

## **Emerging mechanistic insights of selective autophagy in hepatic diseases**

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## Supplementary Table 1 and Related References and Supplementary Fig.1 legend

Table 1 S

Actions of selective autophagy (mitophagy and lipophagy) in liver physiology and pathophysiology.

<b>Mechanistic events</b>	<b>Experimental model with references</b>
<b><i>Defective autophagy in NAFLD steatosis/NASH</i></b>	
Hepatic steatosis suppresses autophagic proteolysis via alteration of lysosomal acidification and proteinase activity of cathepsin B/L	Obese (ob/ob) mice [Inami et al., 2011; Niture et al., 2021]
Changes in membrane lipid composition reduce autophagosome-lysosomal fusion up to 70%	treatment with methyl- $\beta$ -cyclodextrin (MBCD) or male B6 mice fed with HFD challenge [Koga et al.2010; Niture et al.2021]
IR and hyperinsulinemia lead to suppress autophagy via FOXO1-mediated downregulation of key autophagy genes. IR also suppresses mitophagy <i>in vitro</i>	HFD fed C57BL/6 mice [Liu et al.2009; Niture et al.2021]
Over-expression of Rubicon impairs autophagy in liver	HFD fed male C57BL/6 mice or steatotic patient samples [Tanaka et al.2016; Niture et al.2021]
Inhibition of SERCA leads to aggregate in cytosolic Ca <sup>2+</sup> levels and reduced autophagic flux	HFD induced obese mice or hepatocytes treated with SFA (e.g., PA) [Park et al.2014]
Activated PP2A leads to inhibit autophagic lipid catabolism that contributes to liver steatosis	Hepatocytes treated with methionine and SAM [Zubiete-Franco et al., 2016]
Decreased expressions of lipid-metabolizing enzymes (CPT1A, PPARA, ACAT1, ACADVL) and reduced autophagic flux reduces lipid oxidation and increases mitochondria swelling in the liver	Genetic ablation of <i>pld1</i> <sup>-/-</sup> (phospholipase D1) mice [Hur et al., 2016]
Increase SIRT3 (a negative regulator of autophagy), inactivate AMPK1 and overactivate MTOR leading to impaired autophagy	Mice fed with an SFA(PA)-rich HFD [ Li et al., 2017]
Activation of TBK1 leads to induce impaired autophagy, aggregate ubiquitinated proteins resulting in lipotoxicity in hepatocytes	Hepatocytes treated with SFAs (PA and SA)[Cho et al.2018]
Upregulation of SQSTM1, LC3-II and accumulation of autophagosomes, suggests a defective autophagic flux.	Mice fed with HFD or MCD; Huh7 cells treated with PA [Gonzalez-Rodriguez et al., 2014]

Activation of IBTK $\alpha$ , a member of UPR results in induction of autophagy and activation of NF $\kappa$ B triggering hepatocyte lipotoxicity	SFA (PA) treated HepG2 cells [ Willy et al., 2017]
Hepatic steatosis induces defective autophagy in the liver and inhibits autophagic proteolysis	In human NAFLD, chronic hepatitis B and chronic hepatitis C patient samples [ Fukuo et al., 2014]
<b><i>Defective autophagy in AFLD</i></b>	
mTOR activation and a decrease levels of TFEB-mediated lysosomal gene expression lead to defective autophagy	Chronic feeding plus acute binge alcohol (“Gao-binge”) in mice [Chao et al., 2018]
Activation of ACAC/ACC activity and increased malonyl CoA content in liver tissues suppress autophagy.	H4IIEC3 hepatoma cells, chronic Lieber-DeCarli alcohol in C57BL/6J mouse model [ You et al., 2004]
Downregulation of BECN1 and ATG5 and upregulation of SQSTM1 in liver tissues impair hepatocellular autophagy	Male Wistar rats fed a Lieber-DeCarli diet alcohol (5%) for chronic exposure [Menk et al., 2018]
Inactivation of RAB7 and DNM2 result in impaired lipophagy.	Alcohol-containing fed Lieber-DeCarli diet in male Wistar rats [Bao et al., 2014; Rasineni et al., 2017]
Activities of LAL decreases in hepatocytes	Chronic ethanol exposure in rats [Kharbanda et al., 1996; Schulze et al., 2017]
<b><i>Defective autophagy in HCC</i></b>	
Higher expressions of SQSTM1 and GPC3 (glypican 3; a tumor marker for HCC) indicate defective autophagy liver cancer.	Patients with HCC cells with HCV infection [Bao et al., 2014]
Impaired autophagy by increasing oxidative stress leads to start hepatocarcinogenesis.	Liver-specific <i>atg5</i> <sup>-/-</sup> mice [Tian et al., 2015]
HuR increased autophagy mRNA expressions leads to impaired autophagy in HCC cells.	Hep3B, Huh7 and THLE-3 cells [Ji et al., 2019]
Impaired autophagy and increased development of liver tumors	Mosaic deletion of <i>Atg5</i> and liver-specific ATG7-deficient mice [Takamura et al., 2011]
Defective autophagy increases the frequency of spontaneous tumors.	Targeted deletion of BECN1 in embryonic stem (ES) cells or mice [ Yue et al., 2003]
<b><i>Actions of mitophagy in liver physiology</i></b>	
The distorted mitochondria associated with $\alpha$ 1-ATD-mediated chronic liver diseases	Liver specimens from patients with an $\alpha$ 1-ATD; liver samples of SERPINA1/ $\alpha$ 1-AT Z variant Tg mice [ Perlmutter, 2002; Teckman et al., 2004]
Age-dependent loss of mitophagy	Aged rat liver tissues [ Donati et al., 2006; Cavallini et al., 2007]
Impaired autophagic clearance of damaged mitochondria	Liver tissue specimens of <i>atg7</i> <sup>-/-</sup> mice [ Komatsu et al., 2005, Ke 2020]
Control the intracellular inclusion body formation	<i>atg7</i> <sup>-/-</sup> <i>sqstm1</i> <sup>-/-</sup> mice [Komatsu et al., 2007; Czaja et al., 2013]
Liver autophagy regulates blood glucose and amino acid levels	Liver-specific <i>atg7</i> <sup>-/-</sup> mice [ Ezaki et al., 2011, Czaja et al., 2013]

Starvation-induced mitophagic degradation	Liver tissue specimens of GFP-LC3 Tg mice [Kim and Lemasters, 2011; Ke 2020]
Loss of BNIP3-dependent mitophagy decrease mitochondrial turnover	Liver tissues and primary hepatocytes from <i>bnip3</i> KO mice [ Glick et al., 2012]
Mitophagic degradation and aging provoke an impairment in Parkin-mediated mitophagy	Rat liver tissues after I/R [ Kim et al., 2008; Li et al.2018; Ke et al.2020]
Starvation-induced mitophagic degradation	Liver specimens from acute liver damage patient [ Kheloufi et al., 2014 ]
Hepatocyte remodeling by mitophagy	Primary rat hepatocytes [ Rodriguez-Enriquez et al., 2009]
Parkin-dependent mitophagy protects against ethanol-induced liver injury by	Liver specimens from GFP-LC3 Tg mice and PMH (ethanol treatment) [Eid et al., 2015; Williams et al., 2015b; Williams and Ding 2015a]
Mitophagy protected against efavirenz (antiretroviral drug)-induced hepatic injury	Hepatocytes, Hep3B cell line and PMH (treated with efavirenz) [Apostolova et al., 2011a; Apostolova et al., 2011b]
Mitophagy protects against Cd-induced hepatotoxicity	The human normal liver cell line, L02 (Cd treated) [Pi et al., 2013]
Protection against APAP-induced liver injury by mitophagy through AMPK activation	Liver tissues from GFP-LC3 Tg mice (APAP treatment) [ Gordon, 1973; Shan et al., 2019; Sun et al., 2019]
Protection against I/R-induced hepatic injury by SIRT1 and PRKN-dependent mitophagy	Liver specimens from human patients, PMH and liver tissues of I/R-treated <i>sirt1</i> <sup>-/-</sup> mice [Rodriguez-Enriquez et al., 2009; Biel et al., 2016; Chun et al., 2018; Hong and Lee, 2018; Ning et al., 2018]
Protection against I/R-induced liver injury by HO-1-induced mitophagy through PGAM5 activation	Liver tissue specimens of I/R-treated mice [Hong and Lee, 2018]
Protected against I/R-induced liver injury by <i>Mir330-3p</i> -induced mitophagy via upregulation of PGAM5 expression	Liver tissue specimens of I/R-treated mice and liver cell line, L02 [ Sun et al., 2019]
<b><i>Actions of mitophagy in liver injury: steatosis and fatty liver diseases</i></b>	
Mitochondrial dysfunction is associated with ALFD and NAFLD	Liver specimens with ALFD and NAFLD patients [ Gordon, 1973; Ke 2020]
Defense against fatty liver by PINK1-PRKN-dependent mitophagy	Rat liver specimens (ethanol treatment) [Eid et at. 2013; Wiliam et al.2015; Eid et al. 2016a,b]
Mitochondrial dysfunction is associated with NAFLD	Liver specimens from patients with NAFLD [ Caldwell et al., 1999; Ke et al.2020]
Defense against NAFLD by PRKN-dependent mitophagy	Liver tissues from <i>alcat1</i> KO mice fed with a HFD [ Wang et al., 2015]
Suppression of NAFLD development by TH-induced mitophagy	HepG2 cells and mouse liver tissues fed with a MCD [ Sinha and Yen, 2016]

Initiation of DRAM-mediated mitophagy in the development of NAFLD	HepG2 cells treated with OA [Pang et al., 2018]
Megamitochondria by defective PRKN-independent mitophagy in fatty liver	Liver tissues from liver-specific <i>dnm1l</i> <sup>-/-</sup> , <i>opa1</i> <sup>-/-</sup> mice and <i>dnm1l</i> <sup>-/-</sup> <i>opa1</i> <sup>-/-</sup> mice with MCD feeding [Yamada et al., 2018]
Increased mitochondrial protein degradation by activated mitophagy	Liver tissues from LDLR KO with a WD feeding [Lee et al., 2018]
Inflammasome activation by inhibition of mitophagy	Mouse liver tissues fed a HFCD and rat primary hepatocyte treated with PA [Zhang et al., 2019]
<b><i>Actions of mitophagy in liver cancer</i></b>	
Suppression of hepatoma cell growth and liver tumor by ConA-activated BNIP3-dependent mitophagy	BALB/c hepatoma cell line ML-1; liver specimens from NOD/SCID mice treated with ConA [Chang et al. 2007; Lei and Chang, 2007]
Activation of Dox-activated cell death of hepatoma cells	HepG2 cells treated with Dox (adriamycin) [Qian and Yang 2009]
Improvement of Dox-activated cell death of HepG2 cells by curcumin	HepG2 cells treated with Dox and curcumin [Qian et 2011]
Cytotoxicity of sorafenib is amplified in hepatoma cells by melatonin-activated mitophagy	HepG2, Hep3B and Huh7 cells treated with melatonin and sorafenib [ Prieto-Domínguez et al.2016]
Cell death of hepatoma cells is triggered by inducing DRAM-dependent mitophagy	HepG2, Hep3B and Huh7 cells [ Liu et al., 2014]
Inhibition of initiation of HCC by FUNDC1-activated mitophagy through suppression of inflammasome	Liver tissue specimens from HCC patients and liver tissues from liver-specific <i>fundc1</i> KO mice [ Li et al., 2019]
Elevation of HCC cell viability by upregulated DNM1L and decreased MFN1 levels	Liver tissue specimens of HCC patients and mouse xenograft models, Bel7402 and SMMC7721 cell lines [ Huang et al., 2016]
Preservation of the stemness of CSCs by activating NANOG and initiation of mitophagy	HepG2, Hep3B and Huh7 cells [ Liu et al., 2017]
<b><i>Actions of mitophagy in viral hepatitis</i></b>	
PINK1-PRKN-dependent mitophagy elevates HCV replication, and HCV-induced mitophagy reduces infected cell apoptosis	Huh7.5.1 cells with HCV infection [Kim et al 2013b; Kim et al., 2014]
HCV-activated mitophagy degrades depolarized mitochondria	Huh7.5.1 cells transfected with HCV NS5A [Jassey et al., 2019]
A continual HCV infection causes mitochondrial damage by inhibiting mitophagy	Huh7 cells with HCV infection and Liver tissues of HCV Tg mice [ Hara et al., 2014]
HBV-induced PINK1-PRKN-mediated mitophagy protects infected cells from apoptosis	Huh7 cells transfected with HBV [ Kim et al., 2013a]
Nutrient deprivation-induced PINK1-PRKN-dependent mitophagy is augmented by regulatory protein, HBx	HepG2 cells and SMMC-7721 cells (transfection of HBx) [ Kim et al., 2013b]

<b><i>Actions of mitophagy in other liver diseases</i></b>	
Melatonin-induced mitophagy protects against CCl <sub>4</sub> -induced liver fibrosis	CCl <sub>4</sub> treated rat liver tissue specimens [ Kang et al., 2016]
CCl <sub>4</sub> activates of PINK1-PRKN-dependent mitophagy in Kupffer cells but TIMD4/TIM-4 suppresses it	CCl <sub>4</sub> treated mouse liver tissue specimens [Wu et al 2020]
Fine particulate matter (PM <sub>2.5</sub> ) activates HSCs and causes liver fibrosis; and inhibition of mitophagy alleviates the fibrosis	LX-2 cells and primary HSCs [Qiu et al., 2019]
Defective mitophagy promotes inflammasome activation in the HSC model	Liver specimens from patients with acute liver failure and mice treated with LPS and LX-2 cells treated with H <sub>2</sub> O <sub>2</sub> , LPS, NAC or FCCP [ Tian et al., 2018]
NR4A1-PRKDC-TP53 axis acts as a signaling pathway for AFLD pathogenesis	Hepatocytes from <i>nr4a1</i> KO mice, and liver-specific <i>prkdc</i> KO mice (ethanol treatment) [ Zhou et al., 2019]
Defective mitophagy increases lipogenesis via upregulation of lipogenic enzymes	Liver tissues from <i>bnip3</i> KO mice and PMH from <i>bnip3</i> -null mice [Glick et al., 2012]
TH-activated mitophagy increases FA $\beta$ -oxidation through inducing CPT1 $\alpha$ expression	Liver specimens from <i>thr</i> KO mice and HepG2 cells [ Singh et al., 2018]
HFD-fed REDD1 KO mice increase CPT1A, BNIP3 and PRKN expression in the livers	Liver specimens from <i>ddit4/redd1</i> KO mice treated with HFD [ Dumas et al., 2020]
Insulin resistance (IR) inhibits mitophagy	Liver specimens from B6 mice with HFD and PMH [ Liu et al., 2009]
Any defect in PRKN-dependent mitophagy does not change in obesity and IR	Liver specimens from <i>prkn</i> KO mice and PMH treated with HFD [Costa et al., 2016; Edmunds et al., 2019]
Loss of FUNDC1-mediated mitochondrial turnover induce adipose tissue-associated macrophage infiltration	Liver specimens from <i>fundc1</i> KO mice and PMH treated with HFD [Wu et al., 2019]

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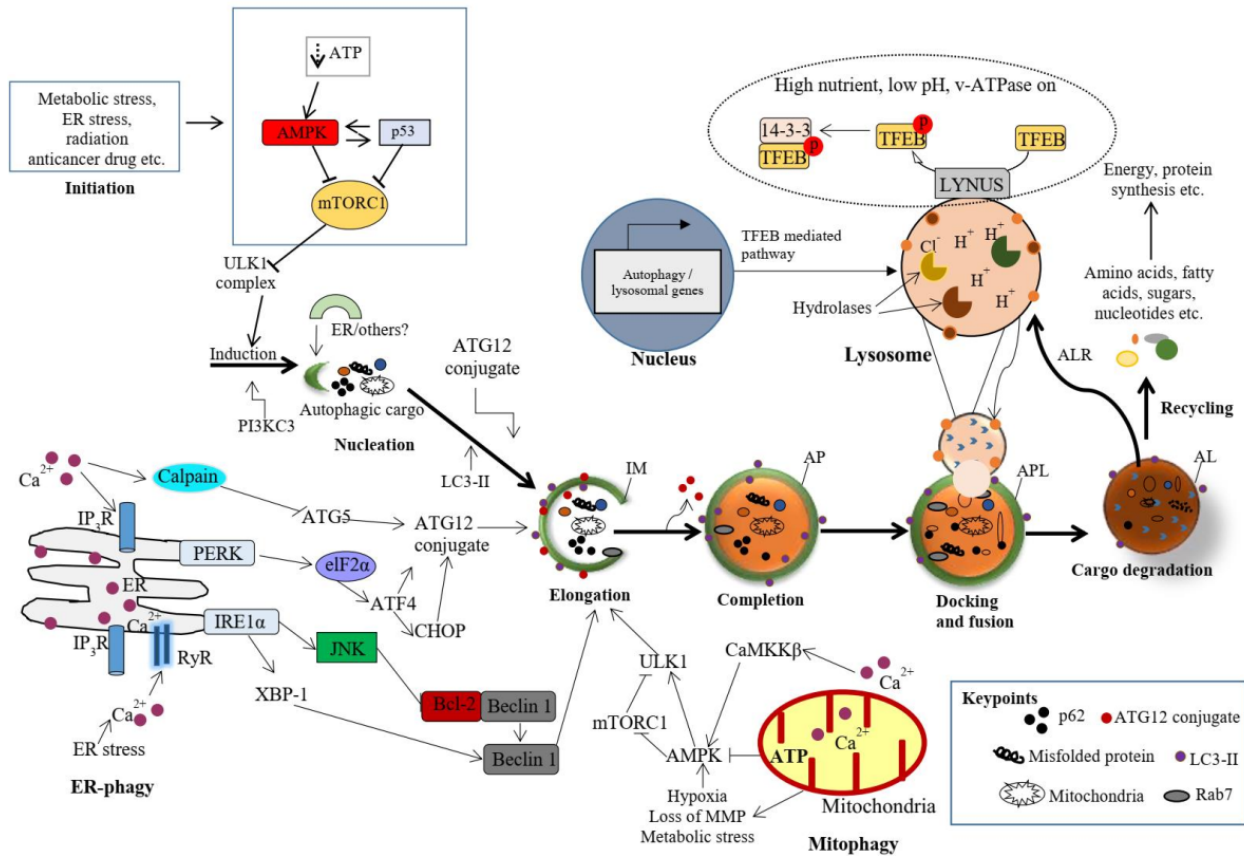
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Supplementary Figure 1



**Figure S1 legend. Molecular mechanisms of various stages of autophagy.** Autophagy is activated in response to various cellular stresses and is triggered by a decrease in rapamycin complex 1 (mTORC1) activity due to the activation of AMP-activated protein kinase (AMPK) or p53 signaling. mTORC1 suppresses the activity of Unc-51-like autophagy activating kinase 1 (ULK1) complex. Therefore, inhibition of mTORC1 causes the initialization of the ULK1-mediated formation of the isolation (autophagosomal) membrane (IM) in association with the class III phosphatidylinositide 3-kinase (PI3K) complex (PI3KC3). The IM expands into an autophagosome (AP) with a double-layer membrane, which can engulf any cellular component, including proteins, damaged organelles, and lipid droplets. The AP merges with the lysosome (via LAMP-1, 2), forming autophagolysosome (APL) or autolysosome (AL), and resulting in the degradation of the cargo by cathepsins and the autophagic lysosome reformation (ALR). The nucleation, elongation and maturation of the IM are dependent on two ubiquitin-like conjugation systems (ATG12 and ATG8), which involve multiple autophagy proteins, including Beclin1, ATG5, ATG16 and MT-associated protein 1 light chain 3 (LC3). The AL provides an acidic milieu for hydrolytic

enzymes to digest the engulfed components. Nuclear localization of transcription factor EB (TFEB) is critical to the formation of lysosomes and to the enhanced expression of autophagy proteins. Importantly, autophagy could be selective of mitochondria (mitophagy) or ER (ER-phagy). (Al-Bari et al 2021, reprinted with permission from IJMS).