Immunosenescenza: cosa è e quanto pesa sulla vita dei nostri pazienti

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LIFE EXPECTANCY

homo sapiens life expectancy at birth **< 30 years**

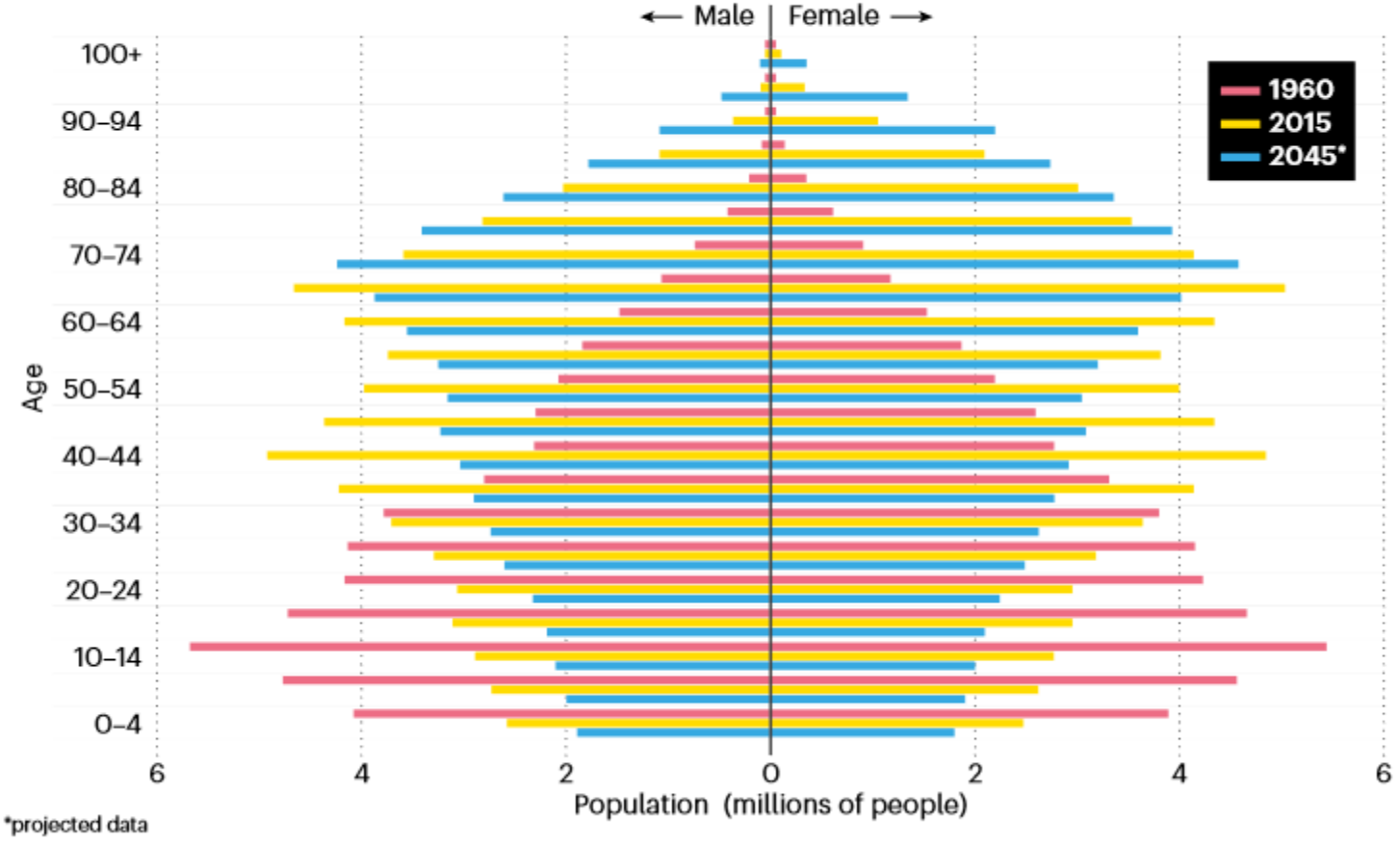
In **Italy** in the **last 4 generations**

From	43y (F)	40y (M)
To	85y (F)	80y (M)

in 2050 >85y about 6.000.000

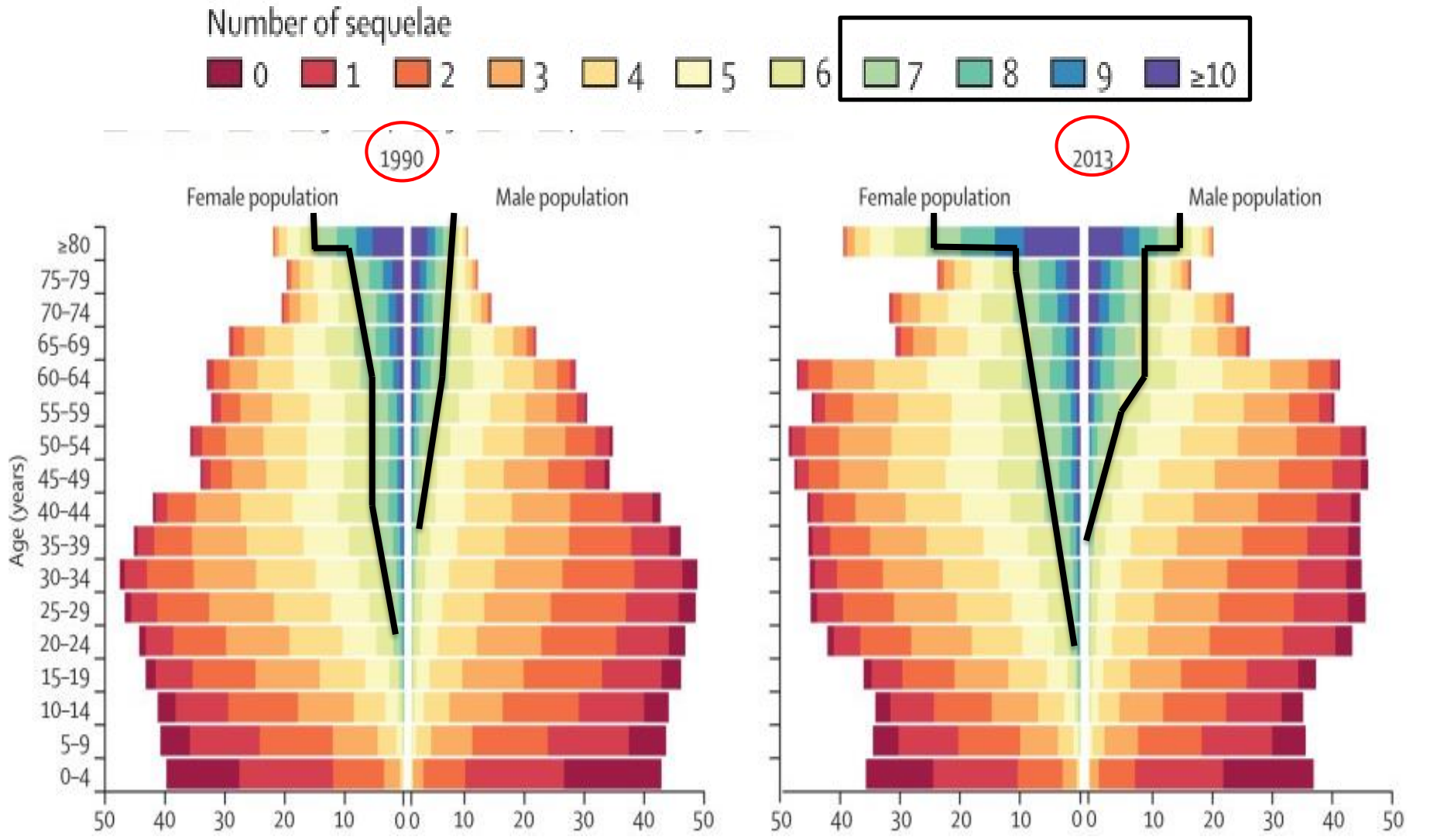
JAPAN'S SUPER-AGED SOCIETY

Japan's demographics are changing rapidly, creating a top-heavy pyramid with more older people than younger people.



*projected data

LIFE EXPECTANCY AND PATHOLOGIES



Population pyramids for developed countries [mln of people]

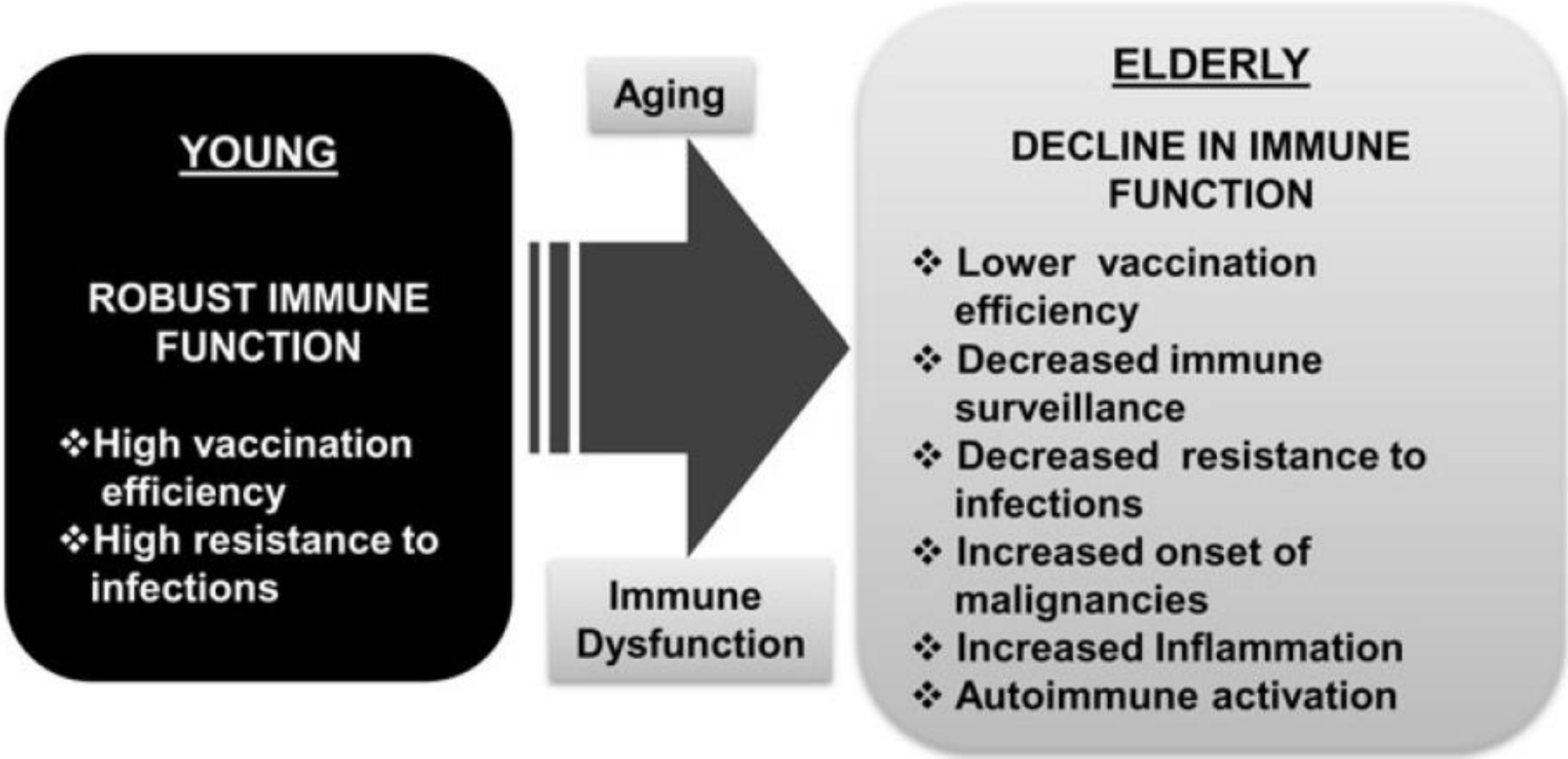
Lancet 2015; 386, 743-800

Pathologies seen in the elderly

Aged Individuals have:

- Increased incidence of INFECTIONS:
  pneumonia, influenza, meningitis, urinary tract infections
- Increased incidence of AUTOIMMUNE DISEASE:
  rheumatoid arthritis, lupus, hepatitis, multiple sclerosis
- Increased incidence of CANCER:
  prostate, breast, lung, colon/, bladder, skin, leukemia, pancreas
- Increased incidence of METABOLIC CONDITIONS:
  hypertension, diabetes, congestive hart failure

IMMUNE SENESCENCE



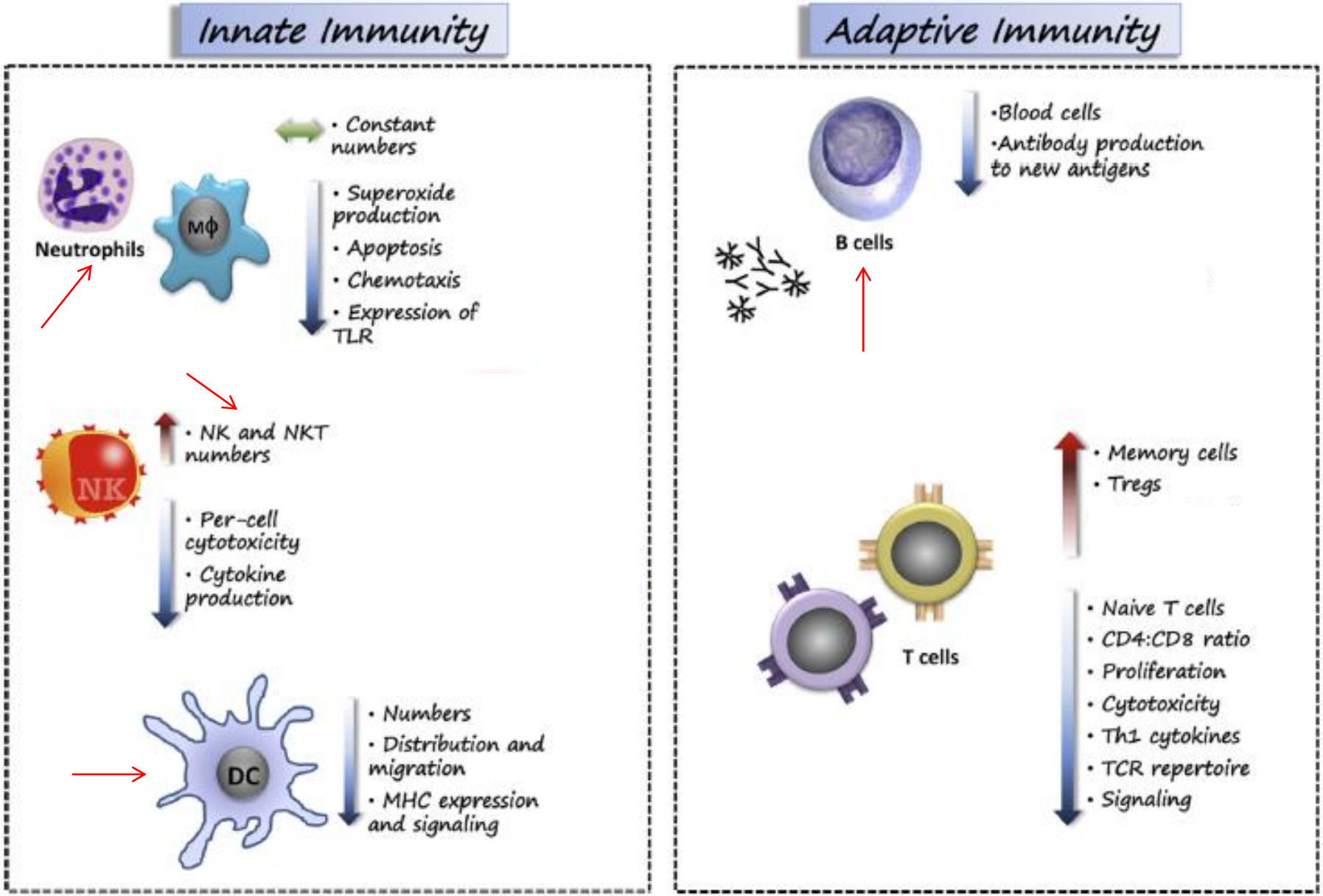
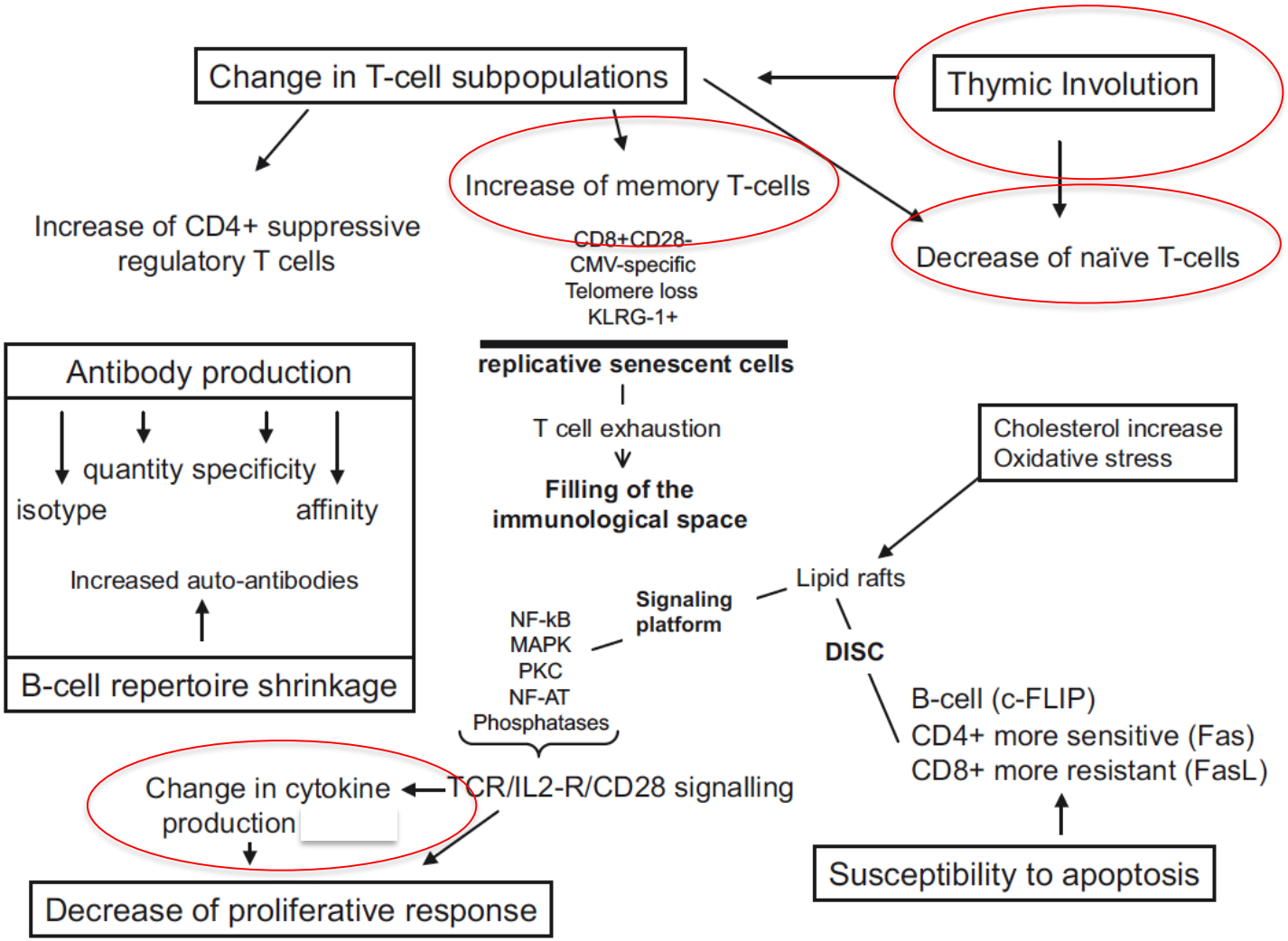


Fig. 1. Summary of major changes reported for human immunosenescence.
DC, dendritic cell; MHC, major histocompatibility complex; TLR, Toll-like receptors; NK, natural killer; Th, helper T cell; TCR, T-cell receptor; Treg, regulatory T cell.

The immune system in the elderly: the functions and parameters which change during aging



Fulop T et al, Clin Interv Aging, 2007

Changes in cytokines production in aging, an extremely complex phenomenon:
Inflammaging

A chronic and smouldering inflammation that characterizes aging and, when excessive, is associated with “accelerated, unhealthy aging”

BALANCE BETWEEN INFLAMMAGING AND ANTI-INFLAMMAGING

Arch. Immunol. Ther. Exp.
DOI 10.1007/s00005-015-0377-3

REVIEW

Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity

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Received: 16 June 2015 / Accepted: 2 November 2015

Abstract Longevity and aging are two sides of the same coin, as they both derive from the interaction between genetic and environmental factors. Aging is a complex, dynamic biological process characterized by continuous remodeling. One of the most recent theories on aging focuses on immune response, and takes into consideration the activation of subclinical chronic low-grade inflammation which as mediators of cytokines. We believe that if inflammaging is a key to understand aging, anti-inflammaging may be one of the secrets of longevity.

Keywords Inflammaging · Anti-inflammaging · Cytokine · Aging · Longevity

The "weight" of pro and anti-inflammatory cytokines in aging and longevity.

↓

Pro-inflammatory cytokines= frailty, age-related diseases, reduced life expectancy.

Balance between pro and anti-inflammatory cytokines= adaptation to the conditions of life, avoidance or delayed onset of diseases, longevity

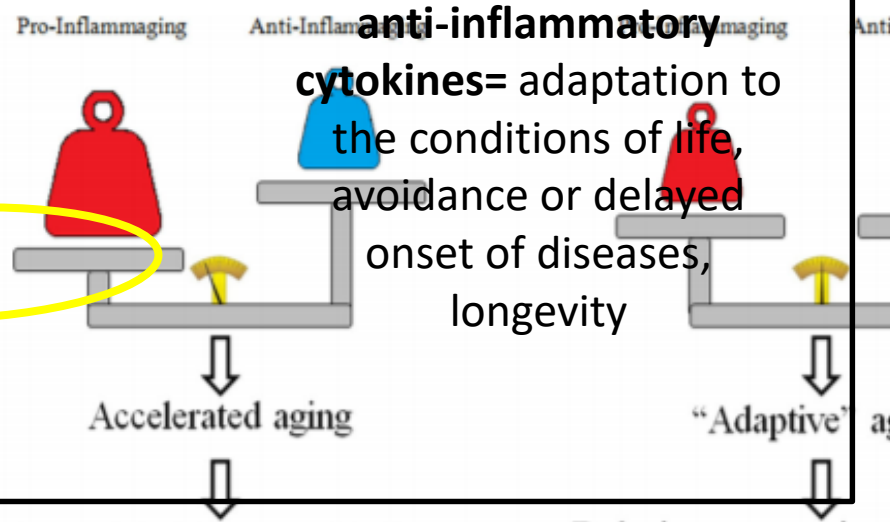


Fig. 1 The "weight" of pro- and anti-inflammatory cytokines in aging and longevity. Increase in pro-inflammatory cytokines promotes frailty and age-related diseases, and reduces life expectancy. The balance between pro-inflammaging and anti-inflammaging favors adaptation to the conditions of life, allows avoidance of diseases or delays onset, and leads to longevity

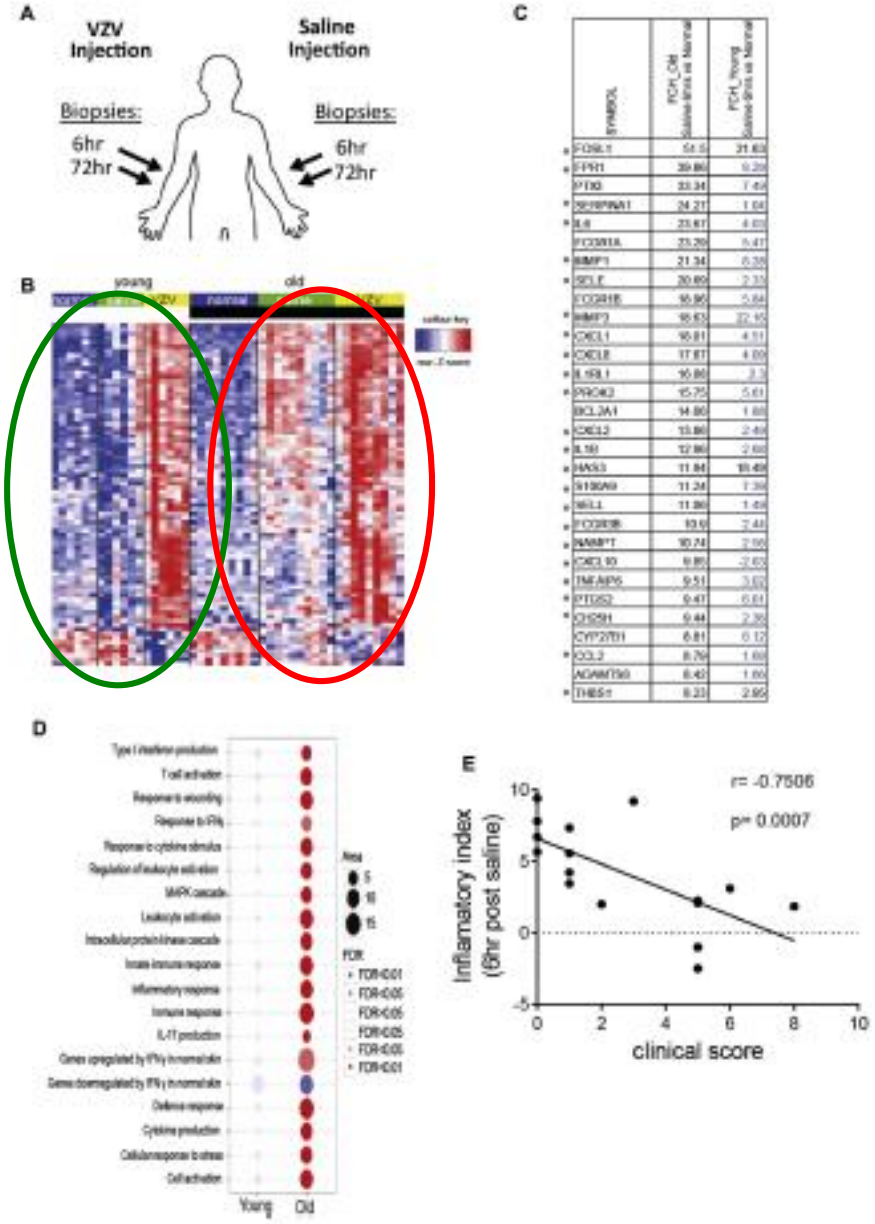
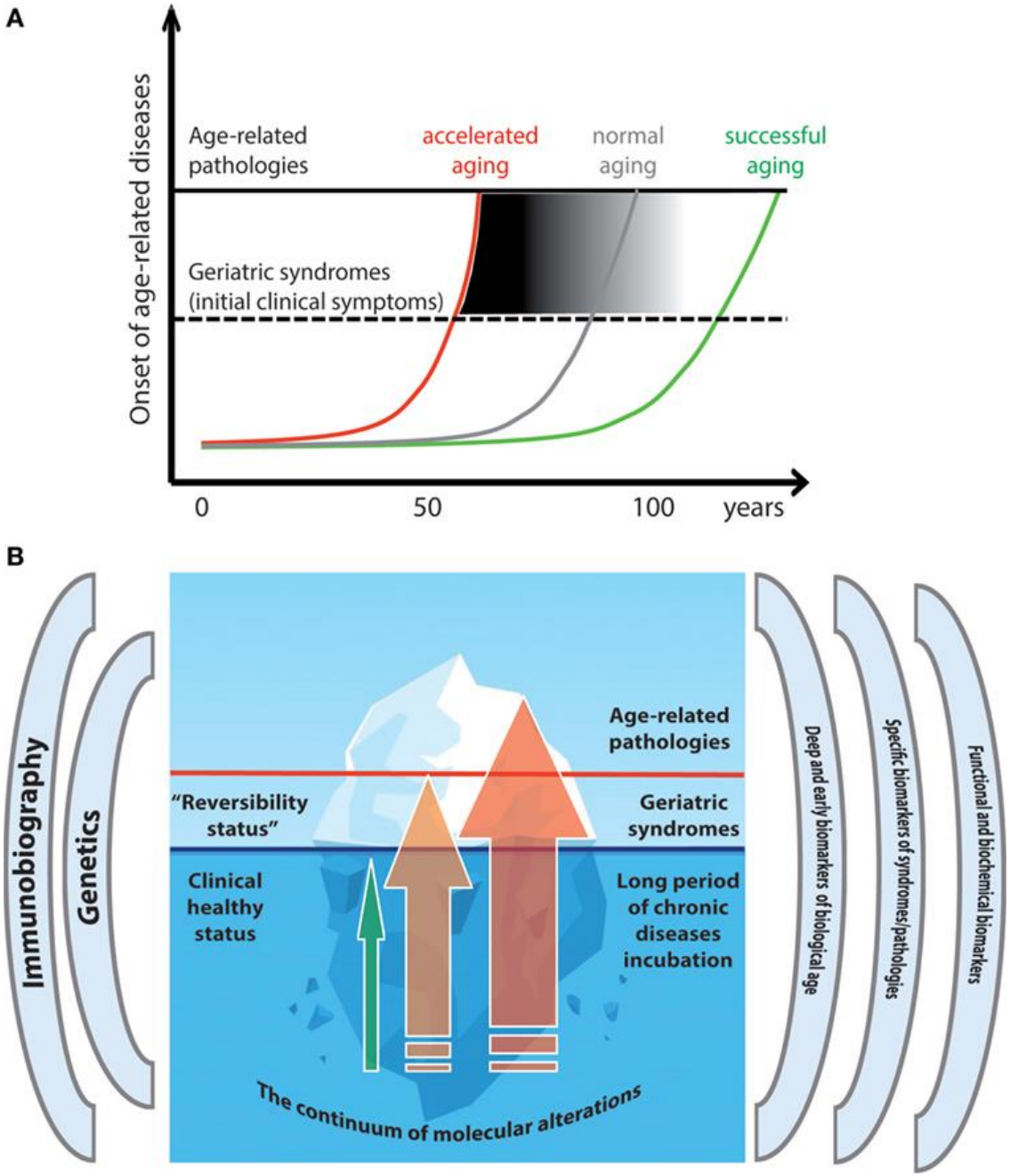
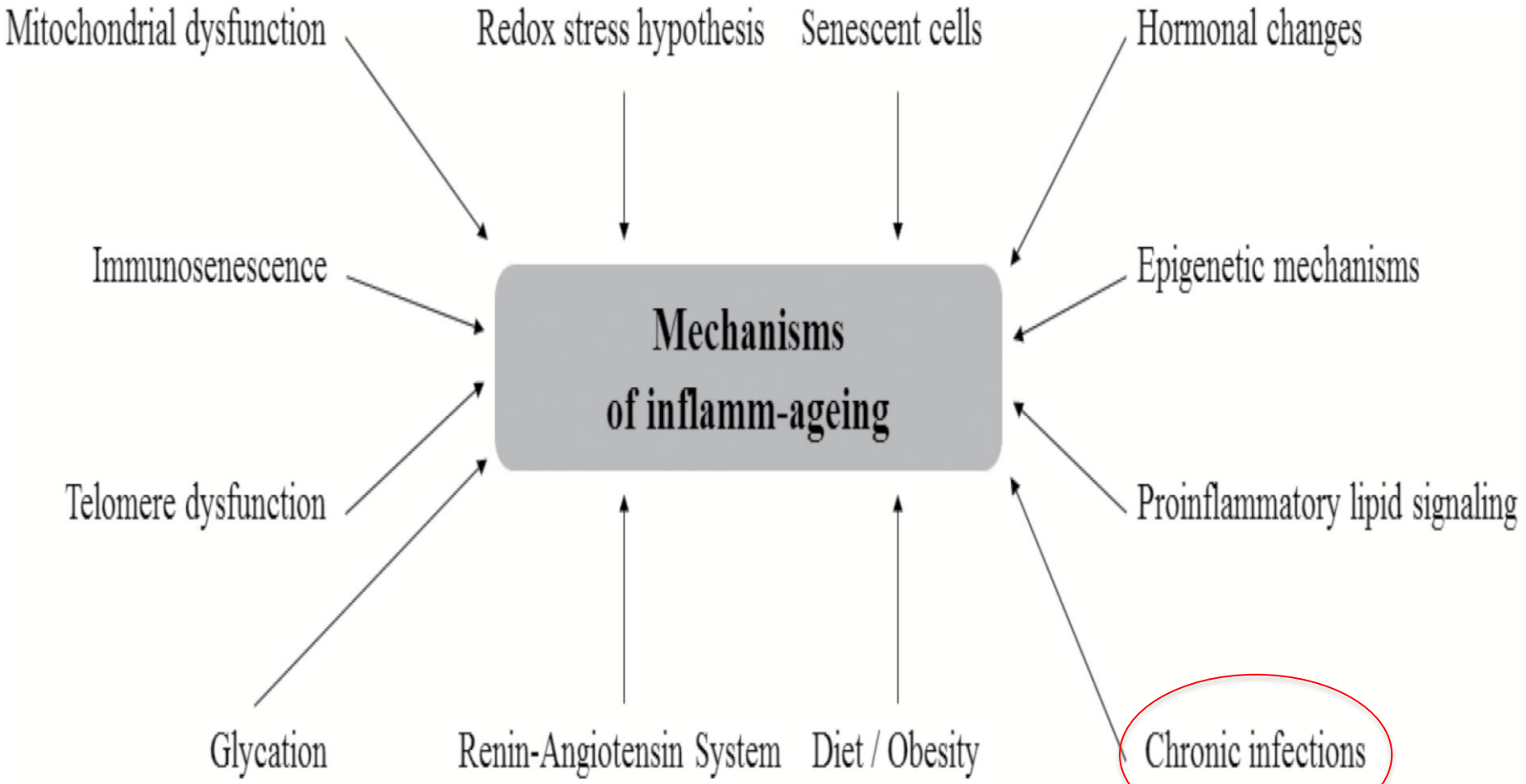


FIG 4. Comparison of global gene expression between normal, saline-injected, and VZV antigen-injected skin. A, Schematic representation of biopsy collection for transcriptional analysis. B, Heat map showing relative expression of DEGs (fold change [FCM] > 2 and false discovery rate > 0.05) between normal skin and saline-injected skin at 6 hours after treatment in young (*left*) and old (*right*) subjects. C, The table shows the top 20 upregulated genes at 6 hours in saline-injected skin from old and young subjects compared with normal skin. Genes not reaching statistical significance are indicated in blue. Asterisks indicate genes

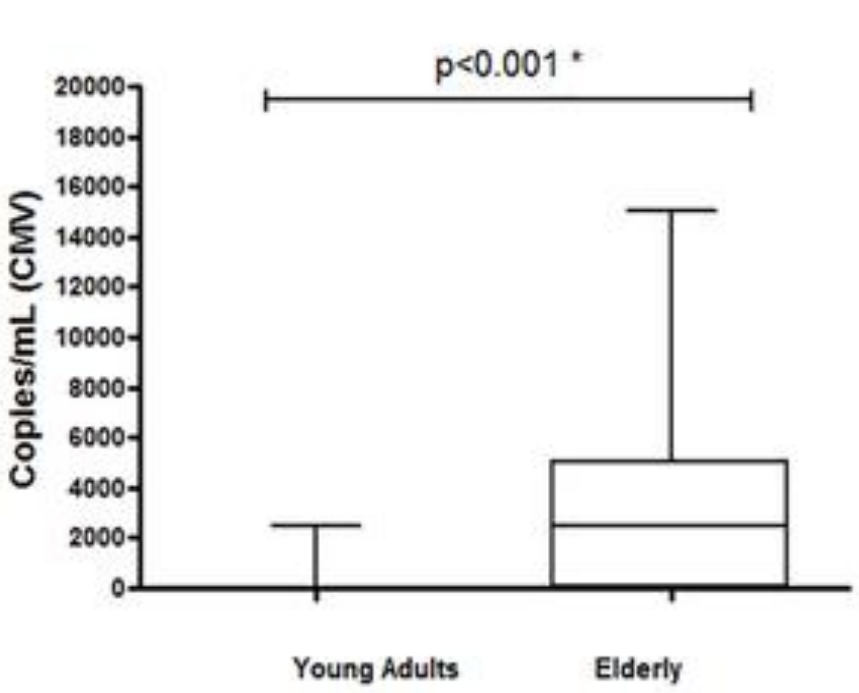

A
Onset of age-related diseases
Age-related pathologies
accelerated aging
normal aging
successful aging
Geriatric syndromes (initial clinical symptoms)
0
50
100
years
B
Immunobiography
Genetics
Age-related pathologies
"Reversibility status"
Geriatric syndromes
Clinical healthy status
Long period of chronic diseases incubation
The continuum of molecular alterations
Deep and early biomarkers of biological age
Specific biomarkers of syndromes/pathologies
Functional and biochemical biomarkers

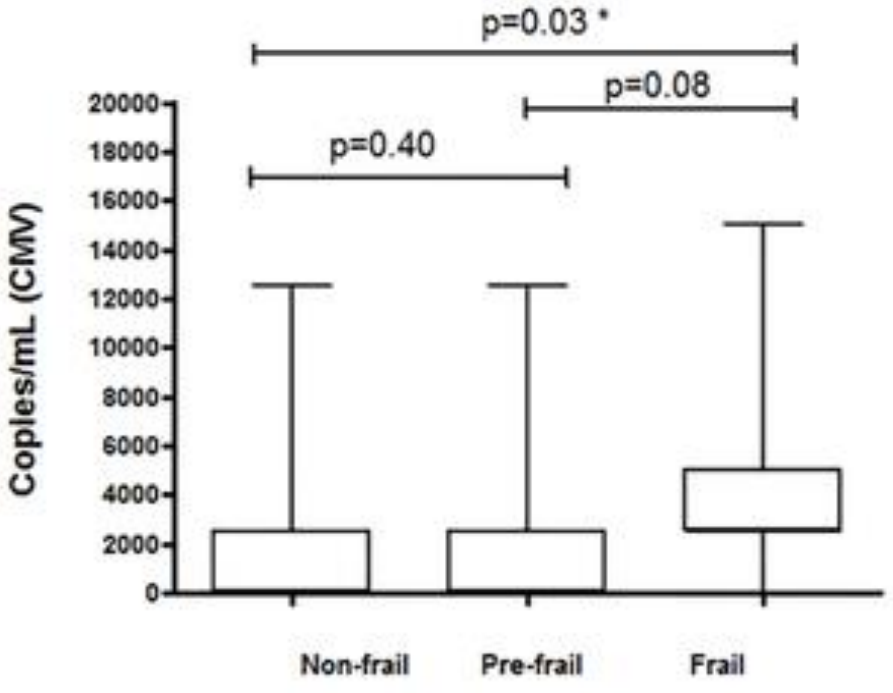


From: Chronic Inflammation: Accelerator of Biological Aging

J Gerontol A Biol Sci Med Sci. 2016;72(9):1218-1225. doi:10.1093/gerona/glw240

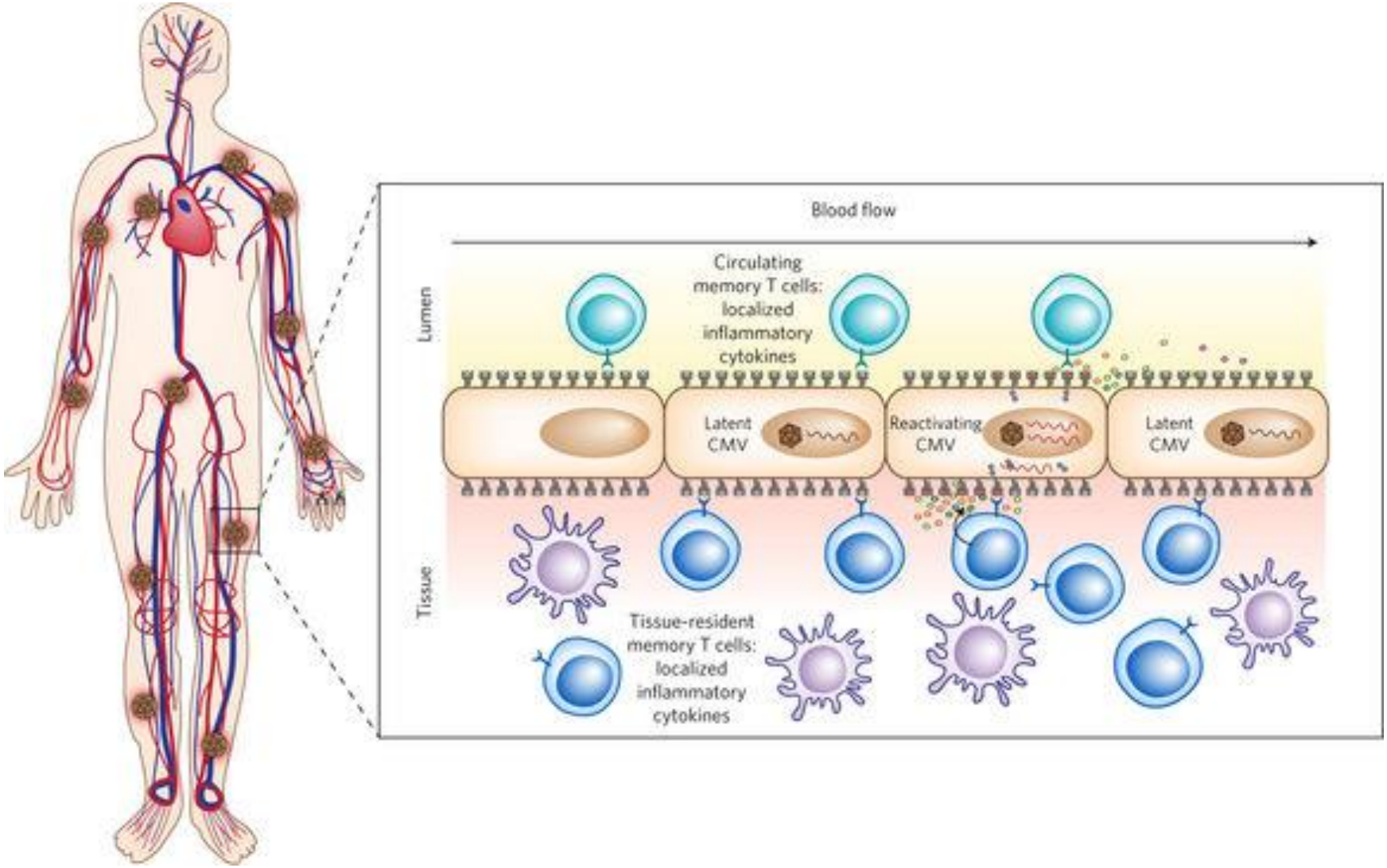
CMV GENOME (COPIES/ML) IN YOUNG AND IN ELDERLY INDIVIDUALS

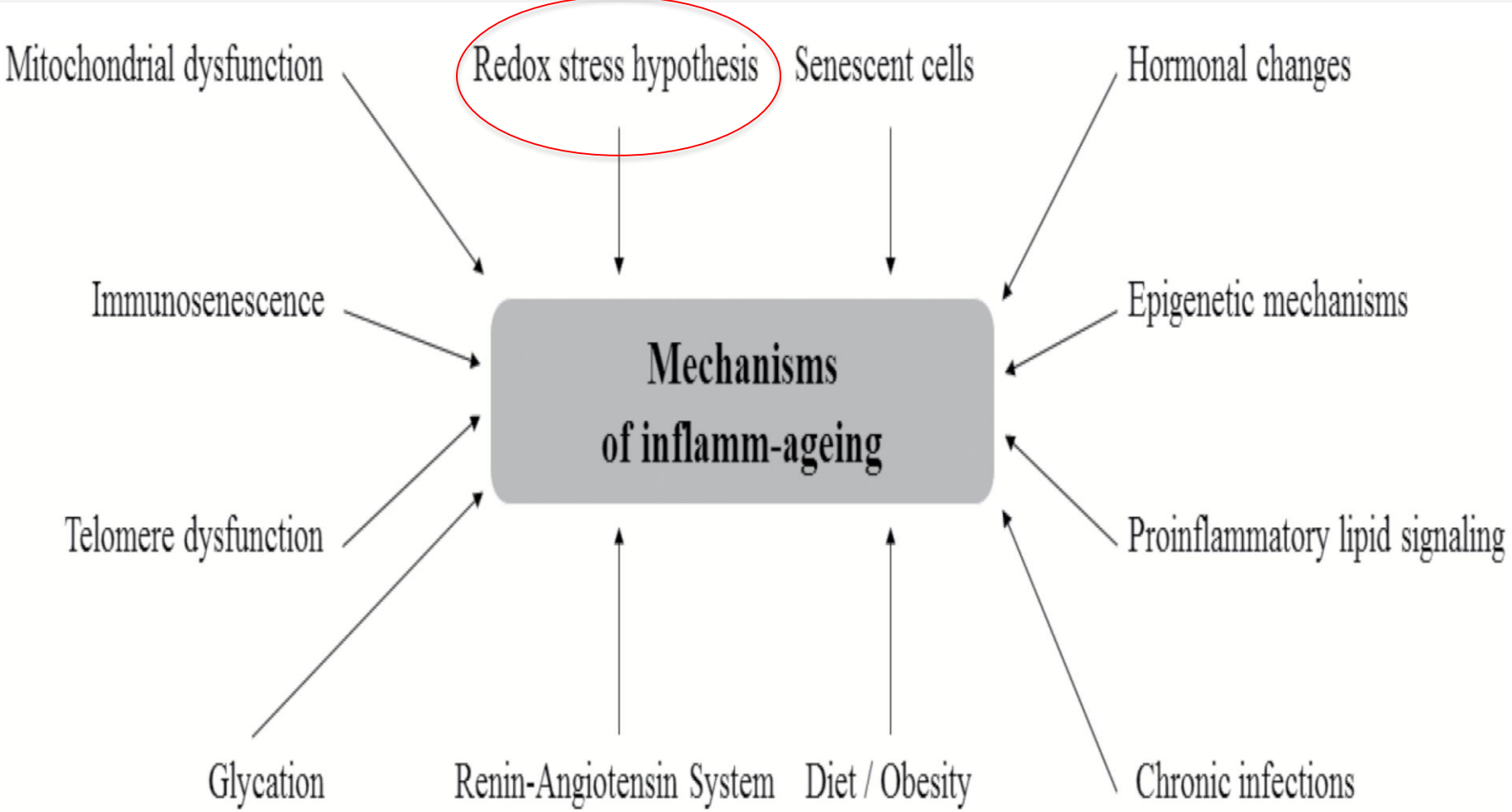




Thomasini RL, Pereira DS, Pereira FSM, Mateo EC, Mota TN, et al. (2017) Aged-associated cytomegalovirus and Epstein-Barr virus reactivation and cytomegalovirus relationship with the frailty syndrome in older women. PLOS ONE 12(7): e0180841. https://doi.org/10.1371/journal.pone.0180841
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0180841

Systemic and tissue-specific consequences of CMV latency, micro-reactivation and full reactivation.





From: Chronic Inflammation: Accelerator of Biological Aging

J Gerontol A Biol Sci Med Sci. 2016;72(9):1218-1225. doi:10.1093/gerona/glw240

Reactive oxygen species (ROS)

- Intermediates of cell metabolism
- Abnormal accumulation of ROS → oxidative stress
- Oxidative stress → cell damage and accelerated cell aging
- ROS increase in aging

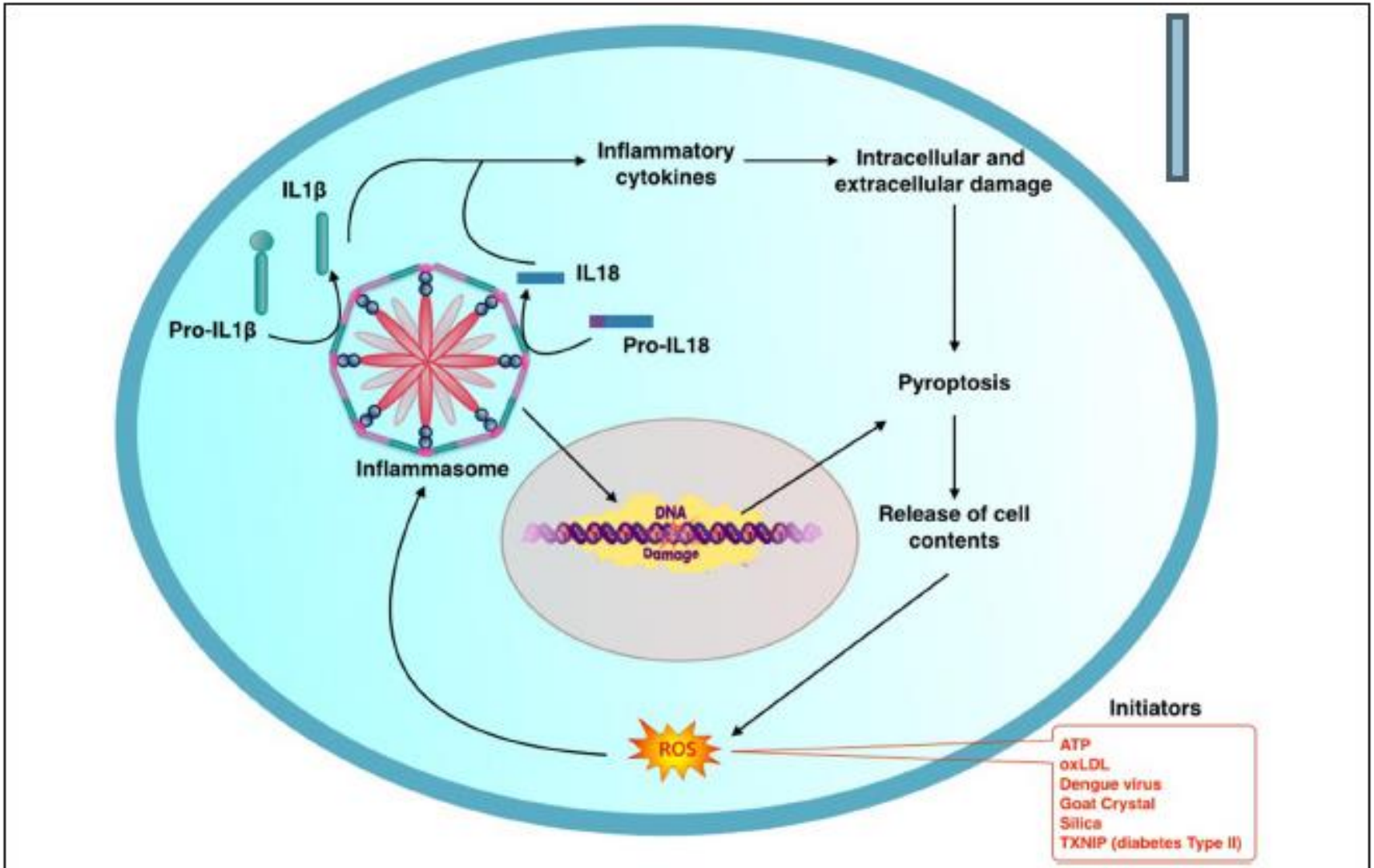
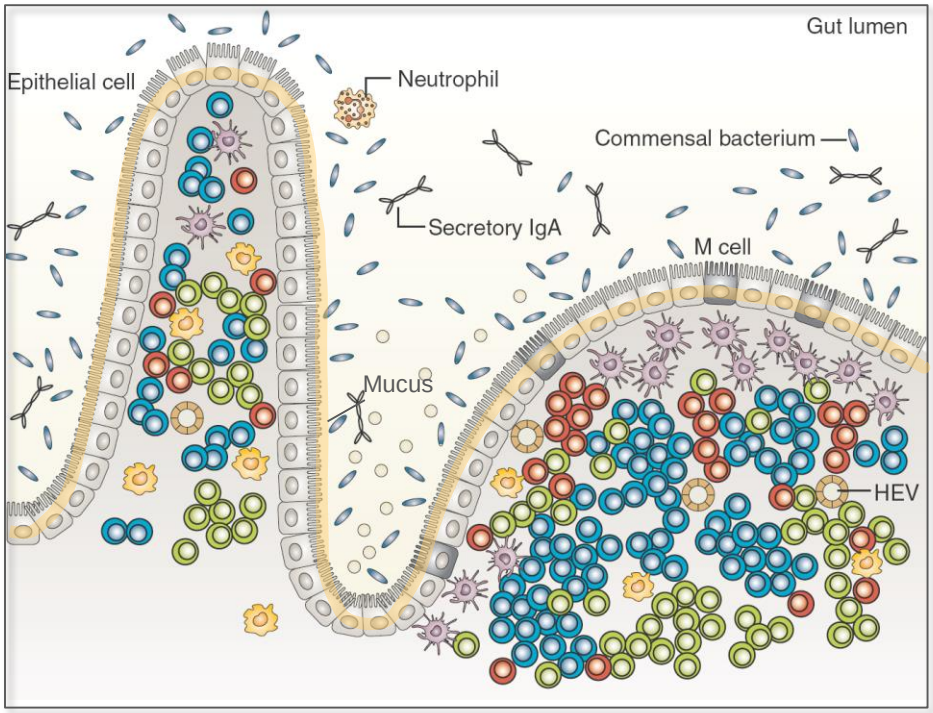
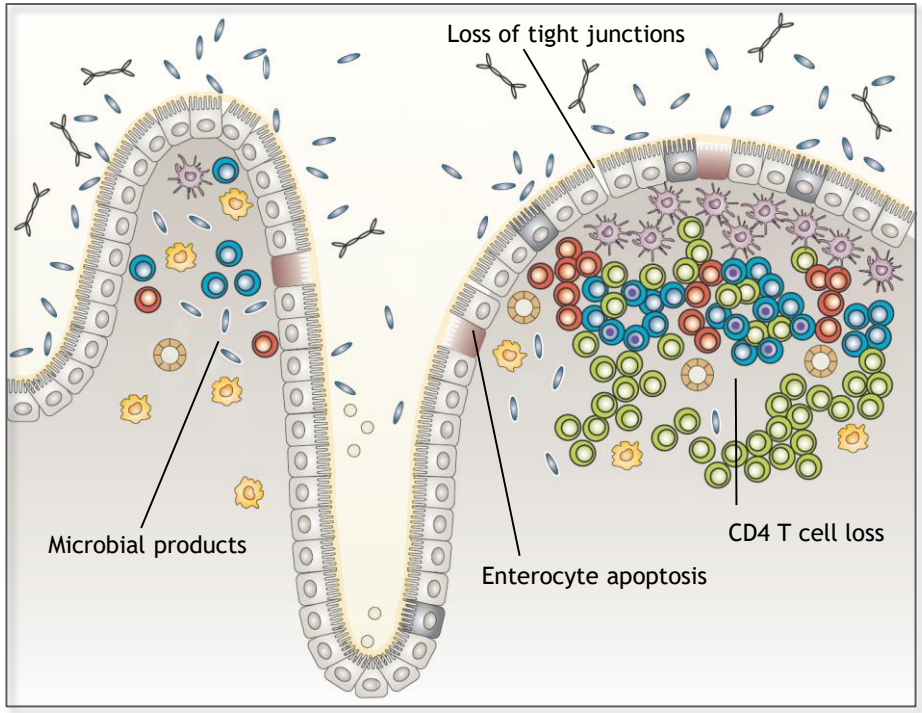


FIGURE 1 | General schema describing the process of activation of inflammasome: initiating factors activate production of reactive oxygen species (ROS) which in turntriggers the inflammasome mediated inflammatory cascade. Oligomerization of components results in assembly of Inflammasome. This in turn activates Il-1β and Il-18 through caspase-1. NLRP3 Inflammasome promotes oxidative DNA damage. Inflammation and DNA damage culminates in pyroptosis releasing contents from the damaged cell. This in turn promotes a vicious cycle of further Inflammasome mediated pathogenic process.

A role for gut alterations in inflammaging?

Aging in the GI Tract





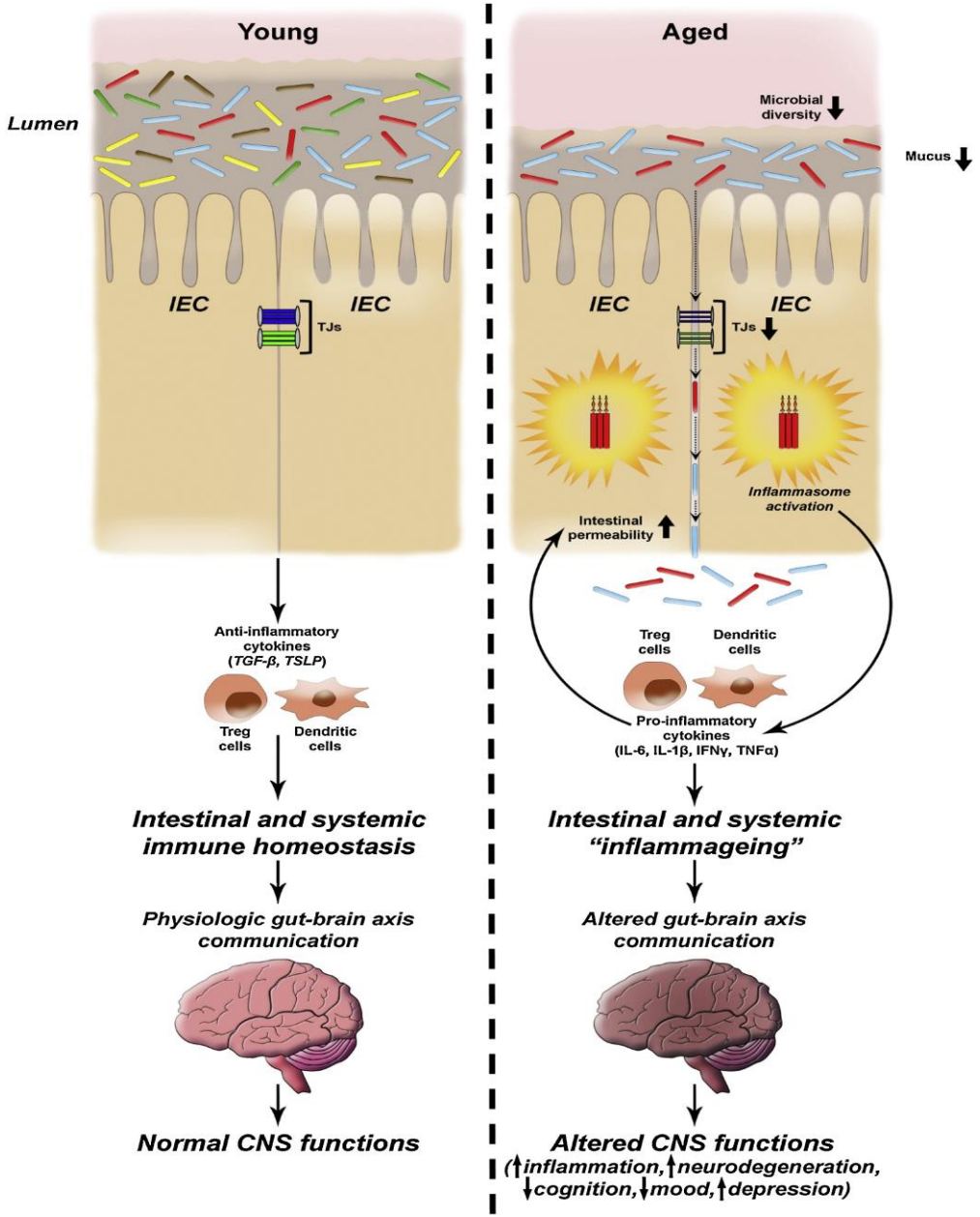
Young Gut

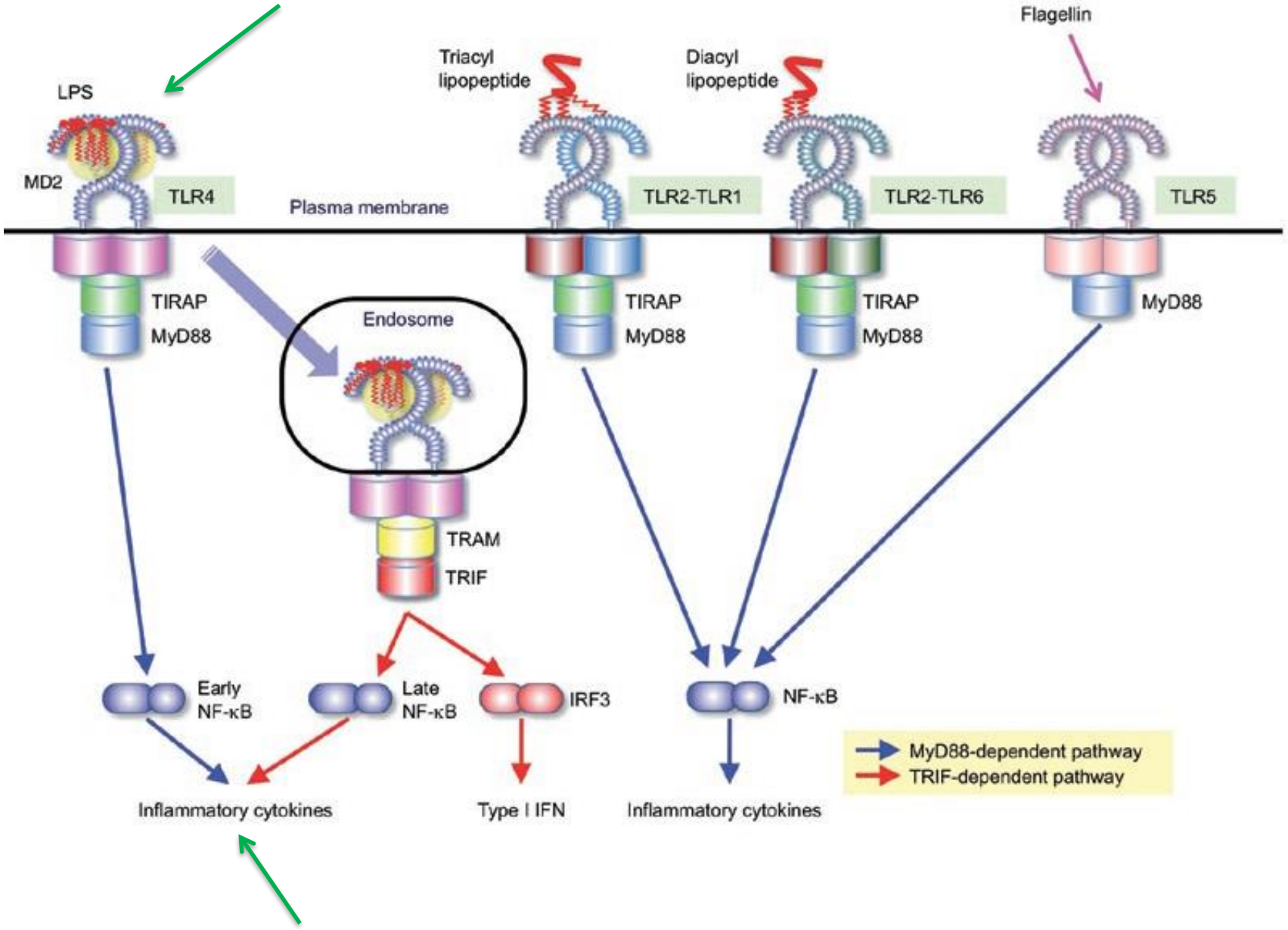
- Tight epithelial junctions, mucus
- Anti-microbial peptides, Abs, cells
- Cross-talk between microbes and epithelial cells and immune cells

Aged Gut

- Fibrosis
- Increased permeability
- **Translocation of microbial products (LPS)** ?
- **Systemic immune activation ?**

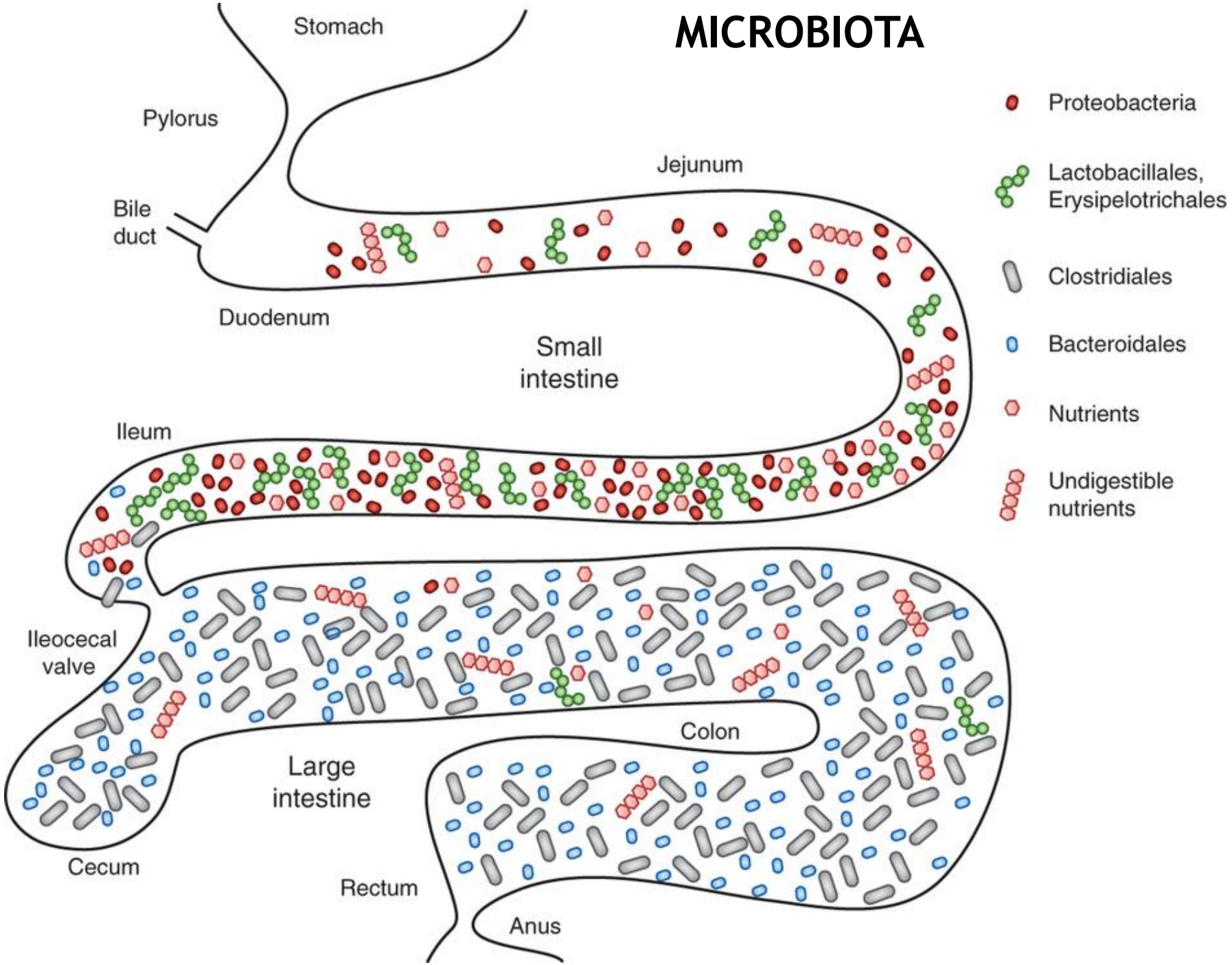
Altered gut permeability and inflammaging

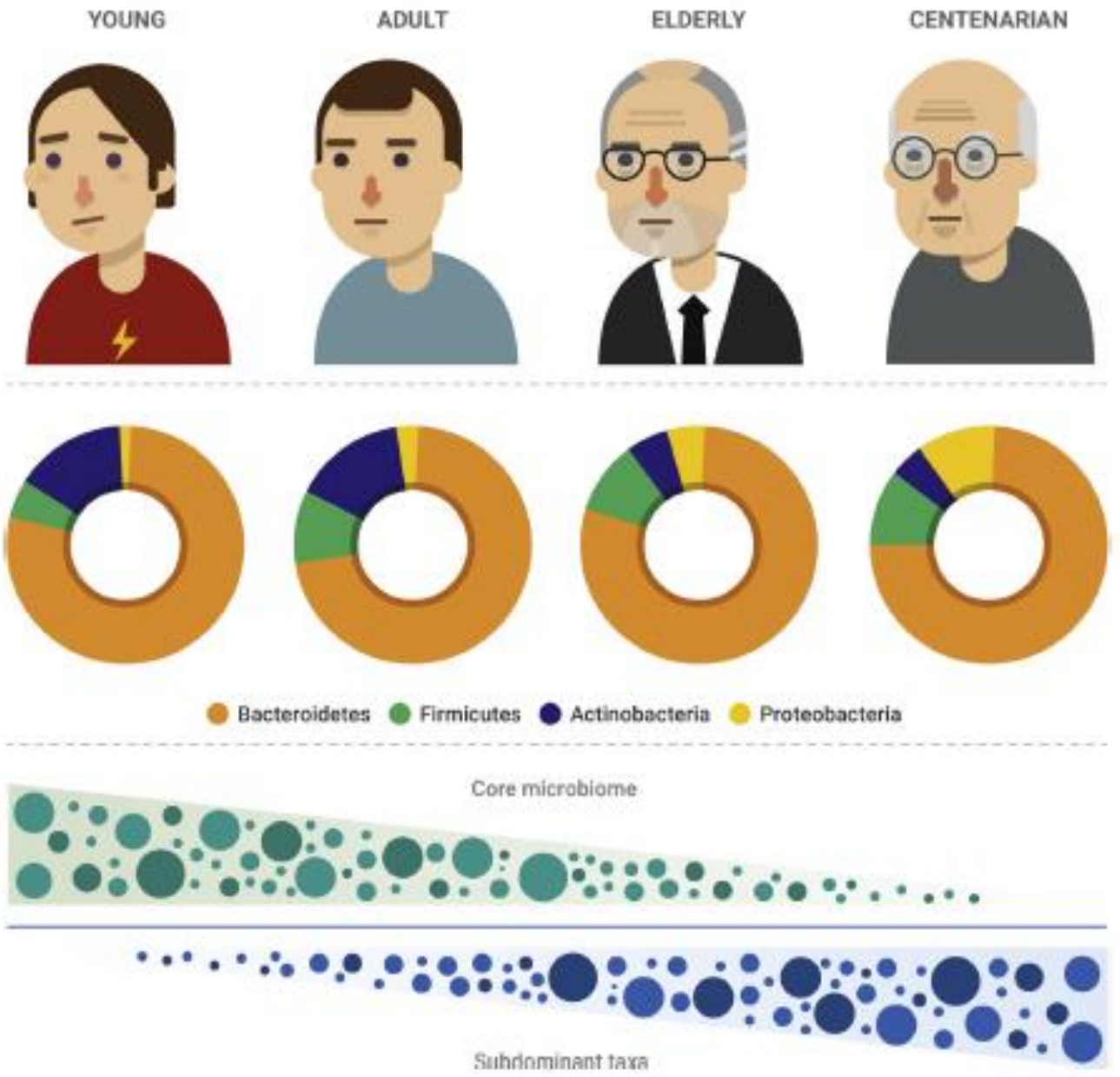



LPS
MD2
TLR4
Plasma membrane
Triacyl lipopeptide
TLR2-TLR1
Diacyl lipopeptide
TLR2-TLR6
Flagellin
TLR5
TIRAP
MyD88
Endosome
TRAM
TRIF
Early NF-κB
Late NF-κB
IRF3
NF-κB
Inflammatory cytokines
Type I IFN
Inflammatory cytokines
MyD88-dependent pathway
TRIF-dependent pathway

A role for alterations of the microbiota in inflammaging?

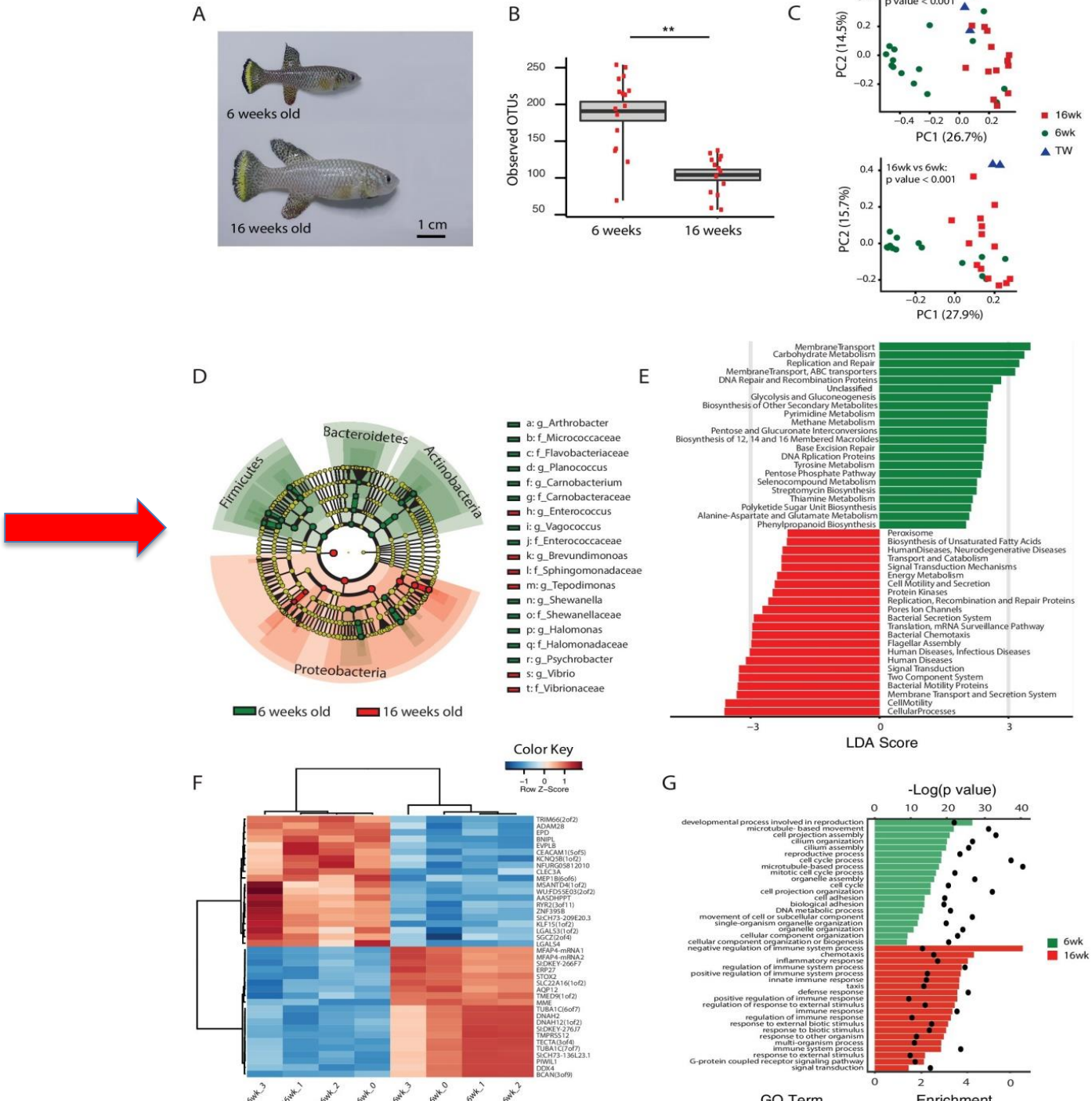
MICROBIOTA

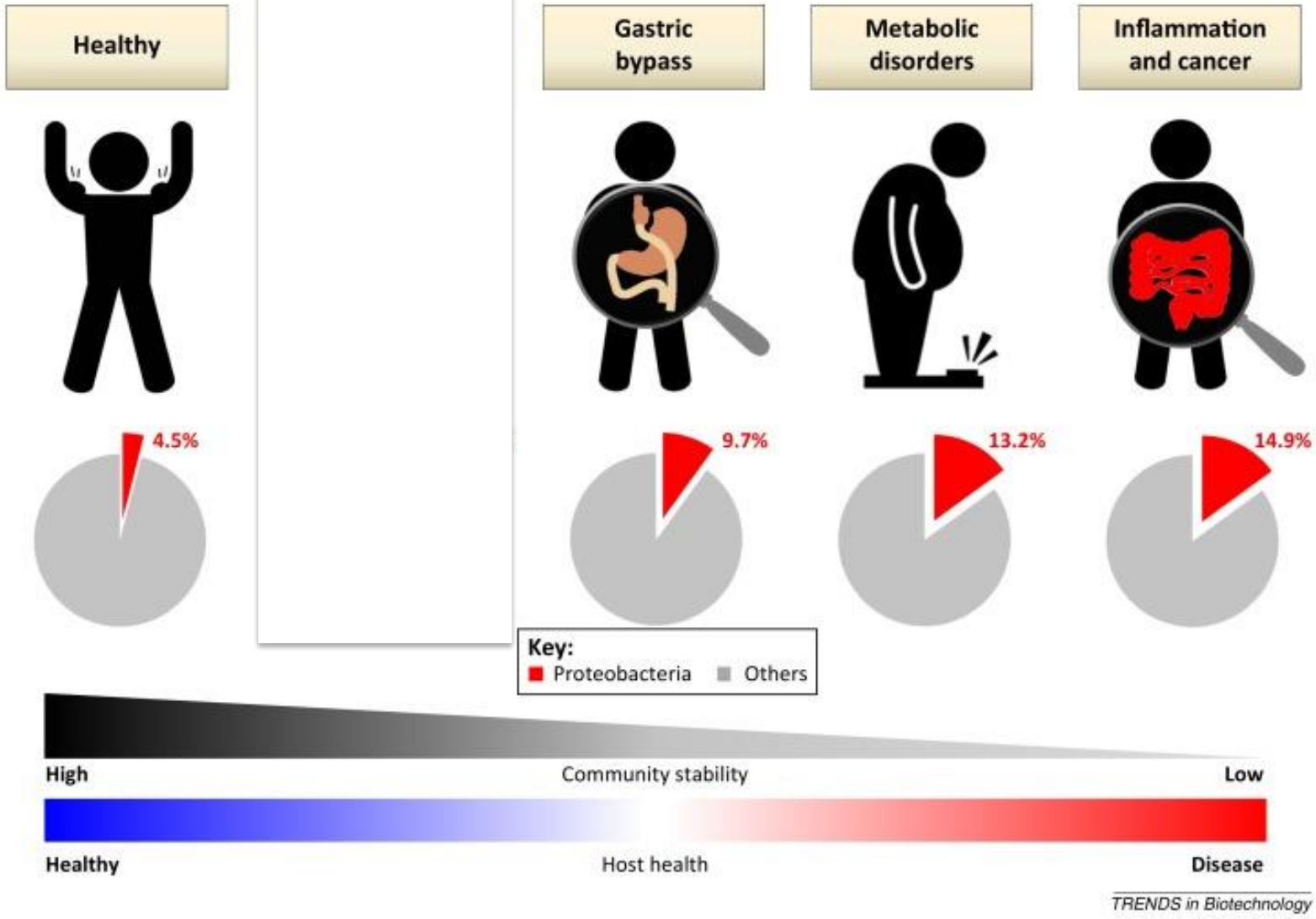

Stomach
Pylorus
Bile duct
Jejunum
Duodenum
Small intestine
Ileum
Ileocecal valve
Colon
Large intestine
Cecum
Rectum
Anus
Proteobacteria
Lactobacillales, Erysipelotrichales
Clostridiales
Bacteroidales
Nutrients
Undigestible nutrients



B ↓
F = o ↓
A ↓
P ↑

Fig. 1. Age-associated changes in human intestinal microbiota composition.


A
6 weeks old
16 weeks old
1 cm
B
**
Observed OTUs
250
200
150
100
50
6 weeks
16 weeks
C
16wk vs 6wk:
p value < 0.001
PC2 (14.5%)
PC1 (26.7%)
16wk
6wk
TW
16wk vs 6wk:
p value < 0.001
PC2 (15.7%)
PC1 (27.9%)
D
Bacteroidetes
Actinobacteria
Firmicutes
Proteobacteria
a: g_Arthrobacter
b: f_Micrococcaceae
c: f_Flavobacteriaceae
d: g_Planococcus
f: g_Carnobacterium
g: f_Carnobacteraceae
h: g_Enterococcus
i: g_Vagococcus
j: f_Enterococcaceae
k: g_Brevundimonoas
l: f_Sphingomonadaceae
m: g_Tepodimonas
n: g_Shewanella
o: f_Shewanellaceae
p: g_Halomonas
q: f_Halomonadaceae
r: g_Psychrobacter
s: g_Vibrio
t: f_Vibrionaceae
6 weeks old
16 weeks old
E
MembraneTransport
Carbohydrate Metabolism
Replication and Repair
MembraneTransport, ABC transporters
DNA Repair and Recombination Proteins
Unclassified
Glycolysis and Gluconeogenesis
Biosynthesis of Other Secondary Metabolites
Pyrimidine Metabolism
Methane Metabolism
Pentose and Glucuronate Interconversions
Biosynthesis of 12, 14 and 16 Membered Macrolides
Base Excision Repair
DNA Rplication Proteins
Tyrosine Metabolism
Pentose Phosphate Pathway
Selenocompound Metabolism
Streptomycin Biosynthesis
Thiamine Metabolism
Polyketide Sugar Unit Biosynthesis
Alanine-Aspartate and Glutamate Metabolism
Phenylpropanoid Biosynthesis
Peroxisome
Biosynthesis of Unsaturated Fatty Acids
HumanDiseases, Neurodegenerative Diseases
Transport and Catabolism
Signal Transduction Mechanisms
Energy Metabolism
Cell Motility and Secretion
Protein Kinases
Replication, Recombination and Repair Proteins
Pores Ion Channels
Bacterial Secretion System
Translation, mRNA Surveillance Pathway
Bacterial Chemotaxis
Flagellar Assembly
Human Diseases, Infectious Diseases
Human Diseases
Signal Transduction
Two Component System
Bacterial Motility Proteins
Membrane Transport and Secretion System
CellMotility
CellularProcesses
−3
0
3
LDA Score
F
Color Key
−1 0 1
Row Z-Score
TRIM66(2of2)
ADAM28
EPD
BNIPL
EVPLB
CEACAM1(5of5)
KCNQ5B(1of2)
NFURG05812010
CLEC3A
MEP1B(6of6)
MSANTD4(1of2)
WLIFDS5E03(2of2)
AASDHPPT
RYR2(3of11)
ZNF395B
SlCH73-209E20.3
KLF15(1of2)
LGALS3(1of2)
SGCZ(2of4)
LGALS4
MFAP4-mRNA1
MFAP4-mRNA2
SlDKEY-266F7
ERP27
STOX2
SLC22A16(1of2)
AQP12
TMED9(1of2)
MME
TUBA1C(6of7)
DNAH2
DNAH12(1of2)
SlDKEY-276J7
TMPRSS12
TECTA(3of4)
TUBA1C(7of7)
SlCH73-136L23.1
PIWIL1
DDX4
BCAN(3of9)
6wk_3
6wk_1
6wk_2
6wk_0
16wk_3
16wk_0
16wk_1
16wk_2
G
-Log(p value)
0
10
20
30
40
developmental process involved in reproduction
microtubule- based movement
cell projection assembly
cilium organization
cilium assembly
reproductive process
cell cycle process
microtubule-based process
mitotic cell cycle process
organelle assembly
cell cycle
cell projection organization
cell adhesion
biological adhesion
DNA metabolic process
movement of cell or subcellular component
single-organism organelle organization
organelle organization
cellular component organization
cellular component organization or biogenesis
negative regulation of immune system process
chemotaxis
inflammatory response
regulation of immune system process
positive regulation of immune system process
innate immune response
taxis
defense response
positive regulation of immune response
regulation of response to external stimulus
immune response
regulation of immune response
response to external biotic stimulus
response to biotic stimulus
response to other organism
multi-organism process
immune system process
response to external stimulus
G-protein coupled receptor signaling pathway
signal transduction
6wk
16wk
0
2
4
0
GO Term
Enrichment


Healthy
Gastric bypass
Metabolic disorders
Inflammation and cancer
4.5%
9.7%
13.2%
14.9%
Key:
Proteobacteria
Others
High
Community stability
Low
Healthy
Host health
Disease
TRENDS in Biotechnology

Proteobacteria support inflammation via a number of mechanisms including:

1. **the activation of the NLRP3 inflammasome,**
2. **the down regulation of IL-10 production,**
3. **the impairment of Treg activity and**
4. **the stimulation of TH17 differentiation**

Multiple possible causes, one outcome: (unhealthy) aging is associated with a smouldering, chronic degree on inflammation

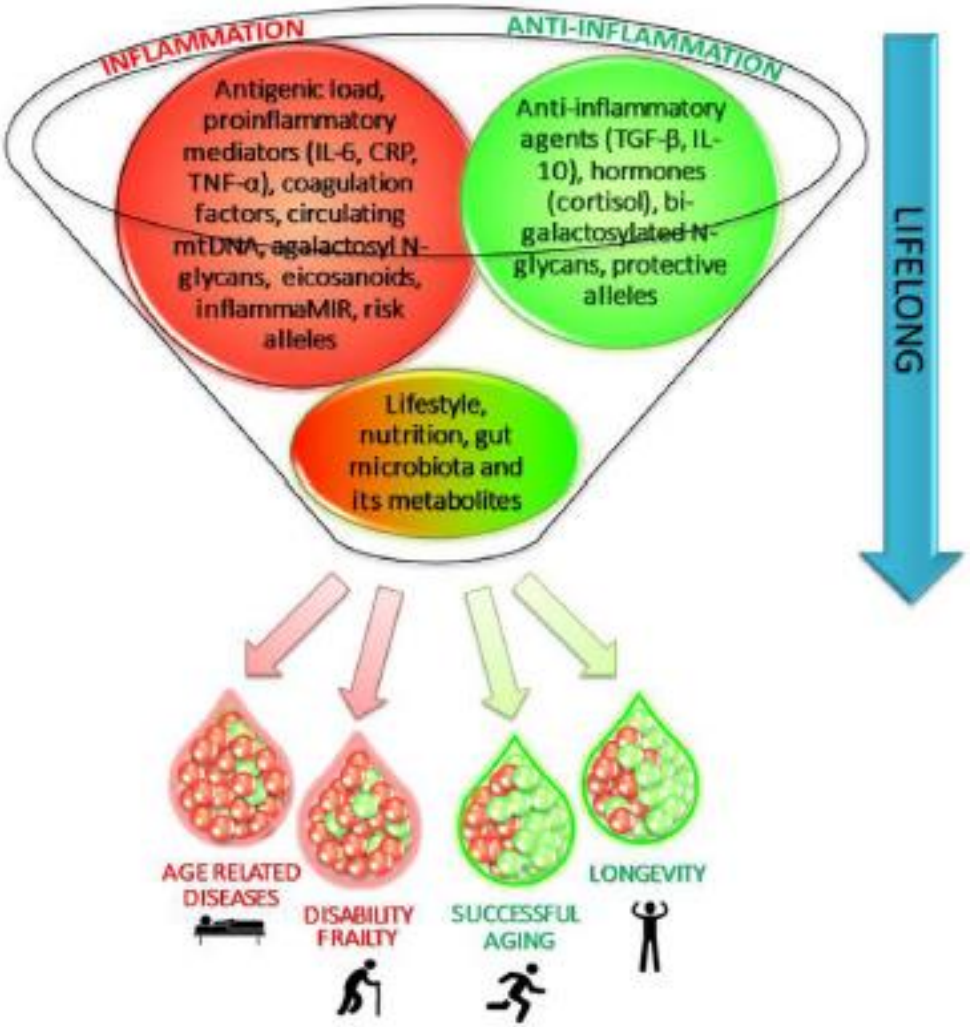


Fig. 2. The major possible endogenous sources of inflammaging and anti-inflammaging combined to lifestyle, nutrition, gut microbiota and its metabolites impinge lifelong to our organism. The theory assumes that an excessive stimulation of pro-inflammatory pathways and an ineffective anti-inflammatory response constitute a driving force for the development of disability/frailty and age-related diseases. On the contrary, the achieving of successful aging and longevity is determined by a lower propensity to mount inflammatory response that should be combined to efficient anti- inflammatory network.

What can we do?

- "Behavioural" approaches
  - Drug therapies
- Modification of the microbiota

What can we do?

- “Behavioural” approaches
  - Drug therapies
- Modification of the microbiota

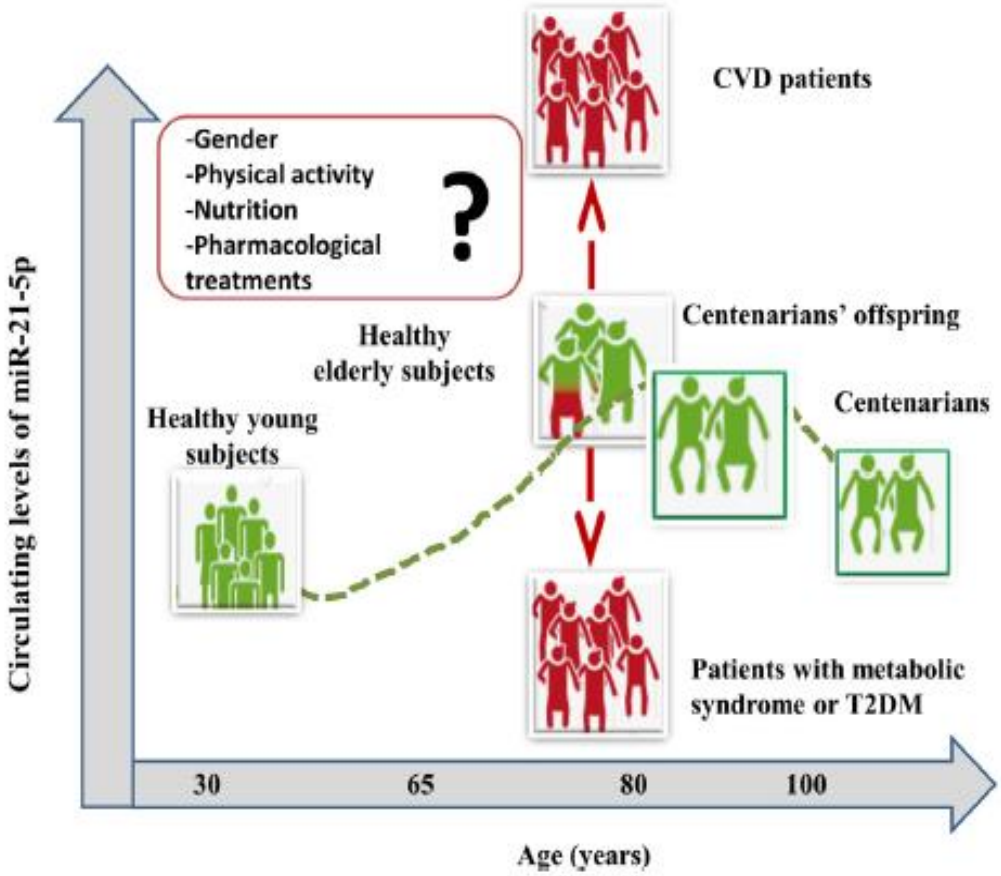


Fig. 2. Trend of circulating miR-21-5p levels: the deviation from a healthy (green) to a non-healthy (red) condition can be monitored by circulating miR-21-5p levels. The figure presents circulating miR-21-5p levels in healthy subjects of different age and in groups of patients suffering from the most common age-related diseases (metabolic syndrome, T2DM, CVD). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1. Some Reported Interventional Strategies Aimed at Increasing Life Span

Factor	*Organism/cell origin*	*Life span extension (yes/no)*
Exercise	*Mus musculus*	No
	Rattus norvegicus	Yes
Heat shock	*Caenorhabditis elegans*	Yes
	Drosophila melanogaster	Yes
Mild repeated heat shock	Human cells	No
Vitamin C	Human cells	Yes
Tea extract	*D. melanogaster*	Yes
N-acetyl cysteine	*D. melanogaster*	Yes
N-t-butyl hydroxylamine	Human cells	Yes
Resveratrol	*Saccharomyces cereviseae*	Yes
Rapamycin	Outbred mice	Yes
Spermidine	Human peripheral blood mononuclear cell	Yes
Caloric restriction	*M. musculus*	Yes
	Homo sapiens	Not assessed

Ponnappan S et al, Antioxidants & Redox Signaling 2011

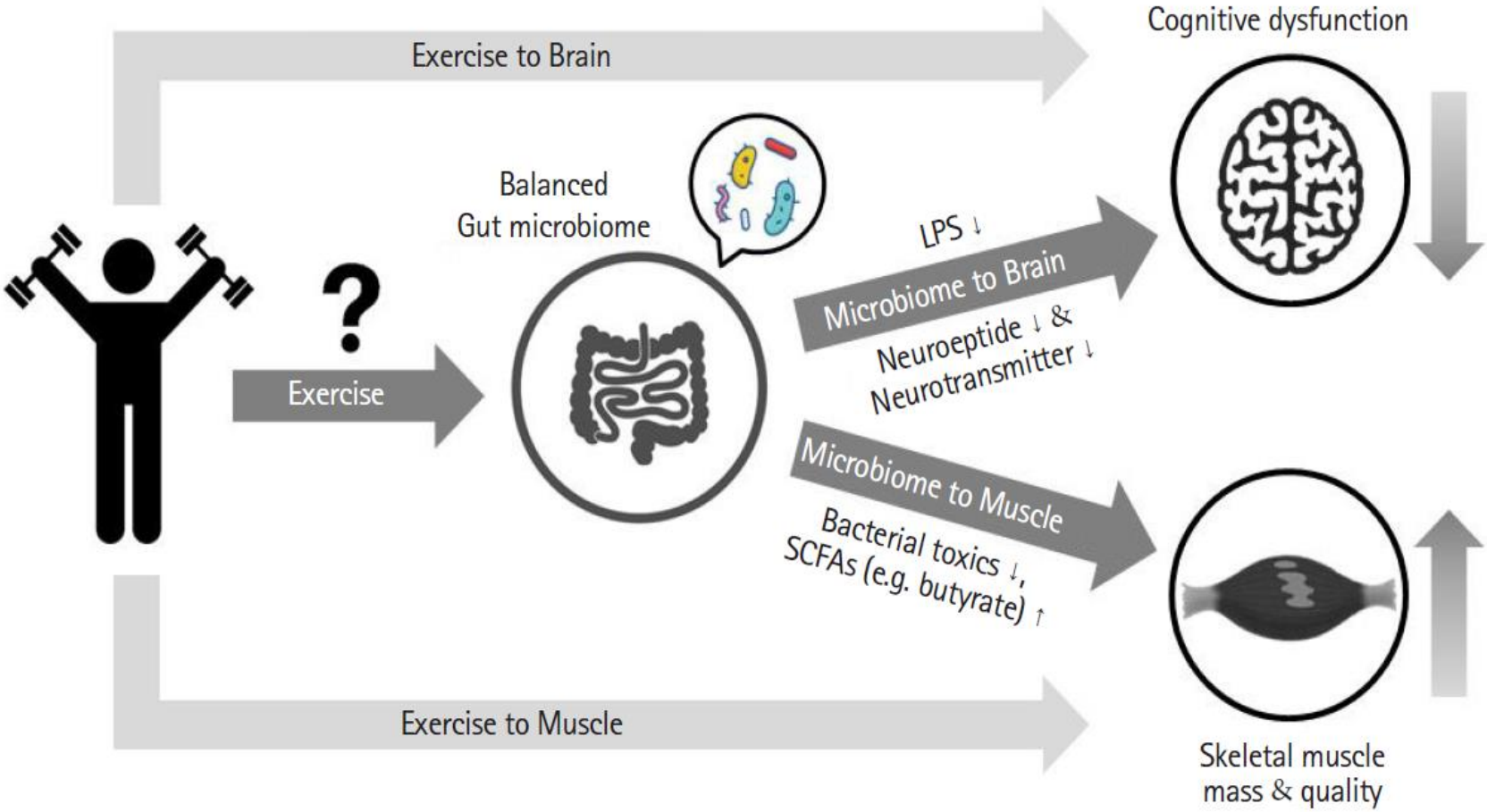
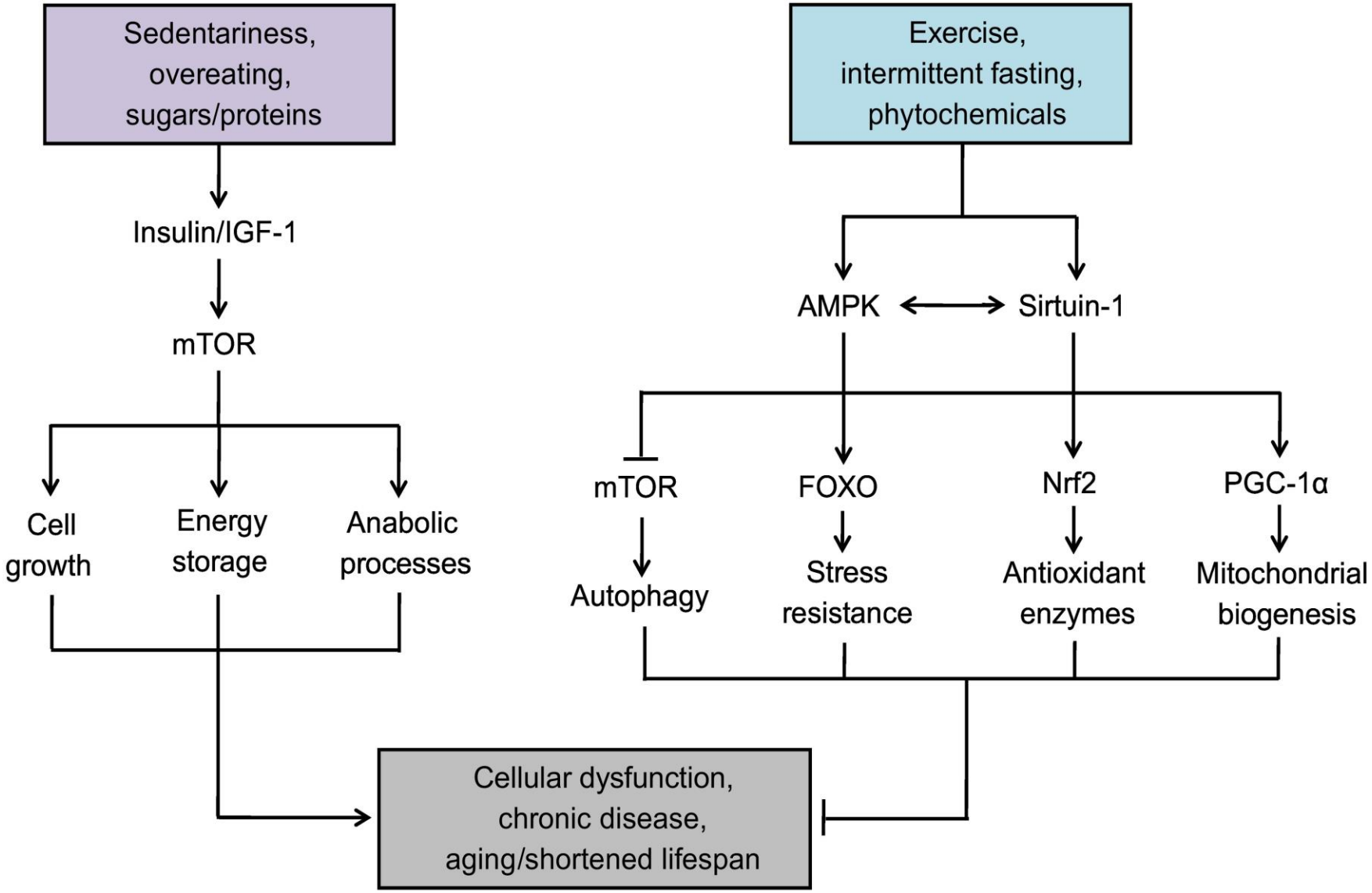


Fig. 1. Study overview: the gut-brain and gut-muscle axes. LPS, lipopolysaccharide; SCFAs, short-chain fatty acids.

Factors contributing to healthy/unhealthy aging

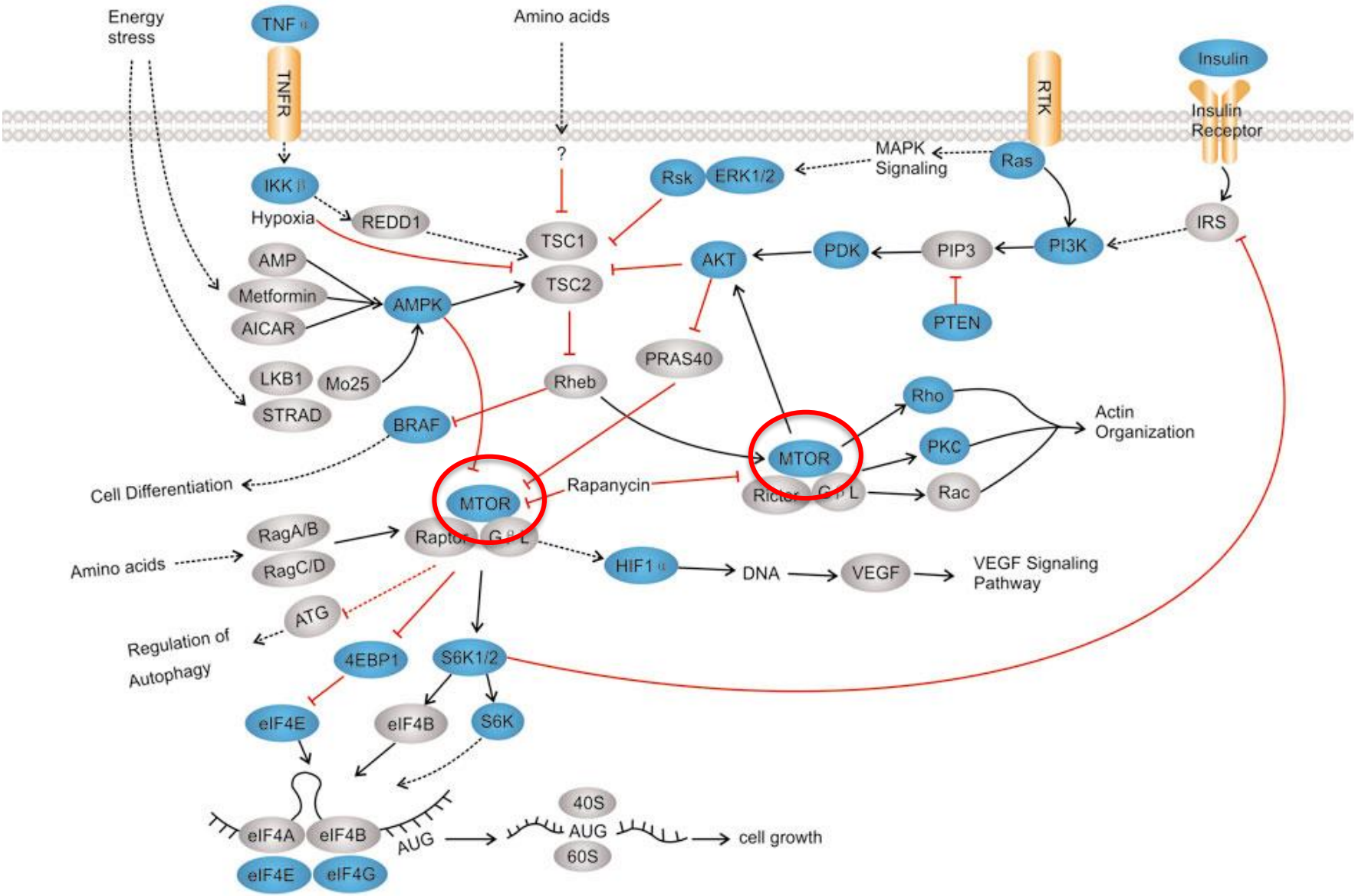


Trends in Endocrinology & Metabolism

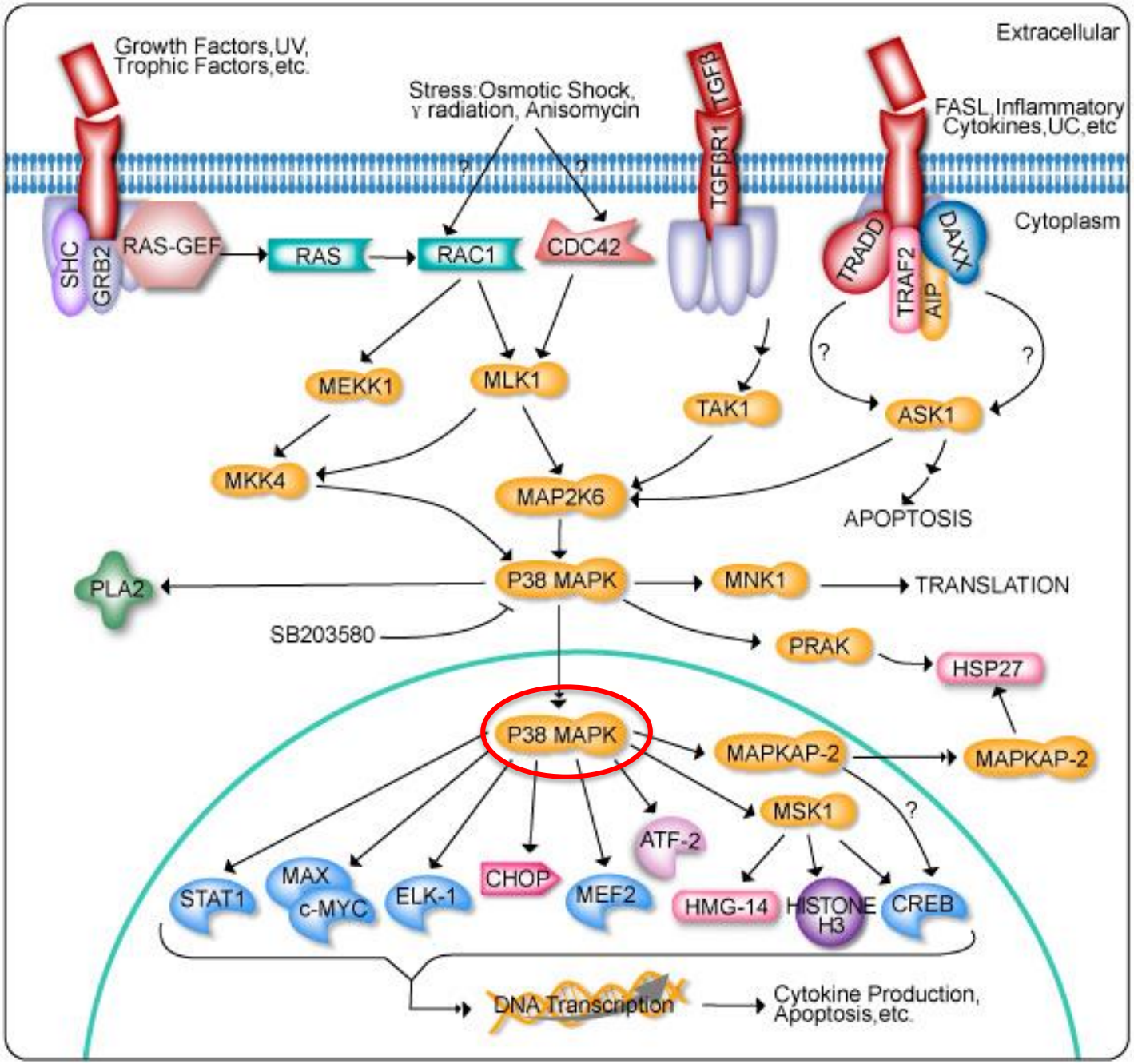
What can we do?

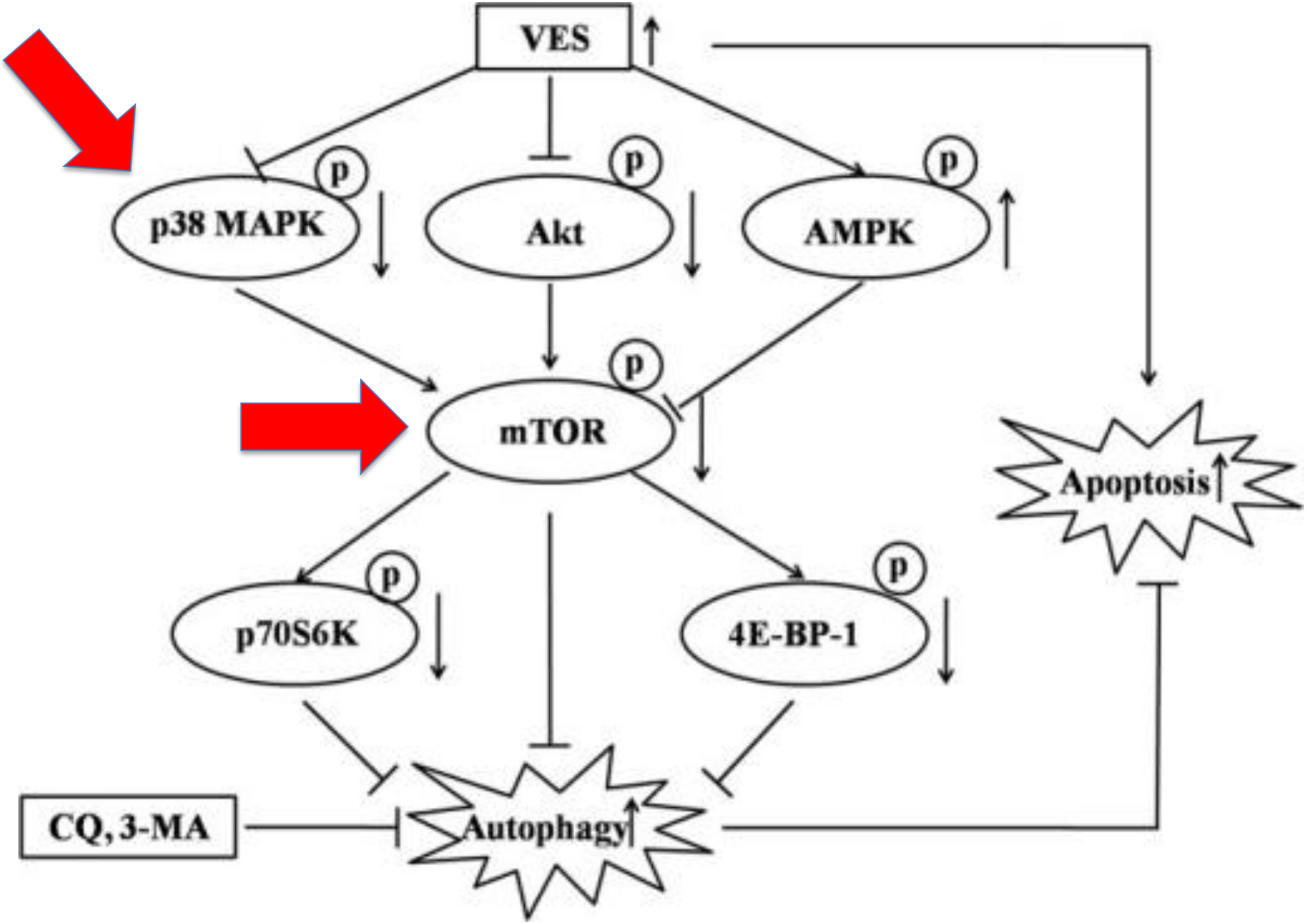
- "Behavioural" approaches
- Drug therapies
- Modification of the microbiota

MTOR Signaling Pathway



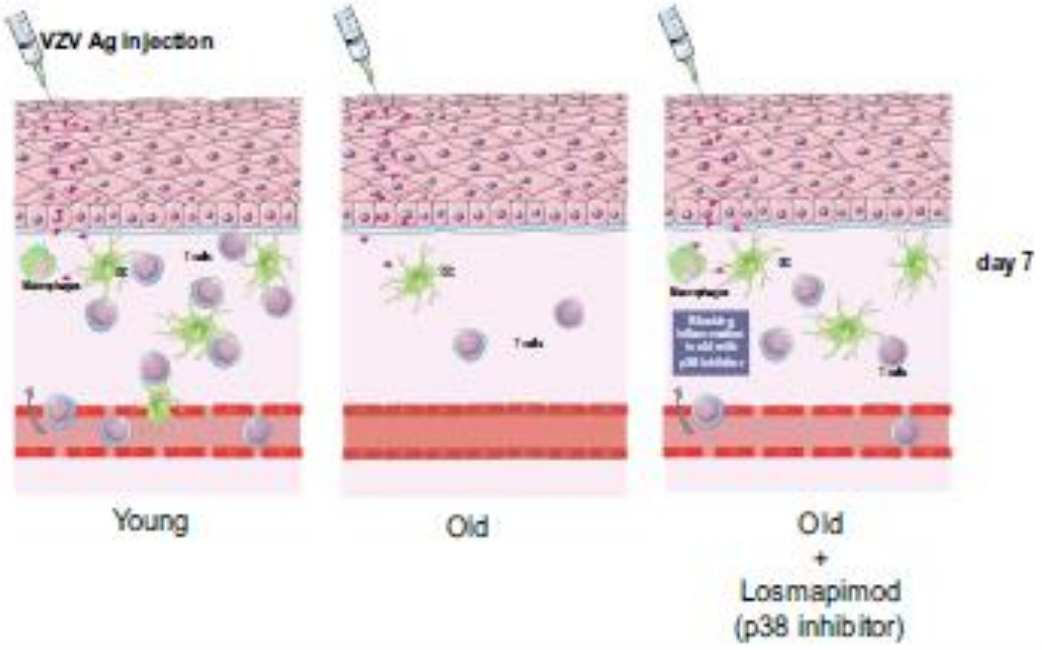
P38MAPK signaling pathway



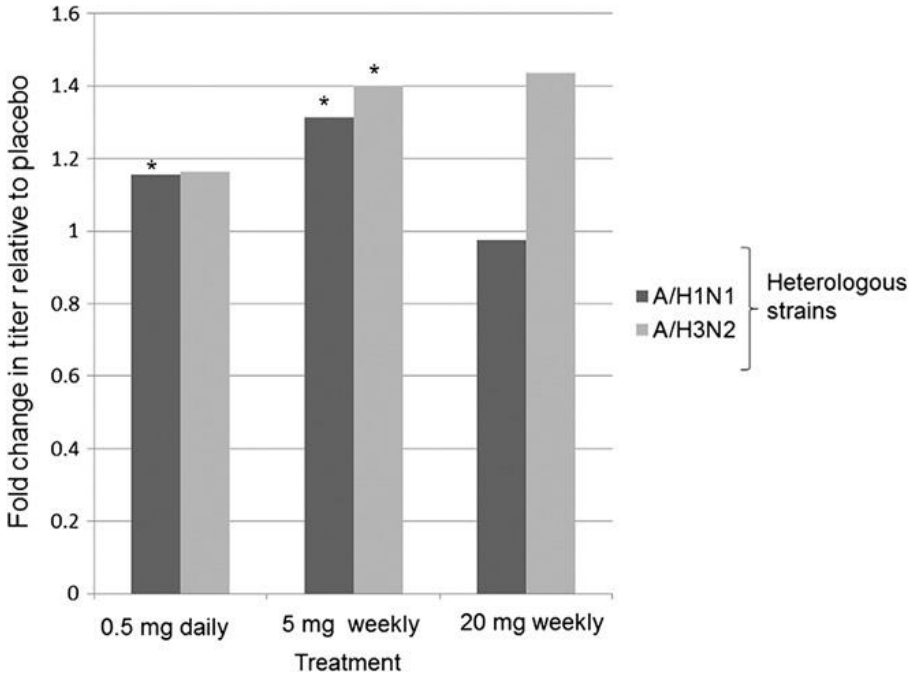

VES
p38 MAPK
Akt
AMPK
mTOR
Apoptosis
p70S6K
4E-BP-1
CQ, 3-MA
Autophagy
p

Varicella-zoster vaccination: effects of p38 MAPK inhibition

GRAPHICAL ABSTRACT



- **Rapamicine (an mTOR inhibitor) improves immune reponses to vaccines in elderly individuals.**
- **Clinical trials based on TOR inhibitors are currently undergoing in humans and in dogs** (***Nature; 552:s57; Dec 2017***)



Science Translational Medicine AAAS

Sci Transl Med 2014;6:268ra179

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

INFECTIOUS DISEASE

TORC1 inhibition enhances immune function and reduces infections in the elderly

Joan B. Mannick[1]*†, Melody Morris[1], Hans-Ulrich P. Hockey[2], Guglielmo Roma[3], Martin Beibel[3], Kenneth Kulmatycki[1], Mollie Watkins[1], Tea Shavlakadze[1], Weihua Zhou[1], Dean Quinn[4], David J. Glass[1], Lloyd B. Klickstein[1]*

Inhibition of the mechanistic target of rapamycin (mTOR) protein kinase extends life span and ameliorates aging-related pathologies including declining immune function in model organisms. The objective of this phase 2a randomized, placebo-controlled clinical trial was to determine whether low-dose mTOR inhibitor therapy enhanced immune function and decreased infection rates in 264 elderly subjects given the study drugs for 6 weeks. A low-dose combination of a catalytic (BEZ235) plus an allosteric (RAD001) mTOR inhibitor that selectively inhibits target of rapamycin complex 1 (TORC1) downstream of mTOR was safe and was associated with a significant ($P = 0.001$) decrease in the rate of infections reported by elderly subjects for a year after study drug initiation. In addition, we observed an up-regulation of antiviral gene expression and an improvement in the response to influenza vaccination in this treatment group. Thus, selective TORC1 inhibition has the potential to improve immune function and reduce infections in the elderly.

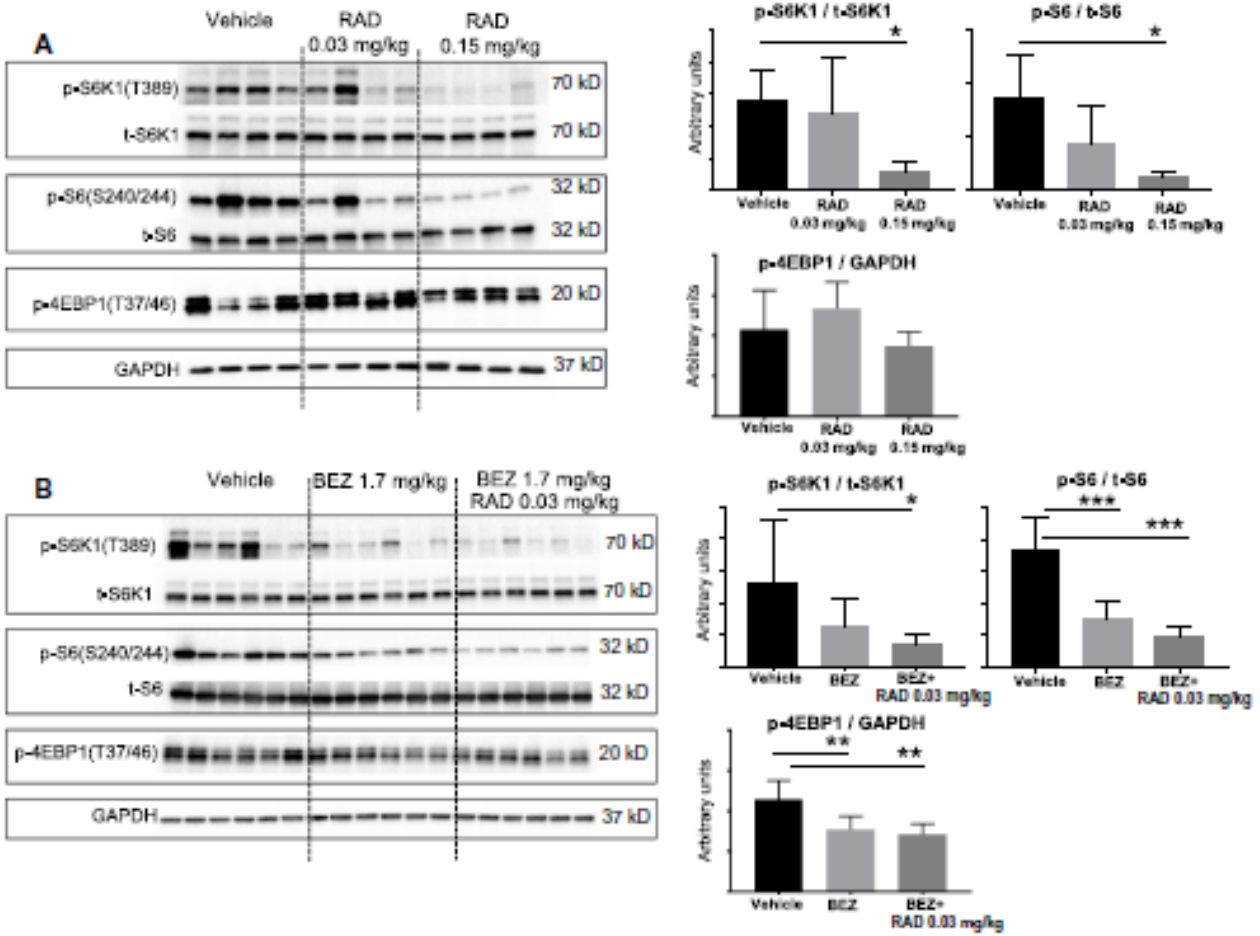


Fig. 3. Low doses of RAD001 and BEZ235 inhibit TORC1. Western blots for phosphorylated (p) and total (t) protein amounts for S6K1, S6, and 4EBP1 in rat livers after 7 days of drug treatment. (**A**) Rats were treated daily for 7 days with RAD001 (RAD) at the dose equivalent of 0.1 mg (0.03 mg/kg) or 0.5 mg (0.15 mg/kg) in humans. (**B**) Rats were treated daily for 7 days with BEZ235 (BEZ) given at the dose equivalent of 10 mg (1.7 mg/kg) in humans alone or in combination with the dose equivalent of RAD001 0.1 mg (0.03 mg/kg). Tissues were collected 4 hours after the last drug dose. Left: Each lane in the immunoblots represents liver tissue from one rat. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a loading control. Right: The amounts of p-S6K1(T389) and p-S6(Ser240/244) on the immunoblots were quantified relative to their respective total protein amounts by densitometry. Amounts of p-4EBP1 (T37/46) were quantified relative to GAPDH. *Y* axes represent arbitrary units. For each group, $n = 4$ to 6 rats. Data are mean ± SD. Data were analyzed with a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, where means from all groups were compared to the vehicle-treated group. In (A), $^{*}P = 0.048$ and $^{*}P = 0.018$ for p-S6K1 and p-S6, respectively. In (B), $^{*}P = 0.015$ for p-S6K1, $^{**}P \leq 0.005$ for p-4EBP1, and $^{***}P \leq 0.001$ for p-S6.

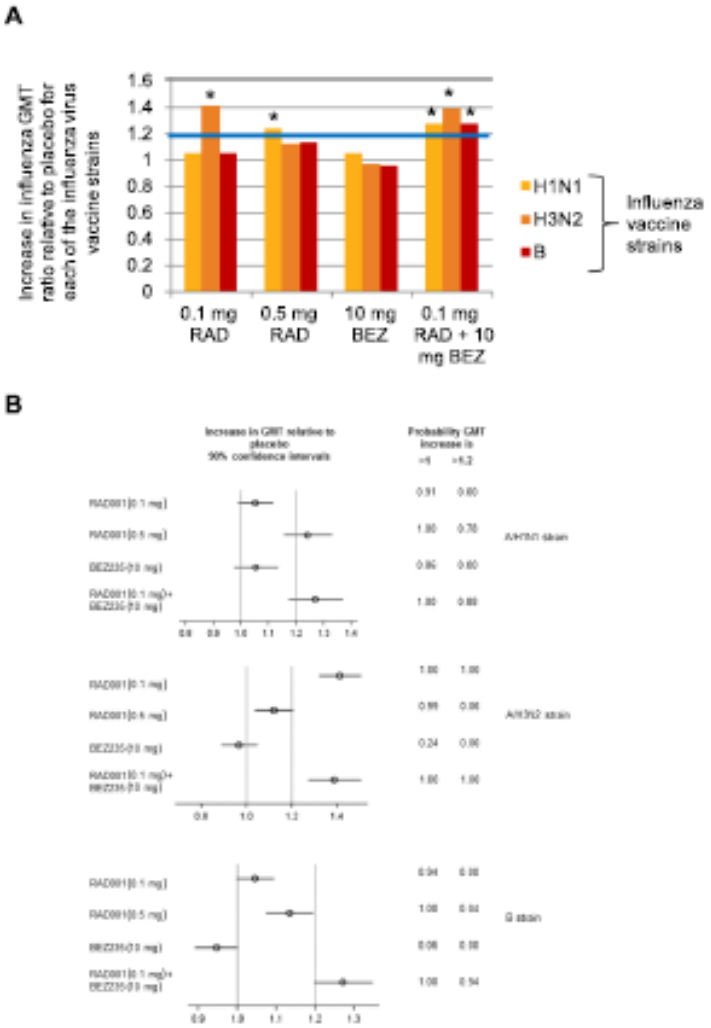


Fig. 2. Increase in antibody titers to influenza virus vaccine strains in mTOR inhibitor treatment groups relative to the placebo group. (**A**) Increase in the ratio (4 weeks after vaccination: baseline) in GMT for each of the three influenza virus vaccine strains in elderly subjects treated with RAD001, BEZ235, RAD001 + BEZ235, or placebo. The three influenza virus vaccine strains used were as follows: A/H1N1 (A/California/7/2009), A/H3N2 (A/Texas/50/2012), and B (B/Massachusetts/2/2012). The blue line indicates the 20% increase in GMT ratios relative to placebo that was required for two of the three influenza virus vaccine strains to meet the primary end point of the study. Asterisks indicate that the probability that the increase in GMT ratio relative to placebo exceeded 1.0 is 100%. (**B**) Forest plots of the data presented in (A) including 90% confidence intervals and probability that the GMT ratio compared to placebo is >1 or >1.2.

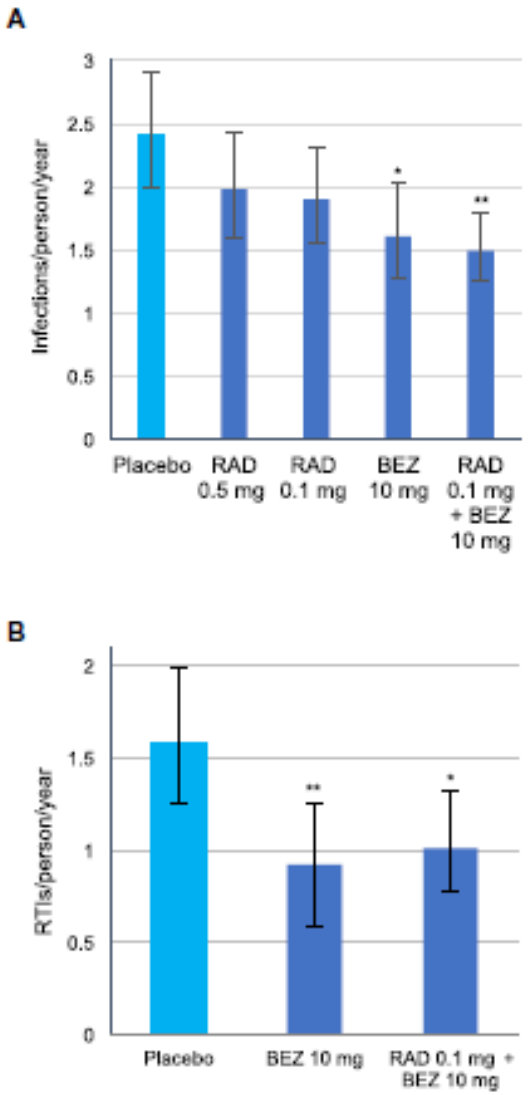


Fig. 4. TORC1 inhibition decreases infection rates in the elderly. (**A**) Fitted annual rates of infections reported per person per year in the 0.1 mg of RAD001, 0.5 mg of RAD001, 10 mg of BEZ235, 0.1 mg of RAD001 + 10 mg of BEZ235, or placebo groups. *$P = 0.008$, **$P = 0.001$ versus placebo. (**B**) Fitted annual rates of respiratory tract infections (RTIs) reported per person per year in the placebo group and in the BEZ235 monotherapy and BEZ235 + RAD001 combination treatment groups. *$P = 0.01$, **$P = 0.008$ versus placebo. In both figures, error bars indicate 95% confidence intervals as determined by Poisson regression modeling.

What can we do?

- "Behavioural" approaches
- Drug therapies
- Modification of the microbiota

How to modify the microbiota

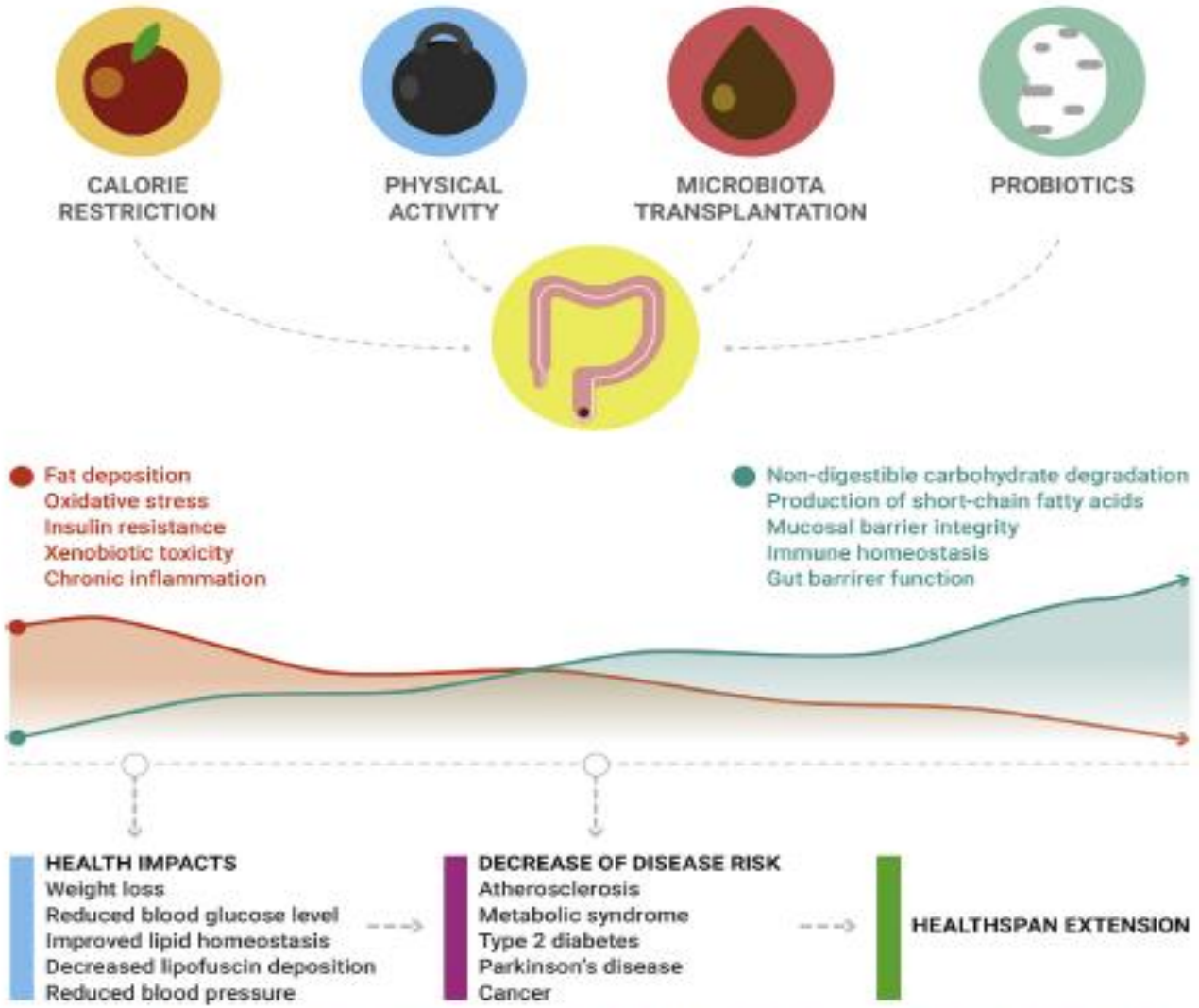


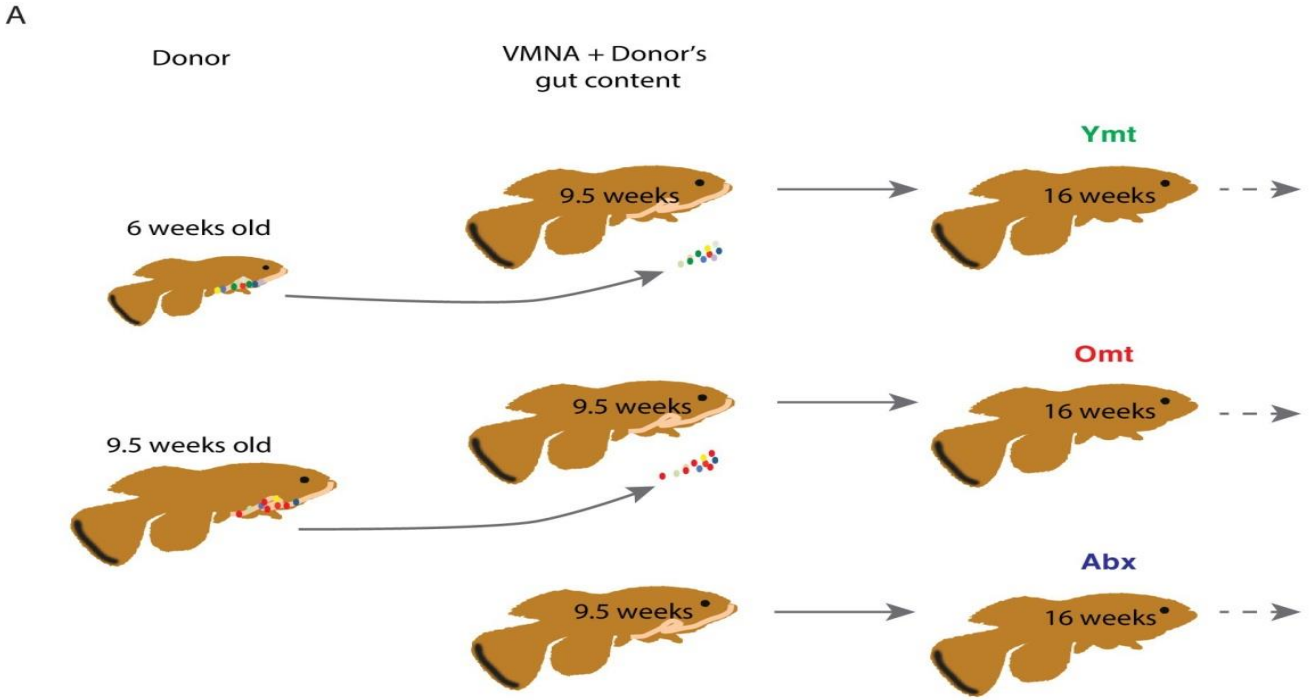
Fig. 2. Schematic representation of potential pathways to extend human healthspan by gut microbiota modulation.

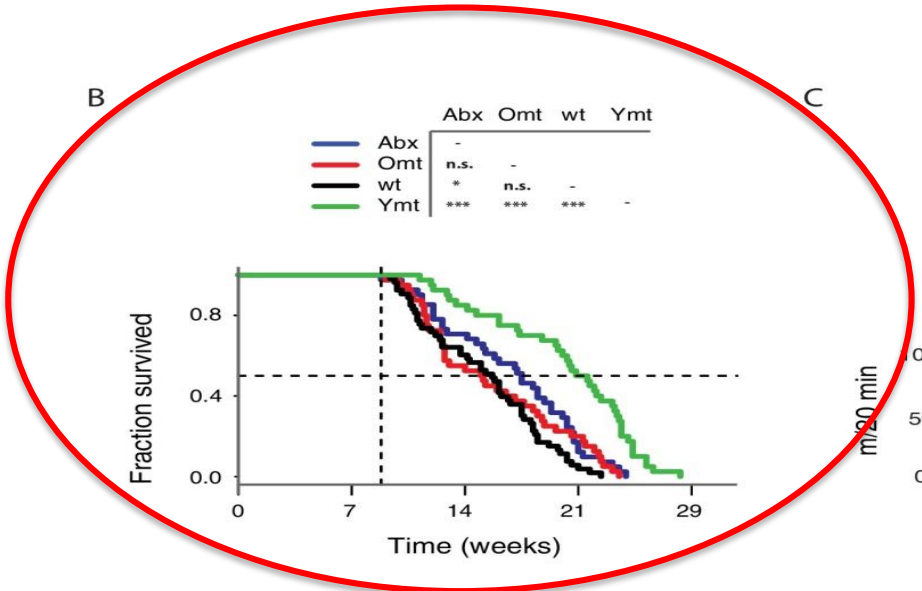
Prebiotics tested for preventing or delaying the gut-related diseases in elderly

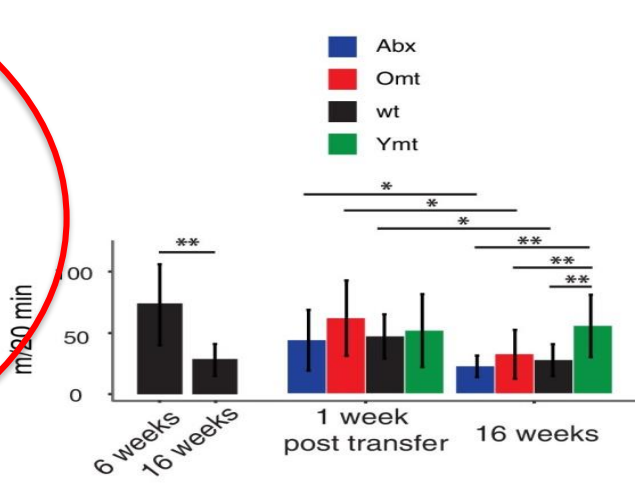
Abbreviation	Prebiotics name	References
MOS	Mannooligosaccharides	[113, 114]
GOS	Galactooligosaccharides	[113, 114]
–	inulin	[115]
–	Lactulose	[115]
FOS	Fructo-oligosaccharides	[117, 118]
POS	Pectic-oligosaccharides	[117, 118]
XOS	Xylooligosaccharides	[119]
TOS	Transgalactosylatedoligosaccharides	[120]

Kumar M et al, Nutrition and Healthy Aging 2016

Fecal transplantation of the microbiota of a young fish into middle aged fish







Conclusions

- A number of reasons (improved sanitary conditions, vaccinations, better health care, etc) explain why worldwide population is getting older
- Aging is associated with complex alterations of the immune system and changes in the composition of the microbiota
- A persistent and smouldering degree of inflammation accompains aging and could be responsible for “unhealthy aging”
- Nutritional and pharmacological interventions, as well as stategies targeting the microbiota are currently investigated in the attempt to achieve “healthy aging”

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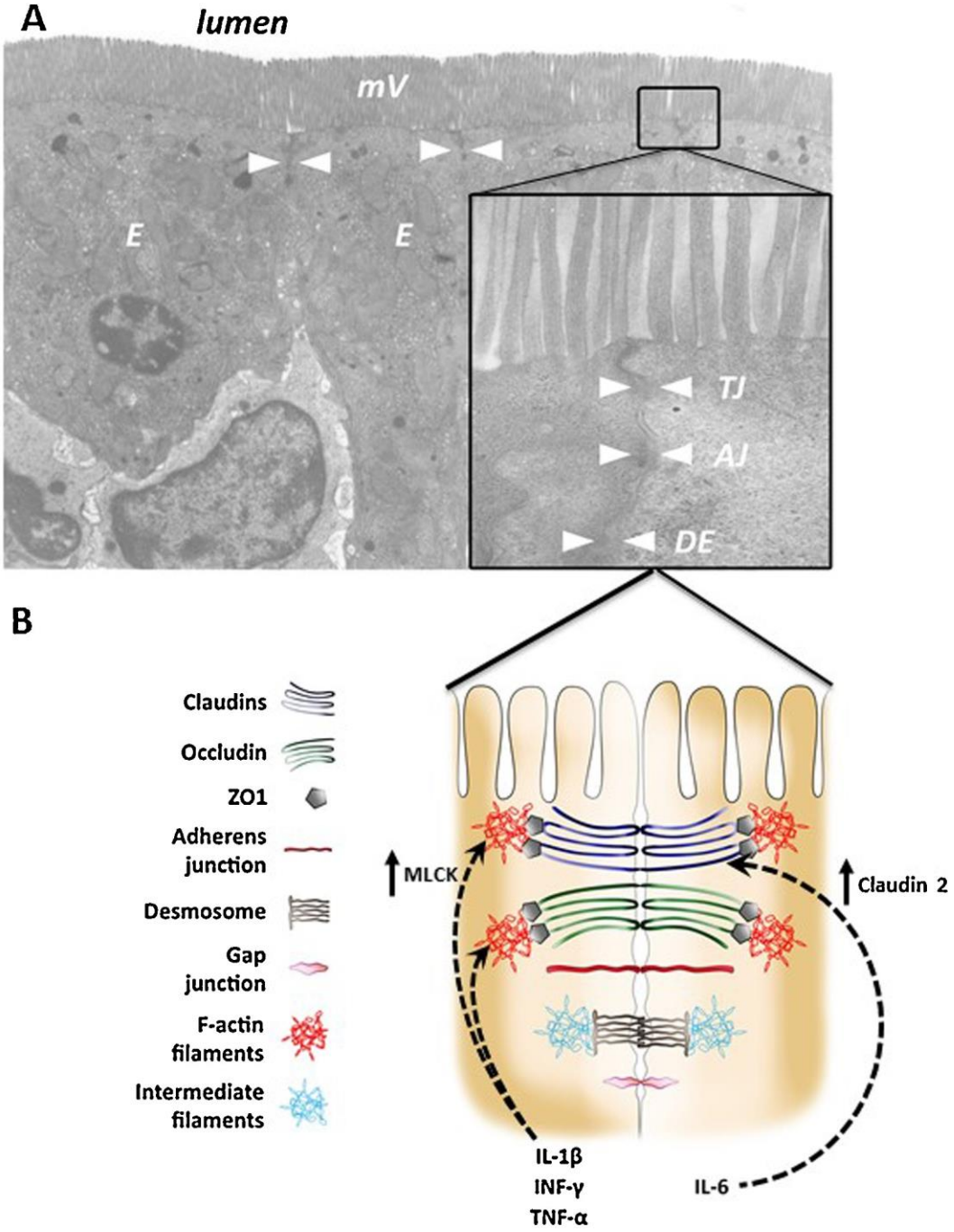
Sant' Agostino


A
lumen
mV
E
E
TJ
AJ
DE
B
Claudins
Occludin
ZO1
Adherens junction
Desmosome
Gap junction
F-actin filaments
Intermediate filaments
MLCK
Claudin 2
IL-1β
INF-γ
TNF-α
IL-6