

Mechanisms of macrophage tissue infiltration

Céline Cougoule, PhD

Institut of Pharmacology
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Team

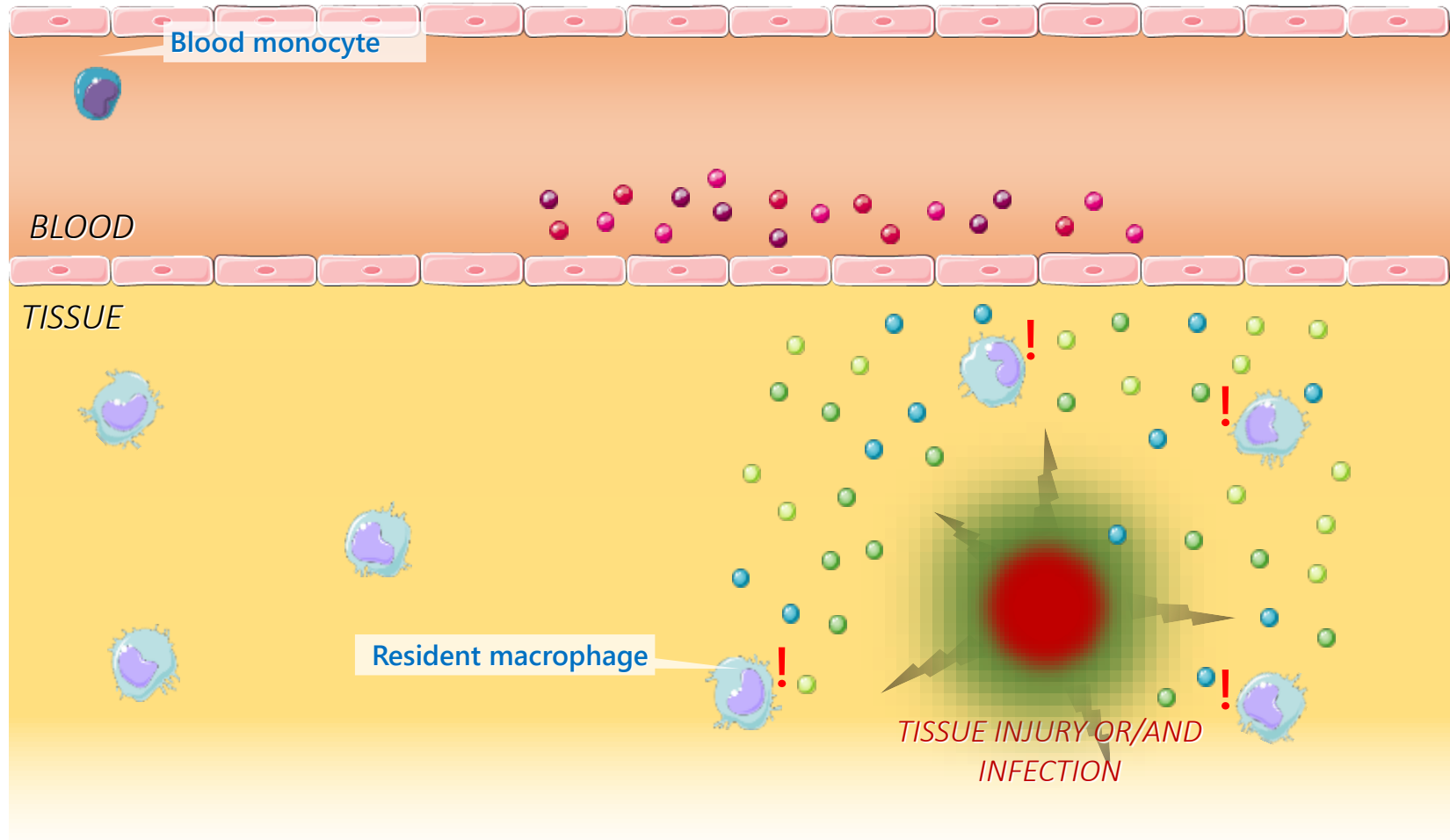
«Migration and Differentiation of Phagocytes »

Dr. Isabelle Maridonneau-Parini

Department

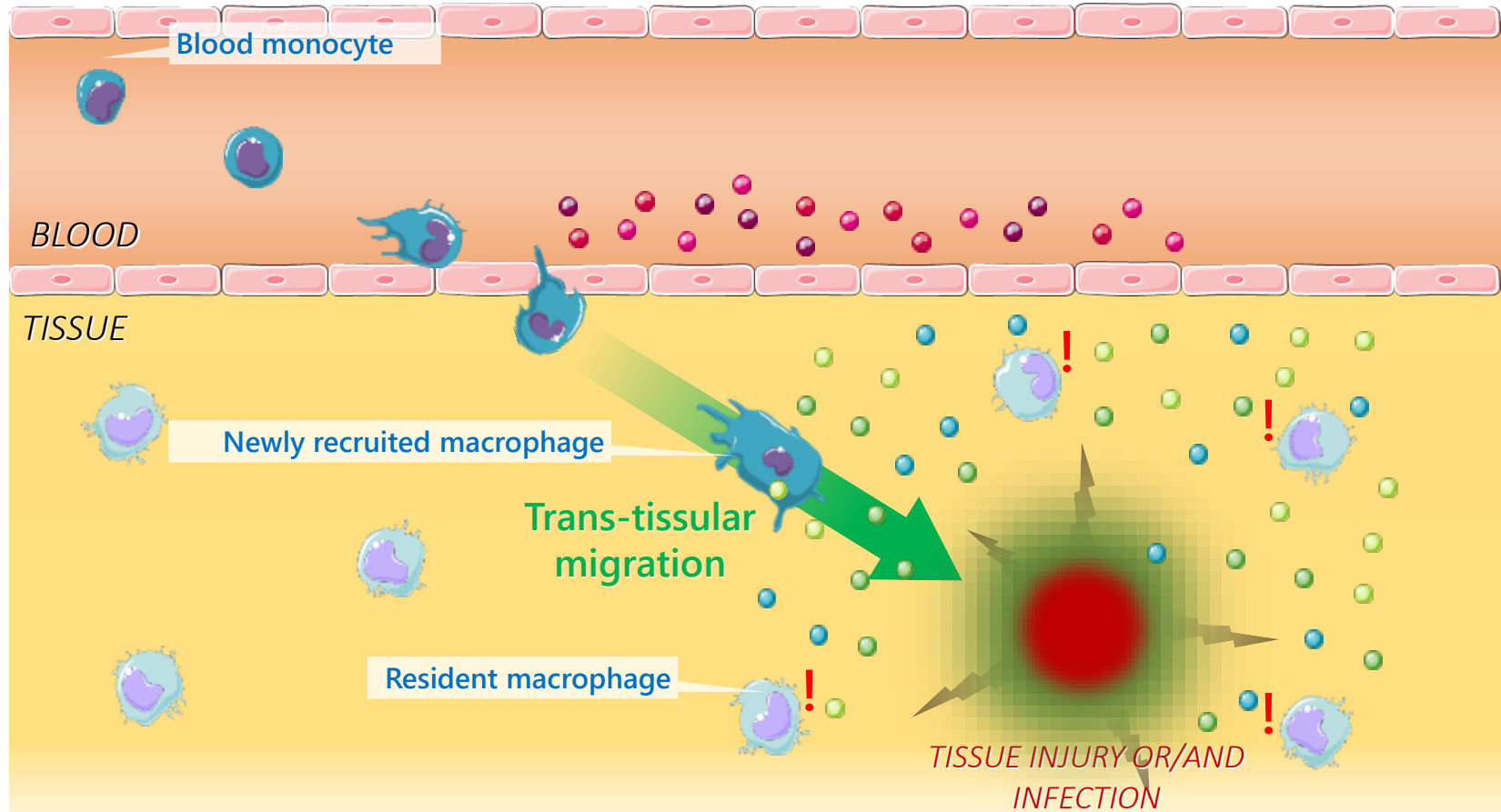
« Tuberculosis and Infection Biology »

Trans-tissular migration of macrophages



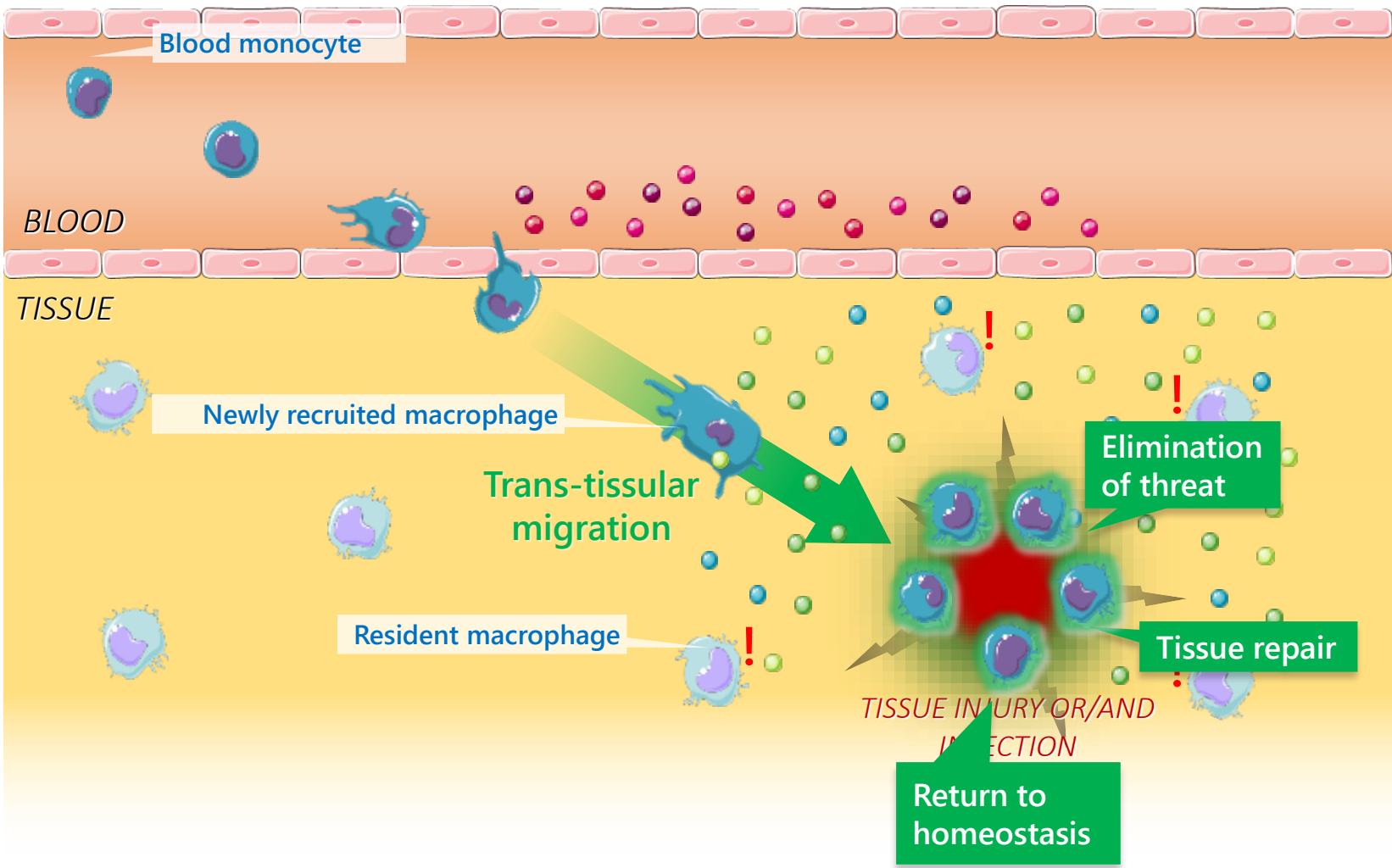
Essential for host immune response and maintenance of tissue homeostasis

Trans-tissular migration of macrophages



Essential for host immune response and maintenance of tissue homeostasis

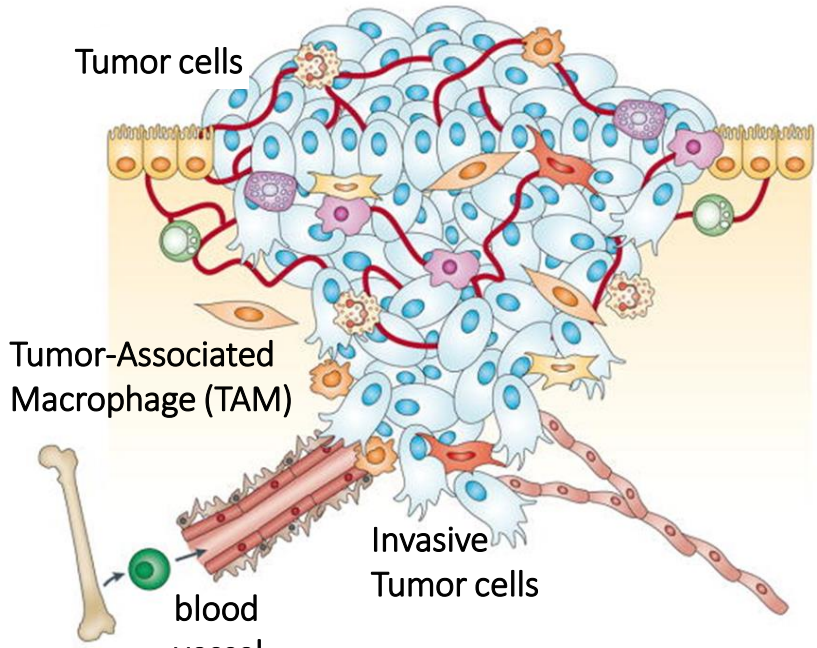
Trans-tissular migration of macrophages



Essential for host immune response and maintenance of tissue homeostasis

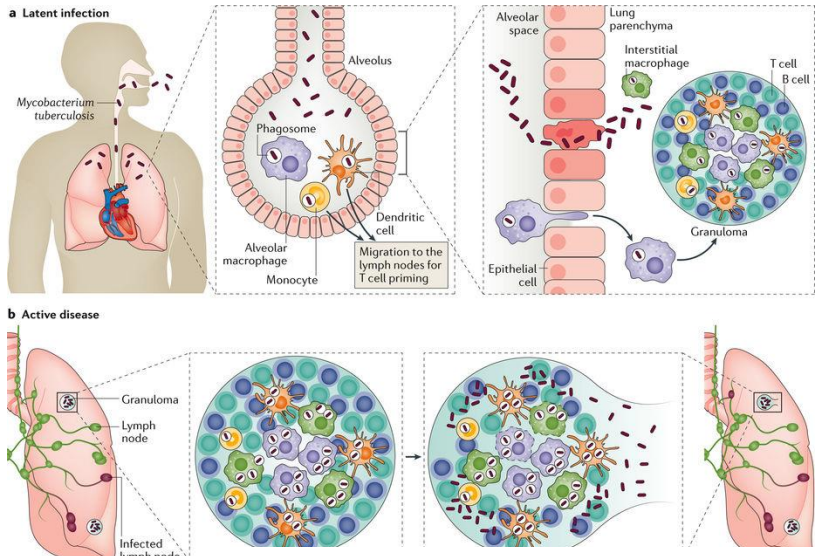
Pathological contexts

Macrophages in cancer



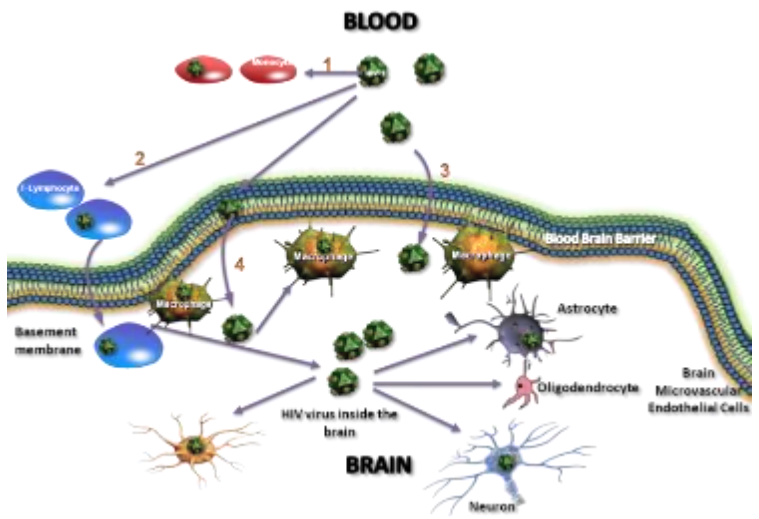
JA Joyce & JW Pollard, Nat Rev Cancer 2009

Macrophages during TB



Pai M et al. Nature Reviews Disease Primers, 2016

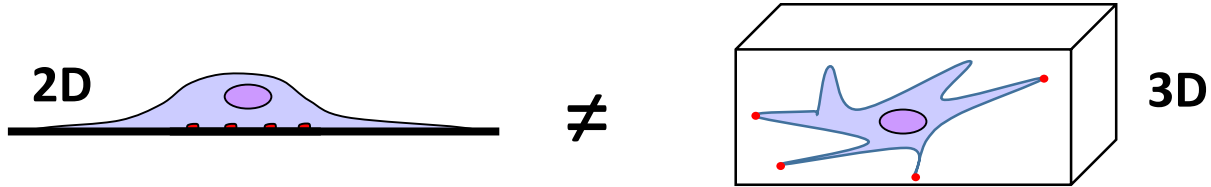
Macrophages in AIDS



*Targeting of macrophage trans-tissular migration
→ new therapeutic strategy*

- 📖 Mackay, 2008. Nature Immunology
- 📖 Ruhrberg, De Palma, 2010 Nature Medicine
- 📖 Qualls, Murray, 2010 Nature Medicine
- 📖 Noy, Pollard, Immunity 2014
- 📖 Andon FT, Simmen Immunol 2017

Trans-tissular migration of macrophages

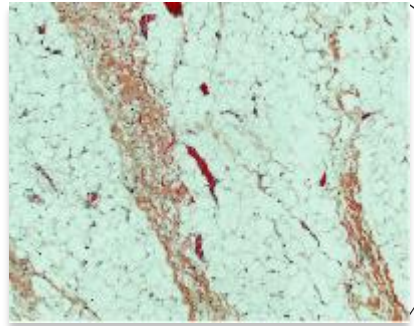


TISSUE HETEROGENEITY

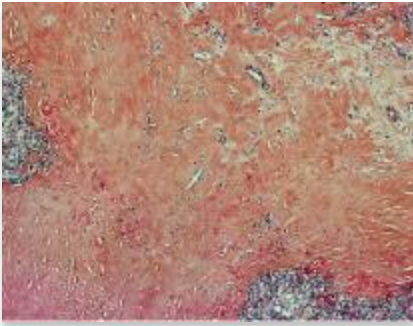


PATHOLOGIC TISSUE DENSIFICATION

Healthy connective tissue



IRM mammary tumor

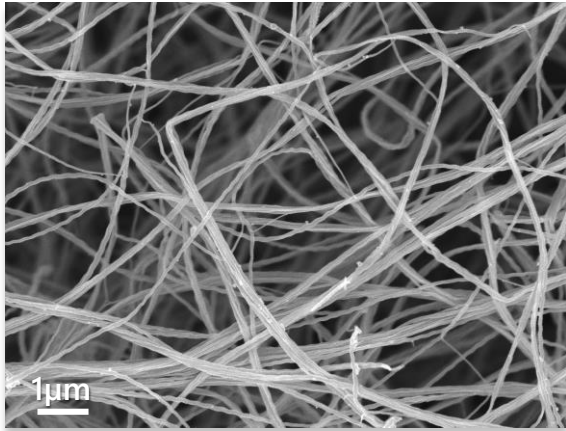


Carcinoma stroma

Characterization of *in vitro* macrophage 3D migration

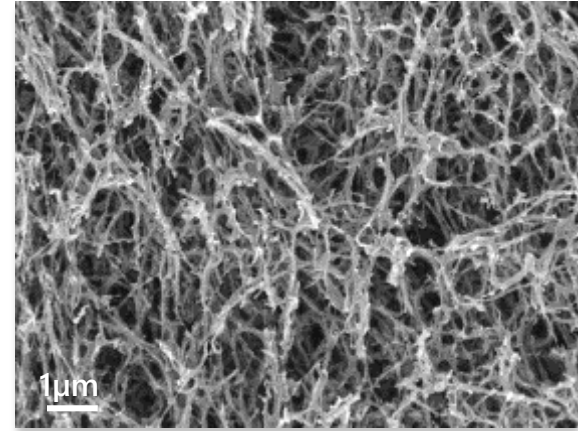
High porosity matrix

(ex: fibrillar collagen I)

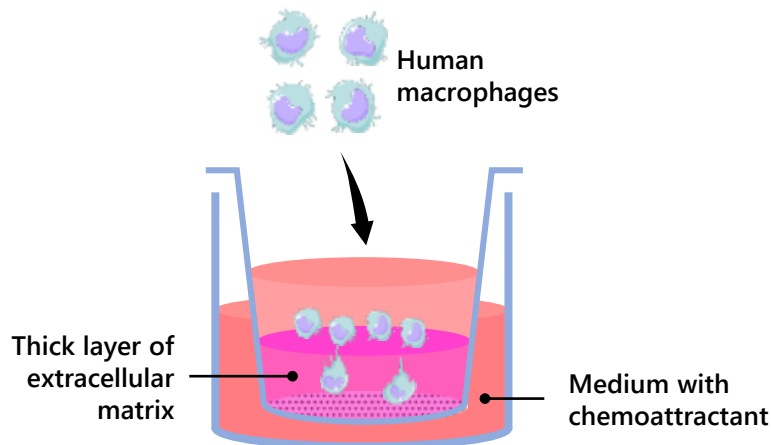


Low porosity matrix

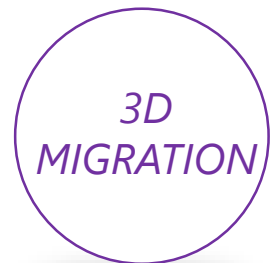
(ex: gelled collagen I or Matrigel™)



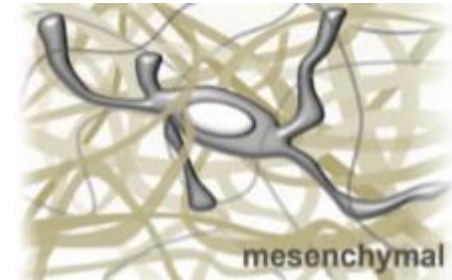
TRANSWELL MIGRATION ASSAY



- Morphology
- Percentage of migration
- Migration distance and speed
- Directionality
- Effect of pharmacological inhibition
- Effect of KO or siRNA gene silencing



Known 3D migration modes from studies on tumor cells



Amoeboid migration

Path finding

Rounded

Contractibility and morphological adaptation

Rho/ROCK-dependent

STRATEGY

CELL MORPHOLOGY

INTERACTION WITH THE EXTRACELLULAR MATRIX

MECHANISM

Mesenchymal migration

Path generating

Elongated and protrusive

Proteolysis and remodeling

Protease-dependent

Wyckoff JB, Pinner SE, Gschmeissner S, Condeelis JS, Sahai E. *Curr Biol*. 2006

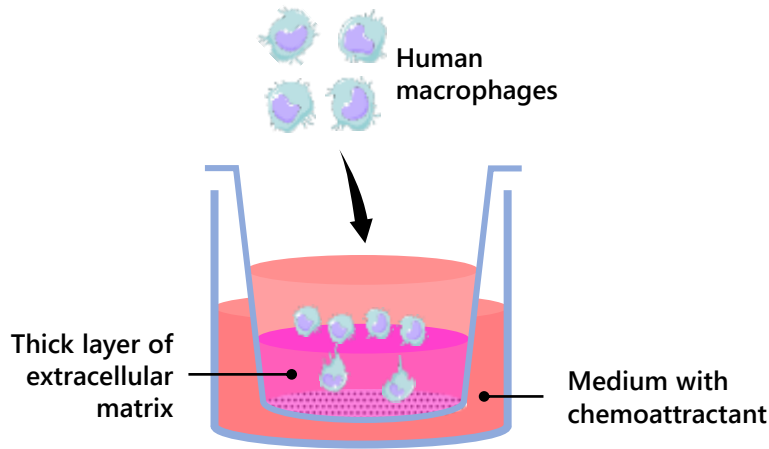
Friedl P, Wolf K. *Biochem Soc Symp*. 2003

Even-Ram S, Yamada KM. *Curr Opin Cell Biol*. 2005

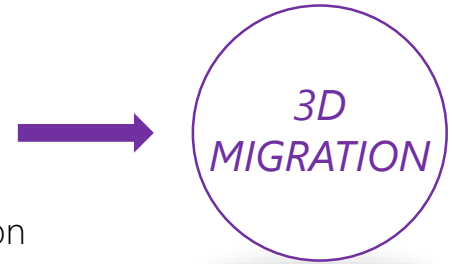
Sabeh F, Shimizu-Hirota R, Weiss SJ. *J Cell Biol*. 2009.

Characterization of *in vitro* macrophage 3D migration

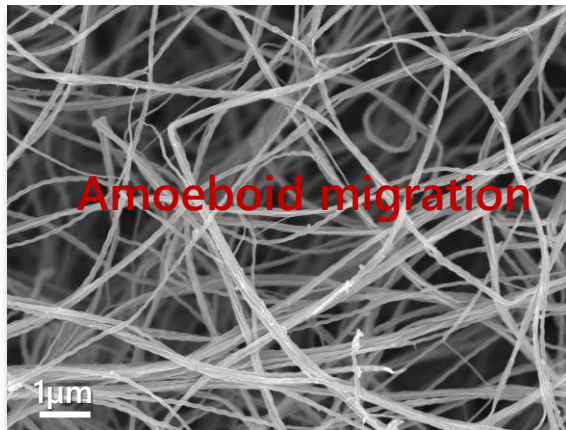
TRANSWELL MIGRATION ASSAY



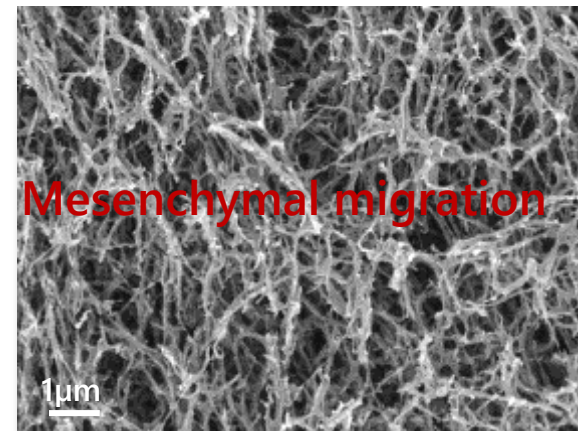
- Morphology
- Percentage of migration
- Migration distance and speed
- Directionality
- Effect of pharmacological inhibition



High porosity matrix (ex: fibrillar collagen I)

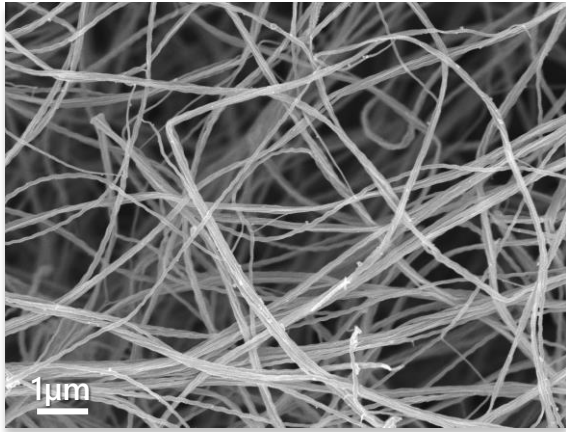


Low porosity matrix (ex: gelled collagen I or Matrigel™)

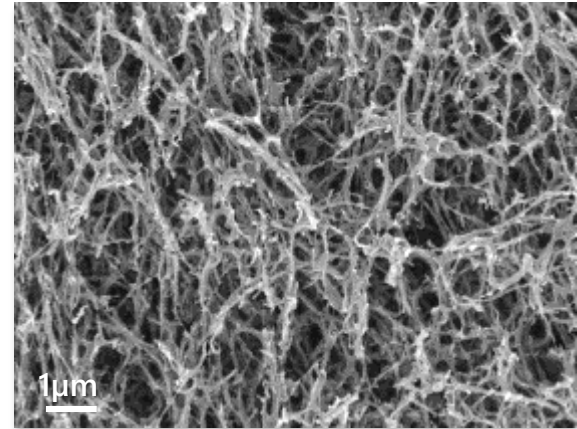


Characterization of *in vitro* macrophage 3D migration

High porosity matrix
(ex: fibrillar collagen I)



Low porosity matrix
(ex: gelled collagen I or Matrigel™)



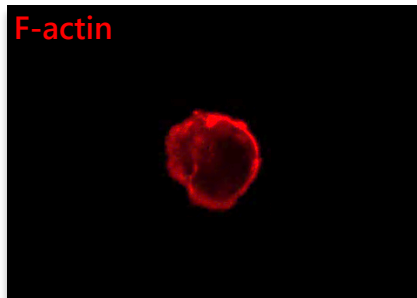
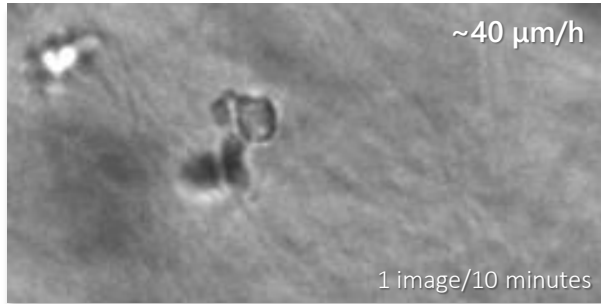
Macrophages → two 3D migration modes in vitro depending on extracellular matrix architecture

Amoeboid migration

Mesenchymal migration

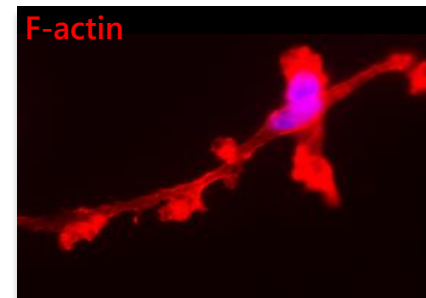
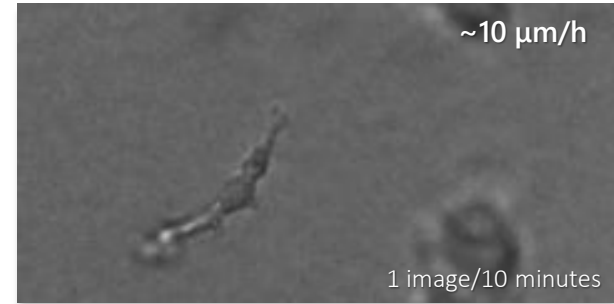
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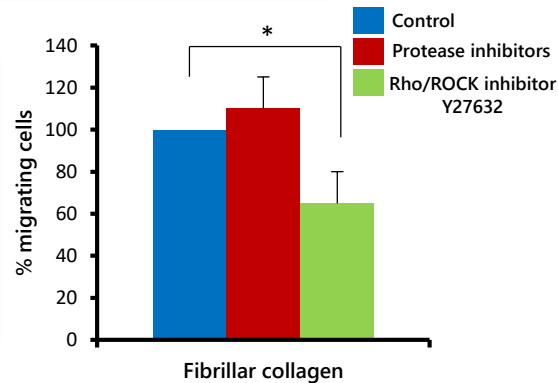
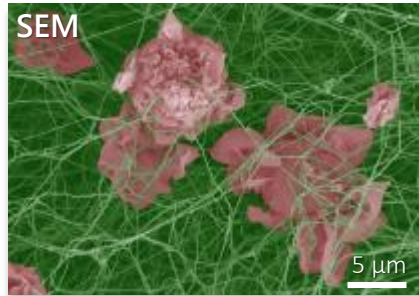
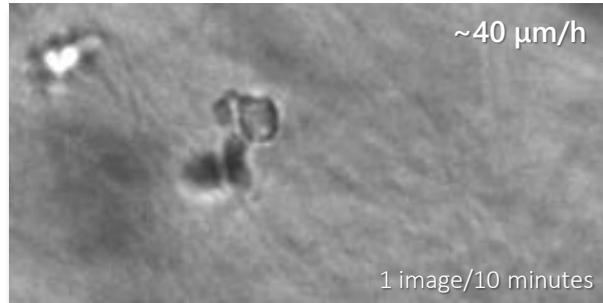
Mesenchymal migration

 Van Goethem et al., J Immunol. 2010

Macrophages → two 3D migration modes *in vitro* depending on extracellular matrix architecture

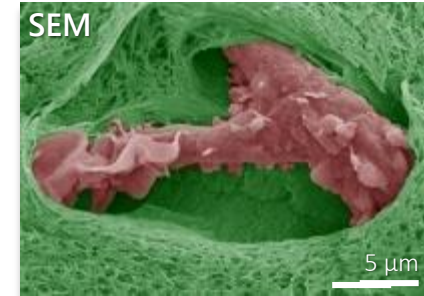
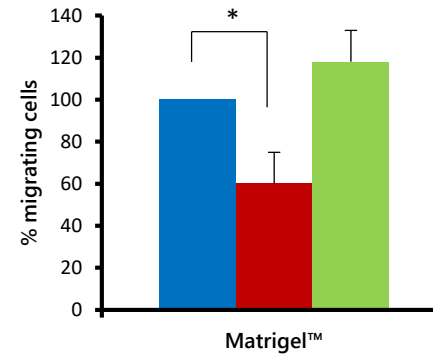
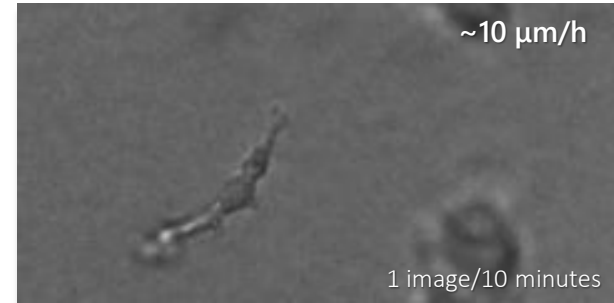
Characterization of *in vitro* macrophage 3D migration

High porosity matrix (ex: fibrillar collagen I)



Amoeboid migration

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Mesenchymal migration

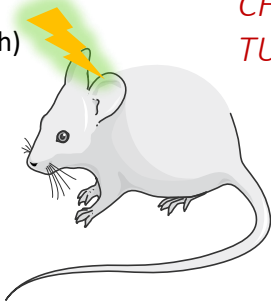
Van Goethem et al., J Immunol. 2010

Macrophages → two 3D migration modes *in vitro* depending on extracellular matrix architecture

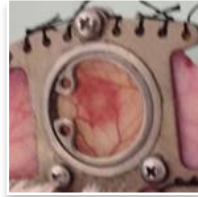
Characterization of macrophage migration mode *in vivo*

MacBlue transgenic mice

(David Hume,
University of Edinburgh)



CHAMBER SURGERY AND
TUMOR INDUCTION



Dr. Véronique Le Cabec, Philippe Gui, Myriam Ben Neji
Dr Elisabeth Bellard, Dr Muriel Golzio

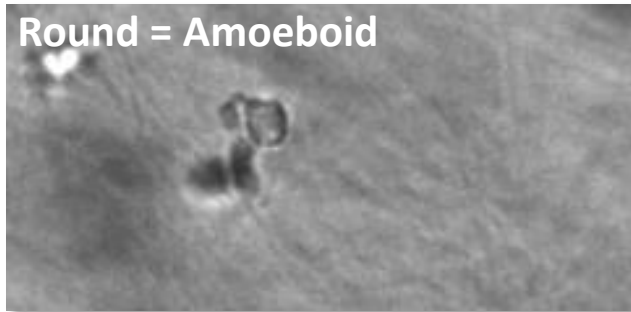


Multiphoton Zeiss 7MP

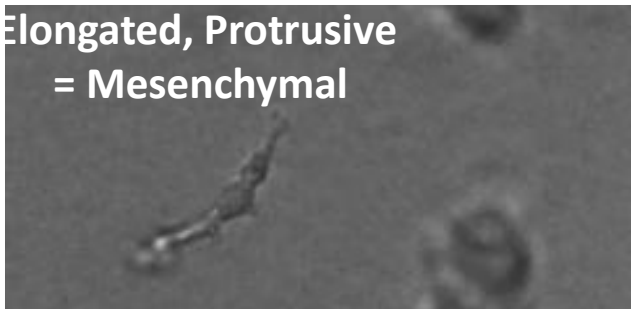


1. Macrophages morphology *in vivo* versus *in vitro*:

Round = Amoeboid



Elongated, Protrusive
= Mesenchymal



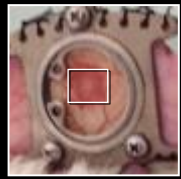
2. Sensitivity to Y27632 or Batimastat

- Dependent on ROCK activity
- Independent on protease activity

=> Inhibited by **Y27632** / not by **Batimastat**

- Dependent on protease activity
- Independent on ROCK activity

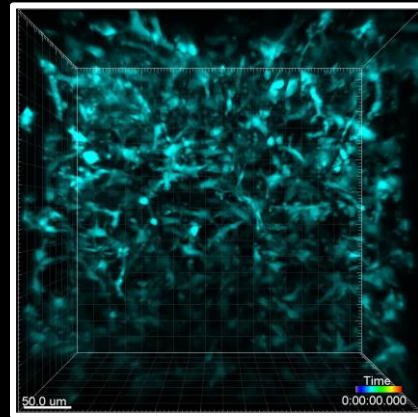
=> Inhibited by **Batimastat** / not by **Y27632**



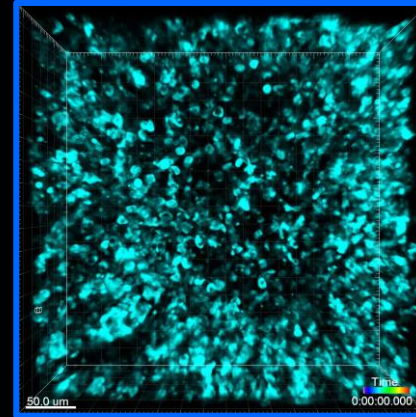
INSIDE THE TUMOR

Time laps

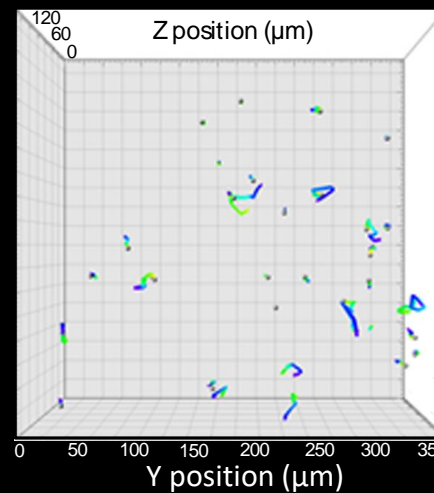
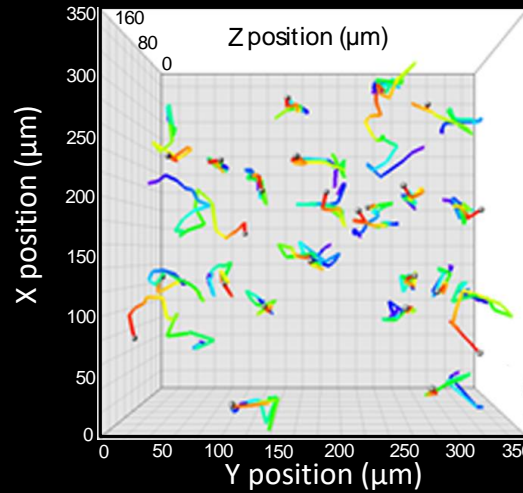
Control



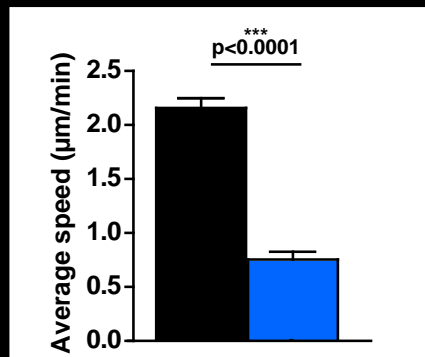
Batimastat



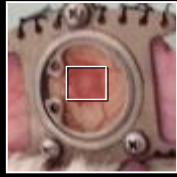
Tracking



Dr. Véronique Le Cabec,
Philippe Gui,
Myriam Ben Neji

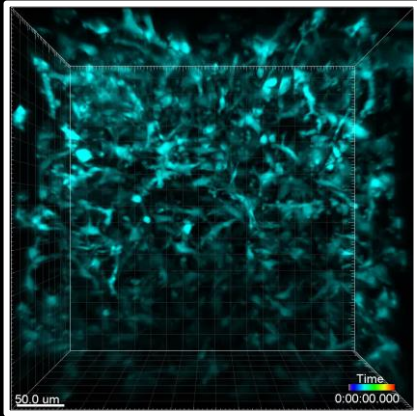


Mean +/- SEM of 6 mice

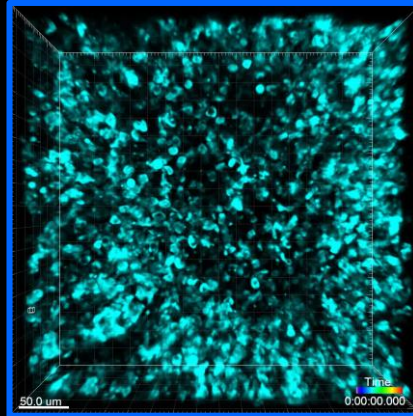


INSIDE THE TUMOR

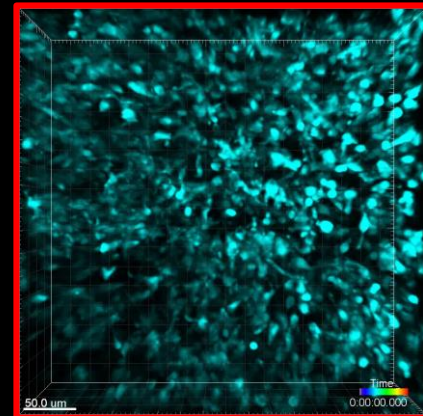
Control



Batimastat



Y27632

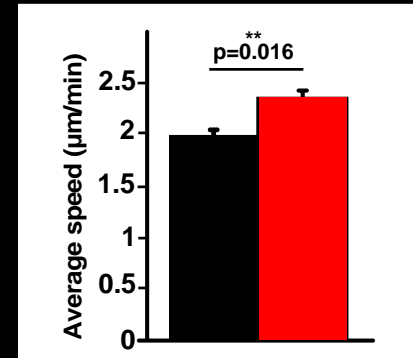
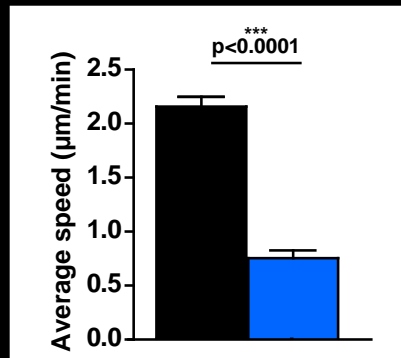


X position (μm)

*Tumor-associated macrophages
=> MESENCHYMAL MIGRATION*

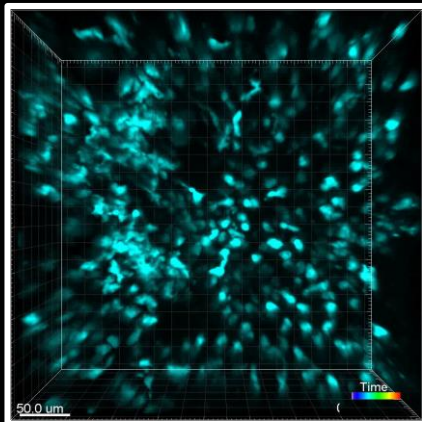
Dr. Véronique Le Cabec,
Philippe Gui,
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Mean \pm SEM of 6 mice

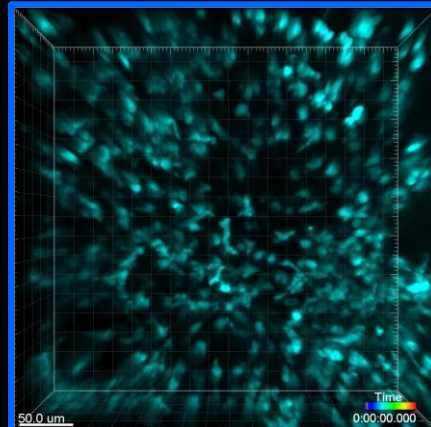




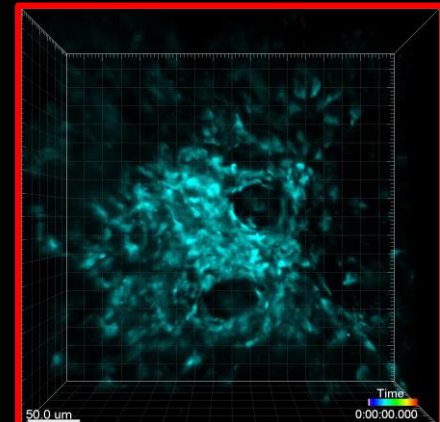
Control



Batimastat



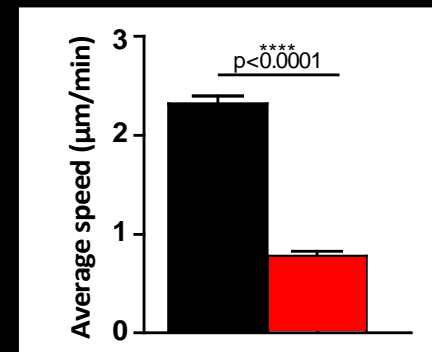
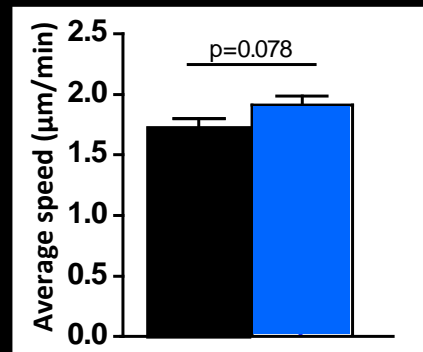
Y27632



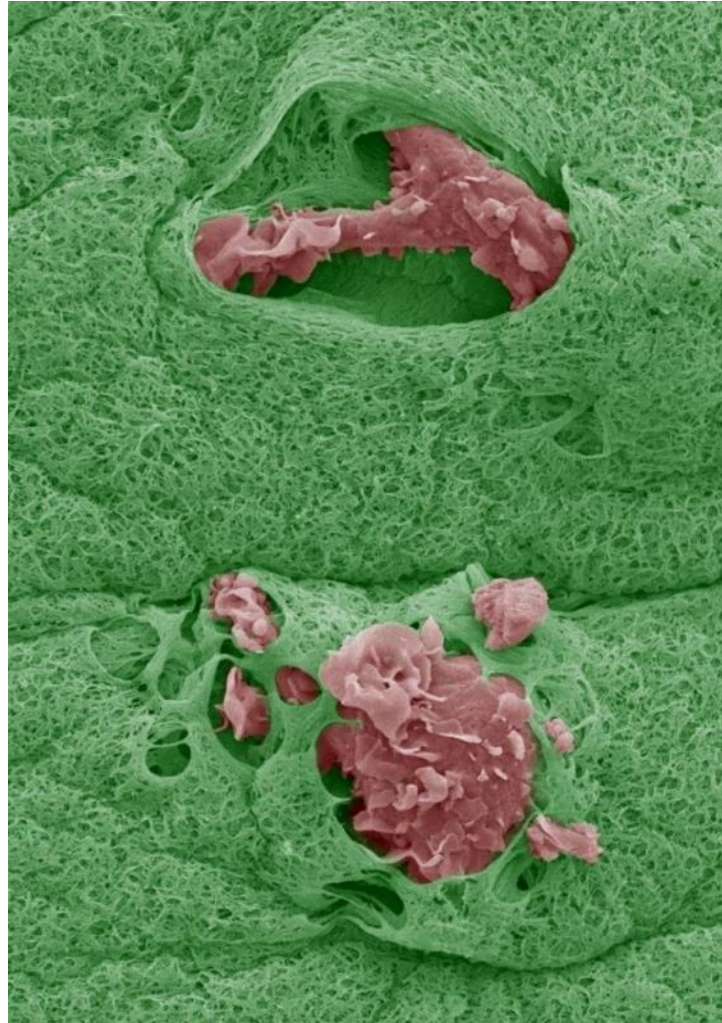
*Macrophages in ear derma
=> AMOEBOID MIGRATION*

Dr. Véronique Le Cabec,
Philippe Gui,
Myriam Ben Neji

Mean+/- SEM of 5 mice

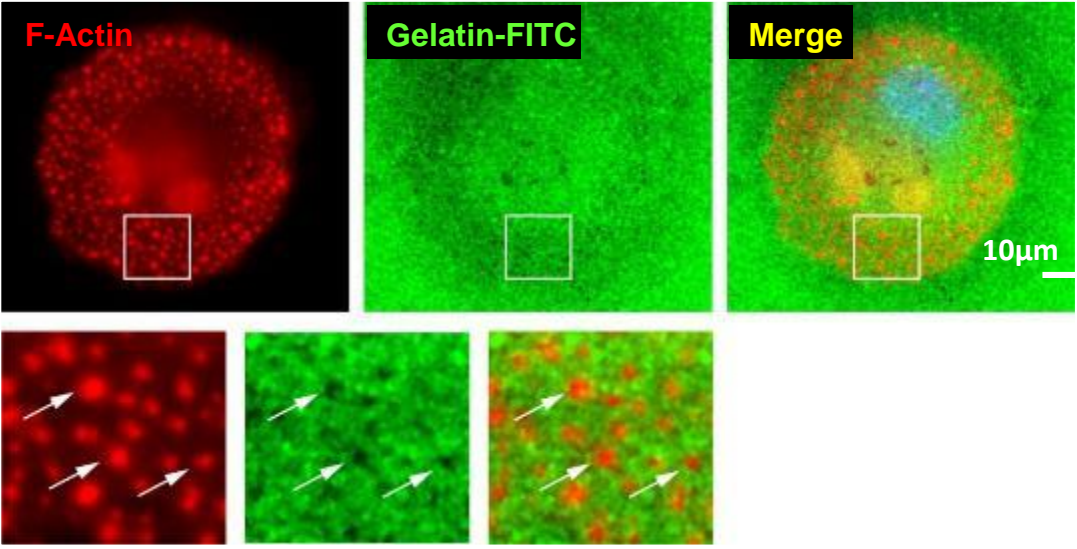
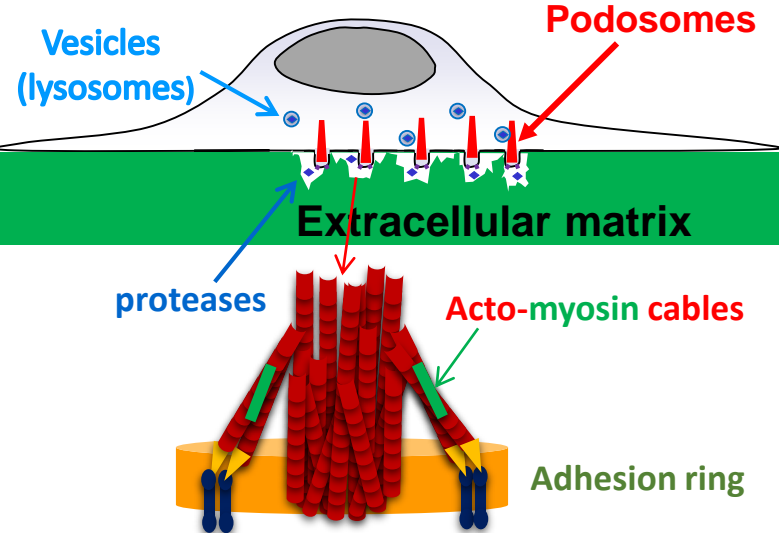


The mesenchymal 3D migration is protease-dependent: role of podosomes



Podosomes: actin-rich structure with ECM proteolytic activity

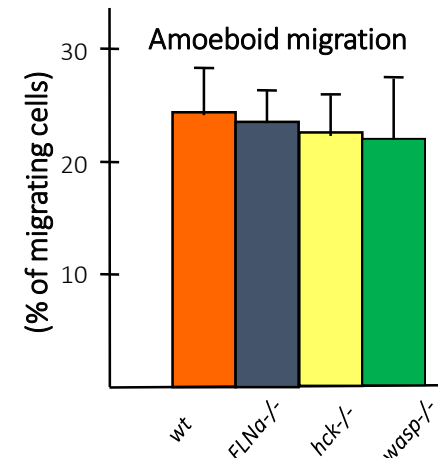
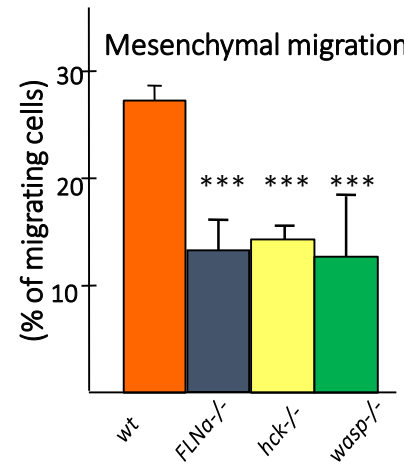
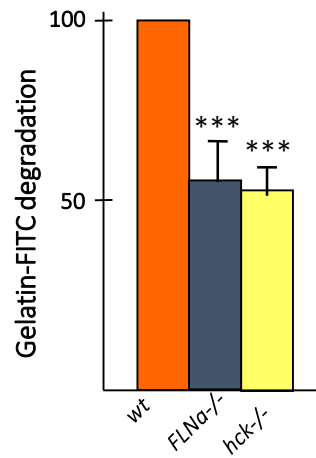
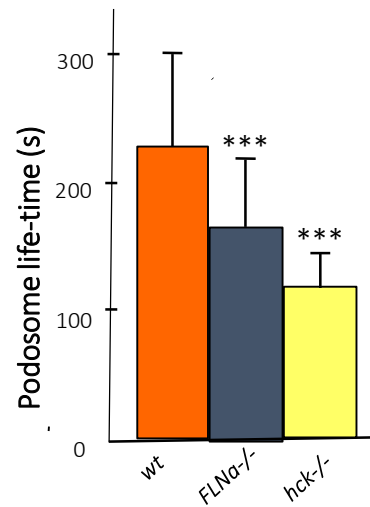
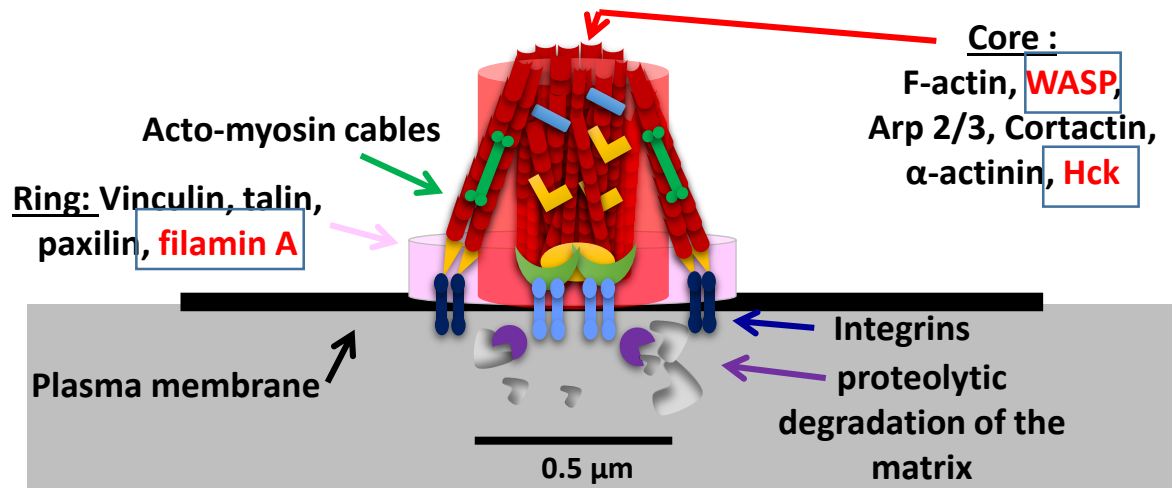
constitutive in macrophages, dendritic cells, osteoclasts



- ✓ Podosomes
- ✓ ECM proteolysis
- ✓ 3D mesenchymal migration

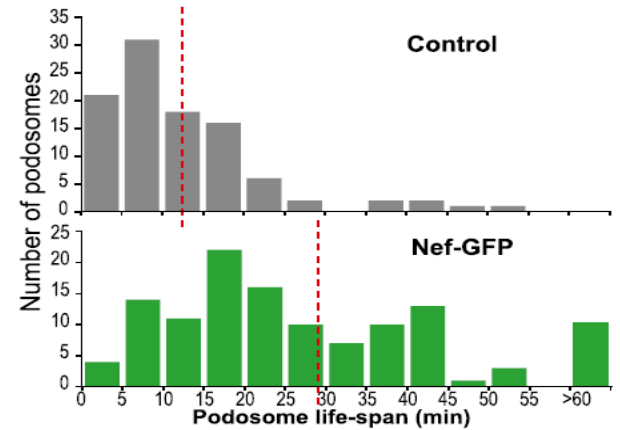
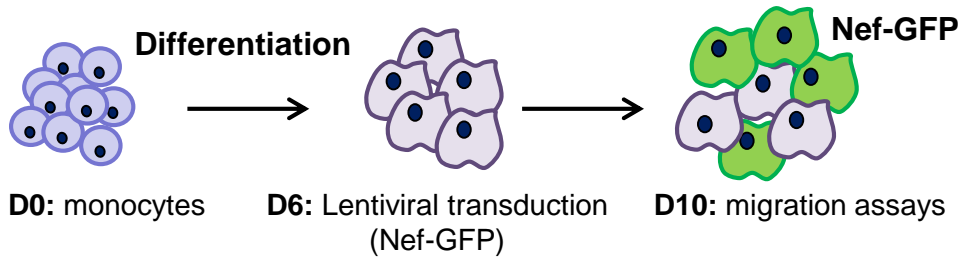
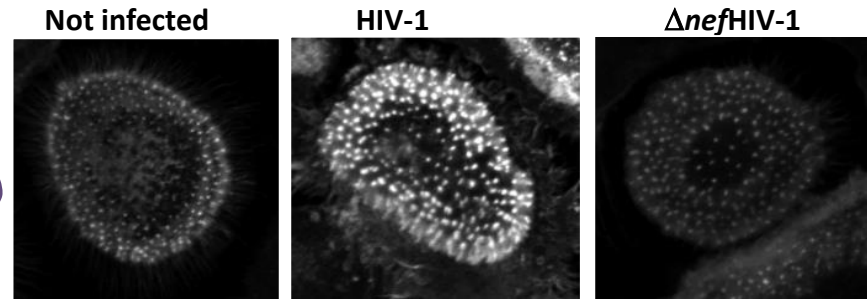
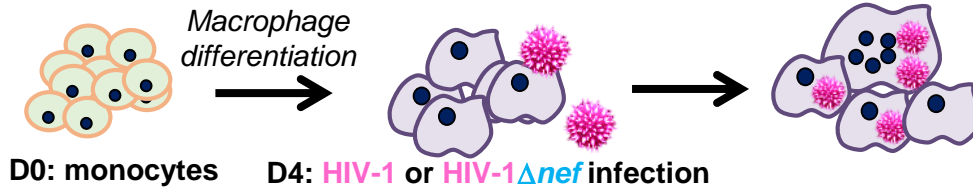
Functionally linked in 3D ?

Role of podosomes in macrophage mesenchymal migration

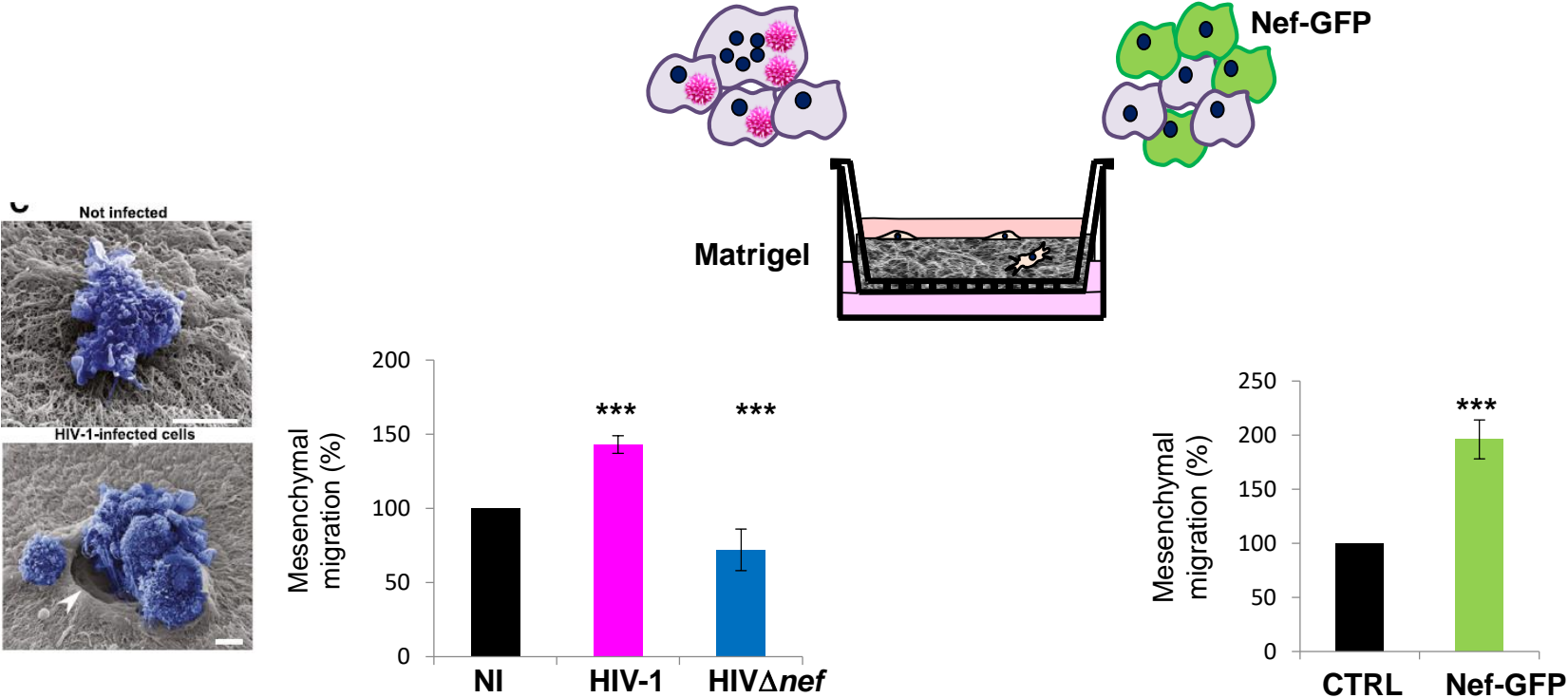
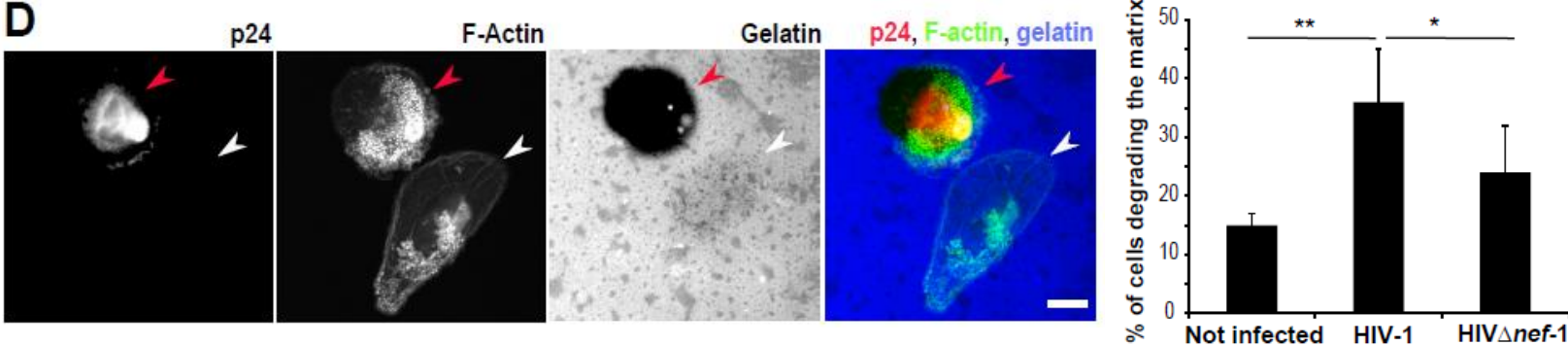


- 📖 Cougoule C et al., Blood 2010
- 📖 Guiet R et al. J. Biol. Chem. 2012
- 📖 Park H et al. J. Biol. Chem. 2014

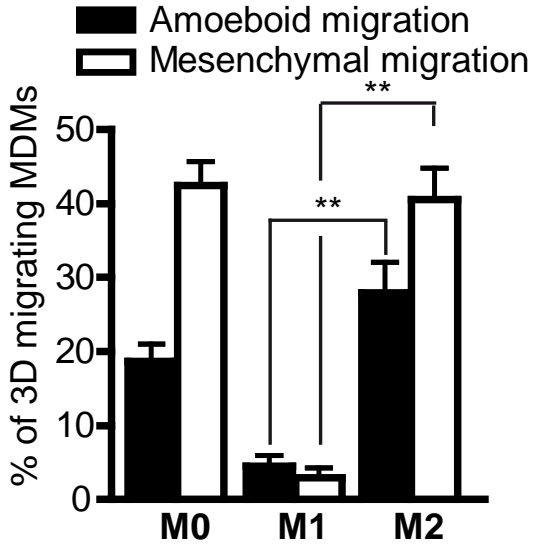
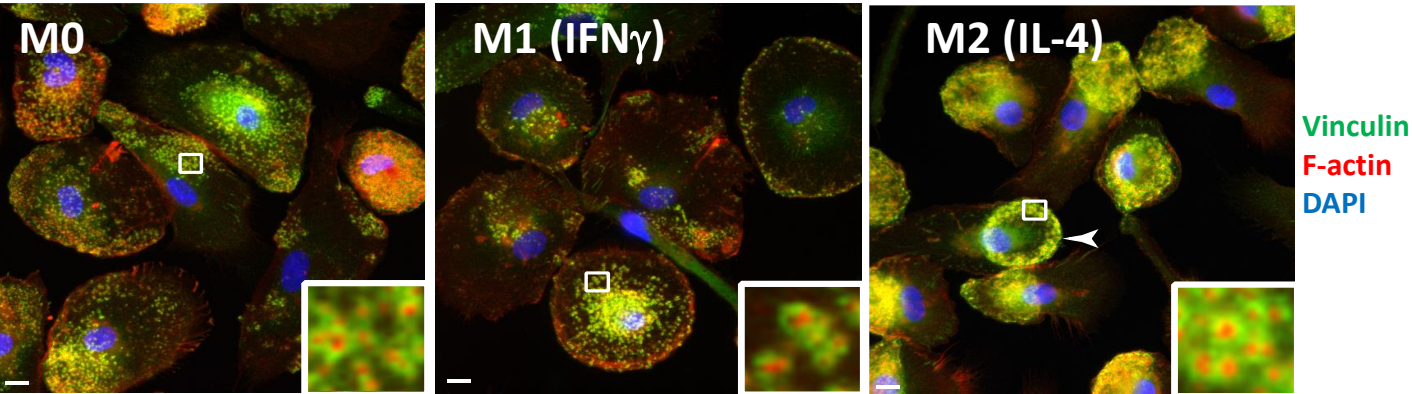
HIV-1 targets podosomes, via Nef



Mesenchymal migration of macrophages is enhanced by HIV-1, via Nef



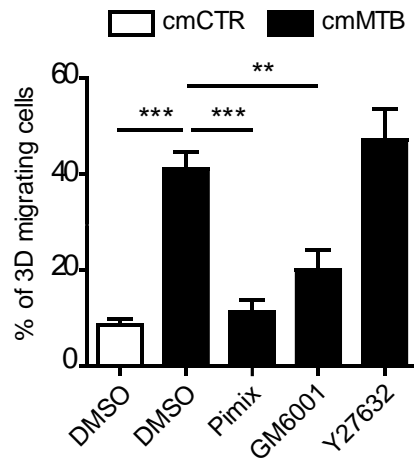
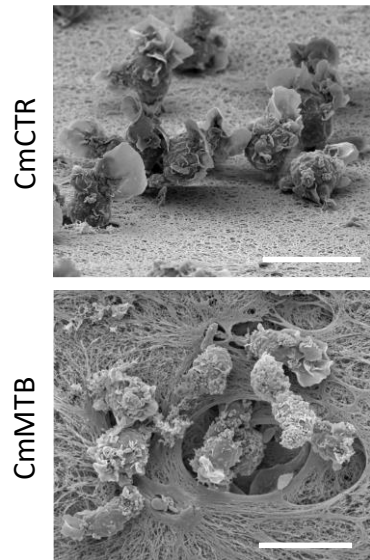
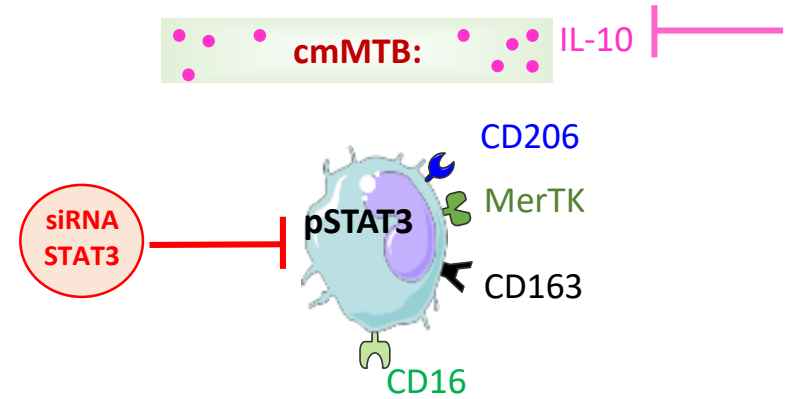
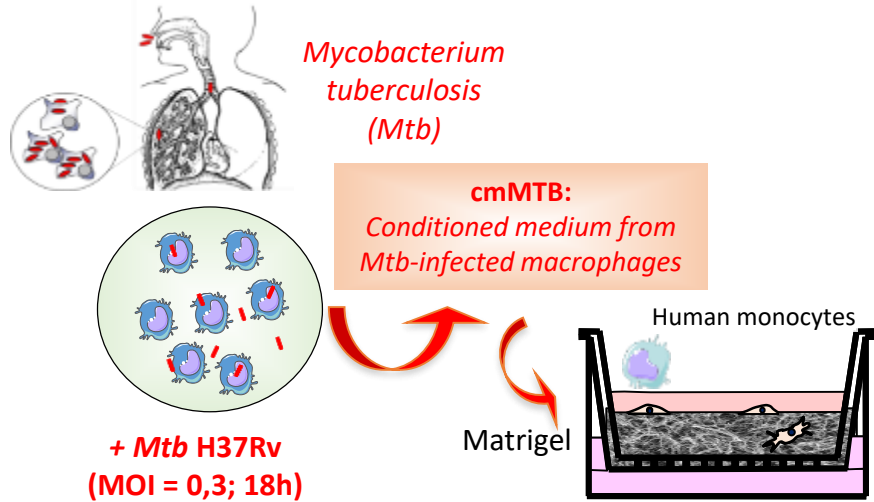
Podosomes in 3D migration: among macrophage diversity



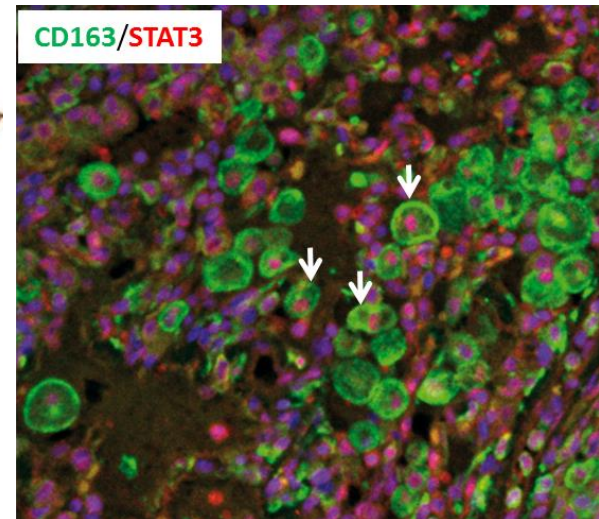
Cougoule et al., Eur. J. Cell Bio. 2012

- M1 macrophages are motionless
- 3D migration is an exclusive property of M2 macrophages.

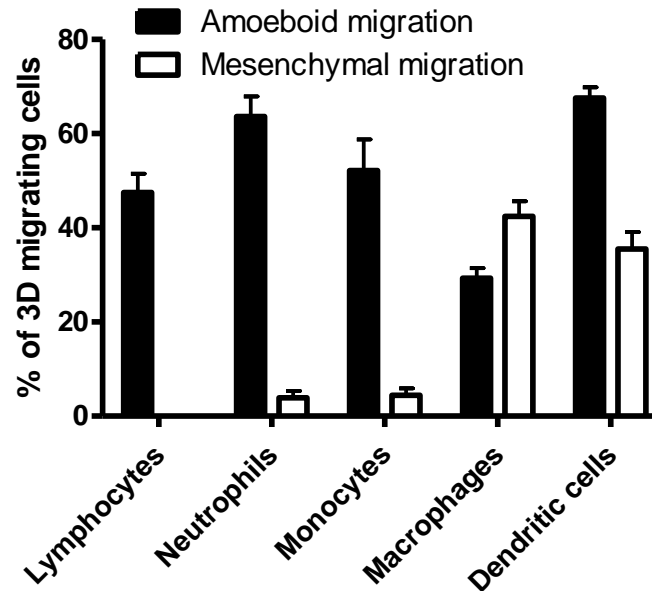
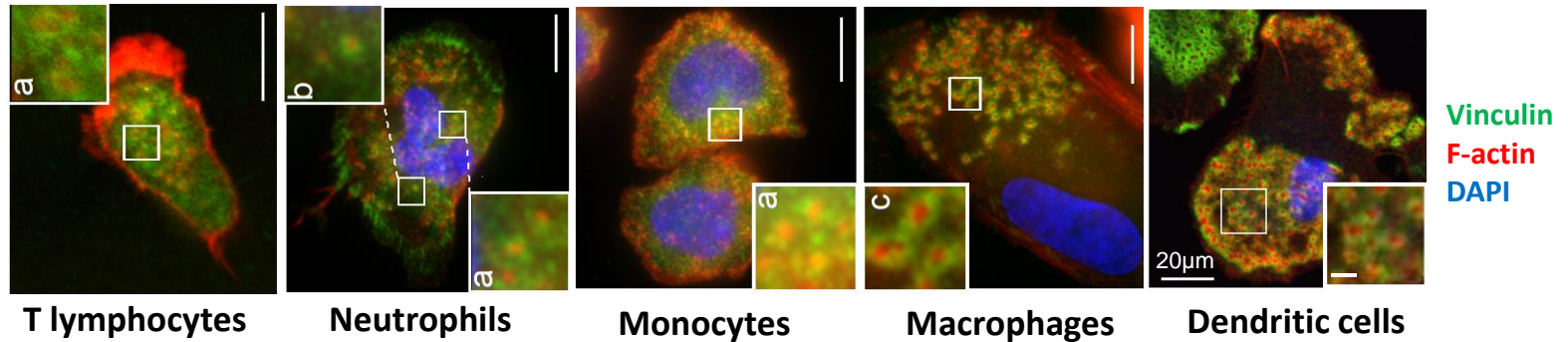
In the context of Mtb infection, monocyte-macrophages display an enhanced motility in dense matrices



Granuloma of Mtb-infected non human primates



Podosomes in 3D migration: other leukocytes



Cougoule et al., Eur. J. Cell Bio. 2012
Cougoule C et al. submitted

- All leukocytes adopt the amoeboid migration mode
- Only macrophages and DC, forming podosomes, use the mesenchymal mode

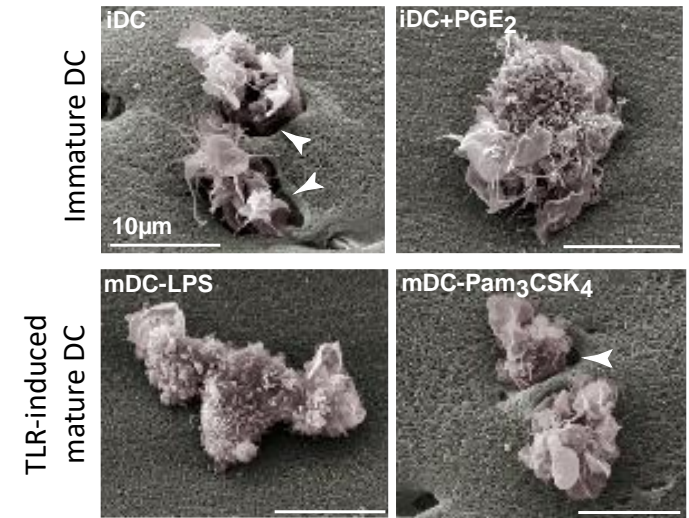
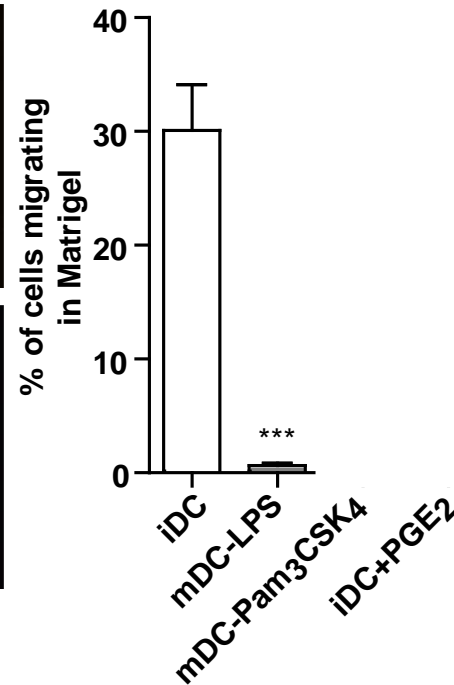
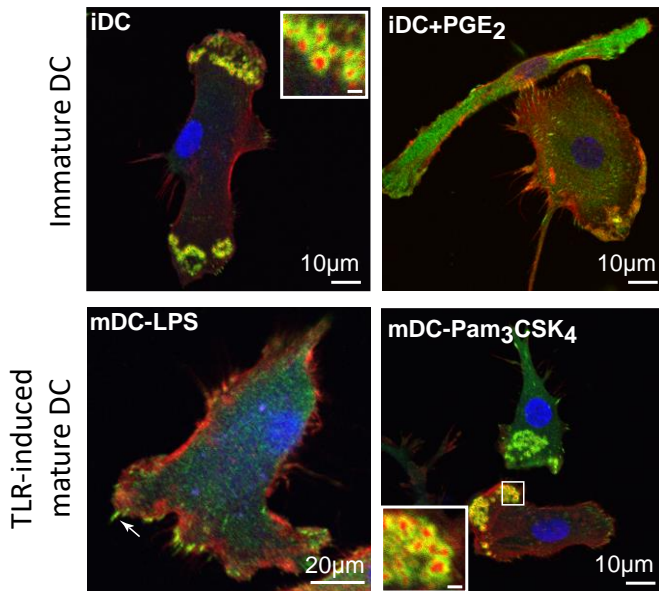
Podosomes in 3D migration: dendritic cells & TLR activation

TLR4-mediated DC maturation induces podosome dissolution: consequences on DC 3D migration ?

📖 Burns et al., 2001; West et al., 2004; van Helden et al., 2010

TLR2-mediated DC maturation maintains podosomes
PGE2 mediates LPS-induced podosome dissolution

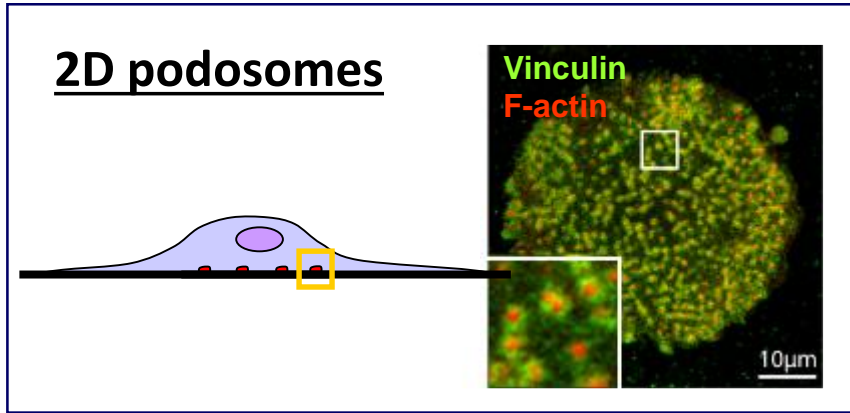
📖 van Helden et al., 2010



📖 Cougoule C et al. submitted

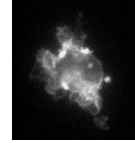
- Human DC adopt the mesenchymal mode, which relies on their capacity to form podosomes, independently of their maturation status,
- TLR activation differentially influences DC 3D migration: consequences on DC migration *in vivo*?

Podosomes in 3D ?

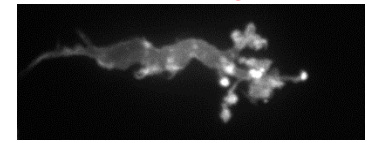


➔ Podosomes in 3D ?

Amoeboid ?

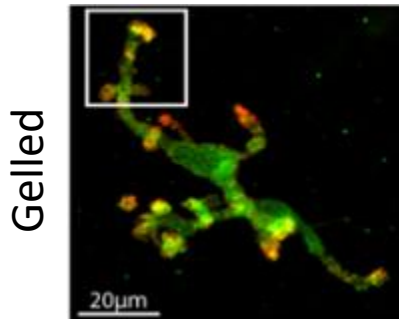


Mesenchymal ?

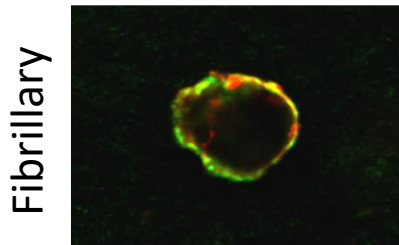
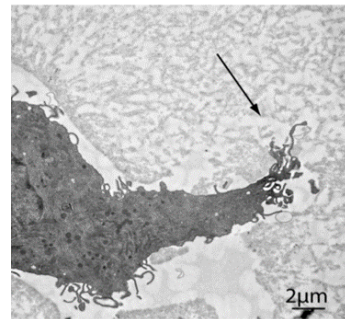


Macrophages in Collagen I matrices

Podosome markers *Proteolytic activity*



Vinculin, F-actin



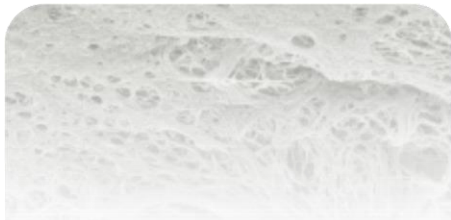
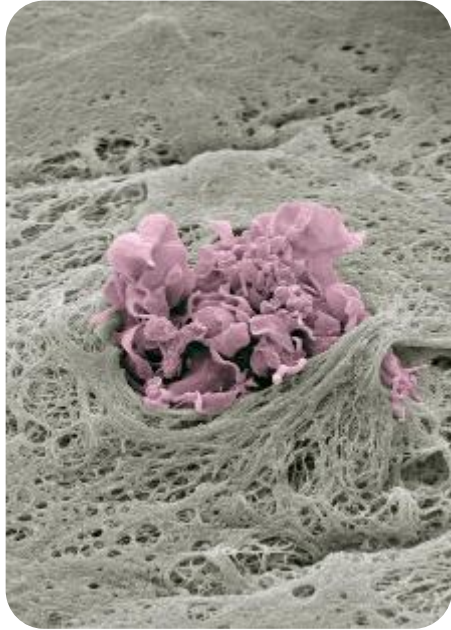
but not the amoeboid migration

➔ 3D podosomes of mesenchymal migration

migration

Macrophage 3D migration: Take-home messages

- Macrophage 3D migration mode depends on the matrix architecture
- Two 3D migration modes *in vitro* and *in vivo* :
 - Amoeboid: protease-independent
 - Mesenchymal: protease-dependent
- Other leukocytes adopt the mesenchymal migration mode when they form podosomes:
 - Dendritic cells and osteoclasts
- Podosomes are functionally linked to mesenchymal 3D migration, but not amoeboid migration
 - Proteolysis of the extracellular matrix
 - Path generating migration
- Mesenchymal 3D migration mode targeted in pathological contexts:
 - *Mycobacterium tuberculosis*
 - HIV-1



The Phagocyte Migration and Differentiation group

<https://phagocytes.weebly.com/>



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Agence Nationale de la Recherche



Agence nationale de recherches sur le sida et les hépatites virales



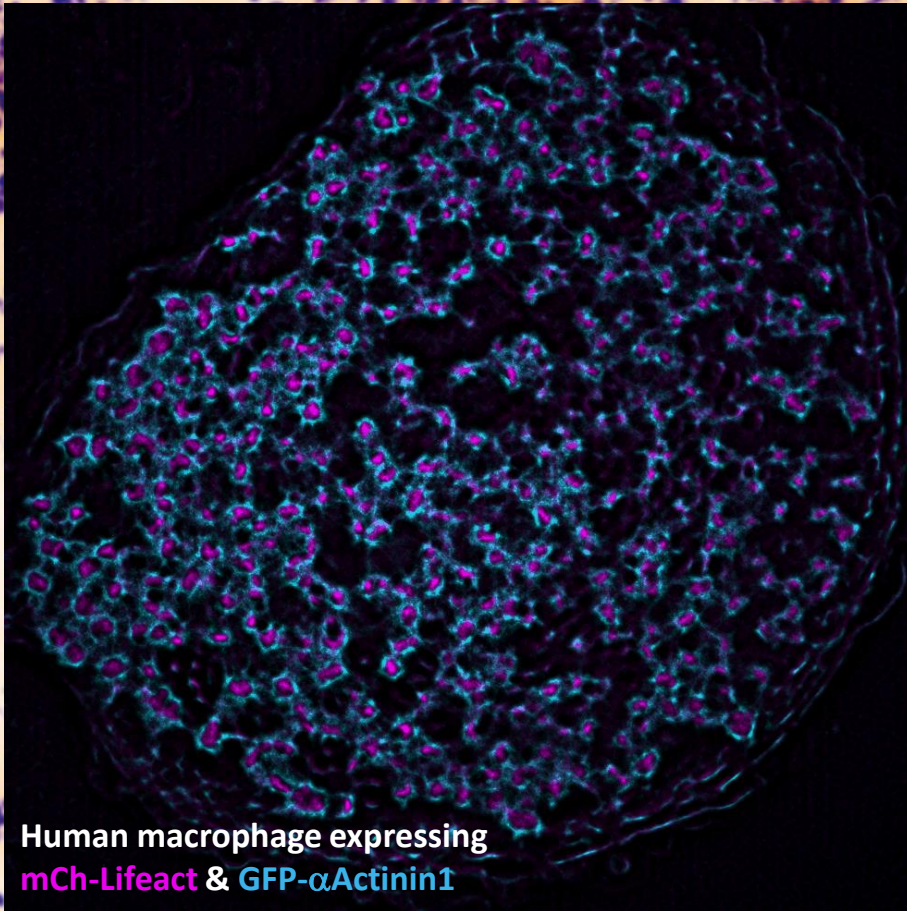
ENSEMBLE CONTRE LE SIDA



Postdoctoral position available !

Architecture/force relationship of macrophage podosomes

For further informations: <https://phagocytes.weebly.com/>
contact imp.job@ipbs.fr



Human macrophage expressing
mCh-Lifect & GFP- α Actinin1

