© 2021. Published by The Company of Biologists Ltd | Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

RESEARCH ARTICLE

THOC4 regulates energy homeostasis by stabilizing *TFEB* mRNA during prolonged starvation

Toshiharu Fujita[1], Sayaka Kubo[2], Tatsuya Shioda[2], Ayaka Tokumura[2], Satoshi Minami[3], Megumi Tsuchiya[4], Yoshitaka Isaka[3], Hidesato Ogawa[4], Maho Hamasaki[1,2], Li Yu[5], Tamotsu Yoshimori[1,2,*] and Shuhei Nakamura[1,2,6,*]

ABSTRACT

TFEB, a basic helix-loop-helix transcription factor, is a master regulator of autophagy, lysosome biogenesis and lipid catabolism. Compared to posttranslational regulation of TFEB, the regulation of *TFEB* mRNA stability remains relatively uncharacterized. In this study, we identified the mRNA-binding protein THOC4 as a novel regulator of TFEB. In mammalian cells, siRNA-mediated knockdown of THOC4 decreased the level of TFEB protein to a greater extent than other bHLH transcription factors. THOC4 bound to *TFEB* mRNA and stabilized it after transcription by maintaining poly(A) tail length. We further found that this mode of regulation was conserved in *Caenorhabditis elegans* and was essential for TFEB-mediated lipid breakdown, which becomes over-represented during prolonged starvation. Taken together, our findings reveal the presence of an additional layer of TFEB regulation by THOC4 and provide novel insights into the function of TFEB in mediating autophagy and lipid metabolism.

KEY WORDS: TFEB, THOC4, Autophagy, Lipid catabolism, mRNA stability

INTRODUCTION

Autophagy is an intracellular bulk degradation system induced by nutrient deprivation. Upon starvation, cytosolic components are sequestered by a double-membrane structure, the autophagosome, and targeted for degradation in lysosomes, thereby ensuring continued nutrient availability (Feng et al., 2014; Mizushima, 2007). Autophagy can also selectively degrade specific substrates including invading pathogens, aggregated proteins, damaged organelles and lipid droplets (Jin et al., 2013). Lipid droplet breakdown by autophagy, called lipophagy, is essential for meeting cellular energy demands, especially during long-term starvation (Minami et al., 2017; Singh et al., 2009).

[1]Department of Genetics, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan. [2]Department of Intracellular Membrane Dynamics, Graduate School of Frontier Biosciences, Osaka University, Osaka 565-0871, Japan. [3]Department of Nephrology, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan. [4]Nuclear Dynamics Group, Graduate School of Frontier Biosciences, Osaka University, Osaka 565-0871, Japan. [5]Department of Biological Science and Biotechnology, Tsinghua University, 100084 Beijing, China. [6]Institute for Advanced Co-Creation Studies, Osaka University, Osaka 565-0871, Japan.

*Authors for correspondence (tamyoshi@fbs.osaka-u.ac.jp; shuhei.nakamura@fbs.osaka-u.ac.jp)

T.F., 0000-0002-0171-7420; S.K., 0000-0003-0511-3787; S.M., 0000-0001-5043-3435; H.O., 0000-0001-9740-9010; T.Y., 0000-0001-9787-3788; S.N., 0000-0002-1488-8317

Handling Editor: Jennifer Lippincott-Schwartz
Received 30 April 2020; Accepted 3 February 2021

Transfection factor EB (TFEB), a member of the MiT/TFE subfamily of basic helix-loop-helix–leucine zipper (bHLH–LZ) transcription factors, was originally identified in association with renal cell carcinoma (Kuiper et al., 2003). It is now known as a master regulator of autophagy, lysosome biogenesis and lipid catabolism (O'Rourke and Ruvkun, 2013; Sardiello et al., 2009; Settembre et al., 2013, 2011). Under normal conditions, TFEB is phosphorylated by mTOR at Ser142 and Ser211 and is retained in the cytosol by interactions with members of the 14-3-3 protein family (Roczniak-Ferguson et al., 2012; Settembre et al., 2012). When cells are exposed to stress, such as starvation or lysosomal dysfunction, TFEB is no longer phosphorylated by mTOR and becomes translocated to the nucleus, where it activates the expression of genes promoting autophagy, lysosome biogenesis and/or lipid catabolism. This posttranslational regulation of TFEB is well characterized, but the mechanisms responsible for regulating the expression of TFEB itself remain to be fully elucidated.

THOC4 is a nuclear mRNA export adaptor. Although some RNAs, such as tRNA and miRNA, are exported from the nucleus by the importin/karyopherin-β-type transport receptor, mRNA uses a receptor called Tap–p15 (also known as NXF1–NXT1). During transcription, THOC4 interacts with the THO complex and RNA helicase UAP56 (also known as DDX39B) to form the TREX complex at the 3′ end of the gene (Strasser et al., 2002). When transcription is terminated, THOC4 is transferred to the mRNA via the TREX helicase activity, resulting in formation of export mRNA–protein complexes (mRNPs). Ultimately, mRNAs are handed over to Tap–p15 for export out of the nucleus. Tap–p15 facilitates mRNA export by directly binding both mRNA and nucleoporins. In both mammals and yeast, siRNA-mediated knockdown of THOC4 results in the nuclear accumulation of poly(A) RNA (Doma and Parker, 2007; Hautbergue et al., 2009; Katahira et al., 2009; Okada et al., 2008; Stubbs and Conrad, 2015). THOC4 also serves as a transcriptional co-activator or co-repressor (Bruhn et al., 1997; Osinalde et al., 2013; Virbasius et al., 1999), and may help to stabilize mRNAs by binding them (Lei et al., 2011; Stubbs et al., 2012).

In this study, we examined the involvement of THOC4 in autophagy and found that THOC4 regulates the level of TFEB by stabilizing the *TFEB* mRNA. TFEB transcriptional activity plays an important role in starvation-induced lipid catabolism by coordinating autophagic activity and lipolysis (Settembre et al., 2013). Expression of TFEB itself was also upregulated by starvation, but the mechanism responsible for this upregulation remains unclear. We also found that the levels of both *TFEB* and *THOC4* transcripts were elevated during prolonged starvation. THOC4 knockdown significantly decreased autophagic activity and lipid breakdown during long-term starvation, highlighting the importance of THOC4-mediated regulation of TFEB for energy

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

homeostasis under prolonged starvation. In addition, we found that THOC4 knockdown increased autophagy activity only in nutrient-rich conditions, independently of TFEB function, suggesting dual roles for THOC4.

RESULTS

THOC4 regulates TFEB at the mRNA level

We examined the role of THOC4 during autophagy using the mRFP–GFP tandem fluorescent-tagged LC3 (tf-LC3) reporter (Kimura et al., 2007). LC3 (also known as MAP1LC3B) is a mammalian homolog of yeast Atg8 and is widely used as an autophagosome marker (Kabeya et al., 2000; Mizushima, 2004). During autophagy, LC3 is recruited to both the inner and outer autophagosome membranes, and it is subsequently degraded when the autophagosome fuses with lysosomes. The tf-LC3 construct distinguishes the autophagosome and autolysosome populations; the GFP signal is quenched in the acidic lysosomal environment, whereas the mRFP is acid-resistant and therefore persists within the lysosome. Accordingly, GFP-positive, RFP-positive LC3 dots and GFP-negative, RFP positive LC3 dots indicate autophagosomes and autolysosomes, respectively. Thus, cells stably expressing tf-LC3 allowed us to monitor autophagic flux.

In nutrient-rich conditions, siRNA-mediated knockdown of THOC4 in HeLa Kyoto cells expressing tf-LC3 increased the population of GFP-negative, RFP-positive autolysosomes relative to that in the control knockdown, indicating an increase in autophagic flux (Fig. S1A,B). In addition, the abundances of LC3 puncta and ATG5 dots, indicating forming autophagosomes (Mizushima et al., 2001), were elevated by THOC4 knockdown in HeLa Kyoto cells, especially in nutrient-rich conditions (Fig. S1C–F). Enhanced autophagic activity following THOC4 knockdown was confirmed by increased LC3 flux in MEF cells (Fig. S1G,H), decreased protein level of autophagy-specific substrate p62 (also known as SQSTM1; Ichimura and Komatsu, 2010) in HEK293 and HeLa Kyoto cells (Fig. S1I–N), and also by the accumulation of autophagic vacuole-like double-membrane structures in HeLa Kyoto cells visualized using electron microscopy (Fig. S2) especially in the nutrient-rich condition, and no such big difference was observed in starved cells. THOC4 knockdown in HeLa Kyoto cells also inhibited the activity of mTOR, a negative regulator of autophagy, as reflected by a reduced level of phosphorylated S6 kinase (Fig. S3A,B) and of UVRAG phosphorylated at Ser498 during the nutrient-rich condition (Fig. S3G,H). Taken together, these data suggested that THOC4 depletion increased autophagic activity by repressing mTOR activity, possibly via the inhibition of UVRAG Ser498 phosphorylation, which reportedly facilitates autophagosome maturation (Kim et al., 2015). Downregulation of mTOR leads to activation of TFEB via dephosphorylation of TFEB on Ser142 and Ser211, resulting in upregulation of autophagy and lysosomal biogenesis at the transcriptional level (Roczniak-Ferguson et al., 2012; Settembre et al., 2011). Surprisingly, however, the level of TFEB protein was dramatically reduced upon THOC4 depletion (Fig. 1A,B; Fig. S5A,B), in the opposite direction to upregulation of autophagy, suggesting that THOC4 may repress autophagy independently of TFEB function. Consistent with this idea, inactivation of mTOR (Fig. S3C,D) and the inhibition of downstream target UVRAG Ser498 phosphorylation (Fig. S3G,H) by THOC4 knockdown was also observed in TFEB knockout (KO) Hela Kyoto cells generated using the CRISPR/Cas9 system (Nakamura et al., 2020). A candidate approach to searching for mTOR regulators by qPCR revealed that mTOR activity might be suppressed by transcriptional upregulation of an upstream mTOR inhibitor, TSC2 (Li et al., 2004), upon THOC4 depletion (Fig. S3I). Importantly, the upregulation TSC2 was observed in TFEB KO cells (Fig. S3J). Furthermore, we found that THOC4 knockdown led to the upregulation of several autophagy-related genes in wild-type (WT) cells (Fig. S4A) and also in TFEB KO cells (Fig. S4B), suggesting that the presence of TFEB-independent autophagy regulation by THOC4, especially in nutrient-rich conditions.

Next, we sought to determine the mechanism responsible for the robust downregulation of TFEB by THOC4 knockdown, as well as the physiological relevance of this phenomenon. The level of TFEB protein decreased by 50% or more upon THOC4 knockdown in HeLa Kyoto cells, whereas the levels of two other MiT/TFE family transcription factors, MITF and TFE3 (Fig. 1A,B), were largely unaffected, suggesting that THOC4 mainly regulates TFEB among the members of the MiT/TFE family. The reduction of TFEB protein was observed for all three siRNAs against THOC4 that we tested, excluding possible off-target effects of siRNA treatment (Fig. S5A,B). To determine the underlying mechanism, we first assessed the involvement of two well-known protein degradation pathways, the ubiquitin–proteasome system (UPS) and autophagy. Neither the proteasome inhibitor MG132 nor bafilomycin A1 (BafA1), an inhibitor of autophagosome–lysosome fusion, reverted the TFEB protein level in THOC4-knockdown cells. Accumulation of polyubiquitylated proteins and LC3, a marker of autophagy, confirmed the efficacy of MG132 and BafA1, respectively (Fig. 1C–F). Therefore, the two major protein degradation pathways are not involved in regulation of TFEB by THOC4.

Next, we tested the possibility that THOC4 regulates TFEB at the mRNA level. Given that THOC4 is an mRNA transport adaptor, it is possible that THOC4 depletion causes mRNA to accumulate in the nucleus, where it cannot be translated. To explore this idea, we fractionated the cell into nuclear and cytosolic fractions and performed qPCR to measure the amount of mRNA in each fraction. Upon THOC4 knockdown, the level of *TFEB* mRNA was reduced in the nucleus as well as in the cytosol (Fig. 2A). Thus, the overall level of *TFEB* mRNA is decreased by THOC4 knockdown, possibly by transcriptional downregulation or mRNA degradation.

THOC4 binds to mRNA and maintains mRNA stability

Next, we assessed the transcriptional regulation of exogenously expressed FLAG- or mNeonGreen (mNG)-tagged TFEB. Transiently transfected TFEB–FLAG (Fig. 2B) and virally transduced TFEB–mNG (Fig. 2C), which do not have the TFEB promoter and UTR, also exhibited dramatic decreases upon THOC4 depletion. This result indicated that THOC4 does not directly bind to a specific sequence in the *TFEB* promoter, ruling out the possibility that THOC4 acts as a transcriptional regulator of the endogenous *TFEB* gene. To support this idea, we examined the binding of THOC4 to the CLEAR element, which is known to be directly bound by TFEB and is located in the *TFEB* promoter (Palmieri et al., 2011; Sardiello et al., 2009; Settembre et al., 2013). Pulldown experiments suggested that THOC4 could not bind to the CLEAR sequence (Fig. S5C). As an alternative explanation, we hypothesized that THOC4 regulates mRNA stability, and that upon THOC4 depletion, the destabilized *TFEB* mRNA is quickly degraded. Hence, we investigated the binding of THOC4 to mRNAs. We confirmed that that THOC4 bound the mRNAs encoding TFEB, other members of the MiT/TFE family, and MondoA (also known as MLXIP), another bHLH transcription factor (Fig. 3A,B), which functions as a glucose sensor and regulates autophagy (Nakamura et al., 2016). While control knockdown did not change the level of *TFEB* mRNA in the presence

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

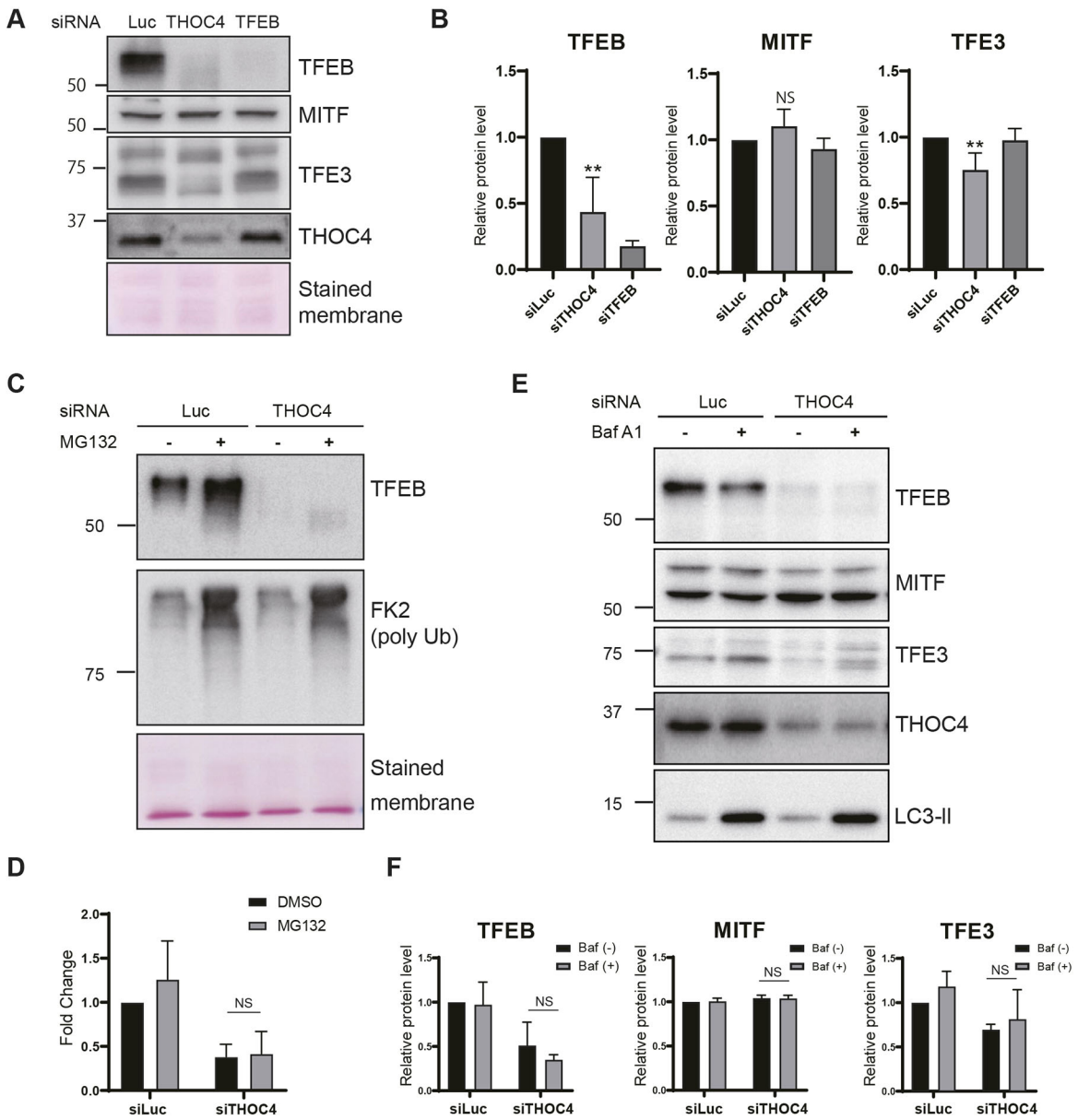

Fig. 1. THOC4 regulates TFEB protein expression. (A) HeLa Kyoto cells were treated with siRNA against THOC4, TFEB or luciferase (Luc; negative control). After 60 h of siRNA treatment, cells were lysed and subjected to western blotting with the indicated antibodies. Ponceau S-stained membrane is shown as a loading control. Blots are representative of four experiments. (B) Relative levels of TFEB, MITF and TFE3 protein, as assayed in A, upon siRNA-mediated THOC4 or TFEB depletion, in comparison with the effects luciferase siRNA (siLuc) treatment. Mean±s.d. (n=4). **P<0.01; NS, not significant (one-way ANOVA and Dunnett's multiple comparisons test). (C,D) HeLa Kyoto cells were treated with siRNA as in A, and 54 h after addition of siRNA, cells were treated with 2.5 µM MG132 or DMSO vehicle for 6 h and subjected to western blotting with antibodies against TFEB and polyubiquitylated (poly Ub) proteins. Ponceau S-stained membrane is shown as a loading control. Mean±s.d. (n=3). NS, not significant (two-way ANOVA and Sidak's multiple comparisons test). (E) HeLa Kyoto cells were treated with siRNA as in A; 58 h after siRNA addition, cells were grown with or without 125 nM bafilomycin A1 (Baf A1) for 2 h and subjected to western blotting with the indicated antibodies. (F) The levels of TFEB, MITF and TFE3 as assayed in E were quantified using ImageJ. Means±s.d. (n=3). NS, not significant (two-tailed unpaired t-test). Size markers in A,C,E are in kDa.

of the transcription inhibitor actinomycin D, THOC4 depletion decreased it by 30% or more (Fig. 3C,D). By contrast, the *MondoA* mRNA was completely stable even after THOC4 depletion. Consistent with this, the protein level of MondoA did not change drastically upon THOC4 knockdown (Fig. S5D,E). Considering the fact that THOC4 knockdown also decreased expression from the exogenously expressed TFEB construct, THOC4 might bind the coding sequence of TFEB for the maintenance of expression.

In general, the primary determinant of mRNA stability is poly(A) tail length. Poly(A) tails of mRNA have an initial length of ∼250 nucleotides and are gradually shortened over the lifetime of the mRNA by deadenylation. Long poly(A) tails inhibit the two main mRNA decay pathways, 3′ exonucleolytic degradation and cap hydrolysis followed by 5′ degradation. Accordingly, mRNAs with short tails have shorter half-lives (Meyer et al., 2004; Parker and Song, 2004). The poly(A) tail of *TFEB* mRNA was shortened upon THOC4 depletion, as reflected by the observation that the proportion of mRNA with a long poly(A) tail was reduced (Fig. 3E,F; see region enclosed by a rectangle).

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

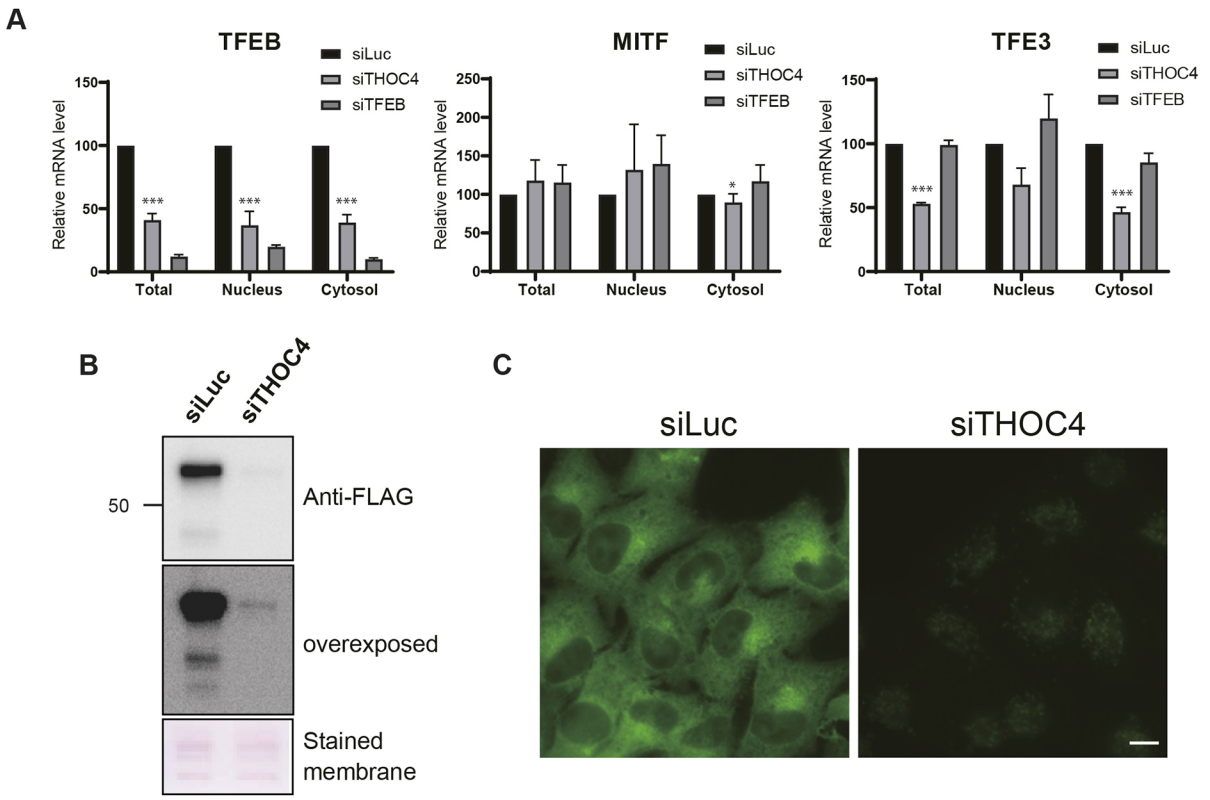

Fig. 2. THOC4 regulates TFEB at the mRNA level. (A) Relative levels of *TFEB*, *MITF* and *TFE3* mRNA in total, nuclear and cytosolic fractions of HeLa Kyoto cells following siRNA-mediated depletion of THOC4 or TFEB, or treatment with luciferase siRNA (siLuc). The level of each mRNA was normalized to the corresponding level of 7SK mRNA. Mean±s.e.m. (n=3). ***P<0.005; *P<0.05 (two-tailed, unpaired t-test). (B) HeLa Kyoto cells were treated with siRNA against THOC4 or luciferase. After 24 h, siRNA was washed out, and cells were transfected with TFEB–FLAG. Cell lysates were collected 24 h after transfection and then immunoblotted with anti-FLAG-M2 antibody. Ponceau S-stained membrane is shown as a loading control. (C) Hela Kyoto cells stably expressing TFEB–mNG were treated with siRNA against THOC4 or luciferase. Scale bar: 10 µm. Data in B and C are representative of three experiments.

Taken together, these results suggest that binding of THOC4 preferentially protects *TFEB* mRNA from degradation by maintaining the poly(A) tail length. Moreover, this effect is mainly observed for TFEB and not for the other bHLH transcription factors assayed.

TFEB regulation by THOC4 is important for maintenance of autophagic activity during prolonged starvation

The levels of THOC4, TFEB and mRNAs regulated by TFEB, such as those encoding p62, LAMP1 and ATP6V1H, were elevated only during long-term (12 h) starvation (Fig. 4A). Importantly, we observed that the exogenously expressed TFEB–mNG was also significantly upregulated during the prolonged starvation, suggesting that this upregulation could partly occur in the absence of CLEAR elements and *TFEB* UTRs (Fig. 4B). Under THOC4 and TFEB depletion, autophagic flux was not affected by a 2 h starvation (Fig. S1G,H), but it was significantly reduced in cells starved for 12 h (Fig. 4C,D). These results suggest that regulation of the TFEB level by THOC4 is important for the maintenance of autophagic activity during prolonged starvation.

TFEB is involved in lipid catabolism by coordinating autophagy and lipolysis in cells starved for prolonged periods; this regulation is also conserved in the nematode *Caenorhabditis elegans* (O'Rourke and Ruvkun, 2013; Settembre et al., 2013). We monitored the turnover of lipid droplets under starvation in worm mutants of *aly* (a worm homolog of THOC4) and *hlh-30* (a worm homolog of TFEB). THOC4 has three homologs in *C. elegans*: *aly-1*, *aly-2* and *aly-3*; the level of HLH-30::GFP was significantly decreased in triple knockdown of all three *aly* genes (Fig. 4E,F). Levels of endogenous *hlh-30* mRNA were also significantly decreased in *aly*-1-deficient worms treated with RNAi against *aly-2* and *aly-3* (Fig. 4G). As in *hlh-30*-deficient mutants, lipid droplets marked by Oil Red O staining were significantly accumulated during starvation in *aly-2*-deficient worms compared to wild-type worms (Fig. 5A,B). Importantly, *hlh-30* knockdown did not further increase the abundance of lipid droplets in an *aly-2*-deficient mutant, suggesting that both genes act in the same pathway (Fig. 5E,F). Lipophagy is known to be induced under prolonged starvation in kidney proximal tubular cells (PTCs; Minami et al., 2017). Consistent with the role of the *C. elegans* THOC4 homolog, we found that BODIPY-stained lipids in PTCs were accumulated to higher levels under prolonged starvation when THOC4 was knocked down (Fig. 5C,D). These data suggest that induction of lipid catabolism under starvation, a known role of TFEB, is regulated by THOC4.

DISCUSSION

The bHLH transcription factor TFEB is a master regulator of autophagy, lysosome biogenesis and lipid catabolism. However, whereas the posttranslational regulation of TFEB has been well studied, the mechanisms involved in regulation of TFEB expression before its translation were largely unclear. In this study, we identified the mRNA-binding protein THOC4 as a novel regulator of TFEB. siRNA-mediated knockdown of THOC4 decreased the level of TFEB protein to a greater extent than other bHLH

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

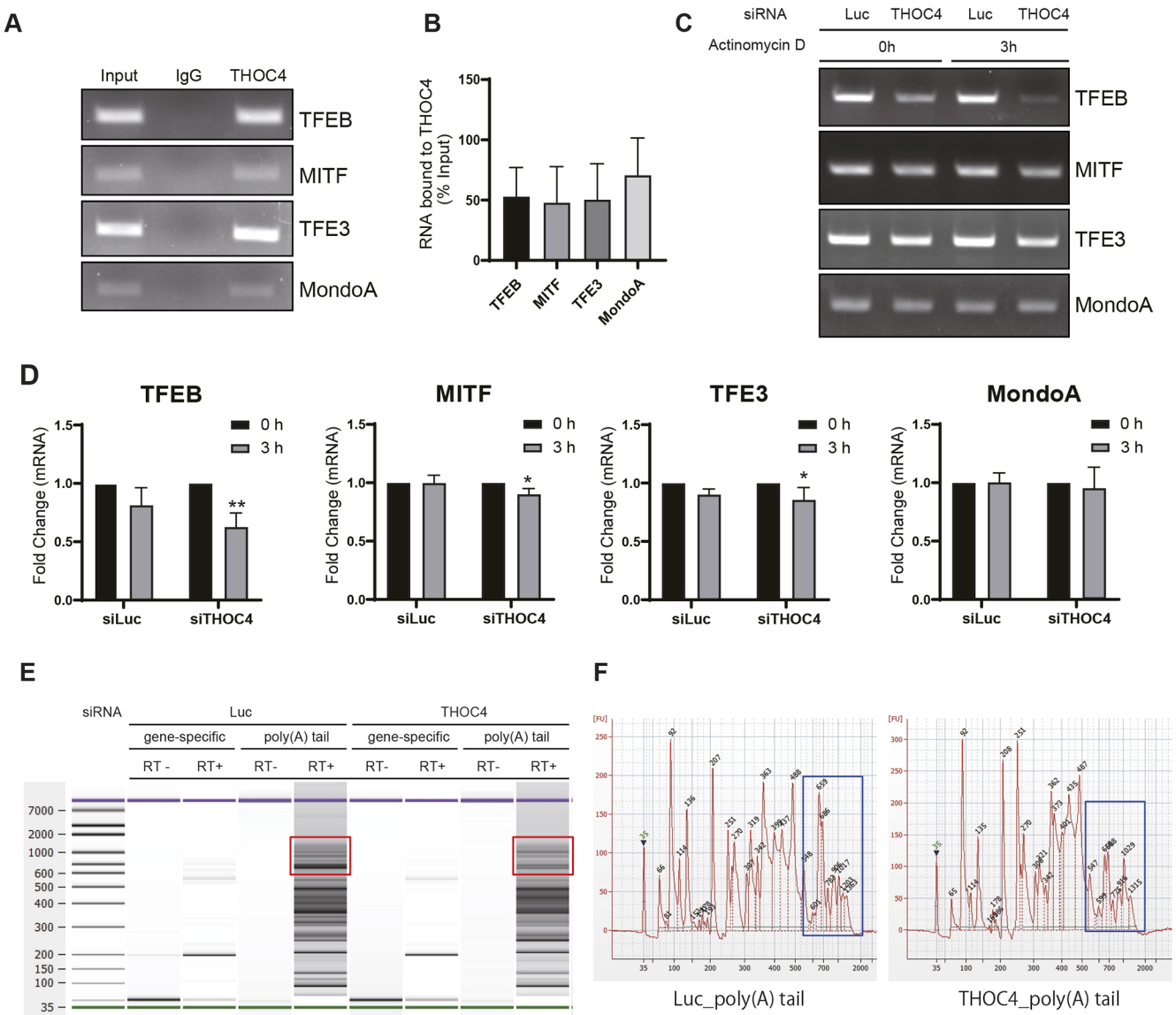

Fig. 3. THOC4 binds to *TFEB* mRNA and maintains its stability. (A,B) The THOC4–RNA complex was pulled down from HeLa Kyoto cells using a RIP assay kit, and bound RNA was amplified by RT-PCR. (A) Representative gels showing amplification of the indicated mRNAs from the input and from THOC4- or control IgG-bound fractions. (B) Quantification of RNA bound to THOC4. Mean±s.e.m. (n=3). (C,D) HeLa Kyoto cells were treated with siRNA against THOC4 or luciferase (siLuc). After 48 h, cells were treated with 1 µg µl^{-1} actinomycin D for 3 h. RNA was purified from cell lysates and subjected to RT-PCR. (C) Representative gels showing RT-PCR of the indicated mRNAs. (D) Band intensities, as in C, were measured using ImageJ and plotted. Mean±s.d. (n=3). $^{**}P$<0.01; $^{*}P$<0.05 (two-way ANOVA and Sidak's multiple comparisons test). (E,F) HeLa Kyoto cells were treated with siRNA against THOC4 or luciferase (Luc), and total RNA was purified from cell lysates. Collected RNAs were subjected to (E) poly(A) length detection and (F) analyzed on an Agilent 2100 Bioanalyzer. Reverse transcription reactions were performed with (+) or without (–) reverse transcriptase enzyme (RT), and PCRs were performed using TFEB-specific or universal poly(A) reverse primers. Rectangles indicate longer (600–2000 bp) size of the products. Size markers in E and x-axis in F are in bp. FU, fluorescence unit. Data are representative of three experiments.

transcription factors. THOC4 bound to *TFEB* mRNA and stabilized it after transcription by maintaining the poly(A) tail length to protect the mRNA from nucleolytic degradation. This mode of regulation was particularly important during prolonged starvation, when TFEB-mediated lipid catabolism is essential for sustaining cell viability. Indeed, during prolonged starvation, the levels of THOC4, TFEB and mRNAs regulated by TFEB were all elevated, presumably due to maintenance of TFEB function via stabilization of its mRNA. Accordingly, THOC4 knockdown resulted in accumulation of lipid droplets similar to that observed for TFEB dysfunction. As we confirmed (Fig. S3E,F), and consistent with a previous report (Yu et al., 2010), mTOR activity is reactivated and autophagic activity decreases when starvation is prolonged. Remarkably, THOC4 (ALY)-mediated TFEB (HLH-30) regulation and its impact on lipid catabolism during starvation are conserved in *C. elegans*.

Intriguingly, THOC4 knockdown in nutrient-rich conditions increased autophagic activity, indicating that THOC4 can serve as either a negative or positive regulator of autophagy depending on the context. Similar dual functions were observed in a study of p53 (also known as TP53; Tasdemir et al., 2008a,b): nuclear p53 transcriptionally activates autophagy, whereas cytoplasmic p53 inhibits autophagy via inactivation of AMPK and activation of mTOR. Despite the dramatic decrease in the level of TFEB, under

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

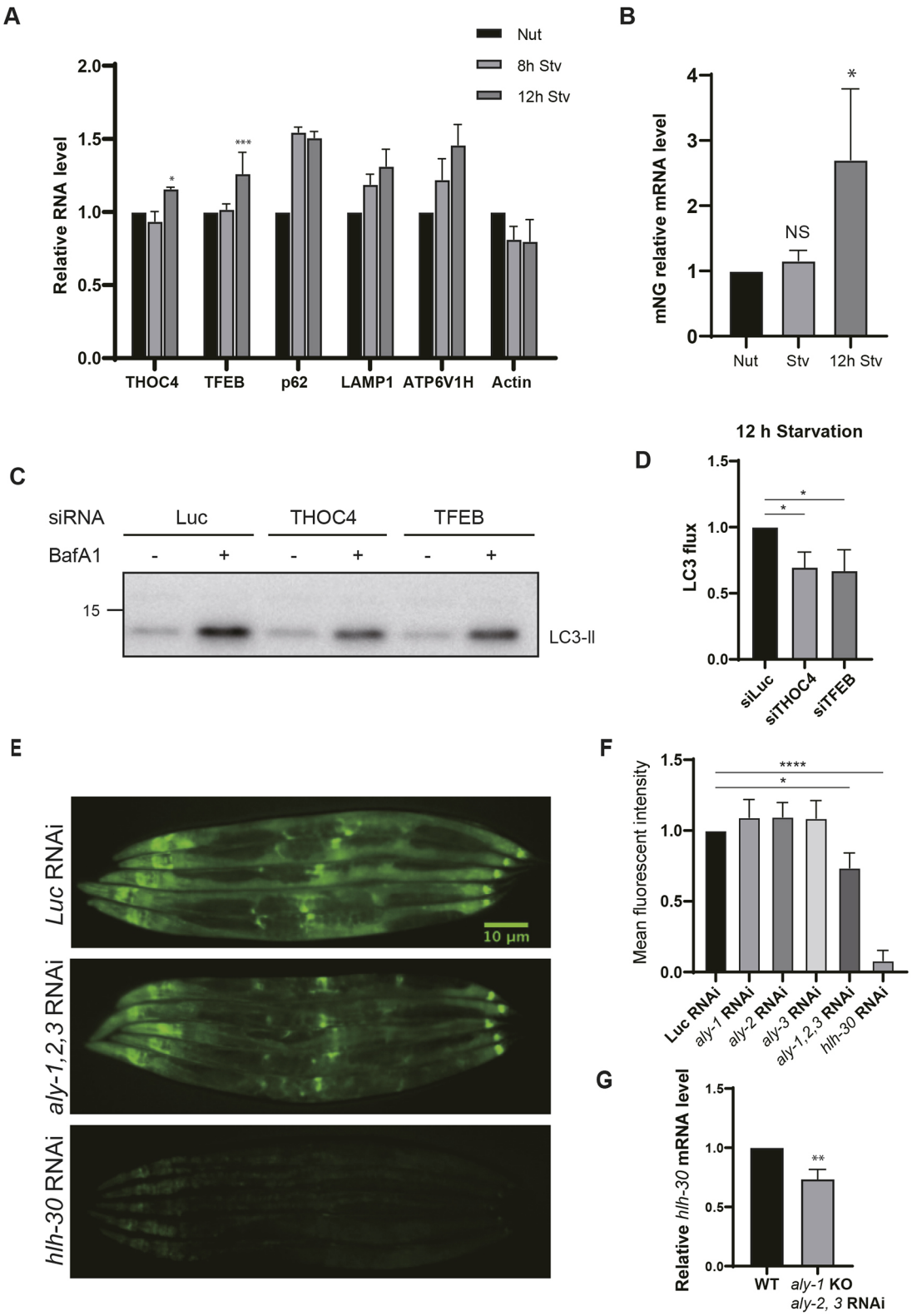

Fig. 4. See next page for legend.

nutrient-rich conditions, autophagic activity was increased by THOC4 knockdown via inactivation of mTOR, implying mTOR-dependent and TFEB-independent regulation of autophagy. mTOR inactivation was possibly due to TFEB-independent transcriptional upregulation of the upstream inhibitor TSC2. Reduction of mTOR activity resulted in the prevention of UVRAG phosphorylation at Ser498. Therefore, THOC4 depletion may facilitate UVRAG-mediated endosome-lysosomal degradation. We also confirmed that THOC4 depletion in nutrient-rich conditions leads to the upregulation of subsets of

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

Fig. 4. TFEB regulation by THOC4 is particularly important for autophagic activity under prolonged starvation. (A) HeLa Kyoto cells were incubated in DMEM (nutrient-rich condition, Nut) or starvation (Stv) medium (EBSS) for 8 or 12 h. Cells were harvested, and RNA was purified to synthesize cDNA. Relative levels of the indicated mRNAs were measured by qPCR. Mean±s.d. (n=3). ***P<0.005; *P<0.05 (two-way ANOVA and Dunnett's multiple comparisons test). (B) HeLa Kyoto cells were transfected with TFEB–mNG. At 24 h after transfection, cells were grown in DMEM or starved in EBSS for 2 h or 12 h. Then, the relative levels of mNG mRNA were measured by RT-qPCR. Mean±s.d. (n=3). *P<0.05; NS, not significant (one-way ANOVA and Dunnett's multiple comparisons test). (C,D) MEF cells were treated with siRNA against THOC4, TFEB, or luciferase (as a control; Luc). After 48 h, cells were grown in DMEM or EBSS for 12 h. Bafilomycin was added for the last 2 h. Cell lysates were collected and subjected to western blotting with anti-LC3 antibody. (C) Representative blot of lysates from 12 h starvation conditions. (D) Quantification of LC3 flux, as in C. Mean±s.d. (n=3). *P<0.05 (one-way ANOVA and Dunnett's multiple comparisons test). (E,F) MAH240 worms expressing HLH-30::GFP were grown from eggs on a plate containing RNAi against the indicated genes, and the GFP fluorescence intensity of second-generation worms was measured on adult day 1. (E) Representative images of worms for each RNAi condition. (F) Measured intensity. Data are expressed as mean±s.d. (n=3). *P<0.05; ****P<0.0001 (one-way ANOVA and Dunnett's multiple comparisons test). (G) WT worms treated with luciferase RNAi or *aly-1* KO worms treated with *aly-2* and *aly-3* RNAi were collected, and RNA was purified. Relative *hlh-30* mRNA level was quantified by RT-qPCR and normalized against *ama-1* mRNA. Mean±s.d. (n=3). **P<0.01 (two-tailed, unpaired t-test)

autophagy genes through currently uncharacterized but clearly TFEB-independent mechanisms. Previous work has shown that BRD4 suppresses autophagy and lysosome function independently of TFEB (Sakamaki et al., 2017). BRD4 transcriptionally regulates gene expression via several pathways (Kanno et al., 2014), and THOC4 may regulate autophagy in concert with those factors independently of TFEB as well.

On the other hand, during prolonged starvation, autophagic activity was reduced upon THOC4 knockdown, even though mTOR activity was inhibited compared to levels in nutrient-rich conditions (Fig. S3E,F), suggesting that TFEB function predominated over the effect of mTOR inhibition. In addition, expression of autophagy-related genes upregulated upon THOC4 depletion was mostly reduced to basal levels during prolonged starvation (Fig. S4C). This may explain the predomination of declined autophagic activity due to decreased TFEB. Alternatively, reduced autophagic activity observed in THOC4-knockdown cells during prolonged starvation might be due to a lack of functional lysosomes. Sustained starvation together with a lack of lysosomal biogenesis following TFEB depletion could consume all the functional lysosomes available to be fused with autophagosomes. A detailed mechanism that explains how THOC4 knockdown represses autophagic activity during long-term starvation needs to be clarified in future studies. The discovery of the role of THOC4 may provide novel insight into TFEB regulation and the control mechanism of autophagy and lipid metabolism by TFEB.

MATERIALS AND METHODS

Reagents and antibodies

Antibodies for THOC4 (1:1000, ab6141) and MITF (1:1000, ab20663) were from Santa Cruz Biotechnology. Antibodies for β-actin (1:10,000, M177-3), p62 (1:10,000, PM045) and LC3 (1:1500, PM036) were from MBL. Antibodies for TFEB (1:1000, #4240), p70 S6 kinase (1:1000, #9202S) and P-p70 S6 kinase (T389) (1:1000, #9205S) were from Cell Signaling Technology. Antibody for TFE3 (1:1000, HPA023881) was from Atlas Antibodies. Antibody for FK2 (1:10,000, 302-06751) was from NIPPON BIO-TEST LABORATORIES INC. MondoA antibody (1:1000, A303-195A) was from Bethyl Laboratories, Inc. HRP-conjugated anti-FLAG M2 antibody (1:10,000, A-8592) and oleic acid (O7501) were from Sigma-Aldrich. Bafilomycin A1 (023-11641), Blasticidin (029-18701) and Oil Red O (154-02072) were from Wako. MG132 (474790) and anti-phospho-UVRAG (Ser498) antibody (1:1000, ABS1600) were from Merck Millipore Ltd. BODIPY 493/503 (D3922) was from Thermo Fisher Scientific. Puromycin (ant-pr-1) was from nacalai tesque.

Cell culture and transfection

MEFs (a kind gift from Sharon A. Tooze, Francis Crick Institute, London, UK), HeLa Kyoto, HEK293 (R70507; Invitrogen) and Plat-E cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM D6429; Sigma-Aldrich) containing 10% fetal bovine serum (FBS), 5 U ml^{-1} penicillin and 50 U ml^{-1} streptomycin. For nutrient starvation, cells were cultured in EBSS (E2888; Sigma-Aldrich). Kidney proximal tubular cells (PTCs) were cultured in low glucose medium (D6046; Sigma-Aldrich) supplied with 5% FBS (nutrient-rich condition) or 0.1% FBS (for starvation). Transient transfection was carried out using Lipofectamine 2000 (Invitrogen). HeLa Kyoto cells were generously provided by Prof. Shuh Narumiya (Kyoto University, Japan; Imai et al., 2016). PlatE cells were generously provided by Toshio Kitamura (University of Tokyo, Japan; Morita et al., 2000). All cells were regularly inspected for Mycoplasma infection.

Plasmids

The p3×FLAG–TFEB was kindly gifted by Andrea Ballabio (University of Naples, Italy). The pMRX-IRES-puro and pMRX-IRES-bsr vectors were kindly provided by S. Yamaoka (Tokyo Medical and Dental University, Tokyo, Japan). pMRX constructs were generated to encode TFEB–mNG (Nakamura et al., 2020), GFP–Atg5 (Fujita et al., 2013) and tandem fluorescent-tagged LC3 (tf-LC3) (Kimura et al., 2007). Retroviruses were prepared as previously described (Saitoh et al., 2003). Stable transformants were selected in growth medium with 1 µg/ml puromycin or 5 µg/ml blasticidin. pcDNA3.1 was purchased from Invitrogen (V79020). The DNA fragment of THOC4 was amplified from HeLa Kyoto cDNA using primers as follows: fw-primer, 5′- AACCAATTCAgtcgacGAGCCGATGCCCG-ATTCCGCGCCCGCCA-3′; and rv-primer, 5′- GTCTAGATATCTCGA-GTGACTGGTGTCCATTCTCGCAT-3′. The fragment was subcloned into the SalI-XhoI sites of pENTR1A (A10462, Invitrogen). pENTR1A-THOC4 was transferred into pcDNA3.1-mCherry using an LR reaction following the manufacturer's protocol (11791043; Invitrogen).

Western blotting

Cells were lysed in sample buffer containing 56 mM Tris-HCl (pH 6.8), 6% (v/v) glycerol, 2% SDS, 0.1 M DTT and 2.4% Bromophenol Blue, and the lysates were heated at 95°C for 5 min. Protein concentrations were measured using a Protein Quantification Assay kit (740967.250; MACHEREY-NAGEL). Equal amounts of cell lysates were analyzed by SDS–PAGE and transferred to polyvinylidene difluoride membranes (IPVH00010, Merck Millipore). Membranes were blocked with Tris-buffered saline (TBS) containing 0.1% Tween-20 and 1% skim milk, and incubated with primary antibodies. Immunoreactive bands were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA), and then with Luminata Forte (WBLUF0100; Merck Millipore) or ImmunoStar LD (290-69904; FUJIFILM) on a ChemiDoc Touch Imaging System (Bio-Rad).

Immunofluorescence and microscopy

Samples were fixed with 4% paraformaldehyde for 10 min, permeabilized with 50 µg/ml digitonin in PCS, blocked with 0.1% gelatin in phosphate-buffered saline (PBS), and then incubated with the indicated primary antibodies. After washing with PBS, the samples were incubated with secondary antibodies and mounted. Images were acquired on an IX83 microscope (OLYMPUS). Secondary antibodies were conjugated to Alexa Fluor 488 or 568. Image acquisition software Metamorph (MOLECULAR DEVICES) was used. The images were adjusted using Fiji (https://imagej.net/Fiji).

Journal of Cell Science (2021) 134, jcs248203. doi:10.1242/jcs.248203

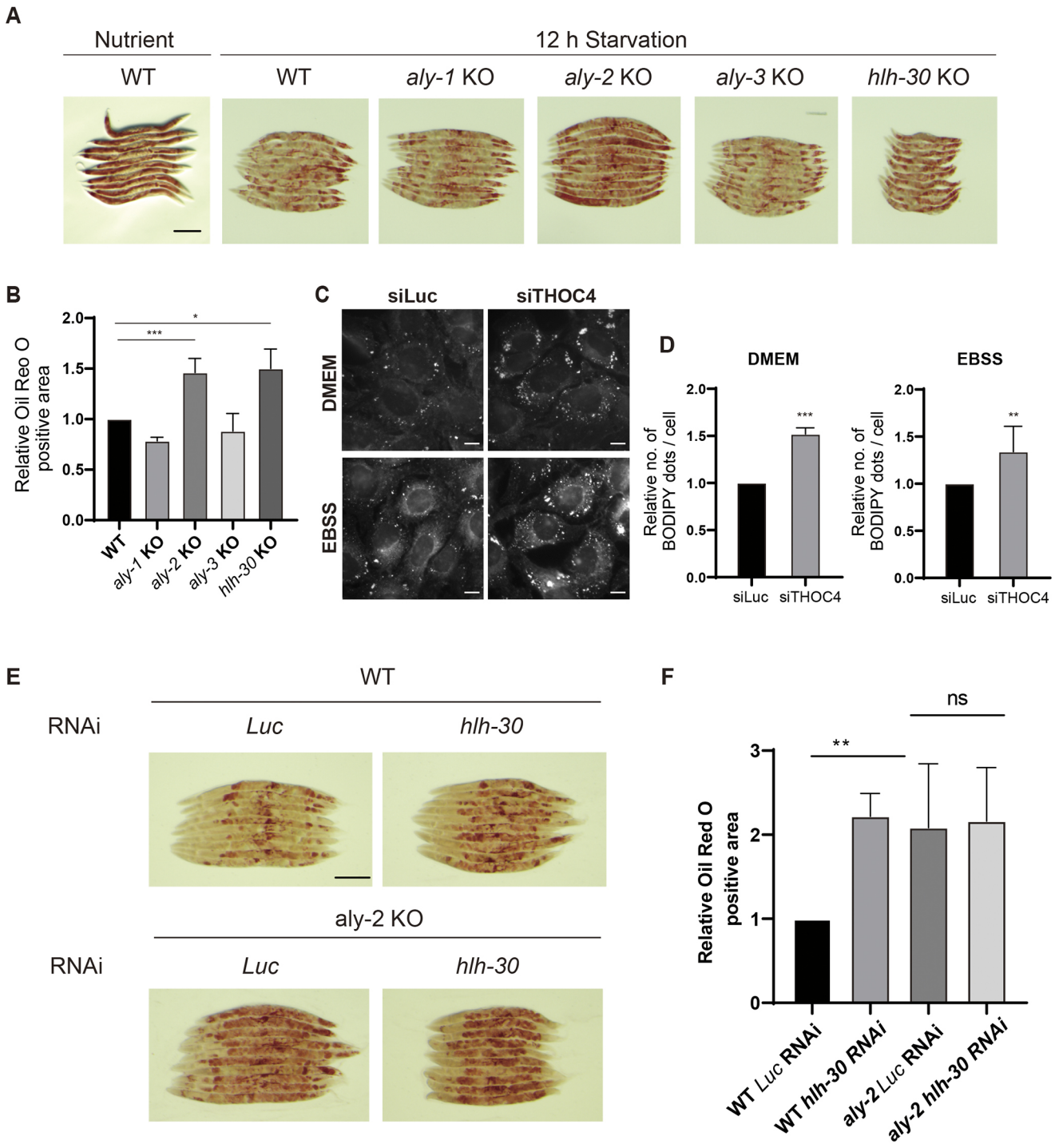

Fig. 5. TFEB regulation by THOC4 is important for lipid metabolism through autophagy under prolonged starvation. (A) WT and the indicated KO worms were either grown in nutrient-rich conditions or starved for 12 h, then stained with Oil Red O. Scale bar: 200 µm. (B) Quantification of relative dyed area, as in A. Data are shown as means±s.d. (n=3). *P<0.05; ***P<0.005 (one-tailed, unpaired t-test). (C,D) Kidney proximal tubular cells (PTCs) treated with siRNA against THOC4 or luciferase (siLuc) were supplied with 250 µM oleic acid (OA) and chased with normal (5% FBS, DMEM) or serum-limited (0.1% FBS, EBSS) medium for 24 h. The samples were stained with BODIPY 493/503 to detect neutral lipids. (C) Representative images of stained cells. Scale bars: 10 µm. (D) BODIPY dots were counted using ImageJ. Mean±s.d. n=3 for nutrient-replete conditions; n=7 for starvation conditions. ***P<0.005; **P<0.01 (two-tailed, unpaired t-test). (E) WT or *aly-2*-mutant worms were grown from eggs on a plate containing RNAi against *hlh-30* or Luciferase (Luc; control). Day 1 adult worms were then starved for 12 h and stained with Oil Red O. Scale bar: 200 µm. (F) Relative values of Oil Red O-positive areas were calculated. Mean±s.d. (n=3). **P<0.01; ns, not significant (two-tailed, unpaired t-test).

LD degradation assay

Kidney proximal tubular cells (PTCs), a kind gift from Yoshitaka Isaka (Osaka University, Osaka, Japan), were incubated with 250 µM oleic acid (OA) for 12 h and then incubated with normal (5% FBS) or serum-limited (0.1% FBS) medium for 24 h. After 4% paraformaldehyde fixation, samples were then stained with 200 ng/ml BODIPY493/503. Images were acquired on an IX83 microscope (OLYMPUS). BODIPY-positive dots were counted using Fiji.

Electron microscopy

For electron microscopy, samples were fixed with 2.5% glutaraldehyde (Wako, 071–01931) and then in a 2% OsO_4 solution. They were embedded in Quetol812 (Nisshin EM), and ultrathin sections (80 nm) were obtained using an Ultracut E ultramicrotome (Reichert-Jung); these were stained with a solution of uranyl acetate and lead and observed using a Hitachi H-7650 transmission electron microscope (Hitachi).

Reverse transcription quantitative PCR

For reverse transcription quantitative PCR (RT-qPCR), total RNA was isolated (RNeasy Plus Mini Kit, QIAGEN), complementary DNA was synthesized (iScript cDNA Synthesis Kit, #170-8891, Bio-Rad), and qRT-PCR (primer sequences in Table S1; designed with Primer Express Software Ver. 3.0.1, Applied Biosystems) was performed on a Quant Studio 7 Flex qPCR machine (Thermo Fisher Scientific). mRNA levels were normalized against the level of 7SK.

RNA interference

siRNA duplex oligomers were designed as follows: 5′-UCGAAGUAUU-CCGCGUACG-3′ (luciferase), 5′-GCAGCUUAGGAACAGCAGA-3′ (human *THOC4* #1), 5′-CAUUCAGCUUGUCACGUCA-3′ (human *THOC4* #2), 5-CGCGGAGGCGCCCGUGGAA-3′ (human *THOC4* #3), 5′-CCGAAACAACUUCCCGACA-3′ (mouse *Thoc4*) and 5′-GUCCCA-UGGCCAUGCUACA-3′ (mouse *Tfeb*), which were purchased from Sigma. For human TFEB, ON-TARGETplus Human TFEB (7942) siRNA (Dharmacon) was used. 20 nM siRNA was introduced to cells using Lipofectamine RNAiMAX (Invitrogen).

RNA immunoprecipitation

Protein–RNA complexes were immuno-precipitated using an RNA immunoprecipitation (RIP) assay kit (RIP Assay Kit; MBL). Two 10-cm dishes of HeLa Kyoto cells were used for each sample. cDNA was synthesized from RNA using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific). PCR primers were as follows: TFEB forward, 5′-GAATGTGTACAGCAGCGACC-3′; TFEB reverse, 5′-GAACATGG-CTGAGCTGGC-3′; MITF forward, 5′-CAACTCCTGTCCAGCCAAC-3′; MITF reverse, 5′-GAACCCGTTCTTGAGAACTG-3′; TFE3 forward, 5′-GACCCATCTGGAGAACCC-3′; TFE3 reverse, 5′-GACAGGATCAAG-GAACTGGG-3′; MondoA forward, 5′-CCACTTCATGGTGTCGTCG-3′; MondoA reverse, 5′-GCTGTATTGGCACAAGCAC-3′; HES5 forward, 5′-CTGCTCAGCCCCAAAGAG-3′; HES5 reverse, 5′-CTGCTGTACCAC-TTCCAGC-3′; ASCL3 forward, 5′-GATGGACAACAGAGGCAAC-3′; ASCL3 reverse, 5′-CCATGCTGACCCTATGTTC-3′; TCF3 forward, 5′-CTCCCTGACCTGTCTCGG-3′; TCF3 reverse, 5′-CAGATGGTGCTTT-CAGCTCC-3′.

Nuclear fractionation

HeLa Kyoto cell monolayers were washed with PBS, trypsinized, and pelleted by centrifugation at 9000 ***g*** for 1 min. The pellet was resuspended and incubated on ice for 10 min in 300 µl PBS containing 0.1% NP-40. The resultant homogenate was subjected to centrifugation at 9000 ***g*** for 10 s. The resultant supernatant and pellet were collected as the cytosolic and nuclear fractions, respectively.

C. elegans growth conditions and strains

All strains were maintained at 20°C using standard techniques on nematode growth medium (3 g NaCl, 2.5 g Bacto Peptone and 17 g Bacto Agar were dissolved in 1 l H_2O supplemented with 1 mM CaCl, 1 mM $MgSO_4$, 40 mM KPO_4 and 5 mg cholesterol) supplied with *E. coli* strain OP50 for *ad libitum* feeding. The following strains were used in this study; N2 as wild-type, MAH235{*sqIs19* [*hlh-30p::hlh-30::GFP*+*rol-6(su1006)*]}, *hlh-30(tm1978)*, *aly-1(ok1920)*, *aly-2(ok1203)* and *aly-3(syb1669)*. *aly-3(syb1669)* was generated at SunyBiotech (https://www.sunybiotech.com). All strains were derived from Caenorhabditis Genetic Center (CGC) except for *aly-3* KO. HLH-30::GFP fluorescence was captured using SZX16 microscopy (OLYMPUS). RNA interference (RNAi) was conducted by feeding HT115 (DE3) bacteria transformed with vector L4440, which produces dsRNA against the targeted gene. Synchronised eggs were placed on the indicated RNAi plates containing IPTG and ampicillin. RNAi clones were obtained from the Ahringer RNAi library. For all RNAi experiments, RNAi knockdown was conducted from egg onward.

Oil Red O staining of worms

Forty to fifty worms were fasted in M9 buffer (100 mM NaCl, 42 mM Na_2HPO_4, 22 mM KH_2PO_4 and 1mM $MgSO_4$) for 12 h, and then fixed with 60% isopropanol for 30 min. Worms were subsequently stained in 60% Oil Red O in isopropanol solution at 22°C for 6 h.

Poly(A) tail length detection

Total RNAs were purified from cell lysates using an RNeasy Plus Mini Kit (74136, QIAGEN). Reverse transcription and PCRs were performed using a poly(A) Tail-Length Assay Kit (76455, Affymetrix). PCR products were analyzed using an Agilent 2100 Bioanalyzer. Gene-specific primers for TFEB were as follows: fw-primer, 5′-cattccatcacctggacttc-3′; and rv-primer, 5′-catggtggacagaagtggatc-3′.

Protein–DNA binding analysis

Protein–DNA binding analysis was performed as previously described (Tsuchiya et al., 2011). Briefly, 3×FLAG-tagged TEFB protein and THOC4–mCherry were expressed in HEK293 cells. The cells were lysed in B400 buffer [20 mM Tris-HCl (pH 8.0), 400 mM KCl, 10% glycerol, 5 mM $MgCl_2$, 0.1% Tween-20 and protease inhibitor cocktail (Nacalai Tesque, Inc., Japan)], then the cell lysate was diluted three times with B0 buffer [20 mM Tris-HCl (pH 8.0), 10% glycerol, 5 mM $MgCl_2$, 0.1% Tween-20 and protease inhibitor cocktail]. An equal quantity of biotinylated sense (5′-bio-accgtcacgtgaccccagggtcacgtgacacc-3′) and unbiotinylated anti-sense (5′-ggtgtcacgtgaccctggggtcacgtgacggt-3′) DNA were mixed and incubated at 95°C for 5 min. This was then cooled down to room temperature to generate double stranded DNA. 20 µl of Dynabeads M-280 Streptavidin (Thermo Fisher Scientific K.K., Yokohama, Japan) was mixed with 100 pmol of double-stranded DNA, incubated at 4°C for 30 min, and then washed with B100 buffer [20 mM Tris-HCl (pH 8.0), 100 mM KCl, 10% glycerol, 5 mM $MgCl_2$, 0.1% Tween-20 and protease inhibitor cocktail]. Next, the agarose beads were incubated with HEK293 cell lysates at 4°C for 3 h then washed three times with B100 buffer. Finally, the proteins bound to the DNA were eluted in LDS sample buffer (Thermo Fisher Scientific K.K.) and analyzed by western blotting with an anti-DDDDK-tag (1:1000; PM020; MBL, Nagoya, Japan) or anti-mCherry antibody (1:2000; 600-401-P16S; Rockland, Limerick, PA, USA).

Acknowledgements

We appreciate the Caenorhabditis Genetic Center (CGC) for worm strains.

Competing interests

T.Y. is the founder of AutoPhagyGO.

Author contributions

Methodology: T.F., S.M., M.T., H.O., M.H., Y.I.; Validation: T.S., M.H., L.Y.; Investigation: T.F., S.K., A.T., M.T.; Writing - original draft: T.F.; Writing - review and editing: T.F., T.Y., S.N.; Supervision: T.Y., S.N.; Project administration: T.Y., S.N.; Funding acquisition: T.Y., S.N.

Funding

T.Y. is supported by the Japan Science and Technology Agency (CREST; JPMJCR17H6), the Japan Agency for Medical Research and Development (AMED; grant numbers JP17gm5010001 and JP17gm0610005), the Japan Society for the Promotion of Science A3 Foresight program and a Human Frontier Science Program research grant. S.N. is supported by the Japan Agency for Medical Research and Development (AMED-PRIME; 17gm6110003h0001); Japan Society for the Promotion of Science KAKENHI; Senri Life Science Foundation; Takeda Science Foundation; The Nakajima Foundation; MSD Life Science Foundation, Public Interest Incorporated Foundation; Astellas Foundation for Research on Metabolic Disorders; and the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

Supplementary information

Supplementary information available online at
https://jcs.biologists.org/lookup/doi/10.1242/jcs.248203.supplemental

Peer review history
The peer review history is available online at
https://jcs.biologists.org/lookup/doi/10.1242/jcs.248203.reviewer-comments.pdf

References

Bruhn, L., Munnerlyn, A. and Grosschedl, R. (1997). ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCRalpha enhancer function. *Genes Dev.* **11**, 640-653. doi:10.1101/gad.11.5.640

Doma, M. K. and Parker, R. (2007). RNA quality control in eukaryotes. *Cell* **131**, 660-668. doi:10.1016/j.cell.2007.10.041

Feng, Y., He, D., Yao, Z. and Klionsky, D. J. (2014). The machinery of macroautophagy. *Cell Res.* **24**, 24-41. doi:10.1038/cr.2013.168

Fujita, N., Morita, E., Itoh, T., Tanaka, A., Nakaoka, M., Osada, Y., Umemoto, T., Saitoh, T., Nakatogawa, H., Kobayashi, S. et al. (2013). Recruitment of the autophagic machinery to endosomes during infection is mediated by ubiquitin. *J. Cell Biol.* **203**, 115-128. doi:10.1083/jcb.201304188

Hautbergue, G. M., Hung, M.-L., Walsh, M. J., Snijders, A. P. L., Chang, C.-T., Jones, R., Ponting, C. P., Dickman, M. J. and Wilson, S. A. (2009). UIF, a new mRNA export adaptor that works together with REF/ALY, requires FACT for recruitment to mRNA. *Curr. Biol.* **19**, 1918-1924. doi:10.1016/j.cub.2009.09.041

Ichimura, Y. and Komatsu, M. (2010). Selective degradation of p62 by autophagy. *Semin. Immunopathol.* **32**, 431-436. doi:10.1007/s00281-010-0220-1

Imai, K., Hao, F., Fujita, N., Tsuji, Y., Oe, Y., Araki, Y., Hamasaki, M., Noda, T. and Yoshimori, T. (2016). Atg9A trafficking through the recycling endosomes is required for autophagosome formation. *J. Cell Sci.* **129**, 3781-3791. doi:10.1242/jcs.196196

Jin, M., Liu, X. and Klionsky, D. J. (2013). SnapShot: selective autophagy. *Cell* **152**, 368-368.e2. doi:10.1016/j.cell.2013.01.004

Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y. and Yoshimori, T. (2000). LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.* **19**, 5720-5728. doi:10.1093/emboj/19.21.5720

Kanno, T., Kanno, Y., LeRoy, G., Campos, E., Sun, H.-W., Brooks, S. R., Vahedi, G., Heightman, T. D., Garcia, B. A., Reinberg, D. et al. (2014). BRD4 assists elongation of both coding and enhancer RNAs by interacting with acetylated histones. *Nat. Struct. Mol. Biol.* **21**, 1047-1057. doi:10.1038/nsmb.2912

Katahira, J., Inoue, H., Hurt, E. and Yoneda, Y. (2009). Adaptor Aly and co-adaptor Thoc5 function in the Tap-p15-mediated nuclear export of HSP70 mRNA. *EMBO J.* **28**, 556-567. doi:10.1038/emboj.2009.5

Kim, Y.-M., Jung, C. H., Seo, M., Kim, E. K., Park, J.-M., Bae, S. S. and Kim, D.-H. (2015). mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. *Mol. Cell* **57**, 207-218. doi:10.1016/j.molcel.2014.11.013

Kimura, S., Noda, T. and Yoshimori, T. (2007). Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy* **3**, 452-460. doi:10.4161/auto.4451

Kuiper, R. P., Schepens, M., Thijssen, J., van Asseldonk, M., van den Berg, E., Bridge, J., Schuuring, E., Schoenmakers, E. F. and van Kessel, A. G. (2003). Upregulation of the transcription factor TFEB in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. *Hum. Mol. Genet.* **12**, 1661-1669. doi:10.1093/hmg/ddg178

Lei, H., Dias, A. P. and Reed, R. (2011). Export and stability of naturally intronless mRNAs require specific coding region sequences and the TREX mRNA export complex. *Proc. Natl. Acad. Sci. USA* **108**, 17985-17990. doi:10.1073/pnas.1113076108

Li, Y., Corradetti, M. N., Inoki, K. and Guan, K.-L. (2004). TSC2: filling the GAP in the mTOR signaling pathway. *Trends Biochem. Sci.* **29**, 32-38. doi:10.1016/j.tibs.2003.11.007

Meyer, S., Temme, C. and Wahle, E. (2004). Messenger RNA turnover in eukaryotes: pathways and enzymes. *Crit. Rev. Biochem. Mol. Biol.* **39**, 197-216. doi:10.1080/10409230490513991

Minami, S., Yamamoto, T., Takabatake, Y., Takahashi, A., Namba, T., Matsuda, J., Kimura, T., Kaimori, J.-Y., Matsui, I., Hamano, T. et al. (2017). Lipophagy maintains energy homeostasis in the kidney proximal tubule during prolonged starvation. *Autophagy* **13**, 1629-1647. doi:10.1080/15548627.2017.1341464

Mizushima, N. (2004). Methods for monitoring autophagy. *Int. J. Biochem. Cell Biol.* **36**, 2491-2502. doi:10.1016/j.biocel.2004.02.005

Mizushima, N. (2007). Autophagy: process and function. *Genes Dev.* **21**, 2861-2873. doi:10.1101/gad.1599207

Mizushima, N., Yamamoto, A., Hatano, M., Kobayashi, Y., Kabeya, Y., Suzuki, K., Tokuhisa, T., Ohsumi, Y. and Yoshimori, T. (2001). Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J. Cell Biol.* **152**, 657-668. doi:10.1083/jcb.152.4.657

Morita, S., Kojima, T. and Kitamura, T. (2000). Plat-E: an efficient and stable system for transient packaging of retroviruses. *Gene Ther.* **7**, 1063-1066. doi:10.1038/sj.gt.3301206

Nakamura, S., Karalay, O., Jäger, P. S., Horikawa, M., Klein, C., Nakamura, K., Latza, C., Templer, S. E., Dieterich, C. and Antebi, A. (2016). Mondo complexes regulate TFEB via TOR inhibition to promote longevity in response to gonadal signals. *Nat. Commun.* **7**, 10944. doi:10.1038/ncomms10944

Nakamura, S., Shigeyama, S., Minami, S., Shima, T., Akayama, S., Matsuda, T., Esposito, A., Napolitano, G., Kuma, A., Namba-Hamano, T. et al. (2020). LC3 lipidation is essential for TFEB activation during the lysosomal damage response to kidney injury. *Nat. Cell Biol.* **22**, 1252-1263. doi:10.1038/s41556-020-00583-9

Okada, M., Jang, S.-W. and Ye, K. (2008). Akt phosphorylation and nuclear phosphoinositide association mediate mRNA export and cell proliferation activities by ALY. *Proc. Natl. Acad. Sci. USA* **105**, 8649-8654. doi:10.1073/pnas.0802533105

O'Rourke, E. J. and Ruvkun, G. (2013). MXL-3 and HLH-30 transcriptionally link lipolysis and autophagy to nutrient availability. *Nat. Cell Biol.* **15**, 668-676. doi:10.1038/ncb2741

Osinalde, N., Olea, M., Mitxelena, J., Aloria, K., Rodriguez, J. A., Fullaondo, A., Arizmendi, J. M. and Zubiaga, A. M. (2013). The nuclear protein ALY binds to and modulates the activity of transcription factor E2F2. *Mol. Cell. Proteomics* **12**, 1087-1098. doi:10.1074/mcp.M112.024158

Palmieri, M., Impey, S., Kang, H., di Ronza, A., Pelz, C., Sardiello, M. and Ballabio, A. (2011). Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. *Hum. Mol. Genet.* **20**, 3852-3866. doi:10.1093/hmg/ddr306

Parker, R. and Song, H. (2004). The enzymes and control of eukaryotic mRNA turnover. *Nat. Struct. Mol. Biol.* **11**, 121-127. doi:10.1038/nsmb724

Roczniak-Ferguson, A., Petit, C. S., Froehlich, F., Qian, S., Ky, J., Angarola, B., Walther, T. C. and Ferguson, S. M. (2012). The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci. Signal.* **5**, ra42. doi:10.1126/scisignal.2002790

Saitoh, T., Nakayama, M., Nakano, H., Yagita, H., Yamamoto, N. and Yamaoka, S. (2003). TWEAK induces NF-kappaB2 p100 processing and long lasting NF-kappaB activation. *J. Biol. Chem.* 278, 36005-36012. doi:10.1074/jbc.M304266200

Sakamaki, J.-I., Wilkinson, S., Hahn, M., Tasdemir, N., O'Prey, J., Clark, W., Hedley, A., Nixon, C., Long, J. S., New, M. et al. (2017). Bromodomain protein BRD4 is a transcriptional repressor of autophagy and lysosomal function. *Mol. Cell* **66**, 517-532.e9. doi:10.1016/j.molcel.2017.04.027

Sardiello, M., Palmieri, M., di Ronza, A., Medina, D. L., Valenza, M., Gennarino, V. A., Di Malta, C., Donaudy, F., Embrione, V., Polishchuk, R. S. et al. (2009). A gene network regulating lysosomal biogenesis and function. *Science* **325**, 473-477. doi:10.1126/science.1174447

Settembre, C., Di Malta, C., Polito, V. A., Garcia Arencibia, M., Vetrini, F., Erdin, S., Erdin, S. U., Huynh, T., Medina, D., Colella, P. et al. (2011). TFEB links autophagy to lysosomal biogenesis. *Science* **332**, 1429-1433. doi:10.1126/science.1204592

Settembre, C., Zoncu, R., Medina, D. L., Vetrini, F., Erdin, S., Erdin, S. U., Huynh, T., Ferron, M., Karsenty, G., Vellard, M. C. et al. (2012). A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J.* **31**, 1095-1108. doi:10.1038/emboj.2012.32

Settembre, C., De Cegli, R., Mansueto, G., Saha, P. K., Vetrini, F., Visvikis, O., Huynh, T., Carissimo, A., Palmer, D., Klisch, T. J. et al. (2013). TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. *Nat. Cell Biol.* **15**, 647-658. doi:10.1038/ncb2718

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A. M. and Czaja, M. J. (2009). Autophagy regulates lipid metabolism. *Nature* **458**, 1131-1135. doi:10.1038/nature07976

Sträßer, K., Masuda, S., Mason, P., Pfannstiel, J., Oppizzi, M., Rodriguez-Navarro, S., Rondón, A. G., Aguilera, A., Struhl, K., Reed, R. et al. (2002). TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* **417**, 304-308. doi:10.1038/nature746

Stubbs, S. H. and Conrad, N. K. (2015). Depletion of REF/Aly alters gene expression and reduces RNA polymerase II occupancy. *Nucleic Acids Res.* **43**, 504-519. doi:10.1093/nar/gku1278

Stubbs, S. H., Hunter, O. V., Hoover, A. and Conrad, N. K. (2012). Viral factors reveal a role for REF/Aly in nuclear RNA stability. *Mol. Cell. Biol.* **32**, 1260-1270. doi:10.1128/MCB.06420-11

Tasdemir, E., Chiara Maiuri, M., Morselli, E., Criollo, A., D'Amelio, M., Djavaheri-Mergny, M., Cecconi, F., Tavernarakis, N. and Kroemer, G. (2008a). A dual role of p53 in the control of autophagy. *Autophagy* **4**, 810-814. doi:10.4161/auto.6486

Tasdemir, E., Maiuri, M. C., Galluzzi, L., Vitale, I., Djavaheri-Mergny, M., D'Amelio, M., Criollo, A., Morselli, E., Zhu, C., Harper, F. et al. (2008b). Regulation of autophagy by cytoplasmic p53. *Nat. Cell Biol.* **10**, 676-687. doi:10.1038/ncb1730

Tsuchiya, M., Ogawa, H., Suzuki, T., Sugiyama, N., Haraguchi, T. and Hiraoka, Y. (2011). Exportin 4 interacts with Sox9 through the HMG Box and inhibits the DNA binding of Sox9. *PLoS ONE* **6**, e25694. doi:10.1371/journal.pone.0025694

Virbasius, C.-M. A., Wagner, S. and Green, M. R. (1999). A human nuclear-localized chaperone that regulates dimerization, DNA binding, and transcriptional activity of bZIP proteins. *Mol. Cell* **4**, 219-228. doi:10.1016/S1097-2765(00)80369-X

Yu, L., McPhee, C. K., Zheng, L., Mardones, G. A., Rong, Y., Peng, J., Mi, N., Zhao, Y., Liu, Z., Wan, F. et al. (2010). Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature* **465**, 942-946. doi:10.1038/nature09076