Regulation of autophagy as a therapeutic approach for COVID-19; the role of phytochemicals

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30 June 2020

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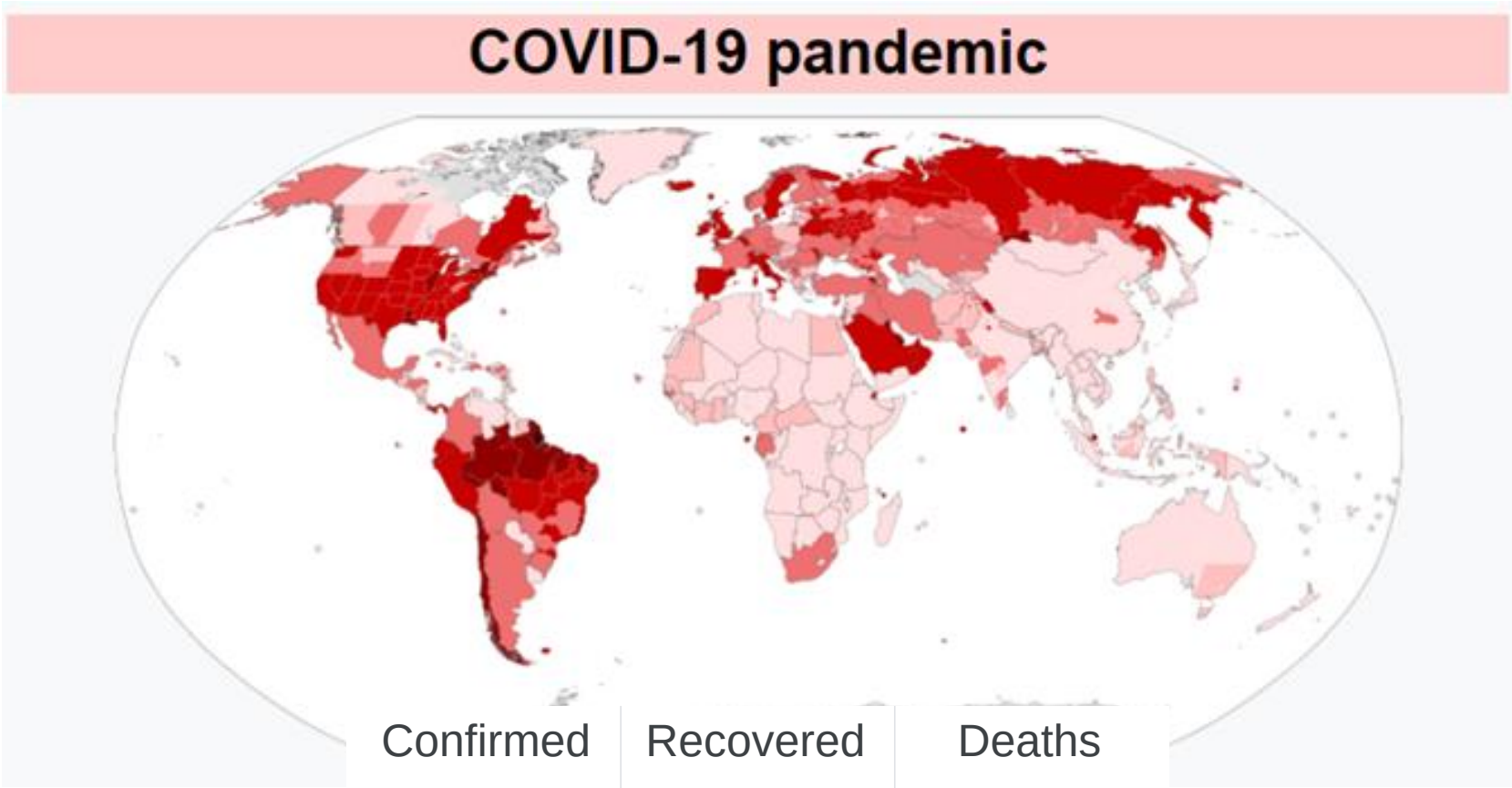
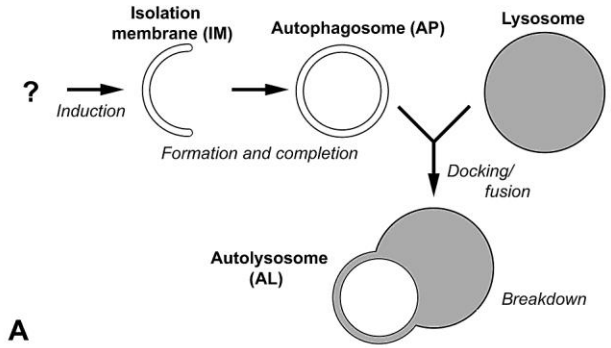
Coronavirus disease 2019 (**COVID-19**) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**).

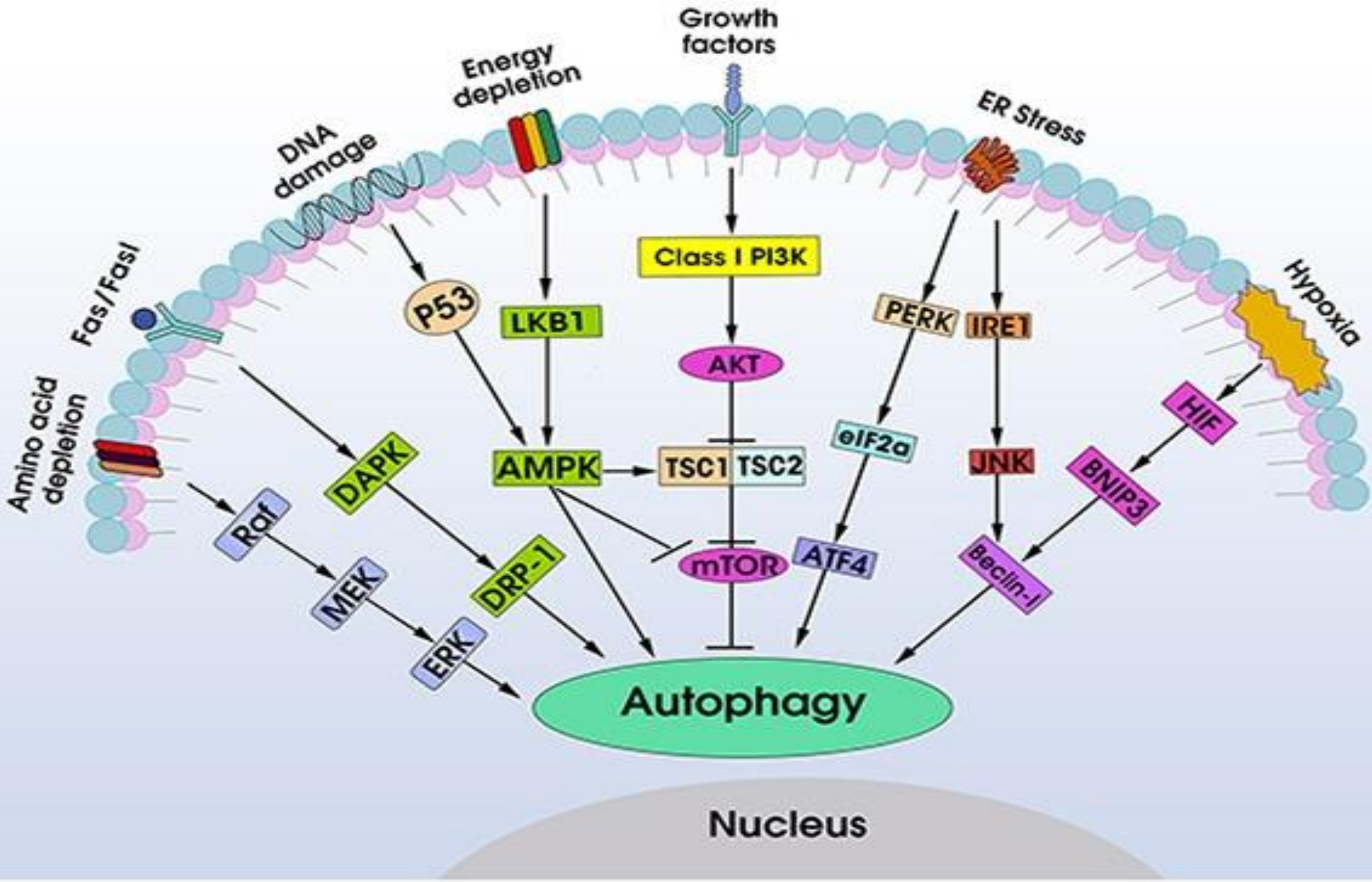


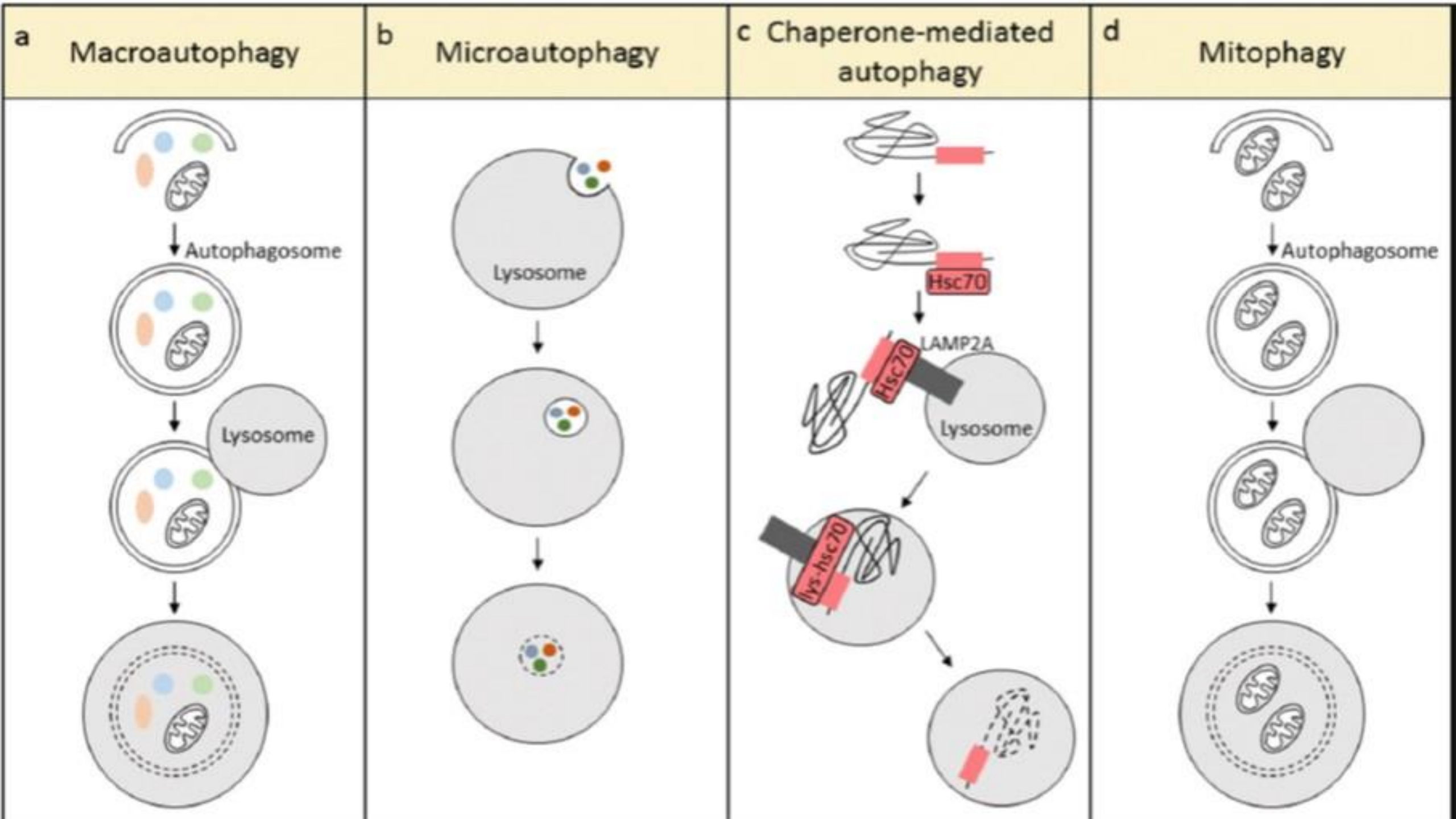
Autophagy (or *autophagocytosis*)



- A **self-digesting mechanism**- removal of long lived proteins, damaged organelles and **invasive microbes**.
- Cell content- delivered to lysosome.
- Induced at nutrient starvation- bulk degradation.

microbial infection, inflammatory disease, immune disease, pulmonary disease, heart and cardiovascular disorders, kidney disease, metabolic disease, and neurodegenerative disorders (Alzheimer's disease, amyotrophic lateral sclerosisnand Parkinson's disease.


Growth factors
Energy depletion
DNA damage
ER Stress
Hypoxia
Fas/FasL
Amino acid depletion
P53
LKB1
Class I PI3K
PERK
IRE1
AKT
HIF
eIF2α
DAPK
AMPK
TSC1 TSC2
JNK
BNIP3
Raf
mTOR
ATF4
Beclin-1
MEK
DRP-1
ERK
Autophagy
Nucleus


a
Macroautophagy
Autophagosome
Lysosome
b
Microautophagy
Lysosome
c
Chaperone-mediated autophagy
Hsc70
LAMP2A
Hsc70
Lysosome
lys-hsc70
d
Mitophagy
Autophagosome

Molecular machinery

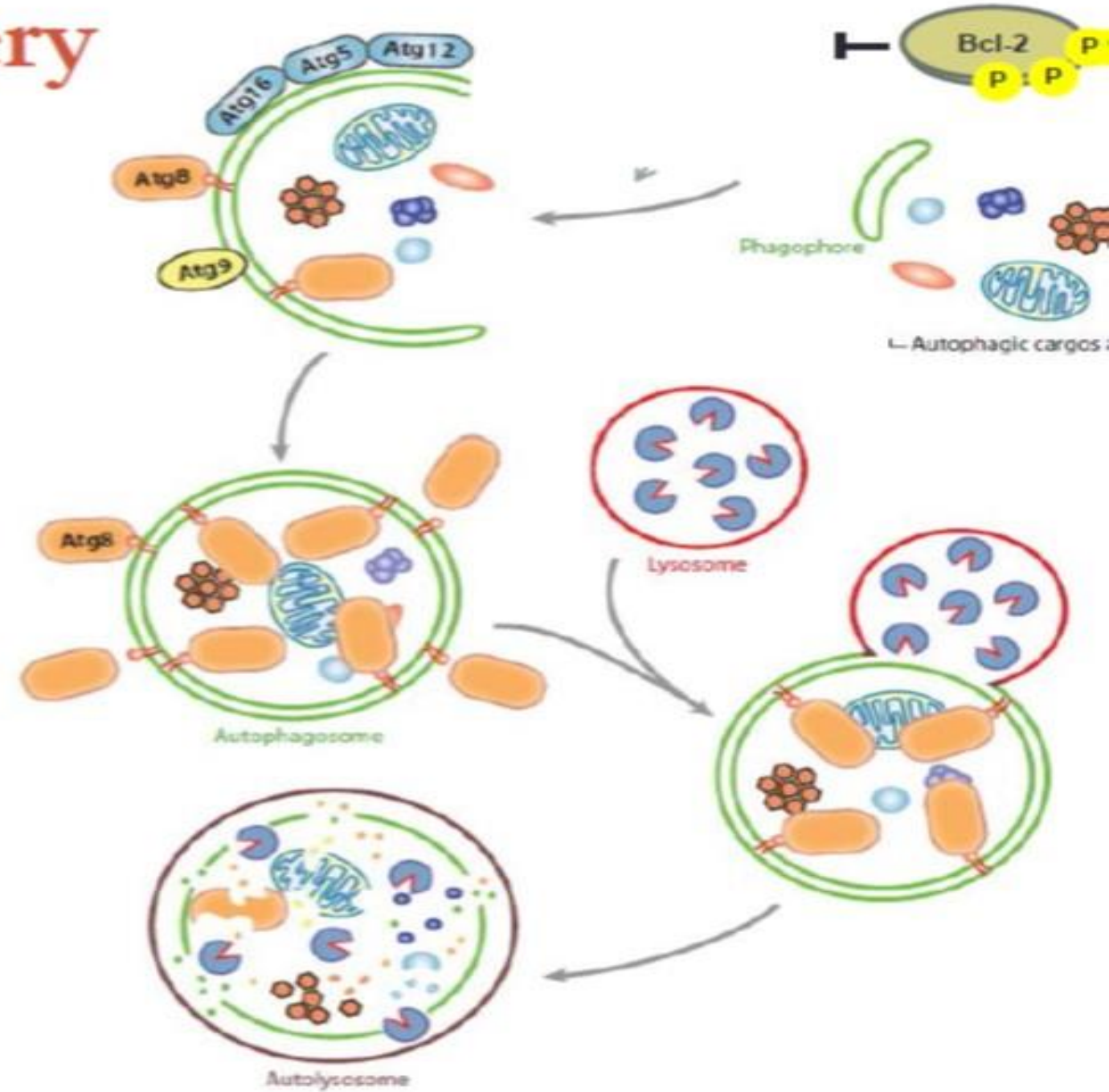
1. **Induction**
   (e.g. low energy, hypoxia, stress, low levels of hormones)

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)

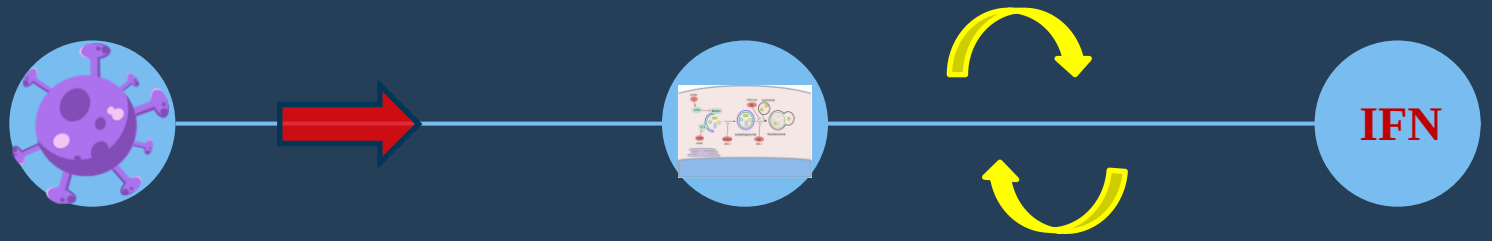


Klionsky D.J., 2009 Annual.Rev.Genet.

When autophagy meets viruses: a double-edged sword with functions in **defense** and **offense**

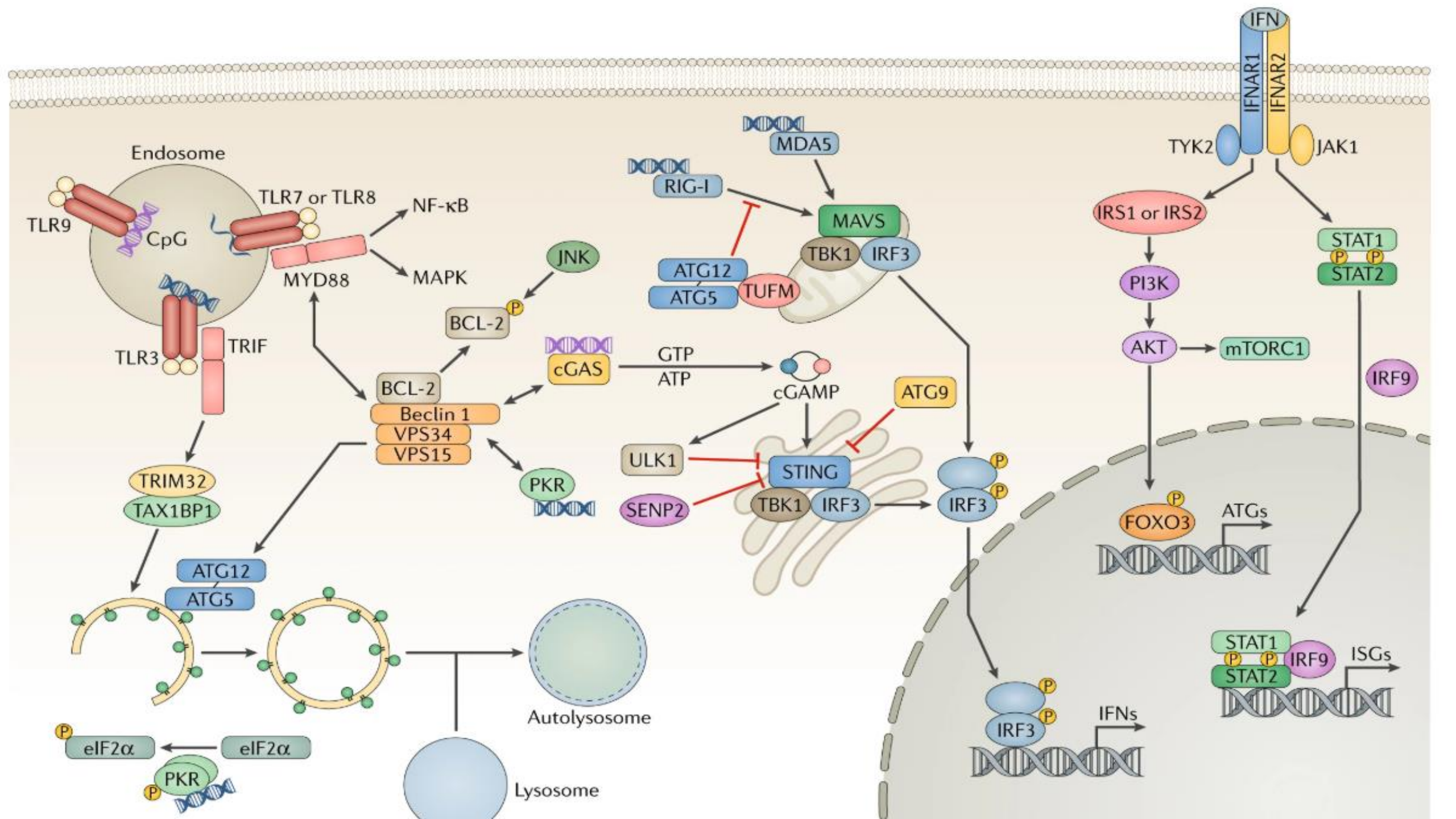
Cross-talk of autophagy and innate immune response

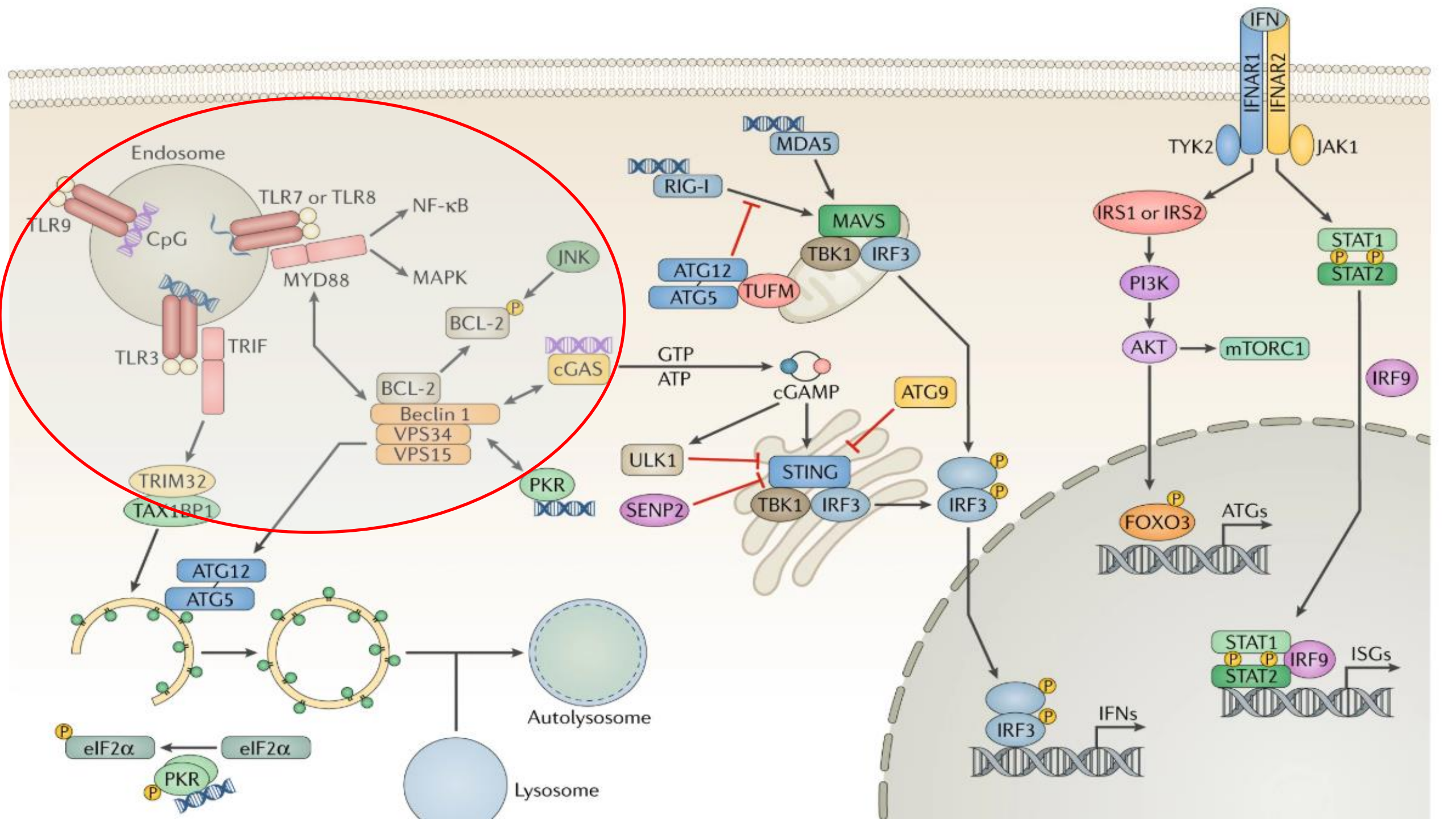
Induction of autophagy



Viral infection

type I interferon (IFN) pathway


Endosome
TLR9
CpG
TLR7 or TLR8
NF-κB
MAPK
MYD88
TLR3
TRIF
TRIM32
TAX1BP1
JNK
BCL-2
BCL-2
Beclin 1
VPS34
VPS15
PKR
cGAS
GTP
ATP
cGAMP
MDA5
RIG-I
MAVS
TBK1
IRF3
ATG12
ATG5
TUFM
ATG9
ULK1
STING
SENP2
TBK1
IRF3
IRF3
ATG12
ATG5
Autolysosome
Lysosome
eIF2α
eIF2α
PKR
IFN
IFNAR1
IFNAR2
TYK2
JAK1
IRS1 or IRS2
PI3K
AKT
mTORC1
STAT1
STAT2
IRF9
FOXO3
ATGs
IRF3
IFNs
STAT1
STAT2
IRF9
ISGs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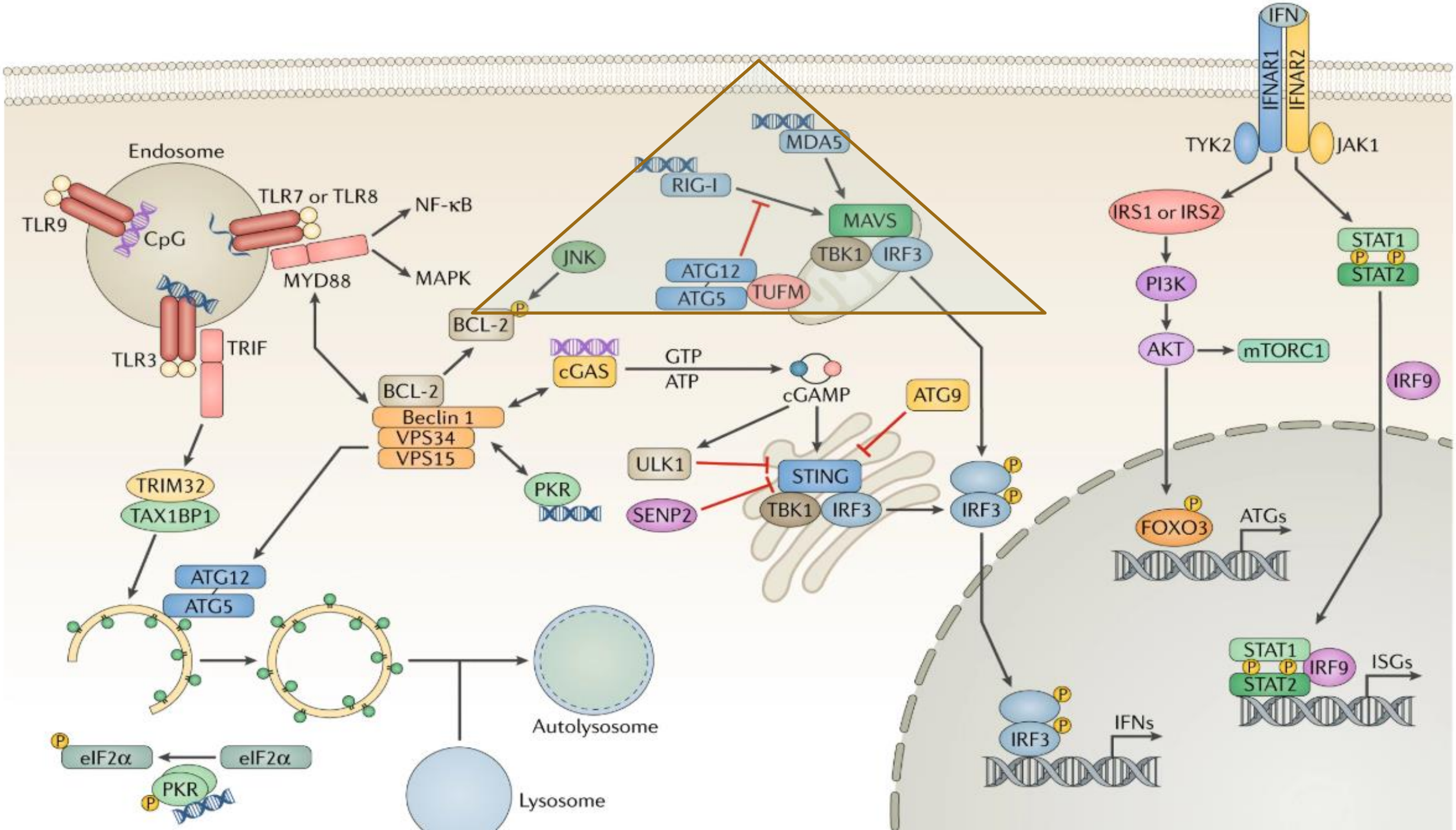
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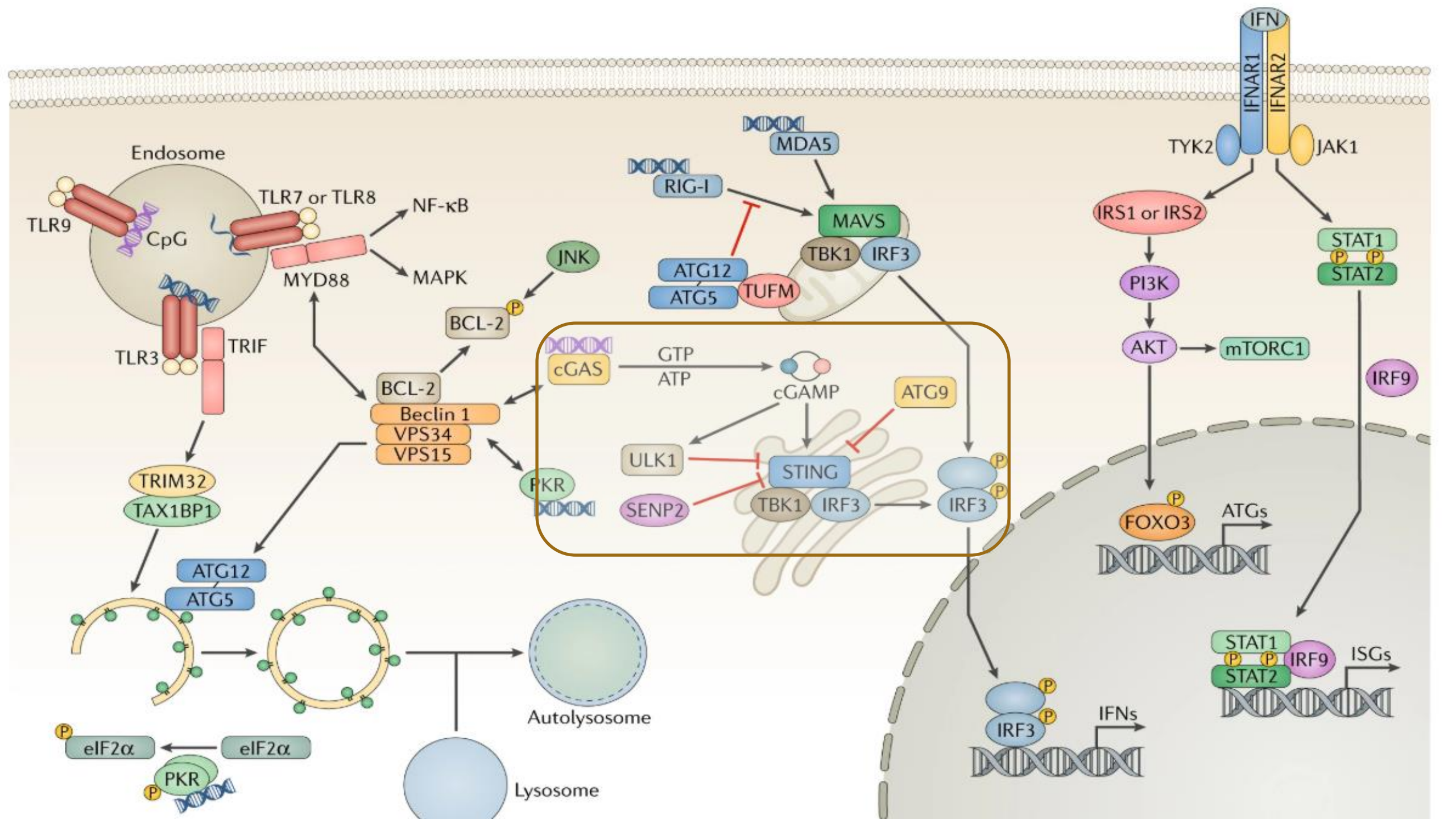
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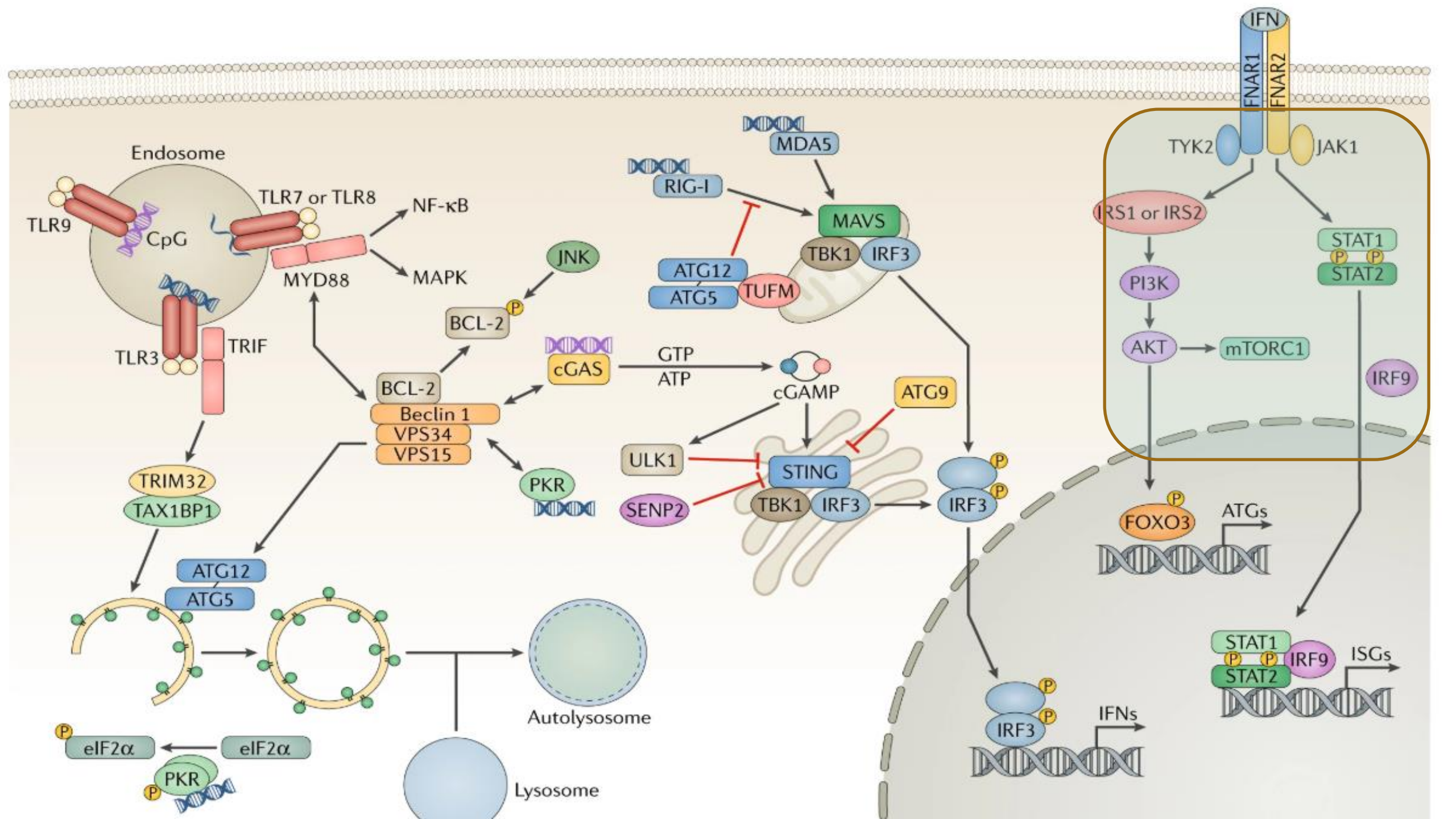
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IRF3
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TUFM
ATG9
ULK1
STING
TBK1
IRF3
SENP2
IRF3
P
IFN
FNAR1
FNAR2
TYK2
JAK1
IRS1 or IRS2
PI3K
AKT
mTORC1
STAT1
STAT2
IRF9
FOXO3
ATGs
ATG12
ATG5
Autolysosome
Lysosome
eIF2α
eIF2α
PKR
IRF3
IFNs
STAT1
STAT2
IRF9
ISGs

Cross-talk between **innate immune response** and **autophagy** leads to generates an **optimal antiviral milieu**.

Autophagy as antiviral defense system

1. Autophagy-mediated **restriction of viral replication**
2. Autophagy-mediated **viral antigen presentation**; adaptive immune response

Autophagy-mediated restriction of viral replication

by **degrading** viral components, viral particles or even host factors

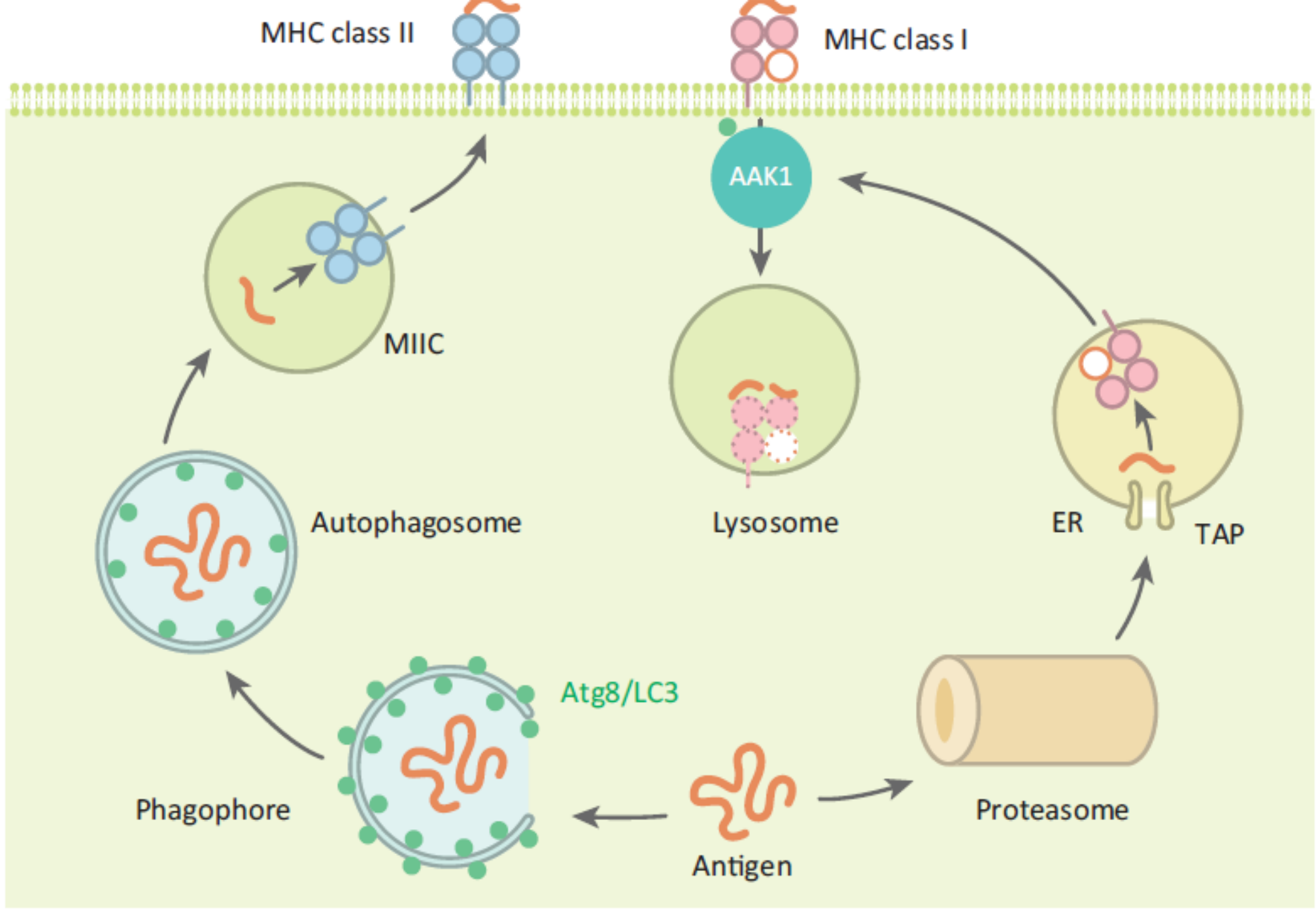
required for viral replication

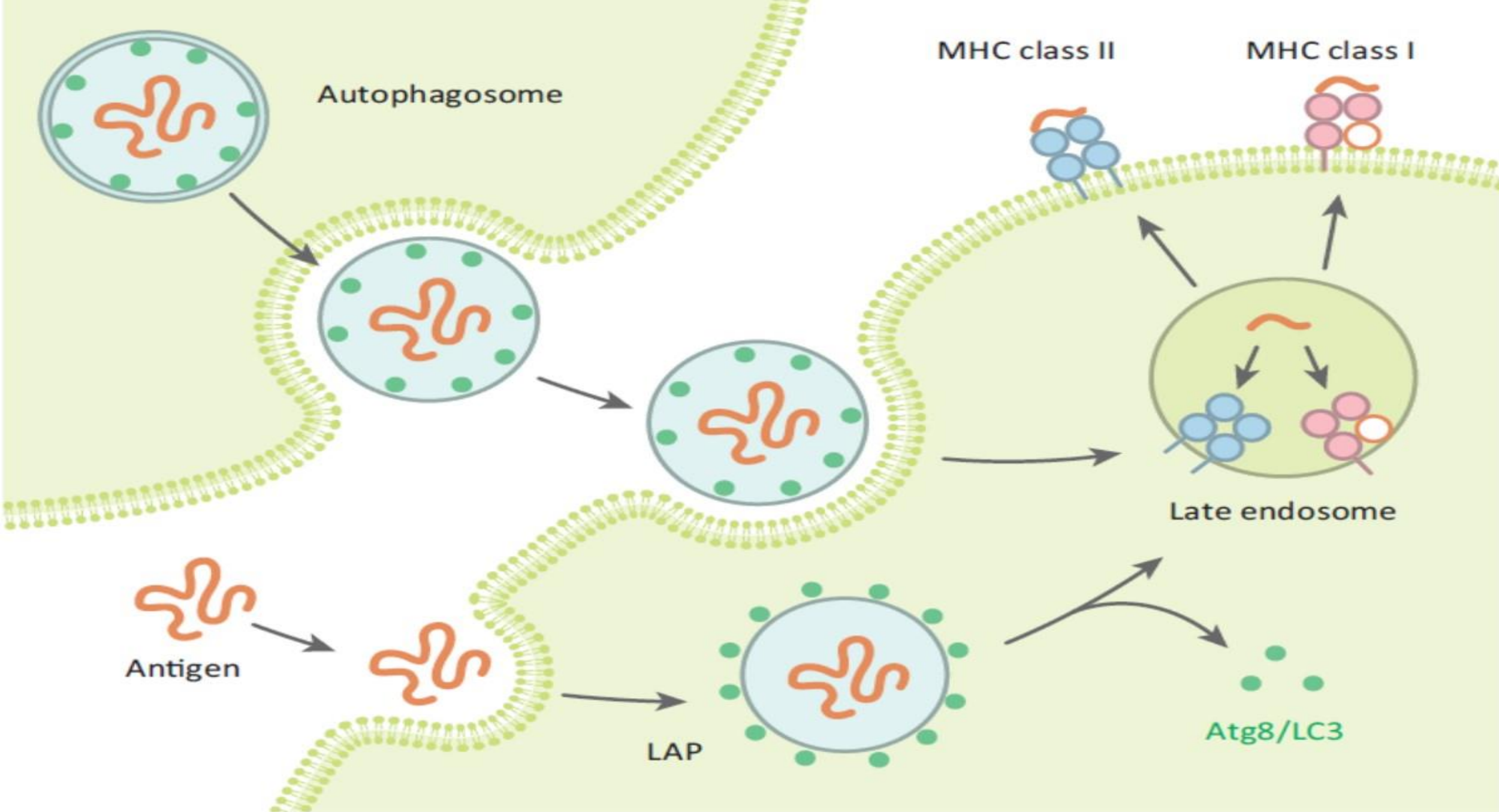
- **Virophagy** targets **neosynthesized** viral components
- **Xenophagy** targets **entire viral particles**

Autophagy-mediated viral antigen presentation

- Autophagy for optimal antigen processing for **MHC class II presentation** and **MHC class I presentation**.

- autophagy-mediated exocytosis of antigens in vesicular compartments **supports cross-presentation on MHC class I molecules**.

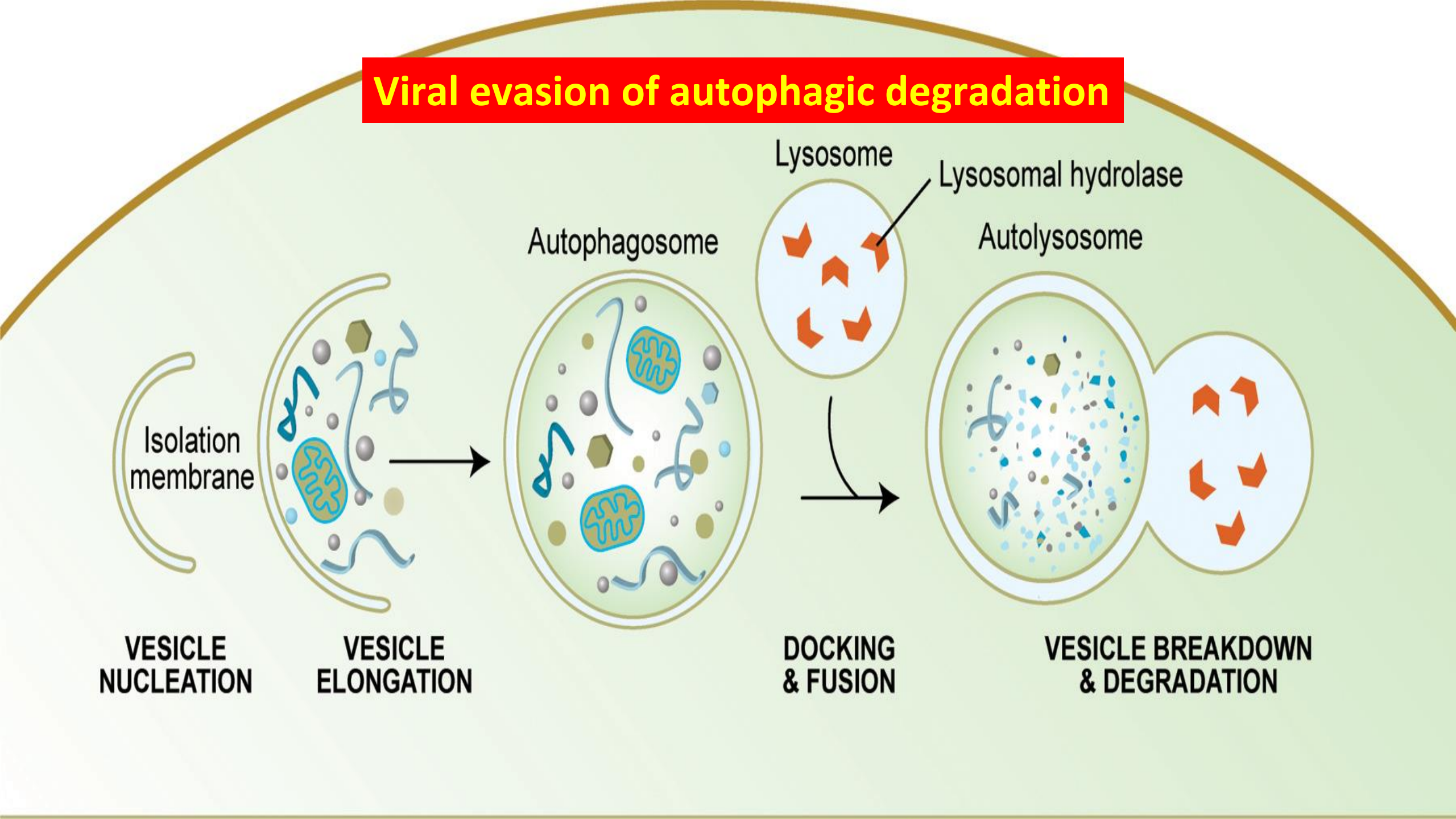

MHC class II
MHC class I
AAK1
MIIC
Lysosome
ER
TAP
Autophagosome
Atg8/LC3
Phagophore
Antigen
Proteasome

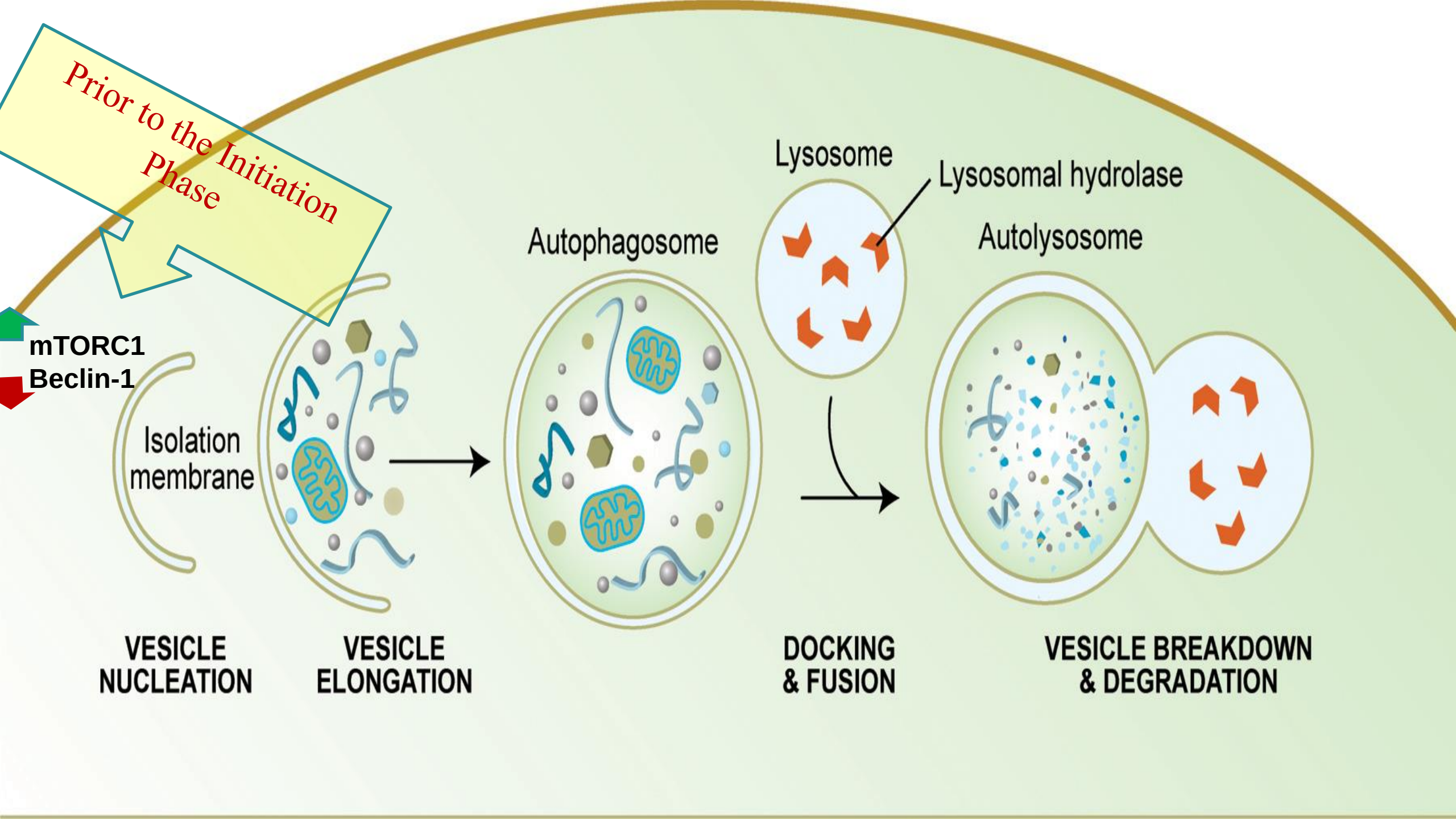

MHC class II
MHC class I
Autophagosome
Late endosome
Antigen
LAP
Atg8/LC3

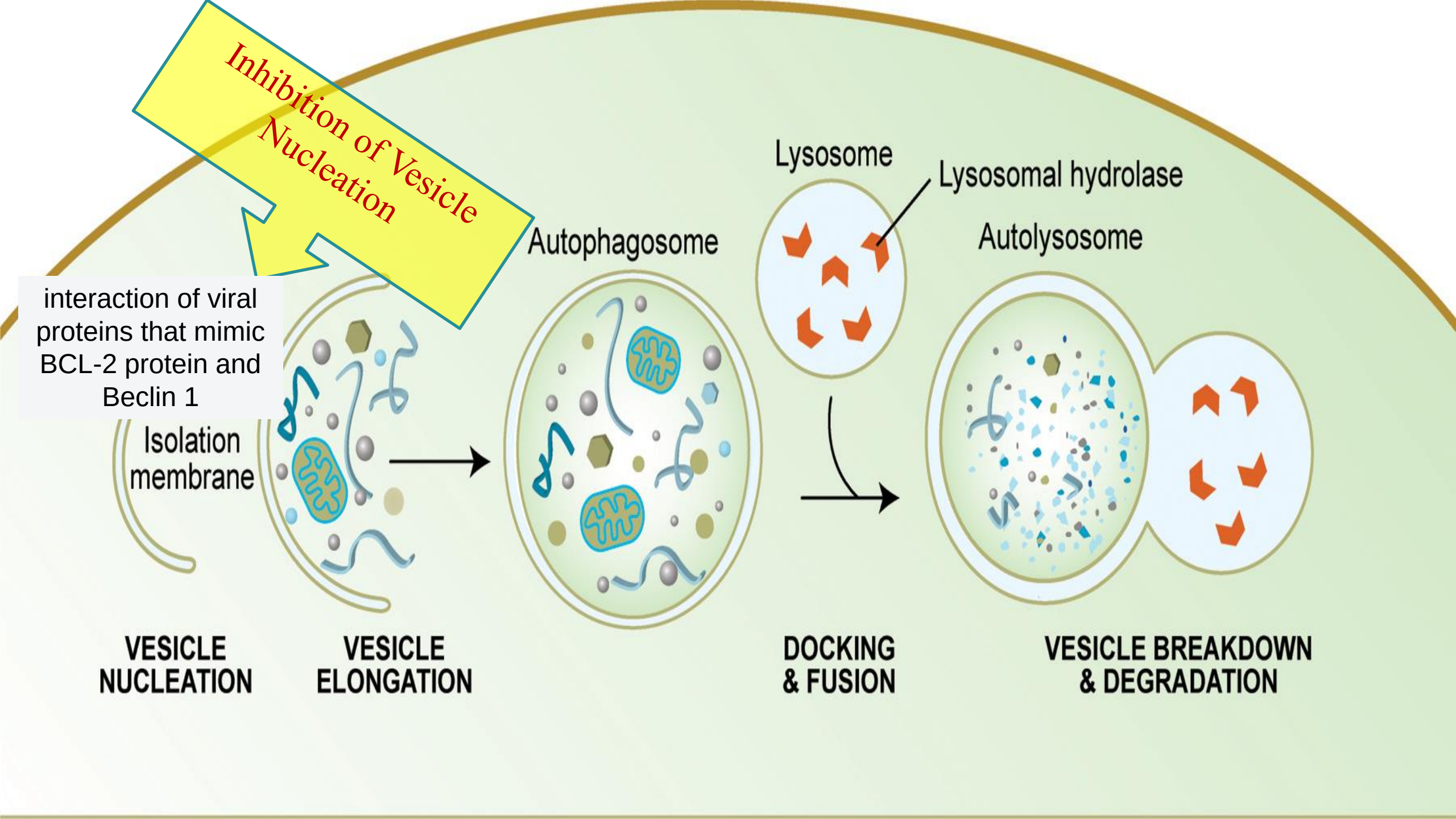
Autophagy under arrest

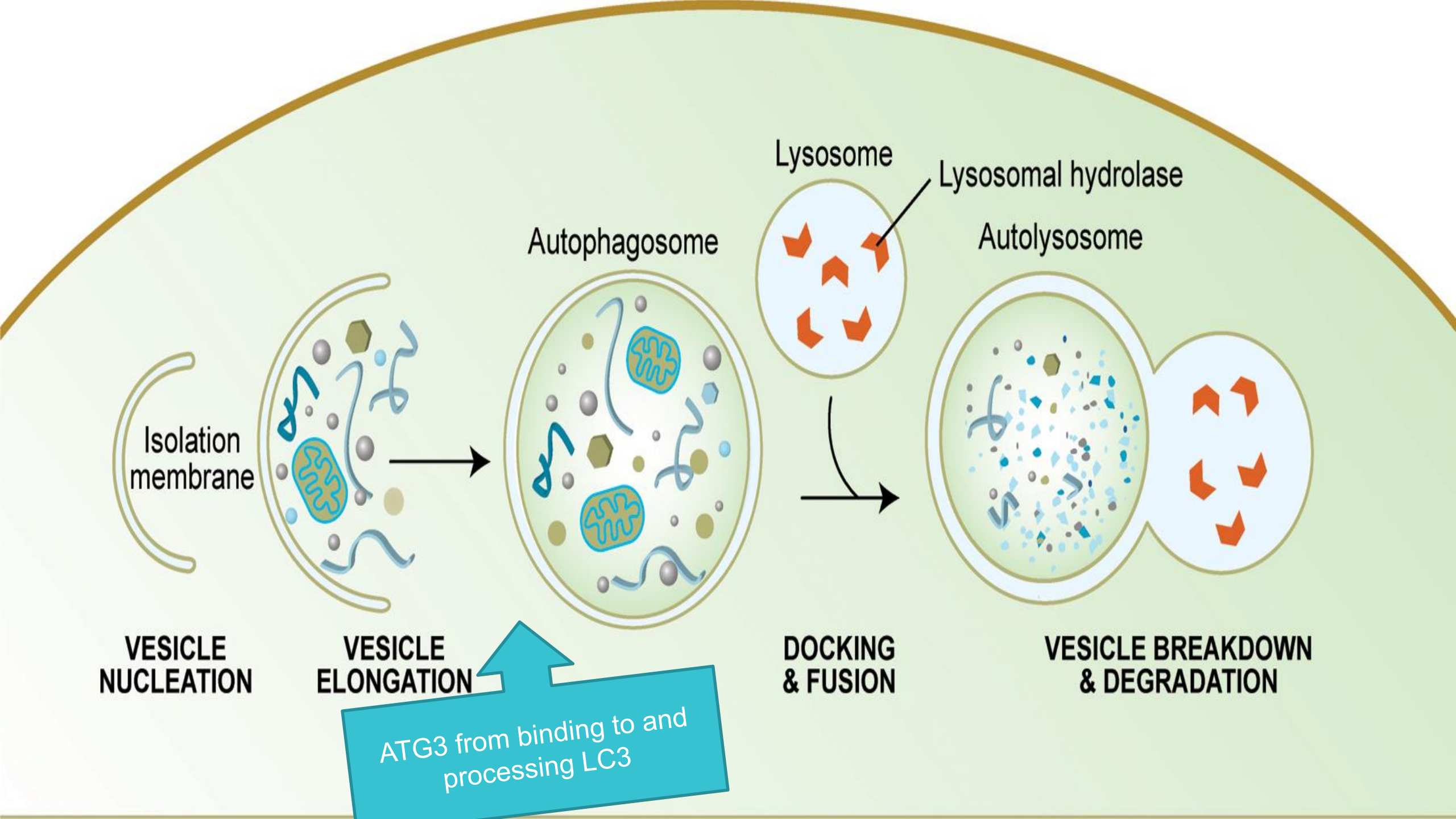
Viruses can escape or take the control of autophagy pathway

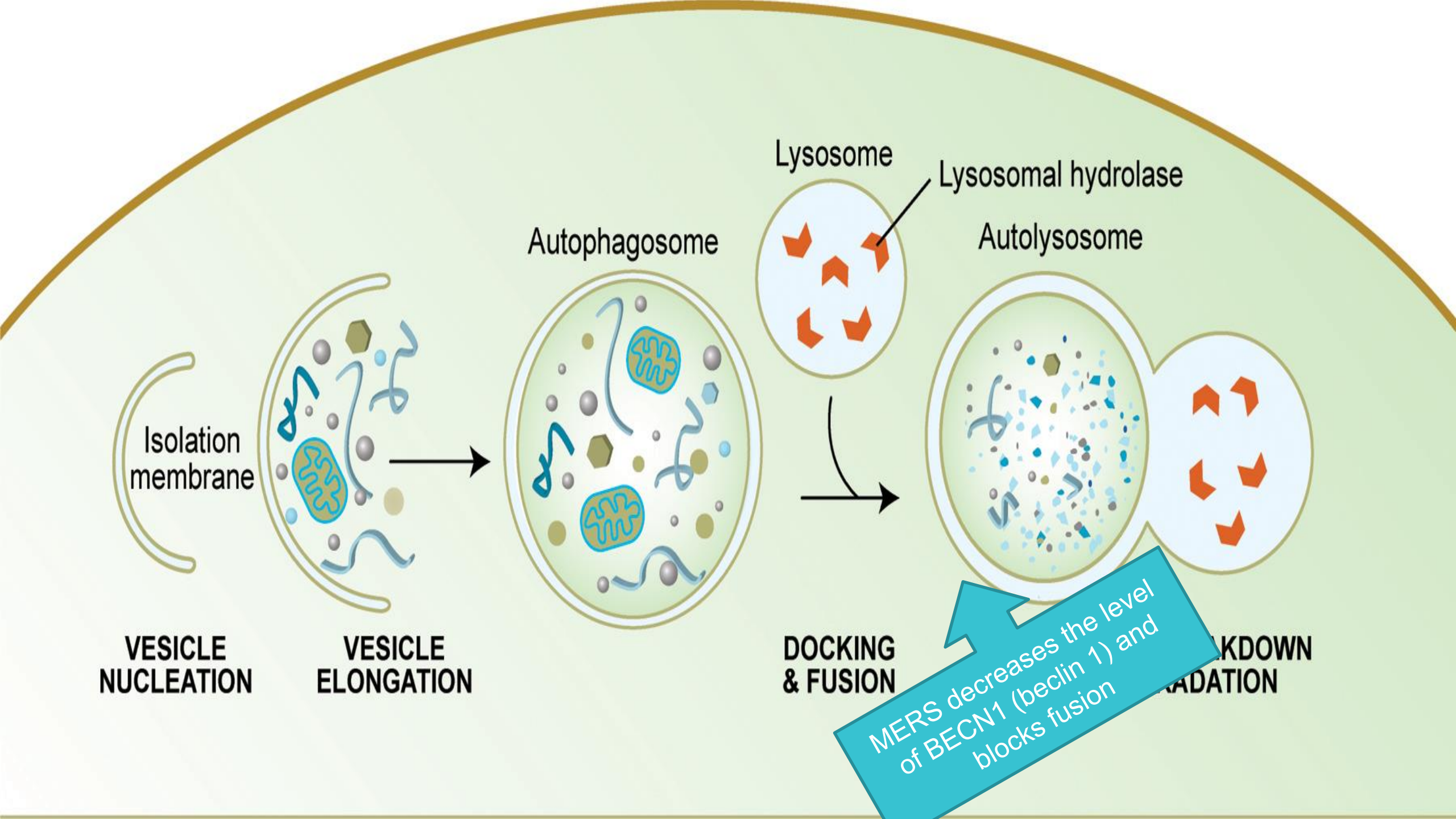
Viral evasion of autophagic degradation




Prior to the Initiation Phase
mTORC1
Beclin-1
Isolation membrane
Autophagosome
Lysosome
Lysosomal hydrolase
Autolysosome
VESICLE NUCLEATION
VESICLE ELONGATION
DOCKING & FUSION
VESICLE BREAKDOWN & DEGRADATION

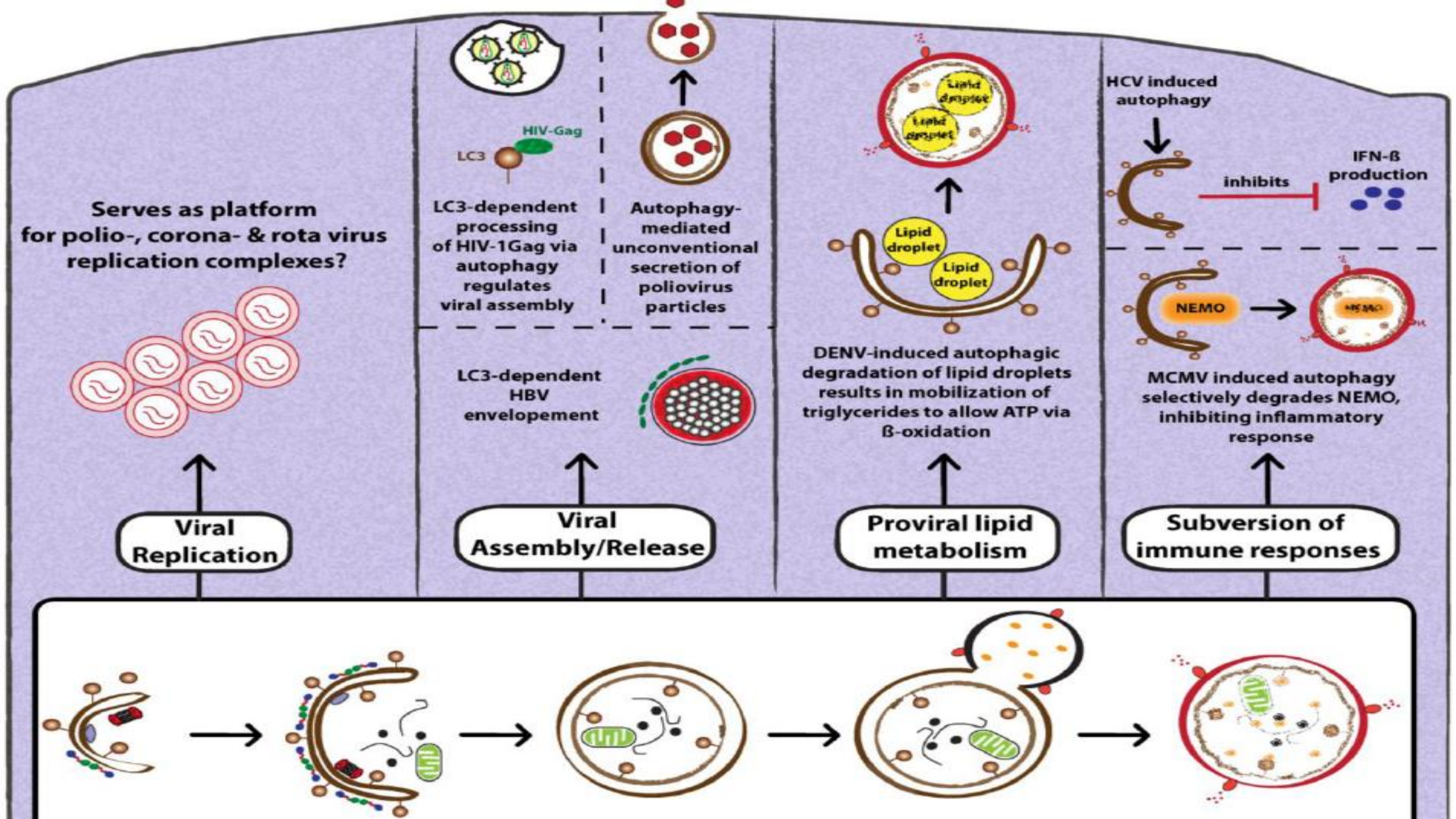

Inhibition of Vesicle Nucleation
interaction of viral proteins that mimic BCL-2 protein and Beclin 1
Isolation membrane
Autophagosome
Lysosome
Lysosomal hydrolase
Autolysosome
VESICLE NUCLEATION
VESICLE ELONGATION
DOCKING & FUSION
VESICLE BREAKDOWN & DEGRADATION


Lysosome
Lysosomal hydrolase
Autophagosome
Autolysosome
Isolation membrane
VESICLE NUCLEATION
VESICLE ELONGATION
DOCKING & FUSION
VESICLE BREAKDOWN & DEGRADATION
ATG3 from binding to and processing LC3


Lysosome
Lysosomal hydrolase
Autophagosome
Autolysosome
Isolation membrane
VESICLE NUCLEATION
VESICLE ELONGATION
DOCKING & FUSION
KDOWN
ADATION
MERS decreases the level of BECN1 (beclin 1) and blocks fusion

Proviral Functions of Autophagy

abetting the enemy


Serves as platform for polio-, corona- & rota virus replication complexes?
Viral Replication
HIV-Gag
LC3
LC3-dependent processing of HIV-1Gag via autophagy regulates viral assembly
Autophagy-mediated unconventional secretion of poliovirus particles
LC3-dependent HBV envelopement
Viral Assembly/Release
Lipid droplet
Lipid droplet
Lipid droplet
DENV-induced autophagic degradation of lipid droplets results in mobilization of triglycerides to allow ATP via ß-oxidation
Proviral lipid metabolism
HCV induced autophagy
inhibits
IFN-ß production
NEMO
MCMV induced autophagy selectively degrades NEMO, inhibiting inflammatory response
Subversion of immune responses

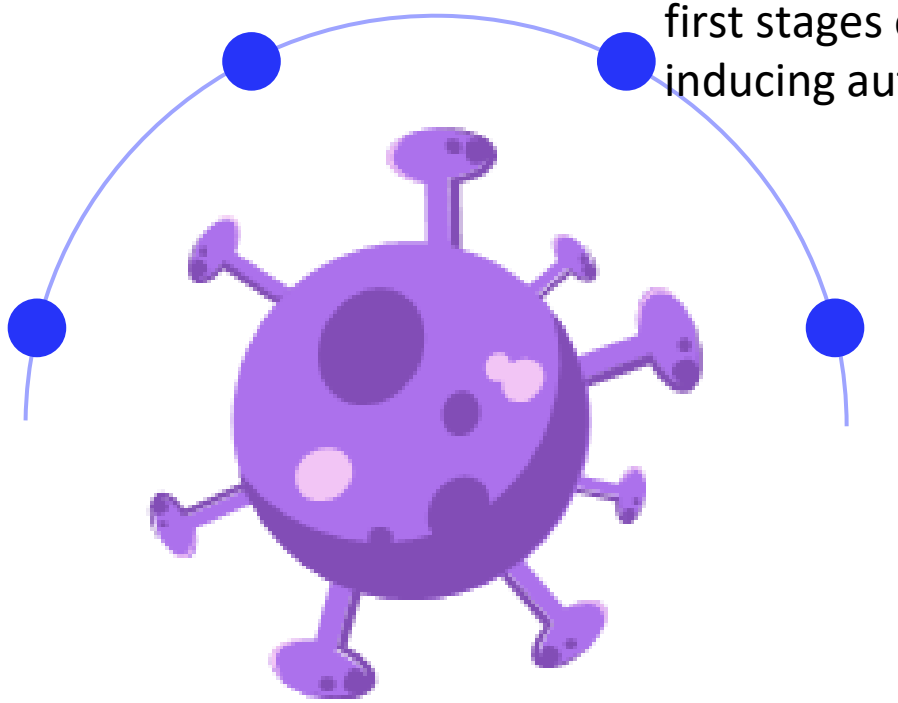
Implication of autophagy in CoVs infection

MERS

decreases the level of beclin 1 and blocks fusion of autophagosomes with lysosomes

SARS-CoV-2

nsp 8 and N-protein are colocalized with LC3 at the first stages of the infection, inducing autophagy pathway

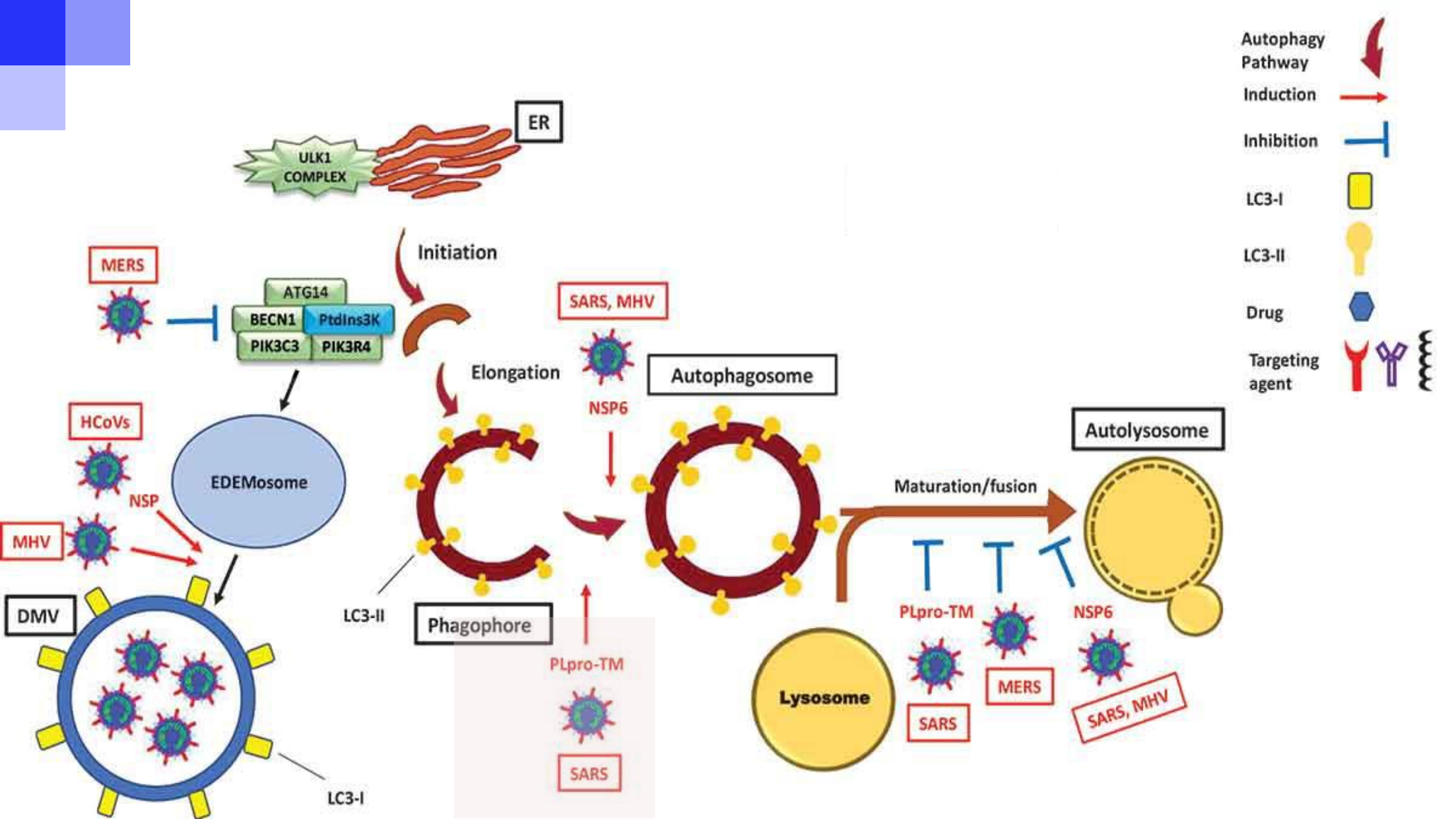


SARS and MHV

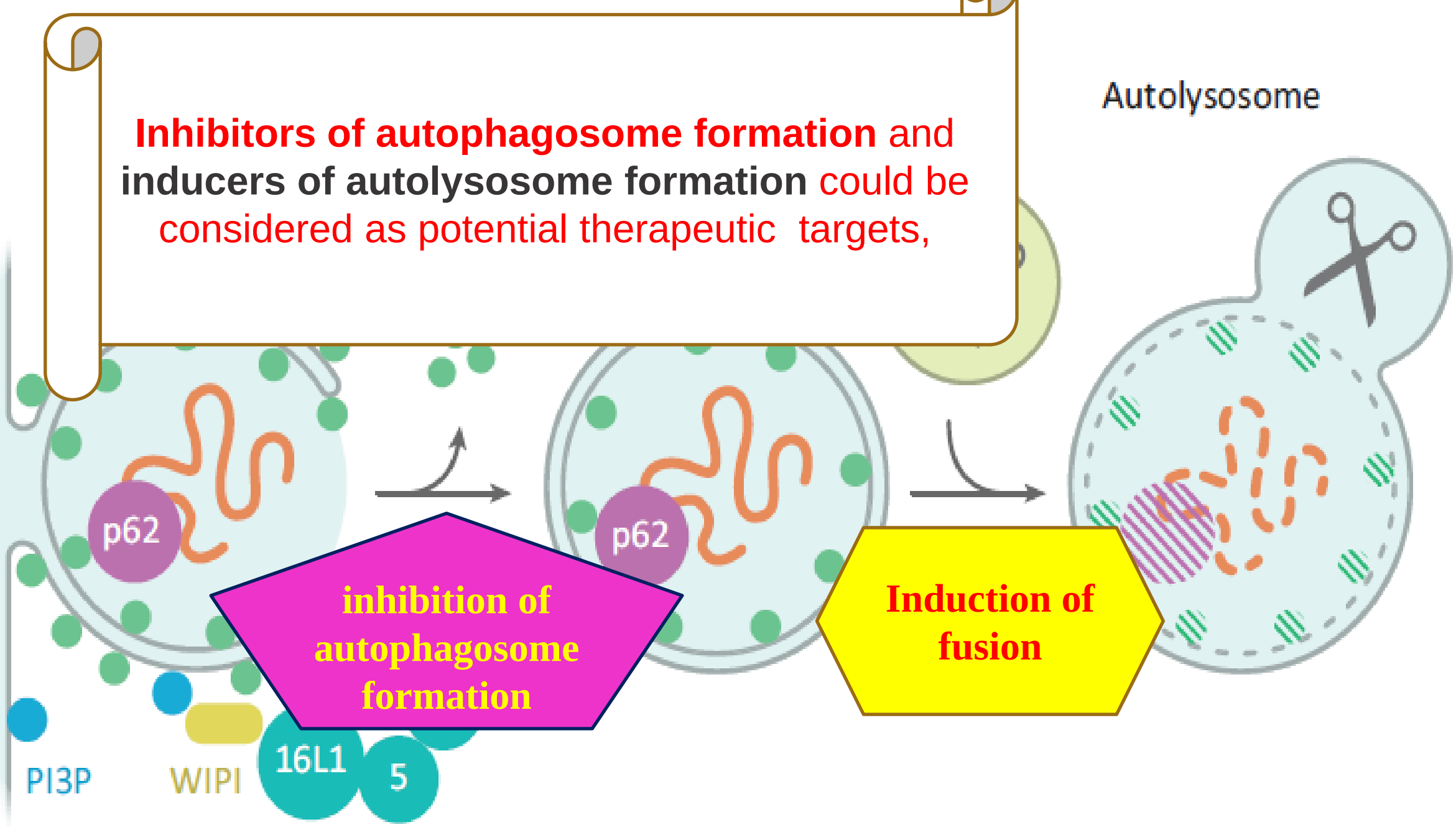
NSP6 protein induces the formation of autophagosomes but confines their expansion and blocks their maturation into autolysosomes

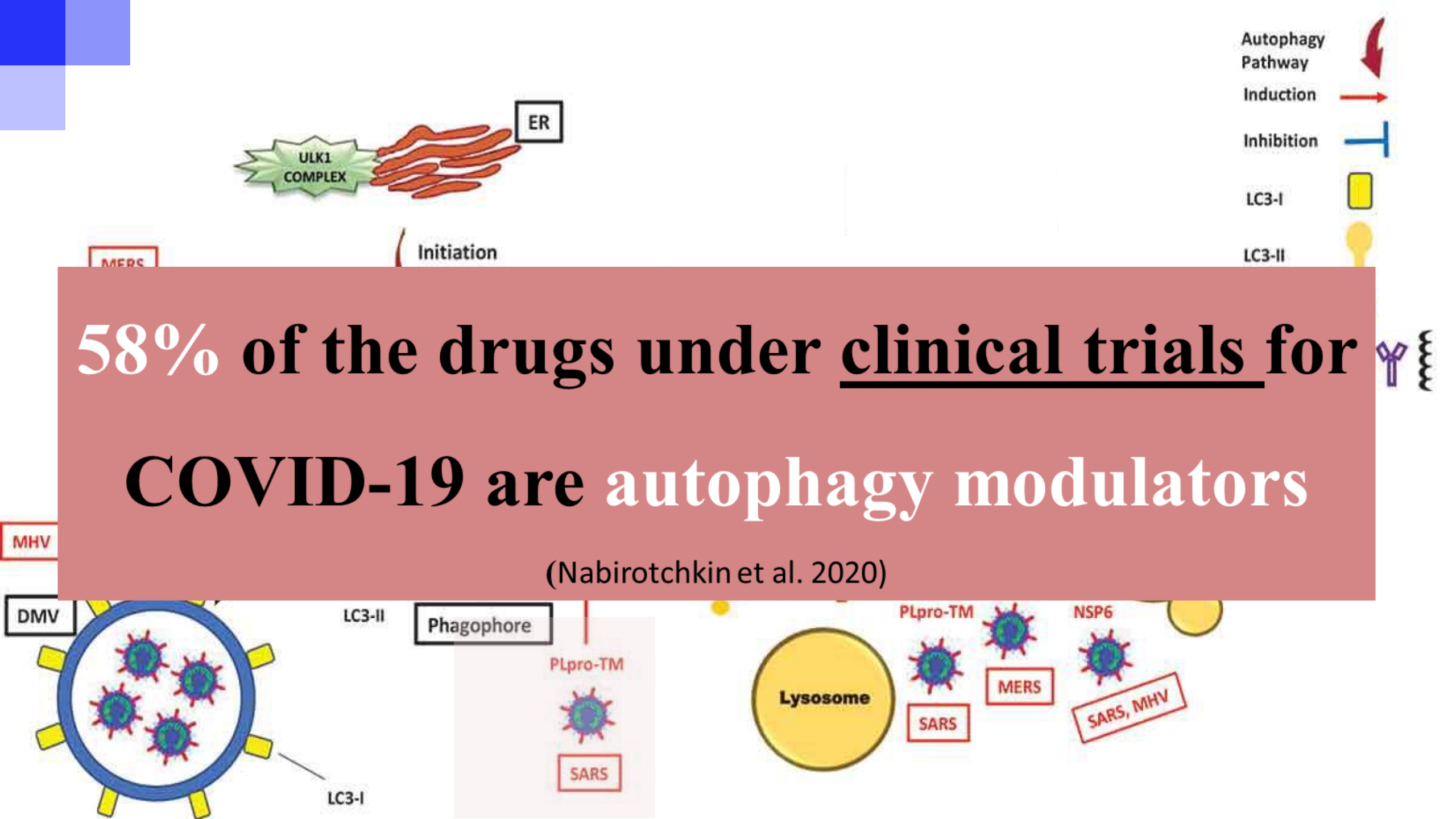
SARS-CoV-2

SARS-CoV-2 infection could possibly induce autophagy via UPR induction in the cells


Autophagy Pathway
Induction
Inhibition
LC3-I
LC3-II
Drug
Targeting agent
ER
ULK1 COMPLEX
Initiation
MERS
ATG14
BECN1
PtdIns3K
PIK3C3
PIK3R4
SARS, MHV
Elongation
Autophagosome
NSP6
HCoVs
EDEMosome
NSP
MHV
DMV
LC3-II
Phagophore
PLpro-TM
SARS
LC3-I
Maturation/fusion
Autolysosome
Lysosome
PLpro-TM
SARS
MERS
NSP6
SARS, MHV

Inhibitors of autophagosome formation and **inducers of autolysosome formation** could be considered as potential therapeutic targets,





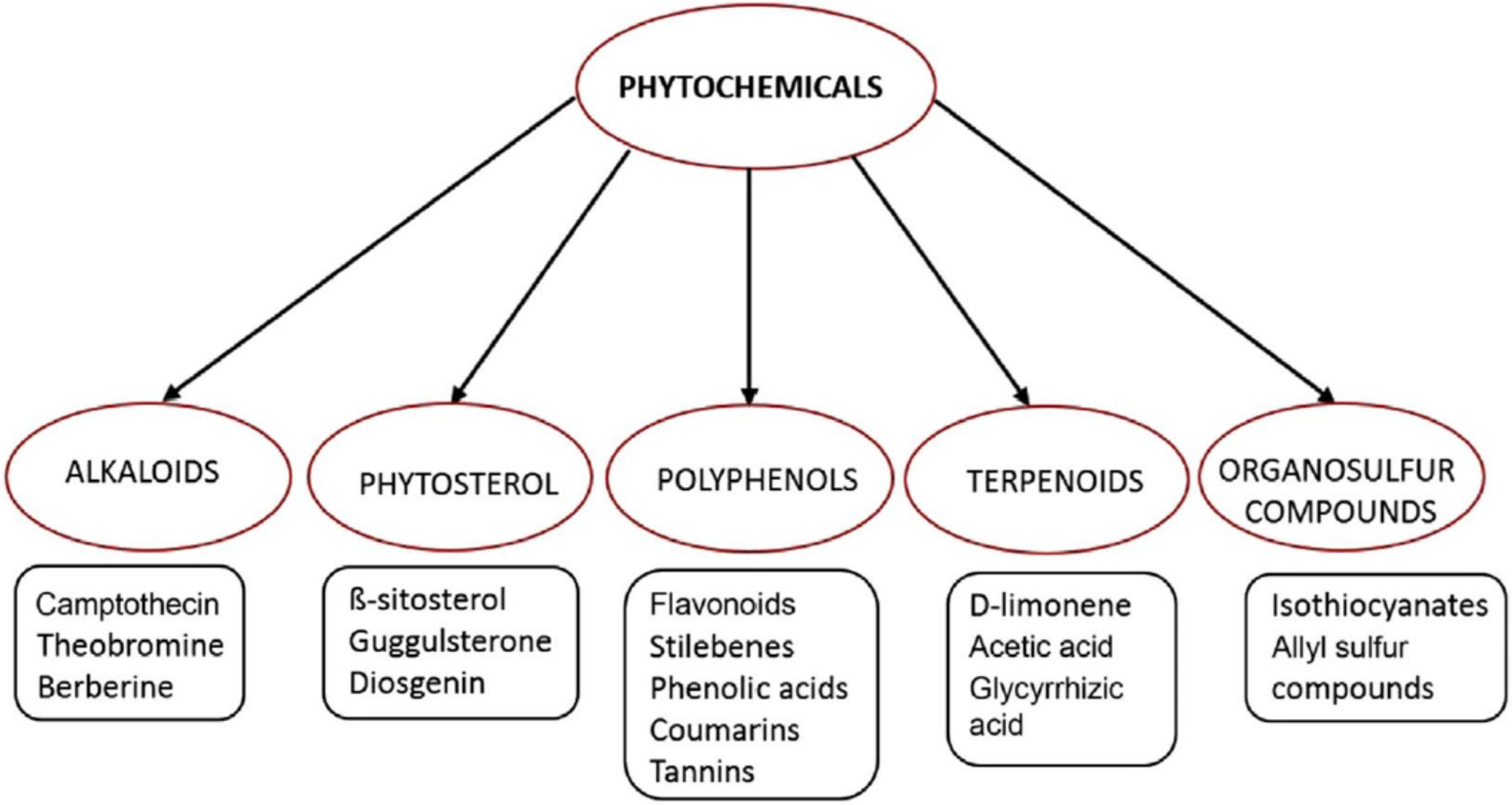
58% of the drugs under clinical trials for COVID-19 are autophagy modulators

(Nabirotchkin et al. 2020)

Drug	Mechanism	Side effects
CQ/HCQ	Inhibits autophagy flux by decreasing autophagosome-lysosome fusion	Retinopathy, gastrointestinal effects, cardiomyopathy, myopathy
Corticosteroids	Inhibits autophagy by blocking LC3 recruitment	Myopathy,osteopenia/osteoporosis,decreased sex hormones
Emtricitabine/Tenofovir	Decreases fusion of autophagosomes with lysosomes	Renal toxicity
Interferon alfa-2b	Induces autophagy and accumulation of autolysosomes	Flu-like symptoms, nausea, anorexia, depression, confusion,
Lopinavir/Ritonavir	Induces autophagosome accumulation	Gastrointestinal effects, headache, diabetes, hyperbilirubinemia,
Ruxolitinib	Downregulates the MTORC1-RPS6KB-EIF4EBP1 pathway, induces accumulation of autophagosomes	Anemia, pancytopenia

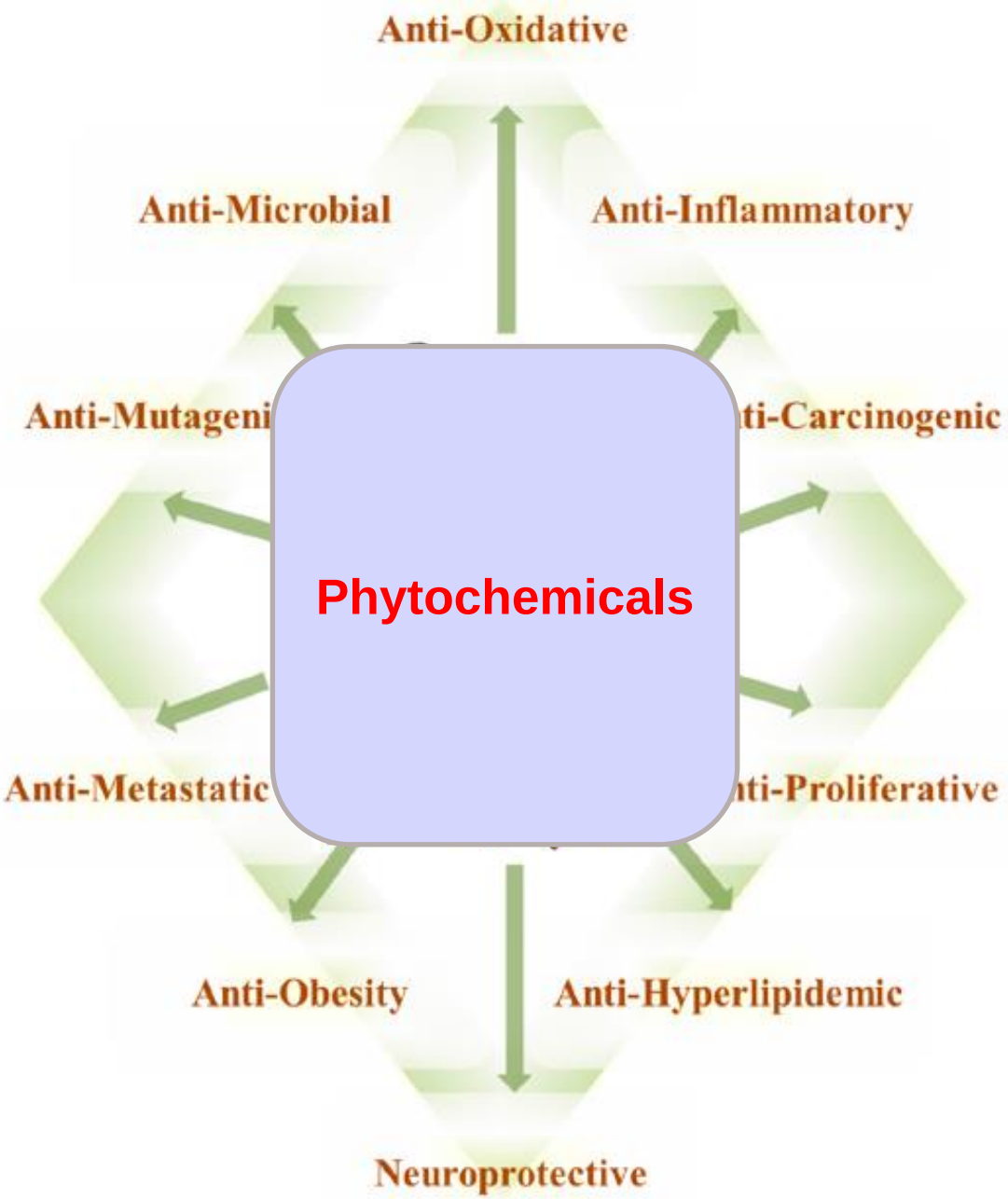
Phytochemicals

are of **plant origin** chemicals produced through **primary or secondary metabolism**



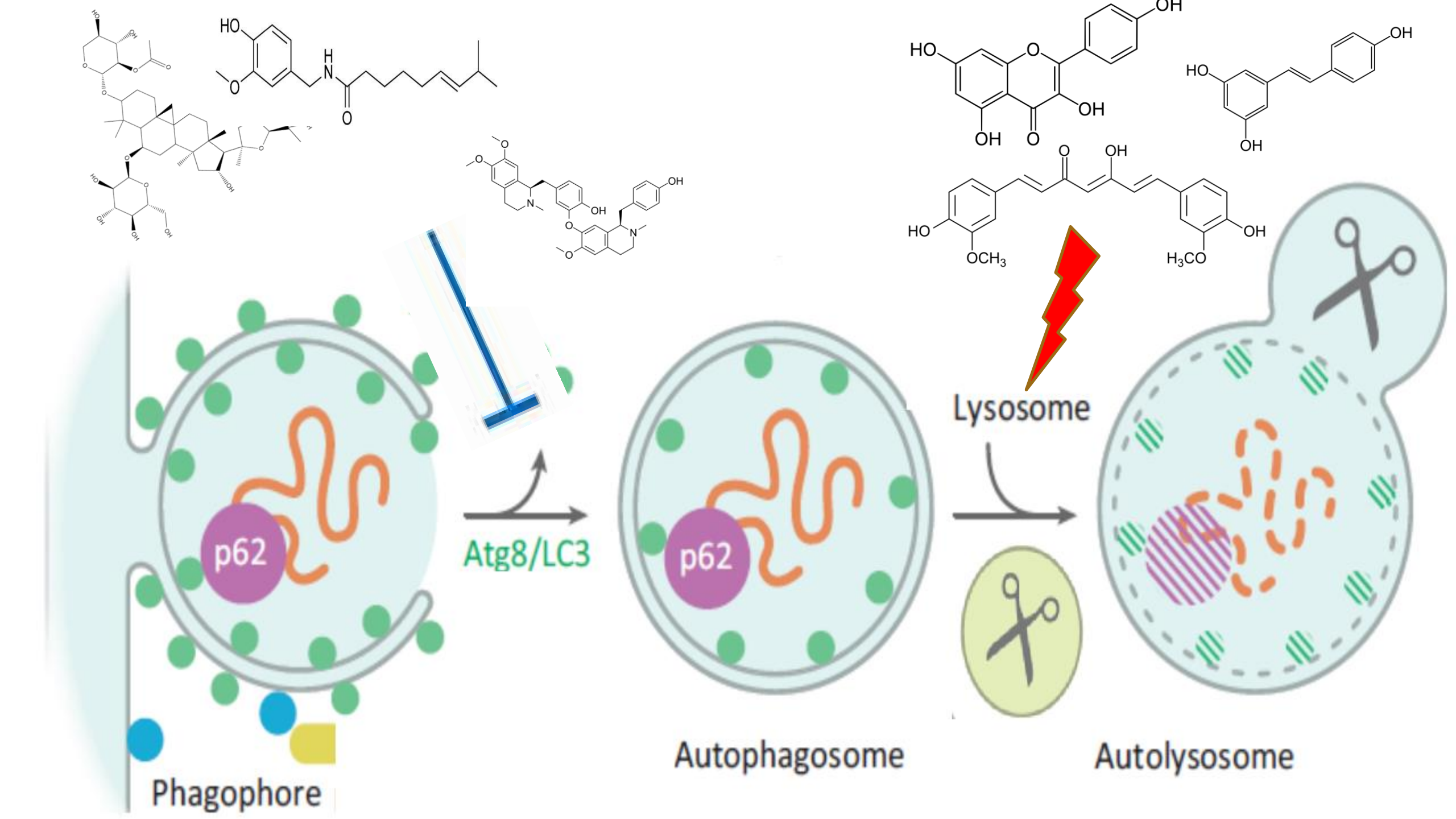
Health Benefits

Cancer preventive, reduce risk of coronary heart disease, Antibacterial, anticancer, antifungal, anti-inflammatory, chemopreventive, hepato-protective, hypolipidemic, hypotensive, and neuroprotective, Anti-allergic, anti-inflammatory, **antioxidants**, and pigments Antispasmodic, chemo-preventive, induce apoptosis, and inhibits breast and ovarian cancers anti-fatigue and anti-stress properties **Anti-carcinogenic**, enhances release of immunogenic cytokines **IL-1 and TNF-alpha**, provide cornea protection against UV light, stimulate DNA repair enzymes, CNS stimulant, and Diuretic, Protect against cancer, bone degeneration, menopausal symptoms (hot flushes) .



Phytochemicals are autophagic modulators

- ✓ **Berberine** from *Coptidis rhizoma* that triggers autophagy (Mohammadinejad et al. 2019)
- ✓ **Kaempferol** treatment enhance the level of the autophagosomal marker LC3-II (Filomeni et al., 2012)
- ✓ **Catechins** increase and decreased the expression of the autophagy marker LC3-II and p62 respectively (Lee et al., 2015)
- ✓ **Curcumin** treatment at 200 and 300 mg/kg/day for 2 weeks increased the expression of LC3-II/LC3-I and beclin-1 protein (Wang et al., 2017)
- ❖ **Oblongifolin C**, a novel autophagic flux inhibitor, (Y Lao et al. 2014)
- ❖ **Deguelin**, a retinoid extracted from Mundulea sericea, inhibits autophagy in human pancreatic cancer cells (XD Xu. Et al 2017)


p62
Atg8/LC3
p62
Lysosome
Phagophore
Autophagosome
Autolysosome

Autolysosome inducers

Compounds	Source	Induction of Autolysosome
kaempferol	*Radix Astragali*	▲LC3-2, LAMP-2
Curcumin	Pepper	▲ conversion of LC3-1 to LC3-1
Resveratrol	*Mundulea sericea*	▲LAMP-1, LAMP-2
Quercetin	Green tea	▲AMP1, LAMP2 and Rab 7

Gene-Eden-VIR/Novirin

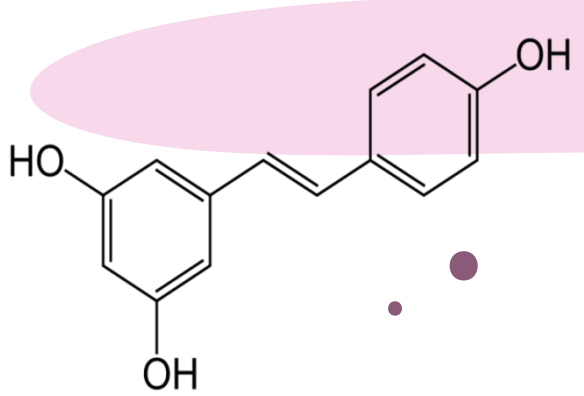
100 mg quercetin, a 150 mg extract of green tea, a 50 mg extract of cinnamon, a 25 mg extract of liquorice and 100 µg of selenium

inhibit the SARS-CoV main protease (3CLpro) with an IC_{50} (50% inhibitory concentration) in vitro of 73 µM (Park et al 2017)

MERS-CoV) 3CLpro protease with IC_{50} values of 52.7, 8.6 and 34.8 µM respectively (Zhuang et al 2009)

Quercetin also modulates the cellular unfolded protein response (UPR). (Brush et al 2006)

Resveratrol



- Completely blocks MERS replication at a concentration of 25 μg/ml (IC50=5.5 μg/ml)
- Nucleocapsid (N) protein essential for MERS-CoV replication was decreased after resveratrol treatment.
- Down-regulates the apoptosis induced by MERS-CoV in vitro (Chao Lin et al 2017)
- Resveratrol as potential treatment adjuncts for SARS-CoV-2/COVID-19 (Marinella 2020)

Autophagy inhibitors

Compounds	**Source**	**Pathways**
Astragaloside II	*Radix Astragali*	PI3K↓, Akt↓
Capsaicin	Pepper	PI3K↓, Akt↓, ROS↑
Deguelin	*Mundulea sericea*	None
(−)-Epigallocatechin-3-O-gallate	Green tea	Atg5↓
20(S)-Ginsenoside	*Panax ginseng*	None
Liensinine	*Nelumbo nucifera Gaevth*	DNM1L↓
Oblongifolin C	*Garcinia yunnanensis hu*	None

Thank you