

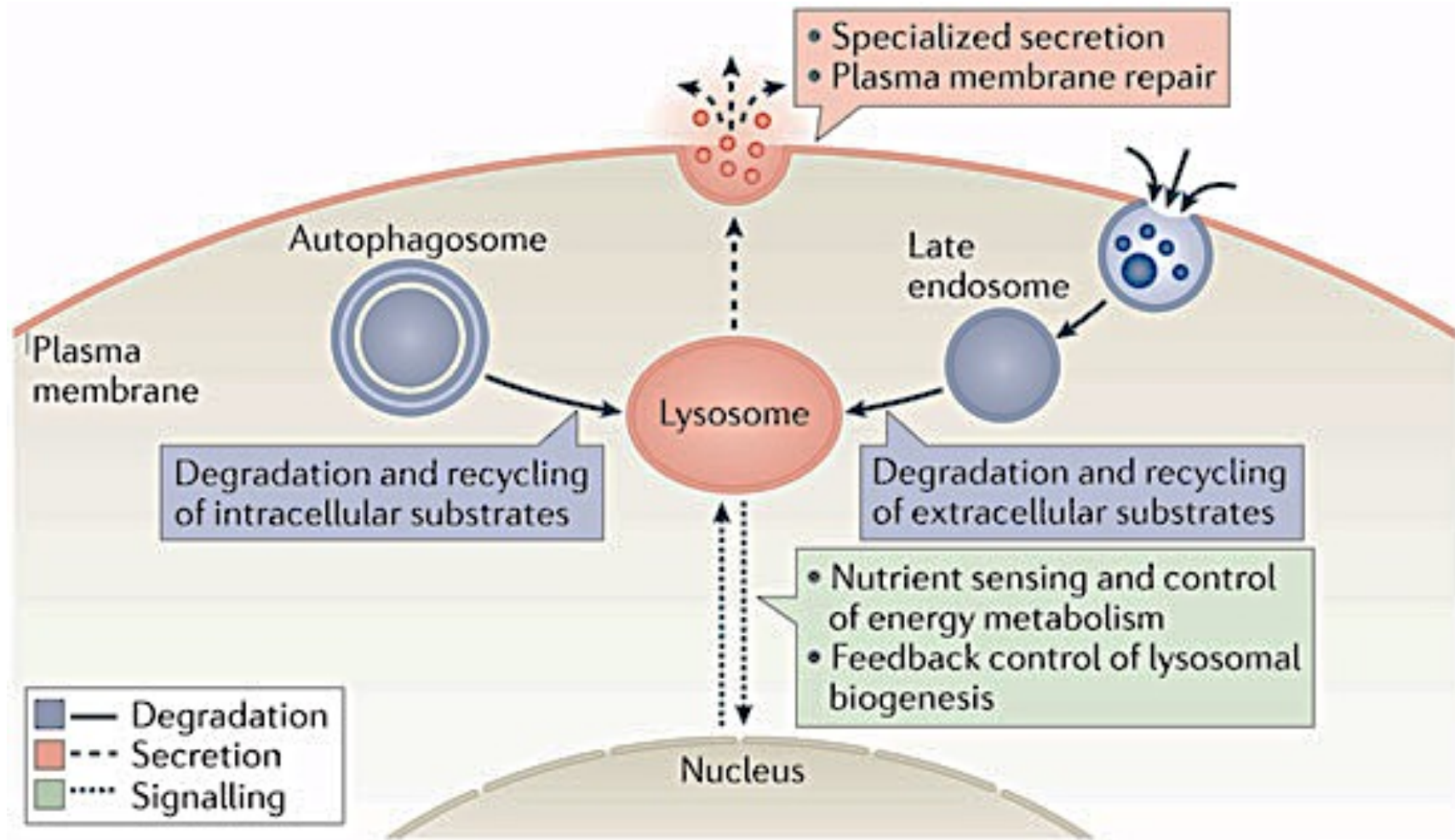
# Overview of autophagy in health and diseases

**CARMINE SETTEMBRE, PhD**

Telethon Institute of Genetics and Medicine  
Medical Genetics Unit, Federico II University



# ROLES OF LYSOSOME



Suggested read:

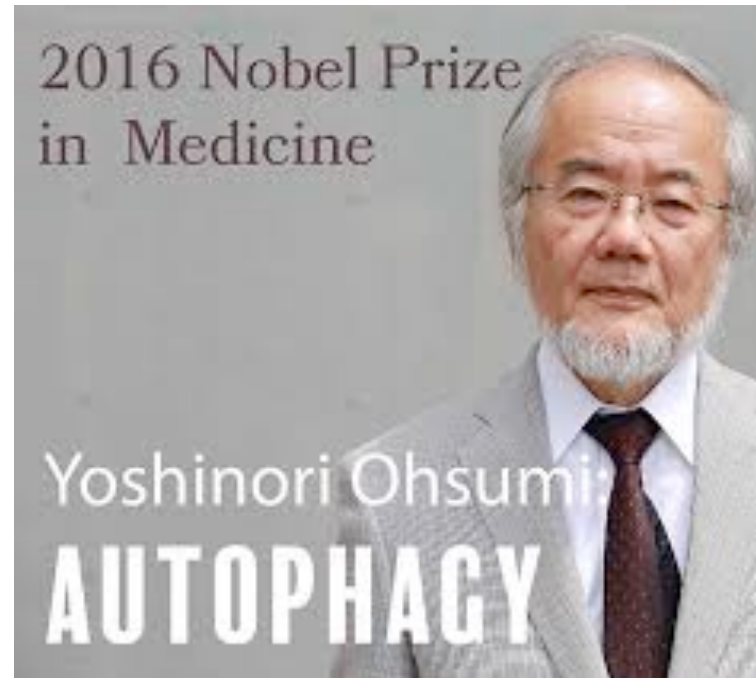
People & Ideas

**Yoshinori Ohsumi:**

**Autophagy from  
beginning to end**

Published April 16, 2012

**JCB**



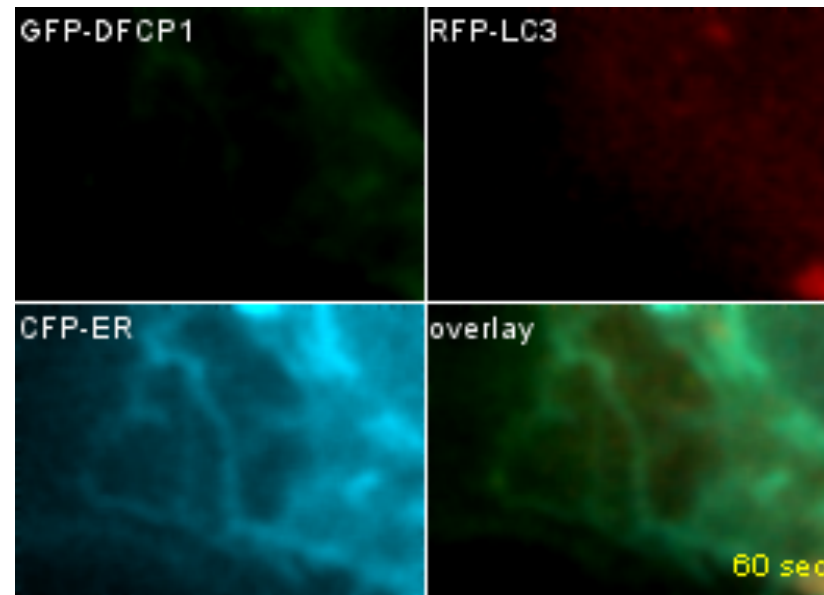
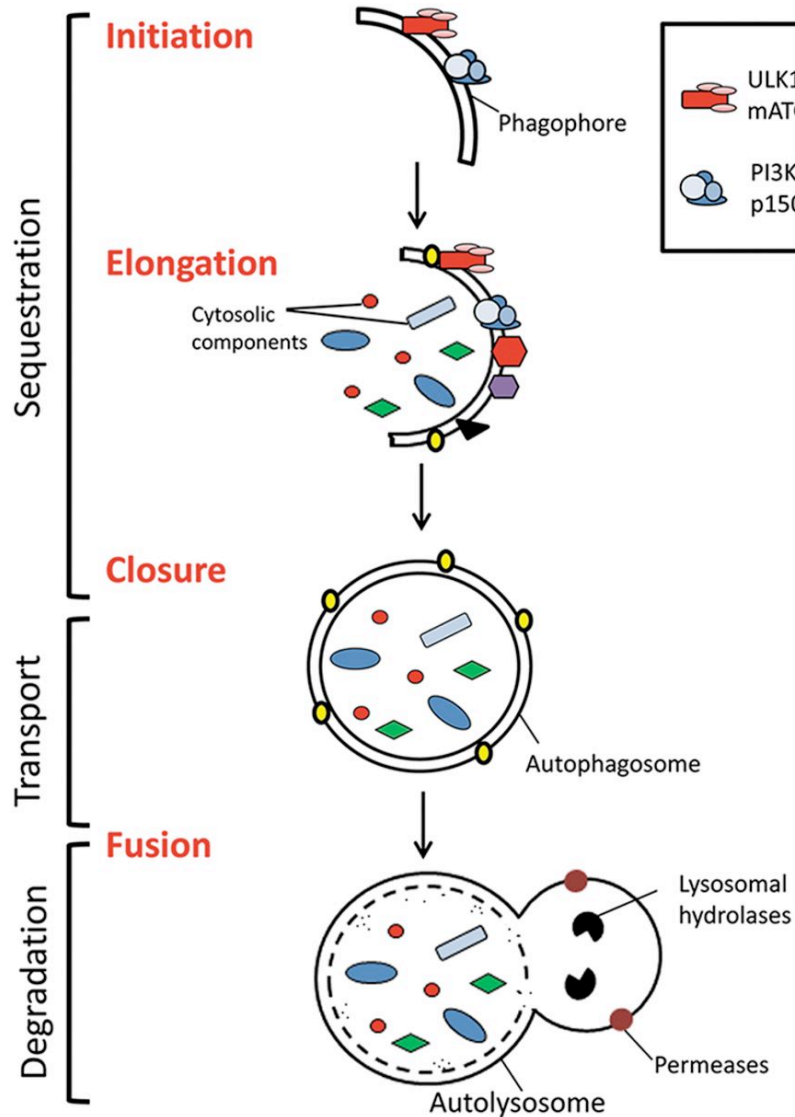
He has identified most of the proteins and pathways involved in the autophagy process, demonstrated how they are regulated by proteins that sense cells' metabolic states

*"I am not very competitive, so I always look for a new subject to study, even if it is not so popular."*

*"If you start from some sort of basic, new observation, you will have plenty to work on."*



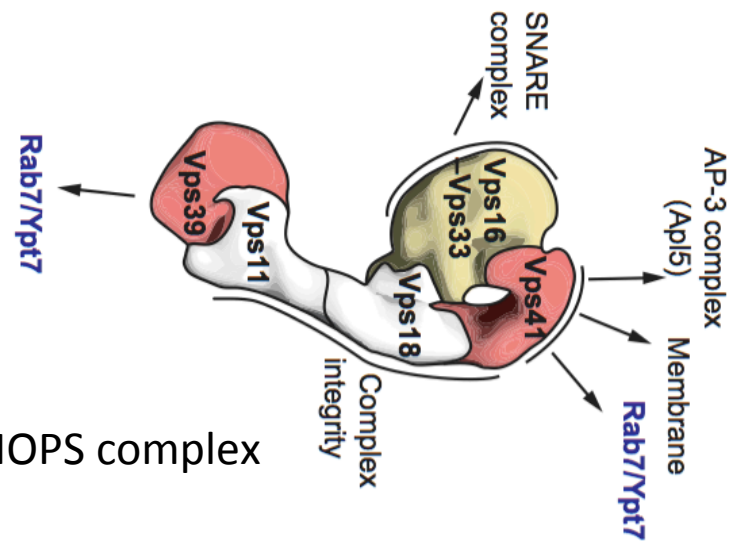
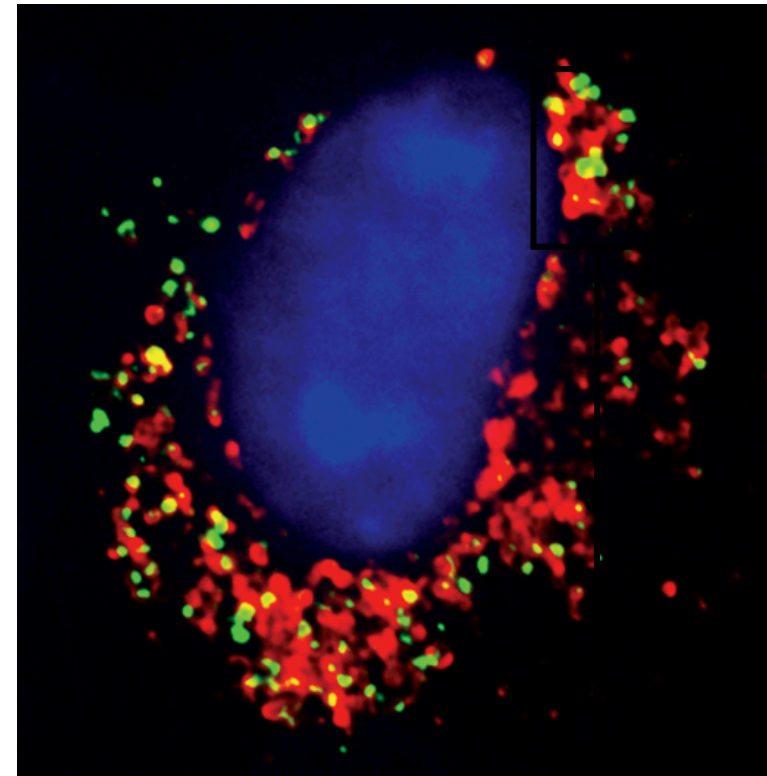
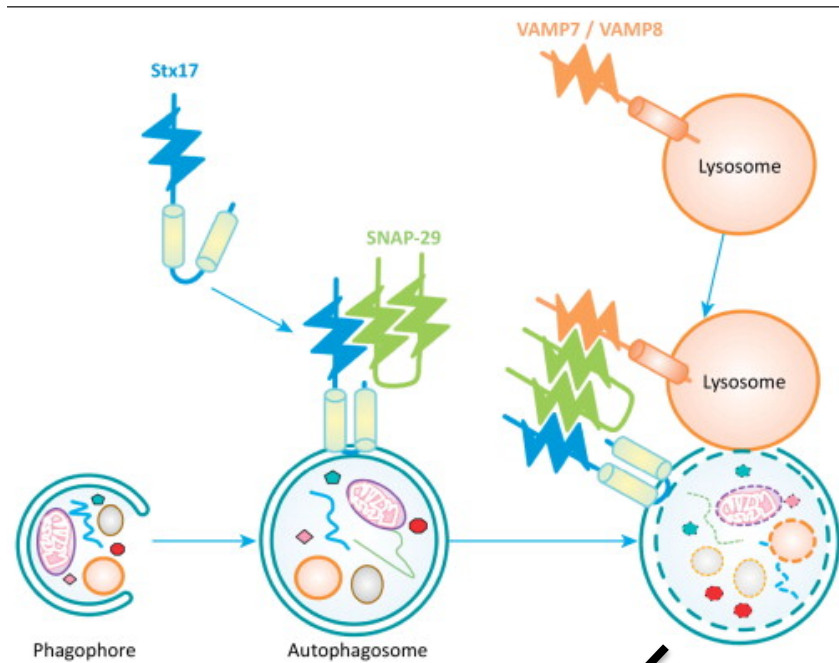
# AUTOPHAGOSOME



Axe... Ktistakis 2008 JCB

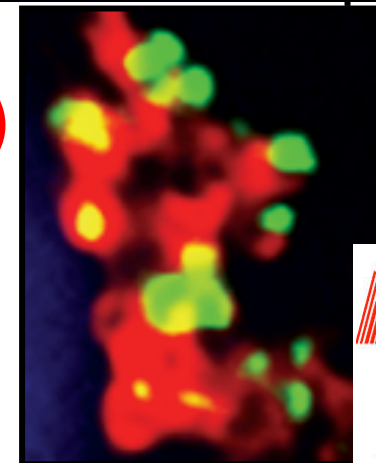


# AUTOPHAGOSOME LYSOSOME FUSION



**LAMP1 (Lys)**

**LC3 (AVs)**





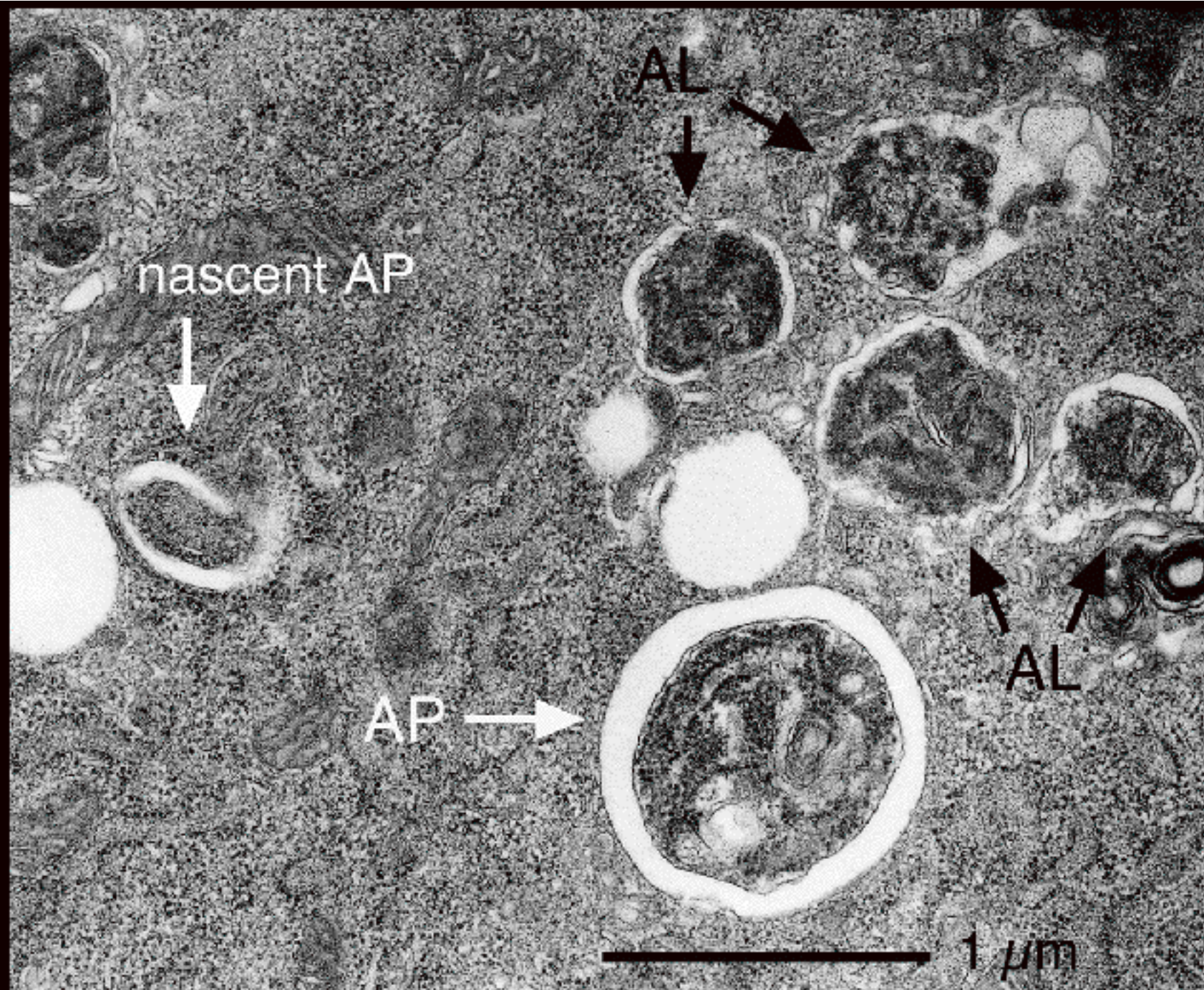
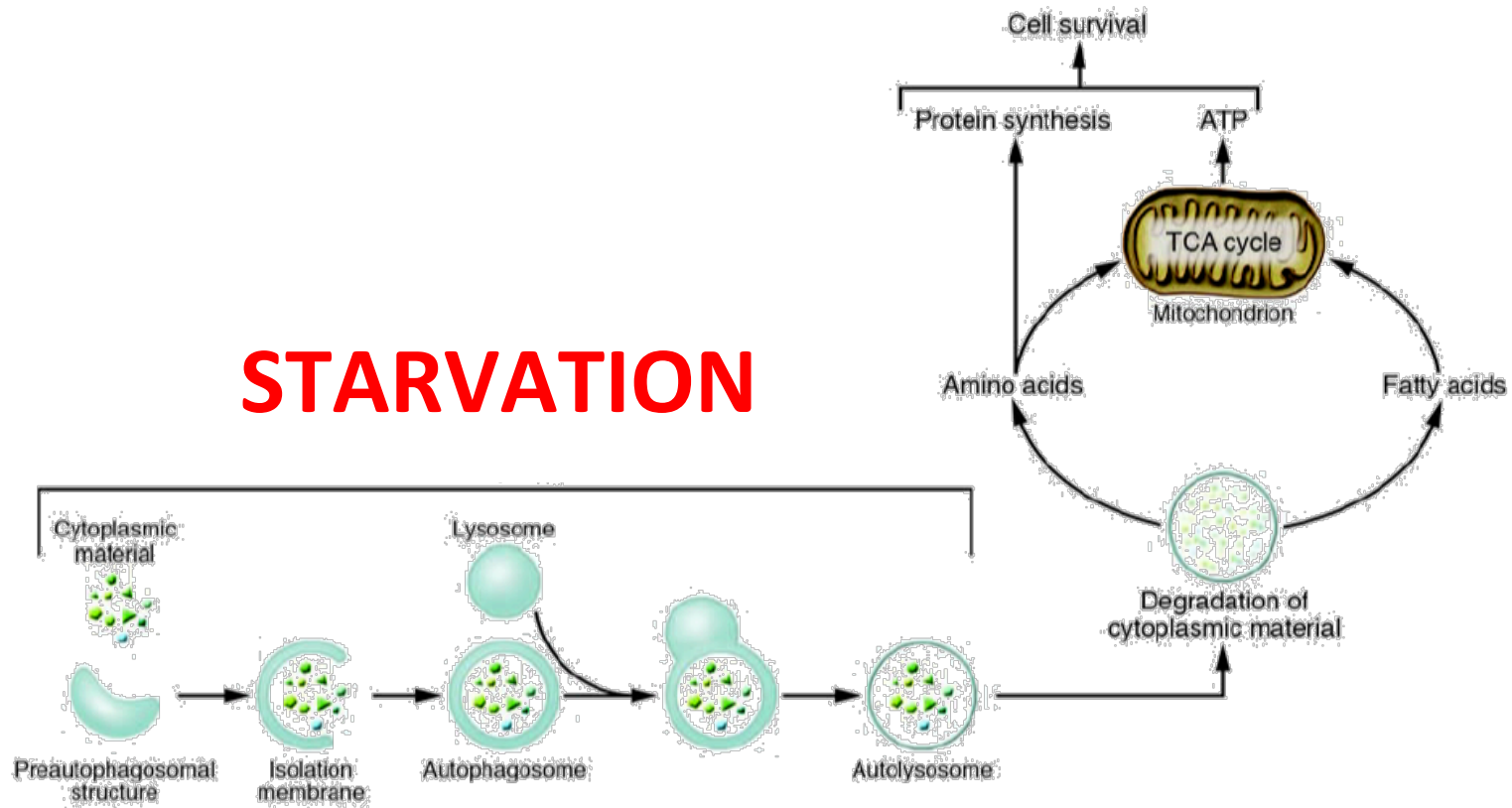


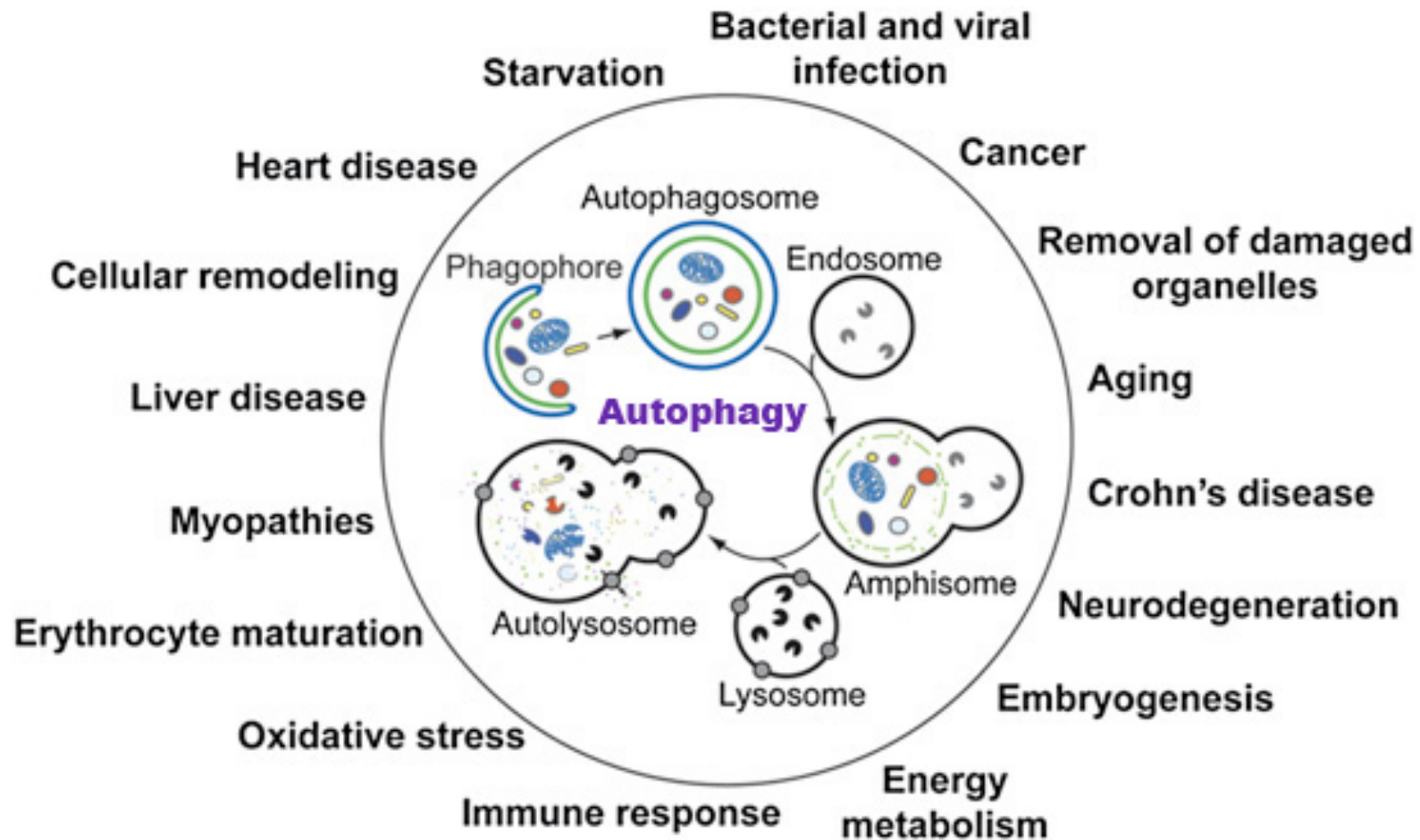
Photo by Dr. Waguri in Fukushima Med. Univ.

# 1) INDUCED, NON SELECTIVE AUTOPHAGY: GENERATES ENERGY DURING NUTRIENT SHORTAGE



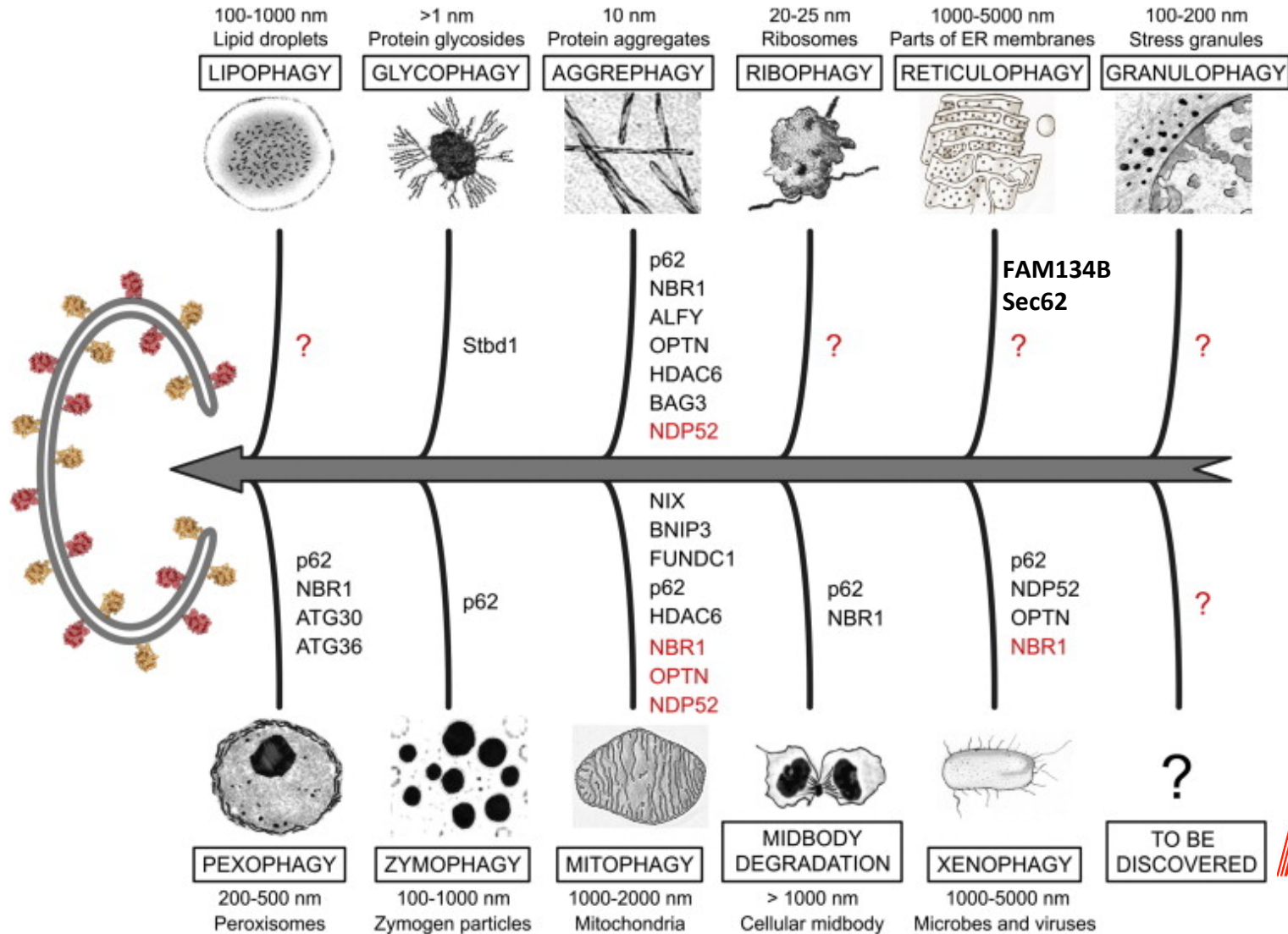


## 2) **BASAL, SELECTIVE AUTOPHAGY:** IS A QUALITY CONTROL PROCESS



Klionsky DJ. The autophagy connection. *Developmental Cell* 19:11-12 (2010)

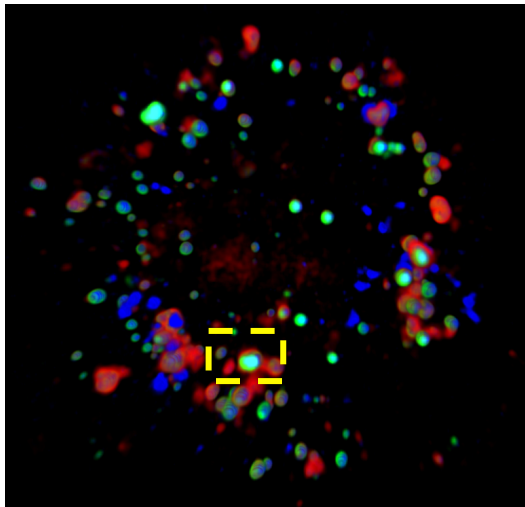
# SUBSTRATES ARE RECOGNIZED VIA RECEPTOR-MEDIATED PROCESSES



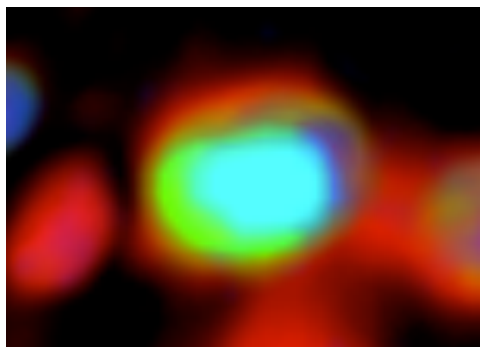
# PC2 IS AN AUTOPHAGY SUBSTRATE

AUTOPHAGOSOME/ COLLAGEN

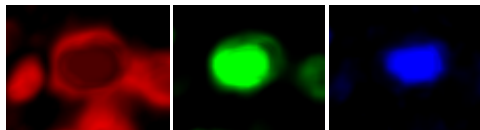
COL2



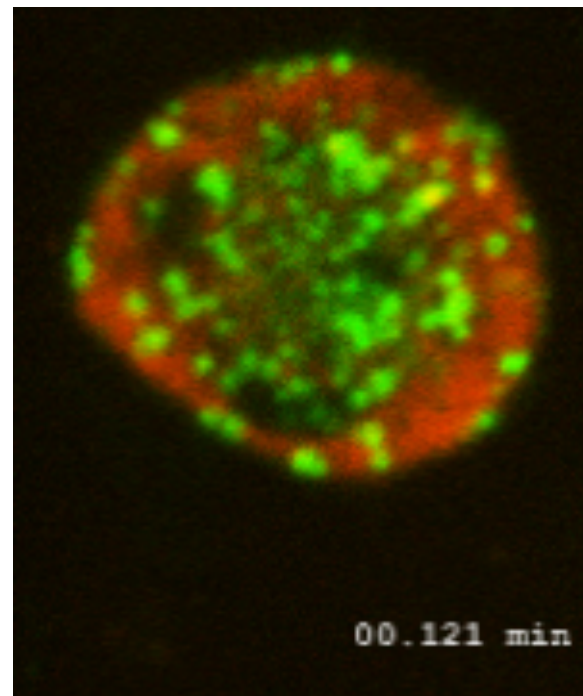
GFP- P62



RFP-LC3

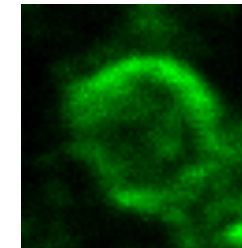
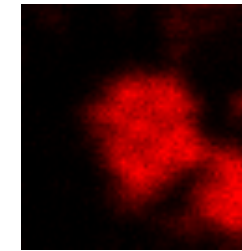
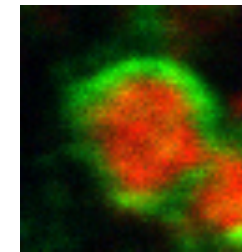


LYSOSOME/ COLLAGEN



COL2

LAMP1



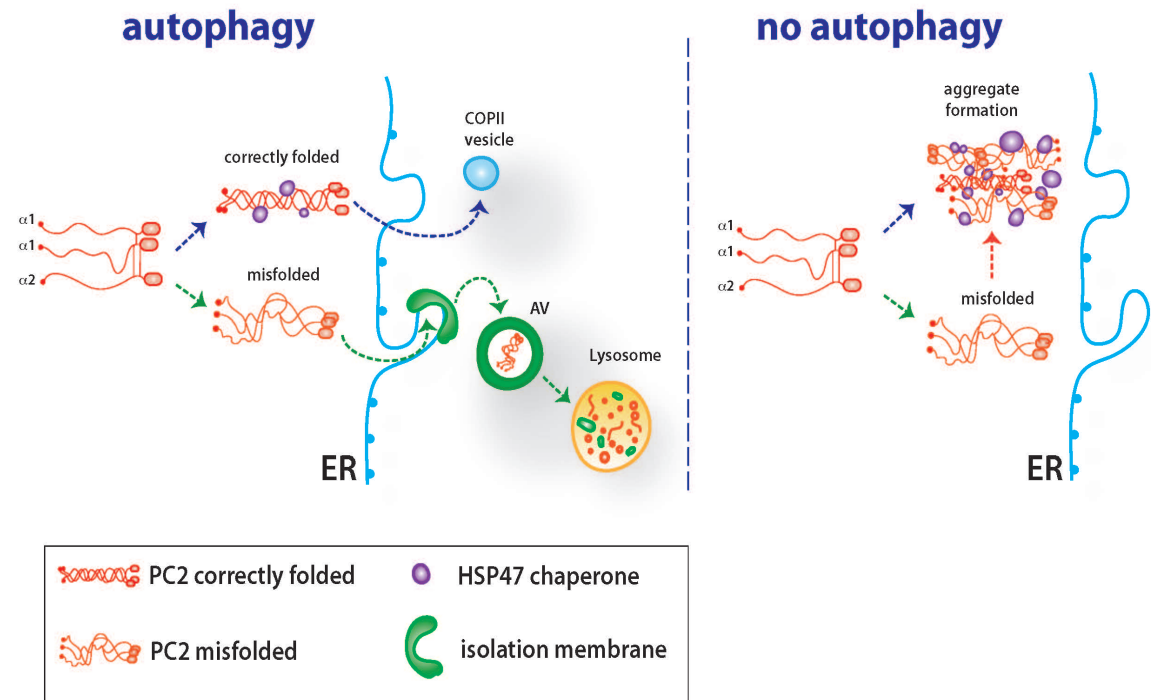
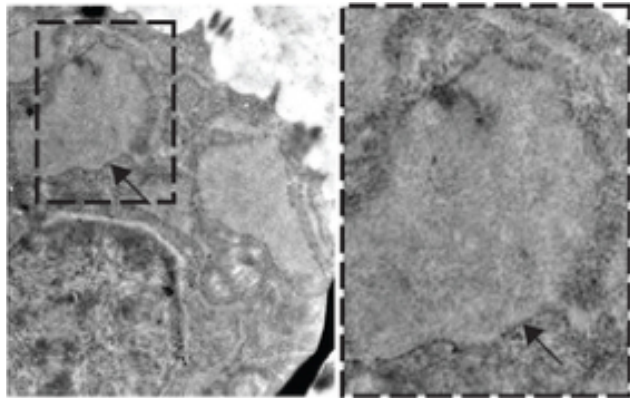
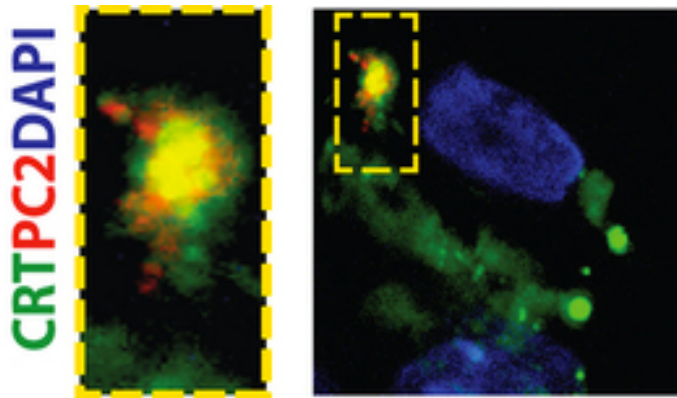
CURRENTLY LOOKING FOR THE COLLAGEN RECEPTOR

Cinque L, Forrester A, et al. *Nature* 2015



# AUTOPHAGY REGULATES COLLAGEN HOMEOSTASIS

## ATG7 KO



Cinque L, Forrester A, et al. *Nature* 2015

# **REGULATION OF LYSOSOMAL AUTOPHAGY PATHWAY**

# SIGNAL

(STARVATION, HYPOXIA, STRESS, DAMAGE, OXIDATION.. ETC)



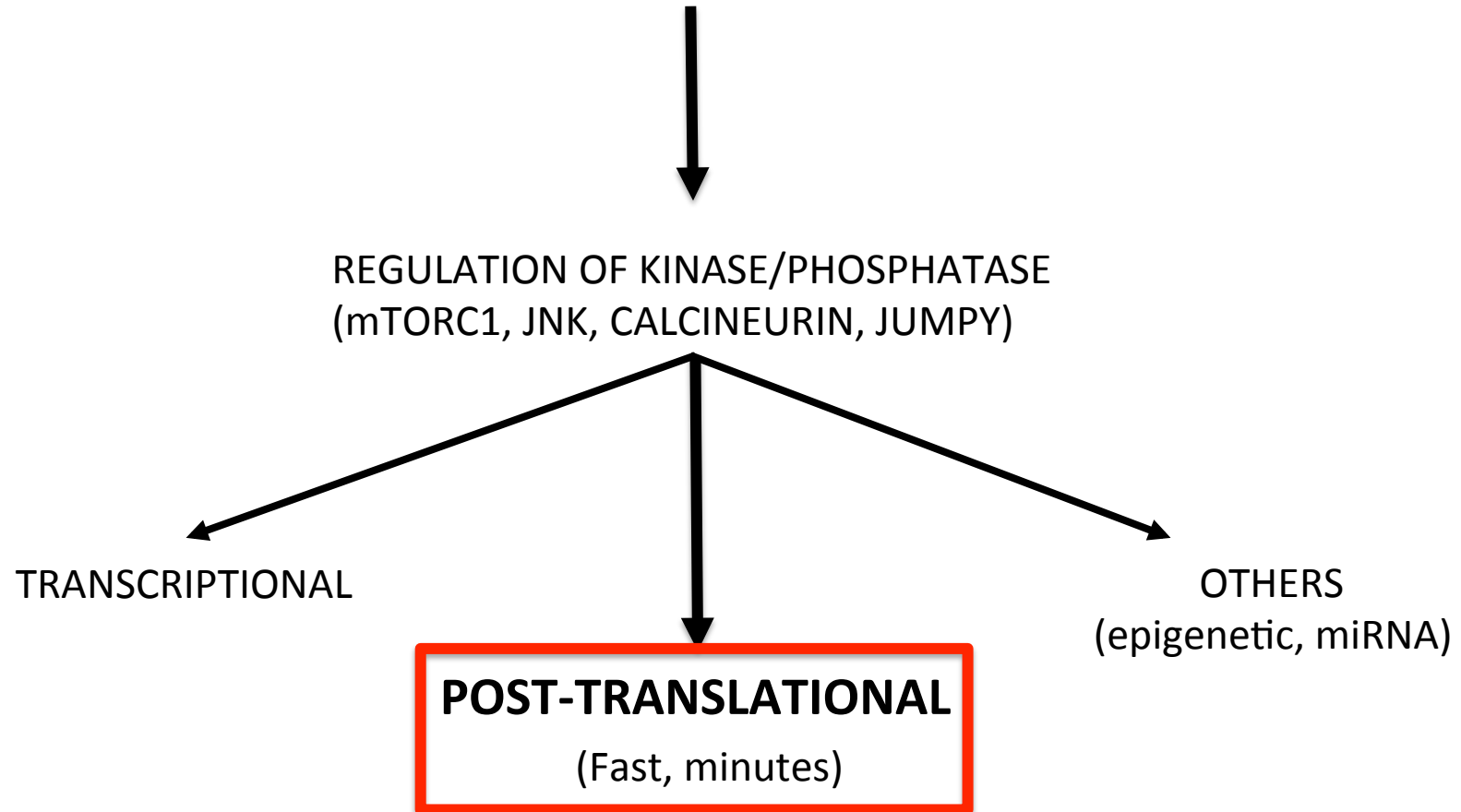
REGULATION OF KINASE/PHOSPHATASE  
(mTORC1, JNK, CALCINEURIN, JUMPY)

TRANSCRIPTIONAL

OTHERS  
(epigenetic, miRNA)

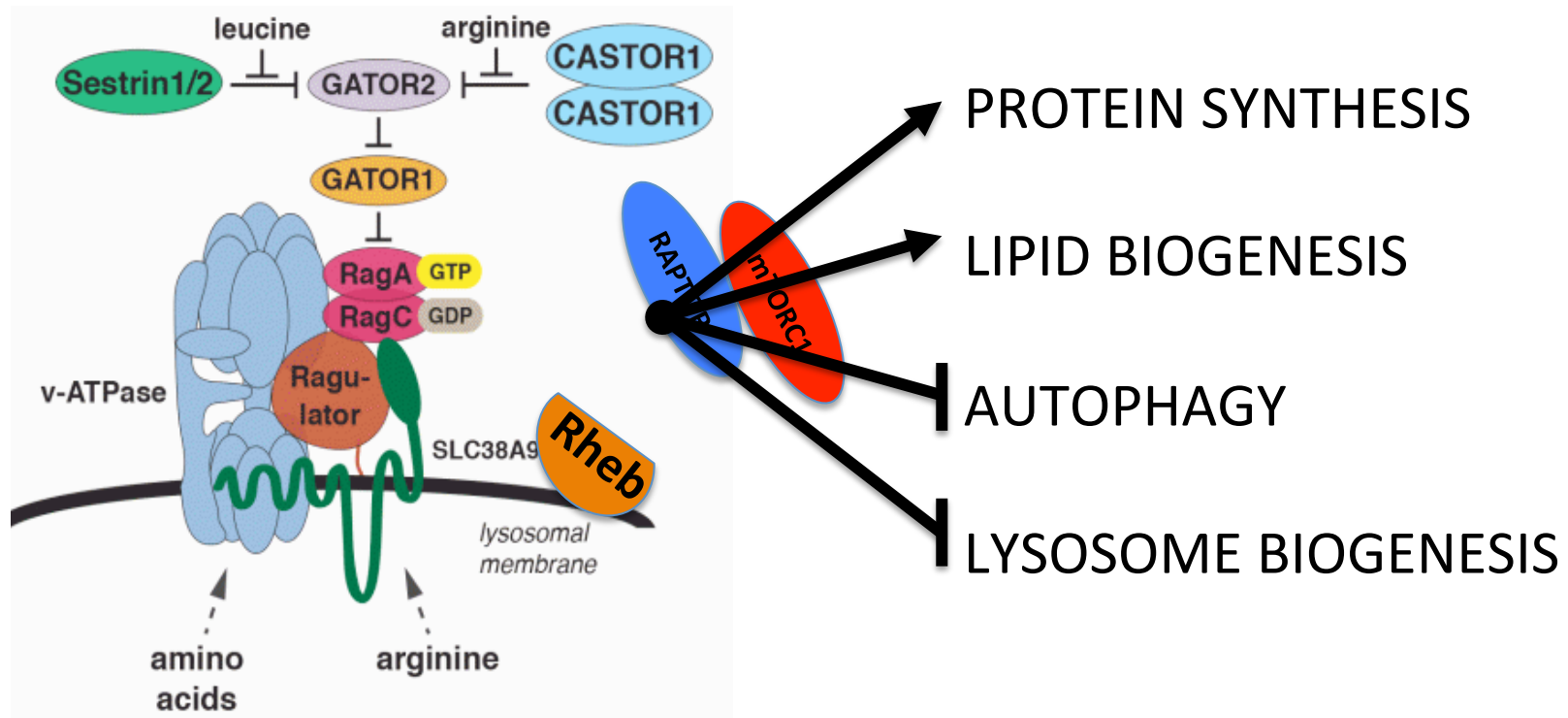
**POST-TRANSLATIONAL**

(Fast, minutes)



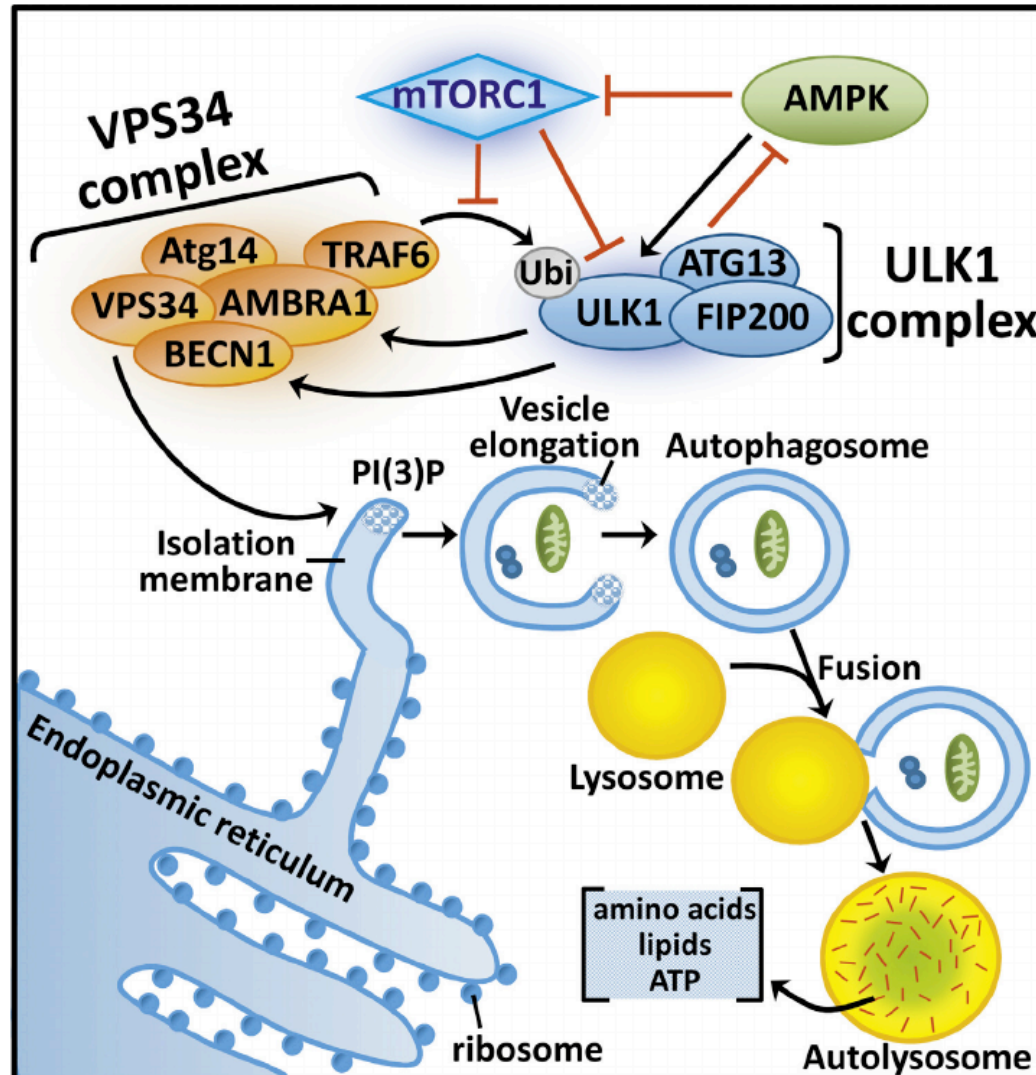


# mTORC1 SIGNALING IS REGULATED BY NUTRIENTS ON LYSOSOMAL SURFACE



works from Sabatini lab

# mTORC1 SUPPRESSES AUTOPHAGOSOME BIOGENESIS

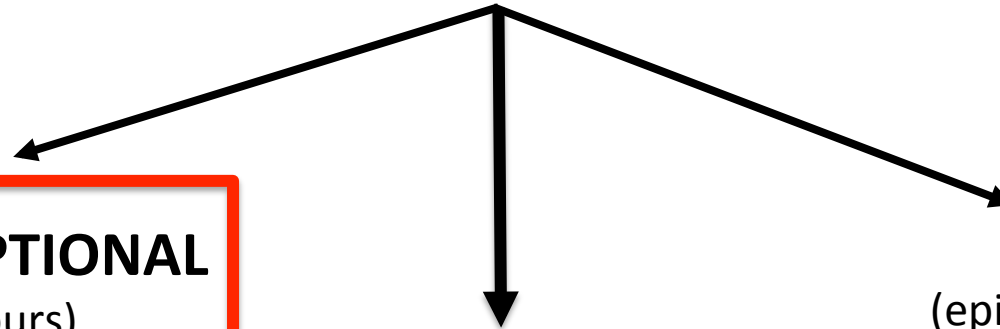


# SIGNAL

(STARVATION, HYPOXIA, STRESS, DAMAGE, OXIDATION.. ETC)



REGULATION OF KINASE/PHOSPHATASE  
(mTORC1, JNK, CALCINEURIN, JUMPY)



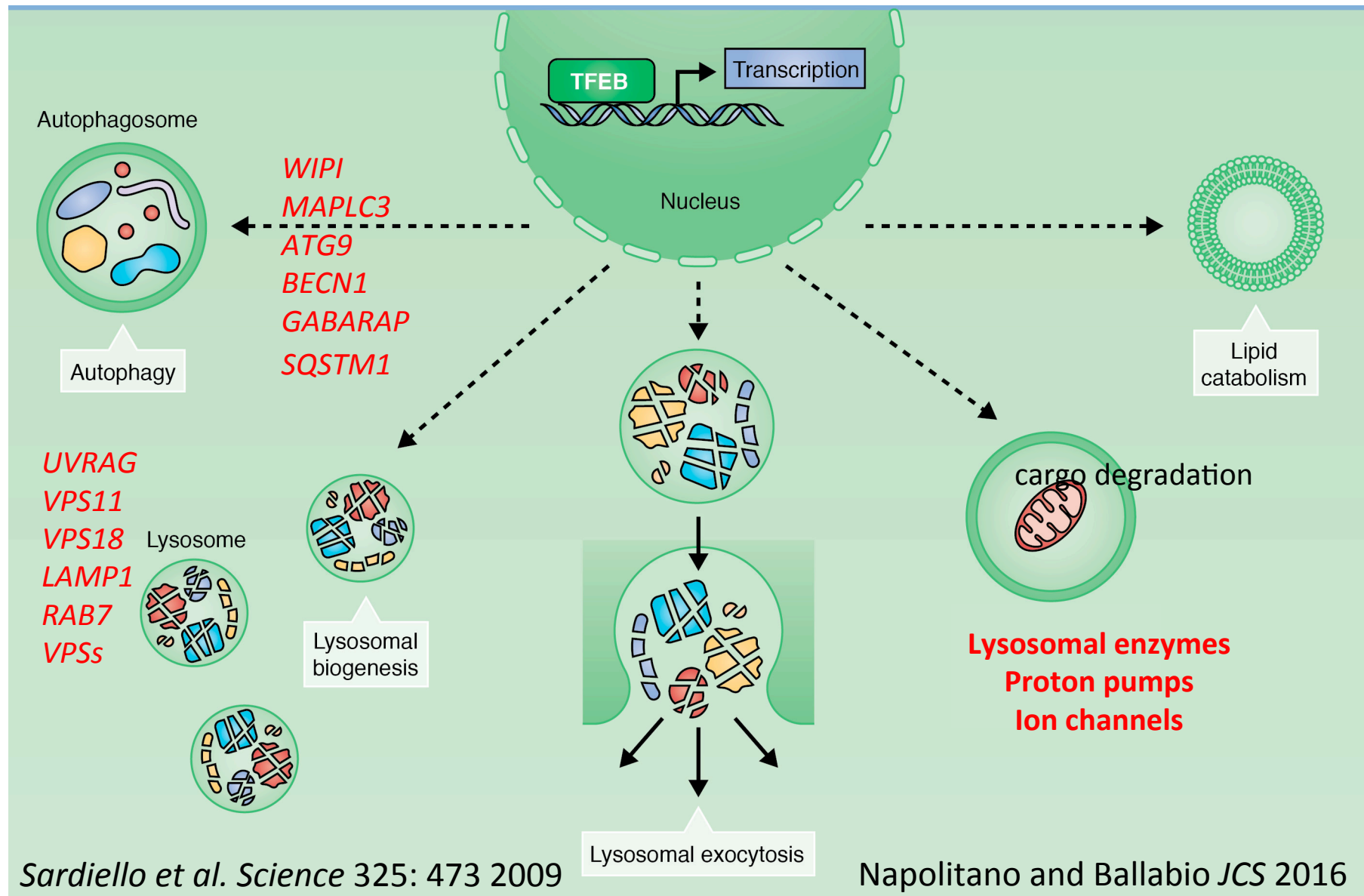
**TRANSCRIPTIONAL**  
(Slow, Hours)

OTHERS  
(epigenetic, miRNA)

POST-TRANSLATIONAL



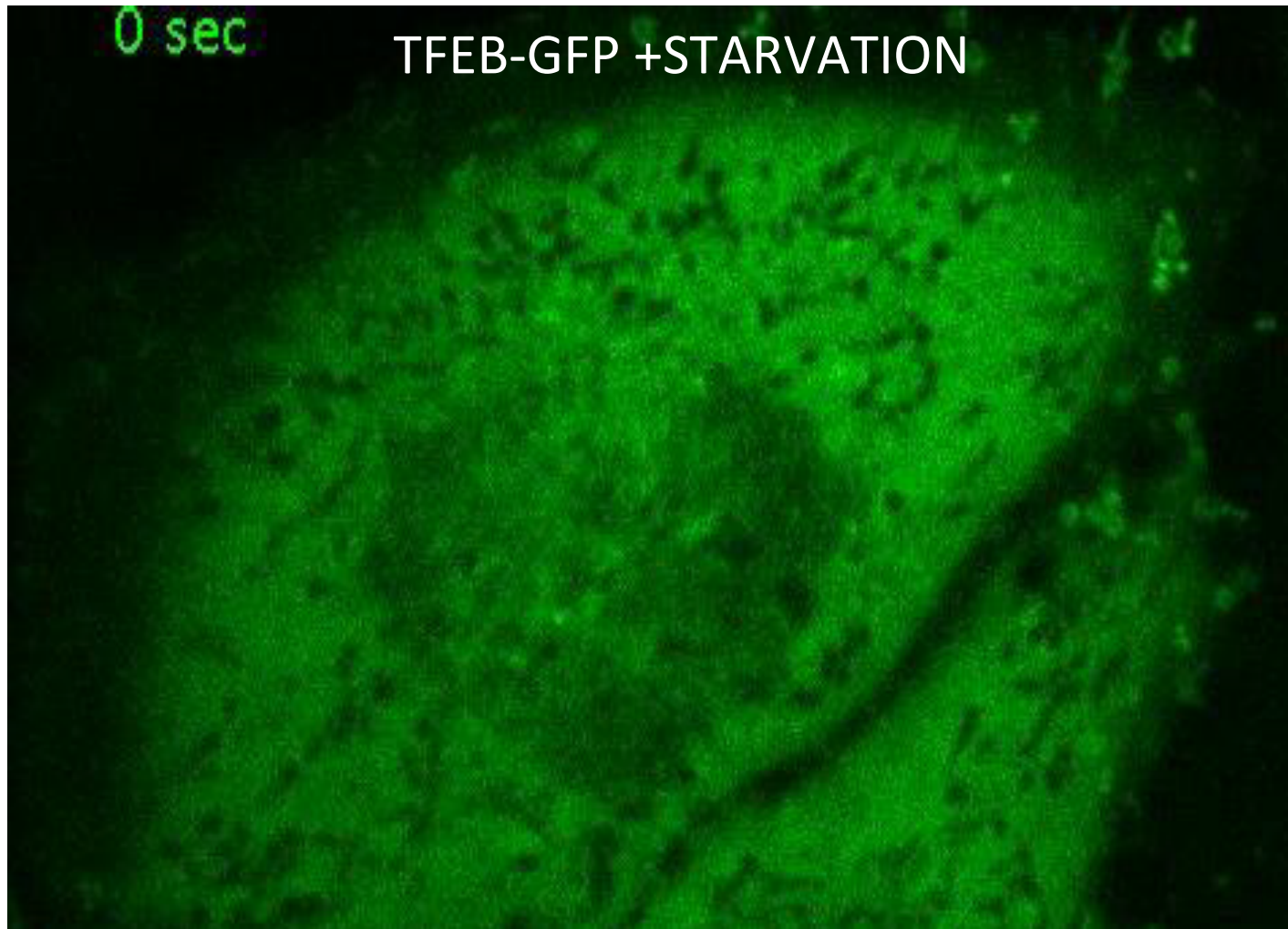
# TFEB: AUTOPHAGY'S TOP CHEF



*Settembre et al. Science 332, 1429 2011*      *Palmieri et al. HMG 20, 11 (2011)*

# STARVATION INDUCES TFEB NUCLEAR TRANSLOCATION

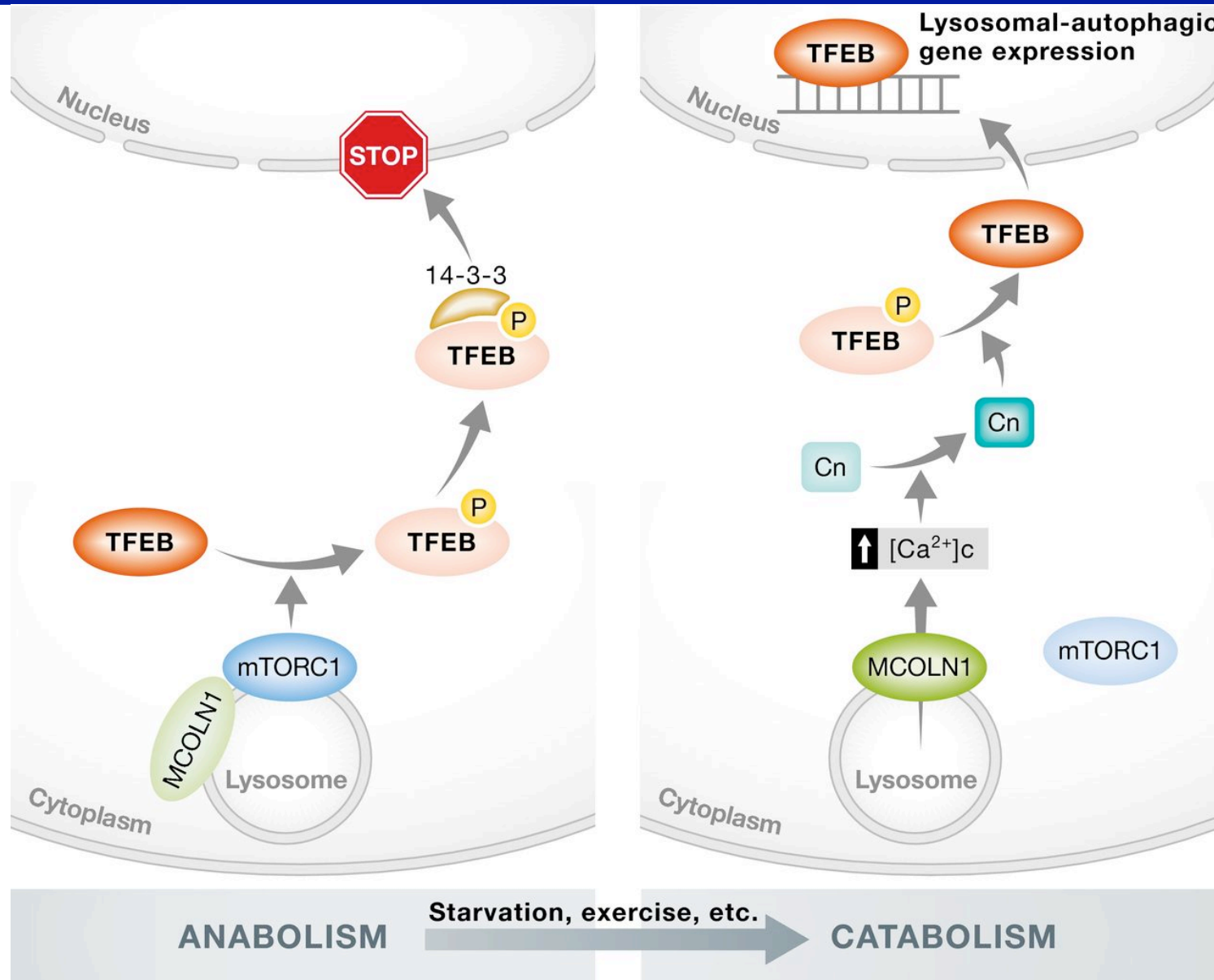
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*Settembre et al. EMBO J 17, 31 (2012)*



# LYSOSOME TO NUCLEUS SIGNALING MECHANISM

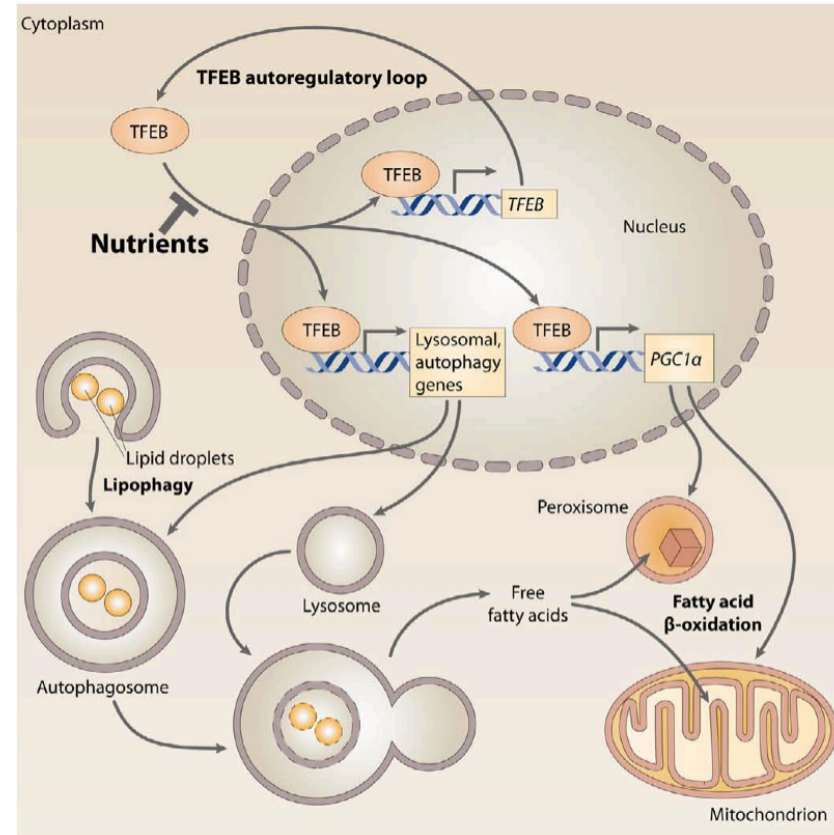
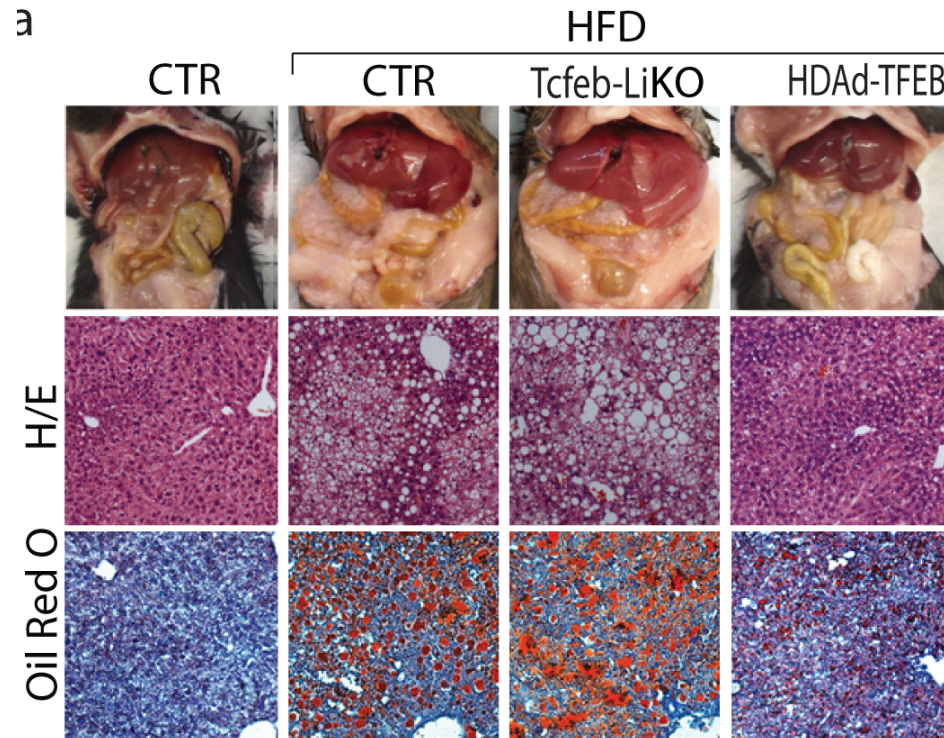








# PHYSIOLOGICAL ROLE OF TRANSCRIPTIONAL REGULATION OF AUTOPHAGY



Settembre et al. *NCB* 2013

# TAKE HOME MESSAGES (1)

---

- Autophagy relies on the biogenesis of two organelles, the lysosome and the autophagosome
- Induced autophagy is required to generate energy during starvation or fasting periods
- Basal autophagy is required for turnover of cellular components and maintains cellular homeostasis
- Autophagy is regulated through both transcriptional and post-transcriptional mechanisms.

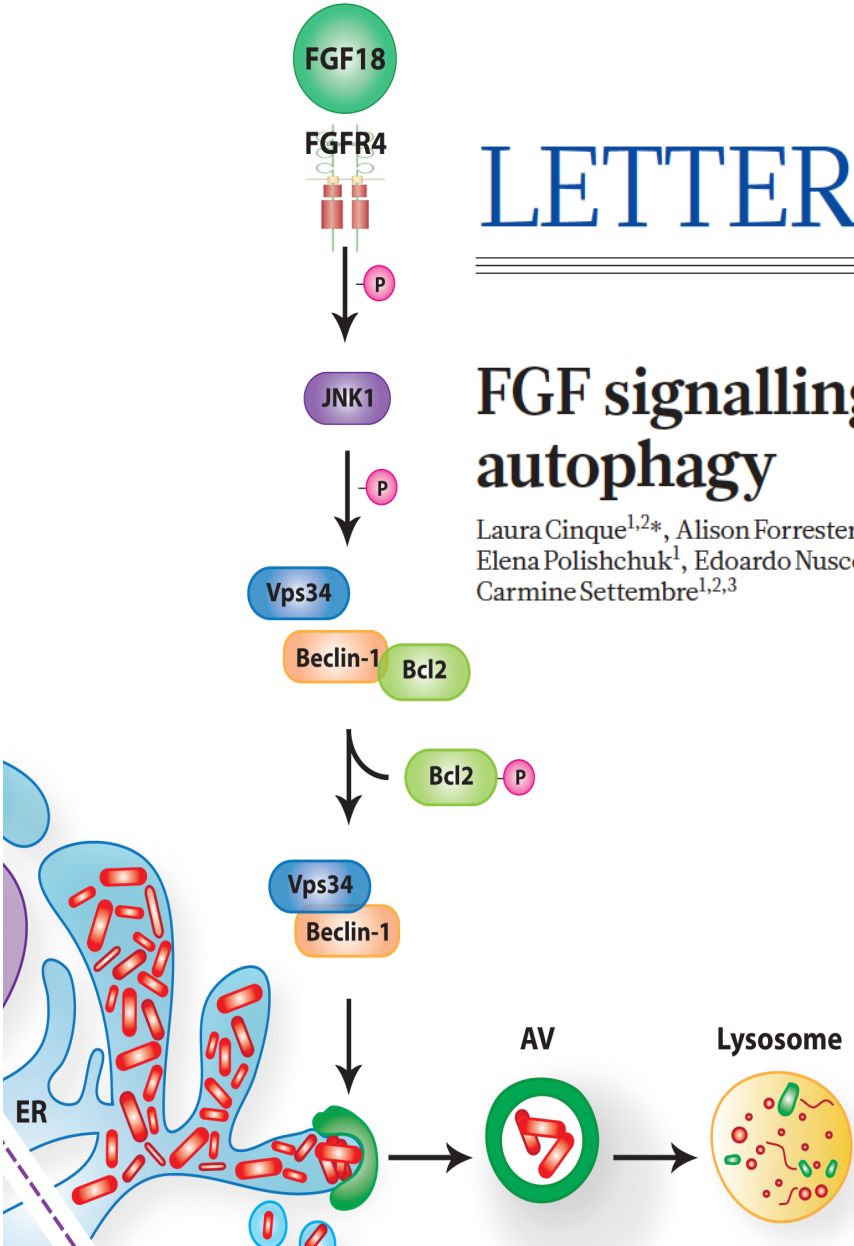
# DEVELOPMENTAL REGULATION OF AUTOPHAGY

LETTER

doi:10.1038/natur

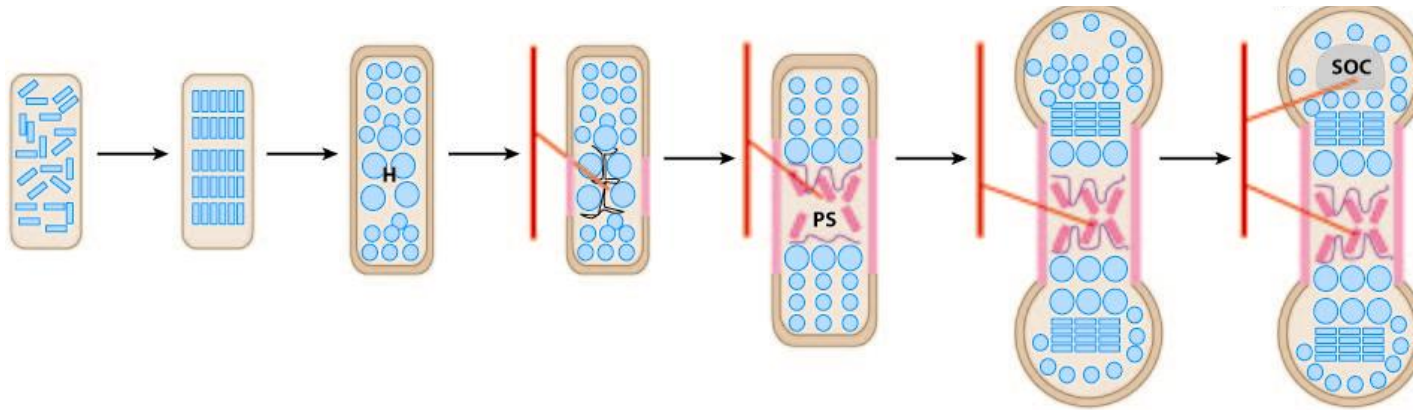
## FGF signalling regulates bone growth through autophagy

Laura Cinque<sup>1,2\*</sup>, Alison Forrester<sup>1,2,3\*</sup>, Rosa Bartolomeo<sup>1,2</sup>, Maria Svelto<sup>1,2,3</sup>, Rossella Venditti<sup>1</sup>, Sandro Montefusco<sup>1</sup>, Elena Polishchuk<sup>1</sup>, Edoardo Nusco<sup>1</sup>, Antonio Rossi<sup>4</sup>, Diego L. Medina<sup>1</sup>, Roman Polishchuk<sup>1</sup>, Maria Antonietta De Matteis<sup>1</sup> & Carmine Settembre<sup>1,2,3</sup>





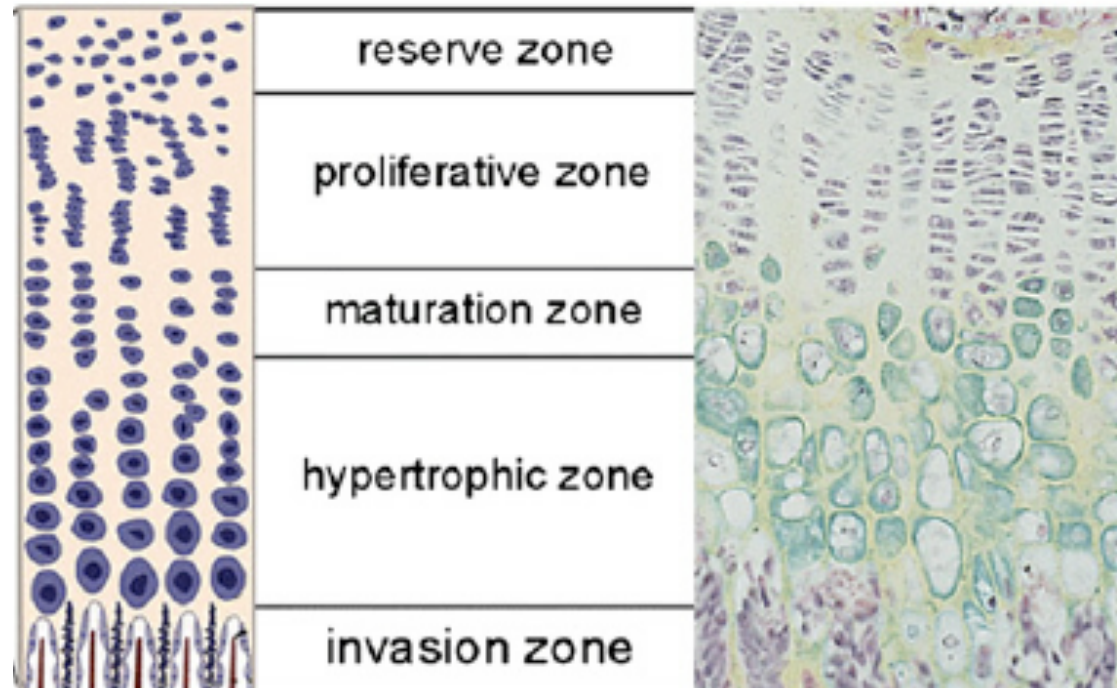
# CHONDROCYTES REGULATE BONE GROWTH



**AR** Karsenty G, et al. 2009.  
Annu. Rev. Cell Dev. Biol. 25:629–48

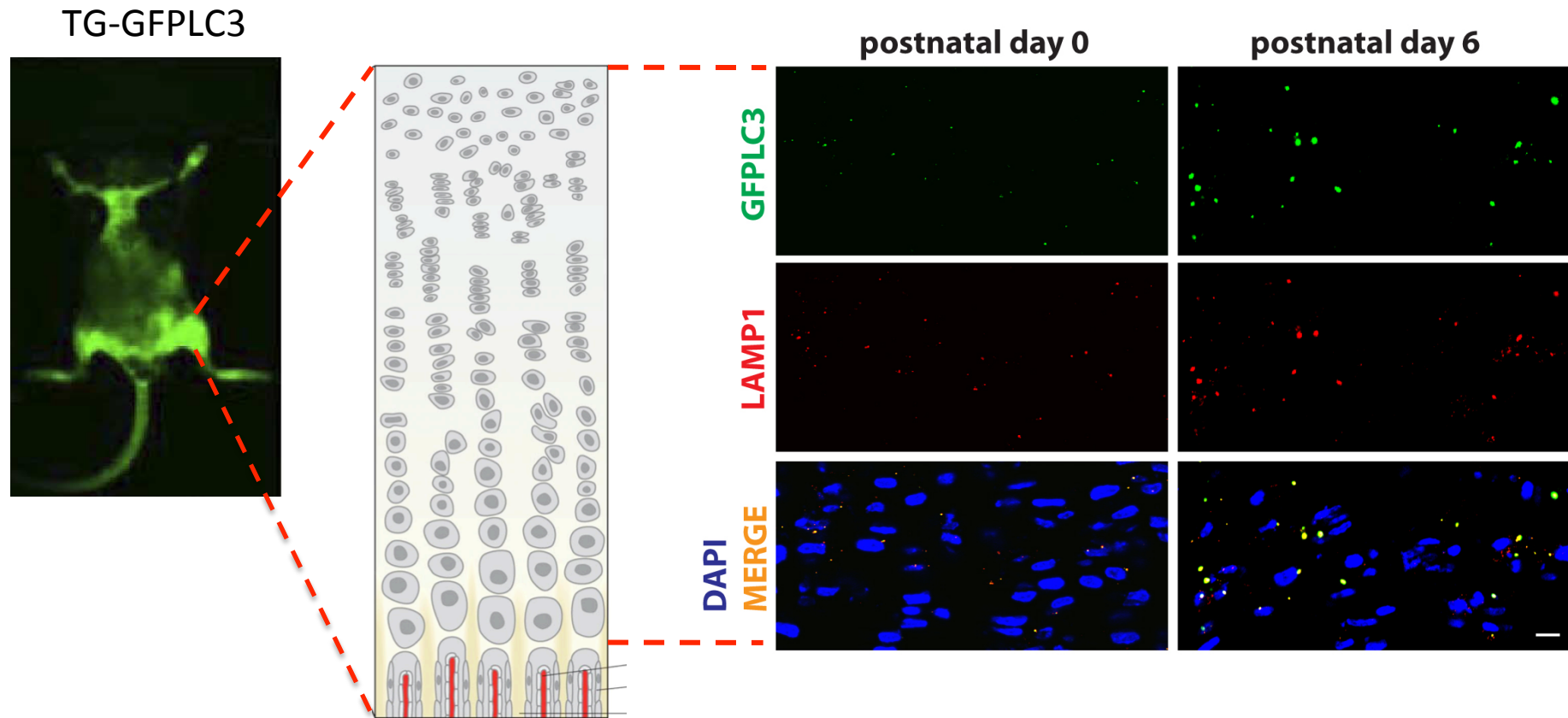


Natural history museum  
(PARIS)



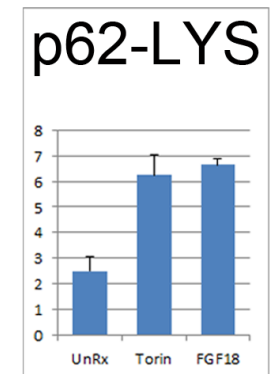
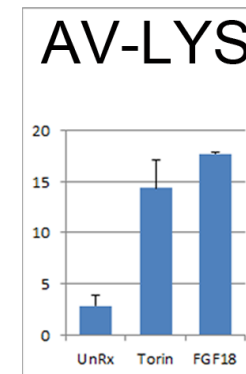
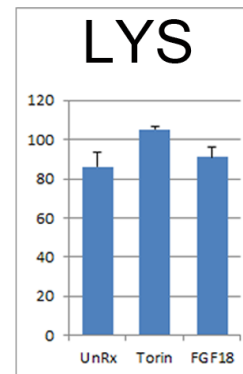
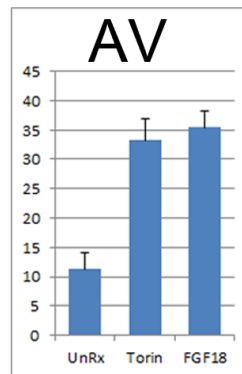
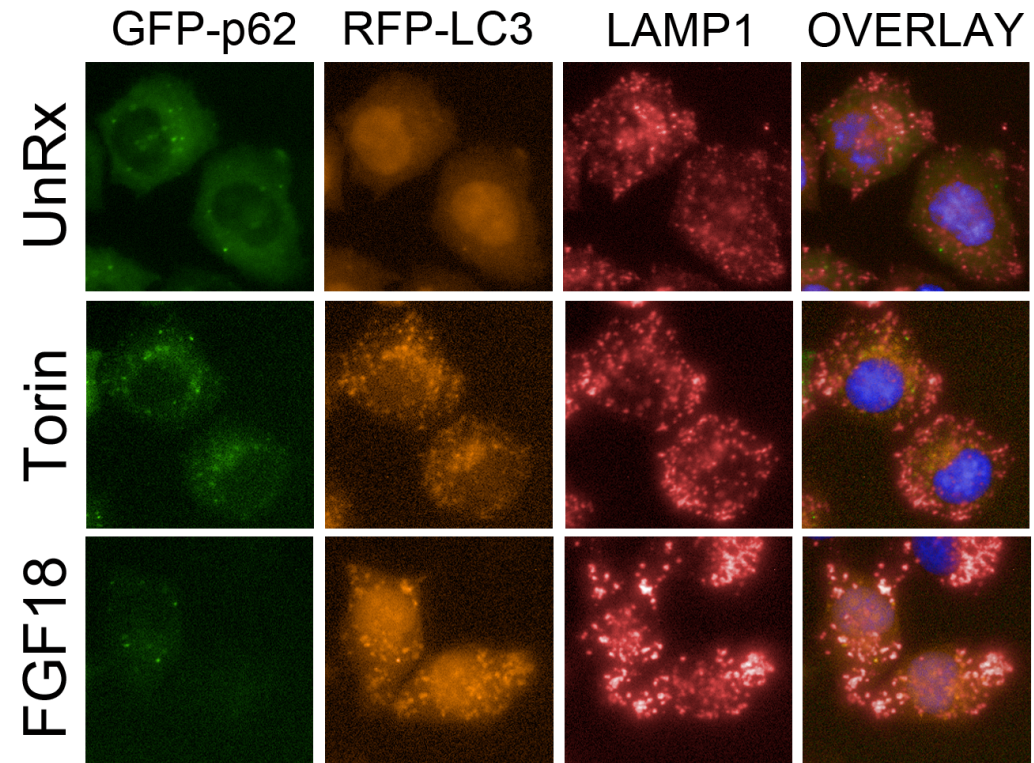


# ACTIVATION OF AUTOPHAGY DURING POST-NATAL BONE GROWTH

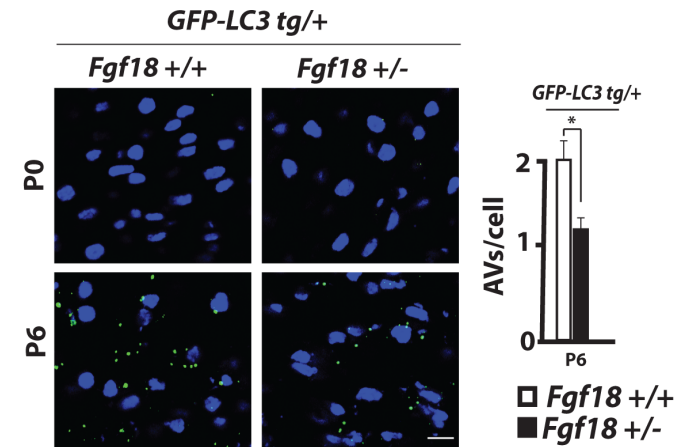
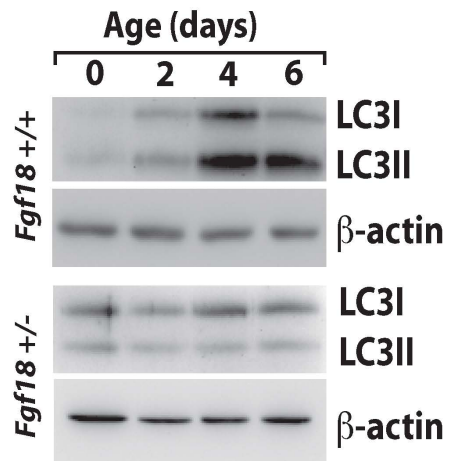
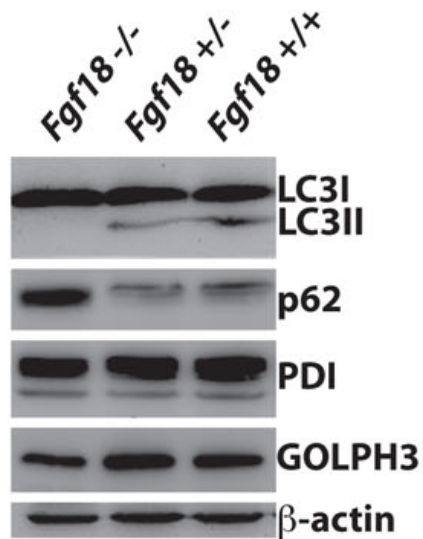


# FGF18 REGULATES AUTOPHAGY *IN VITRO*

**TIGEM HIGH CONTENT  
SCREENING FACILITY**

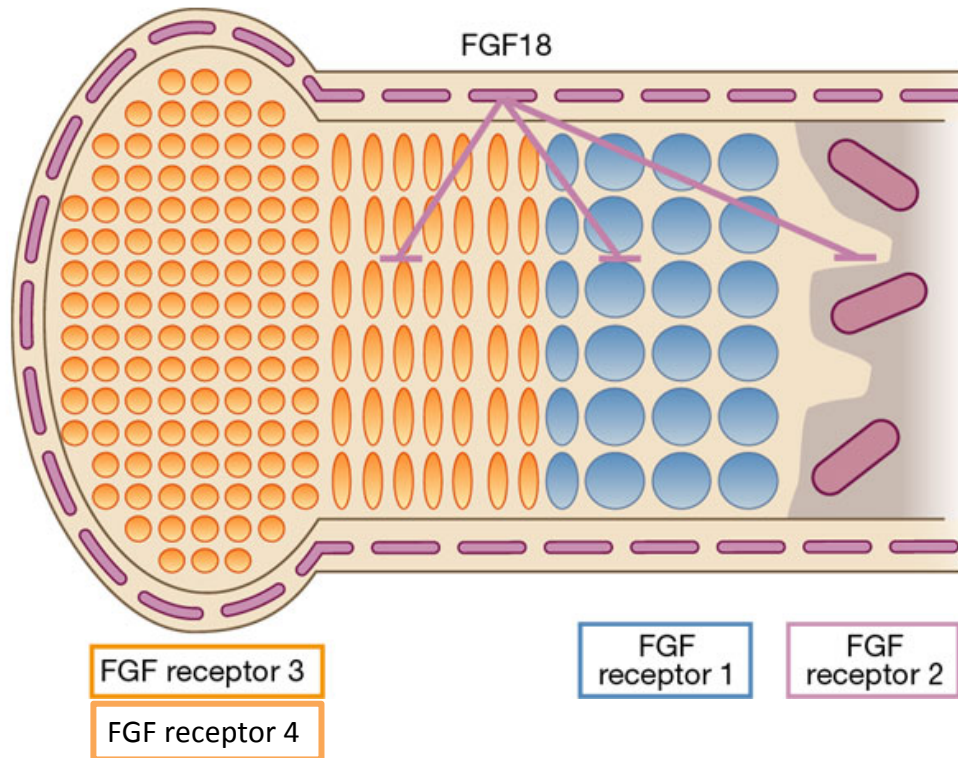


# FGF18 REGULATES AUTOPHAGY *IN VIVO*





# FGF SIGNALING IN THE GROWTH PLATE



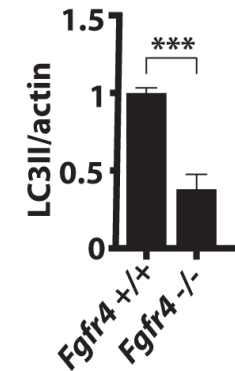
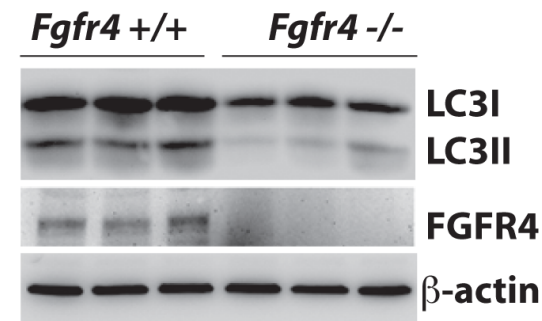
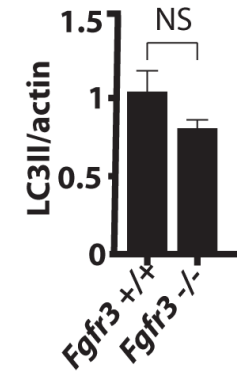
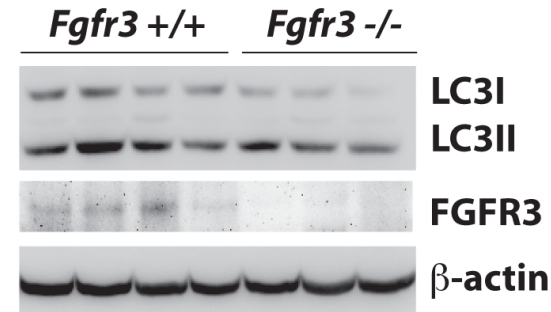
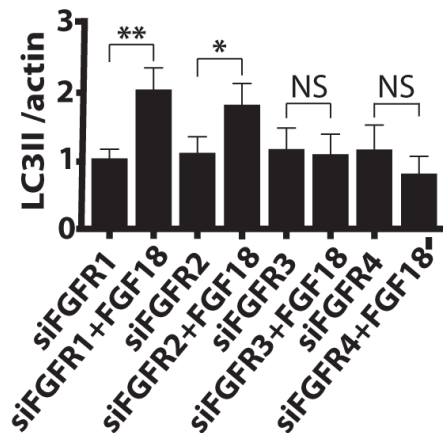
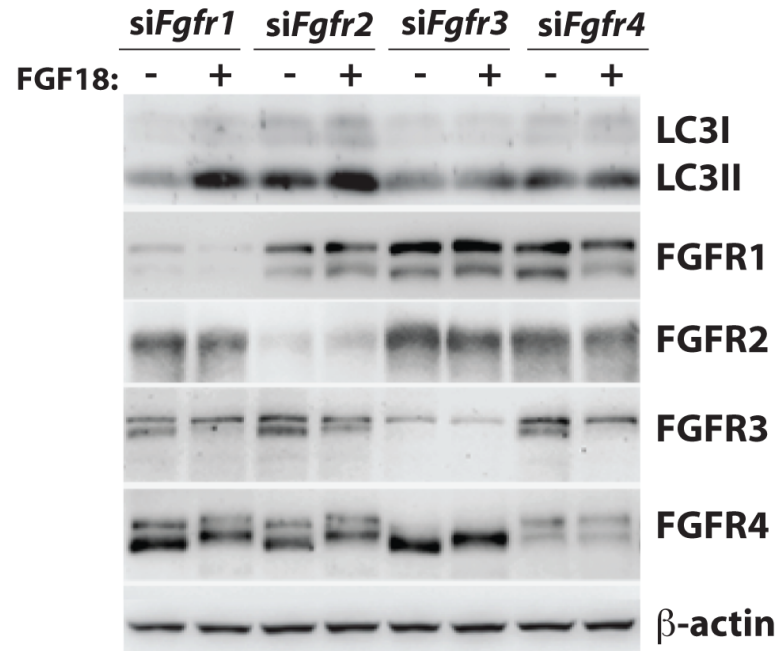
CHONDROCYTE PROLIFERATION

CHONDROCYTE DIFFERENTIATION

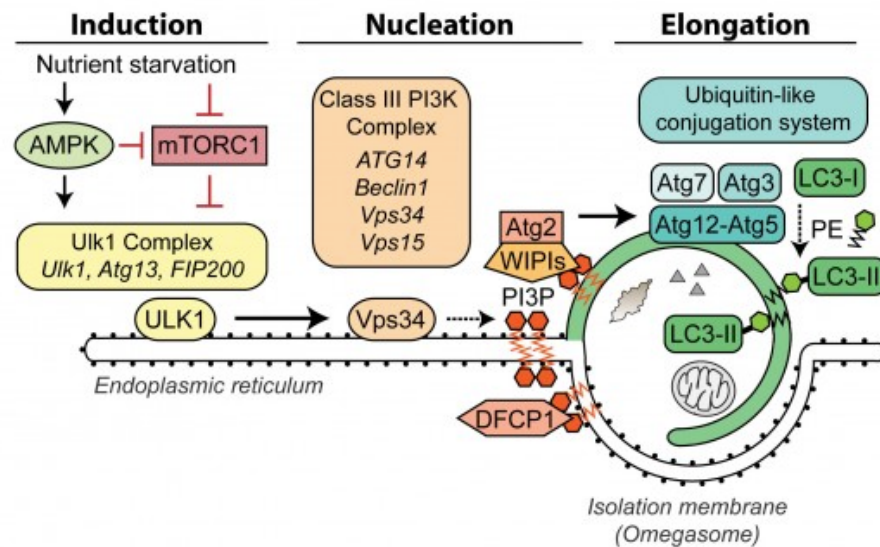
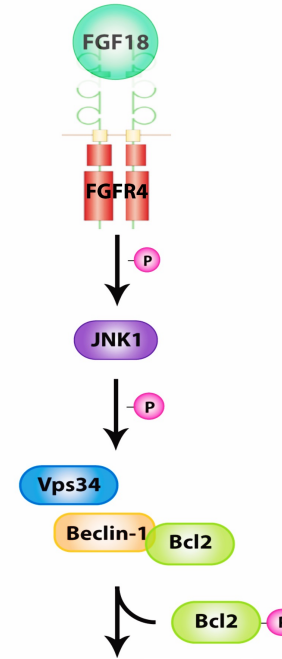
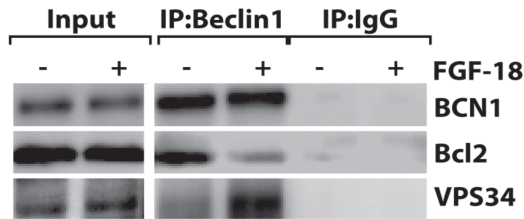
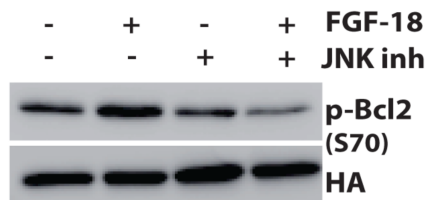
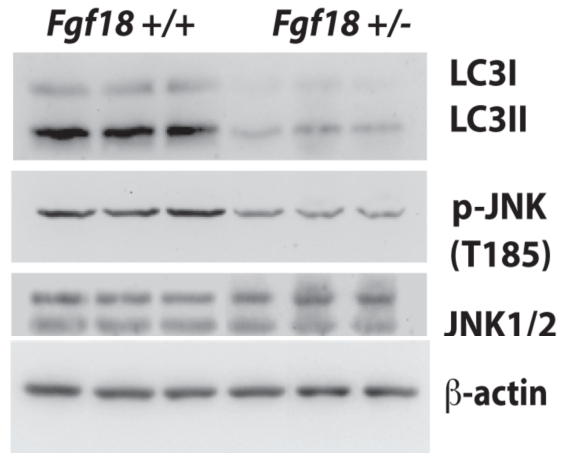
ECM MATRIX PRODUCTION

OSTEOBLAST DIFFERENTIATION

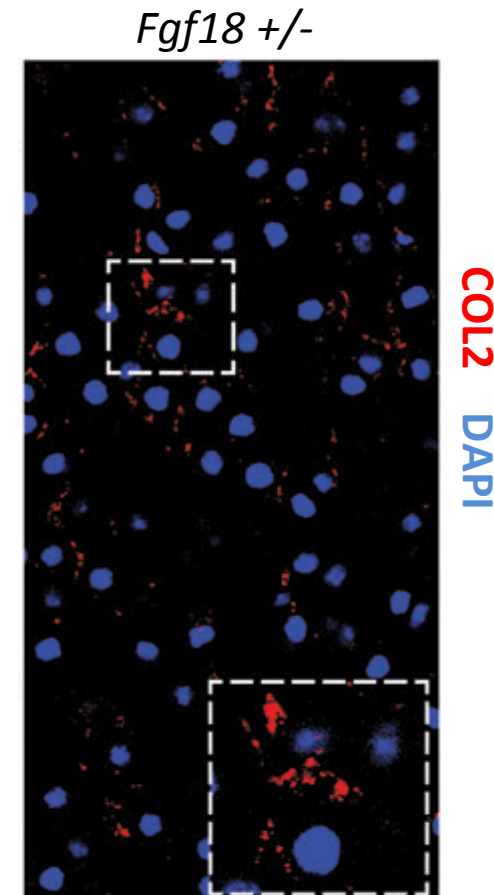
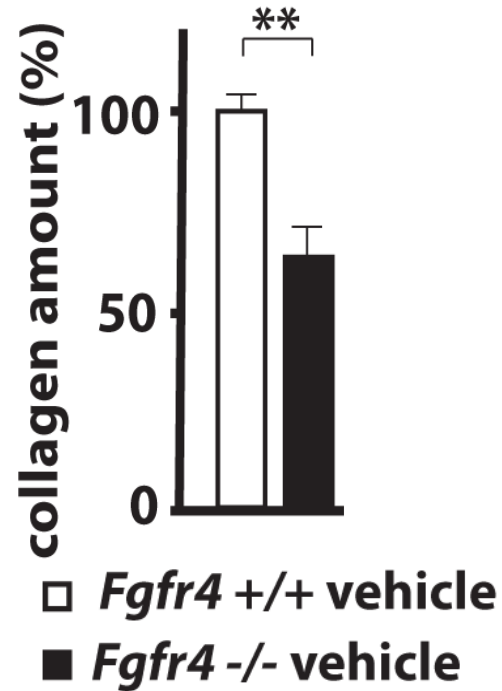
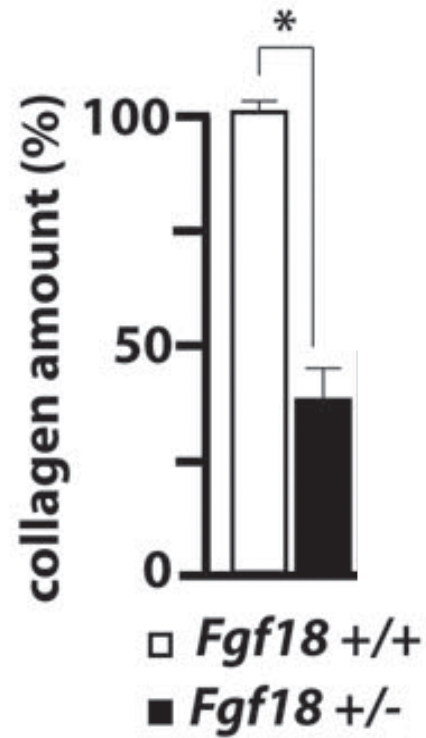
# FGF18 REGULATES AUTOPHAGY THROUGH FGFR4



# FGF18 REGULATES BECLIN 1/VPS34 COMPLEX ACTIVITY

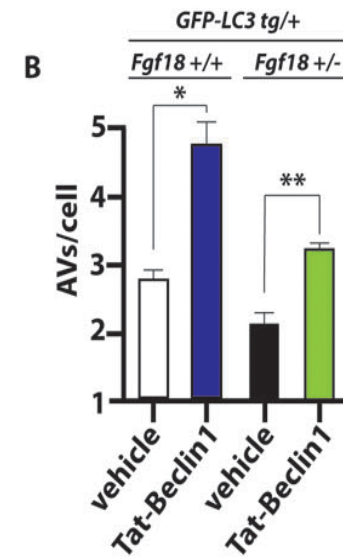
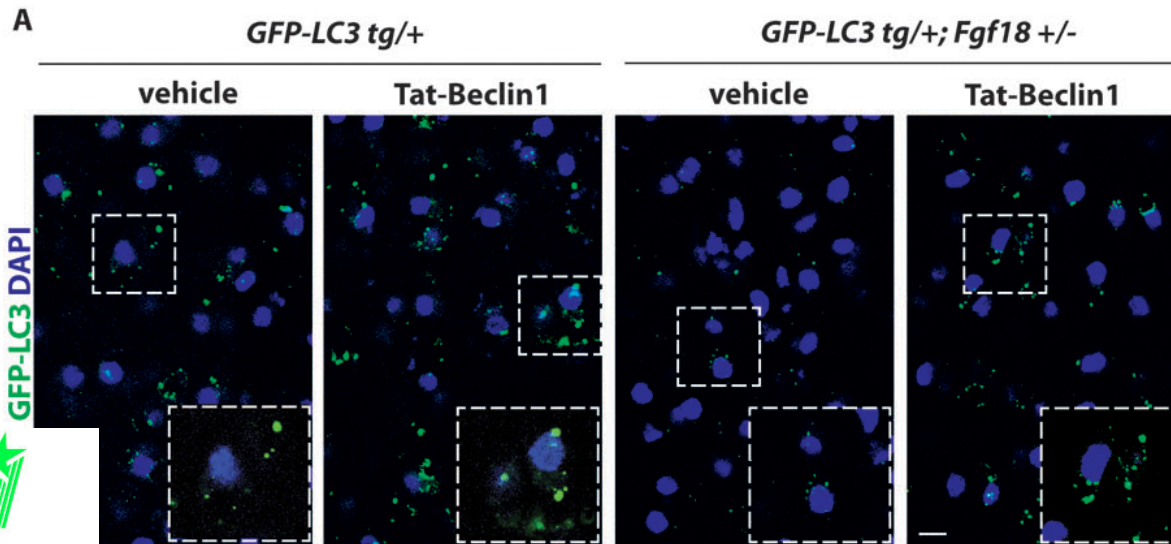
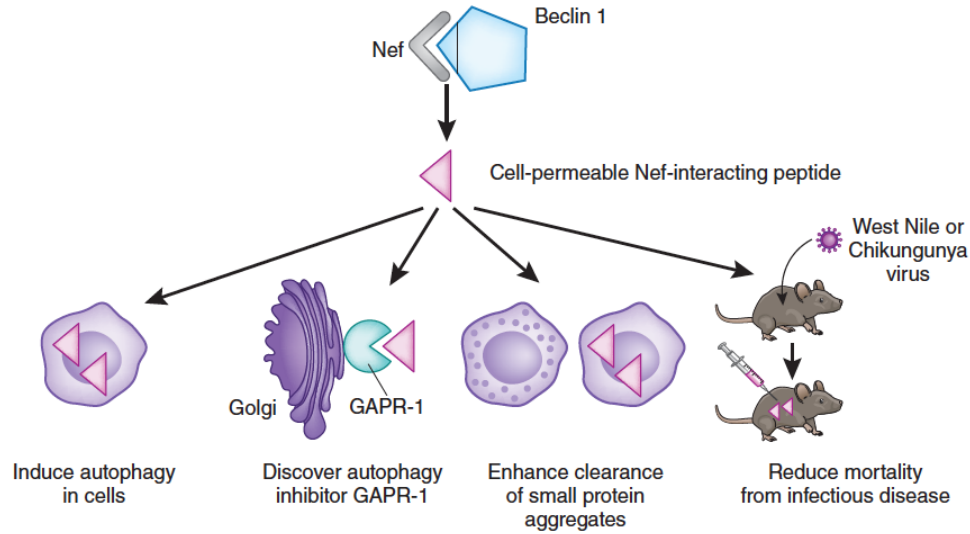


# DEFECTIVE COL2 IN *Fgf18*<sup>+/-</sup> AND *Fgfr4*<sup>-/-</sup> GROWTH PLATES

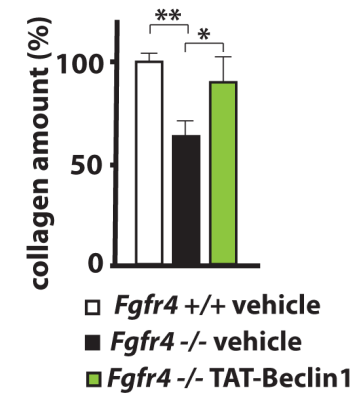
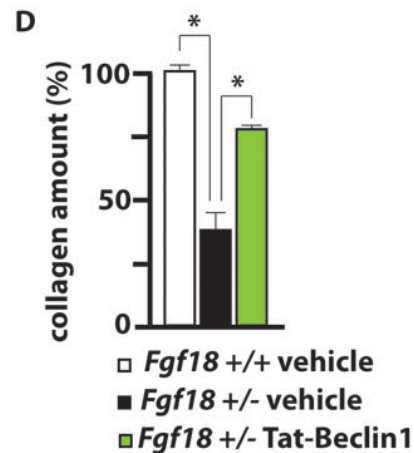
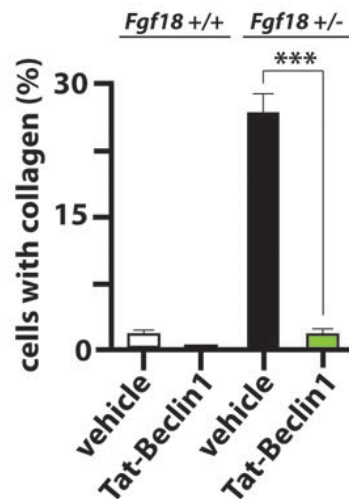
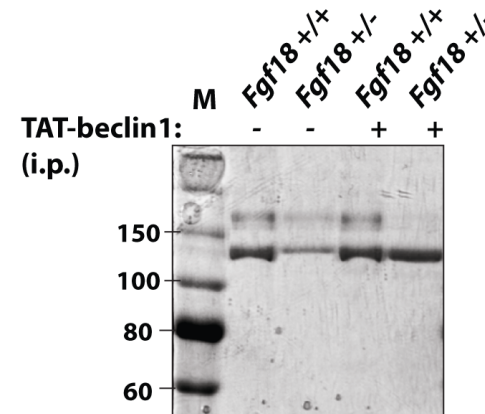
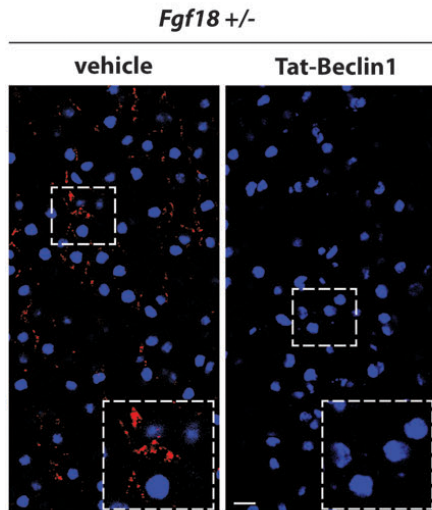




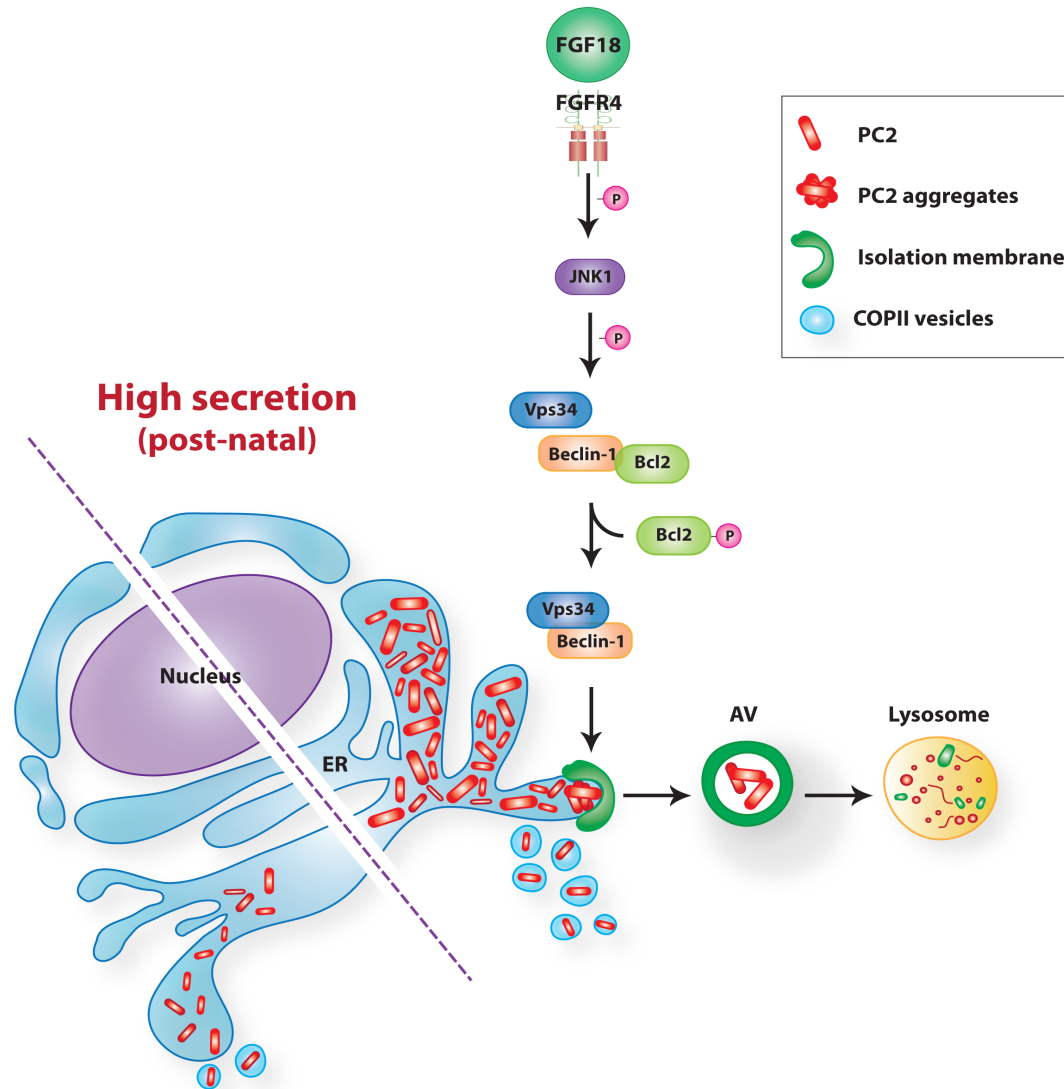
# BECLIN 1 ACTIVATION RESCUES AUTOPHAGY IN *Fgf18 +/-* GROWTH PLATES



# BECLIN 1 ACTIVATION RESCUES COLII DEFECTS IN *Fgf18* +/- AND *Fgfr4* -/- GROWTH PLATES



# FGF SIGNALING REGULATES BONE GROWTH THROUGH AUTOPHAGY



## TAKE HOME MESSAGES (2)

---

- Autophagy is a developmentally regulated process
- Growth factor signaling can modulate autophagy
- Post natal induction of autophagy controls Col2 levels in cartilage.
- Autophagy may be implicated in the pathogenesis of skeletal diseases



# ACKNOWLEDGEMENT



## OPEN POST DOCTORAL POSITIONS

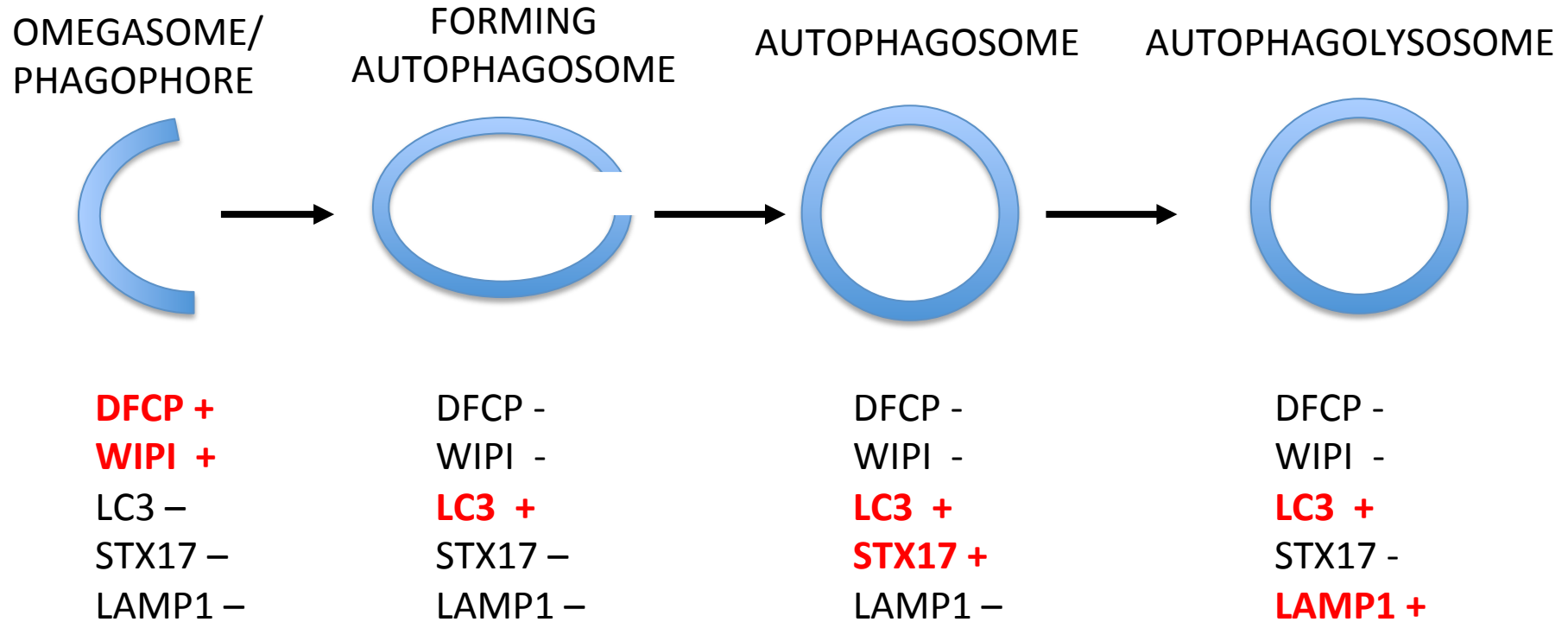
*Laura Cinque  
Alison Forrester  
Rose Bartolomeo*

*Carmen Lanzara  
Maria Svelto  
Anna Chiara Salzano  
Chiara De Leonibus*

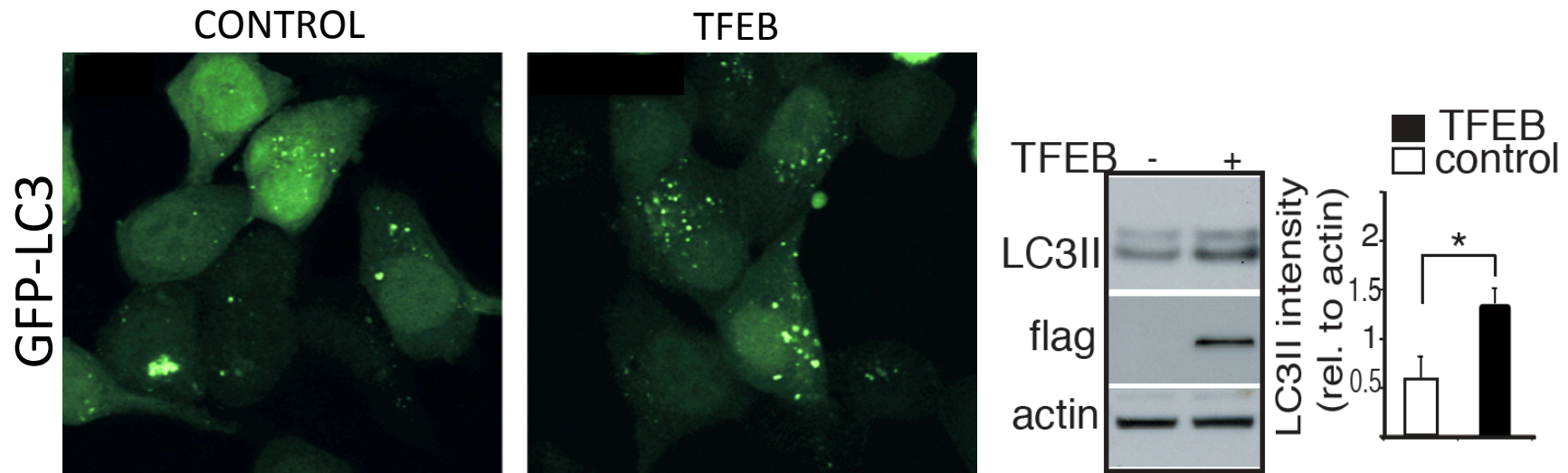
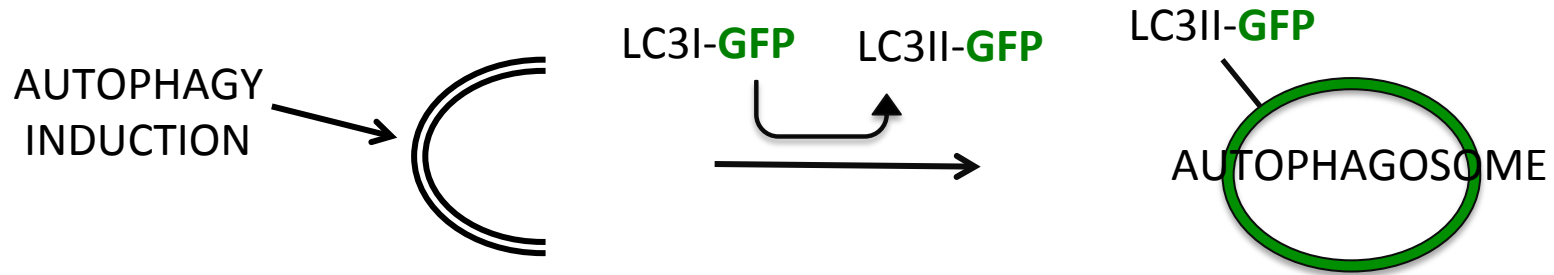


# **HOW TO MONITOR AUTOPHAGY**

# AUTOPHAGIC MARKERS



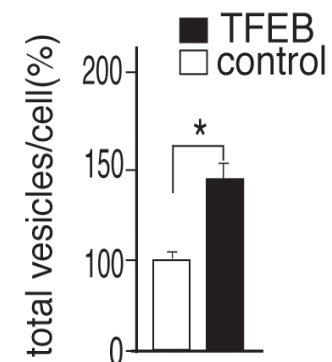
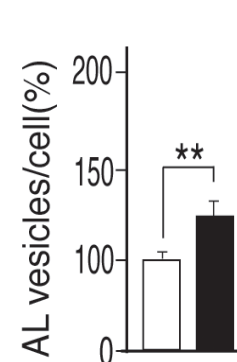
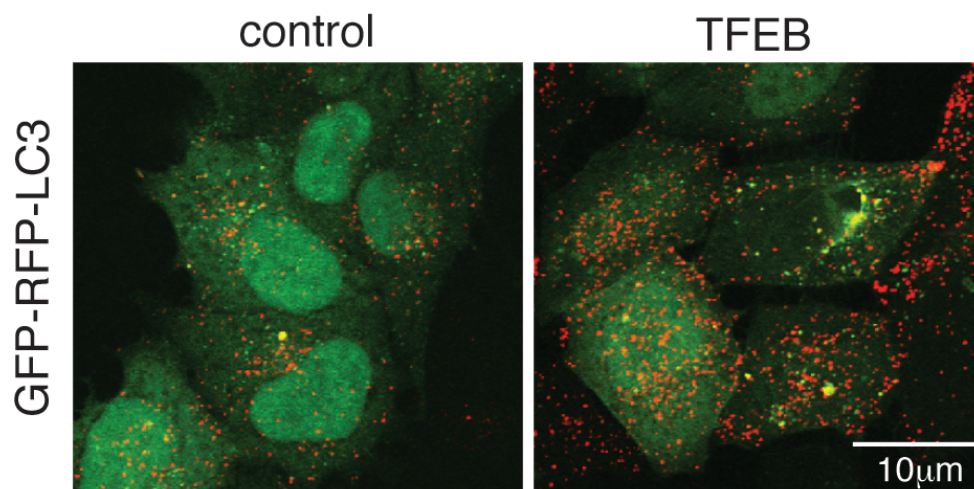
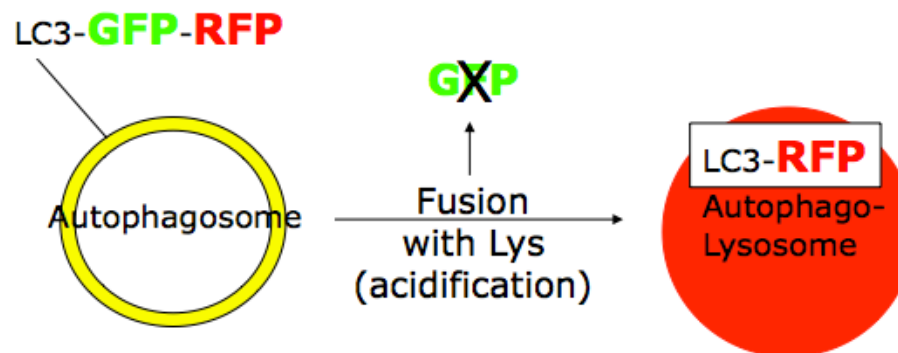
# LC3 PUNCTA FORMATION



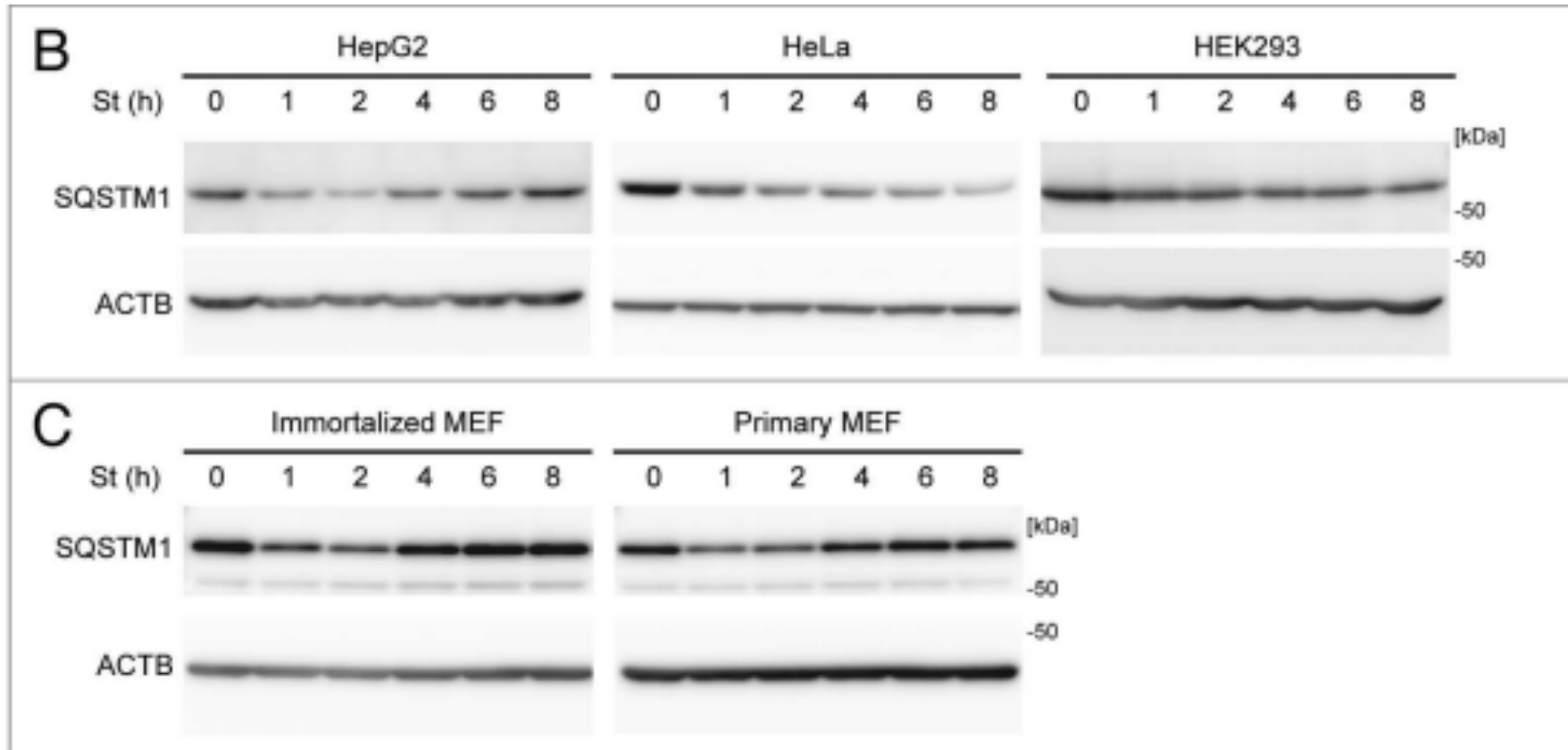
GFP-LC3 plasmid or endogenous LC3



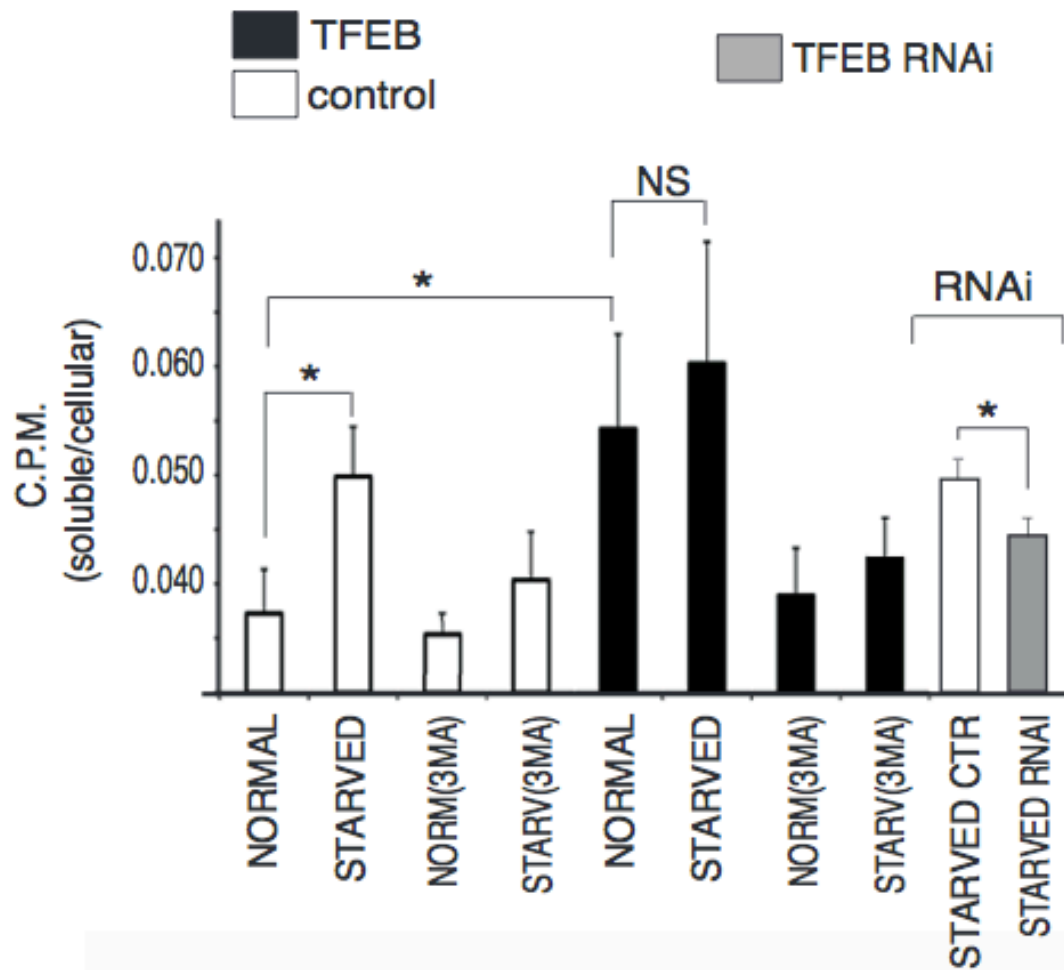
# MONITORING AV-LYS FUSION *in vivo*



# MONITORING AUTOPHAGY BY P62/SQSTM1 DEGRADATION



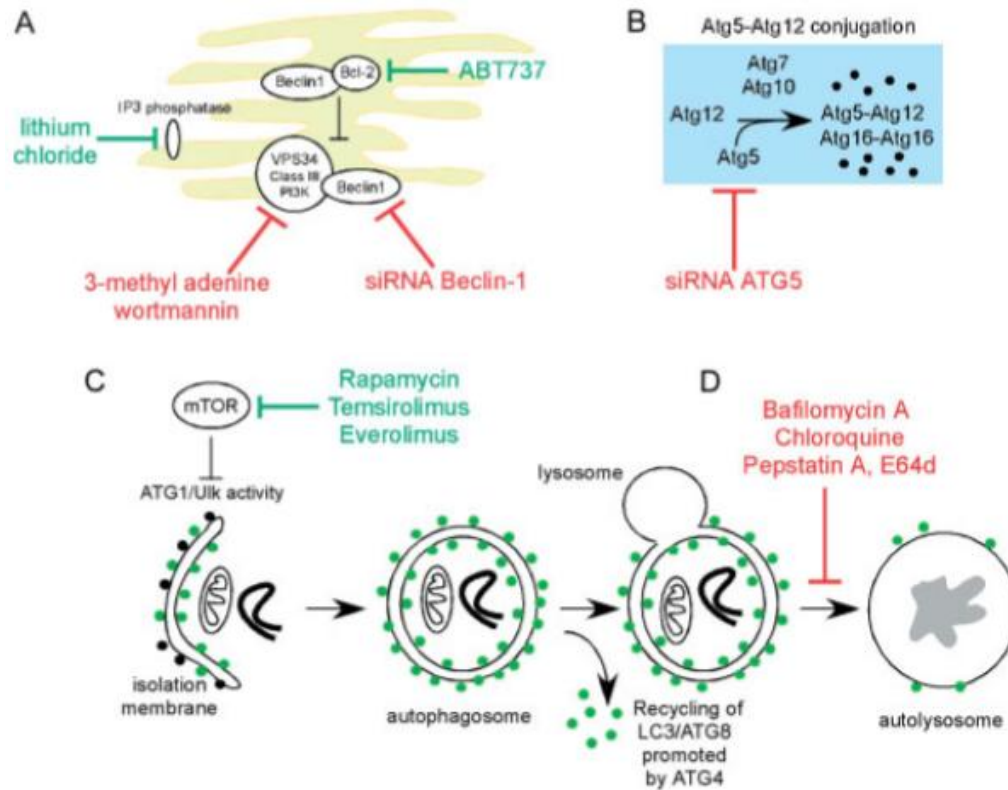
# MONITORING AUTOPHAGY BY LONG LIVED PROTEIN DEGRADATION



# **HOW TO MODULATE AUTOPHAGY**



# PHARMACOLOGICAL MODULATION OF AUTOPHAGY



New modulators (**MORE SPECIFIC**):

TAT-BECLIN1 (inducer) (**ALSO IN VIVO**)

SPAUTIN1 (inhibitor)

VPS34 inhibitors

ULK1 inhibitors