# How flotillin expression influence cell fate by acting on cell-cell adhesion and cell migration properties

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Team : « **Cytoskeleton and membrane trafficking dynamics in cellular adhesion"**

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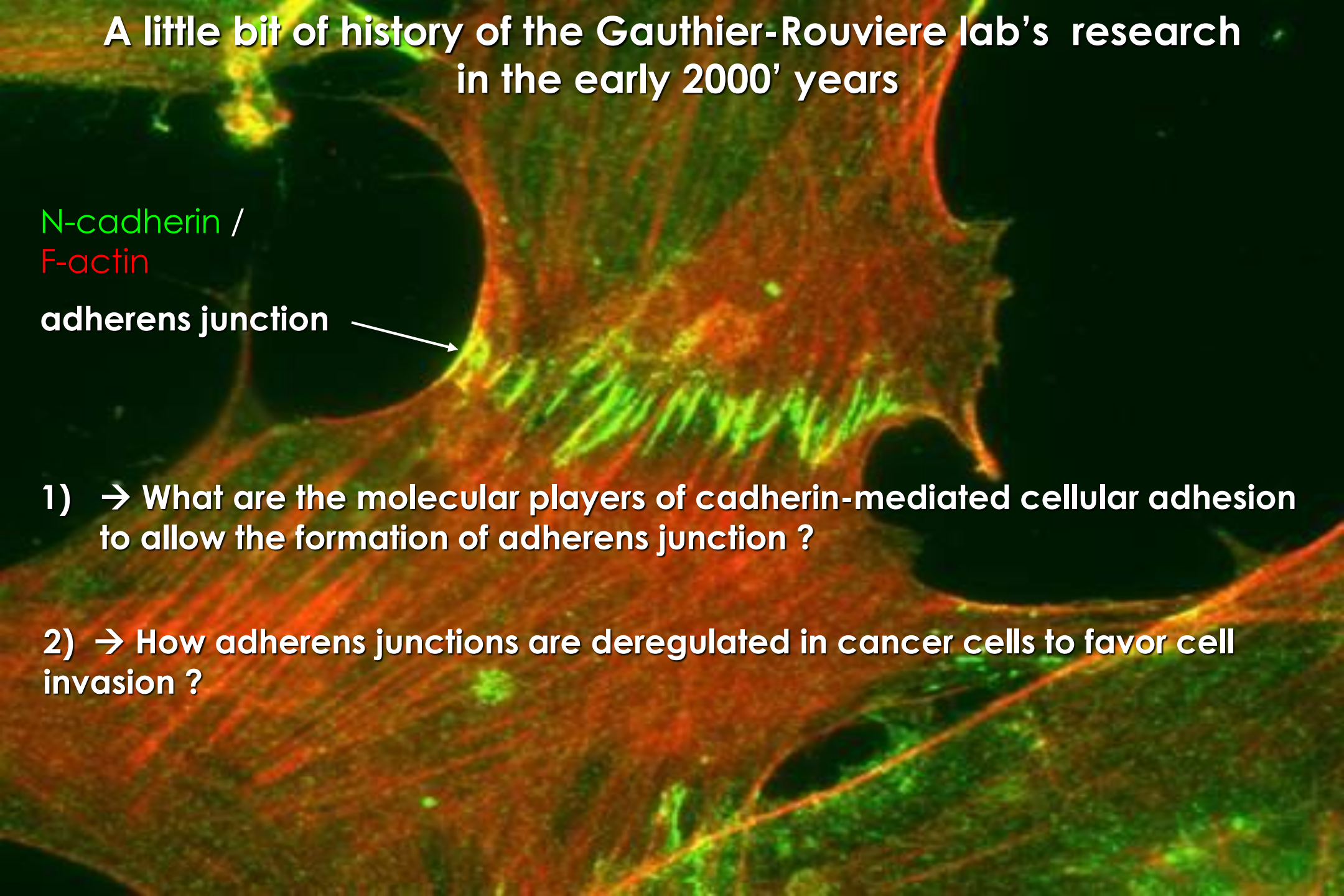
# A little bit of history of the Gauthier-Rouviere lab's research in the early 2000' years

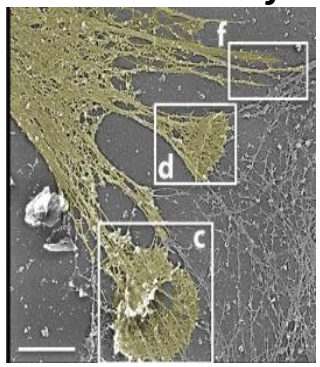


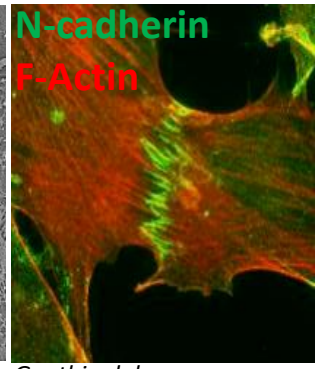

1) → What are the molecular players of cadherin-mediated cellular adhesion to allow the formation of adherens junction ?

2) → How adherens junctions are deregulated in cancer cells to favor cell invasion ?

# *Cadherin complexes require a cholesterol and sphingolipid enriched environment to be stabilized and to allow the formation of adherens junction(CCJs)*

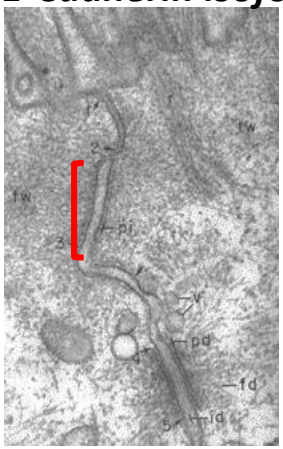
***In mesemchymal cells, N-Cadherin isoform is involved***



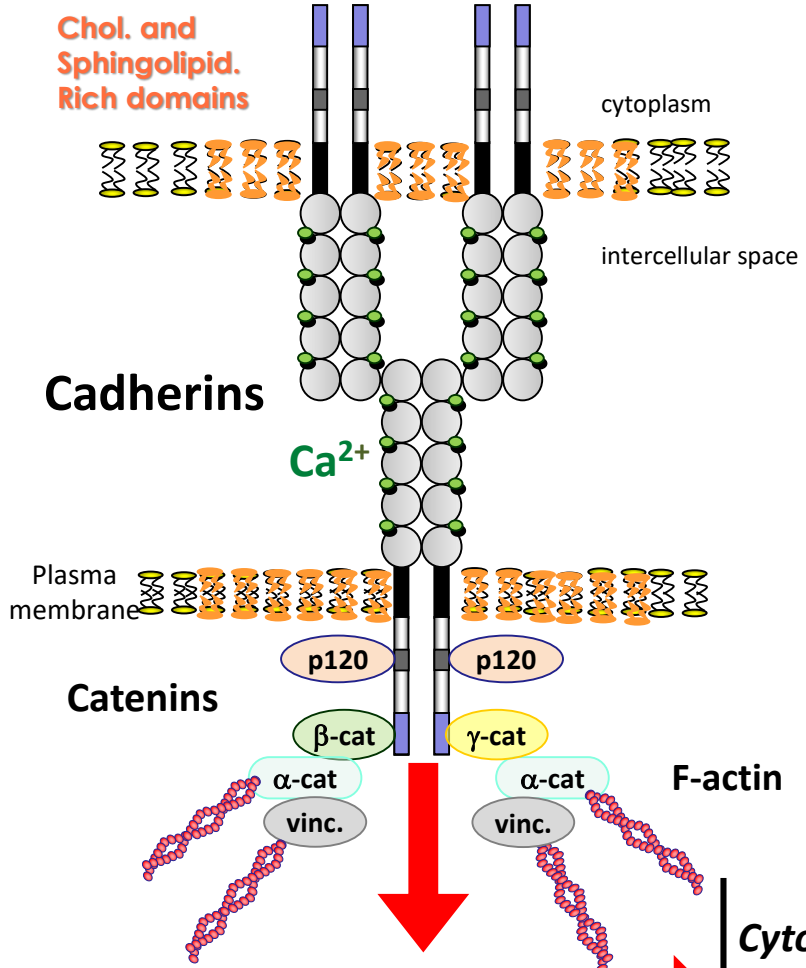




*Hoezle et al. MBoC 2012* *Gauthier lab.*

***In epithelial cells, E-Cadherin isoform is involved***




*Zeidelbar lab.*




**Cholesterol-and sphingolipid-rich microdomains**

**Stabilisation of Cadherin/p120 catenin complexes**

**Maturation of adherens junctions**

*Causeret et al., MoBC, 2005*
*Taulet et al., J Biol Chem 2009*

*Chartier et al., Exp Cell Res, 2011*
*Gentil-dit-Maurin et al., Exp Cell Res 2010*

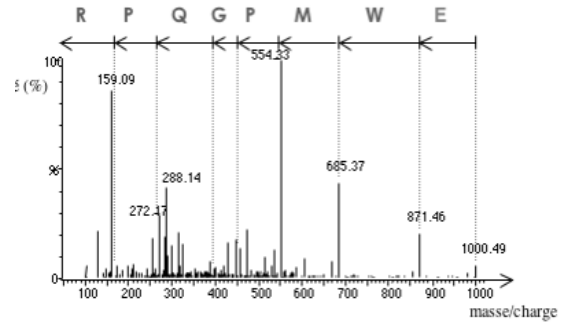
***Cytoskeleton organisation***
***Integrin mediated adhesion***

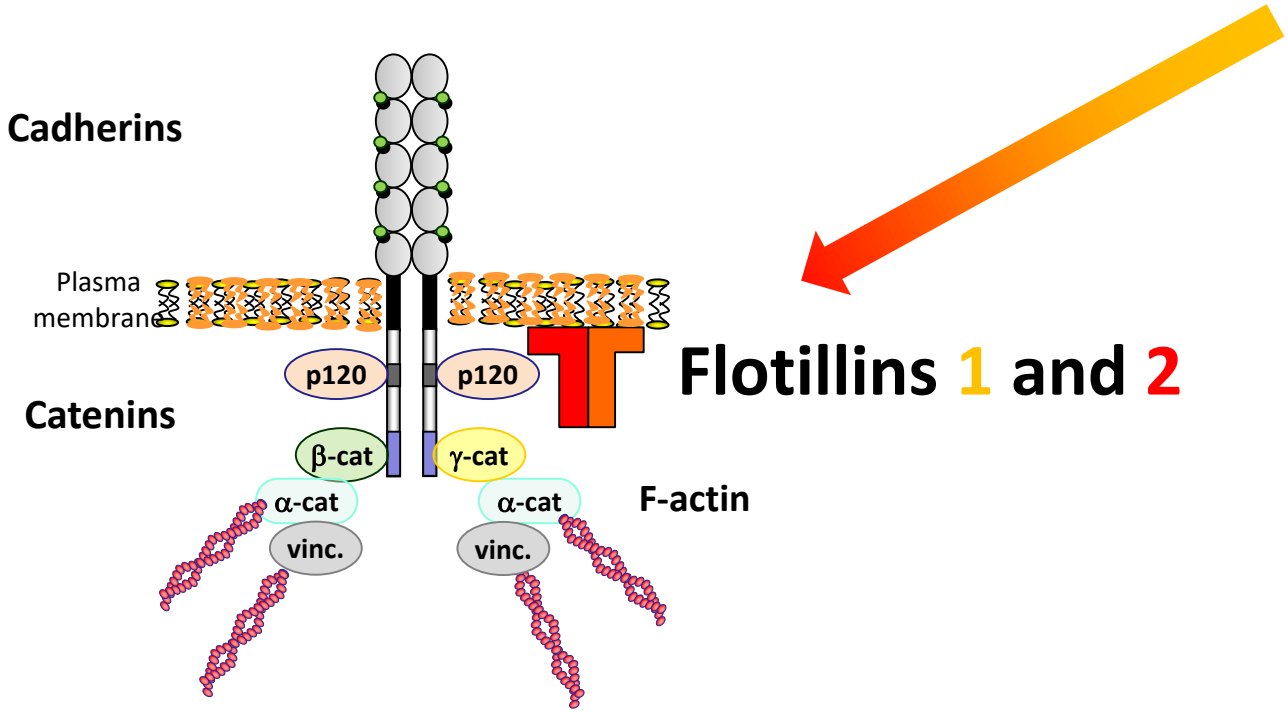
***Cellular morphology and motility***

# *How cadherin complexes are recruited and stabilized into cholesterol rich microdomains ?*

## Searching for new protein partners for cadherin

Imunoprecipitation of cadherins from confluent cells → Mass. Spectrometry analysis

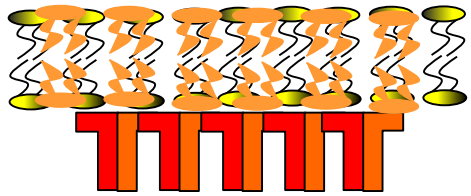


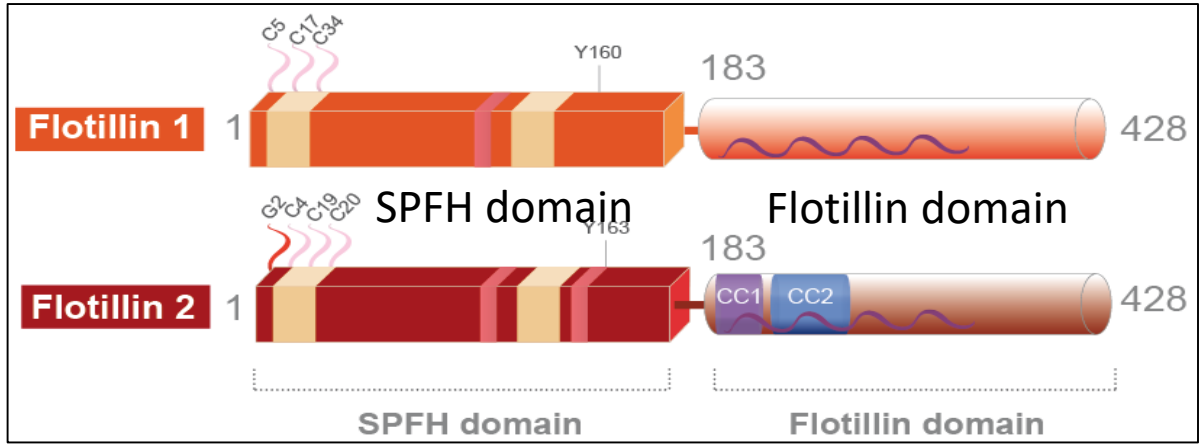

**Flotillins 1 and 2**

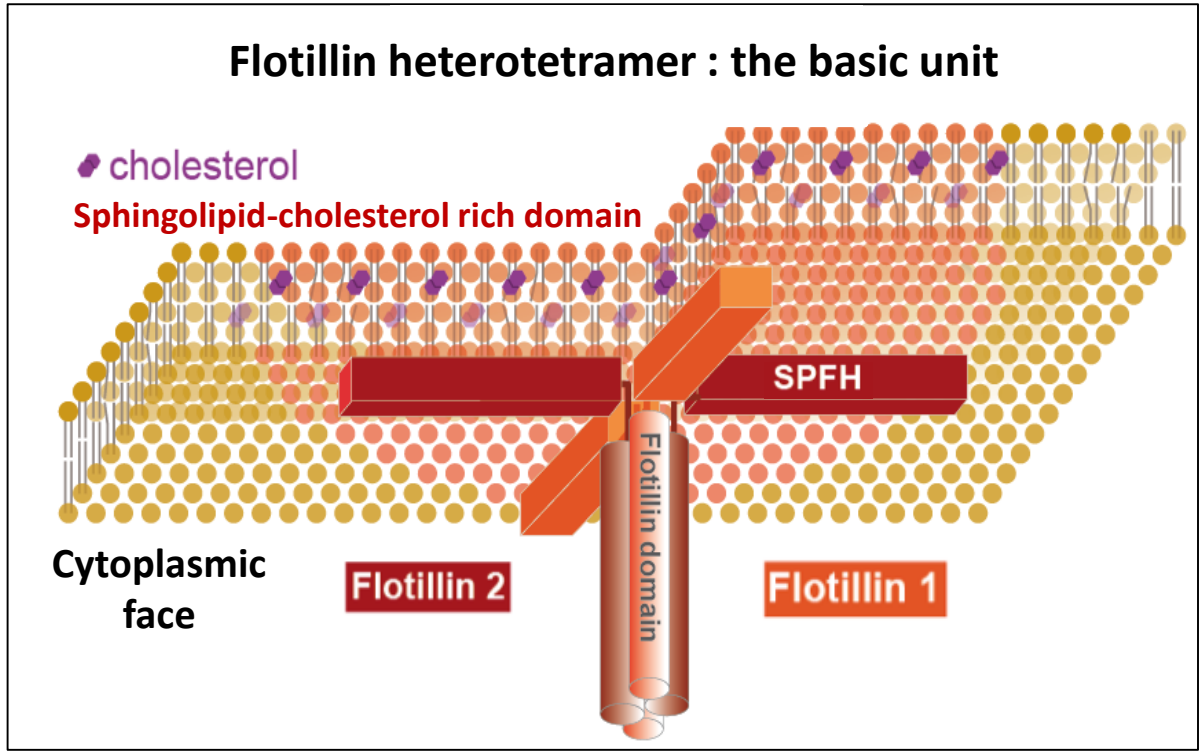
# *Flotillins*

- **Flotillins 1 and 2** *50% identity*
- **Ubiquitously expressed**
- **Highly conserved along evolution**
- **Part of the Family of SPFH domain containing proteins *(Stomatin, Prohibitin, …)***
- **Peripheral membrane proteins linked to the inner leaflet, known to be enriched in cholesterol and sphingolipid rich domains**



**Flotillins 1 & 2**

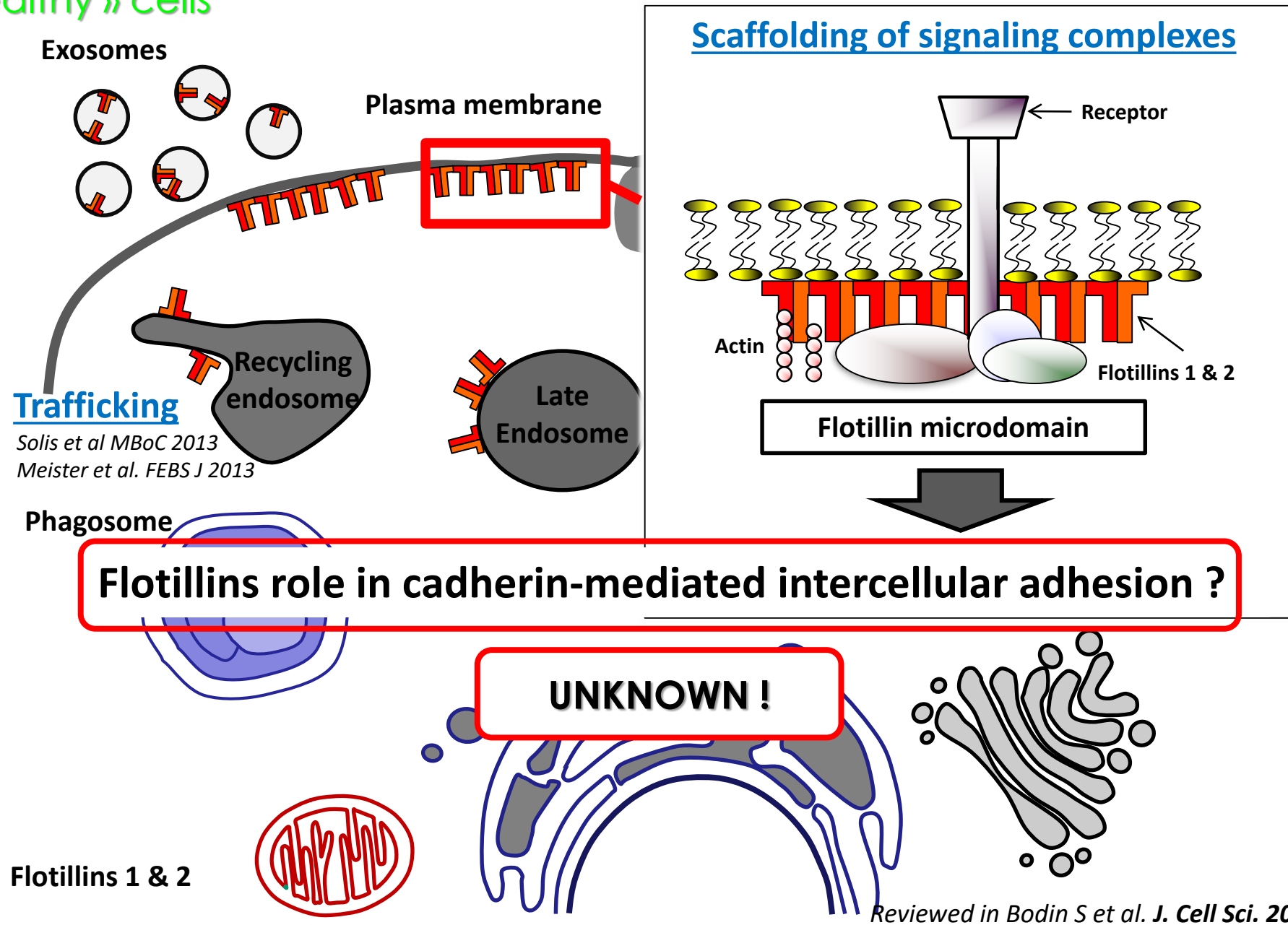



*Bodin S et al.* ***J. Cell Sci. 2014***

# Known localizations and functions of Flotillins around 2008-2010

In « healthy » cells



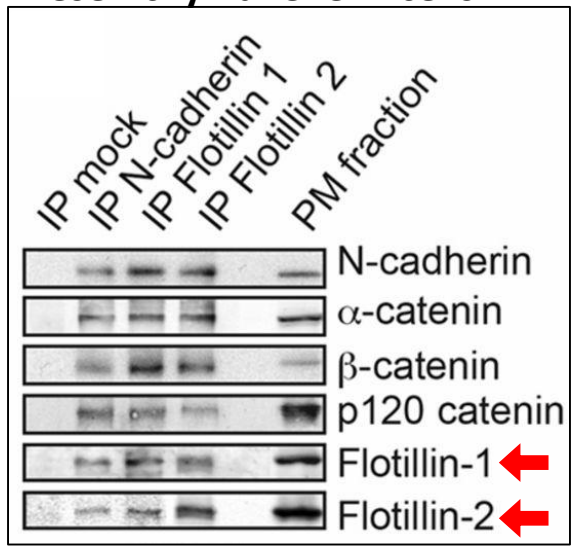

Reviewed in Bodin S et al. ***J. Cell Sci. 2014***

# Validation of flotillins 1 and 2 as new partners of cadherin complexes

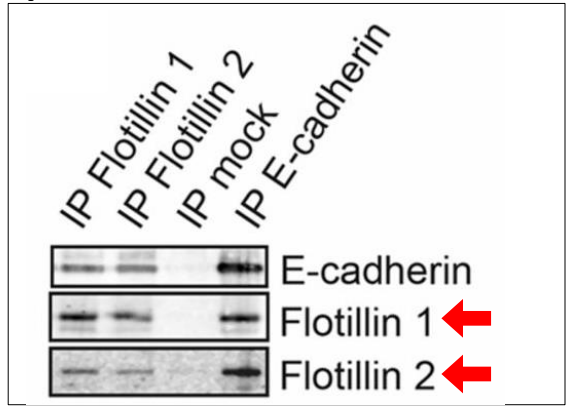
Emilie Guillaume

## Co-Immunoprecipitations Flotillin-cadherins

**Mesemchymal C2C12 cells**

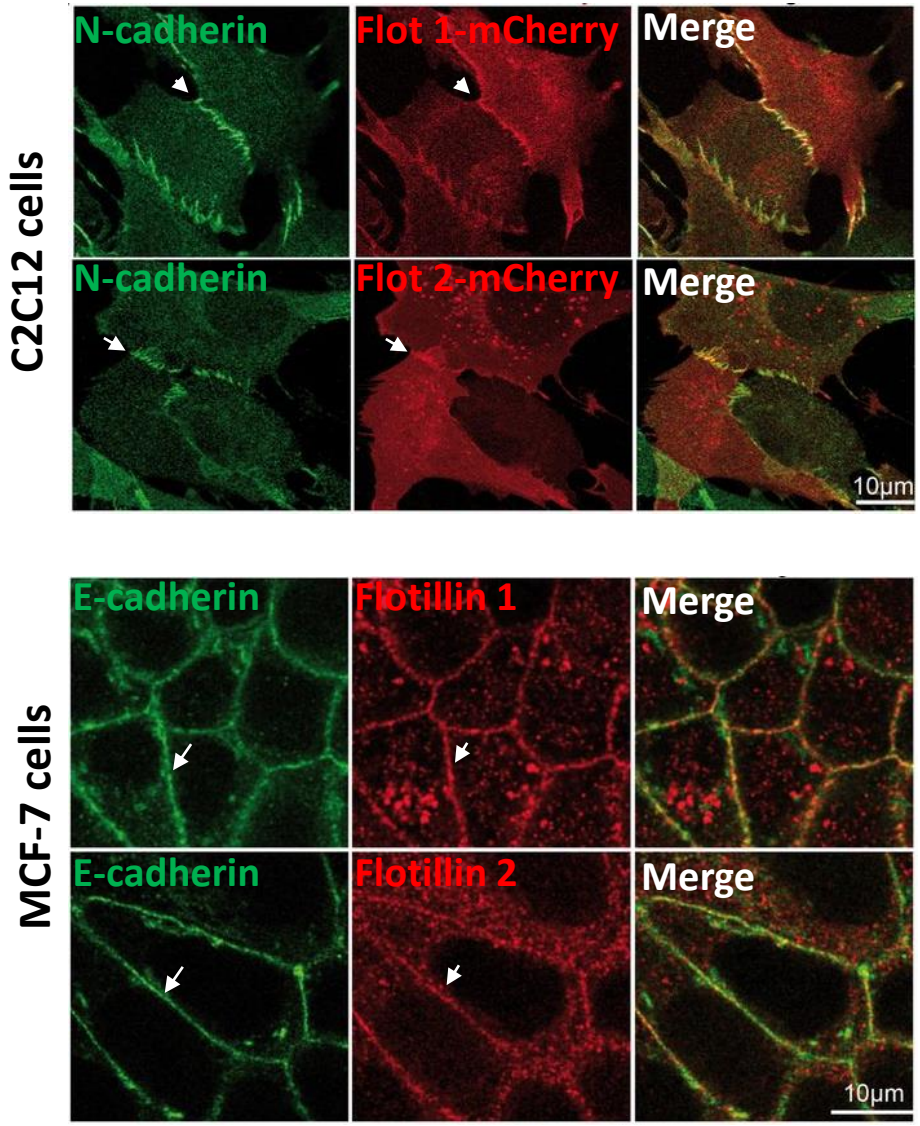



**Epithelial MCF-7 cells**




*Guillaume et al. J Cell Science 2013*

## Accumulation of Flotillins at cell-cell junctions :



# *Flotillin 2 / E-cadherin co-accumulation in MCF-7 epithelial cells*

***3D-reconstruction of the colocalization of E-cadherin and Flotillin 2 signals in epithelial MCF7 cells***

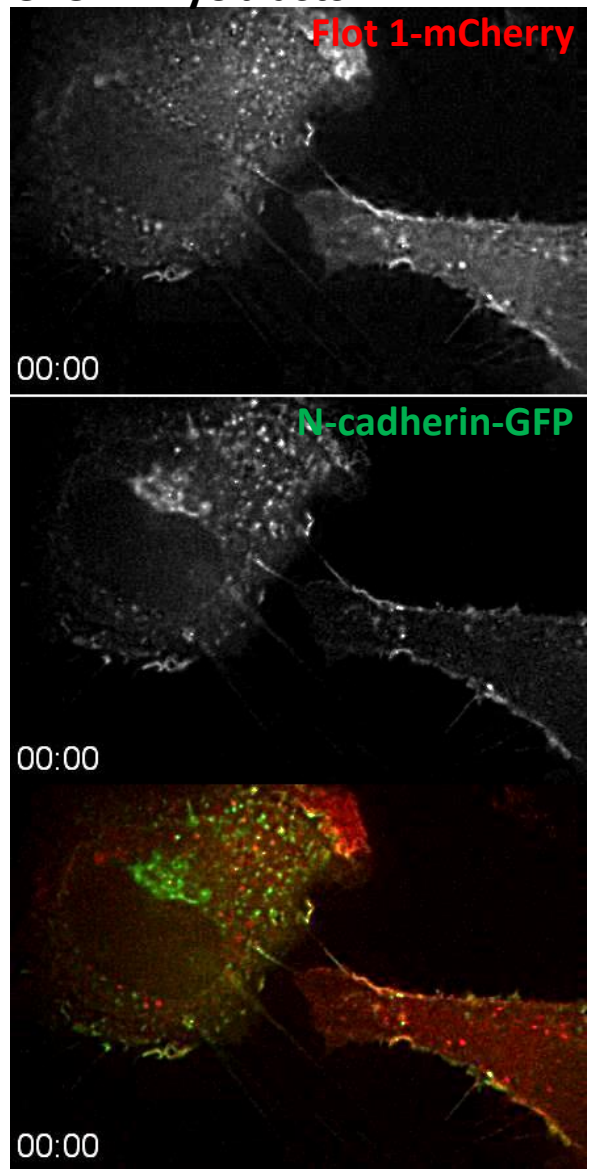
# *When are flotillins recruited to adherens junctions ?*

**Fluorescent –video microscopy**

→ Co-Accumulation of Cadherins and flotillins **since the initial steps of the formation of adherens-junction.**

→ Flotillins remained present in mature adherens junctions.

**C2C12 myoblasts**

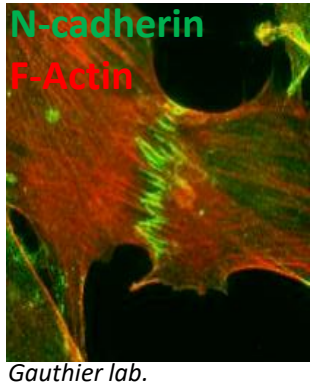



***Guillaume et al. J Cell Science 2013***

# *Flotillins colocalized with Cadherins as « strongly » as the well known direct cadherin-partner p120 catenin*

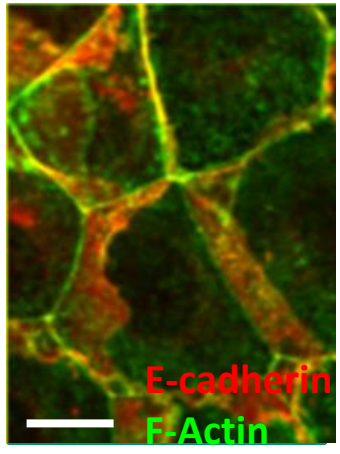
## Super-resolution 3D structural Illumination (3D-SIM) fluorescent microscopy

*In mesenchymal cells*



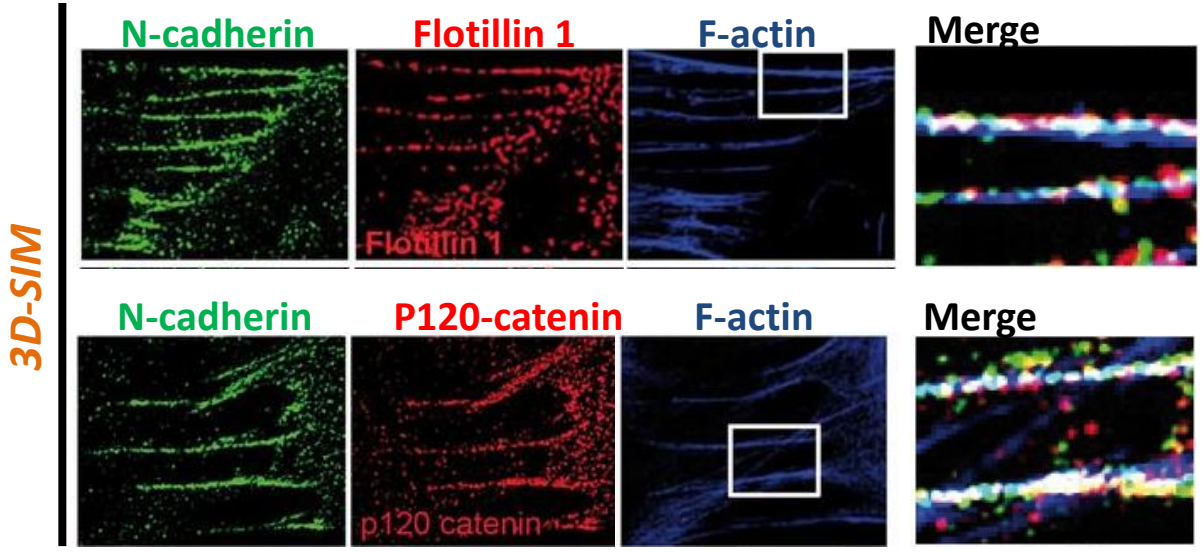

*Gauthier lab.*

*In epithelial cells*

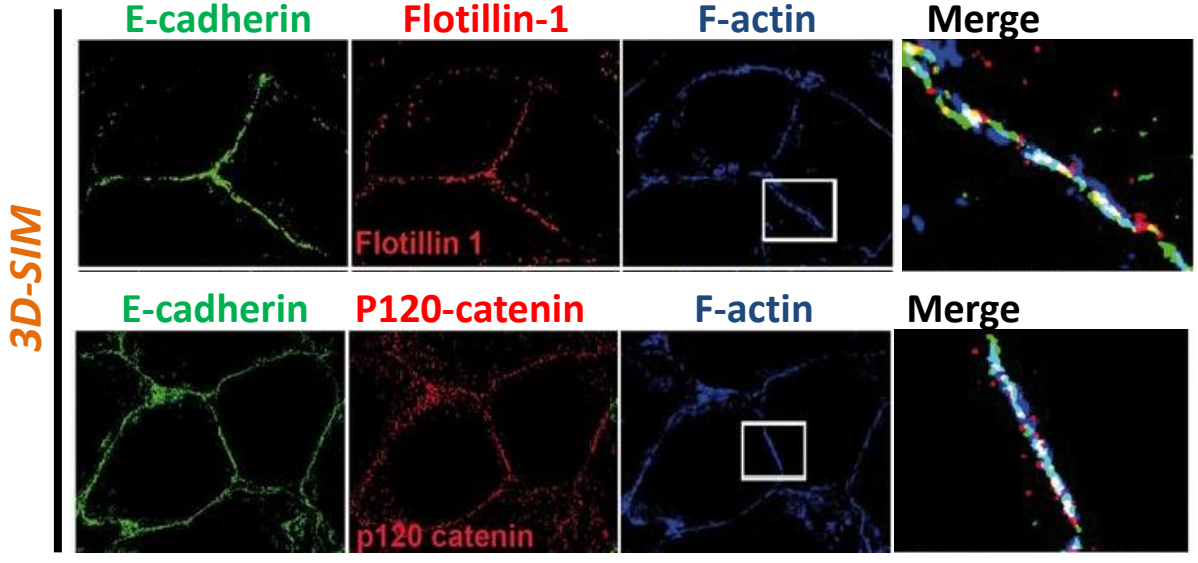



*Zeidelbar lab.*

### Mature Cell-Cell Junction in mesenchymal C2C12 cells

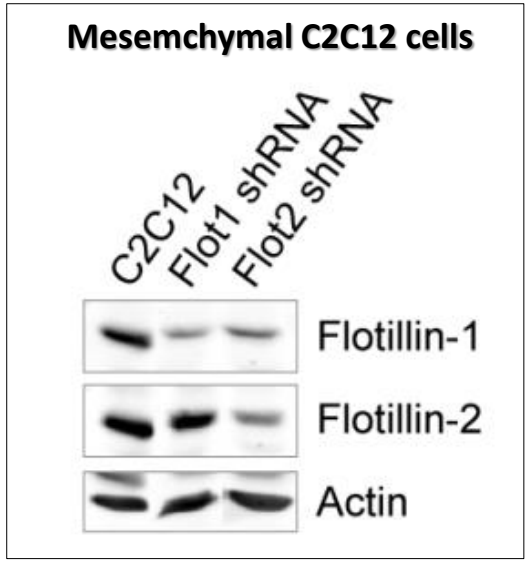



### Mature Cell-Cell Junction in epithelial MCF7 cells



# *Are flotillins required for the formation of cadherin-mediated cell-cell junctions in vitro ?*

**Flotillins Knock down**

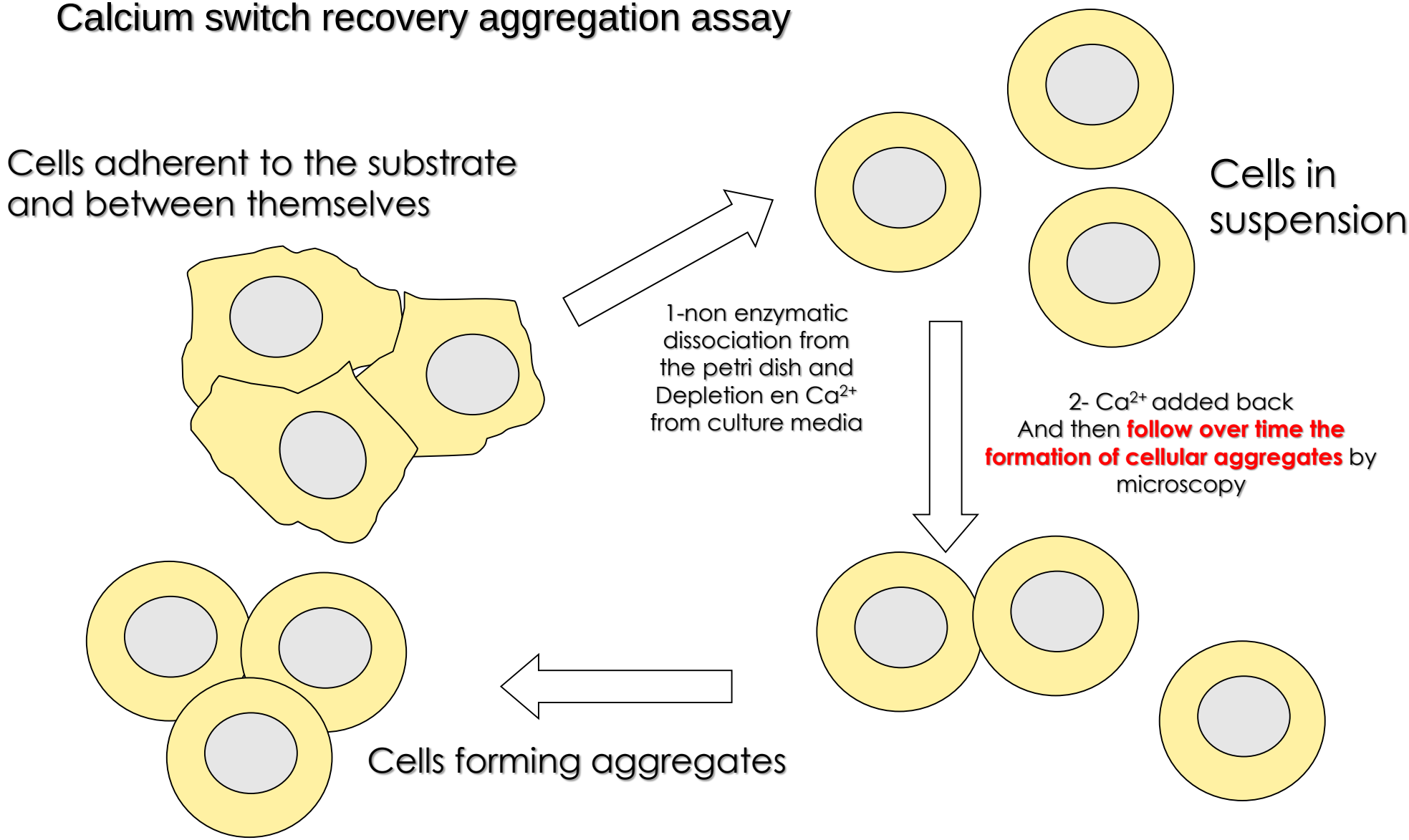



***Guillaume et al. J Cell Science 2013***

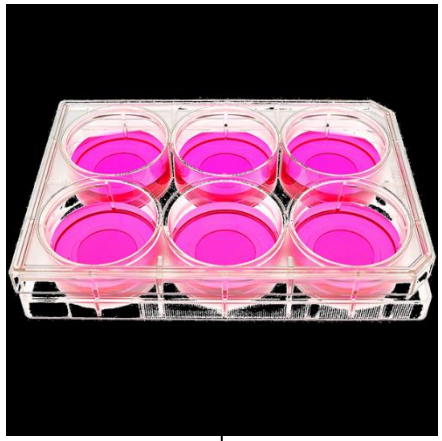
Are cells still able to generate Cadherin dependent cell-cell adhesion in absence of flotillins ?

# Measuring by microscopy the ability of cells to aggregate in a cadherin dependent manner

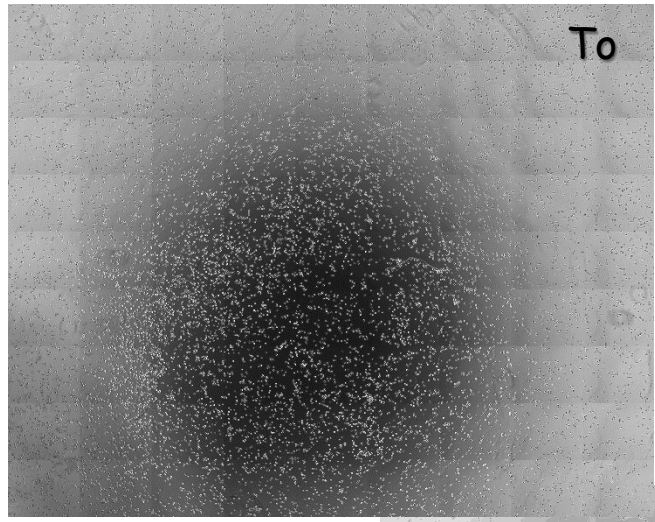
Calcium switch recovery aggregation assay

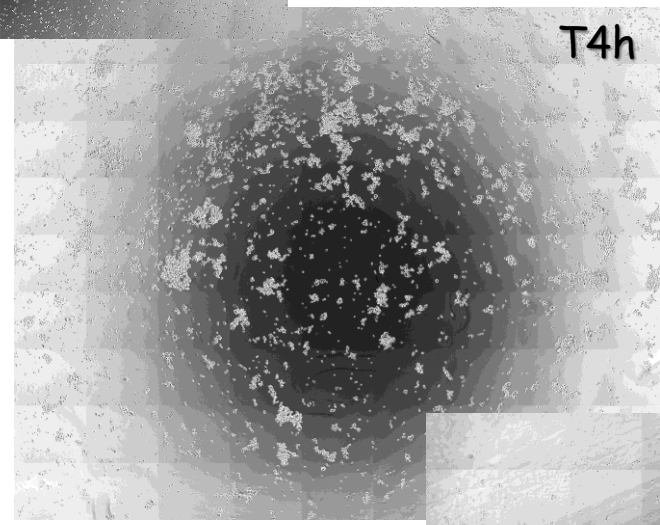


# $Ca^{2+}$ recovery aggregation assay



Low magnification live automatised-imaging of a whole well containing living cells

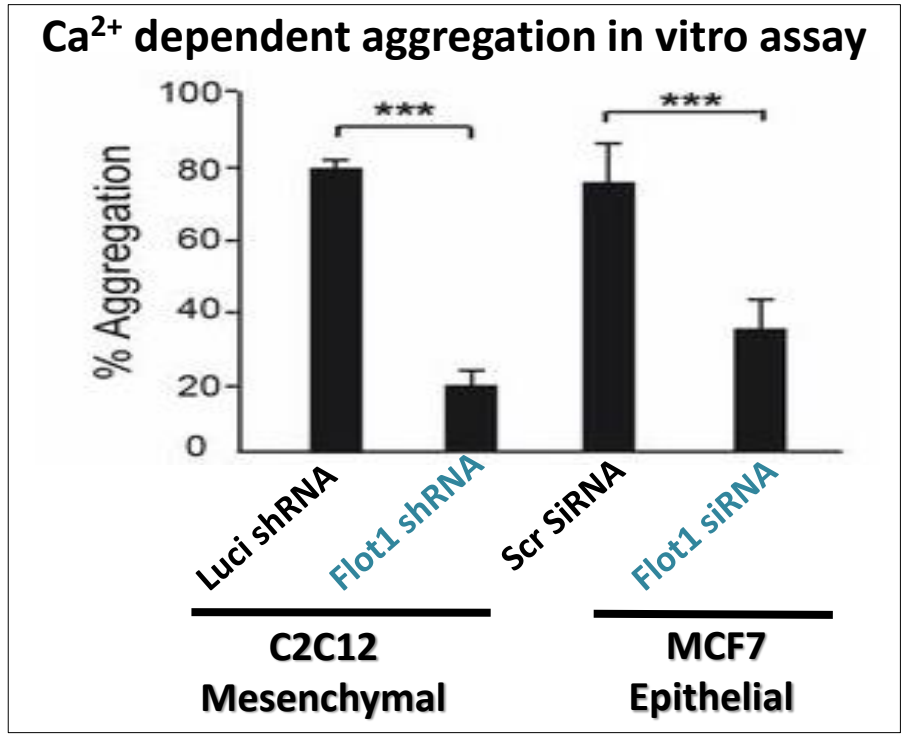




Quantitative analysis of the size and number of aggregates using Fiji /Image J software

# *Are flotillin required for the formation of cadherin-mediated cell-cell junctions ?*




↘ **Flotillins** ⇨ ↘ **cadherin-mediated cellular adhesion**

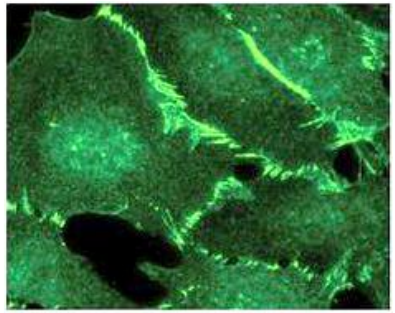
*Guillaume et al. J Cell Science 2013*

# *Flotillins are required for the establishment of functional cell-cell junctions*

## *Mesemchymal C2C12 cells*

### N-cadherin

shLuci shRNA



Flot1 shRNA

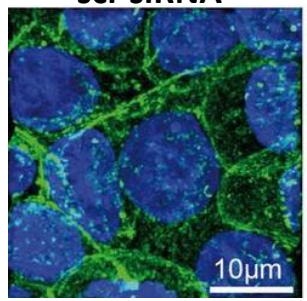
Flot2 shRNA

*Same for α-, β-, γ- and p120 catenin*

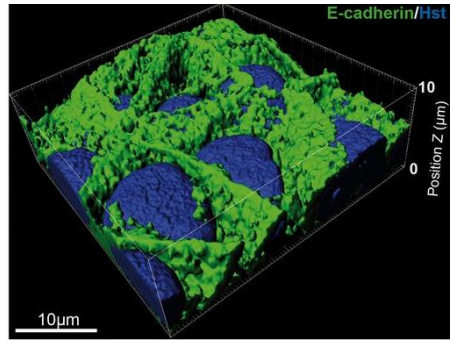
## *Epithelial MCF7 cells*

### E-cadherin

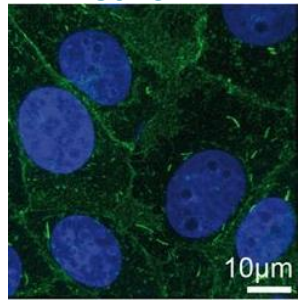
scr siRNA



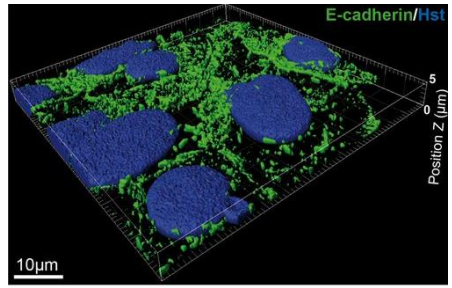

scr siRNA



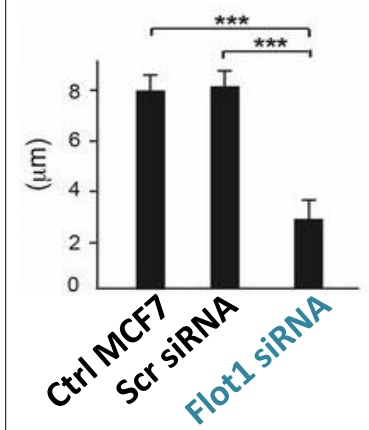

Flot1 siRNA



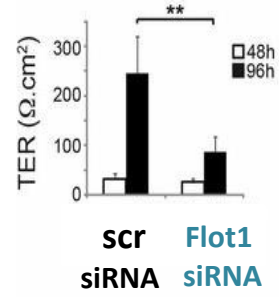

Flot1 siRNA




Height of monolayer (µm)




Transepithelial resistivity



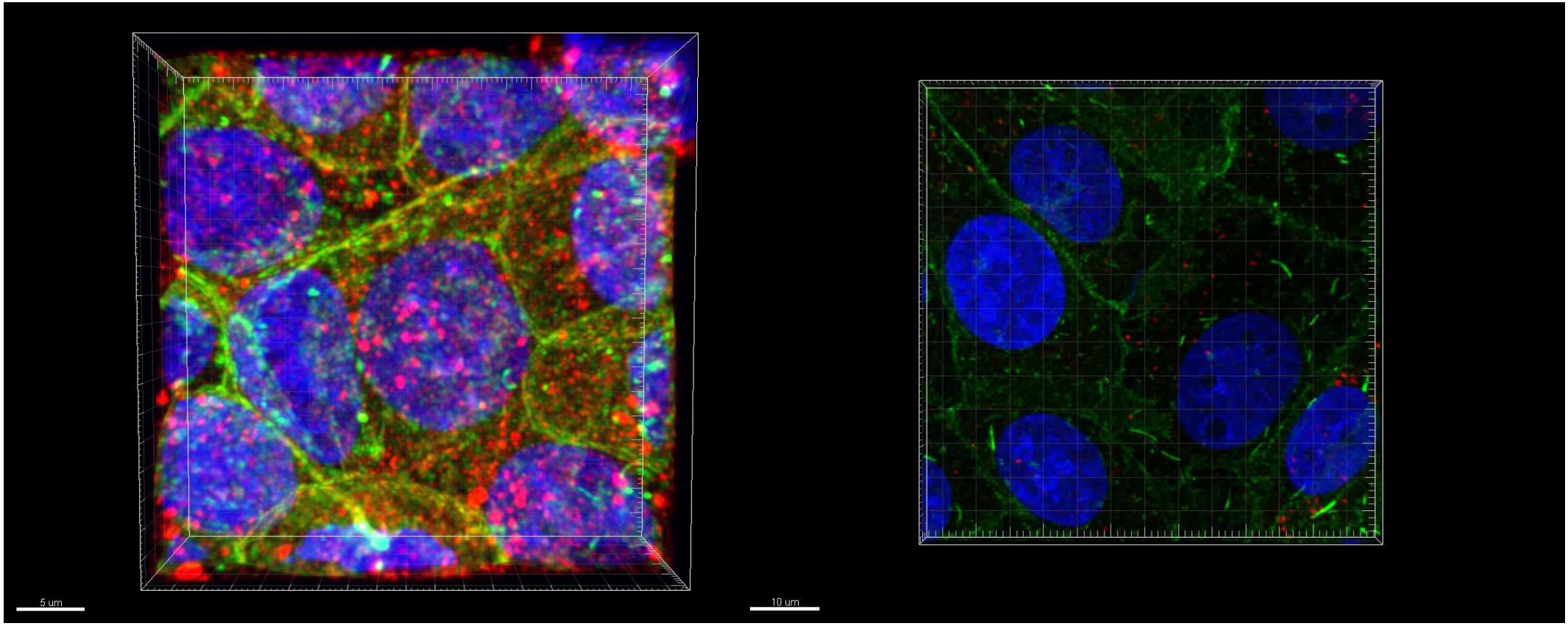

↘ **Flotillins** ⇨ **Loss of cadherin accumulation at CCJ and epithelial polarization**

*Guillaume et al. J Cell Science 2013*

# E-cadherin and Flotillin 1

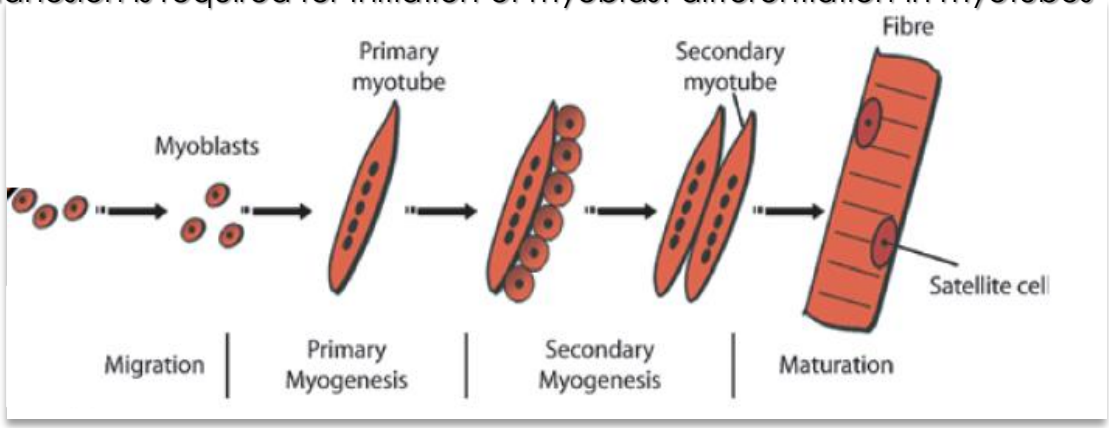
***Control MCF7 cells***

***siRNA Flot1 MCF7 cells***

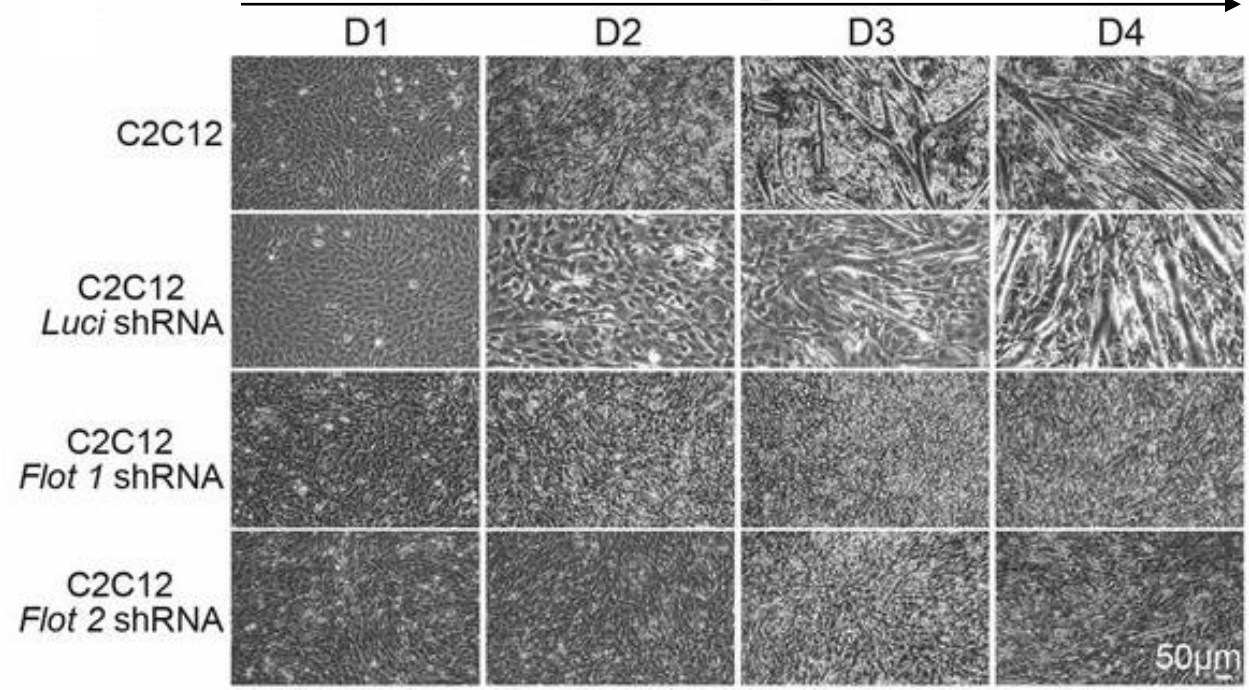


# *Flotillins are required for C2C12 myoblast differentiation*

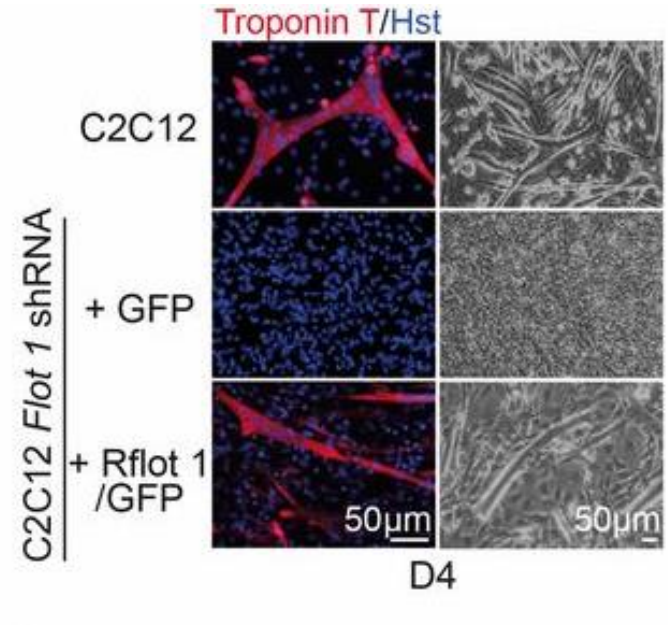
N-cadherin dependent adhesion is required for Initiation of myoblast differentiation in myotubes




Day after inducing differentiation*



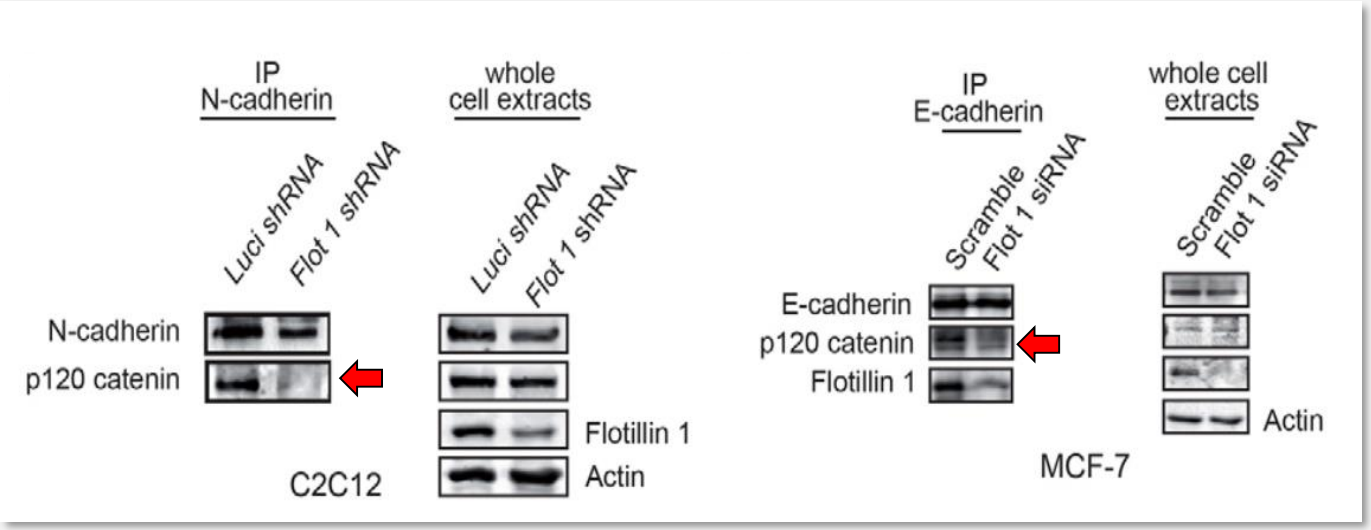

Rescue experiment




* By lowering serum concentration

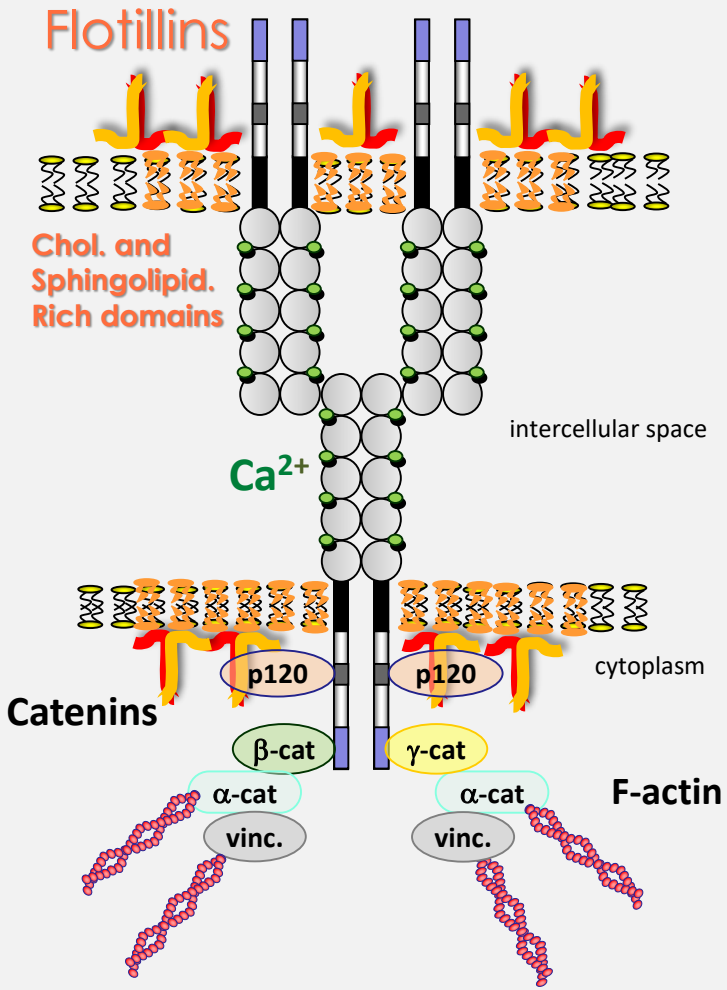
# *Flotillins are required for Cadherin / p120-catenin interaction*

## *Mesemchymal C2C12 cells*




## *Epithelial MCF7 cells*

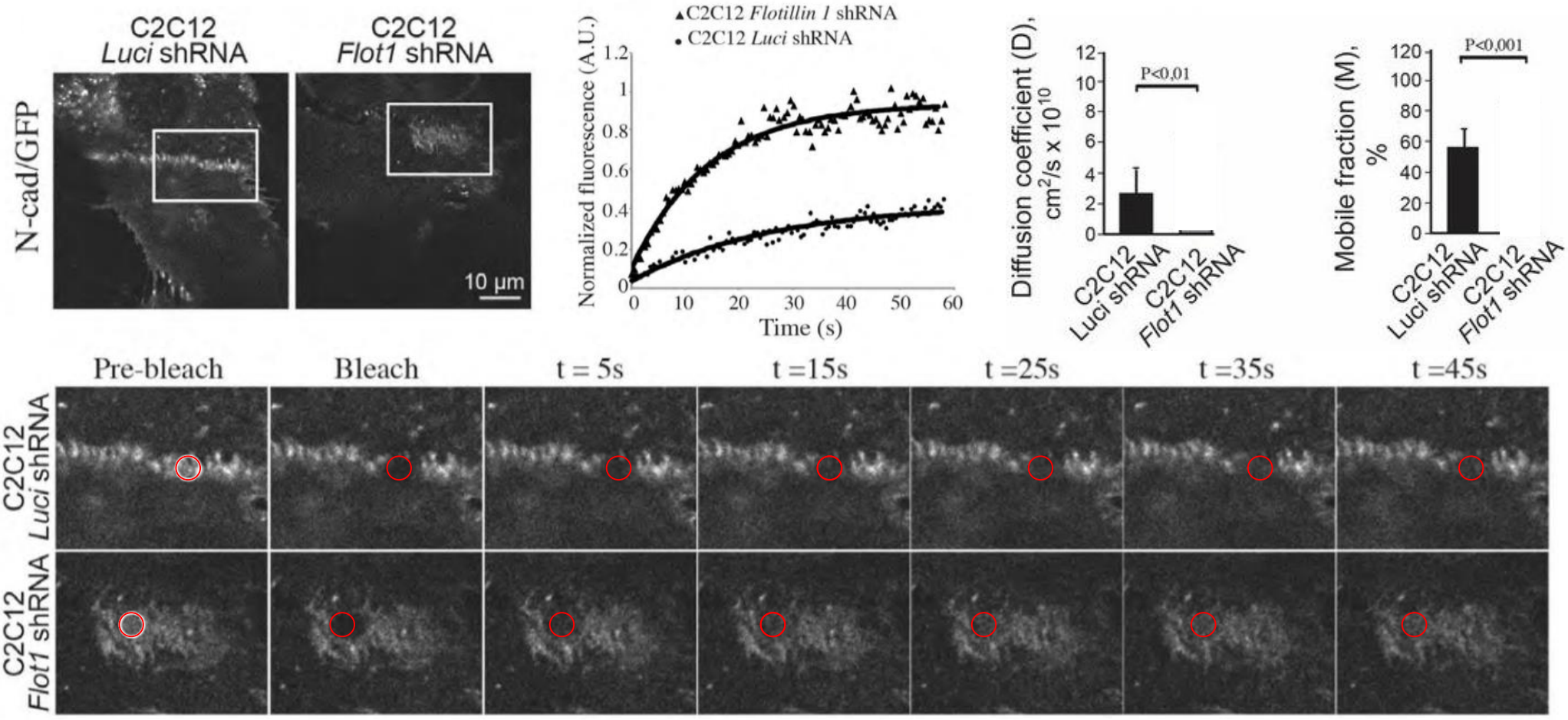


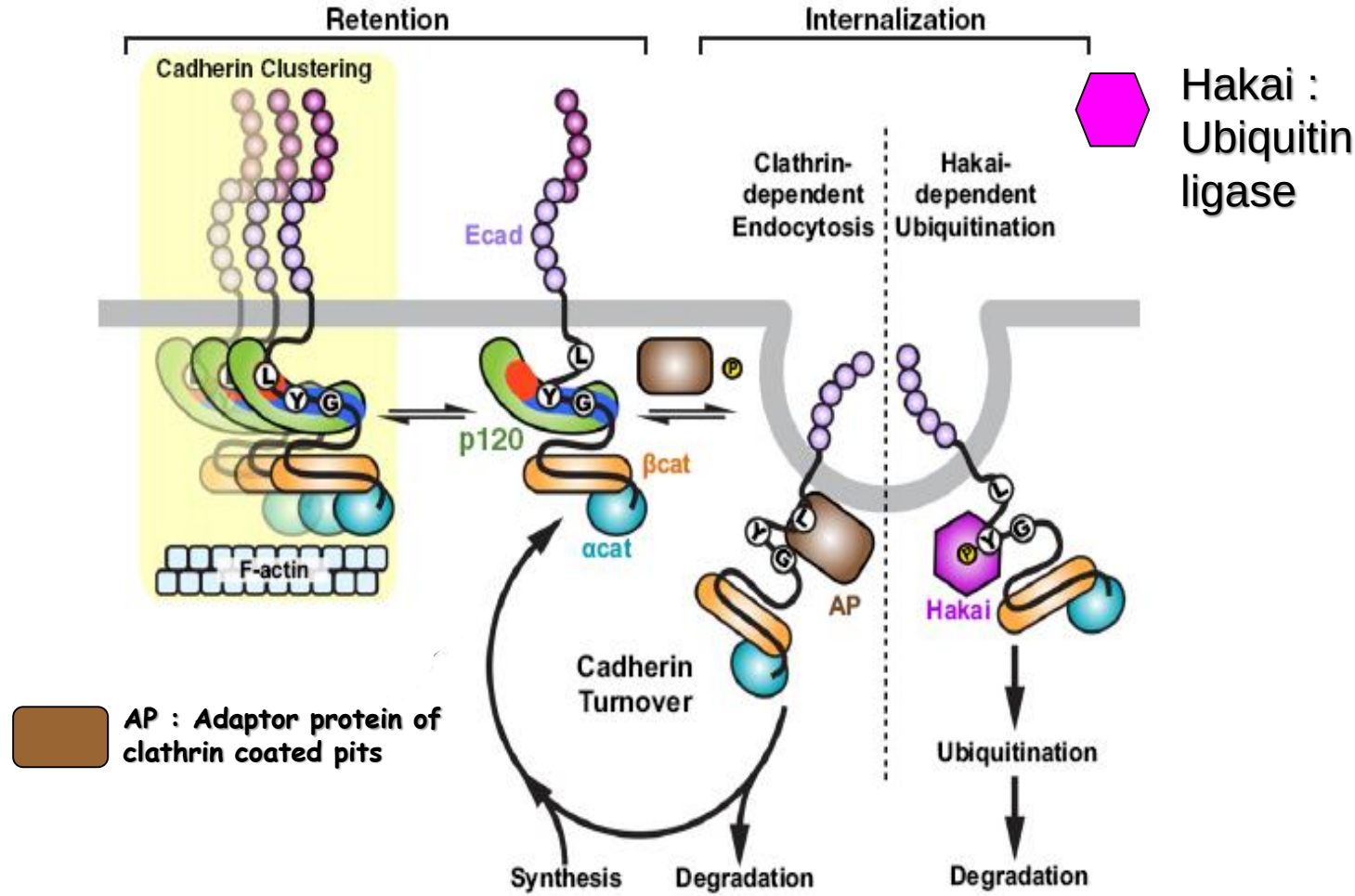

→ Are Cadherin complexes « unstable » in absence of flotillins ?

# *Flotillins are required to stabilize the lateral diffusion of cadherins at cell-cell contacts*

Measurement of cadherin lateral stability by Fluorescent recovery after photobleaching (FRAP) microscopy.



# *p120 catenin, a partner known to regulate Cadherin endocytosis*

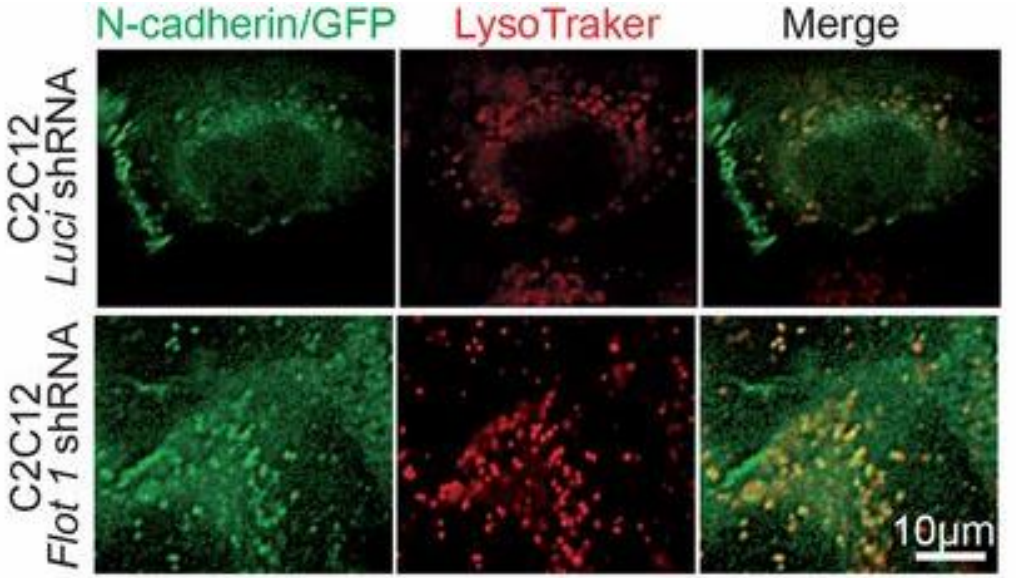



*From Ishiyama et al. Cell 2010*

→ In asbence of flotillins, because the p120-catenin/Cadherin interaction is disrupted, could we observe an increase in Cadherin endocytosis and degradation ?

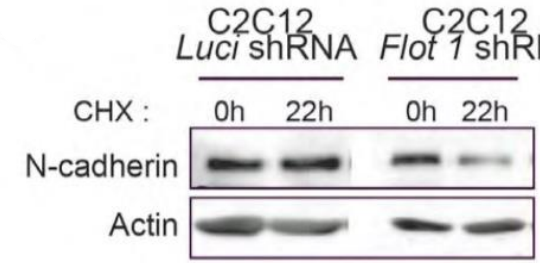
# *In absence of flotillins, cadherins are more subjected to endocytosis and degradation*

In asbence of flotillins, Cadherins are more frequently found in lysosomes (labelled with lysotracker)

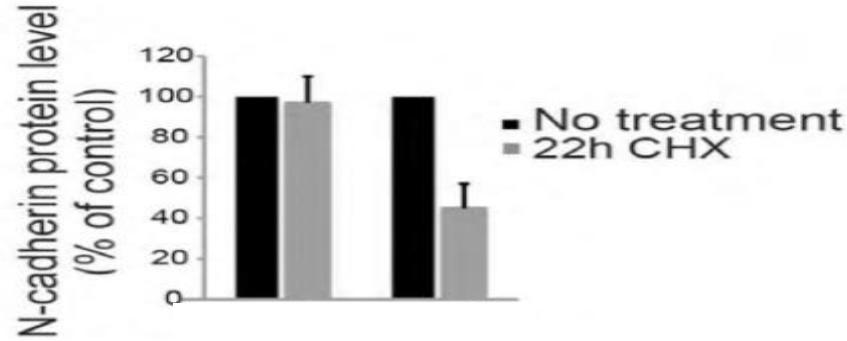



In asbence of flotillins, cadherin degradation is facilitated

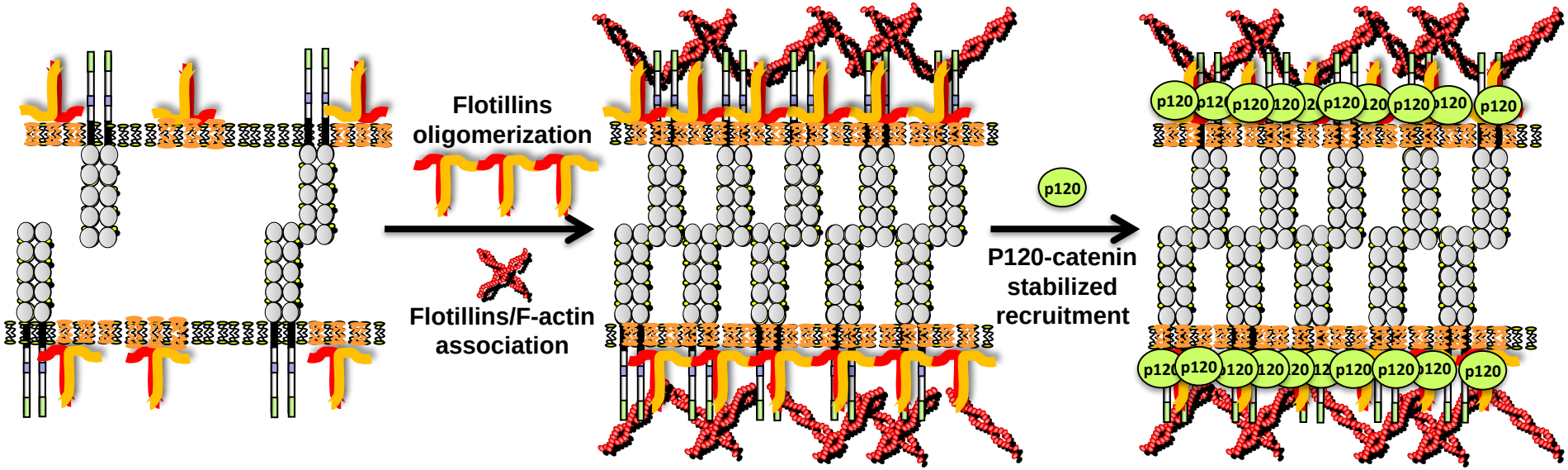

**C2C12 whole cell exctracts**




CHX : Cycloheximide (inhibitor of protein synthesis)



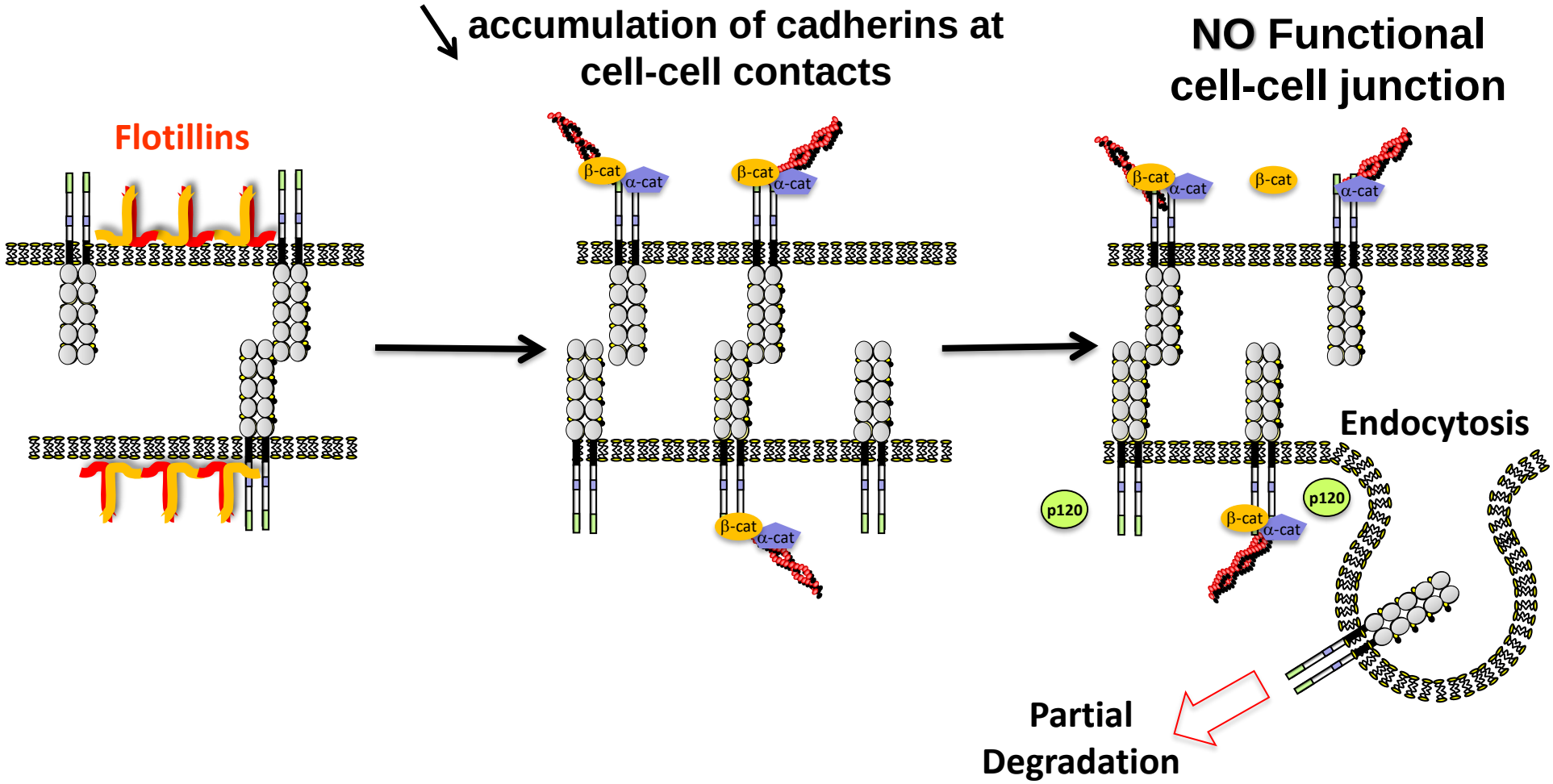
# *Working Model, in presence of flotillins…*




**Cadherin complex stabilization**

**Functional cell-cell junction**

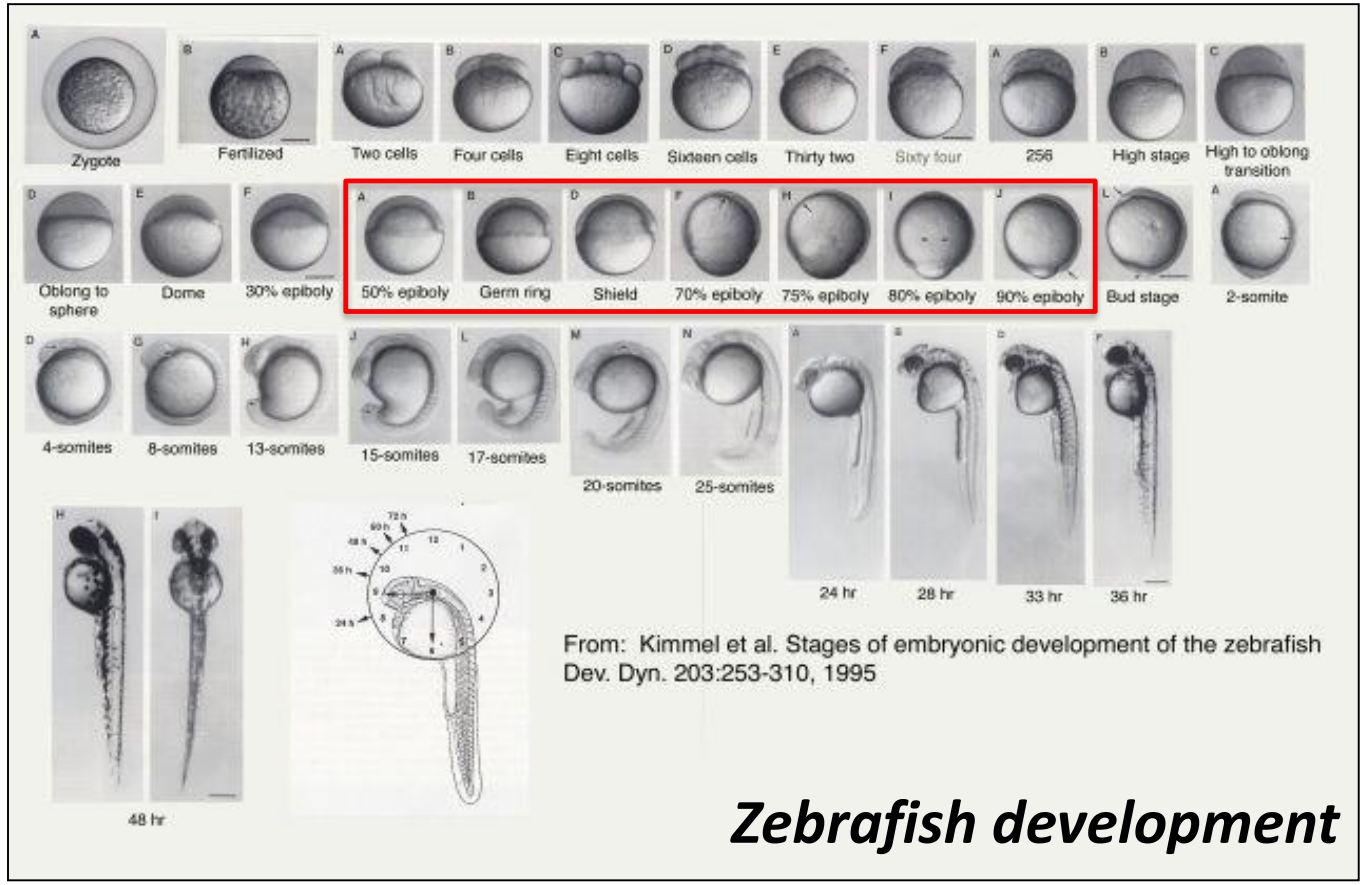
# *Working model, when flotillin level is decreased.*



# *What about the roles of flotillins in cadherin mediated intercellular adhesion <u>in vivo</u> ?*

***Eduardo Rios-Morris***

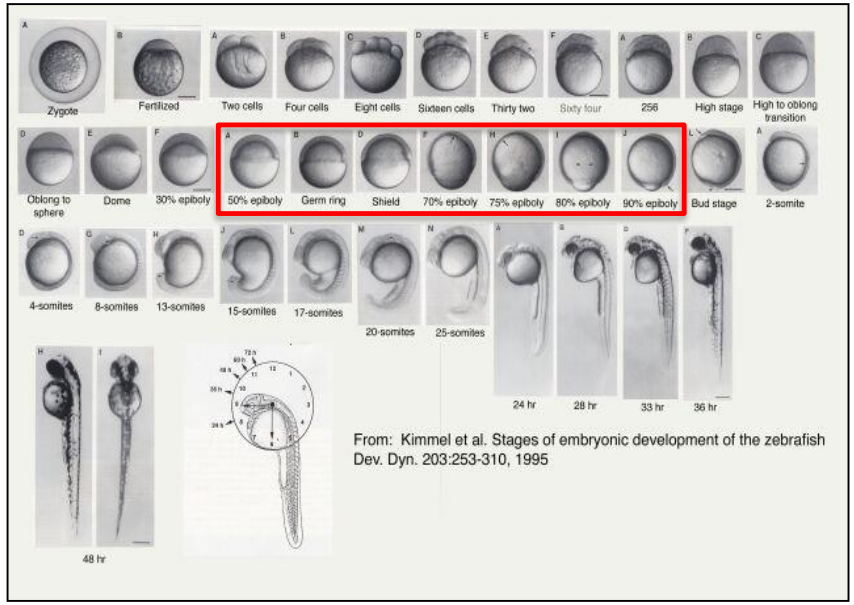
**Gastrulation**



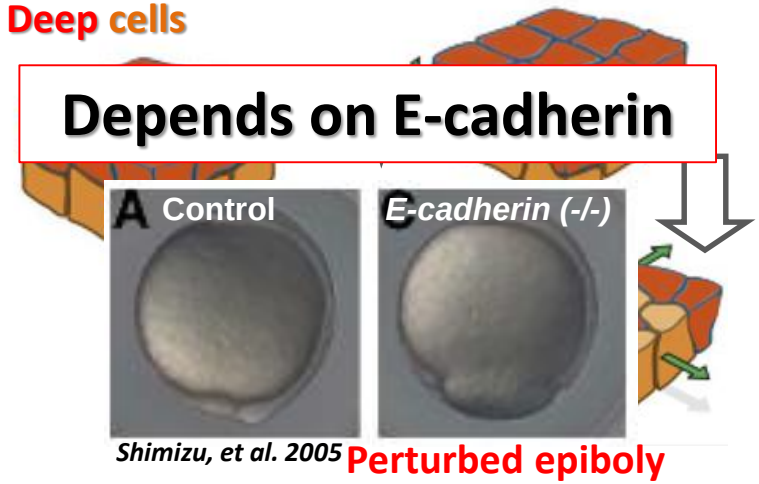

***Zebrafish development***

# *Zebrafish development*

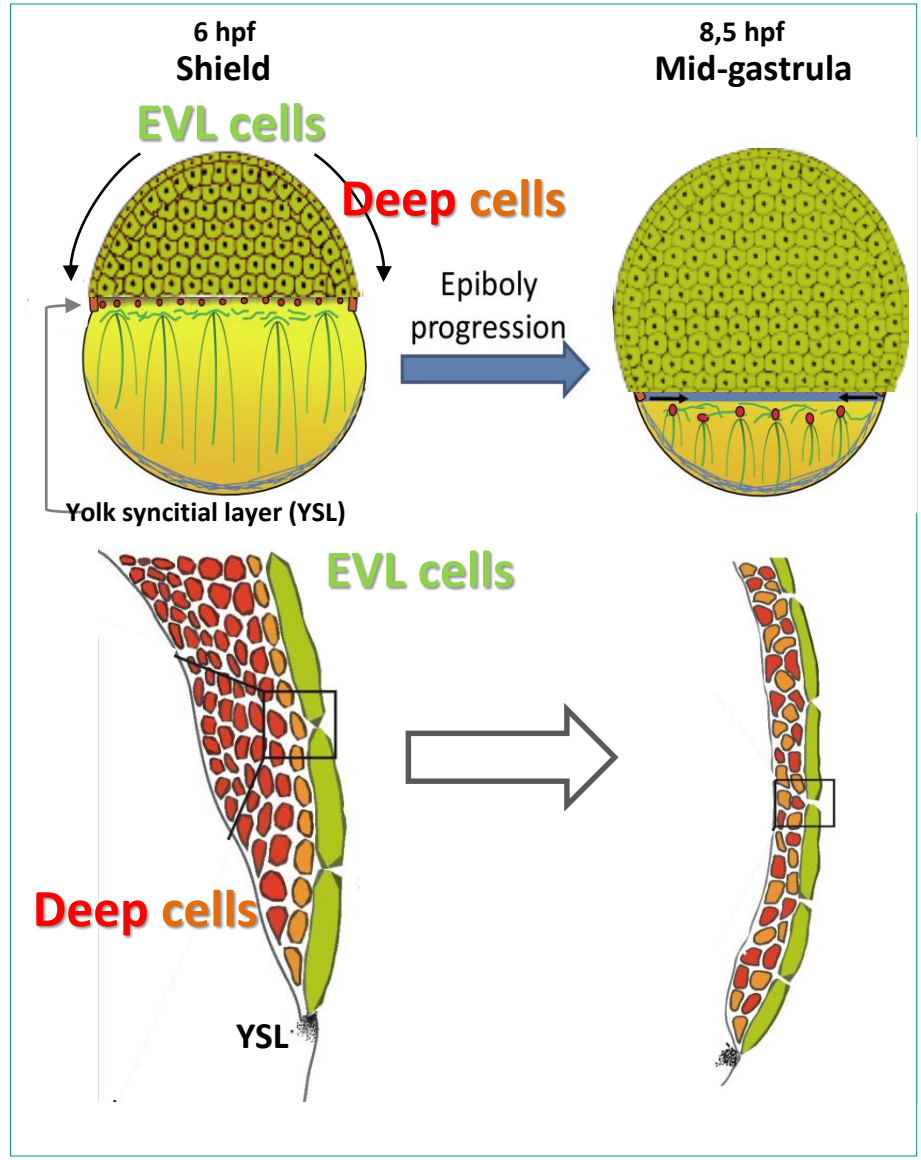
## Gastrulation



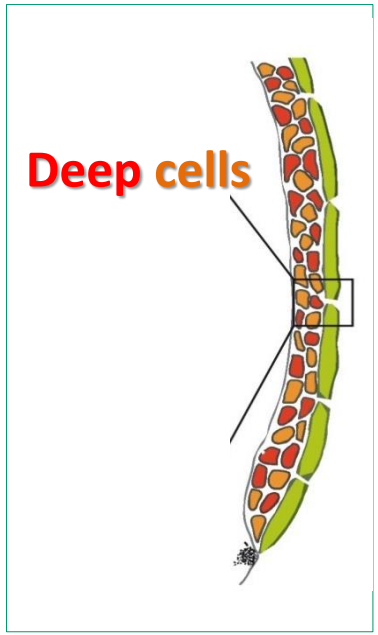

## Radial Cell Intercalation

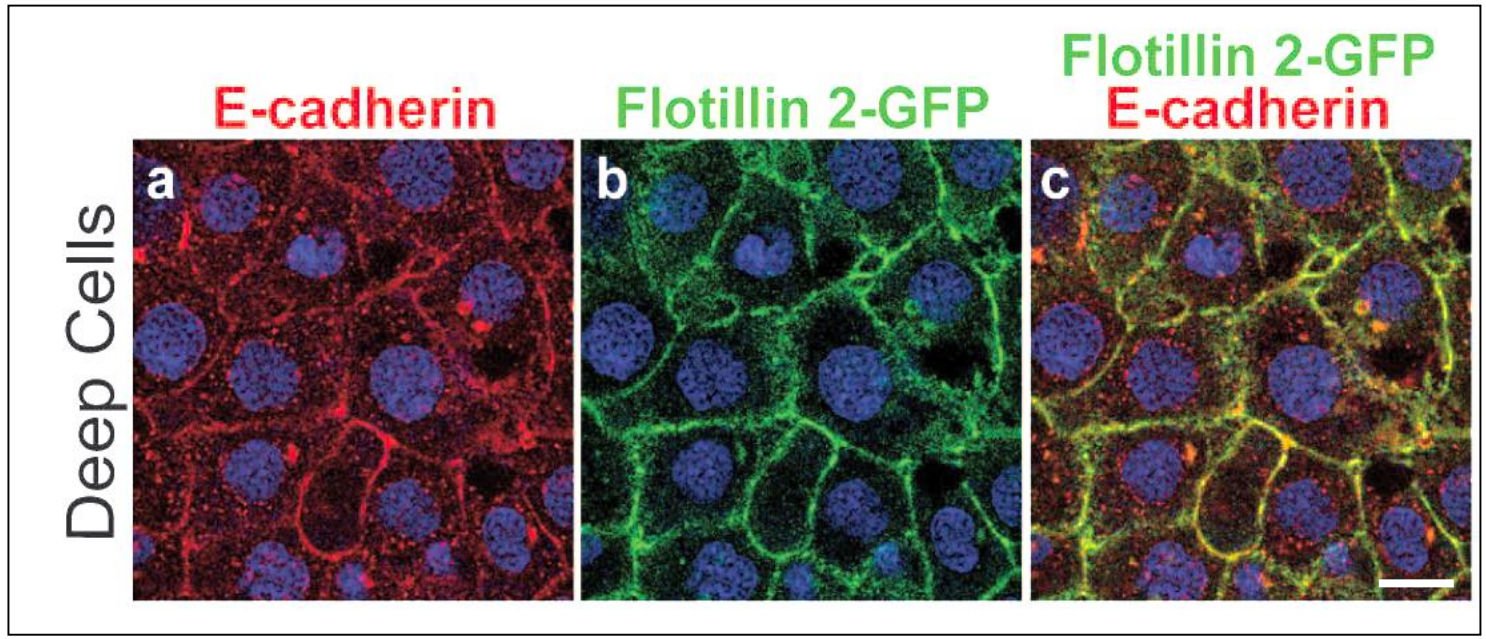



## Epiboly

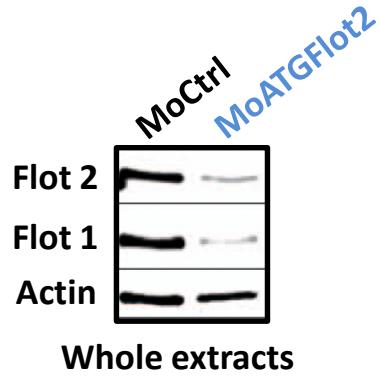


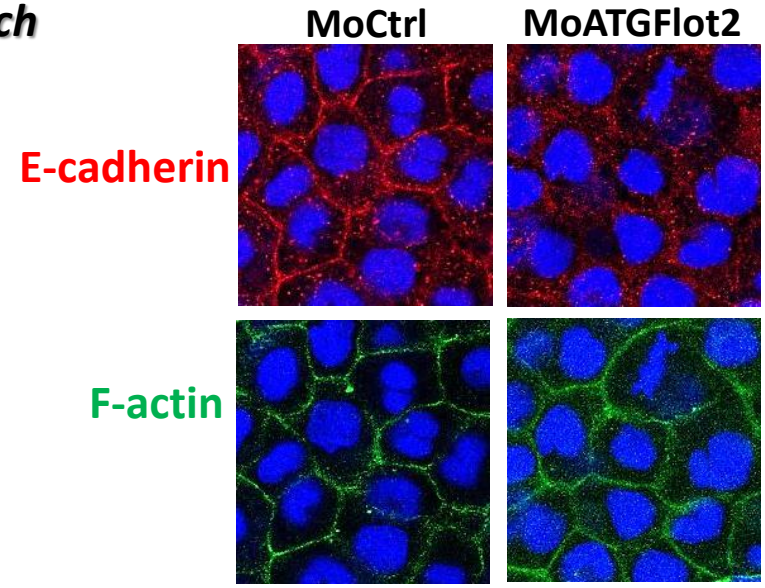
# *In vivo, Flotillins accumulate at cadherin mediated cellular junctions and are required for cadherin accumulation at cell-cell contacts*



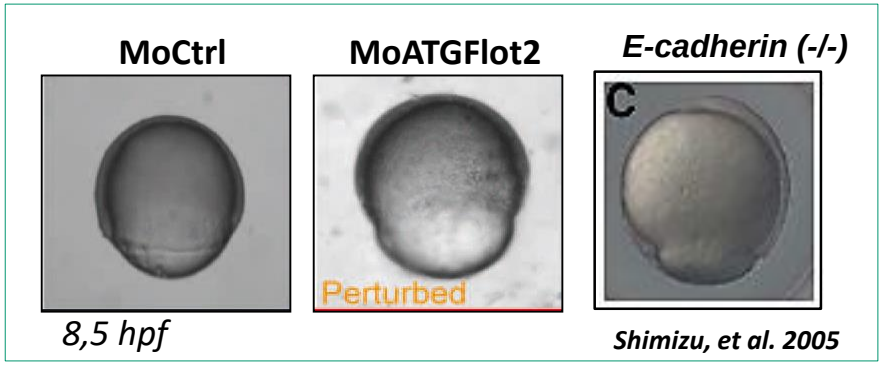




***Flotillins-knock down by morpholino approach***



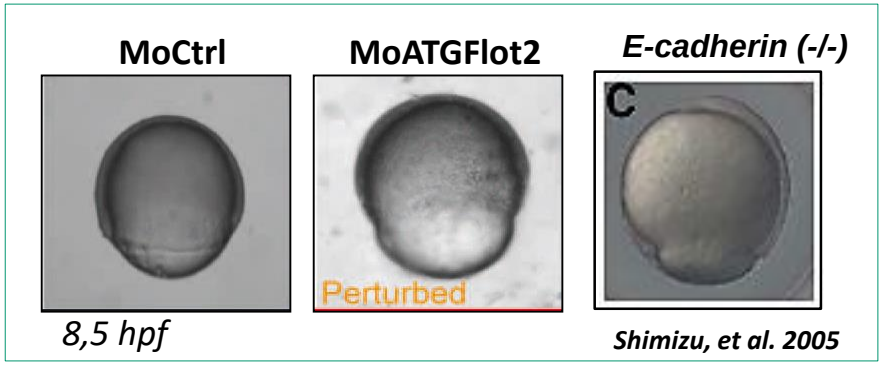



# *Flotillins knock-down phenocopies E-cadherin deficiency by delaying epiboly and preventing deep cells intercalation*

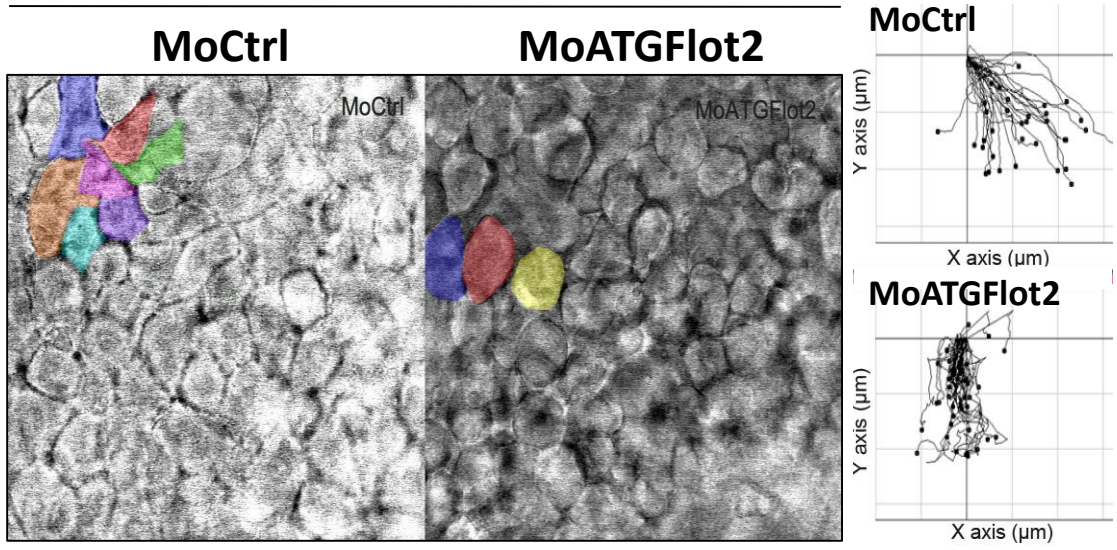



**Flotillins are required for epiboly**

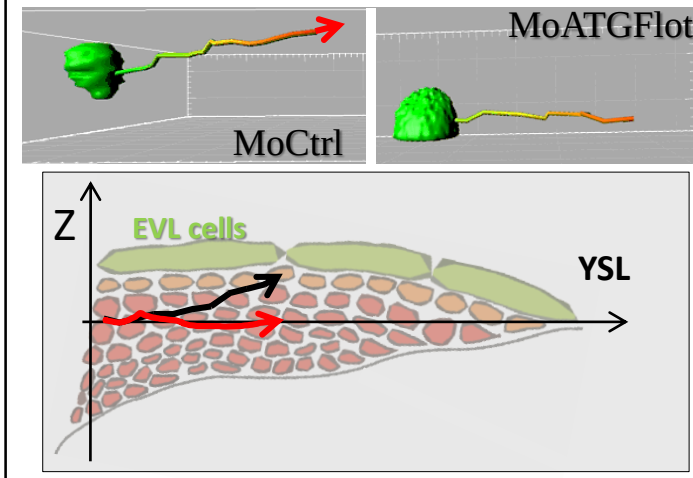
# *Flotillins knock-down phenocopies E-cadherin deficiency by delaying epiboly and preventing deep cells intercalation*

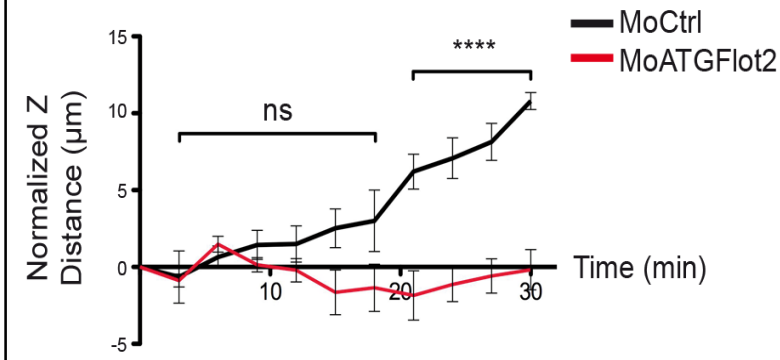



## Deep cells 2D movement analysis

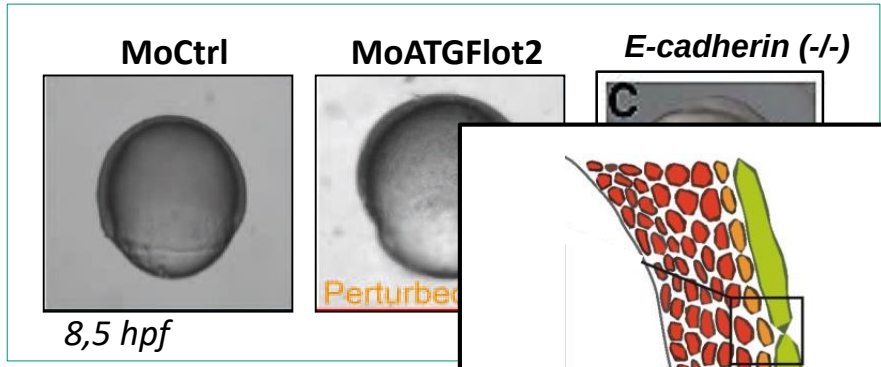


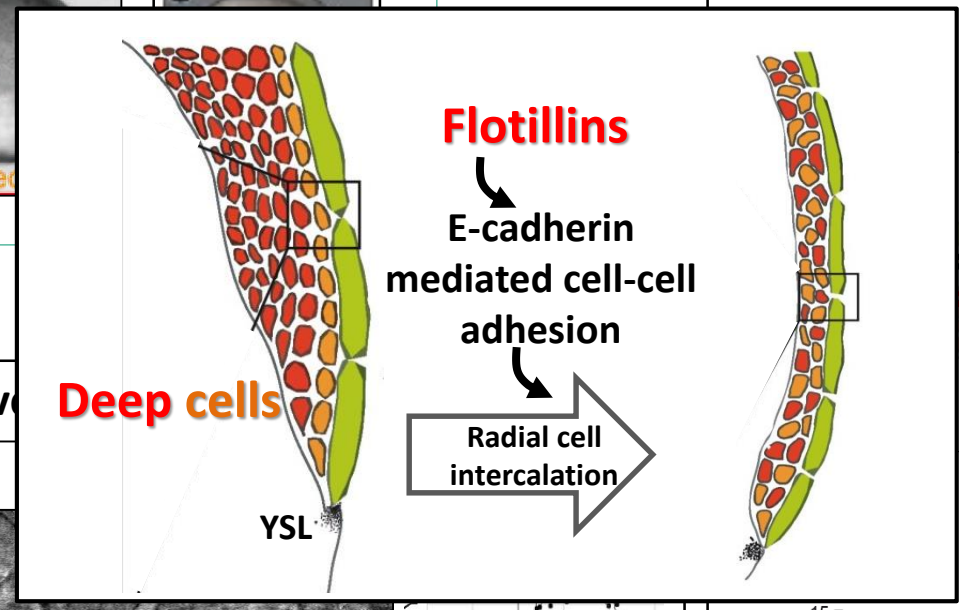

## Deep cells 3D movement analysis

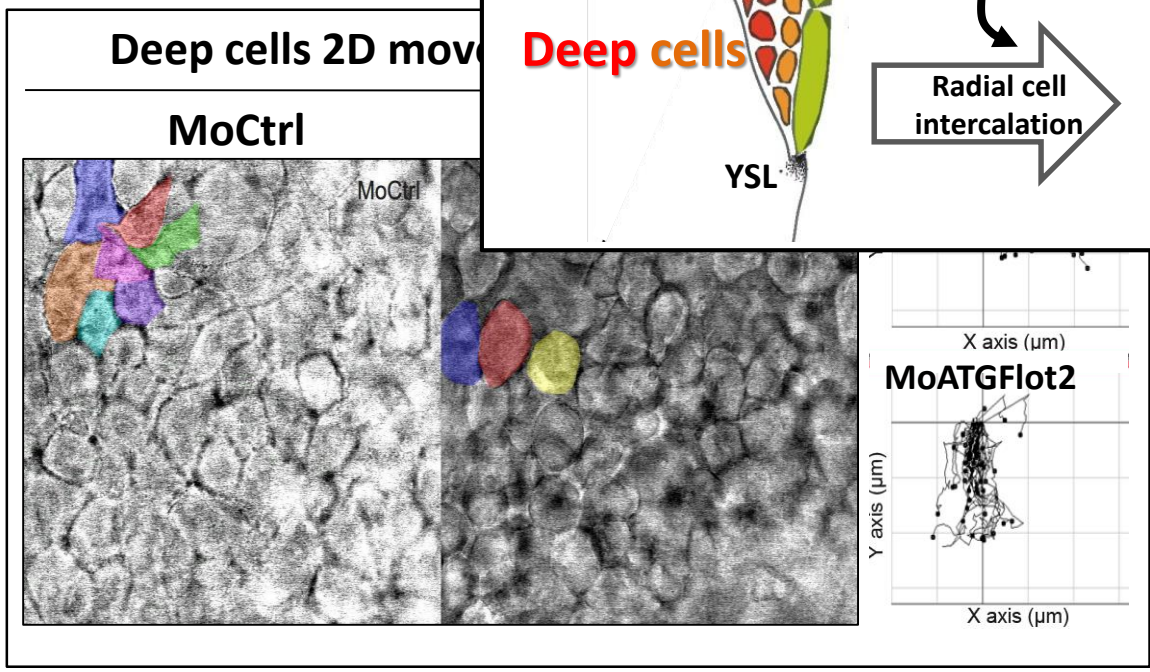


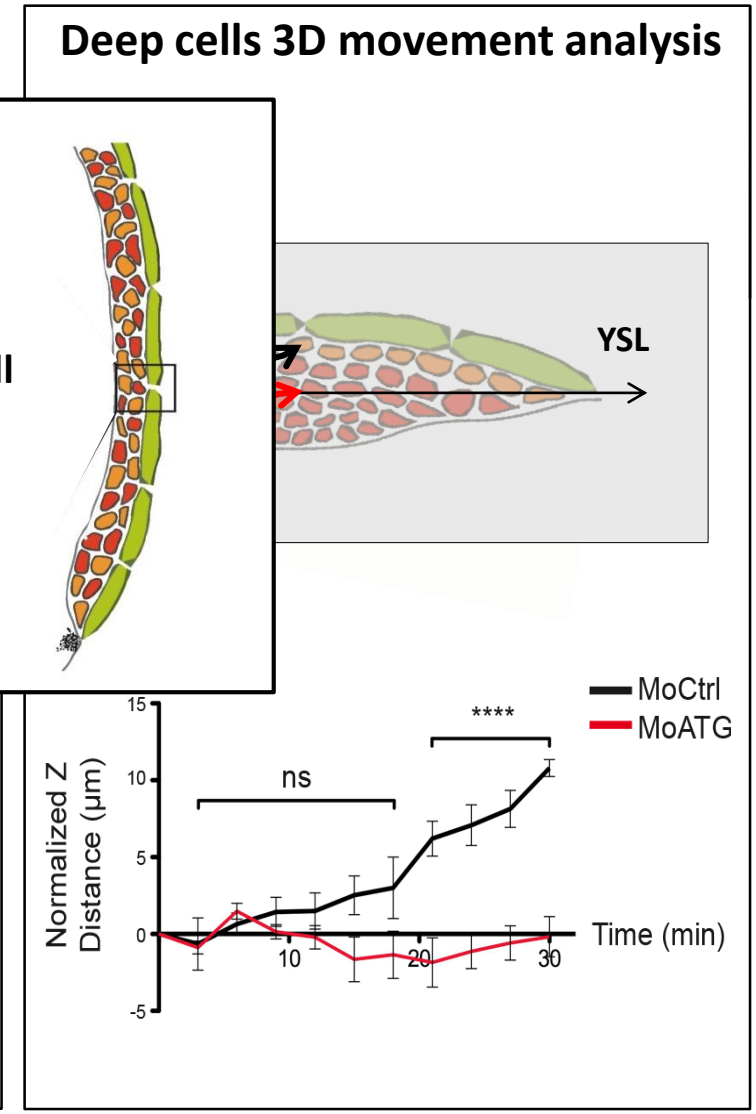



# *Flotillins knock-down phenocopies E-cadherin deficiency by delaying epiboly and preventing deep cells intercalation*



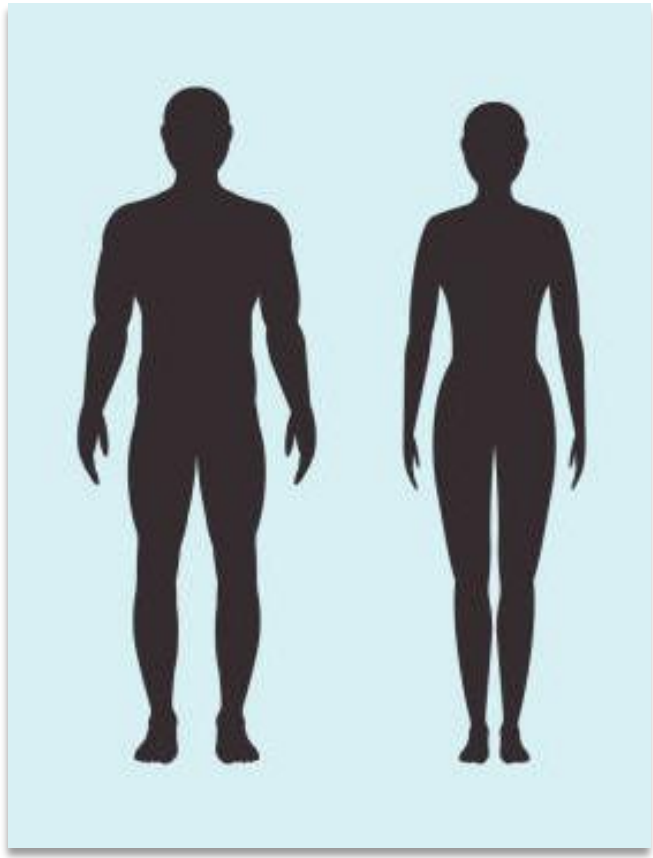



# Flotillins are required for cellular adhesion in vivo, but not in all models …


Flotillin KO mice exist, seem happy, and do not exhibit major phenotypic defect !

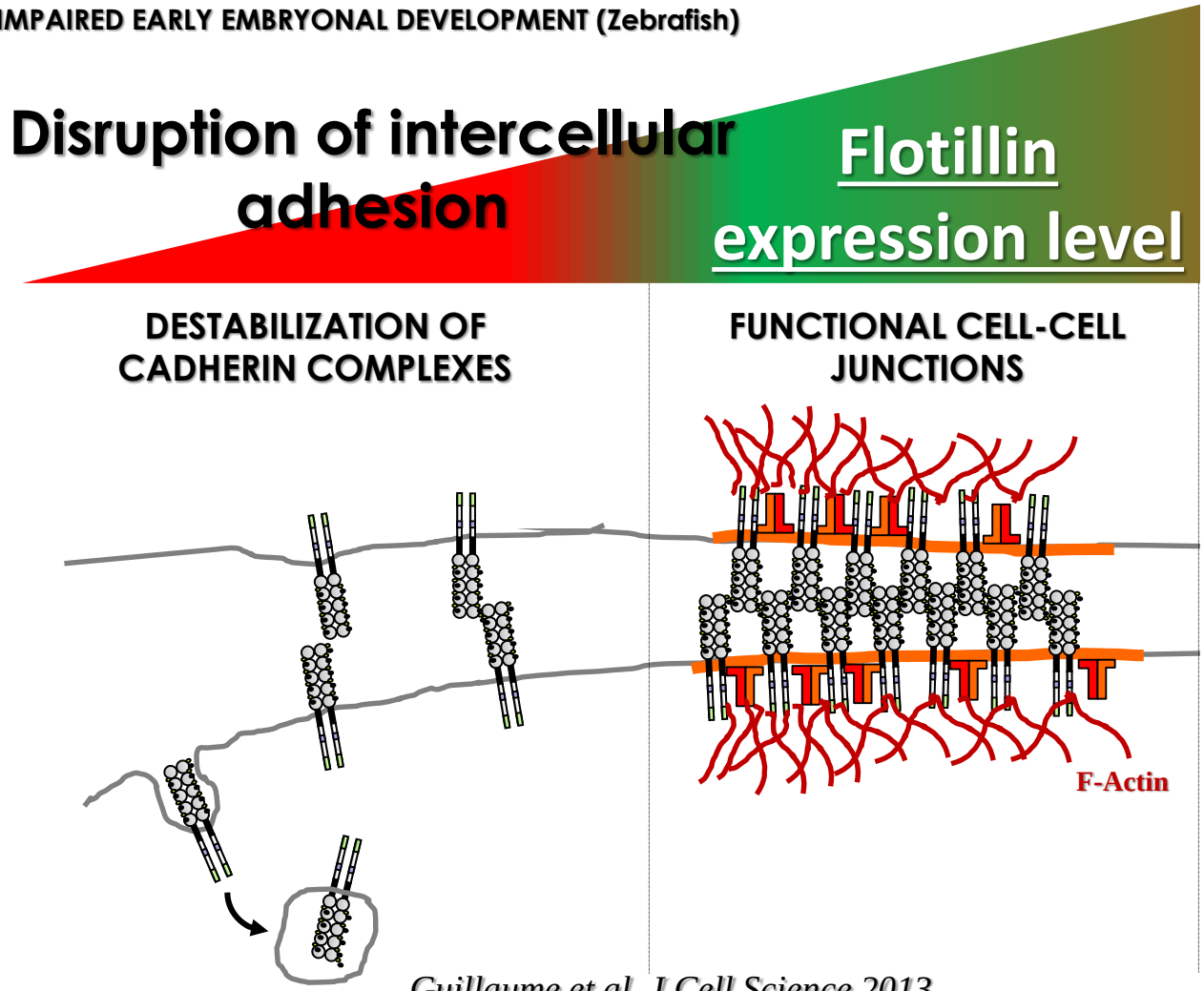


… but deficiency in flotillin expression in human has never been reported so far …

IMPAIRED EARLY EMBRYONAL DEVELOPMENT (Zebrafish)

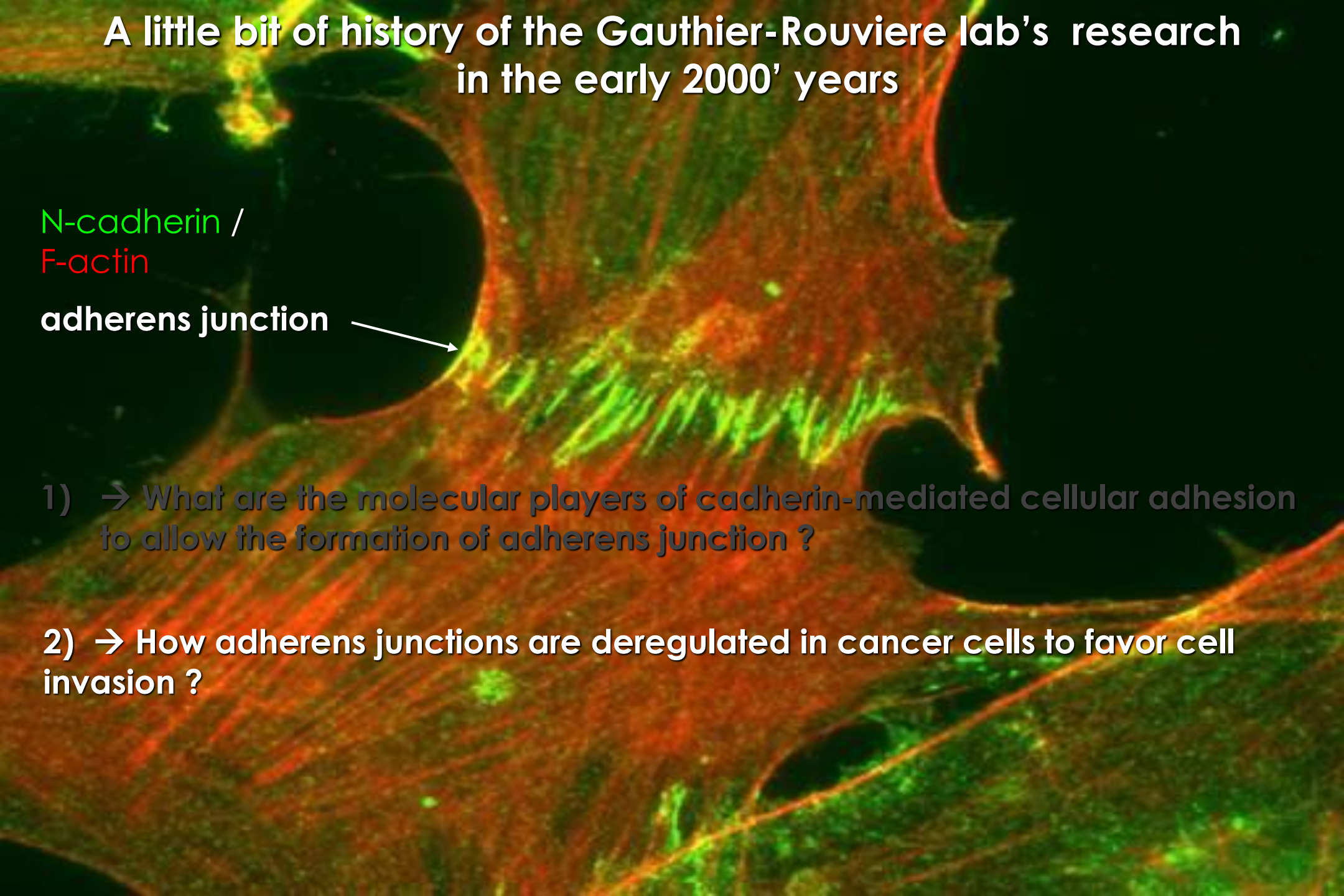
**Disruption of intercellular adhesion**

**Flotillin expression level**




*Guillaume et al. J Cell Science 2013*
*Rios-Morris, Biology of the Cell, 2017*

# A little bit of history of the Gauthier-Rouviere lab's research in the early 2000' years




1) → What are the molecular players of cadherin-mediated cellular adhesion to allow the formation of adherens junction ?

2) → How adherens junctions are deregulated in cancer cells to favor cell invasion ?

IMPAIRED EARLY EMBRYONAL DEVELOPMENT (Zebrafish)

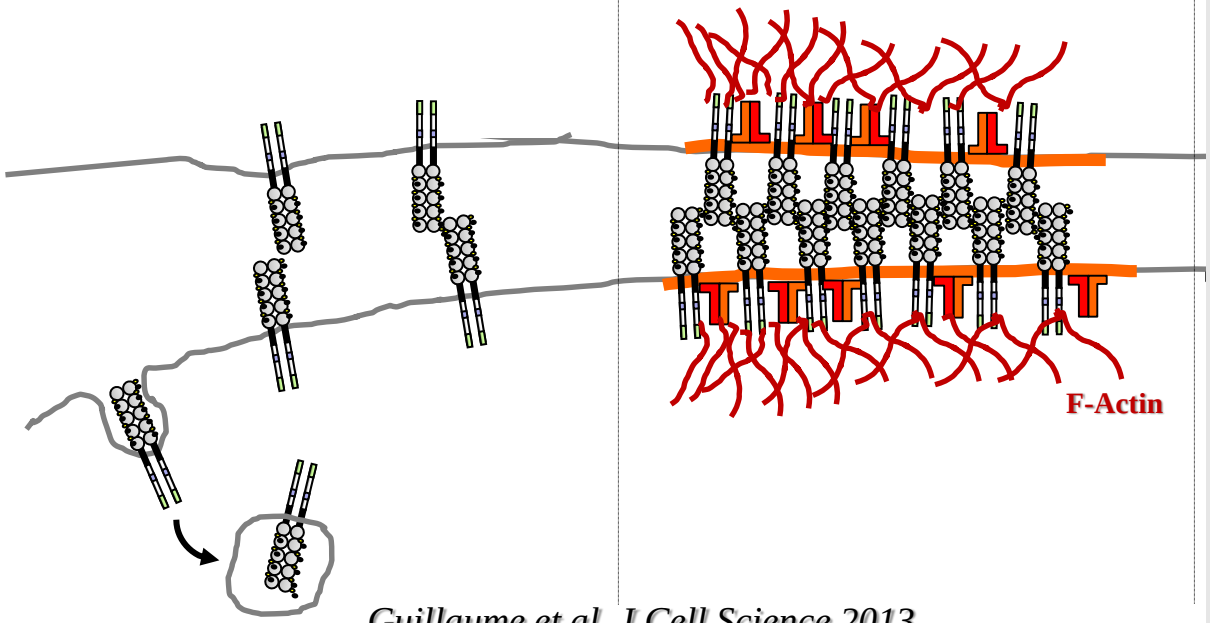
**Disruption of intercellular adhesion**

**Flotillin expression level**

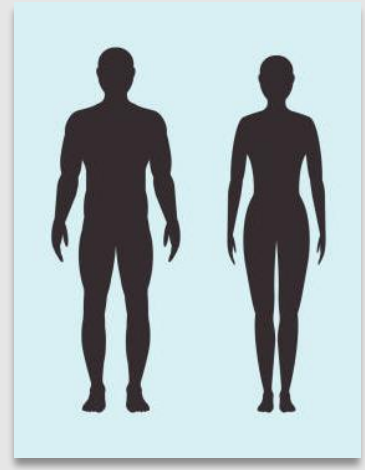
**Deregulated Intercellular adhesion**

**DESTABILIZATION OF CADHERIN COMPLEXES**

**FUNCTIONAL CELL-CELL JUNCTIONS**



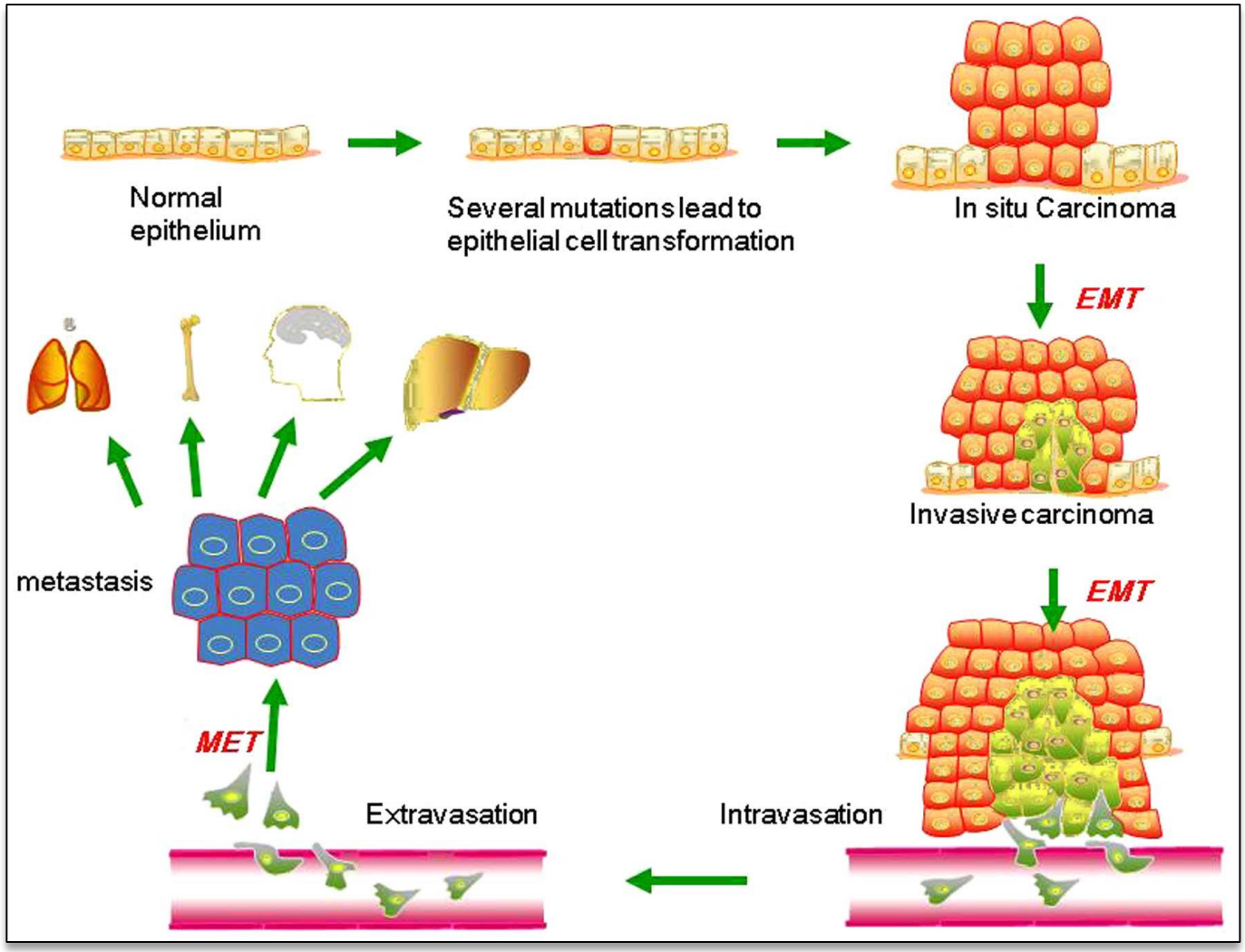

*Guillaume et al. J Cell Science 2013*
*Rios-Morris, Biology of the Cell, 2017*



**Epithelio-mesenchymal transition (EMT)**

**Cancer cell invasion**

# Epithelio-mesenchymal transition (EMT), a key early step in the invasive tumoral development of many carcinomas




## Acquisition of mesenchymal properties :

- **Switch in Cadherin expression**

↘ E-cadherin

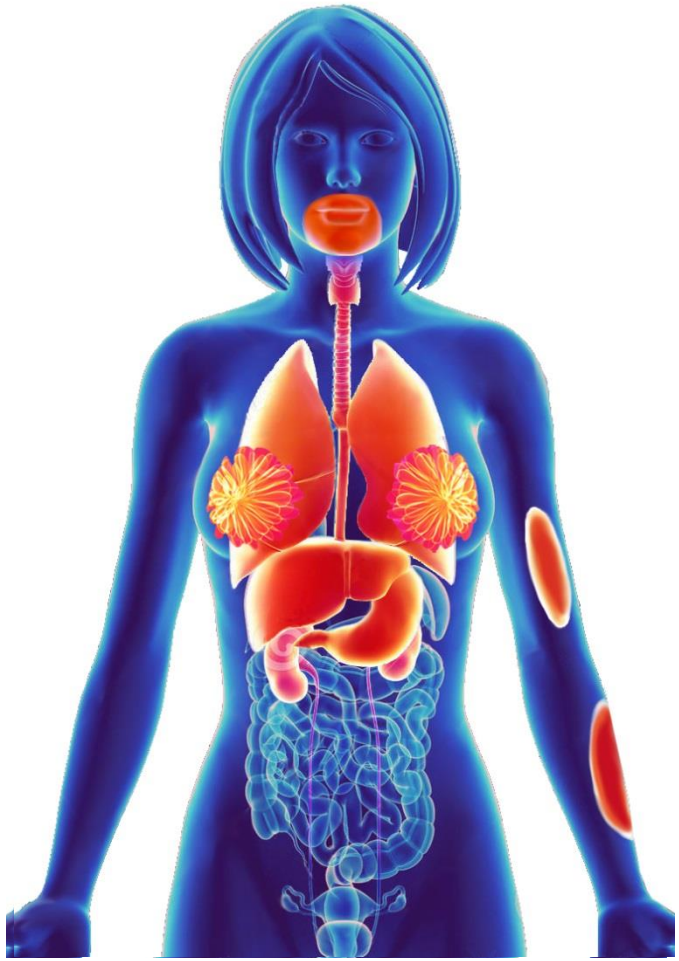
↗ N-cadherin

- **Modified cytoskeleton organisation**

- **Modified morphology**

- **Increased migration**

# Flotillins 1 & 2, two proteins upregulated in many invasive cancers

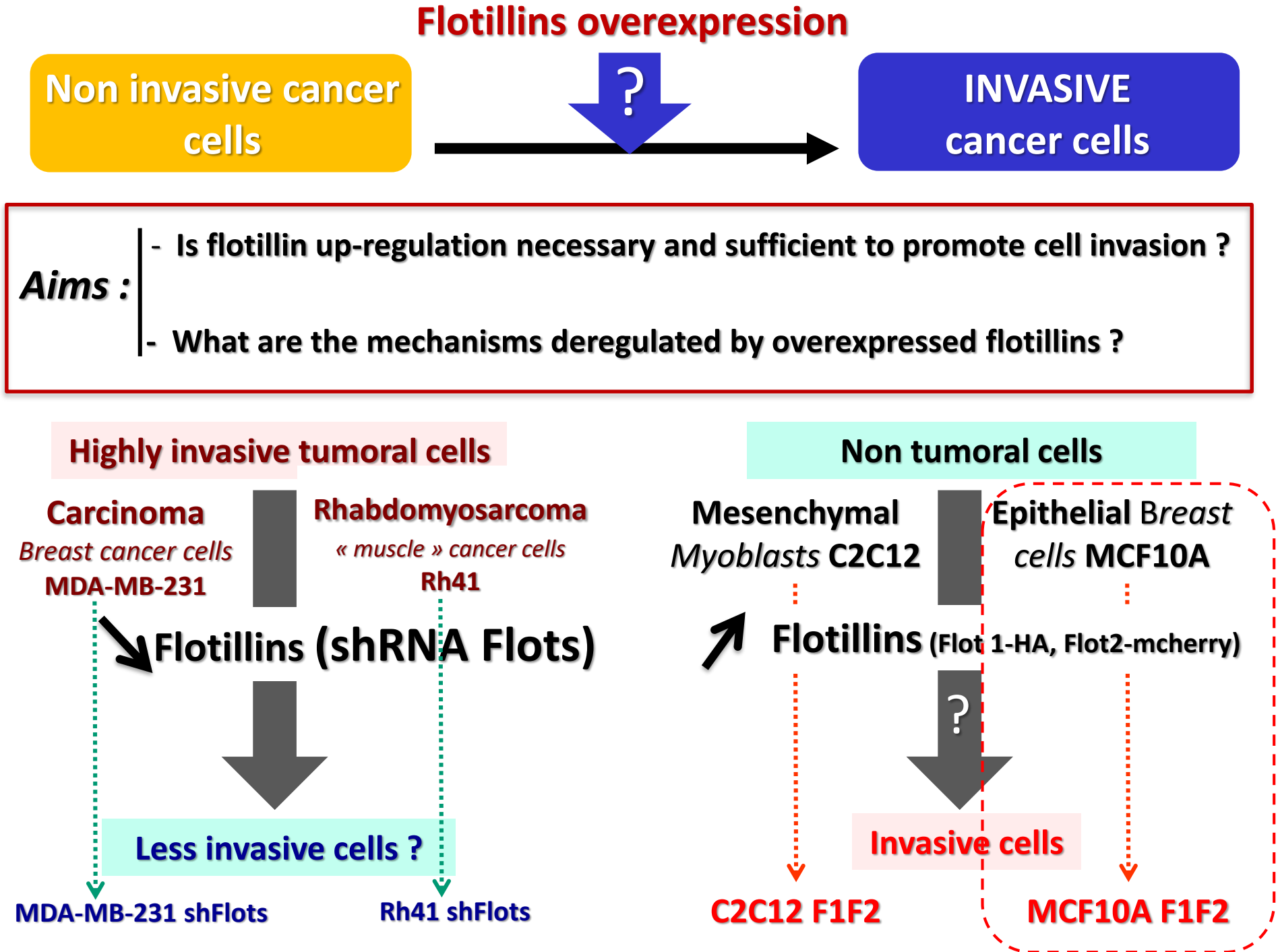


- **Esophageal cancer**
  Gong H et al, *Clin Cancer Res. 2013*
- **Lung cancer**
  Li H et al, *Tumour Biol. 2014*
- **Kidney cancer**
  Zhang Y et al, *Mol Med Rep. 2014*
- **Stomach cancer**
  Cao K et al, *Oncol Res. 2014*
- **Liver cancer**
  Zhang SH et al, *PLoS One. 2013*
- **Melanoma**
  Doherty SD et al, *Melanoma Res. 2006*
- **Oral Cancer**
  Wen Q et al, *Int J Clin Exp Pathol. 2015*
- **Breast cancer**
  May C et al, *Clin Cancer Res. 2011*
  *Planchon D et al. J Cell Science 2018*
- **Rhabdomyosarcoma**
  Planchon D et al, *unpublished data*

↗ **FLOTILLINS**

**Marker of poor prognosis**

**Promote metastasis tumoral development**


Flotillins overexpression
Non invasive cancer cells
?
INVASIVE cancer cells
Aims :
- Is flotillin up-regulation necessary and sufficient to promote cell invasion ?
- What are the mechanisms deregulated by overexpressed flotillins ?
Highly invasive tumoral cells
Carcinoma
Breast cancer cells
MDA-MB-231
Rhabdomyosarcoma
« muscle » cancer cells
Rh41
Flotillins (shRNA Flots)
Less invasive cells ?
MDA-MB-231 shFlots
Rh41 shFlots
Non tumoral cells
Mesenchymal
Myoblasts C2C12
Epithelial Breast cells MCF10A
Flotillins (Flot 1-HA, Flot2-mcherry)
?
Invasive cells
C2C12 F1F2
MCF10A F1F2

# 1▪ How much the overexpression of flotillins contributes to invasion ?

*A reciprocal approach :*

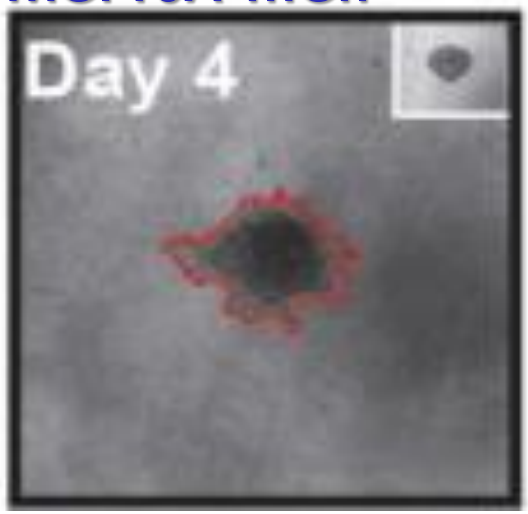
| | CELLULAR INVASION (*in vitro and in vivo*) |
|---|---|
| *Invasive breast carcinoma cells (MDA-MB-231, endogenous high flotillin levels)* | **+++** |
| *Carcinoma cells knocked-down for flotillins (MDA-MB-231 shFlots)* | **-** |
| *Non tumoral mammary epithelial cells (MCF10A)* | **-** |
| *Non tumoral mammary epithelial cells upregulated for flotillins (MCF10AF1F2)* | **+++** |

*Planchon et al. J Cell Science, 2019*

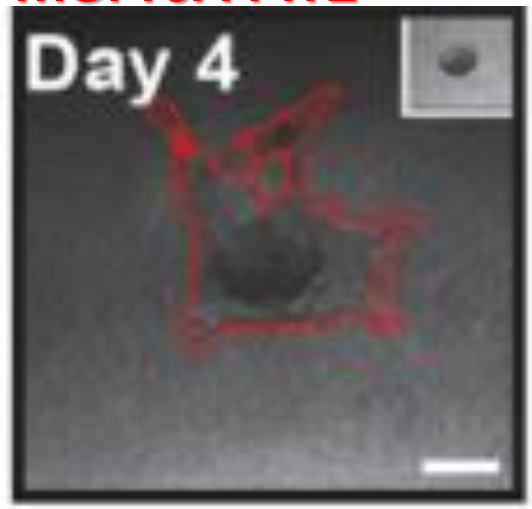
# The upregulation of both flotillins is sufficient to increase invasive properties of non tumoral MCF10 cells

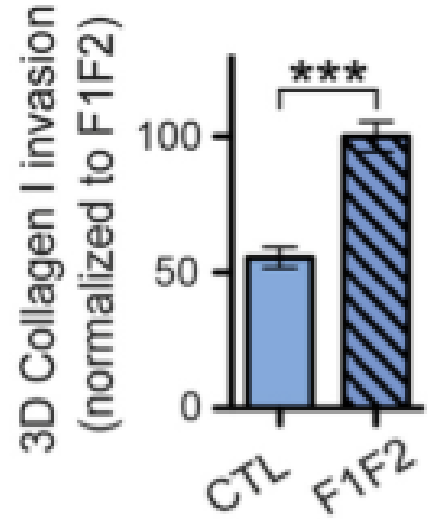
## 3D- spheroïd invasion assay

**MCF10A-mCh**




**MCF10A F1F2**



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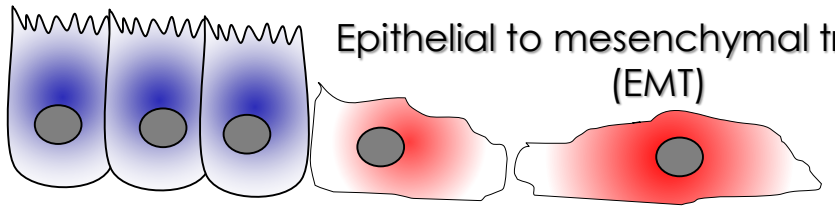
| *Non tumoral mammary epithelial cells (MCF10A)* | - |
| *Non tumoral mammary epithelial cells upregulated for flotillins (MCF10AF1F2)* | +++ |

*Planchon et al. J Cell Science, 2019*

*MCF10A* *MCF10AF1F2*

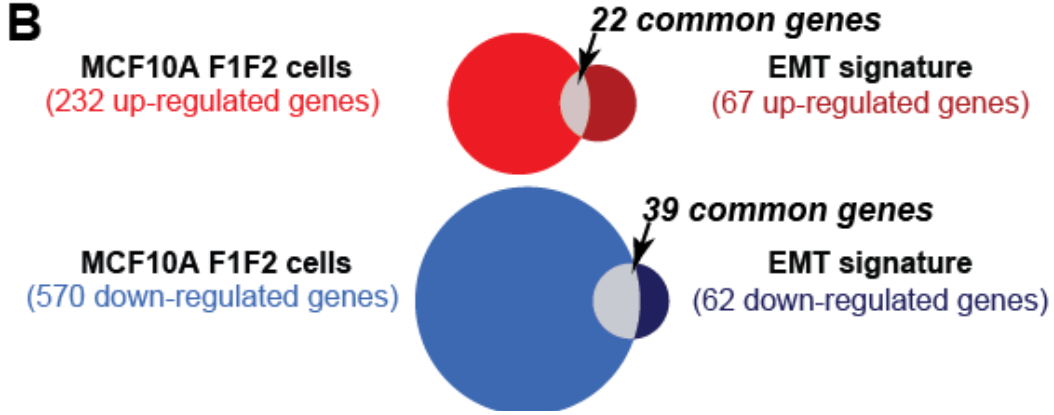



*Genest et al. BiorXiv, 2021*

Does flotillin upregulation promotes EMT ?

# Comparative transcriptomic analysis between MCF10A cells and MCF10F1F2 cells revealed that flotillin upregulatoin induces a transcriptomic signature

**B**




**D**

*Top upregulated genes*

| | Symbol | Fold up | *P-value* |
|---|---|---|---|
| 1 | CTGF | 14,811 | 3.81E-40 |
| 2 | SERPINE2 | 12,754 | 3.88E-33 |
| 3 | SRGN | 5,557 | 3.74E-19 |
| 4 | ZEB1 | 5,462 | 1.38E-16 |
| 5 | FN1 | 17,147 | 2.21E-13 |
| 6 | MAP1B | 12,223 | 5.92E-13 |
| 7 | NRZF1 | 6,466 | 5.59E-12 |
| 8 | MMP2 | 8,789 | 7.51E-12 |
| 9 | HAS2 | 9,963 | 9.37E-12 |
| 10 | VIM | 3.529 | 2.12E-11 |
| 11 | FBLN5 | 8.92 | 8.18E-11 |
| 12 | FBN1 | 7.881 | 1.93E-10 |
| 13 | SPOCK1 | 8.295 | 1.56E-9 |
| 14 | FGF2 | 3.053 | 2.70E-9 |
| 15 | DCN | 6.926 | 3.01E-7 |
| 16 | DLC1 | 3.997 | 1.09E-6 |
| 17 | CDH2 | 3.288 | 1.96E-6 |
| 18 | PTX3 | 4.352 | 7.84E-5 |
| 19 | WTN5A | 3.132 | 2.11E-4 |
| 20 | LUM | 4.123 | 3.52E-4 |

N-Cadh →

*Top downregulated genes*

| | Symbol | Fold down | *P-value* |
|---|---|---|---|
| 1 | DSG3 | 0.017 | 1.48E-107 |
| 2 | EPCAM | 0.020 | 3.77E-94 |
| 3 | CDH1 | 0.006 | 3.13E-90 |
| 4 | MPZL2 | 0.077 | 4.99E-62 |
| 5 | LAD1 | 0.006 | 5.19E-56 |
| 6 | TSPAN1 | 0.026 | 2.57E-54 |
| 7 | SLC27A2 | 0.037 | 1.52E-51 |
| 8 | FGFR2 | 0.071 | 3.76E-30 |
| 9 | CDS1 | 0.134 | 9.03E-30 |
| 10 | FGFR3 | 0.113 | 3.53E-29 |
| 11 | LSR | 0.043 | 3.29E-28 |
| 12 | CXADR | 0.057 | 5.02E-28 |
| 13 | OCLN | 0.034 | 3.83E-25 |
| 14 | PRSS8 | 0.043 | 7.19E-25 |
| 15 | KRT17 | 0.066 | 1.96E-19 |
| 16 | RAPGEF5 | 0.105 | 1.17E-17 |
| 17 | ST6GALNAC2 | 0.088 | 1.47E-16 |
| 18 | IFI30 | 0.199 | 1.60E-16 |
| 19 | ANK3 | 0.180 | 4.03E-15 |
| 20 | SPINT1 | 0.252 | 6.98E-15 |

E-Cadh →

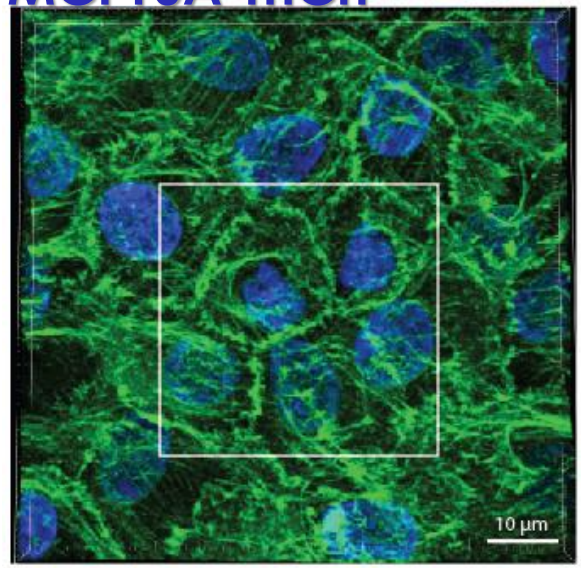
**↗ Flotillins → EMT**

*Mallory Genest*

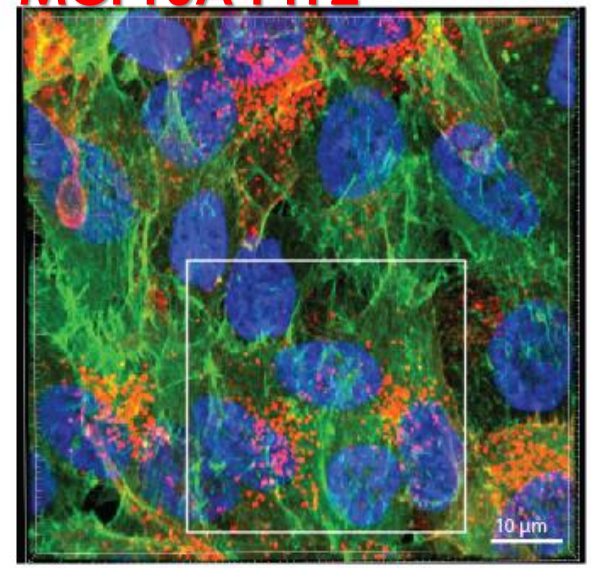
# The upregulation of both flotillins is sufficient to induce EMT in non-tumoral mammary epithelial cells

**Typical EMT-related changes in actin cytoskeleton organization**

MCF10A-mCh



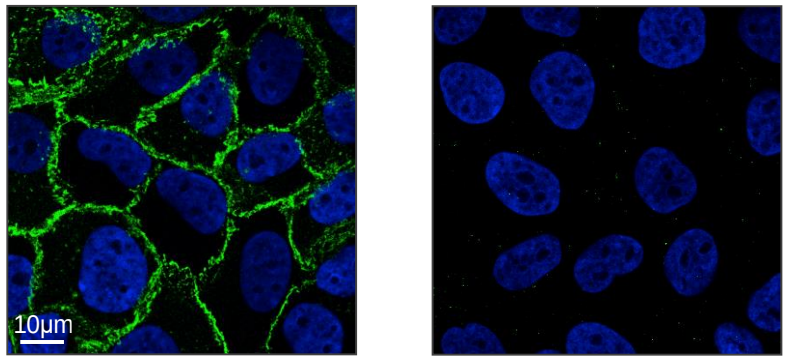

MCF10A F1F2



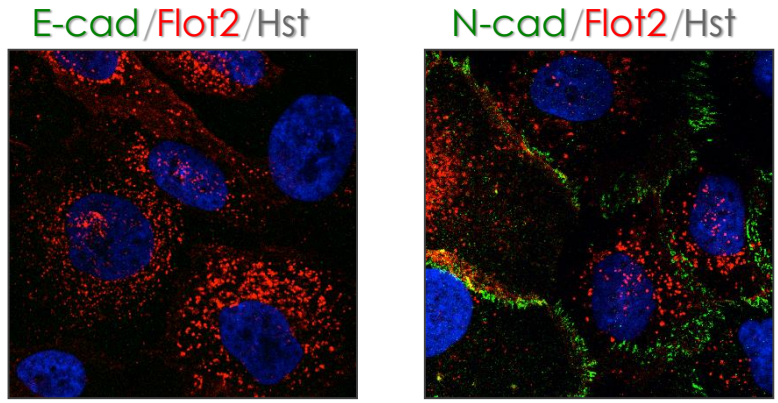
# Flotillin-upregulation induces EMT

## E- to N- cadherin switch

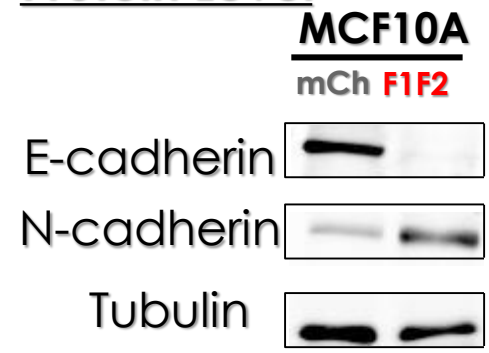
### MCF10A-mCh



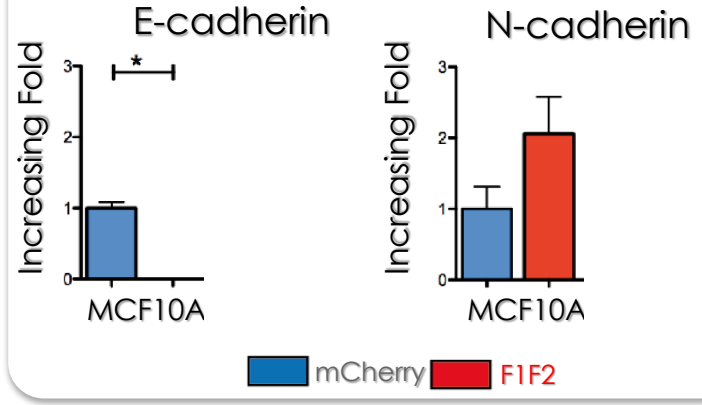

### MCF10A F1F2




### Protein Level

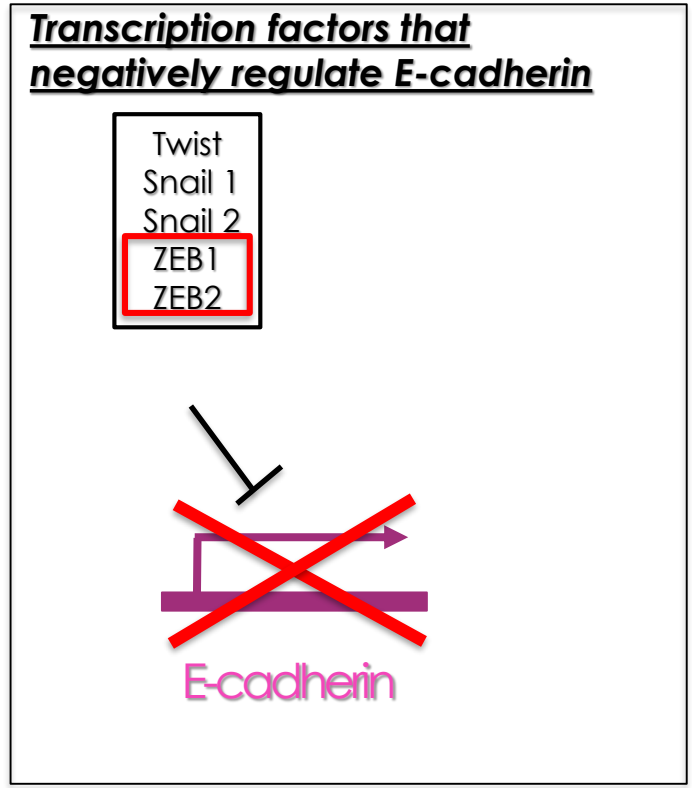



### mRNA Level



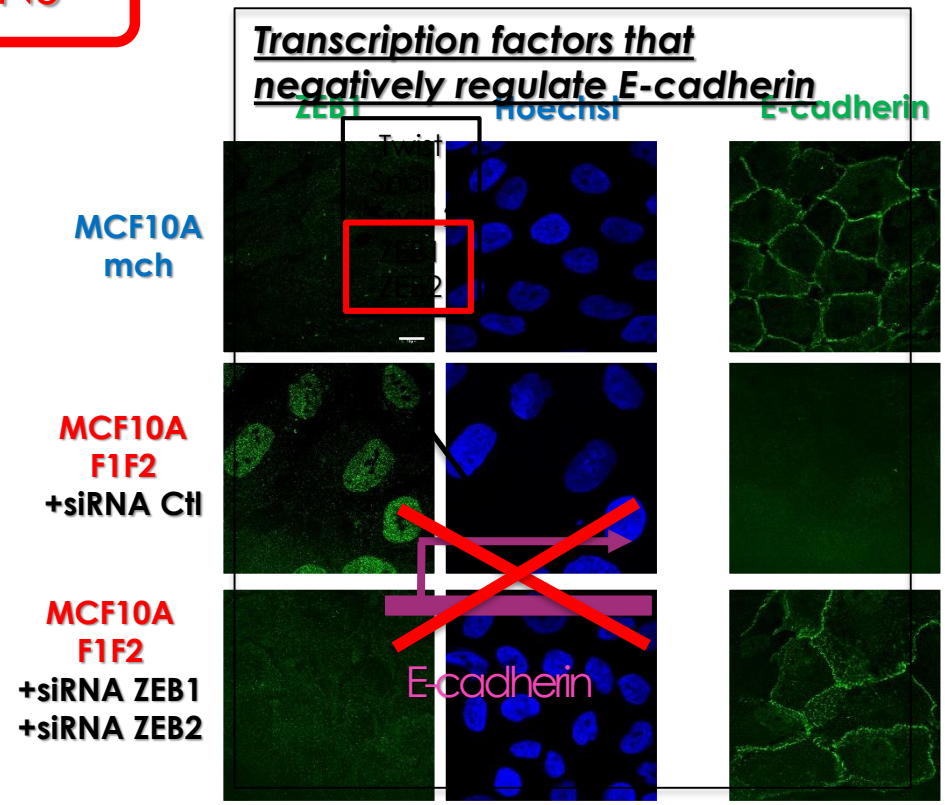
# How Flotillin-upregulation induces E-cadherin down-regulation ?

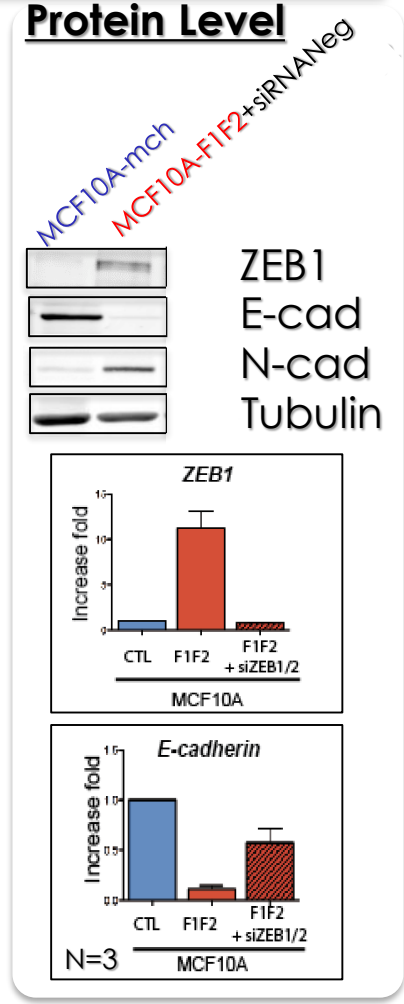
↗ FLOTILLINS



# How Flotillin-upregulation induces E-cadherin down regulation ?

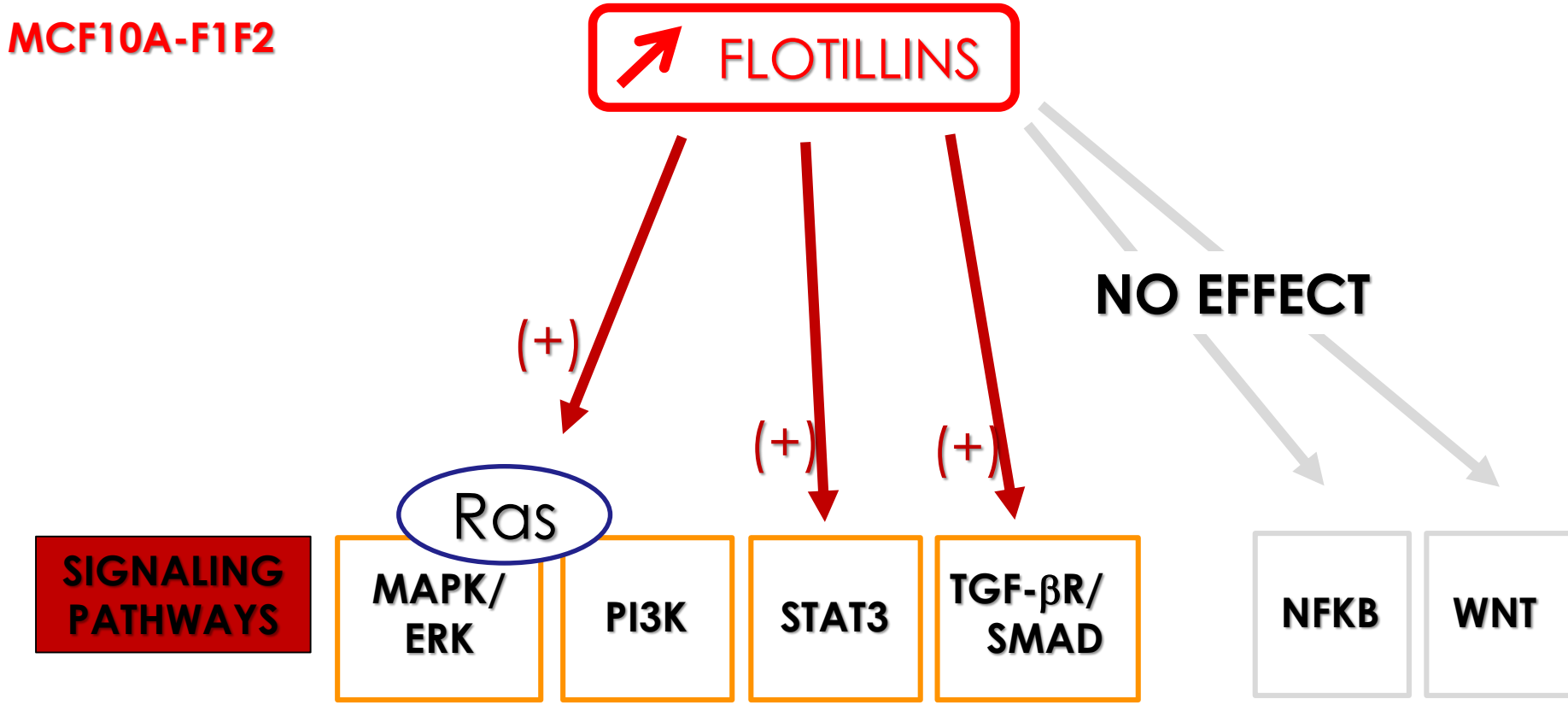
FLOTILLINS




**Flotillin-upregulation induced the down-regulation of E-cadherin in a ZEB1/2 dependent manner**

# Signalling pathways promoting EMT activated downstream Flotillin-upregulation in MCF10A cells



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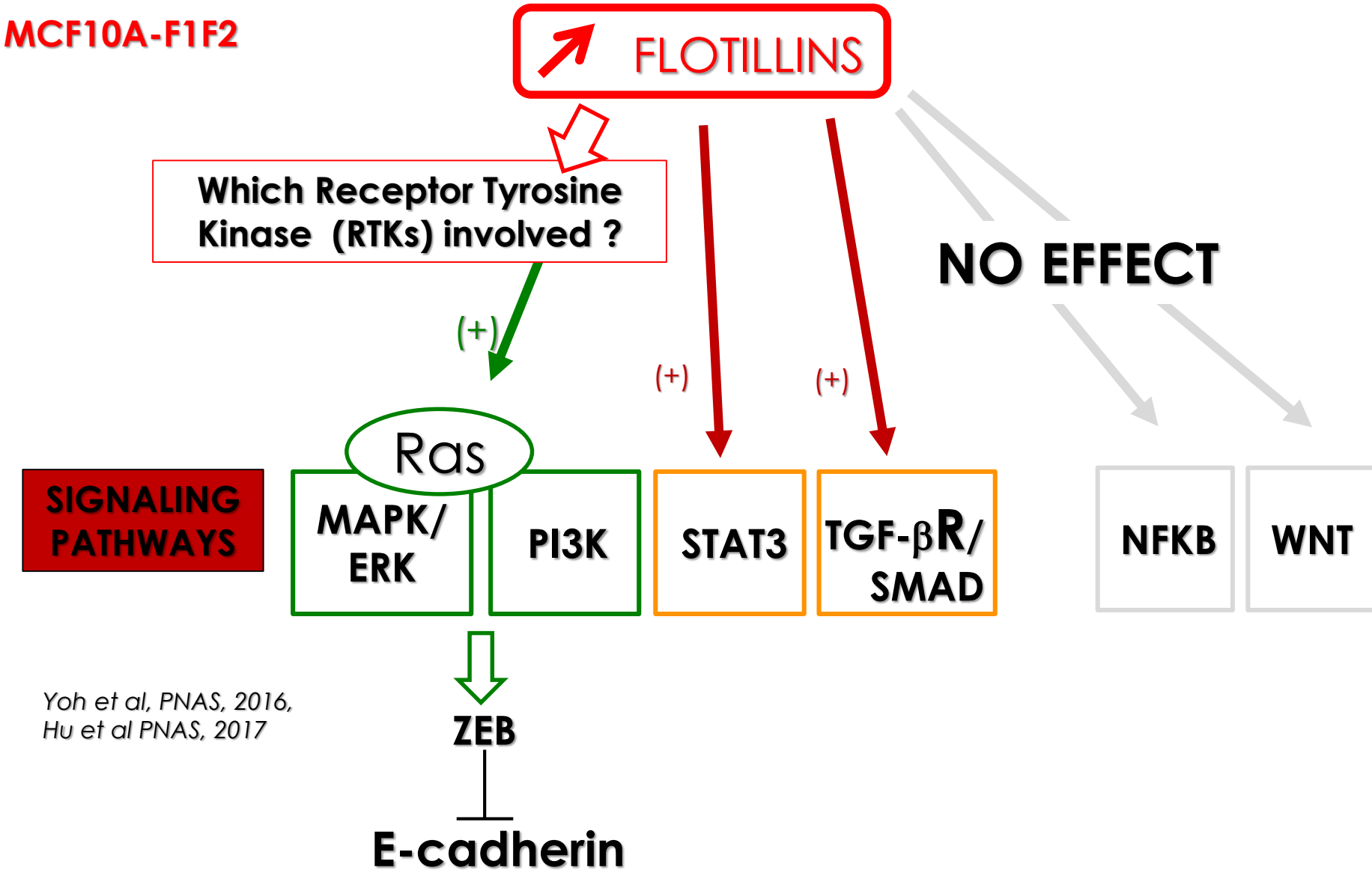
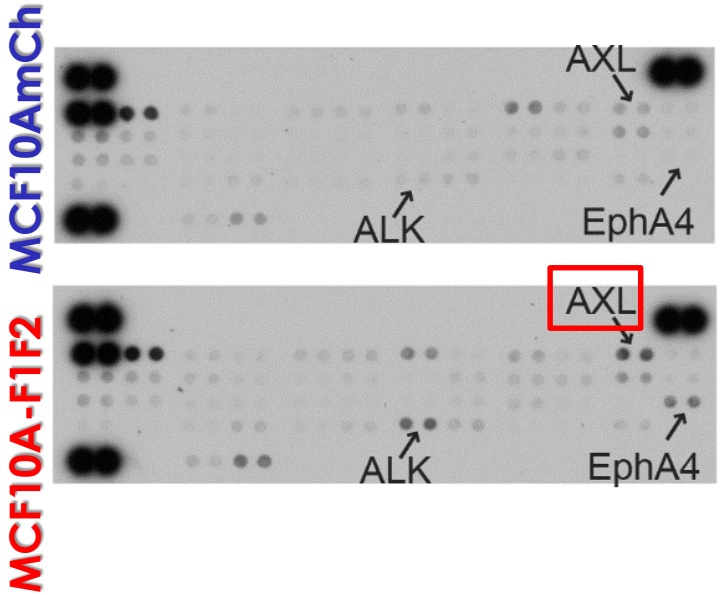



*Yoh et al, PNAS, 2016,*
*Hu et al PNAS, 2017*

# Flotillin-upregulation increases the level of activated RTKs, in particular AXL

***Phospho-RTK array***

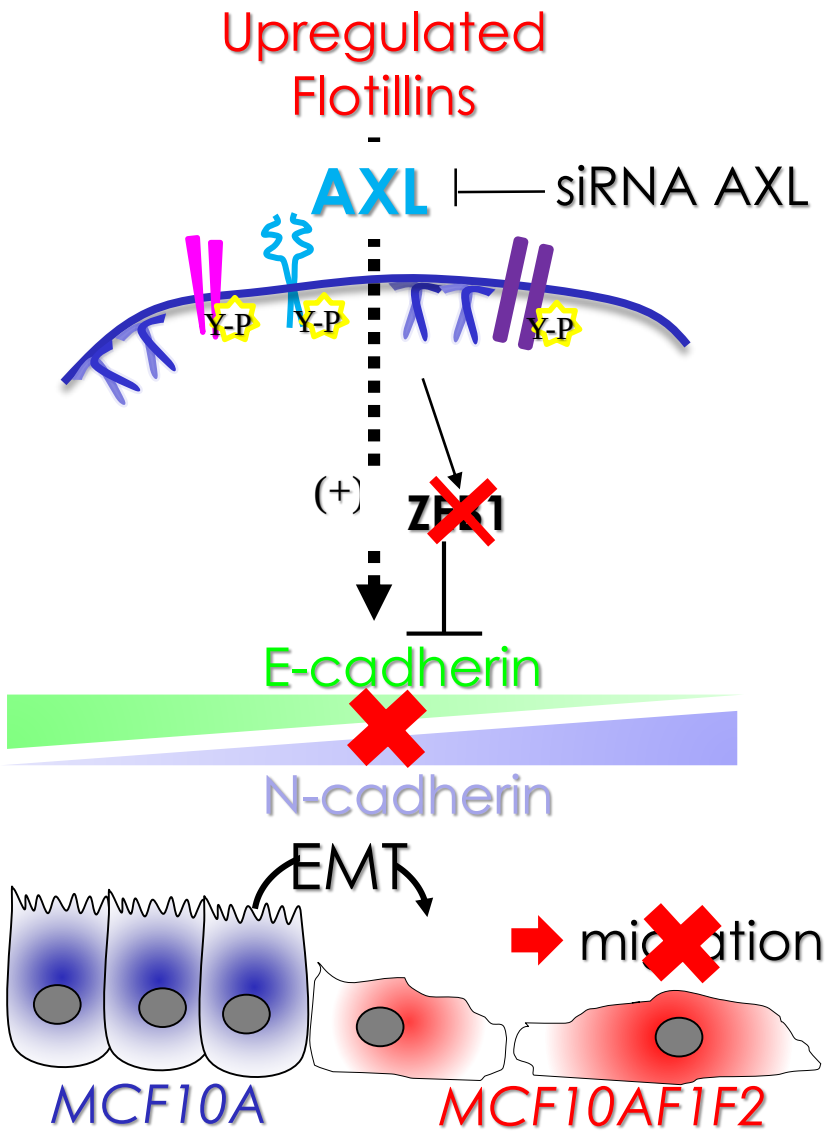
*Simultaneous comparison of the Y-phosphorylation state of 49 RTKs*

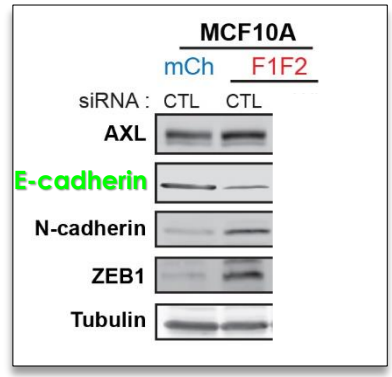


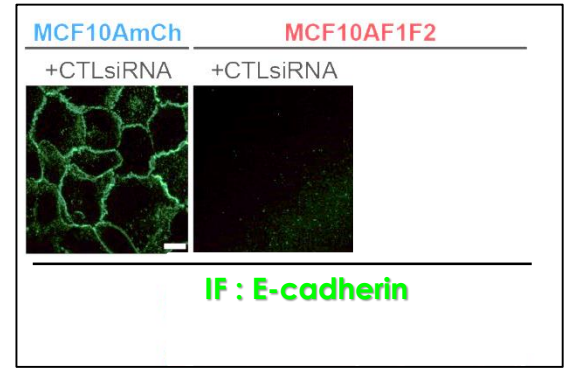

RTKs « up-phosphorylated in MCF10AF1F2 cells compared to Control MCF10A cells

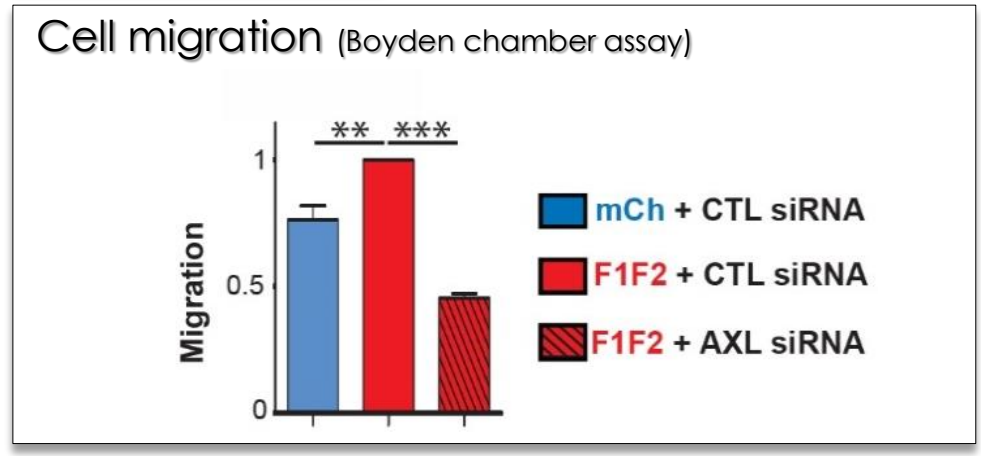
| RTK List | Ratio (F1F2/mCh) |
|---|---|
| EphA4 | 12.28 |
| ALK | 4.92 |
| AXL | 2.80 |
| FGFR3 | 2.41 |
| MSP-R | 2.24 |
| TrkC | 1.85 |
| TrkB | 1.70 |
| TIE-1 | 1.50 |
| c-Ret | 1.49 |
| DTK | 1.44 |
| SCF-R | 1.43 |

# The receptor tyrosine kinase AXL is involved in EMT and increased migration induced by upregulated-Flotillins



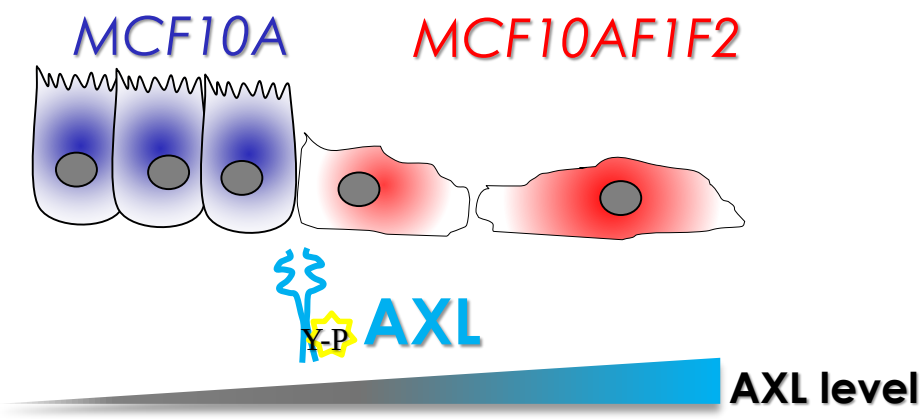

- *AXL is overexpressed in various cancers*

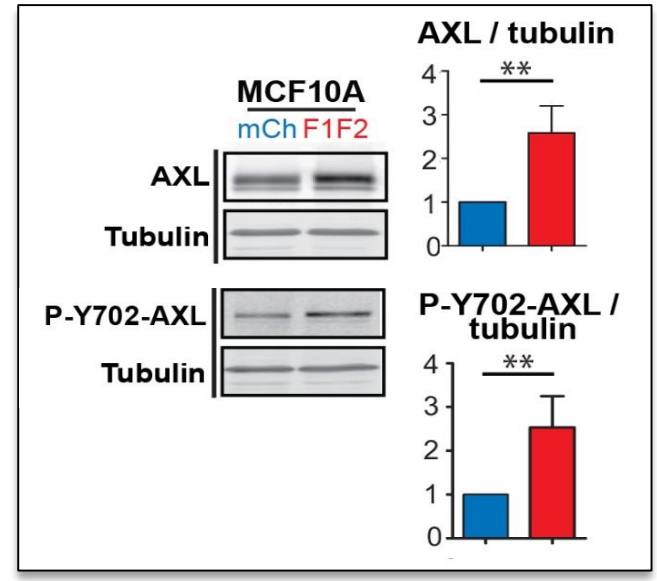
  ↳ *Promotes EMT, invasion, metastasis*

*- The mechanisms responsible for AXL overexpression are still poorly known.*

*Genest et al. BiorXiv, 2021*

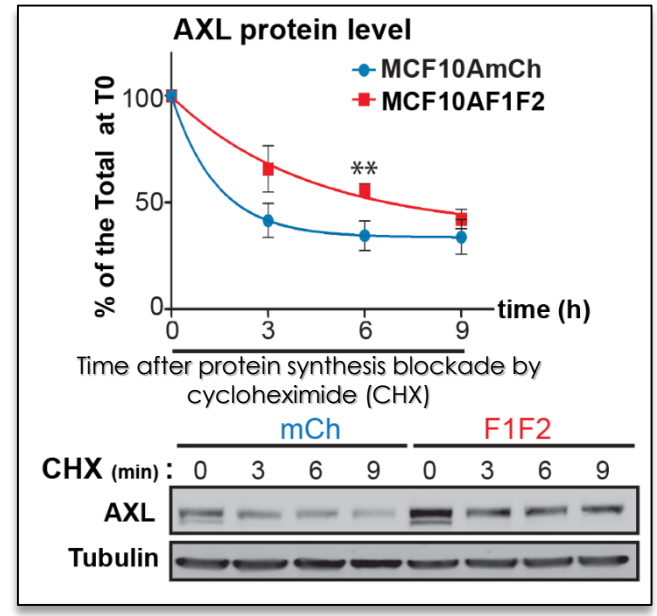
# Flotillin-upregulation increases AXL level by promoting its stabilization




… not due to an upregulation of AXL mRNA

… but due to an **increase in AXL stability**




*Genest et al. BiorXiv, 2021*

# HOW IS AXL STABILIZED BY FLOTILLIN OVEREXPRESSION ?

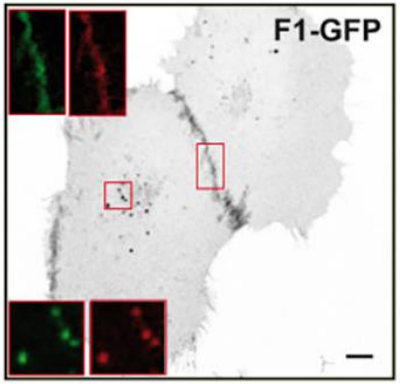
- Our Hypothesis : AXL vesicular trafficking is deregulated in a way that it is protected from degradation.

# WHAT SUPPORTS THIS HYPOTHESIS ?

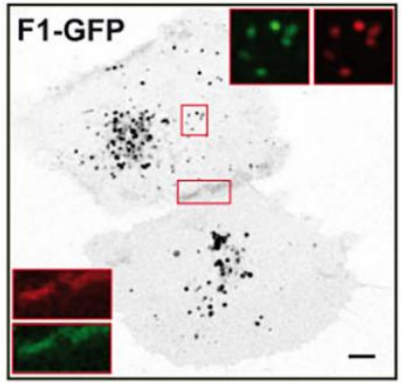
**Non tumoral mammary epithelial MCF10A cells K.O. for Flotillins**

**Ectopic expression of Flotillin 1-GFP / Flotillin 2-mCherry :**

**At moderate and physological level**

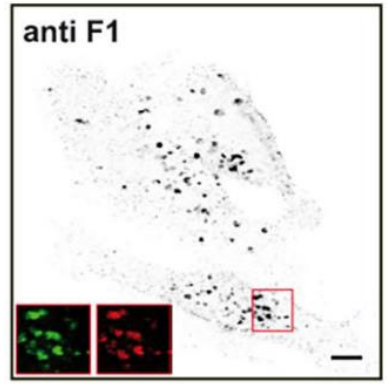



**At high level, similar to tumor cells**




**Invasive tumor MDA-MB-231 cells**

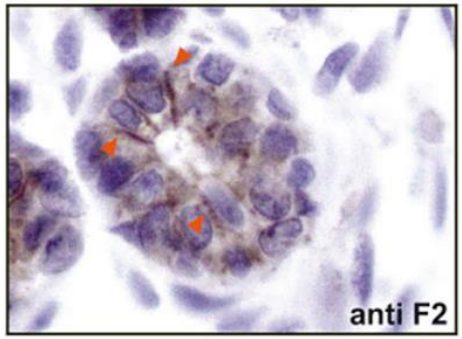
**Endogenous high expression level of Flotillin 1 / Flotillin 2**




## Flotillins staining in tumors

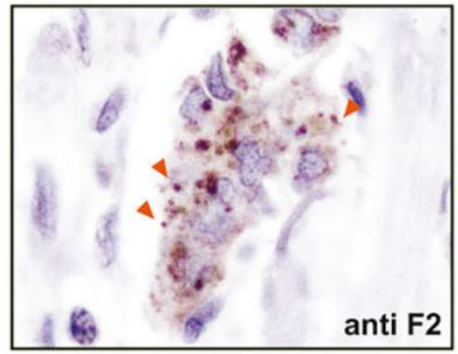
**adjacent peritumoral breast epithelium tissue**

*Normal Acini*




**Invasive tumor cells**

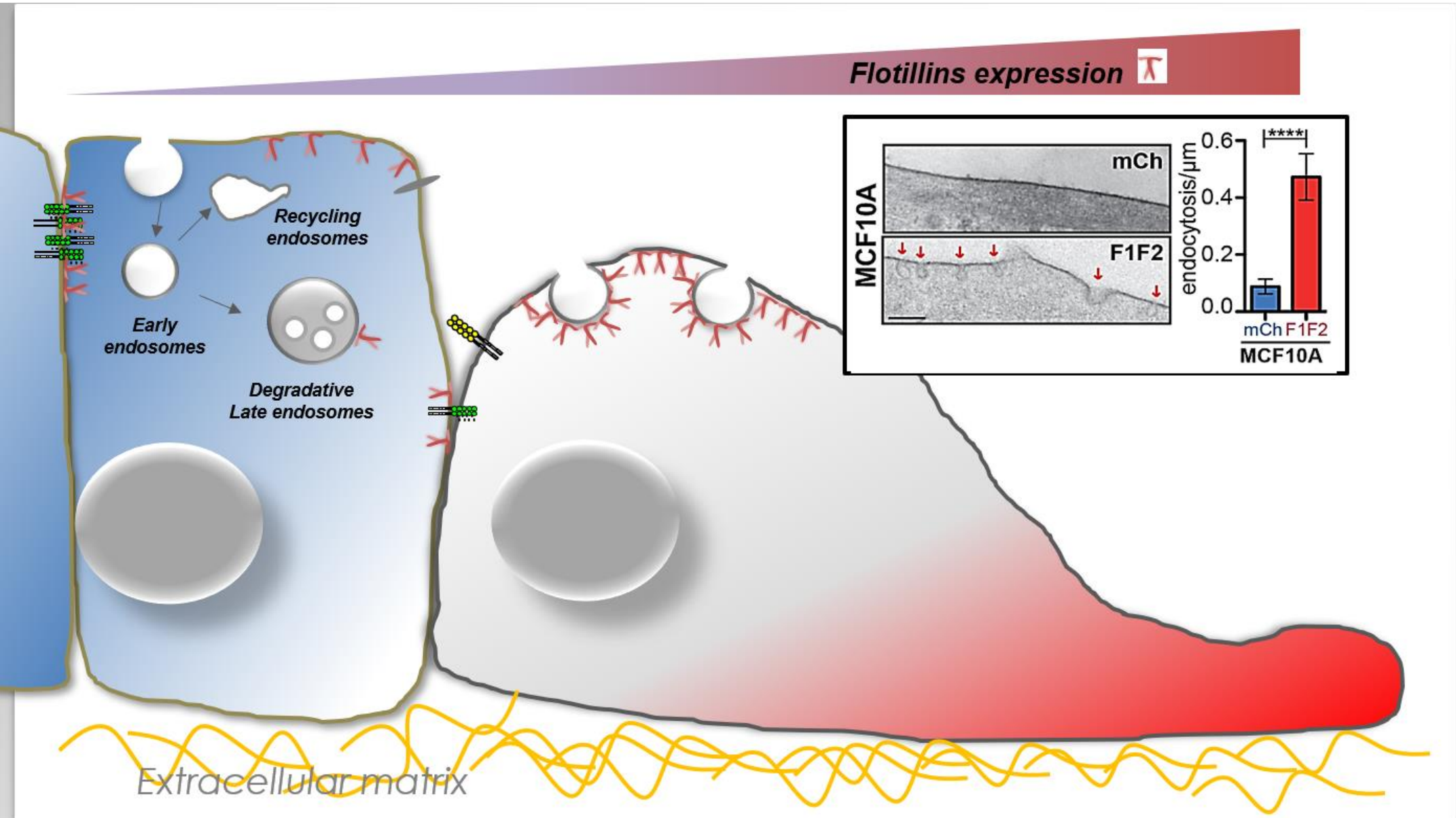
*Invasive Carcinoma*




**When flotillins are overexpressed, there cellular distribution changes drastically, characterized by a strong accumulation in intracellular vesicles.**

# Flotillin upregulation promotes a deregulation of the vesicular trafficking

## The upregulated-Flotillin induced trafficking (UFIT) pathway




Planchon et al. J Cell Science, 2019

# Flotillin upregulation promotes a deregulation of the vesicular trafficking

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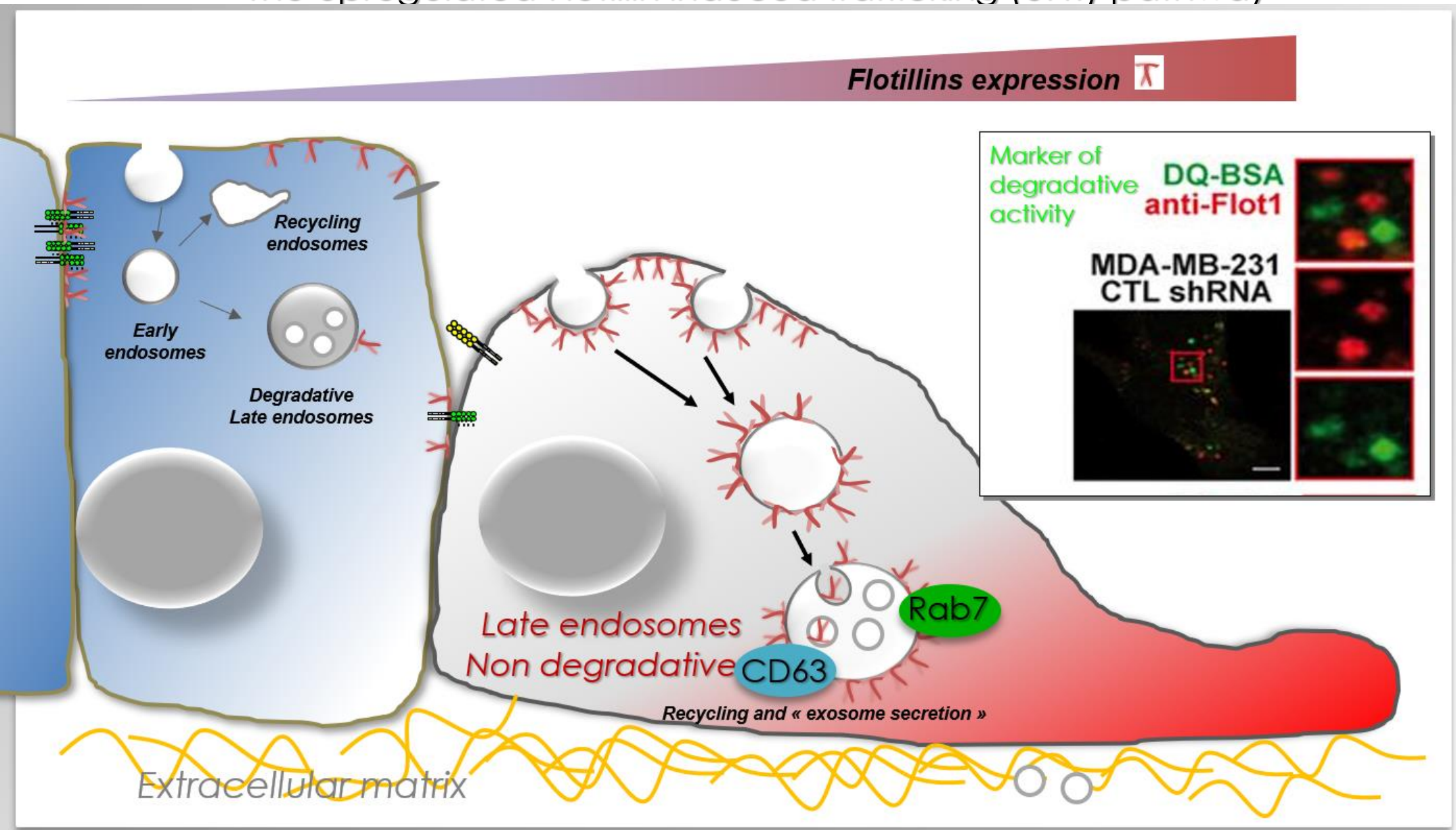


Planchon et al. J Cell Science, 2019

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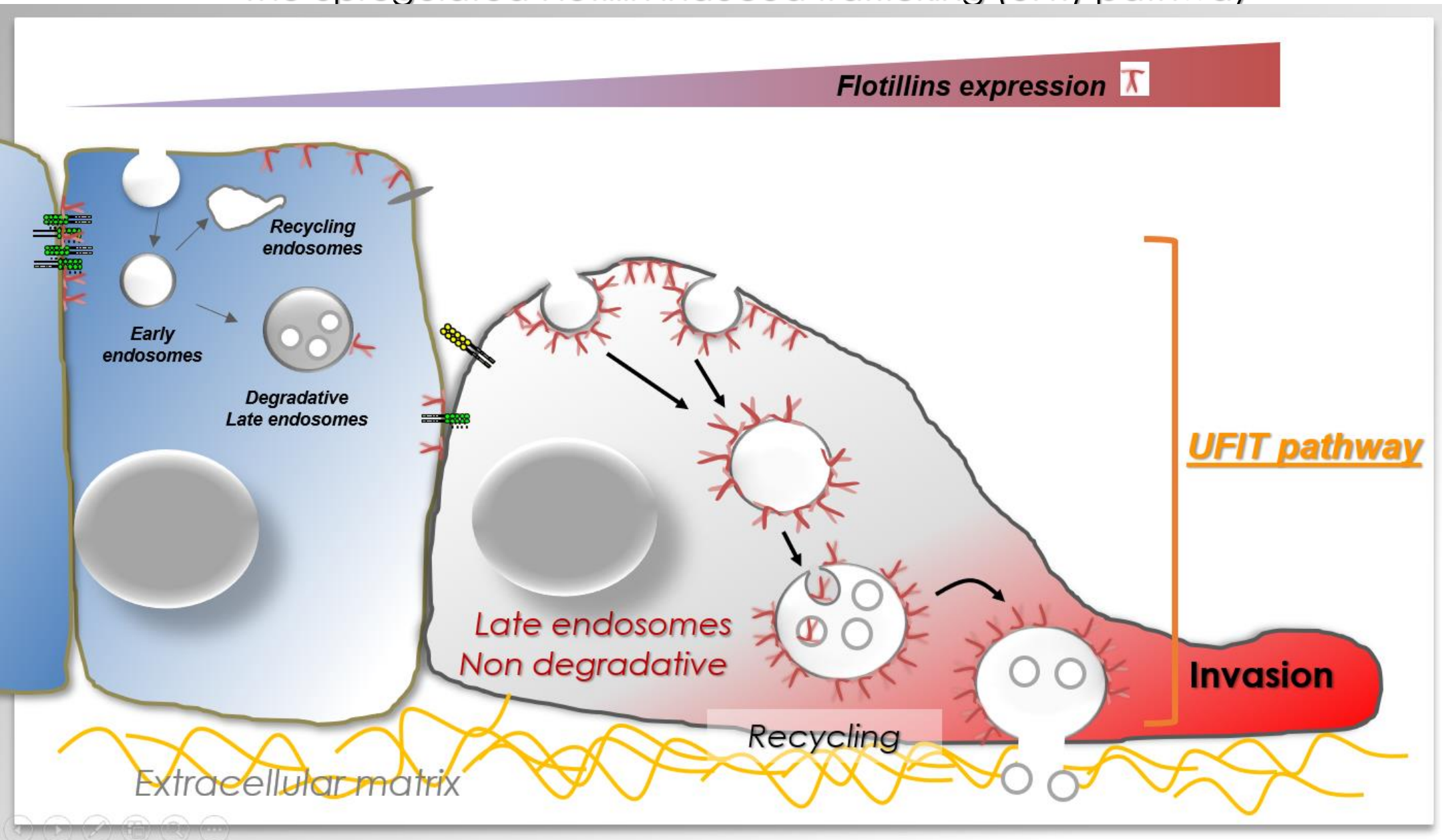
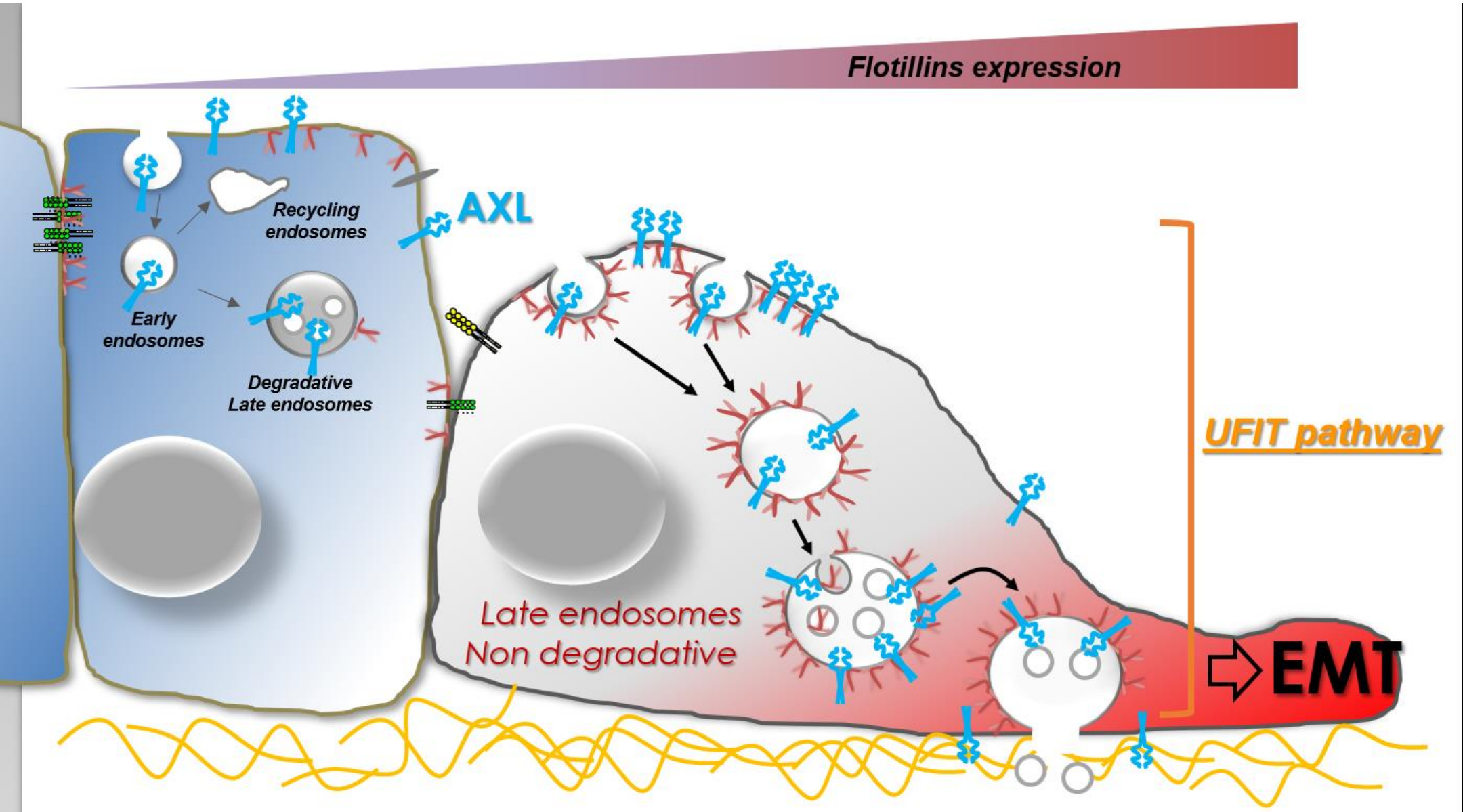
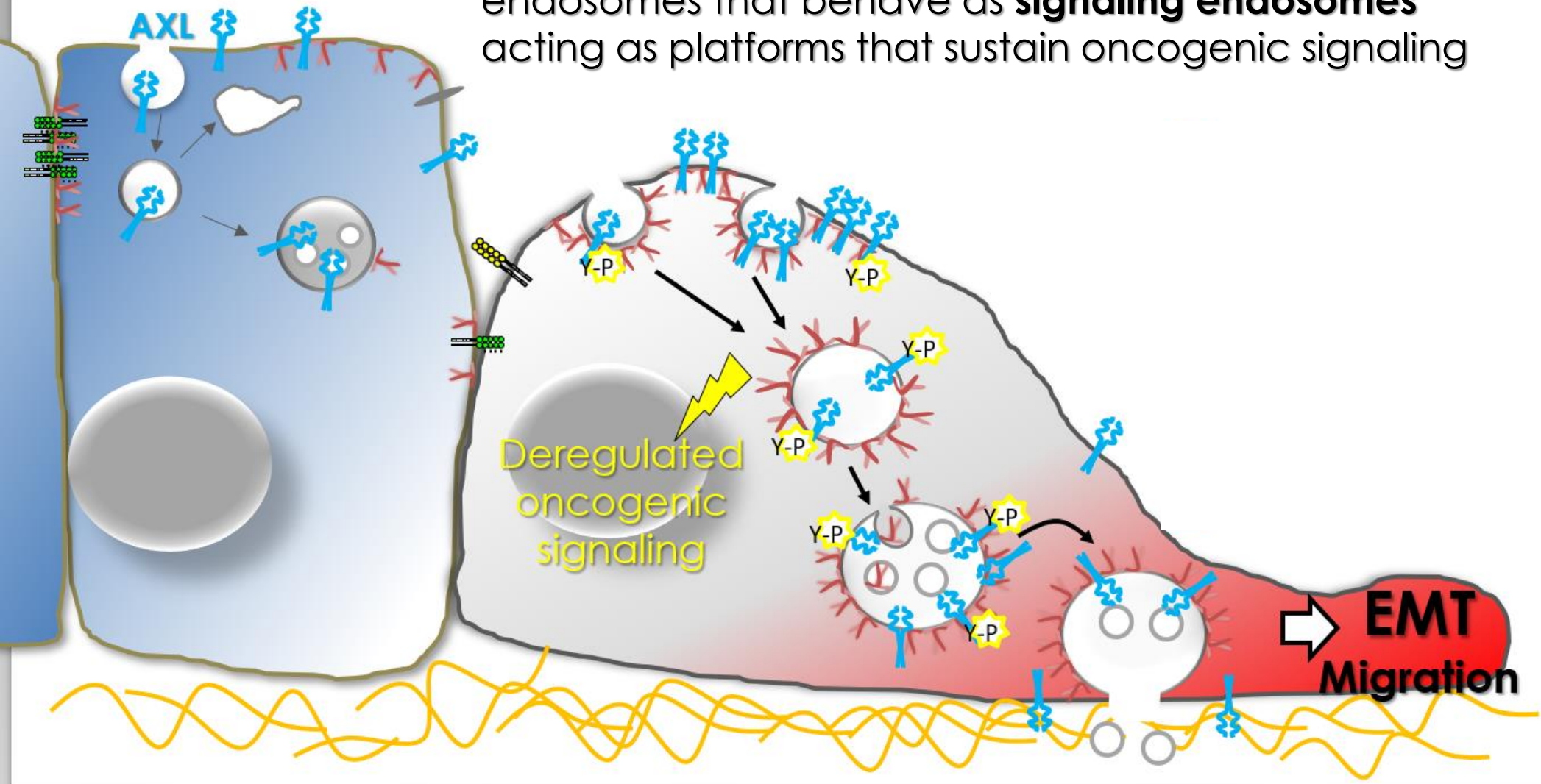


# AXL is diverted from its « normal » trafficking in cells overexpressing flotillins

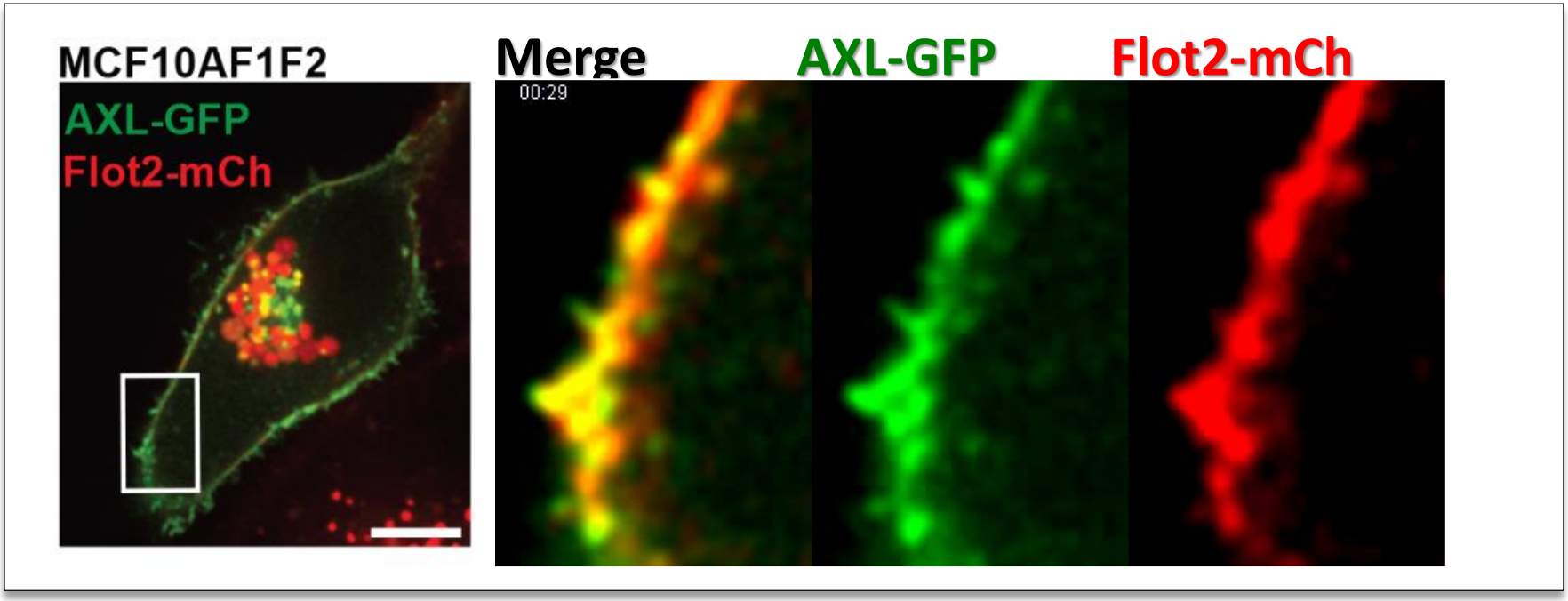


# AXL is diverted from its « normal » trafficking in cells overexpressing flotillins

AXL is endocytosed in non-degradative late endosomes that behave as **signaling endosomes** acting as platforms that sustain oncogenic signaling

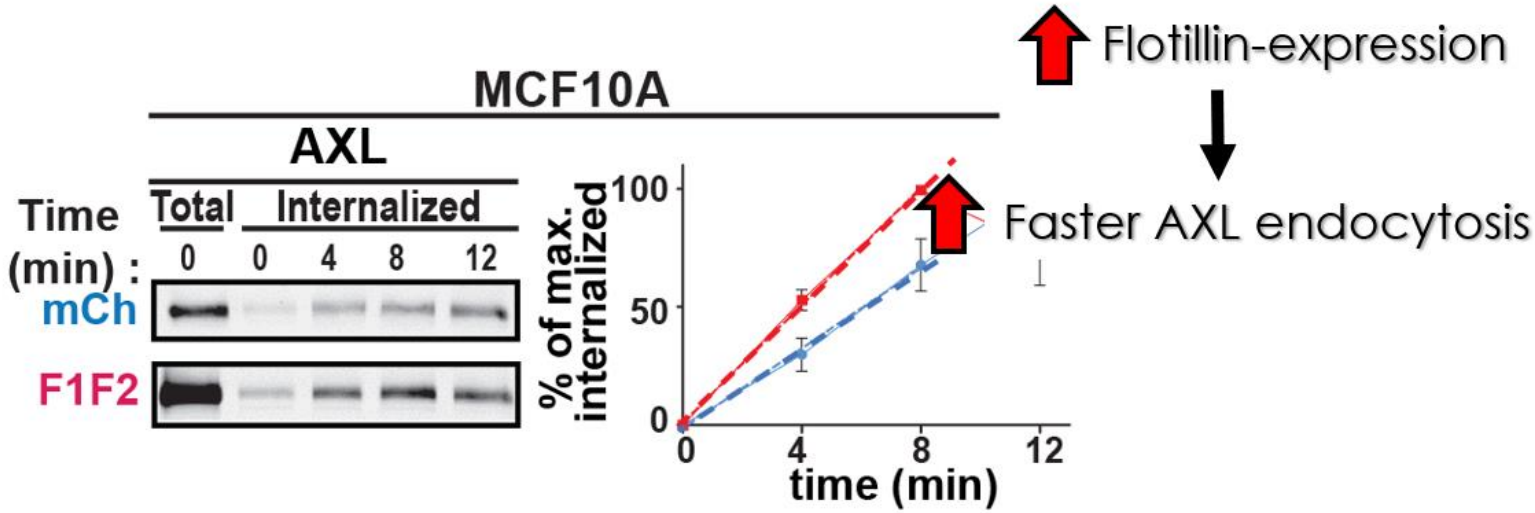


# AXL is co-endocytosed with flotillins



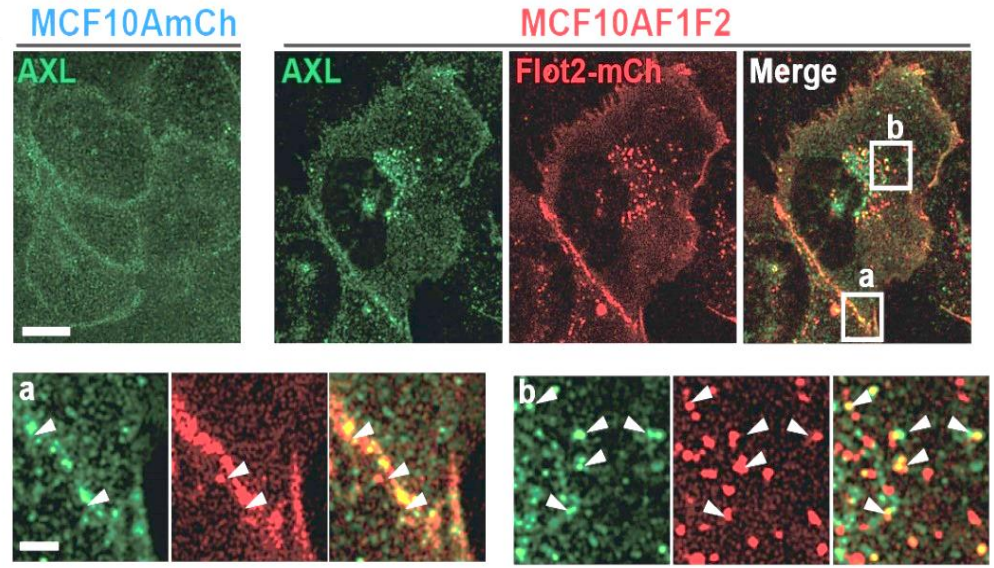
# Flotillin-upregulation accelerates AXL endocytosis

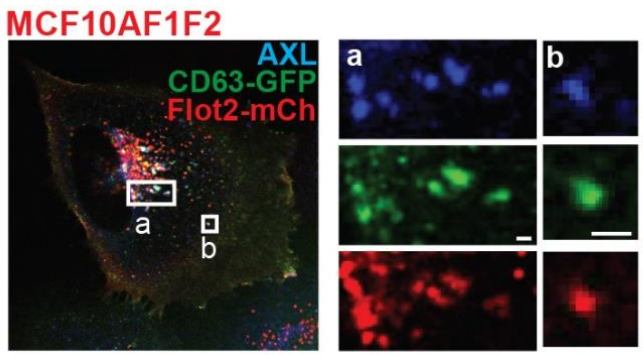
Comparative analysis of AXL internalization *(surface biotinylation assay)*

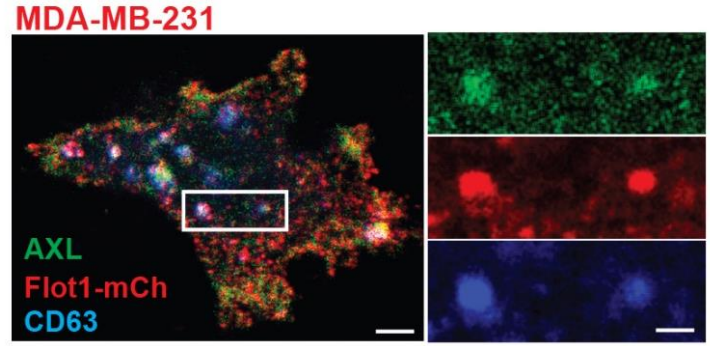



*Genest et al. BiorXiv, 2021*

# AXL is present in flotillin-positive late endosomes

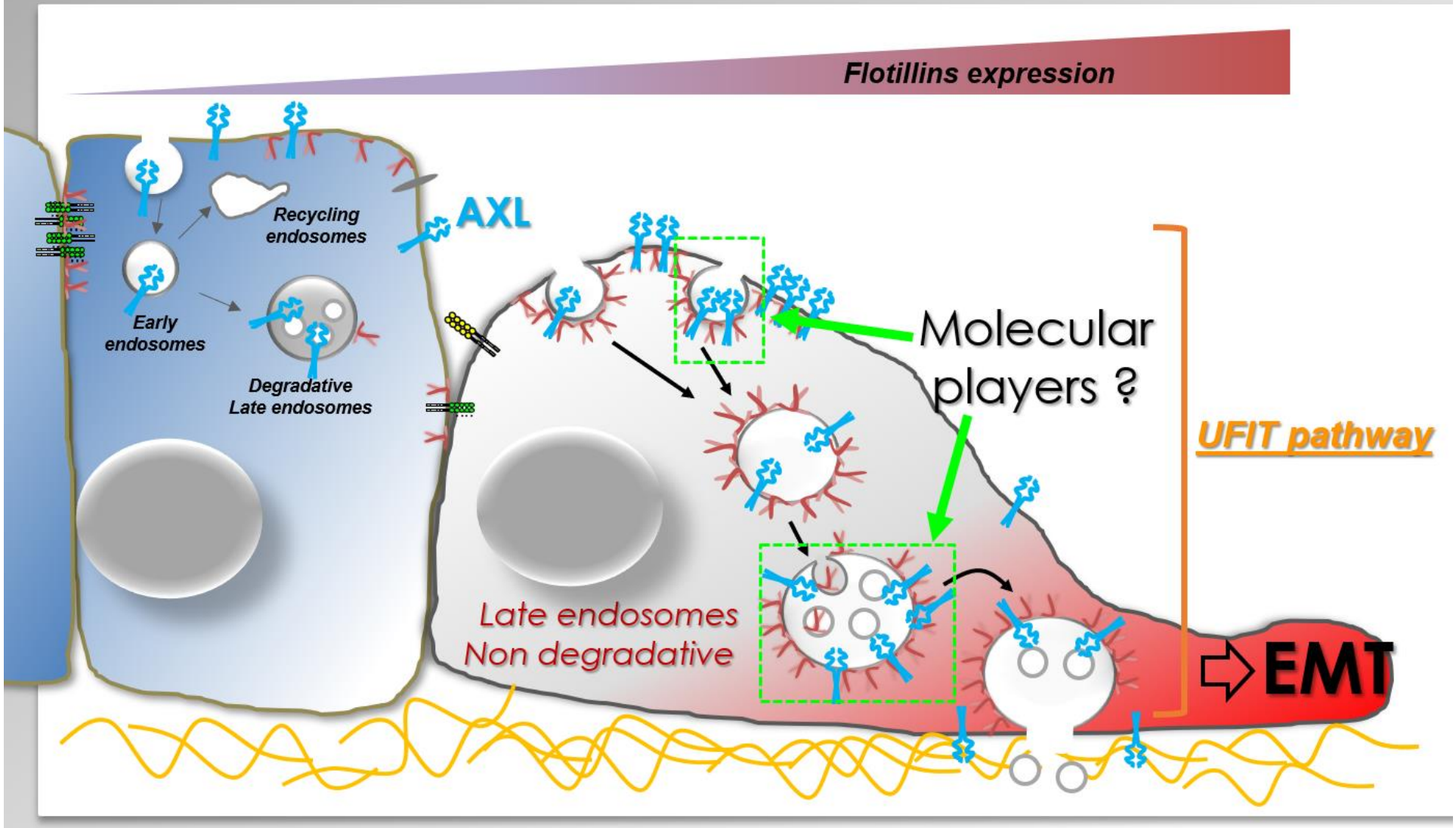



*Genest et al. BiorXiv, 2021*

# Molecular players of the UFIT pathway ?



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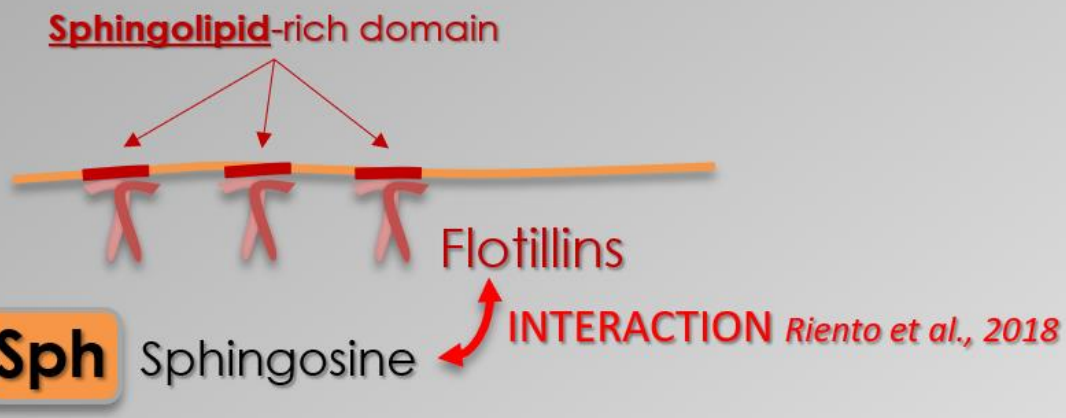

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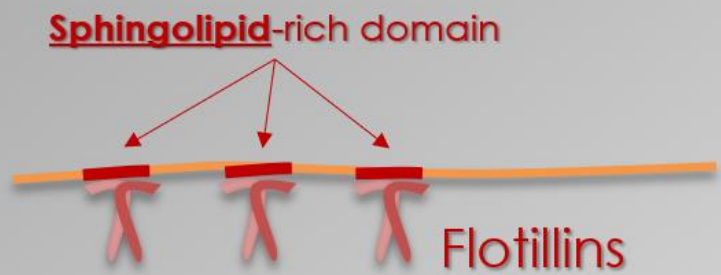


**Sph** Sphingosine ⟷ INTERACTION *Riento et al., 2018*

↓

*Sphingosine kinases (SphK 1 & 2)*

↓

**S1P** Sphingosine 1-phosphate

⇩

*Endocytic events*

*Shen et al. Nat Cell Biol. 2014, Lima et al. J Biol Chem 2017 (De Camilli and Spiegel groups)*

*Membrane remodeling of late endosomes*

*Kajimoto et al, Nat com 2013 ; J Biol Chem 2018 (Nakamura group)*

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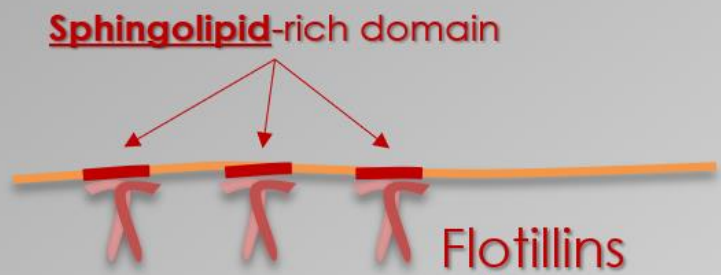


**Sph** Sphingosine ⟷ INTERACTION *Riento et al., 2018*

↓

*Sphingosine kinases (SphK 1 & 2)*

↓

**S1P** Sphingosine 1-phosphate ⇒ High level in invasive cancer cells

⇓

*Endocytic events*

*Shen et al. Nat Cell Biol. 2014, Lima et al. J Biol Chem 2017 (De Camilli and Spiegel groups)*

*Membrane remodeling of late endosomes*

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# Molecular players of the UFIT pathway ?

**HYPOTHESIS**

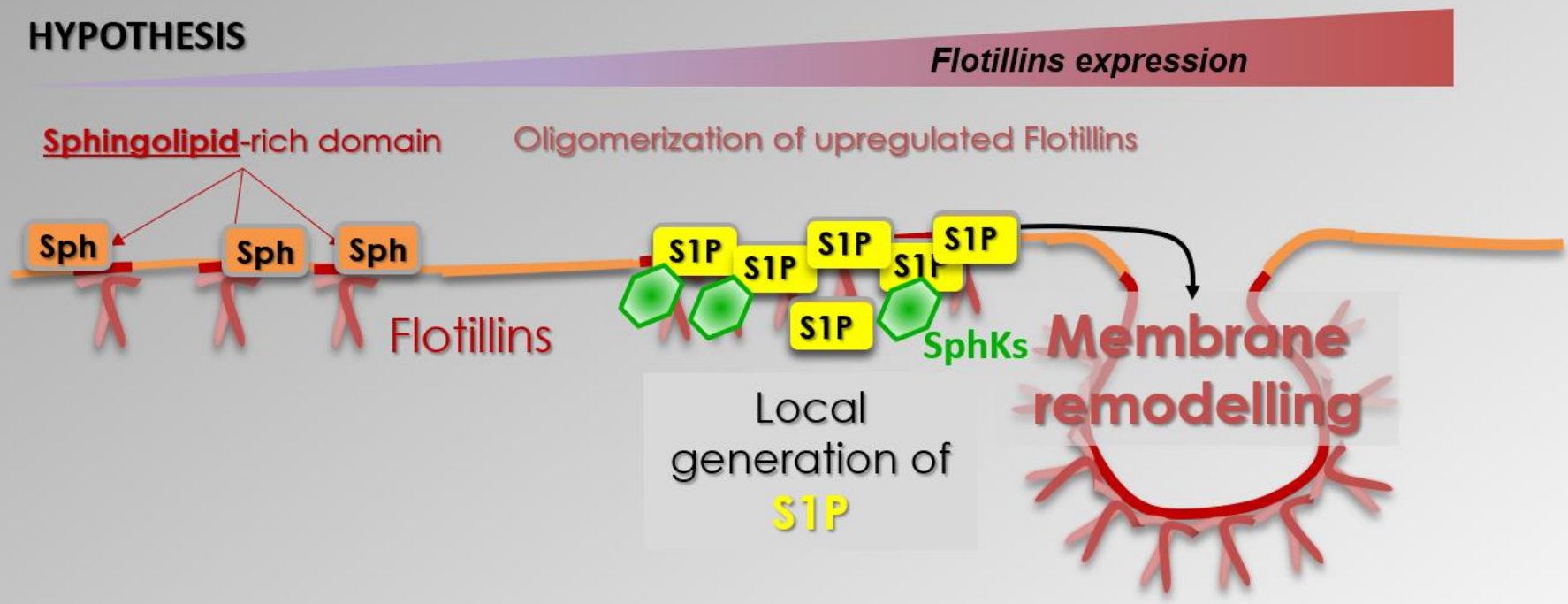
***Flotillins expression***

**Sphingolipid**-rich domain

Oligomerization of upregulated Flotillins

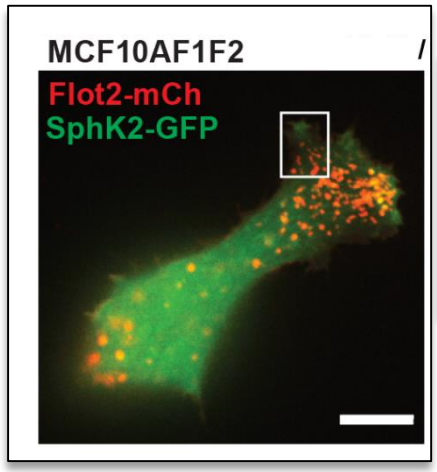

High local concentration of **sphingosine**

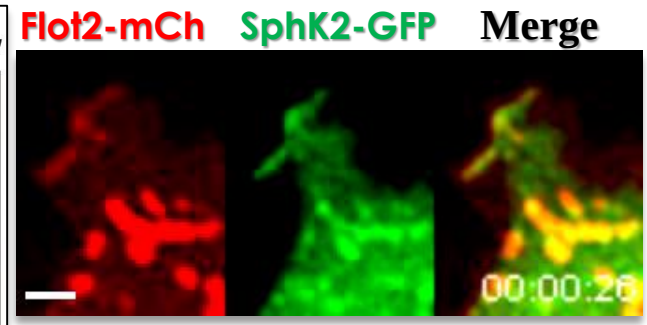
# Molecular players of the UFIT pathway ?

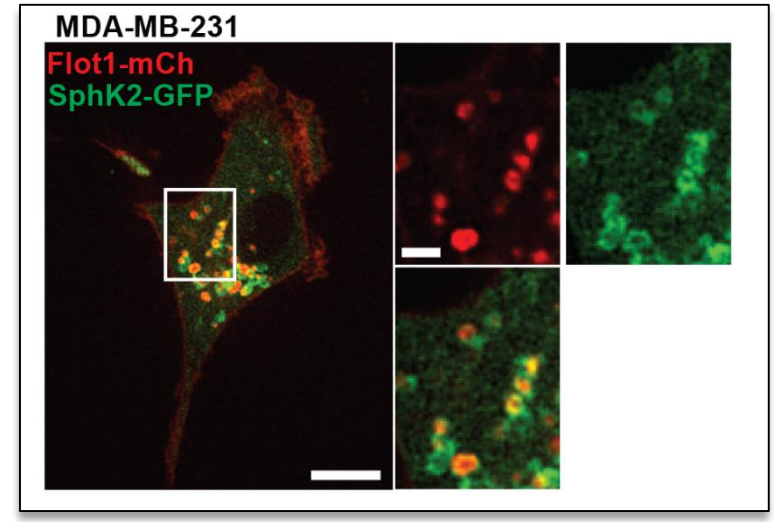


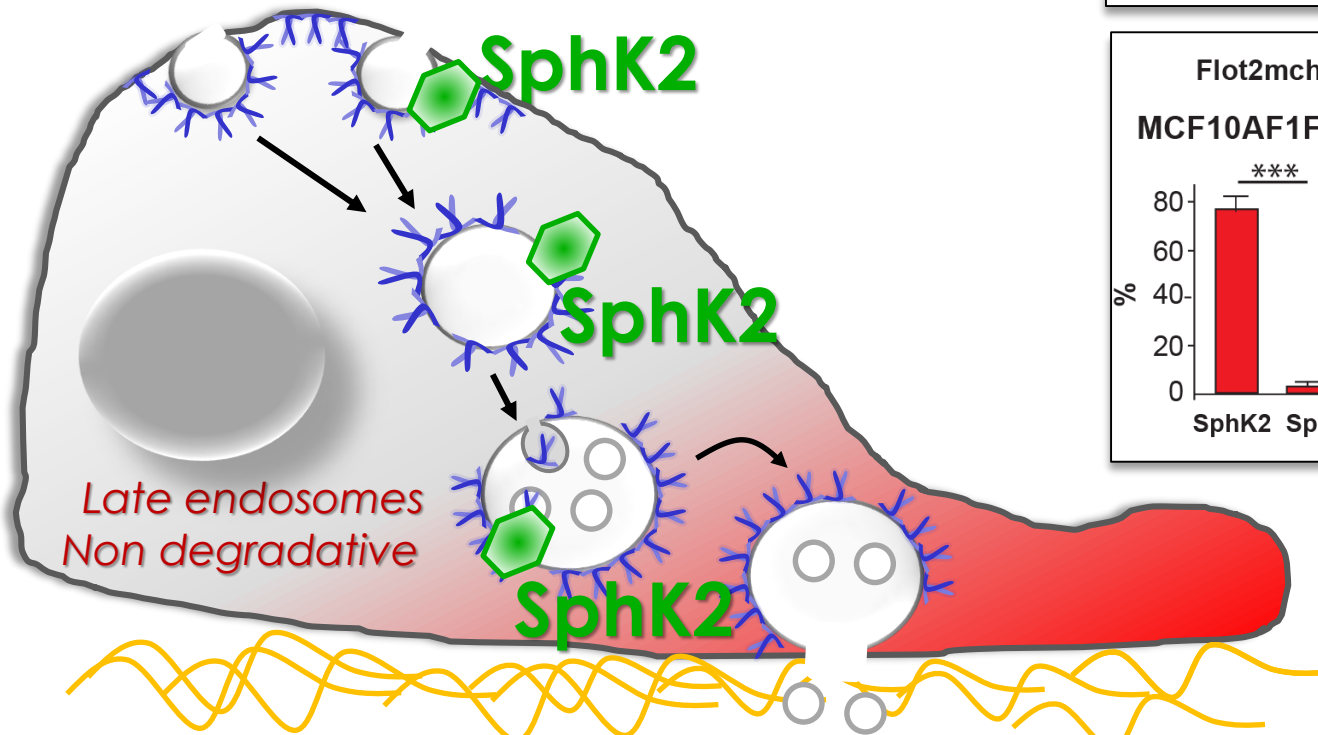

⇨ Do sphingosines Kinases play a key role in the UFIT pathway ?

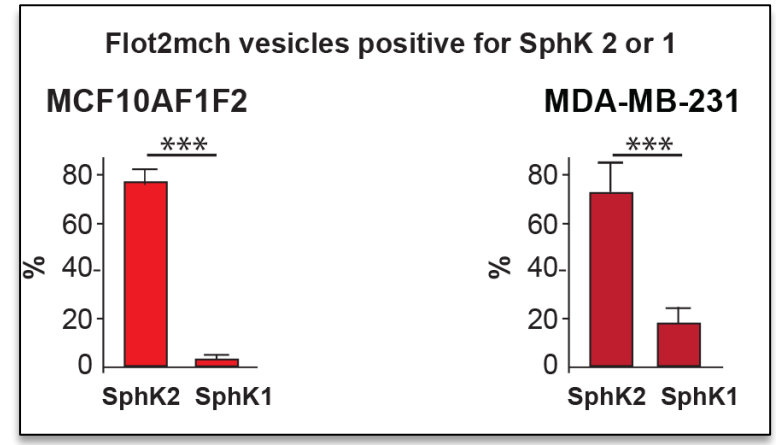
# Sphingosine Kinase 2 ( but not 1) localizes with flotillins at endocytic sites and in flotillin-positive endosomes



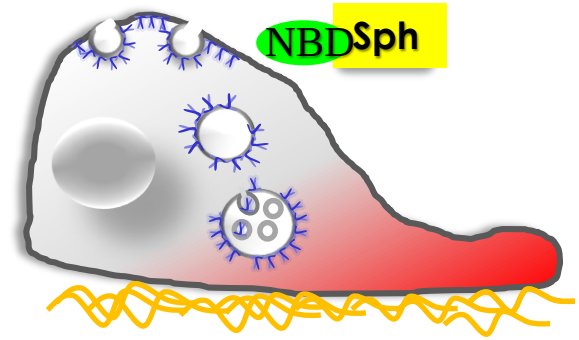




*Genest et al. BiorXiv, 2021*

# Flotillin-positive late endosomes accumulate exogenous sphingosine

NBD-**Sphingosine** directly added to culture media in cells expressing Flot1/2-mCherry




Dune Noly

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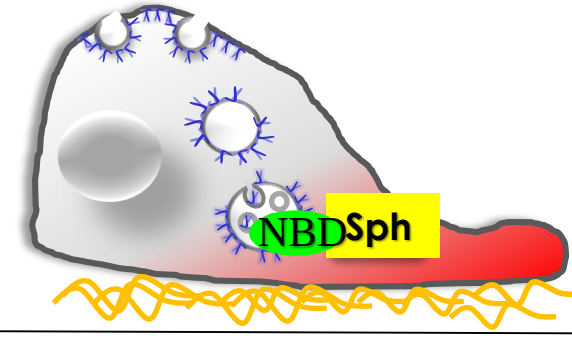
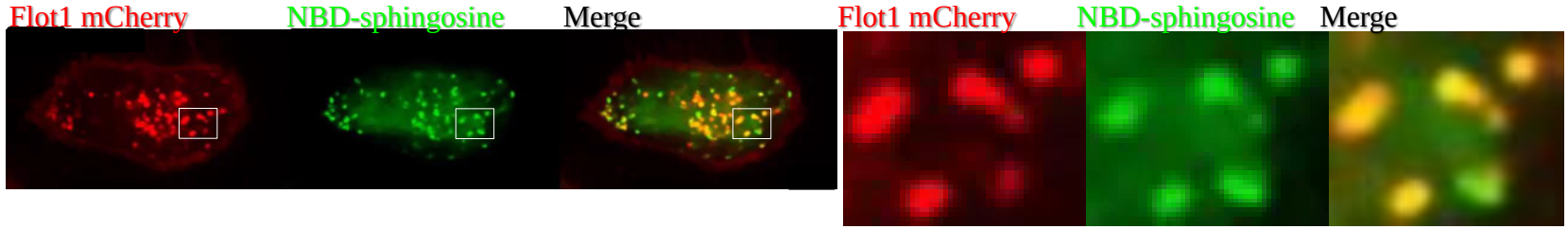
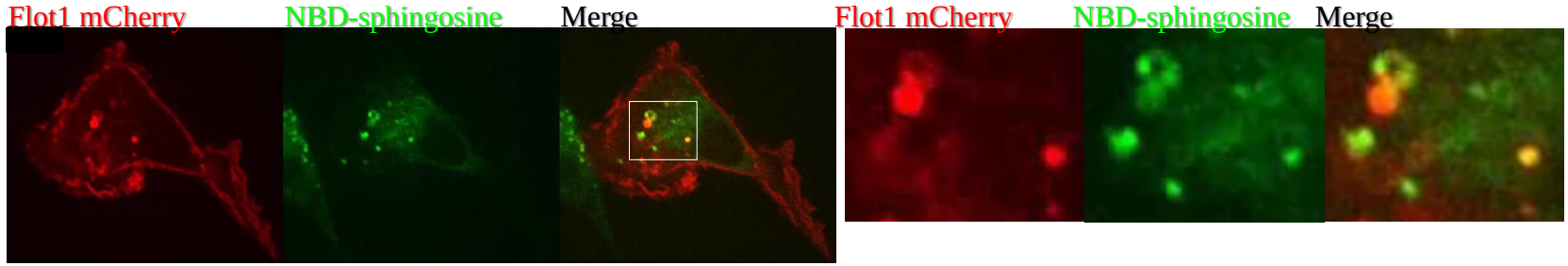



Dune Noly

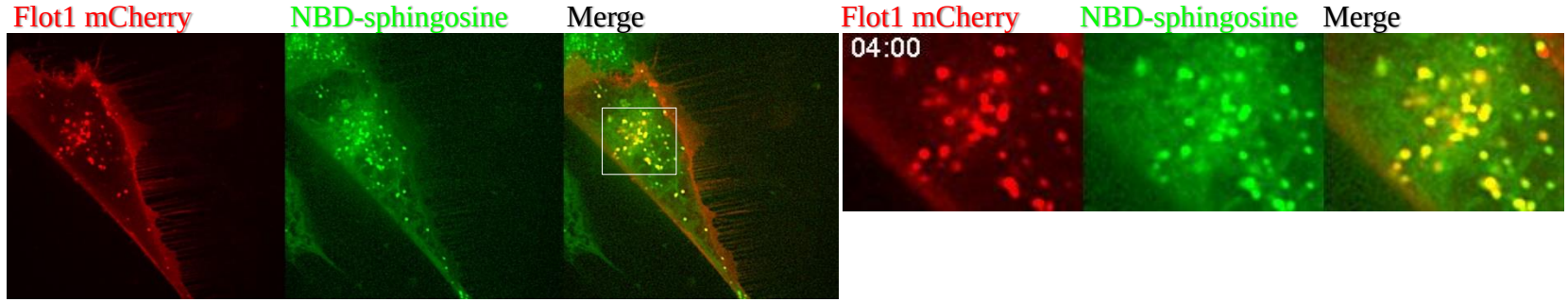
**MCF10AF1F2**




**MDA-MB-231**



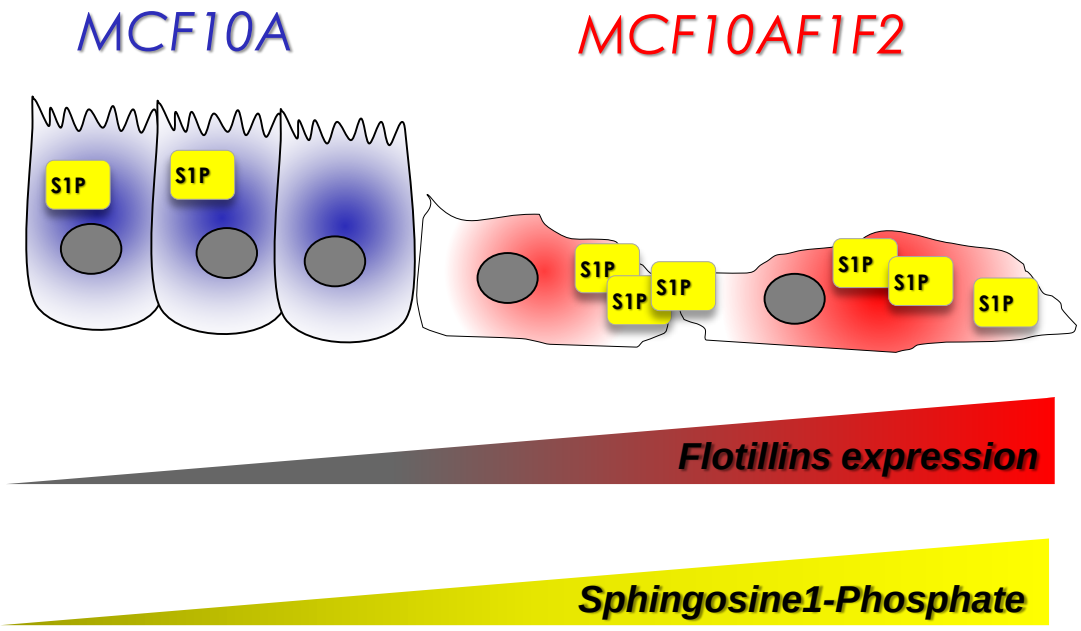

**HS-578T**



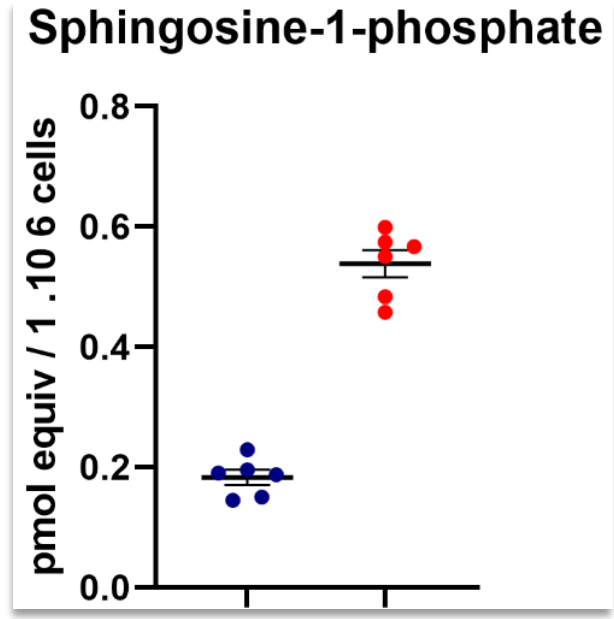

*(Not observed with NBD-PE)*

# Upregulation of Flotillins in MCF10A cells increases the level of Sphingosine 1-phosphate

Sphingosine (Sph) —Sph-kinases→ Sphingosine (S1P)



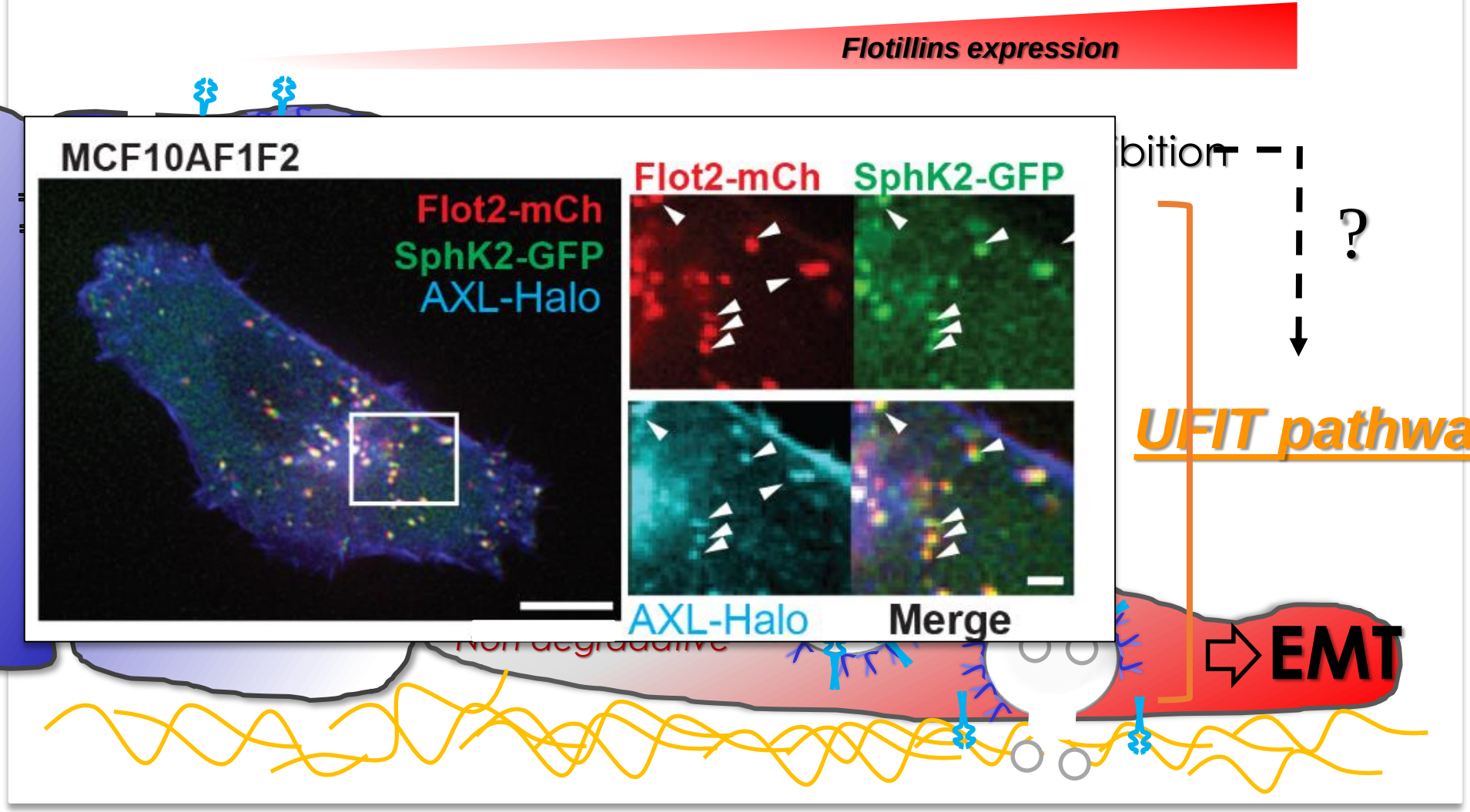

- Mass spectrometry analysis




*Collaboration with Josefina Casas iQAC / RUBAM, Barcelona*

# Is SphK2 a key player of the UFIT-pathway ?

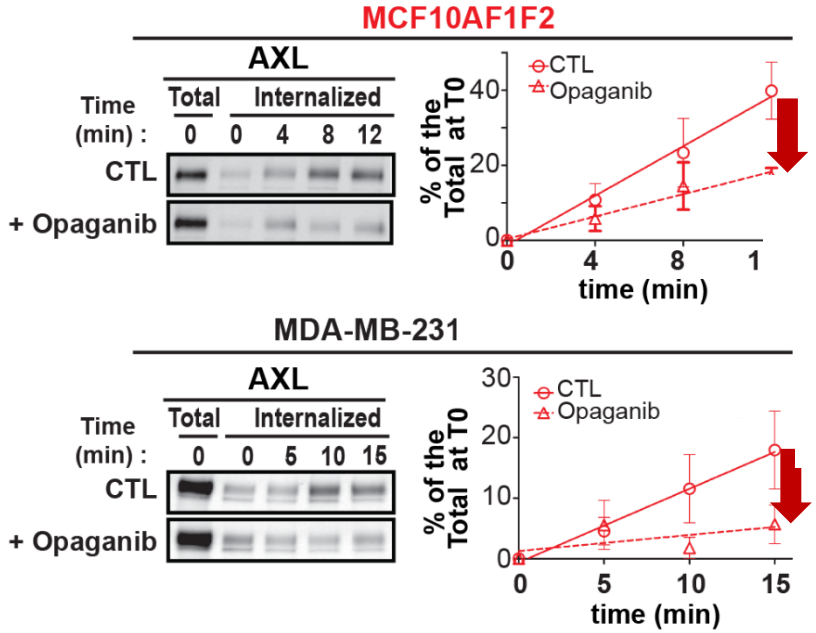
Is SphK2 required for the accelerated endocytosis and the stabilisation of AXL?



# SphK2 participates in AXL endocytosis in cells upregulated for flotillins

Opaganib : specific inhibitor of the catalytic activity of SphK2

- Analysis of AXL internalization



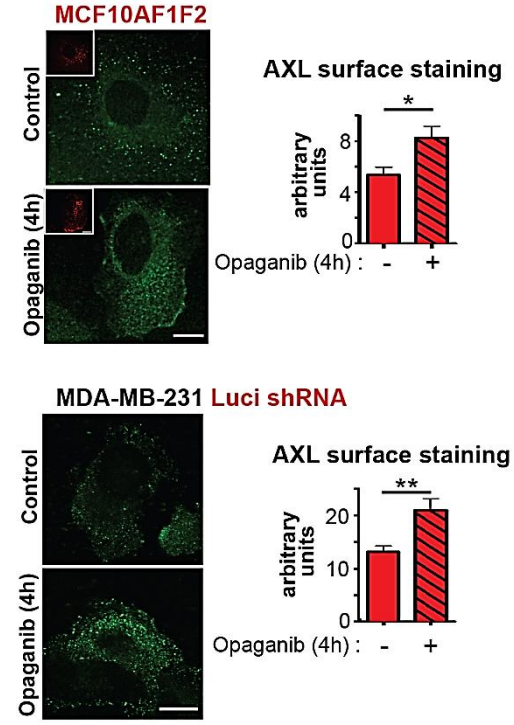

SphK2 inhibition

Slow-down AXL endocytosis

&

Increases AXL at the cell surface

- Analysis of AXL level at the cell surface

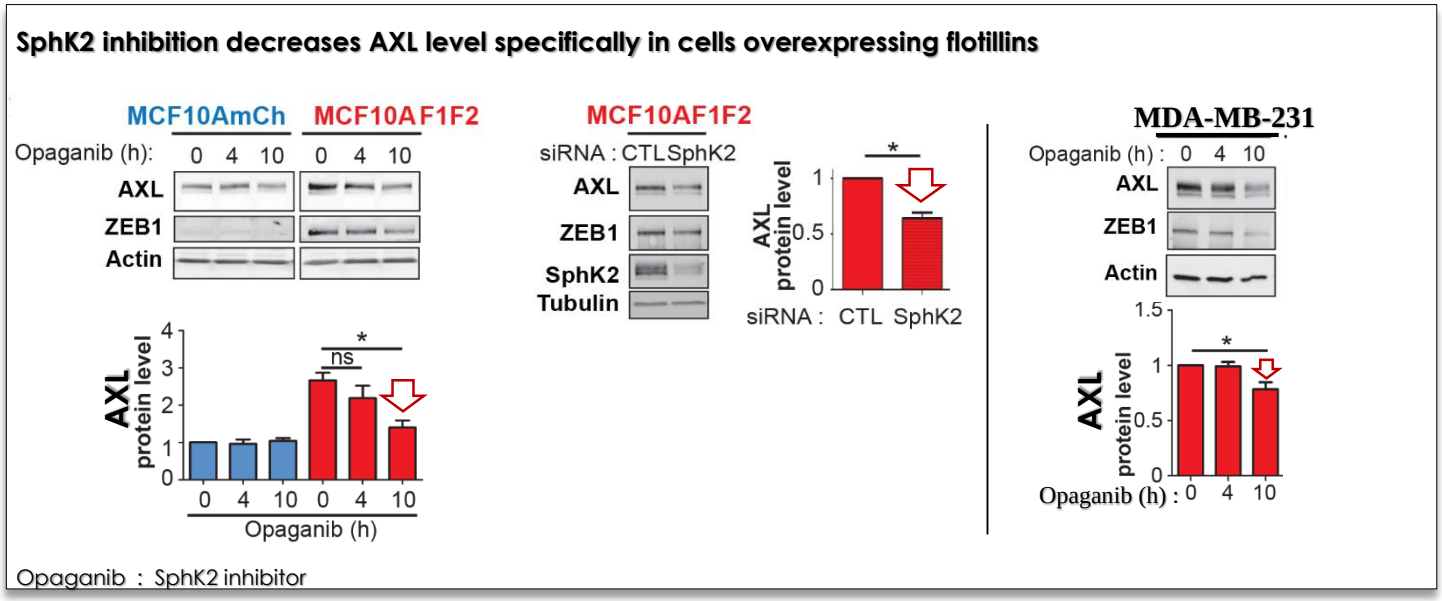


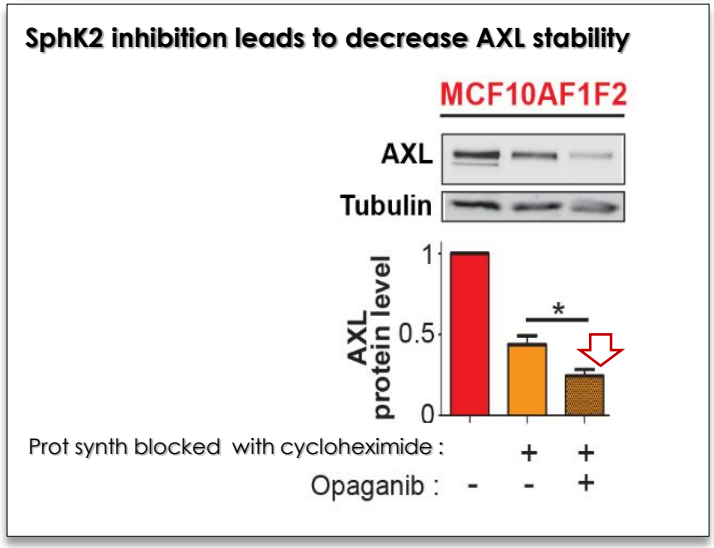

*(Control : no impact on CD71 endocytosis)*

Upregulated Flotillins ..... **SphK2** ..... **Fast AXL endocytosis**

*Genest et al. BiorXiv, 2021*

# SphK 2 is involved in AXL stabilization in cells upregulated for flotillins




Upregulated Flotillins

**SphK2**

**AXL stability and level**

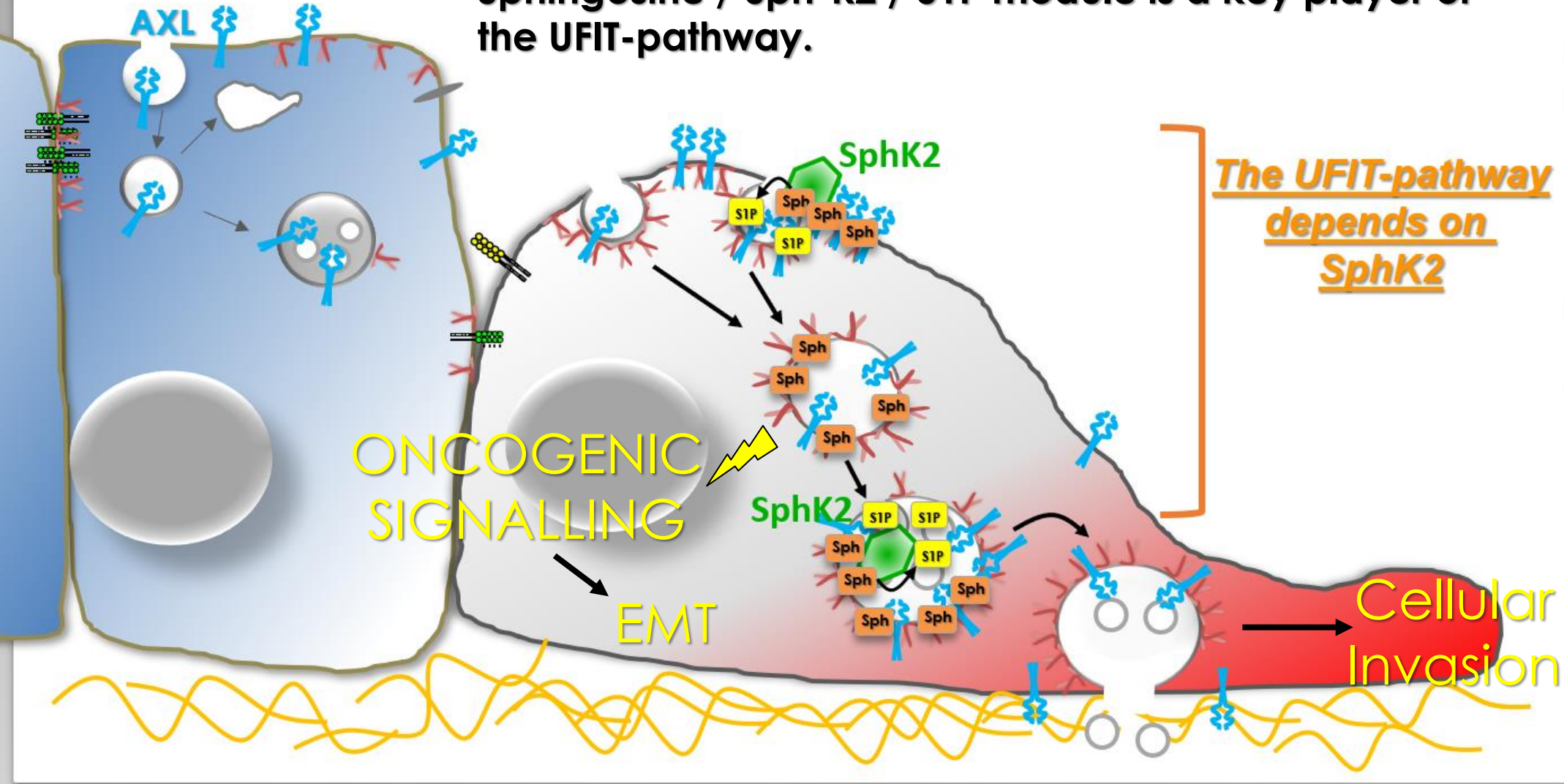
Cell migration

ZEB1

*Genest et al. BiorXiv, 2021*

# Model

**The Sphingolipid metabolism, in particular the Sphingosine / Sph-K2 / S1P module is a key player of the UFIT-pathway.**



# TAKE HOME MESSAGE

Overexpression of 2 proteins : Flotillins

↓

Deregulation of lipid metabolism (increase in S1P)

↓

Deregulation of vesicular trafficking

(endocytosis / Late endosomes function)

↓

Deregulation of cellular signalling

↓

Change in cell fate (EMT and invasion)

# Acknowledgements

**Gauthier / Blangy Team**

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*Alumni*
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*Jean Casanova*

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*Sylvain de Rossi*
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*Volker Baker*
*Chantal Cazevieille*

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*L Linares*

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*I Bièche*
*S Vacher*

**IAB, Grenoble**
*O Destaing*

**CRCT, Toulouse**
*N Andrieu-Dabadie*

**RUBAM, Barcelona**
*J Casas*

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*L Tashireva*
*E V Denisov*

**Yale school of Medecine, U.S.A**
*P de Camilli*

## Fundings

FONDATION ARC POUR LA RECHERCHE SUR LE CANCER
Reconnue d'utilité publique

FONDATION RECHERCHE MÉDICALE