ARHGEF18 participates in Endothelial Cell Mechano-sensitivity in Response to Flow

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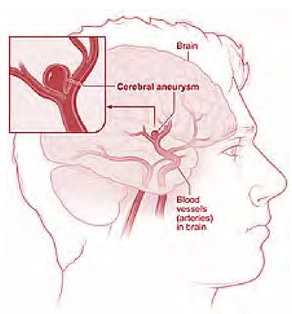
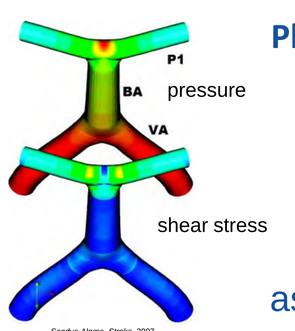
INTRODUCTION



Intracranial aneurysms: vascular abnormalities occurring at bifurcations of cerebral arteries affecting 3% of the general population.

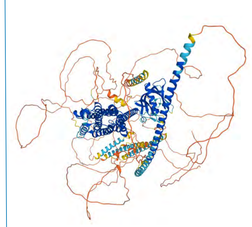


Physiopathology:
- endothelial dysfunction
- inflammation
- molecular mechanisms at play mostly unknown

associated with **altered hemodynamics**

Identification of flow sensitive proteins

ARHGEF18



Guanine nucleotid exchange factors (GEF)
Activator of Rho proteins

Well described in epithelial cells
Cell-cell adhesion
Matrix adhesion
Cell migration
ROS formation

AIM : understand the role of a mechanosensitive GEF, ARHGEF18, in endothelial cell biology

METHODS

IN VITRO

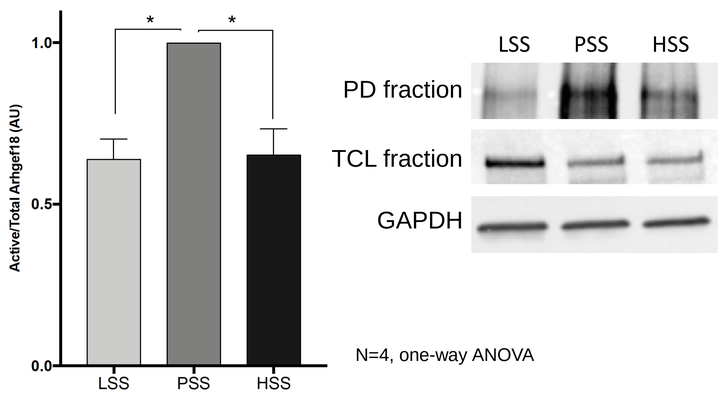
source of ECs: HUVECS
ARHGEF18 silencing : siRNA and shRNA
ARHGEF18 Y260 mutation : lentiviral infection and silencing of endogenous ARHGEF18

Adhesion: Impedance measurement
Migration: wound closure assay

Flow exposure: low (LSS) 3dyn/cm2
24h physiological (PSS) 16dyn/cm2
high (HSS) 36dyn/cm2

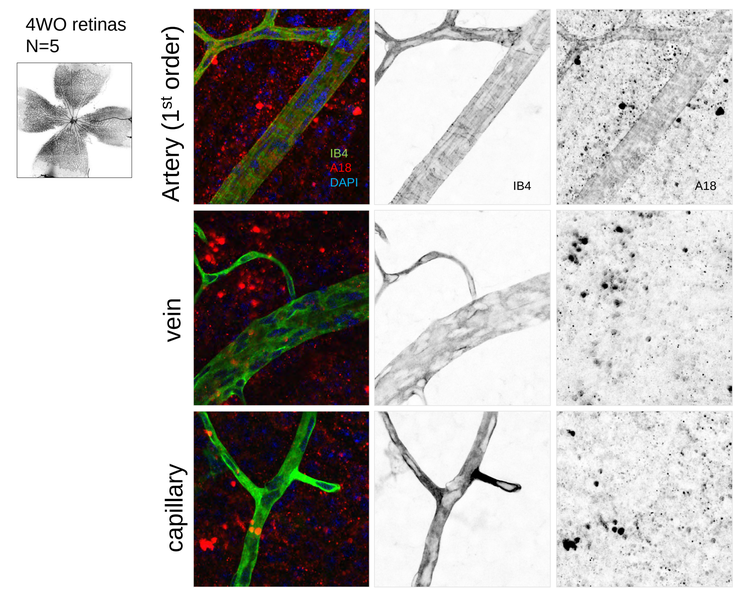
ARHGEF18 activity is flow sensitive

Pull Down assay (Rho interaction)



ARHGEF18 bounds to RhoA but not Rac1
its activity is downregulated by pathological shear stress

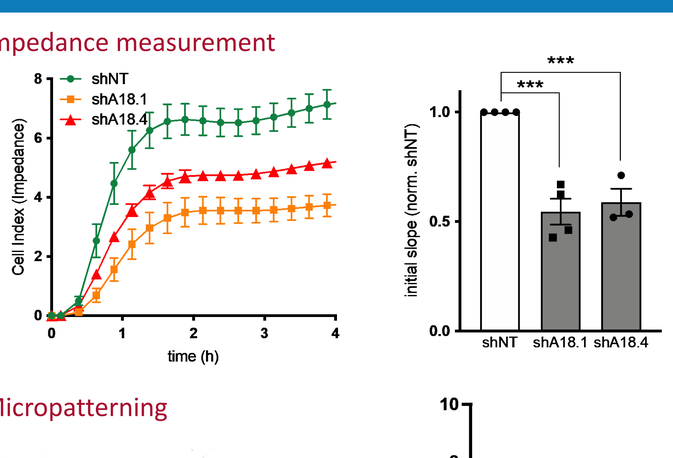
ARHGEF18 expression is restricted to arteries



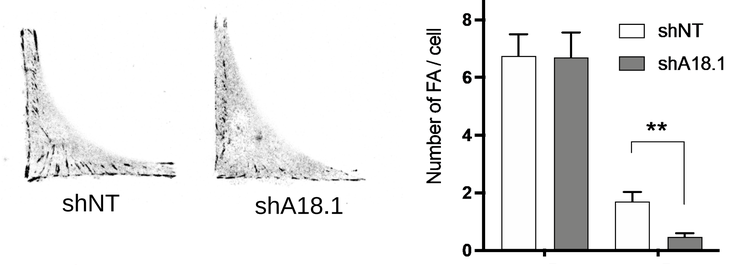
ARHGEF18 is expressed in arteries but not in veins and capillaries

ARHGEF18 controls EC adhesion and migration

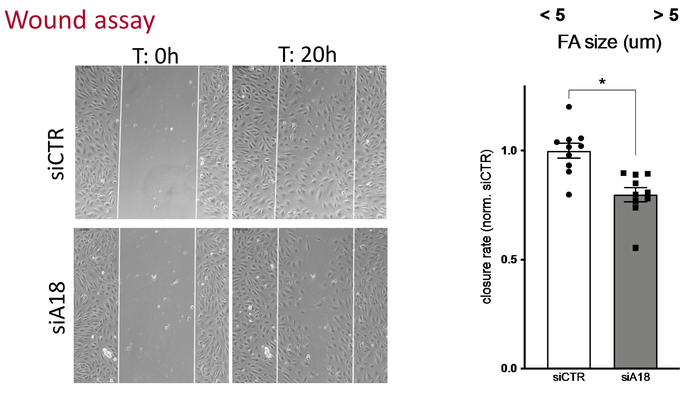
Impedance measurement



Micropatterning

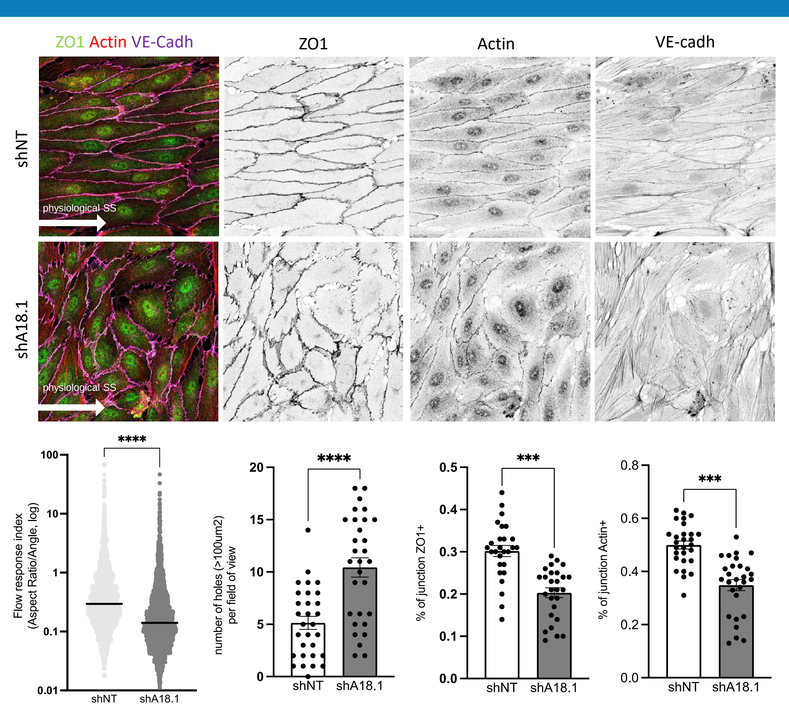


Wound assay

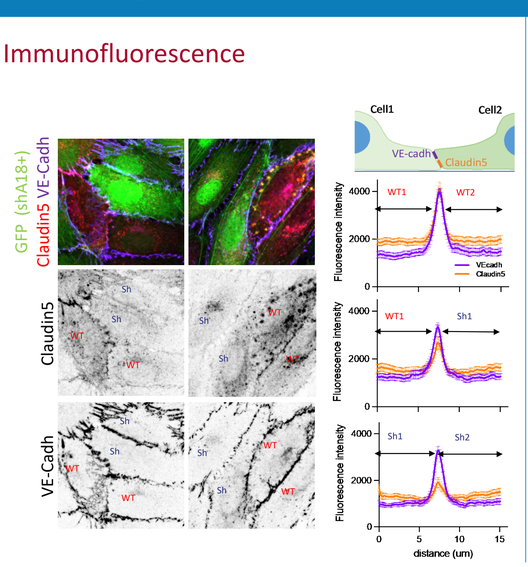


ARHGEF18 deficient ECs have a reduced adhesion and less mature focal adhesion.
They migrate slower than control ECs.

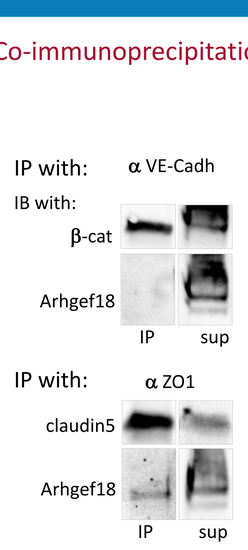
ARHGEF18 contributes to EC alignment with the flow and tight junction formation



Immunofluorescence



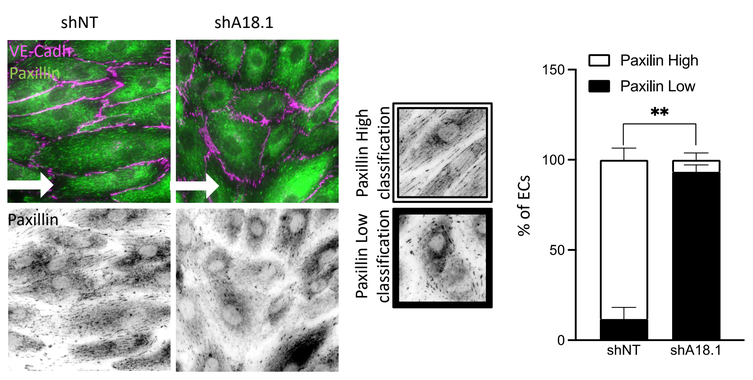
Co-immunoprecipitation



ARHGEF18 deficient ECs fail to elongate with the flow (PSS) and to recruit Z0-1 and Claudin5 at junction

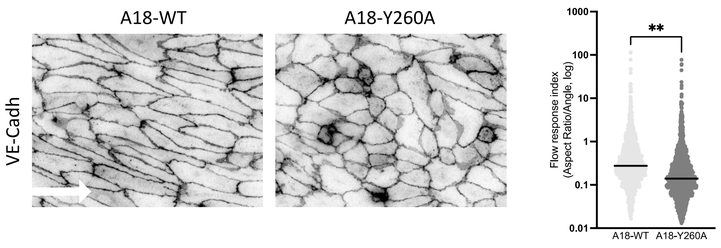
ARHGEF18 contributes to focal adhesion site formation

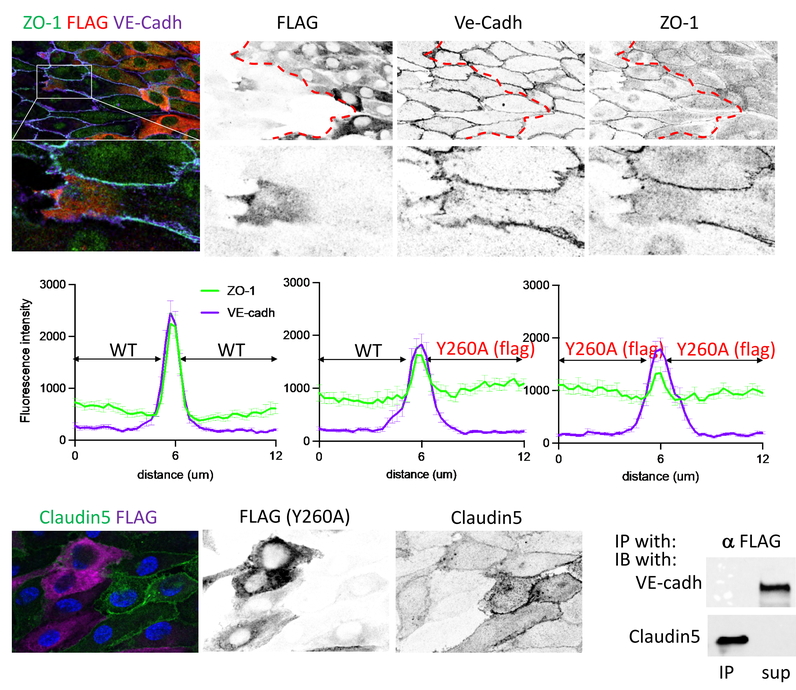
Immunofluorescence



ARHGEF18 deficient ECs fail to form long and oriented focal adhesion under physiological shear stress

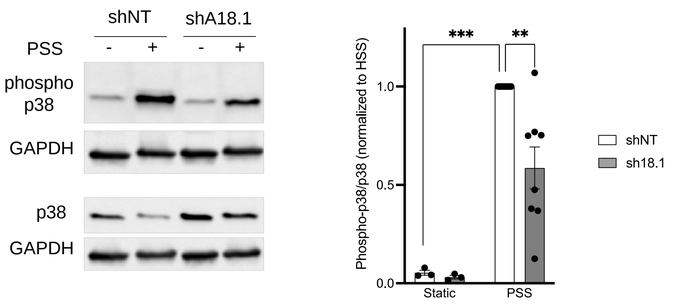
ARHGEF18 nucleotide exchange activity is required for EC alignment and tight junction formation





inactive ARHGEF18 expression in ECs recapitulate ARHGEF18 silencing for alignment and tight junction

ARHGEF18 participates in p38 activity



ARHGEF18 deficient ECs have a reduced p38 phosphorylation

p38 inhibition leads to loss of ECs alignment, reduced ZO-1 at junction and focal adhesion

CONCLUSION

- ARHGEF18 is a mechanosensitive GEF which participate in RHOA activity and interact with ZO-1 and claudin5
- ARHGEF18 is especially active under physiological SS and participates in ECs response to flow.
- ARHGEF18 contributes to tight junction assembly and focal adhesion formation under physiological SS

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