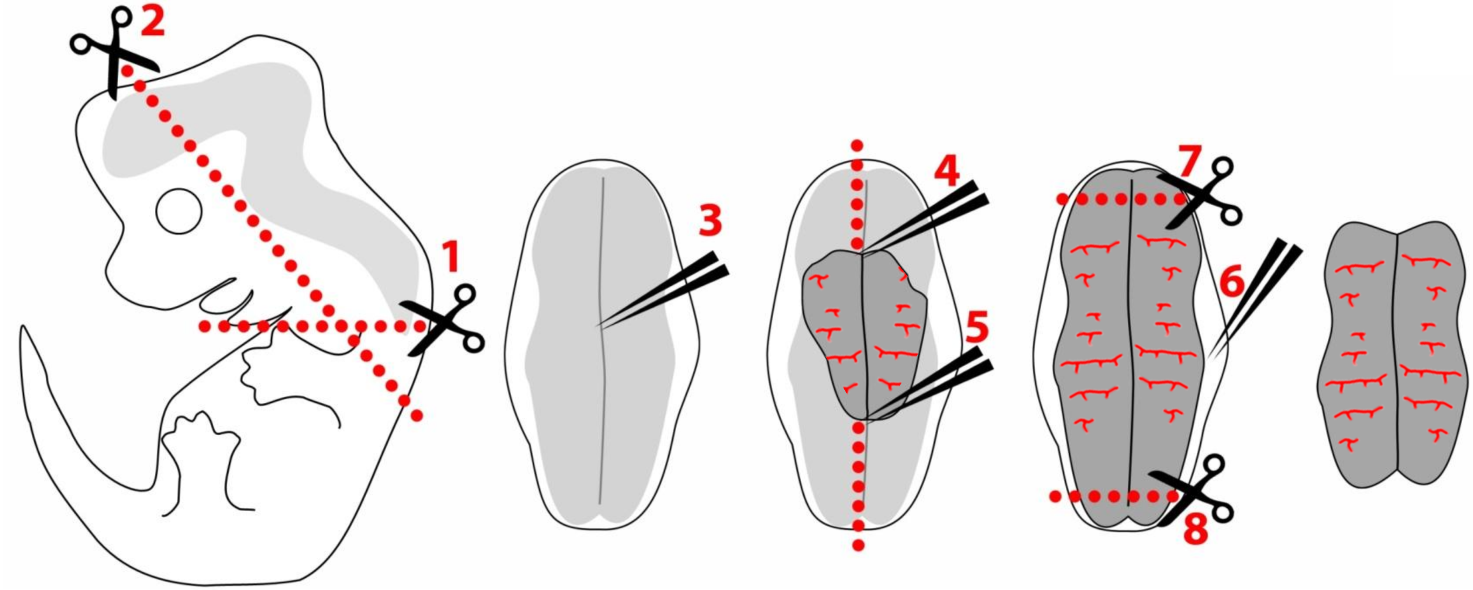
2004: Bevacizumab (Avastin) is FDA approved for the treatment of advanced colorectal cancer. At the time of bevacizumab's approval, FDA Commissioner Mark McClellan declares antiangiogenic therapy "the fourth modality for cancer treatment."

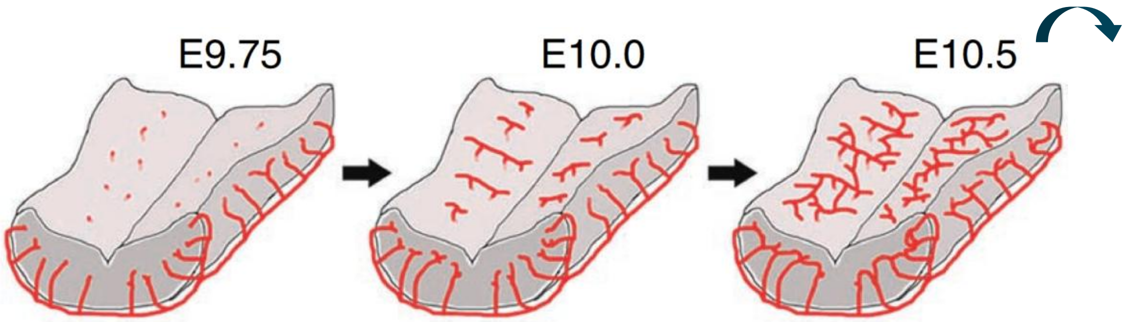
2004: Pegaptanib (Macugen), an anti-VEGF aptamer, becomes the first anti-VEGF drug to be FDA approved for the treatment of age-related macular degeneration.

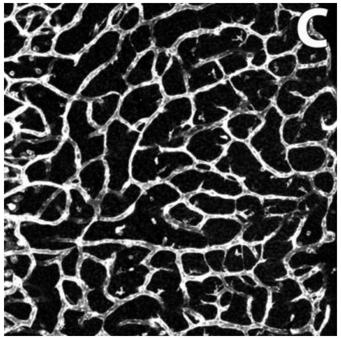
2006: Ranibizumab (Lucentis), a fragment of the bevacizumab molecule, is FDA approved for the treatment of age-related macular degeneration.

Angiogenesis in experimental models

Vascularisation of the mouse embryo hindbrain: sprouting angiogenesis





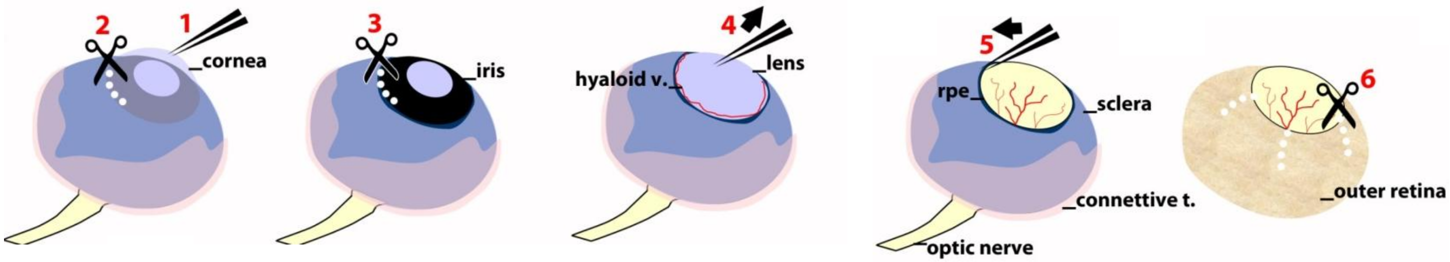


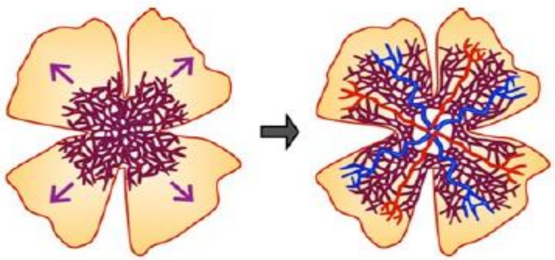
- Vascularisation occurs early in development
- Precise timing
- Limited remodelling

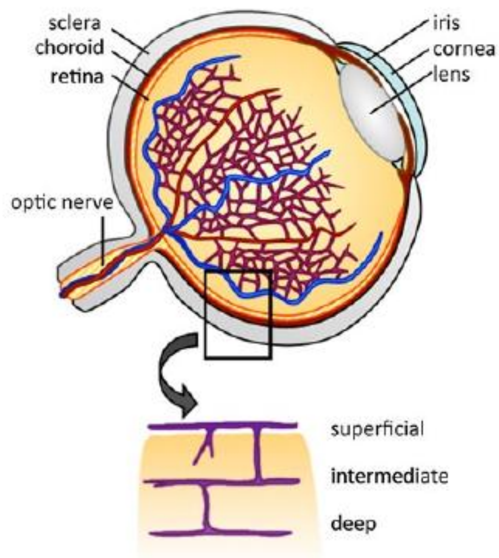
Ruhrberg et al., *Genes Dev* 2002
Fantin et al., *Nat Protoc* 2013
Tillo et al., *JOVE* 2014

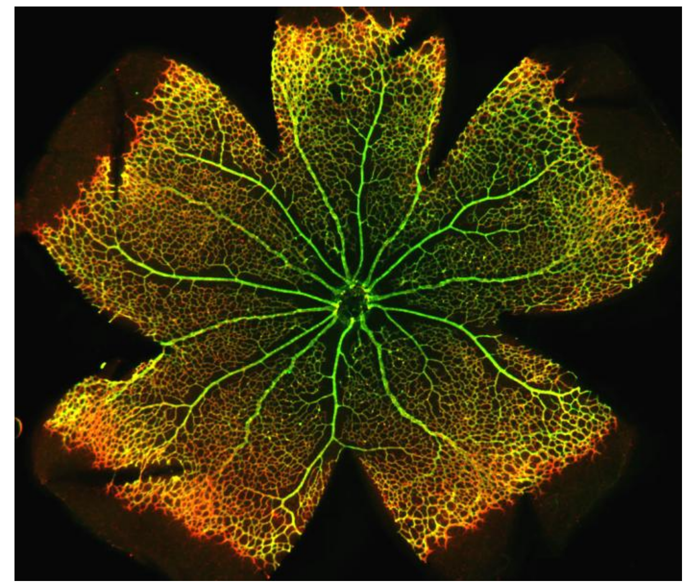
Angiogenesis in experimental models

Vascularisation of the mouse perinatal retina: sprouting angiogenesis









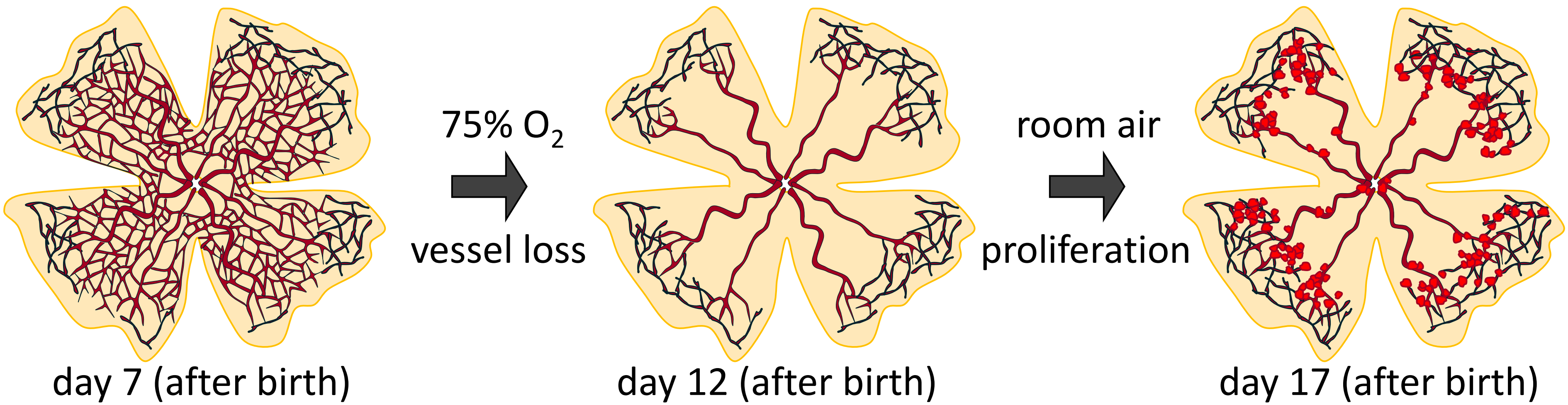
- ➢ Vascularisation occurs after birth
- ➢ Concomitant remodelling

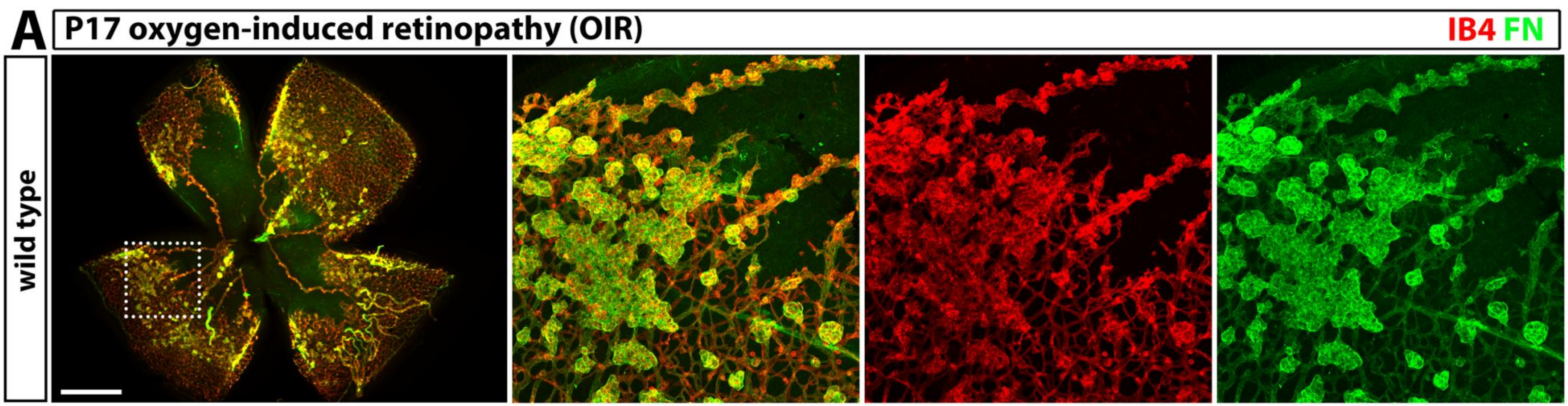
(SMC recruitment, a/v differentiation, pruning/regression)

e.g. Bautch & Rurhberg, 2013

Angiogenesis in experimental models

Vessel growth in oxygen-induced retinopathy (OIR): pathological angiogenesis



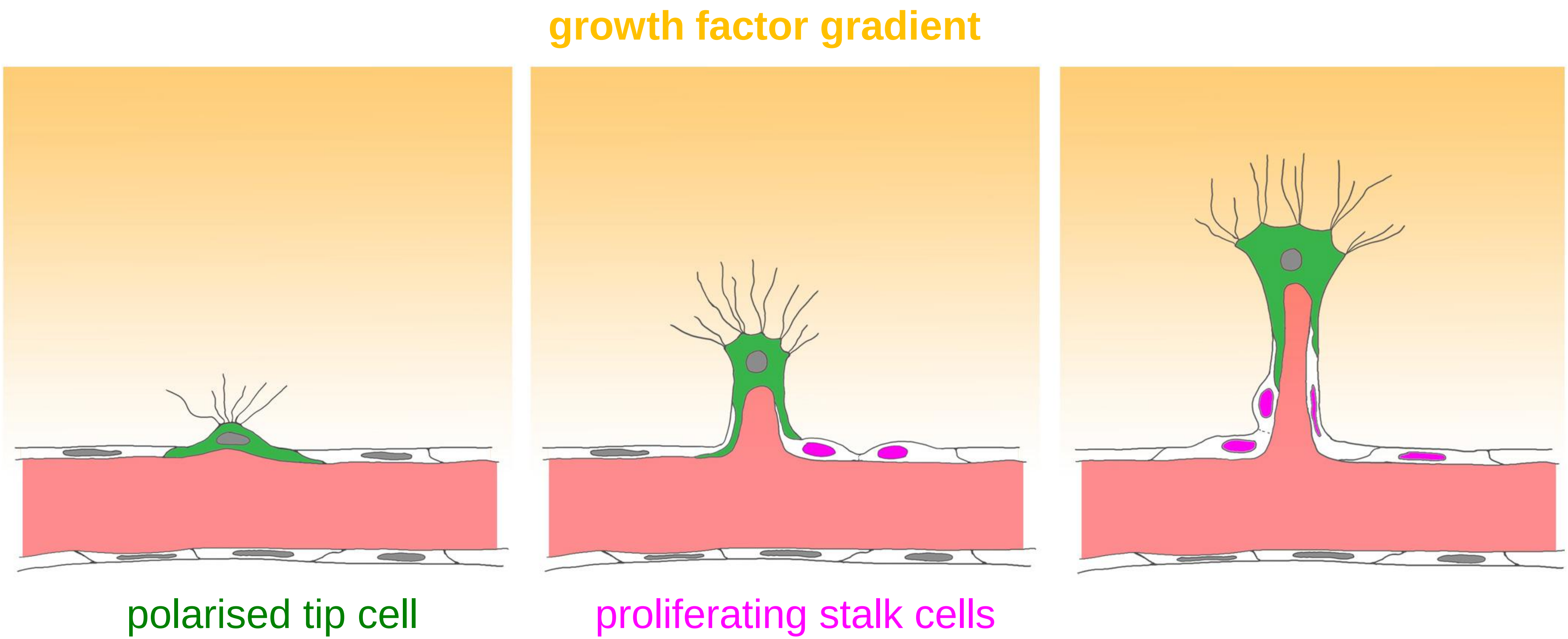


➢ model for retinopathy of prematurity (ROP) and proliferative diabetic retinopathy (PDR)

Raimondi, Fantin et al., *JEM* 2014

Mechanism of angiogenesis:

How do new sprouts form?

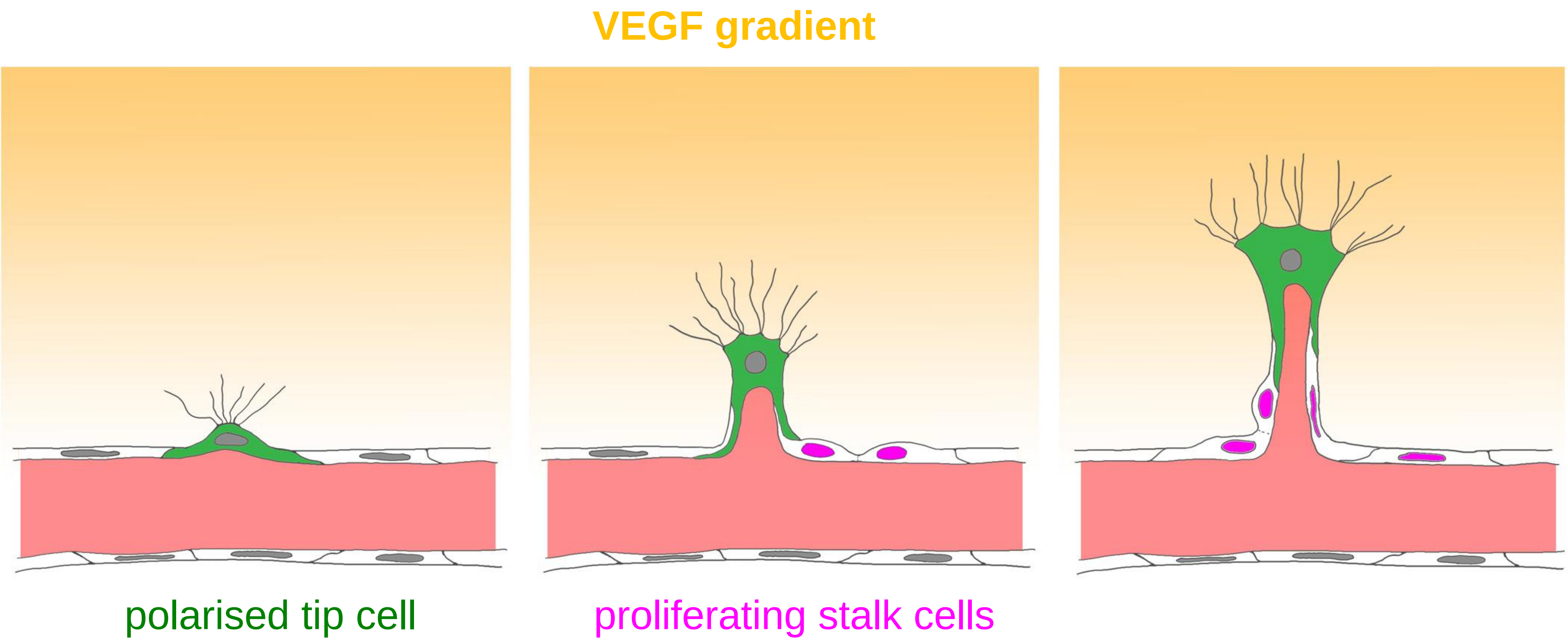


- ➢ Sprouting requires migration and proliferation of ECs
- ➢ Lateral inhibition (DLL4→notch) to restrict tip cell selection

Mechanism of angiogenesis:

How do new sprouts form?

Vascular Endothelial Growth Factor (VEGF, VEGF-A):

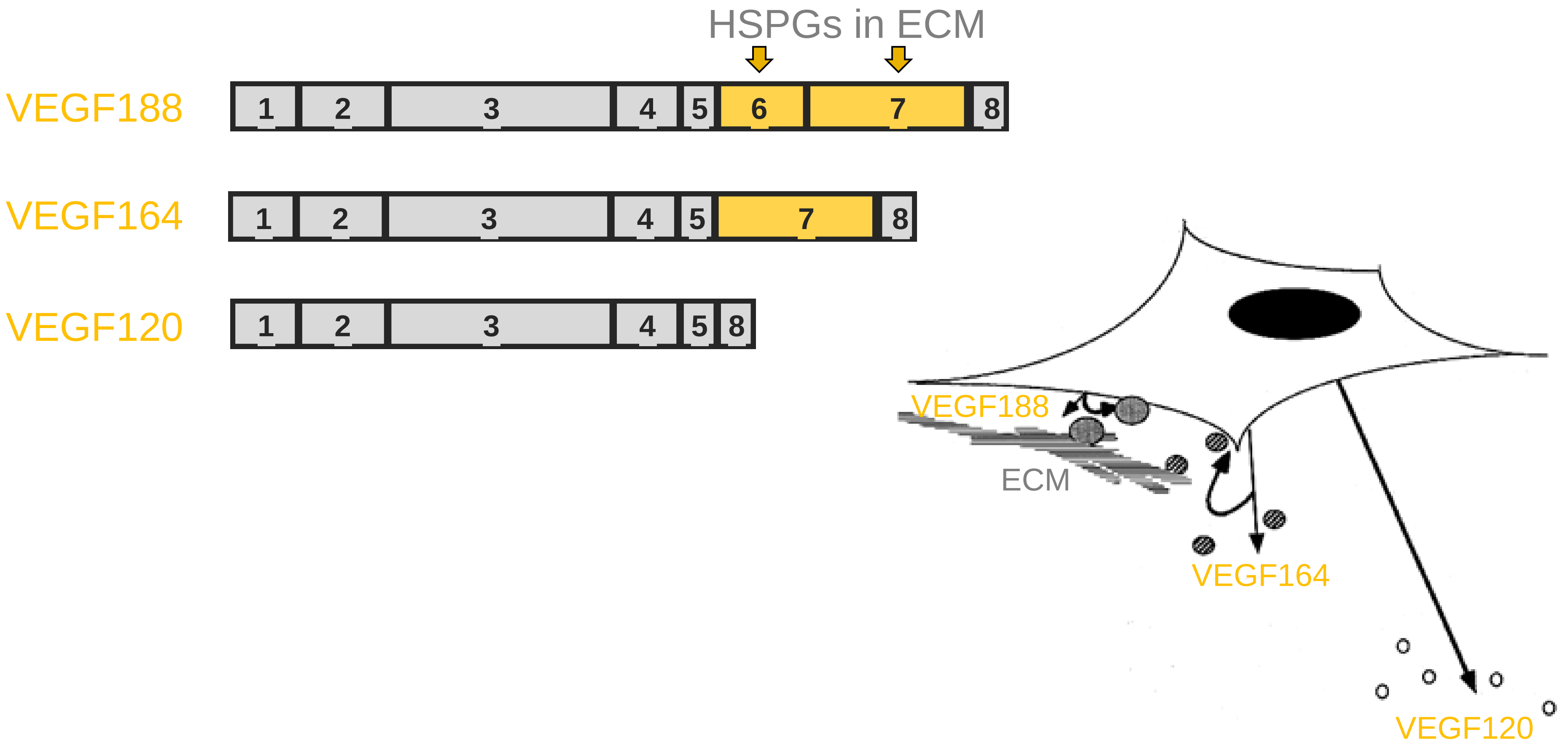


- VEGF forms chemoattractive gradients for vessel growth
- VEGF promotes DLL4/notch signalling to restrict tip cell selection

- VEGF+/- mice are haploinsufficient and embryonic lethal

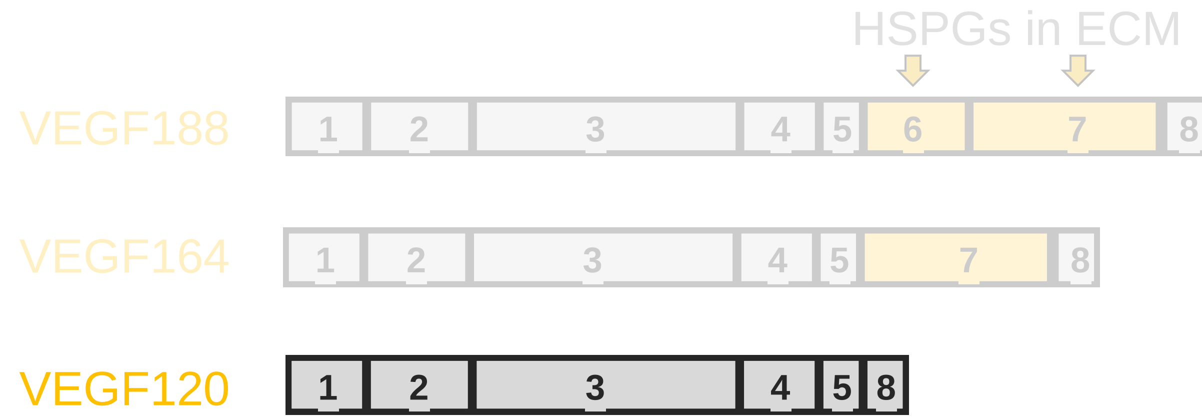
Carmeliet et al., *Nat* 1996

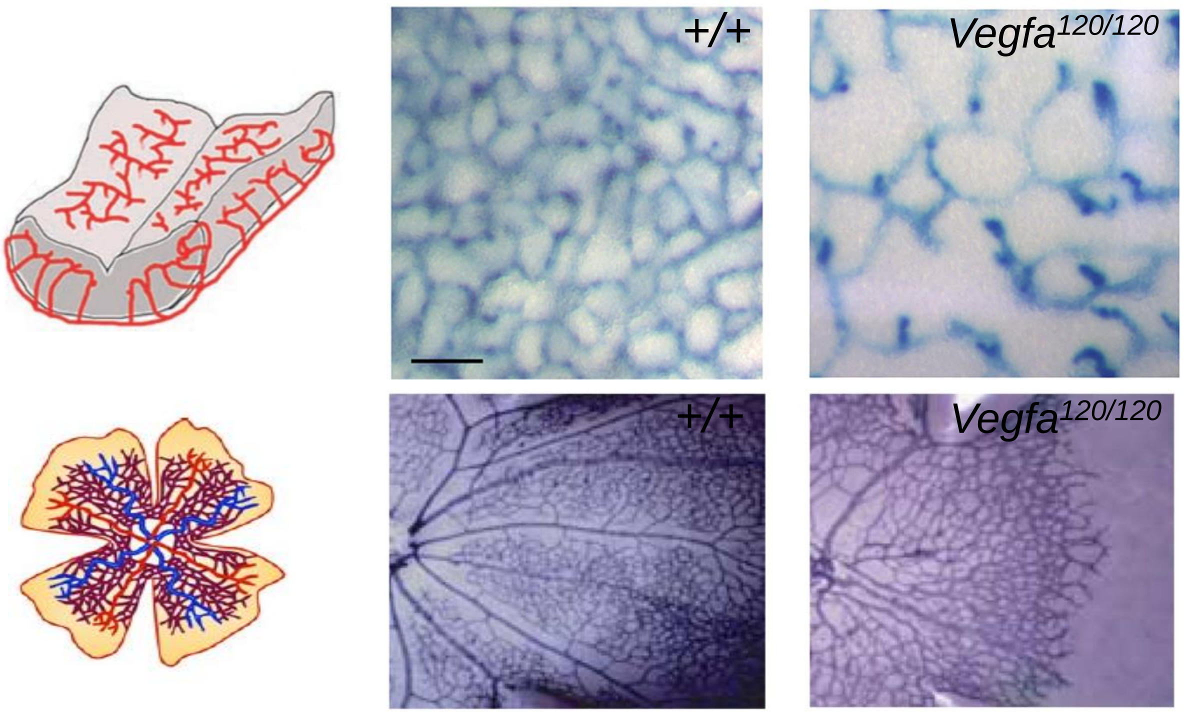
Mechanism of angiogenesis: VEGF



- ➢ VEGF is expressed in several splice forms with different ECM affinity

Mechanism of angiogenesis: VEGF





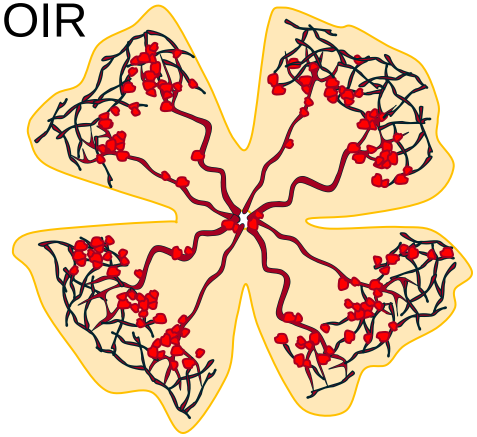
Ruhrberg et al., *Genes Dev* 2002

Carmeliet and Tessier-Lavigne, *Nat* 2005

Loss of VEGF gradients reduces vascular complexity in brain and retina

Mechanism of angiogenesis: VEGF

P17 OIR

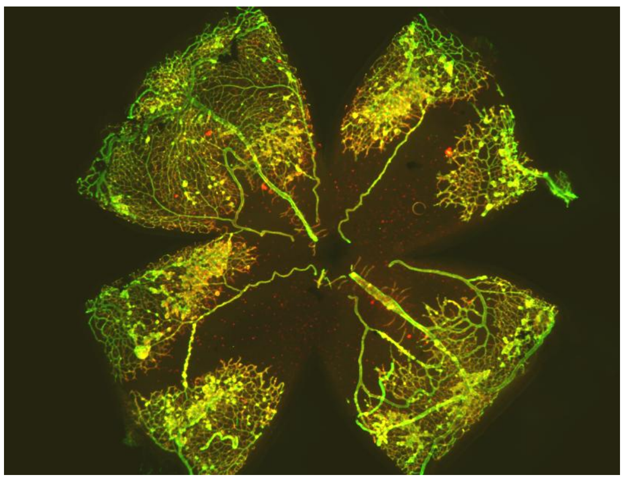


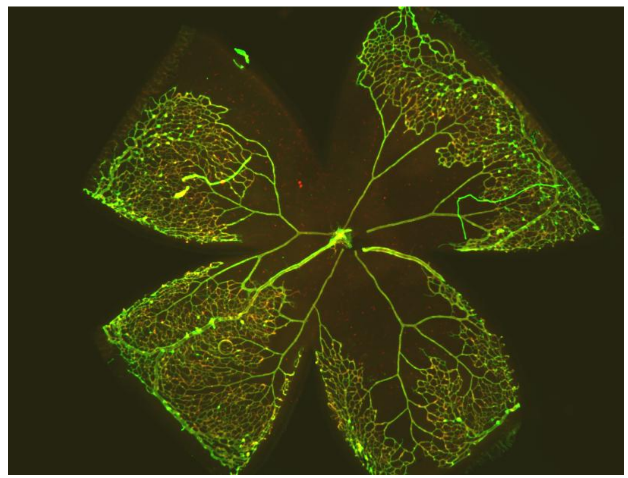
Pdgfb-iCre;Vegfr2$^{fl/fl}$

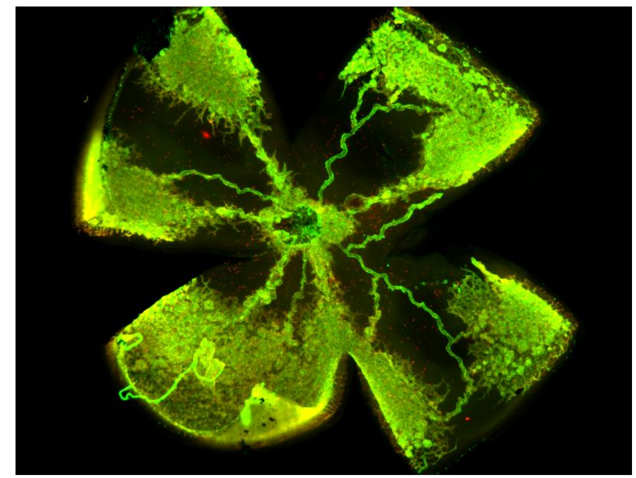
tamoxifen at P13-16

Vegf$^{+/IRES\text{-}LacZ}$

control	VEGF LOF (VEGFR2 KO in ECs)	VEGF GOF (stable VEGF mRNA





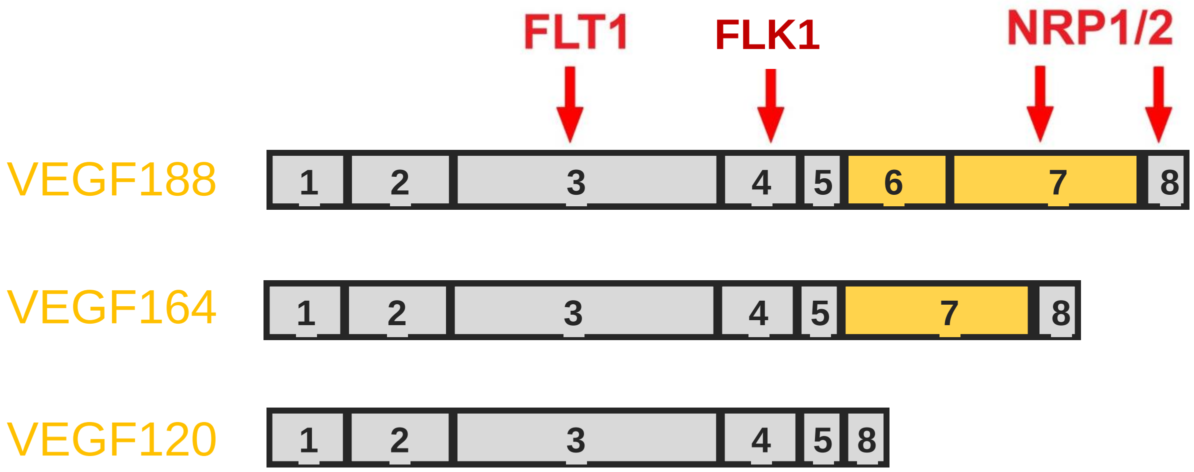


VEGF is essential for EC proliferation during pathological angiogenesis

Fantin, unpublished

Mechanism of angiogenesis: VEGF

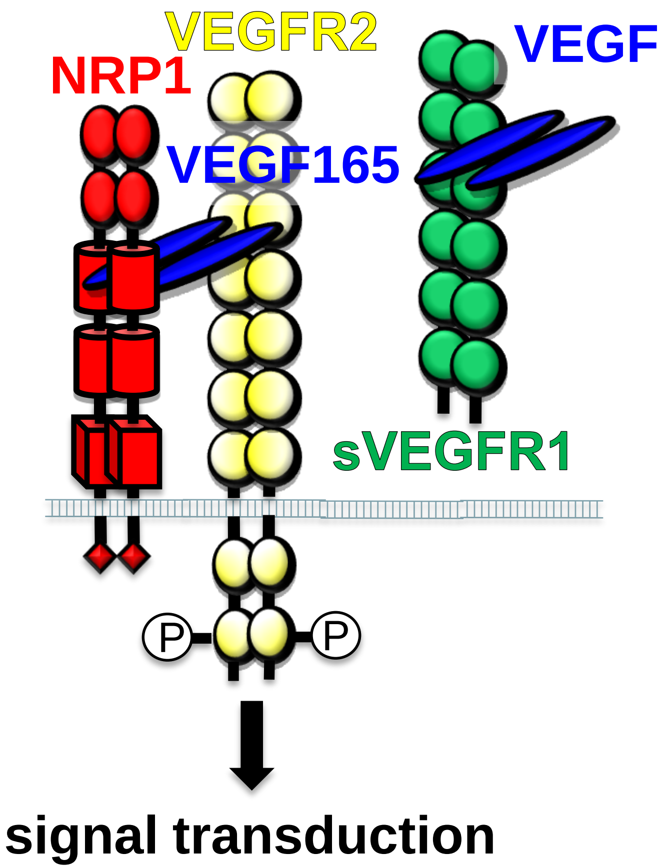
Differential receptor affinity:



FLT1 = VEGFR1

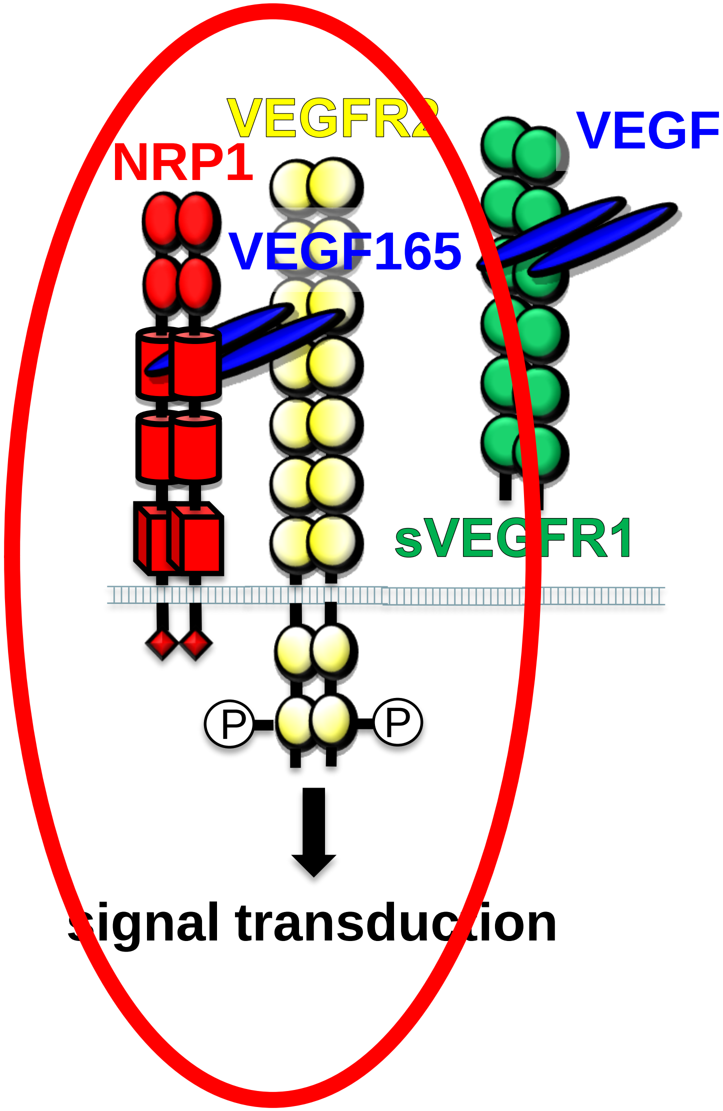
FLK1 = VEGFR2 = KDR

VEGF-A signalling in EC – general model:



- VEGFR1 mainly act as a VEGF decoy through its soluble isoform.
  e.g. Ho et al., 2012; Fong et al., 1995
- VEGF165 bridges NRP1 and VEGFR2.
  e.g. Whitaker et al., 2001; Soker et al., 2002
- NRP1 increases VEGF165-induced VEGFR2 phosphorylation to enhance signalling.
  e.g. Becker et al., 2005; Soker et al., 1998

VEGF-A signalling in EC – current model:



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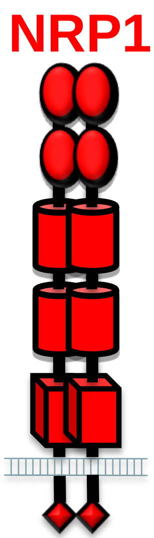
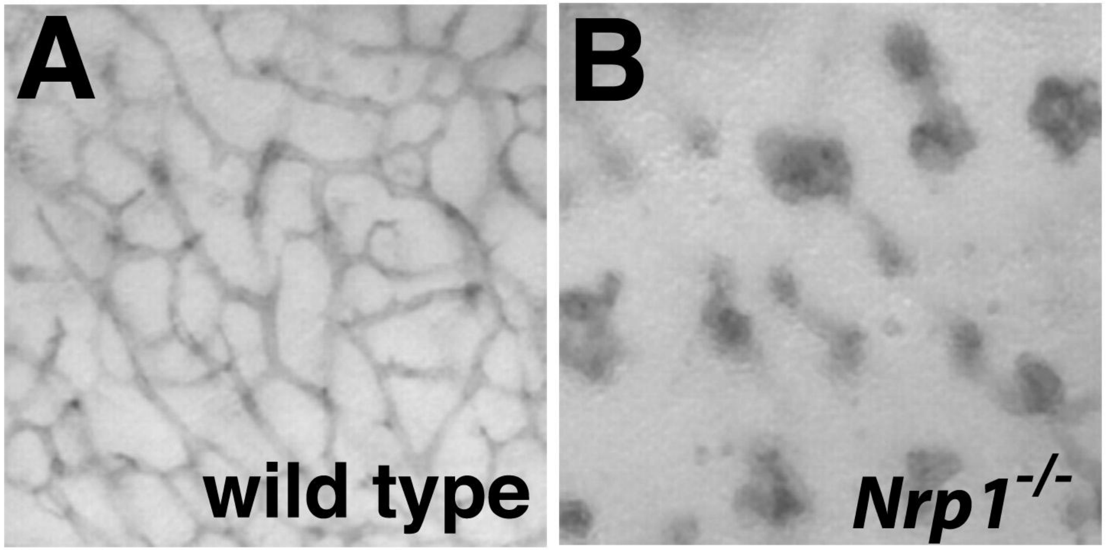
  e.g. Becker et al., 2005; Soker et al., 1998

Does this model apply to angiogenesis?

NRP1 is essential in ECs for angiogenesis

- *Nrp1*-null mice die at midgestation with severe cardiovascular defects (Kawasaki et al., *Dev* 1999);
- NRP1 acts cell autonomously in ECs(Gu et al., *Dev Cell* 2003; Fantin et al., *Blood* 2013)





How does NRP1 promote angiogenesis?

Is VEGF binding to NRP1 required for angiogenesis?

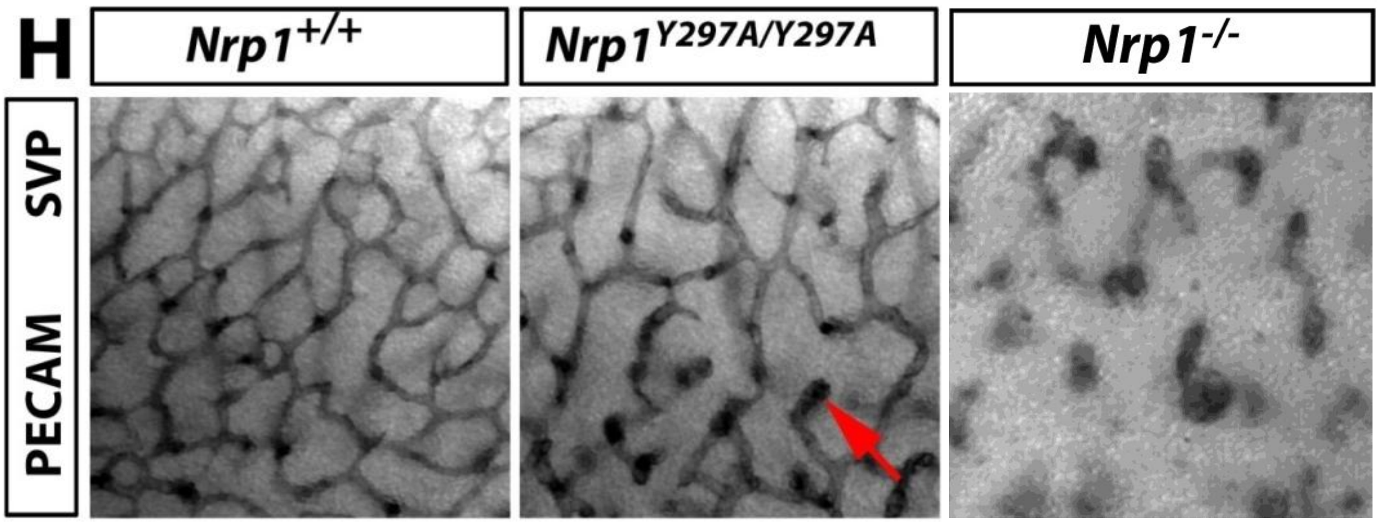
Is VEGF binding to NRP1 required for angiogenesis?

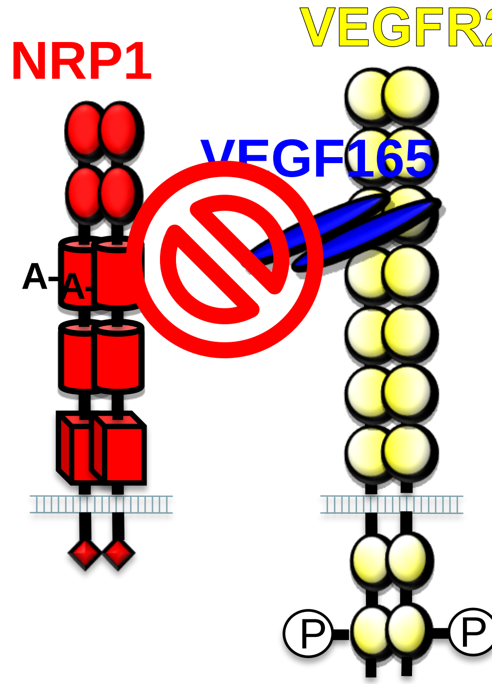
 | Development (2014) 141, 556-562 doi:10.1242/dev.103028

The Company of Biologists

Neuropilin 1 (NRP1) hypomorphism combined with defective VEGF-A binding reveals novel roles for NRP1 in developmental and pathological angiogenesis

Alessandro Fantin[1,*], Birger Herzog[2,*], Marwa Mahmoud[2,‡], Maiko Yamaji[2,‡], Alice Plein[1], Laura Denti[1], Christiana Ruhrberg[1,§] and Ian Zachary[2,§]

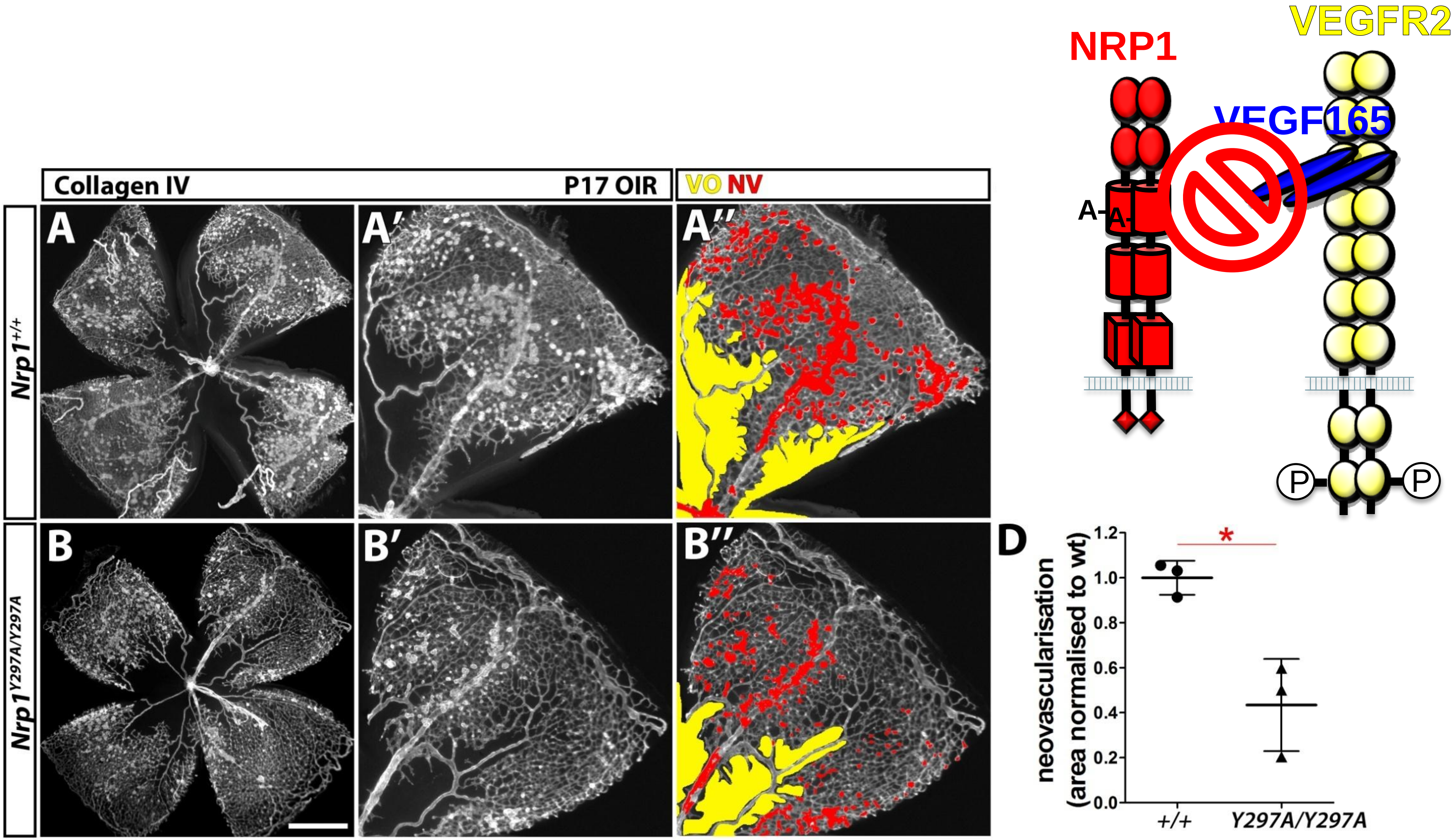




VEGF-binding to NRP1 is not important for NRP1 function in embryonic angiogenesis

- ✧ VEGF-binding to NRP1 plays a role in postnatal angiogenesis and arteriogenesis.
- ✧ Corroborated by Gelfand et al., *eLIFE* 2014 with different mutation in same locus.

Is VEGF binding to NRP1 required for angiogenesis?



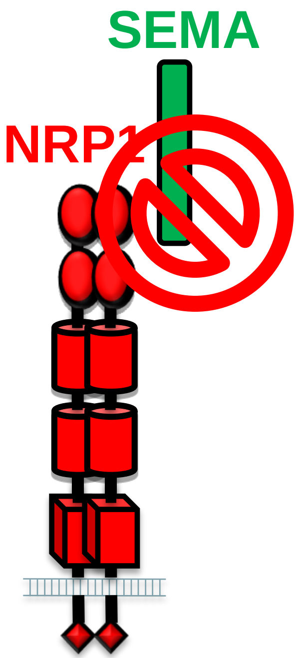
✧ VEGF-binding to NRP1 is not essential in embryonic angiogenesis

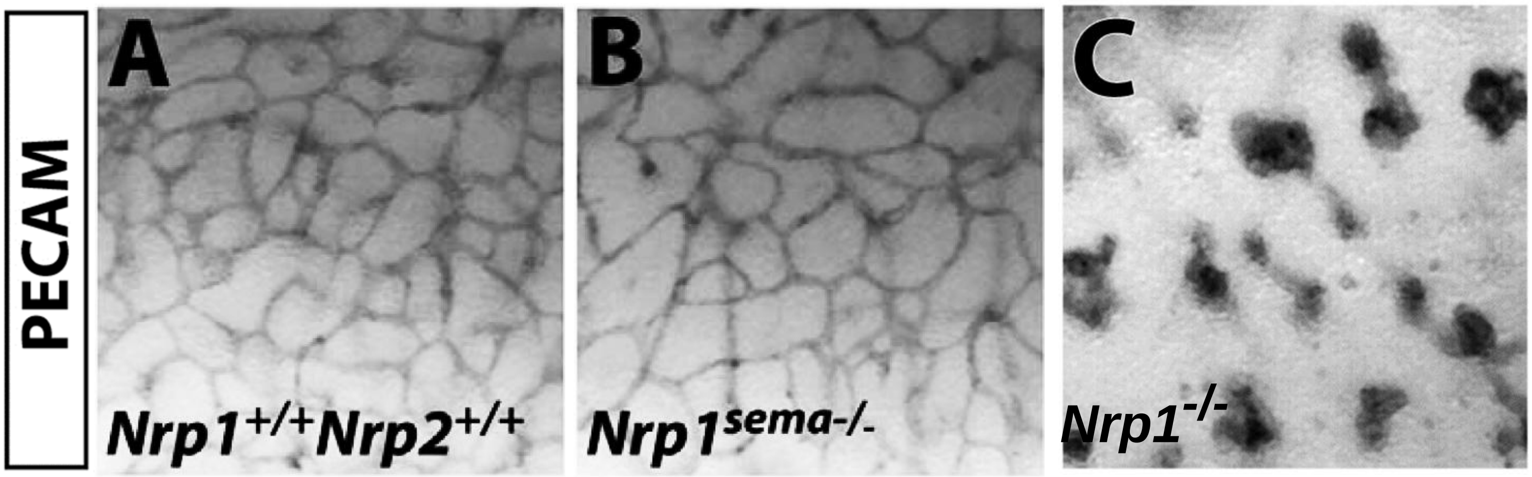
✧ VEGF-binding to NRP1 plays important role in pathological angiogenesis

What is the angiogenic NRP1 ligand that cooperates with VEGF?

What is the angiogenic NRP1 ligand that cooperates with VEGF?

- NRP1 is also a receptor for semaphorins
- *Nrp1* mutants with defective semaphorin binding are viable, with normal developmental angiogenesis (Gu et al., *Dev Cell* 2003; Vieira et al., *Dev* 2007)



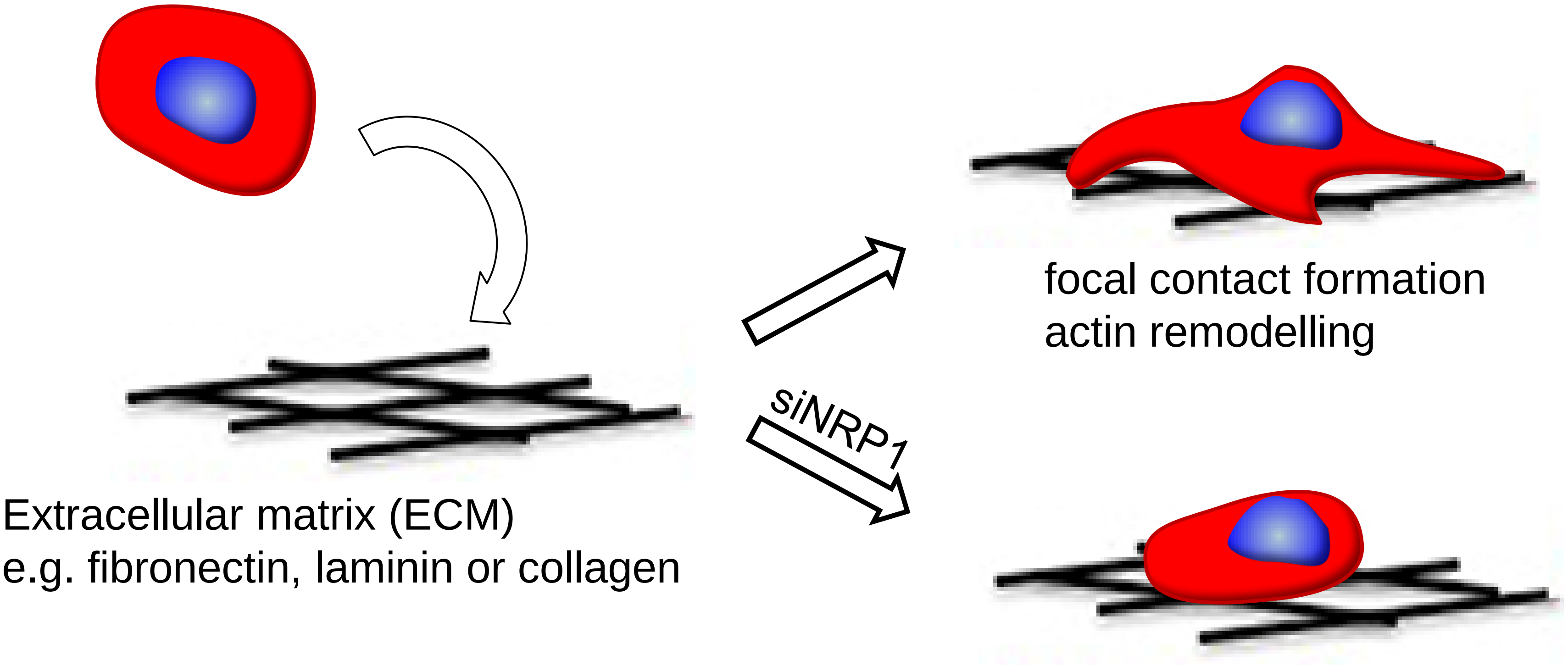


Vieira et al., *Dev* 2007

Semaphorins are NOT main NRP1 ligands during angiogenesis

What is the angiogenic NRP1 ligand that cooperates with VEGF?

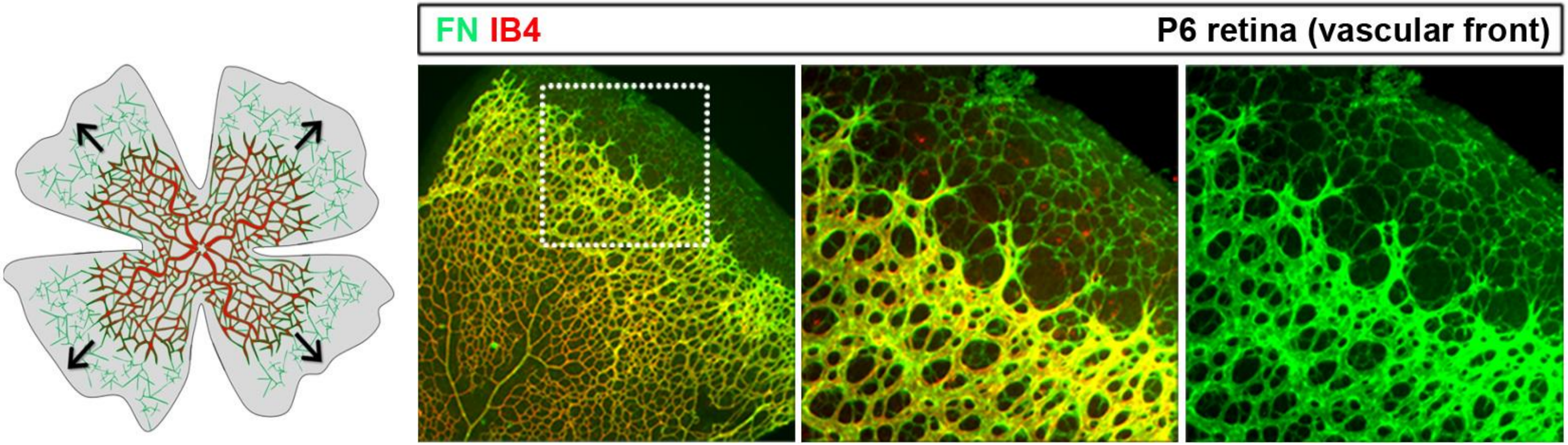
✧ NRP1 interacts with ITGB1 & ITGB3, two fibronectin receptors.



➢ NRP1 promotes ECM-induced ABL1 signalling and actin remodelling independently of VEGFR2

Raimondi, Fantin et al., *JEM* 2014

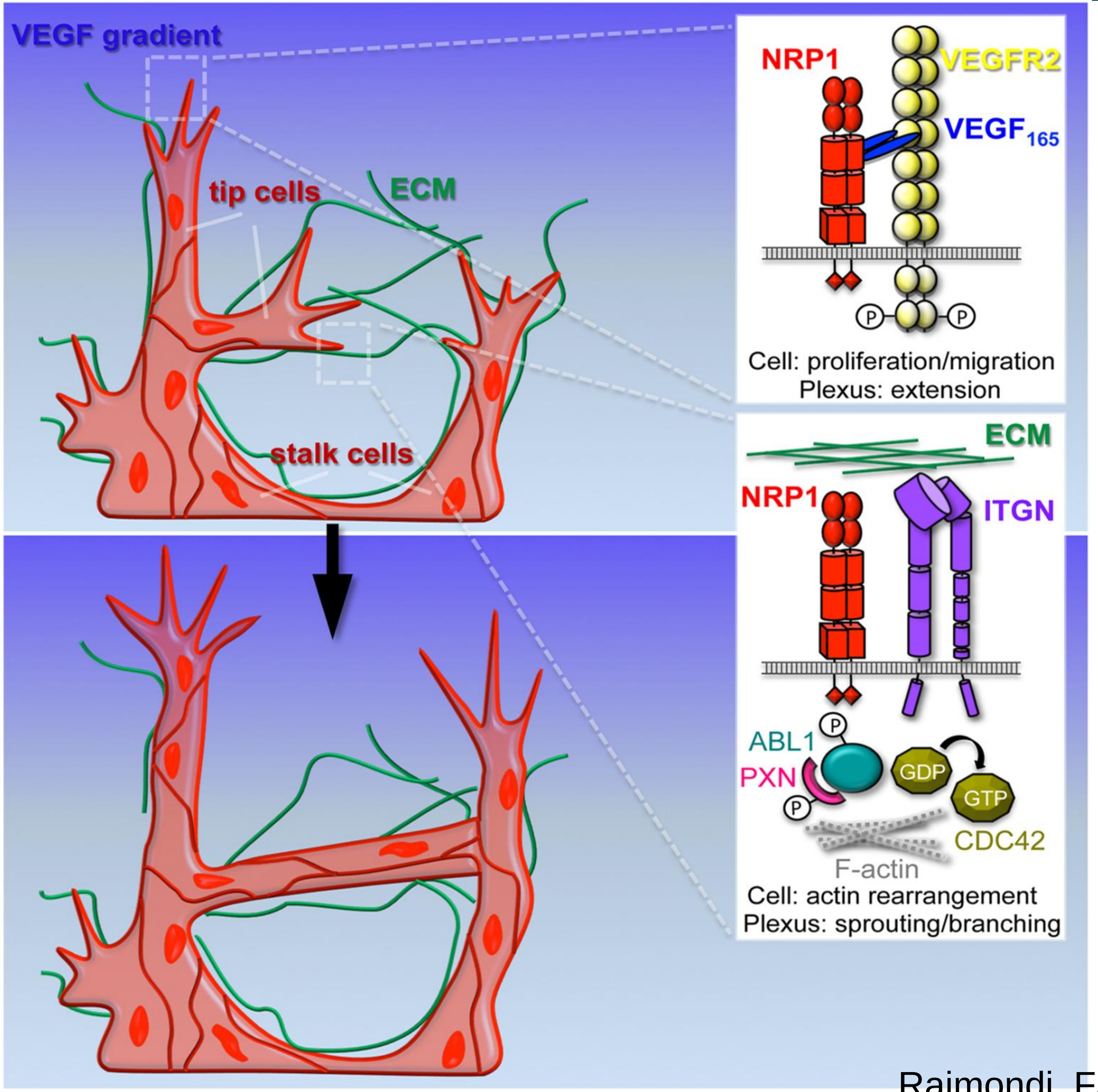
ECM signalling in EC during angiogenesis *in vivo*



- FN is expressed by astrocytes ahead of the vascular front;
- FN is deposited around growing vasculature.

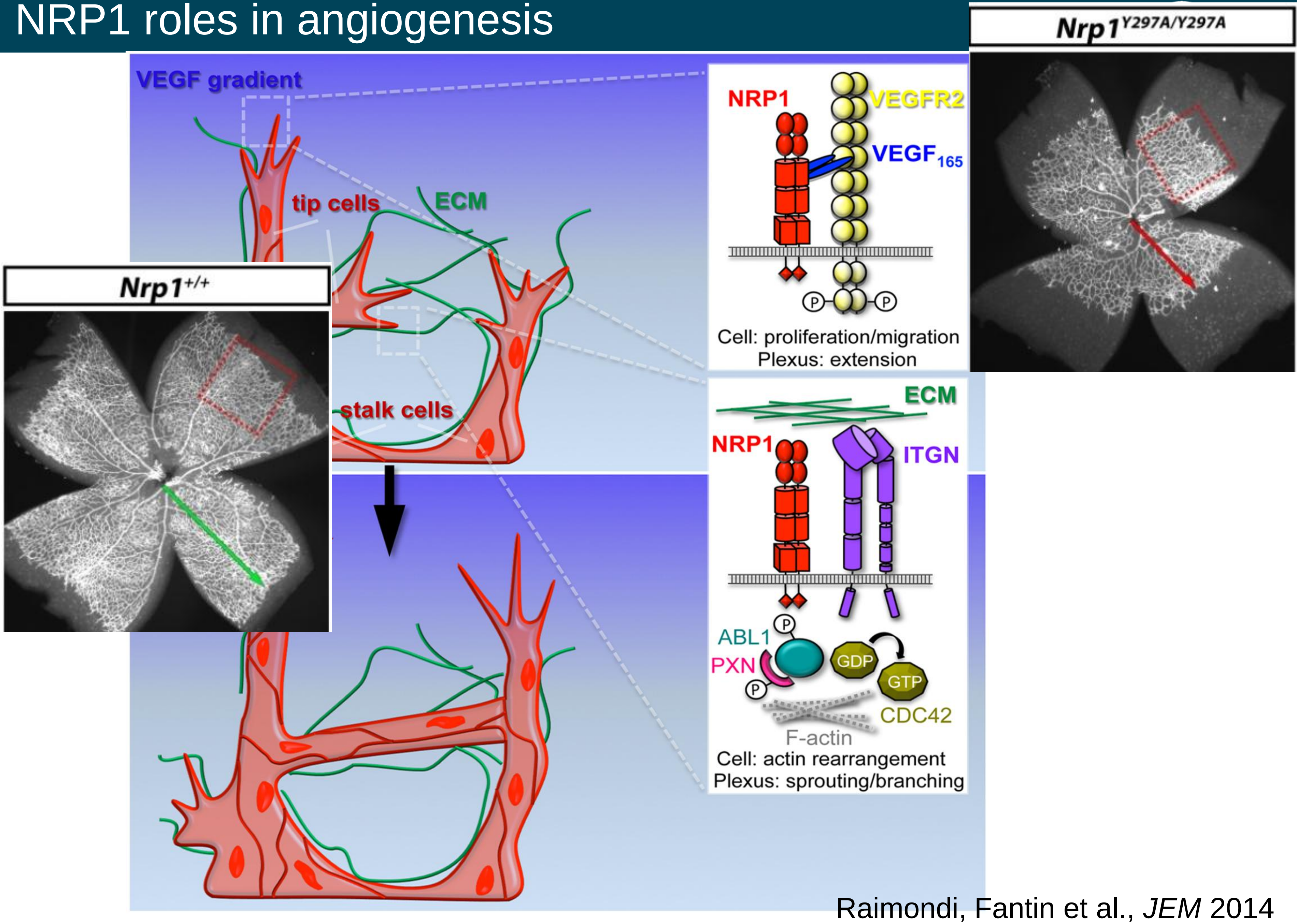
Raimondi, Fantin et al., *JEM* 2014

NRP1 roles in angiogenesis



Raimondi, Fantin et al., *JEM* 2014
Fantin et al., *Cell Reports* 2015

NRP1 roles in angiogenesis



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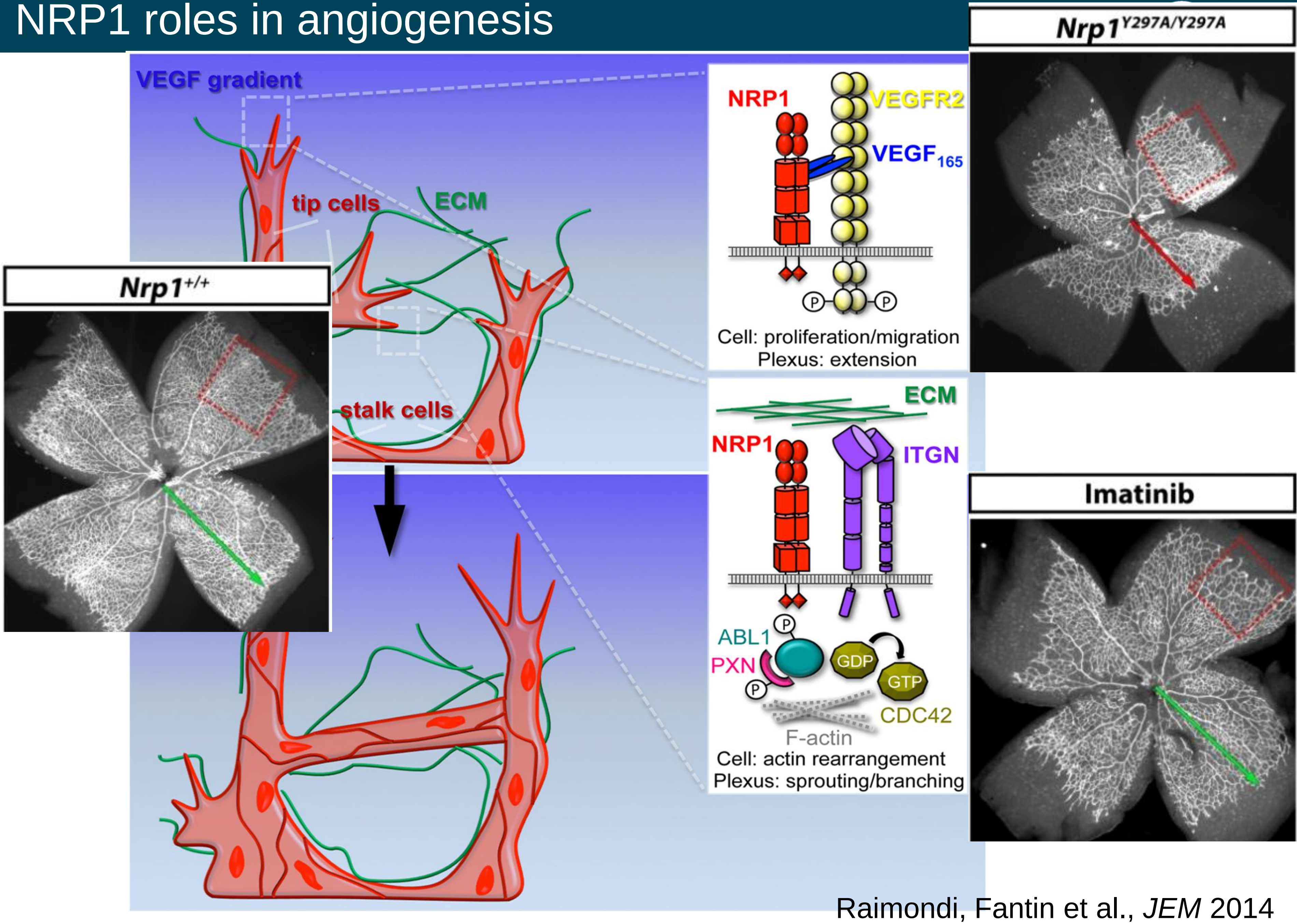

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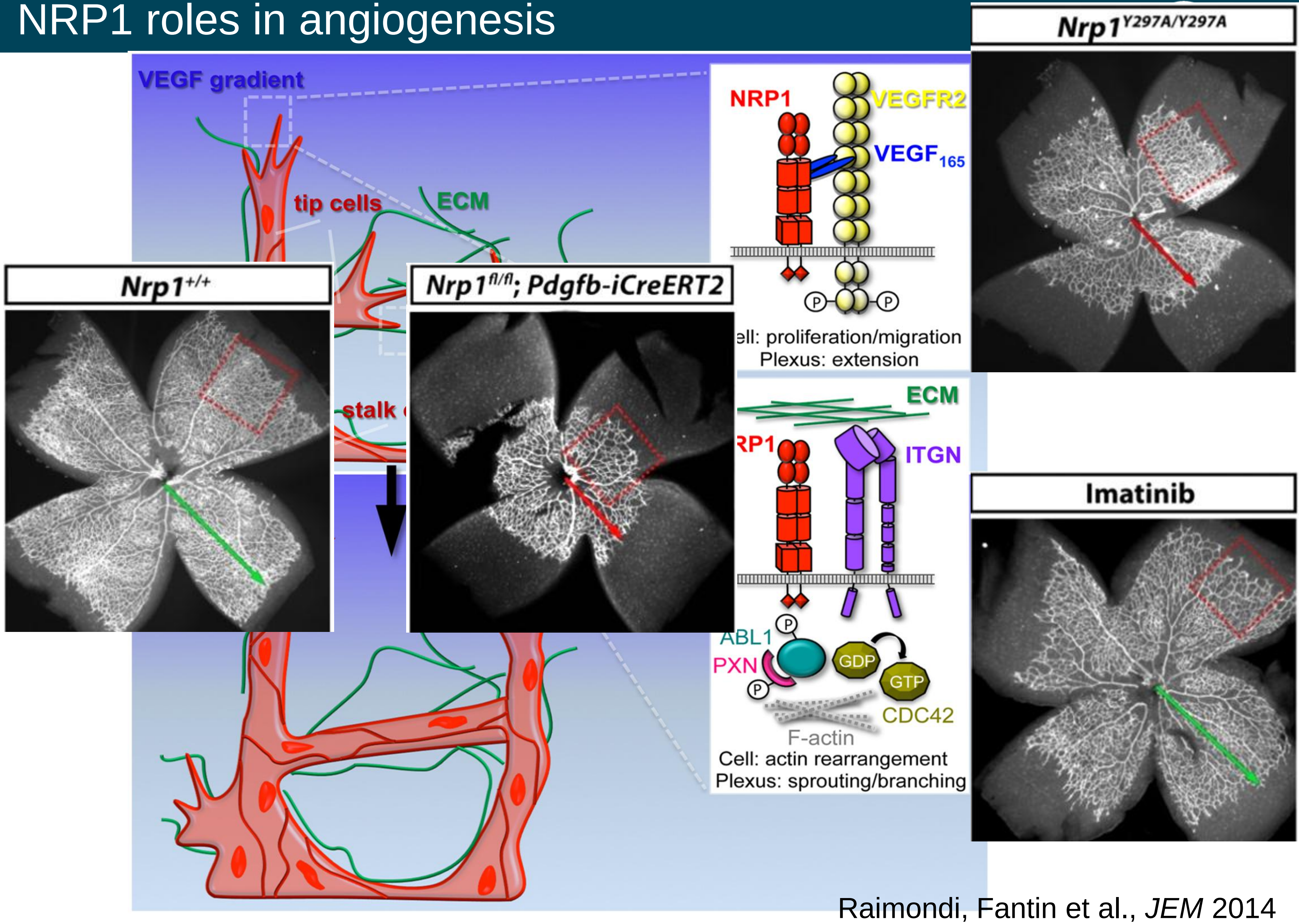
NRP1 roles in angiogenesis



Raimondi, Fantin et al., *JEM* 2014
Fantin et al., *Cell Reports* 2015

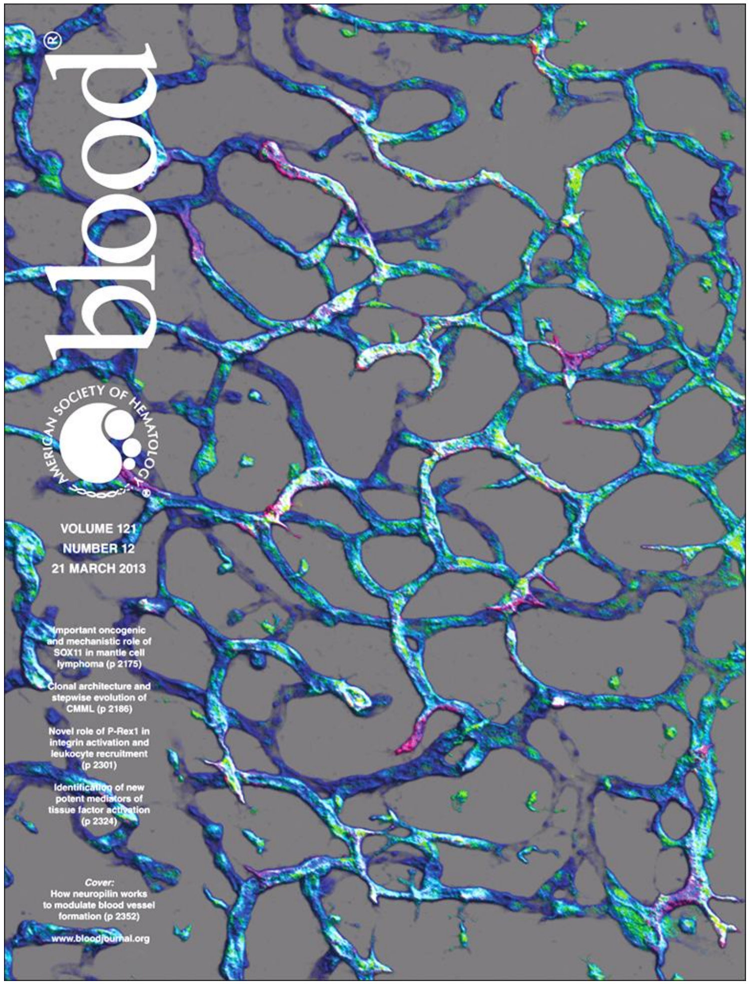
- NRP1 mediates different signalling pathways to control sprout growth and branching during angiogenesis

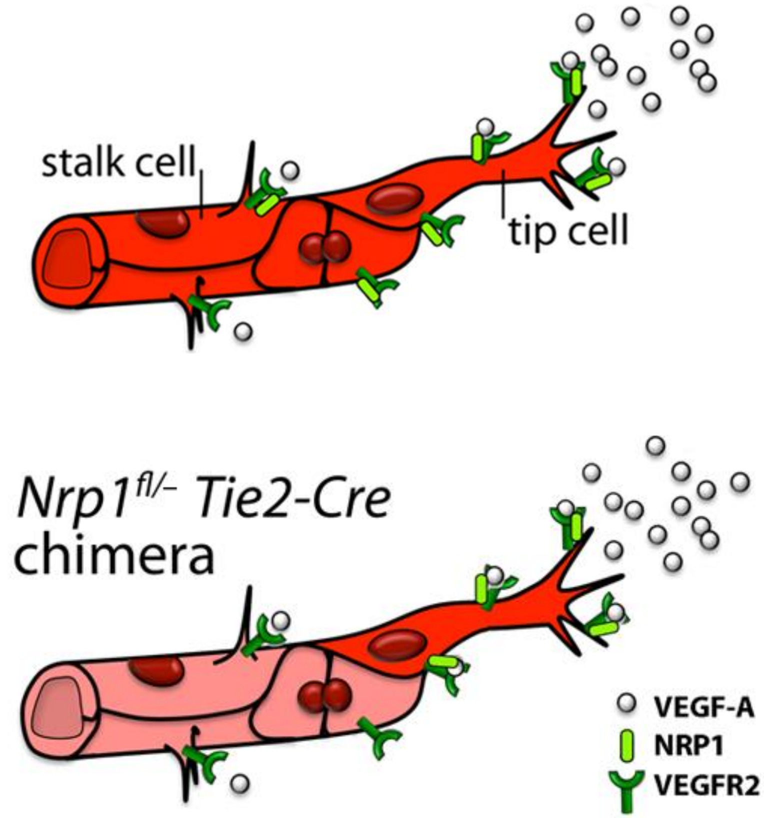
- What is the exact cellular function of NRP1?

NRP1 controls endothelial tip cell formation

NRP1 acts cell autonomously in endothelium to promote tip cell function during sprouting angiogenesis

Alessandro Fantin,[1] Joaquim M. Vieira,[1] Alice Plein,[1] Laura Denti,[1] Marcus Fruttiger,[1] Jeffrey W. Pollard,[2] and Christiana Ruhrberg[1]

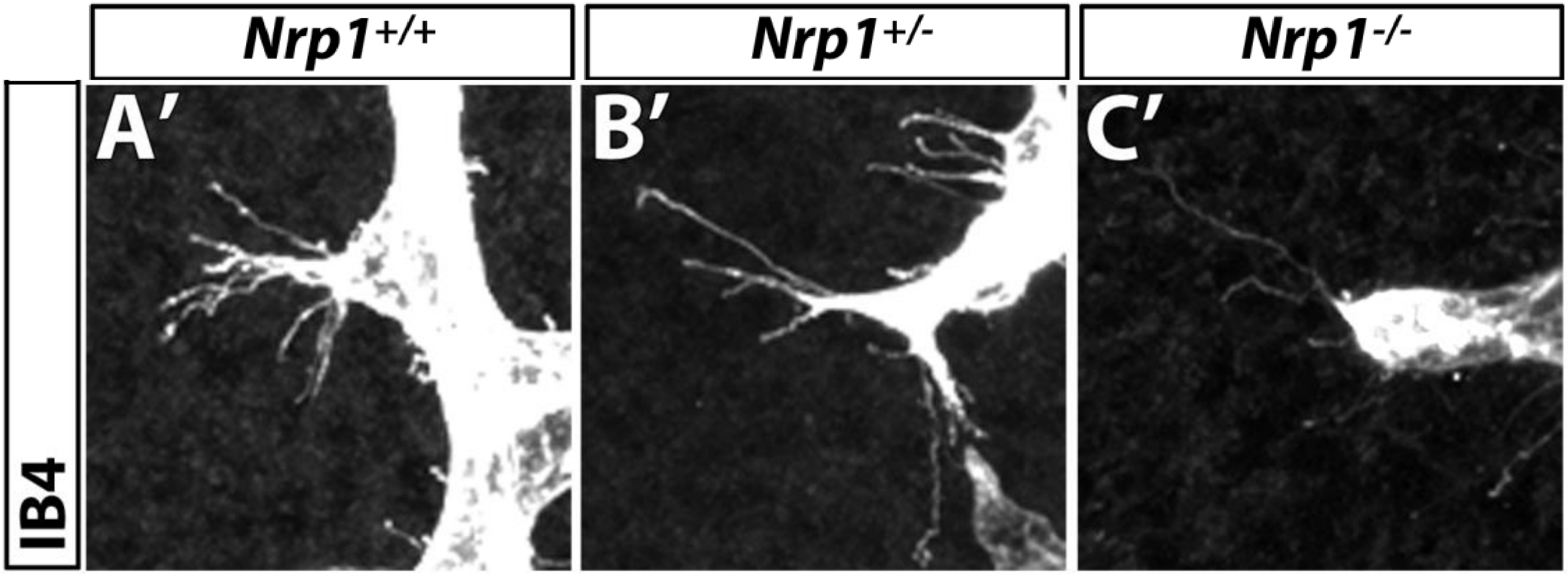




NRP1 low NRP1 high

NRP1 vessels

NRP1 controls filopodia formation

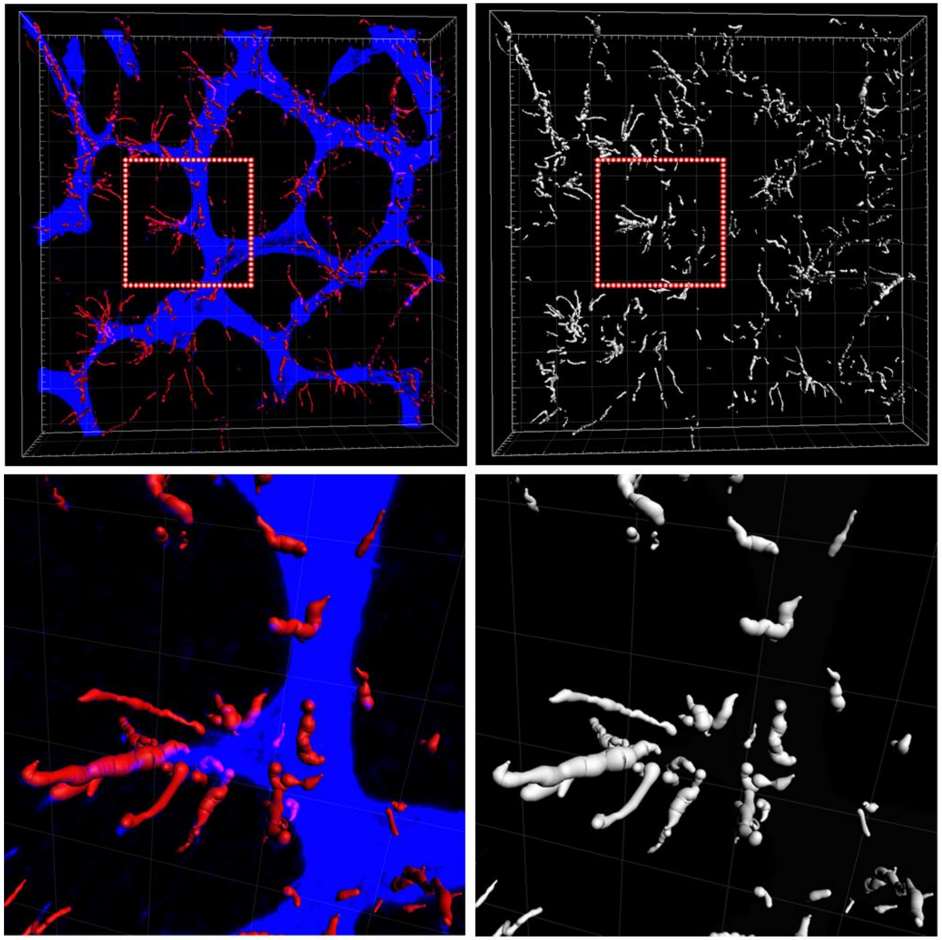


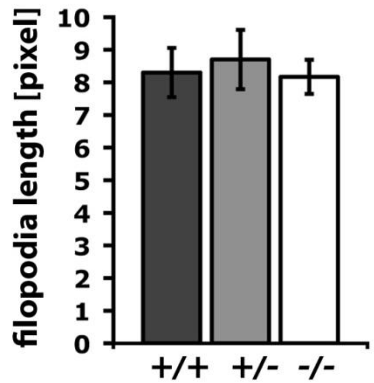
✧ Fewer tip cells in homozygous mutants
✧ NRP1 dosage-dependent reduction in filopodia

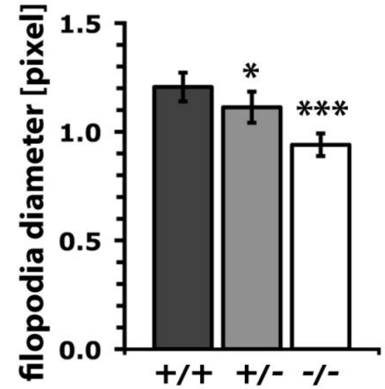
Fantin et al., *Cell Reports* 2015

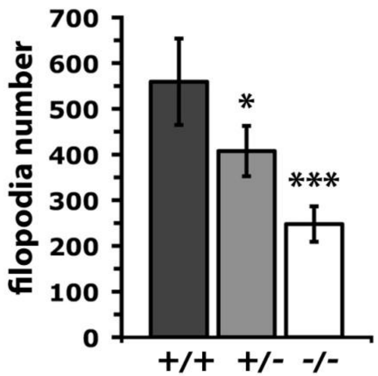
NRP1 controls filopodia formation

Imaris filament tracer







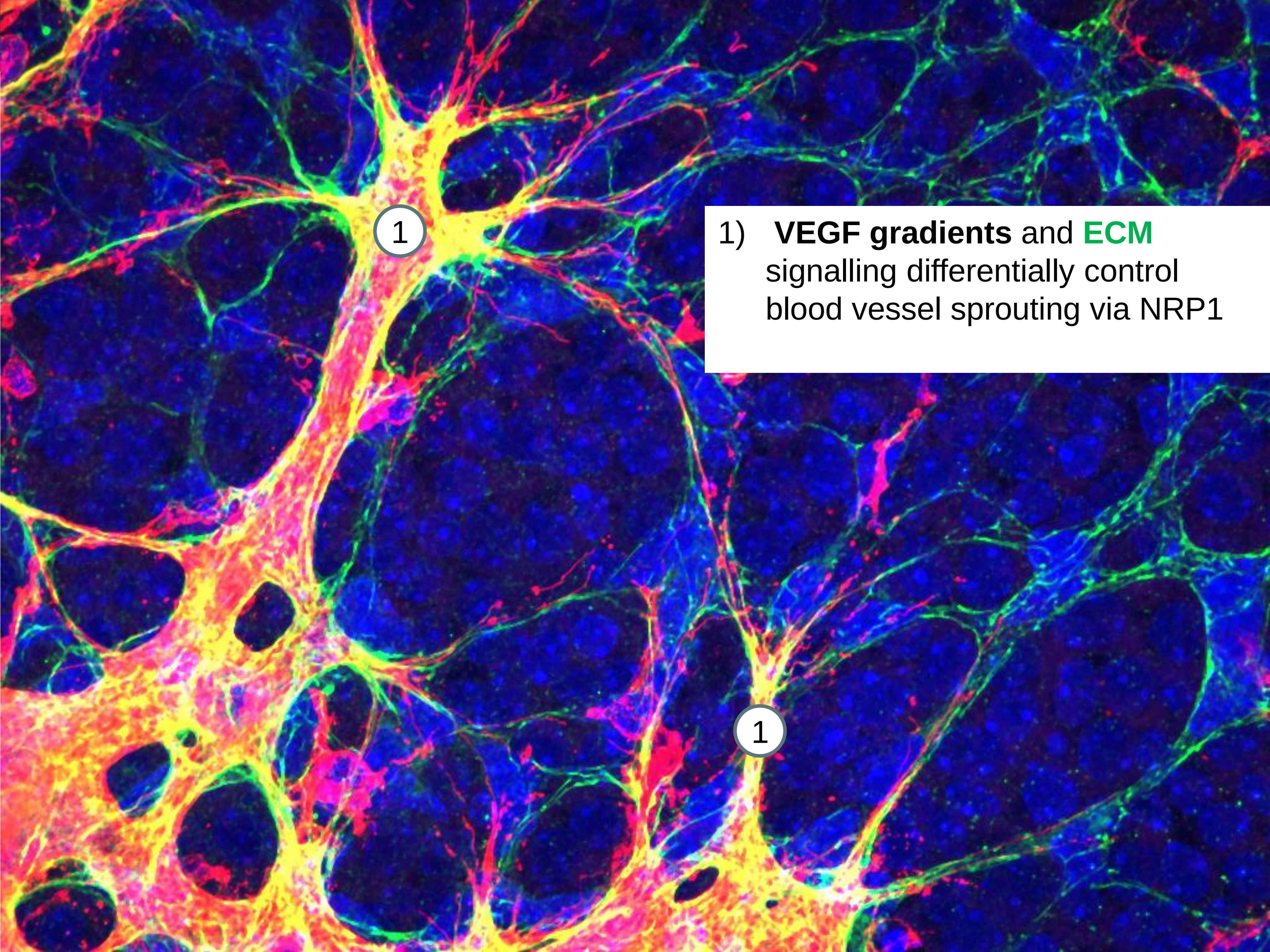


✧ normal filopodia length

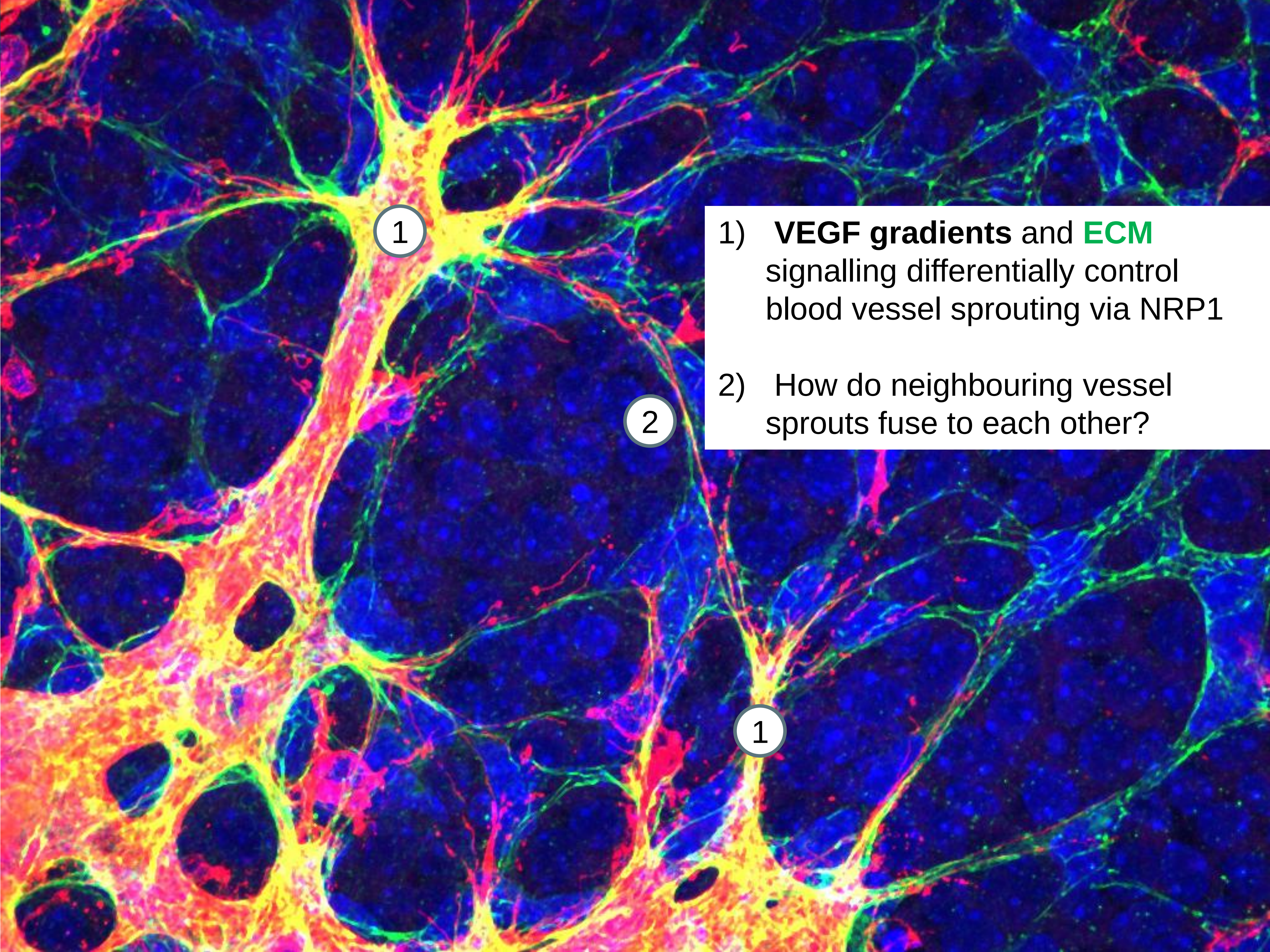
✧ reduced filopodia number and diameter

➢ due to reduced CDC42 activation

Fantin et al., *Cell Reports* 2015



1) **VEGF gradients** and **ECM** signalling differentially control blood vessel sprouting via NRP1

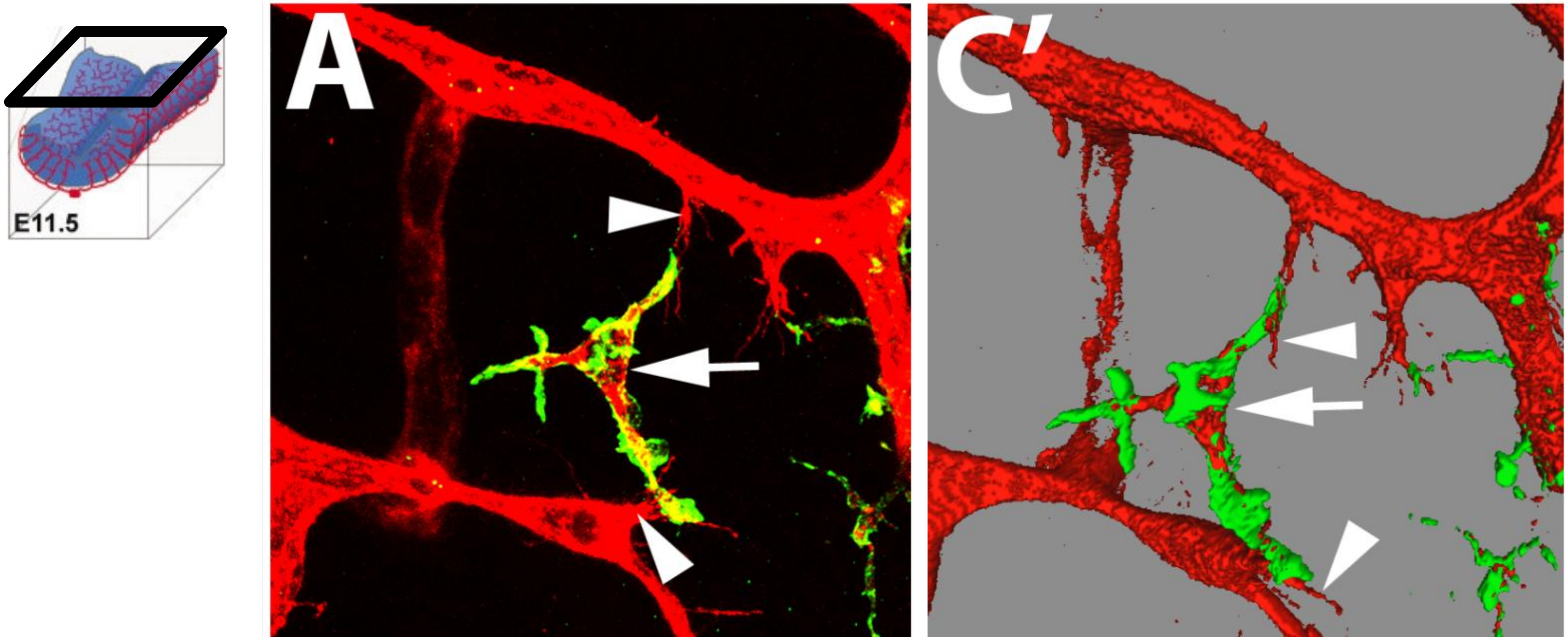


1) **VEGF gradients** and **ECM** signalling differentially control blood vessel sprouting via NRP1

2) How do neighbouring vessel sprouts fuse to each other?

MΦs interact with endothelial tip cells:

IB4: vessels & MΦs
F4/80: MΦs (microglia)



MΦs often interact with filopodia on opposing tip cells, as if to align them in preparation for fusion.

Fantin et al., *Blood* 2010

MΦs interact with endothelial tip cells:

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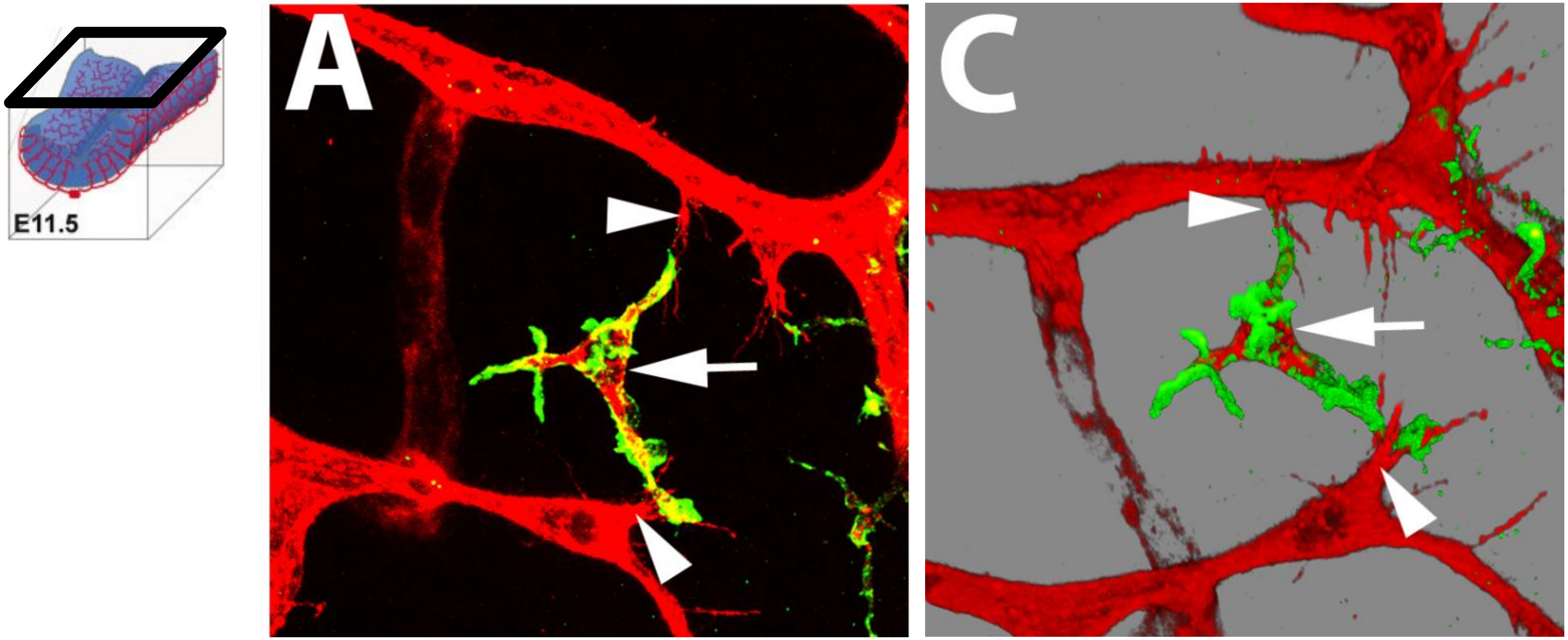

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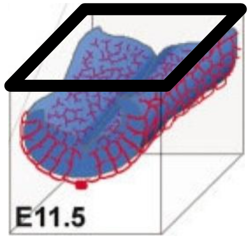

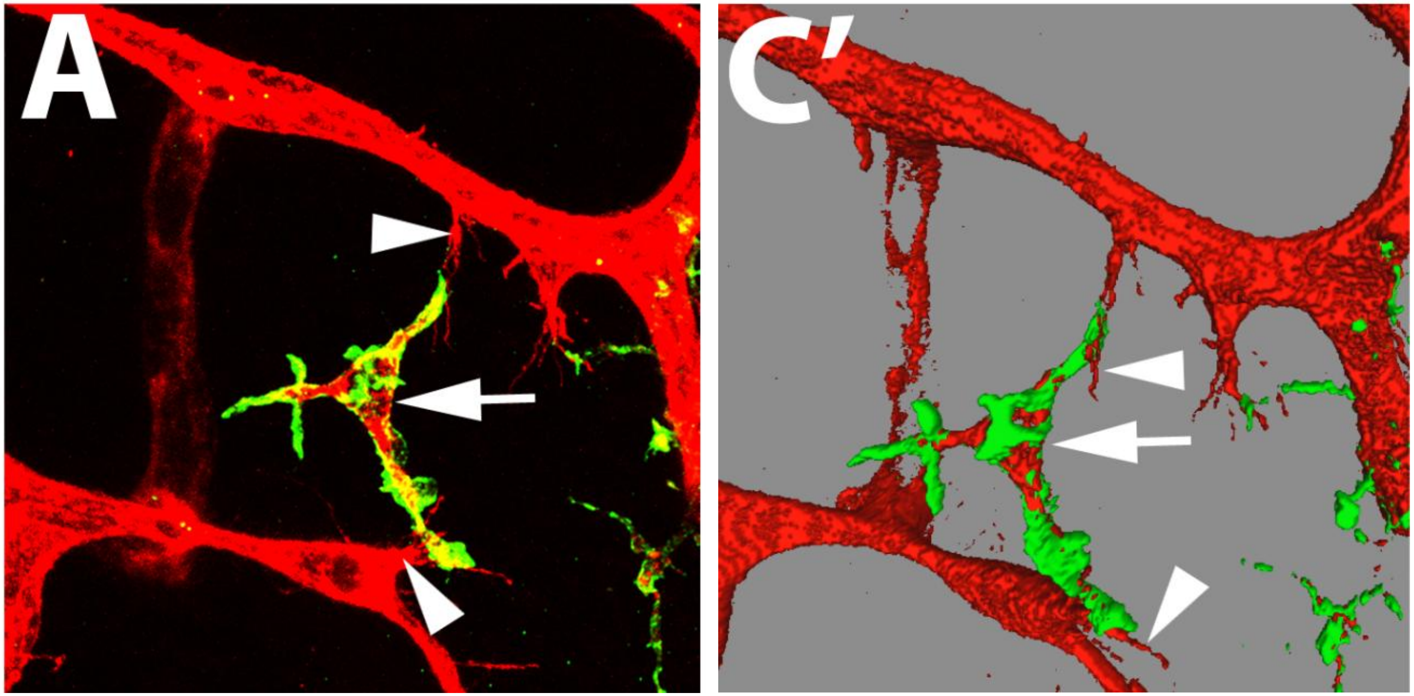

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Fantin et al., *Blood* 2010

MΦs embrace vessel junctions:

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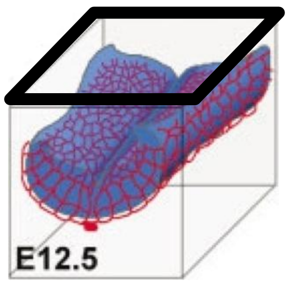

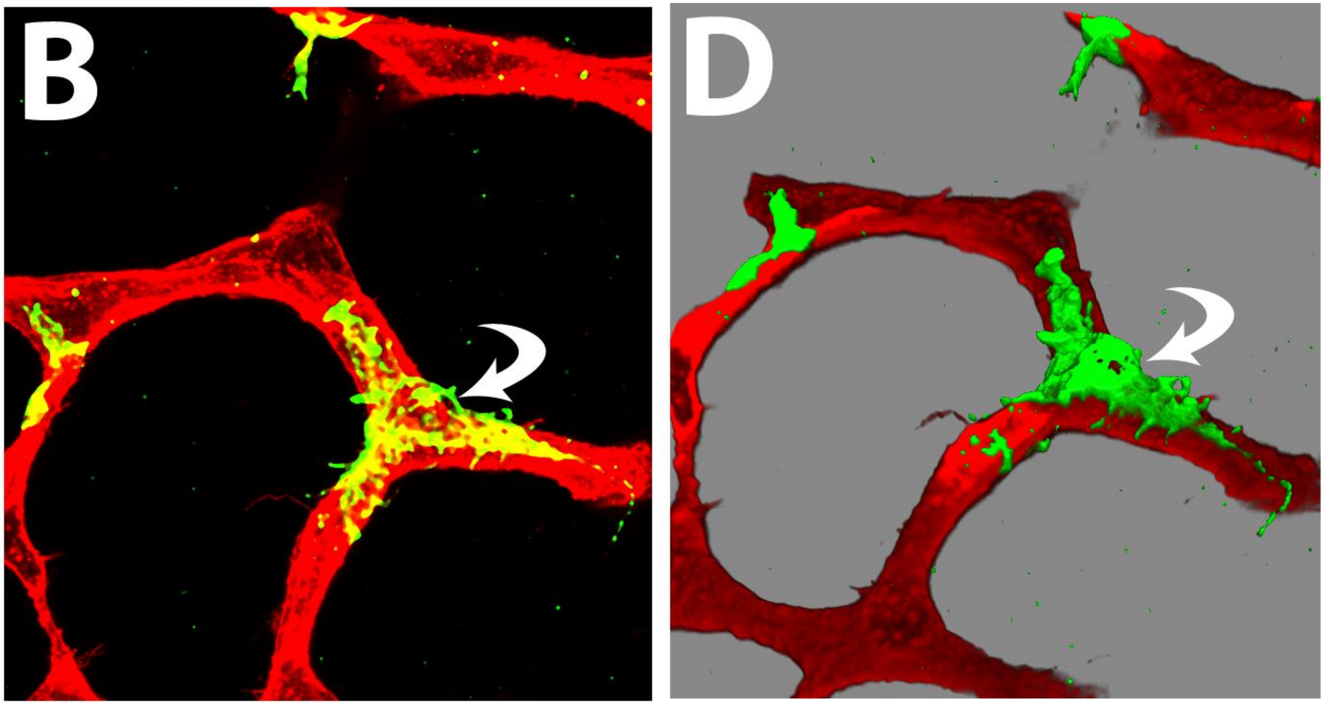

MΦs remain in contact with vessel junctions

Fantin et al., *Blood* 2010

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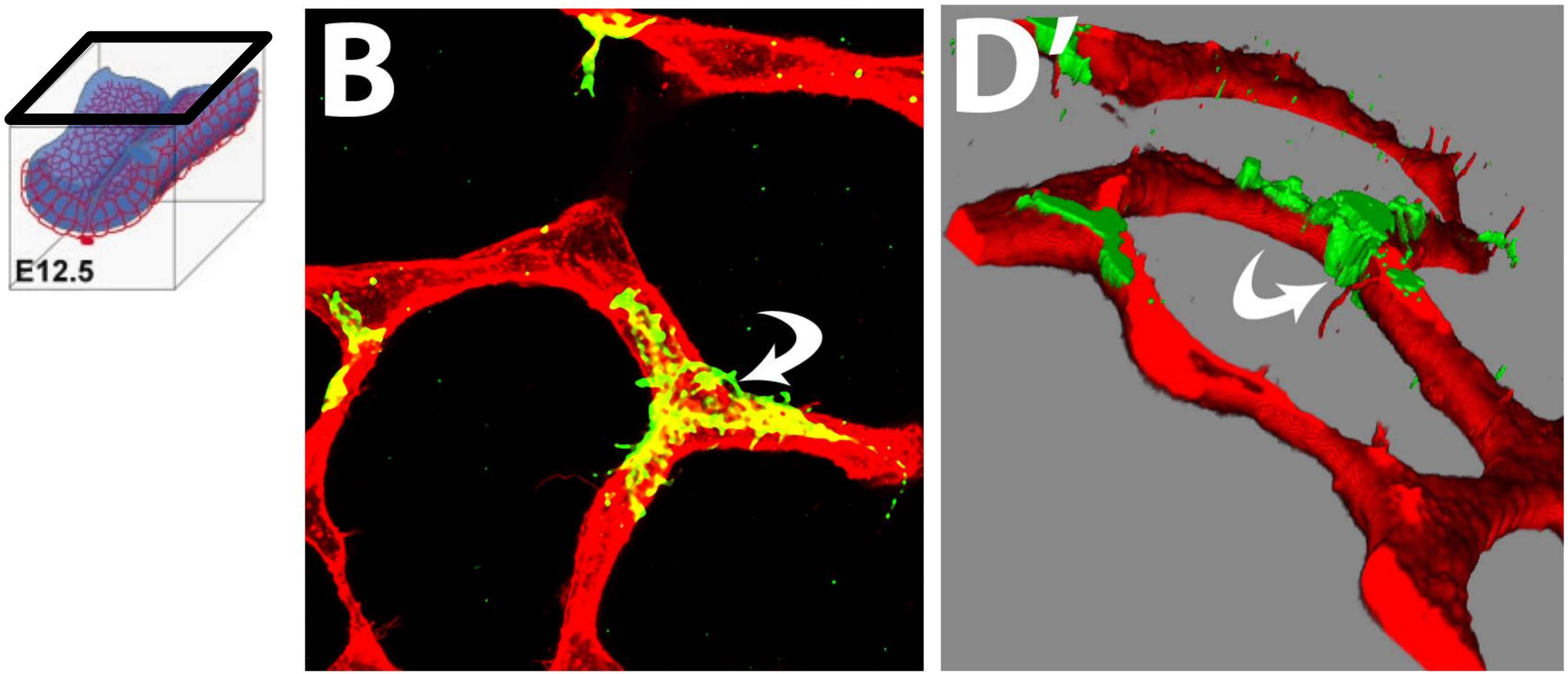
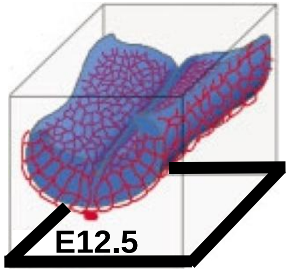
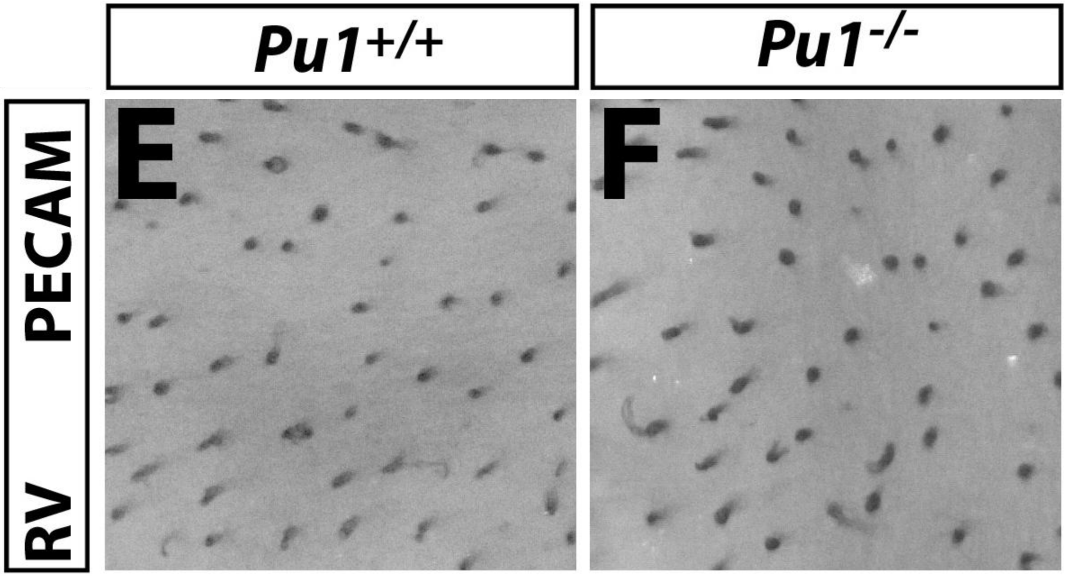


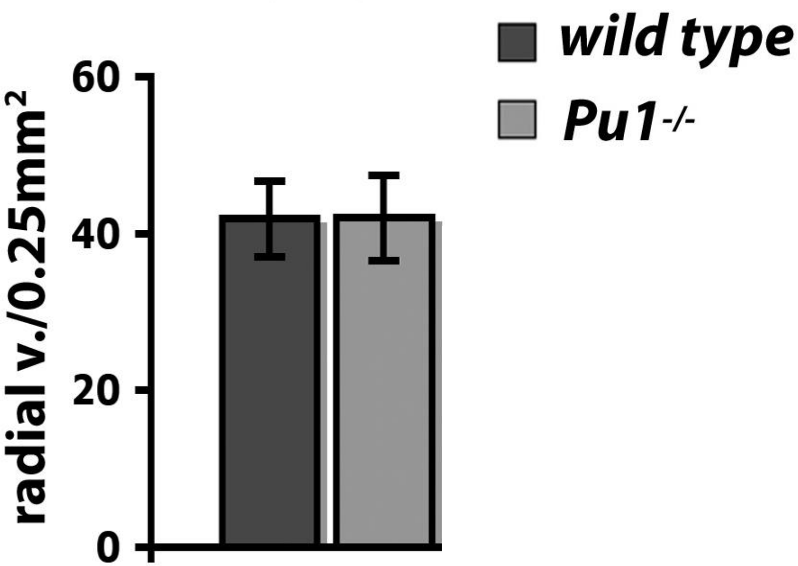
MΦs remain in contact with vessel junctions

Fantin et al., *Blood* 2010

MΦ deficiency does not impair vessel sprouting:







Fantin et al., *Blood* 2010

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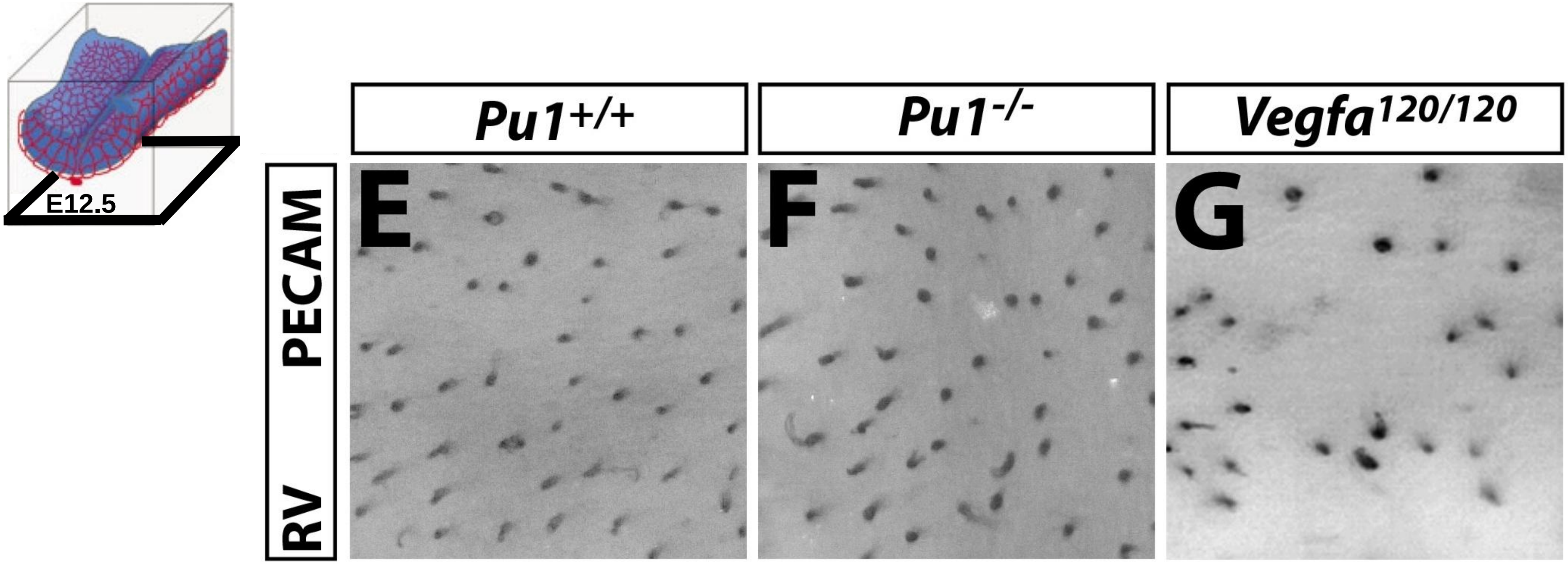
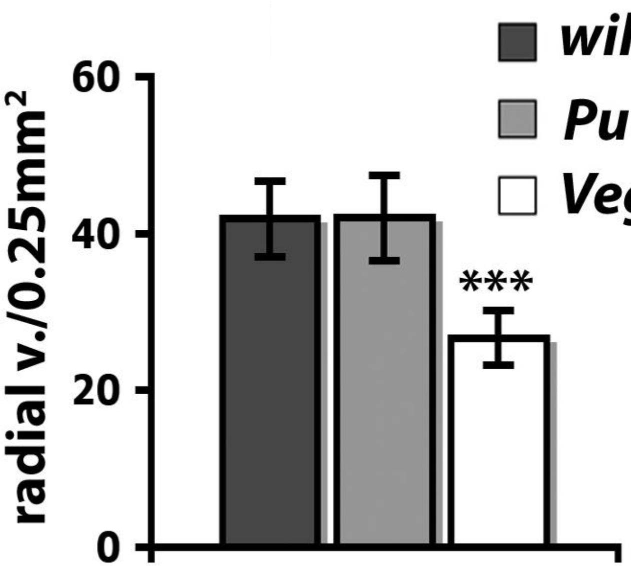
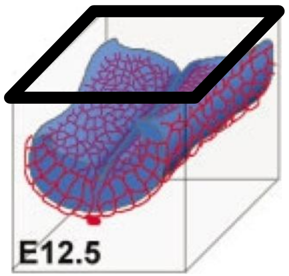


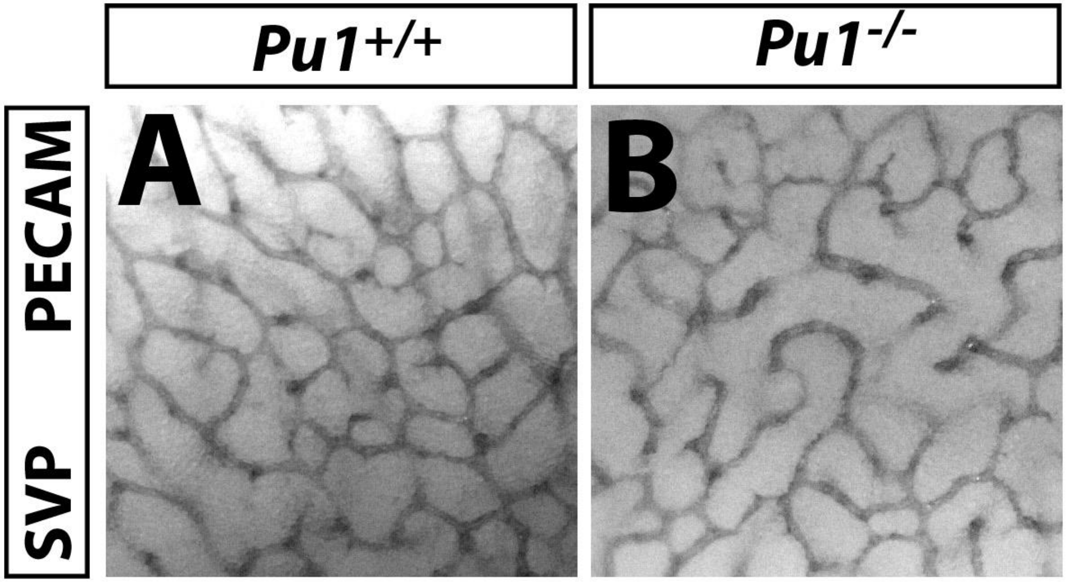


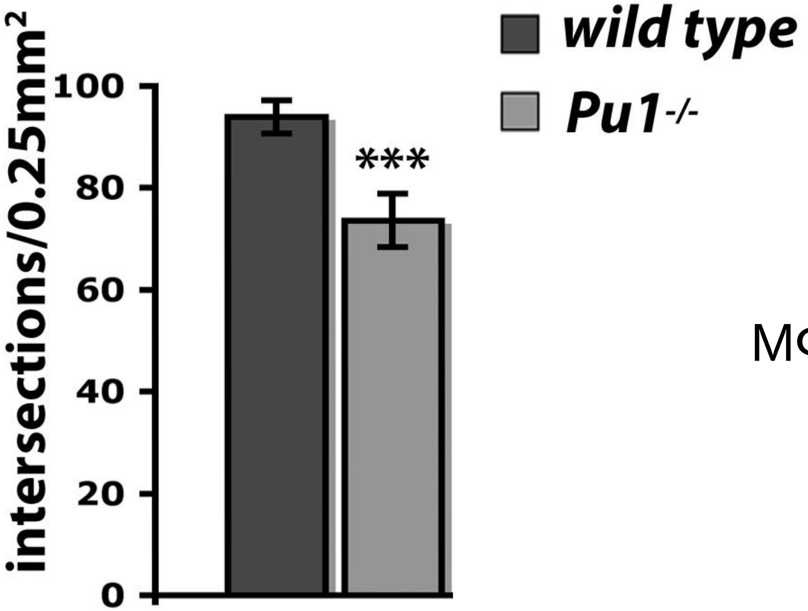
...but the loss of VEGF gradients impairs vessel sprouting into the brain.

Fantin et al., *Blood* 2010

MΦ loss impairs branching in the SVZ:



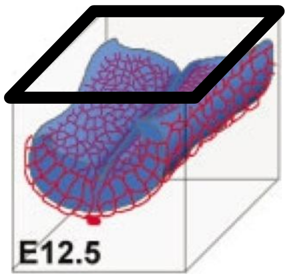


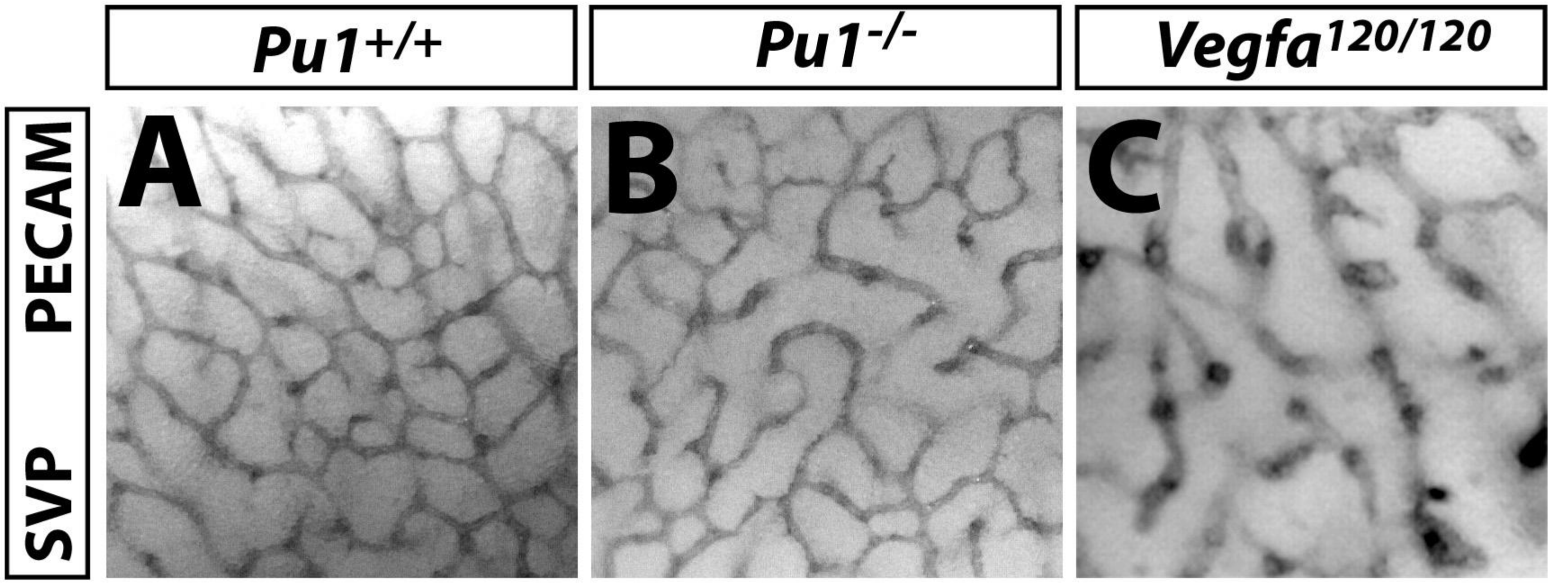


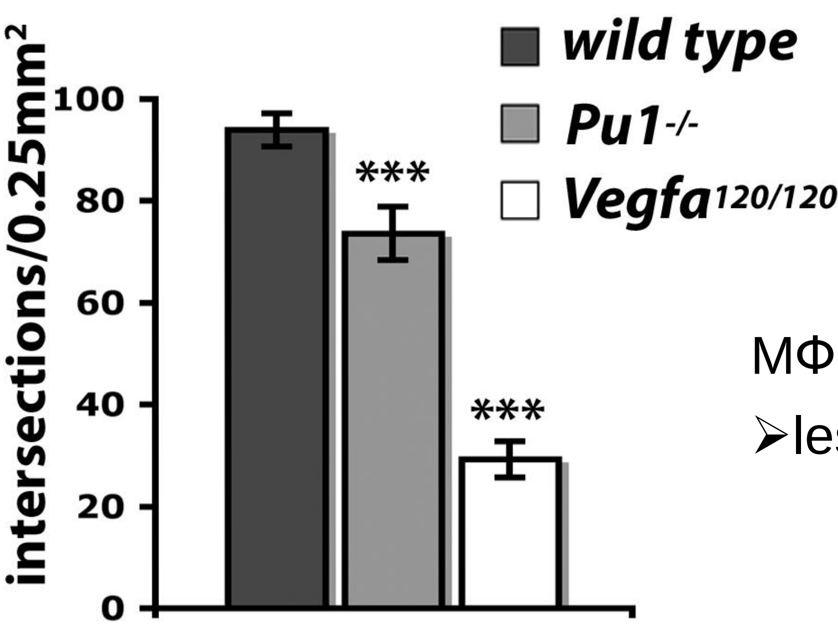
MΦ deficiency reduces vascular complexity:

Fantin et al., *Blood* 2010

MΦ loss reduces network complexity:







MΦ deficiency reduces vascular complexity:

➢less obvious than losing VEGF gradients

Fantin et al., *Blood* 2010

Vegfa$^{120/120}$: reduced sprouting→reduced anastomosis
Pu1$^{-/-}$: normal sprouting→reduced anastomosis

- MΦs are not required for sprouting, but to optimise vascular complexity.

Fantin et al., *Blood* 2010

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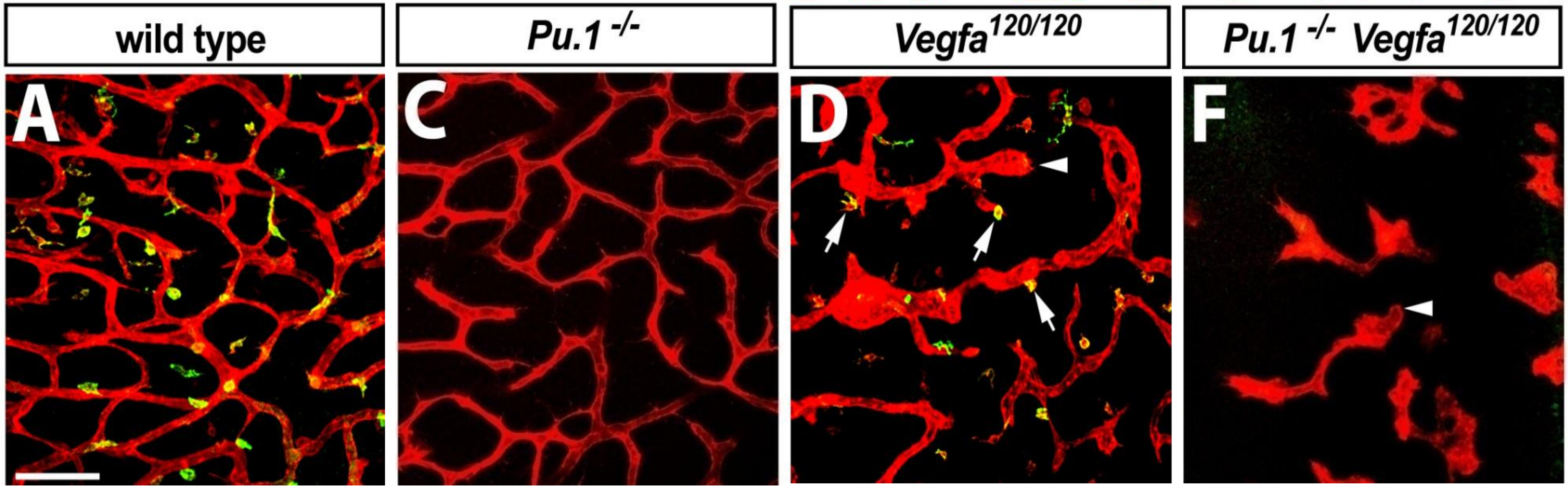
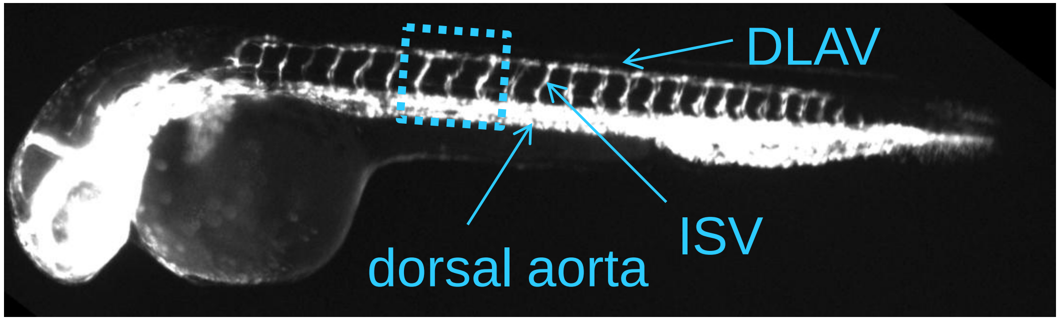


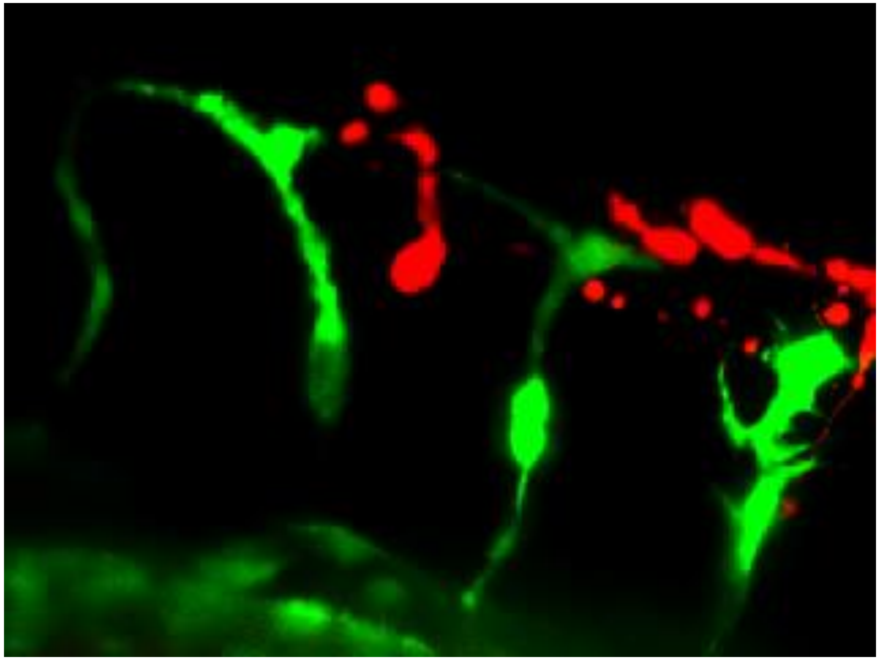
VEGF-induced sprouting and MΦ-mediated sprout fusion are discrete, but complementary steps in brain angiogenesis

Fantin et al., *Blood* 2010

Live imaging of MΦ behaviour during vessel fusion

28-32 hpf zebrafish embryo



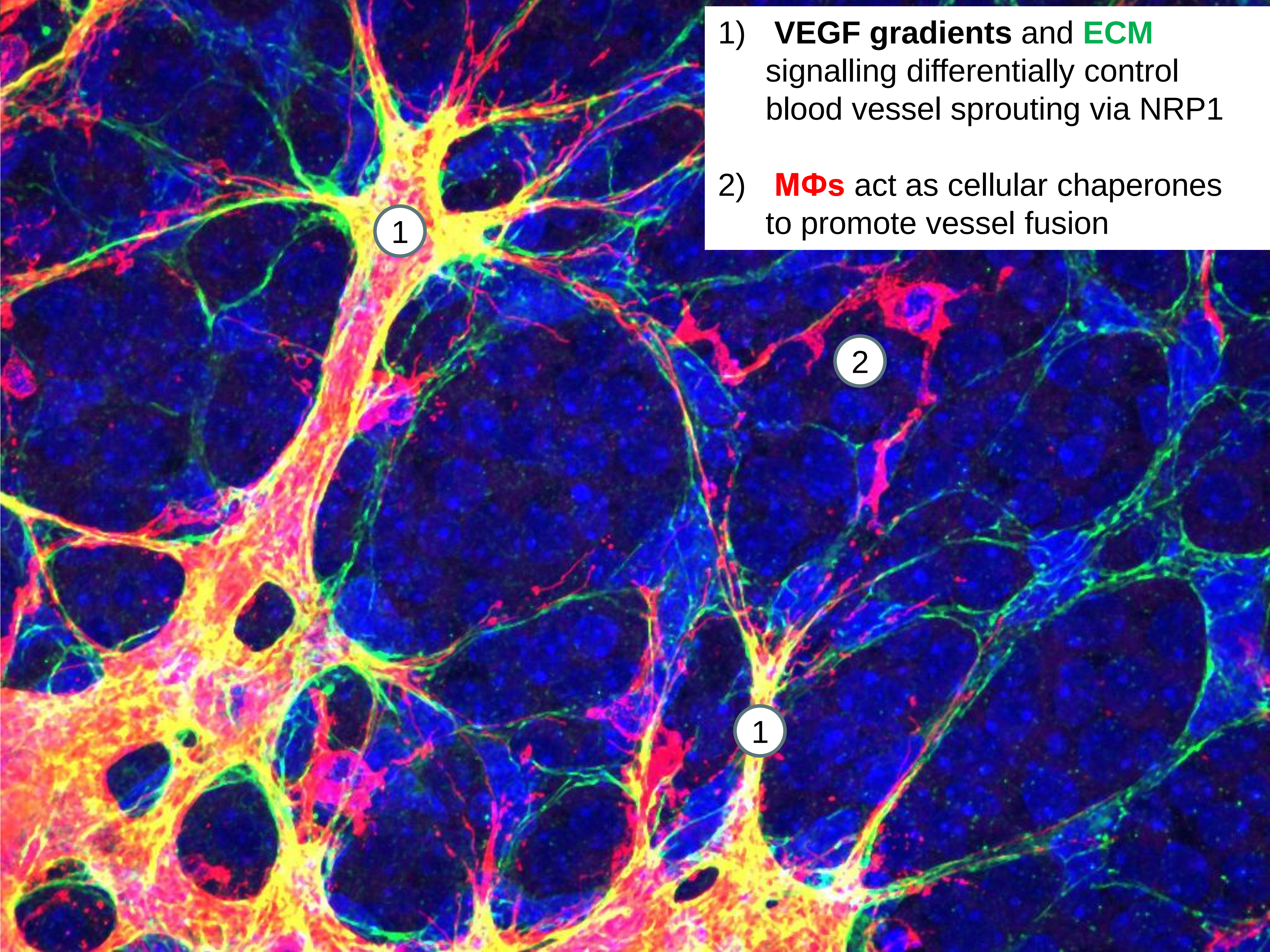


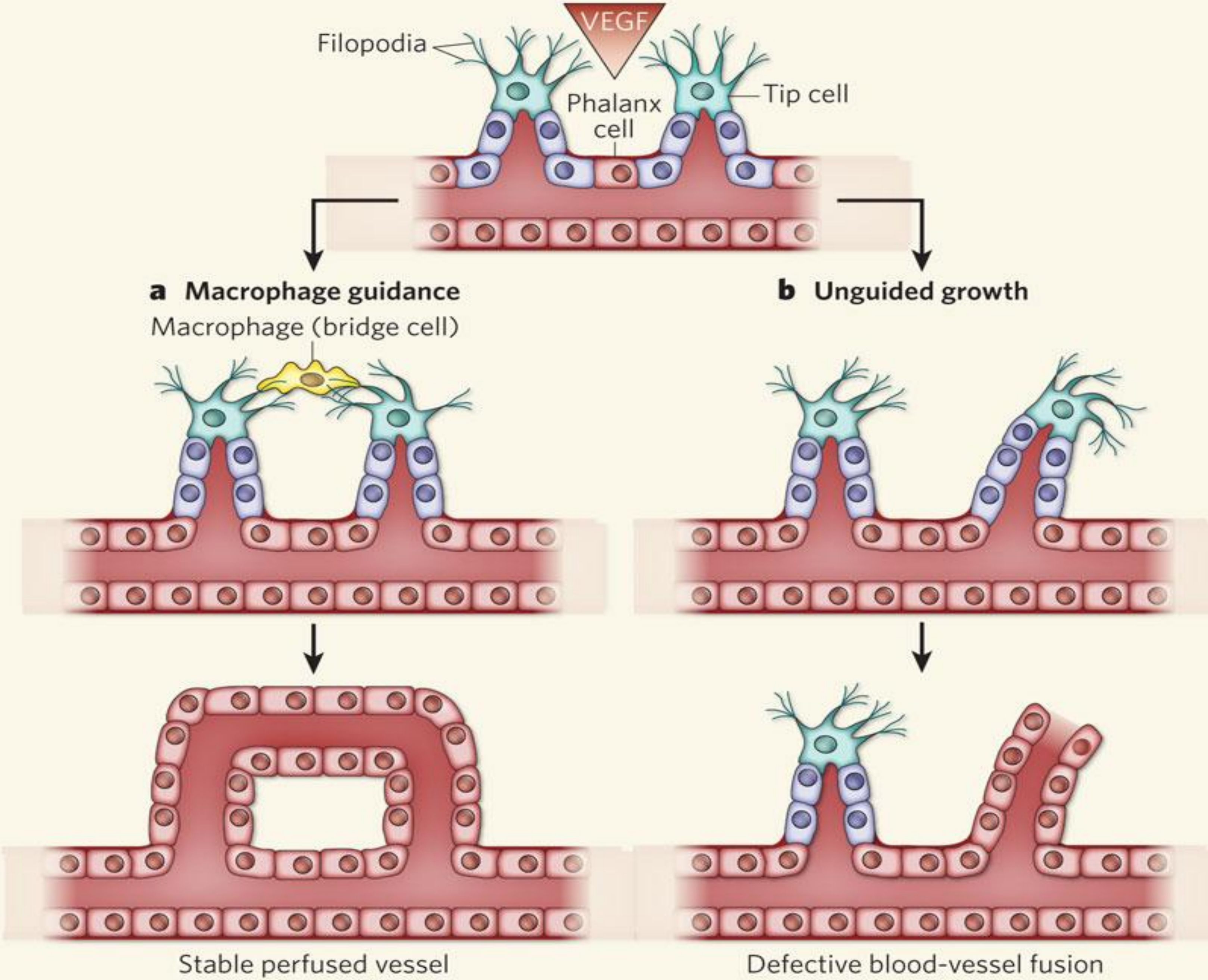
Tg(fli1a:EGFP)^{y5} Tg(pu1:Gal4-UAS-TagRFP)

Fantin et al., *Blood* 2010

1) **VEGF gradients** and **ECM** signalling differentially control blood vessel sprouting via NRP1

2) **MΦs** act as cellular chaperones to promote vessel fusion





Schmidt and Carmeliet, *Nature* (News &Views) 2010

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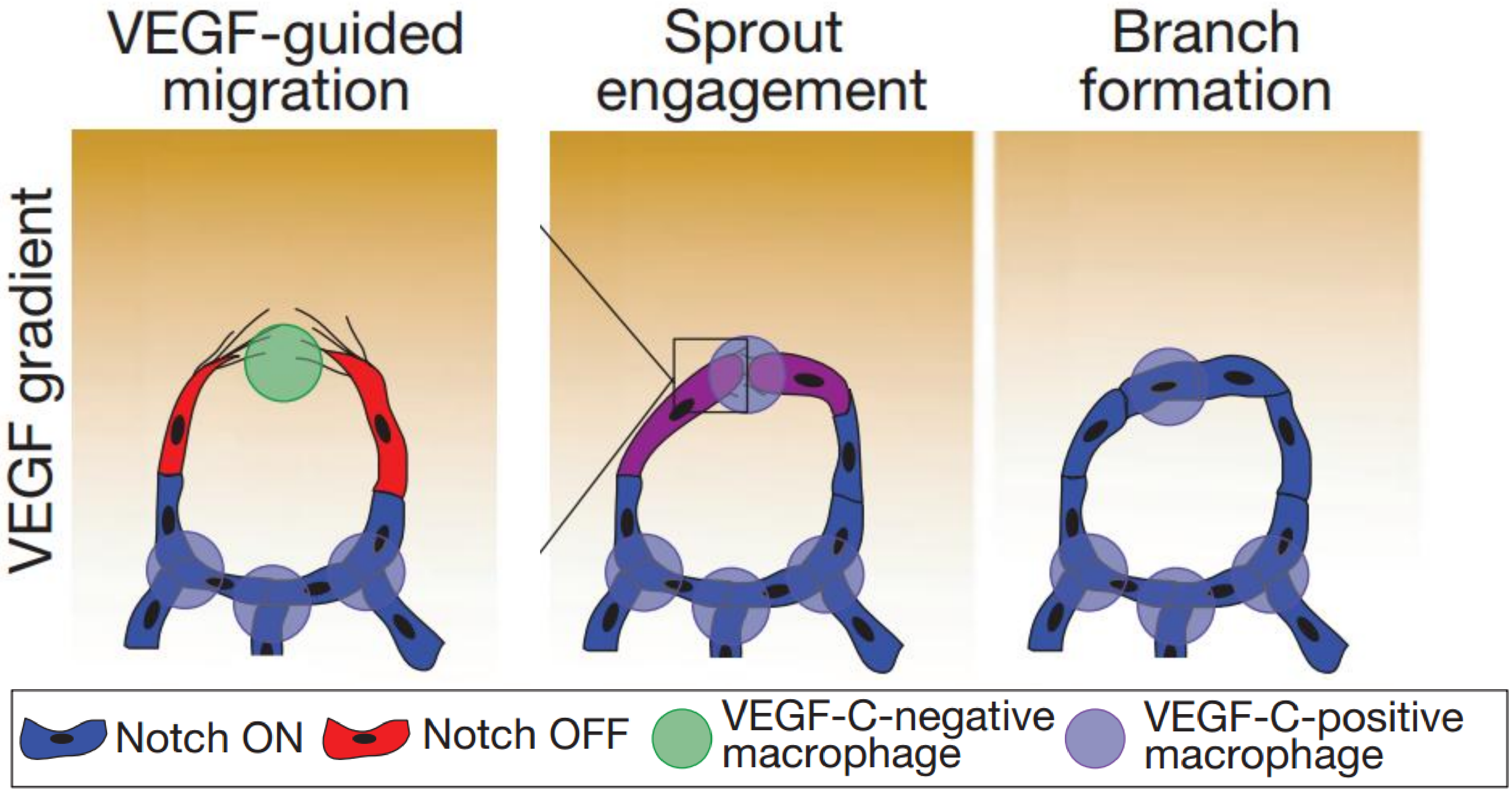
I Zachary (UCL)
G Gestri & S Wilson (UCL)
J Bainbridge (UCL)
H Augustin (Heidelberg)

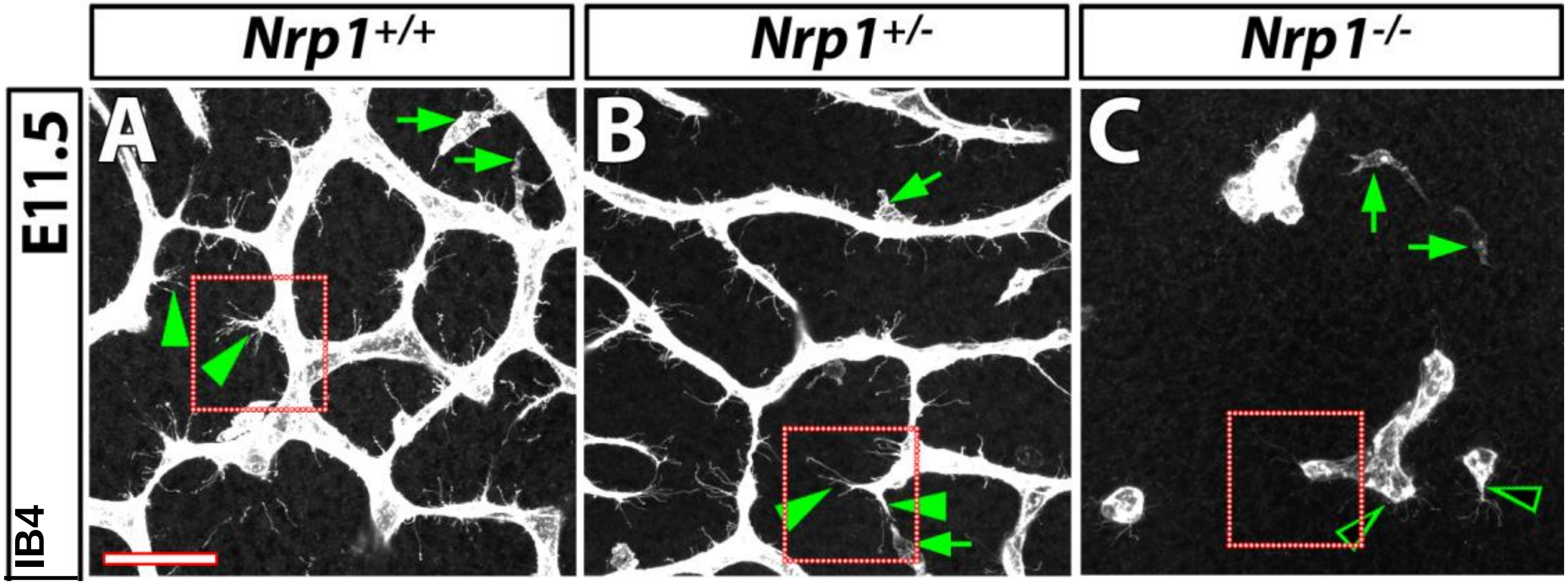
UCL INSTITUTE OF OPHTHALMOLOGY

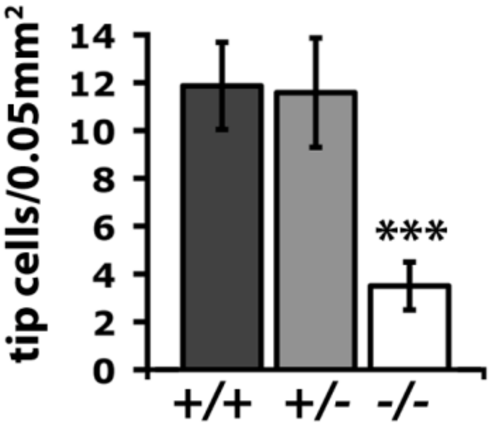
MΦs express VEGF-C (Tammela et al., Nat Cell Biol 2011)



1. 2 tip cells that lead vascular sprouts are chaperoned to fuse by a MΦ
2. VEGF-C expression ensues in the MΦ, activating VEGFR-3 in the tip cells
3. Increased Notch signalling decreases sensitivity to the VEGF gradient in the cells
4. Tip cells are stabilised in stalk or quiescent ECs

NRP1 controls endothelial tip cell formation





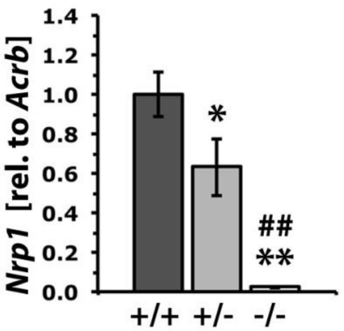
➢ Tip cell formation is defective in *Nrp1* mutants

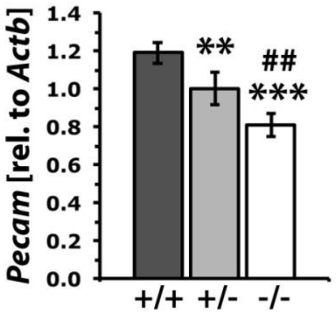
Fantin et al., *Cell Reports* 2015

Does NRP1 control tip cell molecular specification?

RNA

Nrp1 *Pecam* *Apln* *Ang2*





Vegfr2 *Dll4* *Hes1* *Hey*

- ➢ *Nrp1* expression is reduced, as expected.
- ➢ *Pecam* levels are reduced – delayed angiogenesis.

Fantin et al., *Cell Reports* 2015

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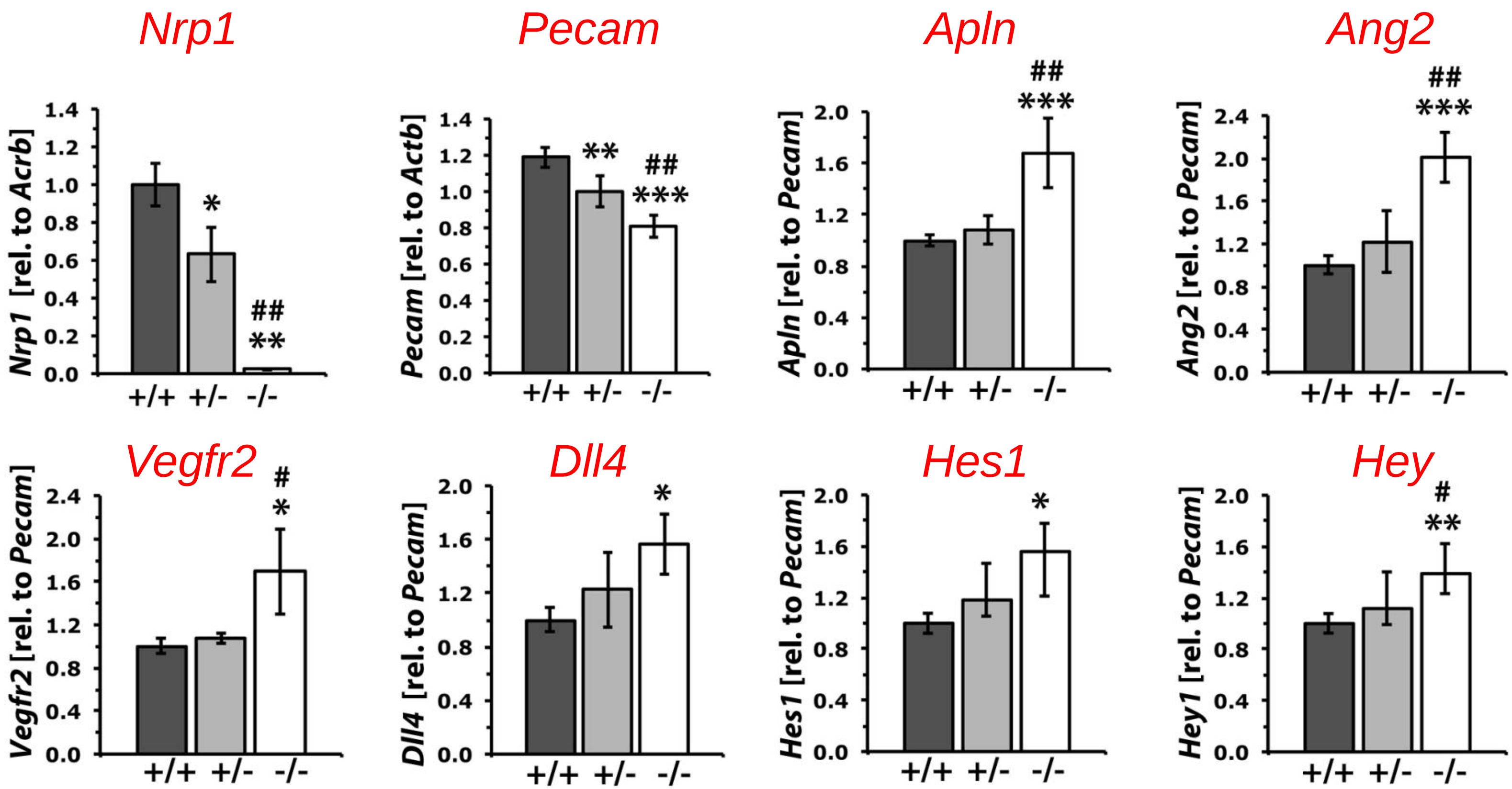

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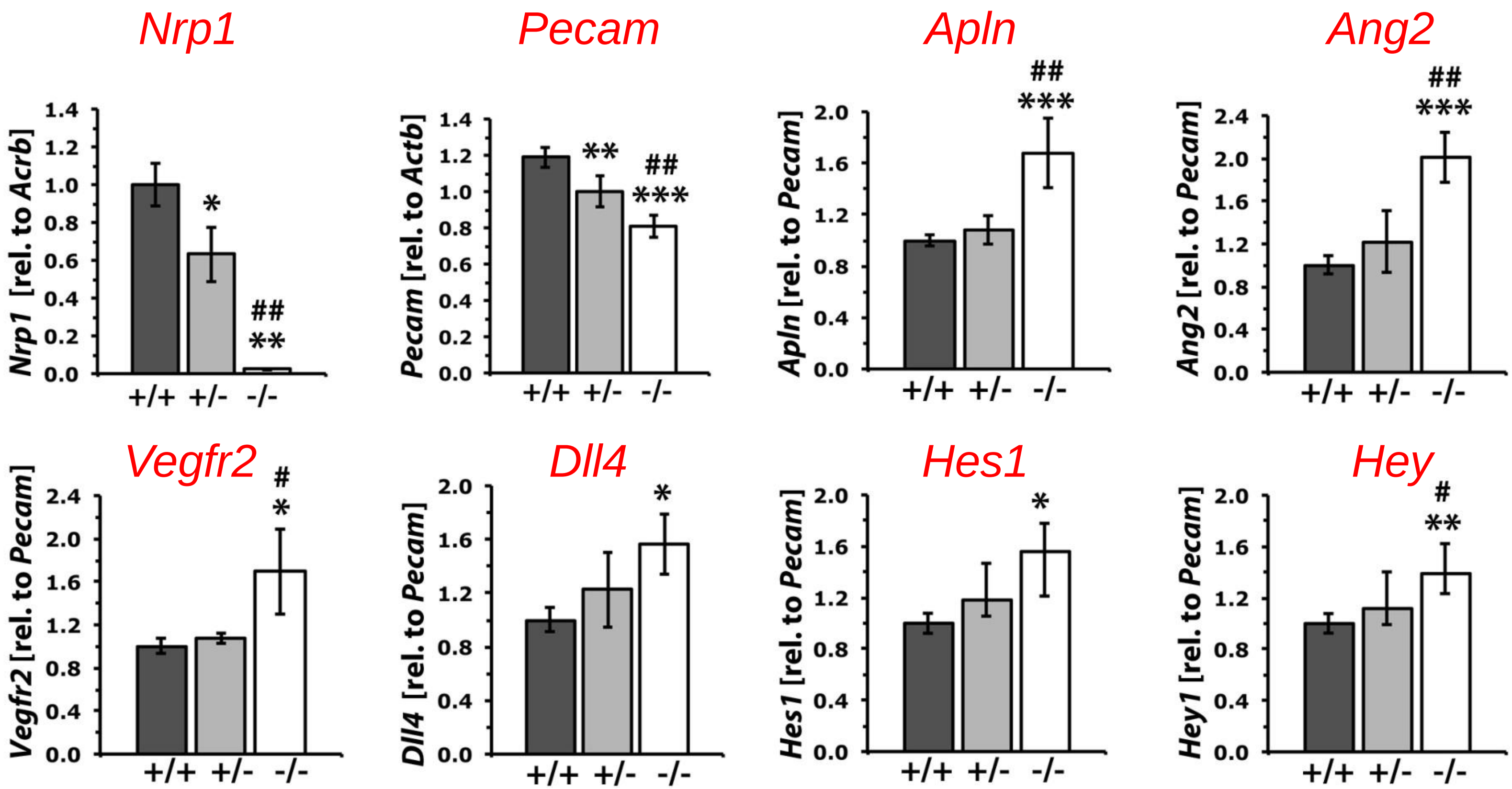
Fantin et al., *Cell Reports* 2015

Does NRP1 control tip cell molecular specification?

REJECTED



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