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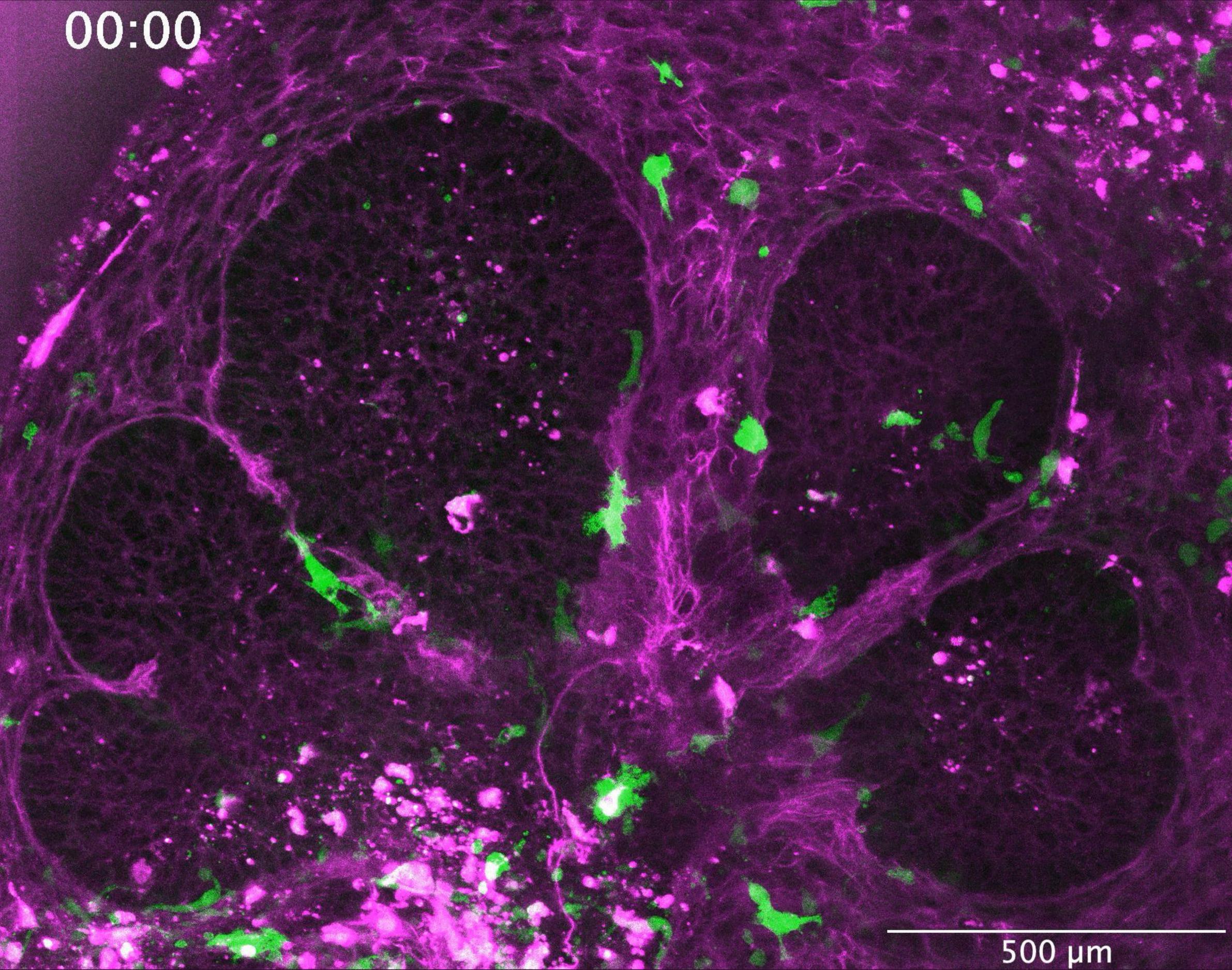
February 9  
GSK course 2026

# Immune cell migration & invasion

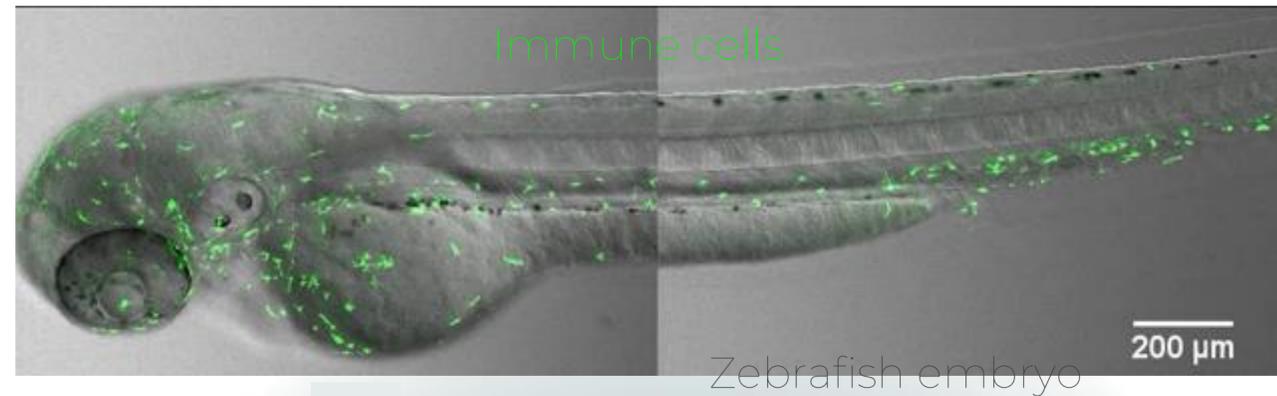
Masha Akhmanova  
Cell Biology Program  
SKI

Salivary gland explant  
from E13 mouse embryo  
Macrophages  
ECM, dead cells

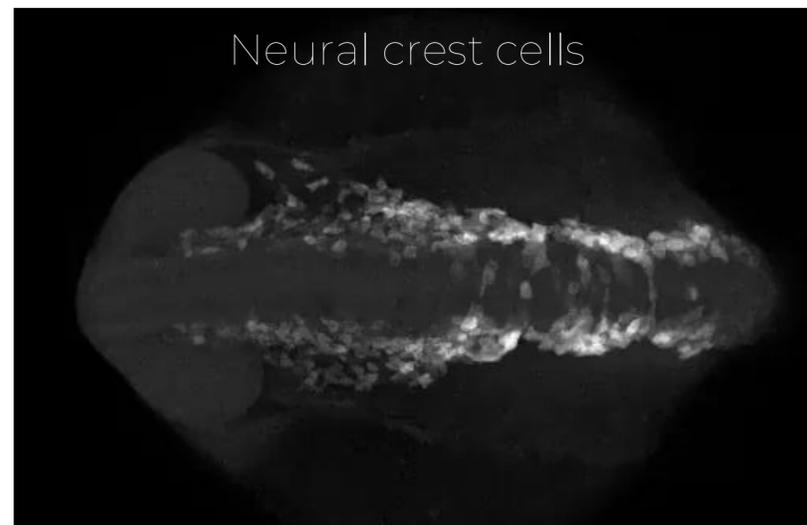
500  $\mu\text{m}$



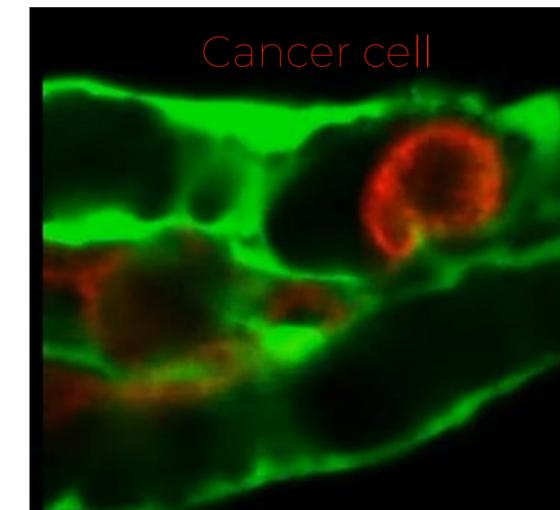
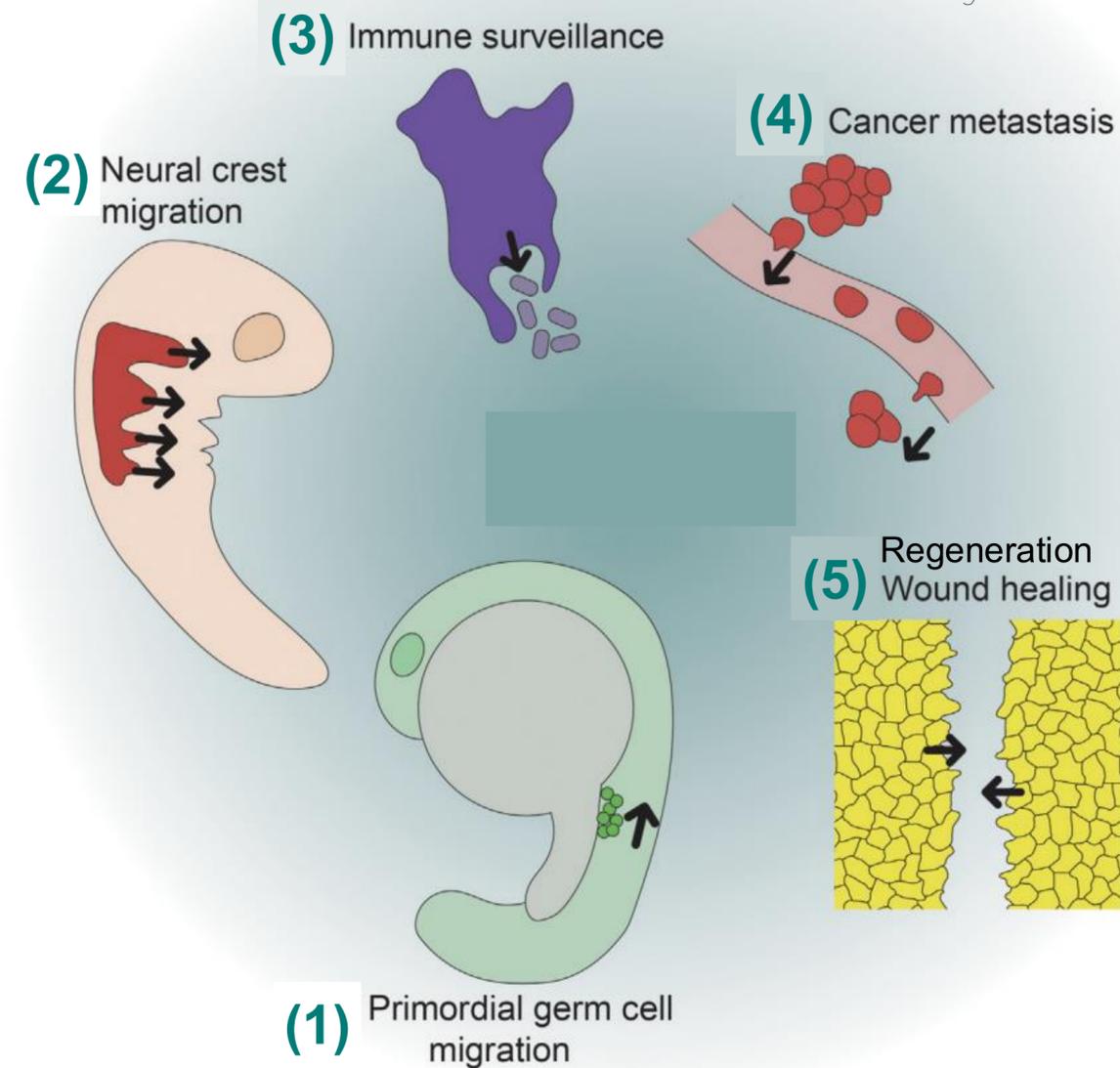
# Cell migration orchestrates key events in development, homeostasis, and disease



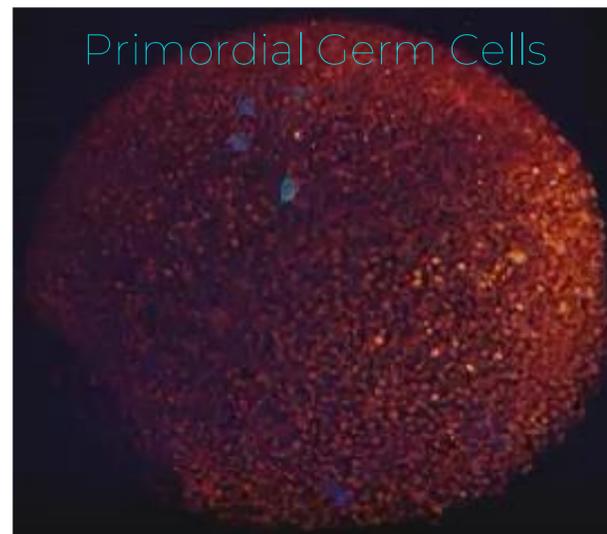
Torraca et al.,  
*Dis Model Mech*, 2014



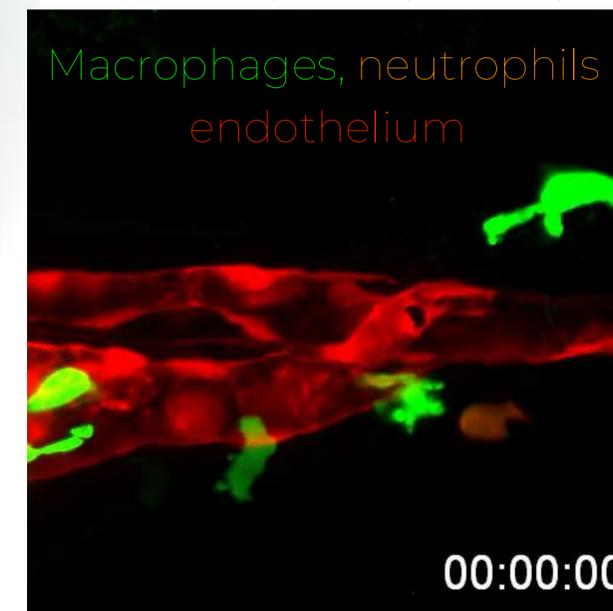
© Peter Fabian



Follain, G. et al., *Dev Cell*, 2018

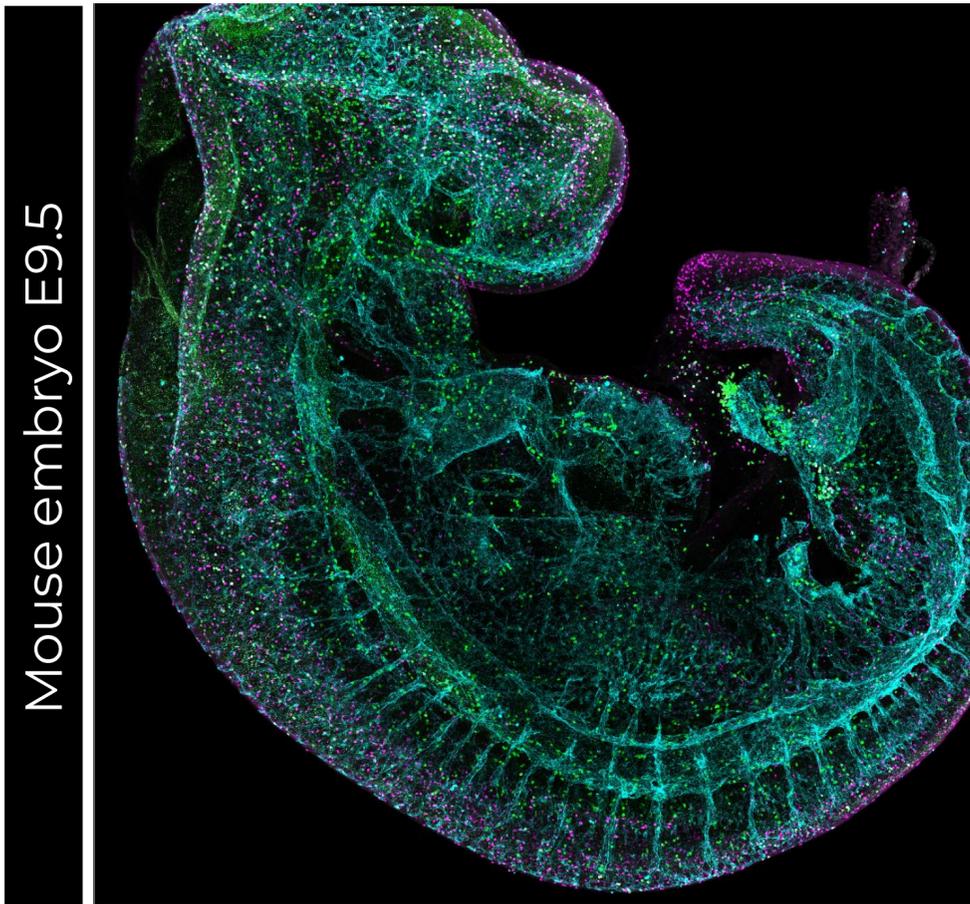


© Azadeh Paksa, Nadine Peyri ras, Erez Raz



(c) Zaza,  
Niethammer lab

# Cell migration orchestrates key events in *development*, homeostasis, and disease

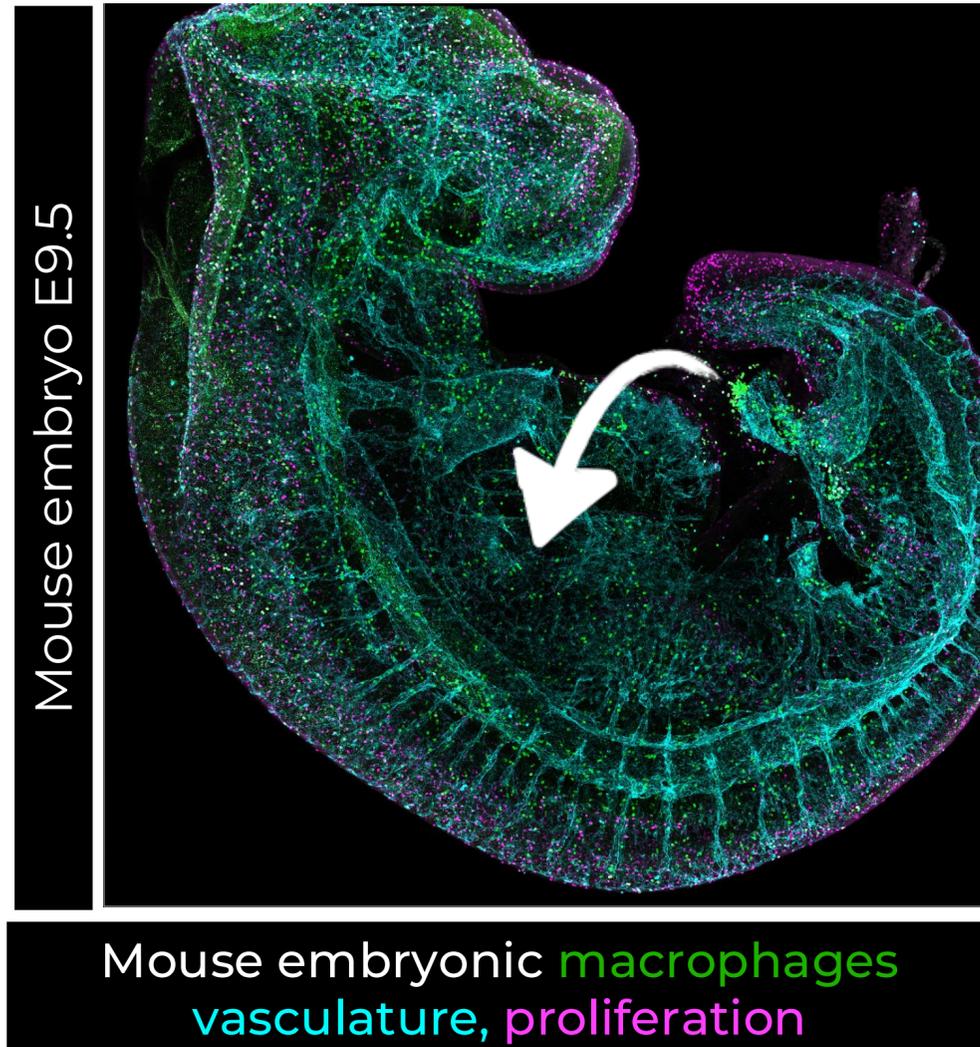


Mouse embryo E9.5

Mouse embryonic **macrophages**  
**vasculature, proliferation**

(c) Frederic Geissmann lab

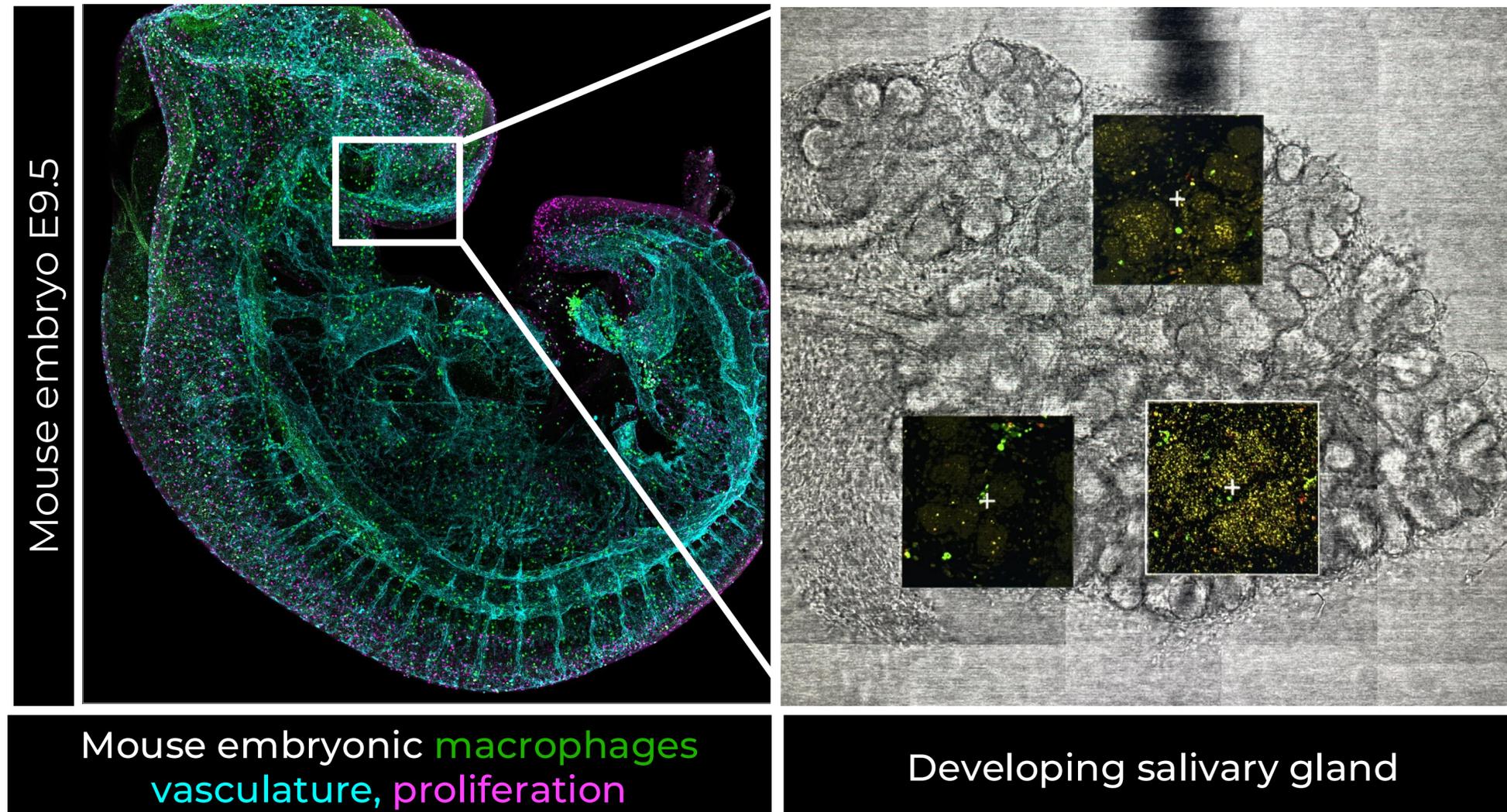
# Cell migration orchestrates key events in *development*, homeostasis, and disease



(c) Frederic Geissmann lab

# Cell migration orchestrates key events in *development*, homeostasis, and disease

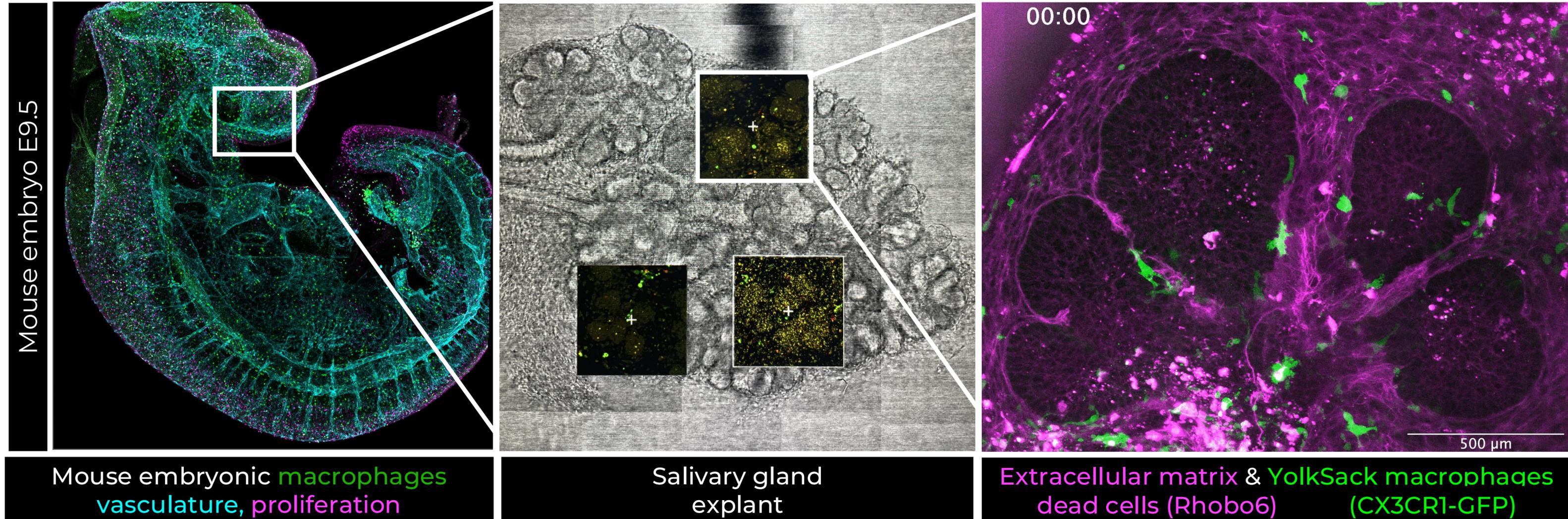
live imaging @ 2P microscope:



(c) Frederic Geissmann lab

# Cell migration orchestrates key events in *development*, homeostasis, and disease

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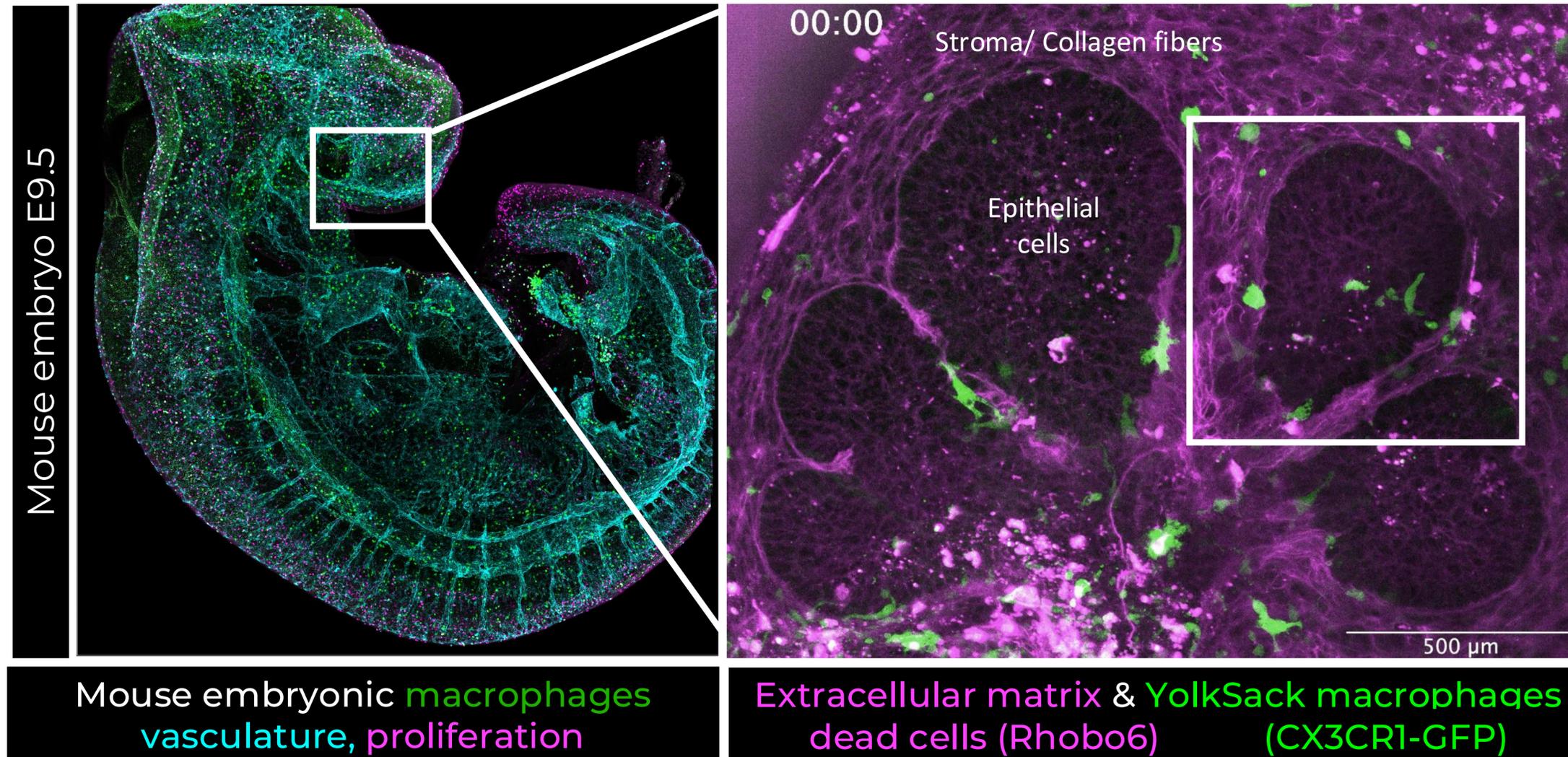


(c) Frederic Geissmann lab

with Ali May  Icahn School of Medicine at  
Mount Sinai

# Cell migration orchestrates key events in *development*, homeostasis, and disease

live imaging @ 2P microscope:

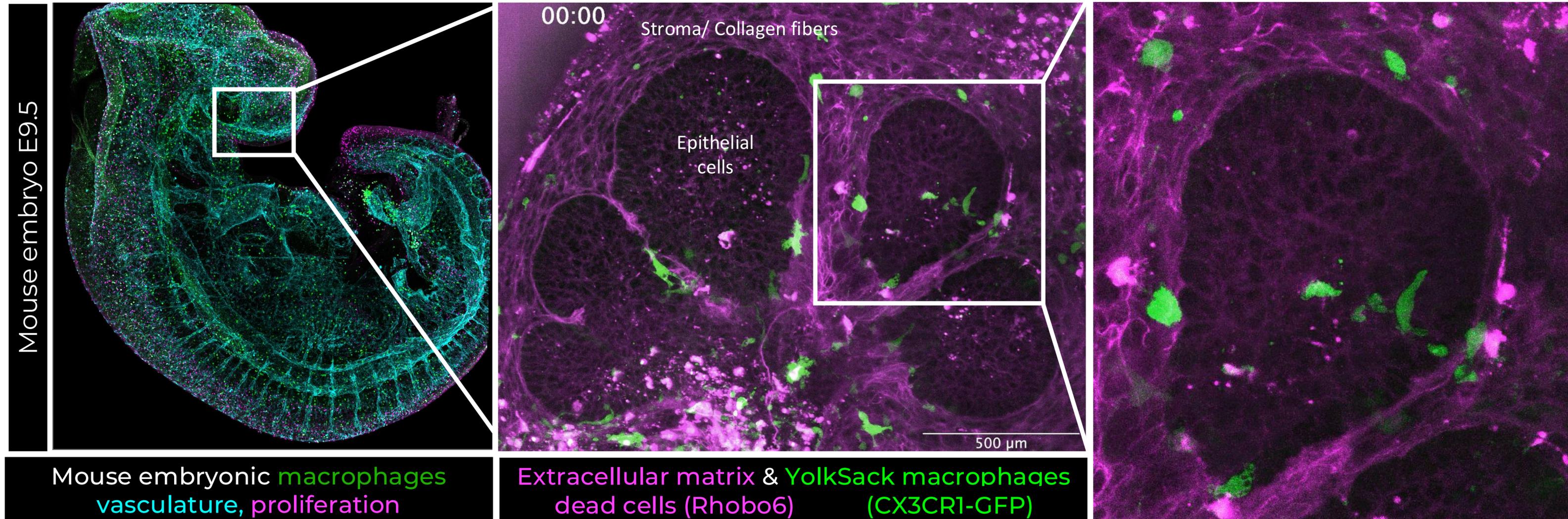


(c) Frederic Geissmann lab

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# Cell migration orchestrates key events in *development*, homeostasis, and disease

live imaging @ 2P microscope:



(c) Frederic Geissmann lab

with Ali May  Icahn School of Medicine at Mount Sinai

Outline:

I. **Overview of** *directed cell migration*

What do cells need to be able to move to their target?

II. *Invasion* **as a special type of directed migration**

How do cells overcome physical barriers on their way?

# Discovery of cell motility



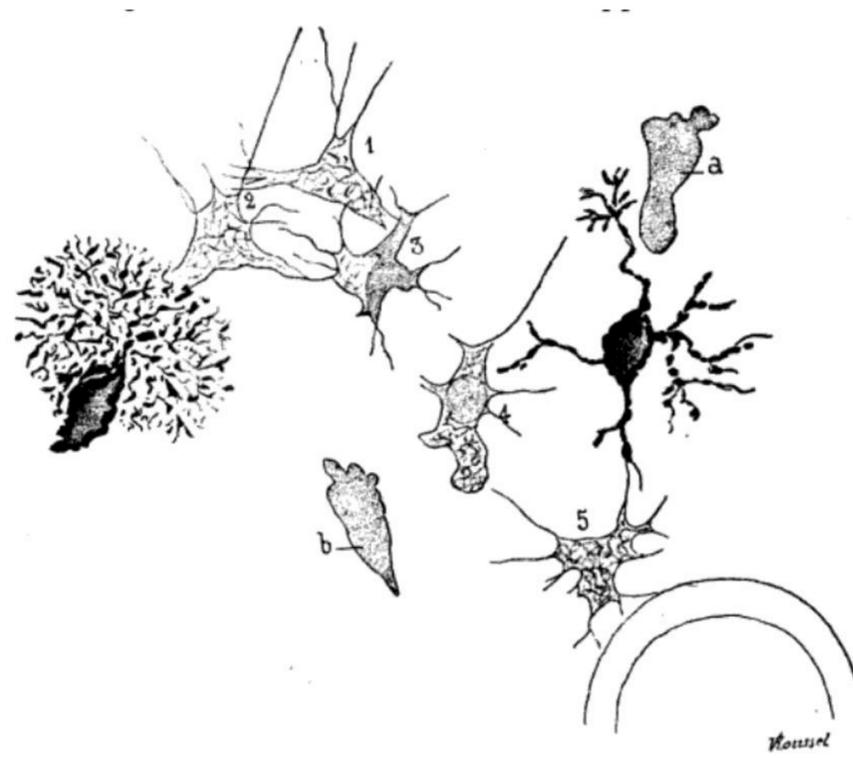
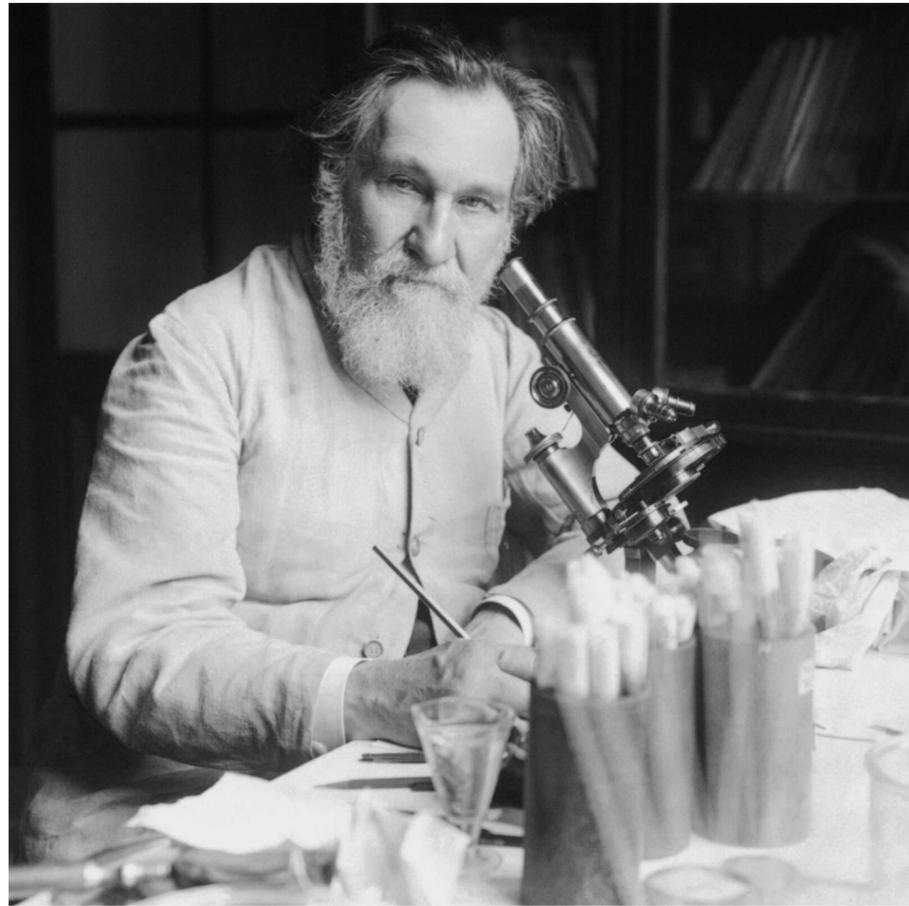
**1882** - Elie Metchnikoff observed motile cells in starfish larva, put rose torn under larval skin and observed chemotaxis and phagocytosis.

Nobel prize in 1908 together with Paul Ehrlich

"in recognition of their work on immunity"

Metchnikoff E. *Lectures on the Comparative Pathology of Inflammation* (1892)

# Discovery of cell motility



15 after stimulation

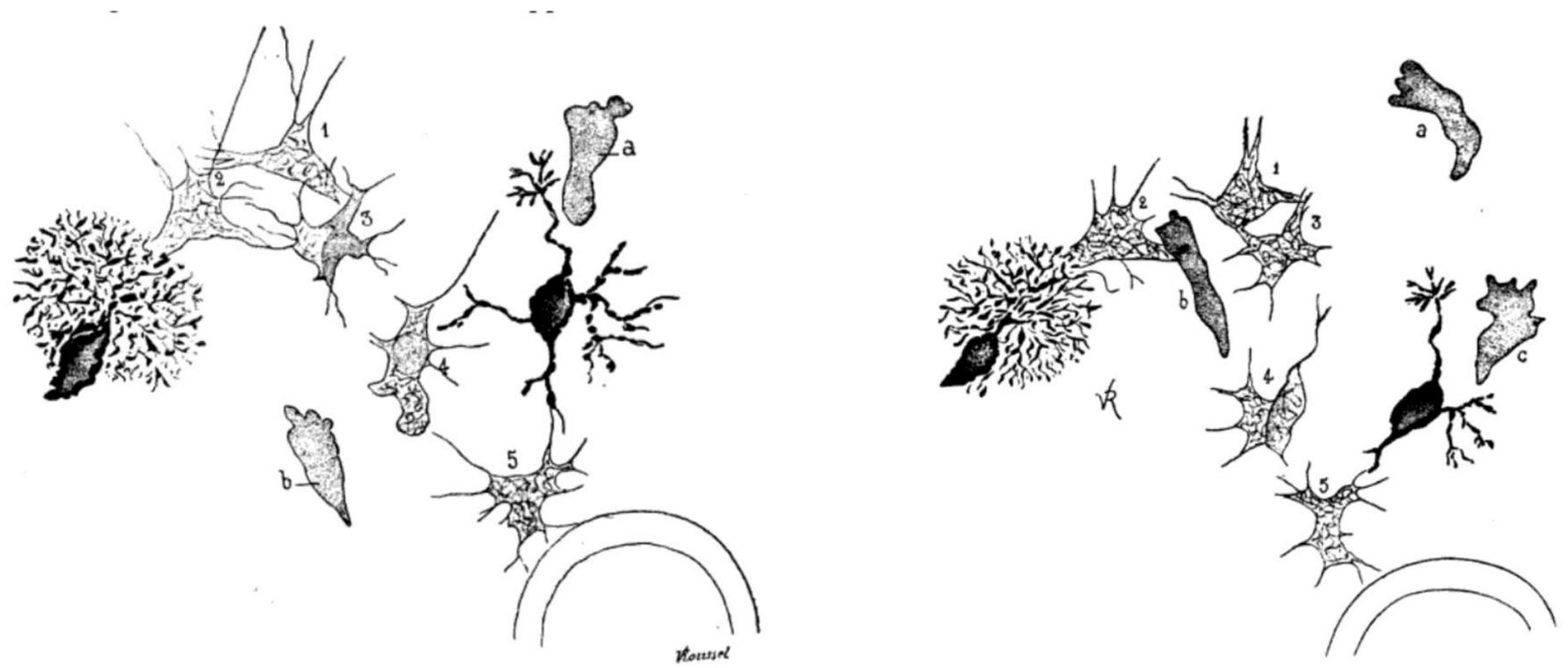
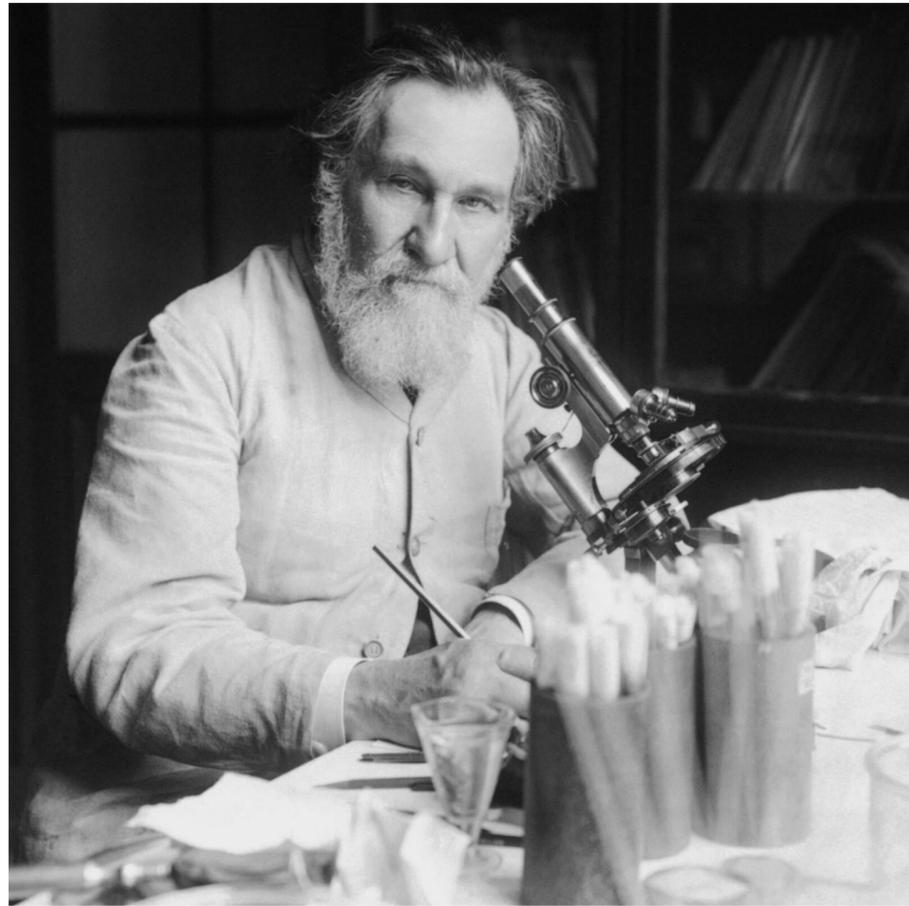
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Metchnikoff E. *Lectures on the Comparative Pathology of Inflammation* (1892)

# Discovery of cell motility



15 after stimulation

30 min later

**1882** - Elie Metchnikoff observed motile cells in starfish larva, put rose thorn under larval skin and observed chemotaxis and phagocytosis.

Nobel prize in 1908 together with Paul Ehrlich

"in recognition of their work on immunity"

*Metchnikoff E. Lectures on the Comparative Pathology of Inflammation (1892)*

# Observing cell motility in 2D...

Neutrophil in a blood drop  
chases and eats a  
*Staphylococcus aureus* bacterium



David Rogers, Vanderbilt University  
in the **1950s**

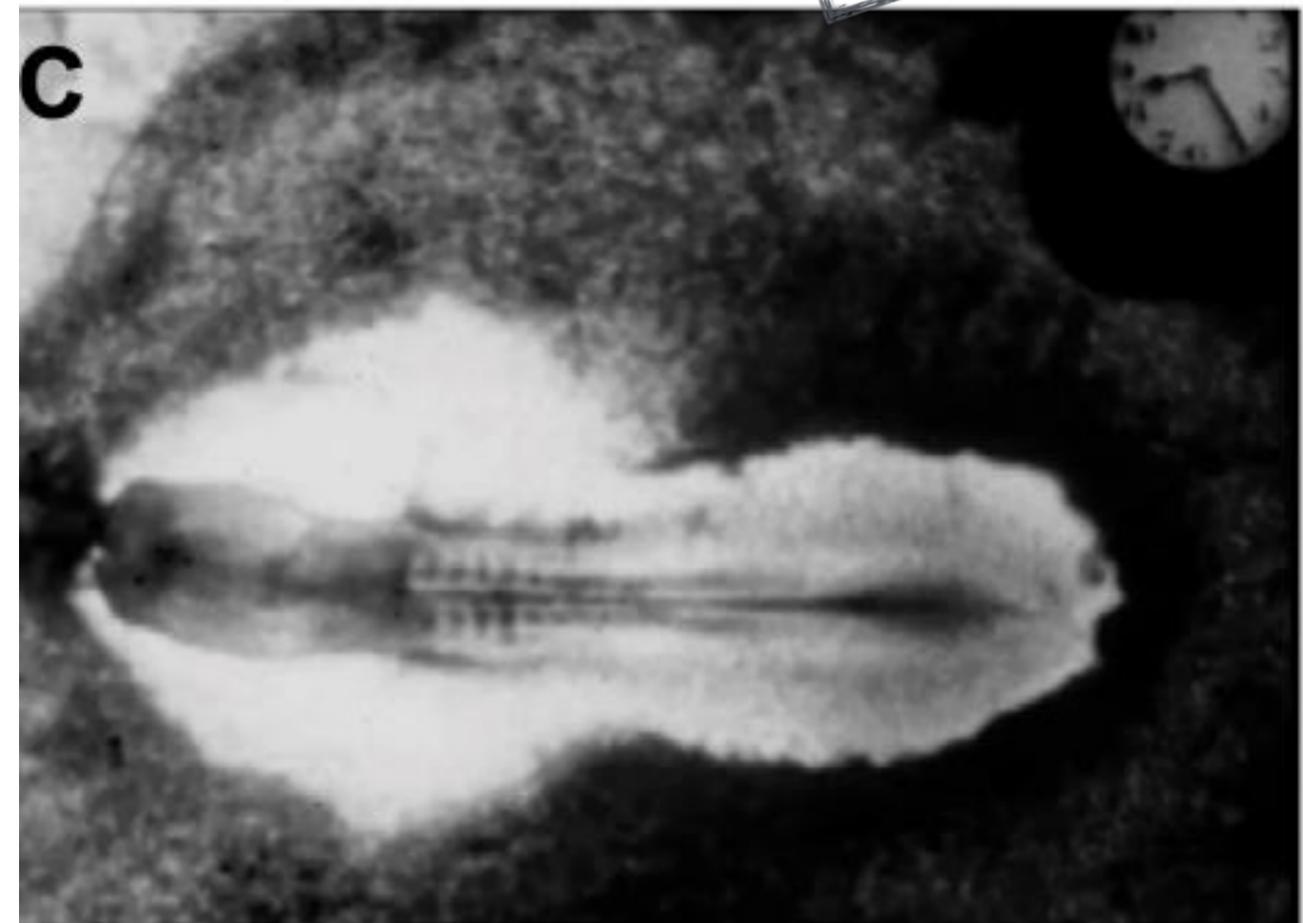
# Observing cell motility in 2D...

Neutrophil in a blood drop  
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*Staphylococcus aureus* bacterium



David Rogers, Vanderbilt University  
in the **1950s**

With time  
stamp!



Cinemicroscopy ~ **1930s**

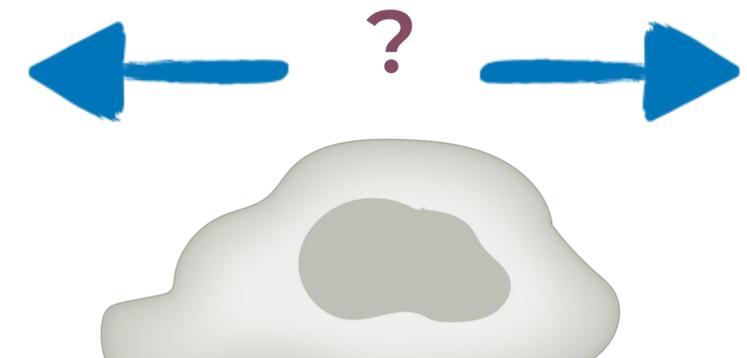
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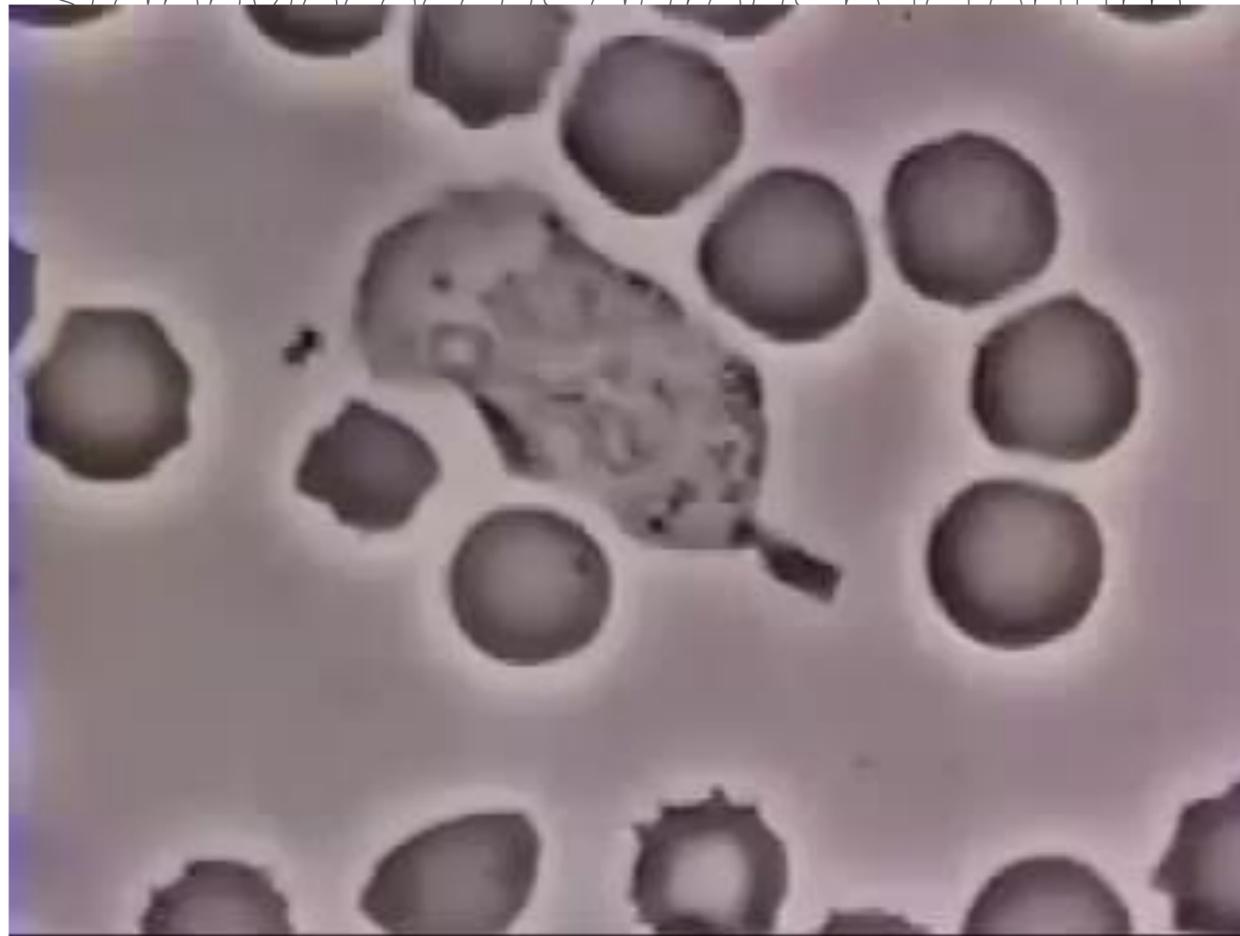
David Rogers, Vanderbilt University  
in the **1950s**

**How does a cell move  
towards it's target?**



# Observing cell motility in 2D...

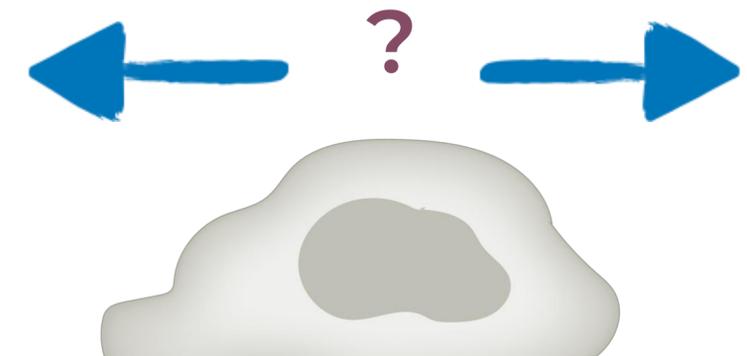
Neutrophil in a blood drop  
chases and eats a  
*Staphylococcus aureus* bacterium



David Rogers, Vanderbilt University  
in the **1950s**

**How does a cell move  
towards it's target?**

**What does a cell *need*  
to move?**



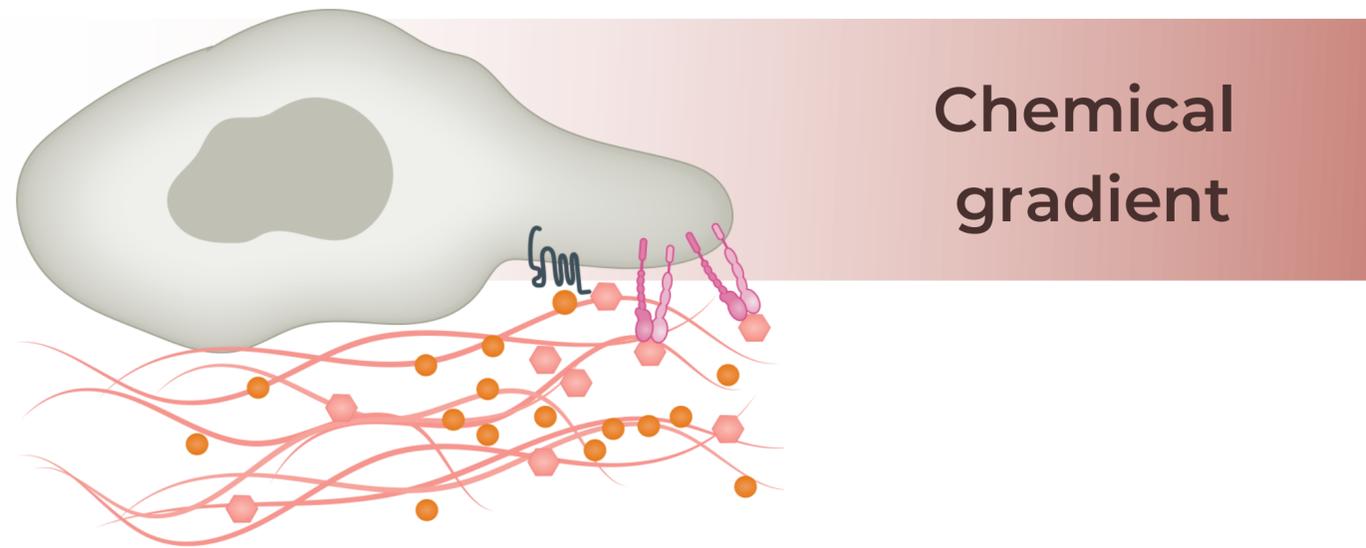
# Principles of directed cell migration

1. Signal - environmental cue
2. Sensing the signal
3. Transmitting the signal inside the cell to define front-back
4. Mechanical response: force generation & transmission

# 1. Signal from the environment

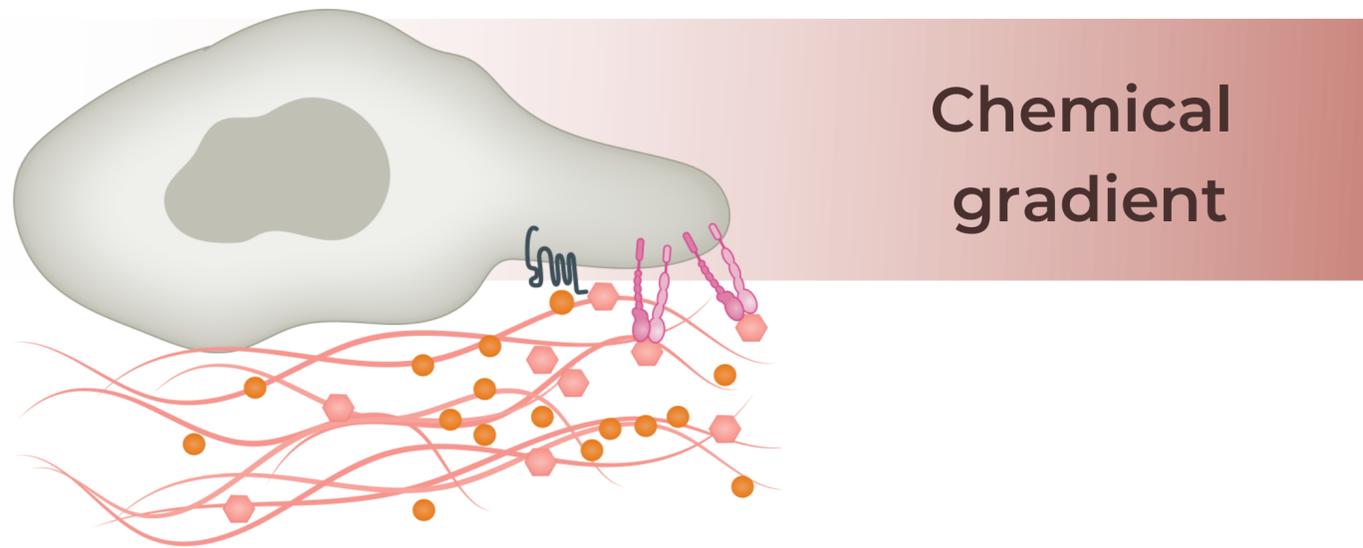
# 1. Signal from the environment

## Chemotaxis



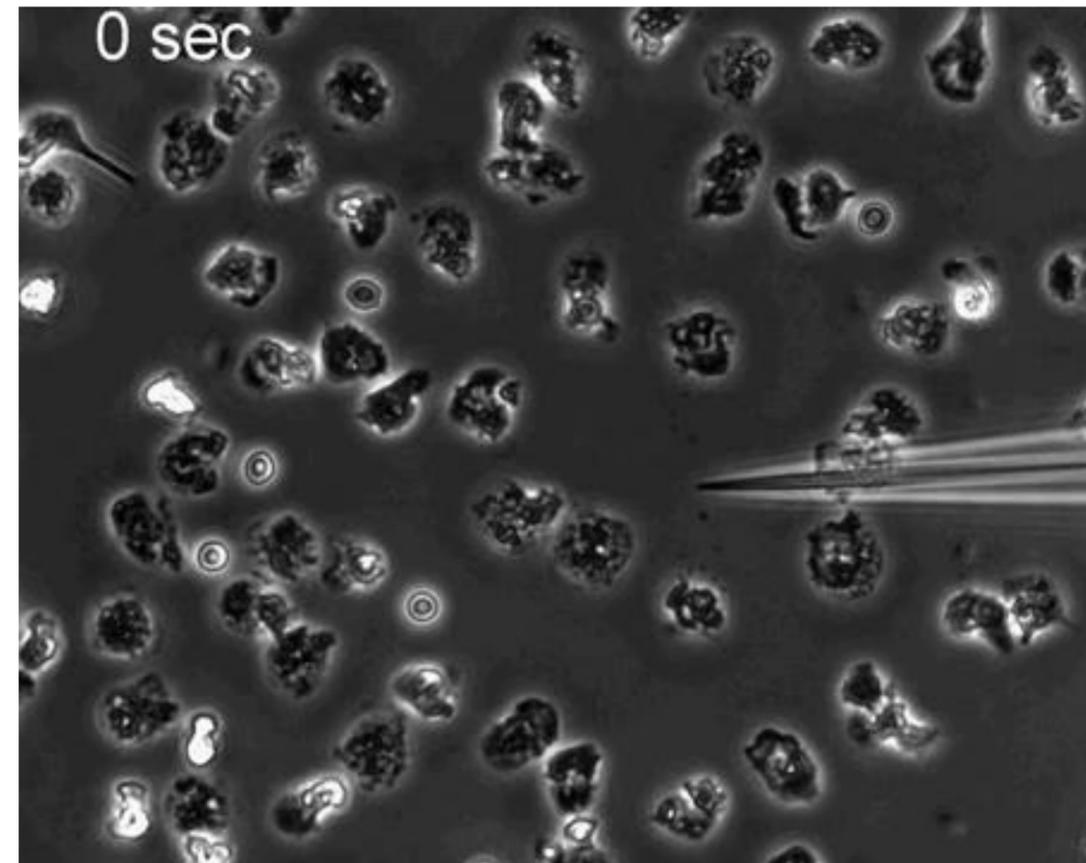
# 1. Signal from the environment

## Chemotaxis



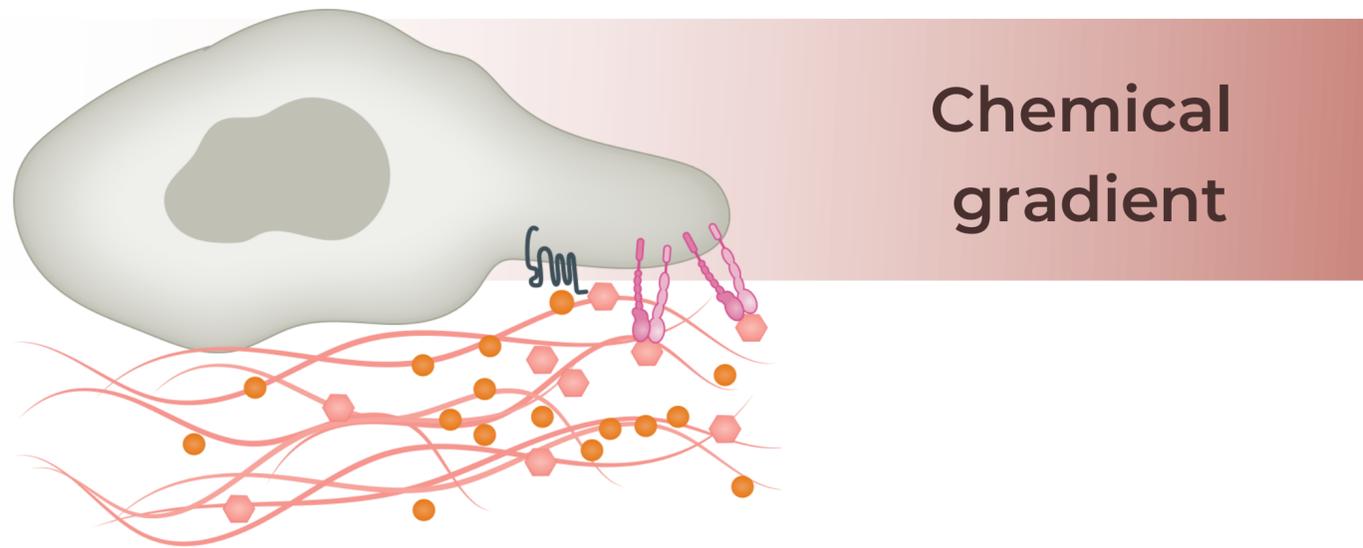
*In vitro:*

Neutrophils migrating towards  
source of bacterial peptide *fMLP*



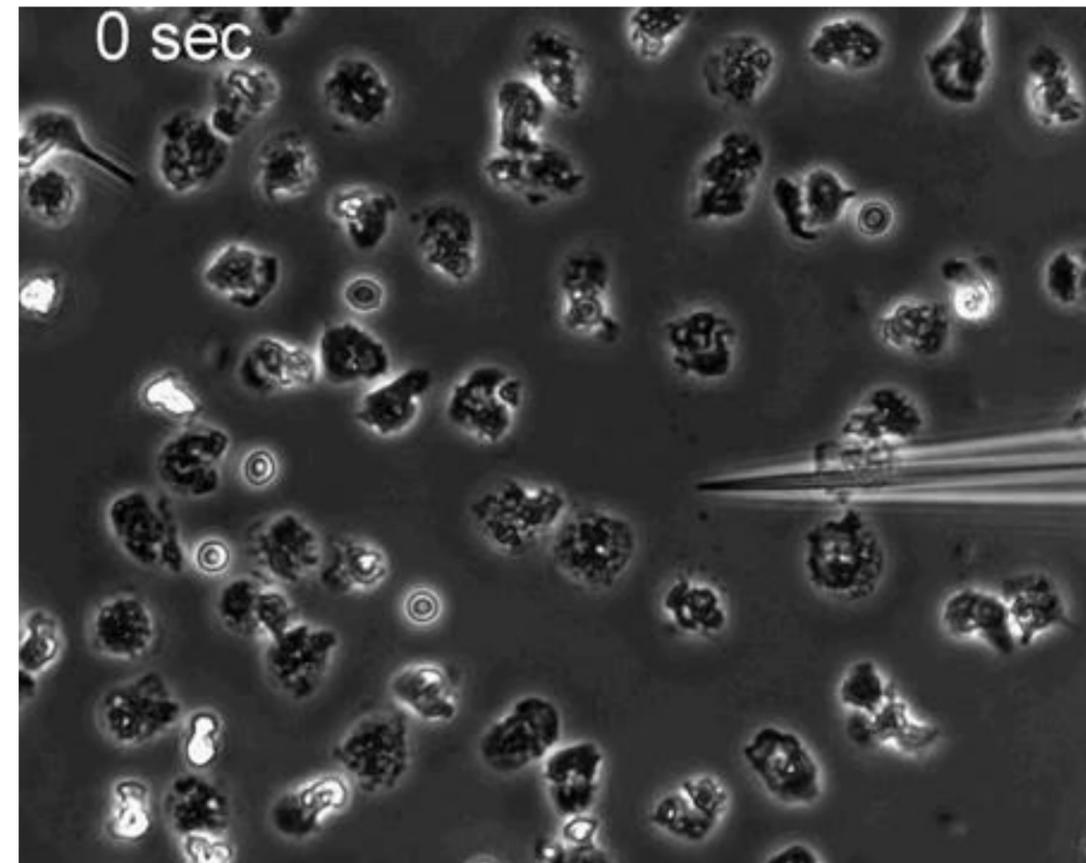
# 1. Signal from the environment

## Chemotaxis



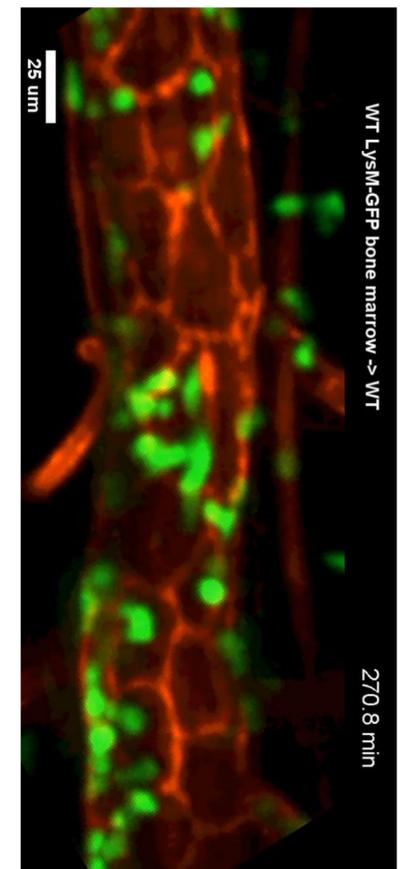
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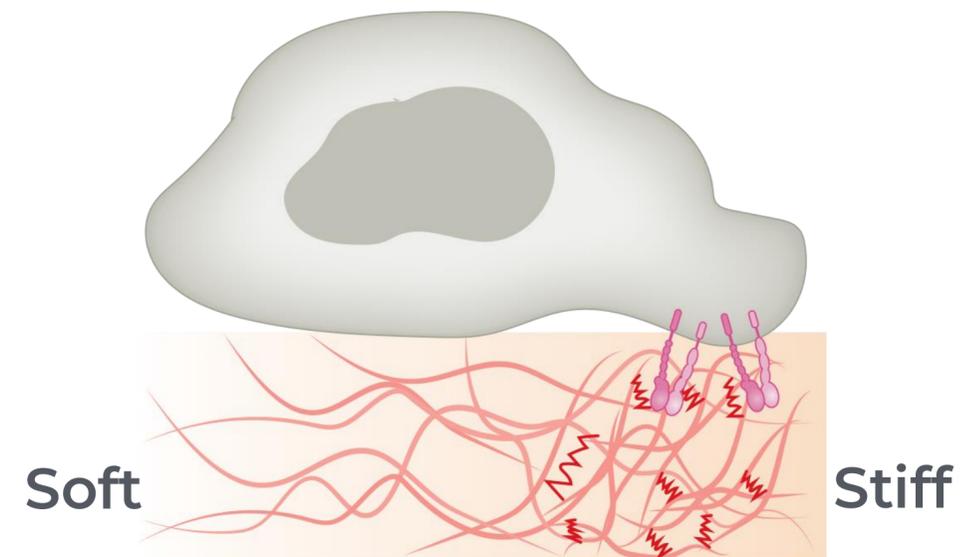
*In vivo:*

Neutrophils, endothelium



# 1. Signal from the environment

## Durotaxis

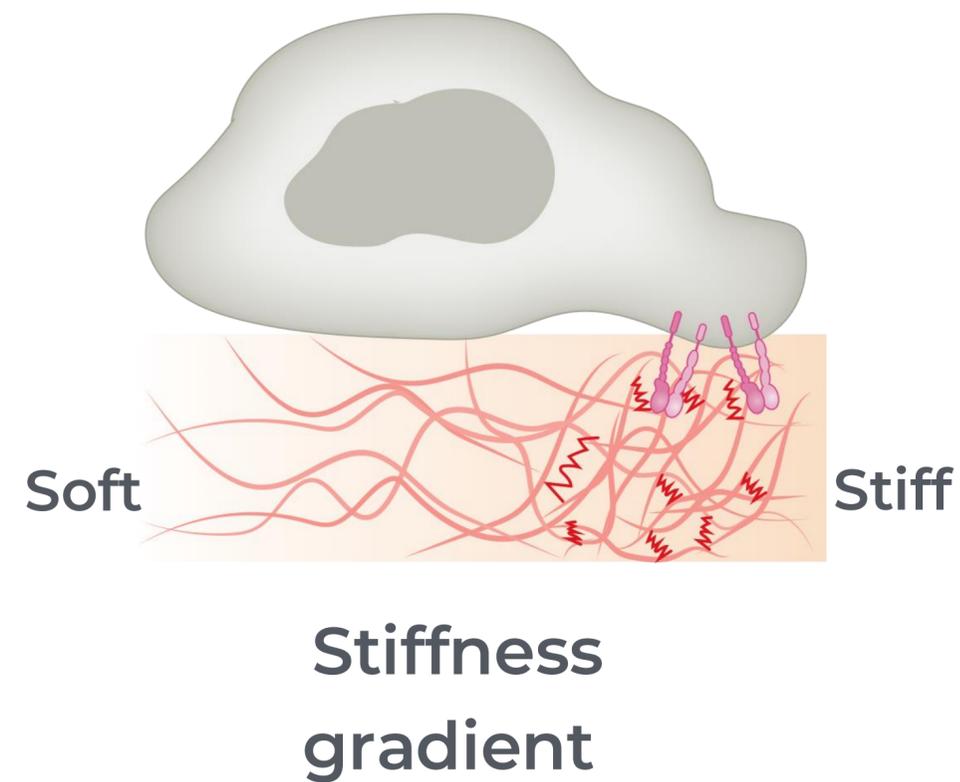


Stiffness  
gradient

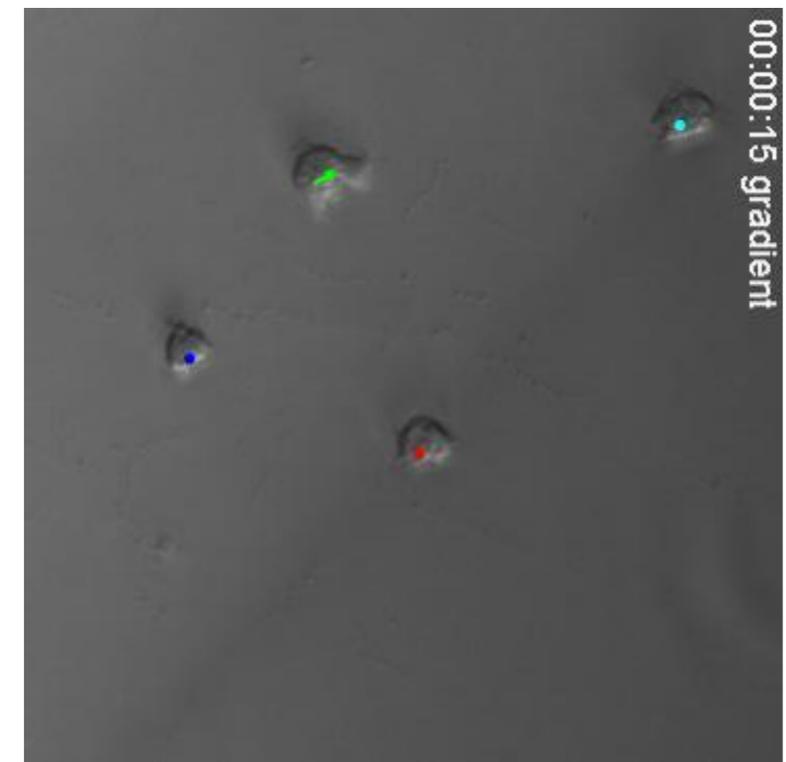
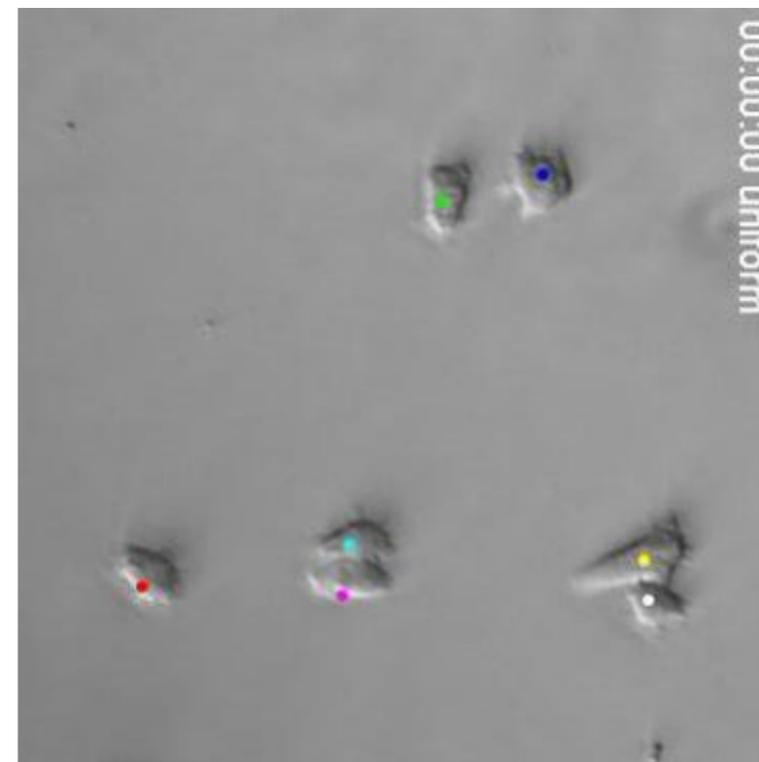
# 1. Signal from the environment

*In vitro:*

## Durotaxis



## Migration of Dicty cells



Uniform stiffness

Softer

Gradient

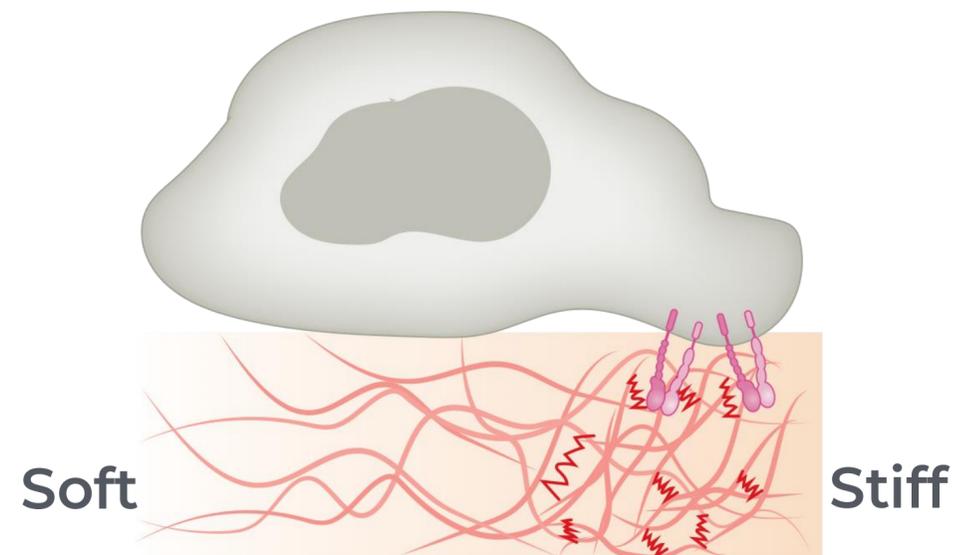
Stiffer

# 1. Signal from the environment

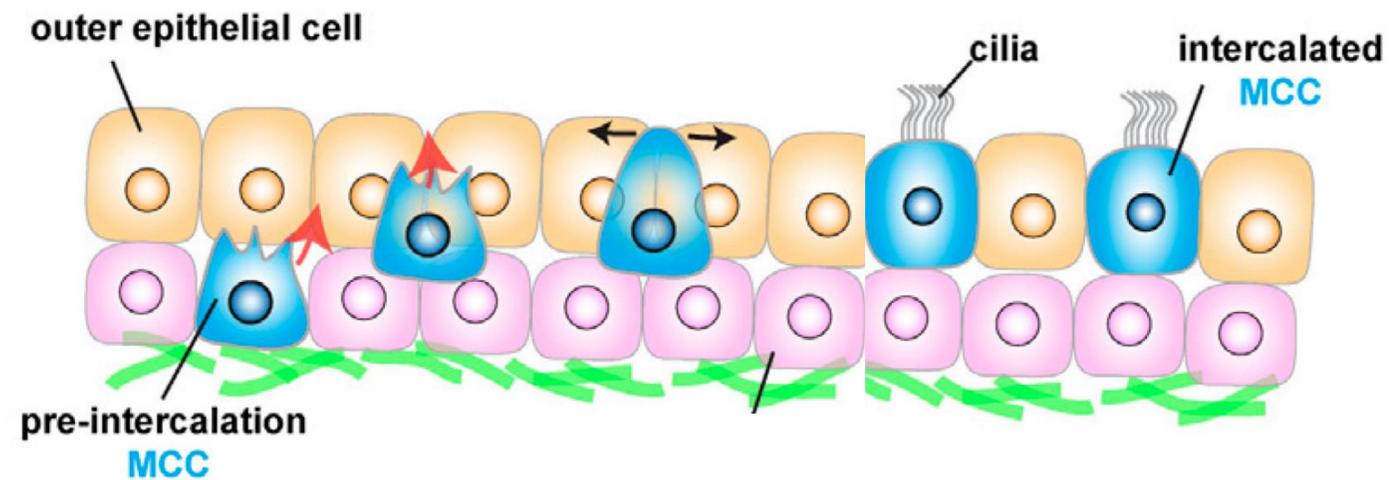
*In vivo*

Integration of multiciliated cells into epithelium in *Xenopus*

## Durotaxis



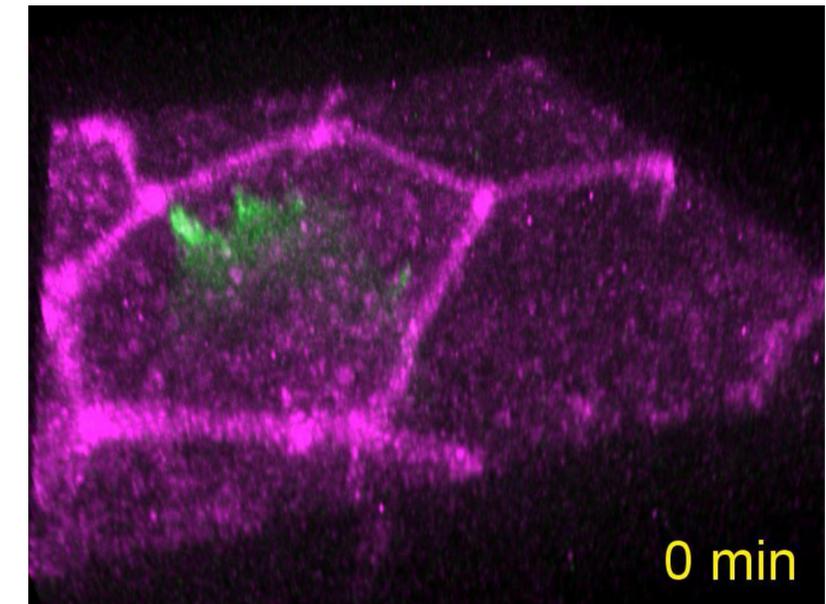
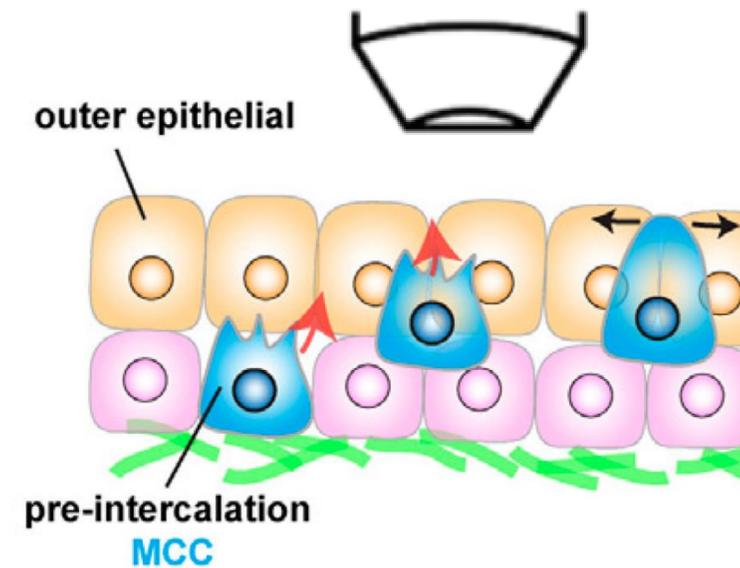
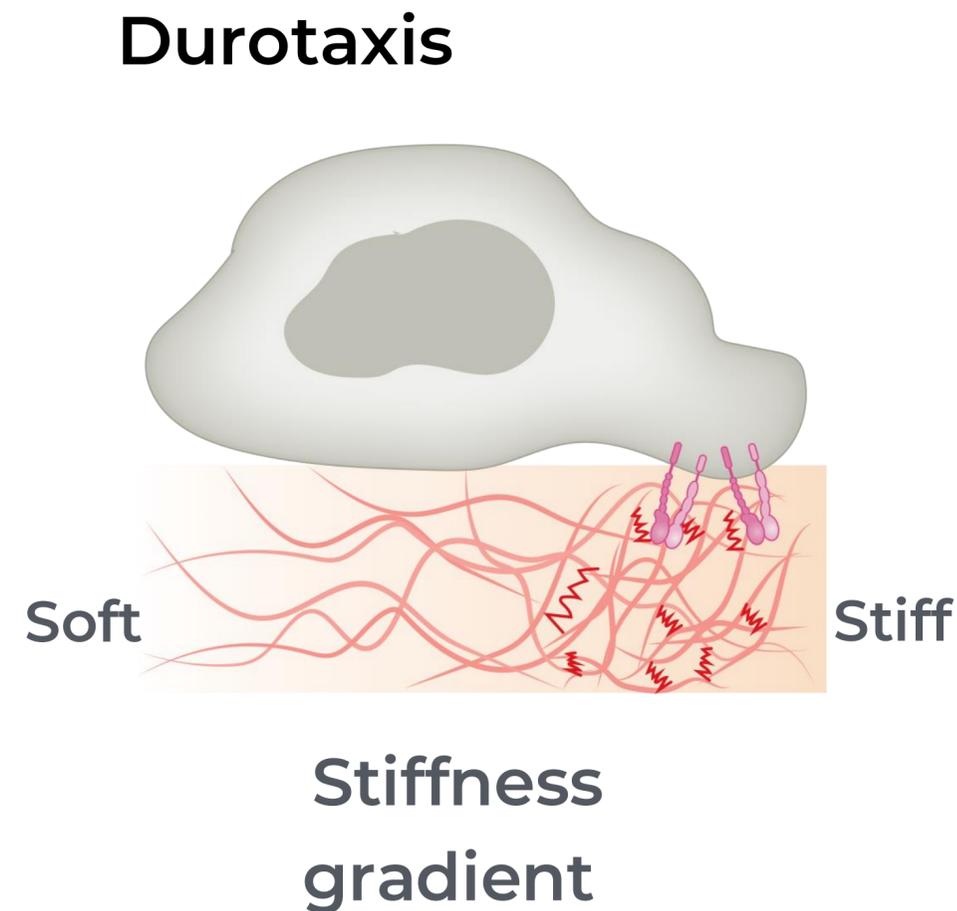
Stiffness  
gradient



# 1. Signal from the environment

*In vivo*

Integration of multiciliated cells into epithelium in *Xenopus*



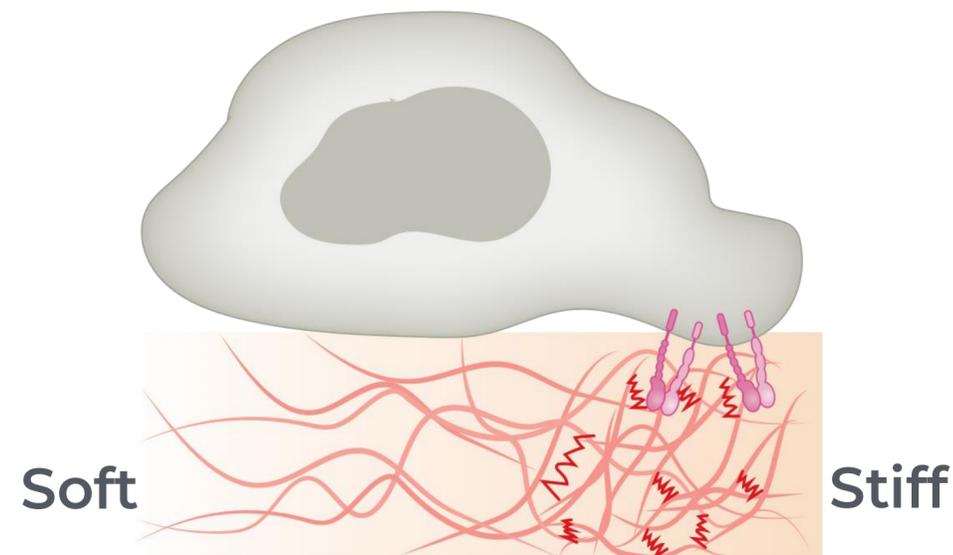
multiciliated cells  
epithelium junctions

# 1. Signal from the environment

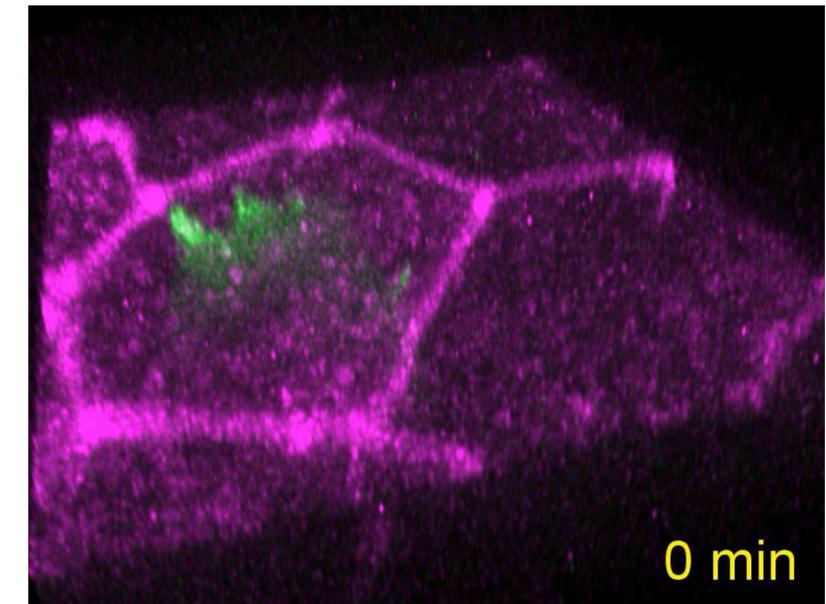
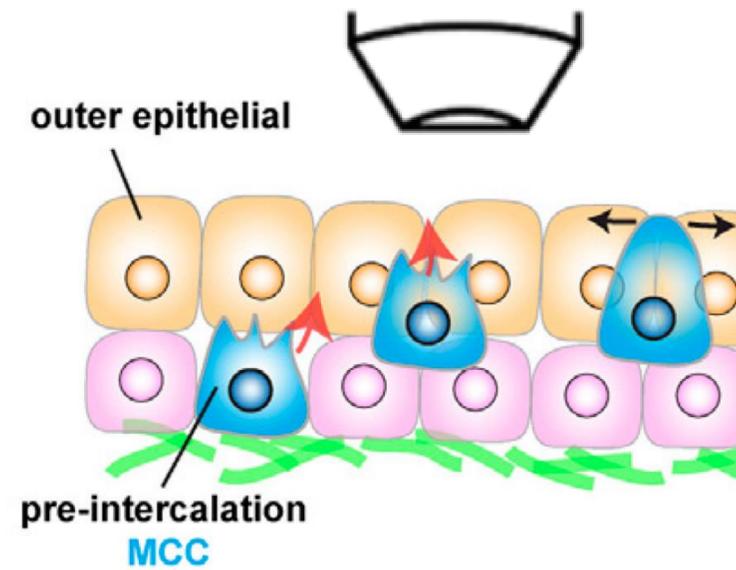
*In vivo*

Integration of multiciliated cells into epithelium in *Xenopus*

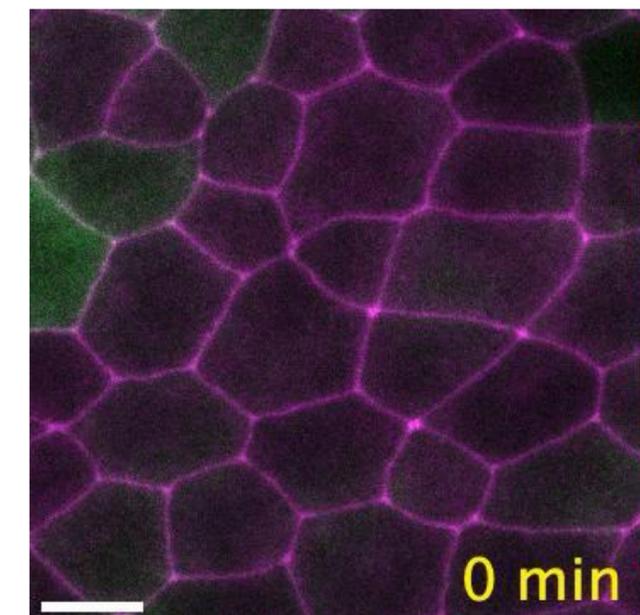
## Durotaxis



Stiffness  
gradient

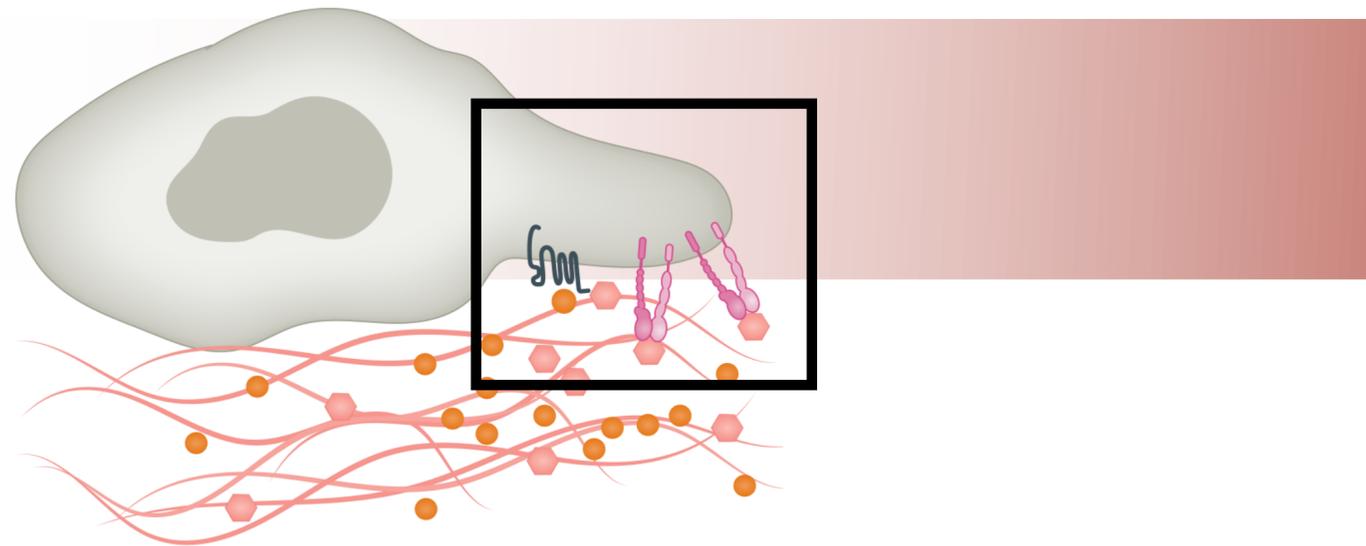


multiciliated cells  
epithelium junctions



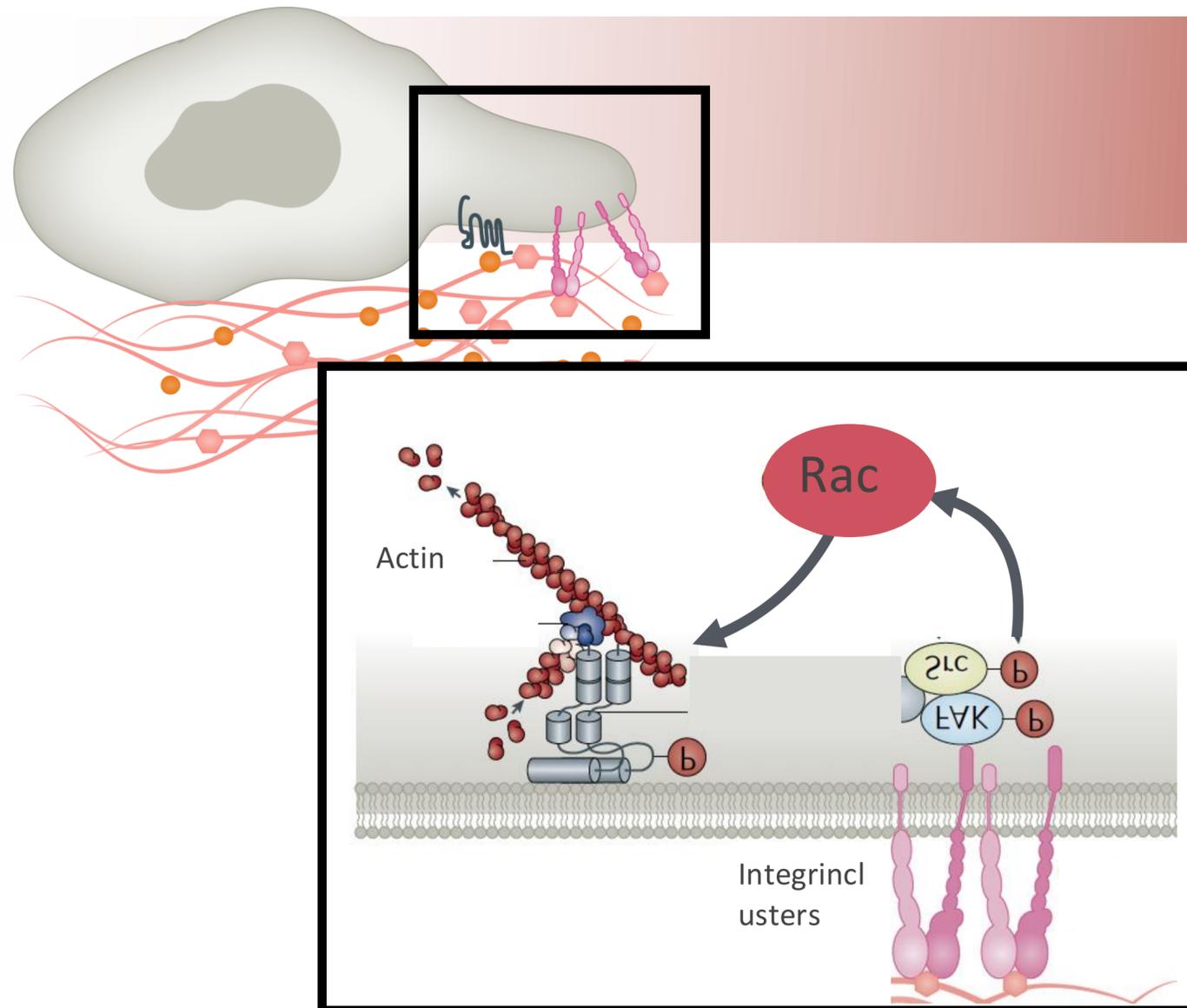
1. Signal from the environment

2. Signal sensing

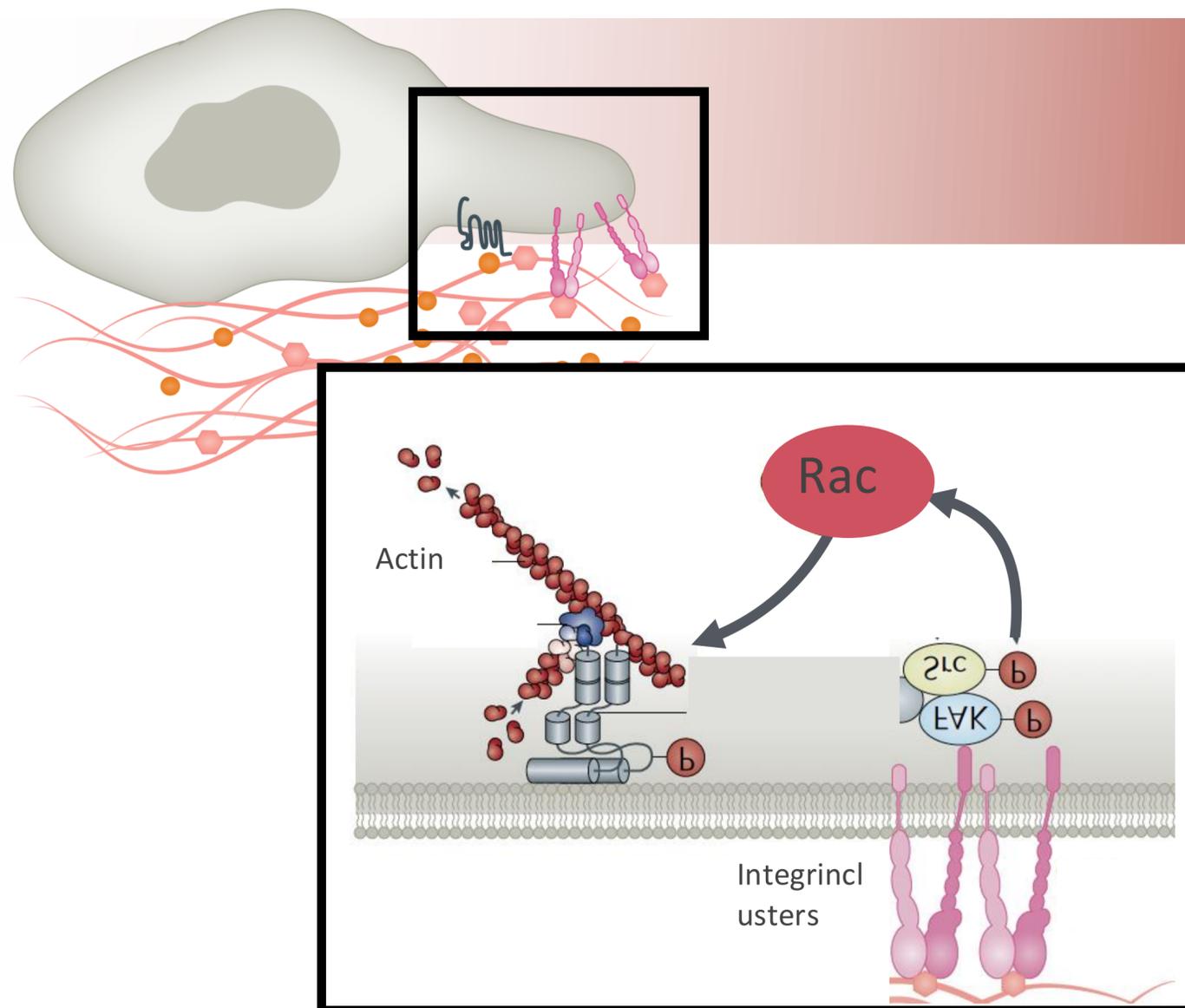


# 1. Signal from the environment

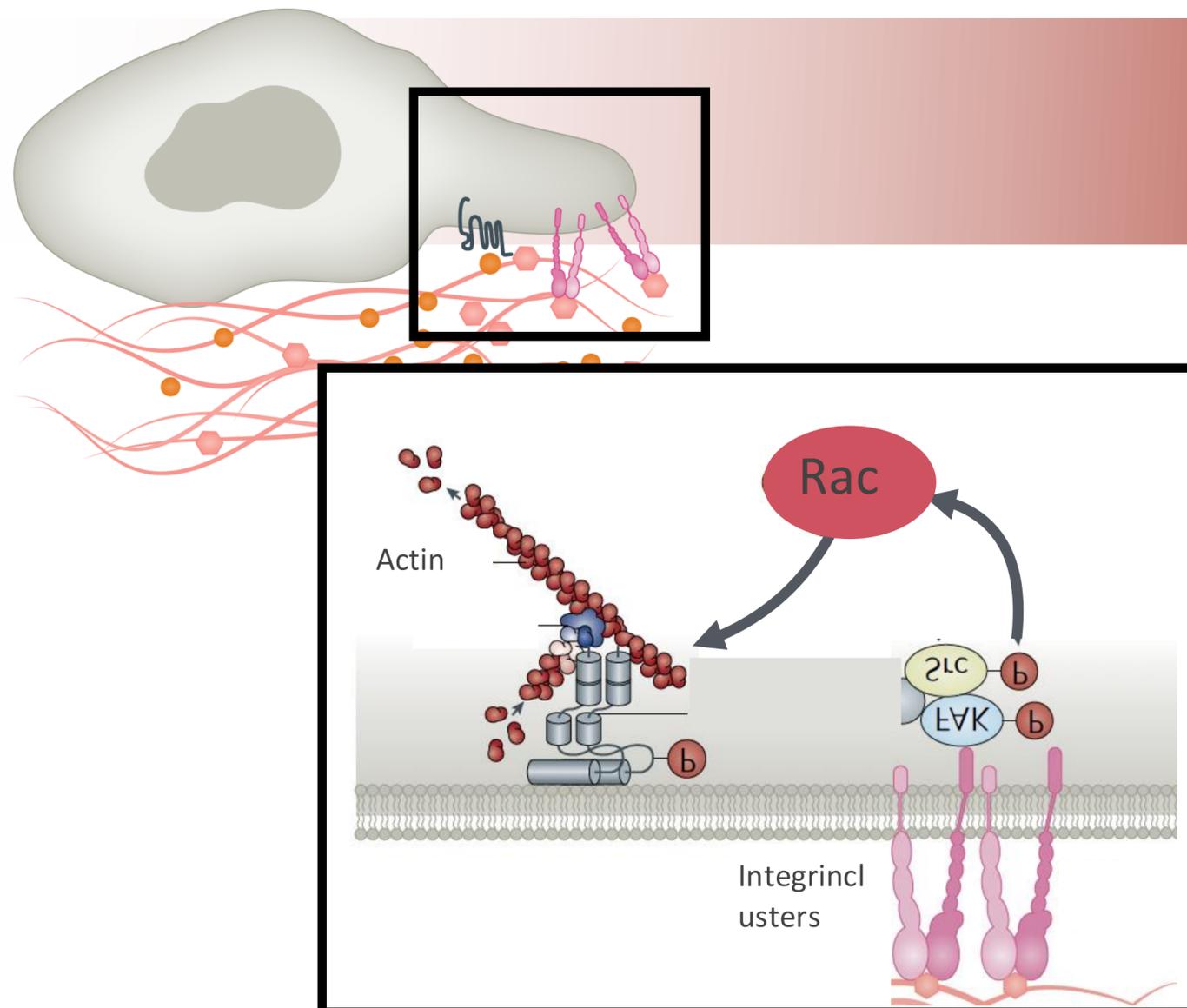
# 2. Signal sensing



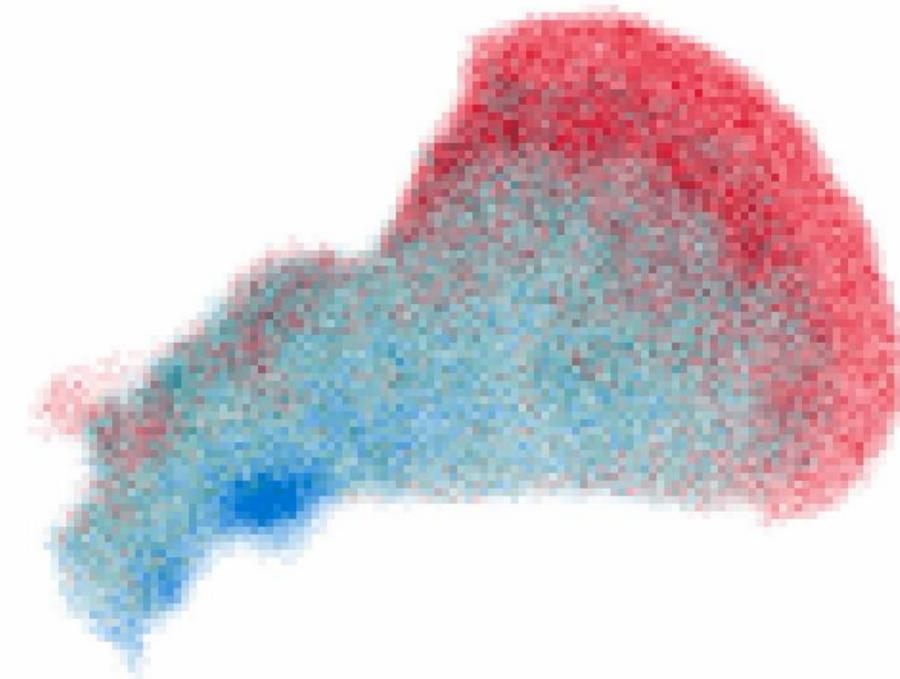
1. Signal from the environment
2. Signal sensing
- 3. Transmitting the signal inside the cell to define front-back**



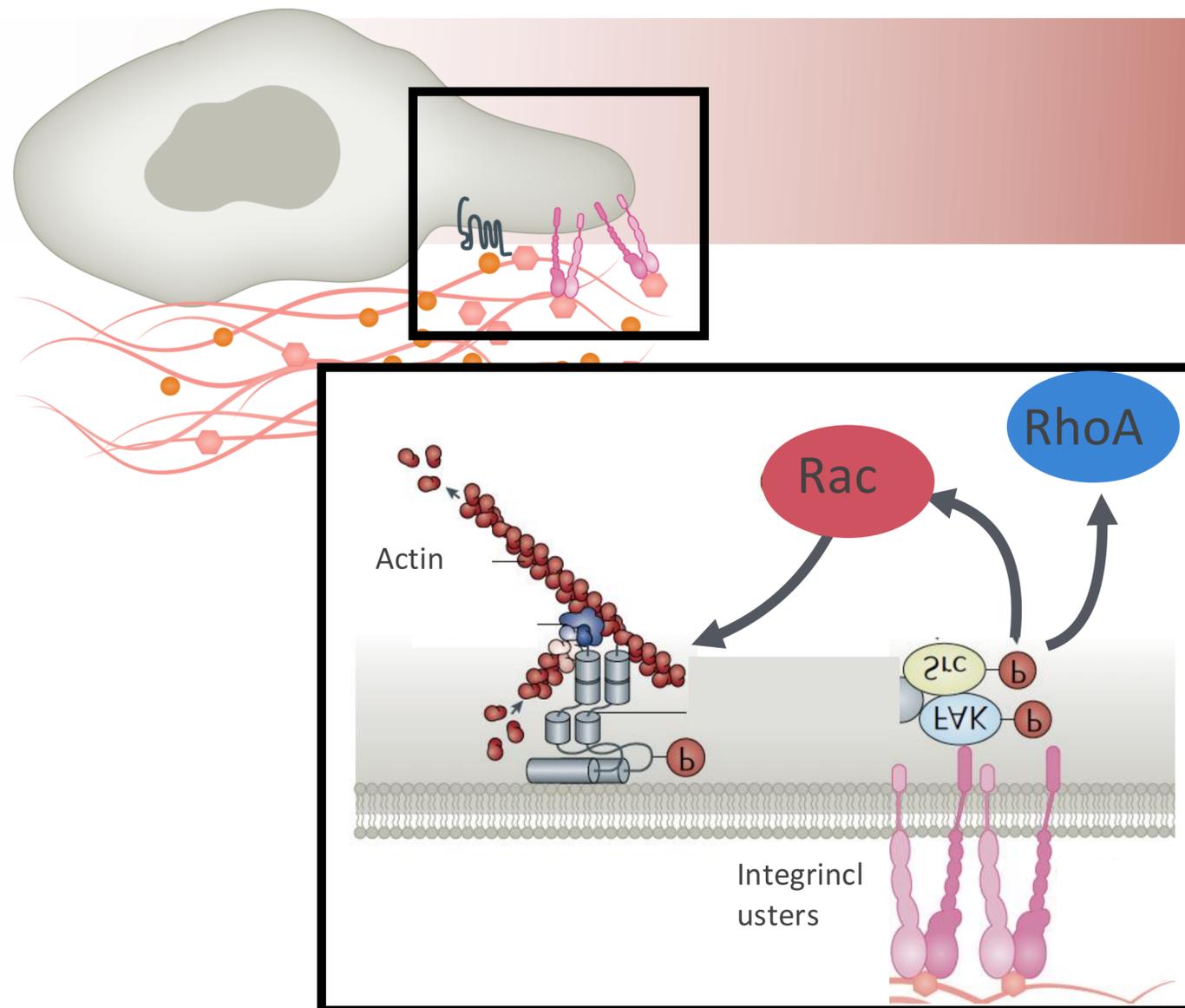
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2. Signal sensing
3. Transmitting the signal inside the cell to define front-back



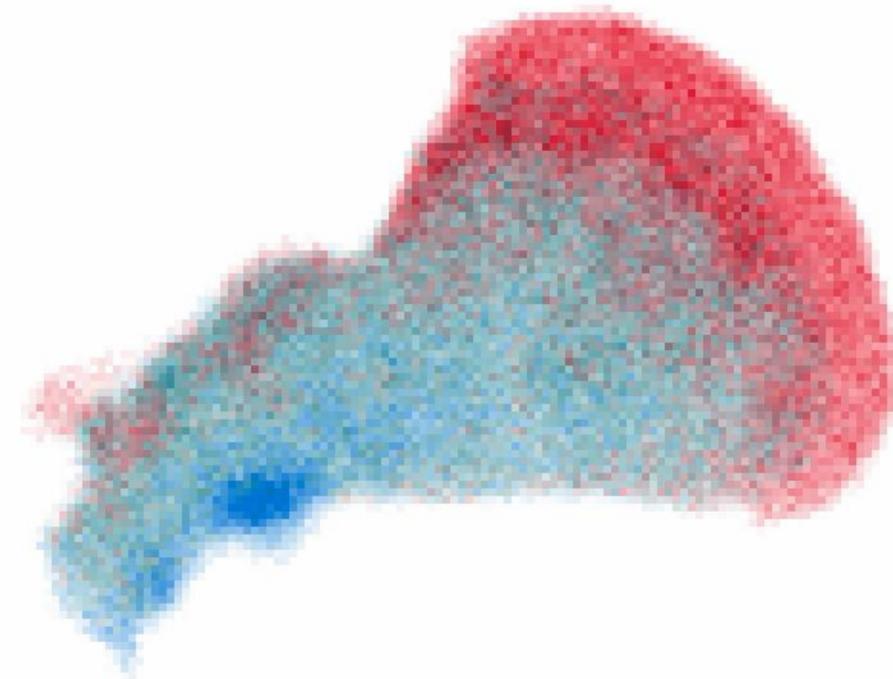
Neutrophil expressing  
**Rac GTPase** in front: actin polymerisation



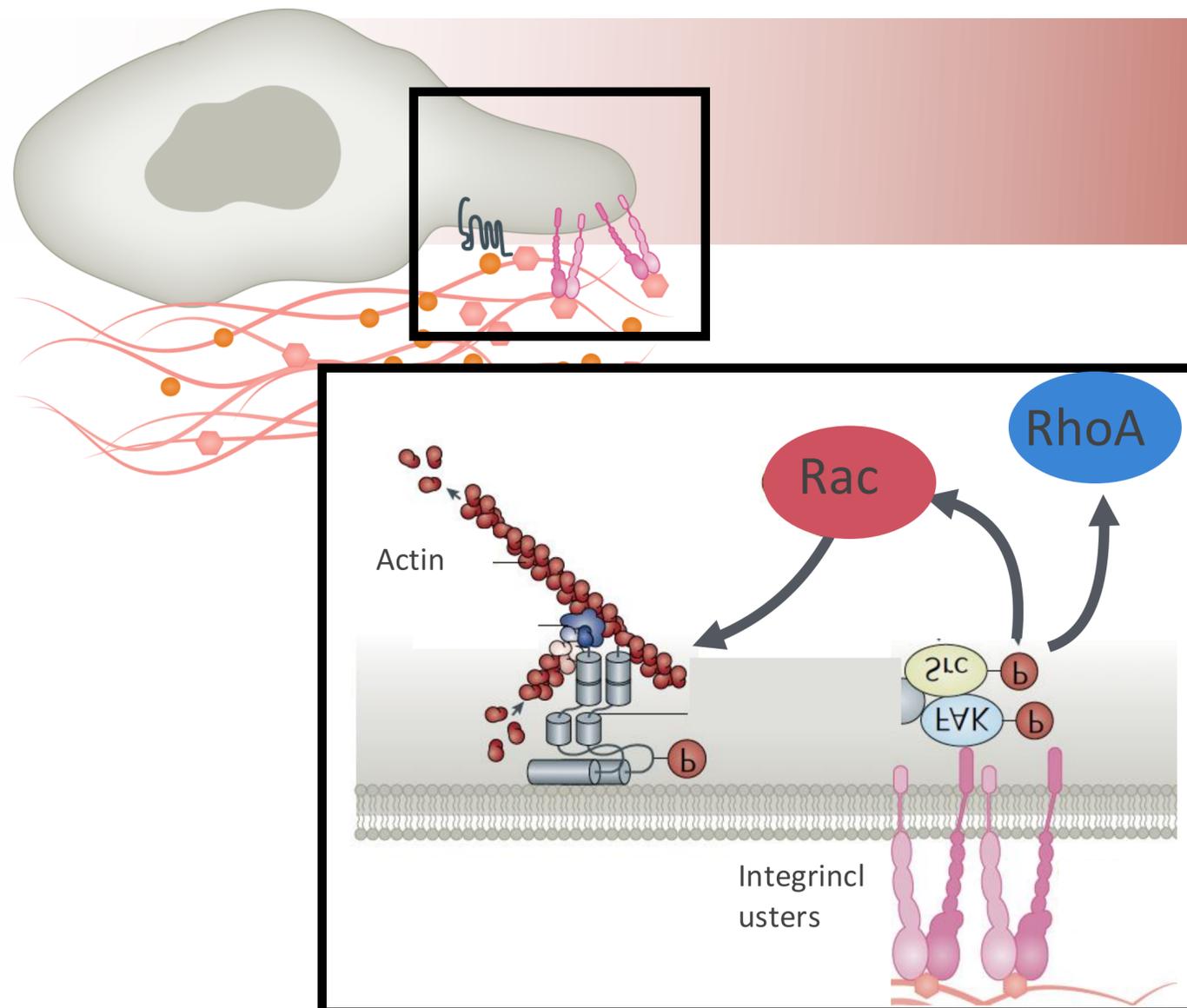
1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back



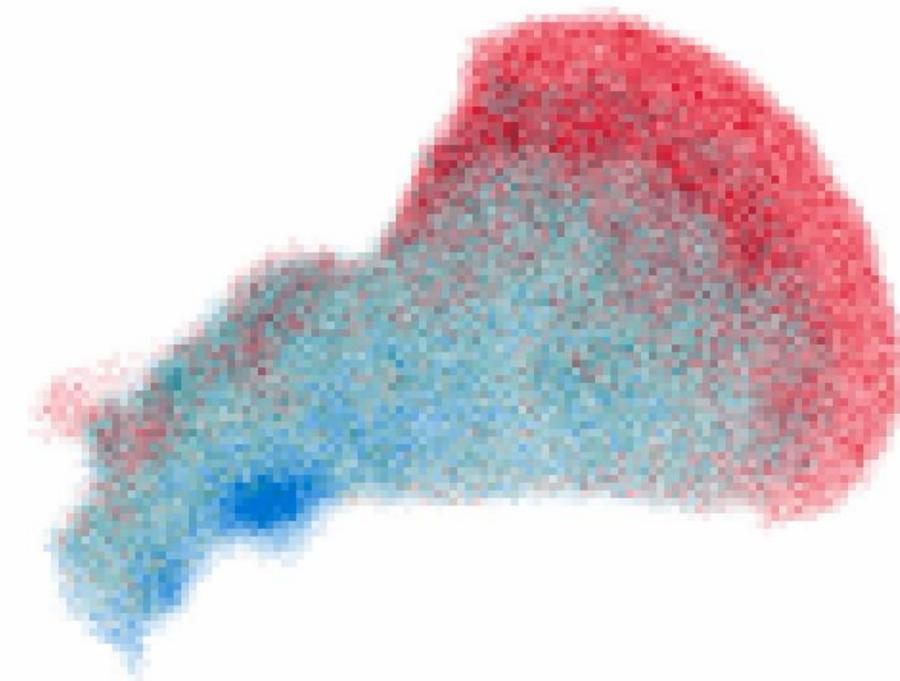
RhoA in the back: myosin activation  
 Neutrophil expressing  
 Rac GTPase in front: actin polymerisation



1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back

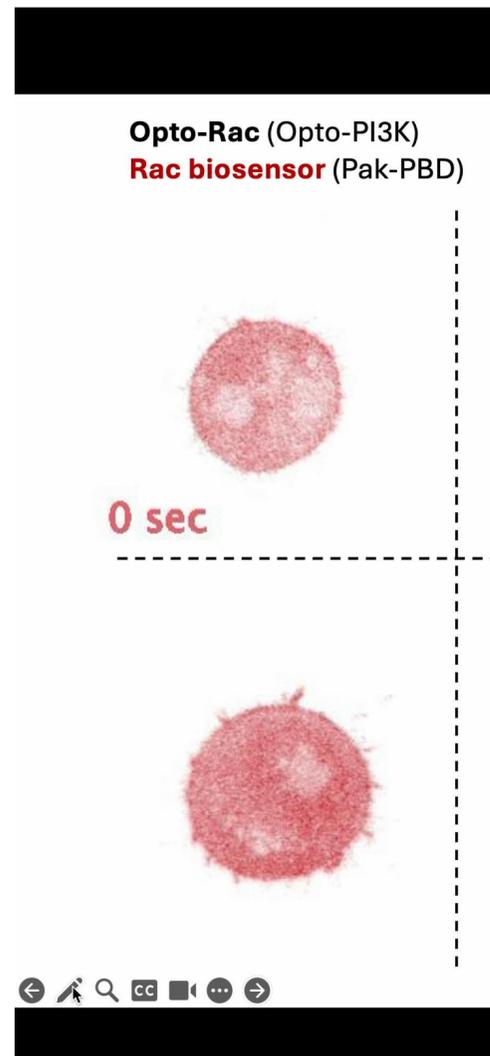


RhoA in the back: myosin activation  
 Neutrophil expressing  
 Rac GTPase in front: actin polymerisation

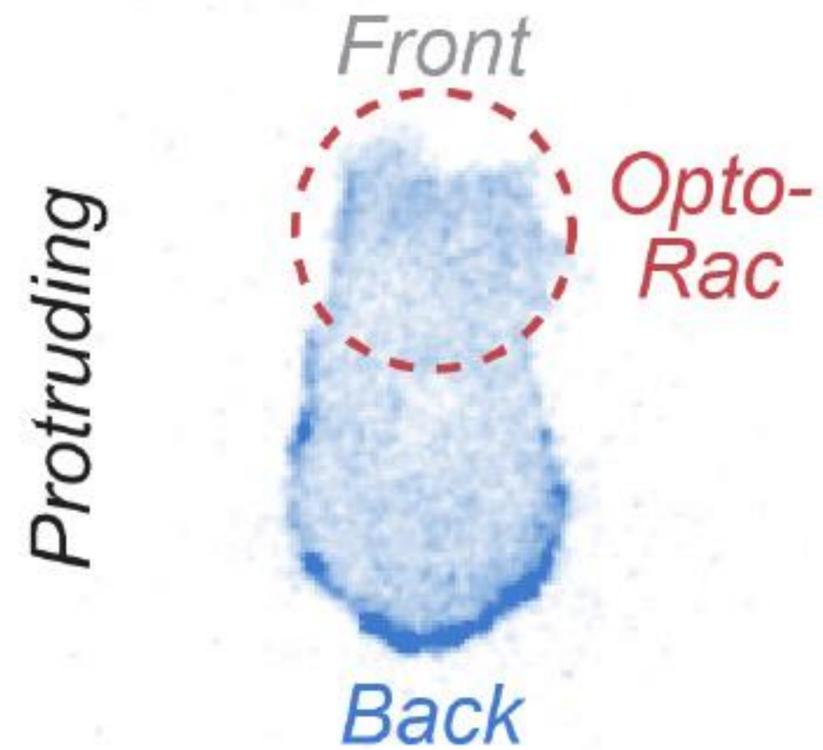
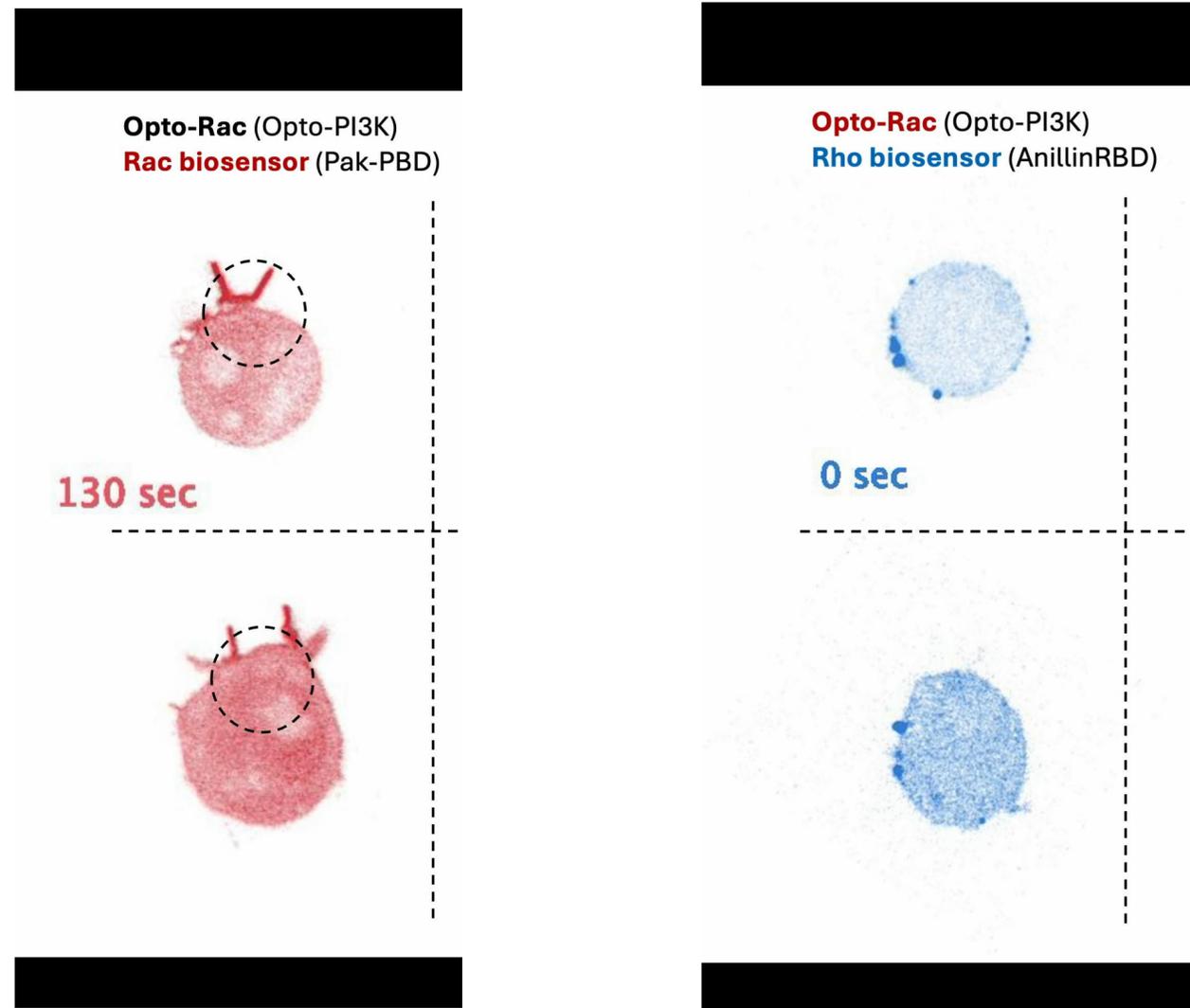


How is RhoA activated in the back?

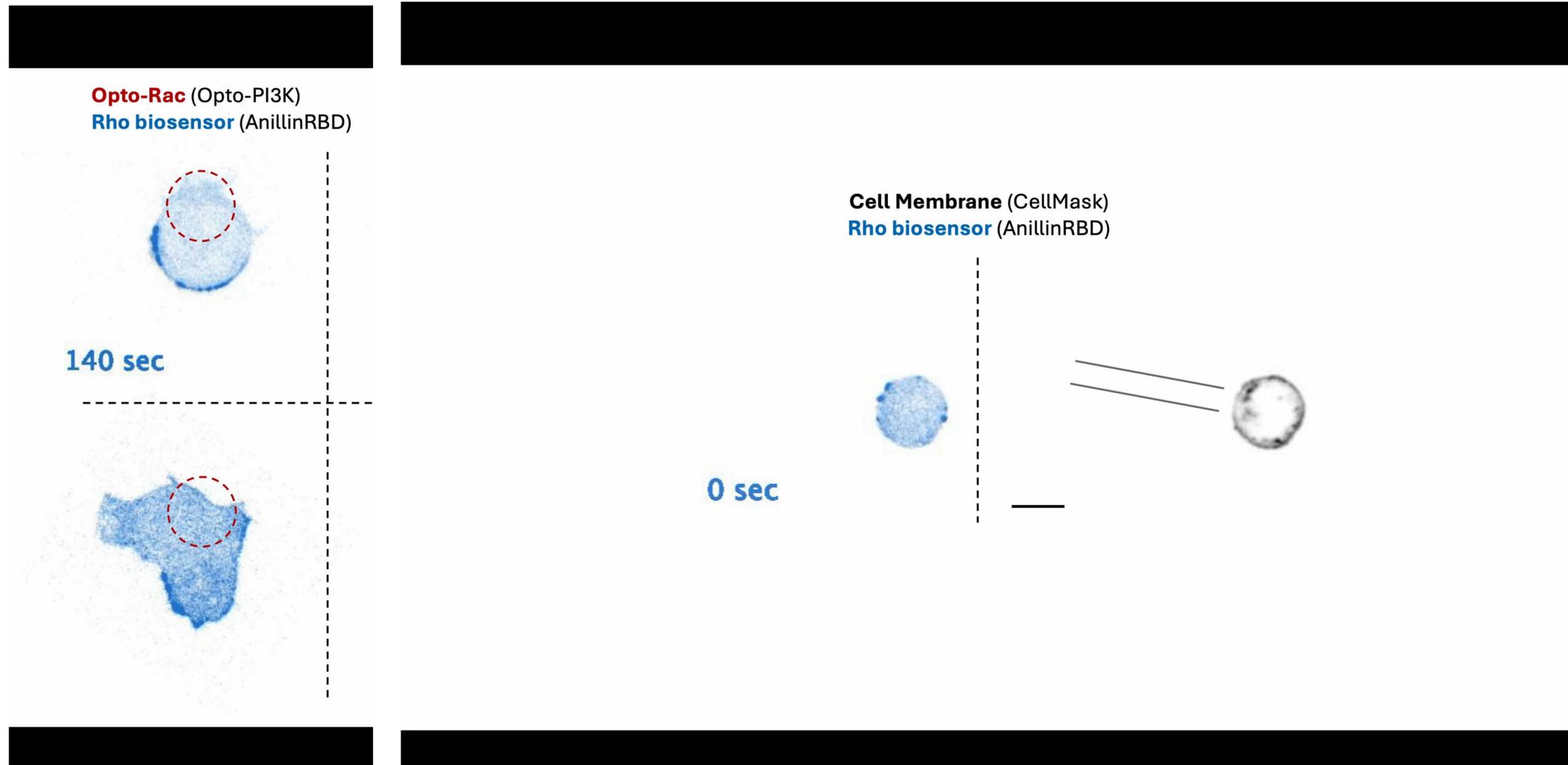
# Q: How is RhoA activated in the back?



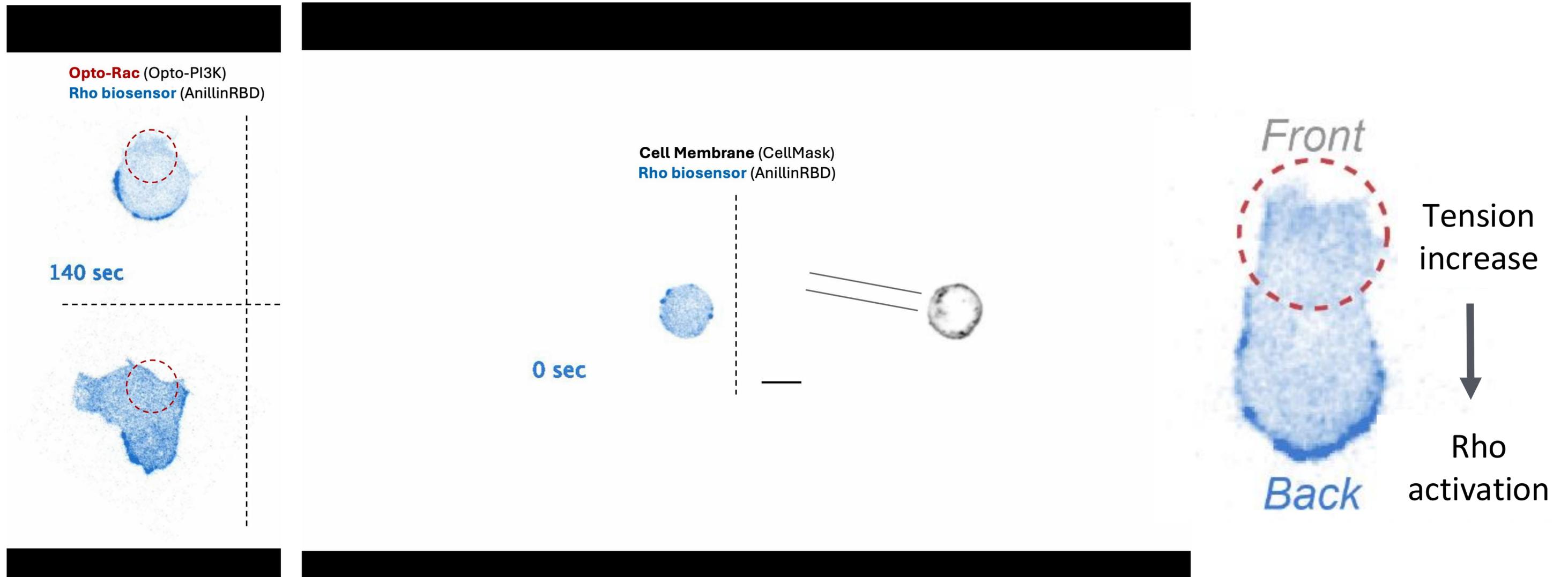
# Q: How is RhoA activated in the back?



# Q: How is RhoA activated in the back?



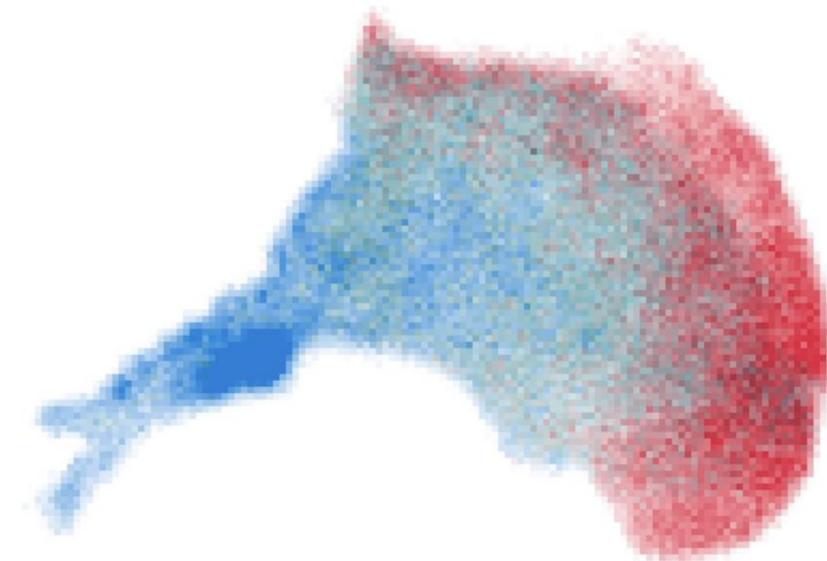
# Q: How is RhoA activated in the back?



A: Cell membrane tension propagates to the back and activates Rho there where it is not inhibited by Rac

1. Signal from the environment
2. Signal sensing
- 3. Transmitting the signal inside the cell to define front-back**

RhoA in the back: myosin activation  
Neutrophil expressing  
Rac GTPase in front: actin polymerisation

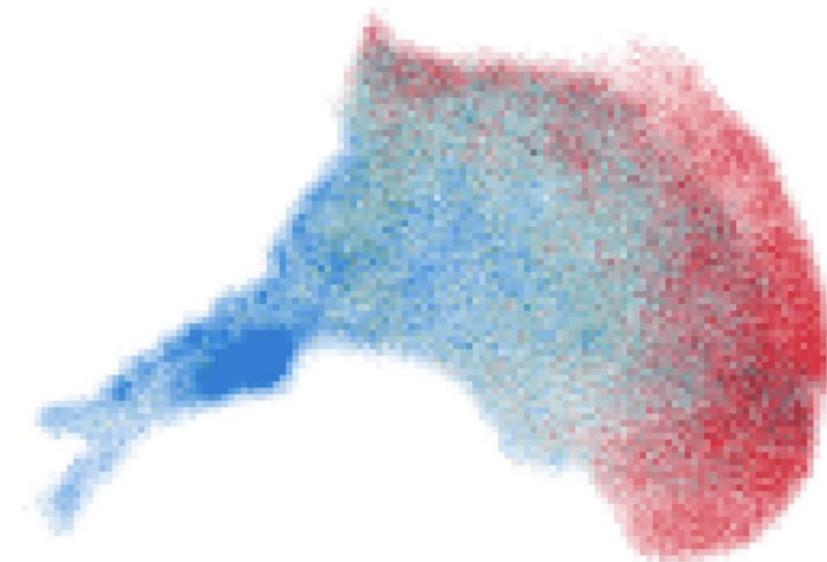
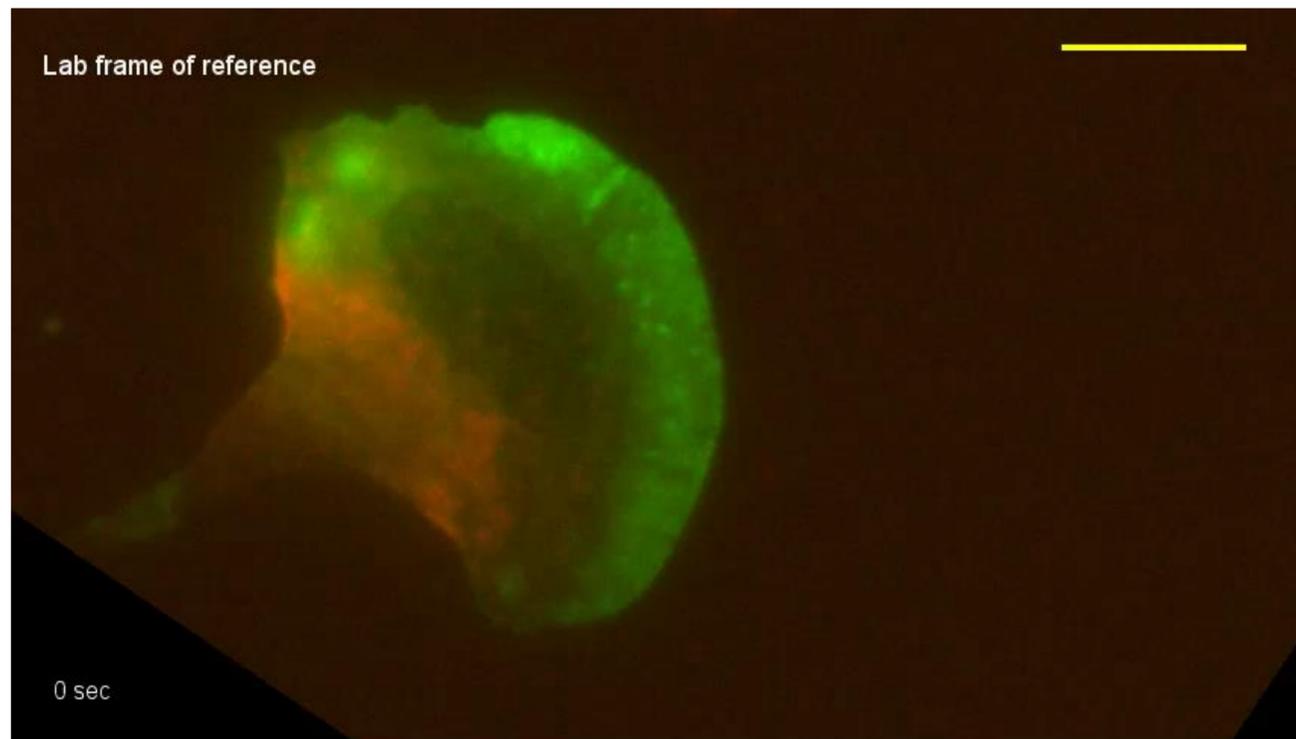


1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation**

Neutrophil expressing **actin**



Neutrophil expressing  
**Rac GTPase** in front: actin polymerisation

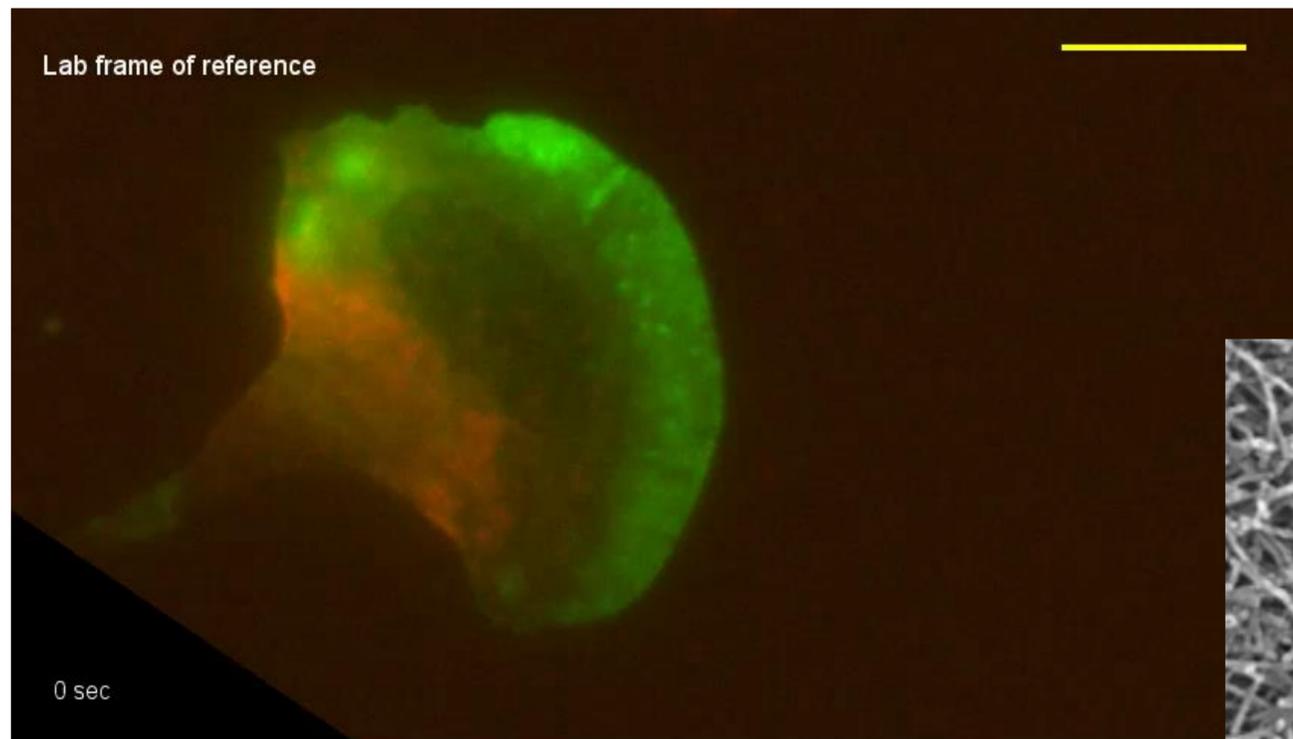


1. Signal from the environment
2. Signal sensing
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4. **Force generation**

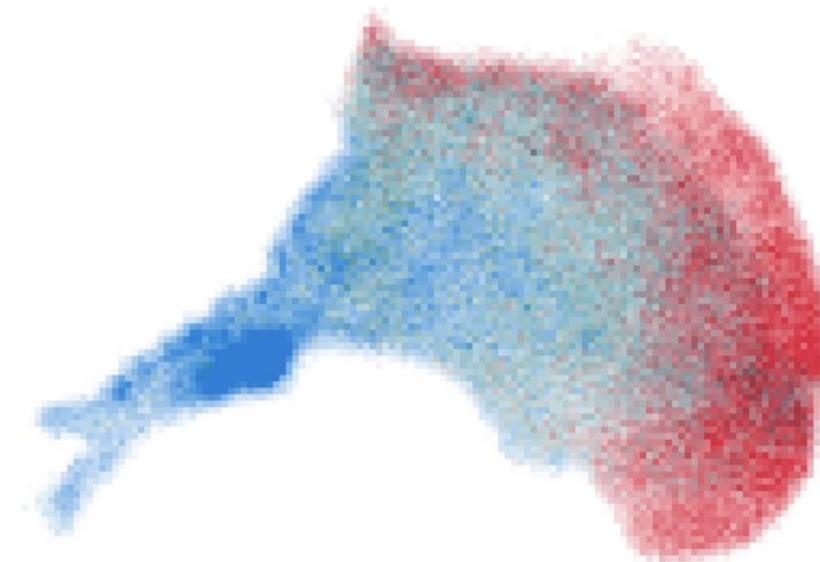
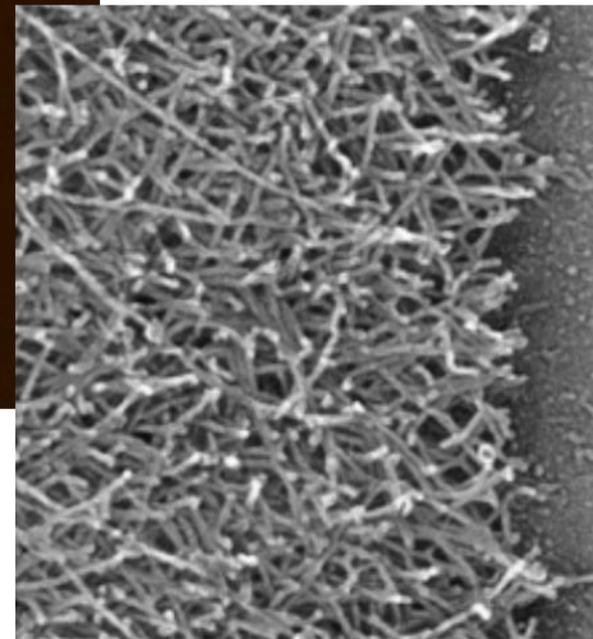
Neutrophil expressing **actin**



Neutrophil expressing  
**Rac GTPase** in front: actin polymerisation



actin network  
pushes forward

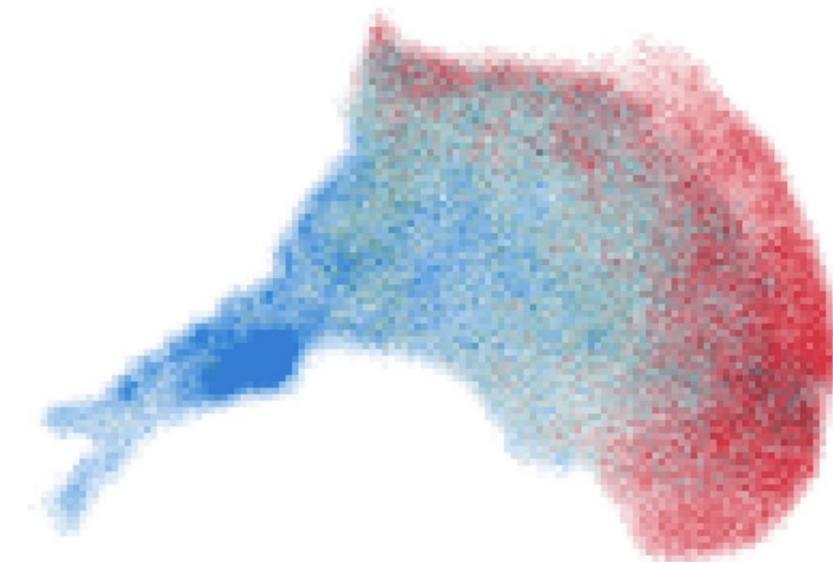
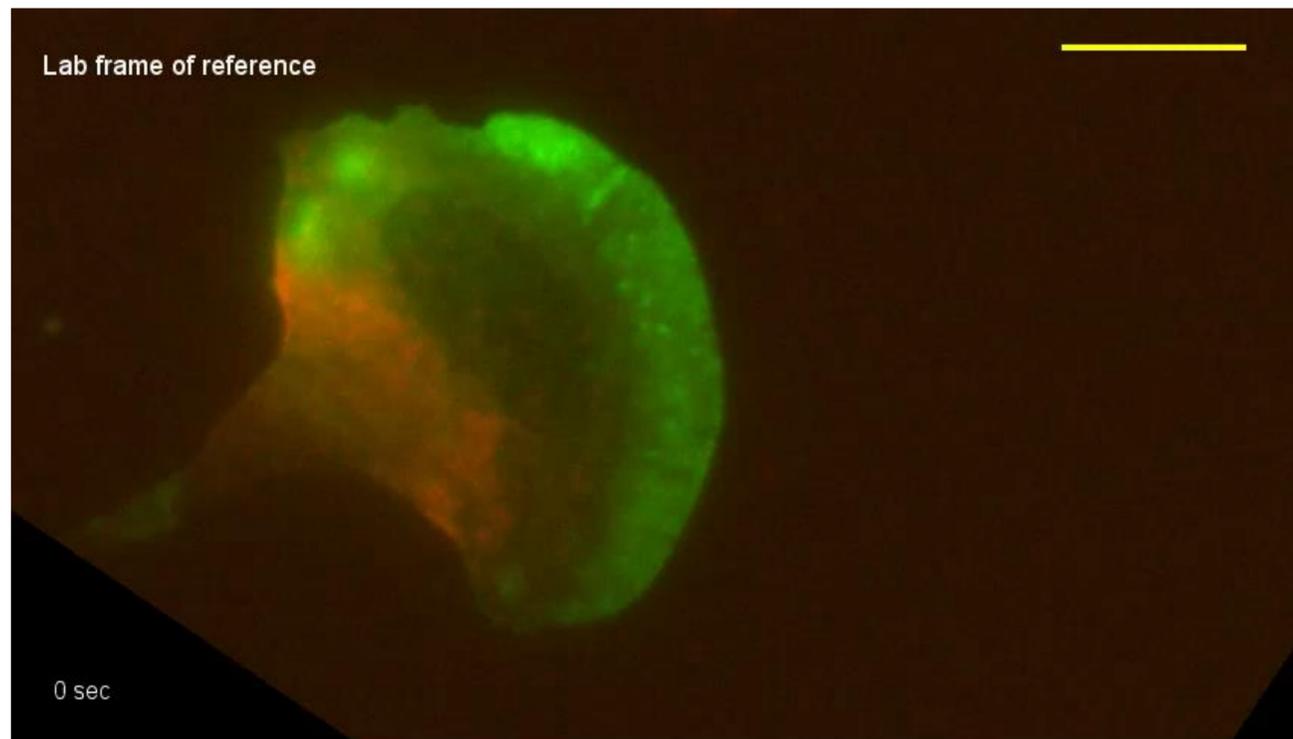


1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation**

Neutrophil expressing **actin**



Neutrophil expressing  
**Rac GTPase** in front: actin polymerisation  
**RhoA** in the back: myosin activation

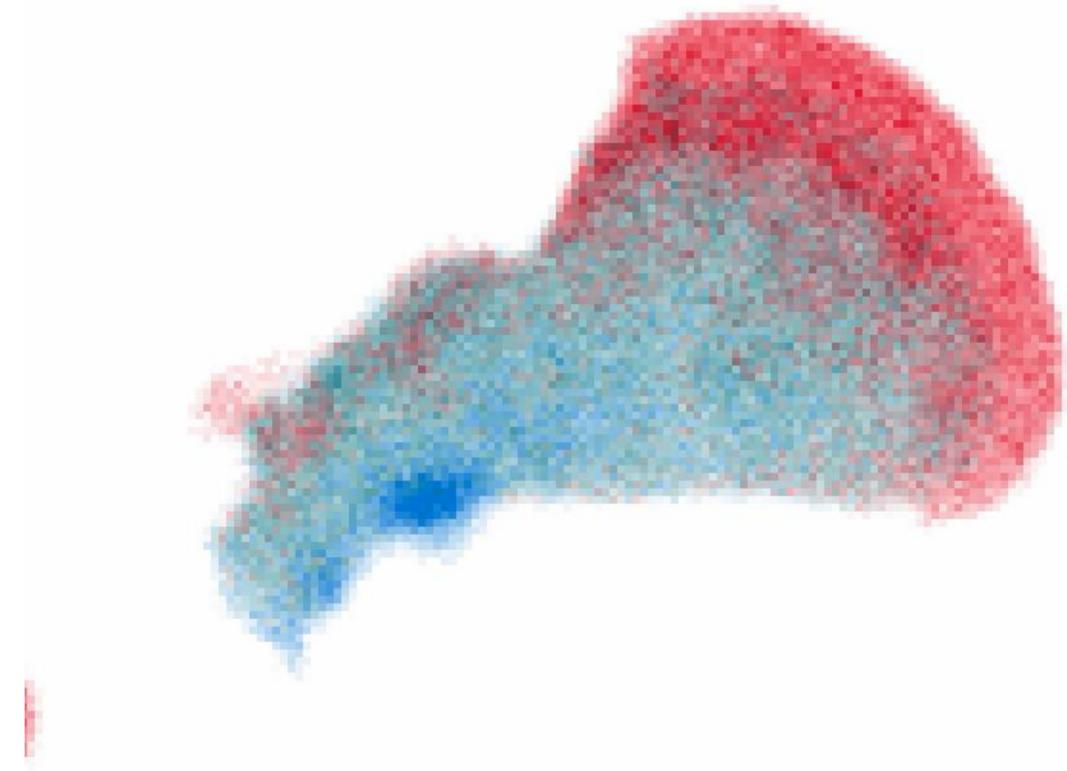
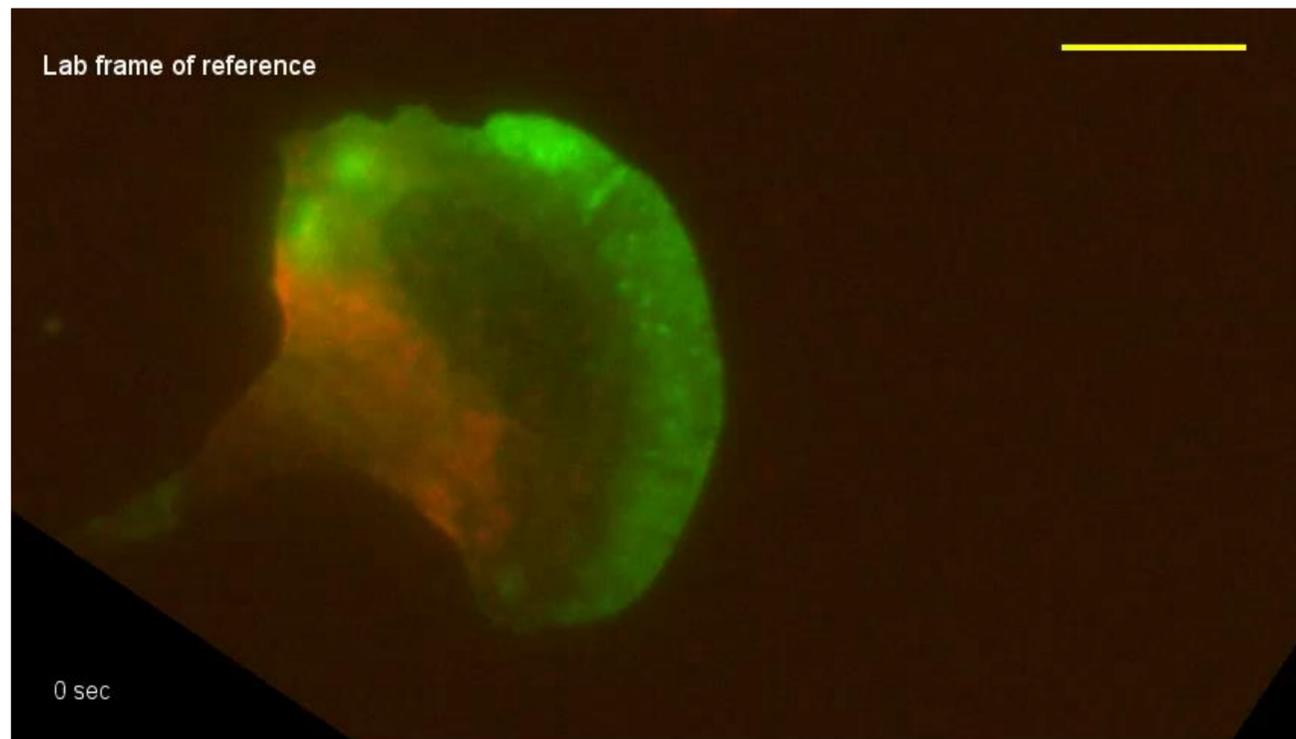


1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation**

Neutrophil expressing **actin**  
and **myosin**

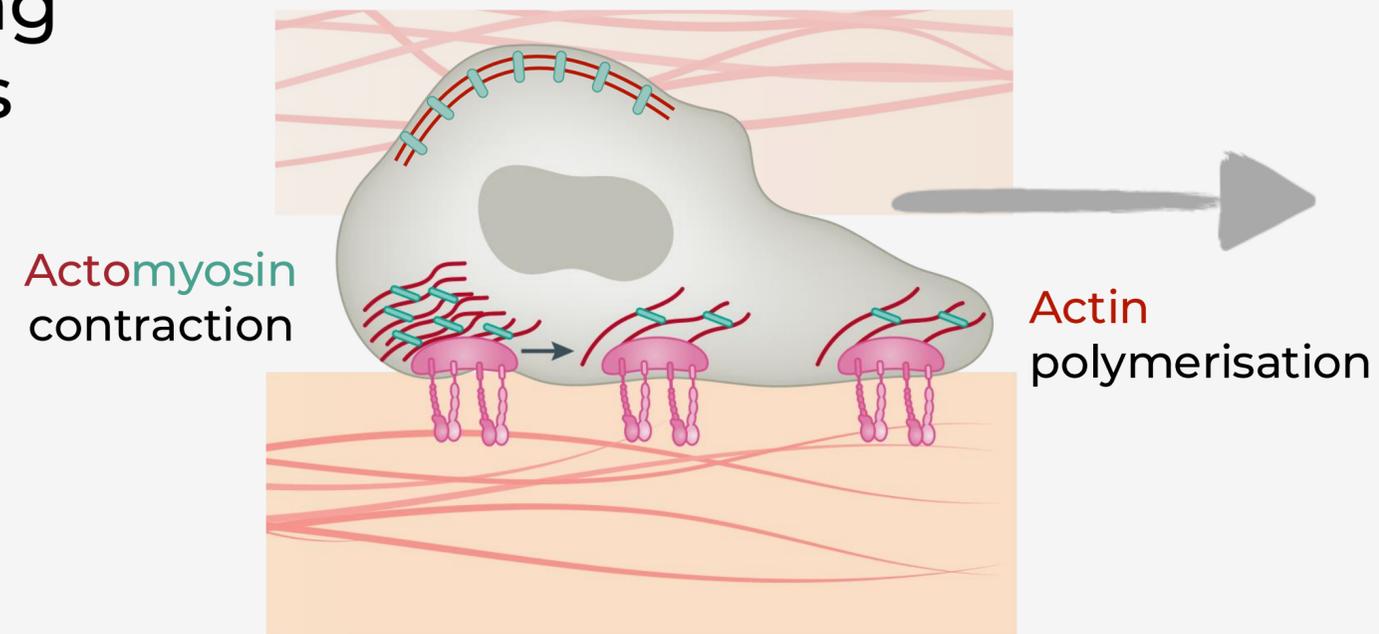


Neutrophil expressing  
**Rac GTPase** in front: actin polymerisation  
**RhoA** in the back: myosin activation

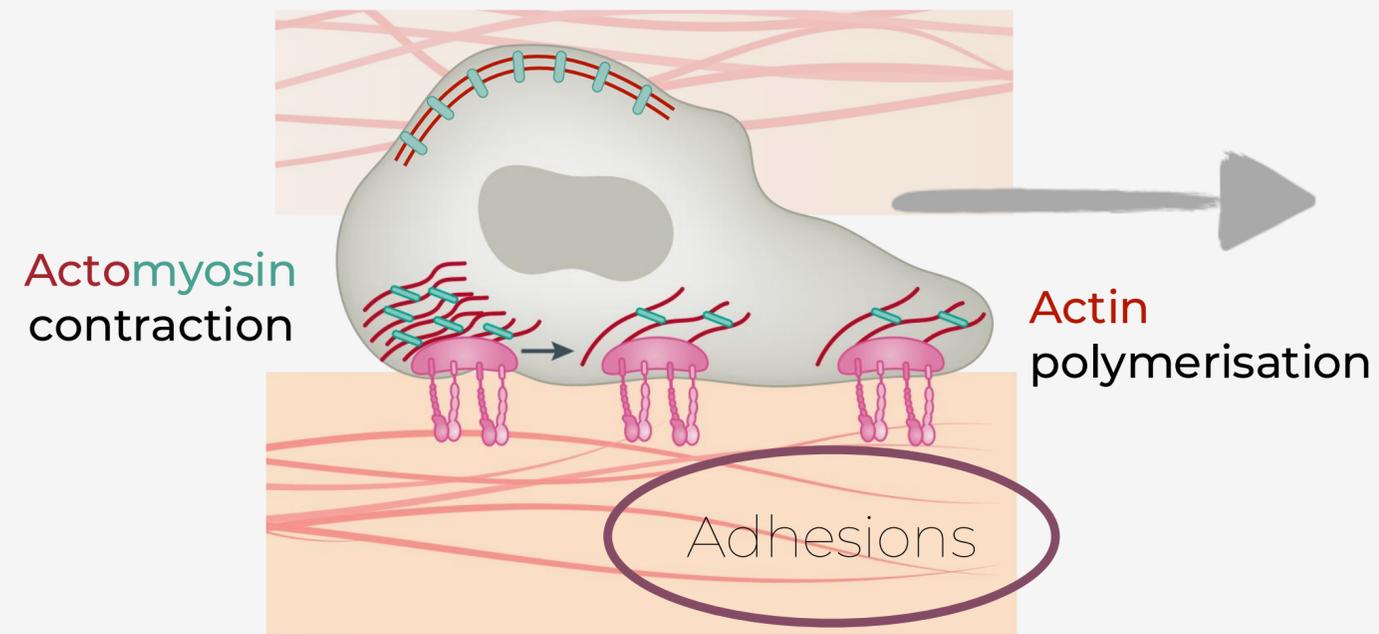


1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
- 4. Force generation**

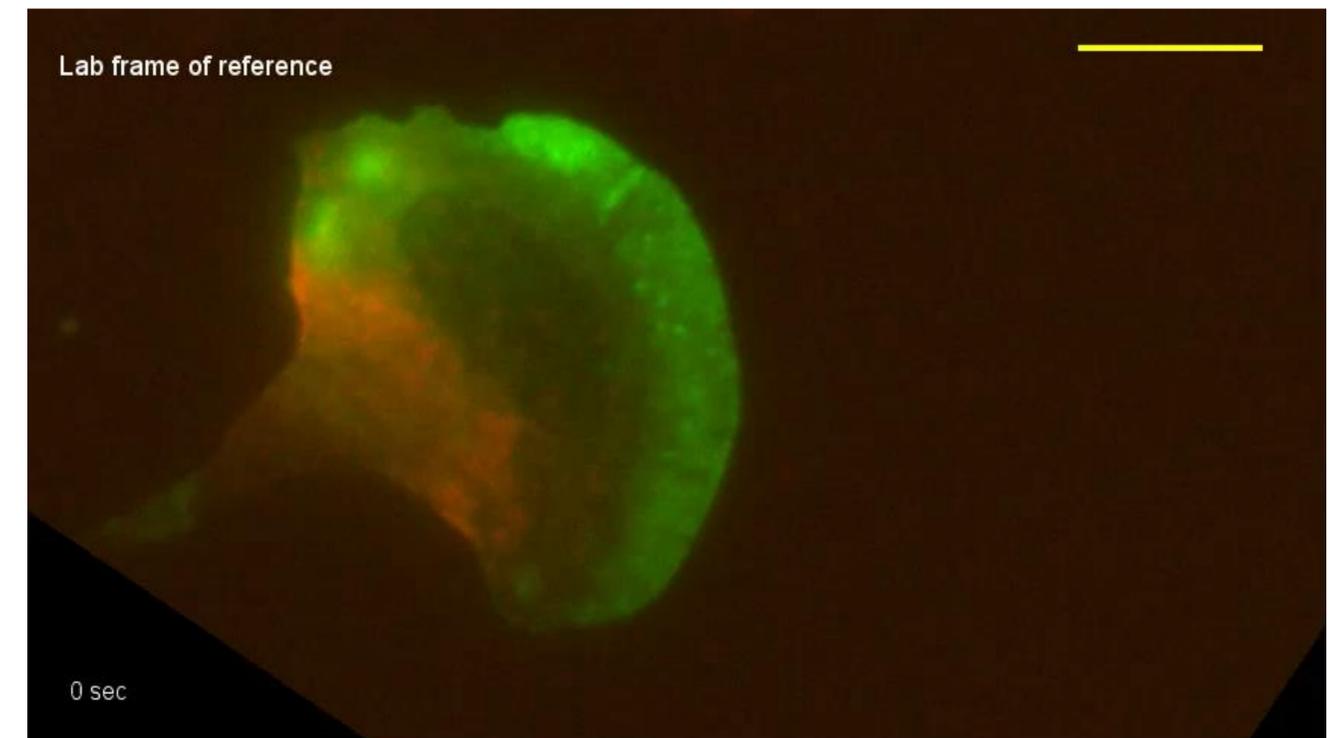
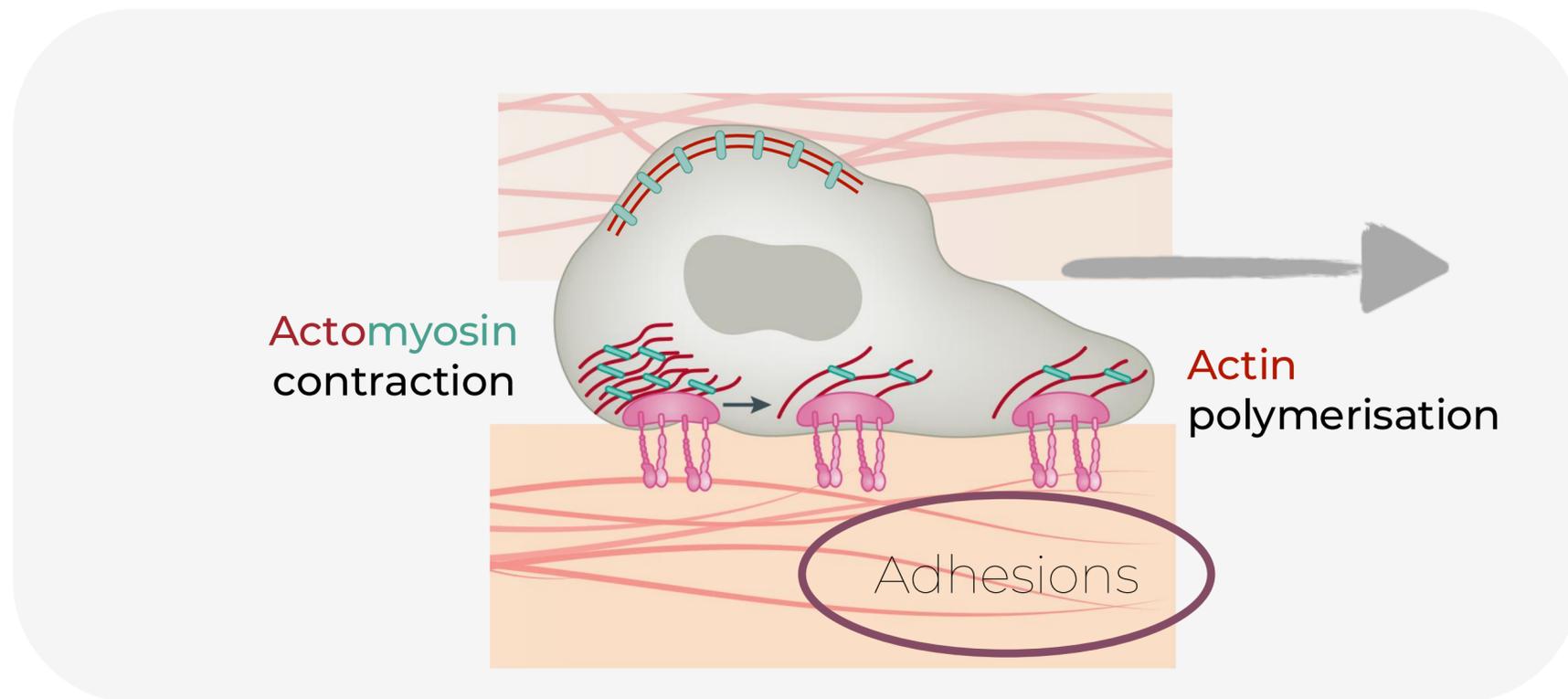
Driving forces



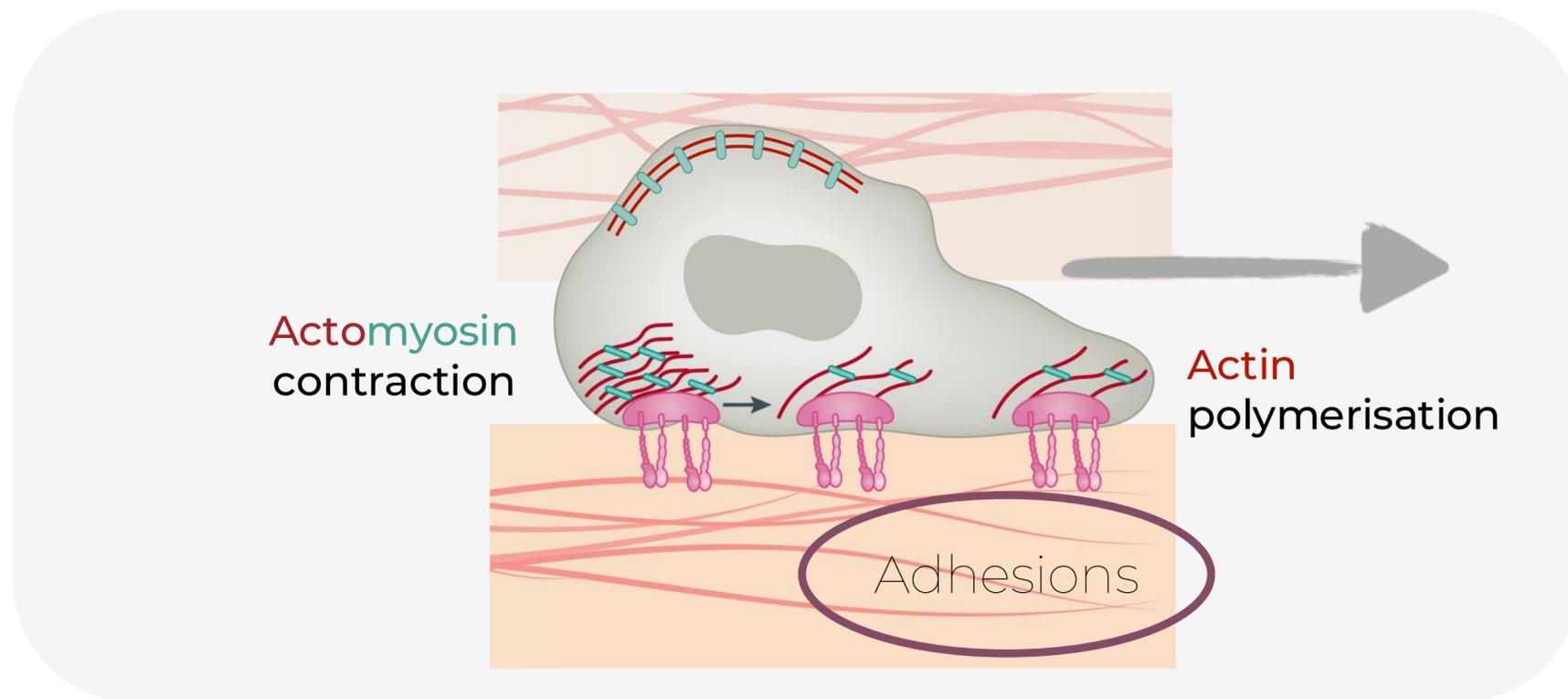
1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation & transmission**



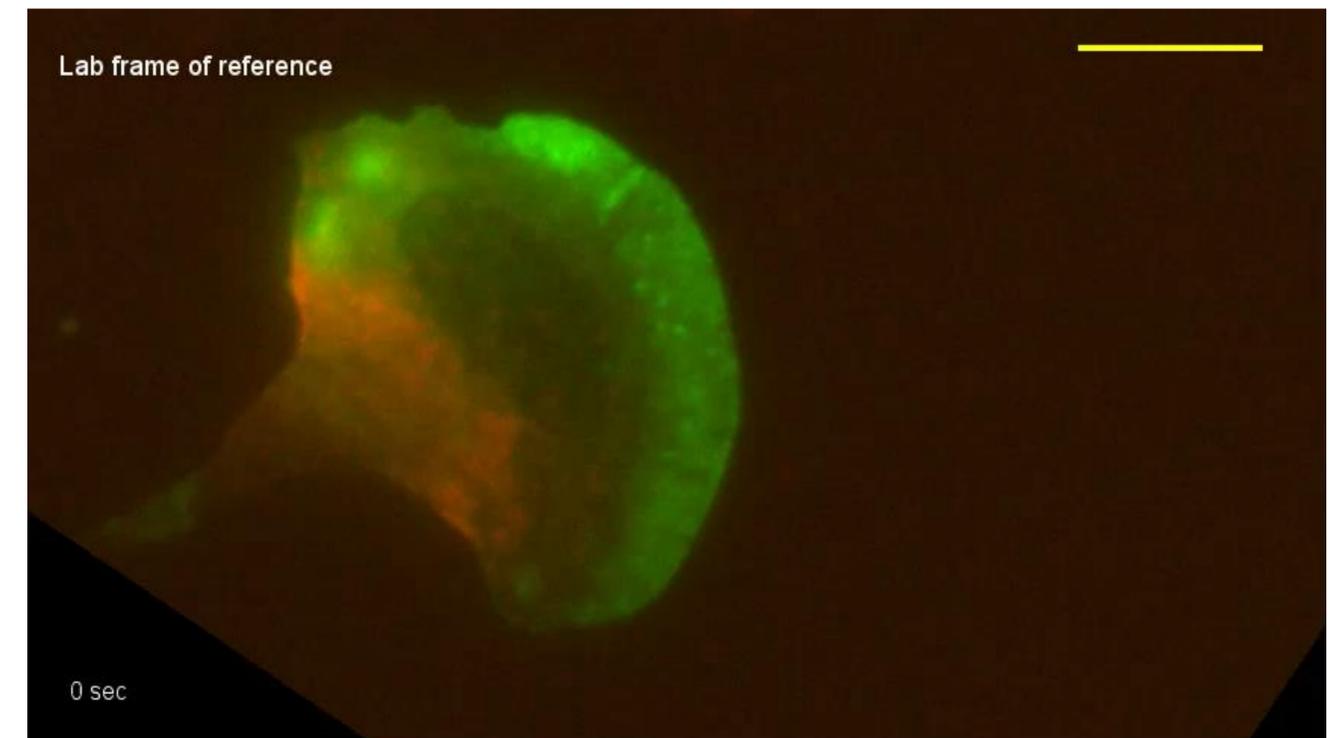
1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation & transmission**



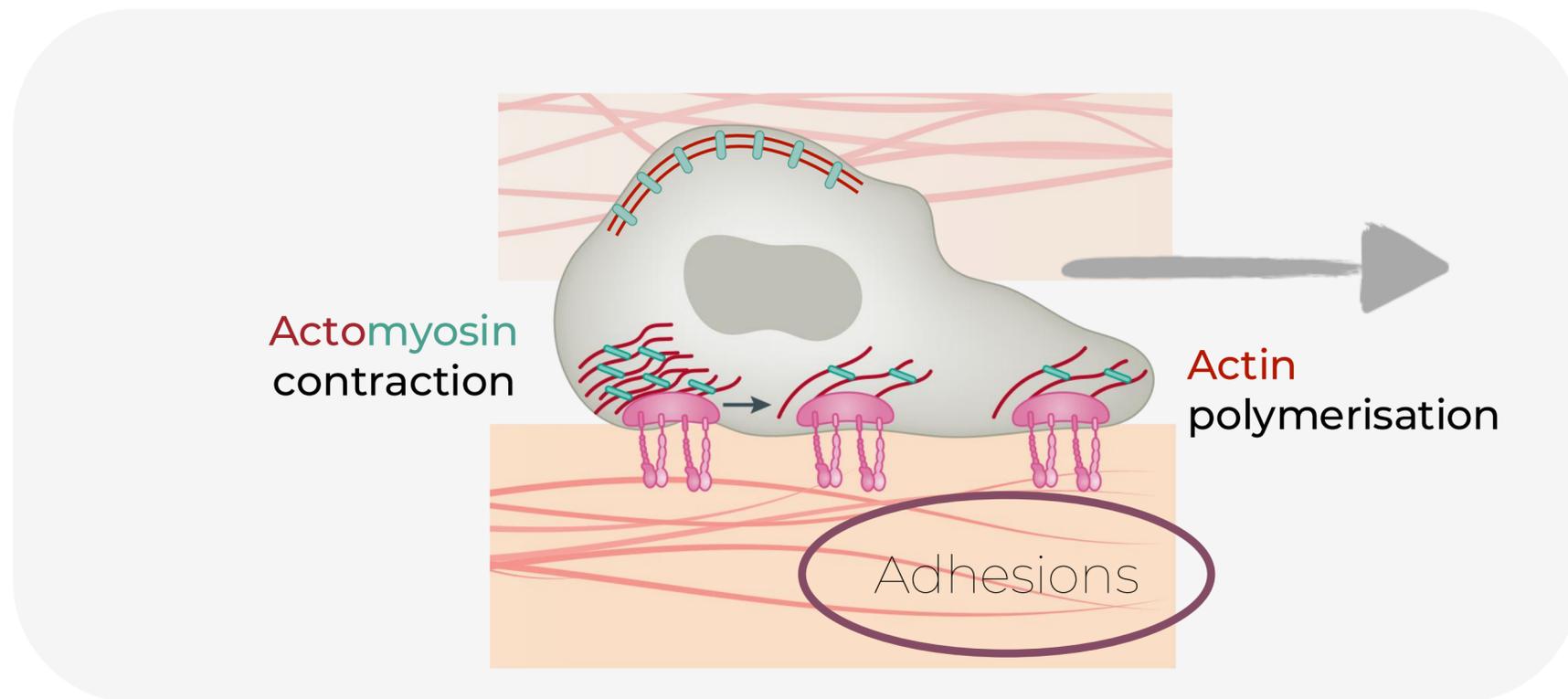
1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation & transmission**



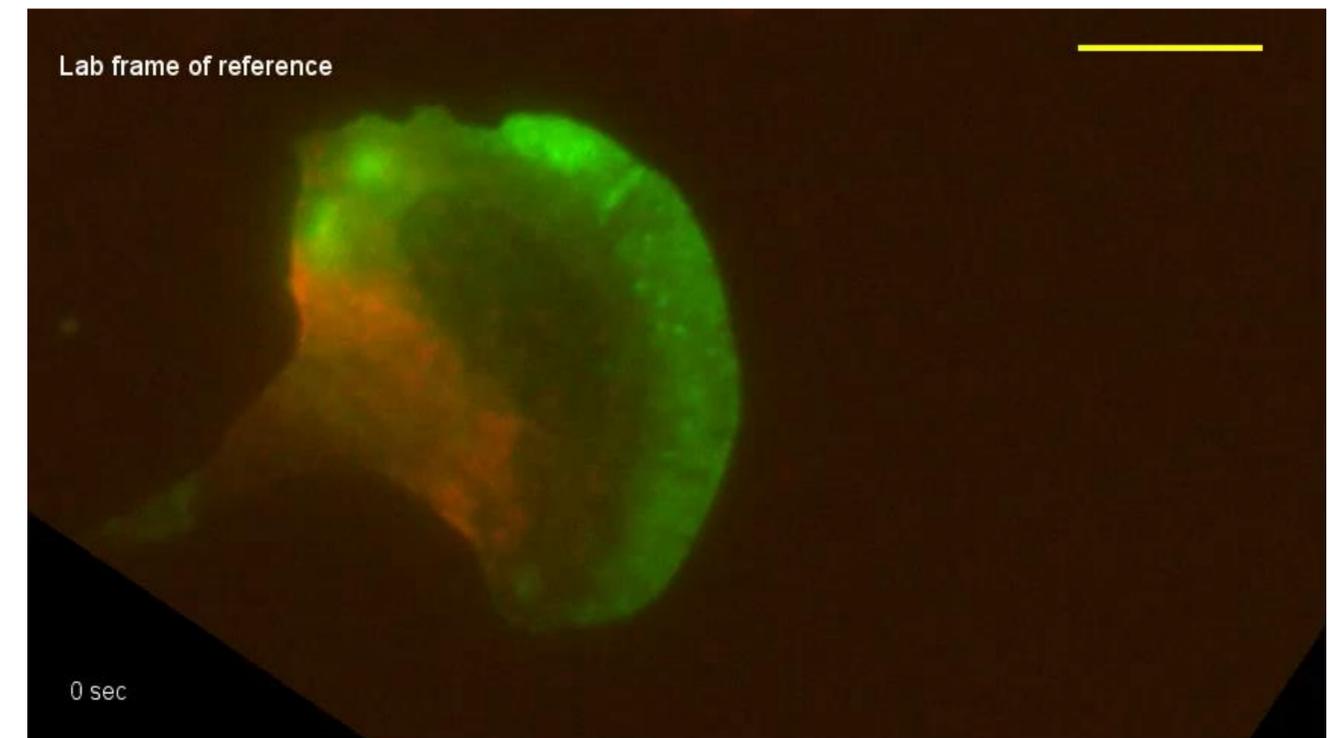
Mesenchymal migration



1. Signal from the environment
2. Signal sensing
3. Transmitting the signal inside the cell to define front-back
4. **Force generation & transmission**

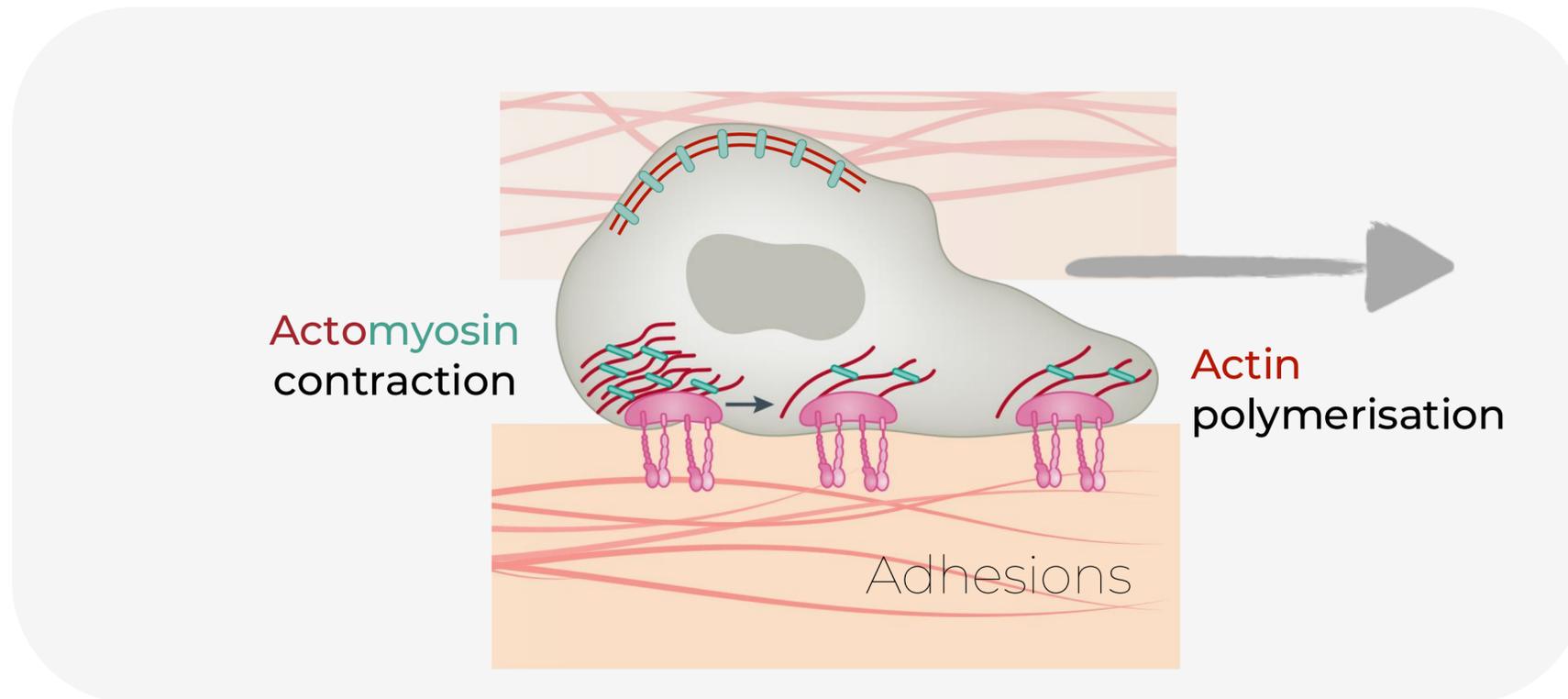


Mesenchymal migration

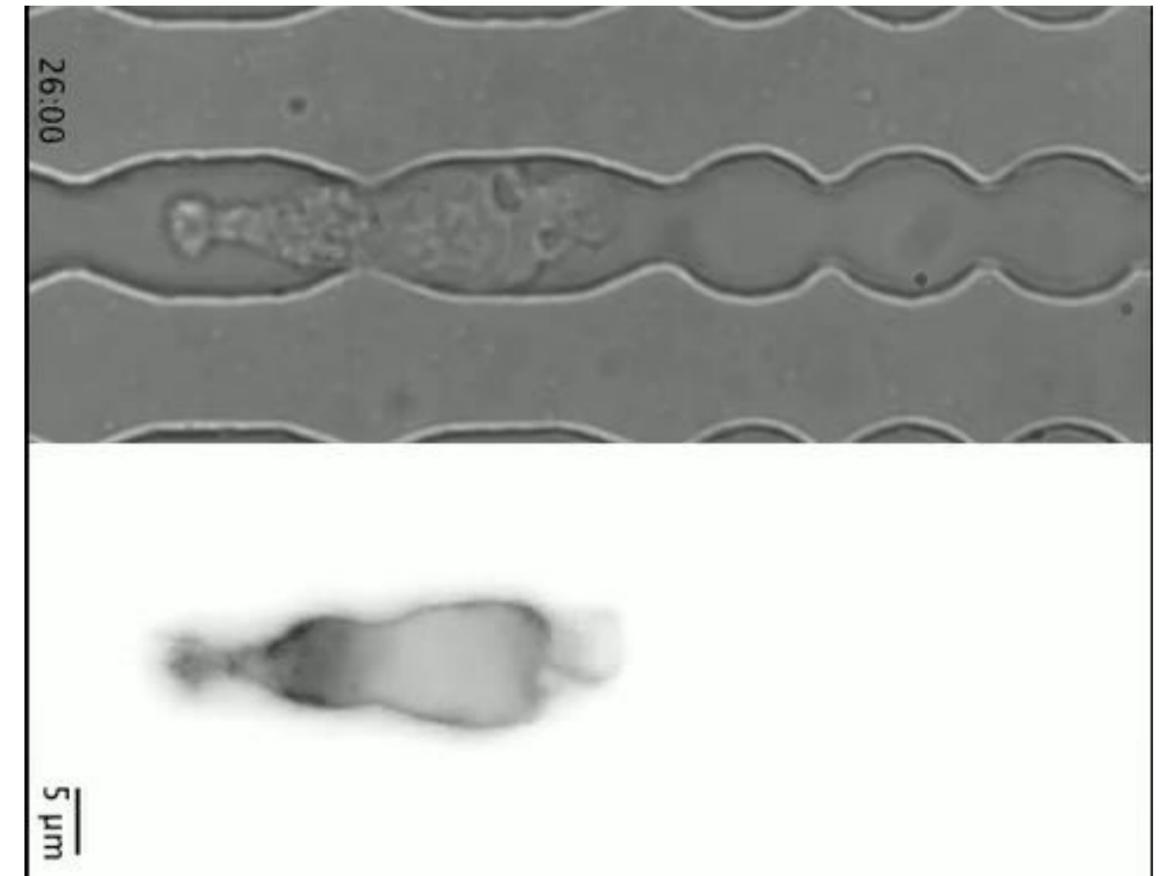


Are *adhesions* required for force transmission?

# Q: Are *adhesions* required for force transmission?

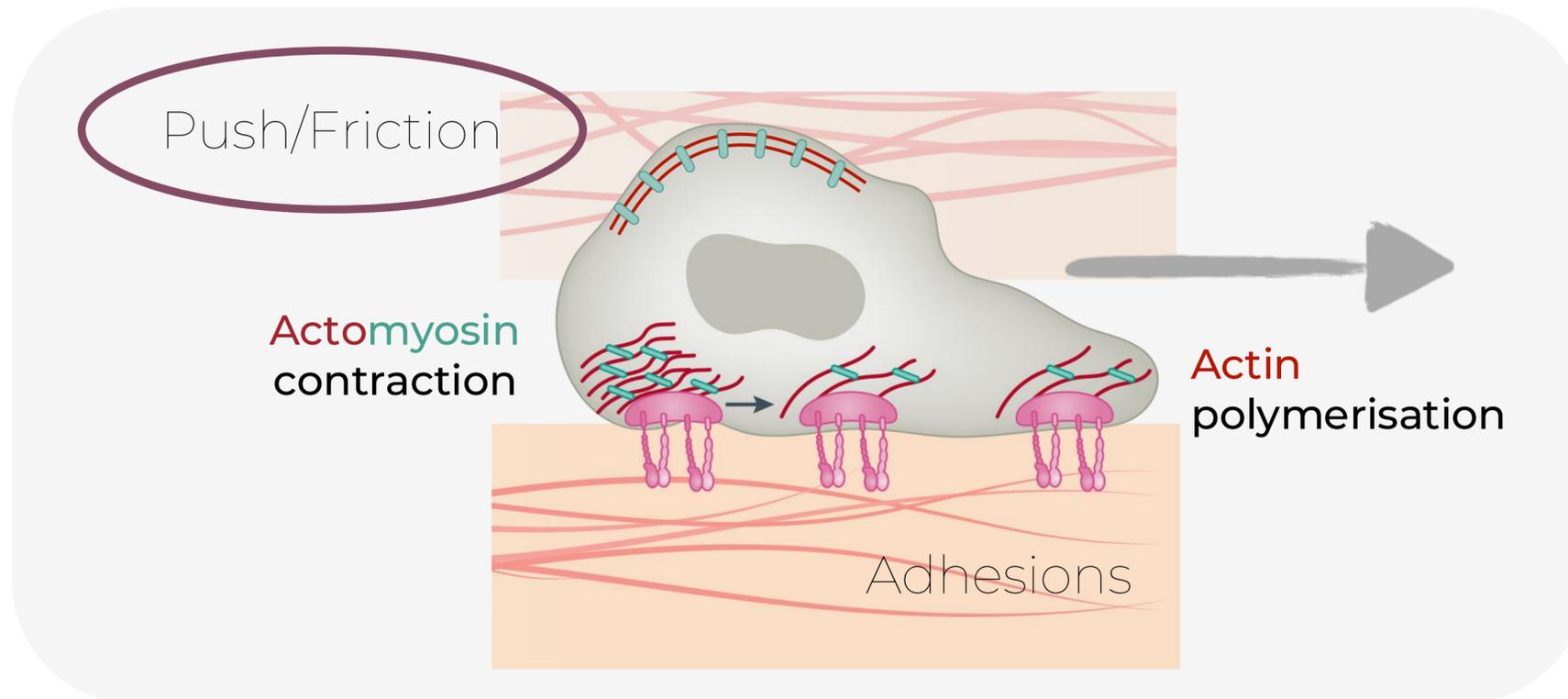


Immune cell moving without adhesion (EDTA, inactivates integrins)

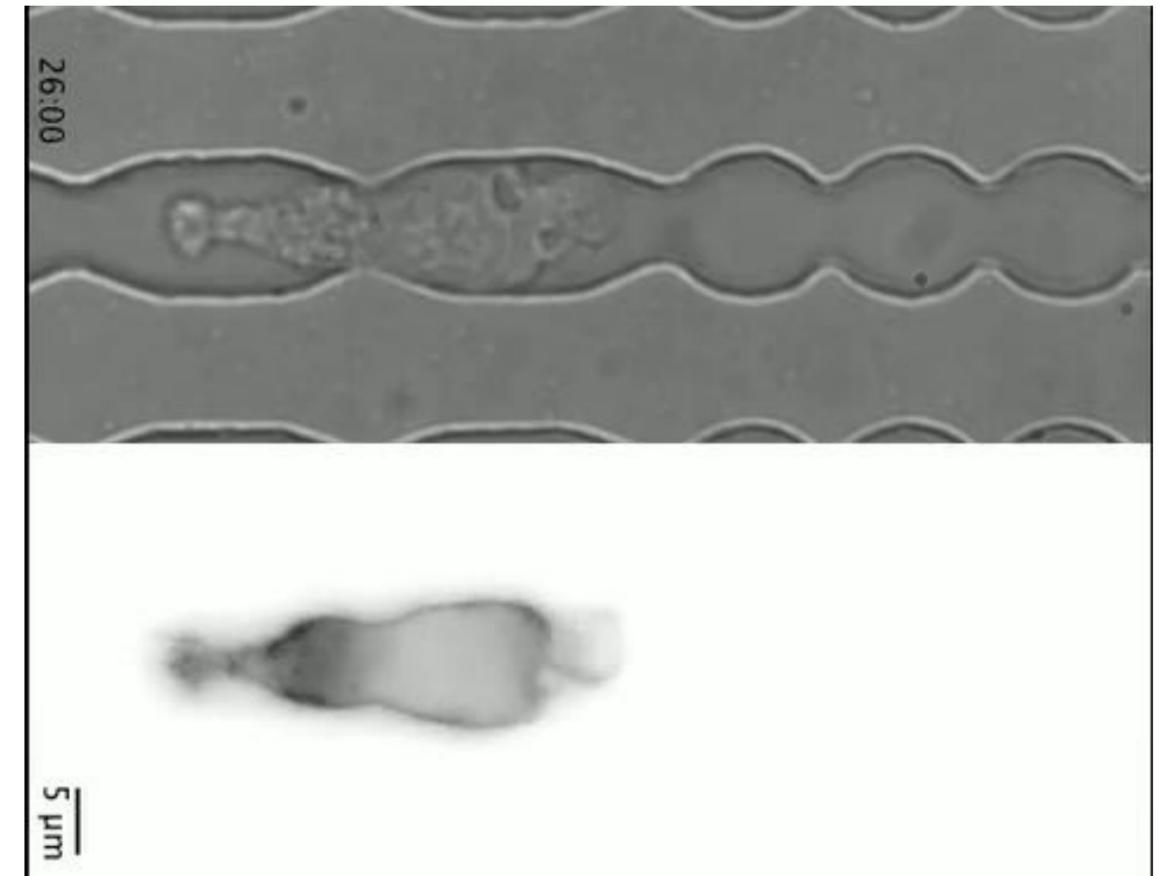


Actin Lifeact-GFP

# Q: Are *adhesions* required for force transmission?

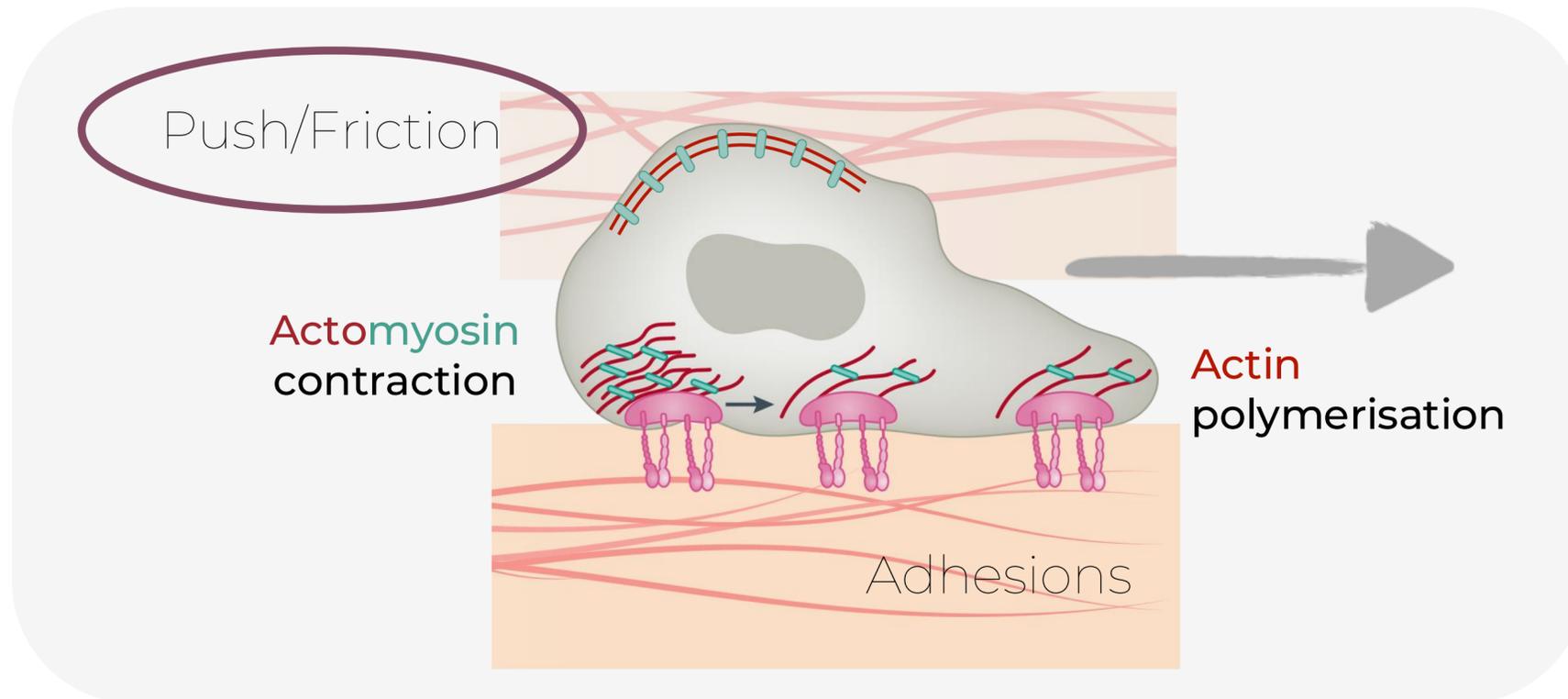


Immune cell moving without adhesion (EDTA, inactivates integrins)



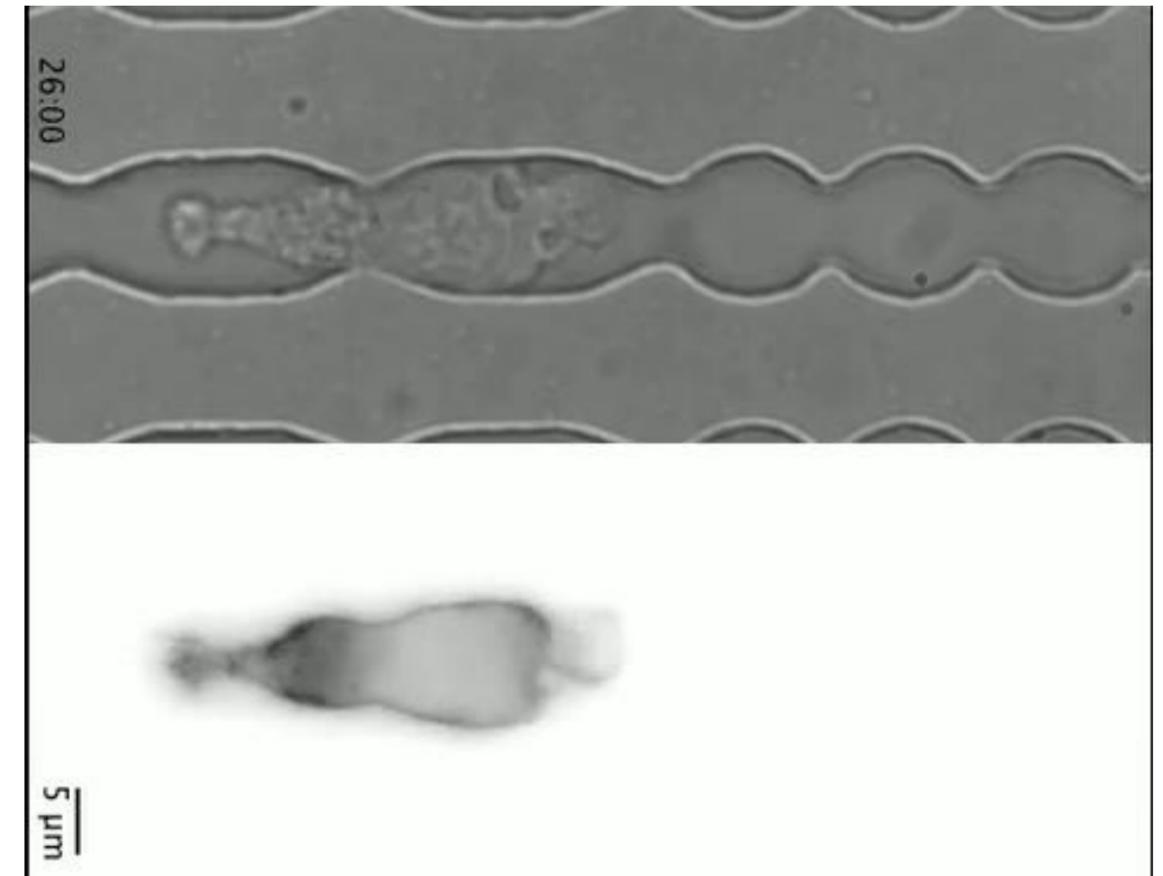
Actin Lifeact-GFP

# Q: Are *adhesions* required for force transmission?



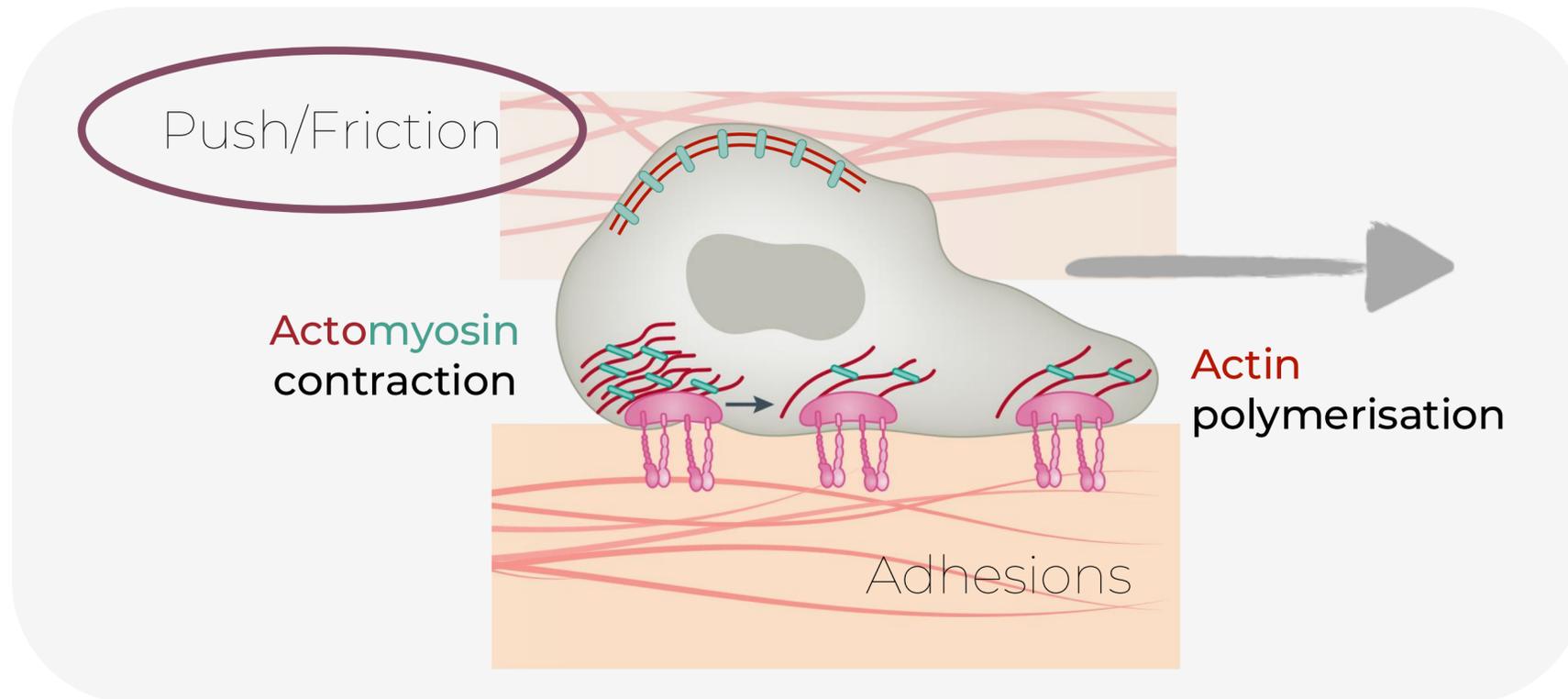
Ameboid migration

Immune cell moving without adhesion (EDTA, inactivates integrins)



Actin Lifeact-GFP

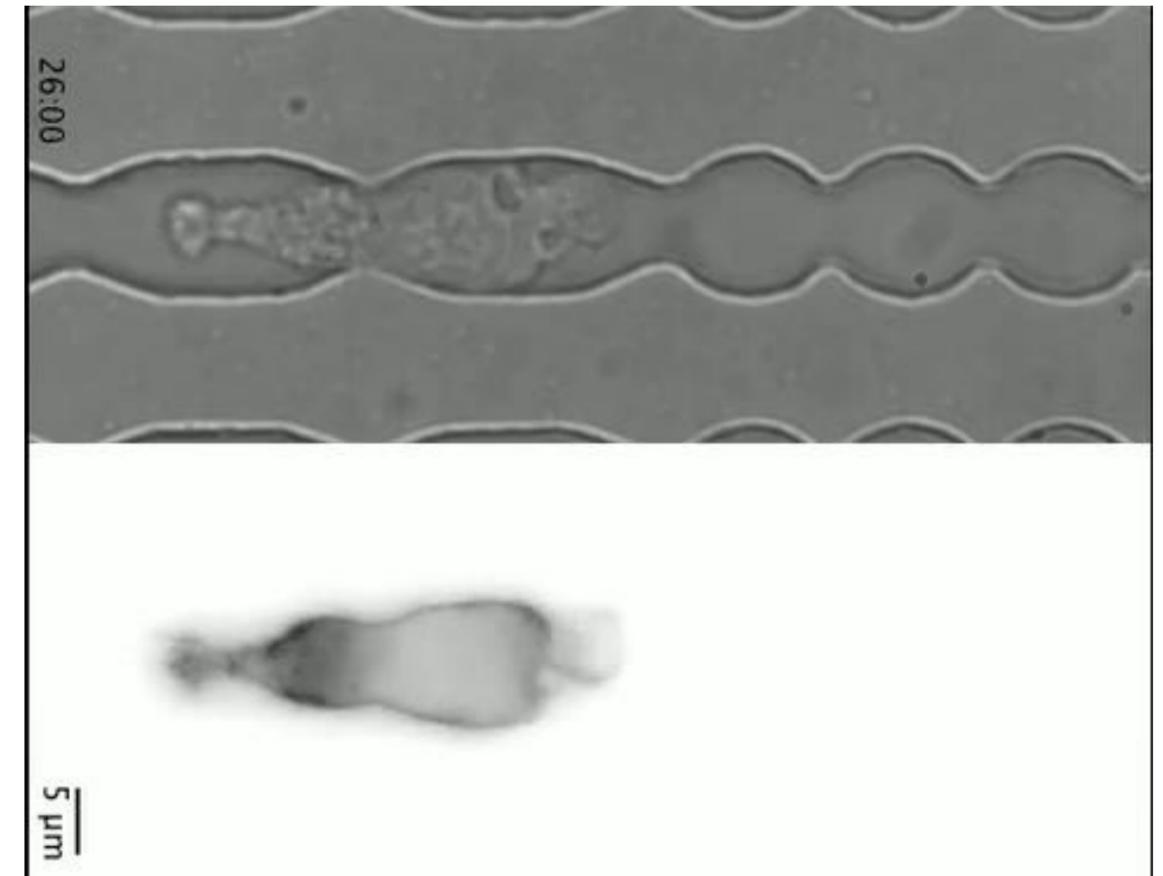
Q: Are *adhesions* required for force transmission?



Ameboid migration

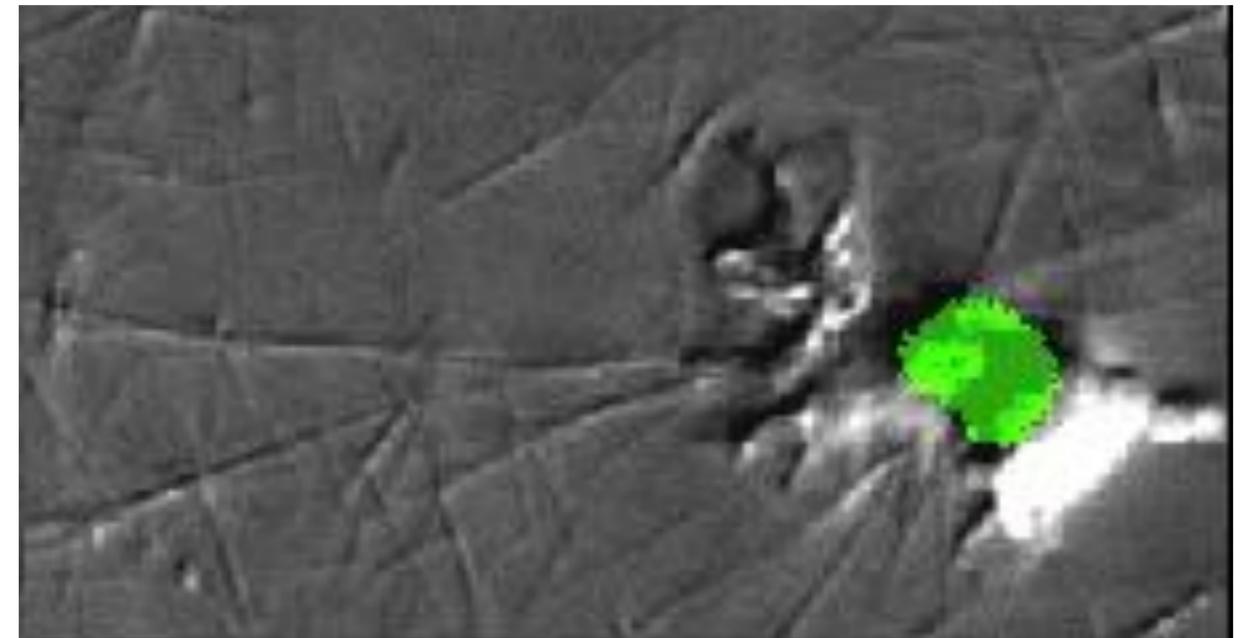
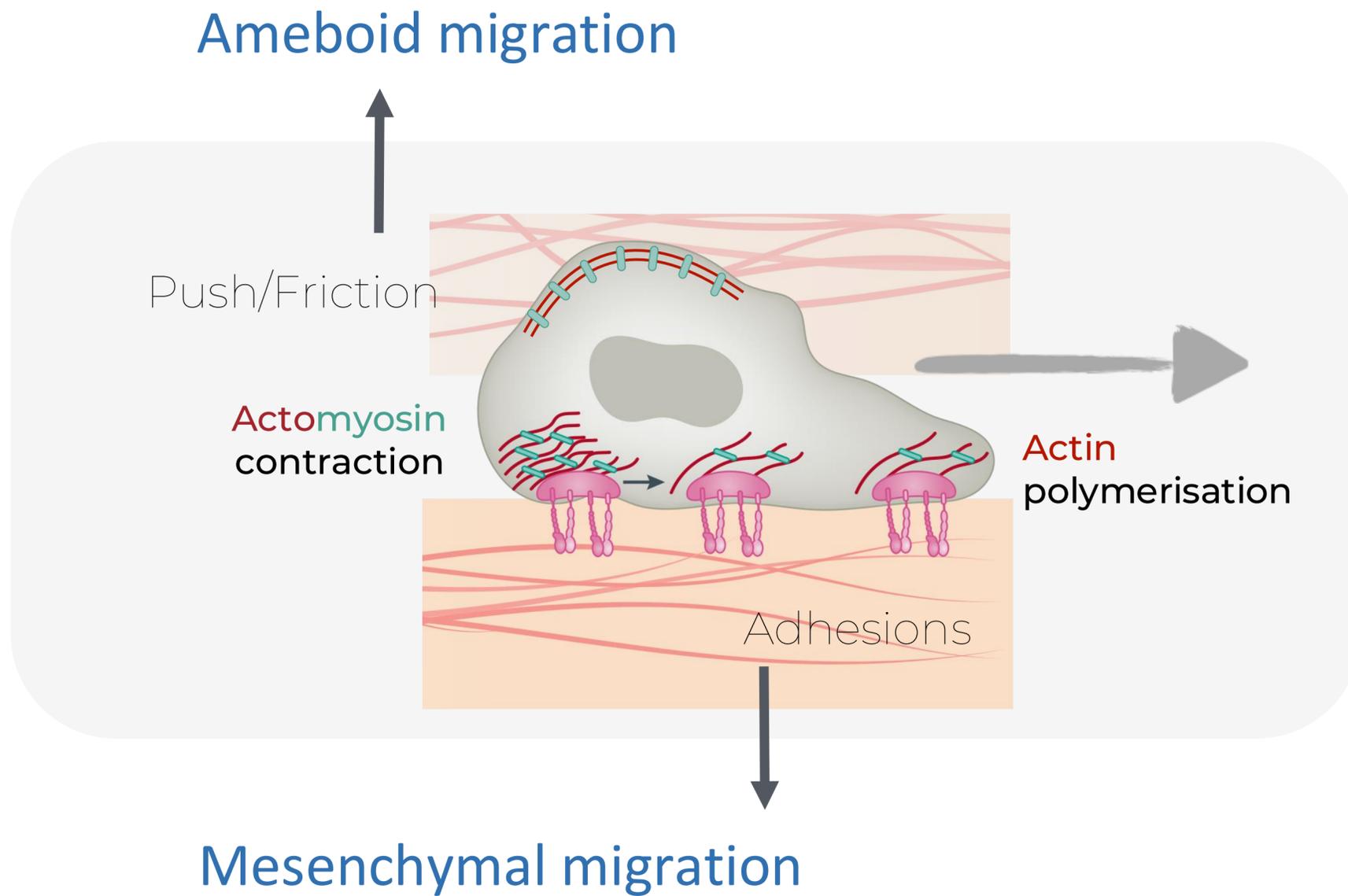
A: No, cells can migrate using friction against substrate as force transmission mechanism

Immune cell moving without adhesion (EDTA, inactivates integrins)



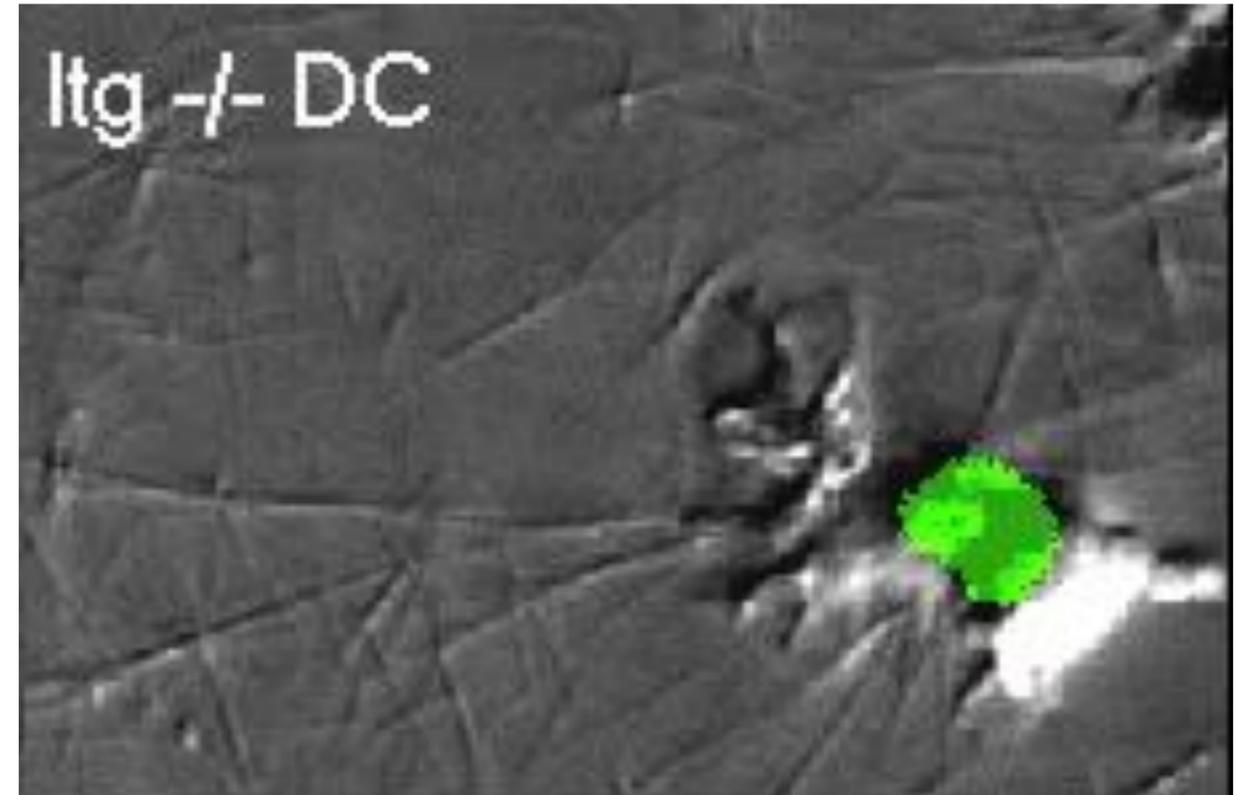
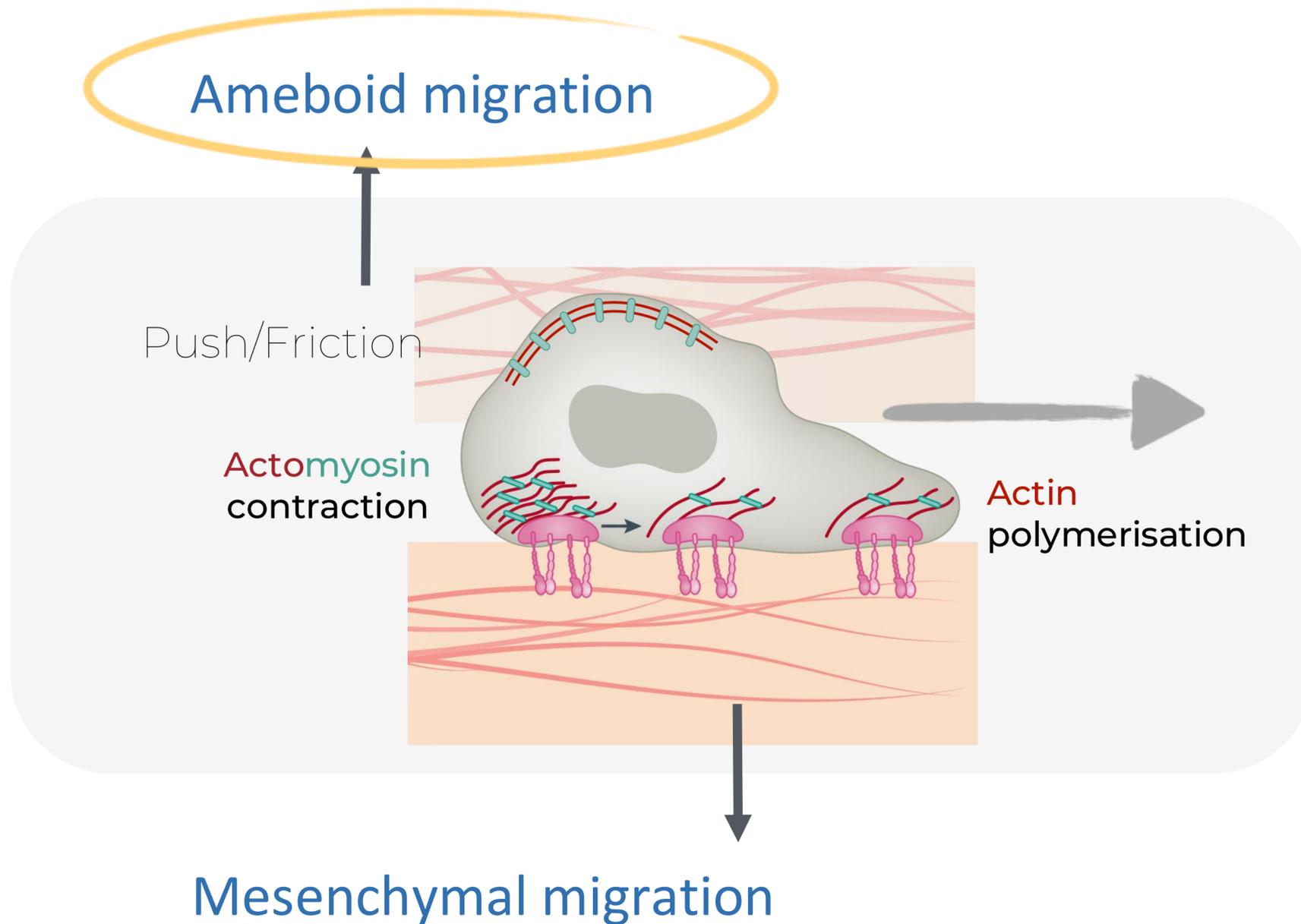
Actin Lifeact-GFP

Guess: Ameboid or mesenchymal?



Directed migration of a dendritic cell towards CCL19 (left) in a 3D collagen gel

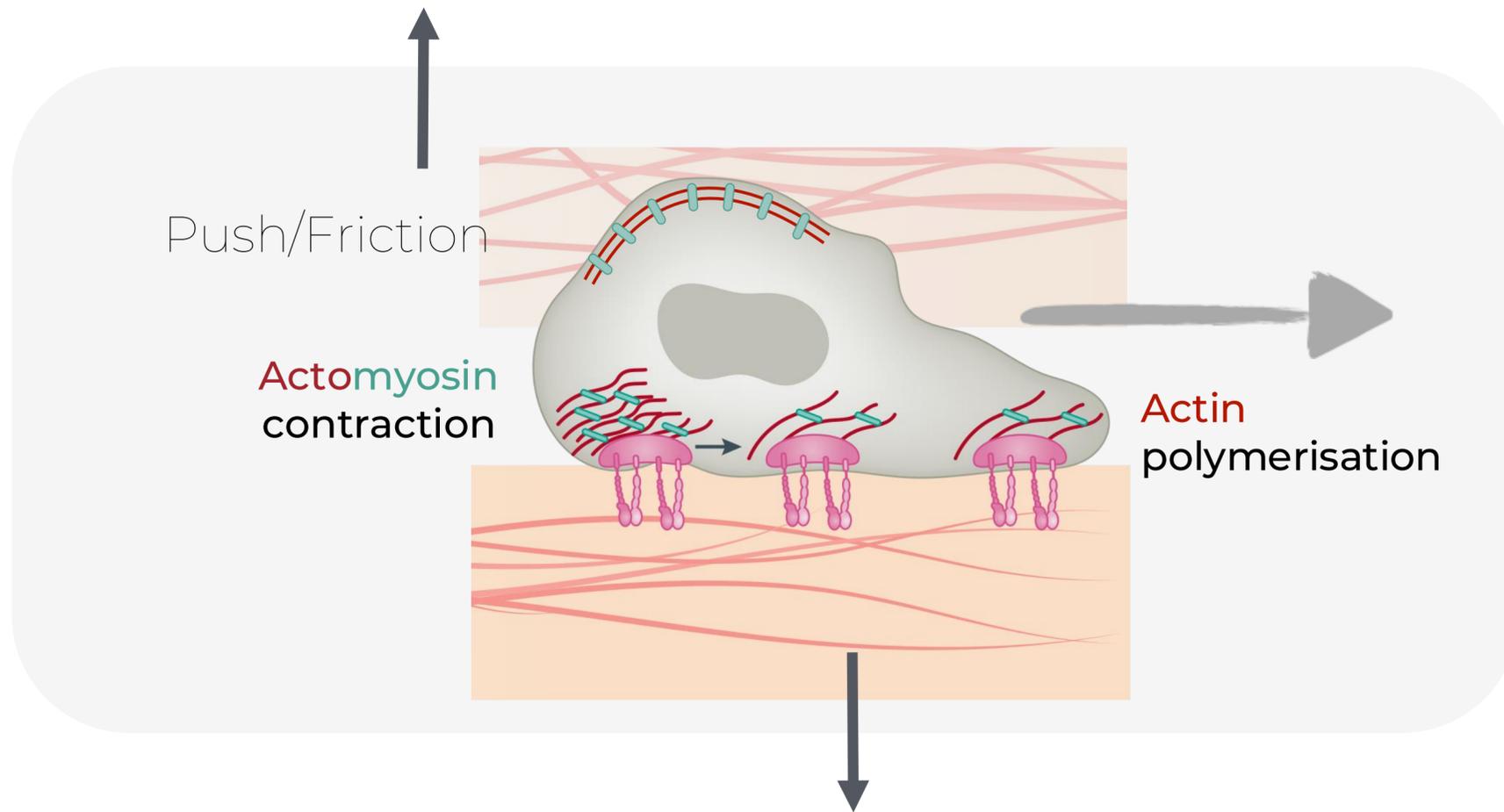
Guess: Ameboid or mesenchymal?



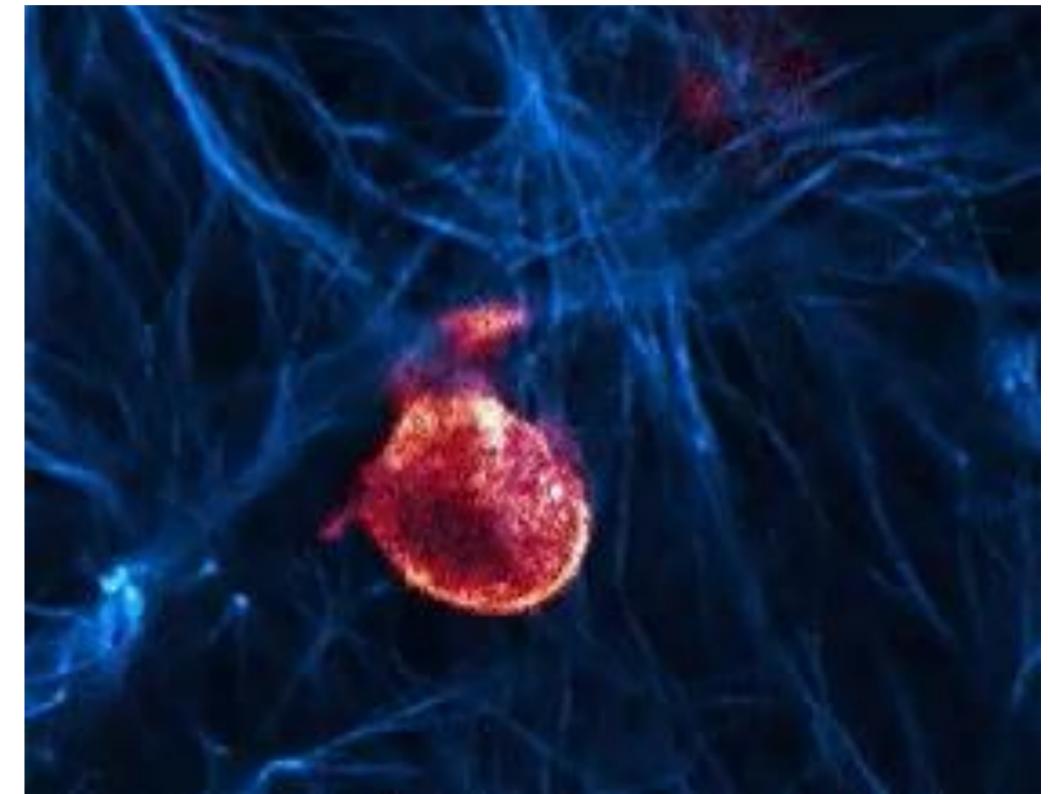
Directed migration of a dendritic cell towards CCL19 (left) in a 3D collagen gel

Guess: Ameboid or mesenchymal?

Ameboid migration



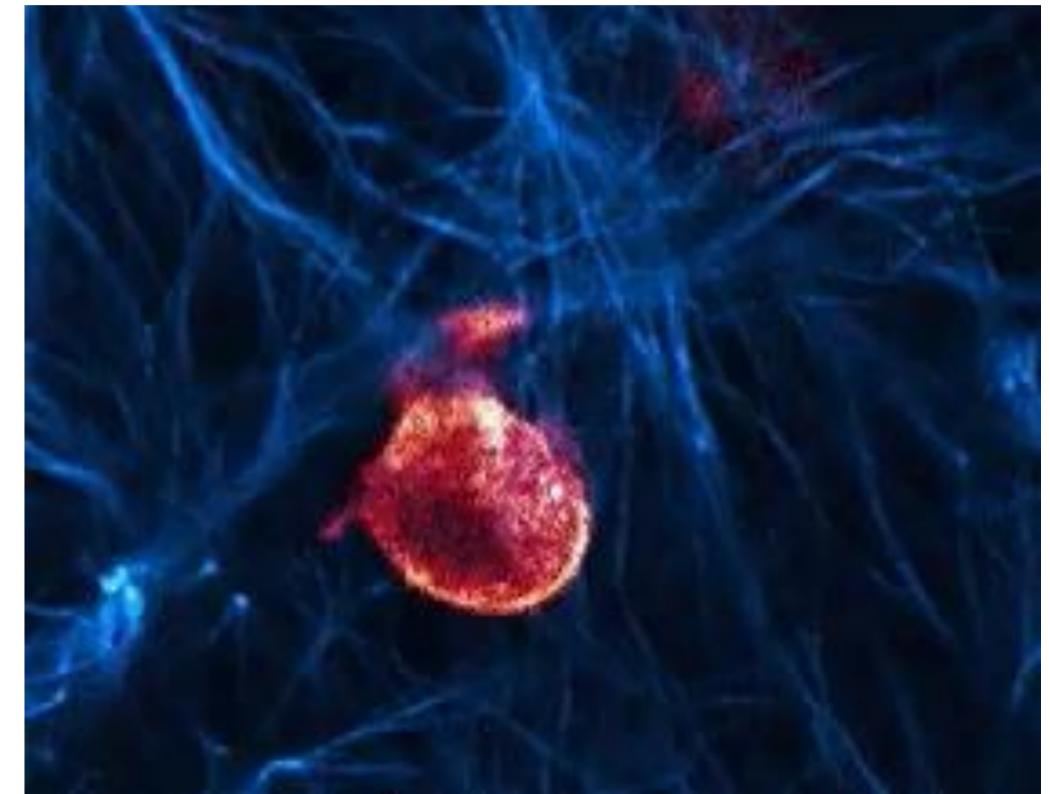
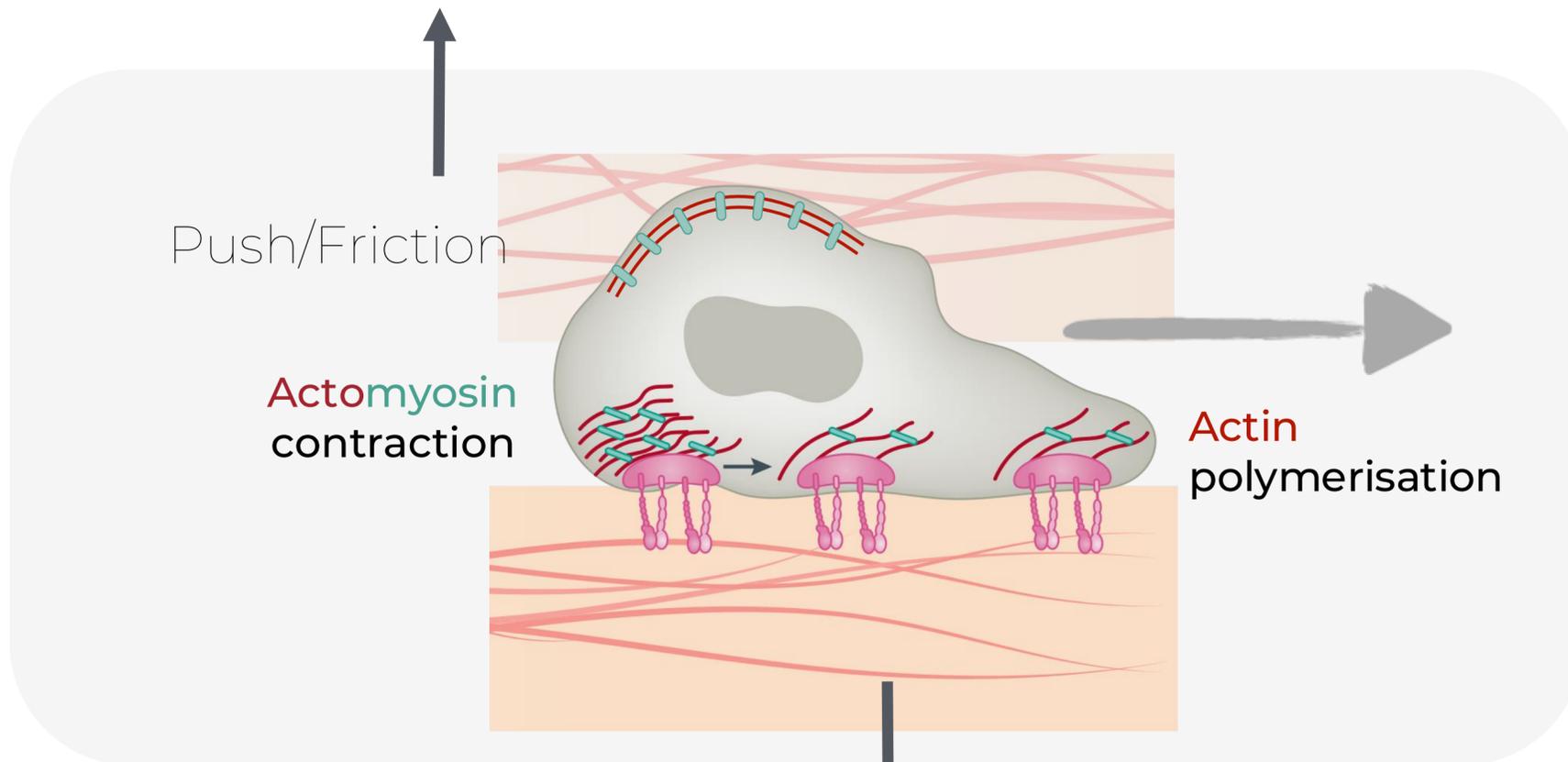
Mesenchymal migration



actin, collagen

Guess: Ameboid or mesenchymal?

Ameboid migration



actin, collagen

Mesenchymal migration

# Speed of vertebrate cell migration

Ameboid

VS

Mesenchymal



# Speed of vertebrate cell migration

In a non adherent channel

Max speed ~ 5  $\mu\text{m}/\text{min}$  \*

Adhesion



No adhesion

T cells

Actin Nucleus

\* neutrophils are super fast 10-30  $\mu\text{m}/\text{min}$ ,  
but they are much smaller

# Speed of vertebrate cell migration

In a non adherent channel

Max speed ~ 5  $\mu\text{m}/\text{min}$



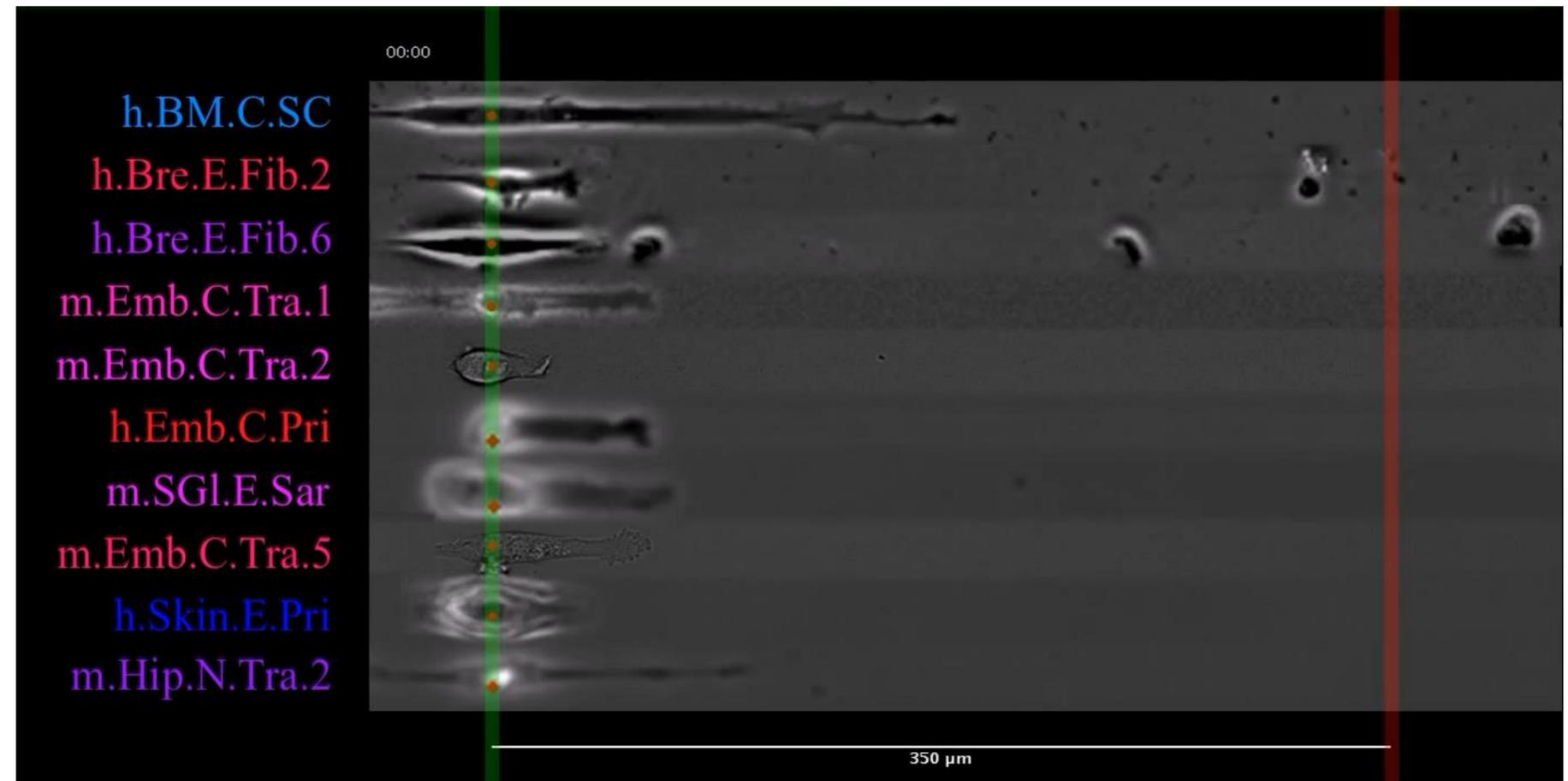
No adhesion

T cells

Nucleus

On a substrate (fibronectin)

~ 1  $\mu\text{m}/\text{min}$



Cancer cells

“World Cell Race” Maiuri P. et al. (2012)

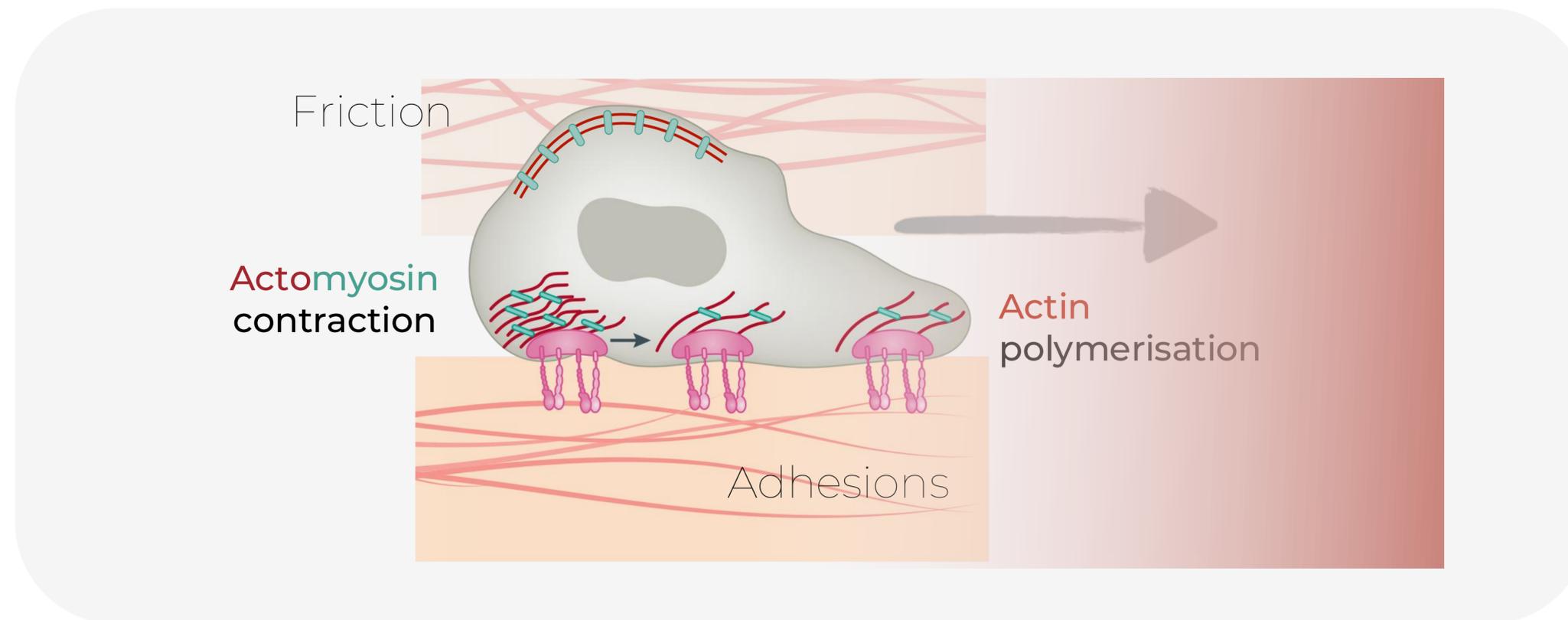
# Principles of directed cell migration

1. Cue

2. Sensing

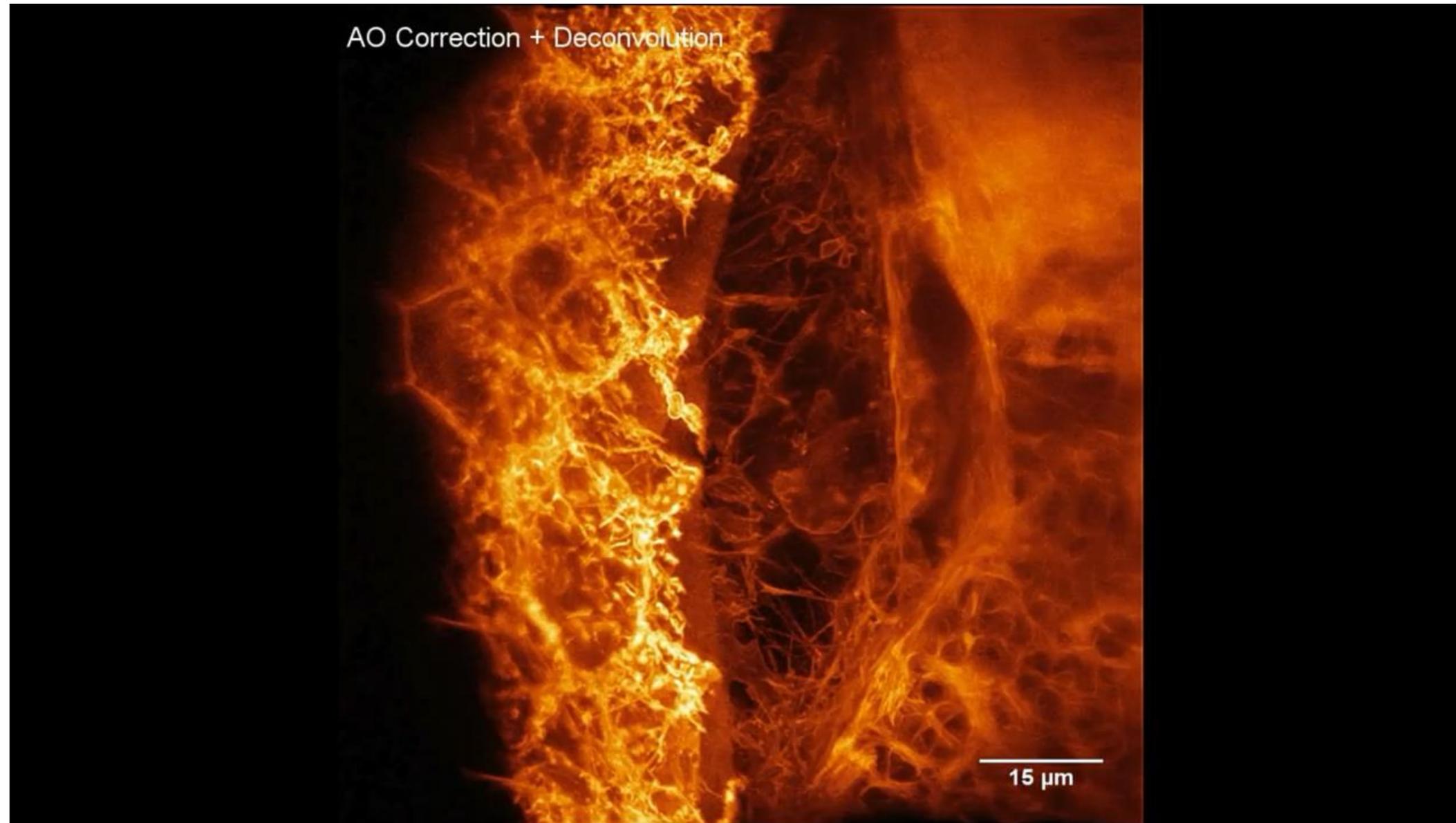
3. Polarisation

4. Motion execution



# Cell motility in vivo in 3D

Immune cells in the inner ear of zebrafish embryo



membrane, particles

# Outline:

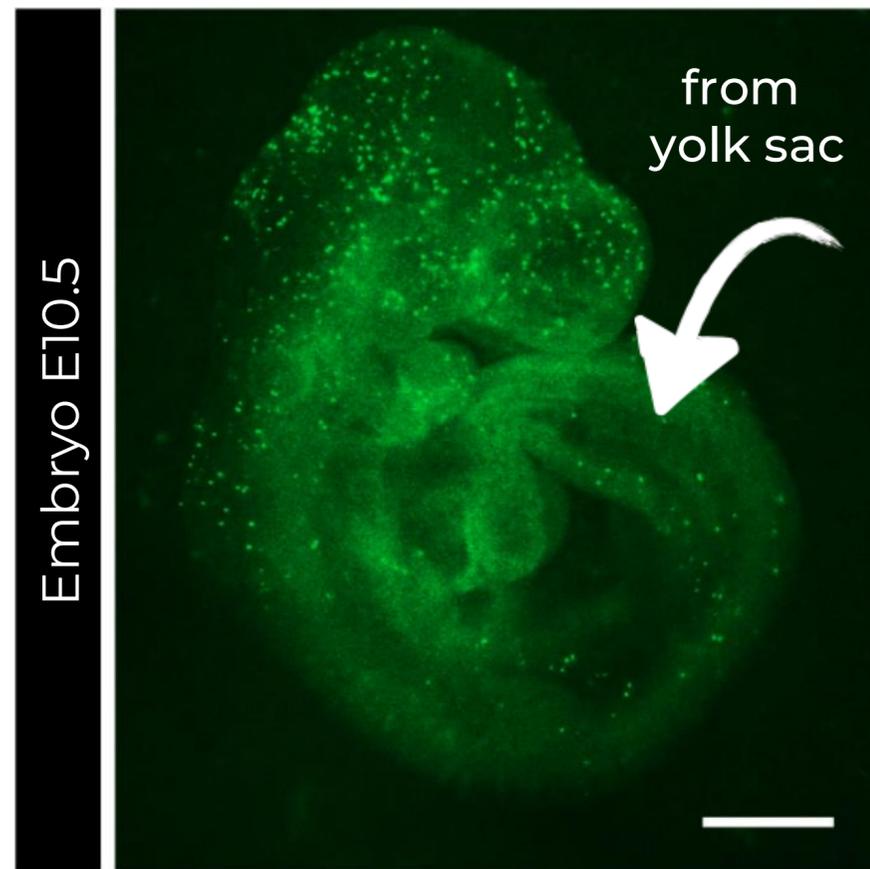
I. Overview of *directed cell migration*

II. *Invasion* as a special type of directed migration

How do cells overcome physical barriers on their way?

# Cell invasion into tissues through *physical barriers* is essential

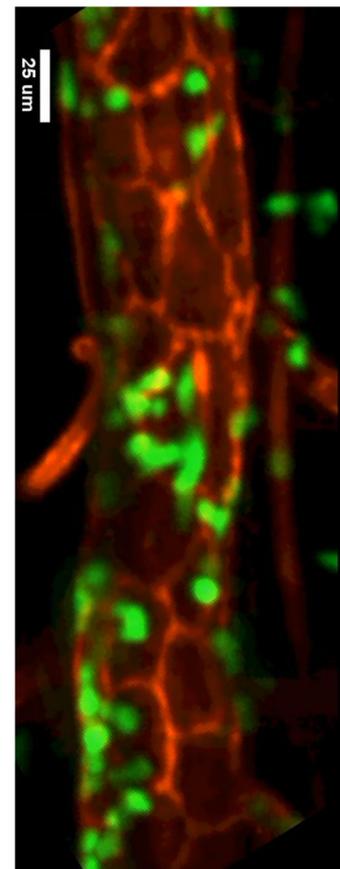
## Development



Mouse embryonic **macrophages**

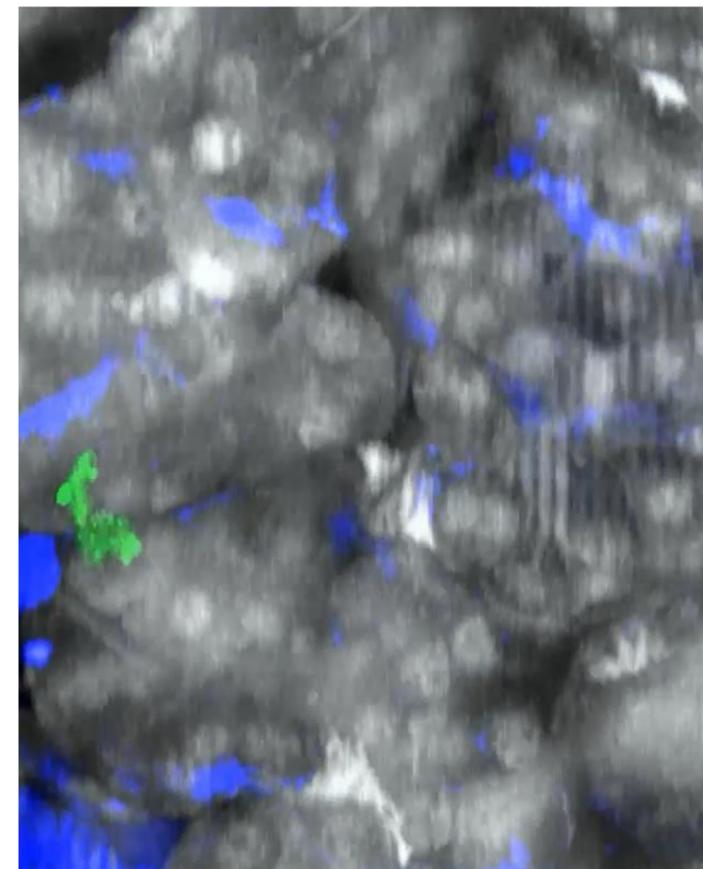
Stremmel C. et al., 2018

## Homeostasis / Immunity



**Neutrophils, endothelium**

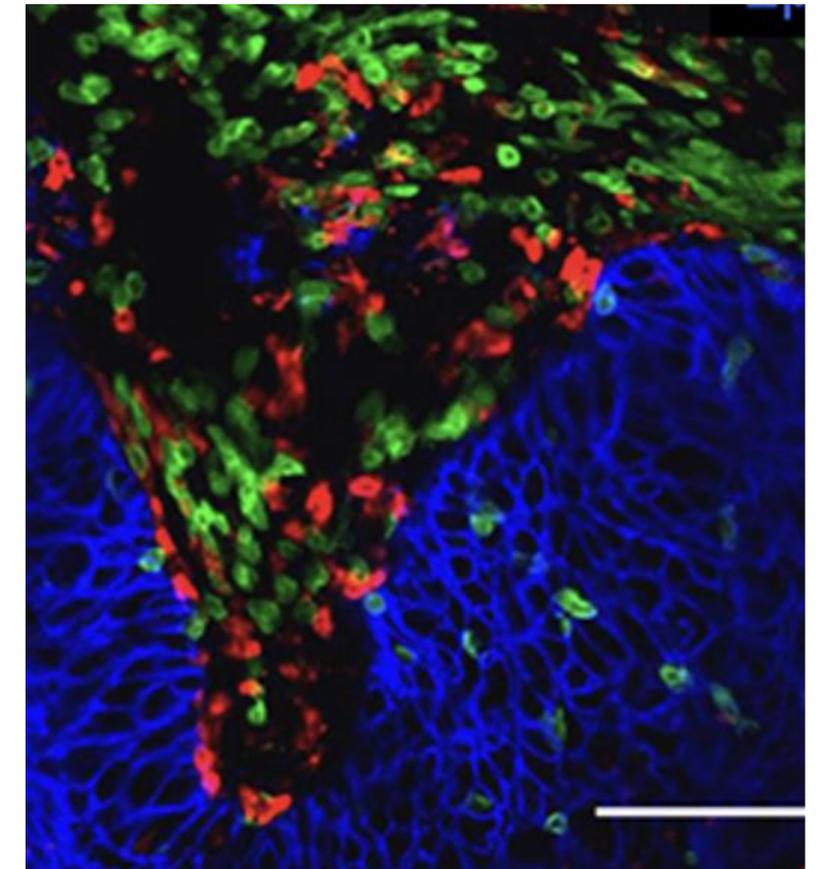
Sullivan D. et al., 2019



**T-cells** in mouse salivary gland

Stolp B., D. et al., 2020

## Disease

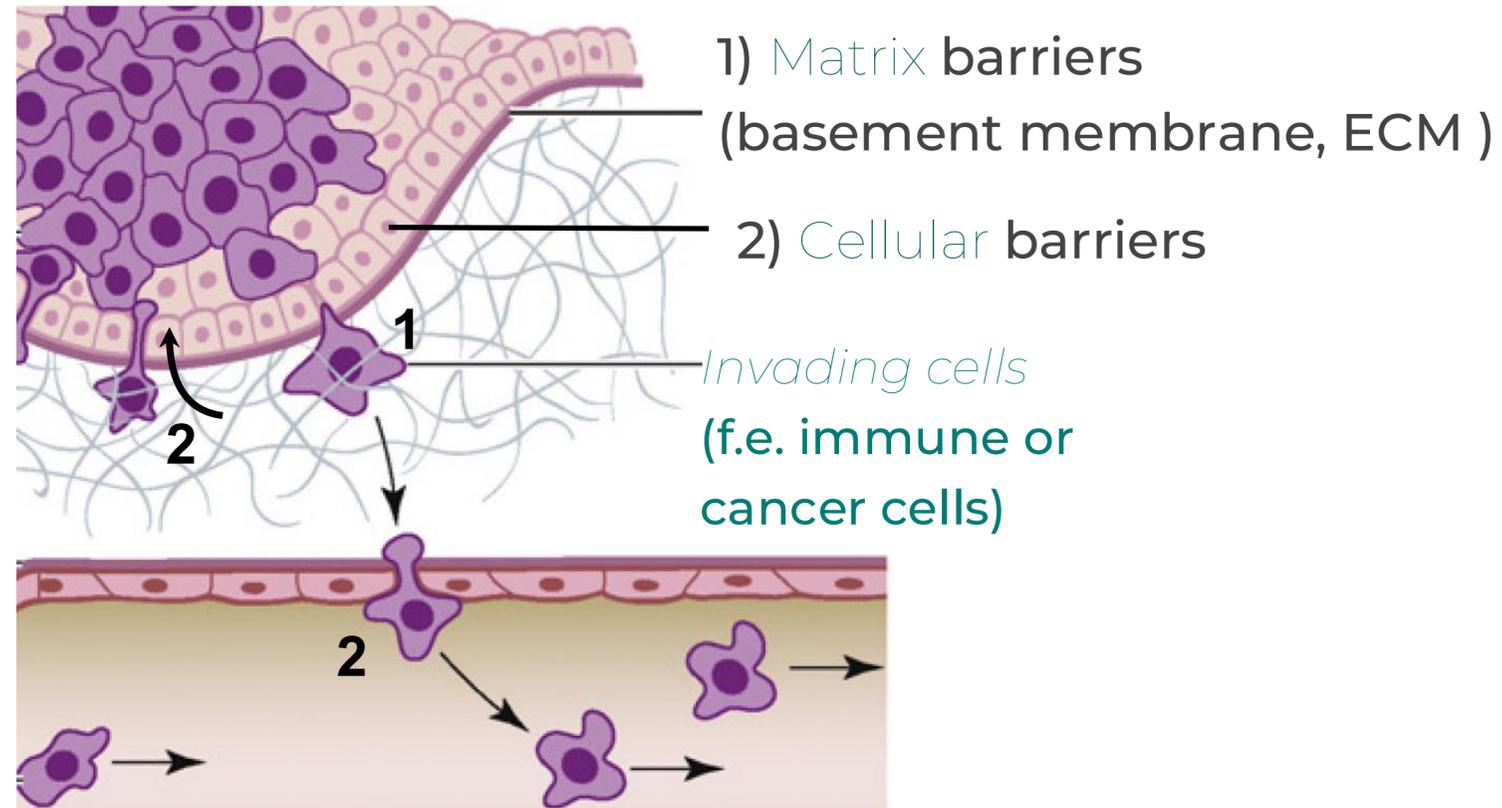


**Macrophages, T-cells** in the tumour

Peranzoni E. et al., 2018

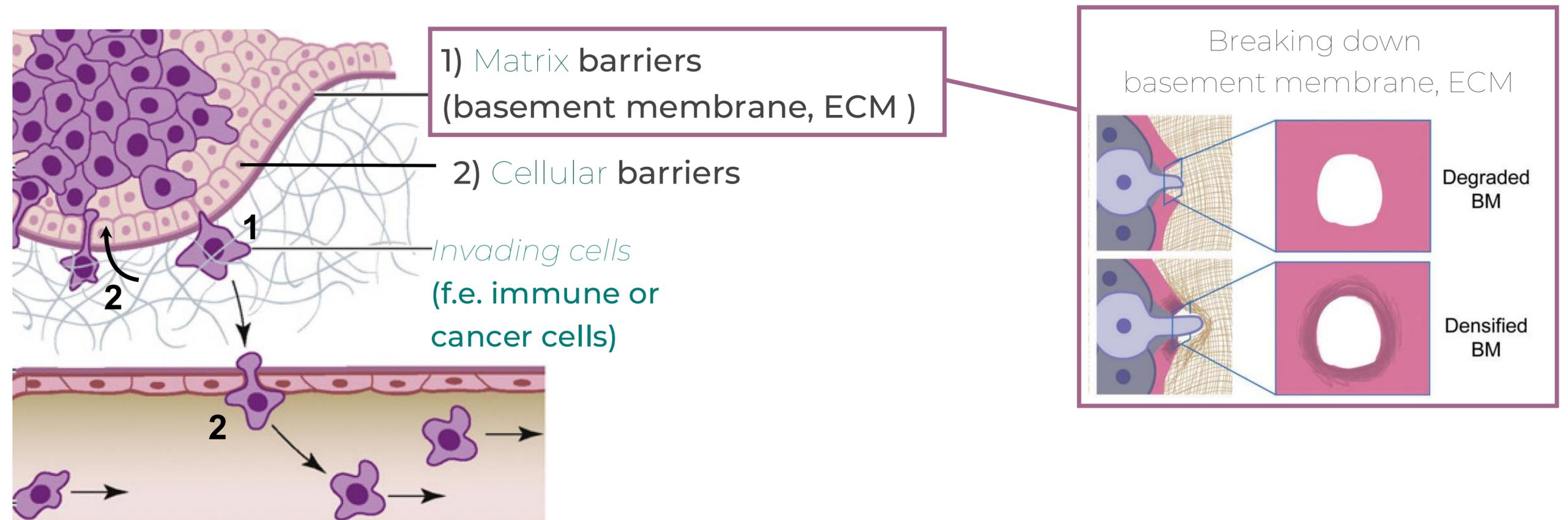
# How do **invading cells** overcome tissue barriers?

What is known



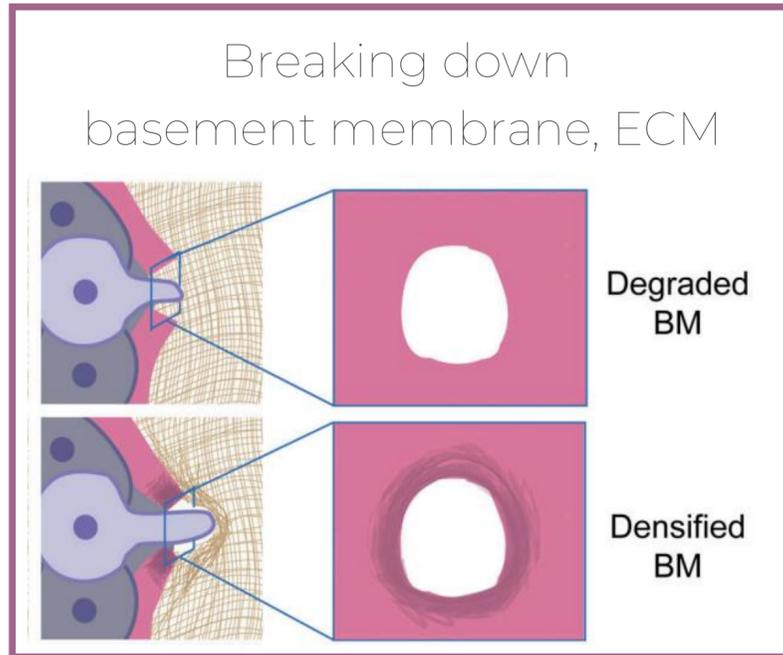
# How do *invading cells* overcome tissue barriers ?

What is known

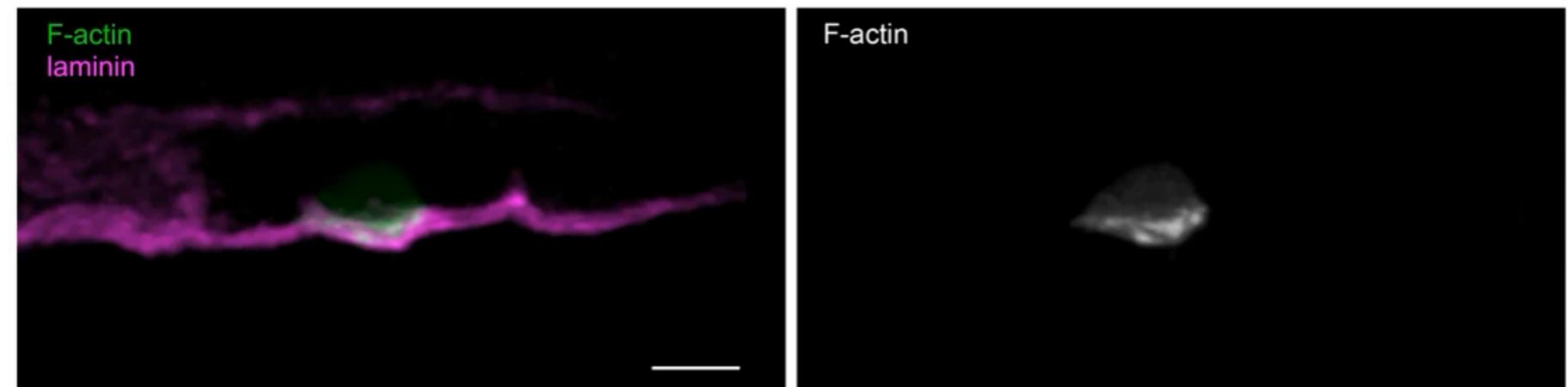
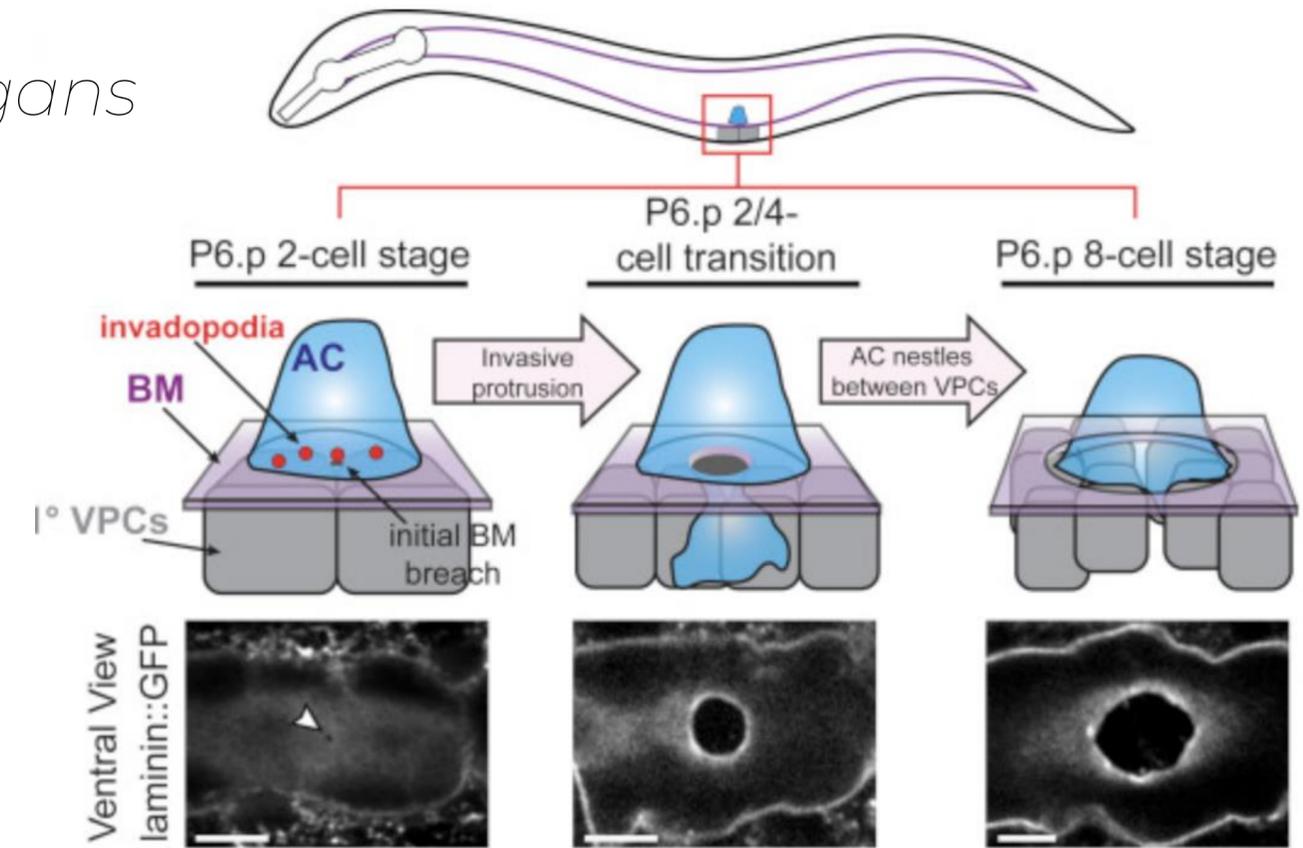


# How do *invading cells* overcome matrix barrier?

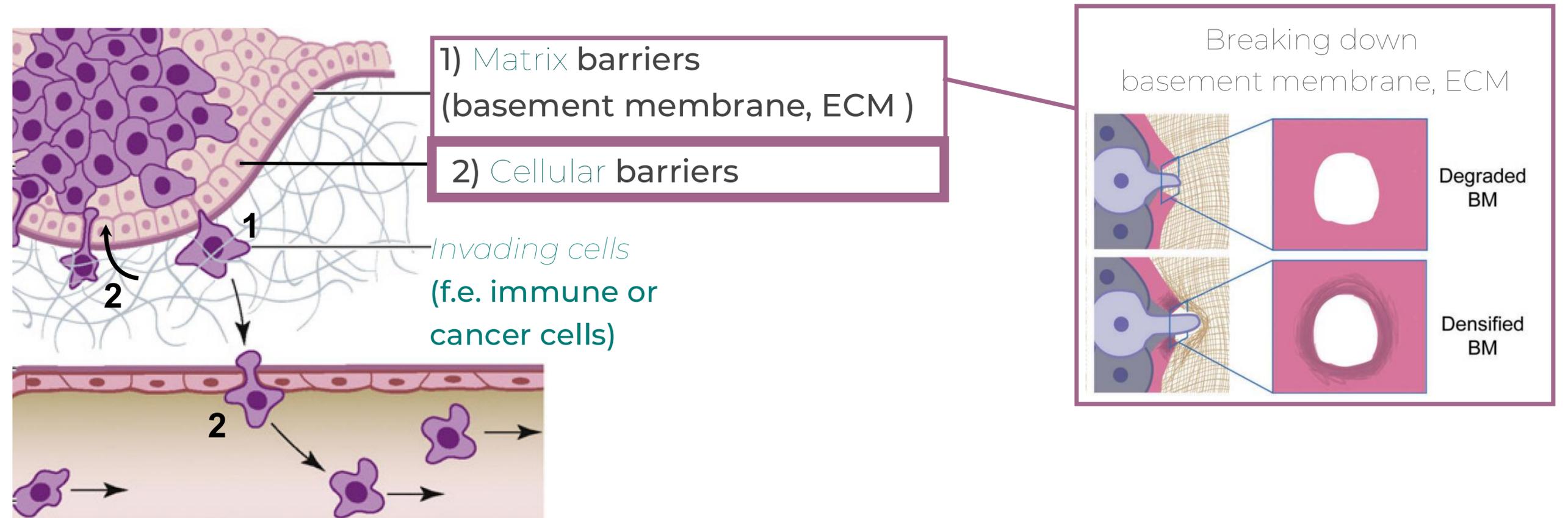
## Anchor cell in *C.elegans*



Protrusion makes initial small hole in BM wider



# How do *invading cells* overcome cellular barriers ?

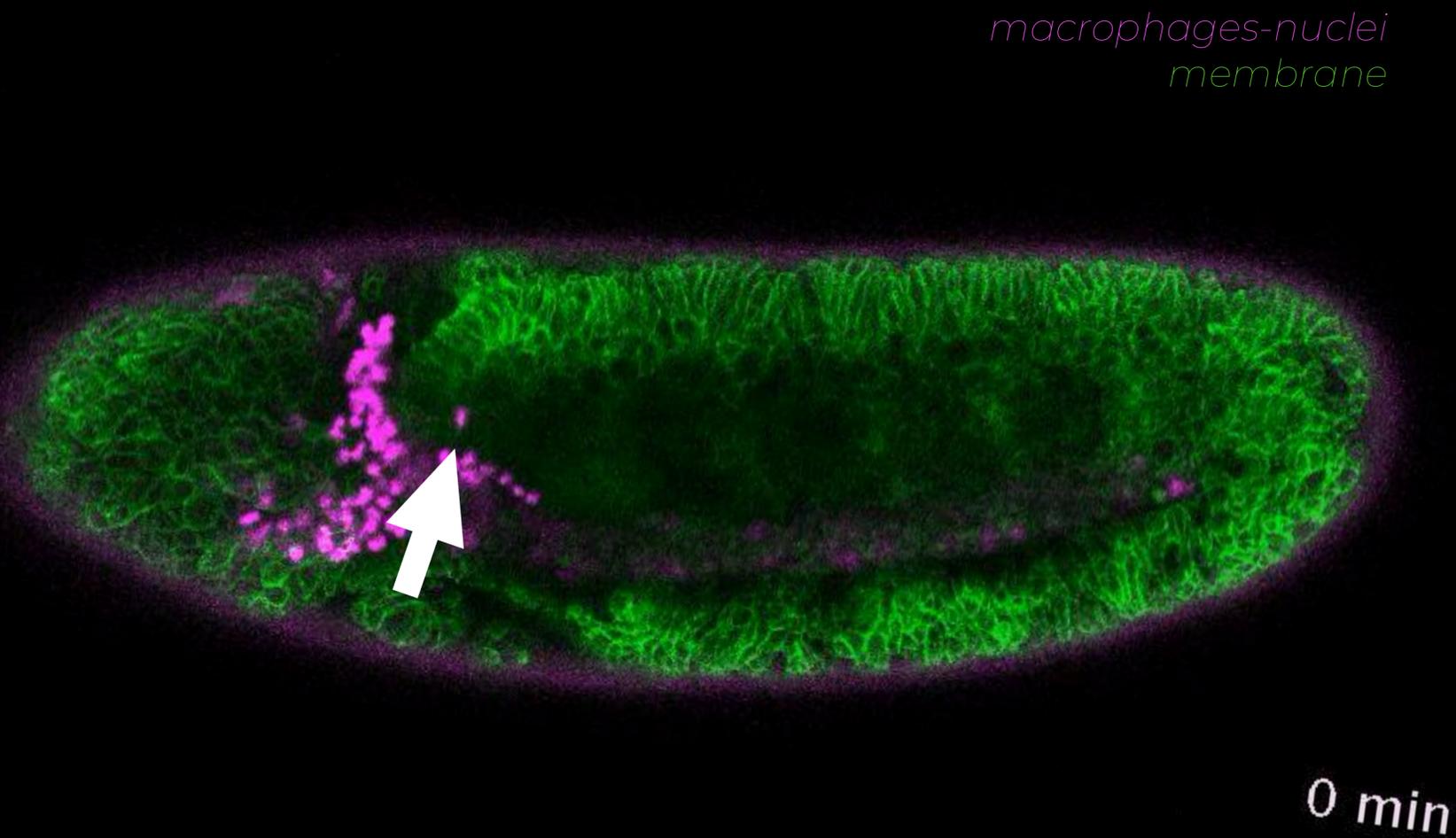


# Macrophage migration in *Drosophila* embryo as *in vivo* model of tissue invasion

at Daria Siekhaus lab

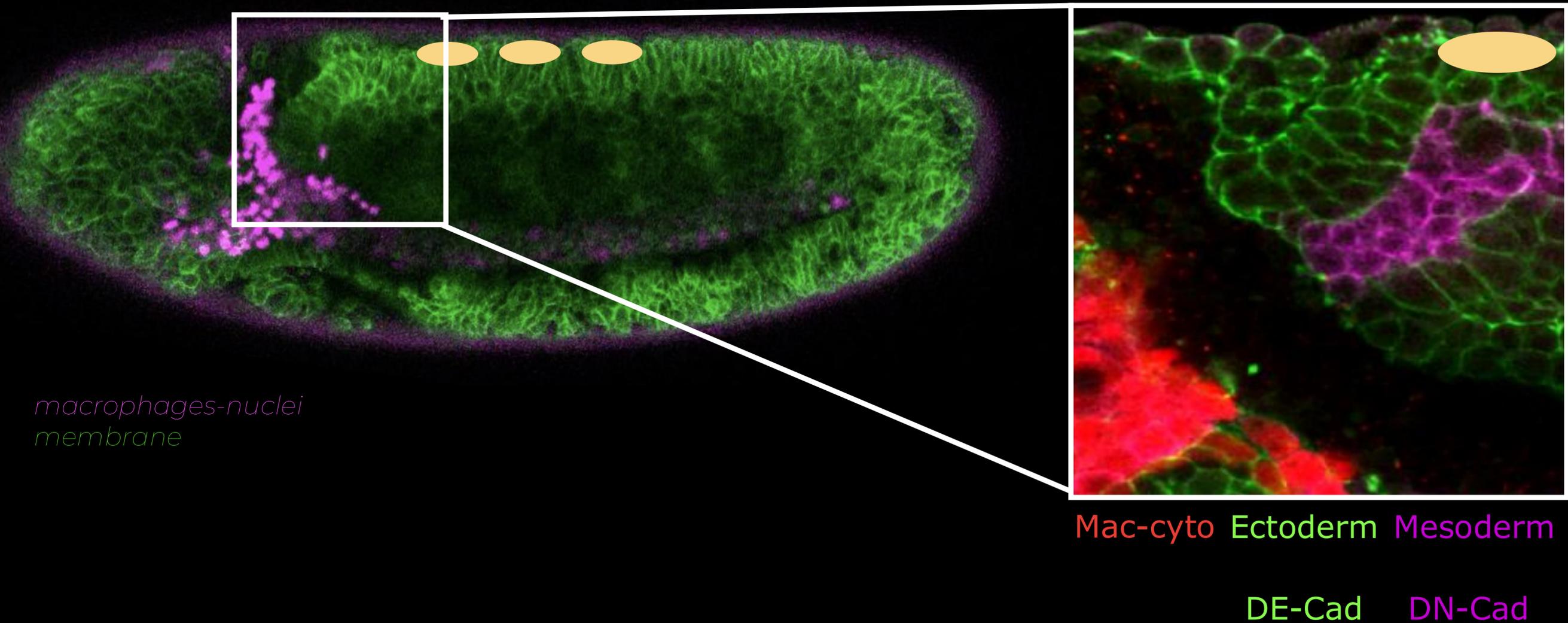


# Macrophage migration in *Drosophila* embryo as *in vivo* model of tissue invasion

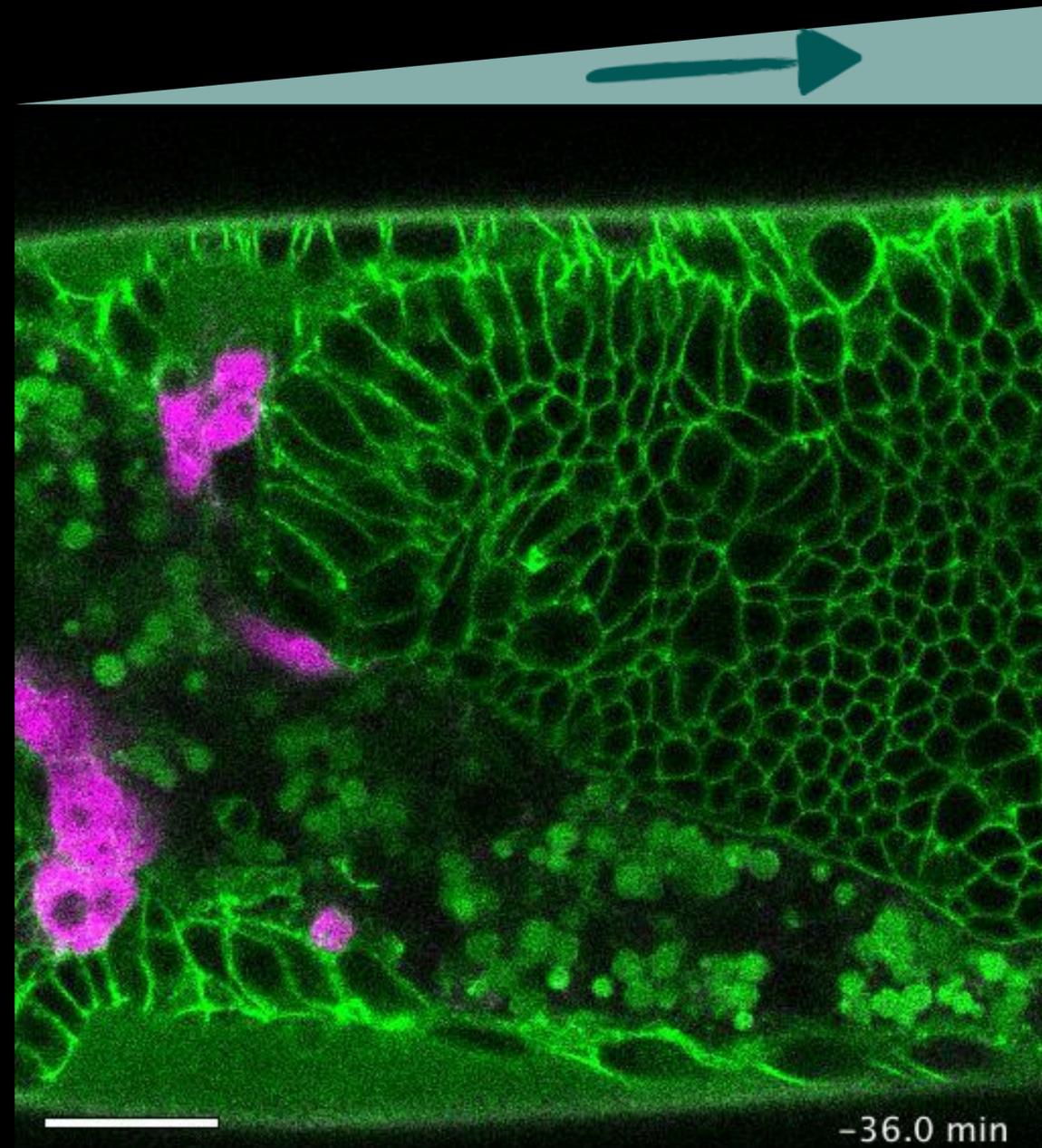


# Macrophage migration in *Drosophila* embryo as *in vivo* model of tissue invasion

Chemoattractant source (VEGF)



# Macrophage migration in *Drosophila* embryo as *in vivo* model of tissue invasion

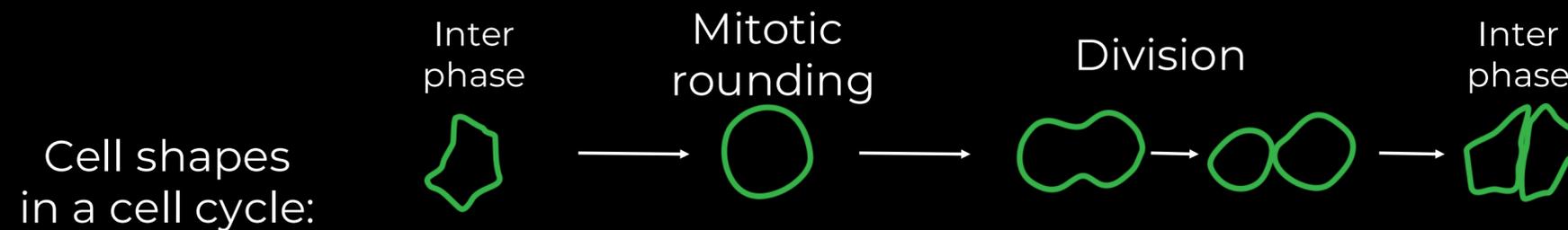
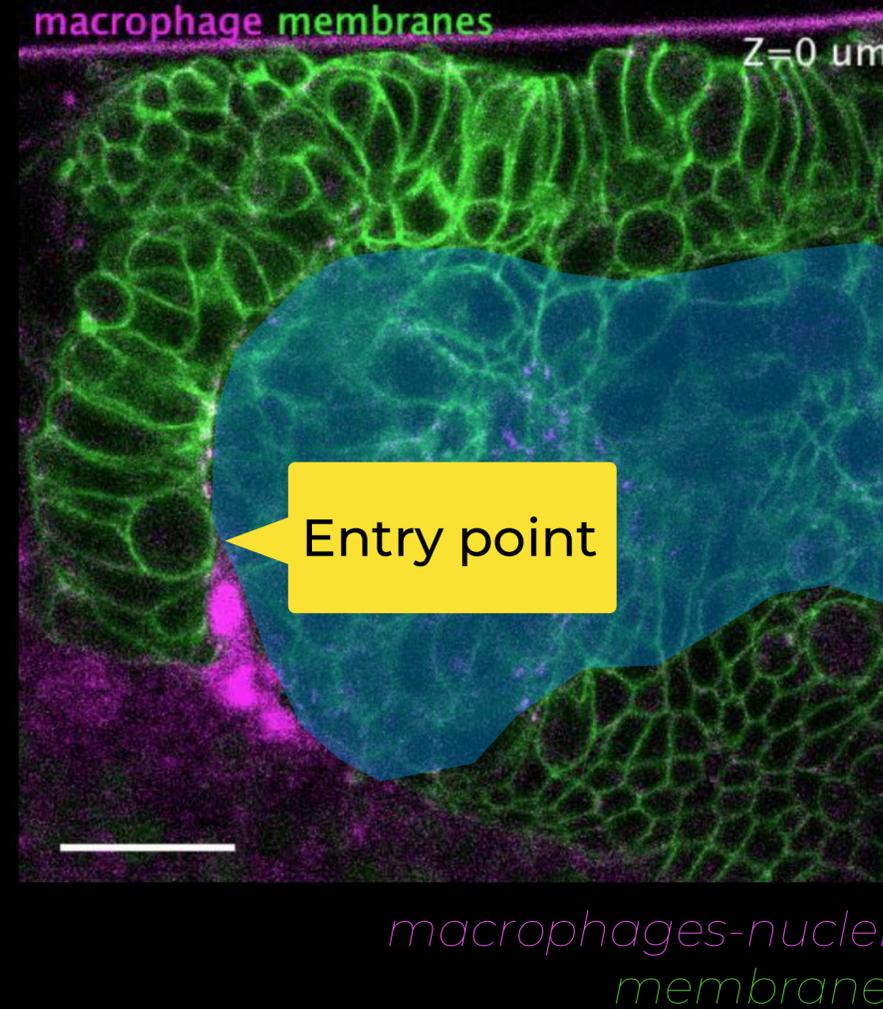
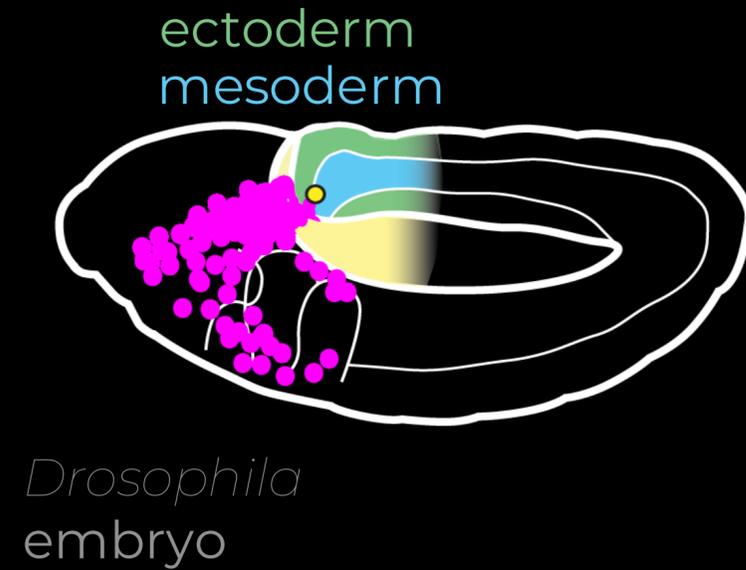


Directional  
cues

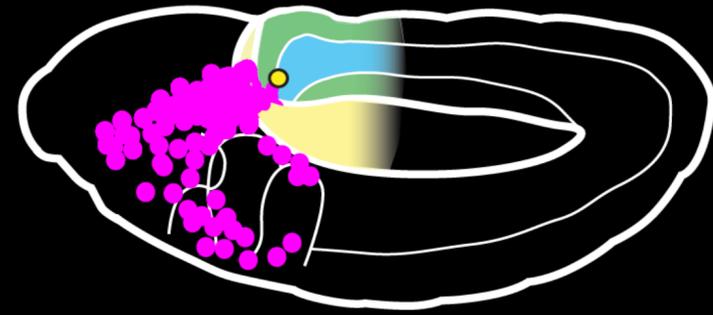


Resistance

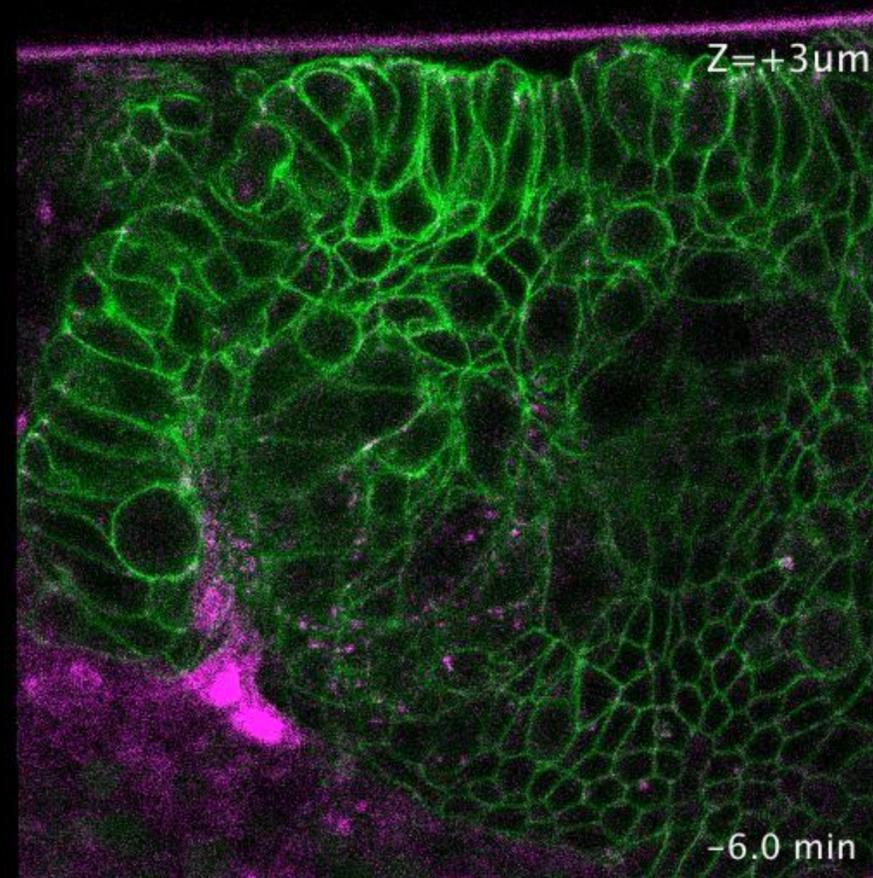
# Tissue cell at the entry point rounds up or divides



# Macrophage entry occurs **only** when adjacent tissue cell rounds up or divides



*Drosophila*  
embryo



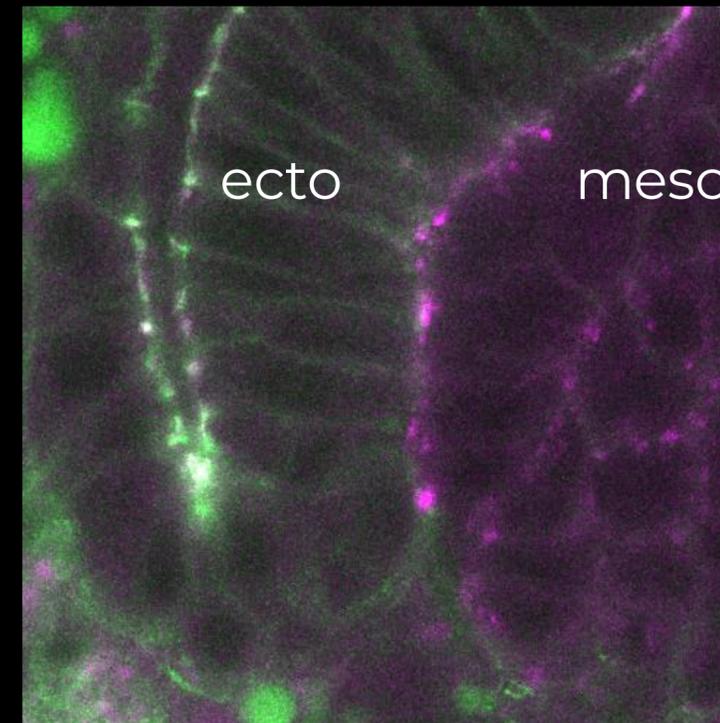
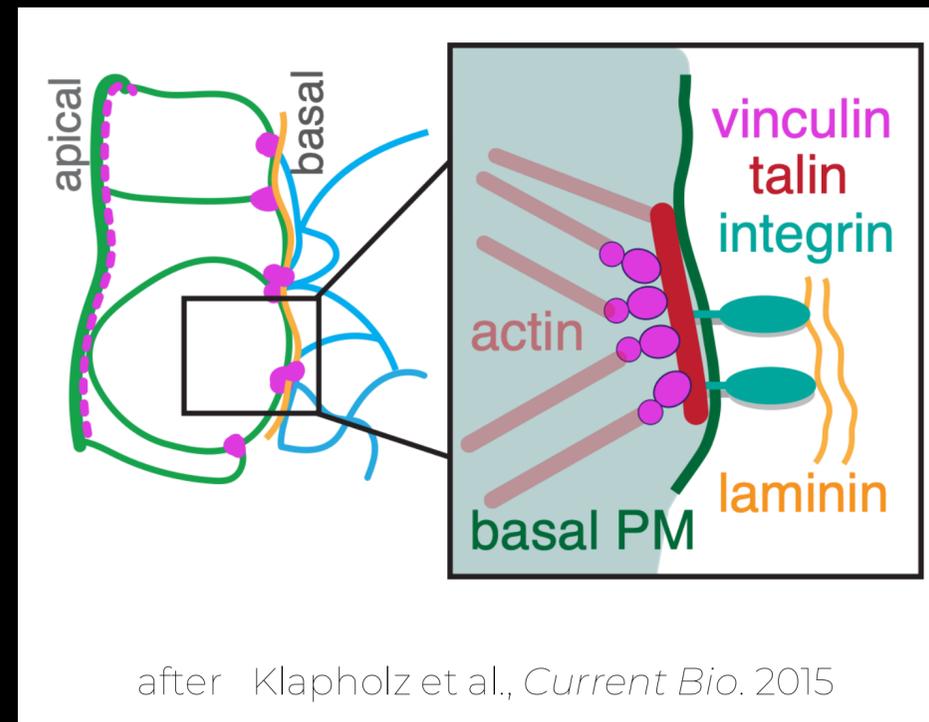
*macrophages-nuclei-cytoplasm*  
*membrane*

Tissue rounding/division  
enables macrophage entry.

**How?**

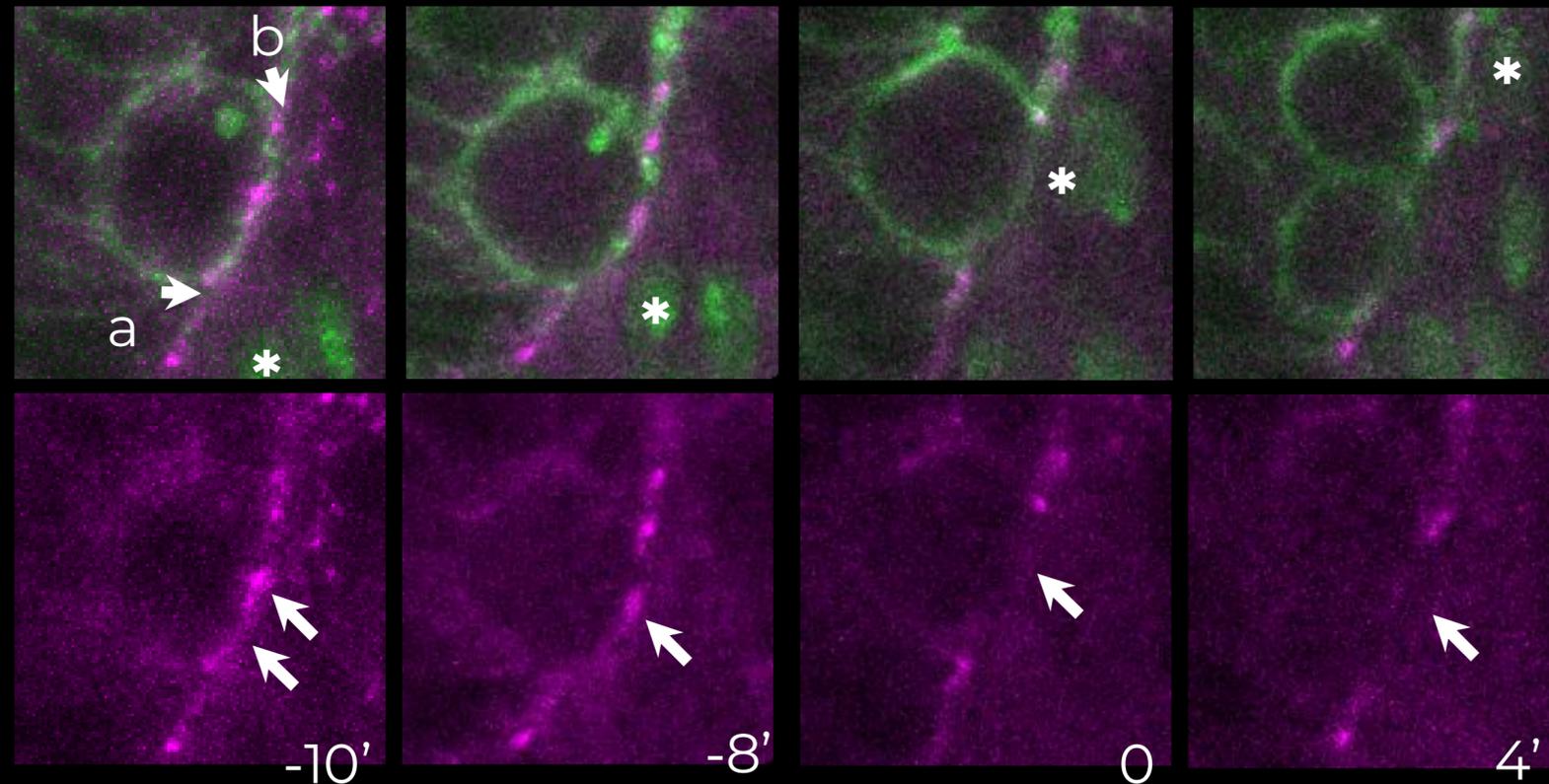
Hypothesis 2: adhesion disassembly in mitosis  
enables invasion

# Focal adhesions seal tissue interface

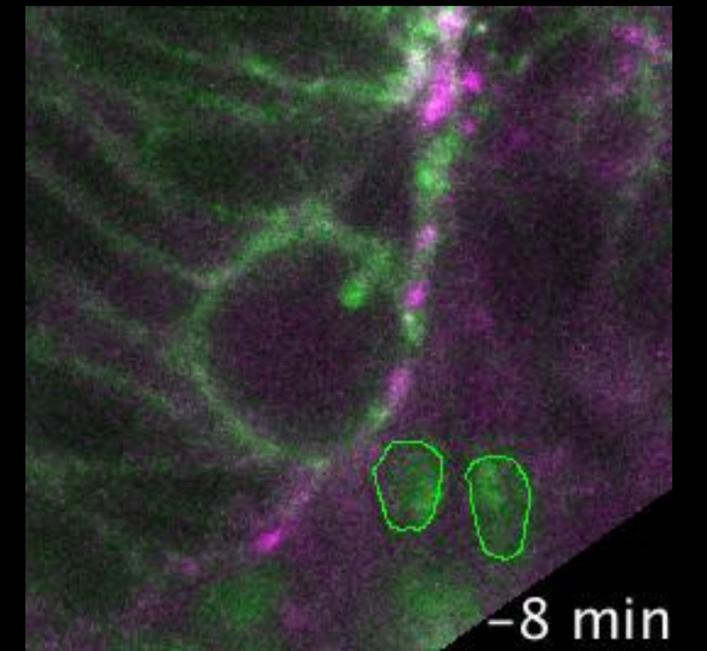
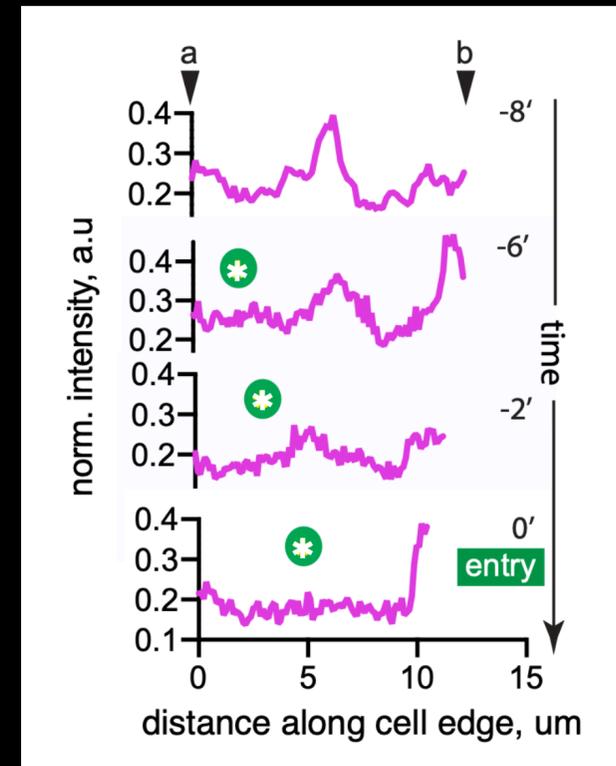


*Vinculin::mCherry*  
*DECad::GFP*

# Macrophages enter when the focal adhesion peak fades away



*Vinculin-mCherry, DECCad-GFP*



*Vinculin-mCherry, DECCad-GFP; coloc mac>nuc-Dendra*

# Conclusion from *Drosophila*: Cell division in tissue enables macrophage infiltration



# Conclusion from *Drosophila*: Cell division in tissue enables macrophage infiltration

Does it fall in  
any of these?

1. Cue
2. Sensing
3. Polarisation
4. Motion execution

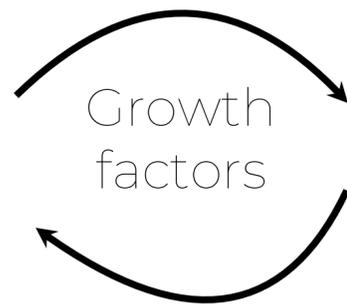
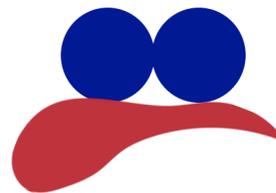


# Invasion is controlled by surrounding tissue cells



Possible  
implications:

Surrounding  
cell division



Macrophage  
localization



Immune cells function

Ways to modulate immune infiltration

Thank you for your attention!



Akhmanova Lab

Emily Pratt  
Alec Tiffany  
Jonah Rosas  
Emily Broutian