

AUTOPHAGY RESEARCH FOCUS

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Introduction

Autophagy is recognized as the main tool to degrade damaged organelles and misfolded proteins. It is the conserved pathway that encapsulates small/large objects in a double membrane vesicle – autophagosome (*Figure 1*). Autophagy plays a critical role in cell homeostasis, during differentiation, nutrient deprivation, and normal growth control. It has also been implicated in different diseases e.g. neurodegeneration disorders, cardiac myopathy, autoimmune disease and cancer.

Autophagic Degradation

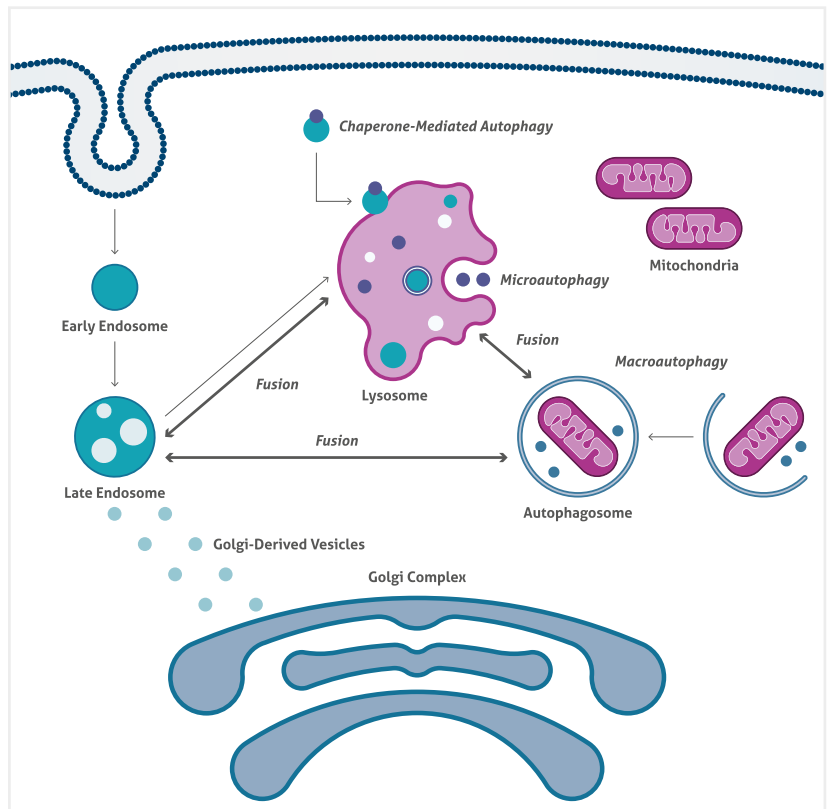


Figure 1: Macroautophagy, microautophagy, and chaperone-mediated autophagy are playing main roles in the lysosomal degradation pathways in mammalian cells.

Single-headed arrows demonstrate the evolution of structures and double-headed arrows trace fusion process.

THE BENCHMARK IN ANTIBODIES

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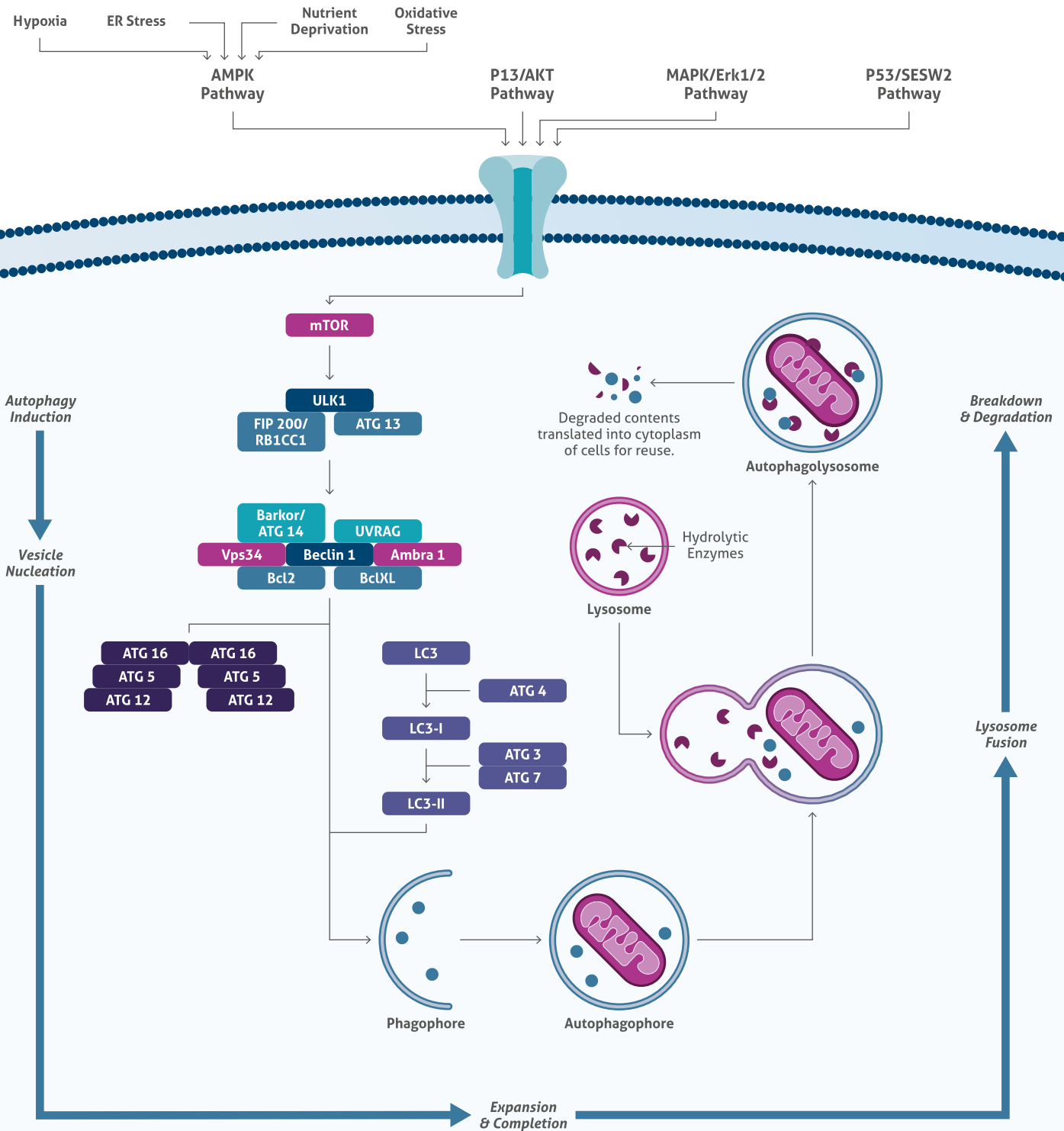
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Autophagy Pathway

Autophagic flux based on five steps: autophagy induction, vesicle nucleation, expansion together with completion, lysosome fusion (autophagolysosome

formation) and breakdown (degradation & recycling). This mechanism is tightly regulated by mTOR, ULK1 complex and ATG molecules.



A Core Component Of The Autophagy Machinery: Beclin 1 (Atg6)

Catalog Number
11306-1-AP

Type
Rabbit Polyclonal

Applications
ELISA, IF, IHC, IP, WB

48 Publications

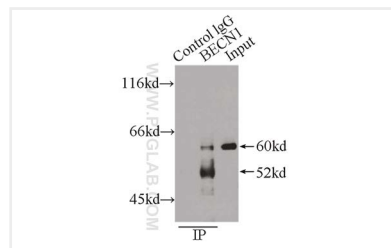
KD/KO Validated

The discoveries of more than 30 autophagy-related (ATG) genes, enable to understand the mechanism of autophagy process. Among the ATG genes, Beclin-1, known also as ATG6 or VPS30 is relatively unique in not being "autophagy-specific." It interacts with various cofactors (e.g. Ambra1, Barkor (Atg14), Rubicon or UVRAG) to regulate the lipid kinase Vps34 and promote formation of the BECLIN1-Vps34-Vps15 complex, hence inducing autophagy. Its function (via BH3 domain) is inhibited by Bcl-2 or Bcl-XL.

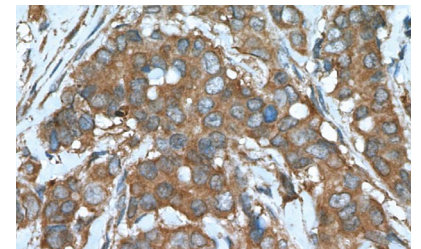
Beclin 1 plays a role as crosstalk between apoptosis and autophagy. It is involved in many disorders, including neurodegeneration and cancer (tumorigenesis). Beclin 1 is a mammalian tumor suppressor. The Beclin 1 gene is monoallelically deleted in even 75% of ovarian, 50% of breast and 40% of prostate cancers. Decreased expression of Beclin1 was also observed in human tumour of brain and lung.

Beclin 1 expression is important for reducing protein aggregates, which supports a general protective role of autophagy. Beclin 1 was also decreased in affected brain regions of patients with Alzheimer disease early in the disease process, that deficiency disrupts neuronal autophagy, modulates APP metabolism, and promotes neurodegeneration. Moreover, gain and loss of Beclin 1 function affect autophagy and death of heart cells.

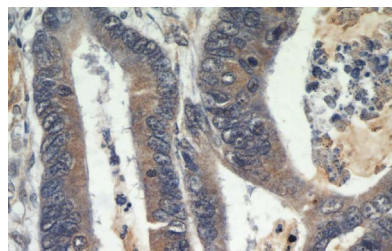
Beclin 1 is a key molecule in the control of autophagic activity, and its activity is regulated by multiple mechanisms, including post-translational modification, protein-protein interaction and subcellular localization. Research on Beclin 1 will provide insight into the mechanisms of autophagy. Targeting Beclin 1 might be a useful and effective future treatment regarding cancer, neurodegeneration or heart disease.



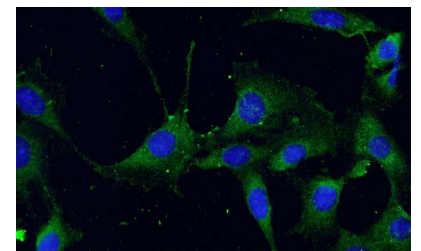
HeLa cells and IP results using Beclin 1 antibody (11306-1-AP; 1:500).



IHC of paraffin-embedded human breast cancer tissue using Beclin 1 antibody (11306-1-AP) at dilution of 1:200; under 40x.



IHC of paraffin-embedded human colon cancer using Beclin 1 antibody (11306-1-AP) at a dilution of 1:50; under 40x.



IF analysis of NIH/3T3 cells labelling Beclin 1 antibody (11306-1-AP; 1:50) and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).

Related Antibodies

Antibody Name	Catalog Number	Type	Applications
ABL2	1 17693-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
AEG-1/ MTDH-Specific	20 13860-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
AMBRA1	1 13762-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
ATG13	1 18258-1-AP	Rabbit Poly	ELISA, IHC, IP, WB
Barkor	1 24412-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
Barkor-Specific	3 19491-1-AP	Rabbit Poly	ELISA, IF, IP, WB

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Related Antibodies

Antibody Name	Catalog Number	Type	Applications
BCL2	184 12789-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, IP, WB
BCL2	7 60178-1-Ig	Mouse Mono	ELISA, FC, IF, IHC, IP, WB
Bcl-XL	2 66020-1-Ig	Mouse Mono	ELISA, IF, IHC, WB
Bcl-XL/S	49 10783-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, IP, WB
Beclin 1	48 11306-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
CALCOCO2	3 12229-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
CISD2-Specific	66082-1-Ig	Mouse Mono	ELISA, IF, IHC, WB
EI24	20456-1-AP	Rabbit Poly	ELISA, WB
LKB1	3 10746-1-AP	Rabbit Poly	ELISA, IHC, IP, WB
MTOR	15 20657-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
PARK7, DJ-1	3 11681-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
PI3K P101	1 13329-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
PI3K P110 (Beta)	5 20584-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
Raptor	4 20984-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
RB1CC1	8 17250-1-AP	Rabbit Poly	ELISA, IF, IHC, IP, WB
Rubicon	21444-1-AP	Rabbit Poly	ELISA, IHC, WB
ULK1	20986-1-AP	Rabbit Poly	ELISA, IHC, WB
UVRAG	19571-1-AP	Rabbit Poly	ELISA, IF, IP, WB
VPS34	2 12452-1-AP	Rabbit Poly	ELISA, IF, IHC, WB

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Both LC3 & GABARAP Are Essential For Autophagosome Formation

Focus Antibody

LC3

Catalog Number

12135-1-AP

Type

Rabbit Polyclonal

Applications

ELISA, IF, IHC, WB

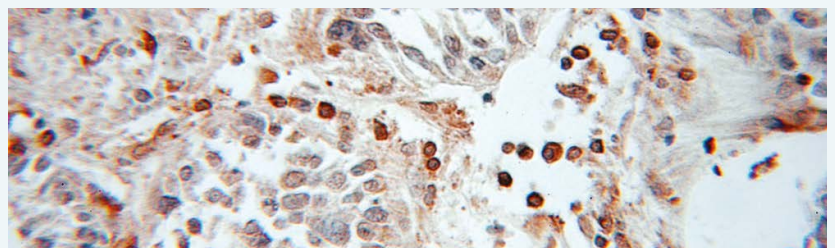
24 Publications

The hallmark of autophagy is the formation of a double-membrane cytosolic vesicle, the autophagosome, which sequesters cytoplasm and delivers it to the lysosomes for degradation.

Autophagy-related 8 proteins (Atg8s) are one of the highly conserved eukaryote-specific protein families. They undergo a unique ubiquitin-like conjugation to phosphatidylethanolamine on the autophagic membrane, what is essential for autophagosome formation. Based on their amino acid sequence homology, at least eight different Atg8 orthologs belonging to two subfamilies (LC3 and GABARAP/GATE-16) occur in mammalian cells.

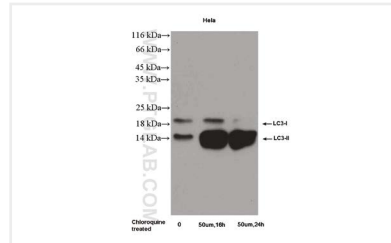
LC3A-C (LC3A has two variants originating from alternative splicing event) belong to the LC3 subfamily and GABARAP, GABARAPL1, GABARAPL2 (GATE-16), and GABARAPL3 are members of the GABARAP/GATE-16 subfamily.

LC3 and GABARAP/GATE-16 subfamilies are both essential in autophagosome biogenesis but act differently. LC3s are involved in elongation of the phagophore membrane and final autophagosome formation whereas the GABARAP/GATE-16 subfamily is crucial for a later stage in autophagosome maturation.

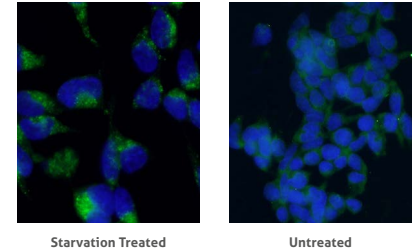


IHC analysis of paraffin-embedded human ovary tumour using GABARAPL1-Specific antibody (ATG8L, 11010-1-AP) 1:50, 40x) at a dilution of 1:50; under 10x.

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Chloroquine treated HeLa cells and WB result of LC3Antibody (12135-1-AP; 1:1000). This antibody recognizes both LC3-I and LC3-II.



IF analysis of fixed HEK-293 cells (ethanol at -20 °C) treated with starvation using LC3 antibody (12135-1-AP; 1:50, 40x) and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).

Related Antibodies

Antibody Name	Catalog Number	Type	Applications
GABARAP	3 18723-1-AP	Rabbit Poly	ELISA, IF, WB
GABARAPL1	1 18721-1-AP	Rabbit Poly	ELISA, FC, IHC, WB
GABARAPL1-Specific	20 11010-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, IP, WB
GABARAPL2	10704-1-AP	Rabbit Poly	ELISA, WB
GABARAPL2-Specific	2 18724-1-AP	Rabbit Poly	ELISA, FC, IHC, WB
LC3	24 12135-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
LC3A/B	10 66139-1-Ig	Mouse Mono	ELISA, FC, IF, IHC, WB
LC3A-Specific	2 18722-1-AP	Rabbit Poly	ELISA, FC, IF, WB
LC3B-Specific	22 18725-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, WB
LC3C-Specific	1 18726-1-AP	Rabbit Poly	ELISA, IF, WB
p62/SQSTM1	58 18420-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, IP, WB
p62/SQSTM1	2 66184-1-Ig	Mouse Mono	ELISA, IF, IHC, WB

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Vesicle Expansion & ATG5, A Protein Containing Ubiquitin Folds

Catalog Number
10181-2-AP

Type
Rabbit Polyclonal

Applications
ELISA, IF, IHC, WB

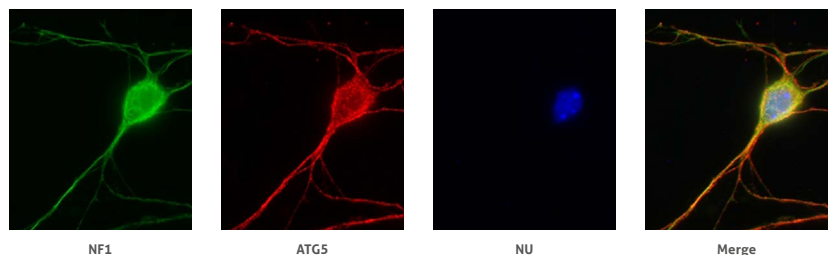
22 Publications

KD/KO Validated

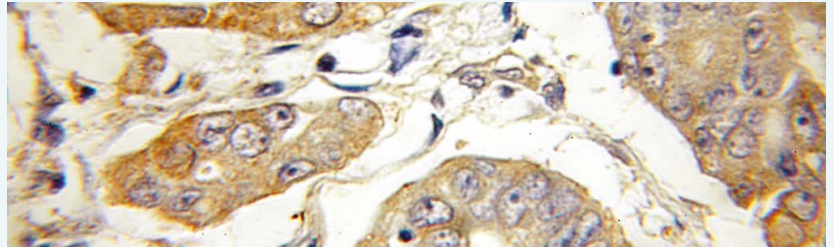
Autophagosome membrane elongation involves two ubiquitin-like conjugation systems: LC3 (mammalian Atg8)-PE (phosphatidylethanolamine) and Atg12-Atg5. Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles.

ATG5 is not only an autophagosome factor. Post-translational modification of ATG5 provides additional insight into the molecular mechanisms of how

autophagy is negatively regulated under normal nutritional conditions and in response to stress stimuli. Together with Beclin1, ATG5 has been found to be cleaved by caspases during apoptosis, blunting the autophagy process to promote the apoptotic response. ATG5 also sensitizes tumor cells to various apoptotic stimuli that are not due to enhanced autophagy.



IF result of anti-ATG5 antibody (10181-2-AP; 1:50) with E15 mouse cortical neurons.



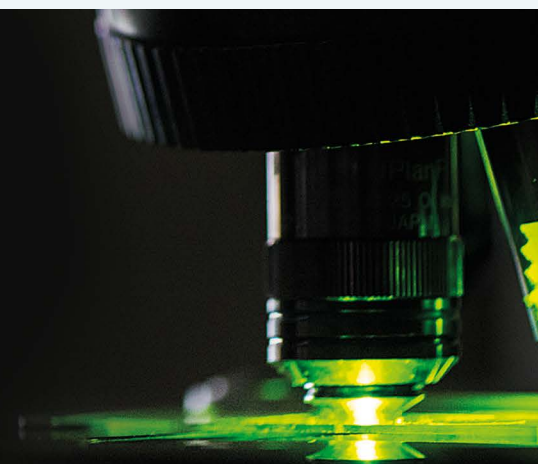
IHC of paraffin-embedded human colon cancer using ATG5 antibody (10181-2-AP) at a dilution of 1:50; under 10x.

Related Antibodies

Antibody Name		Catalog Number	Type	Applications
ATG12	3	11122-1-AP	Rabbit Poly	ELISA, IHC, IP, WB
ATG16L1	1	19812-1-AP	Rabbit Poly	ELISA, IP, WB
ATG16L2		24322-1-AP	Rabbit Poly	ELISA, IP, WB
ATG3		11262-2-AP	Rabbit Poly	ELISA, IF, IHC, WB
ATG4B	1	15131-1-AP	Rabbit Poly	ELISA, IHC, IP, WB
ATG4C		20382-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
ATG4D		16924-1-AP	Rabbit Poly	ELISA, WB
ATG5	22	10181-2-AP	Rabbit Poly	ELISA, IF, IHC, WB
ATG5		60061-1-Ig	Mouse Mono	ELISA, WB
ATG7	5	10088-2-AP	Rabbit Poly	ELISA, IHC, WB
GOPC		12163-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
NBR1	11	16004-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, IP, WB
NPC1	3	13926-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
OPTN	11	10837-1-AP	Rabbit Poly	ELISA, FC, IF, IHC, IP, WB
RAB24	1	11445-1-AP	Rabbit Poly	ELISA, IHC, IP, WB
VMA21	1	21921-1-AP	Rabbit Poly	ELISA, IHC, IP, WB
VPS15		17894-1-AP	Rabbit Poly	ELISA, IF, WB
WDR45		19194-1-AP	Rabbit Poly	ELISA, IF, IHC, WB
WIPI1		25204-1-AP	Rabbit Poly	ELISA, WB
WIPI2		15432-1-AP	Rabbit Poly	ELISA, WB

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