

BAT3 modulates p300-dependent acetylation of ATG7 and p53 during autophagy

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Abstract

Autophagy is regulated by post-translational modifications including acetylation. Here, we show that BAT3 is essential for basal and starvation-induced autophagy in E18.5 *BAT3*^{-/-} mouse embryos and in Mouse Embryonic Fibroblasts (MEFs) through the modulation of p300-dependent acetylation of p53 and ATG7. Specifically, BAT3 increases p53 acetylation and pro-autophagic p53-target gene expression. On the contrary, BAT3 limits p300 dependent-acetylation of ATG7, a mechanism known to inhibit autophagy. In the absence of BAT3 or when BAT3 is exclusively located in the cytosol autophagy is abrogated, ATG7 is hyper-acetylated, p53 acetylation is abolished and p300 accumulated in the cytosol indicating that BAT3 regulates the nuclear localization of p300. In addition, the interaction between BAT3 and p300 is stronger in the cytosol than in the nucleus and, during starvation, p300 amount decreases in the cytosol but increases in the nucleus only if BAT3 is present. We conclude that BAT3 tightly controls autophagy by modulating p300 intracellular localization affecting p300 accessibility to its substrates, p53 and ATG7.

Significance Statement

Autophagy allows the lysosomal degradation of intracellular material. It is a tightly regulated process controlled, among others, by the tumor suppressor gene p53. In this study, we discovered a new regulator of autophagy called BAT3. Thus, BAT3 modulates the intracellular localization of the enzyme p300, an enzyme that add some acetyl residues on targets proteins (acetylation) to modulate their activity. To stimulated autophagy, BAT3 allows the acetylation of p53 by p300 in the nucleus, but, limits the p300-dependent acetylation of ATG7, a protein specific for autophagy, in the cytosol. All together, BAT3 acts both on the cytosol and the nucleus to tightly modulate autophagy.

Introduction

Autophagy allows the lysosomal degradation of intracellular macromolecules and organelles after their sequestration in a vacuole known as the autophagosome (1). Basal autophagy is as a cytoplasmic quality control mechanism that limits the production of reactive oxygen species and genomic instability. Autophagy is also induced to improve cell survival under stress. Signaling pathways involved in its regulation have been widely studied (2). Autophagy is modulated at two levels: (1) the molecular machinery involved in autophagosomes biogenesis and dependent on specific genes known as *Atg* (AuTophagy) genes, and (2) the upstream signaling pathways (PI3K, MAPK, mTOR i.e.) that act on ATG proteins. Post-translational modifications are crucial for the regulation of autophagy. The first example came from Y. Oshumi's laboratory, with the discovery of the conjugation systems for the ATG5-ATG12 and ATG8-phosphatidylethanolamine complexes in yeast (3). Phosphorylation is probably the most thoroughly investigated post-translational event in autophagy. It appears recently that the modulation of acetylation also affects *Atg* gene expression or activity. For instance, acetylation of the ULK1 kinase (mammalian homolog of ATG1) by the acetylase TIP60 induces autophagy after growth factor deprivation (4). In yeast, ESA1-dependent acetylation of ATG3 is essential for its interaction with ATG8 and ATG8 lipidation (5). Conversely, ATG5, ATG7, LC3 and ATG12 acetylation by the acetyltransferase p300 inhibits autophagy (6) whereas their deacetylation by SIRT1 stimulates autophagy (7).

BAT3 (HLA-B associated transcript 3) is a nucleo-cytoplasmic shuttling protein that contains, among others, a Nuclear Export Signal (NES) and a Nuclear Localization Signal (NLS). BAT3 has nuclear and cytosolic functions. In the cytosol, BAT3 plays roles, such as quality control (8) or apoptosis (9, 10, 11, 12). In the nucleus, thanks to its NLS domain (13), BAT3

modulates the methylase activity of SET1A and DOT1L playing a role in transcription and DNA damage response, respectively (14, 15). BAT3 is also involved in p53 activation by modulating its p300-dependent acetylation during apoptosis (16).

Here, we show that BAT3 is essential for basal and starvation-induced autophagy in E18.5 mouse embryos and Mouse Embryonic Fibroblasts (MEFs) through the regulation of p300 intracellular localization. In the presence of BAT3, p300 is mainly nuclear and stimulates p53 acetylation during starvation while maintaining low level of ATG7 acetylation. In contrast, in the absence of BAT3, p300 accumulates in the cytosol, ATG7 is hyper-acetylated and p53 acetylation during starvation is abolished leading to autophagy inhibition. We propose a new function for BAT3 in the regulation of autophagy through modulation of p300 intracellular localization and the subsequent acetylation of p53 and ATGs.

Results

BAT3 is essential for basal autophagy in mice

BAT3 homozygous deletion in mice is lethal, leading to major cell death and proliferation defects (17). Staining with an anti-LC3 antibody (a marker of autophagosomes) showed that BAT3 is essential for basal autophagy in E18.5 embryos. Indeed, the number of LC3-positive dots was significantly lower in the liver and the lung of *BAT3*^{-/-} embryos compared to WT (Figure 1).

BAT3 is essential for basal and starvation-induced autophagy in MEFs

We then studied the role of BAT3 in autophagy in WT and *BAT3*^{-/-} MEFs. By transmission electron microscopy, we observed that (i) starvation increased the number of autophagosomes in WT MEFs and (ii) the number of autophagosome is strongly reduced in Earle's balanced salt solution (EBSS) treated *BAT3*^{-/-} MEFs compared to WT (Figures 2A and 2B). We also tested the maturation of the autophagosome marker LC3-I (16kDa, cytosolic staining) into LC3-II (18 kDa, punctuated staining). LC3 maturation was considerably reduced in *BAT3*^{-/-} MEFs grown in complete media (CM) or switched to EBSS, suggesting that BAT3 is required for basal and starvation-induced autophagy (Figure 2C). The significant lower number of GFP-LC3 dots per cell in *BAT3*^{-/-} than in WT MEFs confirmed that BAT3 is essential for autophagy (Figures 2D and 2E). In addition, autophagic rates in *BAT3*^{-/-} MEFs are similar to *ATG7*^{-/-} MEFs allowing us to conclude about the essential role of BAT3 in autophagy (Figure S1). Similar results were also obtained with other *BAT3*^{-/-} MEF clones (Figure S2). To confirm that the induction of autophagy observed in WT MEFs during starvation was due to the stimulation of the autophagic pathway, rather than increases in both the formation of autophagosomes and their consumption by the lysosome, we measured the autophagic flux by

two independent assays, the GFP-LC3 assay by using chloroquine, an inhibitor of lysosomal acidification, and the measurement of long-lived protein degradation. Chloroquine treatment led to GFP-LC3 dots accumulation only in WT MEFs compared to BAT3 deficient cells suggesting a blockage in autophagosome formation in the absence of BAT3 (Figures 2D and 2E). In *BAT3*^{-/-} MEFs, the absence of BAT3 abrogates protein degradation during starvation compared to WT MEFs (Figure 2F). Taken together, these data suggest that BAT3 is essential for the early steps of autophagy.

BAT3 is responsible for p53 acetylation by p300 during starvation

We next investigated how BAT3 modulates autophagy. BAT3 is essential for the p300-mediated acetylation of p53 on lysine 373 during genotoxic stress (16). To investigate whether p53 acetylation was involved in autophagy, we transfected p53-deficient H1299 cells with peGFP-LC3 and empty vector, or with constructs encoding WT p53, the p53^{K372/373R} mutant in which lysines 372 and 373 cannot be acetylated by p300, the p53^{K319/320/321R} mutant in which lysines 319, 320 and 321 that are targeted by PCAF cannot be acetylated, or the p53^{K6R} mutant in which all the important lysines in the C-terminus are replaced by arginines (Figure 3A, lower panel). We observed an increase in the number of GFP-LC3 dots/cell after transfection of WT p53 compared to empty vector in H1299 cells starved for 2h (Figure 3A, upper panel). Similar results were obtained with the p53^{K319/320/321R} mutant. Conversely, transfection of the p53^{K372/373R} or p53^{K6R} mutant totally abolished starvation-induced autophagy observed in cells transfected with WT p53 (Figure 3A, upper panel). These results strongly suggest that p53 acetylation on lysines 372 and 373, known to be acetylated by p300, is essential for starvation-induced autophagy.

Next, we investigated the role of BAT3 in p53 acetylation during autophagy using antibody that recognizes p53 acetylated at lysine 373. Starvation for 30 min and 1h significantly

increased p53 acetylation at lysine 373, a site acetylated by p300, of 60% and 40%, respectively in WT MEFs, but not in *BAT3*^{-/-} MEFs (Figure 3B), indicating that BAT3 is essential for p53 acetylation during starvation. As a control, we confirmed that p53 acetylation at lysine 320, a site targeted by PCAF, was not modified during starvation (Figure S3). As p53 enhances the expression of pro-autophagic genes, we checked whether p53 dependent-stimulation of autophagy was accompanied by increased expression of p53 target genes during starvation. Indeed, in WT MEFs, the expression of SESTRIN1, p21 and Puma was significantly higher during starvation than in CM (Figure 3C). The qPCR data in *BAT3*^{-/-} MEFs revealed that BAT3 was required for SESTRIN1 up-regulation. In conclusion, BAT3 is essential for p53 pro-autophagic activity by modulating its acetylation by p300 and the expression of pro-autophagic target genes.

BAT3 inhibits p300-dependent acetylation of ATG7

P300 inhibits autophagy by stimulating the p300-mediated acetylation of specific ATG proteins, such as ATG5, ATG7, ATG12 and LC3 (6). We first investigated whether BAT3 affected the acetylation of endogenous ATG7. Immunoprecipitation using an anti-acetylated lysine antibody followed by immunoblotting with an anti-ATG7 antibody showed, as expected, that ATG7 acetylation was higher in WT MEFs grown in CM (-) than in EBSS (Figure 4A). In *BAT3*^{-/-} MEFs, ATG7 acetylation was markedly increased in CM and EBSS compared to WT MEFs. This result may explain autophagy inhibition observed in basal conditions both in *BAT3*^{-/-} mice and MEFs (Figure 1 and 2). In the absence of BAT3, we also observed an increase in ATG5 and LC3-I acetylation without affecting ATG12 acetylation (Figure S4). To confirm the role of p300 in the BAT3 dependent acetylation of ATG7, we lowered p300 expression using a shRNA in *BAT3*^{-/-} MEFs and observed a decrease in ATG7 acetylation in CM (Figure 4B). The remaining level of ATG7 acetylation may be due to

incomplete inhibition of p300 expression. We also checked whether BAT3 modulated the interaction between endogenous p300 and ATG7 by co-immunoprecipitation and observed a higher interaction in *BAT3*^{-/-} than in WT MEFs (Figure 4C). Similar results were obtained using another *BAT3*^{-/-} MEF clone (Figure S5).

BAT3 intracellular localization is important for autophagy

Our data indicate that BAT3 stimulates autophagy via two opposite mechanisms: (i) the stimulation of p53 acetylation during starvation and (ii) the inhibition of ATG7 acetylation in basal conditions and during starvation. ATG proteins are located in the cytosol, whereas active p53 is in the nucleus. As BAT3 is a shuttling nuclear-cytosolic protein, we investigated the role of BAT3 localization by co-transfecting *BAT3*^{-/-} MEFs with peGFP-LC3 and BAT3-FL, the BAT3ΔNLS mutant which is exclusively located in the cytosol since the NLS was deleted, or empty vector (13) (Figure 5A). Ectopic expression of BAT3-FL restored autophagy in *BAT3*^{-/-} MEFs whereas BAT3ΔNLS did not (Figure 5B and 5C). Moreover, ATG7 acetylation was increased in BAT3ΔNLS compared to BAT3 FL-expressing cells (Figure 5D), whereas p300-dependent acetylation of p53 was reduced in cells expressing empty vector or BAT3ΔNLS compared to BAT3-FL (Figure 5E). Thus, BAT3 absence or its cytosolic localization results in ATG7 hyper-acetylation, repression of p53 acetylation during starvation and inhibition of autophagy. This finding highlights the importance of BAT3 nuclear localization for its pro-autophagic activity.

BAT3 regulates p300 cellular localization

BAT3 is involved in the nuclear localization of p21 during cell cycle (18). We thus analyzed whether BAT3 had a role in p300 intracellular localization by fractionating WT and *BAT3*^{-/-} MEFs. In WT MEFs, p300 was almost entirely located in the nucleus, whereas BAT3 was

present in both the nucleus and cytosol. Conversely, in *BAT3*^{-/-} MEFs, p300 amount was greatly increased in the cytosol (Figure 6A). This finding was confirmed by quantifying the cytosol fluorescence in WT and *BAT3*^{-/-} MEFs following staining with an anti-p300 antibody (Figures S6A and S6B). We next investigated the interaction between endogenous BAT3 and p300 in the cytosol and in the nucleus of WT MEFs by co-immunoprecipitation. Although p300 was mainly located in the nucleus, BAT3-p300 interaction was stronger in the cytosol than in the nucleus (Figure 6B). In addition, by cell fractionation, we noticed that the ectopic expression of the BAT3 Δ NLS mutant in *BAT3*^{-/-} MEFs induces a cytosolic accumulation of p300 such as the absence of BAT3 (Figure 6C and 6D) suggesting that BAT3 is responsive for the nuclear shuttling of p300. Thus, we asked whether BAT3 modify p300 intracellular localization during starvation by quantifying the cytosolic fluorescence signal in WT and *BAT3*^{-/-} MEFs stained with an anti-p300 antibody at different time points after switching to EBSS. As previously observed (Figures 6A, 6C, 6D, S6A and S6B), p300 accumulated in the cytosol of *BAT3*^{-/-} MEFs (Figure 6E). In addition, short-term starvation (15 min) induced a significant decrease in the p300 cytosolic pool in WT MEFs, but not in *BAT3*^{-/-} cells (Figure 6E). By cell fractionation, we also observed a relocation of p300 into the nucleus following 15 minutes of starvation (Figures 6F & G). To conclude, we propose a model of BAT3 role in autophagy (Figure 7). In starved WT MEFs, BAT3 allows p300 relocation in the nucleus to induce autophagy by targeting p53 and lowering ATG7 acetylation. In the absence of BAT3, or when BAT3 is exclusively cytosolic, the starvation-induced p300 nuclear shuttling is inhibited resulting in its accumulation in the cytosol where it acetylates ATG7, thus inhibiting autophagy. We speculate that BAT3 is in charge of shuttling a small pool of p300 between nucleus and cytoplasm. In the cytosol, this p300 pool inhibits autophagy whereas it stimulates autophagy after its relocation in the nucleus during starvation.

Discussion

Here, we showed that BAT3 is a regulator of autophagy through its capacity to modulate the p300-dependent acetylation of p53 and ATG7.

First, we showed that BAT3 is involved in the p300 dependent-acetylation of p53 on its lysine 373 during starvation and for the subsequent transcriptional activity of p53 allowing SESTRIN1 up-regulation. The role of p53 in autophagy is still under debate in the field. It depends, among others, on its intracellular localization. Cytoplasmic p53 was initially shown to inhibit autophagy whereas nuclear p53 stimulates this process (19). Controversially, it has been reported that the acetylation of p53 on its lysine 120 by TIP60 and sumoylation on its lysine 386, leads to the cytoplasmic accumulation of p53 and enhancement of autophagy (20). This result supports that stimuli and subsequent modifications of p53 are important for the modulation of autophagy. In HCT116 colon cancer cells, 6-hour starvation stimulates p53 acetylation at lysine 382, a site acetylated by SIRT1 (21). In our work, we detected that p53 acetylation at lysine 373 occurs mainly 30 min after starvation. Duration of starvation is a critical parameter in the regulation of autophagy and long term starvation may reactivate mTor to inhibit autophagy and preserve lysosomal homeostasis (22).

Our findings also demonstrate that BAT3 is involved in the regulation of p300-dependent acetylation of ATG7, the E1 enzyme involved in the ATG5-ATG12 and ATG8-phosphatidylethanolamine conjugation systems. The mechanism by which ATG7 inhibits autophagy once acetylated and the lysine targeted by p300 are still unknown, but few data are available for other ATG proteins. In yeast, the acetylation of ATG3 by ESA1P (yeast homolog of the acetyltransferase TIP60) on lysine 19, 48 and 183, is essential for LC3

lipidation during starvation (5). In mouse cells, the kinase GSK3, activated by serum starvation, phosphorylates TIP60, increasing its affinity for ULK1 (mammalian ortholog of ATG1), which is then acetylated at lysines 162 and 606, leading to autophagy (4). Interestingly, TIP60, like p300, is mainly located within the nucleus, but has also cytoplasmic activities that involve ATG proteins. The deacetylase SIRT1 is also essential for autophagy through two mechanisms: (i) it deacetylates ATG5, ATG7 and LC3, allowing autophagy induction, and (ii) it deacetylates the transcription factor forkhead box O3a (FOXO3a), leading to increased expression of BNIP3 (7, 23). Interestingly, both a deacetylase activator (resveratrol) and an acetylase inhibitor (spermidine) induce autophagy. The acetylproteome of cells treated with these drugs showed that a general cytosolic deacetylation and a nuclear acetylation occur during autophagy (24). These findings agree with ours showing that BAT3 inhibits the acetylation of cytosolic ATG7 and stimulates the acetylation of nuclear p53 to induce autophagy.

The relative importance of p300-p53 and p300-ATG7 pathways in autophagy would be of interest. In H1299, the role of p53 seems predominant. However, ATG7 acetylation is essential since it is the last mandatory step of autophagy and p300 downregulation stimulates autophagy through the inhibition of ATG7 acetylation (6). Interestingly, it has been shown that ATG7 regulates p53 activity during starvation (25).

BAT3 stimulates autophagy by having opposite effects on p300-dependent acetylation of p53 and ATG7. As BAT3 is a nucleo-cytoplasmic protein, we propose that it differentially modulates autophagy through the regulation of p300 localization. Indeed, in the absence of BAT3, the cytoplasmic accumulation of p300 may favor its interaction with ATG7. In addition, we observed a relocation of p300 from the cytosol to the nucleus during starvation. BAT3 is also involved in p21 nuclear translocation during G2/M phases followed by its

degradation during the transition between the G1 and S phases (18) but the mechanism responsible for BAT3 nuclear shuttling remains elusive. A recent study showed that BAT3 variants are generated by alternative splicing of exon11B, leading to a different pattern of expression and intracellular localization. Thus, BAT3 may affect autophagy depending on its variant expression (26).

In conclusion, our study demonstrates that BAT3 is essential for autophagy through the regulation of p300 intracellular localization, thus controlling access to its pro-autophagic substrates.

Materials and Methods

Cells and plasmids

Wild type (WT) and *BAT3*^{-/-} MEFs were obtained as previously described (17). Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (CM) or in depleted medium (EBSS) (Invitrogen, Life Technologies, Carlsbad, CA, USA) for the indicated time. Plasmids encoding human WT and p53 mutants (pcDNA p53 WT, pcDNA p53^{K372/373R}, pcDNA p53^{K319/320/321R} and pcDNA p53^{K6R}) were obtained as previously described (28). Plasmids encoding full length (FL) and mutant BAT3 (PCI-HA-BAT3-FL and PCI-HA-BAT3ΔNLS) were obtained as previously described (13).

Autophagy measurement

WT and *BAT3*^{-/-} E18.5 mouse embryo tissue sections obtained as described (17), were stained using an anti-LC3B antibody (28). Electron microscopy sections were analyzed by counting the number of autophagosome per field. The maturation of LC3 was tested by immunoblotting. The GFP-LC3 assay was performed after transfection of rat peGFP-LC3, kindly provided by T. Yoshimori (Osaka University, Japan) (29). For the measurement of long-lived protein degradation, cells were incubated with L-[14C]valine. Chase was performed with cold valine and degradation was determined as acid-soluble radioactivity recovered from both cells and medium.

Immunoprecipitation

The acetylation of ATG proteins as determined by western blot after performing an immunoprecipitation with an antibody directed against acetyl-lysine (Cell Signaling Technology, Boston, MA, USA). Endogenous p300 was immunoprecipitated with a rabbit

polyclonal anti-p300 antibody (1:1000; N15). BAT3 or ATG7 were then detected in the immunoprecipitation by immunoblotting.

Cell fractionation

The Subcellular Protein Fractionation Kit was used (Thermo Fisher Scientific Inc., Rockford, IL, USA).

Real-Time PCR

PCR were performed with the primers described in Supplemental Experimental Procedures.

Staining of endogenous p300

WT and *BAT3*^{-/-} MEFs were submitted to starvation then were immuno-stained by using an anti p300 antibody (1:200, N15).

Statistical analysis

Quantified data were analyzed using the Student's t-test. A P value lower than 0.001, 0.01 or 0.05 versus controls was considered to be significant and was noted ***, ** or *. The results are the mean \pm standard deviation (SD), with error bars to show the SD.

Please, see Supplemental Experimental Procedures for additional information on autophagy assays, immunoprecipitation, retroviral infection and qPCR primers,

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Figure Legends

Figure 1: Autophagy is impaired in liver and lungs of E18.5 *BAT3*^{-/-} mouse embryos

(A and B) - Light-microscopy quantification of autophagy in liver (A) and lung (B) sections of WT and *BAT3*^{-/-} E18.5 mouse embryos immunostained with an anti-LC3B antibody. Results are the mean (SD) of the autophagosome number per field (around 200 fields were counted per organ) in liver and lungs. ***: $p \leq 0.001$ versus WT embryo. (C and D)- Representative images of LC3 staining in liver (C) and lungs (D). Arrows indicate LC3 dots.

Figure 2: BAT3 is essential for autophagy in MEFs

(A) - Representative ultrastructural images of WT and *BAT3*^{-/-} MEFs in CM or EBSS for 2h. Arrows indicate autophagosomes. C: cytosol ; N: nucleus (B) - Quantification of the autophagosome number per field as shown in A. *** $p \leq 0.001$ versus WT MEFs EBSS (C) - WT and *BAT3*^{-/-} MEFs were cultured in CM (-) or EBSS for 2h, then immunoblotted as indicated. The densitometric analysis of LC3-I and LC3-II /Actin ratio were arbitrarily set at 1 for WT MEFs in CM and represents the mean (SD) of five independent experiments. (D and E) - Quantification of the number of autophagosome per cell (D) and representative images (E) of cells transfected with the peGFP-LC3 plasmid and incubated in CM or EBSS for 2h, with or without 20 μ M chloroquine (CQ) for 6h. Results are the mean (SD) of three independent experiments. *** $p \leq 0.001$. (F) - Measurement of long-lived protein degradation in WT and *BAT3*^{-/-} MEFs cultured in CM or EBSS for 2h.

Figure 3: BAT3 is essential for p53 activity during autophagy

(A) - Quantification of autophagy in H1299 cells co-transfected with GFP-LC3 and the indicated plasmids and grown in CM or EBSS for 2h. Results are the mean (SD) of three independent experiments. ***: $p \leq 0.001$ versus cells with WT p53. The expression of p53 and ERK2 was assessed by western blotting (bottom panel). (B) - Upper panel: Western blot analysis of p53 acetylation at lysine 373 in WT and *BAT3*^{-/-} MEFs using antibodies against p53 acetylated at lysine 373 and Actin. Cells were grown in CM (-) or switched to EBSS for the indicated time. Lower panel: Densitometric quantification for five independent experiments ***: $p \leq 0.001$ and * $p \leq 0.05$ versus starved WT MEFs (C) - Relative mRNA expression in WT and *BAT3*^{-/-} MEFs cultured in EBSS or CM for 4 hours (expression value set at 1 for the CM condition). Results are representative of three independent experiments.

Figure 4: BAT3 inhibits ATG7 acetylation and its interaction with p300

(A) - Western blot of ATG7 expression in protein lysates or after immunoprecipitation (IP) using an anti-acetylated lysine antibody. WT and *BAT3*^{-/-} MEFs were grown in CM (-) or EBSS (+) for 2h. Control IP (CT) was performed using a rabbit IgG (top panel) and loading was checked using an anti-rabbit antibody (bottom panel). Results are representative of three independent experiments. (B) - Co-immunoprecipitation of ATG7 and p300 using an anti-p300 antibody in cells grown in CM (-) or EBSS (+) for 2h. The controls are the same as in (A). Results are representative of three independent experiments. (C) - Western blot analysis of ATG7 expression and acetylation in *BAT3*^{-/-} MEFs in presence of sh-p300 or sh-luciferase (sh-luc) cultured in CM or EBSS for 2h as in (A).

Figure 5: The BAT3 Δ NLS mutant does not restore autophagy in *BAT3*^{-/-} MEFs

(A) - Western blot analyses of BAT3 and Actin expression in *BAT3*^{-/-} MEFs transfected with empty vector, BAT3-FL, or BAT3 Δ NLS. (B) - Quantification of the number of autophagosomes per cell in *BAT3*^{-/-} MEFs co-transfected with peGFP-LC3 and indicated plasmid. Results are the mean (SD) of three independent experiments. ***: $p \leq 0.001$ versus empty vector. (C) - Representative images of (B). (D) - Immunoprecipitation of acetylated lysine residues followed by western blotting using an anti-ATG7 antibody in *BAT3*^{-/-} MEFs transfected with empty vector, BAT3-FL, or BAT3 Δ NLS. Control immunoprecipitation (CT) was carried out using a rabbit IgG. Expression of BAT3 and ATG7 was assessed by western blotting. Results are representative of three independent experiments. (E) - Western blot analysis of p53 acetylation at lysine 373 in *BAT3*^{-/-} MEFs stably transfected with indicated vector and incubated in CM or EBSS for 30 min. Expressions of p53, BAT3 and Actin are also shown. Results are representative of three independent experiments.

Figure 6: BAT3 modulates p300 intracellular localization

(A) - Western blot of the subcellular fractionation in WT and *BAT3*^{-/-} MEFs using anti-p300, -BAT3, -GAPDH (cytosol fraction), -LAMP2 (membrane fraction) and -H2B (soluble nuclear fraction) antibodies. Results are representative of five independent experiments. (B) - Co-immunoprecipitation of BAT3 and p300 in cytosolic and nuclear extracts of WT MEFs in CM or EBSS for 2h. This result is representative of three independent experiments. (C) - Western blot analysis of the subcellular localization of p300 in *BAT3*^{-/-} MEFs stably expressing empty vector, BAT3-FL, or BAT3 Δ NLS. (D) - Densitometric quantification of cytosolic p300 assessed in (C) and arbitrarily set at 100 for *BAT3*^{-/-} MEF expressing BAT3-FL. (E) - Quantification of the fluorescence intensity in the cytosol of WT and *BAT3*^{-/-} MEFs after immunostaining of p300 and during a starvation kinetic as indicated below x axis. Results are

the mean (SD) cytosol fluorescence intensity (in arbitrary units) of 100 cells (three independent experiments). ***: $p \leq 0.001$ versus WT MEFs in CM (F) - Western blot analysis of the subcellular localization of p300 and BAT3 in the cytosol and the nucleus of WT and *BAT3*^{-/-} MEFs during starvation during a starvation kinetic. Controls as in (A). (G) - Densitometric quantification of the p300 level in the nucleus assessed by western blot in (F). Unit was arbitrarily set at 1 for WT and *BAT3*^{-/-} MEFs in CM.

Figure 7: Model of autophagy regulation by BAT3

BAT3 regulates autophagy by modulating p300 localization and p300-dependent acetylation of p53 and ATG7. BAT3, allows p300 shuttling into the nucleus, where it targets p53 to stimulate autophagy. In contrast, when BAT3 is absent or exclusively cytosolic, p300 nuclear shuttling is inhibited, leading to its accumulation in the cytosol and ATG7 hyper-acetylation.