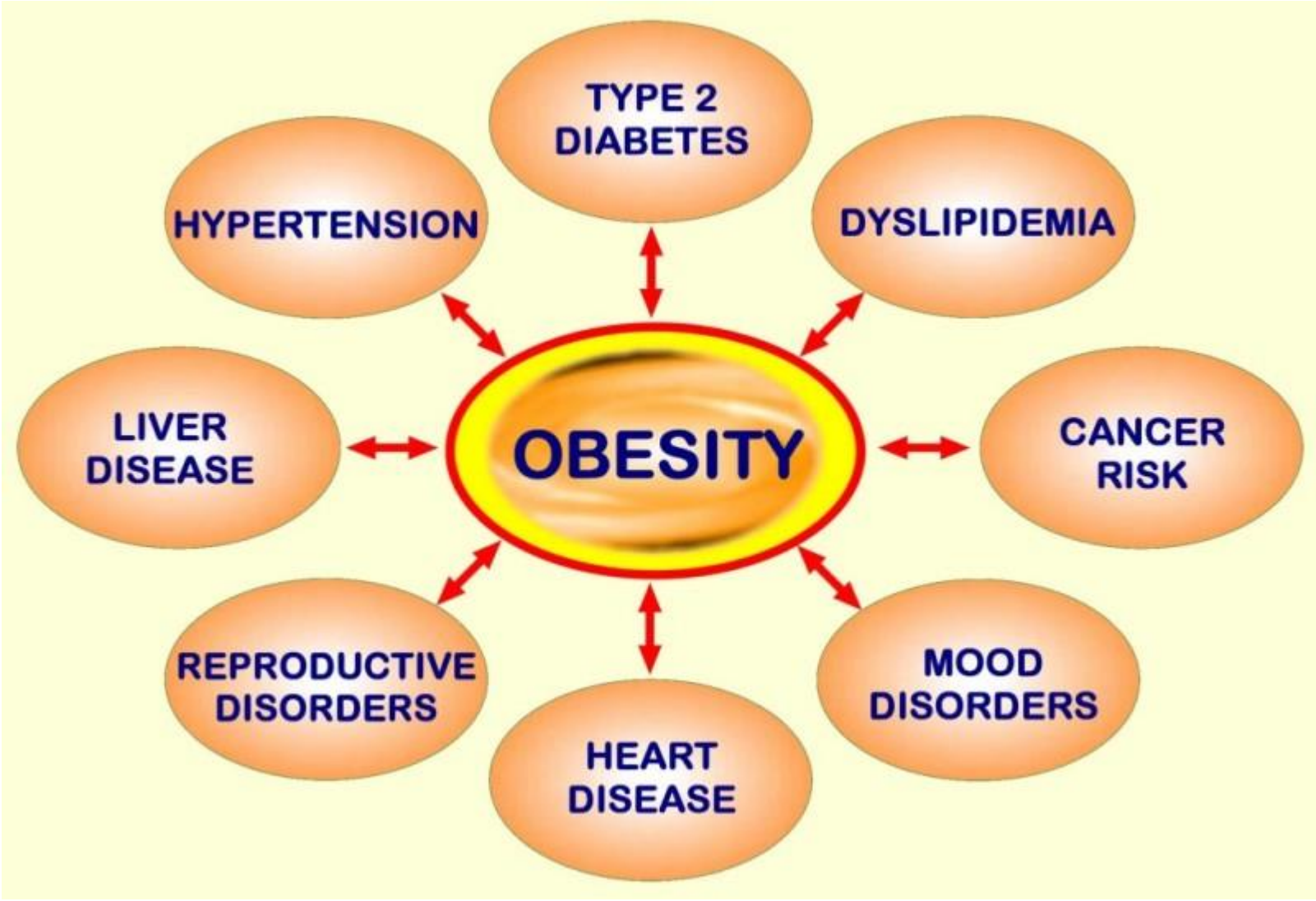
HELLENIC REPUBLIC
National and Kapodistrian
University of Athens

Metabolic dysregulation
Overview of molecular mechanisms

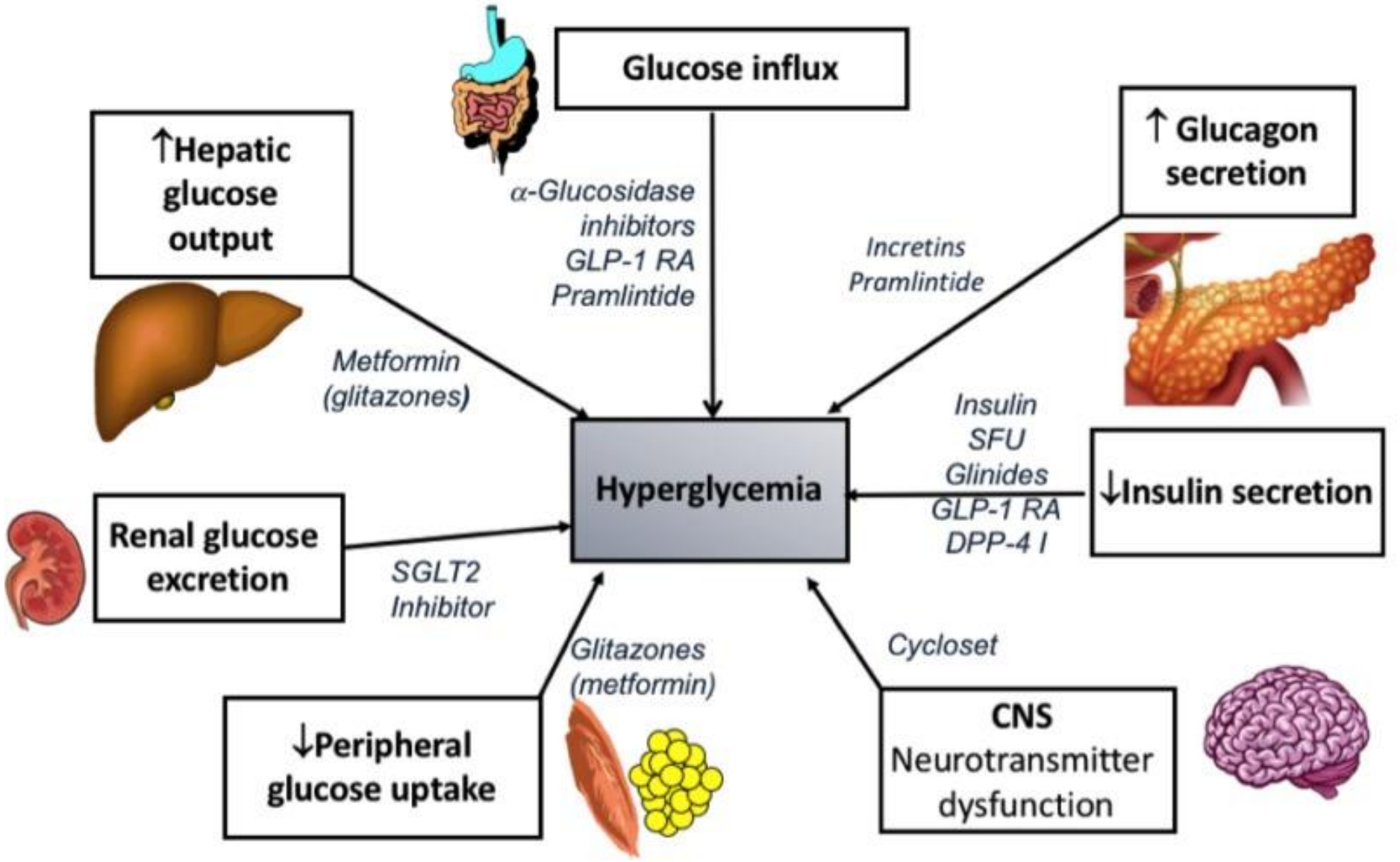
Antonios Chatzigeorgiou (BSc, MD, PhD)
Assistant Professor
Department of Experimental Physiology - Medical School
National and Kapodistrian University of Athens, Greece



Kyrou et. al. Endotext, Bookshelf NCBI, updated Jan 2018

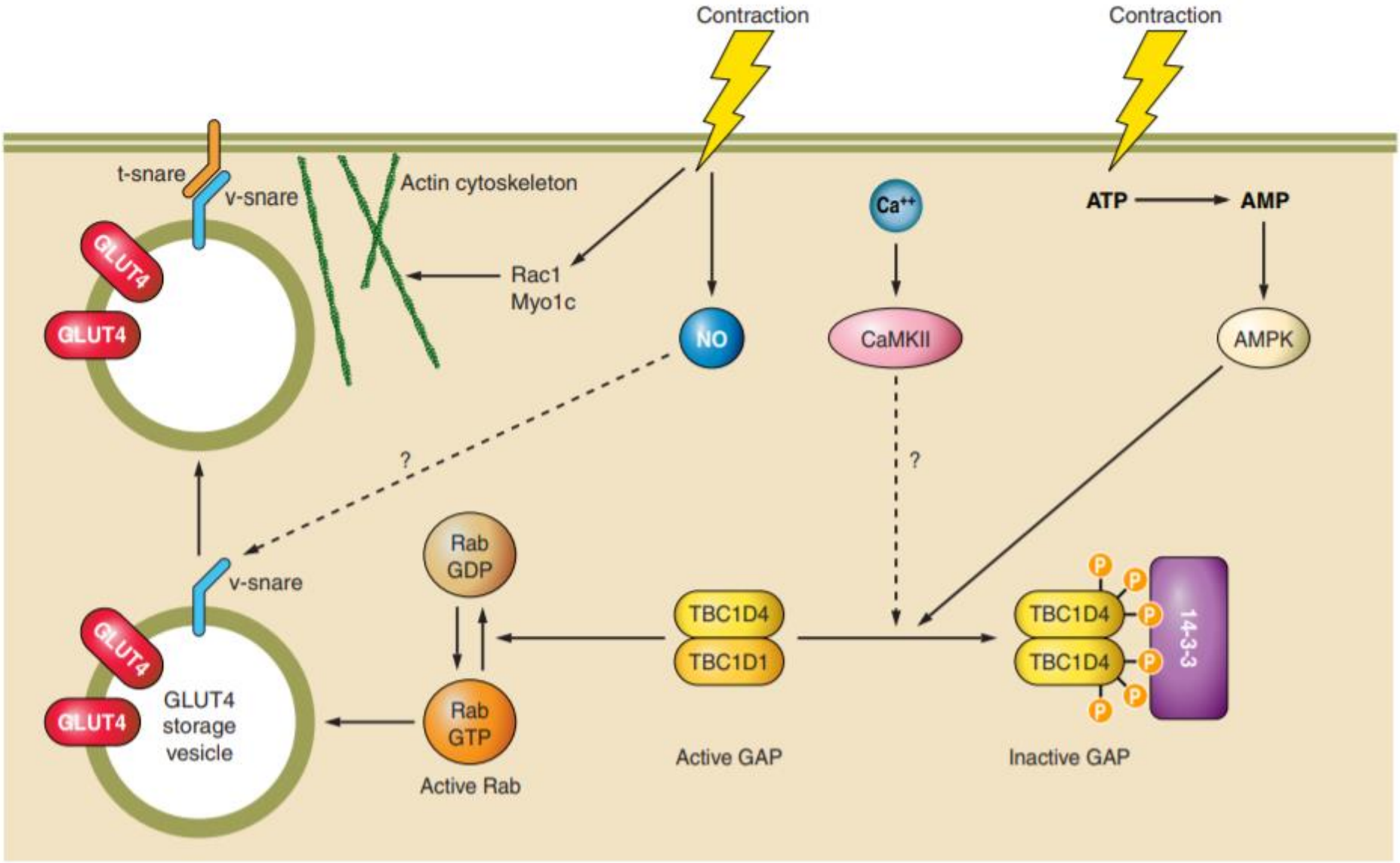
Kyrou et. al. Endotext, Bookshelf NCBI, updated Jan 2018

Current therapies

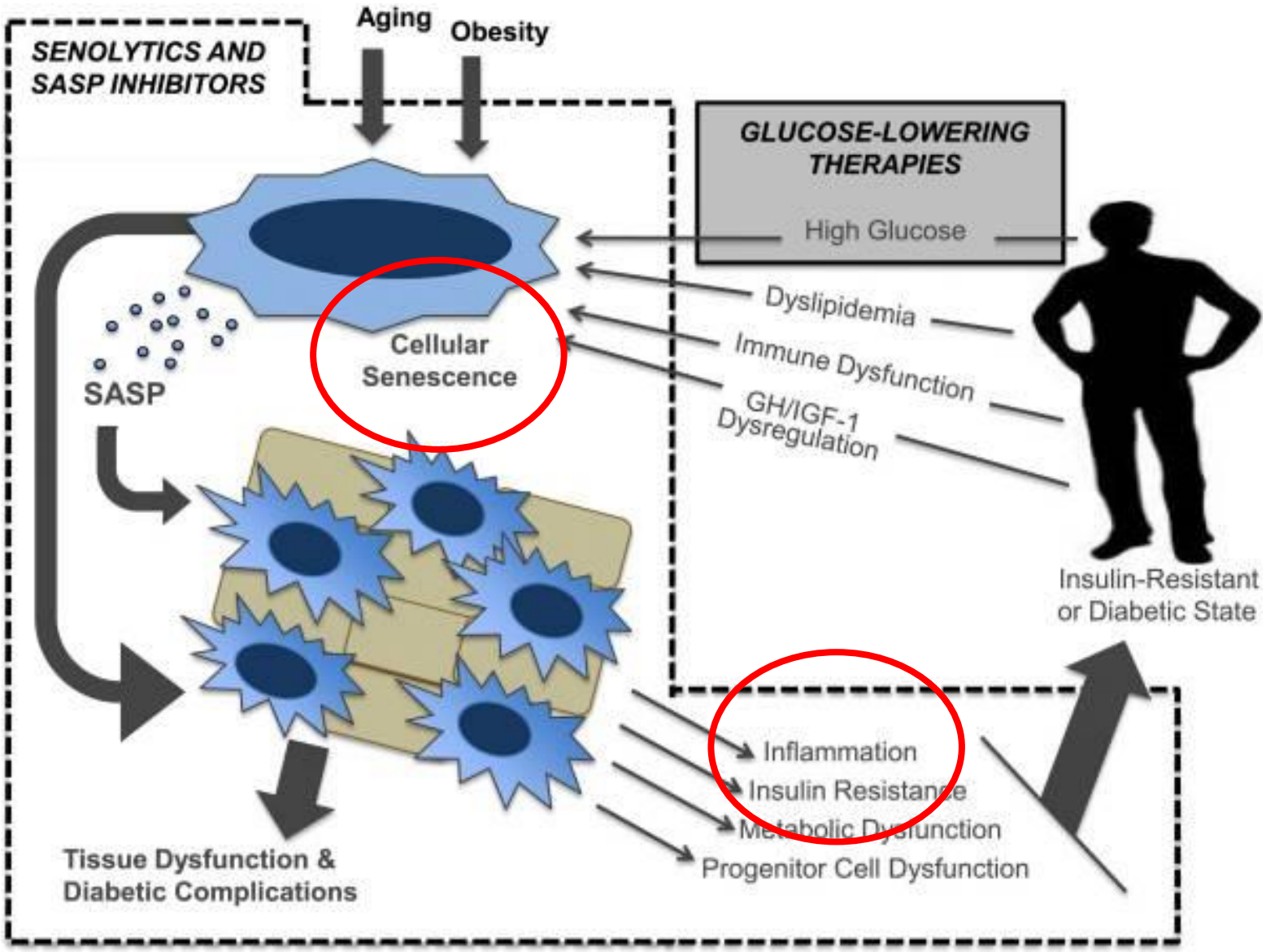


Santos Cavaiola et. al. Endotext, Bookshelf NCBI, updated 2018

Exercise and GLUT4 translocation

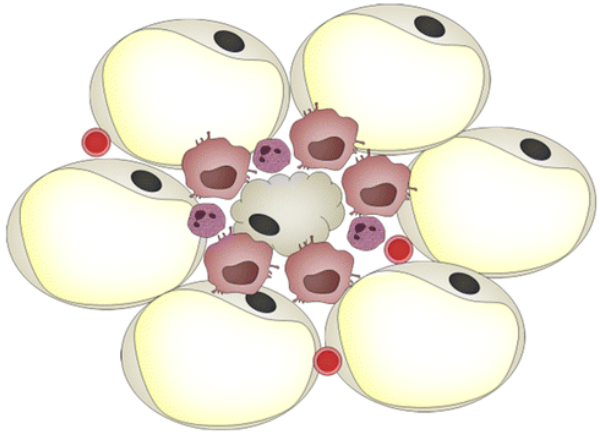


Vicious cycle of senescence and inflammation in metabolic dysregulation



Palmer et al. Diabetes 2015

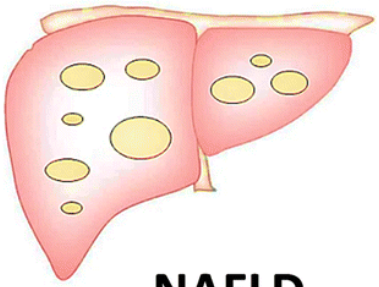
Obese adipose tissue (AT)



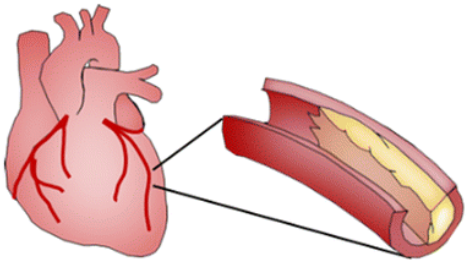
IL-6 TNF IL-1β
Leptin PAI-1

Systemic inflammation

dyslipidemia



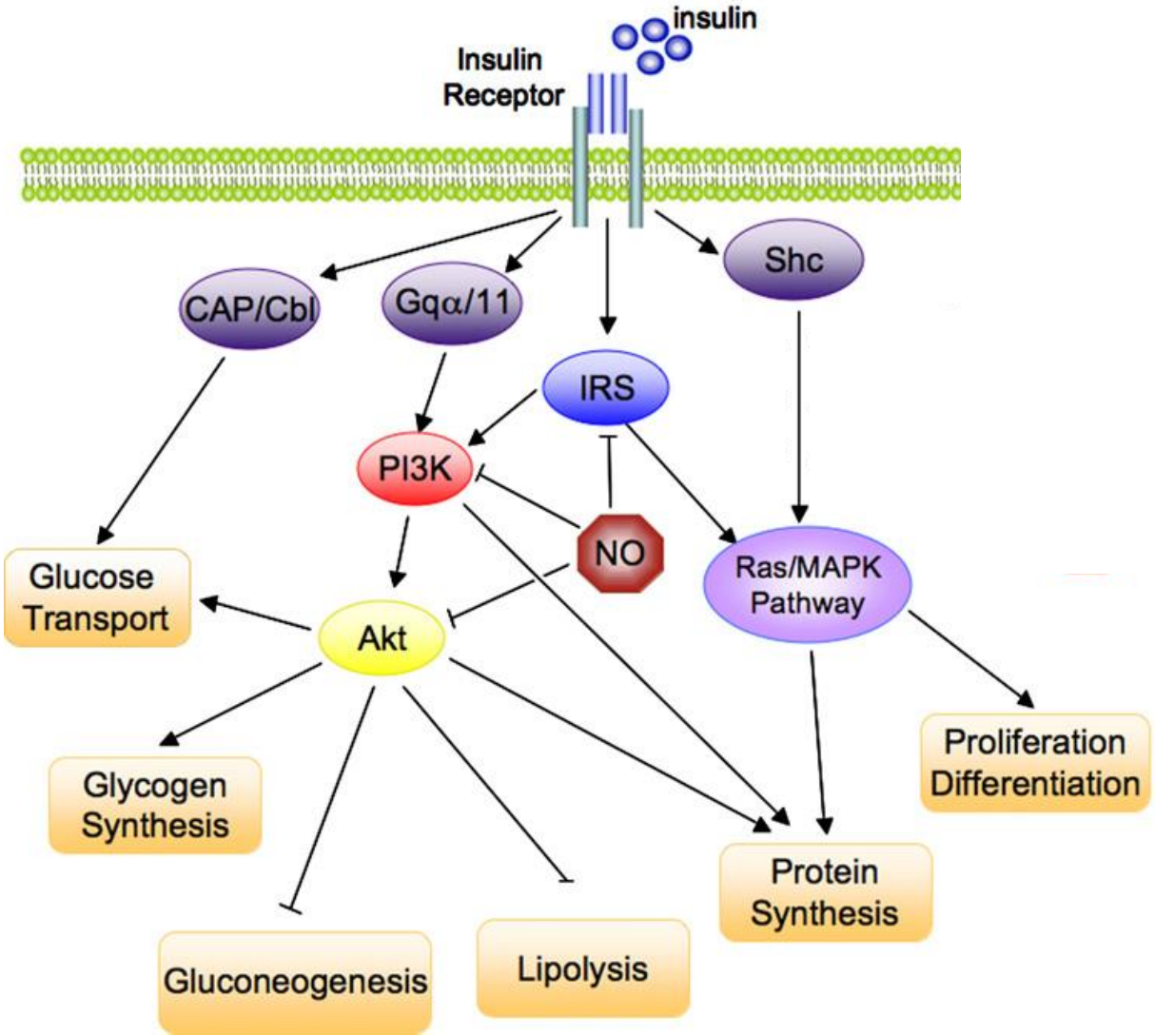
NAFLD



Cardiovascular disease

Adapted from Nati et. al. Rev. End. Metab.Dis. 2018

Molecular pathways at the interface between obesity and inflammation



Olefsky et. al, FEBS Letters, 2008

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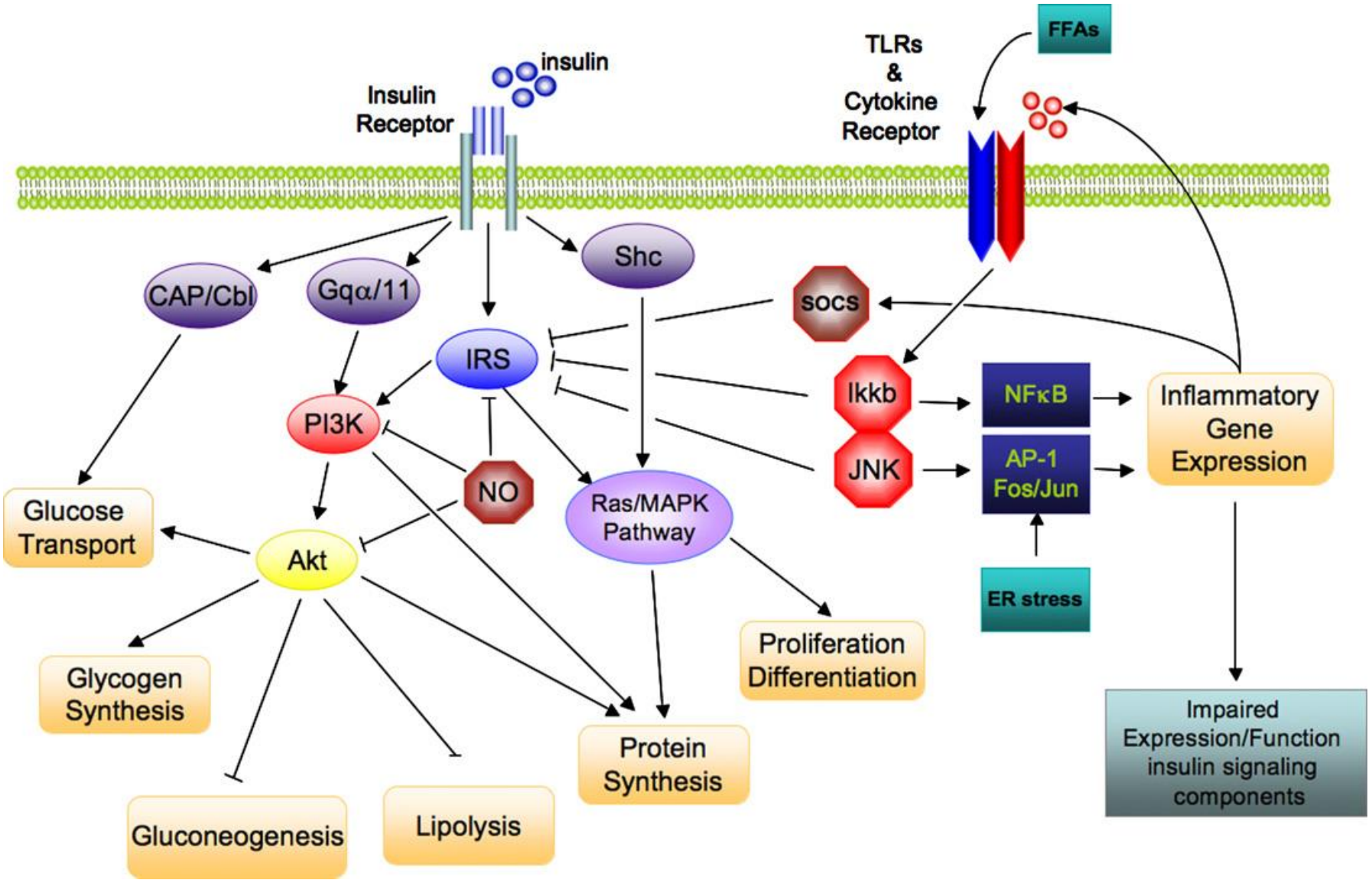


Olefsky et. al, FEBS Letters, 2008

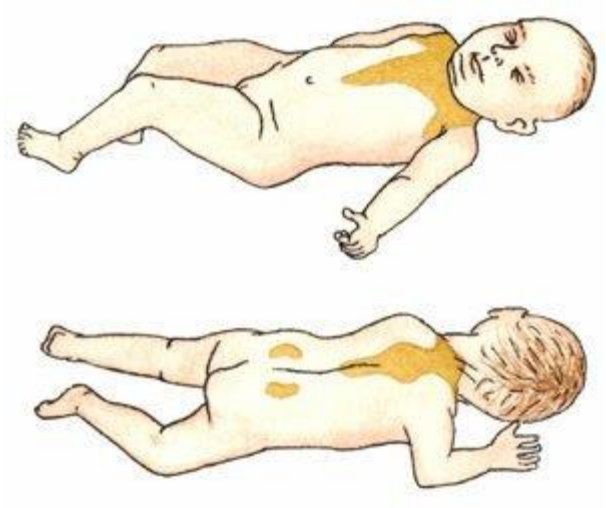
New concepts in metabolic homeostasis
Two Types of Adipose Tissue

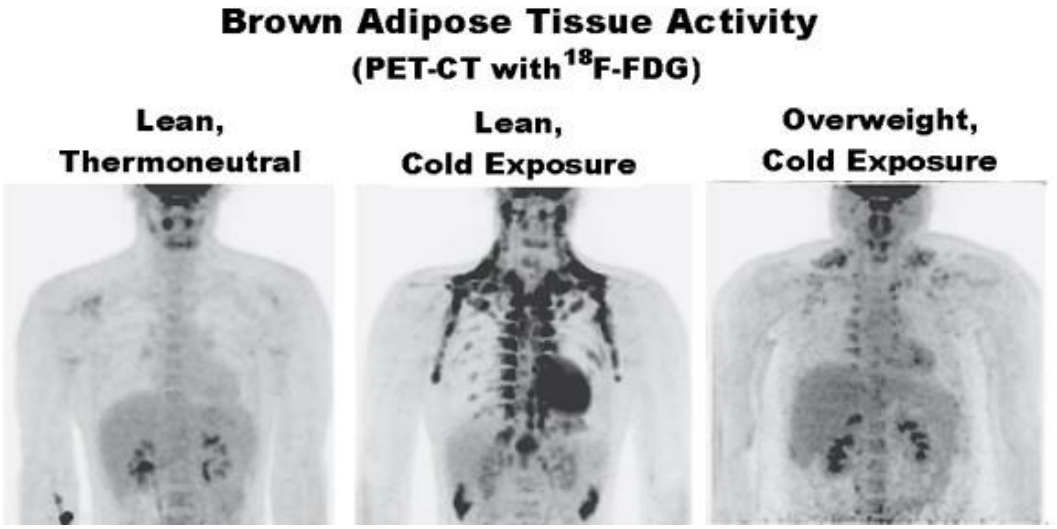
a. White Adipose Tissue (WAT) ⟶ Energy storage and thermal isolation

b. Brown Adipose Tissue (BAT) ⟶ Non-shivering thermogenesis

Mainly present in infants.

Also present in adults, especially after exposure to cold.

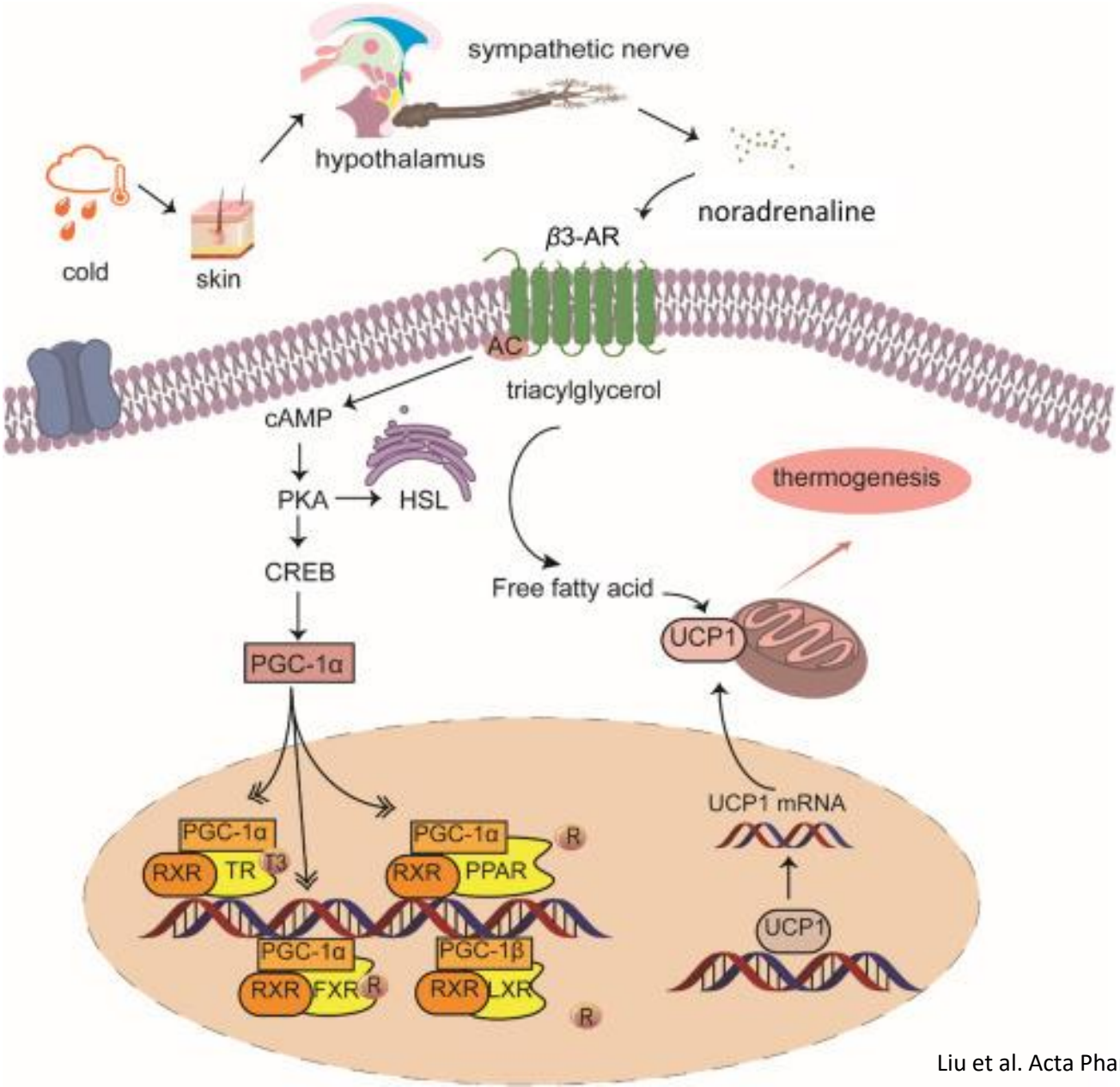




Van Marken Lichtenbelt et al., NEJM 2009

Cypess N Engl J Med. 2009
van der Lans et al., J Clin Invest. 2013
Lidell et al. Nat Med. 2013

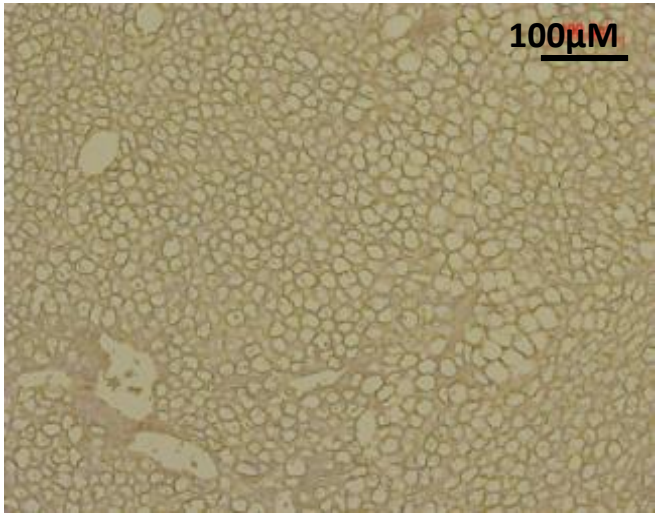
UCP1 (Uncoupling Protein 1) expression in BAT
Cold exposure is a major stimulus for UCP1 expression



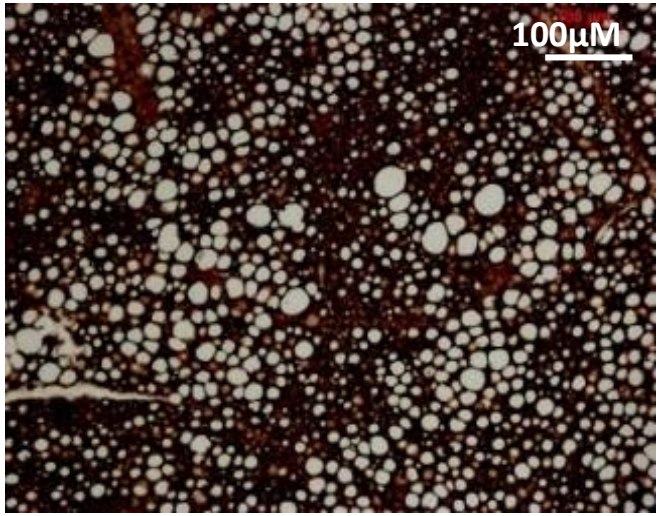
Liu et al. Acta Pharmaceutica Sinica 2020

UCP1 expression in BAT

25°C

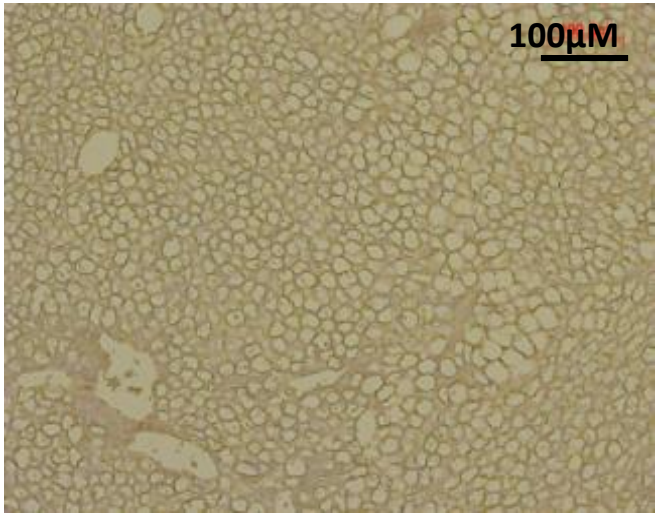


12hrs 4°C

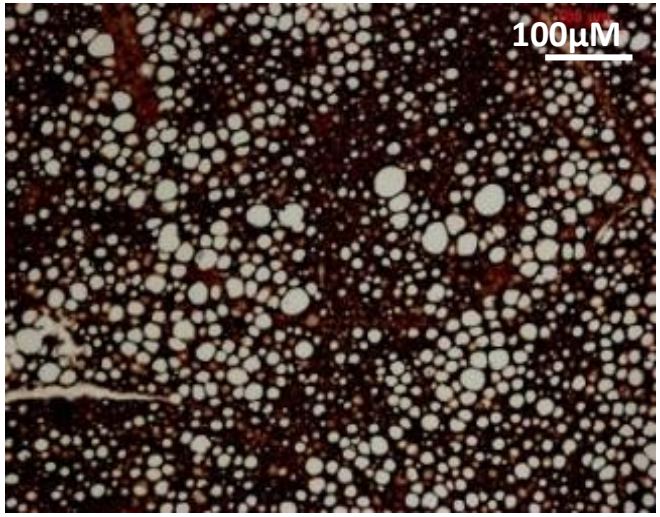


UCP1 expression in BAT

25°C



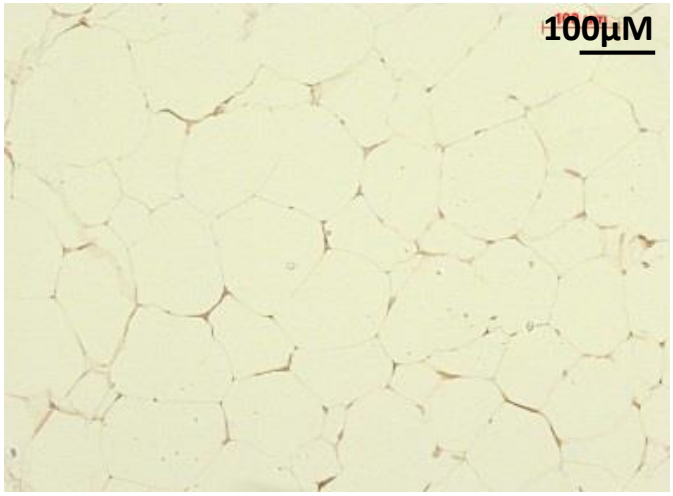
12hrs 4°C



Brown in White (Brite) or Beige AT: WAT that expresses UCP1

Wu et al., Genes Dev. 2013
Harms, Seale. Nat Med. 2013

25°C



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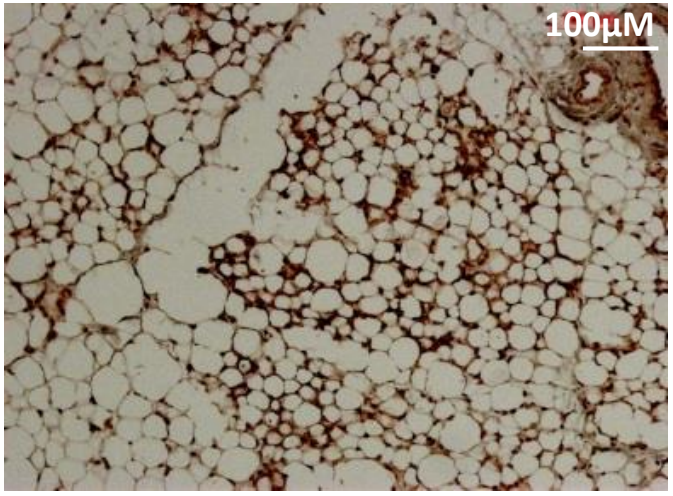
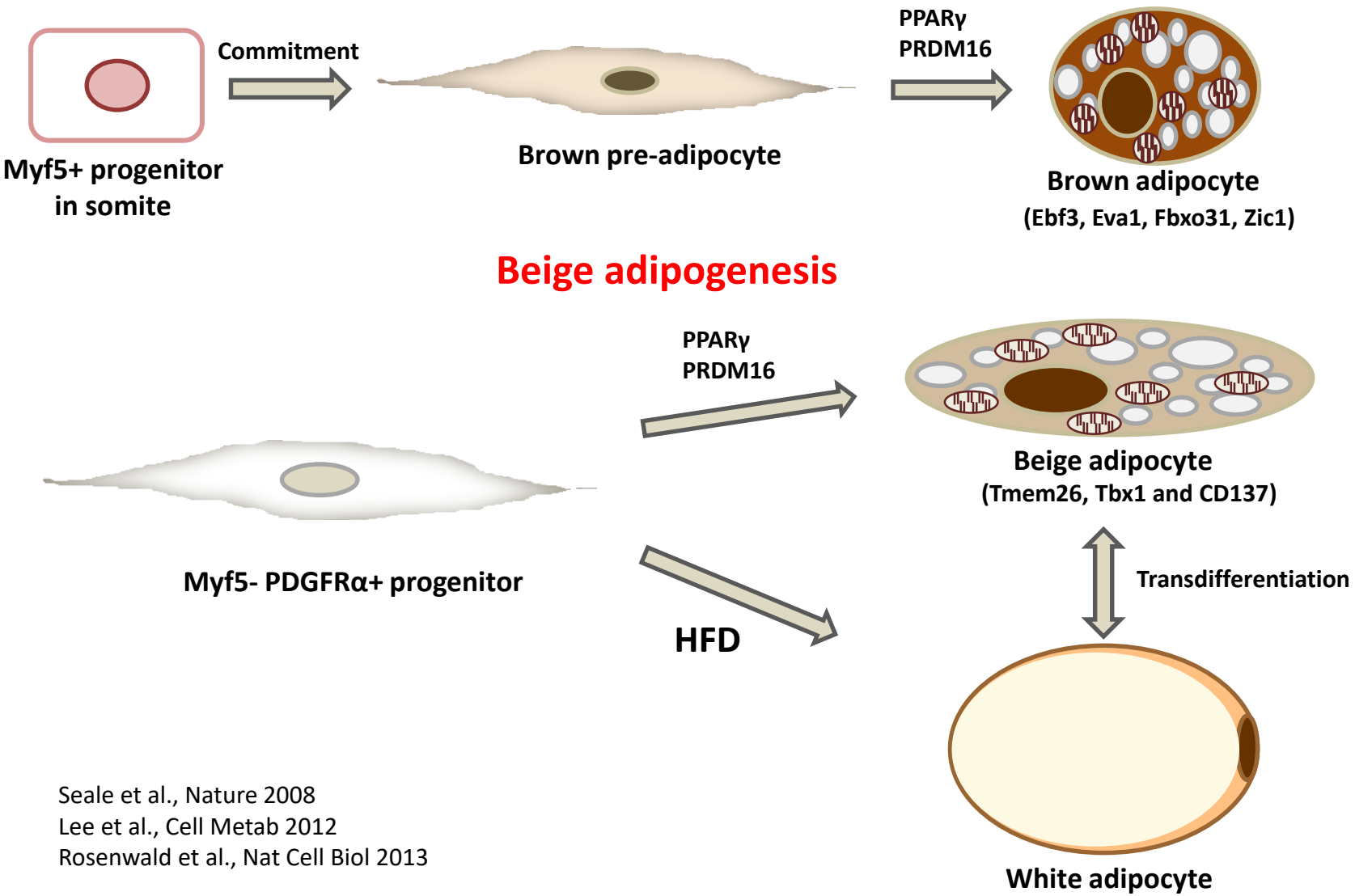


Brown and beige adipocytes differ in their origin



Seale et al., Nature 2008
Lee et al., Cell Metab 2012
Rosenwald et al., Nat Cell Biol 2013

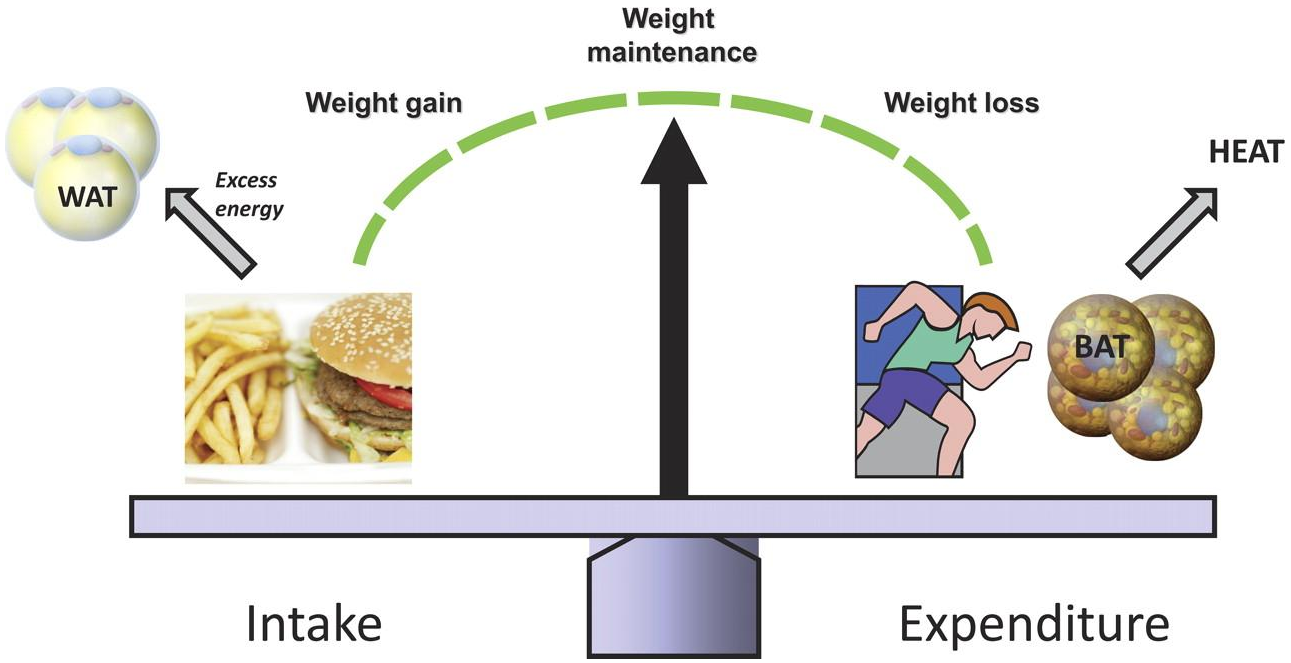


Table 1 Thermogenic regulators in clinical trials.

Name	Identifier	Condition	Phase
Propranolol	NCT03379181	Hyperthyroidism	4
	NCT01791114	Insulin sensitivity, obesity	–
Prednisone	NCT03269747	BAT activity	4
Fluvastatin	NCT03189511	Brown fat activity, insulin resistance	4
RZL-012	NCT03171415	Obesity	2
Caffeine, ephedrine	NCT02048215	Obesity	3
β3-AR agonist	NCT01783470	Obesity	2
Caffeine	NCT00781586	Energy expenditure	4
Zantrex-3	NCT02937298	Diet-induced thermogenesis, obesity	–
Metobes-compound	NCT00302276	Obesity	2 and 3
Tyrosine, green tea, caffeine	NCT02937298	Diet-induced thermogenesis, obesity	1

–Not applicable.

Liu et al. Acta Pharmaceutica Sinica 2020

‚Personalized' thermogenesis

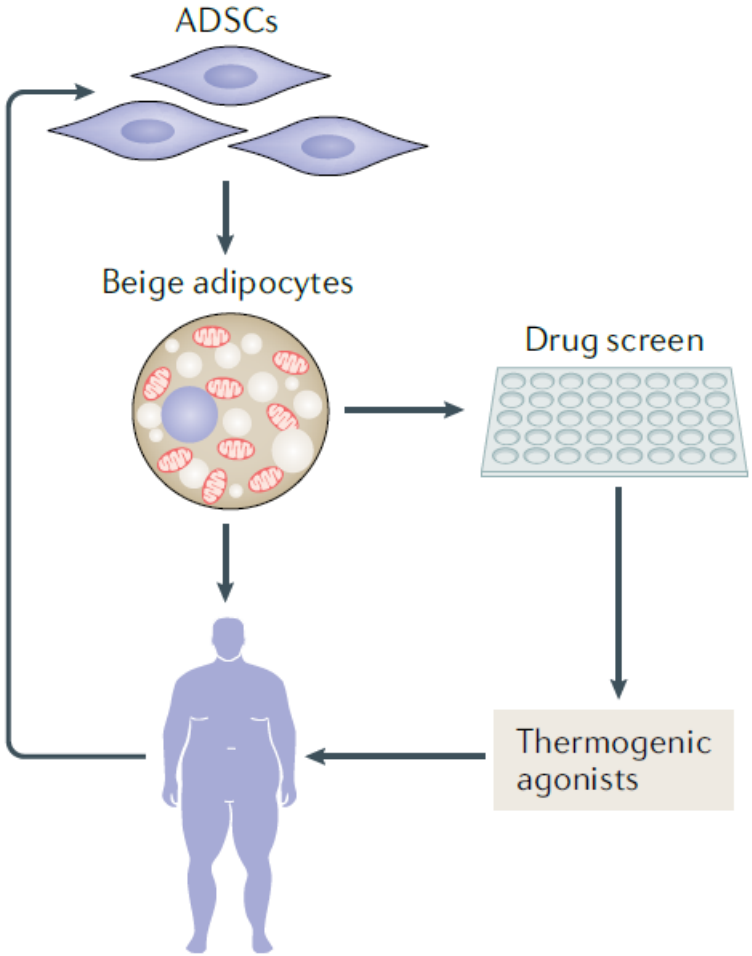
nature COMMUNICATIONS

ARTICLE

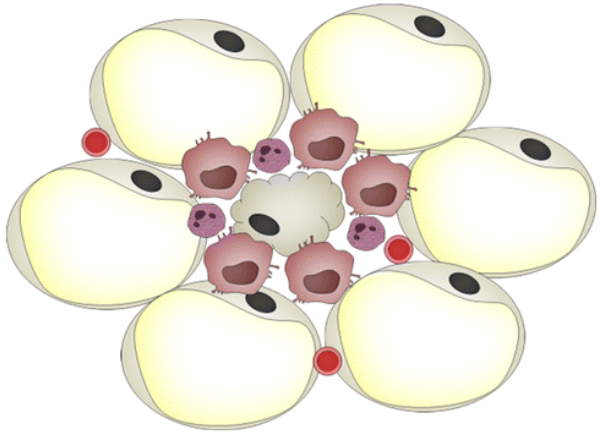
https://doi.org/10.1038/s41467-020-16340-3 OPEN

Human beige adipocytes for drug discovery and cell therapy in metabolic diseases

Amar M. Singh[1,5], Liang Zhang[1,5], John Avery[1,5], Amelia Yin[1], Yuhong Du[2], Hui Wang[3], Zibo Li[3], Haian Fu[2,4], Hang Yin[1] & Stephen Dalton[1✉]



Obese adipose tissue (AT)



IL-6 TNF IL-1β
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Systemic inflammation

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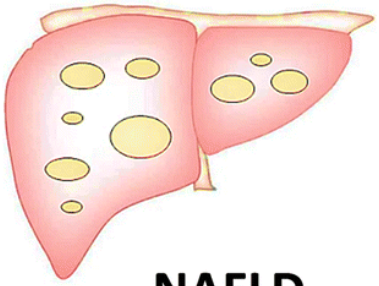
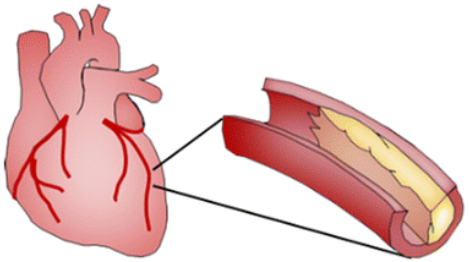


NAFLD



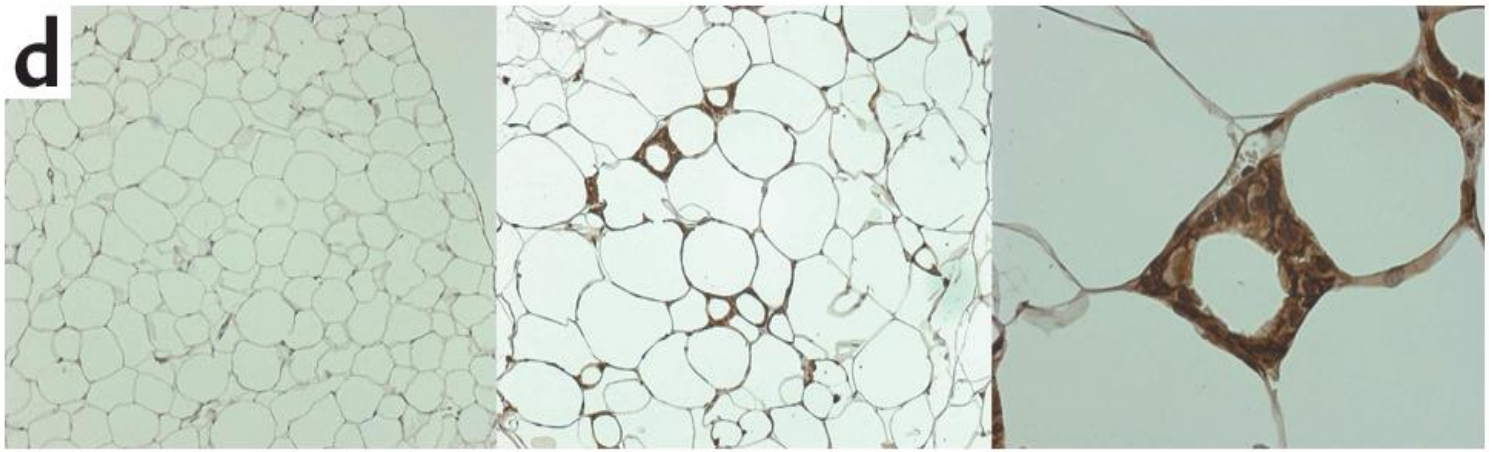
Cardiovascular disease

Adapted from Nati et. al. Rev. End. Metab.Dis. 2018

Insulin resistance - Inflammation

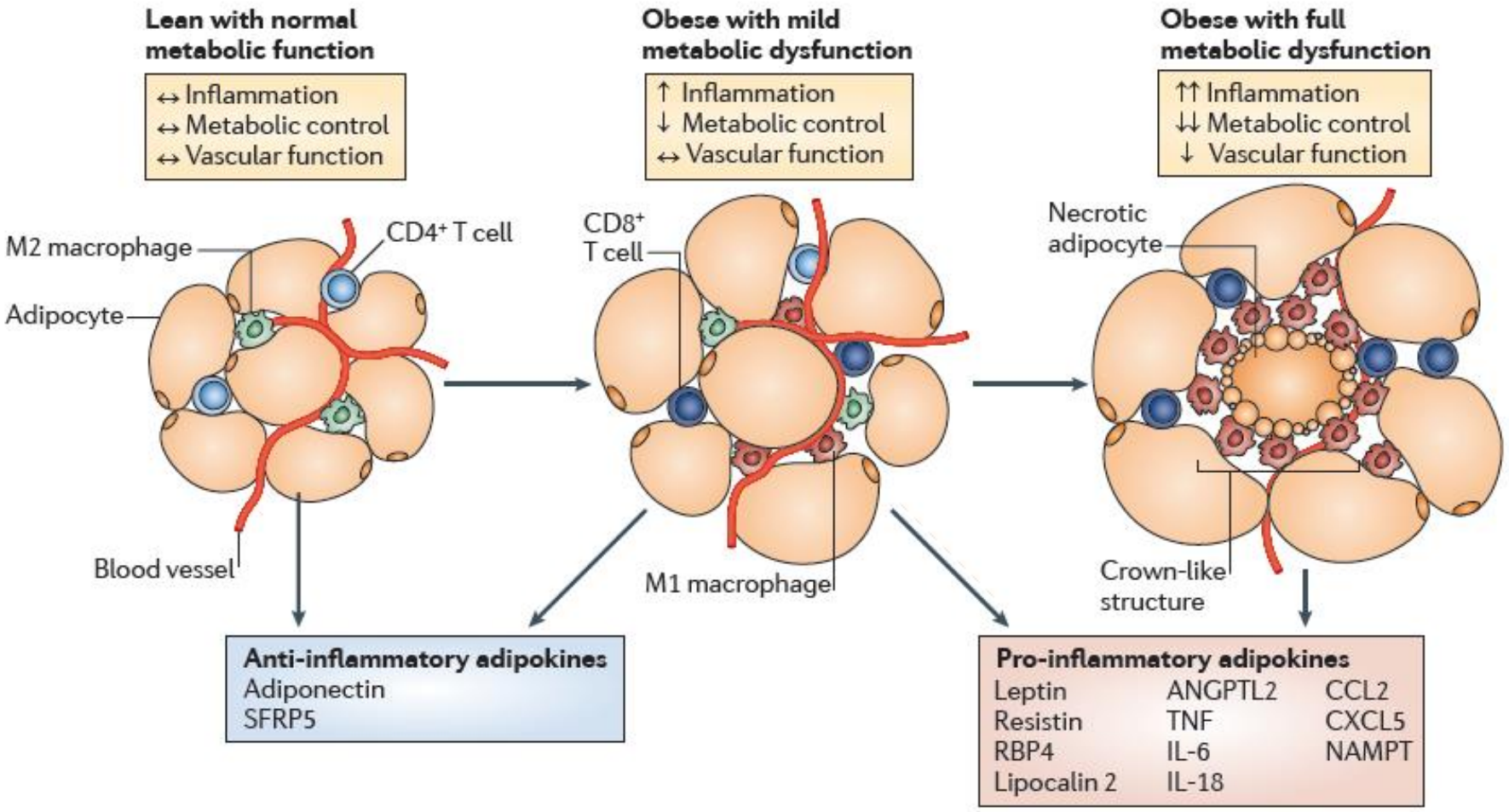
Macrophage infiltration and activation in adipose tissue are causally linked to obesity-induced insulin resistance.

F4/80 macrophages



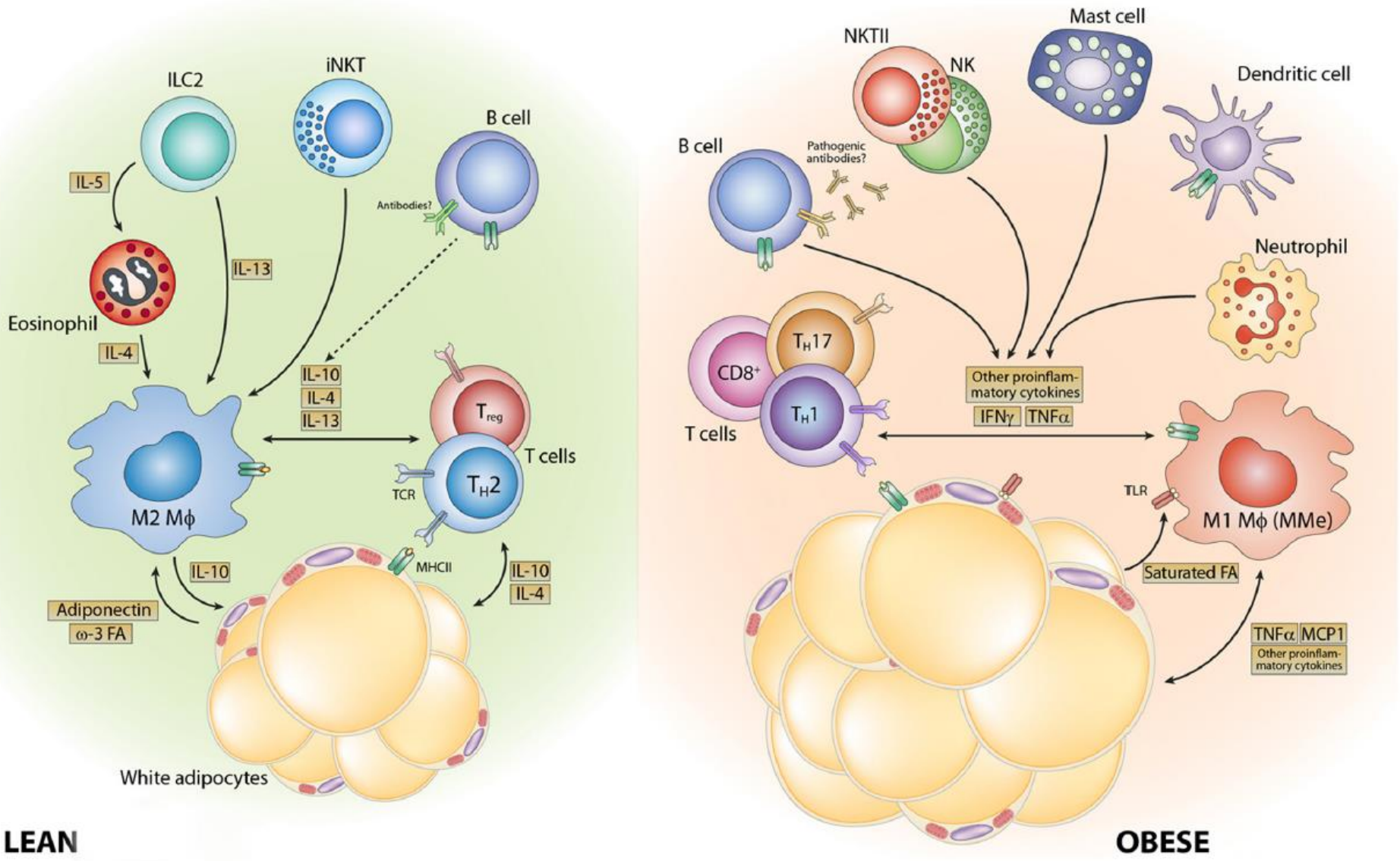
Xu et al., J. Clin. Invest. 112:1821–1830 (2003).
Weisberg et al., J. Clin. Invest. 112:1796–1808 (2003).

Phenotypic modulation of AT in obesity



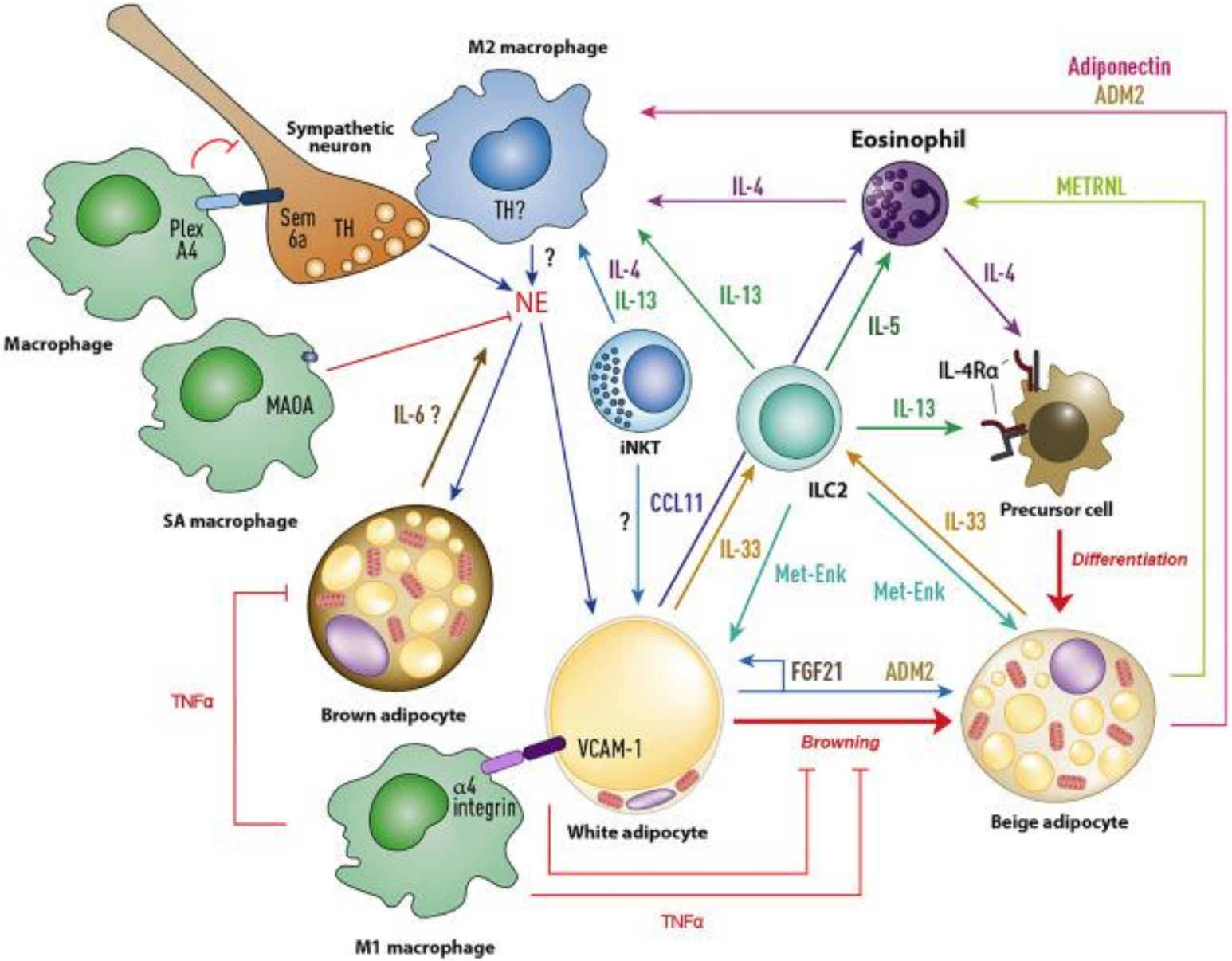
Ouchi et al Nat.Rev.Immunol. 2011

Interplay between type 1/type 2 immunity in maintaining adipose tissue homeostasis



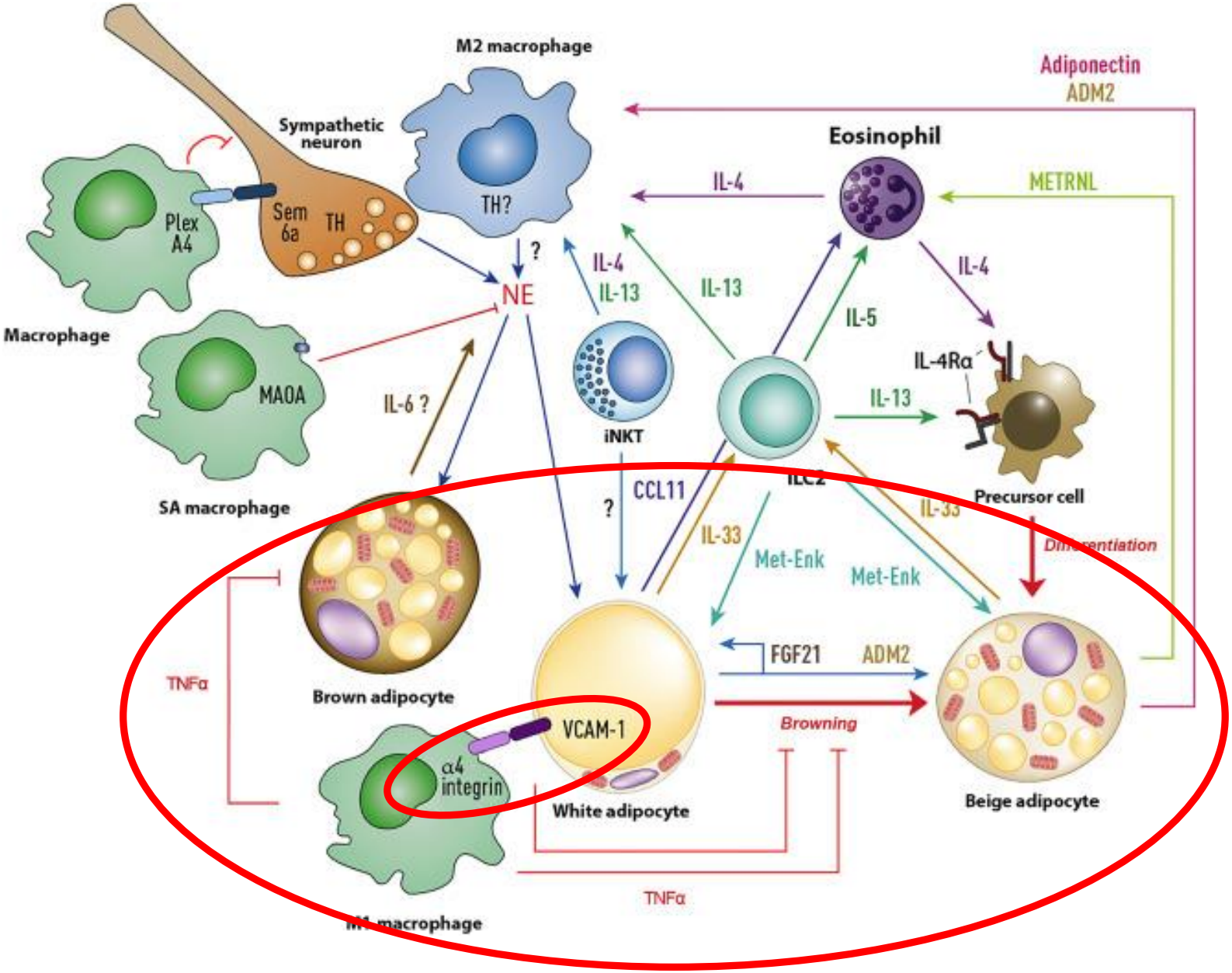
Villarroya et al J Internal Med. 2018

Cell-cell interactions in adipose tissue during obesity



*Villarroya et al Cell. Metab.*2018

Cell-cell interactions in adipose tissue during obesity



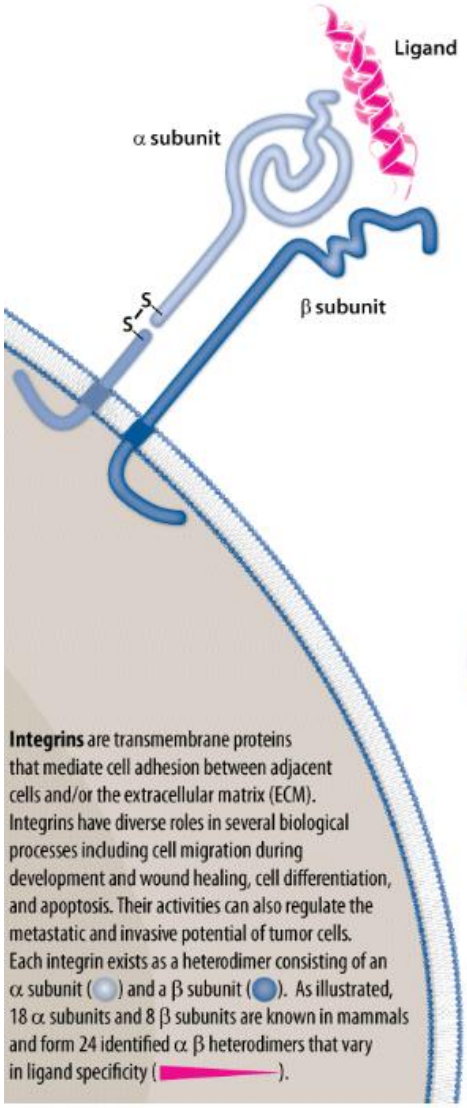
*Villarroya et al Cell. Metab.*2018

AIMS

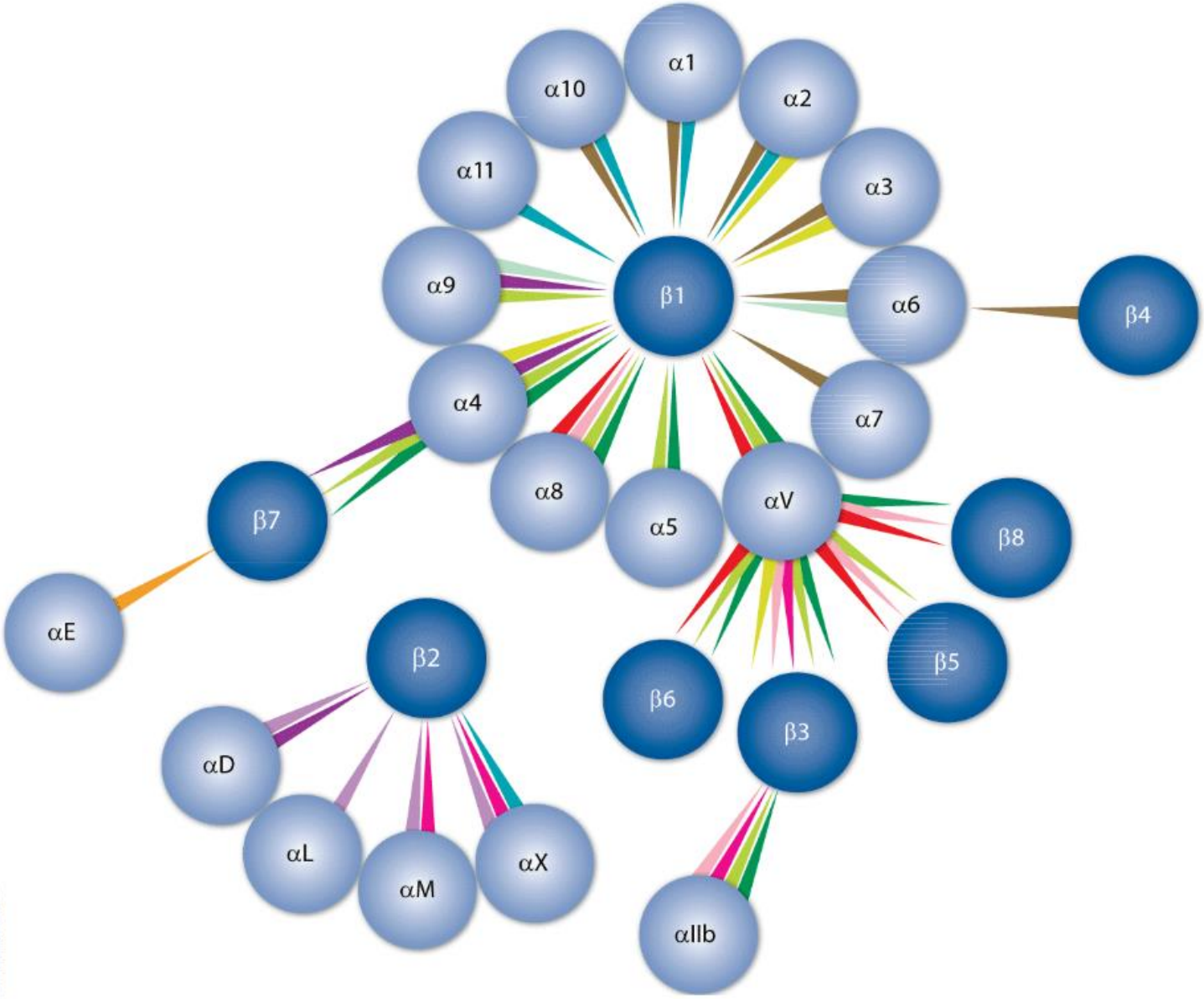
What mediates macrophage accumulation/retention in the obese AT?

Does increased macrophage accumulation and retention in the adipose tissue inhibit beige adipogenesis?

Integrins

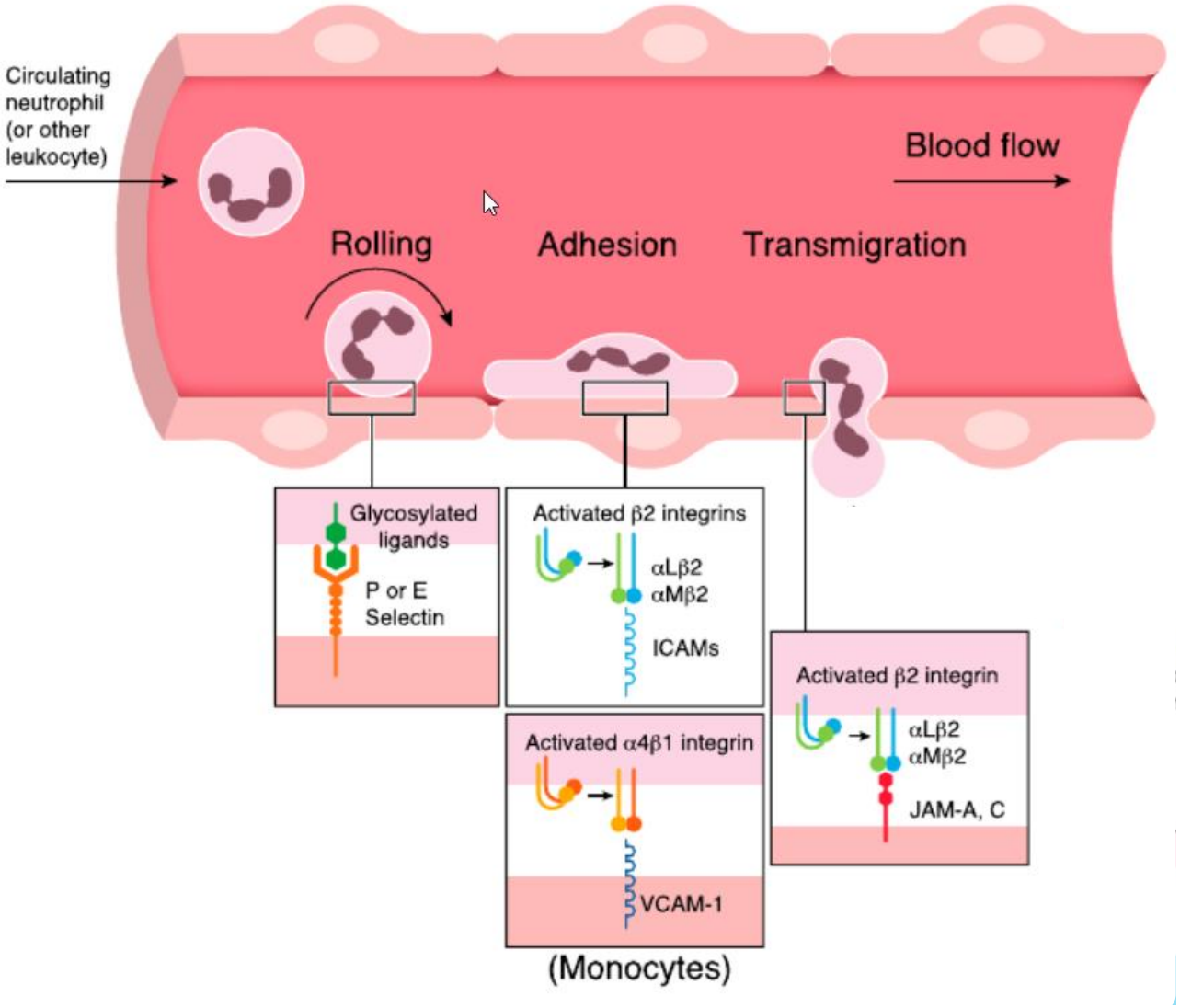


Integrins are transmembrane proteins that mediate cell adhesion between adjacent cells and/or the extracellular matrix (ECM). Integrins have diverse roles in several biological processes including cell migration during development and wound healing, cell differentiation, and apoptosis. Their activities can also regulate the metastatic and invasive potential of tumor cells. Each integrin exists as a heterodimer consisting of an α subunit ( ) and a β subunit ( ). As illustrated, 18 α subunits and 8 β subunits are known in mammals and form 24 identified α β heterodimers that vary in ligand specificity ( ).



Integrin Subunit Interactions

Leukocyte extravasation



Adhesive interactions mediating inflammatory cell accumulation in the adipose tissue?

Adapted from Kourtzelis et. al. J. Leuk.Biol 2017

Macrophage retention assay in the adipose tissue

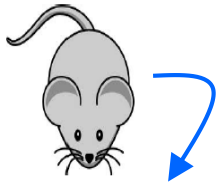
Donor mice:

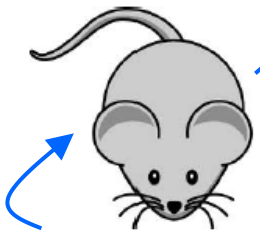
WT (8 weeks old)

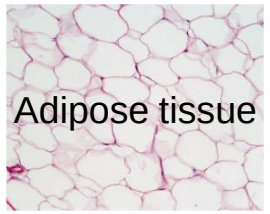
KO (8 weeks old) : VLA4 KO

Recipient mice:

Obese WT (21 weeks HFD)





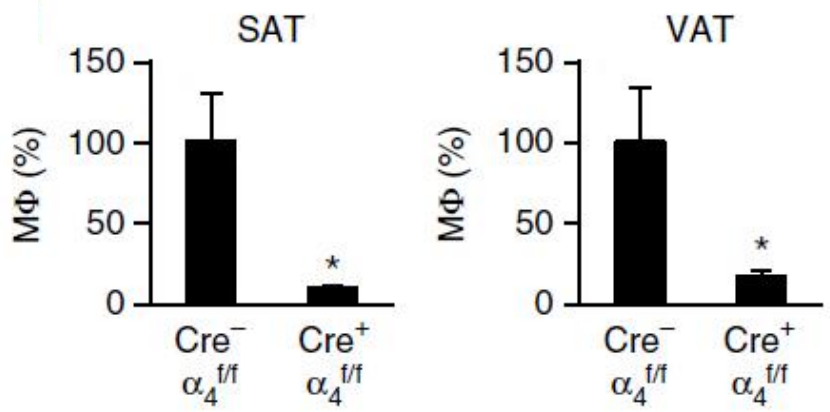


Cell isolation	Labelling	Injection	Analysis
Monocytes (CD11b+Ly6G-)	Wt PKH67 (Green dye) KO PKH26 (Red dye)	Wt (50%) KO (50%)	1:1 → 7 days

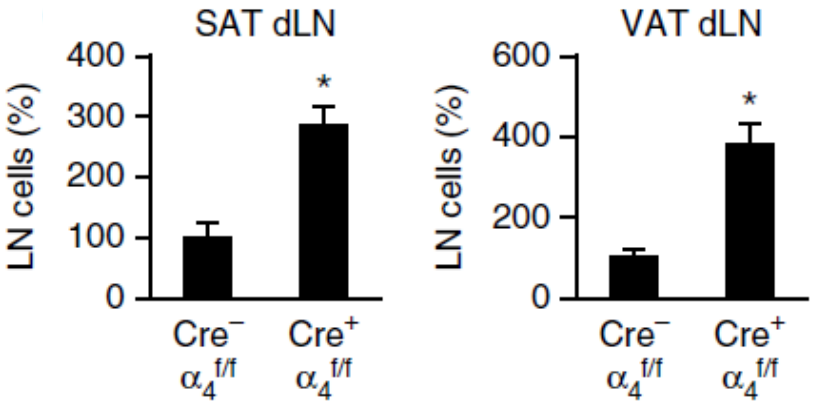
Chung , Chatzigeorgiou*et. al. Nat. Immunol. 2017*

VLA-4-integrin mediates macrophage retention in the obese AT

SAT: subcutaneous AT
VAT: visceral AT

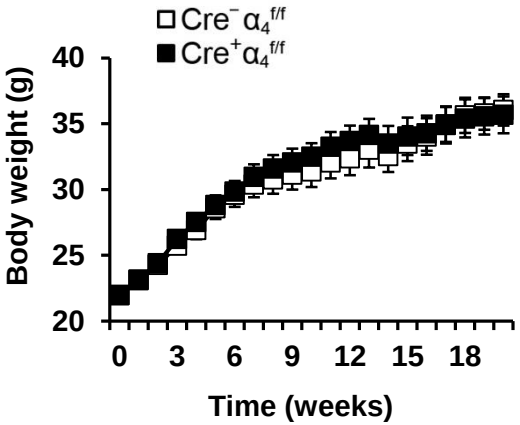


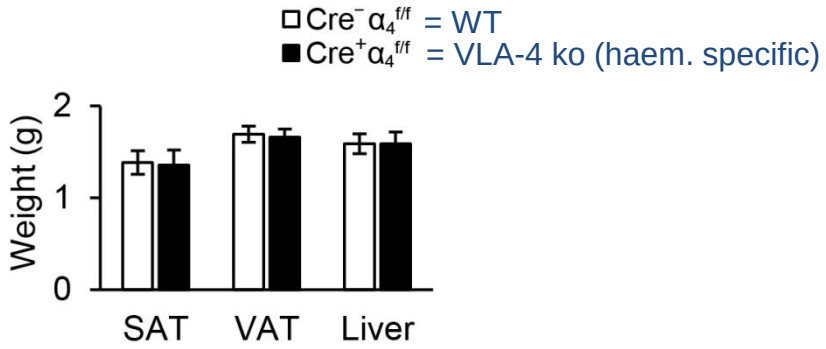
→ **VLA-4-deficient macrophages are not retained in the SAT or VAT but egress into the draining lymph nodes**

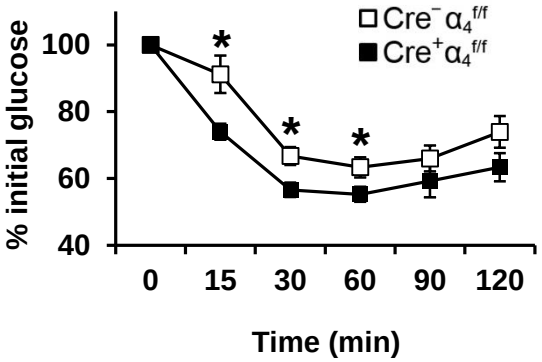


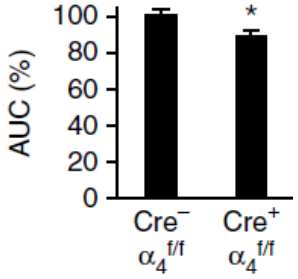
Chung , Chatzigeorgiou*et. al. Nat. Immunol. 2017*

No difference in weight in DIO but improved insulin resistance due to hematopoietic alpha4 (VLA-4) deletion







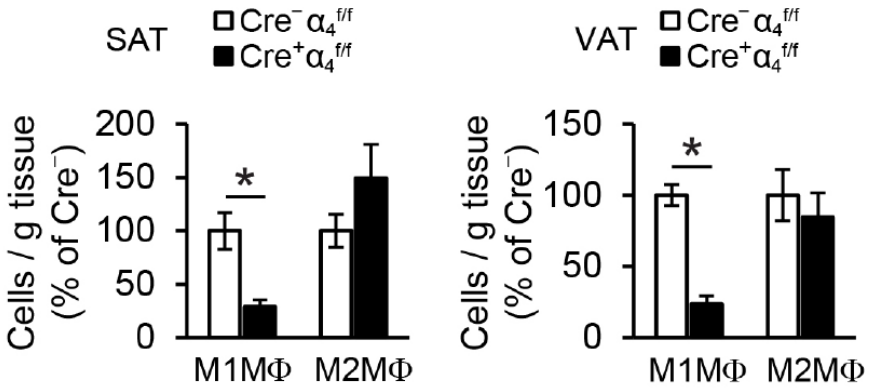


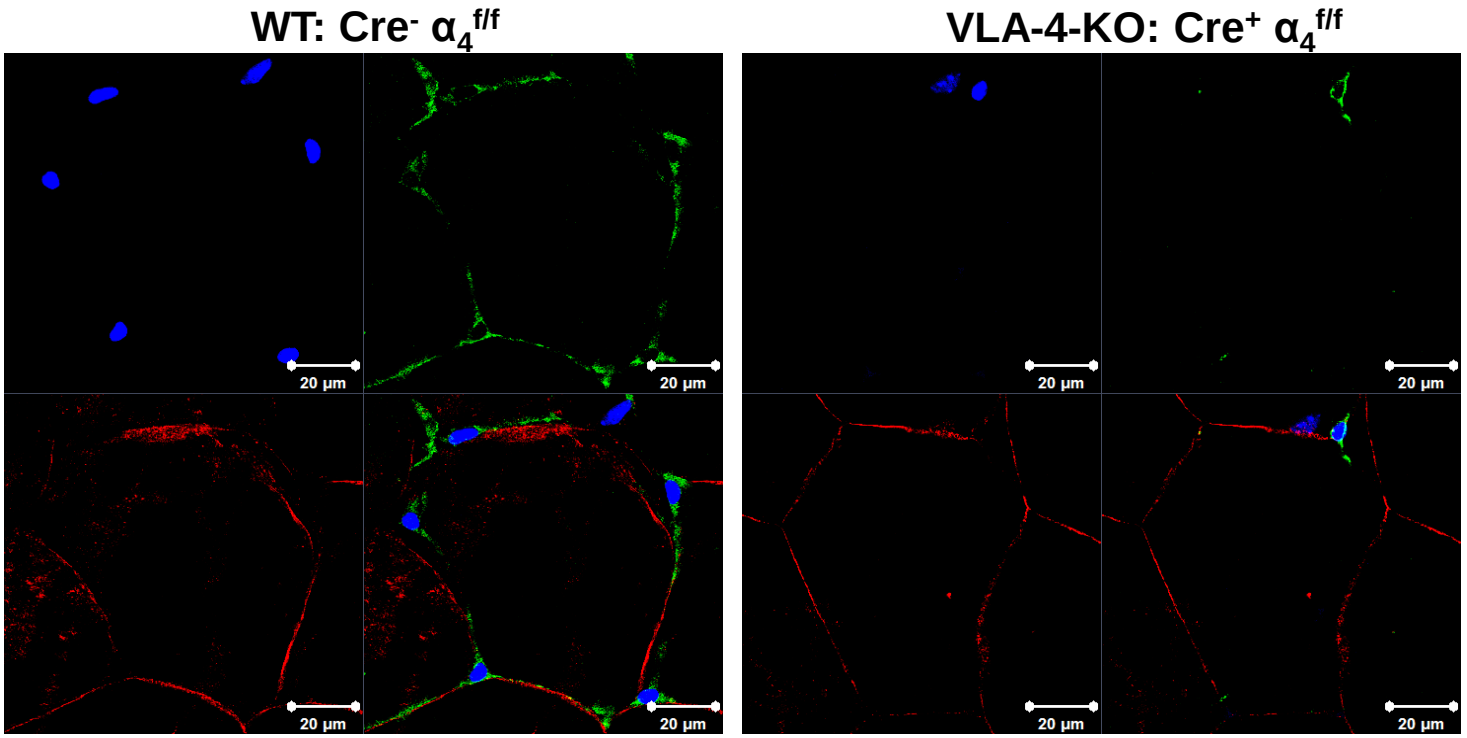
Chung , Chatzigeorgiou*et. al. Nat. Immunol. 2017*

VLA-4 deletion reduces M1 macrophage numbers in the AT

M1MΦ: F4/80$^+$CD11b$^+$iNOS$^+$CD206$^-$

M2MΦ: F4/80$^+$CD11b$^+$iNOS$^-$CD206$^+$

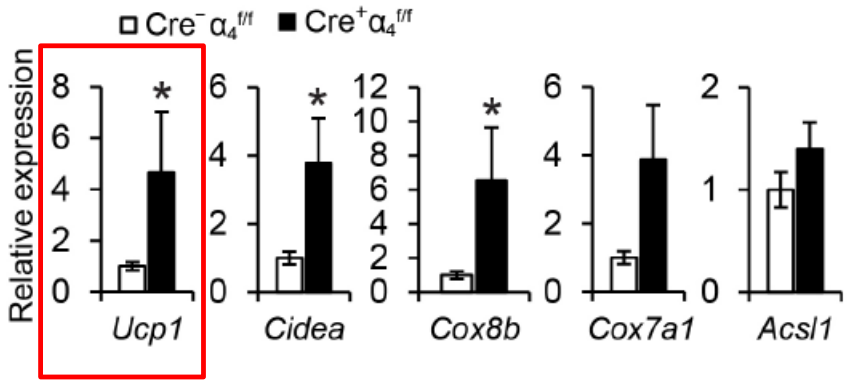


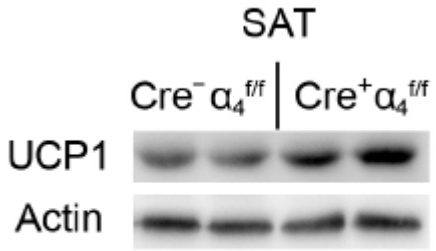


F4/80: macrophages Caveolin: adipocytes

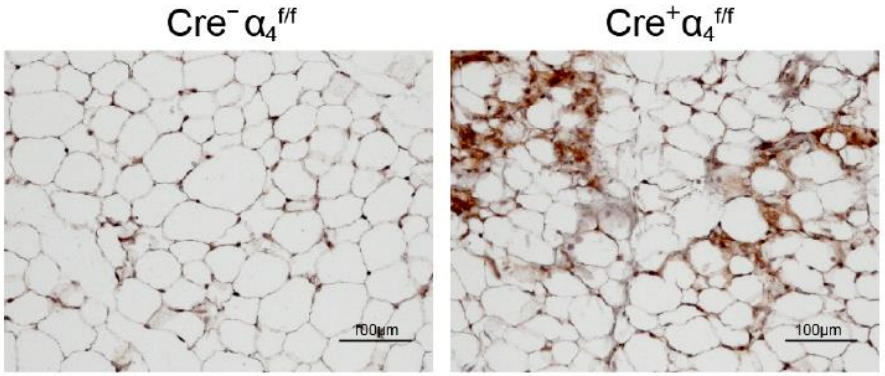
Chung , Chatzigeorgiou*et. al. Nat. Immunol. 2017*

Increased UCP1 expression and beige adipogenesis in obese mice with haematopoietic VLA-4 deletion

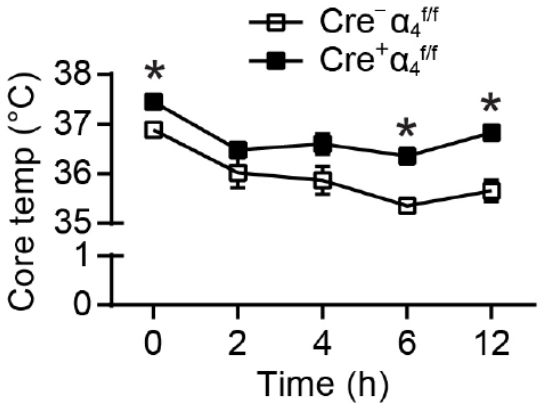




UCP1



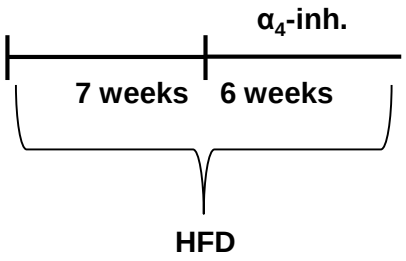
Increased cold resistance

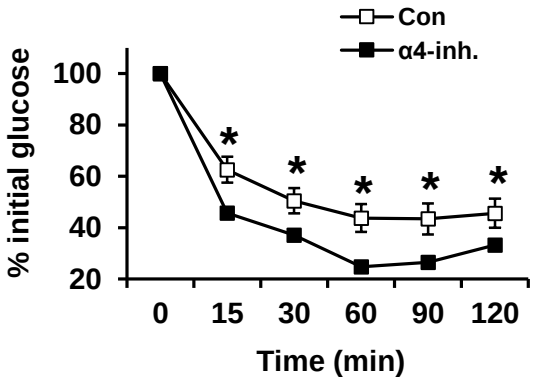


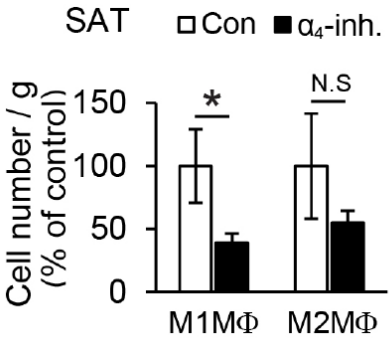
Chung , Chatzigeorgiou*et. al. Nat. Immunol. 2017*

biogen idec.

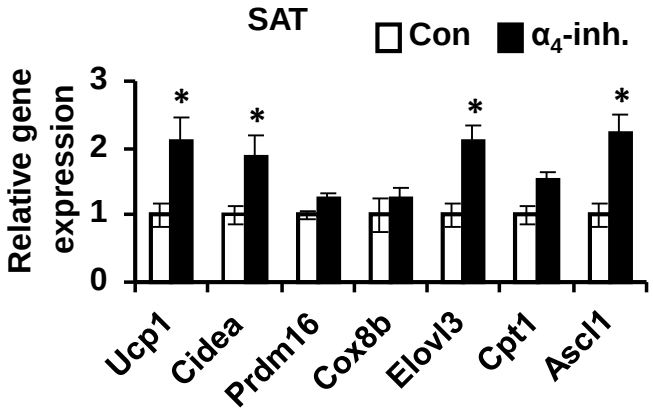
ELND002 : Small molecule alpha 4 integrin inhibitor
→ Improves insulin sensitivity and reduces AT inflammation

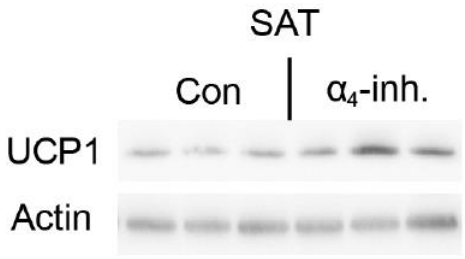


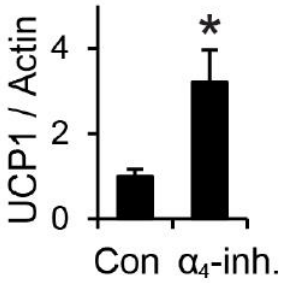




→ Improves beige adipogenesis in obese mice

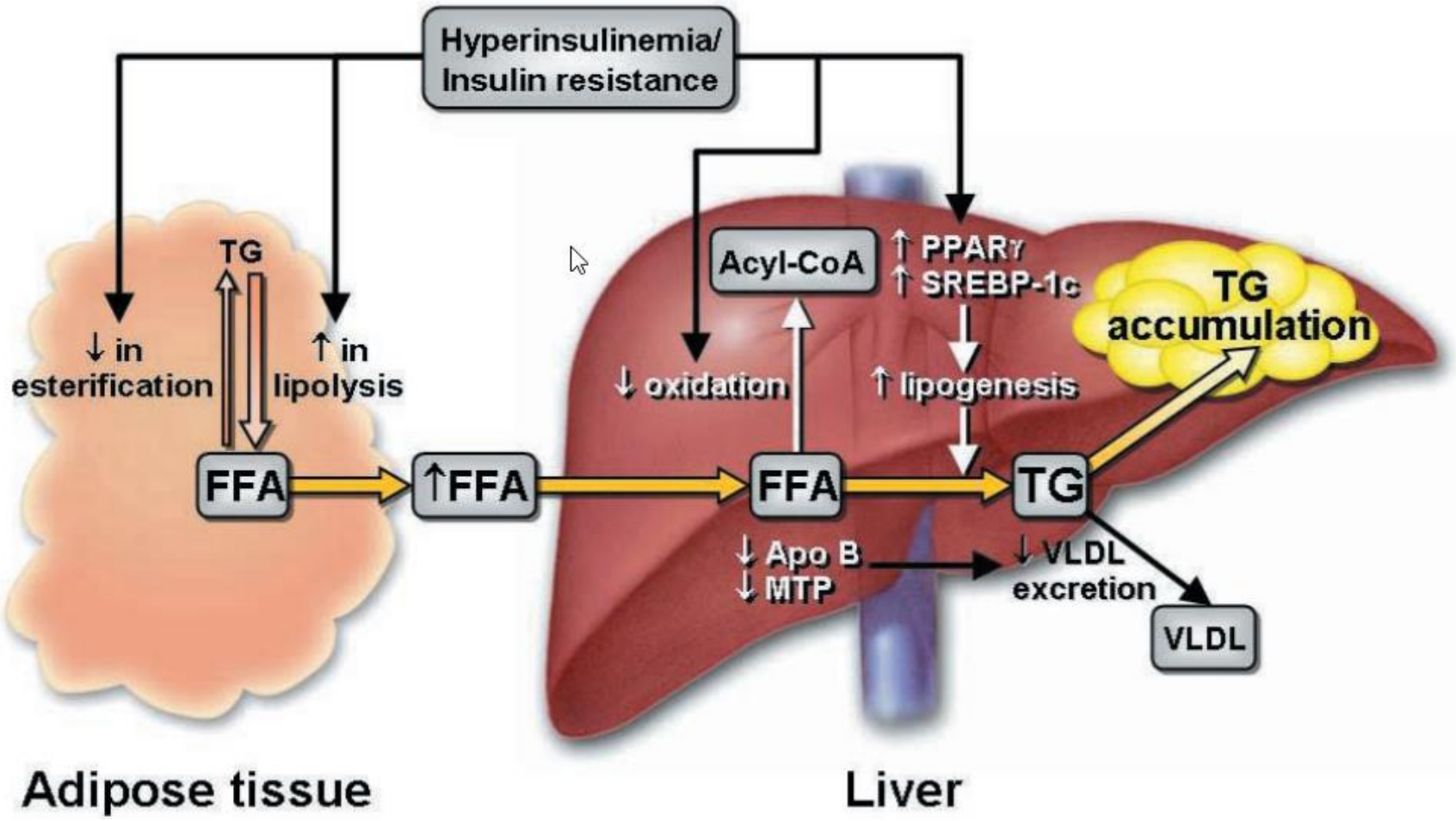






Chung , Chatzigeorgiou*et. al. Nat. Immunol. 2017*

Obesity and non-alcoholic fatty liver disease



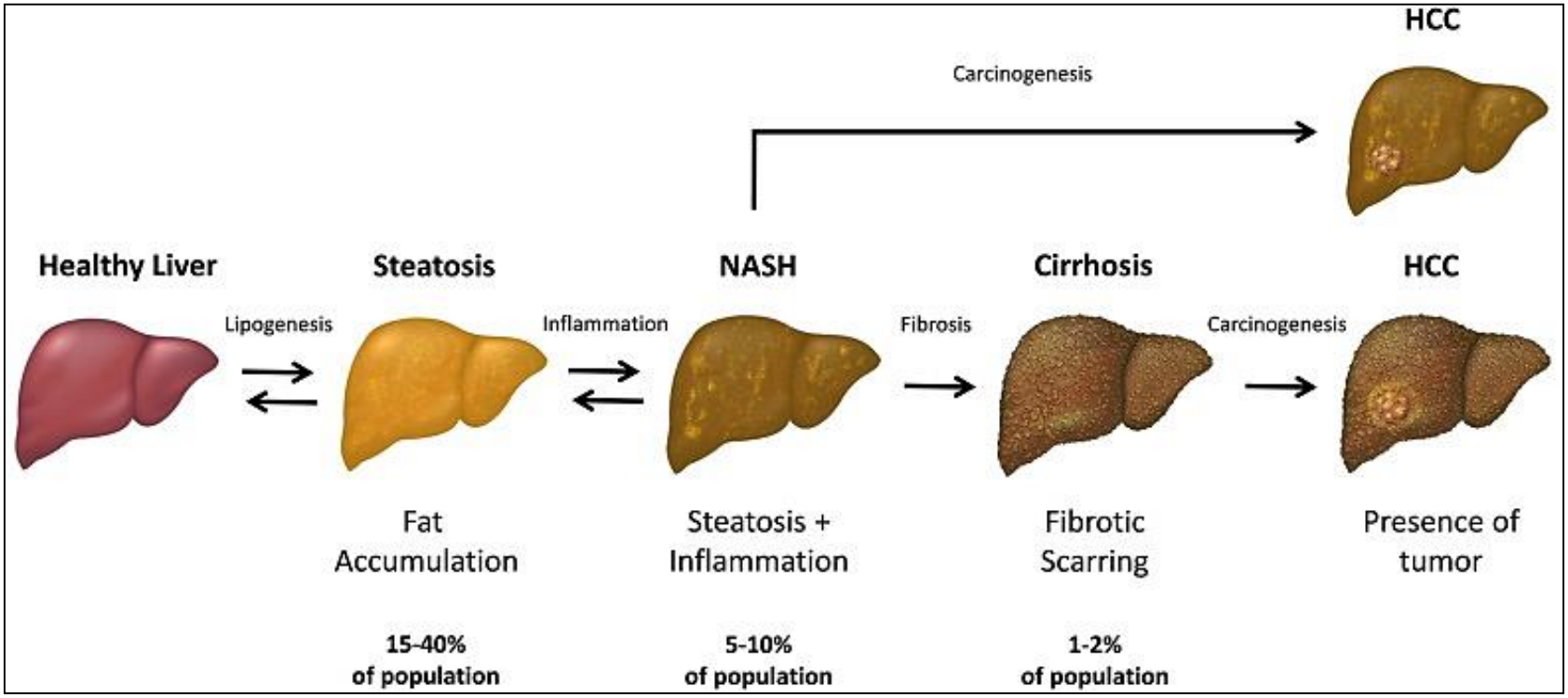
Adams et al, CMAJ , 2005

Non-alcoholic fatty liver disease (NAFLD)

- Most common cause of liver disease in Western countries
- Fat accumulation in the liver exceeding 5-10% by weight of subjects with absent or low (<20-30g/day) alcohol consumption
- Hepatic manifestation of the metabolic syndrome (visceral obesity, insulin resistance, dyslipidemia and hypertension)

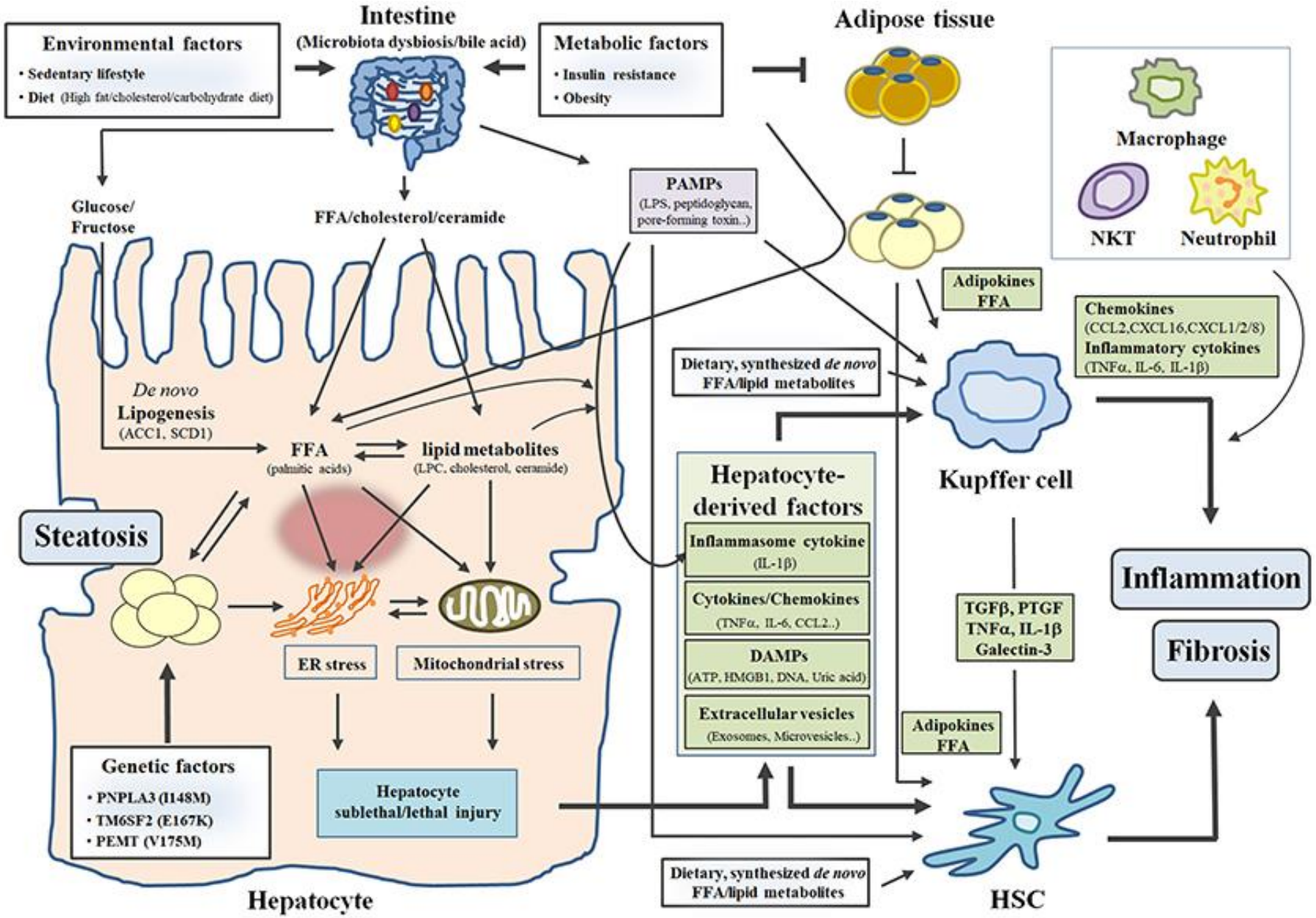
NASH (Non-alcoholic steatohepatitis)

fatty infiltration - hepatocyte damage – inflammation - fibrosis



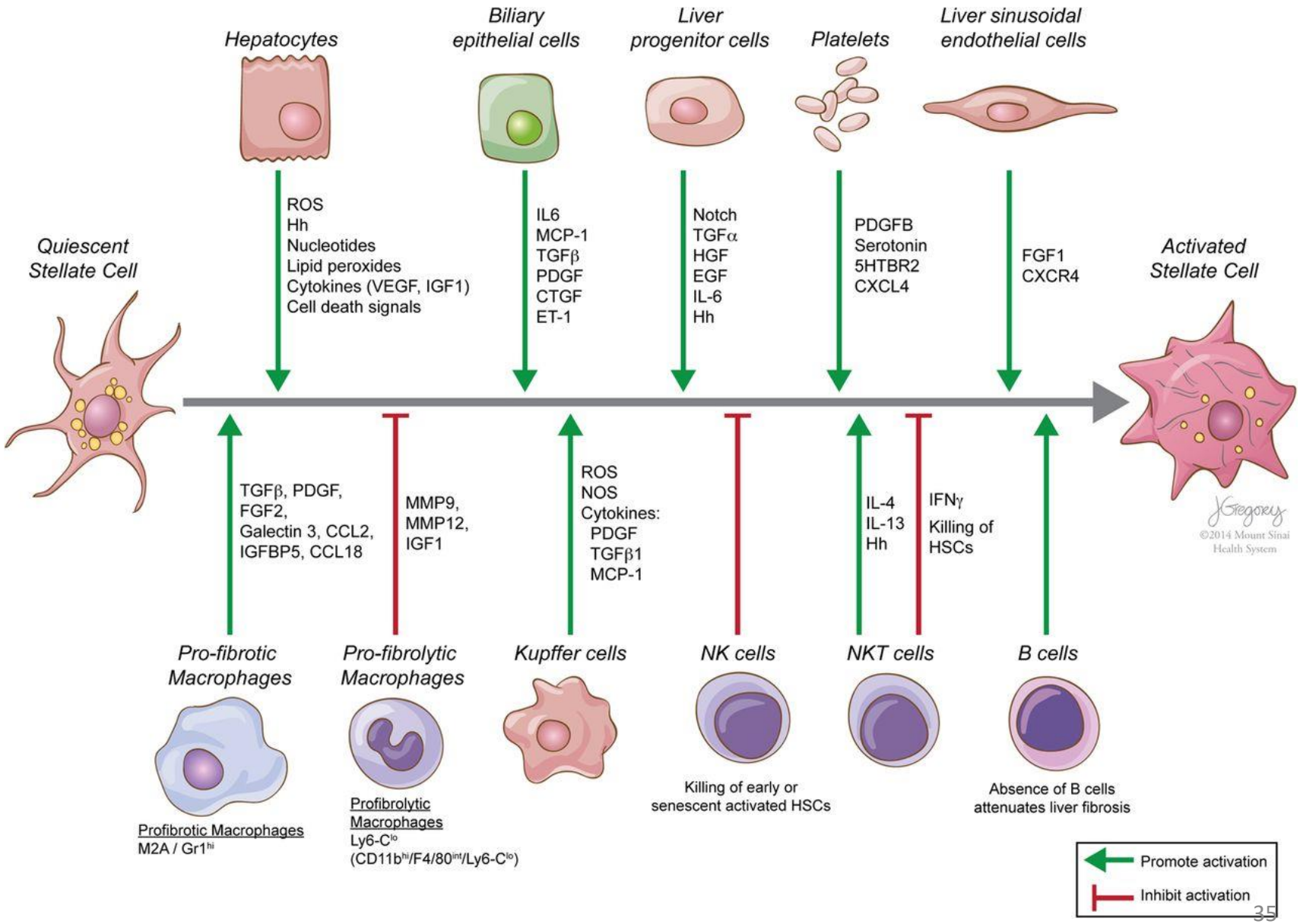
Turchinovich et. al. Front Physiol. 2018

Cell interactions in NASH



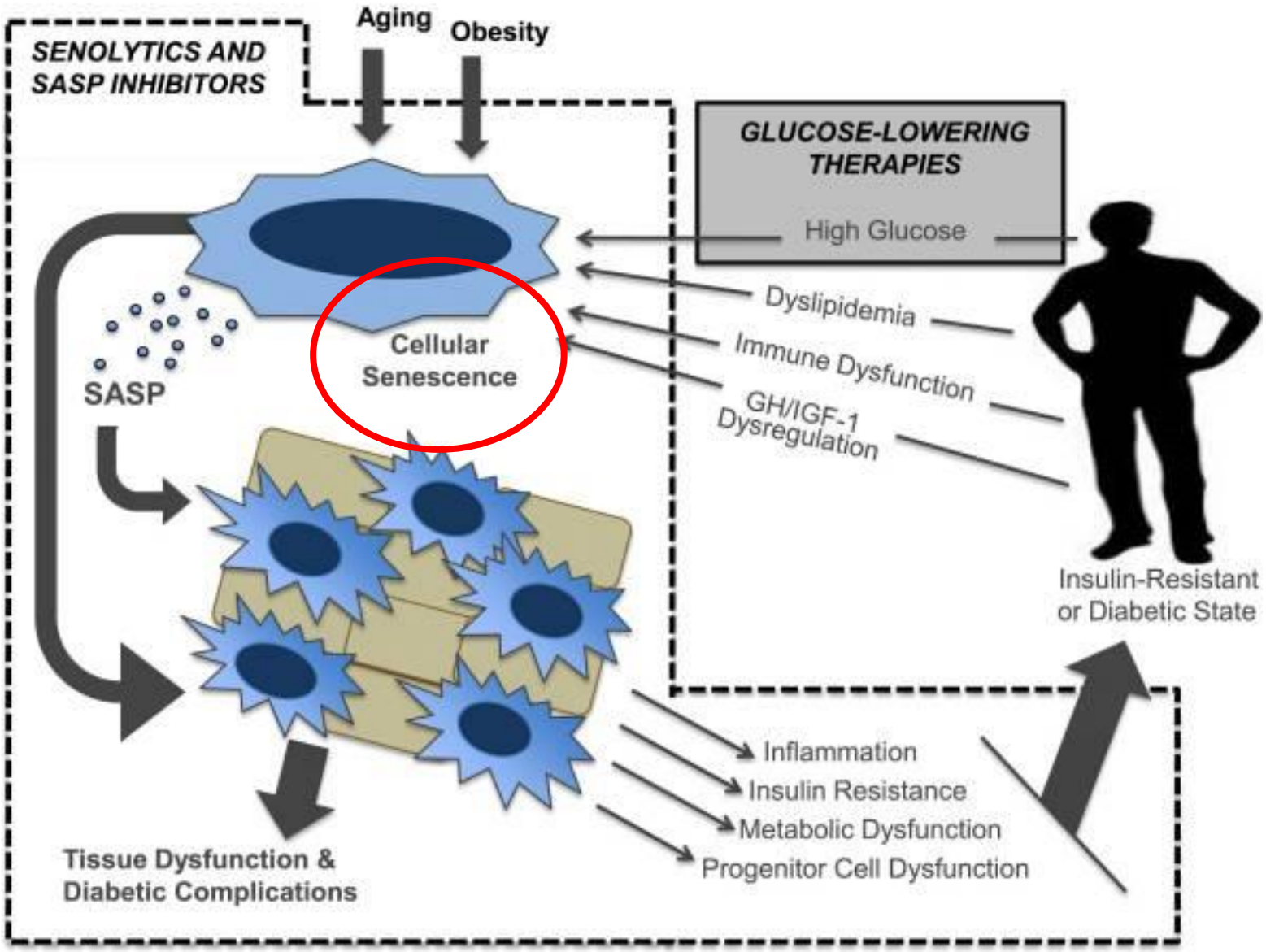
Kim et al Front. Endocrinol . 2018

Liver fibrosis



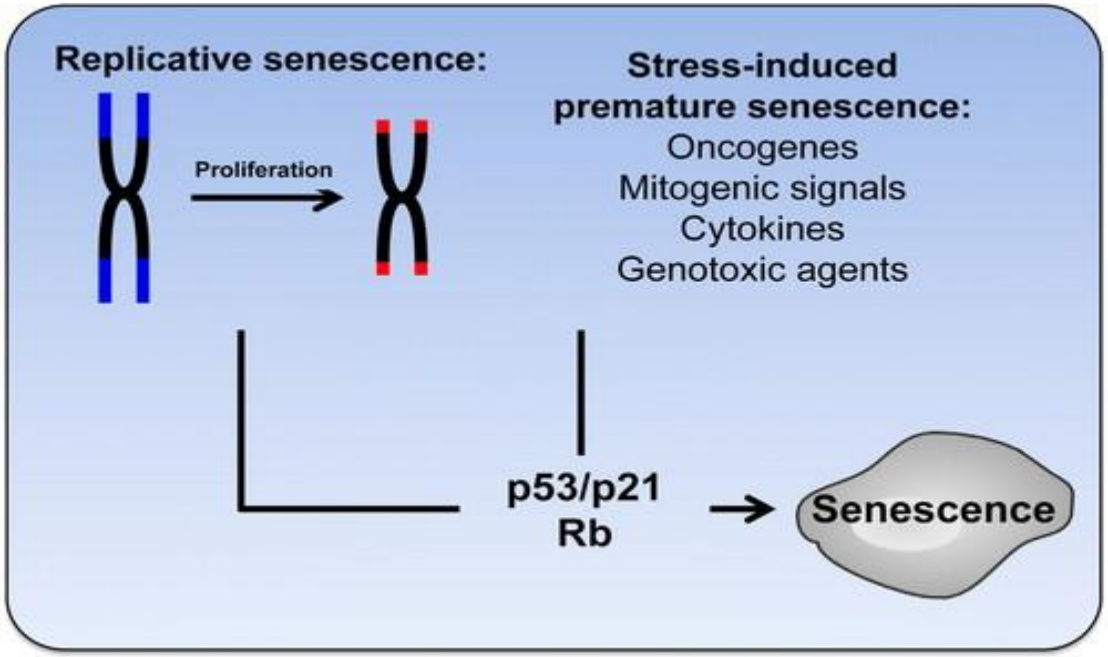
Lee et al Gut 2015

Vicious cycle of senescence and inflammation in metabolic dysregulation



Palmer et al. Diabetes 2015

Types of senescence

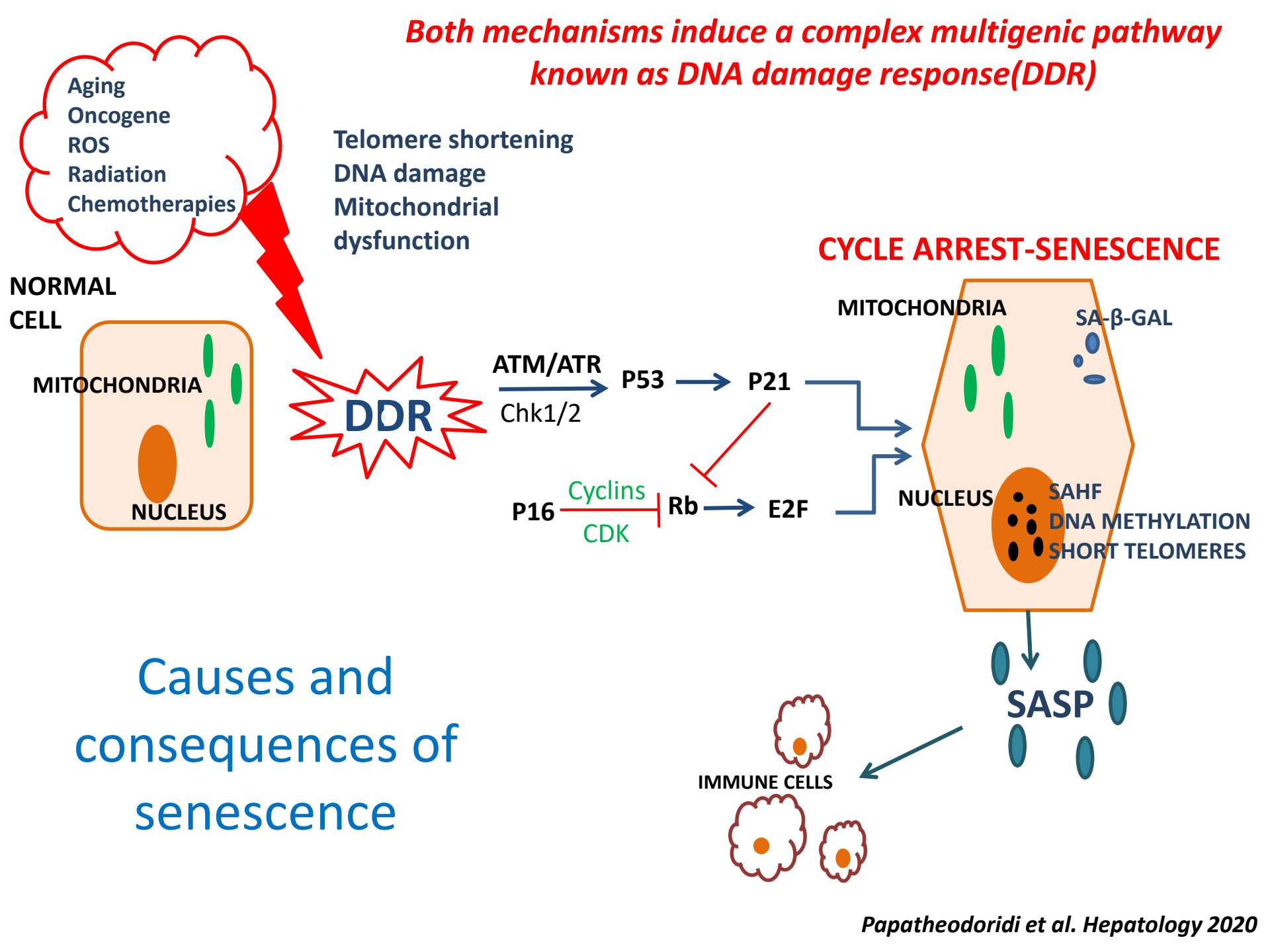


There are two major mechanisms of cellular senescence

- replicative senescence which depends on telomere shortening or erosion, predominantly upon aging, and

- stress-induced premature senescence which is mostly telomere-independent and refers to intracellular or environmental stress factors leading to DNA damage

Blasco, Circ. Res. 2012


Both mechanisms induce a complex multigenic pathway known as DNA damage response(DDR)
Aging
Oncogene
ROS
Radiation
Chemotherapies
Telomere shortening
DNA damage
Mitochondrial dysfunction
CYCLE ARREST-SENESCENCE
NORMAL CELL
MITOCHONDRIA
NUCLEUS
DDR
ATM/ATR
Chk1/2
P53
P21
P16
Cyclins
CDK
Rb
E2F
MITOCHONDRIA
SA-β-GAL
NUCLEUS
SAHF
DNA METHYLATION
SHORT TELOMERES
SASP
IMMUNE CELLS
Causes and consequences of senescence
Papatheodoridi et al. Hepatology 2020

Cellular Senescence Signature

Senescent cells have well defined features, which include:

- Cell cycle arrest
- Morphological change such that cells are enlarged and flattened with enlarged nuclei
- Expression of senescence-associated β-galactosidase (SA-β-GAL) or lipofuscin
- Accumulation of DNA damage foci
- Acquisition of the senescence-associated secretary phenotype (SASP)
- These changes are known collectively as the 'cellular senescence signature'

Table 2. Senescence-Associated Secretory Phenotype (SASP) Components

Class	Component
Interleukins	IL-6; IL-7; IL-1; IL-1b; IL-13; IL-15
Chemokines	IL-8; GRO-a, -b, -g; MCP-2; MCP-4; MIP-1a; MIP-3a; HCC-4; eotaxin; eotaxin-3; TECK; ENA-78; I-309; I-TAC
Other inflammatory molecules	TGFβ; GM-CSE; G-CSE; IFN-γ; BLC; MIF
Growth factors; regulators	Amphiregulin; epiregulin; heregulin; EGF; bFGF; HGF; KGF (FGF7); VEGF; angiogenin; SCF; SDF-1; PlGF; NGF; IGFBP-2, -3, -4, -6, -7
Proteases and regulators	MMP-1, -3, -10, -12, -13, -14; TIMP-1; TIMP-2; PAI-1, -2; tPA; uPA; cathepsin B
Receptors; ligands	ICAM-1, -3; OPG; sTNFRI; sTNFRII; TRAIL-R3; Fas; uPAR; SGP130; EGF-R
Non-protein molecules	PGE2; nitric oxide; ROS
Insoluble factors	Fibronectin; collagens; laminin

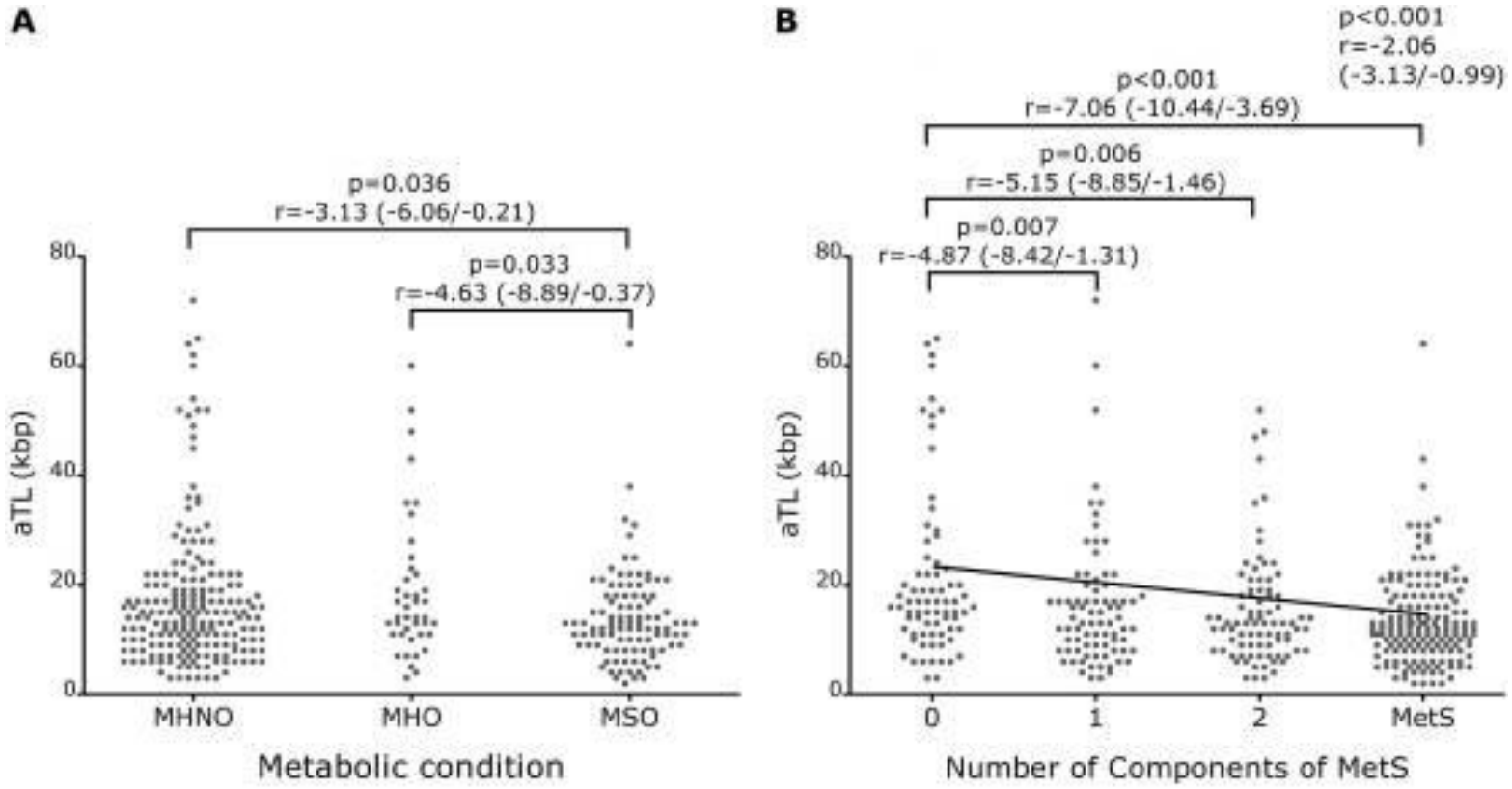
Aravinthan et al., Exp. Gerontology 2014

Gorgoulis et. al . Cell 2019

Role of senescence in NAFLD?

Cause or consequence?

Vicious cycle of senescence and metabolic dysregulation

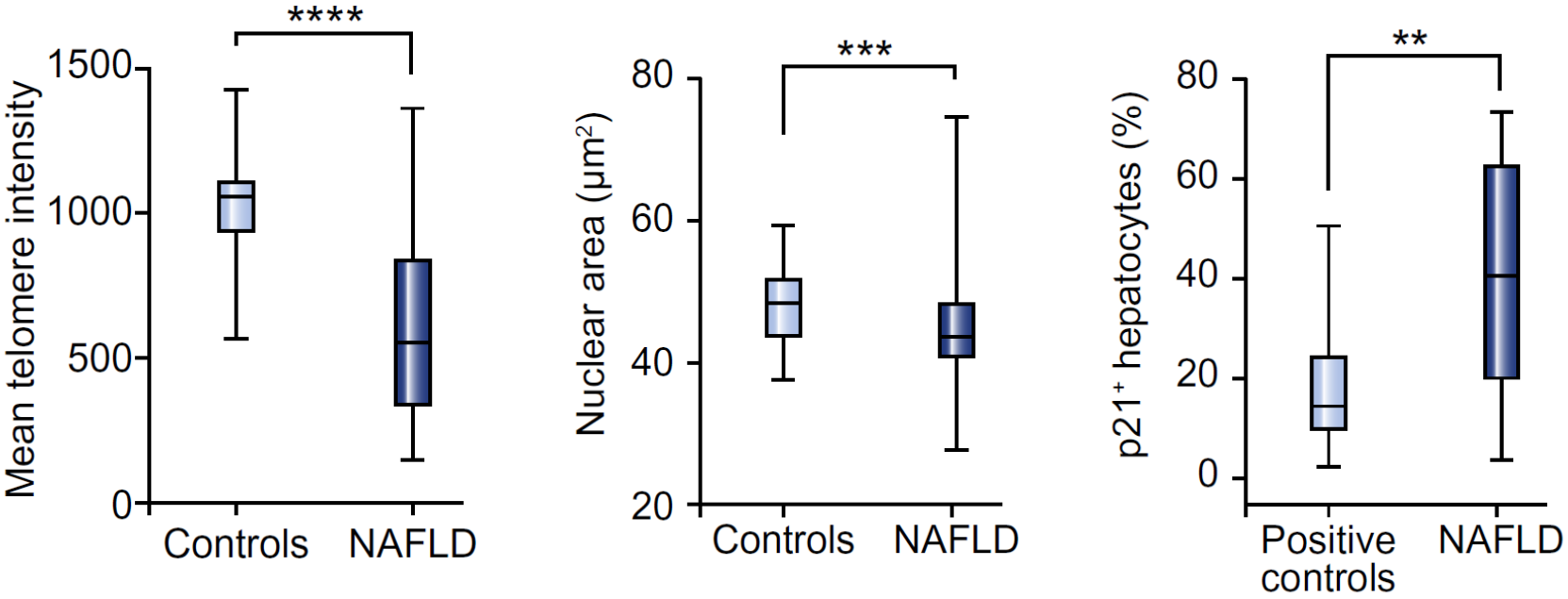


Telomere length according to the metabolic condition (A) and according to the number of components of metabolic syndrome (B).

Covariates: age, pack-years smoked and physical activity. aTL: Absolute telomere length; MHNO: Metabolically Healthy Non-obese individuals; MHO: Metabolically Healthy Obese individuals; MSO: Obese individual with Metabolic Syndrome; MetS: Metabolic Syndrome (individual with three or more components-central obesity-TGs-blood pressure-fasting glucose-waist circumference).

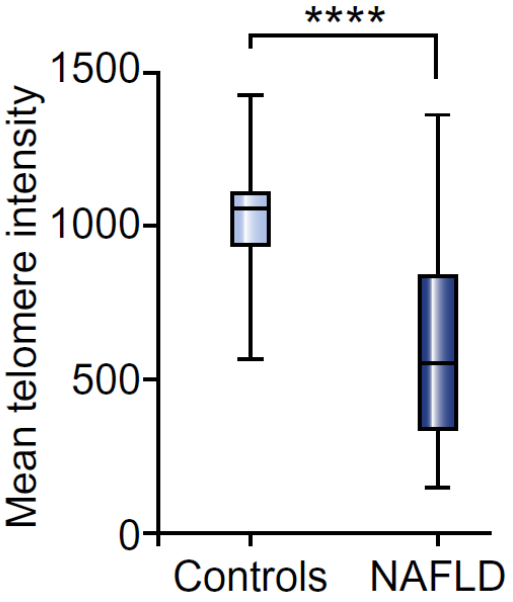
Palmer et al. Diabetes 2015

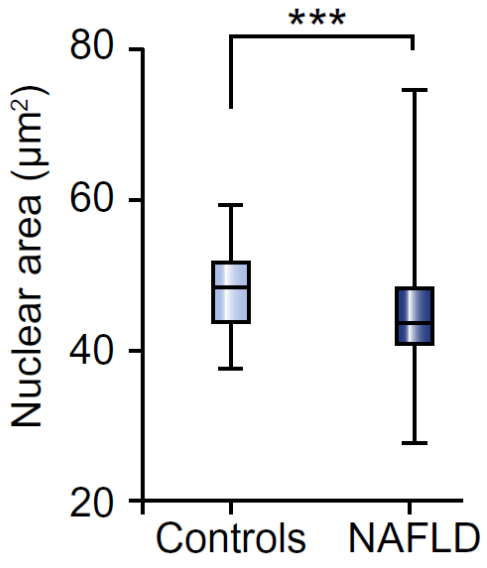
Hepatocyte senescence predicts progression in NAFLD

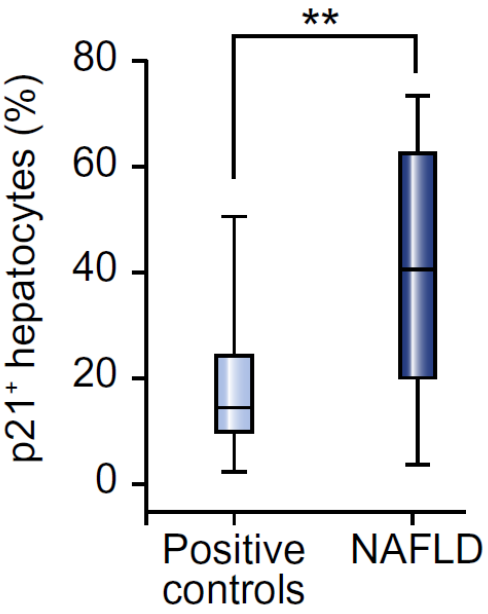


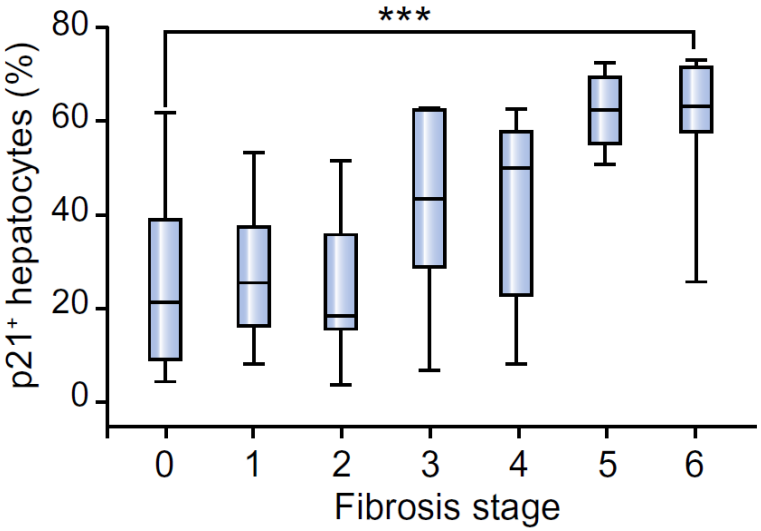
Aravithan et al J. Hepatol. 2013
Aravithan et al Cell Cycle 2014

Hepatocyte senescence predicts progression in NAFLD









Nevertheless in a second biopsy only p21 and nuclear area could predict disease progression while a specific p21 SNP (rs762623) was associated with this phenomenon

Aravithan et al J. Hepatol. 2013
Aravithan et al Cell Cycle 2014

Hepatocyte senescence in NAFLD
Cause or consequence ?

nature COMMUNICATIONS

ARTICLE

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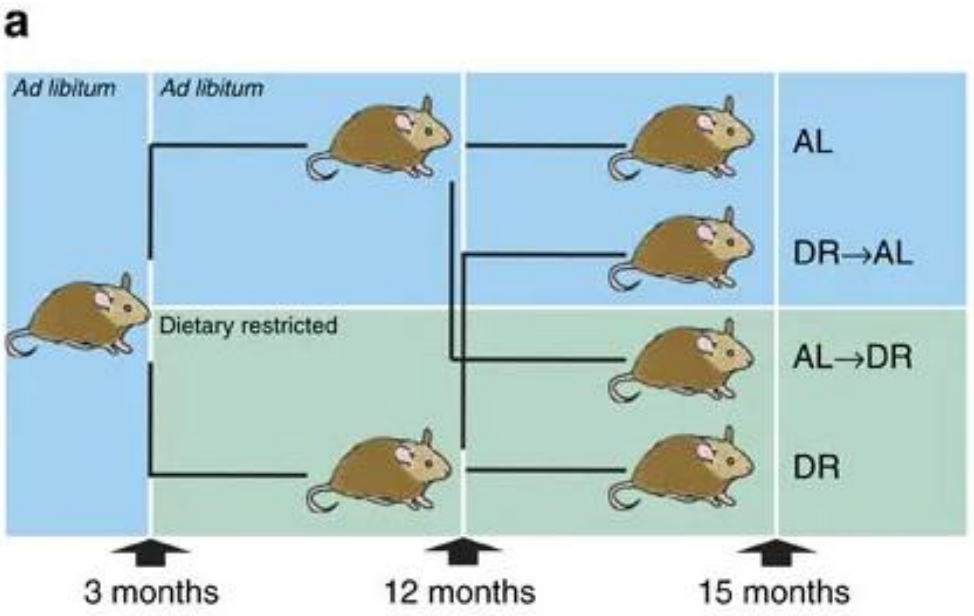
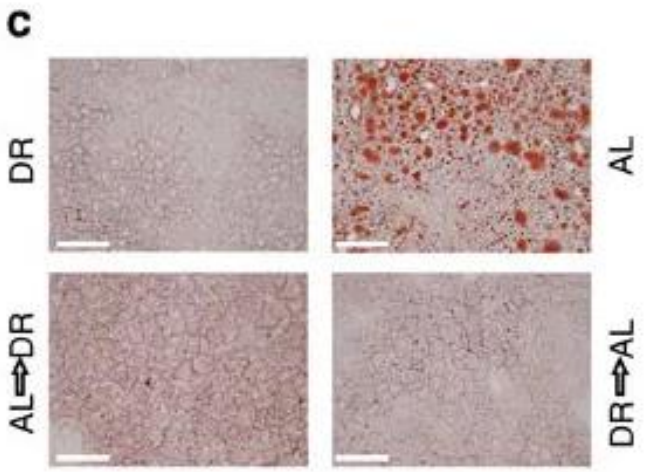
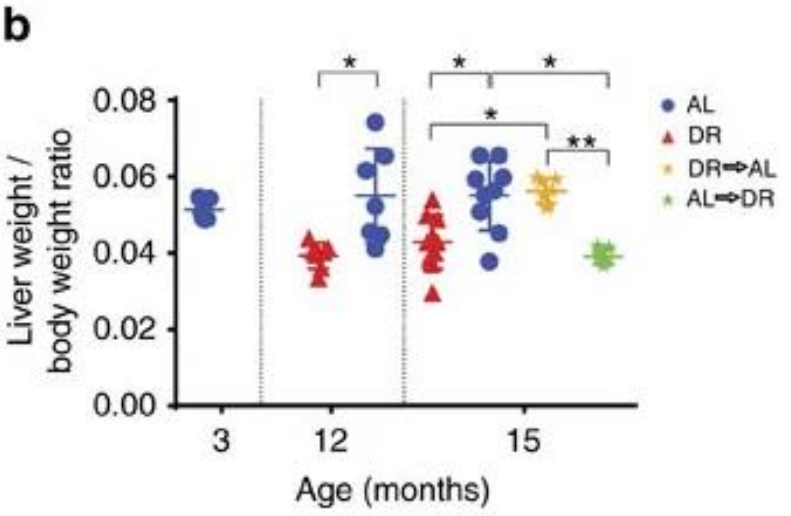
Cellular senescence drives age-dependent hepatic steatosis

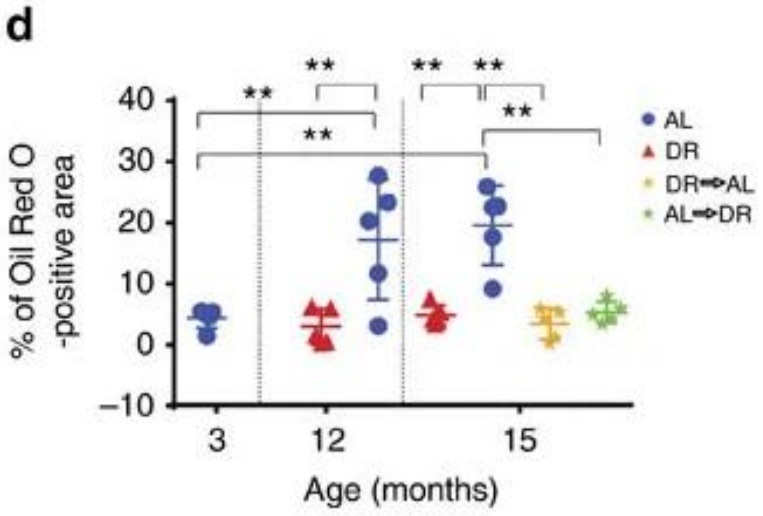
Mikolaj Ogrodnik[1], Satomi Miwa[1], Tamar Tchkonia[2], Dina Tiniakos[3,4], Caroline L. Wilson[3], Albert Lahat[5], Christopher P. Day[3,6], Alastair Burt[3,7], Allyson Palmer[2], Quentin M. Anstee[3], Sushma Nagaraja Grellscheid[5], Jan H.J. Hoeijmakers[8,9], Sander Barnhoorn[8], Derek A. Mann[3], Thomas G. Bird[10,11], Wilbert P. Vermeij[8], James L. Kirkland[2], João F. Passos[1], Thomas von Zglinicki[1] & Diana Jurk[1]

Senescence drives age-dependent steatosis

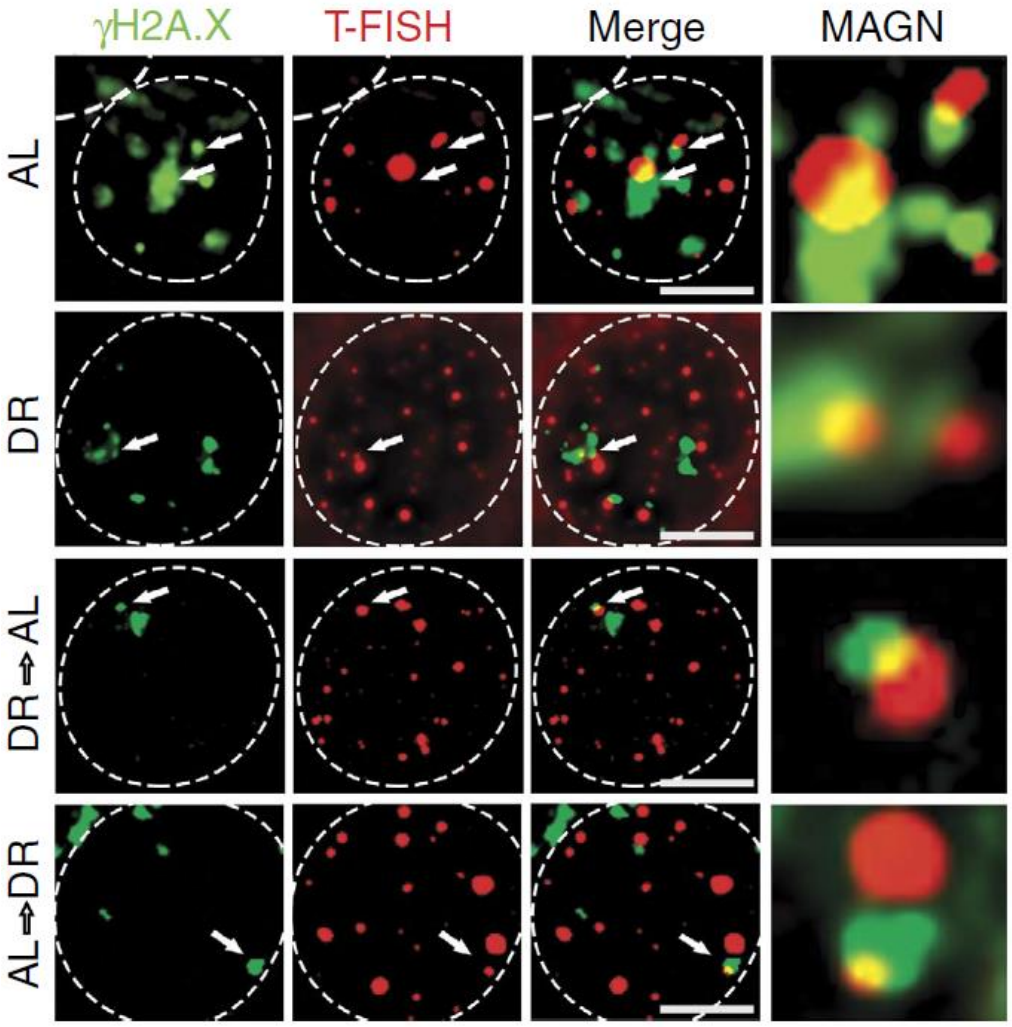


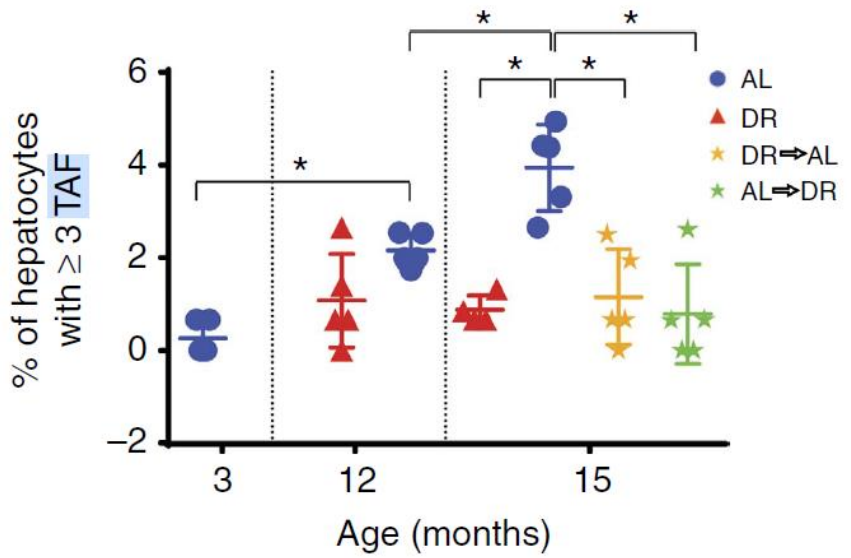


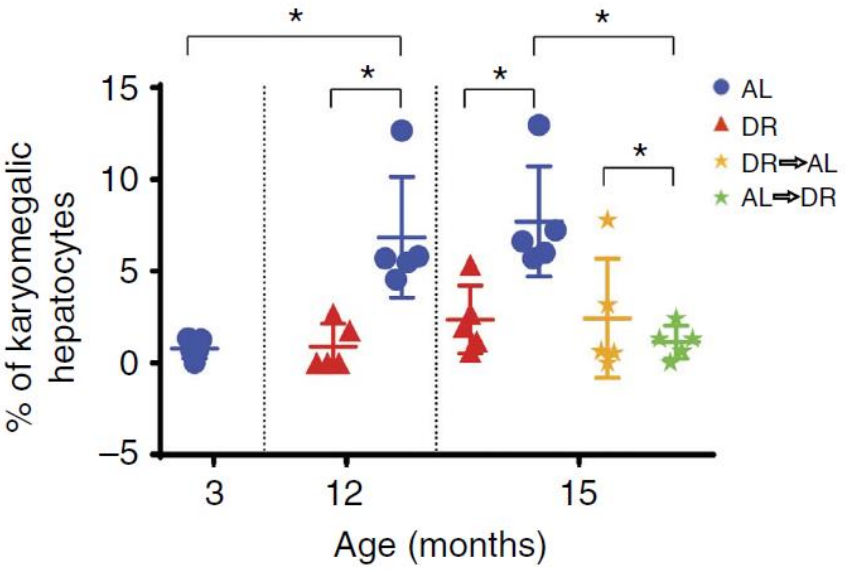




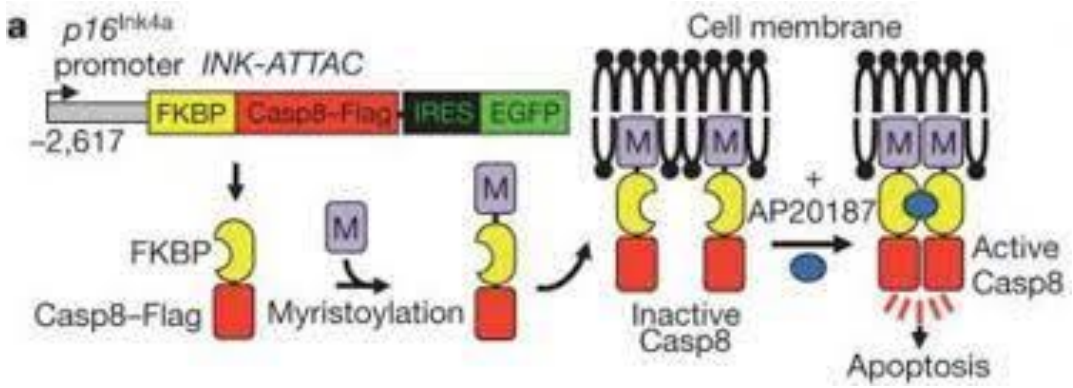
Senescence drives age-dependent steatosis



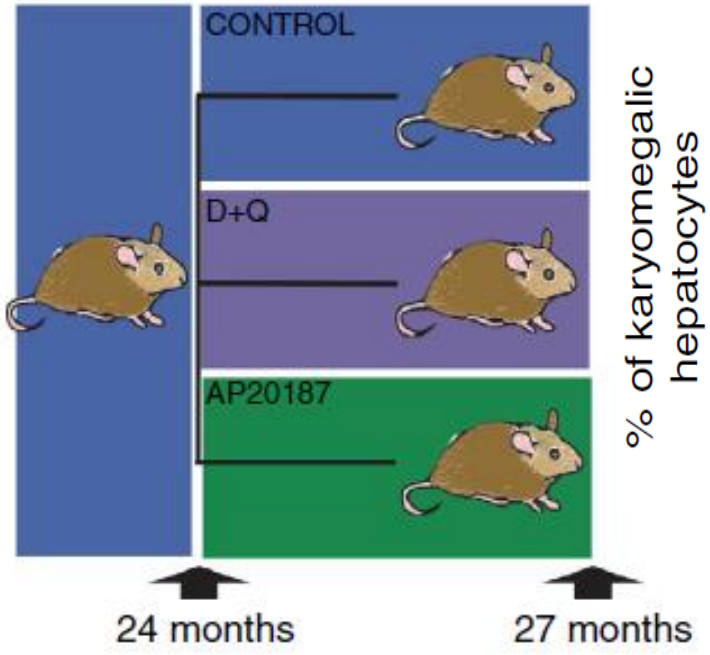


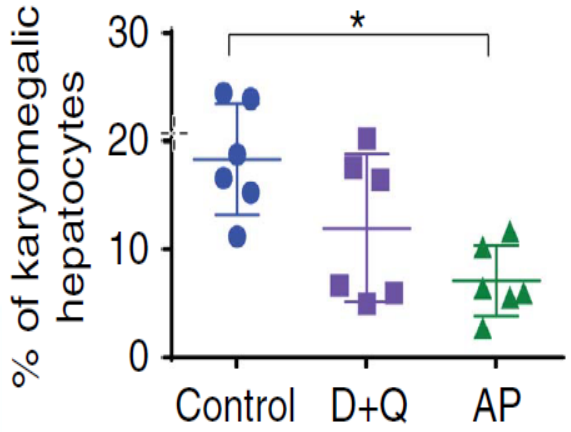


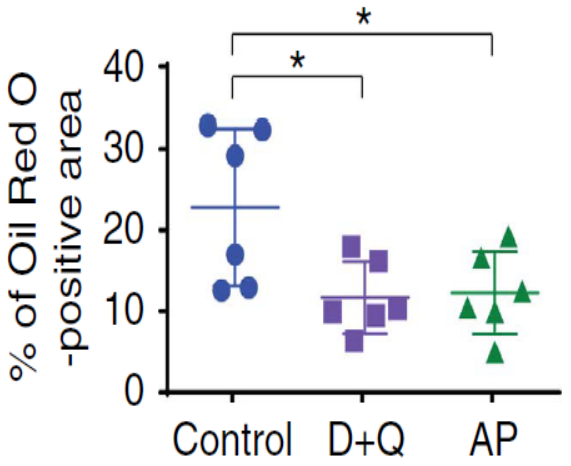
Senescence drives age-dependent steatosis



INK-ATTACK transgenic mice (INK-linked apoptosis through targeted activation of caspase) contain an inducible suicide gene in the CDKNA2 locus, which encodes p16, a key molecule in senescent cells







D+Q: dasatinib and quercetin

Ogrotnik et al Nat. Comm. 2017

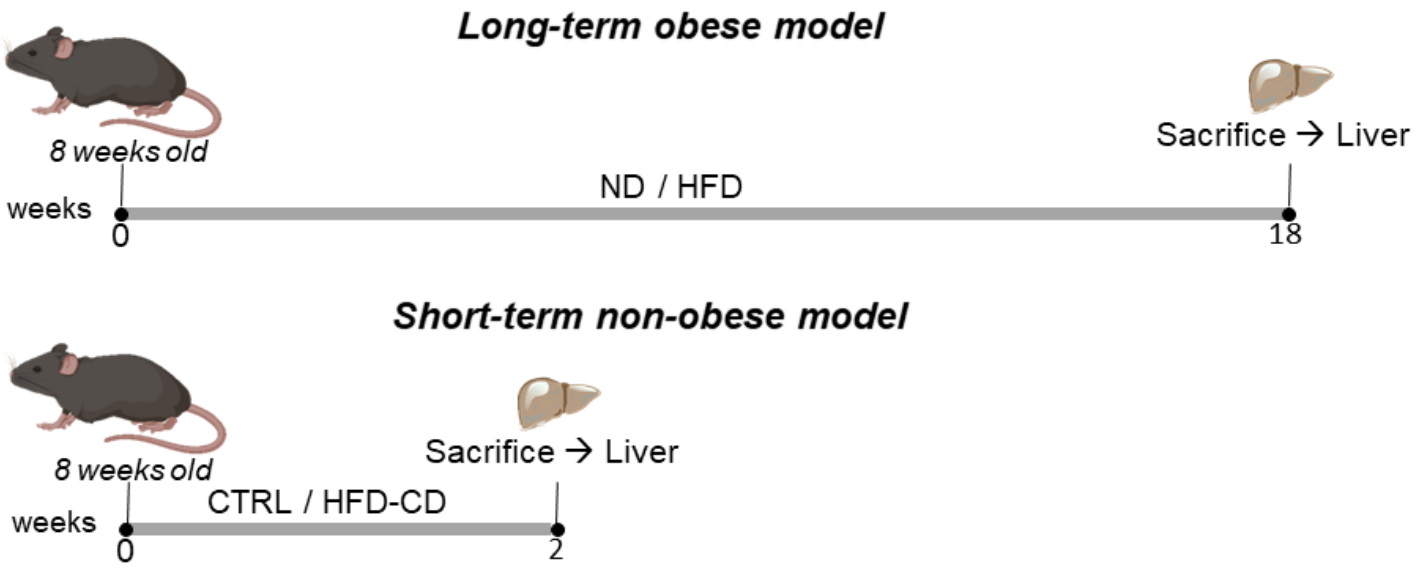
International Journal of
Molecular Sciences

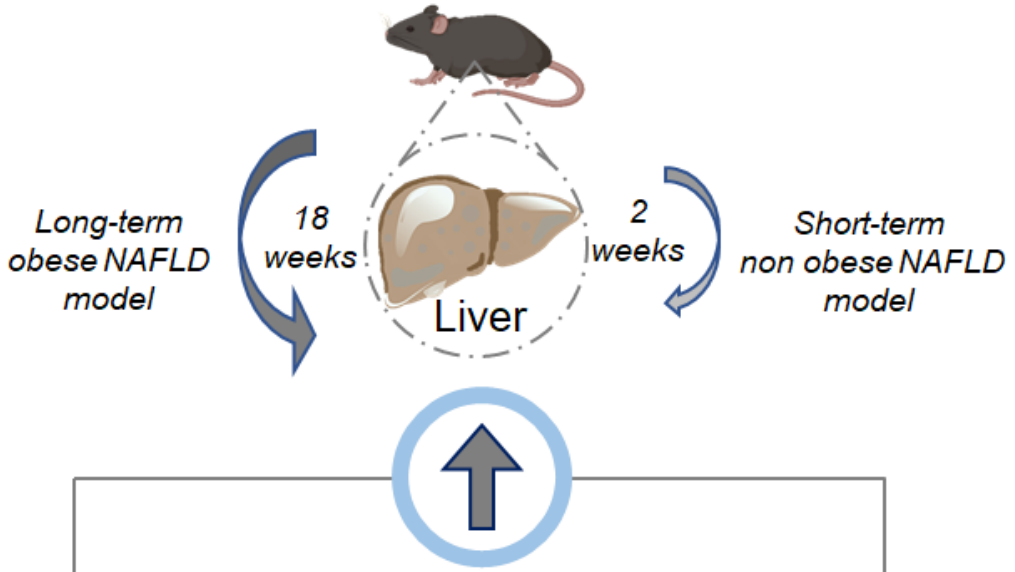
MDPI

Article

Hepatic Senescence Accompanies the Development of NAFLD in Non-Aged Mice Independently of Obesity

Ioannis I. Moustakas [1], Angeliki Katsarou [1], Aigli-Ioanna Legaki [1], Iryna Pyrina [2], Konstantinos Ntostoglou [3], Alkistis-Maria Papatheodoridi [1], Bettina Gercken [2], Ioannis S. Pateras [3], Vassilis G. Gorgoulis [3,4,5,6], Michael Koutsilieris [1], Triantafyllos Chavakis [2] and Antonios Chatzigeorgiou [1,2,*]





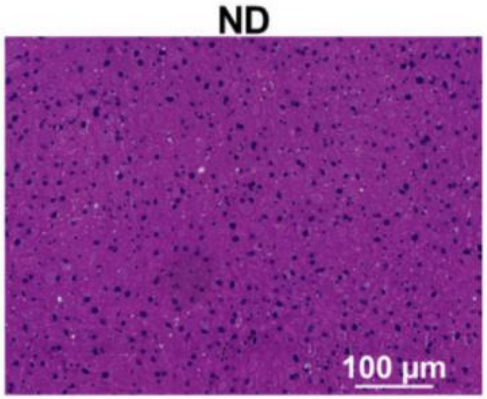
Steatosis

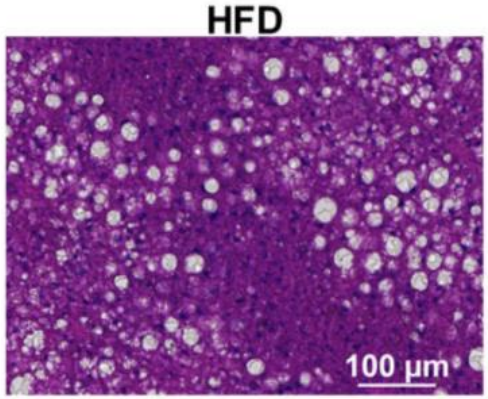
- Liver triglycerides
- Steatosis-related gene expression
- Histological assessment of hepatic steatosis

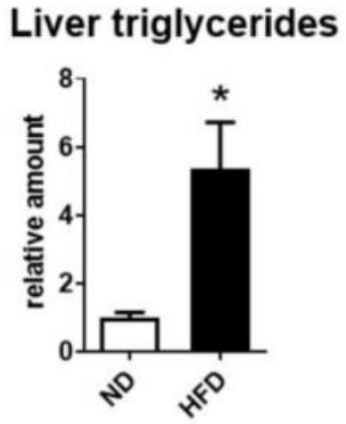
Senescence

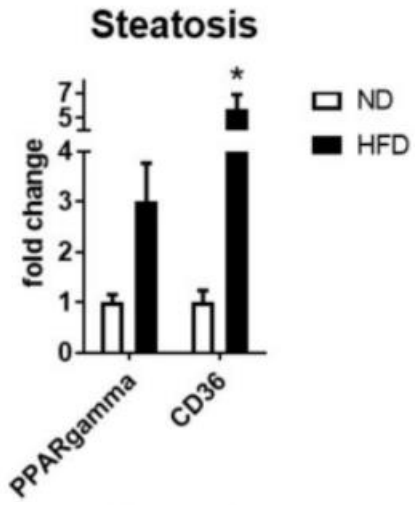
- SASP & inflammation gene expression
- Senescence gene expression
- Senescence staining
- Telomere length
- Global DNA methylation
- MDA oxidative stress

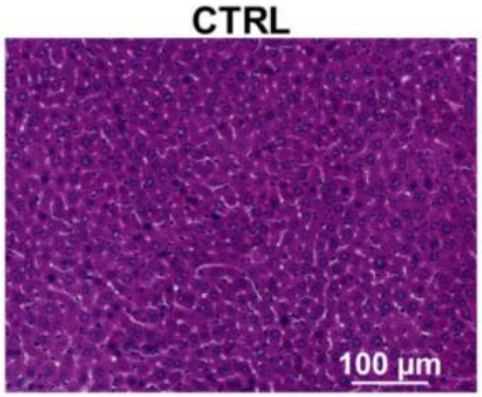
Both models of NAFLD developed steatosis of similar grade

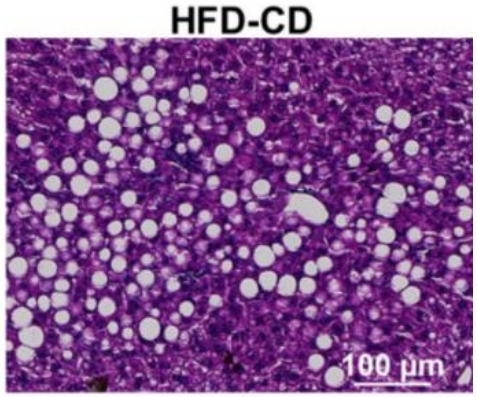


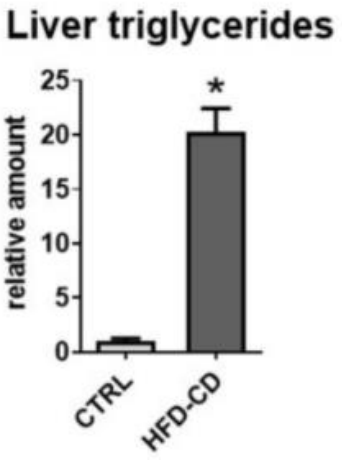


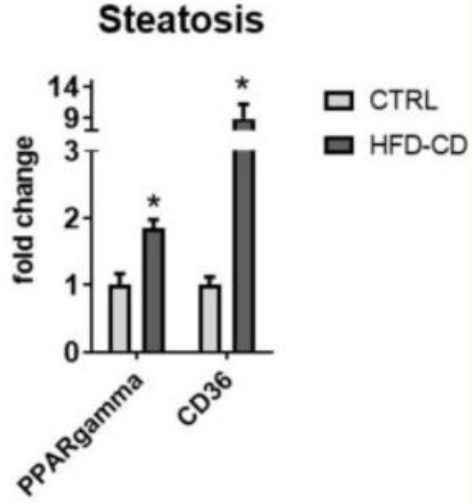




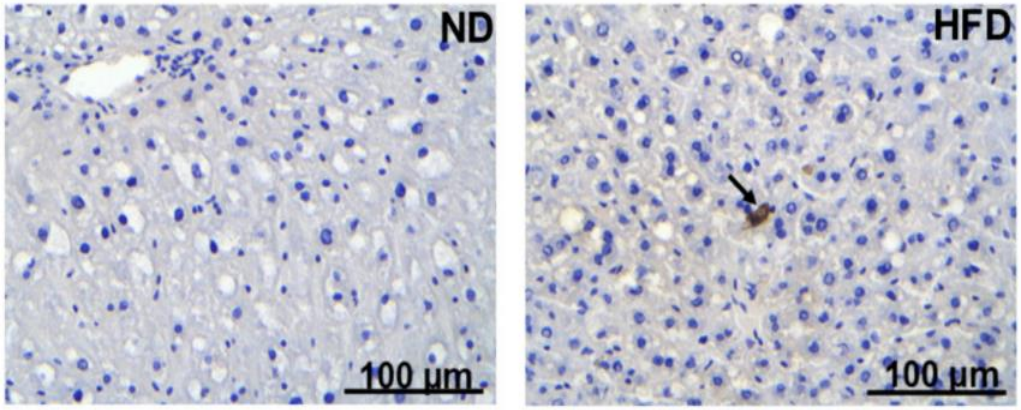


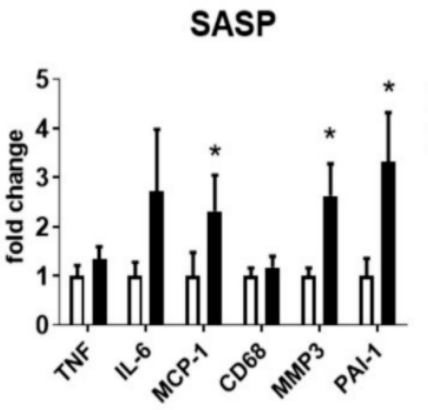


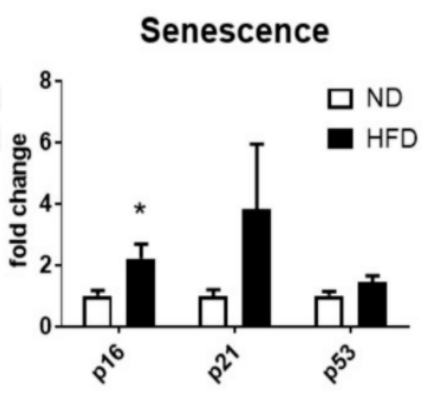


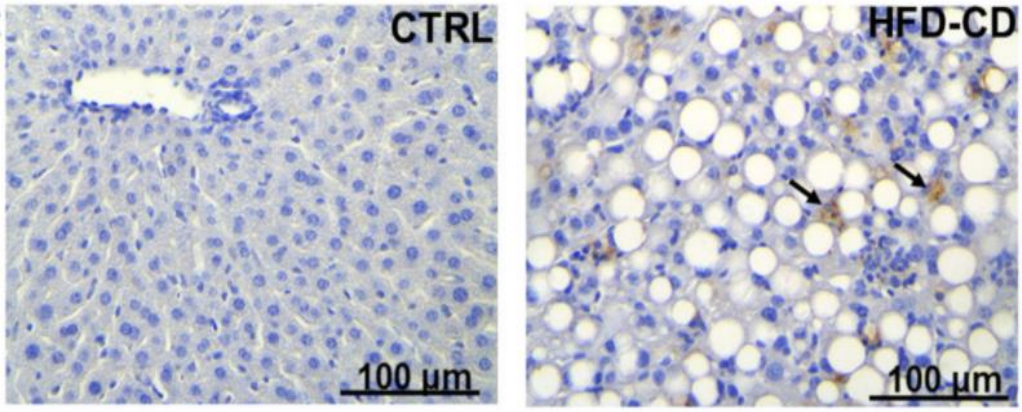


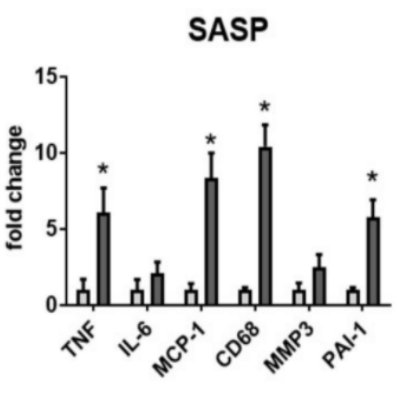
Both models of NAFLD were characterised by increased markers of senescence

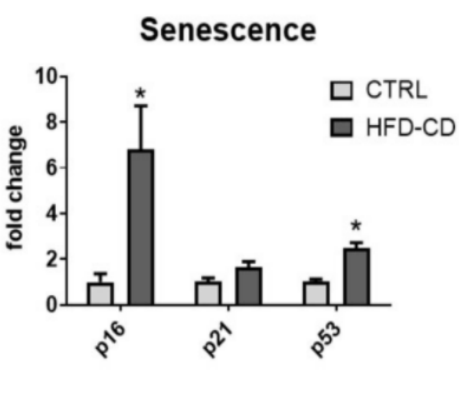




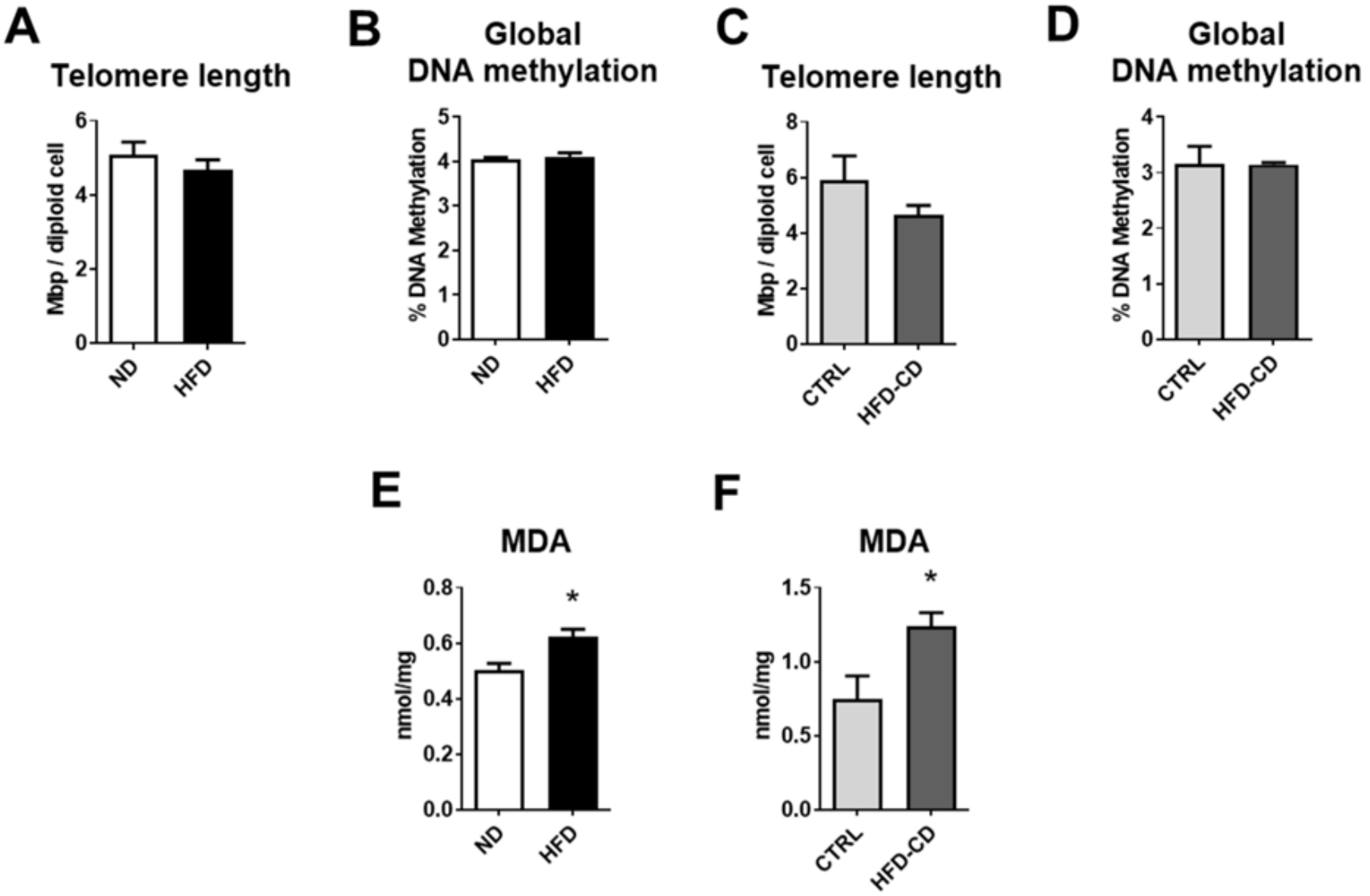






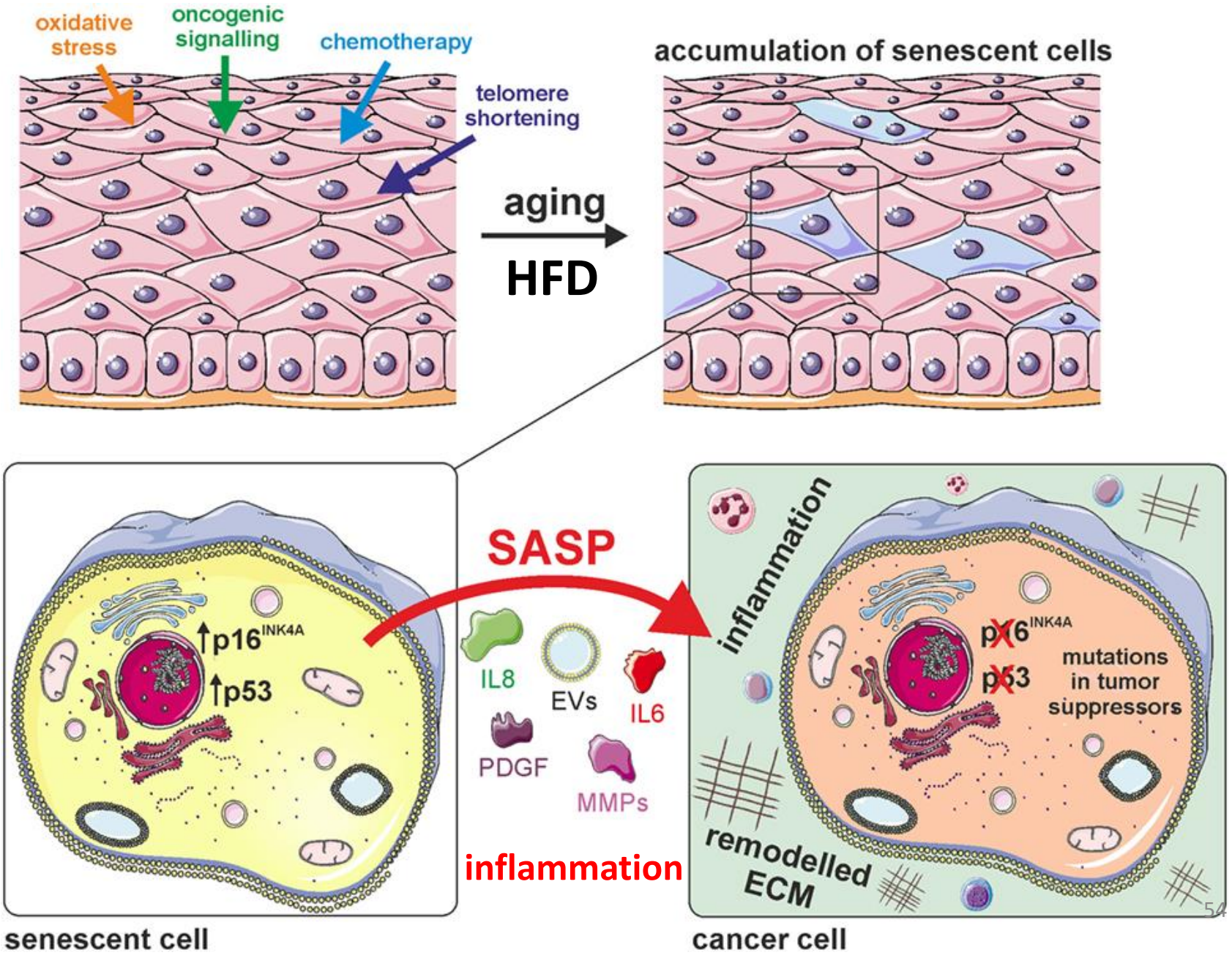


Stress-induced senescence and NAFLD induction in young mice



NAFLD / NASH to HCC transition

Senescence-induced pro-tumorigenic microenvironment in NASH



Several interesting studies but..... mostly several open questions

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ORIGINAL ARTICLE

Aging Cell WILEY

Liver osteopontin is required to prevent the progression of age-related nonalcoholic fatty liver disease

Clinics and Research in Hepatology and Gastroenterology (2020) 44, 513–523

ORIGINAL ARTICLE

Active vitamin D impedes the progression of non-alcoholic fatty liver disease by inhibiting cell senescence in a rat model

RESEARCH ARTICLE

Molecular Nutrition Food Research
www.mnf-journal.com

Saturated Fatty Acids Promote Hepatocytic Senecence through Regulation of miR-34a/Cyclin-Dependent Kinase 6

Experimental Cell Research
journal homepage: www.elsevier.com/locate/yexcr

Lipid accumulation-induced hepatocyte senescence regulates the activation of hepatic stellate cells through the Nrf2-antioxidant response element pathway

SCIENTIFIC REPORTS

OPEN

IGF-I induces senescence of hepatic stellate cells and limits fibrosis in a p53-dependent manner

Senescence of Activated Stellate Cells Limits Liver Fibrosis

Cell

Valery Krizhanovsky,[1] Monica Yon,[1,3] Ross A. Dickins,[1] Stephen Hearn,[1] Janelle Simon,[1,2] Cornelius Miething,[1] Herman Yee,[4] Lars Zender,[1,5] and Scott W. Lowe[1,2,*]

cause or consequence ?
Age vs metabolic dysregulation ?
Immune senescence? – Other cell
Senescence and fibrosis ?
Senescence and HCC ?
Senolytics and HCC chemotherapy ?

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DZD German Center for Diabetes Research

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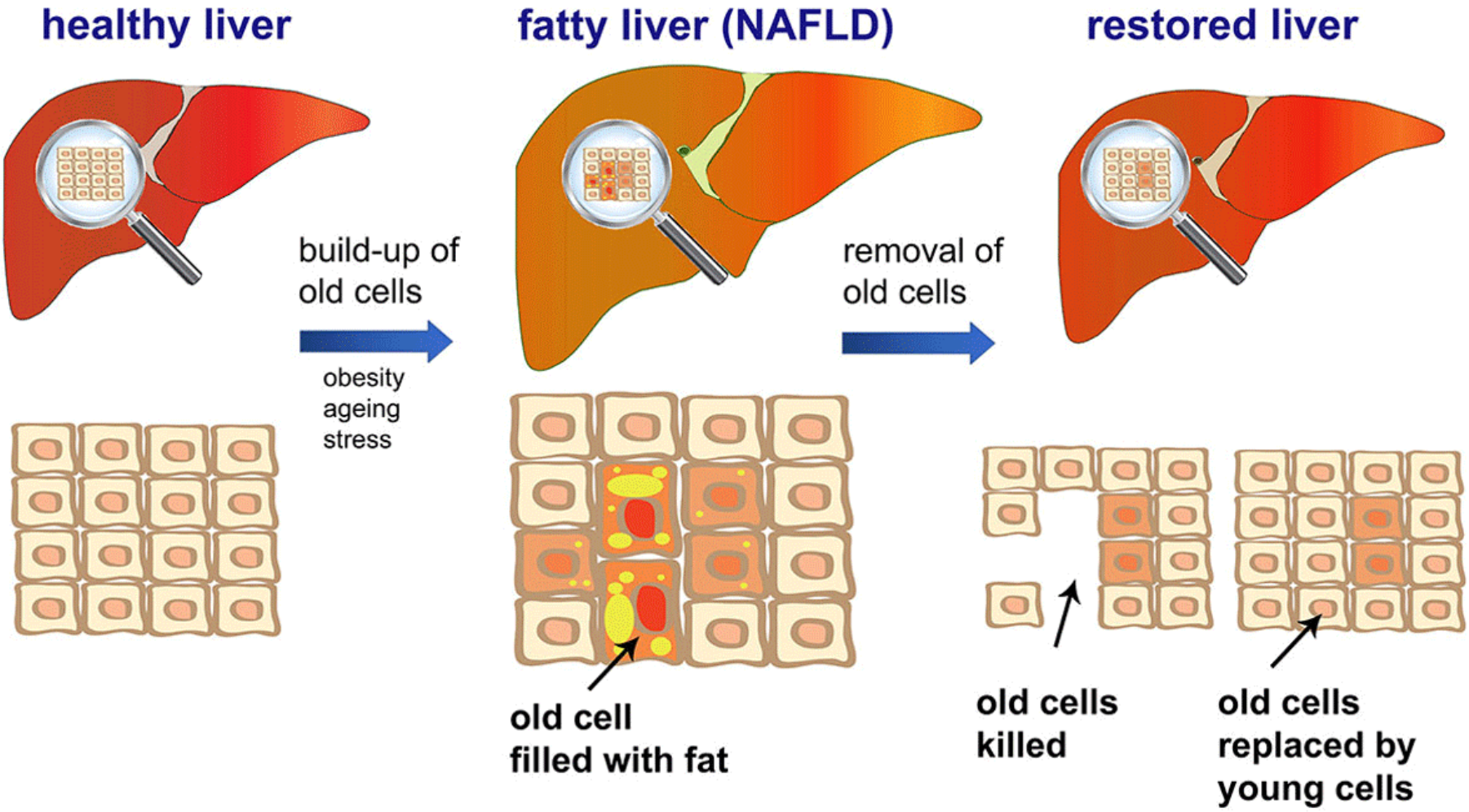
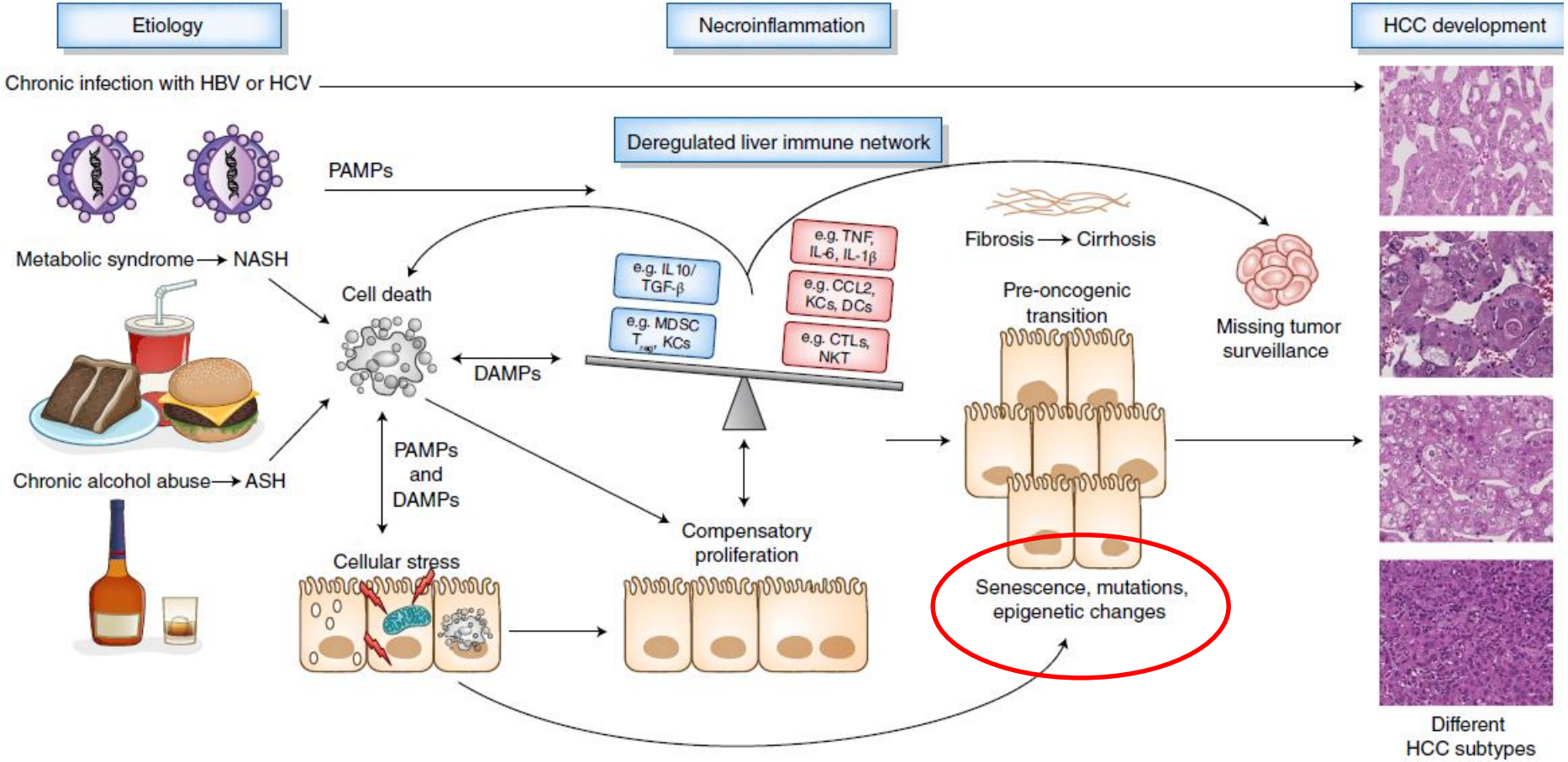
H.F.R.I. Hellenic Foundation for Research & Innovation

EFSD European Foundation for the Study of Diabetes

Thank you for your attention !!


healthy liver
fatty liver (NAFLD)
restored liver
build-up of old cells
obesity
ageing
stress
removal of old cells
old cell filled with fat
old cells killed
old cells replaced by young cells

NAFLD / NASH to HCC transition



Ringelhan et al Nat. Immunol 2018