Autophagy

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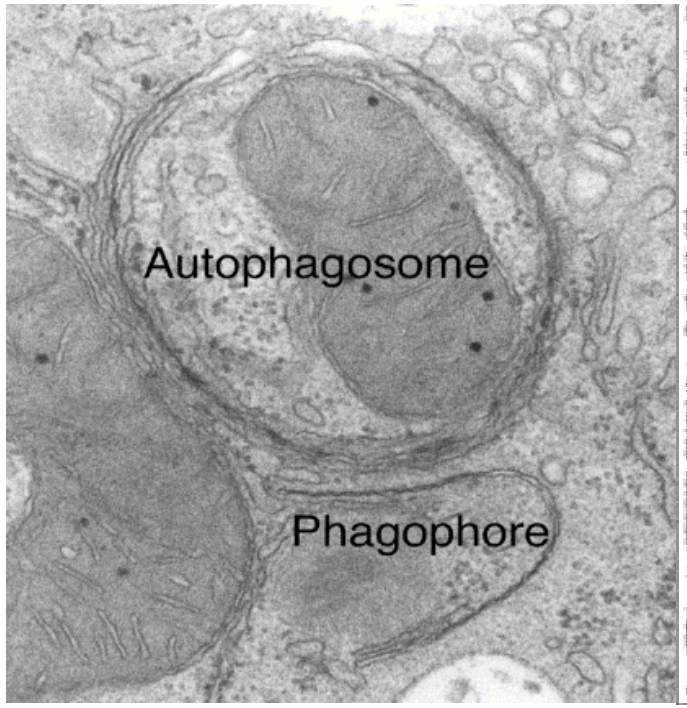
Plan

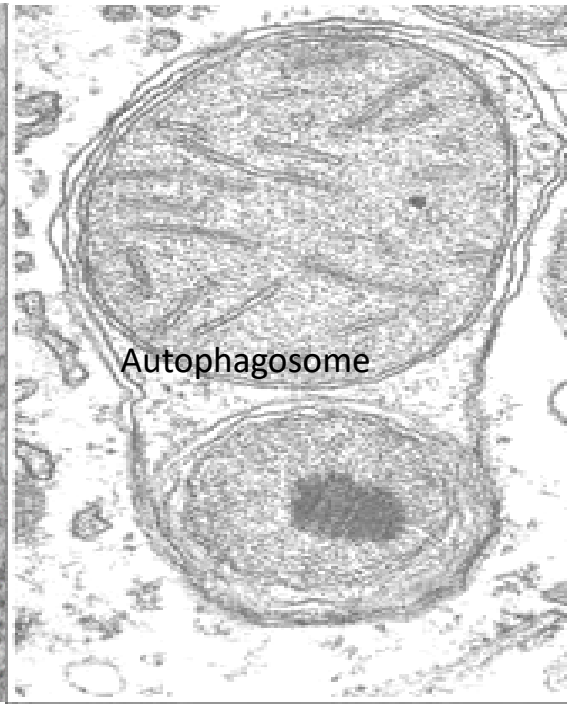
- Autophagy
  - Description
  - Machinery and regulation
  - Biological Functions and associated pathologies
  - Physiopathology
  - Escape and hijacking of autophagy by viruses

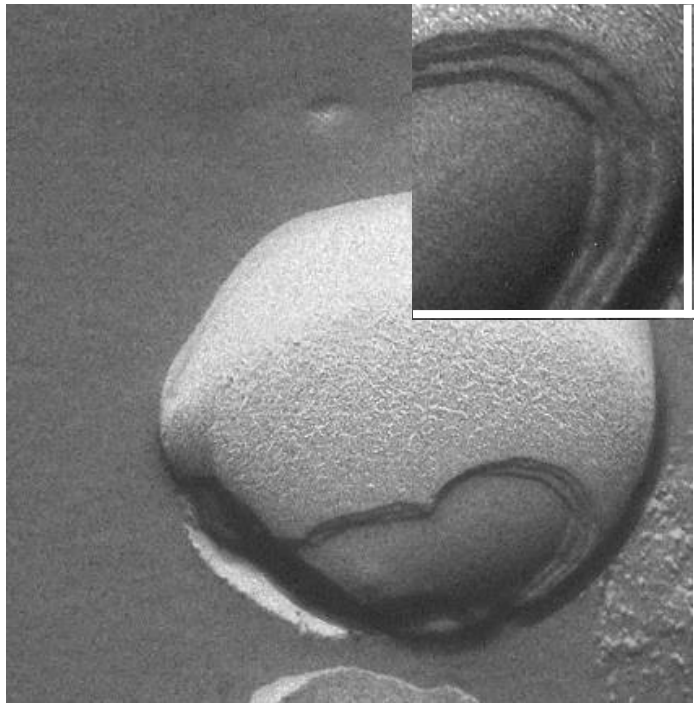
Autophagy

- Cellular constitutive membrane-trafficking mechanism
- Homeostatic lysosomal degradation of large organelles and long-lived proteins
  - Stimulation of autophagy in stress situation

→ Mechanism of cellular survival and adaptation

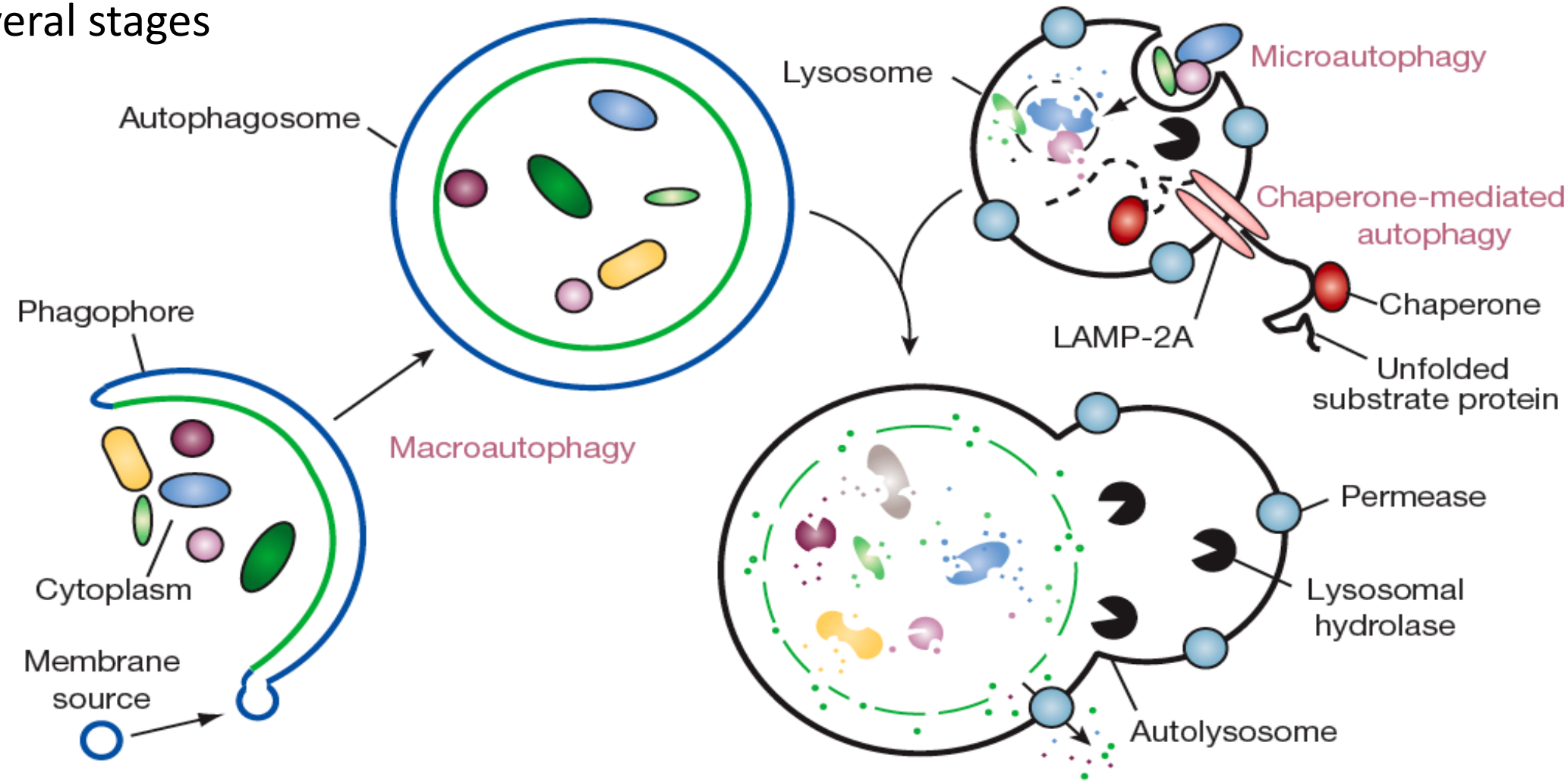






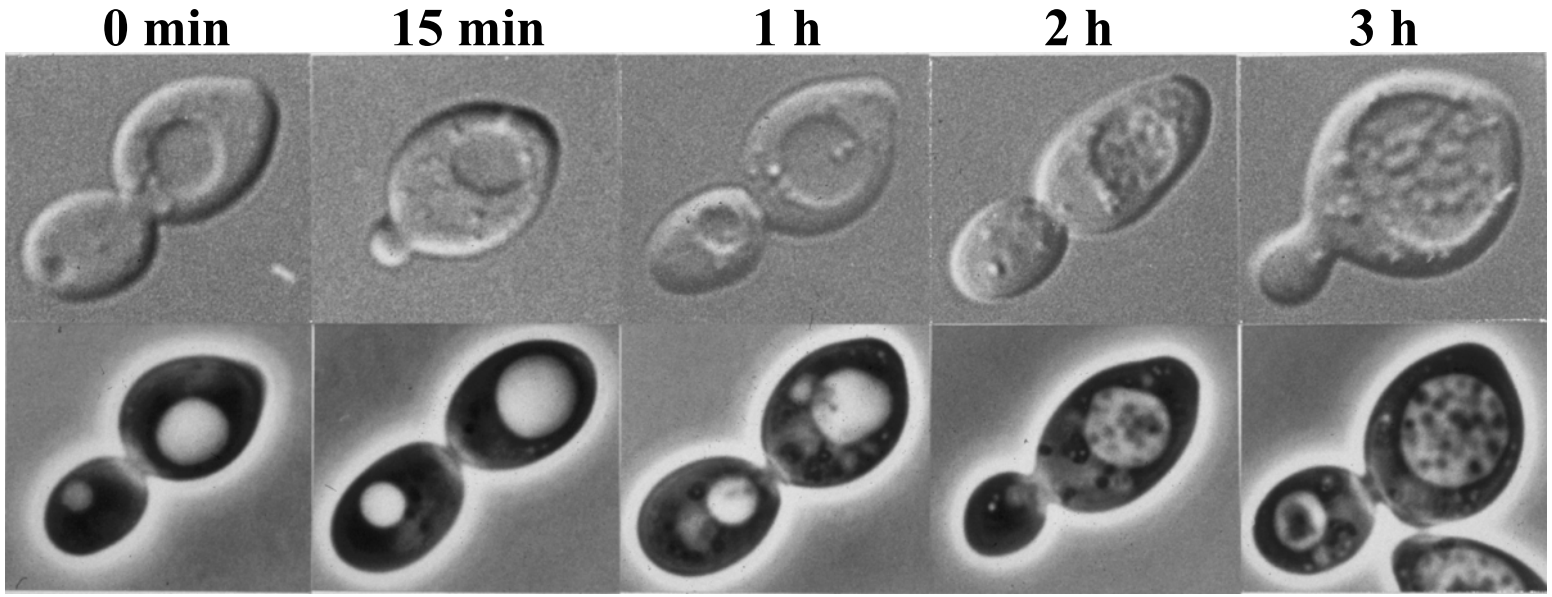
Autophagy

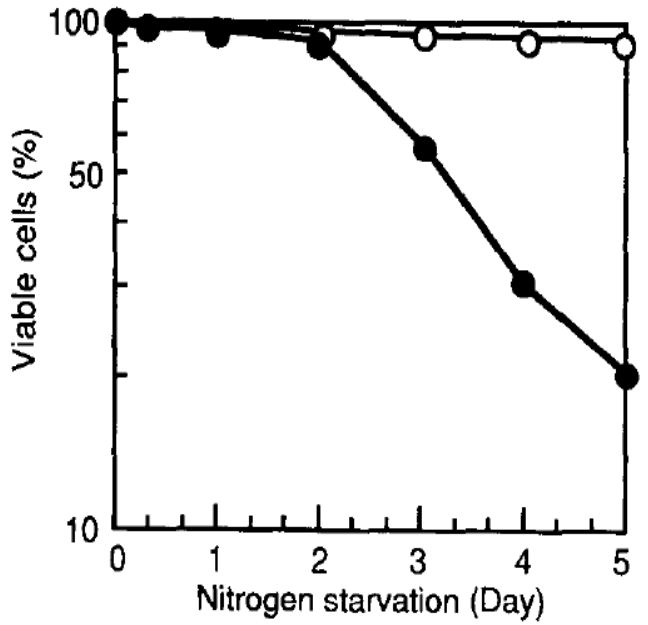
- Different types of autophagy
- Several stages



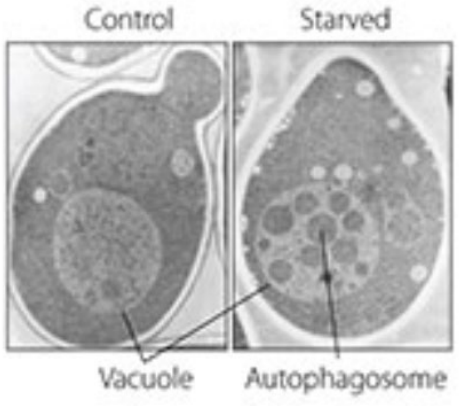
Mizushima, 2008

Autophagy in yeast: Discovery of ATG genes





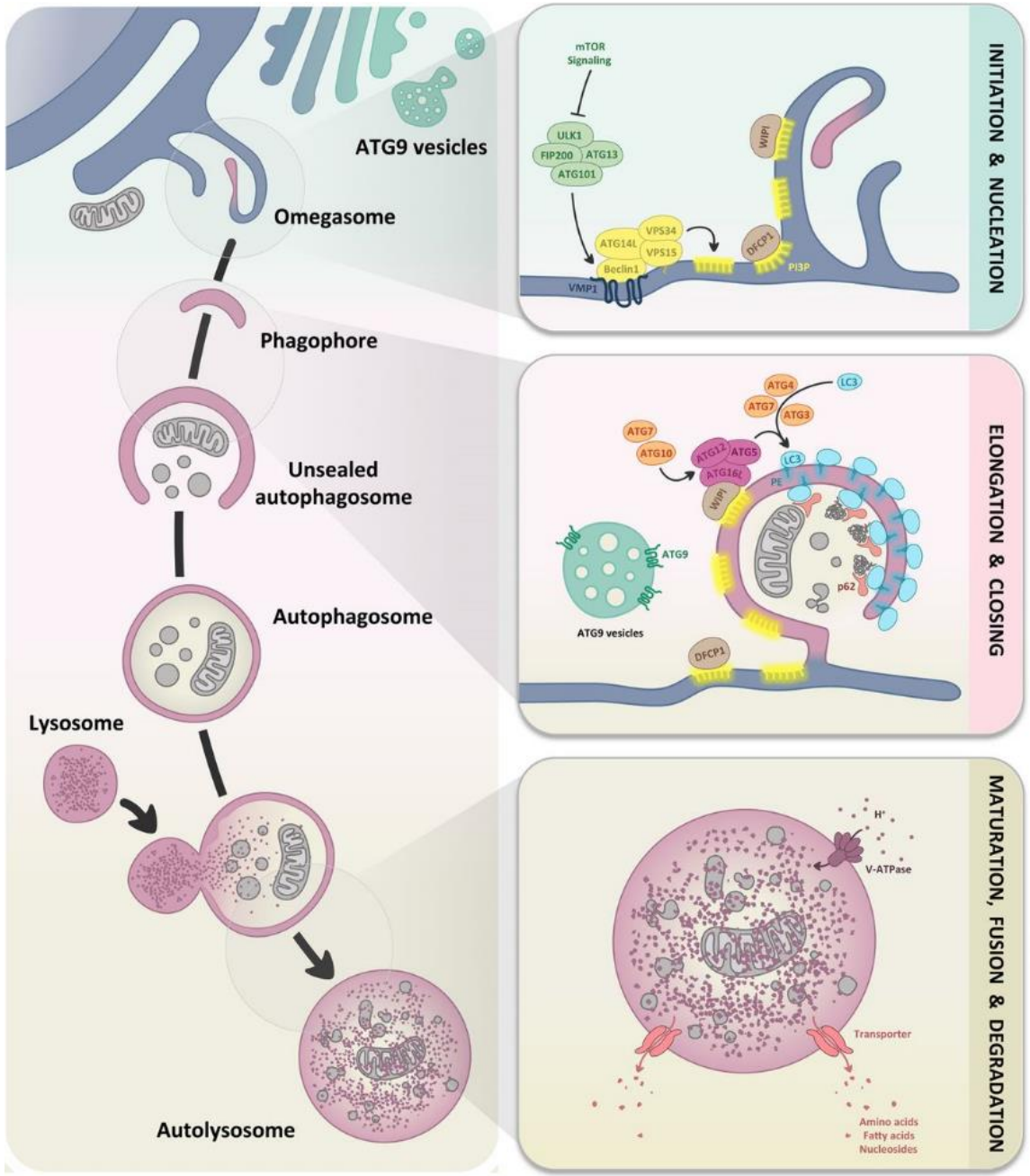
Yoshinori Ohsumi Nobel Prize 2016



The vacuole in yeast corresponds to the lysosome in mammalian cells

J Cell Biol 1992, FEBS letters 1993

Autophagic machinery : role of Atg proteins

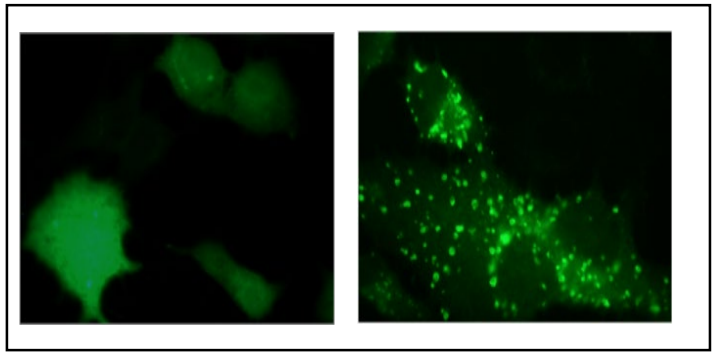


Almost 40 Atg proteins identified

4 major functional groups for the biogenesis of AP

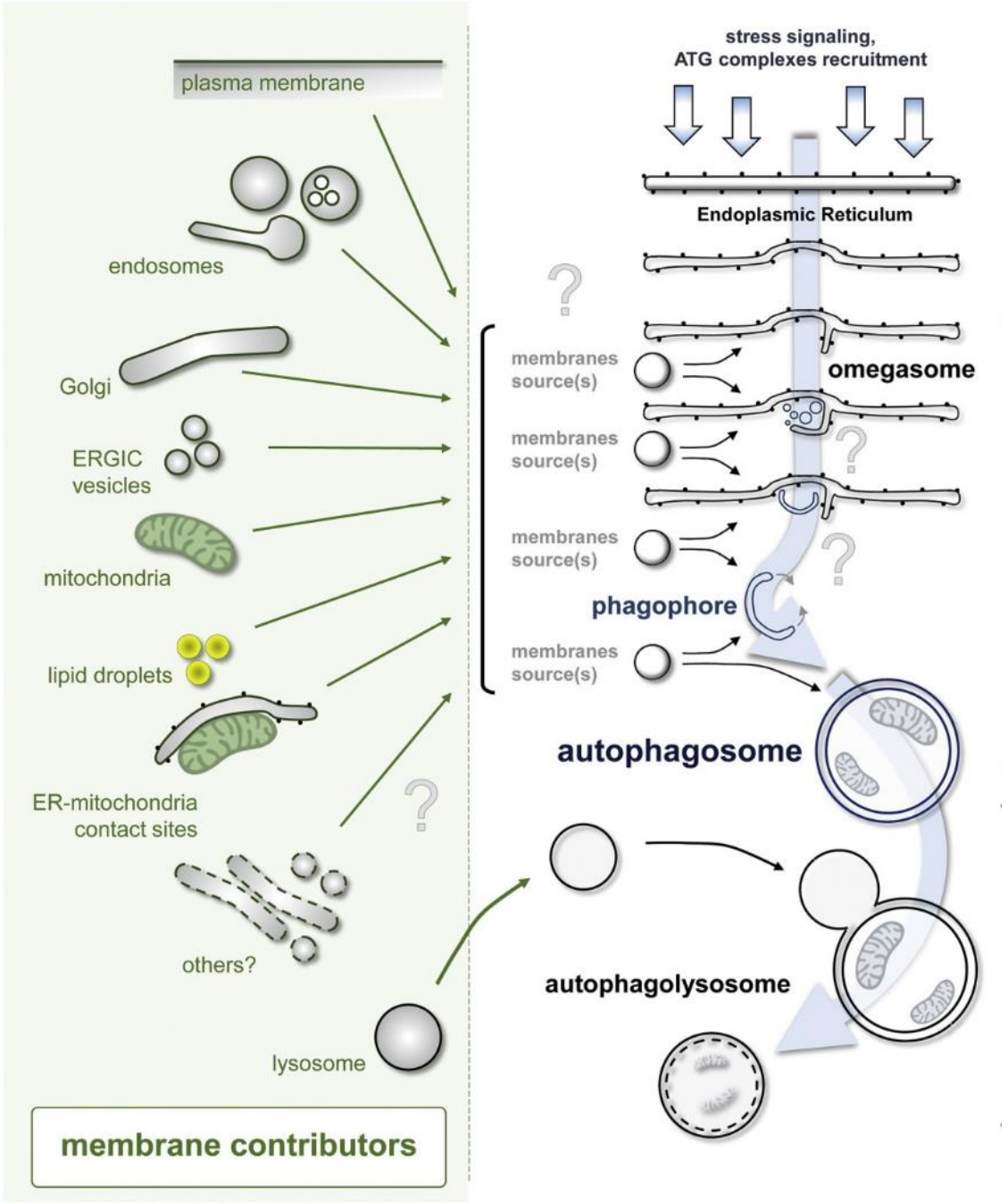
1) ULK1 complex
2) PI3K / Beclin 1 complex
3) The ubiquitin-like system (Atg12-Atg5, LC3-PE)
4) Atg9 vesicles and lipid transfer protein ATG2A

Atg8 / LC3



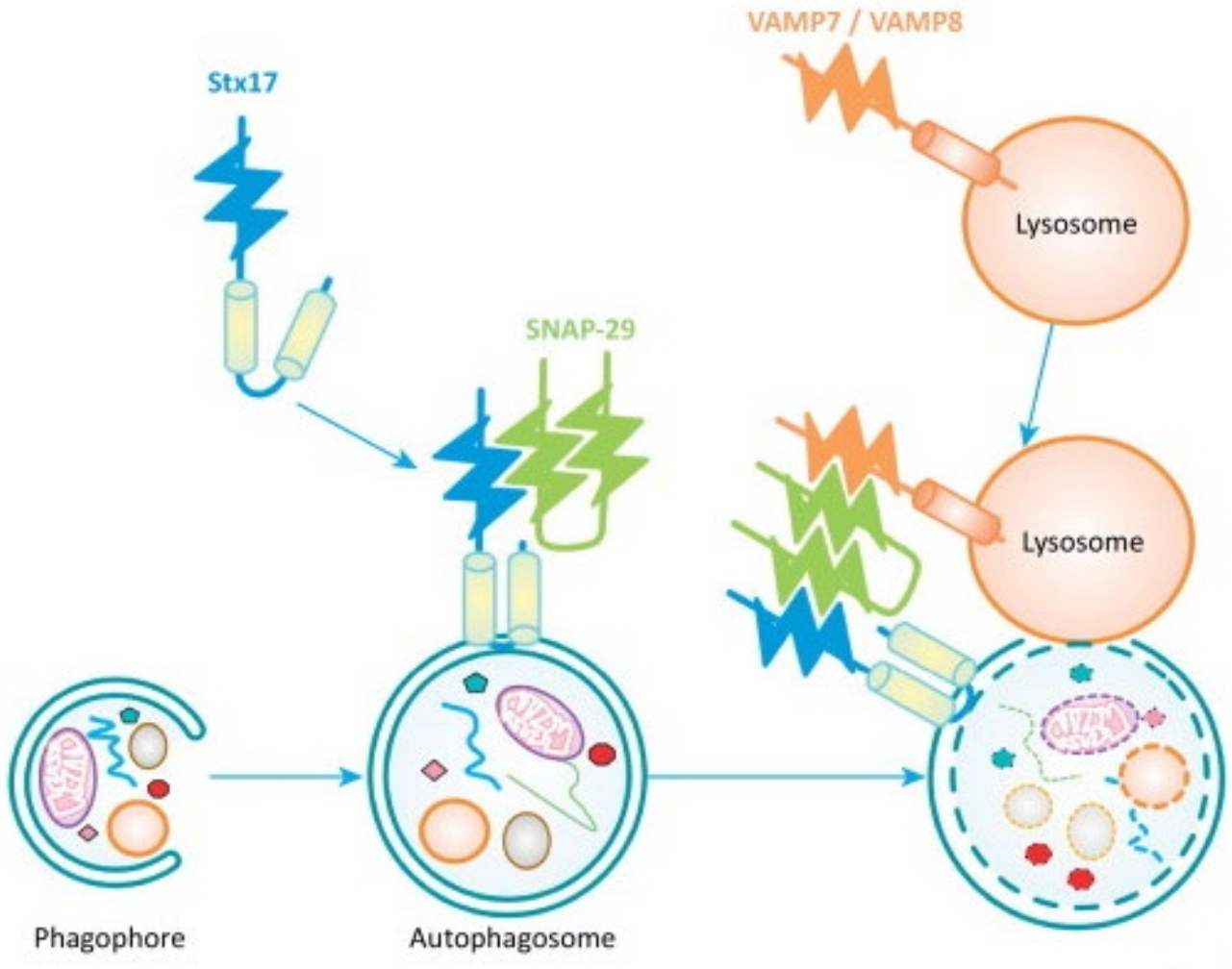
Essays Biochem Volume 61 Issue 6 2017 649-661 10.1042/EBC20170025

Proposed membrane sources for autophagosomal membranes in non selective autophagy



Molino et al 2017

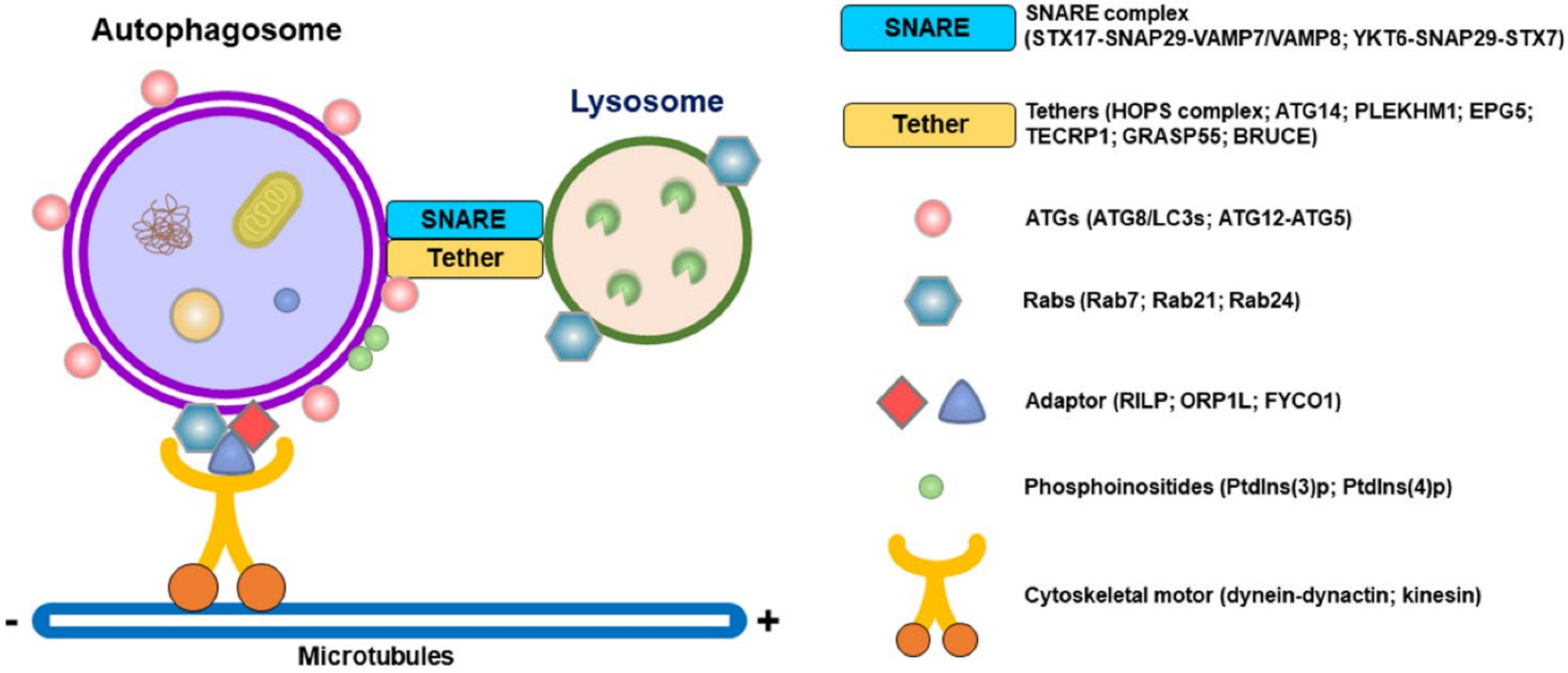
Fusion of autophagosomes with lysosomes



Recruitment of a SNARE complex to allow membrane fusion

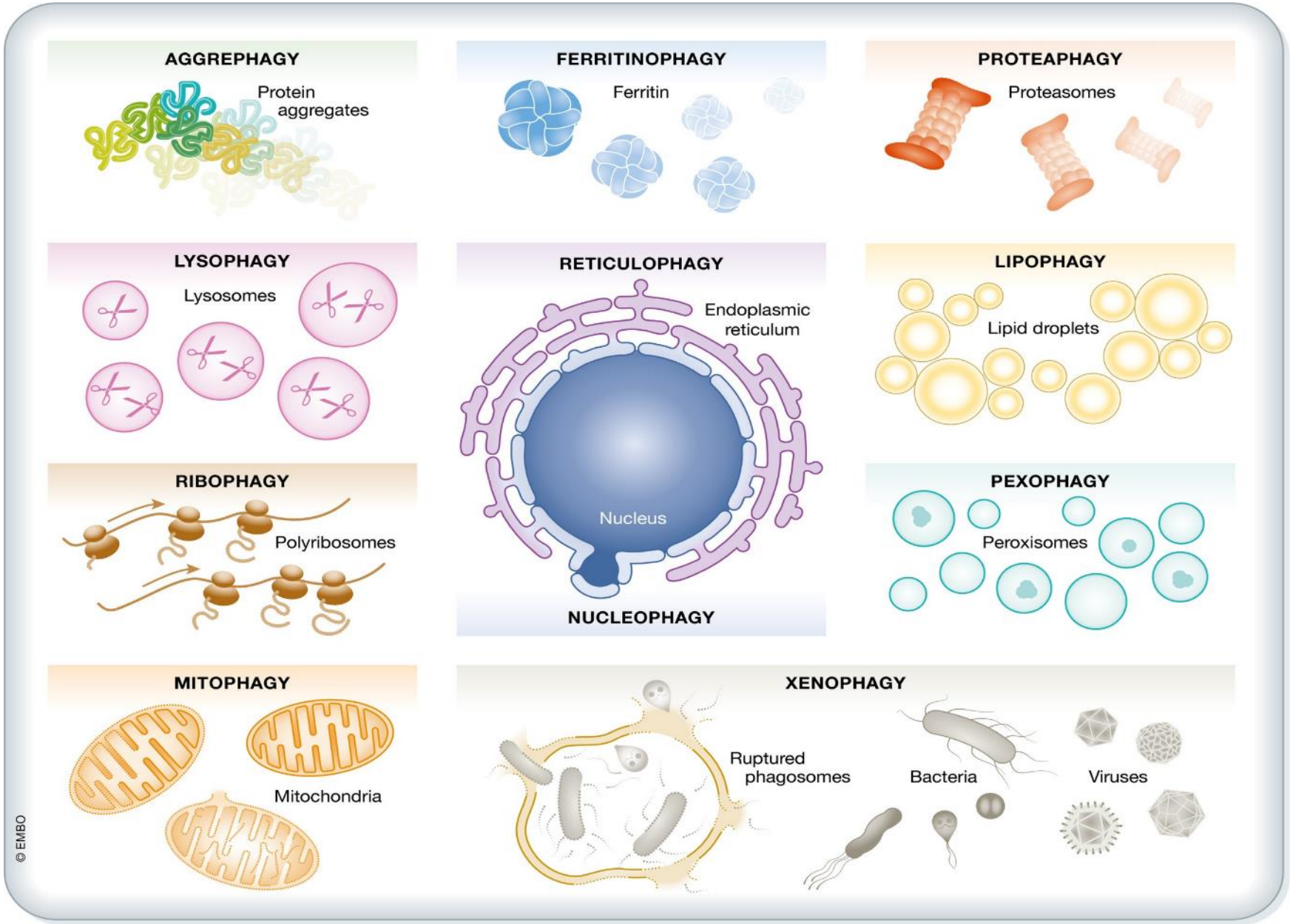
Han-Ming Shen · Noboru Mizushima, Trends, 2014, Po-Yuan Ke, Pathogens, 2024

Fusion of autophagosomes with lysosomes



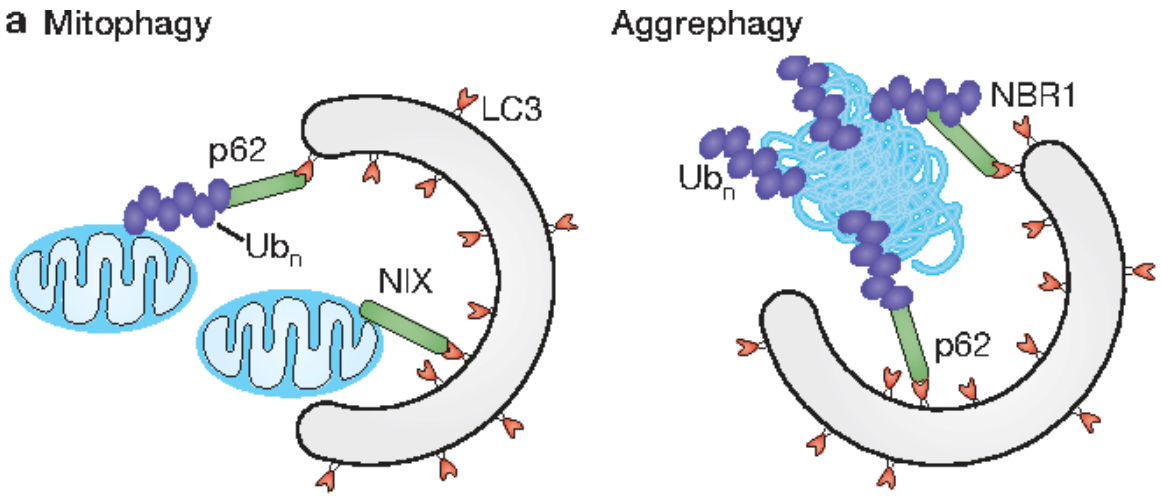
Han-Ming Shen · Noboru Mizushima, Trends, 2014, Po-Yuan Ke, Pathogens, 2024

Selective autophagy

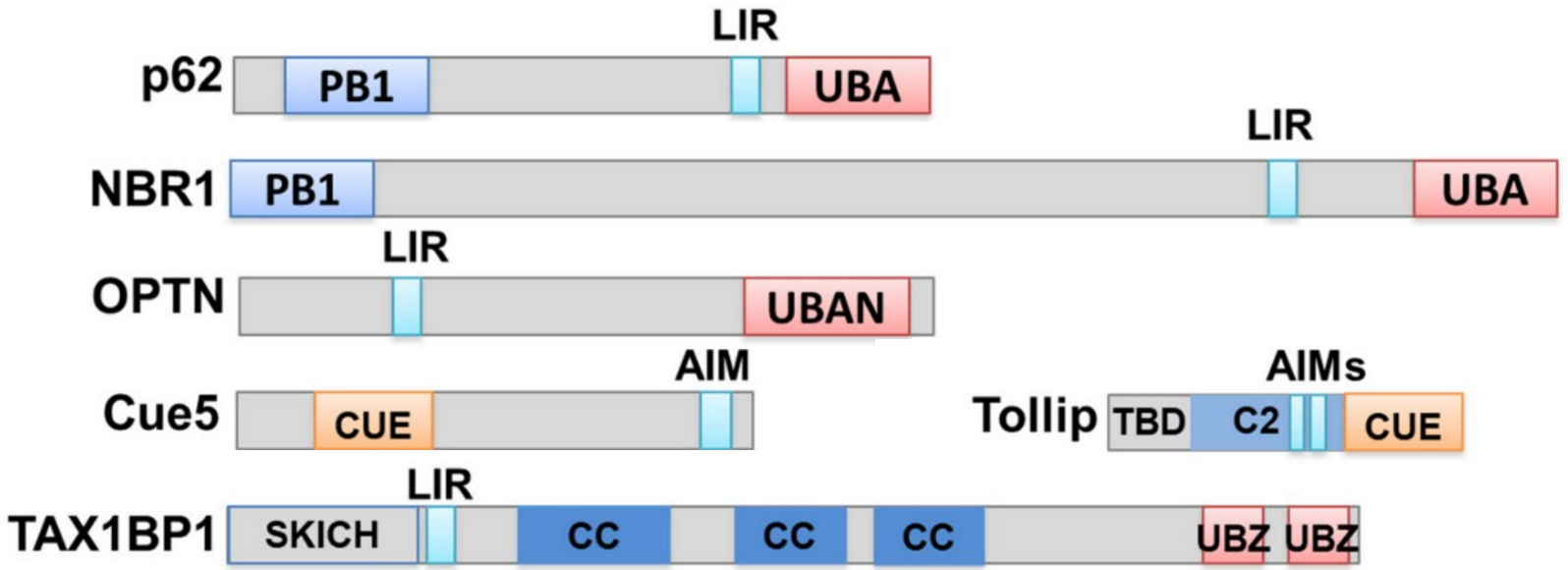


EMBO J, Volume: 36, Issue: 13, Pages: 1811-1836, First published: 08 June 2017, DOI: (10.15252/embj.201796697)

Selective autophagy receptors

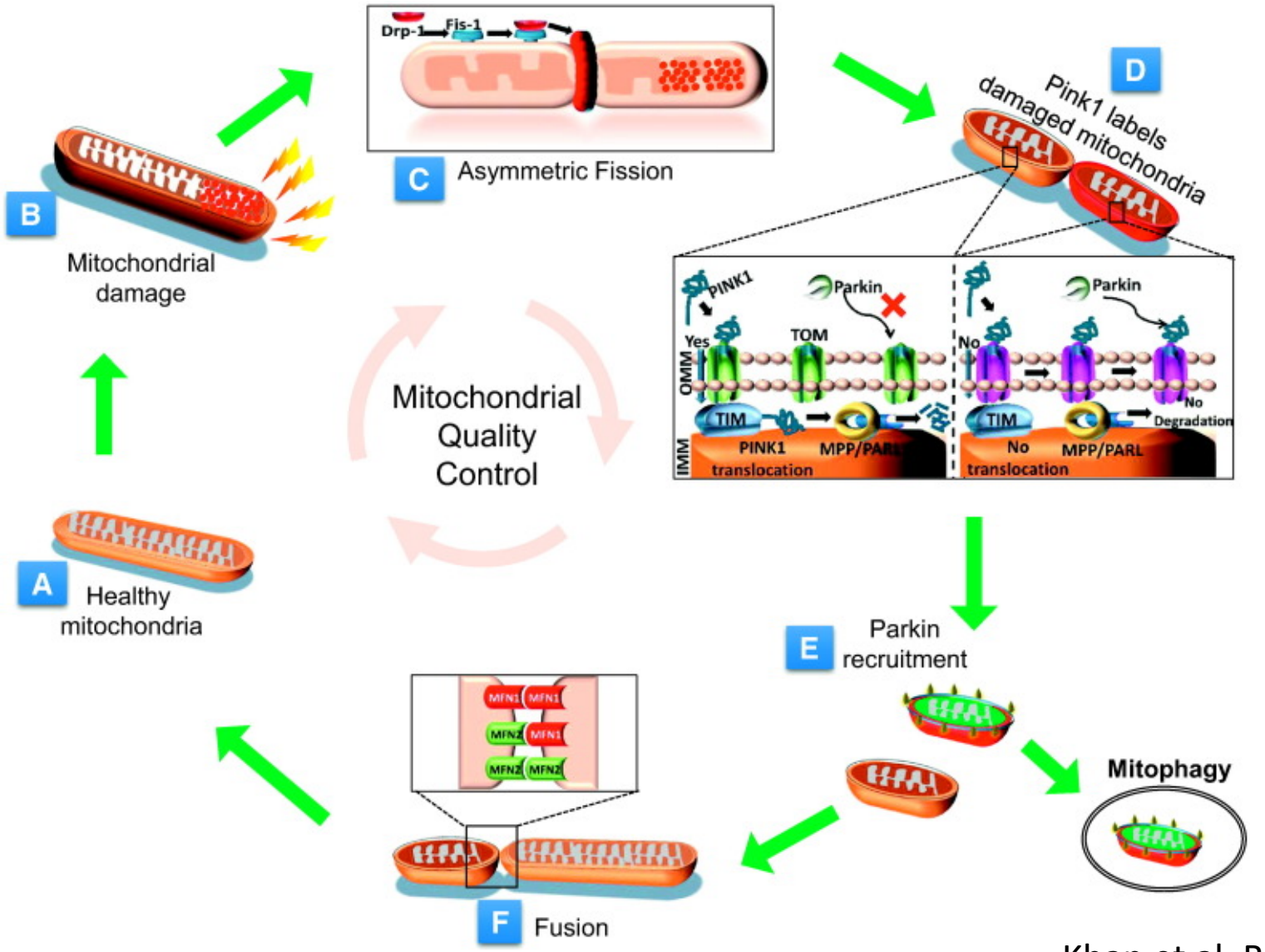


LIR, LC3-interacting region
UBA, ubiquitin-associated domain
UBAN, ubiquitin-binding domain in ABIN proteins and NEMO
AIM, Atg8-interacting motif
UBZ, ubiquitin-binding zinc finger.



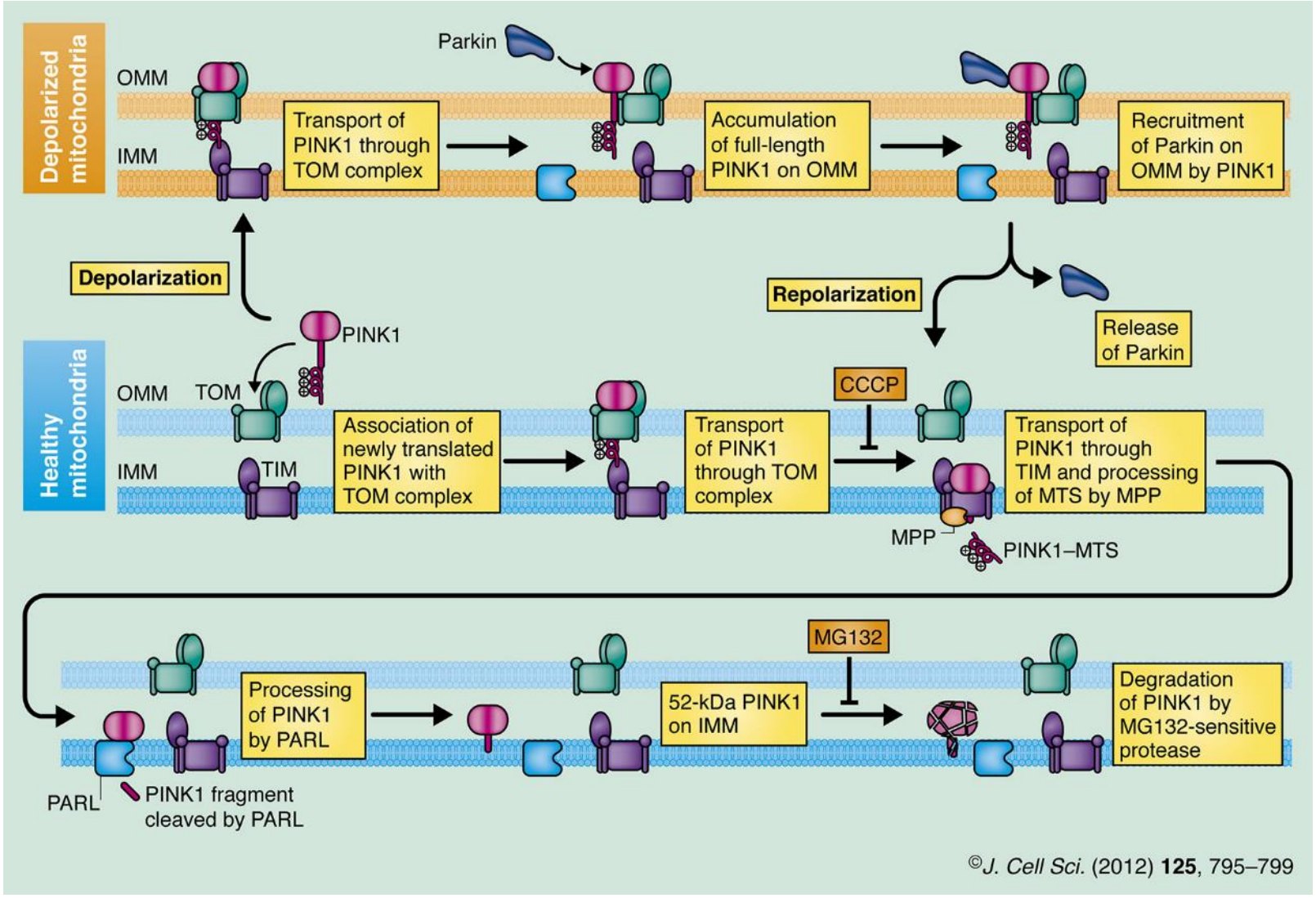
See: Beau et al., 2008, Chen Cells, 2021

Damage can trigger PINK1- Parkin dependent mitophagy

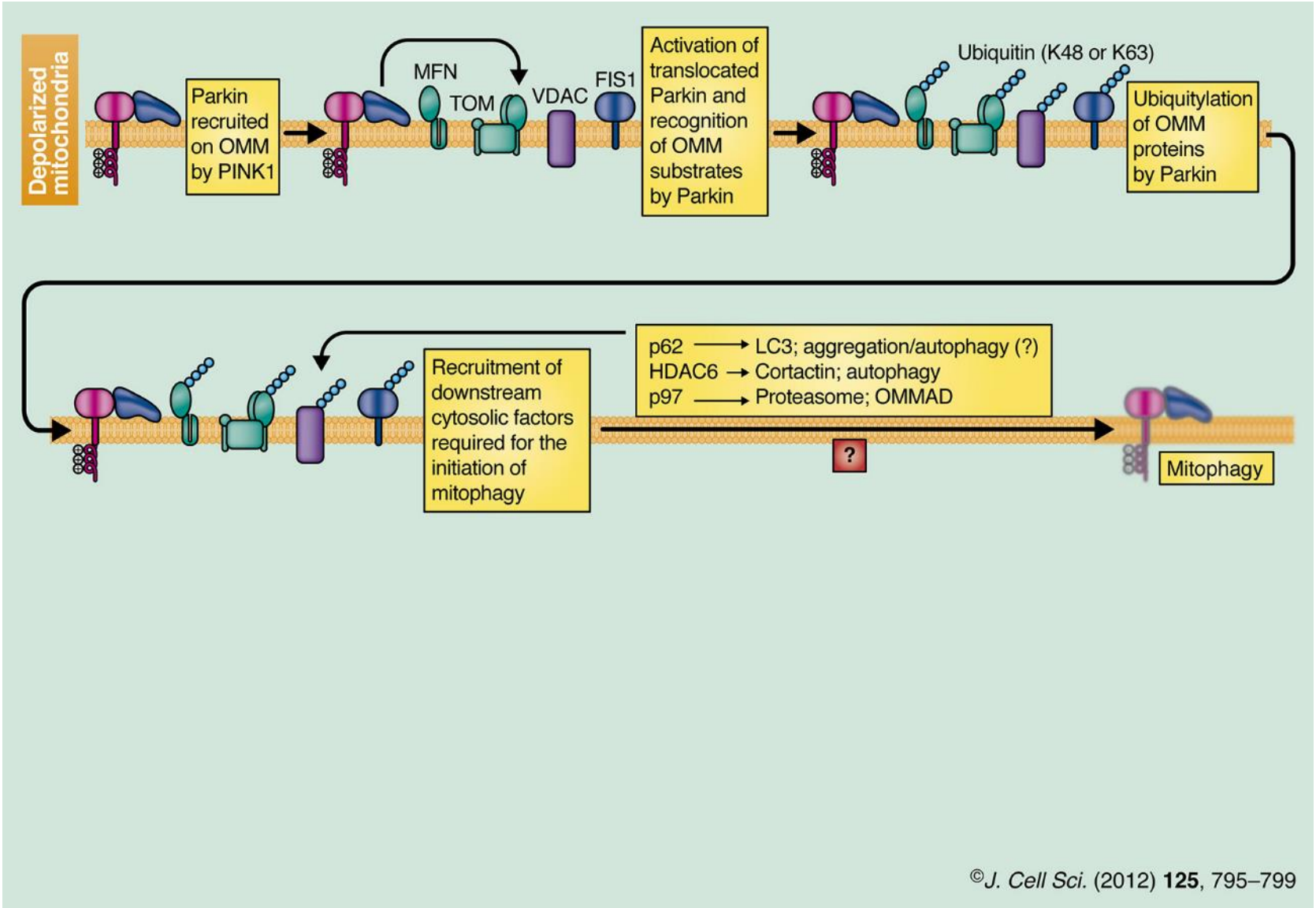


Khan et al, BBA, 2015

Sensing of damaged mitochondria by PINK1



Commitment to mitophagy mediated by Parkin



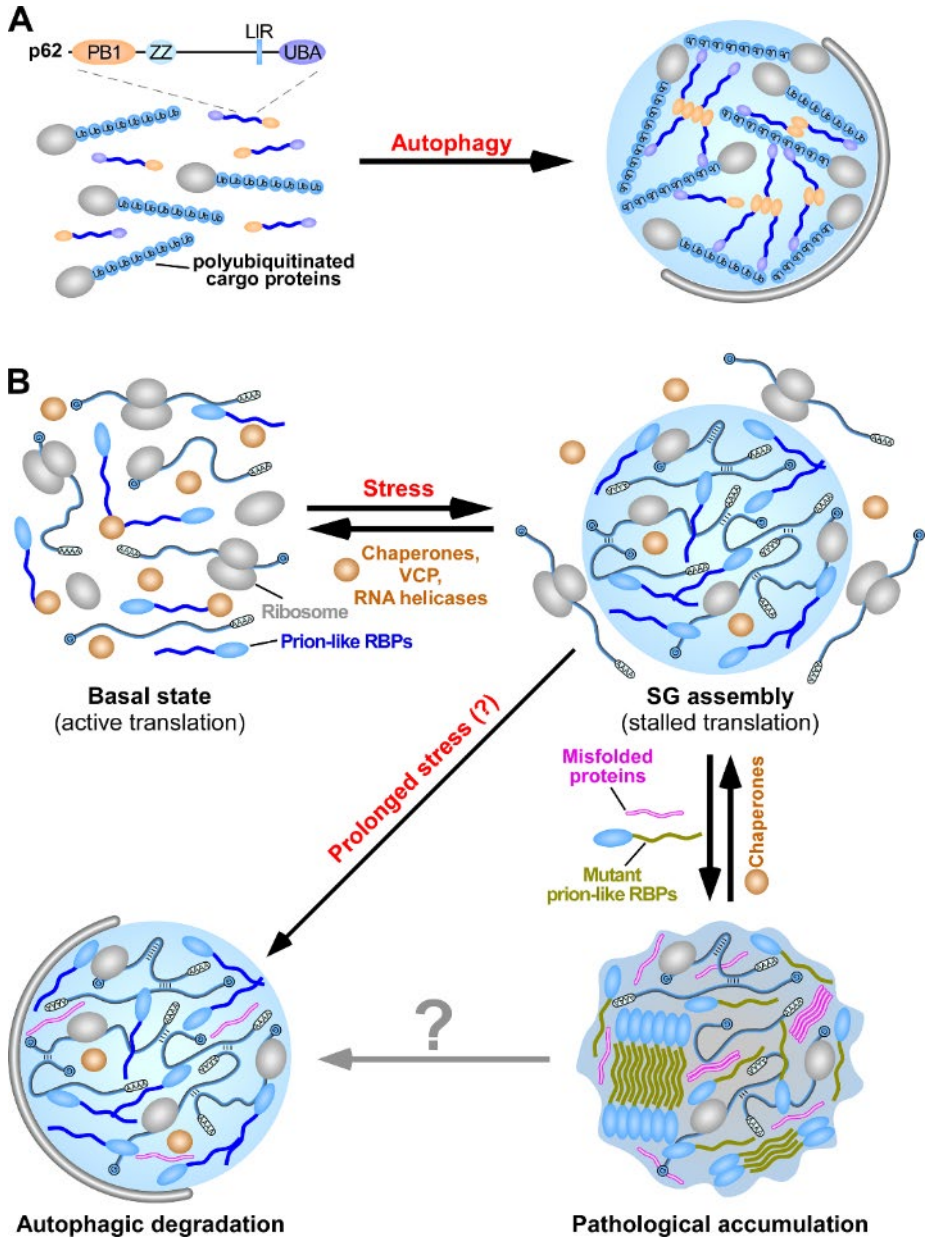
Liquid–liquid phase separation in selective autophagy

(A) Phase separation occurs when p62 is mixed in vitro with substrates carrying two or more ubiquitin chains
p62 aggregates are selectively enclosed by autophagosomal membranes

(B) Stress granules

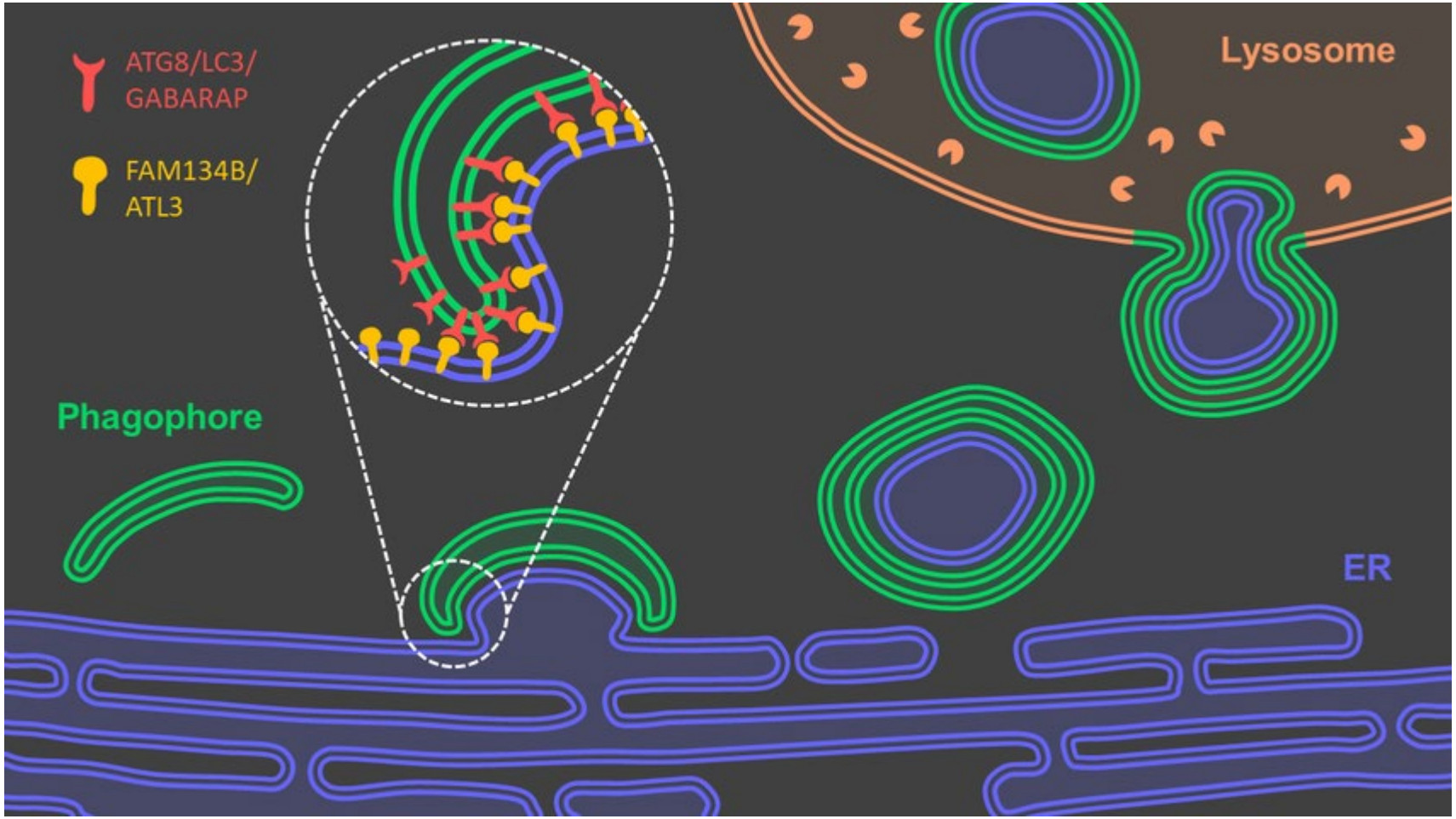
SGs are assembled via LLPS and can rapidly disassemble.

A subset of SGs that fail to disassemble are subjected to autophagic degradation.



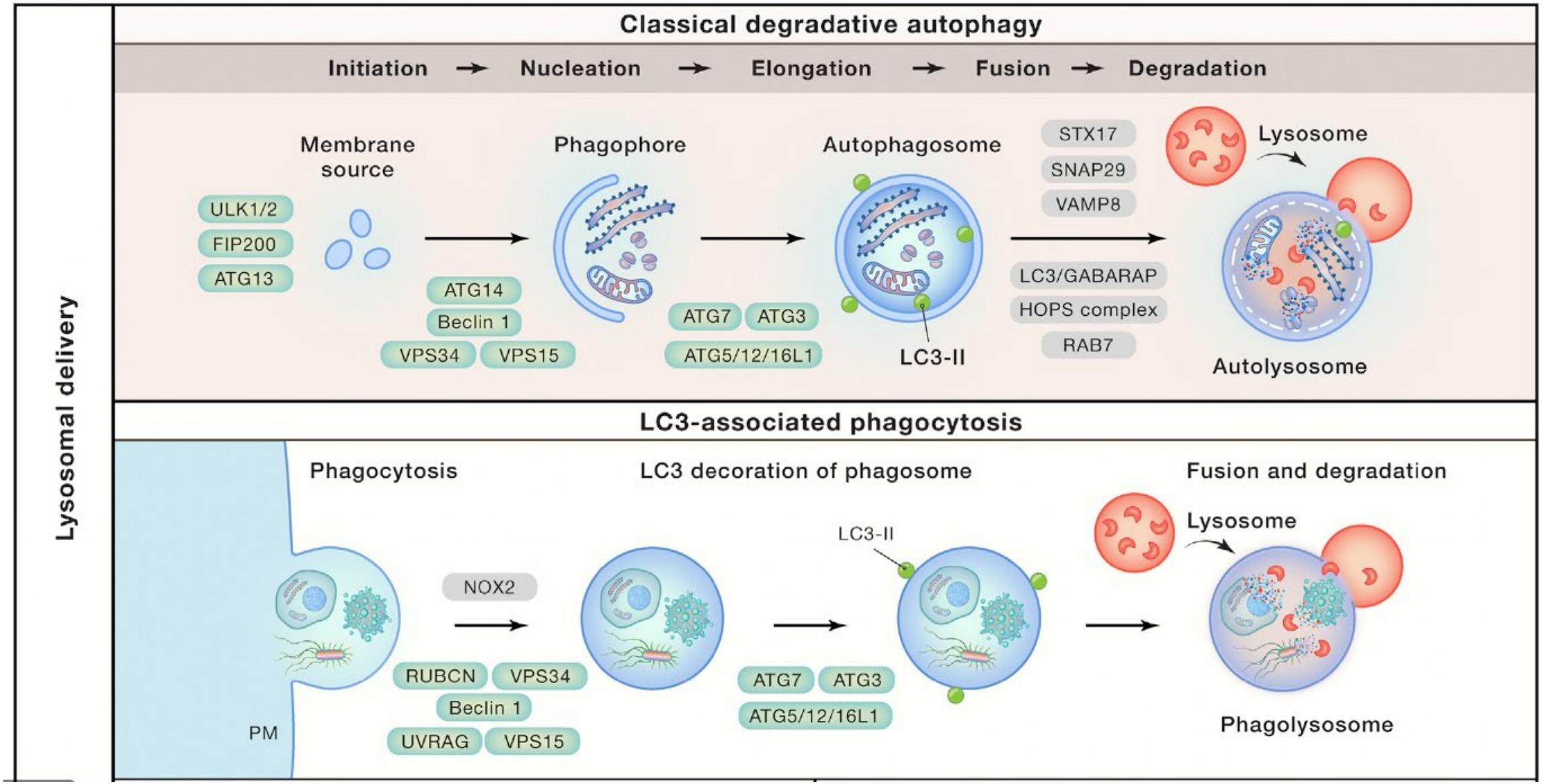
J Cell Biol. 2020;219(8). doi:10.1083/jcb.202004062

Autophagic degradation of ER: reticulophagy



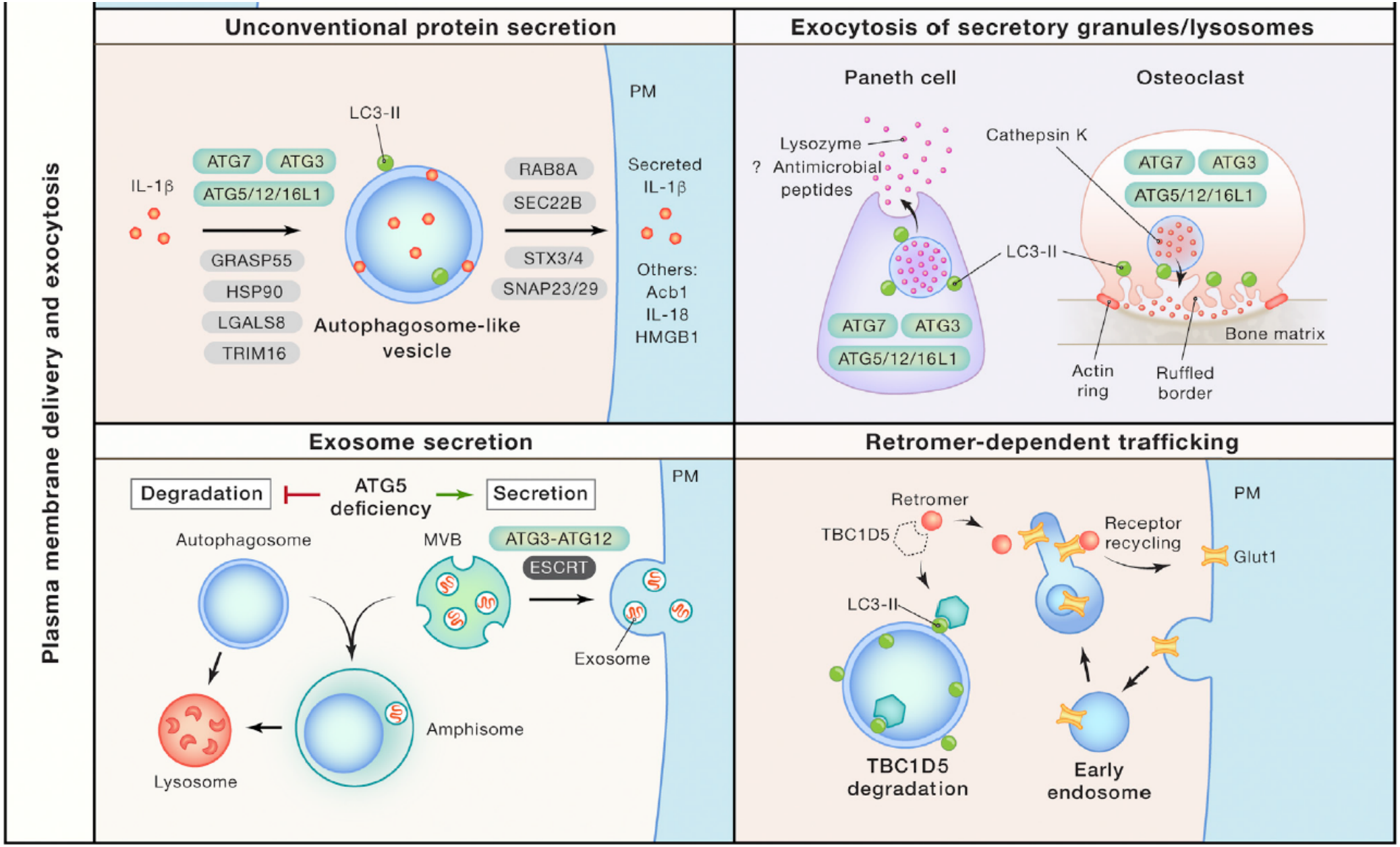
- Transmembrane SARs stably integrated within organelle membranes
- Macro and micro-reticulophagy

ATG gene-dependent pathways: lysosomal delivery



Levine and Kroemer, Cell 176, January 10, 2019

Non-autophagic functions and signaling pathways controlled by ATG proteins



Levine and Kroemer, Cell 176, January 10, 2019

Plan

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  - Machinery and regulation
  - Biological Functions
    - Cell Survival
    - Quality control
    - Development
    - Longevity
    - Immunity
  - Physiopathology
  - Escape and hijacking of autophagy by viruses

Biological functions

Survival

Quality control

Development

Cellular death

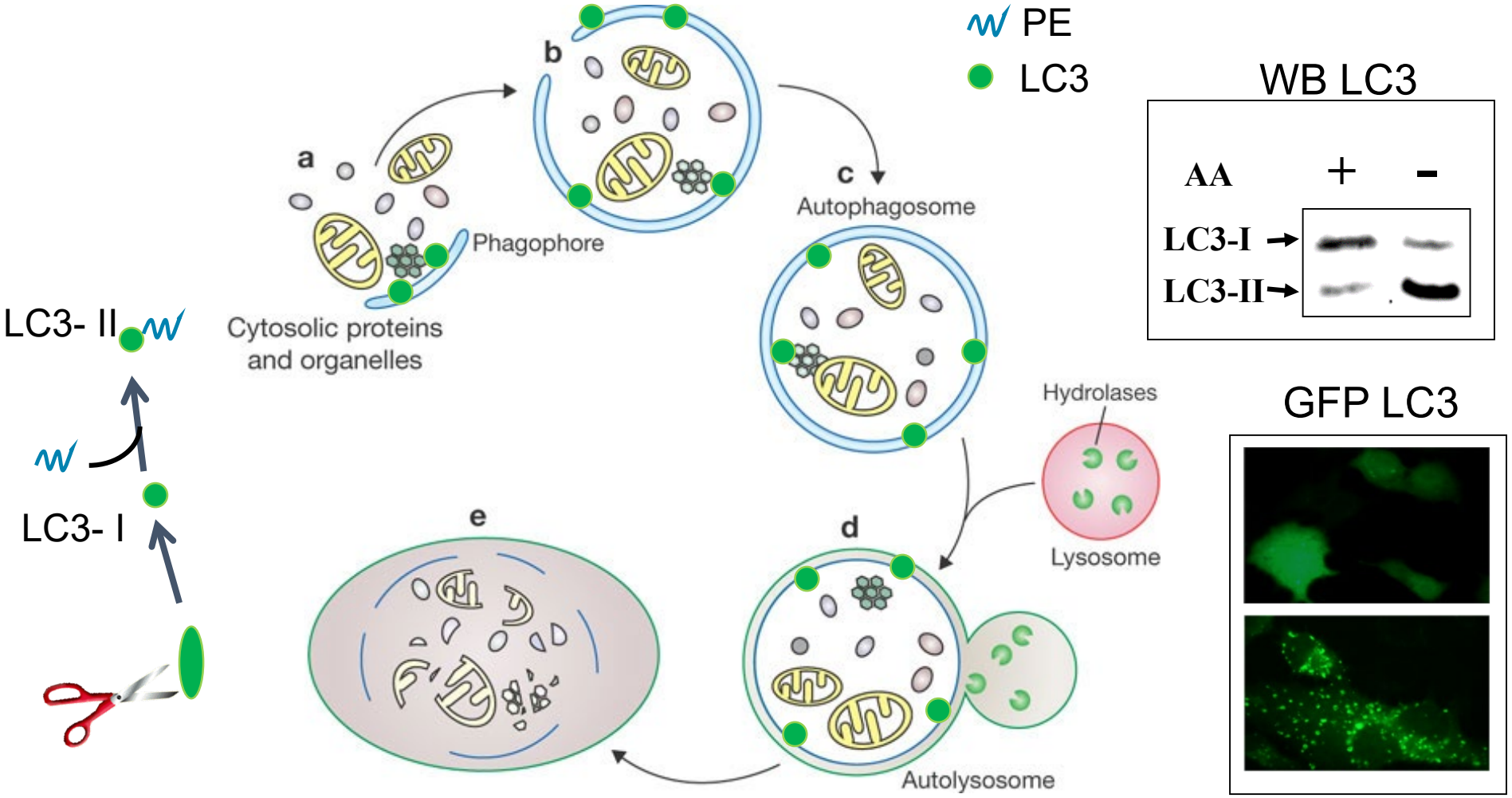
Autophagy is everywhere…

Longevity

Innate immunity

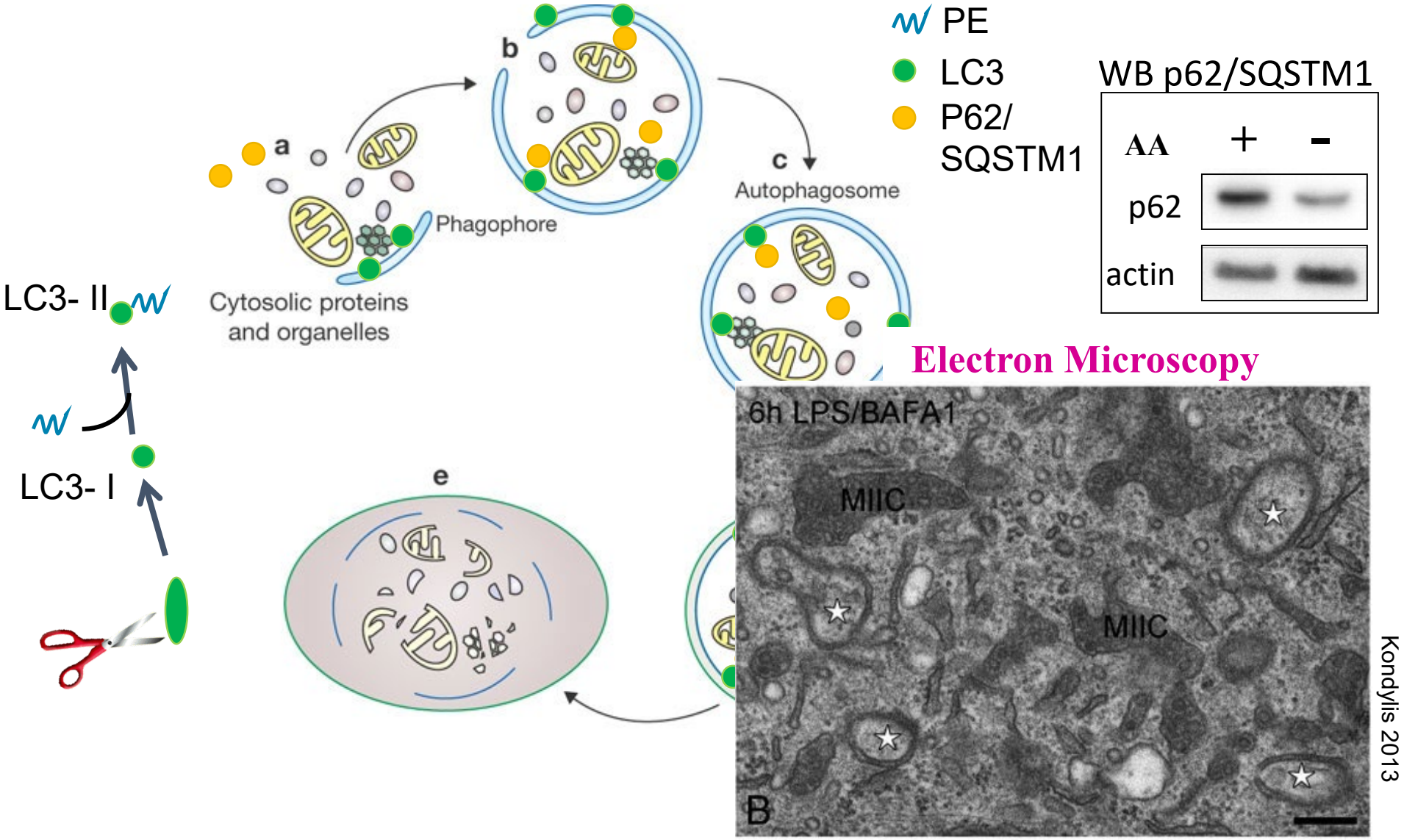
Adaptive immunity

Methods for monitoring autophagy



Modified from Xie and Klionsky, 2007

Methods for monitoring autophagy



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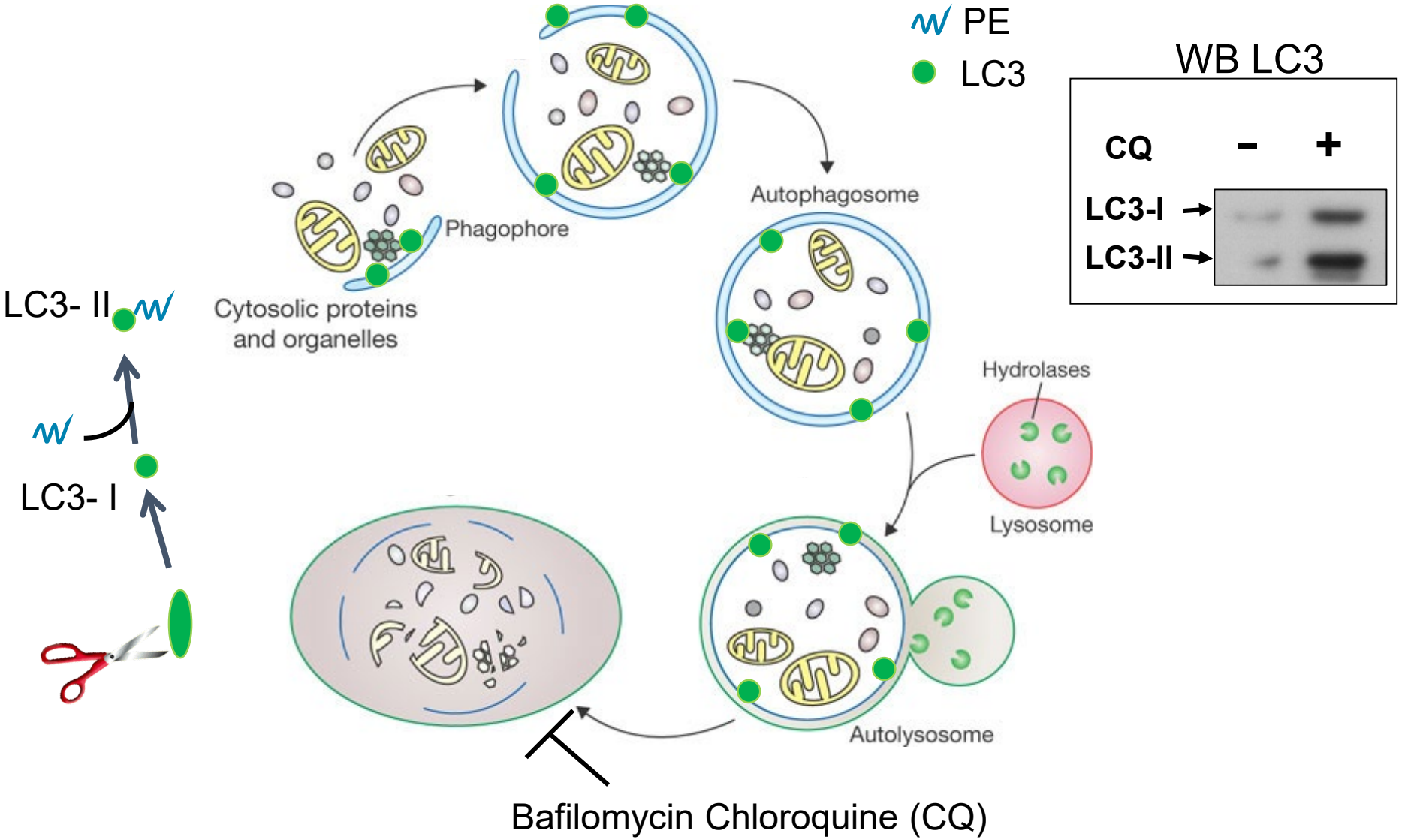
Monitoring the autophagic flux



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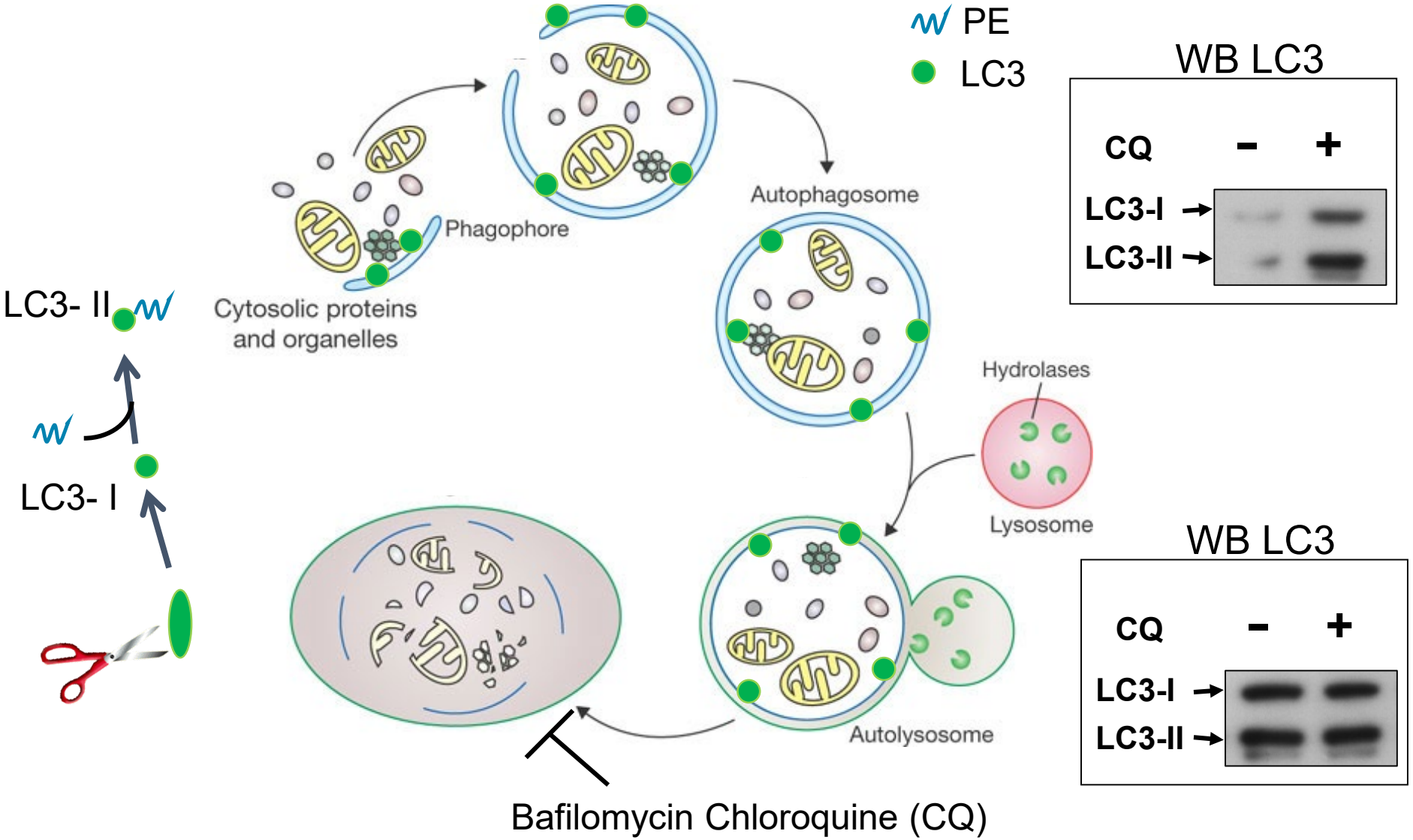
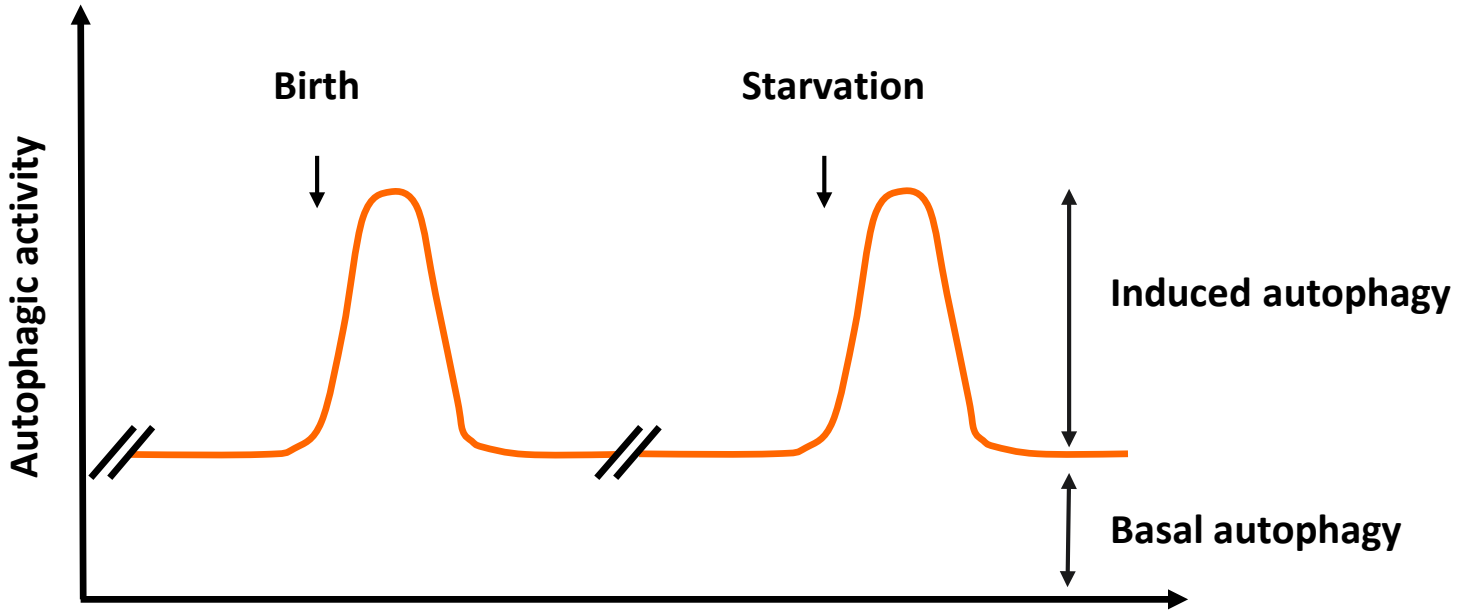


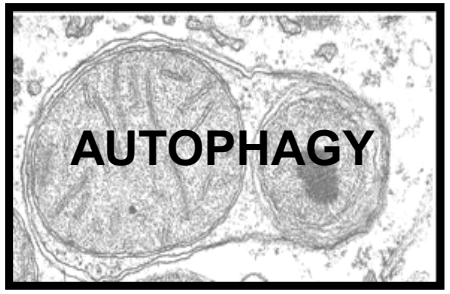
Modified from Xie and Klionsky, 2007

Roles of induced autophagy and basal autophagy:

ENERGY PRODUCTION and/or HOUSEKEEPING



Autophagy and cytoplasm quality control (*atg7*-deficient mice)

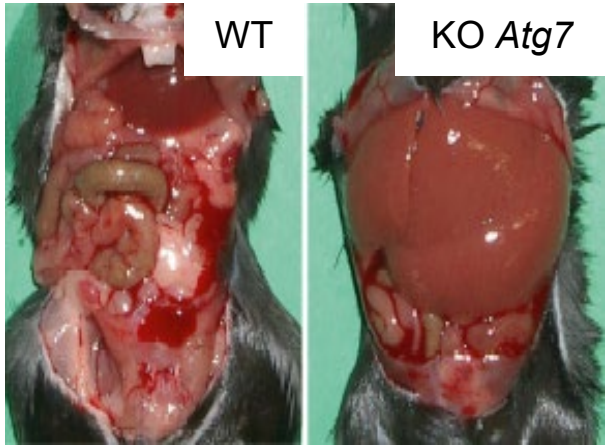
Basal autophagy → 

→ Quality control of cytoplasm
(elimination of protein aggregates and damaged organelles)

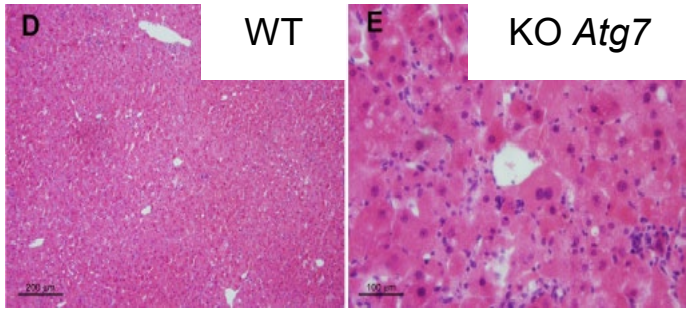
↓

Limits ROS production

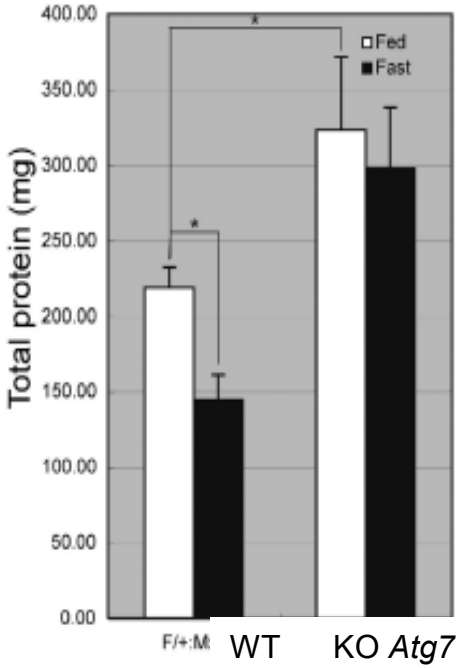
Degradation of cytoplasmic material and recycling

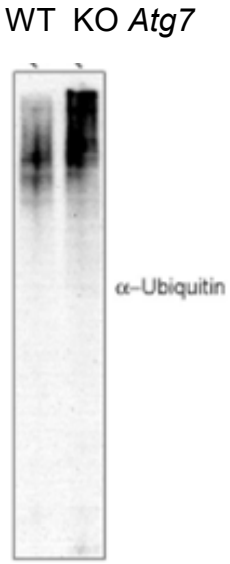


Hepatomegaly

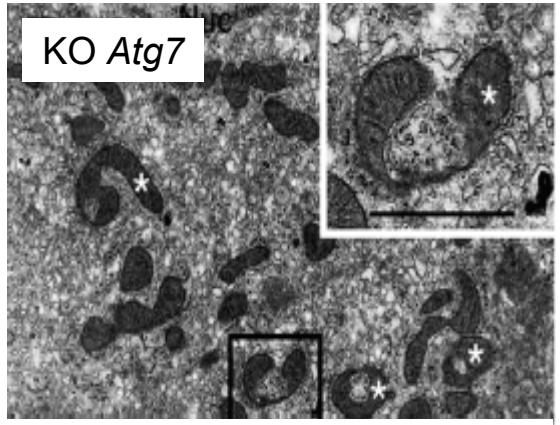


Hepatic cell swelling





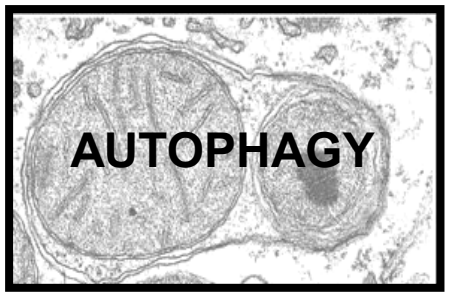
Accumulation of ubiquinylated protein aggregates



Accumulation of organelles

Komatsu et al., 2005

Functions of autophagy

Basal autophagy → 

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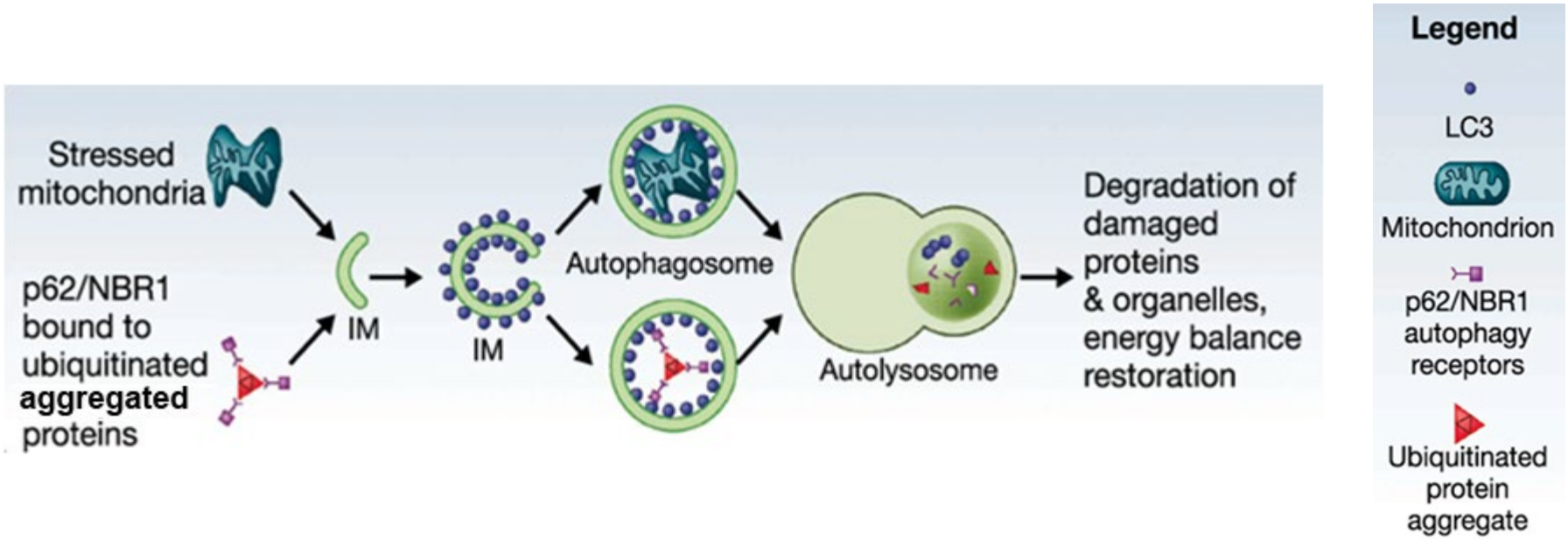

Roles of induced autophagy and basal autophagy:

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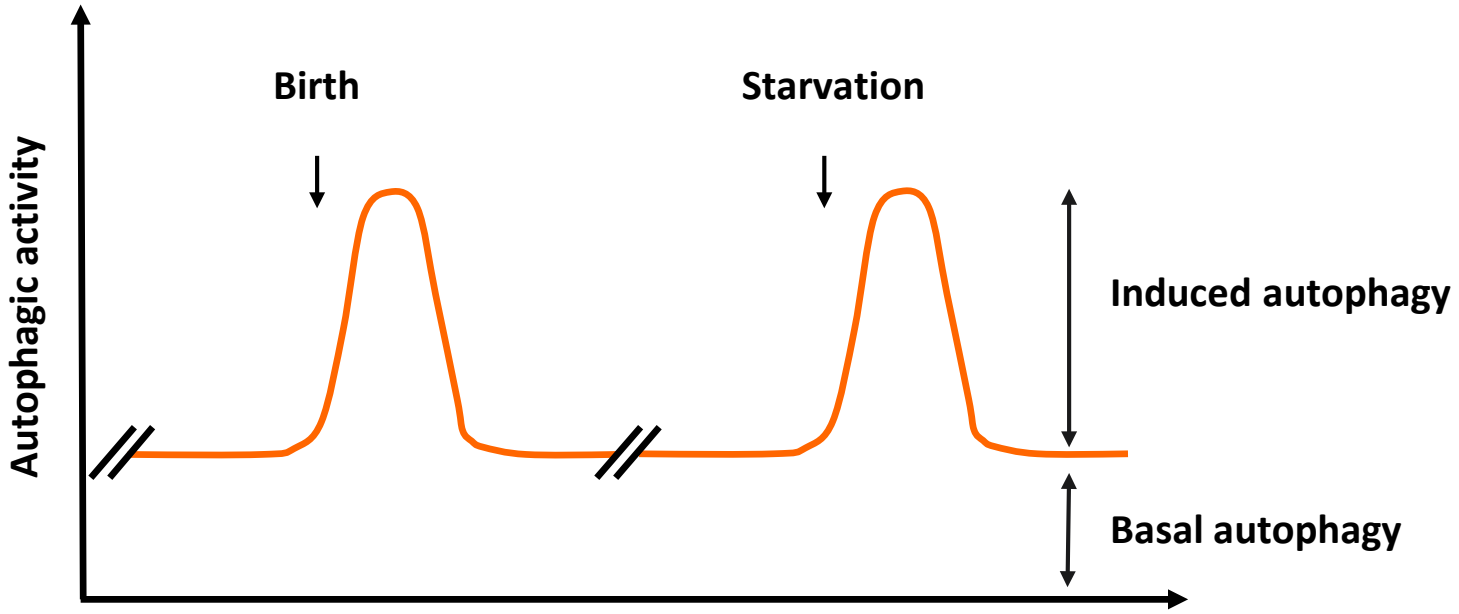
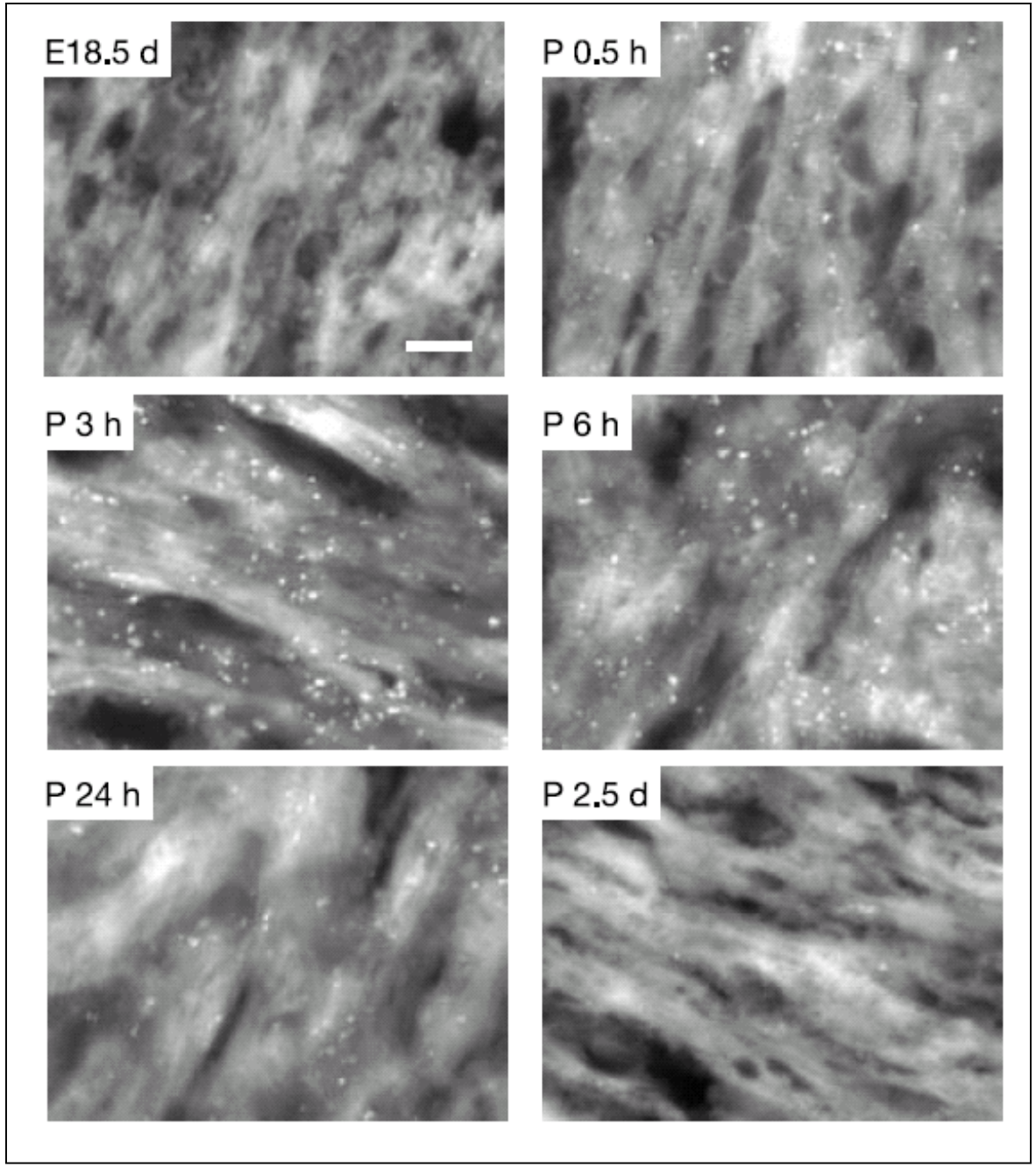


Autophagy activation occurs in heart soon after birth

The role of autophagy during the early neonatal starvation period

Akiko Kuma[1,2,5,7], Masahiko Hatano[2,4], Makoto Matsui[5,6,7], Akitsugu Yamamoto[8], Haruaki Nakaya[3], Tamotsu Yoshimori[9], Yoshinori Ohsumi[5,6], Takeshi Tokuhisa[2] & Noboru Mizushima[1,5,7]

GFP-LC3 mice



Kuma, Nature 2004

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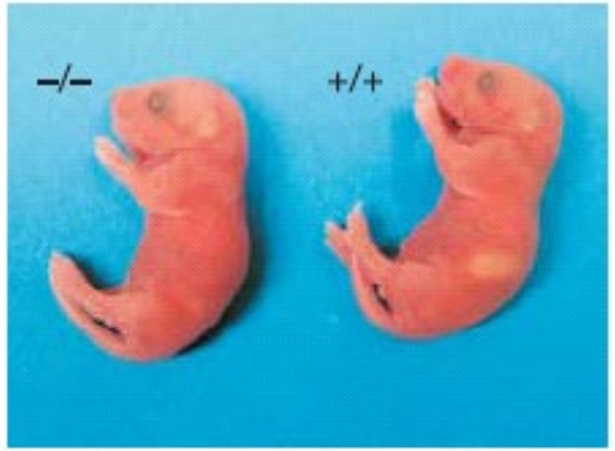
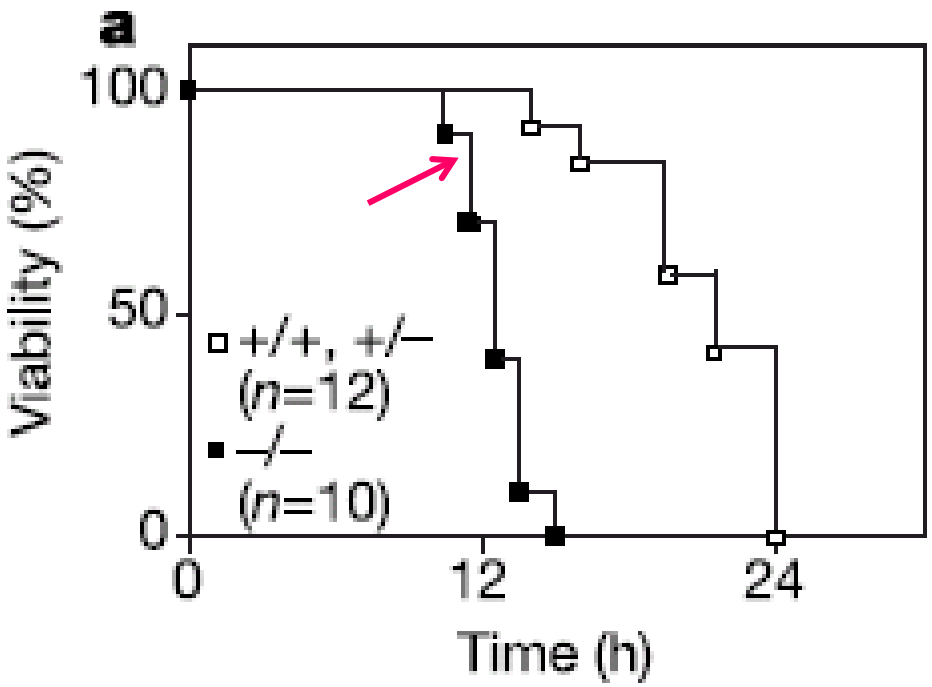
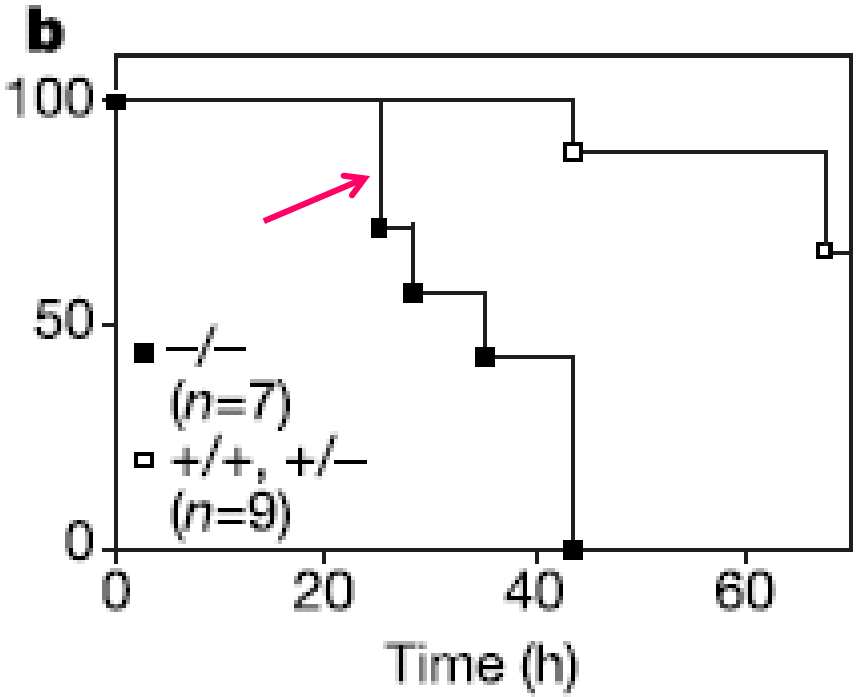
atg5



Without milk feeding

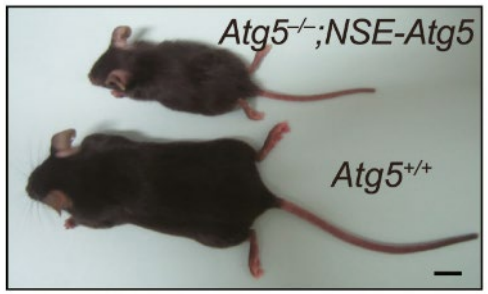


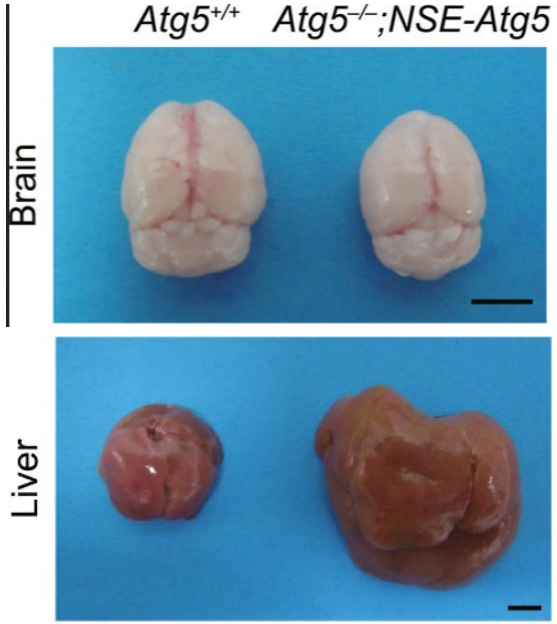
With artificial milk feeding

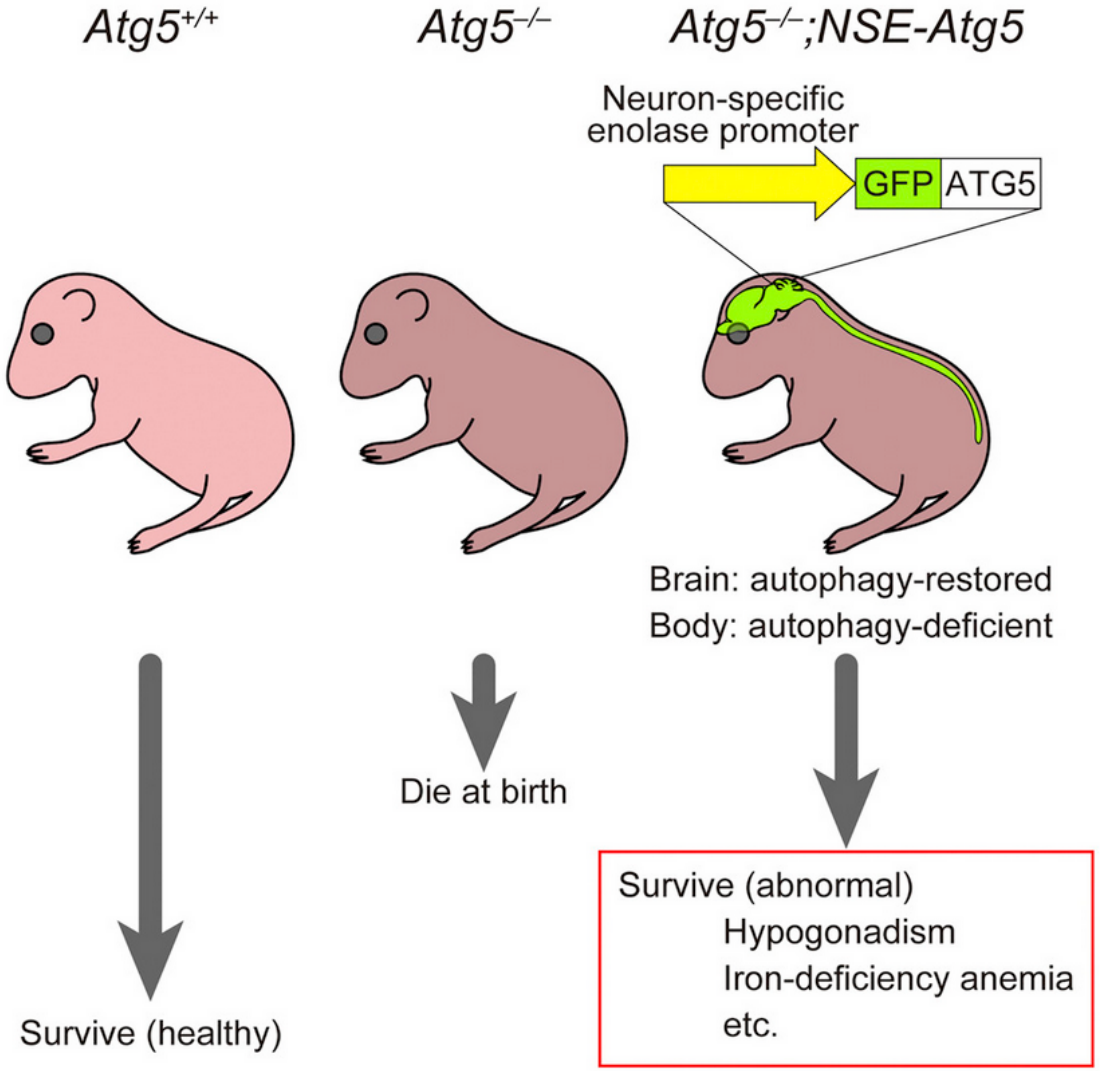


Autophagic deficiency is associated with neuronal dysfunctions

- overexpression of *Atg5* in the neuronal compartment rescues perinatal mortality of *atg5*$^{-/-}$ mice
- neuronal dysfunction, including suckling failure, is the primary cause of the death of Atg5-null neonates

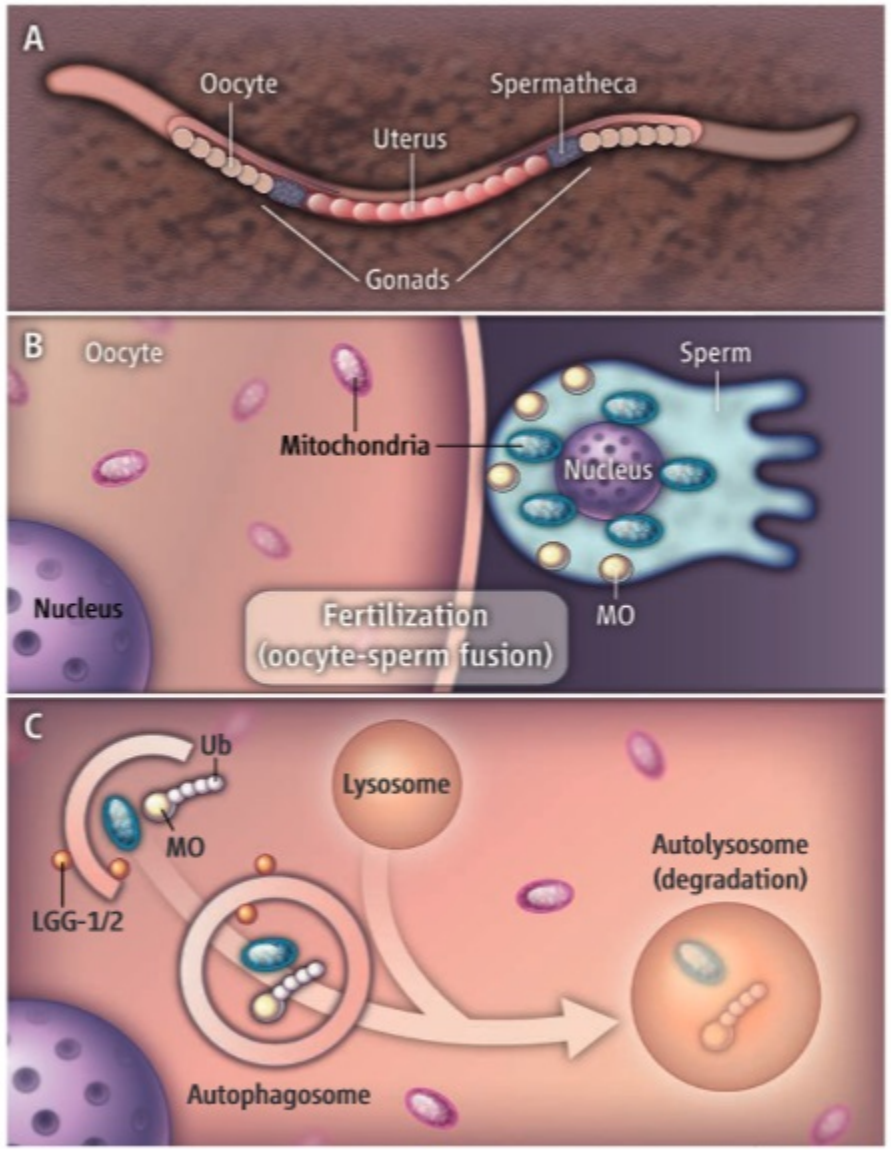






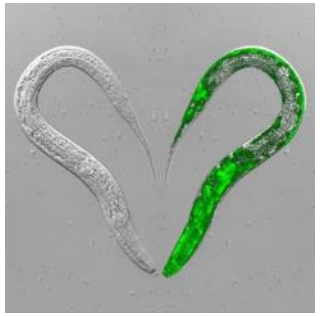
Yoshii *et al*, 2016

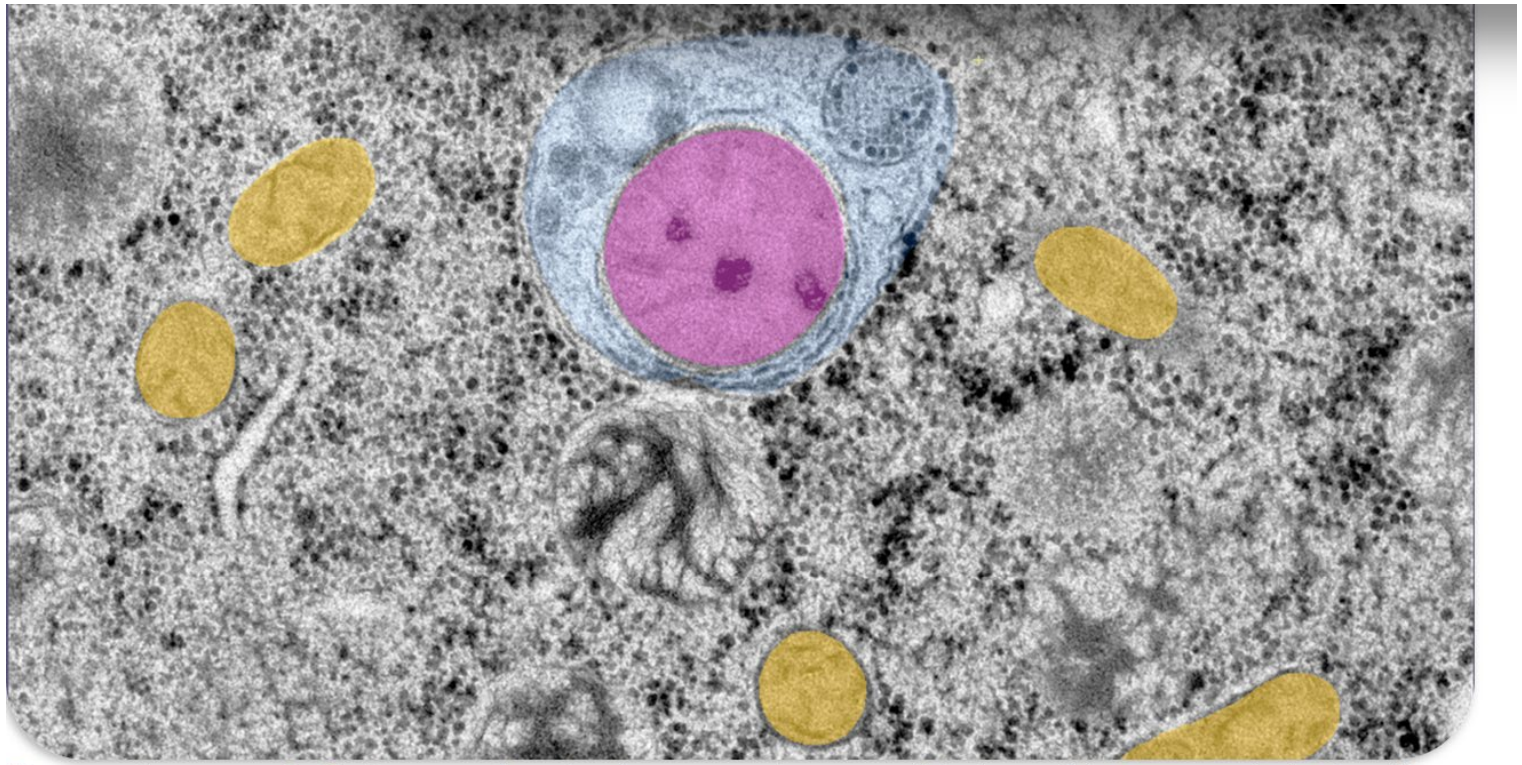
Autophagy allows C elegans to select maternal mitochondria



Selective autophagy of paternal mitochondria. (A) The *C. elegans* hermaphrodite reproductive tract is shown. **(B)** Fusion

Selective autophagy of paternal mitochondria



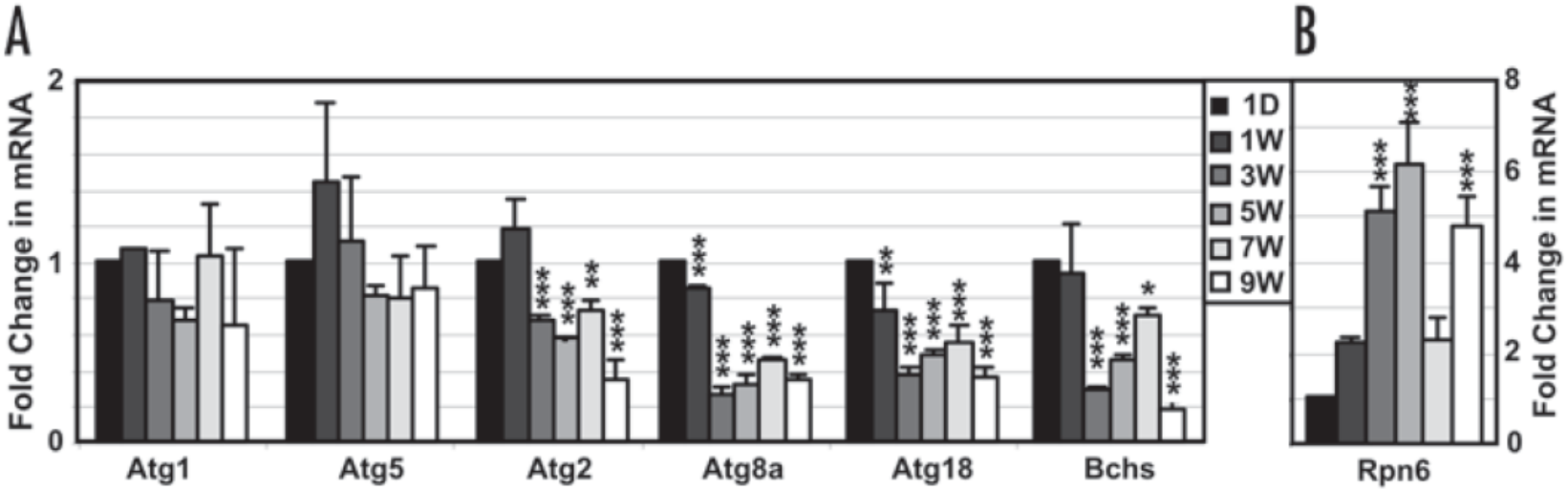


Electron microscopy of a C. elegans embyo showing an autophagosome (blue) containing a paternal mitochondria (pink) and surrounded by maternal mitochondria (yellow)

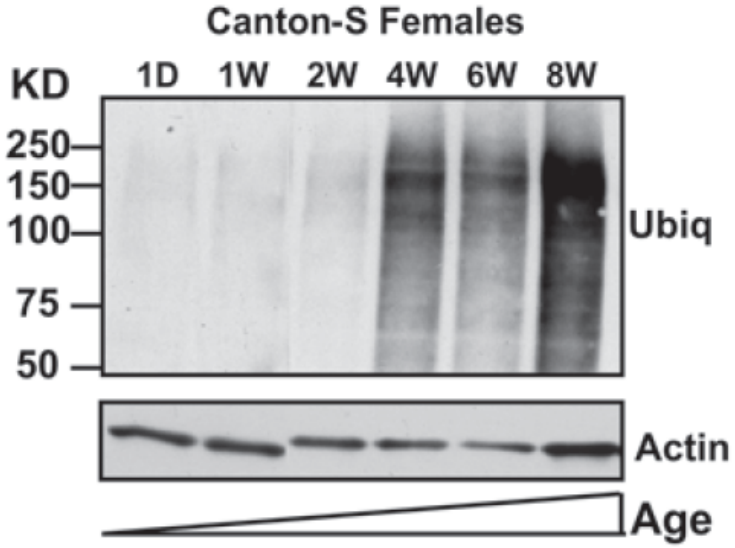
Renaud Legouis, I2BC, Gif sur Yvette

Autophagy and longevity

A decline in autophagy over time



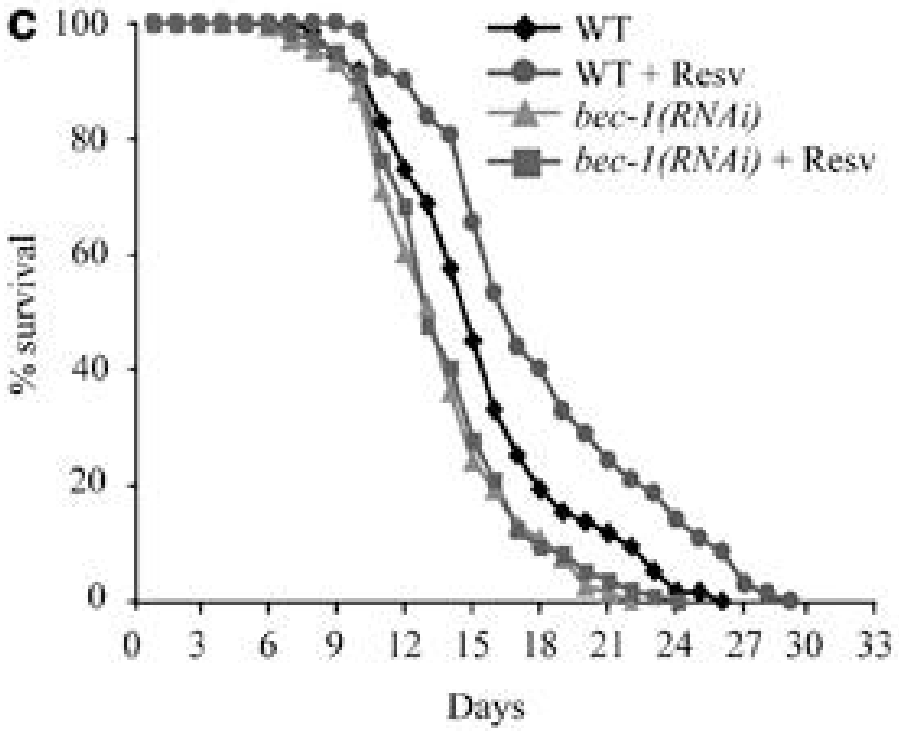
A decrease expression of several ATG genes in flies



This decrease causes an accumulation of autophagic structures and possibly limits autophagic capacity to maintain cellular homeostasis

Simonsen et al, Autophagy 2008

Caloric restriction and resveratrol promote longevity of flies



Resveratrol is an autophagy inducer

Bec-1 deficiency suppresses longevity conferred by treatment with 100 µg/ml resveratrol (Resv)

(lifespan values: WT control mean 14.6±0.7, max 26.0; WT grown on Resv mean 16.2±1.0, max 29.0; bec-1(RNAi) mean 13.1±1.2, max 22.0; bec-1(RNAi) grown on Resv mean 12.9±1.2, max 24.0)

Morcelli et al, CDD, 2010

Autophagy inducers and anti-aging

Life span can be extended by nutritional, pharmacological or genetic manipulations
Yeasts, worms, flies, mice

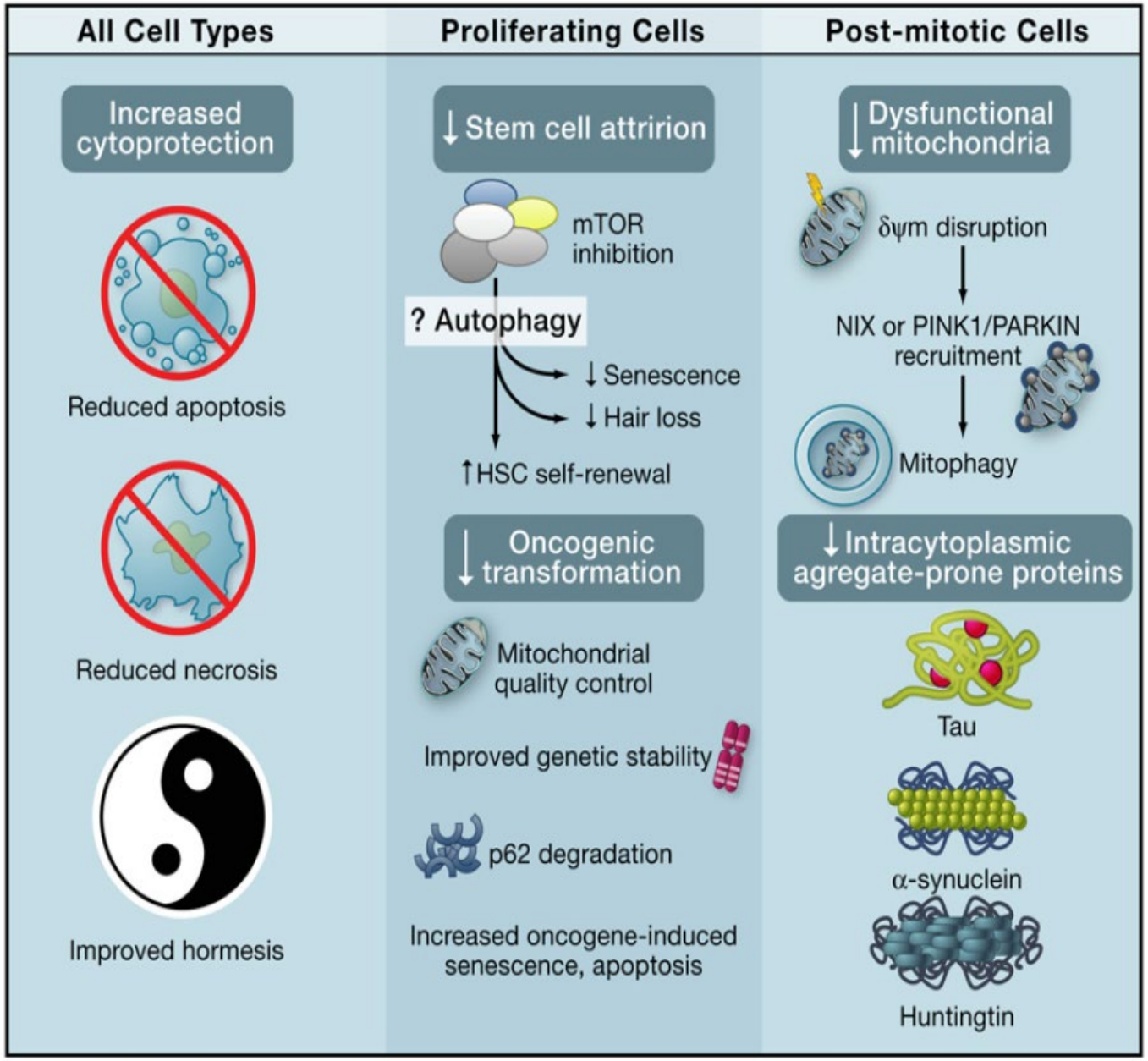
Table 1. Examples of autophagy-dependent life span extension

Genetic/pharmacologic intervention	Species	Anti-aging phenotype	Anti-aging phenotype abolished by	Reference
Loss of IGF signaling due to mutated *daf-2*	*C. elegans*	Elevated autophagy levels and dauer formation, life span extended by 70%	Knockdown of *bec-1, atg-7, unc-51* (*ATG1* homolog), or *lgg-1* (LC3 homolog)	29
CR mutant *eat-2*	*C. elegans*	Enhanced autophagy, life span extended by 28%	Knockdown of *bec-1* or *atg-7*	33
Null-mutant of *cnb-1* (calcineurin)	*C. elegans*	Enhanced autophagy, mean life span extended by 31%	Knockdown of *bec-1* or *atg-7*	30
Overexpression of HLH-30	*C. elegans*	Increased autophagic activity, life span extended by up to 20%	Knockdown of *atg-18*	55
Resveratrol treatment	*C. elegans*	Elevated autophagy levels, life span extended by 11%	Knockdown of *bec-1*	7
Spermidine treatment	*C. elegans*	Elevated autophagy levels, life span extended by 15%	Knockdown of *bec-1*	32
Muscle-specific transgenic expression of FOXO	*D. melanogaster*	Autophagy partially enhanced proteostasis, life span extended by 21%	Proteostasis impaired by knockdown of *Atg7*	27
Brain-specific overexpression of Atg8a	*D. melanogaster*	Enhanced neuronal autophagy, life span extended by >50% in female flies	Not reported	44
Rapamycin treatment	*D. melanogaster*	Increased autophagy levels, decreased translation, median life span extended by 22%	Knockdown of *Atg5* or null mutant of 4E-BP	68
Spermidine treatment	*D. melanogaster*	Elevated autophagy levels, mean life span extended by <30%	Knockout of *Atg7*	32
Transgenic overexpression of ATG5	Mouse	Enhanced autophagy, improved insulin sensitivity and motor function, life span extended by 17%	Not reported	54
CR	Mouse	Life span extended by 51%	Homozygous knockout of *Sirt1*	19

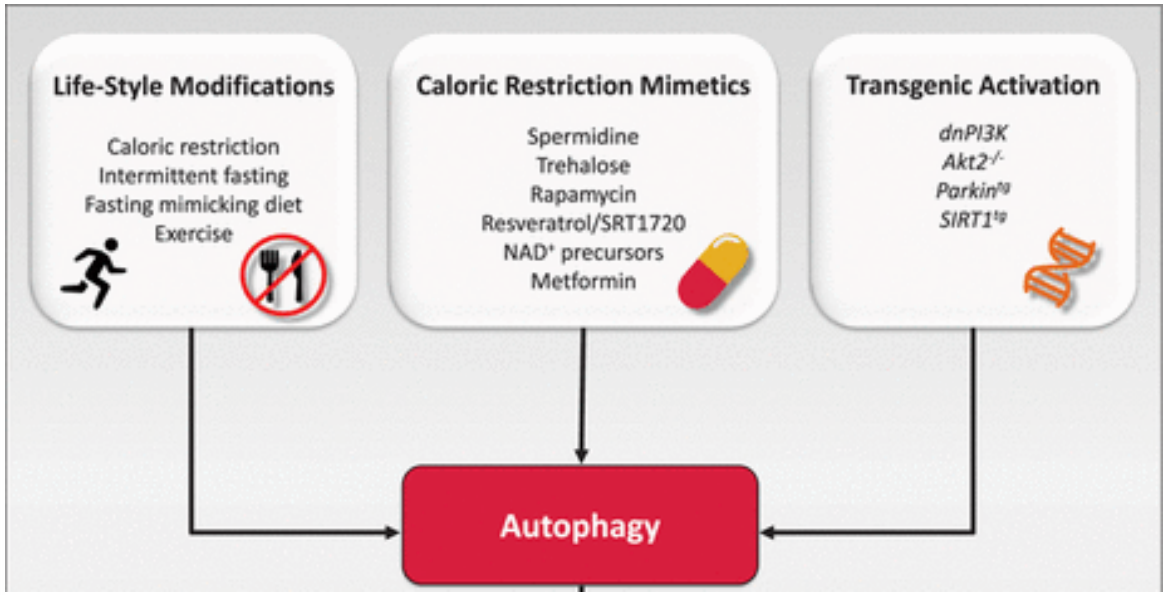
Caloric restriction

From Madeo, Maiuri and Kroemer J Clin Invest 2015

Cell-Autonomous anti-aging effects of autophagy



Rubinsztein et al 2011 Cell 146, 682



Autophagy in Cardiovascular Aging

Mahmoud Abdellatif, Simon Sedej, Didac Carmona-Gutierrez, Frank Madeo and Guido Kroemer

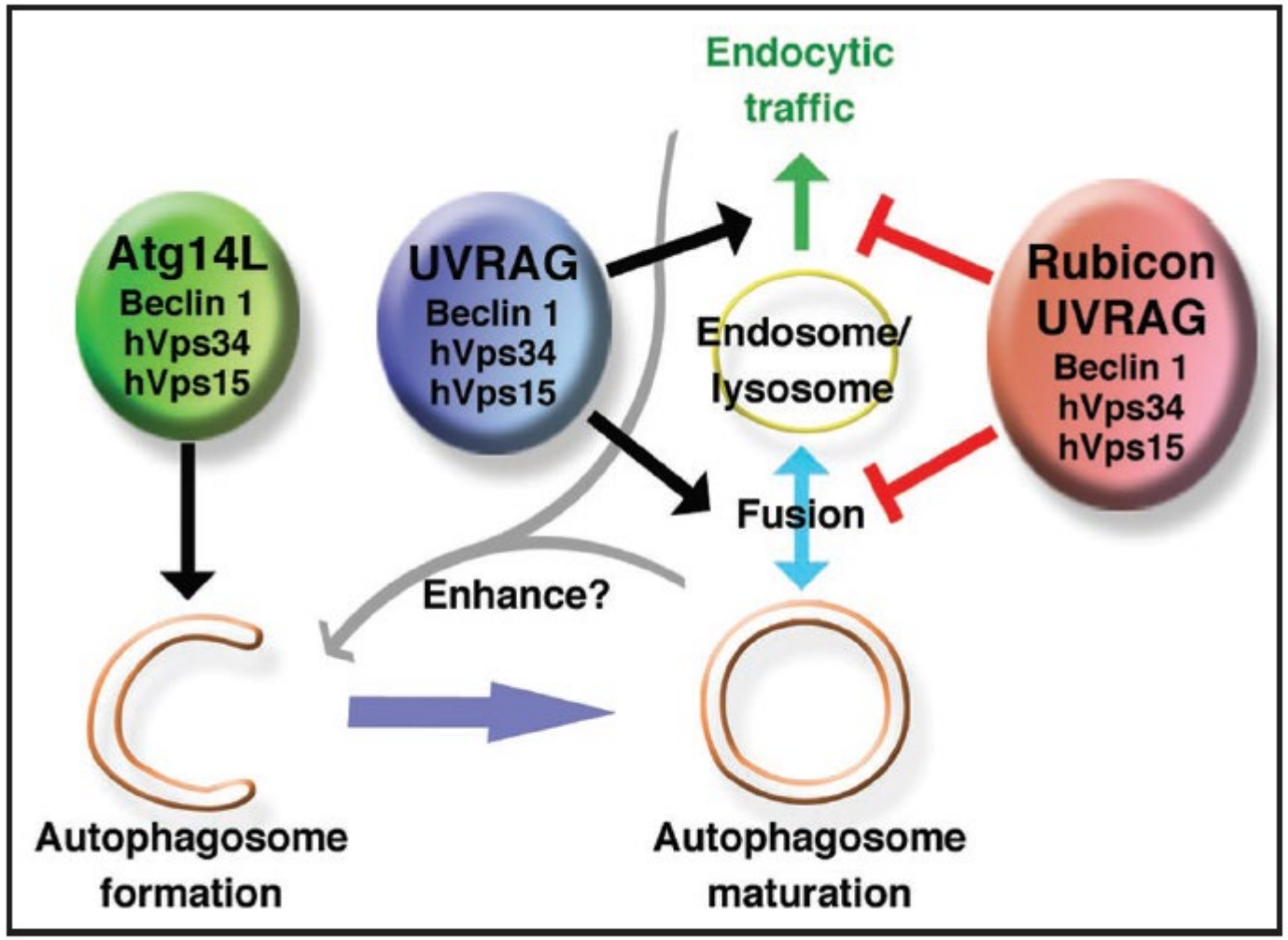
Originally published 13 Sep 2018
https://doi.org/10.1161/CIRCRESAHA.118.312208
Circulation Research. 2018;123:803–824

ARTICLE

https://doi.org/10.1038/s41467-019-08729-6 OPEN

Suppression of autophagic activity by Rubicon is a signature of aging

Rubicon negatively regulates autophagy



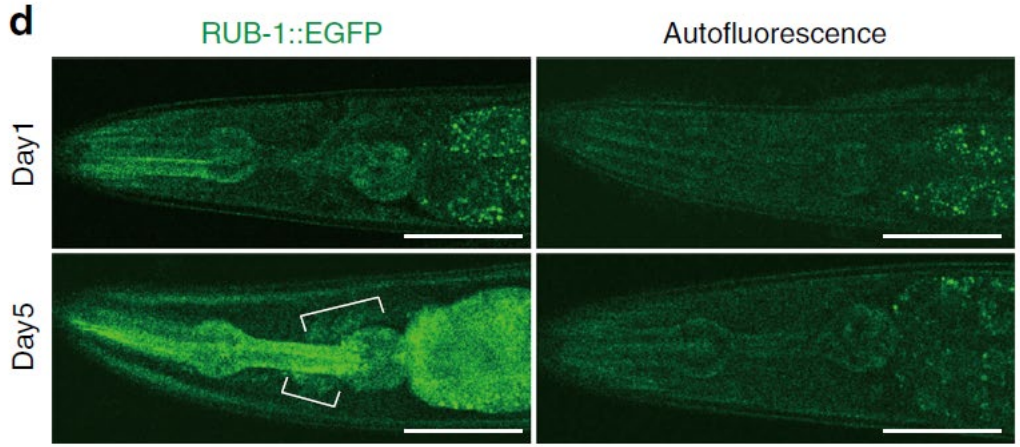
Matsunaga et al, Nature cell biology, 2009

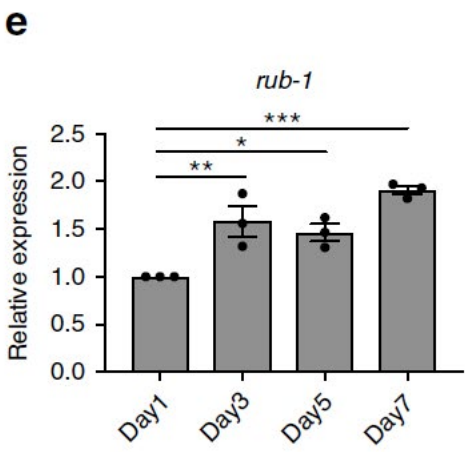
nature COMMUNICATIONS

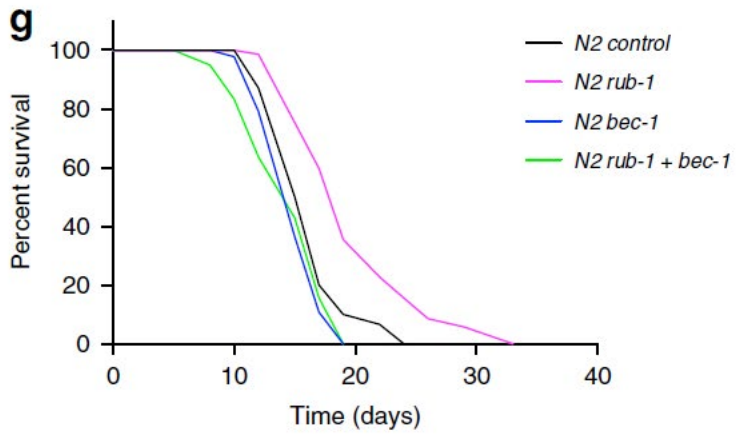
ARTICLE

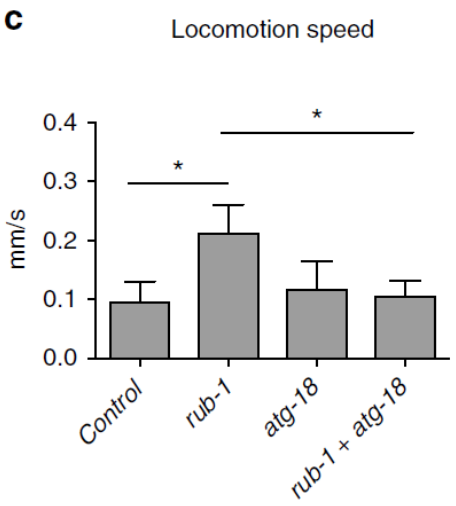
https://doi.org/10.1038/s41467-019-08729-6 OPEN

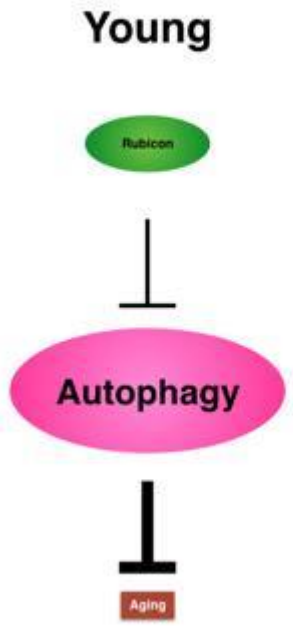
Suppression of autophagic activity by Rubicon is a signature of aging

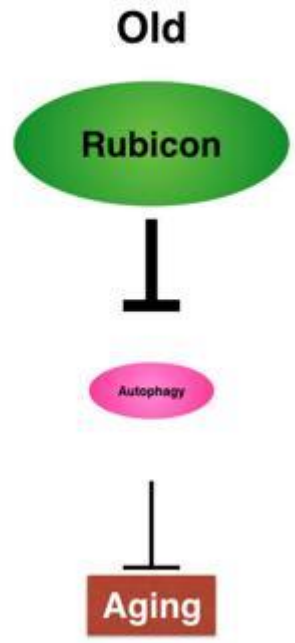












Rubicon expression is increased in older worms

KD of rub-1 extends lifespan and ameliorates age-associated phenotypes

WORMS

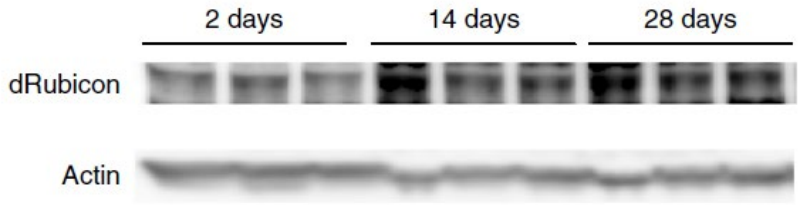
Nakamura et al, Nature Comm 2019

nature COMMUNICATIONS

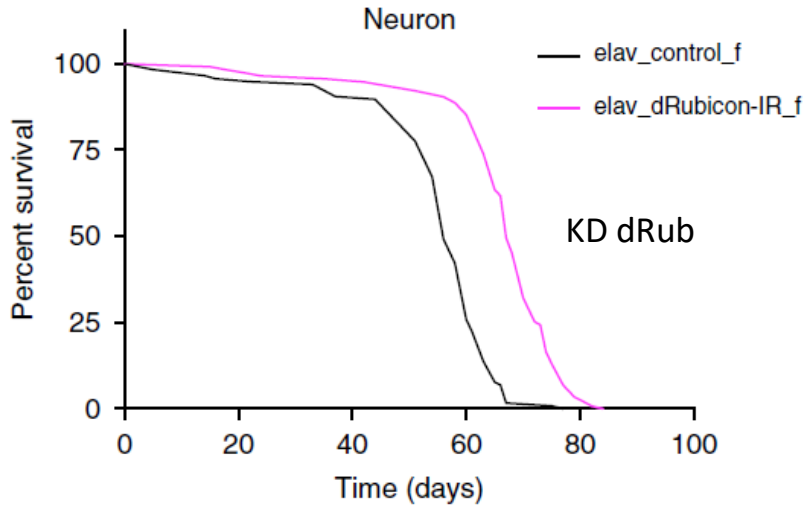
ARTICLE

https://doi.org/10.1038/s41467-019-08729-6 OPEN

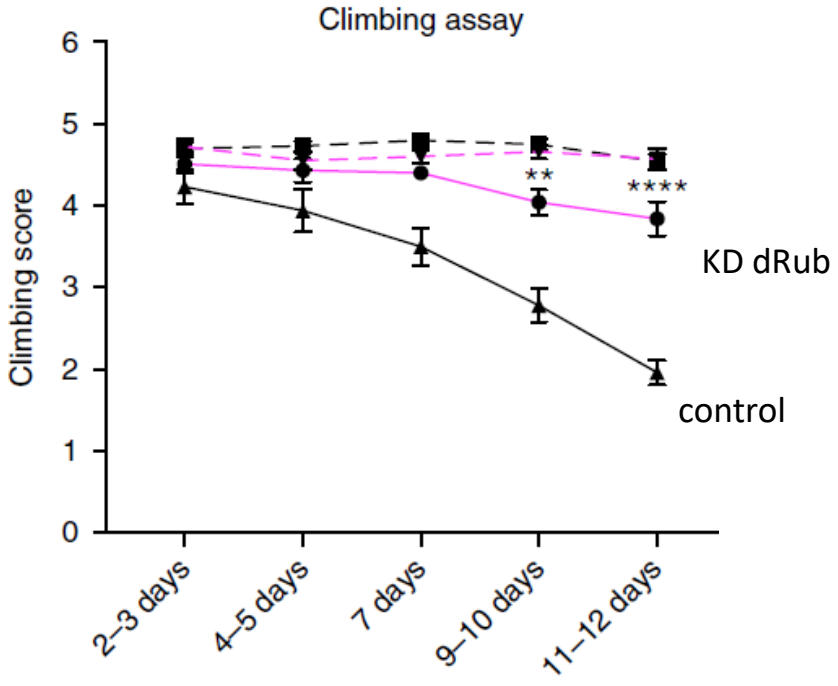
Suppression of autophagic activity by Rubicon is a signature of aging



dRubicon expression is increased in older flies



Neuronal KD of dRubicon extends lifespan



Neuronal KD of dRubicon ameliorates the locomotor dysfunction by polyQ expression

FLIES

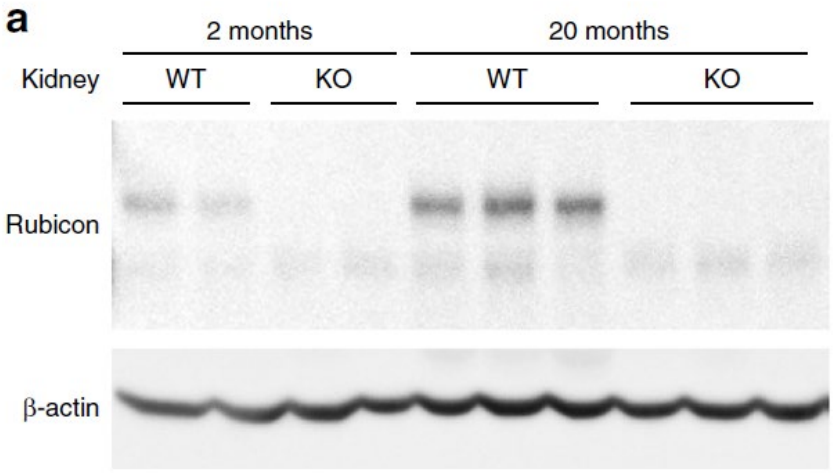
Nakamura et al, Nature Comm 2019

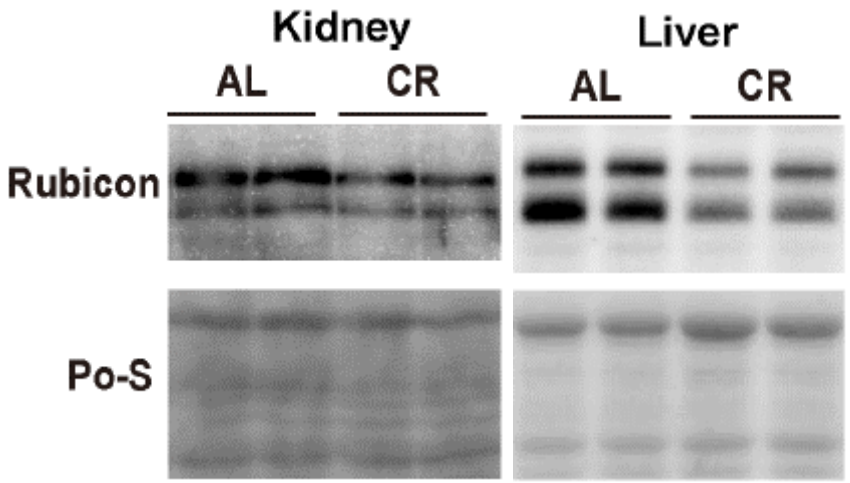
nature COMMUNICATIONS

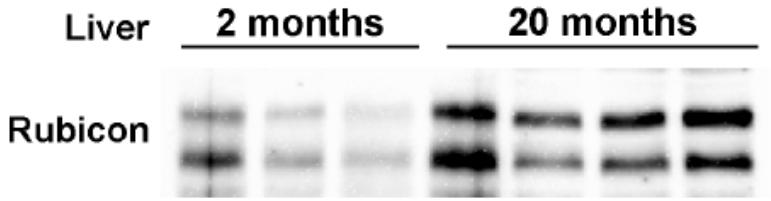
ARTICLE

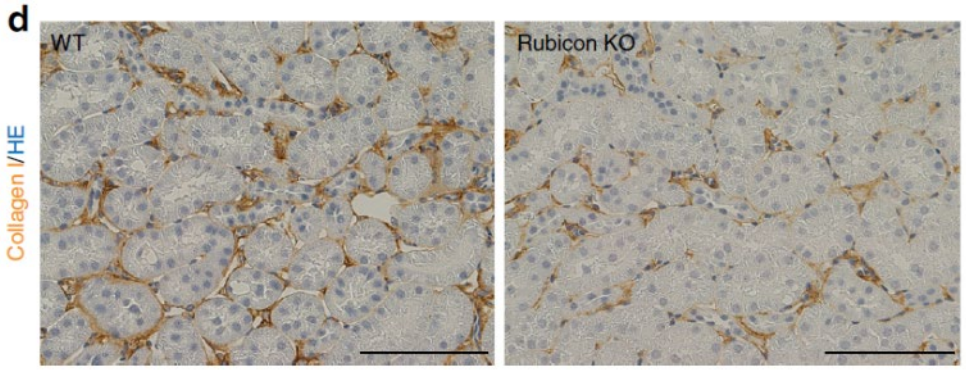
https://doi.org/10.1038/s41467-019-08729-6 OPEN

Suppression of autophagic activity by Rubicon is a signature of aging









Rubicon expression is higher in kidney and in liver of older mice than in juvenile

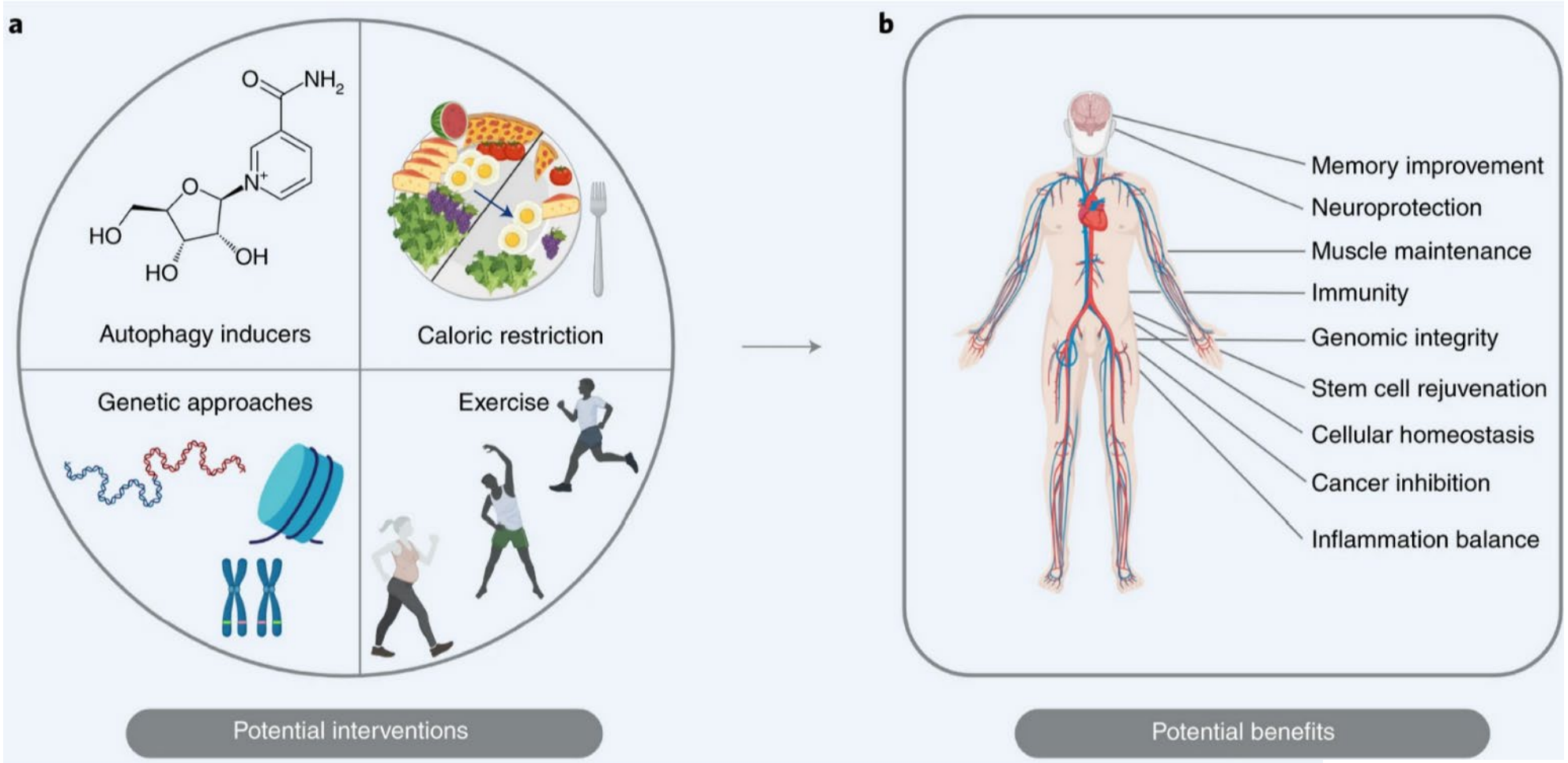
9-month caloric restriction (CR) reduced rubicon expression compared to freely feeding (AL ad libitum)

Less age-associated fibrosis (labelled with collagen) in the kidney of KO rubicon mice.

MICE

Nakamura et al, Nature Comm 2019

Anti-aging effects of autophagy



REVIEW ARTICLE
https://doi.org/10.1038/s43587-021-00098-4
nature aging

Autophagy in healthy aging and disease

Yahyah Aman[1,2,22], Tomas Schmauck-Medina[1,22], Malene Hansen[3], Richard I. Morimoto[4], Anna Katharina Simon[5], Ivana Bjedov[2,6], Konstantinos Palikaras[7], Anne Simonsen[8,9], Terje Johansen[10], Nektarios Tavernarakis[11,12], David C. Rubinsztein[13,14], Linda Partridge[2,15], Guido Kroemer[16,17,18,19,20], John Labbadia[2] and Evandro F. Fang[1,21]

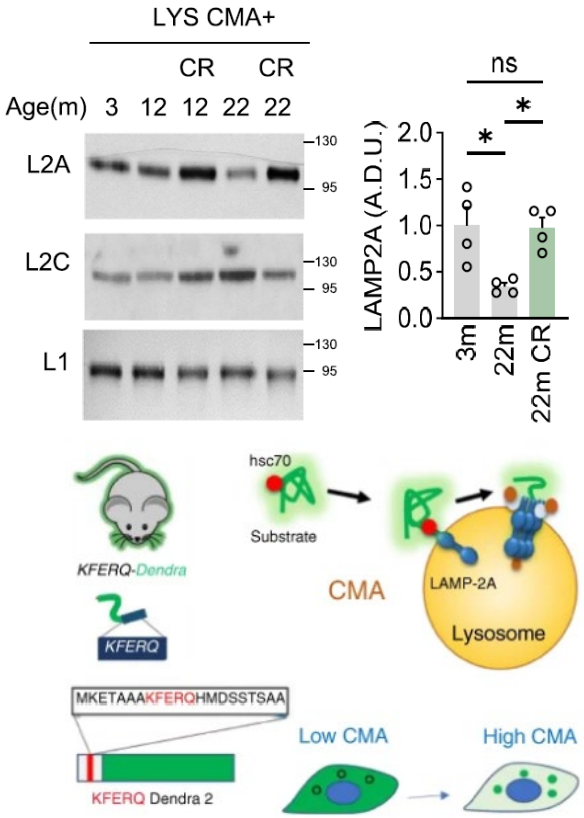
Caloric restriction activates Chaperone-mediated autophagy (CMA)

- CMA decreases with age

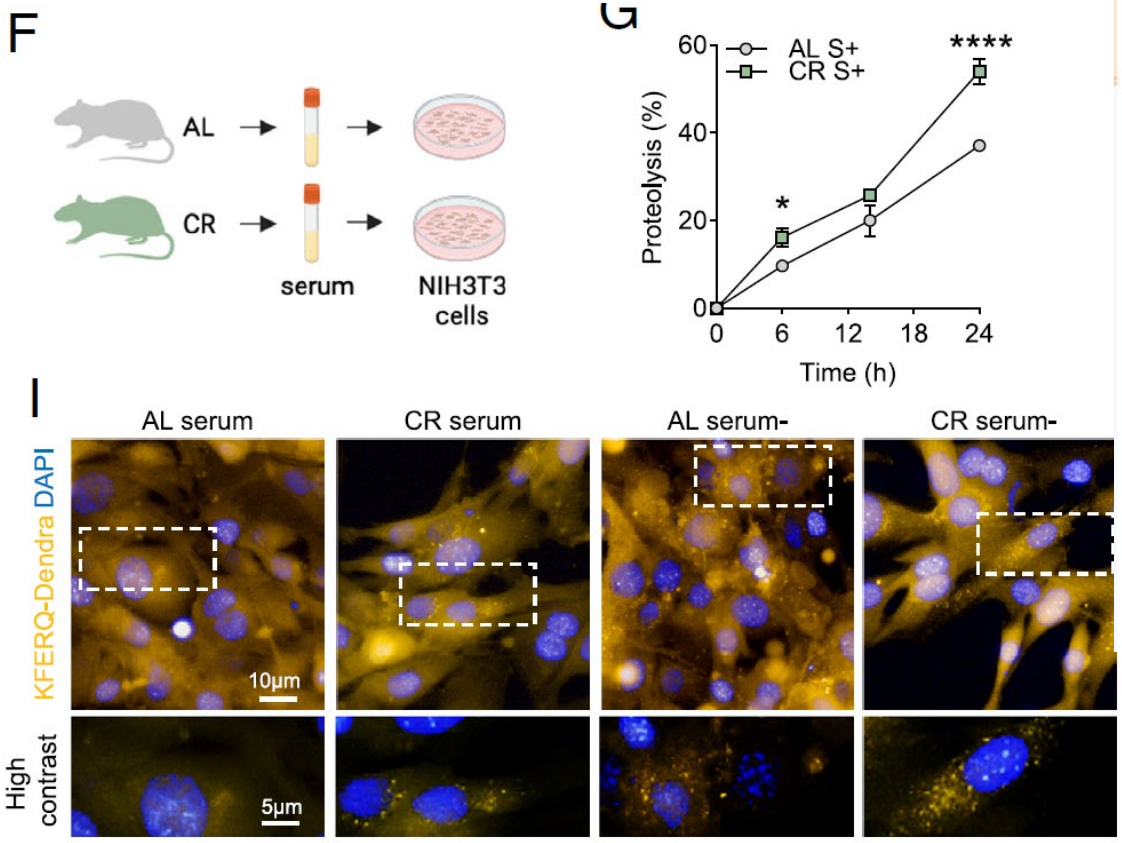
(decrease of LAMP2A)

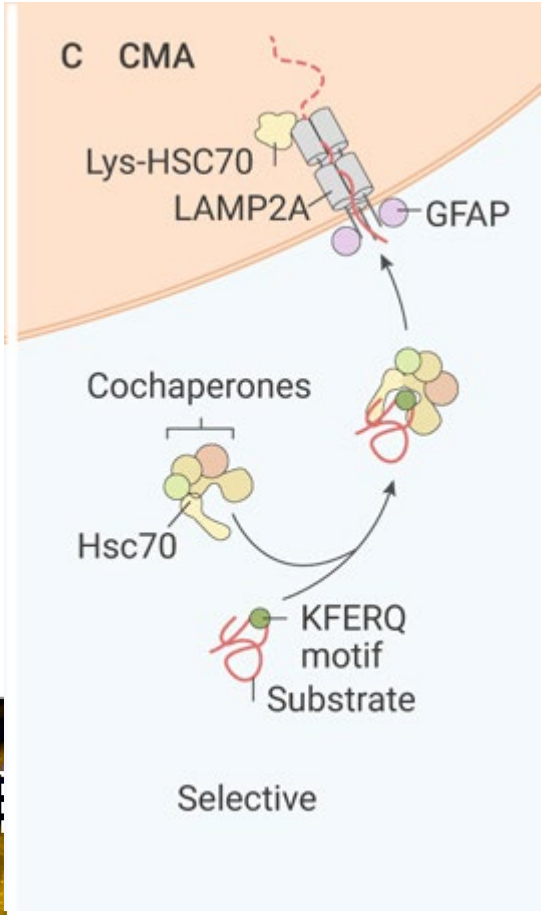
- CR activates CMA

(stabilizes Lysosomal LAMP2A)



caloric restriction (CR)
freely feeding (AL ad libitum)

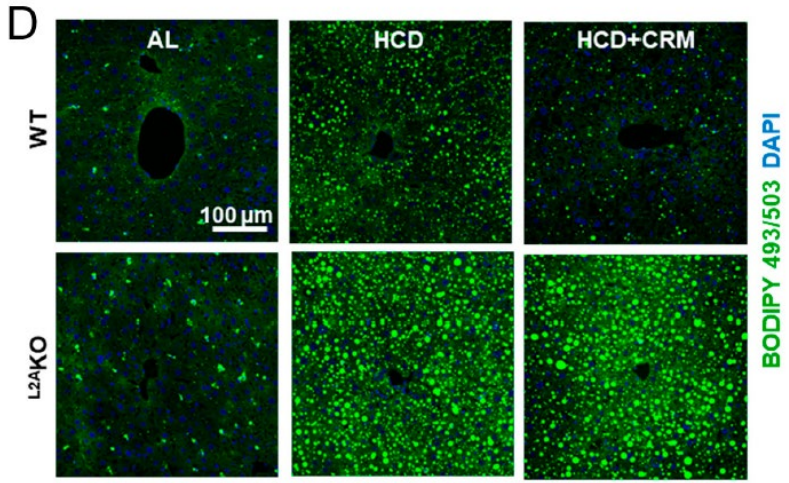


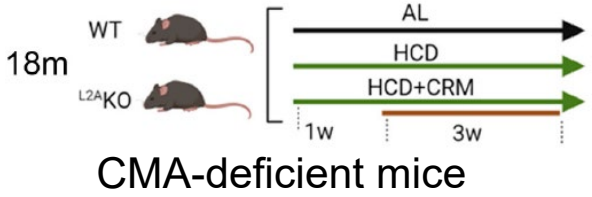


Jafari et al 2024 PNAS

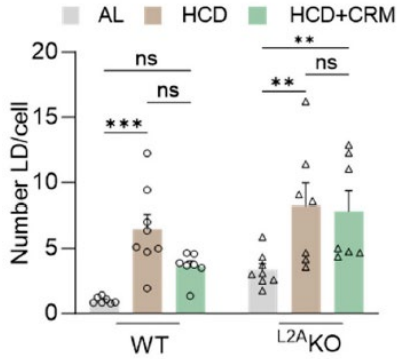
CRMs activate CMA *in vitro* and *in vivo*

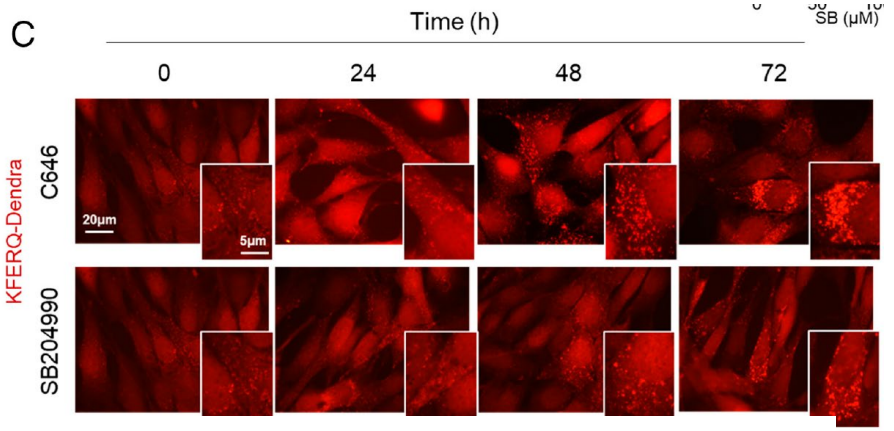
- CRM (CR mimetic)) = pharmacologically active substances that reproduce the biochemical and functional effects of CR
- Acute treatment of old mice with CRMs robustly activates CMA in several tissues
- Beneficial effects of CRM are CMA dependent

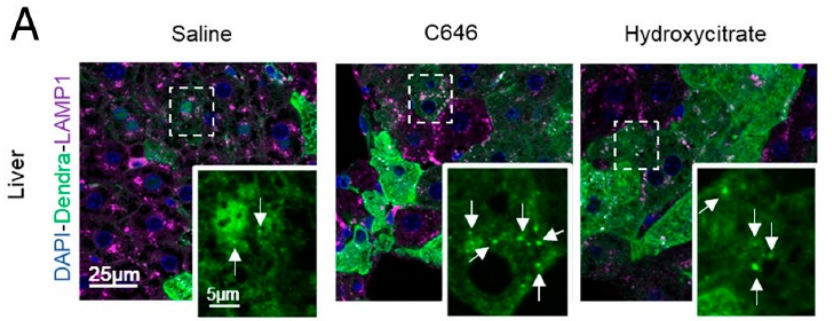


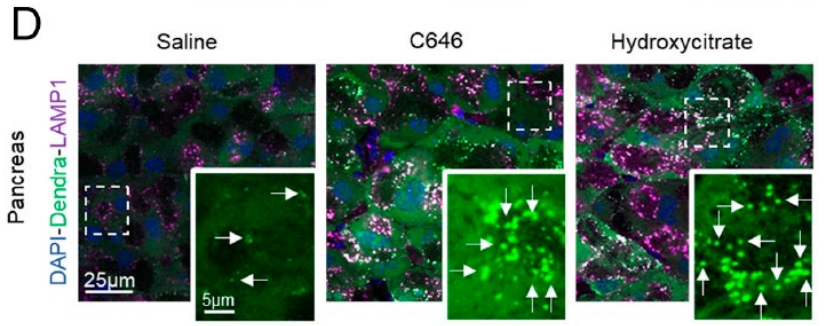


CMA-deficient mice





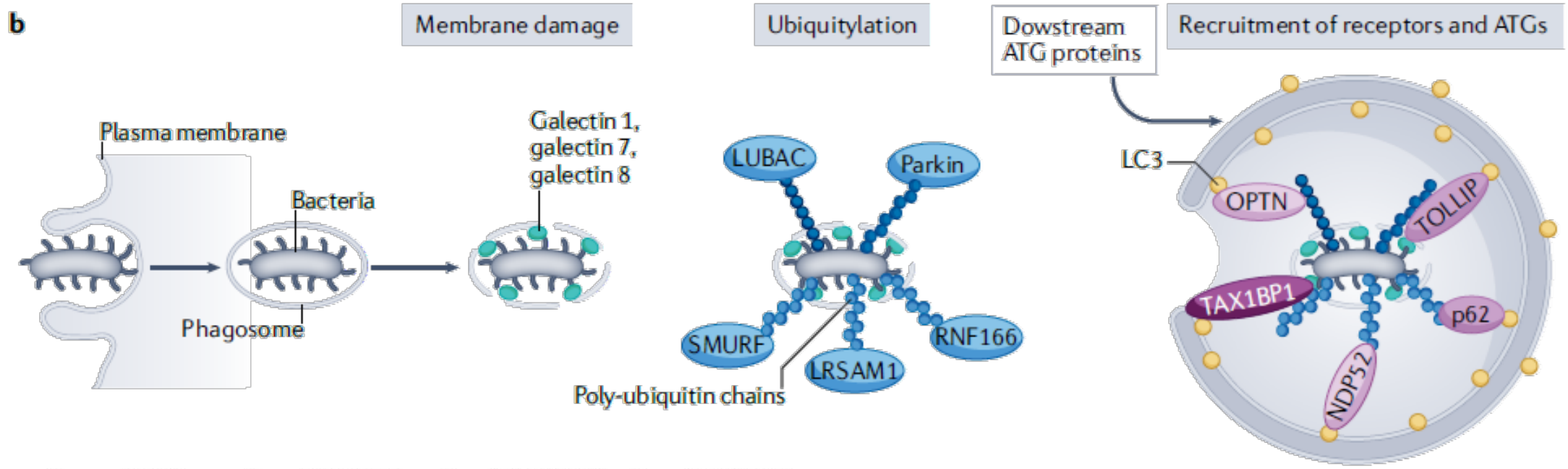


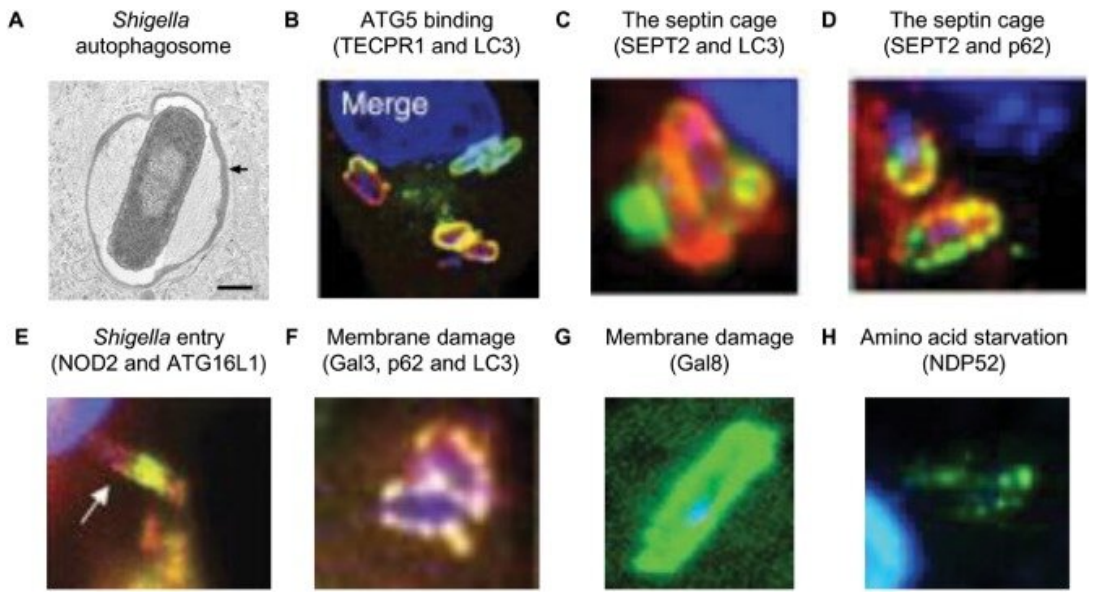


Jafari et al 2024 PNAS

Autophagy and immunity

Xenophagy : degradation of microorganisms by autophagy

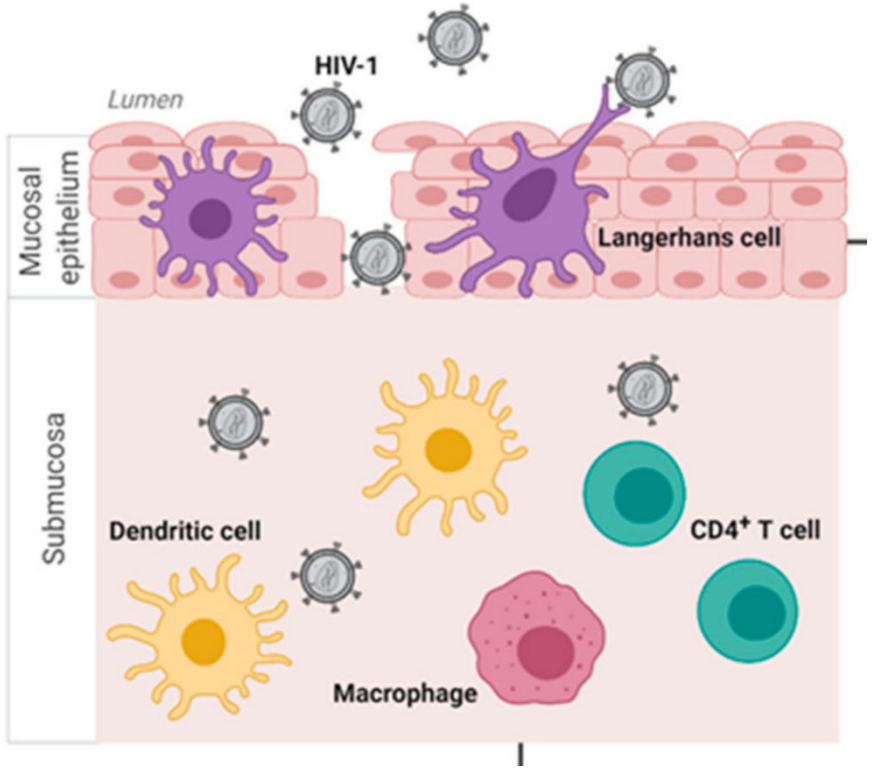




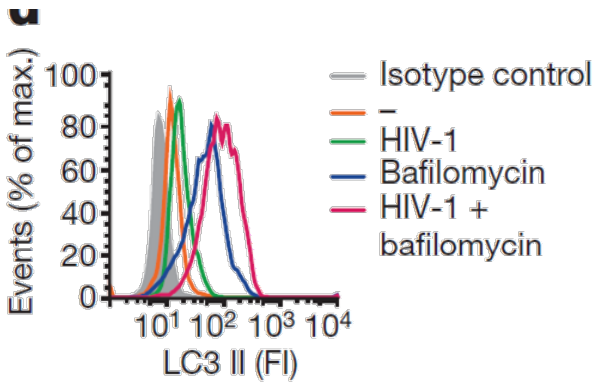
Mostowy 2012

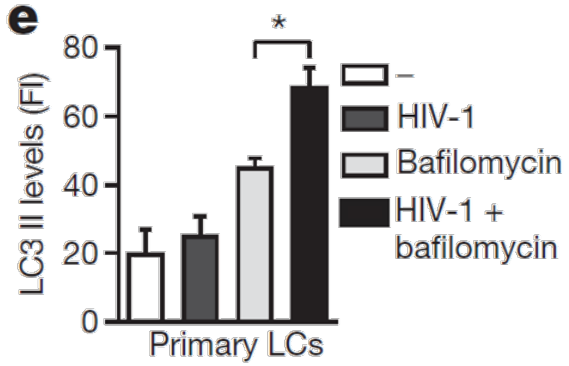
Vargas et al, Nature Review , 2023

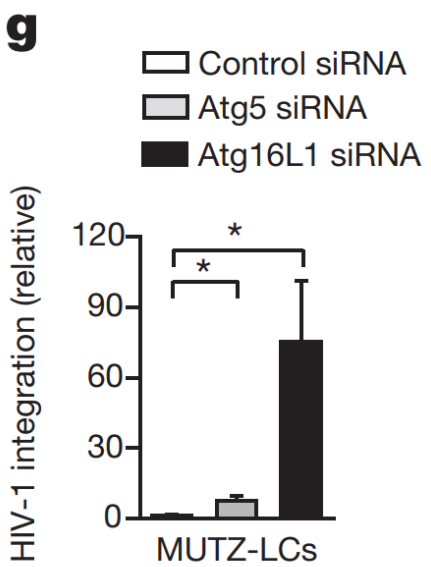
HIV, autophagic degradation in Langerhans cells

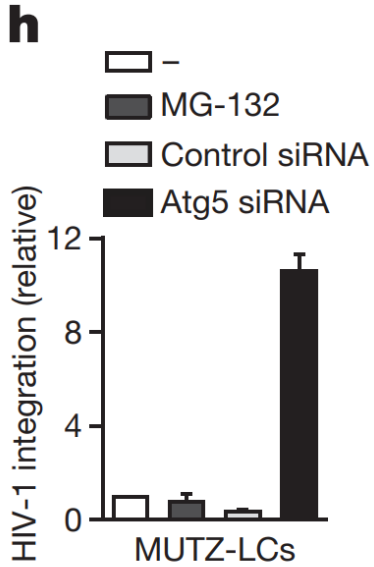


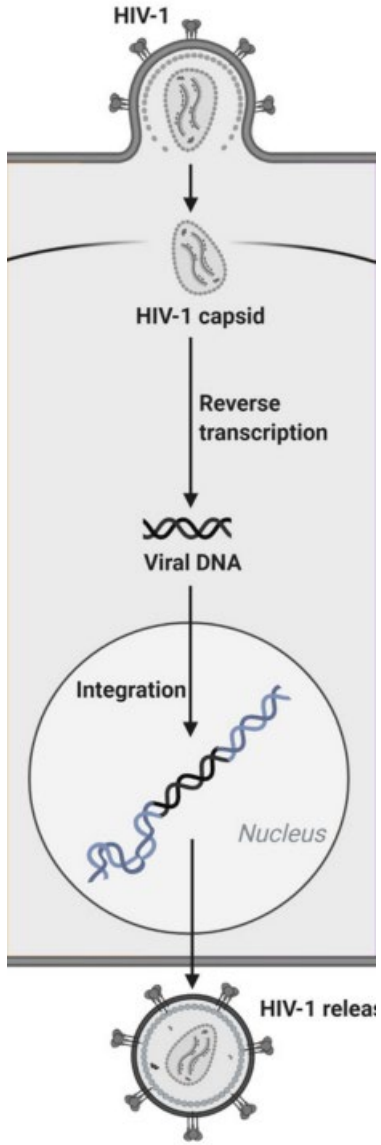
- HIV infects different cell types
- Langerhans cells intrinsically resistant to HIV-1
- Silencing of ATG5 or ATG16L1 in primary human Langerhans cells results in increased HIV-1 integration





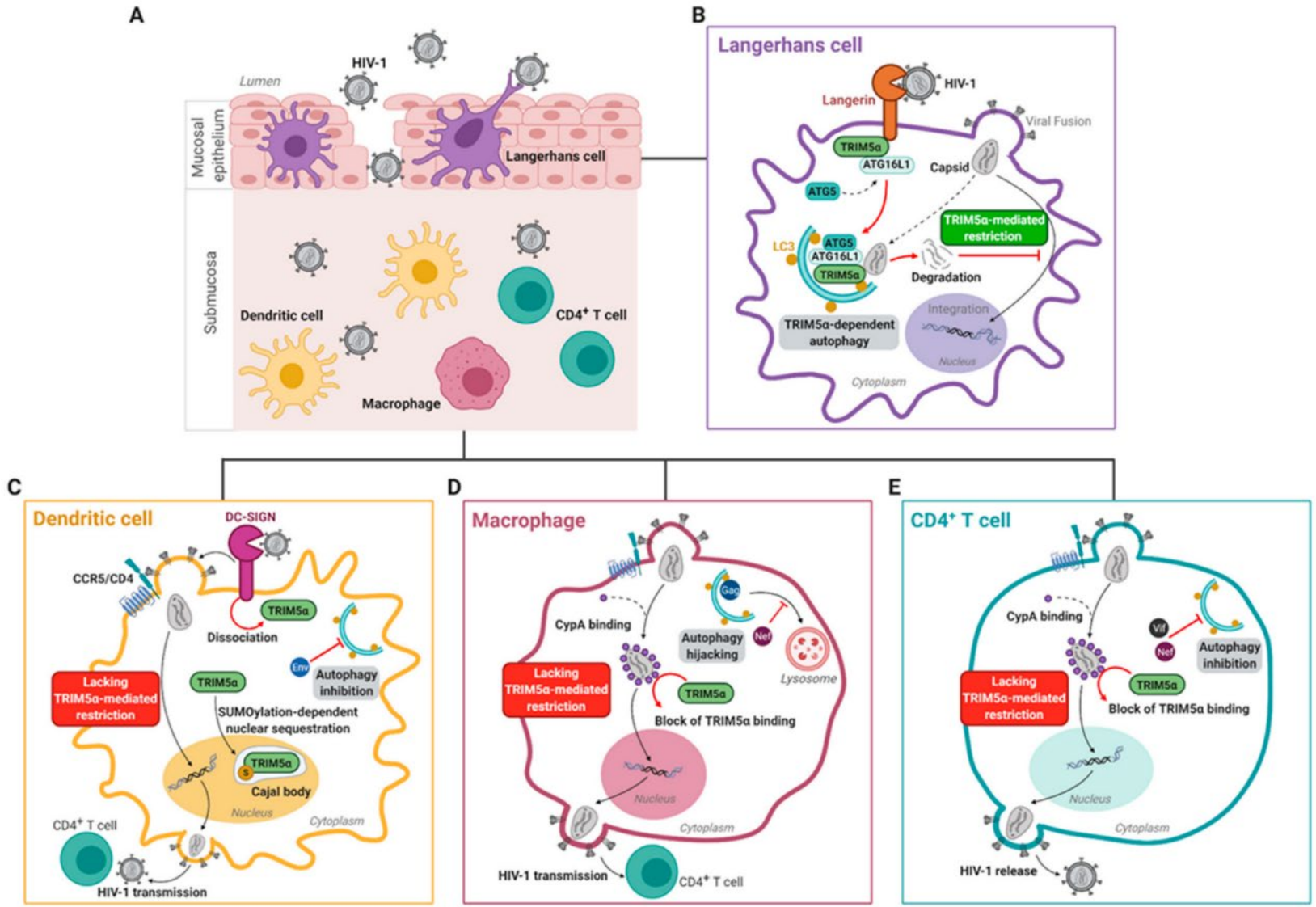




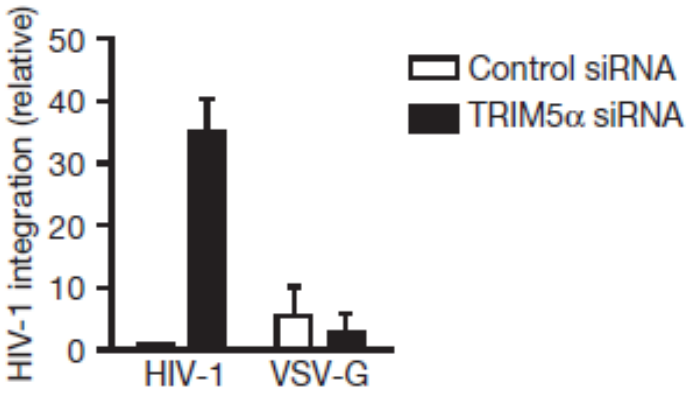


Cloherty et al, Viruses, 2021; Ribeiro et al, Nature, 2016

HIV, autophagic degradation in Langerhans cells



- the role of autophagy is dependent of TRIM5α a restriction factor
- TRIM5α is a cell-specific restriction factor
- No restriction in DC, macrophages and CD4+ T cells



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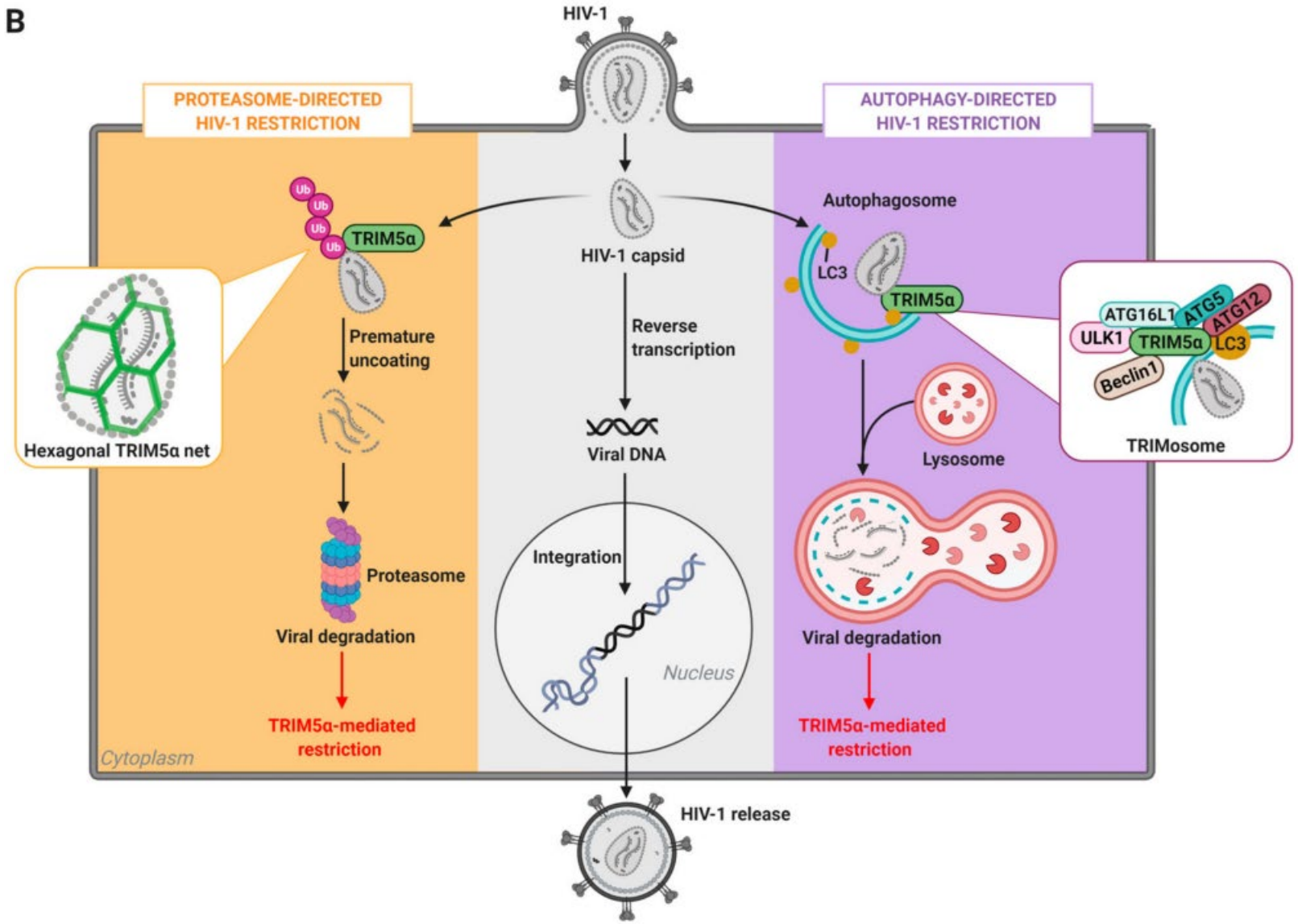
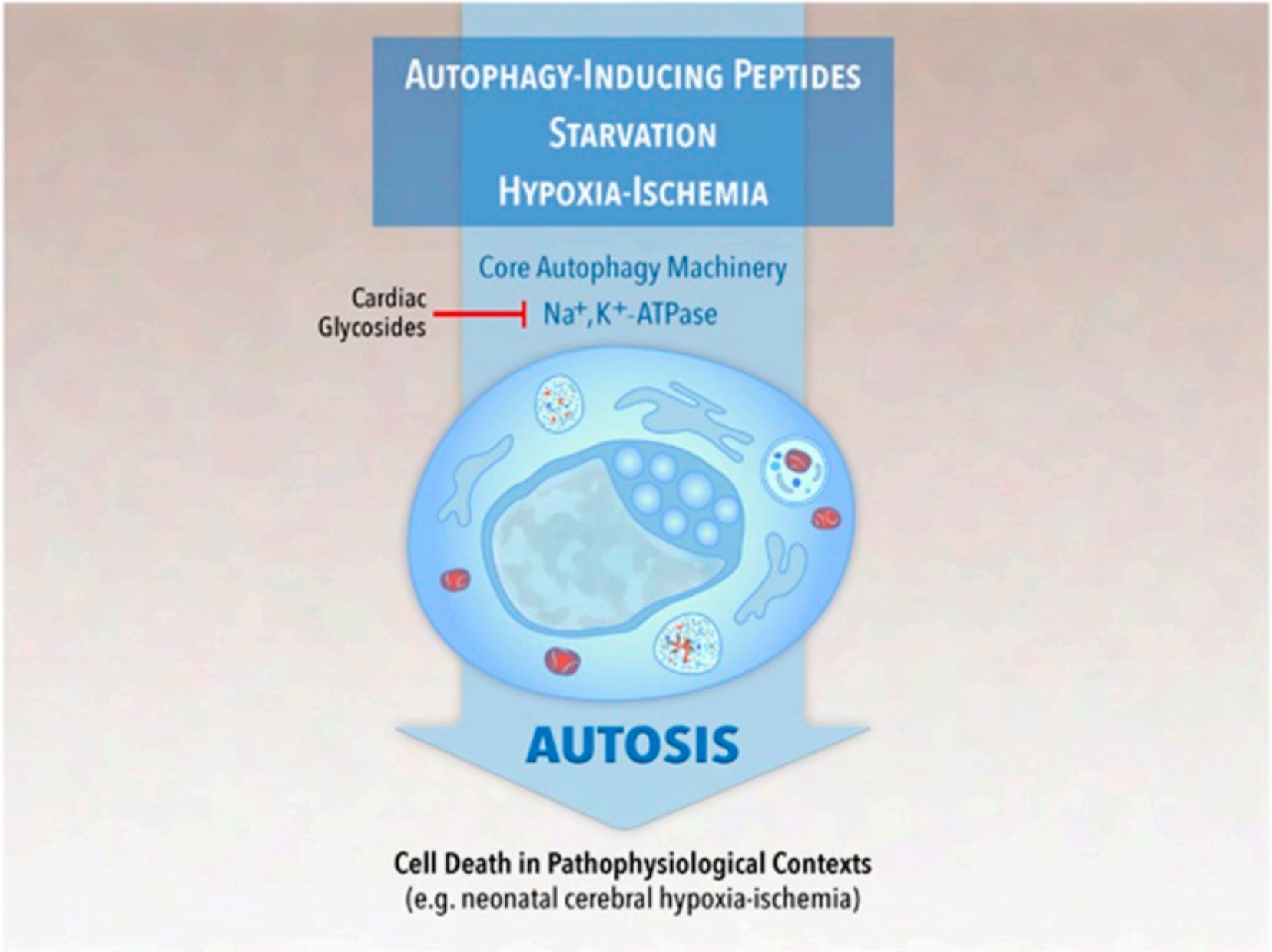


- TRIM5α is a restriction factor
- Two mechanisms
  - Proteasome-directed restriction
  - Autophagy-directed restriction

Cloherty et al, Viruses, 2021; Ribeiro et al, Nature, 2016

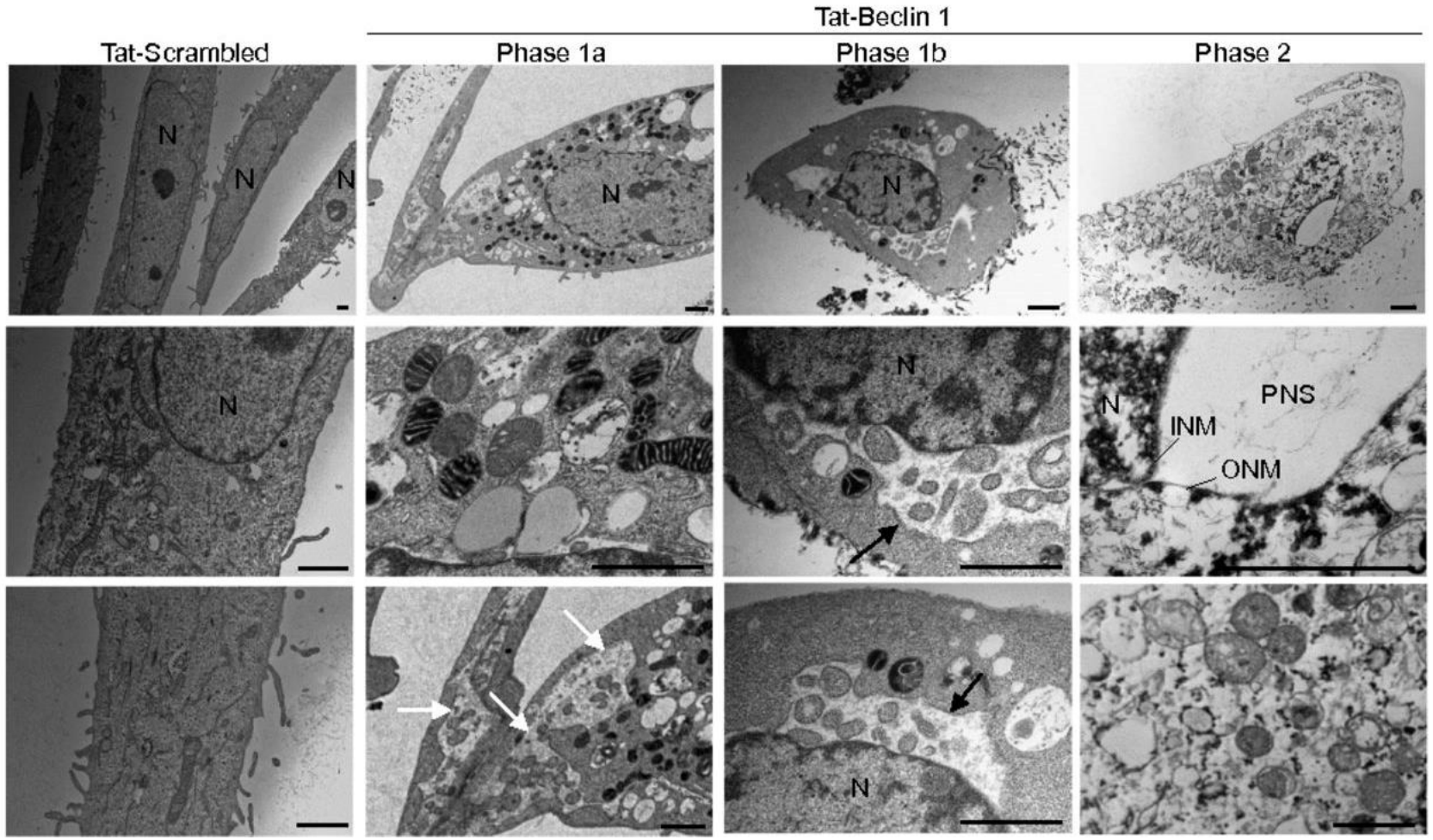
Autophagy and cell death

Autosis, a Form of Non-Apoptotic Cell Death



Yang Liu et al. PNAS 2013;110:20364-20371

Morphological features of Tat-Beclin 1-induced autosis



Tat is a cell-penetrating peptide derived from HIV

Nuclear membrane convolution and shrinkage; focal concavity of the nuclear surface; focal ballooning of perinuclear space

Mild-moderate chromatin condensation

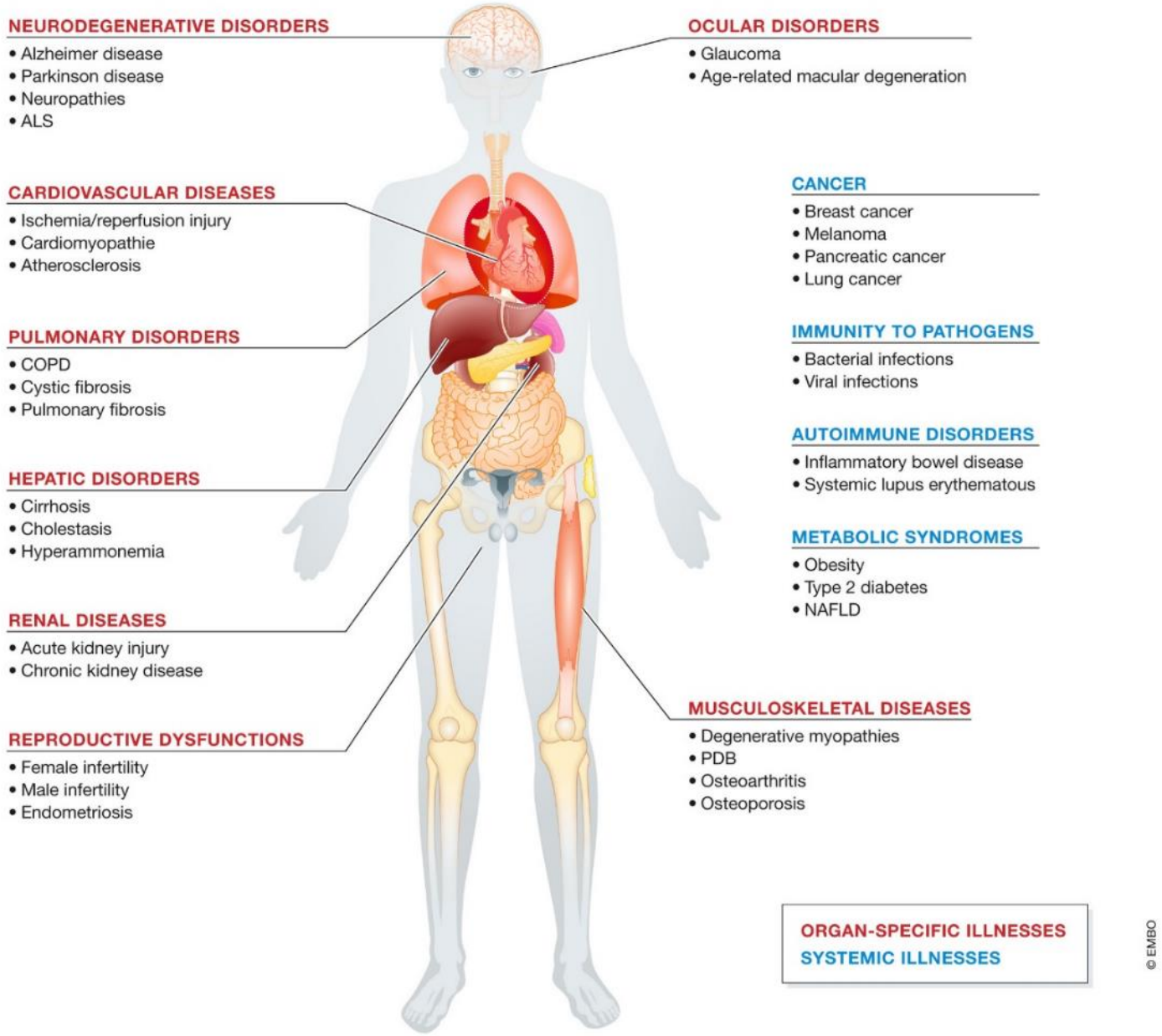
Numerous autophagosomes and autolysosomes (early-mid stages) with disappearance in final stages

Electron-dense mitochondria with abnormal internal structure (early); swollen mitochondria (late); dilated and fragmented ER (early) and ER disappearance (late)

Yang Liu et al. PNAS 2013;110:20364-20371

Autophagy and pathologies

Physiological roles and associated pathologies



Klionsky D et al, EMBO J (2021)

Plan

- Autophagy
  - Discovery and Description
  - Machinery and regulation
  - Biological Functions
  - Physiopathology
    - Neurodegenerative diseases
    - Cancers
    - Infections
  - Escape and hijacking of autophagy by viruses

Neurodegenerative diseases

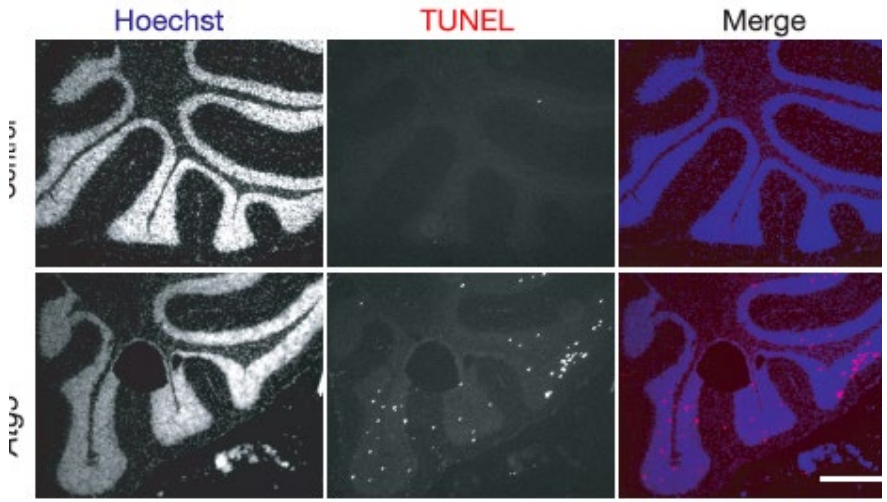
Accumulation of ubiquitinylated proteins are observed in autophagy-deficient cells

Control

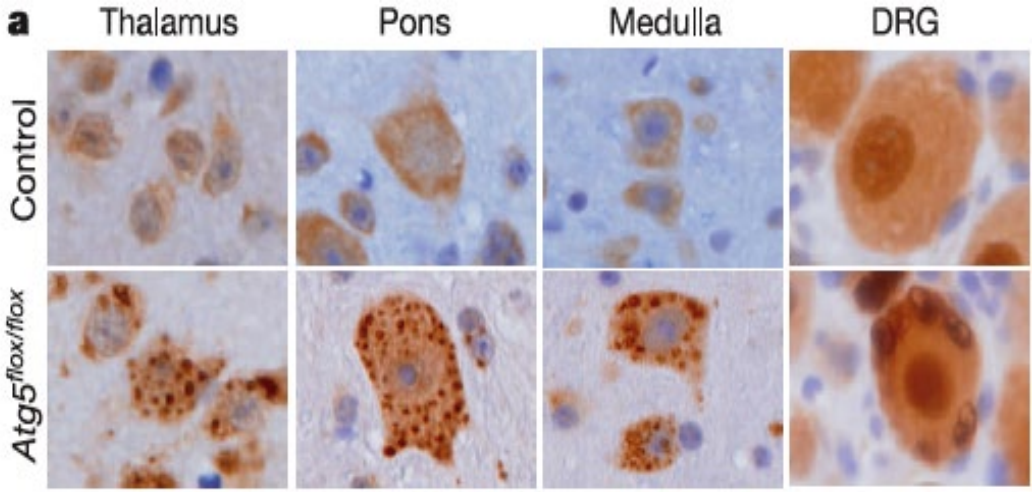
Atg5-conditional

abnormal limb-clasping reflexes and reduction of coordinated movement

Mice deficient for Atg5 specifically in neural cells



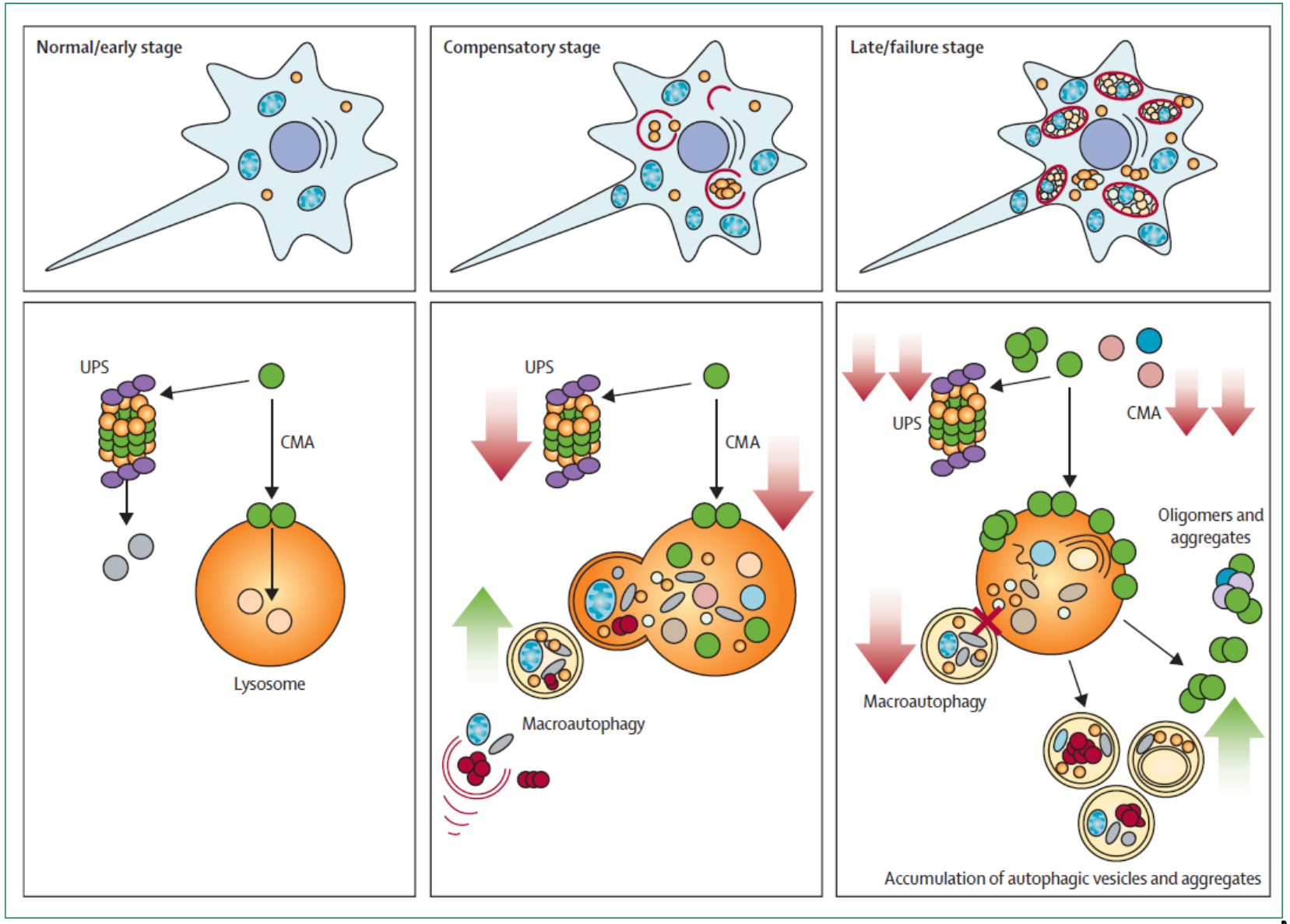
Progressive deficits in motor function



Accumulation of ubiquitin aggregates in the brain

Hara et al 2006 Nature

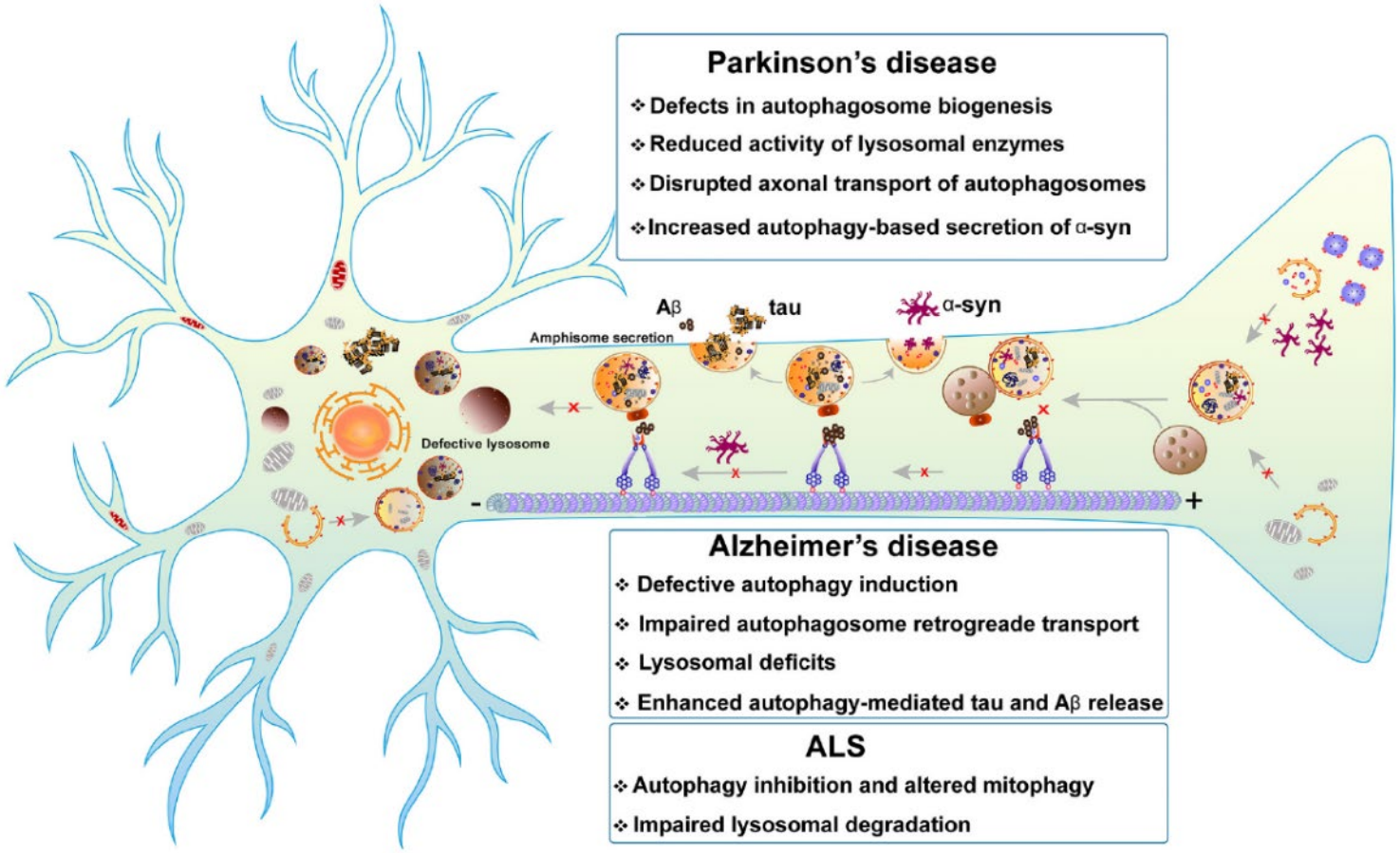
Role of autophagy in the progression of neurodegenerative diseases



CMA Chaperone-mediated autophagy

Martinez Vicente and Cuervo, Lancet Neurol 2007

Autophagy defects in neurodegenerative diseases



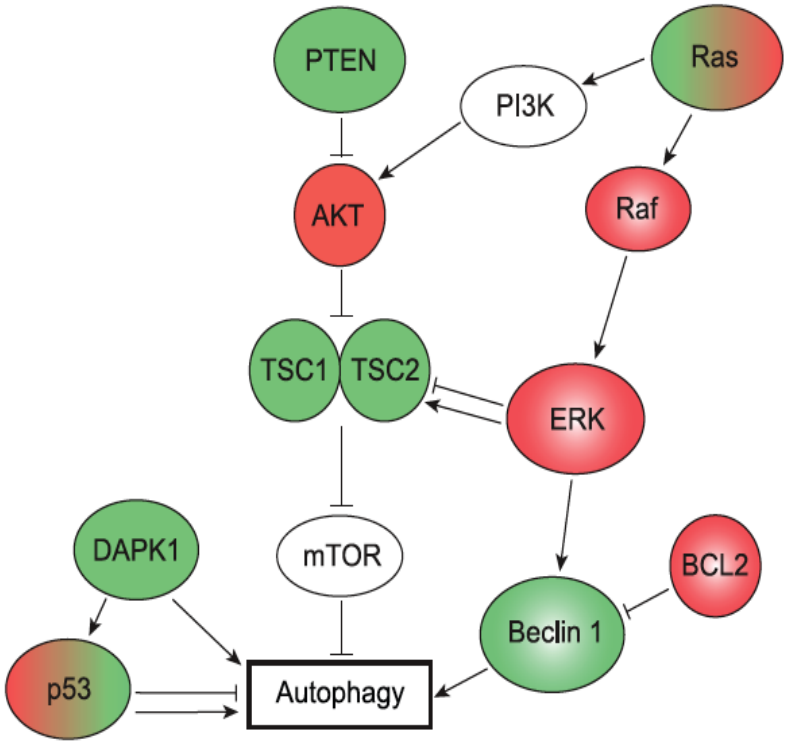
Amyotrophic lateral sclerosis

- Massive accumulation of autophagosomes = prominent feature in the patient brains
- In PD, autophagy-based unconventional secretion of α–synuclein
- In AD, unconventional secretion of Aβ
- amphisomes fuse with the plasma membrane and trigger the extracellular release of Aβ

Cai and Ganesan, 2022

Cancer

Oncoproteins and tumor suppressors in autophagy



Tumor suppressor

Oncoprotein

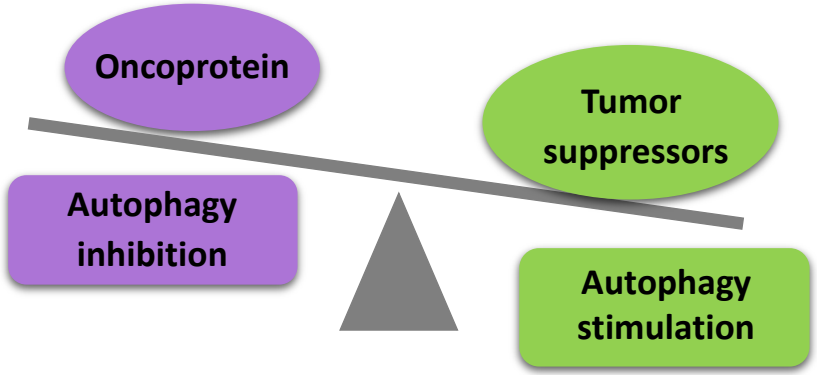


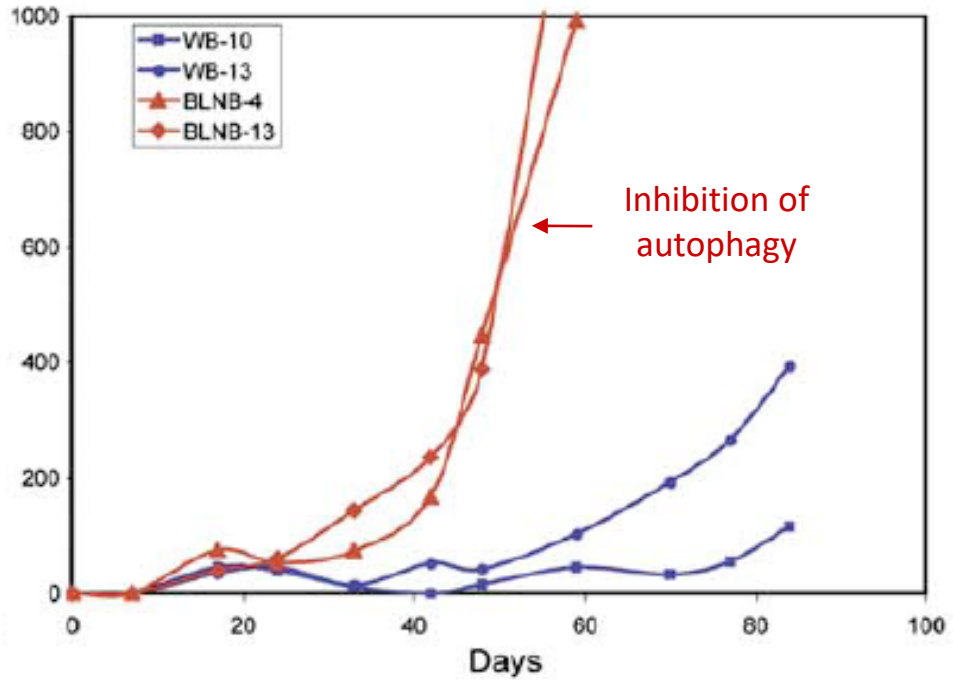
Table 1. Autophagy effector genes are frequently mutated in human cancer

Gene name(s)	Stage of autophagy	Type of human cancers	Type of mutation in cancer	References
BECN1	Initiation	Breast, ovarian and prostate cancer	Monoallelic deficiency	(Aita et al., 1999)
UVRAG	Initiation	Colorectal and gastric cancer	Monoallelic deficiency as a result of frameshift mutation	(Ionov et al., 2004; Goi et al., 2003; Kim et al., 2008)
SH3GLB1 (Bif-1)	Initiation	Gastric and prostate cancer	Decreased expression	(Takahashi et al., 2007)
ATG2B, *ATG5*, *ATG9B* and *ATG12*	Elongation	Gastric and colorectal cancer	Frameshift mutation	(Kang et al., 2009)
RAB7A	Fusion	Leukaemia	Gene rearrangement and deletion	(Kashuba et al., 1997)

Anti-tumor role of autophagy : protection against necrosis and tumor inflammation

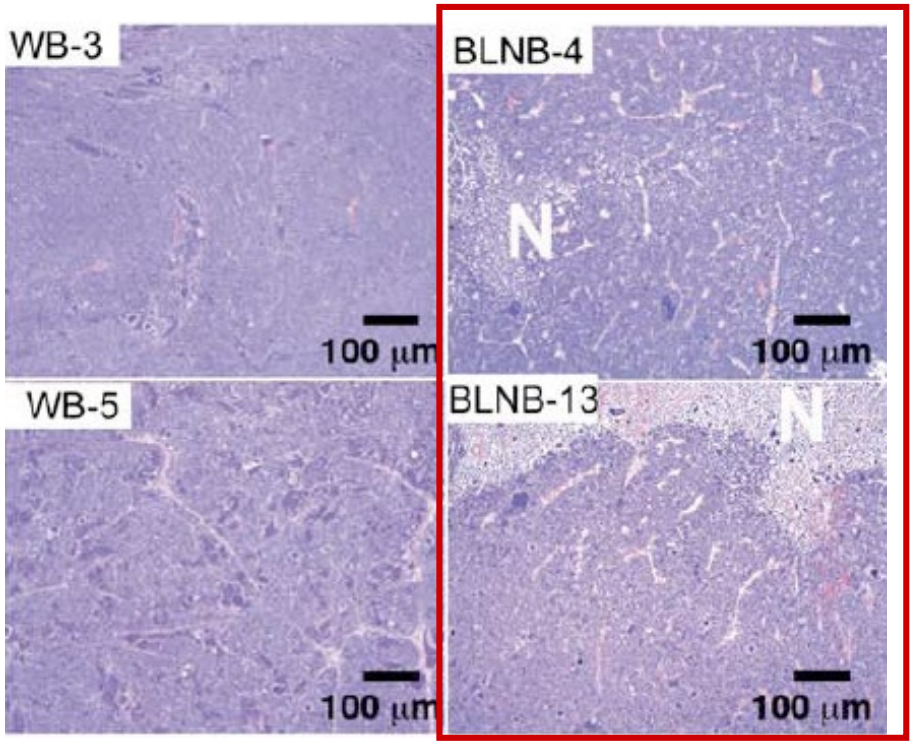
Autophagy deficiency promotes tumor growth and necrosis in apoptosis defective tumors

tumor growth



Apoptotic-deficient cancer cells injected to mice in order to study tumor growth

Necrotic areas



Inhibition of autophagy

Numerous necrotic regions

Degenhardt et al., 2006

Anti-tumor role of autophagy : protection against necrosis and tumor inflammation

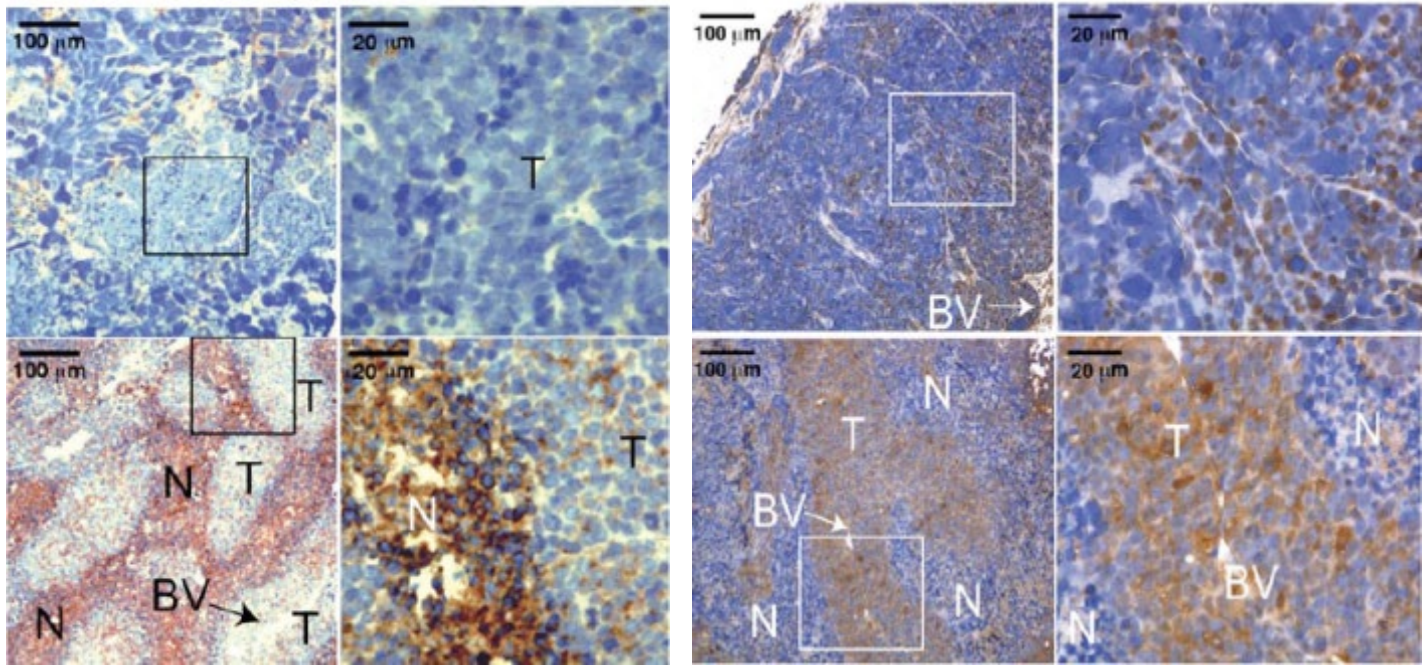
Inflammation markers

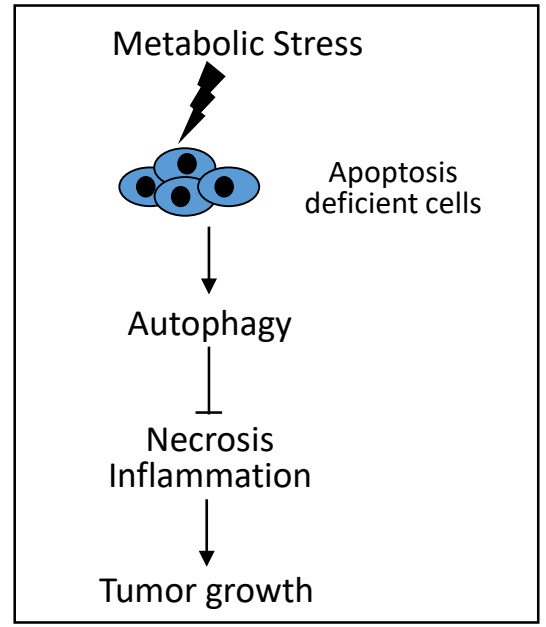
Macrophages

NF-κB

Non necrotic tumors

necrotic tumors

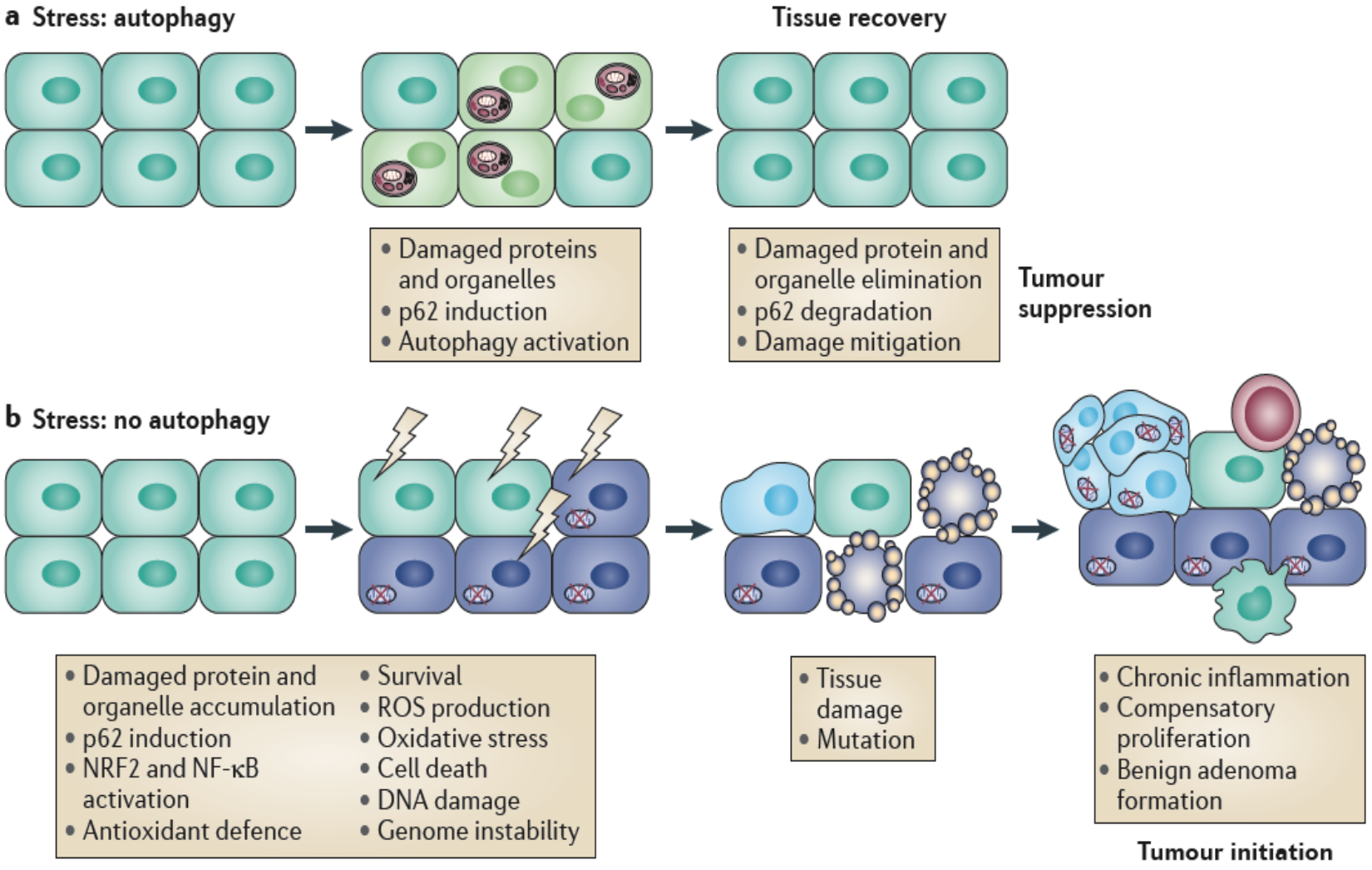




Tumor necrosis is associated with macrophage infiltration and cytokine and chemokine production

Degenhardt et al., 2006

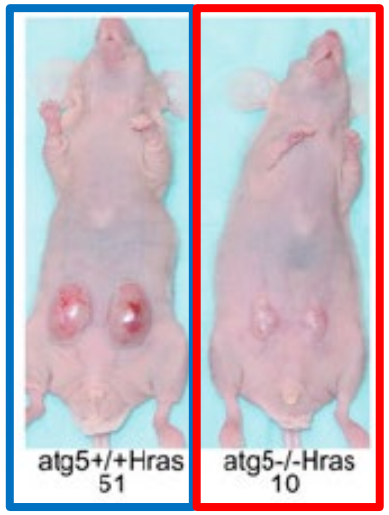
Mechanism of autophagy-mediated tumor suppression

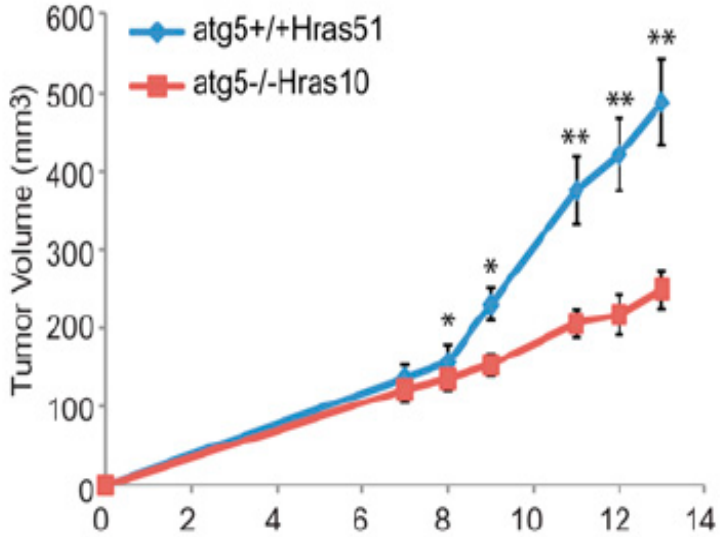


Pro-tumor role of autophagy

- **Deficient mice for *ATG5* or *ATG7***
  - ↳ development of benign tumors only

- **Tumors and cancer lines show a high rate of autophagy**
  - ↳ induced by oncogenes (ex : *RAS*)
  - ↳ autophagy-dependent
  - ↳ inhibition of autophagy : limits tumor development or leads to tumor regression
  - ↳ example : support of tumoral development induced by oncogene RAS



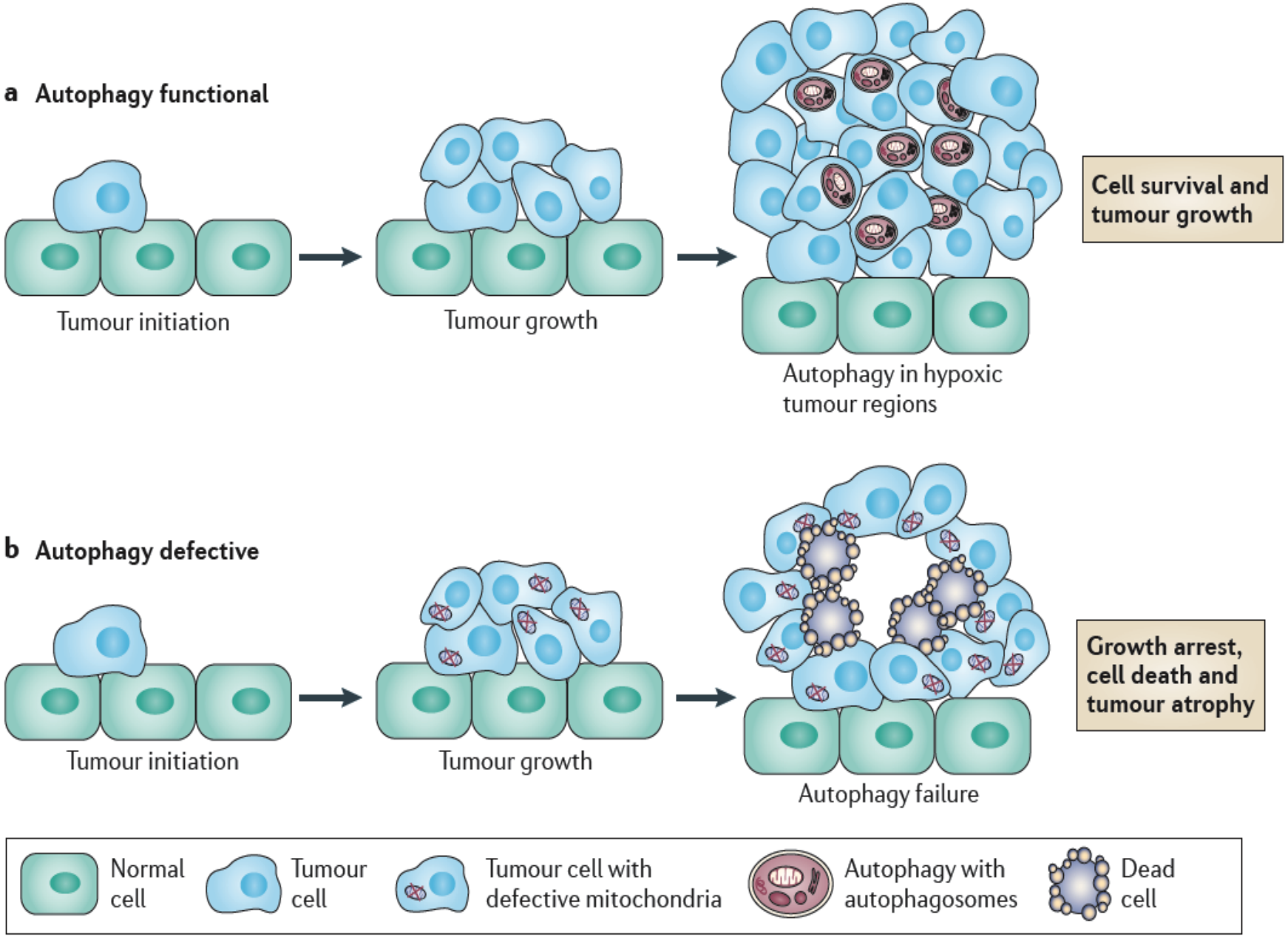


Guo et al., 2011, Genes Dev 25, 460

➡ **Autophagy = Mechanism required for tumor development**

How does autophagy play a pro-tumor role?

The role of autophagy in supporting the growth of agressive cancers



Infectious diseases

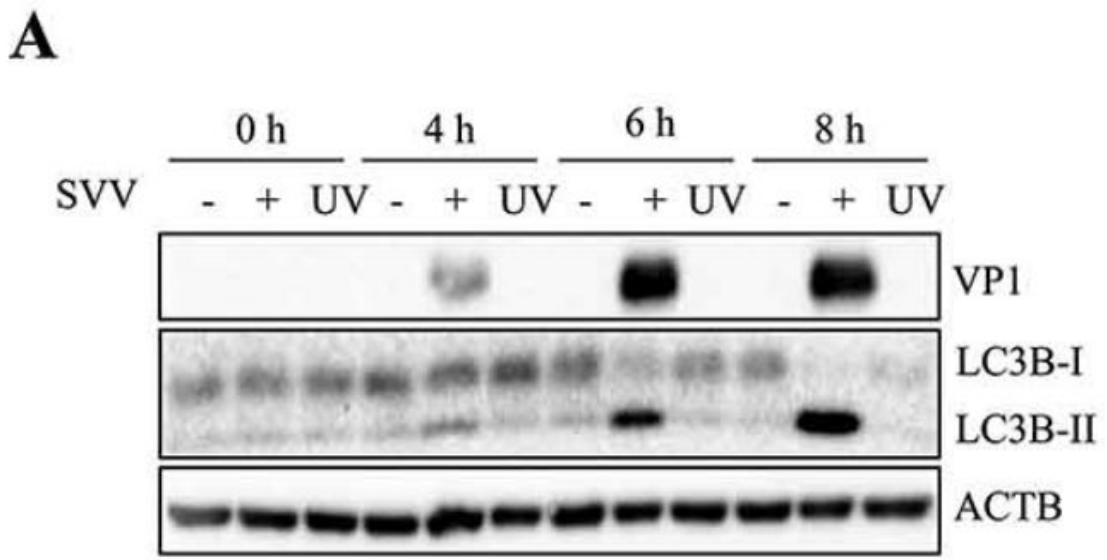
Selective autophagy and viruses

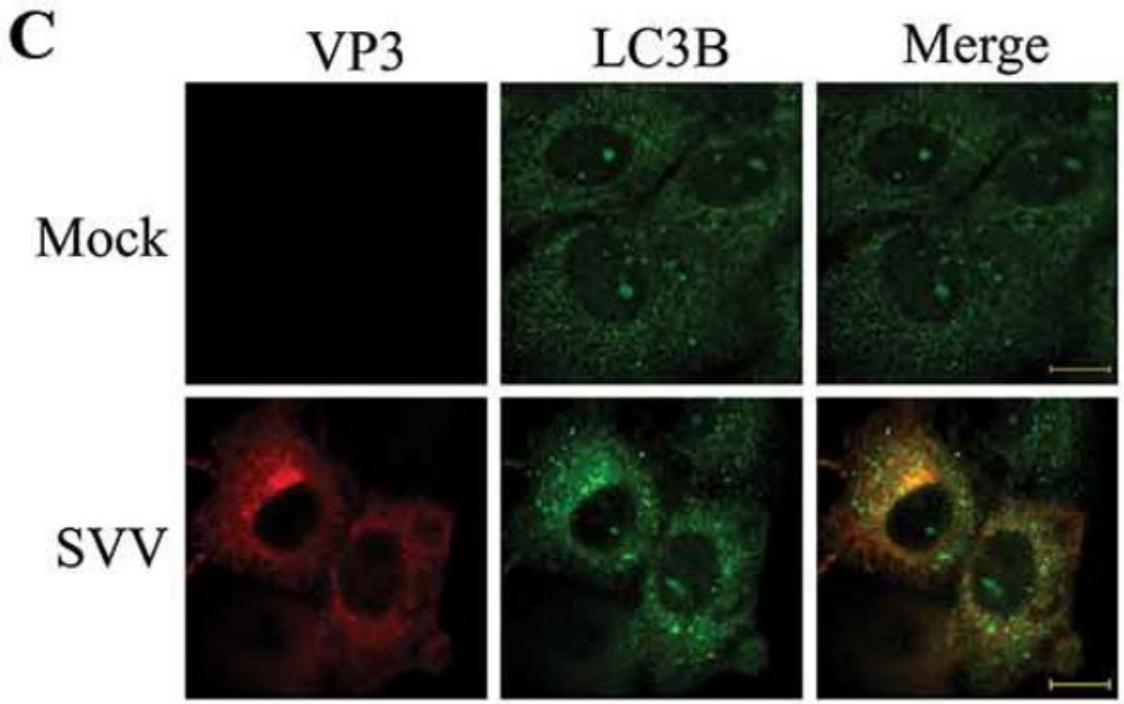
Virophagy = specific degradation of viral proteins by autophagy

Virus	Targets	Receptors
SINV	Capsid protein	SQSTM1
CHIKV	Ubiquitinated capsid	SQSTM1
VIH-1	Tat	SQSTM1
VIH-1	capsid	TRIM5
HTLV-1	Tax	?
Avibirnavirus	capsid protein VP2	SQSTM1
Cauliflower mosaic virus	capsid protein and assembled capsid	NBR1

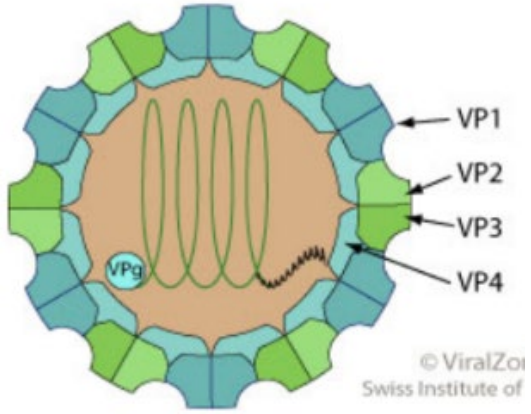
Selective autophagy and Seneca Valley virus

SVV = *Picornaviridae*



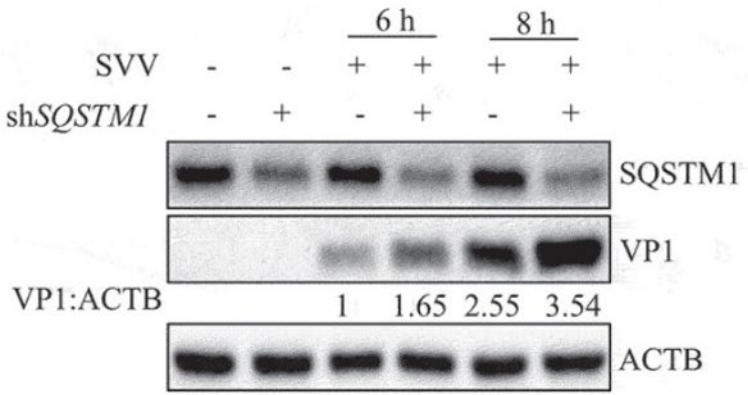


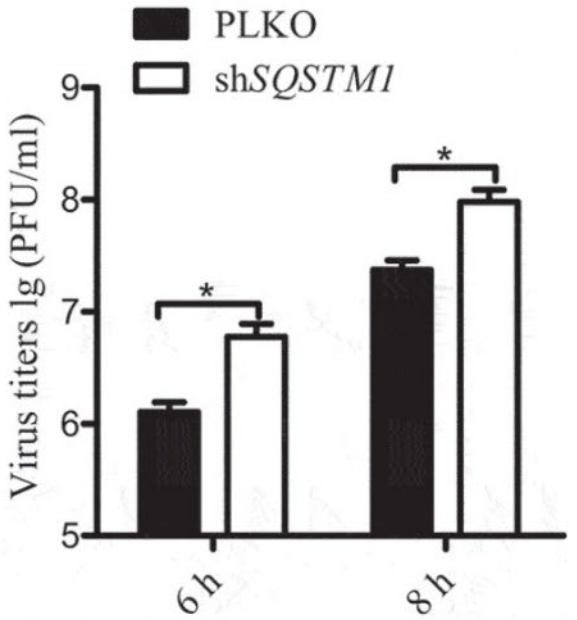
SVV induces autophagy



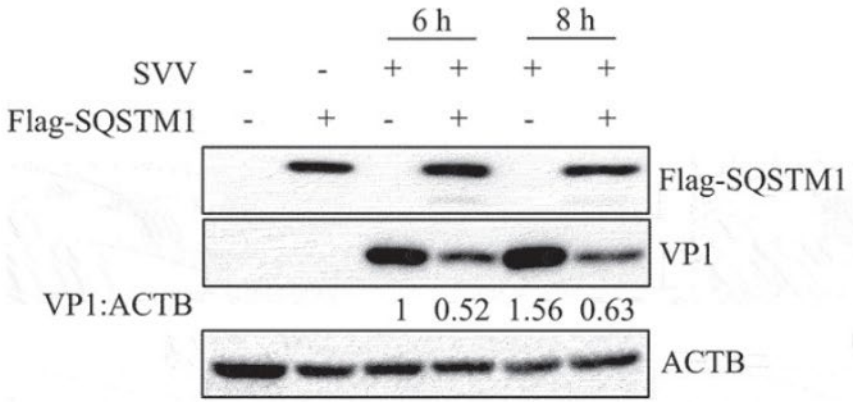
Wen et al, Autophagy 2021

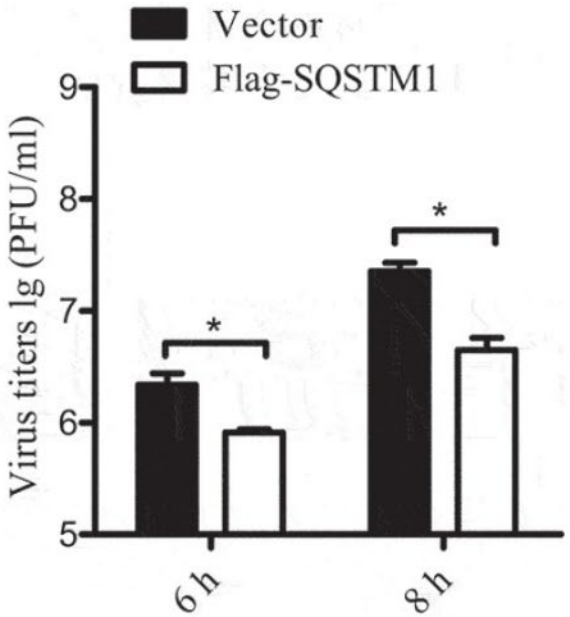
Selective autophagy and Seneca Valley virus





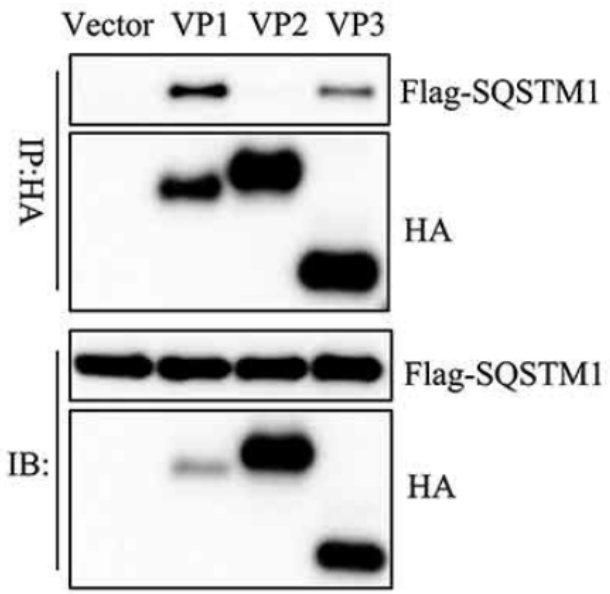
SQSTM1 blocks SVV multiplication

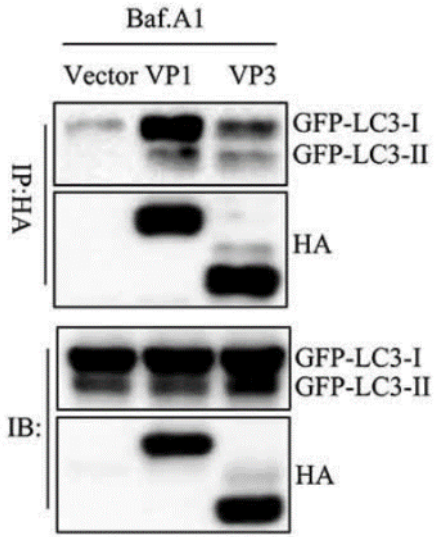


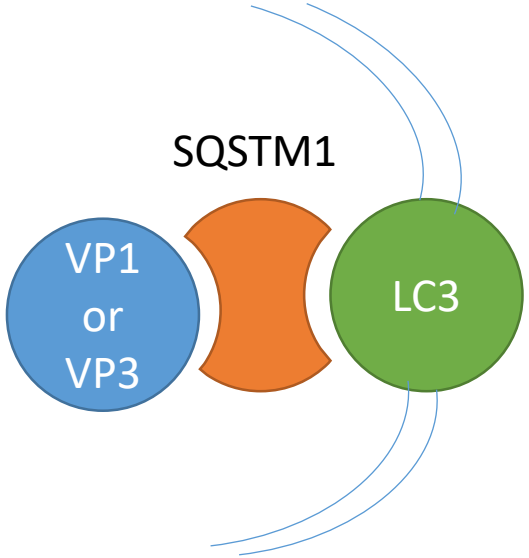


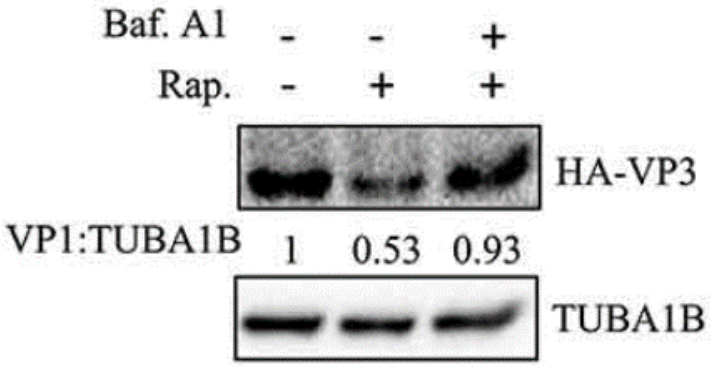
Wen et al, Autophagy 2021

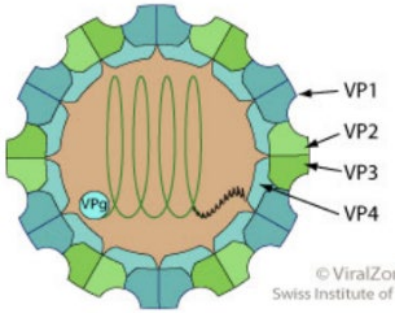
SQSTM1 targets SVV VP1 and VP3 for degradation









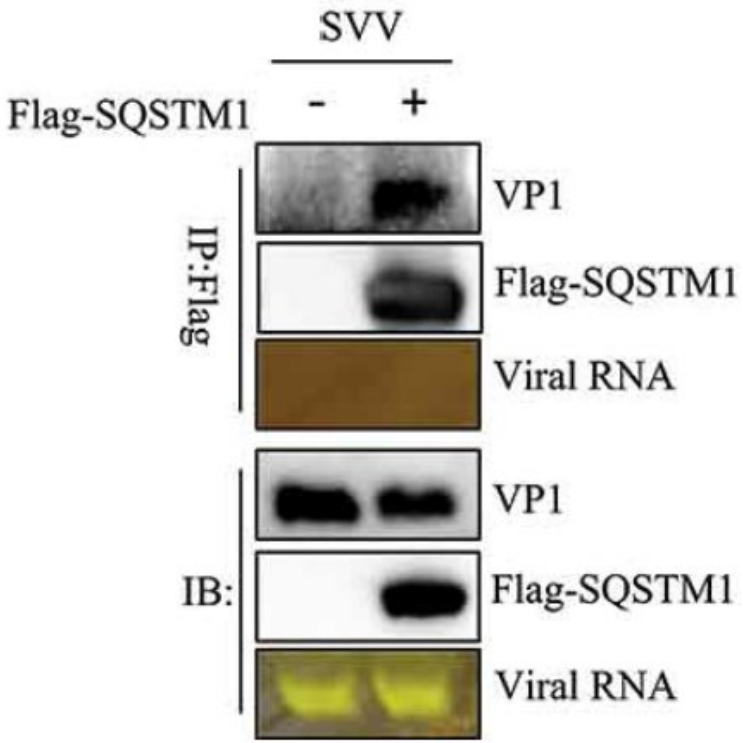


SQSTM1 interacts with VP1 and VP3 but not VP2

Rapamycin induces autophagy
and Bafilomycin A1 blocks it

Wen et al, Autophagy 2021

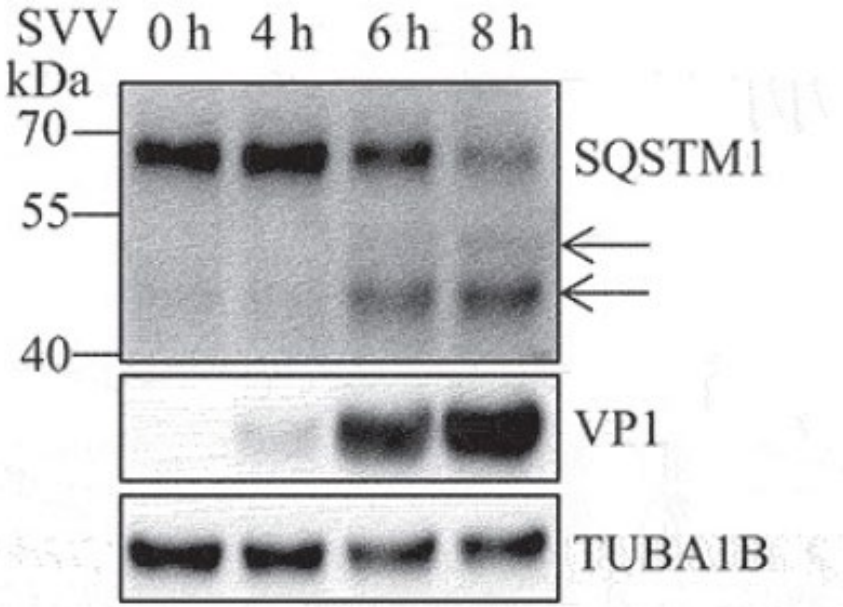
SQSTM1 did not target SVV particles

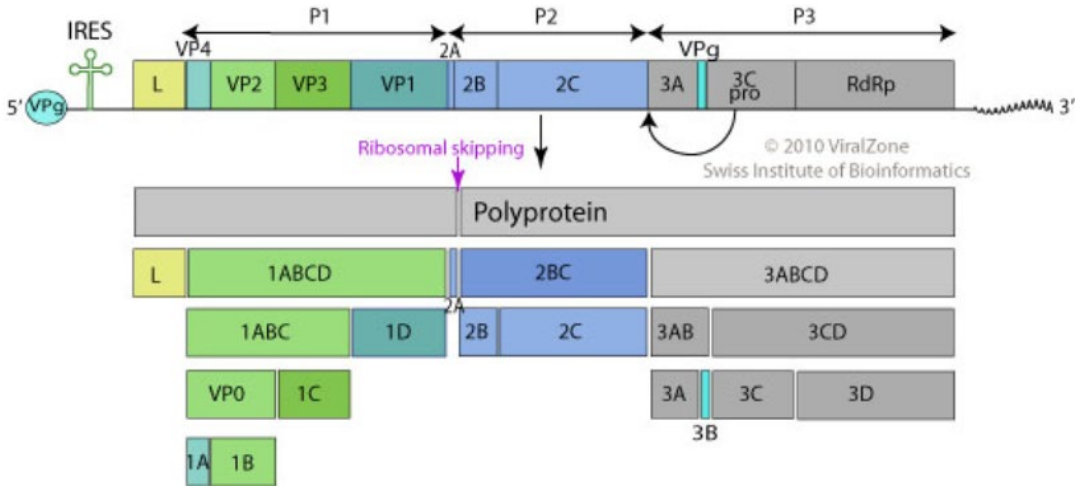


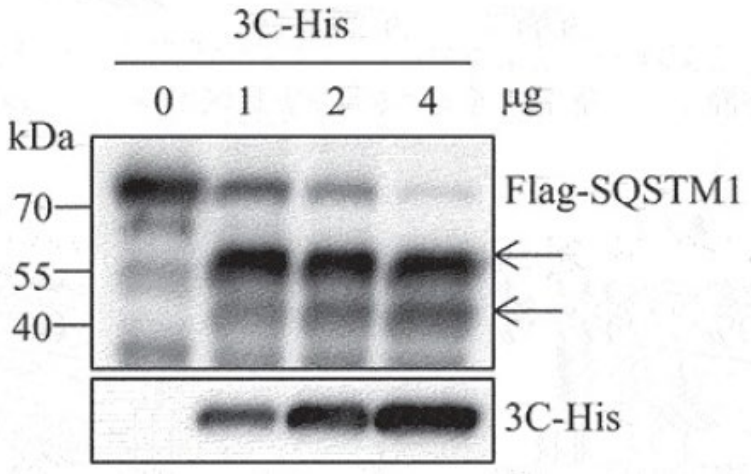
Viral RNA was detected by PCR using primers specific for SVV 3D

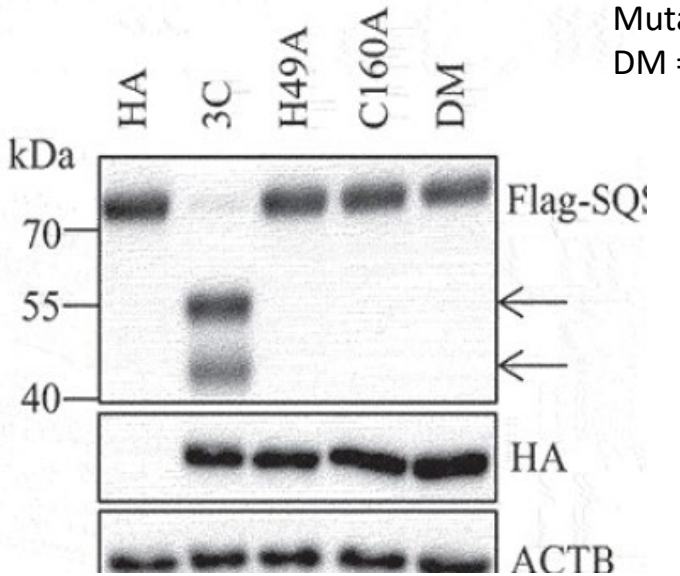
Wen et al, Autophagy 2021

SVV 3Cpro targets SQSTM1 for cleavage through its protease activity





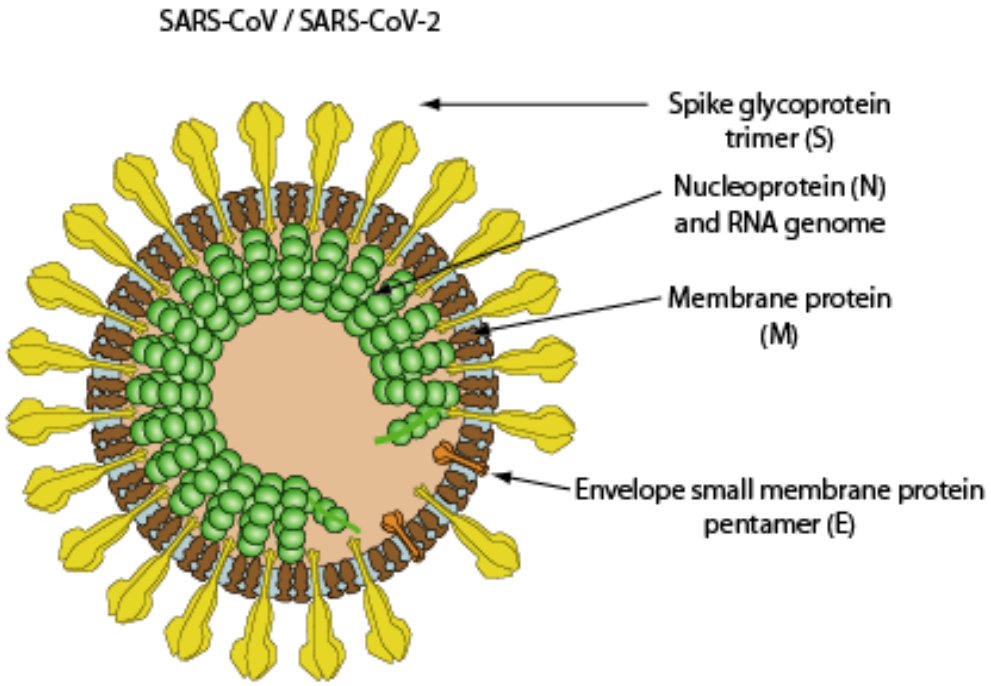




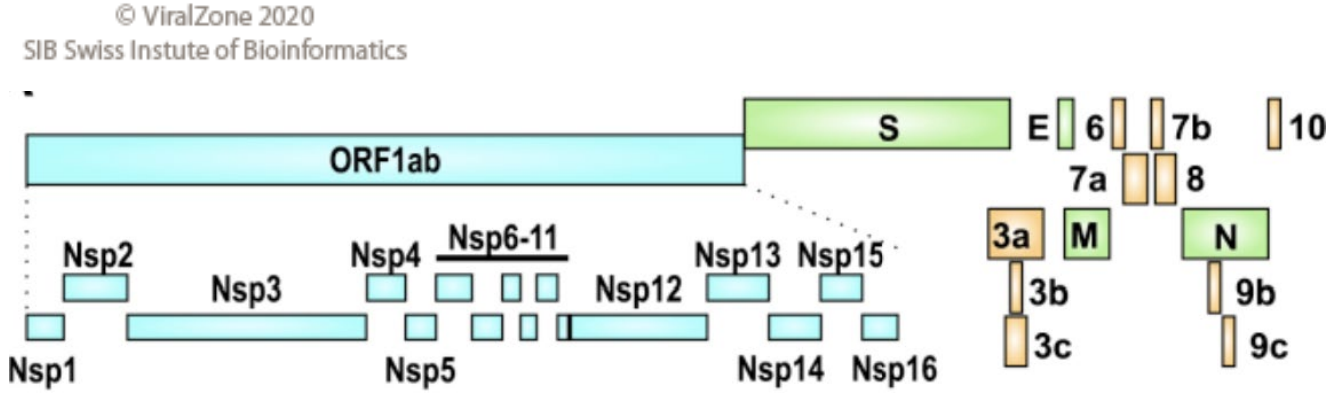
Mutations catalytically inactive
DM = double mutant

Wen et al, Autophagy 2021

Coronavirus SARS-CoV-2

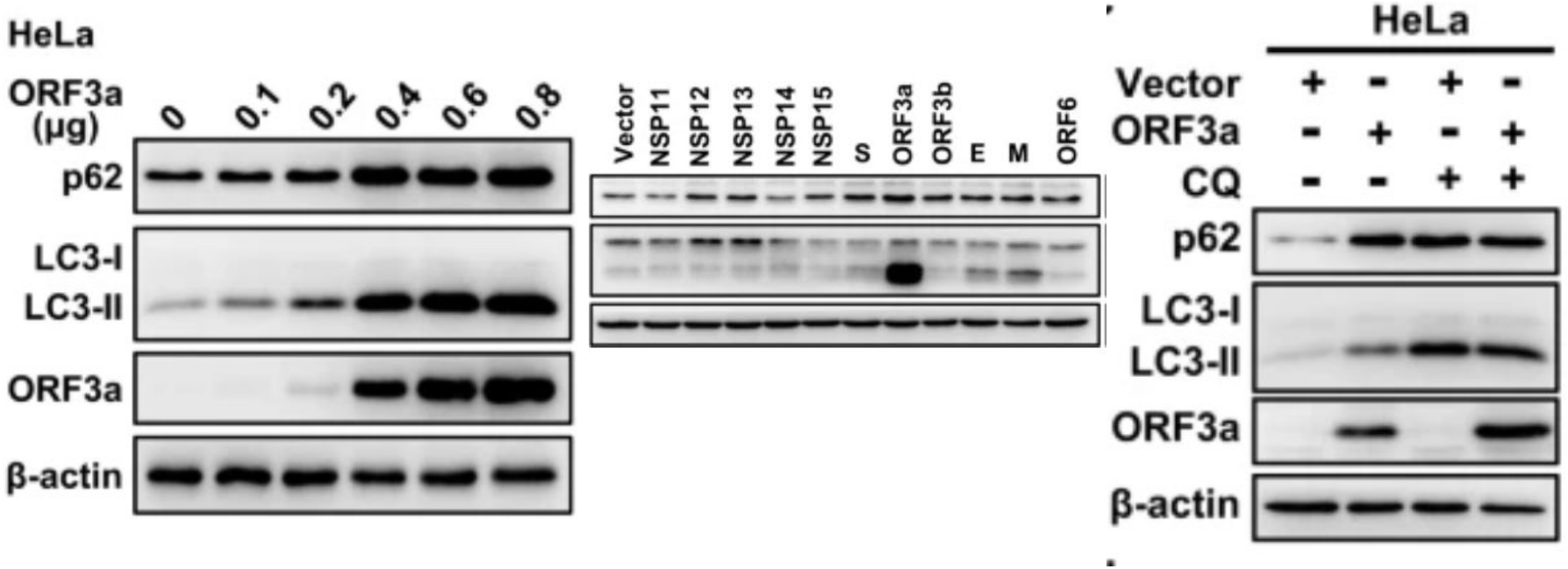


- Single-stranded positive-sense linear RNA
- ~30 kbp
- Helical capsid (N)
- Envelope
- Spike S (trimer)
- Pentamer E
- M link between capsid and envelope



SARS-CoV-2 modulates autophagy

- SARS-CoV-2 ORF3a blocked autophagy and caused the accumulation of autophagosomes



Zhang et al, Cell Discovery 2021, Miao, Dev Cell, 2021, Hayn et al, Cell Reports, 2021

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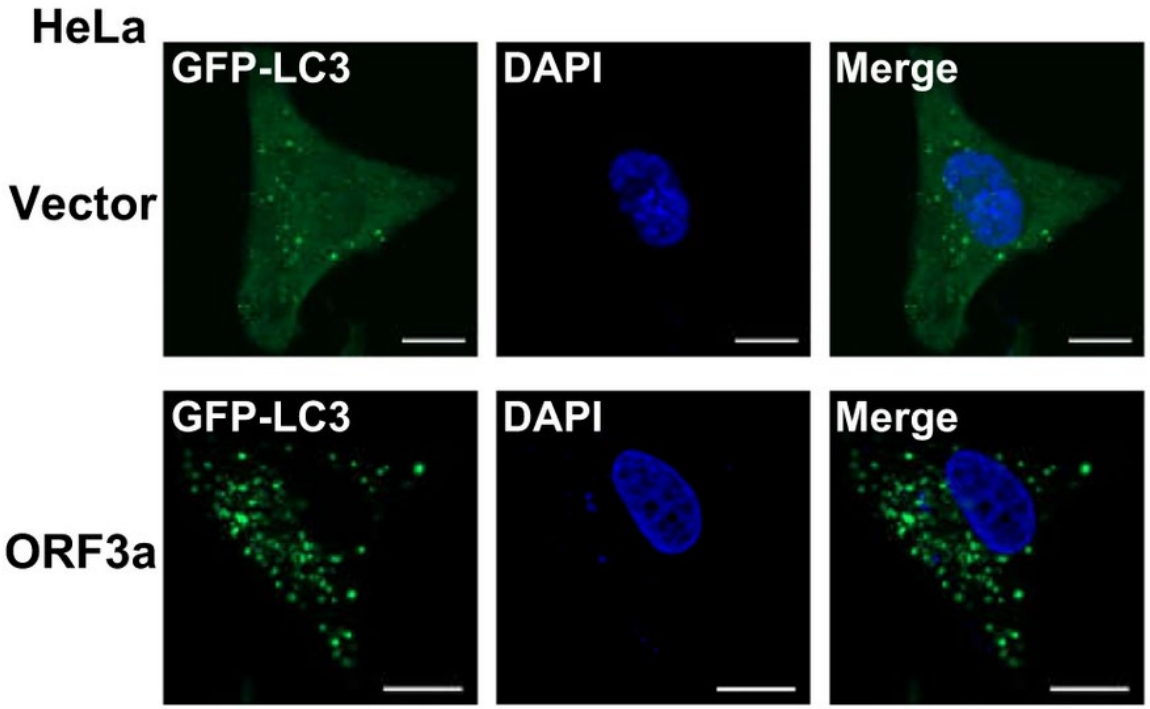
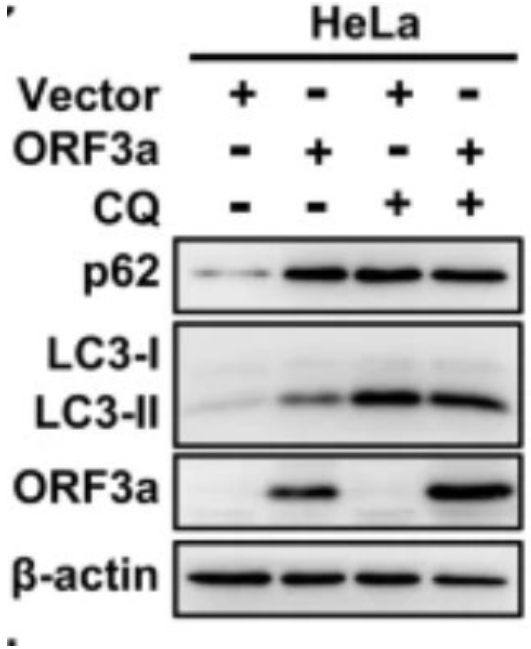
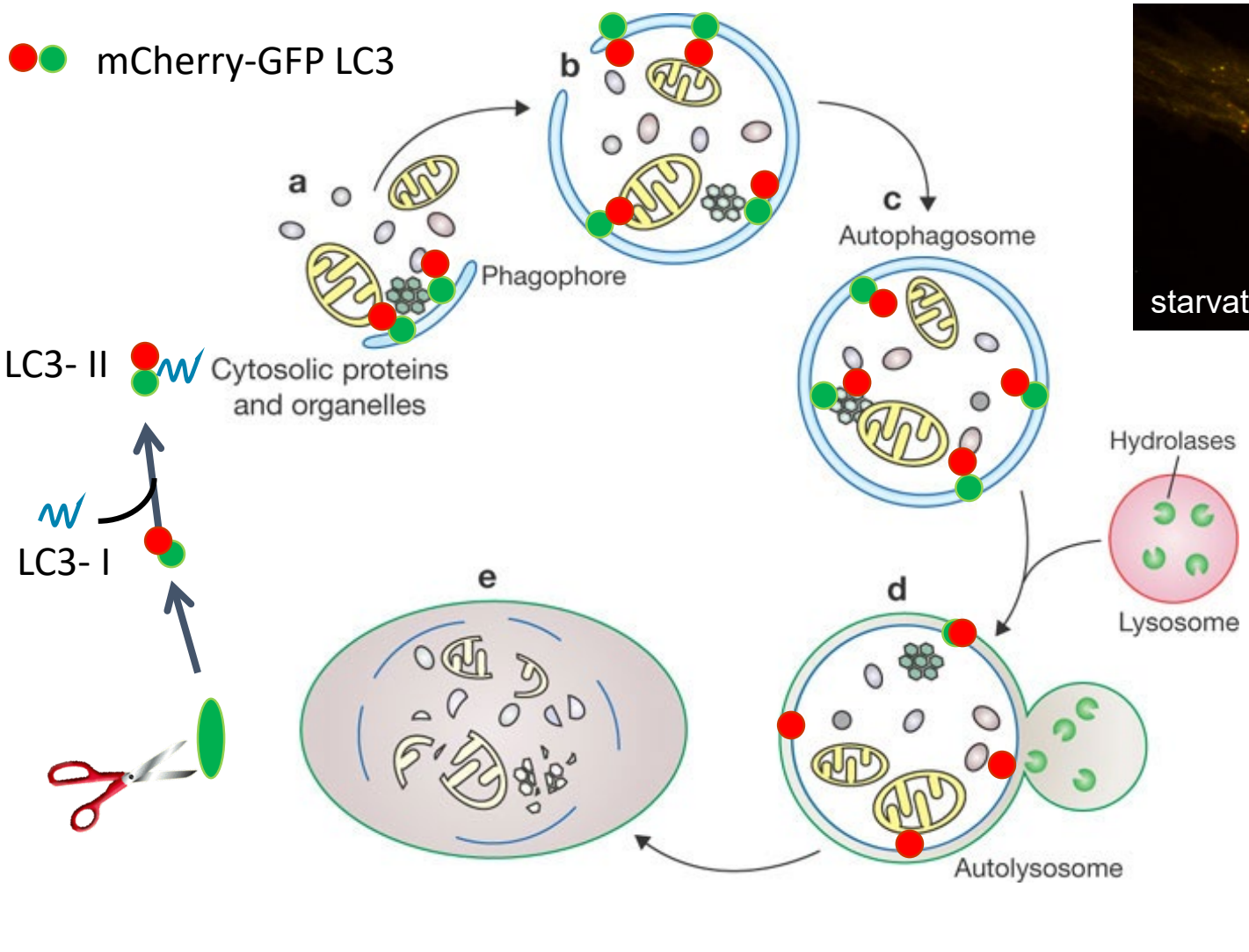
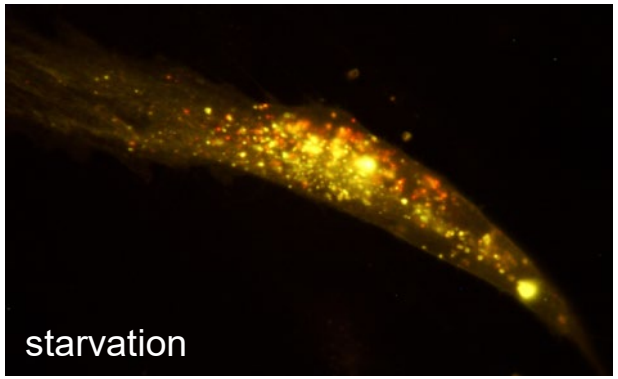


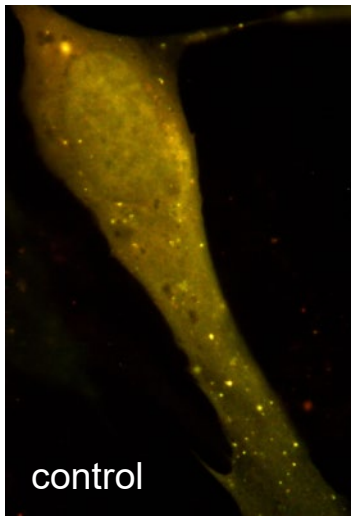


Zhang et al, Cell Discovery 2021, Miao, Dev Cell, 2021, Hayn et al, Cell Reports, 2021

Lysosomal turnover of LC3 : tandem tagged LC3



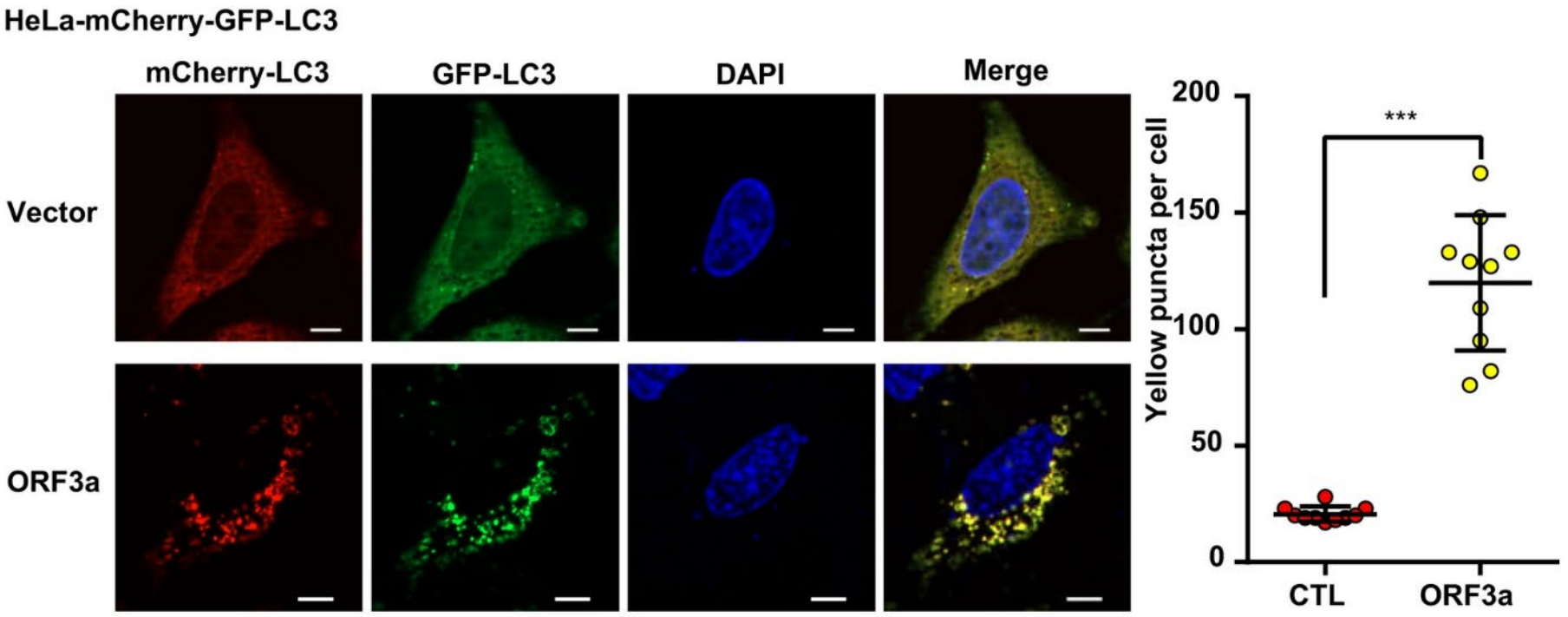




Modified from Xie and Klionsky, 2007

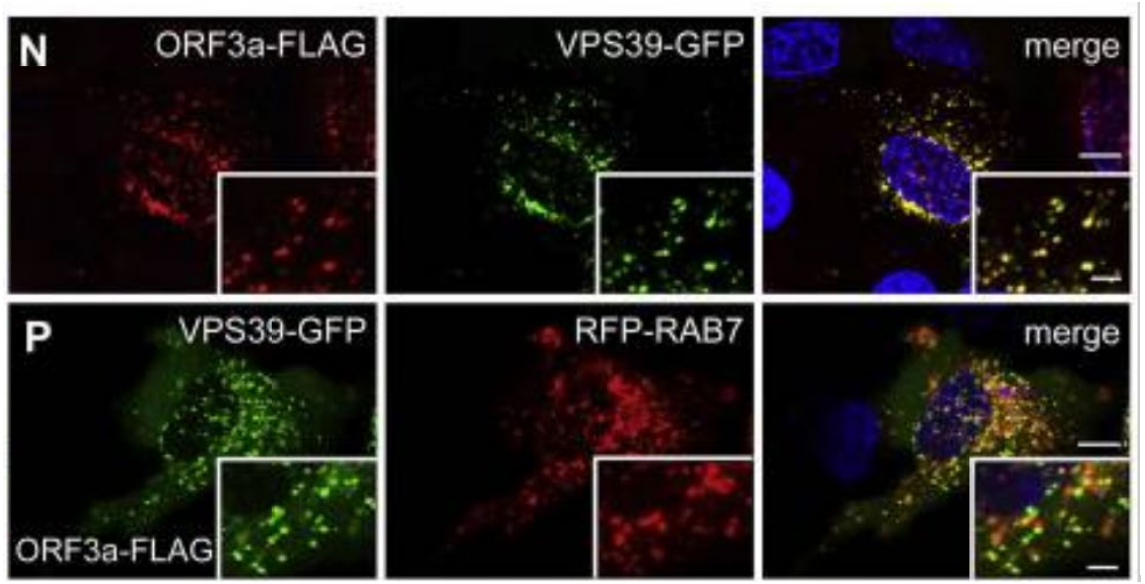
See Kimura et al, 2009

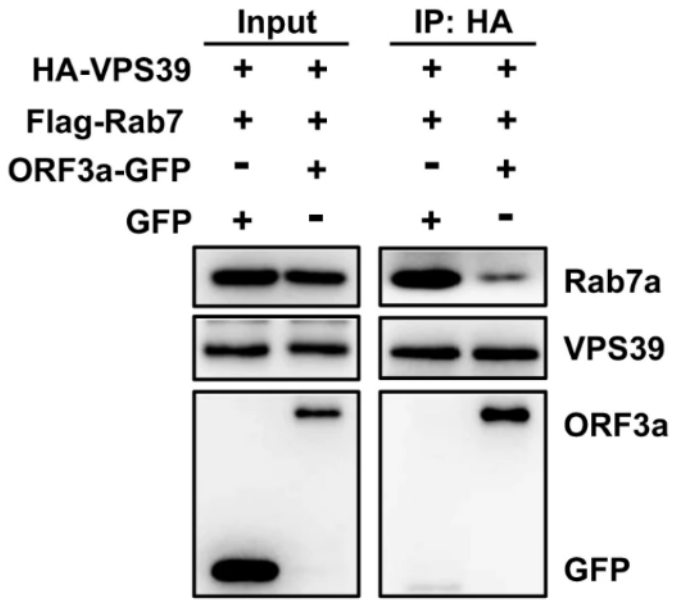
ORF3a blocks autophagosome–lysosome fusion

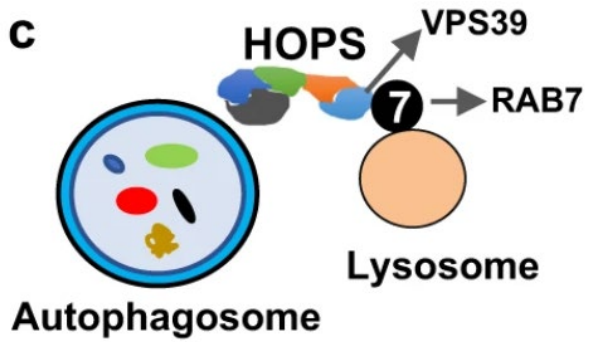


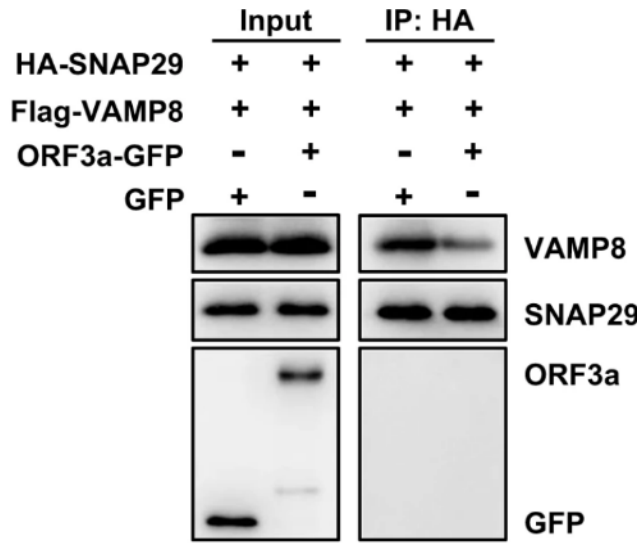
Zhang et al, Cell Discovery 2021, Miao, Dev Cell, 2021, Hayn et al, Cell Reports, 2021

ORF3a localizes to late endosomes/lysosomes and interacts with components of the HOPS complex

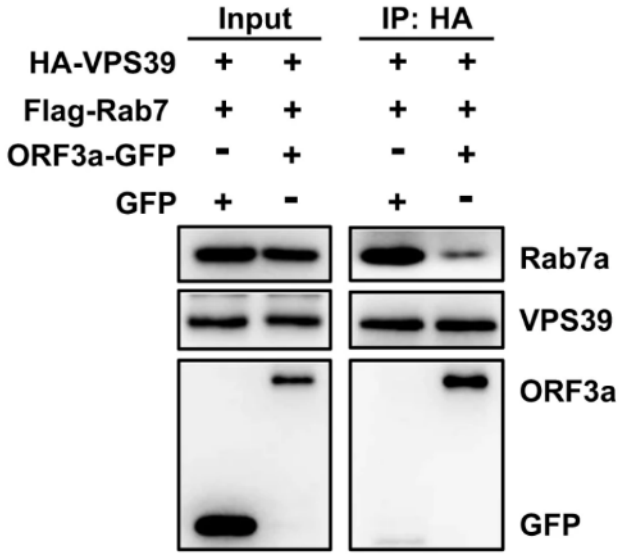


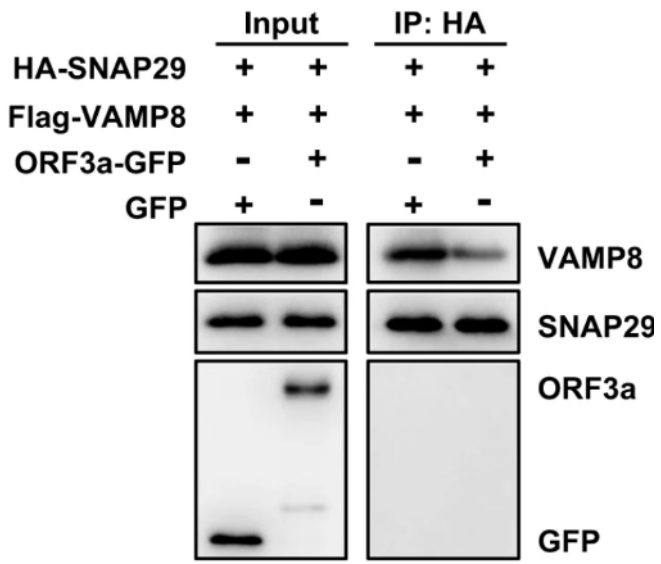


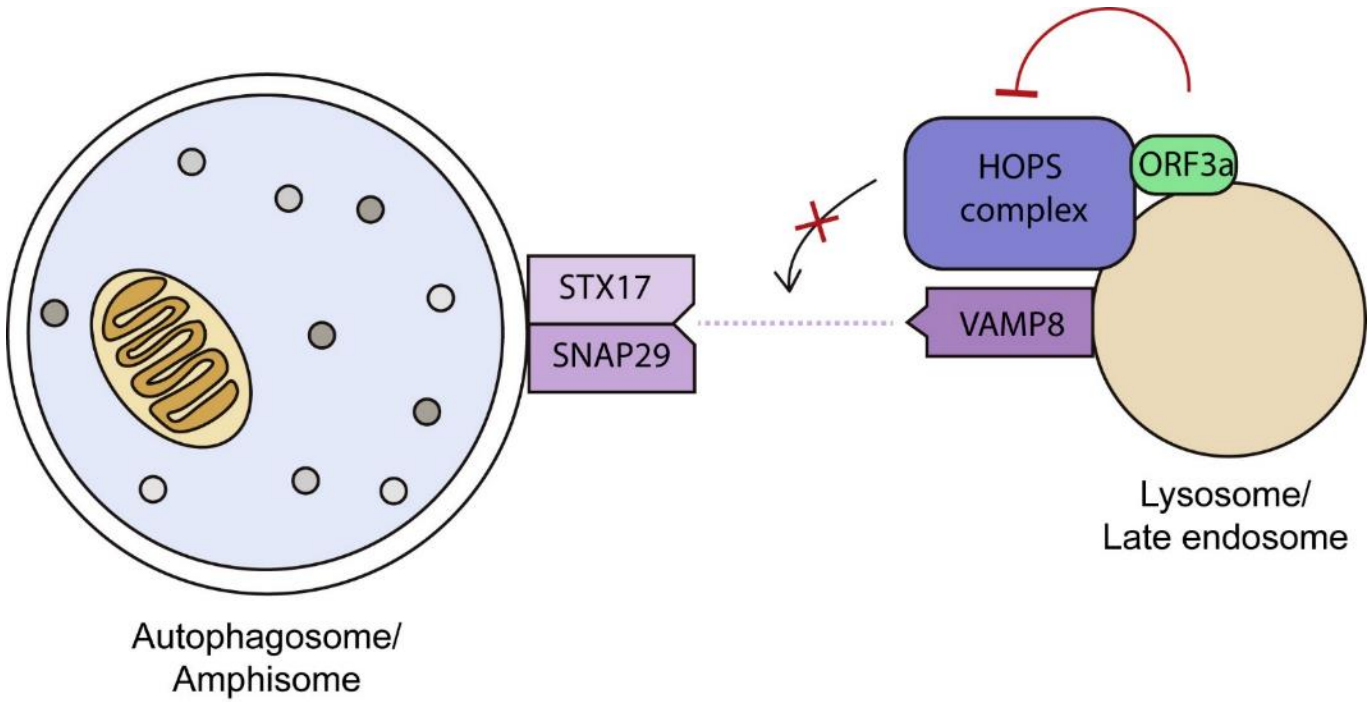




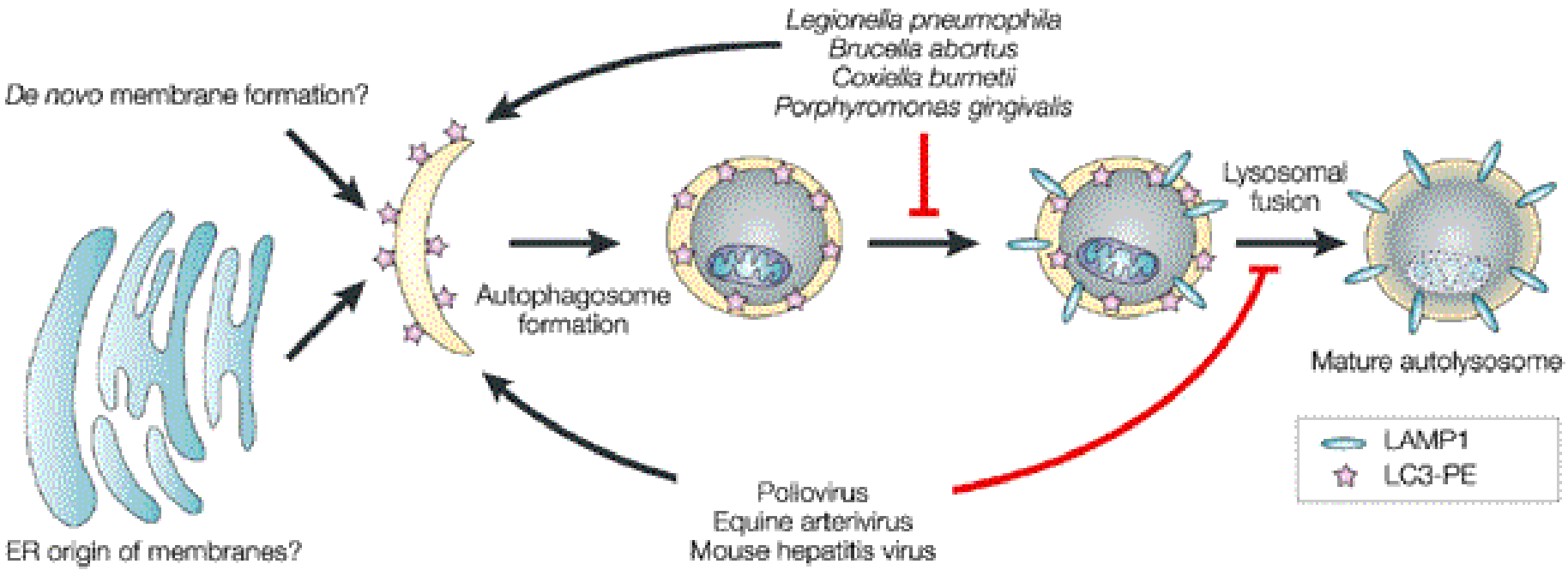
Zhang et al, Cell Discovery 2021, Miao, Dev Cell, 2021, Hayn et al, Cell Reports, 2021


Input
IP: HA
HA-VPS39 + + + +
Flag-Rab7 + + + +
ORF3a-GFP - + - +
GFP + - + -
Rab7a
VPS39
ORF3a
GFP


Input
IP: HA
HA-SNAP29 + + + +
Flag-VAMP8 + + + +
ORF3a-GFP - + - +
GFP + - + -
VAMP8
SNAP29
ORF3a
GFP


STX17
SNAP29
HOPS complex
ORF3a
VAMP8
Autophagosome/
Amphisome
Lysosome/
Late endosome

Subversion of the cellular autophagic pathway

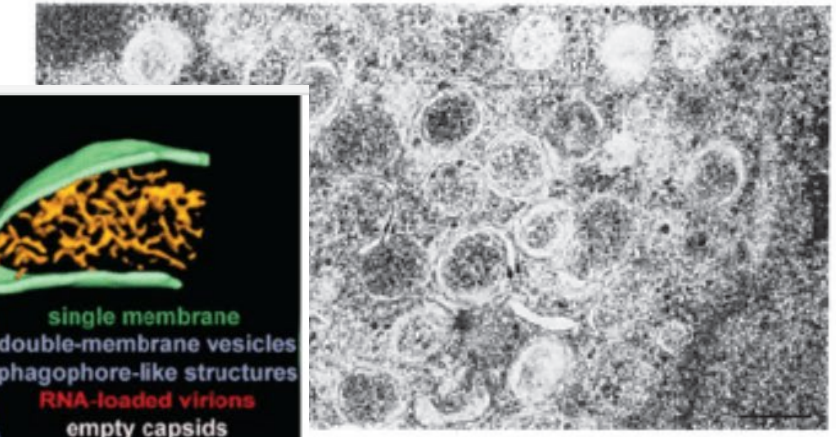


- **Pathogens are able to use autophagy to their own profit**
- **Some viruses block fusion with the lysosome and therefore degradation of autophagosomes and their contents**
- **Some viruses induce a functional autophagy (measles virus, dengue)**

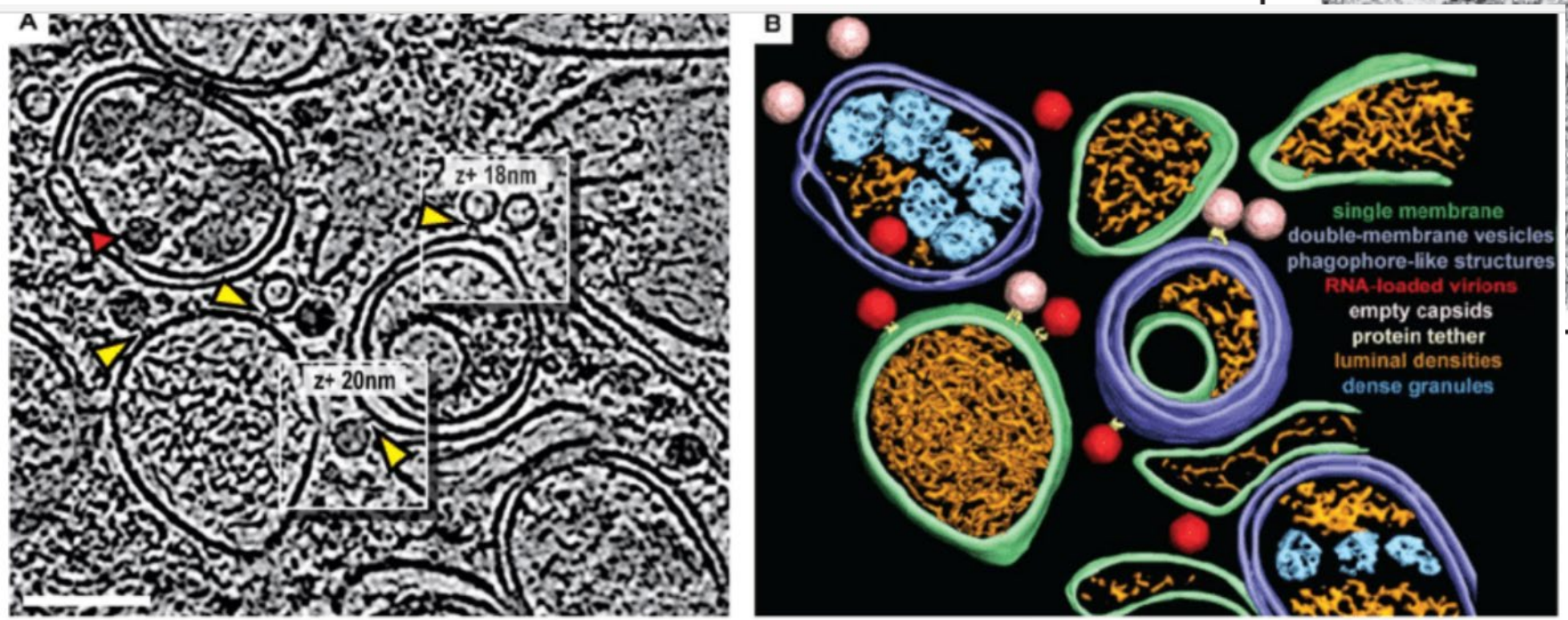
Subversion of autophagy by poliovirus
Replication at the surface

RNA positive virus
Replication at the surface of double-membrane vesicles
Similarity with autophagosomes
Activation of autophagy stimulates intracellular viral production

AUTOPHAGOSOMES	VIRUS-INDUCED VESICLES
Double membranes	
Cytosolic lumen	
LC3, LAMP 1 co-localization	
Increased LC3-II formation	
Average diameter 800 nm	Average diameter 200–400 nm
Induced by many stresses	Induced by viral proteins
Mature in 30 minutes	2BC and 3A
	Persist in "immature" form



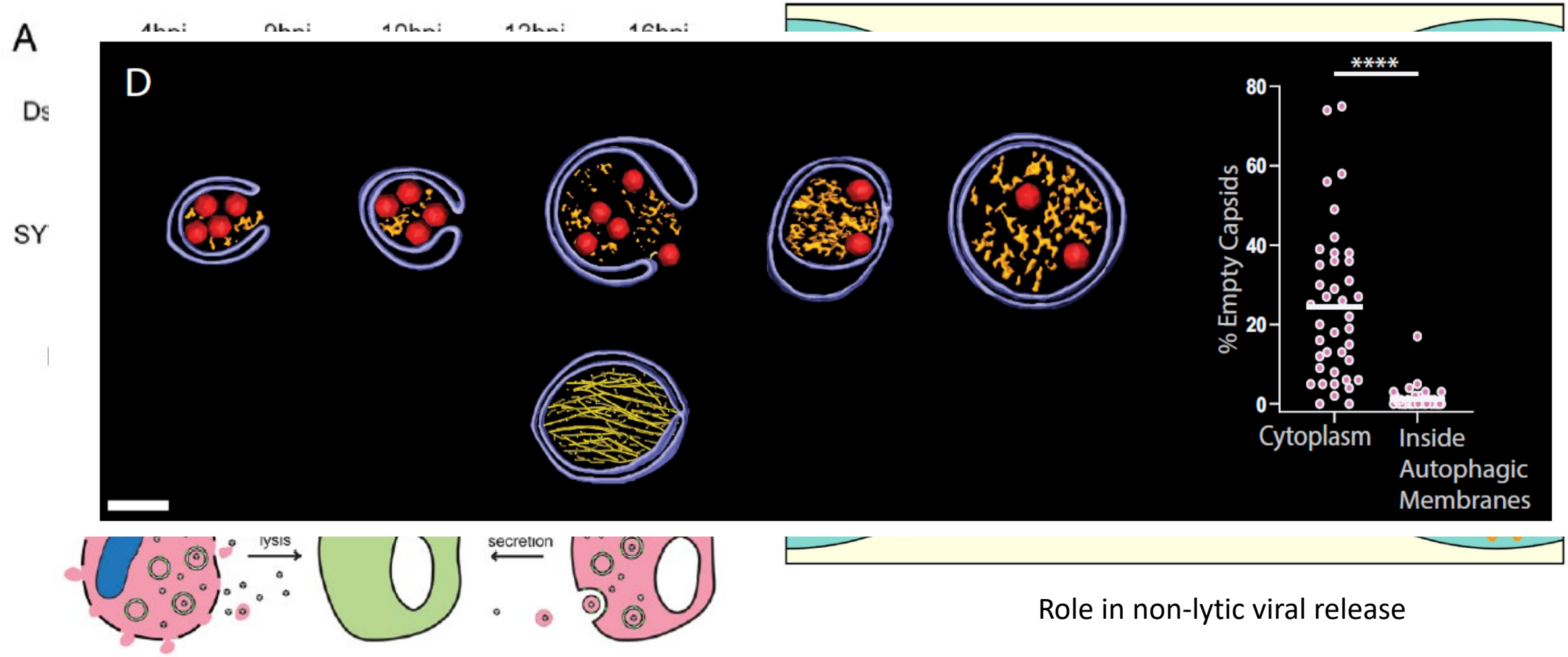
Taylor, 2008



Cryo-electron tomography

Dahmane et al, Nat Comm, 2022 *Taylor et al, Autophagy, 2009* *Taylor et al, J Virol, 2009*

Subversion of autophagy by poliovirus
non lytic release of viral particle



Role in non-lytic viral release

Dahmane et al, Nat Comm, 2022

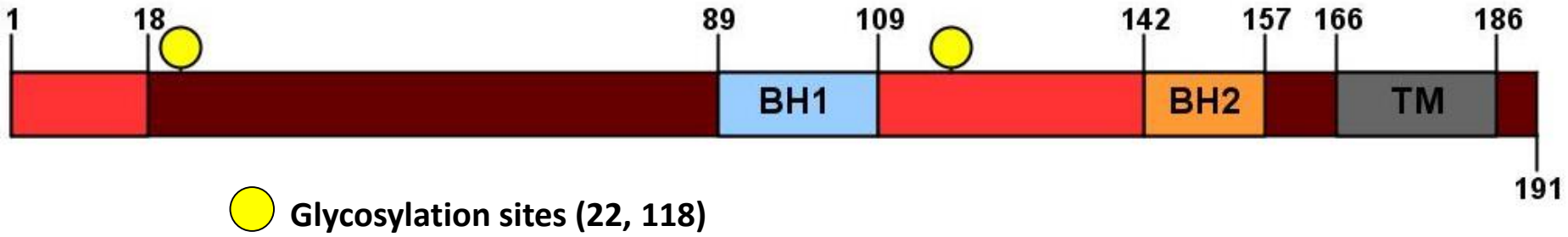
Dahmane et al, Autophagy, 2022

Bird et al, J Virol, 2014

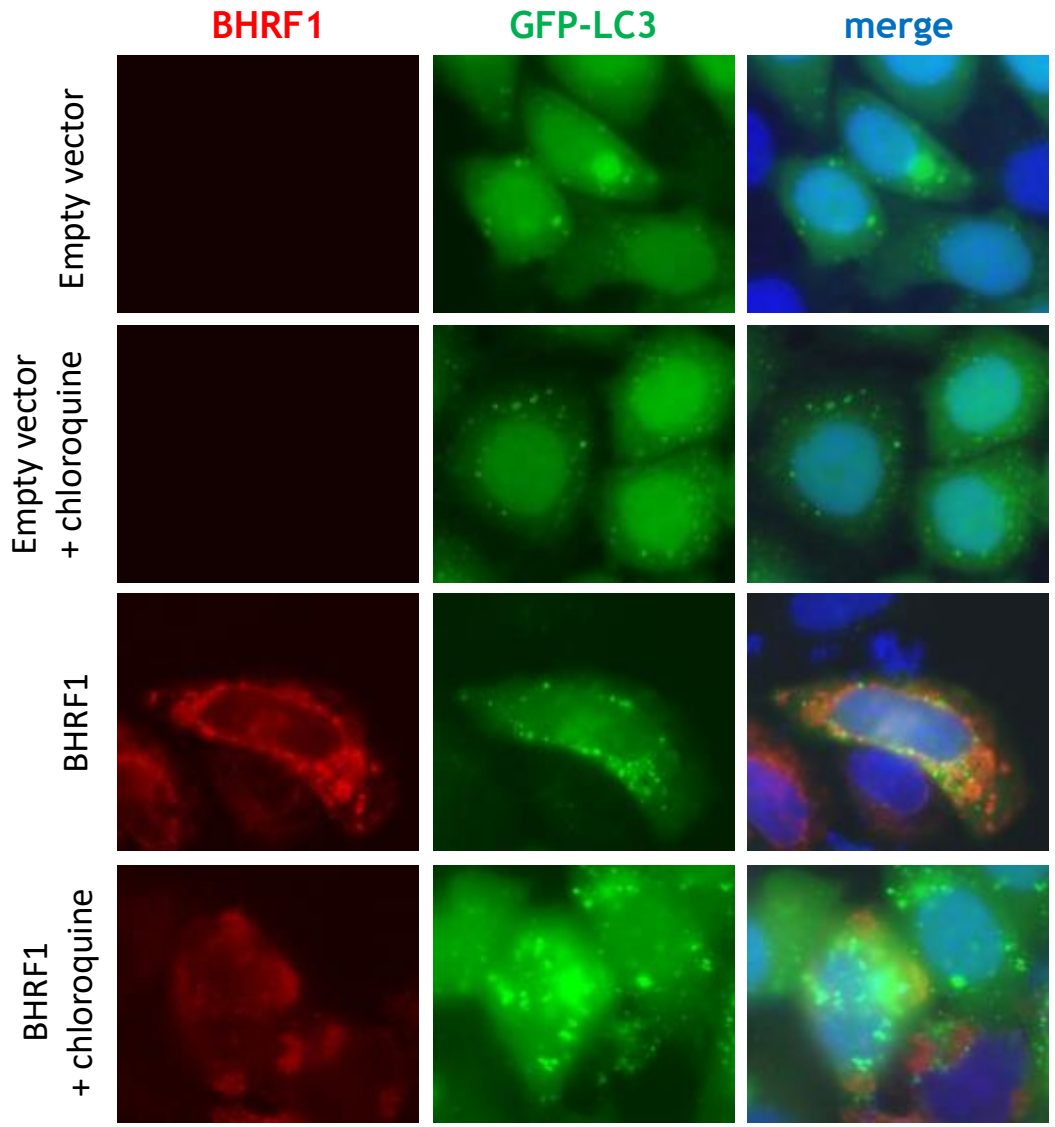
Mutsafi et al, Viruses, 2018

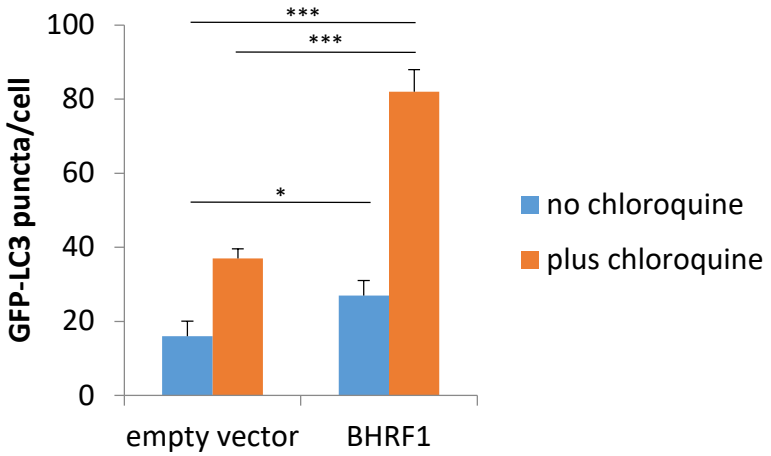
BHRF1 of Epstein-Barr Virus

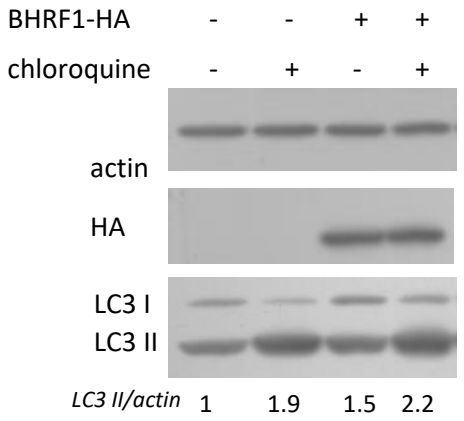
- EBV = Herpesvirus
- BHRF1 = homolog of Bcl-2, two homolog domains BH1 BH2
- Transmembrane protein
- 191 aa
- Early protein during productive cycle and expressed during some latency programs (Kelly et al, Plos Path, 2009)
- Known function: Anti-apoptotic (Kvansakul et al, Plos Path 2010)
- Mitochondrial localization (Henderson et al, PNAS, 1993; Hickish et al, Cancer Research, 1994)



BHRF1 induces accumulation of GFP-LC3 labelled autophagosomes

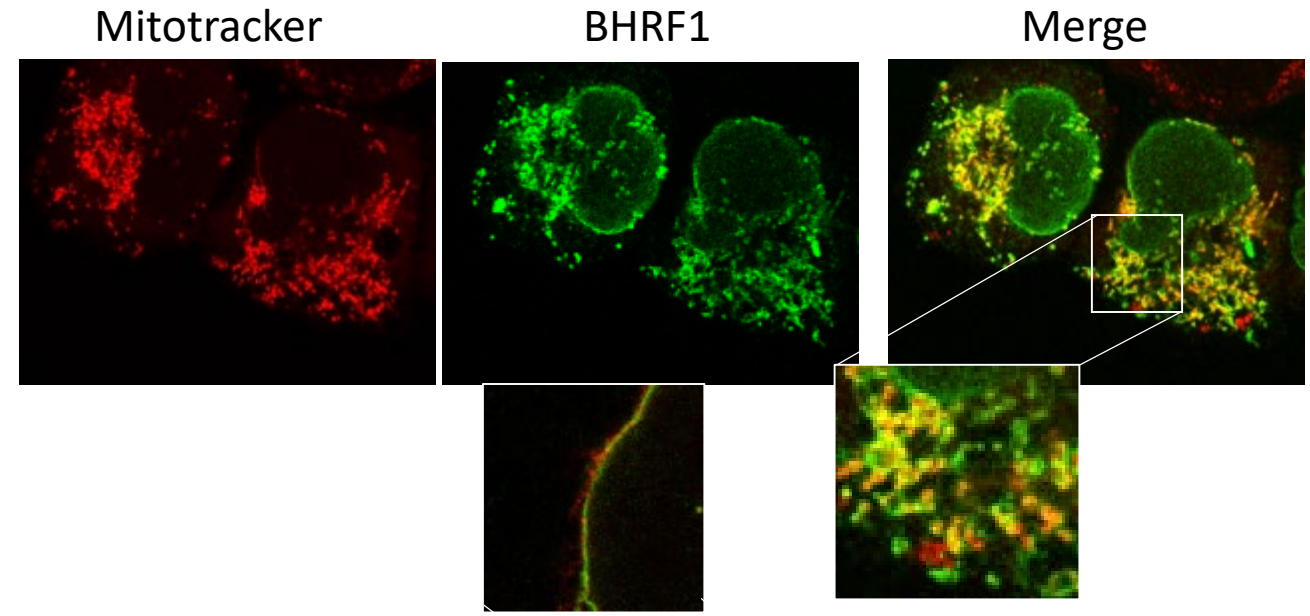


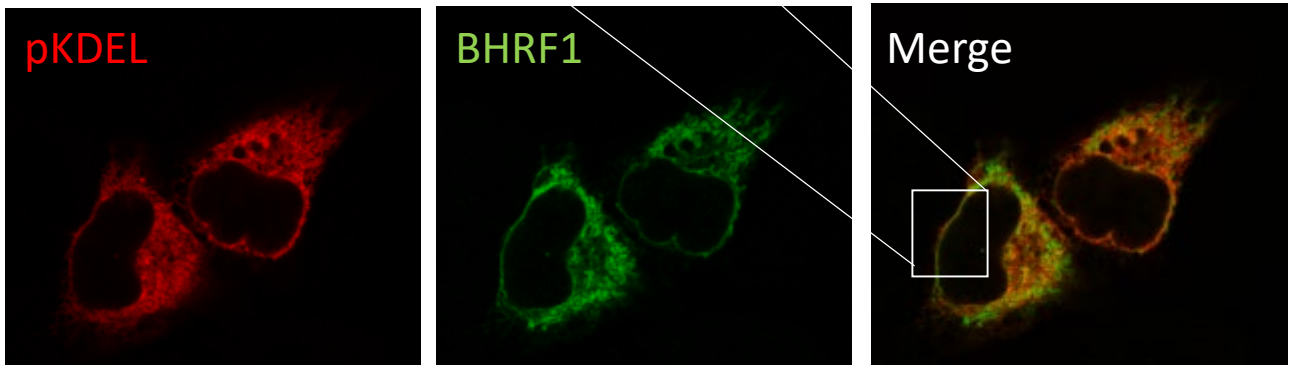




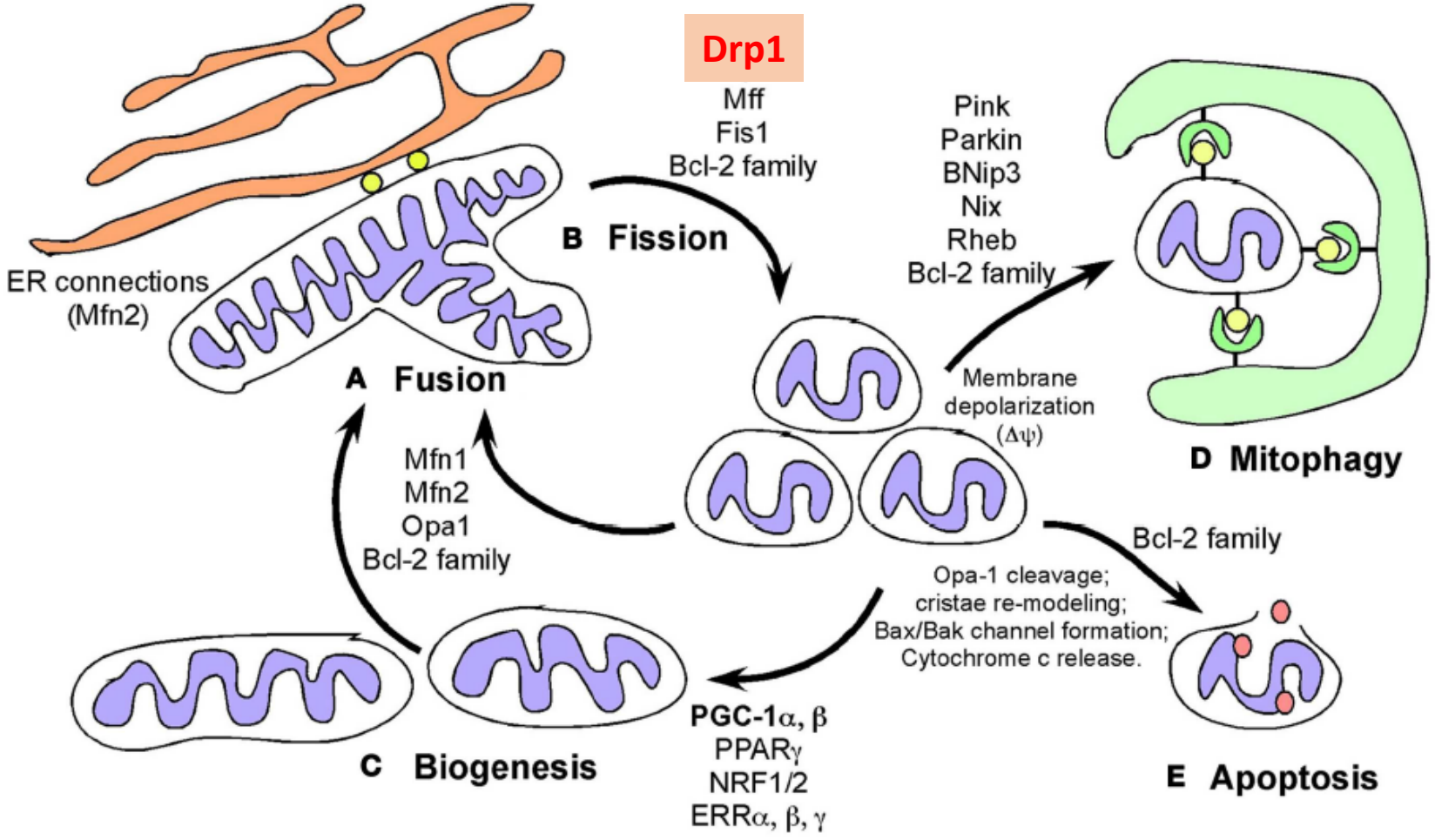
Vilmen, Glon* et al. Autophagy, 2021*

BHRF1 localizes to mitochondria and ER



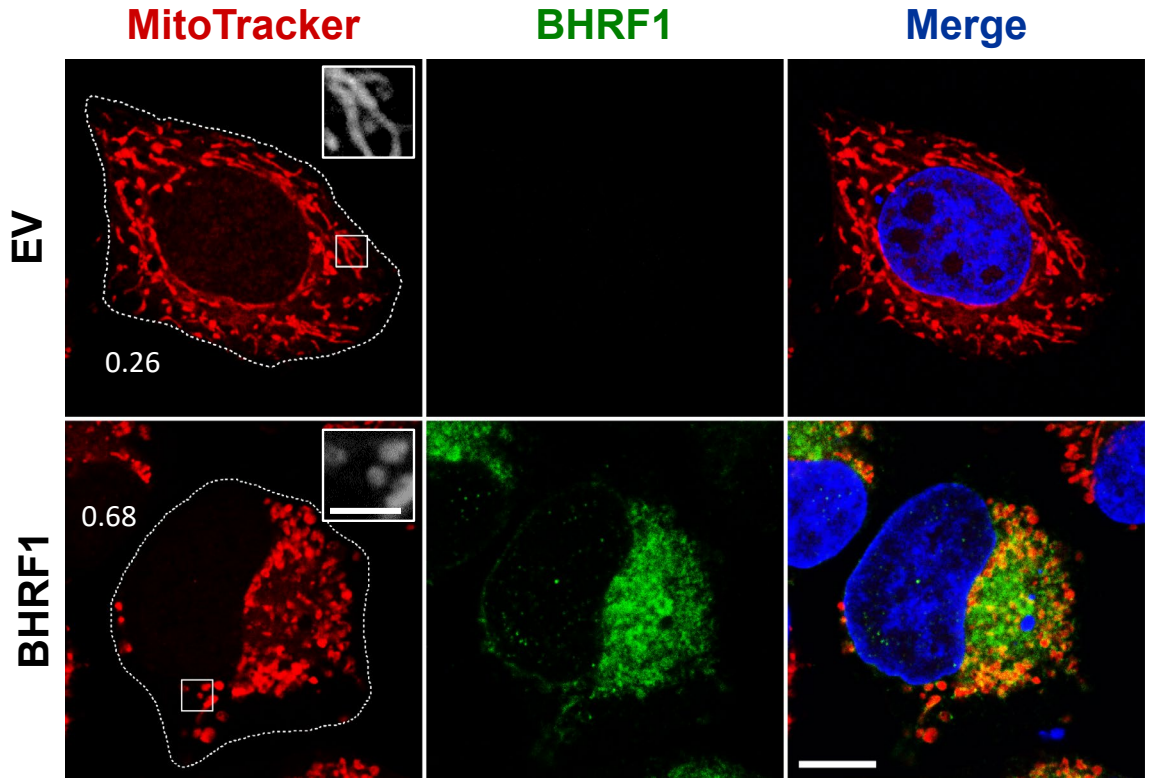


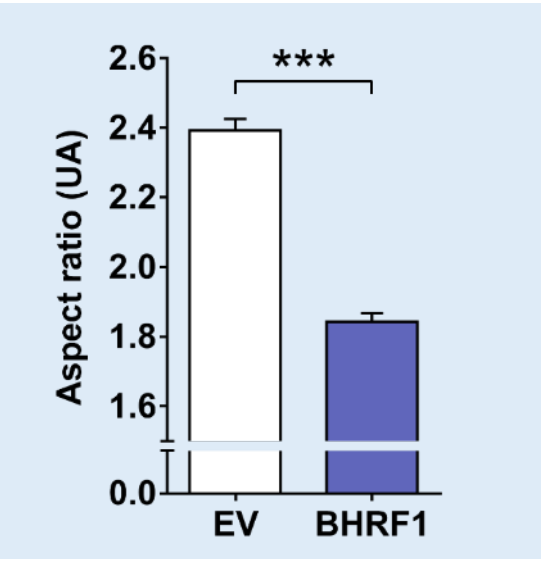
Mitochondria are highly dynamic

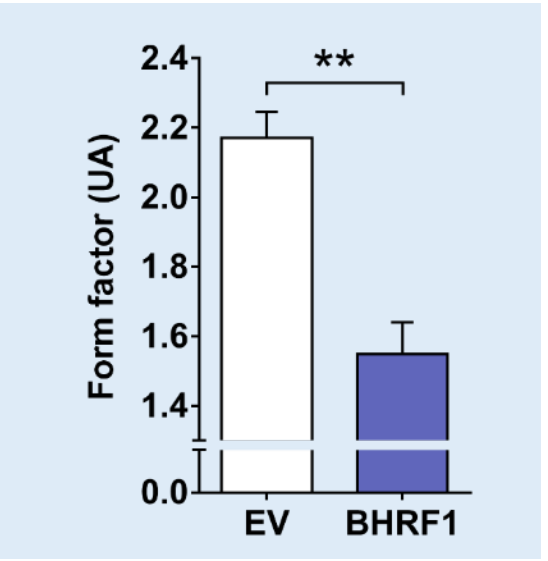


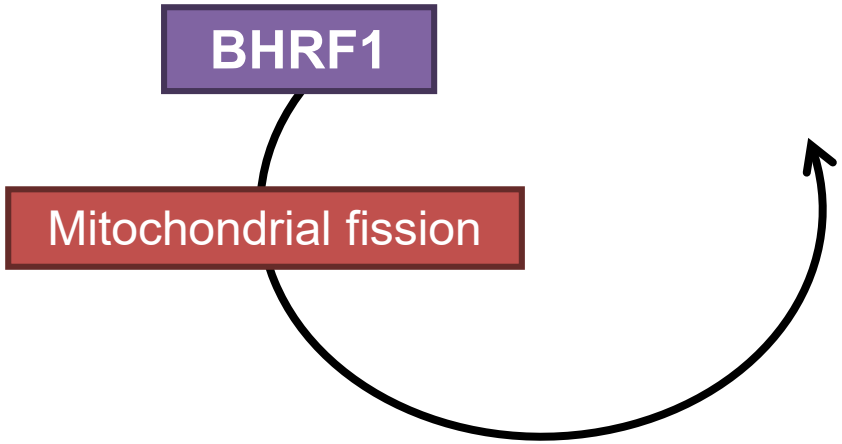
Boland et al, Frontiers in Oncology, 2013

BHRF1 induces mitochondrial fission



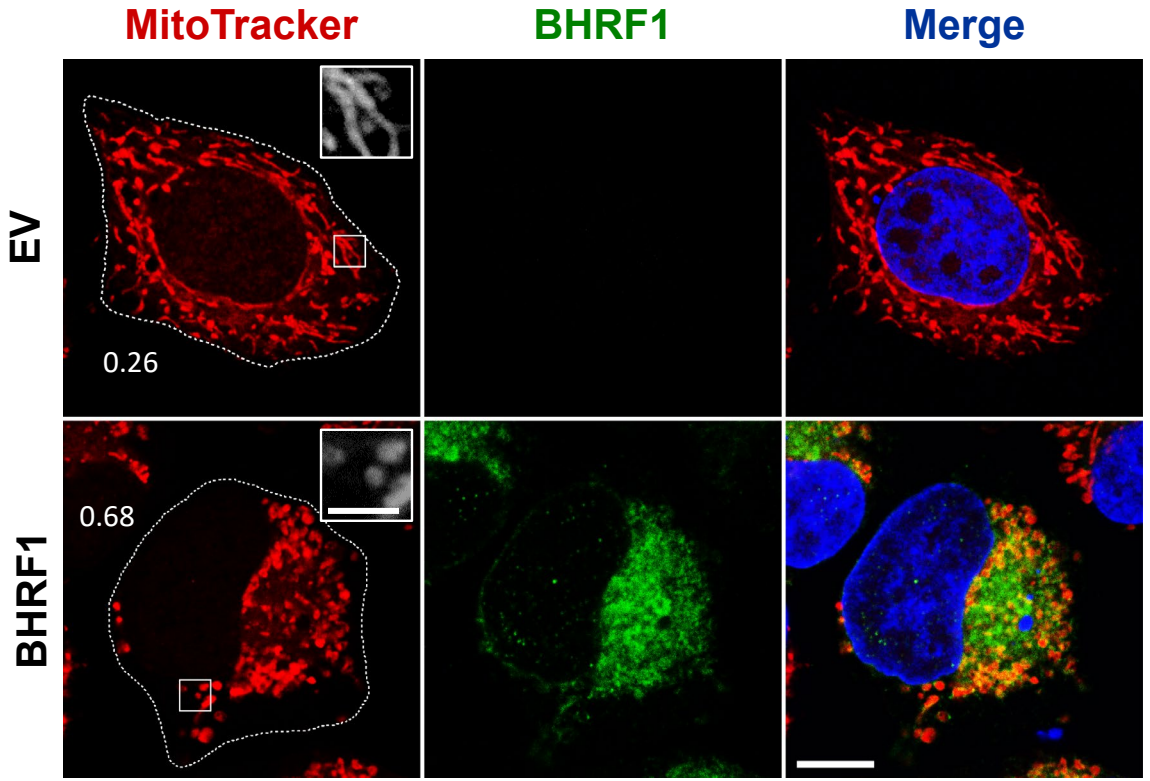


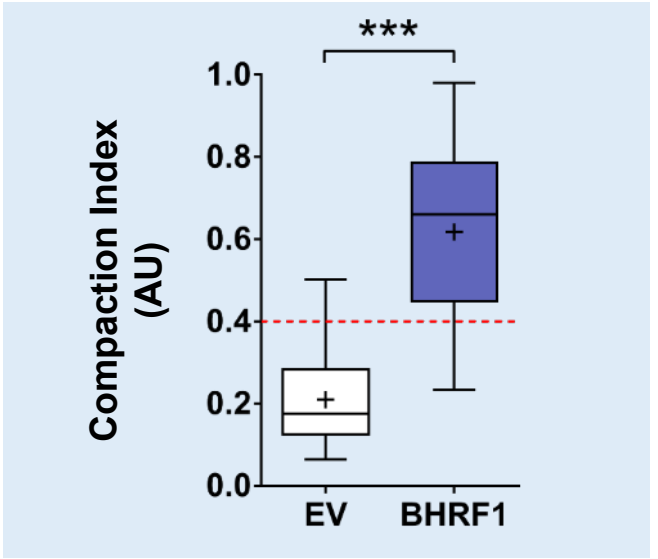


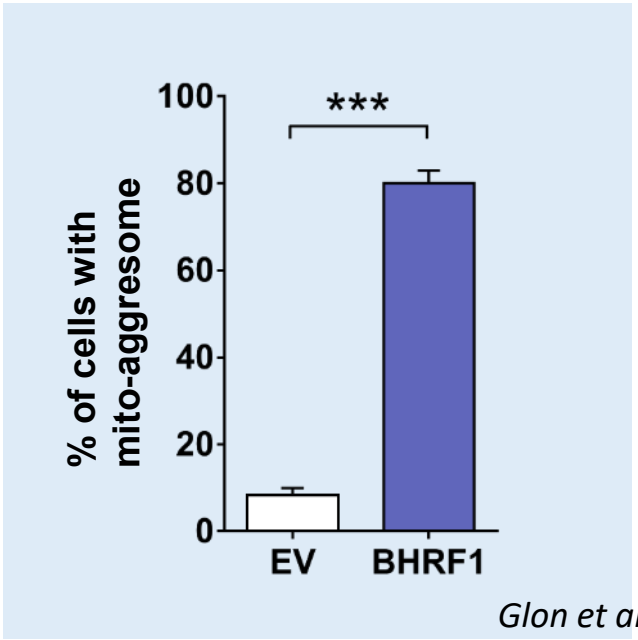


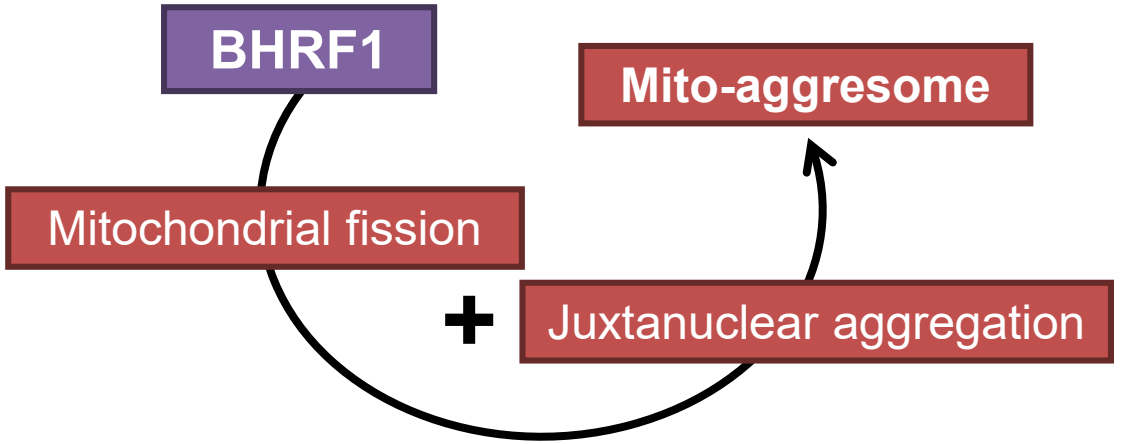
Glon et al. Plos Pathogens, 2022

BHRF1 induces mitochondrial fission and mito-aggresome formation



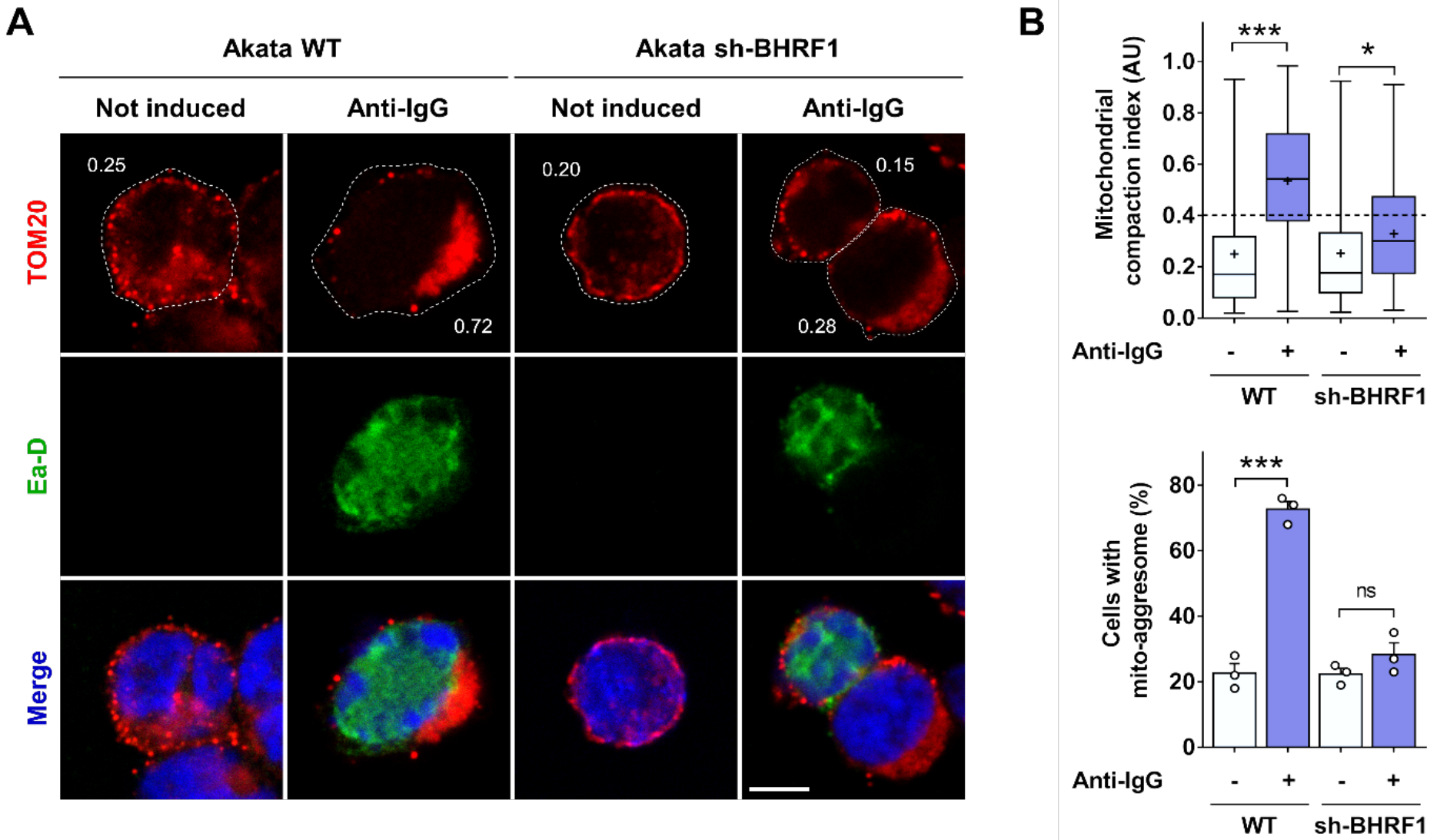






Glon et al. Plos Pathogens, 2022

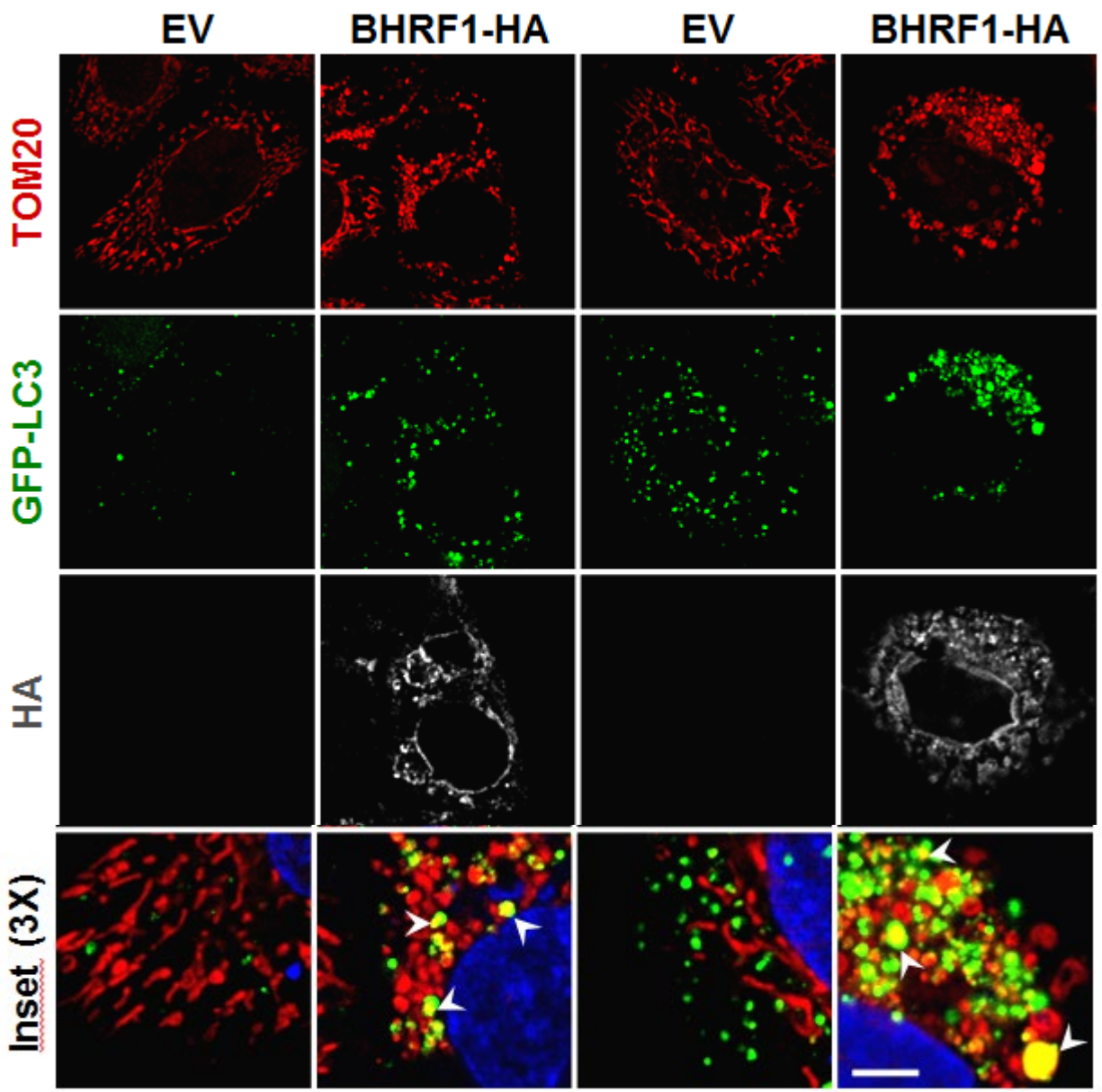
EBV induces mito-aggresome formation in B cells

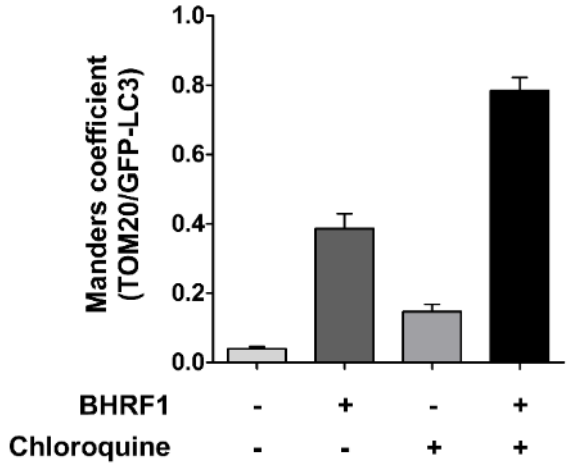


BHRF1 is essential for mito-aggresome formation in infected cells

Glon et al, Plos Pathogens, 2022

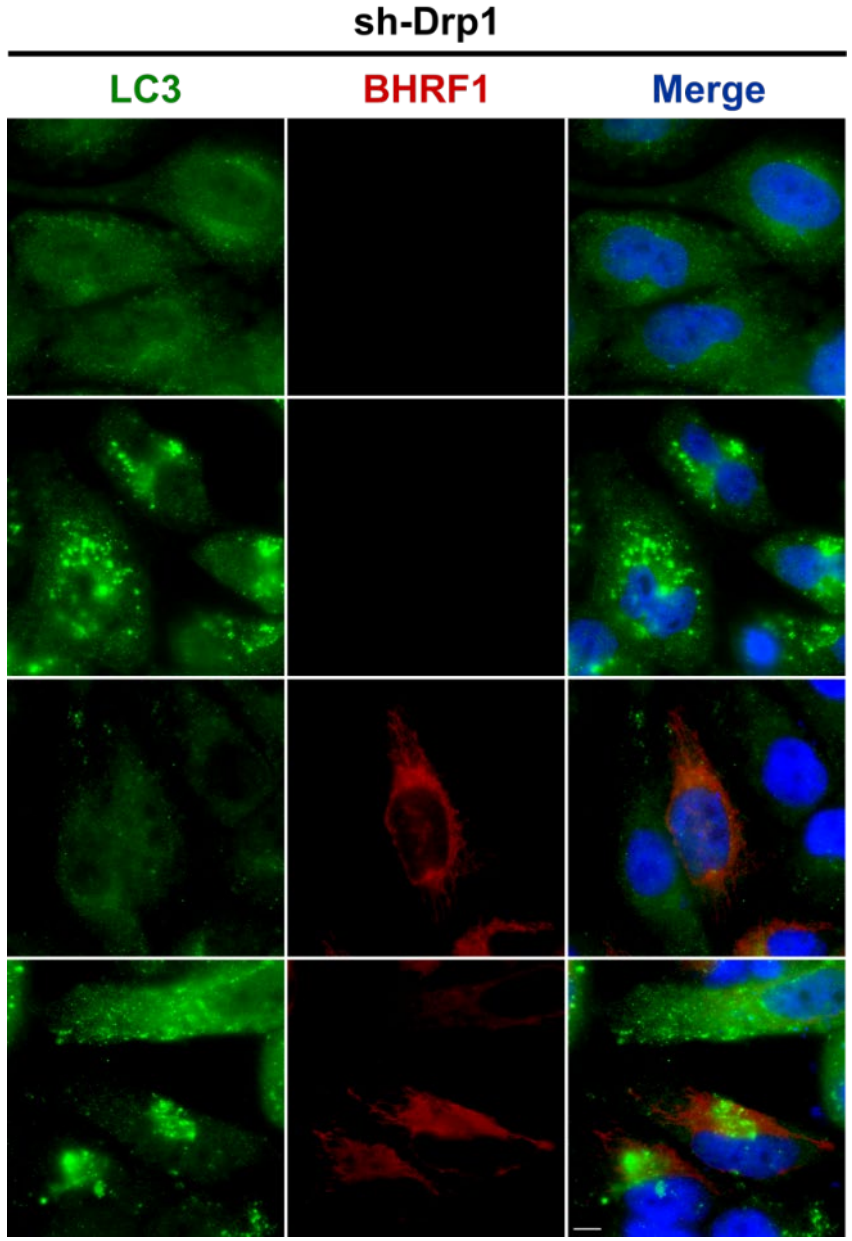
BHRF1 induces mitophagy

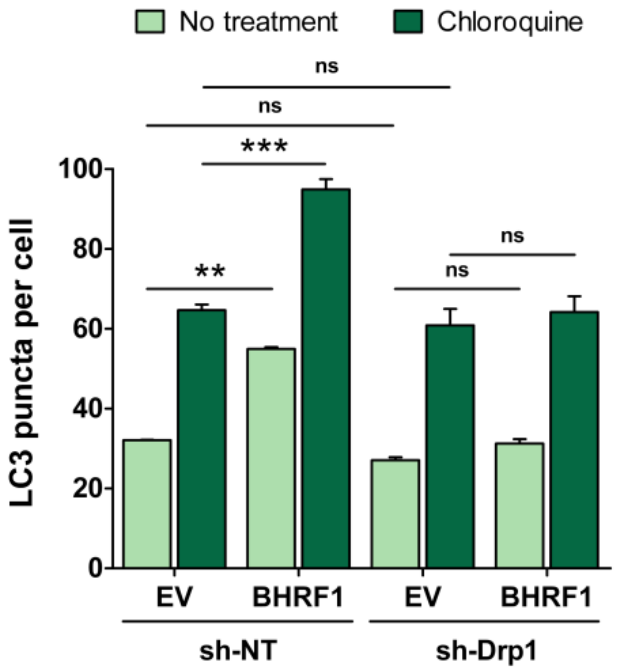




(Vilmen et al, 2020, Autophagy)

Fission is essential for BHRF1-induced autophagy

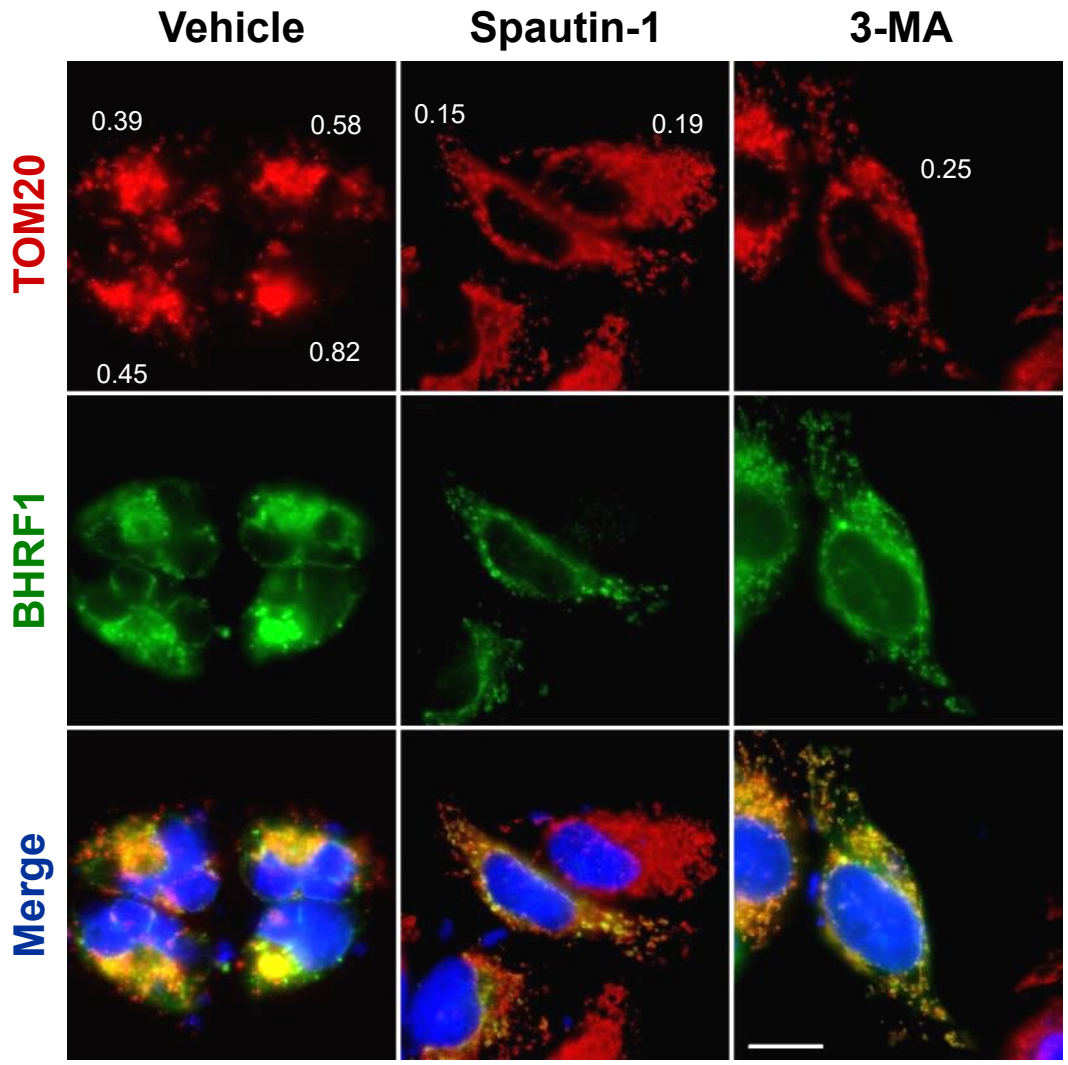


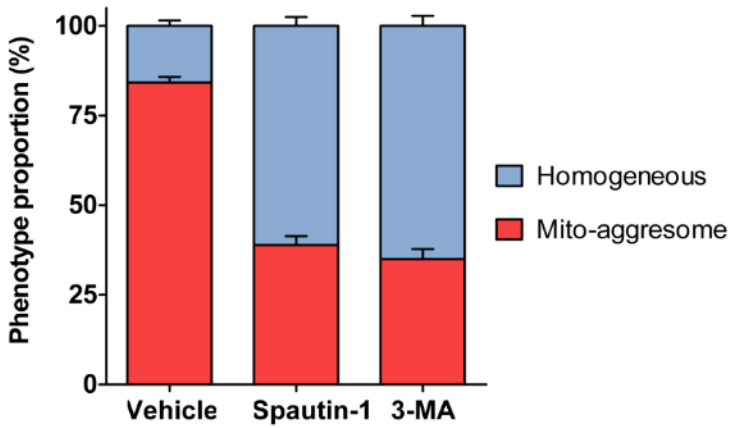


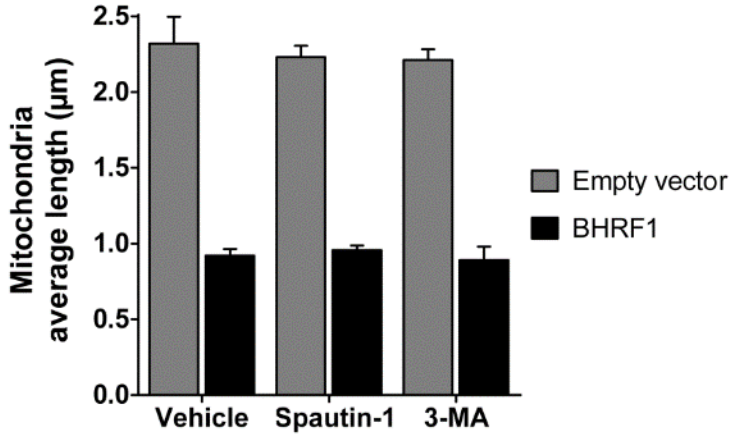
Without mitochondrial fission,
BHRF1 does not induce autophagy

Vilmen, Glon* et al. Autophagy, 2021*

Autophagy is essential for mito-aggresome formation

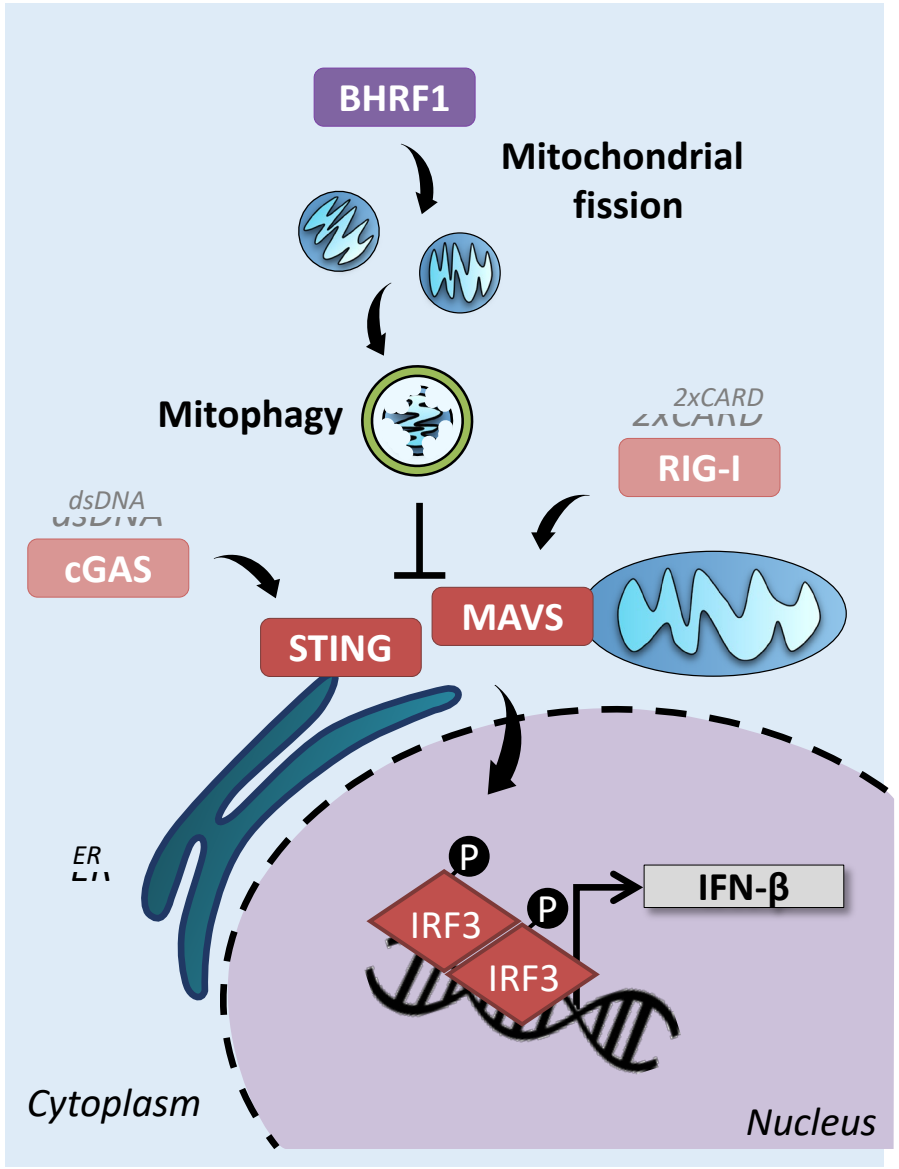


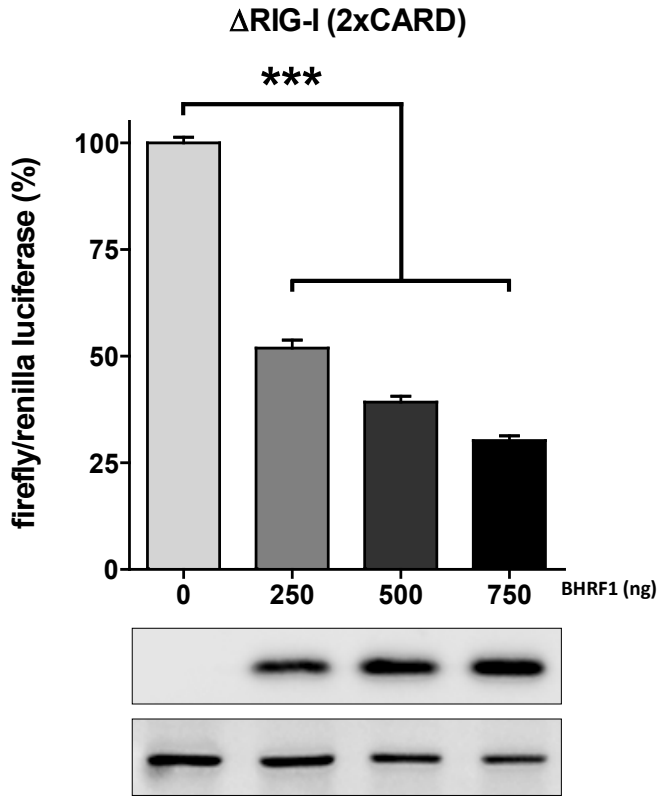


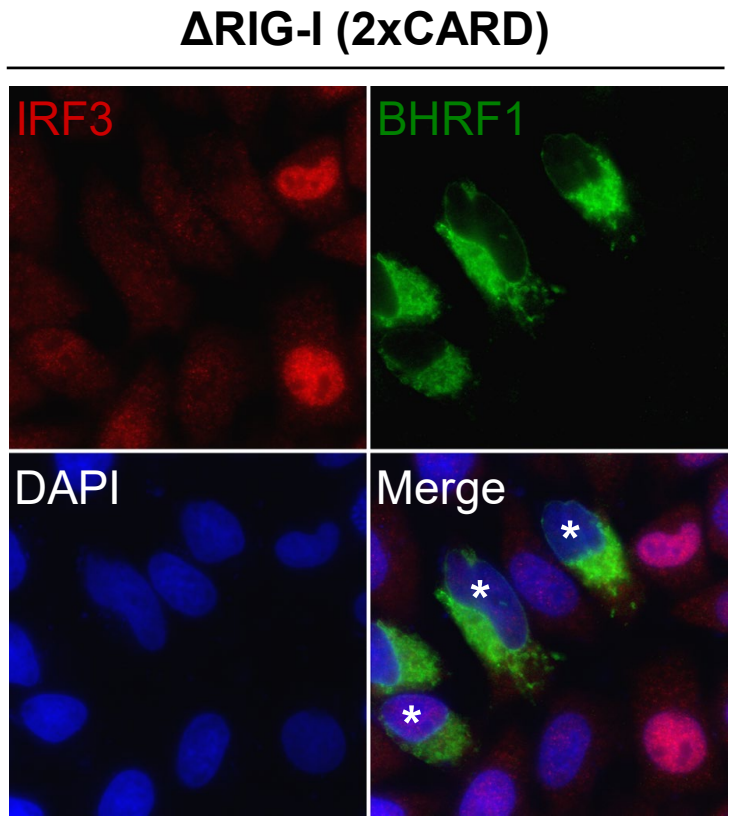


Inhibition of autophagy by Spautin or 3MA blocks formation of mito-aggresome but not the fission

BHRF1 blocks activation of IFN-I production



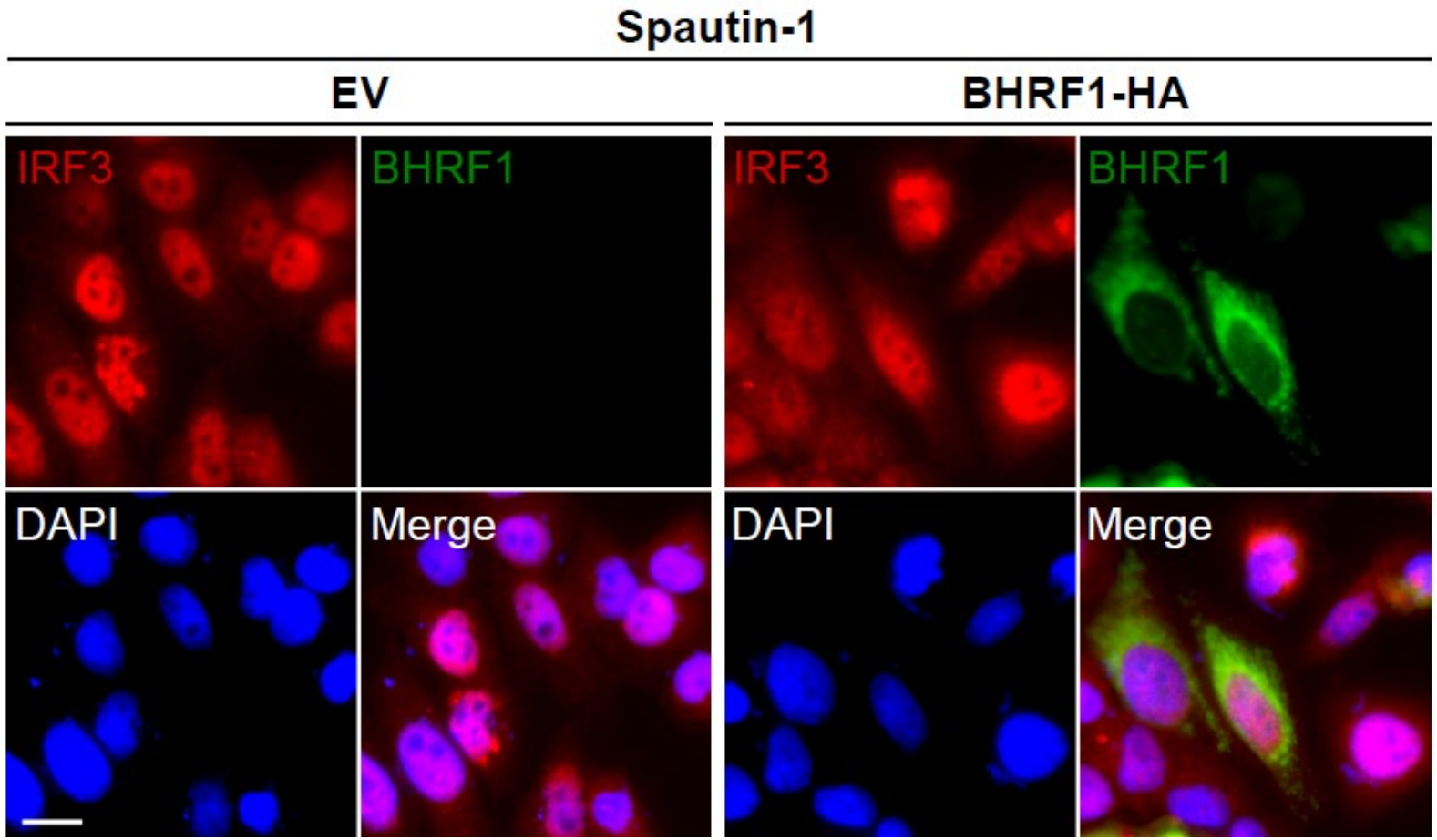


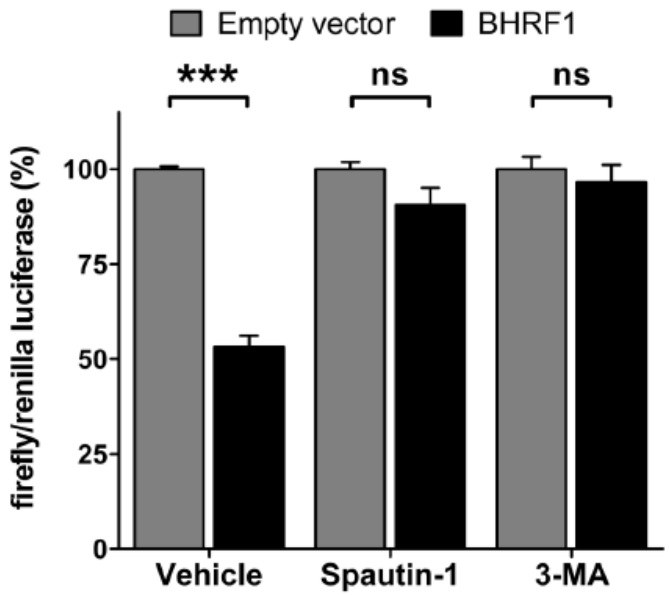


BHRF1 bloque l'activation, dépendante de MAVS et STING, du promoteur de l'IFN-β

Vilmen, Glon* et al. Autophagy, 2021*

Autophagy is required to block IFN production





Vilmen, Glon* et al. Autophagy, 2021*

BHRF1 blocks IFN response via MT acetylation and degradation of mitochondria by autophagy