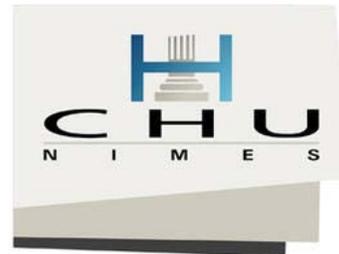


The Role of Endothelium in Haemostasis.

Jean-Christophe Gris, *M.D., Ph.D.*,

*Department of Haematology, University Hospital, Nîmes and University of Montpellier;
INSERM U1318, Montpellier; France.*



Athens, October 2025

Disclosures

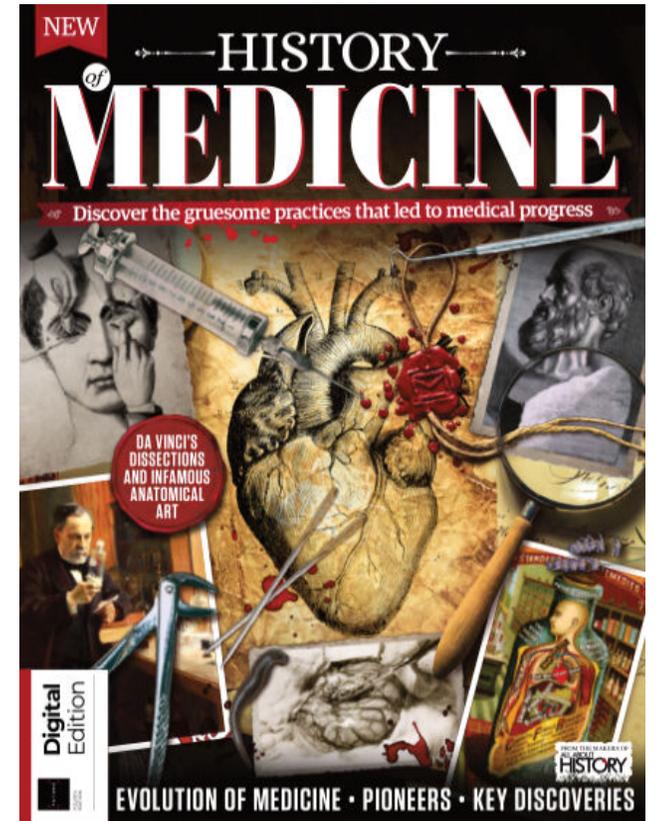
- **Grants:**
 - Bayer Healthcare SAS, Sanofi SA, Stago
- **Scientific Advisory Boards:**
 - Sanofi SA, LFB, Novo Nordisk, Therapeutics
- **Speaker / speaker bureau member, supports:**
 - Alexion Pharma France, Amgen, Astra-Zeneca, Bayer, Bristol-Myers-Squibb, Boehringer-Ingelheim, Daïchi-Sankyo, Fumouze diagnostics-Sofibel, Glaxo-Smith-Kline, Horiba ABX SAS, Laboratoire Français des Biotechnologies (LFB), Léo Pharma, Novo Nordisk, Oséus, Pfizer, Sanofi SA, Shire, Stago



General framework

- Some evidences on the endothelial polymorphism
- Some formal data on endothelium and haemostasis

Historical And Basic Facts



- **Endothelium:**

- *From the ancient Greek:*

- ἐνδον (say éndon: *within*)

- and

- θηλή (say thele: *nipple*).

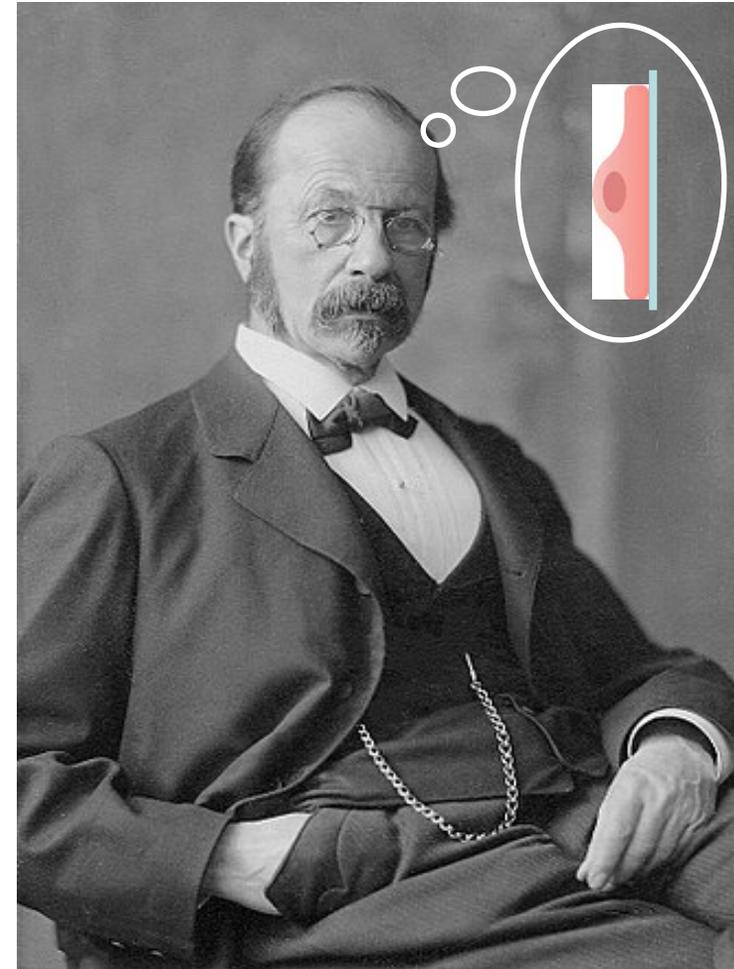
- « *A thin layer of epithelial cells that lines blood vessels, lymph vessels,..* »

- Term coined by the Swiss anatomist **Wilhem His** (1865) to distinguish between the inner layer of body cavities and the epithelium (outer layer);

- afterwards: reduced to just the blood and lymphatic vascular system.*

The cellular interface between blood and tissues.

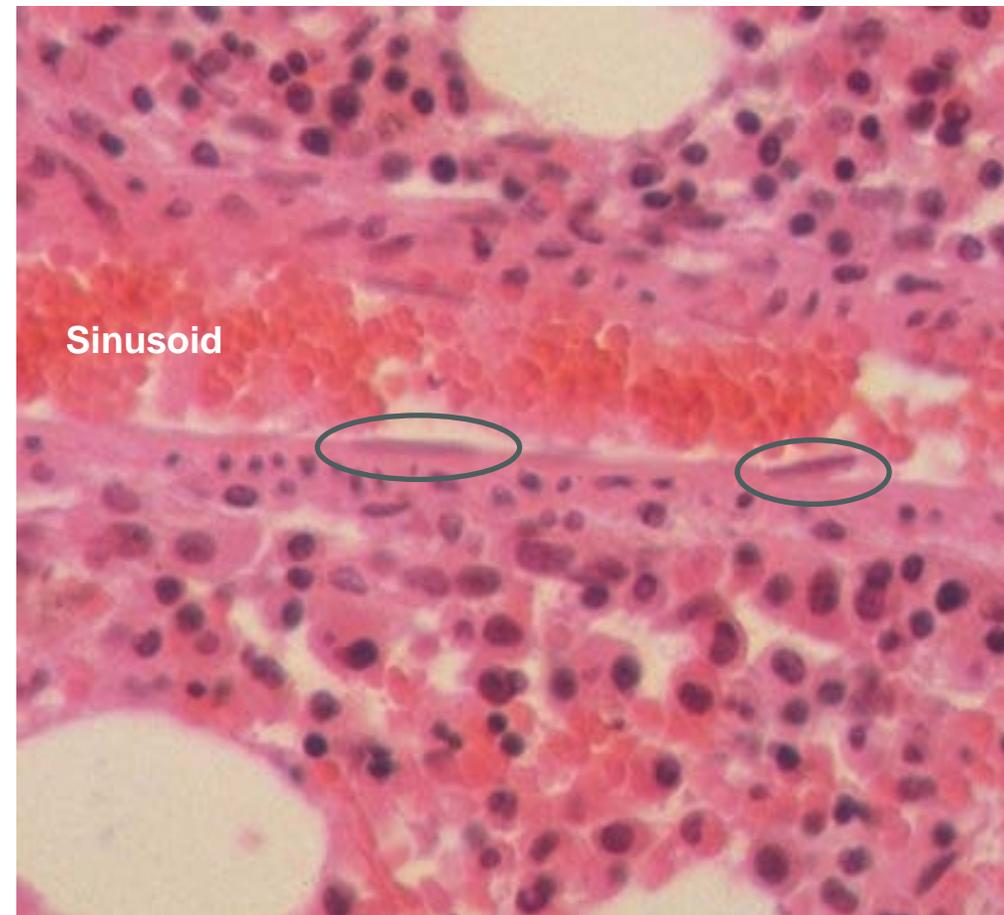
Wilhelm His, 1831-1904.



CENSORED

The early stages of describing the endothelium

- Endothelial cells initially identified by classical microscopy;
- first half of the 20th century...
...« *a sheet of nucleated cellophane* ».



Bone marrow biopsy

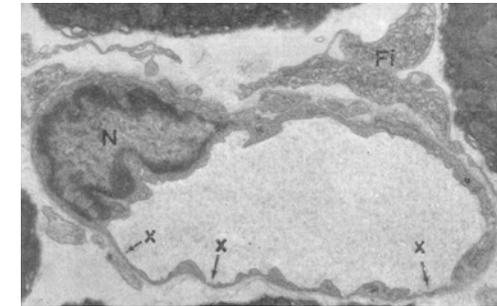
The youth of endothelium description

- After the WWII: electron microscope

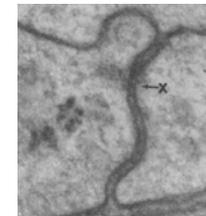
rons long by 10 microns
 joined to one another to
 d and lymphatic channels.
 bounds I shall deal only
 ose from the rat and the

Although little can b
 found in endothelial cell
 that if particles such as
 iron oxide are injected
 way into them in all the
 How these marker part:

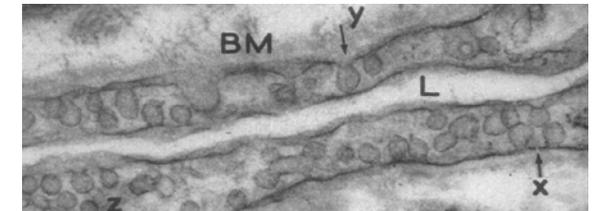
Br Med J 1966; 2: 487-90



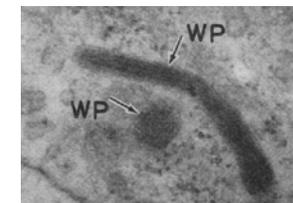
Capillary from the pancreas



A junction



Cardiac capillaries: *caveolae intercellulares*



Aortic endothelium:
 « *bodies similar to this
 were described
 by Edward Weibel
 and George Palade, 1964* »

* Howard Walter Florey, 1998-1968: Nobel price in Physiology and Medicine
 with Ernst Chain and Sir Alexander Fleming for his role in the development of penicillin

The youth of endothelium description

- After the WWII: electron microscope
 - *initial focus on permeability*

« *Caveolae intercellulares:*

may facilitate the **passage of substances through endothelium.**

All endothelial cells so far examined

seem to be able

to take up particles of molecular size:

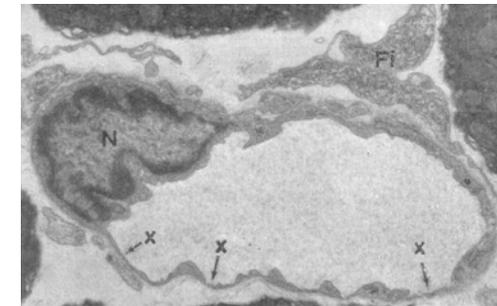
behaviour of virus, of foreign particles?

Initial phases of the development of atherosclerosis? »

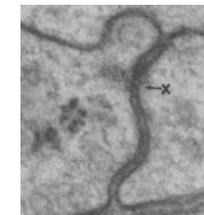
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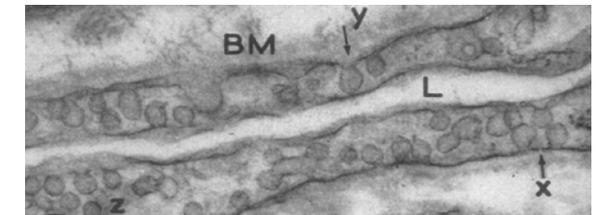
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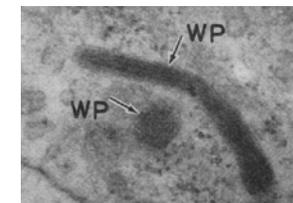
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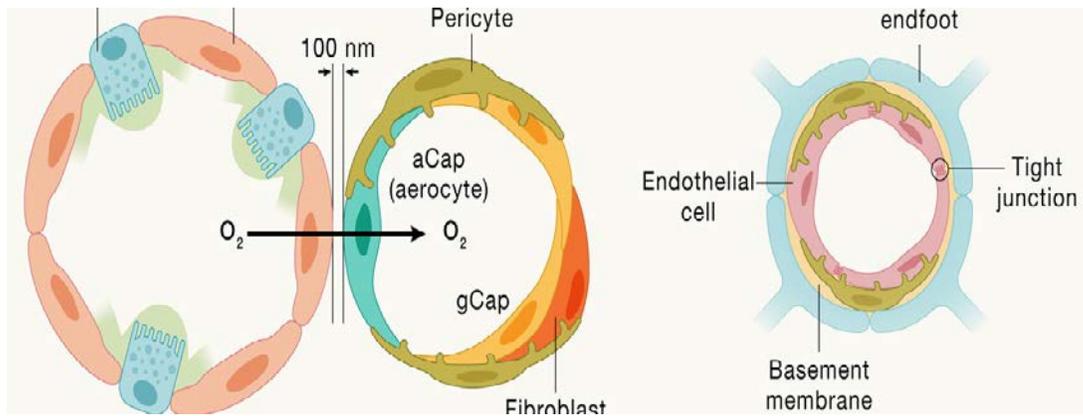
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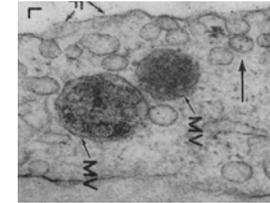
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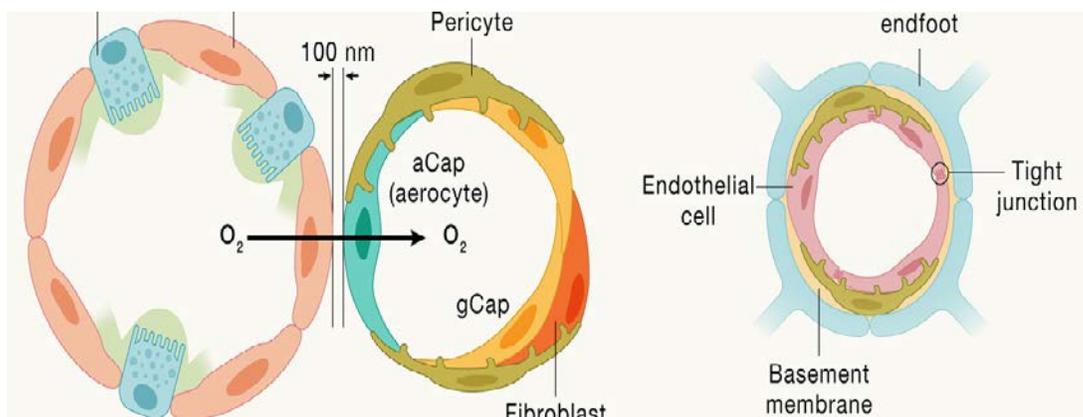
Permeability



- **Structural components of the endocytic pathway**
(targets macromolecules to the lysosomal compartment for degradation; non-specific, fluid-phase process or via receptor-dependent pathways)
 - Clathrin-coated pits
 - Clathrin-coated vesicles
 - Multivesicular bodies
 - Lysosomes

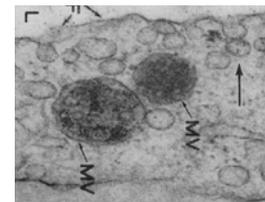


Permeability



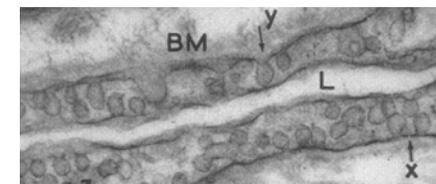
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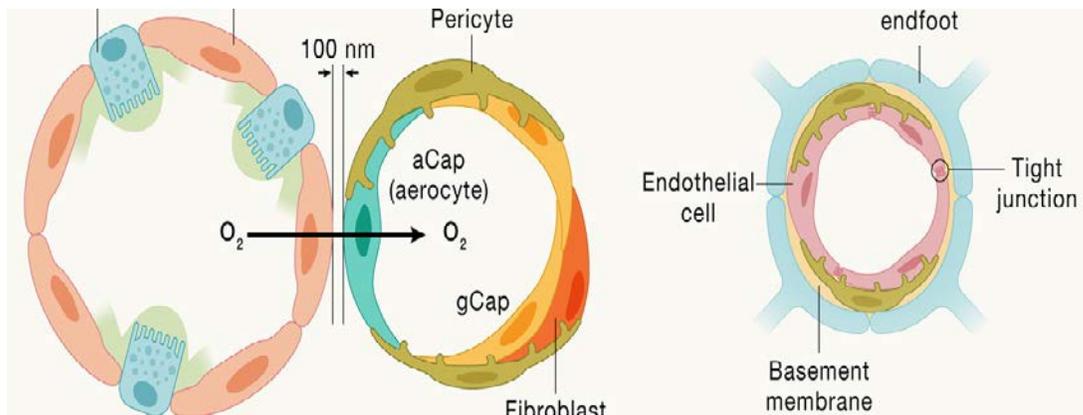


- **Transcytosis** (*governs the transcellular transfer of molecules across the endothelium; specialised structures*)

- Caveolae, 70 nm, plasmalemmal protein-1 (PV-1) positive
- Vesiculo-vacuolar organelles

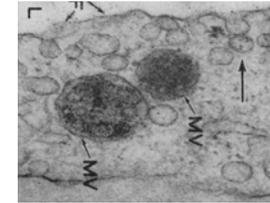


Permeability



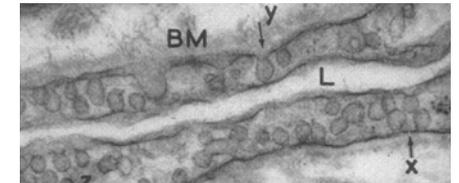
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- Vesiculo-vacuolar organelles



- **Intercellular junctions**

- Tight junctions (*zona occludens*)
 - Apical regions of the intercellular clefts
 - Barrier to paracellular transport; maintain cell polarity between luminal and abluminal sides
- Adherens junctions (*zona adherens*)

Beginnings of modern vascular biology:

endothelial cell culture

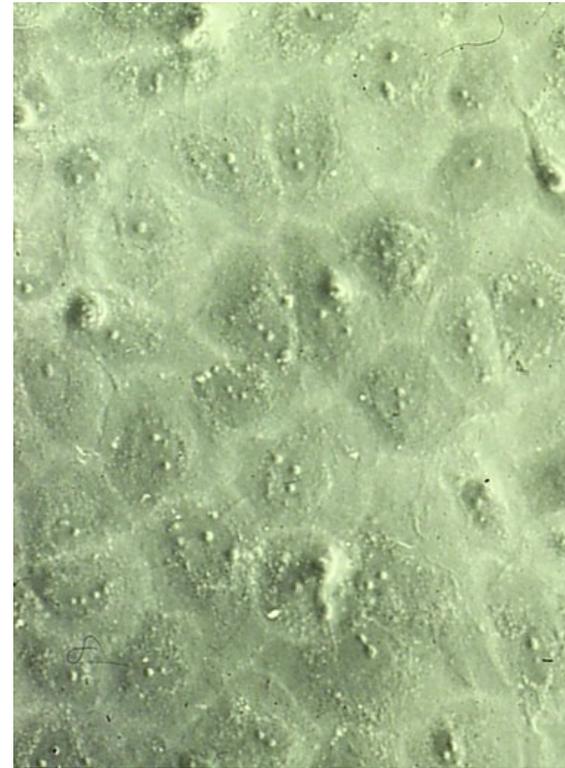
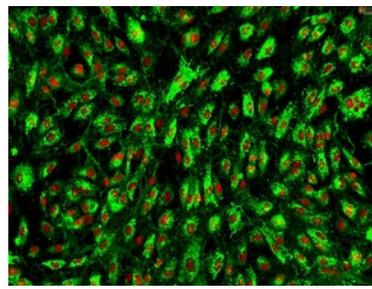
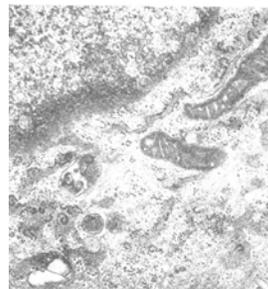
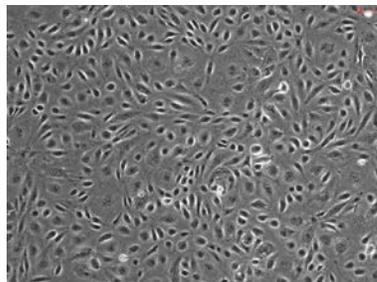
h muscle cells grew as overlapping layers of parallel rays of slender, spindle-shaped cells. By transmission electron microscopy, cultured endothelial cells were found to contain cytoplasmic inclusions (Weibel-Palade bodies) characteristic of *in situ* endothelial cells. These inclusions were also found in endothelial cells lining umbilical veins but were not seen in smooth muscle or fibroblasts in culture or *in situ*. Cultured endothelial cells contained abundant quantities of smooth muscle actomyosin. Cultured endothelial cells also contained ABO antigens appropriate to the tissue donor's type; these antigens were not detectable on cultured smooth muscle cells or fibroblasts. These studies

ability to maintain endothelial cells in pure culture for reasonable periods of time; (b) inability to identify cultured cells as endothelium.

Maruyama (20) cultured what were presumed to be endothelial cells from umbilical veins, but the characterization of these cells in culture was incomplete. In this paper we report on the long-term *in vitro* culture of cells derived from human umbilical veins. These cells have been identified as endothelial cells by morphological and immunologic criteria.

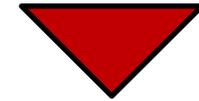
METHODS

From human umbilical cord veins;
long-term culture, periods up to 5 months



CRITERIA

- Cell Morphology +
- Weibel – Palade bodies +
- Smooth Muscle actomyosin -
- ABO antigens +
- Factor VIII Antigen +



+

+

-

+

+

Traditional calling card of endothelial cells

(for naive people, TV presenters and some politicians)

Average-size human:

60 trillions (*i.e.* 10^{12} : billions of billions) cells

total weight: approx. 1 kg

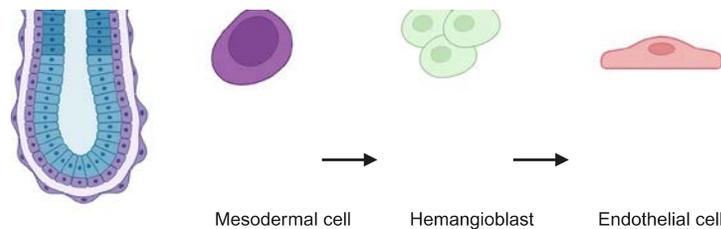
total surface area: 4,000-7,000 m²

polygonal, elongated

length 25-50 μm , width 10-15 μm , thickness: 0.1-1 μm

flat, sometimes plump or cuboidal

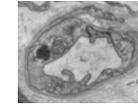
Ontogeny:



Criminal record:

thrombosis, haemorrhage,

cancer progression, vascular malformations,...



Endothelial cells

www.vessel_intimacy.org

BREAKING NEWS

1 MIND THE GAP

WHEN BIG NEWS BREAKS, EVERYONE WANTS THE DETAILS, but verified information can be tough to find. **BE MINDFUL** that a deluge of unconfirmed, misleading, and completely bogus content often rushes in to fill the gap between what's available and what the public wants to know.

2 BEWARE BAD ACTORS

BAD ACTORS NEED ATTENTION TO THEM, so they often capitalize on breaking news situations to spread sensationalized or false content for cheap likes and shares. In times of war, natural disasters and upheaval, misinformation also acts as a tool to advance certain narratives and shape public opinion.

DON'T BE EXPLOITED. Slow down and resist the urge to share unconfirmed claims or visuals.

CHECKLIST

What to do when information is scarce, falsehoods are rampant and the story is still unfolding

6 PRACTICE PATIENCE

QUALITY JOURNALISM AND VERIFICATION TAKE TIME. Journalists have to interview people, gather documentation and confirm details with multiple sources. It might take days or even weeks to untangle initial accounts or investigate claims involving a major story. **BE READY** to follow news developments over time, knowing that the story – and your understanding of it – will likely evolve as more verified information comes to light.

REMEMBER Early details in news reports may turn out to be incorrect. What's important is how a news organization handles these mistakes. **Correcting errors** promptly and transparently is a strong signal of a news source's commitment to accuracy.

3 DO A QUICK SEARCH

A QUICK INTERNET SEARCH IS OFTEN THE BEST DEFENSE AGAINST MISINFORMATION. Hop off social media and do a simple search to investigate unsubstantiated claims and unfamiliar sources. **BEWARE** of stocking news updates circulating among ordinary social media users but not in news coverage. Credible news sources will cover major updates.

TIP It's common for old or unrelated visuals to circulate out of context in breaking news situations. **Reverse image searches** can help put repurposed visuals back in their original context.

4 LOOK FOR EVIDENCE

CLAIMS THAT MAKE BOLD ASSERTIONS BUT PROVIDE NO LINKS OR OTHER EVIDENCE should always be **APPROACHED WITH CAUTION**, especially screenshots. If a social media post does include an outside link – to a news report, study or some other source – **make sure the link actually supports claims in the original post.**

TIP Credible news sources aspire to **ethical guidelines and standards**, including accuracy, transparency and independence. Look for evidence that a source follows such standards.

5 SEEK CREDIBLE SOURCES

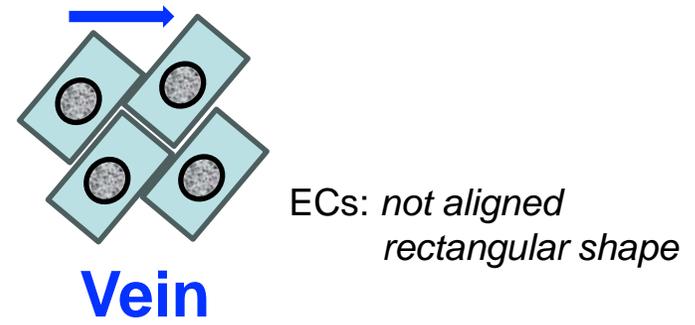
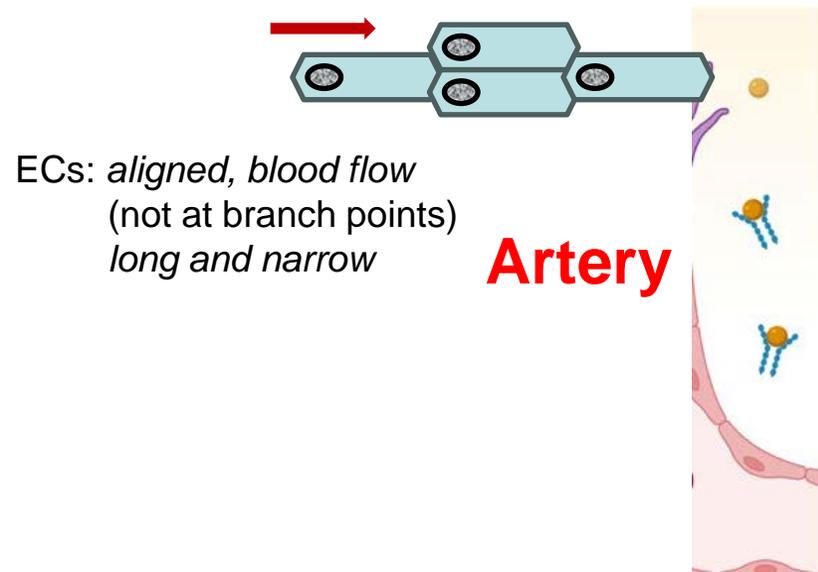
IT'S IMPORTANT TO **ACTIVELY SEEK OUT NEWS FROM CREDIBLE SOURCES** and not rely on social media algorithms to bring you updates. **BE INTENTIONAL** about following professional journalists on the ground.

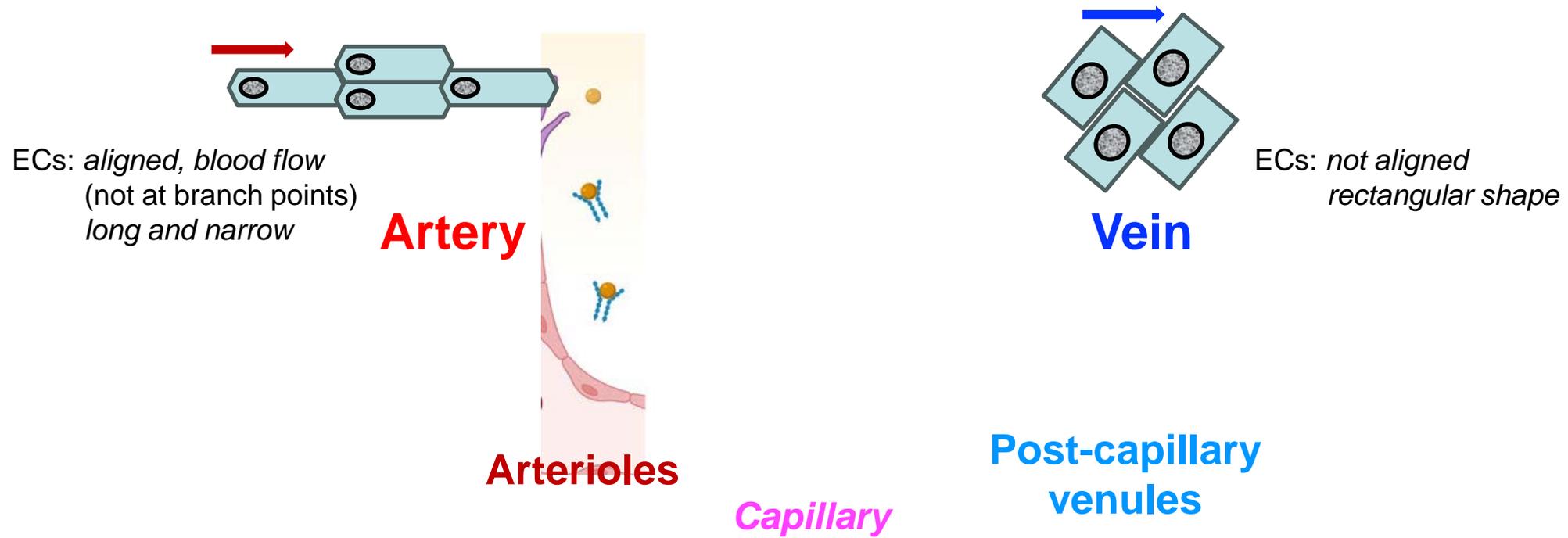
Look for **reputable news organizations and fact-checkers debunking falsehoods in real time**. Compare coverage across multiple credible news outlets and pay special attention to attribution. Is information attributed to sources in a position to know? Does the report independently verify disputed details or simply repeat information from officials?

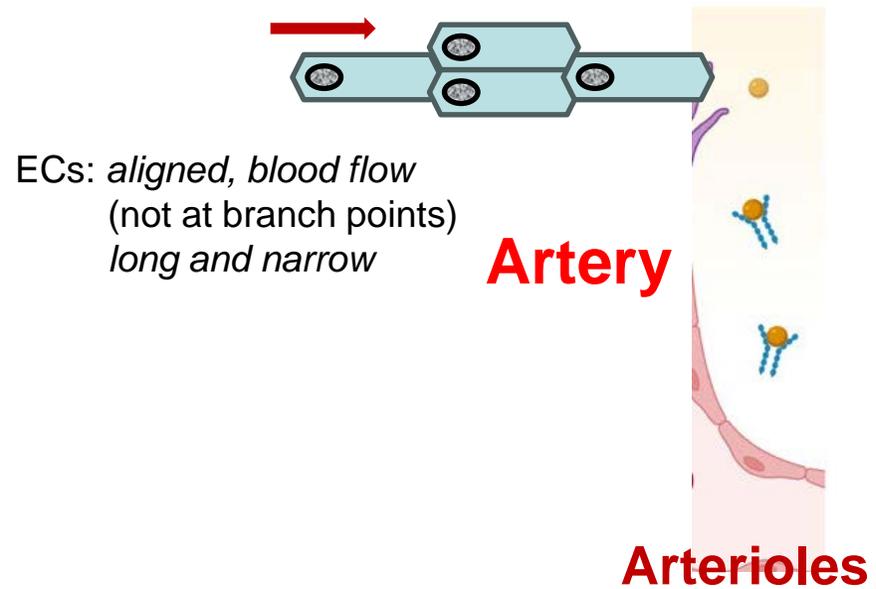
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Endothelium: *not a single homogeneous entity*

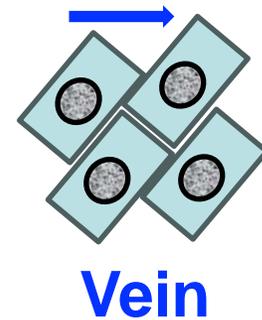
Endothelial heterogeneity:
structural data





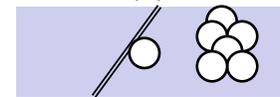


Capillary



**Post-capillary
venules**

Intercellular
Cleft (1)



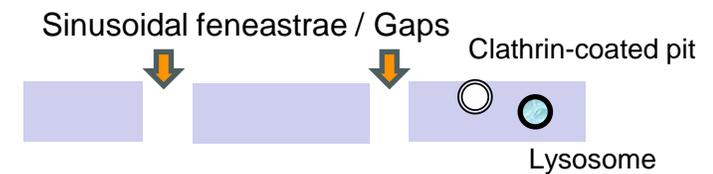
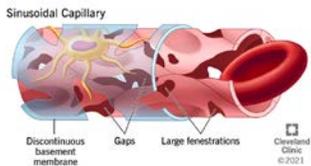
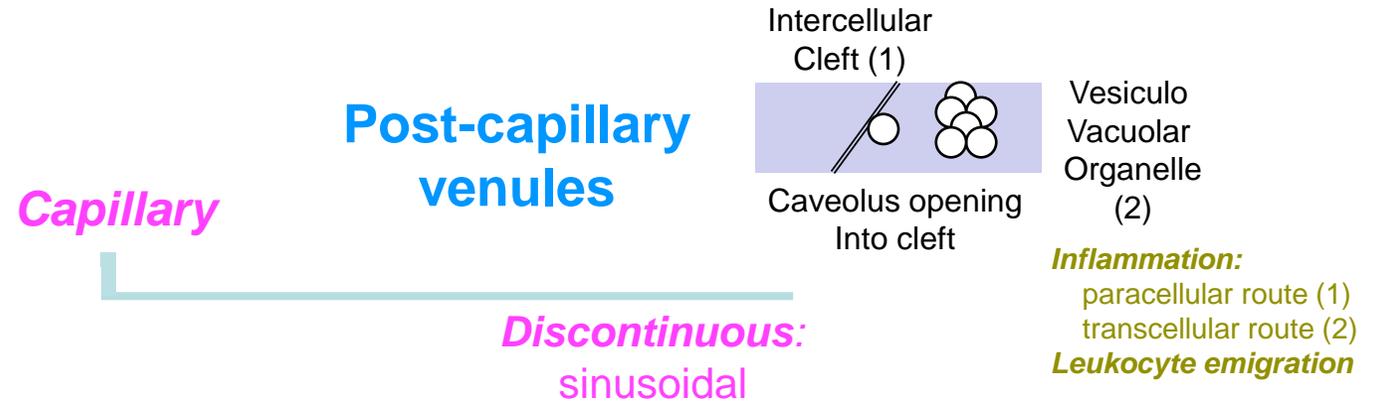
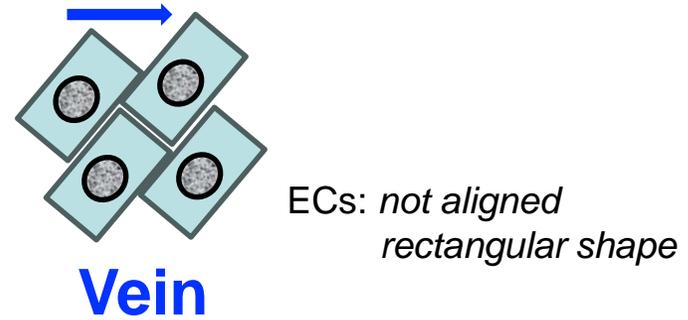
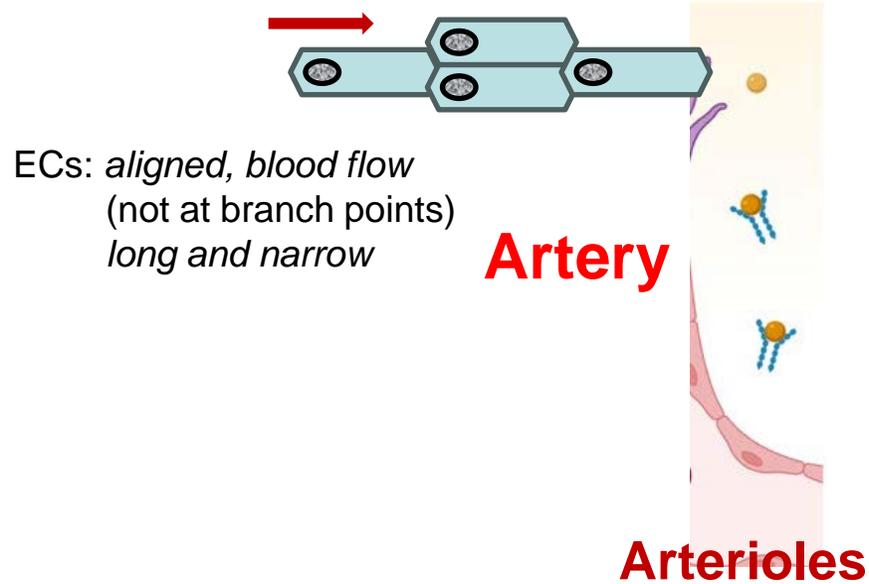
Caveolus opening
Into cleft

Vesiculo
Vacuolar
Organelle
(2)

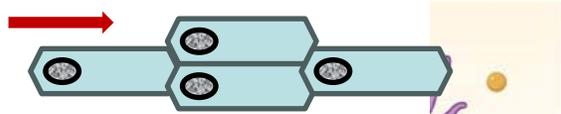
Inflammation:

- paracellular route (1)
- transcellular route (2)

Leukocyte emigration

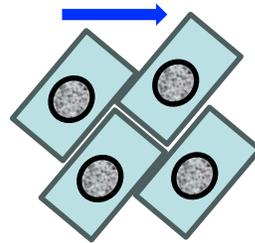


Liver, bone marrow, spleen



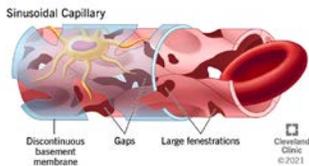
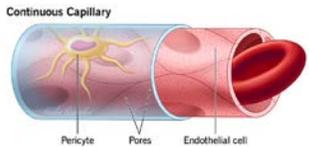
ECs: *aligned, blood flow*
(not at branch points)
long and narrow

Artery



ECs: *not aligned*
rectangular shape

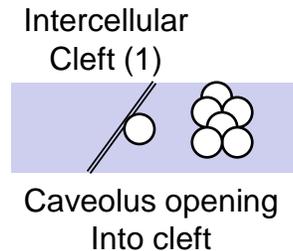
Vein



Arterioles

Capillary

**Post-capillary
venules**



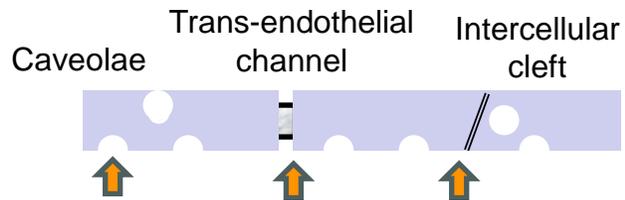
Vesiculo
Vacuolar
Organelle
(2)

Inflammation:
paracellular route (1)
transcellular route (2)
Leukocyte emigration

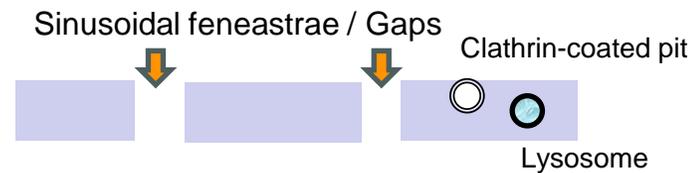
Continous

Non-fenestrated

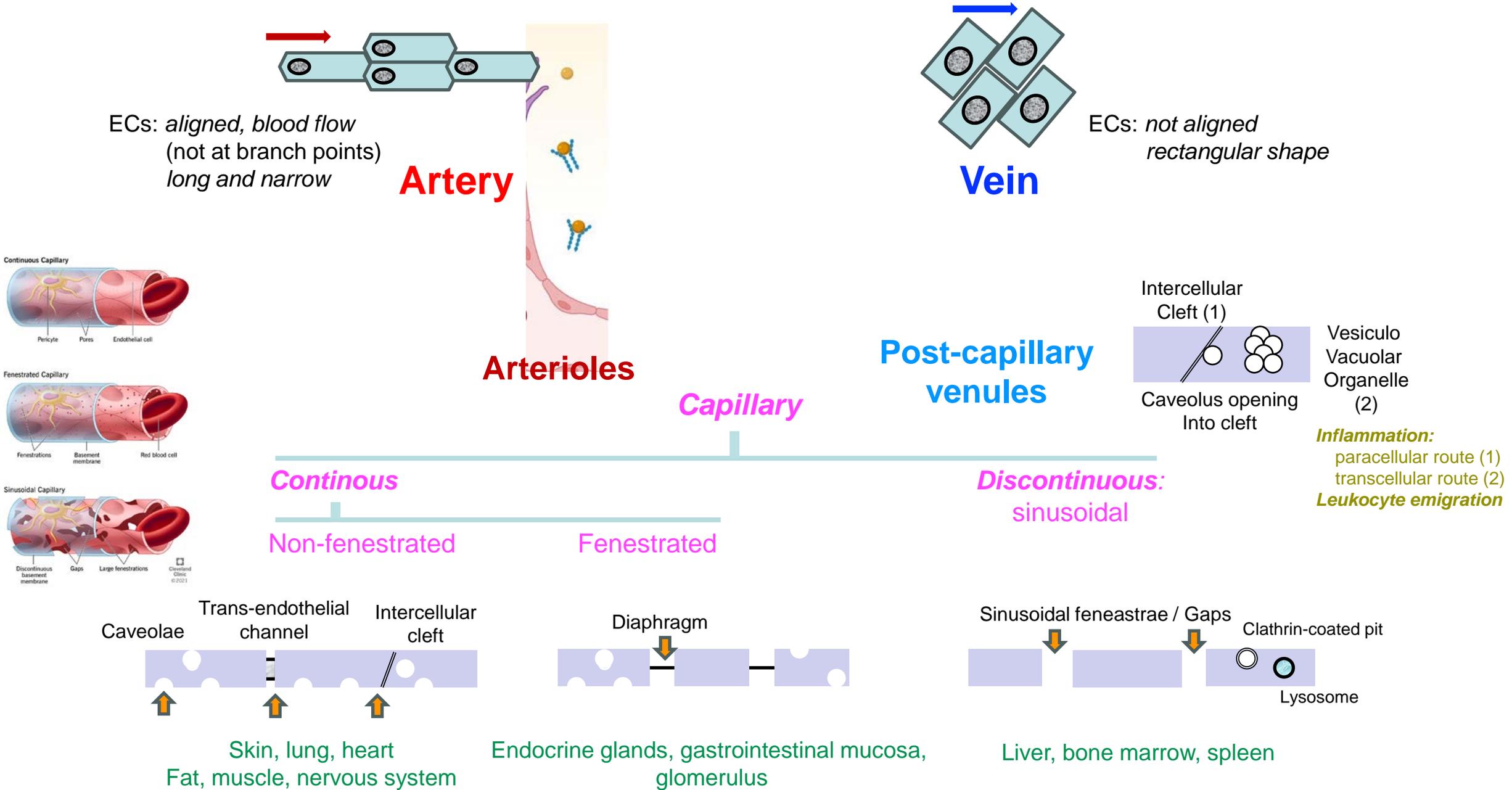
**Discontinuous:
sinusoidal**



Skin, lung, heart
Fat, muscle, nervous system



Liver, bone marrow, spleen



ECs: *aligned, blood flow*
(not at branch points)
long and narrow

Artery

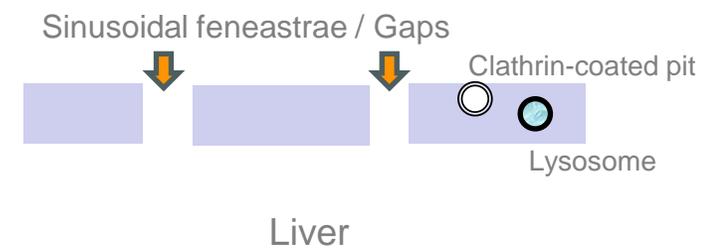
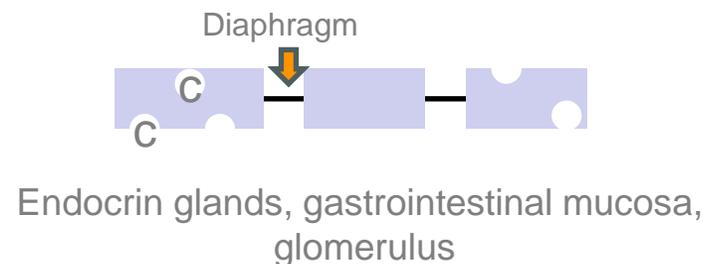
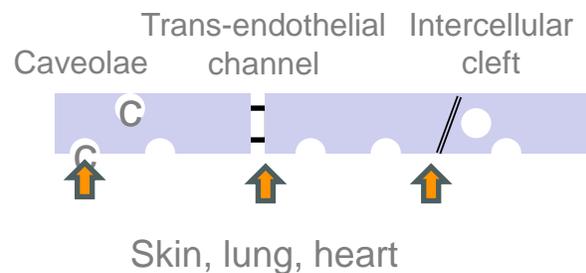


Vein

ECs: *not aligned*
rectangular shape

« There are many kinds of endothelial cells which differ from one another substantially in structure, and to some extent in function »

HW Florey, 1967



ulo
lar
elle
tion:
ular route (1)
ular route (2)

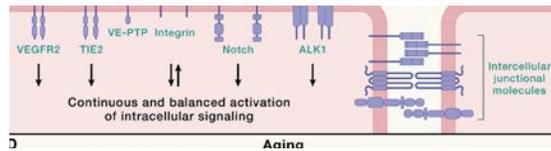
Endothelial heterogeneity:

intra-organ variations

Vascular endothelial cell development and diversity

Emily Trimm^{1,2} and Kristy Red-Horse^{3,4,5}✉

Nat Rev Cardiol 2023; 20(3):197-210



Arterial or Venous differentiation?

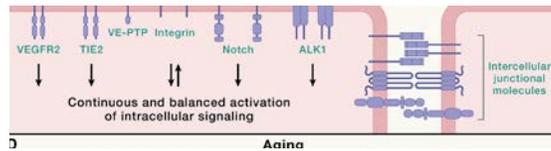
Embryogenesis: blood vessels first arise de novo by *vasculogenesis*, i.e. *rapid proliferation of ECs which form vascular tubes*.

Mechanisms:
involve the effect
on
**cell cycle
control**

Vascular endothelial cell development and diversity

Emily Trimm^{1,2} and Kristy Red-Horse^{3,4,5}✉

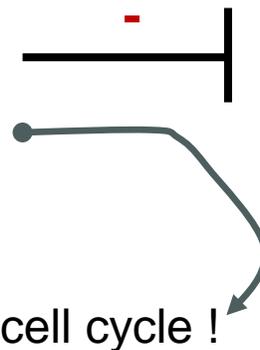
Nat Rev Cardiol 2023; 20(3):197-210



Arterial or Venous differentiation?

Embryogenesis: blood vessels first arise de novo by *vasculogenesis*,
i.e. rapid proliferation of ECs which form vascular tubes.

Increased **VEGF-A:**
VEGF-A-VEGFR2 signalling;
blood flow, shear stress induced
Notch signalling;
support
arterial specification



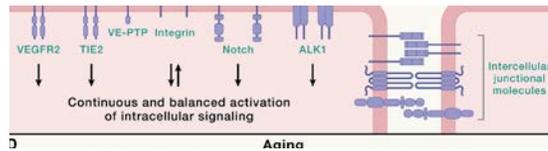
Mechanisms:
involve the effect
on
**cell cycle
control**

ECs **exit** the cell cycle !

Vascular endothelial cell development and diversity

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Nat Rev Cardiol 2023; 20(3):197-210



Arterial or Venous differentiation?

Embryogenesis: blood vessels first arise de novo by **vasculogenesis**,
i.e. rapid proliferation of ECs which form vascular tubes.

Increased **VEGF-A**:
VEGF-A-VEGFR2 signalling;
blood flow, shear stress induced
Notch signalling;
support
arterial specification

ECs **exit** the cell cycle !

Mechanisms:
involve the effect
on
**cell cycle
control**

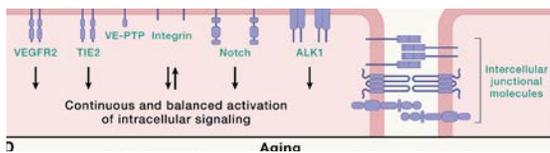
COUP transcription factor 2 (Nr2f2)
COUP-TFII signalling:
restricts arterial specification
(*downregulates Notch signalling*);
required for
venous specification

Actively cycling ECs

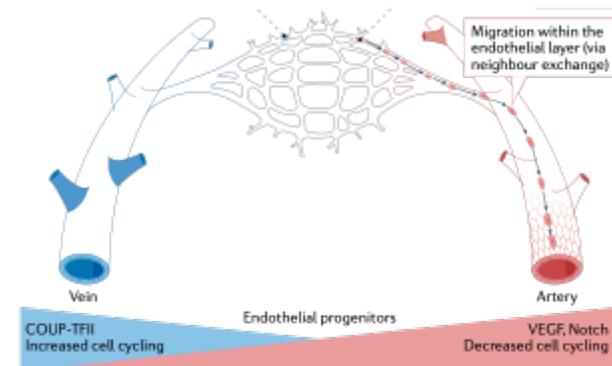
Vascular endothelial cell development and diversity

Emily Trimm^{1,2} and Kristy Red-Horse^{3,4,5}✉

Nat Rev Cardiol 2023; 20(3):197-210

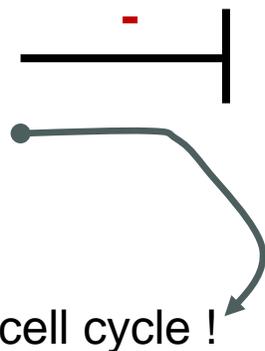


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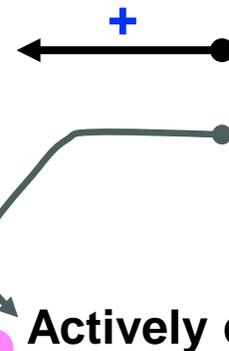
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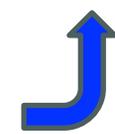
Arteries grow by recruiting ECs from veins and capillaries that migrate against the direction of flow

Mechanisms:
involve the effect
on
cell cycle control



Actively cycling ECs

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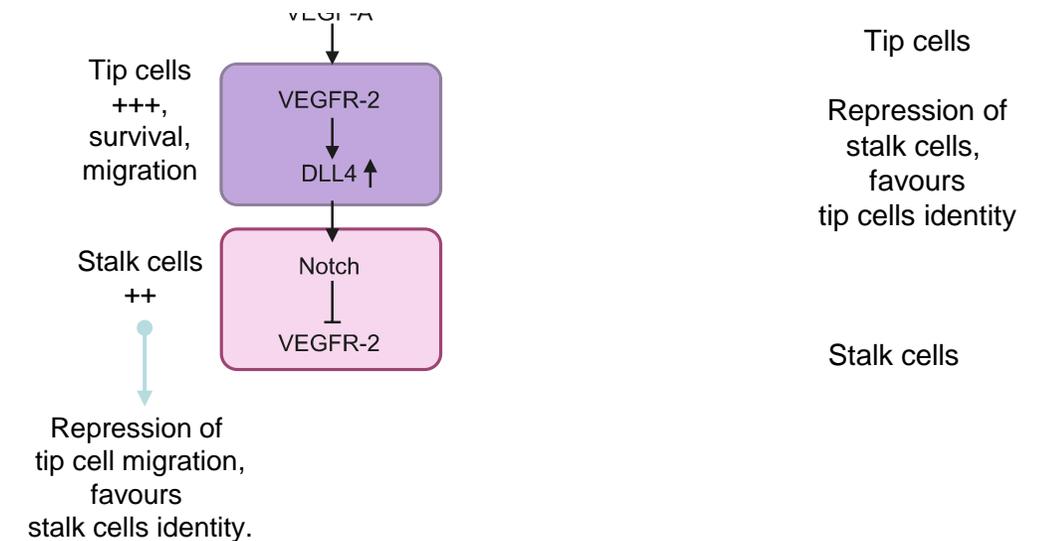
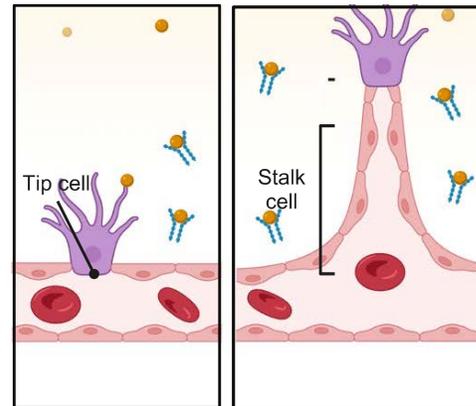
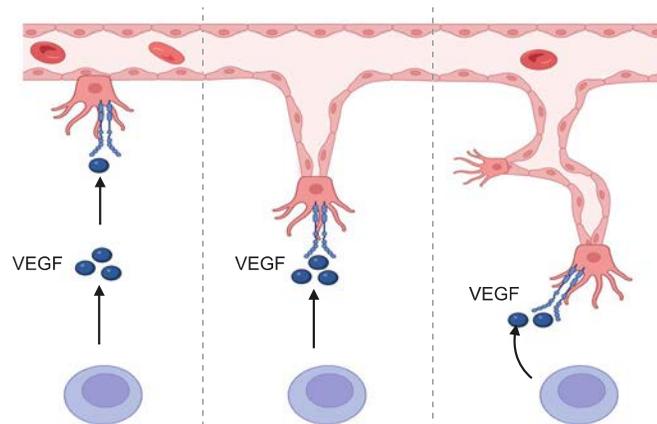
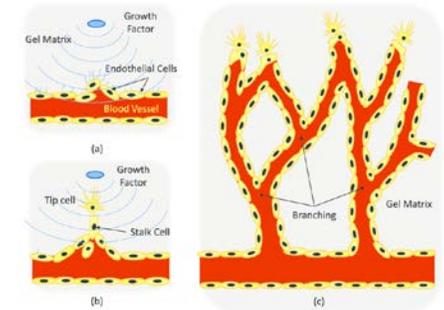
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Nat Rev Cardiol 2023; 20(3):197-210

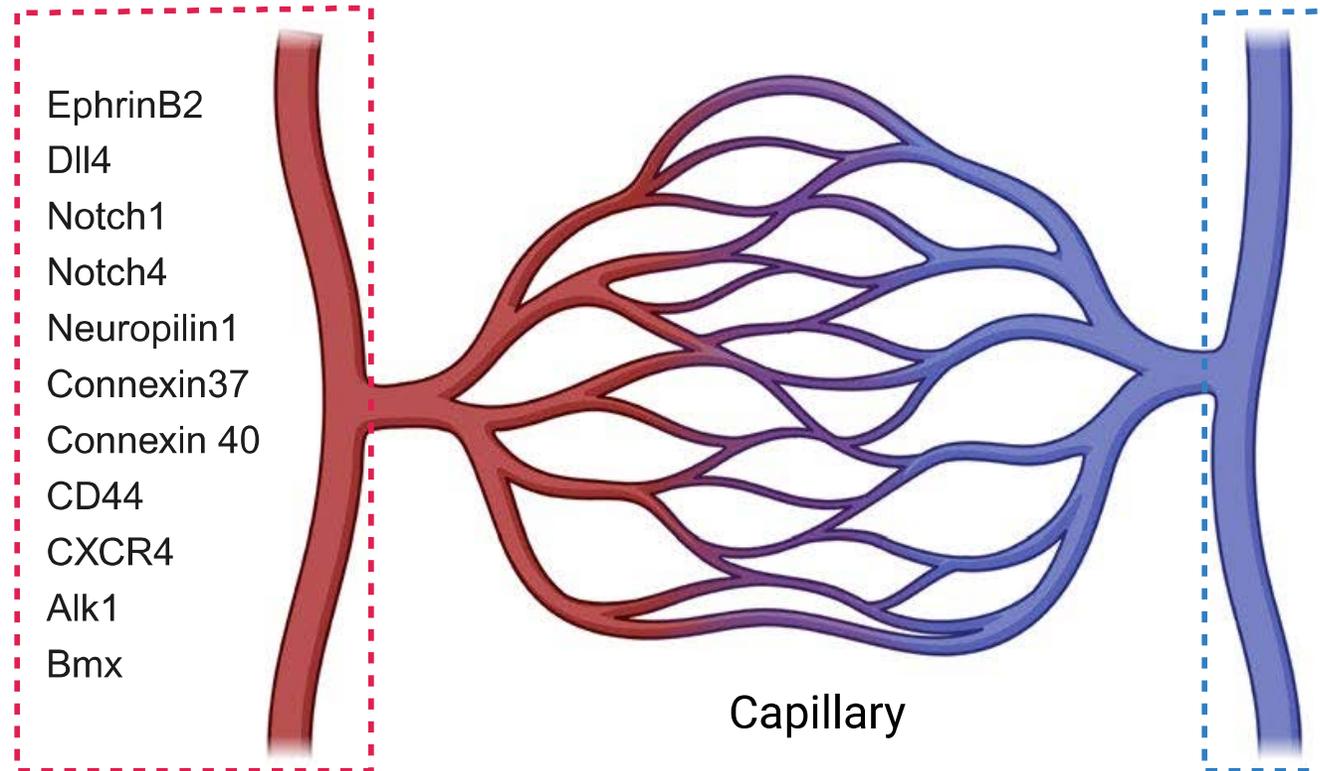
Sprouting angiogenesis:

hypoxia induces tissue resident cells release VEGF-A to induce growth of blood vessels toward hypoxia region...

differentiation of tip and stalk ECs: key process expanding the vascular network



Markers of ECs intra-organ diversity



Molecular markers
for **arterial** or **venous ECs**

Vascular endothelial cell development and diversity

Emily Trimm^{1,2} and Kristy Red-Horse^{3,4,5}✉

Nat Rev Cardiol 2023; 20(3):197-210

Endothelial heterogeneity:

intra-organ variations

localised additional modulations

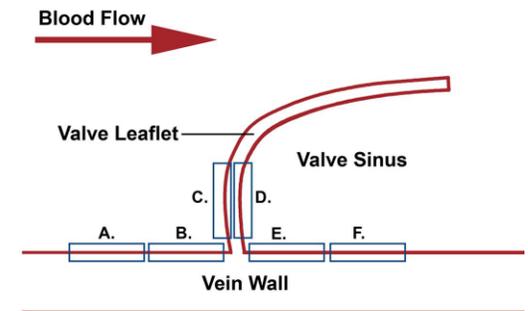
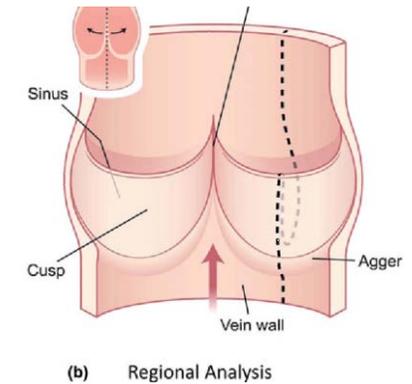
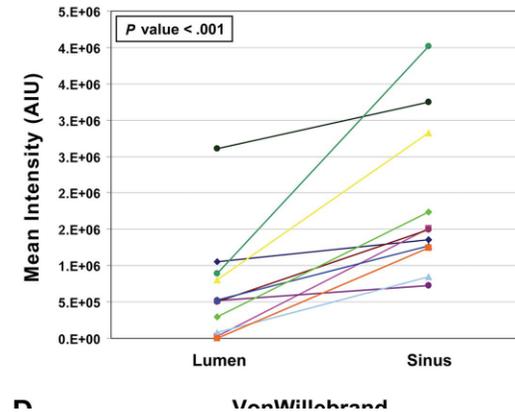
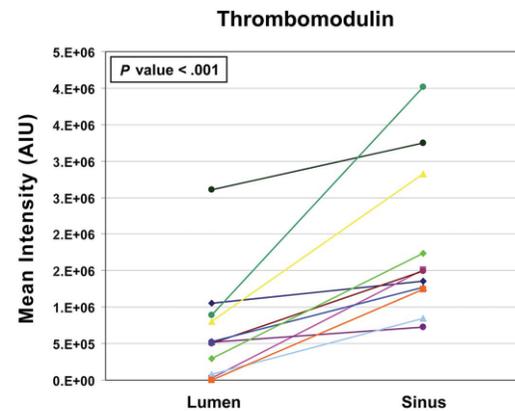
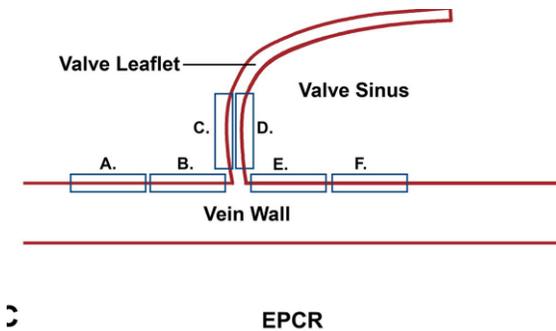
For a given vessel type in a given organ: *local modulation of the ECs phenotype*

Valves of the deep venous system: an overlooked risk factor

*Erin G. Brooks,¹ *Winifred Trotman,¹ Marilyn P. Wadsworth,¹ Douglas J. Taatjes,¹ Mark F. Evans,¹ Frank P. Ittleman,² Peter W. Callas,³ Charles T. Esmon,⁴ and Edwin G. Bovill¹

Blood 2009;114:1276-1279.

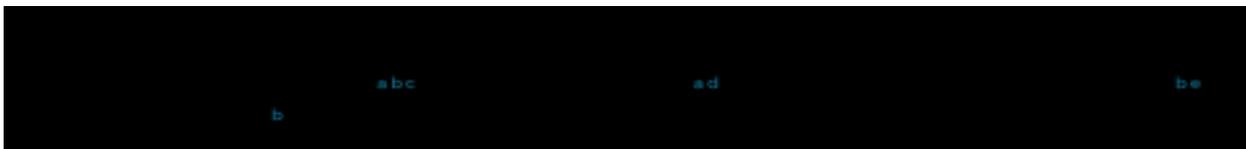
Great saphenous veins collected during coronary artery bypass grafting, n=9
Immunofluorescence; *VWF, thrombomodulin THBD, Endothelial Protein C Receptor EPCR*



Increased expression of THBD and EPCR in the valvular sinus ECs as opposed to the vein luminal ECs, the opposite for VWF:

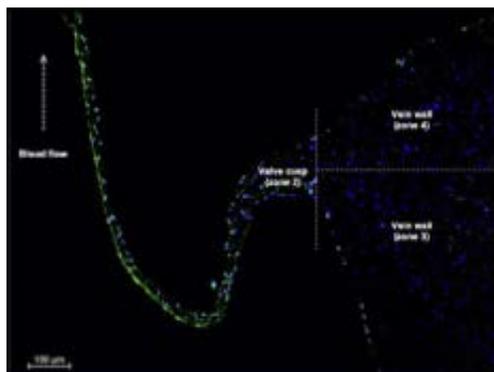
valvular sinus ECs shifted to a more thromboresistant phenotype.

*And remarkable inter-individual variations... **impact of complex rheology** around the valvular sinus?*

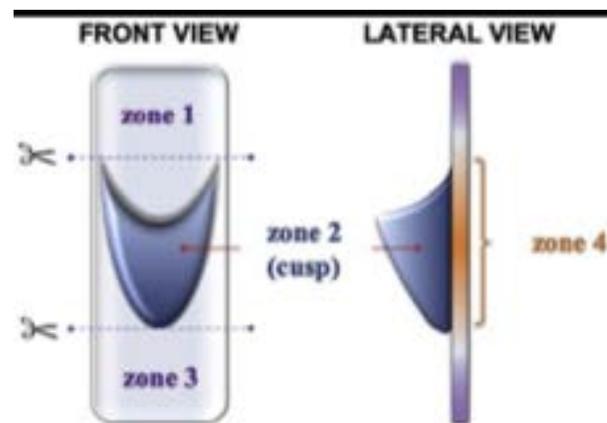


J Vasc Surg Venous Lymphat Disord 2022; 10(1):241-7.

17 Valve-containing segments of competent human deep veins, *above-knee amputation*

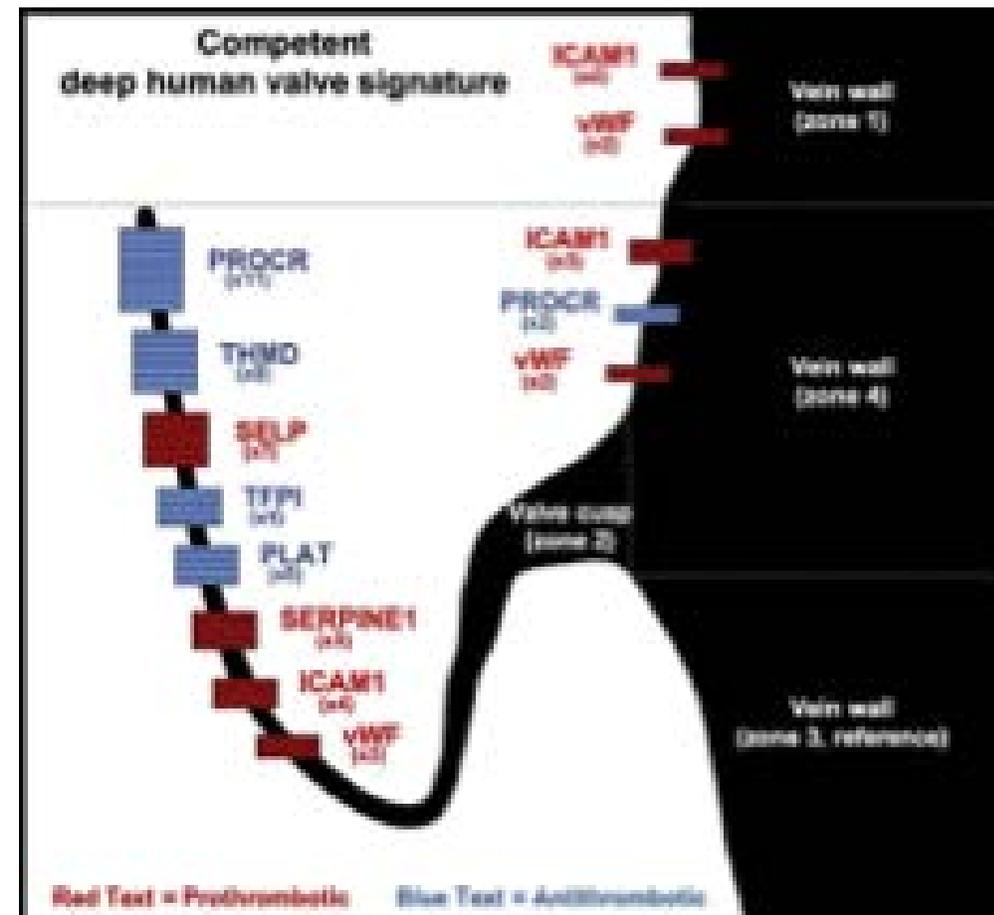
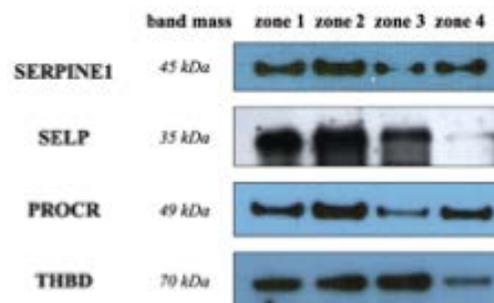


EPCR labelling (green)



The valve cusp,
vein wall within the valve cusp,
and vein wall beyond the valve cusp...

have substantial differences
in expression of genes
underlying function
in coagulation, fibrinolysis and inflammation.



Hemodynamic regulation of perivalvular endothelial gene expression prevents deep venous thrombosis

John D. Welsh,¹ Mark H. Hoofnagle,² Sharika Bamezai,¹ Michael Oxendine,³ Lillian Lim,² Joshua D. Hall,⁴ Jisheng Yang,² Susan Schultz,² James Douglas Engel,⁵ Tsutomu Kume,² Guillermo Oliver,² Juan M. Jimenez,⁴ and Mark L. Kahn¹

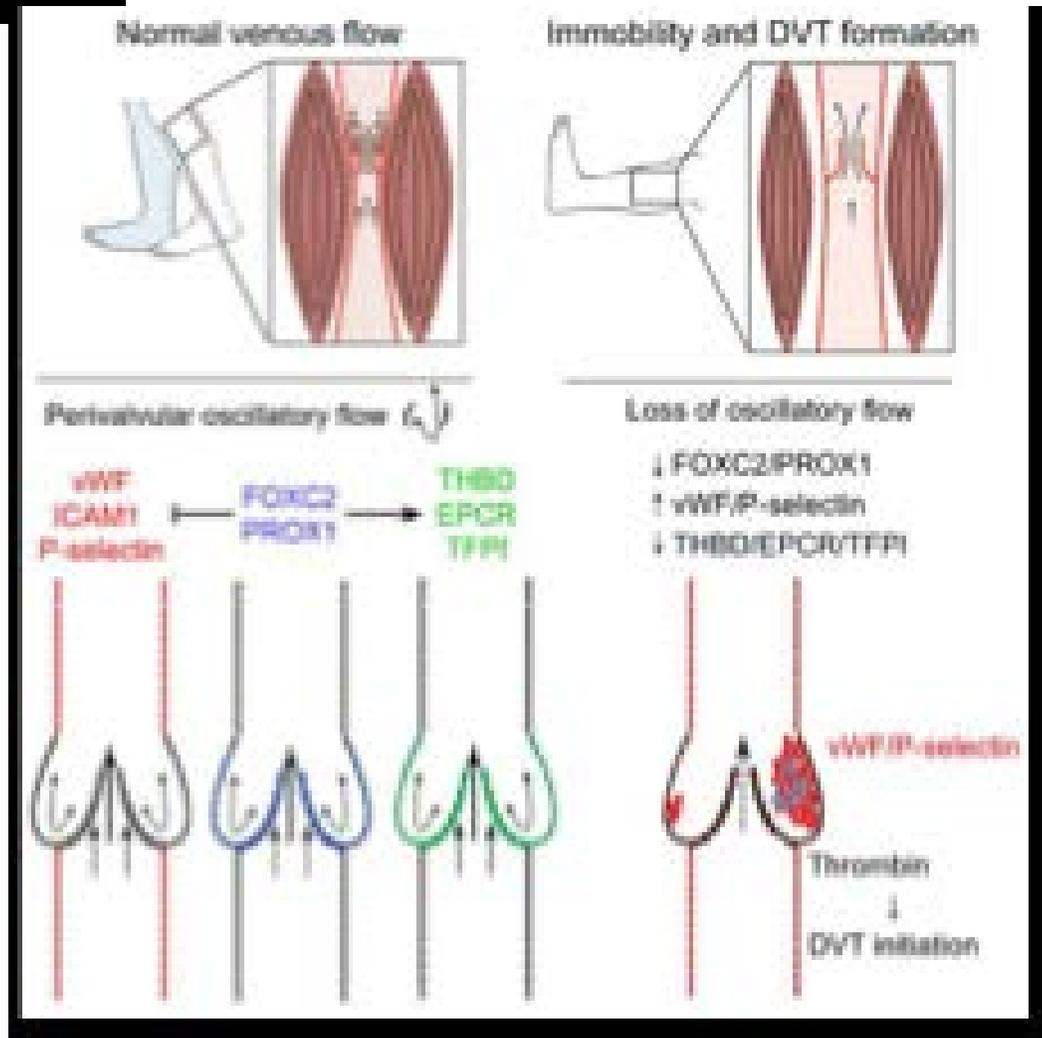
J Clin Invest. 2019;129(12):5489–5500.

ECs surrounding the venous valves express high levels of the transcription factors **FOXC2** and **PROX1** that can be activated by oscillatory shear stress.

They induce

low levels of VWF, P-selectin and ICAM1, high levels of THBD, EPCR and TFPI.

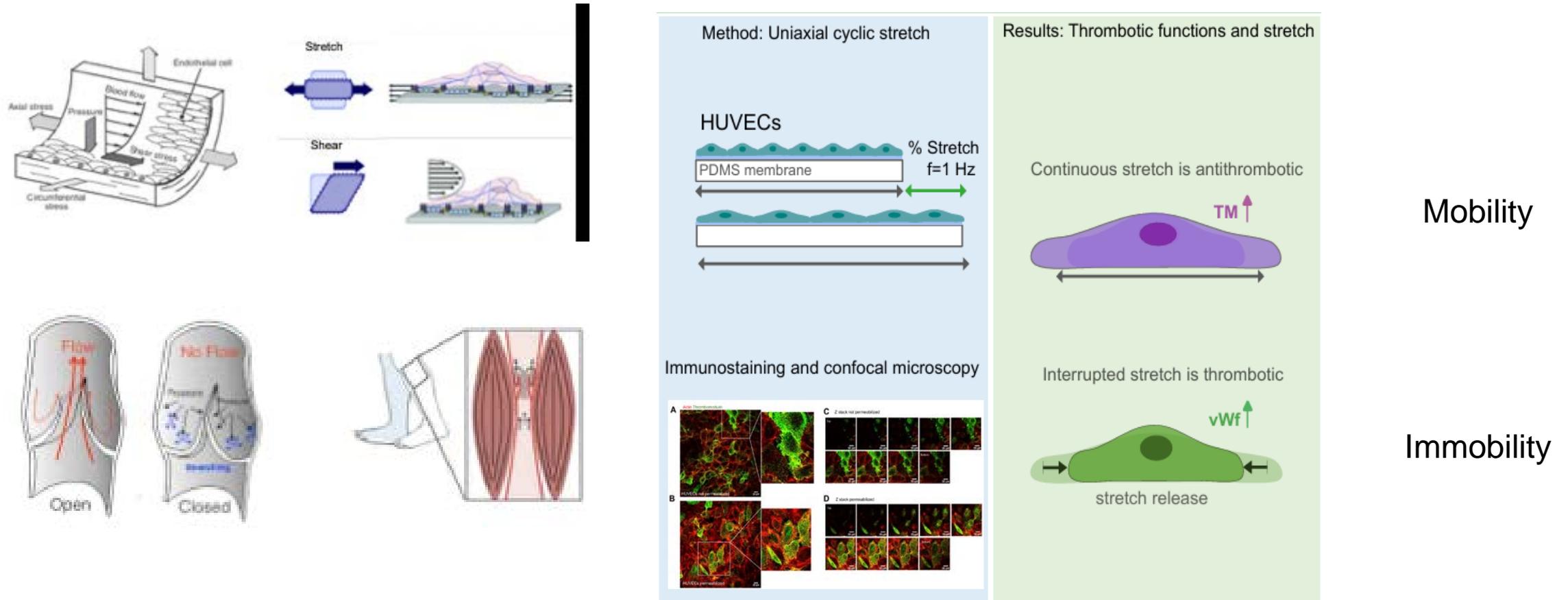
The loss of oscillatory flow inhibits FOXC2 and PROX1, with a loss of the perivalvular antithrombin phenotype favouring valvular thrombogenesis.



Uniaxial cyclic stretch regulates the thrombotic phenotype of confluent venous endothelial cells

Arianna Giannetti, Elisabeth Génot, Antonia Perez Martin, Elsa Faure, Jean-Christophe Gris, Maïda Cardoso, Christophe Goze-Bac, Christine Benistant and Manouk Abkarian

Blood VTH 2025, *accepted*.



Continuous uniaxial cyclic stretch at 10% increases the expression of Thrombomodulin on HUVECs

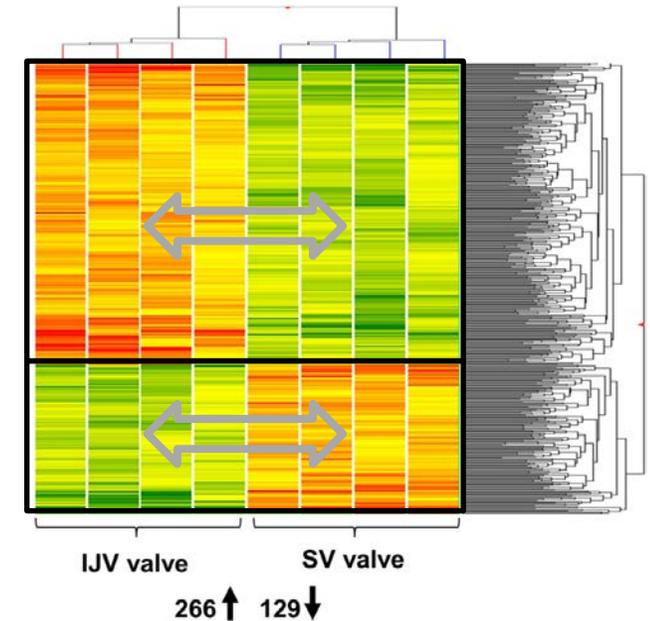
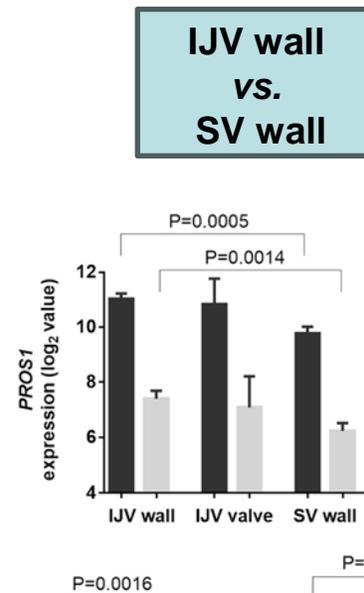
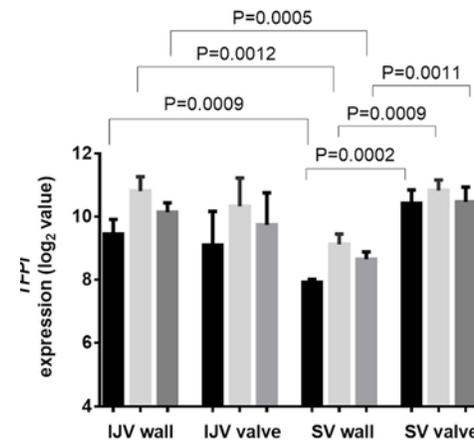
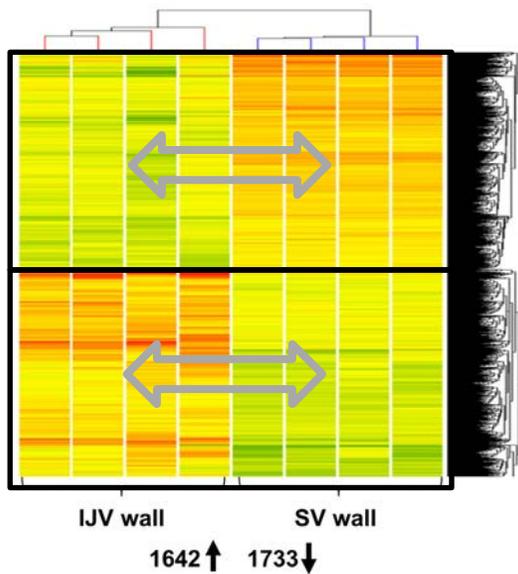
Interrupted cyclic strain increases the protein expression of VWF

The modalities of cyclic stretch modulate the expression of antithrombotic / prothrombotic molecules

Expression profiles of the internal jugular and saphenous veins: Focus on hemostasis genes

Nicole Ziliotto^a, Silvia Meneghetti^a, Erica Menegatti^b, Marcello Baroni^a, Barbara Lunghi^a, Fabrizio Salvi^c, Manuela Ferracin^d, Alessio Branchini^a, Donato Gemmati^e, Francesco Mascoli^f, Paolo Zamboni^b, Francesco Bernardi^{a,*}, Giovanna Marchetti^e

Surgical reconstruction of Internal Jugular Vein IJV by autologous great Saphenous Vein SV: *microarray-based transcriptome analysis in wall and valve specimens.*

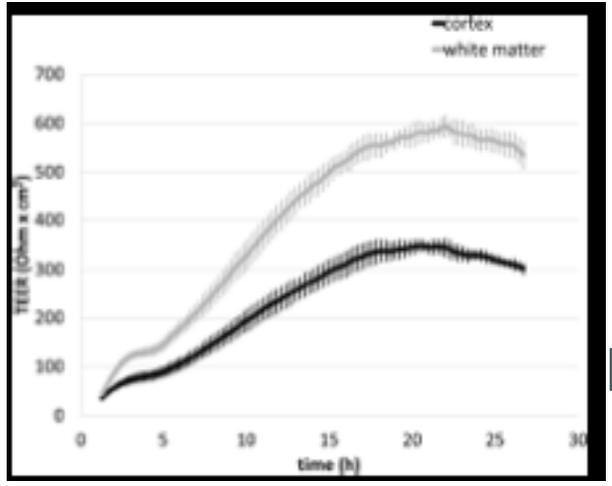


The IntJV and SV rely on differential expression of many hemostasis genes: *to balance local hemostasis, differences in vulnerability to thrombosis?*

Differences in the molecular structure of the blood-brain barrier in the cerebral cortex and white matter: an in silico, in vitro, and ex vivo study

Ádám Nyúl-Tóth,^{1*} Maria Suciu,^{2*} Judit Molnár,¹ Csilla Fazakas,¹ János Haskó,¹ Hildegard Herman,² Attila E. Farkas,¹ József Kaszaki,² Anca Hermenean,² Imola Wilhelm,² and István A. Krizbai^{1,2}

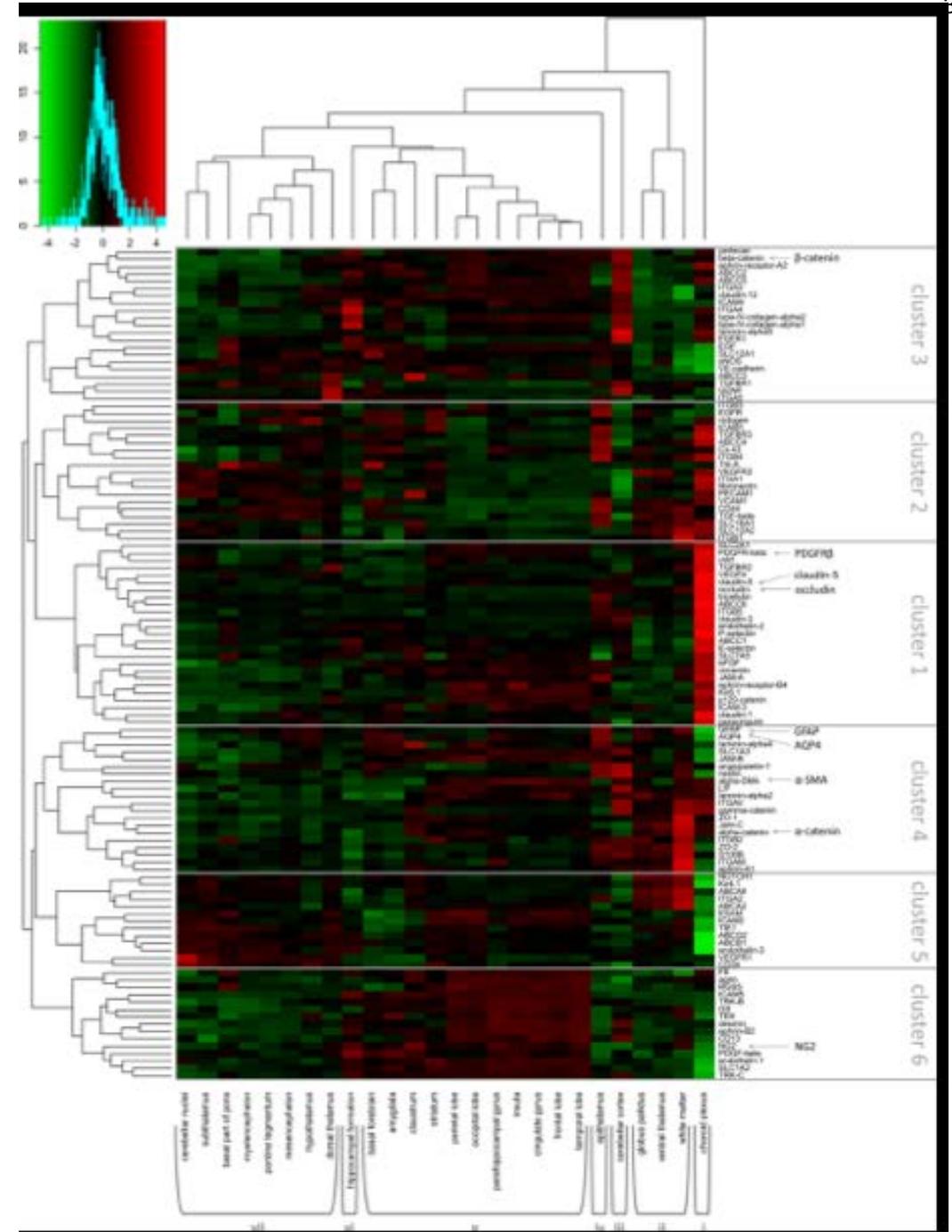
Am J Physiol Heart Circ Physiol 310: H1702–H1714, 2016.



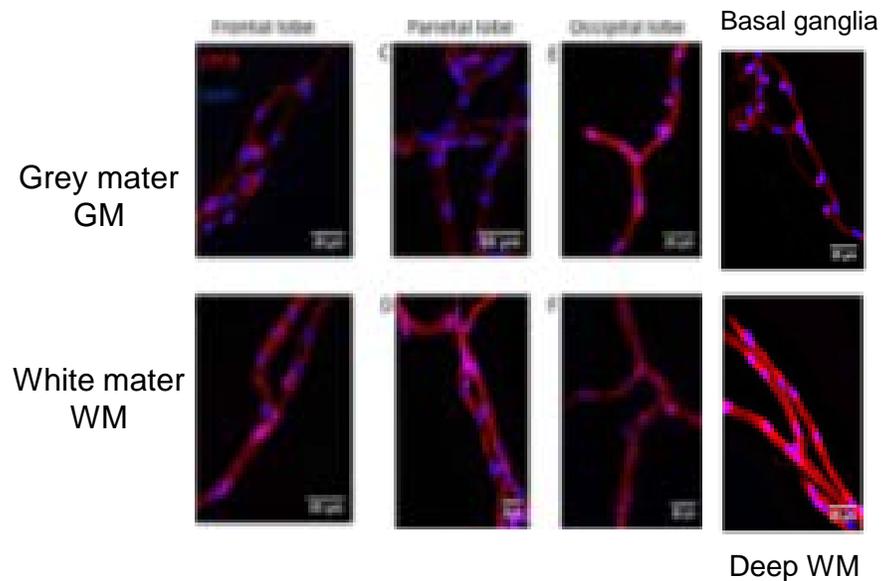
Transendothelial electrical resistance

Endothelial cells from white matter WM and from grey matter GM *have differences in molecular structure*

Barrier properties of ECS from WM are superior to the one of ECs from GM

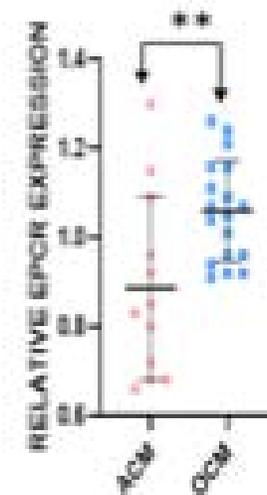


Basal level of **EPCR** expression (red):
heterogeneous in the WM compared to the GM



In vitro brain ECs culture:

oligodendrocyte (predominance: *white mater*) conditioned medium OCM
vs.
 astrocyte (predominance: *grey mater*) conditioned medium ACM



**Differences in EPCR expression
 are influenced by cells adjacent to ECs**

Endothelial heterogeneity:

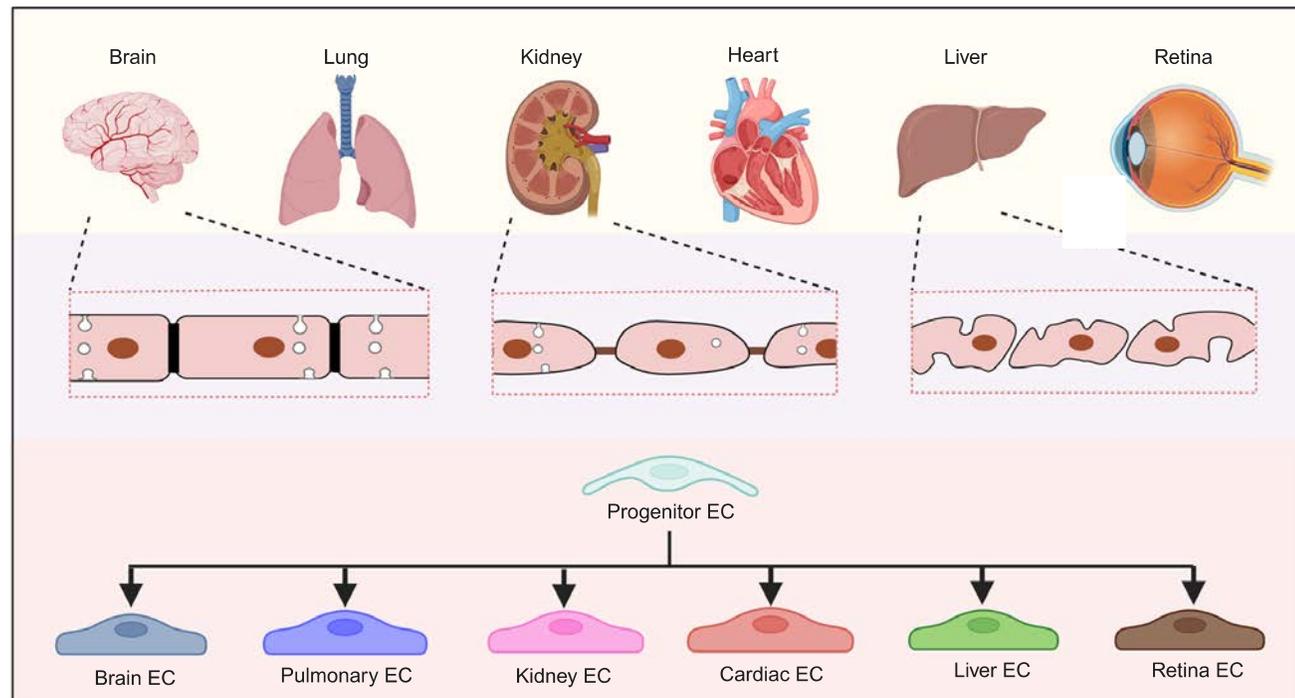
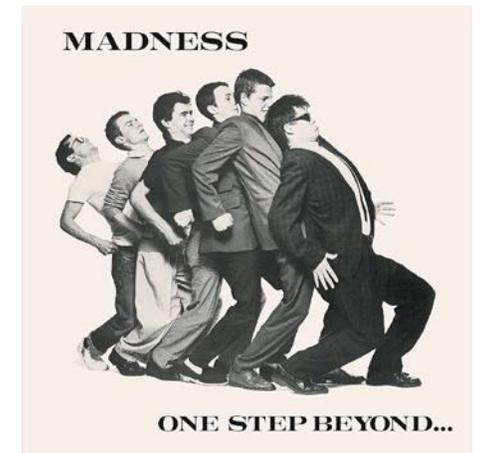
inter-organ variations

One step beyond: ECs inter-organ diversity

The vasculature of each organ is adapted to meet the physiological functions of that tissue.

Organ-specific specialisation of ECs:

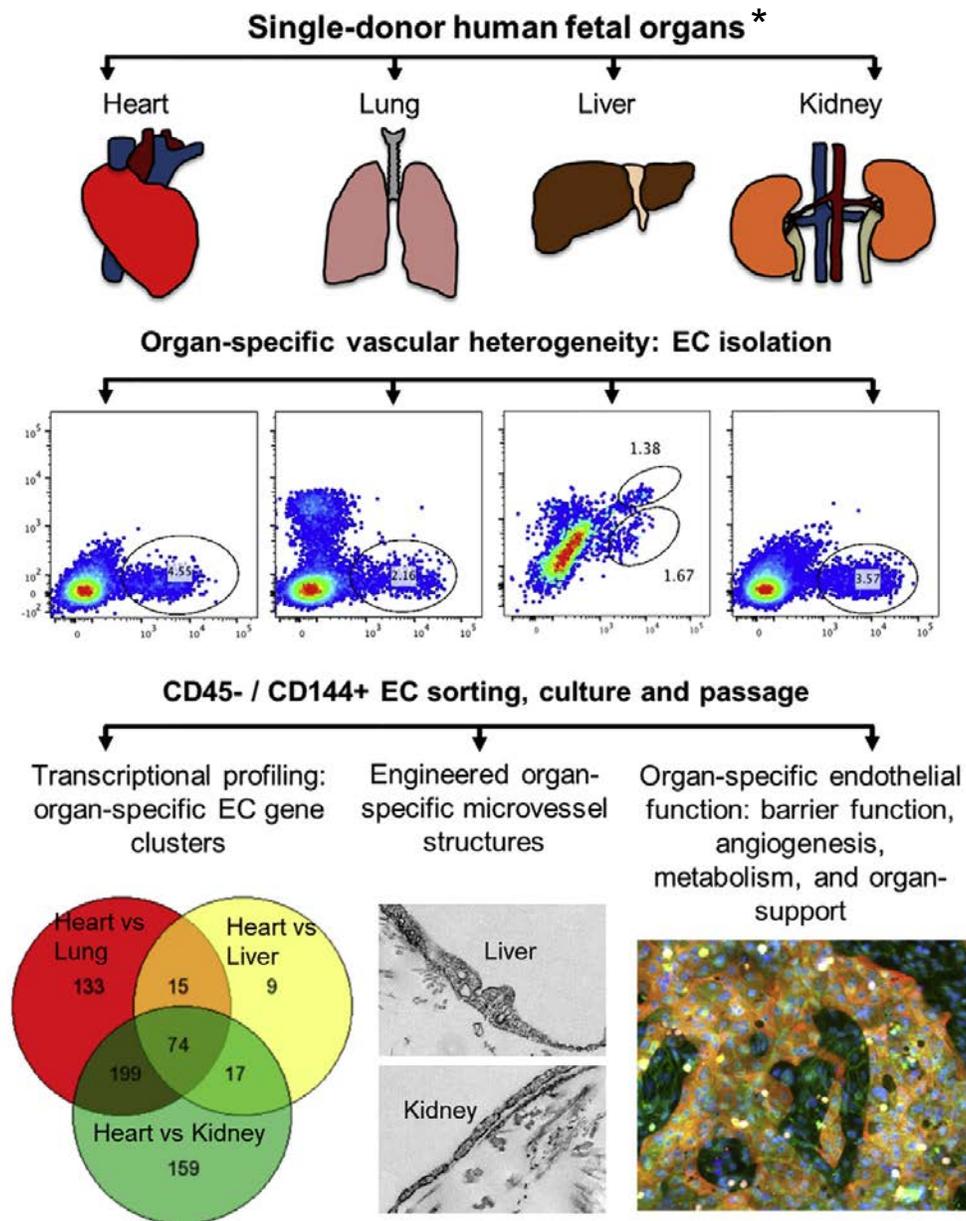
reflected in structure, function and molecular expression profiles of ECs, as well as in the composition of perivascular cells and the surrounding extracellular matrix



Human Organ-Specific Endothelial Cell Heterogeneity

Raluca Marcu,^{1,8} Yoon Jung Choi,^{1,8} Jun Xue,¹ Chelsea L. Fortin,³ Yuliang Wang,^{2,4} Ryan J. Nagao,¹ Jin Xu,¹ James W. MacDonald,⁵ Theo K. Bammler,⁵ Charles E. Murry,^{1,3,4,6} Kimberly Muczynski,⁶ Kelly R. Stevens,^{1,3,4} Jonathan Himmelfarb,^{6,7} Stephen M. Schwartz,³ and Ying Zheng^{1,4,7,9,*}

iScience 2018;4:20-35.



* 3 months' gestation

1- Organ-specific ECs have **distinct expression patterns of gene clusters**

2-ECs display **distinct**

barriers properties, angiogenic potential, metabolic rate, gene/RNA expression

and **support specific organ functions.**

Selecting marker genes for human-specific, organ-specific vascular EC subtypes *is now possible...*

Trimm E, Red-Horse K, Nat Rev Cardiol 2023;20(3):197-210.

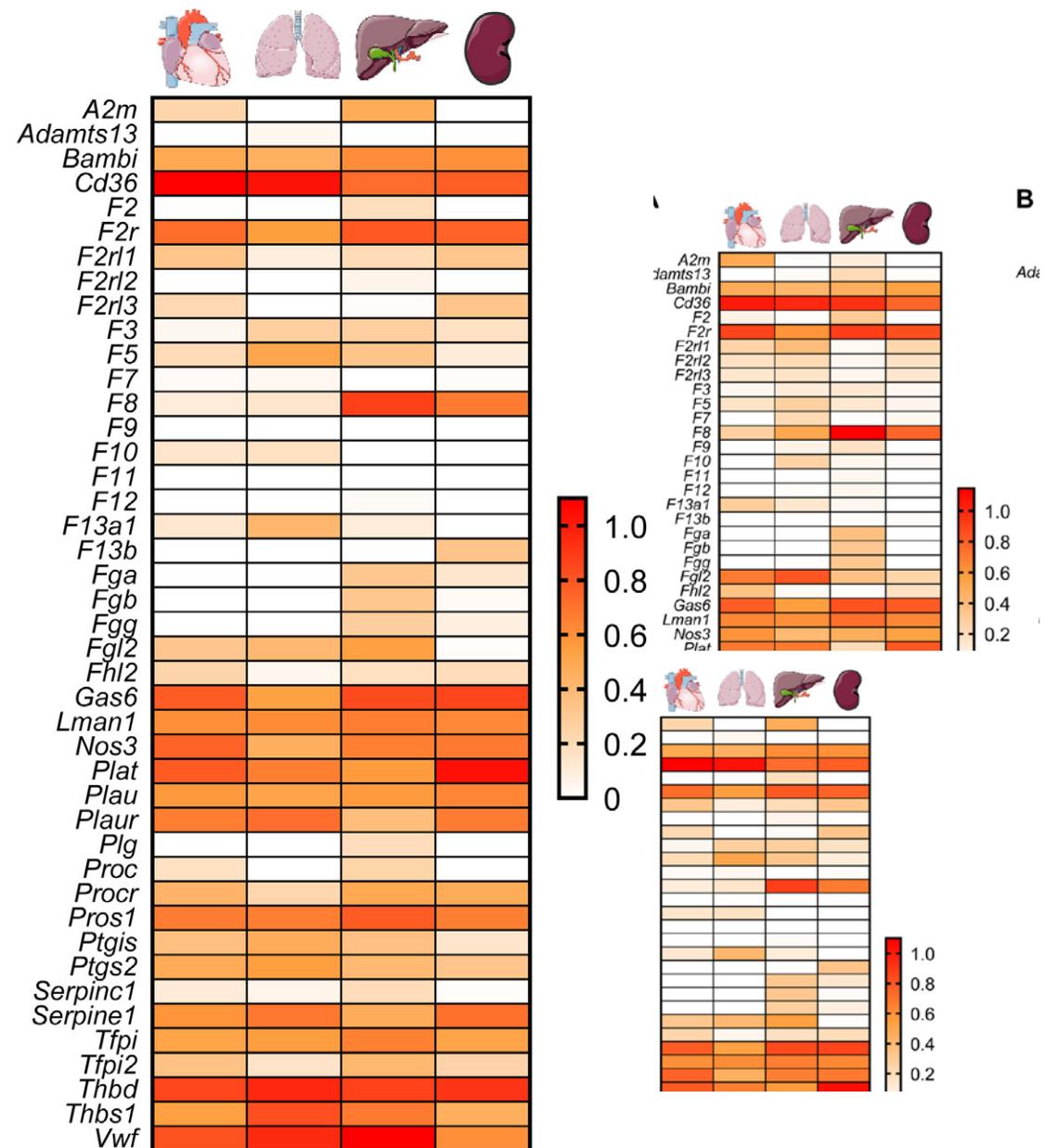
Organ system	Developmental stage	Vascular EC subtype	Marker genes
Multi-organ	Embryonic or adult	Pan-endothelial	PECAM1, CDH5, VWF, KDR, FLT1, TEK, CLDN5
		Artery	GJA4, GJA5, HEY1, GATA2, CXCR4, SOX17, MECOM
		Vein	ACKR1, NR2F2, PLVAP
Heart	Embryonic	Artery	GABBR2, GRIA2, SSUH2, JAG1
		Vein	ACKR1, LHX6, SELE
		Capillary	PRDM1, INMT, APLNR, CA4
	Adult	Tissue-specific EC	SLC14A1
		Artery	SEMA3G, EFNB2, DLL4,
		Vein	NR2F231, ACKR1
		Capillary	RGCC, CA4
Lung	Adult	Tissue-specific EC	VIPR1
		Artery	DKK2, SERPINE2
		Vein	CPE, PTGDS, C7, PLA1A
		Bronchial EC	VWA1, HSPG2, PLVAP, MYC, HBEGF
		Capillary (aCap)	ADIRF, S100A4, EMCN, HPGD, EDNRB, SOSTDC1, IL1RL1, APLN
		Capillary (gCap)	FCN3, EDN1, SLC6A4, GPIHBP1, CD36, IL7R, VWF, PTPRB, PLVAP
Brain	Adult	Large artery	LTBP4
		Artery	INTS6, HSPA1A, JUNB, MECOM, ARL15, TXNIP, MGP, ADAMTS1
		Arteriole	VEGFC, ARL15, BMX, EFNB2, WISR, AIF1L, CD320
		Venule	TSHZ2, ADGRG6, SLC38A5, LRRC1, BNC2, ETV6, TMEM132C, ATP10A, JAM2, PRCP, PRSS23, RAMP3
		Vein	ACKR1, IL1R1, TSHZ2, PTGDS, POSTN, DNASE1
		Large vein	CCL2
		Capillary	MFSD2A, SLC7A5, TFRC, SLC38A5, SRARP, RGCC, SLC3A2, BSG, SLC16A1, SLC01A2
Kidney	Embryonic	Tissue-specific EC	NOTCH4
	Paediatric or adult	Glomerular EC	SEMA3G, CLDN5
Liver	Adult	Tissue-specific EC	OIT3
		Portal vein	AQP1, CD9, IFITM1, RAMP3, INMT, DNASE1L3, LIFR
		LSEC	CLEC4G, STAB1, STAB2, CD14
		LSEC zone 1	MGP, SPARCL1, TM4SF1, CLEC14A, BTNL9, ANPEP
		LSEC zone 2 or 3	CCL14, CLEC1B, FCN2, S100A13, LYVE1, FCN3
		Central vein	SELP, SELE, VWF

Haemostatic genes?

Randi AM, Jones D, Peghaire C, Arachchillage DJ.

J Thromb Haemost. 2023;21:3056–3066

Organotypic expression of **coagulation genes** in mouse ECs.

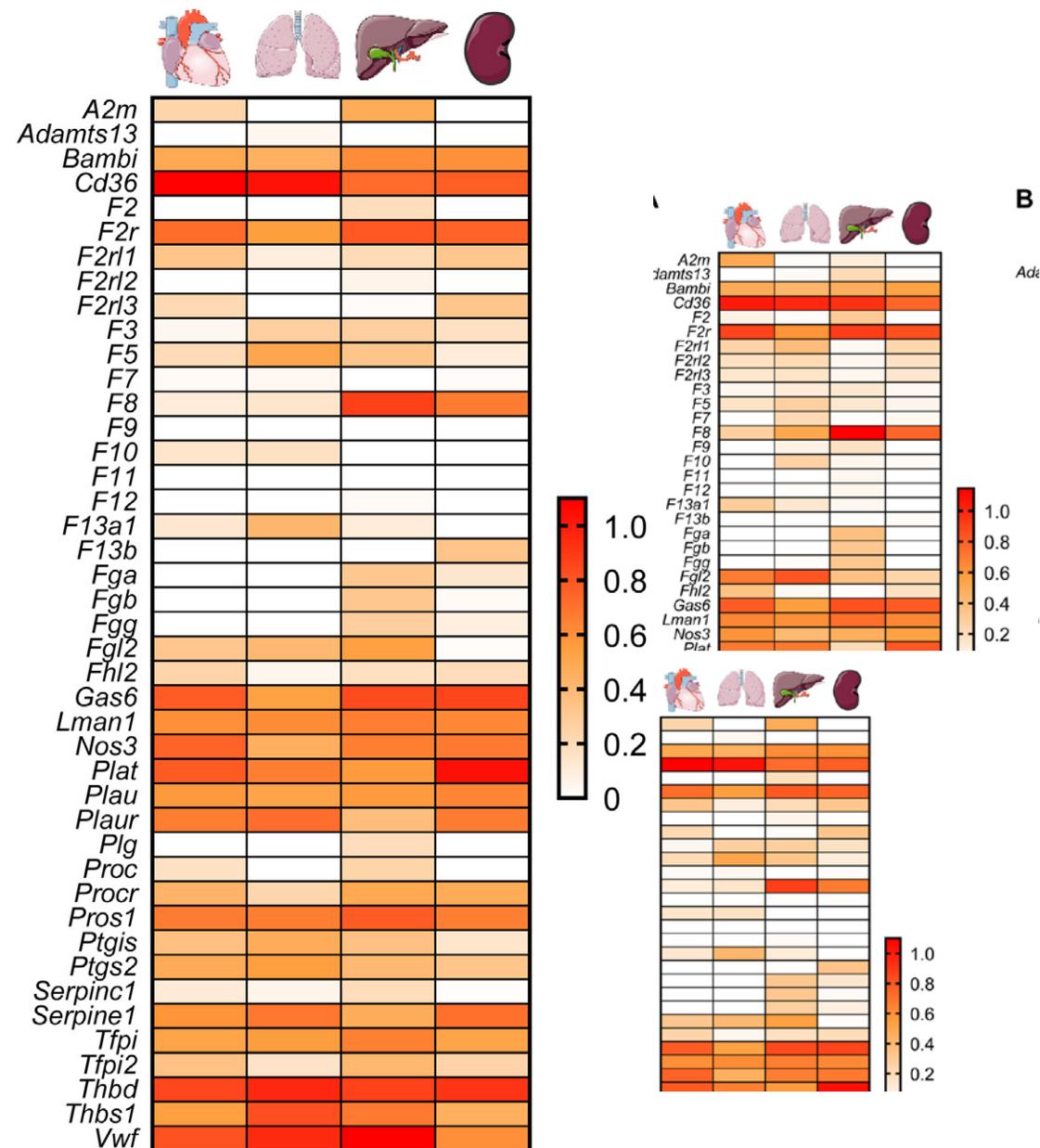


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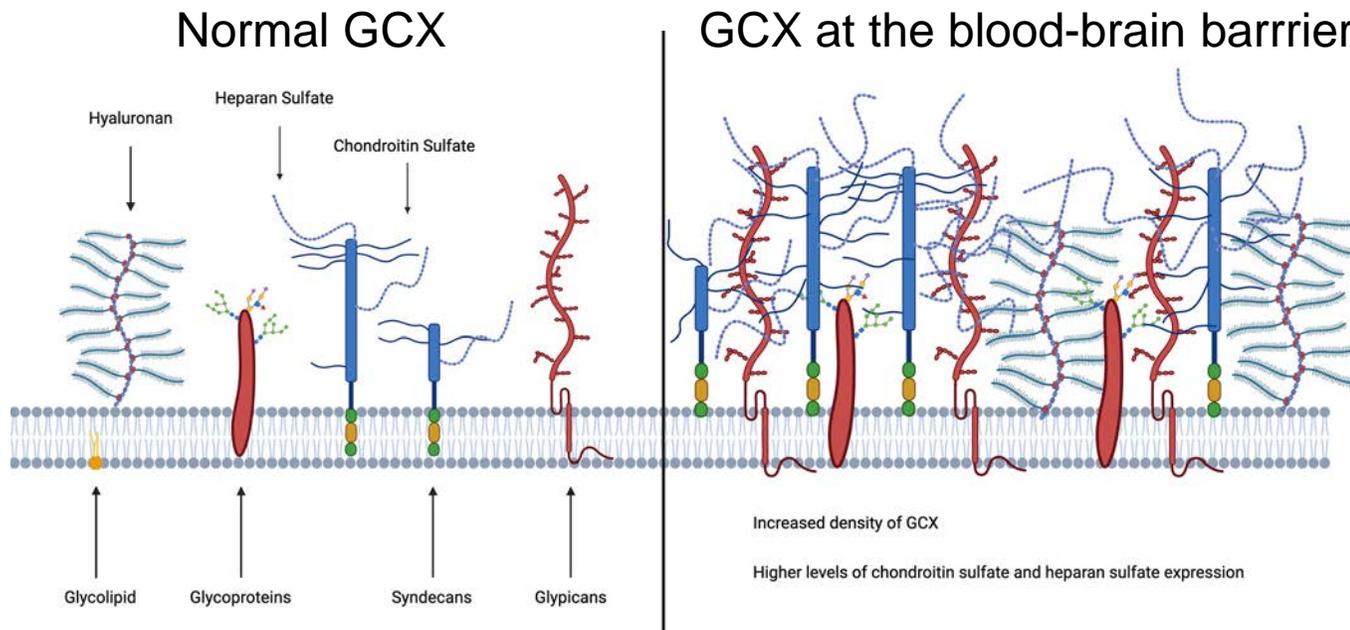
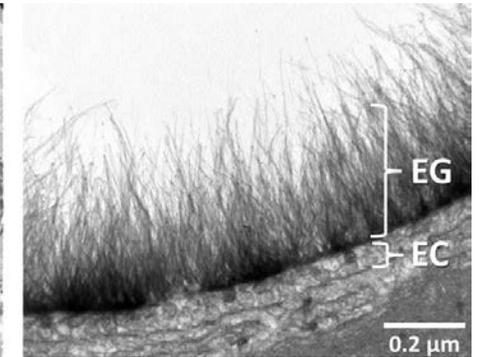
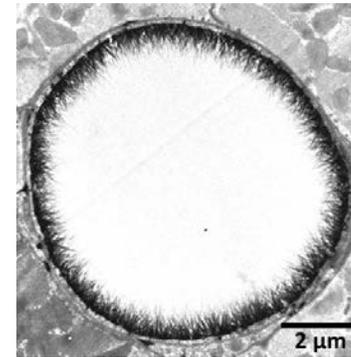


And even the glycocalyx!

Glycocalyx GCX, also known as the pericellular matrix and cell coat: layer of glycoproteins and glycolipids which surround the cell membranes (epithelial cells, endothelial cells, bacteria).

Apical surface of ECs which line the lumen.

Reservoir for a wide range of regulatory glycoproteins.



Endothelium is a dreamed concept,

- Federal Europe? -

multiple endothelia are the underlying reality

(intra-organ and inter-organ diversities)...

- Europe of Nations! -

*and their temporal dynamics
at the cellular level
is the functional rule.*

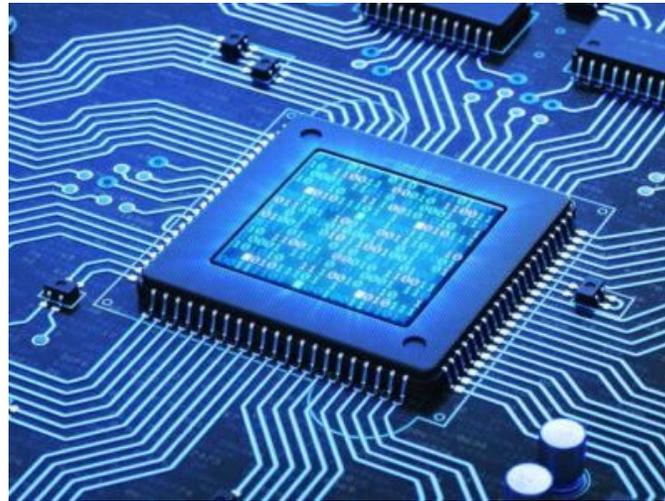


More:

ECs are in fact *Input-Output Devices*

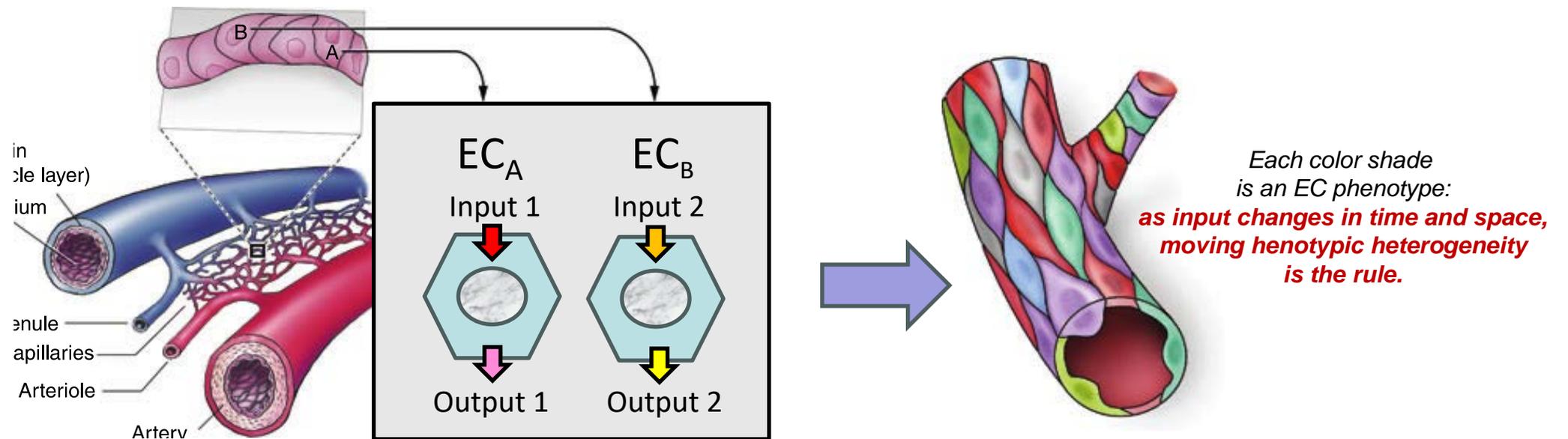
that constantly sense their extracellular environment (mechanosensors, receptors,...)
to produce a moving biological answer

Highly elaborate non-linear array of dynamic signal transduction pathways
that couple signals at the membrane surface to cellular response or phenotype;
input varies even in a micro-scale.



ECs are in fact *Input-Output Devices*

that constantly sense their extracellular environment (mechanosensors, receptors,...)
to produce a biological answer



Input

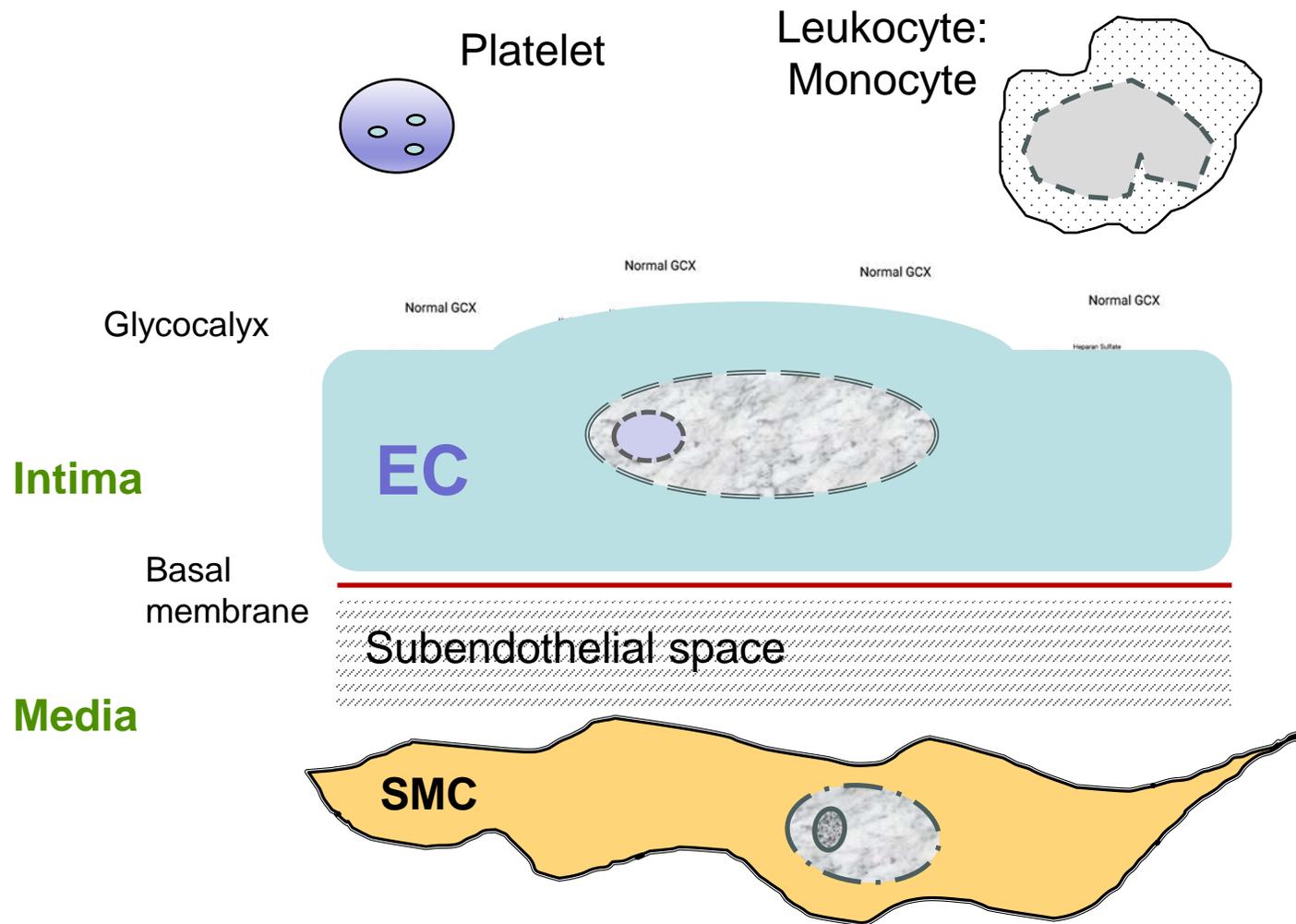
- **Biomechanical**
 - Circumferential stress and strain
 - Longitudinal stress and strain
 - Shear stress
- **Biochemical**
 - Hypoxia, Glucose, Acid base balance
 - Hyperthermia / hypothermia
 - Pathogens
 - Growth factors
 - Extracellular matrix
 - Sex hormones
 - Serine proteases
 - Chemokines, Cytokines
 - Nucleosides
 - Sphingolipids
 - Lipoproteins
 - Contact system
 - Nitric oxide, autacoids
 - Cell-cell interactions
 - Microvesicles, exosomes

Output

- **Single cell level**
 - Cell shape
 - Calcium flux
 - Gene expression
 - Protein translation
 - Post-translational modifications
 - Proliferation
 - Migration
 - Apoptosis
- **Cell monolayer level**
 - Barrier function
 - Leukocyte adhesion
- **Blood vessel / organ / organism**
 - Vasomotor tone
 - Angiogenesis
 - Inflammation
 - Activation of coagulation with fibrin deposition



Endothelia and Haemostasis



Regulation (activation/inhibition):

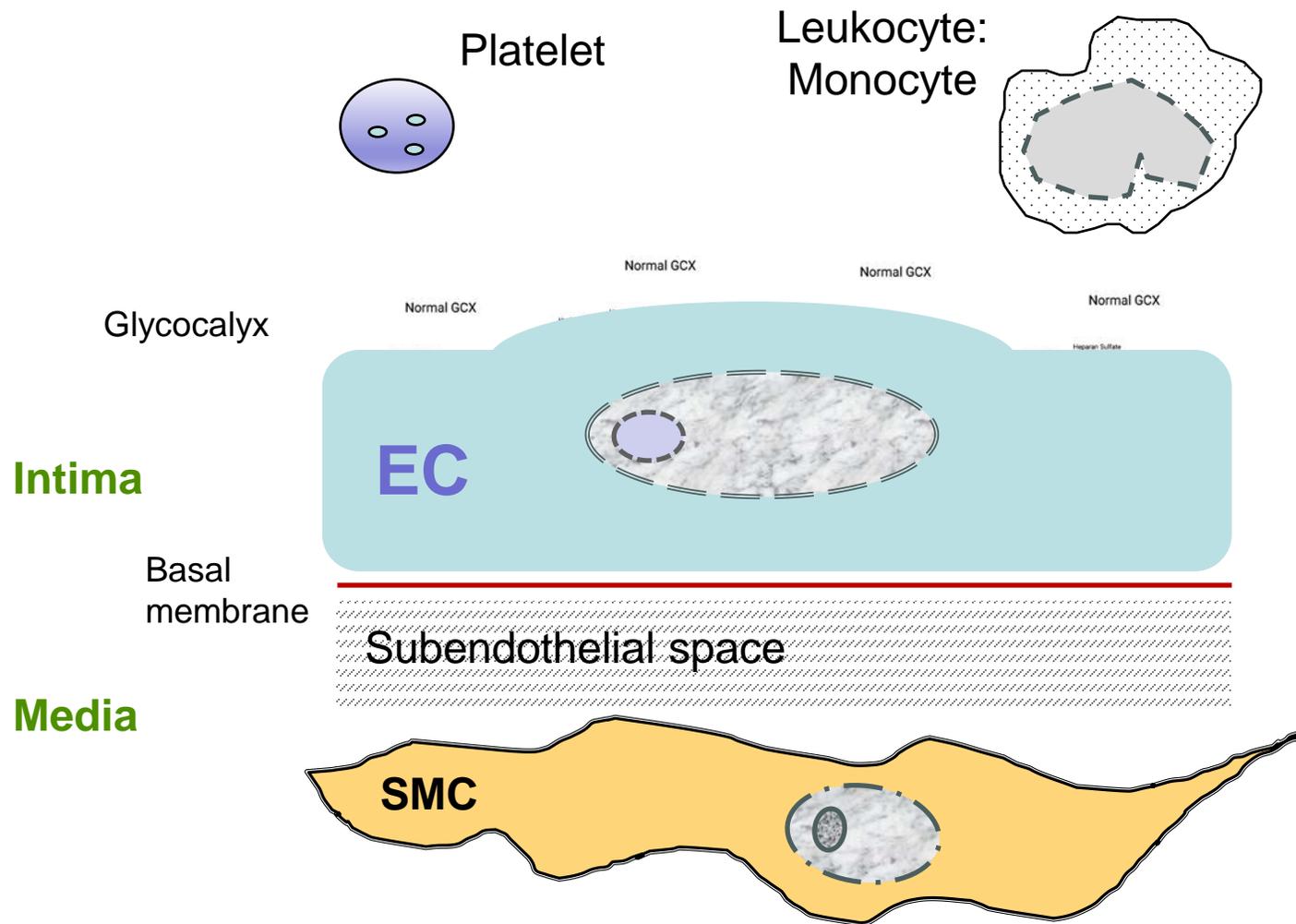
Vascular tone (SMCs)

Primary haemostasis (platelets)

Coagulation

Fibrinolysis

Adhesion of leukocytes,...



Regulation (activation/inhibition):

Vascular tone (SMCs)

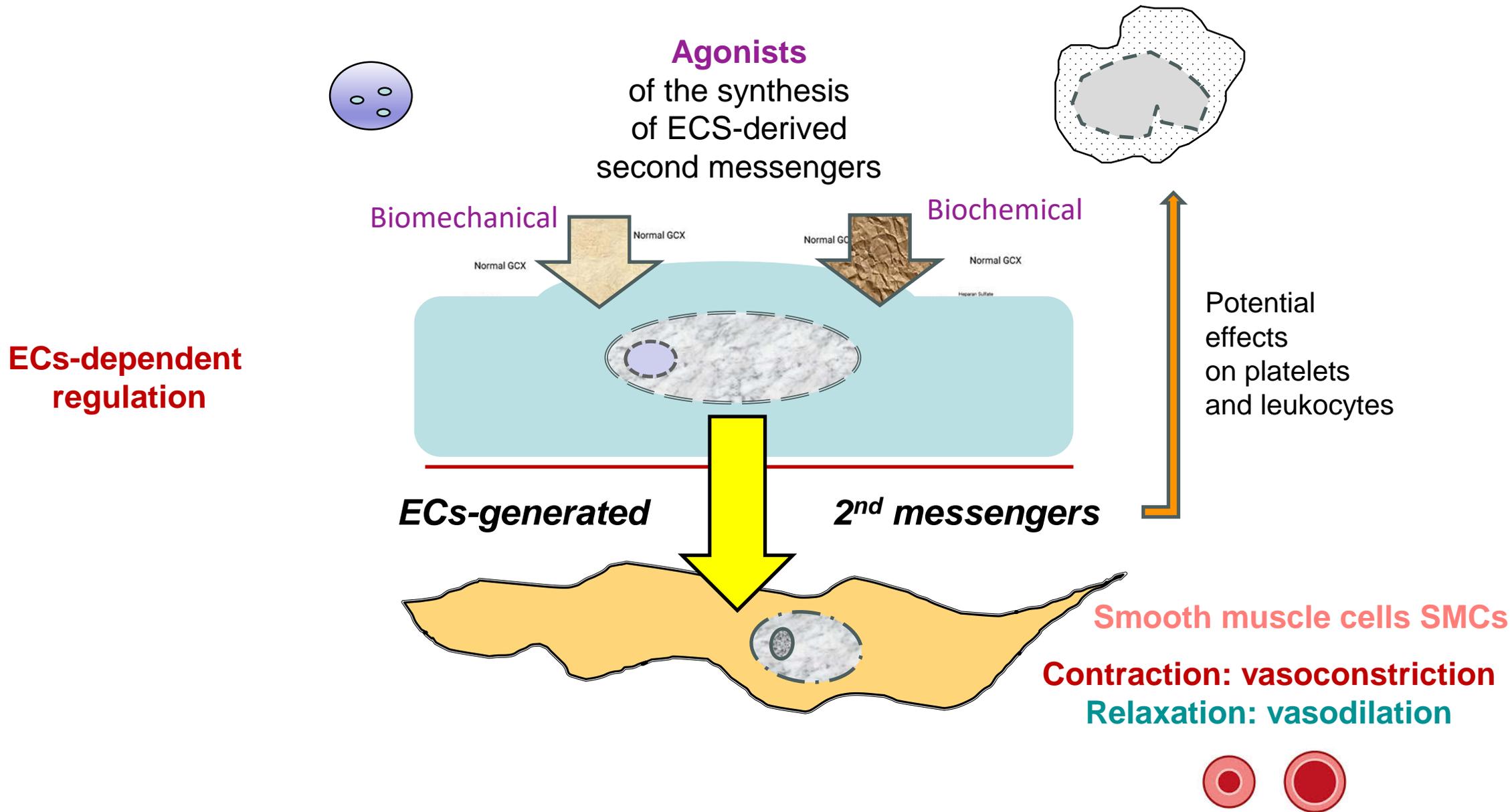
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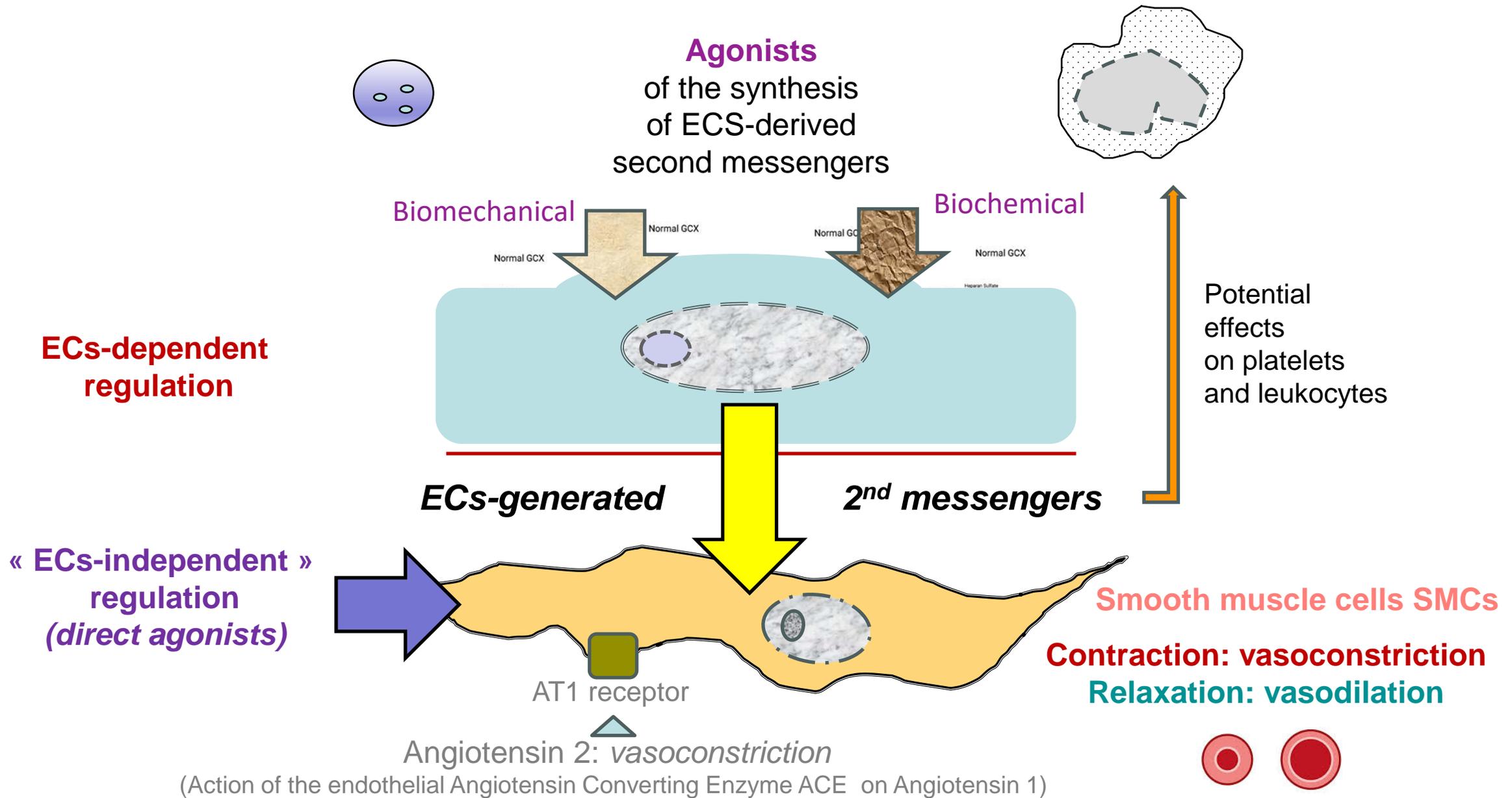
Fibrinolysis

SMC: smooth muscle cell; EC: endothelial cell

Vasoactive role of ECs

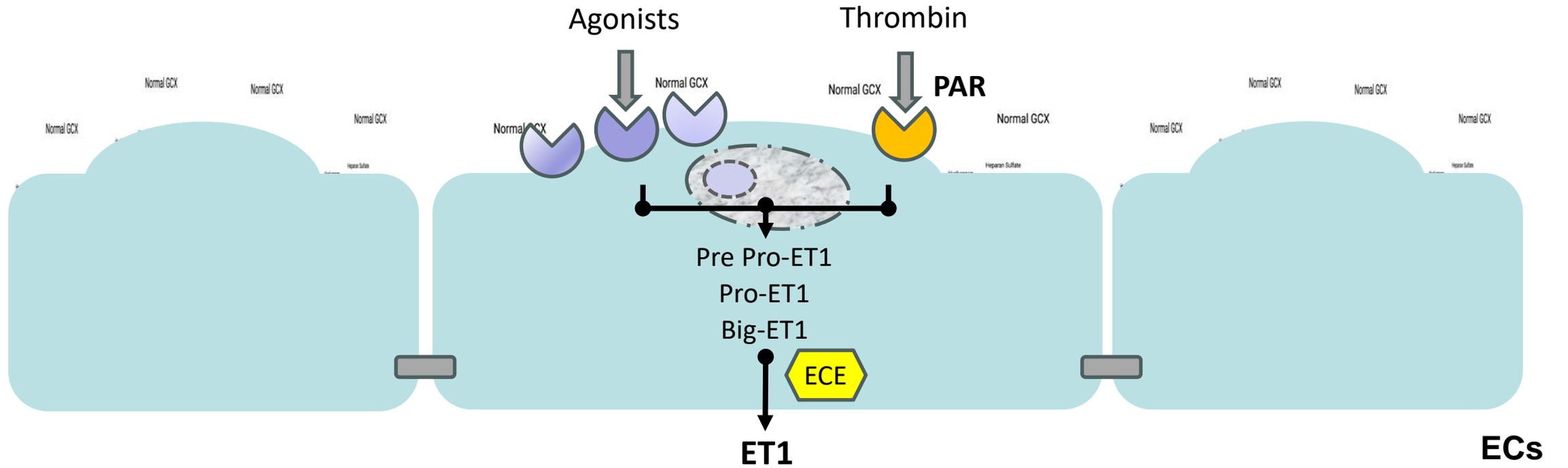


Vasoactive role of ECs

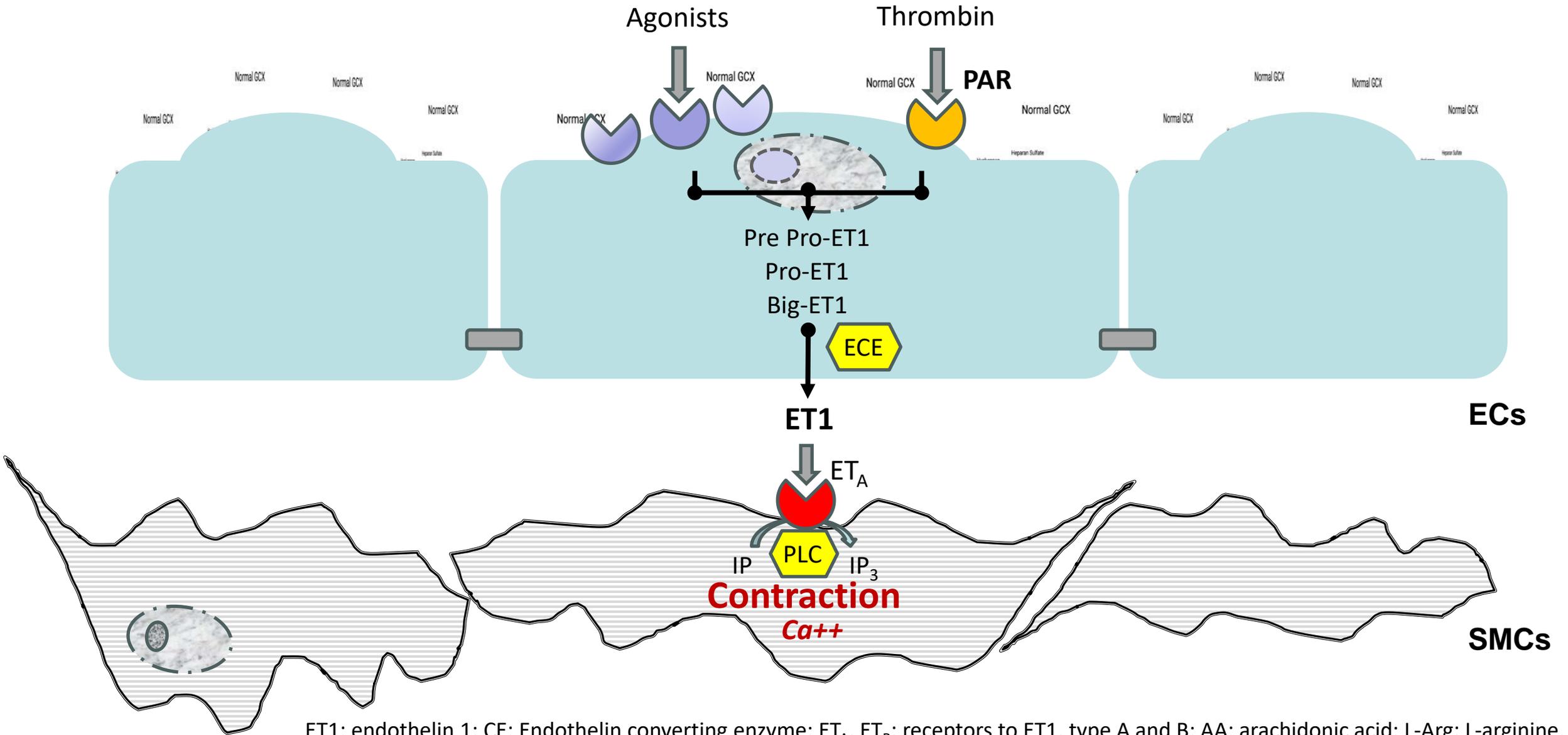


Main ECs-derived vasoactive compounds

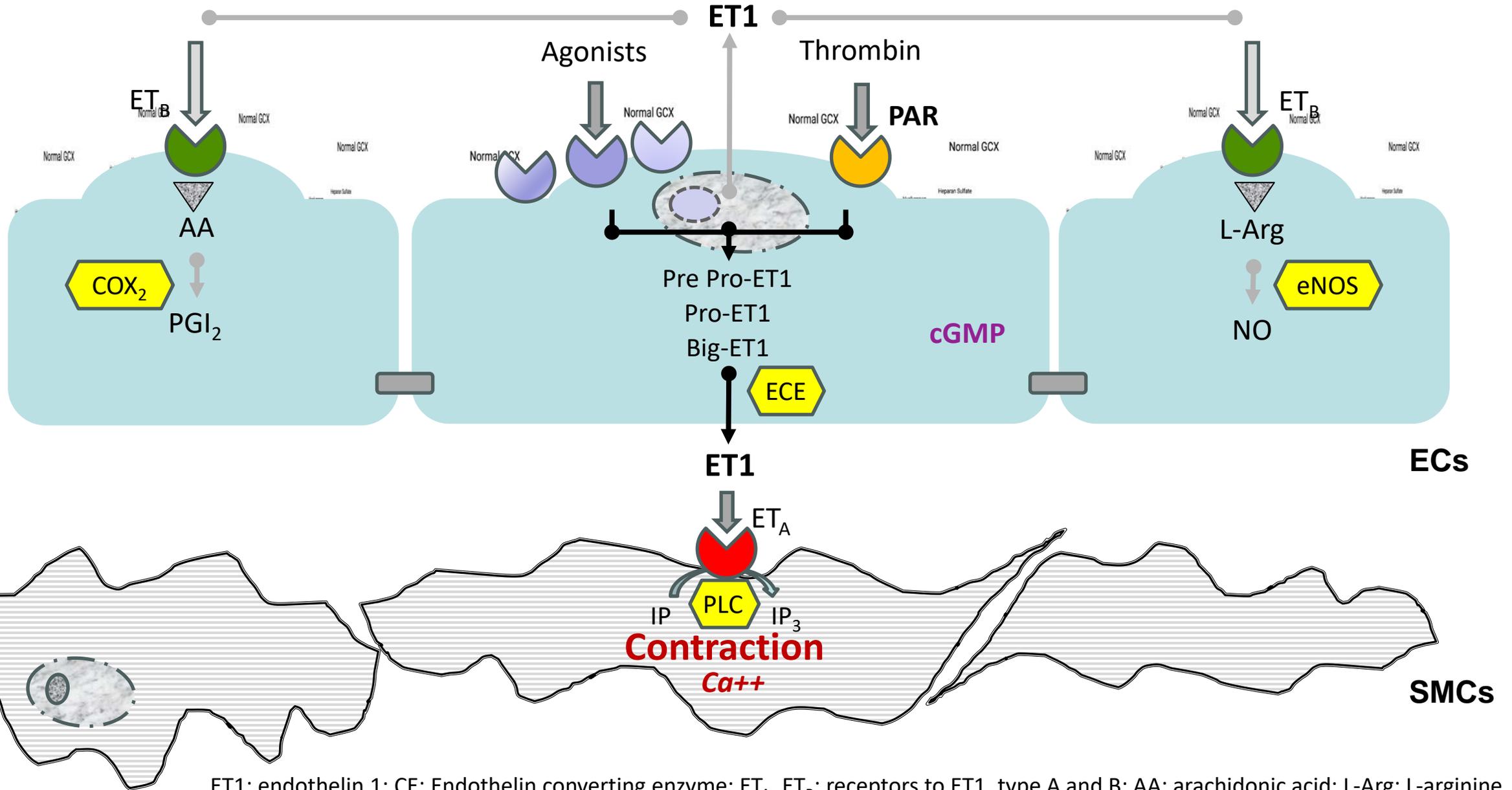
	Vasodilators	Vasoconstrictors
Prostanoids	<p>Acid Arachidonic derived (COX1, COX2 enzymes): PGI₂, prostacyclin. PGI₂ receptor on SMCs: IP; <i>PTGIR</i> gene. Stimulates adenylate cyclase; increases cAMP. Also inhibits platelet activation and leukocyte adhesion. Very short action.</p>	
Gases	<p>L-arginine derived (eNOS enzyme, : NOS-3, constitutive): endogenous NO, nitric oxide (initially: EDRF): Stimulates guanylate cyclase; increases cGMP. Also inhibits platelet activation and leukocyte adhesion. Very short action, inhibited by free Hbα chains.</p>	
Peptides		<p>21 amino acids: Endothelin 1, ET-1. Strong and long-lasting vasoconstriction. Prépro ET derived (ET converting enzyme). Receptors: ET_A, <i>EDNRA</i> gene. ET_B found on ECs: results in vasodilation mediated by PGI₂ and NO release. Increase of intracellular Ca⁺⁺ from the extravascular space.</p>



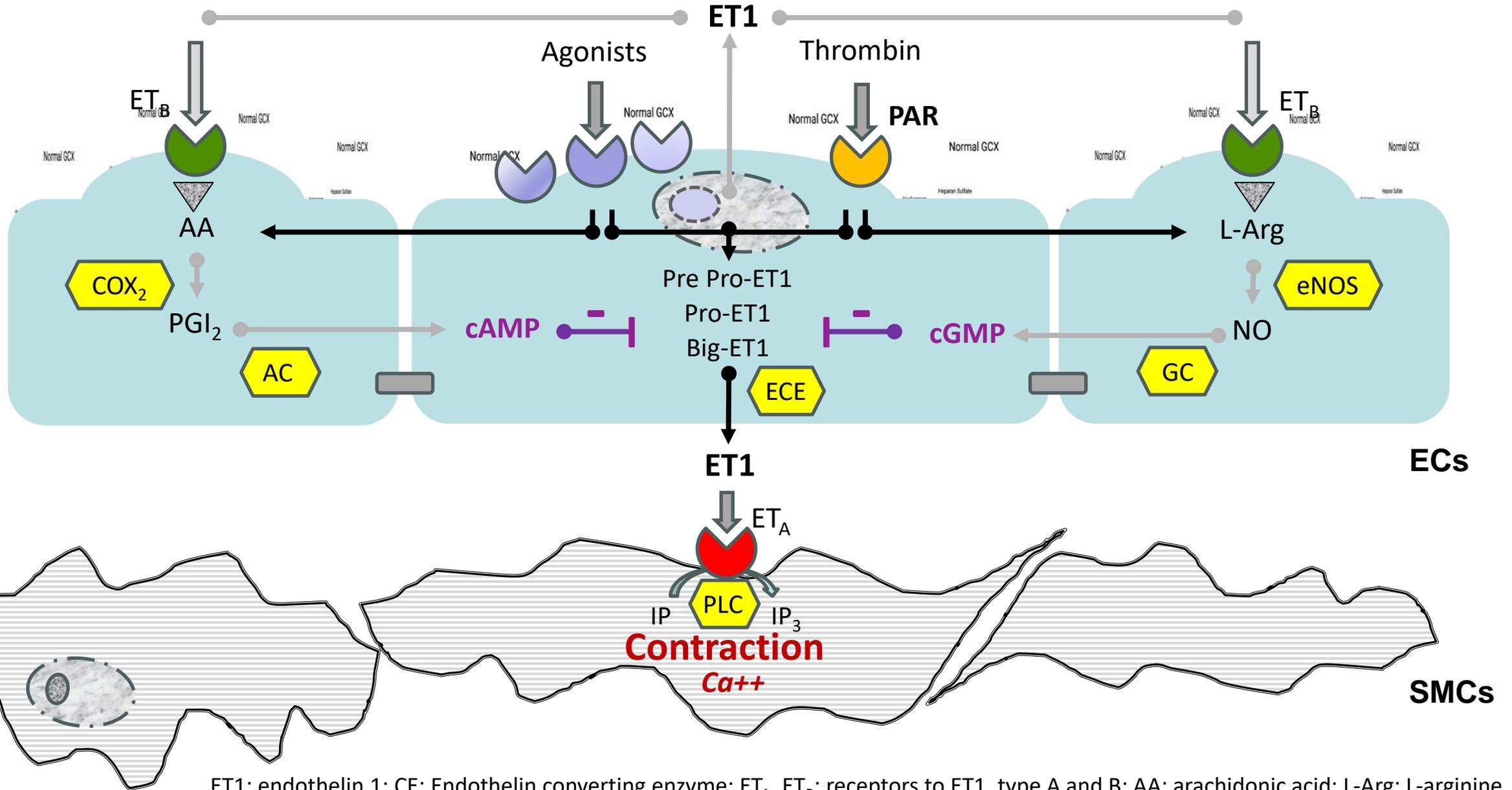
ET1: endothelin 1; CE: Endothelin converting enzyme; ET_A, ET_B: receptors to ET1, type A and B; AA: arachidonic acid; L-Arg: L-arginine, eNOS: endothelial nitric oxide synthase (NOS-3); COX2: type 2 cyclooxygenase; IP: receptor to PGI₂; sGC: receptor to NO; AC: adenylate cyclase; GC: guanylate cyclase; PLC: phospholipase C.



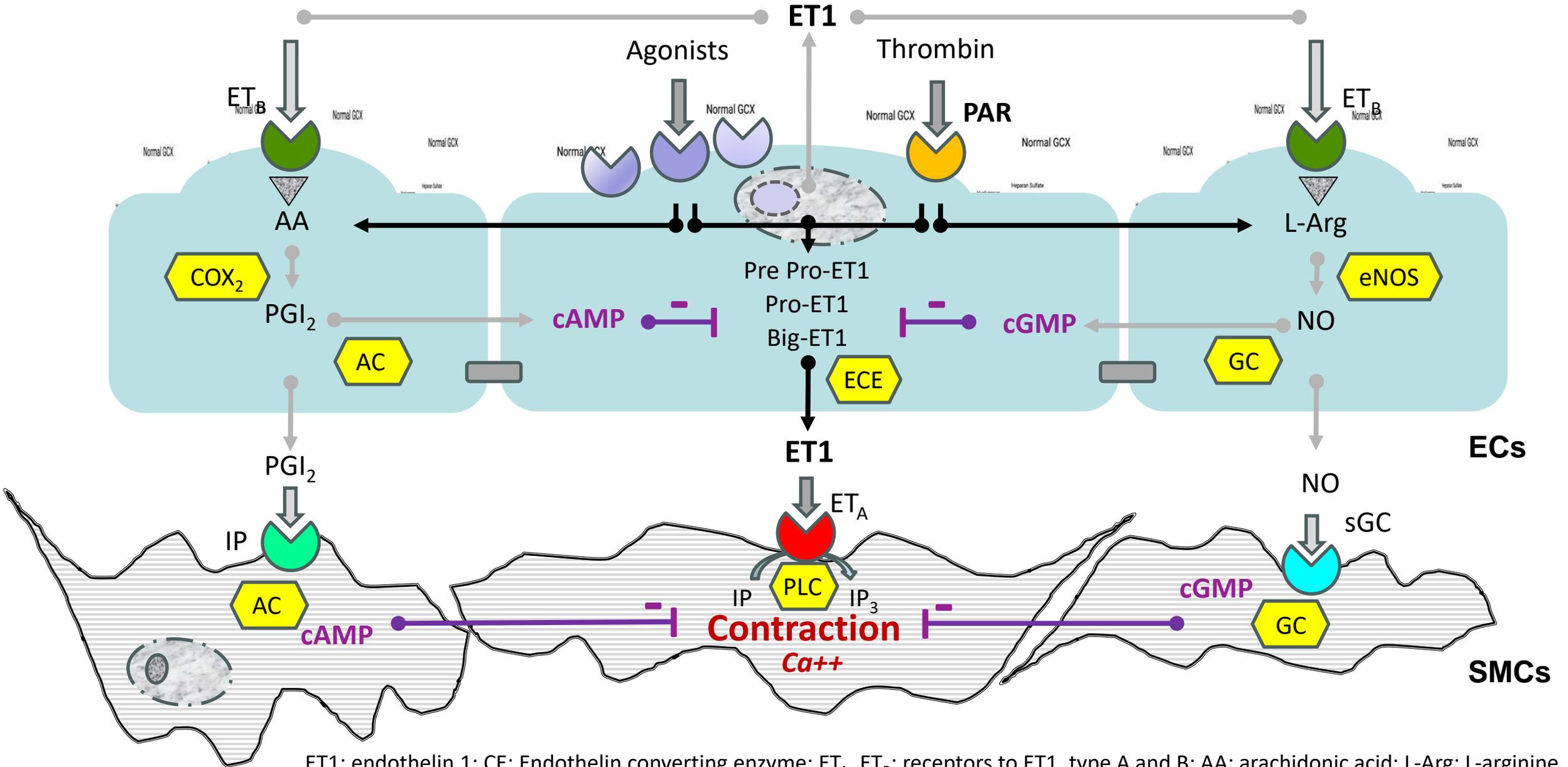
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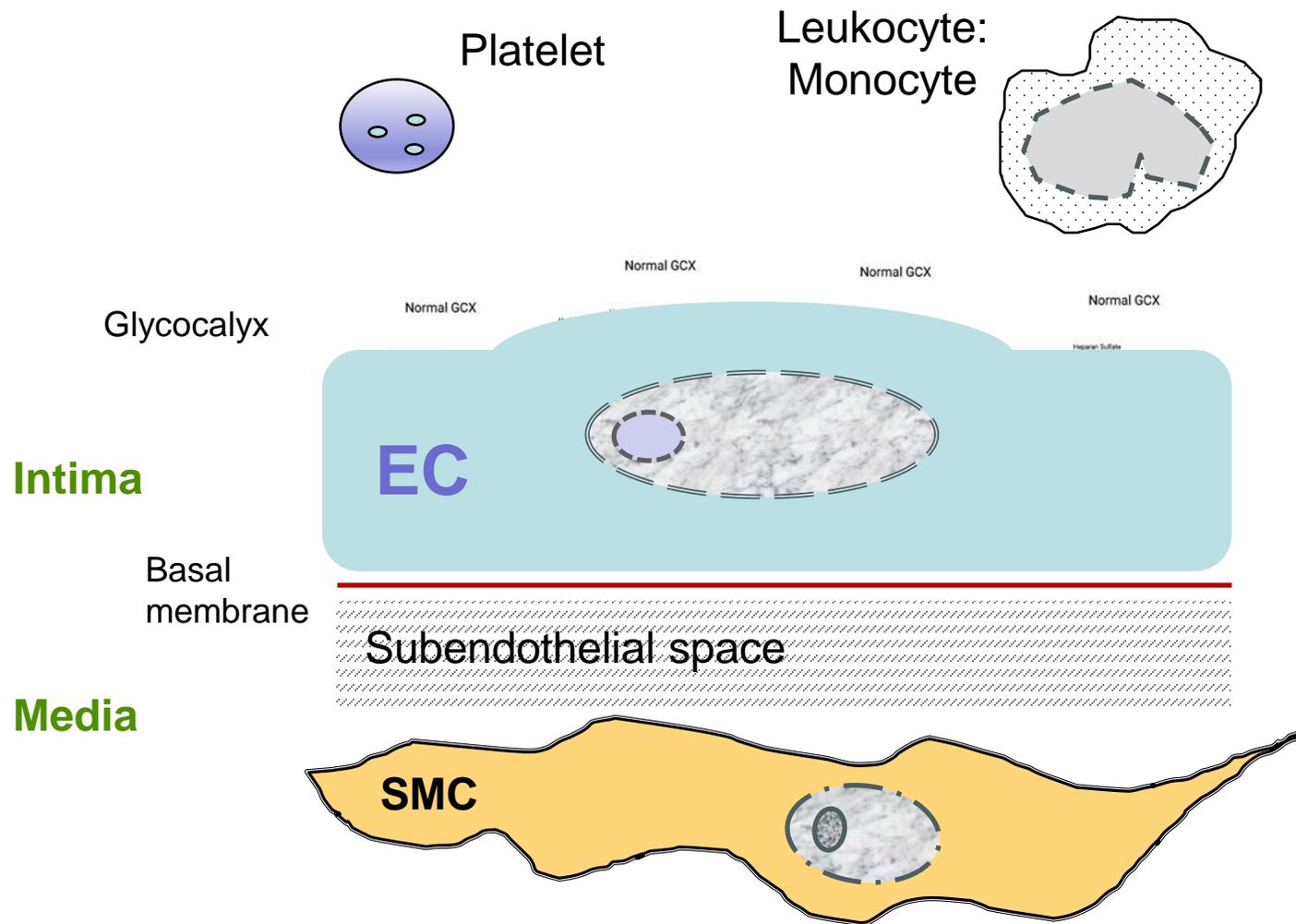
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Regulation (activation/inhibition):

Vascular tone (SMCs)

Primary haemostasis (platelets)

Coagulation

Fibrinolysis

ECs and primary haemostasis

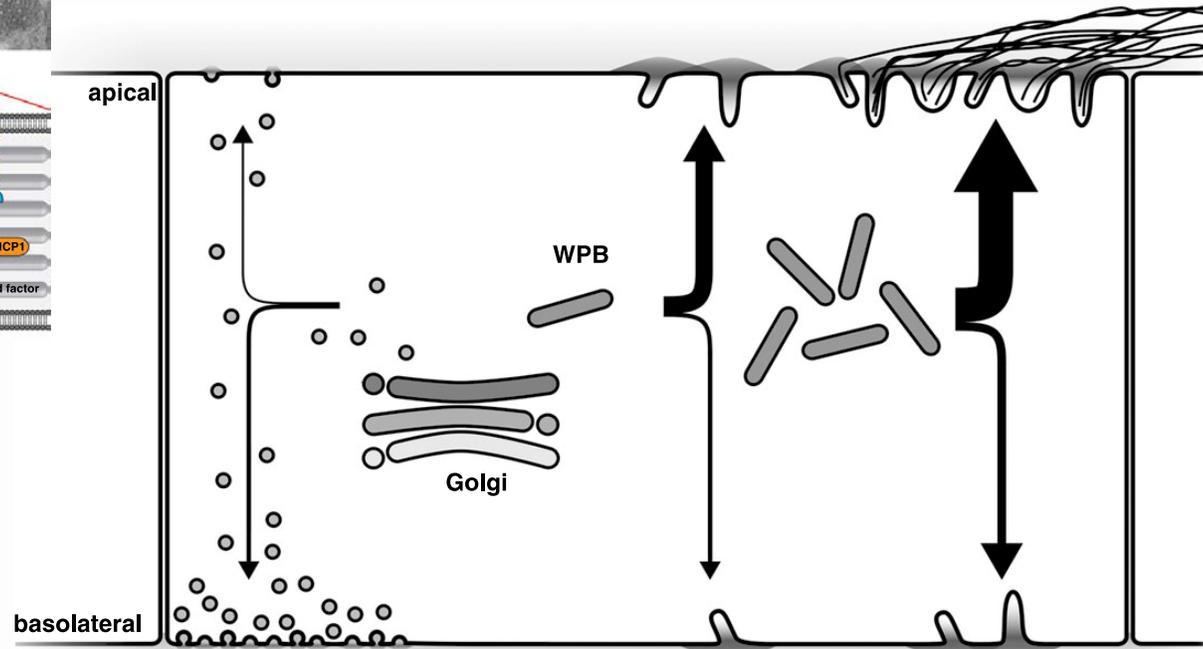
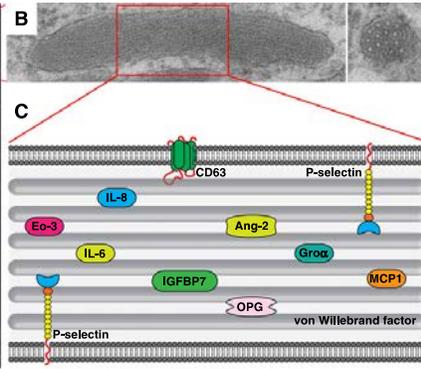
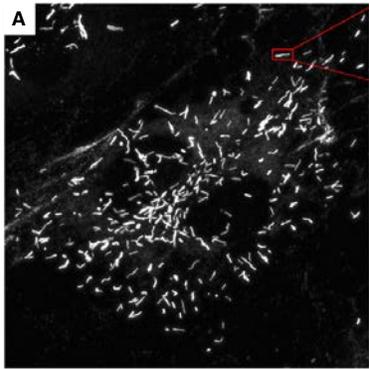
Activation

- **Von Willebrand factor, VWF**
 - **Weibel-Palade bodies, WPb**
 - Rod-shaped storage granules, 0.5 to 5 μm in length
 - Member of lysosome-related organelles
 - Membrane CD63 tetraspanin
 - Haemostatic cargo: *vascular emergency kit*
 - **High-molecular weight VWF, FVIII**
 - **P-selectin**
 - **t-PA**
 - Formation *entirely driven by the expression of VWF*
 - Rapidly released following agonist-induced triggering of intracellular signalling pathways (*exocytosis*)

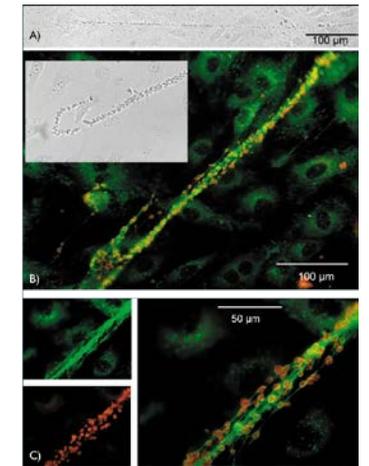
Inhibition

- **PGI₂ and NO** (*cf.*)
 - *Inhibit platelet activation and leukocyte adhesion*
- **Ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD-1 / CD39)**
 - *Membrane-bound enzyme*
 - *Converts ATP and ADP, a potent agonist of platelet activation / aggregation, into AMP (CD73 > adenosine)*
 - *Limits the prothrombotic signals*
 - *Gene: **ENTPD1***
- **ADAMTS13**
 - *Proteolytic cleavage of ultralarge VWF multimers*
 - *Also down-regulation of inflammation, regulation of angiogenesis and degradation of extracellular matrix*
 - *Can tether to the glycocalyx*
 - *Gene: **ADAMTS13***
 - *Also synthesised by the megacaryocytes, the hepatic stellate cells (perisinusoidal: Ito cells)*

A central role for WPb exocytosis



*Ultra-large
VWF multimers;
VWF strings
on the apical side
of ECs*



VWF secretion: 3 pathways;

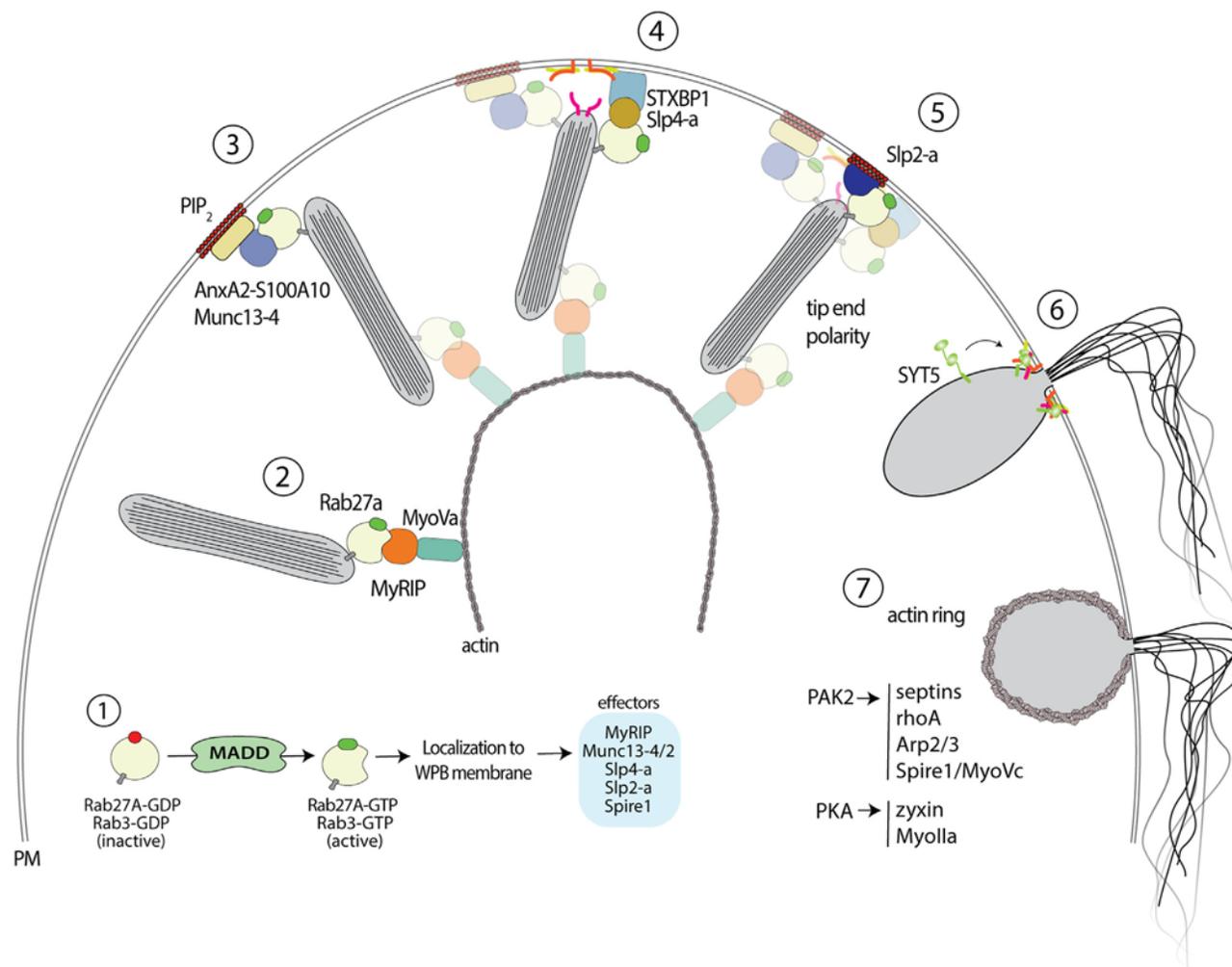
Constitutive secretion: low molecular weight VWF; mainly basolateral side of ECs

Basal secretion

Regulated secretion

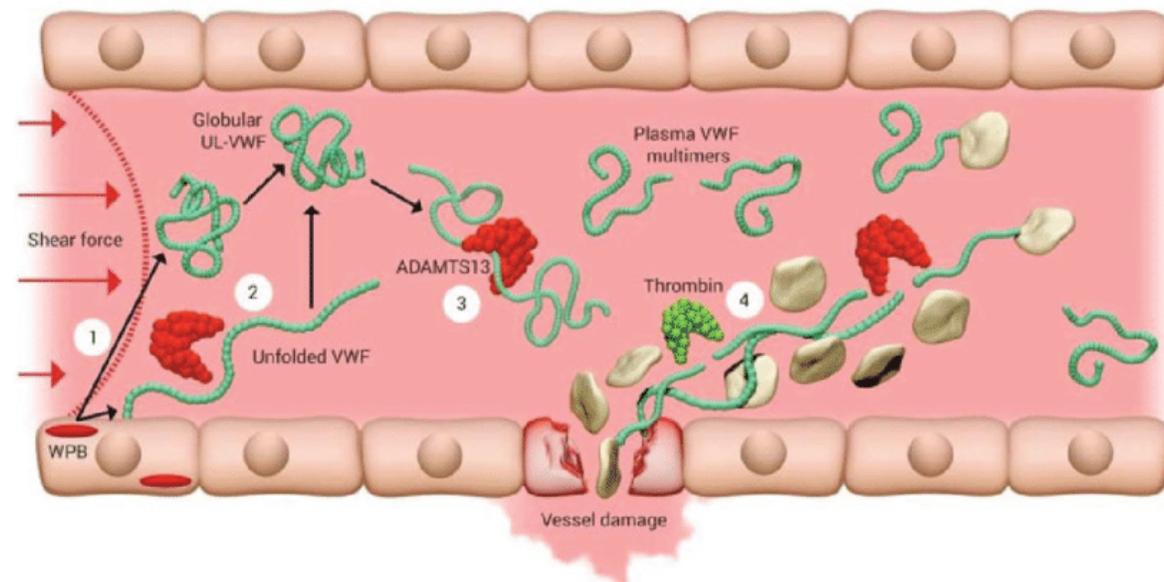
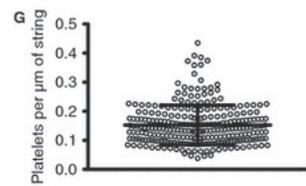
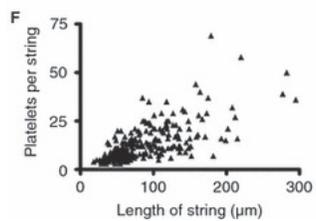
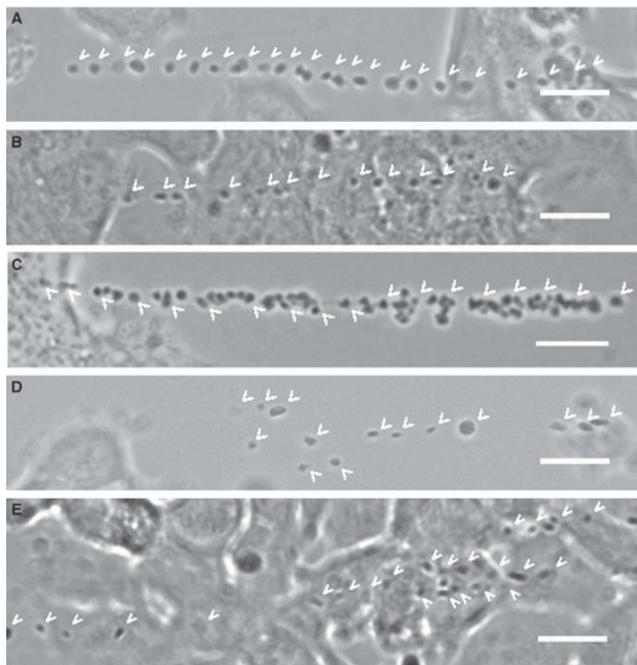
} high molecular weight VWF from WPb; primarily towards apical surface

A central role for WPb exocytosis

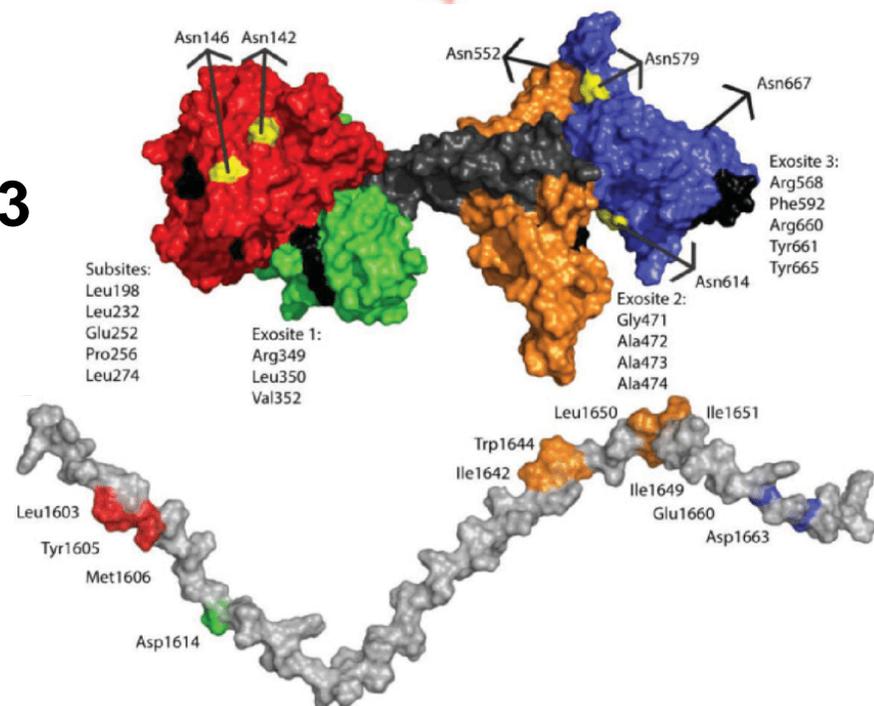


- 1- Activation of soluble factors (RabGTPases)
- 2- Anchoring to the actin skeleton (MyRIP)
- 3- Tethering to the plasma membrane (AnxA2-S100A10)
- 4- Docking to the plasma membrane (Synaptogamin-like protein 4a – syntaxin)
- 5- Polarisation of the WPb (Condensation of Slp2-a)
- 6- Membrane fusion between WPb and plasma membrane; expulsion of content (soluble NSF – synaptogamin 5)
- 7- Subset of events: actin ring formation.

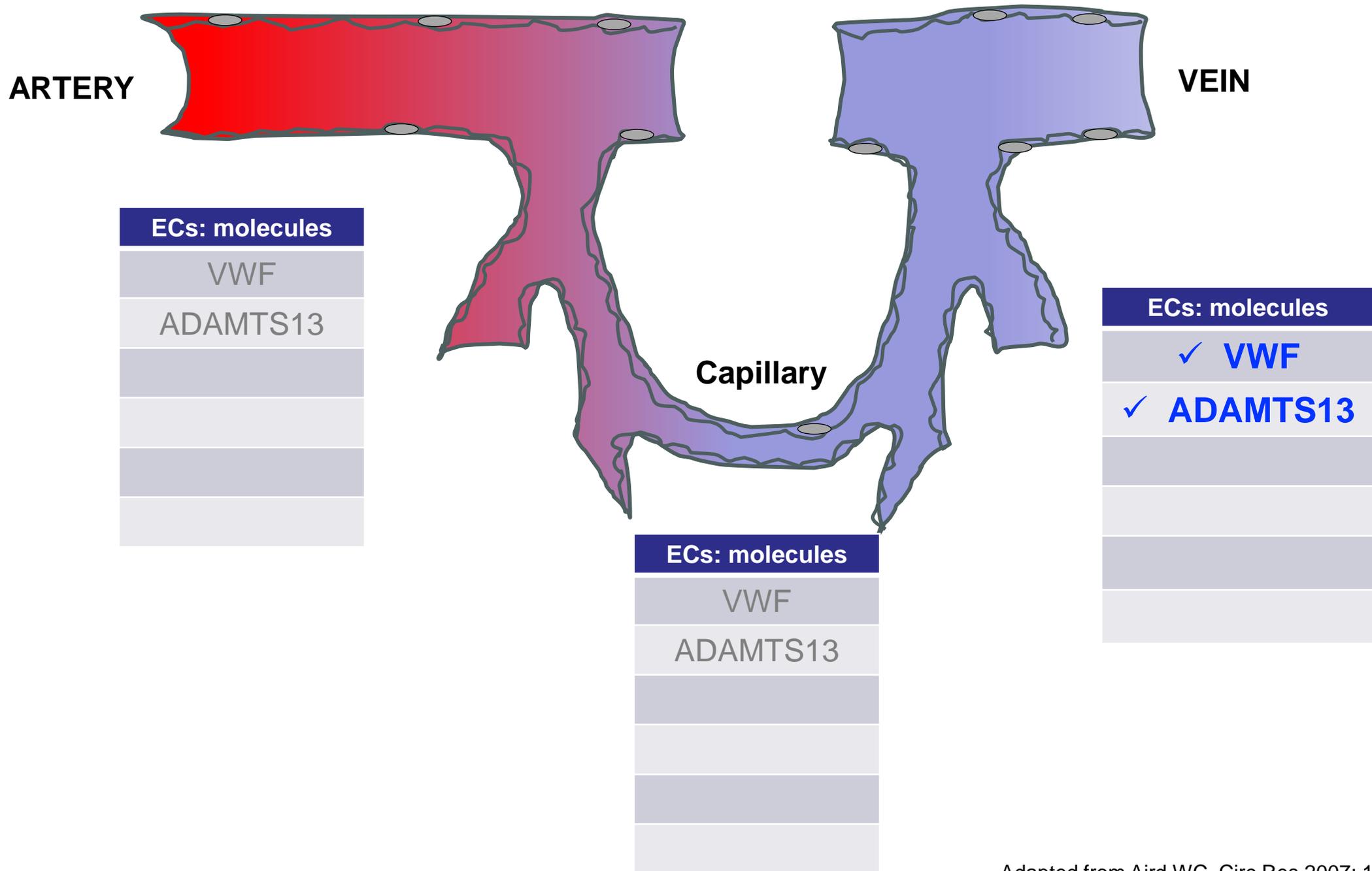
Complex mechanism for WPb exocytosis...



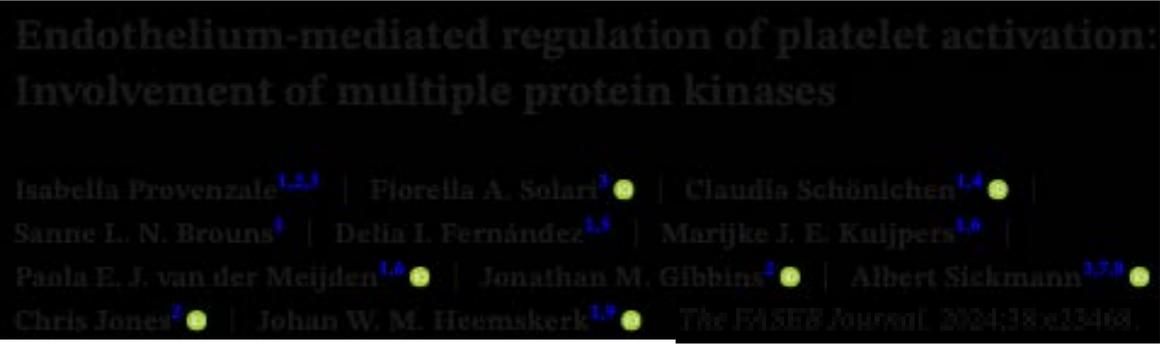
ADAMTS13



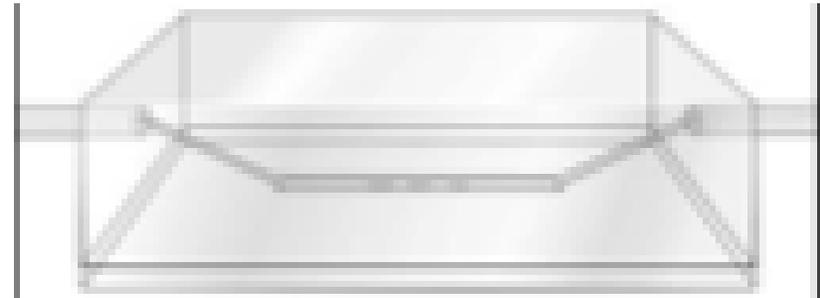
VWF



ECs-mediated regulation of platelet activation: *something else?*



High-shear, whole-blood, micro-engineered endothelialised microfluidic model:
microchannels co-coated with collagen and TF and then overgrown by HUVEC until sub-confluence.

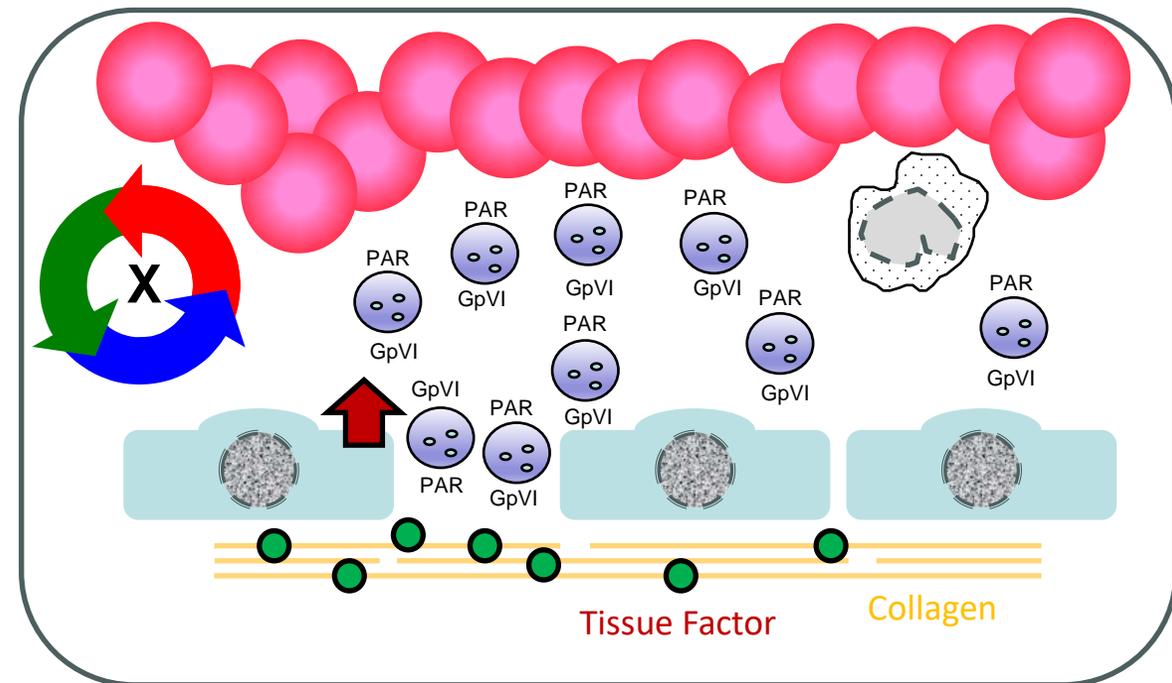


ECs-mediated regulation of platelet activation: *something else!*

Endothelium-mediated regulation of platelet activation:
Involvement of multiple protein kinases

Isabella Provenzale^{1,2,3} | Fiorella A. Solari³ | Claudia Schönichen^{1,4} |
Sanne L. N. Brouns⁵ | Delia I. Fernández^{1,3} | Marijke J. E. Kuijpers^{1,6} |
Paula E. J. van der Meijden^{1,6} | Jonathan M. Gibbins⁷ | Albert Sickmann^{3,7,8} |
Chris Jones⁹ | Johan W. M. Heemskerk^{1,3} | *The FASEB Journal*, 2024;38:e23468.

**Partly confluent HUVEC (60%)
strongly suppress platelet adhesion and activation
via the collagen receptor GpVI
and the PAR receptors for thrombin;
and no major improvement of inhibition
by prostacyclin or nitric oxide.**



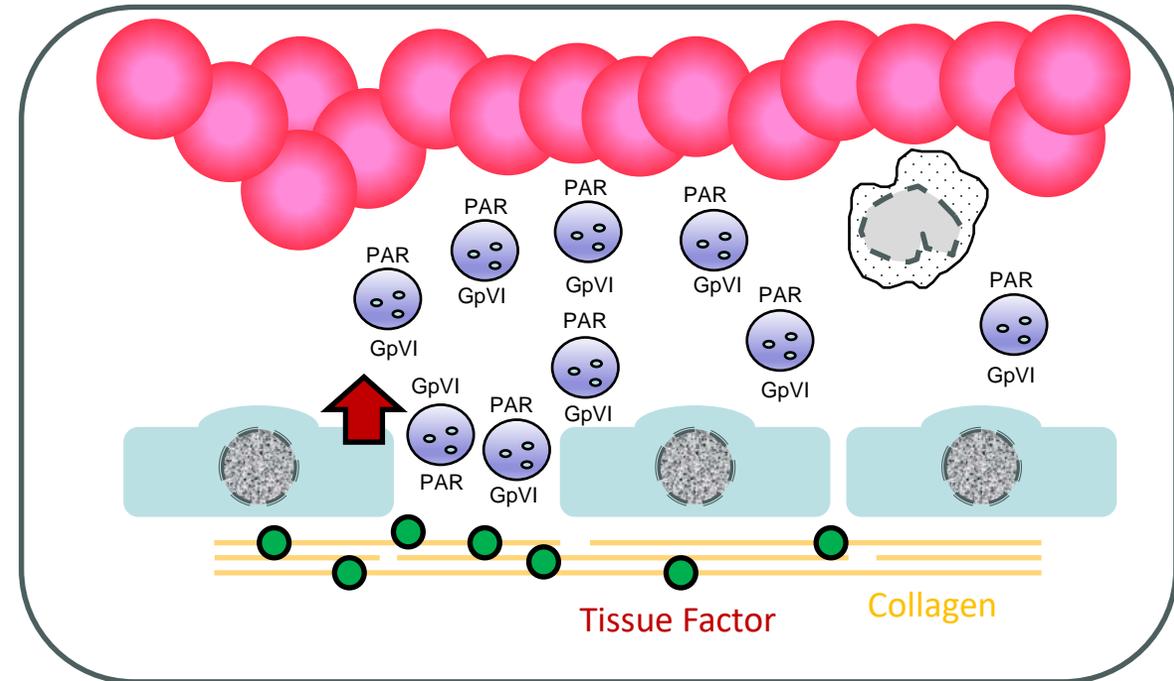
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HUVEC reduce platelet activation
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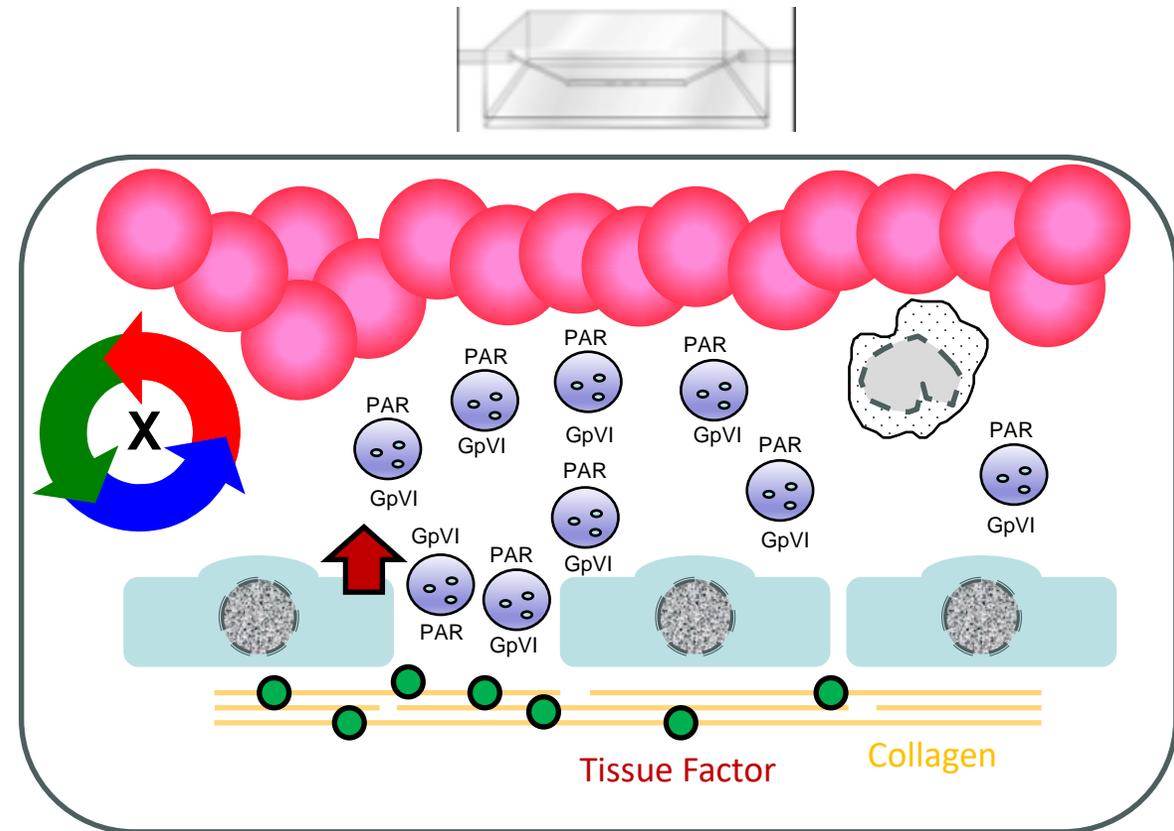


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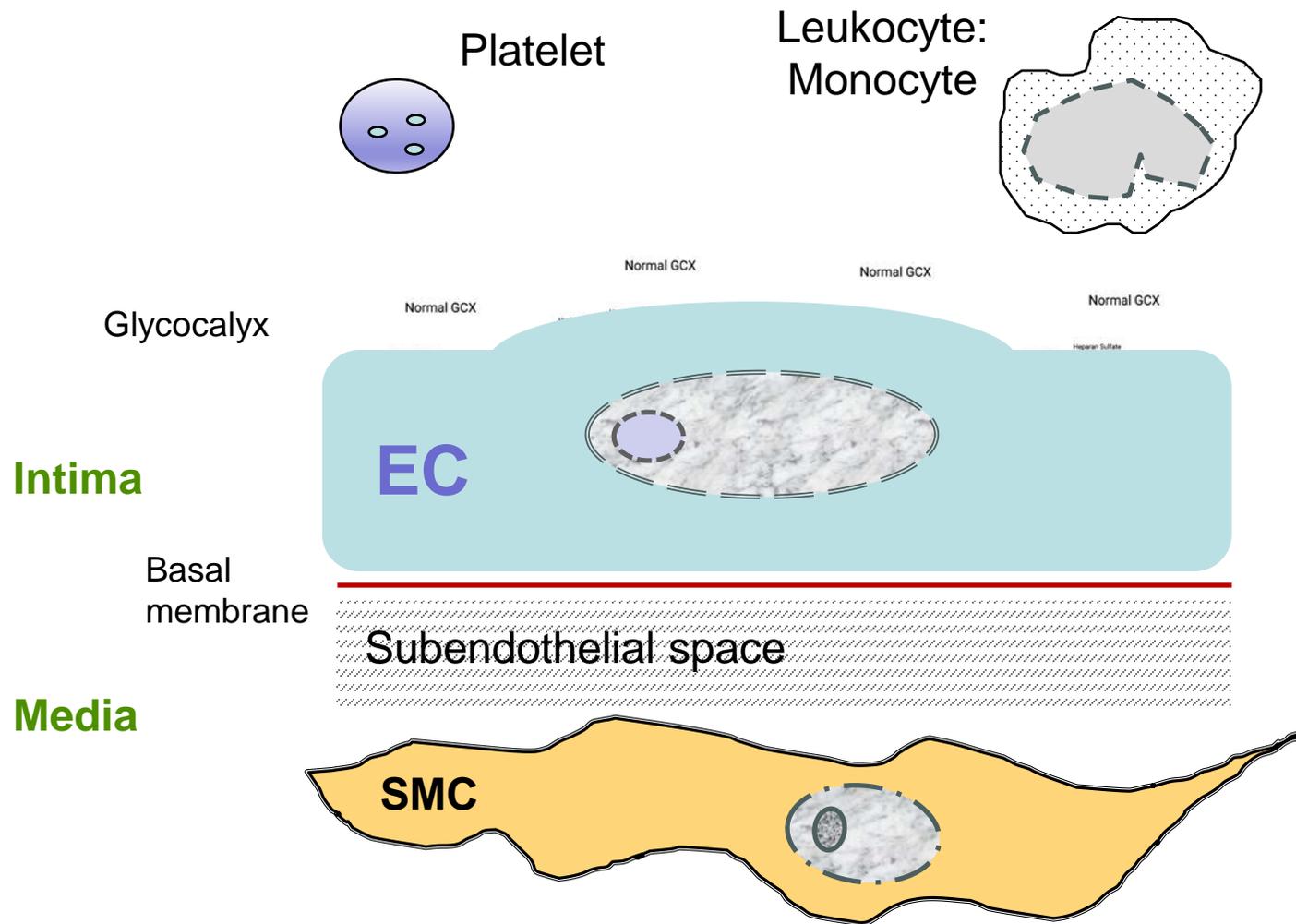


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by prostacyclin or nitric oxide.**

Similarly, under stasis,
HUVEC reduce platelet activation
by collagen-related peptide and thrombin .



**Substrate analysis: major roles of some platelet protein kinase classes
(MAPK, CDK, DYRK, STK, PKC members).**



Regulation (activation/inhibition):

Vascular tone (SMCs)

Primary haemostasis (platelets)

Coagulation

Fibrinolysis

ECs and Coagulation

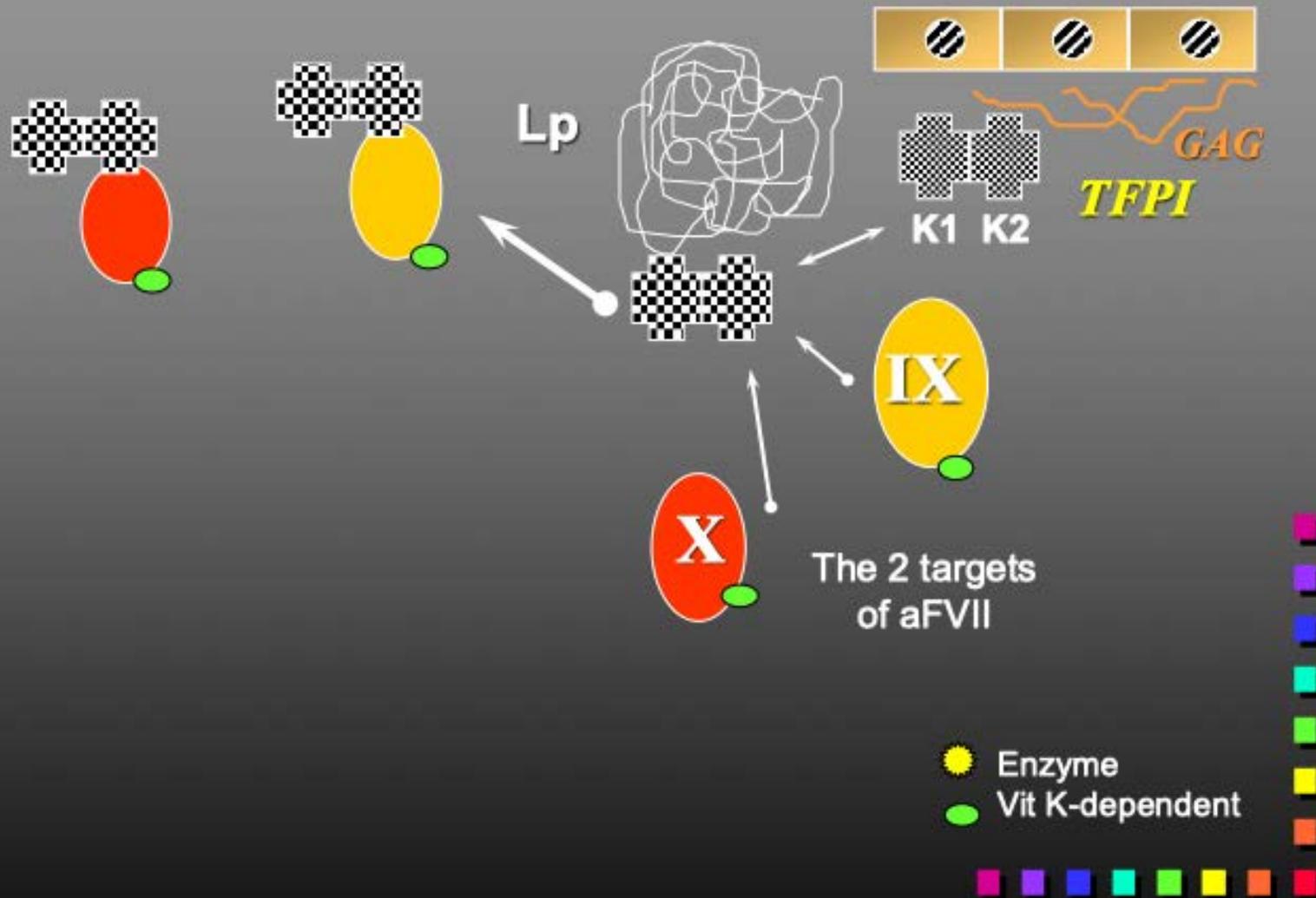
Activation

- **Human ECs: Tissue Factor TF?**
 - HUVECs: *not in basal conditions.*
 - Can only be induced after stimulation
 - Coagulation or Inflammation?
 - *Paucity of evidence that shows that TF is expressed by ECs **in vivo**...*
- **Phospholipid scramblases**
 - Phosphatidylserine externalisation
 - *Endothelial procoagulant activity*
 - **TMEM16** (Transmembrane Member 16: *also anoctamins*; family members E and F)
 - Schmaier AA et al., JCI 2023; 133:e163808
 - Ca⁺⁺ activated
 - *ANO* genes

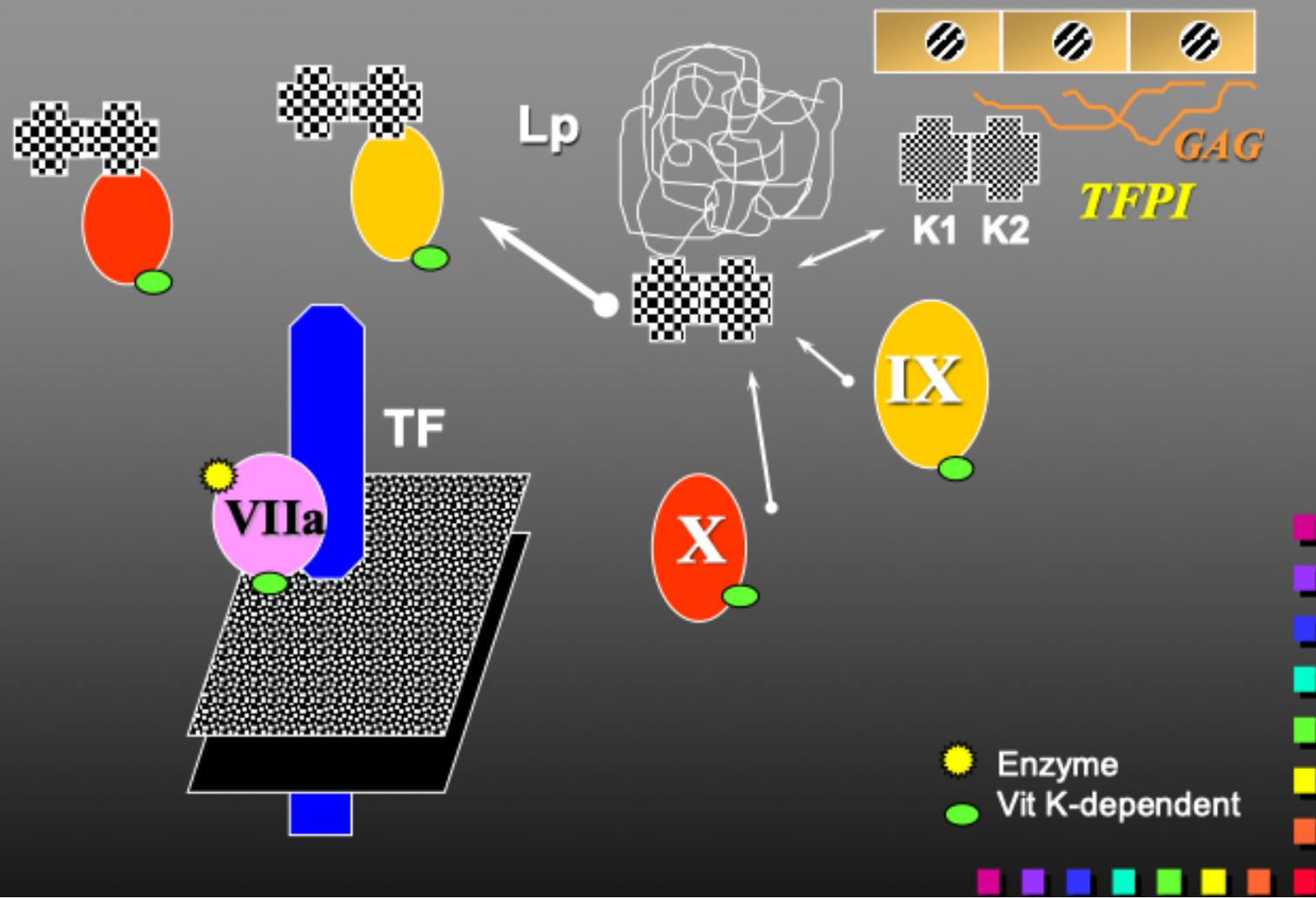
Inhibition

- **Glycocalyx**
 - **Antithrombin AT**
 - *SERPINC1* gene
 - **Tissue Factor Pathway Inhibitor TFPI:**
 - *TFPI* gene
 - *One of the 2 alternatively spliced isoforms*
 - TFPI β : endothelial surface, glycocalyx
- **Receptors for the Protein C system**
 - **Thrombomodulin THBD**
 - Thrombin
 - *THBD* gene
 - **Endothelial Protein C Receptor EPCR**
 - Protein C
 - *PROCR* gene

TFPI, Tissue Factor Pathway Inhibitor



TFPI, Tissue Factor Pathway Inhibitor







Protein C system



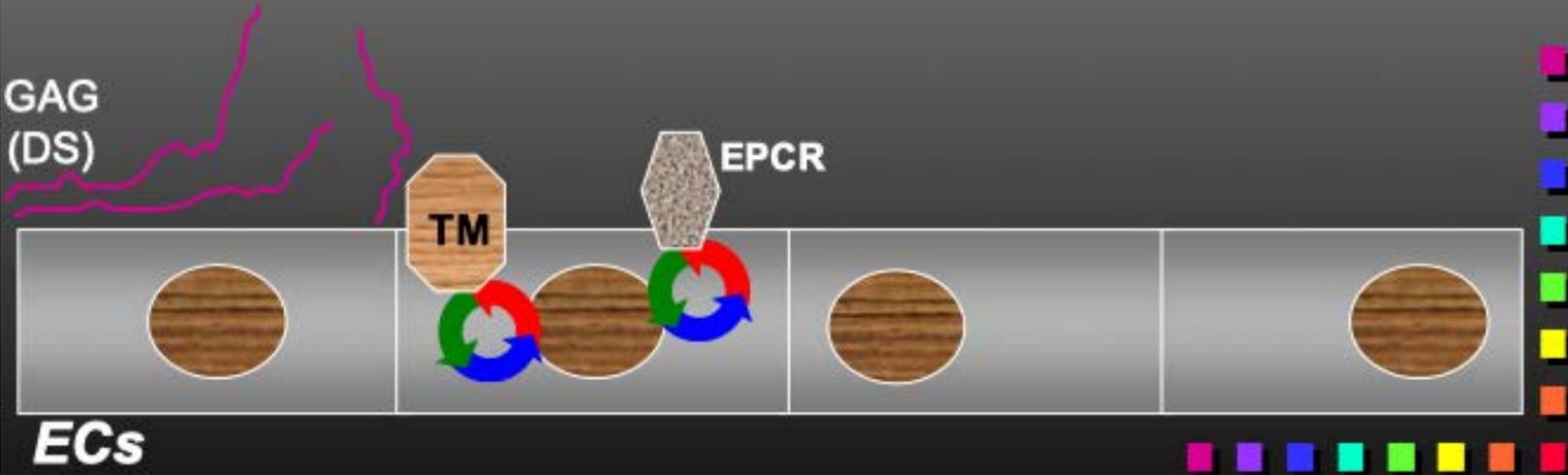
Enzyme

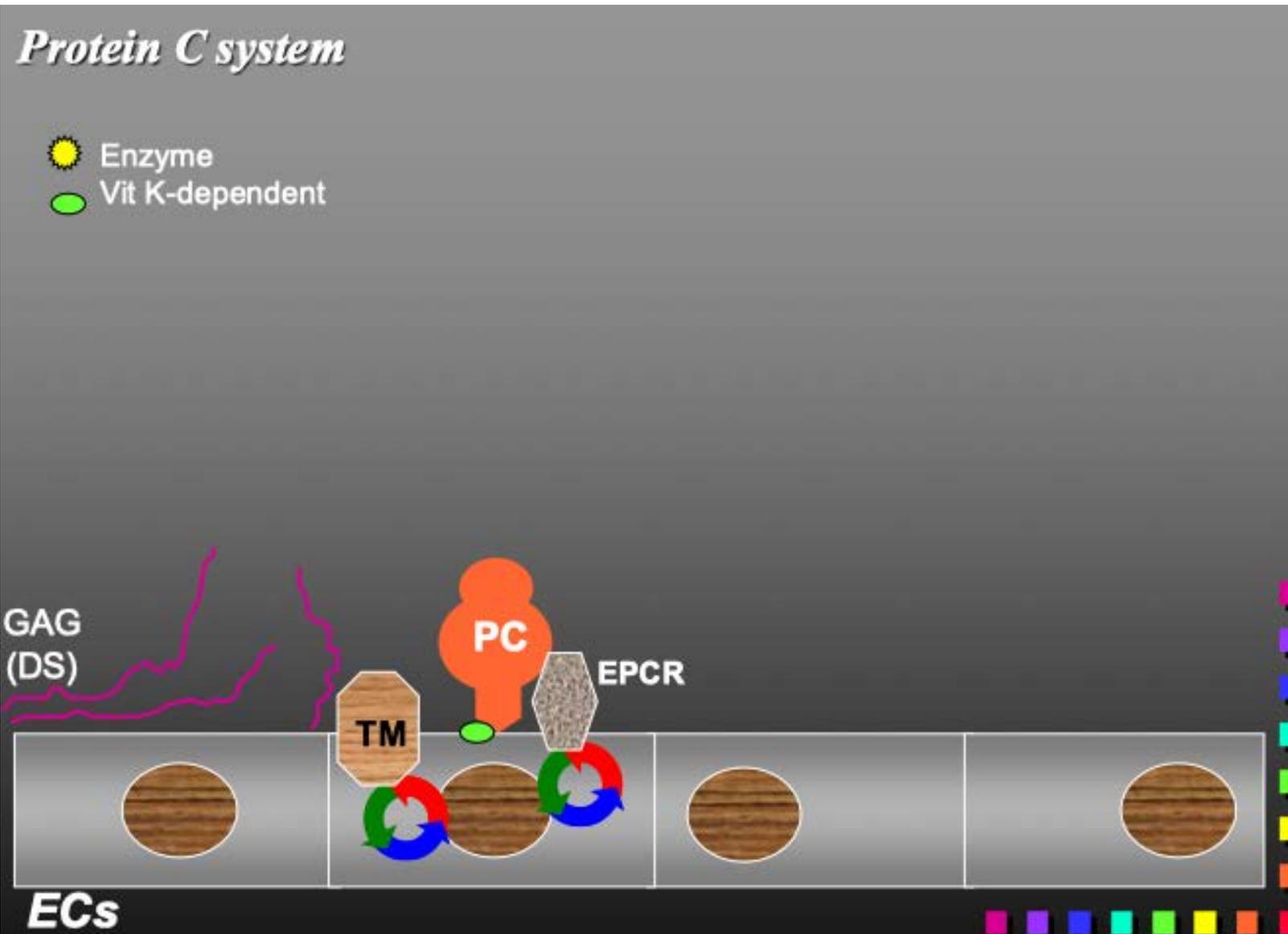


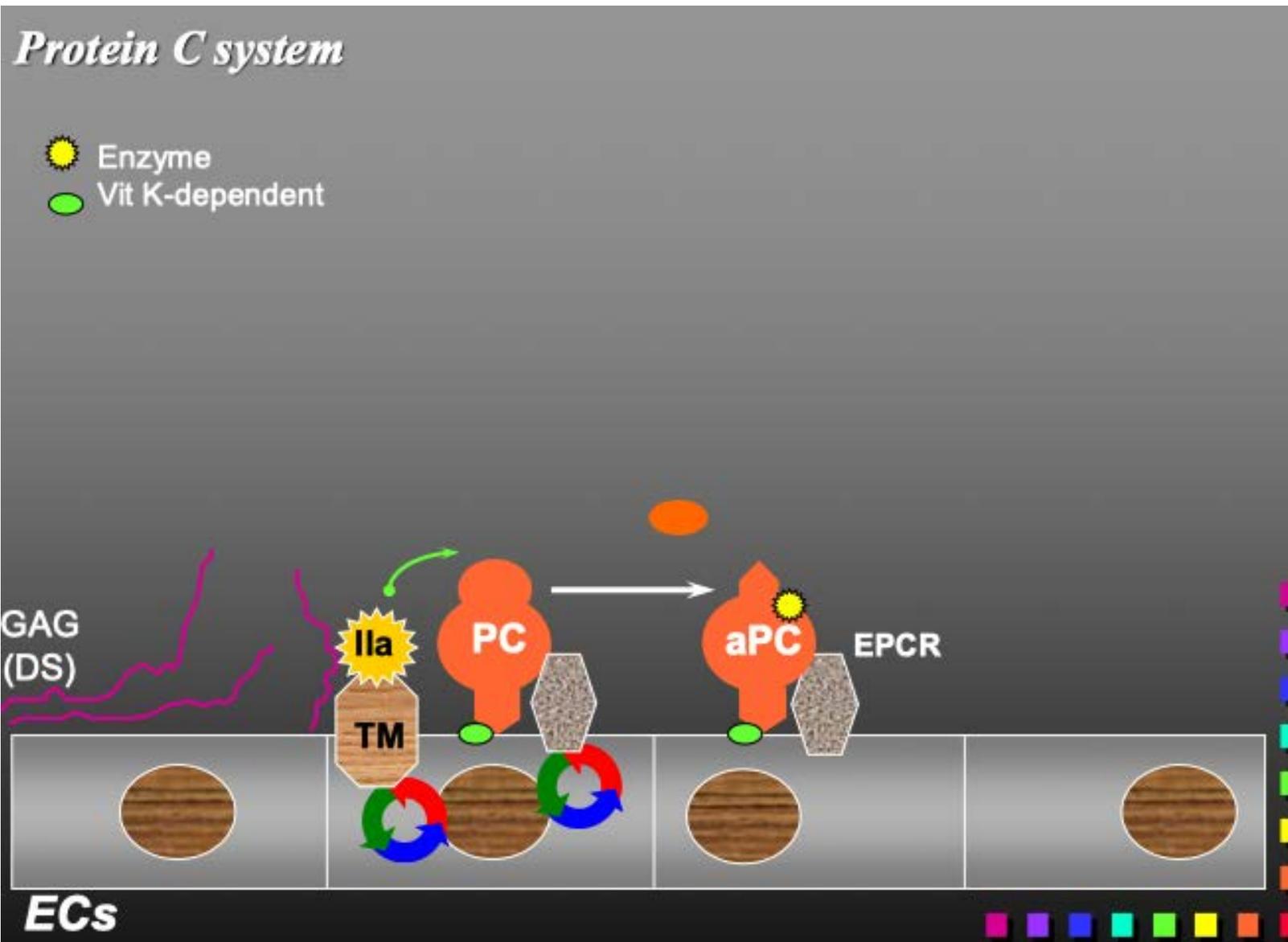
Vit K-dependent

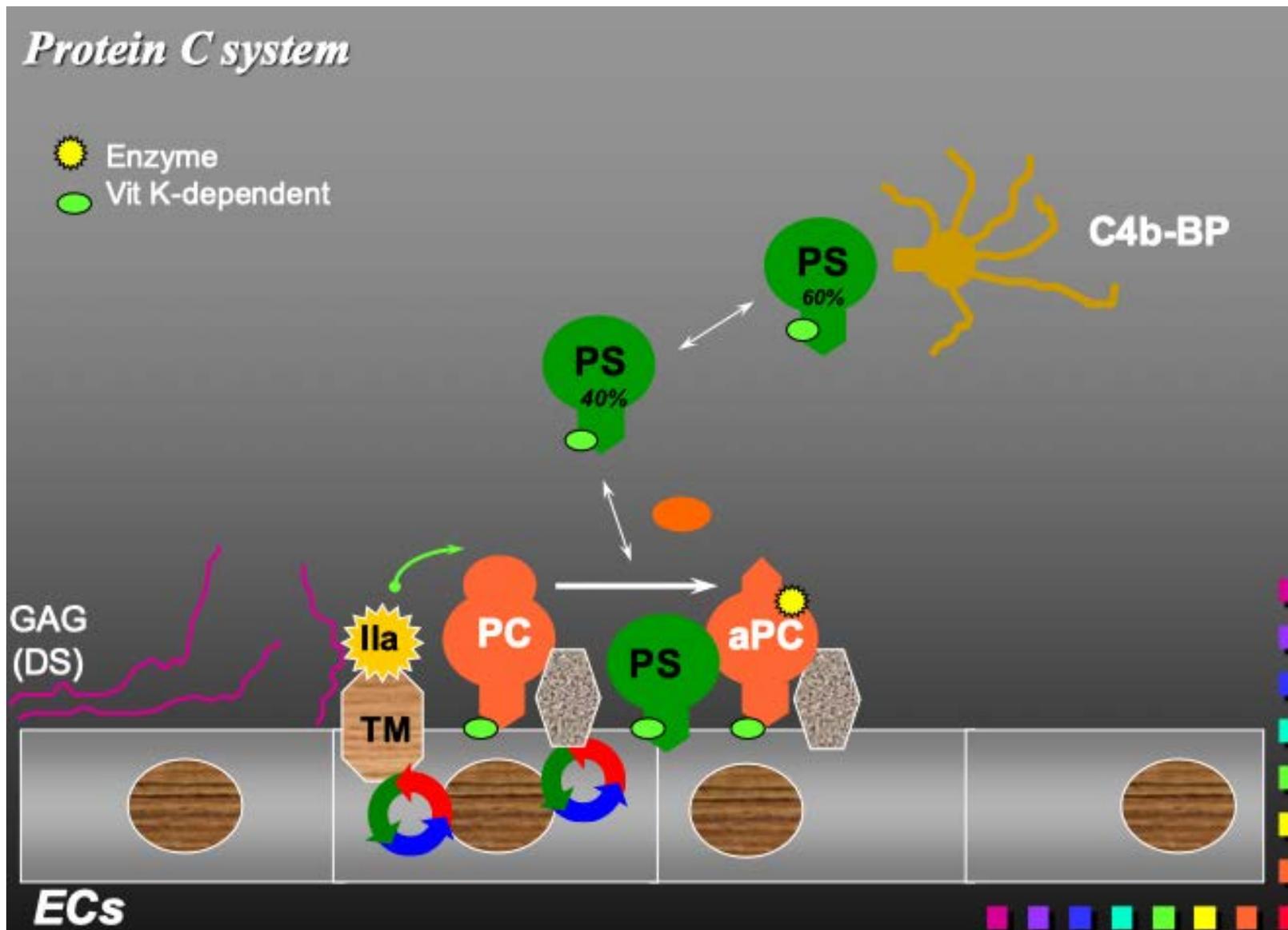
TM: thrombomodulin

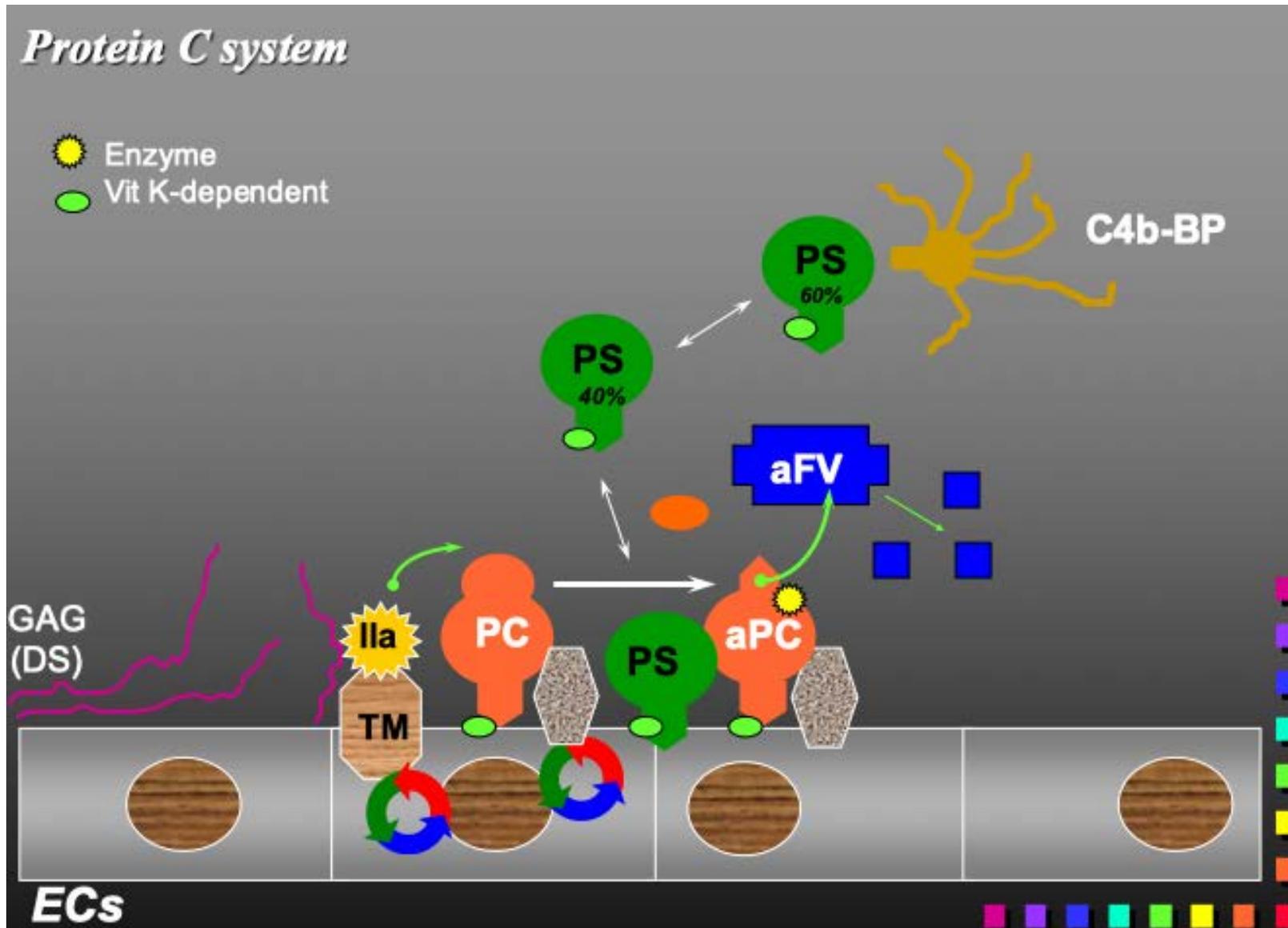
EPCR: Endothelial Protein C Receptor

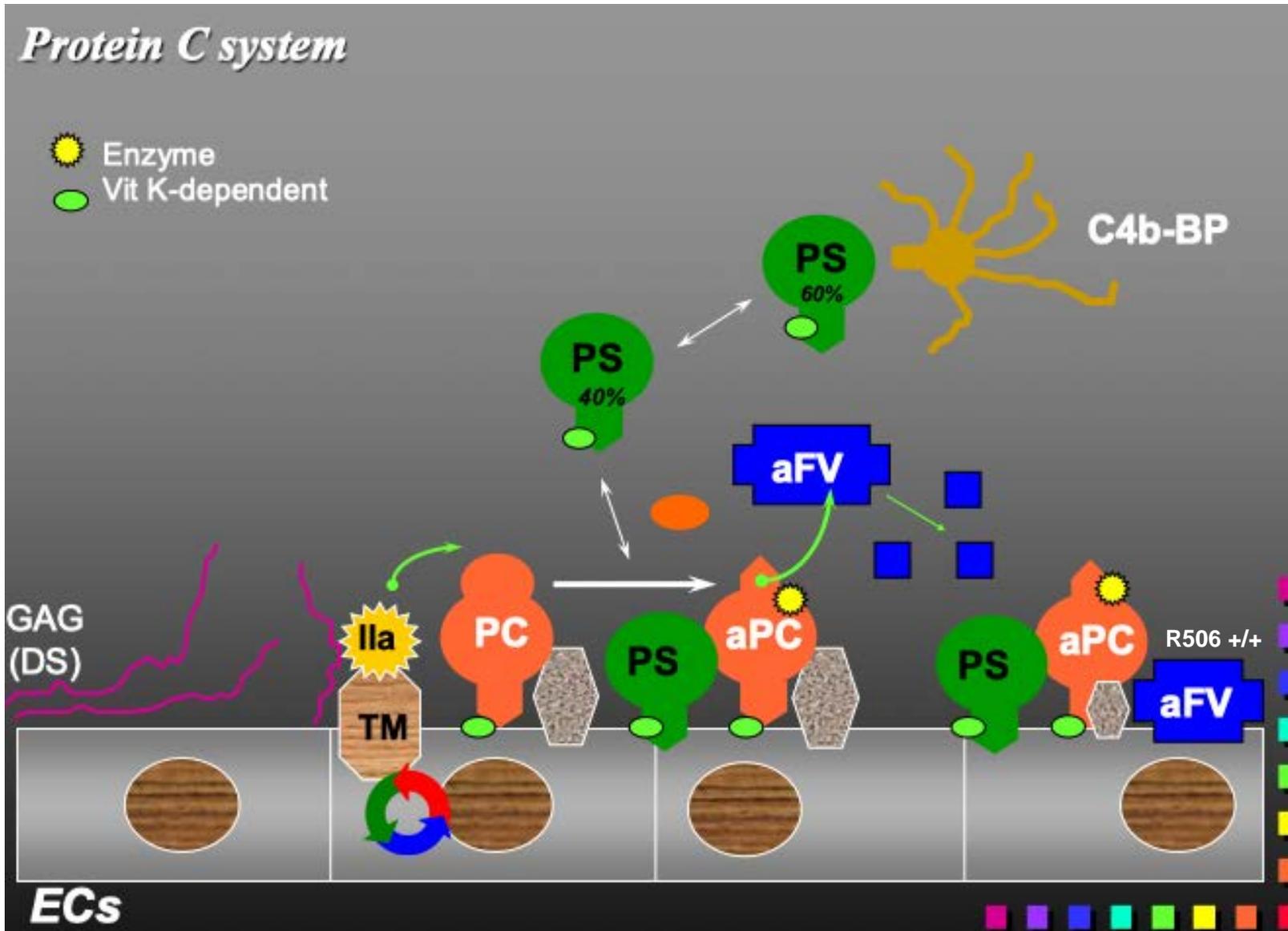


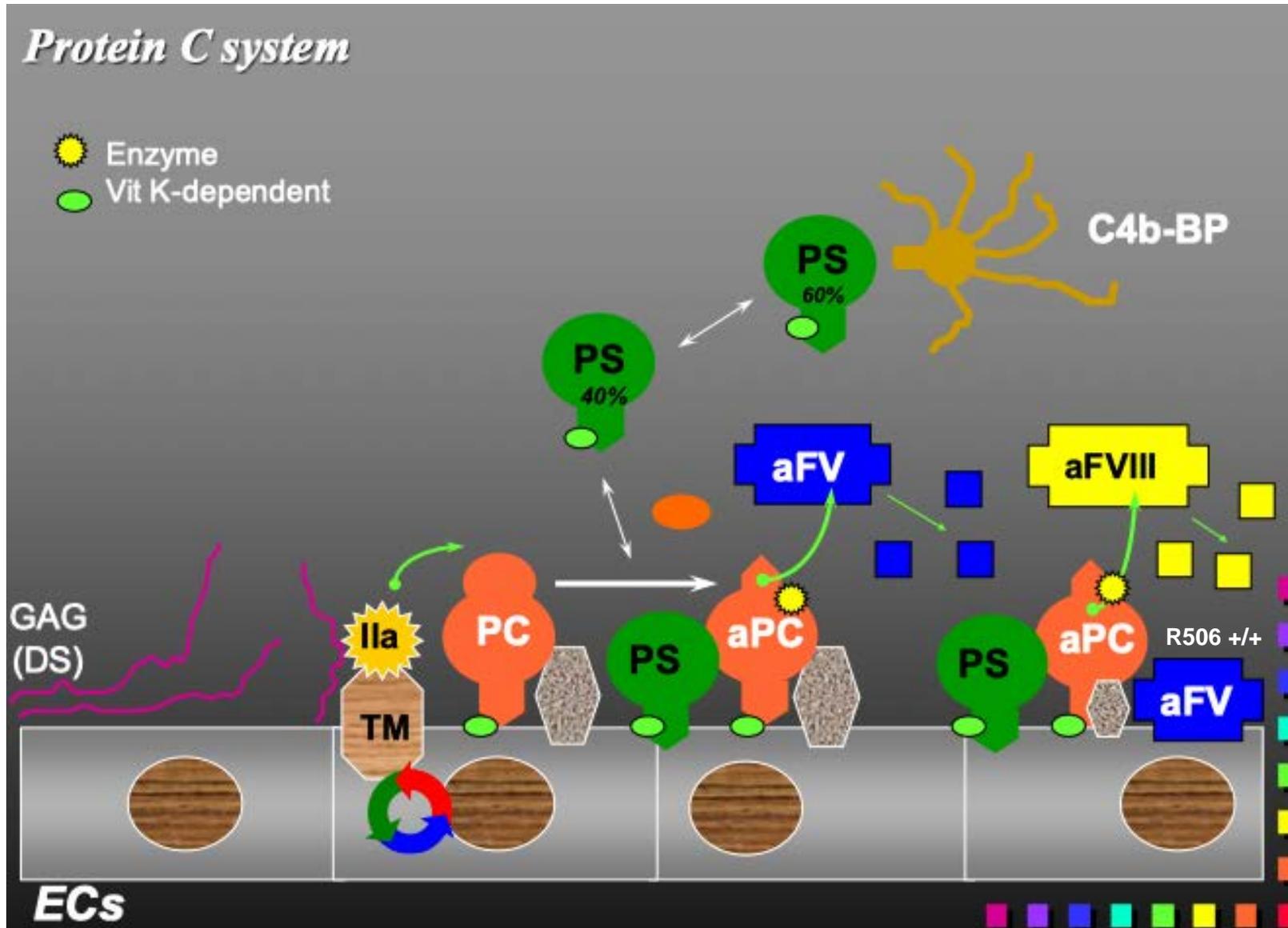






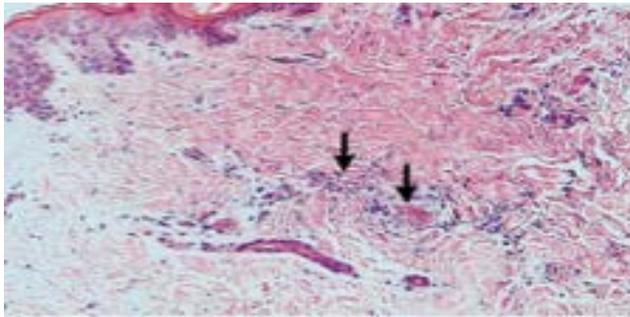




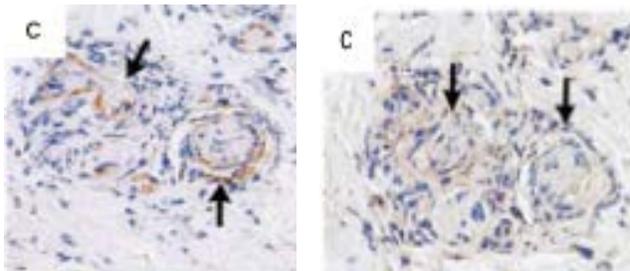


N Engl J Med 2001;345(6):408-16.

Skin lesions in meningococcaemia:



Thrombosed dermal microvessels

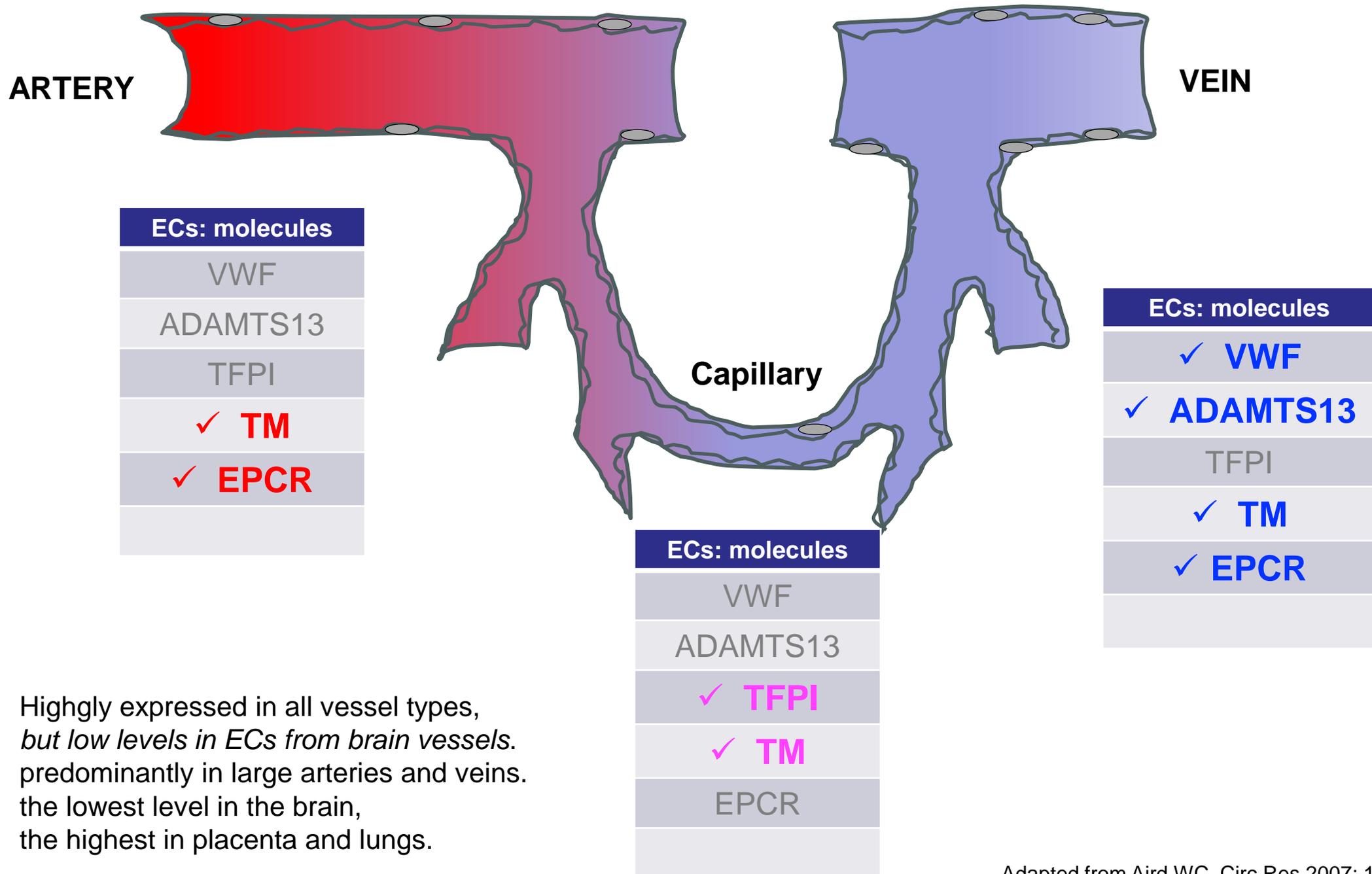


*Reduced immunostaining
for
TM and EPCR*

Skin necrosis also seen in...

- 1- Homozygous PC deficiency
- 2- Homozygous PS deficiency
- 3- Heterozygous PC deficiency starting VKA treatment
- 4- aPL Abs inhibiting the PC system

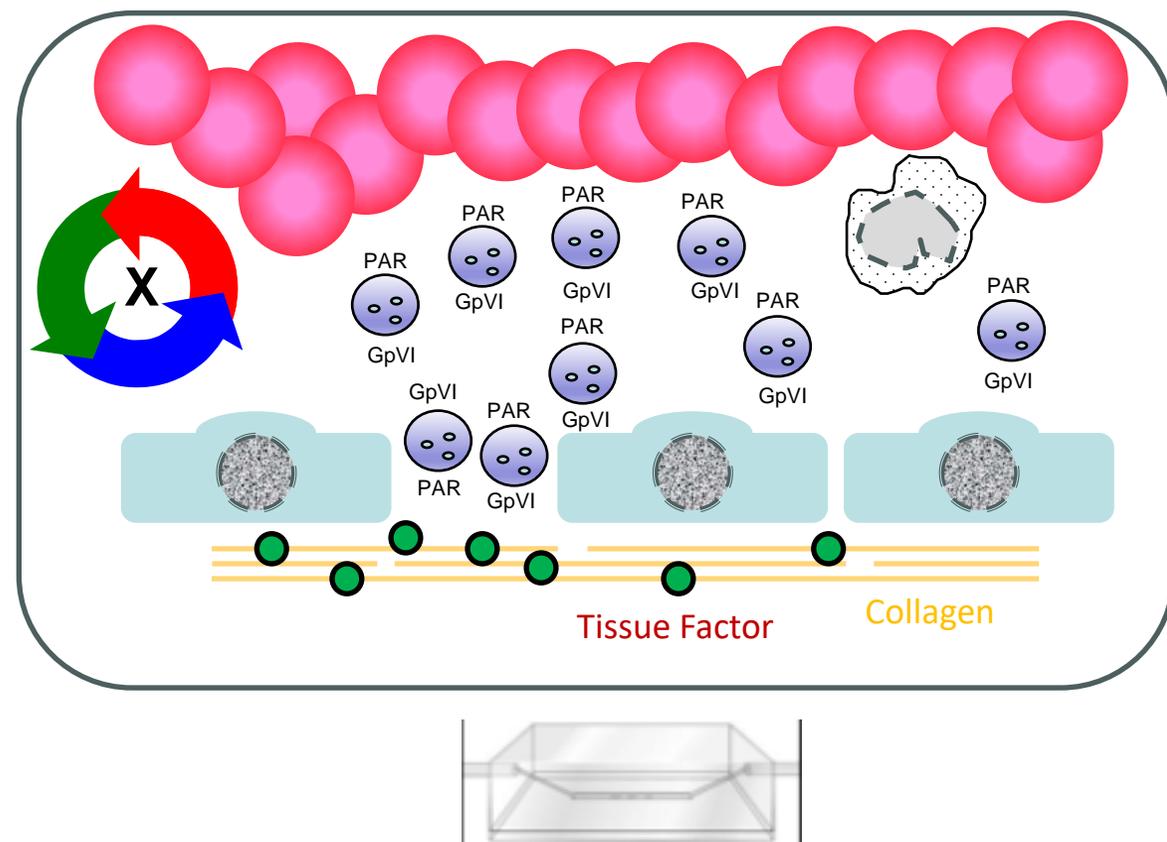
**The PC-PS-TM-EPCR system
is the dominant coagulation inhibiting system
in the microcirculation**



Localized endothelial-based control of platelet aggregation and coagulation under flow: A proof-of-principle vessel-on-a-chip study

Sanne L.N. Brouns | Isabella Provenzale | Johanna P. van Geffen |
Paola E.J. van der Meijden | Johan W.M. Heemskerk

Thromb Haemost. 2020;18:931-941



Vessel-on-a-chip system

with patches of HUVECs on thrombogenic surfaces;

the coagulant activity under flow is regulated by:

(a) the residual **exposure of trigger** (collagen / TF)

(b) the **endothelial glycocalyx**

(c) to a lesser degree the *endothelial thrombomodulin*.

a b

c

e

e

e

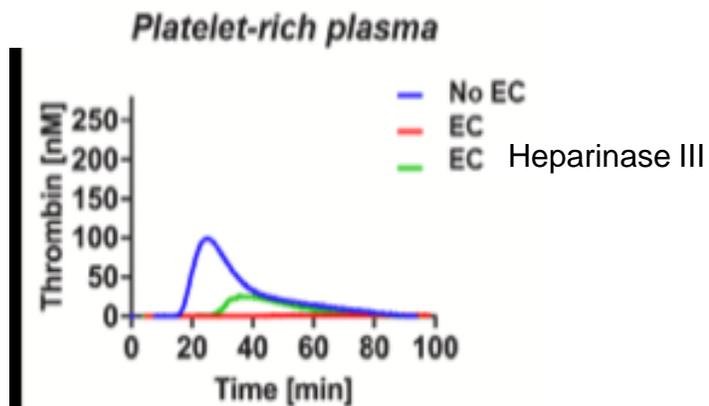
a c⁺e⁺

c

Thrombosis Research 244 (2024) 109208

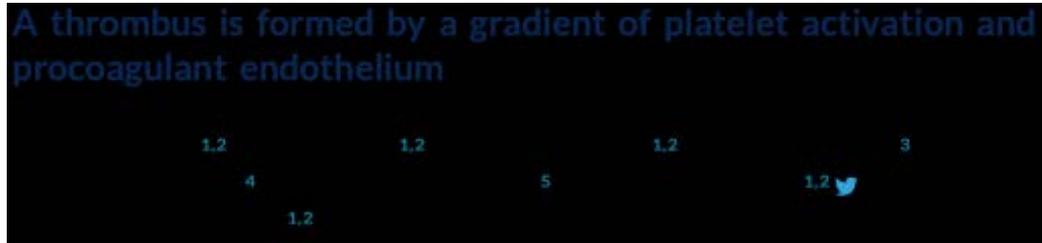
HUVECs cultured in 96-well plates for thrombin generation measurement in whole-blood or plasma systems

- 1- **ECs strongly delay and suppress thrombin generation induced by low-dose TF** (whole blood, platelet-rich plasma and platelet-poor plasma)
- 2- **ECs' anticoagulant activity is dependent of GAGs and of AT**
- 3- **Inhibition of TFPI: revert the endothelial effect.**



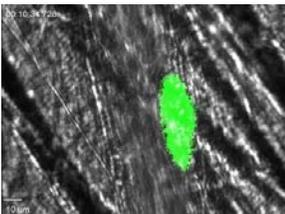
Additive anticoagulant mechanisms of HUVECs through surface-expressed heparin-like GAGs / AT and TFPI, both contributing to thrombin inhibition.

Endothelial-dependent or platelet-dependent procoagulant activity?

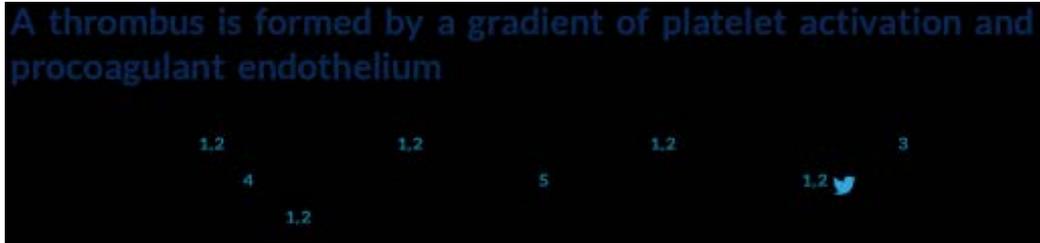


The « Furie model of thrombosis »

Mice,
 intravital video-microscopy of the cremaster muscle microcirculation,
 dye-based pulsed nitrogen laser endothelium activation,
 no endothelial lesion,
 independent of the interaction of collagen with platelets



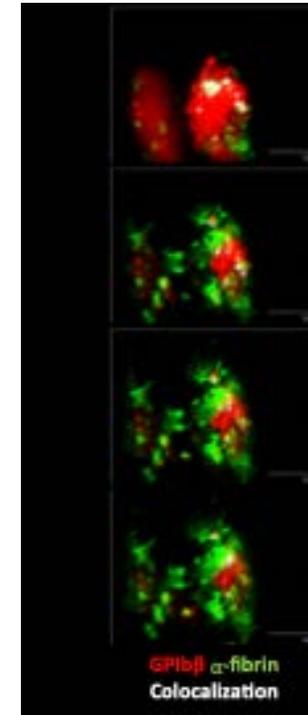
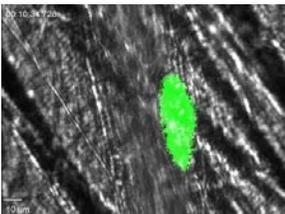
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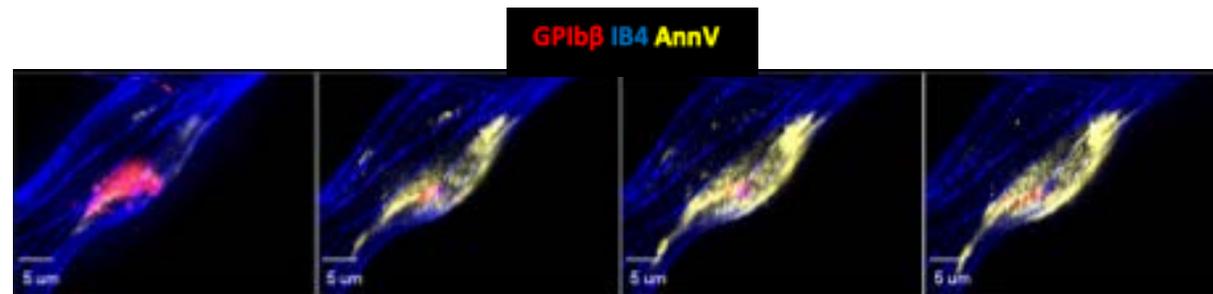
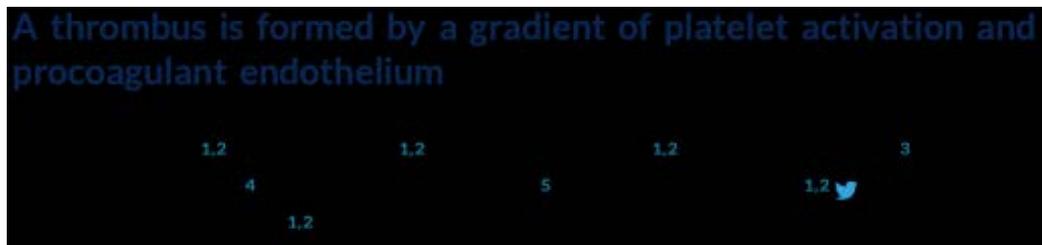
**Platelets are not necessary
for the activation of the coagulation cascade
and the generation of fibrin in vivo.**

The « Furie model of thrombosis »

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intravital video-microscopy of the cremaster muscle microcirculation,
dye-based pulsed nitrogen laser endothelium activation,
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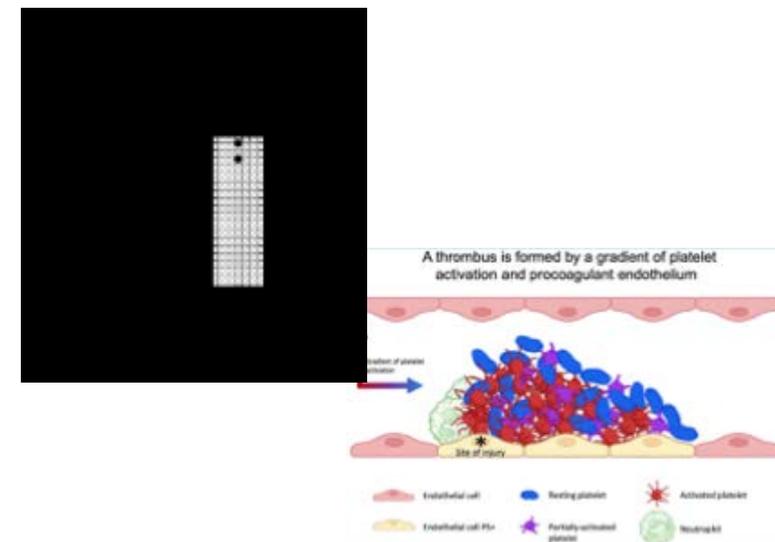
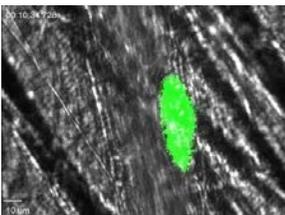
Endothelial-dependent or platelet-dependent procoagulant activity?



The activated endothelium (IB4) is the catalytic surface rich in negative phospholipids on which the activation of the coagulation cascade occurs

The « Furie model of thrombosis »

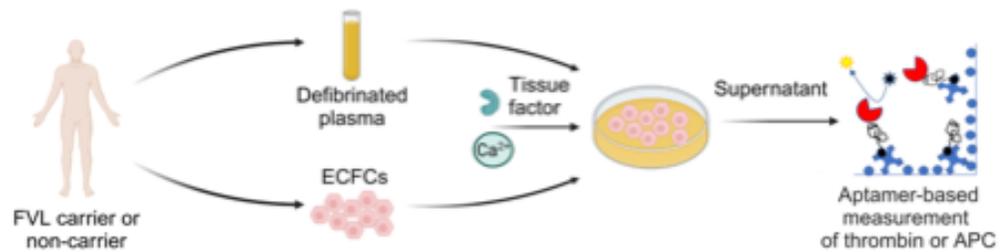
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Endothelium Modulates the Prothrombotic Phenotype of Factor V Leiden: Evidence From an Ex Vivo Model

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Arterioscler Thromb Vasc Biol. 2025;45:412–423.

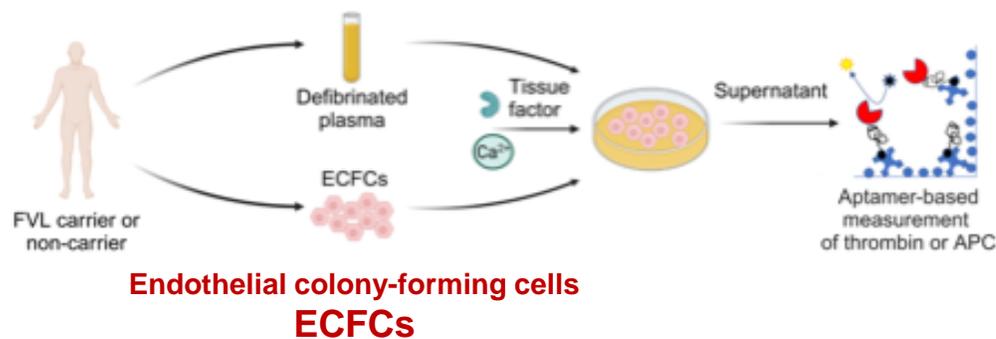


**Endothelial colony-forming cells
ECFCs**

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Autologous plasma + ECFCs

FVL pos plasma + ECFCs
from VTE neg individuals:

lower thrombin generation and *higher aPC generation*

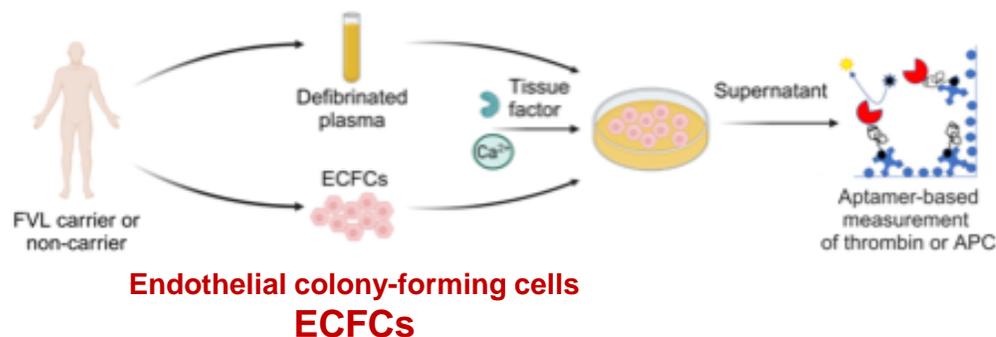
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Autologous plasma + ECFCs

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Cross-over experiments:

increased aPC reponse rates

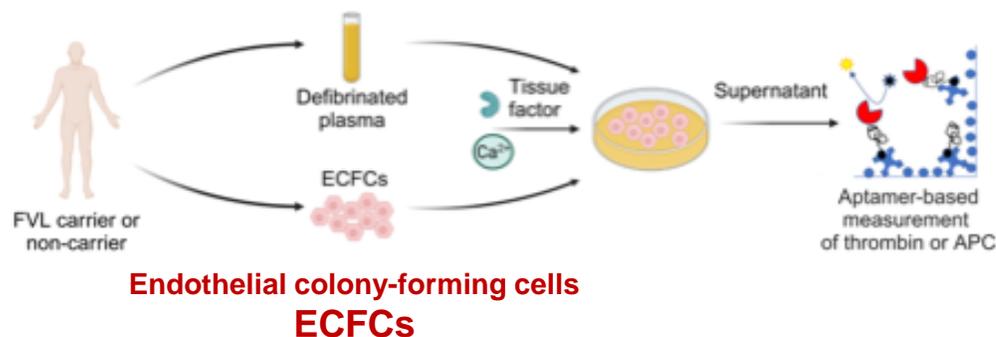
using ECFCs from VTE neg, FVL pos individuals,
regardless of the plasma component.

No difference on THBD and EPCR expression.

Endothelium Modulates the Prothrombotic Phenotype of Factor V Leiden: Evidence From an Ex Vivo Model

Nadine Schwarz, Jens Müller, Hannah L. McRae, Sara Reda, Behnaz Pezeshkpour, Johannes Oldenburg, Bernd Pötzsch, Heiko Rüh

Arterioscler Thromb Vasc Biol. 2025;45:412–423.



Evidence of an unidentified disease-modulating role of the endothelium in FVL carriers at the molecular levels

Autologous plasma + ECFCs:

FVL pos plasma + ECFCs
from VTE neg individuals:

lower thrombin generation and higher aPC generation

than

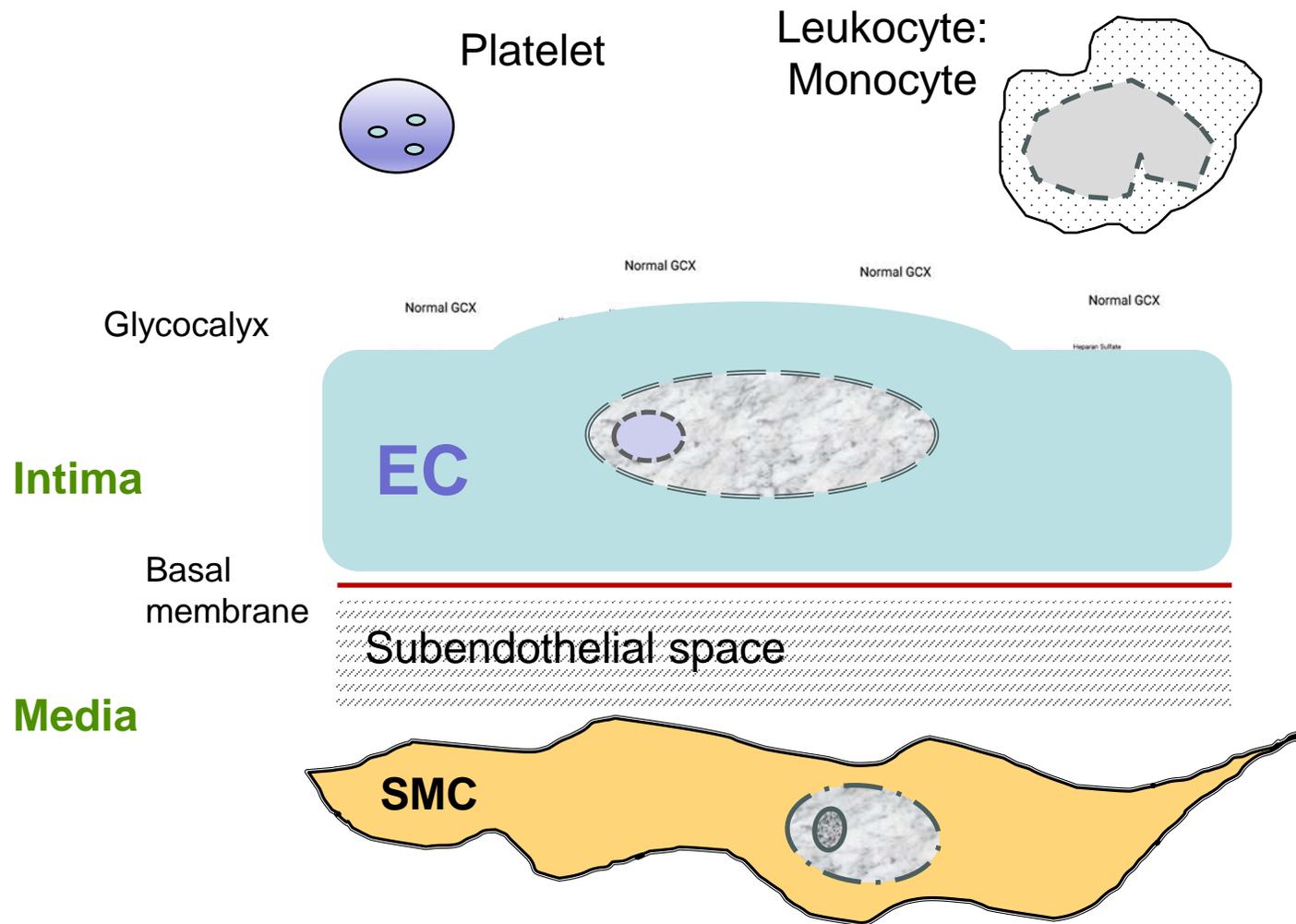
FVL pos plasma + ECFCs
from VTE pos patients.

Cross-over experiments:

increased aPC reponse rates

using ECFCs from VTE neg, FVL pos individuals, regardless of the plasma component.

No difference on THBD and EPCR expression.



Regulation (activation/inhibition):

Vascular tone (SMCs)

Primary haemostasis (platelets)

Coagulation

Fibrinolysis

ECs and Fibrinolysis

Activation

• Receptors

- Plasminogen receptors
 - Heterogeneous; Annexin A2, $\alpha V\beta_3$,...
- Receptors to Plasminogen activators
 - uPA receptors: uPARs, CD87 (*PLAUR*)
 - Glycosylphosphoinositol GPI anchor
 - tPA receptors
 - Annexin A2; cerebral microvascular ECs >>>

• Plasminogen activators PAs

- **Tissue-type: tPA**
 - ECs: WPbs and small storage
 - Gene: *PLAT*
- Urokinase-type: uPA
 - Gene: *PLAU*

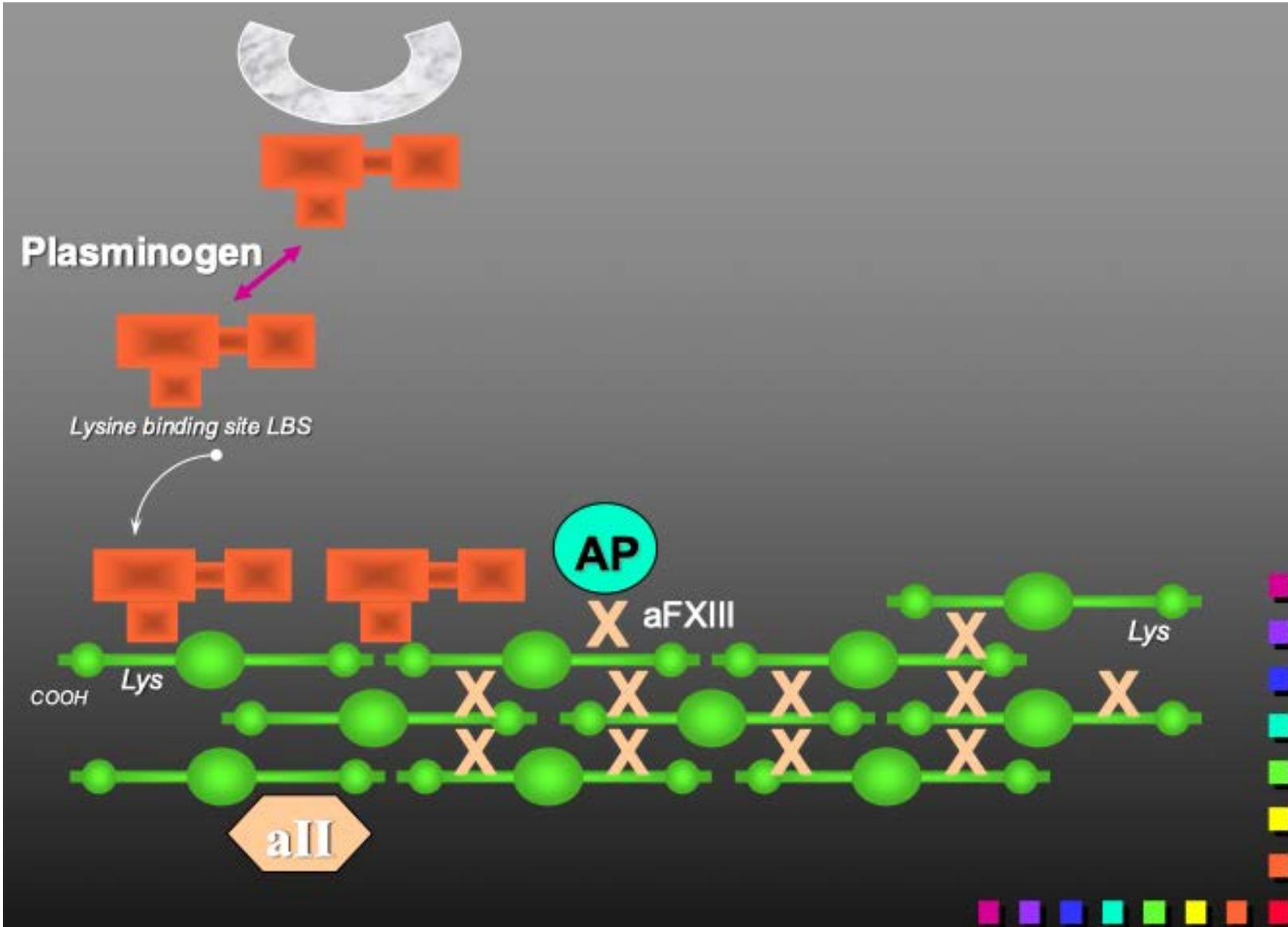
Inhibition

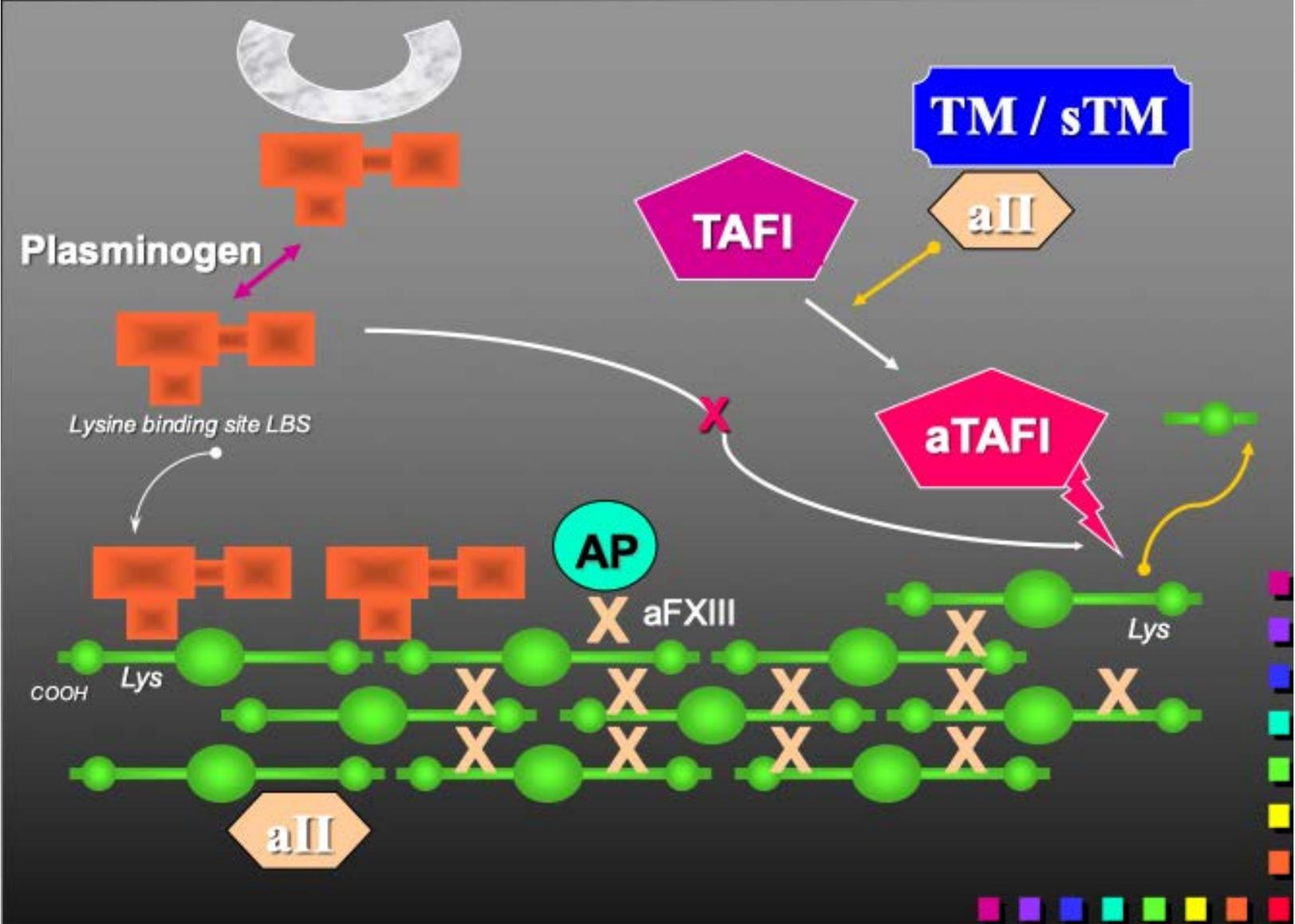
• THBD / sTHBD

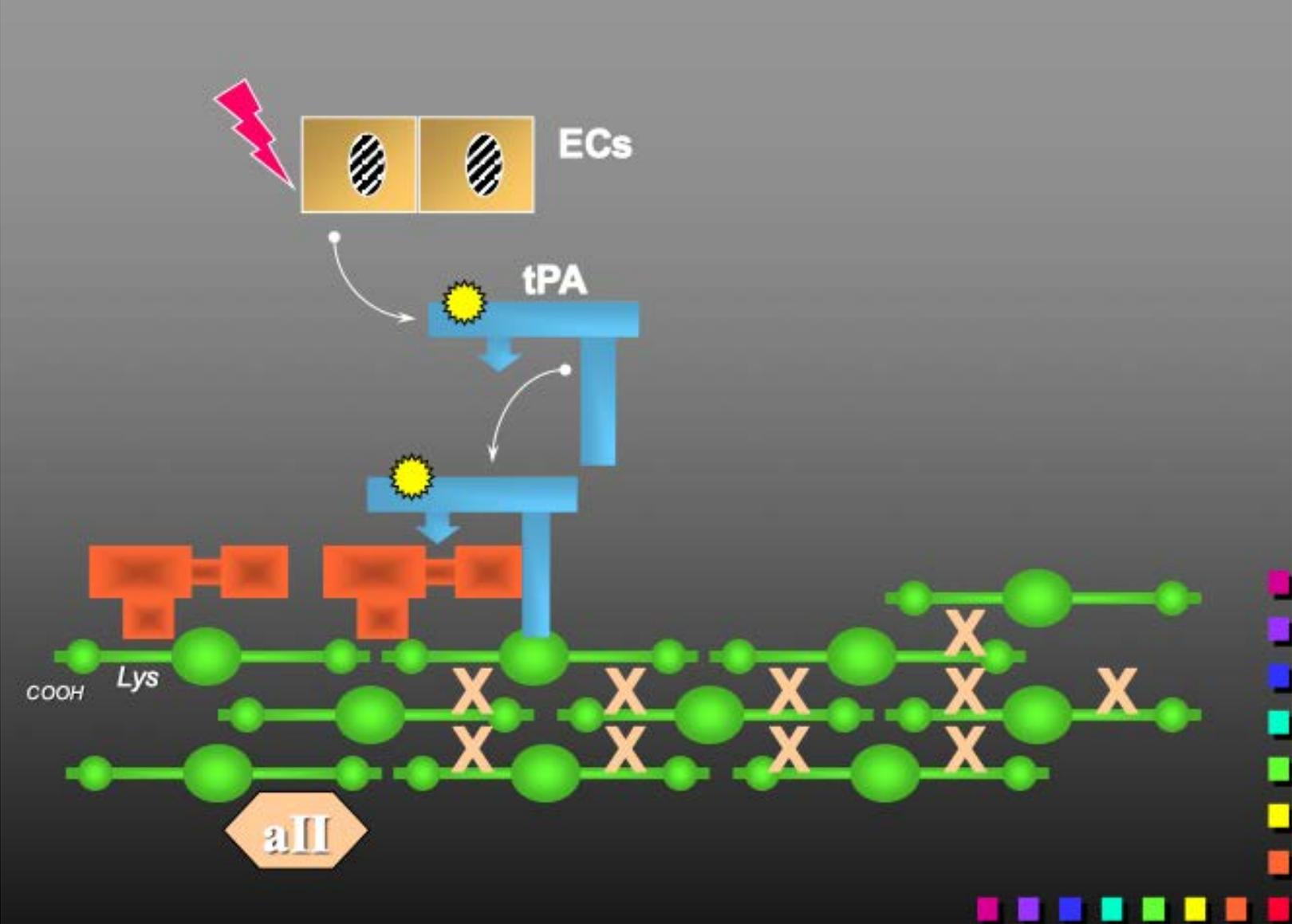
- Thrombin-mediated activation of **TAFI**
 - Removes C-terminal lysines from fibrin
 - No plasminogen binding and activation

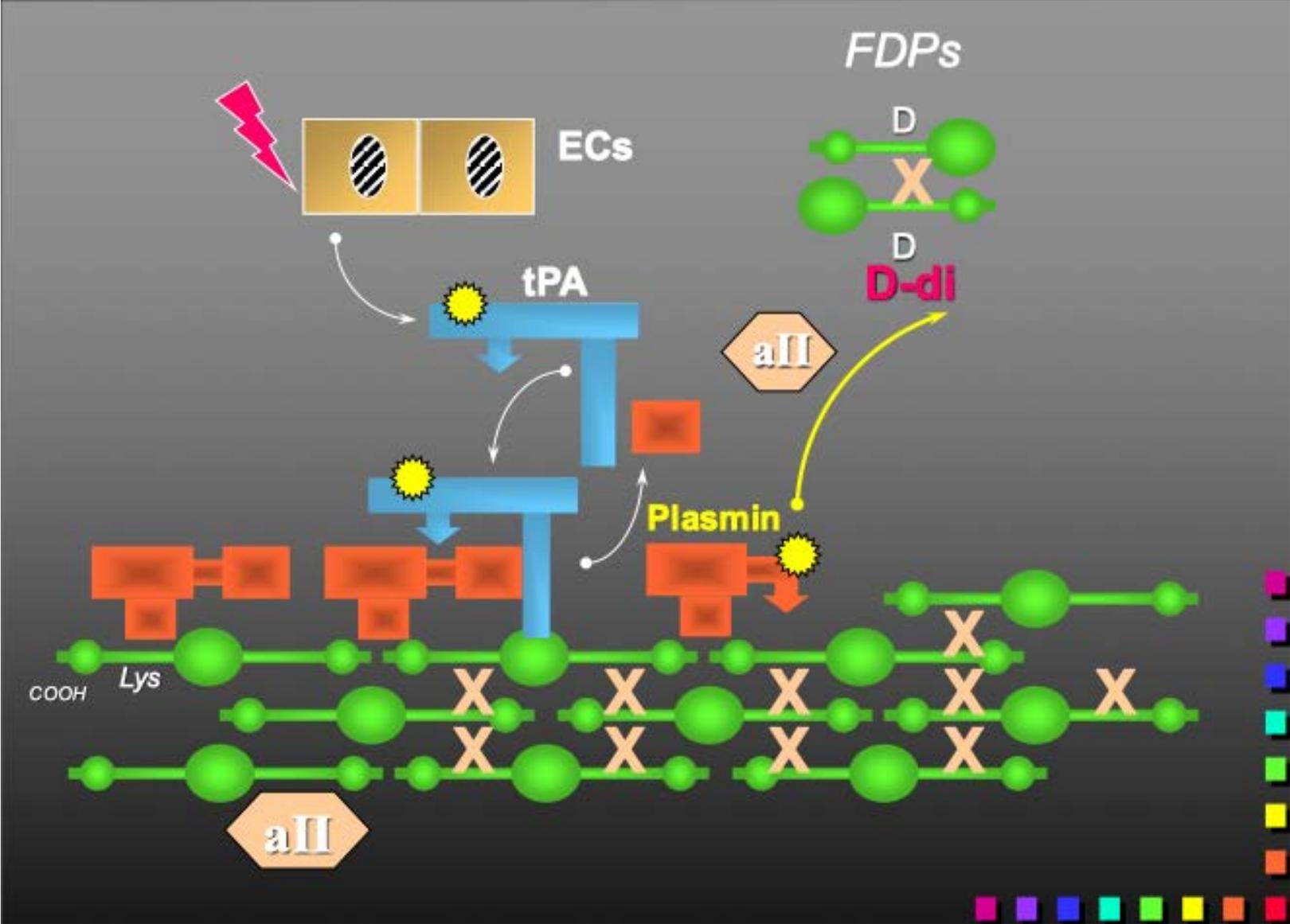
• Plasminogen activator inhibitors PAI

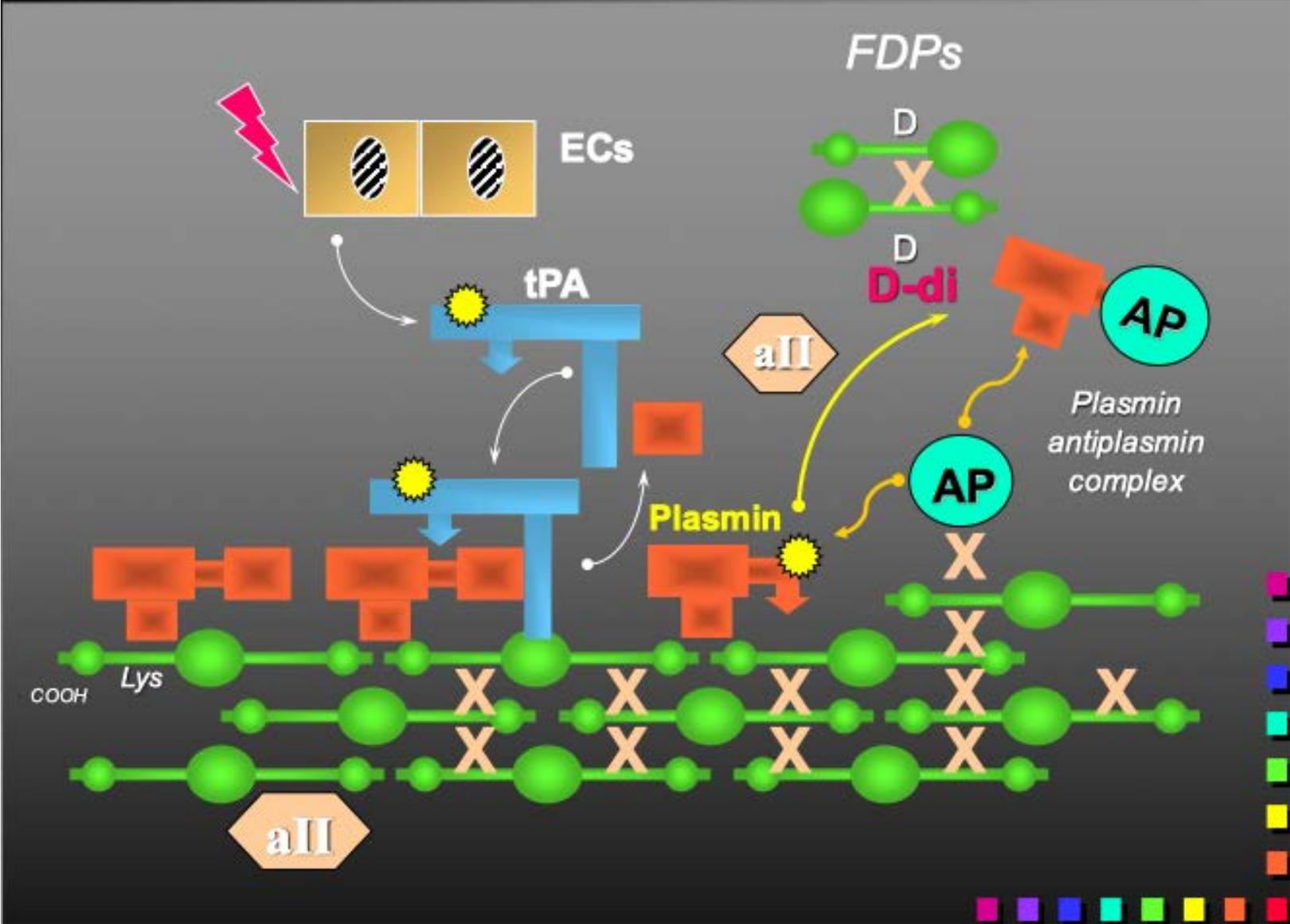
- **PAI-1**
 - Gene: *SERPINE1*

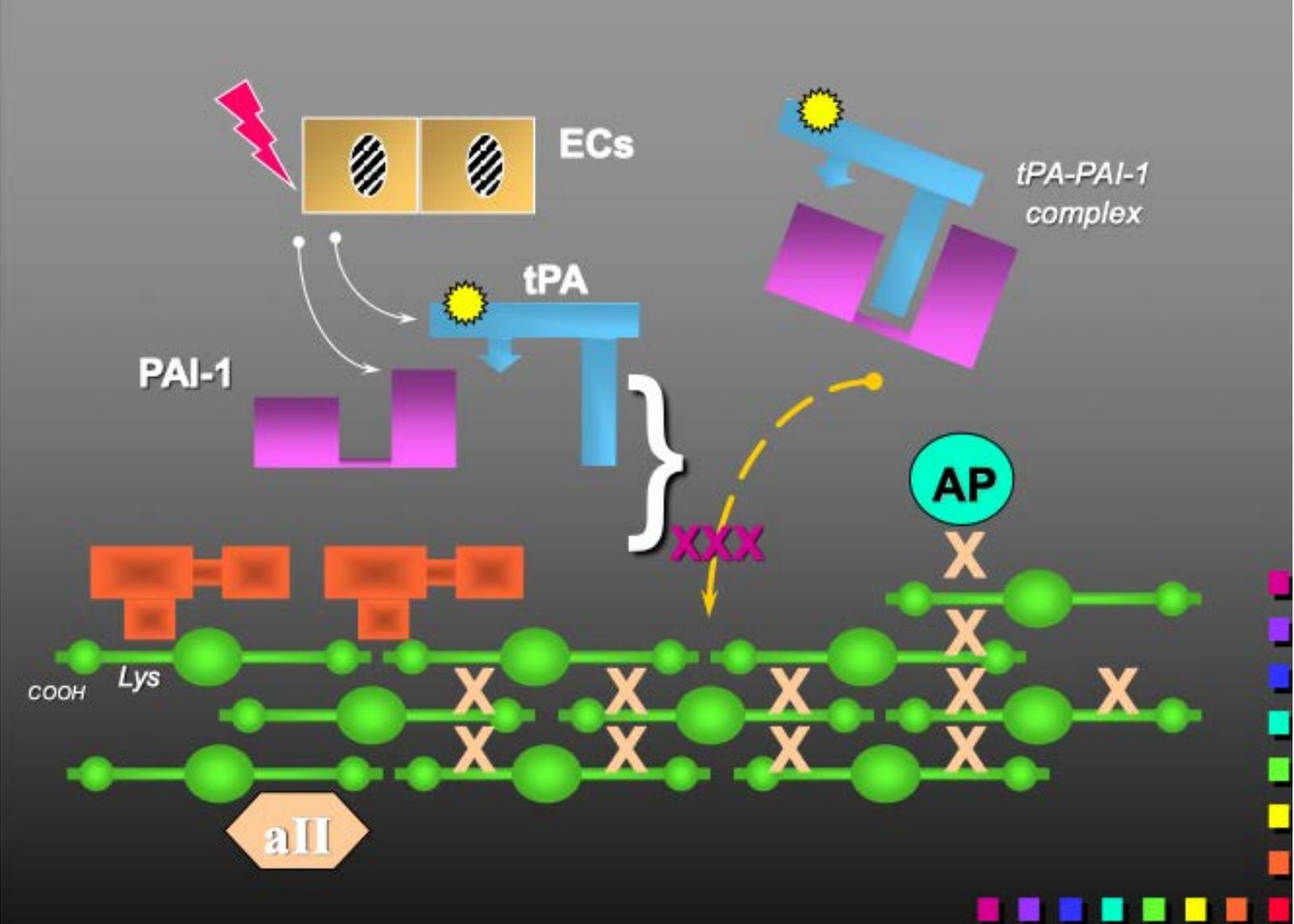


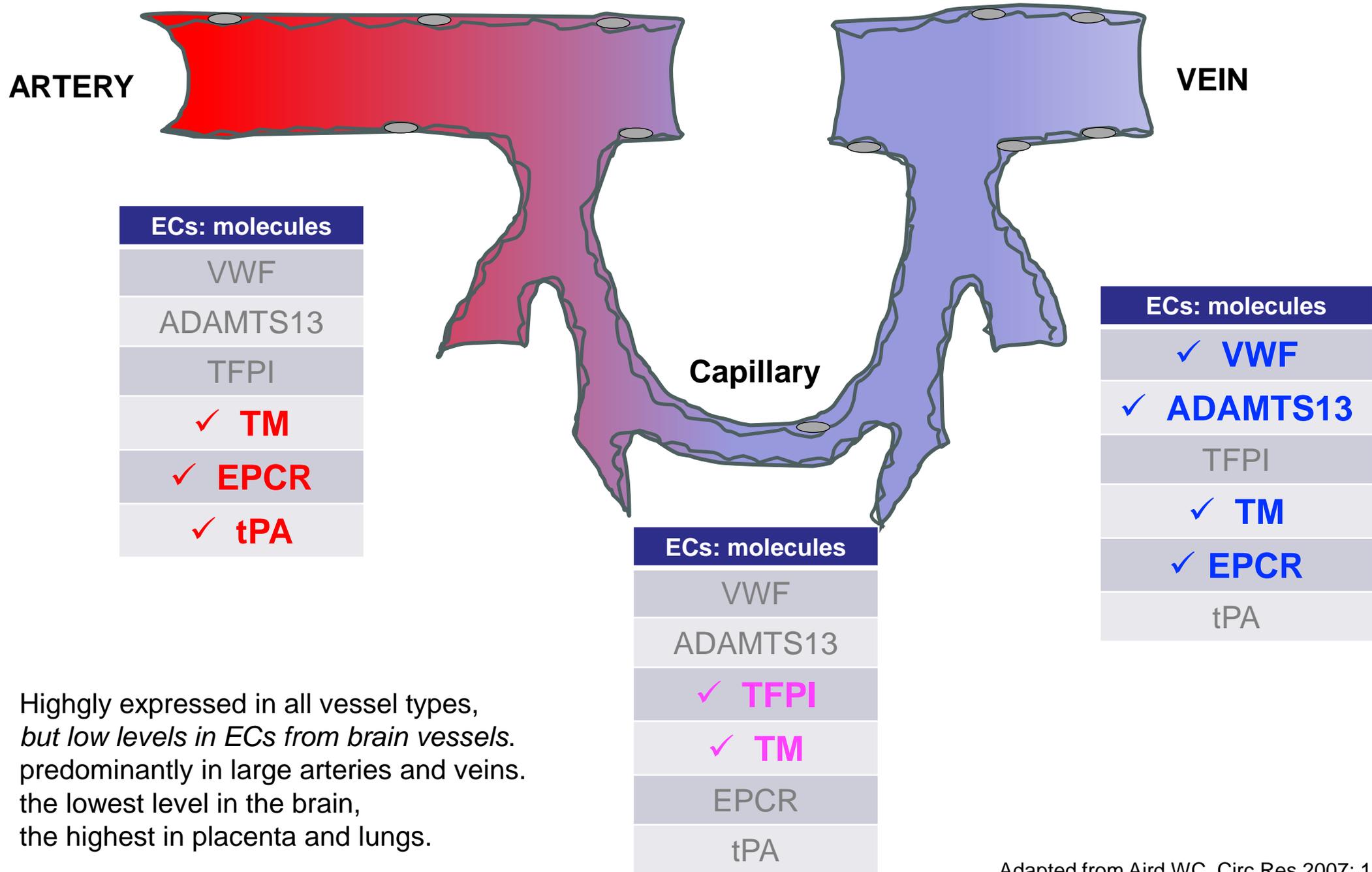












TM: Highly expressed in all vessel types, *but low levels in ECs from brain vessels.*

EPCR: predominantly in large arteries and veins.

TFPI: the lowest level in the brain, the highest in placenta and lungs.

ECs' receptors for the fibrinolytic system...

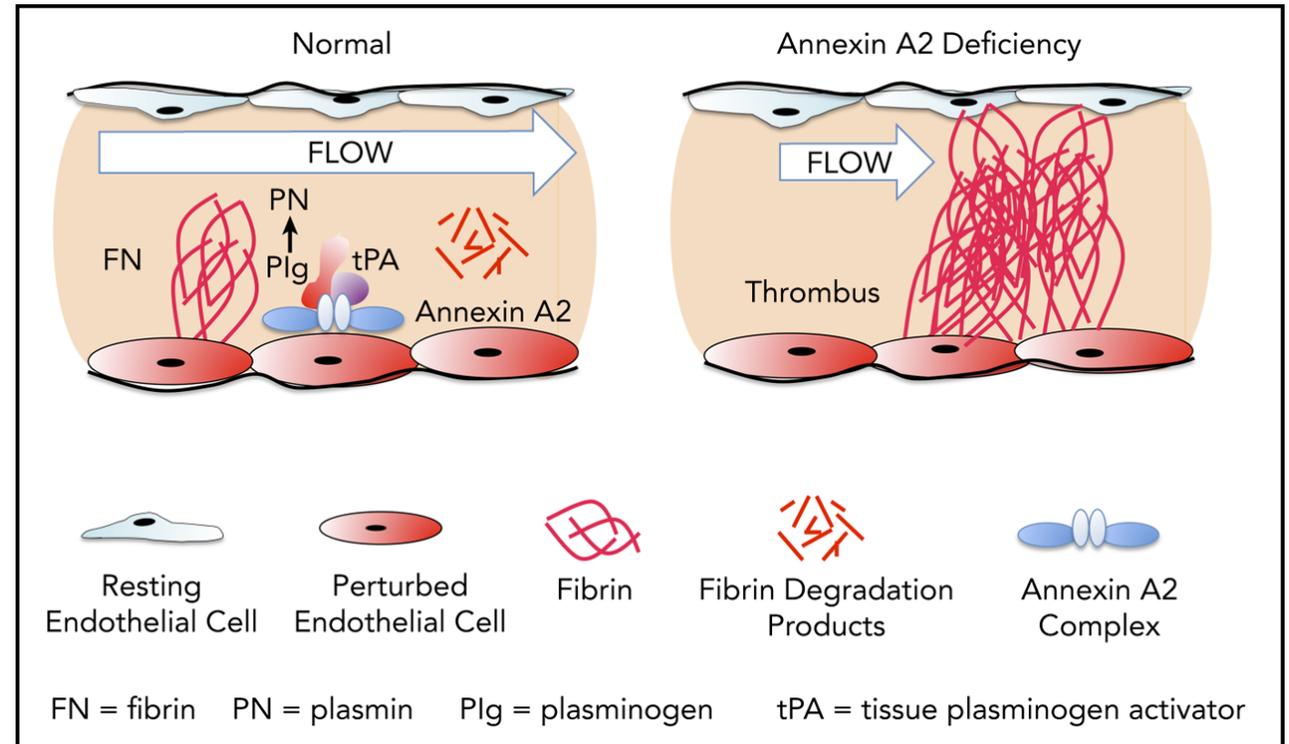
Reduced expression of annexin A2 is associated with impaired cell surface fibrinolysis and venous thromboembolism

Blood 2021; 137:2221-2230

40% decrease in cell-surface t-PA dependent fibrinolytic capacity
Annexin A2 decreased in average by 70%

Adults with VTE vs. healthy controls

Annexin A2 expression and function
on blood mononuclear cells
« as a surrogate of ECs »



Any haemostatic role for the ECs-derived extracellular vesicles (EVs)?

ECs-derived extracellular vesicles (EVs)



0.1-1 μm

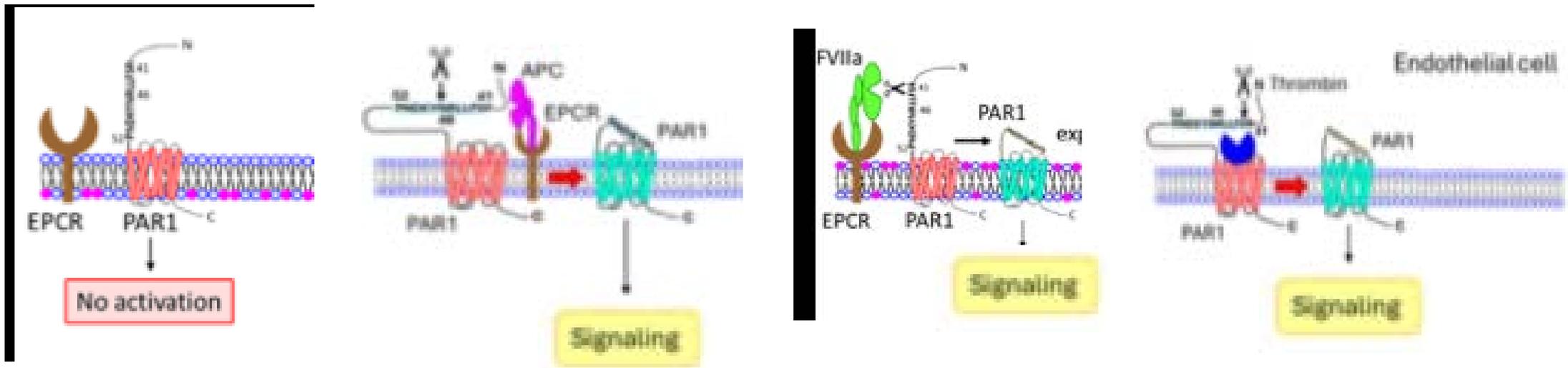
Normal whole blood, ECs-derived EVs (PECAM-1 pos, $\alpha\text{v}\beta\text{3}$ pos):
10% of the total population; around 40,000 per μL
(Combes V *et al.*, J Clin Invest 1999;104:93-102)

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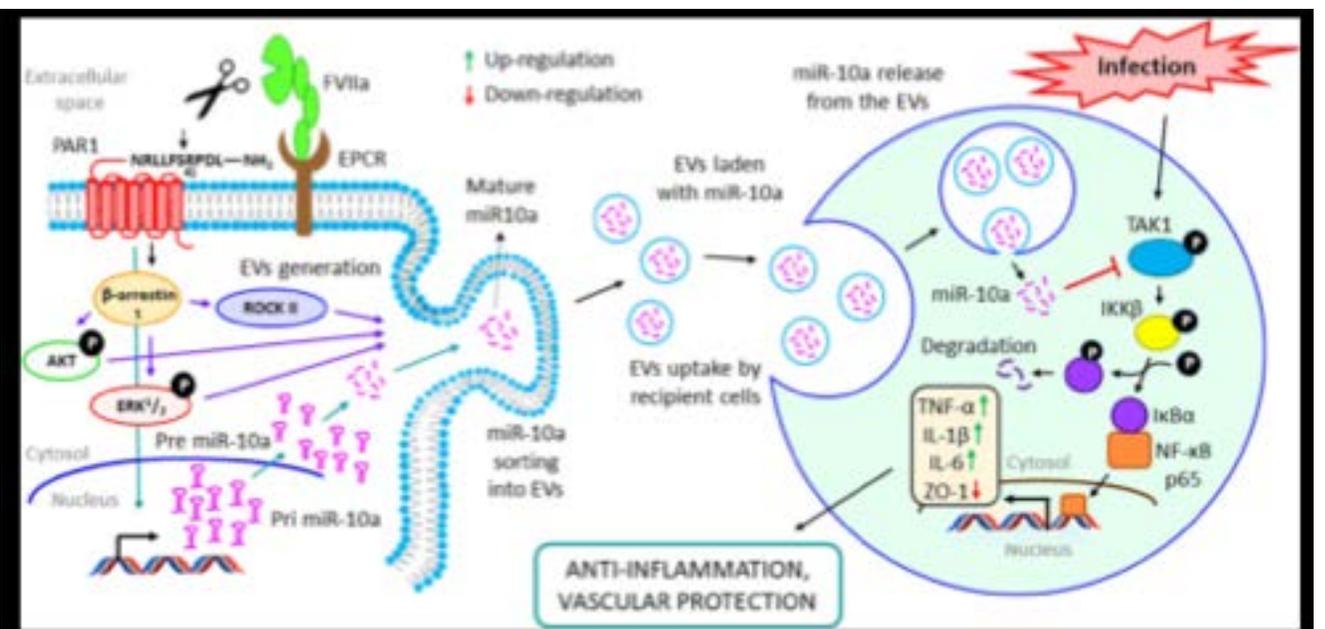
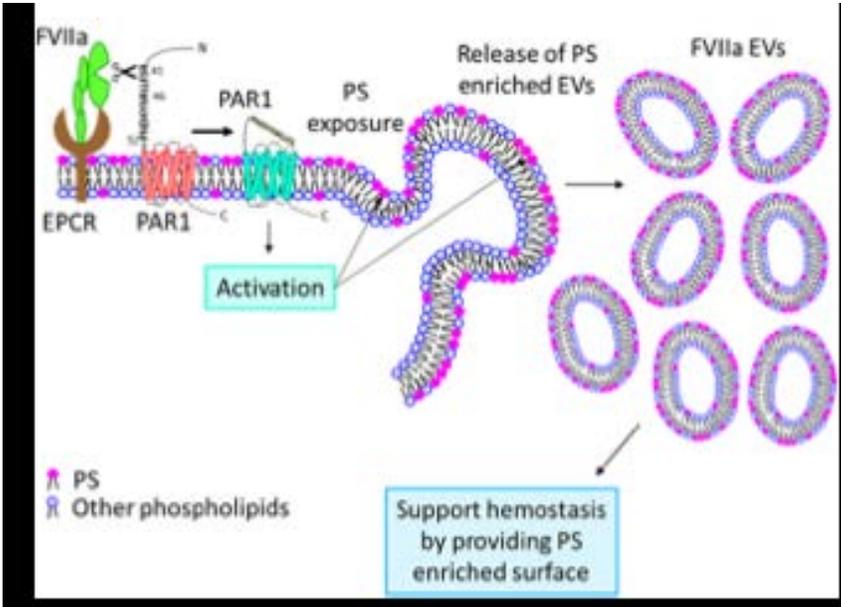
Coagulation proteases (aPC, FVIIa, FXa, thrombin):
release EVs from ECs...*in vitro*;
induce **specific signaling pathways**
via activation of ECs' protease-activated receptors PARs





J Thromb Haemost 2024;22:2976–2990

A complex interplay between **activation of haemostasis** and **vascular protection**; *FVIIa-induced EVs:*

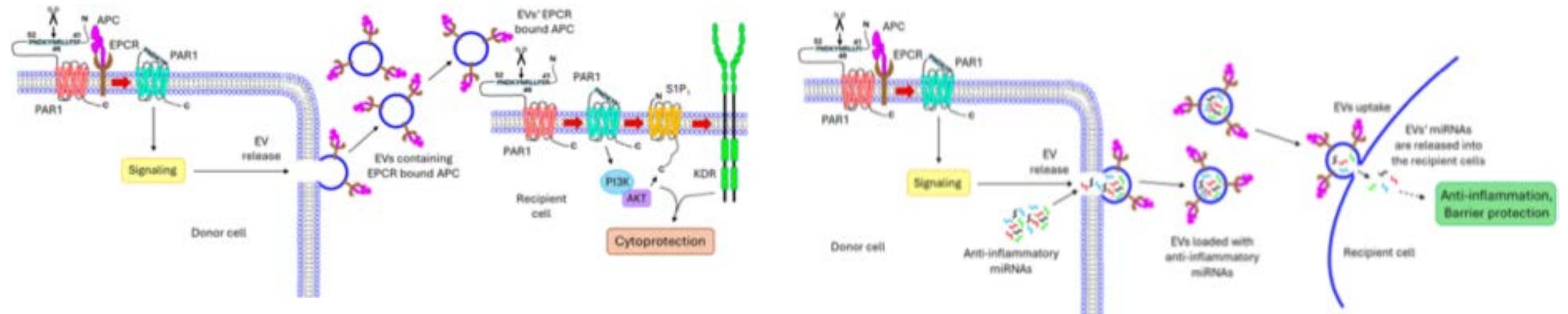


Participation to the endothelial procoagulant activity

Cytoprotective effects via microRNA10a (miR10a)



A complex interplay between *protease-dependent* and *protease-independent* effects;
aPC-induced EVs:

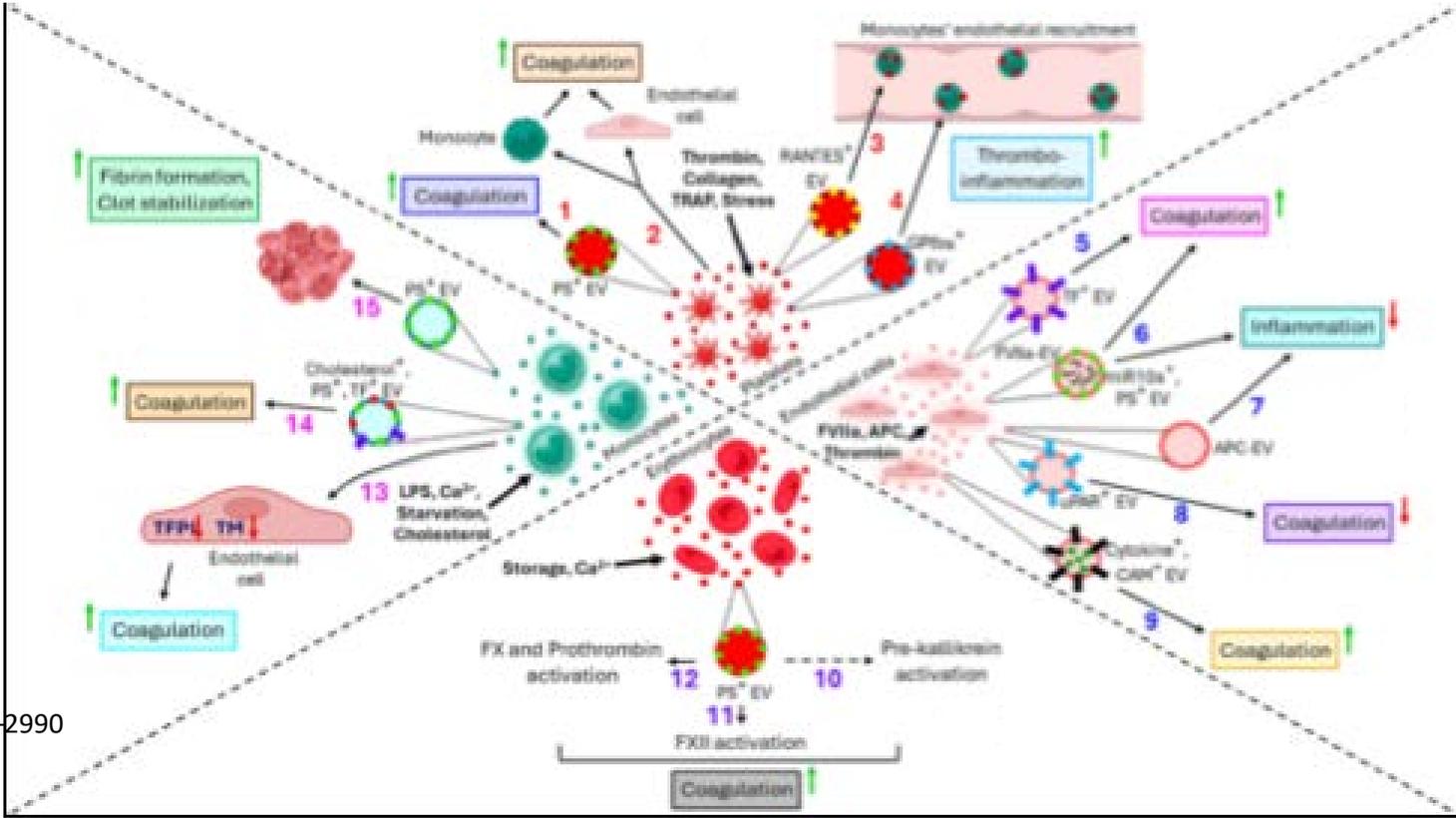


aPC-dependent effect

aPC-independent effects

More globally:

EVs originated from different blood cells can apparently impact on ECs and haemostasis /vascular biology
(platelets, monocytes, erythrocytes and endothelial cells).



J Thromb Haemost 2024;22:2976–2990

Very complex...
relevant clinical situations need to be individually studied.

Role of endothelium-derived vascular thiol isomerases VTIs?

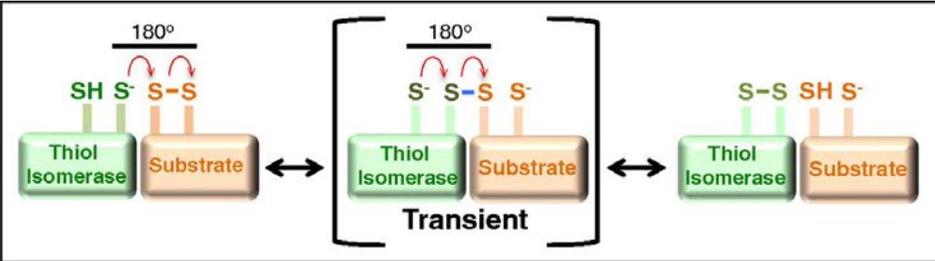
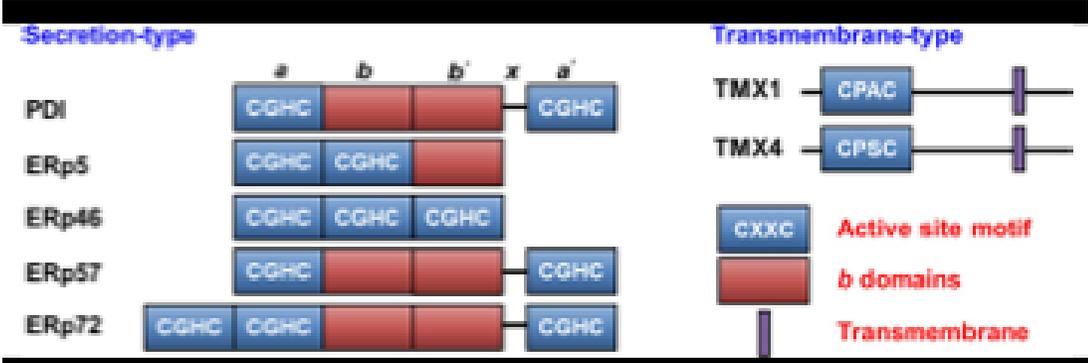
Vascular Thiol Isomerases (VTIs):

enzymes mainly localized within the endoplasmic reticulum;

ECs: localized to vesicles distinct from WPBs.

Oxidoreductase, isomerase, and chaperone functions.

Facilitate the formation of native disulfide bonds in newly synthesized proteins.



Vascular injury:

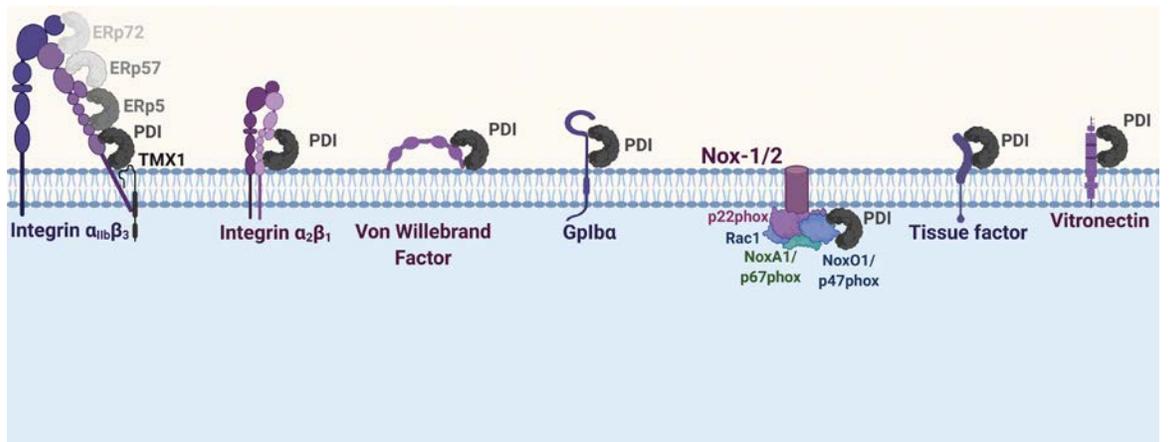
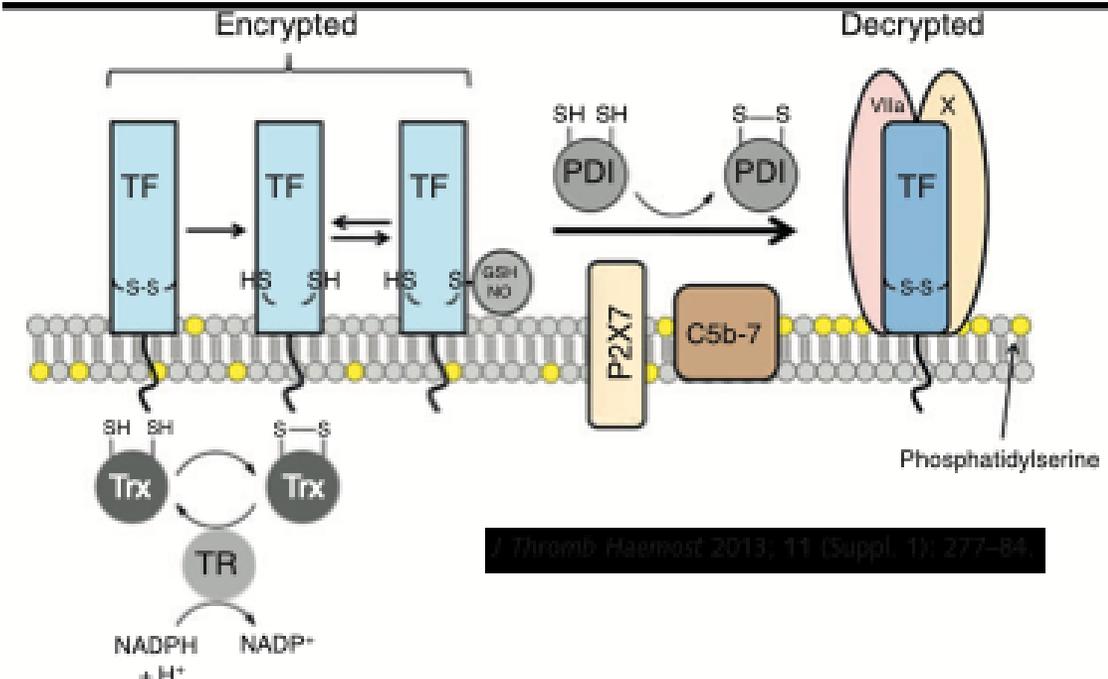
VTIs are released by **ECs** and platelets onto cell surfaces and into circulation;

significant influence on thrombus formation in animal models

by modulating the activities of vascular cell receptors, adhesive proteins and coagulation factors.

One vascular thiol isomerase: Phosphodiesterase PDI

- directly modulates the activation of coagulation factors:
 - Tissue factor (*TF decryption*), factor XI and factor V.
- Regulates platelet and endothelial cell-dependent coagulant activity, adhesive properties,...

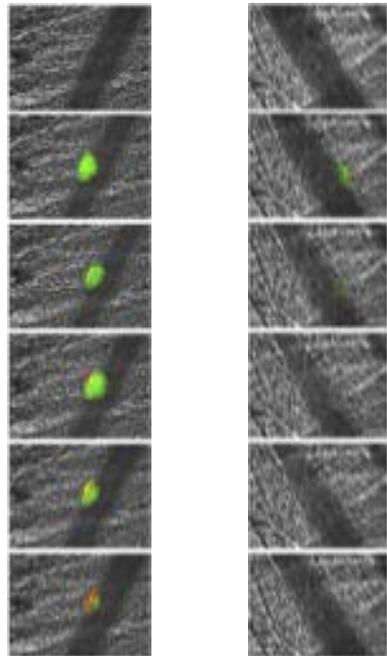


Prothrombotic targets of DPI

TF: mostly in a non-coagulant or cryptic form;
 acute events lead to local decryption of TF and FX activation.
 (myeloid cells: activation of a purinergic receptor or complement system
 decrypt TF with the help of PDI)

Endothelium-derived but not platelet-derived protein disulfide isomerase is required for thrombus formation in vivo
 Heema Jasuja,^{1,2} Bruce Furie,^{1,2} and Barbara C. Furie^{1,2}

Blood 2010;116:4665-74.



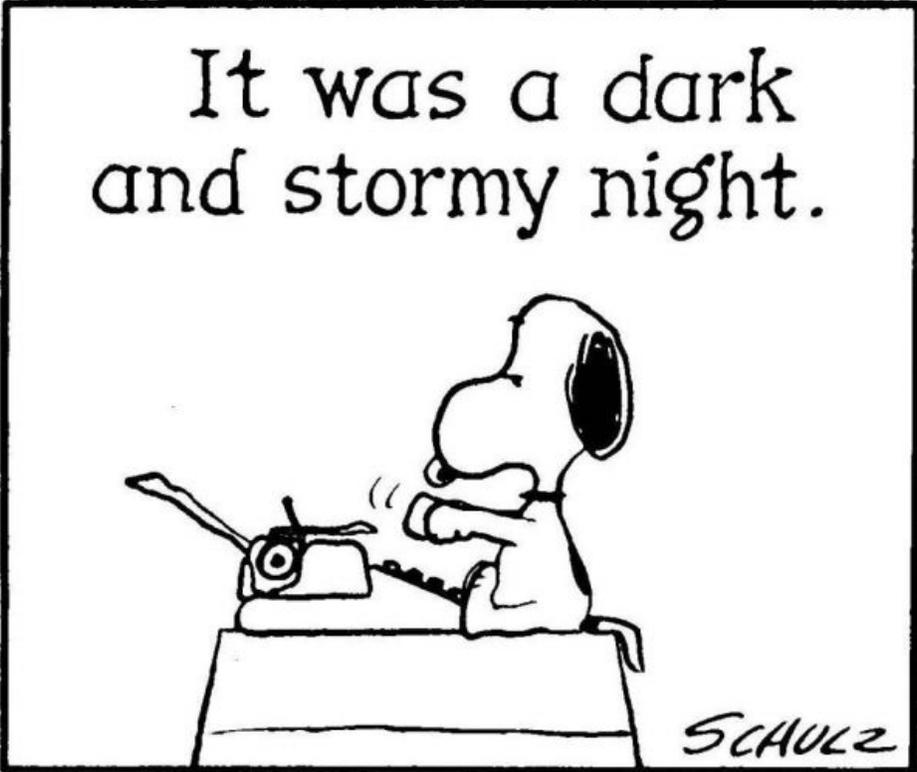
PDI: green
 Fibrin: Red
 Platelet inhibitor
 Anti-PDI mAb

Although both platelets and endothelial cells secrete PDI after laser-induced injury, **PDI from endothelial cells is required for fibrin generation in vivo.**

B. Furie, Exp Rev Hematol 2018; 11:437-48:

« We suggest that absence of extracellular vascular thiol isomerases in the circulation suppresses thrombus formation »

« The thiol isomerase system provides the regulatory switch by which the normal vasculature is protected under physiologic conditions from generation of thrombi »



Conclusion

Endothelial cells and Haemostasis

- A major component of haemostasis, *usually told in a global, caricatured way.*

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- The clinical impact of this variability in function is still difficult to assess.
- “Markers” tested in a peripheral venous blood sample cannot easily reflect this intra-organ and inter-organ variability
- Standardised dynamic tests for stimulation/modulation of the endothelium/haemostasis link *are lacking*.
- Understanding and being able to explore localised, constitutional or acquired anomalies in the endothelium/haemostasis link in relation to thrombotic risk and haemorrhagic risk *is the major clinical challenge of tomorrow*.

University of Montpellier



University Hospital of Nîmes



Sechenov University, Moscow



- Haematology
- UMR 1318 INSERM
- Gynaecology Obstetrics
- Oncology

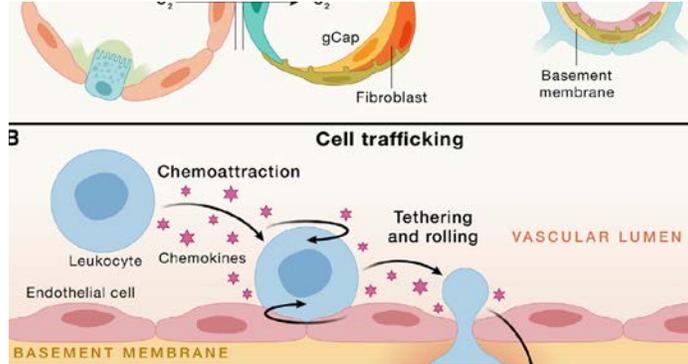
The NOHA network



- Intensive Care Unit
- Vascular Medicine
- Internal Medicine
- Clinical Research Unit

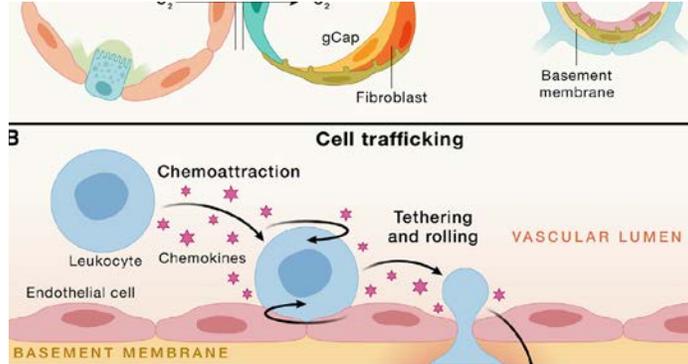
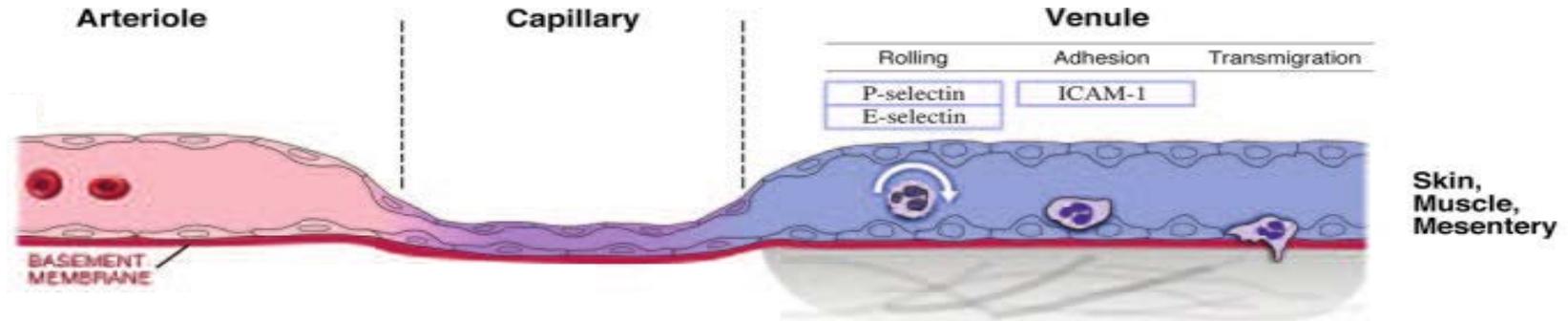
Example of inter-organ diversity:

*ECs
and
leukocytes
trafficking*



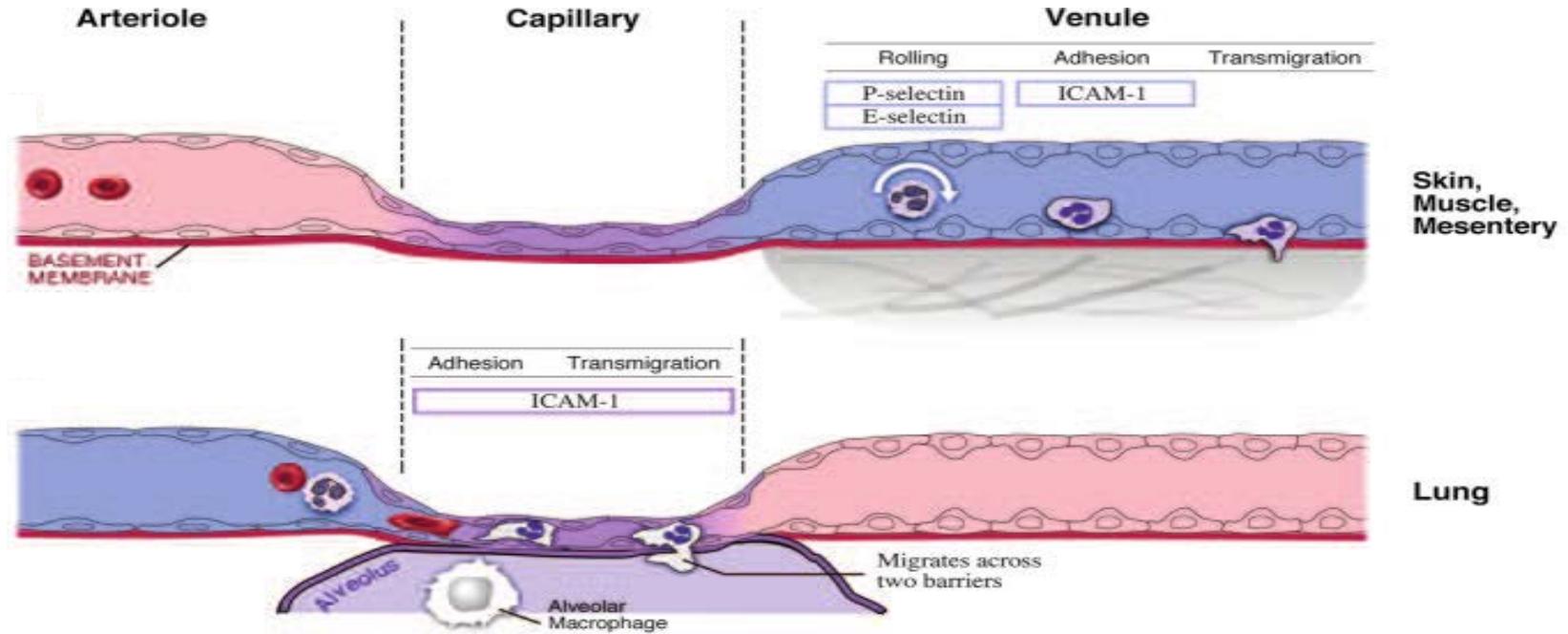
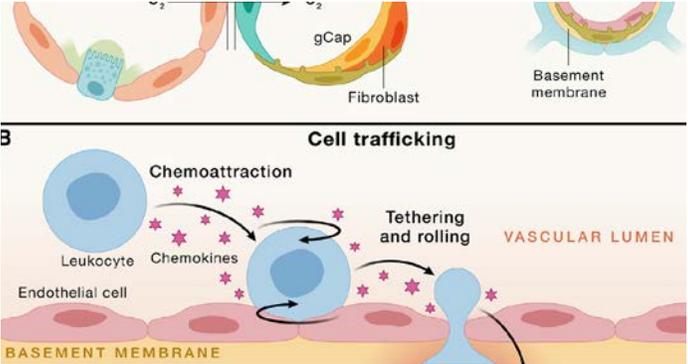
Example of inter-organ diversity:

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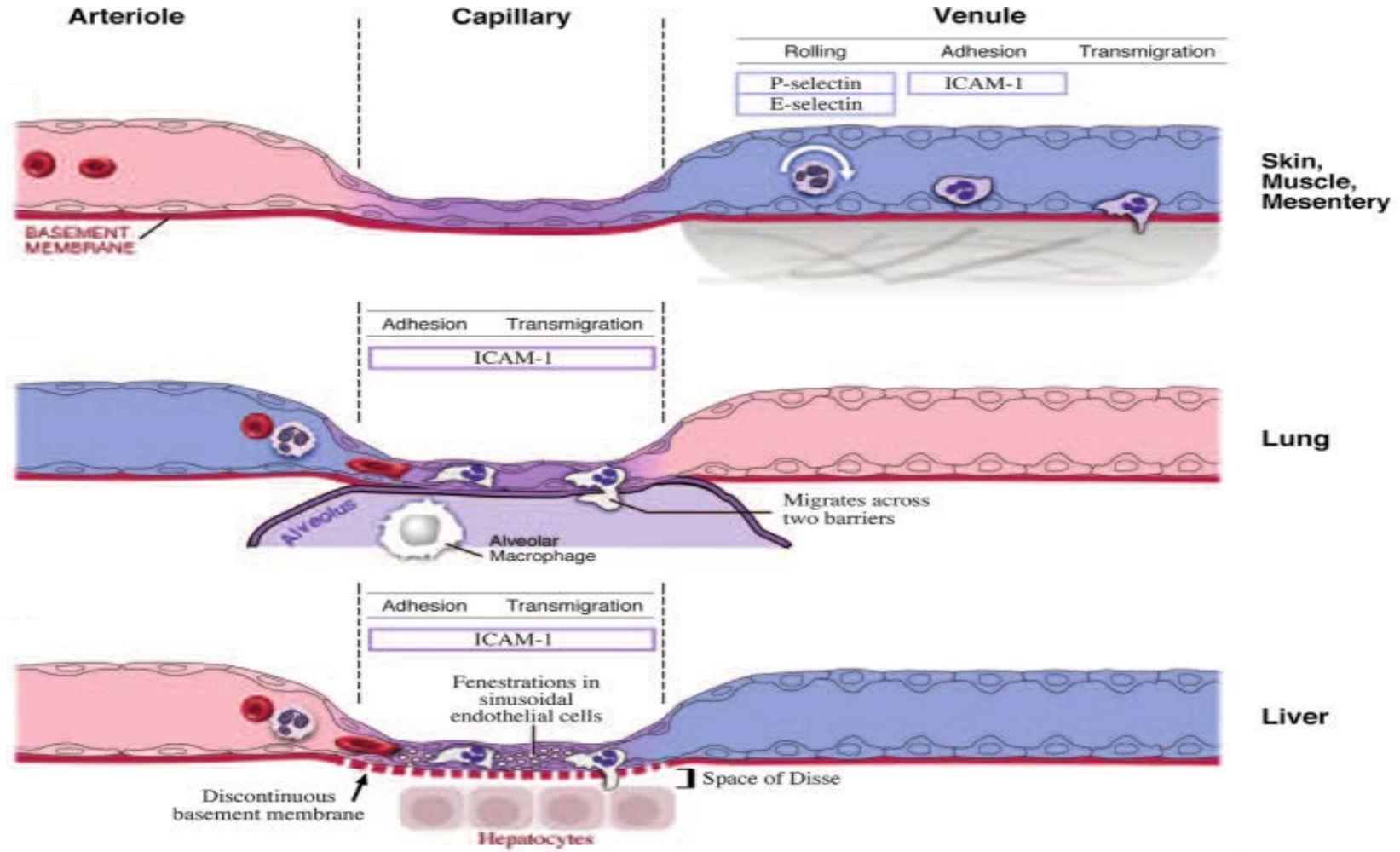
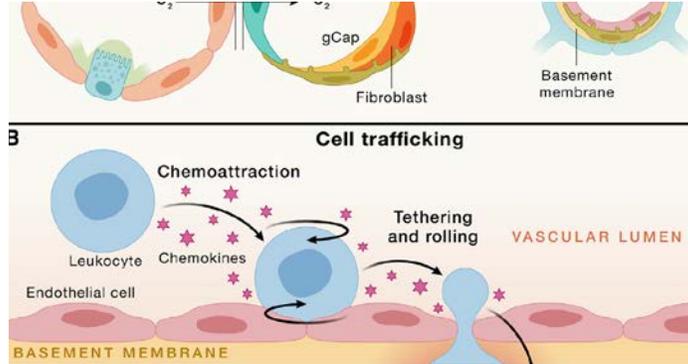
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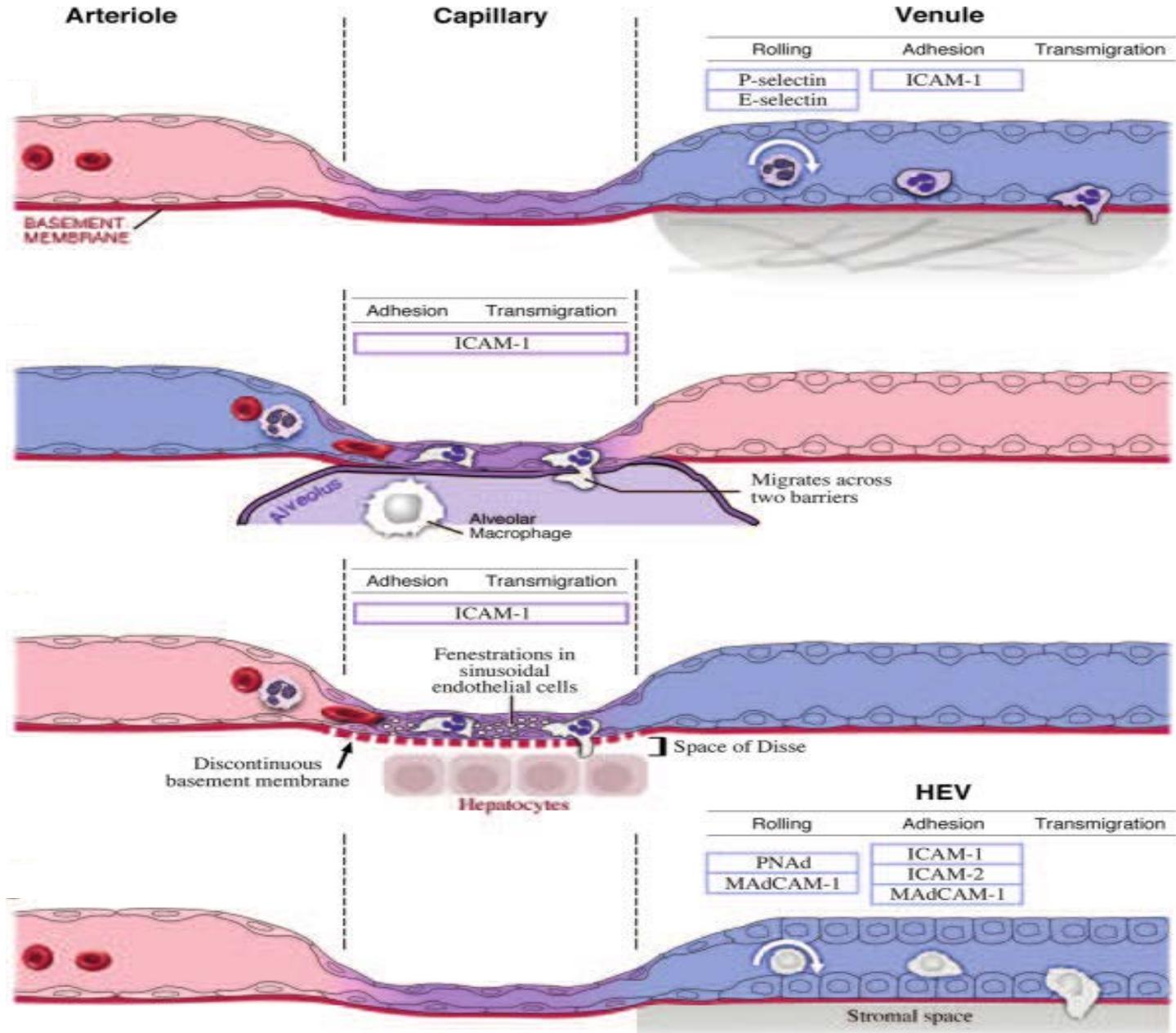
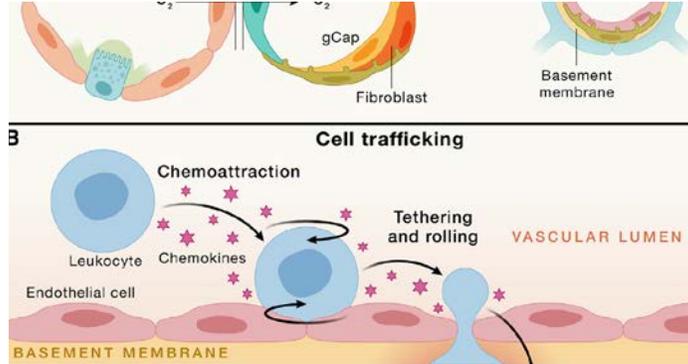
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Example of inter-organ diversity:

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Skin, Muscle, Mesentery

Lung

Liver

Mesenteric lymph node