HELLENIC REPUBLIC
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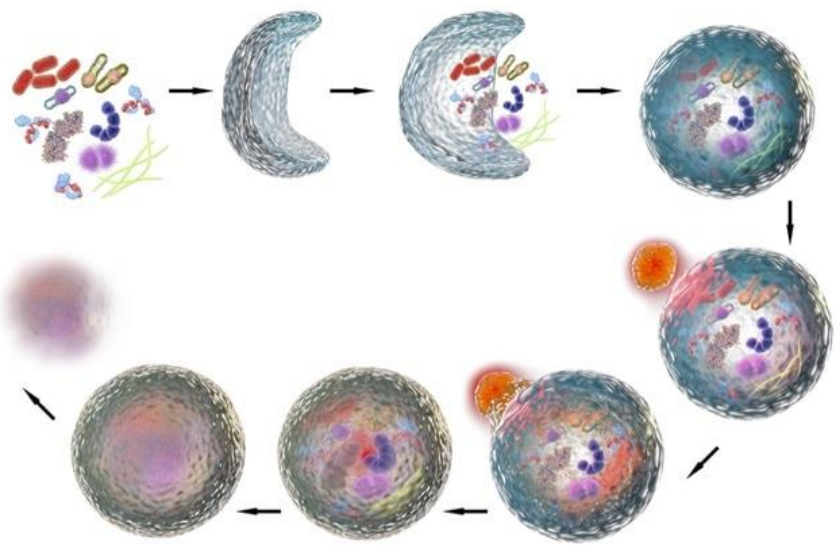
Autophagy – Mitophagy in human health and disease



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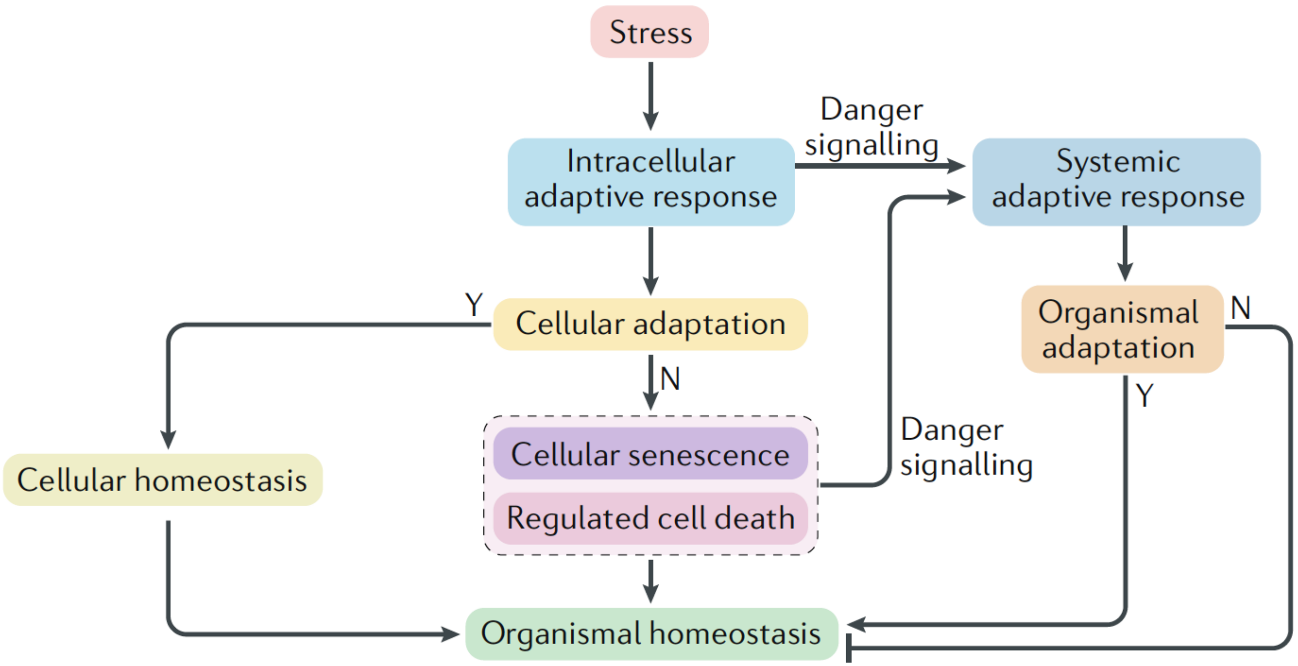
National and Kapodistrian University of Athens

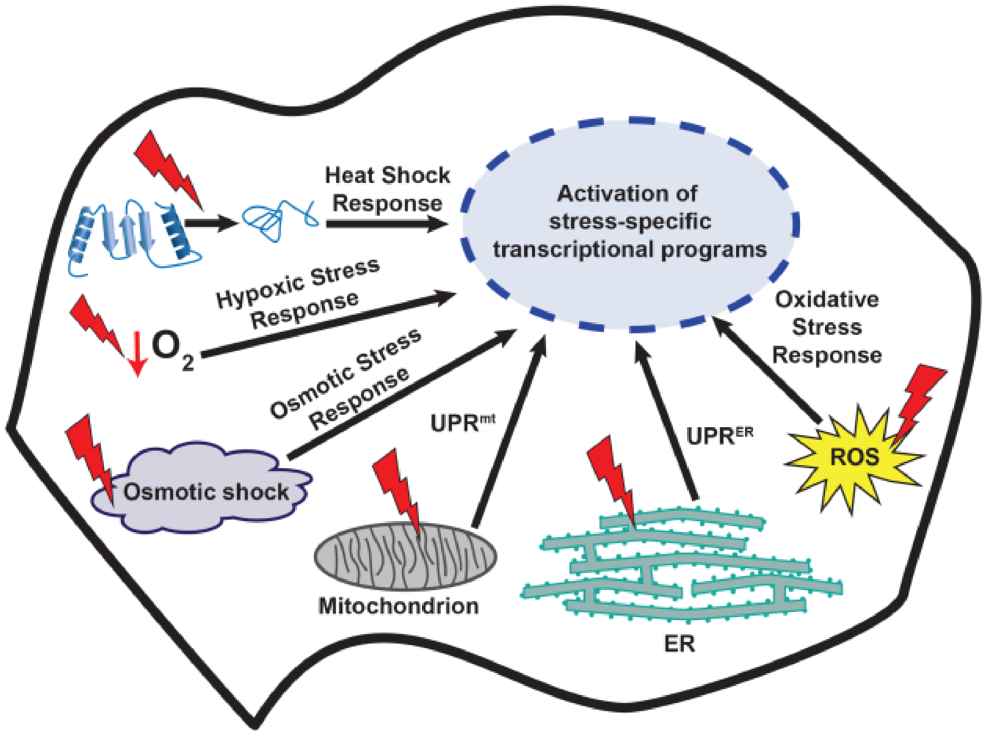
9th October 2021

Heraklion, Crete

Maintenance of organismal homeostasis: integration of cellular & systemic stress responses

- Our cells are constantly exposed to damaging stress from both external sources (e.g. UV rays, temperature fluctuations), as well as internal sources, including free radicals produced by damaged mitochondria.





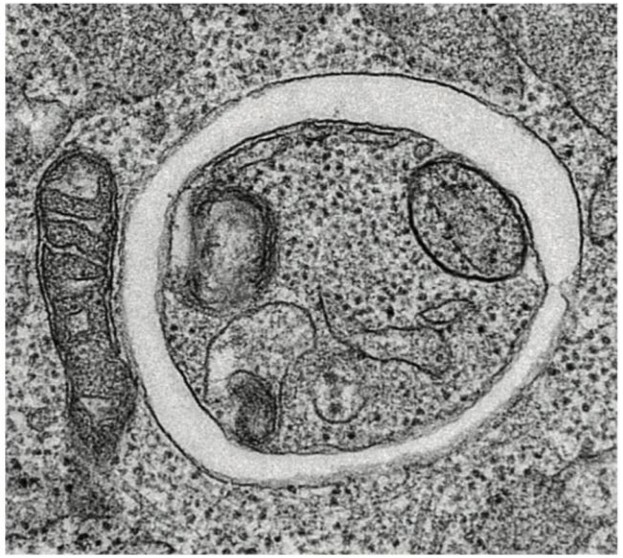
- Environmental stresses are ubiquitous and unavoidable to all living things.
- Organisms respond and adapt to stresses through defined regulatory mechanisms that drive changes in gene expression, organismal morphology, or physiology.
- Adaptation is a genetic variation that allows an organism to better survive in its environment.

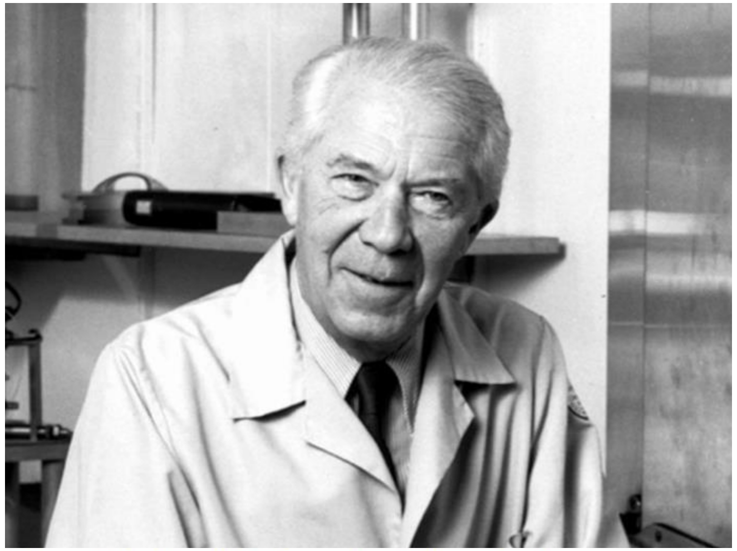
Himanen and Sistonen, 2019 *J Cell Science*
Galluzzi et al., 2018 *Nat Rev Mol Cell Biol*

Autophagy: stress response to maintain cellular homeostasis

- ❖ Auto-phagy = Self- eating
- ➢ First time observed in rat livers

  (de Duve C, Wattiaux R (1966) *Annu Rev Physiol* 28:435–492)
- ➢ High regulated, lysosome mediated catabolic process





The Nobel Prize in Physiology or Medicine 1974

Photo from the Nobel Foundation archive.
Albert Claude
Prize share: 1/3

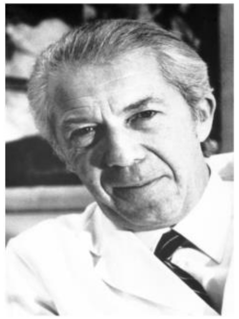
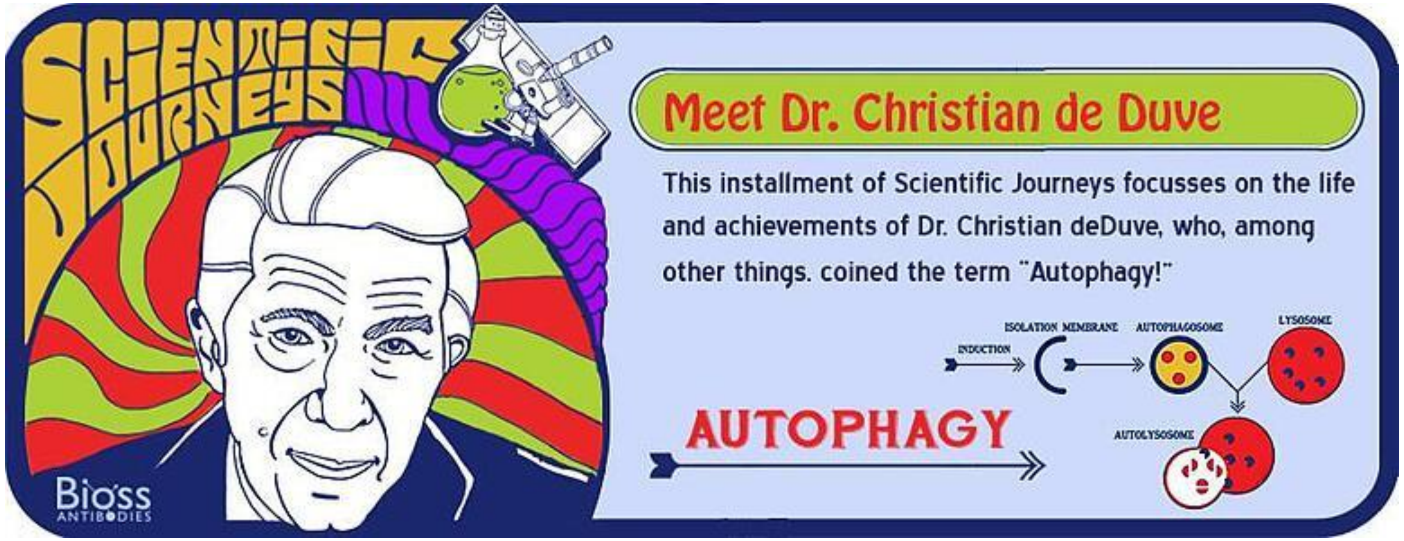


Photo from the Nobel Foundation archive.
Christian de Duve
Prize share: 1/3

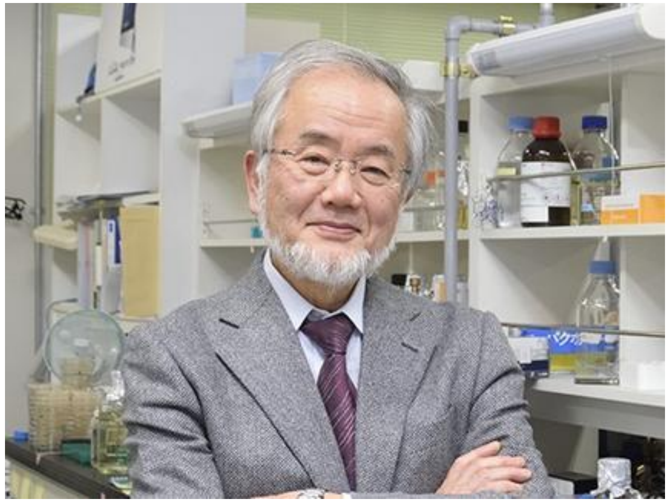
Photo from the Nobel Foundation archive.
George E. Palade
Prize share: 1/3

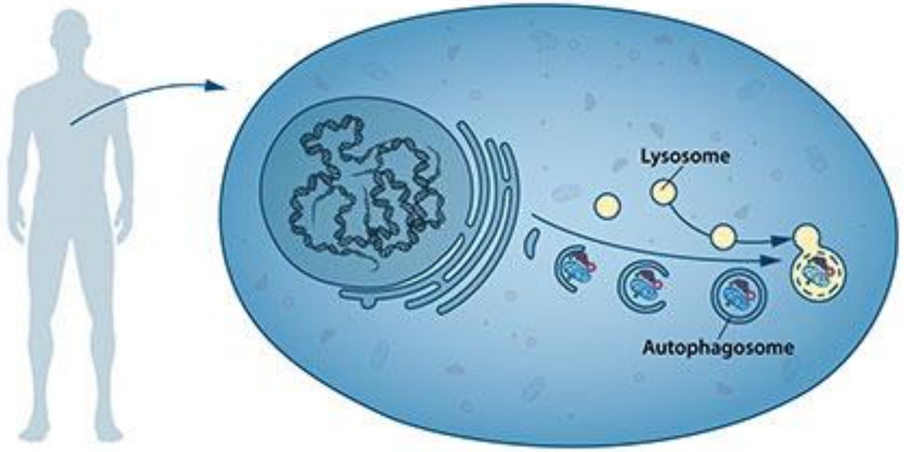


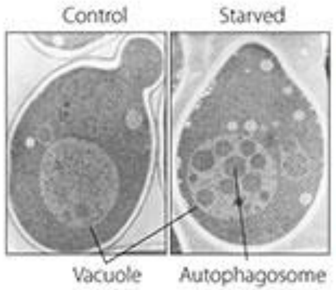
Klionsky et al., (2021) *EMBO J*
Choi *et al.*, (2013) *N Engl J Med*
Sabatini and Adensik, (2013) *PNAS*

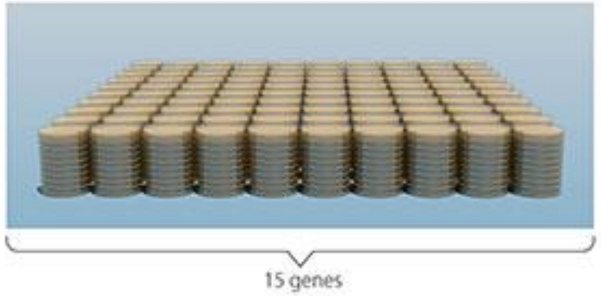
AUTOPHAGY: Nobel prize in Physiology and Medicine 2016

Yoshinory Ohsumi









In yeast a large compartment called the *vacuole* corresponds to the lysosome in mammalian cells. Ohsumi generated yeast lacking vacuolar degradation enzymes. When these yeast cells were starved, autophagosomes rapidly accumulated in the vacuole. His experiment demonstrated that autophagy exists in yeast. As a next step, Ohsumi studied thousands of yeast mutants and identified 15 genes that are essential for autophagy.

- How autophagy is regulated and executed at the molecular level have been made in yeast.
- 32 different autophagy-related genes (Atg)
- Many of these genes are conserved in plants, worms, flies, fish and mammals.

Ichimura *et al.*, (2000) *Nature, 408, 488-492*

Mizushima *et al.*, (1998) *Nature 395, 395-398*

Tsukada and Ohsumi (1993) *FEBS Letters 333, 169-174*

Takeshige *et al.*, (1992) *Journal of Cell Biology 119, 301-311*

Phases of the autophagic pathway

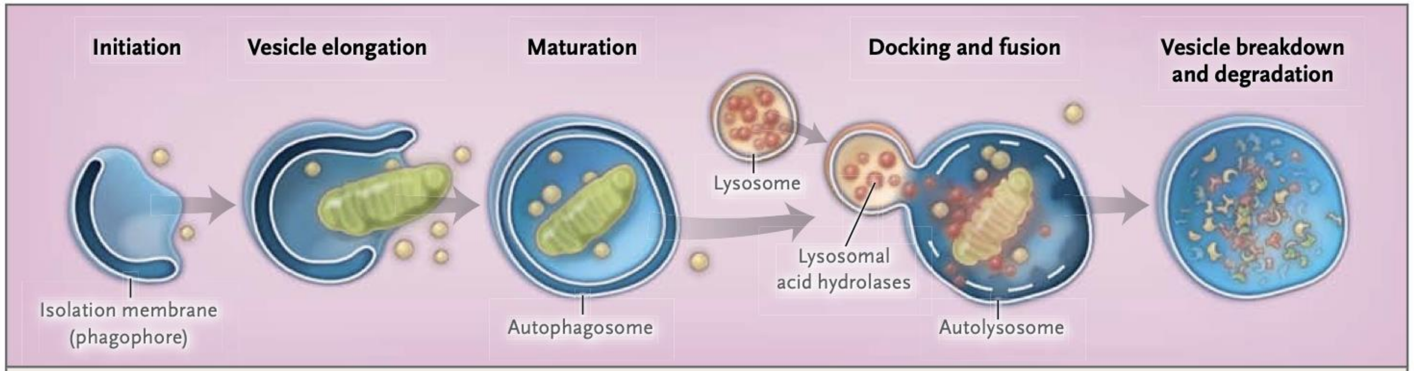


Figure 1. Phases of the Autophagic Pathway.
The autophagic pathway proceeds through several phases, including initiation (formation of a preautophagosomal structure leading to an isolation membrane, or phagophore), vesicle elongation, autophagosome maturation and cargo sequestration, and autophagosome–lysosome fusion. In the final stage, autophagosomal contents are degraded by lysosomal acid hydrolases and the contents of the autolysosome are released for metabolic recycling.

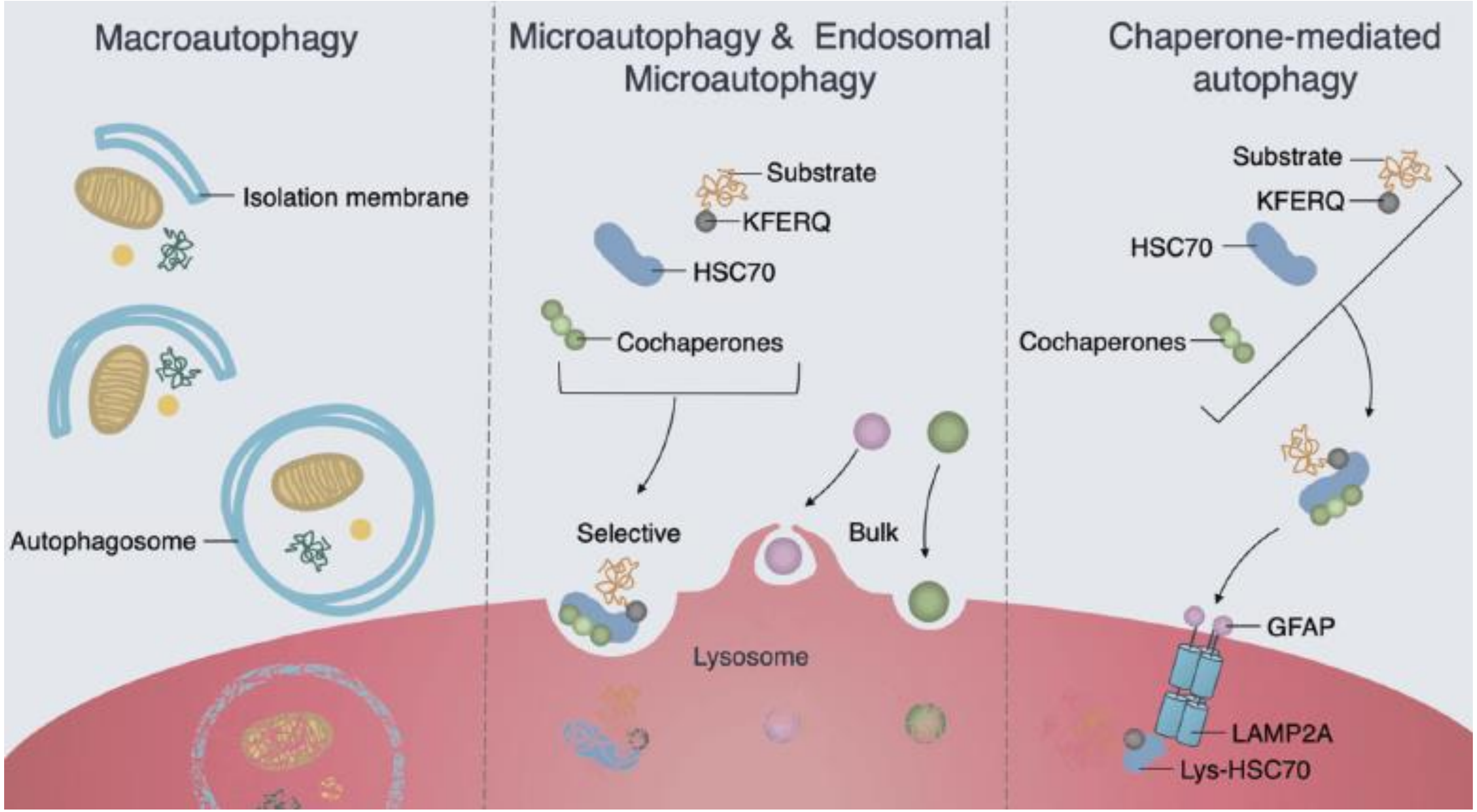
Involves the formation of the autophagosome, which contains and deliver sequestered cytoplasmic material into lysosomes for degradation

The main functions of autophagy:

❖Housekeeping and quality control of proteins and organelles

Aman et al., (2021) *Nature Aging*
Klionsky et al., (2021) *EMBO J*
Choi *et al.*, (2013) *N Engl J Med*

Overview of the autophagic pathways



General feature for all of them : proteolytic degradation of cytosolic components at the lysosome

Allen and Baehrecke, (2020) *Cell Death & Differentiation*
Boya, Reggiori & Patrice Codogno, (2013) *Nat Cell Biol*

Types of selective autophagy in mammals

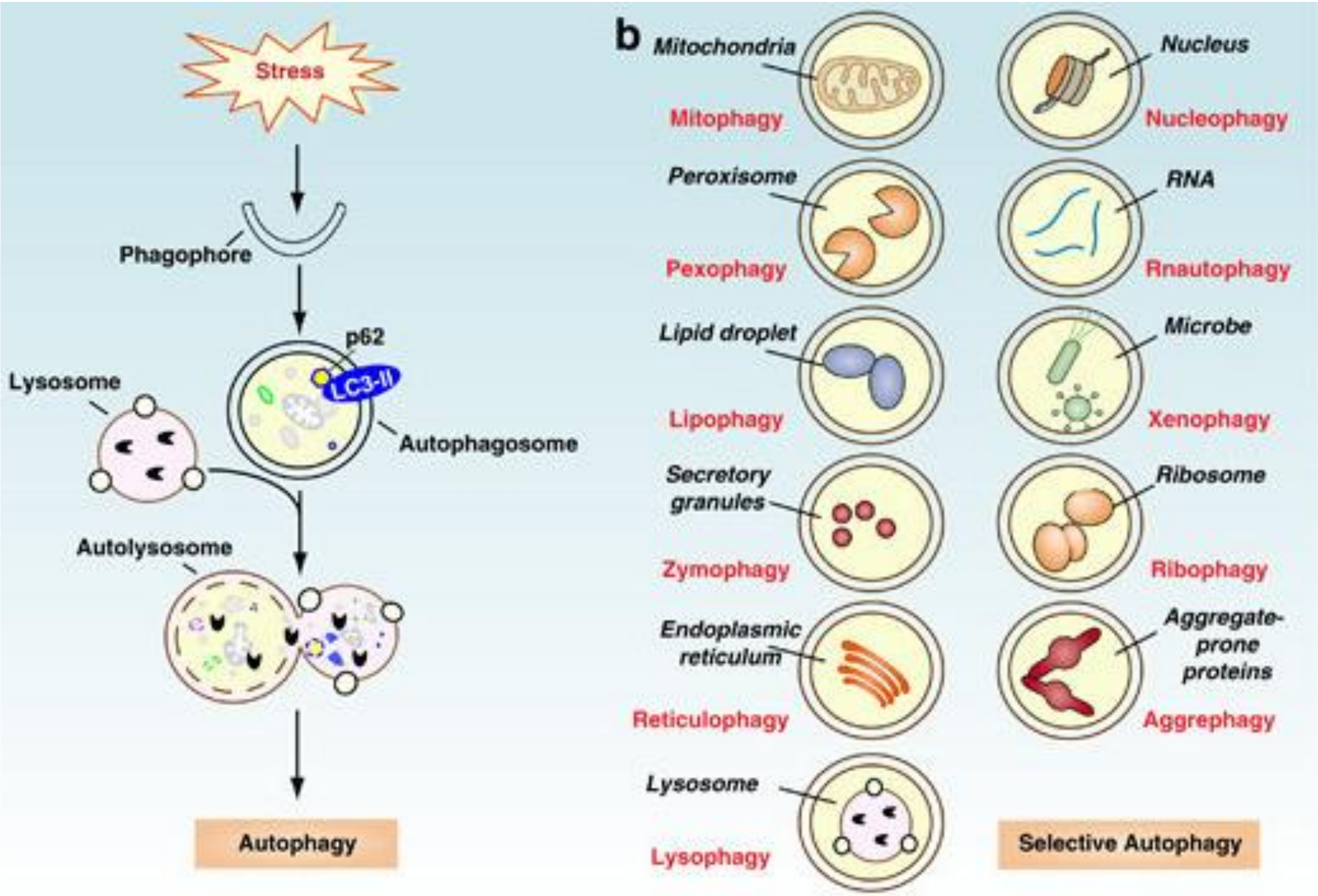
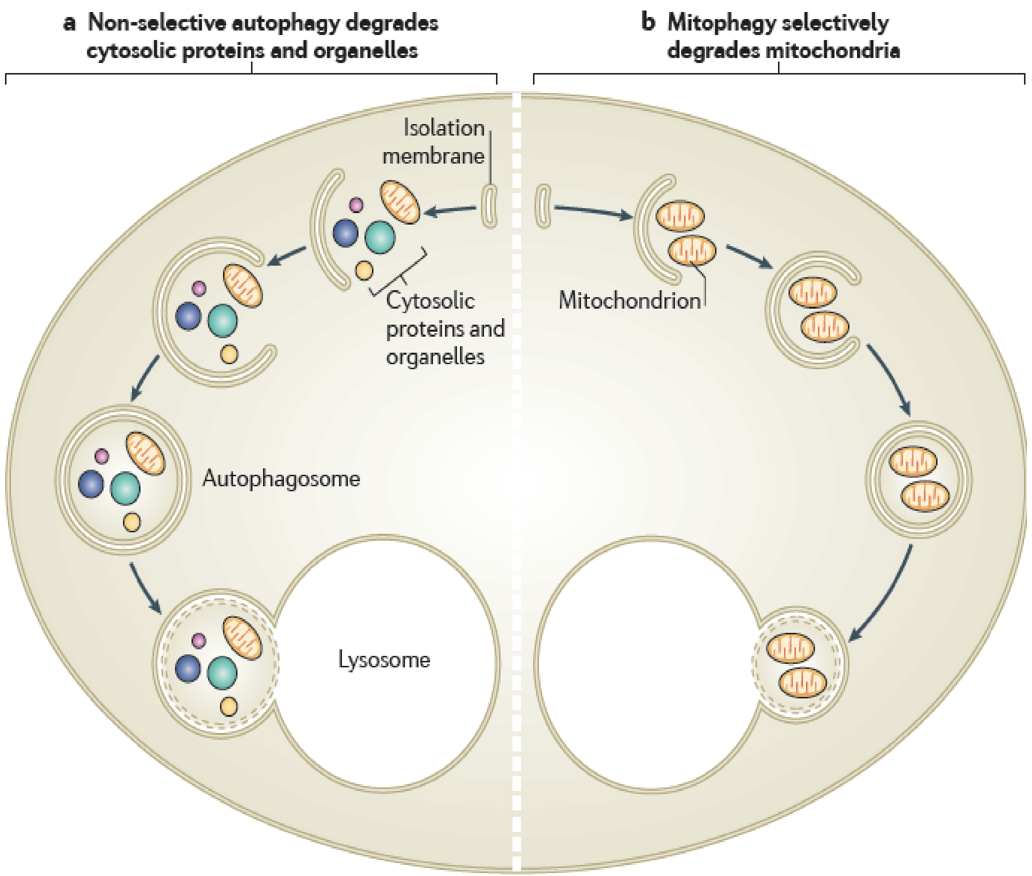


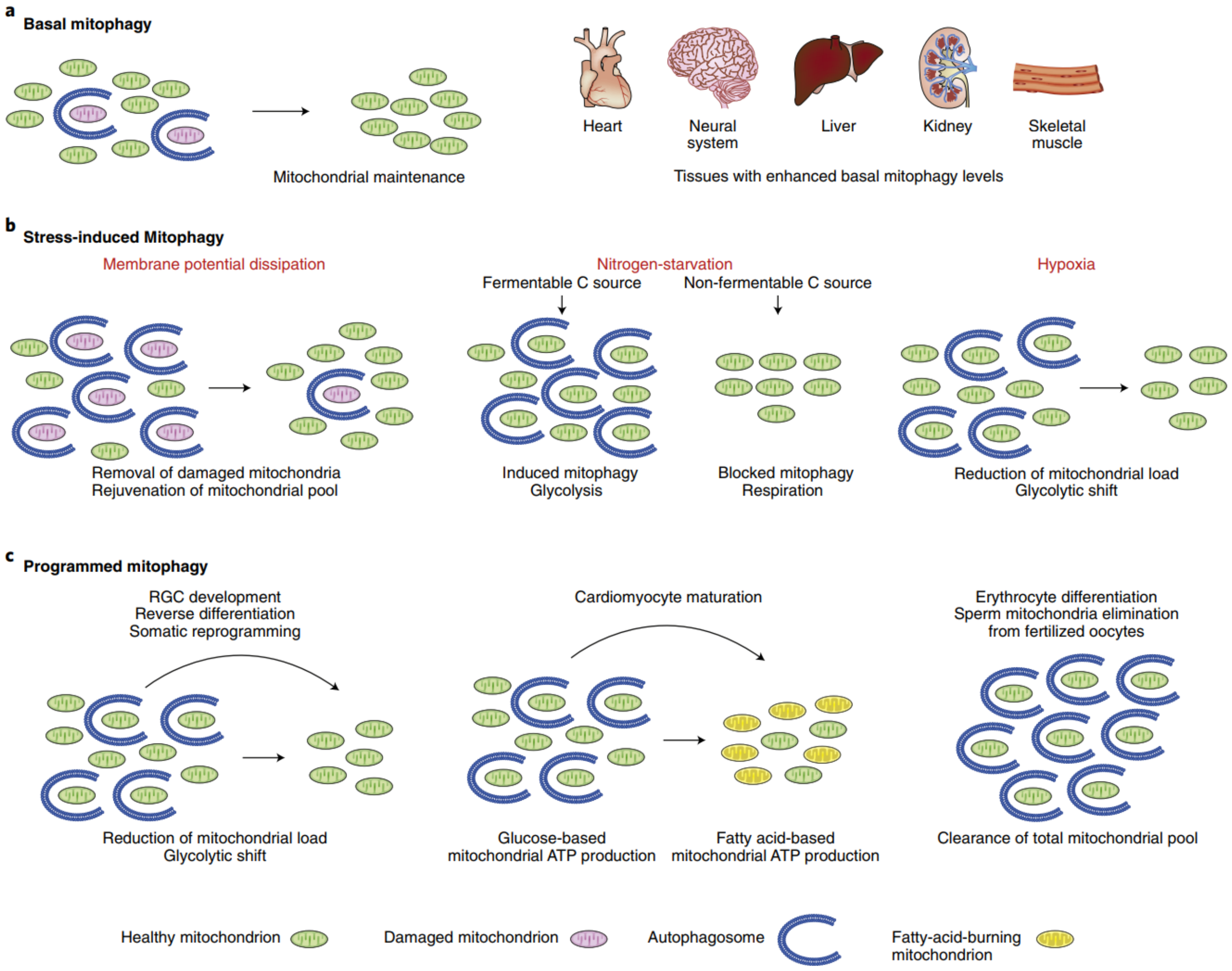
Table 1. Overview of Receptors and Substrates in Selective Autophagy Pathways

Pathway	Receptor	Substrate	Refs
Ub-dependent			
Aggrephagy	p62, NBR1, OPTN, Cue5, TOLLIP	Protein aggregates	[32–36]
Mitophagy	OPTN, NDP52, Tax1BP1	Mitochondria	[41–43]
Xenophagy	p62, NDP52, OPTN	Bacteria	[37–39]
Pexophagy	NBR1	Peroxisomes	[40]
Zymophagy	p62	Zymogen	[16]
Proteaphagy	RPN10	Proteasomes	[24]
Midbody disposal	p62, NBR1	Midbody	[15,44]
Nucleic acid disposal	p62, NDP52	Nucleic acids	[18,45]
Ub-independent			
Mitophagy	NIX, BNIP3, FUNDC1, Atg32	Mitochondria	[84–89]
ER-phagy	FAM134B, Atg40	ER	[93,95]
Nucleophagy	Atg39	Nuclear envelope	[95]
Ferritinophagy	NCOA4	Ferritin	[12,13]
Pexophagy	NBR1, Atg30, Atg36	Peroxisomes	[40,90,91]
Glycophagy	Stbd1	Glycogen	[92]
Signalophagy	c-Cbl	Src	[19]
Cvt targeting	Atg19, Atg34	Ape1, Ams1	[82,83]
Lysophagy	Galectin-8	Lysosomes	[97]
Xenophagy	Galectin-8	Bacteria	[97]
Virophagy	TRIM5α, SMURF1	Viral components	[17,20]
Fatty acid synthase (FAS) disposal	FAS	FAS	[21]
Undefined			
Lipophagy	–	Lipid droplets	[8]
Ribophagy	–	Ribosomes	[7]
Granulophagy	–	Stress granules	[11]
Myelinophagy	–	Myelin	[25]

Kitada and Koya, (2021) *Nature Reviews Endocrinology*
Khaminets et al., (2016) *Trends Cell Biol*
Rogov et al., (2014) *Molecular Cell*

Mitophagy: mitochondrial selective autophagy

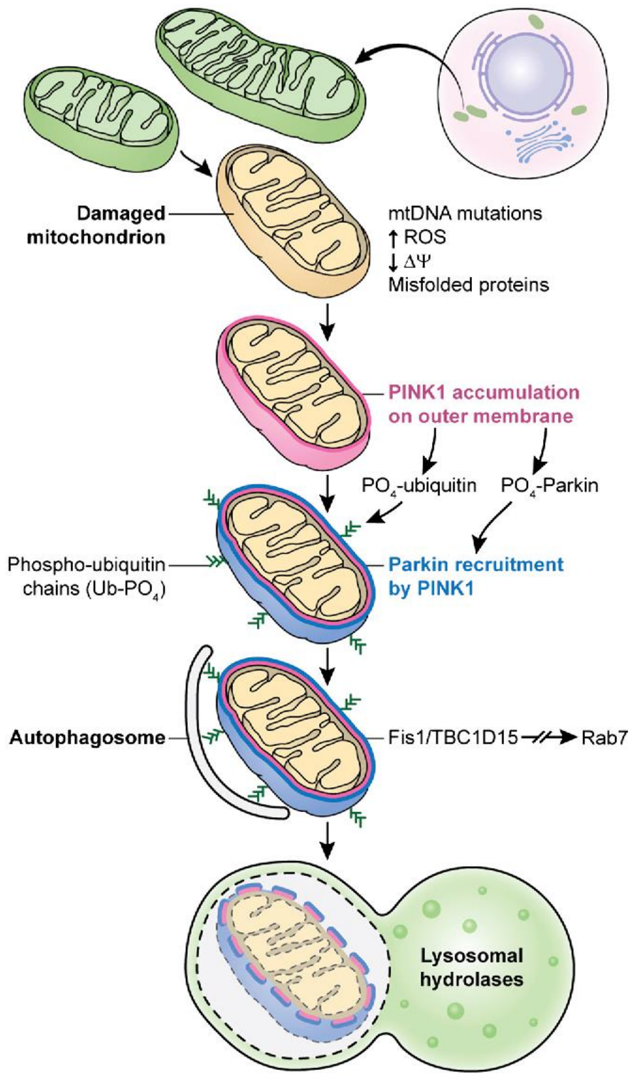




Palikaras et al., (2018) *Nat Cell Biol*
Youle & Narendra, (2011) *Nat Rev Mol Cell Biol*

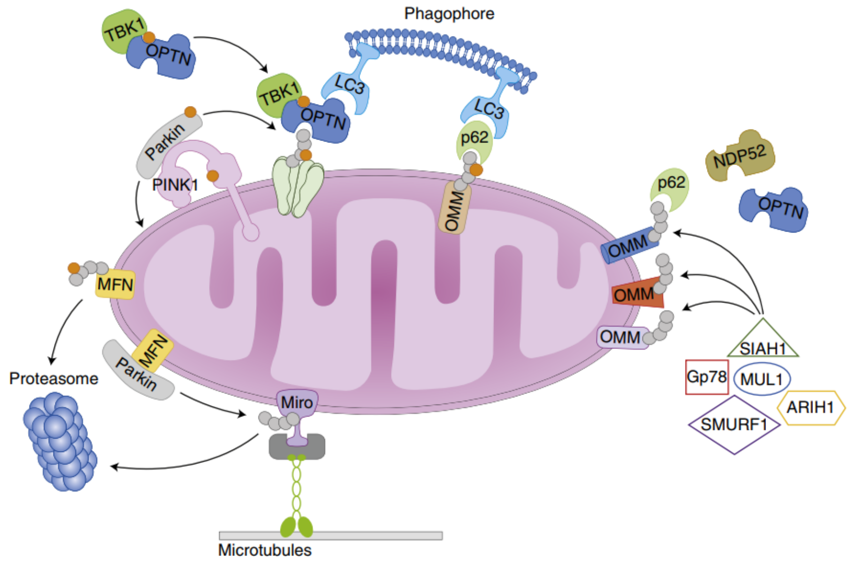
Degradation of dysfunctional mitochondria

Model of Parkin-induced mitophagy

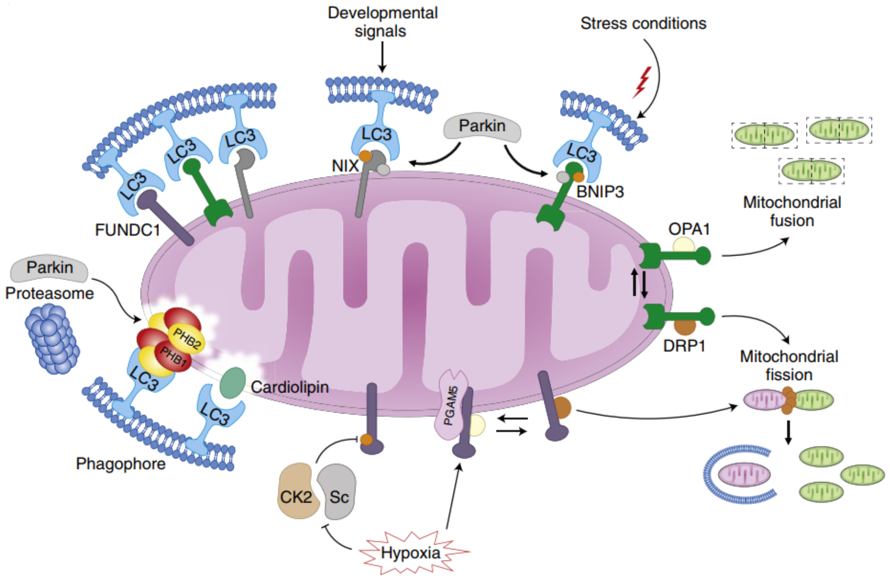


Molecular mechanisms of mitophagy execution

PINK1/Parkin – mediated mitophagy



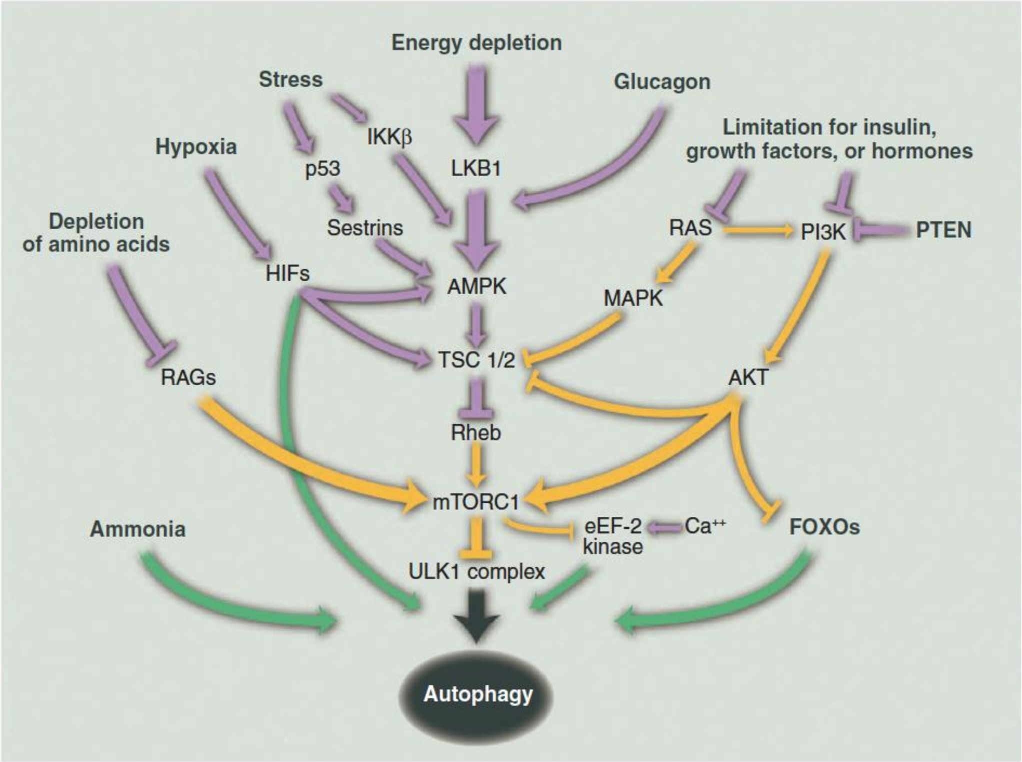
Receptor – mediated mitophagy

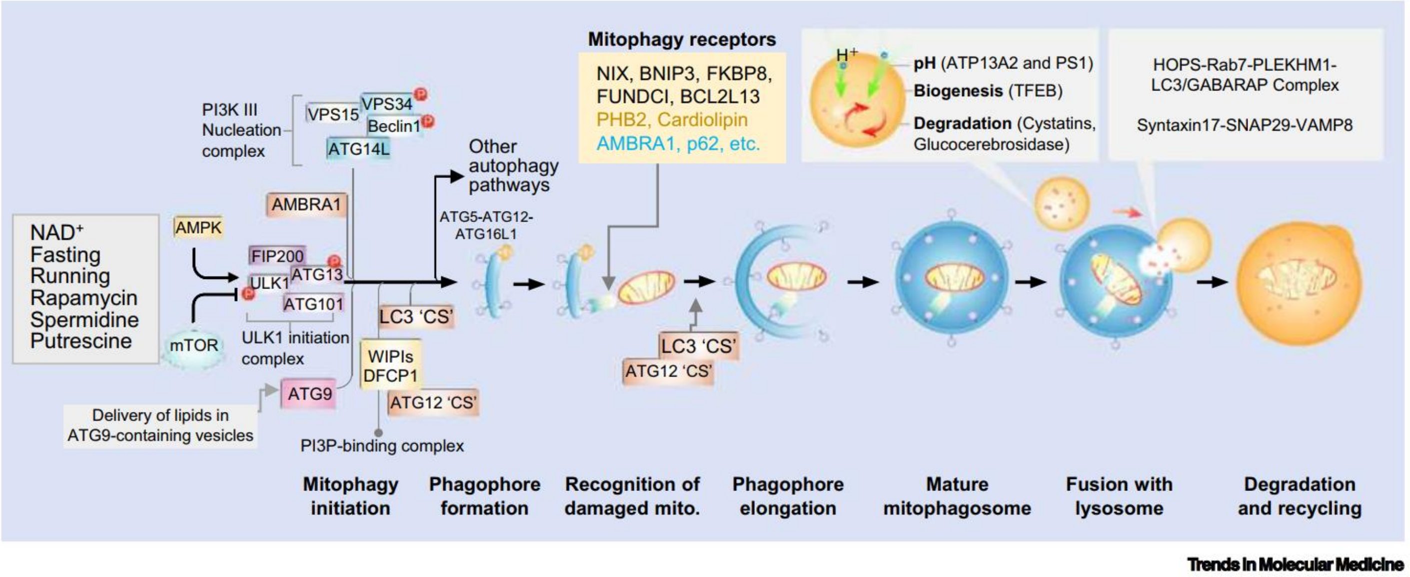


Palikaras et al., (2018) *Nature Cell Biology*
Pickrell & Youle, (2015) *Neuron*

Signaling pathways that regulate autophagy

- Common nutrient, growth factor, hormone, and stress signals regulate autophagy.

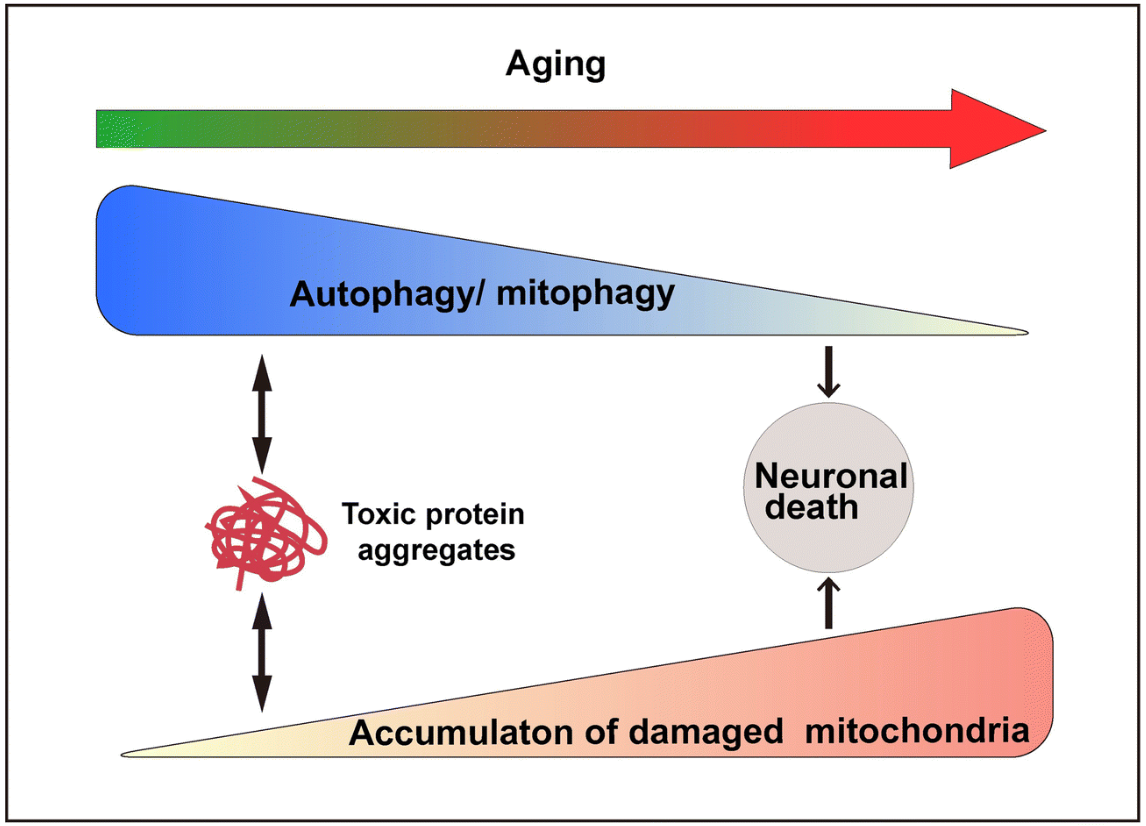


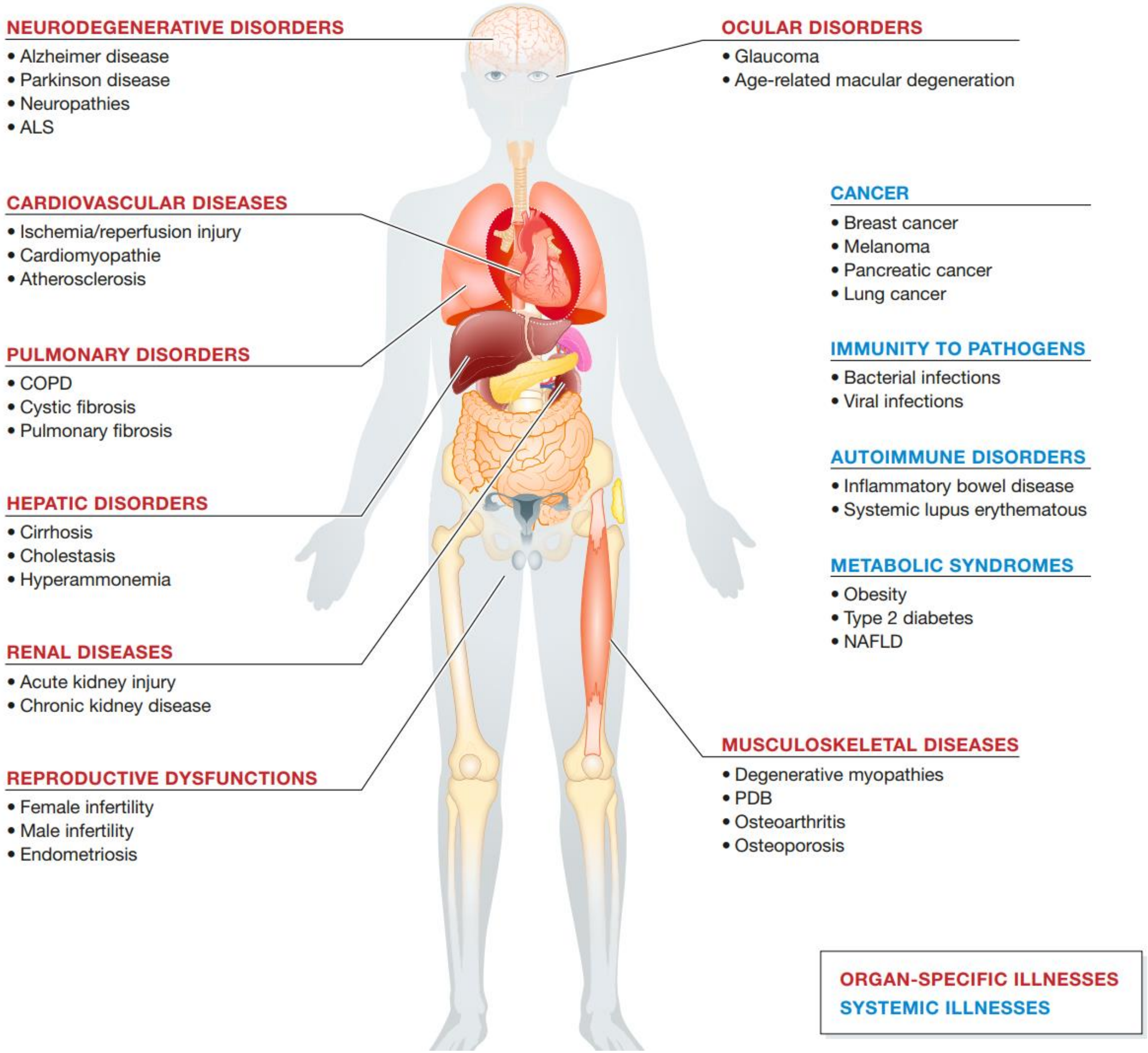


IKKb, inhibitor of nuclear factor kB kinase b; PI3K, phosphatidylinositol-3 kinase; PTEN, phosphatase and tensin homolog; MAPK, mitogen-activated protein kinase; TSC1/2, tuberosclerosis complexes 1 and 2; and EF, elongation factor.

Rabinowitz and White, 2010 *Science*
Leidal et al., 2018 *Nature Cell Biology*
Lou et al., 2019 *Trends Mol Med*

Autophagy and mitophagy efficiency decline with age



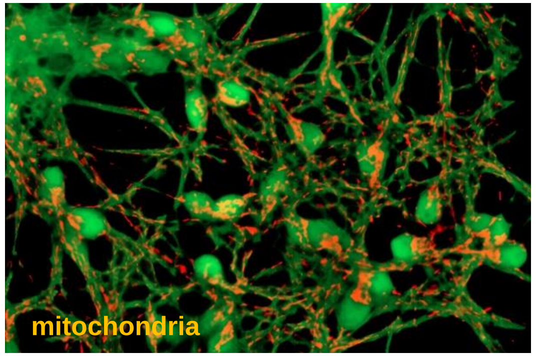


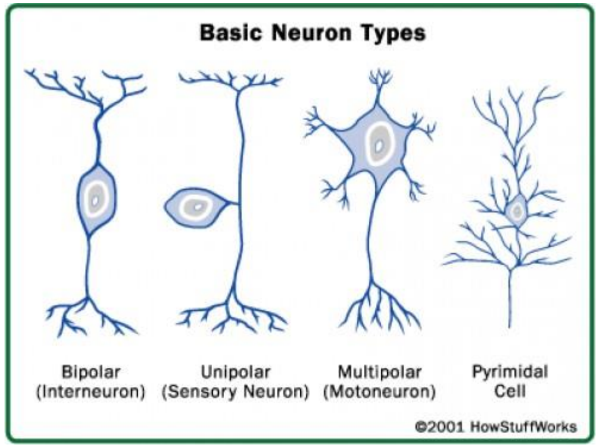
Klionsky *et al.*, 2021 *EMBO J*
Song *et al.*, *2021 Molecular Neurobiology*
Leidal et al., 2018 *Nat Cell Biol*

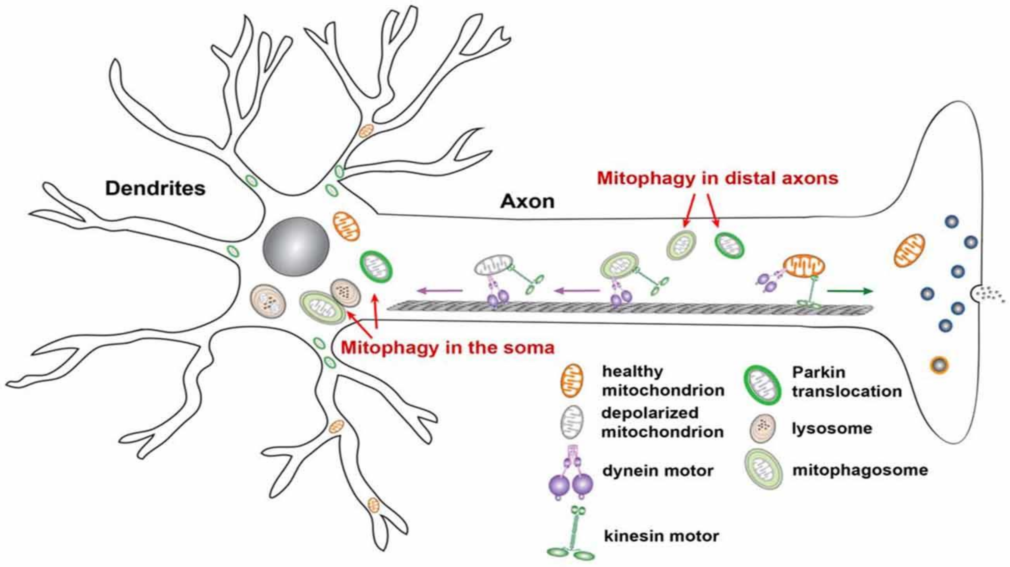
➢ **Autophagy & mitophagy dysfunction: a common denominator in age-related diseases**

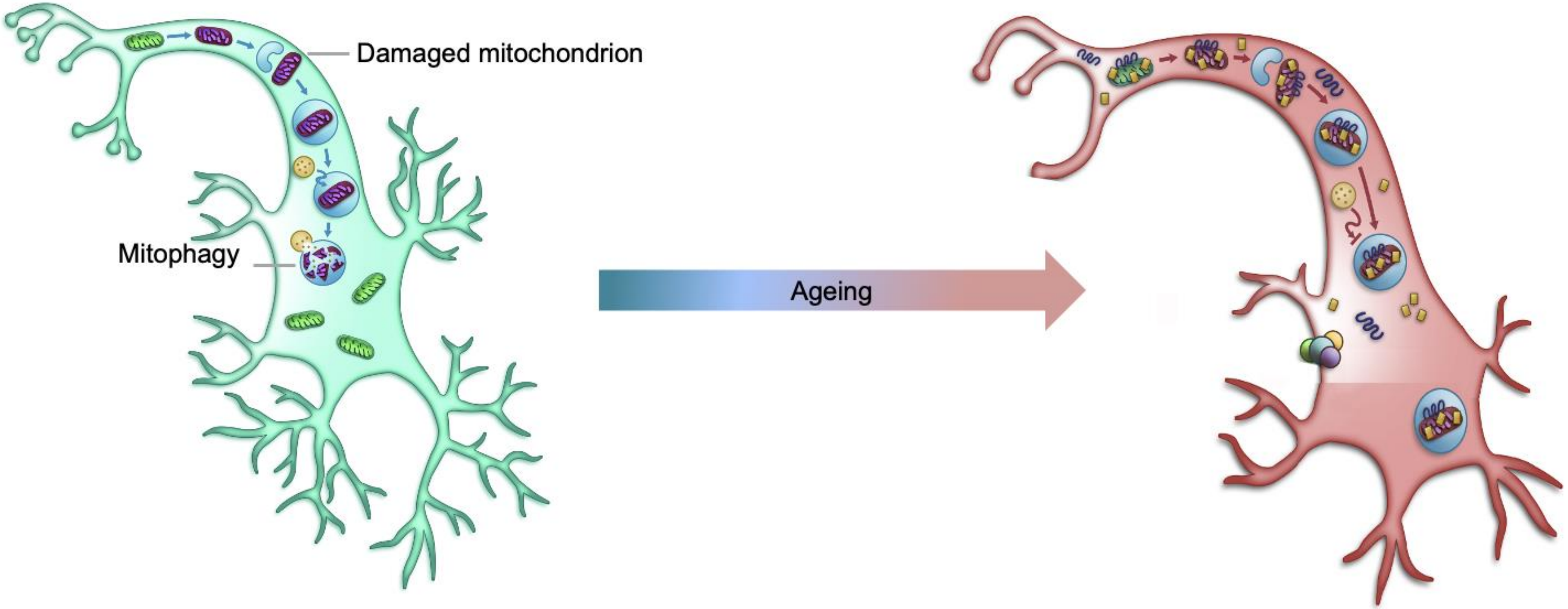
Ageing & mitophagy deficiency lead to increased prevalence of neurodegeneration

Increased mitochondrial population



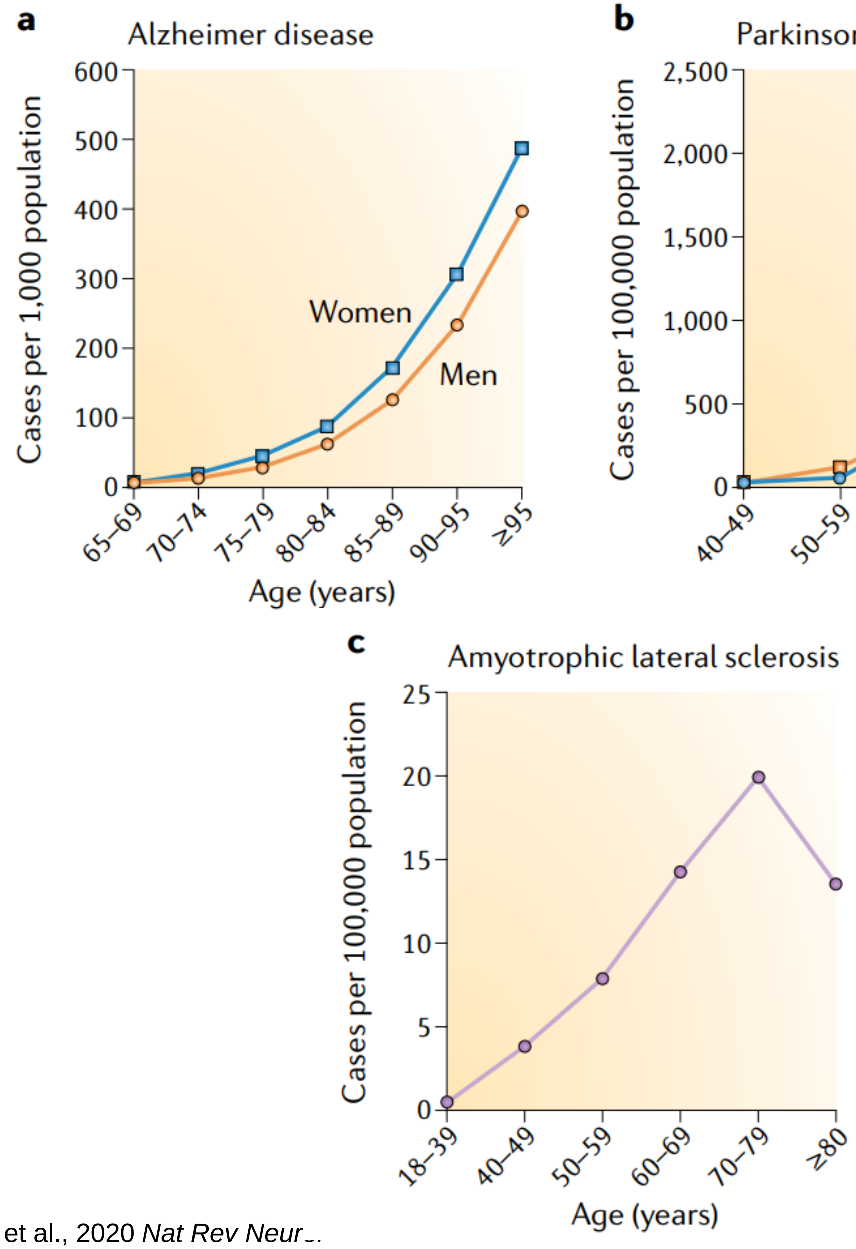


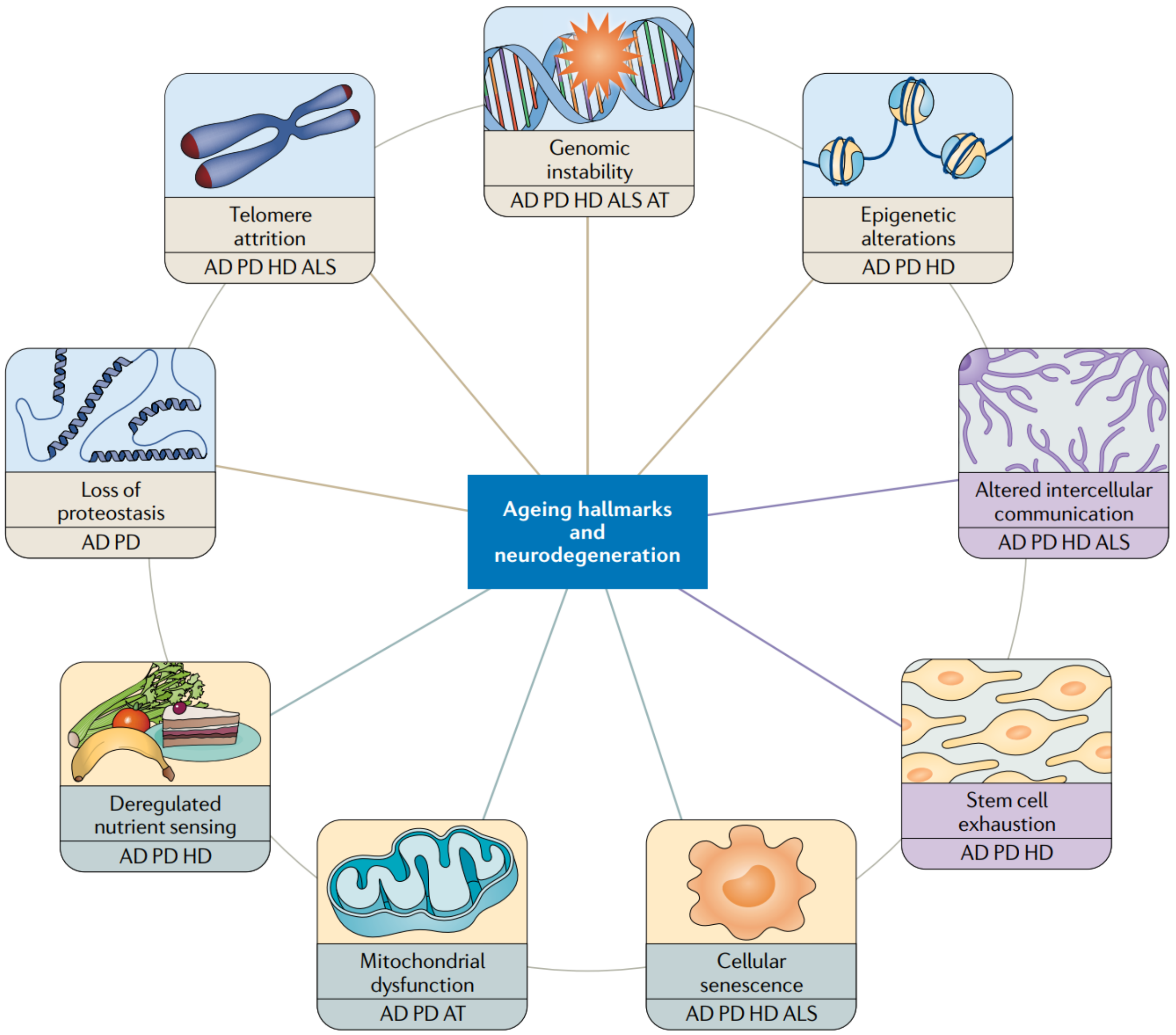




Sheng, 2014 *Journal of Cell Biology*
Kerr et al., 2017 *Trends in Neurosciences*
Lou*, Palikaras* et al., 2019 *Trends in Molecular Medicine*

Ageing & mitophagy deficiency lead to increased prevalence of neurodegeneration

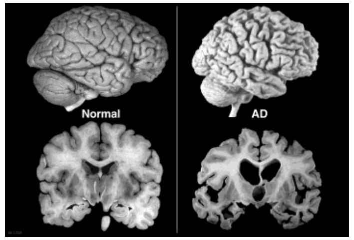


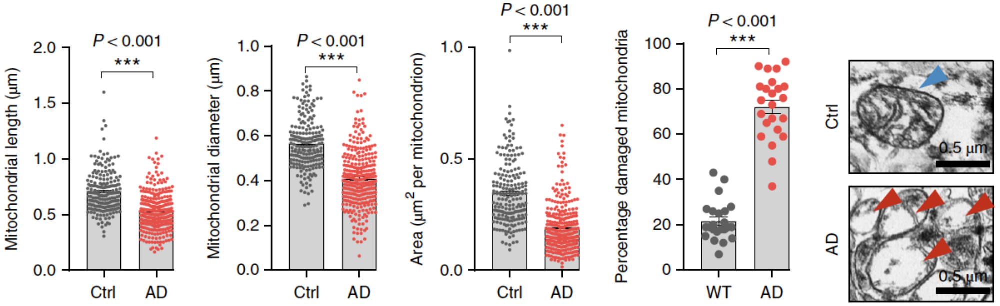


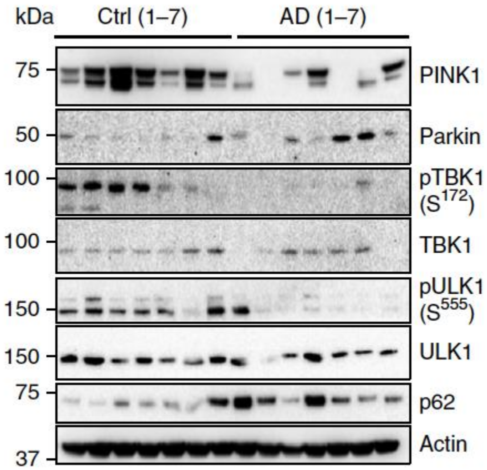
Hou et al., 2020 *Nat Rev Neur*

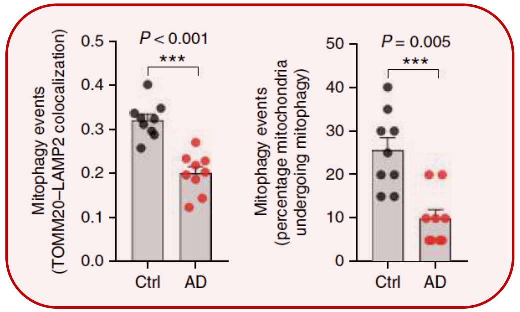
Mitophagy deregulation in the pathogenesis Alzheimer’s disease

Increased mitochondrial dysfunction in AD brain tissue







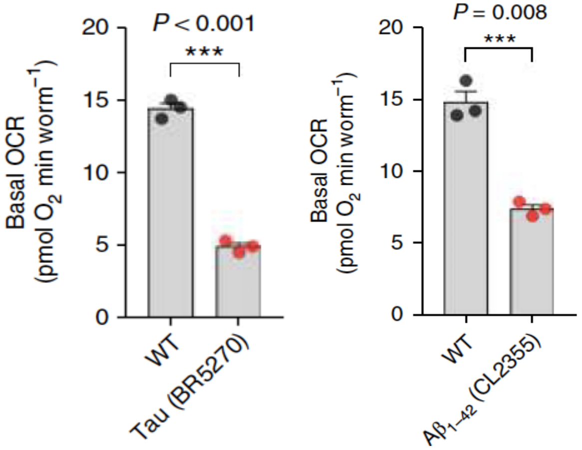


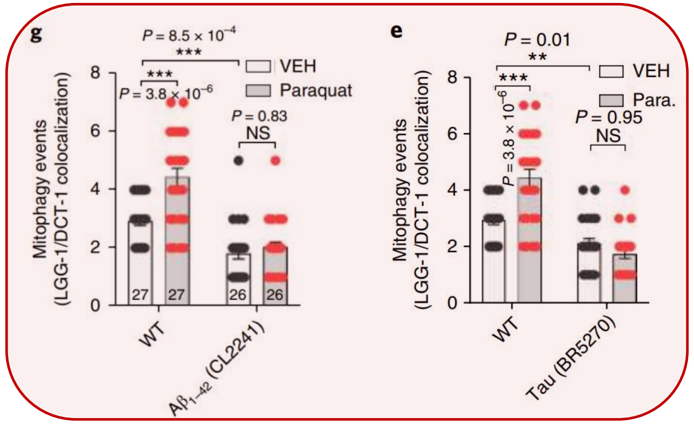
Mitophagy levels

C. elegans **Alzheimer disease model**

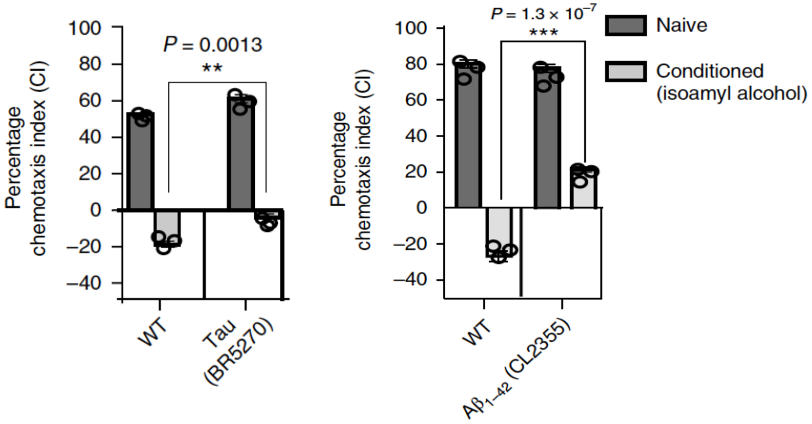
BR5270: $p_{rab\text{-}3}$Tau
CL2355: $p_{snb\text{-}1}$Aβ$_{1\text{-}42}$

Mitophagy levels



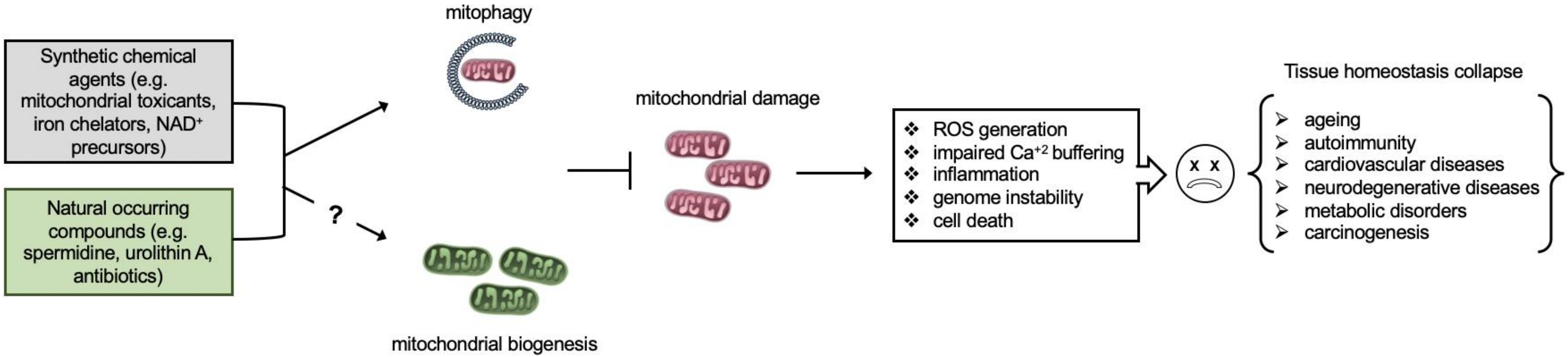


Reduced associated learning abilities in AD nematodes



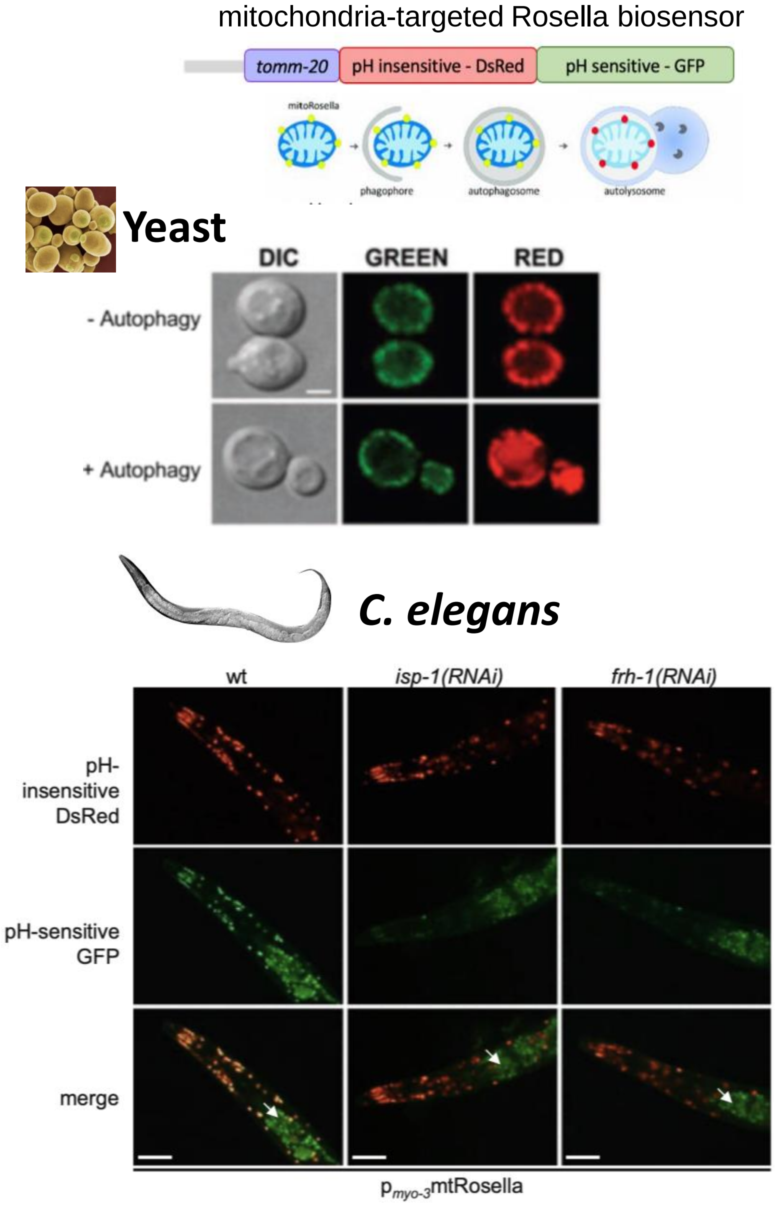
Fang*, Hou*, Palikaras* *et al., 2019 Nature Neuroscience*
*equal contribution

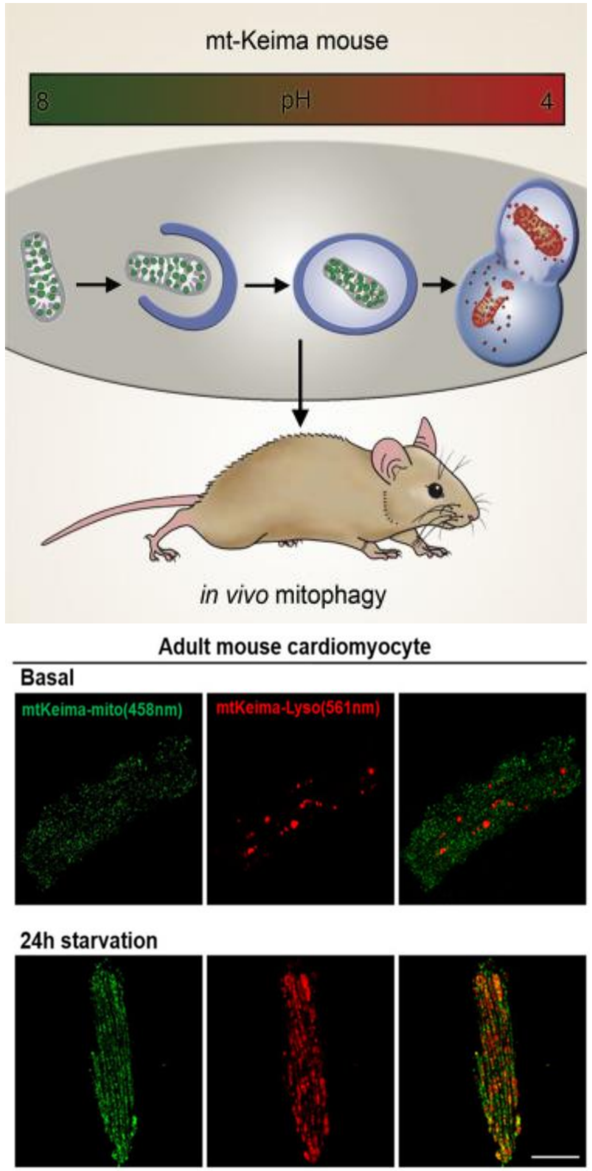
Pharmacological intervention to modulate neuronal mitophagy

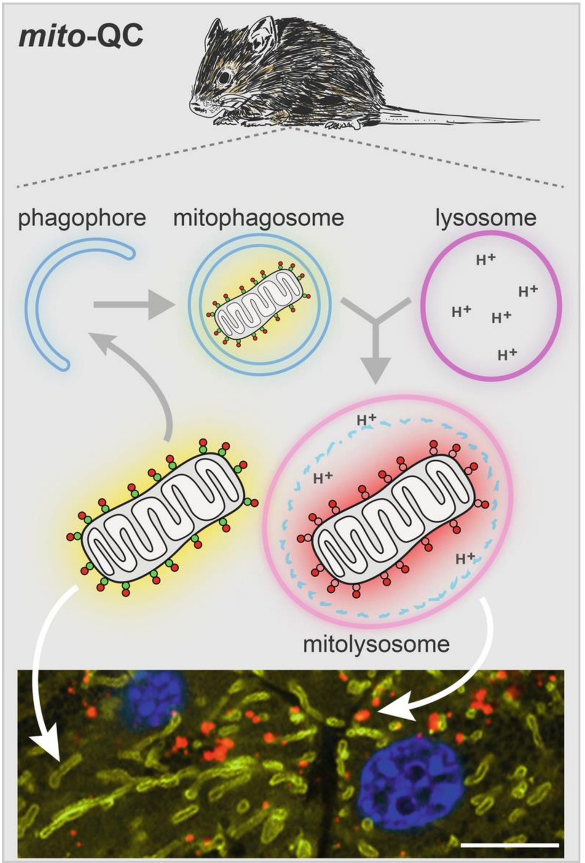


Palikaras and Tavernarakis, (2019) *Encyclopedia of Biomedical Gerontology*

In vivo assessment of mitochondrial selective autophagy

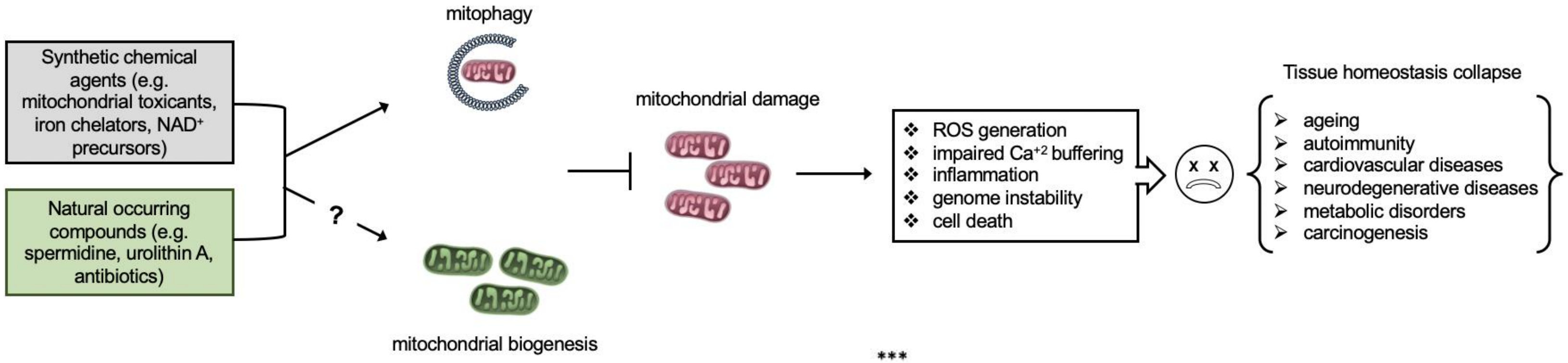


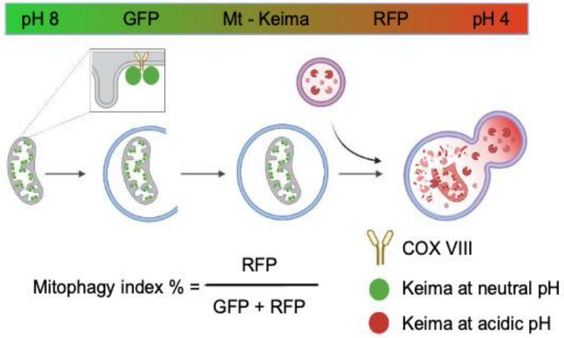


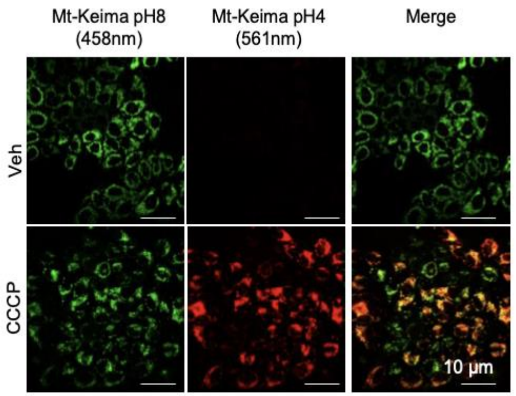


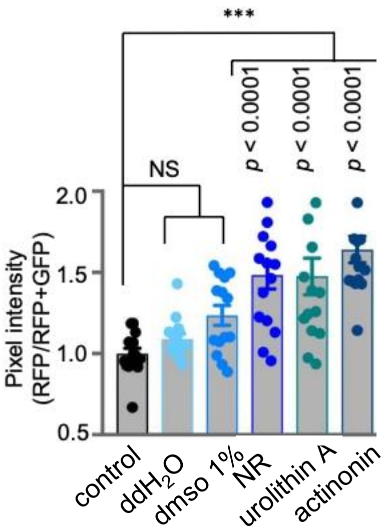
Rosado et al., (2008) *Autophagy*
Palikaras et al., (2015) *Nature*
Sun et al., (2015) Molecular Cell
McWilliam and Ganley, (2019) *Autophagy*

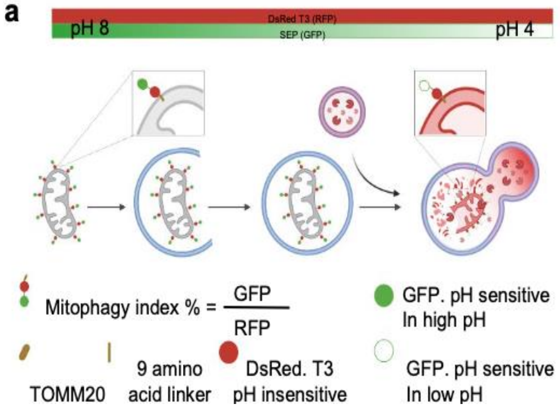
Pharmacological intervention to modulate neuronal mitophagy

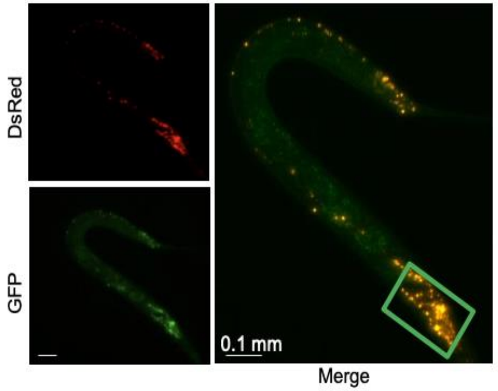


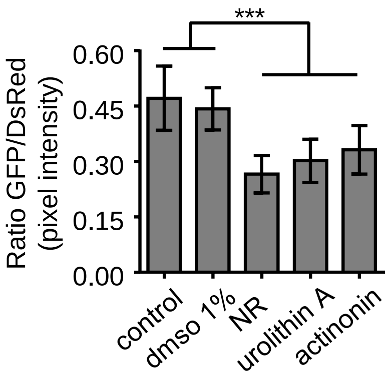










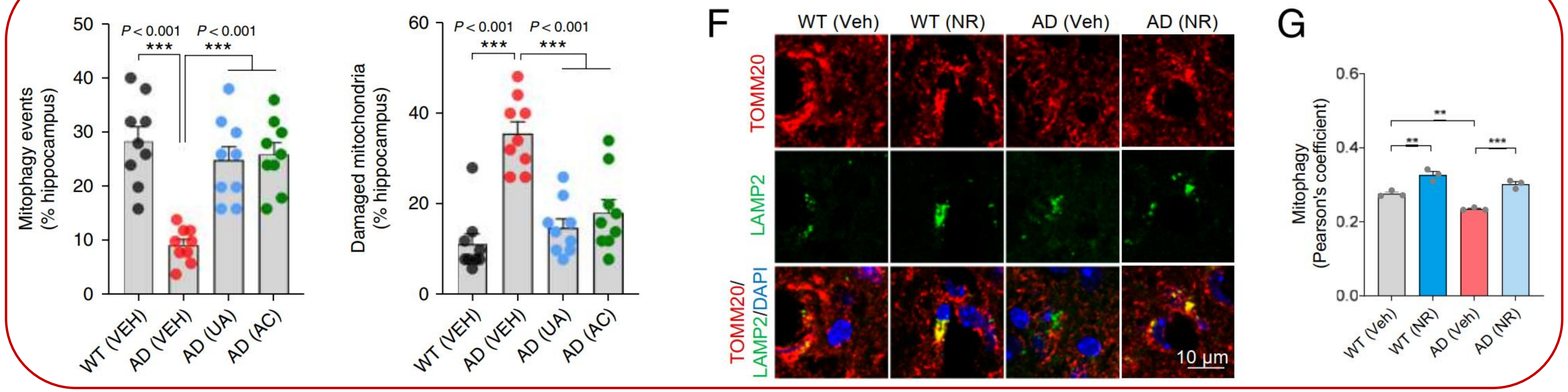


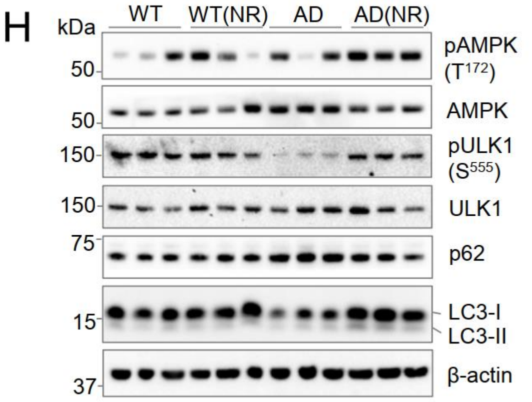
- ***Urolithin A***: naturally occurring compound that triggers mitophagy, thereby increasing lifespan in worms and improving muscle function in rodents and humans.
- ***Nicotinamide riboside (NR)***: NAD+ precursor molecule promoting energy homeostasis and lifespan.
- ***Actinonin***: actinonin, a naturally occurring antibacterial agent

Andreux et al., 2019 *Nature Metabolism*
Mitchell et al., 2018 *Cell Metabolism*
Ryu et al., 2016 *Nature Medicine*

Fang*, Hou*, Palikaras* *et al.*, (2019) *Nature Neuroscience*
Palikaras and Tavernarakis, (2019) *Encyclopedia of Biomedical Gerontology*

Mitophagy induction ameliorates Aβ pathology and cognitive decline in APP/PS1 AD mice

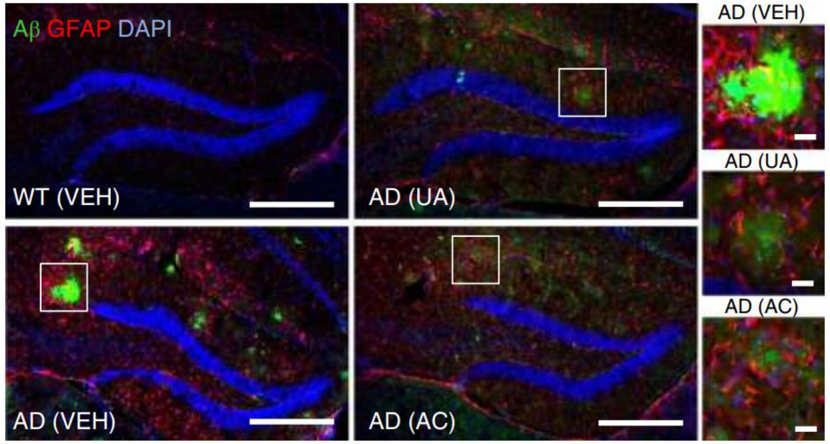


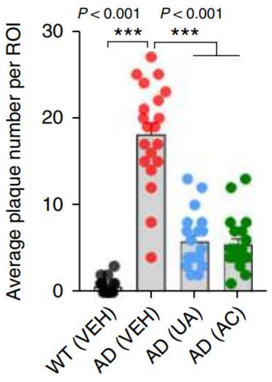


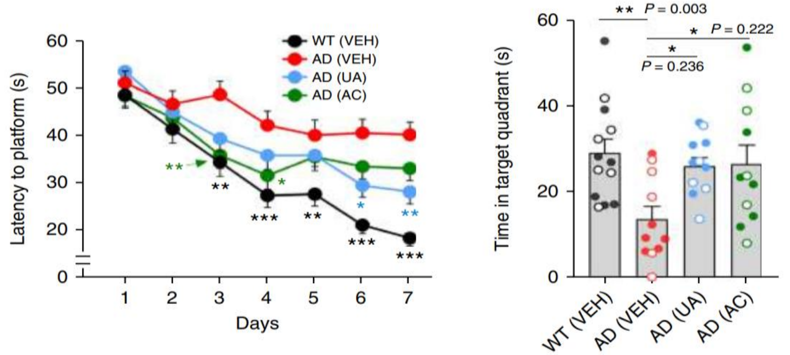
Mitophagy levels

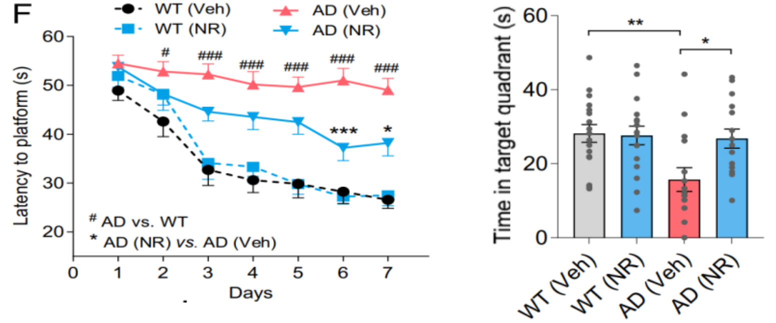
Improved cognitive function upon mitophagy induction

Reduced Aβ plaques formation upon UA and AC treatment





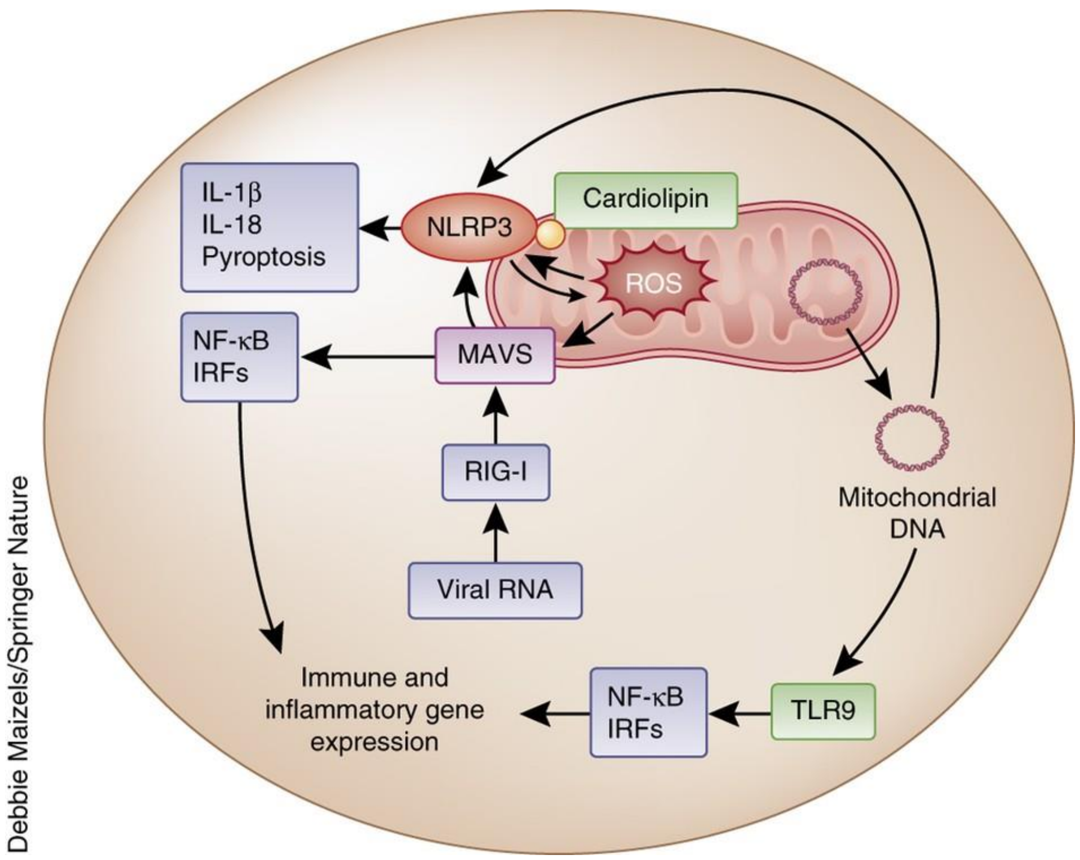


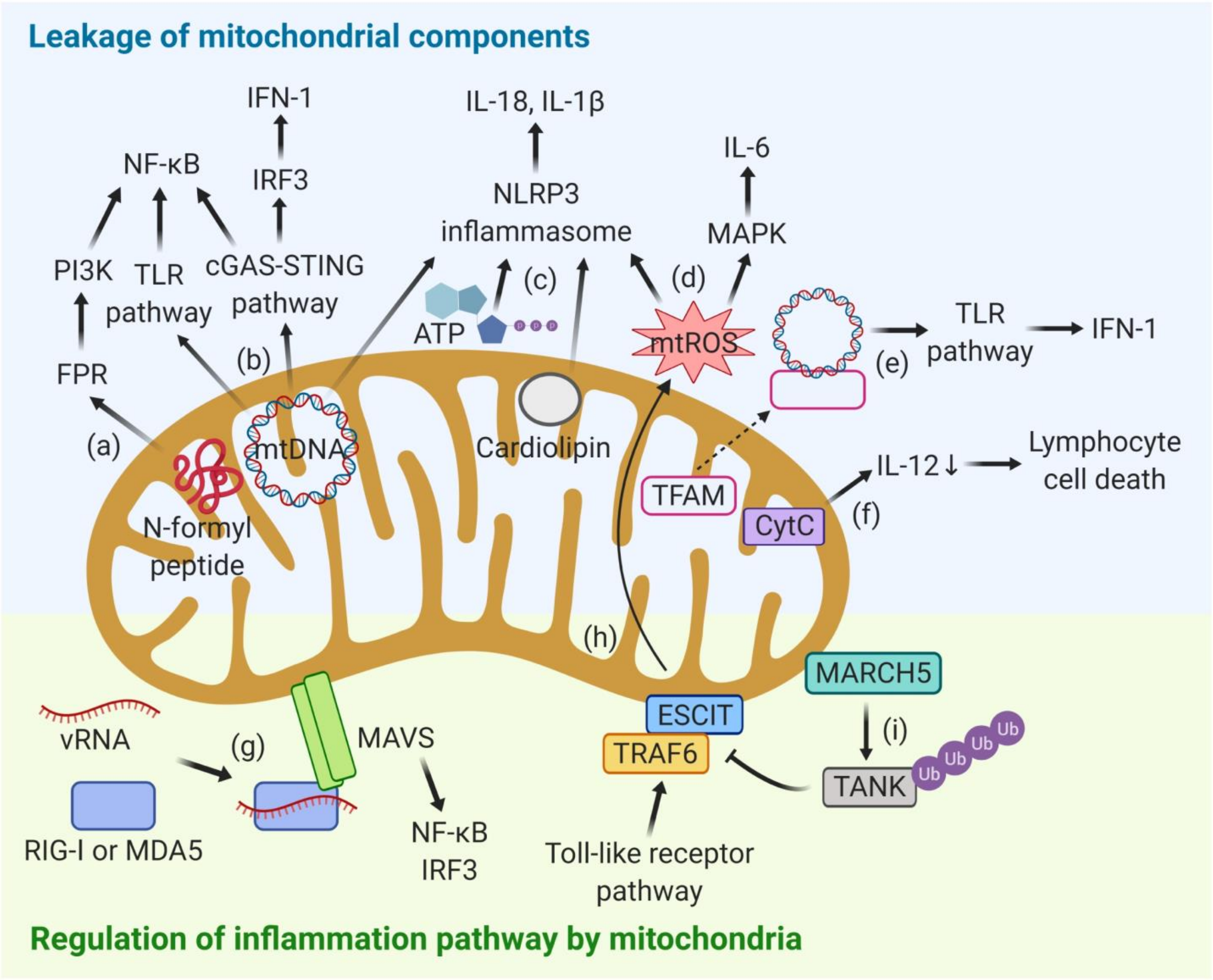


Hou et al.., (2021) *PNAS*

Fang*, Hou*, Palikaras* *et al.,* (2019) *Nature Neuroscienc*

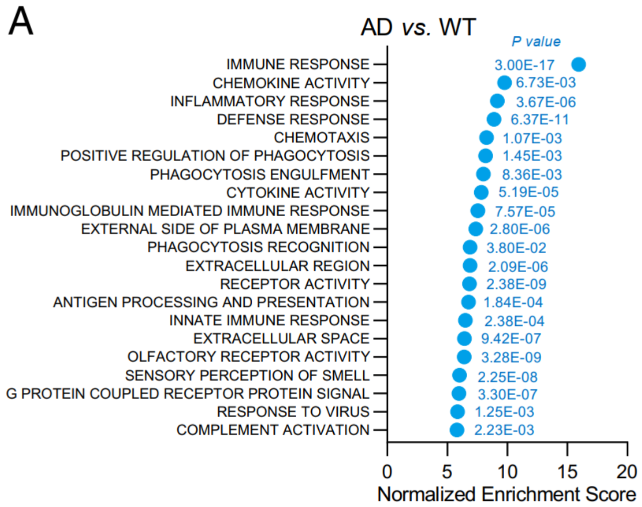
Mitochondria as a signaling hub of immunity

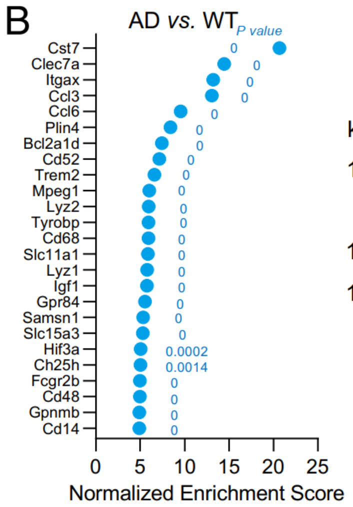




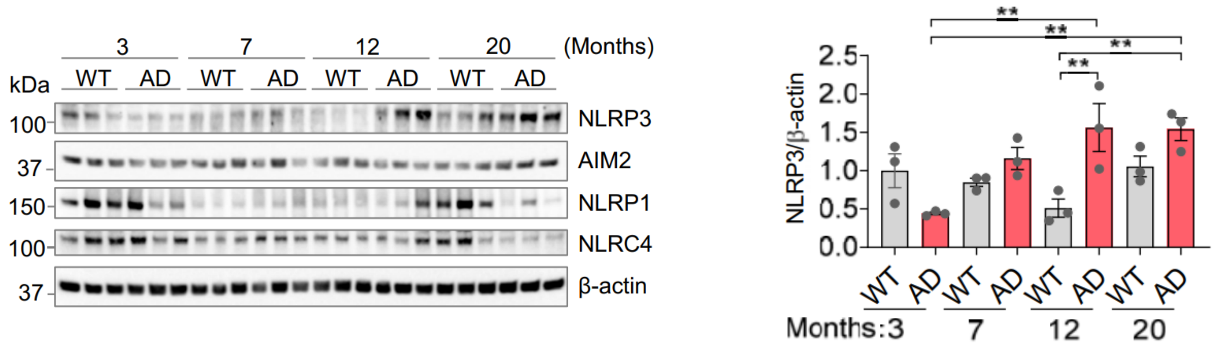
Yoo et al.., (2020) *BMB Reports*
Mills *et al.*, (2017) *Nature Immunology*

Mitophagy induction inhibits neuronal inflammation in APP/PS1 AD mice

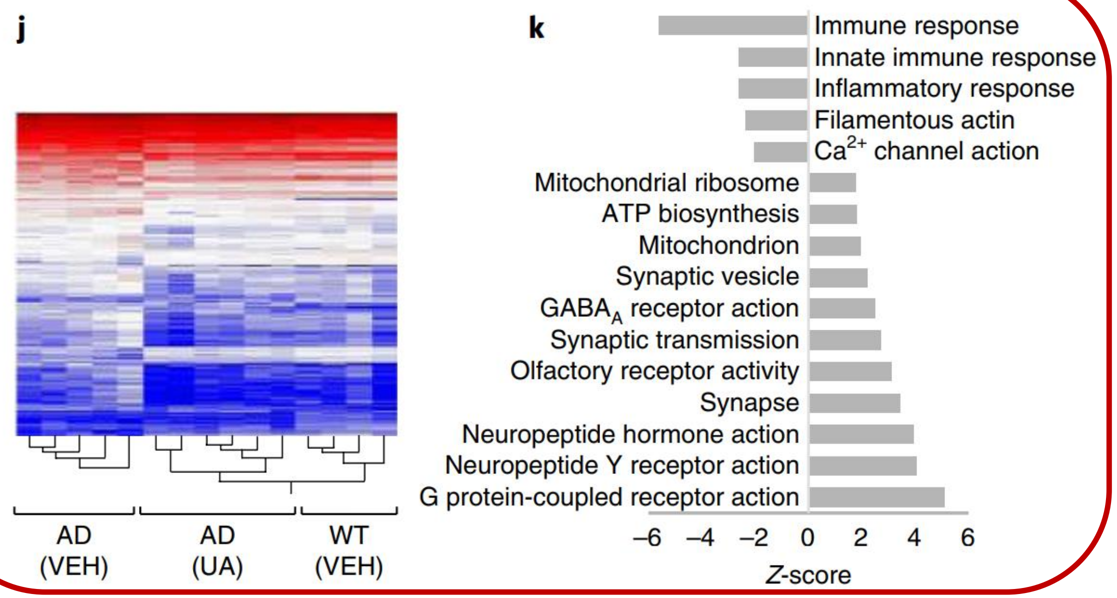




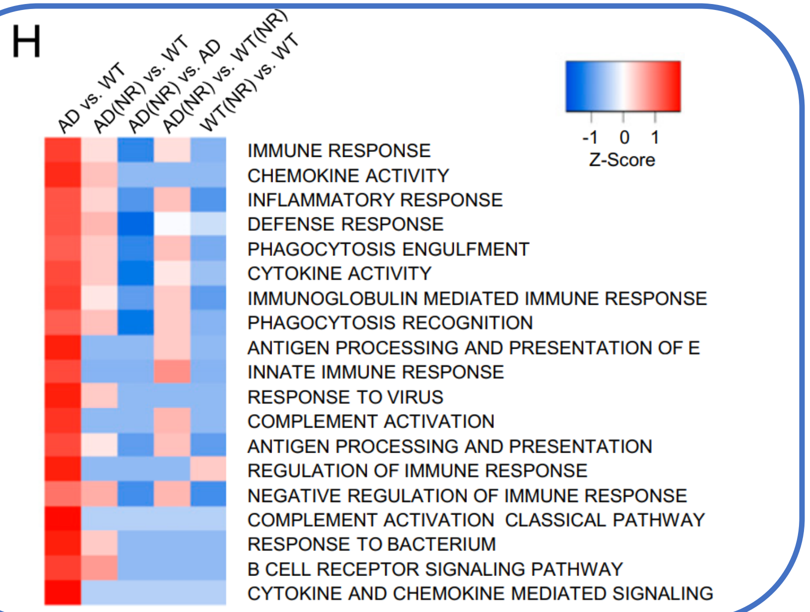
Age-dependent activation of inflammasome



UA treatment



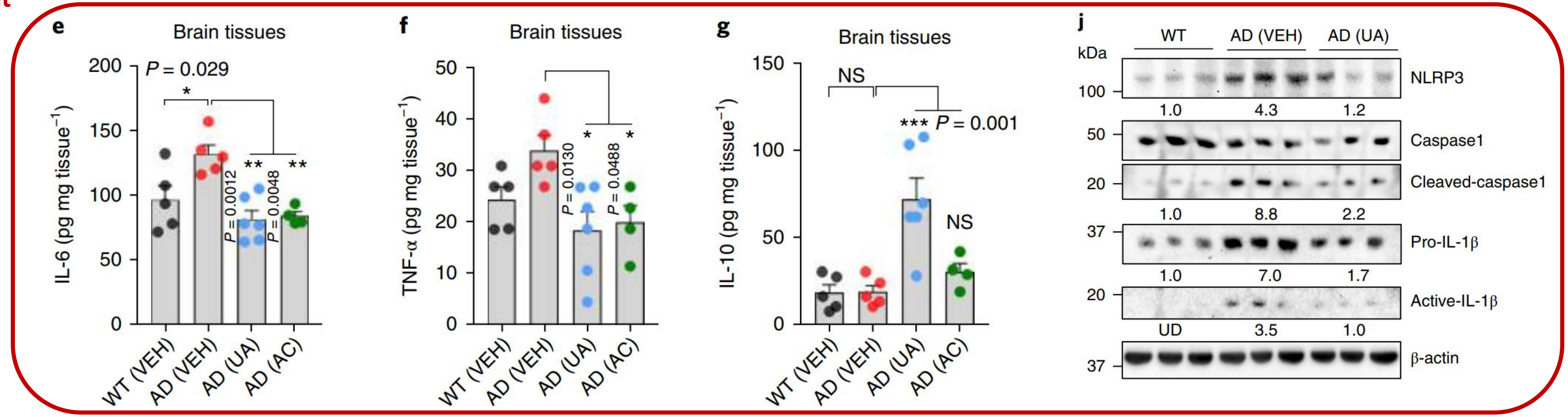
NR treatment



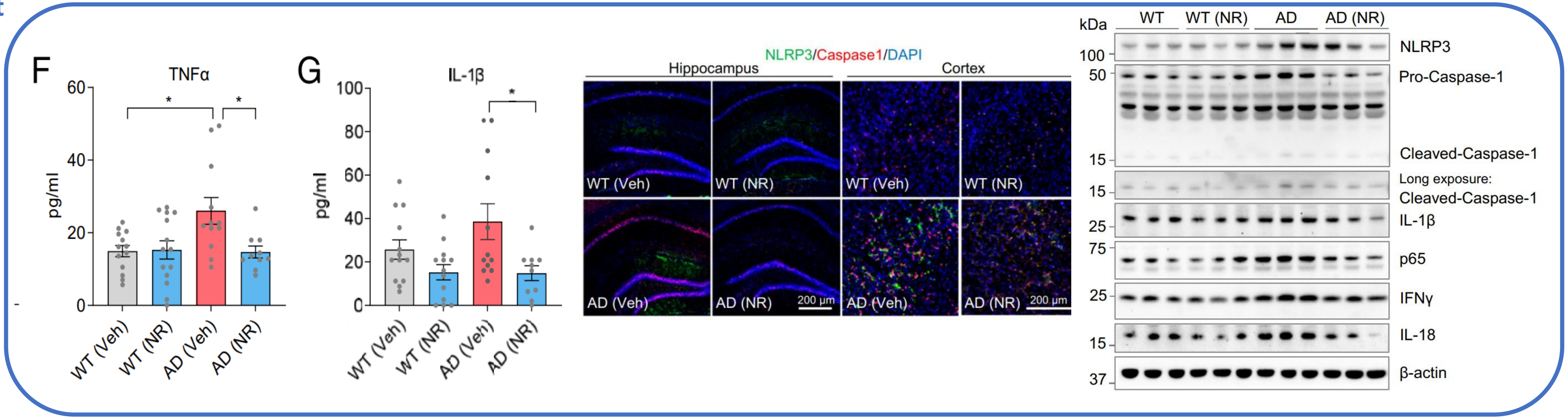
Hou et al.., (2021) *PNAS*
Fang*, Hou*, Palikaras* *et al.*, (2019) *Nature Neuroscience*

Mitophagy induction inhibits neuroinflammation in APP/PS1 AD mice

UA treatment



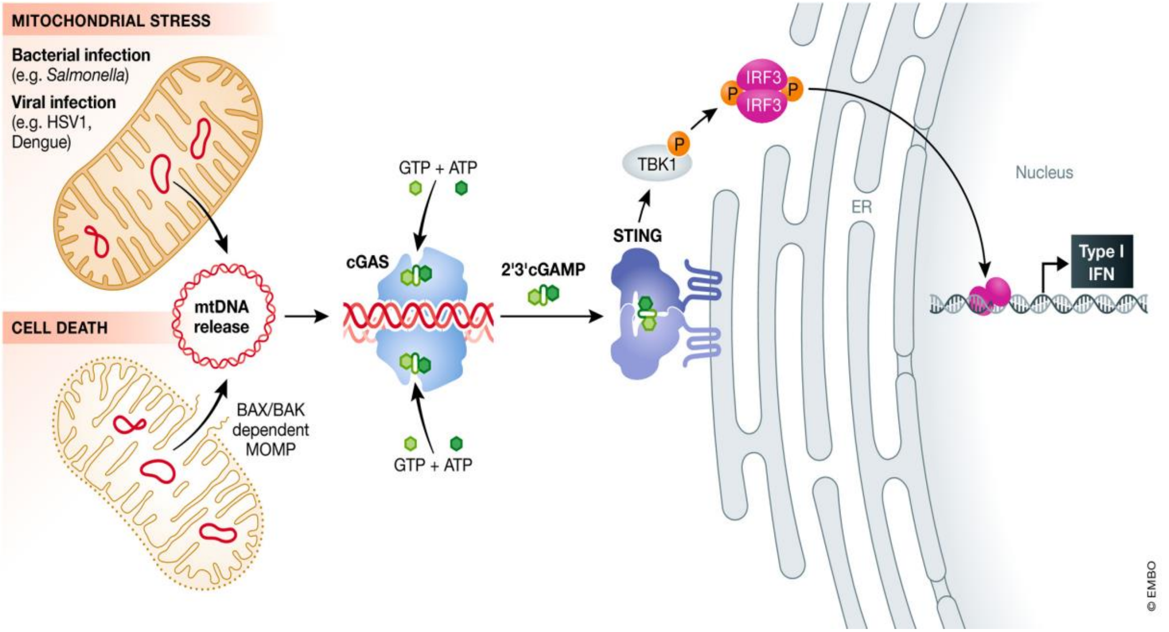
NR treatment



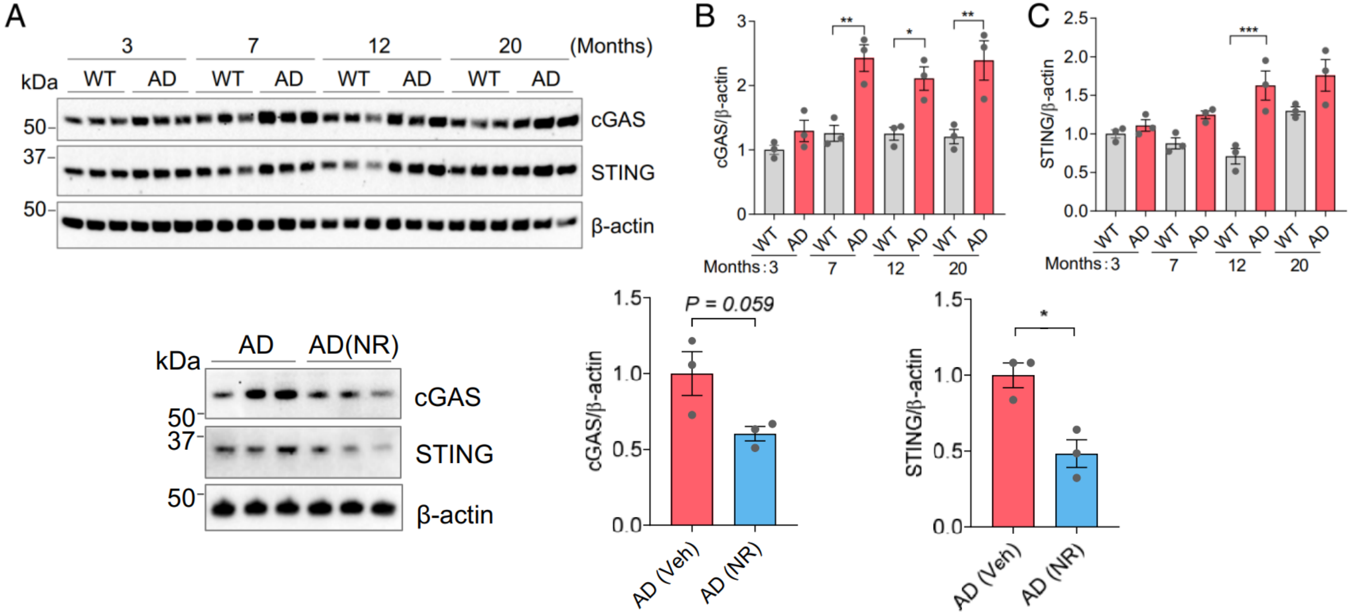
Hou et al.., (2021) *PNAS*
Fang*, Hou*, Palikaras* *et al.*, (2019) *Nature Neuroscience*

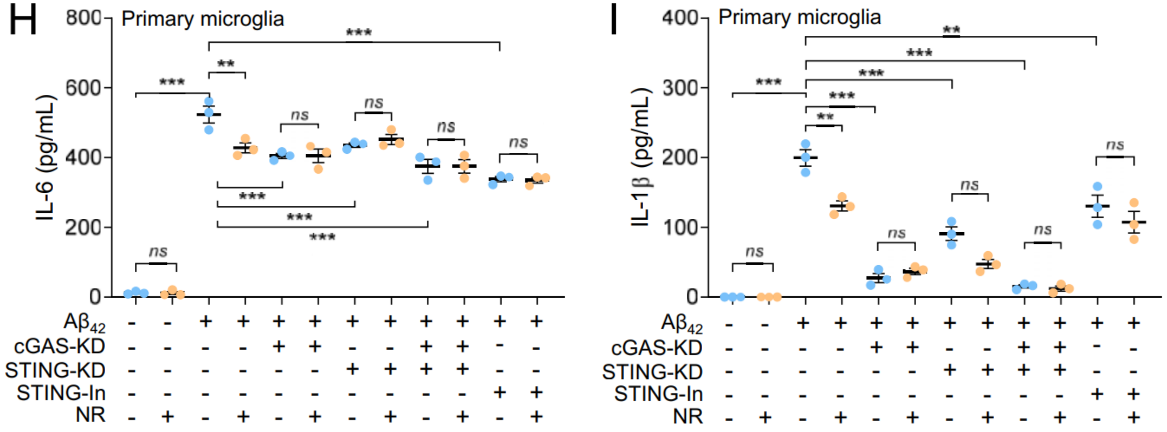
Mitochondrial damage triggers cGAS-STING pathway in AD mice

1. Cytoplasmic mtDNA binds the DNA sensing protein cGAS that catalyses the production of the secondary messenger 2′3′ cyclic GMP–AMP (2′3′cGAMP) from ATP and GTP.
2. cGAMP binds the adaptor molecule STING on the ER leading to activation of TBK1 kinase.
3. Active TBK1 phosphorylates the transcription factor IRF3 initiating a type I interferon response.



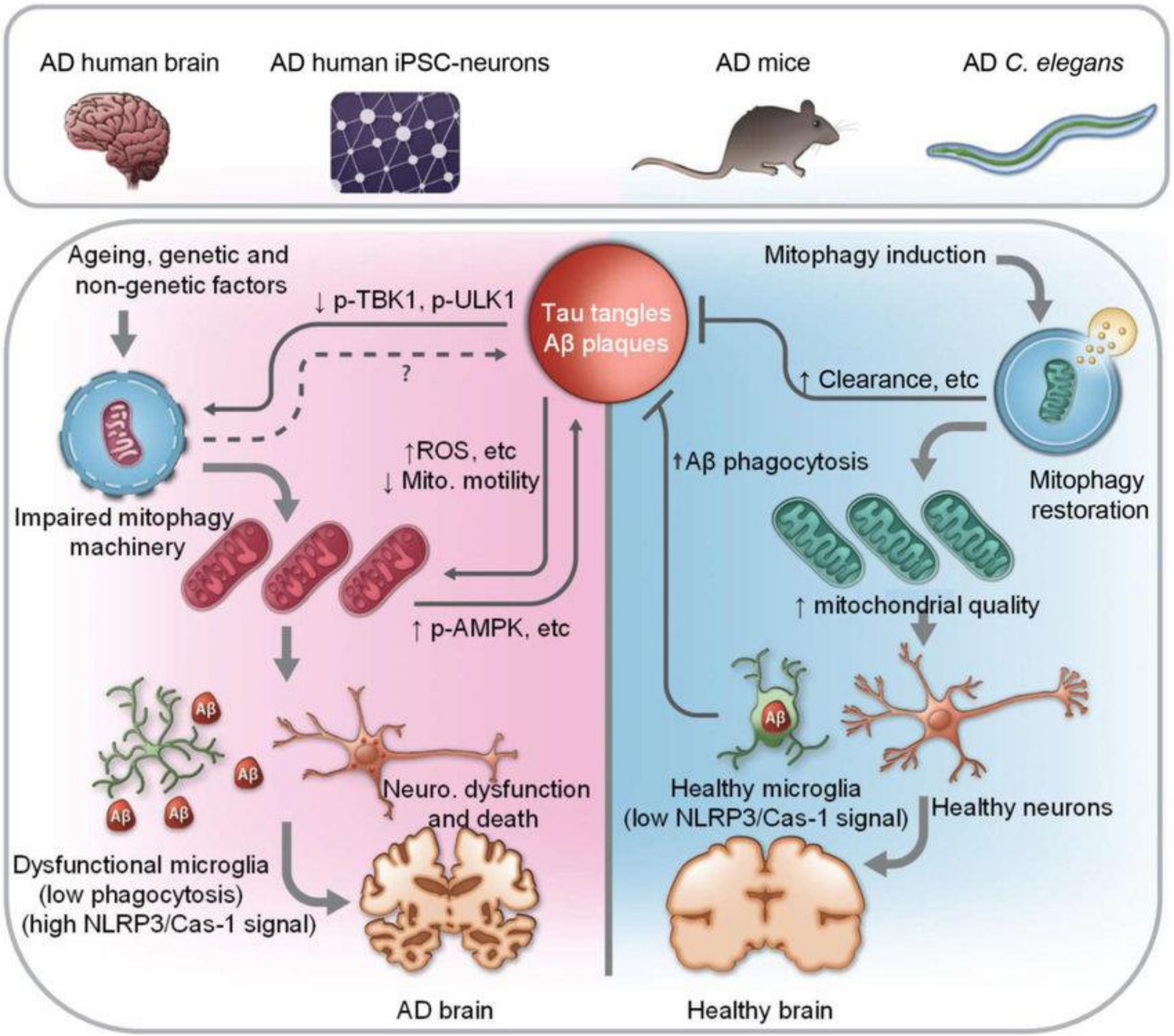
Age-dependent activation of cGAS-STING pathway





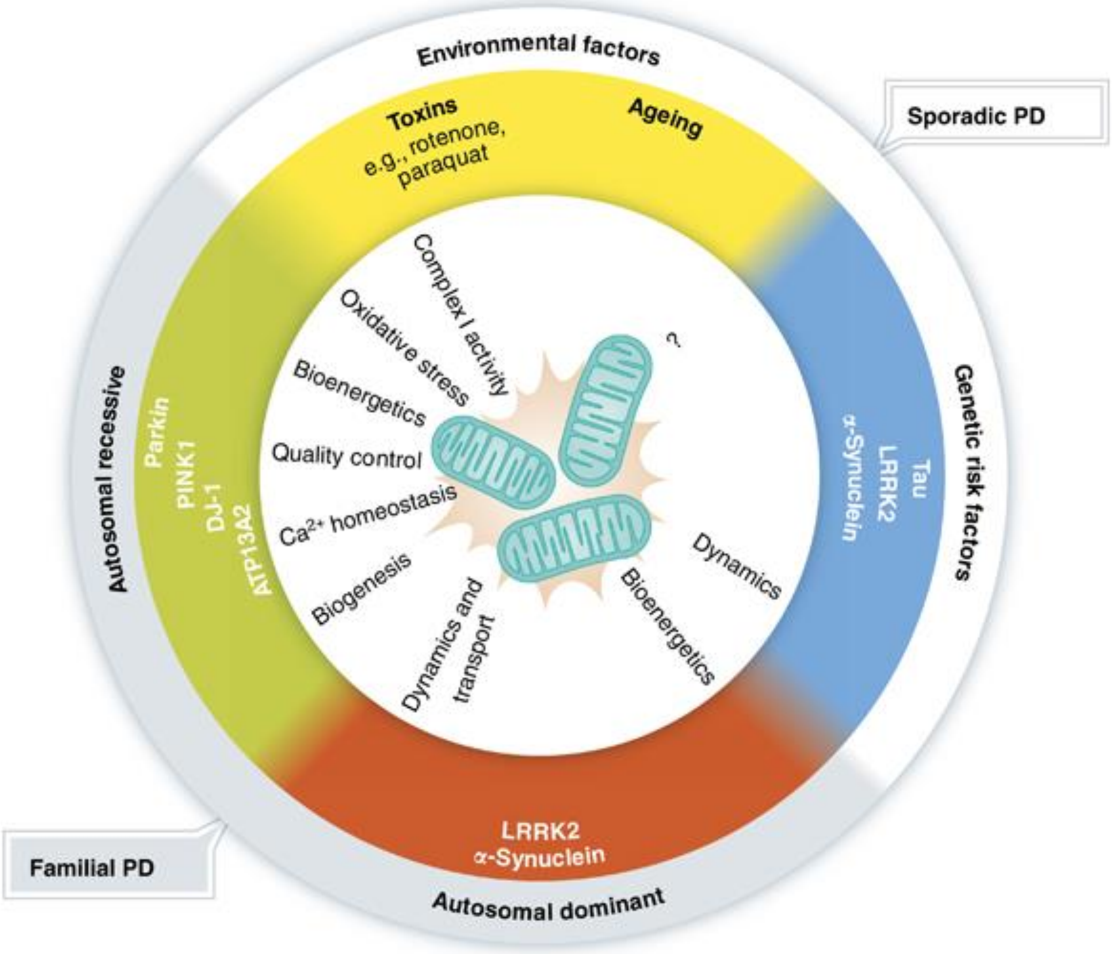
Hou et al., 2021 *PNAS*
Riley and Tait, 2020 *EMBO Reports*

Impaired mitophagy: a hallmark in AD pahtophysiology

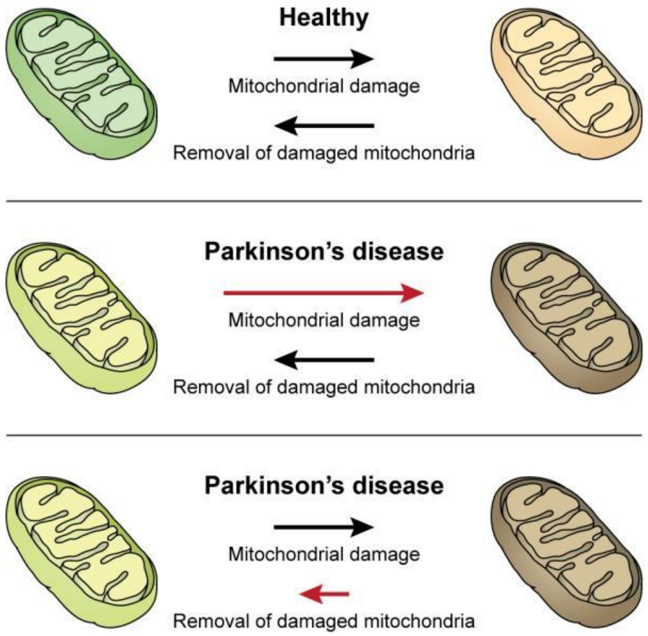


Fang*, Hou*, Palikaras* *et al., 2019 Nature Neuroscience*

Mitochondrial dysfunction in Parkinson's Disease



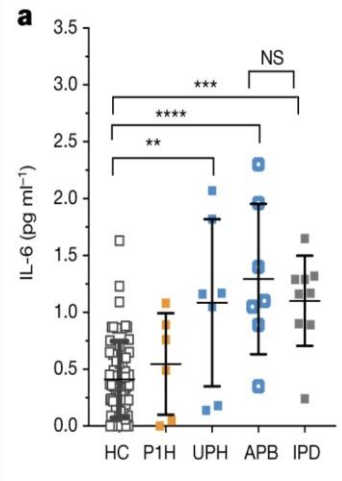
- Kitada, T. et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392, 605–608 (1998).
- Valente, E. M. et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304, 1158–1160 (2004).



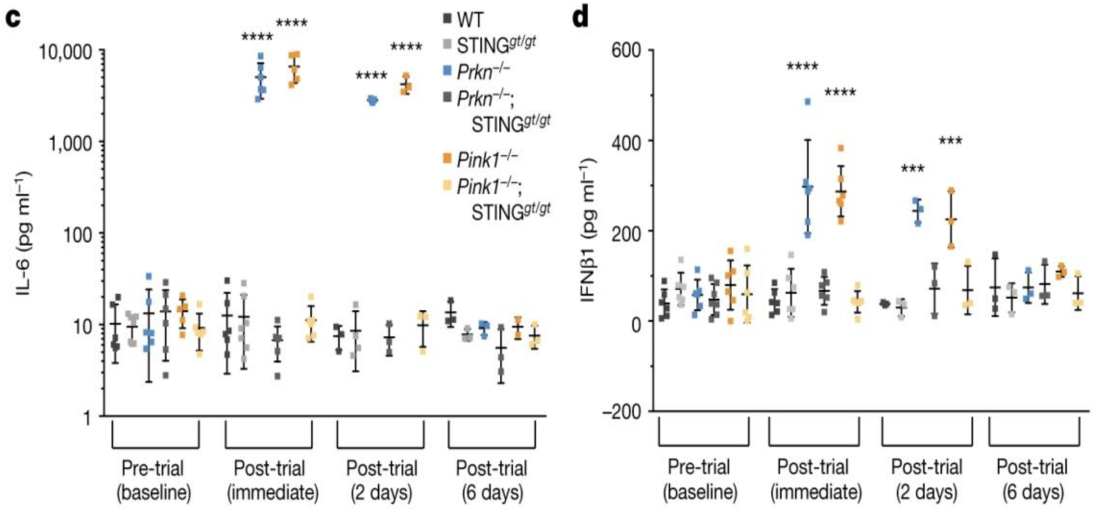
Exner et al., 2012 *EMBO J*
Pickrell & Youle, 2015 *Neuron*

cGAS-STING induced inflammation in Parkinson's Disease

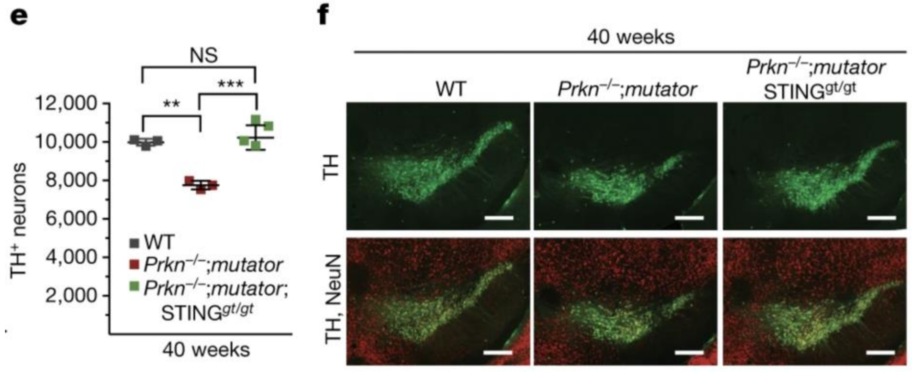
Serum IL-6 concentration in humans



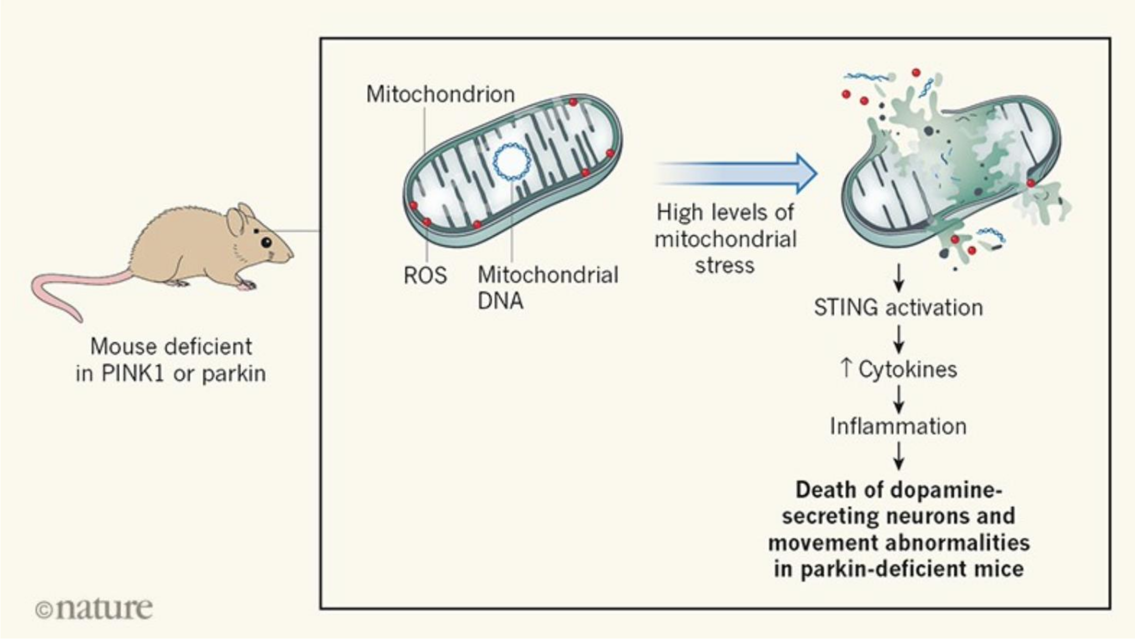
Serum IL-6 & IFNβ1 concentration in mice



Neuronal loss in PD mice

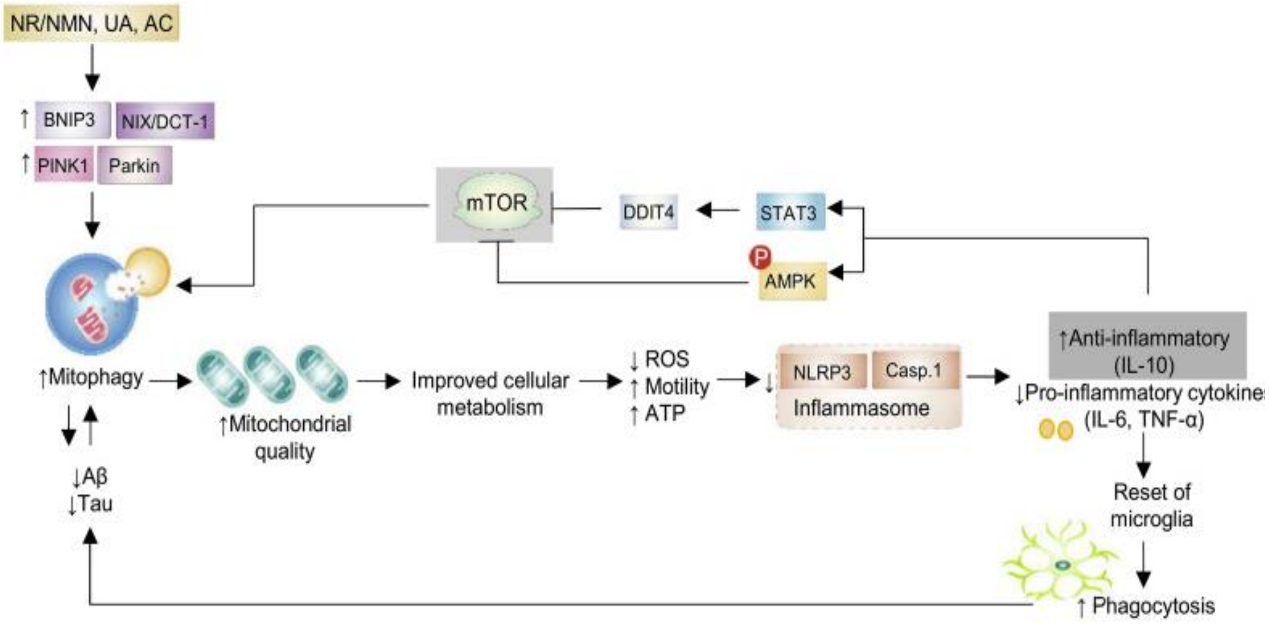


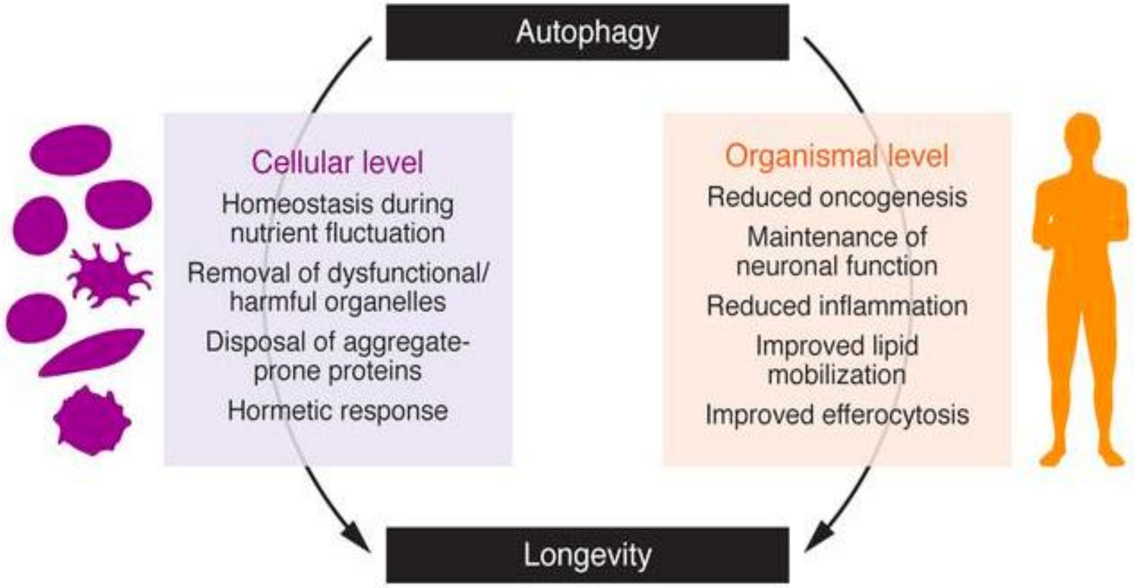
HC: human control
P1H: *PINK1* heterozygotes
UPH: unaffected *PRKN* heterozygotes
APB: affected *PRKN* biallelic mutants
IPD: patients with idiopathic Parkinson's disease

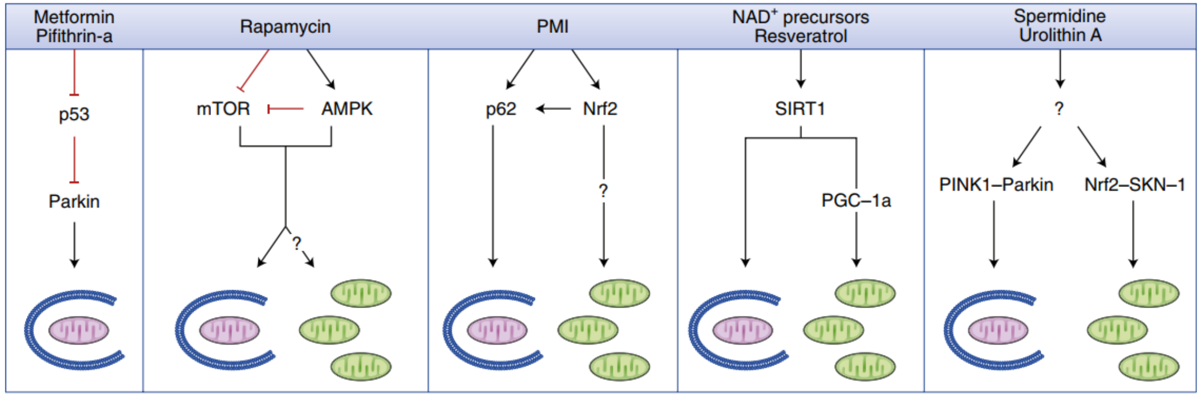


Stolz and Dikic, (2018) *Nature*
Sliter *et al.*, (2018) *Nature*

Pharmacological upregulation of mitophagy to treat neuroinflamation



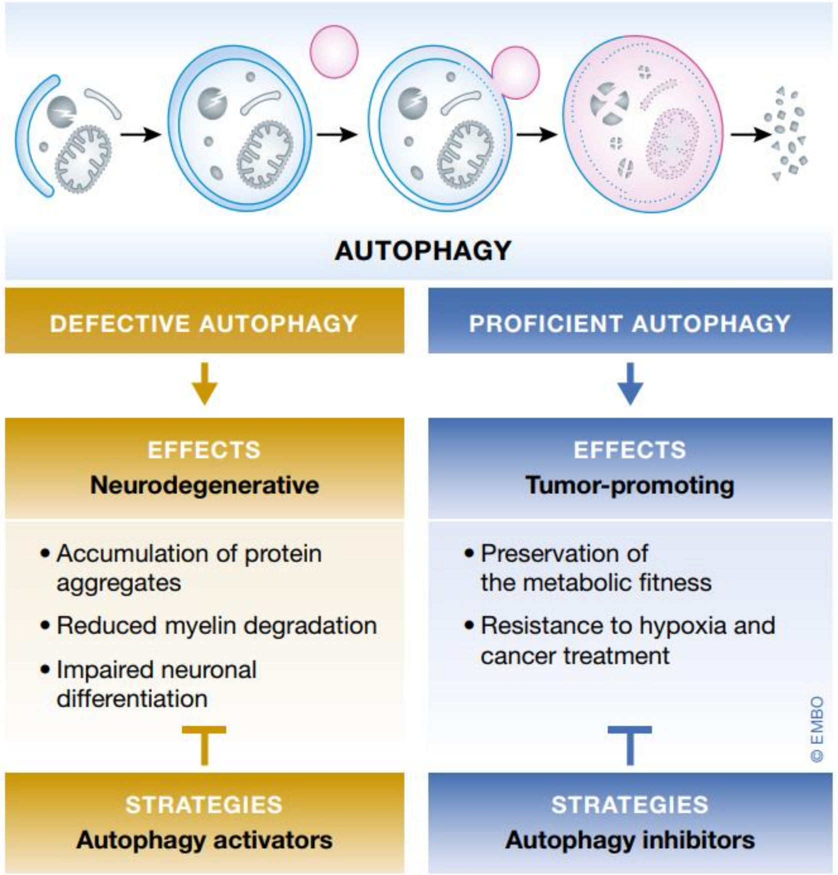


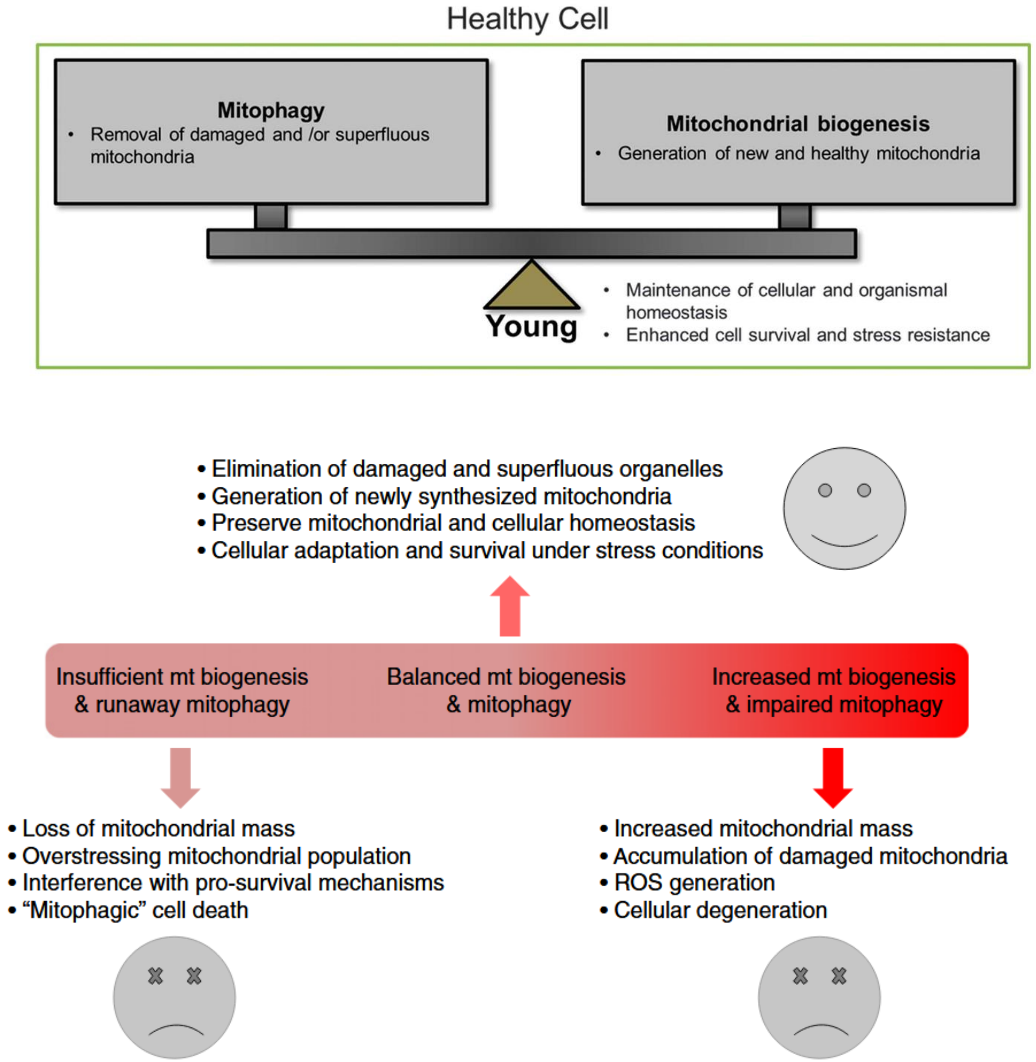


- Several pharmacologic and dietary interventions activate autophagy/mitophagy signaling and thereby promote beneficial effects at the cellular and organismal levels, contributing to prolonged life span and health span.

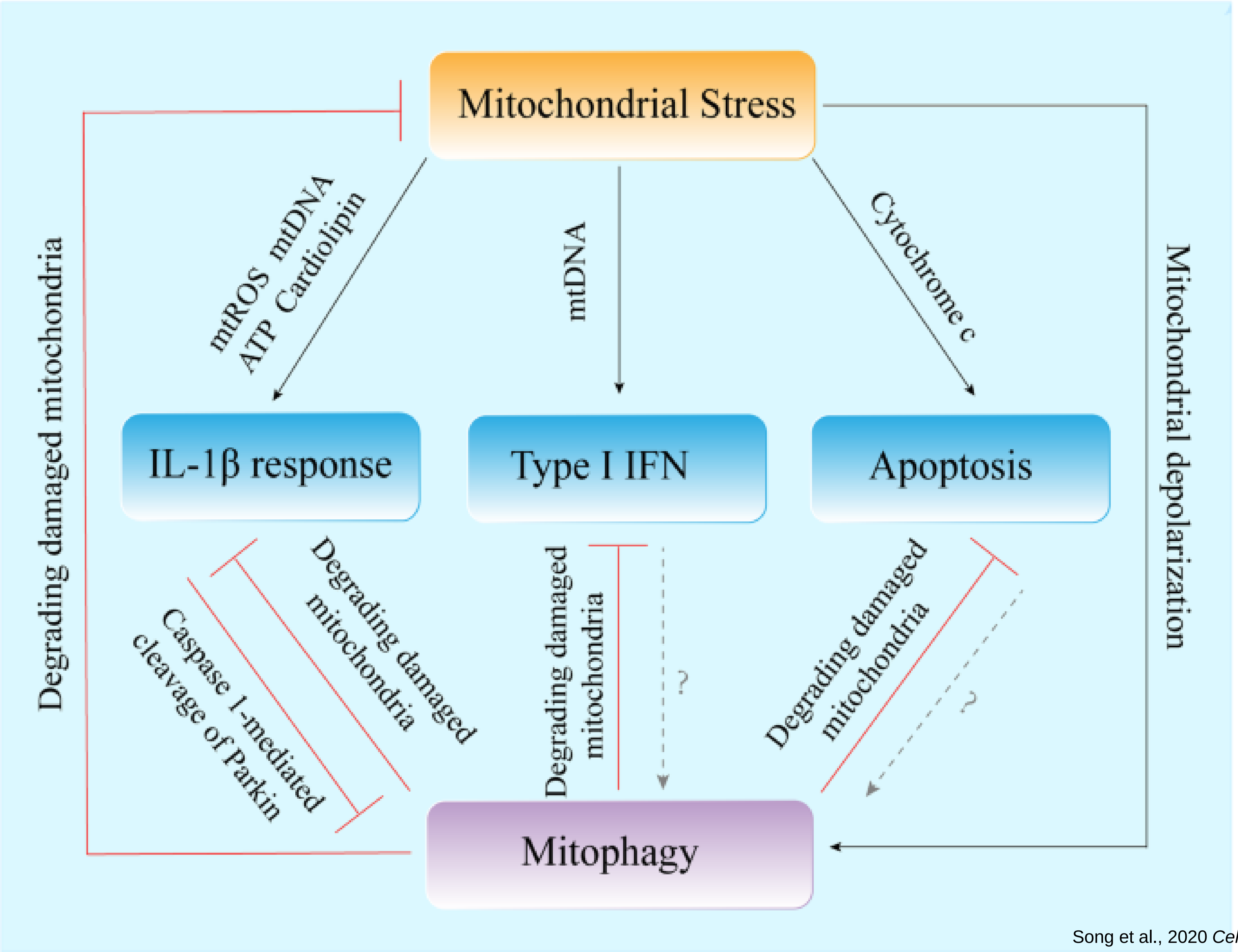
Aman et al., (2021) *Nature Aging*
Lautrup et al., (2019) *Neurochemistry International*
Palikaras et al., (2018) *Nature Cell Biology*

Autophagy/Mitophagy: Double edged sword in cellular physiology





Klionsky *et al.*, 2021 *EMBO J*
Patergnani et al., 2021 *PNAS*
Zaninello et al., 2021 *Cell Death & Differentiation*
Zaninello et al., 2020 *Nature Communications*
Doxaki and Palikaras, 2020 *Frontiers Cell Dev Biol*
Palikaras et al., 2018 *Nature Cell Biology*



Song et al., 2020 *Cell Communication & Signaling*

Potent autophagy inducers against pathological conditions

Agent	Developmental status	Mechanism of autophagy induction
ABT-199 (also known as Venetoclax)	Approved for the treatment of chronic lymphocytic leukaemia (CLL)	BH3 mimetic and Beclin-1 activator
ABT-263 (also known as Navitoclax)	Phase I/II clinical trials for cancer	BH3 mimetic and Beclin-1 activator
ABT-737	In preclinical development	BH3 mimetic and Beclin-1 activator
Alvespimycin (also known as 17-DMAG)	Discontinued from clinical tests (hepatotoxicity)	HSP90 inhibitor and inhibition of Akt/mTOR/p70S6K signalling?
Beclin-1-derived peptide	In preclinical development	Beclin-1 activator
Carbamazepine	Approved for treatment of seizures and bipolar disorders	Reduction in Ins(1,4,5)P_3 and inositol levels
Clonidine and Rilmenidine	Approved for the treatment of hypertension	Reduction in cAMP levels
Caloric restriction	Not available	Multiple
Everolimus (also known as RAD001)	Approved for cancer therapy	Inhibition of mTORC1
Geldanamycin	Discontinued from clinical tests (hepatotoxicity)	Inhibition of Akt/mTOR/p70S6K signalling?
Hydroxycitrate	Nutritional supplement	CRM and AMPK activation
Lithium	Approved for treatment of bipolar disorders	Reduction in Ins(1,4,5)P_3 and inositol levels
Metformin	Approved for type II diabetes	CRM and AMPK activation
Perhexiline	Approved for angina	CRM, AMPK activation and Acetyl-CoA reduction
Physical exercise	Not available	Multiple
Rapamycin (also known as sirolimus)	Approved for immunosuppression and cancer therapy	Inhibition of mTORC1
Resveratrol	Nutritional supplement	CRM and SIRT1 activation
Statins	Approved for obesity	Depletion of geranylgeranyl disphosphate, AMPK activation and mTORC1 inhibition
Spermidine	Nutritional supplement	CRM and EP300 deacetylase inhibitor
Tanespimycin (also known as 17-AAG)	Discontinued from clinical tests	HSP90 inhibitor and inhibition of Akt/mTOR/p70S6K signalling?
Temsirolimus (also known as CCI-779)	Approved for cancer therapy	Inhibition of mTORC1
Torins	Experimental agent	Inhibition of mTORC1
Trehalose	Nutritional supplement, Phase I/II clinical trials for bipolar disorder and vascular aging	Glucose transporter inhibition and AMPK activation
Trifluoperazine	Approved for schizophrenia	Dopamine agonist and unknown

Leidal et al., 2018 *Nat Cell Biol*

Urolithin A: First-in-class mitophagy enhancer

Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents

Dongryeol Ryu, Laurent Mouchiroud, Pénélope A Andreux, Elena Katsyuba, Norman Moullan, Amandine A Nicolet-dit-Félix, Evan G Williams, Pooja Jha, Giuseppe Lo Sasso, Damien Huzard, Patrick Aebischer, Carmen Sandi, Chris Rinsch & Johan Auwerx

Nature Medicine **22**, 879–888 (2016) | Cite this article

The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans

Pénélope A. Andreux, William Blanco-Bose, Dongryeol Ryu, Frédéric Burdet, Mark Ibberson, Patrick Aebischer, Johan Auwerx, Anurag Singh & Chris Rinsch

Nature Metabolism **1**, 595–603 (2019) | Cite this article

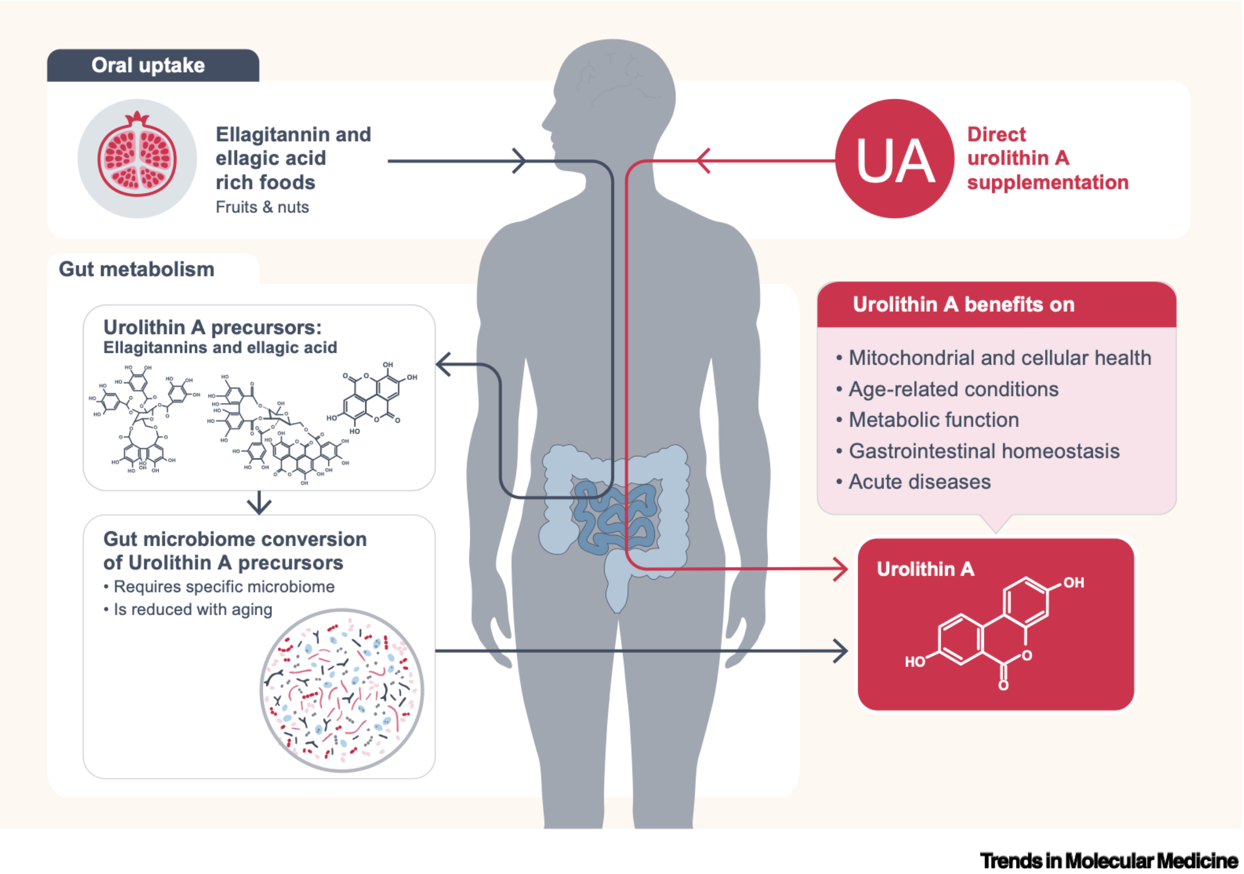
RESEARCH ARTICLE | MUSCULAR DYSTROPHY

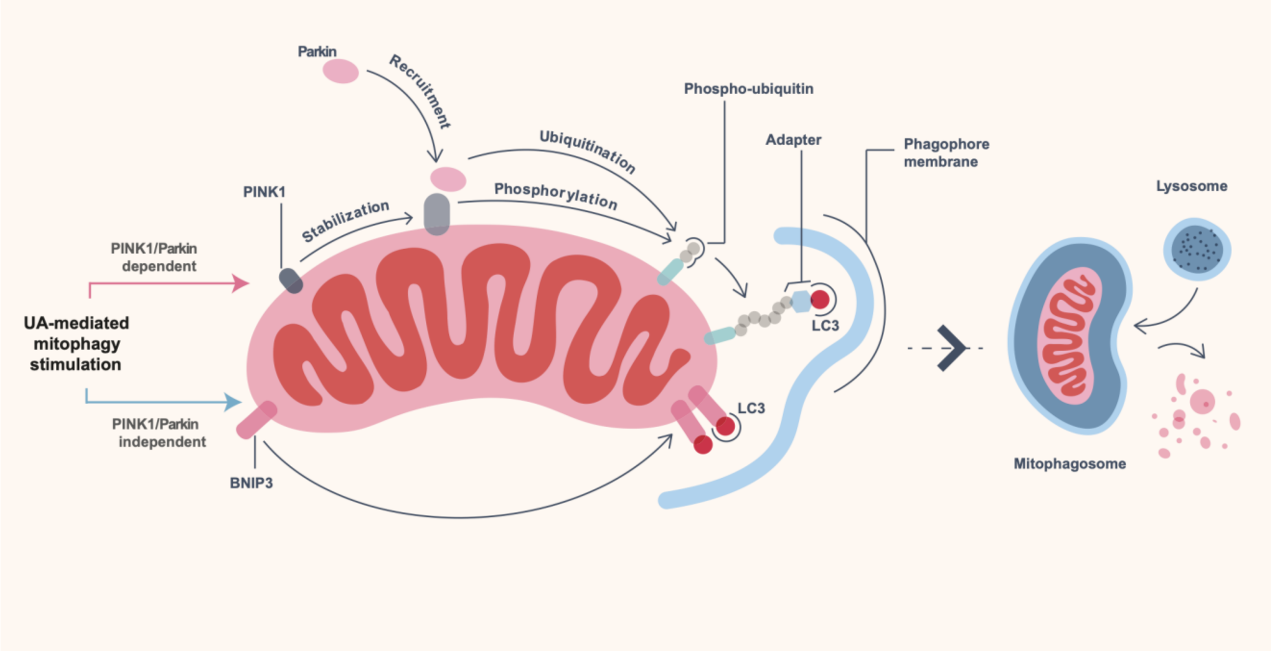
Urolithin A improves muscle function by inducing mitophagy in muscular dystrophy

PEILING LUAN, DAVIDE D'AMICO, PÉNÉLOPE A. ANDREUX, PIRKKA-PEKKA LAURILA, MARTIN WOHLWEND, HAO LI, TANES IMAMURA DE LIMA, NICOLAS PLACE, CHRIS RINSCH, NADÈGE ZANOU, AND JOHAN AUWERX fewer Authors Info & Affiliations

SCIENCE TRANSLATIONAL MEDICINE • 7 Apr 2021 • Vol 13, Issue 588 • DOI: 10.1126/scitranslmed.abb0319

Urolithin A: First-in-class mitophagy enhancer





D'Amico et al., 2021 *Trends Mol Med*

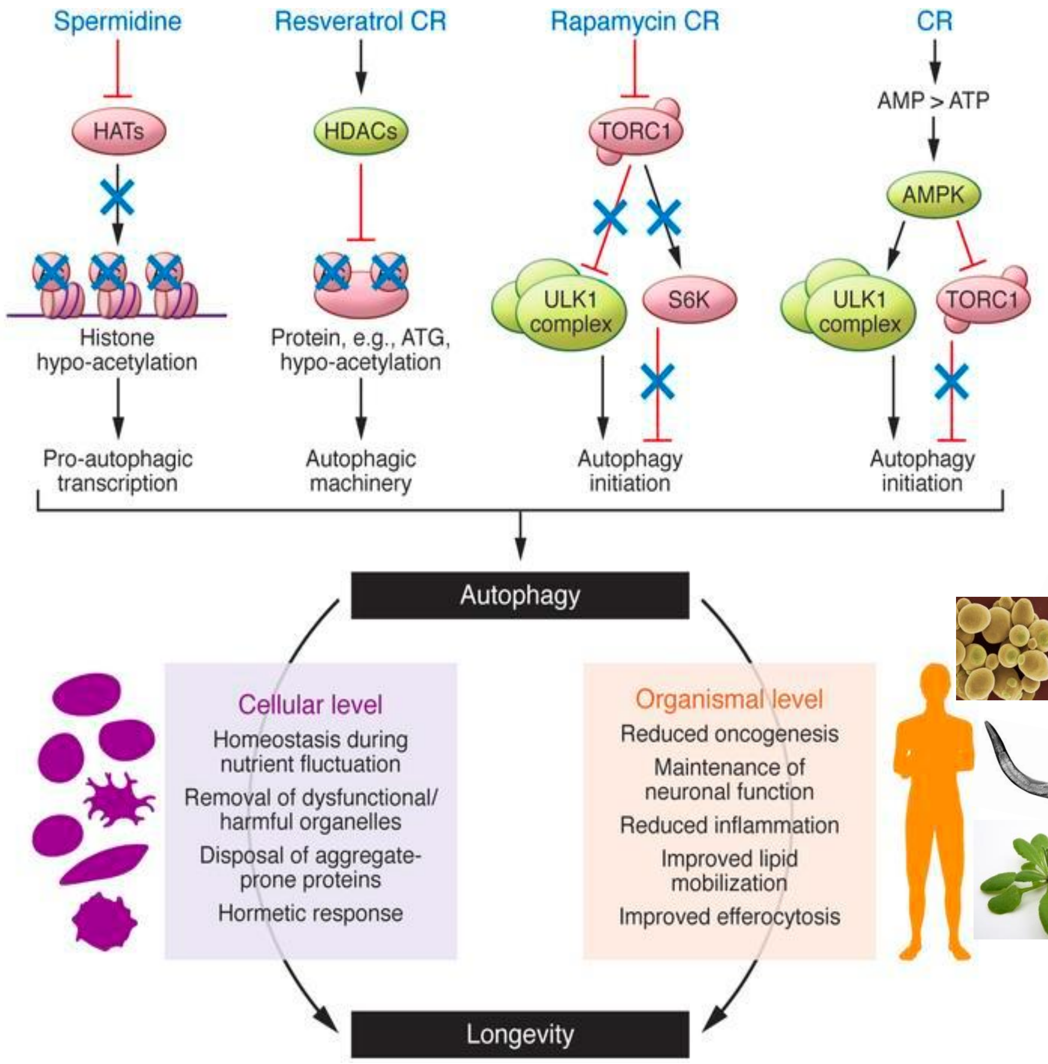
Immunomodulatory functions of Urolithin A

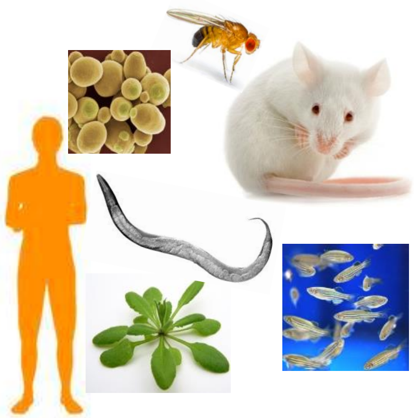
Table 1. Immunomodulatory function of UroA.

Category	Test Model	Disease Type/Treatment	Dose (UroA)	Metabolic Response	Ref.
Immune	C57BL/6 mice BMDM	Edema	40 mg/kg/BW orally 10 µM (BMDM)	↓ MPO activity and LPO activity with iron chelation ↓ ear edema weight (mg)	[26]
Immune	Ex vivo human neutrophils	LPS inflammation	20 µM	↓ IL-18 production (%), MMP-9 production (%), MPO release (%) ↓ ROS release (%) ↓ Superoxide anion production activity (%) and ↑ uric acid production activity (%)	[27]
Immune	RAW265 murine macrophages and peritoneal macrophages	LPS inflammation	2–40 µM	↓ TNFα, IL-6, nitrite, iNOS ↓ DNA binding response to LPS, LPS induced translocation of p65 ↑ IkBα ↓ AP-1 DNA binding activity, c-JUN and p-c-JUN ↓ p-Akt, p-JNK, p-p38 ↓ NOX (ROS)	[28]
Immune	Human osteoarthritis chondrocytes DMM mouse model	Osteoarthritis	3–30 µM 20 mg/kg/day intragastric administration	↓ Il-1β, iNOS, Cox-2, NO generation, PG32 ↓ IkBα degradation and translocation of P65 to nucleus ↓ PI3K/Akt signaling pathway ↓ p-PI3K and p-AKT-positive chondrocytes in mouse ↓ p65-positive nuclei in UA mouse chondrocytes; milder narrowing of joint space compared to OA group	[29]
Immune	Rat chondrocytes	Osteoarthritis	1–15 µM	↓ MMP13, MMP3, iNOS, Cox2, ADAMST4, MMP9 ↑ Col2a1 ↑ Collagen II, Aggrecan, Sox9 ↓ p65, p-ERK1/2, p-JNK, p-P38	[30]
Immune	U937 cell THP-1	LPS inflammation	1.5, 30 µM	↓ Tnfα ↓ NFκB signaling, p50 and p60 subunits	[31]
Immune	RAW 264.7 murine macrophages	LPS inflammation	2–40 µM	↓ NO production, nitrite, iNOS ↓ NF-κB p65 nuclear translocation ↓ binding to NFκB p50 binding ↓ IL-1β, TNFα, IL-6	[32]

Toney et al., 2021 *Biomedicine*

Autophagy modulators in lifespan extension



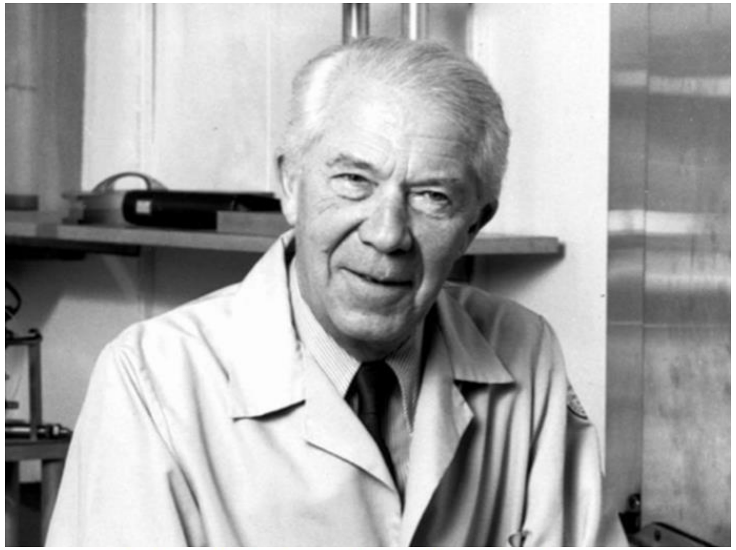


- Several pharmacologic and dietary interventions activate autophagy signaling and thereby promote beneficial effects at the cellular and organismal levels, contributing to prolonged life span and health span.

Georgakopoulos et al., (2017) *Nature Chemical Biology*
Hansen et al., (2018) *Nature Reviews Molecular Cell Biology*
Madeo *et al.,* 2015 *The Journal of Clinical Investigation*

Autophagy: stress response to maintain cellular homeostasis

The field of autophagy research has developed rapidly since the first description of the process in the **1960s** and the identification of autophagy genes in the **1990s**



The Nobel Prize in Physiology or Medicine 1974

Photo from the Nobel Foundation archive.
Albert Claude
Prize share: 1/3

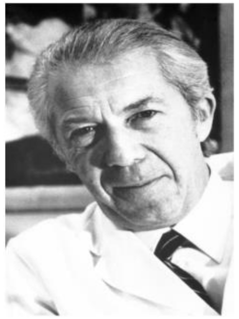


Photo from the Nobel Foundation archive.
Christian de Duve
Prize share: 1/3

Photo from the Nobel Foundation archive.
George E. Palade
Prize share: 1/3

Sabatini and Adensik, 2013 *PNAS*

The father of AUTOPHAGY → self-eating