UiT
THE ARCTIC UNIVERSITY OF NORWAY

Faculty of Health Science

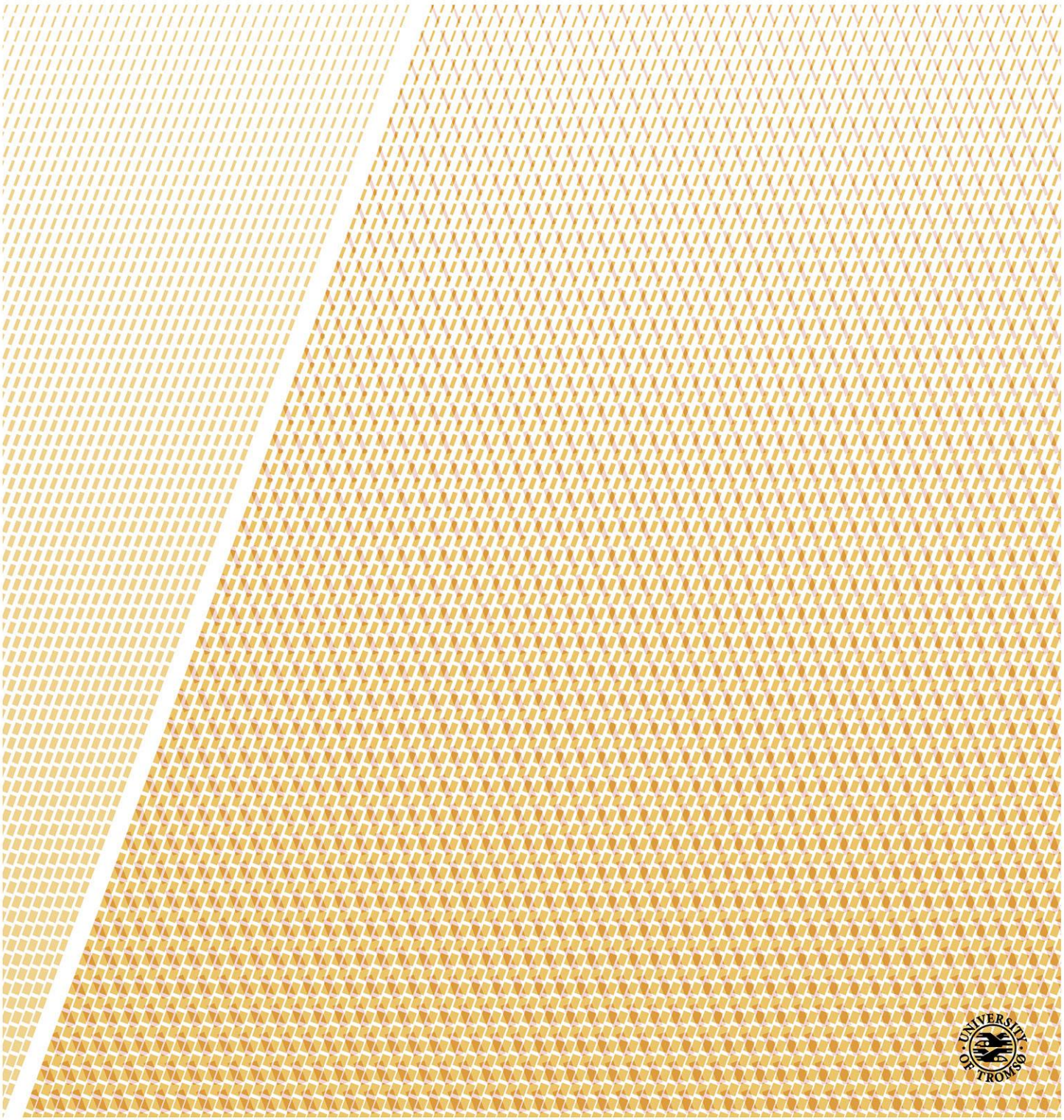
Department of Medical Biology

# FKBP8 and the autophagy-inducing Class-III PI3K Complex

## Roles of LIR dependent interactions

**Zambarlal Bhujabal**

*A dissertation for the degree of Philosophiae Doctor – June 2017*



UiT / NORGES ARKTISKE UNIVERSITET

# FKBP8 and the autophagy-inducing Class-III PI3K Complex

## Roles of LIR dependent interactions

**Zambarlal Baban Bhujabal**

**A dissertation for the degree of Philosophiae Doctor**


UiT The Artic University of Norway

Faculty of Health Science

Department of Medical Biology

Molecular Cancer Research Group

**June 2017**

# Table of Contents

Acknowledgement ..... I
Abbreviation ..... II
Summary ..... IV
List of papers ..... V
Introduction ..... 1
Ubiquitin Proteasome System (UPS) ..... 1
Autophagy ..... 2
Initiation ..... 4
Nucleation ..... 5
Elongation ..... 10
Phagopore closure and fusion ..... 13
ATG8s and the LIR motif ..... 14
Selective autophagy ..... 16
Sequestosome Like Receptors (SLRs) ..... 17
Membrane receptors ..... 19
LIR motifs in autophagy mediator proteins ..... 20
Mitochondria dynamics ..... 21
Mitochondria ..... 21
Mitochondrial Fission Machinery ..... 23
Mitochondrial Fusion Components ..... 24
Mitochondrial protein degradation ..... 25
Mitophagy ..... 26
Membrane receptor mediated mitophagy ..... 27
PINK1-Parkin dependent mitophagy ..... 31
Mitophagy by an alternative autophagy pathway ..... 34
FKBP8 ..... 35
AIM ..... 37
Summary of Papers ..... 38
Discussion ..... 40
Discussion Paper – I and II ..... 40
Discussion Paper – III ..... 46
References ..... 51

# Acknowledgement

I would like to express my sincere gratitude to my main supervisor Terje Johansen for the given opportunity to work in his group. I really appreciate the depth of your knowledge and the way you develop a scientific interest in a person. I would like to thank Trond for his constructive criticism and scientific inputs, Eva for her encouragement, for being supportive and for being strong driving force to achieve my PhD goal. Åsa deserves a special mention here for her support and supervision throughout my work in this lab and listening to my freaky speculations all the time.

Indeed, I appreciate the technical assistance and guidance from Kenneth, Aud, Gry and Hanne. Noteworthy to mention Aud for her expert advice while working on the cell experiments and Kenneth for the colorful imaging part of this work.

Many thanks to all my former and present colleagues, one way the other, everyone has given a helping hand throughout my presence in this lab, especially Birendra, Steingrim, Hallvard, Mads and Mutugi.

Since, I am not workaholic, some of my friends also keep me smiling in this ‘Paris of the North’ city Tromsø. I would like to thank to all my friends, Swapnil, Shripati, Chandra and others for endless enjoyment outside the work.

Last but not the least, I am grateful to my family members for their unconditional support, my parents, my wife Sujata and my little bundle of joy Ishani.

# Abbreviation

| | |
|---|---|
| Ambra1 | Activating molecule in beclin-1-regulated autophagy |
| AIM | Autophagy interacting motif |
| AMPK | Protein kinase AMP-activated catalytic subunit alpha1 |
| ANKMY2 | Ankyrin repeat and mynd containing protein 2 |
| ATG | Autophagy related |
| Bcl-2 | B-cell/lymphoma 2 |
| Bcl-2-L13 | Bcl-2 like protein 13 |
| Bcl-XL | Bcl-2-like 1 |
| BNIP3 | Bcl-2/adenovirus E1b interacting protein 3-like |
| CCCP | Carbonyl cyanide m-chlorophenyl hydrazone |
| CL | Cardiolipins |
| CMA | Chaperone mediated autophagy |
| CVT | Cytoplasm to vacuole transport |
| DFCP1 | Double FYVE containing protein 1 |
| DRP1 | Dynamin related protein 1 |
| ER | Endoplasmic Reticulum |
| FCCP | Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone |
| FIP200 | Focal adhesion kinase family interacting protein 200 kD |
| FKBP | FK506 binding protein |
| FUNDC1 | Fun 14 domain containing protein 1 |
| FYCO1 | FYVE and coiled-coil domain containing 1 |
| GABARAP | Gamma-amino butyric receptor associated protein |
| GATE16 | Golgi associated atpase enhancer of 16 kda |
| GTP | Guanosine triphosphate |
| HOPS | Homotypic fusion and vacuolar protein sorting |
| IMM | Inner mitochondrial membrane |
| LIR | LC3 interacting region |
| LDS | LIR docking site |
| MAP1-LC3 | Microtubule associated protein light chain 3 |
| MAPK | Mitogen-activated protein kinase |
| MDV | Mitochondria derived vesicles |

| | |
|---|---|
| MEF | Mouse embryonic fibroblast |
| Mff | Mitochondrial fission factor |
| Mfn | Mitofusin |
| MiD49/51 | Mitochondrial dynamics protein of 49/51 kda |
| MTC | Multisubunit tethering complex |
| mTOR | Mammalian target of rapamycine |
| NBR1 | Neighbor of brca1 gene 1 |
| NDP52 | Nuclear dot protein-52 |
| OMM | Outer mitochondrial membrane |
| OPA1 | Optic atrophy protein 1 |
| PAS | Preautophagosomal structures |
| PI3-K | Phosphatidylinositol 3-kinase |
| PI3P | Phosphatidylinositol-3-phosphate |
| PINK1 | PTEN-induced kinase 1 |
| RILP | Rab interacting lysosomal protein |
| SLR | Sequestosome like receptors |
| SNAP29 | Synaptosome associated protein 29 |
| SNARES | Soluble *n*-ethylmaleimide-sensitive factor-attachment protein receptors |
| SQSTM1 | Sequestosome-1 |
| STX17 | Syntaxin 17 |
| SYNJBP2 | Synaptojanin 2 binding protein |
| TAX1BP1 | Tax1 binding protein 1 |
| TBC1D15 | Tre2, Bub2, Cdc16 (TBC) 1 domain containing family member 15 |
| UBL | Ubiquitin-like proteins |
| ULK1 | Uncordinated-51-like kinase 1 |
| UPS | Ubiquitin proteosome system |
| UVRAG | UV irradiation resistance associated gene |
| VMP1 | Vacuole membrane protein-1 |
| VPS | Vacuolar protein sorting |
| WIPI | WD-repeat protein interacting with phosphoinoside |

## Summary

This thesis evolves around functional and structural characterisation of LC3 interacting regions (LIRs) in the multifunctional protein FKBP8 and the autophagy specific Class-III PI3K Complex-I. FKBP8 is predominantly localized on the outer mitochondrial membrane and is implicated in a variety of cellular processes. It regulates cell survival through interactions with the anti-apoptotic proteins Bcl-2 and Bcl-XL, cell-proliferation via interaction with the mTOR complex I, protein degradation through interactions with the 26S proteasome, and viral infection via direct binding of Hepatitis C virus protein 5A. The Class-III PI3K Complex-I is localized at the omegasome nucleation site, and is essential for autophagosome formation.

In the first paper, a yeast two-hybrid screen identified FKBP8 as a binding partner of LC3B. We identified a single LIR (LC3 interacting region) motif in the N-terminal of FKBP8 mediating specific interactions with the ATG8 homologues *in-vitro* and *in-vivo*. Further confocal imaging analysis together with biochemical assays revealed that FKBP8 specifically recruits LC3A to damaged mitochondria, and together they induced mitochondrial degradation (mitophagy). The induction was found to be totally dependent on the LIR motif in FKBP8, but independent of the PINK1-Parkin mitophagy pathway. Thus, this study identifies FKBP8 as a mitophagy receptor, specifically recruiting LC3A to damaged mitochondria to promote mitophagy.

The second paper identifies FKBP8 as a regulator of autophagy, and indicates that it is implicated at several stages of the autophagy process. Analysis of FKBP8 knock-out cell lines reconstituted with Myc-FKBP8 showed that FKBP8 inhibits mTOR activity and significantly increases the amount of autophagosomes during nutrient rich conditions. Interestingly, we found FKBP8 to interact directly with Beclin-1, a Class-III PI3K Complex-I protein. Both ER and mitochondria localized FKBP8 bound to Beclin-1. This suggests a role for FKBP8 in recruiting Beclin-1 to the mitochondria membrane contact sites, which are shown to be the site for autophagosome formation.

In Paper III we identified LIR motifs in the Class-III PI3K Complex-I proteins VPS34, Beclin-1 and ATG14L. Notably, they all exerted preference for the GABARAP family proteins. We derived crystal structure of their LIR motifs in complex with the GABARAPs, and revealed that the structural determinants for their ATG8 specificity lies in the amino acid residues C-terminal to the LIRs. Interestingly, confocal imaging and autophagy assays indicated that the LIR motifs are important for the PI3K Complex-I mediated nucleation of the phagophore.

# List of papers

***Paper-I***

**FKBP8 recruits LC3A to mediate Parkin-independent mitophagy.**

Bhujabal Z., Birgisdottir ÅB., Sjøttem E., Brenne HB., Øvervatn A., Habisov S., Kirkin V., Lamark T., Johansen T.

EMBO reports (2017) 18, 947-961

***Paper-II***

**FKBP8 interacts with Beclin-1 and promotes autophagy.**

Bhujabal Z., Sjøttem E., Birgisdottir ÅB., Øvervatn A., Brenne HB., Lamark T., & Johansen T.

*Manuscript*

***Paper-III***

**Members of the autophagy-inducing class III PI3K complex interact preferentially with GABARAP-family proteins via LIR motifs.**

Birgisdottir ÅB., Mouilleron S., Bhujabal Z., Wirth M., Lee R., O'Reilly N., Tooze SA., Lamark T., & Johansen T.

*Manuscript*

# Introduction

Eukaryotic cells require building blocks of life such as amino acids, lipids and carbohydrates for growth and activity. To fulfil their needs, cells are dependent on nutrient uptake and recycling of building blocks. The fine balance between anabolic and catabolic processes and maintenance of cellular homeostasis is defined by tightly regulated mechanisms for biosynthesis and turnover or degradation (Olson, 1997; Schoenheimer et al., 1939). Through degradation mechanisms cells get rid of the undesirable intracellular components and recycle back nutrients or macromolecules. Almost all proteins produced in the cell have their defined half-life, which may range from few minutes to several hours or days (Ganschow and Schimke, 1969). It is well accepted that the degradation process is equally important as the uptake of the nutrients, especially during nutrient deprivation. Also, it is important for quality control of proteins and organelles. There are two major pathways to facilitate the degradation of cellular components and recycling of macromolecules. The ubiquitin/proteasome pathway that degrades single proteins and smaller protein complexes and the lysosomal pathway that degrades cytosolic aggregates and cellular components like mitochondria, peroxisomes etc. (Ciechanover, 2015; De Duve and Wattiaux, 1966).

## Ubiquitin Proteasome System (UPS)

The ubiquitin proteasomal system of degradation depends upon a 76-amino acid long, highly conserved molecule, known as ubiquitin. Target proteins are marked for degradation by conjugation to ubiquitin. Ubiquitination is achieved by three types of ubiquitin enzymes. The first one is ‘ubiquitin activating enzyme’ known as E1. E1 activates ubiquitin in the presence of ATP and converts it to ubiquitin-E1 thiol ester. E1 is then recognised by ‘ubiquitin-conjugating enzyme’ known as E2. E2 is recognised by a third enzyme ‘ubiquitin ligase’, known as E3, by binding through its conserved core. Specificity for the target protein is achieved by the E3 enzyme. E3 links at least four ubiquitin monomers to the target protein to be recognised by the lid like structure on the barrel shaped chamber known as the proteasome (Fig.1A). The proteasome consists of two subunits, 20S and 19S. The 20S protein subunit forms the barrel shaped structure and two 19S protein subunits sit on the ends of the barrel shaped unit. The target protein is recognised, unfolded and propelled by the 19S subunit into the barrel shaped 20S subunit for further proteolysis. (Pickart and Cohen, 2004; Tanaka, 2009).

## Autophagy

Autophagy is a conserved catabolic process through which the bulk degradation of cytoplasmic components by the lysosomal/vacuolar system occurs. Christian De Duve, who first described the '*digestive body*' or lysosome, coined the term 'Autophagy' meaning 'eating of self' (De Duve, 1963; De Duve et al., 1955). Depending upon the delivery of the cellular component to the lysosome, autophagy is divided into three types (Fig.1B to D). Microautophagy, involves direct invagination of the membrane to engulf substrate into the lysosome or endosome (Fig.1D) (Mijaljica et al., 2011). In chaperon mediated autophagy (CMA), the protein to be degraded is recognised by the chaperon 'Heat Shock Cognate-70' (Hsc70), following recognition of a degenerate penta-peptide 'KFERQ' like motif within the target and transported to the lysosomal membrane glycoprotein LAMP-2A to be degraded by the lysosome (Fig.1C). Macroautophagy (hereafter called as 'autophagy') is a highly conserved, selective, multistep process that plays a major role in degrading larger cellular components and complexes (Mizushima, 2007). Microautophagy and CMA remain little studied as compared to macroautophagy (autophagy). The multistep '*de novo*' process of autophagy is initiated with formation of the isolation membrane (phagophore) which engulfs the targeted cargo into a double membrane vesicle (autophagosome) that fuses with the lysosome to degrade its contents (Autolysosome). Some of the autophagosomes fuse with early or late endosomes generating amphisomes that eventually fuse with the lysosome (Fig.1B) (Klionsky, 2007; Mizushima, 2007).

Historically, Christian De Duve and his colleagues were studying the autophagy process at the fusion step with the lysosome using electron microscopy. Later P. O. Selgen's laboratory was studying early and intermediate steps of autophagy and identified the phagophore as a sequestering organelle (Gordon and Seglen, 1988). De Duve observed that autophagy is regulated by glucagon, while U. Pfeifer found that autophagy is strictly regulated by nutrient conditions (Pfeifer and Strauss, 1981). In yeast, a morphologically similar pathway was reported as 'Cytoplasm to vacuole transport' (*CVT*) pathway (Harding et al., 1995). Here the vacuole contains the acid hydrolases, resembling the characteristics of the lysosomal compartment, and is enriched with cellular components under nitrogen starvation (Takeshige et al., 1992). Since the vacuole was filled up with autophagic bodies after starvation, mutants were obtained that failed to accumulate autophagic bodies under nitrogen starvation. This simple and elegant approach was used by the group of Ohsumi to pioneer the identification of autophagy genes. The first mutant for an autophagy gene they isolated was named *apg1*

(Tsukada and Ohsumi, 1993). In parallel, the group of Thumm and Klionsky also initiated genetic screening in yeast for autophagy mutants known as *aut* mutants and *CVT* mutants, respectively (Thumm et al., 1994). A unified nomenclature of autophagy mutants was established, known as ATG (AuTophaGy related) mutants. Further genetic screening revealed several autophagy mutants such as *atg5*, *atg6*, and *atg13*. The yeast genetic approaches led to a search for conserved genes in eukaryotes, and identification of their role in the mammalian autophagy system. So far a total of 41 putative ATG proteins have been identified in yeast (Yao et al., 2015). Several of the *ATG* genes identified in mammalian systems linked the role of autophagy to bacterial infection, cellular differentiation, development, cancer and neurodegeneration (Jiang and Mizushima, 2014).

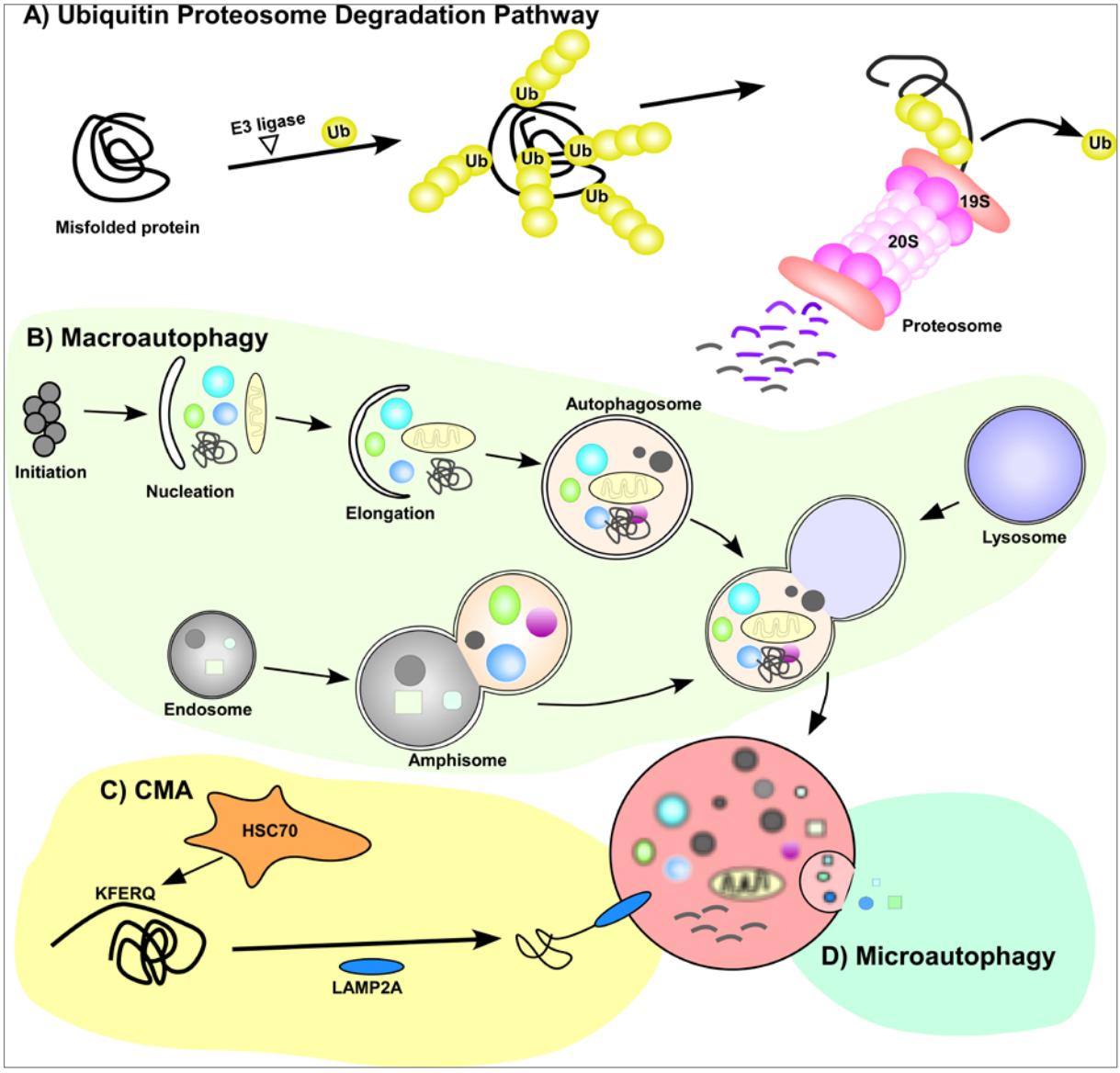



**Fig.1 Eukaryotic degradation systems**: **(A)** Ubiquitin Proteosomal Degradation. A misfolded protein or a targeted component is ubiquitinated by an E3 ligase and transferred to the

proteosome where it is degraded by proteolysis. **(B - D**:) Autophagic pathways. **(B)** Macroautophagy – successive steps in autophagy; Gathering of protein complexes (initiation), generation of the double membrane phagophore (nucleation), expansion of the phagophore along with docking of a targeted organelle or proteins (elongation). The phagophore then closes to become a vacuole-like structure termed the autophagosome that fuses with the lysosome. Some autophagosomes fuse with endosomes resulting in amphisomes prior to fusion with the lysosome. **(C)** Chaperone Mediated Autophagy: misfolded proteins are recognized by Heat shock cognate protein 70 (HSC70) and transported directly into the lysosome by LAMP2A. **(D)** Microautophagy involves direct invagination of cytosolic material into the lysosome.

**Initiation**

The yeast Atg1 (ULK1 in mammals), is a highly regulated serine-threonine protein kinase interacting with yeast Atg13, constituting the protein complex essential for autophagy initiation (Fig.2A) (Funakoshi et al., 1997; Matsuura et al., 1997). Atg13 is highly phosphorylated by the phosphatidylinositol kinase-related kinase, TOR (Target of Rapamycin) under normal growth condition. Upon starvation, Atg13 undergoes rapid de-phosphorylation (Kamada et al., 2000). The dephosphorylated Atg13 has enhanced affinity towards Atg1 and it also binds to Atg17, thus making a complex of Atg1-Atg13-Atg17 (Kabeya et al., 2005). This study established the first mechanistic link to indicate that TOR negatively regulates starvation induced autophagy. Another sub-complex of Atg17 (Atg17-Atg29-Atg31 complex) also participates in autophagosome biogenesis during starvation conditions. Upon induction of autophagy, the Atg17-Atg29-Atg31 complex undergoes dimerization and assembles with Atg1 and Atg13 to initiate phagophore formation, further recruiting other downstream autophagy components (Ragusa et al., 2012).

In mammalians, a study of mTOR (mammalian Target of Rapamycin) activity in response to L-glutamine showed that autophagy is negatively regulated by mTORC1 (mTOR Complex-I) (Nicklin et al., 2009). Upon amino acid deprivation, mTORC1 inactivation leads to rapid dephosphorylation of ULK1 (Uncordinated-51-like kinase 1), ULK2 and ATG13. The mammalian ATG13 binds to both ULK1 and ULK2 and mediates the interaction between ULK1/2 and FIP200 (focal adhesion kinase family interacting protein 200 kD). The binding of ATG13 to ULK proteins stabilizes and accelerates the ULK kinase activity leading to phosphorylation of FIP200 (analogous to yeast Atg17), essential for autophagy initiation (Jung et al., 2009). Thus, under the negative regulation of mTOR, autophagy initiation is driven by the protein complex ULK1-ATG13-FIP200 similar to the yeast complex Atg1-Atg13-Atg17 (Fig.2B). In mammals, ATG101 binds to ULK1 in an ATG13 dependent manner. This protects ATG13 from proteasomal degradation and is important for recruitment of downstream effector

proteins. ATG101 is not homologues to yeast Atg29 or Atg31 but presumably contributes in a similar manner as Atg29 and Atg31 (Mercer et al., 2009; Noda and Mizushima, 2016).

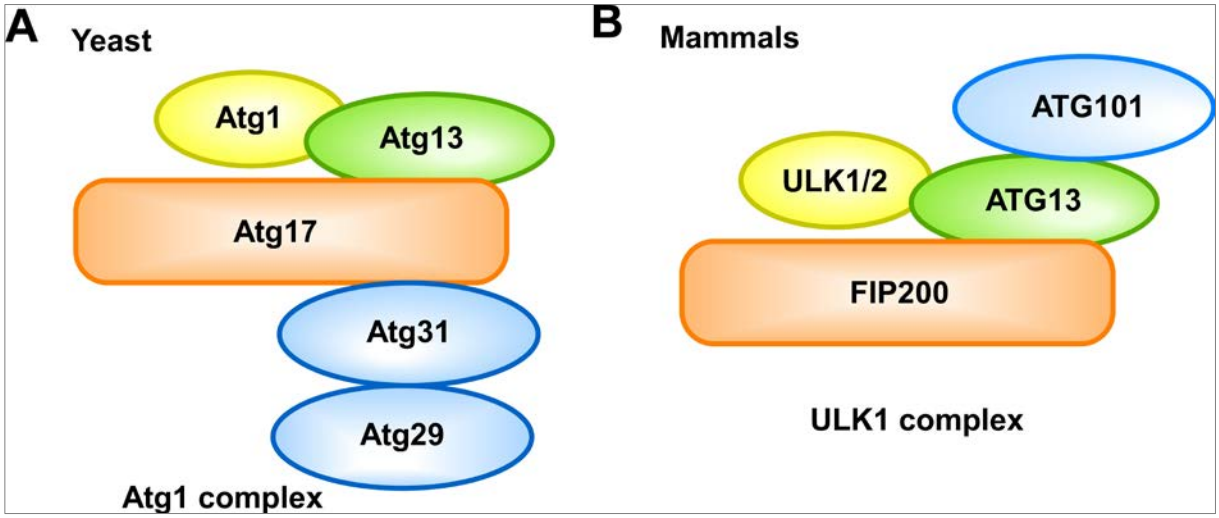



**Fig. 2 Components of the Initiation complex in yeast and mammals**. **(A)** The Atg1- Atg13-Atg17-Atg29-Atg31 complex in *Saccharomyces cerevisiae* (yeast) is formed in response to nutrient deprivation. **(B)** In mammals, the initiation complex is formed by ULK1/2-ATG13-FIP200-ATG101assembly.

**Nucleation**

Ohsumi's group identified the PI3K (phosphatidylinositol 3-kinase) complex in yeast, another complex crucial for autophagy (Kametaka et al., 1998). In mammals, three types of PI3K´s are identified, namely Class-I, -II and –III, depending upon their lipid substrate specificity. The Class-III kinase is a multi-subunit complex involved in intracellular membrane trafficking. Its catalytic subunit Vps34 (vacuolar protein sorting 34) utilizes phosphatidylinositol (PI), as a substrate, phosphorylates the 3'-position of the inositol head group resulting in phosphatidylinositol-3-phosphate (PI3P).

The class-III PI3K complex is highly conserved throughout the lower eukaryotes, plants and mammals (Schu et al., 1993). In yeast, Vps34 is found in two types of class-III PI3K complexes; Complex-I consists of Vps34-Vps15-Vps30/Atg6 and Atg14; Complex-II consists of Vps38 instead of Atg14 (Vps34-Vps15-Vps30/Atg6 and Vps38) (Fig.3A, B). Complex-I is essential for autophagy while Complex-II is essential for the Vps pathway (Vacuolar protein sorting) (Kihara et al., 2001; Obara et al., 2006). Another component was reported in Complex-I, known as Atg38. Atg38 physically interacts with Atg14 and Vps34 via its N-terminus. In the absence of Atg38, Complex-I is dissociated in two sub complexes; Vps15-Vps34 and Atg14-

Vps30. Atg38 dimerises through its C-terminus and serves as a physical linkage between Vps15-Vps34 and Atg14-Atg6, facilitating formation of Complex-I (Araki et al., 2013).

In mammals, the class III PI3K complex consists of VPS34, p150/VPS15, Beclin-1 (homologue of yeast Atg6) and ATG14L (Barkor or ATG14) (Fig.3C, D). Similar to yeast, VPS34 is a part of Complex-I consisting of VPS34-p150/VPS15-Beclin-1-ATG14L (Fig.3C), and Complex-II built of VPS34-p150/VPS15-Beclin-1-UVRAG (UV irradiation resistance associated gene) (Fig.3D). In resemblance to yeast Vps38, UVRAG interacts with VPS34, forming Complex-II (UVRAG complex) (Fig.3E), primarily involved in endosomal transport and later steps in autophagosome maturation (Itakura et al., 2008). In mammals, a sub-population of the UVRAG complex is associated with Rubicon (Run domain protein and as Beclin-1 interacting and cysteine-rich containing) known as the Rubicon complex (Fig.3F). Knock down of Rubicon enhances autophagy, suggesting that it may function as a negative regulator of autophagy (Matsunaga et al., 2009). Another subpopulation of the UVRAG complex interacts with the Endophilin B family member, known as Bif-1 (also known as SH3GLB1, SH3 domain containing GRB2 like endophilin B1 protein). Bif-1 associates with UVRAG via its C-terminal SH3 domain and positively regulates autophagy (Takahashi et al., 2007). Beclin-1 is known to interact with the antiapoptotic protein Bcl-2 (B-Cell CLL/lymphoma 2). Under nutrient deprived conditions, Beclin-1 loses its interaction with Bcl-2 and forms interactions with VPS34. Furthermore, Beclin-1 interacts with ATG14L via its coiled-coil domain, and this step is necessary for autophagy. ATG14L detects membrane curvature of the nascent autophagosome using its C-terminal BATS (Barkor/ATG14L autophagosome targeting sequence) domain. This interaction mediates recruitment of the whole complex to the active site of autophagosome biogenesis (Fan et al., 2011). Furthermore, it has been reported that Beclin-1 harbours a C-terminal domain that mediates ATG14L-independent membrane docking. Beclin-1 is phosphorylated in the presence of ATG14L and these phosphorylation events are critical for efficient autophagy (Fogel et al., 2013). The ULK1 kinase activates the VPS34 complex through phosphorylation of Beclin-1, leading to generation of PI3P (Russell et al., 2013). The regulatory subunit of the ULK1 complex, ATG13, interacts with PI3P, suggesting that the ULK1 complex stabilizes and supports the VPS34 complex at the phagophore site (Karanasios et al., 2013). Two other proteins, Ambra1 (activating molecule in Beclin-1-regulated autophagy) and VMP1 (vacuole membrane protein-1) interact with Beclin-1 to regulate the autophagosome formation (Fimia et al., 2007; Ropolo et al., 2007).

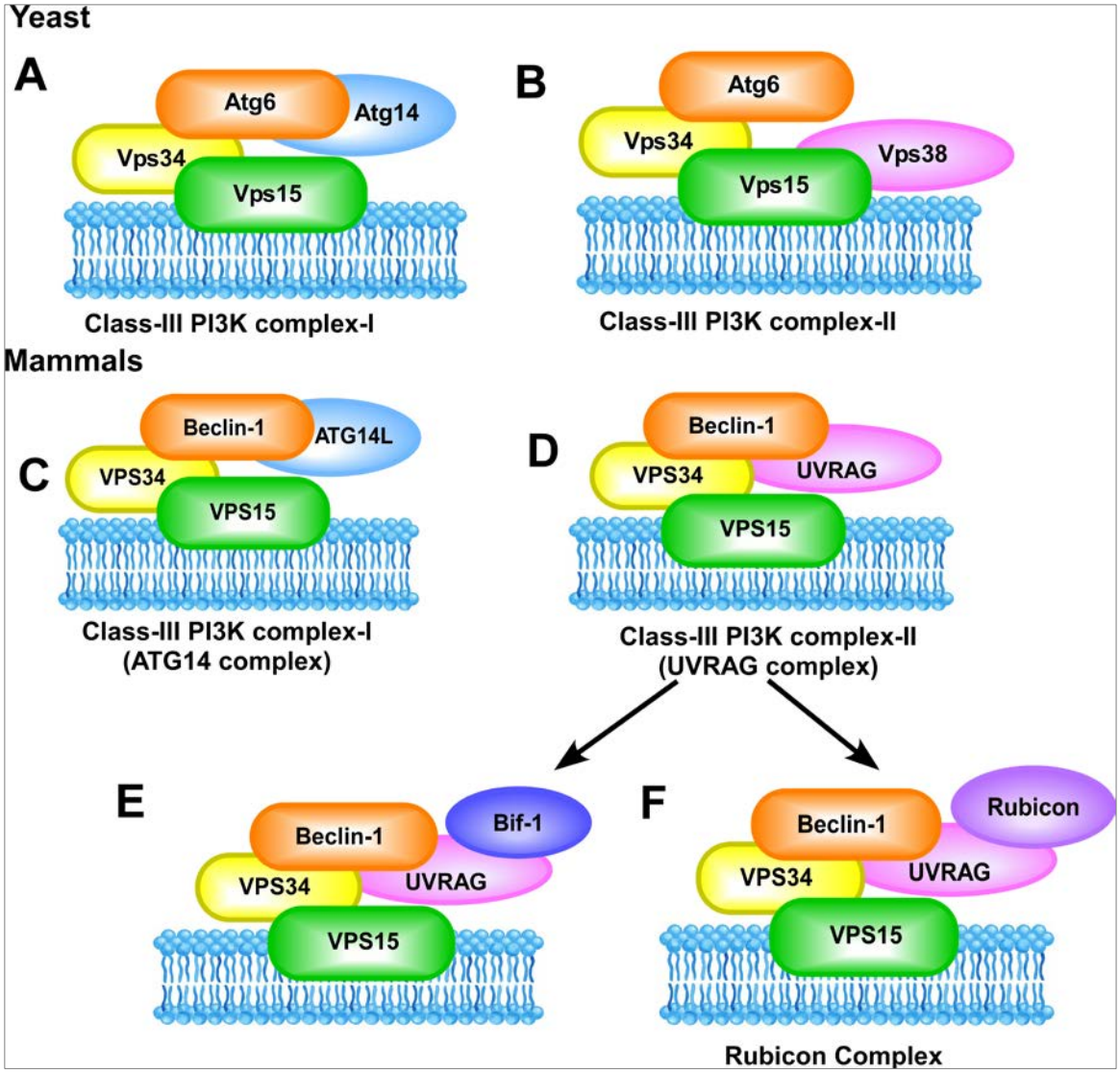



**Fig. 3 Class-III PI3K complexes in yeast and mammals**. In yeast, there are two class-III PI3K complexes formed downstream of the initiation complex, **(A)** Complex-I, Vps34-Vps15-Atg6-Atg14, and **(B)** Complex-II, Vps34-Vps-15-Atg6-Vps38. Complex-I regulates autophagy and Complex-II regulates vacuolar protein sorting. In mammals, the corresponding complexes regulating autophagy are, **(C)** The ATG14 complex and **(D)** The UVRAG complex, The UVRAG complex engages in two other complexes **(E)** UVRAG complex with Bif-1, positively regulating autophagy and **(F)** Rubicon complex, containing additional Rubicon via UVRAG, negatively regulating autophagy. Figure adapted with modification from Kang, Zeh et al. 2011.

In mammals, the PI3P effector proteins are WIPI1-4 (WD-repeat protein interacting with phosphoinoside) and DFCP1 (Double FYVE containing protein 1) (Fig.4). WIPI1 and WIPI2 are homologues to yeast Atg18 and WIPI3 and 4 are closer towards yeast Ygr223. The function of the yeast Ygr223c is speculated to be in micronucleophagy (Krick et al., 2008). DFCP1 is ubiquitously localized on ER and Golgi membranes, but after starvation it concentrates on the ER membrane via its FYVE domain. DFCP1 binds to PI3P via its FYVE domain and provides a platform for expansion of the isolation membrane and separation of the

completed autophagosome. The autophagy specific Class-III PI3K Complex-I at the ER acquires the Ω-like shape known as omegasomes (Axe et al., 2008). The Class-III PI3K Complex-I can also be targeted to these omegasomes by ATG14L via its N-terminal ER localisation signal independent of DFCP1 (Matsunaga et al., 2010). WIPI1 and WIPI2 are found to be colocalised with ATG14L, and formation of WIPI dots are dependent on PI3K activity. DFCP1 is observed adjacent to the WIPI dots (Fig.4) (Polson et al., 2010).

Atg9 is a transmembrane protein (spanning the membrane six times) required both for the autophagy and the *CVT* pathway. Morphological analyses indicate that Atg9 is localised in cytoplasmic dot structures, recruited to the preautophagosomal structures (PAS) or phagophore assembly site where autophagosome or *CVT* vesicle formation begins (Noda et al., 2000). Atg9 dot structures localised to the PAS is dependent on Atg17 (Sekito et al., 2009). Cytoplasmic dots of Atg9 (Atg9 vesicles) are derived from the Golgi apparatus, involving Atg23 and Atg27. Atg9 vesicles assembled on the preautophagosomal membrane are thought to deposit vesicles and lipids on the membrane of the autophagosome for expansion (Yamamoto et al., 2012). The mammalian homologue of yeast Atg9, ATG9L1 localises on omegasomes, *trans*-Golgi network and also on endosomes (Young et al., 2006).

**Class-III PI3K Complex-I**

As described above, the class-III PI3K Complex-I consists of VPS34, VPS15, Beclin-1 and ATG14L. The function of the PI3K Complex-I is regulated by several other interacting proteins, majorly through modifications of Beclin-1 by phosphorylation or ubiquitination.

Under nutrient deprivation, the energy sensor kinase AMPK activates the ULK1 complex (Egan et al., 2011). AMPK also phosphorylates Beclin-1 at S93/S96 and VPS34 T163/165, where the Beclin-1 phosphorylation is dependent on ATG14L to induce autophagy (Fogel et al., 2013; Kim et al., 2013). AMPK senses glucose starvation and phosphorylates ULK1, Beclin-1 and VPS34 in the absence of glucose only. Two members of the p38 mitogen-activated protein kinase (MAPK) signalling pathways, MAPKAPK2 (MK2) and MAPKAPK3 (MK3) phosphorylate Beclin-1 at S90 under amino acid starvation (Wei et al., 2015). To constitutively activate autophagy, ULK1 further phosphorylates Beclin-1 at S15. In addition, ATG13, a subunit of the ULK1 complex, recruits the PI3K Complex-I via its HORMA domain and directly interacts with ATG14L. ULK1 then phosphorylates ATG14L at S29, which is important for phagophore formation under nutrient deprivation or mTOR inhibition, regulating the lipid kinase activity of the class-III PI3K Complex-I (Jao et al., 2013; Park et al., 2016).

The PI3K complex is needed for ULK1/2 complex stabilization at the omegasome and facilitates the recruitment of the PI3K effector proteins WIPI1/2 (Karanasios et al., 2013; Koyama-Honda et al., 2013). WIPI2 translocates to PI3P lipids and binds to ATG16L in the ATG12-ATG5-ATG16L conjugation complex. In addition, FIP200 in the ULK1 complex binds to ATG16L (Dooley et al., 2014; Nishimura et al., 2013). The expansion of the phagophore is assisted by the ATG12-ATG5-ATG16L conjugation system (Kraft et al., 2012; Nakatogawa et al., 2007). Each member of the ULK1 complex (except ATG101) possesses a LIR motif (LC3 interacting region, explained in later section) presumably used for stabilization of the ULK1 complex on the phagophore and to drive the expansion (Alemu et al., 2012).

The crystal structure of Complex-I (VPS30/VPS15/Beclin-1/ATG14L) adopts a 'V' shaped structure where VPS15 holds together and bridges the other two proteins, VPS34 and Beclin-1 (Fig.4A, B). VPS34 is arranged on the right arm of the 'V'-shaped structure. Its C-terminal kinase subunit is on the tip of the right arm while the N-terminus is near the curve of the 'V' shaped structure. The VPS15 kinase is arranged throughout the right arm, forms the base of the 'V' shape and extends towards the left arm. The N-terminal kinase domain of the VPS15 is near the C-terminal kinase domain of VPS34. The HEAT repeat domain adopts a fork-like shape giving the base for the V-shape structural arrangement and the C-terminal WD40 domain makes a donut shaped region on the left arm. The left arm is made up of Beclin-1 where the N-terminus is facing towards the junction of the V-shape and the C-terminal part is on the tip of arm. ATG14L is arranged in parallel to Beclin-1 making a dimer through the coiled-coil domain, unlike the previously described antiparallel dimer (Baskaran et al., 2014; Li et al., 2012).

The crystal structure of the homologues yeast Complex-II (Vps34-Vps15-Vps30 and Vps-38) indicated a similar Y-shaped structure, with two arms and a short hook like base (Rostislavleva et al., 2015). Thus the details of yeast Complex-II most likely share similar features as human Complex-I. Vps15 and Vps34 are arranged in an antiparallel fashion on the right side arm of the V-shaped structure, where the N-terminal kinase domain of Vps15 interacts with the C-terminal kinase domain of Vps34. Vps34 and Vps15 are interdependent on each other, where Vps34 is needed for integrity of Vps15 and Vps15 is needed for membrane recruitment of Vps34 (Kihara et al., 2001; Stack et al., 1993). The left arm is built of Vps30 and Vps38 arranged in parallel where the C-terminal BARA domain of Vps30 is on the tip of the arm together with the C-terminal BARA2 domain of Vps38. The N-terminal domains of Vps30 and Vps38 meet at the base of the complex where they primarily contact Vps15. The proposed structure shows that the short hook like base is entirely made up of the N-terminal

domains of Vps30 and Vps38. In addition, arrangement of Vps38 in parallel with the Vps30 supports the ATG14L/Beclin-1 parallel heterodimer, like proposed in the mammalian Complex-I (Rostislavleva et al., 2015).

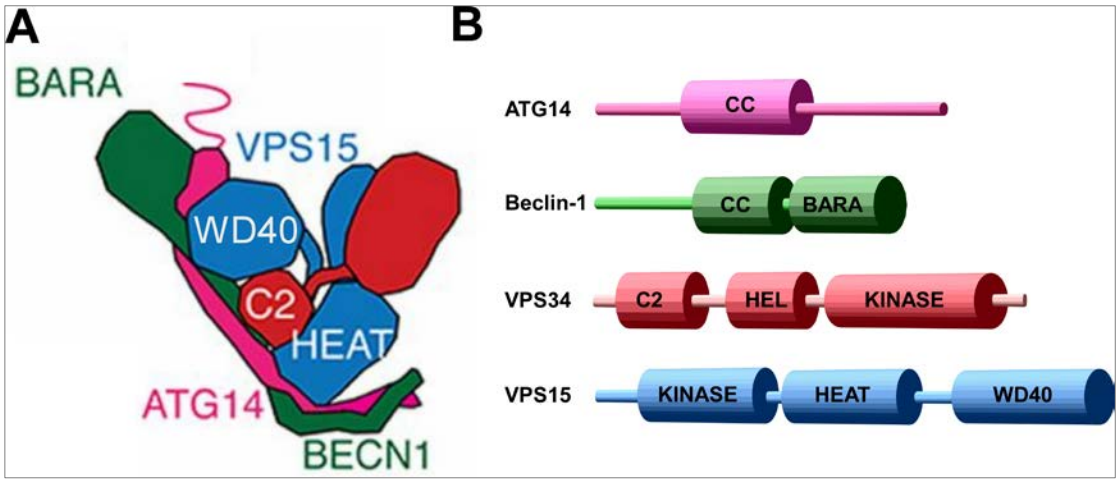



**Fig. 4 Structural arrangement of the PI3K Complex-I. (A)** Schematic representation of the structural arrangement of the four subunits of Class-III PI3K Complex-I. Structure adopts V-shape, base is formed by the VPS34 and VPS15, extending contacts and making right arm, whereas the left arm indicate the dimer made by Beclin-1 and ATG14L. Figure adapted with modification from Young L. et. al. 2016. **(B)** Domain architecture of ATG14L (in Purple, Coiled Coil domain); Beclin-1 (in Green, indicating coiled coil and BARA-(β-α repeated autophagy specific) domain); VPS34 (Red, lipid binding C2 domain, HEL (helical domain) and Kinase domain); VPS15 (in Blue, Kinase domain, HEAT repeat and WD40 repeat).

**Elongation**

ULK and Class III PI3K complexes contribute to the formation and stabilization of phagophore curvature through formation of PI3P. Structural analysis of yeast Atg1 indicates that it forms a crescent shaped dimer at the ER site. Upon autophagy initiation, a crescent shaped phagophore is formed at the ER site. Its formation further involves the membrane uptake from organelles of the endomembrane system like mitochondria, ER and Golgi through exchange of vesicles. Elongation of the phagophore is assisted by two autophagosomal ubiquitin like conjugation systems, Atg12 conjugation and Atg8 lipidation (Reviewed in (Lamb et al., 2013; Shibutani and Yoshimori, 2014)).

Proteins that display resemblance with ubiquitin in their three dimensional structure and function, are referred to as ubiquitin-like proteins (UBLs). UBLs are conjugated to proteins or substrates in a similar way as ubiquitin in the ubiquitin signalling cascade (Hochstrasser, 2009). In the yeast Atg12 conjugation system, a C-terminal Glycine residue of Atg12 is conjugated with a lysine residue of Atg5. Atg7 acts as an E1 enzyme, transferring Atg12 to Atg10 (E2

enzyme) (Fig.5). Atg7 activates the C-terminal carboxyl group of Atg12 by making a thioester bond with the sulfhydryl group by ATP consumption. The catalytic cysteine is further transferred to Atg10 and makes isopeptide binding with the lysine residue in Atg5 (Mizushima et al., 1998). The Atg12-Atg5 conjugate binds to Atg16, generating the Atg12-Atg5-Atg16 complex (Ishibashi et al., 2011). This complex localises to the isolation membrane curvature and dissociates after completion of the autophagosome. Its localization on the membrane depends on Atg16 (Suzuki et al., 2007). In mammals a similar complex ATG12-ATG5-ATG16L1 is known. There are two orthologues of ATG16, L1 and L2, but mainly ATG16L1 is active in the conjugation system. Interaction between ATG16L1 and FIP200 might provide a localisation signal for the ATG12-ATG5 conjugate to the isolation membrane (Gammoh et al., 2013; Nishimura et al., 2013).

Following the Atg12 conjugation system, Ohsumi's group identified the Atg8 conjugation system. They reported the cellular re-localisation of Atg8 to the autophagosome membranes upon starvation (Kirisako et al., 1999). Biochemical analysis further demonstrated that Atg8 works in a unique ubiquitin-like modifier system. Atg7 also acts as an E1 enzyme for the Atg8 conjugation system, where Atg3 is the E2 enzyme (Noda et al., 2011; Taherbhoy et al., 2011). The yeast Atg8 is processed by the cysteine protease Atg4, exposing a C-terminal glycine residue (glycine-119), which is further recognised by Atg7 (Fig.5) (Kirisako et al., 2000). Atg7 transfers the processed Atg8 to the E2 like enzyme, Atg3, in a *trans* thiolation reaction. Atg16 interacts with Atg5 by its N-terminal α-helical domain, making the multimeric complex Atg12-Atg5-Atg16 (Mizushima et al., 1999). This complex further serves as E3 enzyme for Atg8, transferring Atg8 to the lipid PE (phosphatidylethanolamine) by thioester bond formation (Hanada et al., 2007; Ichimura et al., 2000). Even though the Atg12-Atg5 conjugate alone possesses E3 like activity, Atg16 is essential to dock Atg5 to the membrane. Thus, Atg16 plays an important role in tethering the Atg8-PE on the phagophore. It enhances the membrane binding ability of Atg12-Atg5 and allows efficient lipid conjugation of Atg8 on the autophagy related membrane production site (Mizushima et al., 1999; Romanov et al., 2012).

In mammals, a homologous conjugation system is identified, making an ATG12-ATG5 conjugate by ATG7 (E1) and ATG10 (E2), generating the ATG12-ATG5-ATG16L1 (Fig.5B). Complete loss of ATG5 reduces the number of autophagosomes and isolation membranes dramatically, indicating that ATG5 is needed for membrane elongation. Furthermore, ATG5 together with ATG12 is necessary for tethering ATG8 on the autophagosome membrane (Mizushima et al., 2001). Mammals contain six homologues of the yeast Atg8 protein,

subdivided into 3 phylogenetic subfamilies: 1) MAP1-LC3A/B/C (microtubule associated protein light chain 3); 2) GABARAP/GABARAP-L1 (gamma-amino butyric receptor associated protein); 3) GATE16 (Golgi Associated ATPase enhancer of 16 kDa) or GABARAP-L2. LC3B (also known as LC3) was the first identified mammalian homologue of Atg8, localised on autophagosomes and autolysosomes. LC3 exists in two different forms, as LC3-I, an unprocessed form and as LC3-II, which is cleaved at the Gly-120 position by the cysteine protease ATG4B (Fujita et al., 2008a; Kabeya et al., 2000). Membrane bound LC3-II resides on both surfaces of the autophagosome, therefore serving as a good marker to measure autophagic flux. ATG5, together with ATG12 and ATG16L, resides on the convex surface of autophagosomes and serves as a guide to mark the site for the ATG3-LC3 conjugate (Fujita et al., 2008b). The ATG3 enzyme senses lipid packing defects by its N-terminal amphipathic helix, which helps ATG8 family protein lipidation on the growing tip of the phagophore (Nath et al., 2014). LC3 interactions play a crucial role in degradation of cellular materials or proteins by docking them onto the growing phagophore. The size of autophagosomes varies depending upon the components docked by LC3 in its concave side and the level of LC3-II. LC3-II on the convex surface of the phagophore recycles back to cytosol by delipidation mediated by the protease ATG4, while LC3-II on the concave side is degraded by the lysosome (Kabeya et al., 2004).

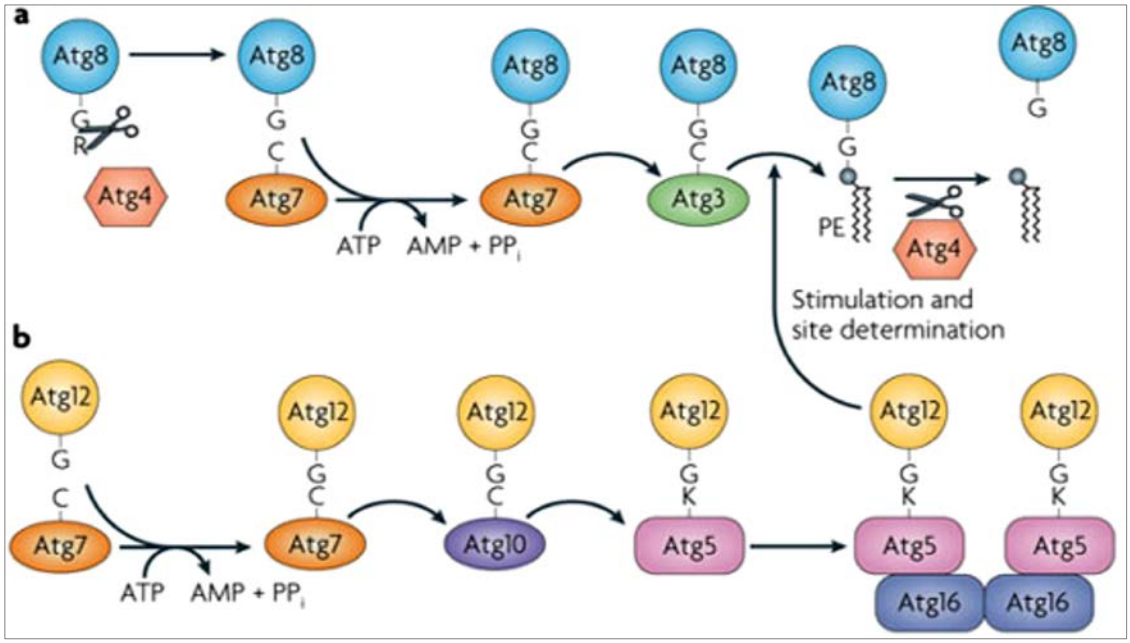



**Fig. 5 The ATG8 and ATG12 Conjugation systems in autophagy. (A)** In ATG8 conjugation, ATG8 is cleaved by the cysteine protease ATG4 exposing a Gly (G) residue that is recognized by ATG7 (as E1), transferred to ATG3 (as E2) and eventually conjugated with phosphotidylethanolamine (PE) by ATG12-ATG5-ATG16 (as an E3 enzyme). ATG4 can also

cleave the PE conjugation off ATG8 in order to release it from the membrane. **(B)** In the ATG12 conjugation system, a glycine residue of ATG12 is conjugated to a lysine residue in ATG7 (as E1), transferred to ATG10 (acts as E2) and finally conjugated to ATG5. The ATG12- ATG5 conjugate then binds to ATG16, resulting in the ATG12-ATG5-ATG16 complex (the E3 enzyme for ATG8 conjugation) Figure reprinted with permission from Nakatogaw et. al. 2009.

**Phagopore closure and fusion**

The cup shaped double membrane phagophore needs to be closed by membrane fusion events before fusion with the lysosome. The mechanism of phagophore closure is not well understood yet. A fraction of autophagosomes are found to fuse with endosomes, making amphisomes, before fusion with lysosomes (Berg et al., 1998). Open autophagosome structures are observed in cells expressing inactive ATG4B. This indicates a role for LC3-II in autophagosome closure (Fujita et al., 2008a). Once autophagosome closure is completed, autophagosomes move bi-directionally along the microtubules to fuse with lysosome (Jahreiss et al., 2008). Autophagosome movement along microtubules is driven by the kinesin and dynein motor family proteins powered by ATP hydrolysis (Stenmark, 2009). Autophagosomes are connected to the microtubules via the small GTPase Rab7. Rab7 mediates trafficking of late endosomes and lysosomes by recruiting the dynein motor through RILP (Rab interacting lysosomal protein) interactions (Jordens et al., 2001). GTP bound Rab7 connects the kinesin motor to the autophagosome by interactions with FYCO1 (FYVE and coiled-coil domain containing 1). FYCO1 interacts with LC3 and PI3P lipids on the autophagosome membrane (Pankiv et al., 2010). Autophagosomes undergo homotypic fusion (fusion of two autophagosome to form a new one, observed in some of the autophagosomes) and/or heterotypic fusion with the lysosome (Jahreiss et al., 2008). Generation of amphisomes can also be categorised as heterotypic fusion. Amphisomes eventually fuse with lysosomes. Fusion events are better studied in the endosome system and autophagosome-lysosome fusion events are predicted to be similar. The endosome-lysosome fusion is mediated by three components, a Rab GTPase, membrane-tethering complexes and SNAREs (soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptors). Rab family proteins activate the tethering complexes by making long range interactions that dock the complex between the endosome and the lysosome. The tethering complex consists of a long coiled-coil domain containing proteins and the multisubunit tethering complex (MTC). MTC bridges the membranes by binding to Rab GTPases and to SNAREs. Additionally, MTC can bind to membranes providing stability to the bridges (Ganley, 2013). In yeast, HOPS (homotypic fusion and vacuolar protein sorting) complex is thought to mediate tethering of endosomes and is shown to bind to the SNAREs

(Starai et al., 2008). SNAREs from the vacuoles and target membranes bring the two lipid bilayers in close proximity by folding into specific four-helix-bundle complexes and mediate fusion by lipid mixing of the bilayers (Brocker et al., 2010). In mammalian SNARE complexes, STX17 and SNAP29 are identified on the autophagosomes, while VAMP7 and VAMP8 are found on lysosomes, mediating the fusion events (Itakura et al., 2012).

The Class-III PI3K complex also plays a role in membrane fusion. The Class-III PI3K Complex-II with UVRAG, activates Rab7 by interacting with VPS16 (Liang et al., 2008). In yeast, VPS16 belongs to the HOPS complex and is presumed to provide membranes and all the subunits are required for the fusion (Wartosch et al., 2015). The Rubicon complex with VPS34 binds UVRAG and negatively regulates autophagosome fusion (Tabata et al., 2010). Activated Rab7 displaces Rubicon from the HOPS complex and facilitates UVRAG binding (Sun et al., 2010). The Class-III PI3K Complex-I member ATG14L binds to the STX17 subunit of the SNARE complex via its coiled-coil domain. The stabilized STX17-SNAP29 complex undergoes homo-oligomerisation that enhances membrane fusion. The STX17 binding deficient mutant of ATG14L retained the membrane tethering ability, but reduced the membrane fusion events, suggesting that the ATG14L interaction is necessary for the fusion activity (Diao et al., 2015). In the mammalian autophagy system, the GABARAP family proteins are important in the later stages of autophagy (Weidberg et al., 2010). Recently reported data suggest that the GABARAP family proteins recruit palmitoylated PI4K-Iiα kinase to generate PI4P lipids on the autophagosome to govern autophagosome-lysosome fusion (Wang et al., 2015).

## ATG8s and the LIR motif

Structural studies of the ATG8 family proteins show that they display high conformational similarities to ubiquitin. The ATG8 proteins consist of a C-terminal ubiquitin core with four β-strands (β1, β2, β3, β4) and two α-helices (α3 and α4) (Fig.6A). Helix α3 is residing in between β2 and β3, while α4 is in between β3 and β4. In addition to the ubiquitin core, each ATG8 protein contains a unique N-terminal domain consisting of two α-helices (α1 and α2) conserved amongst all the six members of the ATG8 family proteins (Noda et al., 2009; Paz et al., 2000; Sugawara et al., 2004). ATG8 bound to the lipid PE on the phagophore recruits additional components of the autophagy machinery via its N-terminal domain, including membrane tethering and hemifusion factors during expansion of the phagophore (Nakatogawa et al., 2007). All the mammalian ATG8 homologues have an exposed β-strand which makes

two hydrophobic pockets. One deep hydrophobic pocket (HP1) is formed between α2 and β2 while the other hydrophobic pocket (HP2) is formed between the central α helix (α3) and β2 of the ubiquitin core (Fig.6B). These two hydrophobic pockets specifically bind to a short linear motif known as the LIR (LC3 Interacting Region) motif, first identified in the structure of the autophagy substrate protein p62/Sequestosome-1 (SQSTM1) bound to LC3B (Fig.6B, C). The core LIR motif consists of four residues, first an aromatic amino acid (W/F/Y) and a fourth hydrophobic amino acid (L/I/V) that are separated by any two amino acids (Birgisdottir et al., 2013; Noda et al., 2010; Pankiv et al., 2007; Rogov et al., 2014). The core LIR motif is also referred to as AIM (Autophagy Interacting Motif) or as LRS (LC3 Recognition Sequence) (Ichimura et al., 2008). The side chain of the aromatic amino acid (W/F/Y) binds deep into the HP1 pocket site, while the hydrophobic residue at position four interacts with the HP2 site. If serine and/or threonine (S/T) residues are preceding the core LIR motif, it may indicate that the LIR mediated interaction is regulated by phosphorylation. The LIR motif in p62 is 'DDDWTHL' where the core LIR motif is preceded by three consecutive acidic residues that strengthen the interaction. The conserved residues of K51, F52, and L53 in the hydrophobic pockets of LC3B are essential for autophagosome formation. Specifically the F52 residue is essential for the LIR motif interaction of p62 (Amar et al., 2006; Shvets et al., 2008). These sites on Atg8 proteins that mediate binding of the substrate LIR motif are known as LDS (LIR docking Sites).

There are numerous examples of proteins that contain LIR motifs interacting with the ATG8 family members. Comparison of LIR motifs has revealed that the ones who have a 'W' residue (W-type LIR) at the first position have higher affinity towards LC3 family proteins while F-type LIRs (with F residue at the first position) seem to have affinity towards the GABARAP family *in vitro* (Alemu et al., 2012; Birgisdottir et al., 2013). In addition to the classical LIR motif, certain proteins harbour an atypical LIR motif which specifically interacts with LC3C. The atypical LIR motif is also known as C-LIR and is found in CALCOCO2/NDP52 (Calcium binding and coiled-coil domain 2/Nuclear Dot protein-52) and TAX1BP1 (Tax1 (human T-cell leukaemia virus type 1) binding protein). The C-LIR identified in NDP52 is L134-V135-V136. Hence, it does not have an aromatic residue in the first position like in a canonical LIR motif, instead it has I133. Since, I133 has a smaller side chain it is not accommodated in the HP1 of the ATG8s. The LIR-LDS interaction of the NDP52 is mediated by the three hydrophobic residues L-V-V, making a flat extensive hydrophobic surface. Even though all of the six ATG8 homologues display similar hydrophobic pockets, the residues that constitute the HP1 and HP2 sites in LC3C are different. LC3C has bulkier side chain residues

in HP2 where V58 and L63 in LC3B are replaced by L64 and F69. These modifications lead to a reduction in the pocket size which is suitable for the flat hydrophobic patch of NDP52. Other differences observed are Q26 and H27 of LC3B, that are replaced by K32 and F33 in LC3C. These residues interact with the V135 of NDP52, giving specificity and strengthen the interaction (Newman et al., 2012; Tumbarello et al., 2015; von Muhlinen et al., 2012).

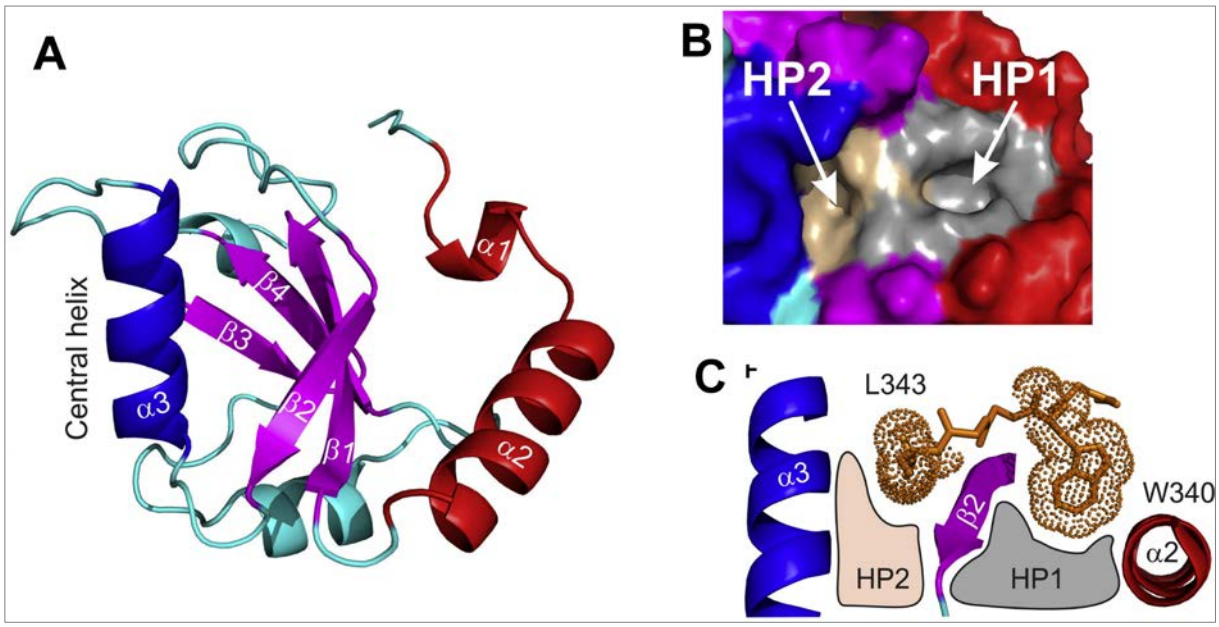



**Fig.6 The structure of LC3B and its interaction with the p62 LIR motif. (A)** The crystal structure of LC3B. The ubiquitin like core consists of four β sheets and two α helices. In addition to the ubiquitin like core, LC3B has two N-terminal α helices, α-1 and α-2. Crystal structure of LC3, PDB ID - 1UGM (Sugawara K. et. al. 2004). **(B)** The hydrophobic pockets of LC3B, HP1 and HP2, are formed by exposed β sheets. HP1 is formed between α-2 and β-2 while that of HP2 is formed between β-2 and α-3. HP1 is shown in light grey and HP2 is shown in light brown in both (B) and (C). **(C)** A schematic picture of the interaction between the hydrophobic pockets of LC3B and the LIR motif of p62. The hydrophobic pockets are shown in ribbon diagrams and the LIR motif of p62 is shown in orange sticks. W340 of the LIR motif interacts with HP1 and L343 with HP2. Crystal structure of the p62-LIR: LC3B complex PDB ID - 2ZJD (Ichimura Y. et. al. 2008.). Figure is adapted from Rogov V. et. al., 2014 with permission:Molecular Cell.

## Selective autophagy

The ATG8 family proteins in mammals are shown to play dual roles in autophagy; membrane fusion and cargo recruitment to the autophagosome. Whether different members of the ATG8 subfamily act on different autophagosomes or whether they collaborate on the same autophagosome is not yet understood. Different types of autophagy are described depending upon which substrate that is sequestered such as; mitophagy (degradation of mitochondria), pexophagy (degradation of peroxisomes), ER-phagy (turnover of endoplasmic reticulum),

aggrephagy (selective protein aggregates degradation), nucleophagy (selective process removing parts of the nucleus), ribophagy (removal of ribosomes), lipophagy (degradation of lipid droplets), glycophagy (delivers glycogen to the lysosome, ferritinophagy (selective turnover of ferritin) and xenophagy (removal of intracellular pathogens/bacteria) (Rogov et al., 2014). Here, mechanism of mitophagy will be discussed in later section.

**Sequestosome Like Receptors (SLRs)**

ATG8 family proteins on the phagophore membrane interact with the substrate directed to degradation via a LDS-LIR mediated interaction. The concept of selective autophagy originated by the characterisation of the ubiquitin (Ub) binding protein p62/SQSTM1 (Sequestosome-1, hereafter p62), which was shown to interact with LC3B and deliver specific substrates into the forming autophagosome (Bjorkoy et al., 2005; Pankiv et al., 2007). The domain architecture of p62 displays an N-terminal self-interacting PB1 domain, followed by ZZ-type zinc-finger domain, a LIR motif and a C-terminal ubiquitin-binding domain (McLelland et al.) (Pankiv et al., 2007). The UBA domain of p62/SQSTM-1 interacts with ubiquitinated cargo, p62 undergoes polymerization via the PB1 domain to form large aggregates, and connects the cargo to the autophagosome through a LIR mediated interaction. In this way, p62 acts as a receptor selecting specific cargo for lysosomal degradation. Furthermore, p62 itself is degraded in the process. In autophagy deficient cells p62 accumulates in ubiquitinated aggregates. The UBA domain of p62 has affinity towards Lys63-polyubiquitin chains, enhanced by UBA phosphorylation at S403 (Pilli et al., 2012). Accumulation of p62 in ubiquitinated aggregates is linked to pathological conditions like neurodegenerative diseases, Lewy bodies in dementia and similar other proteinopathies (Bjorkoy et al., 2005; Komatsu et al., 2007; Kuusisto et al., 2001; Wooten et al., 2008). The turnover of p62 is dependent on its LIR motif and of the autophagy process.

Further discoveries in the field of selective autophagy revealed other receptors following similar mechanisms as p62. Since their cargo recognition mechanism via LIR interactions mimic p62, these are classified as Sequestosome-1 like receptors (SLRs) (Fig.7). Until now four additional SLRs have been identified; NBR1 (neighbor of BRCA1 gene 1), NDP52/CALCOCO2, OPTN/Optineurin and TAX1BP1. These receptors have common mechanistic properties and can be considered as functional homologues. SLRs recognise ubiquitinated substrates, undergo polymerization, interact directly with ATG8 homologues via a LIR-LDS interaction, degrade the substrate in lysosomal compartments and get degraded

themselves with the substrate (Kirkin et al., 2009a; Newman et al., 2012; Thurston et al., 2009; Wild et al., 2011).

NBR1 was described as a receptor for selective autophagy soon after p62. It has similar domain architecture and polymerising ability as p62. NBR1 interacts with p62 via its PB1 domain and makes oligomers via its CC1 domain. Degradation of NBR1 is mediated via the LIR motif and independent of p62 (Kirkin et al., 2009a; Kirkin et al., 2009b). NBR1 has been identified as a major autophagy receptor for peroxisomes (Deosaran et al., 2013), indicating that SLRs play a role in targeting individual organelles for degradation. NBR1 has specificity towards peroxisomes via its amphipathic α-helical J-domain and UBA domain. NBR1 undergoes homo-oligomerization via the CC1 domain and mediates pexophagy in a LIR dependent manner. Presence of p62 increases the efficiency of pexophagy, suggesting that SLRs can cooperate. In plants, NBR1 is essential during stress conditions where it acts as a SLR (Deosaran et al., 2013; Svenning et al., 2011; Zhou et al., 2013).

NDP52 and TAX1BP1 are similar in their domain architecture, possessing a coiled-coil region, a C-LIR motif and a C-terminal UBZ (Ubiquitin binding zinc finger) domain. NDP52 recognises the heavily ubiquitinated intracellular pathogen *Salmonella* through its UBZ, binds directly to LC3C via the C-LIR and directs bacteria for degradation by autophagy (Newman et al., 2012; Thurston et al., 2009; von Muhlinen et al., 2012). NDP52 is reported to play a role in signalling by promoting aggregation of TRAF6 when the Ub-editing enzyme A20 is absent. NDP52 is also reported to mediate degradation of DICER, AGO2, signalling adaptor TRIF and MyD88 (Gibbings et al., 2012; Inomata et al., 2012). The functional role of TAX1BP1 is proposed in mitophagy (Lazarou et al., 2015). The glaucoma and amyotrophic lateral sclerosis linked protein Optineurin, contains several coiled-coil domains mediating oligomerization, a functional LIR motif and a C-terminal UBA as well as UBZ domain. Optineurin binds to ubiquitinated misfolded proteins and mediates aggrephagy. Optineurin collaborates with p62/NDP52 in degradation of *Salmonella enterica* serotype *Typhimurium*. Another study demonstrated that Optineurin binds to ubiquitinated Parkin and mediates efficient degradation of mitochondria where the LIR motif tethers the mitochondria to the autophagosome (Cemma et al., 2011; Mostowy et al., 2011; Wild et al., 2011; Wong and Holzbaur, 2014). NDP52 and Optineurin have recently been reported to play a role in the selective degradation of mitochondria where they are recruited to the mitochondria by the internal mitochondrial protein PINK1 (PTEN-induced kinase 1). Furthermore, these SLRs recruit the autophagy initiation machinery and the ATG8s for degradation of mitochondria (Lazarou et al., 2015).

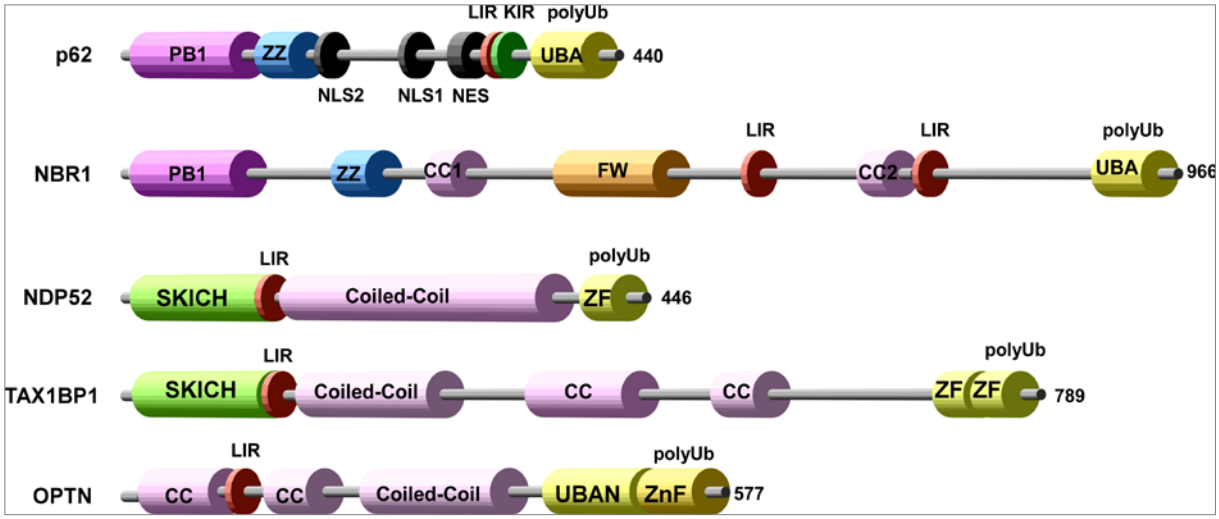



**Fig.7 The domain architecture of Sequestosome like receptors (SLRs).** The domain description shown is: PB1- Phox and Bem1 domain; ZZ, ZZ-type zink finger domain; CC, coiled-coil domain; NLS1 and NLS2, nuclear localization signals 1 and 2; NES, nuclear export signal; LIR, LC3-interacting region; KIR, Keap interacting region; UBA, ubiquitin-associated domain; UBAN, ubiquitin binding in ABIN and NEMO (NF-κB Essential Modulator) domain. The figure is adapted from Birgisdottir A. B. et. al. 2013.

**Membrane receptors**

Besides the SLRs, other proteins are found to mediate specific organelle degradation. Interestingly, also these involve interactions with the ATG8s. Initial screening in yeast, identified Atg32 as a specific mitochondrial protein mediating mitophagy. Atg32 displays LIR dependent interaction with Atg8 and LIR independent interaction with Atg11. The suggested mechanism for Atg32 in mitophagy is that it recruits Atg8 and Atg11 to the mitochondria for a PAS-like assembly to recruit the isolation membrane and surround the mitochondria (Kanki et al., 2009; Okamoto et al., 2009). The mammalian mitophagy system is more complicated. Recently, the mammalian homologue of Atg32 was reported to be Bcl-2-L13 (Bcl-2 like 13). Bcl-2-L13 mediates partial mitochondrial clearance via a LIR dependent LC3B interaction (Murakawa et al., 2015). In addition, there are other outer mitochondrial membrane proteins reported to initiate partial mitophagy in a similar manner. These are BNIP3L/NIX (Bcl-2/adenovirus E1B 19kDa interacting protein 3-like), BNIP3 and FUNDC1 (FUN 14 domain containing protein 1) (Hanna et al., 2012; Liu et al., 2012; Novak et al., 2010). The mechanisms of these receptors are briefly discussed below in the mitophagy section. Other examples of membrane receptors directing degradation of specific intracellular membranous organelles are Atg39 and Atg40 in yeast. Both have a LIR motif dependent interaction with Atg8, and direct ER membranes for degradation. In mammals, the FAM134B (family with sequence similarity 134, member B) protein shows similarity to Atg40 and serves as an ER-phagy receptor

(Khaminets et al., 2015; Mochida et al., 2015). Furthermore, yeast Atg30 via interaction with Atg11 directs pexophagy (Burnett et al., 2015; Farre et al., 2013). In general, membrane receptors that bind to ATG8 proteins directly, may recruit the phagophore membrane and surround the organelle to ensure engulfment by autophagy. The membrane receptor proteins themselves may or may not be autophagy substrates, but these criteria are largely unclear and need further study.

## LIR motifs in autophagy mediator proteins

The first unravelled autophagy gene, the serine/threonine kinase, Atg1 is reported to interact with Atg8 via its LIR motif. Disruption of the core LIR motif in Atg1 results in impaired transport of the autophagosome into the vacuole/lysosome, but the autophagy initiation remains unaffected (Kraft et al., 2012; Nakatogawa et al., 2012). The mammalian homologues of Atg1, ULK1 and ULK2, have been identified to contain LIR motifs essential for targeting ULK1 on the autophagosome. Another member of the same complex, Atg13, co-operates with Atg8 to regulate the function of Atg1 during autophagosome maturation and/or fusion with the lysosome. The mammalian homologue member of this complex, ATG13 and FIP200 have core LIR motifs with preference towards GABARAP family proteins. Since GABARAP family proteins are reported to play a role in later stages of starvation induced autophagy, possibly ATG13 and FIP200 use their LIR motif to anchor them during autophagosome maturation or fusion (Alemu et al., 2012; Suzuki et al., 2014). In the conjugation machinery for autophagosome biogenesis, Atg3 receives Atg8 from Atg7. Atg3 interacts with Atg7 via its flexible E2 core region and handle region (HR), seemingly both regions are needed for interaction with Atg8 (Yamada et al., 2007). Detailed NMR structural studies identified a LIR motif in the HR of Atg3. The interaction mode highly resembles the Atg19-Atg8 and p62-LC3 interactions. Further *in vitro* studies demonstrated that the Atg3-Atg8 interaction is necessary for transfer of Atg8 from the Atg3-Atg8 thioester intermediate to the PE (Noda et al., 2008; Sakoh-Nakatogawa et al., 2015; Yamaguchi et al., 2010). The crystal structure studies of the mammalian cysteine protease ATG4B-LC3B complex indicate that side chains of Y8 and L11 in ATG4B interact with the hydrophobic pockets in LC3B. Binding of LC3B leads to conformational changes in ATG4B, which gives access of LC3B to the catalytic site. These interactions with LC3B stabilize N-terminal conformational changes in ATG4B, allowing access to the membranes for later steps in autophagy (Satoo et al., 2009).

TBC1D25 (Tre2, Bub2, Cdc16 (TBC) 1 domain containing family member 25) is a LIR motif containing protein recruited on the isolation membrane via ATG8 family interactions. TBC1D25 isinvolved in autophagosome lysosome fusion and inhibits Rab33B activity, allowing Atg16L to be on the autophagosome (Itoh et al., 2011). Another member of the Rab-GAP family proteins, TBC1D5 (TBC1 domain family member 5), interacts with VPS29 which is a subunit of the retromer complex mediating retrograde traffic from endosomes to Golgi for cargo recycling. TBC1D5 has two LIR motifs. The N-terminal LIR is important for interaction with the retromer complex and transport of cargo. Upon starvation, TBC1D5 is re-localised to LC3 indicating a switch from the endosome. The C-terminal LIR is used to bridge the endosome and autophagosome, affecting autophagosome maturation (Popovic et al., 2012; Seaman et al., 2009). Yet another member of the Rab-GAP family proteins, TBC1D15 (TBC1 domain family member 15) contains a LIR motif and is a mitochondrial Rab-GAP protein associated with mitochondria via binding with FIS1 (Fission mitochondrial 1), and with the isolation membrane through the LIR motif. TBC1D15 makes homodimers and heterodimers with TBC1D17, that inhibit Rab7 activity and mediate proper encapsulation of mitochondria in autophagosomes, working downstream of Parkin activation (Yamano et al., 2014).

## Mitochondria dynamics

### Mitochondria

The initial observation of mitochondria in the cell was described by Richard Altmann back in the 19$^{th}$ century defining them as ‘Bioblast’ present nearly in all cell types. Later on, Carl Benda coined the term ‘mitochondria’ from the Greek word ‘mitos’ (thread) and ‘chondrion’ (granules) because of their appearance in the cell after osmium staining. According to the endosymbiosis theory mitochondria are believed to originate from bacteria, and hypothesized to be adapted into the eukaryotic cell. (Ernster and Schatz, 1981; Taylor, 1979; Wallin, 1926).

Mitochondria serve as powerhouses of the cell, generating energy through series of oxidative phosphorylations. Mitochondria are involved in many other cellular functions like signalling, cellular differentiation and cell death. They are composed of an outer membrane and an inner membrane, organised to make compartment like structures known as intermembrane space (space between outer and inner membrane), cristae (infolding of inner membrane) and mitochondrial matrix (space within the inner membrane). The outer membrane of mitochondria

is made up of a lipid bilayer containing integral membrane proteins known as porins. The porins on the outer membrane form channels that allow transfer of larger proteins from one side of the membrane to the other. The inner membrane of the mitochondria is made up of phospholipids, and displays high protein to phospholipid ratio. The inner membrane proteins are components of the electron transport chain and redox reaction, the ATP synthase complex and the mitochondrial fusion-fission machinery. The cristae structures provide increased space and surface area to enhance the ability of energy production. The mitochondrial matrix consists of numerous enzymes, mitochondrial ribosomes, tRNA and the mitochondrial DNA genome. Pyruvate, the product of glucose breakdown in the cytosol via glycolysis, is transported into the mitochondrial membrane space for energy production. Mitochondria can also import fatty acids for energy production purpose. Pyruvate and fatty acid are converted into Acetyl-CoA catalysed by enzymes in the matrix. Acetyl-CoA is the starting material for the citric acid cycle, oxidizing Acetyl-CoA to $CO_2$ and releasing energy in form of ATP. The conversion of Acetyl-CoA to $CO_2$ leads to the generation of two molecules NADH and $FADH_2$ by reducing $NAD^+$ and $FAD^{++}$, respectively. The electron rich molecules NADH and FADH undergo oxidation, transferring electron to molecular oxygen ($O_2$) via the electron transport chain, reducing it to water molecules. Transfer is carried out by the protein complexes known as NADH dehydrogenase, cytochrome-c reductase, succinate dehydrogenate and cytochrome-c oxidase. The resulting energy is used to pump protons into the intermembrane space. The transfer of protons across the membrane generates a chemiosmosis gradient flow that is used by ATP synthase to generate ATP from ADP (Ernster and Schatz, 1981; Huang and Manton, 2004; King et al., 2006).

During the constant process of electron transfer, a small percentage of the electrons prematurely reduce oxygen molecules generating highly reactive molecules called reactive oxygen species (ROS). The reduction of oxygen molecules produces superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical (-OH). These by-products from oxidative phosphorylation damage the mitochondrial DNA, lipids and proteins giving rise to oxidative stress within mitochondria that eventually damage the organelle. Damaged mitochondria further release $Ca^{2+}$ and cytochrome C into the cytosol, which in turn induce apoptosis (Parsons and Green, 2010; Wallace, 2005).

Mitochondria are highly dynamic organelles that continuously undergo fusion and fission processes to maintain their homeostasis. The fission process generates several small daughter mitochondria from tubular mother mitochondria. The fusion process reconnects healthy mitochondria by mixing their contents and helps the cell to overcome metabolic or

environmental stress (Liesa et al., 2009). During biogenesis, mitochondria replicate through generating two healthy daughter organelles from one healthy parent organelle. Recently, this process has been termed as 'symmetrical fission' (Shirihai et al., 2015). The new daughter mitochondria grow by adding new components generated through mitochondrial biogenesis via mitochondrial genes. These daughter mitochondria further fuse with other healthy mitochondria. Upon cellular/mitochondrial stress like the above mentioned ROS or other damage to mitochondria, parent organelles produce smaller daughter mitochondria which are functionally dissimilar. Functionally dissimilar mitochondria are defined as the mixture of those who have depolarized membrane potential and equal or higher membrane potential compared with healthy mitochondria. This process is termed 'asymmetrical fission' (Shirihai et al., 2015). After asymmetrical fission, the daughter mitochondrion with equal membrane potential fuses back to the healthy mitochondrion. The other daughter mitochondrion with reduced/depolarised membrane potential is either repaired, or in case of excessive damage, is removed. Such reduced/depolarised daughter mitochondria contain dysfunctional mitochondrial components including oxidized or old proteins, making them unable to recover the membrane potential. Such mitochondria become autophagy substrates and are degraded. Thus, asymmetrical fission becomes the essential event prior to removal of damaged mitochondria (Twig et al., 2008).

A perfect balance between the fusion and fission processes is necessary for preserving the integrity of mitochondrial DNA, maintenance of the electrochemical gradient during oxidative phosphorylation, for appropriate cellular response to apoptotic stimuli, and also for cell division to properly partition the mitochondria into daughter cells (Mishra and Chan, 2014). In mammals, mitochondrial fusion and fission is mediated by members of the dynamin related GTPase family proteins.

**Mitochondrial Fission Machinery**

The majority of mitochondrial fission in mammals is controlled by the DRP1 protein (Dynamin related protein 1). It is a ubiquitously expressed cytosolic protein containing a GTPase domain at its N-terminus, followed by a middle domain and a C-terminal GED (GTPase effector domain). The cytosolic DRP1 translocate to the mitochondrial outer membrane to promote fission. Here, the GED domain interacts with the middle domain of another DRP1 molecule to assemble into multimeric ring-like structures, and to divide the mitochondria in a GTP dependent manner (Smirnova et al., 2001; Smirnova et al., 1998). DRP1 as such does not have any transmembrane domain, so to translocate to the mitochondria, it needs to interact with other OMM (outer mitochondrial membrane) proteins. Initially, it was

thought to interact with FIS-1 (Fission-1) (Yoon et al., 2003), but in mammals FIS-1 inhibition did not reduce the translocation of DRP1. Instead, it depends on another interaction partner on OMM known as Mff (Mitochondrial fission factor) (James et al., 2003; Otera et al., 2010). In addition to Mff, other components on OMM recruiting DRP1 are identified, known as MiD49 and MiD51 (mitochondrial dynamics protein of 49 and 51 kDa respectively) (Fig.8). Knock down of MiD49/51 leads to reduced DRP1 translocation to mitochondria. On the other hand, overexpression of MiD49/51 sequestered the DRP1, prevented it from functioning at mitochondria and resulted in fused tubules associated with actin (Palmer et al., 2011; Zhao et al., 2011). Mitochondria and ER exhibit extensive contacts where ER tubules play crucial roles in defining the sites for constriction. It has been observed that DRP1 is recruited to sites where ER tubules contact mitochondria and constrict the membrane (Friedman et al., 2011). Even though DRP1 is a major fission component, DRP1 deficient embryonic fibroblasts display fragmentation of mitochondria during mitosis, indicating that DRP1 independent mechanisms exists. Another component involved in mitochondrial fission is TBC1D15 that interacts with the TPR (tetratricopeptide repeats) domain of FIS-1 on OMM. Furthermore, FIS-1 and TBC1D15 are involved in mitophagy. (Onoue et al., 2013; Otera et al., 2010). Since mitochondria are able to divide during mitosis independent of DRP1, it could be possible that mitochondrial fission events are controlled differently during different cellular stages and stresses.

**Mitochondrial Fusion Components**

In a similar way to fission, mitochondrial fusion also requires certain GTPase family proteins, named Mfn1 and Mfn2 (mitofusin 1 and 2). Mfn1 and Mfn2 contain an N-terminal GTPase domain, two hydrophobic heptad repeats coiled-coil domains (HR1 and HR2) and a transmembrane domain. Mfn1/2 are OMM proteins with the N-terminal GTPase domain and heptad repeats facing towards cytosol. The heptad repeats of Mfn1 and Mfn2 on daughter mitochondria form intermolecular antiparallel coiled coil structures that tether the adjacent mitochondria (Fig.8). These intermolecular interactions between mitofusins can be homotypic (Mfn1-Mfn1 or Mfn2-Mfn2) or heterotypic (Mfn1-Mfn2), where the heterotypic interactions are shown to be more efficient (Hoppins et al., 2011; Koshiba et al., 2004; Santel and Fuller, 2001). The coiled coil structure formation pulls the membranes close together, further initiating lipid bilayer mixing. The GTPase activity is thought to provide energy for biomechanical outer membrane fusion (Fritz et al., 2001; Rojo et al., 2002). Once the outer membranes are fused together, IMMs (Inner mitochondrial membranes) need to fuse to preserve oxidative

phosphorylation. IMM fusion is mediated by another mitochondrial dynamin-like GTPase, OPA1 (optic atrophy protein 1) anchored on IMM and facing towards the intermembrane space (Olichon et al., 2002). Loss of OPA1 results in heterogeneous mitochondria with impaired cellular respiration, indicating that IMMs were unable to fuse. Still, in absence of OPA1, OMM can fuse through Mfn1 and Mfn2, indicating that these two fusion events are independent of each other (Olichon et al., 2003). In addition to the fusion of IMM, OPA1 is essential in maintaining proper crista morphology and functioning of the electron transport chain (Frezza et al., 2006).

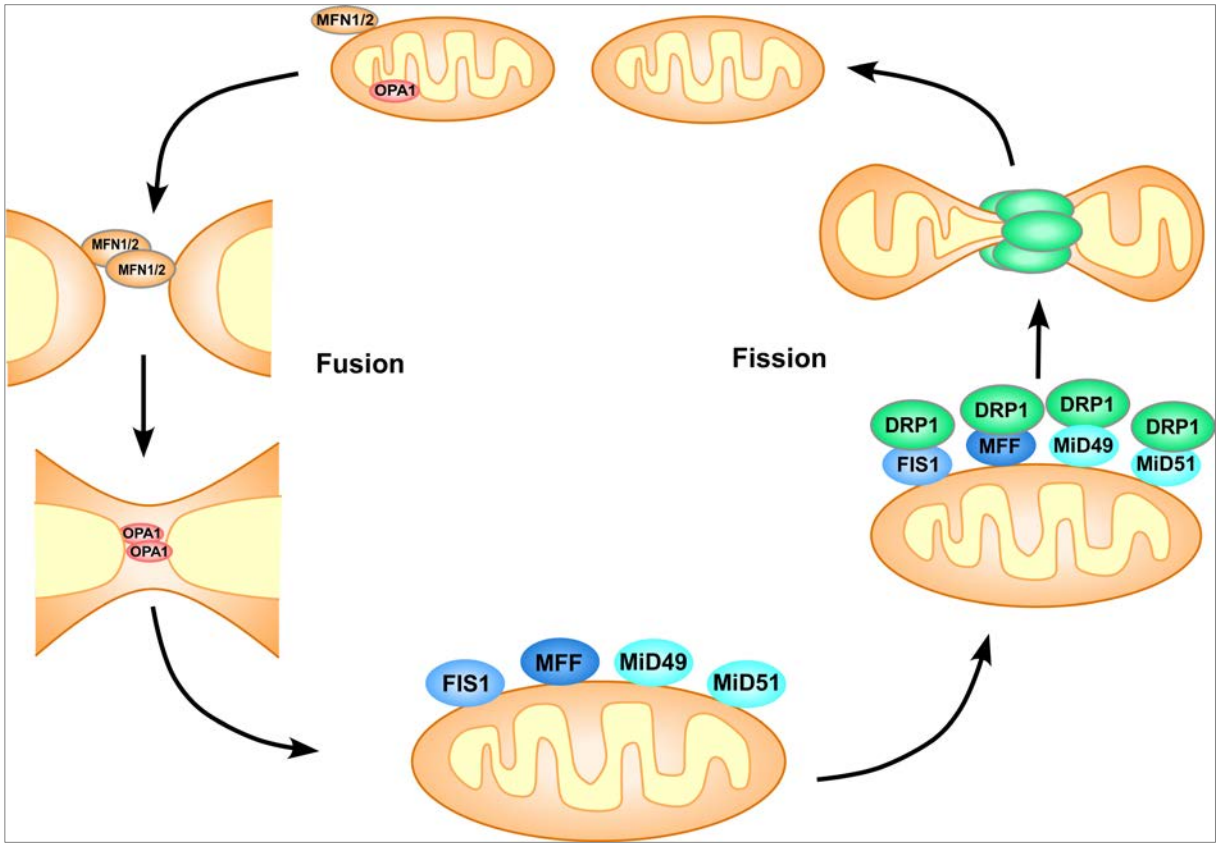



**Fig.8 Mitochondrial Fisson and Fusion**. During fission, the GTPase protein DRP1 translocates from the cytosol to the OMM where it interacts with FIS1, MFF, and MiD49/MiD51. DRP1 molecules assemble on to the mitochondria in a ring-like structure around the mitochondria, resulting in constriction that eventually divides the mitochondria. Fusion of mitochondria is a two-step process, First, OMM fusion is mediated by the mitofusin proteins MFN1 and MFN2 that undergoe homotypic and heterotypic dimerization. Then IMM fusion occurs, where OPA1 is the IMM GTPase responsible for the fusion. The figure is adapted and modified from Mishra P., and Chan D. 2014 with permission.

**Mitochondrial protein degradation**

Mitochondria overcome the ROS generated damage of proteins by exerting their own proteolytic system. Two AAA (ATPase associated with number of cellular activities) protease

complexes, constitute the first line of defence. The two AAA proteases degrade misfolded proteins within the inner membrane space, and hence play a crucial role in mitochondrial biogenesis (Langer et al., 2001; Leonhard et al., 1996). The second line of defence in mitochondrial quality control is the ubiquitin/proteasome degradation system, involving the mitochondrial integral membrane protein PINK1 and the E3 ubiquitin ligase Parkin. Some of the OMM proteins, like the mitofusins, are degraded by the ubiquitin/proteasome degradation pathway, inhibiting mitochondrial fusion and promoting fission (Karbowski and Youle, 2011). An emerging pathway known as the MDV (mitochondria derived vesicles) pathway is highlighted in mitochondrial quality control, where vesicles budding from mitochondria sequester the mitochondrial proteins and transport them to the lysosome for degradation (Sugiura et al., 2014). In the initial stages of ROS generation or damage to mitochondria, MDVs are reported to be part of the second line of defence. However, under extreme stress, the entire dysfunctional mitochondria must be removed (McLelland et al., 2014). The first and second line defence pathways are active mainly to degrade damaged mitochondrial proteins, while bulk degradation of the entire mitochondria is mediated via mitophagy.

## Mitophagy

Depending upon the mechanisms that activate mitophagy and the proteins involved, mitophagy can roughly be categorised into three pathways; namely membrane receptor mediated mitophagy (Fig.9B), PINK1-Parkin dependent mitophagy (Fig.9A), and mitophagy by alternative autophagy, independent of ATG5-ATG7.

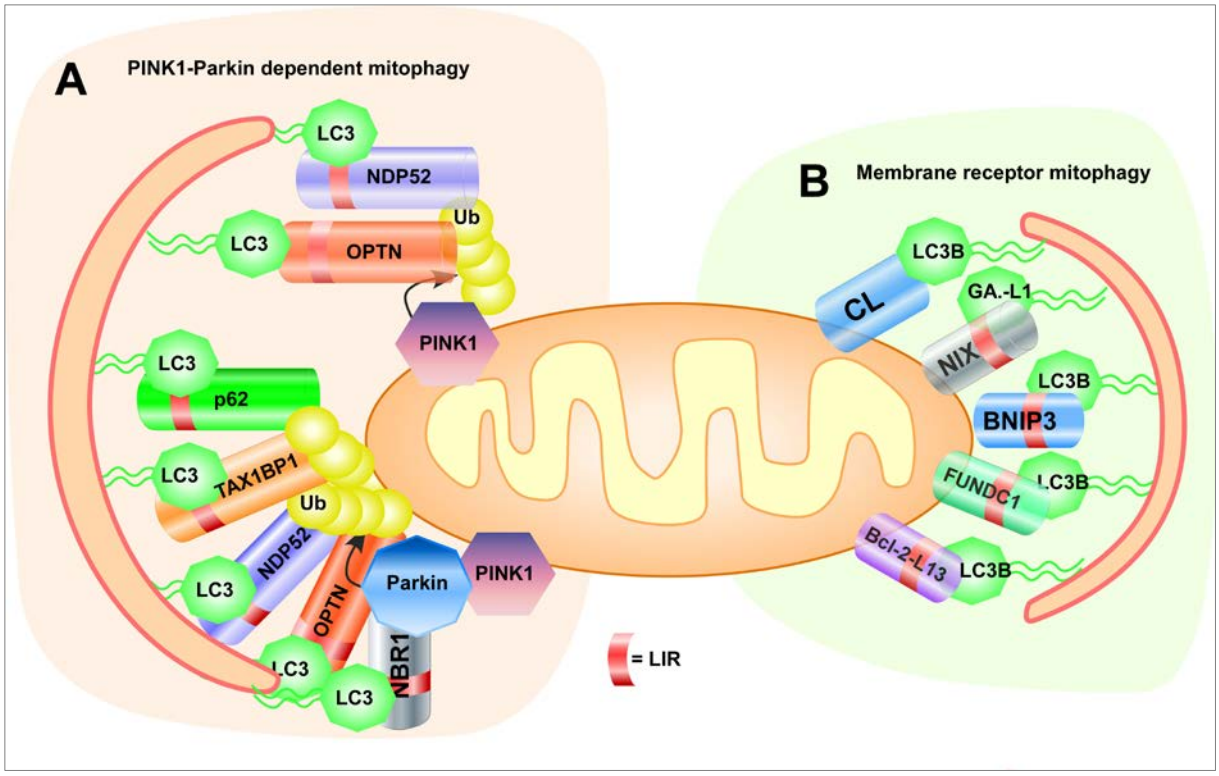



**Fig.9 Two major pathways of Mitophagy**. **(A) PINK1-Parkin dependent mitophagy**. Here, PINK1 is translocated and stabilized on the OMM. Stabilized PINK1 on the outer membrane, phosphorylates cytosolic Parkin at S65, makes a homodimer, and undergoes auto-phosphorylation at S228 and S402 residue resulting in a highly active kinase promoting Parkin translocation to mitochondria. Parkin translocation further hyper-ubiquitinate mitochondria generating different ubiquitin chains recognized by the SLRs, eventually targeting the mitochondria for degradation on the growing phagophore via LIR binding. PINK1 on the OMM phosphorylate ubiquitin at S65, generating structurally unique phosphor-ubiquitin, which further specifically recruits OPTN and NDP52 on mitochondria. NDP52 and OPTN then recruit the autophagy initiation machinery and dock mitochondria on the phagophore through LIR binding. **(B) Membrane receptor mediated mitophagy**, Receptors in the OMM (NIX, BNIP3, Bcl-2-L13 and FUNDC1) interact directly with the ATG8 family. Each of the receptors prefers a specific ATG8 homologue, recruiting it to the OMM thereby directly tethering the mitochondria on the phagophore for degradation. Cardiolipins (CL) are unique in their activation mechanism, residing in IMM, externalized under specific stress, further interact with LC3B via N-terminal basic patches thereby targeting mitochondria for degradation.

## Membrane receptor mediated mitophagy

As described in the membrane receptor section, Atg32 in yeast is an OMM protein inducing mitophagy via Atg8. Atg32 possesses uniqueness of a dual interaction with Atg8 and Atg11 that is not found in mammalian mitophagy yet. In mammalian mitophagy, some of the OMM proteins display interaction with ATG8 family proteins via a LIR motif interaction.

**NIX/BNIP3L**

NIX was identified as a dimeric pro-apoptotic mitochondrial protein that interacted with Bcl-2 and adenovirus E1B 19kDa (Chen et al., 1997). NIX contains a Bcl-2 homology domain 3, hence classified as BH3 only protein, inserted into the outer mitochondrial membrane by its C-terminal transmembrane domain. The N-terminus is exposed to cytosol (Matsushima et al., 1998). Apart from the pro-apoptotic activity, NIX was later identified as crucial for erythroid maturation, activating mitophagy independent of BAX and BAK (Aerbajinai et al., 2003; Schweers et al., 2007). Elimination of mitochondria is an essential step for red blood cell maturation where NIX is highly induced. NIX knock out mice develop anaemia due to retention of mitochondria and reduced life span *in vivo* (Sandoval et al., 2008). Furthermore, NIX was implicated in an interaction with GABARAP, mediating possible crosstalk between autophagy and apoptosis (Schwarten et al., 2009). Until 2009, the mechanism of specific mitochondria removal by NIX was not fully understood. Novak et al., 2010, identified a highly conserved LIR motif in NIX ['WxxL'] in the N-terminus exposed towards cytosol. The NIX LIR motif interacts with ATG8 family proteins and recruits specifically GABARAP-L1 during mitochondrial depolarisation, mediating partial clearance of mitochondria (Novak et al., 2010). NIX deficient MEFs are more resistant to mitochondria uncouplers like CCCP (Carbonyl cyanide m-chlorophenyl hydrazone) where NIX promotes the CCCP induced mitochondrial depolarization. In addition, NIX is required for mobilization of the autophagy machinery during mitophagy (Ding et al., 2010) and has been shown to promote hypoxia induced autophagy. NIX is upregulated during hypoxic conditions where HIF-1 (hypoxia inducible factor 1) binding on the NIX promoter is enhanced, leading to its upregulation (Mazure and Pouyssegur, 2009). Upregulated NIX displaces Beclin-1 from Bcl-2 and Bcl-XL complexes, promoting autophagy induction (Bellot et al., 2009). In the Parkin dependent mitophagy pathway, NIX is required under CCCP induced Parkin recruitment to mitochondria, a process termed mitochondrial priming (Ding et al., 2010).

**BNIP3**

BNIP3 is a homologous protein to NIX/BNIP3L, BH3 only protein, sharing 53-56 % amino acid identity with NIX. BNIP3 contains a C-terminal transmembrane domain, inserted into the OMM exposing the N-terminus towards cytoplasm (Chen et al., 1997). In a similar manner as NIX, BNIP3 is a pro-apoptotic protein, heterodimerizes with Bcl-2/Bcl-XL independent of its BH3 domain and induces cell death (Ray et al., 2000). BNIP3 has been

proposed to induce a necrosis like-cell death by opening the mitochondrial permeability transition (MPT) pore (Vande Velde et al., 2000). Further experiments demonstrated that BNIP3 induces autophagy under hypoxic conditions (Hamacher-Brady et al., 2007; Tracy et al., 2007). In cardiac myocytes, overexpression of BNIP3 leads to mitochondrial fragmentation, translocation of DRP1 to mitochondria, and assisted by Parkin translocation, induces mitophagy (Lee et al., 2011). This mechanism of mitophagy mediated by BNIP3 involves a LIR motif localised in the N-terminus, interacting directly with LC3B in a LIR-dependent manner to mediate mitochondrial degradation. BNIP3, if compartmentalised at ER, promotes ER-phagy dependent on the LIR motif, indicating that its autophagy function is independent of mitochondrial targeting. Disruption of LC3B binding by mutating the LIR motif in BNIP3 impairs its autophagy function, but not its pro-apoptotic function (Hanna et al., 2012). The LIR motif in BNIP3 is identified as '18-WxxL-21', preceded immediately by a serine residue (S17) and followed by another serine at (S24). As described above, the residues flanking a LIR motif can regulate its function (Matsumoto et al., 2011). BNIP3 displays such regulation of the LIR motif by phosphorylation of these two serine residues. The S17 residue phosphorylation is essential for binding with LC3B, however strong binding with LC3B and GABARAP-L2 is observed under mimicked phosphorylation of both the S17 and the S24 residues (Zhu et al., 2013). Similar to NIX, BNIP3 is also upregulated during hypoxia where HIF-1A (HIF-1 alpha subunit) binding to its promoter is enhanced (Guo et al., 2001). In addition to hypoxia, BNIP3 is reported to be upregulated during starvation by Foxo3 (fork head box O3) transcription factor, binding to the promoter of BNIP3 and NIX in skeletal muscles (Mammucari et al., 2007). Interestingly, the expression level and transcriptional level of Foxo3 is upregulated during erythroid maturation where NIX is upregulated (Marinkovic et al., 2007).

**FUNDC1**

FUNDC1 is another integral mitochondrial membrane protein containing a N-terminal LIR motif. FUNDC1 regulates mitophagy via LIR motif interaction with LC3B. The LIR motif in FUNDC1 is identified as 13-SxxxS**Y**xx**L**-21, where the first core LIR motif residue is Tyrosine (Y), similar to the LIR motif in NBR1. The core LIR motif is regulated by phosphorylation where Y18 is phosphorylated by Src kinase and the serine residue preceding the LIR motif (S13) is phosphorylated by CK2 (Casein kinase-2). In contrast to NIX and BNIP3 LIR motifs, phosphorylation of these two residues negatively regulate the LIR mediated interaction with LC3B. Also FUNDC1 plays a role in hypoxia induced mitophagy, but must be dephosphorylated at the Y18 residue. During hypoxic conditions, Src kinase is constitutively

inactivated leading to de-phosphorylation of FUNDC1 at Y18 that increases interaction with LC3B (Chen et al., 2014; Liu et al., 2012). FUNDC1 is also regulated by Bcl-XL where Bcl-XL interacts with PGAM-5, a mitochondrial serine/threonine phosphatase, to prevent dephosphorylation of FUNDC1 at S13 (Wu et al., 2014a). ULK1 translocates to mitochondria upon hypoxia induced mitophagy or FCCP (Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone) mediated mitochondrial damage, and phosphorylates the FUNDC1 S17 residue immediately preceding to LIR motif. The phosphorylation of S17 increases FUNDC1 binding to LC3B and induces mitophagy (Wu et al., 2014b).

**Cardiolipin (CL)**

Cardiolipins (CLs) are mitochondrial phospholipids found only in mitochondria, residing in the inner membrane, supporting cristae structures and stabilizing the respiratory chain complexes (Beyer and Nuscher, 1996; Schlame, 2008). Cardiolipins are found to be implicated in autophagy and cell death (Singh et al., 2010). CL externalises on mitochondria in response to bacterial toxin treatment like rotenone or 6-OHDA (6-hydroxydopamine). The degree of externalisation of CL is mediated greater by toxins than by the mainly used mitochondrial uncouplers like FCCP/CCCP, indicating distinct activation mechanisms. Furthermore, PINK-1 is not upregulated by toxin mediated mitochondrial stress. The externalised CL then directly interacts with the LC3 family proteins to induce mitophagy. This interaction is mediated by the N-terminal basic patches that are specific for LC3B and not found on the GABARAP family proteins. Thus, this indicates specific LC3 interaction involving residues R10 and R11 of LC3B (Chu et al., 2013).

**Bcl2-L-13**

Bcl2-L13 or Bcl-rambo is the first homologue of yeast Atg32 identified in mammalian cells. Bcl2-L-13 (Bcl-2 like protein 13) is an OMM protein, anchored on mitochondria by a C-terminal transmembrane domain, preceded by four Bcl-2 homology domains (BH1-4) and a functional LIR motif identified as 273-WxxL-276. Initially, Bcl-rambo was identified as a pro-apoptotic protein. However, overexpression studies conducted by Murakawa et. al. did not lead to apoptosis in HEK293 cells (Kataoka et al., 2001; Murakawa et al., 2015). Instead, Bcl-2-L13 overexpression induced mitochondrial fragmentation independent of the DRP1 fission mechanism, and induced mitophagy that was dependent on its LIR motif interaction with LC3B, even in Parkin deficient cells. The BH1-4 domains of Bcl-2-L13 are essential for mitochondrial

fragmentation, the pre-requisite step for mitophagy. Furthermore, the LIR motif is preceded by a Serine residue (S272), where phosphorylation enhances LC3B binding and mitophagy (Murakawa et al., 2015). However, the mitochondrial fragmentation mechanism independent of DRP1 is not fully understood and the kinase phosphorylating the S272 residue is not known yet. Even though Bcl-2-L13 is an Atg32 homologue, the characteristic interaction with Atg11 or mammalian Atg11 homolog remains to be identified.

In addition to these above mentioned receptors, a new member joins the mitophagy receptor family, the PHB2 (Prohibitin-2) protein recently identified by Beth Levin and colleagues. PHB2 is an IMM (Inner Mitochondrial membrane) protein. It harbors a LIR motif, and works in the degradation process of paternal mitochondria first identified in *C. elegance*. According to the report, PHB2 becomes recognizable after Parkin mediated proteasomal degradation of the mitochondrial outer membrane. Thereafter it follows the canonical LIR binding pathway and sequesters mitochondria for lysosomal degradation (Wei et al., 2017).

## PINK1-Parkin dependent mitophagy

Mammalian mitophagy is promoted by the E3 ubiquitin ligase Parkin, first reported by Richard Youle and colleagues. Parkin dependent mitophagy requires PINK-1, a serine threonine kinase within mitochondria (Narendra et al., 2008; Narendra et al., 2010b). PINK-1 displays an N-terminal mitochondrial targeting sequence (MTS), a transmembrane domain (Koshiba et al.) and a Ser/Thr kinase domain. The C-terminus contains a highly conserved non-catalytic domain with an unknown function. PINK1 has been indicated to play a role in mitochondrial dynamics. Studies with overexpression of PINK-1 indicate enhanced fission while loss of PINK1 leads to fusion events (Yang et al., 2008). Fission of mitochondria is suggested to be upstream of mitophagy. The level of PINK1 is usually kept below a detectable level under normal conditions by constant degradation. PINK-1 is inserted into mitochondria via its MTS signal, where the import pathway is dependent on the translocase of the outer membrane (TOM) and the translocase of the inner membrane to travel across the mitochondrial membranes. The imported PINK1 is then subsequently cleaved between its A103 – F104 residues by a mitochondrial rhomboid protease, presenilin-rhomboid like (PARL) protein (Deas et al., 2011; Meissner et al., 2011; Whitworth et al., 2008). Cleaved PINK1 dissociates from mitochondria and is degraded by the ubiquitin proteasome system through the N-end rule pathway (Yamano and Youle, 2013). During loss of the mitochondrial membrane potential, destabilization of the proteolytic PARL presumably causes PINK1 to escape from proteolytic

cleavage. Furthermore, PINK1 with its TM domain associated with the TOM can also escape from N-end rule proteasomal degradation (Jin et al., 2010). Stabilized PINK1 on the outer membrane phosphorylates cytosolic Parkin at S65, makes a homodimer, and undergoes auto-phosphorylation at S228 and S402 residues. This results in a highly active kinase promoting Parkin translocation to mitochondria (Kondapalli et al., 2012; Okatsu et al., 2012; Okatsu et al., 2013).

The domain architecture of Parkin displays an N-terminal Ubiquitin like domain (UBL), three really interesting new gene (RING0, RING1 and RING2) (Fiesel et al.) domains and in between RING domain (IBR) where RING2 is C-terminally separated from RING1 by the IBR domain. Parkin belongs to the ring between ring (RBR) families of E3 ligases. The RBR family proteins contain a RING motif for binding to E2, and additional catalytic cysteine residue to make ubiquitin transfer from E2 to substrate. In case of Parkin, the catalytic cysteine residue, C431, is essential for mitophagy (Riley et al., 2013; Wenzel et al., 2011; Zheng and Hunter, 2013). Parkin is in an inactive state via its UBL domain binding to the RING1 domain, negatively regulating its own E3 ligase activity (Chaugule et al., 2011; Ham et al., 2016). PINK1 phosphorylates Parkin on the UBL domain at S65, resulting in conformational changes that allow binding to the E2 conjugating enzymes like UBE2D, UBE2L3 and UBE2N (Fiesel et al., 2014; Geisler et al., 2014). PINK1 also phosphorylates ubiquitin at S65, and phospho-S65 ubiquitin binds to phospho-S65 Parkin, inducing the E3 ligase activity of Parkin that is essential for mitophagy (Kane et al., 2014; Koyano et al., 2014). The amount of phospho-S65 ubiquitin chains on mitochondria is highly increased during mitochondrial depolarisation in a PINK1-Parkin dependent manner. This indicates that mitochondrial ubiquitin chains serve as receptors for Parkin translocation, where PINK1 has already accelerated Parkin activity (Okatsu et al., 2015; Ordureau et al., 2014; Shiba-Fukushima et al., 2014).

Following activation and translocation on mitochondria, Parkin ubiquitinates the OMM proteins generating different ubiquitin chains like K48, K63, K6 and K11 (Cunningham et al., 2015; Ordureau et al., 2014). The detailed role of these chains is not well understood. The Parkin-mediated PINK1-dependent ubiquitination mediates degradation of proteins like mitofusins 1/2 and MIRO (also known as RHOT, ras homolog family member -T1 and -T2) via the proteasome. In initial events, Parkin serves in mitochondrial quality control together with PINK1, and also to enhance fission by degrading fusion responsible proteins prior to mitophagy (Chen and Dorn, 2013; Wang et al., 2011). The K63 linked polyubiquitin chains made by Parkin are recognised by the selective autophagy receptor p62 connecting the mitochondria to the autophagosomes via LC3 interactions facilitating their degradation (Narendra et al., 2008). The

OMM protein VDAC1 (voltage dependent anion channel 1) was found to be ubiquitinated and recognised by p62, and to be indispensable for p62 recruitment. Furthermore, p62 aggregates damaged mitochondria by polymerising via its PB1 domain prior to degradation (Narendra et al., 2010a). However, p62 is not essential for mitophagy since p62 KO MEFs are able to degrade mitochondria, indicating the existence of other mitophagy pathways (Okatsu et al., 2010). The SLRs Optineurin and NDP52 are recruited to polyubiquitinated mitochondria upon PINK1-Parkin activation (Heo et al., 2015). The recognition of ubiquitinated mitochondria is achieved by the UBA domain in p62, UBAN and ZFN domain in Optineurin and UBZ domain in NDP52. Interestingly, PINK1-Parkin activation leads to TBK1 (TANK binding kinase 1) phosphorylation at TBK1 S172 residue, which in turn efficiently recruits and phosphorylates p62, Optineurin and NDP52 (Heo et al., 2015; Wong and Holzbaur, 2014). A detailed study of PINK1-Parkin mediated mitophagy, by knocking out all five SLRs and restoring them back selectively, was conducted by R. Youle and colleagues, highlighting a novel activation mechanism. PINK1 alone generates phosphor-ubiquitin chains and recruits Optineurin and NDP52, but not p62, on mitochondria independent of Parkin. Once NDP52 and Optineurin are recruited to mitochondria, they recruit the autophagy initiation machinery proteins ULK1, DFCP1 and WIPI1, and later LC3 is recruited. However, Parkin together with PINK1 enhances mitophagy by generating more ubiquitin chains than PINK1alone (Lazarou et al., 2015).

A recent study reports another mitophagy pathway, partially overlapping with Parkin dependent mitophagy (Hammerling et al., 2017). The damaged mitochondria are ubiquitinated by Parkin are recognized by the Rab5 positive early endosomes via ESCRT (endosomal sorting complexes required for transport) complex. The mitochondria are invaginated into the endosome which fuses with the Rab7 positive late endosome. The Rab5 endosomal pathway appeares to be independent of Atg5. The Rab5 endosomal pathway is distinct from the Mitochondria Derived Vesicles pathway (MDV). MDVs are about 70 to 150 nm in size while Rab5 endosomes carries about 500 nm vesicles. Another distinguishable factor is the fusion. MDVs are directly transported to the lysosome whereas these Rab5 vesicles fuses to the late endosomes and then to the lysosome. Taken together, mitophagy exerts in multiple ways to counteract damage and cellular stress, and to maintain mitochondrial homeostasis (Hammerling et al., 2017; McLelland et al., 2014).

## Mitophagy by an alternative autophagy pathway

As described above, ATG7 and ATG5 are essential in the ATG8-PE conjugation in the autophagy pathway. Absence of ATG7 or ATG5 leads to inability to elongate the phagophore membrane, inability of ATG8 to be membrane bound and leads to dramatic reduction in autophagosome formation. However, autophagy deficient $Atg5^{-/-}$ or $Atg7^{-/-}$ mice are healthy during their embryonic stages, indicating that there are other compensatory mechanisms activated. In 2009, Shigeomi Shimizu and his colleagues observed a typical double membrane Lamp2 positive autolysosomal structures under certain cytotoxic stress in $Atg5^{-/-}$ MEFs (Nishida et al., 2009). Those structures were regulated by ULK1 and Beclin-1, indicating that an alternative ATG5 independent form of autophagy exists. These autophagic structures seem to be generated from vesicles derived from trans-Golgi and endosomes in a RAB9 (member of RAS oncogene family) dependent manner. Further studies in $Atg7^{-/-}$ mice indicated that mitochondria are partially cleared through an Atg7 independent mechanism during erythrocyte maturation (Zhang et al., 2009). These reports indicate that two parallel autophagy pathways are involved, a canonical Atg5/Atg7 dependent pathway and Atg5/Atg7 independent alternative pathway. Mitophagy during erythrocyte maturation in $Ulk1^{-/-}$ mice is largely impaired compared to $Atg5^{-/-}$ mice where, an Ulk1 dependent and Atg5 independent mechanism seems to be dominant (Honda et al., 2014; Kundu et al., 2008). Hence, ULK1 activation is essential in both conventional and alternative autophagy. In relation to mitophagy, ULK1 is shown to be phosphorylated and acetylated by AMPK (AMP-activated protein kinase) (Egan et al., 2011). In addition, ULK1 is regulated by heat shock protein HSP90 and CDC37 (cell division cycle 37) chaperons. HSP90-CDC37 chaperone complex binds to ULK1, leading to stabilization and activation of ULK1 during erythroid differentiation, inducing mitophagy (Joo et al., 2011). Consistent with the previous observation, knockdown of ATG7 and ATG12 does not supress starvation-and hypoxia-induced mitophagy. Instead, knock down of RAB9 and/or UBA1 (ubiquitin like modifier activating enzyme 1) which are required for alternative autophagy severely supress the hypoxia induced mitophagy. Furthermore, $Ulk1^{-/-}$ MEFs display severely supressed starvation-and hypoxia-induced mitophagy (Chang et al., 2013; Hirota et al., 2015; Nishida et al., 2009). Taken together, alternative autophagy has an essential role in clearance of mitochondria but under very specific conditions or distinct cellular stage.

## FKBP8

FKBP8 or FKBP38 (FK506 binding protein 38) is a member of the immunophilin family of proteins. The immunosuppressant drugs CsA (Cyclosporin A), Tacrolimus/FK506 and rapamycin, the macrolide type of immunosuppressants, exert their immune suppression by binding to immunophilins. These drugs are mainly used during organ transplantations for prevention of T-cell activation and to lower the risk of organ rejection. Depending upon the drug affinity, the immunophilins are categorised as cyclosporins (cyclophilin binding) and FK506 binding proteins (FKBPs). The FK506 binding proteins are also known to bind to rapamycin. Tacrolimus/FK506 inhibits T-cell activation through the FKBPs. During T-cell activation, the signalling cascade initiated leads to influx of calcium ions. The upregulated calcium ions, together with calmodulin, bind to the calcium-calmodulin-dependent phosphatase, calcineurin. Calcineurin dephosphorylates the transcription factor NF-AT (nuclear factor of activated T-cells), that translocates to the nucleus and mediates the transcriptional upregulation of several cytokines like IL-2 (interleukin2), resulting in further proliferation of T-cells. The tacrolimus/FK506 drug binds to the peptidyl-propyl isomerase domain (PPIase) of FKBP (mainly studied in FKBP12) that further inhibits the calcineurin activity, leading to reduced T-cell activation (Kino et al., 1987; Ruhlmann and Nordheim, 1997; Thomson et al., 1995).

The mammalian family of FKBPs contains 14 members, each of them consisting of one or more PPIase domain. The FKBPs are cytoplasmic, nuclear and some contain a transmembrane domain docking on ER or mitochondria. A few members of the family possess multiple TPR (tetratricopeptide repeat) domains like FKBP36, FKBP38/FKBP8, FKBP51 and FKBP52, with distinct biological functions (Blackburn and Walkinshaw, 2011). The domain architecture of FKBP8 displays an N-terminal Glu-rich domain, preceding the PPIase domain, three TPR domains, calmodulin binding domain and a C-terminal transmembrane domain (Koshiba et al.) (Fig.10). FKBP8 is anchored in the mitochondrial outer membrane via its TM domain with the N-terminus pointing towards cytosol (Lam et al., 1995; Shirane and Nakayama, 2003). The N-terminal extension of FKBP8 is not common for other FKBPs and its significance is not very well understood yet. It might work as an auto-inhibitory arm for the PPIase activity, of which inhibition is released after $Ca^{++}$ saturation (Kang et al., 2013). The Sonic hedgehog signalling pathway (Shh) regulates neural patterning during development, where Shh signalling is activated by ANKMY2 (Ankyrin repeat and MYND containing protein 2). FKBP8 binds to the $Zn^{++}$ finger motif of ANKMY2 via its N-terminal extension, inhibiting the ANKMY2

activity and thereby preventing inappropriate activation of the Shh-pathway (Cho et al., 2008; Saita et al., 2014). FKBP8 is unique amongst the FKBPs because of its reduced property to bind the FK506 drug and to have constitutively inactive PPIase domain. The PPIase domain of FKBP12 possesses a hydrophobic drug binding property where W59, F36 and H87 are important residues. The sequence alignment of the PPIase domain in FKBP8 andFKBP12 displays that these conserved residues are substituted with L, V and R residues in FKBP8, respectively. This narrows the active site for FKBP8 interaction with FK506 (Galat, 2000; Sich et al., 2000). FKBP8 displays PPIase activity only in presence of $Ca^{++}$ due to the formation of the heterodimeric complex with $Ca^{++}$ sensor calmodulin (CaM) leading to allosteric rearrangement of FKBP8. (Edlich et al., 2007). The N-terminal extension of FKBP8 auto-inhibits the isomerase activity where auto-inhibition was reversed by CaM/$Ca^{++}$ (Kang et al., 2013). Loss of FKBP8 in mice results in several deformities of neural tube closure (Spina Bifida), and the mice die early because of frequent apoptosis and abnormal extension of nerve fibres. The abnormal extension of nerve fibres in FKBP8 $^{-/-}$ mice is due to hyper-phosphorylation of Protrudin (also known as ZFYVE27, zinc finger FYVE type containing 27). FKBP8 binds to Protrudin and prevents its hyperphosphorylation, thereby regulating neurite extension (Shirane et al., 2008).

The TPR domains of FKBP8 facilitate multiple protein-protein interactions. FKBP8 interacts with Bcl-2 and Bcl-XL via the TPR domain surface, protecting Bcl-2 from caspase dependent degradation. This results in Bcl-2 accumulation on mitochondria that inhibits apoptosis (Choi et al., 2010; Haupt et al., 2012). FKBP8 exerts chaperone function by interaction with Hsp90 and Hsp70 via a TPR domain, regulating the folding of CFTR (cycstic fibrosis transmembrane conductance regulator) at the ER site (Banasavadi-Siddegowda et al., 2011; Hutt et al., 2012). FKBP8 also interacts with the NS5A (Non-structural protein 5 A, viral protein essential for viral replication) of the swine fever and HCV (Hepatitis C virus). Interaction with NS5A and Hsp90 is modulated by the S100 calcium binding proteins, where they compete for binding to a TPR domain of FKBP8 (Li et al., 2016; Shimamoto et al., 2014; Tani et al., 2013).

The mTOR (mammalian target of rapamycin), a known negative regulator of autophagy, consists of two major complexes, mTORC1 and mTORC2. FKBP8 binds to the mTORC1 complex and inhibits its activity. The Ras like GTPase, Rheb, is activated during nutrient rich conditions, binds to FKBP8 and Bcl-2 and releases them from mTORC1. While during nutrient deprivation Rheb remains inactive and facilitates FKBP8 inhibition of mTORC1 (Bai et al., 2007; Ma et al., 2010; Zou et al., 2013). Phosphatidic acid (PA) is a key mediator of the

mitogenic mTOR activation during cell proliferation. PA is generated by phospholipase D, cleaving phosphatidylcholine into PA and choline (Fang et al., 2001). The mechanism of how PA activates mTOR is suggested as; PA competes with FKBP8, disrupts FKBP8 binding to mTORC1 and thereby stimulates mTOR kinase activity in the absence of FKBP8 (Yoon et al., 2011). This is the upstream regulation of mTOR kinase activity where FKBP8 needs to be displaced from the complex. As FKBP8 is a mitochondrial outer membrane protein, it has been studied during Parkin dependent mitophagy. The study showed that FKBP8 translocates from the mitochondria to ER during mitochondrial degradation. This is mediated by its dual targeting TM domain capabilities. In addition, Bcl-2 does also evacuate from mitochondria during mitophagy and is translocated to the ER. At the ER, FKBP8 continues its anti-apoptotic function together with Bcl-2. The translocation fate of these proteins during mitophagy is decided by the reduced basicity of their C-terminal TM domain. The TM domain of both, FKBP8 and Bcl-2, is flanked by only one strong basic residue arginine or lysine. Substitution of the terminal residue with a strong basic residue at the TM domain gives increased basicity, leads to constraining of FKBP8 and Bcl-2 on mitochondria and their degradation during mitophagy. While other OMM proteins, such as SYNJBP2 (Synaptojanin 2 binding protein also known as OMP25) and Bcl-XL, have three strongly basic residues flanking the TM domain and are unable to escape from mitochondria during mitophagy. (Saita et al., 2013).

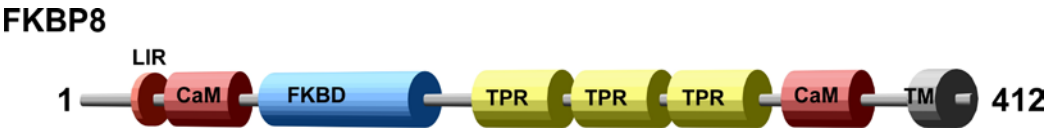



**Fig. 10. Domain architect of FKBP8.** LIR, LC3 Interacting Region, FKBD-FK506 Binding domain; TPR-tetratricopeptide repeats; CaM-Calmodulin binding domain; TM-Transmembrane domain.

# AIM

The overall aim of this study was to characterize the interactions of FKBP8 and the components of the PI3K Complex-I with the ATG8 family proteins. FKBP8 was known to be a mitochondria associated protein, and an inhibitor of mTORC1 complex. The aim of the FKBP8 project was to I) Elucidate if the interaction of FKBP8 with the ATG8 proteins could play a role in autophagy mediated degradation of mitochondria and II) Investigate if FKBP8 was a regulator of the autophagy process. The aim of the PI3K complex project was to investigate the specificity and functionality of the ATG8 interacting motifs in the initial steps of autophagosome formation.

# Summary of Papers

***Paper-I***

**FKBP8 recruits LC3A to mediate Parkin-independent mitophagy.**

Bhujabal Z., Birgisdottir ÅB., Sjøttem E., Brenne HB., Øvervatn A., Habisov S., Kirkin V., Lamark T., Johansen T.

EMBO reports (2017) 18, 947-961

In this paper we have identified FKBP8 to act as a mitophagy receptor harbouring an N-terminal single LIR motif. Initially, FKBP8 was identified as a LC3B interacting protein in a yeast two hybrid screen. Further *in-vitro* interaction assays revealed that FKBP8 binds to all ATG8 homologues, though indicate stronger binding with LC3A and GABARAP-L1. Overexpression of FKBP8 in Hek293 and HeLa cell lines promoted mitochondrial fission, which is upstream of mitophagy. Furthermore, confocal imaging and mitophagy assays revealed that FKBP8 recruits LC3A to damaged mitochondria, and together they induced mitochondrial degradation. Interestingly, the mitophagy induction was found to be exclusively dependent on the N-terminal LIR motif. In line with previous reports, upon induction of mitophagy FKBP8 extraordinarily makes its own escape and translocate back to ER, probably to continue apoptosis inhibition. Importantly, we found LC3A to also be recruited to ER-localised FKBP8, indicating that their induced interaction is independent on their localisation. Hence, this paper shows that under mitochondrial damaged condition, FKBP8 recruits lipidated LC3A to the mitochondria, and together they direct them into the lysosome for degradation.

***Paper-II***

**FKBP8 interacts directly with Beclin-1 and promotes autophagy**

Bhujabal Z., Sjøttem E., Birgisdottir ÅB., Øvervatn A., Brenne HB., Lamark T., & Johansen T.

*Manuscript*

In Paper-I we identified FKBP8 as an outer mitochondrial membrane receptor recruiting LC3A on mitochondria to promote mitophagy. In this manuscript, we establish the first link of evidence that FKBP8 promotes multiple steps of the autophagy pathway. We established FKBP8 KO cell lines reconstituted with Myc-tagged FKBP8, and monitored the effect of FKBP8 on mTOR activity and autophagosome formation. Interestingly, we found FKBP8 inhibits mTOR activity and promotes autophagy under nutrient rich conditions. However, starvation induced mTOR inhibition and autophagy induction was not dependent on FKBP8,

endogenous LC3B puncta formation was induced only under normal nutrient conditions. In a protein-protein interaction screen of FKBP8 and various autophagy proteins, we identified Beclin-1 and TBC1D15 as putative partners of FKBP8. The Beclin-1 interaction was verified further by both *in-vitro* and *in-vivo* assays. Hence this manuscript suggest that FKBP8 can promote autophagy by acting at three steps, first to inhibit upstream mTOR activity, second to recruit Beclin-1 to ER-mitochondria membranes, and third in later stages by recruiting ATG8 family protein.

***Paper-III***

**Members of the autophagy-inducing class III PI3K complex interact preferentially with GABARAP-family proteins via LIR motifs**

Birgisdottir ÅB., Mouilleron S., Bhujabal Z., Wirth M., Lee R., O'Reilly N., Tooze SA., Lamark T., & Johansen T.

*Manuscript*

In this study we identified functional LIR motifs in VPS34, Beclin-1 and ATG14 of the Class III PI3K Complex-I. The LIR motifs in PI3K Complex-I bind preferentially to the GABARAP family proteins, GABARAP and GABARAP-L1. The crystal structures of the LIR motif in VPS34, Beclin-1 and ATG14L bound to GABARAP and/or to GABARAP-L1 were obtained to help understand their interaction and binding preference. Variation in residues comprising part of hydrophobic pocket 2 (HP2) mediating contacts with residues C-terminal to the core LIR motif, help to explain the specificity of VPS34, Beclin-1 and ATG14L LIRs for the GABARAP family. In addition, focusing on the autophagy-specific component ATG14L, we found that a functional LIR motif was required for efficient autophagosome formation. Thus, we observed reduction in the PI3K-effector protein-WIPI2 dots upon starvation. The LIR mutant of ATG14L also did not co-localize with LC3B punctae. We suggest a model where the GABARAP-preferring LIR motifs in VPS34, Beclin-1 and ATG14L are involved via coincidence detection mechanisms in scaffolding the Class III PI3K Complex-I on membranes for efficient autophagosome formation.

# Discussion

## Discussion Paper – I and II

### FKBP8 as a novel mitophagy receptor

In this paper, we identified FKBP8 as a novel mitophagy receptor, recruiting specifically LC3A on depolarized mitochondria to mediate Parkin-independent mitophagy. FKBP8 was detected in a yeast two hybrid screen, using LC3B/GABARAP as bait. In the same screen, conjugation defective mutants of LC3/GABARAP (LC3B-ΔG120 and GATE-16-ΔG) failed to pick up FKBP8 (Kirkin et al., 2009b). This was the first hint towards FKBP8 involvement in autophagy. Thus, we initiated studies using a series of biochemical methods such as peptide array, 2D peptide array, GST-pull down assays (from HEK293 cell lysate and *in vitro* translated FKBP8) and co-immunoprecipitation assays to find the LIR motif in FKBP8. We identified a single N-terminal LIR motif in FKBP8, which mediates interaction with the ATG8 homologues. Furthermore, mutations of the core LIR motif residues and residues involved in LDS dependent binding with the ATG8 homologues confirmed the presence of a canonical and functional LIR motif in FKBP8. The mammalian FKBP8 has a transmembrane domain (TM domain) that harbours mitochondrial- and ER-localisation signals. FKBP8 is predominantly inserted in the outer mitochondrial membrane (OMM). The presence of a functional LIR motif indicated involvement of FKBP8 in mitophagy, in a similar manner to other previously identified OMM mitophagy receptors such as BNIP3 and NIX (Hanna et al., 2012; Novak et al., 2010). To address the functional role of the LIR motif in FKBP8, we performed imaging analyses with overexpression of FKBP8 together with the ATG8 homologues, narrowing down the search for functional partner *in vivo*. LC3A was efficiently translocated to mitochondria upon FKBP8 overexpression in a LIR dependent manner. This translocation tendency of LC3A by preferential interaction with FKBP8 was confirmed in cells with CRISPR/CAS9 knock out of FKBP8.

### Specificity towards LC3A

The recruitment of LC3A to mitochondria through FKBP8 to mediate PINK1-Parkin independent mitophagy is a novel observation. Such an ATG8 homolog preference and specificity has been reported before. NIX has preference towards GABARAP-L1; BNIP3, FUNDC1 and Bcl2-L-13 display preference towards LC3B to mediate mitophagy (Hanna et al., 2012; Liu et al., 2012; Murakawa et al., 2015; Novak et al., 2010). The residues flanking

the core LIR motif play a crucial role in regulation and specificity for the ATG8 homologues (Olsvik et al., 2015). The LIR motif in BNIP3 is preceded by an acidic residue (serine), which after phosphorylation leads to stronger LC3B binding and mitochondrial sequestration. The serine residue following the LIR motif of BNIP3 leads to enhanced binding with GATE-16 after phosphorylation (Zhu et al., 2013). Similar regulation is seen by phosphorylation of a serine preceding the LIR motif in Bcl2-L-13 (Murakawa et al., 2015). FUNDC1, on the other hand, shows LIR motif regulation in the opposite way. Dephosphorylation of Y18 of the core LIR motif enhances binding with LC3B (Liu et al., 2012). In case of FKBP8, we do not find any putative candidates for regulating the LIR motif. Thus, the membrane mitophagy receptors, except for FKBP8, are regulated by phosphorylation. In addition to these reports, a crystal structure study of the p62 LIR peptide bound to LC3B indicates that the three acidic residues N-terminal to the LIR motif of p62 interact with the basic surface of α-helix-1 (R10-R11) of LC3B (Ichimura et al., 2008). Taken together, residues preceding the LIR motif contribute to their specificity and their regulation. The LIR motif in FKBP8 is preceded by two acidic residues that may interact with the basic surface of α-helix-1 of both LC3A and LC3B. The ATG8 family proteins differ in their electrostatic surface potential distribution on the N-terminal arm. The α1-helix is basic and α2-helix is acidic in LC3A and -B. The same α1-helix is acidic in both GABARAP and GABARAP-L2. The α2-helix is basic in GABARAP and neutral in GABARAP-L2 (Sugawara et al., 2004). However, without x-ray or NMR data it is impossible to determine why FKBP8 binds better to LC3A than LC3B. While BNIP3 and NIX are upregulated under hypoxic conditions, FUNDC1 enhances its LC3B binding by dephosphorylation of Y18 under hypoxia. We could not detect any upregulation of FKBP8 under hypoxic conditions (data not shown), and were not successful in finding any other upregulation stress/event so far.

**The possible role of FKBP8 in mitochondrial fission**

During our study of FKBP8 overexpression, we consistently observed a striking change in mitochondrial morphology. FKBP8 overexpression resulted in fragmented mitochondria. In order to pursue the role of FKBP8 in mitochondria fission, we tested its possible interaction with the fission regulator GTPase DRP1 and TBC1D15. The cytosolic TBC1D15 interacts with the TPR domain of FIS1, translocates to OMM and causes mitochondrial fragmentation independent of DRP1. DRP1 translocates to OMM with multiple fission related proteins whereas TBC1D15 is identified to interact with FIS1 (Onoue et al., 2013). Apparently, FKBP8 does exhibit interaction with TBC1D15 *in-vitro* (Paper-II, Fig.2B) and *in-vivo* (data not shown)

but not with DRP1. TBC1D15 does efficiently co-immunoprecipitate with FKBP8, even though FKBP8 is targeted to the ER. The binding efficiency is comparable to the known binding partner FIS1 (Onoue et al., 2013). In parallel, another group studying TBC1D15 reported that, together with FIS1 it gains access to mitochondria. TBC1D15 then interacts with LC3 via its LIR motif and mediates mitochondria encapsulation by regulating Rab7 activity at the mitochondria and isolation membrane interface. However, knockdown of TBC1D15 did not have any effect on mitochondrial morphology and neither did knockout of FIS1 (Yamano et al., 2014). The mitochondrial fission is enhanced by proteasomal degradation of the fusion related proteins following Parkin ubiquitination during mitochondrial homeostasis and during mitophagy (Tanaka et al., 2010; Ziviani et al., 2010). The TPR domains of FKBP8 interact with the S4 subunit of the 26S proteasome (made up of the 20S proteolytic core particle sandwiched between two 19S regulatory particles) tethering the proteasome to the organelle membranes. Loss of FKBP8 resulted in reduced abundance and activity of the membrane bound proteasome (Nakagawa et al., 2007; Voges et al., 1999). Thus the possible degradation of the mitochondrial fusion proteins such as OPA1 or MFN1/2 during homeostasis and/or mitophagy needs further investigation.

**Cross talk between PINK1-Parkin dependent mitophagy and receptor-mediated mitophagy**

Crosstalk between PINK1-Parkin dependent mitophagy and membrane receptor mitophagy might exist. NIX is the only member of the membrane mitophagy receptors known to have a role in PINK1-Parkin mitophagy. In NIX knockout cells mitochondria are more resistant to CCCP-damage and NIX is needed for Parkin recruitment (Bellot et al., 2009; Ding et al., 2010). Even though degradation of mitochondria is mediated through both pathways, their possible interdependence is not known yet. Also, exactly how dysfunctional mitochondria are actually engulfed by autophagic membranes is very little understood. TBC1D15 coordinates the sequestration of mitochondria together with FIS1 but whether it coordinates with other membrane receptors remains to be determined. TBC1D15 is identified as a substrate of Parkin and controls autophagosome morphology downstream of Parkin translocation together with FIS1 during mitophagy (Sarraf et al., 2013; Yamano et al., 2014). It would be interesting to see whether FKBP8 coordinates with TBC1D15 similar to FIS1 downstream of Parkin. In addition, Beclin-1 and AMBRA1 are reported to directly interact with Parkin where Beclin-1 plays a dual role in initiating mitophagy and translocation of Parkin for Mfn2 ubiquitin degradation (Choubey et al., 2014; Tanaka et al., 2010; Ziviani et al., 2010). Even though FKBP8 does not

exhibit direct interaction with Parkin, other interactions bridging FKBP8 and Parkin such as Beclin-1 need to be elucidated further in the future.

**Escape of FKBP8 from mitochondria to ER**

While we were characterizing FKBP8 for its functional role in autophagy, we observed that endogenous FKBP8 itself was not degraded by autophagy under autophagy inducing conditions. When we induced mitophagy by overexpression of FKBP8 together with LC3A, FKBP8 itself did not co-localize with acidified mitochondria (red only structures) (Paper-I, Fig.6A). FKBP8 has been reported to indeed escape from mitochondria during mitophagy (due to reduced basicity of its TM domain) and translocate to the ER (Saita et al., 2013). Mitophagy induced in this study was however solely dependent on Parkin and CCCP treatment. Furthermore, the pathway for such translocation is independent of Mfn2 tethering of mitochondria and ER and suggested to depend on microtubule transport. The focus of the study by Saita et al. was entirely on the FKBP8 TM domain and its basicity mediating the escape while the importance of the N-terminus of FKBP8 was not addressed. The LIR motif of FKBP8 resides in the N-terminus and the role of the N-terminal arm in FKBP8 is interesting since this region is also speculated to induce mitochondrial fission phenotype (Saita et al., 2013) and cause auto inhibition of PPIase activity (Kang et al., 2013). The mechanism of how FKBP8 does escape while mitochondria are docked on forming autophagosomes remains to be elucidated.

**Interactions of FKBP8 with autophagy-mediating proteins**

In PINK1-Parkin mediated mitophagy, proteins in the OMM are ubiquitinated by Parkin, recognized by SLRs interacting with ATG8 homologues, and the mitochondria then dock onto the growing autophagosomal membrane. Investigation of the mechanism of PINK1-Parkin mediated mitophagy revealed a new mode of activation by PINK1 alone. Optineurin and NDP52 are recruited on the mitochondria downstream of the PINK1 stabilisation, but not p62. NDP52 and Optineurin then specifically recruit LC3B on mitochondria during mitophagy. TAX1BP1, upon PINK1-Parkin activation can recruit LC3C on the mitochondria (Lazarou et al., 2015). Interestingly, NDP52 harbours an atypical C-type LIR motif with preference for LC3C during clearance of *Salmonella* (von Muhlinen et al., 2012). Apparently, the interaction preference is shifted towards LC3B during NDP52-mediated mitophagy (Lazarou et al., 2015). In addition, NDP52 and Optineurin recruit ULK1 to initiate the mitophagy independent of the Parkin activation (Lazarou et al., 2015). In our interaction studies of FKBP8, we observed that

FKBP8 directly interacts with Beclin-1 *in-vivo* and *in-vitro* (Paper-II, Fig.3) and with NDP52 (Paper-II, Fig.2C). However, FKBP8 does not interact with p62, Optineurin, PINK1 or Parkin. We have seen that FKBP8 acts as membrane receptor for mitophagy independent of Parkin. Herein, the most intriguing question is how FKBP8 orchestrates the initial events in mitophagy. Beclin-1 is involved in autophagy initiation and NDP52 recruits the initiation complex via ULK1 downstream of PINK1 activation. In our mitophagy assay, mitochondria were labelled with mCherry-EGFP-OMP25. The green fluorescence of EGFP is quenched at low pH resulting in mitochondria under degradation appearing as coloured red only in the confocal microscope. Our assay indicates that mitophagy is efficiently induced by FKBP8 in comparison with other two known membrane mitophagy receptors, BNIP3 and NIX. An important point is that degradation of mitochondria is dependent on overexpression of the membrane receptors together with their preferred ATG8 homolog. At the moment we can only speculate that, the interactions with Beclin-1 and NDP52 mentioned above might contribute to enhanced mitophagy induced by FKBP8 compared to BNIP3 and NIX. But certainly, the detailed mechanism is not known and needs further investigation. To add more complexity, FKBP8 is a known endogenous inhibitor of mTOR activity and it also interacts with Bcl-2 (Bai et al., 2007; Choi et al., 2010; Haupt et al., 2012).

**FKBP8 as an inhibitor of mTOR activity**

Since FKBP8 is reported to act as an inhibitor of mTOR activity, it is a potential regulator of autophagy. In this study, autophagy activity was monitored as LC3B puncta formation. We found that reintroduction of Myc-FKBP8 in FKBP8 knock-out cell lines strongly induced autophagy in nutrient rich conditions. Furthermore, the mTOR activity in these cells, measured by quantitation of phosphorylated Thr389 residue of S6 kinase, was reduced. This shows that FKBP8 is a strong inhibitor of mTOR, and promotes autophagy during nutrient rich conditions. Starvation of the Myc-FKBP8 expressing FKBP8 KO cells did not enhance autophagy further, or inhibit mTOR further. Moreover, mTOR activity was inhibited and autophagy induced in the FKBP8 KO cells. This indicates that FKBP8 is not necessary for starvation induced mTOR inhibition and autophagy activation. However, FKBP8 clearly acts as an inhibitor of mTOR, leading to induction of autophagy, under nutrient rich conditions. We also found FKBP8 to act as a mitophagy receptor, inducing mitophagy upon mitochondrial damage. Whether FKBP8 also stimulates mitophagy via mTOR inhibition upon mitochondrial damage will be interesting to address in further studies, since the initial events to activate mitophagy are not very well understood yet.

The autophagosome biogenesis begins at the mitochondria-ER interface. Here, recruitment of the PI3K complex and nucleation of the omegasome takes place both upon starvation induced autophagy and during induction of mitophagy (Gelmetti et al., 2017; Hamasaki et al., 2013). The core member of the PI3K complex, Beclin-1, re-localizes to MAM structures to induce mitophagy (Gelmetti et al., 2017). We found Beclin-1 to colocalize with FKBP8, and to co-immunoprecipitate from both mitochondria and ER bound FKBP8. Hence, FKBP8 can potentially recruit Beclin-1 to both ER and mitochondria membranes following autophagy/mitophagy induction. An interesting question to address in further studies is whether the recruitment of Beclin-1 does occur together with the PI3K complex or alone at the MAM structures. In this context, it will be interesting to investigate whether FKBP8 associates with the PI3K complex via Beclin-1. To be a part of the PI3K complex, Beclin-1 needs to be free from Bcl-2. Hence, whether the FKBP8 interaction with Beclin-1 displaces Bcl-2 and thereby induces PI3K complex formation needs to be studied further.

In addition to the previously described symmetrical fission process for mitochondrial homeostasis, a new model has been proposed highlighting mitophagy initiation and its role in mitochondrial homeostasis under normal physiological condition (Yamashita et al., 2016). According to this model, upon induction of mitophagy the autophagy isolation membrane forms on the mitochondria, and the mitochondria buds off the impaired part which is enwrapped by the isolation membrane. The encapsulated mitochondria then proceed into the canonical autophagy pathway, resulting in a mitophagosome (mitochondria containing autophagosome). In this proposed model for mitochondrial fission/budding off, the nucleation complex (Class-III PI3K Complex-I) is needed to enwrap the mitochondrial fragment and to generate the omegasome like structures. The process occurs independent of the mitochondrial division factor DRP1. Interestingly, in our mitophagy study FKBP8 induces mitochondrial fragmentation independent of DRP1. Thus, whether FKBP8, via its Beclin-1 interaction, may mediate docking of the PI3K Complex-I on damaged mitochondria, needs to be further addressed. Especially since mitophagy is an important regulator of mitochondrial homeostasis under normal physiological conditions and during age related increased mitochondrial impairment (Palikaras et al., 2017).

The active mTORC1 complex is sitting on the lysosome together with GTPase like Rheb, needed for the activation of mTORC1 during excess of amino acids (Sancak et al., 2010; Sancak et al., 2008). The active Rheb antagonises the FKBP8 mediated mTORC1 inhibition in response to growth factors or nutrients by displacing it from the mTOR complex (Bai et al., 2007; Ma et al., 2010). In this context, loss of FKBP8 may lead to aberrant mTOR activity on

the lysosomal compartment. Moreover, Bcl-2/Bcl-XL compete with mTORC1 for FKBP8 binding. Hence, there is a crosstalk between mTOR mediated cellular proliferation, autophagy induction and apoptosis. An interesting question is to evaluate how FKBP8 is involved in the crosstalk between these processes.

**Discussion Paper – III**

**LIR motifs in the Class-III PI3K Complex-I**

In this paper, we identified LIR motifs in members of the Class-III PI3K Complex-I, namely VPS34, Beclin-1 and ATG14L. Using peptide array strategy, we detected two putative LIR motifs in both VPS34 and ATG14L and one LIR motif in Beclin-1. The *in vitro* interaction studies identified one main functional LIR motif in VPS34 (LIR-F250) and ATG14L (LIR-W435), respectively, and verified a functional LIR motif in Beclin-1. VPS34, Beclin-1 and ATG14L all bind to the ATG8s homologues, displaying preference for the GABARAP subfamily. All the LIR motifs are canonical and correlate with previously described crucial requirements for LIR motifs as ϕ–X–X–η where ϕ is an aromatic amino acid (W/F/Y) and η is a hydrophobic amino acid (I/L/V). Furthermore, we obtained high-resolution crystal structures of the LIR interactions for VPS34, Beclin-1 and ATG14L with GABARAP/GABARAP-L1. To assess the functional role of the LIR motifs in the Class-III PI3K Complex-I in autophagy we focused on the ATG14 LIR motifs. ATG14L represents the sole autophagy specific member of the complex. Our data indicate the importance of the ATG14L LIR interaction for initiation of autophagy.

**Structural insights into LIR interactions; explaining preference towards the GABARAP subfamily**

The specificity towards a certain subfamily of ATG8 is achieved by residues in the LIR motif communicating with residues in α–helices and β–sheets of ATG8s. The hydrophobic pocket 2 (HP2) is formed between the central α helix (α3) and β2 of the ATG8s ubiquitin core. Multiple alignment of the ATG8 homologues shows that the residues between the α3 and β2 of LCA/B differ from that of the GABARAP family proteins. In LC3A/B, residues H57, V58 and L63 form the base of HP2 while these corresponding residues in the GABARAP subfamily are replaced by D54, L55 and F60; and in LC3C replaced by E63, L64 and F68 respectively. For most LIR containing proteins, residues of HP2 establish electrostatic interactions with residues flanking the core LIR motif. Structural studies of the FYCO1 (FYVE and coiled-coil protein 1)

LIR motif, show that an aspartic acid residue (D1285) C-terminal to the core LIR interacts with H57 in LC3B and thus achieves specific binding to LC3A/B. The corresponding residue of H57 in LC3B, is replaced by the aspartic acid residue (D54) in the GABARAP subfamily. The presence of D54 in GABARAP reduces binding due to charge repulsion (Olsvik et al., 2015). Conversely, an interaction between D54 of GABARAP and a tyrosine residue (Y3351; corresponding to the aspartic acid in FYCO1) C-terminal to the core LIR motif of ALFY (autophagy-linked FYVE protein) mediates specificity for the GABARAP subfamily (Lystad et al., 2014). In our study, the high-resolution crystal structures of the LIR motifs in the PI3K complex 1 bound to GABARAP/GABARAP-L1 indicate the importance of the HP2 residues D54, L55 and F60 (Fig.11, indicated by *). We propose that these three residues are responsible for the observed subfamily specificity of the LIR motifs. Residues L55 and F60 are engaged in hydrophobic interactions with position +3 as well as positions +4 to +6 C-terminal to the core LIR motifs. Replacements with the shorter residues V58 and L63 in LC3A/B would potentially lead to the weakening of these hydrophobic interactions. Furthermore, H57 of LC3A/B imposes a clash with residues in position +4 to +6 of each of the LIR motifs, highlighting the importance of D54 in GABARAPs/LC3C to enable binding. Our results demonstrate the importance of including residues C-terminal to the core LIR motif. In previously solved crystal structures of LIR interactions the LIR peptides used sometimes lack the residues C-terminal to the core motif (Suzuki et al., 2014). This should be taken into consideration for future structural studies of LIR interactions.


**Fig.11. Alignment of the mammalian homologues of ATG8 proteins.** The asterisks indicate the residues important for the GABARAP specific interactions. Alignment generated by the ClustalOmega (EMBL-EBI) and BOXSHADE.

The initial events of autophagosome formation are regulated by the ULK1 complex and the PI3K Complex-I. We have previously identified functional LIR motifs in four of the proteins constituting the mammalian ULK1 complex, i. e. ULK1, ULK2, ATG13 and FIP200, and all of these LIR motifs displayed a binding preference for the GABARAP subfamily (Alemu et al., 2012). Our data in paper-III showed that three of the proteins in PI3K Complex-

I have functional LIR motifs, and these motifs similarly have a preference for GABARAP and GABARAPL1.

Recently, GABARAP was shown to be essential for the recruitment and activation of ULK1 at the very early step of autophagy induction, prior to any covalent conjugation of ATG8s to a growing phagophore (Joachim et al., 2015). In this study, they identified a centrosomal pool of unlipidated GABARAP that is released upon autophagy induction to induce a LIR mediated recruitment of ULK1. It remains to be tested whether this pool of unlipidated GABARAP similarly recruits the PI3K Complex-I to autophagosome formation sites. However, the striking similarity in sequence and binding preference seen for LIR motifs in these two complexes suggests a scenario where these two complexes are recruited together.

Another proposed role of LIR interactions is to facilitate a continuous recruitment of core autophagy proteins to the rim of the growing phagophore. This has been demonstrated *in vitro* for the composite LIR motif of yeast Atg12 (Kaufmann et al., 2014). Initially, PI3K Complex-I catalyses the formation of PI3P, and this is needed for the initial recruitment of autophagy proteins like WIPI proteins and ATG16-ATG5-ATG12 and initiation of phagophore formation. As a next step, ATG8 proteins then become covalently attached to both surfaces of the growing phagophore, where they act as a scaffold for the recruitment of cargos to the inner surface or core autophagy components and various effector proteins to the outer surface. It remains to be shown if the ULK1 complex or the PI3K Complex-I is recruited via LIR interactions to the outer surface (or the rim) of the growing phagophore. However, our data showed that the LIR motifs in ULK1 (Alemu et al., 2012) and ATG14L (Paper-III) were essential for the co-localization of these proteins with WIPI2 puncta and LC3B puncta induced by autophagy induction. From this, we conclude that a sustained assembly of the ULK1 and PI3K complexes at growing phagophores or autophagosomes depends on LIR motifs, and most likely these motifs perform their roles by interacting with GABARAP and/or GABARAP-L1.

Finally, there is increasing evidence for that individual core autophagy proteins may have roles associated either with the closure of autophagosomes or their fusion with endosomes or lysosomes. Earlier studies have suggested that GABARAPs are important during later stages of autophagosome formation (Weidberg et al., 2010), while ATG14L has been shown to facilitate the fusion of autophagosomes with endosomes or lysosomes (Diao et al., 2015; Wang et al., 2015). Interestingly, it is not known if these later roles of GABARAPs depend on PI3K Complex-I. The autophagosome fusion role of ATG14L is mediated by the interaction with STX17, but whether ATG14L interacts along with Complex-I while sitting on the phagophore is not known yet. In this scenario Complex-I can be envisioned sitting on the outer membrane

to enhance membrane tethering and fusion events. Moreover, phosphatidylinositol (4, 5) $P_2$ (PtdIns 4, 5 $P_2$) has been identified to regulate autophagy by binding to ATG14L through its membrane sensing BATS domain. We speculate in paper-III that the role of ATG14L during the fusion event may depend on a coincident binding of the BATS domain to PtdIns 4, 5 $P_2$ and the LIR motif to GABARAP or GABARAPL1.

Another question is whether the LIR motifs in PI3K Complex-I proteins are used for selective autophagy of the respective proteins. We have not tested if proteins of the PI3K Complex-I are degraded by selective autophagy, so this needs to be addressed in future studies. There is no evidence for that the mammalian ULK1 complex uses its LIR motifs for its own degradation by selective autophagy (Alemu et al., 2012), but Atg1 complexes in Arabidopsis and yeast are partially degraded by LIR mediated selective autophagy (Kraft et al., 2012; Nakatogawa et al., 2012; Suttangkakul et al., 2011).

**General Discussion**

In this study, our focus was to find the functional role of the described proteins in the mechanistic steps of autophagy. The common trait for all the proteins studied was the identification of functional LIR motifs shown to be important for their role in autophagy. In Paper-I we initially had to rely on protein overexpression and chemical stresses to investigate the role of FKBP8 in mitophagy. Since mitophagy is not easily detectable under normal cellular conditions, overexpression helped us to narrow down our focus on certain stages of mitophagy, and especially to establish the specificity *in vivo*. Later, we were able to address endogenous protein-protein interactions and co-localisations. Other membrane mitophagy receptors like BNIP3, NIX, Bcl-2-L13 and FUNDC1 were also initially identified upon overexpression. Recently, they were found to be upregulated upon hypoxia (BNIP3, FUNDC1) and to induce mitophagy in hypoxic cells (Bellot et al., 2009). NIX, on the other hand, was found to be important for mitophagy during erythrocyte maturation (Sandoval et al., 2008). We have not observed any significant changes in the level of FKBP8 expression upon cellular stresses. However, its localisation changes at least upon mitochondrial damage, suggesting that one way to regulate FKBP8 activity is by subcellular localisation. Taken together, a prominent marker to detect the mitophagy under normal physiological conditions is still missing.

Next we found FKBP8 to interact with multiple autophagy proteins (paper-II), suggesting a regulatory role in several steps of the autophagy process. Hence, paper-II introduced FKBP8 as a new member joining the autophagy club, containing a functional LIR motif and involved in upstream autophagy signalling. Intriguingly, FKBP8 interacts with

Beclin-1, and the importance of this interaction in autophagy is an interesting question to address in further studies.

In paper-III, we used the ATG14L LIR motifs to investigate the functional role of the identified LIR motifs in PI3KC3 Complex-I. To assess the individual roles of the LIR motifs in VPS34 and Beclin-1 is challenging, since both are involved in many different complexes, in which some negatively regulate autophagy, such as the Rubicon complex (Matsunaga et al., 2009). Since the Class-III PI3K Complex-I consists of four members, overexpression might override the endogenous complex function in uncertain direction. To address the roles of the LIRs in each of the complex components, gene editing tools such as CRISPR/CAS9 could be used to mutate the LIR motifs in each member and hereafter address their impact on the autophagy process.

# References


Aerbajinai, W., M. Giattina, Y.T. Lee, M. Raffeld, and J.L. Miller. 2003. The proapoptotic factor Nix is coexpressed with Bcl-XL during terminal erythroid differentiation. *Blood*. 102:712-717.

Alemu, E.A., T. Lamark, K.M. Torgersen, A.B. Birgisdottir, K.B. Larsen, A. Jain, H. Olsvik, A. Overvatn, V. Kirkin, and T. Johansen. 2012. ATG8 family proteins act as scaffolds for assembly of the ULK complex: sequence requirements for LC3-interacting region (LIR) motifs. *J Biol Chem*. 287:39275-39290.

Amar, N., G. Lustig, Y. Ichimura, Y. Ohsumi, and Z. Elazar. 2006. Two newly identified sites in the ubiquitin-like protein Atg8 are essential for autophagy. *EMBO Rep*. 7:635-642.

Araki, Y., W.C. Ku, M. Akioka, A.I. May, Y. Hayashi, F. Arisaka, Y. Ishihama, and Y. Ohsumi. 2013. Atg38 is required for autophagy-specific phosphatidylinositol 3-kinase complex integrity. *J Cell Biol*. 203:299-313.

Axe, E.L., S.A. Walker, M. Manifava, P. Chandra, H.L. Roderick, A. Habermann, G. Griffiths, and N.T. Ktistakis. 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol*. 182:685-701.

Bai, X., D. Ma, A. Liu, X. Shen, Q.J. Wang, Y. Liu, and Y. Jiang. 2007. Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. *Science*. 318:977-980.

Banasavadi-Siddegowda, Y.K., J. Mai, Y. Fan, S. Bhattacharya, D.R. Giovannucci, E.R. Sanchez, G. Fischer, and X. Wang. 2011. FKBP38 peptidylprolyl isomerase promotes the folding of cystic fibrosis transmembrane conductance regulator in the endoplasmic reticulum. *J Biol Chem*. 286:43071-43080.

Baskaran, S., L.A. Carlson, G. Stjepanovic, L.N. Young, J. Kim do, P. Grob, R.E. Stanley, E. Nogales, and J.H. Hurley. 2014. Architecture and dynamics of the autophagic phosphatidylinositol 3-kinase complex. *Elife*. 3.

Bellot, G., R. Garcia-Medina, P. Gounon, J. Chiche, D. Roux, J. Pouyssegur, and N.M. Mazure. 2009. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol*. 29:2570-2581.

Berg, T.O., M. Fengsrud, P.E. Stromhaug, T. Berg, and P.O. Seglen. 1998. Isolation and characterization of rat liver amphisomes. Evidence for fusion of autophagosomes with both early and late endosomes. *J Biol Chem*. 273:21883-21892.

Beyer, K., and B. Nuscher. 1996. Specific cardiolipin binding interferes with labeling of sulfhydryl residues in the adenosine diphosphate/adenosine triphosphate carrier protein from beef heart mitochondria. *Biochemistry*. 35:15784-15790.

Birgisdottir, A.B., T. Lamark, and T. Johansen. 2013. The LIR motif - crucial for selective autophagy. *J Cell Sci*. 126:3237-3247.

Bjorkoy, G., T. Lamark, A. Brech, H. Outzen, M. Perander, A. Overvatn, H. Stenmark, and T. Johansen. 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol*. 171:603-614.

Blackburn, E.A., and M.D. Walkinshaw. 2011. Targeting FKBP isoforms with small-molecule ligands. *Curr Opin Pharmacol*. 11:365-371.

Brocker, C., S. Engelbrecht-Vandre, and C. Ungermann. 2010. Multisubunit tethering complexes and their role in membrane fusion. *Curr Biol*. 20:R943-952.

Burnett, S.F., J.C. Farre, T.Y. Nazarko, and S. Subramani. 2015. Peroxisomal Pex3 activates selective autophagy of peroxisomes via interaction with the pexophagy receptor Atg30. *J Biol Chem*. 290:8623-8631.

Cemma, M., P.K. Kim, and J.H. Brumell. 2011. The ubiquitin-binding adaptor proteins p62/SQSTM1 and NDP52 are recruited independently to bacteria-associated microdomains to target Salmonella to the autophagy pathway. *Autophagy*. 7:341-345.

Chang, T.K., B.V. Shravage, S.D. Hayes, C.M. Powers, R.T. Simin, J. Wade Harper, and E.H. Baehrecke. 2013. Uba1 functions in Atg7- and Atg3-independent autophagy. *Nat Cell Biol*. 15:1067-1078.

Chaugule, V.K., L. Burchell, K.R. Barber, A. Sidhu, S.J. Leslie, G.S. Shaw, and H. Walden. 2011. Autoregulation of Parkin activity through its ubiquitin-like domain. *EMBO J*. 30:2853-2867.

Chen, G., Z. Han, D. Feng, Y. Chen, L. Chen, H. Wu, L. Huang, C. Zhou, X. Cai, C. Fu, L. Duan, X. Wang, L. Liu, X. Liu, Y. Shen, Y. Zhu, and Q. Chen. 2014. A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. *Mol Cell*. 54:362-377.

Chen, G., R. Ray, D. Dubik, L. Shi, J. Cizeau, R.C. Bleackley, S. Saxena, R.D. Gietz, and A.H. Greenberg. 1997. The E1B 19K/Bcl-2-binding protein Nip3 is a dimeric mitochondrial protein that activates apoptosis. *J Exp Med*. 186:1975-1983.

Chen, Y., and G.W. Dorn, 2nd. 2013. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science*. 340:471-475.

Cho, A., H.W. Ko, and J.T. Eggenschwiler. 2008. FKBP8 cell-autonomously controls neural tube patterning through a Gli2- and Kif3a-dependent mechanism. *Dev Biol*. 321:27-39.

Choi, B.H., L. Feng, and H.S. Yoon. 2010. FKBP38 protects Bcl-2 from caspase-dependent degradation. *J Biol Chem*. 285:9770-9779.

Choubey, V., M. Cagalinec, J. Liiv, D. Safiulina, M.A. Hickey, M. Kuum, M. Liiv, T. Anwar, E.L. Eskelinen, and A. Kaasik. 2014. BECN1 is involved in the initiation of mitophagy: it facilitates PARK2 translocation to mitochondria. *Autophagy*. 10:1105-1119.

Chu, C.T., J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, V.A. Tyurin, N. Yanamala, I.H. Shrivastava, D. Mohammadyani, K.Z. Qiang Wang, J. Zhu, J. Klein-Seetharaman, K. Balasubramanian, A.A. Amoscato, G. Borisenko, Z. Huang, A.M. Gusdon, A. Cheikhi, E.K. Steer, R. Wang, C. Baty, S. Watkins, I. Bahar, H. Bayir, and V.E. Kagan. 2013. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat Cell Biol*. 15:1197-1205.

Ciechanover, A. 2015. The unravelling of the ubiquitin system. *Nat Rev Mol Cell Biol*. 16:322-324.

Cunningham, C.N., J.M. Baughman, L. Phu, J.S. Tea, C. Yu, M. Coons, D.S. Kirkpatrick, B. Bingol, and J.E. Corn. 2015. USP30 and parkin homeostatically regulate atypical ubiquitin chains on mitochondria. *Nat Cell Biol*. 17:160-169.

De Duve, C. 1963. The lysosome. *Sci Am*. 208:64-72.

De Duve, C., B.C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans. 1955. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem J*. 60:604-617.

De Duve, C., and R. Wattiaux. 1966. Functions of lysosomes. *Annu Rev Physiol*. 28:435-492.

Deas, E., H. Plun-Favreau, S. Gandhi, H. Desmond, S. Kjaer, S.H. Loh, A.E. Renton, R.J. Harvey, A.J. Whitworth, L.M. Martins, A.Y. Abramov, and N.W. Wood. 2011. PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet*. 20:867-879.

Deosaran, E., K.B. Larsen, R. Hua, G. Sargent, Y. Wang, S. Kim, T. Lamark, M. Jauregui, K. Law, J. Lippincott-Schwartz, A. Brech, T. Johansen, and P.K. Kim. 2013. NBR1 acts as an autophagy receptor for peroxisomes. *J Cell Sci*. 126:939-952.

Diao, J., R. Liu, Y. Rong, M. Zhao, J. Zhang, Y. Lai, Q. Zhou, L.M. Wilz, J. Li, S. Vivona, R.A. Pfuetzner, A.T. Brunger, and Q. Zhong. 2015. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. *Nature*. 520:563-566.

Ding, W.X., H.M. Ni, M. Li, Y. Liao, X. Chen, D.B. Stolz, G.W. Dorn, 2nd, and X.M. Yin. 2010. Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. *J Biol Chem*. 285:27879-27890.

Dooley, H.C., M. Razi, H.E. Polson, S.E. Girardin, M.I. Wilson, and S.A. Tooze. 2014. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. *Mol Cell*. 55:238-252.

Edlich, F., M. Maestre-Martinez, F. Jarczowski, M. Weiwad, M.C. Moutty, M. Malesevic, G. Jahreis, G. Fischer, and C. Lucke. 2007. A novel calmodulin-Ca2+ target recognition activates the Bcl-2 regulator FKBP38. *J Biol Chem*. 282:36496-36504.

Egan, D.F., D.B. Shackelford, M.M. Mihaylova, S. Gelino, R.A. Kohnz, W. Mair, D.S. Vasquez, A. Joshi, D.M. Gwinn, R. Taylor, J.M. Asara, J. Fitzpatrick, A. Dillin, B. Viollet, M. Kundu, M. Hansen, and R.J. Shaw. 2011. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science*. 331:456-461.

Erbil, S., O. Oral, G. Mitou, C. Kig, E. Durmaz-Timucin, E. Guven-Maiorov, F. Gulacti, G. Gokce, J. Dengjel, O.U. Sezerman, and D. Gozuacik. 2016. RACK1 is an Interaction Partner of ATG5 and a Novel Regulator of Autophagy. *J Biol Chem*.

Ernster, L., and G. Schatz. 1981. Mitochondria: a historical review. *J Cell Biol*. 91:227s-255s.

Fan, W., A. Nassiri, and Q. Zhong. 2011. Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). *Proc Natl Acad Sci U S A*. 108:7769-7774.

Fang, Y., M. Vilella-Bach, R. Bachmann, A. Flanigan, and J. Chen. 2001. Phosphatidic acid-mediated mitogenic activation of mTOR signaling. *Science*. 294:1942-1945.

Farre, J.C., A. Burkenroad, S.F. Burnett, and S. Subramani. 2013. Phosphorylation of mitophagy and pexophagy receptors coordinates their interaction with Atg8 and Atg11. *EMBO Rep*. 14:441-449.

Fiesel, F.C., E.L. Moussaud-Lamodiere, M. Ando, and W. Springer. 2014. A specific subset of E2 ubiquitin-conjugating enzymes regulate Parkin activation and mitophagy differently. *J Cell Sci*. 127:3488-3504.

Fimia, G.M., A. Stoykova, A. Romagnoli, L. Giunta, S. Di Bartolomeo, R. Nardacci, M. Corazzari, C. Fuoco, A. Ucar, P. Schwartz, P. Gruss, M. Piacentini, K. Chowdhury, and F. Cecconi. 2007. Ambra1 regulates autophagy and development of the nervous system. *Nature*. 447:1121-1125.

Fogel, A.I., B.J. Dlouhy, C. Wang, S.W. Ryu, A. Neutzner, S.A. Hasson, D.P. Sideris, H. Abeliovich, and R.J. Youle. 2013. Role of membrane association and Atg14-dependent phosphorylation in beclin-1-mediated autophagy. *Mol Cell Biol*. 33:3675-3688.

Frezza, C., S. Cipolat, O. Martins de Brito, M. Micaroni, G.V. Beznoussenko, T. Rudka, D. Bartoli, R.S. Polishuck, N.N. Danial, B. De Strooper, and L. Scorrano. 2006. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell*. 126:177-189.

Friedman, J.R., L.L. Lackner, M. West, J.R. DiBenedetto, J. Nunnari, and G.K. Voeltz. 2011. ER tubules mark sites of mitochondrial division. *Science*. 334:358-362.

Fritz, S., D. Rapaport, E. Klanner, W. Neupert, and B. Westermann. 2001. Connection of the mitochondrial outer and inner membranes by Fzo1 is critical for organellar fusion. *J Cell Biol*. 152:683-692.

Fujita, N., M. Hayashi-Nishino, H. Fukumoto, H. Omori, A. Yamamoto, T. Noda, and T. Yoshimori. 2008a. An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. *Mol Biol Cell*. 19:4651-4659.

Fujita, N., T. Itoh, H. Omori, M. Fukuda, T. Noda, and T. Yoshimori. 2008b. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell*. 19:2092-2100.

Funakoshi, T., A. Matsuura, T. Noda, and Y. Ohsumi. 1997. Analyses of APG13 gene involved in autophagy in yeast, Saccharomyces cerevisiae. *Gene*. 192:207-213.

Galat, A. 2000. Sequence diversification of the FK506-binding proteins in several different genomes. *Eur J Biochem*. 267:4945-4959.

Gammoh, N., O. Florey, M. Overholtzer, and X. Jiang. 2013. Interaction between FIP200 and ATG16L1 distinguishes ULK1 complex-dependent and -independent autophagy. *Nat Struct Mol Biol*. 20:144-149.

Ganley, I.G. 2013. Autophagosome maturation and lysosomal fusion. *Essays Biochem*. 55:65-78.

Ganschow, R.E., and R.T. Schimke. 1969. Independent genetic control of the catalytic activity and the rate of degradation of catalase in mice. *J Biol Chem*. 244:4649-4658.

Geisler, S., S. Vollmer, S. Golombek, and P.J. Kahle. 2014. The ubiquitin-conjugating enzymes UBE2N, UBE2L3 and UBE2D2/3 are essential for Parkin-dependent mitophagy. *J Cell Sci*. 127:3280-3293.

Gelmetti, V., P. De Rosa, L. Torosantucci, E.S. Marini, A. Romagnoli, M. Di Rienzo, G. Arena, D. Vignone, G.M. Fimia, and E.M. Valente. 2017. PINK1 and BECN1 relocalize at mitochondria-associated membranes during mitophagy and promote ER-mitochondria tethering and autophagosome formation. *Autophagy*. 13:654-669.

Gibbings, D., S. Mostowy, F. Jay, Y. Schwab, P. Cossart, and O. Voinnet. 2012. Selective autophagy degrades DICER and AGO2 and regulates miRNA activity. *Nat Cell Biol*. 14:1314-1321.

Gordon, P.B., and P.O. Seglen. 1988. Prelysosomal convergence of autophagic and endocytic pathways. *Biochem Biophys Res Commun*. 151:40-47.

Guo, K., G. Searfoss, D. Krolikowski, M. Pagnoni, C. Franks, K. Clark, K.T. Yu, M. Jaye, and Y. Ivashchenko. 2001. Hypoxia induces the expression of the pro-apoptotic gene BNIP3. *Cell Death Differ*. 8:367-376.

Ham, S.J., S.Y. Lee, S. Song, J.R. Chung, S. Choi, and J. Chung. 2016. Interaction between RING1 (R1) and the Ubiquitin-like (UBL) Domains Is Critical for the Regulation of Parkin Activity. *J Biol Chem*. 291:1803-1816.

Hamacher-Brady, A., N.R. Brady, S.E. Logue, M.R. Sayen, M. Jinno, L.A. Kirshenbaum, R.A. Gottlieb, and A.B. Gustafsson. 2007. Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death Differ*. 14:146-157.

Hamasaki, M., N. Furuta, A. Matsuda, A. Nezu, A. Yamamoto, N. Fujita, H. Oomori, T. Noda, T. Haraguchi, Y. Hiraoka, A. Amano, and T. Yoshimori. 2013. Autophagosomes form at ER-mitochondria contact sites. *Nature*. 495:389-393.

Hammerling, B.C., R.H. Najor, M.Q. Cortez, S.E. Shires, L.J. Leon, E.R. Gonzalez, D. Boassa, S. Phan, A. Thor, R.E. Jimenez, H. Li, R.N. Kitsis, G.W. Dorn Ii, J. Sadoshima, M.H. Ellisman, and A.B. Gustafsson. 2017. A Rab5 endosomal pathway mediates Parkin-dependent mitochondrial clearance. *Nat Commun*. 8:14050.

Hanada, T., N.N. Noda, Y. Satomi, Y. Ichimura, Y. Fujioka, T. Takao, F. Inagaki, and Y. Ohsumi. 2007. The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. *J Biol Chem*. 282:37298-37302.

Hanna, R.A., M.N. Quinsay, A.M. Orogo, K. Giang, S. Rikka, and A.B. Gustafsson. 2012. Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to

selectively remove endoplasmic reticulum and mitochondria via autophagy. *J Biol Chem*. 287:19094-19104.

Harding, T.M., K.A. Morano, S.V. Scott, and D.J. Klionsky. 1995. Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. *J Cell Biol*. 131:591-602.

Haupt, K., G. Jahreis, M. Linnert, M. Maestre-Martinez, M. Malesevic, A. Pechstein, F. Edlich, and C. Lucke. 2012. The FKBP38 catalytic domain binds to Bcl-2 via a charge-sensitive loop. *J Biol Chem*. 287:19665-19673.

Heo, J.M., A. Ordureau, J.A. Paulo, J. Rinehart, and J.W. Harper. 2015. The PINK1-PARKIN Mitochondrial Ubiquitylation Pathway Drives a Program of OPTN/NDP52 Recruitment and TBK1 Activation to Promote Mitophagy. *Mol Cell*. 60:7-20.

Hirota, Y., S. Yamashita, Y. Kurihara, X. Jin, M. Aihara, T. Saigusa, D. Kang, and T. Kanki. 2015. Mitophagy is primarily due to alternative autophagy and requires the MAPK1 and MAPK14 signaling pathways. *Autophagy*. 11:332-343.

Hochstrasser, M. 2009. Origin and function of ubiquitin-like proteins. *Nature*. 458:422-429.

Honda, S., S. Arakawa, Y. Nishida, H. Yamaguchi, E. Ishii, and S. Shimizu. 2014. Ulk1-mediated Atg5-independent macroautophagy mediates elimination of mitochondria from embryonic reticulocytes. *Nat Commun*. 5:4004.

Hoppins, S., F. Edlich, M.M. Cleland, S. Banerjee, J.M. McCaffery, R.J. Youle, and J. Nunnari. 2011. The soluble form of Bax regulates mitochondrial fusion via MFN2 homotypic complexes. *Mol Cell*. 41:150-160.

Huang, H., and K.G. Manton. 2004. The role of oxidative damage in mitochondria during aging: a review. *Front Biosci*. 9:1100-1117.

Hutt, D.M., D.M. Roth, M.A. Chalfant, R.T. Youker, J. Matteson, J.L. Brodsky, and W.E. Balch. 2012. FK506 binding protein 8 peptidylprolyl isomerase activity manages a late stage of cystic fibrosis transmembrane conductance regulator (CFTR) folding and stability. *J Biol Chem*. 287:21914-21925.

Ichimura, Y., T. Kirisako, T. Takao, Y. Satomi, Y. Shimonishi, N. Ishihara, N. Mizushima, I. Tanida, E. Kominami, M. Ohsumi, T. Noda, and Y. Ohsumi. 2000. A ubiquitin-like system mediates protein lipidation. *Nature*. 408:488-492.

Ichimura, Y., T. Kumanomidou, Y.S. Sou, T. Mizushima, J. Ezaki, T. Ueno, E. Kominami, T. Yamane, K. Tanaka, and M. Komatsu. 2008. Structural basis for sorting mechanism of p62 in selective autophagy. *J Biol Chem*. 283:22847-22857.

Inomata, M., S. Niida, K. Shibata, and T. Into. 2012. Regulation of Toll-like receptor signaling by NDP52-mediated selective autophagy is normally inactivated by A20. *Cell Mol Life Sci*. 69:963-979.

Ishibashi, K., N. Fujita, E. Kanno, H. Omori, T. Yoshimori, T. Itoh, and M. Fukuda. 2011. Atg16L2, a novel isoform of mammalian Atg16L that is not essential for canonical autophagy despite forming an Atg12-5-16L2 complex. *Autophagy*. 7:1500-1513.

Itakura, E., C. Kishi-Itakura, and N. Mizushima. 2012. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. *Cell*. 151:1256-1269.

Itakura, E., C. Kishi, K. Inoue, and N. Mizushima. 2008. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell*. 19:5360-5372.

Itoh, T., E. Kanno, T. Uemura, S. Waguri, and M. Fukuda. 2011. OATL1, a novel autophagosome-resident Rab33B-GAP, regulates autophagosomal maturation. *J Cell Biol*. 192:839-853.

Jahreiss, L., F.M. Menzies, and D.C. Rubinsztein. 2008. The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. *Traffic*. 9:574-587.

James, D.I., P.A. Parone, Y. Mattenberger, and J.C. Martinou. 2003. hFis1, a novel component of the mammalian mitochondrial fission machinery. *J Biol Chem*. 278:36373-36379.

Jao, C.C., M.J. Ragusa, R.E. Stanley, and J.H. Hurley. 2013. A HORMA domain in Atg13 mediates PI 3-kinase recruitment in autophagy. *Proc Natl Acad Sci U S A*. 110:5486-5491.

Jiang, P., and N. Mizushima. 2014. Autophagy and human diseases. *Cell Res*. 24:69-79.

Jin, S.M., M. Lazarou, C. Wang, L.A. Kane, D.P. Narendra, and R.J. Youle. 2010. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol*. 191:933-942.

Joachim, J., H.B. Jefferies, M. Razi, D. Frith, A.P. Snijders, P. Chakravarty, D. Judith, and S.A. Tooze. 2015. Activation of ULK Kinase and Autophagy by GABARAP Trafficking from the Centrosome Is Regulated by WAC and GM130. *Mol Cell*. 60:899-913.

Joo, J.H., F.C. Dorsey, A. Joshi, K.M. Hennessy-Walters, K.L. Rose, K. McCastlain, J. Zhang, R. Iyengar, C.H. Jung, D.F. Suen, M.A. Steeves, C.Y. Yang, S.M. Prater, D.H. Kim, C.B. Thompson, R.J. Youle, P.A. Ney, J.L. Cleveland, and M. Kundu. 2011. Hsp90-Cdc37 chaperone complex regulates Ulk1- and Atg13-mediated mitophagy. *Mol Cell*. 43:572-585.

Jordens, I., M. Fernandez-Borja, M. Marsman, S. Dusseljee, L. Janssen, J. Calafat, H. Janssen, R. Wubbolts, and J. Neefjes. 2001. The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. *Curr Biol*. 11:1680-1685.

Jung, C.H., C.B. Jun, S.H. Ro, Y.M. Kim, N.M. Otto, J. Cao, M. Kundu, and D.H. Kim. 2009. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell*. 20:1992-2003.

Kabeya, Y., Y. Kamada, M. Baba, H. Takikawa, M. Sasaki, and Y. Ohsumi. 2005. Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy. *Mol Biol Cell*. 16:2544-2553.

Kabeya, Y., N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, and T. Yoshimori. 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J*. 19:5720-5728.

Kabeya, Y., N. Mizushima, A. Yamamoto, S. Oshitani-Okamoto, Y. Ohsumi, and T. Yoshimori. 2004. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci*. 117:2805-2812.

Kamada, Y., T. Funakoshi, T. Shintani, K. Nagano, M. Ohsumi, and Y. Ohsumi. 2000. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J Cell Biol*. 150:1507-1513.

Kametaka, S., T. Okano, M. Ohsumi, and Y. Ohsumi. 1998. Apg14p and Apg6/Vps30p form a protein complex essential for autophagy in the yeast, Saccharomyces cerevisiae. *J Biol Chem*. 273:22284-22291.

Kane, L.A., M. Lazarou, A.I. Fogel, Y. Li, K. Yamano, S.A. Sarraf, S. Banerjee, and R.J. Youle. 2014. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol*. 205:143-153.

Kang, C., H. Ye, J. Chia, B.H. Choi, S. Dhe-Paganon, B. Simon, U. Schutz, M. Sattler, and H.S. Yoon. 2013. Functional role of the flexible N-terminal extension of FKBP38 in catalysis. *Sci Rep*. 3:2985.

Kanki, T., K. Wang, Y. Cao, M. Baba, and D.J. Klionsky. 2009. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev Cell*. 17:98-109.

Karanasios, E., E. Stapleton, M. Manifava, T. Kaizuka, N. Mizushima, S.A. Walker, and N.T. Ktistakis. 2013. Dynamic association of the ULK1 complex with omegasomes during autophagy induction. *J Cell Sci*. 126:5224-5238.

Karbowski, M., and R.J. Youle. 2011. Regulating mitochondrial outer membrane proteins by ubiquitination and proteasomal degradation. *Curr Opin Cell Biol*. 23:476-482.

Kataoka, T., N. Holler, O. Micheau, F. Martinon, A. Tinel, K. Hofmann, and J. Tschopp. 2001. Bcl-rambo, a novel Bcl-2 homologue that induces apoptosis via its unique C-terminal extension. *J Biol Chem*. 276:19548-19554.

Kaufmann, A., V. Beier, H.G. Franquelim, and T. Wollert. 2014. Molecular mechanism of autophagic membrane-scaffold assembly and disassembly. *Cell*. 156:469-481.

Khaminets, A., T. Heinrich, M. Mari, P. Grumati, A.K. Huebner, M. Akutsu, L. Liebmann, A. Stolz, S. Nietzsche, N. Koch, M. Mauthe, I. Katona, B. Qualmann, J. Weis, F. Reggiori, I. Kurth, C.A. Hubner, and I. Dikic. 2015. Regulation of endoplasmic reticulum turnover by selective autophagy. *Nature*. 522:354-358.

Kihara, A., T. Noda, N. Ishihara, and Y. Ohsumi. 2001. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae. *J Cell Biol*. 152:519-530.

Kim, J., Y.C. Kim, C. Fang, R.C. Russell, J.H. Kim, W. Fan, R. Liu, Q. Zhong, and K.L. Guan. 2013. Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell*. 152:290-303.

King, A., M.A. Selak, and E. Gottlieb. 2006. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. *Oncogene*. 25:4675-4682.

Kino, T., H. Hatanaka, S. Miyata, N. Inamura, M. Nishiyama, T. Yajima, T. Goto, M. Okuhara, M. Kohsaka, H. Aoki, and et al. 1987. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. *J Antibiot (Tokyo)*. 40:1256-1265.

Kirisako, T., M. Baba, N. Ishihara, K. Miyazawa, M. Ohsumi, T. Yoshimori, T. Noda, and Y. Ohsumi. 1999. Formation process of autophagosome is traced with Apg8/Aut7p in yeast. *J Cell Biol*. 147:435-446.

Kirisako, T., Y. Ichimura, H. Okada, Y. Kabeya, N. Mizushima, T. Yoshimori, M. Ohsumi, T. Takao, T. Noda, and Y. Ohsumi. 2000. The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J Cell Biol*. 151:263-276.

Kirkin, V., T. Lamark, T. Johansen, and I. Dikic. 2009a. NBR1 cooperates with p62 in selective autophagy of ubiquitinated targets. *Autophagy*. 5:732-733.

Kirkin, V., T. Lamark, Y.S. Sou, G. Bjorkoy, J.L. Nunn, J.A. Bruun, E. Shvets, D.G. McEwan, T.H. Clausen, P. Wild, I. Bilusic, J.P. Theurillat, A. Overvatn, T. Ishii, Z. Elazar, M. Komatsu, I. Dikic, and T. Johansen. 2009b. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol Cell*. 33:505-516.

Klionsky, D.J. 2007. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol*. 8:931-937.

Komatsu, M., S. Waguri, M. Koike, Y.S. Sou, T. Ueno, T. Hara, N. Mizushima, J. Iwata, J. Ezaki, S. Murata, J. Hamazaki, Y. Nishito, S. Iemura, T. Natsume, T. Yanagawa, J. Uwayama, E. Warabi, H. Yoshida, T. Ishii, A. Kobayashi, M. Yamamoto, Z. Yue, Y. Uchiyama, E. Kominami, and K. Tanaka. 2007. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell*. 131:1149-1163.

Kondapalli, C., A. Kazlauskaite, N. Zhang, H.I. Woodroof, D.G. Campbell, R. Gourlay, L. Burchell, H. Walden, T.J. Macartney, M. Deak, A. Knebel, D.R. Alessi, and M.M. Muqit. 2012. PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol*. 2:120080.

Koshiba, T., S.A. Detmer, J.T. Kaiser, H. Chen, J.M. McCaffery, and D.C. Chan. 2004. Structural basis of mitochondrial tethering by mitofusin complexes. *Science*. 305:858-862.

Koyama-Honda, I., E. Itakura, T.K. Fujiwara, and N. Mizushima. 2013. Temporal analysis of recruitment of mammalian ATG proteins to the autophagosome formation site. *Autophagy*. 9:1491-1499.

Koyano, F., K. Okatsu, H. Kosako, Y. Tamura, E. Go, M. Kimura, Y. Kimura, H. Tsuchiya, H. Yoshihara, T. Hirokawa, T. Endo, E.A. Fon, J.F. Trempe, Y. Saeki, K. Tanaka, and N. Matsuda. 2014. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature*. 510:162-166.

Kraft, C., M. Kijanska, E. Kalie, E. Siergiejuk, S.S. Lee, G. Semplicio, I. Stoffel, A. Brezovich, M. Verma, I. Hansmann, G. Ammerer, K. Hofmann, S. Tooze, and M. Peter. 2012. Binding of the Atg1/ULK1 kinase to the ubiquitin-like protein Atg8 regulates autophagy. *EMBO J*. 31:3691-3703.

Krick, R., S. Henke, J. Tolstrup, and M. Thumm. 2008. Dissecting the localization and function of Atg18, Atg21 and Ygr223c. *Autophagy*. 4:896-910.

Kundu, M., T. Lindsten, C.Y. Yang, J. Wu, F. Zhao, J. Zhang, M.A. Selak, P.A. Ney, and C.B. Thompson. 2008. Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. *Blood*. 112:1493-1502.

Kuusisto, E., A. Salminen, and I. Alafuzoff. 2001. Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. *Neuroreport*. 12:2085-2090.

Lam, E., M. Martin, and G. Wiederrecht. 1995. Isolation of a cDNA encoding a novel human FK506-binding protein homolog containing leucine zipper and tetratricopeptide repeat motifs. *Gene*. 160:297-302.

Lamb, C.A., T. Yoshimori, and S.A. Tooze. 2013. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol*. 14:759-774.

Langer, T., M. Kaser, C. Klanner, and K. Leonhard. 2001. AAA proteases of mitochondria: quality control of membrane proteins and regulatory functions during mitochondrial biogenesis. *Biochem Soc Trans*. 29:431-436.

Lazarou, M., D.A. Sliter, L.A. Kane, S.A. Sarraf, C. Wang, J.L. Burman, D.P. Sideris, A.I. Fogel, and R.J. Youle. 2015. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature*. 524:309-314.

Lee, Y., H.Y. Lee, R.A. Hanna, and A.B. Gustafsson. 2011. Mitochondrial autophagy by Bnip3 involves Drp1-mediated mitochondrial fission and recruitment of Parkin in cardiac myocytes. *Am J Physiol Heart Circ Physiol*. 301:H1924-1931.

Leonhard, K., J.M. Herrmann, R.A. Stuart, G. Mannhaupt, W. Neupert, and T. Langer. 1996. AAA proteases with catalytic sites on opposite membrane surfaces comprise a proteolytic system for the ATP-dependent degradation of inner membrane proteins in mitochondria. *EMBO J*. 15:4218-4229.

Li, H., C. Zhang, H. Cui, K. Guo, F. Wang, T. Zhao, W. Liang, Q. Lv, and Y. Zhang. 2016. FKBP8 interact with classical swine fever virus NS5A protein and promote virus RNA replication. *Virus Genes*. 52:99-106.

Li, X., L. He, K.H. Che, S.F. Funderburk, L. Pan, N. Pan, M. Zhang, Z. Yue, and Y. Zhao. 2012. Imperfect interface of Beclin-1 coiled-coil domain regulates homodimer and heterodimer formation with Atg14L and UVRAG. *Nat Commun*. 3:662.

Liang, C., J.S. Lee, K.S. Inn, M.U. Gack, Q. Li, E.A. Roberts, I. Vergne, V. Deretic, P. Feng, C. Akazawa, and J.U. Jung. 2008. Beclin-1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol*. 10:776-787.

Liesa, M., M. Palacin, and A. Zorzano. 2009. Mitochondrial dynamics in mammalian health and disease. *Physiol Rev*. 89:799-845.

Liu, L., D. Feng, G. Chen, M. Chen, Q. Zheng, P. Song, Q. Ma, C. Zhu, R. Wang, W. Qi, L. Huang, P. Xue, B. Li, X. Wang, H. Jin, J. Wang, F. Yang, P. Liu, Y. Zhu, S. Sui, and Q. Chen. 2012. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat Cell Biol*. 14:177-185.

Lystad, A.H., Y. Ichimura, K. Takagi, Y. Yang, S. Pankiv, Y. Kanegae, S. Kageyama, M. Suzuki, I. Saito, T. Mizushima, M. Komatsu, and A. Simonsen. 2014. Structural determinants in GABARAP required for the selective binding and recruitment of ALFY to LC3B-positive structures. *EMBO Rep*. 15:557-565.

Ma, D., X. Bai, H. Zou, Y. Lai, and Y. Jiang. 2010. Rheb GTPase controls apoptosis by regulating interaction of FKBP38 with Bcl-2 and Bcl-XL. *J Biol Chem*. 285:8621-8627.

Mammucari, C., G. Milan, V. Romanello, E. Masiero, R. Rudolf, P. Del Piccolo, S.J. Burden, R. Di Lisi, C. Sandri, J. Zhao, A.L. Goldberg, S. Schiaffino, and M. Sandri. 2007. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metab*. 6:458-471.

Marinkovic, D., X. Zhang, S. Yalcin, J.P. Luciano, C. Brugnara, T. Huber, and S. Ghaffari. 2007. Foxo3 is required for the regulation of oxidative stress in erythropoiesis. *J Clin Invest*. 117:2133-2144.

Matsumoto, G., K. Wada, M. Okuno, M. Kurosawa, and N. Nukina. 2011. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol Cell*. 44:279-289.

Matsunaga, K., E. Morita, T. Saitoh, S. Akira, N.T. Ktistakis, T. Izumi, T. Noda, and T. Yoshimori. 2010. Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. *J Cell Biol*. 190:511-521.

Matsunaga, K., T. Saitoh, K. Tabata, H. Omori, T. Satoh, N. Kurotori, I. Maejima, K. Shirahama-Noda, T. Ichimura, T. Isobe, S. Akira, T. Noda, and T. Yoshimori. 2009. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol*. 11:385-396.

Matsushima, M., T. Fujiwara, E. Takahashi, T. Minaguchi, Y. Eguchi, Y. Tsujimoto, K. Suzumori, and Y. Nakamura. 1998. Isolation, mapping, and functional analysis of a novel human cDNA (BNIP3L) encoding a protein homologous to human NIP3. *Genes Chromosomes Cancer*. 21:230-235.

Matsuura, A., M. Tsukada, Y. Wada, and Y. Ohsumi. 1997. Apg1p, a novel protein kinase required for the autophagic process in Saccharomyces cerevisiae. *Gene*. 192:245-250.

Mazure, N.M., and J. Pouyssegur. 2009. Atypical BH3-domains of BNIP3 and BNIP3L lead to autophagy in hypoxia. *Autophagy*. 5:868-869.

McLelland, G.L., V. Soubannier, C.X. Chen, H.M. McBride, and E.A. Fon. 2014. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. *EMBO J*. 33:282-295.

Meissner, C., H. Lorenz, A. Weihofen, D.J. Selkoe, and M.K. Lemberg. 2011. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J Neurochem*. 117:856-867.

Mercer, C.A., A. Kaliappan, and P.B. Dennis. 2009. A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy*. 5:649-662.

Mijaljica, D., M. Prescott, and R.J. Devenish. 2011. Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. *Autophagy*. 7:673-682.

Mishra, P., and D.C. Chan. 2014. Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat Rev Mol Cell Biol*. 15:634-646.

Mizushima, N. 2007. Autophagy: process and function. *Genes Dev*. 21:2861-2873.

Mizushima, N., T. Noda, and Y. Ohsumi. 1999. Apg16p is required for the function of the Apg12p-Apg5p conjugate in the yeast autophagy pathway. *EMBO J*. 18:3888-3896.

Mizushima, N., T. Noda, T. Yoshimori, Y. Tanaka, T. Ishii, M.D. George, D.J. Klionsky, M. Ohsumi, and Y. Ohsumi. 1998. A protein conjugation system essential for autophagy. *Nature*. 395:395-398.

Mizushima, N., A. Yamamoto, M. Hatano, Y. Kobayashi, Y. Kabeya, K. Suzuki, T. Tokuhisa, Y. Ohsumi, and T. Yoshimori. 2001. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J Cell Biol*. 152:657-668.

Mochida, K., Y. Oikawa, Y. Kimura, H. Kirisako, H. Hirano, Y. Ohsumi, and H. Nakatogawa. 2015. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature*. 522:359-362.

Mostowy, S., V. Sancho-Shimizu, M.A. Hamon, R. Simeone, R. Brosch, T. Johansen, and P. Cossart. 2011. p62 and NDP52 proteins target intracytosolic Shigella and Listeria to different autophagy pathways. *J Biol Chem*. 286:26987-26995.

Murakawa, T., O. Yamaguchi, A. Hashimoto, S. Hikoso, T. Takeda, T. Oka, H. Yasui, H. Ueda, Y. Akazawa, H. Nakayama, M. Taneike, T. Misaka, S. Omiya, A.M. Shah, A. Yamamoto, K. Nishida, Y. Ohsumi, K. Okamoto, Y. Sakata, and K. Otsu. 2015. Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. *Nat Commun*. 6:7527.

Nakagawa, T., M. Shirane, S. Iemura, T. Natsume, and K.I. Nakayama. 2007. Anchoring of the 26S proteasome to the organellar membrane by FKBP38. *Genes Cells*. 12:709-719.

Nakatogawa, H., Y. Ichimura, and Y. Ohsumi. 2007. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell*. 130:165-178.

Nakatogawa, H., S. Ohbayashi, M. Sakoh-Nakatogawa, S. Kakuta, S.W. Suzuki, H. Kirisako, C. Kondo-Kakuta, N.N. Noda, H. Yamamoto, and Y. Ohsumi. 2012. The autophagy-related protein kinase Atg1 interacts with the ubiquitin-like protein Atg8 via the Atg8 family interacting motif to facilitate autophagosome formation. *J Biol Chem*. 287:28503-28507.

Narendra, D., L.A. Kane, D.N. Hauser, I.M. Fearnley, and R.J. Youle. 2010a. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy*. 6:1090-1106.

Narendra, D., A. Tanaka, D.F. Suen, and R.J. Youle. 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol*. 183:795-803.

Narendra, D.P., S.M. Jin, A. Tanaka, D.F. Suen, C.A. Gautier, J. Shen, M.R. Cookson, and R.J. Youle. 2010b. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol*. 8:e1000298.

Nath, S., J. Dancourt, V. Shteyn, G. Puente, W.M. Fong, S. Nag, J. Bewersdorf, A. Yamamoto, B. Antonny, and T.J. Melia. 2014. Lipidation of the LC3/GABARAP family of autophagy proteins relies on a membrane-curvature-sensing domain in Atg3. *Nat Cell Biol*. 16:415-424.

Newman, A.C., C.L. Scholefield, A.J. Kemp, M. Newman, E.G. McIver, A. Kamal, and S. Wilkinson. 2012. TBK1 kinase addiction in lung cancer cells is mediated via autophagy of Tax1bp1/Ndp52 and non-canonical NF-kappaB signalling. *PLoS One*. 7:e50672.

Nicklin, P., P. Bergman, B. Zhang, E. Triantafellow, H. Wang, B. Nyfeler, H. Yang, M. Hild, C. Kung, C. Wilson, V.E. Myer, J.P. MacKeigan, J.A. Porter, Y.K. Wang, L.C. Cantley, P.M. Finan, and L.O. Murphy. 2009. Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell*. 136:521-534.

Nishida, Y., S. Arakawa, K. Fujitani, H. Yamaguchi, T. Mizuta, T. Kanaseki, M. Komatsu, K. Otsu, Y. Tsujimoto, and S. Shimizu. 2009. Discovery of Atg5/Atg7-independent alternative macroautophagy. *Nature*. 461:654-658.

Nishimura, T., T. Kaizuka, K. Cadwell, M.H. Sahani, T. Saitoh, S. Akira, H.W. Virgin, and N. Mizushima. 2013. FIP200 regulates targeting of Atg16L1 to the isolation membrane. *EMBO Rep*. 14:284-291.

Noda, N.N., H. Kumeta, H. Nakatogawa, K. Satoo, W. Adachi, J. Ishii, Y. Fujioka, Y. Ohsumi, and F. Inagaki. 2008. Structural basis of target recognition by Atg8/LC3 during selective autophagy. *Genes Cells*. 13:1211-1218.

Noda, N.N., and N. Mizushima. 2016. Atg101: Not Just an Accessory Subunit in the Autophagy-initiation Complex. *Cell Struct Funct*. 41:13-20.

Noda, N.N., Y. Ohsumi, and F. Inagaki. 2009. ATG systems from the protein structural point of view. *Chem Rev*. 109:1587-1598.

Noda, N.N., Y. Ohsumi, and F. Inagaki. 2010. Atg8-family interacting motif crucial for selective autophagy. *FEBS Lett*. 584:1379-1385.

Noda, N.N., K. Satoo, Y. Fujioka, H. Kumeta, K. Ogura, H. Nakatogawa, Y. Ohsumi, and F. Inagaki. 2011. Structural basis of Atg8 activation by a homodimeric E1, Atg7. *Mol Cell*. 44:462-475.

Noda, T., J. Kim, W.P. Huang, M. Baba, C. Tokunaga, Y. Ohsumi, and D.J. Klionsky. 2000. Apg9p/Cvt7p is an integral membrane protein required for transport vesicle formation in the Cvt and autophagy pathways. *J Cell Biol*. 148:465-480.

Novak, I., V. Kirkin, D.G. McEwan, J. Zhang, P. Wild, A. Rozenknop, V. Rogov, F. Lohr, D. Popovic, A. Occhipinti, A.S. Reichert, J. Terzic, V. Dotsch, P.A. Ney, and I. Dikic. 2010. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep*. 11:45-51.

Obara, K., T. Sekito, and Y. Ohsumi. 2006. Assortment of phosphatidylinositol 3-kinase complexes--Atg14p directs association of complex I to the pre-autophagosomal structure in Saccharomyces cerevisiae. *Mol Biol Cell*. 17:1527-1539.

Okamoto, K., N. Kondo-Okamoto, and Y. Ohsumi. 2009. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev Cell*. 17:87-97.

Okatsu, K., F. Koyano, M. Kimura, H. Kosako, Y. Saeki, K. Tanaka, and N. Matsuda. 2015. Phosphorylated ubiquitin chain is the genuine Parkin receptor. *J Cell Biol*. 209:111-128.

Okatsu, K., T. Oka, M. Iguchi, K. Imamura, H. Kosako, N. Tani, M. Kimura, E. Go, F. Koyano, M. Funayama, K. Shiba-Fukushima, S. Sato, H. Shimizu, Y. Fukunaga, H. Taniguchi, M. Komatsu, N. Hattori, K. Mihara, K. Tanaka, and N. Matsuda. 2012. PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nat Commun*. 3:1016.

Okatsu, K., K. Saisho, M. Shimanuki, K. Nakada, H. Shitara, Y.S. Sou, M. Kimura, S. Sato, N. Hattori, M. Komatsu, K. Tanaka, and N. Matsuda. 2010. p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes Cells*. 15:887-900.

Okatsu, K., M. Uno, F. Koyano, E. Go, M. Kimura, T. Oka, K. Tanaka, and N. Matsuda. 2013. A dimeric PINK1-containing complex on depolarized mitochondria stimulates Parkin recruitment. *J Biol Chem*. 288:36372-36384.

Olichon, A., L. Baricault, N. Gas, E. Guillou, A. Valette, P. Belenguer, and G. Lenaers. 2003. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. *J Biol Chem*. 278:7743-7746.

Olichon, A., L.J. Emorine, E. Descoins, L. Pelloquin, L. Brichese, N. Gas, E. Guillou, C. Delettre, A. Valette, C.P. Hamel, B. Ducommun, G. Lenaers, and P. Belenguer. 2002.

The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett*. 523:171-176.

Olson, R.E. 1997. The dynamic state of body constituents (Schoenheimer, 1939). *J Nutr*. 127:1041S-1043S.

Olsvik, H.L., T. Lamark, K. Takagi, K.B. Larsen, G. Evjen, A. Overvatn, T. Mizushima, and T. Johansen. 2015. FYCO1 Contains a C-terminally Extended, LC3A/B-preferring LC3-interacting Region (LIR) Motif Required for Efficient Maturation of Autophagosomes during Basal Autophagy. *J Biol Chem*. 290:29361-29374.

Onoue, K., A. Jofuku, R. Ban-Ishihara, T. Ishihara, M. Maeda, T. Koshiba, T. Itoh, M. Fukuda, H. Otera, T. Oka, H. Takano, N. Mizushima, K. Mihara, and N. Ishihara. 2013. Fis1 acts as a mitochondrial recruitment factor for TBC1D15 that is involved in regulation of mitochondrial morphology. *J Cell Sci*. 126:176-185.

Ordureau, A., S.A. Sarraf, D.M. Duda, J.M. Heo, M.P. Jedrychowski, V.O. Sviderskiy, J.L. Olszewski, J.T. Koerber, T. Xie, S.A. Beausoleil, J.A. Wells, S.P. Gygi, B.A. Schulman, and J.W. Harper. 2014. Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis. *Mol Cell*. 56:360-375.

Otera, H., C. Wang, M.M. Cleland, K. Setoguchi, S. Yokota, R.J. Youle, and K. Mihara. 2010. Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *J Cell Biol*. 191:1141-1158.

Palikaras, K., I. Daskalaki, M. Markaki, and N. Tavernarakis. 2017. Mitophagy and age-related pathologies: Development of new therapeutics by targeting mitochondrial turnover. *Pharmacol Ther*.

Palmer, C.S., L.D. Osellame, D. Laine, O.S. Koutsopoulos, A.E. Frazier, and M.T. Ryan. 2011. MiD49 and MiD51, new components of the mitochondrial fission machinery. *EMBO Rep*. 12:565-573.

Pankiv, S., E.A. Alemu, A. Brech, J.A. Bruun, T. Lamark, A. Overvatn, G. Bjorkoy, and T. Johansen. 2010. FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. *J Cell Biol*. 188:253-269.

Pankiv, S., T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Overvatn, G. Bjorkoy, and T. Johansen. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem*. 282:24131-24145.

Park, J.M., C.H. Jung, M. Seo, N.M. Otto, D. Grunwald, K.H. Kim, B. Moriarity, Y.M. Kim, C. Starker, R.S. Nho, D. Voytas, and D.H. Kim. 2016. The ULK1 complex mediates MTORC1 signaling to the autophagy initiation machinery via binding and phosphorylating ATG14. *Autophagy*. 12:547-564.

Parsons, M.J., and D.R. Green. 2010. Mitochondria in cell death. *Essays Biochem*. 47:99-114.

Paz, Y., Z. Elazar, and D. Fass. 2000. Structure of GATE-16, membrane transport modulator and mammalian ortholog of autophagocytosis factor Aut7p. *J Biol Chem*. 275:25445-25450.

Pfeifer, U., and P. Strauss. 1981. Autophagic vacuoles in heart muscle and liver. A comparative morphometric study including circadian variations in meal-fed rats. *J Mol Cell Cardiol*. 13:37-49.

Pickart, C.M., and R.E. Cohen. 2004. Proteasomes and their kin: proteases in the machine age. *Nat Rev Mol Cell Biol*. 5:177-187.

Pilli, M., J. Arko-Mensah, M. Ponpuak, E. Roberts, S. Master, M.A. Mandell, N. Dupont, W. Ornatowski, S. Jiang, S.B. Bradfute, J.A. Bruun, T.E. Hansen, T. Johansen, and V. Deretic. 2012. TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. *Immunity*. 37:223-234.

Polson, H.E., J. de Lartigue, D.J. Rigden, M. Reedijk, S. Urbe, M.J. Clague, and S.A. Tooze. 2010. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy*. 6:506-522.

Popovic, D., M. Akutsu, I. Novak, J.W. Harper, C. Behrends, and I. Dikic. 2012. Rab GTPase-activating proteins in autophagy: regulation of endocytic and autophagy pathways by direct binding to human ATG8 modifiers. *Mol Cell Biol*. 32:1733-1744.

Ragusa, M.J., R.E. Stanley, and J.H. Hurley. 2012. Architecture of the Atg17 complex as a scaffold for autophagosome biogenesis. *Cell*. 151:1501-1512.

Ray, R., G. Chen, C. Vande Velde, J. Cizeau, J.H. Park, J.C. Reed, R.D. Gietz, and A.H. Greenberg. 2000. BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites. *J Biol Chem*. 275:1439-1448.

Riley, B.E., J.C. Lougheed, K. Callaway, M. Velasquez, E. Brecht, L. Nguyen, T. Shaler, D. Walker, Y. Yang, K. Regnstrom, L. Diep, Z. Zhang, S. Chiou, M. Bova, D.R. Artis, N. Yao, J. Baker, T. Yednock, and J.A. Johnston. 2013. Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. *Nat Commun*. 4:1982.

Rogov, V., V. Dotsch, T. Johansen, and V. Kirkin. 2014. Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Mol Cell*. 53:167-178.

Rojo, M., F. Legros, D. Chateau, and A. Lombes. 2002. Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J Cell Sci*. 115:1663-1674.

Romanov, J., M. Walczak, I. Ibiricu, S. Schuchner, E. Ogris, C. Kraft, and S. Martens. 2012. Mechanism and functions of membrane binding by the Atg5-Atg12/Atg16 complex during autophagosome formation. *EMBO J*. 31:4304-4317.

Ropolo, A., D. Grasso, R. Pardo, M.L. Sacchetti, C. Archange, A. Lo Re, M. Seux, J. Nowak, C.D. Gonzalez, J.L. Iovanna, and M.I. Vaccaro. 2007. The pancreatitis-induced vacuole membrane protein 1 triggers autophagy in mammalian cells. *J Biol Chem*. 282:37124-37133.

Rostislavleva, K., N. Soler, Y. Ohashi, L. Zhang, E. Pardon, J.E. Burke, G.R. Masson, C. Johnson, J. Steyaert, N.T. Ktistakis, and R.L. Williams. 2015. Structure and flexibility of the endosomal Vps34 complex reveals the basis of its function on membranes. *Science*. 350:aac7365.

Ruhlmann, A., and A. Nordheim. 1997. Effects of the immunosuppressive drugs CsA and FK506 on intracellular signalling and gene regulation. *Immunobiology*. 198:192-206.

Russell, R.C., Y. Tian, H. Yuan, H.W. Park, Y.Y. Chang, J. Kim, H. Kim, T.P. Neufeld, A. Dillin, and K.L. Guan. 2013. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol*. 15:741-750.

Saita, S., M. Shirane, T. Ishitani, N. Shimizu, and K.I. Nakayama. 2014. Role of the ANKMY2-FKBP38 axis in regulation of the Sonic hedgehog (Shh) signaling pathway. *J Biol Chem*. 289:25639-25654.

Saita, S., M. Shirane, and K.I. Nakayama. 2013. Selective escape of proteins from the mitochondria during mitophagy. *Nat Commun*. 4:1410.

Sakoh-Nakatogawa, M., H. Kirisako, H. Nakatogawa, and Y. Ohsumi. 2015. Localization of Atg3 to autophagy-related membranes and its enhancement by the Atg8-family interacting motif to promote expansion of the membranes. *FEBS Lett*. 589:744-749.

Sancak, Y., L. Bar-Peled, R. Zoncu, A.L. Markhard, S. Nada, and D.M. Sabatini. 2010. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 141:290-303.

Sancak, Y., T.R. Peterson, Y.D. Shaul, R.A. Lindquist, C.C. Thoreen, L. Bar-Peled, and D.M. Sabatini. 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*. 320:1496-1501.

Sandoval, H., P. Thiagarajan, S.K. Dasgupta, A. Schumacher, J.T. Prchal, M. Chen, and J. Wang. 2008. Essential role for Nix in autophagic maturation of erythroid cells. *Nature*. 454:232-235.

Santel, A., and M.T. Fuller. 2001. Control of mitochondrial morphology by a human mitofusin. *J Cell Sci*. 114:867-874.

Sarraf, S.A., M. Raman, V. Guarani-Pereira, M.E. Sowa, E.L. Huttlin, S.P. Gygi, and J.W. Harper. 2013. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature*. 496:372-376.

Satoo, K., N.N. Noda, H. Kumeta, Y. Fujioka, N. Mizushima, Y. Ohsumi, and F. Inagaki. 2009. The structure of Atg4B-LC3 complex reveals the mechanism of LC3 processing and delipidation during autophagy. *EMBO J*. 28:1341-1350.

Schlame, M. 2008. Cardiolipin synthesis for the assembly of bacterial and mitochondrial membranes. *J Lipid Res*. 49:1607-1620.

Schoenheimer, R., S. Ratner, and D. Rittenberg. 1939. The Process of Continuous Deamination and Reamination of Amino Acids in the Proteins of Normal Animals. *Science*. 89:272-273.

Schu, P.V., K. Takegawa, M.J. Fry, J.H. Stack, M.D. Waterfield, and S.D. Emr. 1993. Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science*. 260:88-91.

Schwarten, M., J. Mohrluder, P. Ma, M. Stoldt, Y. Thielmann, T. Stangler, N. Hersch, B. Hoffmann, R. Merkel, and D. Willbold. 2009. Nix directly binds to GABARAP: a possible crosstalk between apoptosis and autophagy. *Autophagy*. 5:690-698.

Schweers, R.L., J. Zhang, M.S. Randall, M.R. Loyd, W. Li, F.C. Dorsey, M. Kundu, J.T. Opferman, J.L. Cleveland, J.L. Miller, and P.A. Ney. 2007. NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc Natl Acad Sci U S A*. 104:19500-19505.

Seaman, M.N., M.E. Harbour, D. Tattersall, E. Read, and N. Bright. 2009. Membrane recruitment of the cargo-selective retromer subcomplex is catalysed by the small GTPase Rab7 and inhibited by the Rab-GAP TBC1D5. *J Cell Sci*. 122:2371-2382.

Sekito, T., T. Kawamata, R. Ichikawa, K. Suzuki, and Y. Ohsumi. 2009. Atg17 recruits Atg9 to organize the pre-autophagosomal structure. *Genes Cells*. 14:525-538.

Shiba-Fukushima, K., T. Arano, G. Matsumoto, T. Inoshita, S. Yoshida, Y. Ishihama, K.Y. Ryu, N. Nukina, N. Hattori, and Y. Imai. 2014. Phosphorylation of mitochondrial polyubiquitin by PINK1 promotes Parkin mitochondrial tethering. *PLoS Genet*. 10:e1004861.

Shibutani, S.T., and T. Yoshimori. 2014. A current perspective of autophagosome biogenesis. *Cell Res*. 24:58-68.

Shimamoto, S., M. Tsuchiya, F. Yamaguchi, Y. Kubota, H. Tokumitsu, and R. Kobayashi. 2014. Ca2+/S100 proteins inhibit the interaction of FKBP38 with Bcl-2 and Hsp90. *Biochem J*. 458:141-152.

Shirane, M., and K.I. Nakayama. 2003. Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis. *Nat Cell Biol*. 5:28-37.

Shirane, M., M. Ogawa, J. Motoyama, and K.I. Nakayama. 2008. Regulation of apoptosis and neurite extension by FKBP38 is required for neural tube formation in the mouse. *Genes Cells*. 13:635-651.

Shirihai, O.S., M. Song, and G.W. Dorn, 2nd. 2015. How mitochondrial dynamism orchestrates mitophagy. *Circ Res*. 116:1835-1849.

Shvets, E., E. Fass, R. Scherz-Shouval, and Z. Elazar. 2008. The N-terminus and Phe52 residue of LC3 recruit p62/SQSTM1 into autophagosomes. *J Cell Sci*. 121:2685-2695.

Sich, C., S. Improta, D.J. Cowley, C. Guenet, J.P. Merly, M. Teufel, and V. Saudek. 2000. Solution structure of a neurotrophic ligand bound to FKBP12 and its effects on protein dynamics. *Eur J Biochem*. 267:5342-5355.

Singh, S.B., W. Ornatowski, I. Vergne, J. Naylor, M. Delgado, E. Roberts, M. Ponpuak, S. Master, M. Pilli, E. White, M. Komatsu, and V. Deretic. 2010. Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol*. 12:1154-1165.

Smirnova, E., L. Griparic, D.L. Shurland, and A.M. van der Bliek. 2001. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell*. 12:2245-2256.

Smirnova, E., D.L. Shurland, S.N. Ryazantsev, and A.M. van der Bliek. 1998. A human dynamin-related protein controls the distribution of mitochondria. *J Cell Biol*. 143:351-358.

Stack, J.H., P.K. Herman, P.V. Schu, and S.D. Emr. 1993. A membrane-associated complex containing the Vps15 protein kinase and the Vps34 PI 3-kinase is essential for protein sorting to the yeast lysosome-like vacuole. *EMBO J*. 12:2195-2204.

Starai, V.J., C.M. Hickey, and W. Wickner. 2008. HOPS proofreads the trans-SNARE complex for yeast vacuole fusion. *Mol Biol Cell*. 19:2500-2508.

Stenmark, H. 2009. Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol*. 10:513-525.

Sugawara, K., N.N. Suzuki, Y. Fujioka, N. Mizushima, Y. Ohsumi, and F. Inagaki. 2004. The crystal structure of microtubule-associated protein light chain 3, a mammalian homologue of Saccharomyces cerevisiae Atg8. *Genes Cells*. 9:611-618.

Sugiura, A., G.L. McLelland, E.A. Fon, and H.M. McBride. 2014. A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. *EMBO J*. 33:2142-2156.

Sun, Q., W. Westphal, K.N. Wong, I. Tan, and Q. Zhong. 2010. Rubicon controls endosome maturation as a Rab7 effector. *Proc Natl Acad Sci U S A*. 107:19338-19343.

Suttangkakul, A., F. Li, T. Chung, and R.D. Vierstra. 2011. The ATG1/ATG13 protein kinase complex is both a regulator and a target of autophagic recycling in Arabidopsis. *Plant Cell*. 23:3761-3779.

Suzuki, H., K. Tabata, E. Morita, M. Kawasaki, R. Kato, R.C. Dobson, T. Yoshimori, and S. Wakatsuki. 2014. Structural basis of the autophagy-related LC3/Atg13 LIR complex: recognition and interaction mechanism. *Structure*. 22:47-58.

Suzuki, K., Y. Kubota, T. Sekito, and Y. Ohsumi. 2007. Hierarchy of Atg proteins in pre-autophagosomal structure organization. *Genes Cells*. 12:209-218.

Svenning, S., T. Lamark, K. Krause, and T. Johansen. 2011. Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. *Autophagy*. 7:993-1010.

Tabata, K., K. Matsunaga, A. Sakane, T. Sasaki, T. Noda, and T. Yoshimori. 2010. Rubicon and PLEKHM1 negatively regulate the endocytic/autophagic pathway via a novel Rab7-binding domain. *Mol Biol Cell*. 21:4162-4172.

Taherbhoy, A.M., S.W. Tait, S.E. Kaiser, A.H. Williams, A. Deng, A. Nourse, M. Hammel, I. Kurinov, C.O. Rock, D.R. Green, and B.A. Schulman. 2011. Atg8 transfer from Atg7 to Atg3: a distinctive E1-E2 architecture and mechanism in the autophagy pathway. *Mol Cell*. 44:451-461.

Takahashi, Y., D. Coppola, N. Matsushita, H.D. Cualing, M. Sun, Y. Sato, C. Liang, J.U. Jung, J.Q. Cheng, J.J. Mule, W.J. Pledger, and H.G. Wang. 2007. Bif-1 interacts with Beclin

1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol*. 9:1142-1151.

Takeshige, K., M. Baba, S. Tsuboi, T. Noda, and Y. Ohsumi. 1992. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol*. 119:301-311.

Tanaka, A., M.M. Cleland, S. Xu, D.P. Narendra, D.F. Suen, M. Karbowski, and R.J. Youle. 2010. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol*. 191:1367-1380.

Tanaka, K. 2009. The proteasome: overview of structure and functions. *Proc Jpn Acad Ser B Phys Biol Sci*. 85:12-36.

Tani, J., S. Shimamoto, K. Mori, N. Kato, K. Moriishi, Y. Matsuura, H. Tokumitsu, M. Tsuchiya, T. Fujimoto, K. Kato, H. Miyoshi, T. Masaki, and R. Kobayashi. 2013. Ca(2+) /S100 proteins regulate HCV virus NS5A-FKBP8/FKBP38 interaction and HCV virus RNA replication. *Liver Int*. 33:1008-1018.

Taylor, F.J. 1979. Symbionticism revisited: a discussion of the evolutionary impact of intracellular symbioses. *Proc R Soc Lond B Biol Sci*. 204:267-286.

Thomson, A.W., C.A. Bonham, and A. Zeevi. 1995. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther Drug Monit*. 17:584-591.

Thumm, M., R. Egner, B. Koch, M. Schlumpberger, M. Straub, M. Veenhuis, and D.H. Wolf. 1994. Isolation of autophagocytosis mutants of Saccharomyces cerevisiae. *FEBS Lett*. 349:275-280.

Thurston, T.L., G. Ryzhakov, S. Bloor, N. von Muhlinen, and F. Randow. 2009. The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. *Nat Immunol*. 10:1215-1221.

Tracy, K., B.C. Dibling, B.T. Spike, J.R. Knabb, P. Schumacker, and K.F. Macleod. 2007. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. *Mol Cell Biol*. 27:6229-6242.

Tsukada, M., and Y. Ohsumi. 1993. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. *FEBS Lett*. 333:169-174.

Tumbarello, D.A., P.T. Manna, M. Allen, M. Bycroft, S.D. Arden, J. Kendrick-Jones, and F. Buss. 2015. The Autophagy Receptor TAX1BP1 and the Molecular Motor Myosin VI Are Required for Clearance of Salmonella Typhimurium by Autophagy. *PLoS Pathog*. 11:e1005174.

Twig, G., A. Elorza, A.J. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, J. Alroy, M. Wu, B.F. Py, J. Yuan, J.T. Deeney, B.E. Corkey, and O.S. Shirihai. 2008. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J*. 27:433-446.

Vande Velde, C., J. Cizeau, D. Dubik, J. Alimonti, T. Brown, S. Israels, R. Hakem, and A.H. Greenberg. 2000. BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. *Mol Cell Biol*. 20:5454-5468.

Voges, D., P. Zwickl, and W. Baumeister. 1999. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem*. 68:1015-1068.

von Muhlinen, N., M. Akutsu, B.J. Ravenhill, A. Foeglein, S. Bloor, T.J. Rutherford, S.M. Freund, D. Komander, and F. Randow. 2012. LC3C, bound selectively by a noncanonical LIR motif in NDP52, is required for antibacterial autophagy. *Mol Cell*. 48:329-342.

Wallace, D.C. 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*. 39:359-407.

Wallin, I.E. 1926. Bacteria and the Origin of Species. *Science*. 64:173-175.

Wang, H., H.Q. Sun, X. Zhu, L. Zhang, J. Albanesi, B. Levine, and H. Yin. 2015. GABARAPs regulate PI4P-dependent autophagosome:lysosome fusion. *Proc Natl Acad Sci U S A.* 112:7015-7020.

Wang, X., D. Winter, G. Ashrafi, J. Schlehe, Y.L. Wong, D. Selkoe, S. Rice, J. Steen, M.J. LaVoie, and T.L. Schwarz. 2011. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell*. 147:893-906.

Wartosch, L., U. Gunesdogan, S.C. Graham, and J.P. Luzio. 2015. Recruitment of VPS33A to HOPS by VPS16 Is Required for Lysosome Fusion with Endosomes and Autophagosomes. *Traffic*. 16:727-742.

Wei, Y., Z. An, Z. Zou, R. Sumpter, M. Su, X. Zang, S. Sinha, M. Gaestel, and B. Levine. 2015. The stress-responsive kinases MAPKAPK2/MAPKAPK3 activate starvation-induced autophagy through Beclin 1 phosphorylation. *Elife*. 4.

Wei, Y., W.C. Chiang, R. Sumpter, Jr., P. Mishra, and B. Levine. 2017. Prohibitin 2 Is an Inner Mitochondrial Membrane Mitophagy Receptor. *Cell*. 168:224-238 e210.

Weidberg, H., E. Shvets, T. Shpilka, F. Shimron, V. Shinder, and Z. Elazar. 2010. LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. *EMBO J*. 29:1792-1802.

Wenzel, D.M., A. Lissounov, P.S. Brzovic, and R.E. Klevit. 2011. UBCH7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. *Nature*. 474:105-108.

Whitworth, A.J., J.R. Lee, V.M. Ho, R. Flick, R. Chowdhury, and G.A. McQuibban. 2008. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. *Dis Model Mech*. 1:168-174; discussion 173.

Wild, P., H. Farhan, D.G. McEwan, S. Wagner, V.V. Rogov, N.R. Brady, B. Richter, J. Korac, O. Waidmann, C. Choudhary, V. Dotsch, D. Bumann, and I. Dikic. 2011. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. *Science*. 333:228-233.

Wong, Y.C., and E.L. Holzbaur. 2014. Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proc Natl Acad Sci U S A.* 111:E4439-4448.

Wooten, M.W., T. Geetha, J.R. Babu, M.L. Seibenhener, J. Peng, N. Cox, M.T. Diaz-Meco, and J. Moscat. 2008. Essential role of sequestosome 1/p62 in regulating accumulation of Lys63-ubiquitinated proteins. *J Biol Chem*. 283:6783-6789.

Wu, H., D. Xue, G. Chen, Z. Han, L. Huang, C. Zhu, X. Wang, H. Jin, J. Wang, Y. Zhu, L. Liu, and Q. Chen. 2014a. The BCL2L1 and PGAM5 axis defines hypoxia-induced receptor-mediated mitophagy. *Autophagy*. 10:1712-1725.

Wu, W., W. Tian, Z. Hu, G. Chen, L. Huang, W. Li, X. Zhang, P. Xue, C. Zhou, L. Liu, Y. Zhu, X. Zhang, L. Li, L. Zhang, S. Sui, B. Zhao, and D. Feng. 2014b. ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. *EMBO Rep*. 15:566-575.

Yamada, Y., N.N. Suzuki, T. Hanada, Y. Ichimura, H. Kumeta, Y. Fujioka, Y. Ohsumi, and F. Inagaki. 2007. The crystal structure of Atg3, an autophagy-related ubiquitin carrier protein (E2) enzyme that mediates Atg8 lipidation. *J Biol Chem*. 282:8036-8043.

Yamaguchi, M., N.N. Noda, H. Nakatogawa, H. Kumeta, Y. Ohsumi, and F. Inagaki. 2010. Autophagy-related protein 8 (Atg8) family interacting motif in Atg3 mediates the Atg3-Atg8 interaction and is crucial for the cytoplasm-to-vacuole targeting pathway. *J Biol Chem*. 285:29599-29607.

Yamamoto, H., S. Kakuta, T.M. Watanabe, A. Kitamura, T. Sekito, C. Kondo-Kakuta, R. Ichikawa, M. Kinjo, and Y. Ohsumi. 2012. Atg9 vesicles are an important membrane source during early steps of autophagosome formation. *J Cell Biol*. 198:219-233.

Yamano, K., A.I. Fogel, C. Wang, A.M. van der Bliek, and R.J. Youle. 2014. Mitochondrial Rab GAPs govern autophagosome biogenesis during mitophagy. *Elife*. 3:e01612.

Yamano, K., and R.J. Youle. 2013. PINK1 is degraded through the N-end rule pathway. *Autophagy*. 9:1758-1769.

Yamashita, S.I., X. Jin, K. Furukawa, M. Hamasaki, A. Nezu, H. Otera, T. Saigusa, T. Yoshimori, Y. Sakai, K. Mihara, and T. Kanki. 2016. Mitochondrial division occurs concurrently with autophagosome formation but independently of Drp1 during mitophagy. *J Cell Biol*. 215:649-665.

Yang, Y., Y. Ouyang, L. Yang, M.F. Beal, A. McQuibban, H. Vogel, and B. Lu. 2008. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci U S A*. 105:7070-7075.

Yao, Z., E. Delorme-Axford, S.K. Backues, and D.J. Klionsky. 2015. Atg41/Icy2 regulates autophagosome formation. *Autophagy*. 11:2288-2299.

Yoon, M.S., Y. Sun, E. Arauz, Y. Jiang, and J. Chen. 2011. Phosphatidic acid activates mammalian target of rapamycin complex 1 (mTORC1) kinase by displacing FK506 binding protein 38 (FKBP38) and exerting an allosteric effect. *J Biol Chem*. 286:29568-29574.

Yoon, Y., E.W. Krueger, B.J. Oswald, and M.A. McNiven. 2003. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol Cell Biol*. 23:5409-5420.

Young, A.R., E.Y. Chan, X.W. Hu, R. Kochl, S.G. Crawshaw, S. High, D.W. Hailey, J. Lippincott-Schwartz, and S.A. Tooze. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J Cell Sci*. 119:3888-3900.

Zhang, J., M.S. Randall, M.R. Loyd, F.C. Dorsey, M. Kundu, J.L. Cleveland, and P.A. Ney. 2009. Mitochondrial clearance is regulated by Atg7-dependent and -independent mechanisms during reticulocyte maturation. *Blood*. 114:157-164.

Zhao, J., T. Liu, S. Jin, X. Wang, M. Qu, P. Uhlen, N. Tomilin, O. Shupliakov, U. Lendahl, and M. Nister. 2011. Human MIEF1 recruits Drp1 to mitochondrial outer membranes and promotes mitochondrial fusion rather than fission. *EMBO J*. 30:2762-2778.

Zheng, X., and T. Hunter. 2013. Parkin mitochondrial translocation is achieved through a novel catalytic activity coupled mechanism. *Cell Res*. 23:886-897.

Zhou, J., J. Wang, Y. Cheng, Y.J. Chi, B. Fan, J.Q. Yu, and Z. Chen. 2013. NBR1-mediated selective autophagy targets insoluble ubiquitinated protein aggregates in plant stress responses. *PLoS Genet*. 9:e1003196.

Zhu, Y., S. Massen, M. Terenzio, V. Lang, S. Chen-Lindner, R. Eils, I. Novak, I. Dikic, A. Hamacher-Brady, and N.R. Brady. 2013. Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3 determines pro-survival mitophagy versus apoptosis. *J Biol Chem*. 288:1099-1113.

Ziviani, E., R.N. Tao, and A.J. Whitworth. 2010. Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A*. 107:5018-5023.

Zou, H., Y. Lai, X. Zhao, G. Yan, D. Ma, N. Cardenes, S. Shiva, Y. Liu, X. Bai, Y. Jiang, and Y. Jiang. 2013. Regulation of mammalian target of rapamycin complex 1 by Bcl-2 and Bcl-XL proteins. *J Biol Chem*. 288:28824-28830.