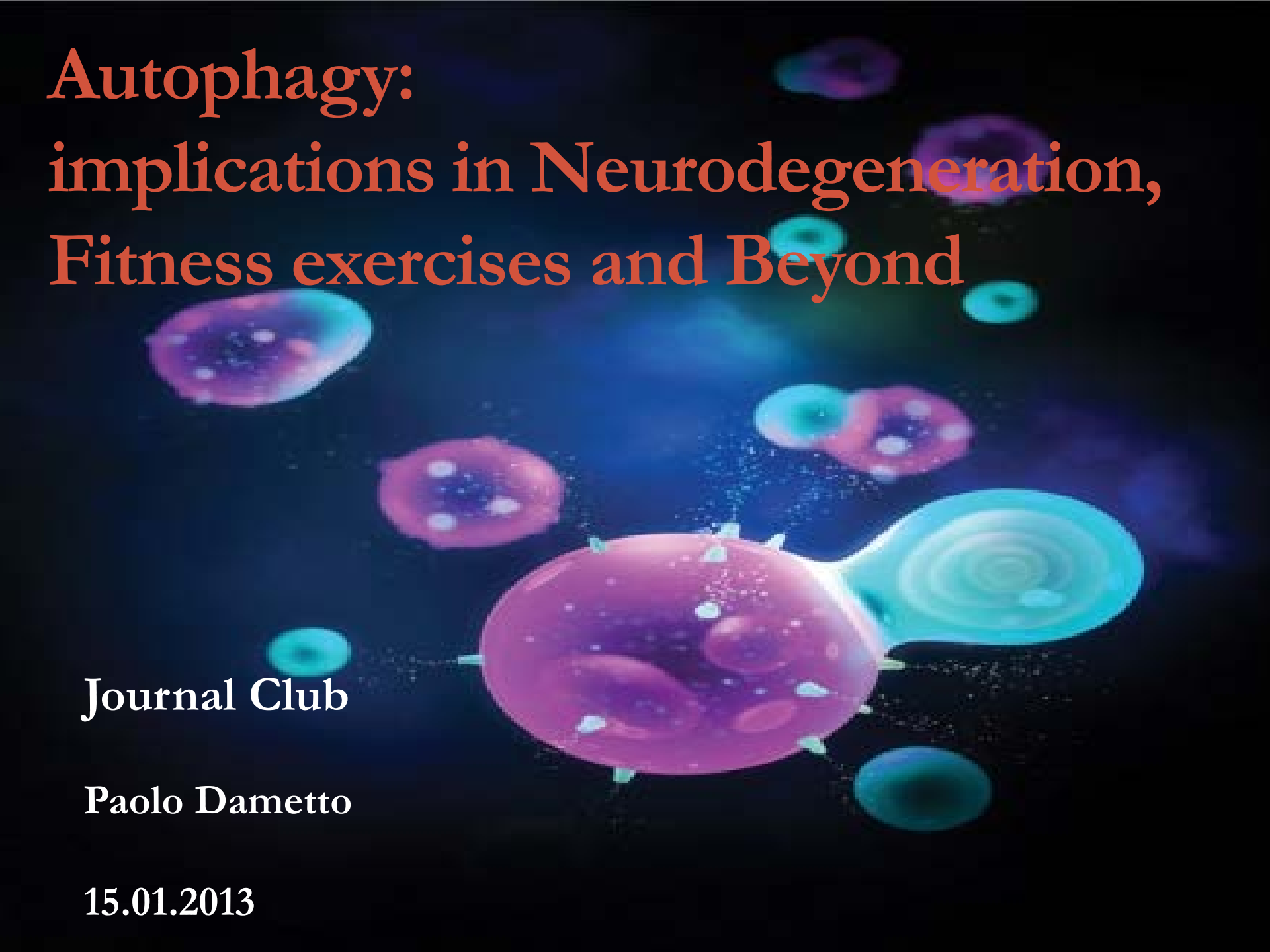
Autophagy: implications in Neurodegeneration, Fitness exercises and Beyond



Journal Club

Paolo Dametto

15.01.2013

Outline

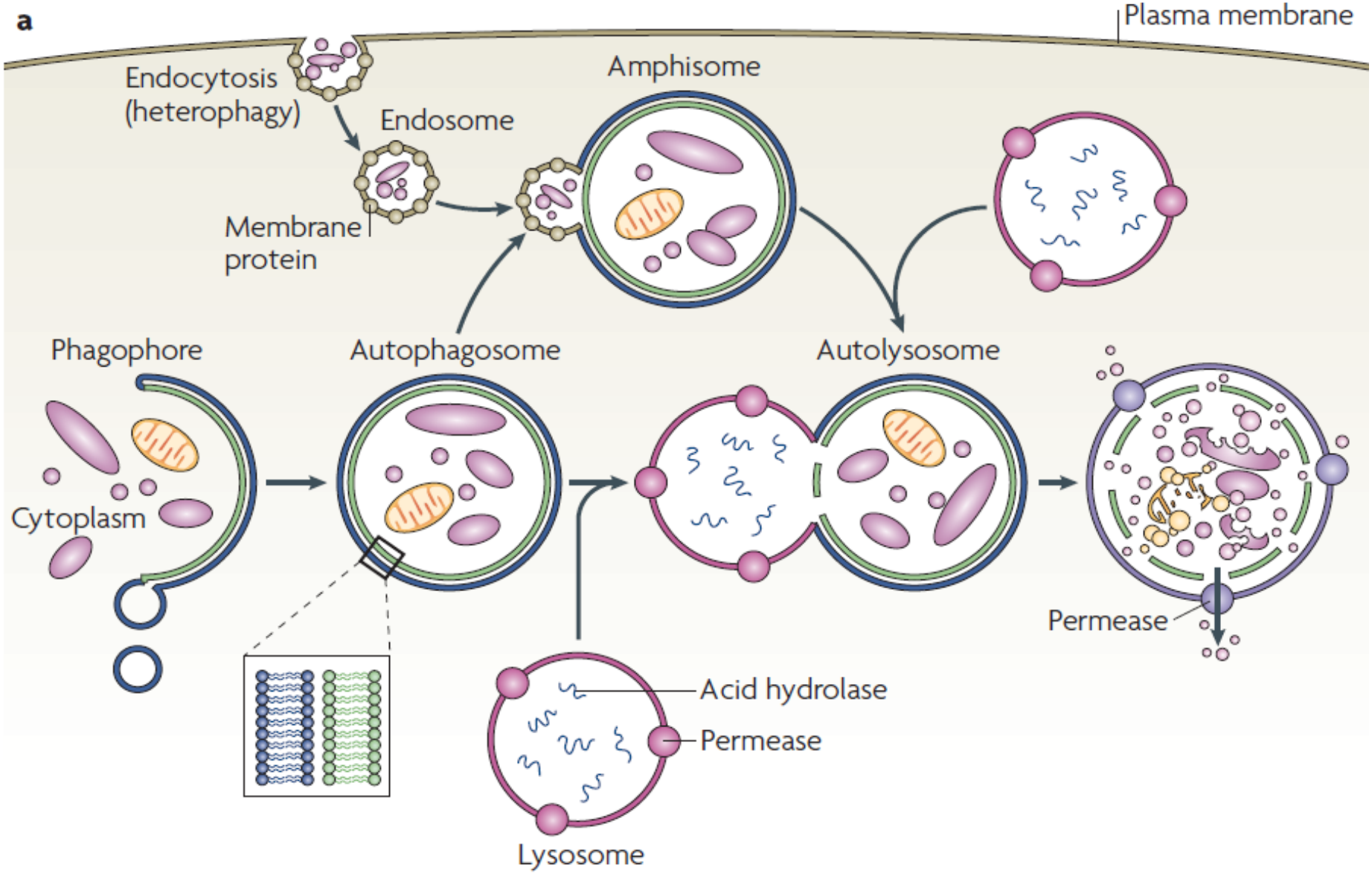
1. Introduction on Autophagy
   - **Definitions, functions, hystory and main players involved**

1. Signaling pathways regulating Autophagy

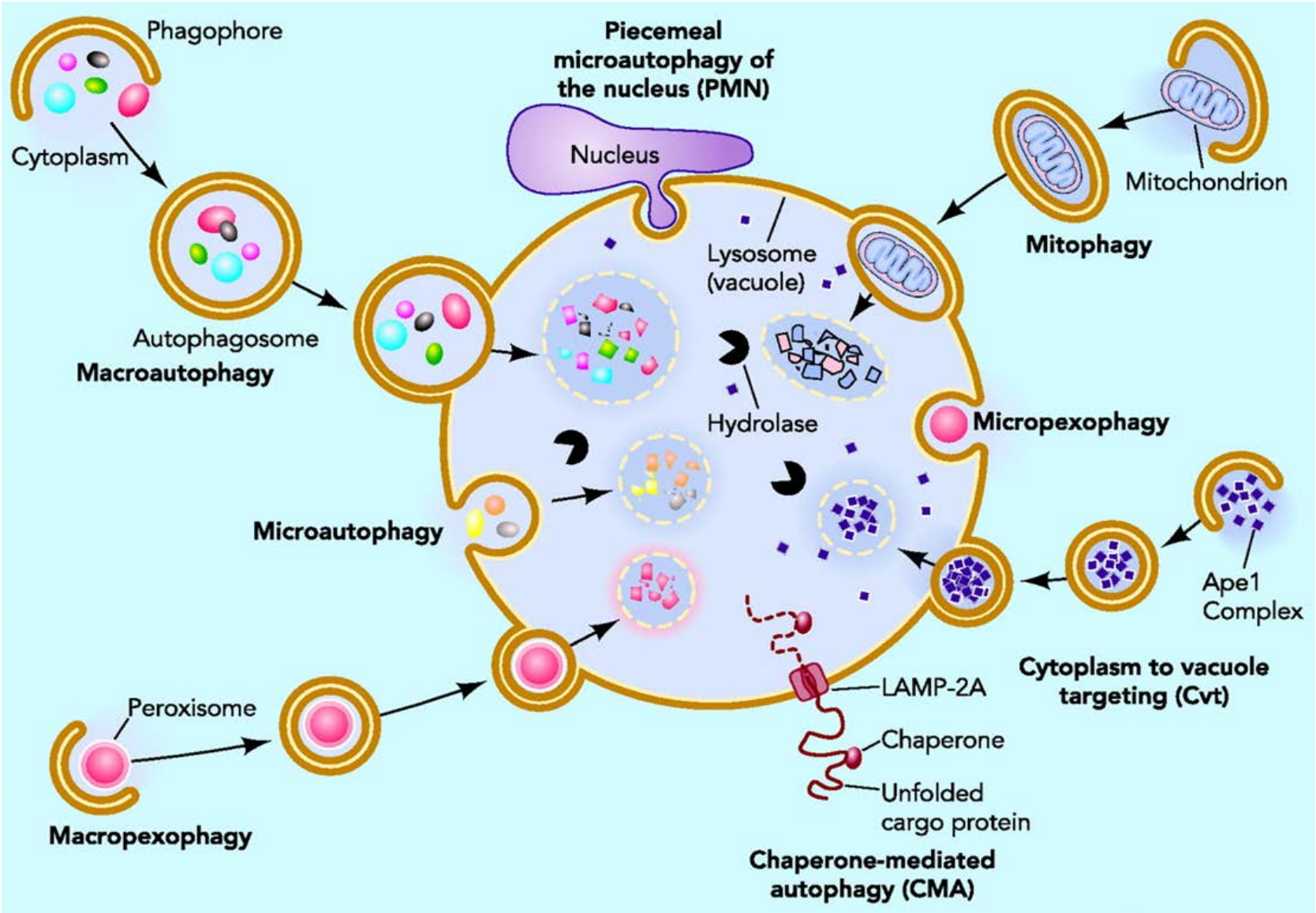
2. Implications in Neurodegeneration
   1. **Lysosomal Proteolysis and Autophagy Require Presenilin 1 and Are Disrupted by Alzheimer-Related PS1 Mutations**
   2. **Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits**
   - **Other neurodegenerative diseases**
   - **Strategies for potential treatments**

3. Implications in Health&Fitness
   3. **Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis**

Autophagy: definitions



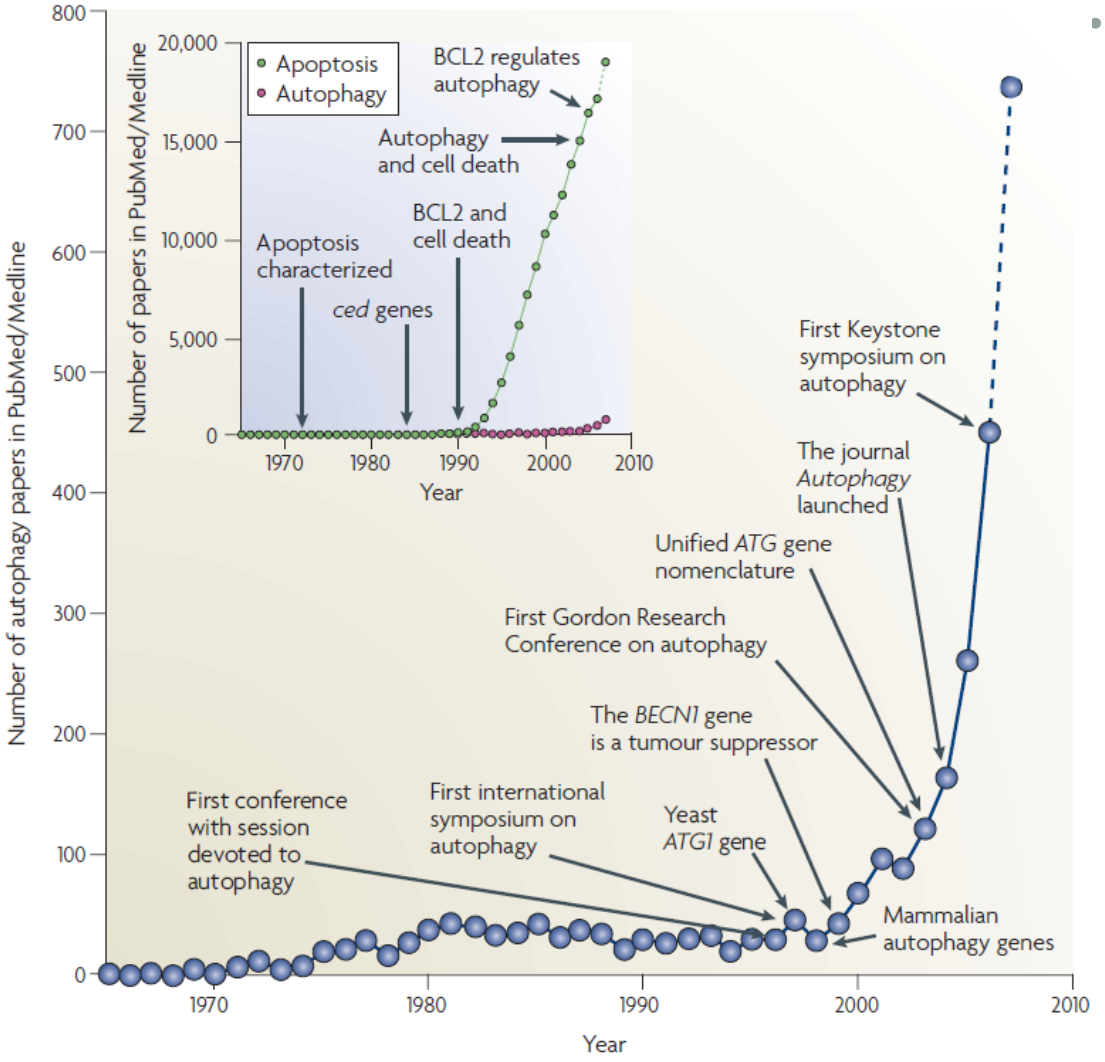
Subtypes of autophagy



Physiological functions

- Autophagy defends against metabolic stress
  - Autophagy is activated as an adaptive catabolic process in response to different forms of metabolic stress, including **nutrient deprivation, growth factor depletion, and hypoxia**. This bulk form of degradation generates free amino and fatty acids that can be recycled in a cell-autonomous fashion or delivered systemically to distant sites within the organism.
- Autophagy works as a cellular housekeeper
  - The repertoire of routine housekeeping functions performed by autophagy includes **the elimination of defective proteins and organelles, the prevention of abnormal protein aggregate accumulation, and the removal of intracellular pathogens**. The autophagy pathway is uniquely capable of degrading entire organelles such as mitochondria, peroxisomes, and ER as well as intact intracellular microorganisms.
- Autophagy in life and death decisions of the cells
  - Autophagy constitutes a stress adaptation pathway that promotes cell survival. An apparent paradox is that autophagy is also considered a form of non-apoptotic programmed cell death called “type II” or “autophagic” cell death. But it is now clear that the mere presence of autophagosomes in dying cells is insufficient to distinguish “cell death with autophagy” from “cell death by autophagy”.
- Autophagy may be a guardian of the genome
  - The autophagic machinery can limit DNA damage and chromosomal instability

Autophagy, the new apoptosis?

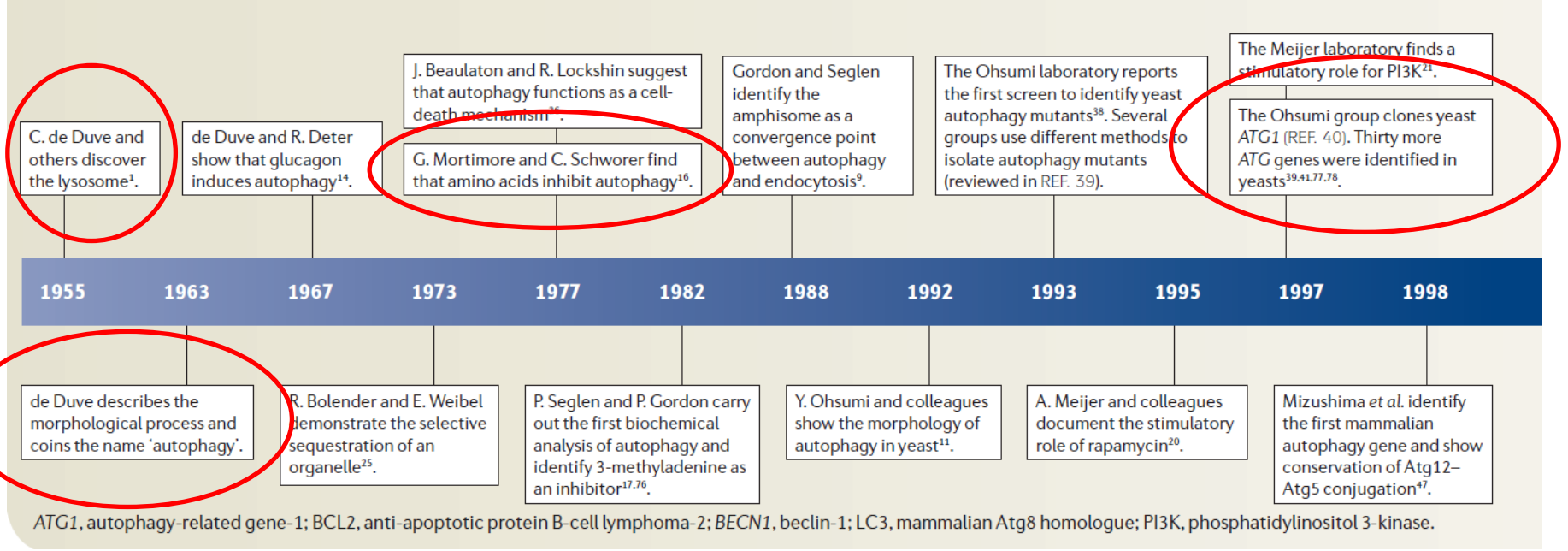


- Although initially considered simply a degradative process, recent studies have revealed an **integral role for autophagy in human pathophysiology.** Accordingly, there has been a tremendous increase in autophagy research in the past 10 years

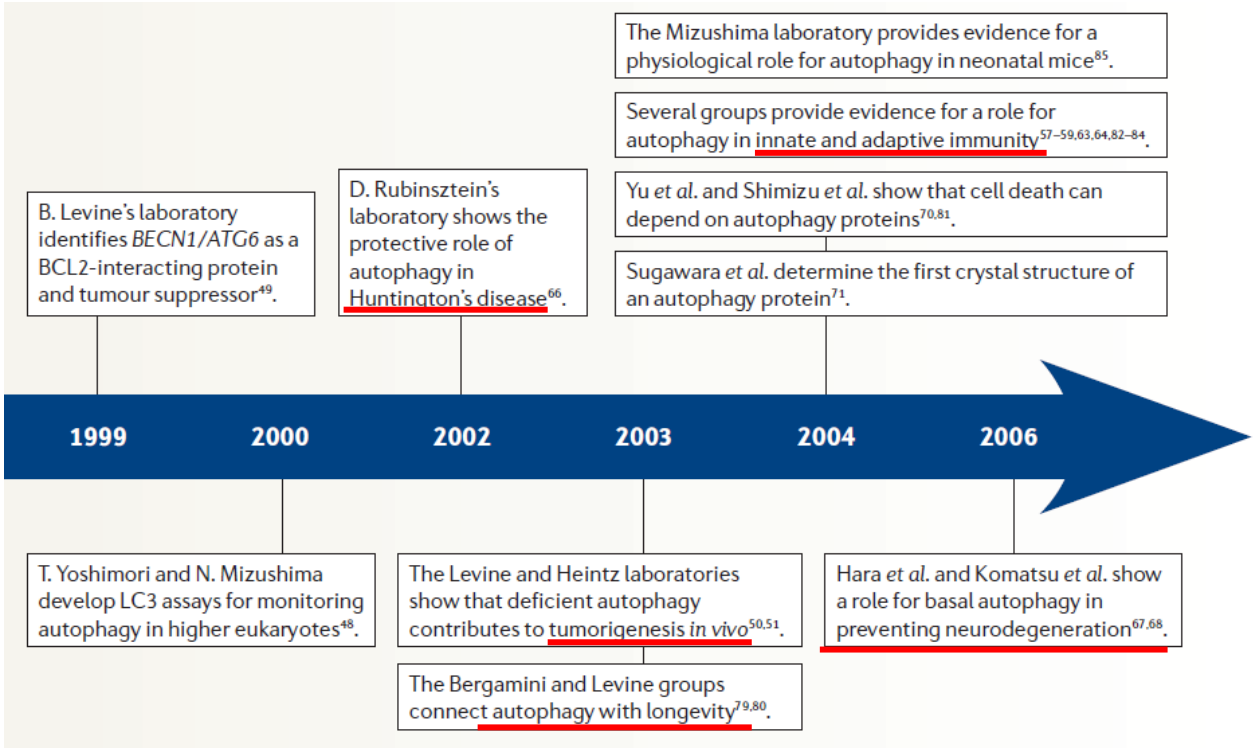
Year	Impact Factor (IF)	Total Articles	Total Cites
2011	7.453	108	4923
2010	6.643	82	4142
2009	6.829	149	3197
2008	5.479	190	1852

Klionsky D.J. et al, 2007 Nature Mol. Cell Biology

History of Autophagy



ATG1, autophagy-related gene-1; BCL2, anti-apoptotic protein B-cell lymphoma-2; *BECN1*, beclin-1; LC3, mammalian Atg8 homologue; PI3K, phosphatidylinositol 3-kinase.



Klionsky D.J. et al, 2007 Nature Mol. Cell Biology

Molecular machinery

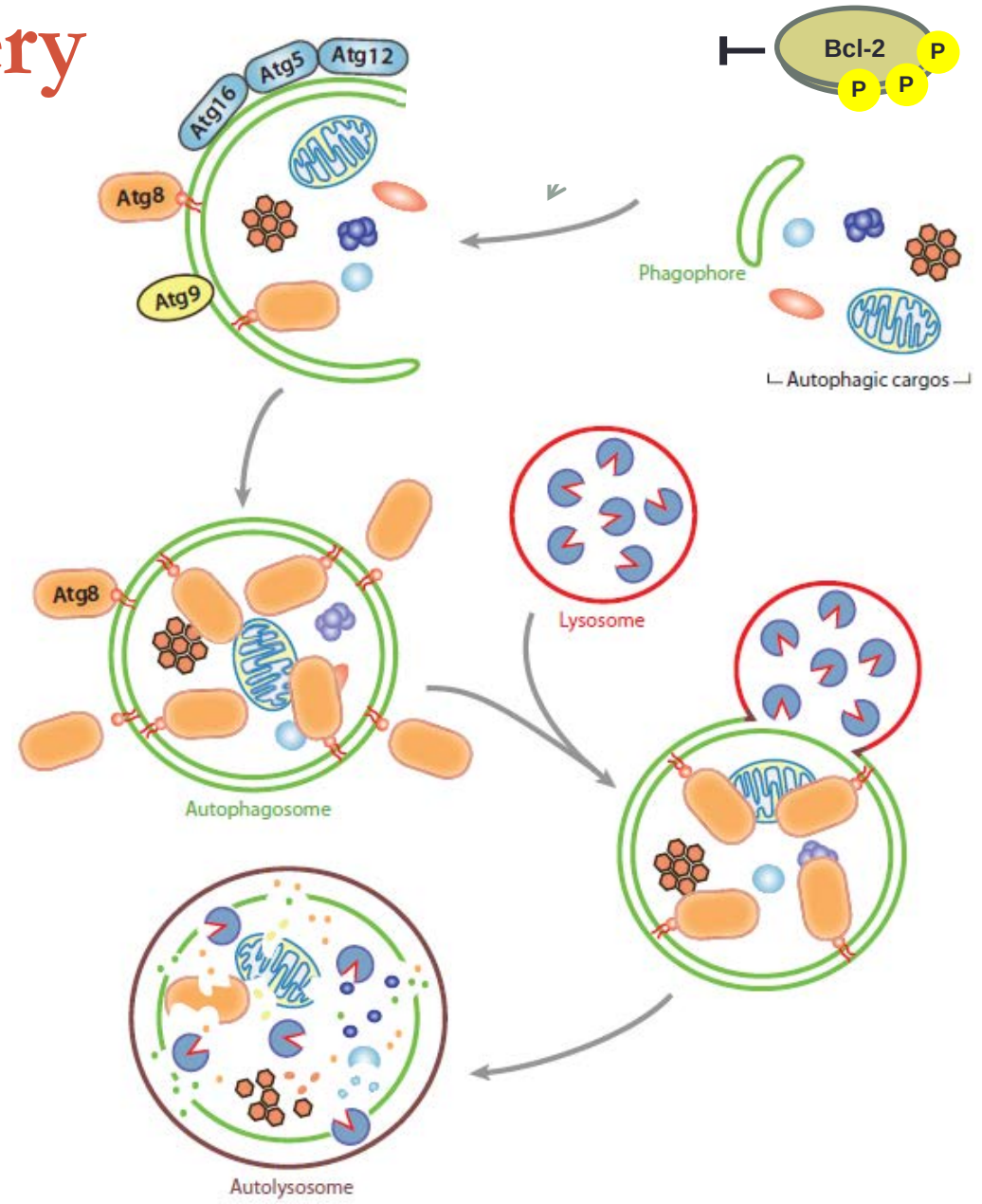
1. **Induction**
   (e.g. low energy, hypoxia, stress, low levels of hormones)

2. **Autophagosome formation**
   (Atg 5,8,9,12,16, Beclin-1)

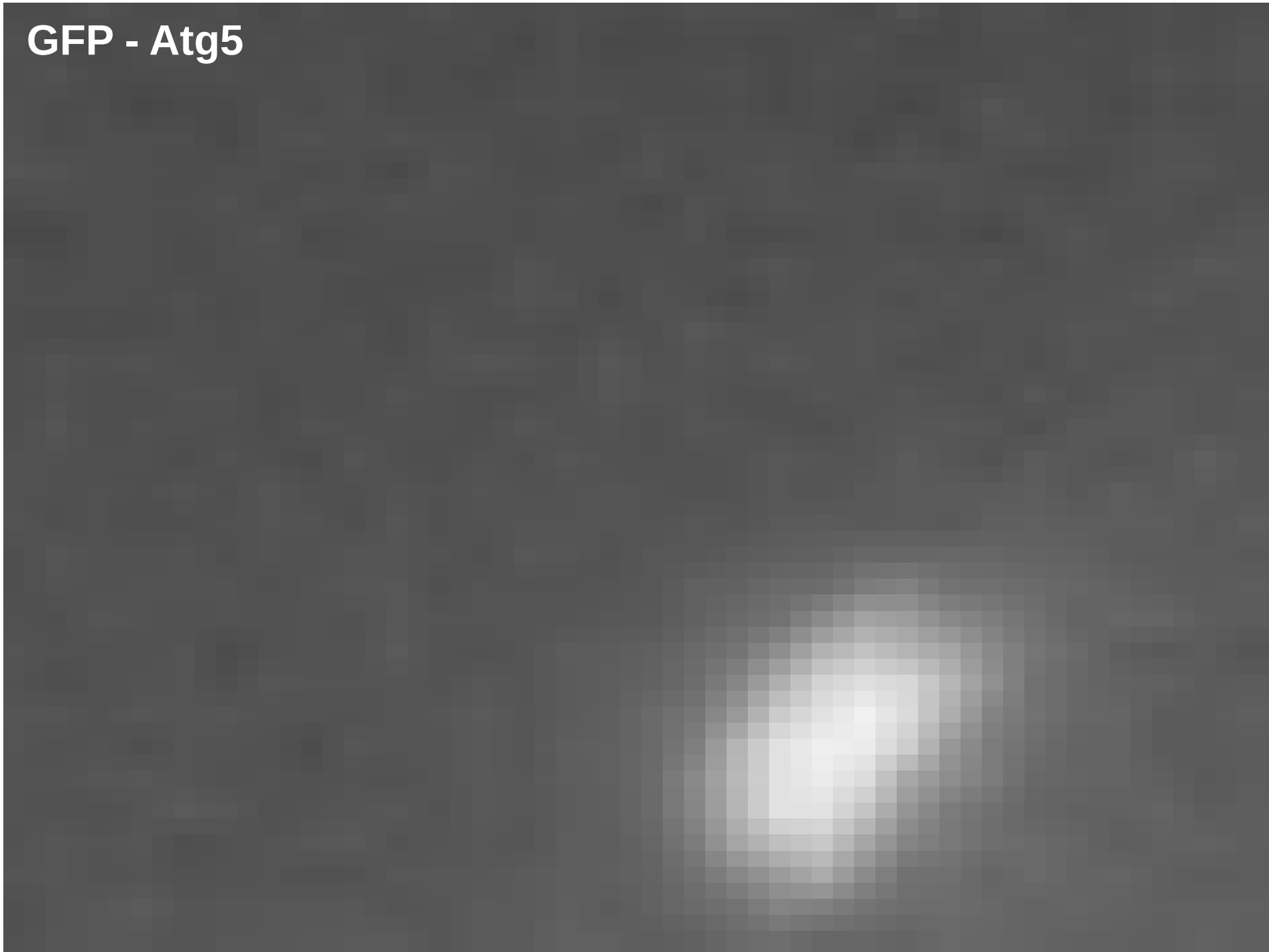
Atg8 = **LC3** in mammals!! Upon authophagy induction, LC3 exists as the lipd-conjugated form (**LC3-II**)

3. **Vesicle fusion and autophagosome break-down**
   LAMP2 and the small GTPase Rab7 are needed for autophagosome-lysosome fusion

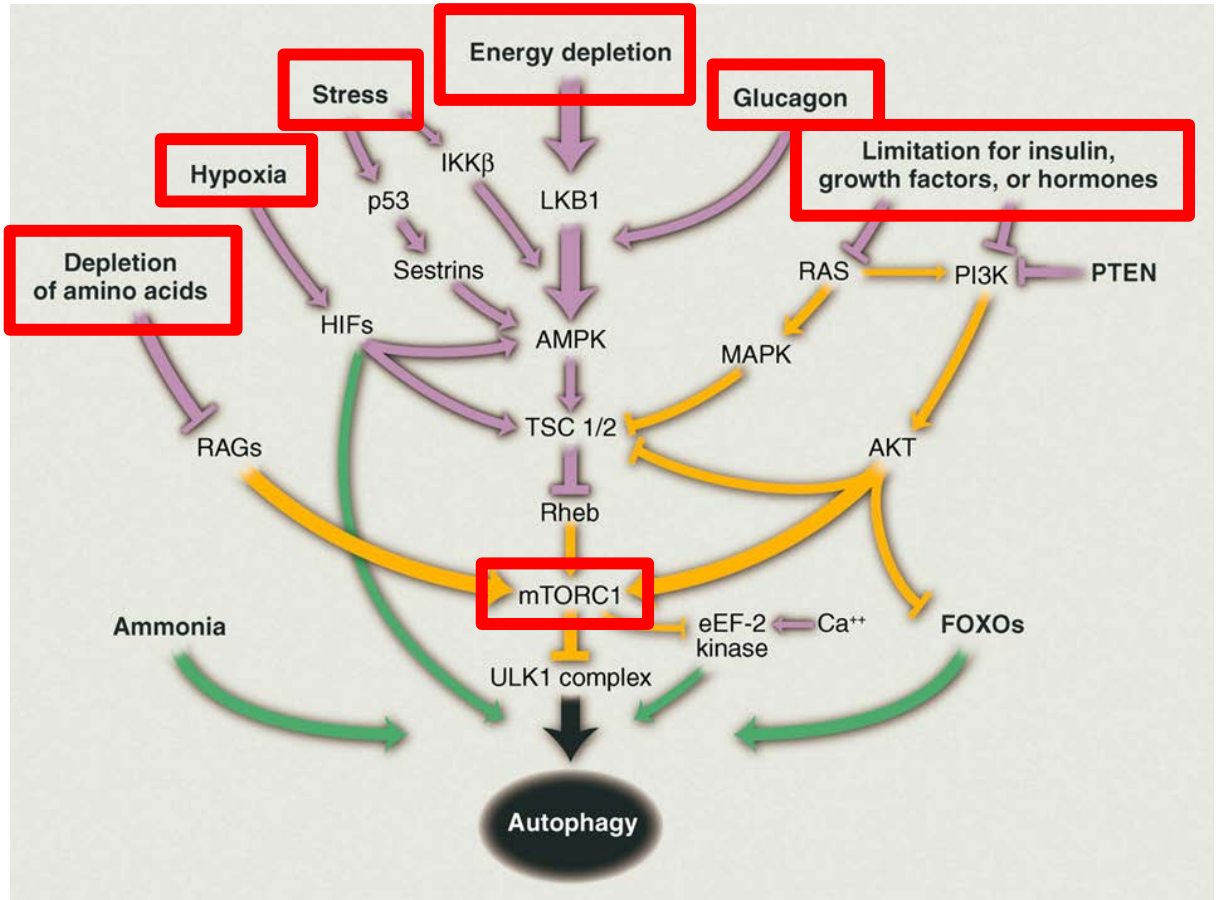
4. **Degradation**
   acid hydrolase degrades the cargos (e.g. cathepsin B, D, L)



Klionsky D.J., 2009 Annual.Rev.Genet.


GFP - Atg5

Signaling pathways regulating Autophagy



- In the presence of abundant nutrients and growth factors including insulin, **mTORC1** promotes cell growth and metabolic activity while suppressing the ULK1 complex and autophagy.
- In deprivation or stress, numerous signaling pathways inactivate mTORC1 kinase activity. This both **suppresses cell growth to reduce energy demand and induces autophagy to enable stress adaptation and survival.**
- Upstream of mTORC1 is the cellular energy–sensing pathway controlled by adenosine monophosphate–activated protein kinase (**AMPK**). High concentrations of AMP signal energy depletion, activate AMPK, and inhibit mTORC1, thus promoting autophagy.
- Hypoxia and activation of hypoxia-inducible factors (HIFs) induce mitophagy.
- Glucagon, a predominant hormone of the fasted state, also triggers autophagy in the liver.

Joshua D. Rabinowitz et al, 2010 Science

Autophagy and Diseases

Neurodegeneration

Pro: Basal autophagy is a homeostatic process that prevents intracellular proteins from accumulating to toxic levels.

Con: Inefficient lysosomal clearance results in intracellular accumulation of autophagosomes, which may process the amyloid precursor protein into toxic forms.

Myopathies

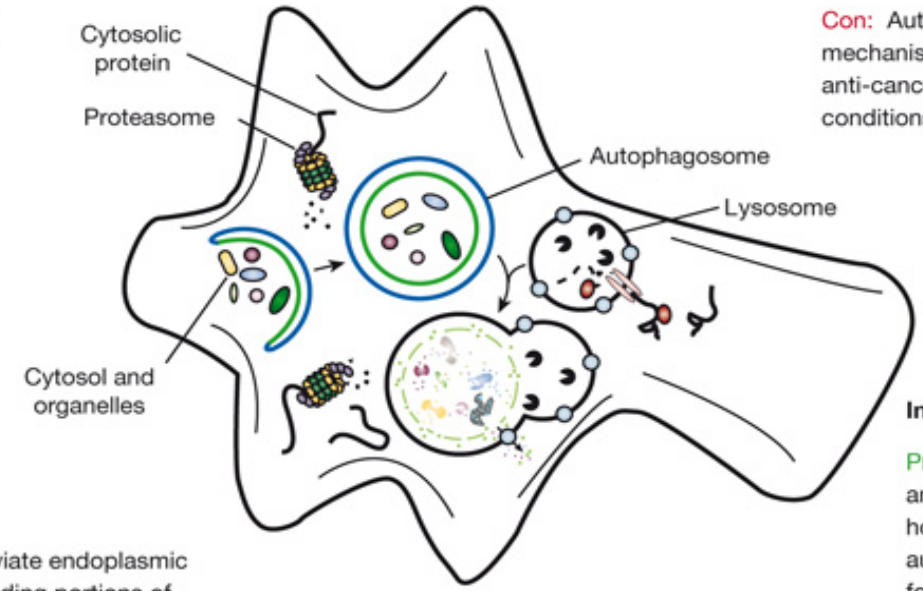
Pro: Autophagy prevents aggregate-prone protein accumulation that leads to physiological dysfunction.

Con: Autophagy may contribute to muscle wasting and defective autophagosome clearance may interfere with cellular function.

Cancer

Pro: Autophagy acts in tumour suppression by removing damaged organelles and possibly growth factors, and reduces chromosome instability.

Con: Autophagy acts as a cytoprotective mechanism that helps cancer cells resist anti-cancer treatments and survive in conditions of low nutrient supply.



Ageing

Pro: Autophagy removes damaged organelles and can limit production of reactive oxygen species.

Liver disease

Pro: Autophagy can alleviate endoplasmic reticulum stress by degrading portions of the organelle containing misfolded proteins.

Con: Excessive autophagy may cause liver damage.

Heart disease

Pro: Autophagy may be protective during ischaemia and pressure overload.

Con: Autophagy is harmful during reperfusion.

Infection and immunity

Pro: Intracellular bacteria, viruses and protozoans are removed from host removed from host cells by autophagy, and antigens are processed for MHC class II presentation. Autophagy may prevent auto-immune and inflammatory diseases.

Con: Some microbes have evolved to subvert autophagy to establish a replicative niche.

Mizushima N. et al, 2008 Nature

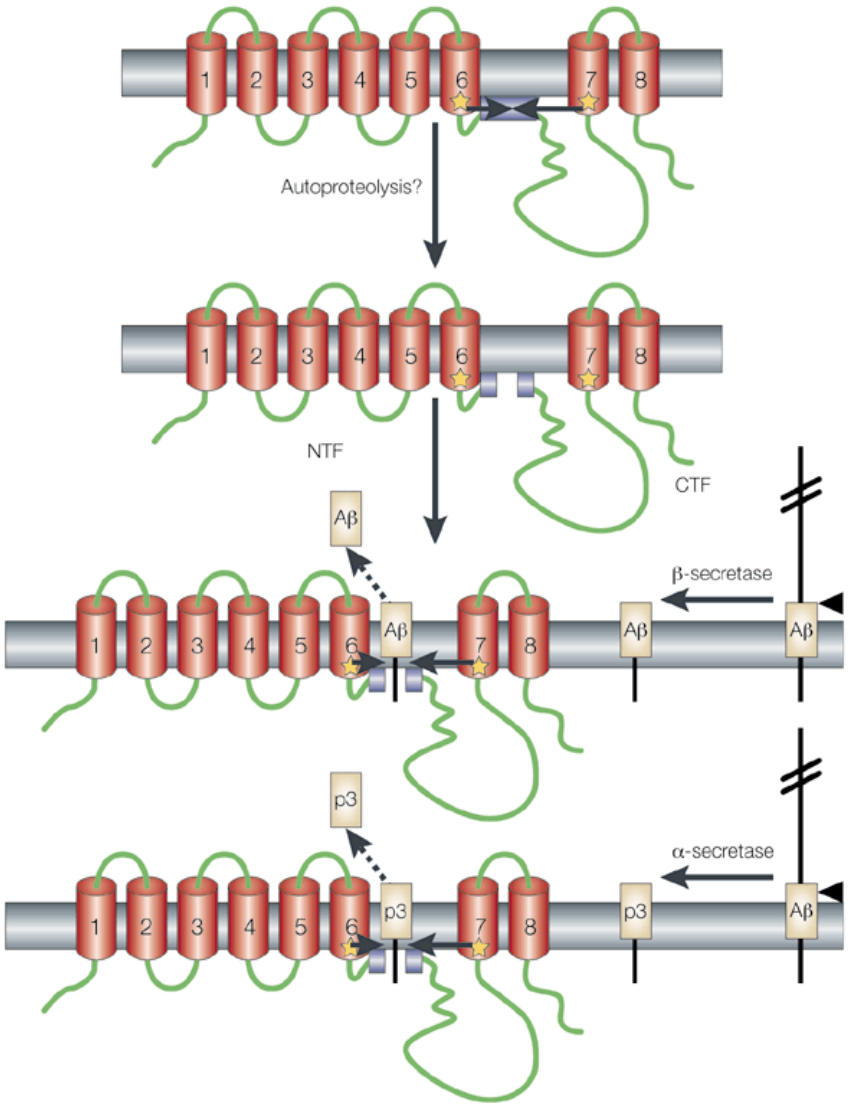
Cell

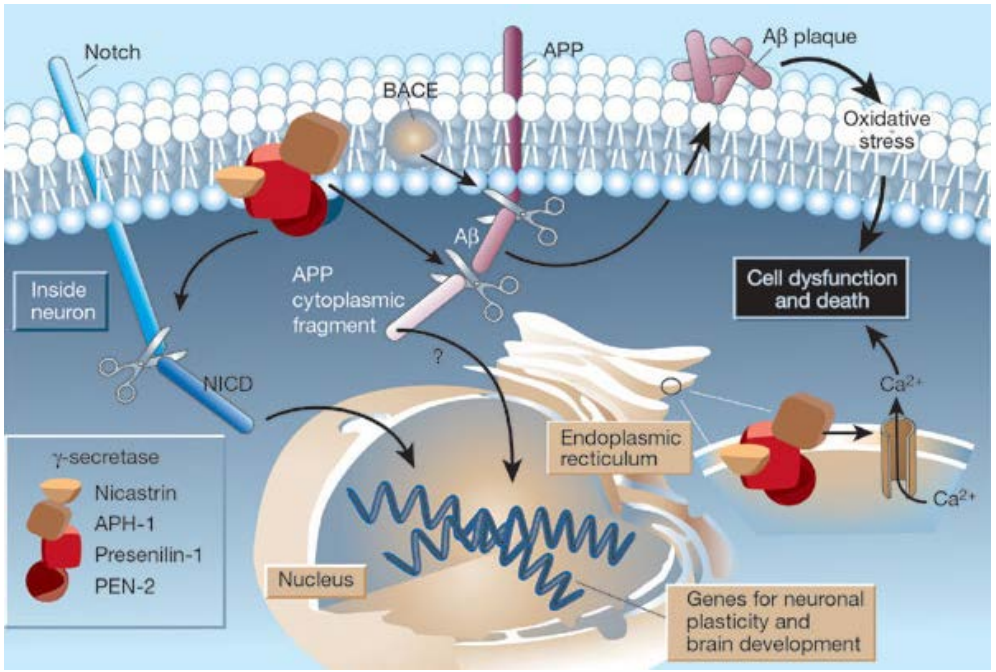
Lysosomal Proteolysis and Autophagy Require Presenilin 1 and Are Disrupted by Alzheimer-Related PS1 Mutations

Ju-Hyun Lee,[1,2] W. Haung Yu,[1,2,9] Asok Kumar,[1,3] Sooyeon Lee,[1,4] Panaiyur S. Mohan,[1,2] Corrinne M. Peterhoff,[1] Devin M. Wolfe,[1] Marta Martinez-Vicente,[6,10] Ashish C. Massey,[6] Guy Sovak,[6,11] Yasuo Uchiyama,[7] David Westaway,[8] Ana Maria Cuervo,[6] and Ralph A. Nixon[1,2,5,*]

- Autophagosome and their contents are cleared upon fusing with lysosomes containing cathepsins, other acid hydrolases, and vacuolar $[H]^+$ ATPase.
- Acidification of autolysosomes is crucial for activating cathepsins and effecting proteolysis of substrates.
- Autophagy pathology in Alzheimer's disease (AD) is exceptionally robust. Autophagic vacuoles (AVs), mostly containing Aβ peptide, collect in massive numbers within grossly distended portions of axons and dendrites of affected neurons >>> **Defective AV clearance.**
- This lysosome-related pathology is greatly accentuated in early-onset familial AD (FAD) due to mutations of Presenilin-1 (PS1).
- Scientific question: **what is the role of PS1 in the context of FAD?**

About PS1

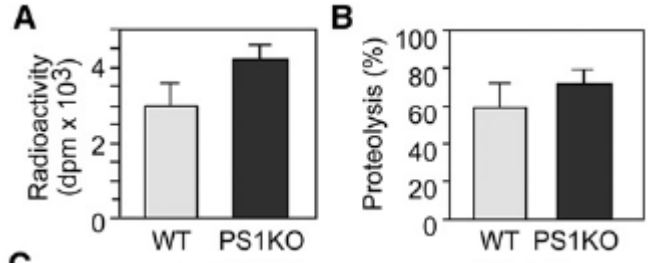


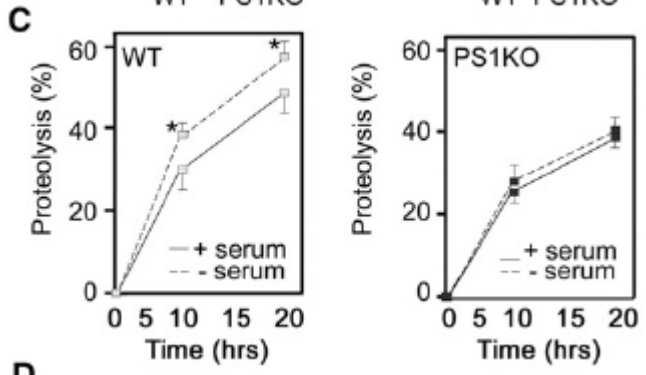


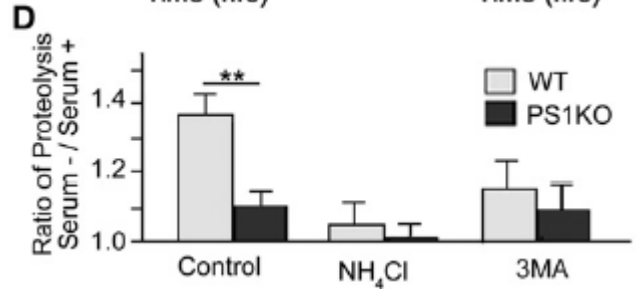
Haass C. et al, 2000 Nature Reviews Mol.Cell B.

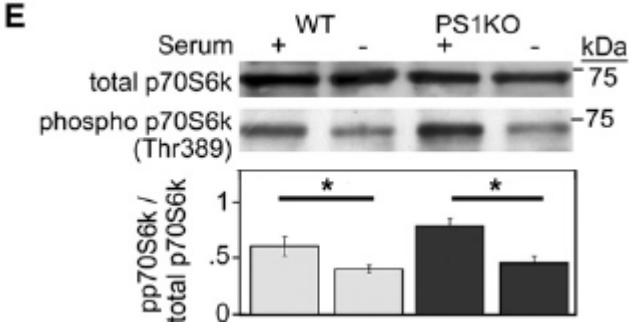
Mattson M., 2003 Nature

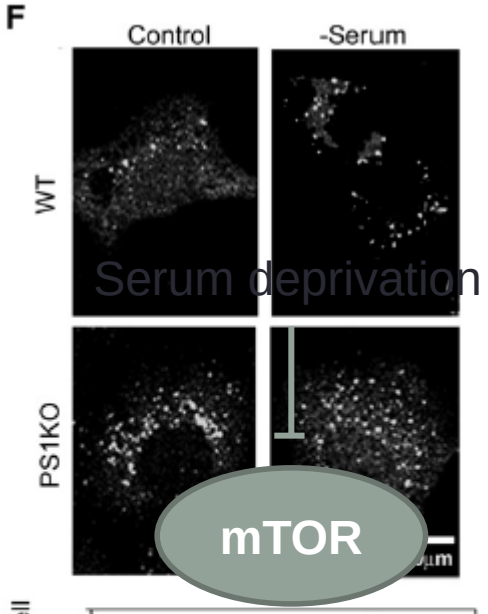
PS1 gene deletion selectively inhibits macroautophagy turnover of proteins

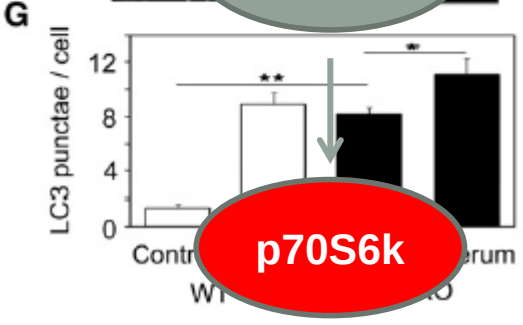


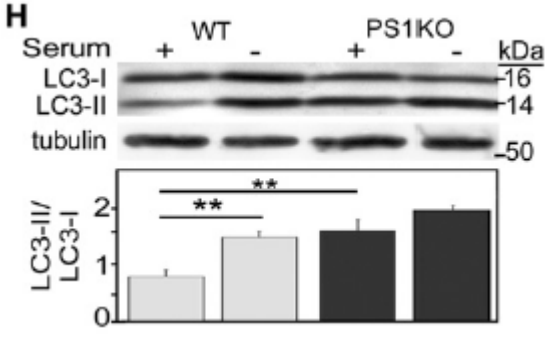


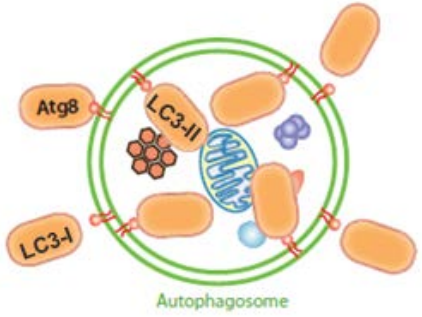






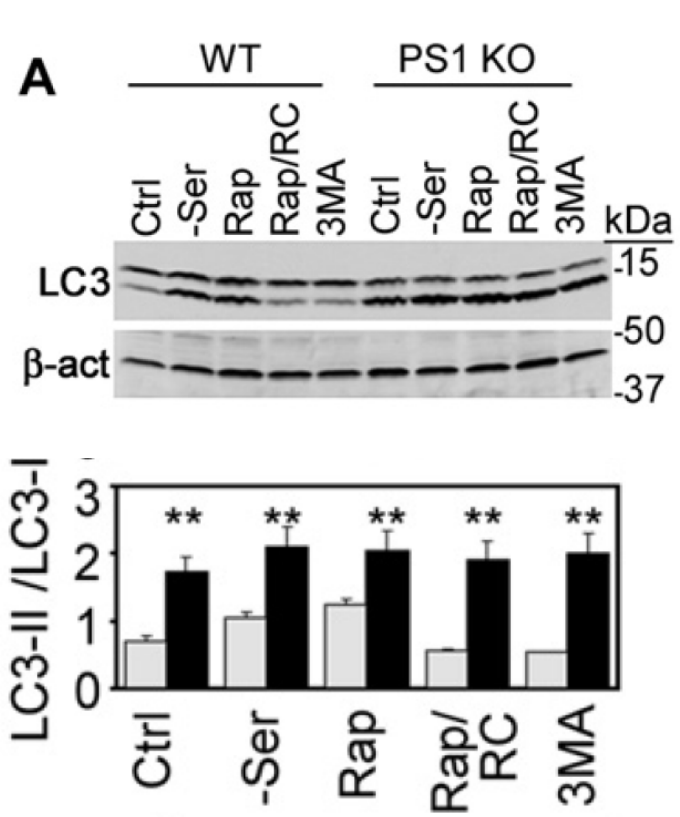


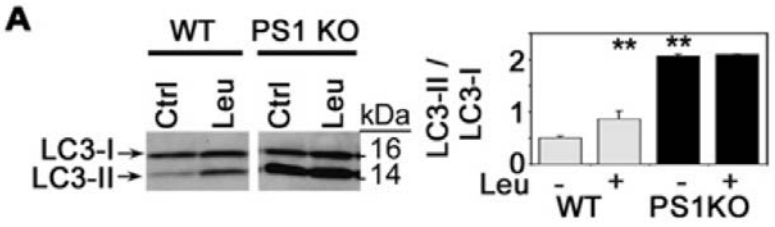


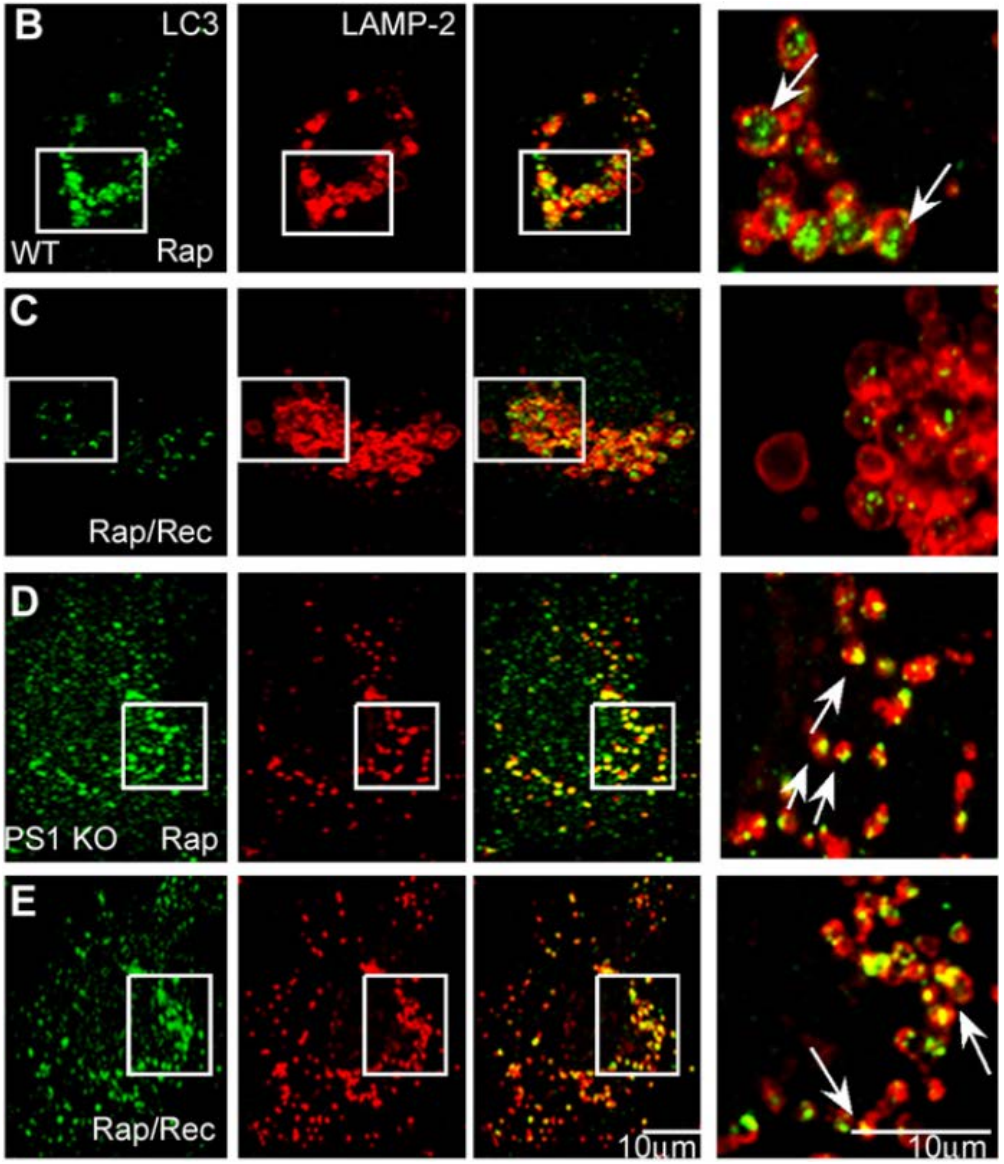


Autophagy inhibitor
3MA = 3-methyladenine

Defective clearance of autophagic vacuoles in PS1 KO blastocysts

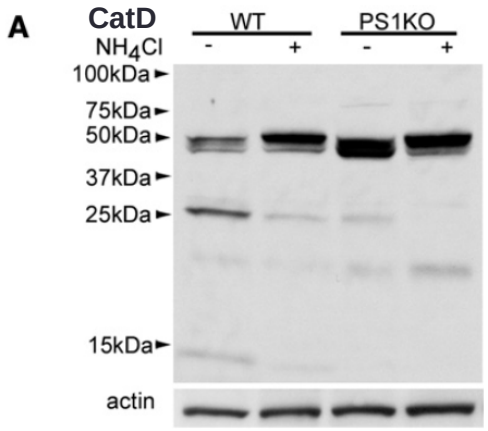


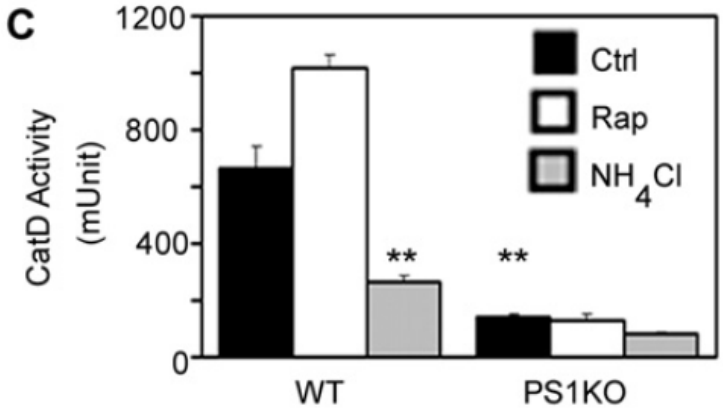


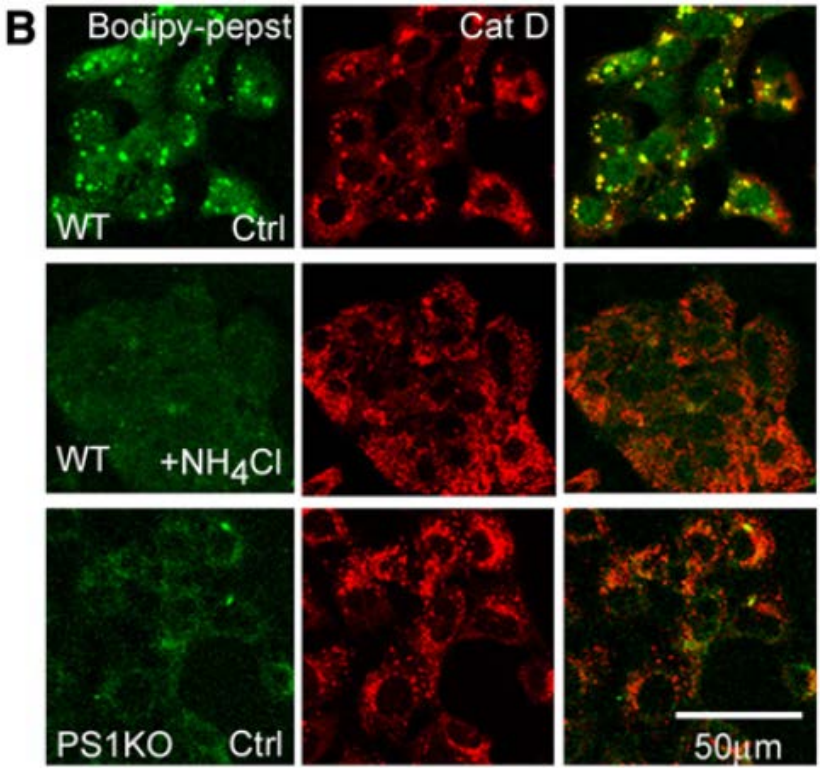


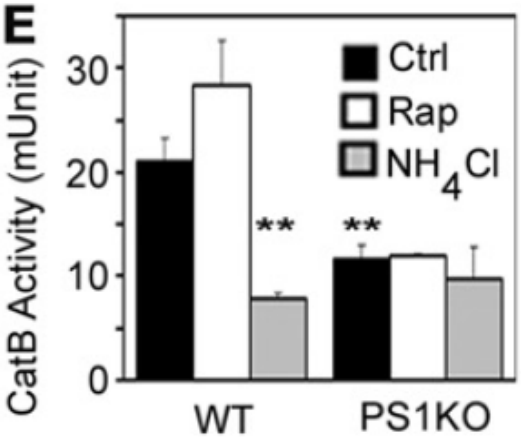
Rap = Rapamycin
RC = Rapamycin Removal

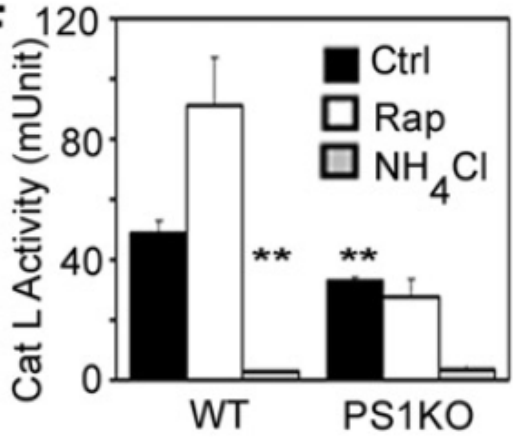
Proteolysis deficits in autolysosomes of PS1 KO blastocysts





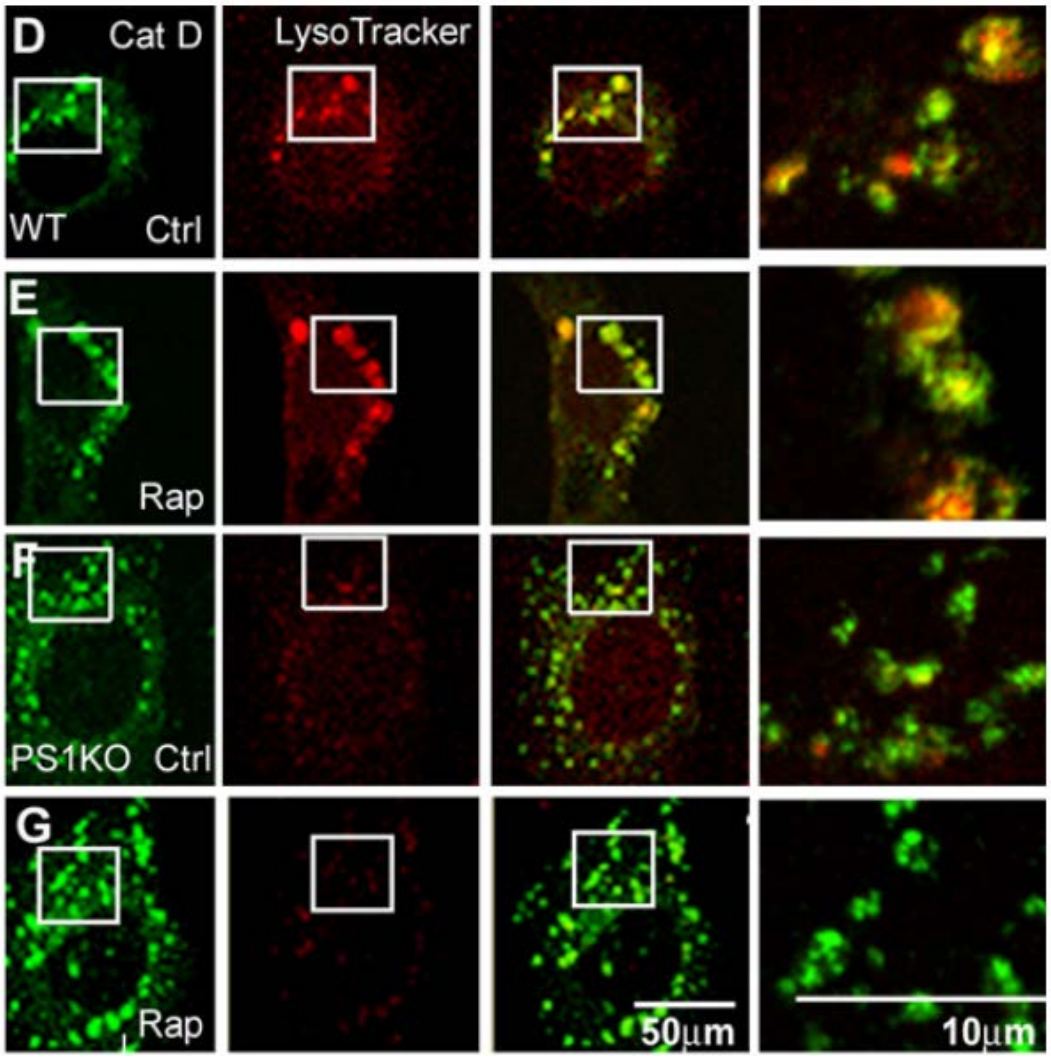


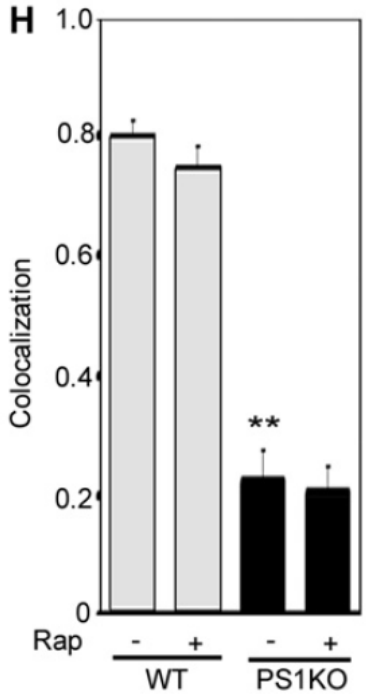


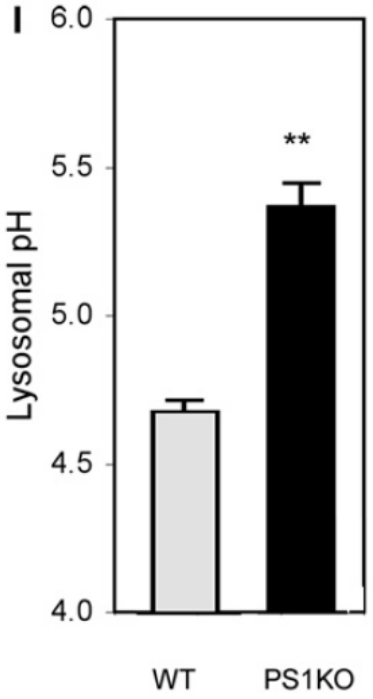


Is lysosome acidification impaired?

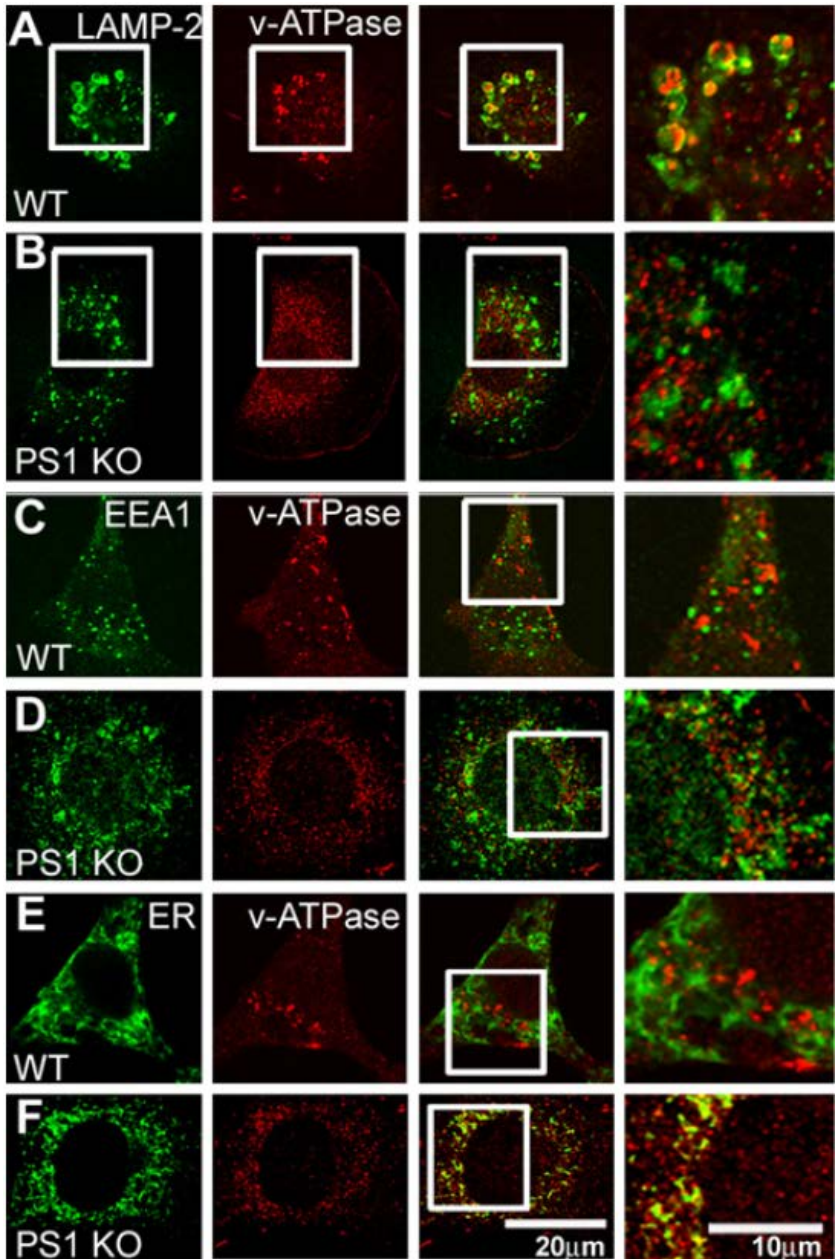
Defective lysosome acidification in PS1 KO blastocysts

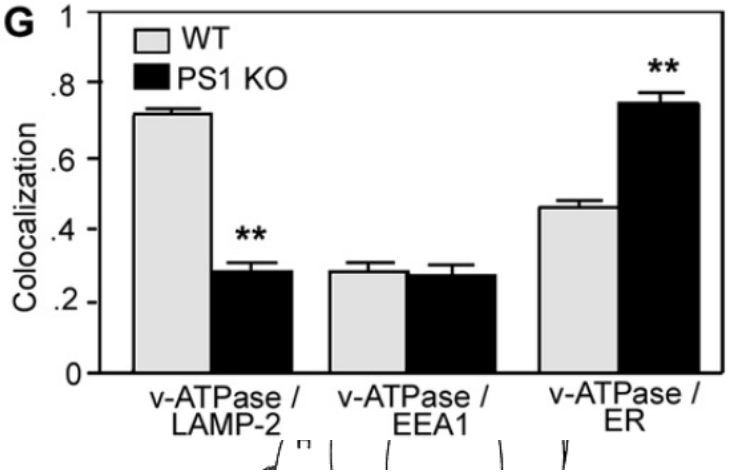


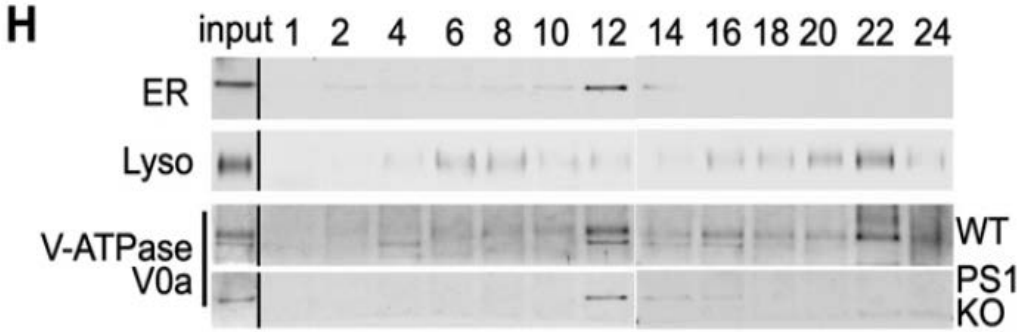


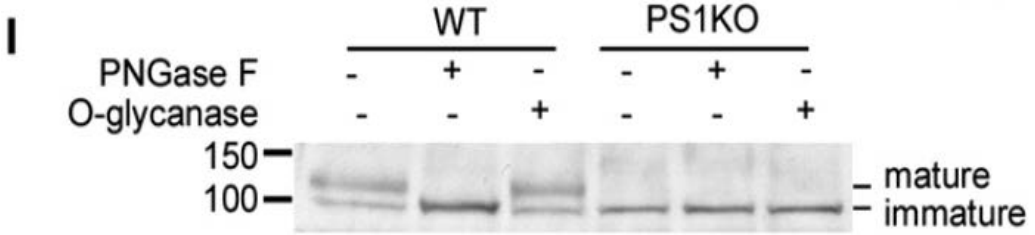


Impaired glycosilation and targeting of the v-ATPase V0a1 subunit in PS1 KO cells



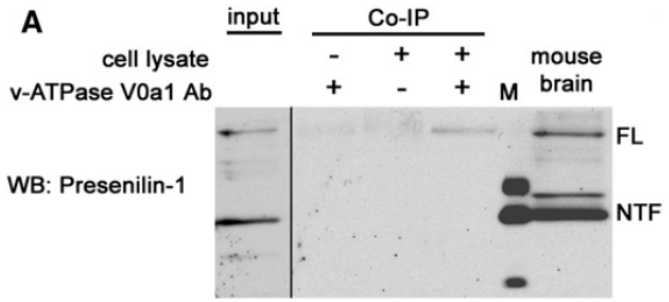


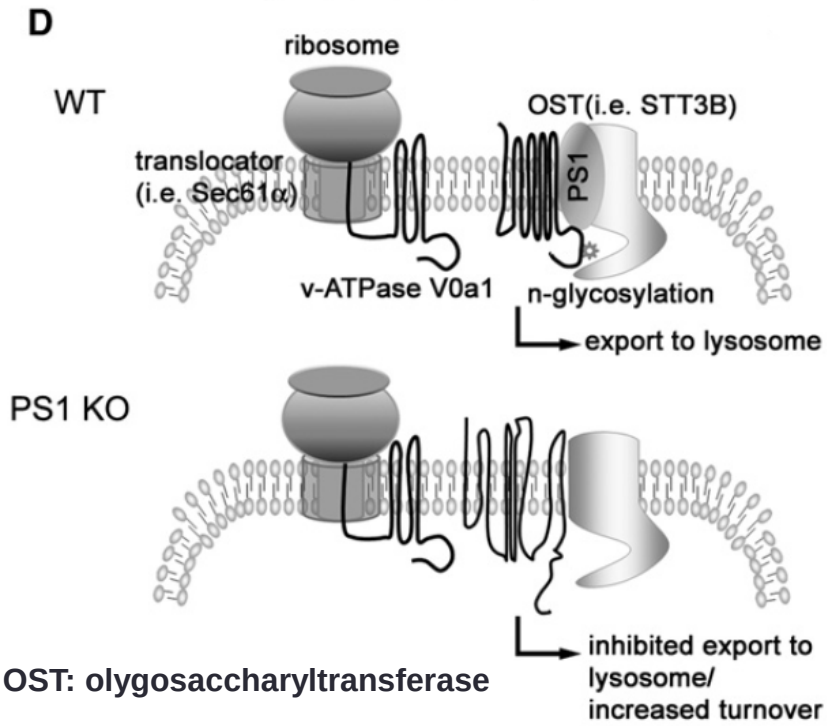




Does PS1 play a role in v-ATPase maturation?

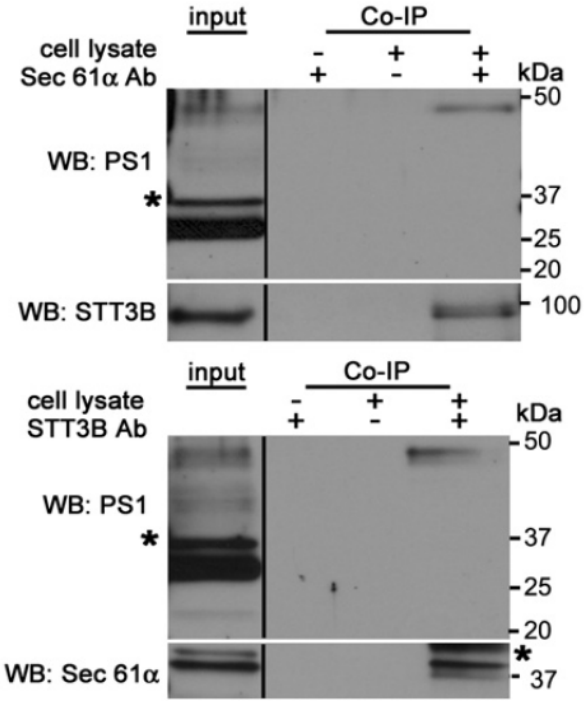
Impaired glycosilation and targeting of the v-ATPase V0a1 subunit in PS1 KO cells





OST: olygosaccharyltransferase

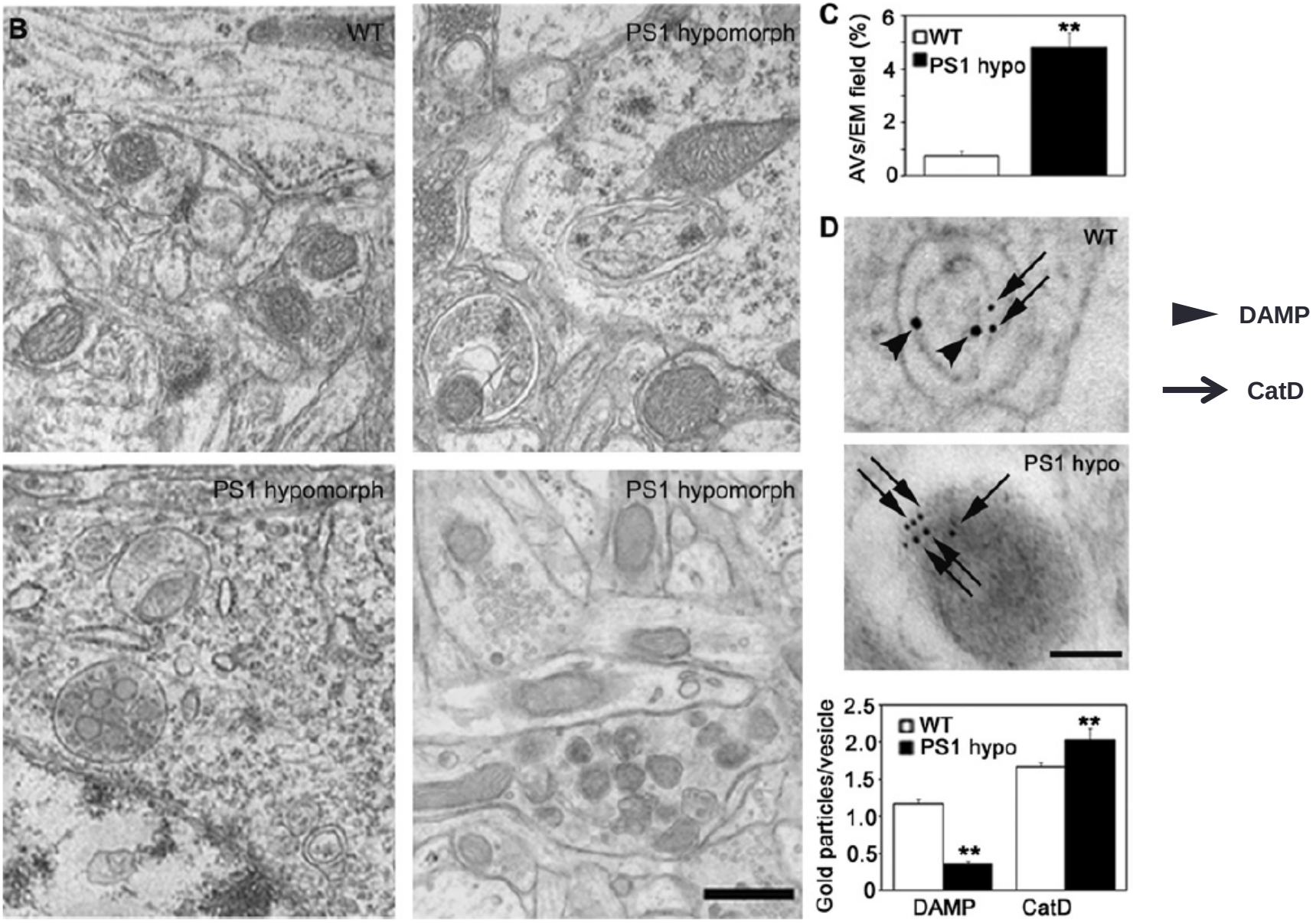
Uncleaved PS1 binds to immature v-ATPase. Does PS1 modulate its maturation in the ER and affect ist delivery to lysosomes?



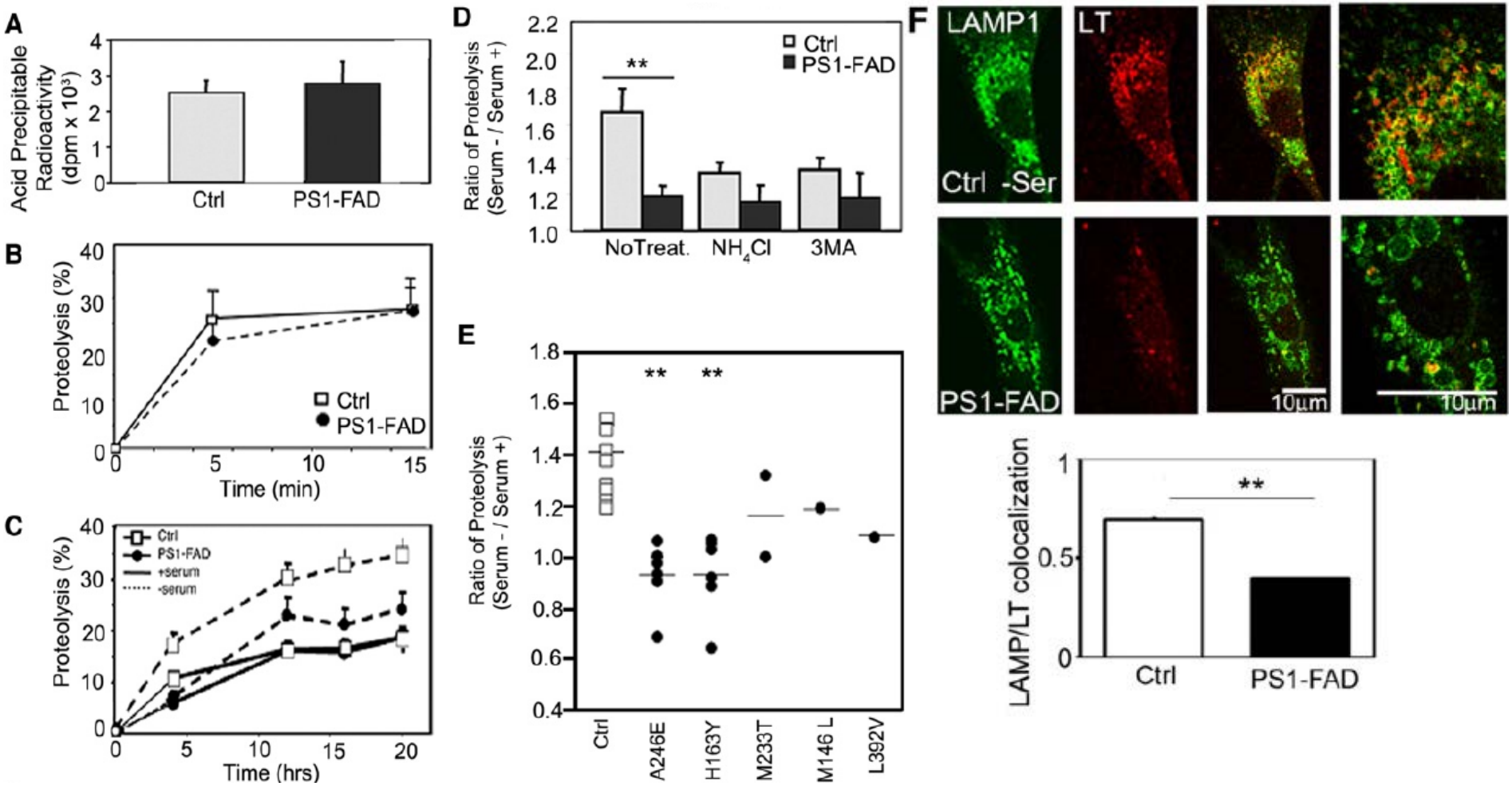
Other ER proteins, such as PDI and GRP94, did not interact

Stable transfection of human PS1 into PS1 KO cells completely restored vesicular compartment acidification, CadD maturation, v-ATPase glycosylation and autophagy response

Defective vesicle acidification and autophagic pathology in neurons of PS1 hypomorphic and PS cKO mice



PS1 mutations impair macroautophagy and v-ATPase targeting in fibroblast from patients with FAD



Conclusions

- This study defines an essential role for PS1 in the maturation and trafficking of the v-ATPase responsible for lysosomal acidification.
- The normal turnover of protein and organelles by autophagy is impaired if PS1 is ablated or mutated.
- The loss of lysosomal function is accountable for the marked acceleration of autophagy-related dysfunction and neuronal cell death associated with PS1-FAD.

Speculations

- Impaired lysosomal clearance could account for reported PS1-mediated increases in Aβ.
- Similarities between the severe autophagy pathology in PS1-FAD and that developing with a later onset in sporadic AD suggests that lysosomal dysfucntion is also a pathogenic mechanism in the common sporadic form of AD.

doi:10.1093/brain/awq341 Brain 2011: 134; 258–277 | 258

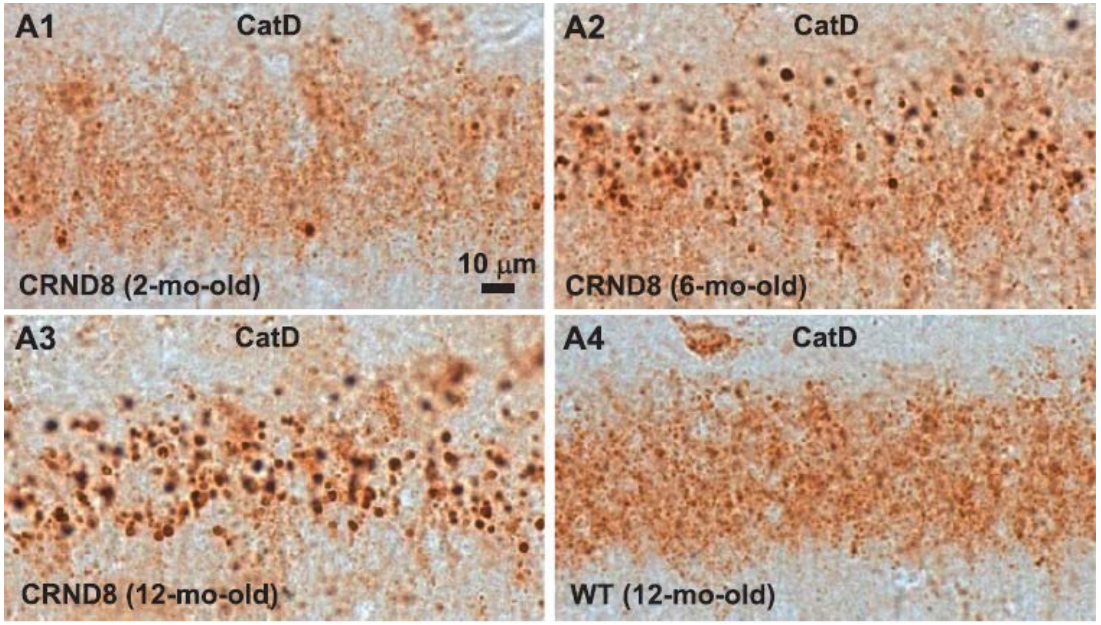
BRAIN
A JOURNAL OF NEUROLOGY

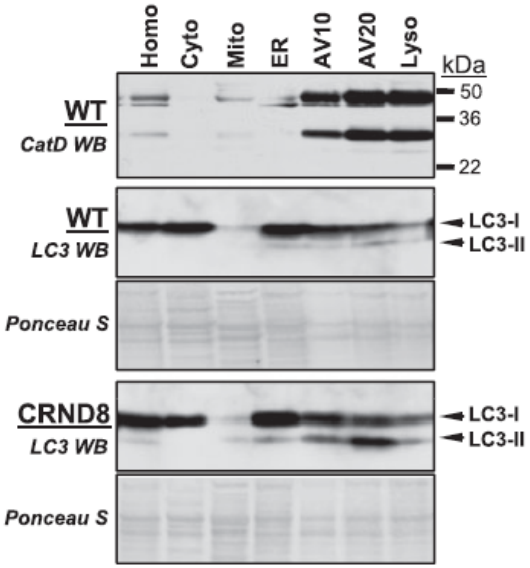
Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits

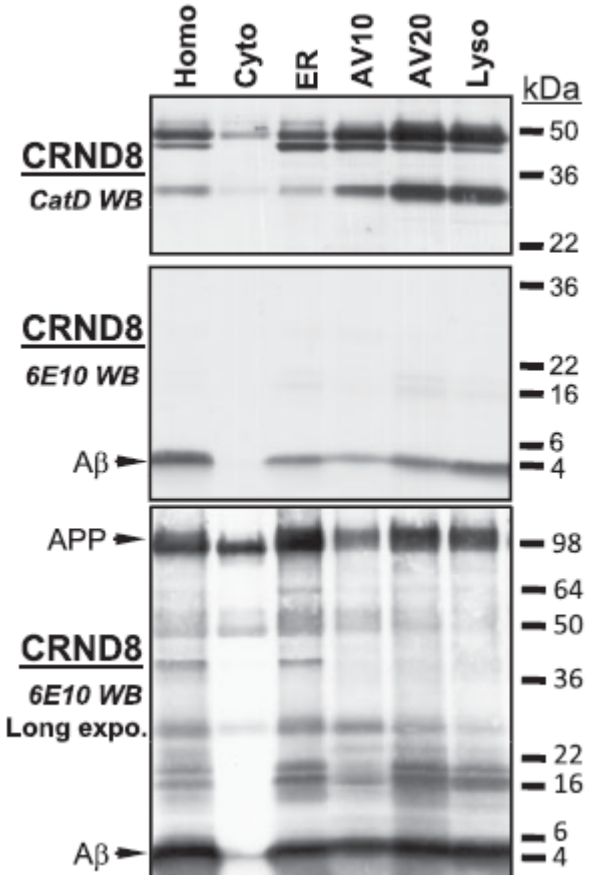
Dun-Sheng Yang,[1,2] Philip Stavrides,[1] Panaiyur S. Mohan,[1,2] Susmita Kaushik,[3] Asok Kumar,[1,2] Masuo Ohno,[1,2] Stephen D. Schmidt,[1] Daniel Wesson,[4,5] Urmi Bandyopadhyay,[3] Ying Jiang,[1,2] Monika Pawlik,[1] Corrinne M. Peterhoff,[1] Austin J. Yang,[6] Donald A. Wilson,[4,5] Peter St George-Hyslop,[7] David Westaway,[8] Paul M. Mathews,[1,2] Efrat Levy,[1,2,9] Ana M. Cuervo[3] and Ralph A. Nixon[1,2,10]

- TgCRND8 mice, overexpressing a version of APP695 including Swe and Ind mutations and producing more Aβ42 than Aβ40, develop lysosomal system pathology, accumulate intraneuronal Aβ and robustly deposit β-amyloid extracellularly in neuritic plaques.
- TgCRND8 mice were crossed with Cystatin B KO mice (CBKO), in order to relieve inhibition of multiple cathepsins, improving lysosomal proteolytic function in TgCRND8 mice.
- Scientific question: **does improved lysosomal activity amielorate AD-related pathologies?**

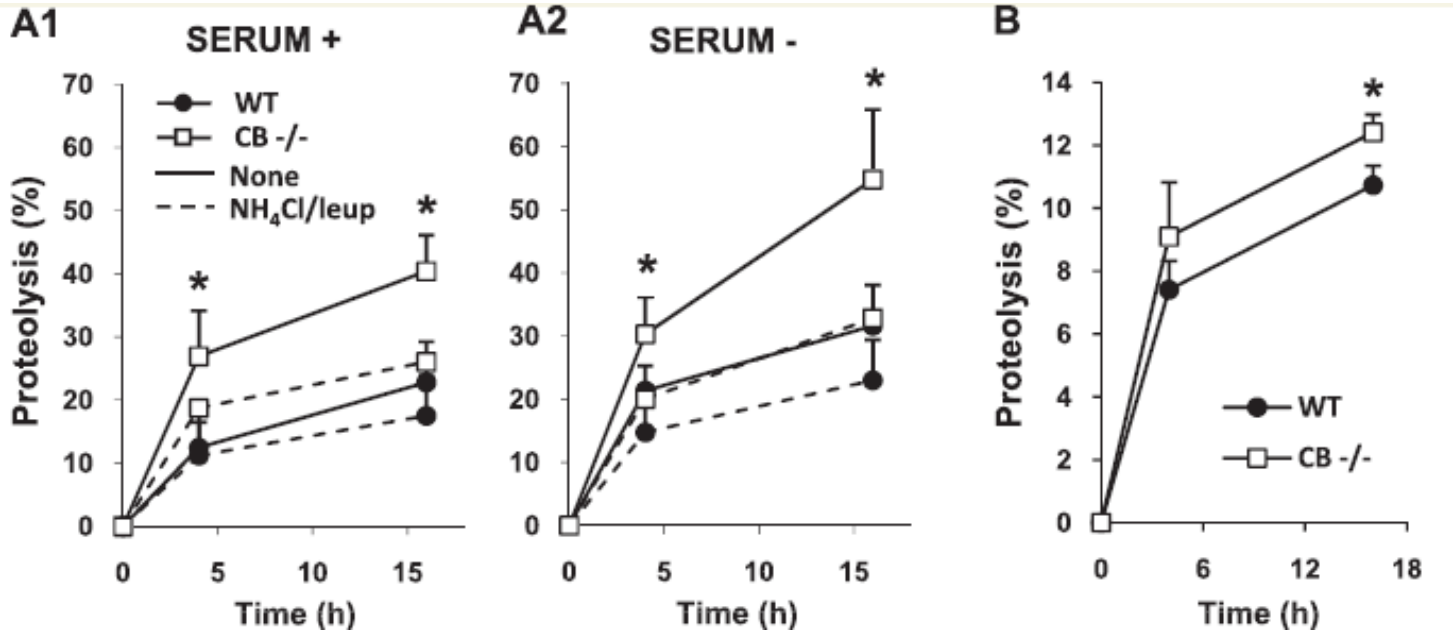
TgCRND8 mice exhibit marked autophagic-lysosomal dysfunction



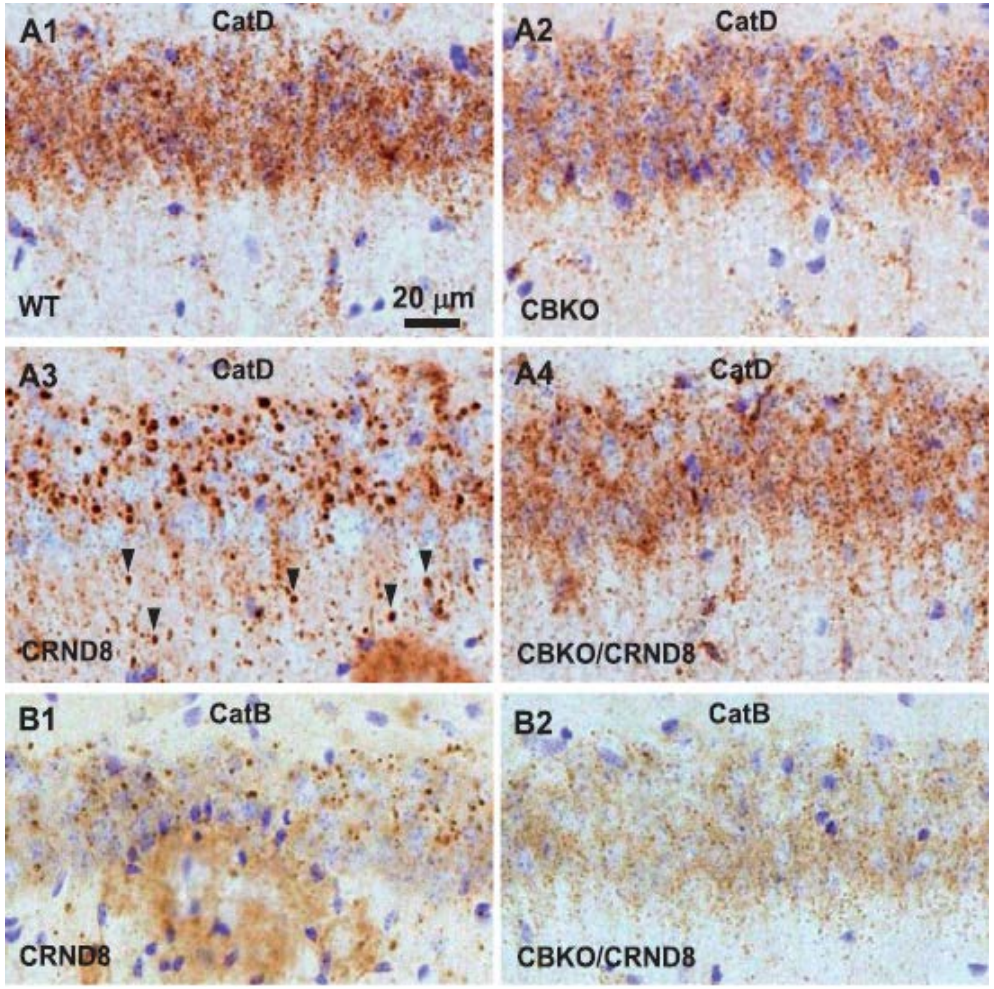


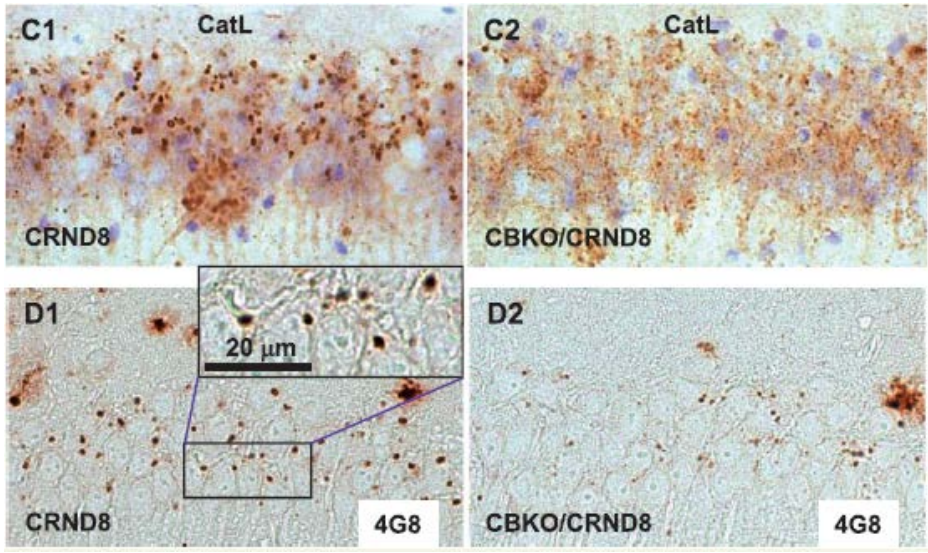


Deletion of cystatin B enhances lysosomal activities and accelerates protein turnover

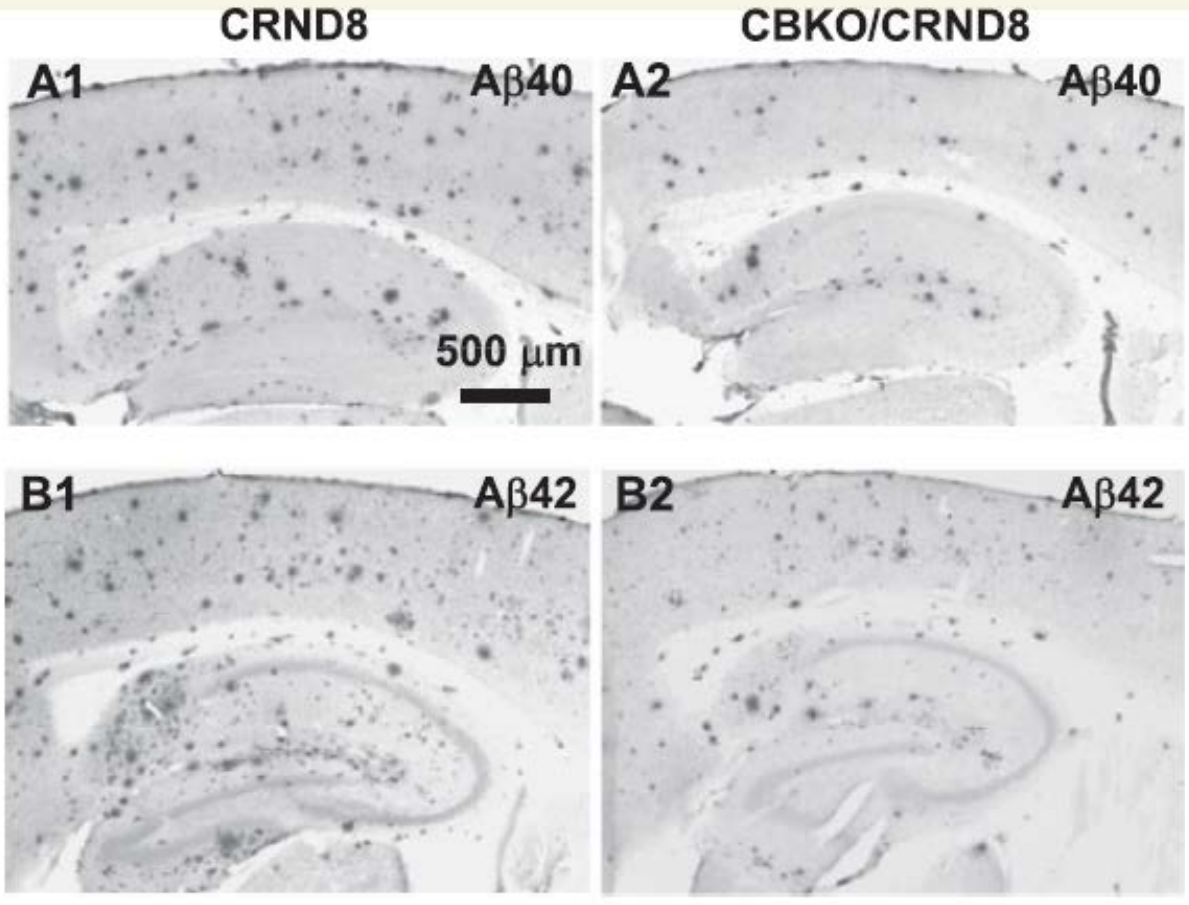


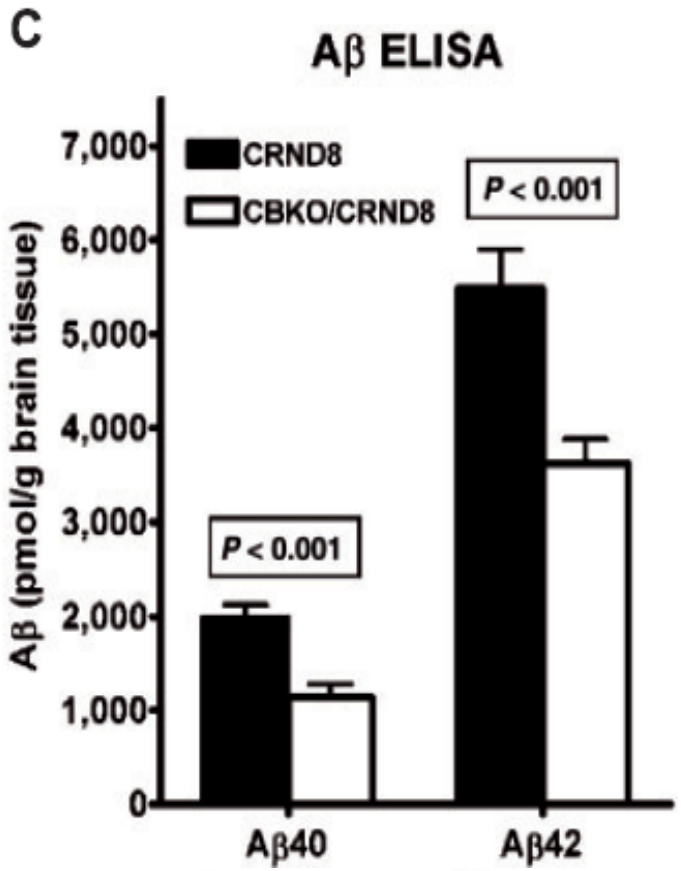
Deletion of cystatin B eliminates giant autolysosomes in the brain of CBKO/TgCRND8 mice



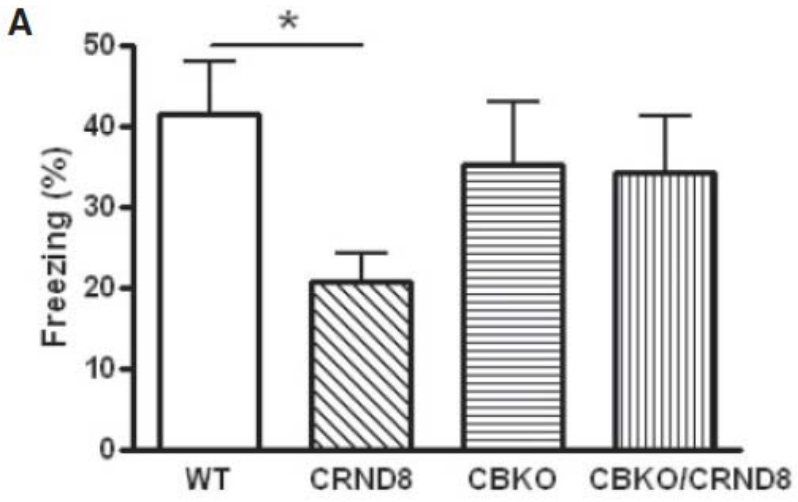


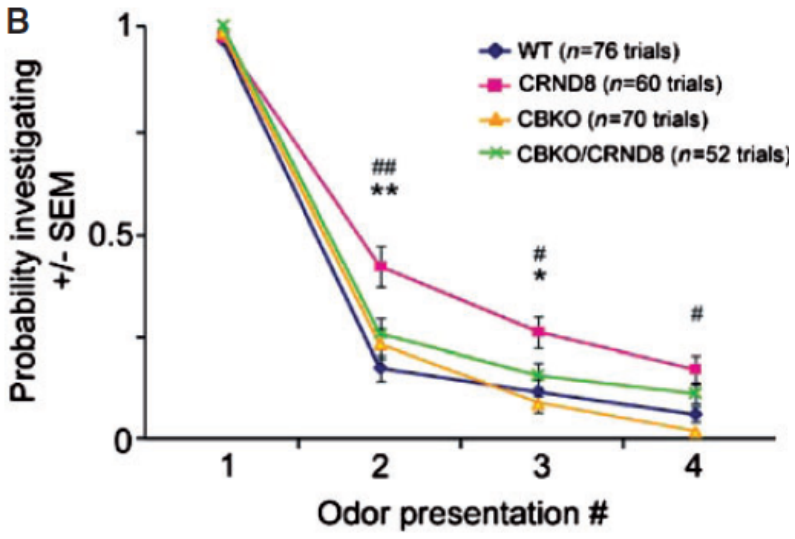
Deletion of cystatin B reduces the amyloid load and Aβ level in the brain of CBKO/TgCRND8 mice





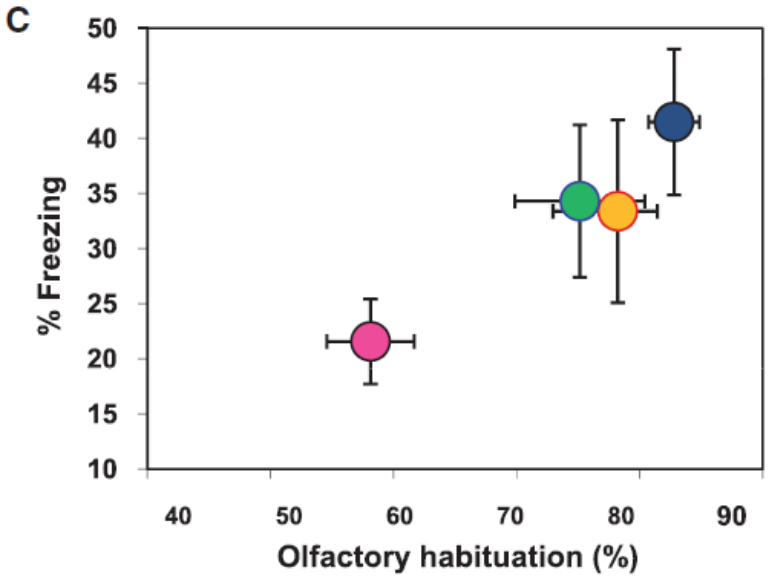
Cystatin B deletion restores learning and memory functions in CBKO/TgCRND8 mice





Hippocampus-dependent contextual fear conditioning

CBKO mice can improve contextual memory deficits found in TgCRND8 mice



Odor habituation test

TgCRND8 mice had an increased latency to habituate to novel odours in comparison to age-matched controls. CBKO/TgCRND8 mice did not differ from wild-type mice

Conclusions

- TgCRND8 mice show aggressive amyloidosis and neuritic plaque development, accompanied by extensive autophagic-lysosomal pathology similar to that seen in AD brain, reflecting defective proteolytic clearance of autophagic substrate.

- Proof of concept: partially restoring of lysosomal proteolytic function in TgCRND8 mice significantly ameliorates lysosomal system pathology, intra-neuronal Aβ accumulation, amyloid plaque formation, and memory and learning deficits in the TgCRND8 model.

- These effects underscore the pathogenic significance of lysosomal system dysfunction in AD and they demonstrate the value of reversing this dysfunction as a potential therapy for AD and other neurodegenerative diseases.

Science
AAAS

Impaired Degradation of Mutant α-Synuclein by Chaperone-Mediated Autophagy

Ana Maria Cuervo *et al.*

Science **305**, 1292 (2004);
DOI: 10.1126/science.1101738

α-Synuclein impairs macroautophagy: implications for Parkinson's disease

JCB: Article

Ashley R. Winslow,[1] Chien-Wen Chen,[1,3] Silvia Corrochano,[4] Abraham Acevedo-Arozena,[4] David E. Gordon,[2] Andrew A. Peden,[2] Maike Lichtenberg,[1] Fiona M. Menzies,[1] Brinda Ravikumar,[1] Sara Imarisio,[1,3] Steve Brown,[4] Cahir J. O'Kane,[3] and David C. Rubinsztein[1]

Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease

nature neuroscience

Marta Martinez-Vicente[1,3,4], Zsolt Talloczy[2–4], Esther Wong[1,4], Guomei Tang[2], Hiroshi Koga[1], Susmita Kaushik[1], Rosa de Vries[2], Esperanza Arias[1], Spike Harris[2], David Sulzer[2] & Ana Maria Cuervo[1]

12396 • The Journal of Neuroscience, September 5, 2012 • 32(36):12396–12405
Neurobiology of Disease

Rapamycin Delays Disease Onset and Prevents PrP Plaque Deposition in a Mouse Model of Gerstmann–Sträussler–Scheinker Disease

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Table 1 | Classification and treatment of disorders of autophagy

Condition	Impairment	Pathway or stage of autophagy	Potential pharmacological treatment
Lafora disease	Loss-of-function mutation in *EPM2A*[37]	Initiation	Rapamycin
Huntington disease	Expanded polyglutamine domain of huntingtin protein[42]	Cargo recognition	Rapamycin, rilmenidine, clonidine, carbamazepine, valproate
Frontotemporal dementia	Mutations in *CHMP2B*[49,50]	Maturation	Unknown*
Amyotrophic lateral sclerosis	Mutations in *DCTN1* and *DNCHC1*[43–46]	Maturation	Unknown*
Lysosomal storage disorders	Dysfunction of lysosomal hydrolases and other lysosomal proteins[54]	Lysosomal fusion and clearance	Unknown*
Familial Alzheimer disease	Mutation in *PSEN1*, *PSEN2* and *APP*[56–61]	Lysosomal fusion and clearance	Unknown*
Parkinson disease	Mutations in *PINK* and *PARK*[70–77]	Mitophagy	Unknown

*Upregulation of autophagy might be unwise in patients who have defective autophagosome degradation.

LETTER

doi:10.1038/nature10758

Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis

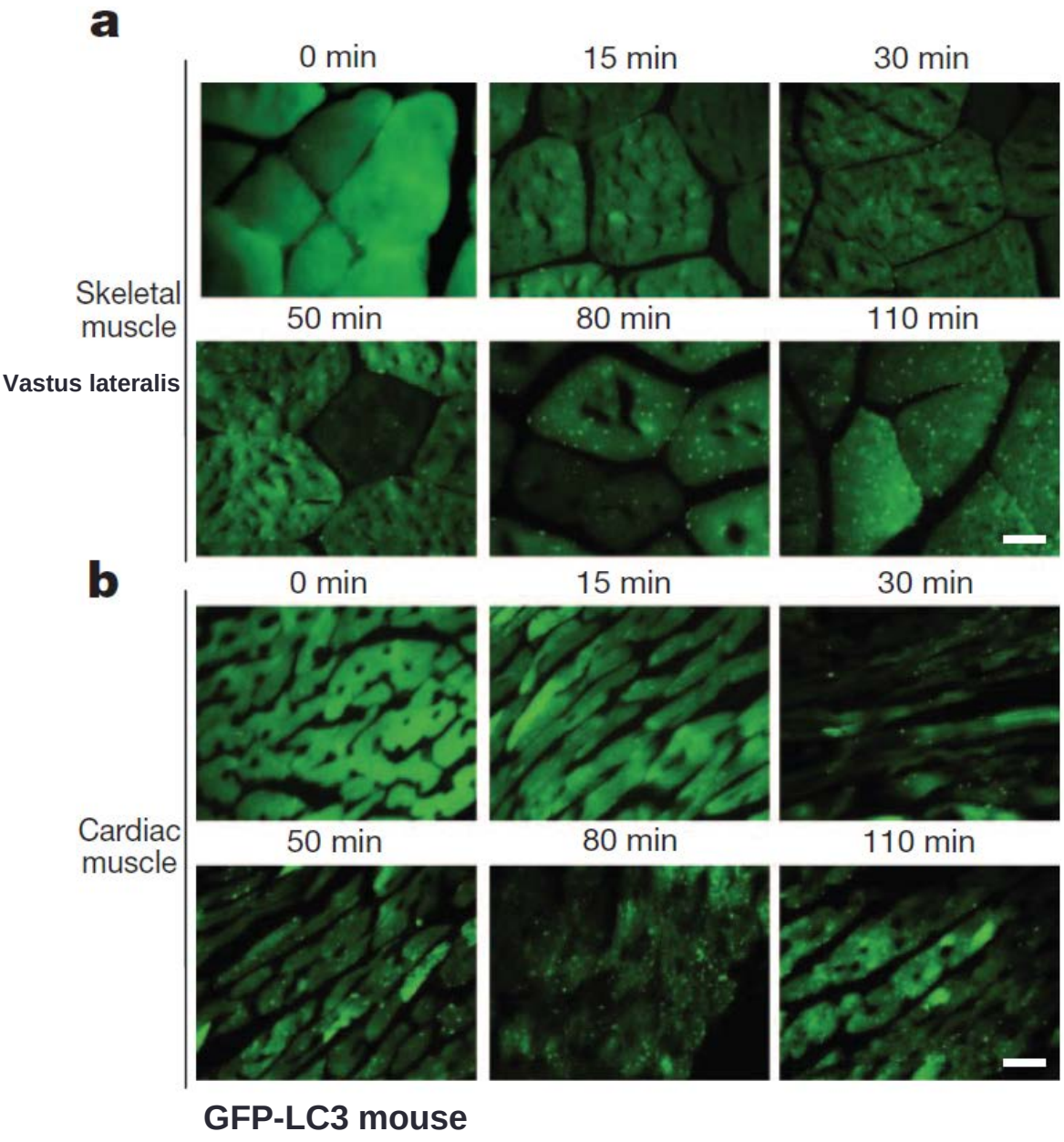
Congcong He[1,2,3]*, Michael C. Bassik[4]†*, Viviana Moresi[5], Kai Sun[2,6], Yongjie Wei[1,2,3], Zhongju Zou[1,2,3], Zhenyi An[1,2], Joy Loh[7], Jill Fisher[4], Qihua Sun[1,2], Stanley Korsmeyer[4]‡, Milton Packer[8], Herman I. May[2], Joseph A. Hill[2], Herbert W. Virgin[7], Christopher Gilpin[9], Guanghua Xiao[8], Rhonda Bassel-Duby[5], Philipp E. Scherer[2,6] & Beth Levine[1,2,3,10]

Exercise has beneficial effects on human health, including protection against metabolic disorders such as diabetes.

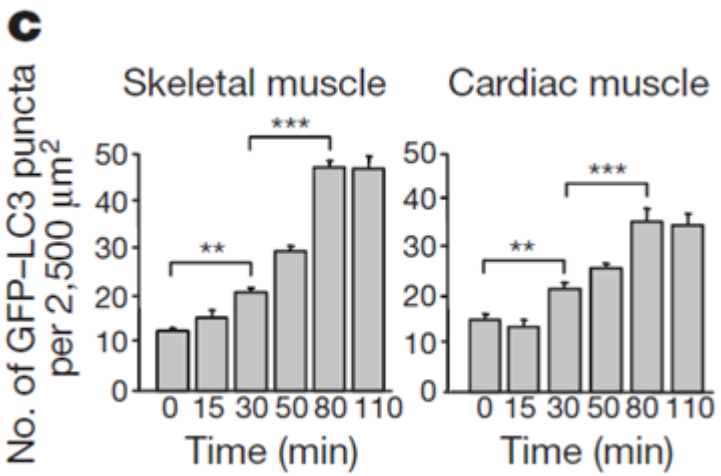
The cellular mechanisms underlying these effects are incompletely understood.

- During stress, increased levels of autophagy permit cells to adapt to changing nutritional and energy demands through protein catabolism.
- In animal models, autophagy protects against diseases such as cancer, neurodegenerative disorders, infections, inflammatory diseases, ageing and insulin resistance.
- Scientific question: **is autophagy implicated in exercise-induced health?**

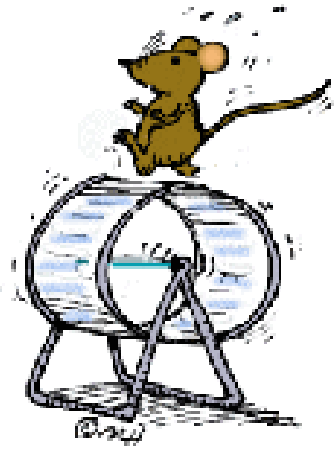
Exercise induces autophagy in skeletal and cardiac muscle



GFP-LC3 mouse



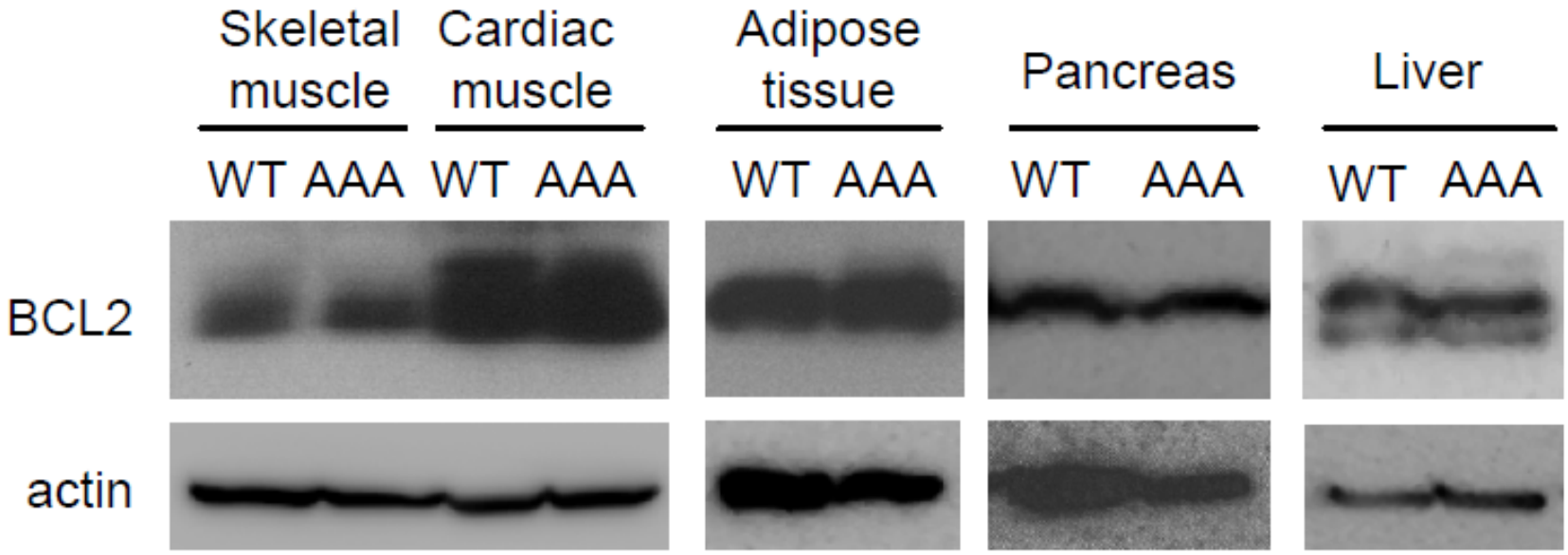
Similar results were observed in solelus, tibialis anterior, extensor digitorum longus, liver and pancreas



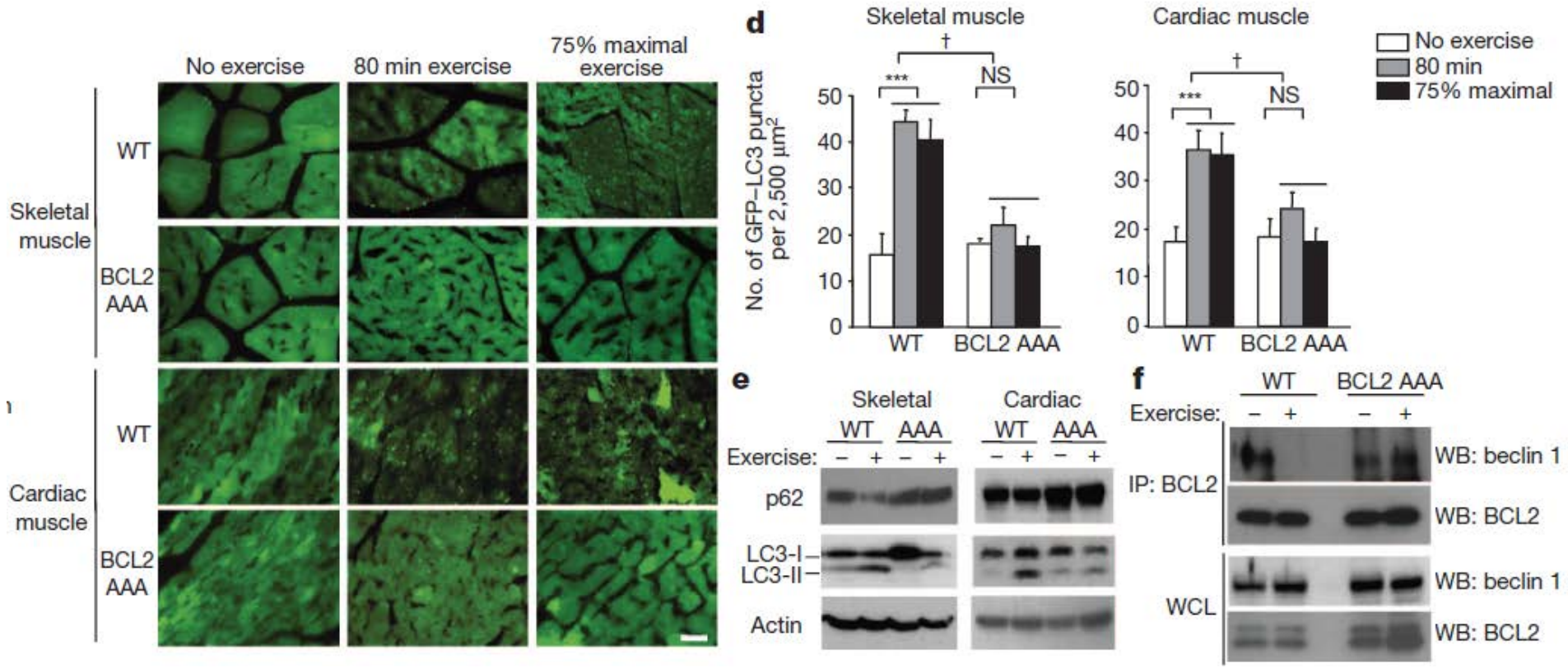
Generation of BCL2 AAA knock-in mice

- Phosphorylation of three sites (Thr69, Ser70, Ser84) in the non-structured loop of human BCL2 is critical for stimulus-induced autophagy

- A knock-in mouse was generated; Thr69, Ser70, Ser84 were replaced with three Ala (AAA) > BCL2 could not free beclin-1



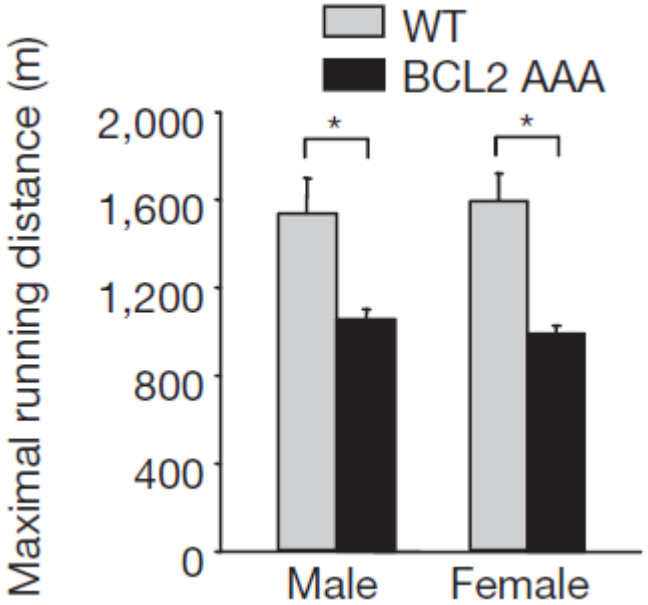
BCL2 AAA mice do not show exercise- and starvation-induced autophagy

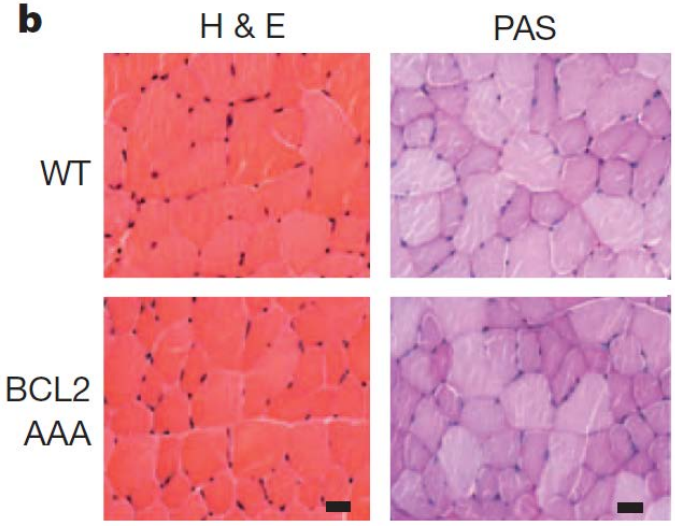


Similar results were observed in liver and pancreas

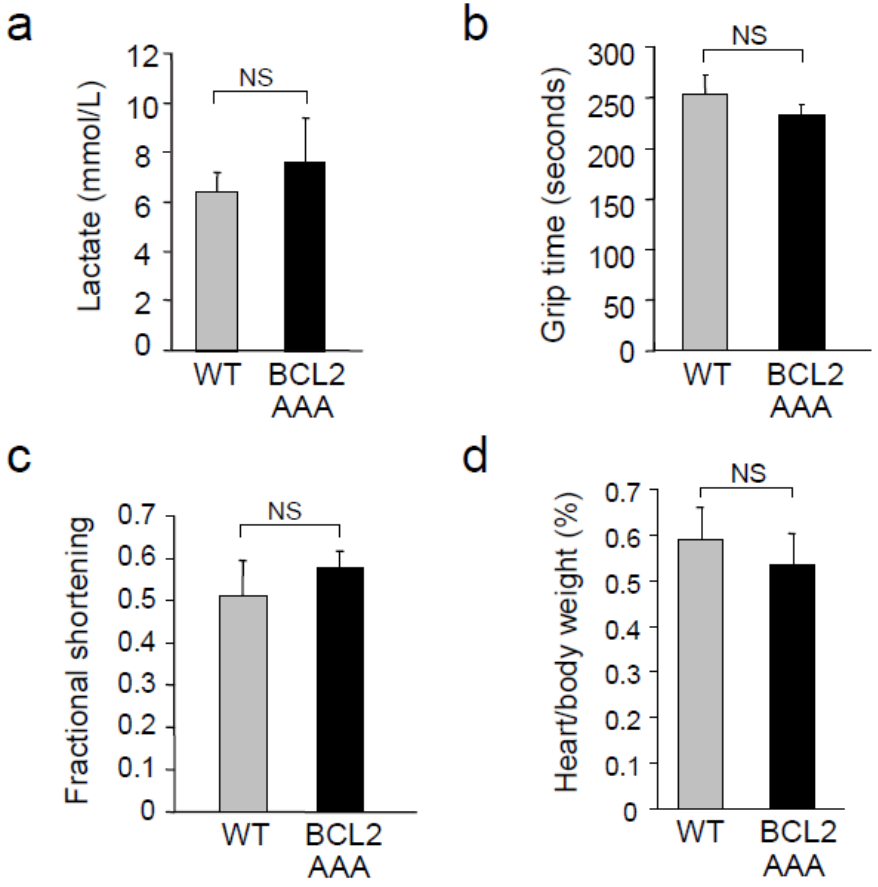
Non-phosphorylatable BCL2 does not alter basal autophagy *in vivo*, but prevents autophagy activation in response to starvation and exercise.

BCL2 AAA mice show deficient exercise endurance



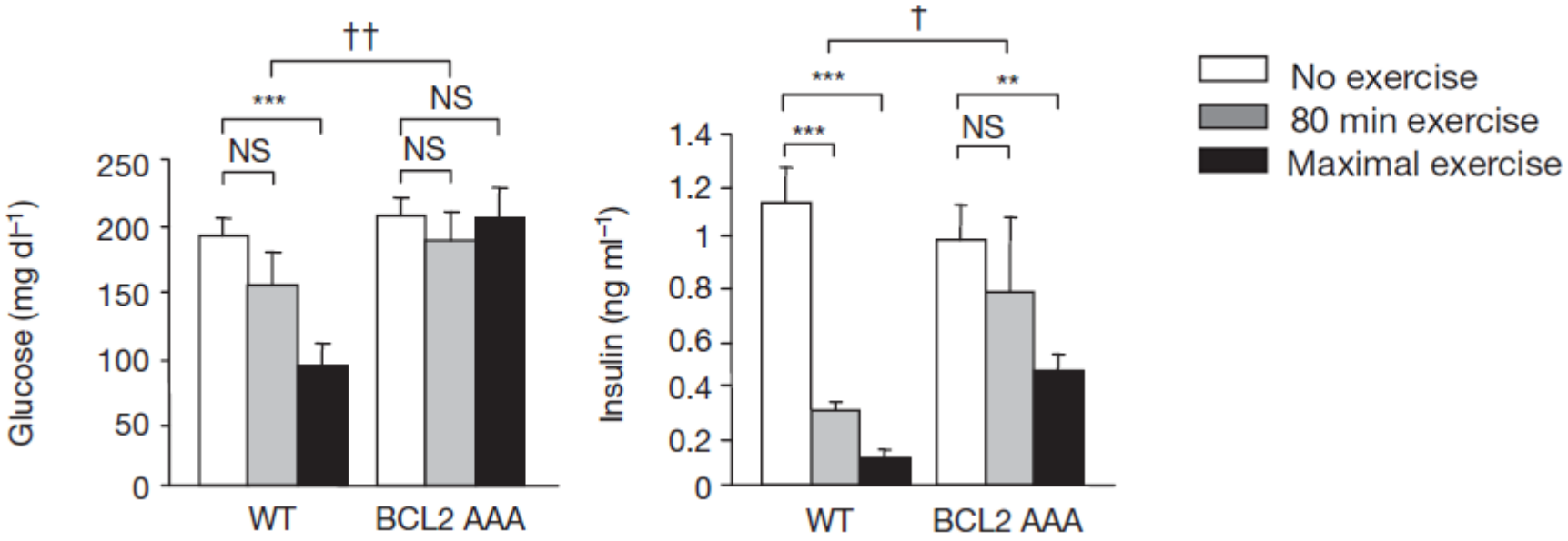


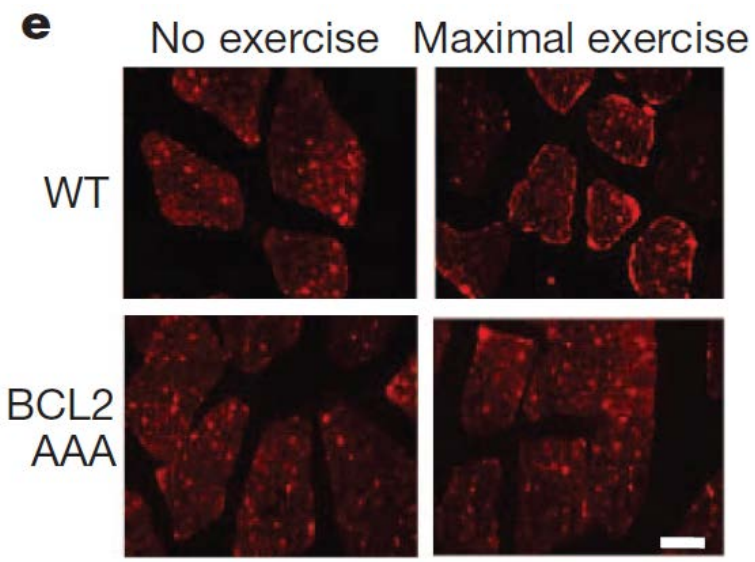
tibialis anterior muscle



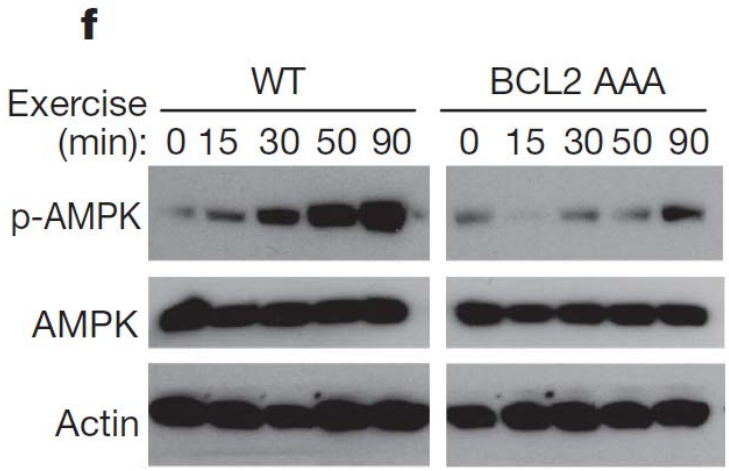
After 80 min exercise, no differences in weight, fibre cross-sectional area, glycogen content, morphology, and mitochondrial content and functionality

BCL2 AAA mice show alteration in muscle glucose metabolism



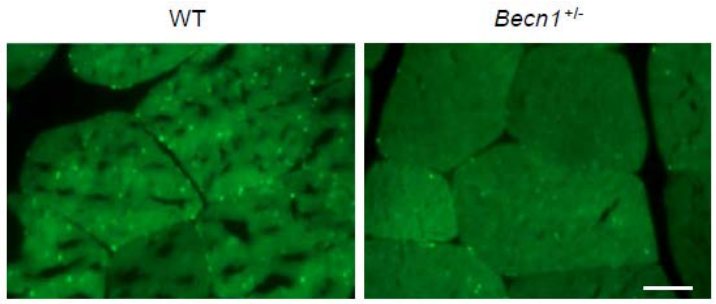


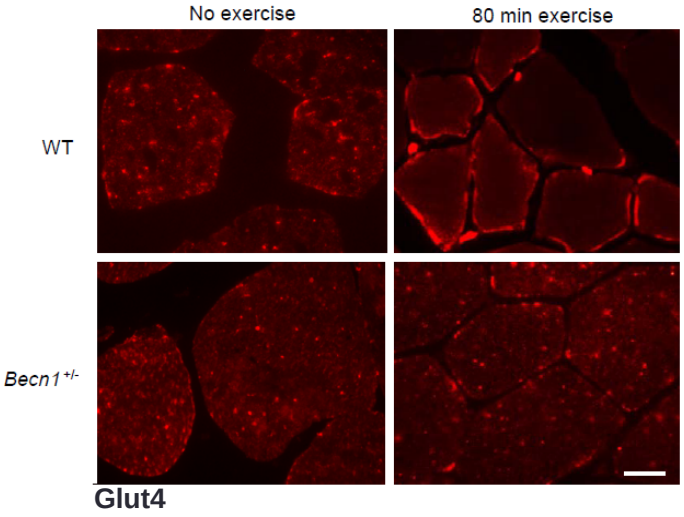
GLUT4 – vastus lateralis muscle

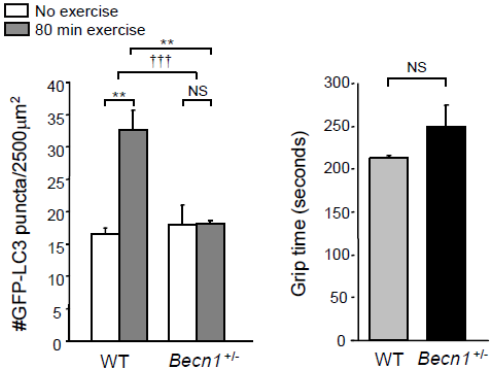


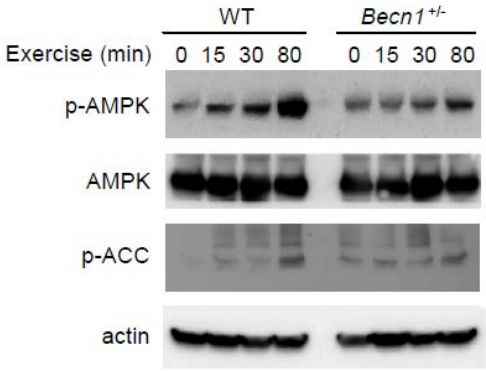
Is defective autophagy directly responsible?

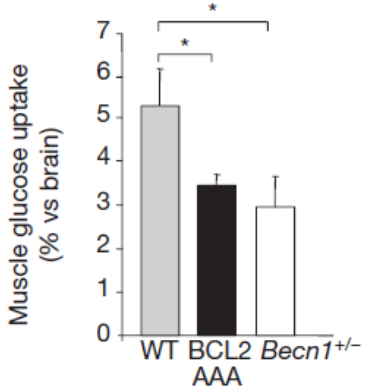
Becn1$^{+/-}$ and Atg16l1^{HM} mice show a phenotype similar to BCL2 AAA mice









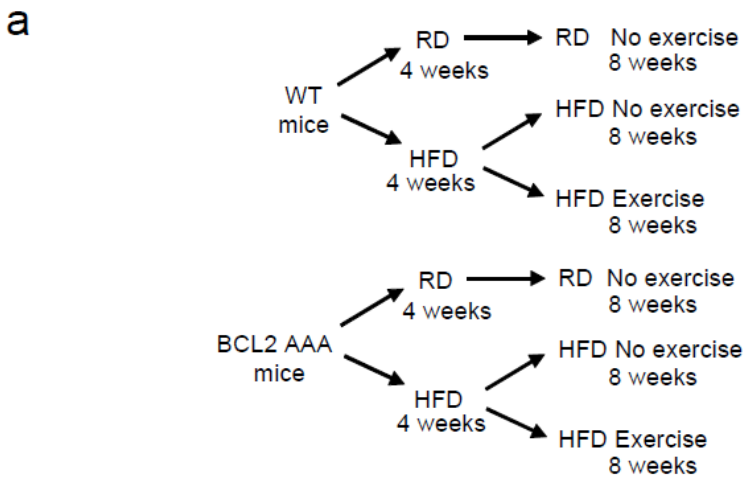


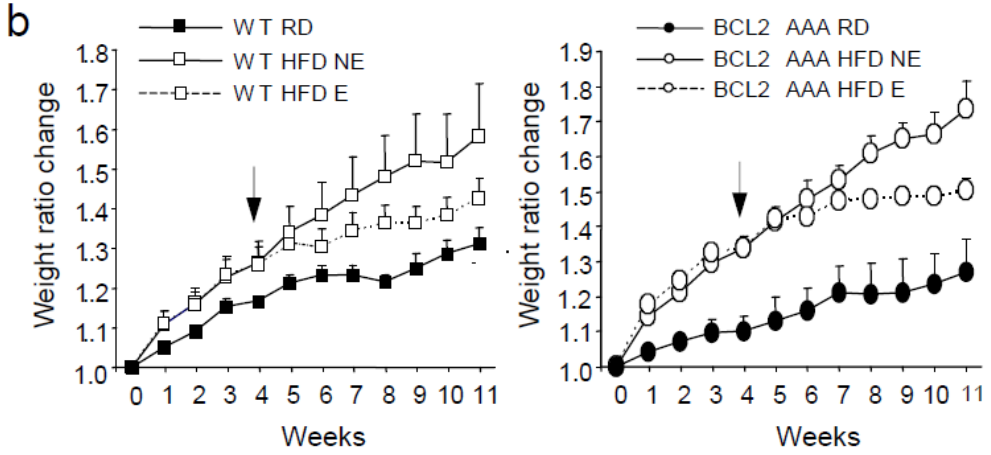
Data provides strong support for a role of deficient beclin 1 activity , rather than other BCL2-regulated functions, in the impairment of exercise endurance, glucose uptake and AMPK activation in BCL2 AAA mice

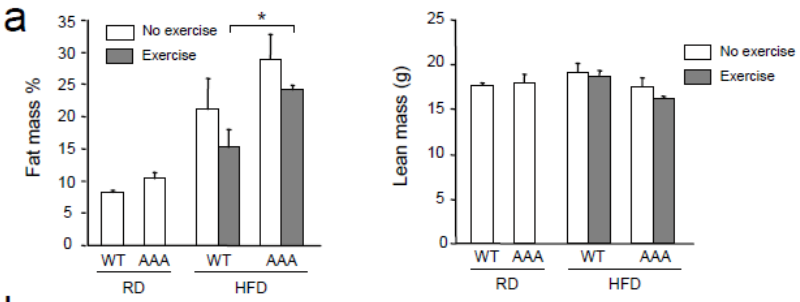
Similar results were provided by *Atg16l1*^{HM} mice (e.g. defects in exercise-induced autophagy associated with decreased AMPK phosphorylation)

Studies in BCL2 AAA, *Becn1*$^{+/-}$ and *Atg16l1*^{HM} suggest that cellular autophagy function is partially required for normal levels of exercise-induced muscle AMPK activation.

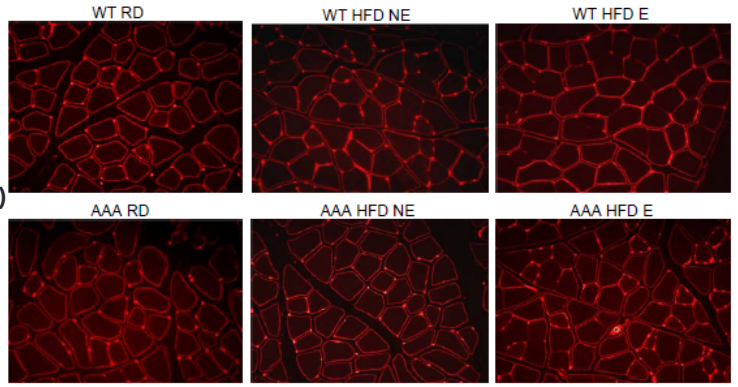
High fat diet(HFD)-induced metabolic abnormalities in BCL2 AAA mice

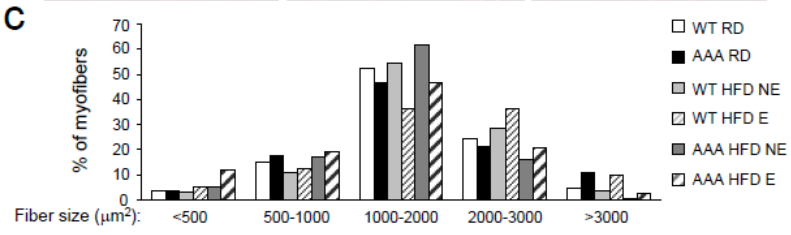


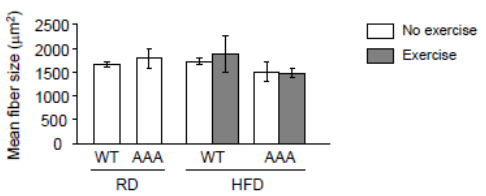




Wheat agglutinin (outline muscle fibers)



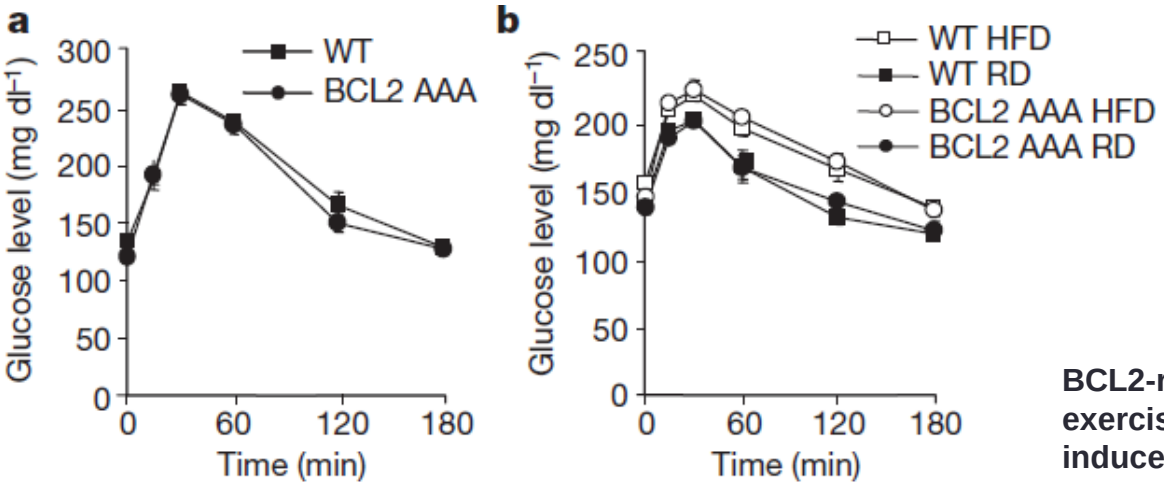




The BCL2 AAA mutation did not alter the response of mice to HFD with respect to muscle fiber size, the morphology of liver and pancreas, or the effect of exercise on HFD-induced obesity

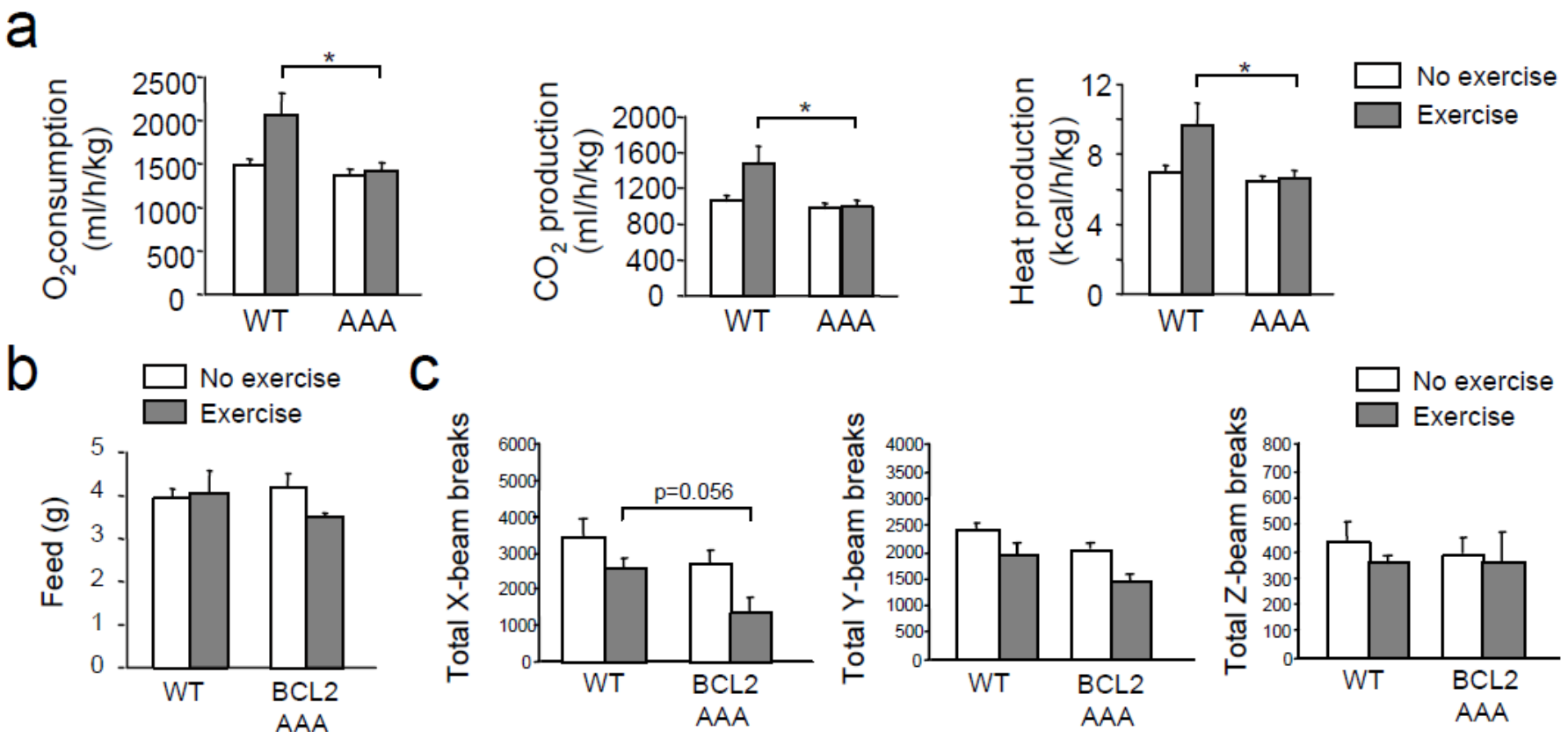
High fat diet(HFD)-induced impaired glucose tolerance in BCL2 AAA mice

Oral Glucose Tolerance Test (OGTT)



BCL2-regulated functions are essential for chronic exercise-mediated protection against HFD-induced glucose intolerance

HFD-fed BCL2 AAA mice are less metabolically active



The HFD study suggests that increased autophagy triggered by exercise may be critical for improving impaired glucose tolerance and metabolism in diet-induced obesity

Conclusions

- This study demonstrates that exercise is a potent inducer of autophagy, and that acute and chronic exercise enhances glucose metabolism in mice capable of inducing autophagy but not in autophagy-deficient mice.
- BCL2 has now previously undescribed essential roles in the *in vivo* regulation of stimulus-induced autophagy as well as glucose metabolism.
- They propose that manipulation of the autophagy pathway and/or the function of the autophagy inhibitory BCL2 protein may be a logical strategy to mimic the health effects of exercise and to prevent or treat impaired glucose metabolism.

Speculation

- On the basis of this newly discovered link between exercise, autophagy and altered metabolism, autophagy may represent a cellular mechanism by which exercise prolongs life and protects against cancer, cardiovascular disorders and inflammatory diseases.

Train, train, train……

….but not too much

THX FOR YOUR ATTENTION!!