

# **Autophagy and Cancer**

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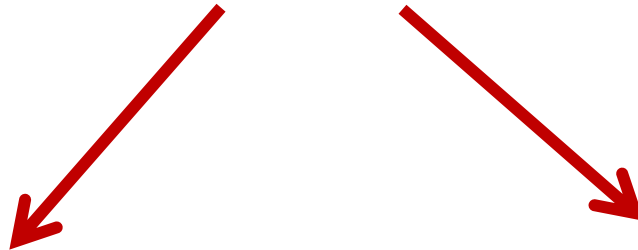
Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR)

Mustansiriyah University

# Cellular Homeostasis

- ❖ constant turnover of continuous synthesis of cellular components
- ❖ clearance of damaged or superfluous proteins and organelles.

## Degradation pathways



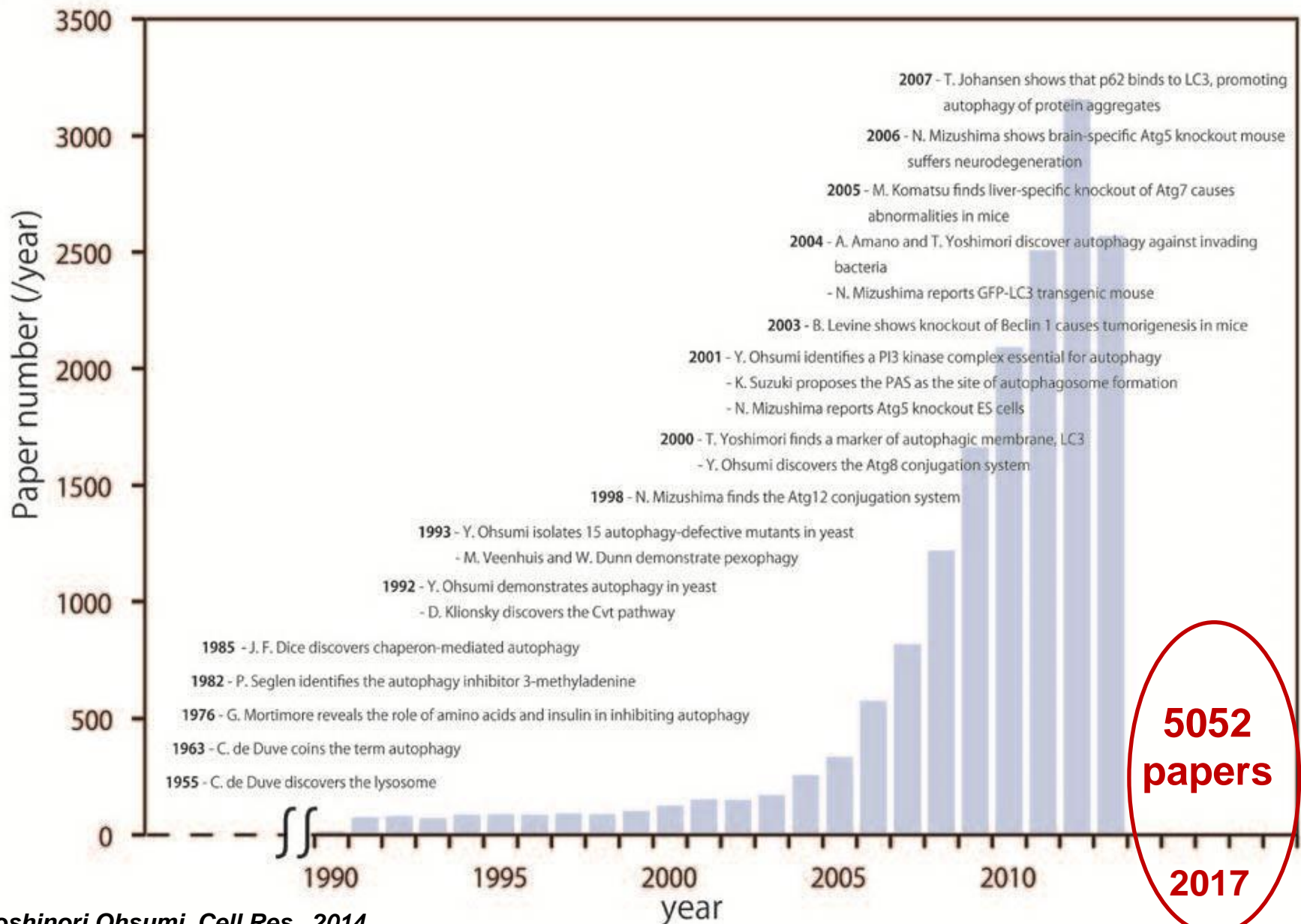
### Ubiquitin-Proteasome System (UPS)

high selectivity  
short-lived proteins

### Lysosomal pathway

Autophagy

# Chronology

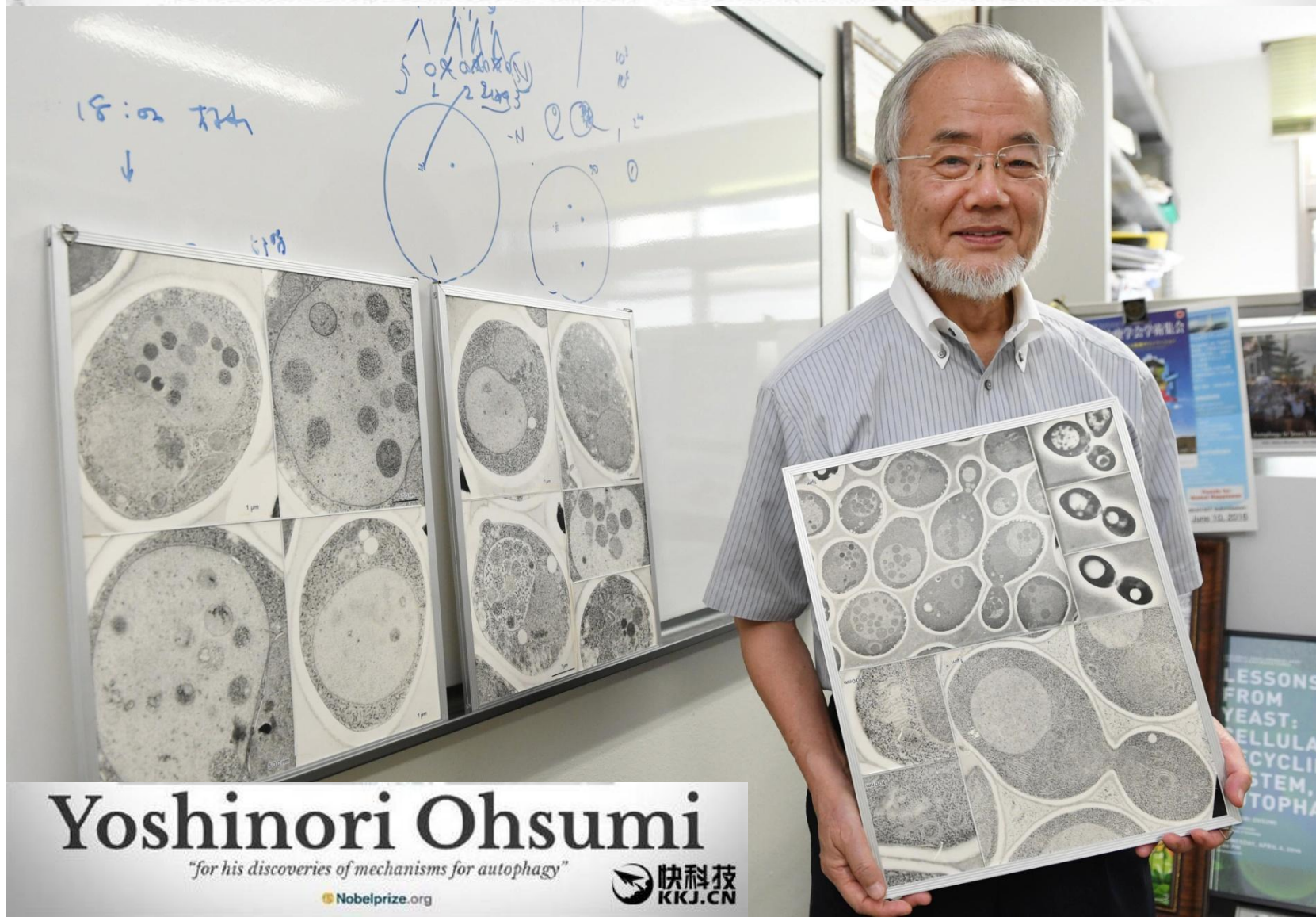


"For the greatest benefit to mankind"  
*Alfred Nobel*



The Nobel Assembly at Karolinska Institutet has today decided to award the

# 2016 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE



## Yoshinori Ohsumi

"for his discoveries of mechanisms for autophagy"

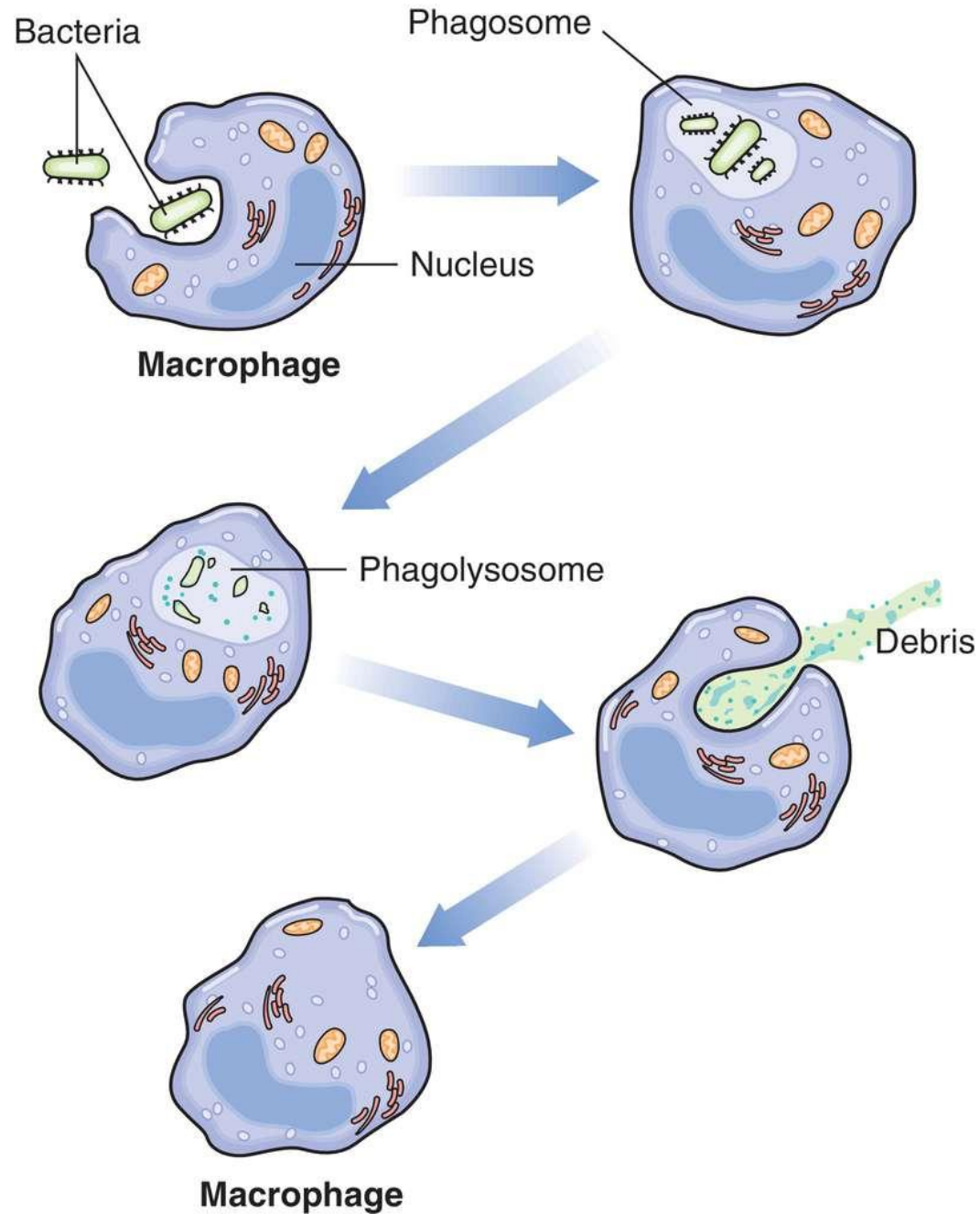
 Nobelprize.org

 快科技  
KKJ.CN



## Phagocytosis

Autophagy is  
totally different



# What is Autophagy?

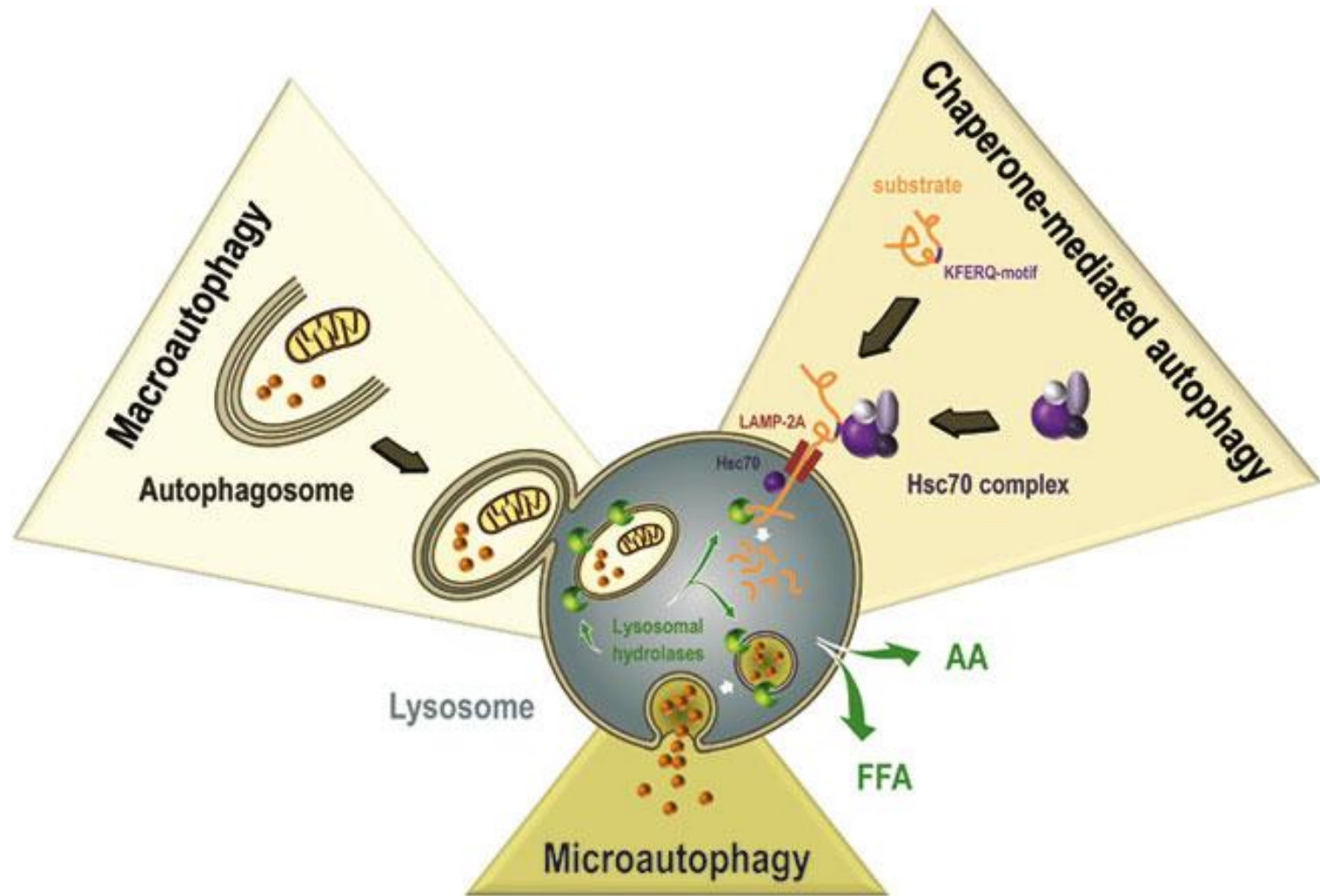
“Self-eating”

From the Greek words, *auto* "self" and *phagein* "to eat"

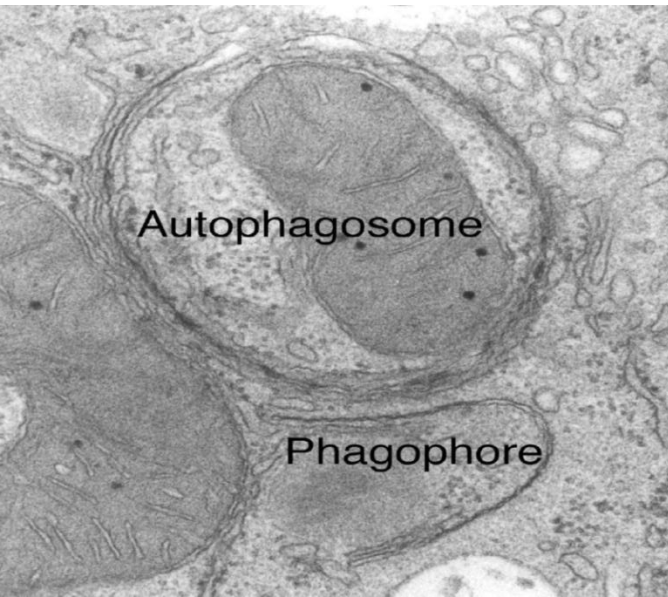
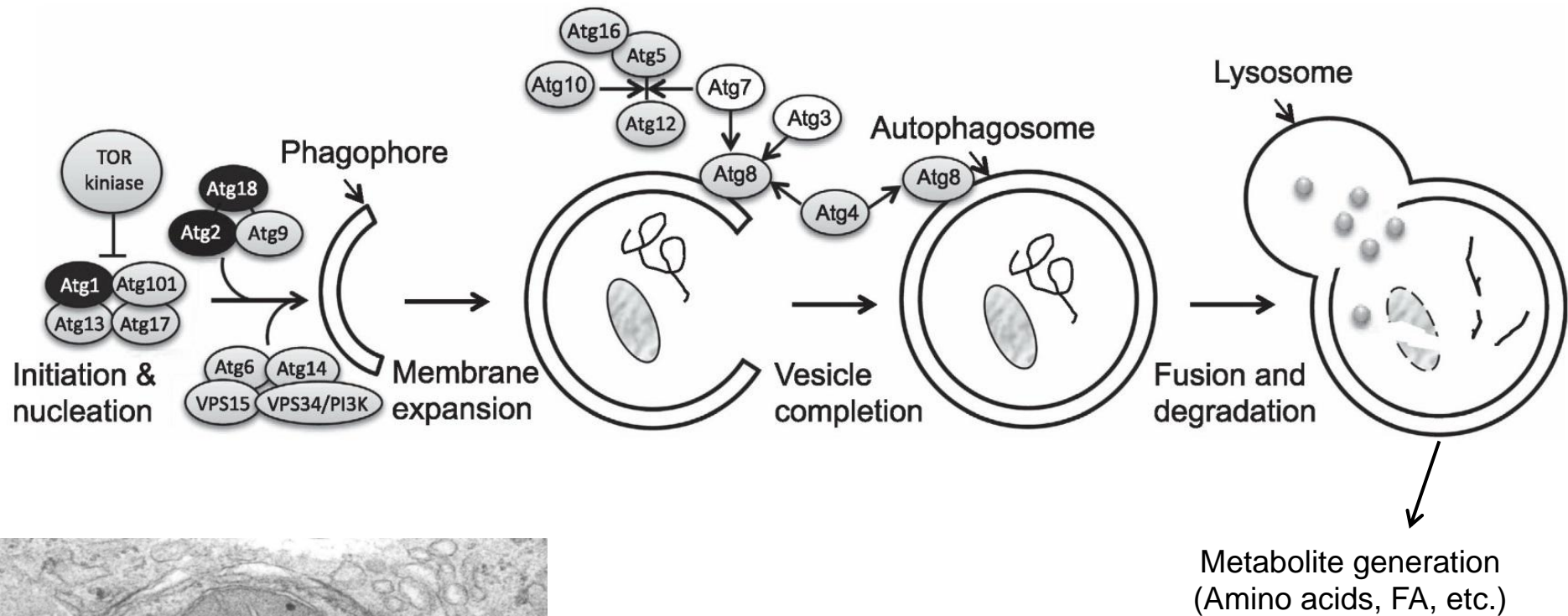
Catabolic process through which the cell recycles its own constituents.

Pathway that lead to the elimination of cytoplasmic components by delivering them into lysosomes.

# Types of Autophagy



# Mechanism of Autophagy



# Multiple Functions of Autophagy

- Occurs in all eukaryotic cells
- Bulk degradative process that ends in lysosomes
- Degradation of intracellular components

Basal autophagy  
Quality control

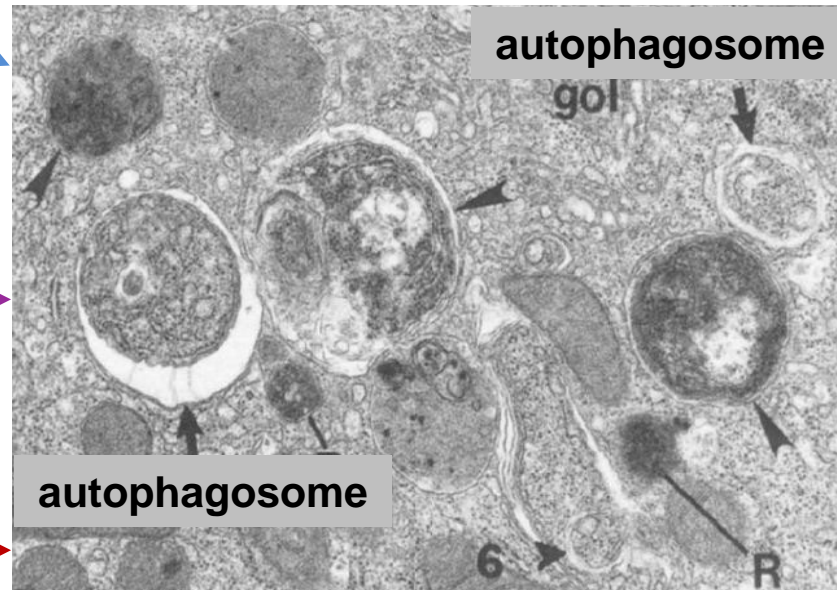
Removal of  
obsolete organelles  
and protein aggregates

Nutrient/  
Growth factor  
deprivation

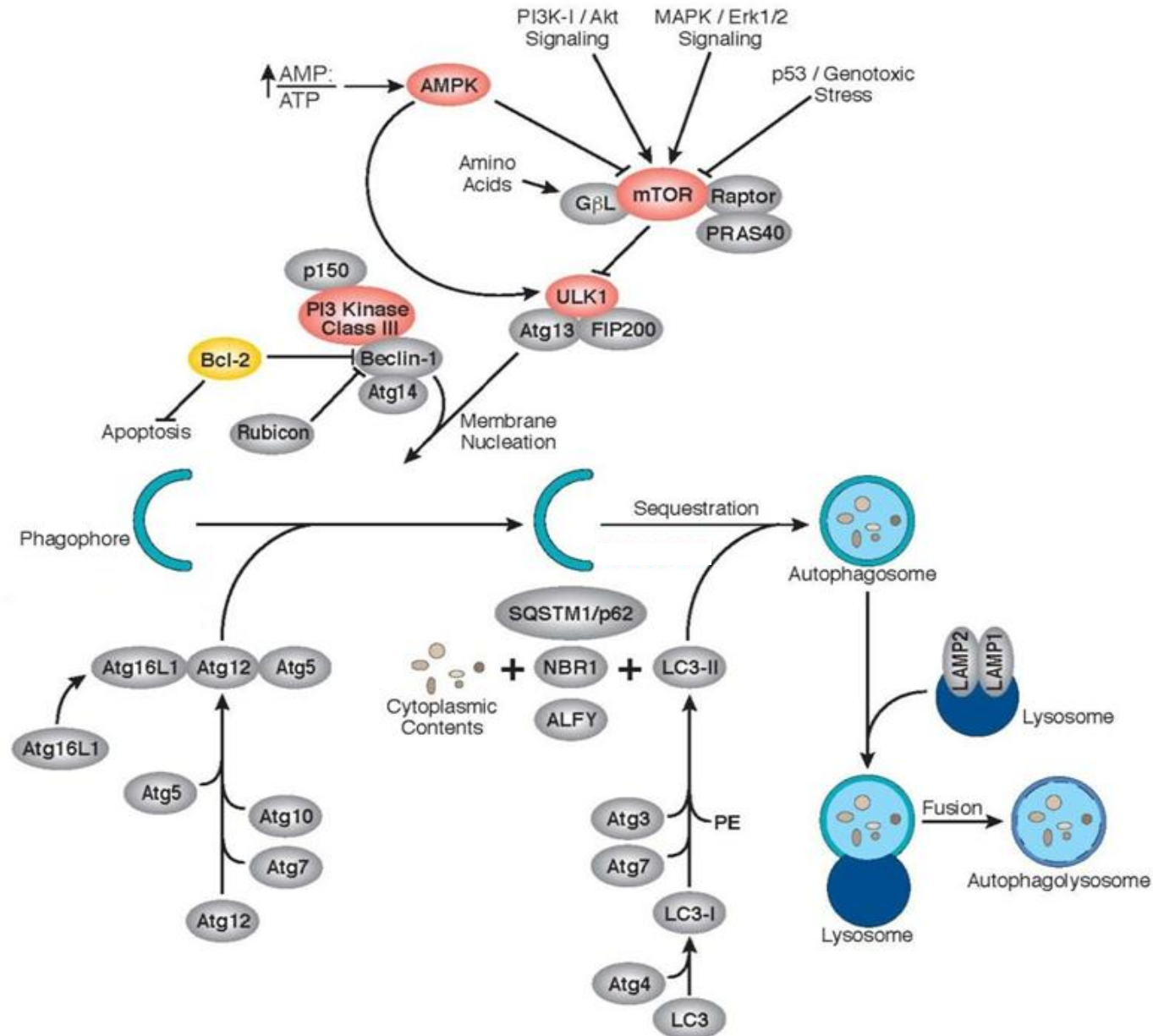
Metabolic substrates  
(energy, nutrient)

Uncontrolled  
autophagy

Autophagic  
cell death

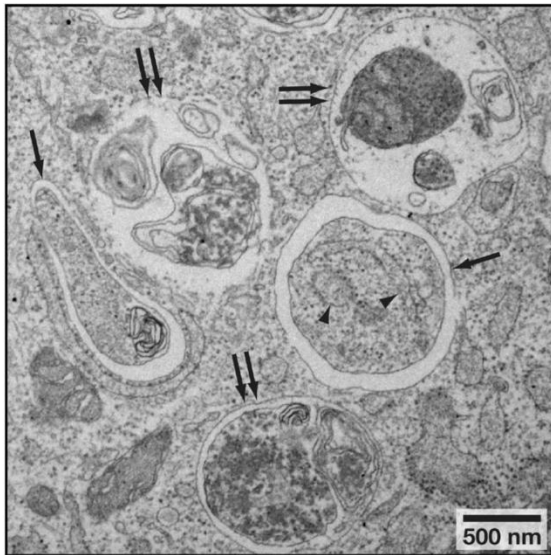


# Autophagy Signalling Pathway

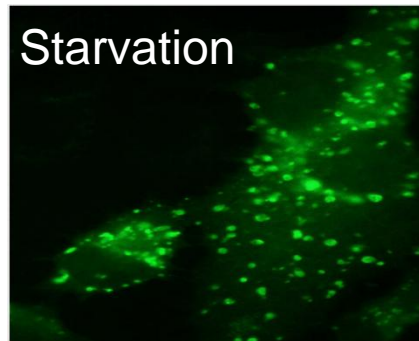
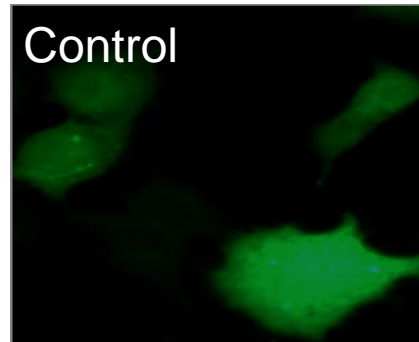


# How can We Monitor Autophagy?

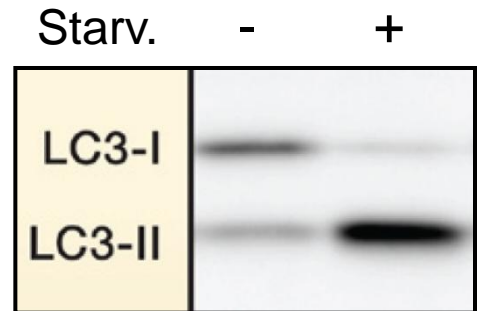
## EM



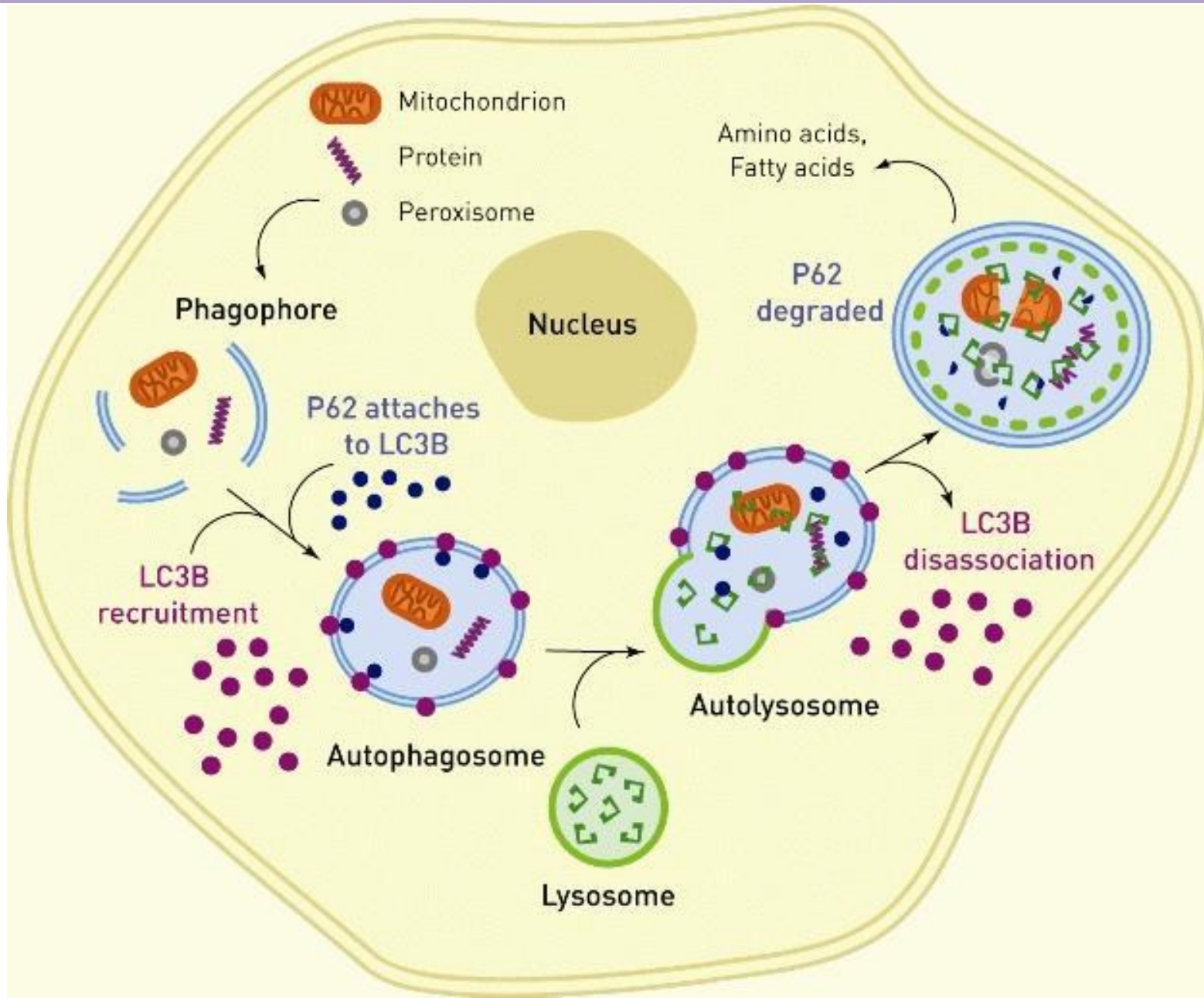
## IF LC3



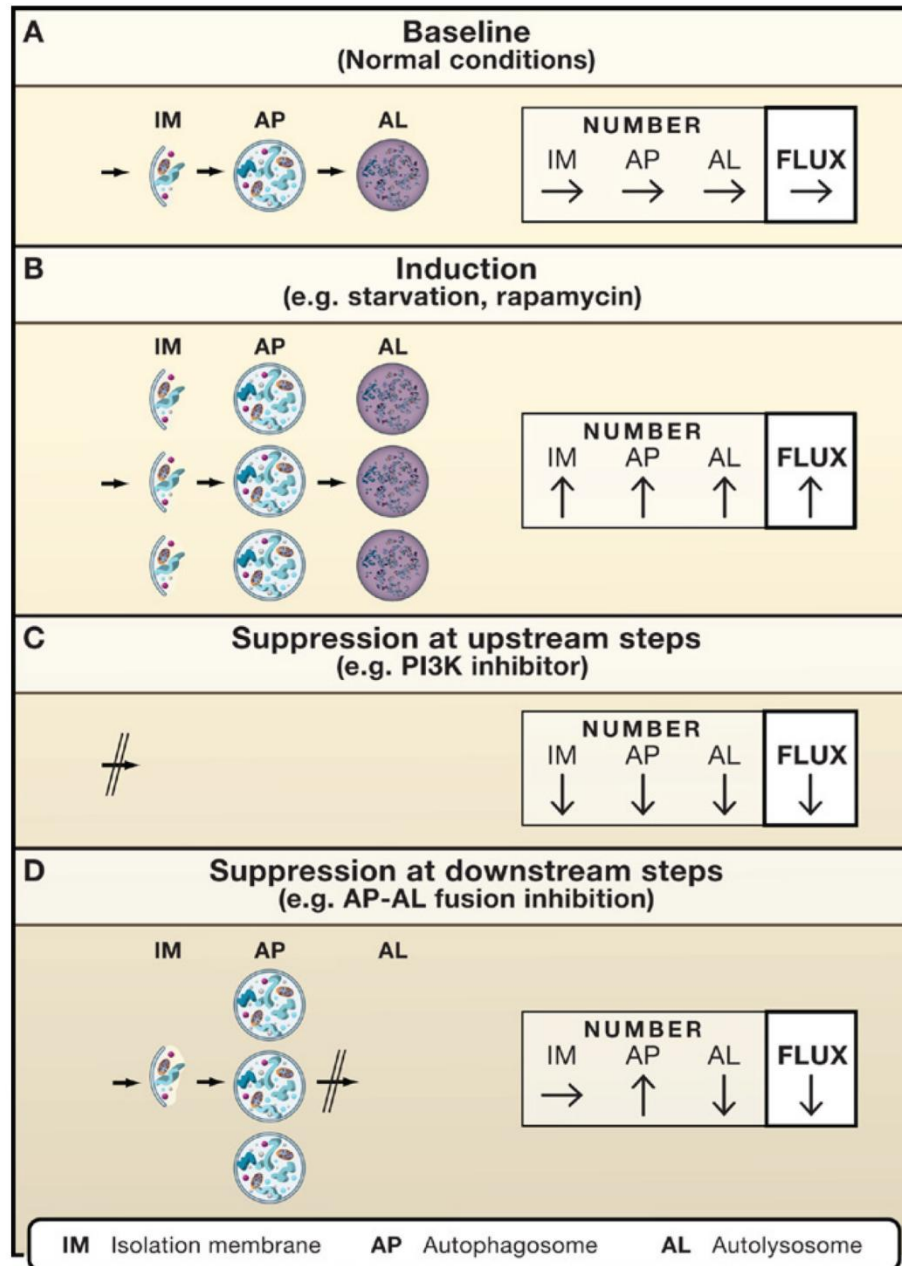
## WB



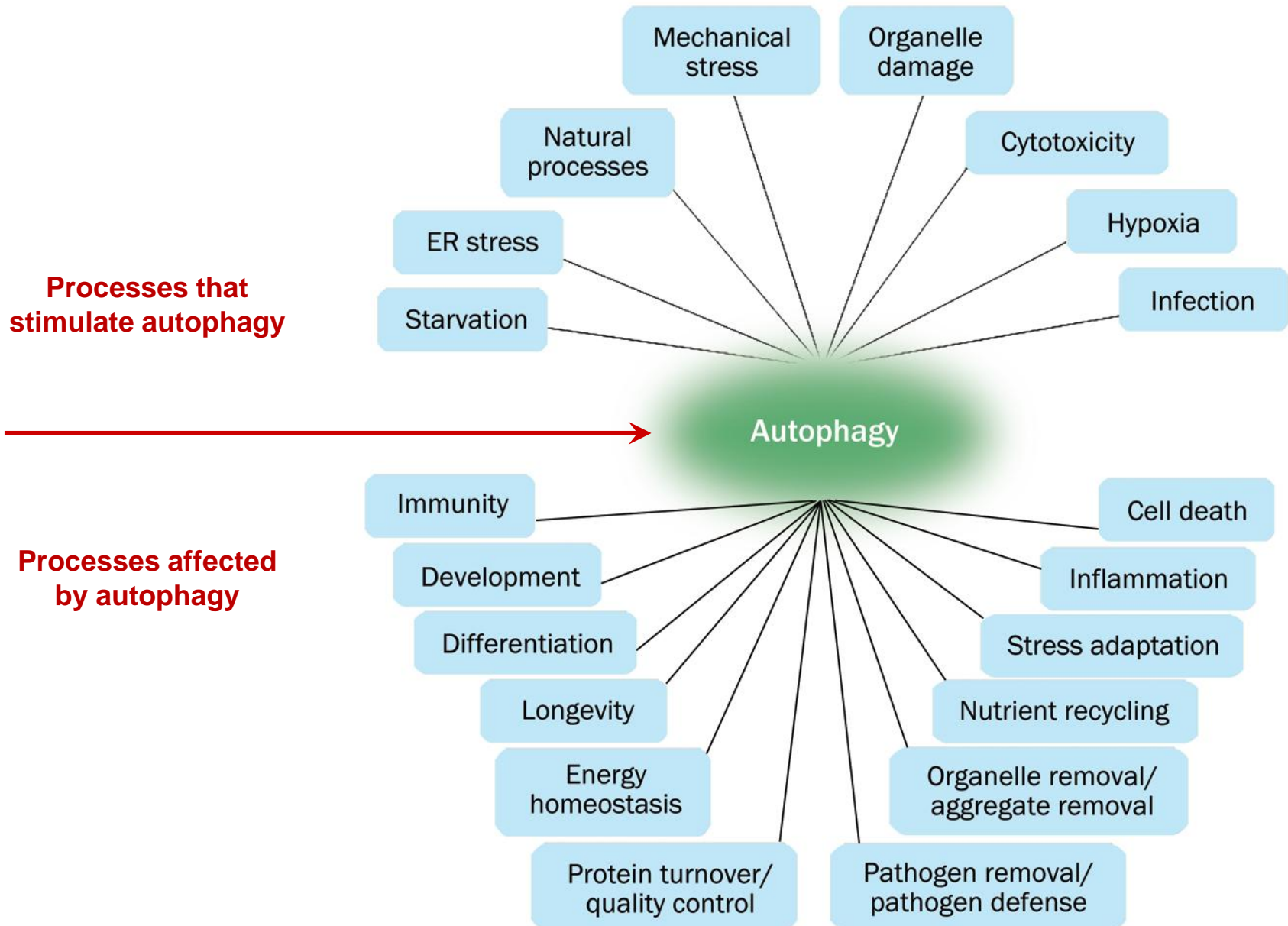
# How can We Monitor Autophagy?



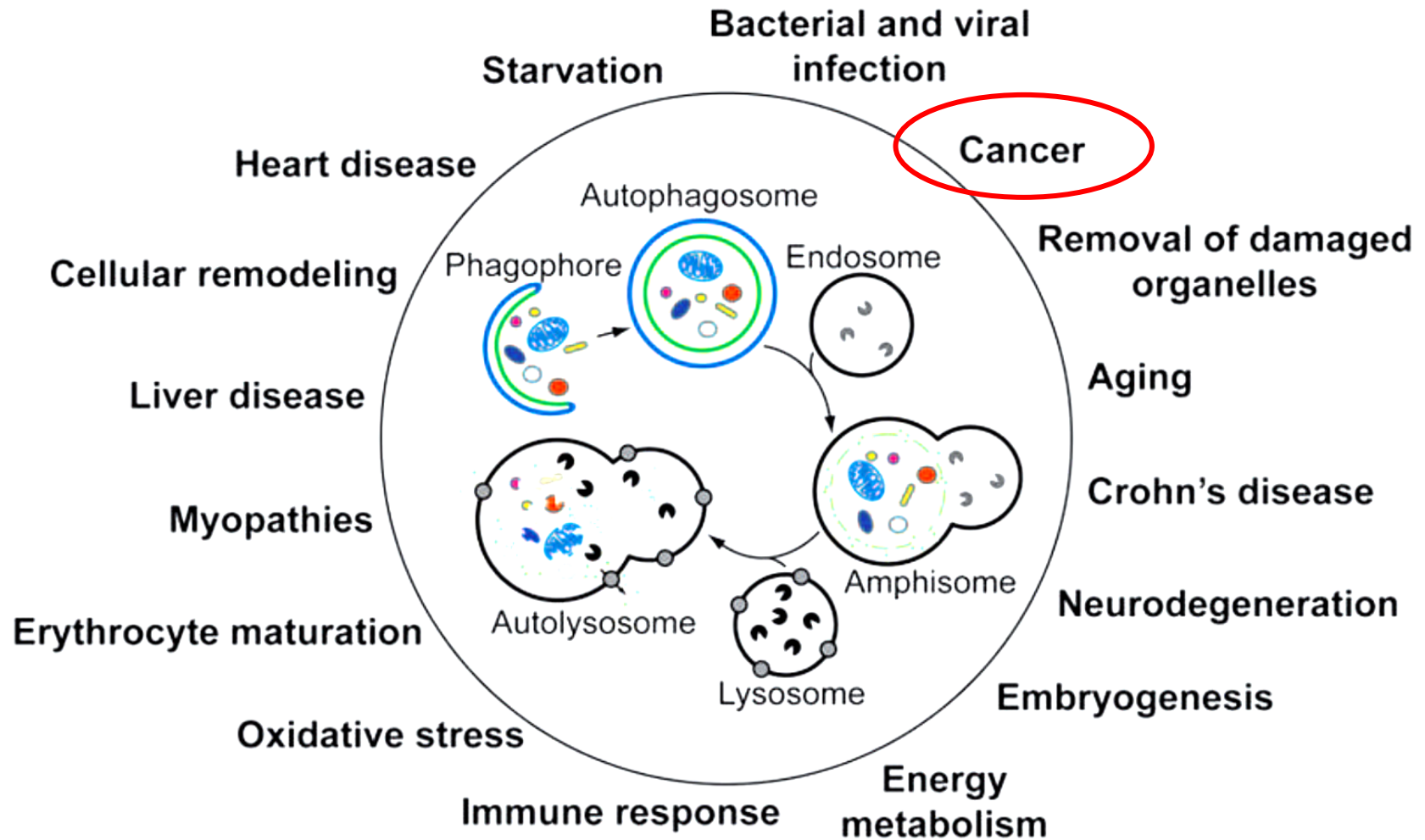
# Dynamic regulation of autophagy



# Induction of Autophagy



# Autophagy and Diseases



# Autophagy and Cancer

The connections between autophagy and cancer occur at two aspects:

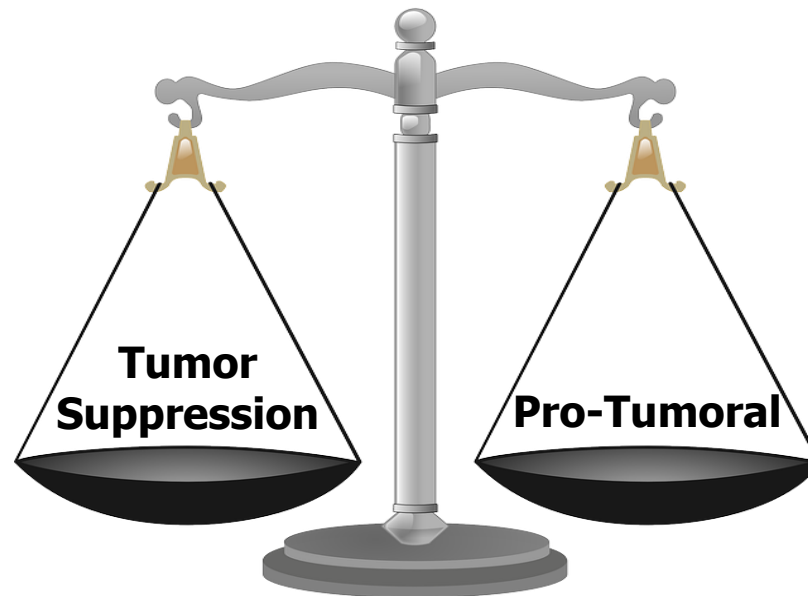
**First** at the level of tumor initiation and progression,

**Second** during cancer treatment.

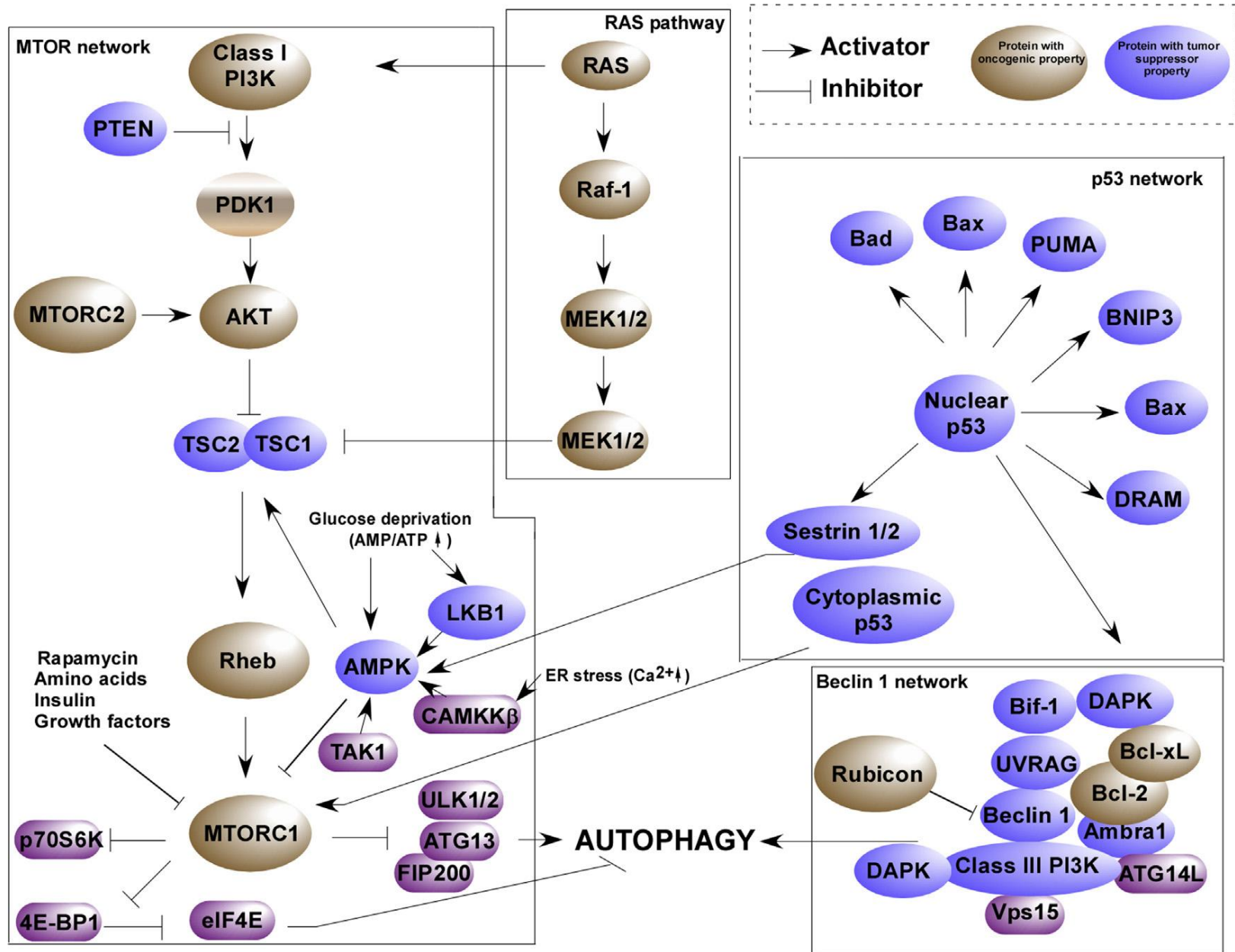
# Autophagy in Tumor Initiation and Progression

The role of autophagy in cancer is complex and likely tissue and genetic context-dependent.

## Dual role of Autophagy



# Autophagy in Tumor Initiation and Progression



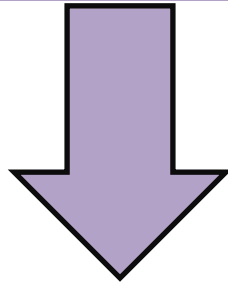
# Autophagy and Cancer

## ❖ Mouse models for autophagy-deficient gene:

- Beclin1: tumor suppression function
- ATG5, ATG7, and FIP200: No malignant tumor development *in vivo*.
- GABARAP: less tumor formation after carcinogen treatment



## Autophagy as Tumor Suppressor Mechanism



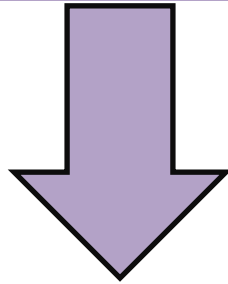
Maintain cellular  
Homeostasis

Prevent DNA  
damage and genomic  
instability

Preventing necrosis  
and subsequent  
inflammation

Prevent tumor  
development by  
regulating the cellular  
level of p62

## Autophagy as Tumor Promoting Mechanism



Allow tumor cells to survive under stressful conditions

Metabolic reprogramming that cancer cells encounter after oncogenic transformation

Support tumor development by maintaining the survival and self renewal of cancer stem cells

Autophagy promotes the tumor cell dissemination and metastasis by protecting them from anoikis.

# Autophagy and Cancer

## Autophagy gene profile in human tumors

Human cancer types	Genetic autophagy modulation
<b>Breast</b>	BECN1 gene monoallelically deleted in up to 50% of cases. Loss of heterozygosis and aberrant methylation of promoter and the intron 2 in breast tumor tissues Association between BECN1 loss and HER2/NEU amplification
<b>Colorectal</b>	Frameshift mutations of ATG2B, ATG5 and ATG9B detected in a subset of MSI-H cases
<b>Gastric</b>	Frameshift mutation of UVRAG detected in 9.4% of MSI-H cases Frameshift mutations of ATG2B, ATG5 and ATG9B detected in a subset of MSI-H cases
<b>Head and neck</b>	ND
<b>Liver</b>	Reduction of Beclin 1 mRNA expression observed in 45.5% of HCC tissues
<b>Leukemia</b>	RAB7A gene rearrangement and deletion
<b>Melanoma</b>	Constitutive formation of autophagosomes detected in invasive and metastatic melanoma cells Melanoma cells actively undergoing autophagy Downregulation of Beclin 1 and LC3 during disease progression
<b>Osteosarcoma</b>	Weaker Beclin 1 IHC staining in tumors than in normal bones
<b>Ovarian</b>	BECN1 gene monoallelically deleted in up to 75% of case
<b>Pancreatic</b>	Positive LC3 IHC staining in 43.7% of cases
<b>Prostate</b>	BECN1 gene monoallelically deleted in up to 40% of cases

# Autophagy in Cancer Treatment

Autophagy induction have been found to spatially localize to:

- 1- Hypoxic tumor regions.
- 2- Poorly vascularized tumor regions.
- 3- Following cytotoxic treatments.



Promotes cancer cell survival under stressful conditions



Treatment resistance mechanism

# Autophagy in Cancer Treatment

Therapeutic Agent	Model	Autophagy Inhibition	Response
Temozolomide	Human malignant glioma cell lines	3-Methyladenine	Decreased cytotoxicity
Cyclophosphamide	Murine Myc-induced lymphoma cancer	Bafilomycin A Chloroquine	Increased cytotoxicity Increased antitumor response
5-Fluorouracil	Human colon cancer cell lines	3-Methyladenine	Increased apoptosis
5-Fluorouracil	Human colon cancer cell lines and xenograft		Increased cytotoxicity
5-Fluorouracil	Human colon cancer cell line (HT29)	Chloroquine	Increased cytotoxicity
5-Fluorouracil	Human hepatic carcinoma cell lines	3-Methyladenine	Increased apoptosis
5-Fluorouracil	Murine colon cancer cell line and tumor xenograft	Chloroquine	Increased apoptosis
5-Fluorouracil	Human NSCLC cell line (A549)	3-Methyladenine	Increased apoptosis
Cisplatin	Esophageal SSC cell line (EC9706)	3-Methyladenine	Increased apoptosis
Cisplatin	Human cholangiocarcinoma cell lines	3-Methyladenine Wortmannin	Increased cytotoxicity
Cisplatin	Human cervical cancer cell line (HeLa)	3-Methyladenine Chloroquine	Increased apoptosis
Cisplatin	Human hepatic carcinoma cell lines	3-Methyladenine	Increased apoptosis
Cisplatin	Laryngeal cancer cells (Hep-2)	3-Methyladenine	Increased apoptosis
Cisplatin	Human NSLC cell line (A549)	3-Methyladenine	Increased apoptosis
Oxaliplatin	Human colon cancer cell lines and xenograft	Chloroquine	Increased cytotoxicity and tumor control
Paclitaxel	Human NSLC cell line (A549)	3-Methyladenine	Increased apoptosis
Etoposide	Human hepatocellular carcinoma cell line (HepG2)	3-Methyladenine	Increased cytotoxicity
Doxorubicin	Human multiple myeloma cell lines, patient-derived multiple myeloma cells, human plasmacytoma xenograft	Hydroxychloroquine 3-Methyladenine	Increased apoptosis
Epirubicin	Human breast cancer cell line (MCF7)	Bafilomycin A	Increased apoptosis
Melphalan	Human multiple myeloma cell lines, patient-derived multiple myeloma cells, human plasmacytoma xenograft	Hydroxychloroquine 3-Methyladenine	Increased apoptosis
Topotecan	Human NSLC cell line (A549)	Chloroquine	Increased cytotoxicity
Camptothecin	Human breast cancer cell lines	Wortmannin 3-Methyladenine Bafilomycin A	Increased apoptosis in selective cell lines

# Autophagy in Cancer Treatment

Therapeutic Agent	Model	Autophagy Inhibition	Response
Imatinib	Human glioma cell lines	3-Methyladenine	Decreased cytotoxicity
Imatinib	Human Philadelphia chromosome positive CML cells	Bafilomycin A	Increased cytotoxicity
HDACi/vorinostat	Human colon cancer cells and xenografts	Chloroquine	Increased cytotoxicity
HDACi/panobinostat	Human triple negative breast cancer cells and xenografts	Chloroquine	Decreased growth
HDACi/SAHA	Human CML cell lines and primary CML cells	Chloroquine	Increased cytotoxicity
HDACi/valproic acid	Human t(8;21) acute myeloid leukemia cells	Chloroquine	Increased cytotoxicity
HSP90i/DMAG	Human multiple myeloma cell lines	3-Methyladenine	Increased cytotoxicity
Erlotinib	Human glioblastoma cell lines	Chloroquine	Increased cytotoxicity
Sorafenib	Human hepatocellular carcinoma cell lines and xenografts	Chloroquine	Increased cytotoxicity and decreased tumor growth
Sorafenib	Human hepatocellular carcinoma cell lines and xenografts	3-Methyladenine	Increased cytotoxicity and decreased tumor growth
Sunitinib	Rat PC12 cells	Chloroquine	Increased cytotoxicity
AKTi/AZD5363	Human prostate cancer cell lines and xenograft	Ammonium chloride	Increased cytotoxicity and decreased tumor growth
METi/PHA665752 and EMD1214063	Human gastric adenocarcinoma cell line	3-Methyladenine	Increased cytotoxicity
Vandetanib	Human glioblastoma cell lines and xenograft	3-Methyladenine	Increased cytotoxicity and decreased tumor growth
Bevacizumab	Human hepatocellular carcinoma xenografts	Chloroquine	Decreased tumor growth
Bortezomib	Human multiple myeloma cell line (U266)	3-Methyladenine	Decreased cytotoxicity
Bortezomib	Human hepatocellular carcinoma cell lines and xenografts	Bafilomycin A	Increased cytotoxicity
		Chloroquine	Increased apoptosis

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Rank	Status	Study
1	Unknown <sup>†</sup>	<p><a href="#">Autophagy Inhibition Using Hydrochloroquine in Breast Cancer Patients</a></p> <p>Condition: Breast Cancer</p> <p>Intervention: Drug: Hydrochloroquine</p>
2	Recruiting	<p><a href="#">Modulation of Autophagy in Patients With Advanced/Recurrent Non-small Cell Lung Cancer - Phase II</a></p> <p>Conditions: Non-small Cell Lung Cancer; Advanced Non-small Cell Lung Cancer; Recurrent Non-small Cell Lung Cancer</p> <p>Interventions: Drug: Paclitaxel; Drug: Carboplatin; Drug: Hydroxychloroquine; Drug: Bevacizumab</p>
3	Active, not recruiting	<p><a href="#">Hydroxychloroquine in Blocking Autophagy in Patients With Prostate Cancer Undergoing Surgery or Active Surveillance</a></p> <p>Condition: Prostate Carcinoma</p> <p>Interventions: Drug: Hydroxychloroquine; Other: Laboratory Biomarker Analysis</p>
4	Completed	<p><a href="#">Chloroquine as an Anti-Autophagy Drug in Stage IV Small Cell Lung Cancer (SCLC) Patients</a></p> <p>Condition: Small Cell Lung Cancer</p> <p>Intervention: Drug: Chloroquine, A-CQ 100</p>

# Tumor suppression in mice lacking GABARAP, an Atg8/LC3 family member implicated in autophagy, is associated with alterations in cytokine secretion and cell death

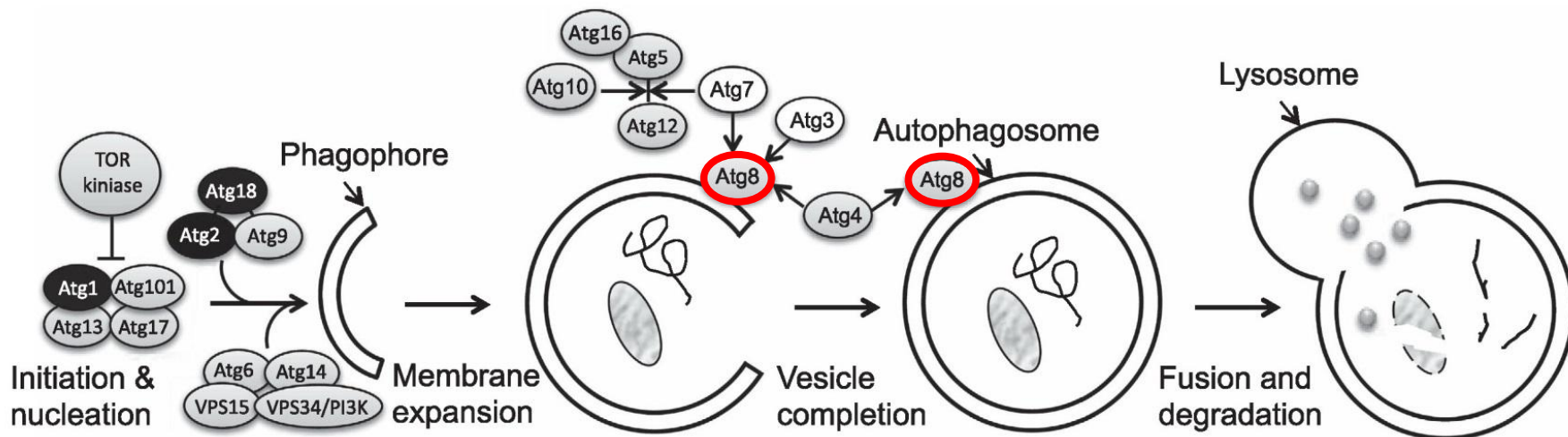
FS Salah<sup>1,2</sup>, M Ebbinghaus<sup>3</sup>, VY Muley<sup>4,5</sup>, Z Zhou<sup>6</sup>, KRD Al-Saadi<sup>2</sup>, M Pacyna-Gengelbach<sup>7</sup>, GA O'Sullivan<sup>8</sup>, H Betz<sup>8,9</sup>, R König<sup>4,5</sup>, Z-Q Wang<sup>6,10</sup>, R Bräuer<sup>1</sup> and I Petersen<sup>\*,1</sup>

GABARAP belongs to an evolutionary highly conserved gene family that has a fundamental role in autophagy. There is ample evidence for a crosstalk between autophagy and apoptosis as well as the immune response. However, the molecular details for these interactions are not fully characterized. Here, we report that the ablation of murine GABARAP, a member of the Atg8/LC3 family that is central to autophagosome formation, suppresses the incidence of tumor formation mediated by the carcinogen DMBA and results in an enhancement of the immune response through increased secretion of IL-1 $\beta$ , IL-6, IL-2 and IFN- $\gamma$  from stimulated macrophages and lymphocytes. In contrast, TGF- $\beta$ 1 was significantly reduced in the serum of these knockout mice. Further, DMBA treatment of these GABARAP knockout mice reduced the cellularity of the spleen and the growth of mammary glands through the induction of apoptosis. Gene expression profiling of mammary glands revealed significantly elevated levels of Xaf1, an apoptotic inducer and tumor-suppressor gene, in knockout mice. Furthermore, DMBA treatment triggered the upregulation of pro-apoptotic (Bid, Apaf1, Bax), cell death (Tnfrsf10b, Ripk1) and cell cycle inhibitor (Cdkn1a, Cdkn2c) genes in the mammary glands. Finally, tumor growth of B16 melanoma cells after subcutaneous inoculation was inhibited in GABARAP-deficient mice. Together, these data provide strong evidence for the involvement of GABARAP in tumorigenesis *in vivo* by delaying cell death and its associated immune-related response.

*Cell Death and Disease* (2016) 7, e2205; doi:10.1038/cddis.2016.93; published online 28 April 2016

Gamma ( $\gamma$ )-aminobutyric acid type A (GABA<sub>A</sub>) receptor-associated protein (**GABARAP**) is an evolutionary highly conserved gene family from yeast to mammals.

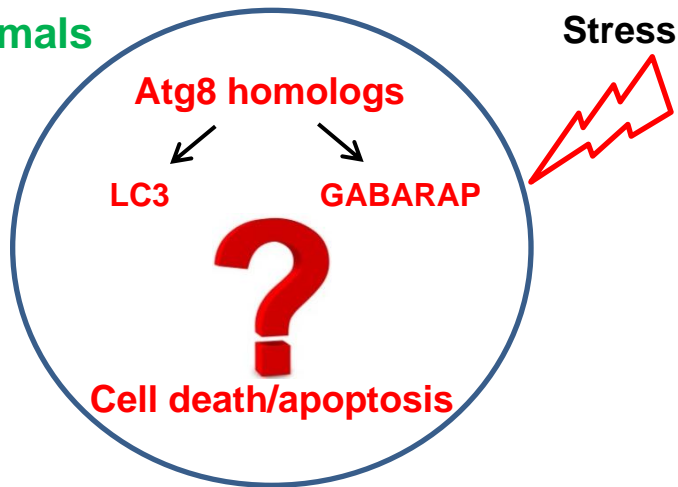
- 100% identity at amino acid level for mammalian forms.
- GABARAP regulates the intracellular trafficking of GABA<sub>A</sub> receptor, a major inhibitory neurotransmitter in cortical neurons.
- In mammals, there are several Atg8 homologues; grouped into two subfamilies:
  - ❖ LC3 (microtubule-associated protein-1 light chain 3)
  - ❖ GABARAP



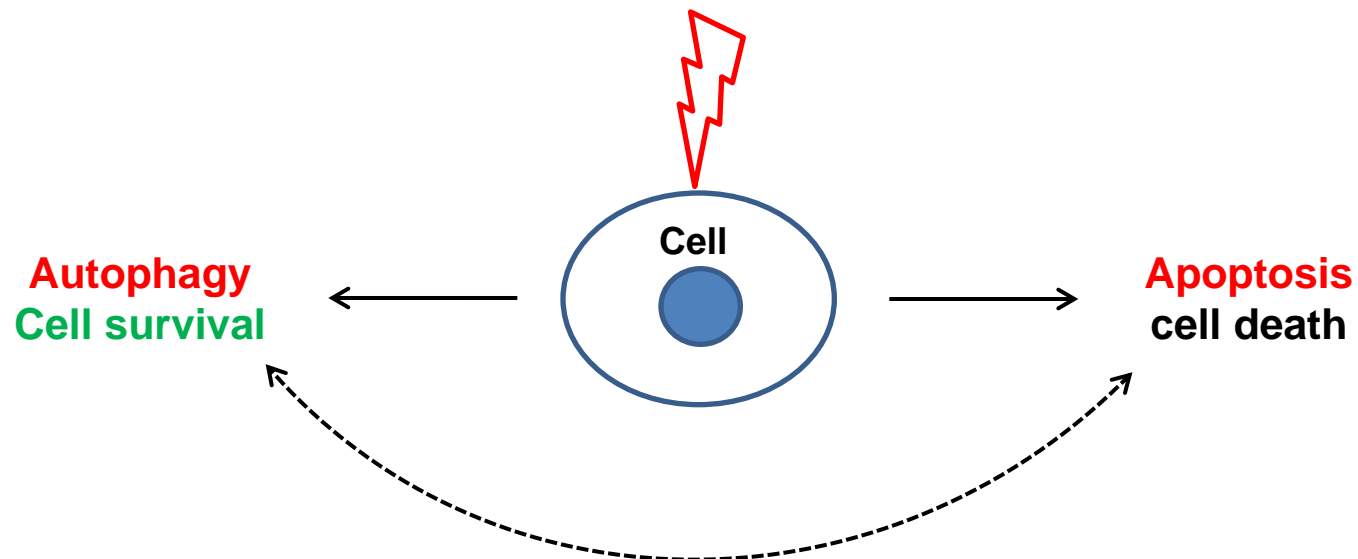
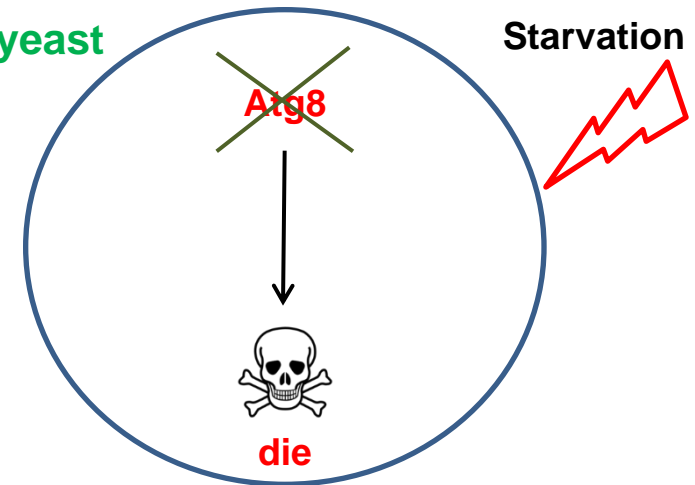
- Neuroblastoma: ↓ GABARAP expression associated with ↓ survival
- Breast tumors: ↓ GABARAP expression
- Thyroid tumors: ↑ GABARAP expression in adenomas and thyroid cancer
- Colorectal tumors: ↑ GABARAP expression associated with a low grade of differentiation and shortened survival

***However, the precise role of GABARAP in tumorigenesis is unknown so far***

In mammals



In yeast



**Explore the role of GABARAP in tumorigenesis by using a knockout mouse model**

- **Treatment of GABARAP knockout mice with carcinogens affect tumorigenesis?**
- **GABARAP knockout mice influence the growth of inoculated tumor cells?**
- **What are the cellular mechanisms being affected by GABARAP deficiency?**
  - **Apoptosis**
  - **Immunity**

Wild-type (C57BL/6) mice  
GABARAP knockout mice (C57BL/6 background)

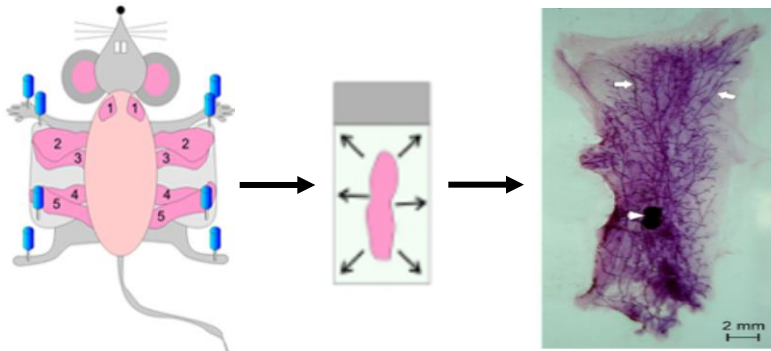
Primary culture (MEFs)  
Protein expression (WB)



Tumor inoculation  
B16 melanoma cell

Carcinogen treatment (DMBA)

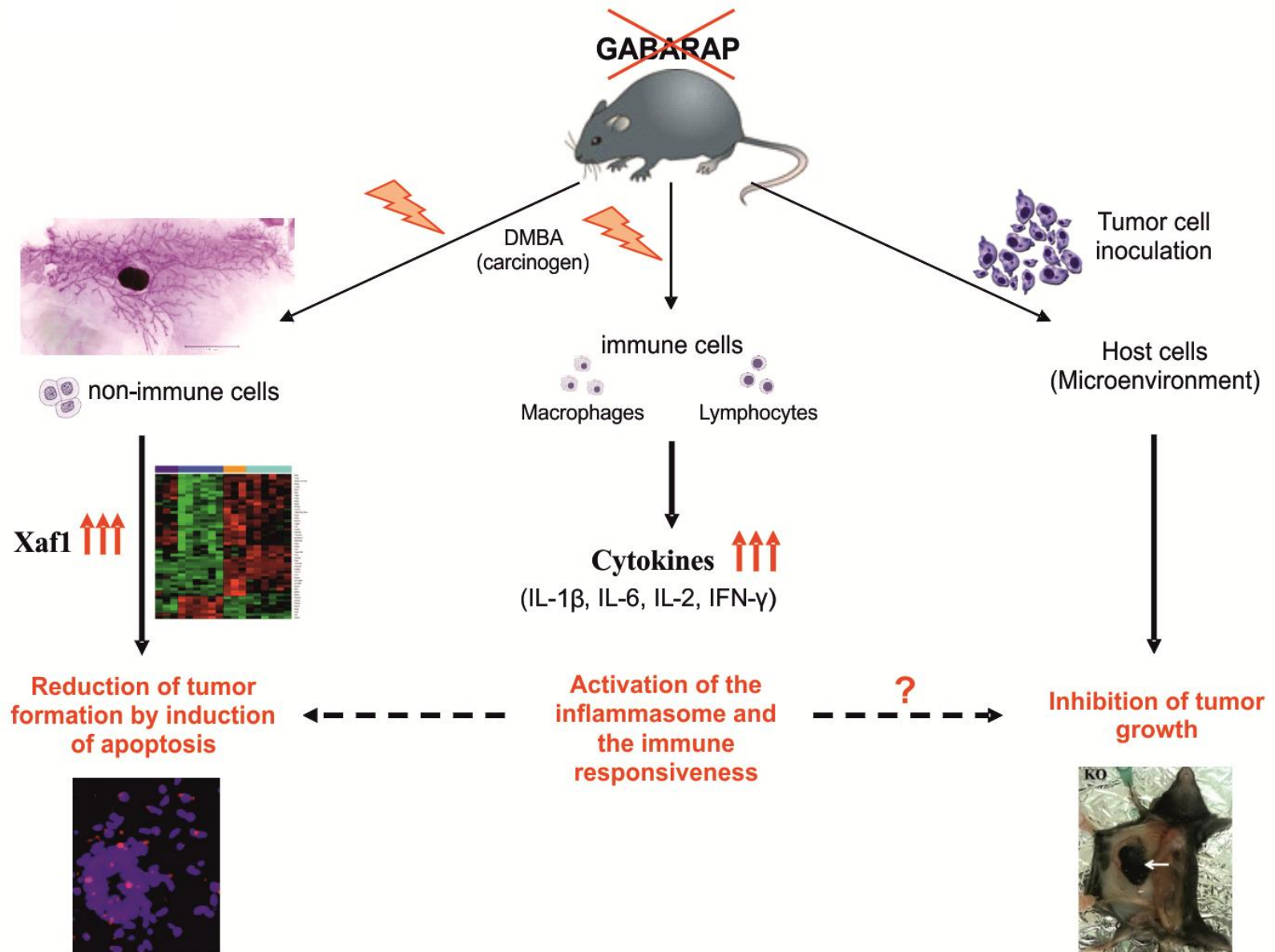
– Whole mount analysis of mammary glands



Immunologic experiments:

- Cell surface marker analysis (FACS)
- Macrophages isolation
- Lymphocytes isolation
- Cytokine analysis (ELISA)

- Proliferation assay (IHC) by using Ki-67
- Apoptosis assay (TUNEL)
- cDNA microarray gene expression profiling
- qRT-PCR





Thank you for  
attention

Jena / Germany