ETH zürich

D-BIOL



Tissue Marcophages
Immunology III HS 2025

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01. Dezember 2025

How to read these slides

! Central concepts — exam-relevant

How we know In Immunology III, we look at the actual experiments behind key ideas—not just textbook summaries. These slides show *where the evidence comes from*. You don't need to memorize the details but use them to think about **how scientists know what they claim** and how data shapes our understanding.

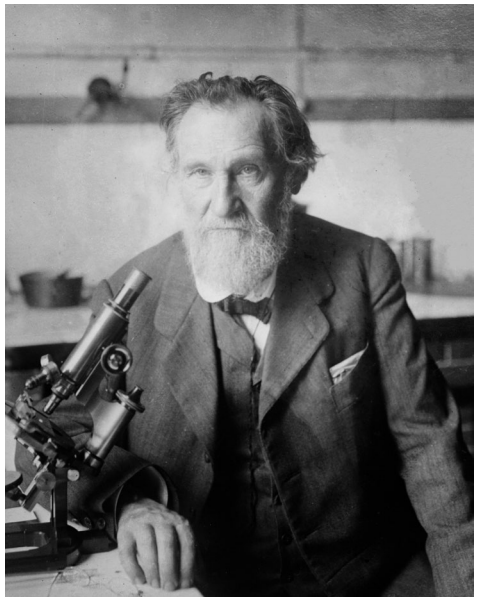
Slides with black background serve as additional illustration from our own research.

Literature (DOI) these slides are mostly based on:

- 10.1038/s41586-023-06002-x
- 10.1016/j.immuni.2016.02.024
- 10.1016/j.cell.2022.10.007
- 10.1038/nri3712

Historical Concepts

- Mechnikov 1892 Discovery of phagocytes
  - Small eaters (**microphages**, today PMN)
  - Large eaters (**macrophages**)
- Aschoff: «**Reticulo Endothelial System**» (RES)
- Late 1960s «**Mononuclear Phagocyte System**» (MPS) premise that **all macrophages derived from monocytes**.
- 1970 Steinman discovery of **dendritic cell** (DC) which was considered the third cell in the MPS
- Cells of the MPS initially thought to derive exclusively from blood monocytes, but with more fate-mapping tools available this concept is now obsolete.



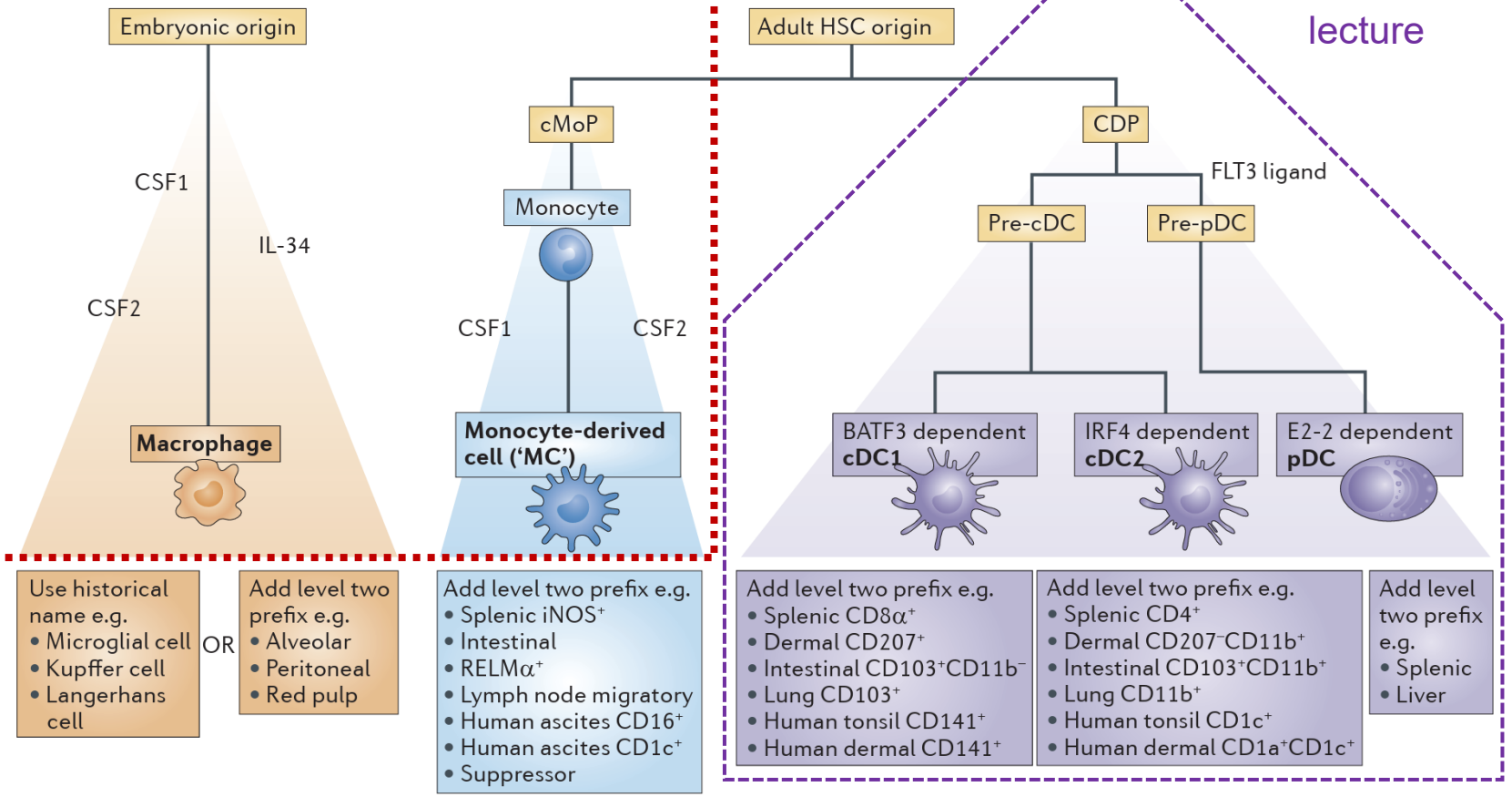
Ilya (Elie) Mechnikov 1892, the father of cellular immunity

Learning Objectives

1. Students can correctly **classify/name cells of the Mononuclear Phagocyte System (MPS)**
    1. First level nomenclature by otogeny
    2. Second level nomenclature by tissue/niche
    3. Second level nomenclature by functional properties/programs.
2. Students understand the **Core Functions** of macrophages in homeostasis
    1. Extracellular milieu: safeguarding tissue structure
    2. Cellular fitness: efferocytosis
    3. Microorganisms: innate immunity
    4. Metabolism
3. Students illustrate the role of macrophages in **specific disease mechanisms**
    1. Primary macrophage defects.
    2. The role of macrophages in non-primary macrophage diseases.

A **decision tree** to facilitate nomenclature for mononuclear phagocytes

First level nomenclature by ontogeny



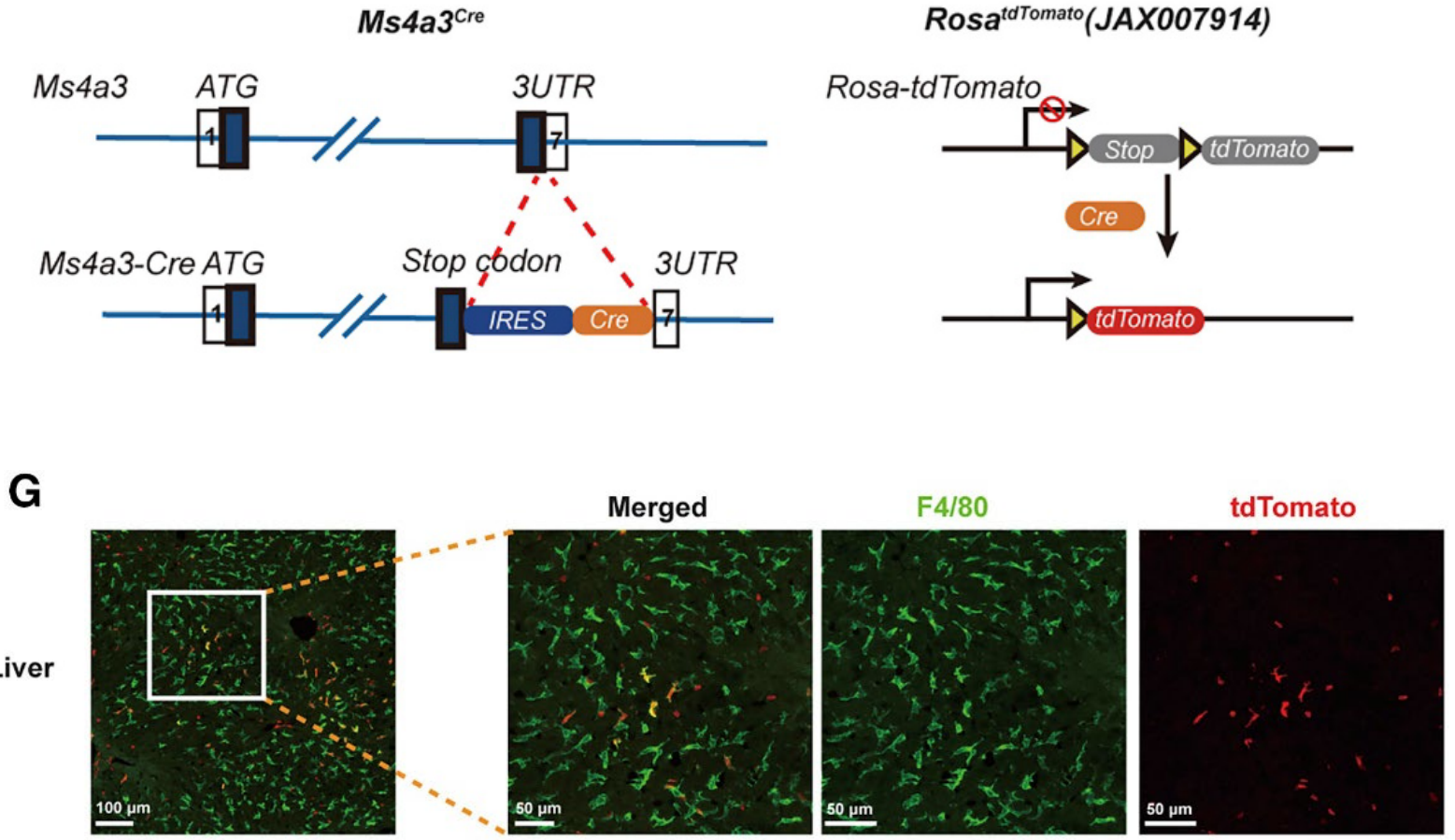
DCs were already covered in separate lecture

10.1038/nri3712

Example of a fate mapping study.

How we know

- In this paper they show first that **Ms4a3^{Cre} Rosa26$^{LSL\ tdTomato}$** is specifically and transiently expressed by Granulo-Monocyte Progenitors (GMP)
- Ms4a3^{Cre}-based models fate-map monocytes and granulocytes.
- Then, they look at many bona fide TRMs over time (**without** inflammatory challenge).
- The contribution of monocytes to TRMs is largely tissue-dependent.



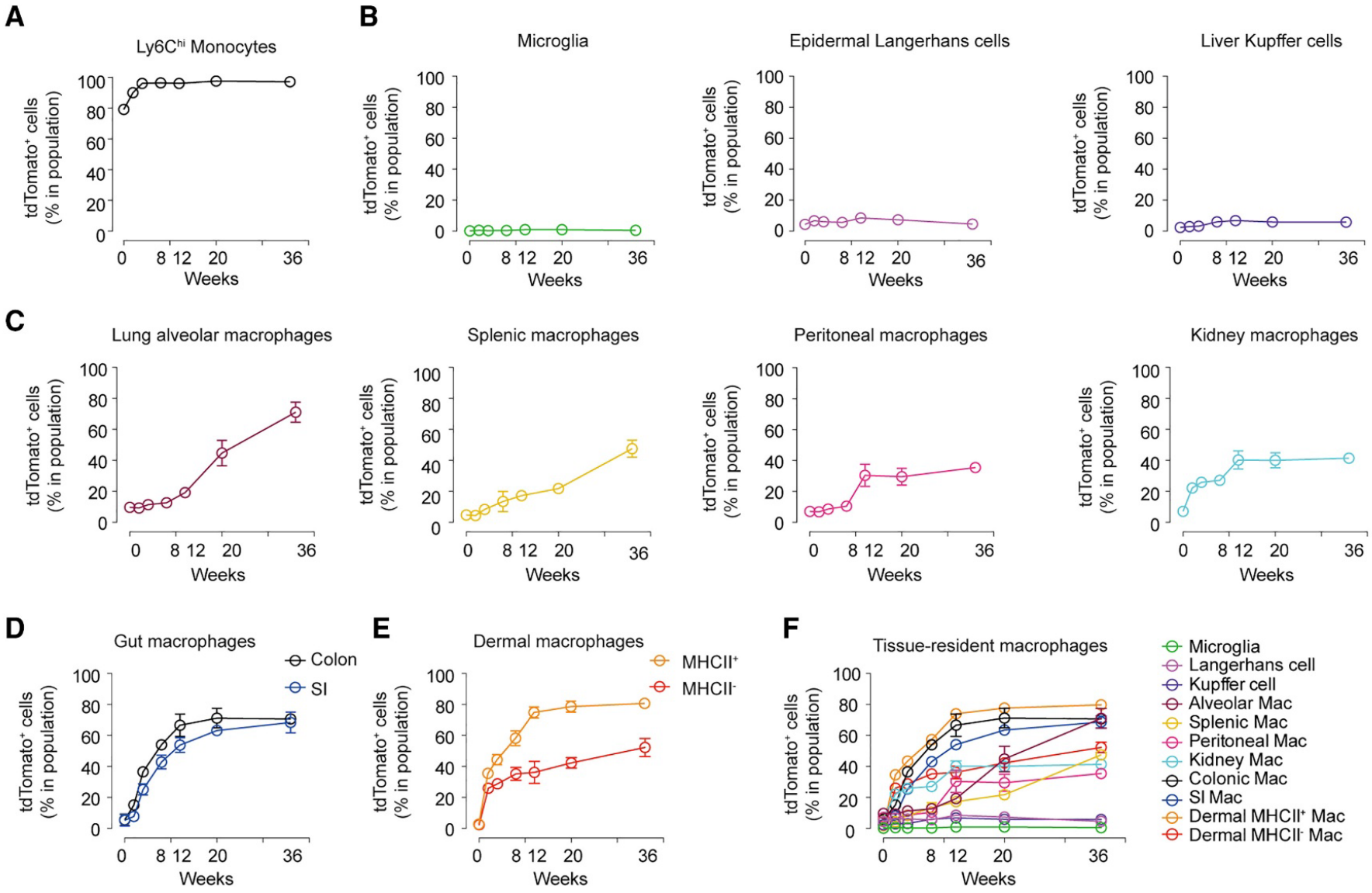
Green = Kupffer cells

Red= Monocyte derived

https://doi.org/10.1016/j.cell.2019.08.009

Example of a fate mapping study.

- These observations are under homeostatic non-inflammatory conditions.
- Importantly, monocytes contribute **signifcantly more under inflammatory challenge (injury)**

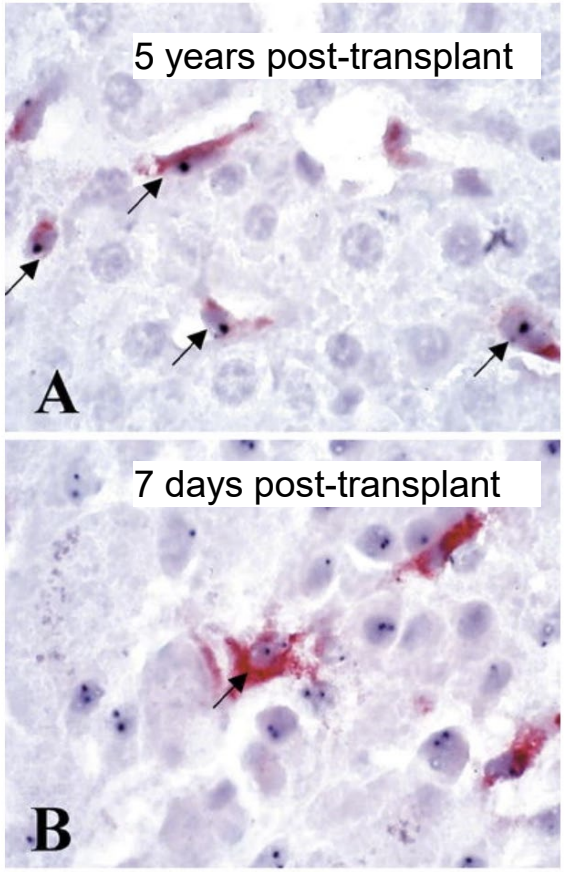


Fate-mapping studies in humans?

How we know

- Mouse paradigm broadly supported in humans, i.e. presence of **long-lived/replicated TRMs that can be replaced by monocytes, especially under injury.**
- After **lung transplant**, alveolar macrophages are largely replaced by monocyte-derived cells (Sex-mismatched donors). Note this is **controversial!** Some authors find no or very late replacement.
- After liver transplant, Kupffer cells are long-live, self-replicating, but also largely replaced over time (HLA-mismatched, Sex-mismatched)
- Brain microglia stay recipient-derived after bone marrow transplantation (even decades later), but replacement possible after injury.

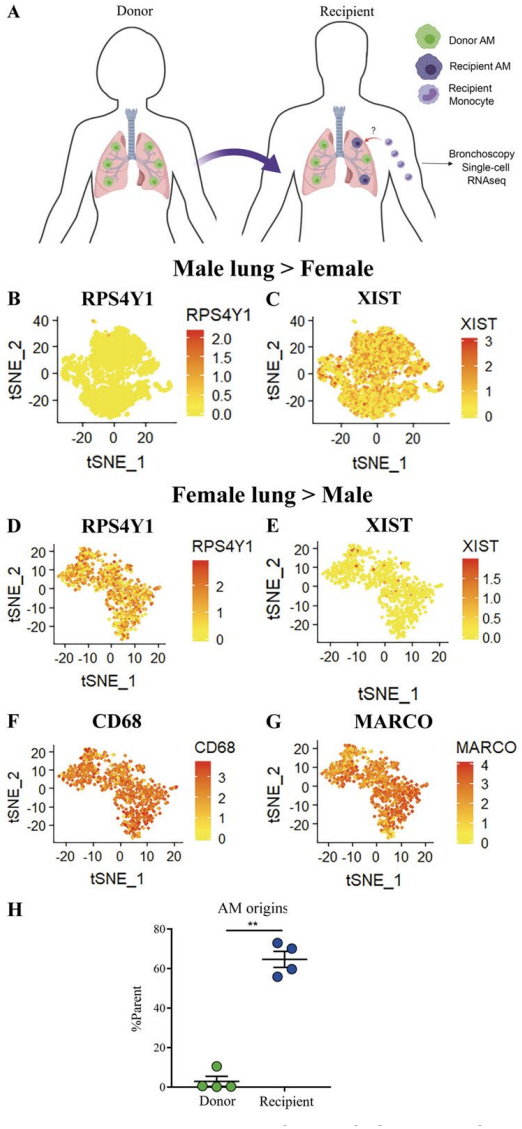
Liver



Signal of Y chromosome (arrow)

Anti-CD68 (Kupffer cell marker)

Lung



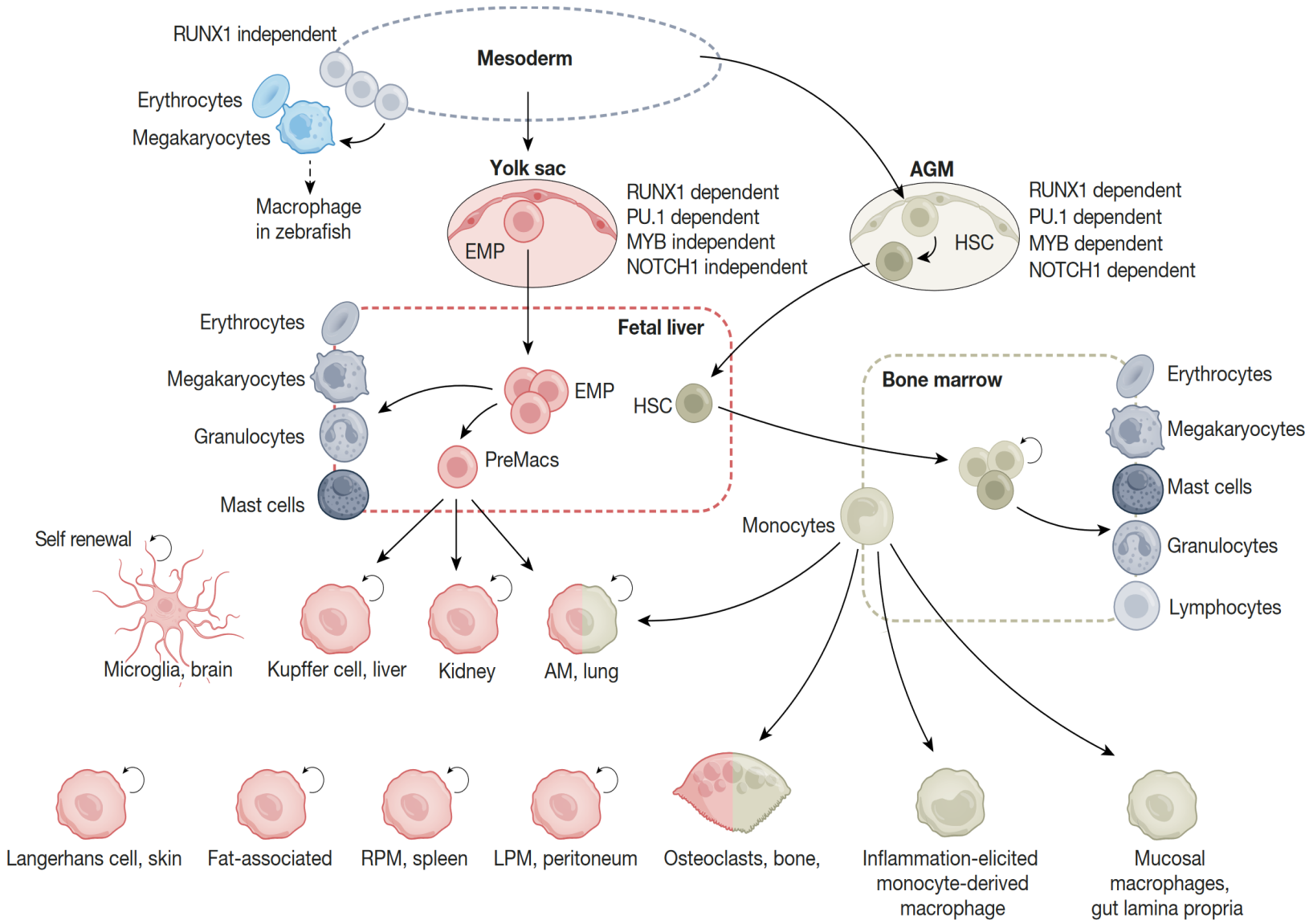
https://doi.org/10.1084/jem.20191236

DOI 10.1007/s00428-003-0861-8

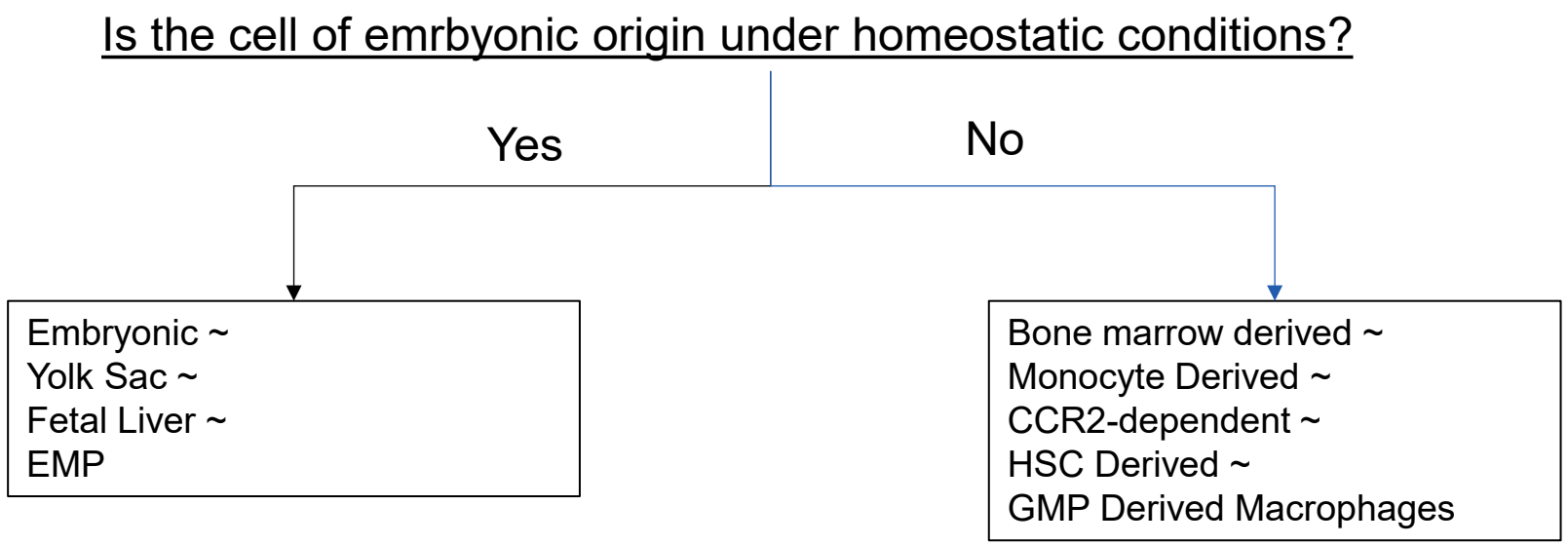
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Otogeny: Developmental Origins

- Using many different fate-mapping tools the different waves of otogeny have been established
- At E8.5 erythro-myeloid progenitors (**EMPs**) arise from the **yolk sac** in a RUNX1-dependent manner and colonize the fetal liver.
- EMP-derived precursor macrophages (preMAcs) colonize the entire embryo during organogenesis, before the emergence of HSCs
- Haematopoietic stem cells (**HSCs**) emerge from the intra-embryonic endothelium and colonize the bone marrow before birth.
- HSC give rise to erythroid, lymphoid, and myeloid cells (including macrophages) throughout life.

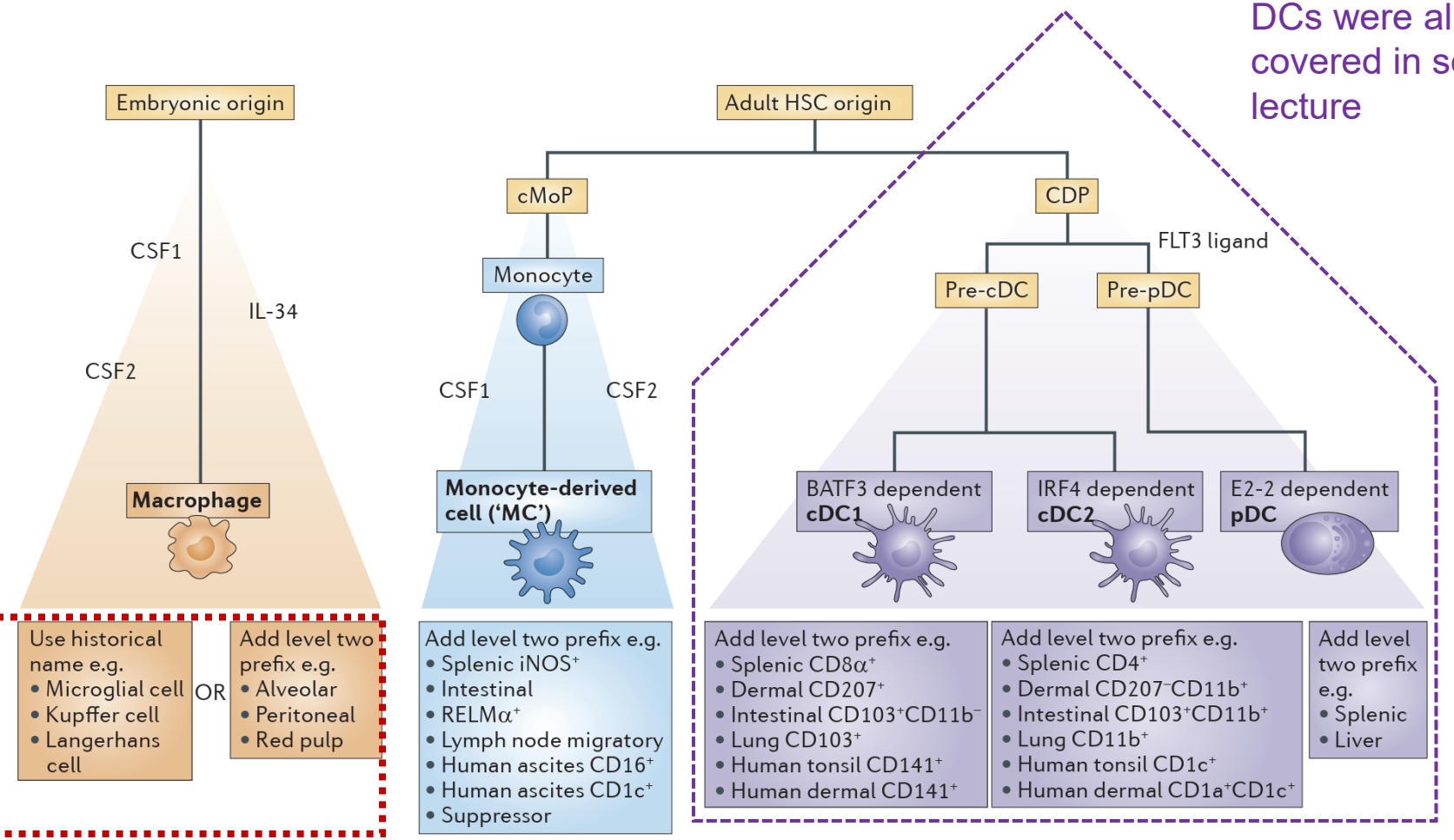


Otogeny: Reduced Complexity Model



Paradigm: **EMP** gice rise to tissue resident macrophages (TRM) that self-renew locally, whereas **HSC**-derived macrophages are short-lived and rely on circulating monocytes and can massively expant.

A **decision tree** to facilitate nomenclature for mononuclear phagocytes

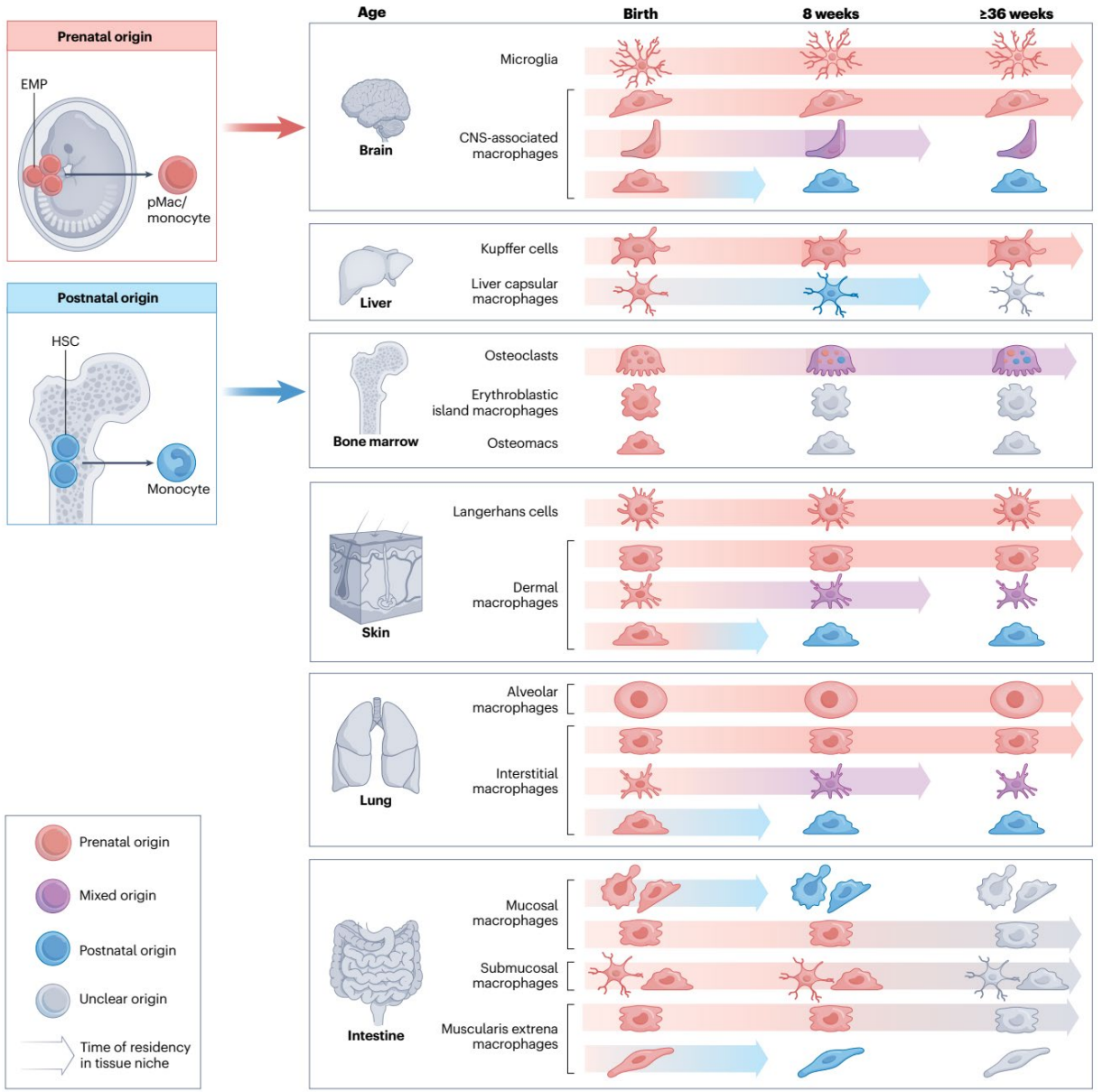


DCs were already covered in separate lecture

Second level nomenclature by tissue/niche

Level II: Name by **Niche (mostly for TRM)**

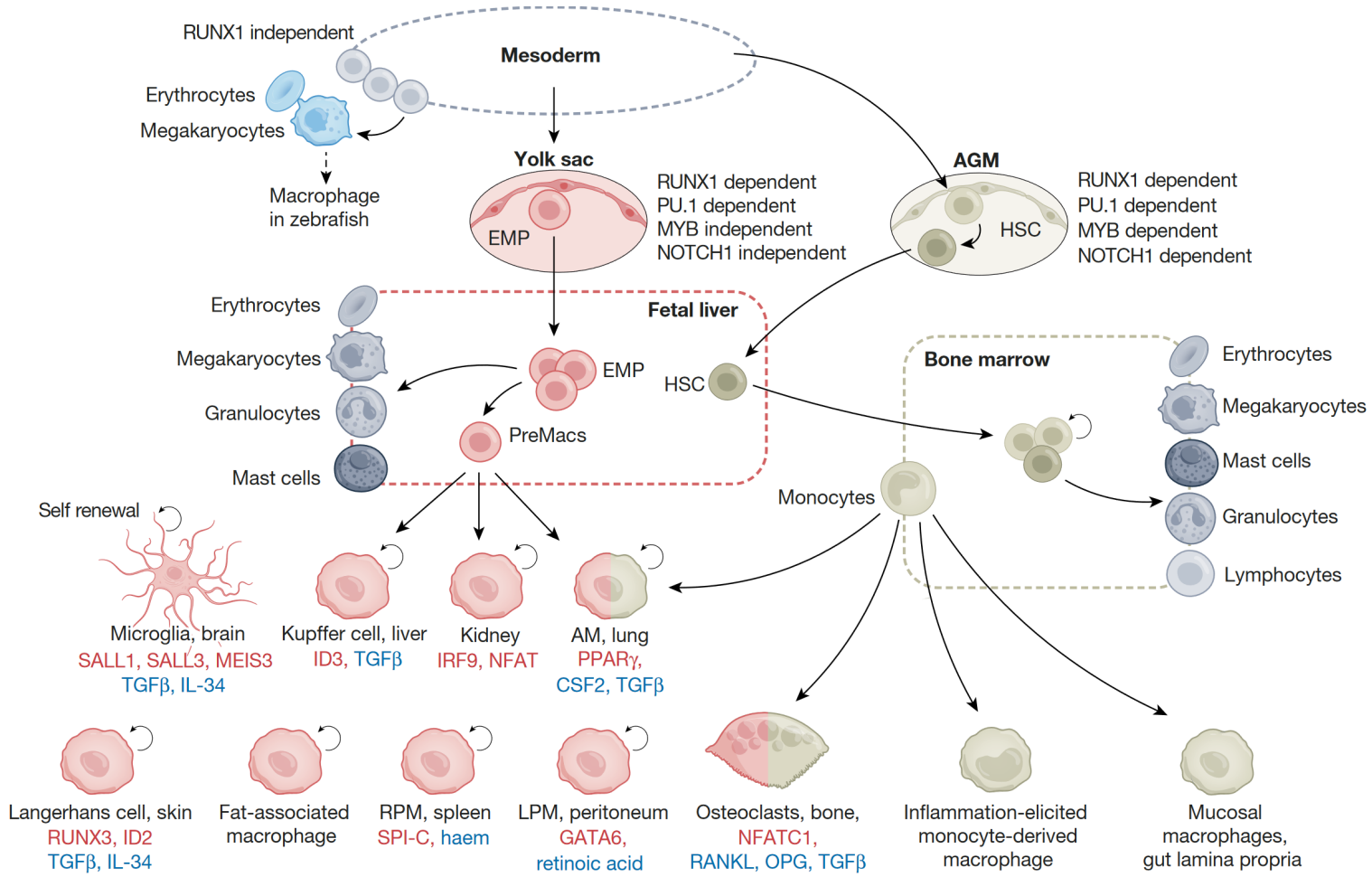
- Second level classification according to niche.
- Either by historic names such as Kupffer cells, Langerhans cells, Microglia, ect.
- Or by adding a 2nd level prefix, e.g. Alveolar Macrophage, Peritoneal Macrophage.
- When we look more closely there are often **several distinct niches per organ.**
- The cell populations in these **subtissular niches** can be of different EMP/MSC origin which can make classification complex.



https://www.nature.com/articles/s41577-023-00848-y

Is the niche shaping the macrophages or the other way arround?

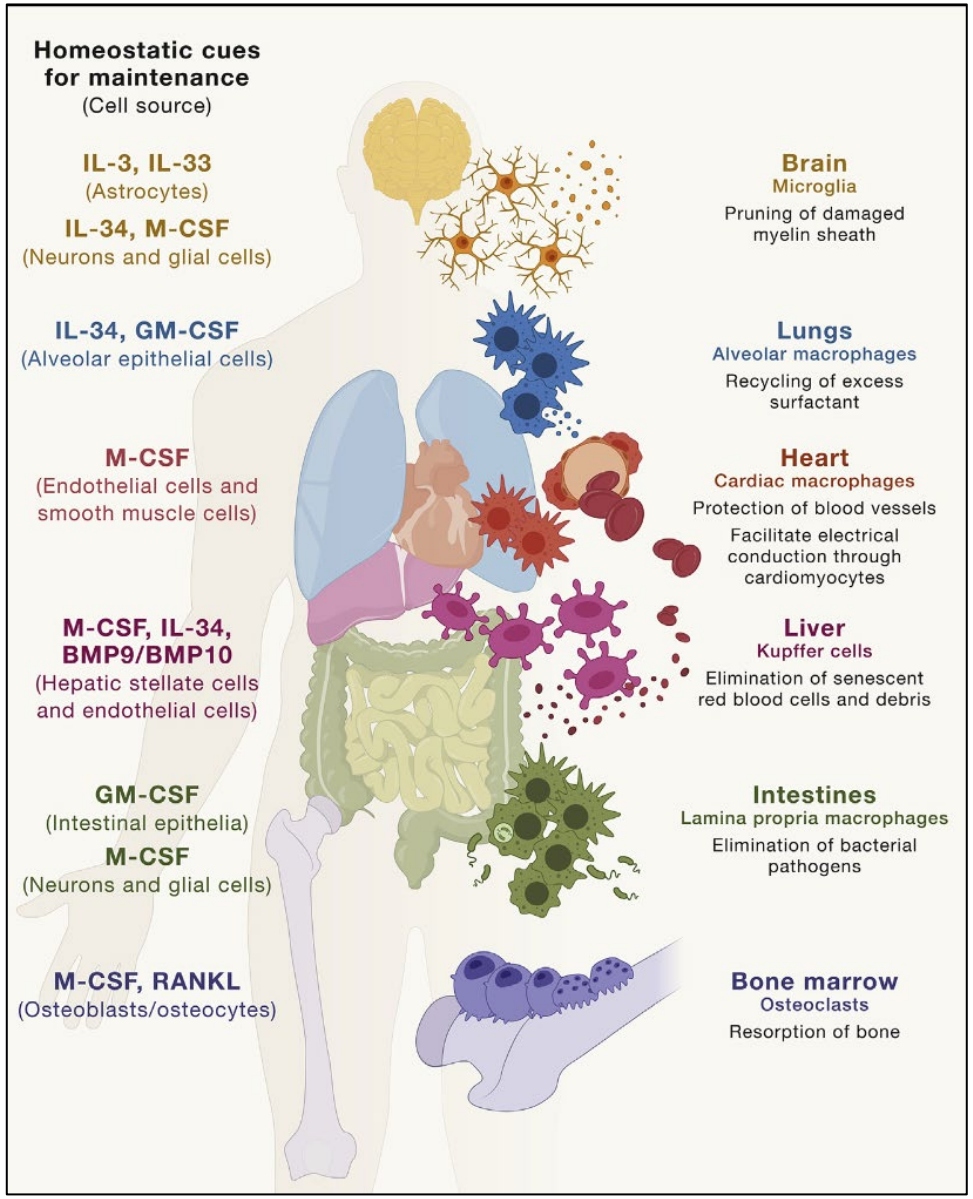
- While preMacs share a core macrophage transcriptional programme (PU.1, cMAF, IRF8), cells start to express **niche-specific lineage marker in their respective destination tissue.**
- Cell-intrinsic lineage Determining Factors (LDFs) comprise **SALL1, ID3, PPARy, SPI-C, GATA6.**
- At the same time, the macrophages receive niche-specific signals that have been shown to induce LDFs: **TGFb, RANKL, Retinoic Acid.**
- The **instructive model** suggests that the specification process is driven by niche-specific signals, while the **stochastic model** suggest that it is driven mainly by cell-intrinsic signals.



Level II: Name by **Niche**

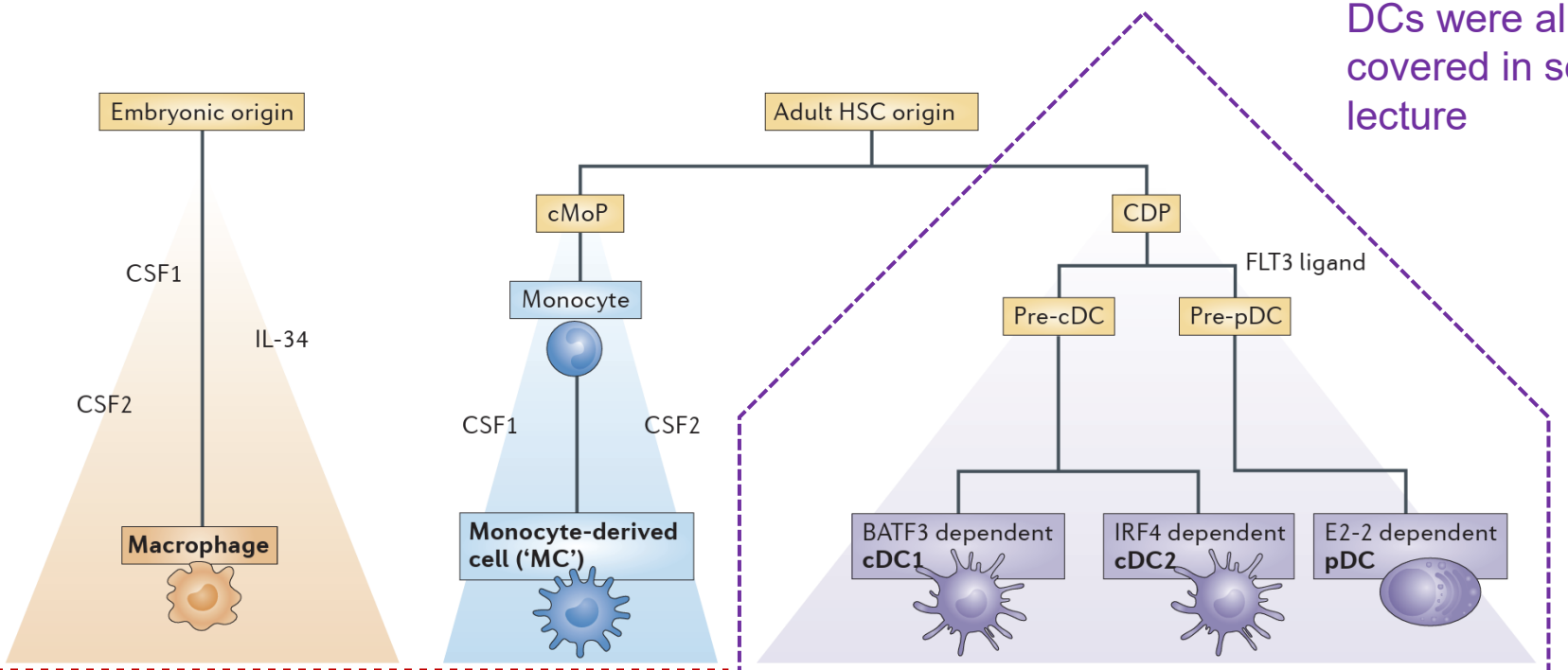
A TRMs niche needs lifelong suvival signals to be maintained. These signals are mostly CSF1R and CSF2R signaling.

- **CSF1$^{-/-}$ mice**: No macrophages except Langerhans cells and Microglia
- **IL34$^{-/-}$ mice**: no Langerhans cells and no Microglia.
- **CSF2$^{-/-}$ mice**: no lung alveolar macrophages
- **CSF2R$^{-/-}$ in humans**: pulmonary alveolar proteinosis (i.e. no alveolar macrophages)
- **Remember this 1234**:
  - CSF**1** = **M**-CSF
  - CSF**2** = **GM**-CSF
  - Il**34** = CSF1R **ligand**



10.1016/j.cell.2022.10.007

A **decision tree** to facilitate nomenclature for mononuclear phagocytes



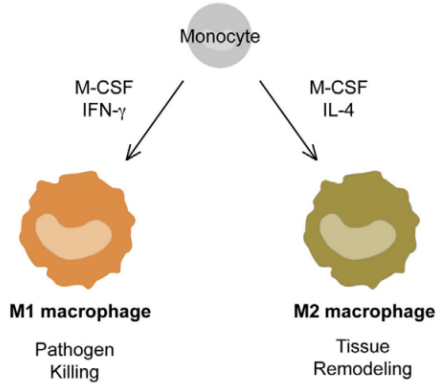
DCs were already covered in separate lecture

Second level nomenclature by function/program

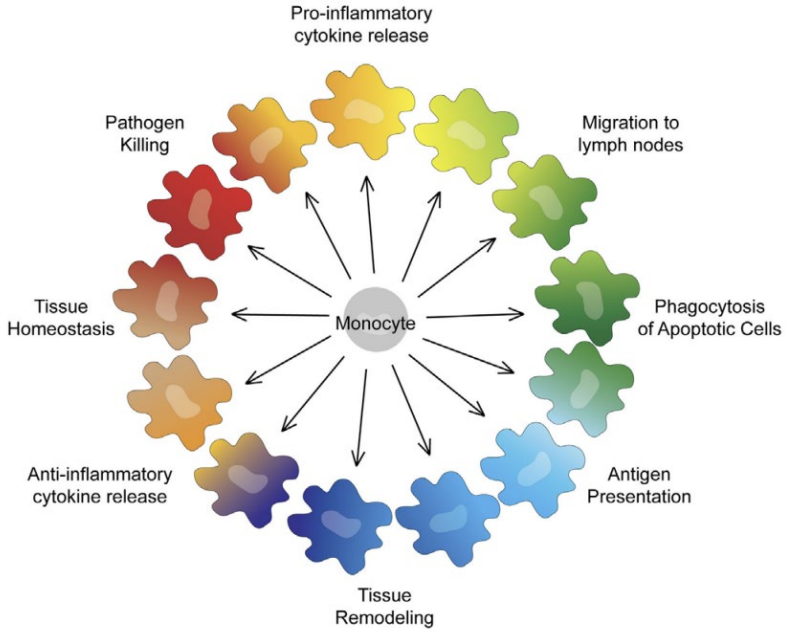
LEVEL II – Name by Functional Properties (**mostly for BMDM**)

- M1/M2 are no longer considered accurate descriptors, historically
  - **M1 «classical»**
  - **M2 «alternative»**
  - **DC**
- This heuristic came mostly from in vitro experiments (Figure left panel).
- It is recommended to retire this heuristic when describing in vivo systems and to think of mac activation as a **modular spectrum**.
- The granularity of this spectrum has increased with scRNA-Seq:
  - Example: Lipid-handling (Trem2^{+}) macrophage core program with disease- and tissue specific modifiers: Fibrosis-associated (FAM), Tumor-associated (TAM), Lipid-associated (LAM), Scar-associated (SAM), NASH-associated (NAM)

(historic) Discrete Polarization Model

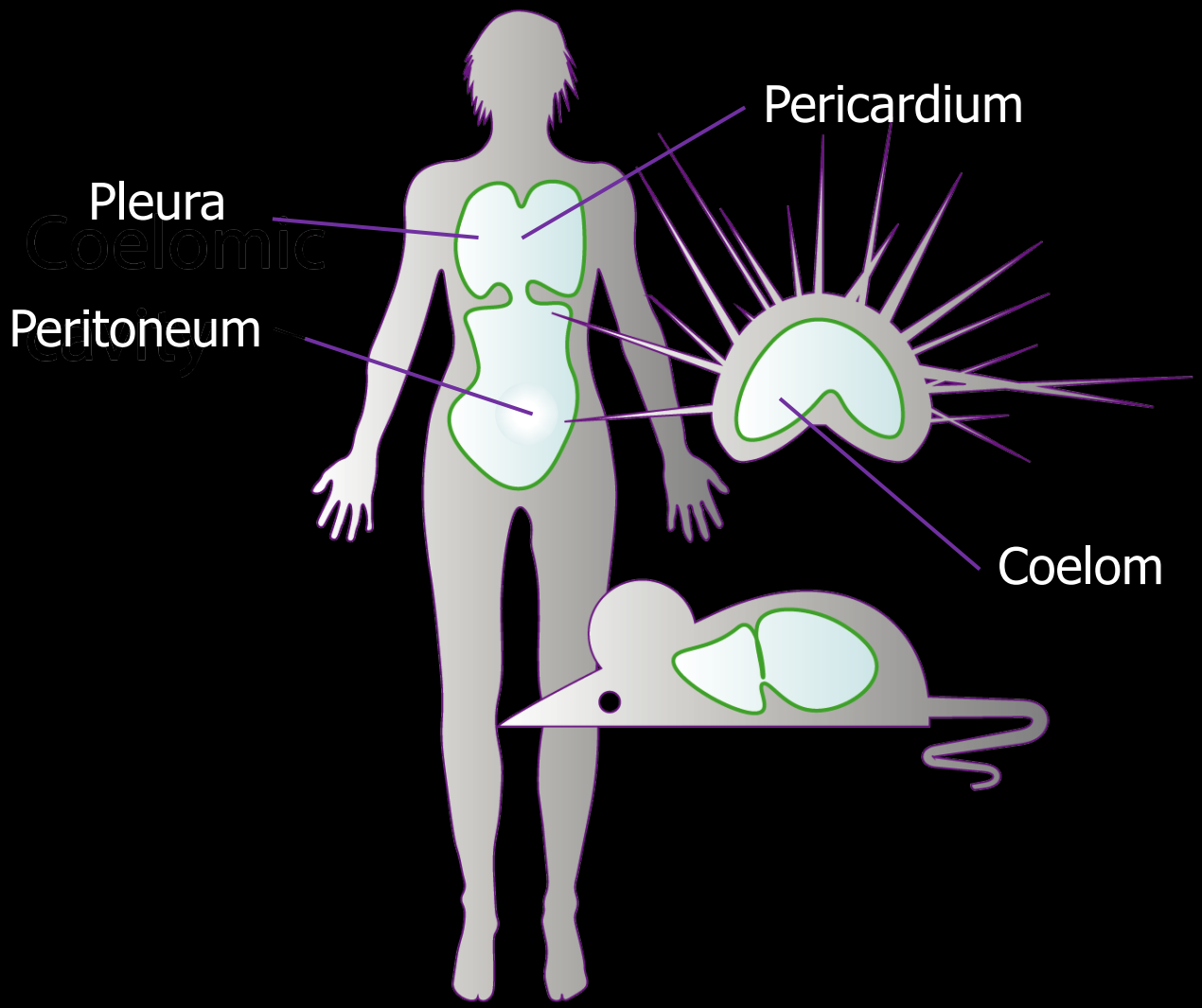


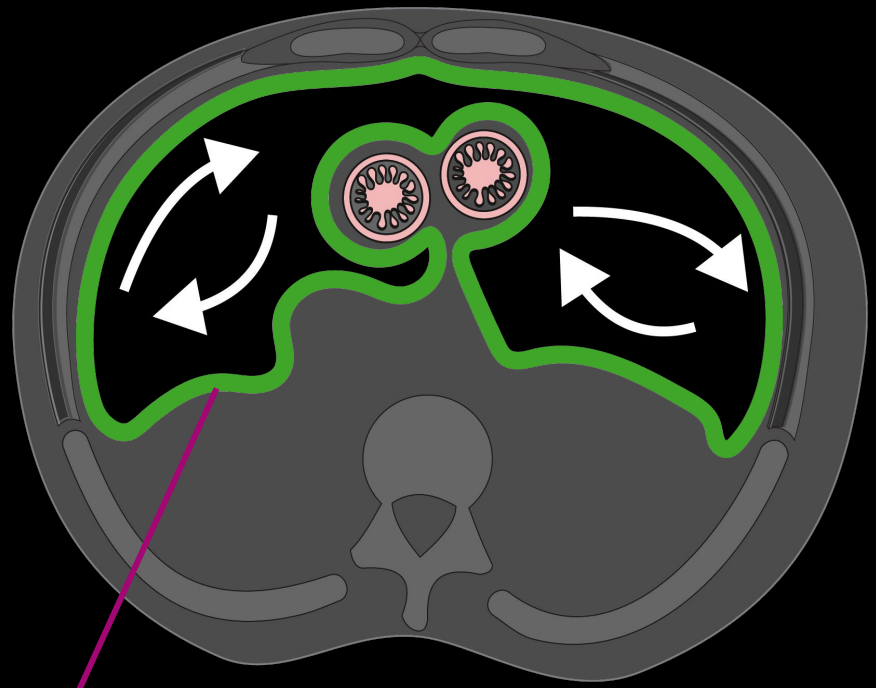
Modular Spectrum Model



Let's look at one niche more closely (from our own research)

How we know





Mesothelium ~ Serosa ~ Peritoneum

Let’s look at one niche more closely (from our own research)

How we know

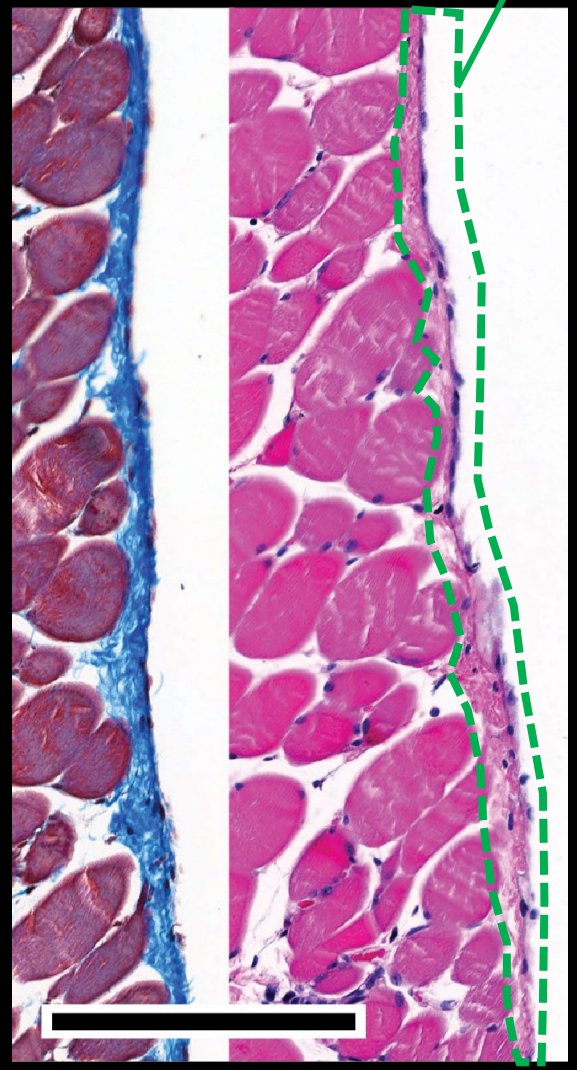




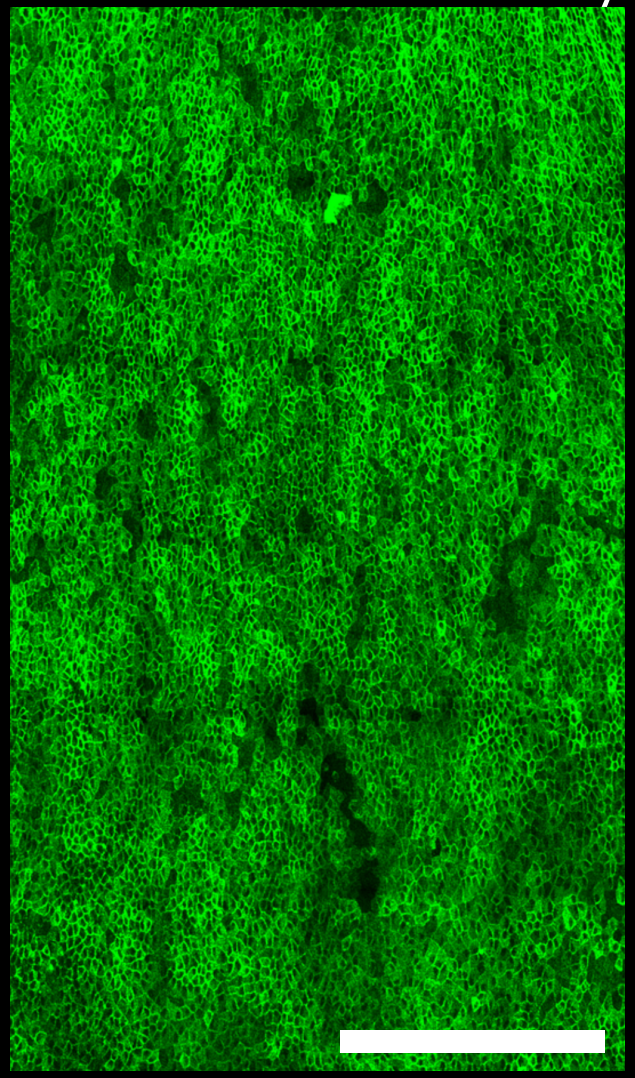
Fair Warning

Let's look at one niche more closely (from our own research)

How we know



Masson



H&E

Anti-Podoplanin

500um

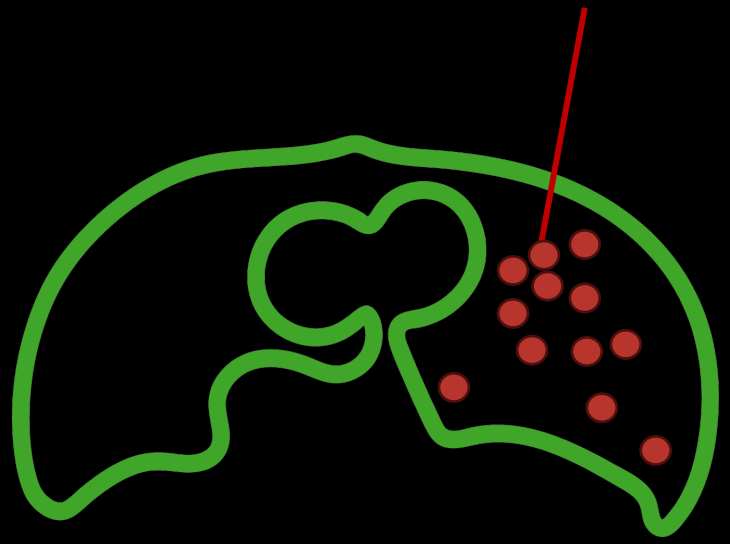
Tissue Resident Macrophages =
Large Peritoneal Macrophages=
GATA6^{+} F4/80^{hi} CD102^{+}

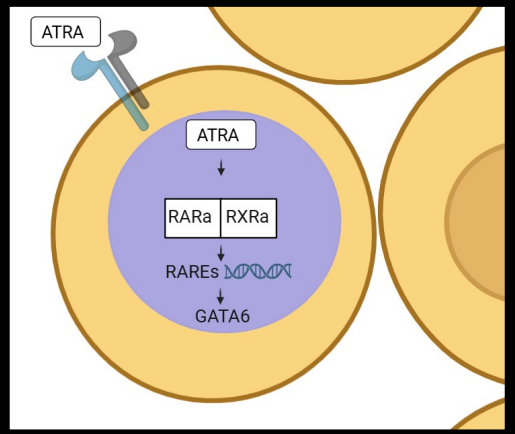


How we know

Modulation of macrophage GATA6 expression

Tissue Resident Macrophages =
Large Peritoneal Macrophages=
GATA6^{+} F4/80^{hi} CD102^{+}

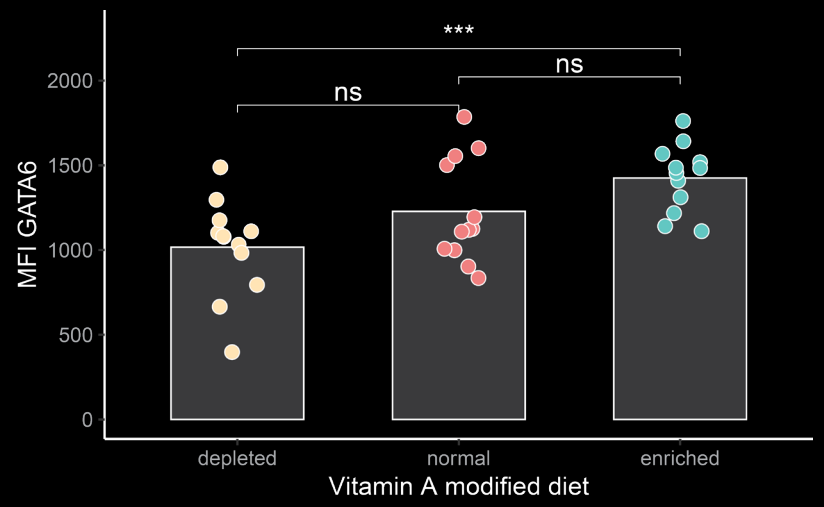




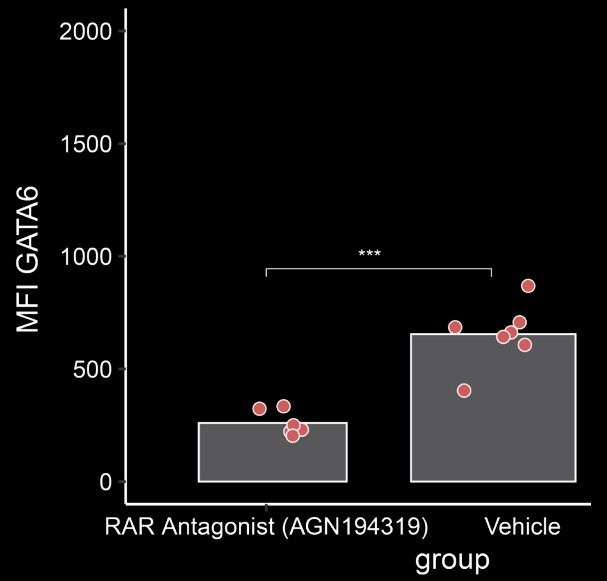
Dietary Vitamin A intake

depleted normal enhanced



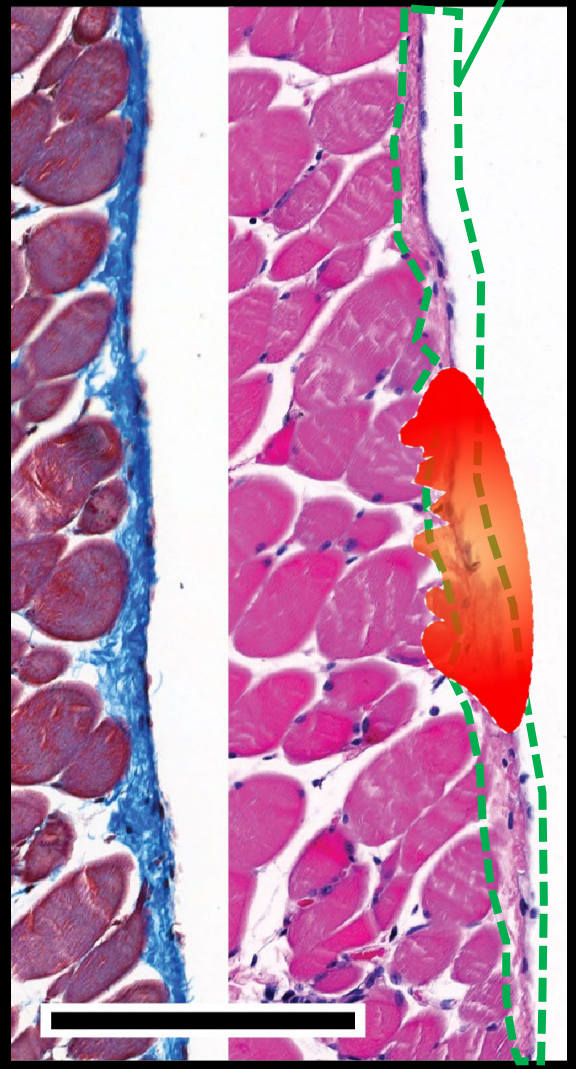


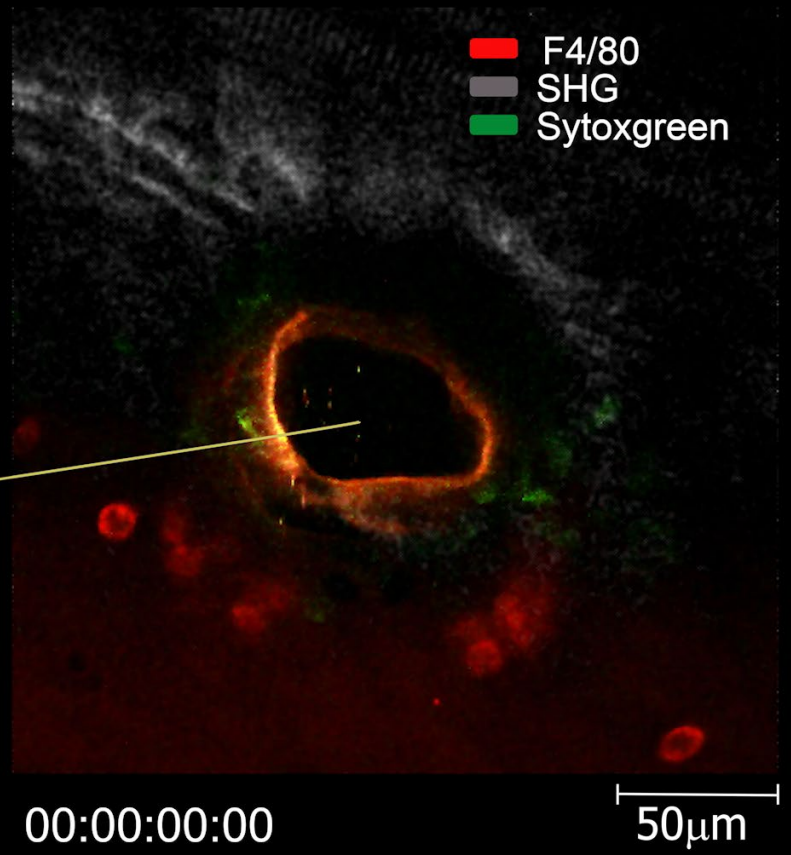
Pharmacological



Let's look at one niche more closely (from our own research)

How we know





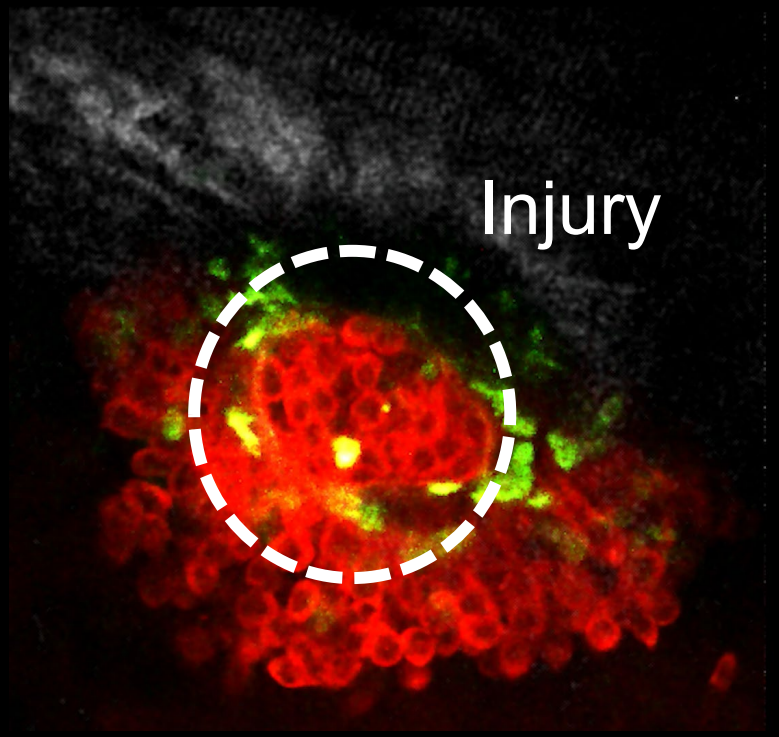
Use case: Intravital Microscopy

How we know

Non-alcoholic beverage

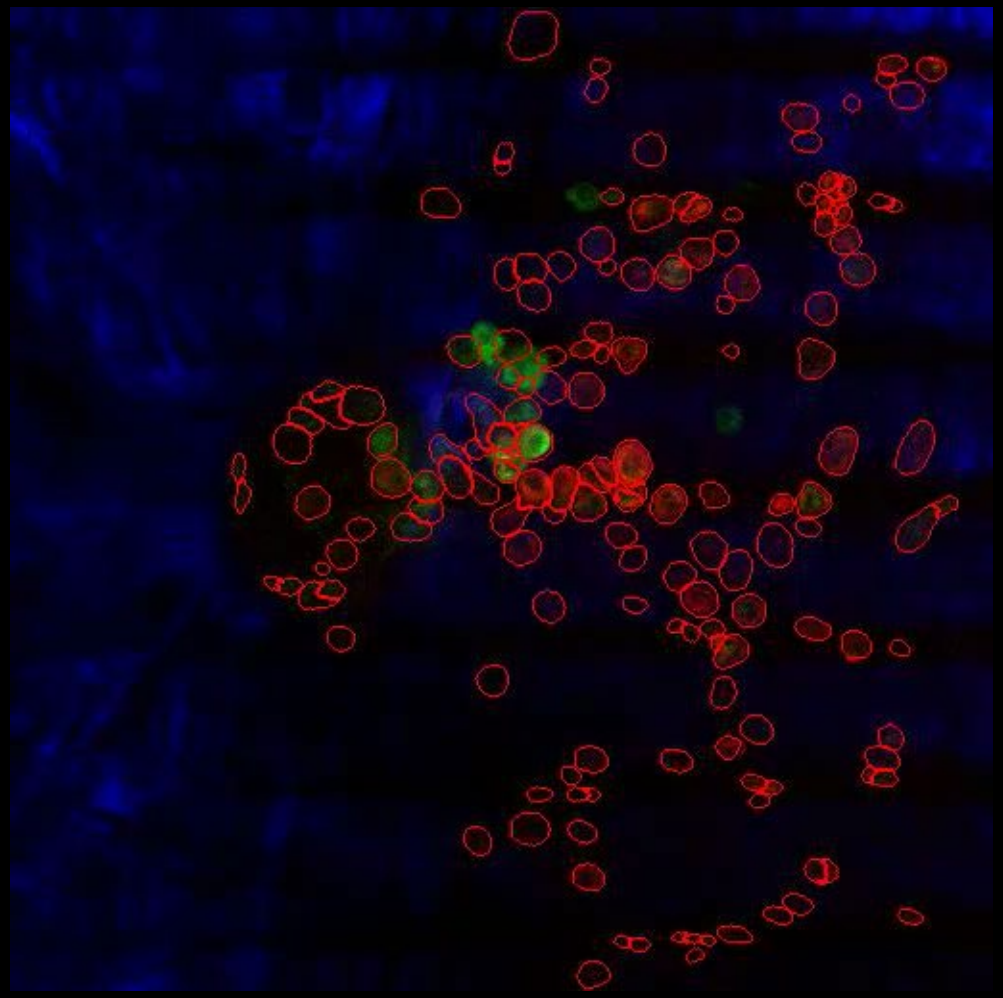


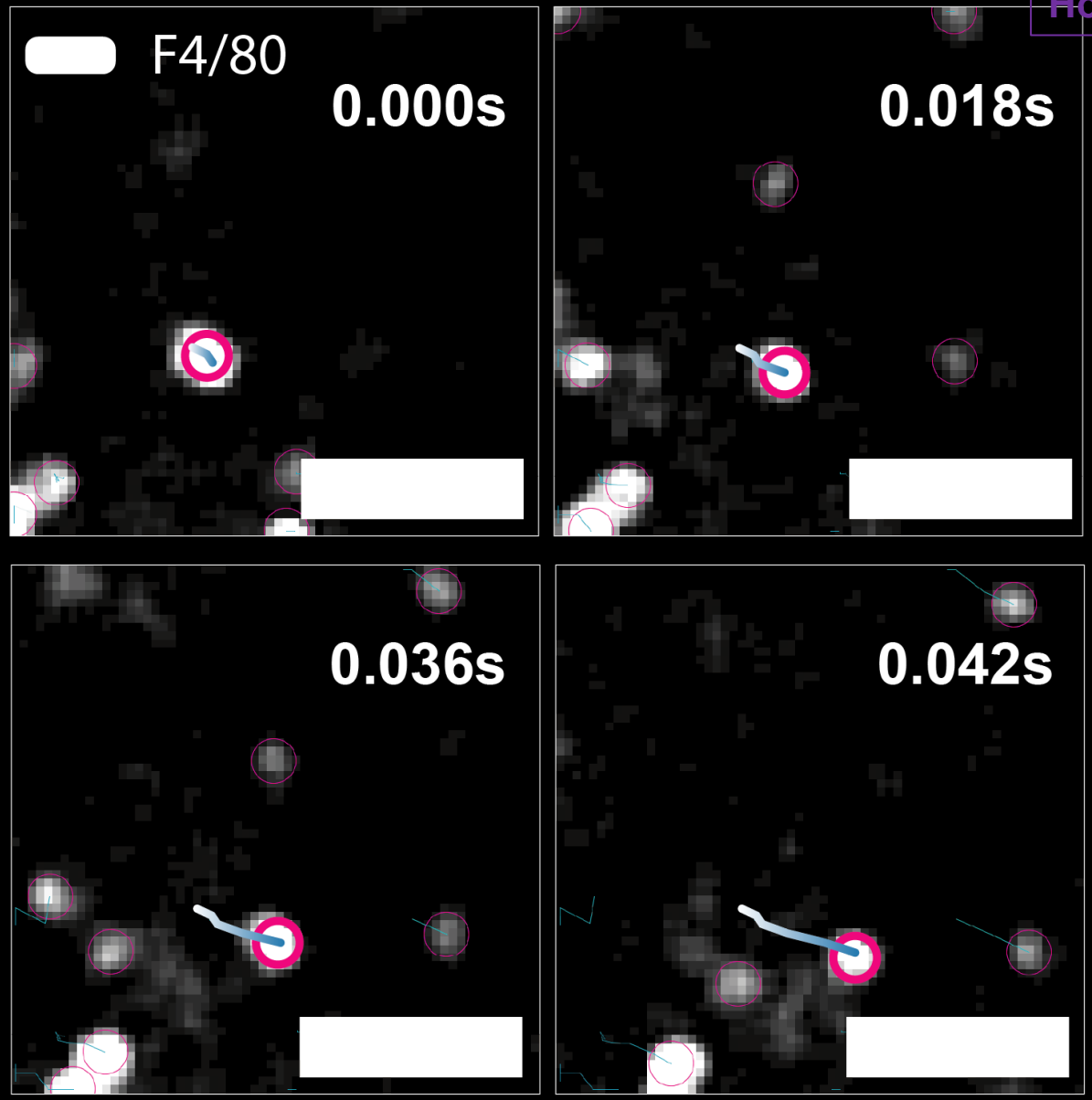
Students



Macrophages

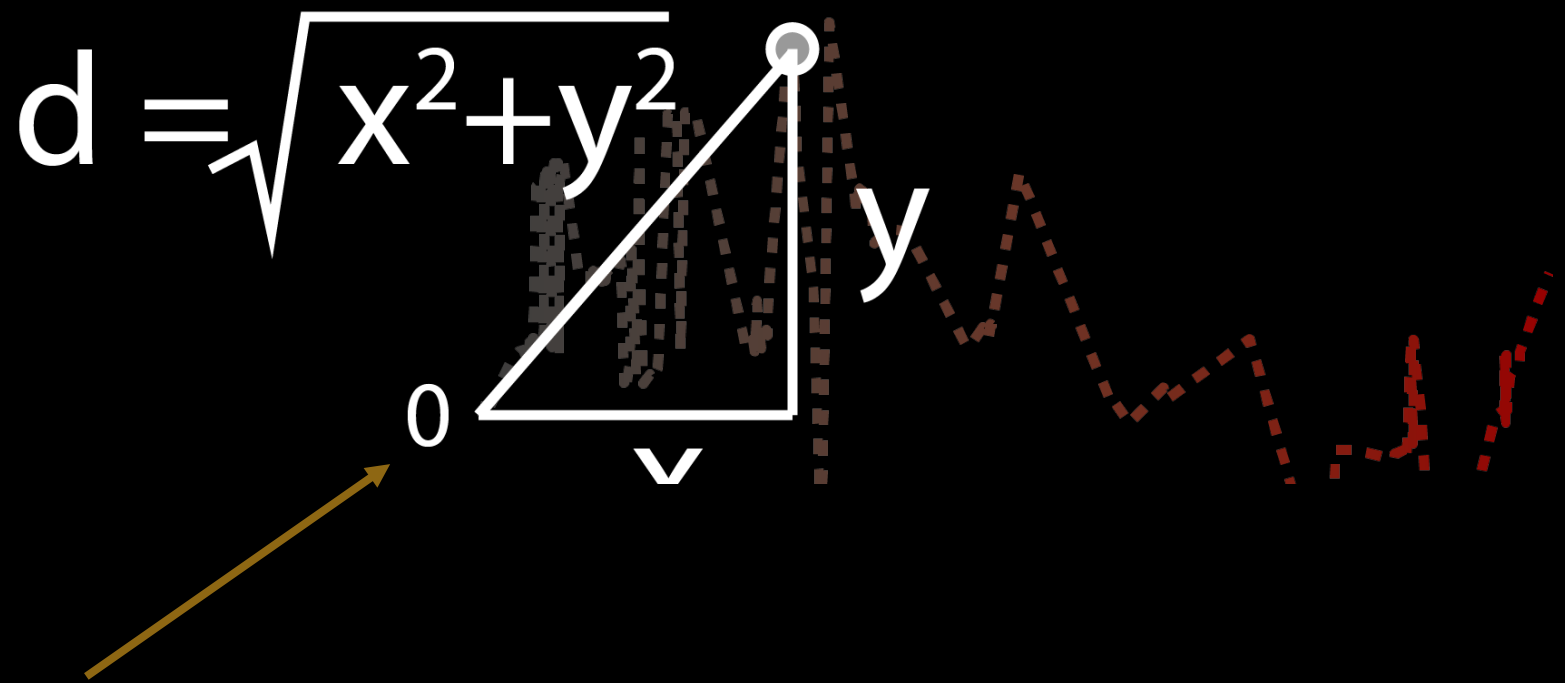
How we know





Tinevez, J. Y., et al. (2017). "TrackMate: An open and extensible platform for single-particle tracking." Methods **115: 80-90.**

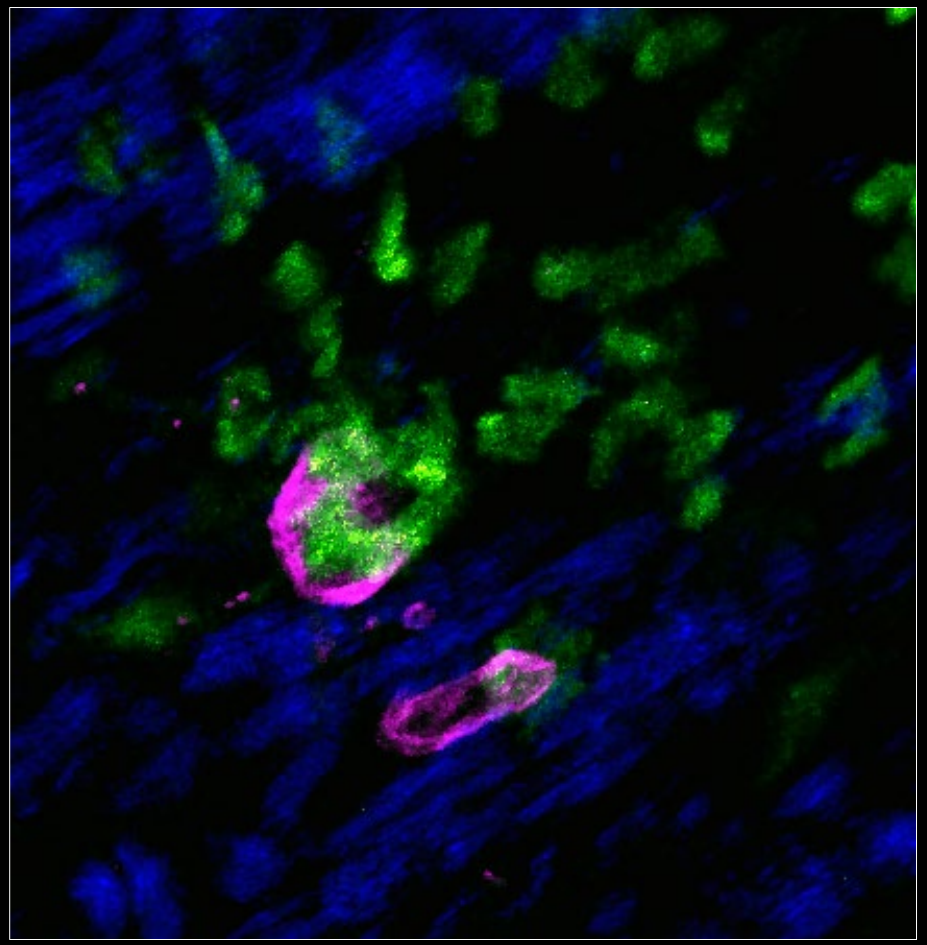
Directed or random movement?

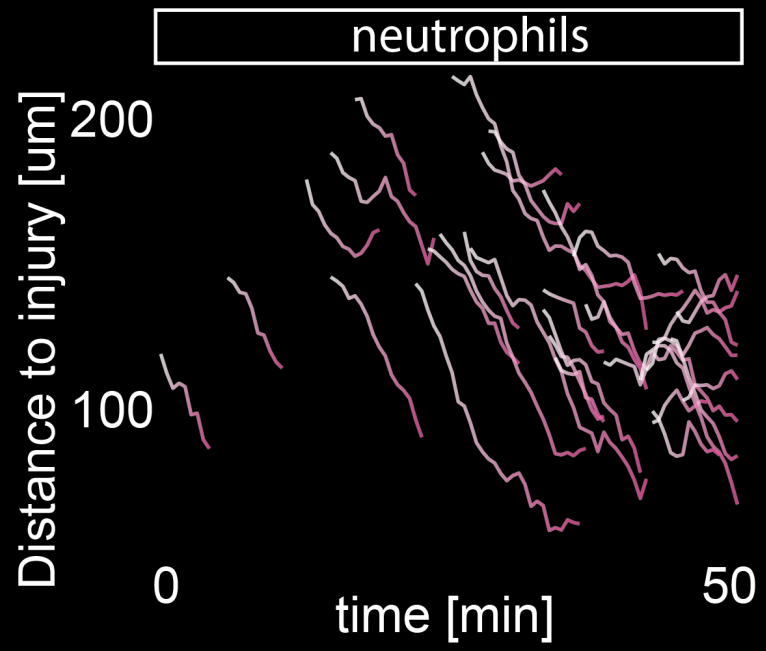


Focal injury

How we know

Canonical example neutrophils



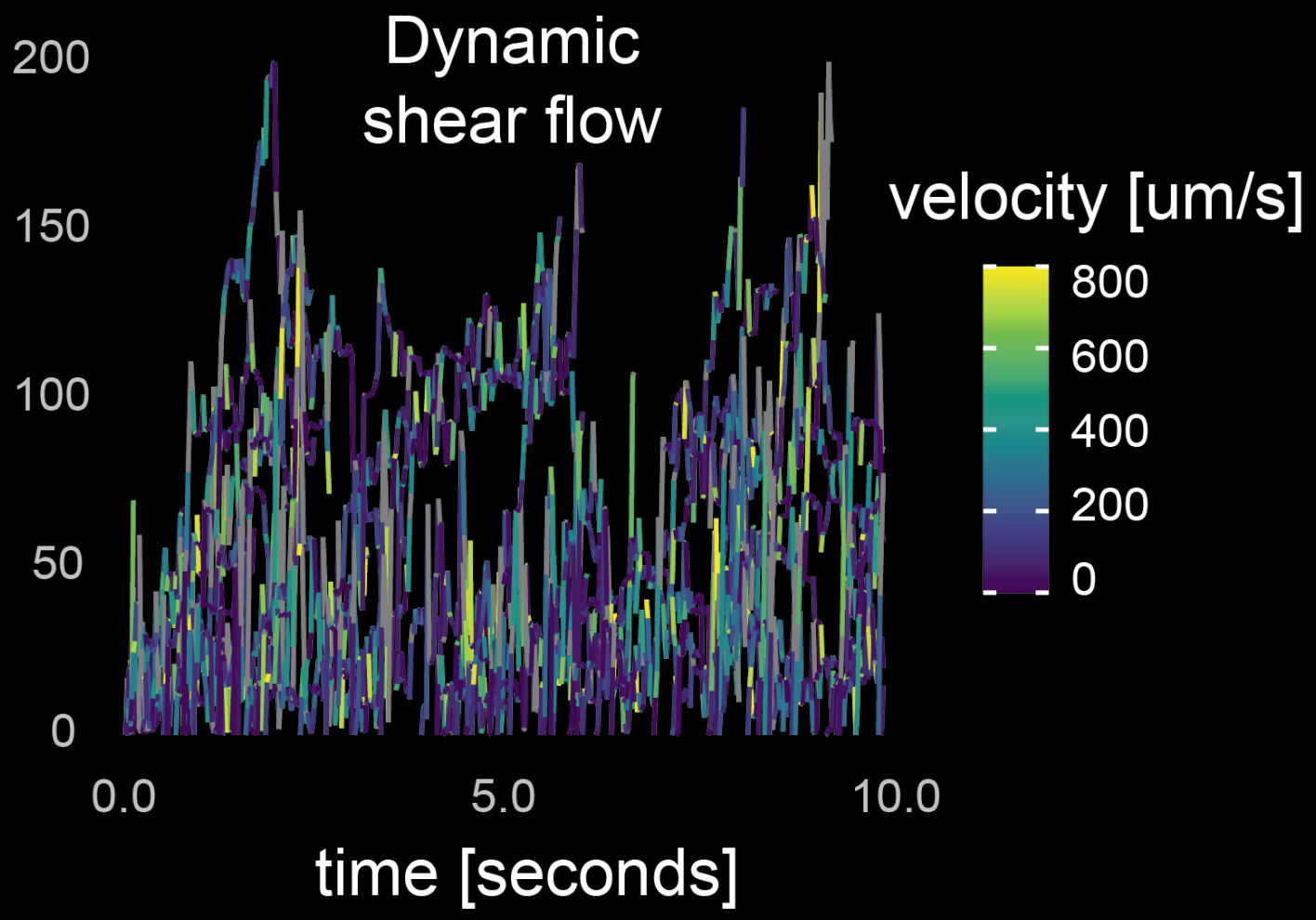


Guys there is free drinks on F-floor



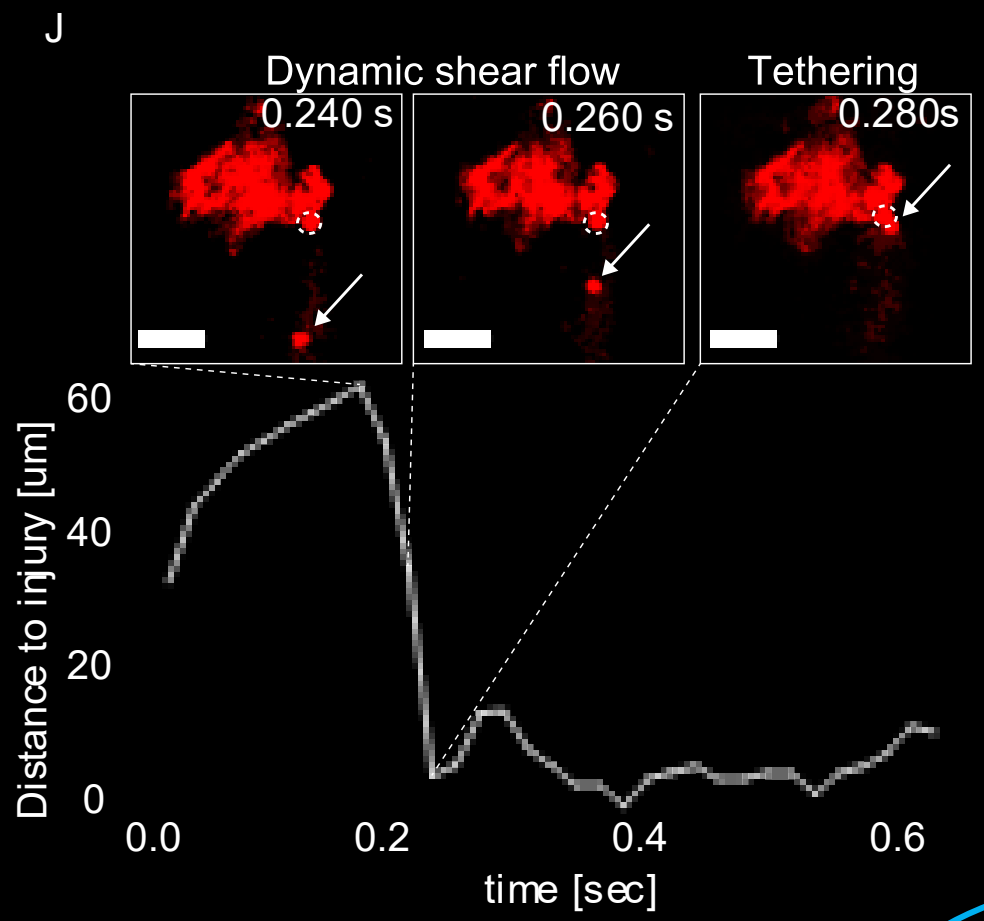
How we know

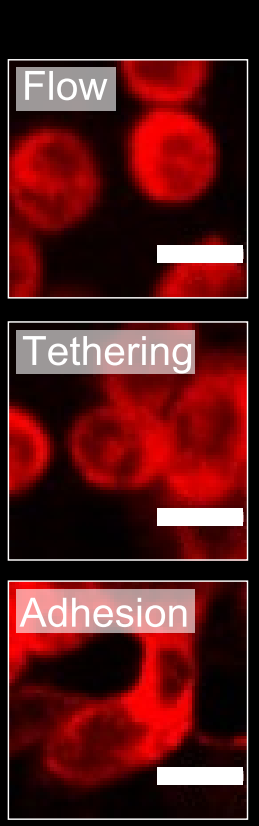
Tracking Macrophage recruitment



Undirected migration

How we know





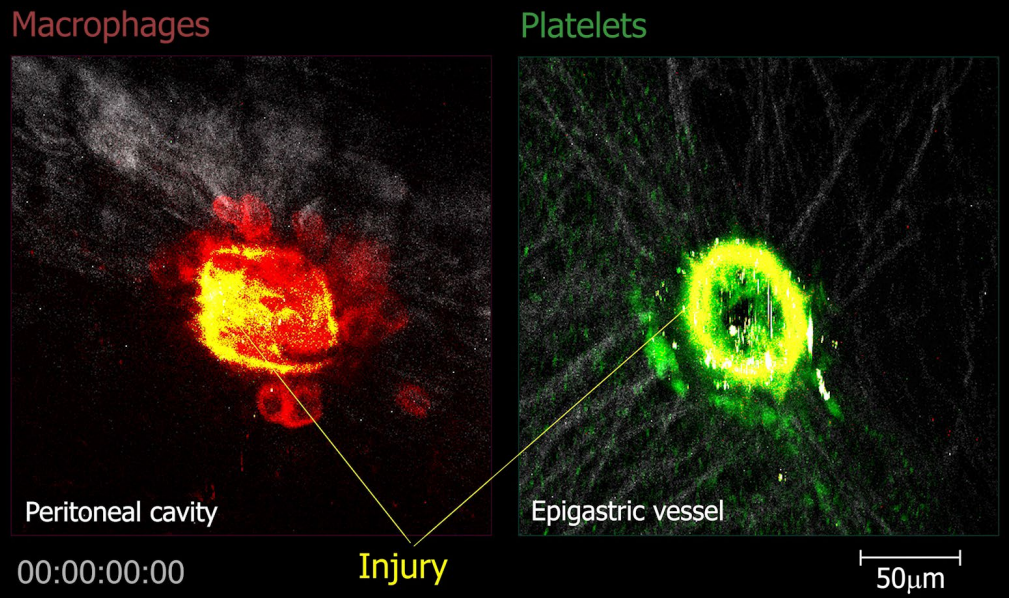


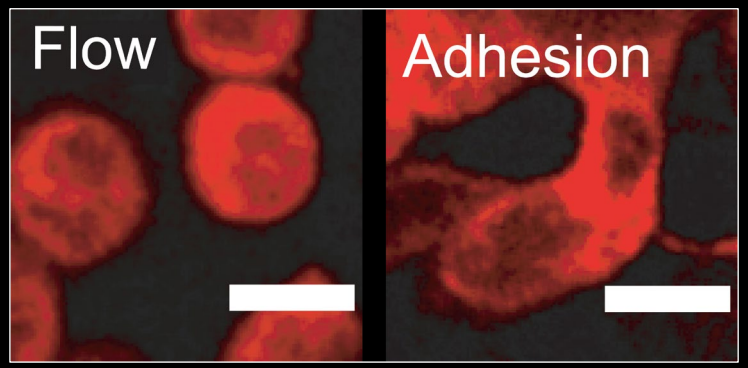
I really like this random bar, let's have a drink here

Macrophages act as platelets

How we know

In vivo (IVM)

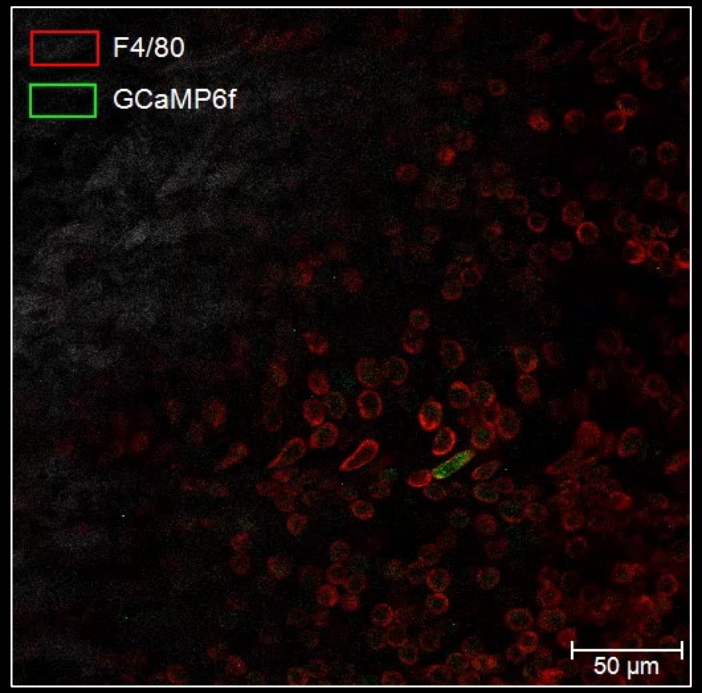




LyzCre: Rosa26$^{LSL\text{-}GCaMP6f}$



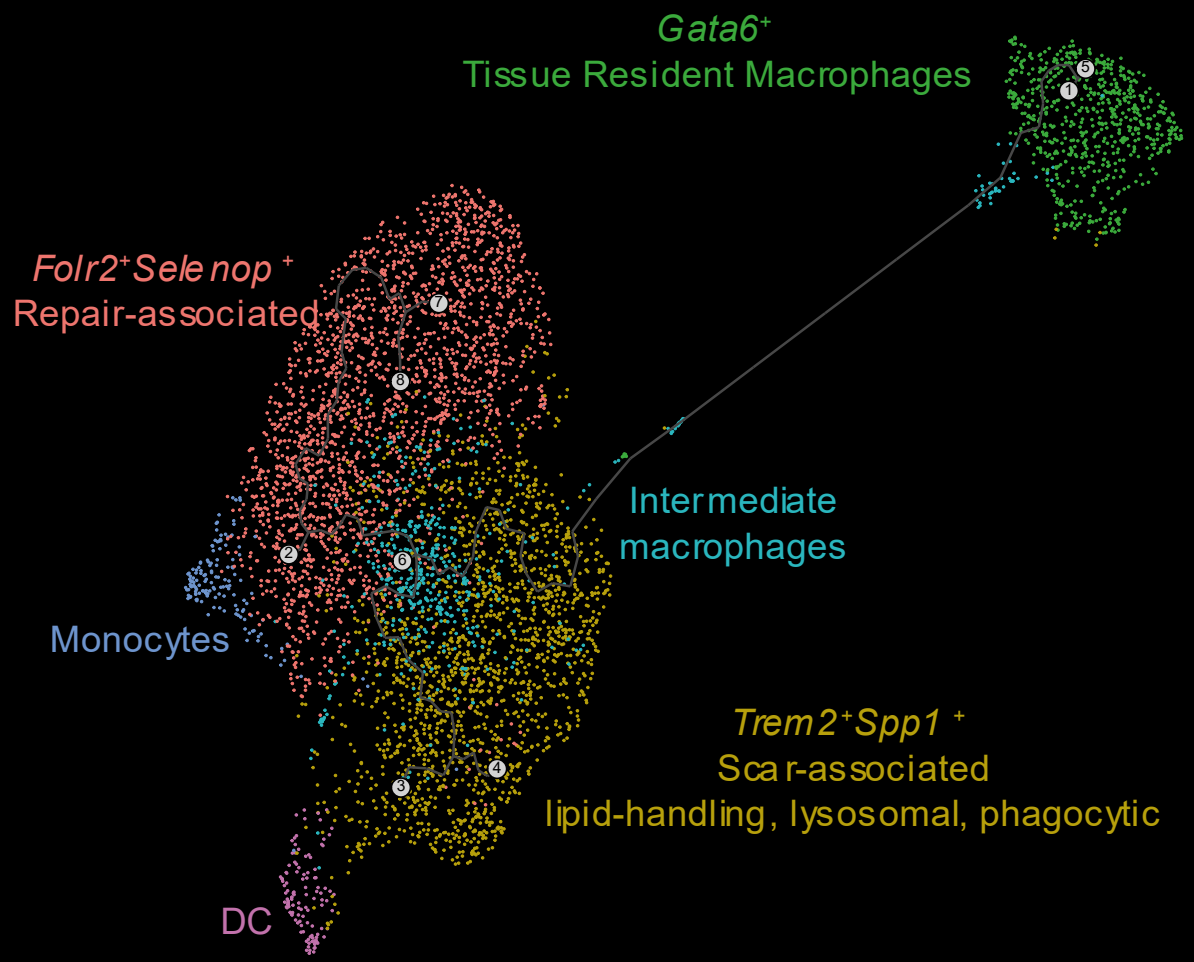
SHG F4/80



SHG F4/80

Let's look at one niche more closely (from our own research)

How we know



Pro-inflammatory cytokine release (used to be M1)

Repair/**Folr2**

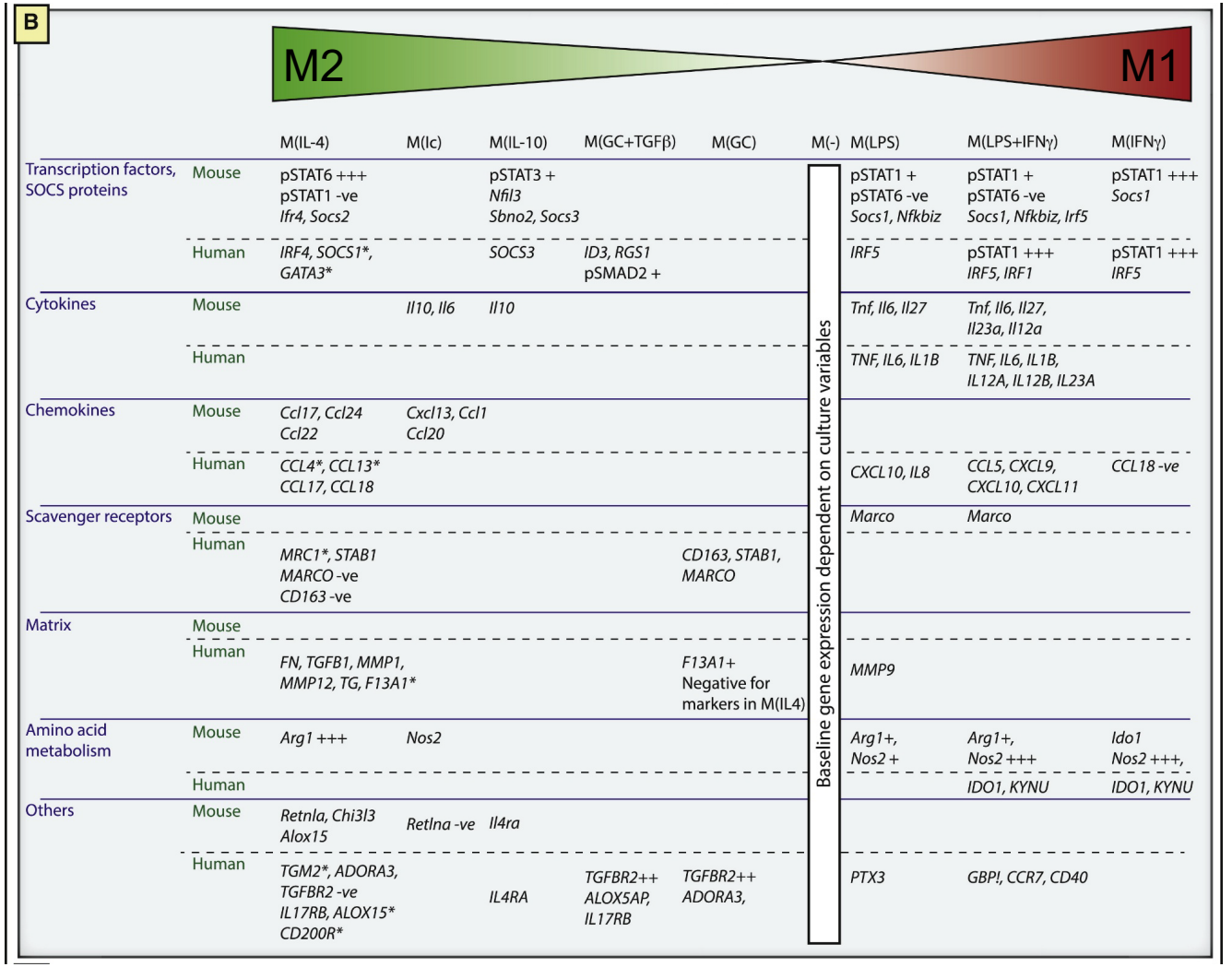
Trem2/Spp1
(lipid/stress)

Il-4 induced (used to be M2)

How we know

Discrete Polarization *in vitro*

- The M1/M2 heuristic can still be used to describe in vitro polarization.
- You may find this useful later, so we'll add the info here (but please don't memorize this!)
- First, the source of macrophages should be described (e.g. bone marrow).
- Second, it is recommended to describe the precise macrophages activation e.g. M(IL-4), or M(IL-10), rather than just the two ends of the spectrum M1/M2.
- The terms «classical» and «alternative» macrophages are misleading and should not be used anymore.

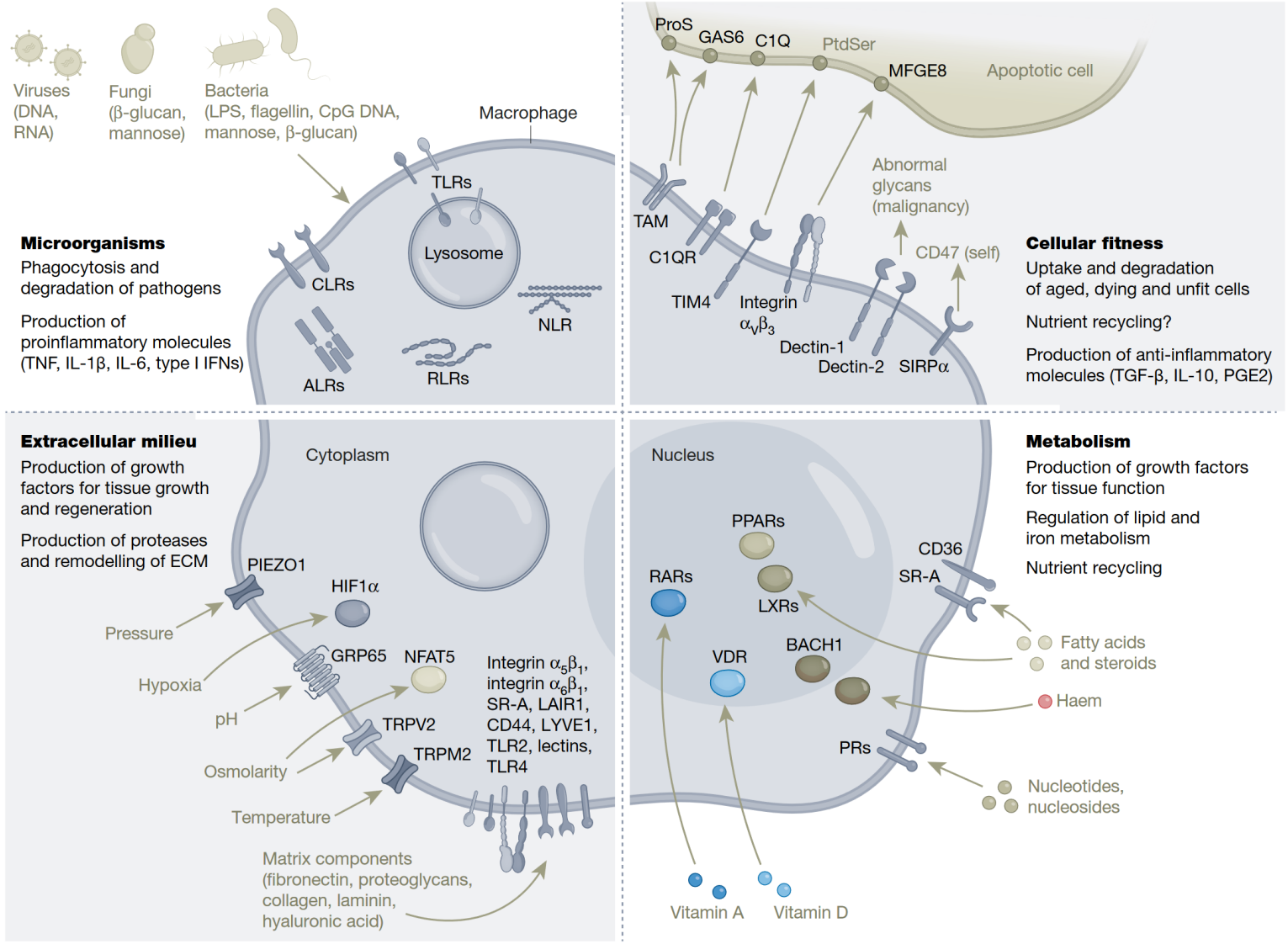


		M(IL-4)	M(Ic)	M(IL-10)	M(GC+TGFβ)	M(GC)	M(-)	M(LPS)	M(LPS+IFNγ)	M(IFNγ)
Transcription factors, SOCS proteins	Mouse	pSTAT6 +++ pSTAT1 -ve *Ifr4, Socs2*		pSTAT3 + *Nfil3* *Sbno2, Socs3*			Baseline gene expression dependent on culture variables	pSTAT1 + pSTAT6 -ve *Socs1, Nfkbiz*	pSTAT1 + pSTAT6 -ve *Socs1, Nfkbiz, Irf5*	pSTAT1 +++ *Socs1*
	Human	*IRF4, SOCS1*, GATA3**		*SOCS3*	*ID3, RGS1* pSMAD2 +			*IRF5*	pSTAT1 +++ *IRF5, IRF1*	pSTAT1 +++ *IRF5*
Cytokines	Mouse		*Il10, Il6*	*Il10*				*Tnf, Il6, Il27*	*Tnf, Il6, Il27, Il23a, Il12a*	
	Human							*TNF, IL6, IL1B*	*TNF, IL6, IL1B, IL12A, IL12B, IL23A*	
Chemokines	Mouse	*Ccl17, Ccl24* *Ccl22*	*Cxcl13, Ccl1* *Ccl20*							
	Human	*CCL4*, CCL13** *CCL17, CCL18*						*CXCL10, IL8*	*CCL5, CXCL9, CXCL10, CXCL11*	*CCL18 -ve*
Scavenger receptors	Mouse							*Marco*	*Marco*	
	Human	*MRC1*, STAB1* *MARCO -ve* *CD163 -ve*				*CD163, STAB1, MARCO*				
Matrix	Mouse									
	Human	*FN, TGFB1, MMP1, MMP12, TG, F13A1**				*F13A1+* Negative for markers in M(IL4)		*MMP9*		
Amino acid metabolism	Mouse	*Arg1 +++*	*Nos2*					*Arg1+,* *Nos2 +*	*Arg1+,* *Nos2 +++*	*Ido1* *Nos2 +++,*
	Human								*IDO1, KYNU*	*IDO1, KYNU*
Others	Mouse	*Retnla, Chi3l3* *Alox15*	*Retlna -ve*	*Il4ra*						
	Human	*TGM2*, ADORA3, TGFBR2 -ve IL17RB, ALOX15* CD200R**		*IL4RA*	*TGFBR2++ ALOX5AP, IL17RB*	*TGFBR2++ ADORA3,*		*PTX3*	*GBP!, CCR7, CD40*	

Learning Objectives

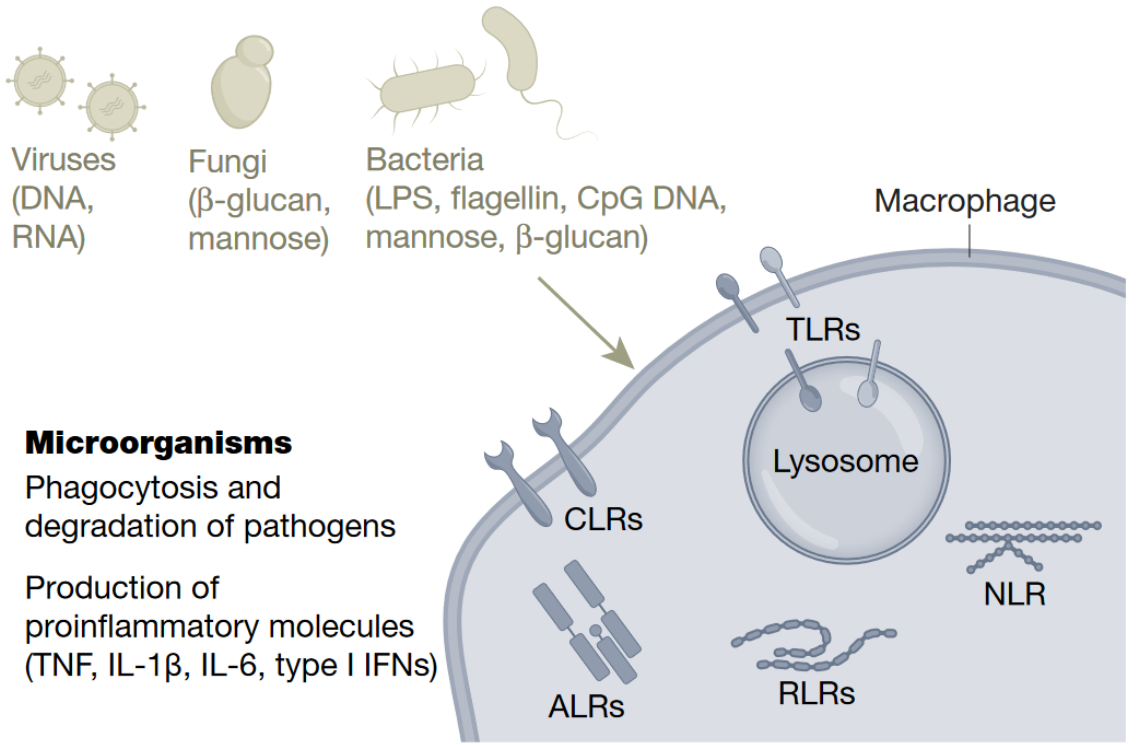
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Core Macrophage Functions



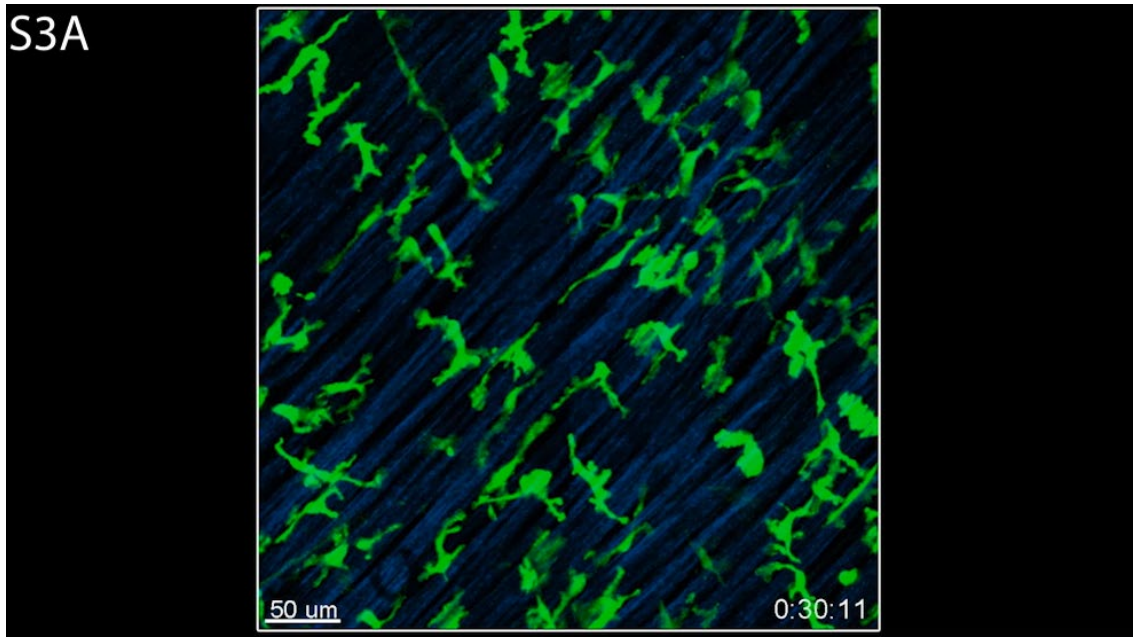
Microorganisms: Innate Immunity (Repetition of Immunology I & II)

- Macrophage moderate importance of direct bacterial clearance. **Neutrophils are the best bacterial killers**.
- Professional Antigen Presenting Cell (APC), especially Th1/th17, but less potent than **Dendritic best APCs**)
- The most important role of macrophages is d**etection/sensing of danger**
- Therefore, all macrophages express germline-encoded **pattern recognition receptors** (PRR)
  - Toll-like receptors
  - RIG-I-like receptors
  - NOD-like receptors
  - AIM2-like receptors
  - C-type lectin receptors



Surveillance

How we know





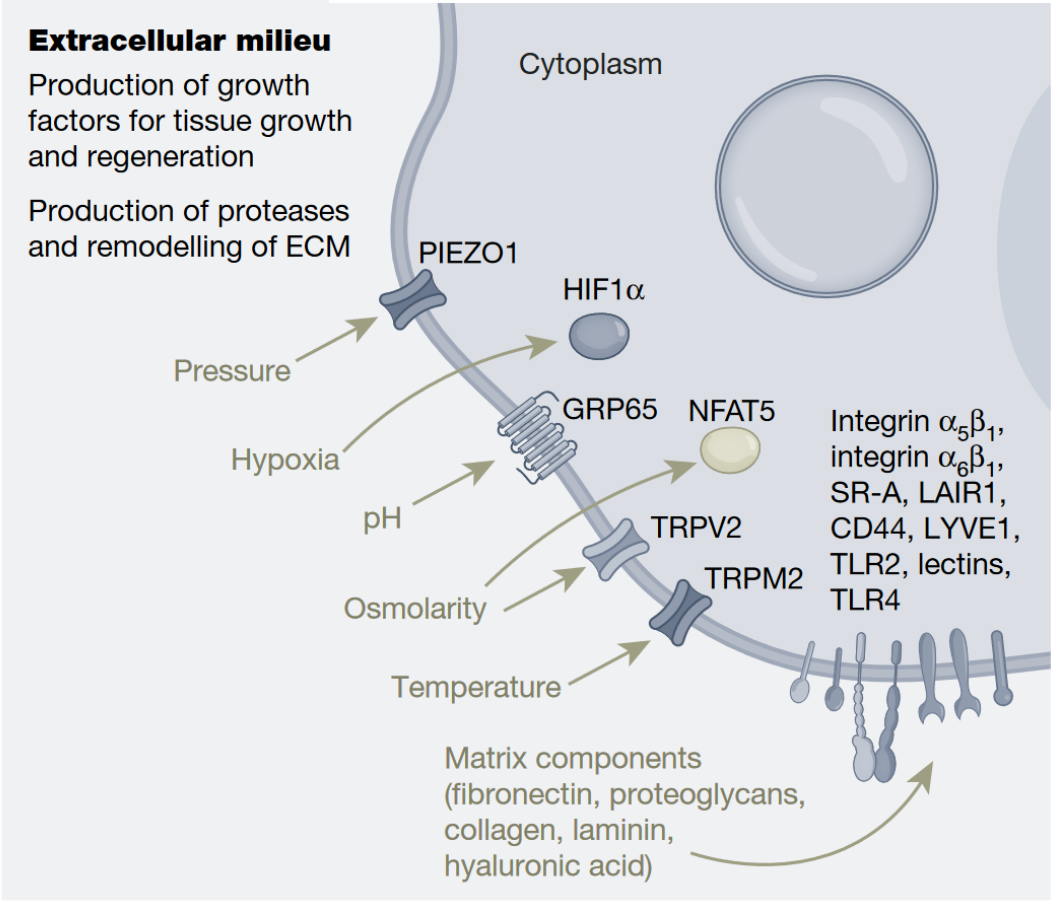
- Macrophages constantly probe environment
- Macrophages are dynamic first reponders to tissue damage.
- Small injuries can be «cloaked» and repaired without inflammation
- If injury exceeds a certain threshold, macrophages induce inflammation signified by neutrophil recruitment.

https://www.cell.com/fulltext/S0092-8674(19)30211-9

Extracellular Milieu: Safeguarding Tissue Structure

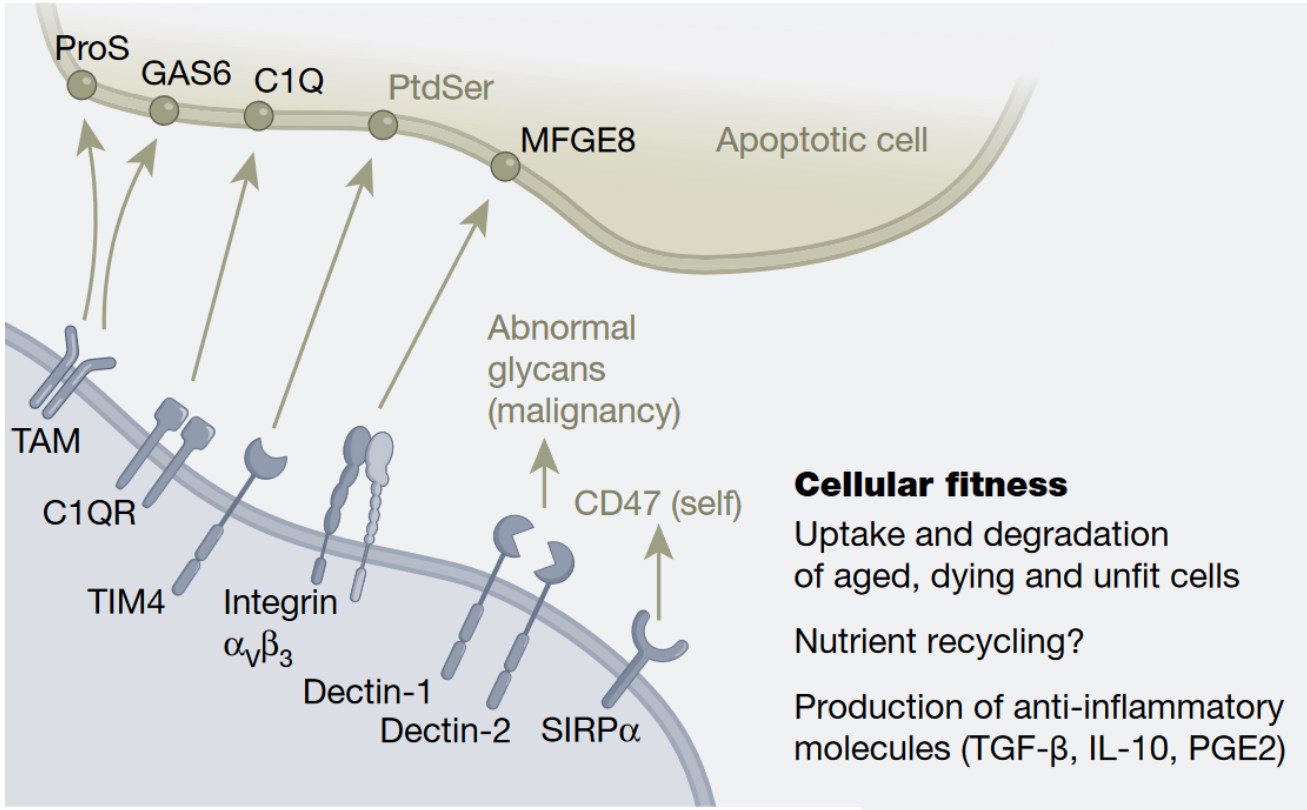
- Macrophages are a key cell type in defining the ***set point*** of a tissue. Mechnikov called them «sensors and healers» and
- Medzhitov uses the term «macrophage fibroblast circuits» where a **reciprocal regulatory loop** (circuit) defines tissue architecture, repair, and steady state.
- **Tissue provides**
  - Csf1
  - Instruction for TRM programming
  - Chemokines (recruitment)
  - Metabolic cues
  - Structure

  Macrophages provide
  - Structure
  - Have many sensors and integrate signals (also danger signals)
  - Production of signals: growth factorts (PDGF, TGFb, IGF), matrix remodelling signals, inflammatory signals, resolution signals.
  - Production of proteases and protease inhibitors



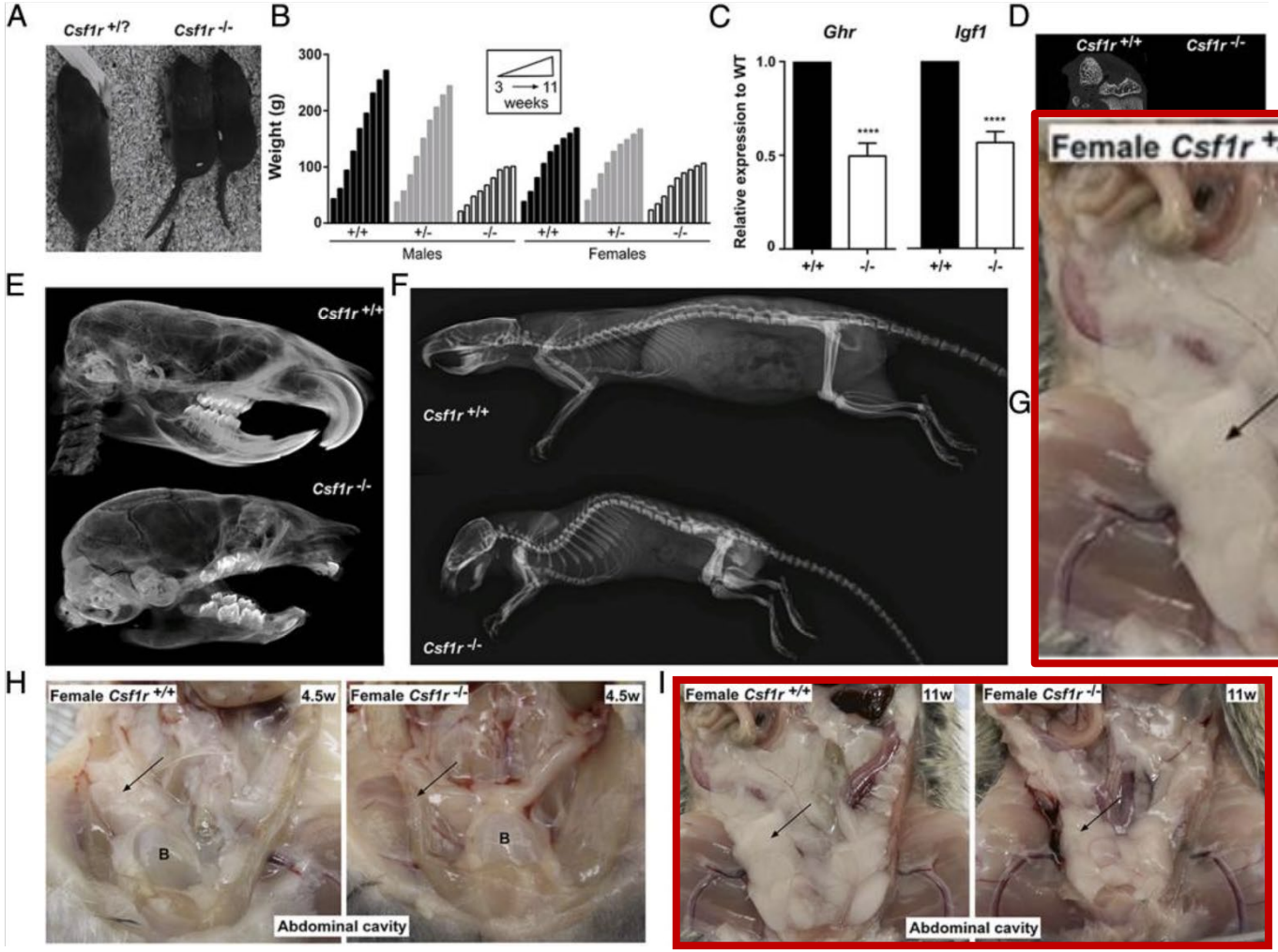
Cellular Fitness: Efferocytosis

- Daily, billions of cells die in the tissue by undergoing apoptosis, yet apoptotic cells are rarely observed in homeostasis.
- This is due to a process called efferocytosis that efficiently clears apoptotic cells without causing inflammation.
- Efferocytosis is a well-defined molecular event (follow link for review). The following elements are worth memorizing:
  - Healthy cells express **CD47**, a «**don't eat me signal**» recognized by **SIRP1α** on phagocytes
  - Apoptotic cells express «**eat me signals**» the most important is **Phosphatidylserine** (PS) which is recognized by **TIM4** and many others (Figure)
- The process is anti-inflammatory characterized by the release of TGFb, IL-10, PGE2 and the inhibition of inflammatory cytokines such as TNF, IL1b, IL6



Csf1R$^{-/-}$ in rats

How we know

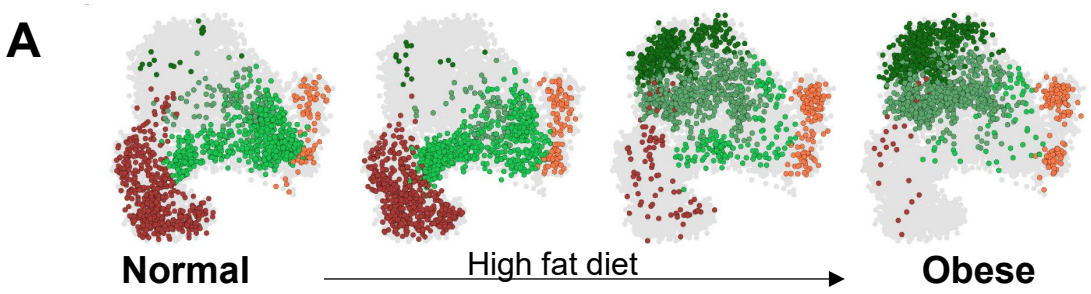


- Almost as an incidental finding the authors report significantly reduced visceral fat in this rat.
- Because of the potential therapeutic implications (and market!) this was taken up by others.

https://doi.org/10.4049/jimmunol.1701783

Trem2^{+} Lipid Associated Macropages (LAM)

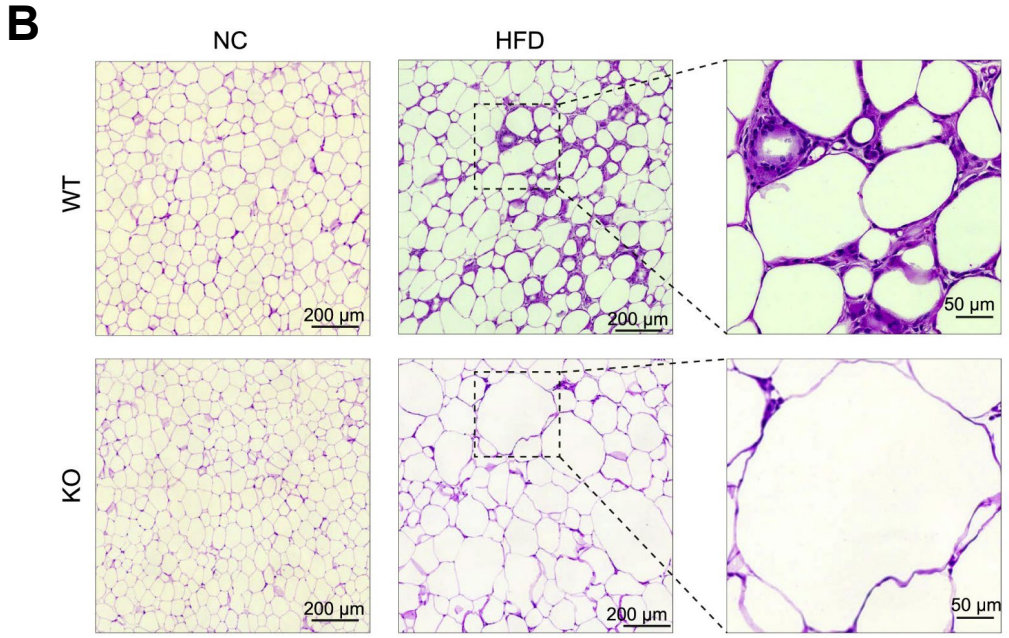
How we know

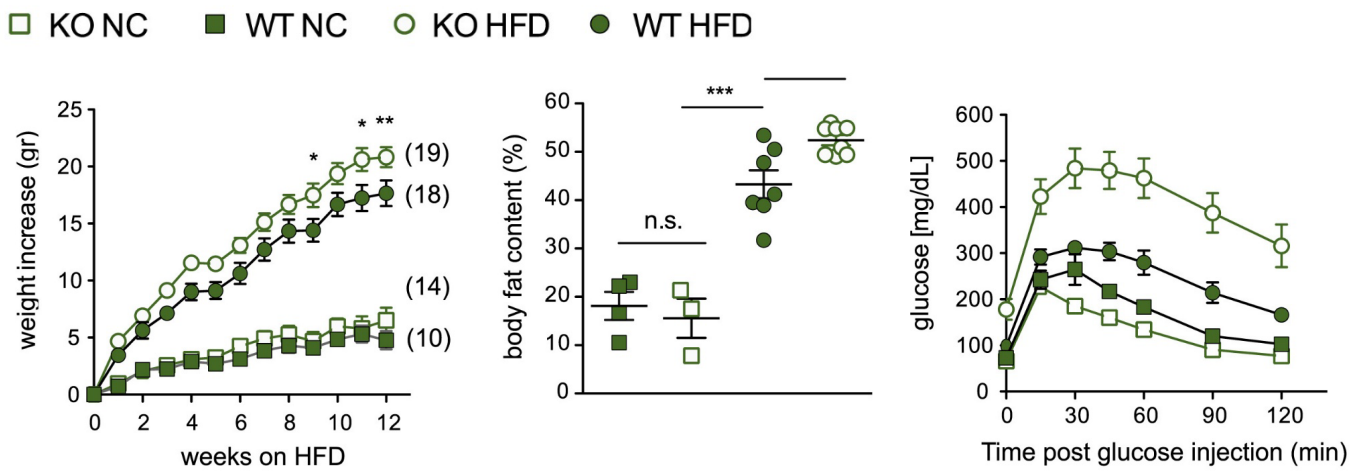


(**A**) A conserved **Trem2** signature characterizes obesity-related macrophages in mice and humans.

(**B**) Trem2$^{-/-}$ results in **adipocyte hypertrophy**

(**C**) Trem2$^{-/-}$ results in **loss of systemic metabolic homeostasis**

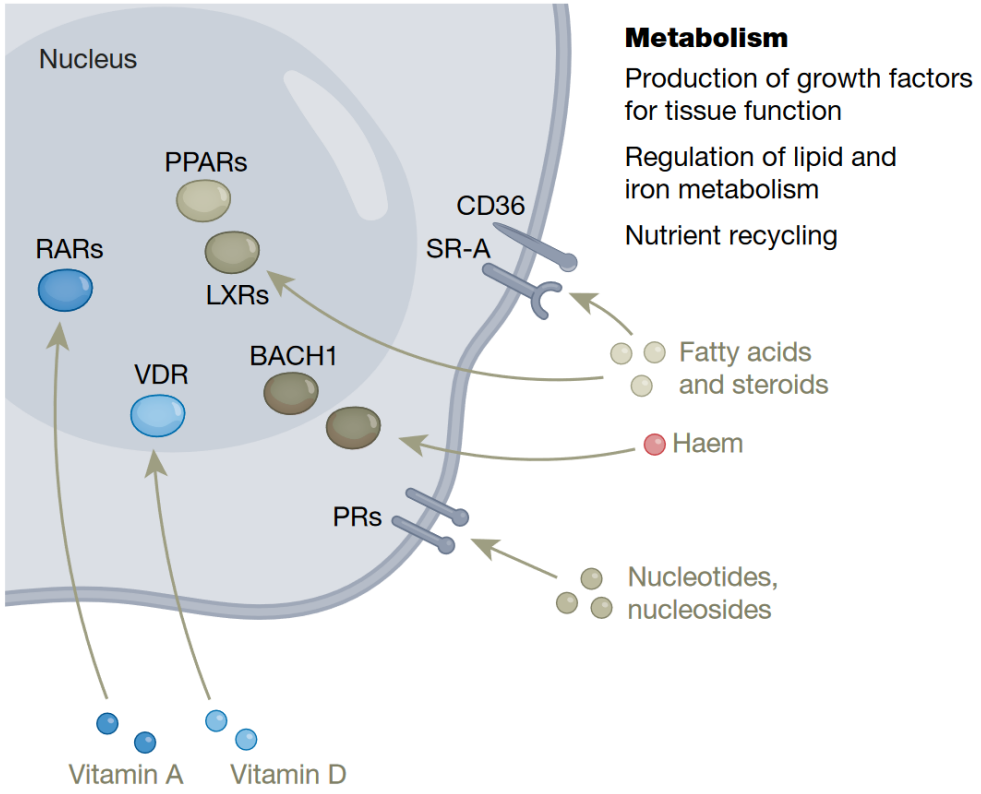




https://doi.org/10.1016/j.cell.2019.05.054

Metabolism

- **Csf1R$^{-/-}$ rats**: loss of visceral adipose tissue!
- CCR2-dependent BMDM promote systemic inflammation and insulin resistance in obese animals.
- **But, (other?) CCR2-dependent Trem2 Lipid Associated Macrophages** (**LAM**) prevent adipocyte hypertrophy and help with systemic metabolic homeostasis.
- Macrophages as «**metabolic sensors**» that **support lipid storage** in white adipocytes through the production of **PDGF-CC**.
- Fat-resident macrophages can also control energy expenditure by promoting browning of white adipose tissue (produce heat from fat).
- May represent therapeutic targets!!

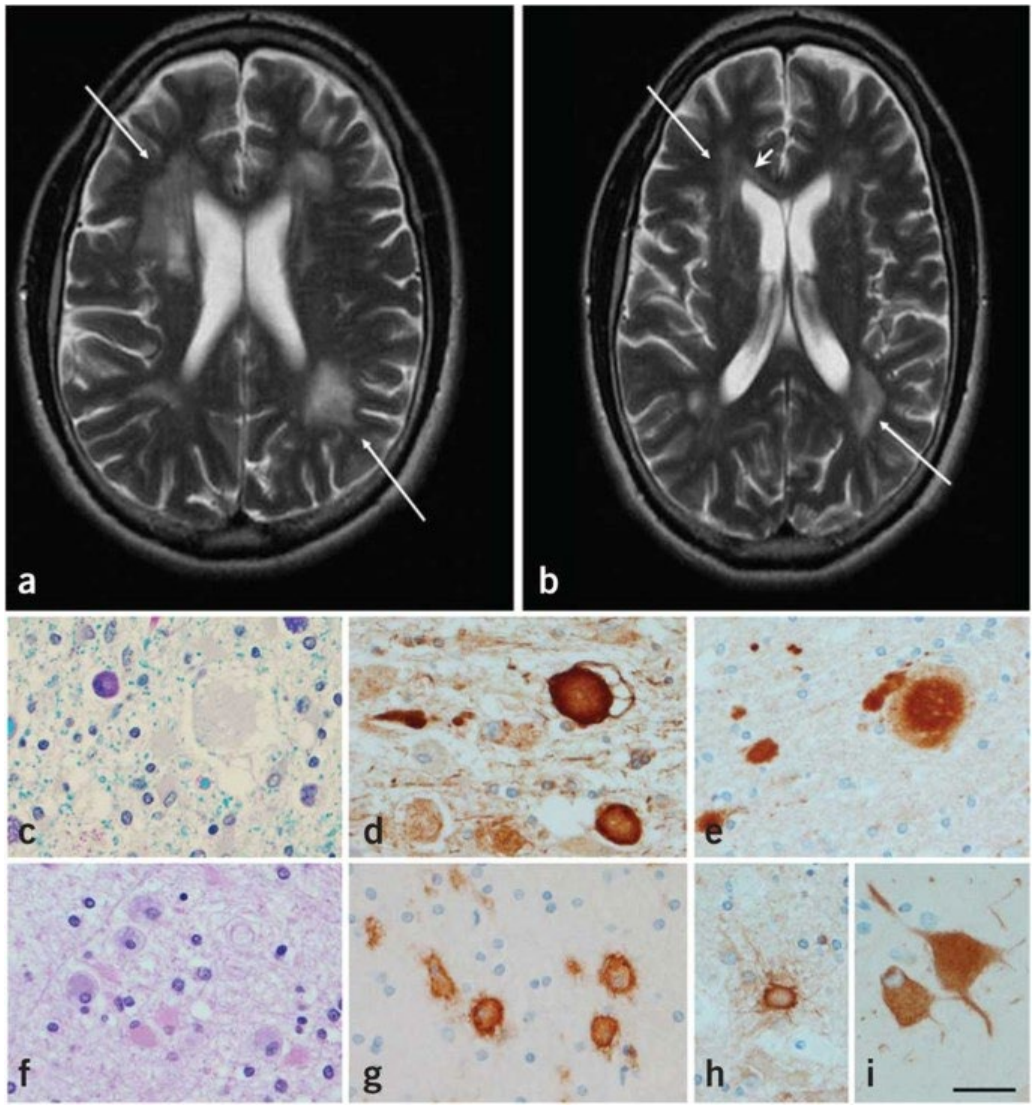


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Leukoencephalopathy and Alzheimer

- Microglia, the main macrophage subset of the CNS, provide trophic support, may regulate neuronal activity.
- Growth factors (IGF, NGF, BDNG, neurotropin-3) important for development and survival of neurons and glial cells.
- Humans with CSF1R mutations present with a spectrum of neurological and skeletal phenotypes.
- Depending on rest activity of **CSF1R** can be adult-onset (Figure), but bi-allelic CSF1R loss-of-function results in paediatric-onset **leukoencephalopathy** with near absence of microglia and death in the first year of life (histology: spheroids).
- Mutations in *TREM2* gene lead to neurodegenerative bone disease with slerosing leukoencephalopathy.
- Population-wide studies identified TREM2 polymorphisms associated with the risk of Alzheimer's disease.



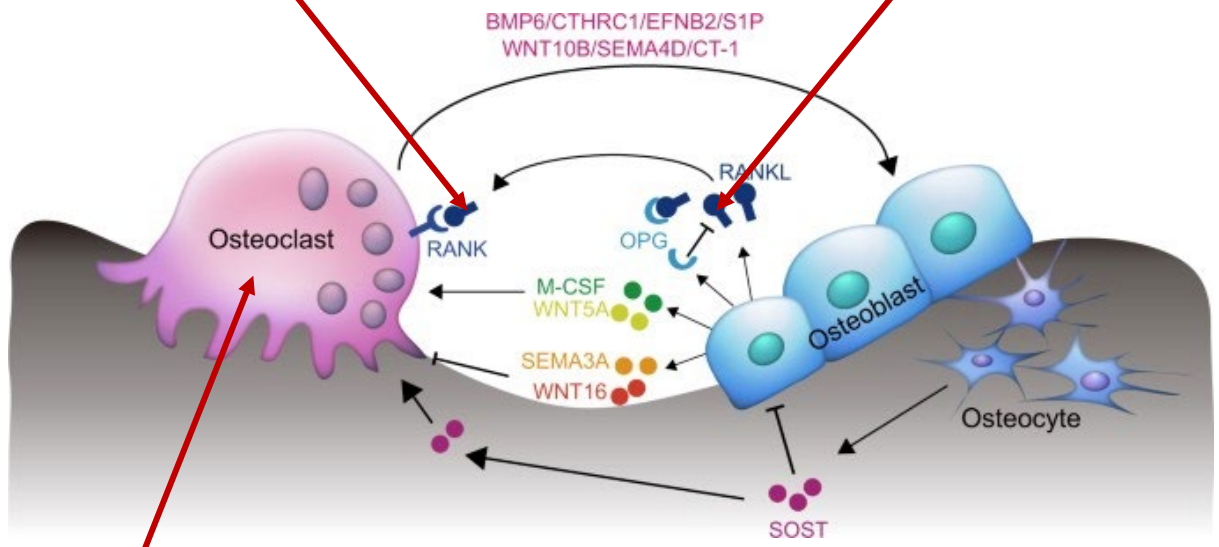
https://www.nature.com/articles/ng.1027

Bone osteoclasts, osteopetrosis, and osteoporosis

- Osteoclasts are a highly specialized form of multinucleated macrophages reside in the bone endosteum.
- They secrete acid and lytic enzymes such as cathepsin K that dissolve bone.
- Osteoclasts secrete growth factors (IGF1, TGFβ) for osteoblasts and vice versa (RANKL, CSF1).
- Loss-of-function mutations in genes that are required for osteoclast formation or function (RANKL, RANK receptor, chloride channels,…) cause osteopetrosis, an extremely rare inherited disorder whereby bones become harder and denser.
- Osteoporosis in turn, can be treated by inhibiting Osteoclasts (examples in red in the figure)

Selective estrogen receptor modulators/hormone therapy: normalize RANKL/OPG ratio

RANKL-Antibody



https://www.nature.com/articles/s41413-018-0019-6

Bisphosphonates (e.g. Clodronate, Alendronate): osteoclast apoptosis

From Osteoporosis to Clodronate Liposomes

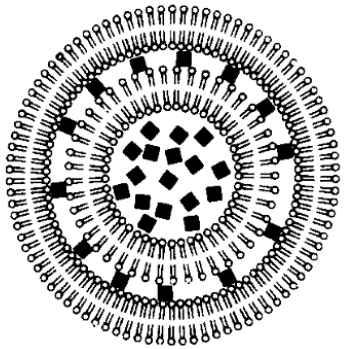
How we know

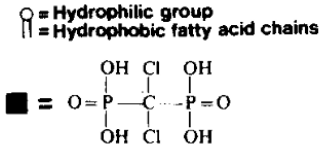
- **A very nerdy link**: Clodronate is also a first-gen bisphosphonate which are used to treat osteoporosis. Except for osteoclasts which resorbe bisphosphonates while resorbing bones, bisphosphonates are membrane-impermeable and as such **non-toxic**.
- If you pack this in a liposome (which is also not toxic alone) it will be taken up by phagocytes and released into the cytosol by lysosomal phospholipase, reaching high concentrations.
- Clodronate -> AppCCl2p (ATP analog) -> mitochondria inhibition -> apoptosis

Osteoporosis drug

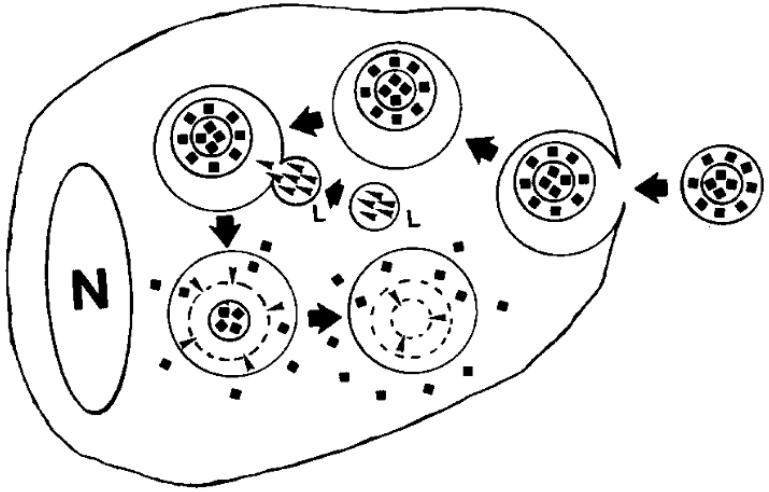


Clodronate Liposomes



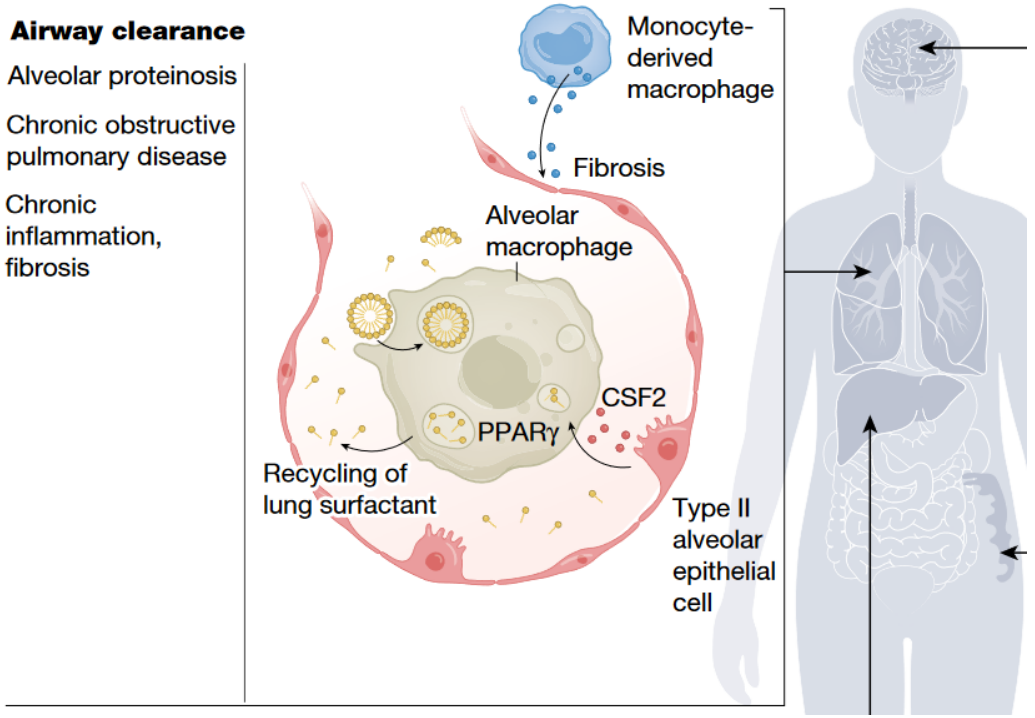


Often-used tool for macrophage depletion studies



Alveolar proteinosis

- Pulmonary Alveolar proteinosis (PAP) is a rare diseease (6-7 per Million)
- >90% autoimmune PAP: **Anti-GM-CSF** auto-Antibodies)
- 5-10% secondary PAP: due to other diseases
- 2% genetic with mutations in
  - **GM-CSFR** mutation of alpha or beta subunit (*CSF2RA*, *CSF2RB*).
  - Other genes involved with surfactant production and clearance.



Link to Otogeny: should genetic PAP patients be transplanted or not?

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journal homepage: www.elsevier.com/locate/rmed

Short communication

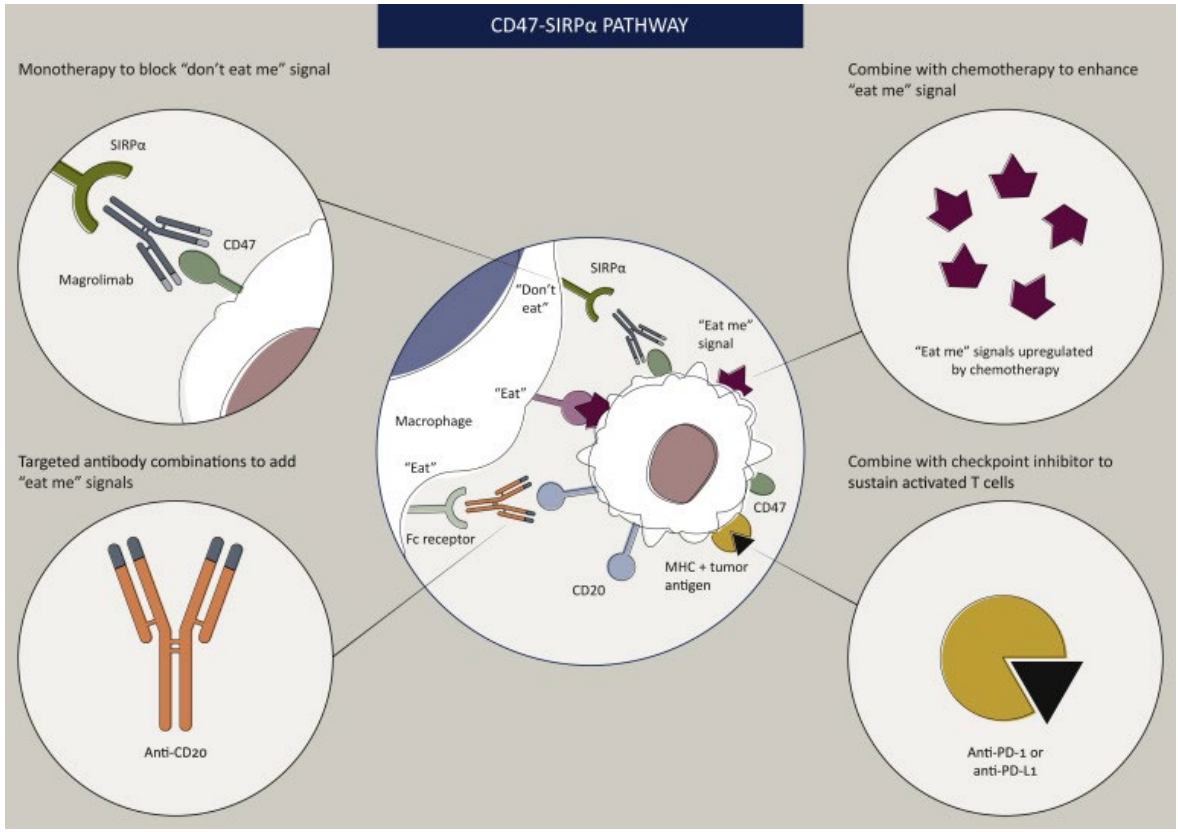
Recurrent pulmonary alveolar proteinosis after lung transplantation: a case series

Dirk Westhölter [a,*], Marcel Opitz [b], Josefine Rawitzer [c], Mark Greer [d], Thomas Wessendorf [a], Sebastian Cordes [a], Ulrich Costabel [a,e], Christian Taube [a], Francesco Bonella [a,e]

https://doi.org/10.1016/j.rmed.2025.108167

Anti-Tumor Immunity

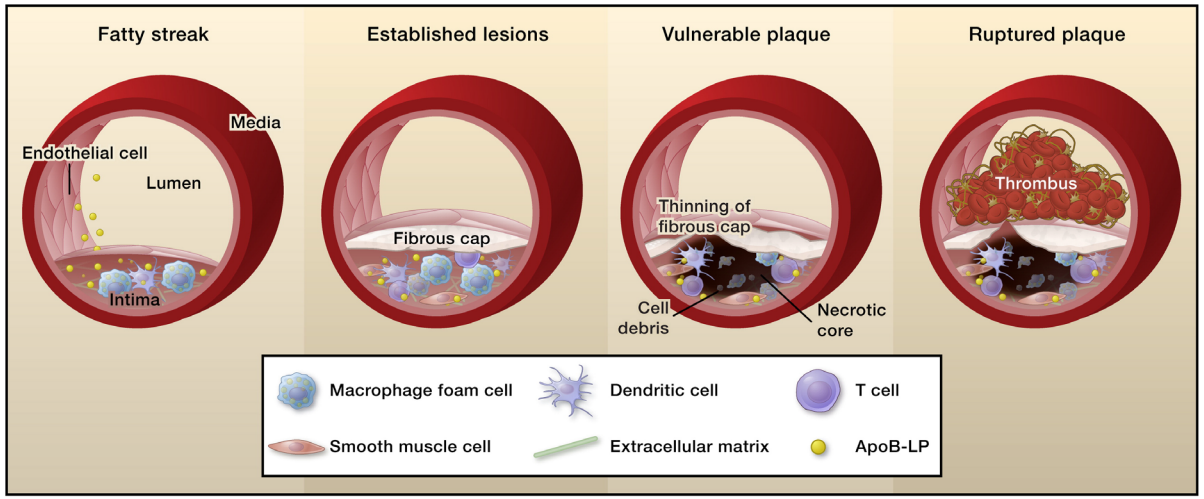
- CD47 is a «**don't eat me**» signal overexpressed on cancer cells
- Blockade of the CD47-SIRPα signaling pathway leads to phagocytosis of tumor cells.
- CD47-SIRPα blockade plus standard treatment shows promising efficacy.
- Clinical trials are angoing
- **Safety concerns also macrophage related**: CD47 is highly expressed on red blood cells, when you block CD47
  - You remove «don't eat me» signal on RBCs
  - Spleen macrophages sort out old RBCs based on CD47 loss
  - Therefore, in all phase I trials anemia was the most common toxicity

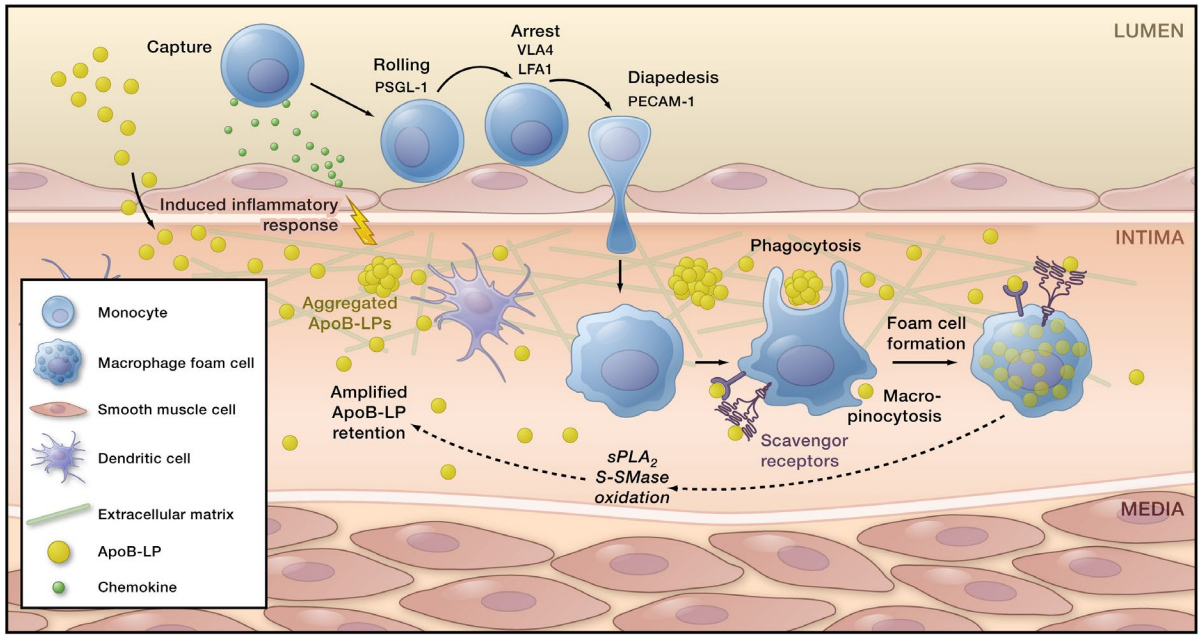


https://doi.org/10.1016/j.iotech.2022.100070

Atherosclerosis/ Cardiovascular Disease

- **Monocyte recruitment** after Endothelial activation (VCAM-1), **monocytes** enter the intima.
- **Differentiation to macrophages.** Macrophages take up **oxidized LDL** through phagocytosis, pinocytosis, **scavenger receptors** play an important role
- **Foam cell formation.** The macrophages «**over-eat**», lipid-loaded macrophages accumulate, formation of fatty streaks.
- **Necrotic core formation.** Impaired efferocytosis leads to cell **death and plaque growth**.
- **Fibrous cap / instability.** The lesion is «**walled-off**» which can lead to stable ischemic symptoms, but when the smooth muscle cap thins risk of rupture which can lead to sudden vascular occlusion



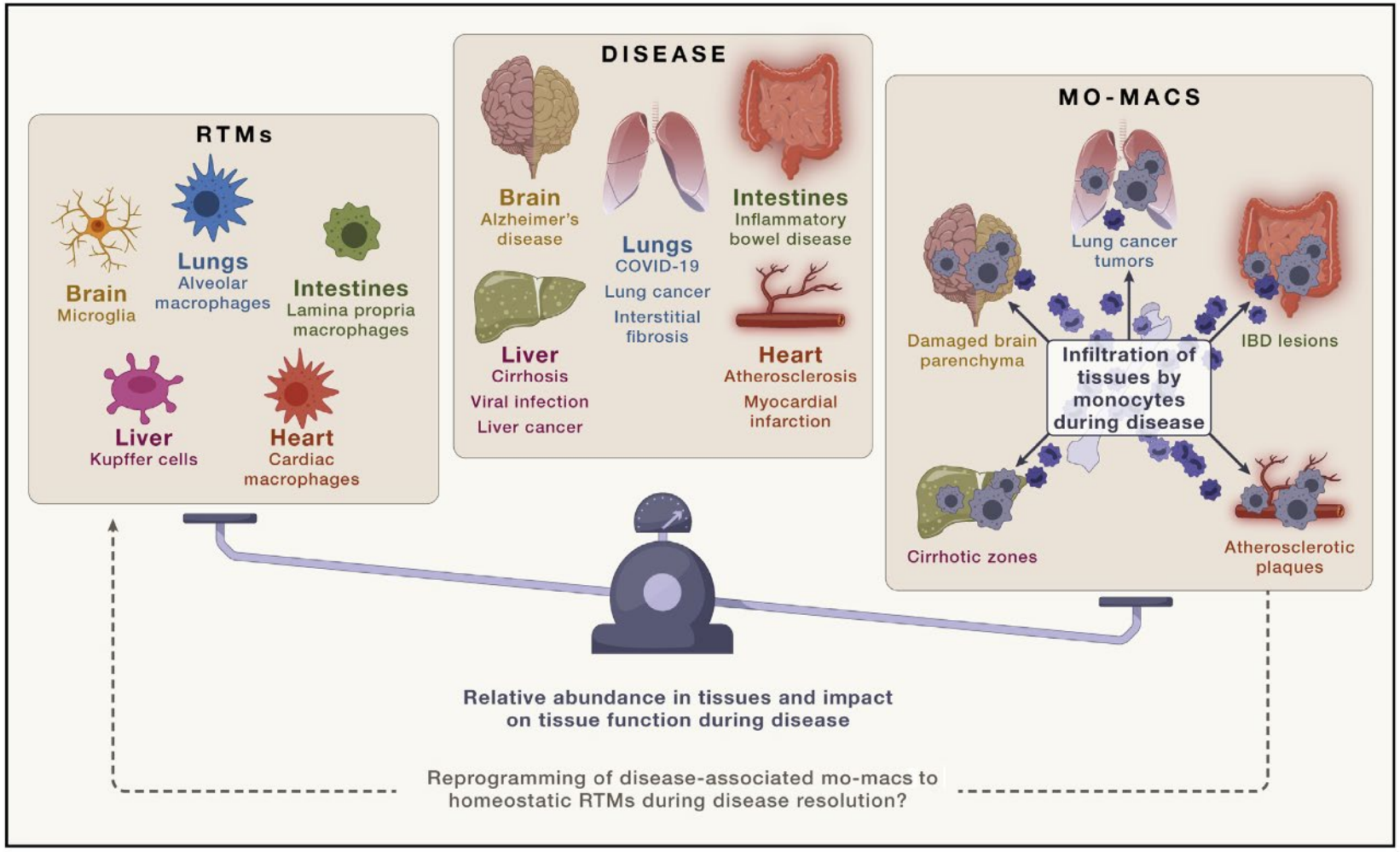


DOI: 10.1016/j.cell.2011.04.005

Macrophages play a role in many more diseases

Rule of thumb

- **TRMs** main players during homeostasis
- **Mo-Macs** main players during disease pathogenesis.



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