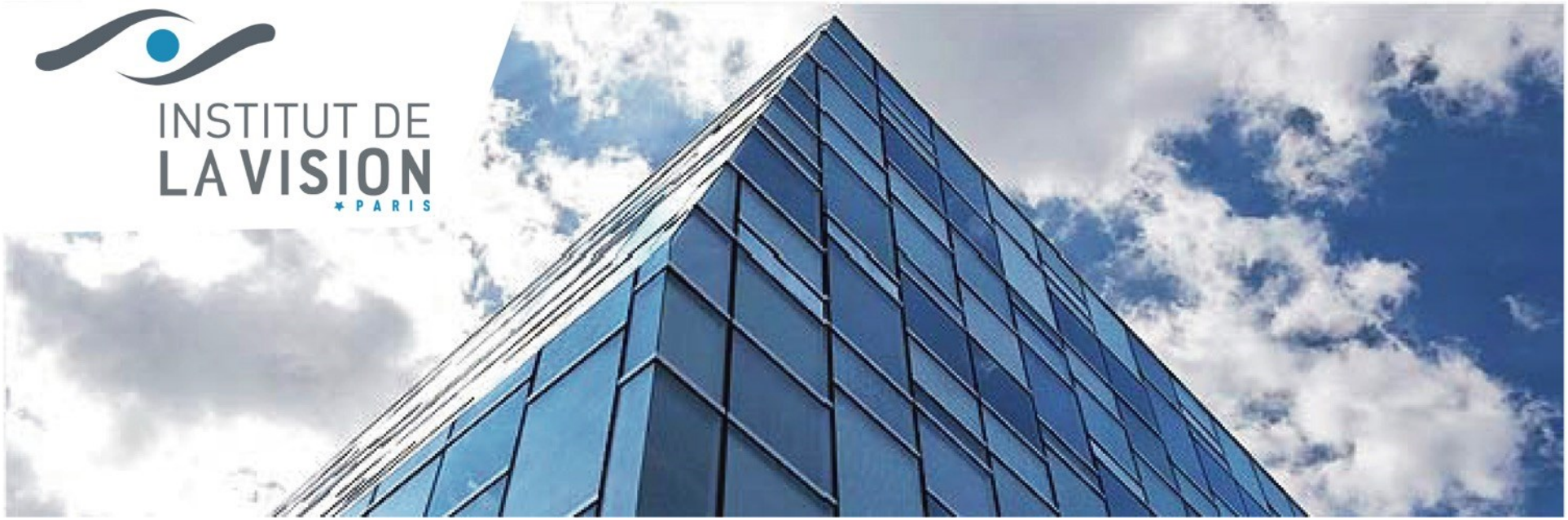


AMD pathophysiology

Florian Sennlaub

Age related Macular Degeneration

•1rst cause of visual impairment

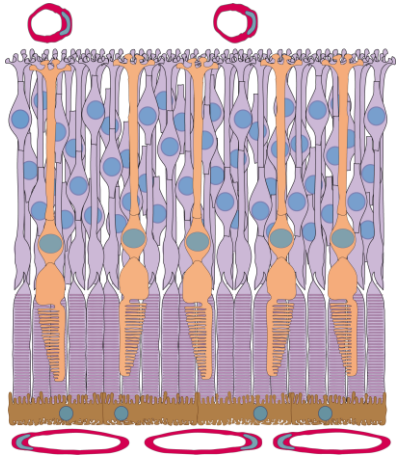
Risk Factors:

- Age
- Tobacco / Obesity / Sleep Apnea
- Family: Genetic Variant CFH 402H
  10q26 haplotype

late AMD

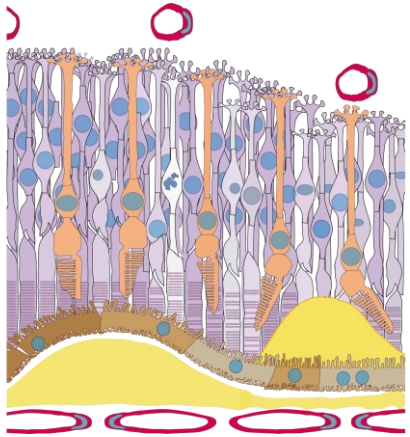
(sight-threatening)

healthy



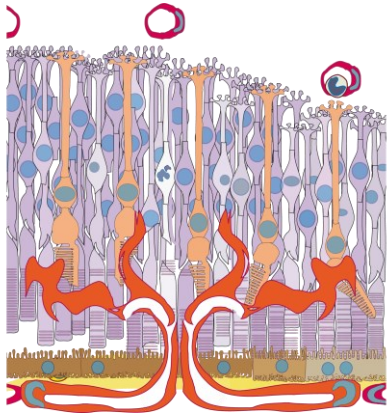
earlyAMD

- Drusen



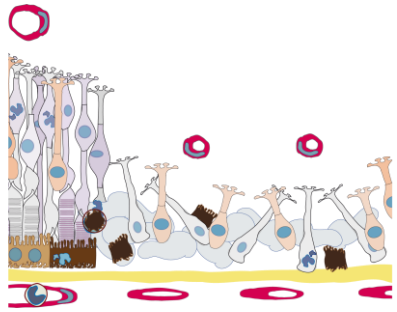
wetAMD

- Neovascularization

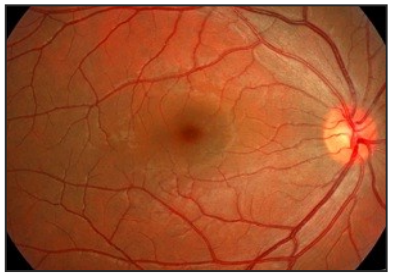


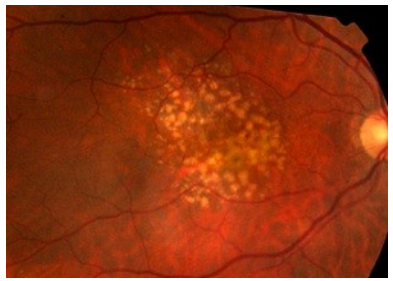
Geographic Atrophy

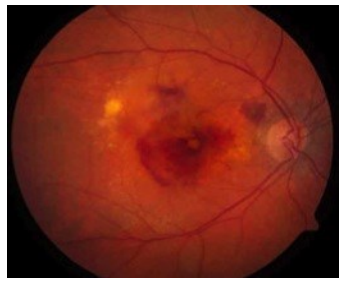
- Degeneration

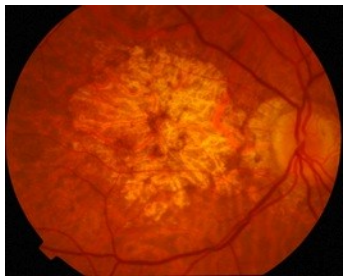


Prevalence: 20% > 75 years 4 % > 75 years 4 % > 75 years







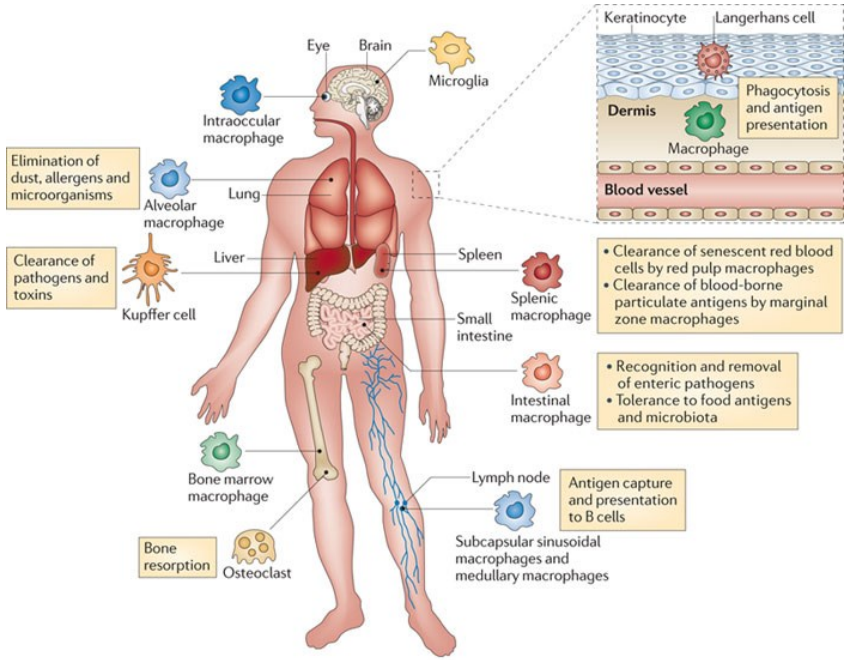


Beaver Dam Study

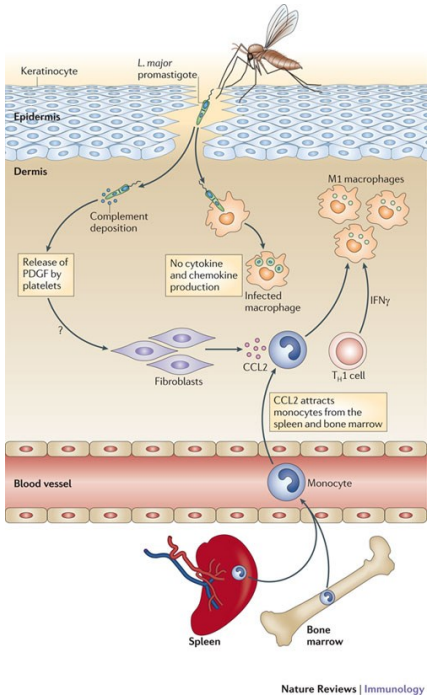
Mononclear Phagocytes

Resident Macrophages

efferocytosis / surveillance



Monocyte-derived Macrophages



- Trauma / Infection
- Inflammatory CCR2^{+}macrophages
  anti-microbial / collateral damage
- Alternative macrophages
  angiogenesis / fibrosis / scar formation
- Inflammation resolution
- Homeostasis

Table 1 | **Body sites and tissues that are immune privileged**

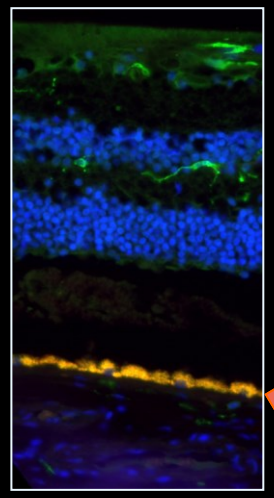
Sites	Tissues
Eye: cornea, anterior chamber, vitreous cavity and subretinal space	Eye: cornea, lens, pigment epithelium and retina
Brain: ventricles and striatum	Brain and spinal cord
Pregnant uterus	Placenta
Ovary	Ovary
Testis	Testis
Adrenal cortex	Liver
Hair follicles	
Hamster cheek pouch	Hamster cheek pouch
Certain tumours	Certain tumours

Immune privilege is a characteristic of specialized tissues and sites in the body. Immune-privileged sites allow foreign grafts to survive for extended, often indefinite intervals, and immune-privileged tissues survive for extended, often indefinite intervals at conventional sites, as described in REFS 3 and 6.

Immune priviledge reduces the inflammatory reaction in organs where potential collateral damage outweighs the risk from infection

Mononuclear phagocyte distribution in Health and AMD

healthy

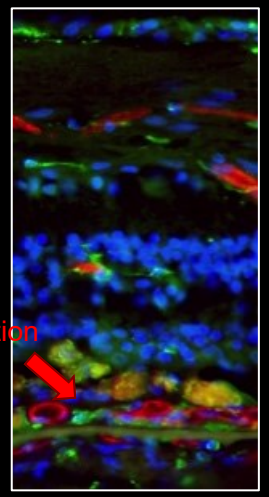


Microglia
only inner retina

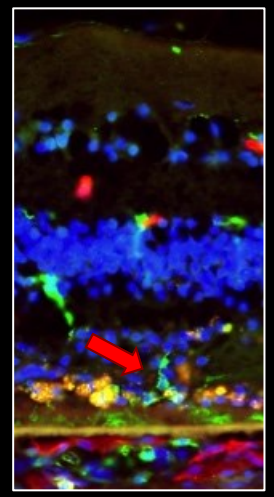
Photoreceptors:
- no Mφs
- no blood vessels
- no lymphatics

RPE:
immuno-supressive

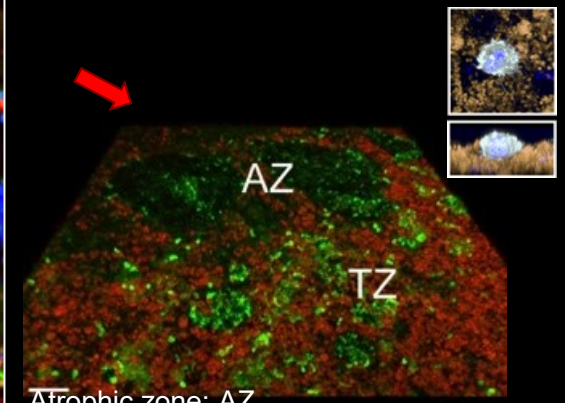
wetAMD



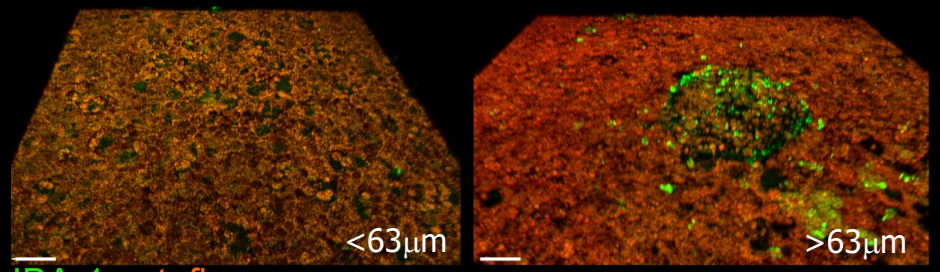
Mφ
infiltration



Geographic Atrophy

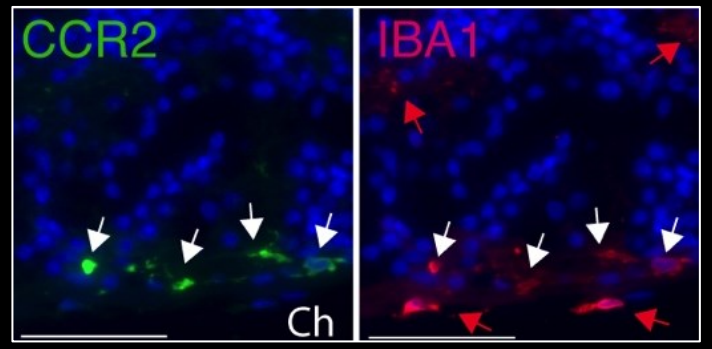


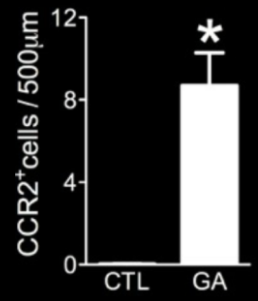
Atrophic zone: AZ
Transitional zone: TZ



IBA-1 autofluorescence

Drusen





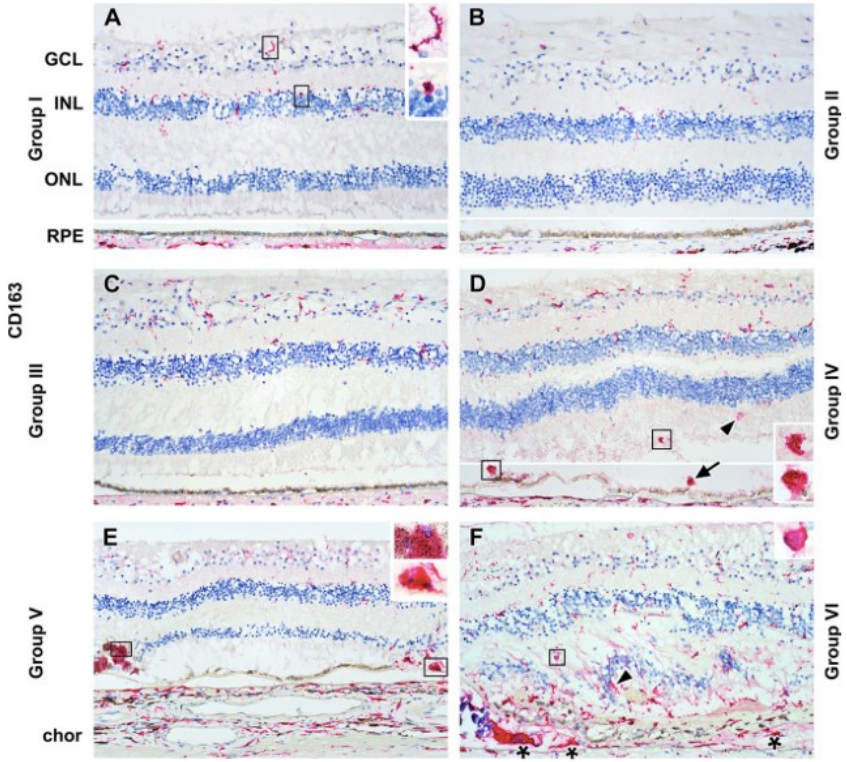
Sennlaub et al. ***EMBO Mol Med.*** 2013

➡ MPs infiltrate and accumulate in AMD

CCR2+Mo-derived Mfs participate in the infiltrate

Abundance of infiltrating CD163+ cells in the retina of postmortem eyes with dry and neovascular age-related macular degeneration

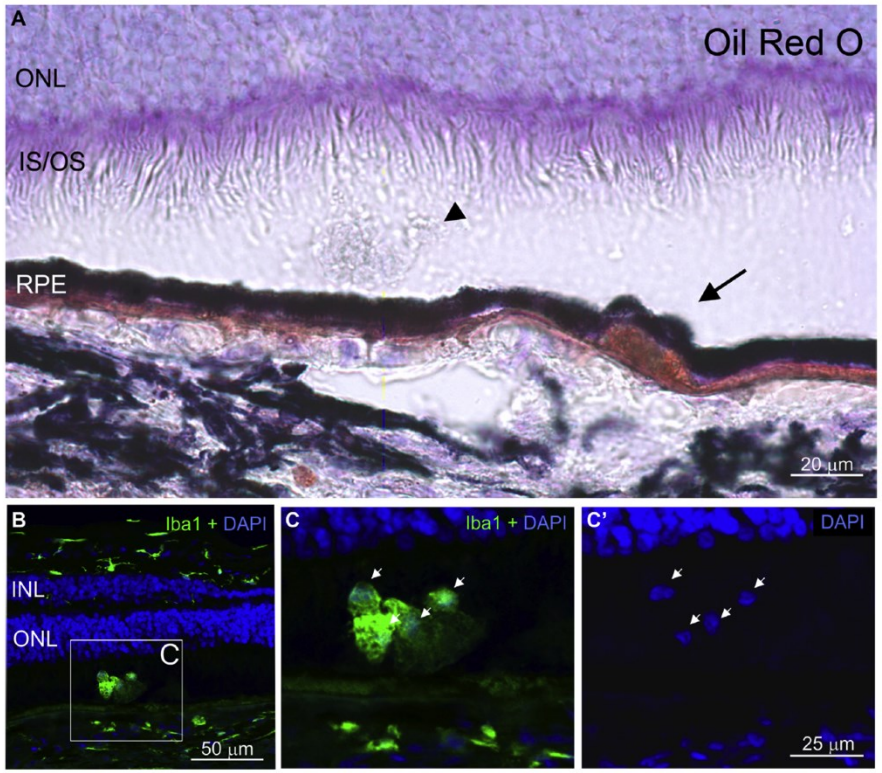
Eleonora M. Lad[1] · Scott W. Cousins[1] · John S. Van Arnam[2] · Alan D. Proia[1,2]



Lad et al. **Graefes Arch Clin Exp Ophthalmol.** 2015

Correlation of Histologic Features with In Vivo Imaging of Reticular Pseudodrusen

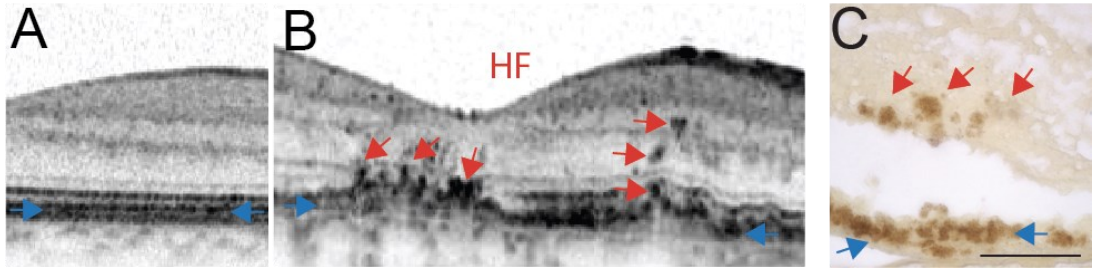
Ursula Greferath, PhD,[1,] Robyn H. Guymer, PhD,[2,3,*] Kirstan A. Vessey, PhD,[1] Kate Brassington, PhD,[2,3] Erica L. Fletcher, PhD[1]*



Greferath et al. **Am Aca of Ophthalmol.** 2016

Melanophages are at the origin of hyperreflective foci in AMD

Hyperreflective foci

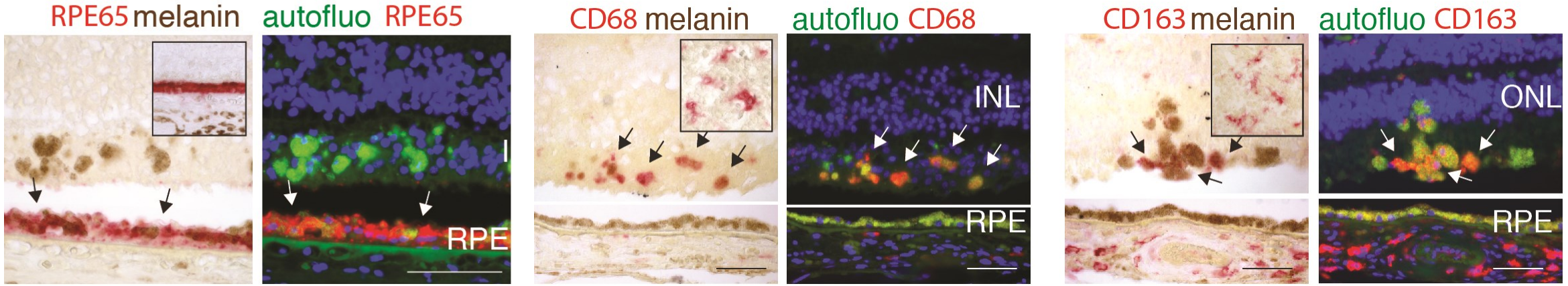


➡ **highly predictive biomarker for progression from intermediate to late AMD**

Chrisenbury et la. IOVS 2013; Leuschen et al. Ophthalmology 2013

➡ **HF are caused by retinal melanosome containing cells**

Balaratnasingam, C., *et al.* Ophthalmology 2017

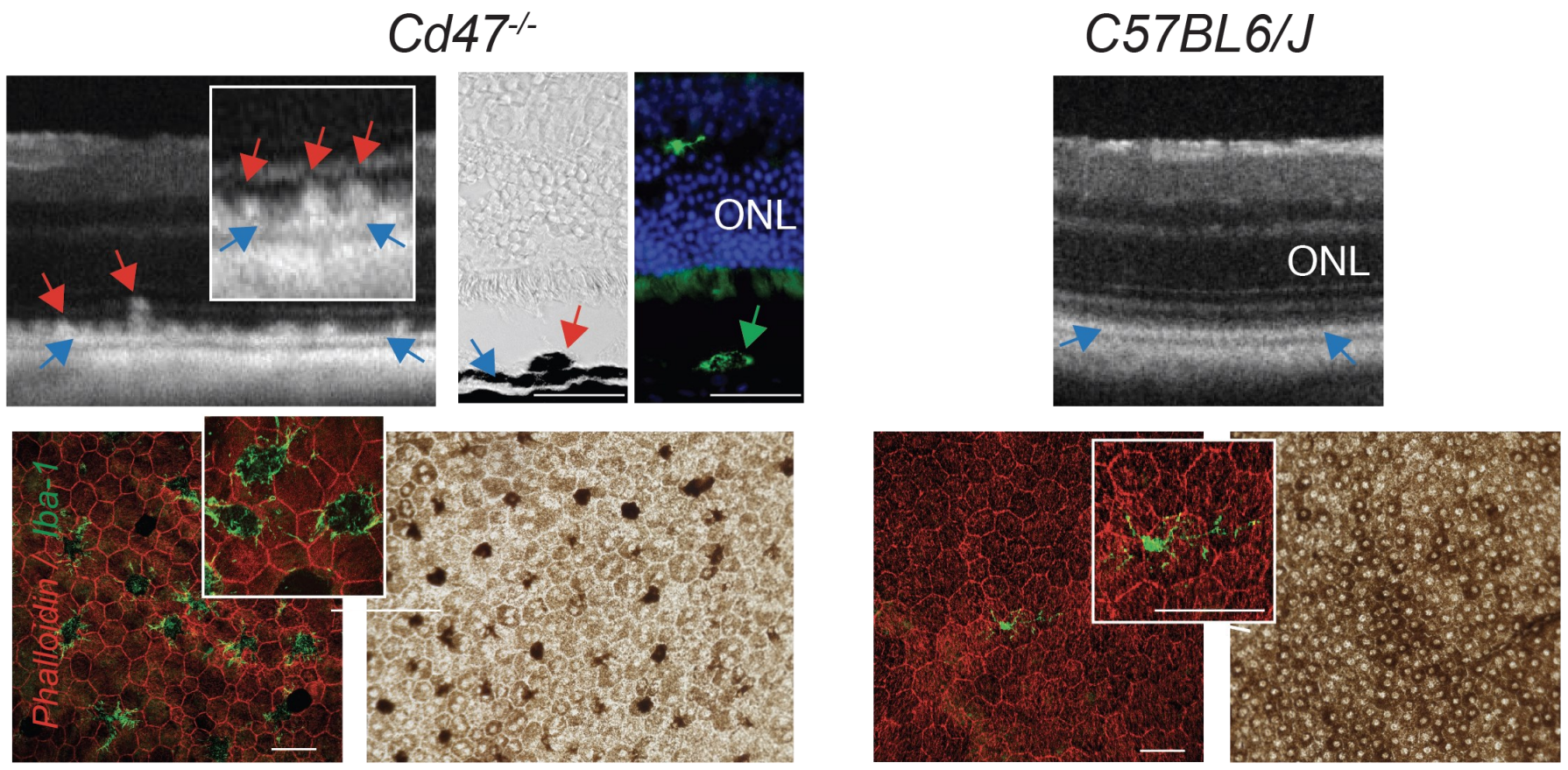


➡ How are melanophages generated

Augustin et al. **J Neuroinfl**. 2023

Aged CD47-/- mice present hyperreflective foci

CD47$^{-/-}$ mouse



➡ Why?

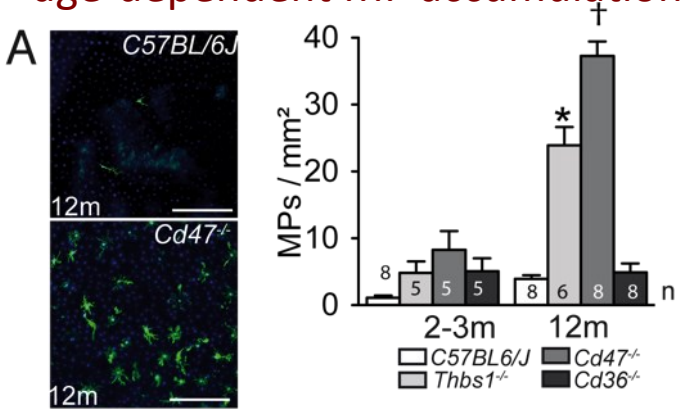
1) CD47= TSP1 receptor

CD47 mediates physiological subretinal immune suppression

Thrombospondin-1-mediated regulation of microglia activation after retinal injury
Lack of thrombospondin 1 and exacerbation of choroidal neovascularization

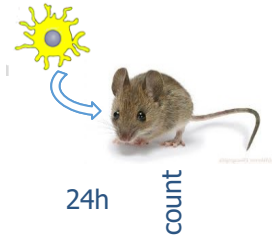
Ng *et al.*, *IOVS*, 2009
Wang *et al.*, *Arch OPhth*, 2012

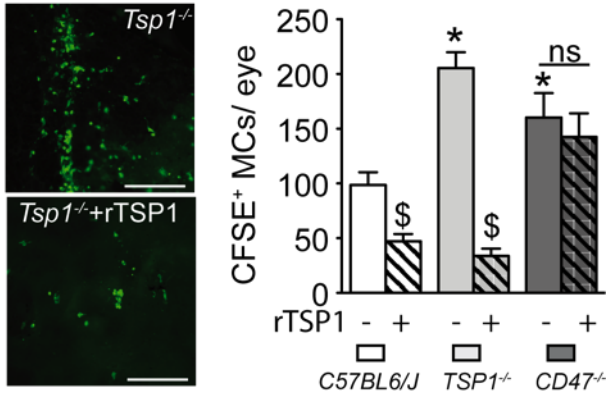
age-dependent MP accumulation



TSP-1 and CD47 deficiency lead to MP accumulation

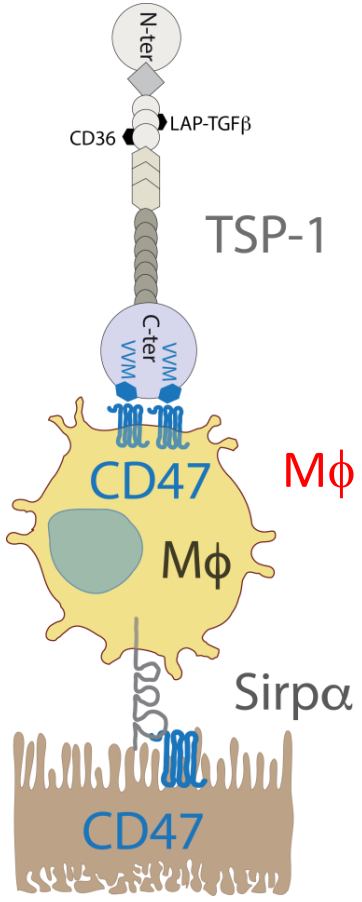
subretinal adoptive-transfer of MCs



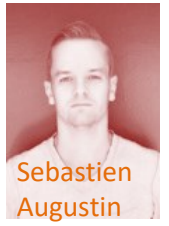


Recombinant TSP-1 reverses TSP1$^{-/-}$ MC elimination deficit (but not CD47$^{-/-}$)

Calippe et al. **Immunity** 2017

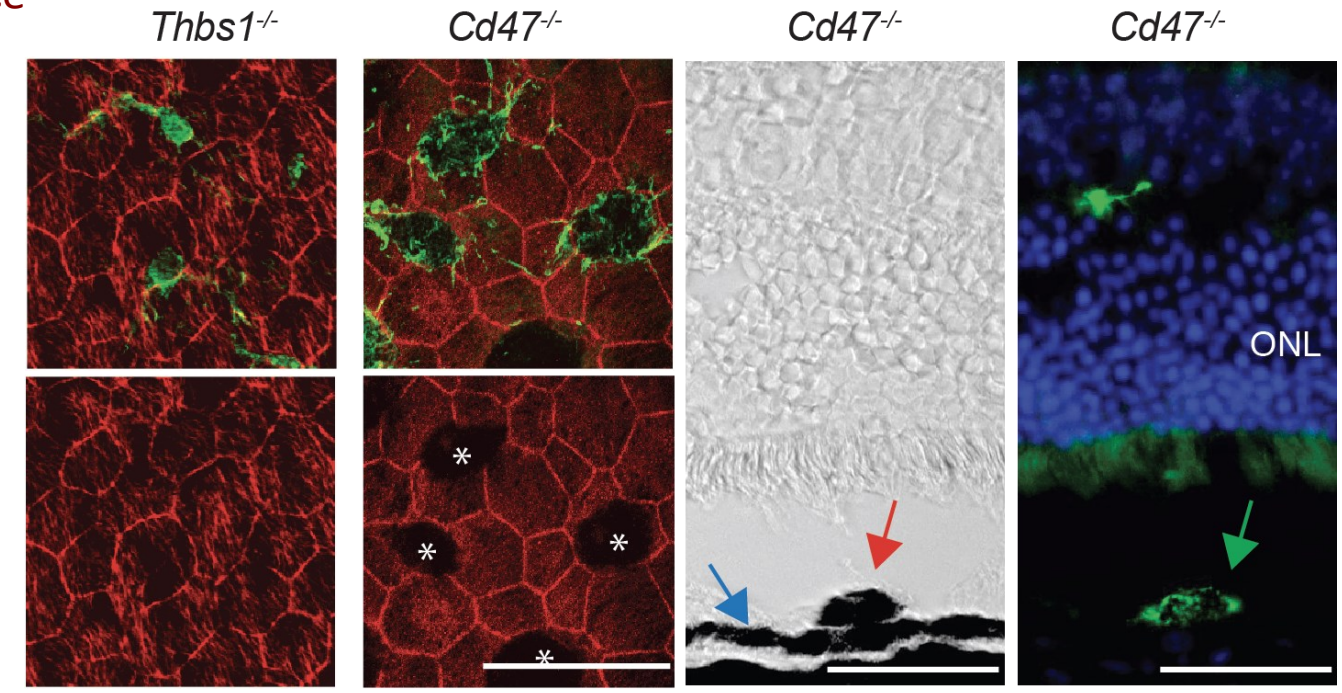


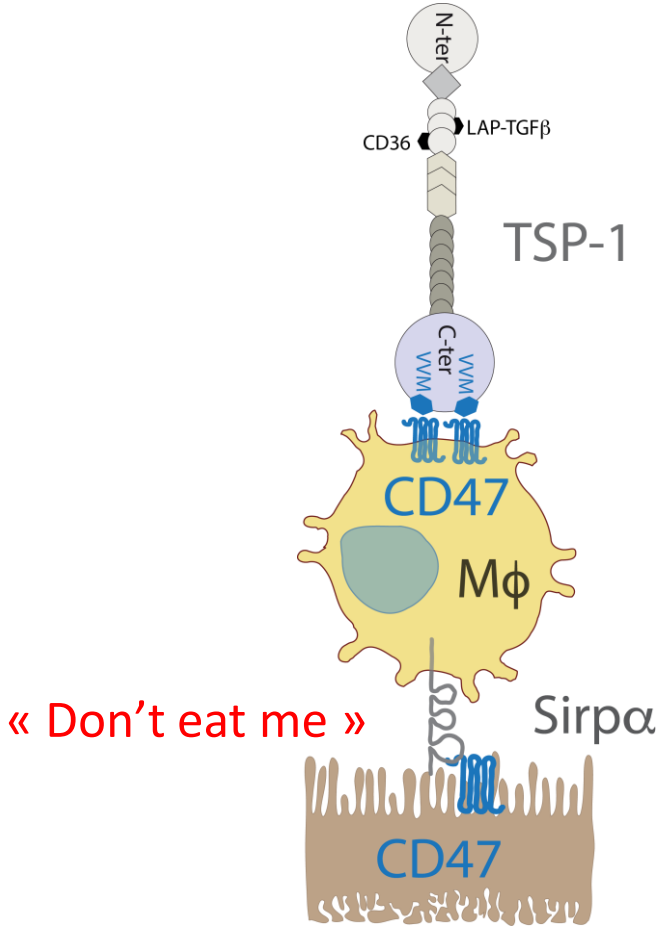
CD47
Receptor: TSP1



2) CD47= ligand for Sirpα receptor
CD47 inhibits trogocytosis of melanolipofuscein from the RPE

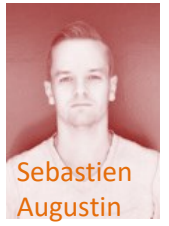
12m old mice



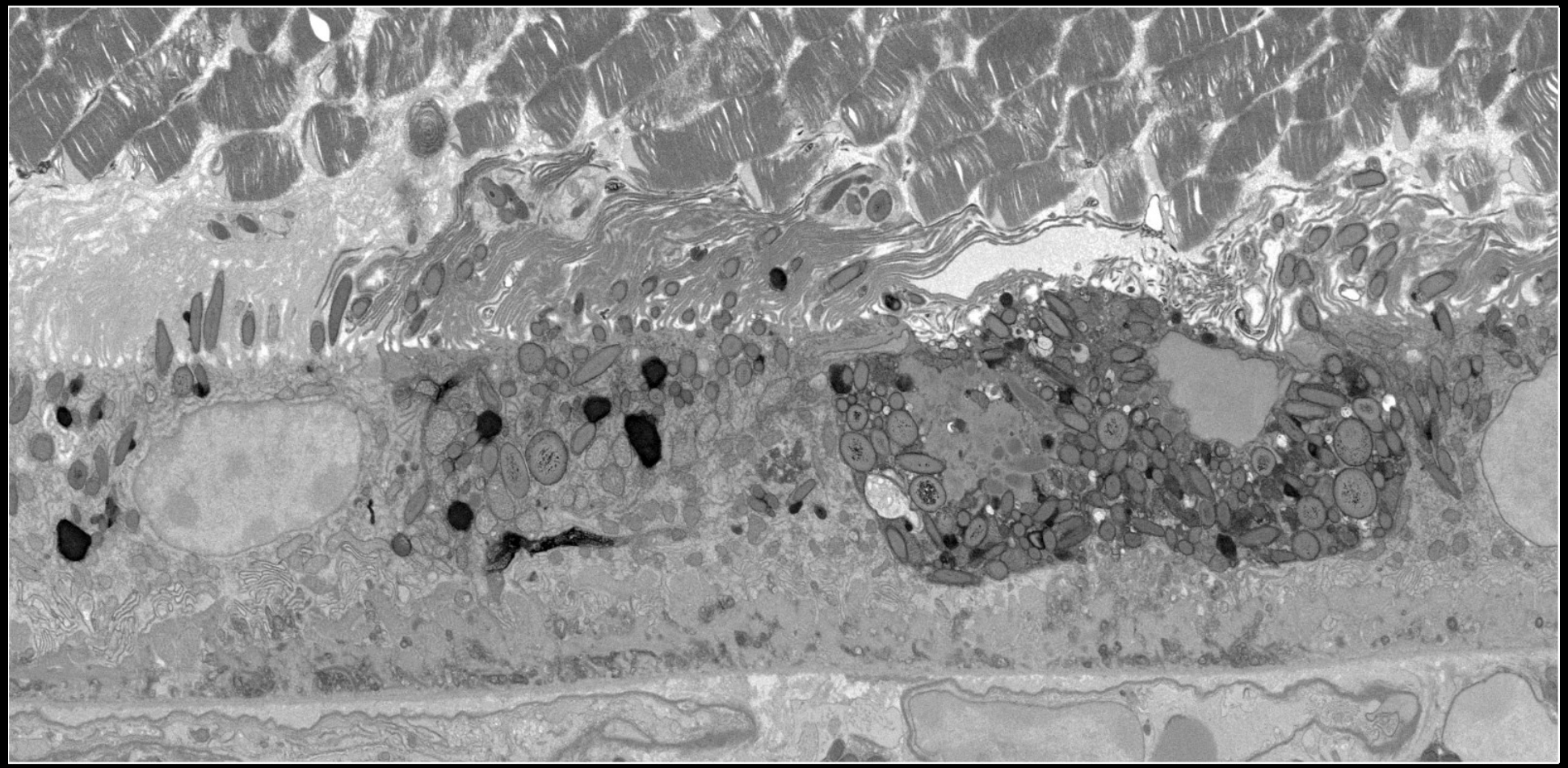


- TSP-1 and CD47 deficiency lead to MP accumulation
- Only subretinal MPs in CD47$^{-/-}$ are pigmented

CD47
Ligand: SIRPα

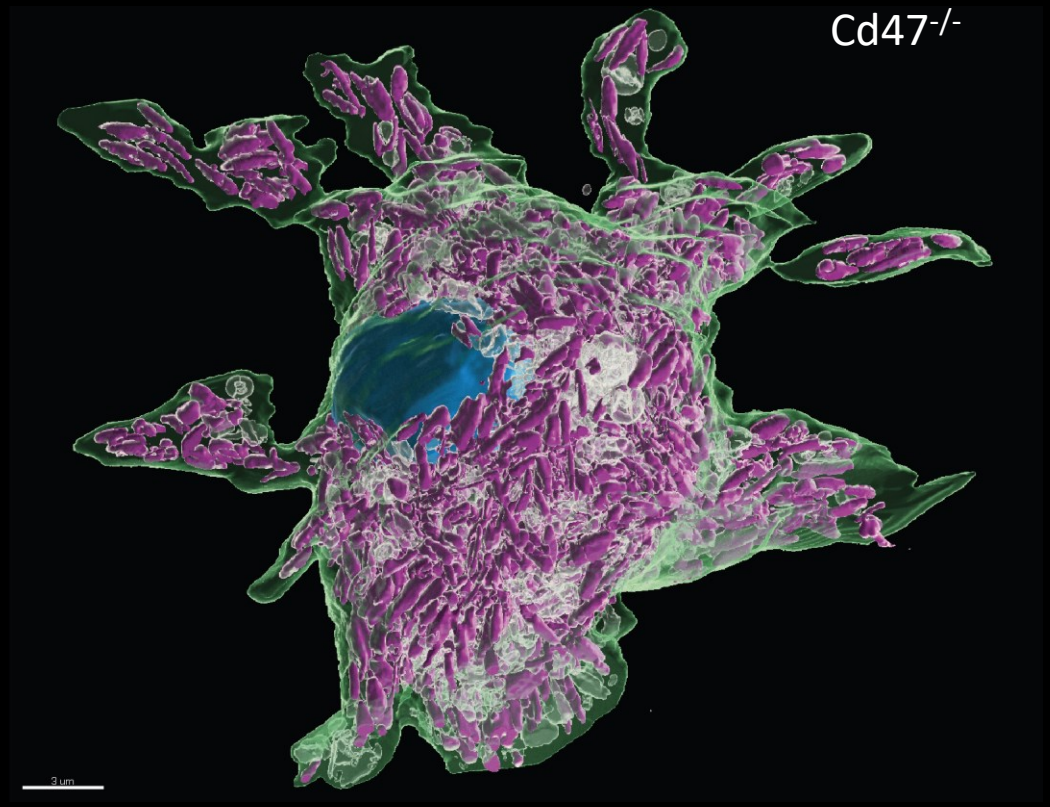


Melanophages in serial block-face scanning electron microscopy

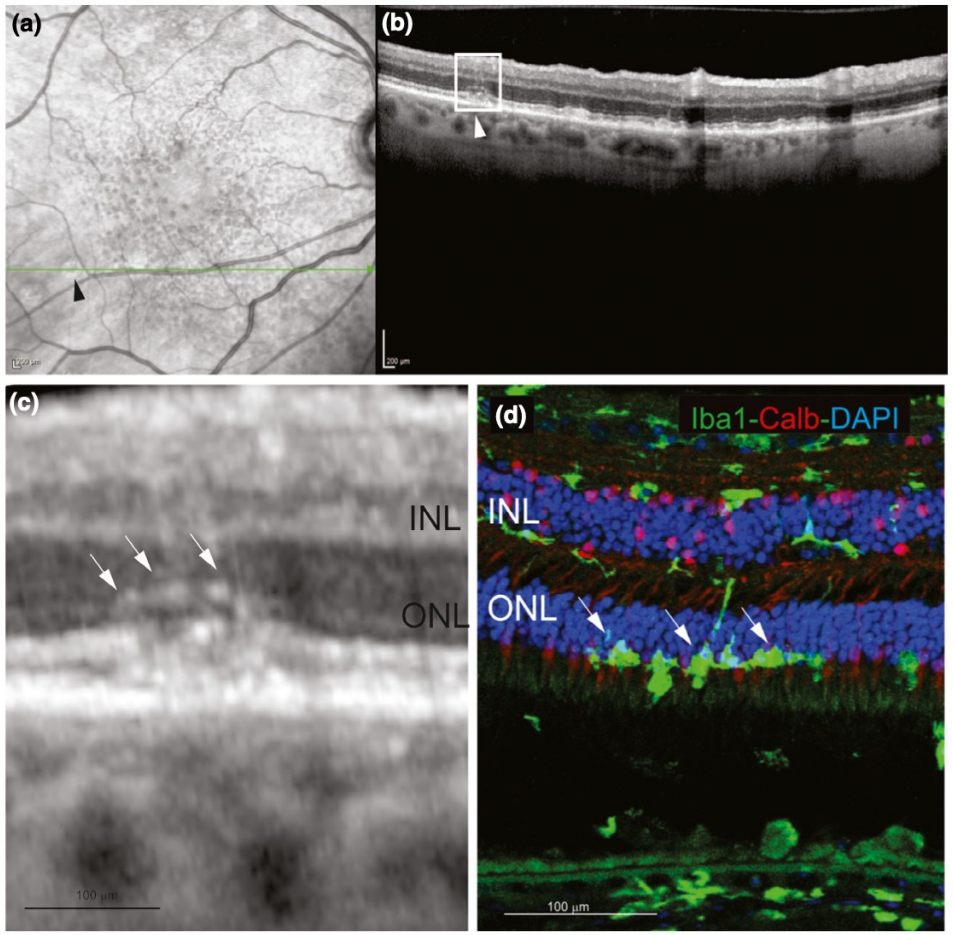


ImagoSeine facility

Melanophages in serial block-face scanning electron microscopy



nucleus
melanosome
other organelles



Fletcher, EL., *et al.* Ophthalmic and Physiological Optics 2020

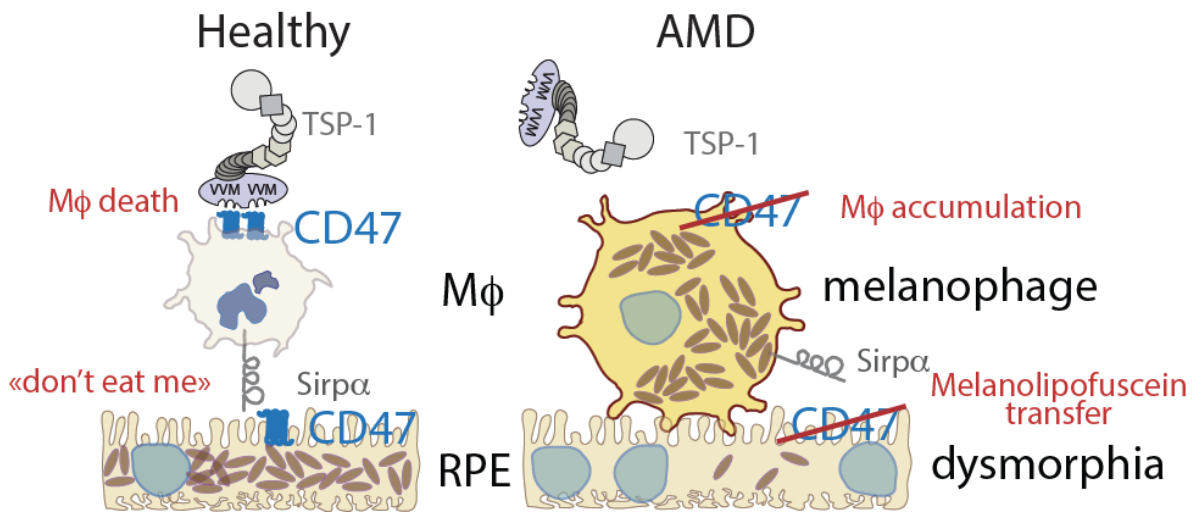
Melanophages are at the origin of hyperreflective foci in AMD

- Melanosome containing cells (MCCs) provoke HF in AMD (C. Balaratnasingam, *Ophthalmology* (2017))
- MCCs in AMD **are never** positive for RPE markers
- The majority of MCCs in AMD are positive for Mϕ markers
- RPE melanosomes transfer to subretinal Mϕ and form Melanophages *in vivo*
- Melanophages in vivo provoke HF in OCT

➡ Melanophages are most likely at the origin of HFs

- Melanosomes are not a specific marker for RPE cells in the context of AMD (Melanophages)
- There is no evidence that RPE cells can « transdifferentiate » into macrophages

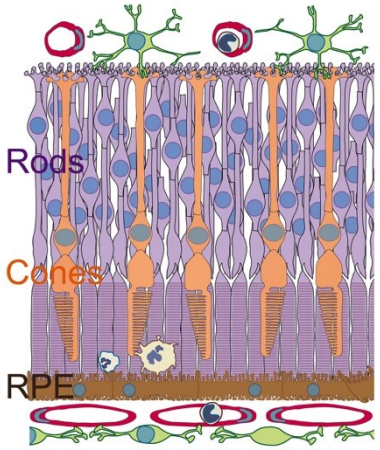
➡ There is no evidence that HFs in AMD are due to migrating RPE cells



AMD pathogenesis: chronic MP accumulation

Risk Factors:
- Age
- Tabacco/Obesity
- Genetic Variant CFH 402H

healthy



- Homeostasis
- Immune suppression

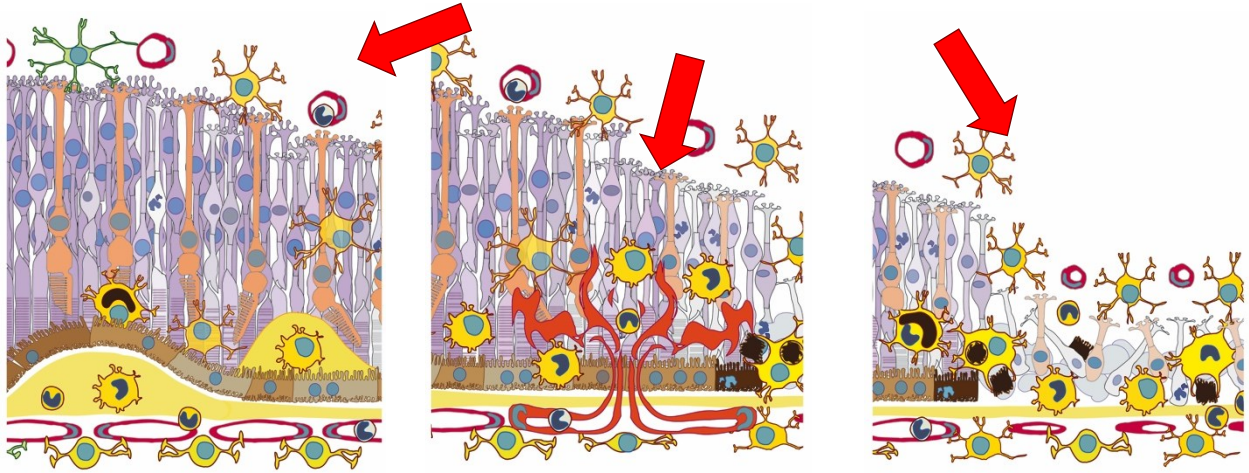
earlyAMD
- Drusen

late AMD

wetAMD
- Neovascularization

Geographic Atrophy
- Degeneration

Macrophage infiltration



- Immune suppression ↘
- **Subretinal Mϕ iniltration**

- **Subretinal Mϕ iniltration**
- Neovascularization

- **Subretinal Mϕ iniltration**
- Degeneration

➡ Just cleaning up?

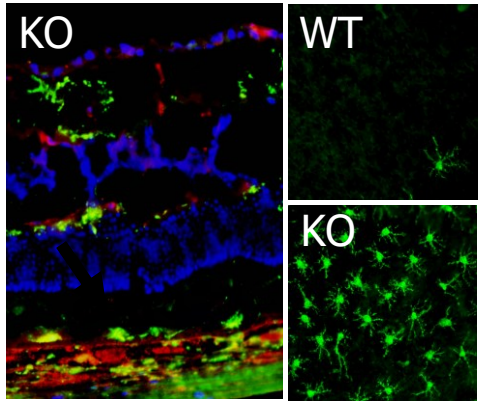
Macrophage-driven degeneration

Cardinal features of age related macular degeneration develop secondary to a CX3CR1-dependent subretinal microglia accumulation

Combadière et al. *J Clin Invest* . Volume 117, issue 10 (Oct, 2007)

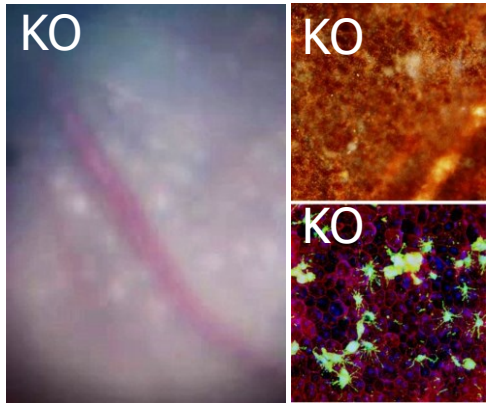
$Cx3cr1^{-/-}$

MC accumulation



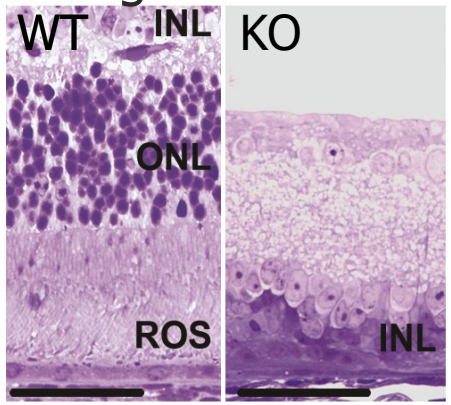
- Age
- Light (albino)
- Laser injury

Drusen



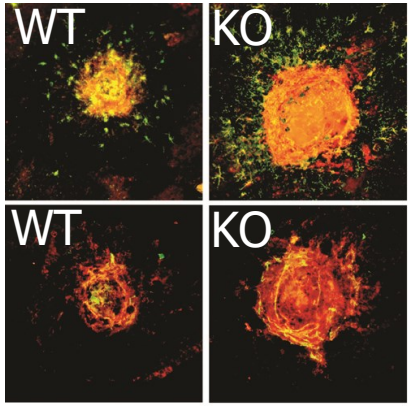
- Age

Degeneration



- Age
- Light (albino)
- Laser injury

Neovasc.



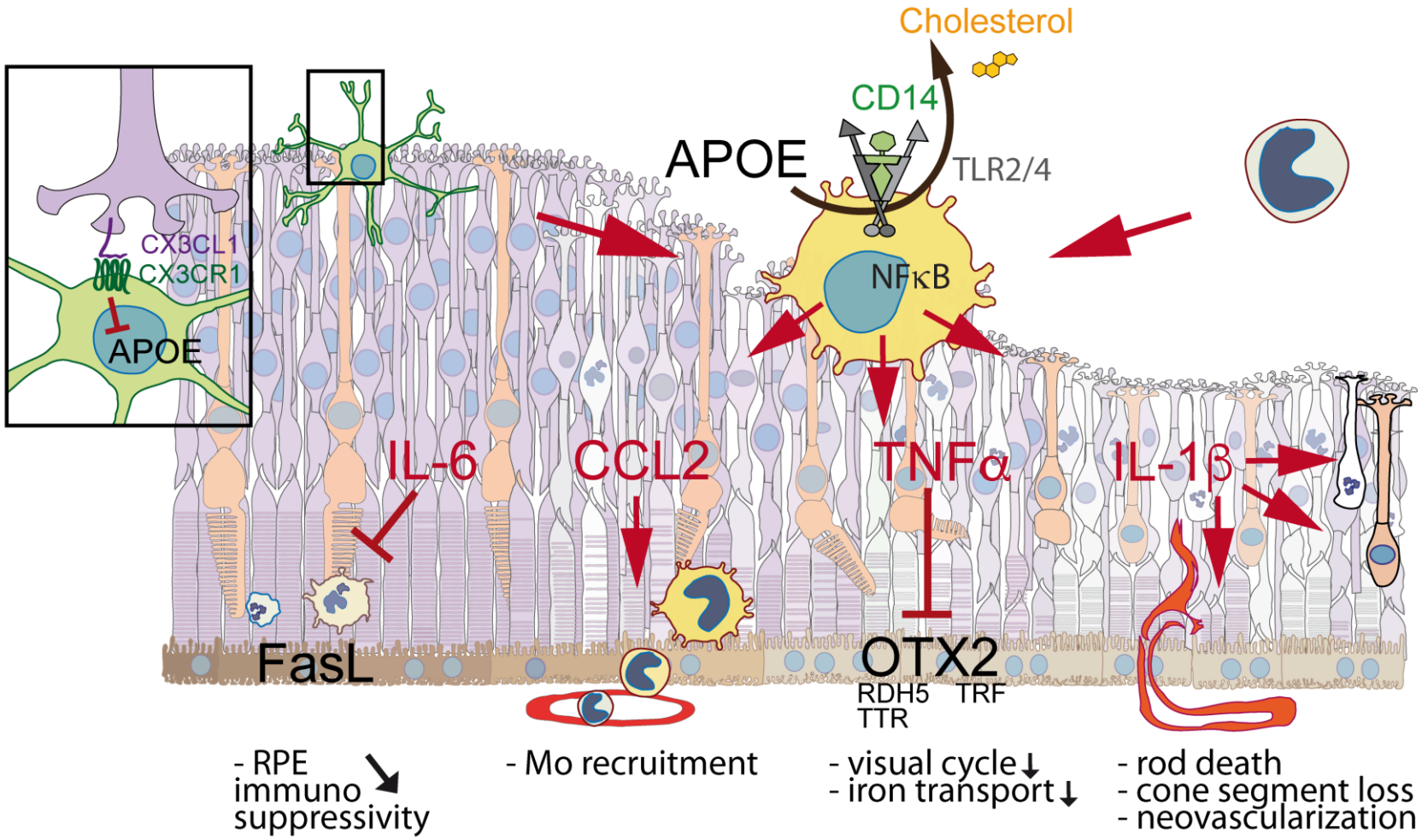
- Laser injury

➡ **A deficit in a macrophage gene** (CX3CR1) **is sufficient to induce age-related inflammation, degeneration, and CNV**

➡ CX3CR1-deficiency aggravates retinal disease

IRD models: Peng et al 2014, Zabel et al. 2016 (rd10); **Diabetic retinopathy:** Beli *et al.* 2016; Cardona et al. 2015; Kezib. Et al. 2013; Mendiola et al; 2016; **Glaucoma:** Wang et al. 2014**; Paraquat-induced retinopathy:** Chen et al. 2013

Consequences of chronic Mϕ activation



"Cytokine storm"

- RPE immuno suppressivity ↘

Levy et al. ***EMBO Mol Med***. 2015

- Mo recruitment

Sennlaub et al. ***EMBO Mol Med***. 2013

- visual cycle↓
- iron transport↓

Mathis et al. ***Aging Cell***. 2016
Touhami et al. **J Neuroinfl**. 2018

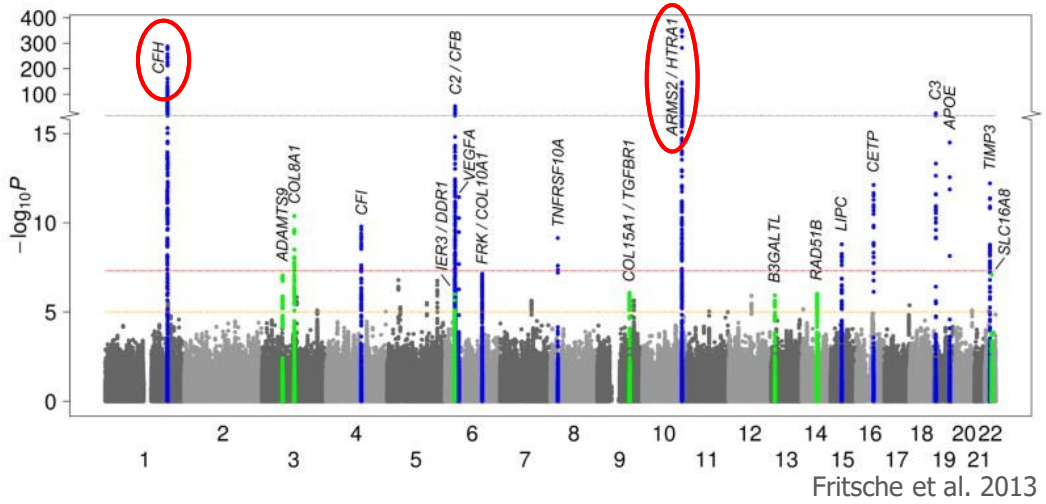
- rod death
- cone segment loss
- neovascularization

Lavalette et al. ***Am J Pathol***. 2011
Hu et al. ***J Neuroscience***. 2015
Eandi et al. ***Elife***. 2016
Charles Messance et al. **J Neuroinfl** 2020

AMD pathogenesis: why certain people?

Why do certain people develop AMD?

AMD: most heritable multifactorial disease



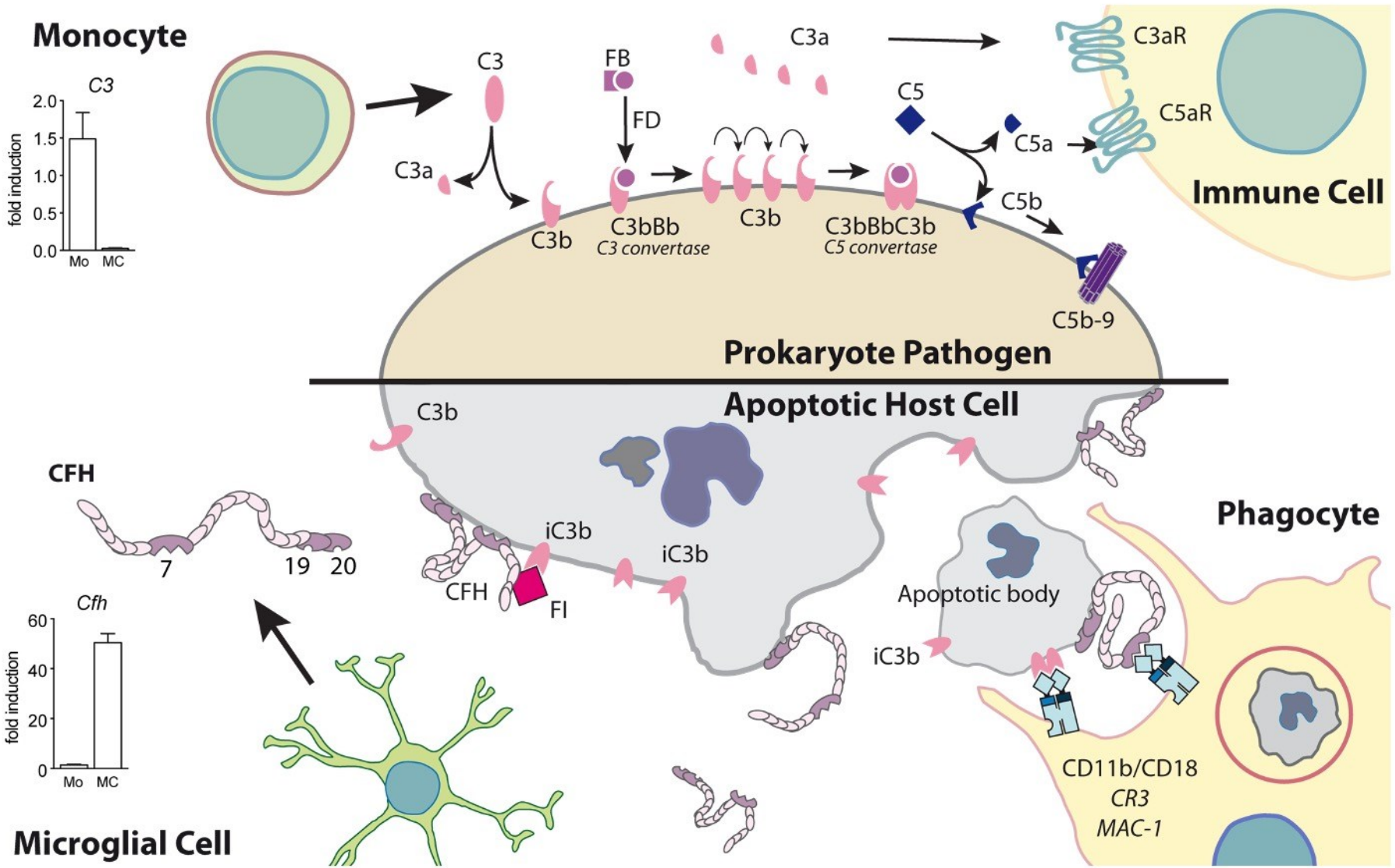
Fritsche et al. 2013

- Homozygosity for CFH402H risk variant
  = 10 fold risk increase for GA and wetAMD
- Homozygosity for 10q26 risk variant
  = 10 fold risk increase for GA and wetAMD

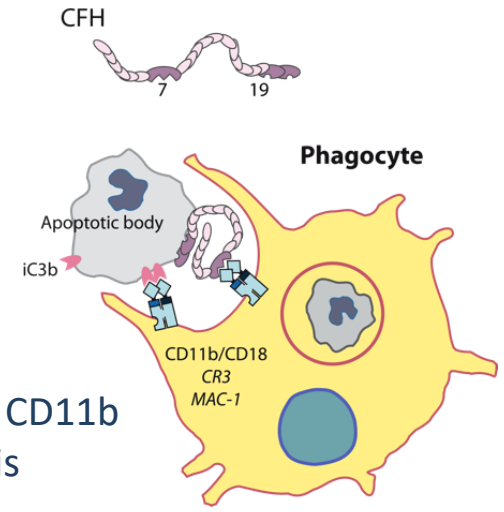
CFH Y402H?

Heterozygosity for the risk CFH 402H variant = 3 fold risk increase for GA
Homozygosity for the risk CFH 402H variant = 10 fold risk increase for AMD

Alternative Complement cascade and Factor H



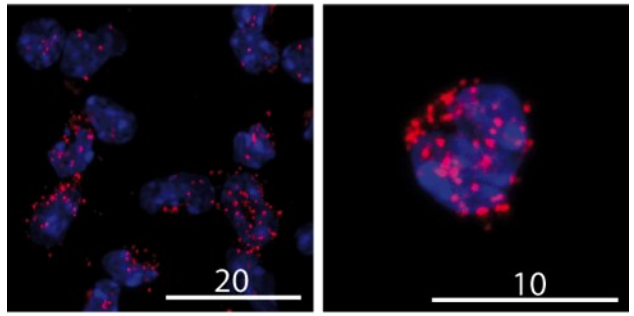
Complement Factor H



CFH's 7th domain binds to CD11b and promotes phagocytosis

Proximity assay

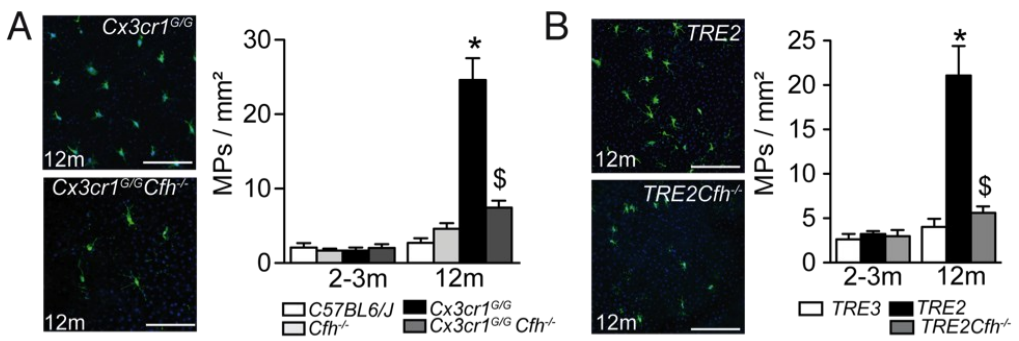
CD11b/CD47 complexes



➡ CD47 and CD11b co-locate on MCs

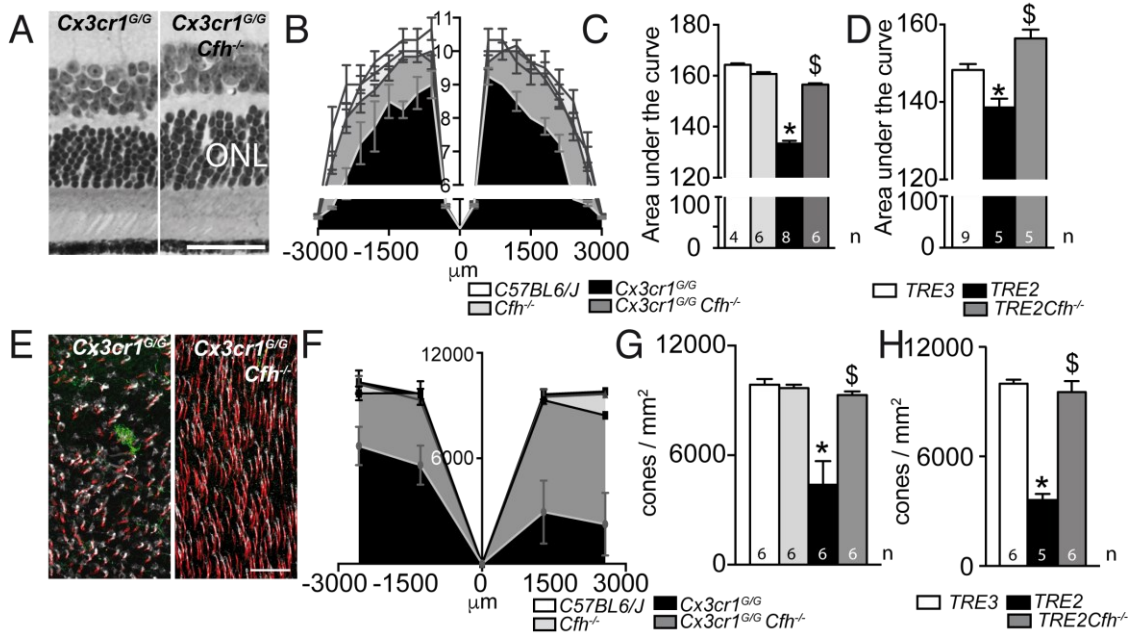
FH is necessary for chronic, pathogenic, age-related MP accumulation

Age-dependent accumulation (200-500lux 12h/24h)



➡ CFH deletion prevents chronic age-related MP accumulation in *Cx3cr1*$^{GFP/GFP}$ and *TRE2 mice*

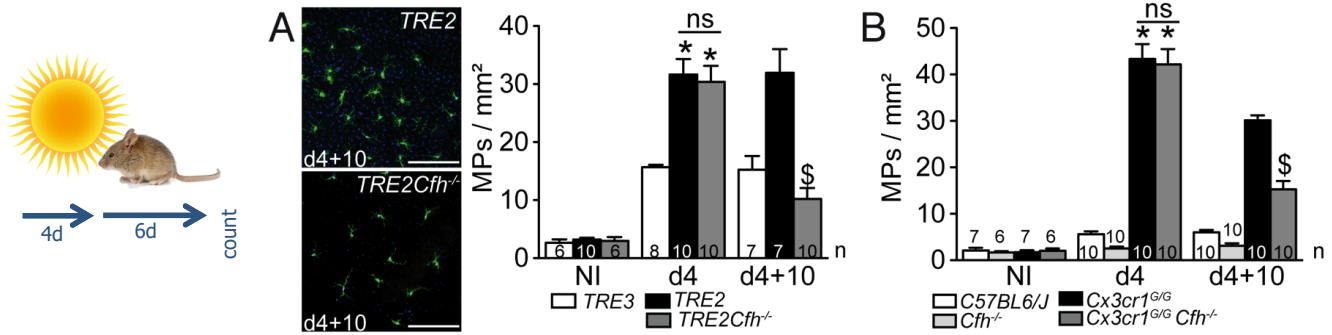
Age-dependent degeneration (200-500lux 12h/24h)



➡ CFH deletion prevents MP-associated degeneration in *Cx3cr1*$^{GFP/GFP}$ and *TRE2 mice*

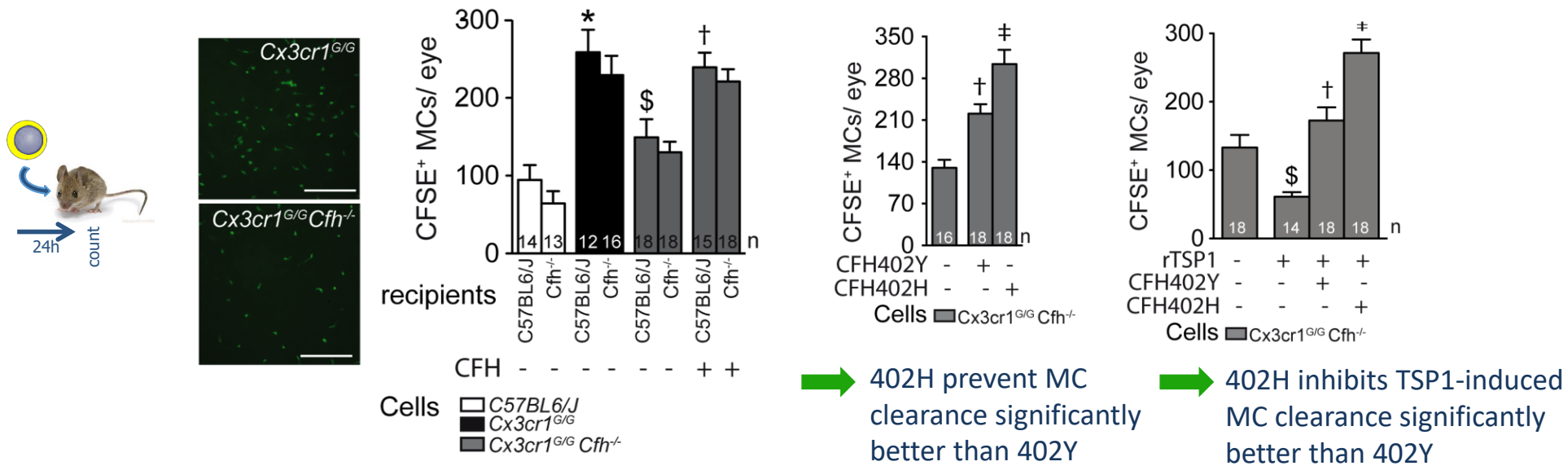
FH is inhibits resolution of light-induced acute inflammation

Light-induced accumulation (4d 4500lux 500nm)



➡ CFH deletion does not affect acute light-induced inflammation but accelerates its resolution in *Cx3cr1*$^{GFP/GFP}$ and *TRE2 mice*

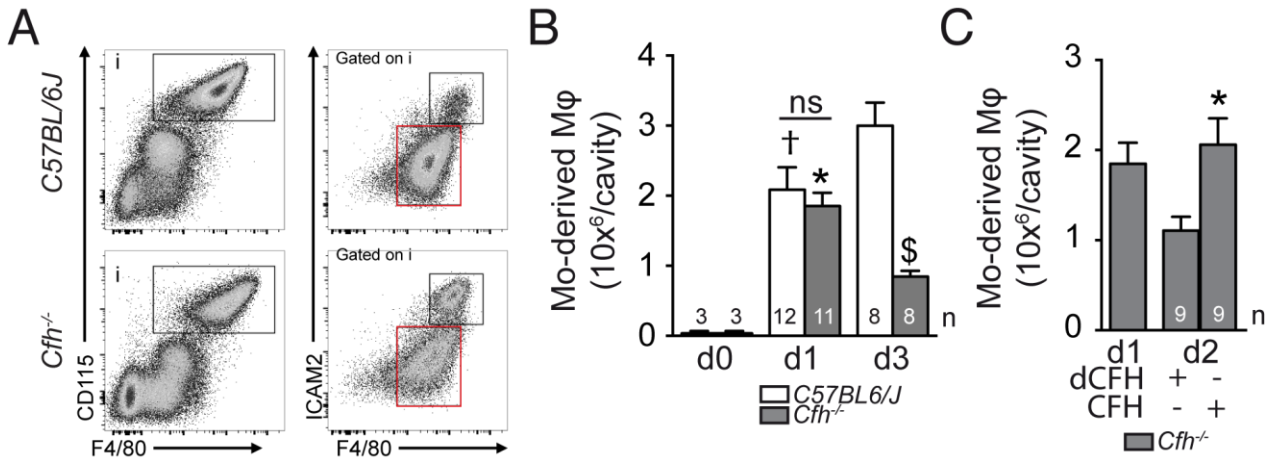
Subretinal adoptive MP transfer



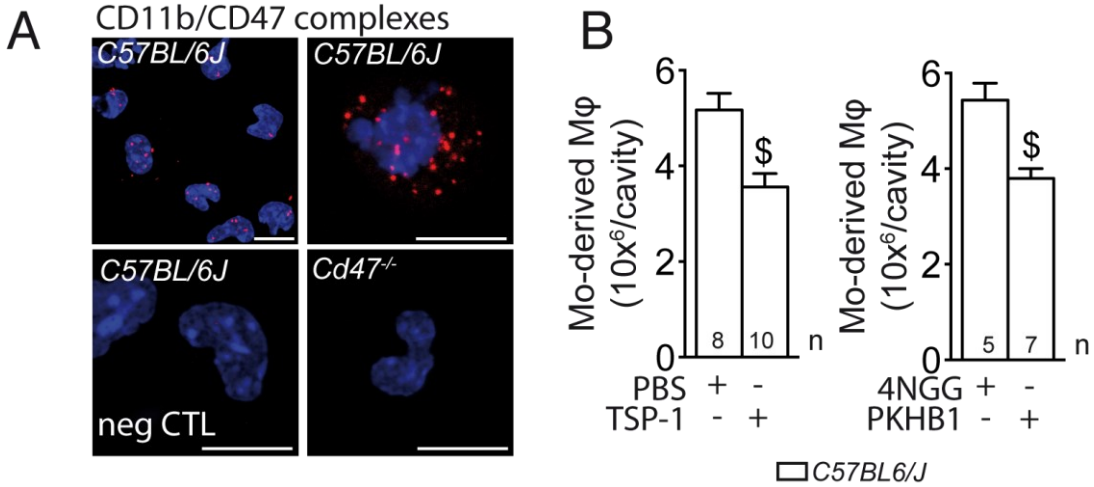
➡ 402H prevent MC clearance significantly better than 402Y

➡ 402H inhibits TSP1-induced MC clearance significantly better than 402Y

FH inhibits inflammation resolution in acute peritonitis



➡ FH inhibbits the elimination of inflammatory Mϕ in acute peritonitis



➡ CD47 and CD11b co-locate on Mϕs

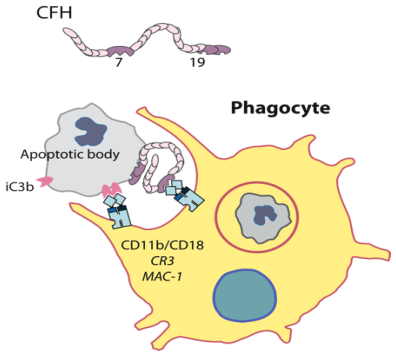
➡ CD47 accelerates Mϕ elimination

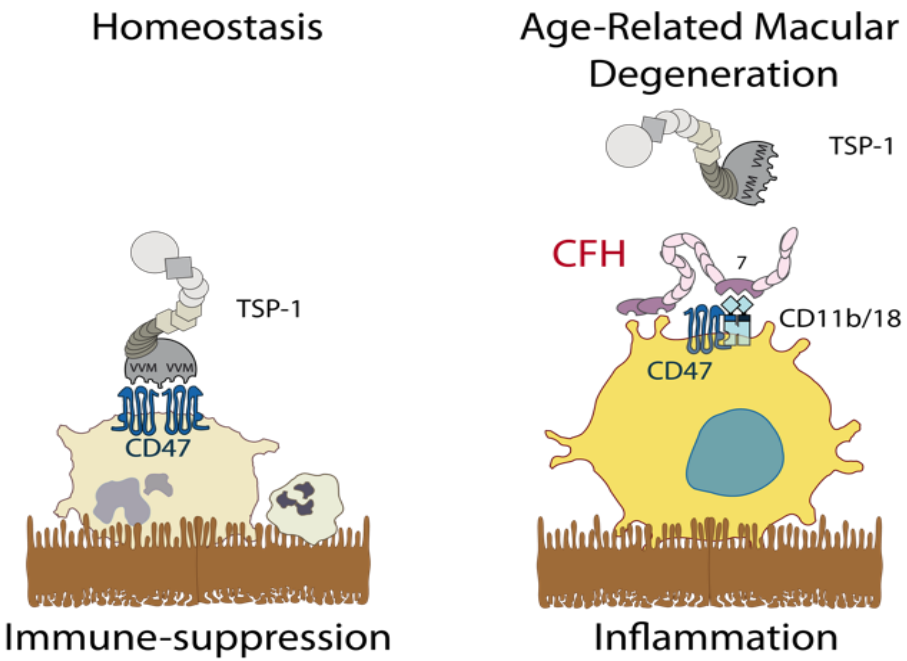
AMD pathogenesis: why certain people ?

AMD: most heritable multifactorial disease

1. Homozygosity for CFH402 risk variant = 10 fold risk increase for GA

Complement factor H inhibits CD47-mediated resolution of inflammation



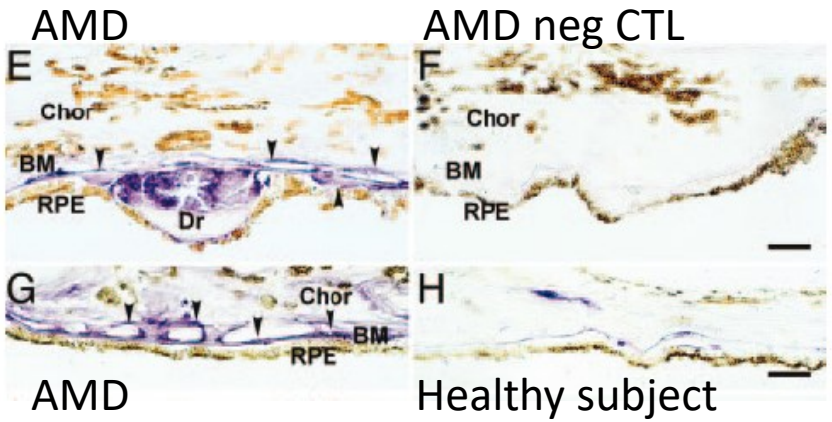


- CFH is necessary for chronic pathogenic subretinal inflammation
- Mononuclear phagocyte-derived CFH inhibits their elimination during inflammation resolution
- CFH binding to CD11b/CD18 interferes with TSP-1 activation of the integrin-associated CD47
- The AMD-associated CFH402H is particularly potent to inhibit the elimination of microglial cells.

Calippe et al. **Immunity** 2017

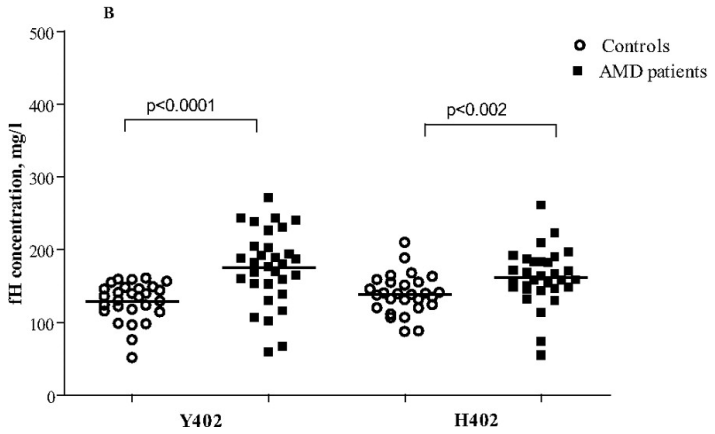
Is there too much CFH in AMD?

CFH immunohistochemistry



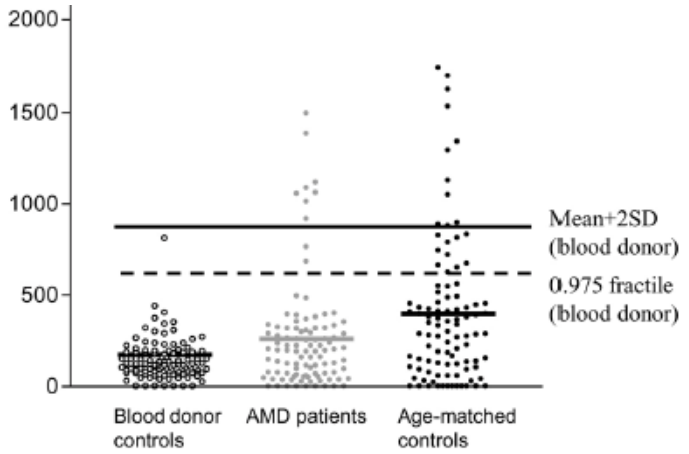
Hagemann et al. ***PNAS*** 2005
Johnson et al. ***PNAS*** 2006
Weismann et al. ***Nature***. 2011
Shaw et al. ***PNAS***. 2012

Plasma CFH



Hakobyan et al. ***IOVS*** 2008

CFH auto-antibodies



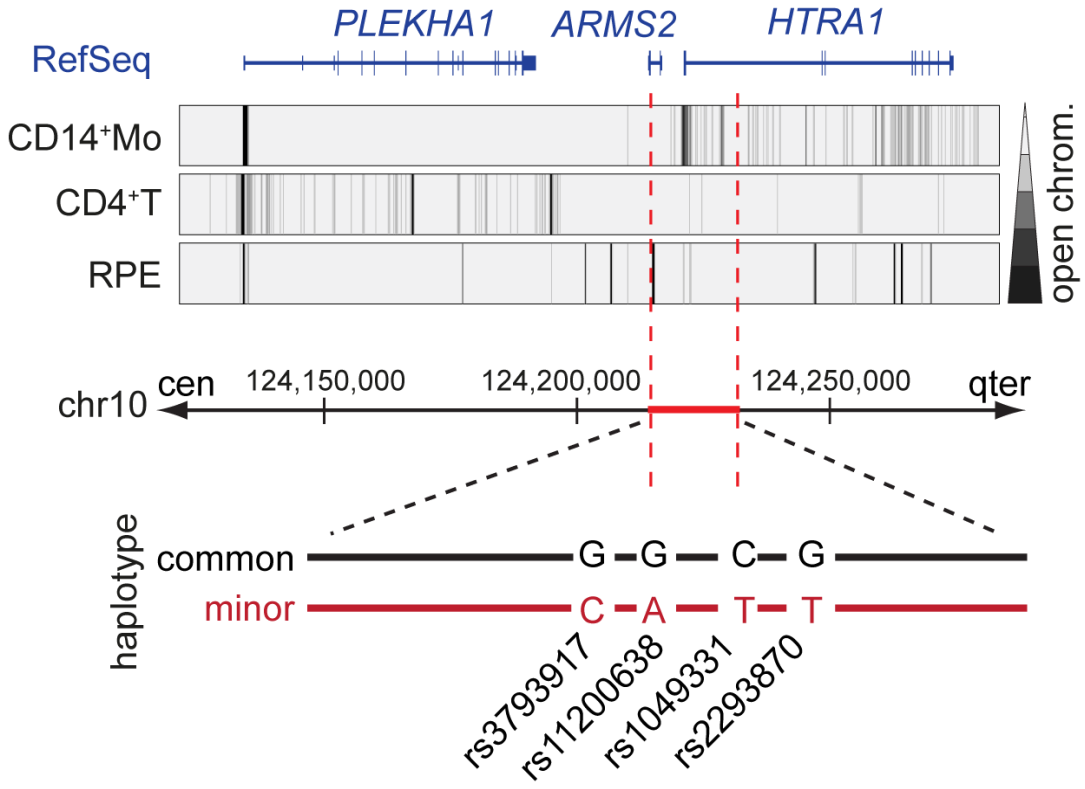
Dhillon et al. ***IOVS*** 2010

What about 10q26?

Heterozygosity for the risk 10q26 variant = 3 fold risk increase for AMD
Homozygosity for the risk 10q26 variant = 10 fold risk increase for AMD

Locus 10q26 and AMD

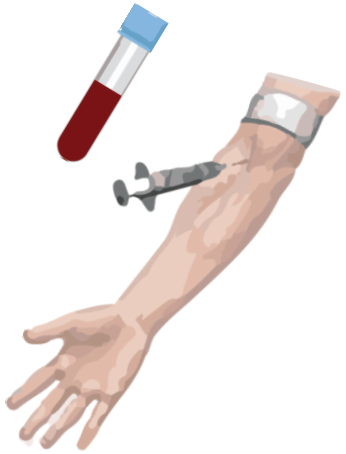
- Minor Haplotype (mH) strongly **associated with GA and wetAMD**
- mH/mH→ **10x increased risk** to develop AMD



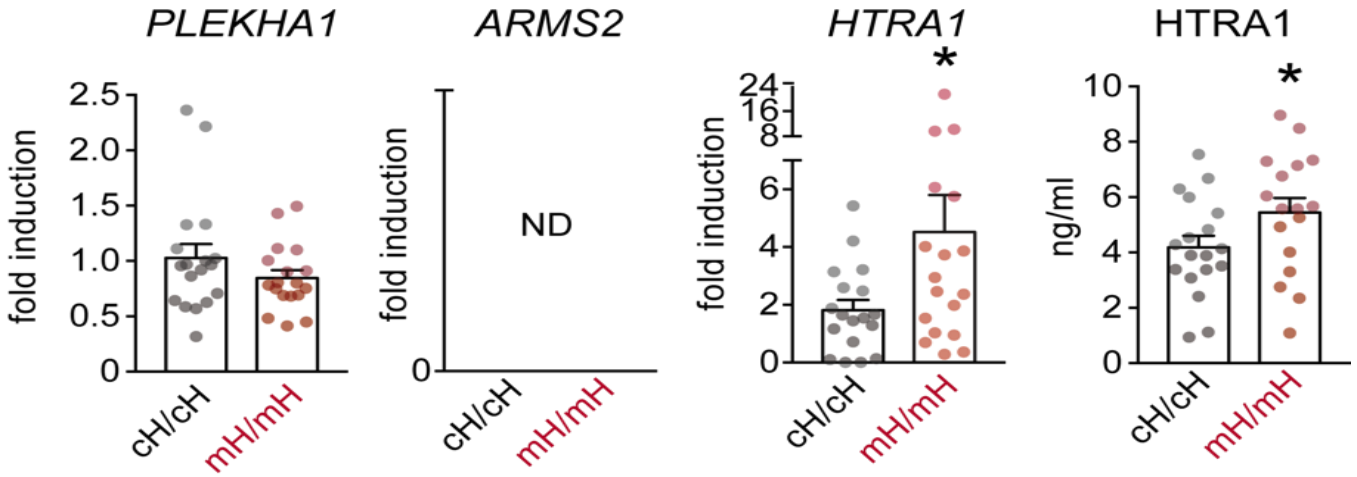
ENCODE

➡ open chromatine sites in HTRA1 promoter mainly in Monocytes

10q26 variant and PLEKHA1, ARMS2 and HTRA1 expression in monocytes?

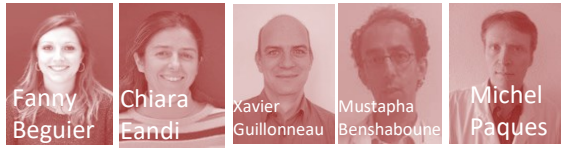


Characteristics	cH/cH (n=18)	mH/mH (n=18)	p value
Age, mean (SD)	80,3 (8,5)	81 (10,2)	0,416 (t student)
Women, n (%)	12 (66,7)	10 (55,6)	0,495 (chi2)
AMD, n (%)	9 (50)	9 (50)	

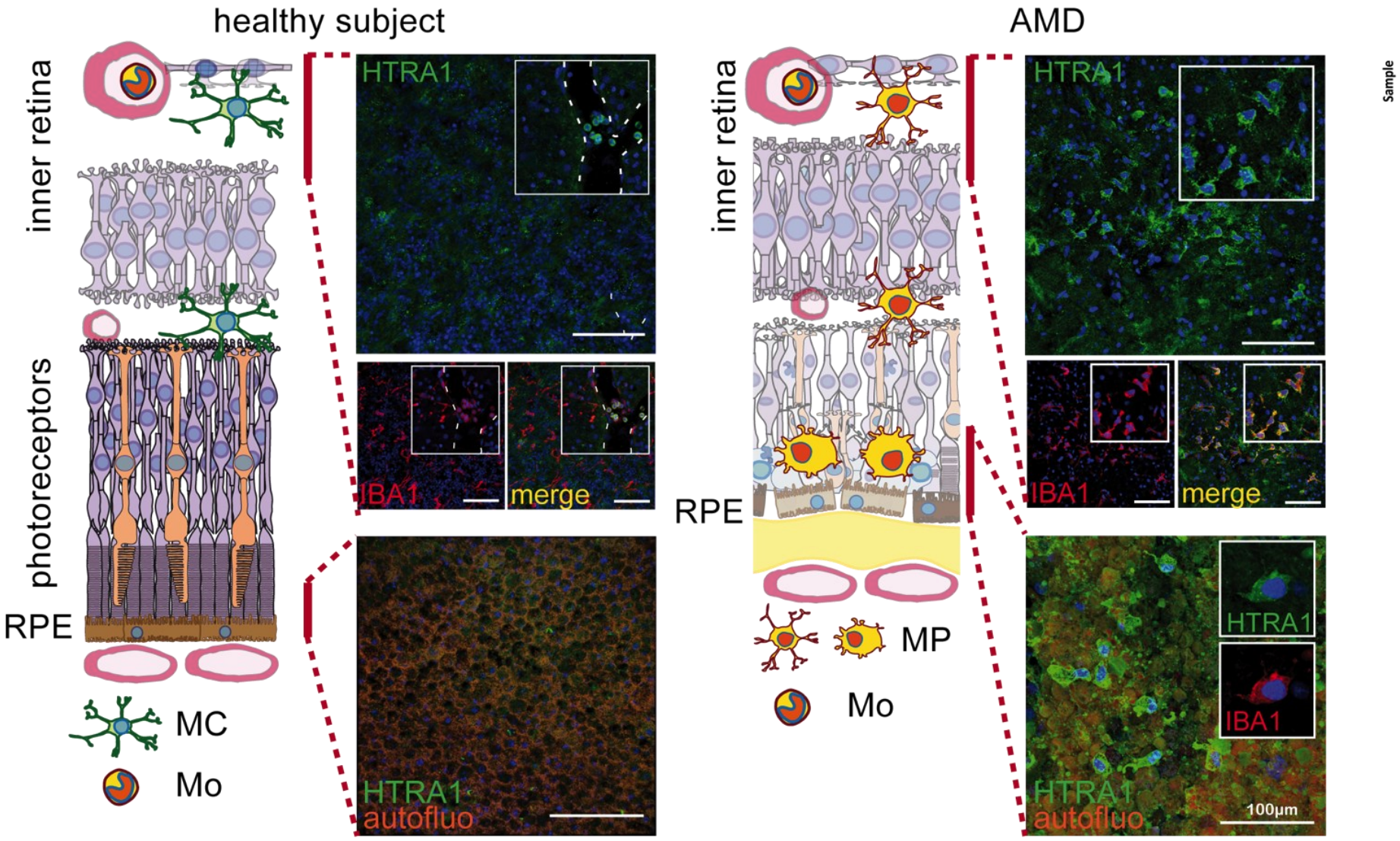


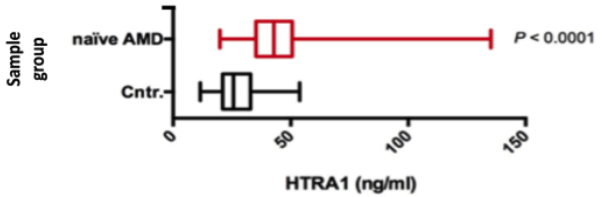
mH/mH monocytes: HTRA1 ↗

Where does HTRA1 locate in AMD eyes?



HTRA1 localisation in healthy and AMD cH/cH donors ?

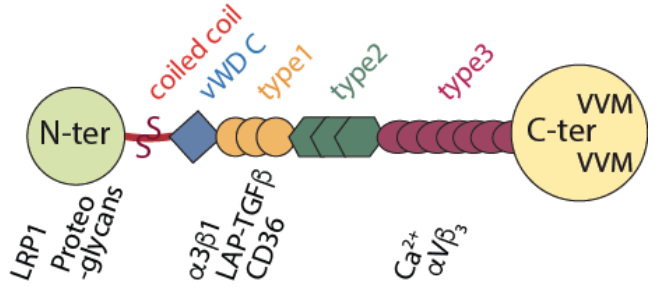


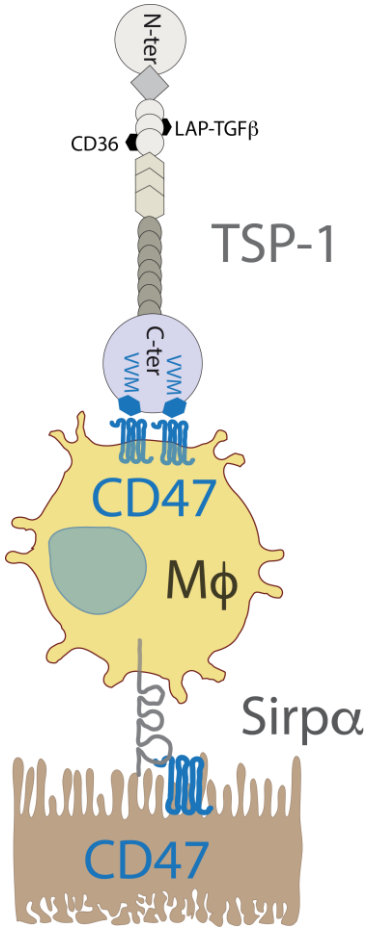


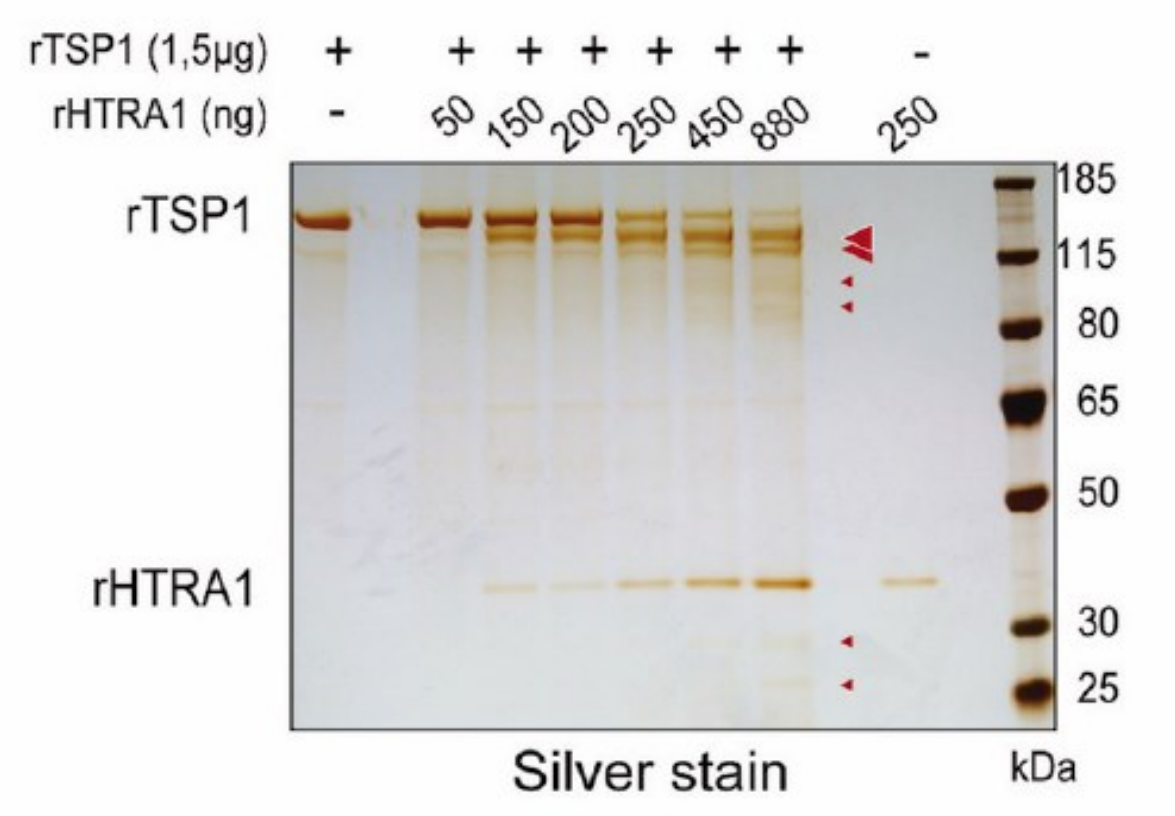
Tosi et al. 2017

What about the Hageman PNAS?

TSP-1 is a substrate of HTRA1



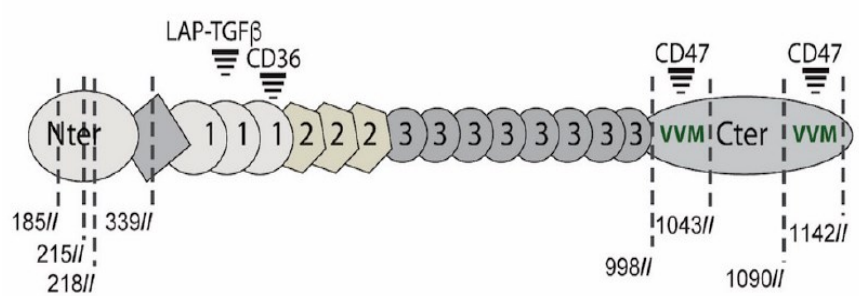


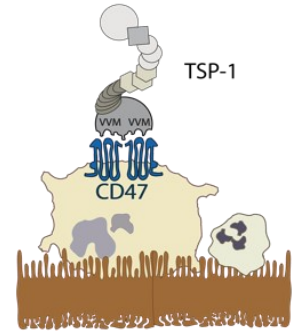


→ Where is TSP-1 cleaved ?

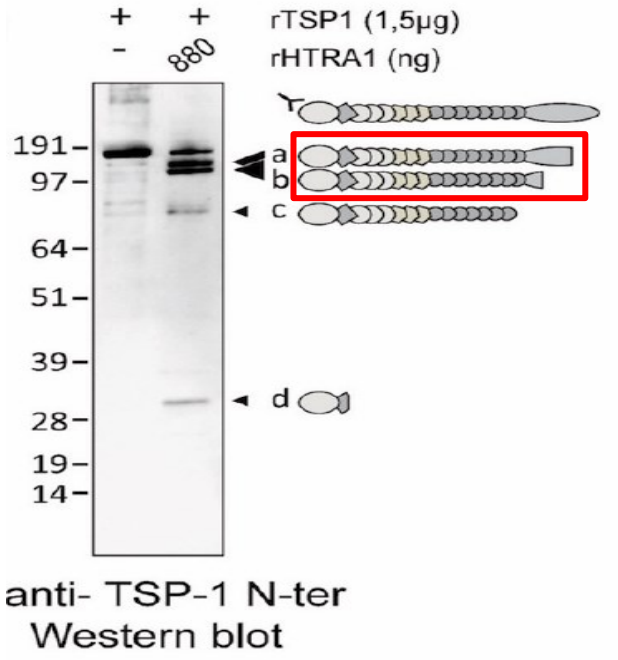
Where does HTRA1 cut TSP-1?

→Mass Spectrometry:
8 potential clivage site candidates

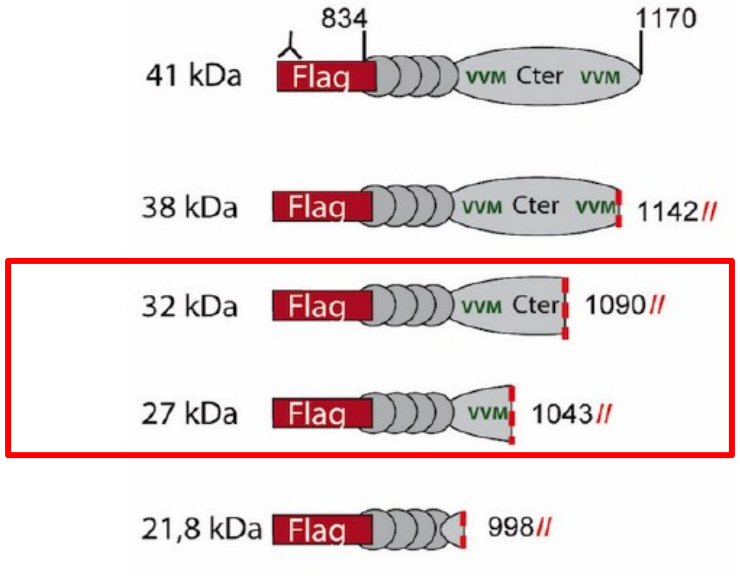


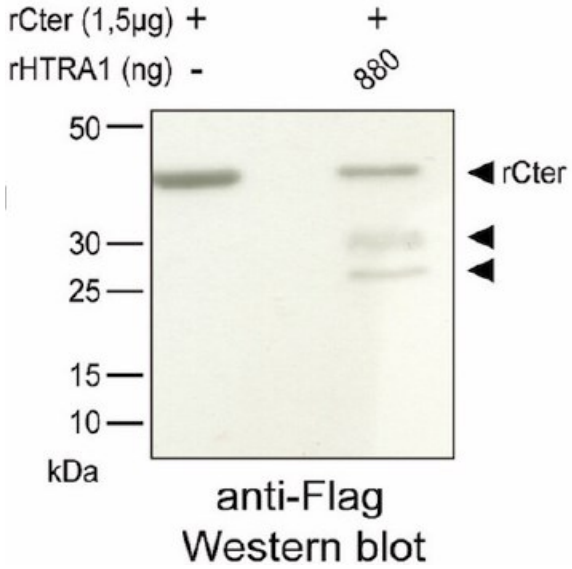


Immune-suppression

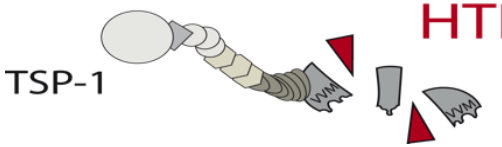


anti- TSP-1 N-ter
Western blot





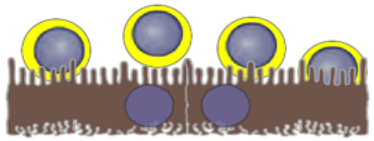
anti-Flag
Western blot

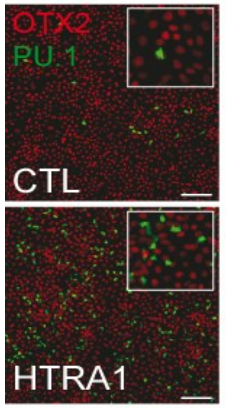


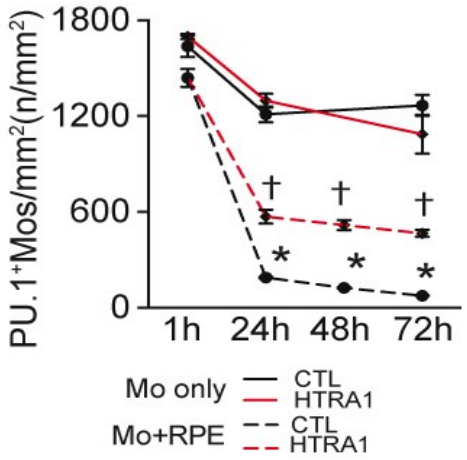
→ HTRA1 separates the two CD47-binding sites of TSP-1

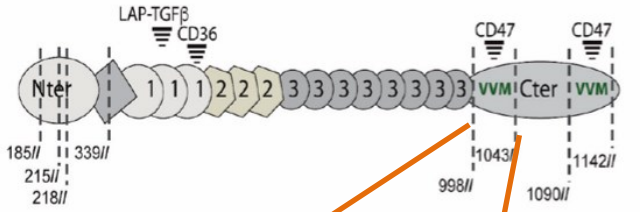
HTRA1 in co-culture of primary RPE and Mo

Automated human Mos / RPE co-culture system



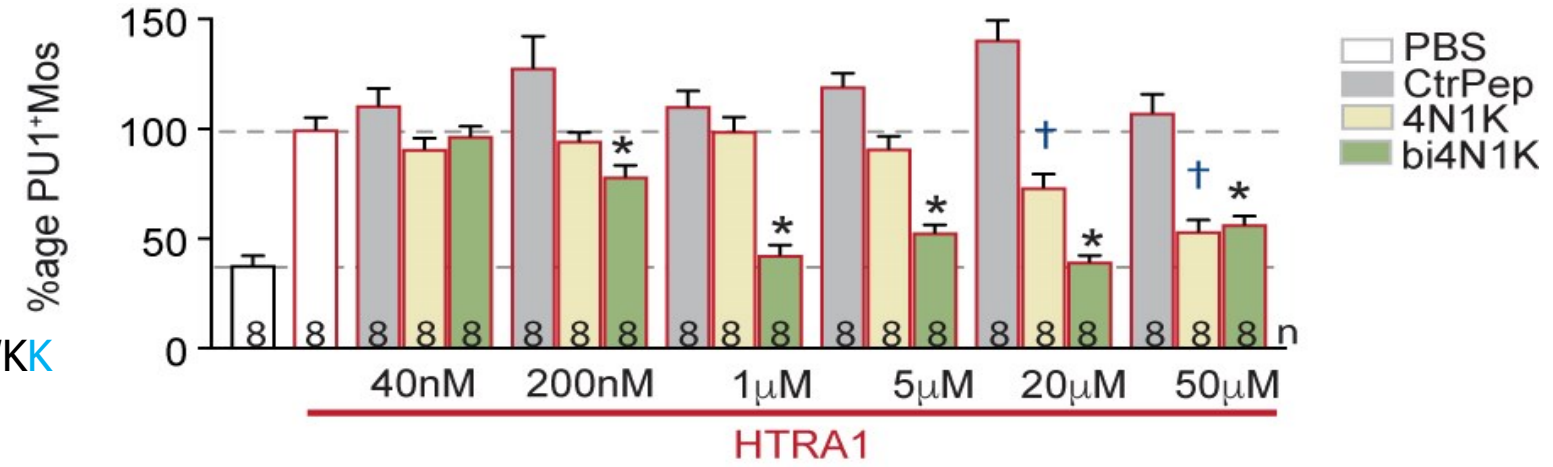






4N1K: KRFYVVMWKK

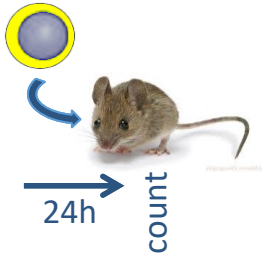
bi4N1K: KRFYVVMWKKGGGGGGGGKRFYVVMWKK

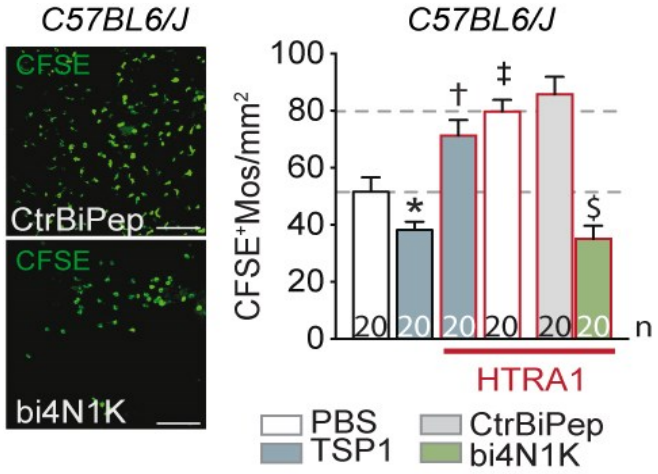


➡ separating the two VVM CD47-binding sites of TSP-1 likely dramatically reduces its CD47-activating capacity

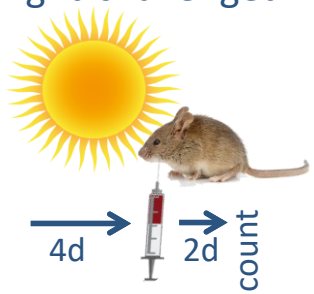
VVM peptide reverses HTRA1 *in vivo*

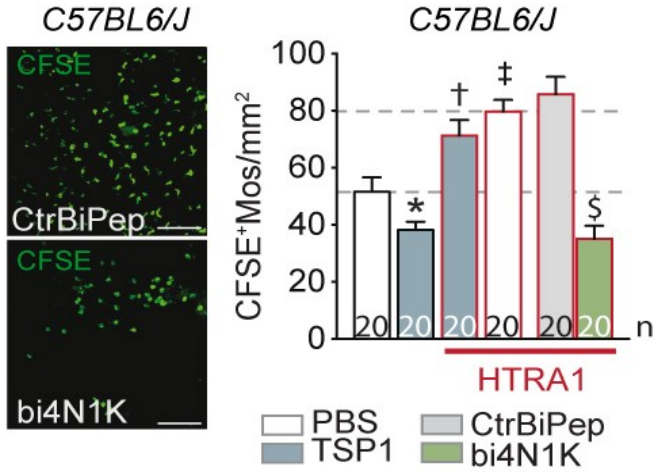
Adoptive Transfer

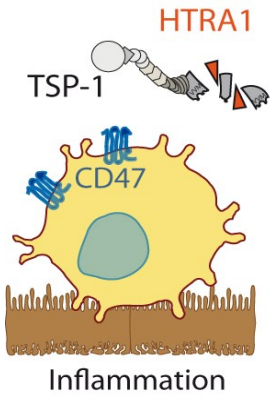


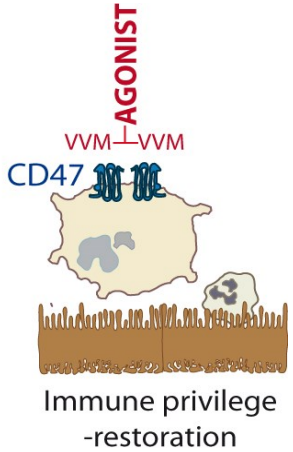


Light-challenged Thbs1$^{-/-}$





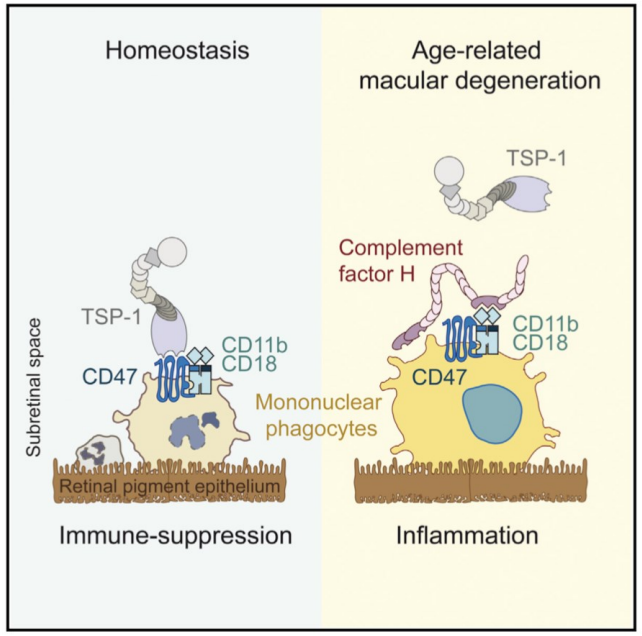




AMD pathogenesis: why certain people ?

CFH H402Y

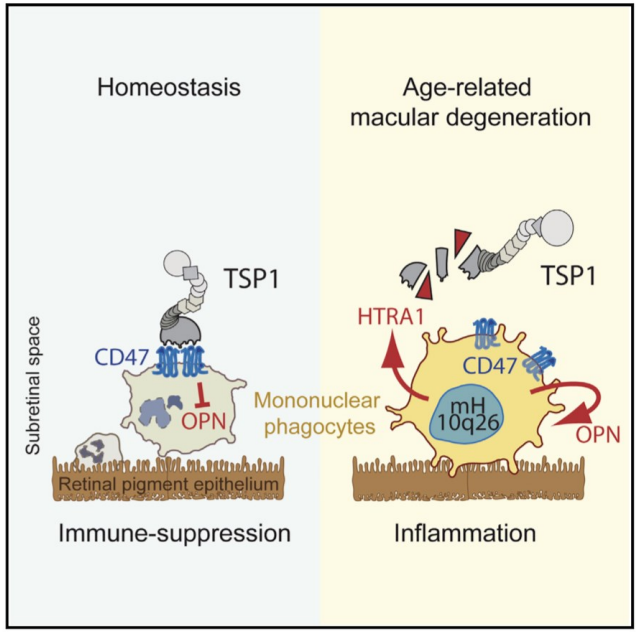
Graphical Abstract



Calippe et al. **Immunity** 2017

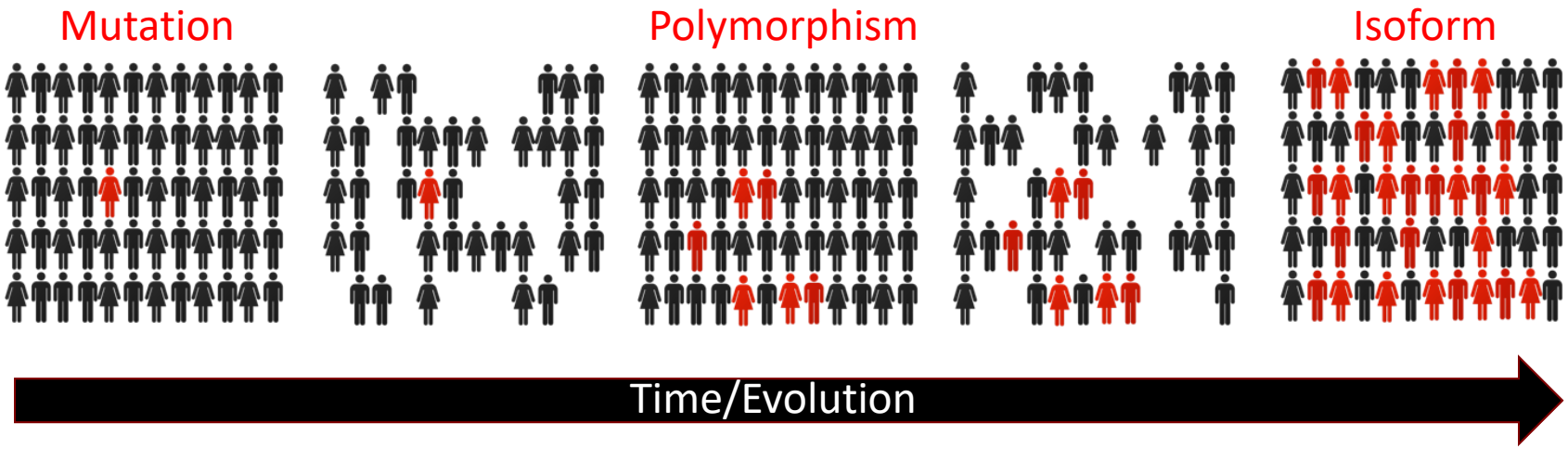
10q26 haplotype

Graphical Abstract



Beguier et al. **Immunity** 2020

Why would the common AMD-polymorphisms play a role in subretinal inflammation?



Infectious diseases

plague cholera tuberculosis syphilis

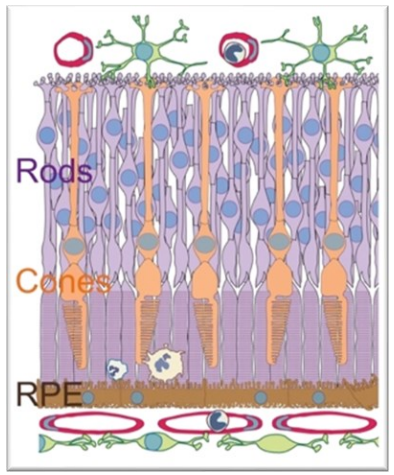
The common AMD-associated polymorphisms might have appeared because they lead to a stronger inflammatory response and better defense against infectious disease.

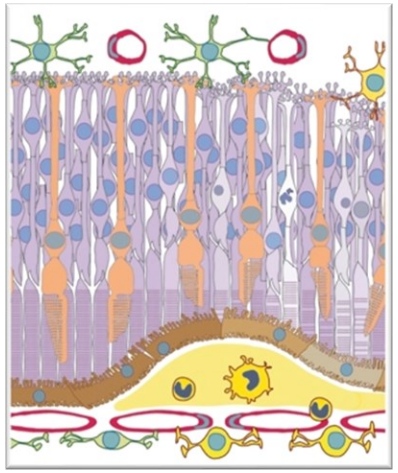
AMD pathogenesis

AMD

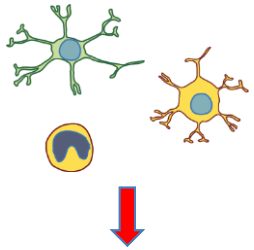
healthy | Drusen | intermediate | GA | wetAMD

excessive macrophage infiltration

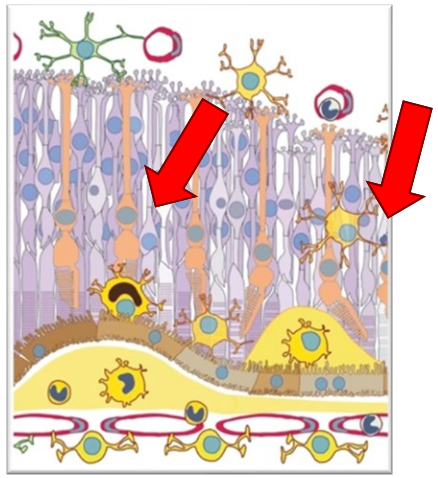


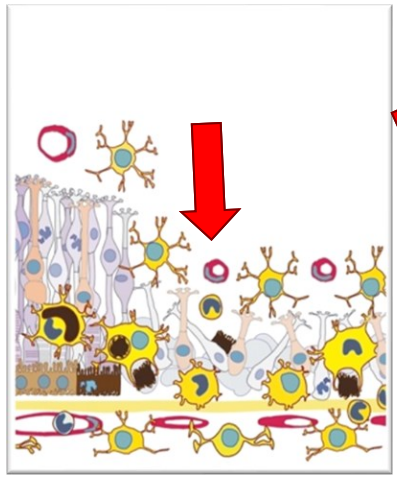


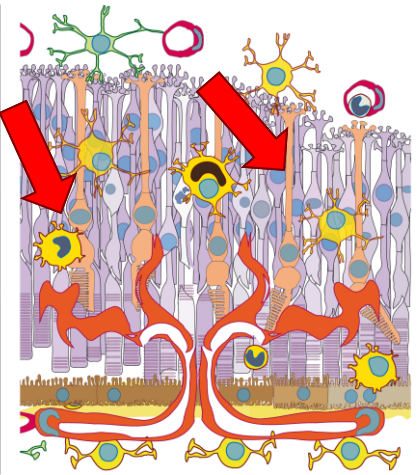
Genetic /environmental risk factors



stronger inflammation







- Homeostasis
- Immune suppression

- Sub-RPE parainflammation (controls Drusen growth)?

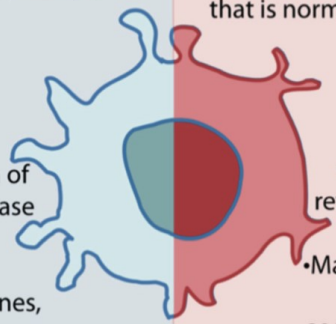
- Hyperreflective Foci
- Reticular drusen
- beginning photoreceptor degeneration

- Photoreceptor degeneration
- RPE degeneration
- neovascularisation

- Photoreceptor degeneration
- neovascularisation

Adaptive responses

- Help maintain retinal physiology, including in perturbed states that are normally subthreshold stressors, such as advanced aging
- Restrict the progression of retinal degenerative disease
- Involve homeostatic microglial checkpoint genes, such as Cx3cr1



Maladaptive responses

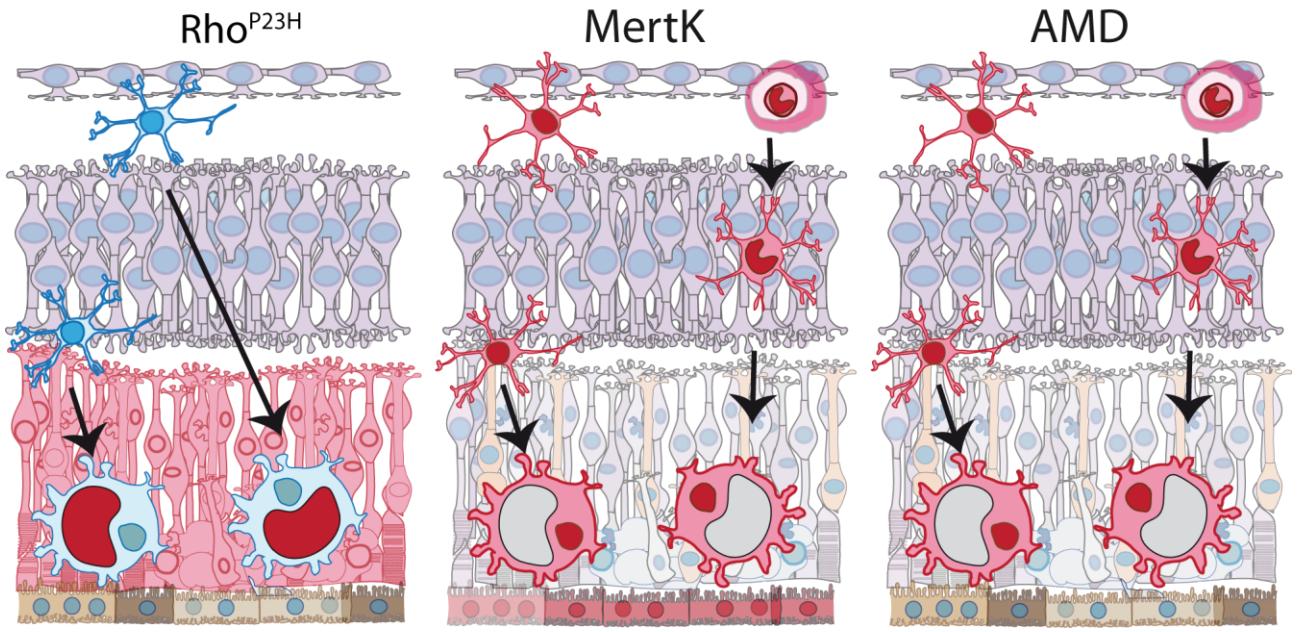
- Can drive a perturbed state that is normally subthreshold, such as advanced aging, into a chronic progressive diseased state
- Accelerate progression of retinal degenerative disease
- May involve genetic variants such as CFH and APOE, as well as lifestyle risk factors (smoking, high-fat diet)

Guillonneau et al. ***PRER*** 2017
Yu et al. ***Trends Neurosci.*** 2020

Mononuclear phagocytes in retinal degeneration

Photoreceptor gene mutations

MP gene variants



Adaptive immune response

1. primary photoreceptor degeneration
2. microglia help contain toxic debris

Maladaptive immune response

1. genetic variants -> hyperinflammatory MPs
2. CCR2+Mo recruitment
3. inflammation-induced degeneration / neovascularization

Inflammation related drug targets for late AMD

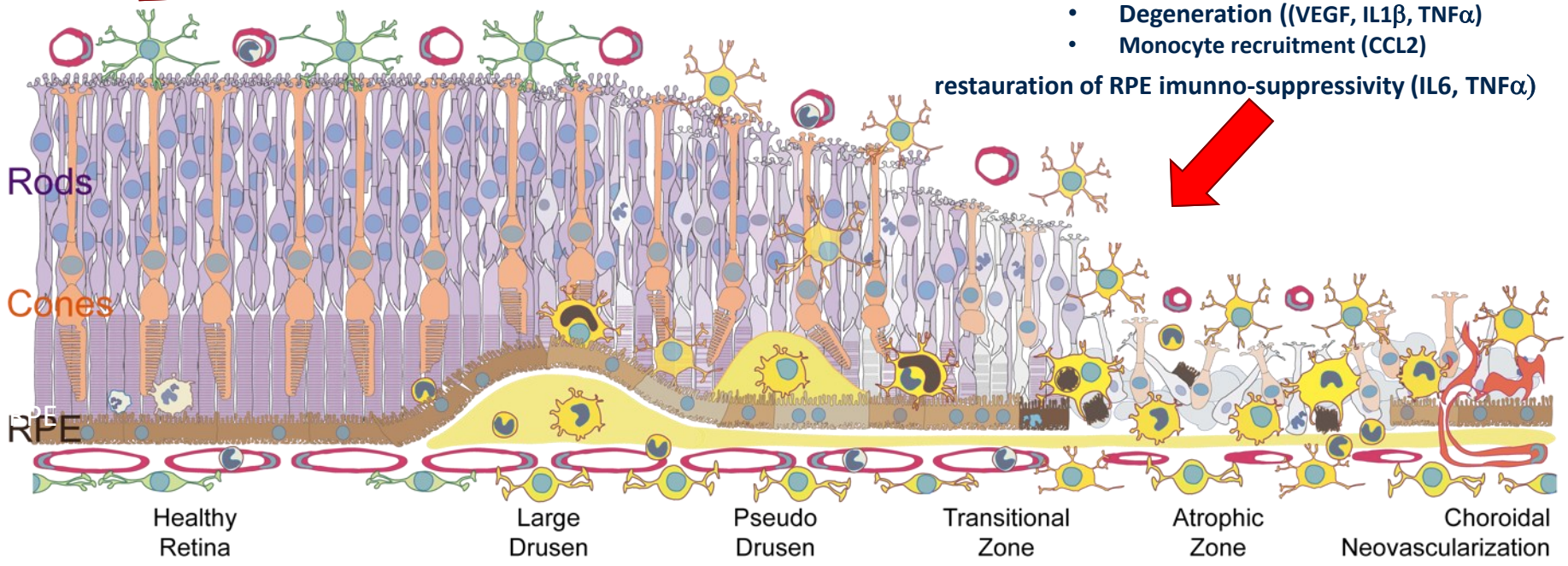
Boost tonic inhibitory signals

IIRC / cytokine inhibition:

inhibition of

- **neovascularization (VEGF, IL1β, IL6, TNFα)**
- **Degeneration ((VEGF, IL1β, TNFα)**
- **Monocyte recruitment (CCL2)**

restauration of RPE imunno-suppressivity (IL6, TNFα)



Reduce the macrophage infiltrate:

- **decreases Mo recruitement**
- **shorten Mphi half life**
- **Boost immuno-supressivity**

Contents lists available at ScienceDirect

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On phagocytes and macular degeneration

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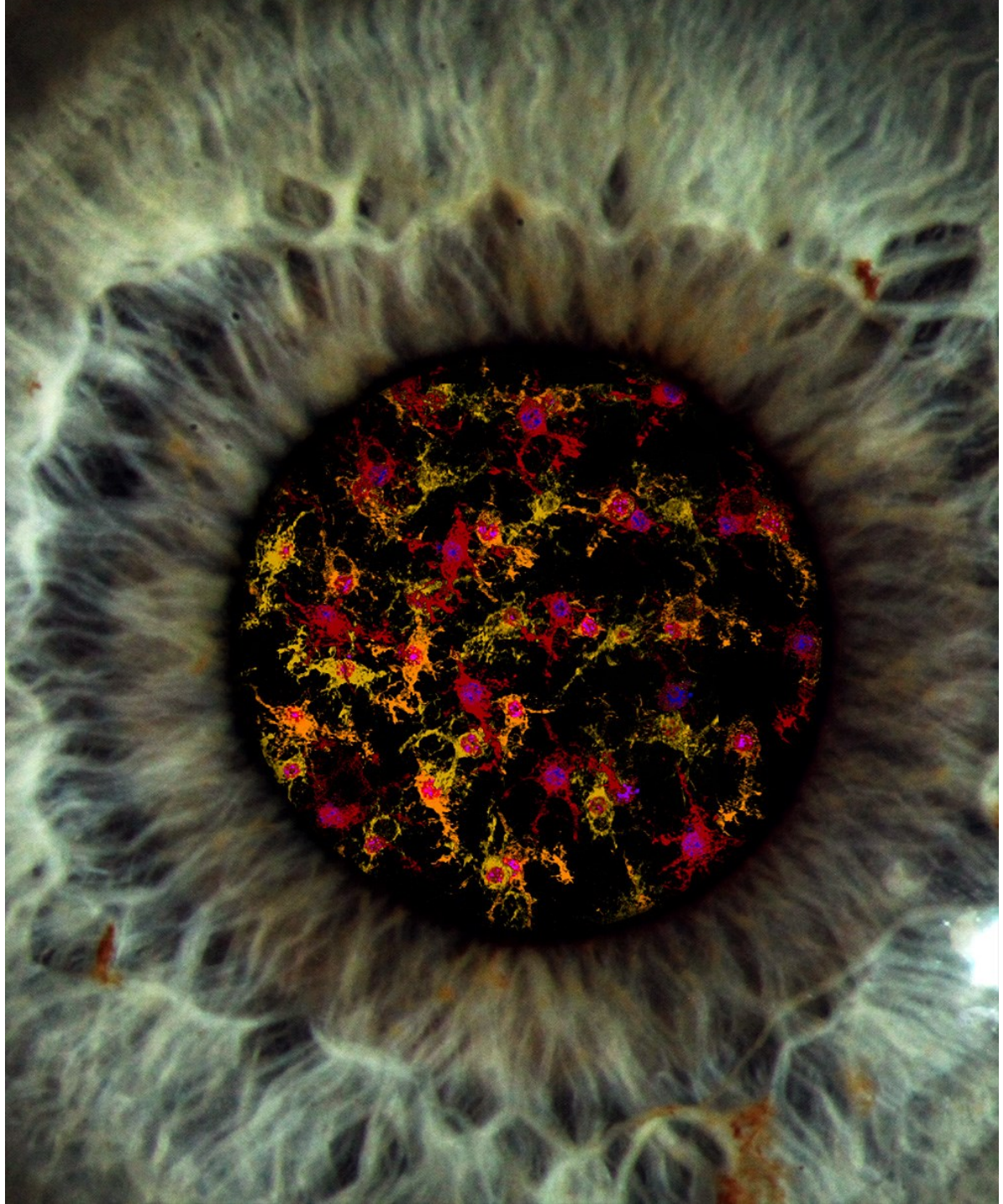

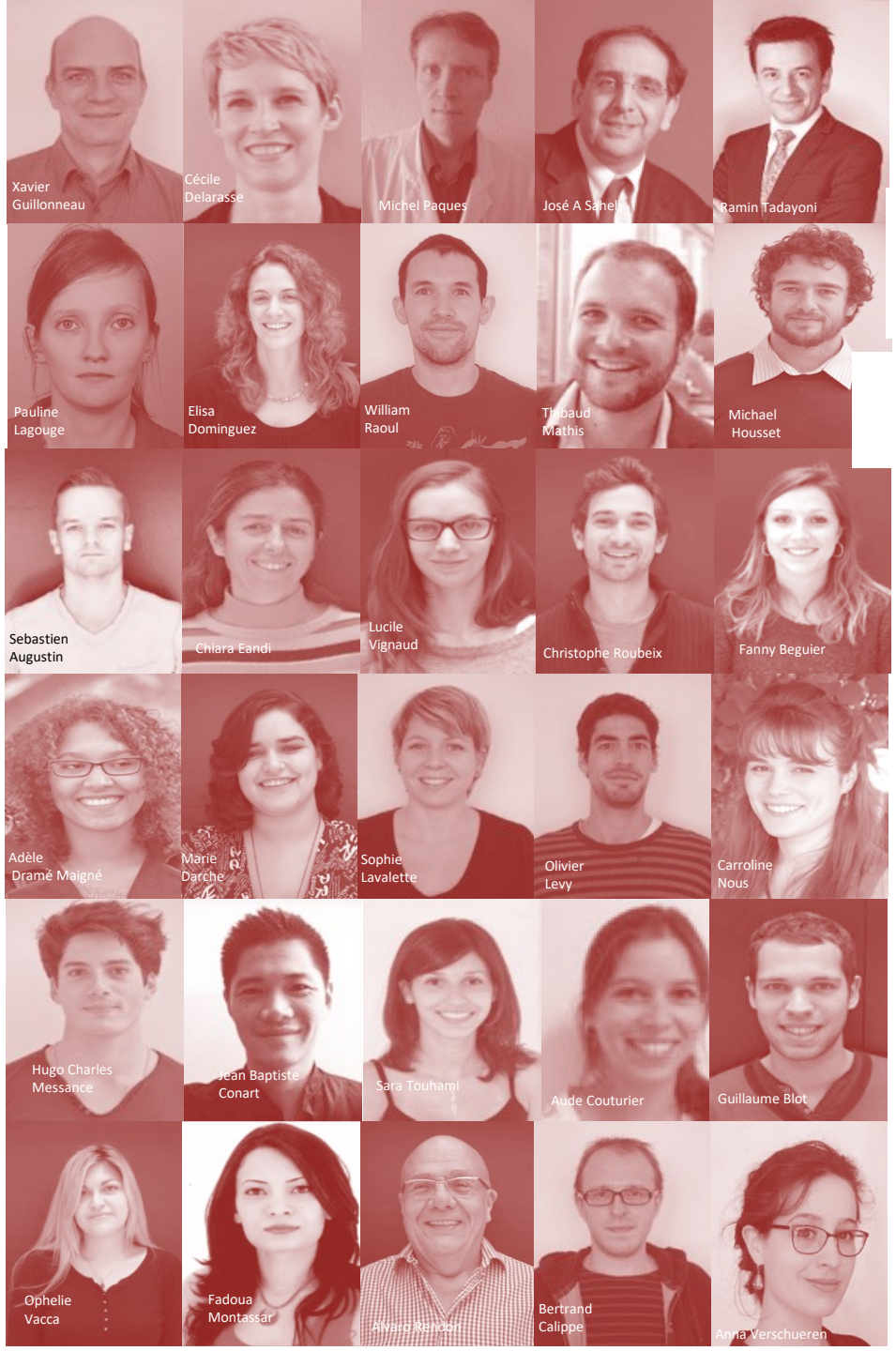
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Ramin Tadayoni
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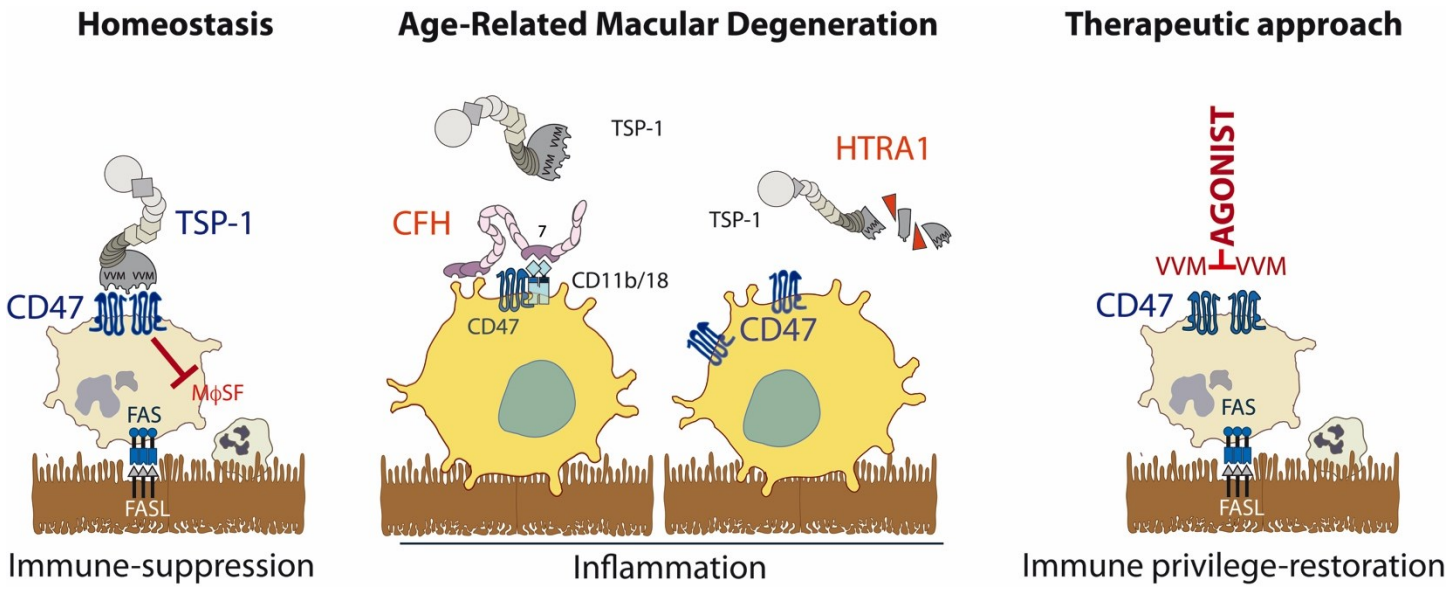
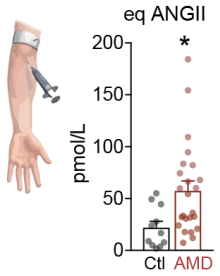
Fadoua Montassar
Alvaro Rendon
Bertrand Calippe
Anna Verschueren

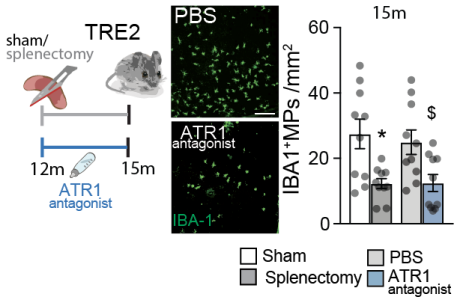
Inflammation related drug targets for late AMD

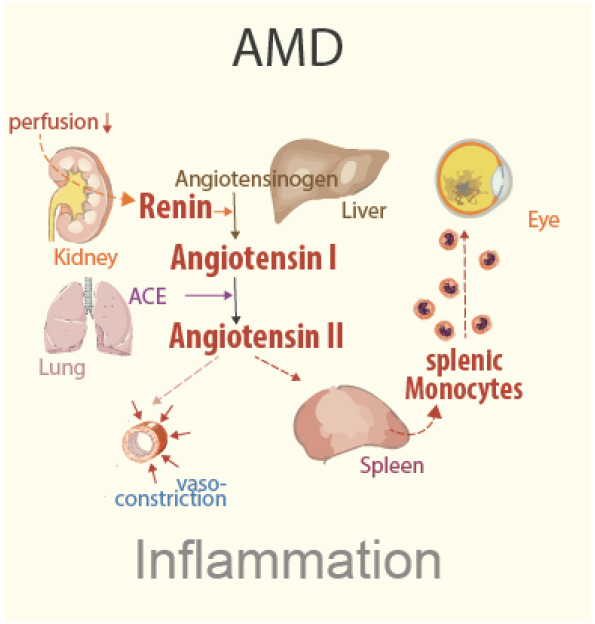


AMD: Splenic monocytes / inflammatory reflex

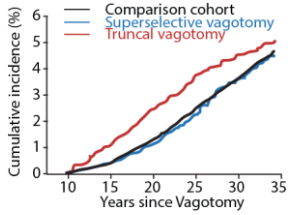
Splenic monocytes in AMD

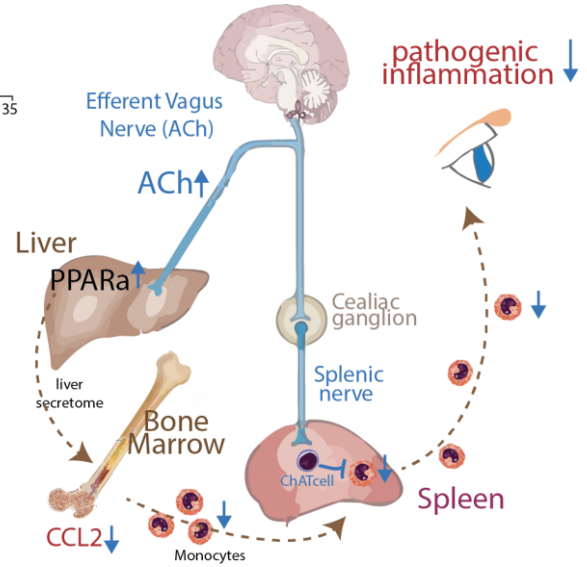


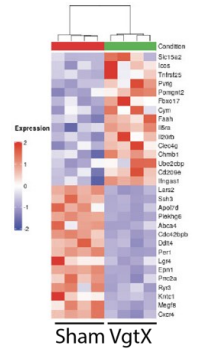


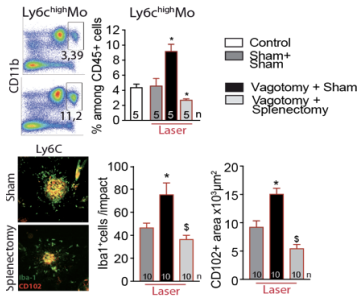


Inflammatory reflex





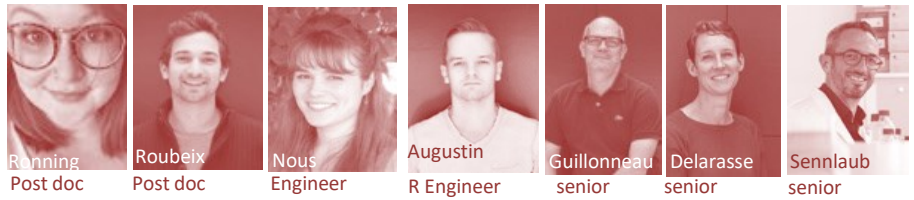




Grants:

Unadev 2018

Einstein Foundation 2014



Grant: ANR 2022

Kevin+