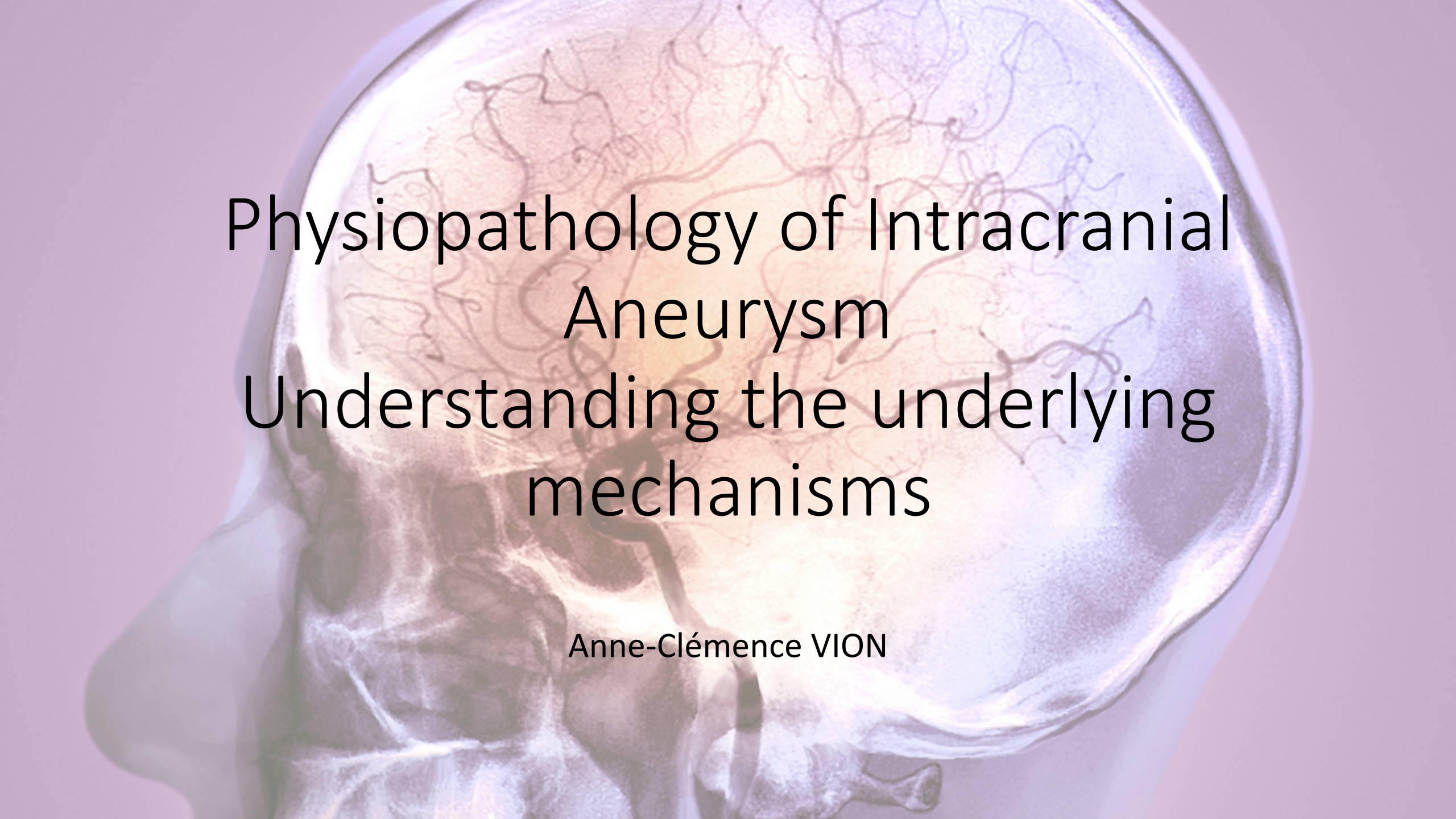


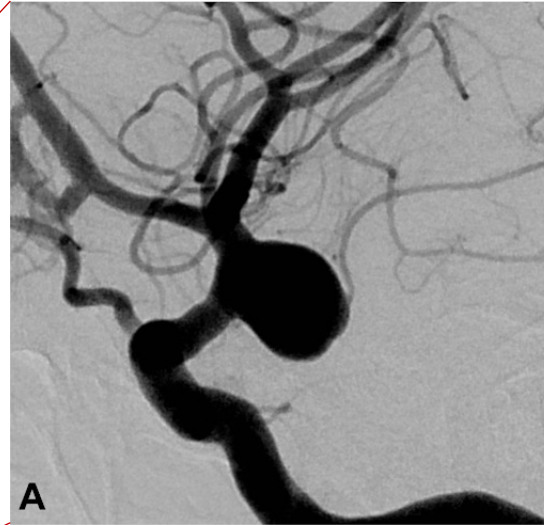
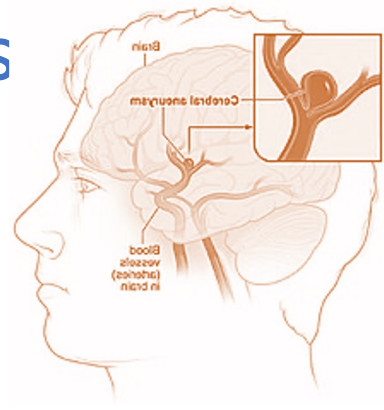


Physiopathology of Intracranial Aneurysm

Understanding the underlying mechanisms

Anne-Clémence VION

Intracranial Aneurysm : causes and consequences



- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture

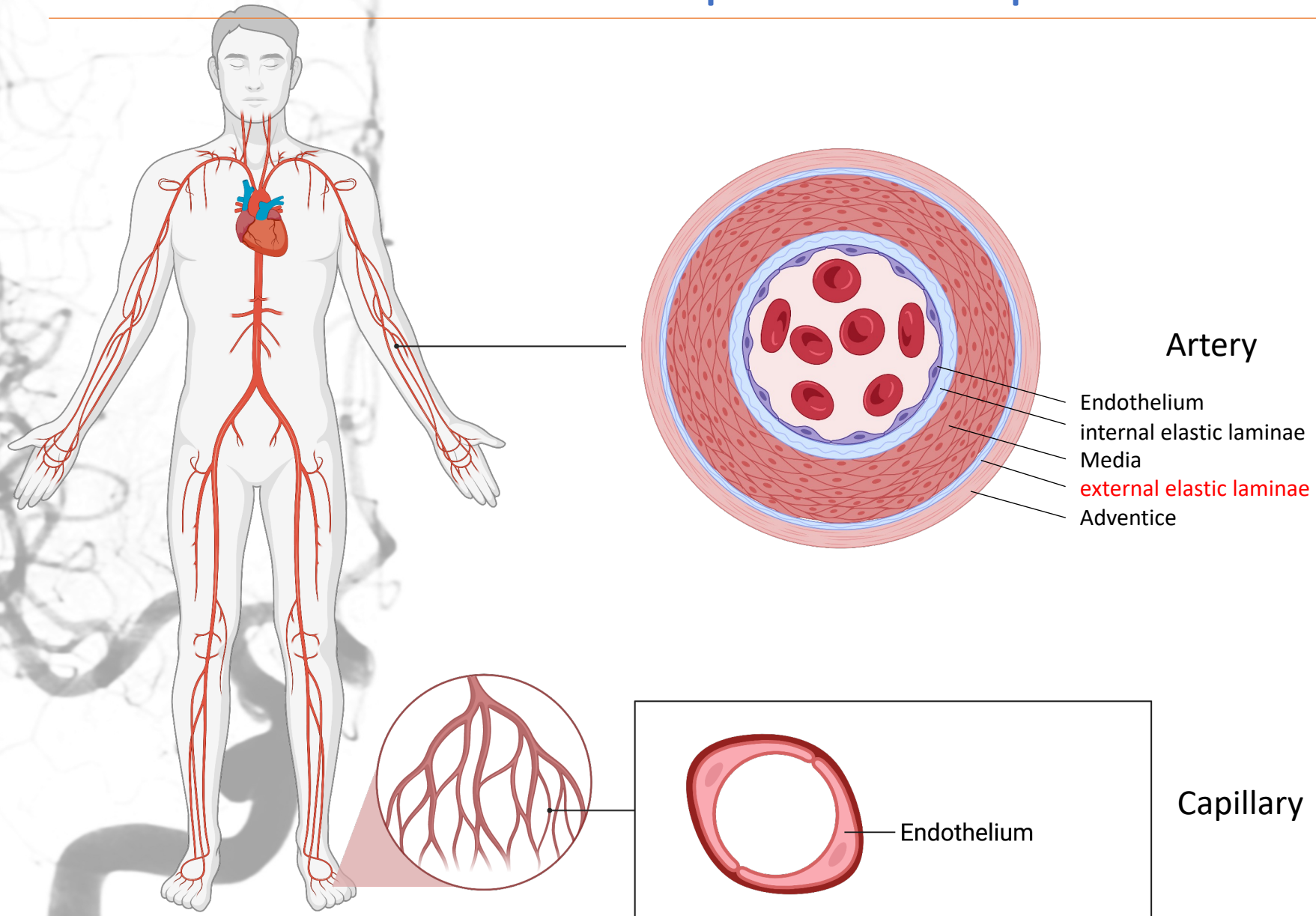
- **Risk factors :**

- Smoking, alcohol consumption, hypertension
- female sex, age, familial history

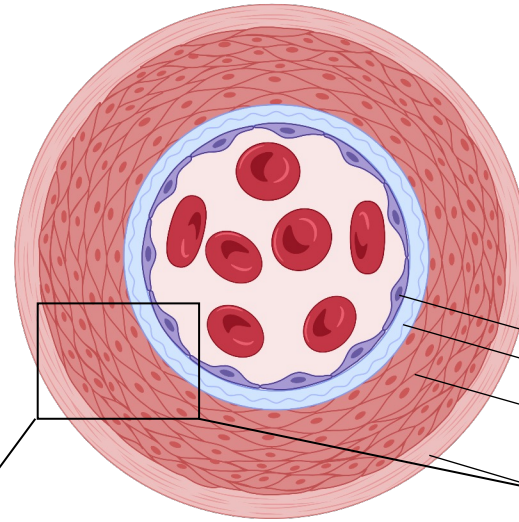
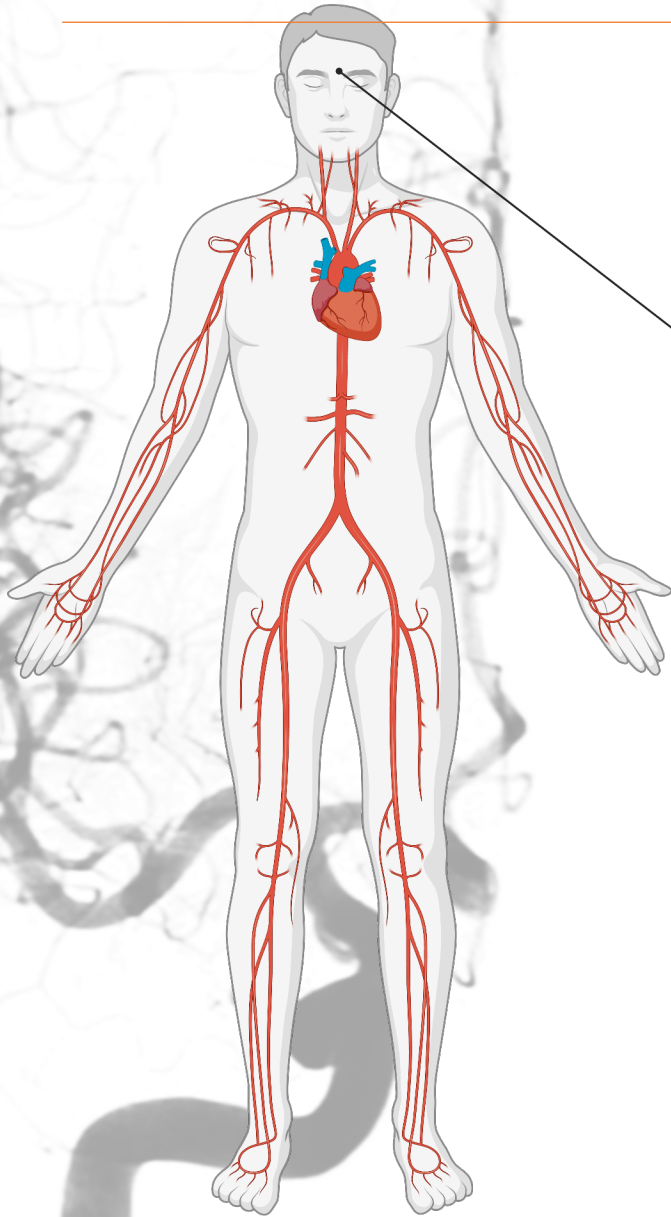
- **physiopathology :**

- endothelial dysfunction
- inflammation
- remodeling of the vascular wall
- molecular mechanisms at play are mostly unknown

Cerebral arteries : a specific composition



Cerebral arteries : a specific composition



NO external elastic laminae

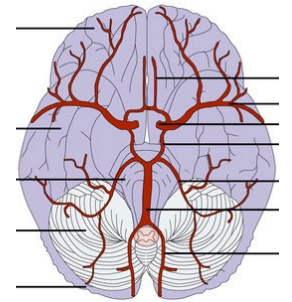
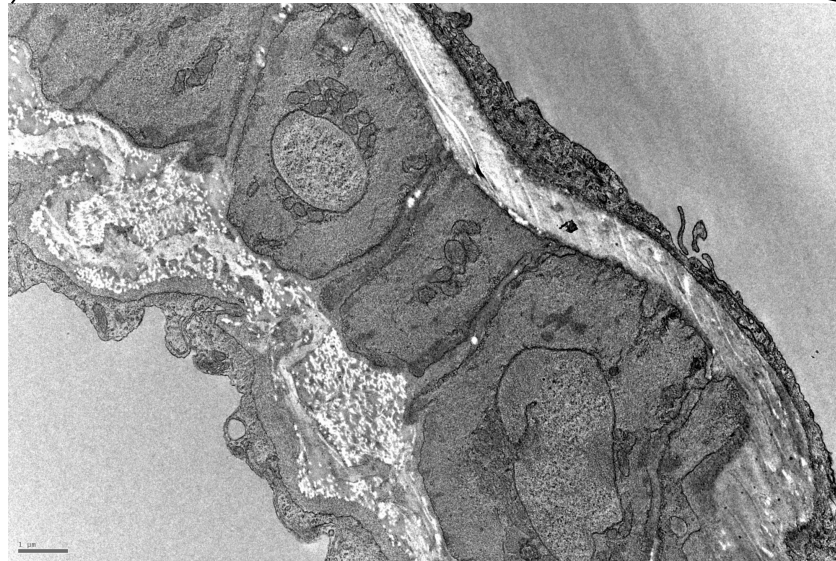
Cerebral artery

Endothelium

internal elastic laminae

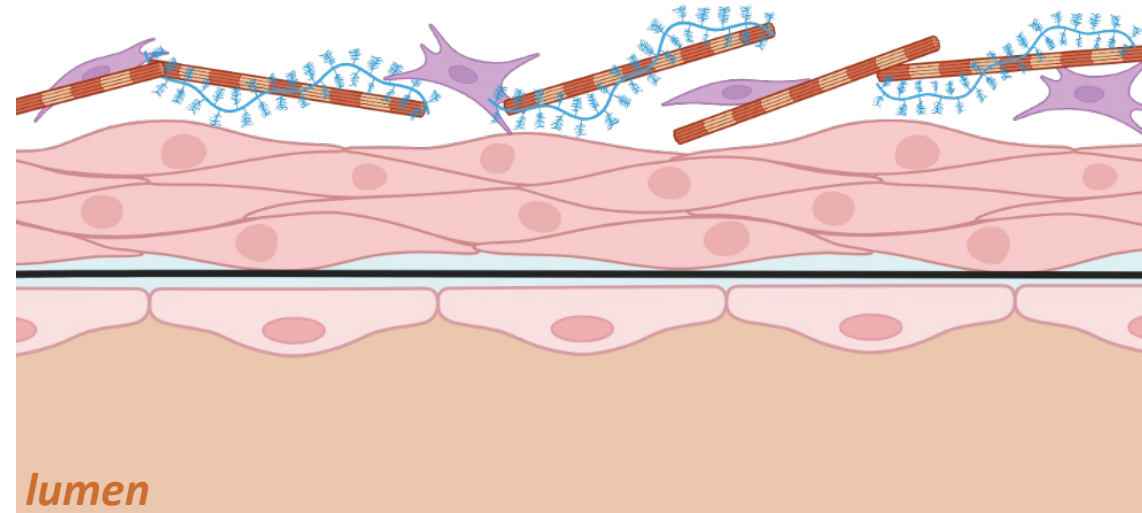
Media

Adventitia



open angle at bifurcation

Natural history of Intracranial Aneurysm



endothelial cell



Fibroblastes



SMC



Proteoglycan



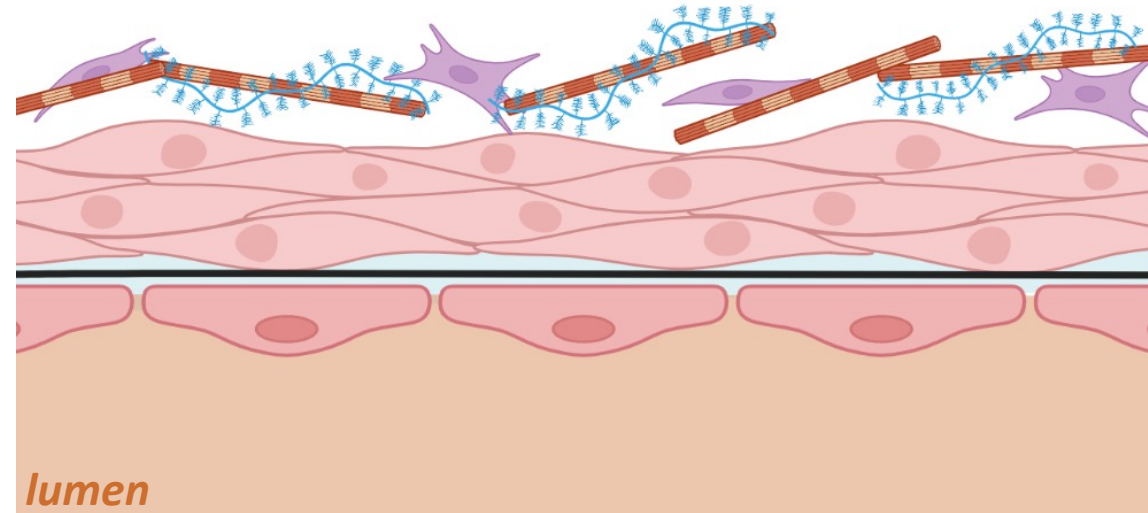
internal elastic laminae



collagen fibers



Natural history of Intracranial Aneurysm



endothelial dysfunction



endothelial cell



Fibroblasts



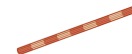
SMC



Proteoglycan

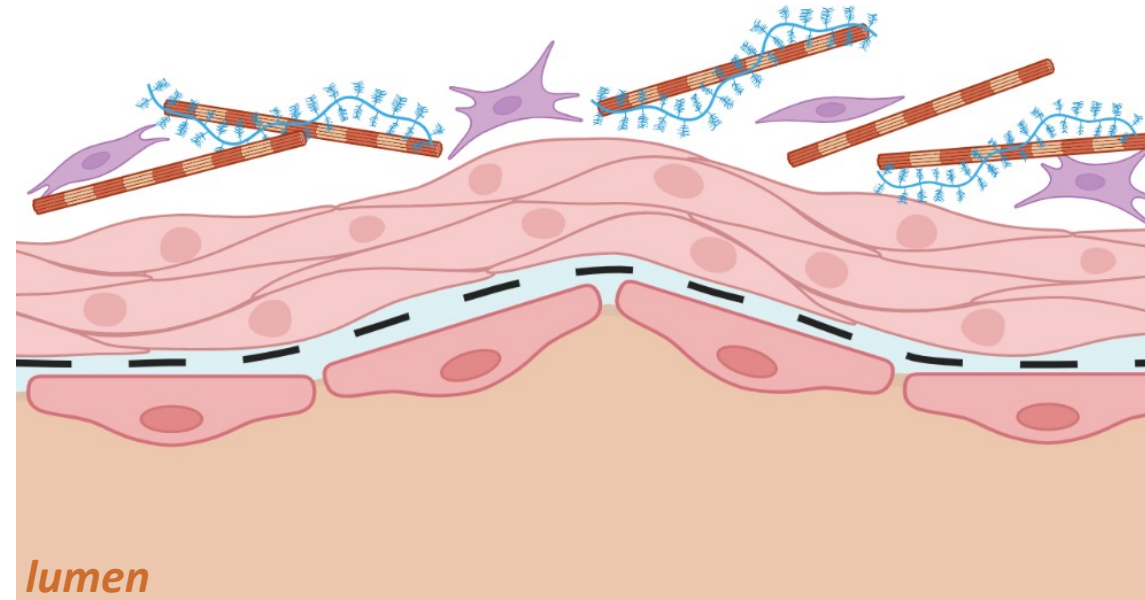


internal elastic laminae



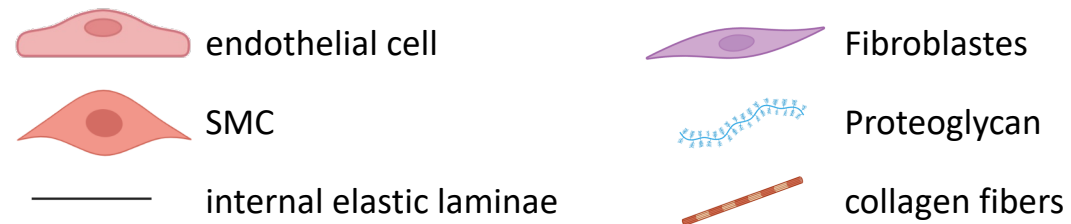
collagen fibers

Natural history of Intracranial Aneurysm



**internal elastic laminae
fragmentation**

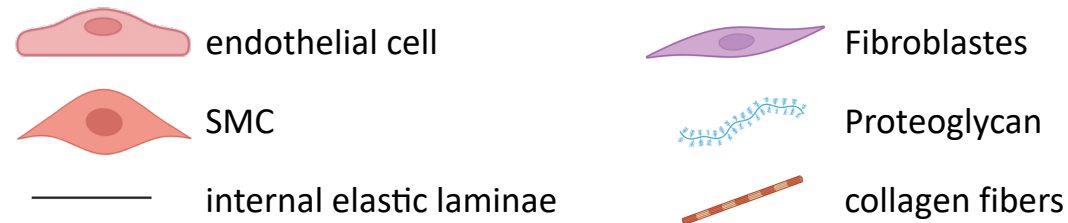
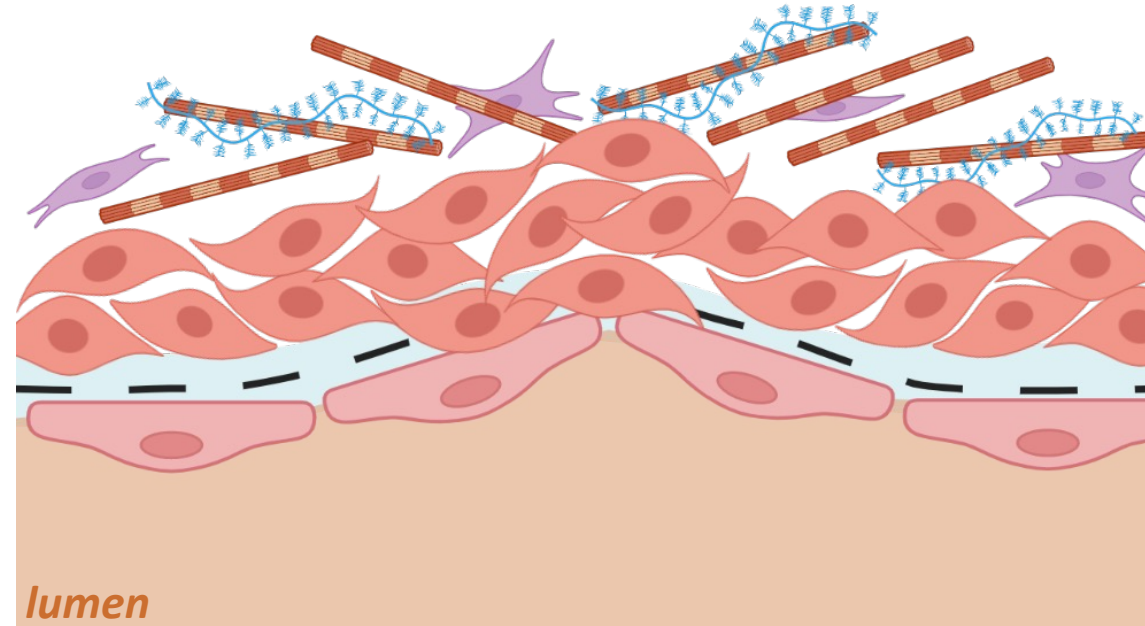
Surgery on rat
Hashimoto model



Natural history of Intracranial Aneurysm

secretory and
proliferative
phenotype

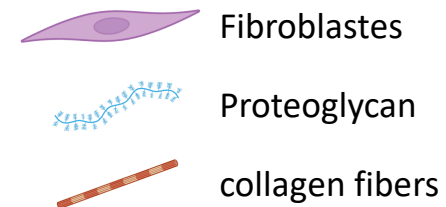
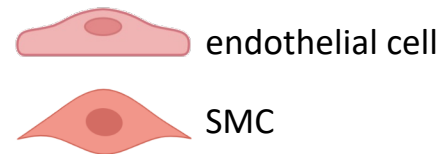
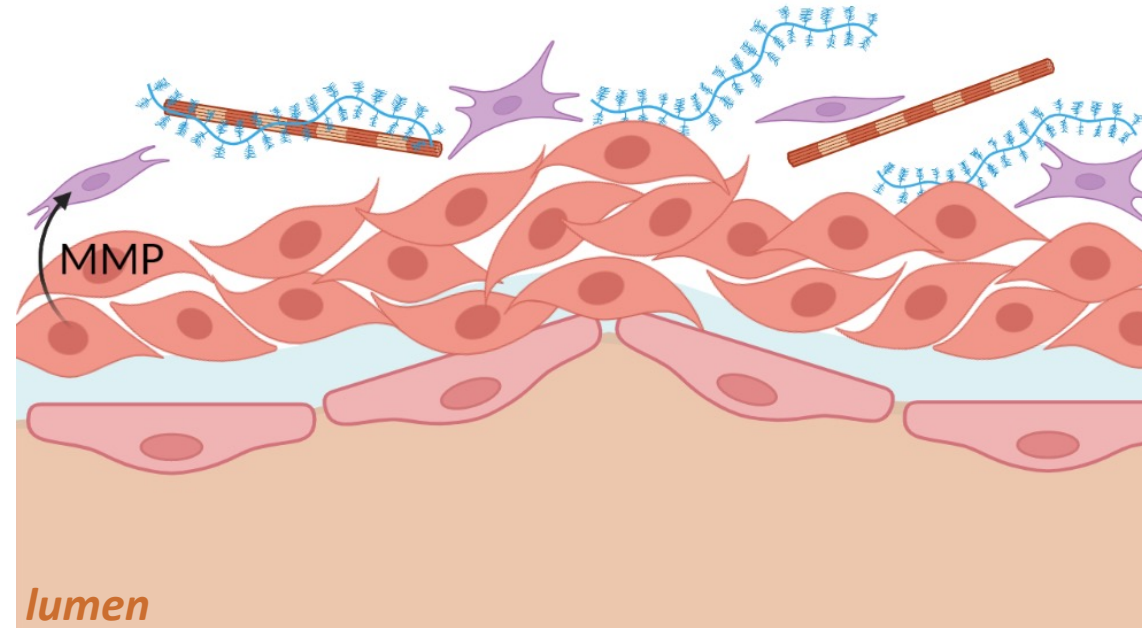
Animal model
Rat with elastase injection



Natural history of Intracranial Aneurysm

matrix
metalproteinases
secretion

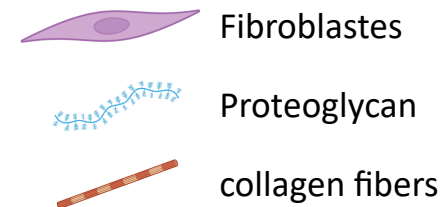
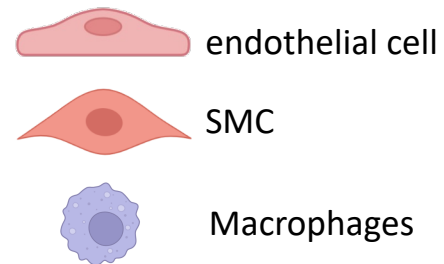
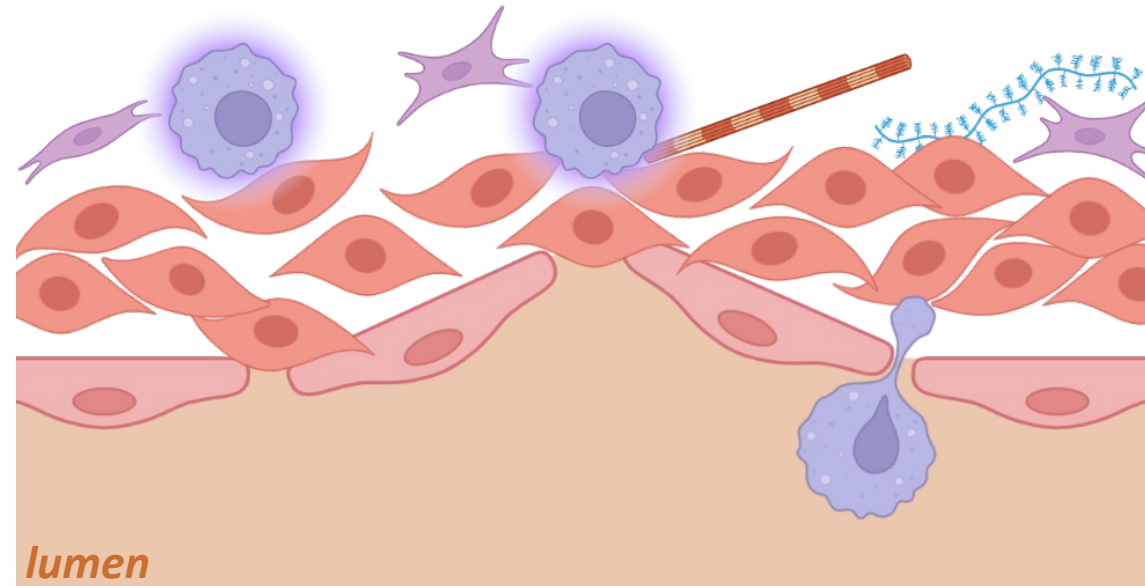
Animal model
Rabbit with CCA ligation



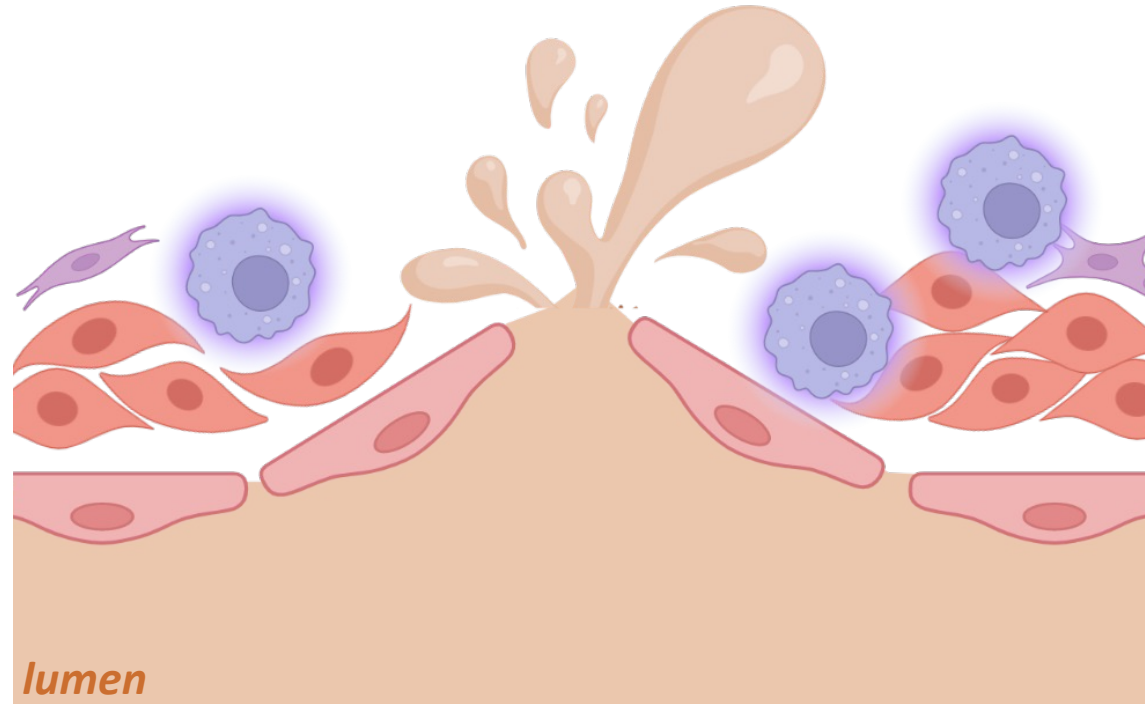
Natural history of Intracranial Aneurysm

Inflammatory cells
recruitment

SMC apoptosis



Natural history of Intracranial Aneurysm



endothelial cell



Fibroblastes



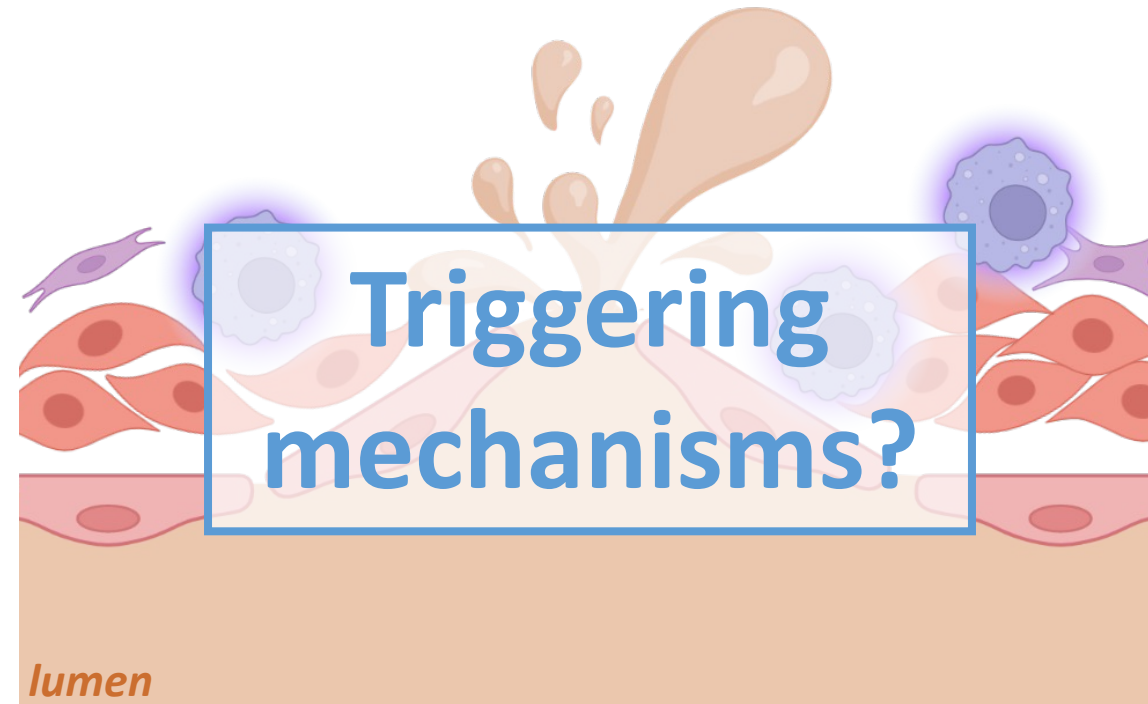
SMC

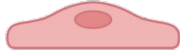


Macrophages



Natural history of Intracranial Aneurysm



 endothelial cell

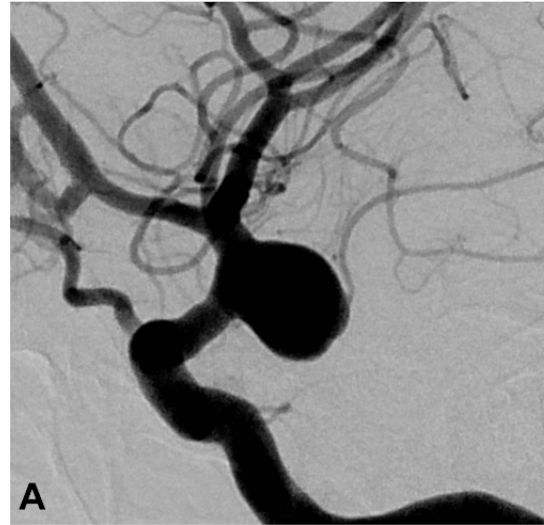
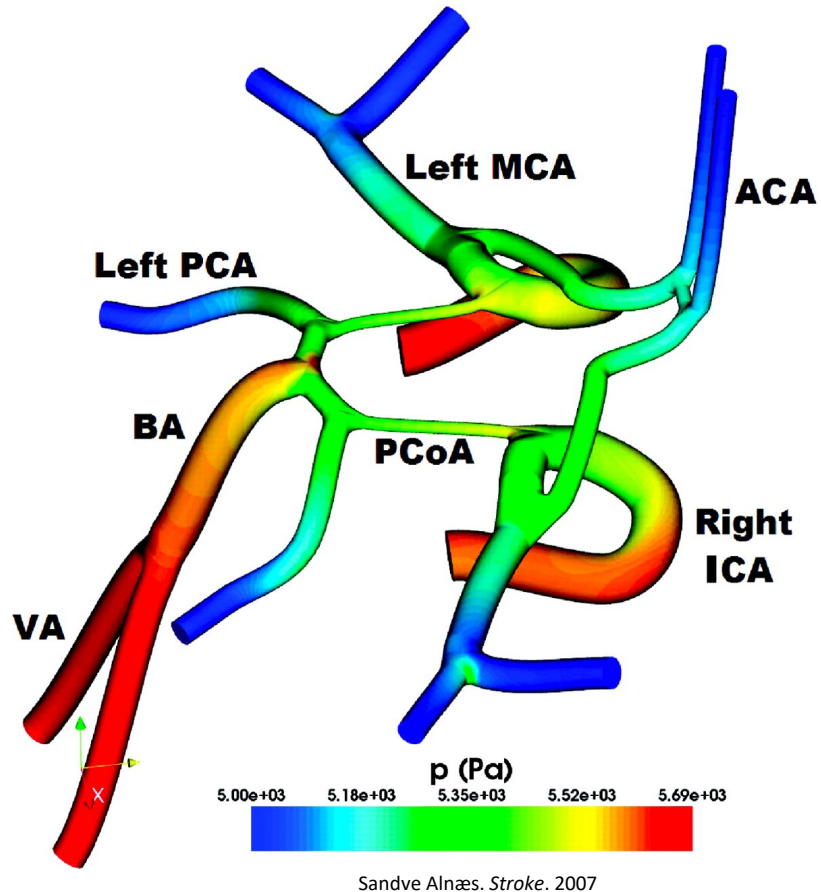
 Fibroblasts

 SMC

 Macrophages

What happens within the cells ?

Intracranial Aneurysm : causes and consequences



- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture

- **Risk factors :**

- Smoking, alcohol consumption, hypertension
- female sex, age, familial history

- **physiopathology :**

- endothelial dysfunction
- inflammation
- remodeling of the vascular wall
- molecular mechanisms at play are mostly unknown
- associated with altered **hemodynamics**

Just 2 slides of physics :

What are hemodynamics ?

It is everything that concern the dynamics of blood flow.

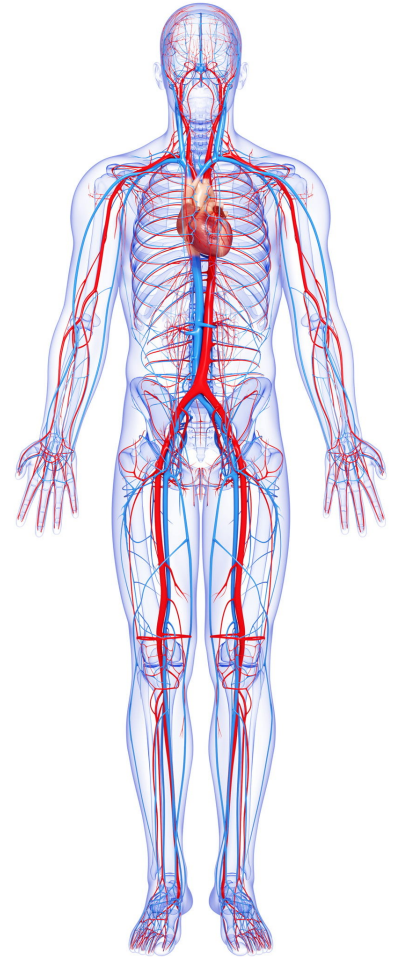
It gathers in one term the physical laws that govern the flow of blood in the blood vessels, ie, in an hydraulic circuit.

As any system it is highly controlled

What blood flow is made to ?

Blood flow ensures the transportation of :
nutrients, hormones, metabolic wastes, O₂ and CO₂ throughout the body

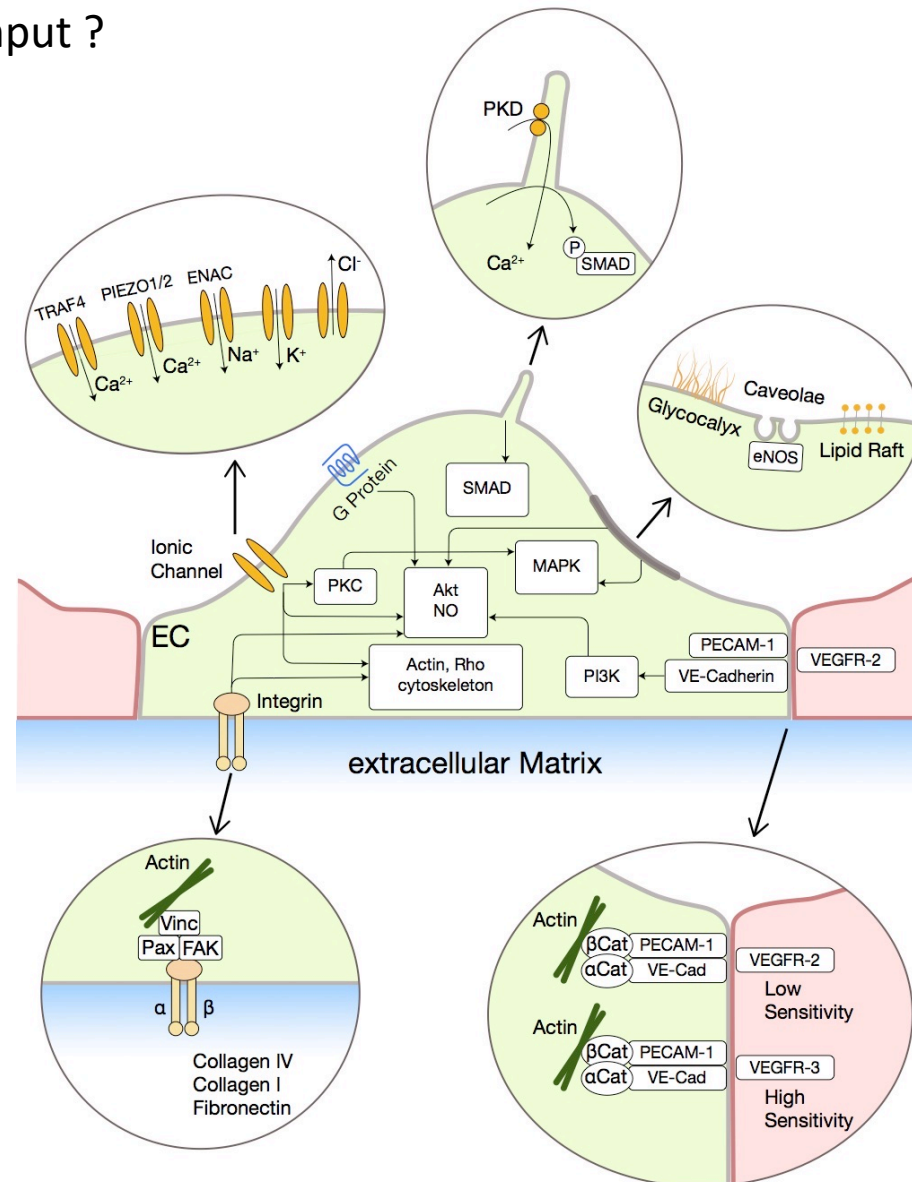
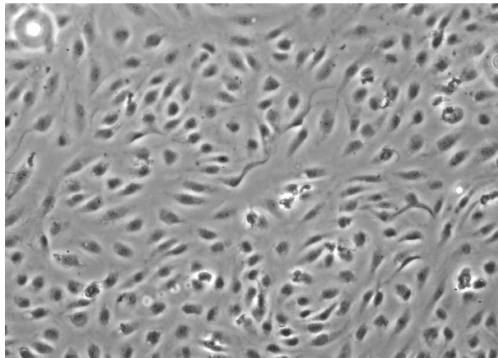
to maintain cell-level metabolism, the regulation of the pH, osmotic pressure and temperature of the whole body, and the protection from microbial and mechanical harms.



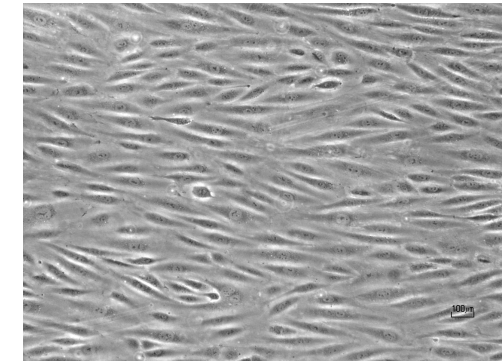
The endothelial cell is dedicated to sense blood flow

How a cell can “feel” a mechanical input ?

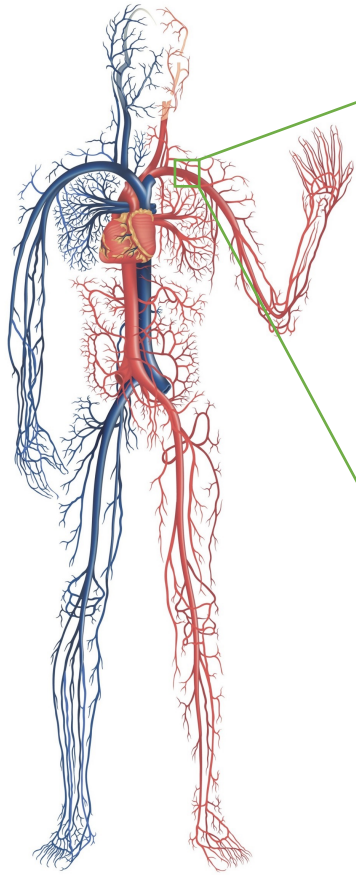
No shear stress



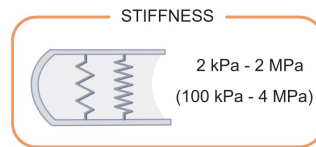
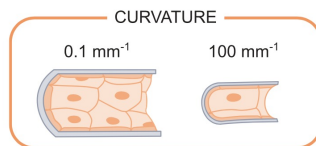
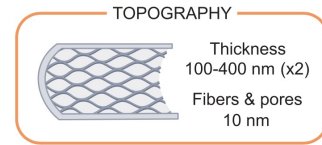
Physiological shear stress



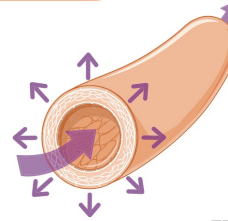
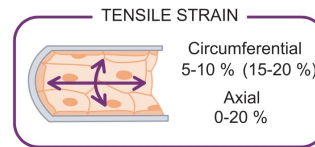
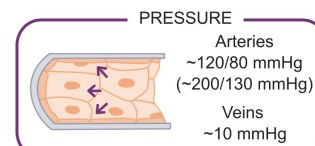
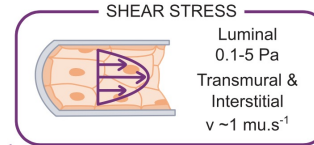
Just 2 slides of physics :



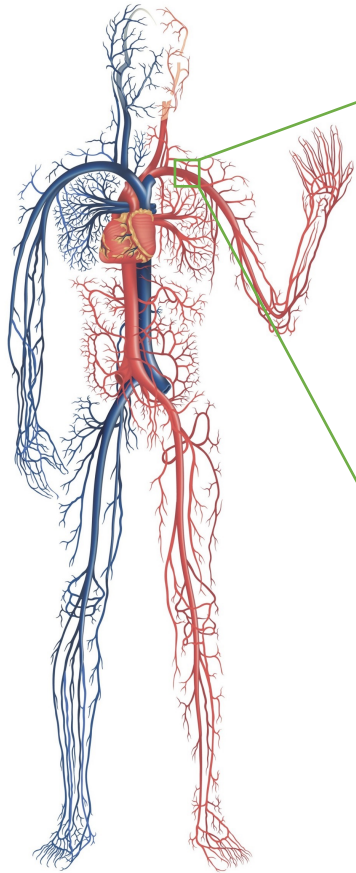
CONTACT-DERIVED STRESSES



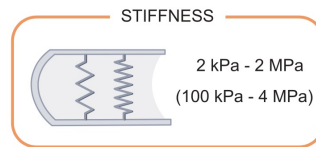
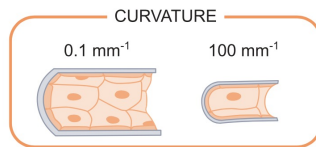
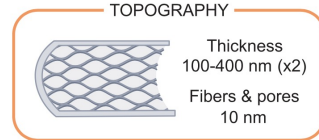
FLOW-DERIVED STRESSES



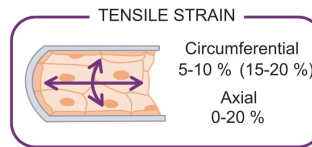
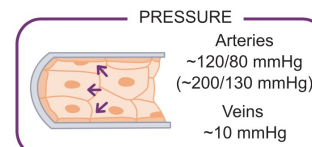
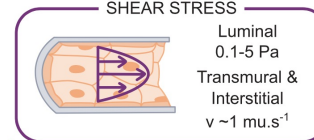
Just 2 slides of physics :



CONTACT-DERIVED STRESSES



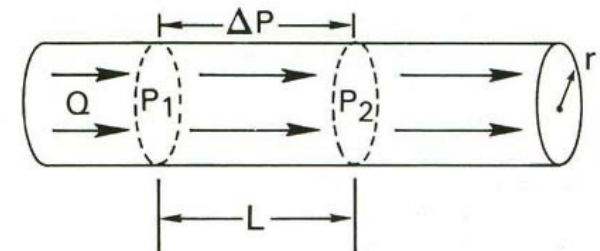
FLOW-DERIVED STRESSES



Shear stress
Hagen-Poiseuille Law

$$\tau = \frac{4 \mu \cdot Q}{\pi \cdot r^3}$$

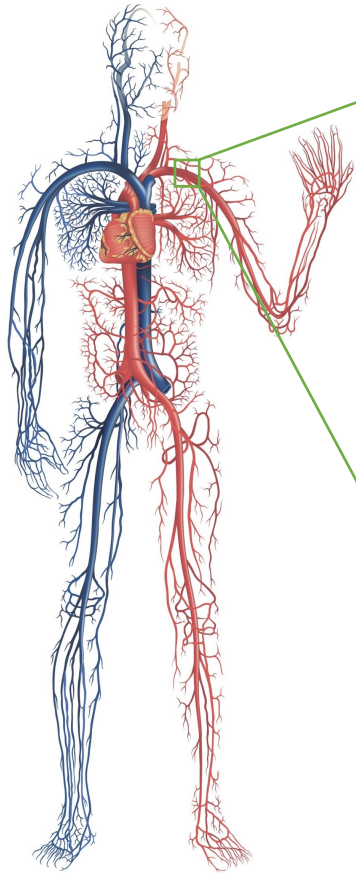
μ = viscosity of the blood
 r = radius of the vessel
 Q = flow rate



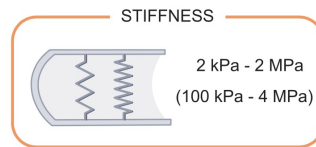
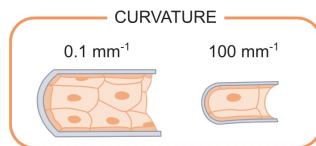
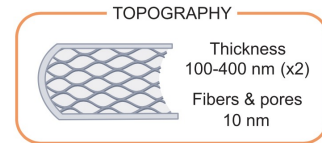
POISEUILLE'S LAW

$$Q = \frac{\Delta P \cdot r^4 \cdot \pi}{\eta L \cdot 8}$$

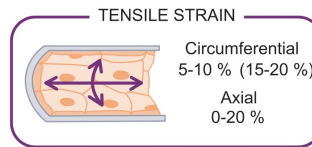
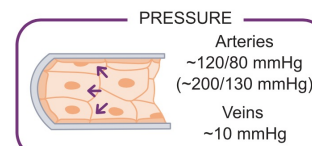
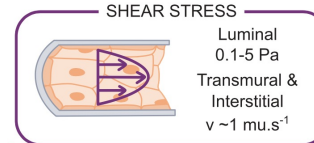
Just 2 slides of physics :



CONTACT-DERIVED STRESSES



FLOW-DERIVED STRESSES

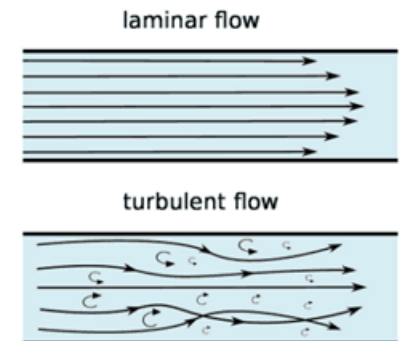
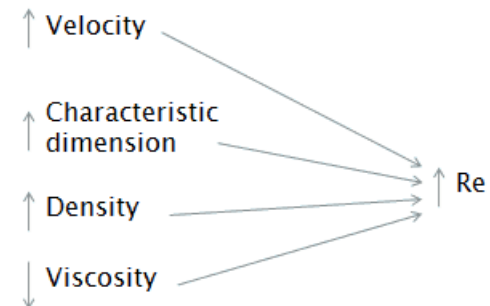


Shear stress
Hagen-Poiseuille Law

$$\tau = \frac{4 \mu \cdot Q}{\pi \cdot r^3}$$

Perturbation of the flow

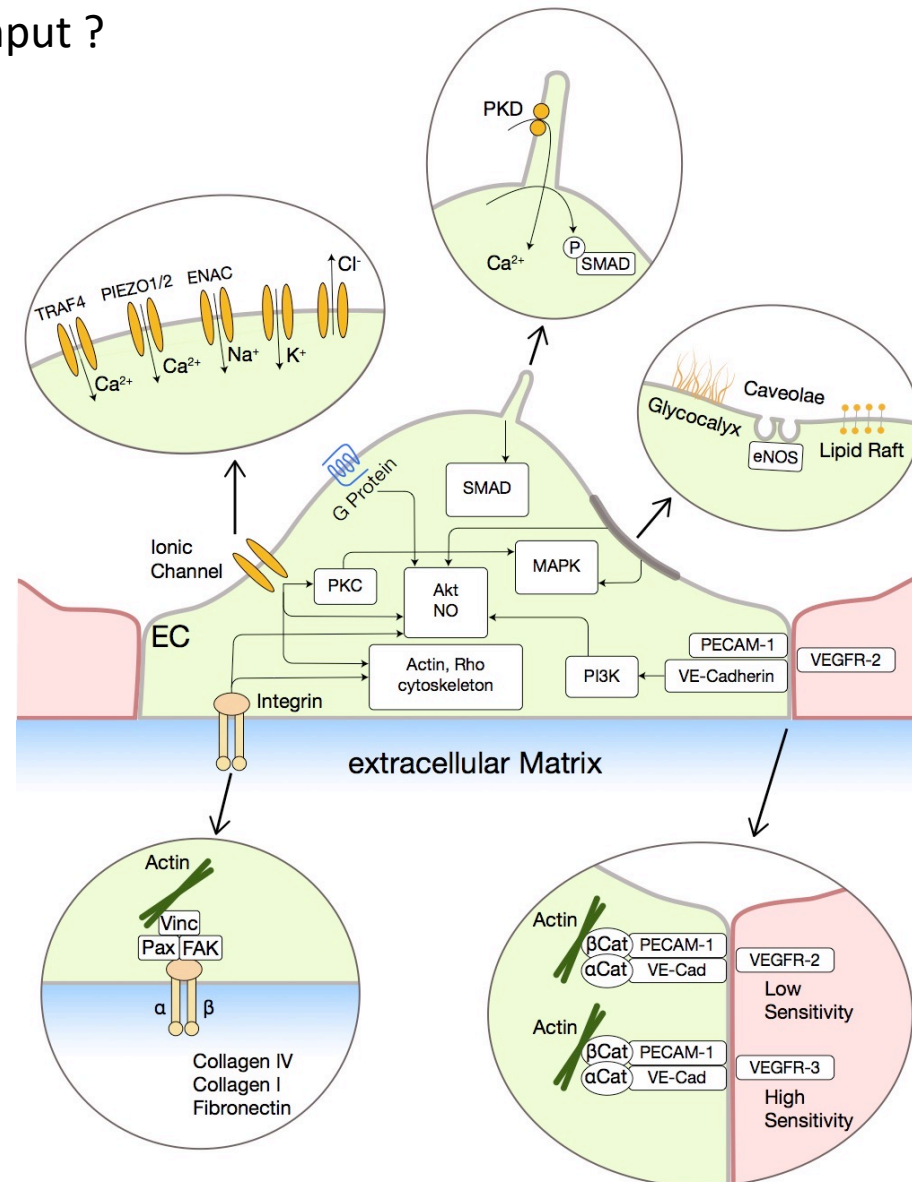
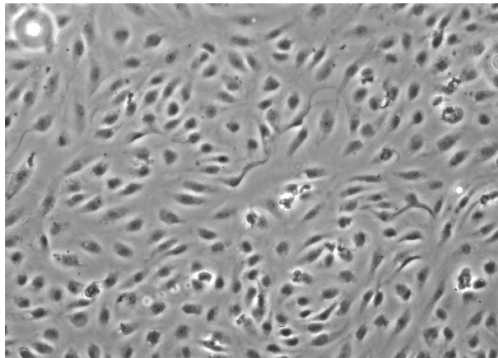
$$Re = \frac{\text{inertia forces}}{\text{viscous forces}} = \frac{\rho \cdot V \cdot D}{\mu}$$



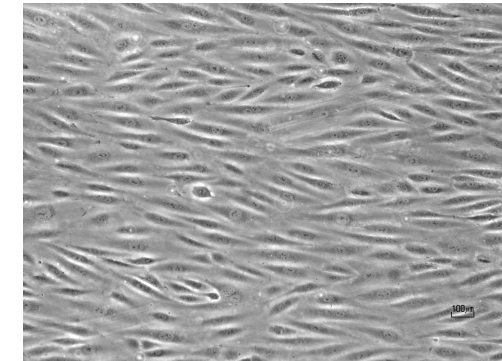
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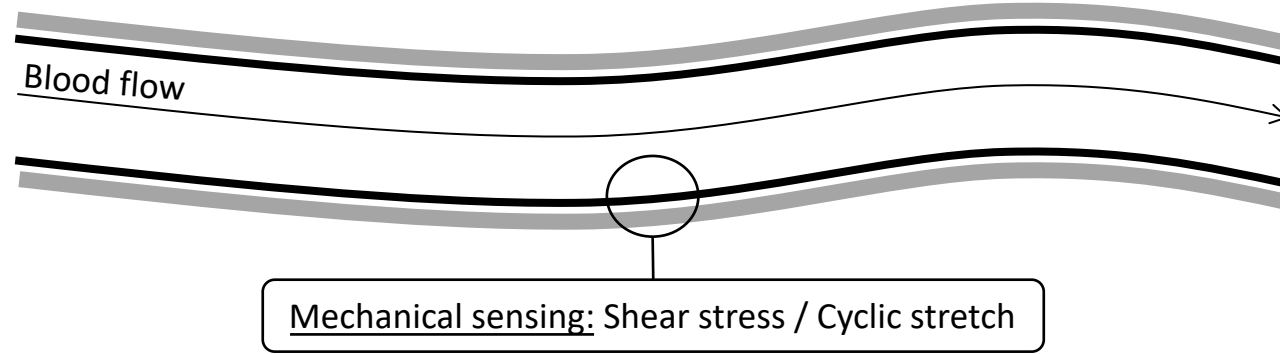
No shear stress



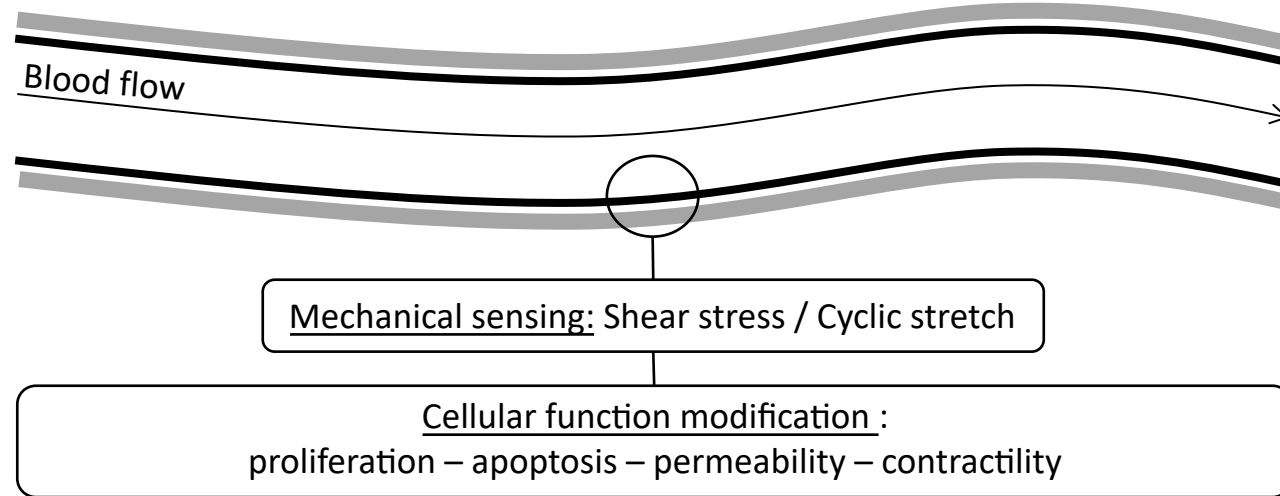
Physiological shear stress



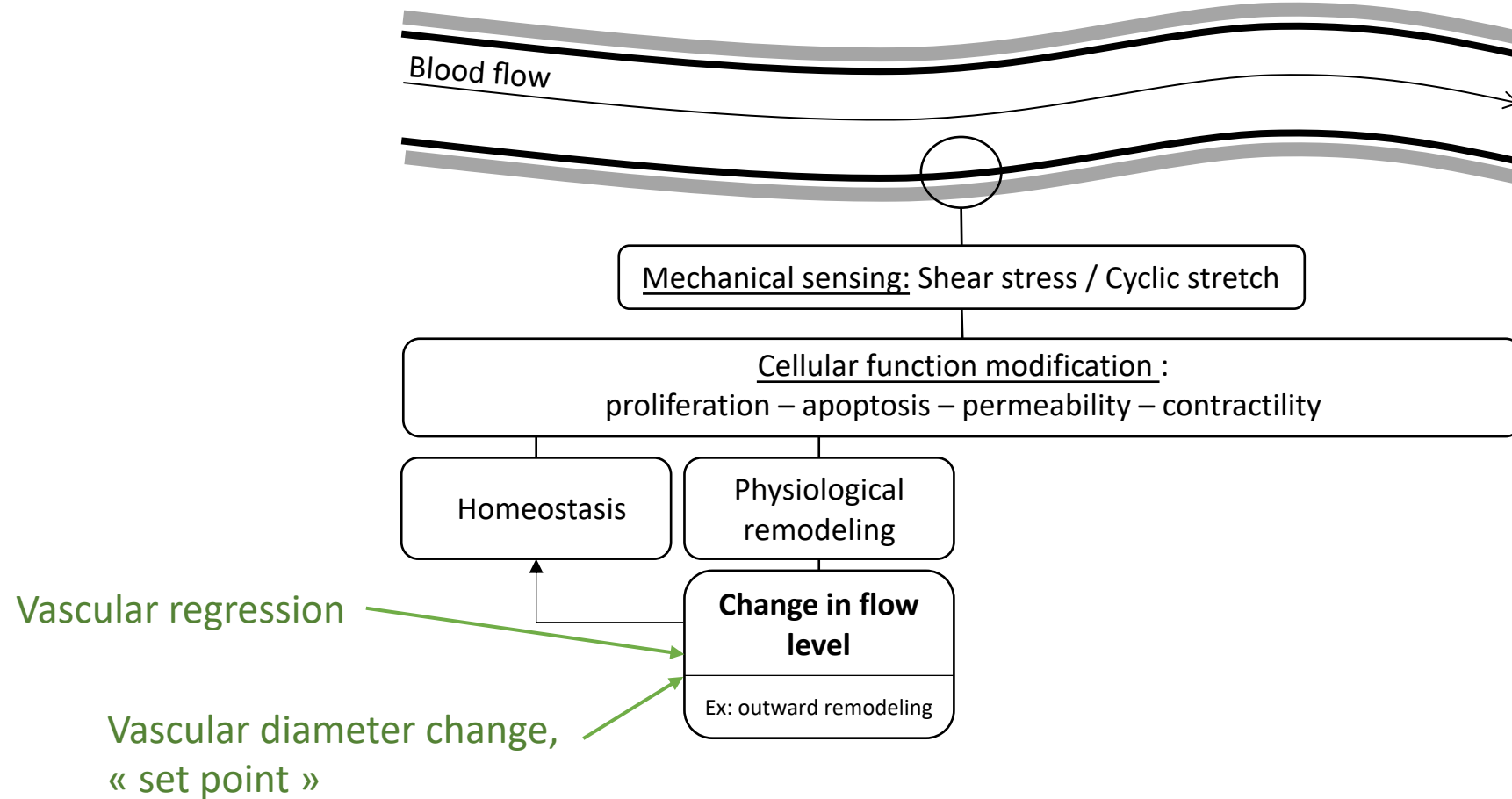
physiological and pathological response to flow



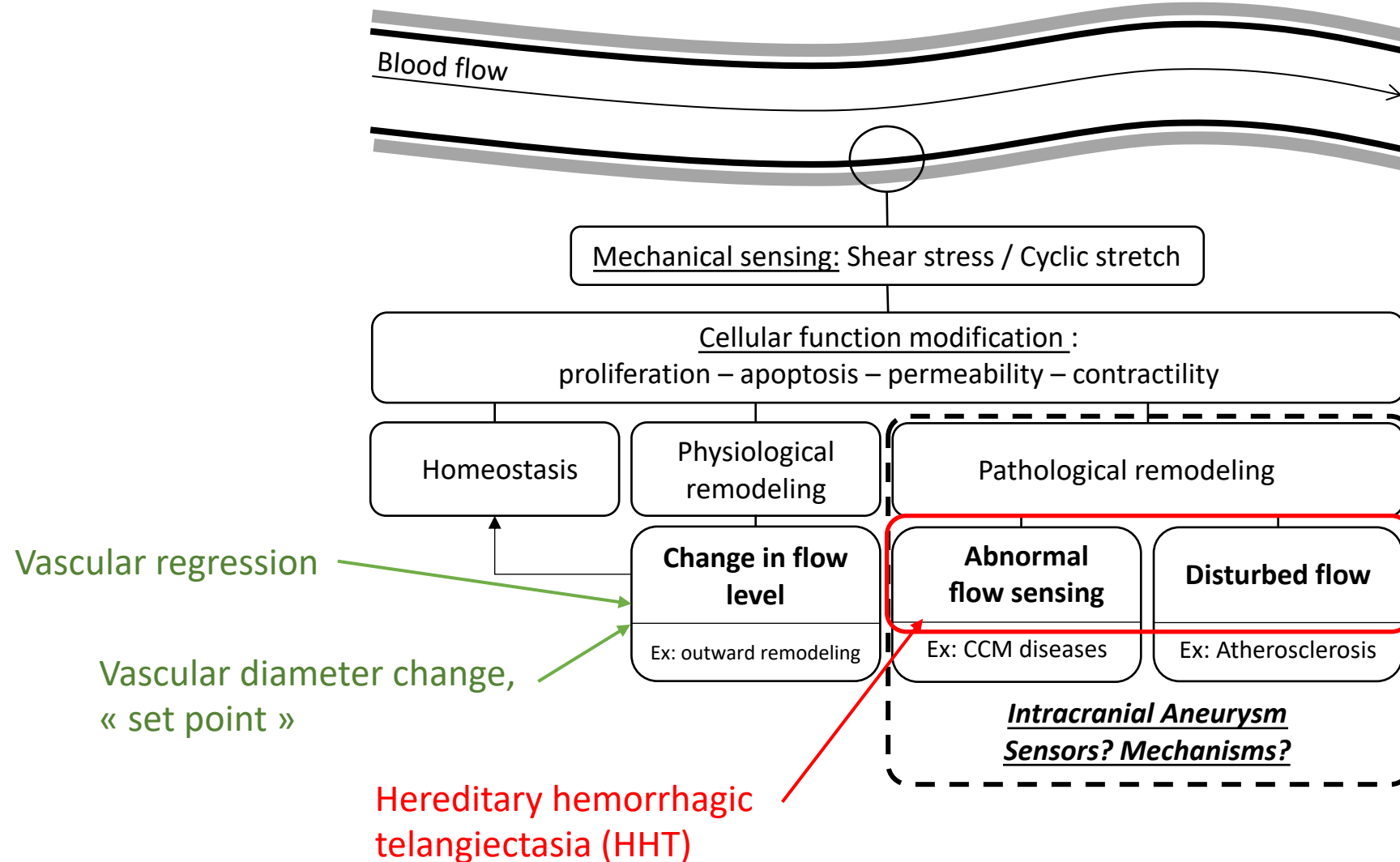
physiological and pathological response to flow



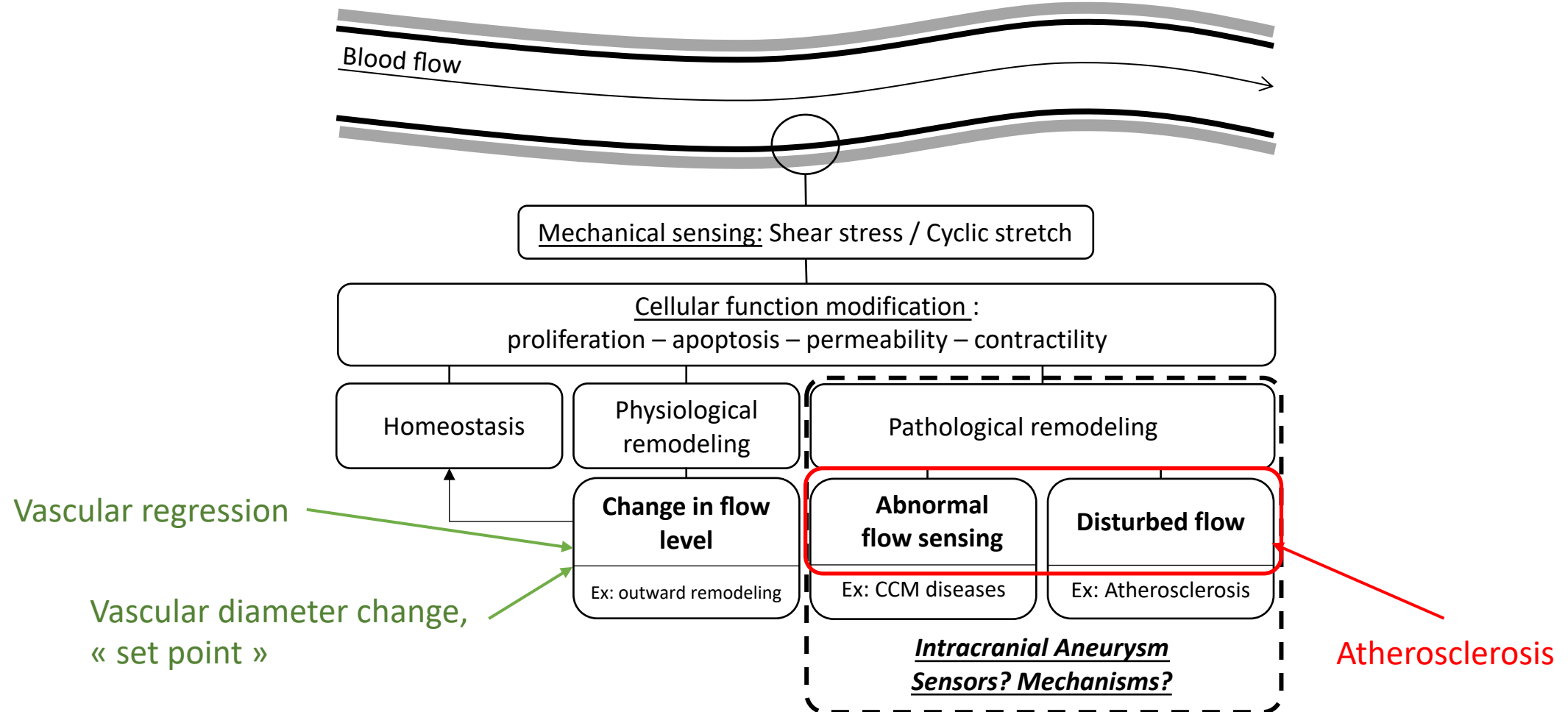
physiological and pathological response to flow



physiological and pathological response to flow

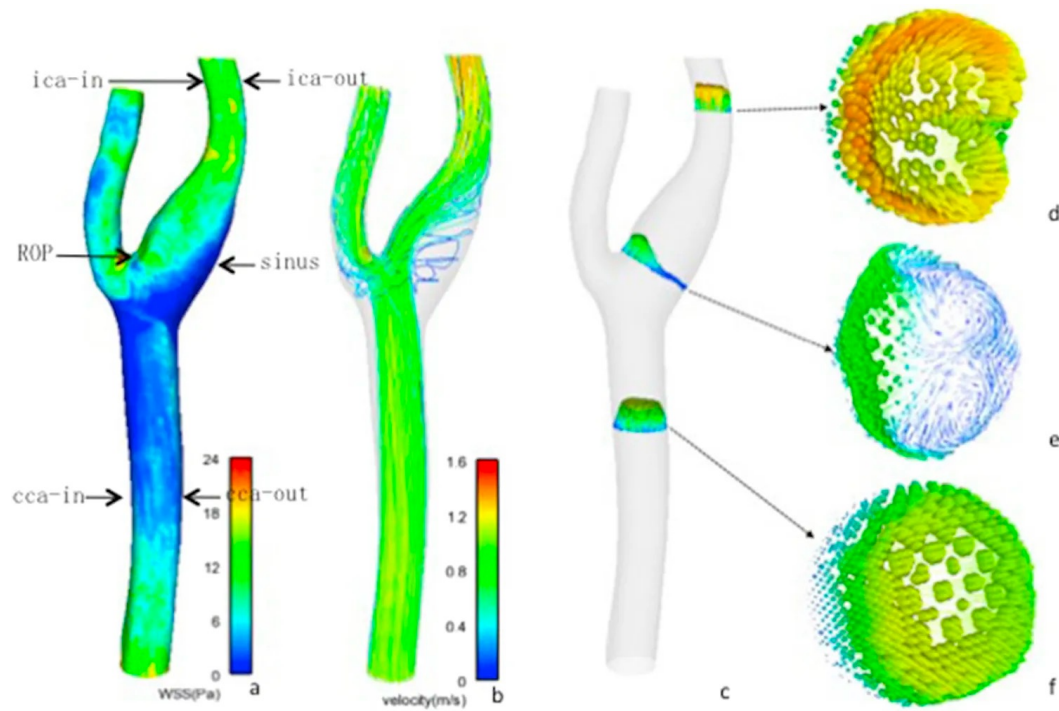


physiological and pathological response to flow

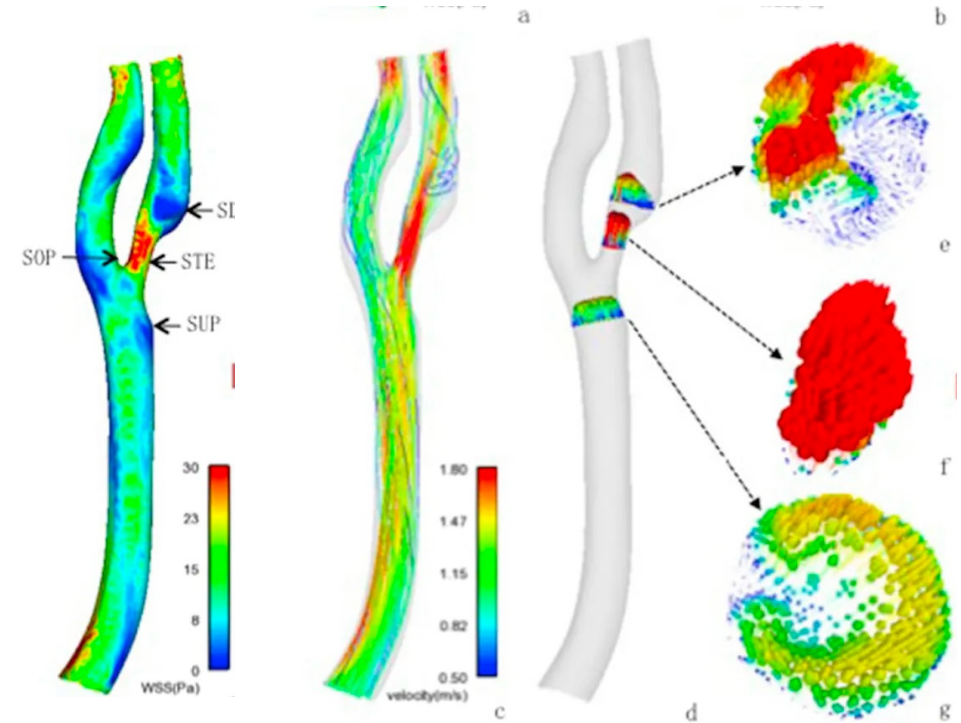


Hemodynamics at bifurcation sites : Carotides and atherosclerosis

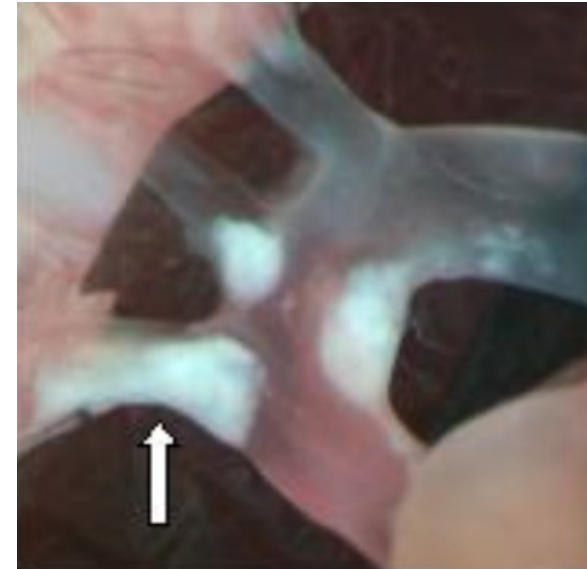
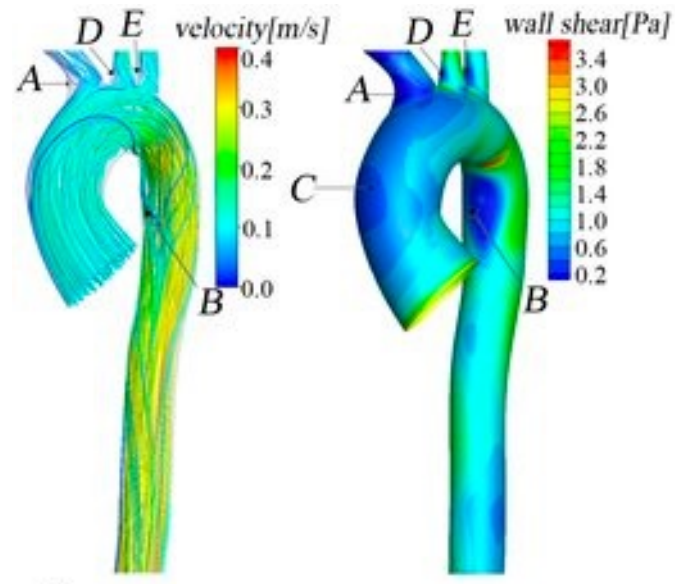
Normal carotid bifurcation



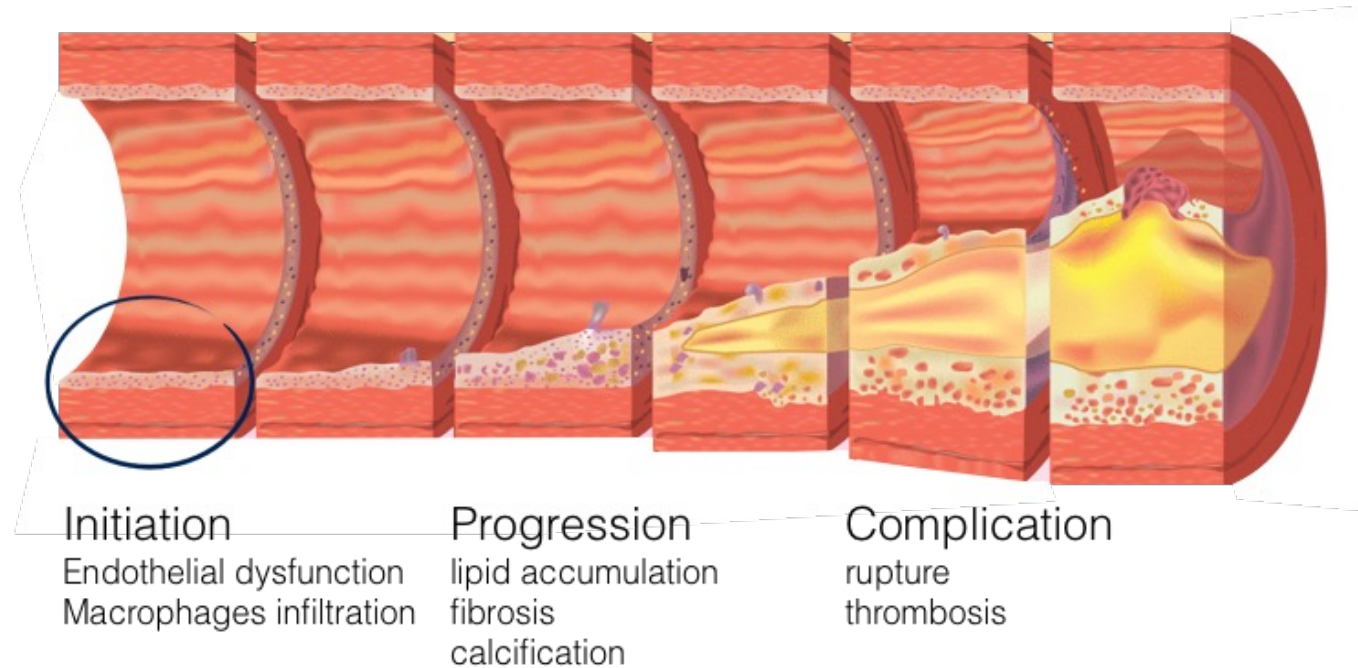
carotid bifurcation with atherosclerosis



Hemodynamics in curvatures: Aortic arch and atherosclerosis



Disturbed flow and atherosclerosis



Branched points / Disturbed Flow / Low shear stress

Endothelial Dysfunction

Vascular tone

Cell shape

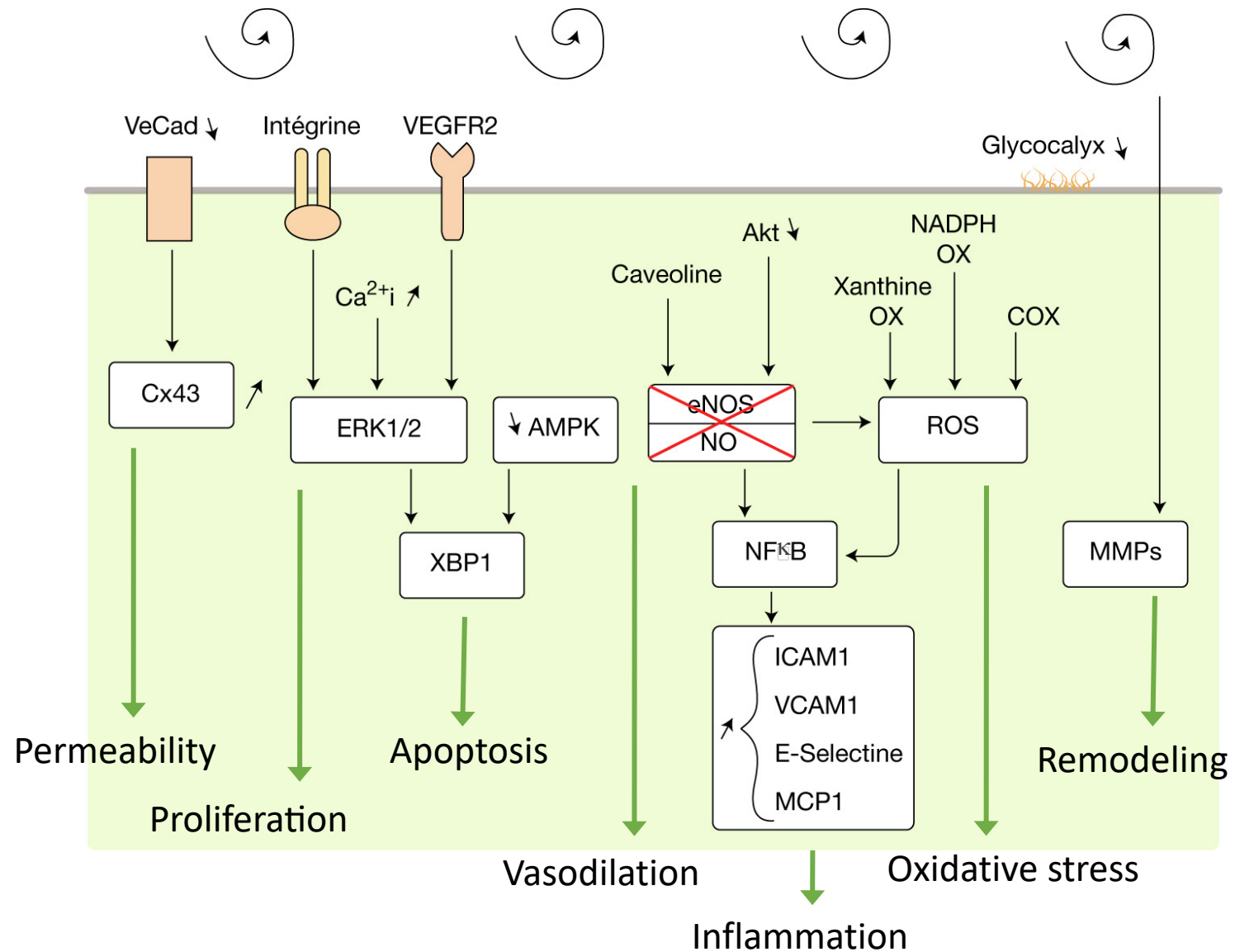
Apoptosis

Proliferation

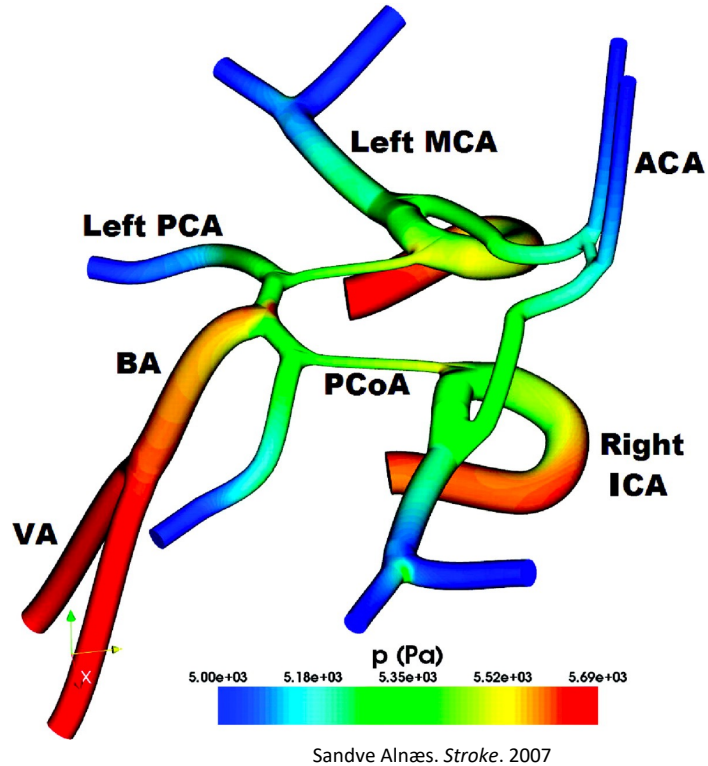
Inflammation

Thrombosis

Disturbed flow and atherosclerosis



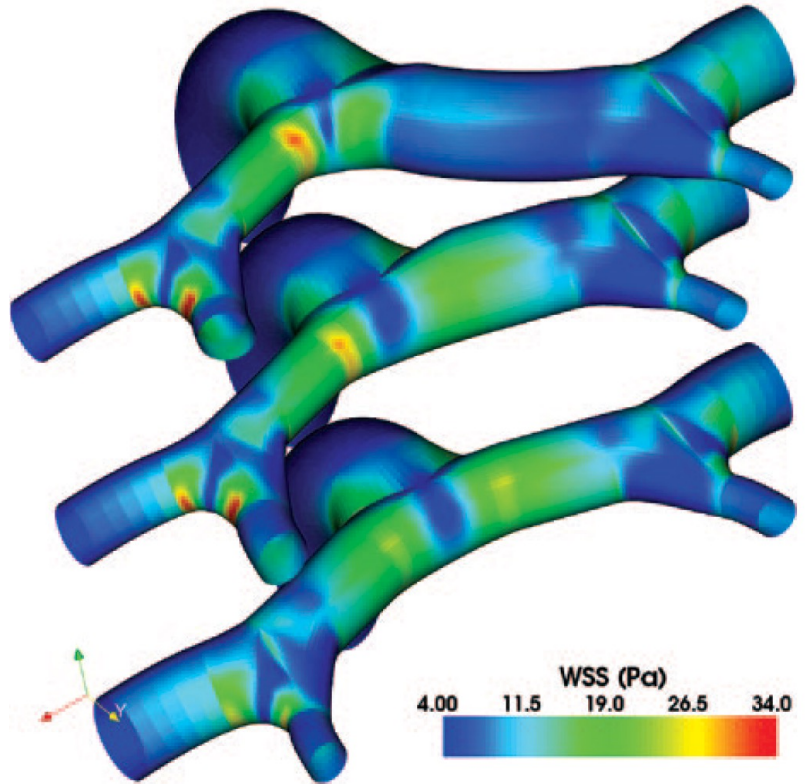
Intracranial Aneurysm : causes and consequences



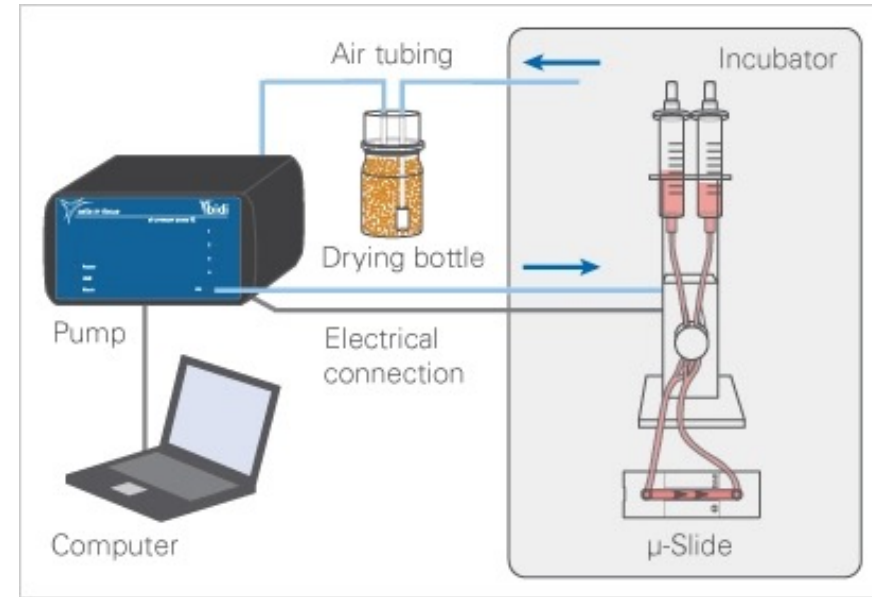
- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture
- **Risk factors :**
 - Smoking, alcohol consumption, hypertension
 - female sex, age, familial history

- **physiopathology :**
 - endothelial dysfunction
 - inflammation
 - remodeling of the vascular wall
 - molecular mechanisms at play are mostly unknown
 - associated with altered **hemodynamics**

Starting from the hemodynamic peculiarity?



Alnaes, MS. Stroke. 2007;38:2500-2505.



Cells analysis

genes

proteins

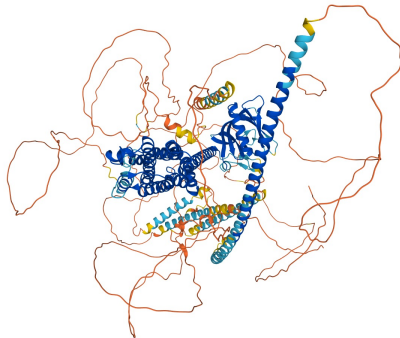
shapes

activation

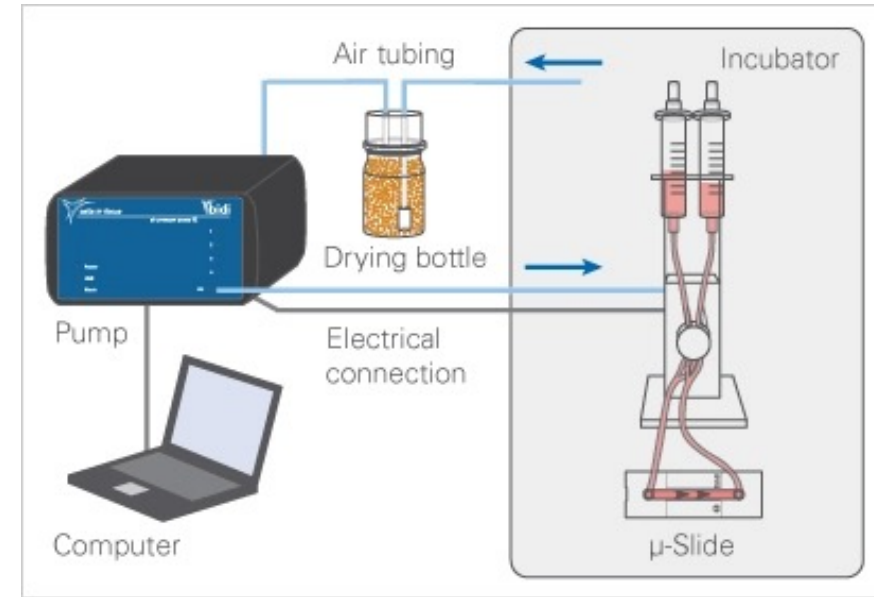
Identification of new mechanosensitive proteins

Identification of a protein sensitive to blood flow velocity variation

ARHGEF18



More active when blood flow is physiological
less active when blood flow is abnormal



Cells analysis

genes

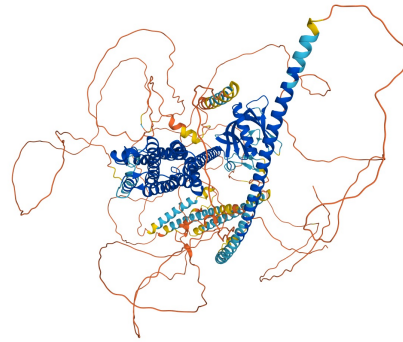
proteins

shapes

activation

ARHGEF18 identification in endothelial cells

ARHGEF18



Well described in epithelial cells

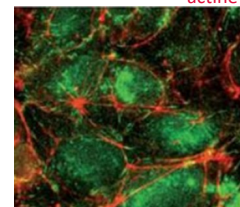
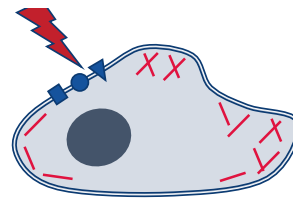
Guanin echange factor -> RhoA activation

Cell-cell adhesion

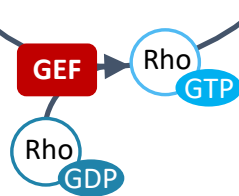
Matrix adhesion and cell migration

ROS formation

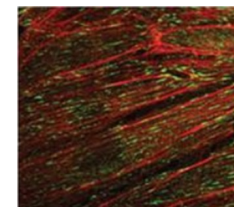
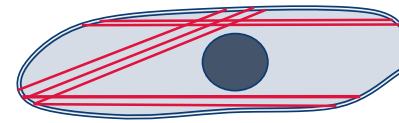
Mechanical stimulus



actine



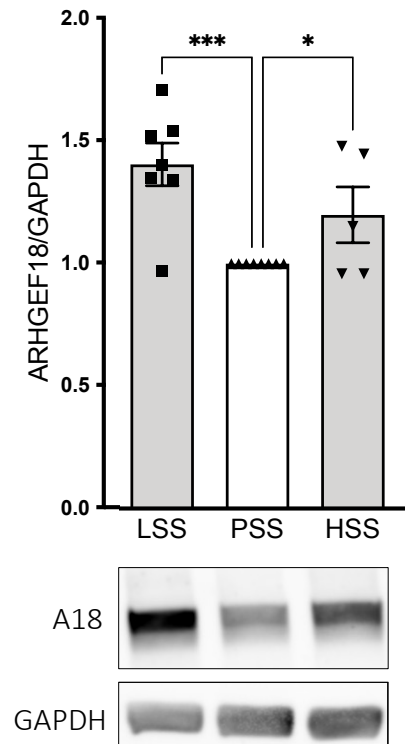
cytoskeleton
reorganization



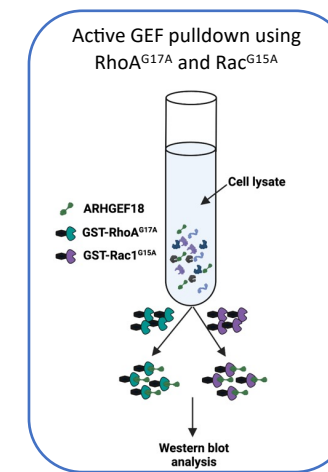
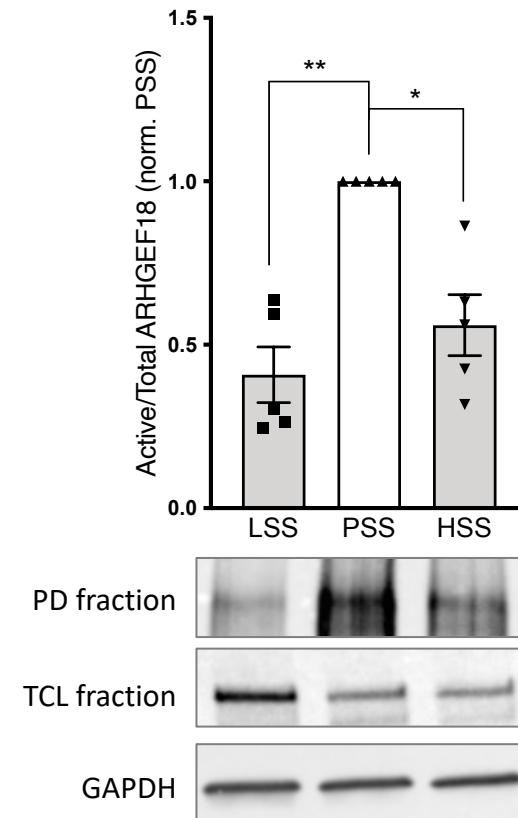
ARHGEF18 expression and activity is flow sensitive



Protein level

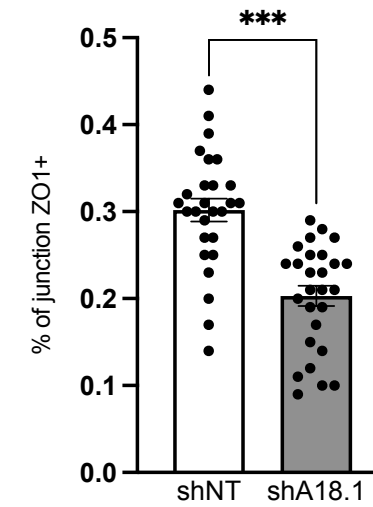
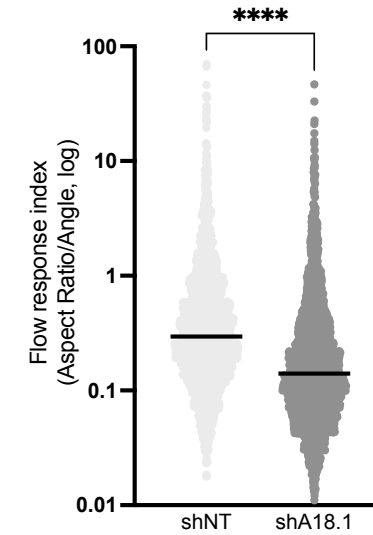
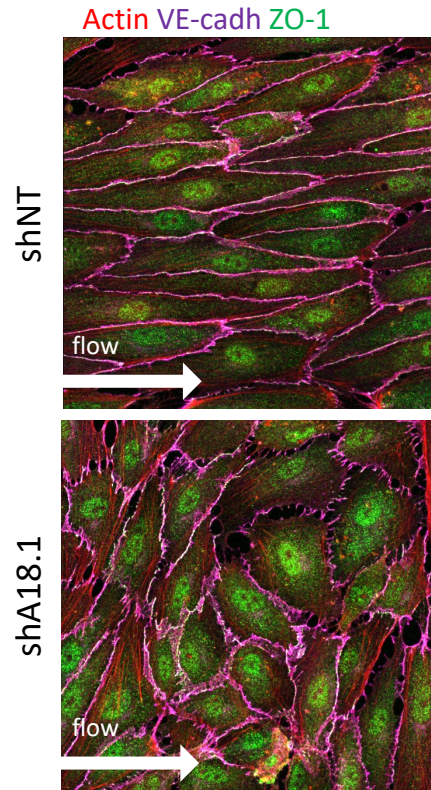


Guanin exchange activity

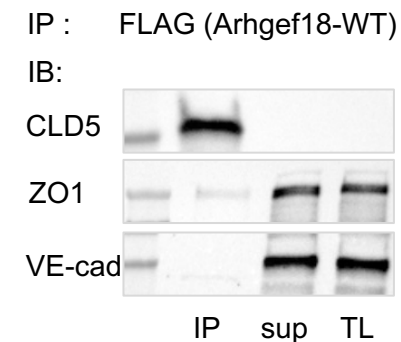
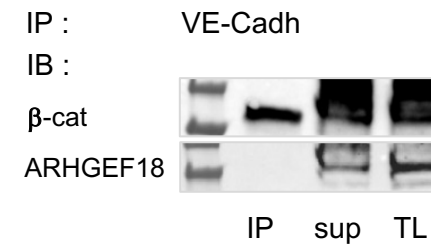
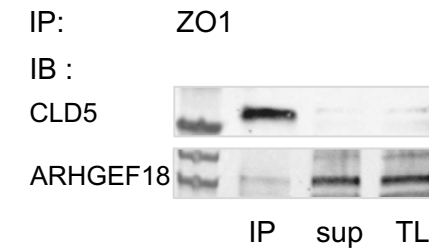
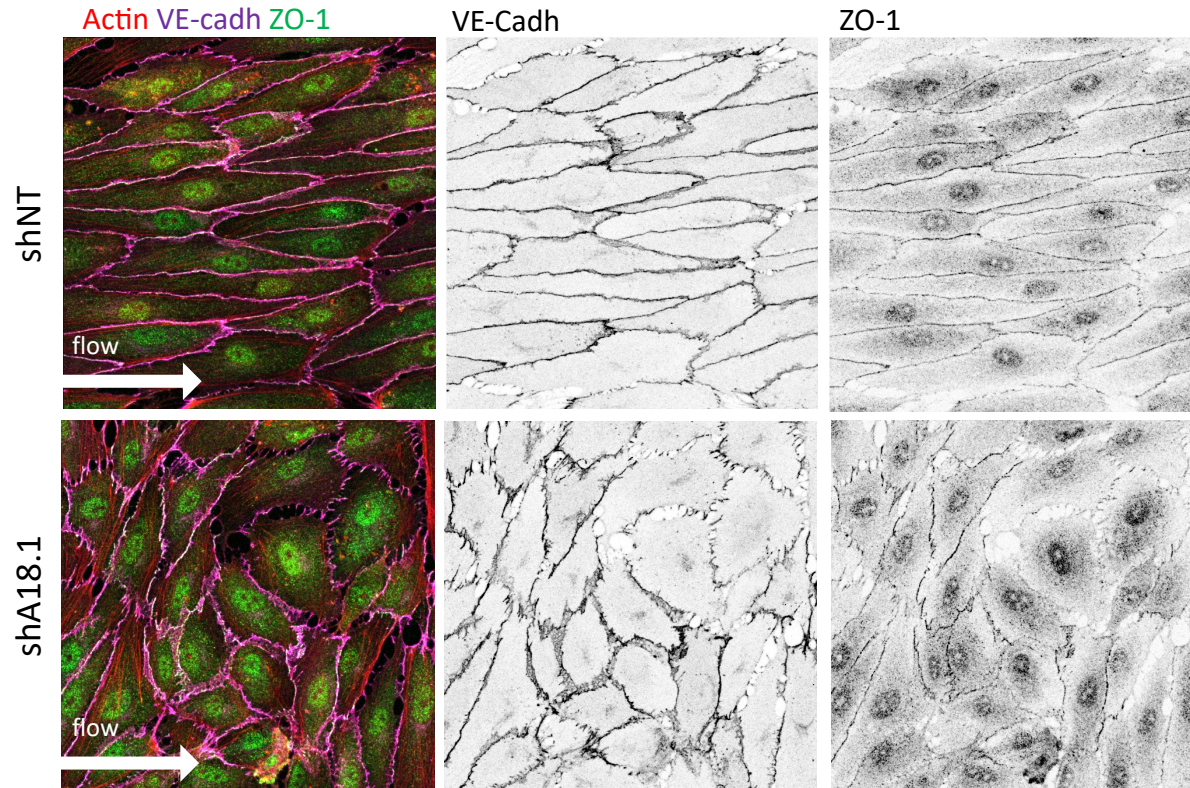


ARHGEF18 binds to RhoA
its activity is downregulated by pathological shear stress

ARHGEF18 contributes in ECs alignment with the flow

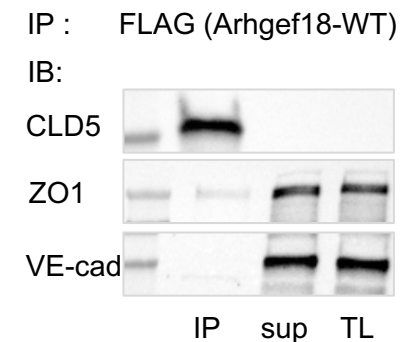
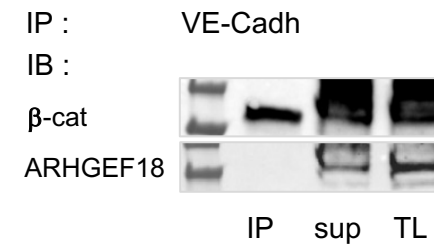
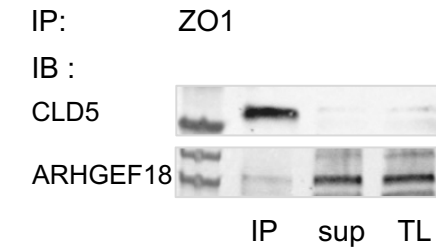
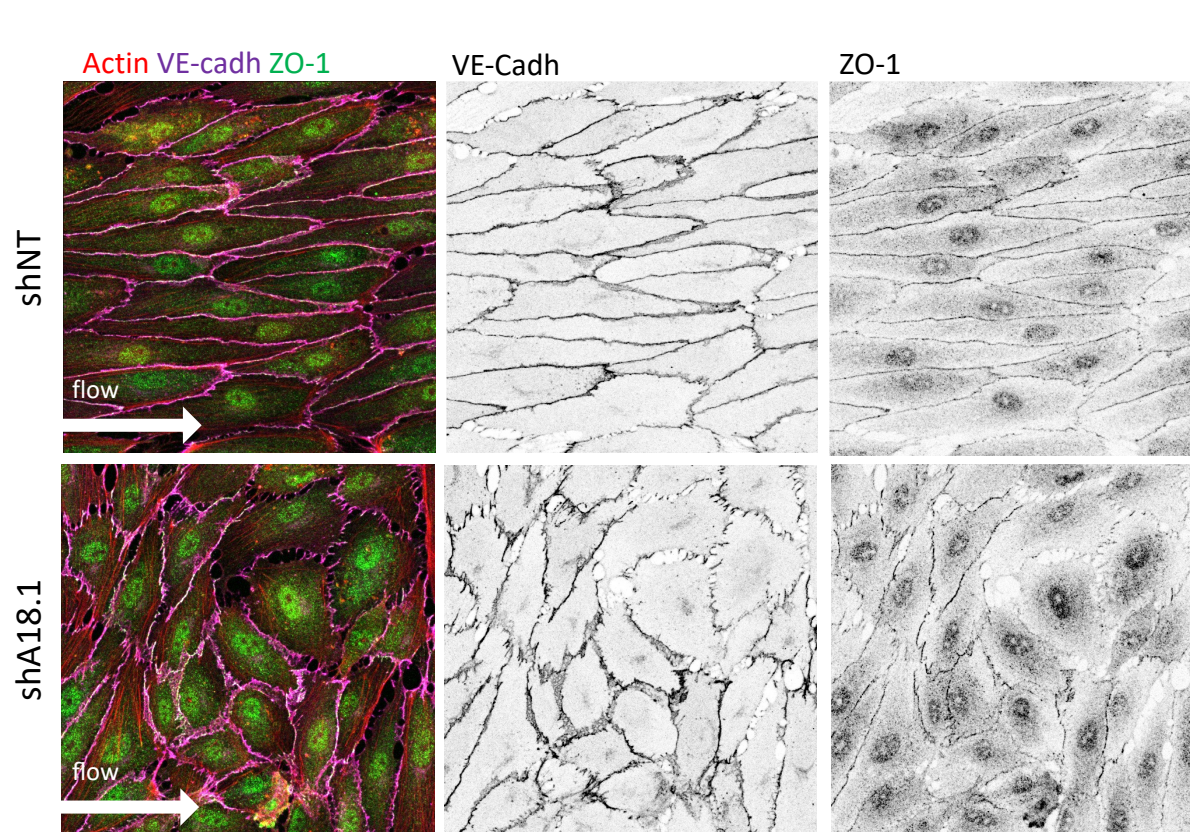


ARHGEF18 interact with Tight Junction proteins

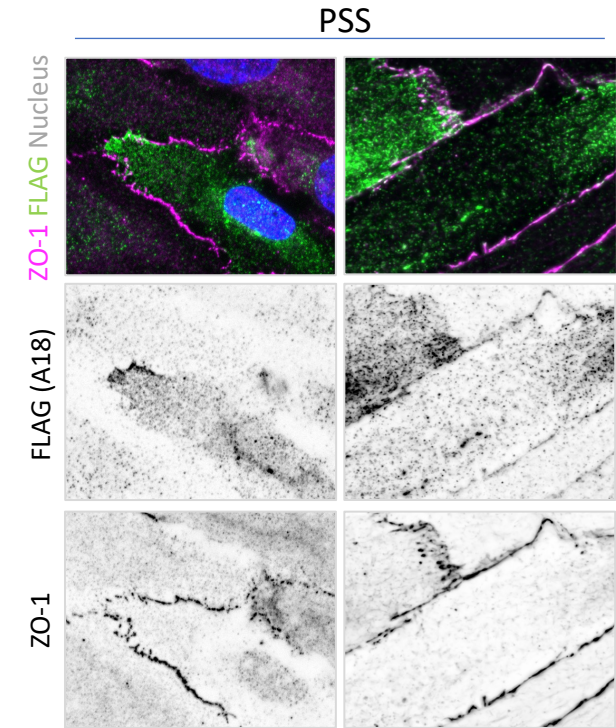


ARHGEF18 interacts with ZO-1 and Claudin5 but not VE-cadherin

ARHGEF18 interact with Tight Junction proteins



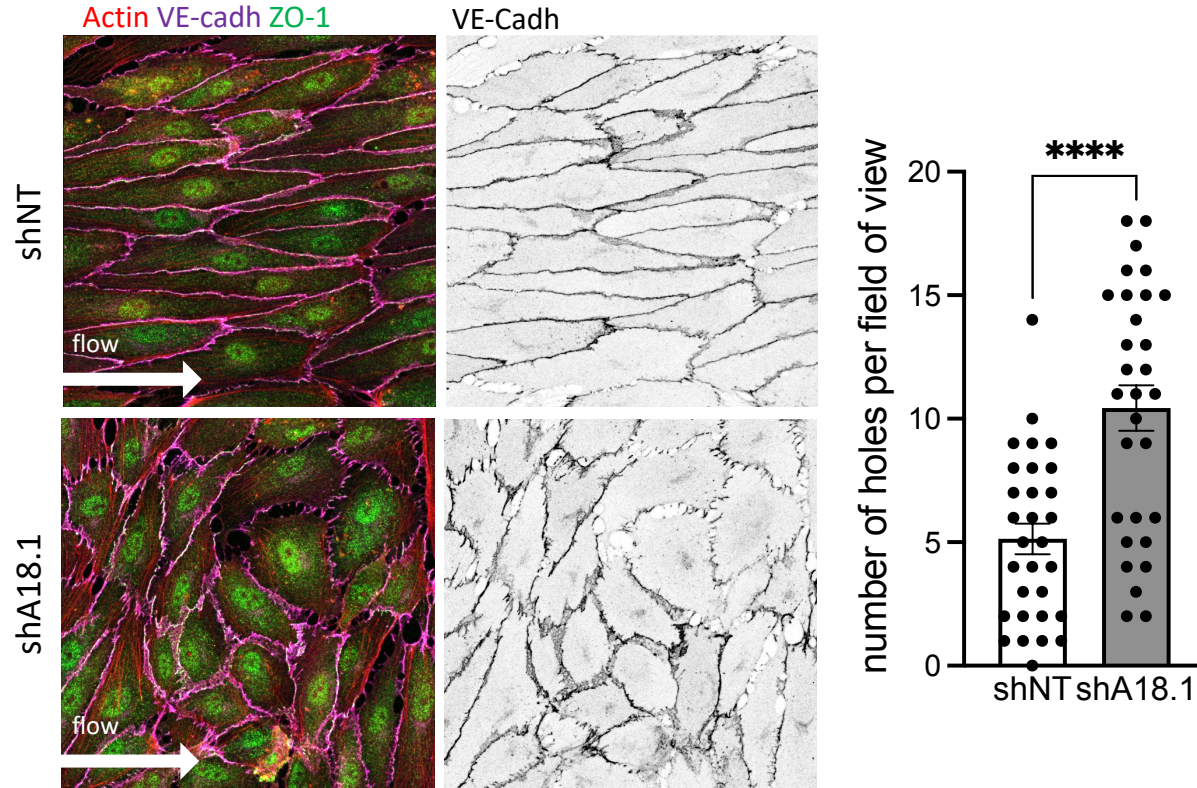
FLAG tag staining



ARHGEF18 interacts with ZO-1 and Claudin5 but not VE-cadherin

Its expression seems localized near to the junction

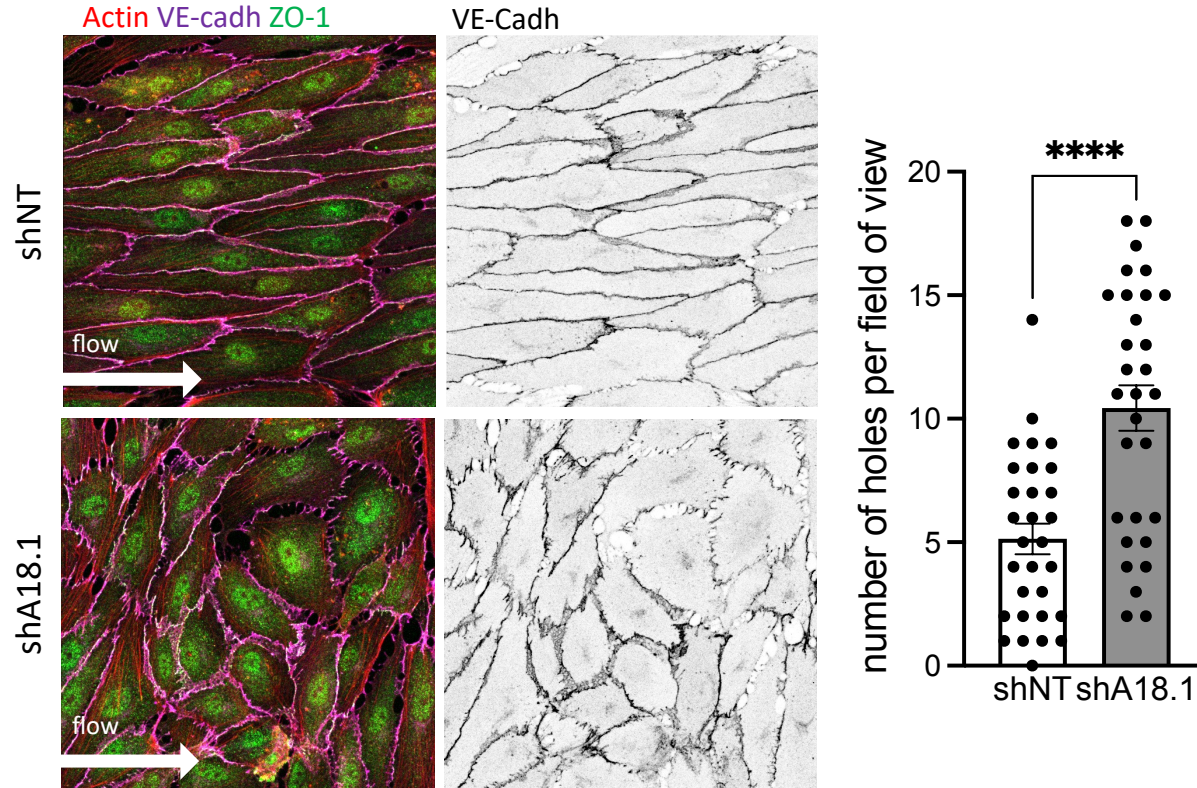
ARHGEF18 favors cell-cell junctions integrity



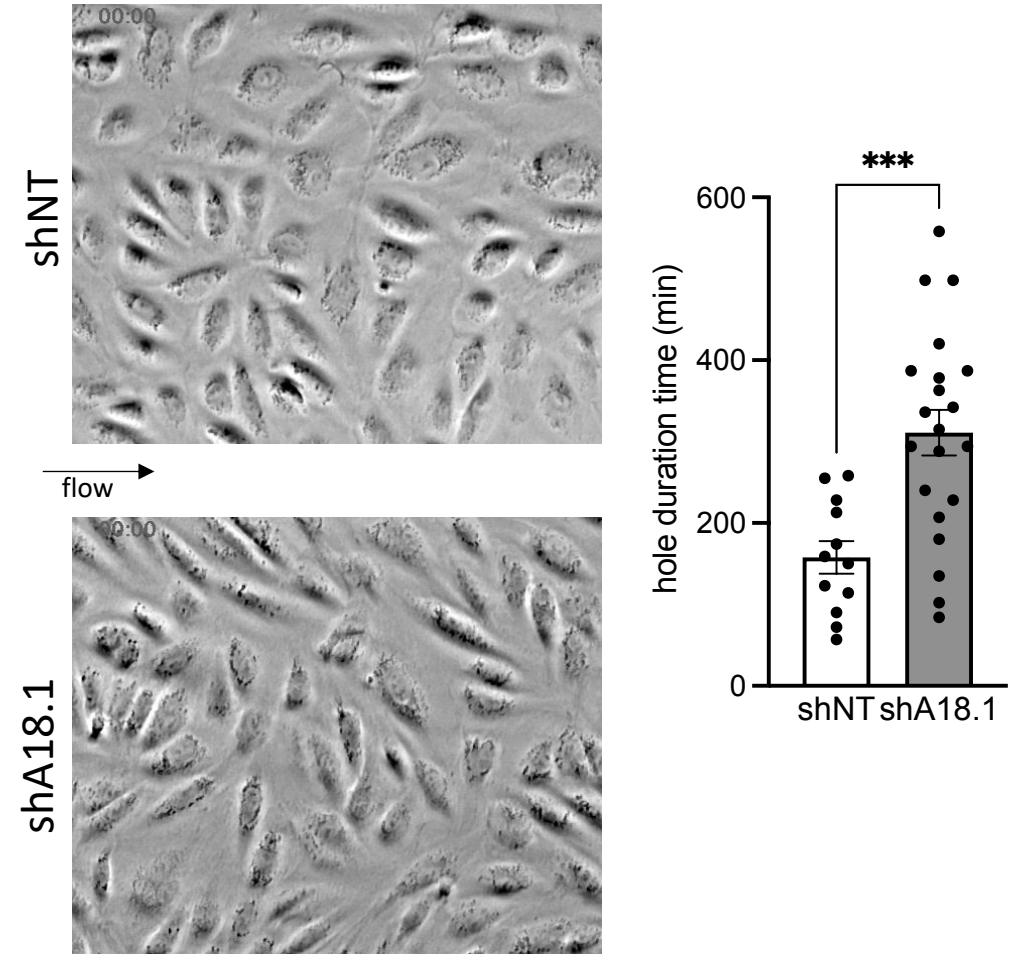
ARHGEF18 deficient ECs present holes in between cells

ARHGEF18 favors cell-cell junctions integrity

Dynamics of holes formation under flow



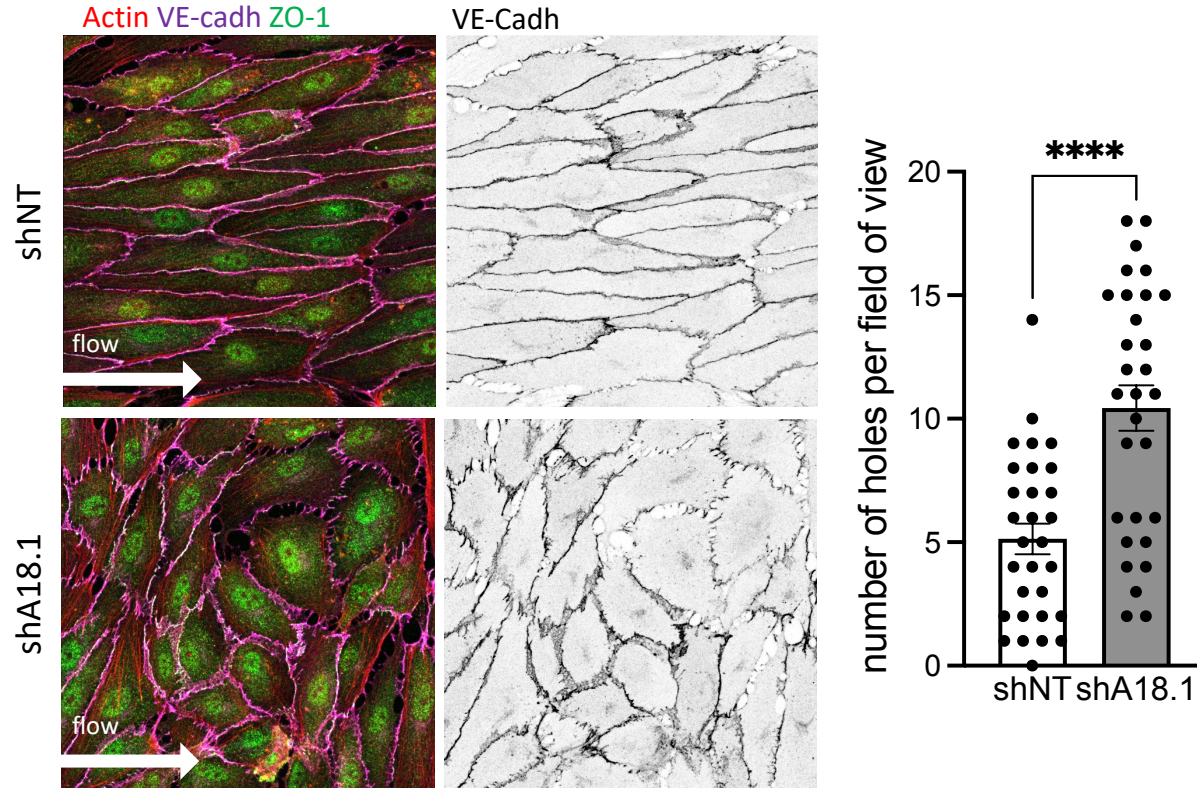
ARHGEF18 deficient ECs present holes in between cells



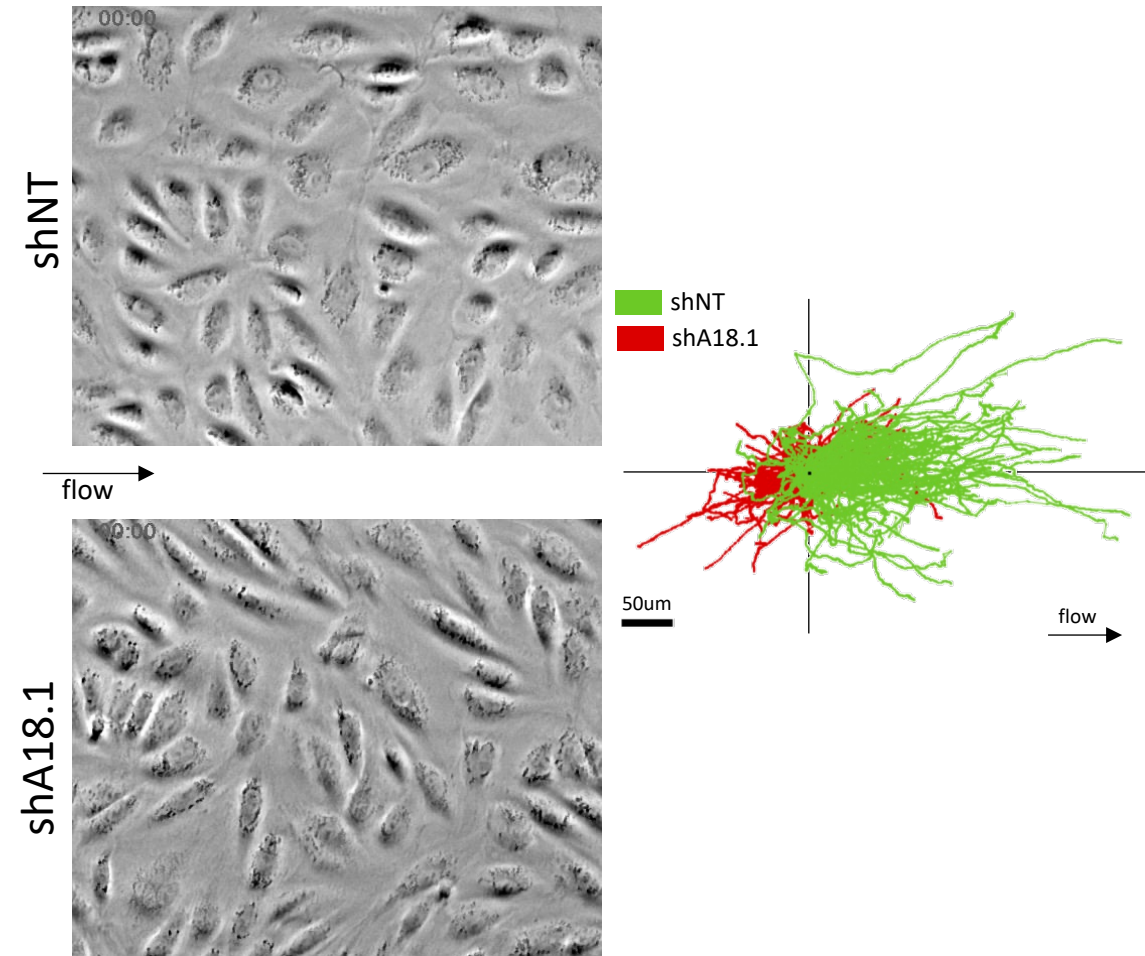
ARHGEF18 deficient ECs fail to close holes under flow
... And to migrate in the direction of the flow

ARHGEF18 favors cell-cell junctions integrity

Dynamics of holes formation under flow

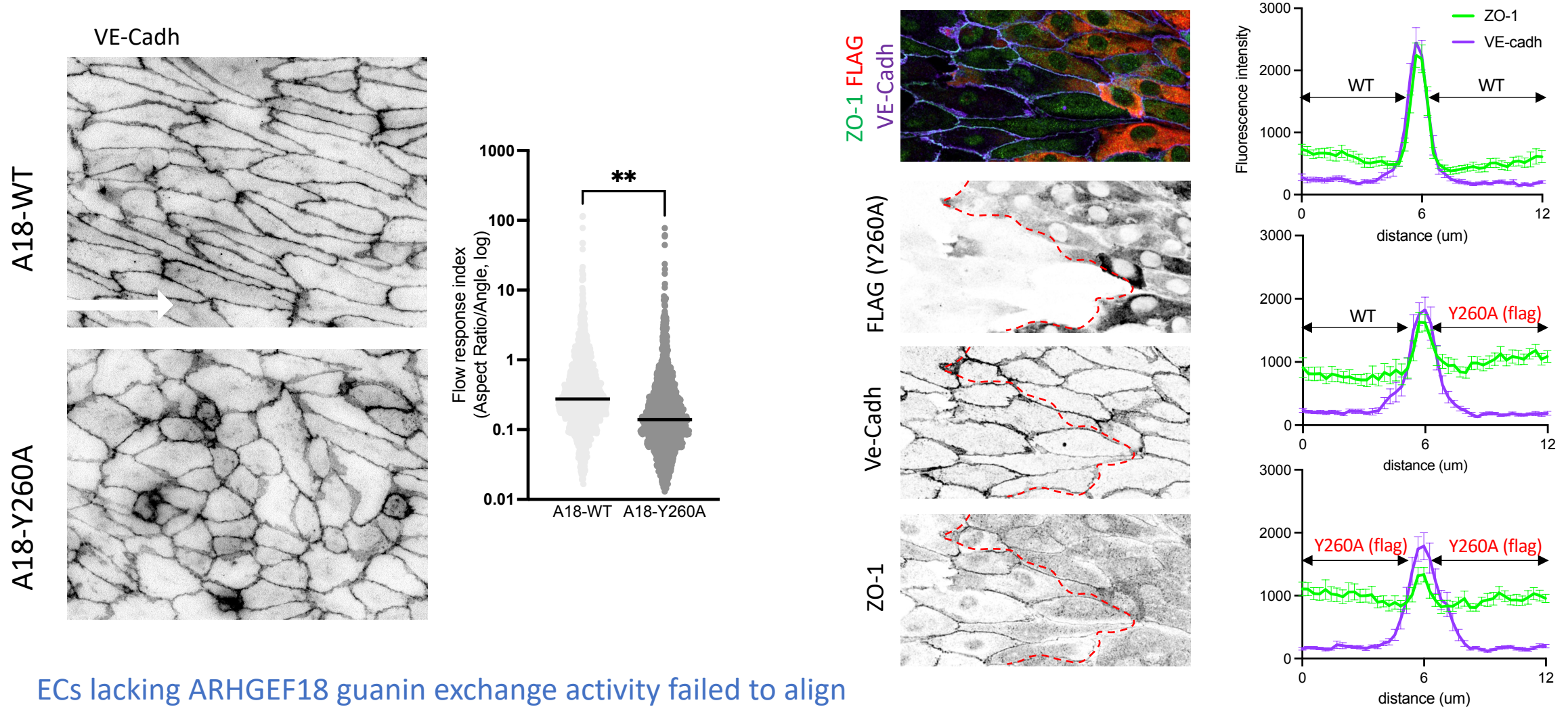


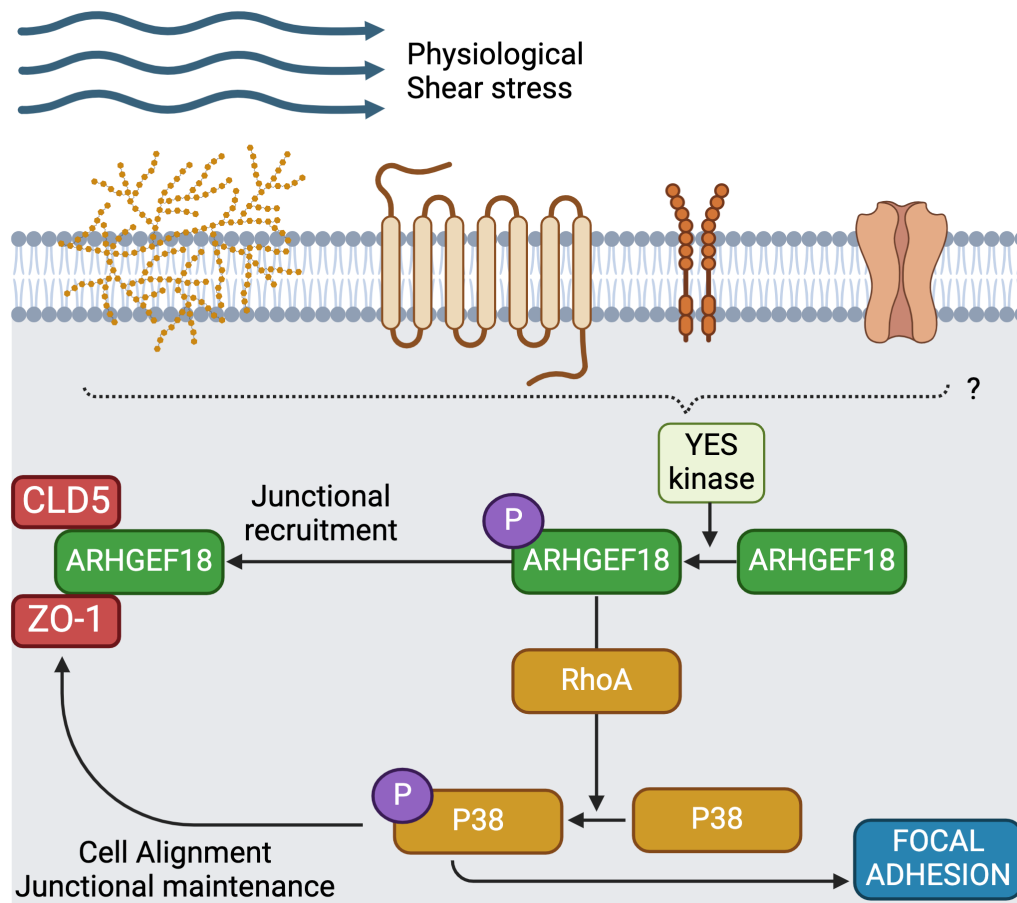
ARHGEF18 deficient ECs present holes in between cells



ARHGEF18 deficient ECs fail to close holes under flow

Nucleotide exchange activity contributes to alignment and tight junctions





In vitro



Orientation and elongation
Migration
Tight Junction / permeability
Basal adhesion

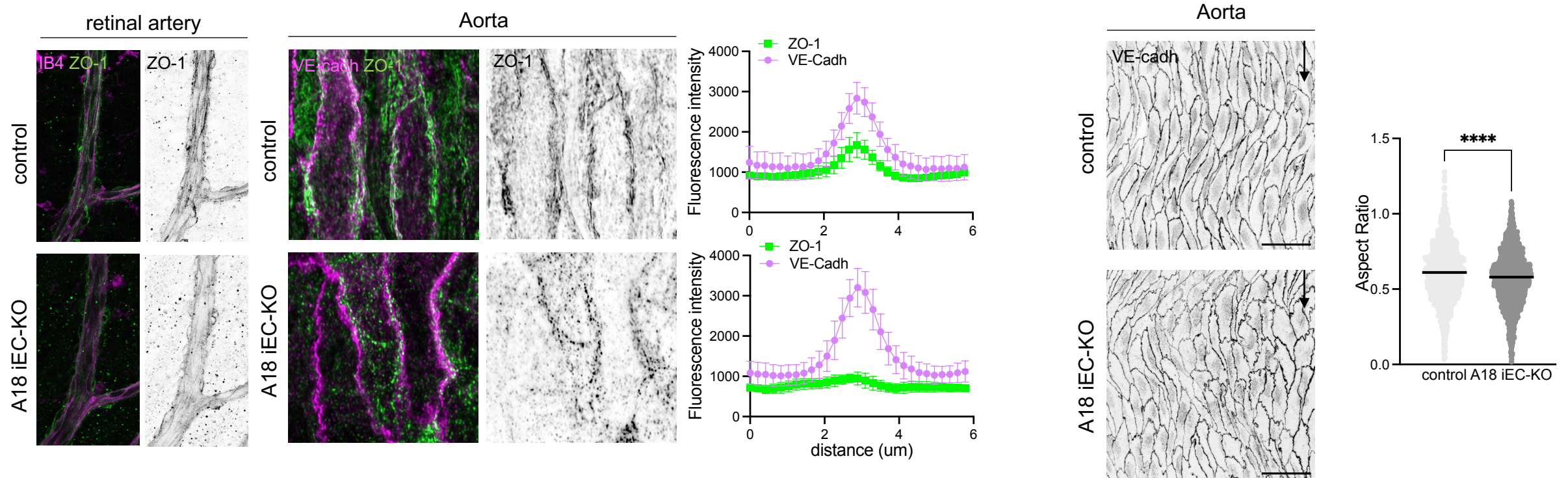


ARHGEF18 contributes to Tight Junction formation in vivo



Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

P6 retinas/aortas



ZO-1 localization at TJs is reduced in Arhgef18-iEC mice compared to control
Elongation in response to flow is altered



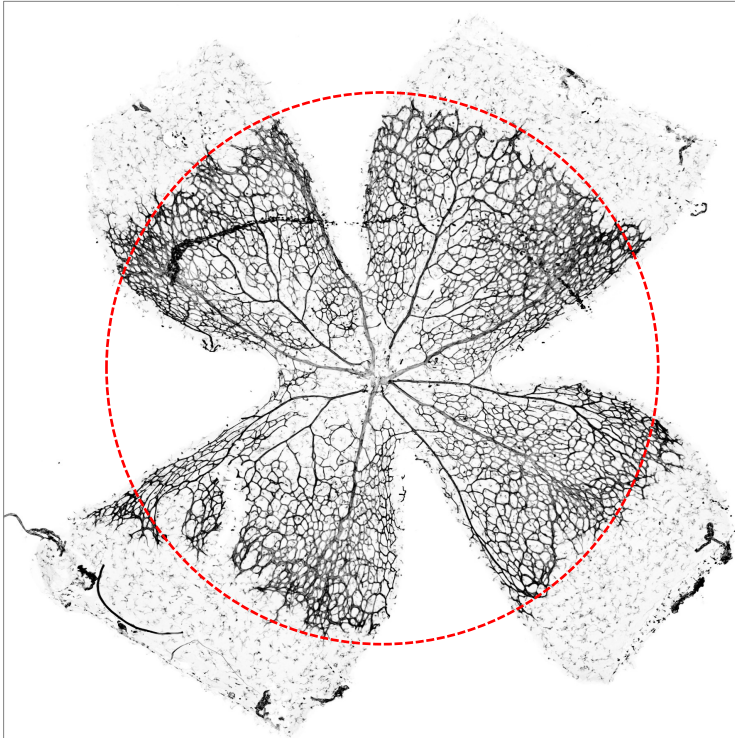
ARHGEF18 participates in developmental angiogenesis



Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

Arhgef18^{lox/lox}

P6 retinas





ARHGEF18 participates in developmental angiogenesis

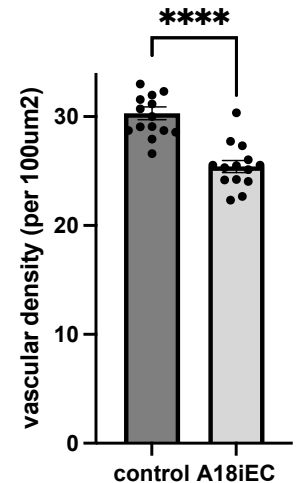
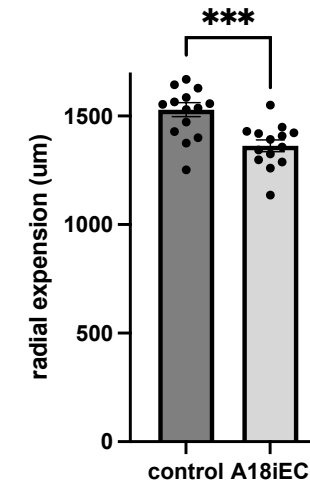
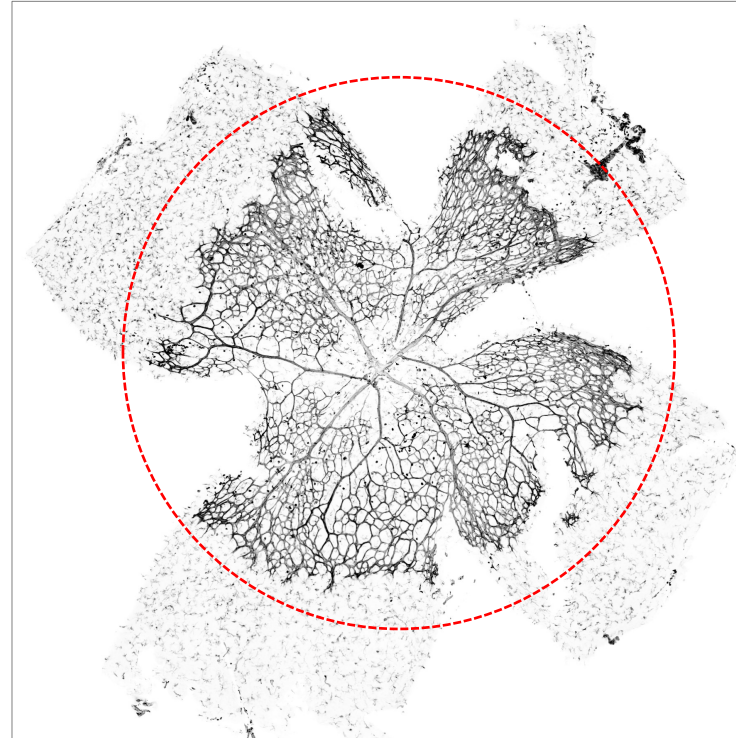
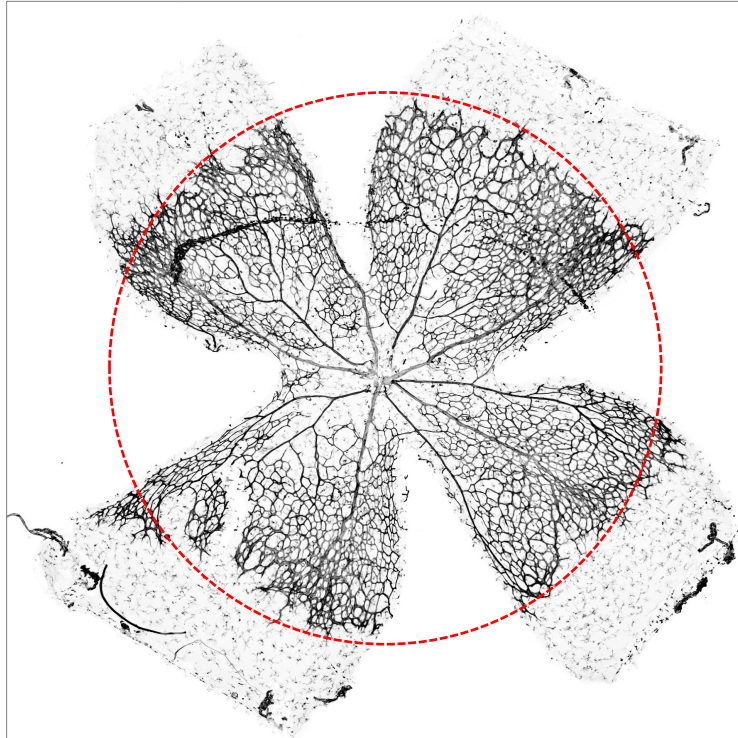


Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

Arhgef18^{lox/lox}

Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

P6 retinas



Vascular expansion is reduced in *Arhgef18*-iEC mice compared to control

ARHGEF18 prevent retinal hemorrhage in vivo

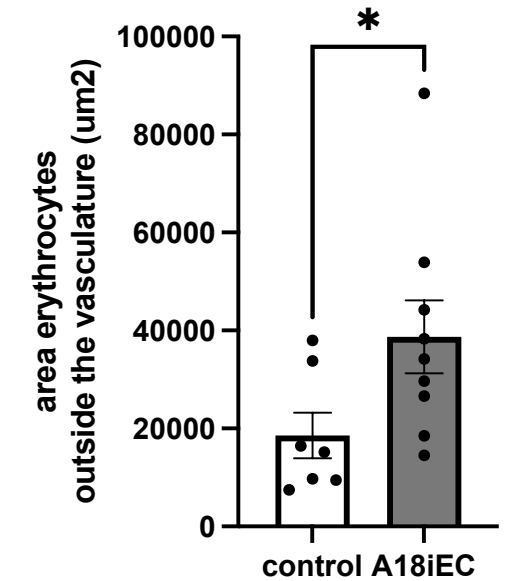
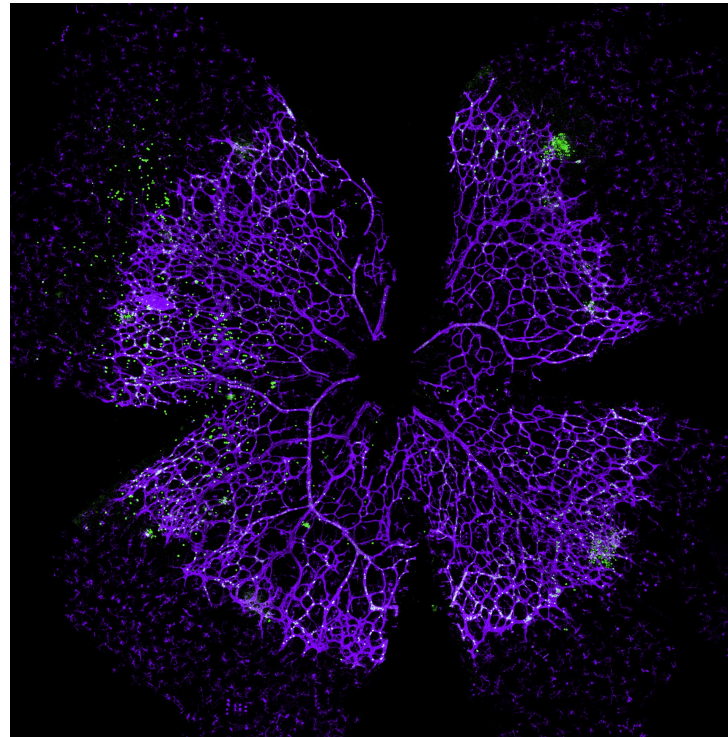
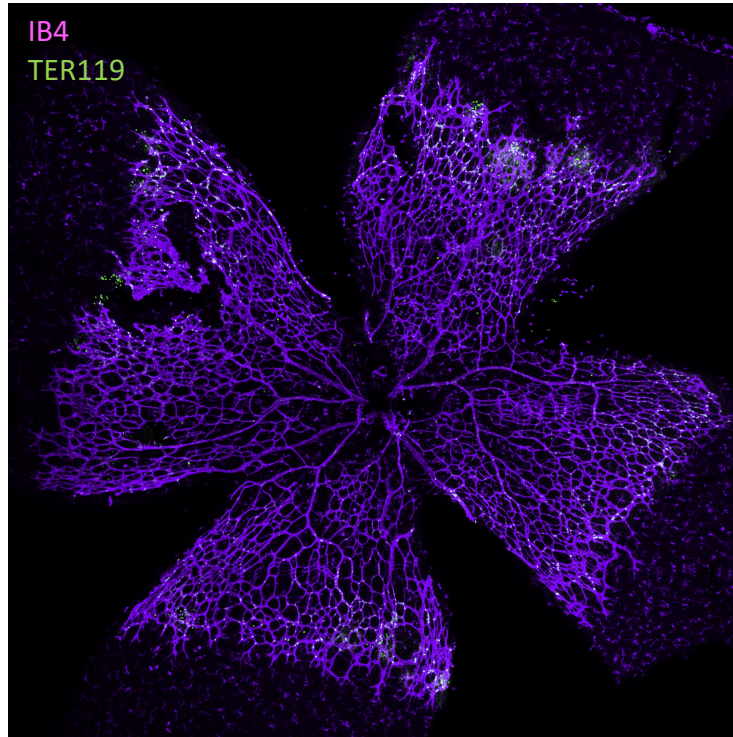


Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

Arhgef18^{lox/lox}

Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

P6 retinas



The Area of Erythrocytes outside the vasculature is reduced in Arhgef18-iEC retinas compared to control

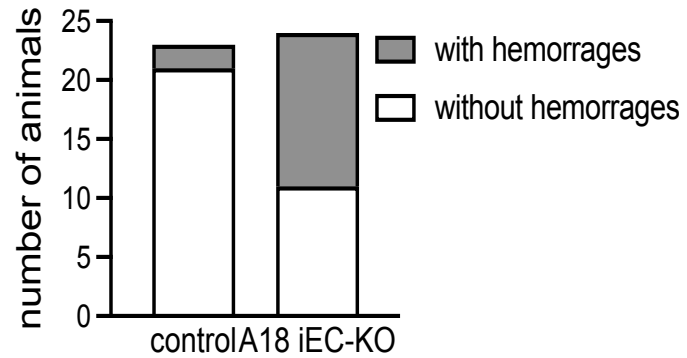


ARHGEF18 prevents brain hemorrhage in vivo

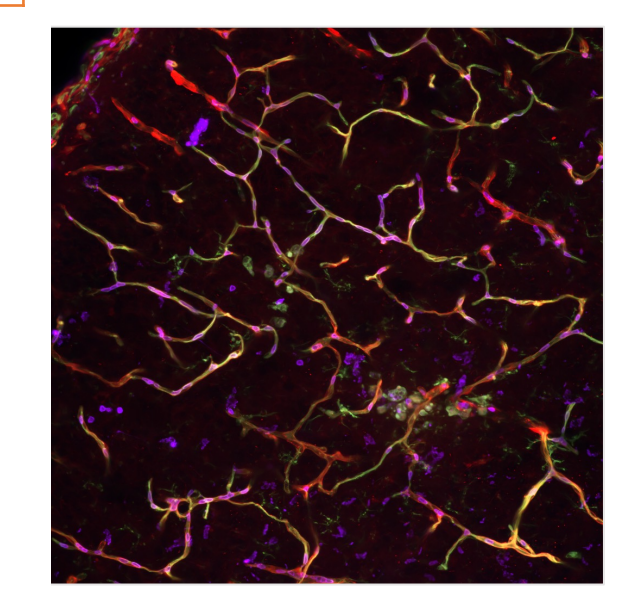
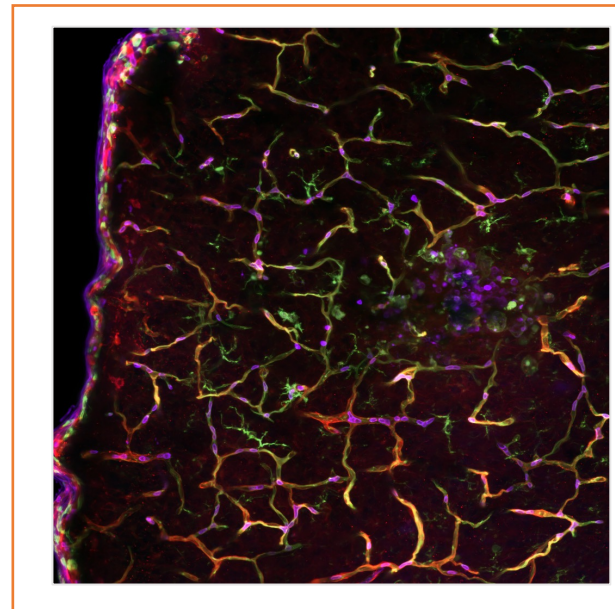
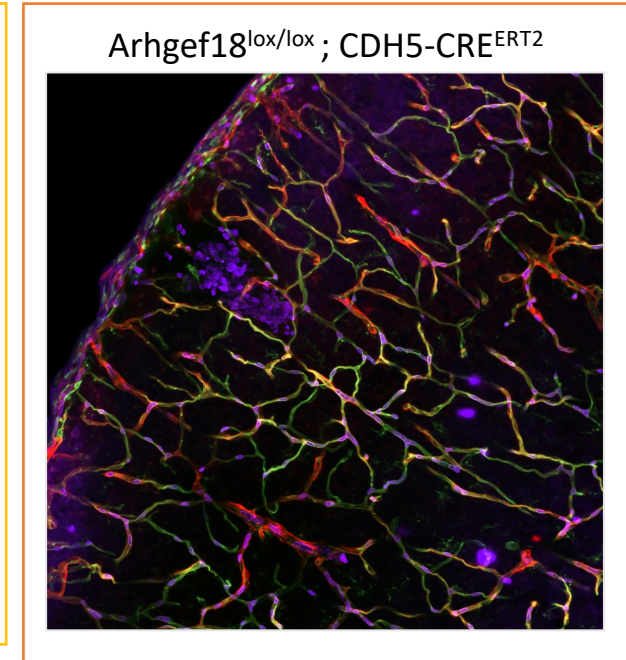
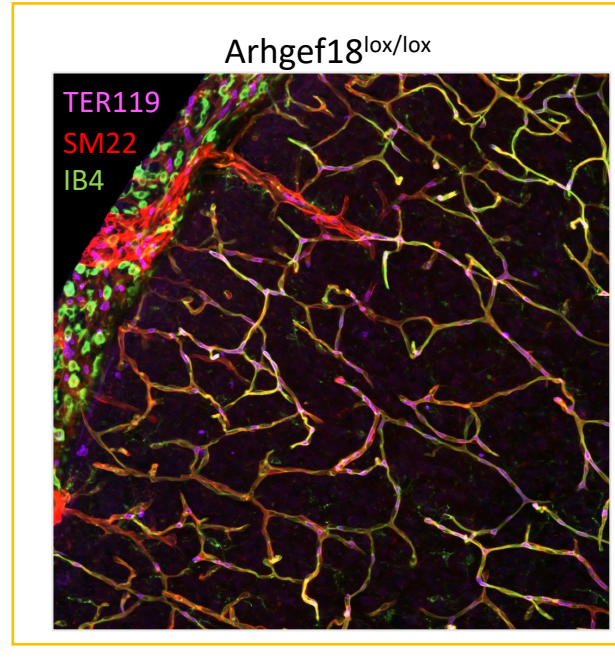


Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

P6 brains



Brains of Arhgef18-iEC pups harbors more visual focal hemorrhages than control





ARHGEF18 prevents vascular leakage in vivo



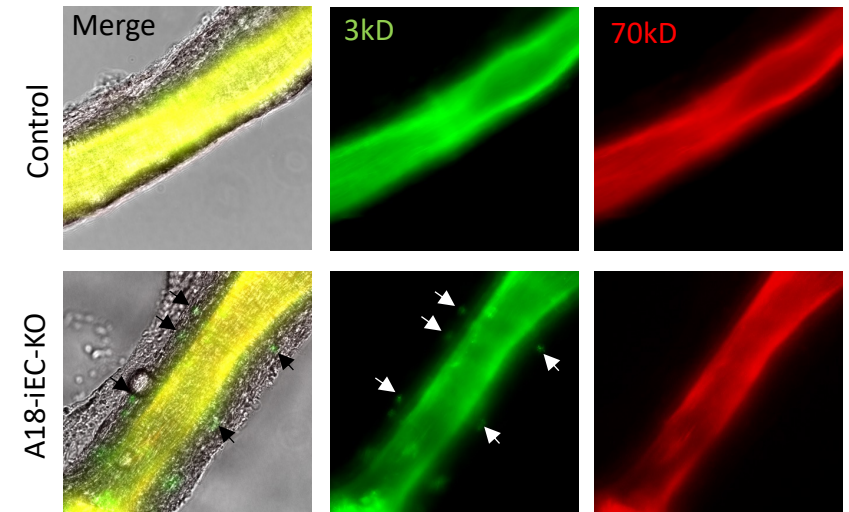
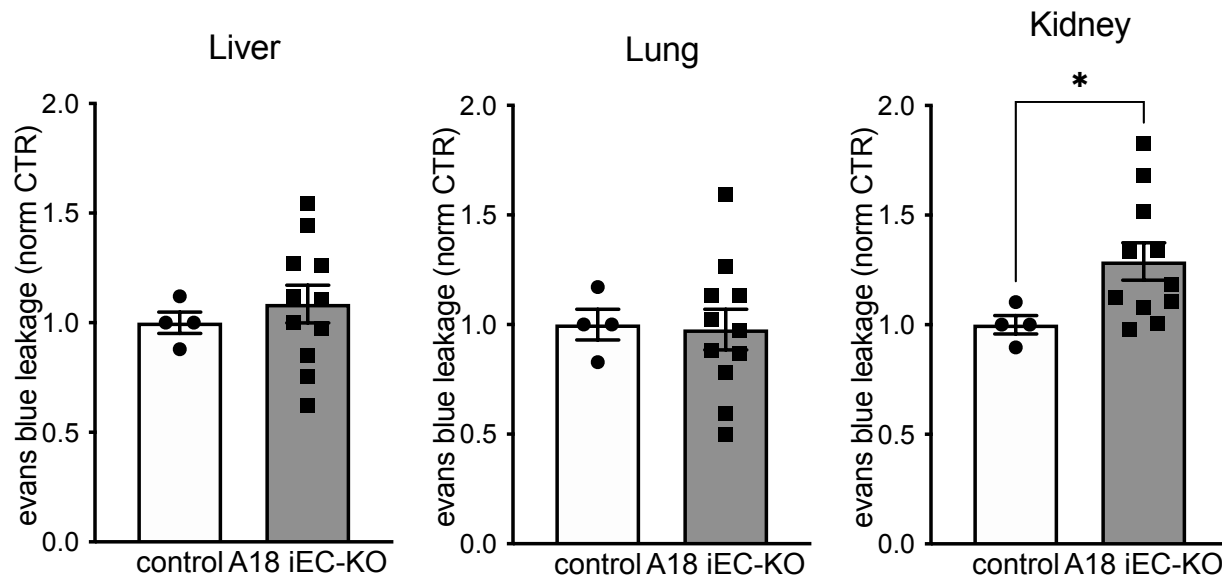
Arhgef18^{lox/lox}; CDH5-CRE^{ERT2}

P6 Brains

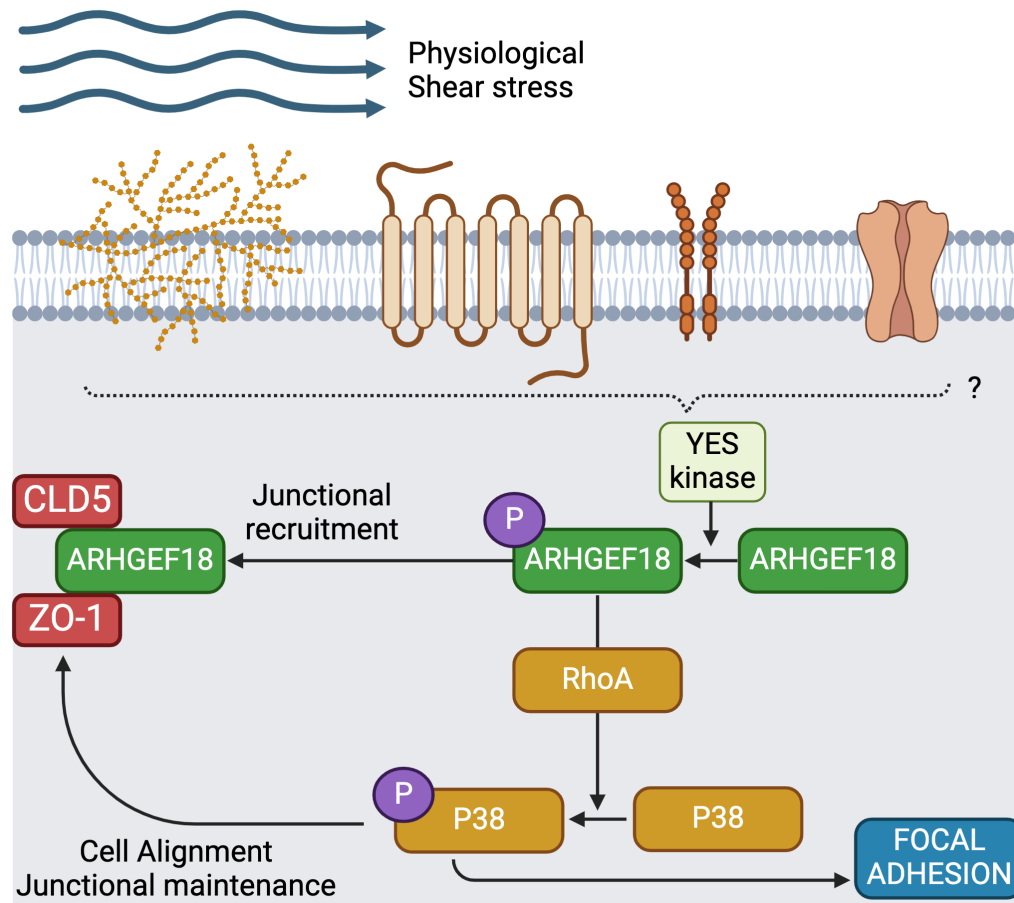


albumin extravasation (Evans Blue)

dextran extravasation



Brains of Arhgef18-iEC pups have increase vascular leakage in the kidney and in the mesenteric arteries



In vitro



Orientation and elongation
Migration
Tight Junction / permeability
Basal adhesion

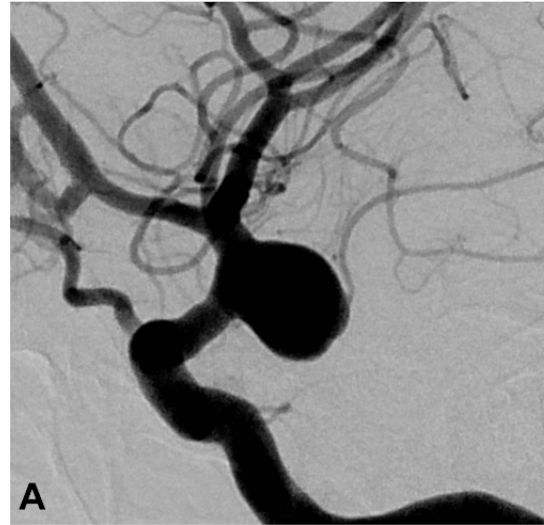
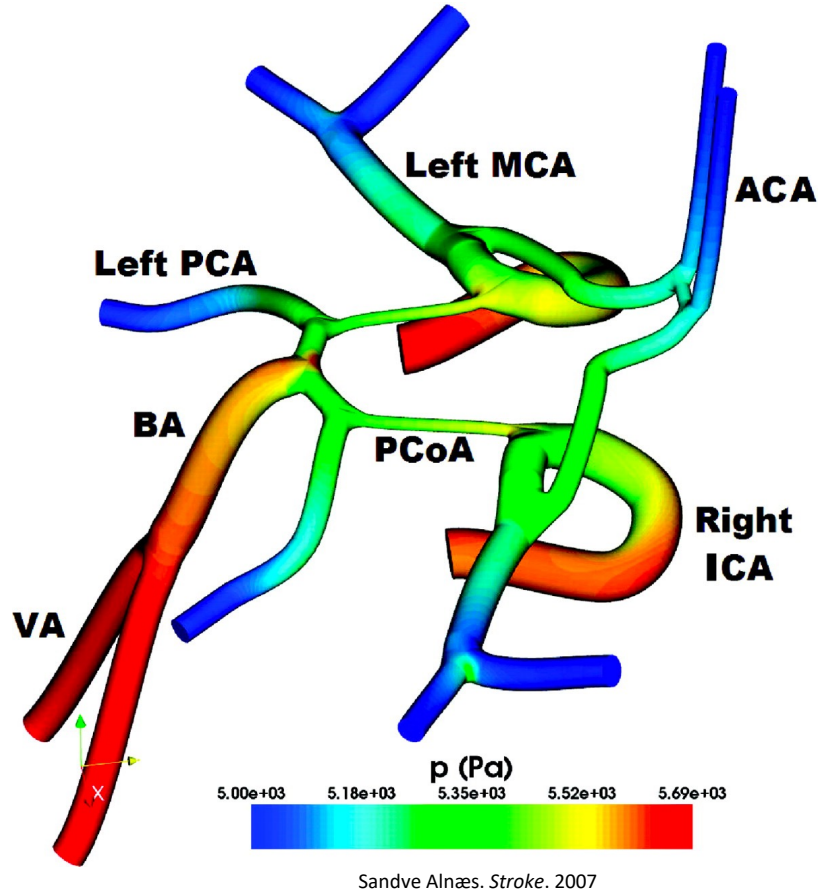
In vivo



conducting arteries :
Elongation/tight junctions

Resistance arteries :
Permeability /vascular leakage

Intracranial Aneurysm : causes and consequences



- 3% of the general population
- unpredictable rupture
- 50% of death in case of rupture

- Risk factors :

- Smoking, alcohol consumption, **hypertension**
- female sex, age, **familial history**

- physiopathology :

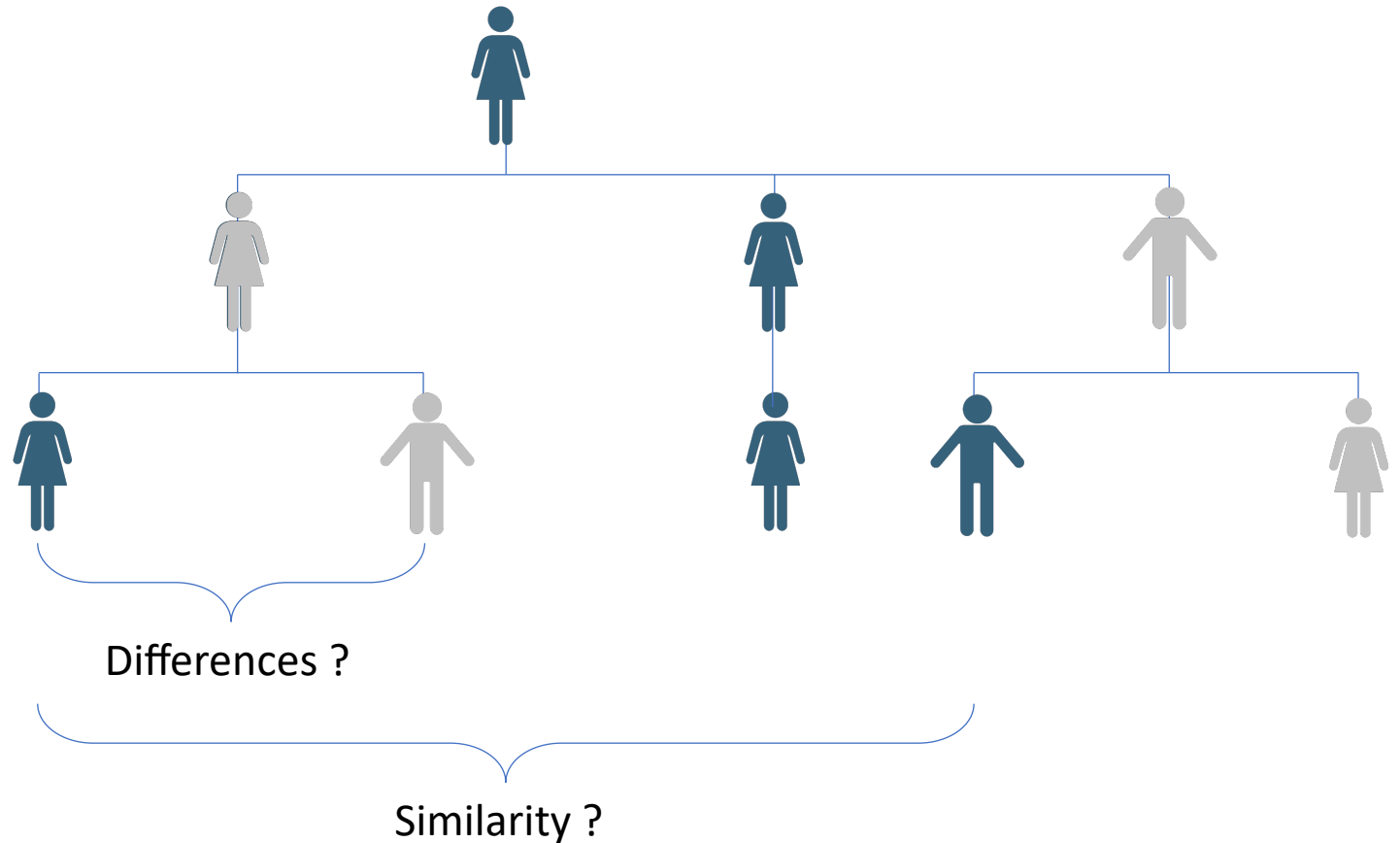
- endothelial dysfunction
- inflammation
- remodeling of the vascular wall
- **molecular mechanisms at play are mostly unknown**
- associated with altered hemodynamics

Using of the familial form ?

What is a familial form ?

A patient with at least two close relatives with the disease

Why are they suffering from the disease ?
How can we use their peculiarity ?



This forms represent 10% of
the total of ICA

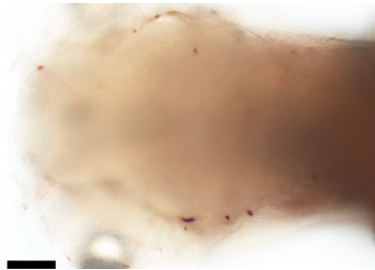
IA and genetics

ADAMTS15
RNF213
THSD1
ARHGEF17
PPIL4

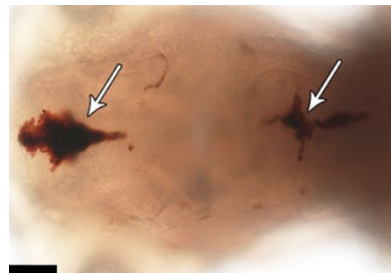
...

zebrafish brain after
adrenaline exposure

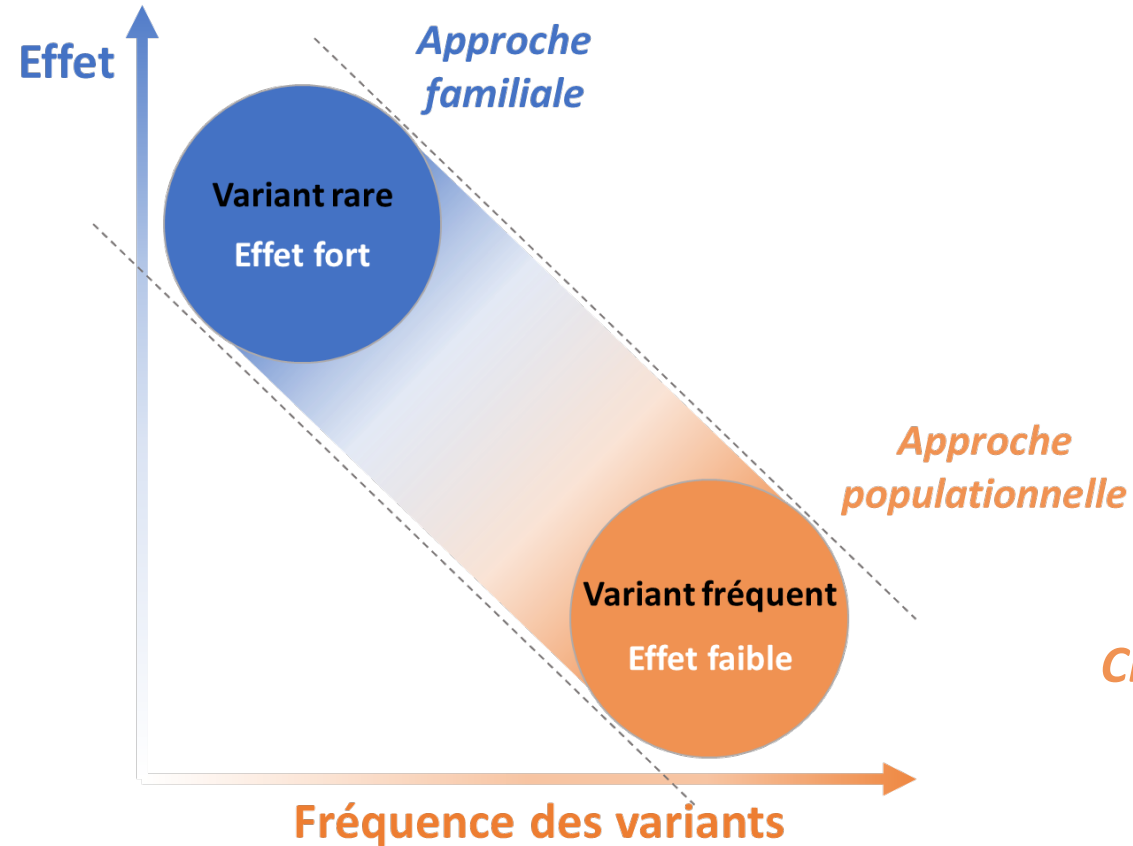
ppil4^{+/+}



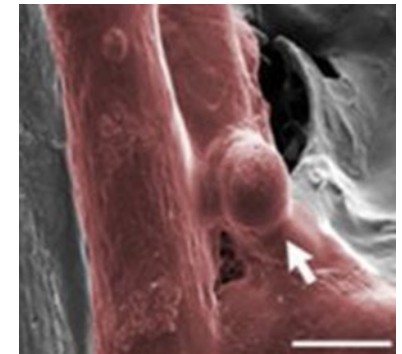
ppil4^{-/-}



Barak *et al.*, Nat Med, 2021



**CoW artery of *Sox17*-KO^{EC}
after angiotensin treatment**



Lee *et al.*, Circulation, 2015

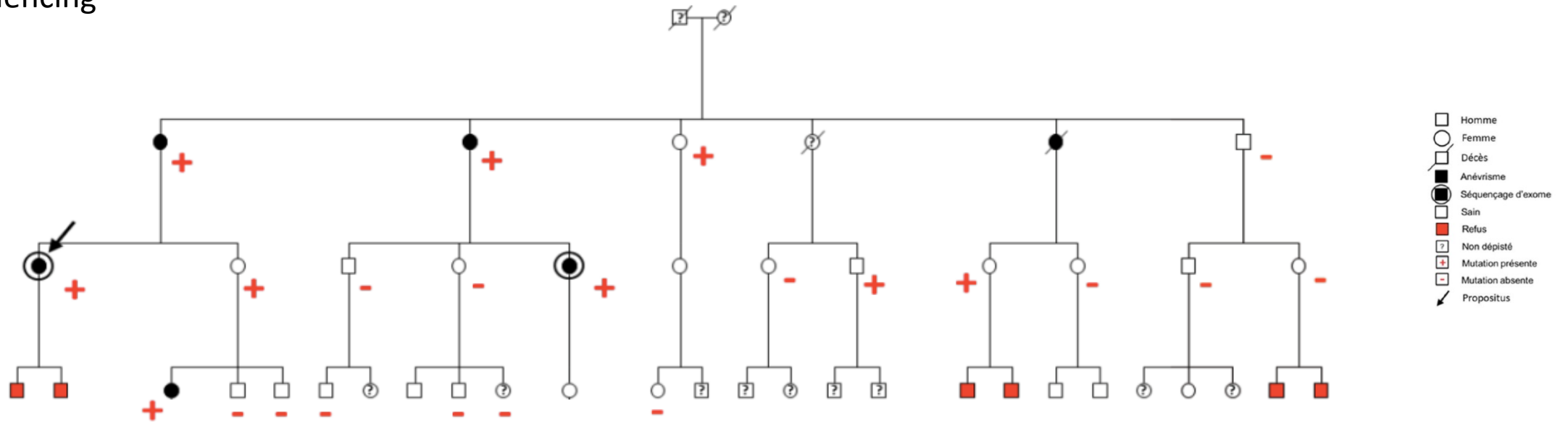
EDNRA
CDKN2A/B/BAS
SOX17

...

Using of the familial form ?

Bourcier *et al.*, Am. J. Hum. Genet., 2018

Exome sequencing



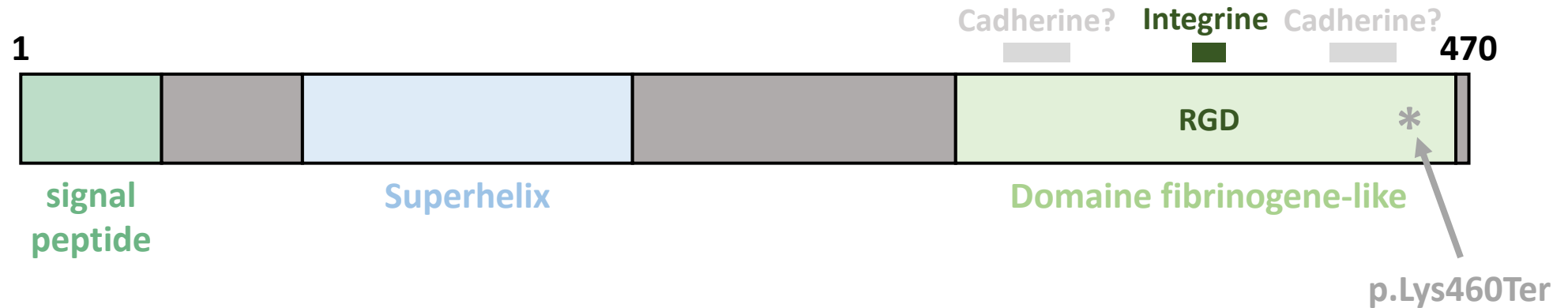
Identification of a mutation in the gene coding for the ANGPTL6 protein:

- Reduced liver production
- less protein in the blood

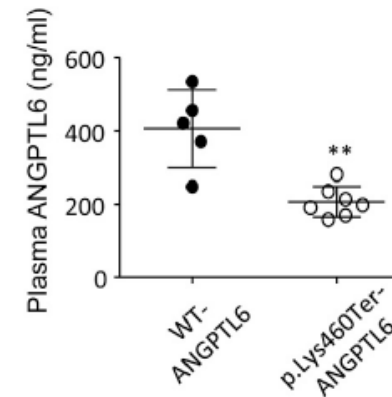
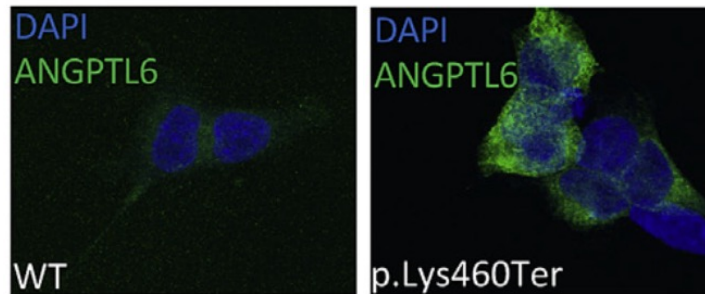
How does this mutation contribute to the formation of intracranial aneurysms?

what would you first do ?

the p.Lys460Ter-ANGPTL6 variant



➡ Rare variant p.Lys460Ter-ANGPTL6 predicted as loss of function



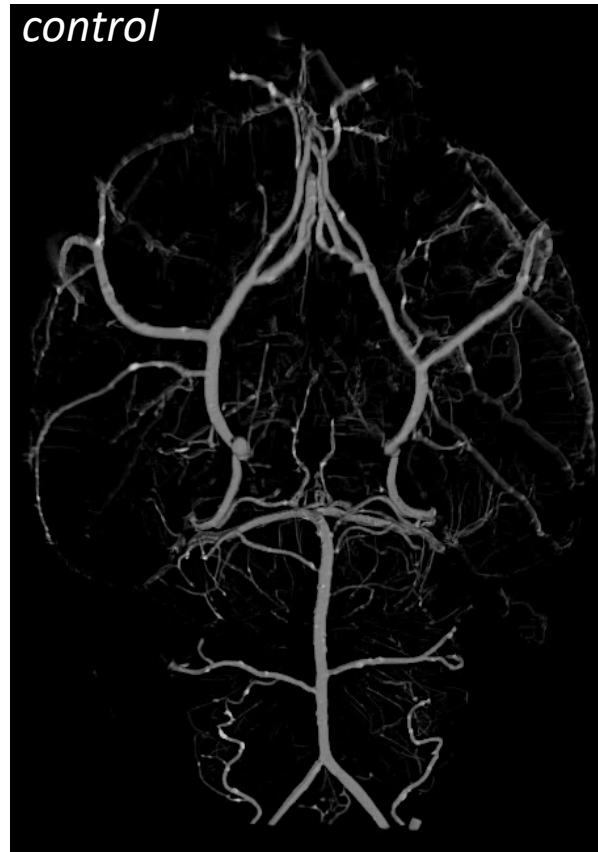
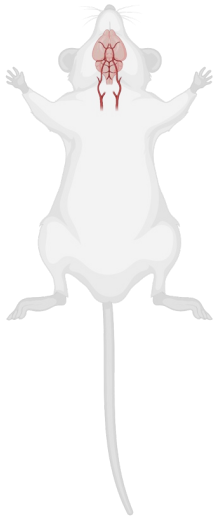
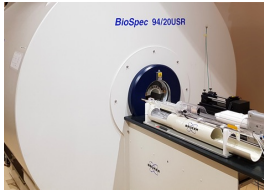
➡ Plasma concentration decreased by half in patients carrying the variant

How can we explore and decipher the role of this mutation in IA formation ?

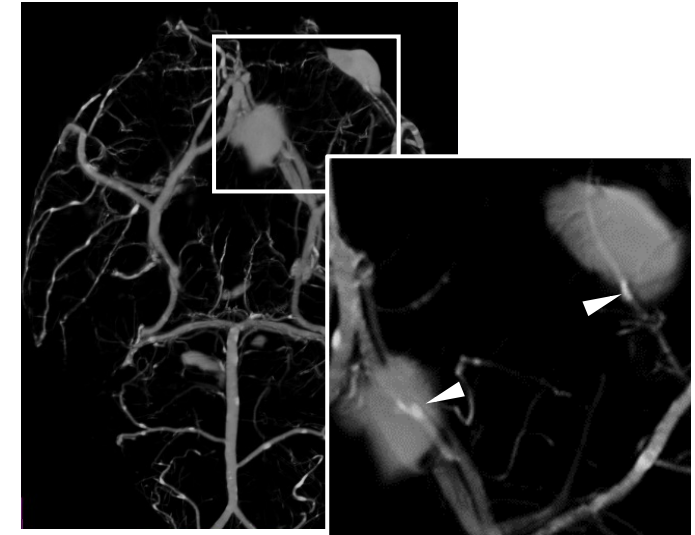
YOUR IDEAS ?

How are the cerebral arteries of mutant mice?

Hypertension
CT scan



Angptl6^{Δ/Δ}

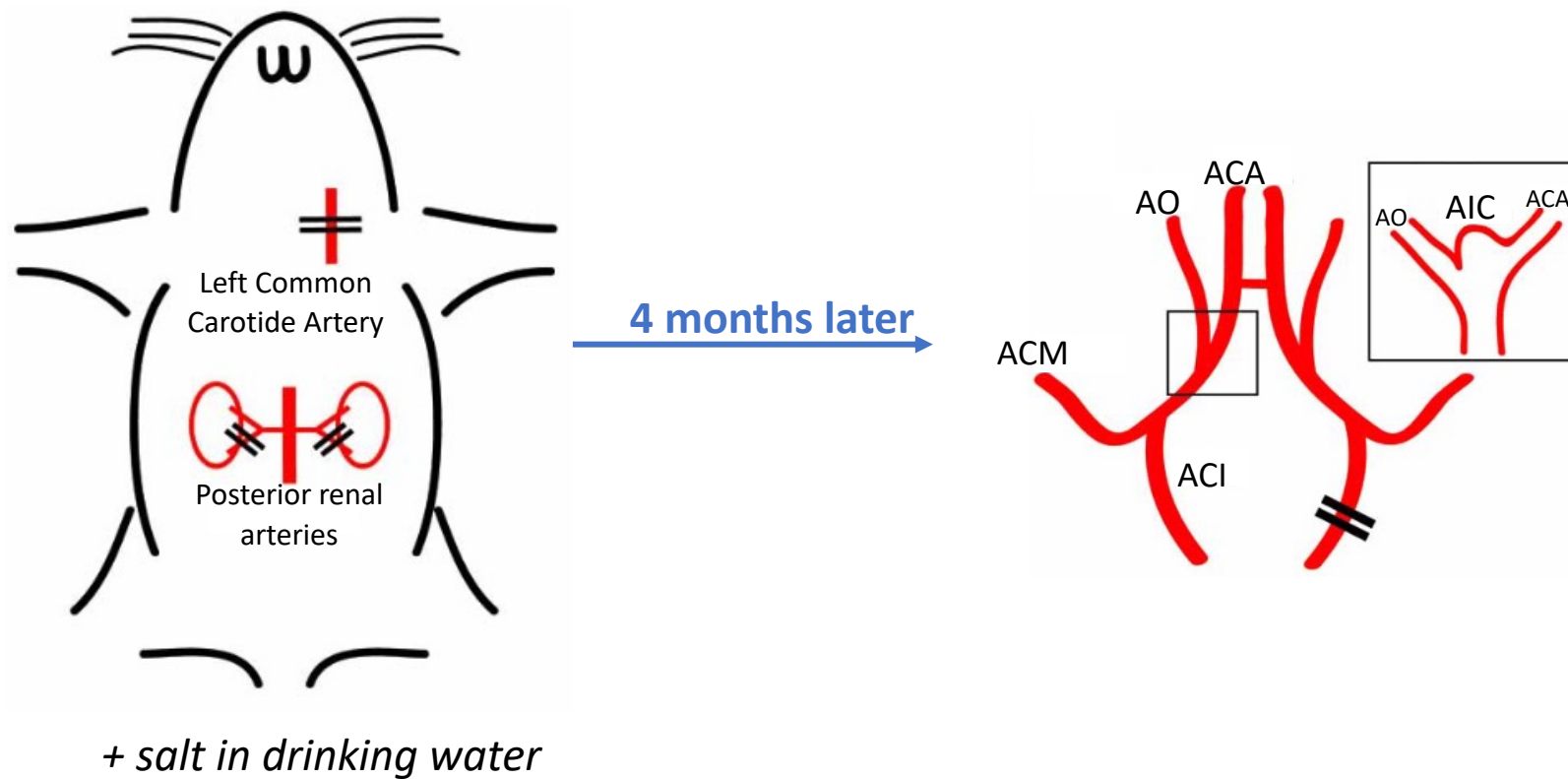


Arteries in mutant mice show local dilatation

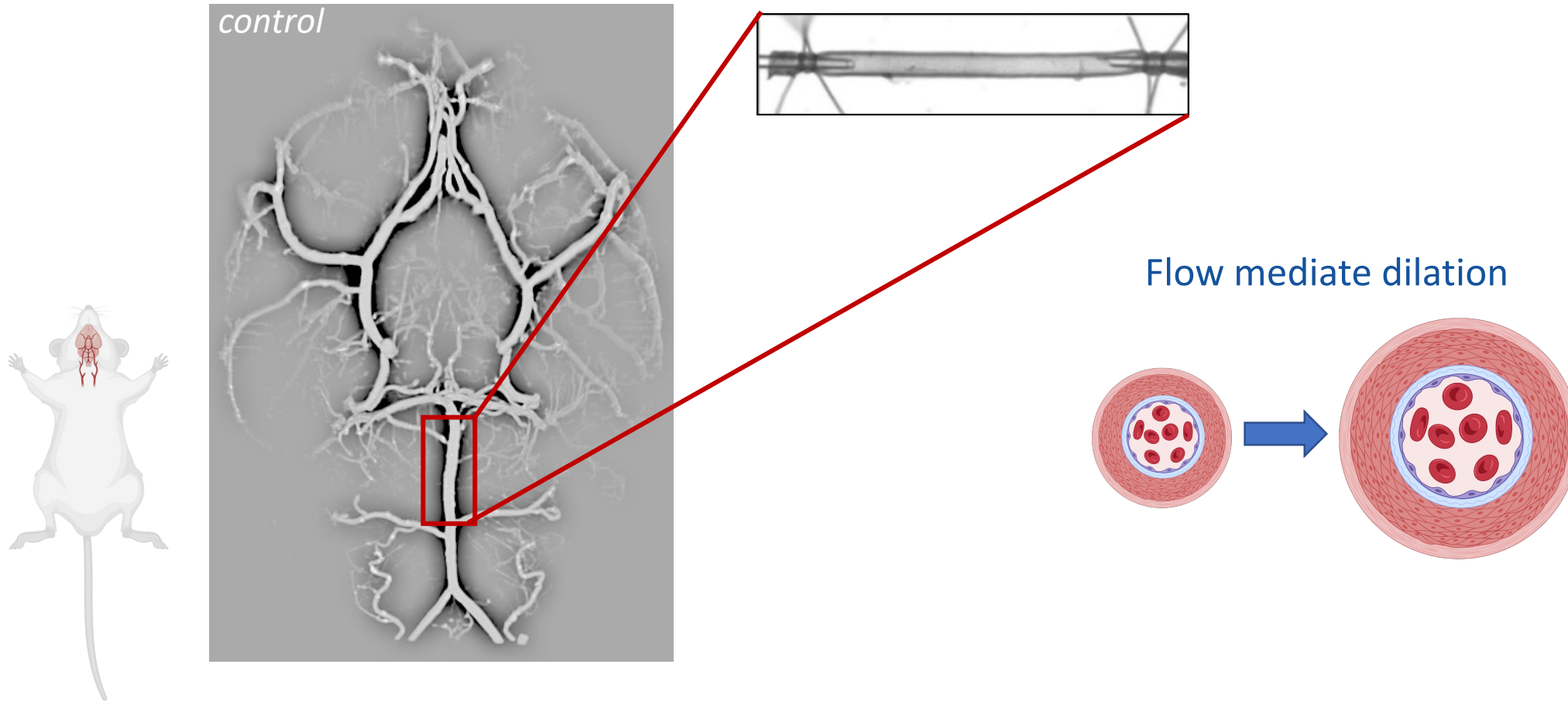
unpublished data

What is the role of the hemodynamics ?

Morimoto model

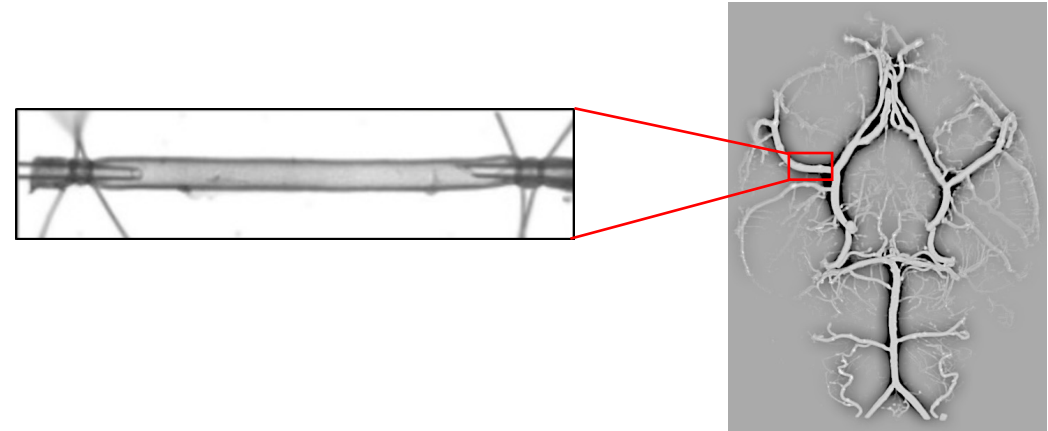
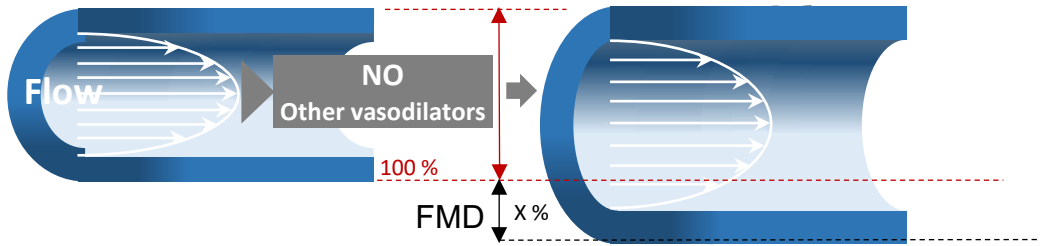


Are the cerebral arteries of those mice able to dilate properly ?



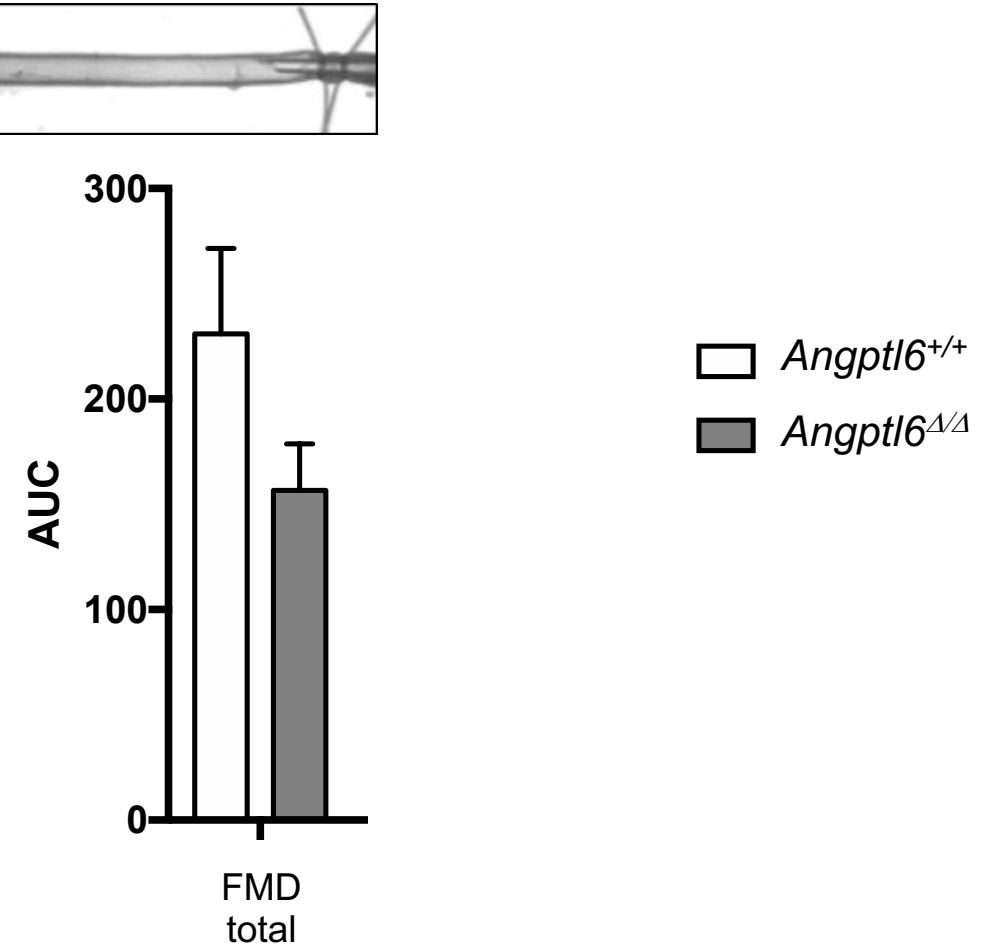
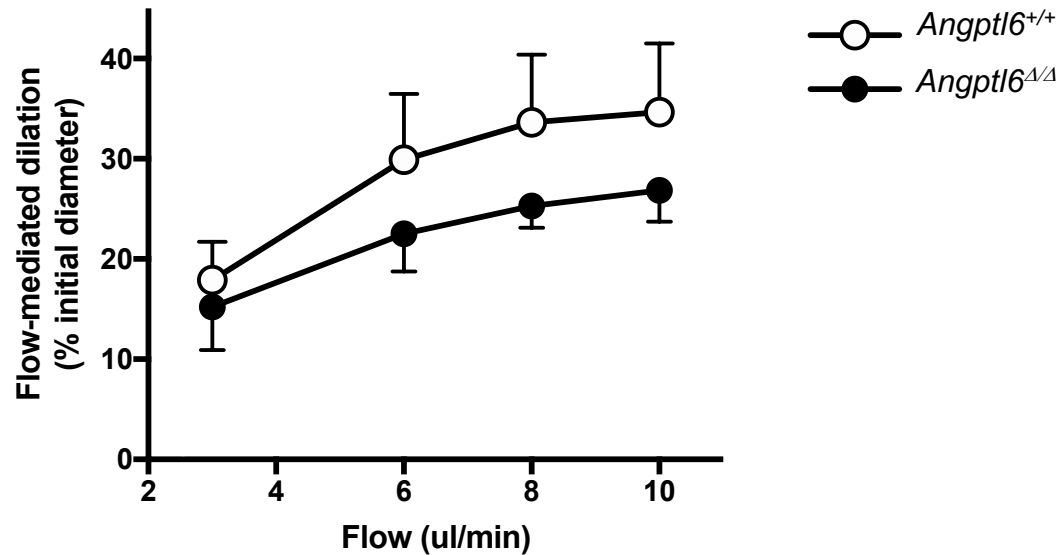
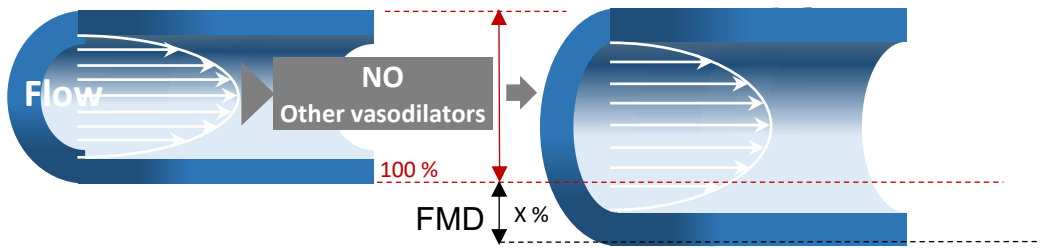
Does *Angptl6* mutation affect brain arteries dilation ?

Flow mediated dilation of brain arteries



Does *Angptl6* mutation affect brain arteries dilation ?

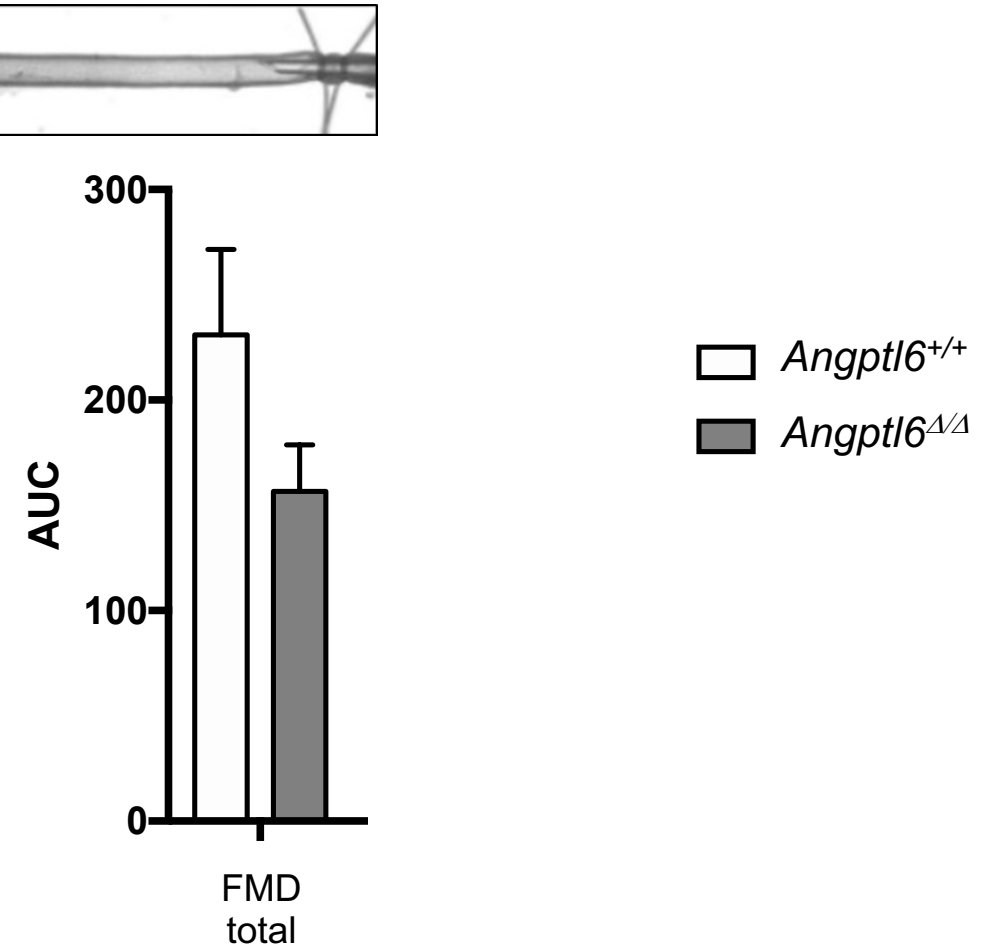
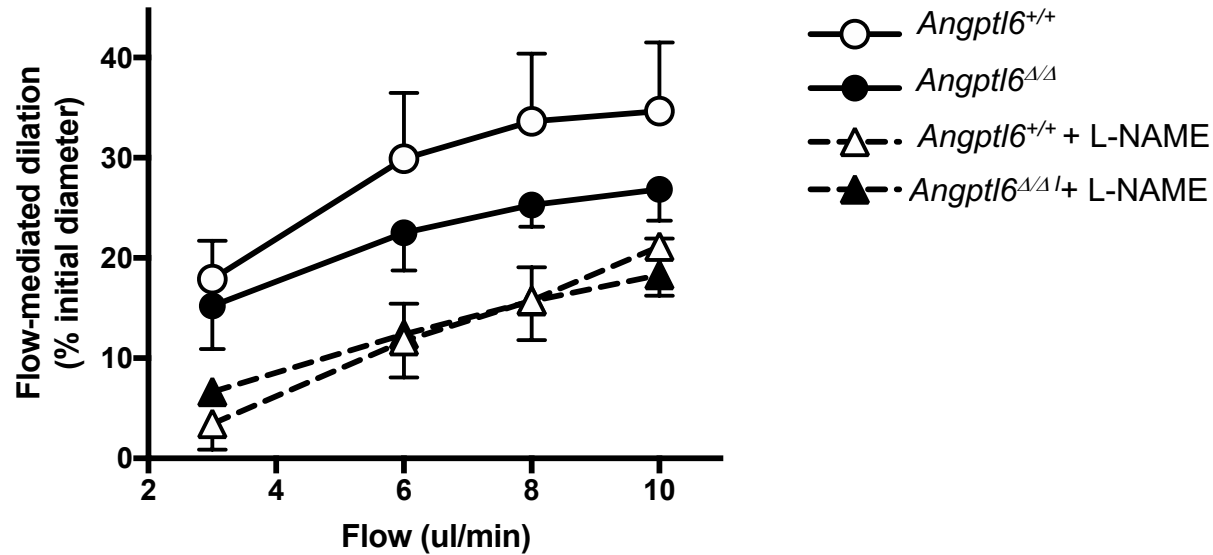
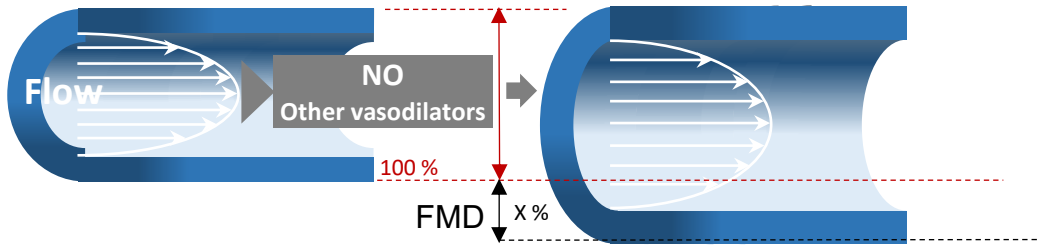
Flow mediated dilation of brain arteries



Arteries in mutant mice dilate less in response to blood flow than control

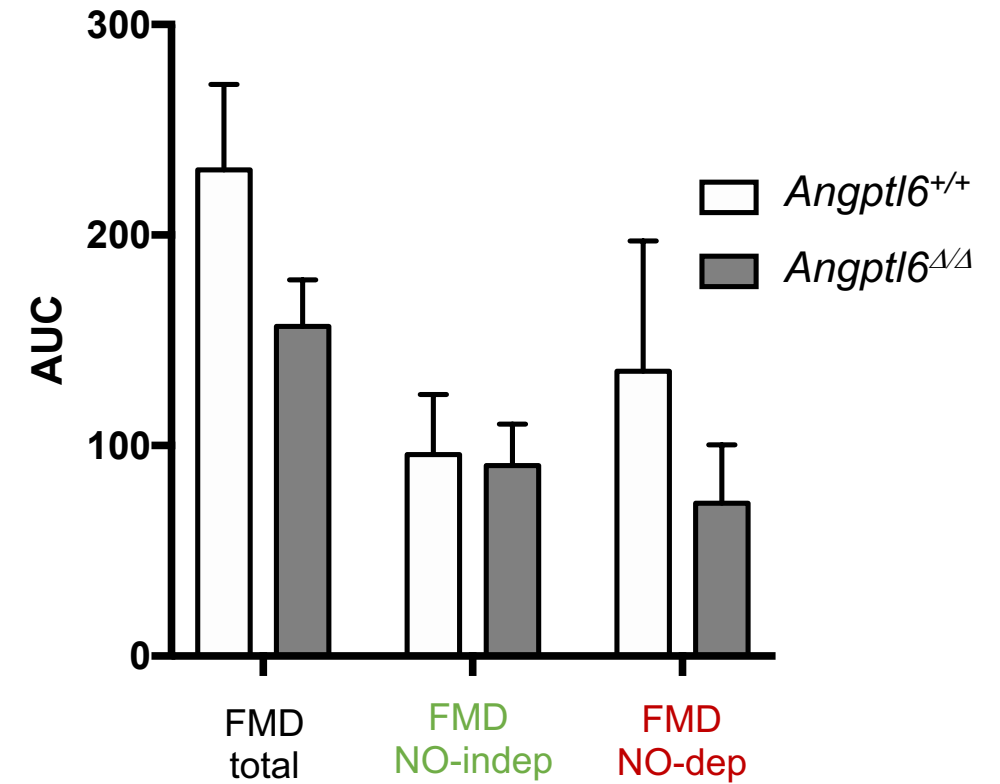
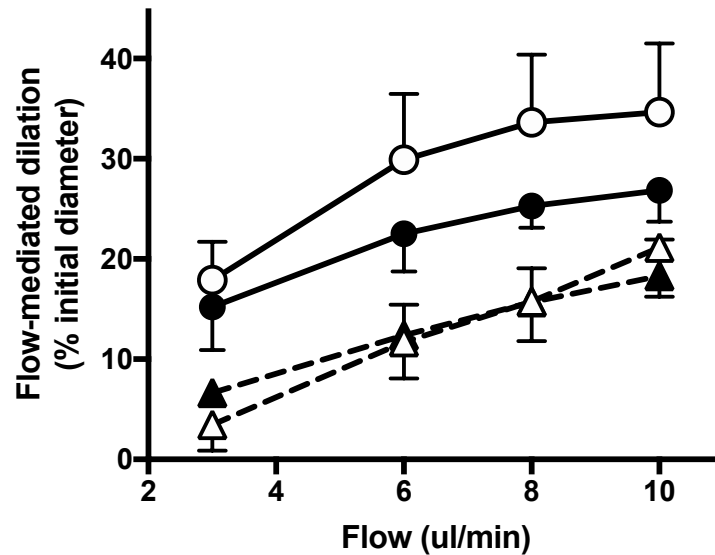
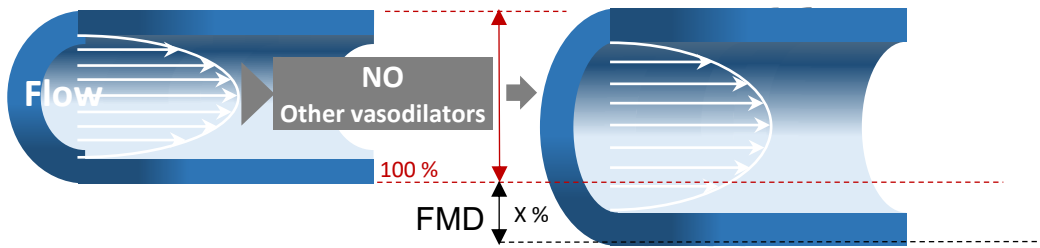
Does *Angptl6* mutation affect brain arteries dilation ?

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Does *Angptl6* mutation affect brain arteries dilation ?

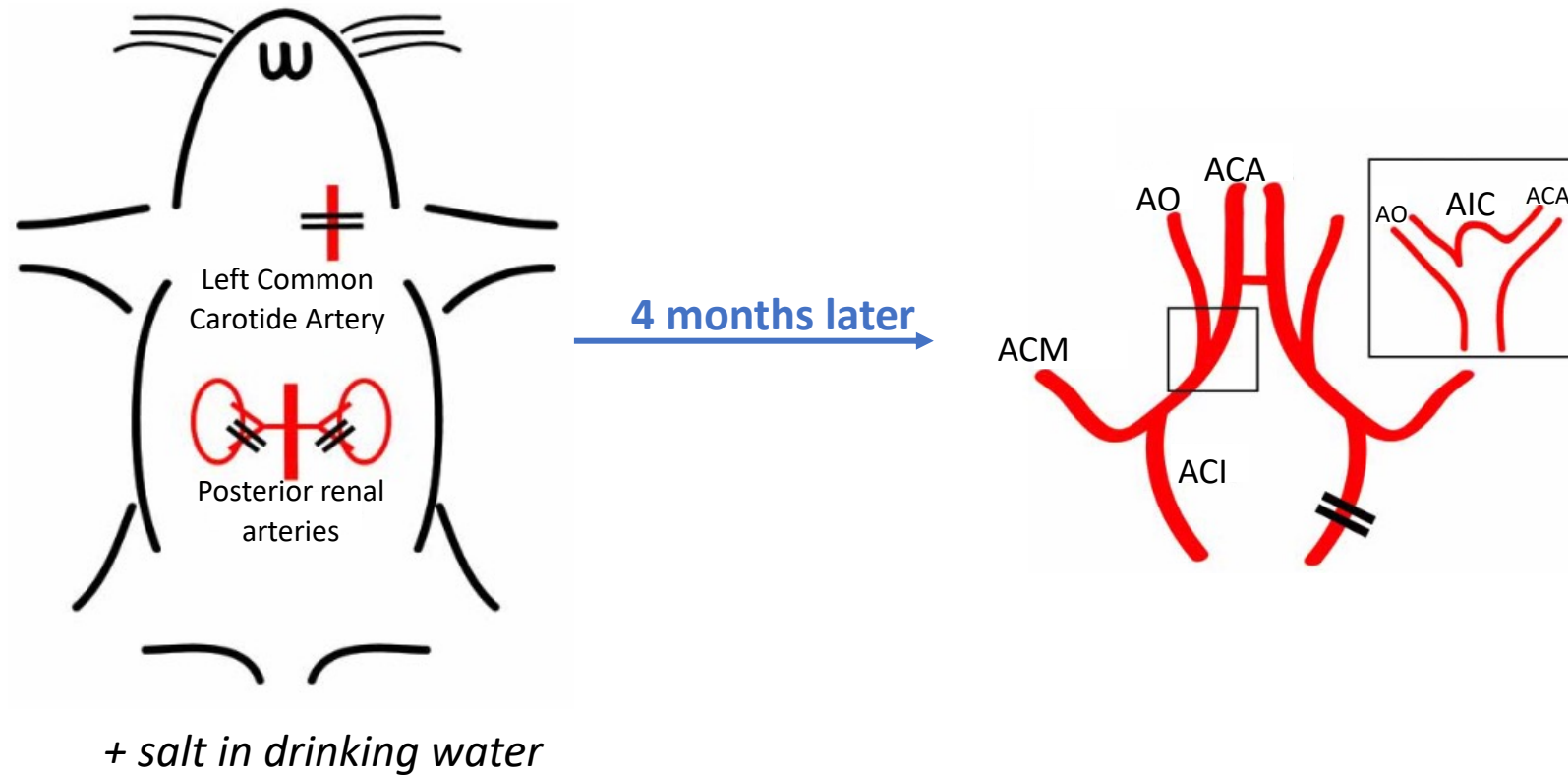
Flow mediated dilation of brain arteries



***Angptl6*^{Δ/Δ} mice have an endothelial dysfunction**

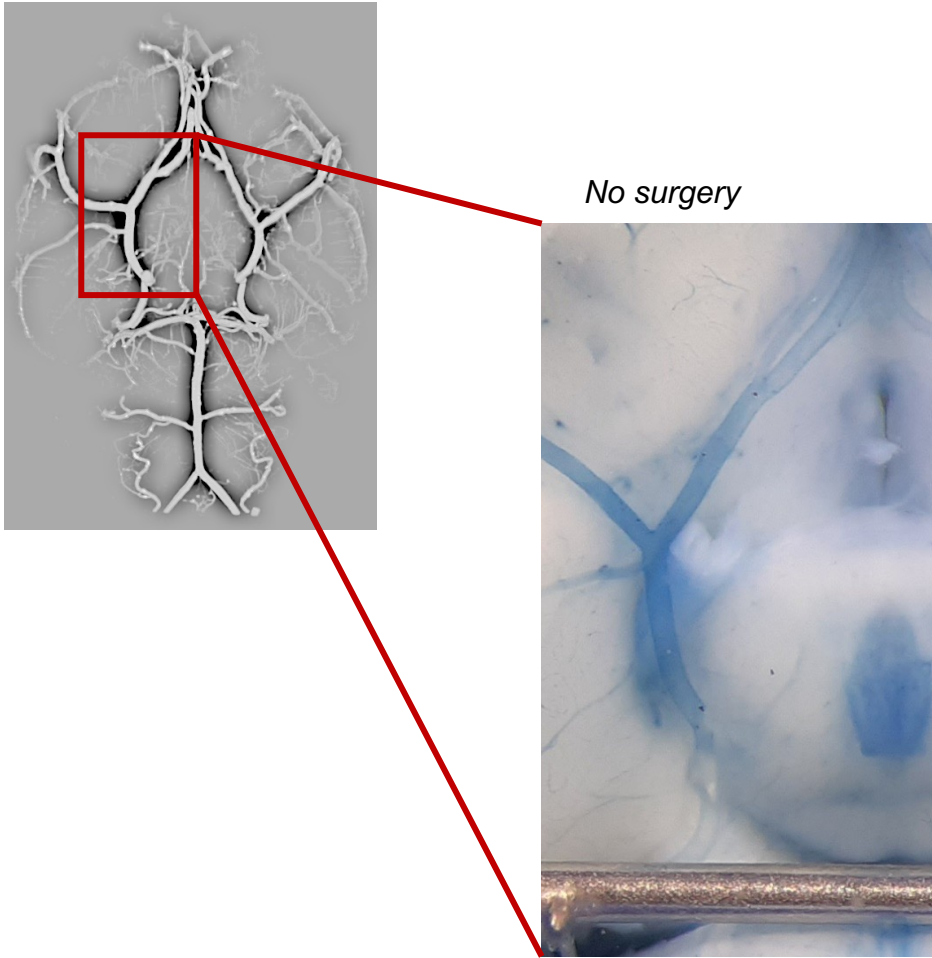
What is the role of the hemodynamics ?

Morimoto model



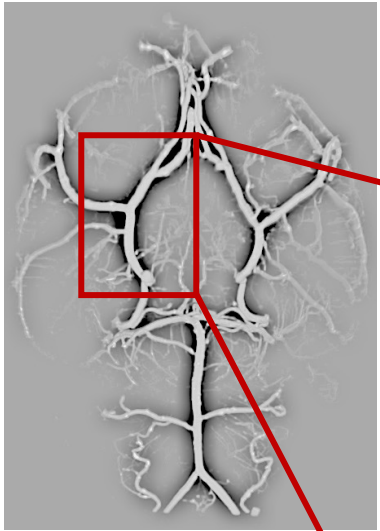
Do mutant mice develop intracranial aneurysms?

Morimoto model

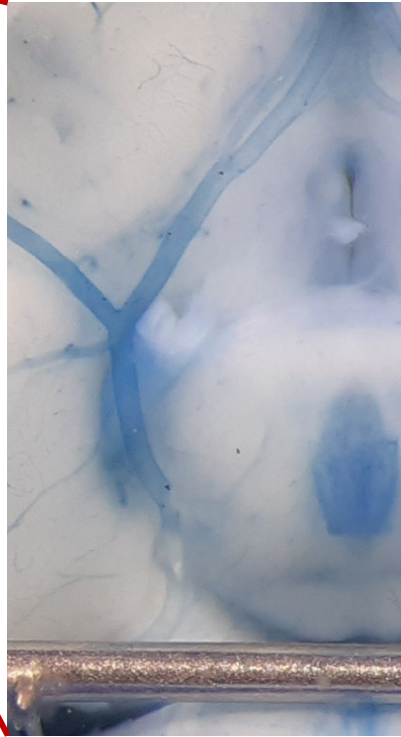


Do mutant mice develop intracranial aneurysms?

Morimoto model



No surgery



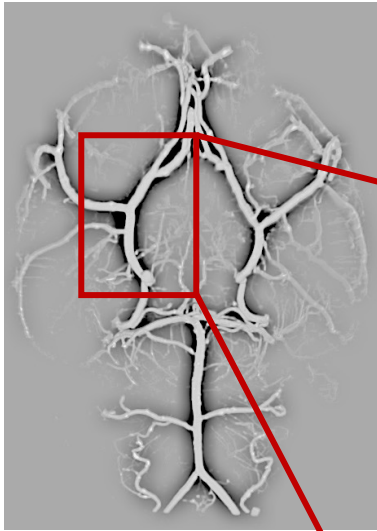
Surgery

control

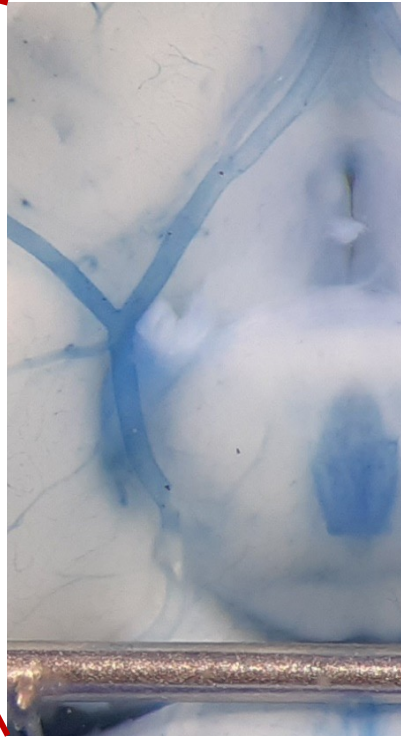


Do mutant mice develop intracranial aneurysms?

Morimoto model



No surgery

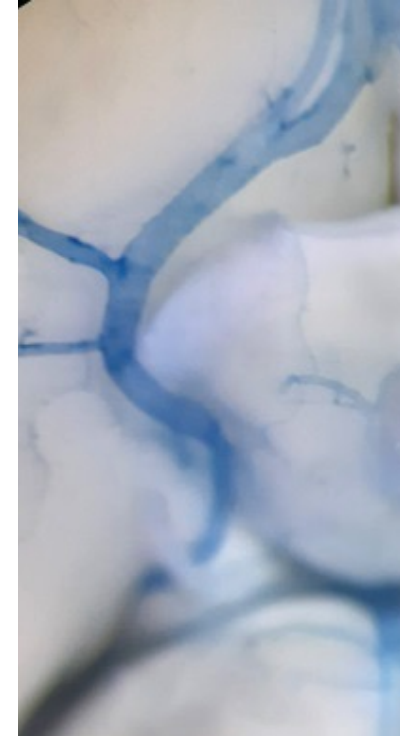


Surgery

control



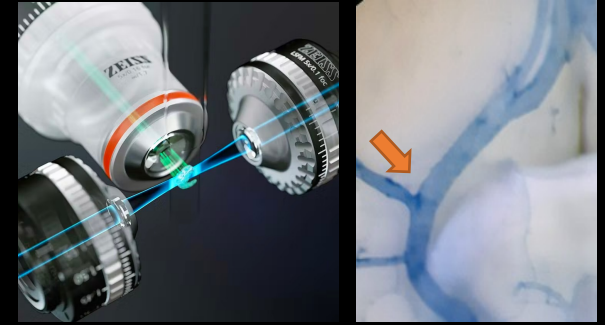
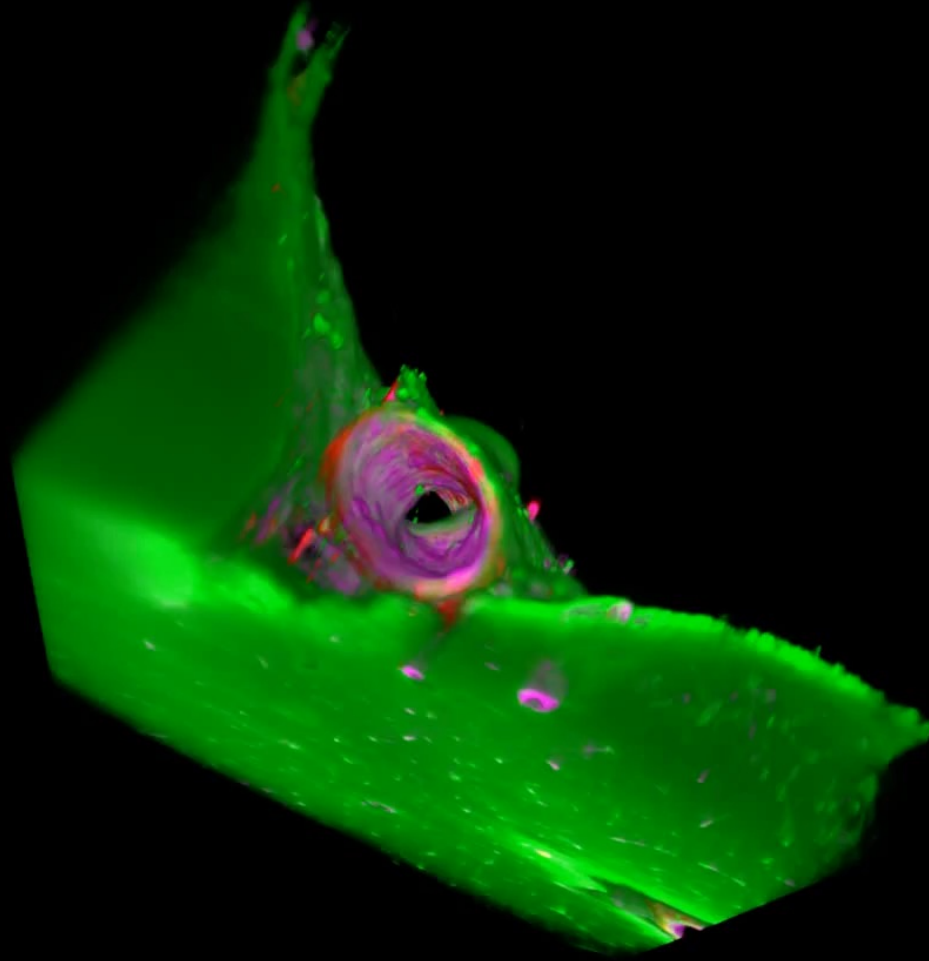
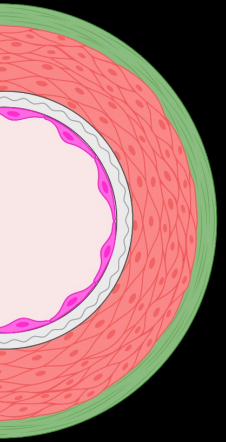
Angptl6^{Δ/Δ}



no aneurysms, but arteries wider and more tortuous than controls

Do mutant mice develop intracranial aneurysms?

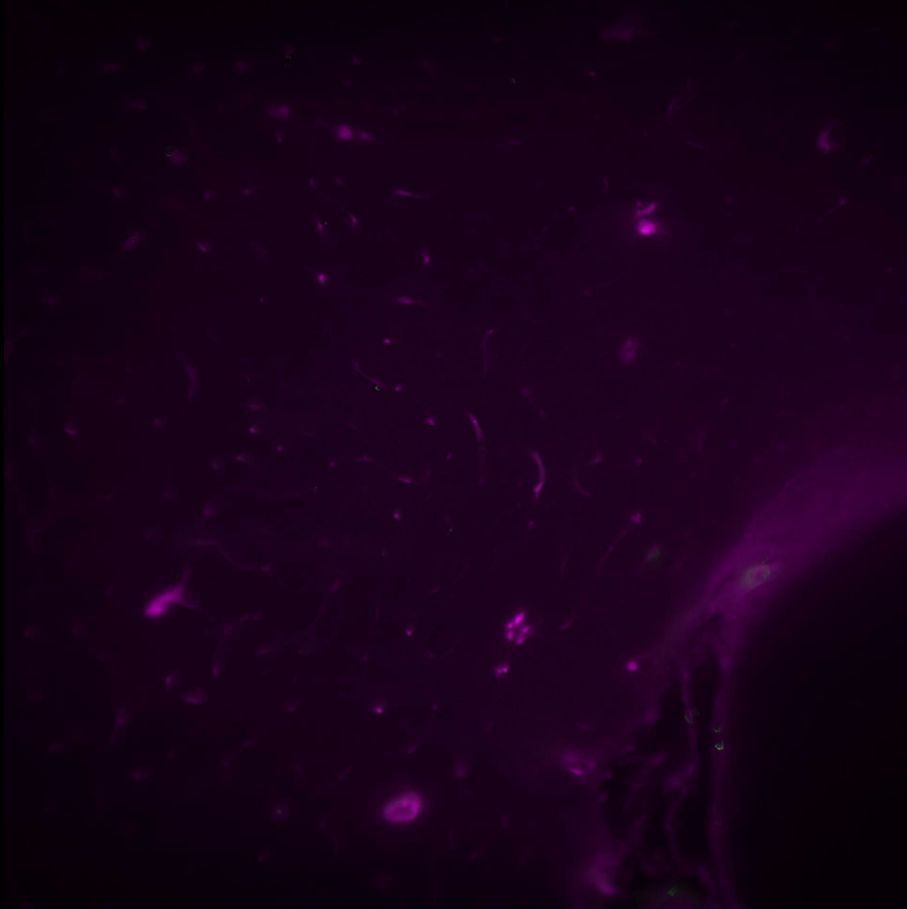
using a more precise method: light sheet microscopy



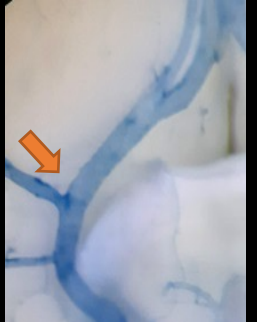
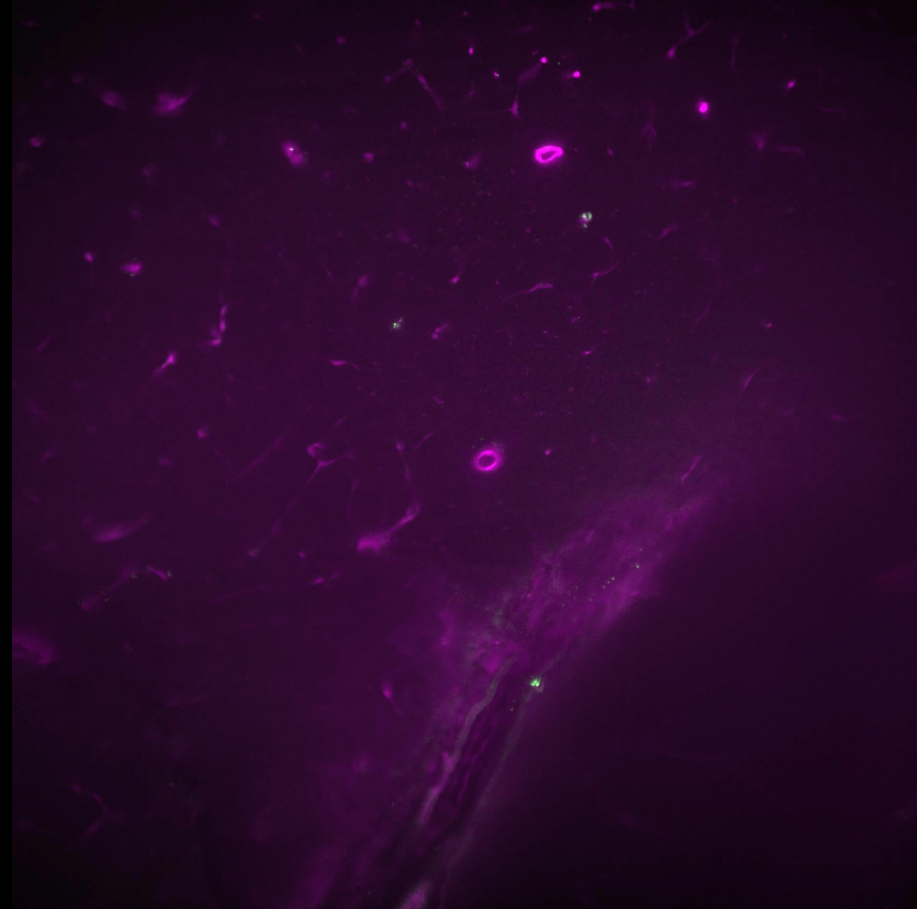
Do mutant mice develop intracranial aneurysms?

using a more precise method: light sheet microscopy

control



Angptl6^{Δ/Δ}

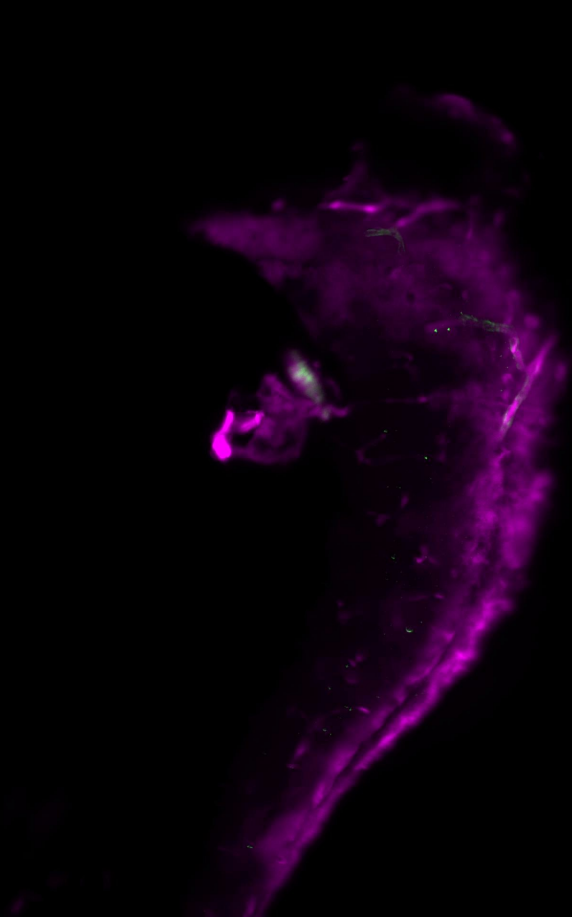


The bifurcations of mutant arteries display some distortions

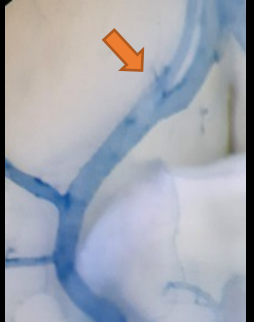
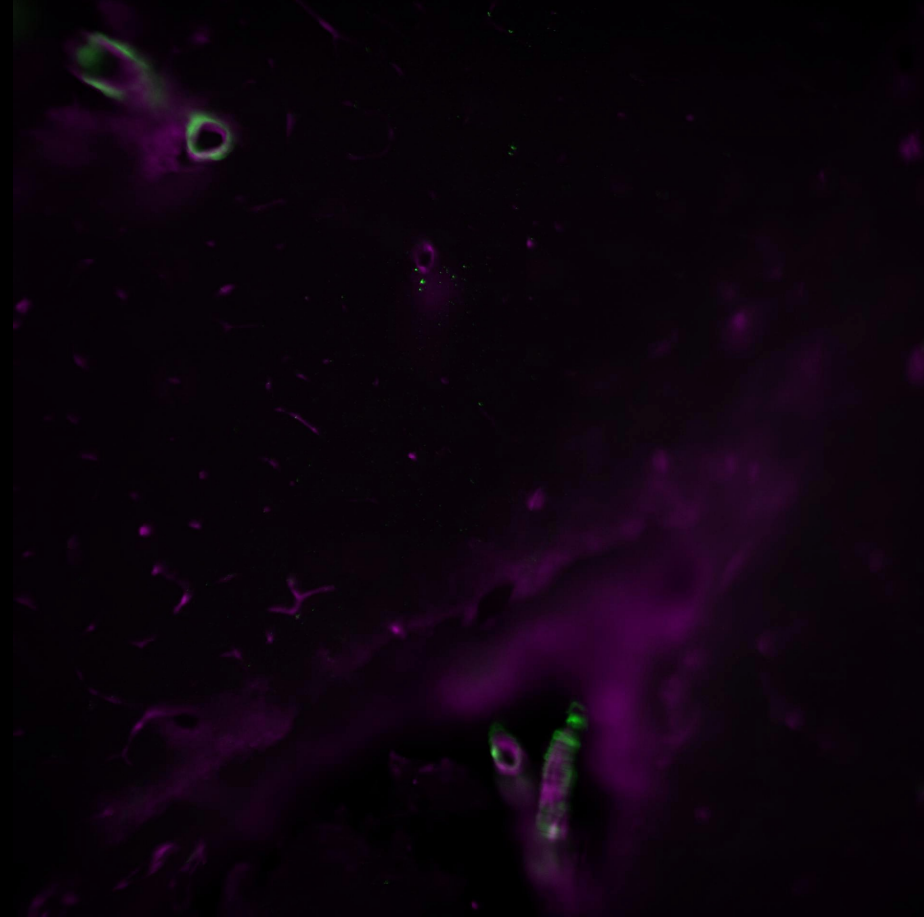
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Angptl6^{Δ/Δ}



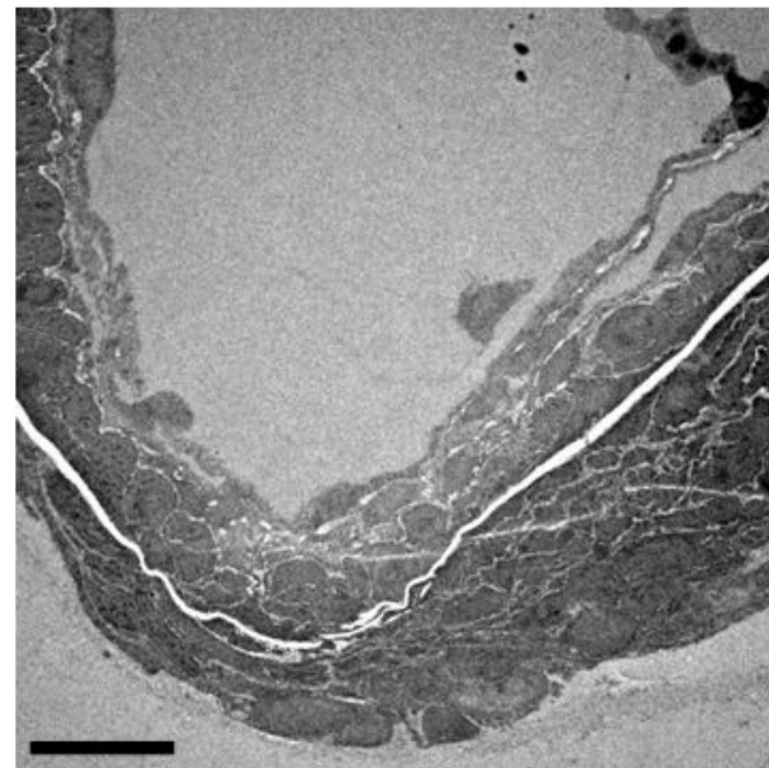
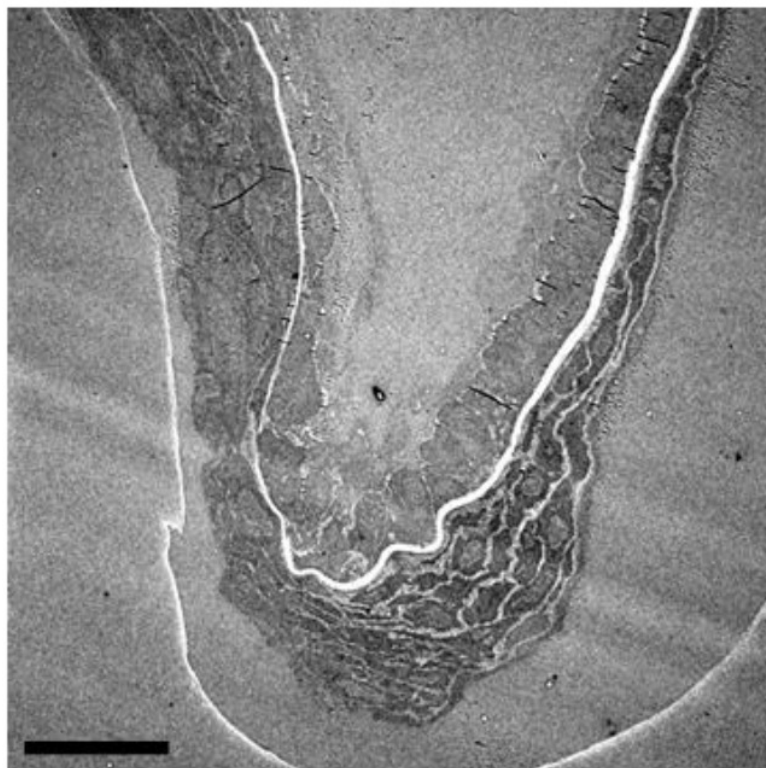
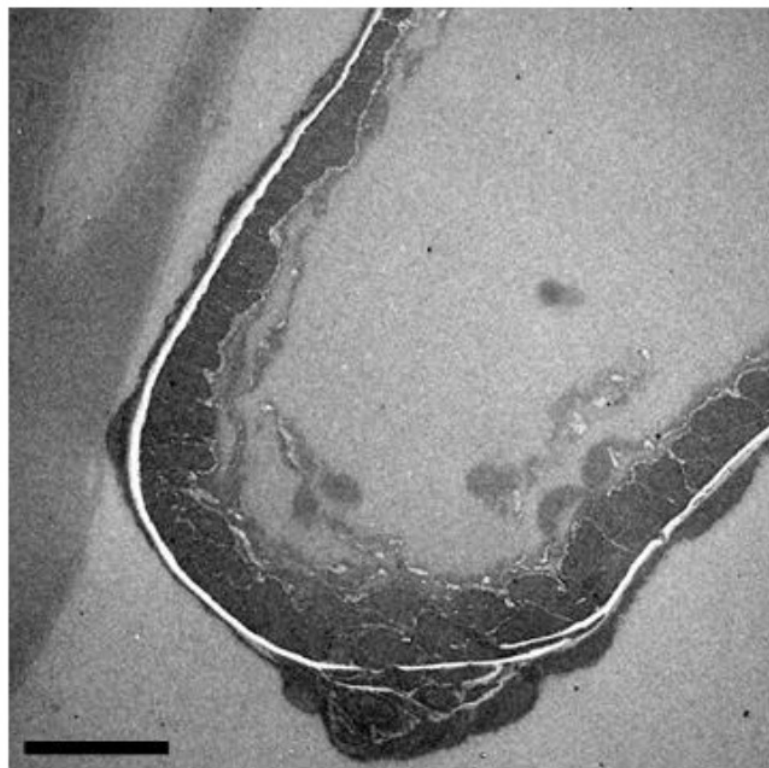
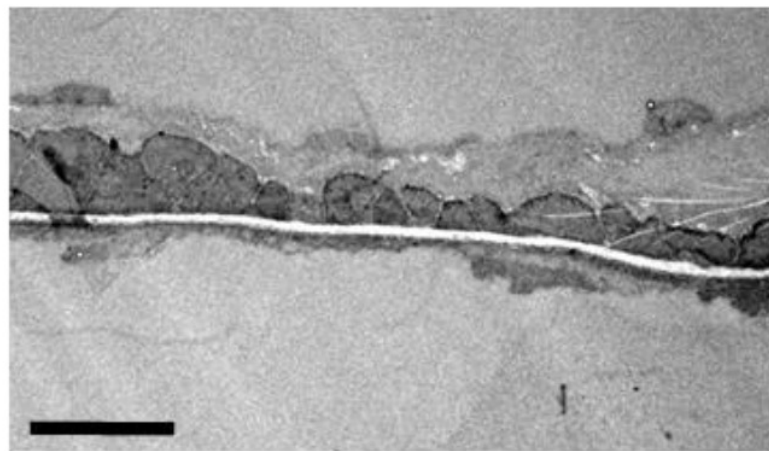
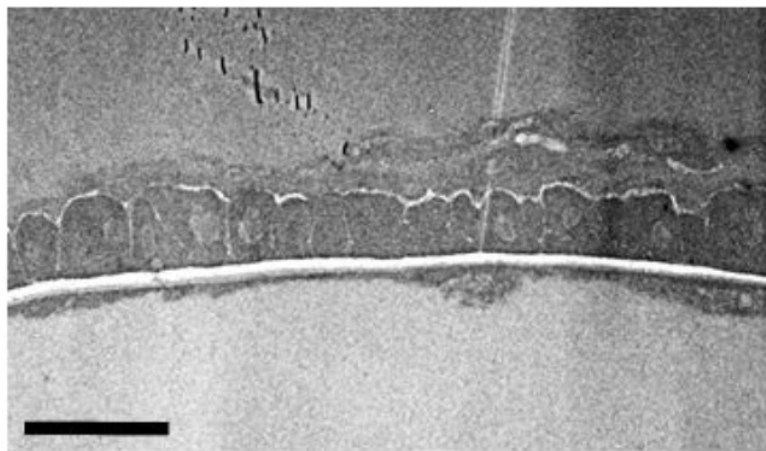
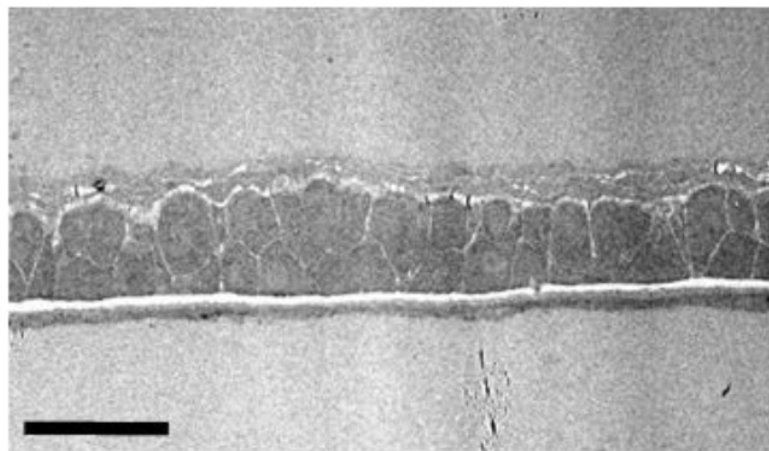
The bifurcations of mutant arteries present a thickening of the wall

Do mutant mice develop intracranial aneurysms?

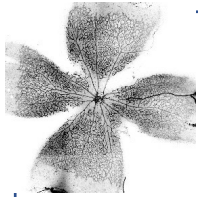


Yes, some mice form aneurysms similar to the patient's ones.

A

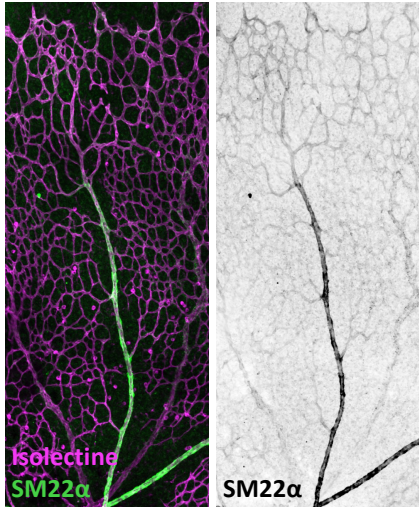
Angptl6^{WT/WT}*Angptl6*^{WT/ Δ} *Angptl6* ^{Δ/Δ} 

Is developmental angiogenesis in Angptl6-KI mice affected?

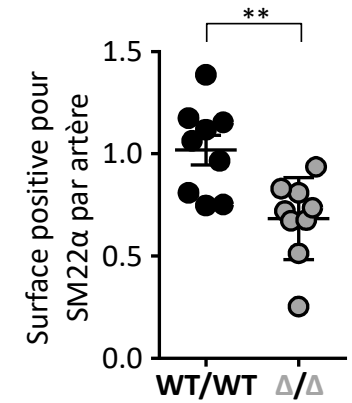
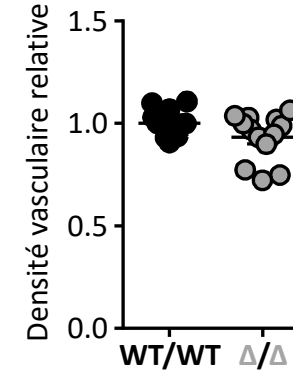
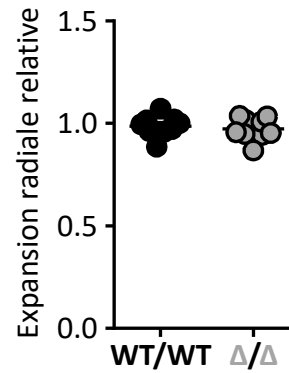
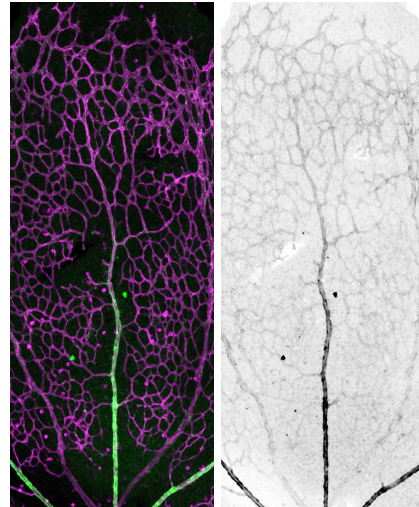


Immunofluorescence on newborn retinas

Angptl6^{WT/WT}



Angptl6^{Δ/Δ}



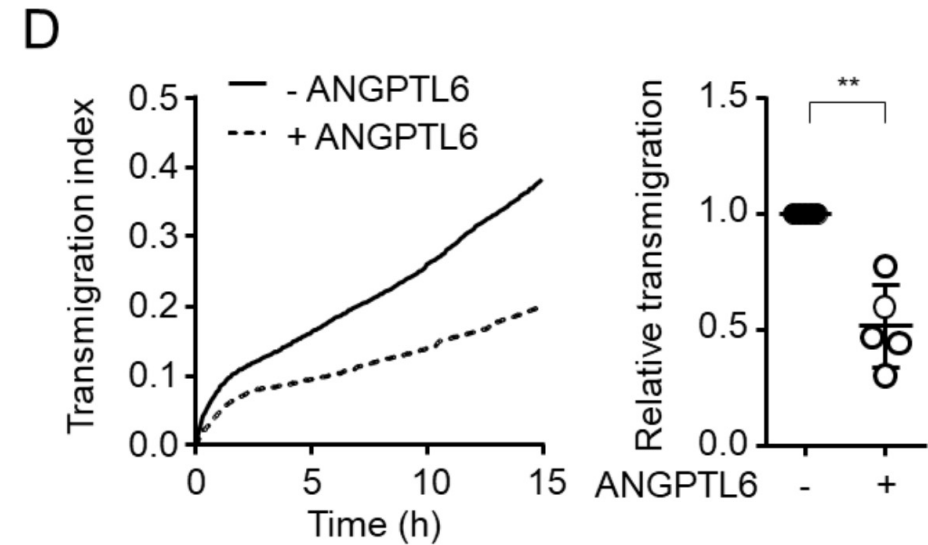
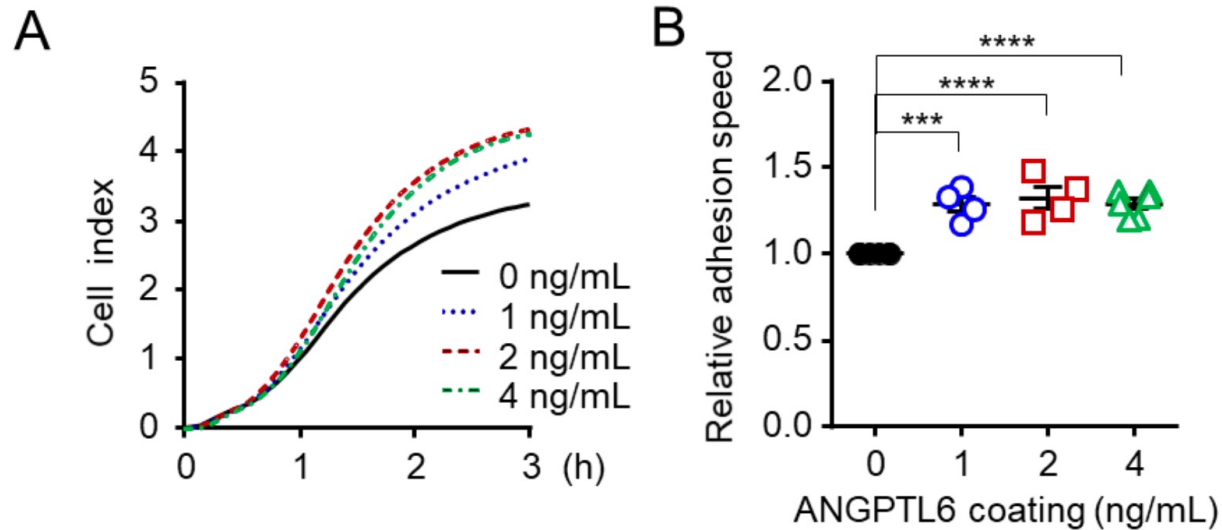
➡ *Angptl6*-KI mice have a reduced SMC coverage during vascular network formation

Does ANGPTL6 modulates SMC adhesion and migration ?

In vitro adhesion measurement by impedance

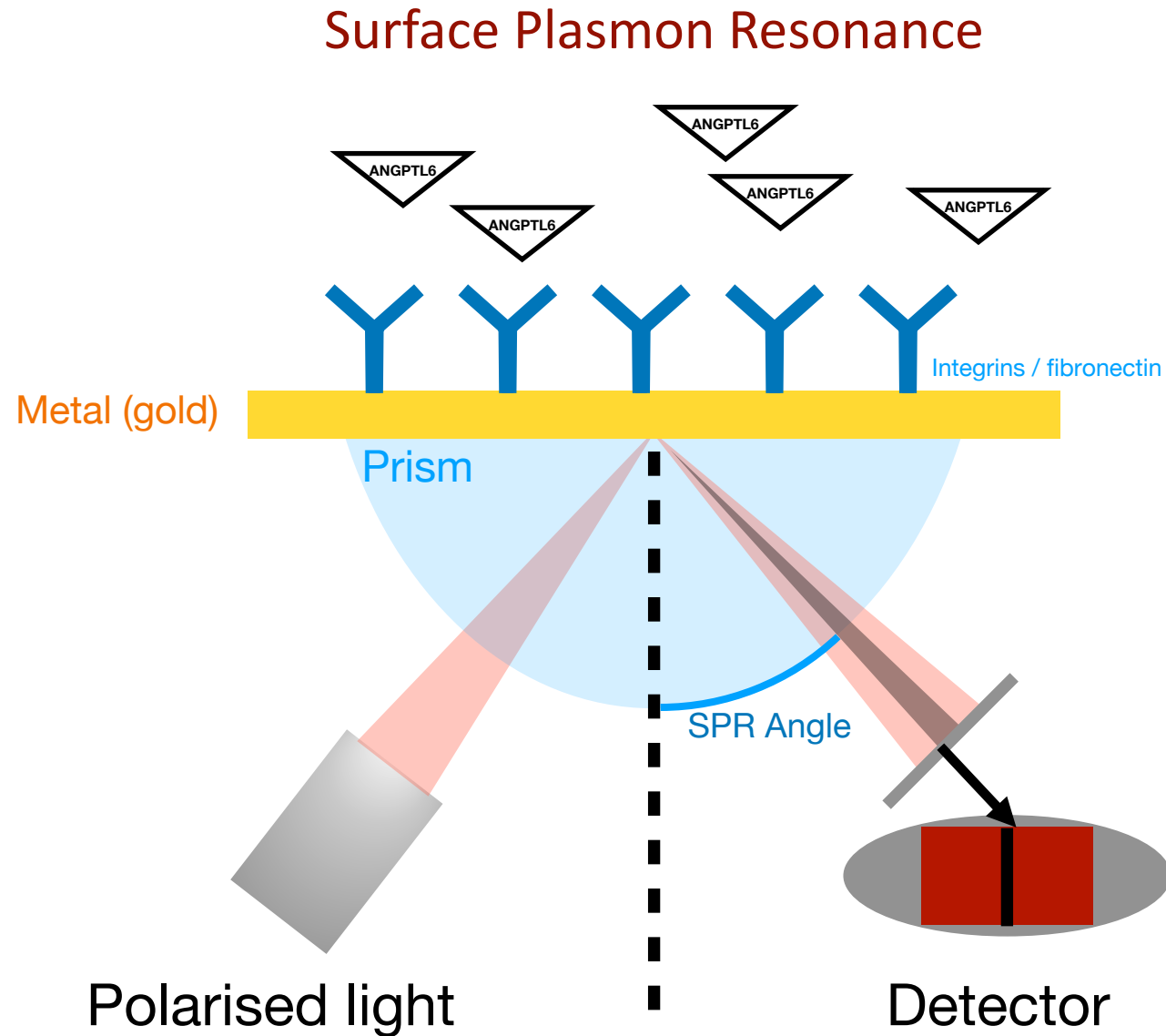


Transmigration experiment

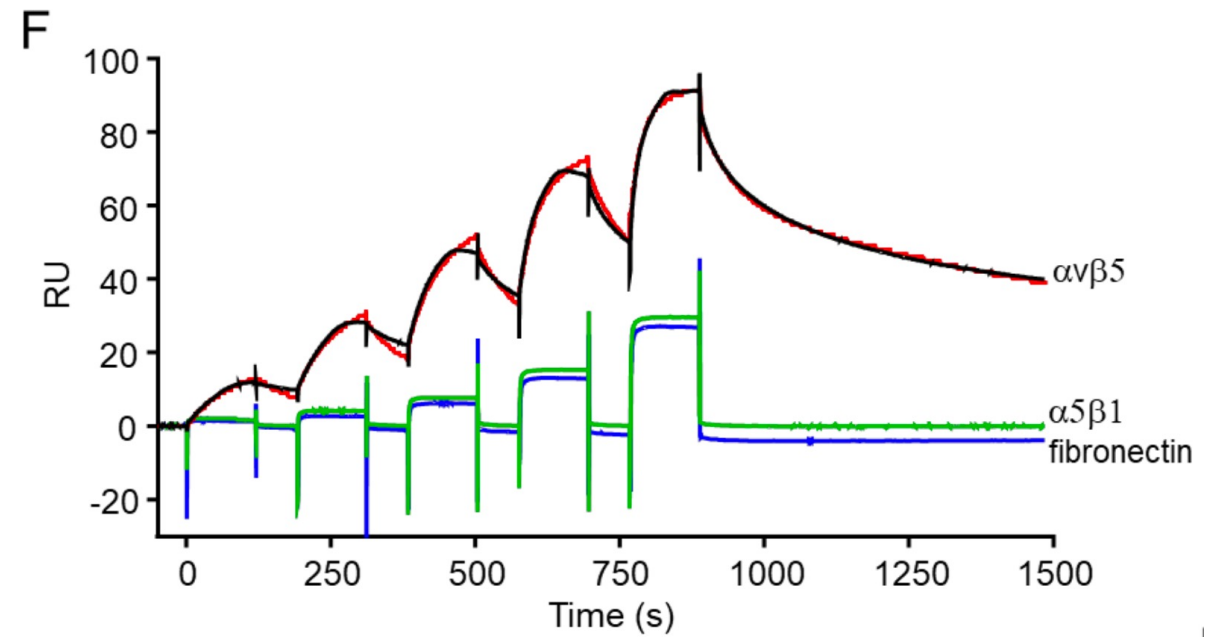
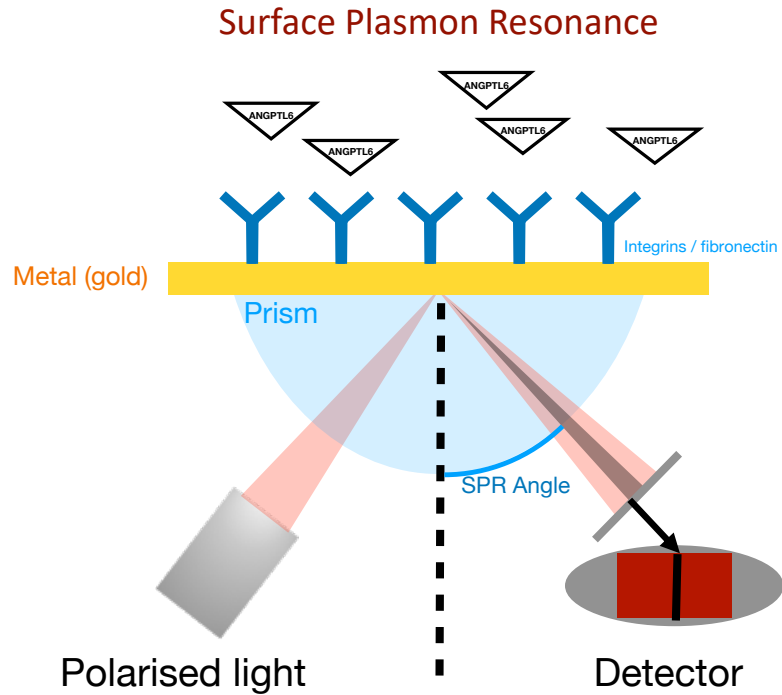


➡ ANGPTL6 increases in SMC adhesion and migration

Assessment of fibronectin and CTSO potential interaction

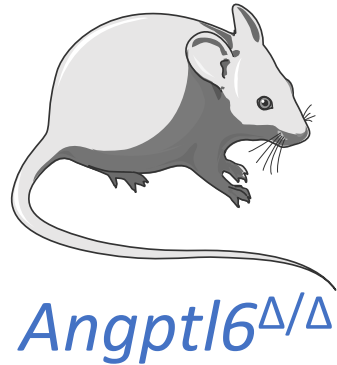


What is the partner of ANGPTL6 ?



➡ ANGPTL6 Binds to $\alpha v \beta 5$ integrins

Using of the familial form ? YES ☺



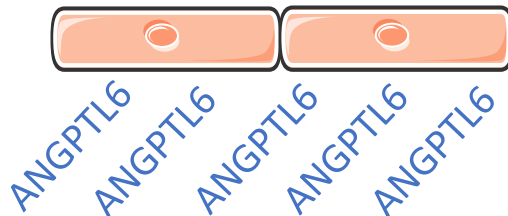
Developmental : Delayed VSMC coverage

Adulthood :

- Hyper-density signal under hypertensive treatment
- Arteries enlargement and tortuosity
- Endothelial dysfunction / Impaired flow mediated dilation

Surgical IA :

- Local vascular wall deformation
- Neo-Intima / IA



In vitro : ANGPTL6 enhances SMC adhesion
prevents SMC transmigration

Team leaders:

Céline Menguy	Ibtissam Babahnini
Gervaise Loirand	Laurent Beck
Vincent Sauzeau	Sarah Beck-Cormier
Maxence Bodet	Hugo Bergereau
Mary-Adel Mrad	Nathan Chate
Corentin Lebot	Marie-Aude Cheminant
Surya Batta	Thibault Quillard
	Manon Marie
	Marc Rio
	Morgane Rousselle
	Virginie Vignard



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L'unité de recherche de l'institute du thorax
Inserm UMR 1087 / CNRS UMR 6291
Nantes, France



CTSO variant in ICA

<https://www.medrxiv.org/content/10.1101/2023.01.31.23285168v1>

ANGPTL6 ICA pphysiopath

<https://www.biorxiv.org/content/10.1101/2025.01.10.632391v1>

ANGPTL6 variant in ICA

<https://www.sciencedirect.com/science/article/pii/S0002929717304950?via%3Dihub>

ARHGEF18 in ECs mechanotransduction

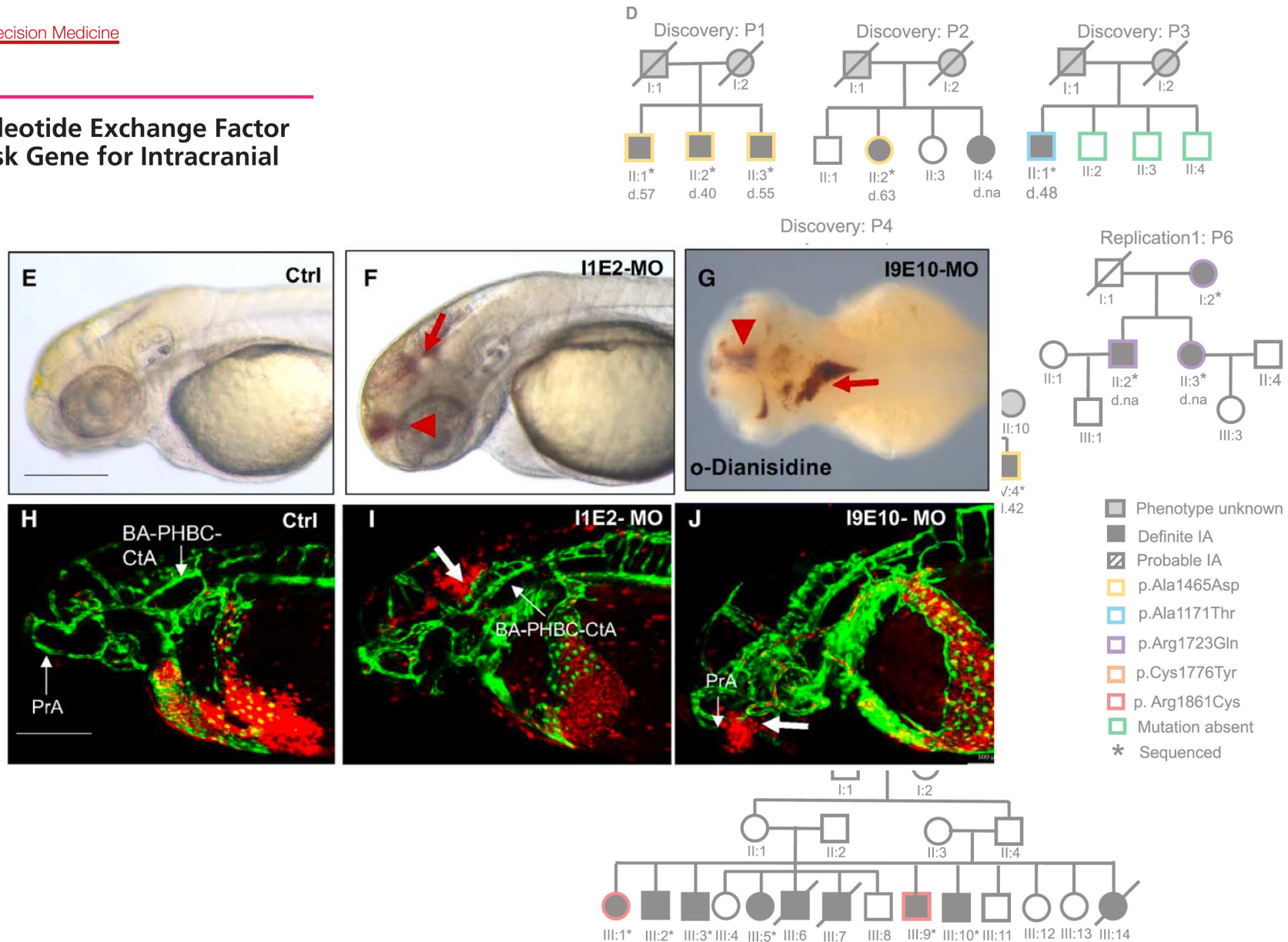
[https://www.cell.com/cell-reports/fulltext/S2211-1247\(25\)00059-2](https://www.cell.com/cell-reports/fulltext/S2211-1247(25)00059-2)

other examples

[Circulation: Genomic and Precision Medicine](#)

ORIGINAL ARTICLE

Rho Guanine Nucleotide Exchange Factor *ARHGEF17* Is a Risk Gene for Intracranial Aneurysms



other examples

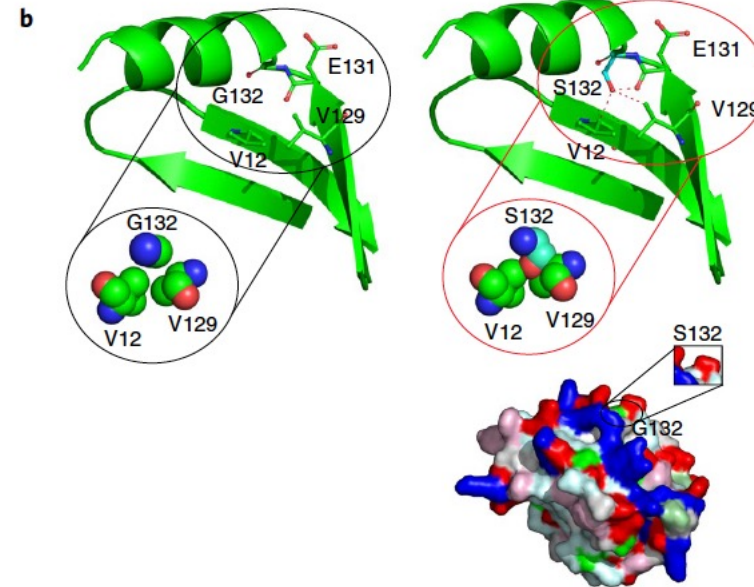
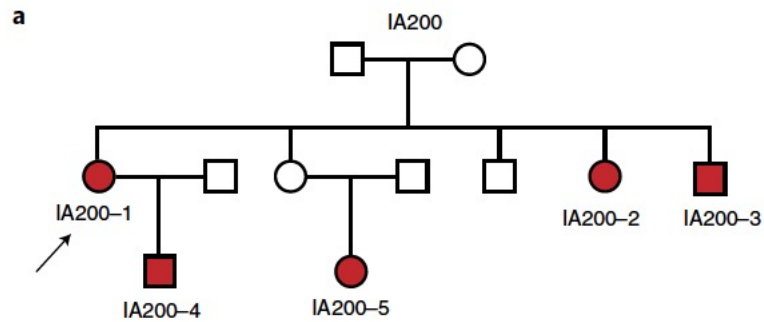
nature
medicine

ARTICLES

<https://doi.org/10.1038/s41591-021-01572-7>



***PPIL4* is essential for brain angiogenesis and implicated in intracranial aneurysms in humans**



other examples

nature
medicine

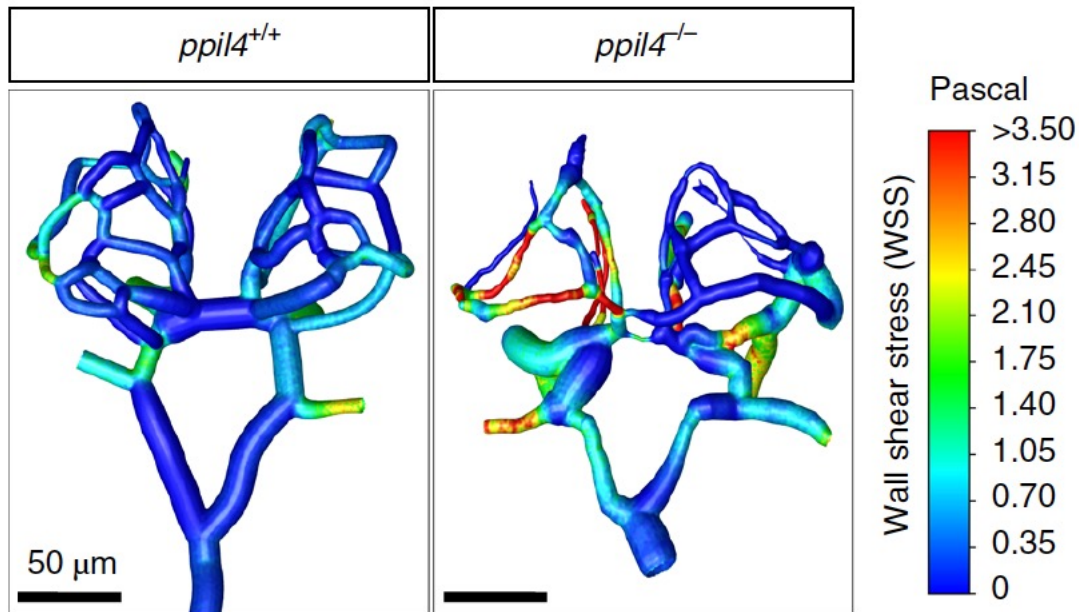
ARTICLES

<https://doi.org/10.1038/s41591-021-01572-7>



***PPIL4* is essential for brain angiogenesis and implicated in intracranial aneurysms in humans**

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