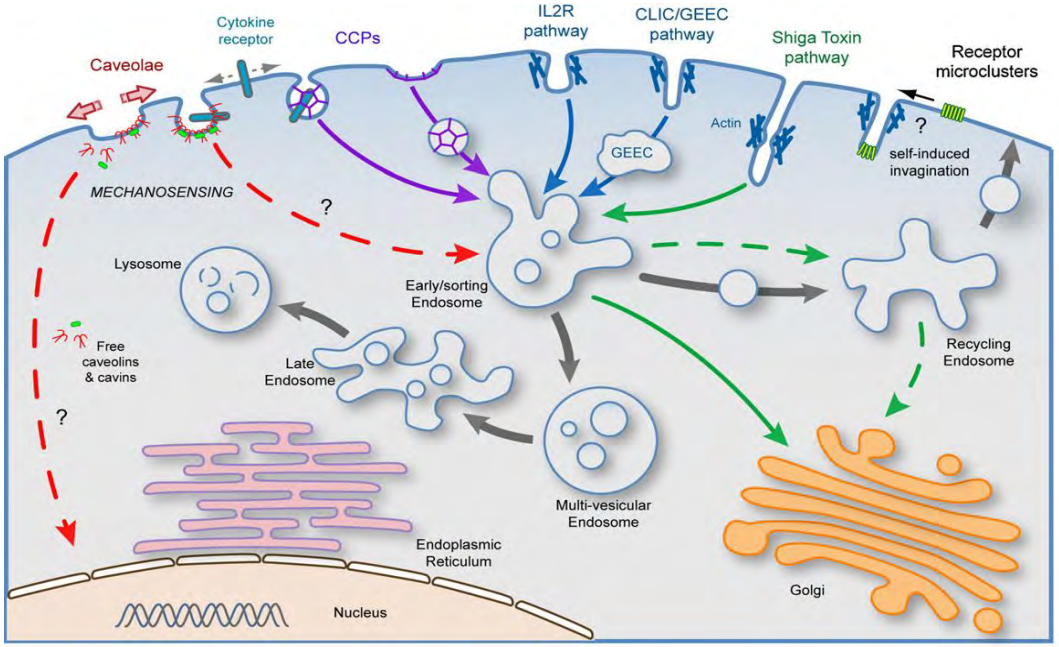
Cavéoles membranaires et mécanique cellulaire

De la structure à la pathologie

Christophe Lamaze

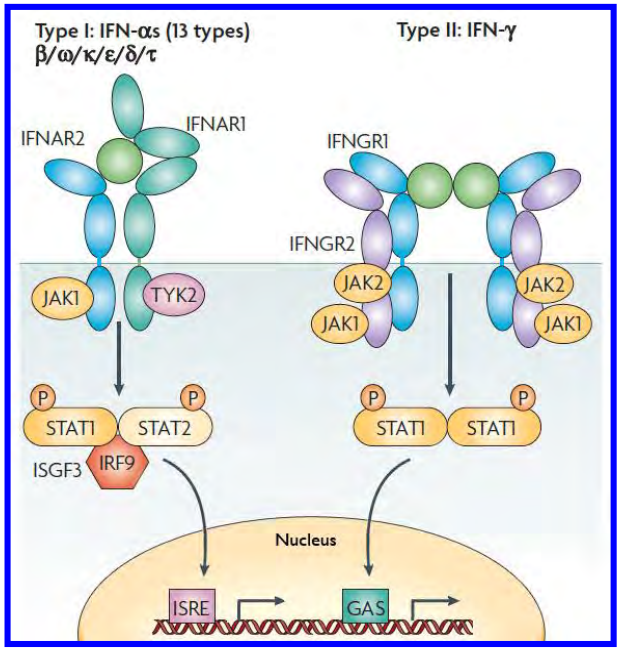
Equipe « Mécanique et Dynamique Membranaires de la Signalisation Intracellulaire »
U1143 INSERM - UMR 3666 CNRS
Institut Curie

Plasticité et complexité des voies d'endocytose….



Gonnord, Blouin, Lamaze. Semin. Cell Dev. Biol. 2012

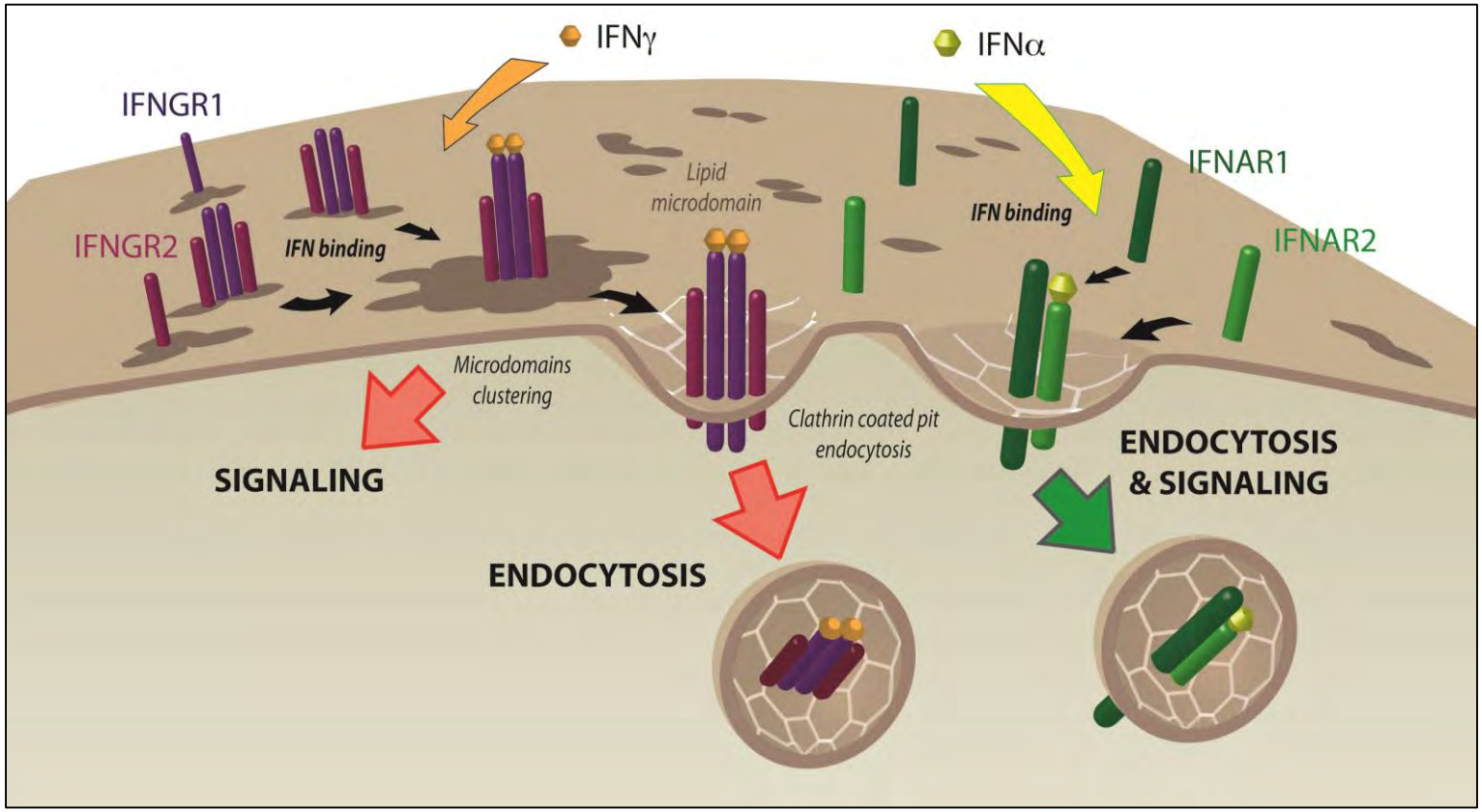
IFN-Rs and the JAK/STAT pathway



IFN-α and IFN-β bind to the same IFNAR and share signaling effectors (JAK/STAT)

C. Lamaze, and S.L. Schmid. 1996. Control of EGF receptor signaling by clathrin-mediated endocytosis. ***Science***.

Le trafic intracellulaire contrôle l'activation de la signalisation JAK/STAT par les interferons



Lamaze and Blouin, *Front Immunol* 2013

Stat-mediated signaling induced by type I and type II interferons (IFNs) is differentially controlled through lipid microdomain association and clathrin-dependent endocytosis of IFN receptors.

(Marchetti et al., *MBoC* 2006)

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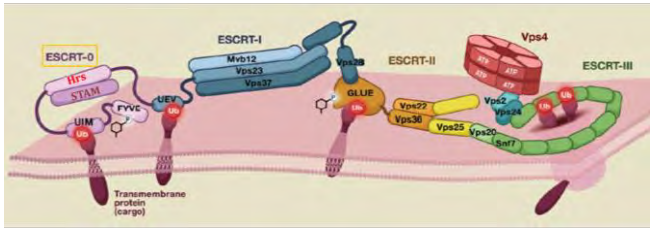
Ensemble, prenons le cancer de vitesse.

Identification de la première machinerie endosomale contrôlant l'activation différentielle de la voie JAK/STAT par les IFNs alpha et beta



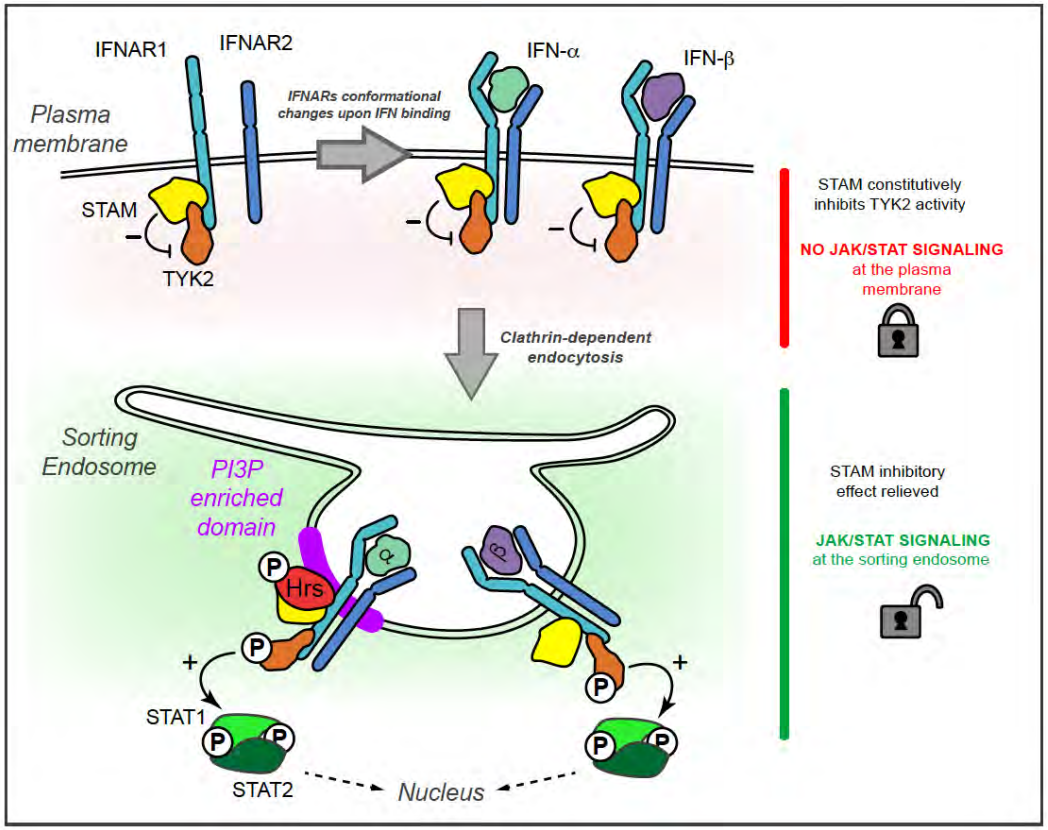
Role of endosomal machineries: (ESCRT, Retromer…)

Spatiotemporal Control of Interferon-induced JAK/STAT Signaling and Gene Transcription by the Retromer Complex.

Chmiest et al. ***Nat Commun* 2016**

STAM Interaction with Hrs Controls JAK/STAT Activation by Interferon-α at the Early Endosome

Zanin N; Blouin C.M. et al *submitted to* ***Science***



Natacha Zanin, Cédric Blouin, Christine Viaris

Rôle de l'organisation nanoscopique de la membrane plasmique dans le contrôle de la signalisation du récepteur de l'IFN gamma

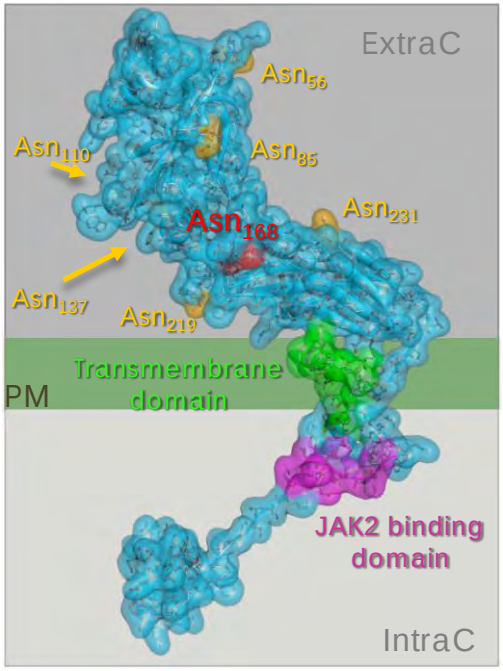
IFN-γR2 point mutation : new asparagine residue at 168 :

(Thr-Ser-Thr-Ala => Asn-Ser-Thr-Ala)

=> creating a new consensus N-glycosylation site

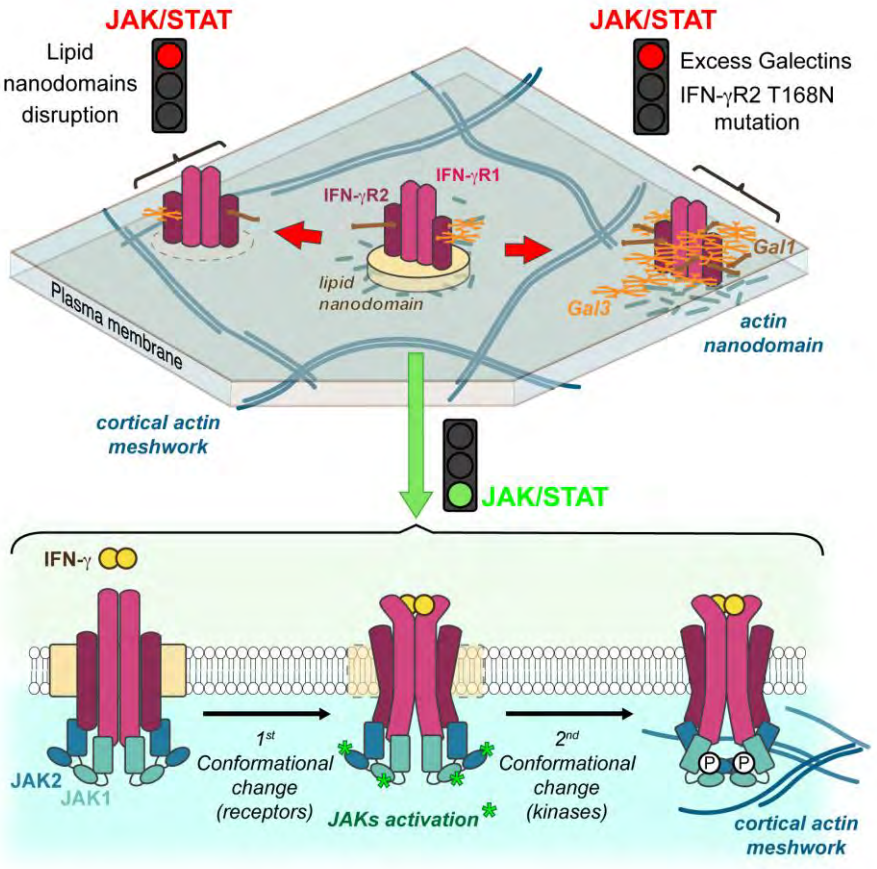
No biological response to IFNγ *(Vogt et al., 2005 Nat. Genet)*

Coll JL Casanova Hop. Necker/Rockefeller Univ.



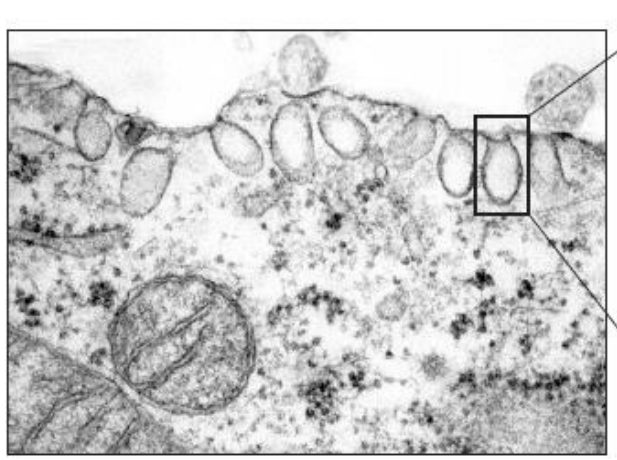
Orange = N-glycosylation

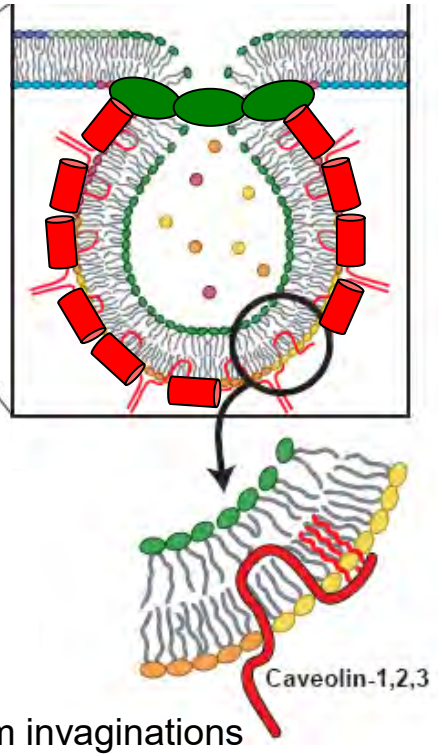
Red = additional N-glycosylation (T168N)



Glycosylation-Dependent IFN-γR Partitioning in Lipid and Actin Nanodomains Is Critical for JAK Activation. Blouin et al. ***Cell*** 2016

Les cavéoles de la membrane plasmique

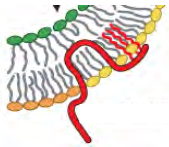




• 50-80 nm invaginations

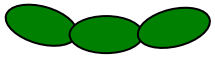
(1953 Palade, 1955 Yamada)

- Caveolin (Cav1, Cav2)



- Cavins (1-4)

- EHD2



- Associated proteins (eNOS, Akt, Src, Rho, Rac etc.)
- Cholesterol, sphingolipids

Cav1 high affinity for cholesterol

Lamaze C., Tardif N., Dewulf M., Vassilopoulos S. and Blouin C.M. *Current Opin Cell Biol*, 2017

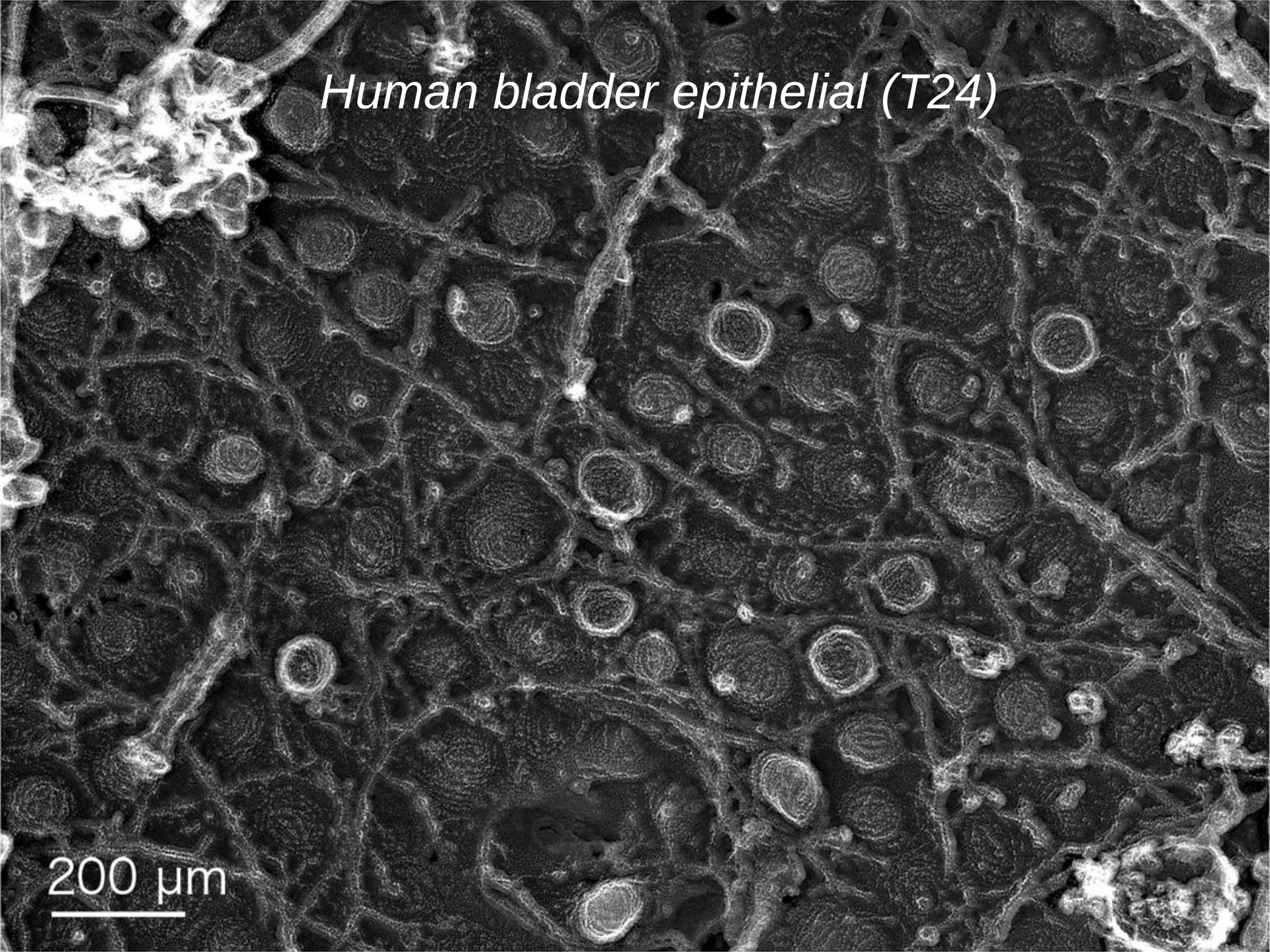
Signaling: eNOS, ERK , Akt activation…., lack of consensus.

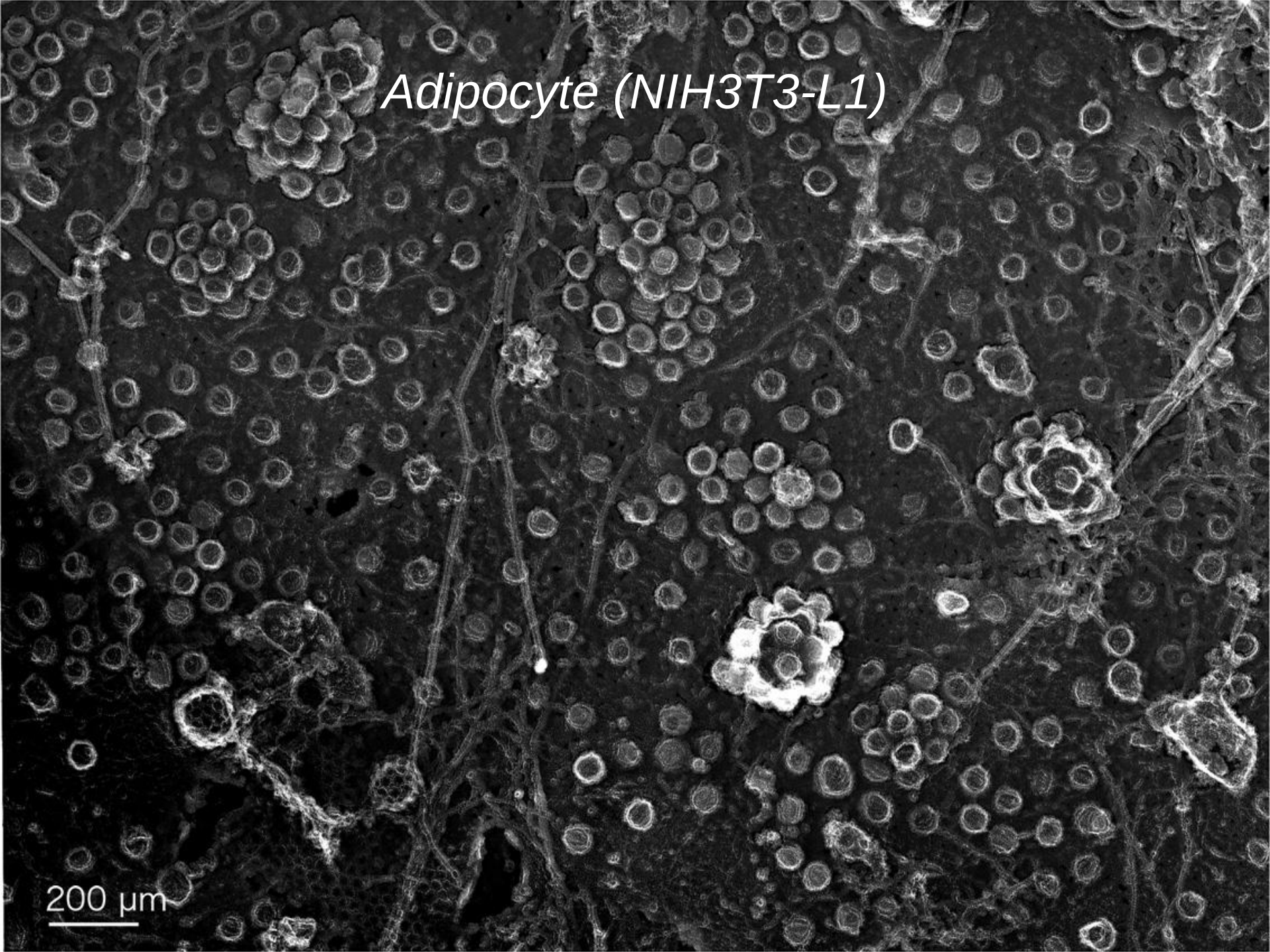
Lipid regulation: Mechanism unclear

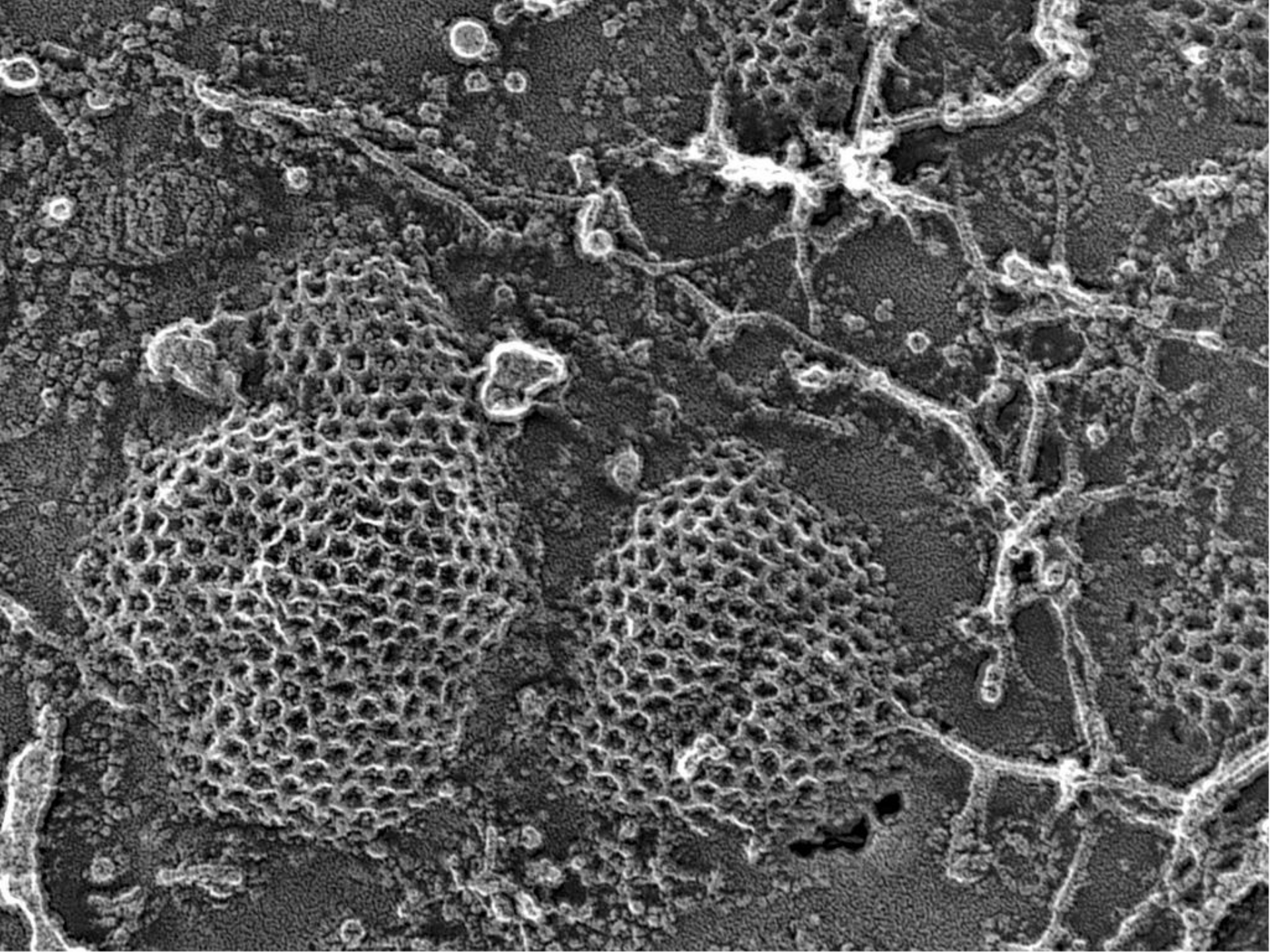
Endocytosis… controversial

Cav1 mutation in humans: Insulin resistance, major lipodystrophy

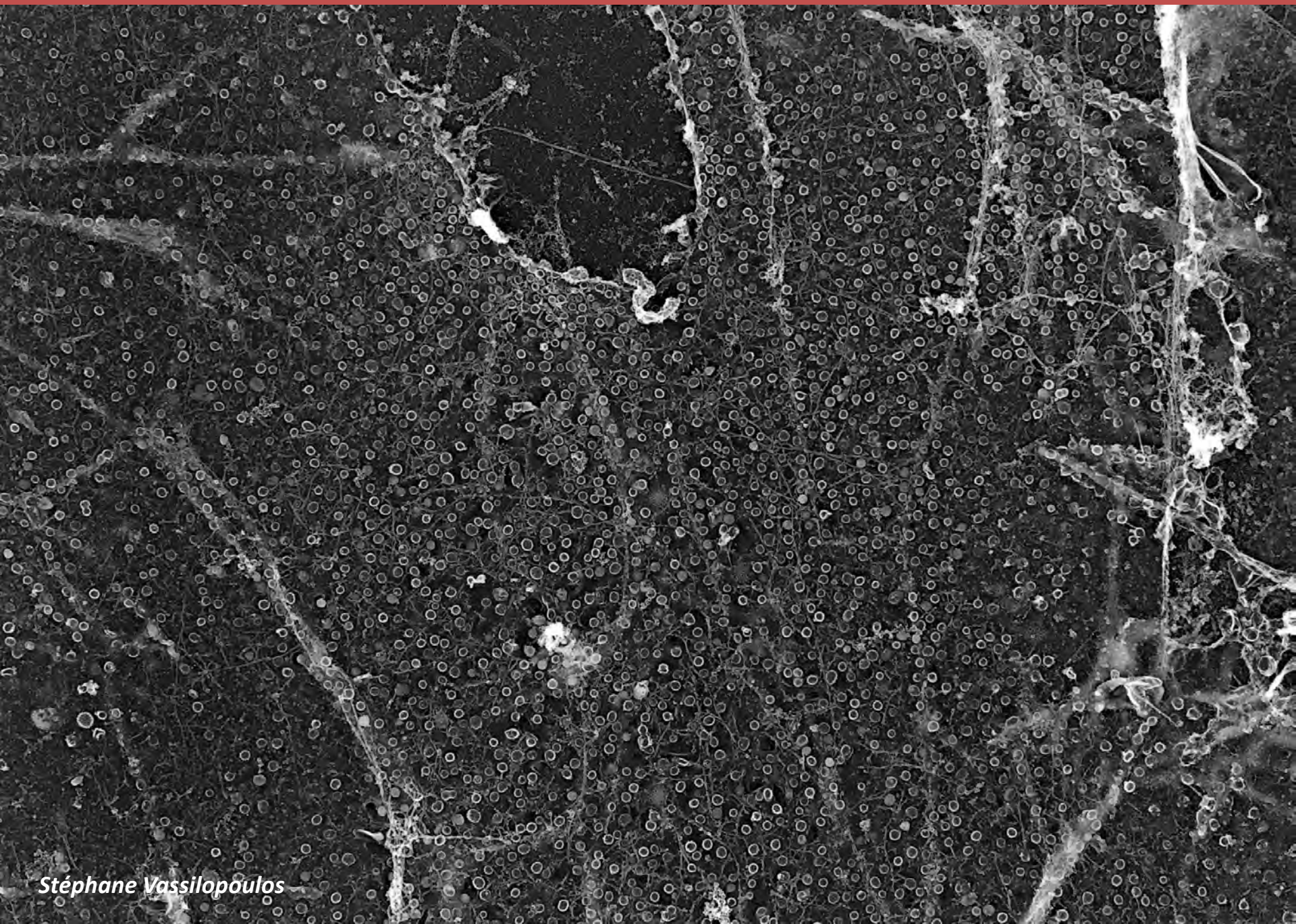
Cav3 mutation in humans: muscular dystrophies


Human bladder epithelial (T24)
200 µm

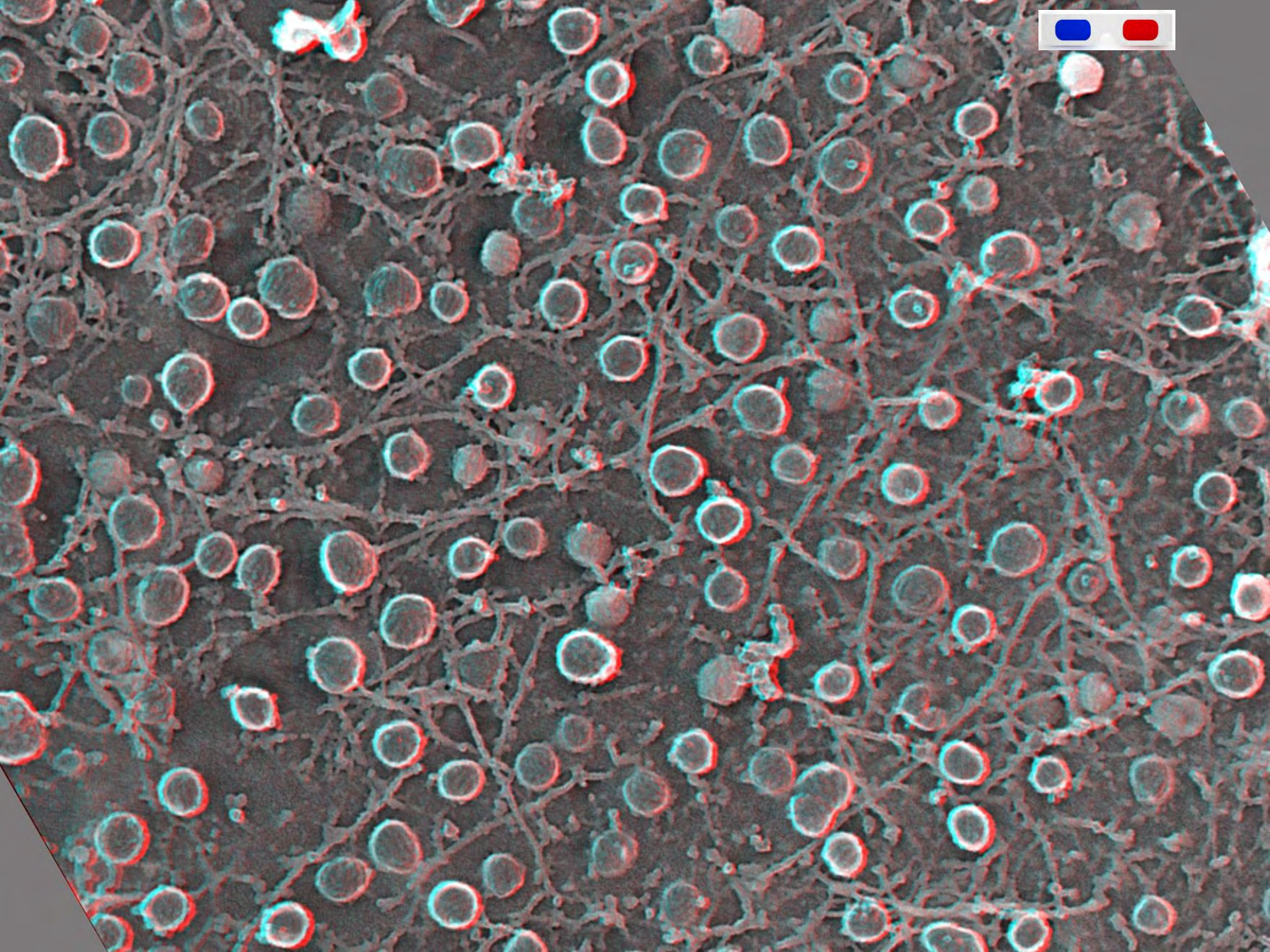

Adipocyte (NIH3T3-L1)
200 µm



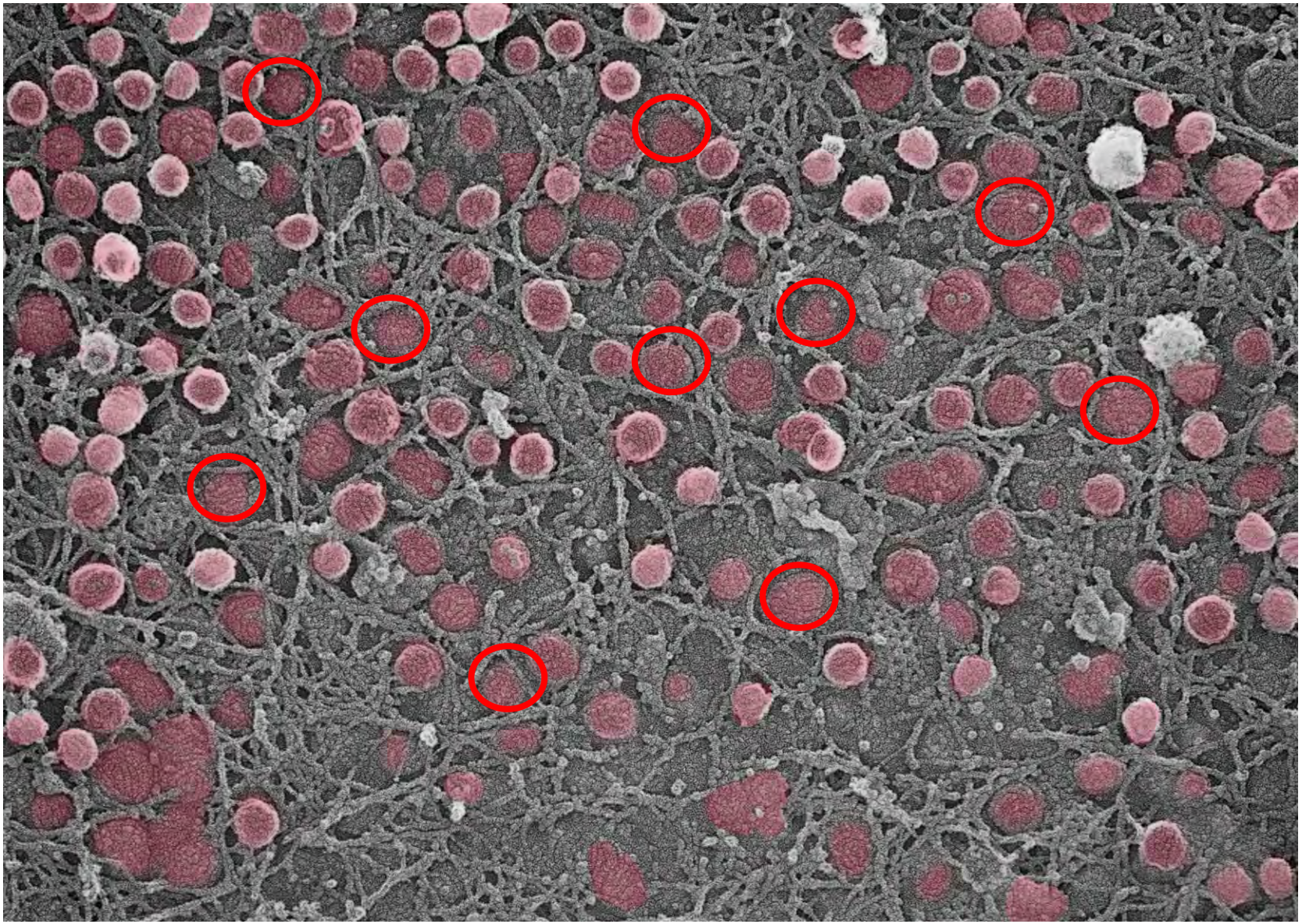
1/ Caveolae in human myoblasts



Stéphane Vassilopoulos



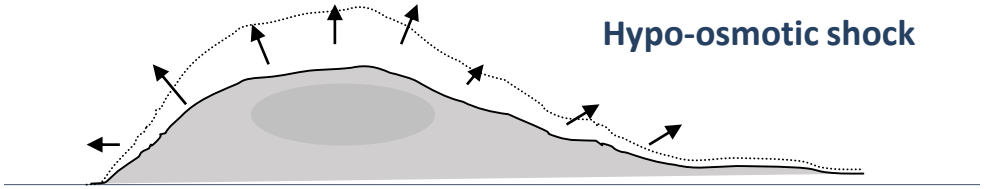
Cavéoles: invaginées ou plates?



Répliques métalliques de cellules Hs578T « ouvertes »

Stéphane Vassilopoulos

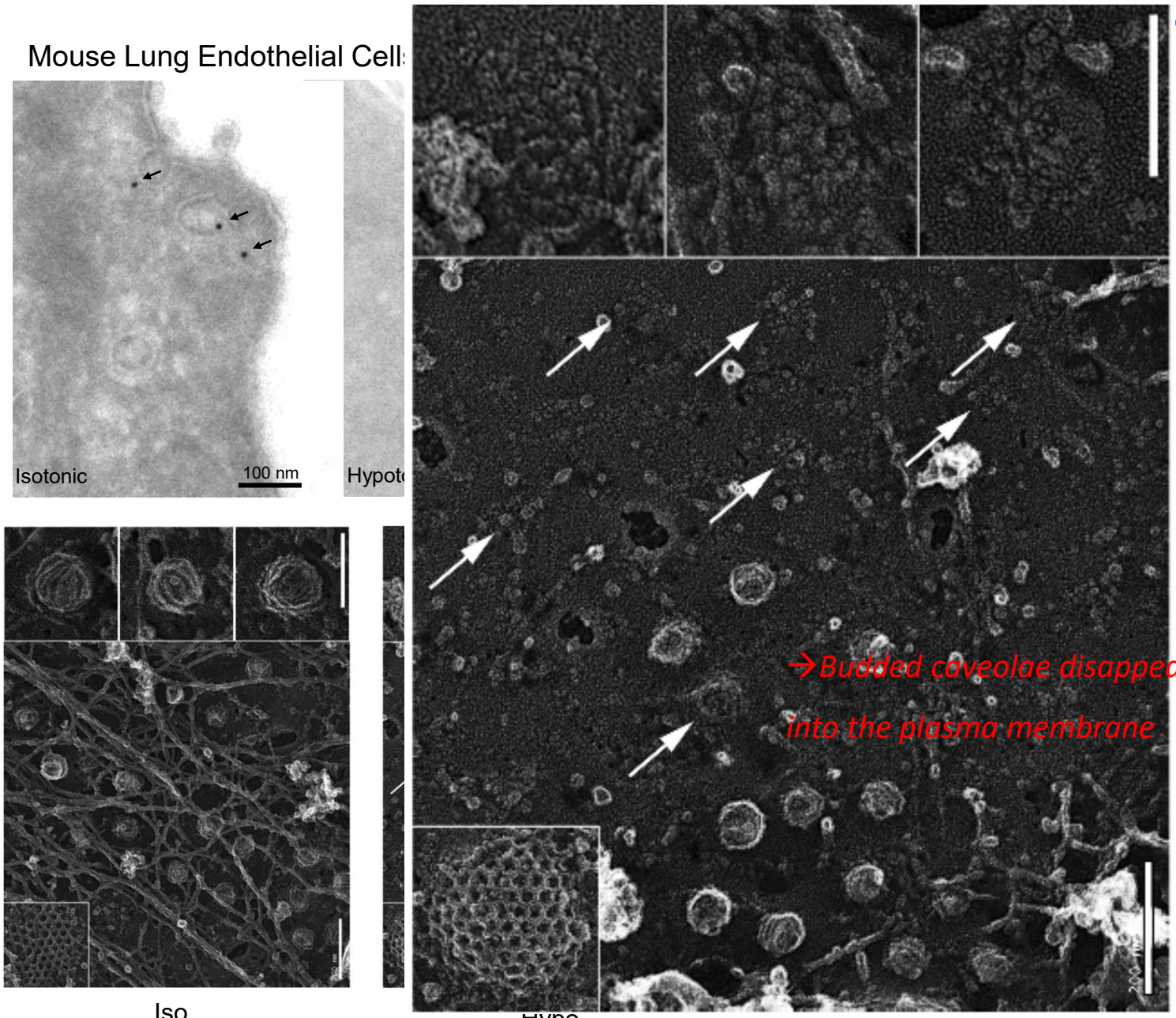
Augmentation aigüe de la tension de membrane par choc hypo-osmotique



Iso-osmolarity: 330mOsm
Hypo-osmolarity: 30mOsm

Weiwei Shen

❖Aplanissement de la structure cavéolaire



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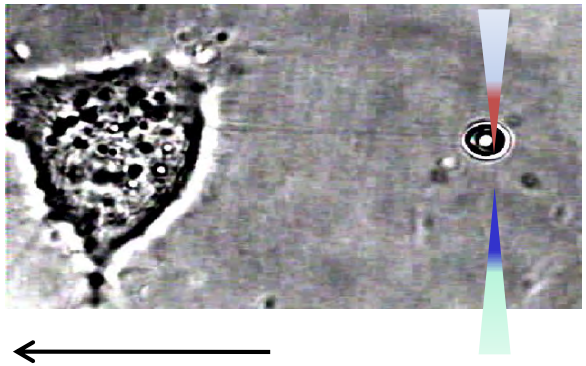
Les cavéoles maintiennent la valeur de la tension de membrane



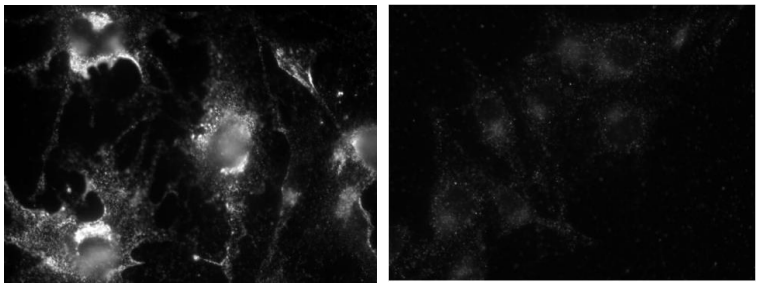
Linear shift of stage Δx ~ Force

Dai, Sheetz et al *J neuroscience* 1998

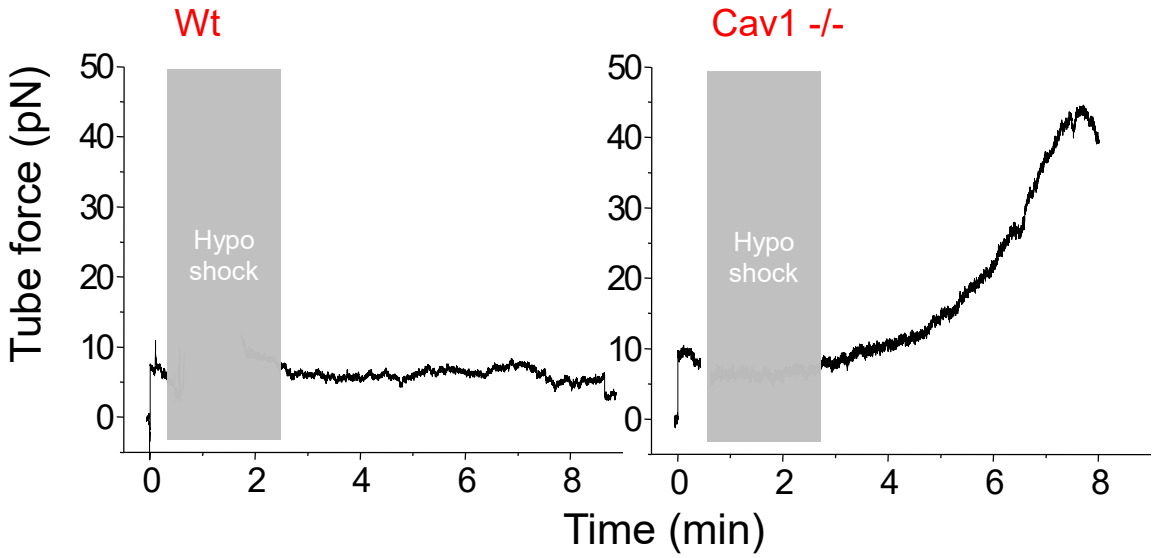
$$f_s = 2\pi\sqrt{2\sigma\kappa}$$

Cellular nanotubes are membrane tension sensors

MLEC



Wild type cav1 -/-



→*Caveolae are required to maintain membrane tension homeostasis during hypo-osmotic shock*

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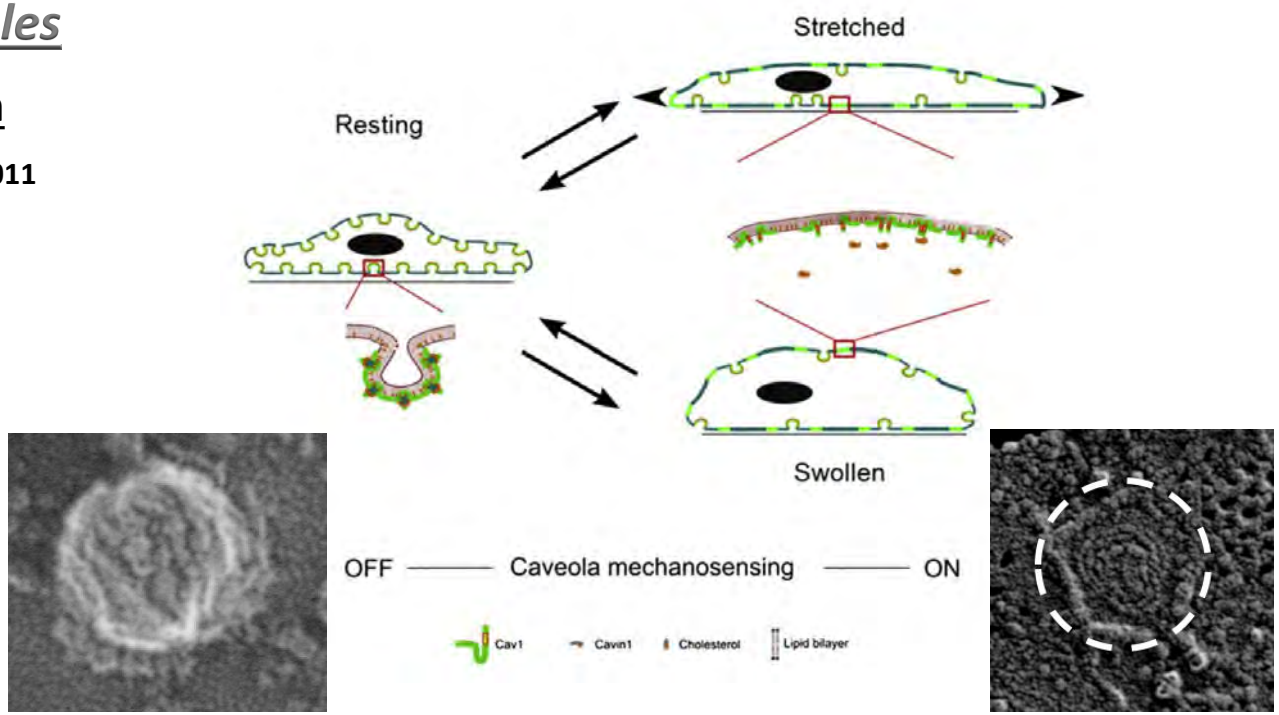
Fonctions des cavéoles

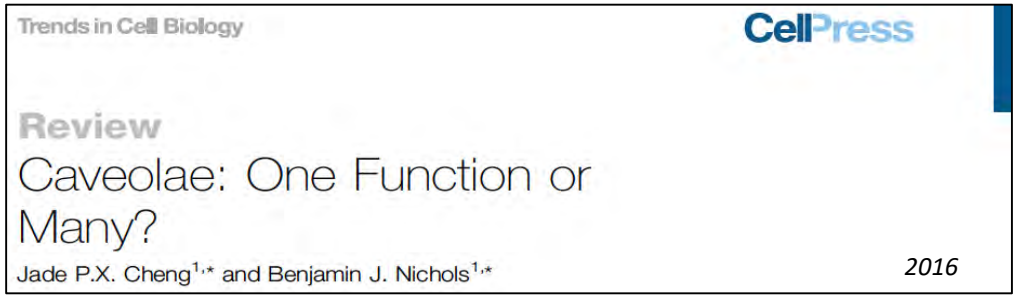
⊙ Mécanoprotection

Sinha et al. *Cell* 2011

Gervasio et al. *J Cell Sci* 2011
Joshi et al. *J Cell Biol* 2012
Ariotti et al. *J Cell Biol* 2014
Cheng et al. *J Cell Biol* 2015
Lo et al. *J Cell Biol* 2015
Lim et al. *Curr Biol* 2017
Garcia et al. *Curr Biol* 2017



- ⊙ Endocytose
- ⊙ Homéostasie des lipides
- ⊙ Signalisation



Un rôle nouveau des cavéoles: mécanique et physiopathologie

→ Molecular regulation of caveolae disassembly/ reassembly?

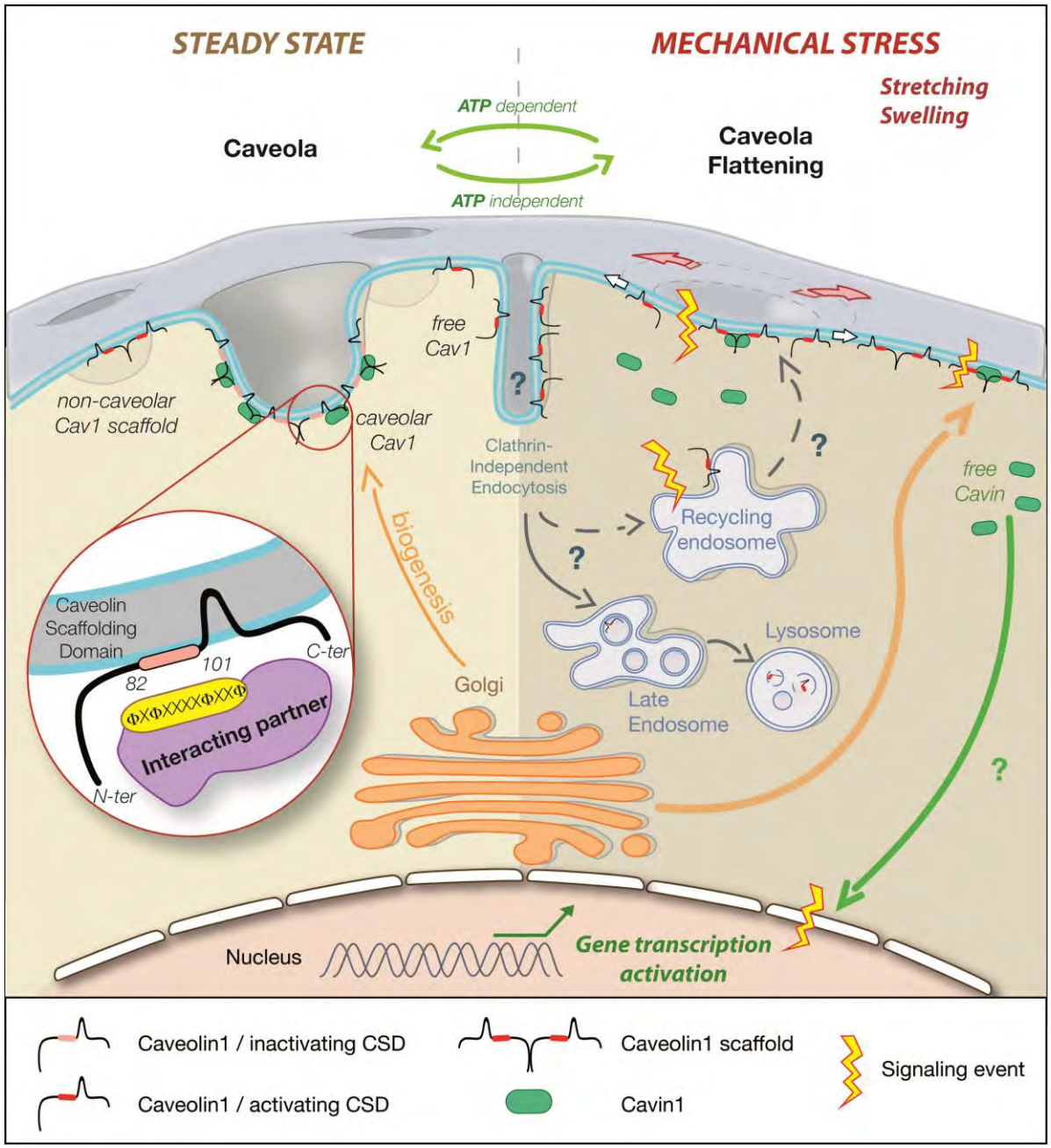
→Role in mechano-transduction

→ Role in human diseases:

→Breast cancers & melanoma

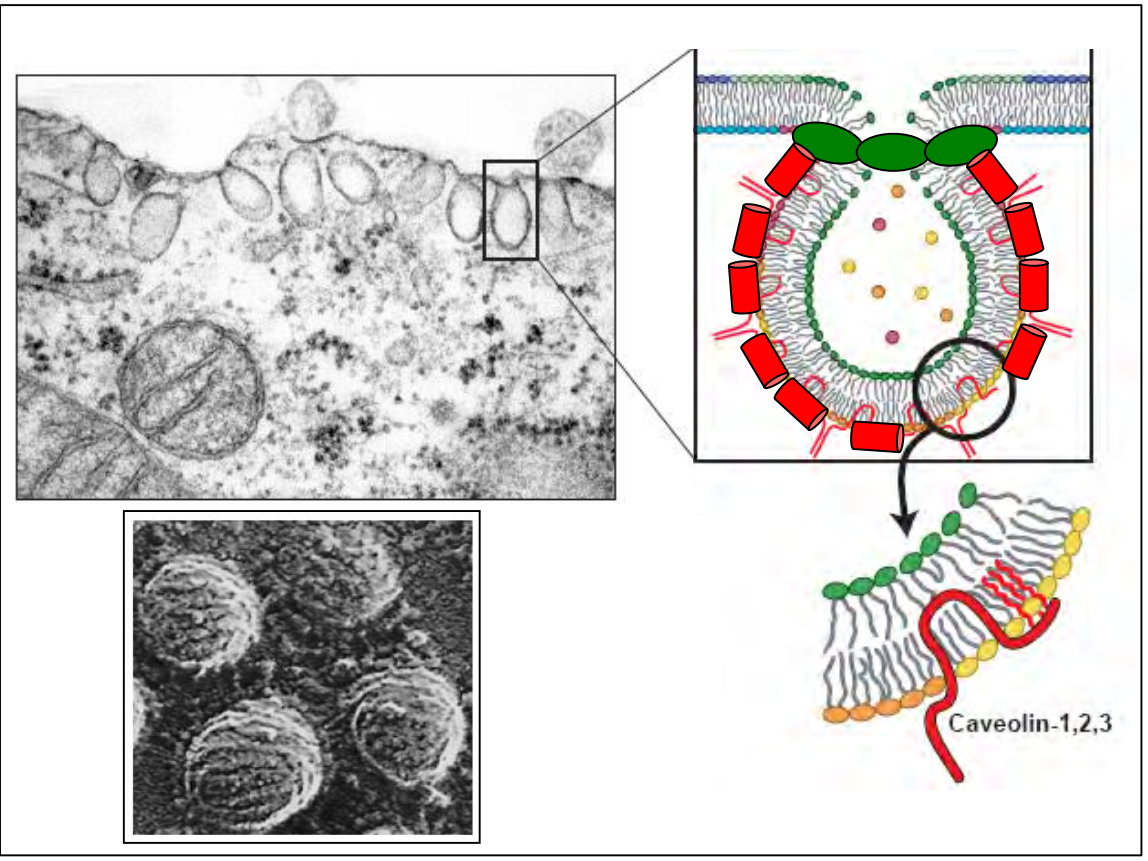
→Muscular dystrophies

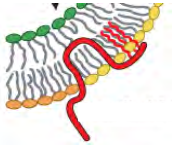
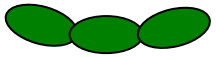
→Atherosclerosis



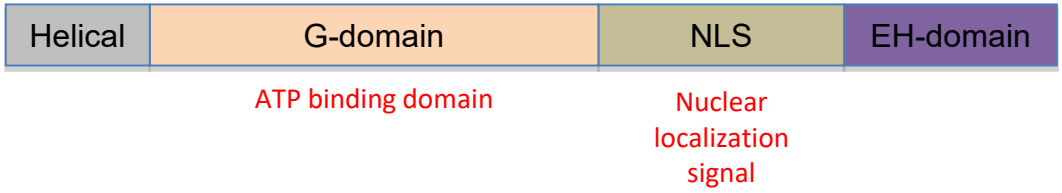
Nassoy and Lamaze, TCB, 2012

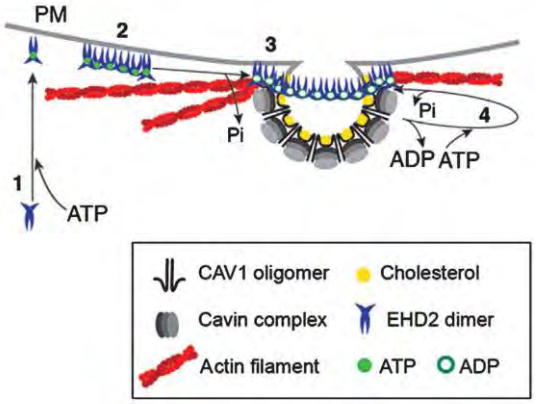
Les Cavéoles de la membrane plasmique



- Cholesterol, sphingolipids
- Caveolins 
- Cavins 
- EHD2 
- Associated proteins (eNOS, Akt, Src, Rho, Rac etc.)

EHD2 ATPase: Eps-15 homology domain-containing protein 2

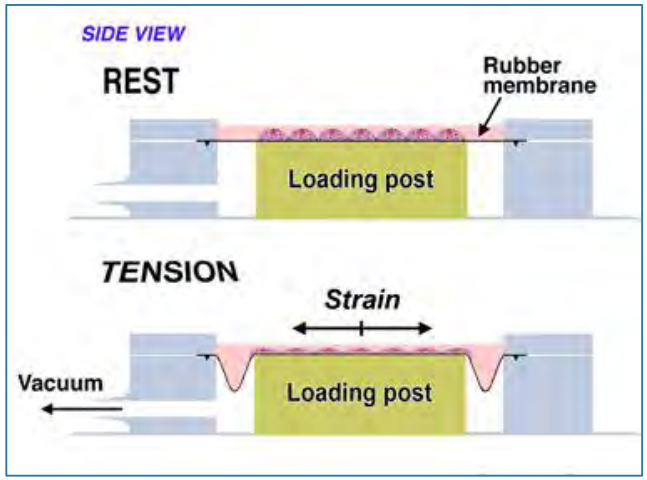


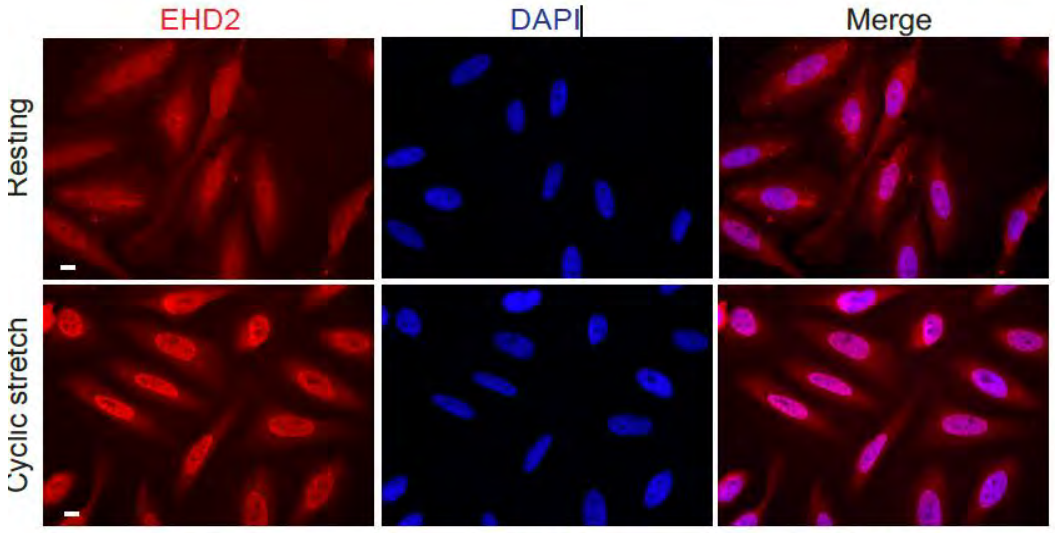


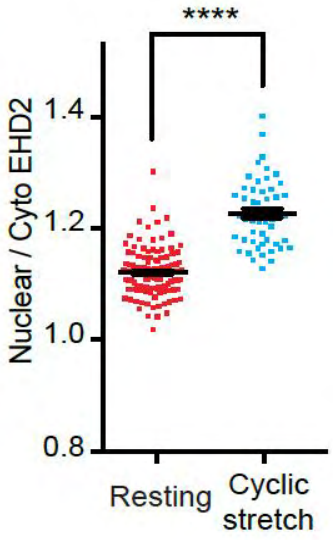
Stoeber et al., Oligomers of the ATPase EHD2 confine caveolae to the plasma membrane through association with actin. *Embo J* 2012

Devenir intracellulaire de EHD2 sous stress mécanique?

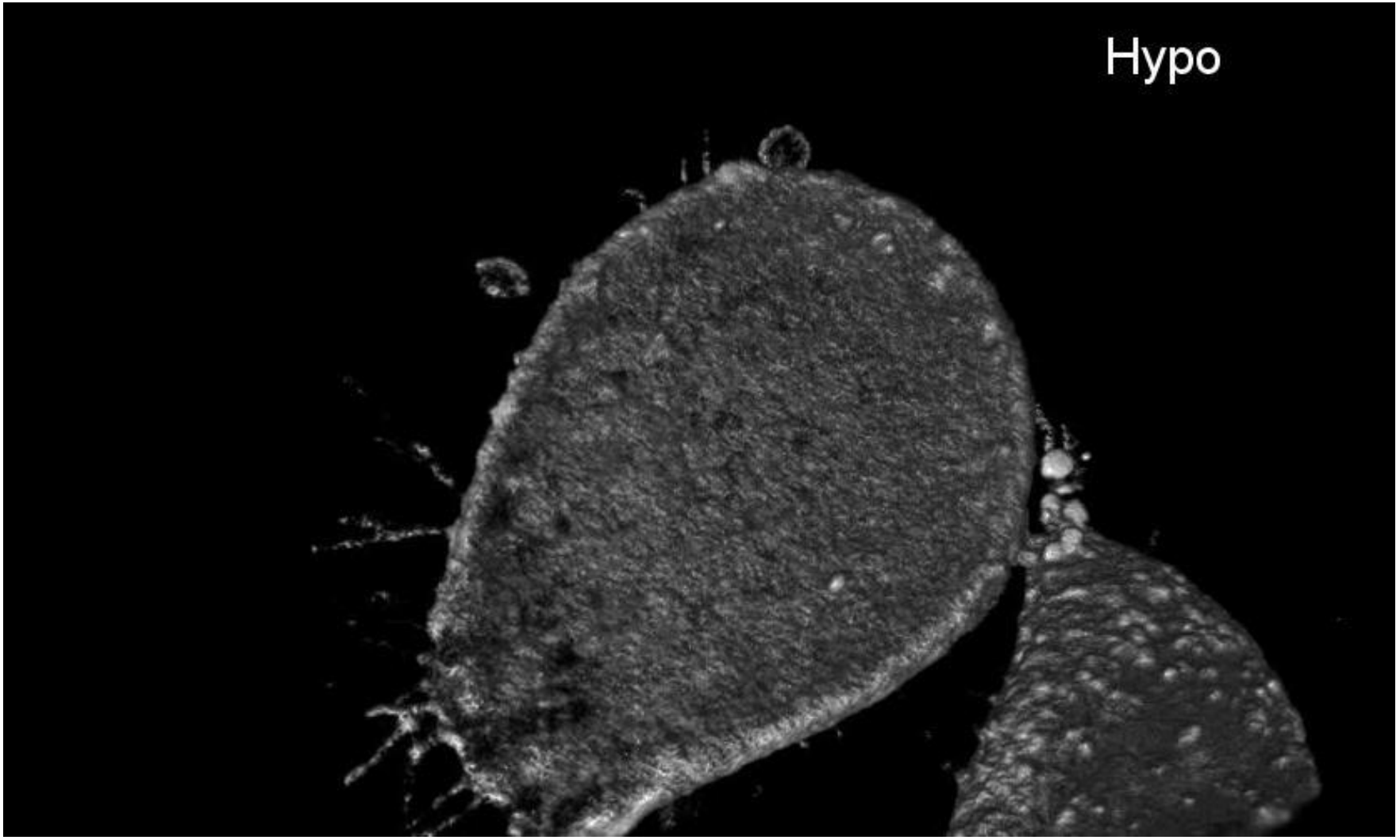
Flexcell : 10% cyclic stretching 30 min (0.5 hertz)







Dynamique de la translocation nucléaire?



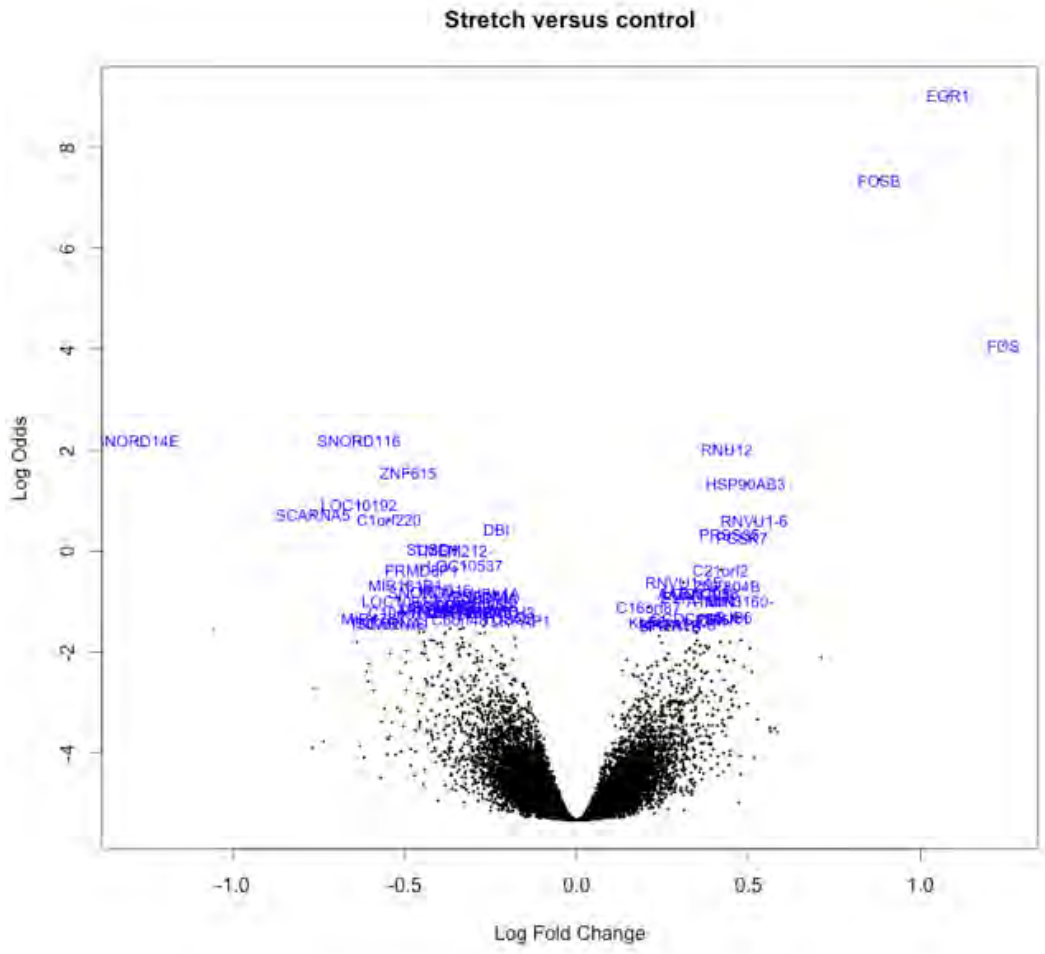
Lattice Light Sheet Microscopy
EHD2-mEmerald

Eric Betzig, Janelia Research Campus
Nobel 2014

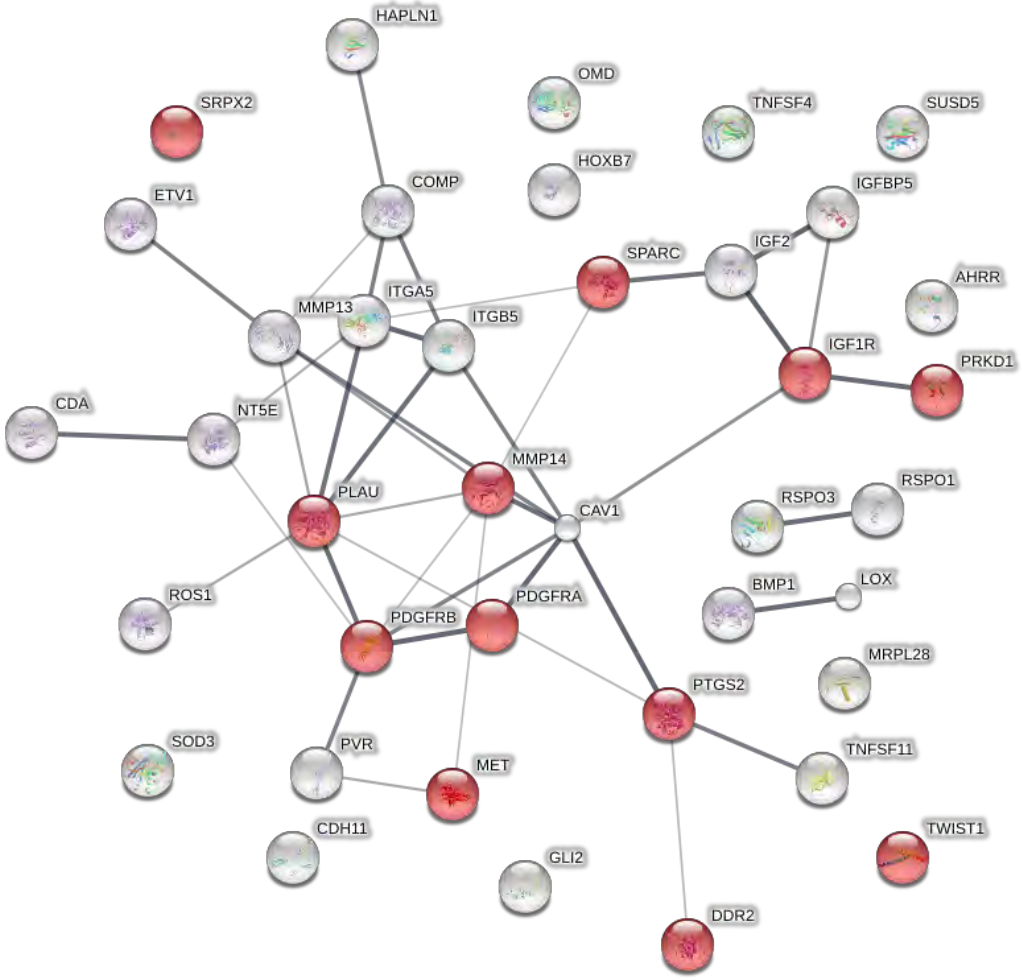
Analyse des gènes impactés par le stretch mécanique

MicroArray analysis (DNA chip)

Hs578T cells @ 10% cyclic stretching for 30 min 0.5 Hz



Vassili Soumélis, Pierre Bost



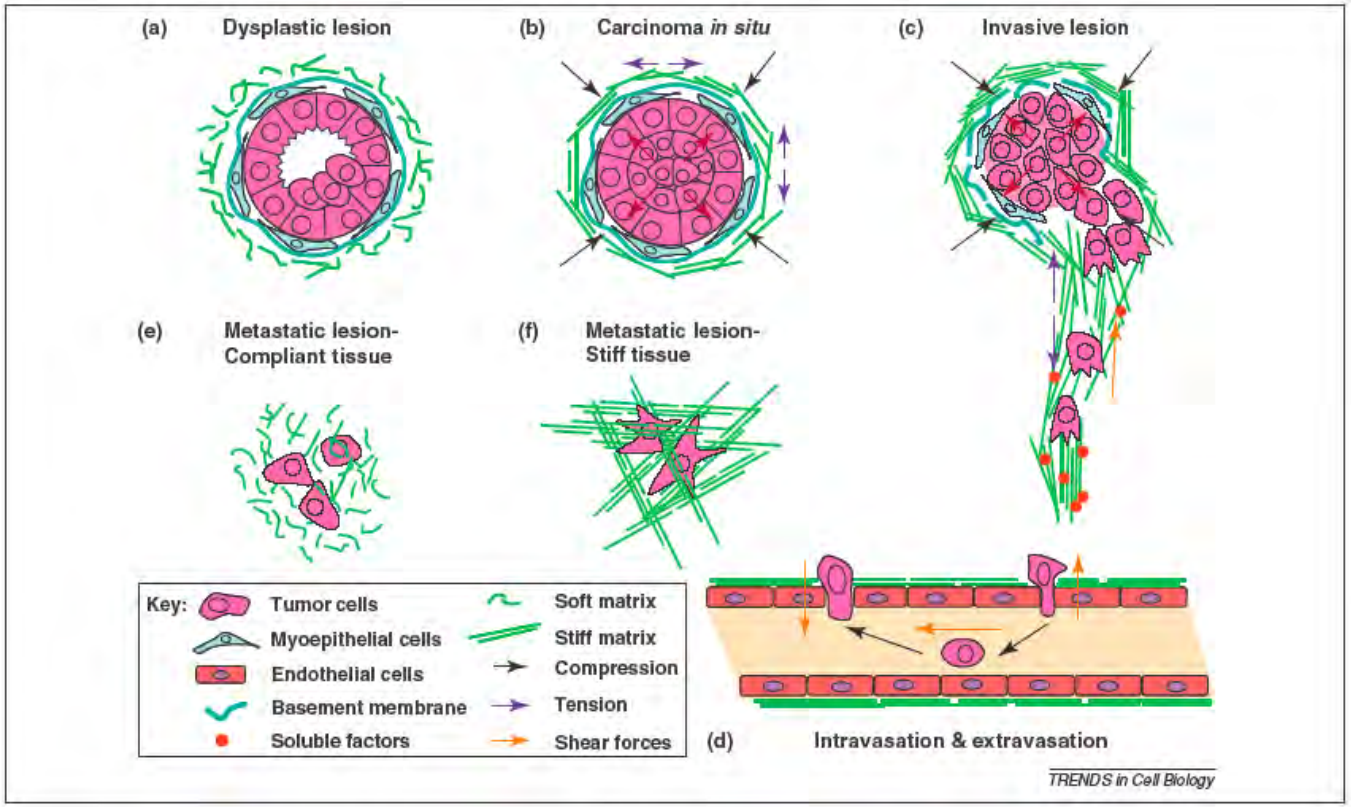
Upregulated when EHD2 is high

positive regulation of cell motility

Weiwei Shen, Sophie Vacher, Yvan Bièche

Role de la mécanotranduction des cavéoles dans la tumorigenèse?

Caveolin1 : involved in several steps of tumorigenesis
(Lamaze and Torrino *Biomed J* 2015)

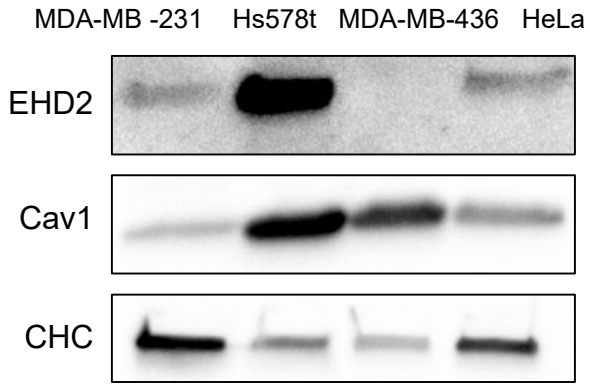


Forcing force and function : biomechanical regulation of tumor evolution
Valerie Weaver UCSF Trends in Cell Biology 2011

Niveaux d'expression de Cav1 and EHD2 dans les cellule de cancer du sein triple négatifs (TNBC)

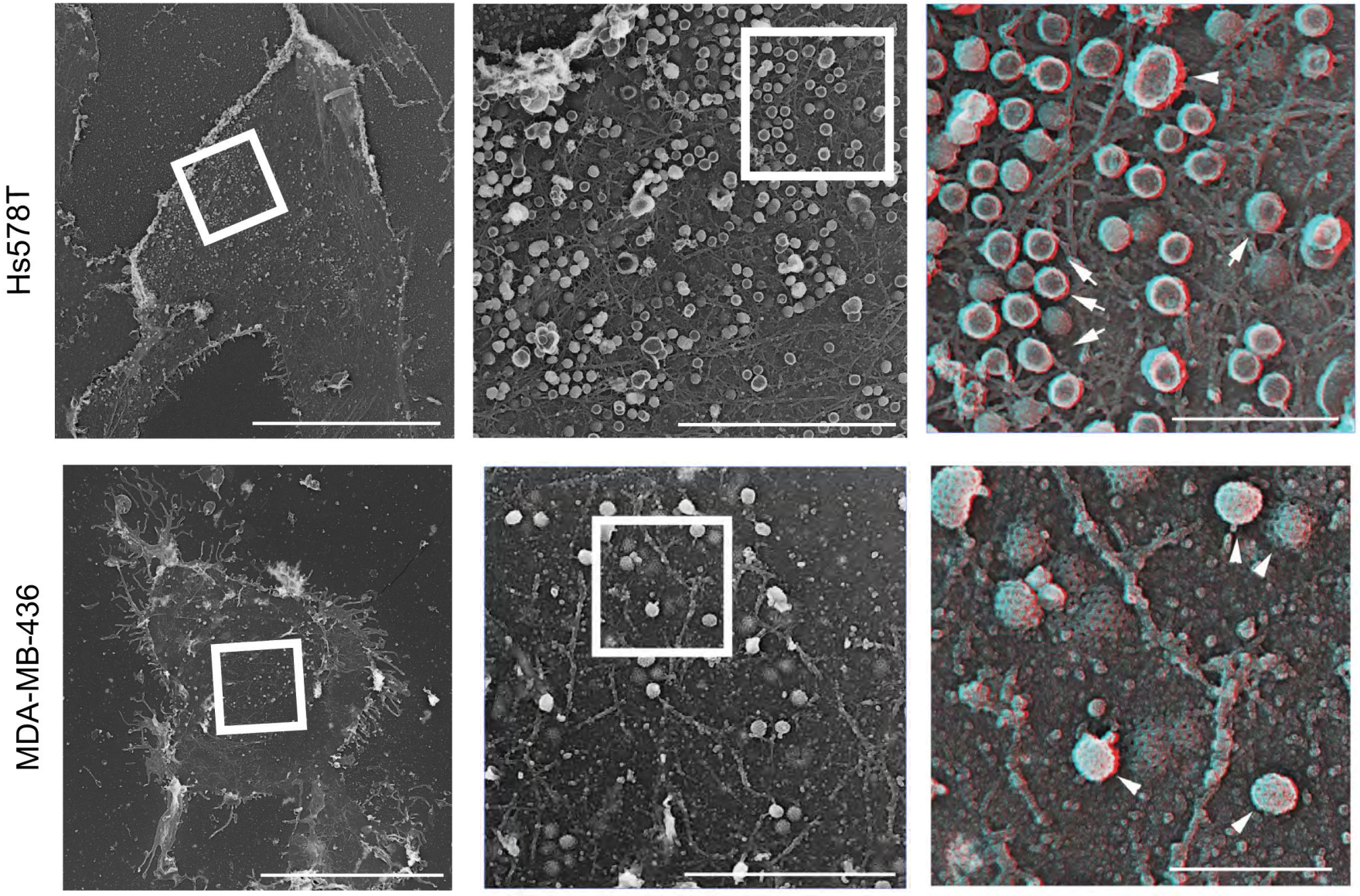
Cell lines	Cav1 expression	EHD2 expression	Aggressiveness
MCF-10A	+	+	Control
MDA-MB-231	+ +	+	+ + +
MDA-MB-436	++	- - -	+++
Hs578T	+ +	+ +	+ +
HCC1187	- - -	- - -	+
MDA-MB-468	- - -	- - -	+

HS578T HIGH EHD2
MDA-MB-231 LOW EHD2
MDA-MB-436 NO EHD2



Collaboration with T. Dubois

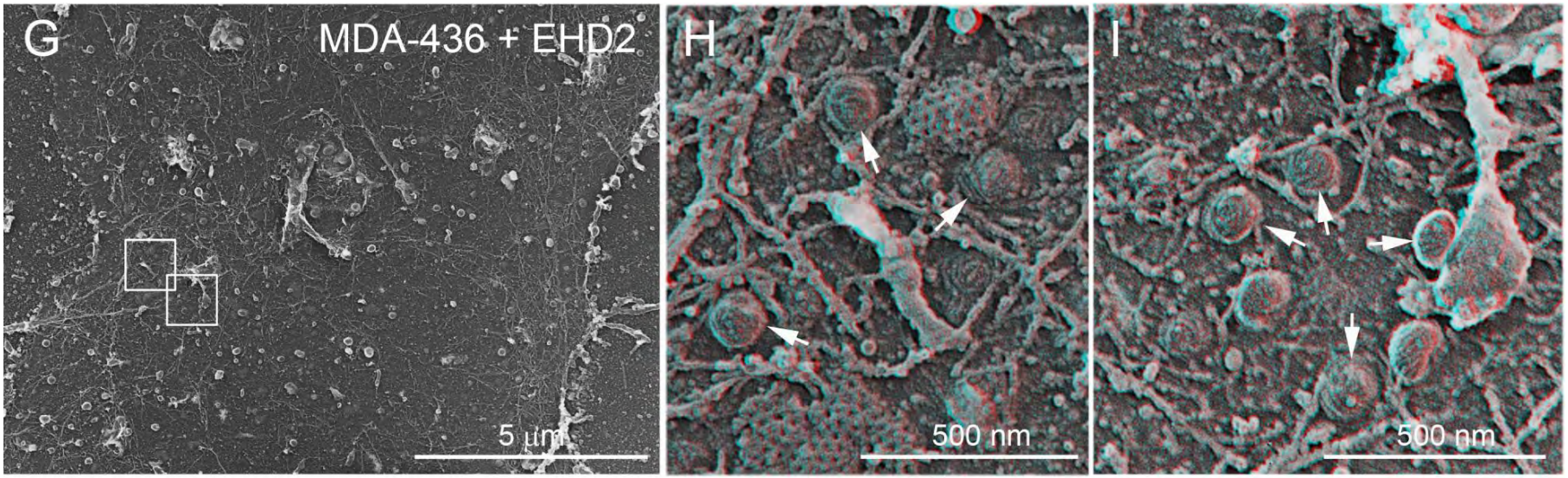
Absence du réservoir de cavéoles dans les cellules deTNBC



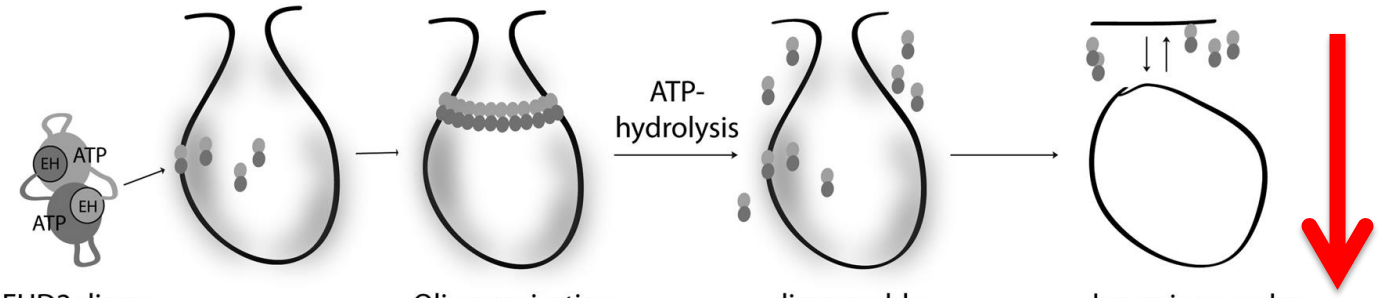
répliques métalliques de cellules « ouvertes »

Stéphane Vassilopoulos

EHD2 stabilise le réservoir de cavéoles dans les TNBC



Stéphane Vassilopoulos



Membrane relaxation
No EHD2

Moren *et al.* 2012 MBoC

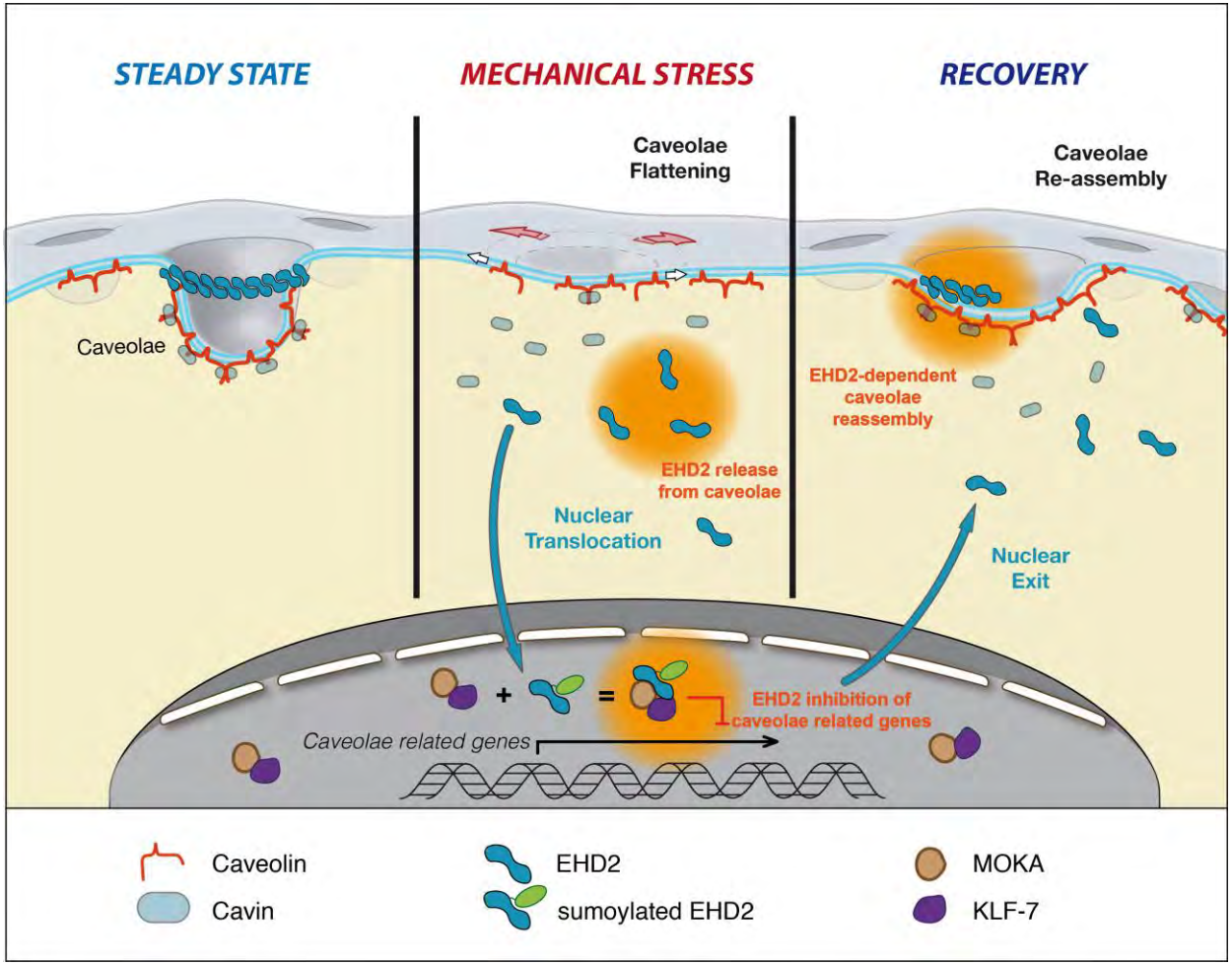
EHD2 est un nouveau mécanotransducteur qui connecte la réponse mécanique des cavéoles à la transcription des gènes

Normal ratio EHD2/Cav1

Normal ratio EHD2/Cav1

Low ratio EHD2/Cav1

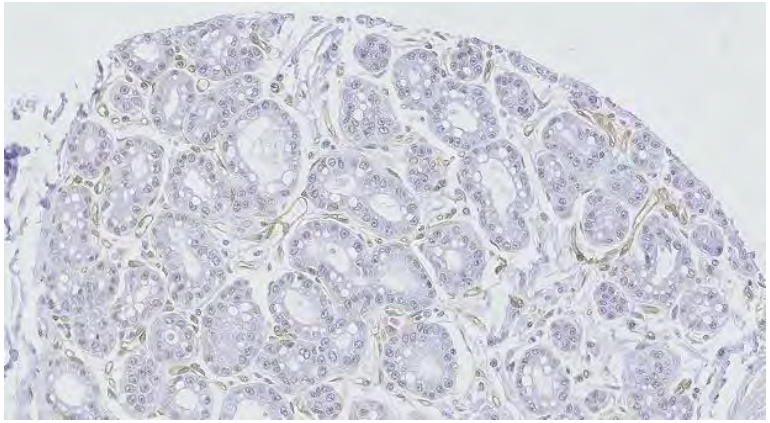
Increased breast cancer cell migration and aggressiveness



Torrino, Shen et al.,
The Journal of Cell Biology, 2018

Rôle d'EHD2 chez les patientes atteintes de cancers du sein

Tissue Micro Arrays (TMA) data from TNBC patient biopsies (Hôpital Curie)

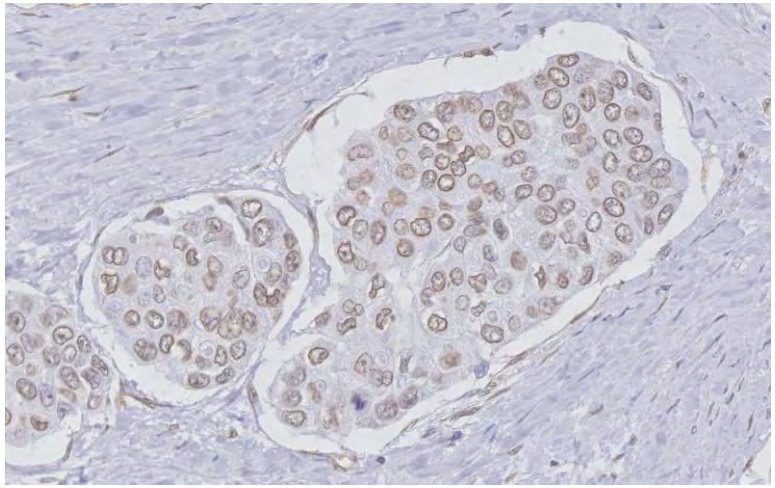


Caveolin in normal breast

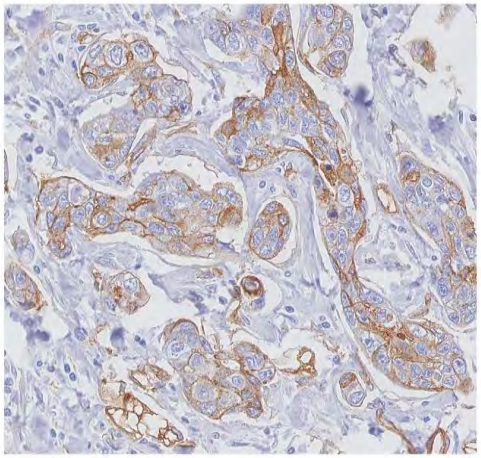
Cohort: **103 TNBC** patients

- 54 HER2+ with 27 N+ (axillar metastasis)
- 114 luminal A with 45N+
- 101 luminal B with 48N+
- 12 luminal B/ HER2+ (OR+ / PR+) with 4N+

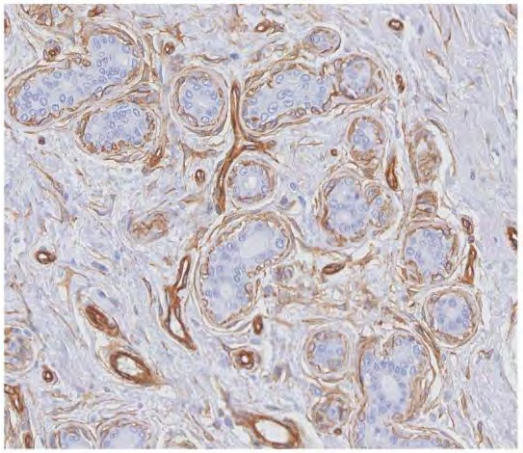
EHD2



Caveolin in invasive carcinoma



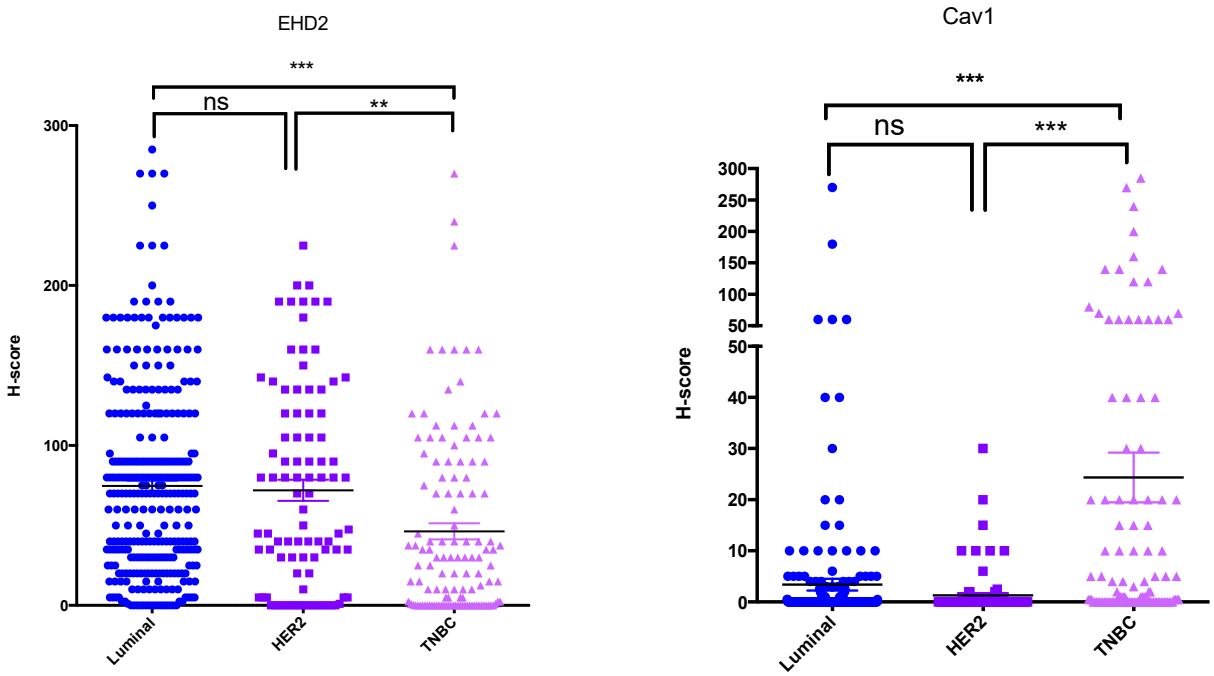
Normal breast



Invasive carcinoma

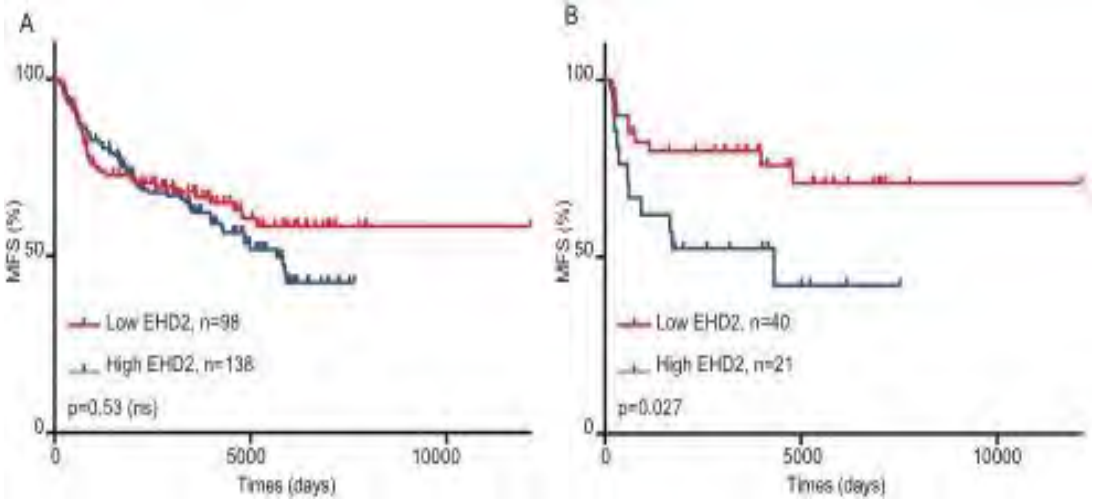
Stéphanie Torrino

Lower EHD2/Cav1 ratio in aggressive TNBC



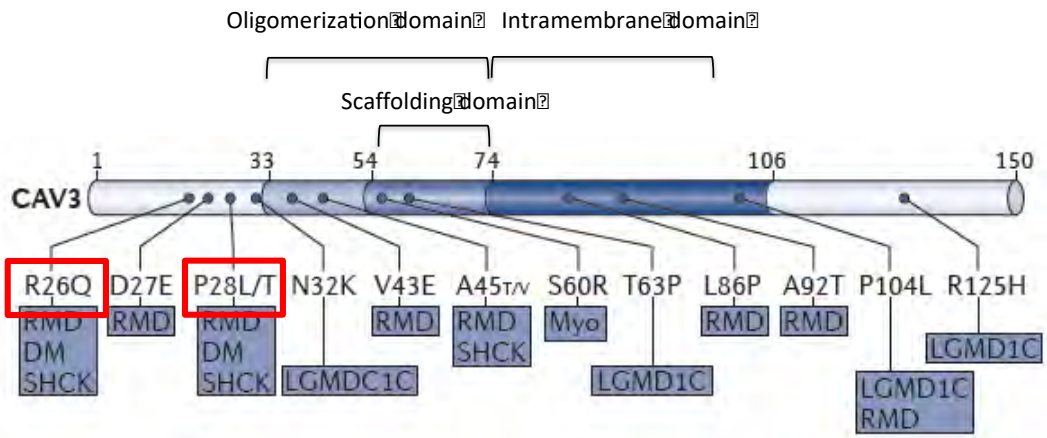
EHD2 is a Predictive Biomarker of Chemotherapy Efficacy in Triple Negative Breast Carcinoma

Submitted Breast Cancer Research



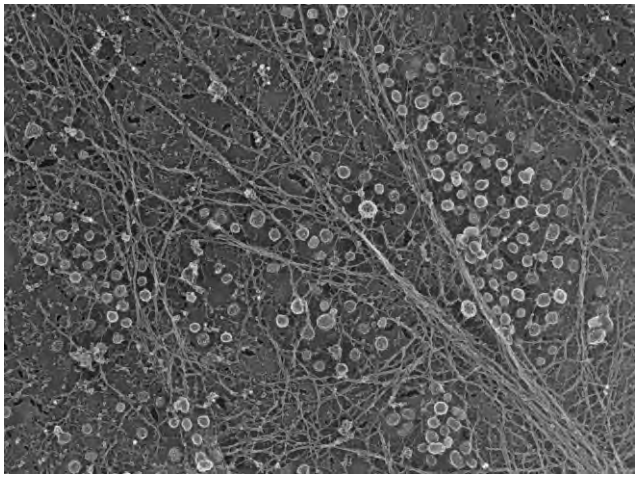
Stéphanie Torrino

Caveolin-3 et dystrophies musculaires



Parton and Del Pozo, 2013

Hayashi et al *JCI* 2009



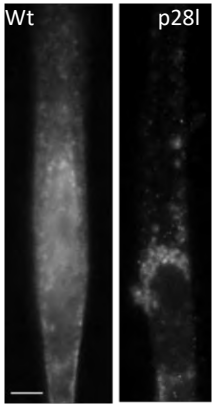
Stéphane Vassilopoulos

Muscular dystrophies, group of diseases

- Defect in muscle proteins
- Lack in membrane integrity and repair
- Progressive death of muscle cells and muscle tissue

RMD : Rippling Muscle Disease
LGMD1C : Limb-Girdle Muscular Dystrophy
FHCK : HyperCKaemia

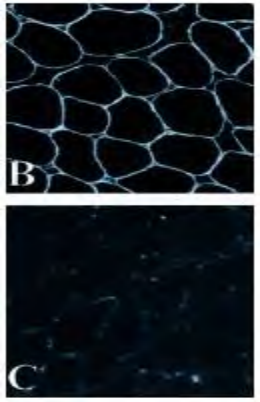
Coll. Dr. Gillian Butler-Browne, Inst de Myologie, Pitié-salpétrière
- Human myoblasts → myotubes



Human FHCK (Cav3 P28L) myotubes
(Famililal HyperCreatine Kinasemia)

Caveolin3

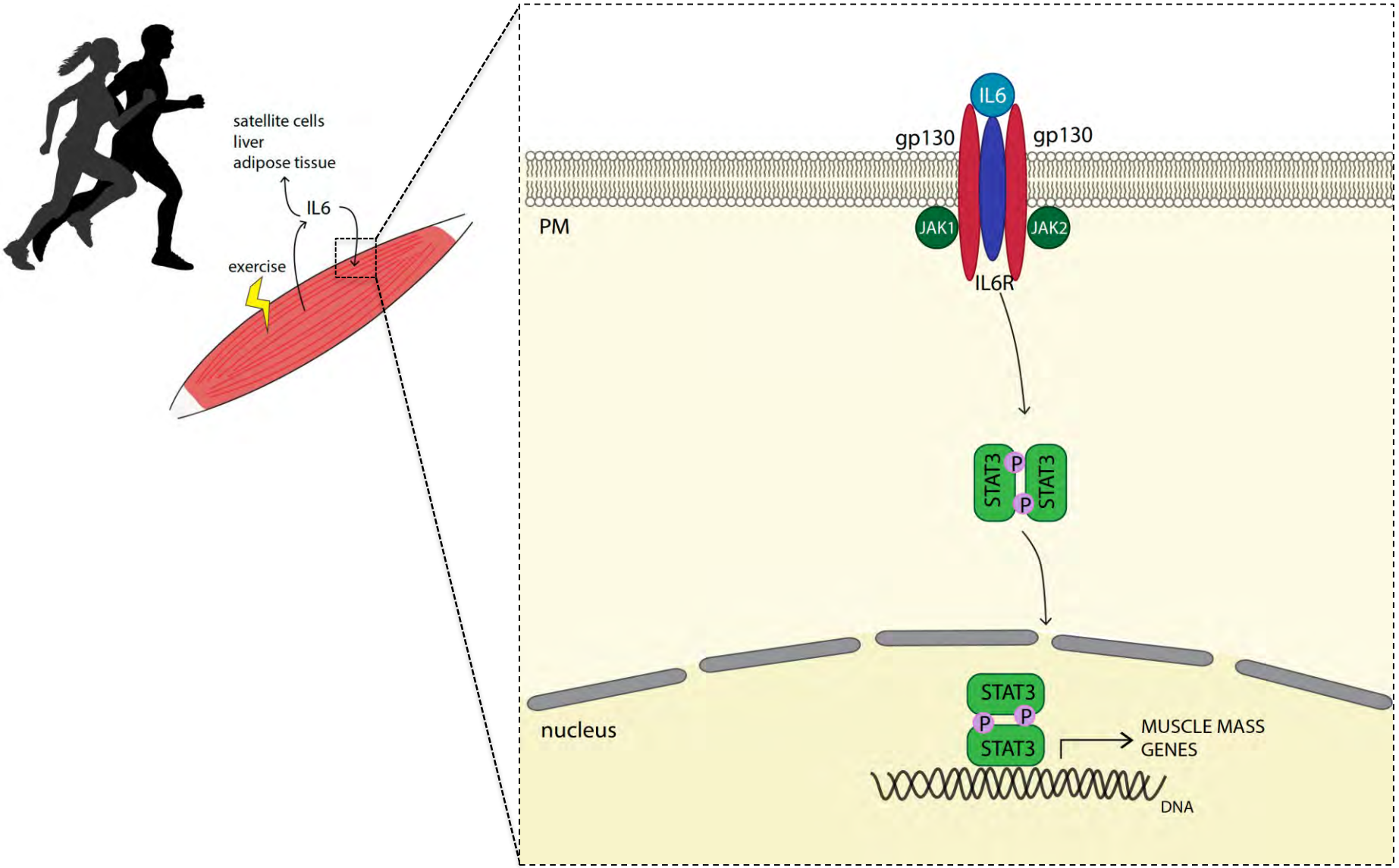
normal muscle cells



LGMD1 patients

Melissa Dewulf

La signalisation de IL6/STAT3 dans le muscle

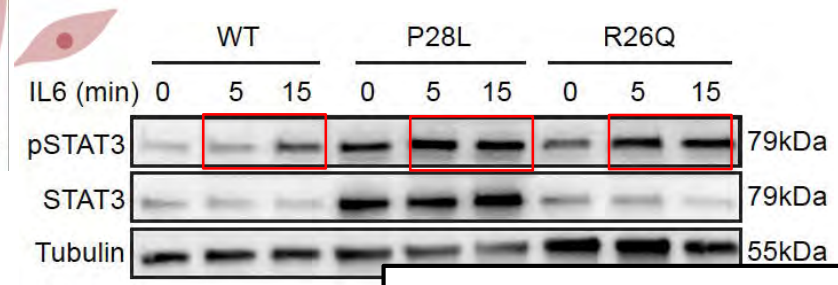


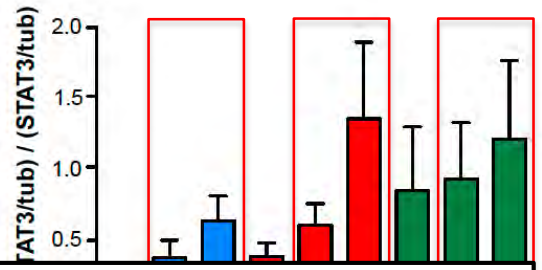
Cédric Blouin, Melissa Dewulf

Lack of IL6/STAT3 mechanosignaling in dystrophy-associated Cav3-mutations

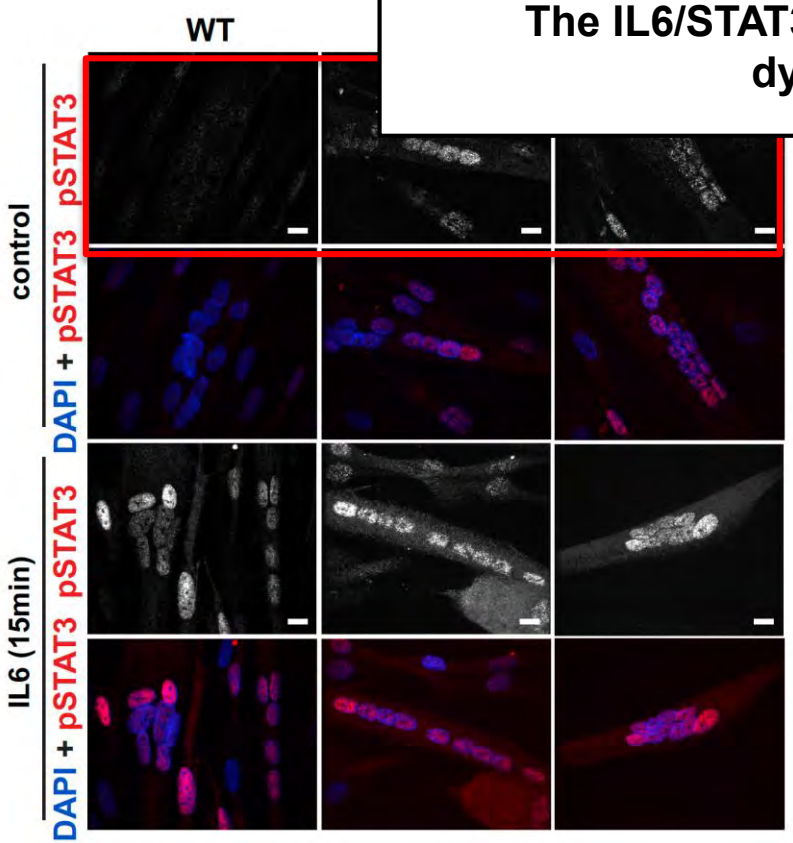
-

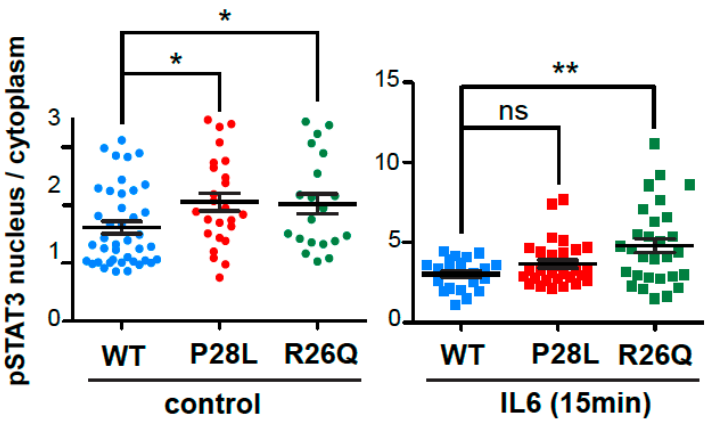
IL6/STAT3 signaling in resting human muscle cells





The IL6/STAT3 pathway is hyperactivated in dystrophic myotubes

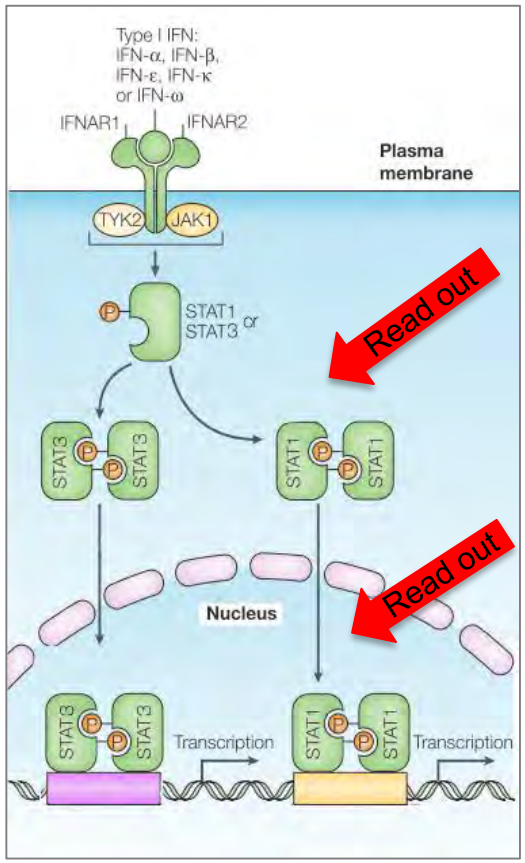


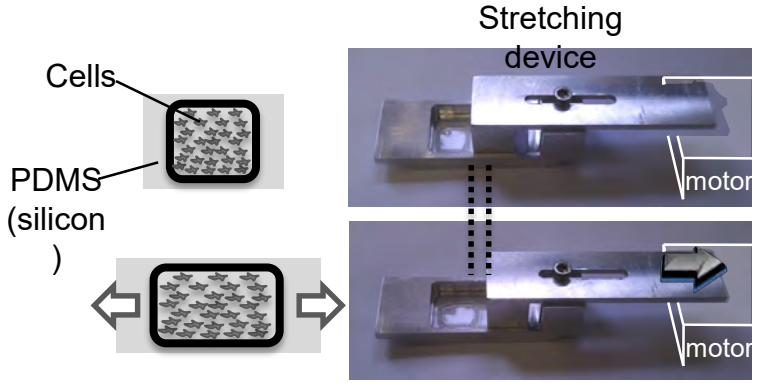


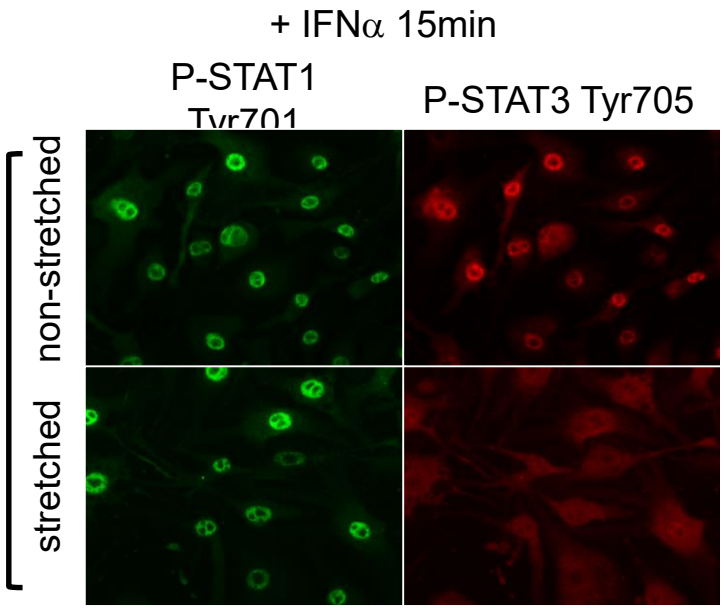
* p < 0.05 ** p < 0.01

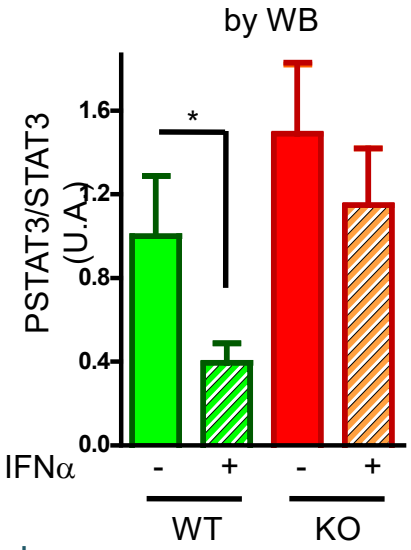
Melissa Dewulf
Nature Commun sous presse

Conséquences du désassemblage mécanique des cavéoles sur la signalisation intracellulaire ?







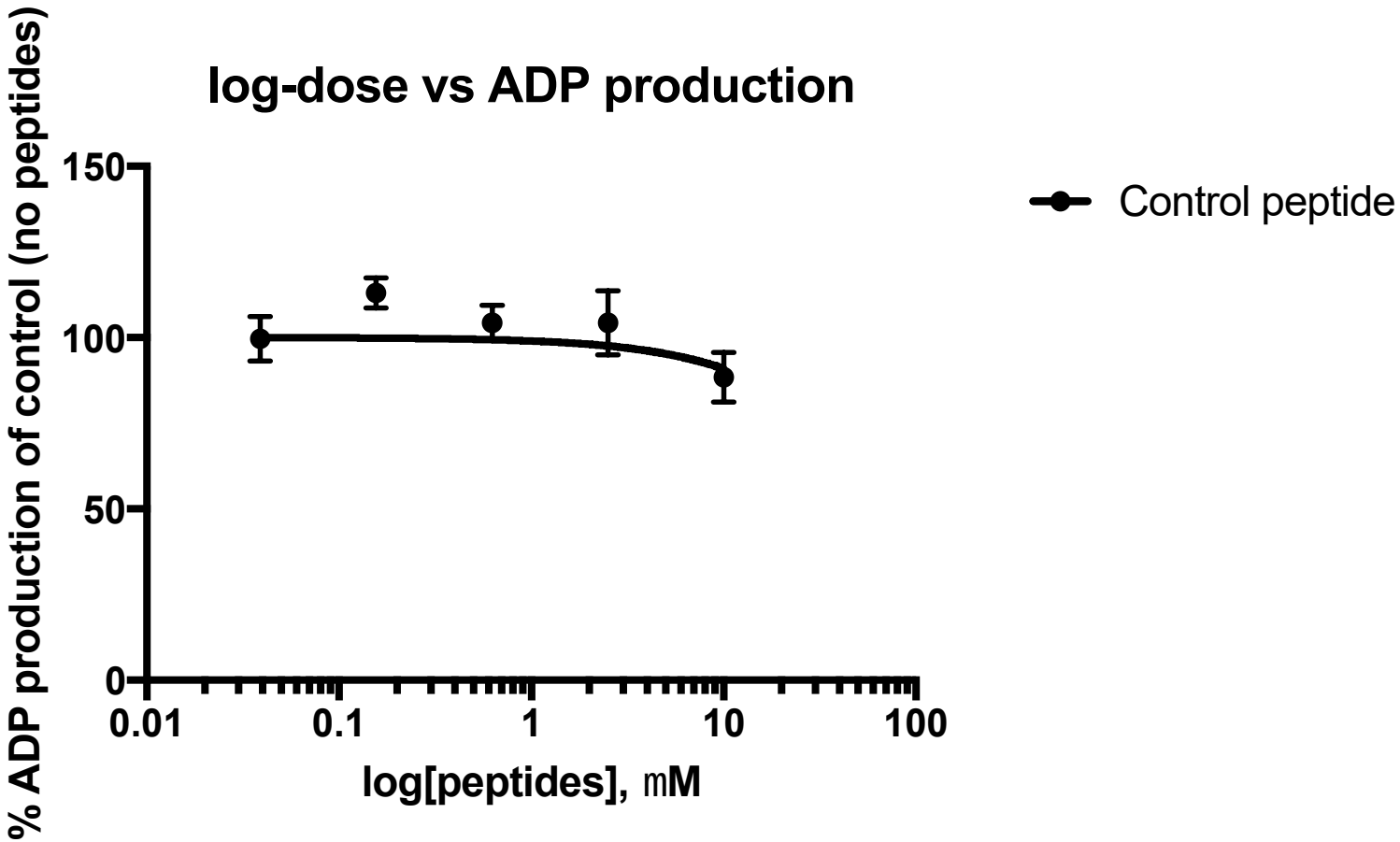


Lack of STAT3 phosphorylation & nuclear translocation in stretched cells

Cédric Blouin, Nicolas Tardif

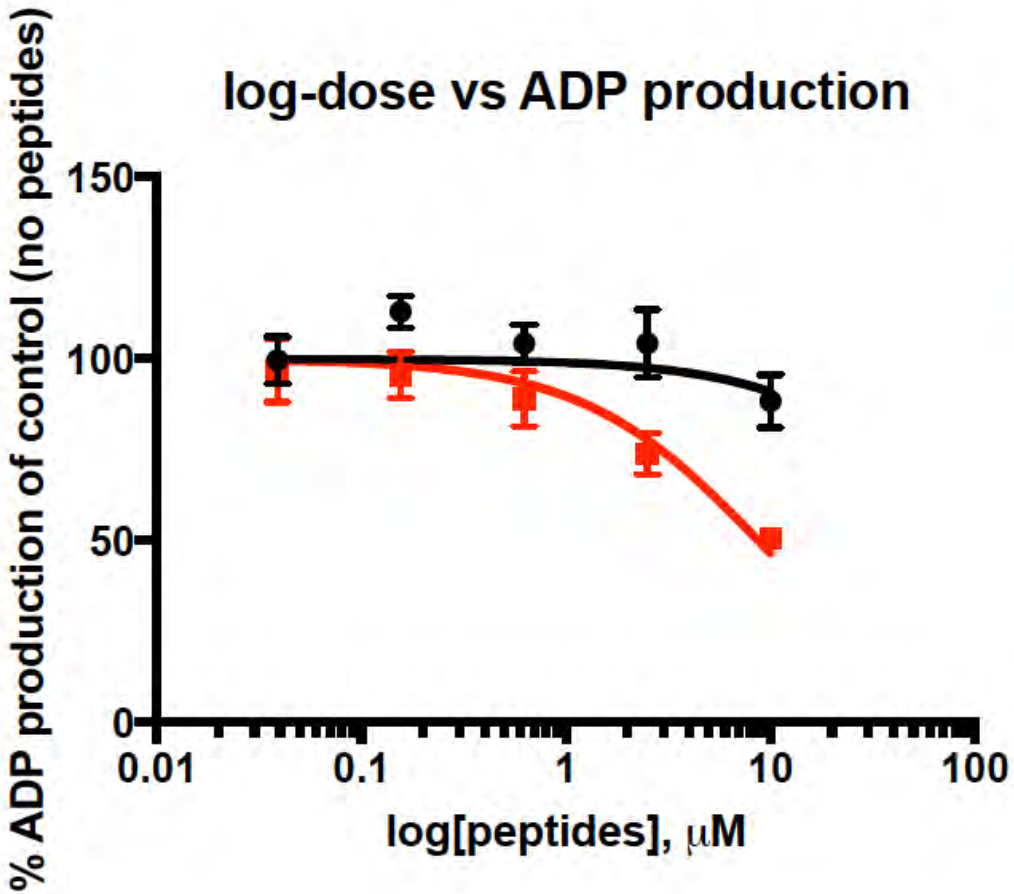
Does the CSD modulte JAK1 catalytic activity ?

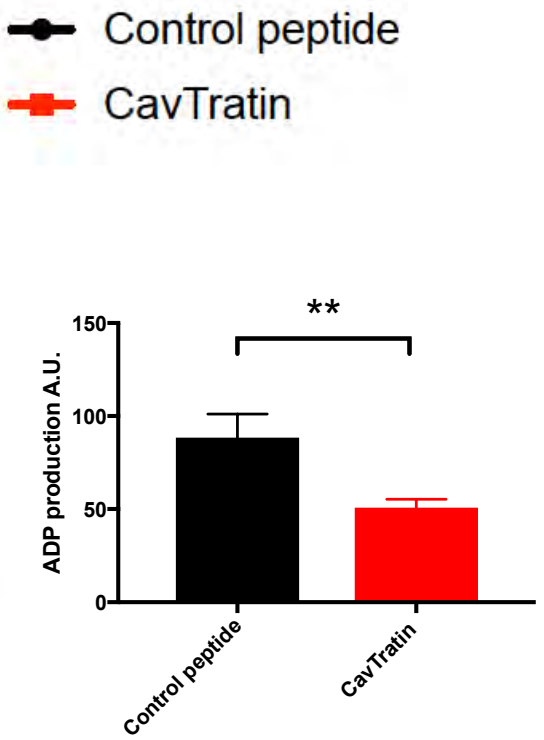
JAK1 aa583-1154 WT = 100 ng
[ATP] = 100 uM
[IRStide] = 80 ug/ml



La cavéoline contrôle directement l'activation de la kianse JAK1

JAK1 aa583-1154 WT = 100 ng
[ATP] = 100 uM
[IRStide] = 80 ug/ml

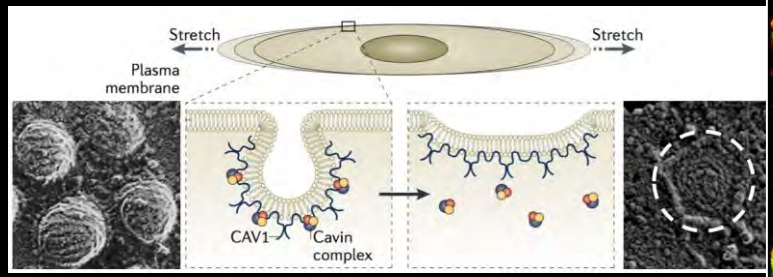




CBM1 = 157-164
CBM2 = 777-784

Nicolas Tardif

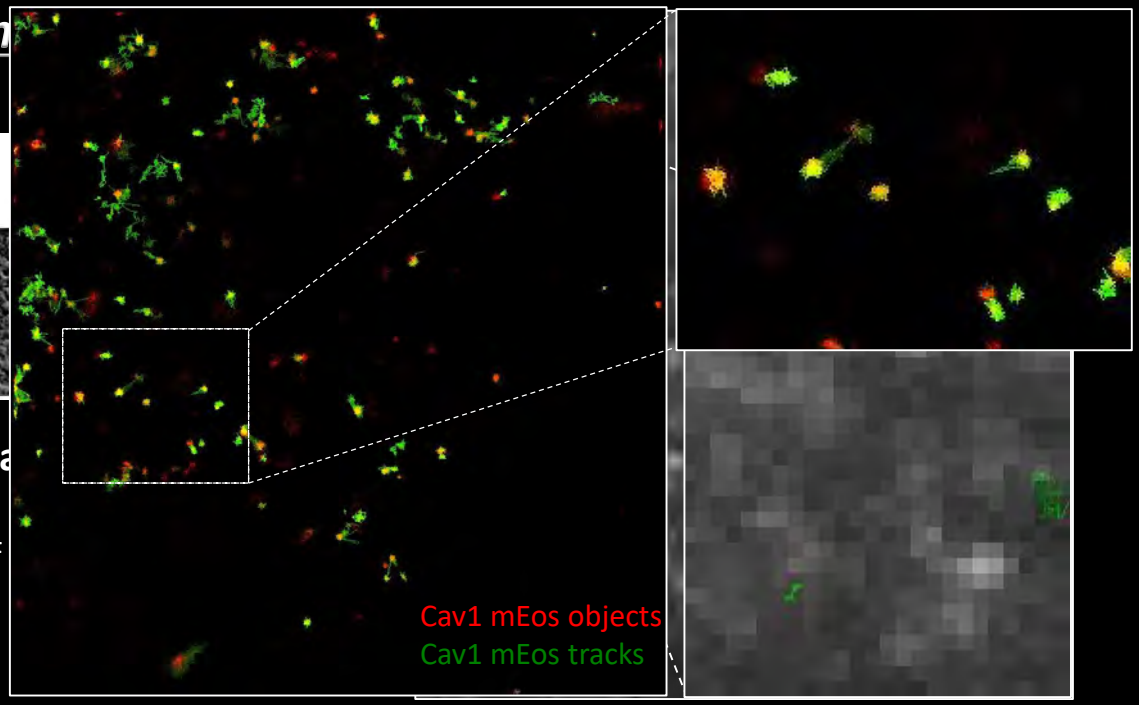
State of Cav1 release after mechan

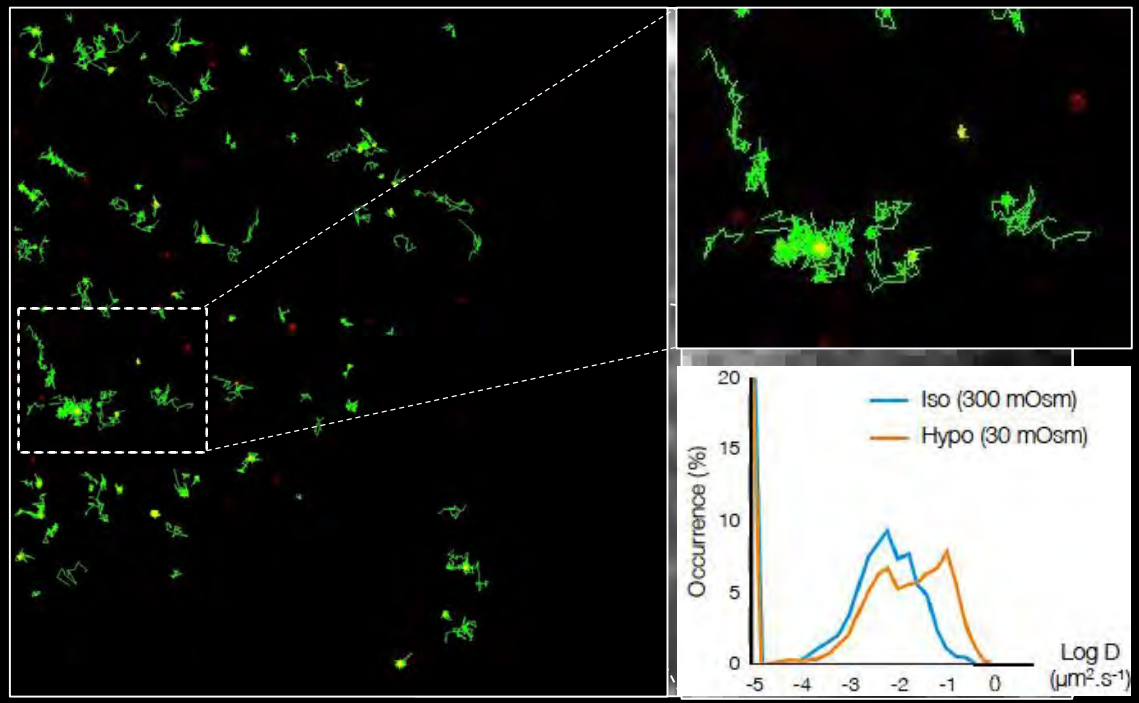


SuperResolution imaging of caveolae dyna

Single Molecule Localization by live cell PALM / TIRF
405/480 nm

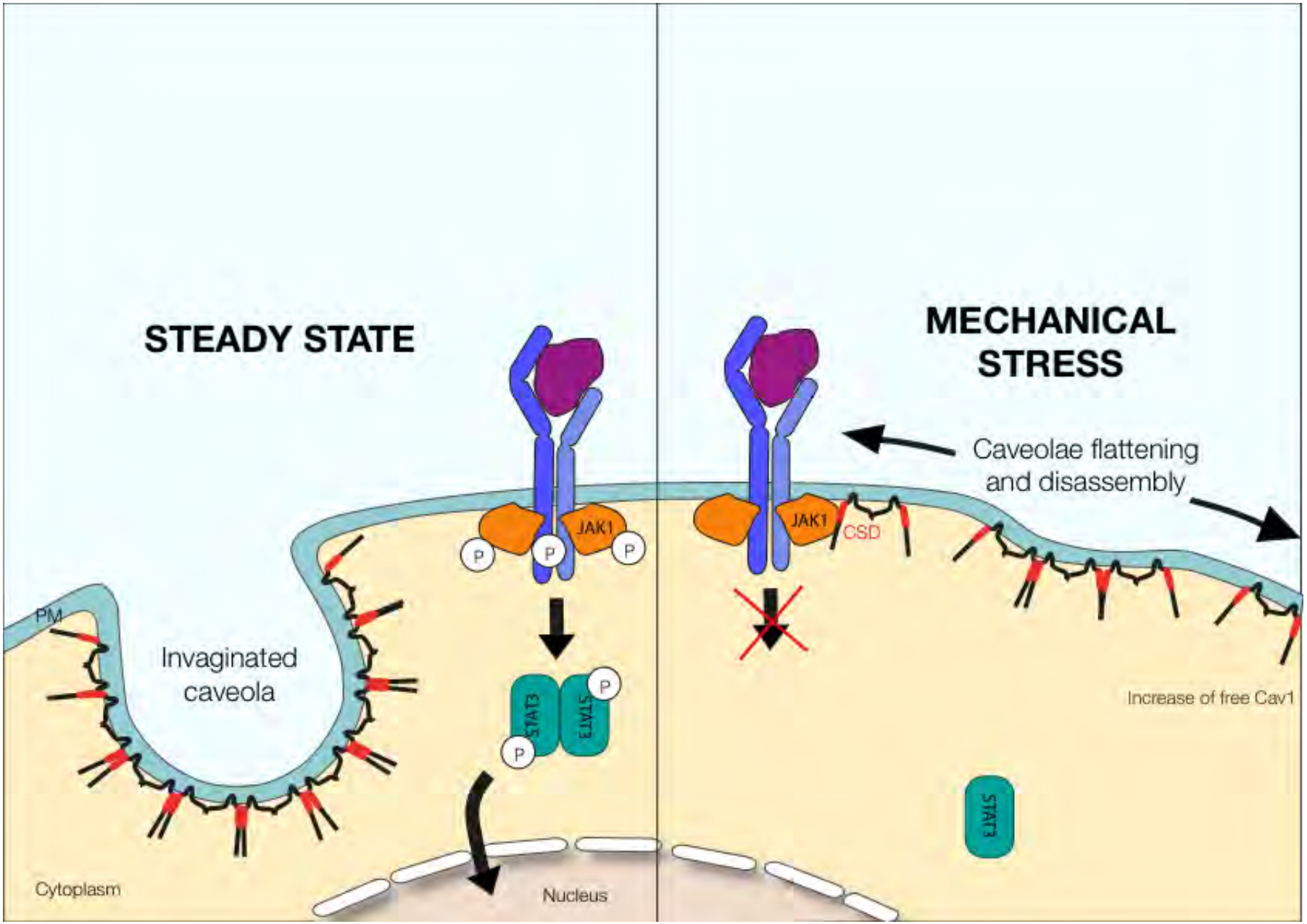
Coll. Pierre Nassoy/Grégory Giannone
Bordeaux University





Nicolas Tardif

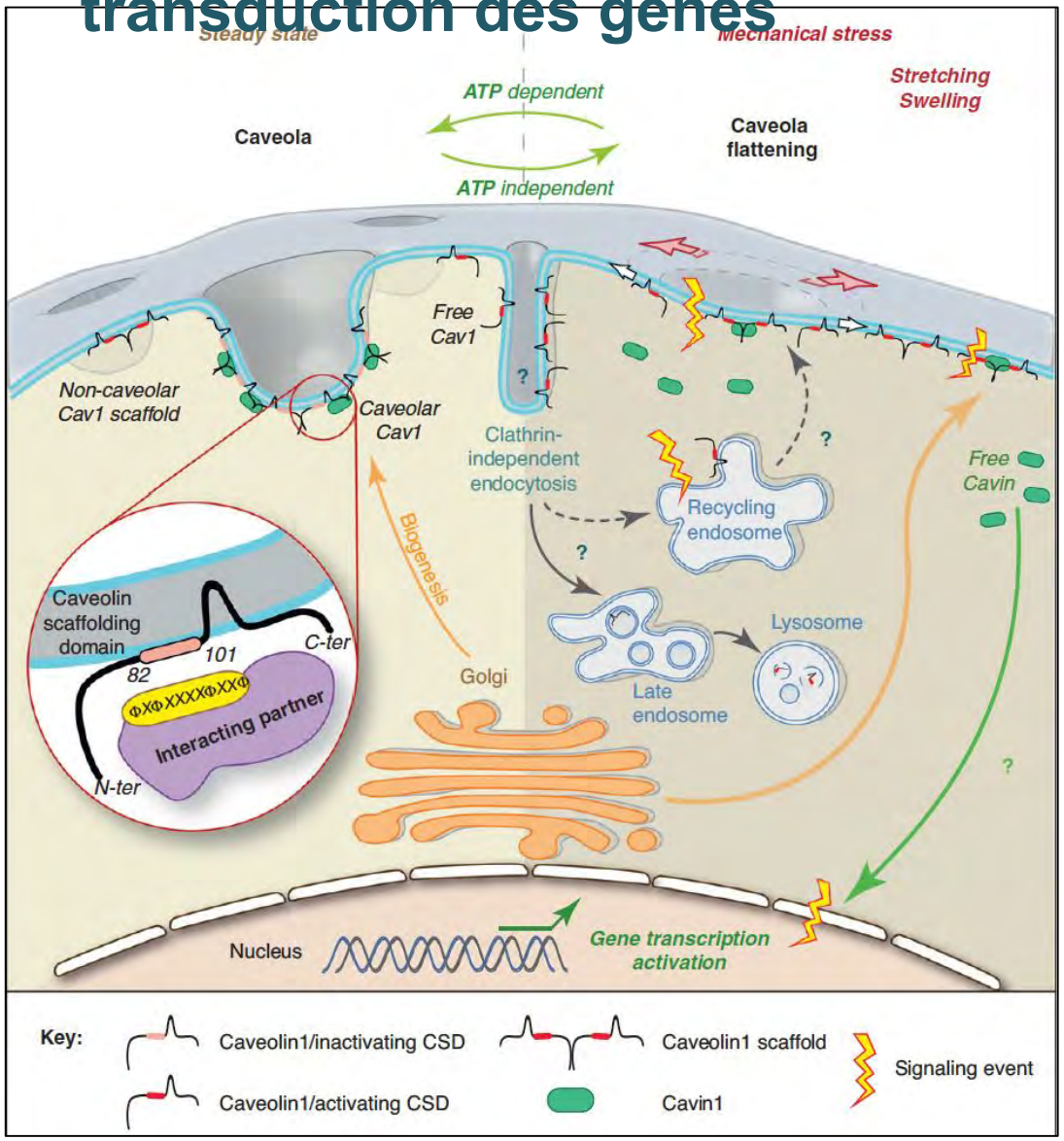
Un nouveau paradigme



Cédric Blouin, Nicolas Tardif

Les cavéoles: des domaines clefs connectant les contraintes mécaniques à la signalisation et la transduction des gènes

- Endocytose
- Homéostasie Lipides
- Signalisation



Team« Membrane Dynamics and Mechanics of Intracellular Signaling

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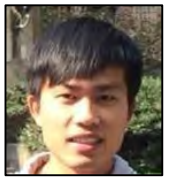
Chemical & Cellular Biology Unit

institutCurie



Present members:

Cédric BLOUIN
Christine VIARIS
Mélissa DEWULF
Carlos URENA MARTIN
Nicolas TARDIF
Satish Kailasam Mani
Joana Podkalicka
Manon Barutaut
Changting Li
Vibha Singh

Past members:

Stéphanie Torrino
Weiwei SHEN

Collaborators

- Stéphane Vassilopoulos (Inst Myologie Paris)
- Gillian Buttler-Browne (Inst Myologie Paris)
- Pierre Nassoy Bordeaux
- G. Giannonne Bordeaux

Association pour la Recherche sur le Cancer
FONDATION DE FRANCE
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INSTITUT NATIONAL DU CANCER
CONTRE LE CANCER LA LIGUE 101 comités
Inserm La science pour la santé From science to health
AFM
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