The autophagy protein Becn1 improves insulin sensitivity by promoting adiponectin secretion via exocyst binding

Highlights
- Autophagy-hyperactive Becn1^{F121A} mice show systemic activation of AMPK
- Becn1 positively regulates the secretion of an adipokine, adiponectin
- Becn1-exocyst interaction is essential for facilitating adiponectin secretion
- Adipose Becn1^{F121A} is sufficient to improve metabolism by elevating serum adiponectin

Authors
Kenta Kuramoto, Yoon-Jin Kim, Jung Hwa Hong, Congcong He

Correspondence
congcong.he@northwestern.edu

In brief
Kuramoto et al. demonstrate a non-degradative and non-cell-autonomous mechanism by which the autophagy protein Becn1 regulates systemic energy metabolism. Becn1 interacts with exocyst proteins in white adipose tissue to facilitate adiponectin secretion, resulting in systemic insulin sensitization via the adiponectin receptor-AMPK signaling pathway in non-adipose tissues.
The autophagy protein Becn1 improves insulin sensitivity by promoting adiponectin secretion via exocyst binding

Kenta Kuramoto,1 Yoon-Jin Kim,1 Jung Hwa Hong,1 and Congcong He1,2,*
1Department of Cell and Developmental Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA
2Lead contact
*Correspondence: congcong.he@northwestern.edu
https://doi.org/10.1016/j.celrep.2021.109184

SUMMARY

Autophagy dysregulation is implicated in metabolic diseases, including type 2 diabetes. However, the mechanism by which the autophagy machinery regulates metabolism is largely unknown. Autophagy is generally considered a degradation process via lysosomes. Here, we unveil a metabolically important non-cell-autonomous, non-degradative mechanism regulated by the essential autophagy protein Becn1 in adipose tissue. Upon high-fat diet challenge, autophagy-hyperactive Becn1 F121A mice show systemically improved insulin sensitivity and enhanced activation of AMP-activated protein kinase (AMPK), a central regulator of energy homeostasis, via a non-cell-autonomous mechanism mediated by adiponectin, an adipose-derived metabolic hormone. Adipose-specific Becn1 F121A expression is sufficient to activate AMPK in non-adipose tissues and improve systemic insulin sensitivity by increasing adiponectin secretion. Further, Becn1 enhances adiponectin secretion by interacting with components of the exocyst complex via the coiled-coil domain. Together, our study demonstrates that Becn1 improves insulin sensitivity by facilitating adiponectin secretion through binding the exocyst in adipose tissue.

INTRODUCTION

Autophagy is a key lysosomal degradation pathway induced by stress, such as fasting and exercise, which breaks down damaged or unnecessary structures by fusion of autophagosome vesicles with lysosomes (Kaur and Debnath, 2015; Rocchi and He, 2017b). Deletion of autophagy genes in mice, either globally or specifically in metabolic tissues, including pancreatic β cells and liver, has been linked with metabolic dysfunctions, such as hyperglycemia and insulin resistance, the hallmarks of type 2 diabetes (T2D) (Ebató et al., 2008; He et al., 2013; Jung et al., 2008; Rocchi and He, 2015; Yang et al., 2010). In contrast, recent data indicate that both physiological and genetic activation of autophagy increases insulin sensitivity (He et al., 2012; Yamamoto et al., 2018). However, the mechanism by which the autophagy machinery regulates insulin sensitivity and T2D pathogenesis remains obscure. Autophagy is generally considered a destruction and degradation process. Although emerging evidence suggests that autophagy may have an unconventional secretory function in immunity and inflammation (Bel et al., 2017; Zhang et al., 2015), whether autophagy-related proteins play non-degradative roles in the regulation of metabolism, as well as the underlying mechanisms, is unknown.

Besides serving as lipid storages, adipose tissue is an important endocrine organ that regulates energy metabolism in various metabolic tissues via secreting adipokines (adipose-derived hormones), including adiponectin. Adiponectin regulates a spectrum of metabolic parameters in the muscle, liver, and vascular system, including maintaining insulin sensitivity, promoting free fatty acid oxidation, reducing hepatic glucose and triglyceride (TG) production, and inhibiting vascular inflammation (Kadowaki et al., 2006; Ouchi et al., 2011; Stern et al., 2016; Yamauchi et al., 2001). The expression and plasma concentration of adiponectin positively correlates with insulin sensitivity and is reduced in obesity and T2D in both animal models and humans (Arita et al., 1999; Hotta et al., 2001; Li et al., 2009). Low blood adiponectin caused by SNPs (single-nucleotide polymorphisms) or mutations in the Adiponectin gene in humans, also known as hypoadiponectinemia, has been associated with insulin resistance and T2D (Hara et al., 2002; Kondo et al., 2002; Menzaghi et al., 2002; Stumvoll et al., 2002). Adiponectin knockout (KO) mice phenocopy the human phenotypes and develop insulin resistance (Kubota et al., 2002; Nawrocki et al., 2006), demonstrating the key role of adiponectin in the prevention of T2D. Thus, understanding factors that regulate the endocrine pathway of adiponectin secretion in adipose tissue will help elucidate the pathogenesis of insulin resistance and metabolic disorders. However, despite the importance of adiponectin in metabolic homeostasis, the molecular mechanism of adiponectin secretion from adipose tissue is unclear. Although extracellular signals, such as insulin, adrenergic stimuli, and fibroblast growth factor 21 (FGF21) (Blümmer et al., 2008; Cong et al., 2007; Geng et al., 2019; Hajri et al., 2011; Holland et al., 2013; Komai et al., 2016; Lim et al., 2015; Lin et al., 2013),
Figure 1. Autophagy-hyperactive Becn1<sup>F121A</sup> mice show enhanced AMPK activation in tissues, caused by factors in circulation

(A) Metabolic tissues of Becn1<sup>F121A</sup> KI mice show increased AMPK activation. Western blot (WB) analysis and quantification of AMPK phosphorylation in liver and muscle of Becn1<sup>F121A</sup> mice. n = 7.
have been reported to increase adiponectin release in adipocytes via corresponding receptors, the intracellular mechanism that controls the secretion of adiponectin after it is folded and packaged in the endoplasmic reticulum (ER) is poorly defined.

Becn1/Beclin 1 is an essential autophagy protein that binds the class III Vps34 phosphatidylinositol 3-kinase (PtdIns3K) complex and regulates its stability and kinase activity during autophagy initiation (He and Levine, 2010). Becn1 is reported to be required for exercise-induced activation of AMPK (AMP-activated protein kinase), a central positive regulator of insulin sensitivity and glucose uptake, in muscle via binding with the Toll-like receptor 9 (TLR9) (He et al., 2012; Liu et al., 2020). We recently established a Becn1 mutant mouse model of hyperactive autophagy, Becn1F121A knockin (KI) mice, to study the role and mechanism of autophagy in metabolic regulation (Rocchi et al., 2017; Yamamoto et al., 2018). The Becn1F121A point mutant cannot be bound and inhibited by the anti-apoptotic and anti-autophagy protein Bcl-2 and leads to high autophagy. Becn1F121A-mediated genetic upregulation of autophagy improves systemic insulin sensitivity, but the mechanism by which autophagy regulates insulin sensitivity is unclear.

In this study, we found that Becn1 regulates metabolism through a previously uncharacterized non-degradative, non-cell-autonomous mechanism by regulating adiponectin secretion from adipocytes. The autophagy-hyperactive Becn1F121A mutation mimics the effect of exercise on AMPK activation and leads to systemic AMPK activation at resting conditions. This effect is exerted by a non-cell-autonomous mechanism mediated by autophagy-induced circulating adiponectin from adipocytes, because adipocyte tissue-specific expression of Becn1F121A is sufficient to increase liver and muscle AMPK activation, circulating adiponectin levels, and systemic insulin sensitivity, which is abolished by reduced adiponectin expression. Mechanistically, we discovered that an interaction between Becn1 and the exocyst components in adipocytes is required for Becn1F121A-induced secretion of adiponectin. Altogether, we establish a model of Becn1-facilitated adiponectin secretion in adipocyte tissue: when the essential autophagy protein Becn1 is released from the inhibitory binding of Bcl-2, it promotes adiponectin secretion via binding to the exocyst complex in adipose tissue, which elevates circulating adiponectin, activates AMPK in target metabolic tissues, and increases insulin sensitivity systemically (Figure 7G).

RESULTS

Autophagy-hyperactive Becn1F121A mice show systemic AMPK activation in metabolic tissues caused by circulator factors

To study how autophagy regulates metabolism, we previously generated an autophagy-hyperactive Kl mouse model carrying a single amino acid substitution (F121A) in the Becn1 protein (Becn1F121A), which disrupts inhibitory binding between Becn1 and Bcl-2 and leads to constitutive activation of autophagy (Rocchi et al., 2017). We found that in response to 8- or 12-week high-fat diet (HFD) feeding, while Becn1F121A mice showed comparable body weight to wild-type (WT) mice, they are less glucose tolerant yet more insulin sensitive than WT mice (Yamamoto et al., 2018) (Figure S1A). Our previous findings suggest that after HFD challenge, the Becn1F121A mice showed excessive autophagic degradation of insulin granule vesicles (vesicophagy) in β cells, leading to impaired insulin secretion and glucose intolerance. However, it does not explain why Becn1F121A mice are paradoxically more insulin sensitive. To solve this puzzle, we analyzed various metabolic pathways in their metabolic tissues and found that compared with WT mice, metabolic tissues (liver and skeletal muscle) of Becn1F121A mice showed enhanced phosphorylation/activation of AMPK, which plays a central beneficial role in energy homeostasis and insulin sensitization (Garcia and Shaw, 2017), after feeding of either regular diet (RD) (Figure 1A) or HFD (Figure 1B). Further, in support of AMPK activation, Becn1F121A mouse tissues showed a spectrum of changes downstream of AMPK, including increased phosphorylation of Raptor (Regulatory-associated protein of mTOR [mechanistic target of rapamycin]), decreased phosphorylation of p70S6K and IRS-1 (Insulin receptor substrate 1), and stabilization of IRS-1 (Figure 1B). AMPK phosphorylates Raptor at S792, which leads to inhibition of mTOR and its substrate p70S6K (Gwinn et al., 2008). The latter can phosphorylate IRS-1 at several serine residues, including S302 and S632/635 (Haruta et al., 2000; Ozes et al., 2001; Ueno et al., 2005; Werner et al., 2004), which promotes IRS-1 degradation via proteasomes (Pederson et al., 2001; Sun et al., 1999; Takano et al., 2001). Because IRS-1 is a key docking protein downstream of the activated insulin receptor (IR) and insulin-like growth factor-1 receptor (IGF-1R) to form scaffolding complexes and initiate insulin signaling cascades, stabilization of IRS-1 by AMPK activation in part enhances insulin sensitivity. Thus, more Raptor S792 phosphorylation, less p70S6K T389 phosphorylation, less IRS-1 S302 and S632/635 phosphorylation, and higher levels of IRS-1 in the metabolic tissues of Becn1F121A mice suggest an activated AMPK signaling pathway in these mice (Figure 1B).

Notably, WT Becn1 is reported to be required for exercise-induced activation of AMPK in muscle via binding with TLR9 (He et al., 2012; Liu et al., 2020). Thus, the hyperactive Becn1F121A mutation mimics the effect of exercise on AMPK activation at resting conditions. Intriguingly, this effect is not due to differences in the level of TLR9 (Figure S1B) and is not limited to muscle (Figures 1A and 1B). To reveal the mechanism underlying AMPK hyperactivation in Becn1F121A mice, we asked whether AMPK activation is caused by a cell-autonomous or non-cell-autonomous mechanism. We added 10% serum collected from either WT or Becn1F121A mice to the medium of WT cells of metabolic tissue origins. We found that when added to cell culture media, serum from Becn1F121A mice also leads to enhanced AMPK activation in various cell lines (including NIH 3T3 fibroblasts, HepG2 and Huh7 hepatocytes, C2C12 (B) WB analysis and quantification of AMPK activation and signaling pathways downstream of AMPK in the liver of HFD-fed WT and Becn1F121A mice. n = 7. (C) Circulating factors in Becn1F121A mice play a role in activating AMPK. WB analysis of AMPK phosphorylation in cell lines cultured for 1 h in medium containing 10% mouse serum from WT or Becn1F121A mice, n = 4. Data represent mean ± SEM, t test. “p < 0.05; **p < 0.01; ***p < 0.001.”
Adiponectin exists in the circulation as different multimer complexes, categorized as high-molecular-weight (HMW) multimers, medium-molecular-weight (MMW) hexamers, and low-molecular-weight (LMW) trimers, with the HMW adiponectin being the most biologically active form (Basu et al., 2007; Lara-Castro et al., 2002; Yoon et al., 2006). To determine whether AMPK activation in Becn1F121A mice is dependent on increased secretion of adiponectin, we asked whether depleting adiponectin receptors in non-adipose cells abolishes AMPK activation induced by Becn1F121A mouse serum. We found that compared with WT mouse serum, serum from Becn1F121A mice led to higher AMPK activation in WT C2C12 myotubes but failed to do so in C2C12 myotubes deleted for the adiponectin receptor AdipoR1 (the major adiponectin receptor expressed in myotubes) by short hairpin RNAs (shRNAs) (Figure 3A). Similarly, we also found that the adiponectin receptors are required for Becn1F121A mouse serum-induced AMPK activation in NIH 3T3 fibroblasts (primarily expressing AdipoR1) (Figure 3B) and 3T3-L1-differentiated adipocytes (expressing both AdipoR1 and AdipoR2) (Figure 3C). These data suggest that adiponectin signaling is required for AMPK activation in target metabolic cells induced by Becn1F121A mouse serum.

The exocyst components Sec5, Sec6, and Sec8 are binding partners of Becn1 and preferentially interact with the hyperactive Becn1F121A mutant compared with WT Becn1

We next aimed to understand how the autophagy machinery cell autonomously regulates adiponectin secretion in adipocytes. Indeed, we found that the WAT of the Becn1F121A mice shows disrupted Becn1–Bcl-2 interaction, assayed by co-immunoprecipitation (coIP) (Figure 4A), as well as increased autophagy activity, evidenced by elevated LC3-II and p62 flux using a lysosomal inhibitor chloroquine (Figure S4A). Nonetheless, we did not detect elevated or accelerated adipogenesis in the WAT of Becn1F121A mice (Figure 4B), in primary adipocytes from Becn1F121A mice (Figure S4B), or in Becn1F121A-expressing 3T3-L1-differentiated adipocytes (Figures 4C and S4C), by analyzing the expression of a variety of adipogenesis markers. The data suggest that upregulating autophagy by Becn1F121A does not affect adipose development and differentiation. We then suspected that autophagosomes may directly sequestrate adiponectin and promote its secretion, because we previously found that under certain conditions hormones can become an autophagy cargo (Kuramoto and He, 2018; Yamamoto et al., 2018). However, our imaging and biochemical data ruled out
this possibility as well. We did not observe significant colocalization between adiponectin and the autophagosome marker LC3 in primary adipocytes of either WT GFP-LC3 or Becn1F121A GFP-LC3 reporter mice by confocal microscopy (Figure S4D). Moreover, using a biochemical method we developed to immunoisolate intact autophagosomes from cells and tissues (Yamamoto et al., 2018), we did not detect adiponectin inside autophagosomes purified from 3T3-L1-differentiated adipocytes (Figure S4E). Thus, these data suggest that adiponectin elevation in Becn1F121A KI mice is not due to altered adipogenesis or enhanced sequestration and secretion of adiponectin directly by autophagosome vesicles.

Nonetheless, we detected colocalization between adiponectin and Becn1 in primary adipocytes and a higher level of colocalization of adiponectin with Becn1F121A than with WT Becn1 (Figure 5A), supporting that Becn1 may play a direct role in regulating adiponectin secretion. To investigate the potential mechanism of Becn1-regulated adiponectin release, we generated a HEK293 cell line stably expressing a tetracycline-inducible cleavable zz tag-Becn1-3XFlag fusion protein (Sun et al., 2008). Using this cell line, we pulled down several subunits of the exocyst as Becn1 binding partners, including Sec5/Exoc2, Sec6/Exoc3, and Sec8/Exoc4 (Figure S5A). In the meantime, when we interrogated published databases and reports of the Becn1 interactome, we found that another exocyst component Exo84/Exoc8 has been reported as a Becn1-binding protein (Bodemann et al., 2011). The exocyst is a multiprotein complex involved in plasma-membrane docking of secretory vesicles,

Figure 3. Non-cell-autonomous activation of AMPK by Becn1F121A mouse serum is mediated by adiponectin signaling

(A and B) C2C12 myotubes (A) or NIH 3T3 fibroblasts (B) stably expressing scrambled (Scr) or shRNAs against adiponectin receptor 1 (AdipoR1) were cultured for 1 h in medium containing 10% mouse serum from WT or Becn1F121A mice, and AMPK phosphorylation was analyzed and quantified by WB studies. AdipoR1 KD efficiency is shown on the right. n = 3 mice.

Data represent mean ± SEM, t test. *p < 0.05; **p < 0.01. NS, not significant.
**A**

IWAT

| IP:       | WT | F121A |
|-----------|----|-------|
| IgG       | 1  | 1     |
| Bcl-2     | 1  | 1     |
| Becn1     | 1  | 1     |
| Bcl-2     | 1  | 1     |
| Input     | 1  | 1     |

**B**

Adipogenesis markers

| gWAT (gonadal) | iWAT (inguinal) |
|----------------|-----------------|
| Mouse #        | WT | F121A | WT | F121A |
| PLIN1          | 1  | 1     | 1  | 1     |
| PLIN2          | 1  | 1     | 1  | 1     |
| FAS            | 1  | 1     | 1  | 1     |
| PPARγ          | 1  | 1     | 1  | 1     |
| C/EBPα         | 1  | 1     | 1  | 1     |
| Actin          | 1  | 1     | 1  | 1     |

**C**

Adipogenesis markers

| 3T3-L1 differentiated adipocytes |
|----------------------------------|
| HA-mBecn1                        |
| WT                               |
| 0  2  4  6  8  10                |
| PLIN1                            |
| ADIPOQ                           |
| FABP4                            |
| FAS                              |
| C/EBPα                           |
| PPARγ                            |
| α-Tubulin                        |

(legend on next page)
containing eight components: Sec3/Exoc1, Sec5/Exoc2, Sec6/Exoc3, Sec8/Exoc4, Sec10/Exoc5, Sec15/Exoc6, Exo70/Exo7, and Exo84/Exoc8 (Heider and Munson, 2012; Wu and Guo, 2013). By coIP, we confirmed the interaction of the key exocyst component Sec6 with both WT Becn1 and Becn1F121A in HEK293 cells (Figure 5B) and found that the hyperactive mutant form, Becn1F121A, shows a higher affinity with Sec6 than WT Becn1 in HEK293 cells stably expressing WT Becn1 or Becn1F121A (Figures 5B and 5C), in primary adipocytes isolated from WT or Becn1F121A mice (Figure 5D), and in 3T3-L1-differentiated adipocytes (Figure S5B). Different from the previous report (Bodemann et al., 2011), we were not able to detect an interaction between Becn1 and the exocyst components Exo84 or Exo70 in HEK293 cells (Figure 5B), but we found that in the presence of Becn1F121A, there is a stronger binding between Sec6 and several other exocyst components, such as Sec5 and Sec8 (Figure 5C). Further, in addition to Sec6, the exocyst components Sec5 and Sec8, as well as PtdIns3K complex subunits ATG14, VPS34, and UVRAG, also show stronger binding with Becn1F121A than with WT Becn1 analyzed by coIP in primary adipocytes (Figure 5D). Together, these data suggest that Becn1 binds the exocyst complex and facilitates exocyst assembly, and the exocyst machinery preferentially interacts with the hyperactive Becn1F121A mutant compared with WT Becn1.

The Becn1-exocyst interaction is essential for promoting adiponectin secretion

To further determine whether the Becn1-Sec6 interaction is important for adiponectin secretion, we aimed to generate a loss-of-Sec6 interaction mutant of Becn1. Becn1 contains three major domains: the BH3 (Bcl-2 homology 3) domain that binds Bcl-2, the coiled-coil domain (CCD), and the evolutionally conserved domain (ECD) (He and Levine, 2010; Levine et al., 2015). We generated deletion mutants of Becn1F121A lacking the N-terminal intrinsically disordered region, the BH3 domain, the CCD, the ECD, or the C-terminal regions, respectively (Figure 5E). We found that the Becn1ΔCCD mutant (lacking the CCD) disrupts the Becn1-Sec6 binding by coIP (Figure 5E), suggesting that the CCD of Becn1 is important for exocyst binding. We subsequently stably expressed full-length Becn1F121A and the loss-of-Sec6 interaction Becn1F121AΔCCD mutant in WT 3T3-L1 preadipocytes, induced adipocyte differentiation of the 3T3-L1 cells, and analyzed adiponectin levels in the conditioned medium. We found that Becn1F121AΔCCD abolishes the increase in the medium adiponectin level induced by Becn1F121A and by WB (Figure 5F) and ELISA (Figure 5G) analyses, suggesting that disrupting Becn1-Sec6 binding interferes with Becn1-regulated adiponectin secretion in adipocytes. Thus, we conclude that Becn1 regulates adiponectin secretion by interacting with the exocyst.

We also demonstrated that the exocyst machinery functions in adiponectin release from adipocytes, which has not been characterized before. We found that shRNA knockdown (KD) of Sec6 significantly decreases the adiponectin level in the conditioned medium of 3T3-L1 preadipocyte-differentiated adipocytes (Figure S5C), suggesting that Sec6 is required for adiponectin secretion in adipocytes. Yet KD of another exocyst component Sec5 does not affect adiponectin secretion (Figure S5D). This is in line with reported phenomena in Drosophila, where mutating Sec5 does not impair the secretion of neurotransmitters (Murthy et al., 2003). Based on the cryoelectron microscopy (cryo-EM) structure of the exocyst (Mei et al., 2018), we speculate that it is possible that Becn1 and Sec5 may be partially functionally redundant in adiponectin secretion, and binding of Becn1 to Sec6 or the exocyst may compensate for the loss of Sec5 in the secretion. We found that KD of Sec8 reduces adiponectin secretion (Figure S5D); yet, depleting Sec8 also reduces the stability of Sec6, consistent with the structural data suggesting that Sec8 forms a paired subcomplex with Sec6 in the exocyst (Mei et al., 2018). These data demonstrate that Sec6 needs to cooperate with Sec8, but not Sec5, to exert its effects on adiponectin secretion.

We also found that Sec6 is partially required for the secretion of another adipokine leptin (Figure S5C). However, intriguingly, we found that different from adiponectin, expressing Becn1F121A or blocking the Becn1-Sec6 binding does not affect the secretion of leptin from 3T3-L1-differentiated adipocytes (Figure 5G). Our microscopy data suggest that this specificity is potentially caused by subcellular compartmentation and distinct trafficking pathways of different types of secretory vesicles. We found that as previously reported (Xie et al., 2008), although adiponectin-containing vesicles are enriched in the internal subcellular pool (center), leptin vesicles mostly localize in proximity to the cell surface (periphery) and show limited colocalization with adiponectin (Figure 5H). In addition, compared with adiponectin vesicles, leptin vesicles have reduced colocalization with Becn1, and such colocalization is not increased by hyperactive Becn1F121A (Figure S5E). Thus, it is likely that various types of vesicles are originated from distinct compartments of the Golgi and trafficked to different subcellular locations that are Becn1 positive or negative. We propose that the Becn1-Sec6 complex forms approximate to adiponectin vesicles at the center of the adipocyte to promote their transport toward the plasma membrane.

Adipose tissue-specific expression of Becn1F121A is sufficient to improve systemic insulin sensitivity, glucose tolerance, and insulin signaling in liver and muscle

To further demonstrate that Becn1 in the WAT is key for adiponectin secretion and metabolic improvement, we compared the metabolic phenotypes of mice expressing Becn1F121A specifically in the WAT or globally in the whole body, to determine whether adipose tissue-specific Becn1F121A expression is sufficient to activate AMPK in non-adipose tissues and improve metabolic phenotypes. We generated a transgenic mouse line, Becn1F121A-ACT4, expressing Becn1F121A specifically in the WAT. We found that adiponectin secretion and metabolic improvement were only improved in the WAT, but not in the global form, Becn1F121A, shows a higher affinity with Sec6 than WT Becn1 analyzed by coIP in primary adipocytes (Figure 5B) and found that the hyperactive mutant

![Figure 4. Increasing autophagy by Becn1F121A does not promote adipogenesis in vivo or in vitro](image-url)

(A) CoIP of endogenous Bcl-2 and Becn1 in the iWAT of WT and Becn1F121A mice. n = 3 mice.
(B) Adipogenesis markers in gWAT and iWAT of RO-fed WT and Becn1F121A mice were analyzed by WB. n = 6 mice. t test. Data represent mean ± SEM.
(C) 3T3-L1 preadipocytes stably expressing hemagglutinin (HA)-tagged WT Becn1 or Becn1F121A were cultured in the adipocyte-differentiation medium for a 10-day time course, and adipogenesis markers were analyzed every 2 days by WB.
insulin sensitivity systemically. We utilized the FLEX switch system to deliver Becn1F121A to adipose tissue via adeno-associated virus 2/8 (AAV2/8) (Jimenez et al., 2013) that can efficiently target adipose tissues. In the FLEX system, the Becn1F121A reading frame is reversed and flanked by loxP and lox2272 sites (Figure 6A). When AAV2/8-FLEX-Becn1F121A is injected into WT mice (without Cre expression), Becn1F121A is not expressed, while when AAV2/8-FLEX-Becn1F121A is injected into an adipose-specific Adiponectin-Cre (Adipoq-Cre) mouse line (Cre driven by the adiponectin promoter), the Becn1F121A reading frame is flipped following Cre-Lox recombination and expresses specifically in adipose tissues. Using this system, we confirmed the expression specificity of Becn1F121A after AAV delivery in both inguinal WAT (iWAT) and gonadal WAT (gWAT), but not liver, of Adipoq-Cre mice (Figures 6A and S6A).

With RD feeding, we found that Adipoq-Cre mice injected with AAV-FLEX-Becn1F121A (Adipoq-Cre AAV-Becn1F121A mice) have similar body weight (Figure S6B), insulin sensitivity (by insulin tolerance test [ITT]), and glucose tolerance (by glucose tolerance test [GTT]) (Figure S6C) as the control (WT AAV-Becn1F121A) mice. Yet, in response to HFD feeding, although Adipoq-Cre AAV-Becn1F121A mice have comparable body weight (Figure S6B) and WAT mass (Figure S6D) with the control mice, they showed significantly improved insulin sensitivity similar to the global Becn1F121A KI mice (Figure 6B), suggesting that adipose-specific expression of Becn1F121A is sufficient to enhance systemic insulin sensitivity. Importantly, different from global expression of Becn1F121A, which improves insulin sensitivity but impairs glucose tolerance as a result of reducing insulin storage and secretion in islets (Figure S1A) (Yamamoto et al., 2016), adipose tissue-specific Becn1F121A expression improves both insulin sensitivity (ITT) and glucose tolerance (GTT) (Figure 6B, compared with Figure S1A), suggesting an anti-diabetic function of Becn1F121A in adipose tissue.

Along this line, we found that after HFD challenge, control mice showed an abnormally high level of insulin in the serum (hyperinsulinemia) under basal conditions (time 0 min), whereas Adipoq-Cre AAV-Becn1F121A mice showed significantly reduced HFD-induced hyperinsulinemia (Figure 6C). Further, different from whole-body Becn1F121A KI (Yamamoto et al., 2018), adipose tissue-specific expression of Becn1F121A does not cause a reduction in the insulinogenic index (a measure of glucose-stimulated insulin secretion) (Figure S6E), further supporting that previously observed Becn1F121A-induced reduction in insulin secretion is due to its role specifically in β cells. In addition, in support of systemic insulin sensitization, we detected higher phosphorylation of Akt at S473, a phosphorylation target downstream of the activated IR, in both liver and muscle of Adipoq-Cre AAV-Becn1F121A mice than in the control mice, after exogenous insulin stimulation after HFD feeding (Figure 6D), suggesting that Becn1F121A in adipose tissue improves insulin signaling in non-adipose tissues. Thus, together, these findings demonstrate that Becn1F121A is metabolically beneficial and improves systemic insulin sensitivity when specifically expressed in the WAT, and highlight that autophagy proteins may play different roles in different metabolic tissues.

Adipose-specific Becn1F121A regulates AMPK activity and lipid metabolism in non-adipose tissues

We further found that similar to the effect of global Becn1F121A expression (Figure 1B), adipose tissue-specific expression of Becn1F121A is sufficient to activate AMPK in the liver (Figure 6E) and muscle (Figure S6F), evidenced by activated AMPK phosphorylation and downstream signaling of AMPK, including increased phosphorylation of Raptor, decreased phosphorylation of Akt at S473, a phosphorylation target downstream of the activated IR, and increased phosphorylation of P70S6K and IRS-1, and stabilization of IRS-1. Notably, upon IR activation, IRS-1 is phosphorylated at Y612, which is essential for downstream PtdIns3K activation and GLUT4 translocation. We detected a higher cellular level of Y612 phosphorylation in IRS-1 in Adipoq-Cre AAV-Becn1F121A mice than in control mice (Figure S6G), further supporting liver insulin sensitization mediated non-cell autonomously by adipose Becn1F121A.

Further, adiponectin systemically regulates lipid metabolism by activating AMPK and peroxisome proliferator-activated receptor (PPAR) α signaling pathways via AdipoR2 in the liver (Kadowaki et al., 2006; Yamamura et al., 2002; Yoon et al., 2006). Activated AMPK inhibits SREBP1 and SREBP2 (sterol regulatory...
element binding proteins), the master transcription factors of fatty acid biosynthesis (Li et al., 2011). Activation of PPARα induces gene expression involved in fatty acid oxidation (Haemmerle et al., 2011; Rakhashandehroo et al., 2010). Thus, as the outcome of elevated circulating adiponectin, decreased fatty acid biosynthesis and increased fatty acid oxidation together will lead to reduced liver TG levels. Indeed, compared with control mice, we found that Adipoq-Cre AAV-Becn1F121A mice have significantly decreased liver TG contents (Figure 6F) and, as a result, a reduced level of the lipid droplet coating protein PLIN2 (Perilipin-2) in the liver (Figure 6G), because PLIN2 is stable only on lipid droplets, and cytosolic PLIN2 is degraded by the proteasome pathway (Masuda et al., 2006; Xu et al., 2005). Such reduction in TG and PLIN2 is not due to higher lipophagy in the liver of these mice because the Becn1F121A expression is adipose specific. Rather, in line with increased circulating adiponectin, in response to HFD feeding, we detected a significantly reduced level of the SREBP-target key lipogenesis enzyme ACC (acetyl coenzyme A carboxylase) by WB analysis (Figure 6G) and a trend of increased expression of PPARα-target fatty acid oxidation genes by qPCR (including peroxisomal acyl-coenzyme A oxidase 1 [Acox1], carnitine palmitoyltransferase 1A [Cpt1a], uncoupling protein 2 [Ucp2], and fatty acid binding protein 1 [Fabp1]) (Figure S6H) in the liver of Adipoq-Cre AAV-Becn1F121A mice compared with control mice. These data support that adipose Becn1 non-cell-autonomously regulates AMPK and PPARα signaling pathways and lipid metabolism in the liver. Together, the findings demonstrate that the activity of Becn1 in the WAT plays an important role in the regulation of systemic glucose and lipid metabolism.

**Adipose-specific Becn1F121A increases adiponectin secretion and non-cell-autonomously regulates systemic insulin sensitivity via enhancing adiponectin secretion**

We further detected a significantly higher level of circulating adiponectin in Adipoq-Cre AAV-Becn1F121A mice than in control mice in response to HFD treatment, measured by WB analysis (Figure 7A) and ELISA (Figure 7B). The circulating adiponectin level is also increased in adipose-specific Becn1F121A mice fed with RD (Figure S7A), which is similar to global Becn1F121A mice (Figure 2A). Consistent with cell culture studies (Figure 5G), we found that except adiponectin, circulating levels of two other adipokines, leptin and resistin, are comparable in control and adipose-specific Becn1F121A mice after HFD treatment (Figure 7B), further supporting a level of specificity of Becn1 in the regulation of adipokine secretion. These data suggest that adipose tissue-specific Becn1F121A expression is sufficient to increase adiponectin secretion, in support of the cell-autonomous effect of Becn1F121A on adiponectin secretion in primary adipocytes (Figure 2E).

To directly illustrate whether the metabolic effects of adipose-specific Becn1F121A are dependent on elevated adiponectin secretion, we asked whether reduced adiponectin expression abolishes Becn1F121A-induced insulin sensitization in response to HFD feeding, using the Adiponectin (Adipoq) KO mice. Consistent with previous literature (Nawrocki et al., 2006), we confirmed that the serum adiponectin level in AdipoqKO heterozygous KO mice is approximately half of the WT level after either RD (Figure S7B) or HFD (Figure S7C) feeding. Thus, we generated heterozygous Adipoq+/−-Cre-/+ mice and then injected them with AAV-Becn1F121A to obtain adipose-specific Becn1F121A mice expressing only one copy of the Adiponectin gene. We used AAV-Becn1F121A-injected Adipoq−/−-Cre-/+ mice as the positive control, and AAV-Becn1F121A-injected Adipoq−/−-KO and WT mice without Cre expression as the negative control. We found that under RD feeding conditions, WT and adipose-Becn1F121A mice expressing both or one copy of Adiponectin showed similar body weight, glucose tolerance, and insulin tolerance (Figures S7D and S7E). After HFD feeding, although all four mouse strains had comparable body weight (Figure S7F), only adipose-Becn1F121A mice expressing both copies of Adiponectin, but not those expressing just one copy of Adiponectin, showed improved glucose tolerance (GTT) and insulin tolerance (ITT) compared with non-Becn1F121A-expressing WT mice (Figure 7C), suggesting that reducing adiponectin in adipose-specific Becn1F121A mice abolishes the systemic insulin-sensitizing effects of adipose Becn1F121A in response to HFD feeding. Indeed, we found that adipose Becn1F121A-induced adiponectin secretion to the circulation is abolished when there is a 50% reduction in adiponectin expression in Adipoq−/− mice (Figure 7D). These data further support that the metabolic-improving effects of Becn1F121A are mediated through the adiponectin pathway. Altogether, these findings demonstrate that adipose Becn1 improves systemic glucose tolerance and insulin sensitivity by promoting adiponectin secretion into the circulation.
Model of Becn1-facilitated adiponectin secretion in adipocytes

In addition to the hyperactive genetic mutant Becn1^{F121A}, we found additional upstream physiological and pharmacological modulators of autophagy that also regulate adiponectin secretion. Exercise is a potent physiological inducer of autophagy that disrupts the Becn1-Bcl-2 inhibitory binding (He et al., 2012; Rocchi and He, 2017a). We found that 90-min exercise increases the level of adiponectin in the serum of both male and female WT mice (Figure S7G), suggesting that adiponectin secretion is enhanced by a single bout of exercise. This result is in line with our earlier finding that chronic exercise also increases circulating adiponectin in WT mice, but not in Becn1-inhibited Bcl-2^{AAA} mutant mice (He et al., 2012). This is likely mediated by exercise-induced release of Becn1 from the inhibitory binding of Bcl-2. Via coIP, we found that exercise disrupts the binding of Becn1 with its inhibitor Bcl-2 in the WAT of WT mice (Figure S7H). Although we also detected weak interactions between Becn1 and TLR9 in both WAT and muscle (Figure S7I), different from Becn1-TLR9 binding in muscle, which is enhanced by exercise (Liu et al., 2020), Becn1-TLR9 interaction in the WAT is constitutive and not regulated by exercise (Figure S7I). Thus, we propose that exercise promotes adiponectin secretion by releasing Becn1 from the inhibitory Bcl-2 binding in the WAT.

Conversely, we found that inhibiting the early phase of autophagy imposes an opposite effect. SBI-0206965 and Spautin-1, two inhibitors that block the initiating step of autophagy via inactivating the ULK1 kinase (Egan et al., 2015) or the Vps34 kinase complex (Liu et al., 2011), respectively, reduce the adiponectin level in the serum of WT mice; yet, inhibiting the late stages of autophagy, such as autophagosome-lysosome fusion or autolysosome degradation by chloroquine, does not significantly alter circulating adiponectin levels, measured by WB (Figure 7E) and ELISA analyses (Figure 7F). Given the lack of detection of adiponectin inside autophagosomes (Figures S4D and S4E), these data suggest that the upstream PtdIns3K-Becn1 complex, rather than the sequestering and degradative steps of autophagy, is important for adiponectin regulation. Because compared with WT Becn1, Becn1^{F121A} shows reduced binding to its inhibitor Bcl-2 in the WAT (Figure 4A), altogether, we propose the model of Becn1-facilitated adiponectin secretion: under autophagy-inducing conditions that release Becn1 from its inhibitor Bcl-2 (by exercise or the active Becn1^{F121A} mutant), Becn1 interacts with Sec6 and other exocyst components to enhance adiponectin secretion from adipose tissue, which activates AMPK in non-adipose metabolic tissues and systemically increases insulin sensitivity via circulation (Figure 7G).

**DISCUSSION**

Although emerging evidence suggests that autophagy abnormality is associated with T2D, the mechanism by which autophagy regulates insulin sensitivity is largely unknown. Autophagy is generally thought to be a cellular self-degradation process via lysosomes. In this study, we discovered a previously unknown secretory function regulated by the essential autophagy protein Becn1 in the WAT, which plays a pivotal role in metabolic regulation. We previously found that autophagy-hyperactive Becn1^{F121A} KI mice are protected from HFD-induced insulin resistance (Figure S1A) (Yamamoto et al., 2018). Through analyzing the metabolic signaling pathways in their metabolic tissues, we found that the global Becn1^{F121A} KI mice have higher activation of AMPK, a central player beneficial for energy homeostasis (Figure 1). It is normally believed that autophagy proteins regulate metabolism through cell-autonomous mechanisms, for example, by alleviation of ER stress or improvement of mitochondrial function, because both have been previously linked with insulin sensitization (Patti and Corvera, 2010; Salvadó et al., 2015; Zhang et al., 2013). However, this seems not to be the case in Becn1^{F121A} mice. We found that when added to cell culture media, serum from Becn1^{F121A} mice also enhances AMPK activation in the cells, suggesting that circulating factors are key for Becn1^{F121A}-induced metabolic benefits. To identify the Becn1-regulated, AMPK-activating, circulating factor, we performed a Luminox metabolic hormonal screen in the serum of WT and Becn1^{F121A} mice and detected a higher circulating level of adiponectin in Becn1^{F121A} mice (Figure 2). Adiponectin is a key insulin-sensitizing adipokine that targets multiple metabolic tissues, and its reduction in circulation is strongly associated with T2D and metabolic syndrome. We found that AMPK activation in Becn1^{F121A} mice is at least partially mediated by increased secretion of adiponectin, because the adiponectin...
receptor of target cells is essential for the AMPK-activating effects of Becn1F121A mouse serum (Figure 3). Based on the findings, we propose that improved insulin sensitivity in Becn1F121A mice is mediated through a non-cell-autonomous mechanism (through the circulating factor adiponectin).

Primary adipocytes from Becn1F121A mice also secrete more adiponectin into medium, suggesting that Becn1F121A-induced adiponectin secretion is cell autonomous. To reveal the underlying mechanism, we carried out a number of different analyses using Becn1F121A-expressing adipocytes. Inhibition of autophagy by disrupting autophagy genes Atg5 or Atg7 in pre-adipocytes impairs normal white adipocyte development and differentiation (Baerga et al., 2009; Singh et al., 2009; Zhang et al., 2009), likely caused by a defect in the removal of organelles (such as mitochondria) during adipogenesis. However, we found that elevating autophagy by Becn1F121A, in contrast, does not increase WAT mass (Figure S2) or accelerate adipogenesis (Figures 4, S4B, and S4C). Instead, we found that Becn1 interacts with the exocyst components, including Sec6, and, compared with WT Becn1, the Becn1F121A mutant interacts more strongly with the exocyst (Figures 5B and 5D). Notably, a previous study performed in HEK293 cells reported that an interaction between Becn1 and another exocyst component Exo84 is important for autophagosome formation during starvation (Bodemann et al., 2011). However, we were not able to detect such interaction in HEK293 cells or adipocytes. In addition, whether and how the Becn1-exocyst interaction plays a role in the regulation of the exocyst pathway and function, especially in organs with endocrine functions (such as WAT), was still unstudied. Using a Becn1 deletion mutant that loses Sec6 binding we identified by mutational studies, we found that the Becn1-Sec6/exocyst binding is essential for Becn1F121A to promote adiponectin secretion (Figures 5E–5G). Given the exocyst complex facilitates the docking of secretory vesicles at the plasma membrane, we proposed a previously unknown role of Becn1 in the regulation of adiponectin secretion via the exocyst. This is a different mechanism from reported unconventional autophagic secretion of the leaderless cargo interleukin-1β (IL-1β) via autophagosome-like vesicles (Zhang et al., 2015) or LC3-dependent secretion of extracellular vesicles that contain RNA-binding proteins (Leidal et al., 2020). It is possible that in addition to Becn1, additional autophagy proteins, especially other PtdIns3K complex subunits, may participate in the regulation of the exocyst assembly and adiponectin secretion in adipocytes. Future loss-of-function studies in both WT and Becn1F121A-expressing cells are needed to test these ideas.

After HFD challenge, global Becn1F121A KI mice show improved insulin sensitivity but impaired glucose tolerance (GTT) (Figure S1A) as a result of lower insulin storage in islet β cells caused by chronic autophagic degradation of insulin granules (Yamamoto et al., 2018). It should be noted that although these mice are glucose intolerant with reduced insulin secretion, their islets are not degenerated or reduced in size; instead, their β cell mass is expanded. We reasoned that this compensatory increase in β cell mass can be partly explained by the elevated level of circulating adiponectin in the Becn1F121A mice, because adiponectin has been reported to play a role in maintaining β cell mass (Holland et al., 2011). Using the AAV system, we were able to dissect and differentiate the diverse functions of Becn1F121A in different metabolic cells, for example, adipocytes versus β cells. Different from global Becn1F121A KI mice, adipose tissue-specific Becn1F121A mice show improved glucose tolerance, as well as improved insulin sensitivity (Figure 6), highlighting different physiological outcomes of autophagy manipulation in different metabolic tissues.

Intriguingly, we found that adipose-specific Becn1F121A expression is sufficient to increase circulating levels of adiponectin, but not two other adipokines, leptin and resistin (Figures 7A and 7B), suggesting that Becn1 has specificity in the regulation of adipokine secretion. This is supported by our finding demonstrating that the Becn1-Exo6 interaction is important for the secretion of adiponectin, but not leptin (Figure 5G). We reason that the specificity of the exocyst-autophagy network in the regulation of adipokine secretion is determined by subcellular compartmentation and distinct trafficking pathways of different types of secretory vesicles. Although it is possible that the autophagy machinery and upstream autophagy modulators may regulate a broader endocrine function of WAT and release of additional adipokines, how specificity is achieved at the cellular level is worthy of further investigation. The use of different autophagy mutant adipocytes and mouse lines will be useful to understand the role of the autophagy machinery in the regulation of the adipocyte secretome.

Besides the hyperactive Becn1F121A genetic mutant, we found that upstream physiological and pharmacological autophagy modulators that alter Becn1-Bcl-2 binding also regulate circulating adiponectin levels. The physiological autophagy inducer exercise and the early-stage autophagy inhibitors have opposite effects on circulating adiponectin. Exercise increases the circulating level of adiponectin (Figure S7G), whereas inhibiting the ULK1 or the Becn1-PtdIns3K complex (notably not the late-stage autolysosomal degradation) decreases serum adiponectin (Figures 7E and 7F). We propose that autophagy inducers, such as acute and chronic exercise, promotes adiponectin secretion by releasing Becn1 from inhibitory Bcl-2 binding in adipose tissue (Figure 7G), rather than through enhancing Becn1-TLR9 binding as previously reported in muscle (Liu et al., 2020), for three reasons: (1) via coIP, we found that exercise disrupts the binding of Becn1 with its inhibitor Bcl-2 in the WAT of WT mice (Figure S7H); (2) Becn1F121A does not change TLR9 levels under either RD or HFD treatment (Figure S1B); and (3) although we detected weak interactions between Becn1 and TLR9 in both WAT and muscle, different from Becn1-TLR9 binding in muscle, which is enhanced by exercise (Liu et al., 2020), Becn1-TLR9 interaction in the WAT seems constitutive and not regulated by exercise (Figure S7I). Altogether, our results unveiled a non-degradative, non-cell-autonomous mechanism of Becn1 in metabolic regulation and demonstrated that Becn1-facilitated adiponectin secretion is an important mechanism by which Becn1 regulates systemic AMPK activity and insulin sensitivity.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:
declared no competing interests.

Received: September 23, 2020
Revised: March 16, 2021
Accepted: May 5, 2021
Published: May 25, 2021

REFERENCES

Anita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoaka, K., et al. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. 257, 79–83.

Baerga, R., Zhang, Y., Chen, P.H., Goldman, S., and Jin, S. (2009). Targeted deletion of autophagy-related 5 (atg5) impairs adipogenesis in a cellular model and in mice. Autophagy 5, 1118–1130.

Basu, R., Pajvani, U.B., Rizza, R.A., and Scherer, P.E. (2007). Selective down-regulation of the high molecular weight form of adiponectin in hyperinsulinemia and in type 2 diabetes: differential regulation from nondiabetic subjects. Diabetes 56, 2174–2177.

Bel, S., Pends, M., Wang, Y., Li, Y., Ruhn, K.A., Hassel, B., Leal, T., Winter, S.E., Xavier, R.J., and Hooper, L.V. (2017). Paneth cells secrete lysozyme via secretory autophagy during bacterial infection of the intestine. Science 357, 1047–1052.

Bligh, E.G., and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. 37, 911–917.

Blümer, R.M., van Roomen, C.P., Meijer, A.J., Houben-Weerts, J.H., Sauerwein, H.P., and Dubbelhuis, P.F. (2008). Regulation of adiponectin secretion by insulin and amino acids in 3T3-L1 adipocytes. Metabolism 57, 1655–1662.

Bodemann, B.O., Orvedahl, A., Cheng, T., Ram, R.R., Ou, Y.H., Formstecher, E., Maiti, M., Hazlett, C.C., Wauson, E.M., Balakireva, M., et al. (2011). RalB and the exocyst mediate the cellular starvation response by direct activation of autophagosome assembly. Cell 144, 253–267.

Cong, L., Chen, K., Li, J., Gao, P., Li, Q., Wu, X., and Zhao, A.Z. (2007). Regulation of adiponectin and leptin secretion and expression by insulin through a P38K-PDE3B dependent mechanism in rat primary adipocytes. Biochem. J. 403, 519–525.

Ebatu, C., Uchida, T., Arakawa, M., Komatsu, M., Ueno, T., Komiyama, K., Azuma, K., Hirose, T., Tanaka, K., Kominami, E., et al. (2008). Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. Cell Metab. 8, 325–332.

Egan, D.F., Chun, M.G., Vamos, M., Zou, H., Rong, J., Miller, C.J., Lou, H.J., Rainev-Paris, D., Yang, C.C., Sheffler, D.J., et al. (2015). Small Molecule Inhibition of the Autophagy Kinase ULK1 and Identification of ULK1 Substrates. Mol. Cell 59, 285–297.

Fung, C., Lock, R., Gao, S., Salas, E., and Debnath, J. (2008). Induction of autophagy during extracellular matrix detachment promotes cell survival. Mol. Biol. Cell 19, 797–806.

Garcia, D., and Shaw, R.J. (2017). AMPK: Mechanisms of Cellular Energy Sensing and Restoration of Metabolic Balance. Mol. Cell 66, 789–800.

Geng, L., Liao, B., Jin, L., Huang, Z., Triggle, C.R., Ding, H., Zhang, J., Huang, Y., Lin, Z., and Xu, A. (2019). Exercise Alleviates Obesity-Induced Metabolic Dysfunction by Enhancing FGFR2 Sensitivity in Adipose Tissues. Cell Rep. 26, 2738–2752.e4.

Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E., and Shaw, R.J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell 30, 214–226.

Haemmerle, G., Moustafa, T., Woelkert, G., Büttner, S., Schmidt, A., van de Weijer, T., Hesselin, M., Jaeger, D., Kienesberger, P.C., Zierler, K., et al. (2011). ATGL-mediated fat catabolism regulates cardiac mitochondrial function via PPAR-α and PGC-1. Nat. Med. 17, 1076–1085.

Hajri, T., Tao, H., Wattacheril, J., Marks-Shulman, P., and Abumrad, N.N. (2011). Regulation of adiponectin production by insulin: interactions with tumor necrosis factor-α and interleukin-6. Am. J. Physiol. Endocrinol. Metab. 300, E350–E360.

Haruta, K., Boutin, P., Mori, Y., Tobe, K., Dina, C., Yasuda, K., Yamauchi, T., Otabe, S., Okada, T., Eto, K., et al. (2002). Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 51, 536–540.

Haruta, T., Uno, T., Kawahara, J., Takano, A., Egawa, K., Sharma, P.M., Olefsky, J.M., and Kobayashi, M. (2000). A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol. Endocrinol. 14, 783–794.
He, C., and Levine, B. (2010). The Beclin 1 interactome. Curr. Opin. Cell Biol. 22, 140–149.

He, C., Bassik, M.C., Moresi, V., Sun, K., Wei, Y., Zou, Z., An, Z., Loh, J., Fisher, J., Sun, Q., et al. (2012). Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature 481, 511–515.

He, C., Wei, Y., Sun, K., Li, B., Dong, X., Zou, Z., Liu, Y., Kinch, L.N., Khan, S., Sinha, S., et al. (2013). Beclin 2 functions in autophagy, degradation of G protein-coupled receptors, and metabolism. Cell 154, 1085–1099.

Heider, M.R., and Munson, M. (2012). Exorcising the exocytosis complex. Traffic 13, 898–907.

Holland, W.L., Miller, R.A., Wang, Z.V., Sun, K., Barth, B.M., Bui, H.H., Davis, K.E., Bikman, B.T., Halberg, N., Rutkowski, J.M., et al. (2011). Receptor-mediated activation of ceramide kinase initiates the pleiotropic actions of adiponectin. Nat. Med. 17, 55–63.

Holland, W.L., Adams, A.C., Brozinick, J.T., Bui, H.H., Myauchi, Y., Kusminski, C.M., Bauer, S.M., Wade, M., Singhal, E., Cheng, C.C., et al. (2013). An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice. Cell Metab. 17, 790–797.

Hotta, K., Funahashi, T., Bodkin, N.L., Singh, E., Cheng, C.C., et al. (2013). Beclin 2 functions in autophagy, degradation of G protein-coupled receptors, and metabolism. Nat. Rev. Mol. Cell Biol. 14, 2026–2028.

Kaur, J., and Debnath, J. (2015). Autophagy at the crossroads of catabolism and anabolism. Nat. Rev. Mol. Cell Biol. 16, 461–472.

Kondo, H., Shimomura, I., Matsukawa, Y., Kume, M., Takahashi, M., Matsuda, M., Ouchi, N., Kihara, S., Kawamoto, T., Sumitani, S., et al. (2002). Association of adiponectin mutation with type 2 diabetes: a candidate gene for diabetes. Diabetes 51, 1228–1233.

Lim, C.Y., Hong, W., and Han, W. (2015). Adiponectin is released via a unique regulated exocytosis pathway from a pre-formed vesicle pool on insulin stimulation. Biochem. J. 471, 381–389.

Liu, Z., Tian, H., Lam, K.S., Lim, S., Hoo, R.C., Konishi, M., Itoh, N., Wang, Y., Bornstein, S.R., Xu, A., and Li, X. (2013). Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metab. 17, 779–789.

Liu, J., Xia, H., Kim, M., Xu, L., Li, Y., Zhang, L., Cai, Y., Norberg, H.V., Zhang, T., Furuya, T., et al. (2011). Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell 147, 223–234.

Liu, Y., Nguyen, P.T., Wang, X., Zhao, Y., Meacham, C.E., Zou, Z., Bordieau, I., Johanss, M., Vertommen, D., Wijshake, T., et al. (2020). TLR9 and beclin 1 crosstalk regulates muscle AMPK activation in exercise. Nature 578, 605–609.

Matsuda, Y., Itohe, H., Odaki, M., Hama, K., Fujimoto, Y., Mori, M., Sasabe, N., Aoki, J., Arair, H., and Takanо, T. (2006). ADRP/adipophilin is degraded through the proteasome-dependent pathway during regression of lipid-storing cells. J. Lipid Res. 47, 87–98.

Mei, K., Li, Y., Wang, S., Shao, G., Wang, J., Ding, Y., Luo, G., Yue, P., Liu, J.J., Wang, X., et al. (2018). Cryo-EM structure of the exocytosis complex. Nat. Struct. Mol. Biol. 25, 139–146.

Menzaghí, C., Ercolino, T., Di Paola, R., Berg, A.H., Warram, J.H., Scherer, P.E., Trischitta, V., and Doria, A. (2002). A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. Diabetes 51, 2306–2312.

Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2000). In vivo adeno-associated viral vector-mediated genetic engineering of white and brown adipose tissue in adult mice. Diabetes 49, 4012–4022.

Moffat, J., Grueneberg, D.A., Yang, X., Kim, S.Y., Kleopfer, A.M., Hinkle, G., Piczani, B., Eisenhaure, T.M., Luo, B., Grenier, J.K., et al. (2006). A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell 124, 1283–1298.

Murdoch, G., Hammarstedt, A., Schneizl, M., Janssøn, P.A., and Smith, U. (2009). Acute hyperinsulinemia differentially regulates intestinal and circulating adiponectin oligomeric pattern in lean and insulin-resistant, obese individuals. J. Clin. Endocrinol. Metab. 94, 4508–4516.

Murthy, M., Garza, D., Scheller, R.H., and Schwarz, T.L. (2003). Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists. Neuron 37, 433–447.

Nawrocki, A.R., Rajala, M.W., Tomas, E., Pajvani, U.B., Saha, A.K., Trumbauer, M.E., Pang, Z., Chen, A.S., Ruderman, N.B., Chen, H., et al. (2006). Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J. Biol. Chem. 281, 2654–2660.

Ouchi, N., Parker, J.L., Lugus, J.J., and Walsh, K. (2011). Adiponectins in inflammation and metabolic disease. Nat. Rev. Immunol. 11, 85–97.

Ozes, O.N., Akca, H., Mayo, L.D., Gustin, J.A., Maehama, T., Dixon, J.E., and Donner, D.B. (2001). A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc. Natl. Acad. Sci. USA 98, 4640–4645.

Pajvani, U.B., Hawkins, M., Combs, T.P., Rajala, M.W., Doebber, T., Berger, J.P., Wagner, J.A., Wu, M., Knopps, A., Xiang, A.H., et al. (2004). Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J. Biol. Chem. 279, 12152–12162.

Patti, M.E., and Corvera, S. (2010). The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr. Rev. 31, 364–395.
Pederson, T.M., Kramer, D.L., and Rondinone, C.M. (2001). Serine/threonine phosphorylation of IRS-1 triggers its degradation: possible regulation by tyrosine phosphorylation. Diabetes 50, 24–31.

Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., Rosen, J., Eskelinen, E.L., Mizushima, N., Ohsumi, Y., et al. (2003). Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J. Clin. Invest. 112, 1809–1820.

Rakshshendehroo, M., Knoch, B., Müller, M., and Kersten, S. (2010). Peroxisome proliferator-activated receptor alpha target genes. PPAR Res. 2010, 612089.

Rocchi, A., and He, C. (2015). Emerging roles of autophagy in metabolism and metabolic disorders. Front. Biol. (Beijing) 10, 154–164.

Rocchi, A., and He, C. (2017a). Activating Autophagy by Aerobic Exercise in Mice. J. Vis. Exp. 2017, 55099.

Rocchi, A., Yamamoto, S., Ting, T., Fan, Y., Sadleir, K., Wang, Y., Zhang, W., Huang, S., Levine, B., Vassar, R., and He, C. (2017). A Becn1 mutation mediates hyperactive autophagic sequestration of amyloid oligomers and improved cognition in Alzheimer’s disease. PLoS Genet. 13, e1006962.

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

Salvador, L., Palomer, X., Barroso, E., and Vázquez-Carrera, M. (2015). Targeting endoplasmic reticulum stress in insulin resistance. Trends Endocrinol. Metab. 26, 438–448.

Sarbassov, D.D., Guertin, D.A., Ali, S.M., and Sabatini, D.M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098–1101.

Singh, R., Xiang, Y., Wang, Y., Baikati, K., Cuervo, A.M., Luu, Y.K., Tang, Y., Pessin, J.E., Schwartz, G.J., and Czaja, M.J. (2009). Autophagy regulates adipose mass and differentiation in mice. J. Clin. Invest. 119, 3321–3333.

Stern, J.H., Rutkowski, J.M., and Scherer, P.E. (2016). Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. Cell Metab. 23, 770–784.

Stewart, S.A., Dykkoohorn, D.M., Palliser, D., Mizuno, H., Yu, E.Y., An, D.S., Sabatini, D.M., Chen, I.S., Hahn, W.C., Sharp, P.A., et al. (2003). Lentivirus-delivered stable gene silencing by RNAi in primary cells. RNA 9, 493–501.

Stumvoll, M., Tschritter, O., Fritsche, A., Staiger, H., Renn, W., Weisser, M., Haffner, S.M., and Londos, C. (2005). Post-translational regulation of adipose differentiation-related protein by the ubiquitin/proteasome pathway. J. Biol. Chem. 280, 42841–42847.

Yamamoto, S., Kuramoto, K., Wang, N., Situ, X., Priyadarshini, M., Zhang, W., Cordoba-Chacon, J., Layden, B.T., and He, C. (2018). Autophagy Differentially Regulates Insulin Production and Insulin Sensitivity. Cell Rep. 23, 3286–3299.

Yang, L., Li, P., Fu, S., Calay, E.S., and Hotamisligil, G.S. (2010). Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. 11, 467–478.

Yoon, M.J., Lee, G.Y., Chung, J.J., Ahn, Y.H., Hong, S.H., and Kim, J.B. (2006). Adiponectin increases fatty acid oxidation by activating AMP-activated protein kinase. Nat. Med. 12, 1288–1295.

Zhang, Y., Goldberg, J.L., Ojo, A.Y., and Mitchell, J.J. (1999). Insulin-induced insulin receptor substrate-1 degradation is mediated by the proteasome degradation pathway. Diabetes 48, 1359–1364.

Zhang, Y., Sun, Q., Fan, W., Chen, K., Ding, X., Chen, S., and Zhong, Q. (2008). Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. Proc. Natl. Acad. Sci. USA 105, 19211–19216.

Takano, A., Usui, I., Haruta, T., Kawahara, J., Uno, T., Iwata, M., and Kobayashi, M. (2001). Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. Mol. Cell. Biol. 21, 5050–5062.

Ueno, M., Carvalheira, J.B., Tambascia, R.C., Bezerra, R.M., Amaral, M.E., Carneiro, E.M., Folli, F., Franchini, K.G., and Saad, M.J. (2005). Regulation of insulin signalling by hyperinsulinaemia: role of IRS-1/2 serine phosphorylation and the mTOR/p70 S6K pathway. Diabetologia 48, 506–518.

Werner, E.D., Lee, J., Hansen, L., Yuan, M., and Shoelson, S.E. (2004). Insulin resistance due to phosphorylation of insulin receptor substrate-1 at serine 302. J. Biol. Chem. 279, 35298–35305.

Wu, B., and Guo, W. (2015). The Exocyst at a Glance. J. Cell Sci. 128, 2957–2964.

Wu, X., Motoshima, H., Mahadev, K., Stalker, T.J., Scala, R., and Goldstein, B.J. (2003). Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes 52, 1355–1363.

Xie, L., O’Reilly, C.P., Chapes, S.K., and Mora, S. (2008). Adiponectin and leptin are secreted through distinct trafficking pathways in adipocytes. Biochim. Biophys. Acta 1782, 99–108.

Xu, G., Sztalryd, C., Lu, X., Tansey, J.T., Gan, J., Dorward, H., Kimmel, A.R., and Londos, C. (2005). Post-translational regulation of adipose differentiation-related protein by the ubiquitin/proteasome pathway. J. Biol. Chem. 280, 42841–42847.

Yamamoto, S., Kuramoto, K., Wang, N., Situ, X., Priyadarshini, M., Zhang, W., Cordoba-Chacon, J., Layden, B.T., and He, C. (2018). Autophagy Differentially Regulates Insulin Production and Insulin Sensitivity. Cell Rep. 23, 3286–3299.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., et al. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipopa-thy and obesity. Nat. Med. 7, 941–946.

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamasita, S., Noda, M., Kita, S., Ueki, K., et al. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. 8, 1288–1295.

Yang, L., Li, P., Fu, S., Calay, E.S., and Hotamisligil, G.S. (2010). Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. 11, 467–478.

Yoon, M.J., Lee, G.Y., Chung, J.J., Ahn, Y.H., Hong, S.H., and Kim, J.B. (2006). Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 55, 2525–2542.

Zhang, Y., Goldberg, J.L., Baerga, R., Zhao, Y., Komatsu, M., and Jin, S. (2009). Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. Proc. Natl. Acad. Sci. USA 106, 19860–19865.

Zhang, W., Hietakangas, V., Wee, S., Lim, S.C., Gunaratne, J., and Cohen, S.M. (2013). ER stress potentiates insulin resistance through PERK-mediated FOXO phosphorylation. Genes Dev. 27, 441–449.

Zhang, M., Kenny, S.J., Ge, L., Xu, K., and Schekman, R. (2015). Translocation of interleukin-1β into a vesicle intermediate in autophagy-mediated secretion. eLife 4, e11205.
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| anti-LC3B          | Novus Biologicals | Cat#NB100-2220; RRID:AB_10003146 |
| anti-Leptin         | Novus Biologicals | Cat#NB300-611; RRID:AB_2136214 |
| anti-TLR9           | Novus Biologicals | Cat#NB2-24729 |
| anti-p62            | BD Biosciences | Cat#61033; RRID:AB_398152 |
| anti-PLIN2          | Progen | Cat#GP40 |
| anti-acetyl-CoA carboxylase | Cell Signaling Technology | Cat#3676; RRID:AB_2219397 |
| anti-fatty acid synthase | Cell Signaling Technology | Cat#3180; RRID:AB_2100796 |
| anti-PPARγ          | Cell Signaling Technology | Cat#2c435; RRID:AB_2166051 |
| anti-C/EBPα         | Cell Signaling Technology | Cat#8178; RRID:AB_11178517 |
| anti-PLIN1          | Cell Signaling Technology | Cat#9349; RRID:AB_10829911 |
| anti-AMPK           | Cell Signaling Technology | Cat#2532; RRID:AB_330331 |
| anti-phospho-AMPK (Thr172) | Cell Signaling Technology | Cat#2535; RRID:AB_331250 |
| anti-phospho-AMPK (Thr172) & anti-Raptor | Cell Signaling Technology | Cat#2531; RRID:AB_330330 |
| anti-phospho-Raptor (Ser792) | Cell Signaling Technology | Cat#2083; RRID:AB_2249475 |
| anti-p70S6K         | Cell Signaling Technology | Cat#2708; RRID:AB_390722 |
| anti-phospho-p70S6K (Thr389) | Cell Signaling Technology | Cat#9234; RRID:AB_2269803 |
| anti-IRS-1          | Cell Signaling Technology | Cat#3407; RRID:AB_2127860 |
| anti-phospho-IRS-1 (Ser302) | Cell Signaling Technology | Cat#2491; RRID:AB_823551 |
| anti-phospho-IRS-1 (Ser636/639) | Cell Signaling Technology | Cat#2388; RRID:AB_330339 |
| anti-Akt            | Cell Signaling Technology | Cat#4691; RRID:AB_915783 |
| anti-phospho-Akt (Ser473) | Cell Signaling Technology | Cat#4060; RRID:AB_2315049 |
| anti-ATG14          | MBL International | Cat#PD026; RRID:AB_1953054 |
| anti-UVRAG         | Millipore Sigma | Cat#SAB4200005; RRID:AB_10603351 |
| anti-GFP           | Millipore Sigma | Cat#G1544; RRID:AB_439690 |
| horseradish peroxidase (HRP)-conjugated anti-Flag M2 | Millipore Sigma | Cat#A8592; RRID:AB_439702 |
| anti-VPS34 antibody | Echelon Biosciences | Cat#Z-R016; RRID:AB_11128208 |
| anti-GAPDH         | Thermo Fisher Scientific | Cat#MA5-15738; RRID:AB_10977387 |
| anti-Phospho-IRS1 (Tyr612) | Thermo Fisher Scientific | Cat#44-816G; RRID:AB_2533768 |
| anti-BECN1         | Santa Cruz Biotechnology | Cat#sc-11427; RRID:AB_2064465 |
| anti-BECN1         | Santa Cruz Biotechnology | Cat#sc-48341; RRID:AB_626745 |
| anti-α-Tubulin     | Santa Cruz Biotechnology | Cat#sc-53029; RRID:AB_793541 |
| anti-β-Actin       | Santa Cruz Biotechnology | Cat#sc-7382; RRID:AB_626736 |
| HRP-conjugated anti-β-Actin | Santa Cruz Biotechnology | Cat#sc-47778 HRP; RRID:AB_2714189 |
| anti-SEC6          | Santa Cruz Biotechnology | Cat#sc-374054; RRID:AB_10916711 |
| anti-SEC5          | Santa Cruz Biotechnology | Cat#sc-393230 |
| anti-SEC8          | Santa Cruz Biotechnology | Cat#sc-14215 |
| anti-EXO84         | Santa Cruz Biotechnology | Cat#sc-515532 |
| anti-EXO70         | Santa Cruz Biotechnology | Cat#sc-365825; RRID:AB_10843358 |
| anti-AdipoR1       | Santa Cruz Biotechnology | Cat#sc-518030 |
| anti-HA            | Cell Signaling Technology | Cat#3724; RRID:AB_1549585 |
| anti-HA            | Santa Cruz Biotechnology | Cat#sc-7392; RRID:AB_627809 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| anti-adiponectin    | Cell Signaling Technology | Cat#2789; RRID:AB_2221630 |
| anti-adiponectin    | Thermo Fisher Scientific | Cat#MA1-054; RRID:AB_557516 |
| HRP-conjugated anti-rabbit IgG | Jackson ImmunoResearch Labs | Cat#111-035-003; RRID:AB_2313567|
| HRP-conjugated anti-mouse IgG | Jackson ImmunoResearch Labs | Cat#115-035-003; RRID:AB_10015289 |
| HRP-conjugated anti-guinea pig IgG | Thermo Fisher Scientific | Cat#A18769; RRID:AB_2535546 |
| Alexa Fluor 594-conjugated anti-mouse IgG | Thermo Fisher Scientific | Cat#A-11005 RRID:AB_2534073 |
| Alexa Fluor Plus 488-conjugated anti-mouse IgG | Thermo Fisher Scientific | Cat#A32723; RRID:AB_2633275 |
| Alexa Fluor Plus 594-conjugated anti-rabbit IgG | Thermo Fisher Scientific | Cat#A32740; RRID:AB_2762824 |
| Mouse IgG Isotype Control | Thermo Fisher Scientific | Cat#02-6502; RRID:AB_2532951 |

Bacterial and virus strains

| Lentivirus: shRNA (pLKO.1) | This paper | N/A |
| Retrovirus: GFP-LC3 | This paper | N/A |
| Adeno-associated virus (AAV): CAG-FLEX-3xFlag-mBECN1<sup>1121A</sup> | This paper | N/A |
| Lentivirus: HA-tagged mouse BECN1 (HA-mBECN1) | This paper | N/A |

Chemicals, peptides, and recombinant proteins

| Dexamethasone | Millipore Sigma | Cat#D4902; CAS: 50-02-2 |
| Polybrene | Millipore Sigma | Cat#TR-1003-G |
| anti-Flag M2 affinity gel | Millipore Sigma | Cat#A2220 |
| Type I collagenase | Worthington Biomedical Corp. | Cat#LS004196 |
| Type II collagenase | Worthington Biomedical Corp. | Cat#LS004176 |
| 3-isobutyl-1-methylxanthine (IBMX) | MP biomedicals | Cat#195262; CAS: 28822-58-4 |
| Rosiglitazone | Tokyo Chemical Industry Co. | Cat#R0106; CAS: 122320-73-4 |
| Halt Protease and Phosphatase inhibitor cocktail | Thermo Fisher Scientific | Cat#78446 |
| ProLong Diamond Antifade Mountant | Thermo Fisher Scientific | Cat#P36961 |
| Lipofectamine 3000 transfection reagent | Thermo Fisher Scientific | Cat#L3000150 |
| PowerUP SYBR Green Master Mix | Thermo Fisher Scientific | Cat#A25742 |
| Protein A/G Plus-Agarose | Santa Cruz Biotechnology | Cat#sc-2003 |
| IgG Sepharose 6 Fast Flow | Cytiva | Cat#17-0969-01 |
| ProTEV Plus | Promega | Cat#V6101 |
| SBI-0209665 | ApexBio | Cat#A8715; CAS: 1884220-36-3 |
| DNase I | Millipore Sigma | Cat#D5025; CAS: 9003-98-9 |
| AICAR | Cayman Chemical | Cat#10010241; CAS: 2627-69-2 |
| Infinity Triglycerides Liquid Stable Reagent | Thermo Fisher Scientific | Cat#TR22421 |
| SapphireAmp Fast PCR Master Mix | Takara Bio Inc. | Cat#RR350B |
| Spautin-1 | AdooQ Bioscience | Cat#A12942; CAS: 1262888-28-7 |
| Chloroquine (phosphate) | Cayman Chemical | Cat#14194; CAS: 50-63-5 |
| Insulin | Millipore Sigma | Cat#I0516-5ML; CAS: 11070-73-8 |
| Histopaque-1077 | Millipore Sigma | Cat#10771 |

Critical commercial assays

| Mouse HMW & Total Adiponectin ELISA | ALPCO | Cat#47-ADPMS-E01 |
| Mouse Adiponectin/Acrp30 DuoSet ELISA | R&D Systems | Cat#DY1119 |
| Ultra Sensitive Mouse Insulin ELISA Kit | Crystal Chem | Cat#909080 |
| Mouse Leptin DuoSet ELISA | R&D Systems | Cat#DY498-05 |
| Mouse Resistin ELISA Kit | RayBiotech, Inc. | Cat#ELM-Resistin-1 |

(Continued on next page)
### REAGENT or RESOURCE SOURCE IDENTIFIER

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Mouse/Rat FGF-21 ELISA Kit | Proteintech | Cat#KE10042 |
| In-Fusion HD cloning Plus kit | Takara Bio Inc. | Cat#38910 |
| BCA protein assay kit | Thermo Fisher Scientific | Cat#23225 |
| High-Capacity cDNA Reverse Transcription kit | Thermo Fisher Scientific | Cat#4368814 |

### Experimental models: Cell lines

| Human: HEK293 cells | ATCC | Cat#CRL-1573 |
| Human: HEK293FT cells | Thermo Fisher Scientific | Cat#R70007 |
| Human: T-REx 293 cells | Thermo Fisher Scientific | Cat#R71007 |
| Human: GP2-293 cells | Takara Bio Inc. | Cat#631458 |
| Human: WI-38 cells | ATCC | Cat#CCL-75 |
| Human: Huh7 cells | JCRB Cell Bank | Cat#JCRB0403 |
| Mouse: 3T3-L1 cells | ATCC | Cat#CL-173 |
| Mouse: HepG2 cells | ATCC | Cat#HB-8065 |
| Mouse: NIH 3T3 cells | ATCC | Cat#CRL-1658 |
| Mouse: C2C12 cells | ATCC | Cat#CRL-1772 |

### Experimental models: Organisms/Strains

| Mouse: Becn1^F121A knockin mice: B6.129(Cg)-Becn1^tm1.1Hec/J | The Jackson Laboratory | Cat#033360 |
| Mouse: Adipoq-Cre: B6.FVB-Tg(Adipoq-cre)1Evd/J | The Jackson Laboratory | Cat#028020 |
| Mouse: Adipoq KO: B6;129-Adipoq^tm1Chan/J | The Jackson Laboratory | Cat#008195 |
| Mouse: Becn1 heterozygous KO mice: B6.129X1-Becn1^tm1Blev/J | The Jackson Laboratory | Cat#018429 |
| Mouse: GFP-LC3 mice: Tg(CAG-EGFP/Mapl1c3b)53Nmz | RIKEN BioResource Research Center | Cat#RBRC00806 |
| Mouse: C57BL/6J mice | The Jackson Laboratory | Cat#000664 |

### Oligonucleotides

| Primers for shRNA, see Table S1 | This paper | N/A |
| Primers for PCR, see Table S2 | This paper | N/A |

### Recombinant DNA

| pcDNA3.1(-) | Thermo Fisher Scientific | Cat#V79520 |
| pCDH-CMV-MCS-EF1-Puro | System Biosciences | Cat#CD510B-1 |
| BECLIN-HA WT | Russell et al., 2013 | Addgene plasmid Cat#46993; RRID:Addgene_46993 |
| pLKO.1 - TRC cloning vector | Moffat et al., 2006 | Addgene plasmid Cat #10878; RRID:Addgene_10878 |
| pLKO.1 scramble shRNA | Sarbassov et al., 2005 | Addgene plasmid Cat #1864; RRID:Addgene_1864 |
| pCMV-VSV-G | Stewart et al., 2003 | Addgene plasmid Cat #8454; RRID:Addgene_8454 |
| psPAX2 | Gift of Didier Trono lab | Addgene plasmid Cat #12260; RRID:Addgene_12260 |
| pBABEpuro GFP-LC3 | Fung et al., 2008 | Addgene plasmid Cat #22405; RRID:Addgene_22405 |
| pAAV-FLEX-GFP | Gift of Edward Boyden lab | Addgene plasmid Cat #28304; RRID:Addgene_28304 |
| pZZ-Flag | Sun et al., 2008 | N/A |
| pAAV-FLEX-3xFlag-mBecn1^F121A | This Paper | N/A |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for reagents and resources should be directed to and will be fulfilled by the lead contact, Congcong He (congcong.he@northwestern.edu).

Materials availability
The plasmids and mice used in this study are available from the lead contact.

Data and code availability
This study did not generate/analyze datasets/code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal
All mouse care and procedures were performed in accordance with animal experimental protocols approved by the Northwestern University Institutional Animal Care and Use Committee (IACUC). All mice were housed on a 14-hr/10-hr light/dark cycle with ad libitum access to chow diet and water. GFP-LC3 mice, Becn1F121A KI mice, and Becn1+/- KO mice were described previously (Mizushima et al., 2004; Qu et al., 2003; Rocchi et al., 2017). C57BL/6J mice (JAX#000664), Adiponectin-Cre (Adipoq-Cre) mice (JAX#028020), and Adiponectin KO (Adipoq KO) mice (JAX#008195) were acquired from the Jackson Laboratory. All strains have been backcrossed for more than 12 generations to C57BL/6J mice. All experiments were performed with sex- and age-matched mice. Diet-induced diabetic mice were generated by HFD (D12492; Research Diets Inc) feeding for 12 weeks. SBI-0206965 (2 mg/kg body weight), Spautin-1 (2 mg/kg body weight) and chloroquine (50 mg/kg body weight) were injected intraperitoneally (i.p.) into mice for 10 consecutive days.

Cell culture
3T3-L1 preadipocytes, HEK293 cells, HEK293FT cells, Flp-In T-REx 293 cells, NIH 3T3 cells, C2C12 myoblasts, HepG2 cells, Huh7 cells, WI-38 cells, GP2-293 cells, and stromal vascular fraction (SVF) cells were cultured in culture medium: Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. For 3T3-L1 preadipocytes differentiation to mature adipocytes, two days after full confluency, 3T3-L1 cells (day 0) were cultured in culture medium supplemented with 0.5 mM IBMX, 1 μM dexamethasone, 1 μg/ml insulin for 2 days. Then cells were cultured in culture medium containing 1 μg/ml insulin and refed every two days. At day 10 of culture, 3T3-L1 adipocytes were utilized for experiments. For C2C12 myoblast differentiation to myotubes, fully confluent C2C12 myoblasts (day 0) were cultured in DMEM containing 2% horse serum, 100 units/ml penicillin and 100 μg/ml streptomycin, and refed with fresh medium every day. At day 6 after differentiation, cells were utilized for experiments.

SVF cells containing fibroblastic preadipocytes were isolated from iWAT of 8 week-old mice by collagenase digestion. Briefly, the iWAT was harvested and washed with ice-cold Hank’s balanced salt solution (HBSS). Then iWAT was minced in HBSS containing 300 U/ml type I collagenase and incubated at 37°C for 1 hour with shaking (150 rpm). The digestion was stopped by adding culture medium. The digest was centrifuged at 500 × g for 5 min, and resuspended in the culture medium. After repeating this wash step once again, the cell suspension was filtered through a 70-μm cell strainer and plated in a gelatin-coated dish. To remove non-preadipocytes including red blood cells and immune cells, cells were washed with PBS after 2 hours incubation and then cultured in fresh culture medium. Two days after full confluence, the SVF-derived fibroblastic preadipocytes were cultured in culture medium supplemented with 0.5 mM IBMX, 1 μM dexamethasone, 2 μM rosiglitazone, 1 μg/ml insulin for 2 days. Cells were then cultured in the same way as 3T3-L1 preadipocyte differentiation.
Primary MEFs were isolated from E13.5 embryos by mechanical and enzymatic dissociation method and cultured in MEF culture medium (DMEM with 15% FBS, 2 mM glutamine, 1 x MEM nonessential amino acid, 0.1 mM 2-mercaptopethanol, 100 unit/ml penicillin, and 100ug/ml streptomycin). Briefly, after the head and red-colored organs were removed from the embryo, tissues were dissociated by pipetting and incubating with 1 mL of 0.05% trypsin-EDTA including 100 kU DNase I (D5025, Millipore Sigma) for 5 min at room temperature. Cell suspension (supernatant) was collected and the digestion was stopped by addition of the MEF culture medium. This digestion step was repeated with the remaining tissues (precipitate). The digest was centrifuged at 300 x g for 5 min and resuspend in the MEF culture medium. The cell suspension was filtered through the 70 μm cell strainer and plated in the culture dish.

Primary hepatocytes were isolated from the adult male mice by perfusing the collagenase solution from the portal vein. Briefly, the mouse was euthanized and perfused with 50 mL of calcium and magnesium ions-free HBSS including 0.5 mM EDTA, followed by 40 mL collagenase solution (HBSS including 200 U/ml type II collagenase [LS004176, Worthington], and 2% BSA) at 5 ml/min using peristaltic pump. After perfusion, the liver was cut into small pieces in the culture medium and passed through the 70 μm cell strainer. The digest was centrifuged at 40 x g for 2 min and resuspend in the culture medium. To eliminate non-parenchymal cells, dead cells and cell debris, the cell suspension was layered over the 1.077 g/ml Histopaque (10771, Millipore Sigma) and centrifuged at 200 x g for 5 min. Then primary hepatocytes were resuspended in the culture medium and plated in the gelatin-coated dish.

Plasmid
Human Sec6 was cloned from cDNA generated by reverse transcription of the total RNA of WI-38 cells. HA-tagged human SEC6 (HA-hSEC6) and Flag-tagged human BECN1 (Flag-hBECN1) were subcloned into the EcoRI restriction enzyme site of the pcDNA 3.1 (+) vector using In-Fusion cloning kit. Flag-tagged human SEC6 (Flag-hSEC6) and HA-tagged human BECN1 (HA-hBECN1) were subcloned into the EcoRI restriction enzyme site of the pCDH-CMV-MCS-EF1-Puro lentivector using In-Fusion cloning kit. HA-tagged mouse BECN1 (HA-mBECN1) was amplified by PCR from BECLIN-HA WT (Addgene plasmid #46993) (Russell et al., 2013) and ligated into the XbaI and EcoRI restriction enzyme sites of pCDH-CMV-MCS-EF1-Puro lentivector. Flag-hBECN1 mutants and HA-mBECN1 mutants were generated by site-direct mutagenesis. Double-strand oligos encoding shRNAs against the target genes (Table S1) were cloned into pLKO.1-TRC cloning vector lentivector (Addgene plasmid #10878) (Moffat et al., 2006). A scramble shRNA lentivector (Addgene plasmid #1864) (Sarbassov et al., 2005) was used as a negative control.

METHOD DETAILS

AICAR treatment
Prior to the AICAR treatment, cells were incubated in DMEM containing 0.5% FBS for 6 hours. Cells were treated with 1 mM AICAR (10010241, Cayman Chemical) for 2 hours. Then cells were washed with PBS and lysed with hot-Laemmli buffer. The cell lysate was heated at 95°C for 5 min and used for immunoblotting.

GTT and ITT
GTT and ITT were performed as previously described (Yamamoto et al., 2018). Briefly, mice were fasted for 6 hours and 4 hours prior to GTT and ITT, respectively. Glucose was i.p. injected at 1.5 g/kg body weight. Insulin was i.p. injected at 0.75 U/kg in RD-fed mice and 0.5 U/kg in HFD-fed mice. Blood was collected from the tail vein, and the blood glucose levels were measured using glucose meters (Counter Next EZ; Ascensia Diabetes Care). The insulinogenic index was calculated as follows: insulinogenic index = (blood insulin at 15 min - blood insulin at 0 min [picomoles per liter])/(blood glucose at 15 min - blood glucose at 0 min [milligrams per deciliter]).

Hormone ELISA
High molecular weight (HMW) adiponectin levels and total adiponectin levels in mouse serum were determined using ELISA (47-ADPMS-E01, ALPCO; and DY1119, R&D Systems), according to the manufacture's protocol. For serum insulin measurement, mice were i.p. injected with glucose (1.5 g/kg) after 6 hours of fasting, sera were collected at 0, 15, and 30 min after glucose injection, and serum insulin levels were measured by ELISA (90080; Crystal Chem) according to the manufacture’s protocol. Leptin levels in the mouse serum and 3T3-L1 medium were measured by the mouse leptin ELISA kit (DY498-05, R&D Systems). Resistin levels were measured using the resistin ELISA kit (ELM-Resistin, RayBiotech, Inc.). Serum FGF-21 levels were determined using the FGF-21 ELISA kit (KE10042, Protientech).

Lentivirus production and infection
HEK293FT cells were transfected with a lentiviral expression vector together with a pCMV-VSV-G (Addgene plasmid #8454) (Stewart et al., 2003) and a psPAX2 (Addgene plasmid #12260, a gift from Didier Trono) using the lipofectamine 3000 transfection reagent. Thirty-six hours after transfection, the medium containing the lentivirus was collected and filtered. 3T3-L1 cells, C2C12 cells and NIH 3T3 cells were infected with lentivirus by cell culture in the lentivirus-containing medium with 8 μg/ml polybrene.
**Retrovirus production and infection**

GP2-293 cells were transfected with pCMV-VSV-G and pBABEpuro GFP-LC3 (Addgene plasmid #22405) (Fung et al., 2008) plasmids using lipofectamine 3000 transfection reagents. Forty-eight hours after transfection, the medium containing the retrovirus was collected and filtered. 3T3-L1 cells were infected with retrovirus by cell culturing in the retrovirus-containing medium with 8 µg/ml polybrene.

**Immunopurification of autophagosomes**

Autophagosomes were immunopurified from GFP-LC3-expressing 3T3-L1 adipocytes as described previously (Yamamoto et al., 2018). Briefly, cells were homogenized in lysis buffer (0.25 M sucrose, 1 mM EDTA, 20 mM HEPES [pH7.4], 1 x Halt protease and phosphatase inhibitor cocktail) by passing through 27G (5 times) and 30G (10 times) needles. Lysates were centrifuged at 1, 000 x g for 10 min at 4°C. The supernatants were recovered and centrifuged at 20, 000 x g for 20 min at 4°C. Pellets were resuspended in lysis buffer and incubated with anti-GFP antibody for 2 hours at 4°C. The beads were washed for 4 times with lysis buffer, and the immunoprecipitated proteins were eluted in Laemmli buffer and detected by immunoblotting.

**Immunofluorescence staining**

Cells were plated on a gelatin-coated cover glass, and were fixed with 4% paraformaldehyde in PBS for 30 min and washed with PBS. The cells were then permeabilized with 0.01% digitonin in PBS for 10 min and incubated with primary antibody in PBS containing 5% normal goat serum. After overnight incubation at 4°C, the cells were washed with PBS and incubated with Alexa Fluor-conjugated secondary antibodies in PBS for 1 hour. After washing with PBS, the cells were mounted in a ProLong Diamond Antifade mountant. Fluorescence images were acquired using a spinning-disc confocal microscope (CSU-W1 spinning disk field scanning confocal system (Yokogawa Electric Corp.) with Hamamatsu Flash 4 camera (Hamamatsu Photonics) mounted to a Nikon Ti2 microscope (Nikon)].

**Tandem affinity purification of the Becn1 complex**

HEK293 cells stably expressing tetracycline-inducible ZZ tag-hBECN1-3 x Flag were generated using the pZZ-3xFlag construct (Sun et al., 2008) and the Flp-In T-REx 293 cell system according to the manufacture’s protocol. Cells were incubated in culture medium containing 1 µg/ml tetracycline for 24 hours. After washing with PBS, the cells were lysed in Triton X-100 cell lysis buffer. The lysate was centrifuged at 15,000 x g for 10 min at 4°C, and the supernatant was incubated with IgG Sepharose for 3 hours at 4°C. The bound proteins were then eluted by TEV protease cleavage. The eluate was incubated with anti-Flag antibody-conjugated beads for 14-16 hours at 4°C, and the bound proteins were eluted in Laemmli buffer and detected by immunoblotting.

**Co-immunoprecipitation (co-IP)**

HEK293 cells transfected with plasmids by the lipofectamine 3000 transfection reagent, SVF-derived adipocytes, or mouse WAT and muscle were lysed in Triton X-100 cell lysis buffer (1% Triton X-100, 20 mM HEPES [pH7.4], 150 mM NaCl, 1 mM EDTA). The lysate was centrifuged at 15,000 x g for 10 min at 4°C, and the supernatant was incubated with the target protein-specific antibody and Protein A/G beads for 14-16 hours at 4°C. After bead washing for 4 times with Triton X-100 cell lysis buffer, immunoprecipitated proteins were eluted in Laemmli buffer and detected by immunoblotting.

**AAV delivery of pAAV-FLEX-3xFlag-mBecn1^{:F121A}**

The EGFP ORF region of the pAAV-FLEX-GFP plasmid (Addgene plasmid #28304) was replaced by 3xFlag-mBecn1^{:F121A} ORF using KpnI and XhoI restriction enzymes (NEB) and the In-Fusion HD Plus cloning kit. The AAV2/8 virus was produced by the Sanford Burnham Prebys Medical Discovery Institute Viral Vector Core Facility. The virus titer was 9 × 10e10 VG per 100 ml phosphate-buffered saline (PBS) virus solution was injected into each iWAT lobe using a 31 G insulin syringe (BD). The skin was closed with an absorbable suture. For postoperative care, mice were injected subcutaneously with meloxicam (0.5 mg/kg body weight) for two consecutive days.

**Total lipid extraction and TG measurement**

Total lipids were extracted from the mouse liver using the Bligh and Dyer method (Bligh and Dyer, 1959). Extracted total lipids were resuspended in isopropanol. TG levels were measured using Infinity Triglycerides Liquid Stable Reagent (TR22421, Thermo Fisher Scientific) according to the manufacturer’s protocol.
Treadmill exercise
Exercise was performed as described previously (He et al., 2012) using a 10° uphill Exer 3/6 open treadmill (Columbus Instruments), with some modifications. Briefly, for acclimation, on day 1, mice ran for 5 min at 8 m/min, and on day 2, mice ran for 5 min at 8 m/min followed by another 5 min at 10 m/min. On day 3, mice were subject to running exercise starting at 12 m/min for 40 min. After 40 min, the treadmill speed was increased at the rate of 1 m/min every 10 min for 30 min and then every 5 min for 20 min.

RT-PCR and qPCR
Total RNA was isolated from the 3T3-L1 adipocytes and the mouse liver using the Trizol Reagent. Two micrograms of total RNA was reverse-transcribed using High-Capacity cDNA Reverse Transcription kit (4368814, Thermo Fisher Scientific) according to the manufacturer’s protocol. RT-PCR (reverse transcription-PCR) was performed using SapphireAmp Fast PCR Master Mix (RR350, Takara Bio Inc.). The amplicons were separated and detected by agarose gel electrophoresis and ethidium bromide staining. The band intensity was measured using ImageJ software. qPCR (quantitative PCR) was performed using the PowerUP SYBR Green Master Mix and QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher Scientific). The relative gene expressions were quantified using the $-2^\Delta\Delta Ct$ method and normalized to Actb. The sequences of the primers used for PCR are listed in Table S2.

Immunoblotting
For Akt phosphorylation in the liver, mice were i.p. injected with insulin (1.5 U/kg) after 4 hours fasting, and tissues were collected 15 minutes after insulin injection. For all immunoblotting analyses, tissues and cells were lysed using RIPA lysis buffer (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% NP-40, 0.1% sodium lauryl sulfate [SDS], 0.5% sodium deoxycholate, 1 mM EDTA, 1 x Halt protease and phosphatase inhibitor cocktail). After centrifugation at 15,000 x g for 10 min at 4°C, the supernatant was collected and protein concentration was determined using a BCA protein assay kit. Proteins were denatured in Laemmli buffer by heating at 95°C for 5 min. Samples containing the equivalent amount of protein were resolved by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane or nitrocellulose membrane. After membrane blocking by 0.5% skim milk in Tris-buffered saline containing 0.05% Tween-20 (TBS-T), the proteins were probed with primary antibody overnight at 4°C. The membrane was then washed with TBS-T for 10 min 3 times, and incubated with HRP-conjugated secondary antibody for 1 hour. After membrane washing with TBS-T, specific bands were visualized by chemiluminescence and myECL imager (Thermo Fisher Scientific). The band intensities were quantified using ImageJ software.

QUANTIFICATION AND STATISTICAL ANALYSIS
All data are shown as mean ± standard errors of the mean (SEM). Data were analyzed by Student’s t test for two groups, and by one-way analysis of variance (ANOVA) followed by Tukey-Kramer test or Dunnett’s test for more than two groups. The difference with a P value of < 0.05 was considered statistically significant. Please note that statistical details are found in the figure legends.
Supplemental information

The autophagy protein Becn1 improves insulin sensitivity by promoting adiponectin secretion via exocyst binding

Kenta Kuramoto, Yoon-Jin Kim, Jung Hwa Hong, and Congcong He
### Table S1. shRNA target sequences. Related to the STAR Methods section.

| Target gene | # | shRNA target sequences |
|-------------|---|------------------------|
| mouse Sec6  | 1 | 5’-GCTGTCATGCAGAAGCGTATT-3’ |
|             | 2 | 5’-CCCACACTGTATGTGCAGTA-3’ |
|             | 3 | 5’-CCTCTTGACATGGGTCTCTAAA-3’ |
| mouse Sec5  | 1 | 5’-GCAGGCTACTTTGATTGGAA-3’ |
|             | 2 | 5’-GCGGAAGAGATAAAGAGATTA-3’ |
| mouse Sec8  | 1 | 5’-CGCATCACTAACTCGAGGAAT-3’ |
|             | 2 | 5’-ATCCCTACTCCACGCAAATTC-3’ |
| mouse Adipor1 | 1 | 5’-GGGATTGCTCTACTGATTATG-3’ |
|             | 2 | 5’-TTCCGTTCCTGGCTCTATTAC-3’ |
| mouse Adipor2 | 1 | 5’-GCAGGAATTTCGTTTCATGAT-3’ |
|             | 2 | 5’-CCATCATGCTATGGAACGAAT-3’ |

### Table S2. Primer sequences for PCR. Related to the STAR Methods section.

| Target gene | Forward primer (5’→3’) | Reverse primer (5’→3’) |
|-------------|-------------------------|------------------------|
| mouse Actb  | GATCTGGCACACACACCTCTCT  | GGGGTGTTGAAGGTCTCAAA   |
| mouse Adipor1 | GGTGCTCTGCCTCACGTCTCTCT | CCAGGAACACCTGCTCTCTT   |
| mouse Adipor2 | GGAGATTTGGAGCCCAGCTT  | GCCCTTCCACACCTTACAA    |
| mouse Acox1  | ACCTTCGAGGGGGGAGAACAC  | GCAGCAATTTCACCAATCTGGC |
| mouse Cpt1a  | GACTCCGCTCGCTCATTCC    | ACCAGTGATGATGGCATTCTTG |
| mouse Ucp2  | GCAGTCGGAGACACGATAGTA  | GGACCTTCAATCGGCAAGA    |
| mouse Fabp1  | GGCAAGTACCAATTGAGAGA   | GCTTGACGACTGCCTTGGACTT |
**Figure S1**

**A**

- **ITT** and **GTT** graphs showing blood glucose levels over time for WT and F121A mice.
- **AUC** graphs comparing WT and F121A for ITT and GTT.

**B**

- **RD** and **HFD** graphs showing Cleaved-TLR9/Actin levels in skeletal muscle for WT and F121A mice.

**C**

- **C2C12 myotubes** graphs showing p-AMPK/AMPK, AMPK, and actin levels for PBS, WT, and F121A serum.
- **3T3-L1 differentiated adipocytes** graphs showing p-AMPK/AMPK, AMPK, and actin levels for PBS, WT, and F121A serum.

**D**

- **Primary MEFs** graphs showing p-AMPK, AMPK, and actin levels for AICAR and PBS, with comparison between WT and F121A.
- **Primary hepatocytes** graphs showing p-AMPK, AMPK, and actin levels for AICAR and PBS, with comparison between WT and F121A.
Figure S1. Improved insulin sensitivity, reduced glucose tolerance, normal TLR9 levels, and circulating factor-induced AMPK activation in global Becn1^{F121A} mice. Related to Figure 1.

(A) Insulin tolerance test (ITT) and glucose tolerance test (GTT) on WT and Becn1^{F121A} mice fed with HFD for 12 weeks. Area under the curve (AUC) of blood glucose is shown. n=5-7. T-test.
(B) Western blot analysis of TLR9 levels in the gastrocnemius muscle of WT and Becn1^{F121A} mice fed with RD or HFD for 12 weeks. n=6. T-test.
(C) Western blot analysis of AMPK phosphorylation in WT C2C12 myotubes and 3T3-L1 differentiated adipocytes cultured for 1 h in medium added with PBS, or 10% mouse serum from WT or Becn1^{F121A} mice. n=4. One-way ANOVA with Tukey Kramer test.
(D) Western blot analysis and quantification of AMPK phosphorylation in primary MEFs and primary hepatocytes isolated from WT and Becn1^{F121A} mice with or without treatment of 1 mM AMPK activator AICAR for 2 h. n=3. One-way ANOVA with Tukey Kramer test.

Data represent mean±SEM. *, P<0.05; **, P<0.01; ***, P<0.001; NS, not significant.
Figure S2

A

|           | Insulin | C-peptide | GIP   | Amylin | MCP1 | IL-6 |
|-----------|---------|-----------|-------|--------|------|------|
| WT F121A | pg/ml   | pg/ml     | pg/ml | pg/ml  | pg/ml| pg/ml|
|           | NS      | NS        | NS    | NS     | NS   | NS   |

B

C

Adiponectin level in gWAT

Mouse #: 1 2 3 4 5 6

WT F121A

Adiponectin

actin

D

Serum FGF-21 ELISA

Mouse #: 1 2 3 4

WT F121A

FGF-21 (ng/ml)

NS

E

Rabbit adiponectin Ab

Mouse #: 1 2 3 4

WT F121A

MMW adiponectin

Whole gel

Ponceau S

F

3T3-L1 differentiated adipocytes

HA-Becn1

Expt #: 1 2 3 4

WT F121A

Medium adiponectin

Ponceau S

Adiponectin

NS

** **
Figure S2. Becn1F121A increases secreted, but not adipose-resident, adiponectin levels. Related to Figure 2.

(A) Magnetic bead Luminex assays on circulating metabolic hormones in the serum of RD-fed WT and autophagy-hyperactive Becn1F121A mice show that the serum level of insulin, C-peptide, GIP (gastric inhibitory polypeptide), amylin, MCP1 (monocyte chemoattractant protein 1), or IL-6, is comparable in WT and Becn1F121A mice. n=15.

(B) Body weight, adipose tissue weight, and adipose tissue percentage in body weight of RD or HFD-fed WT and Becn1F121A mice. iWAT, inguinal white adipose tissue; gWAT, gonadal white adipose tissue. HFD WT, n=19; HFD Becn1F121A, n=10; RD WT, n=15; RD Becn1F121A, n=16.

(C) The adiponectin level in the gWAT (gonadal white adipose tissue) of WT and Becn1F121A mice was measured by Western blot analysis. n=6.

(D) ELISA analyses of circulating FGF-21 in HFD-fed WT and Becn1F121A knock-in mice. WT, n=8; F121A, n=9.

(E) The medium-molecular weight (MMW) form of adiponectin is increased in Becn1F121A mice. Western blot and quantification of the MMW adiponectin in the serum of WT and Becn1F121A mice using a rabbit anti-adiponectin antibody (Cell Signaling #2789) that detects MMW but not HMW adiponectin. Total protein loading is shown by Ponceau S staining. n=4.

(F) Adiponectin secreted into the conditioned medium by 3T3-L1 differentiated adipocytes stably expressing vector, WT Becn1, or Becn1F121A was analyzed and quantified by Western blot after 24 hr cell culture. n=4.

(A-E) T-test. (F) One-way ANOVA with Tukey-Kramer test. Data represent mean±SEM. *, P<0.05; **, P<0.01; NS, not significant.
Figure S3. Adiponectin receptors are required for Becn1<sup>F121A</sup> mouse serum-induced AMPK activation in cultured adipocytes. Related to Figure 3. 3T3-L1 differentiated adipocytes stably expressing scrambled (Scr) or shRNAs against adiponectin receptors (AdipoR1/2) were cultured for 1 h in medium containing 10% mouse serum from WT or Becn1<sup>F121A</sup> mice, and AMPK phosphorylation was analyzed and quantified by Western blot. n=6 mice. shRNA knockdown efficiency was analyzed by RT-PCR. Data represent mean±SEM. One-way ANOVA with Tukey-Kramer test. ***, P<0.001; NS, not significant.
Figure S4

A

|       | WT  | F121A |
|-------|-----|-------|
| iWAT  |     |       |
| LC3B-I|     |       |
| LC3B-II|    |       |
| SQSTM1/p62| |       |
| Actin |     |       |

![Graphs showing LC3B-II and SQSTM1/p62 levels with and without CQ](image)

B

**Adipogenesis markers**

SVF (day 10 differentiation)

|       | WT  | F121A |
|-------|-----|-------|
| PLIN1 |     |       |
| ADIPOQ|     |       |
| FABP4 |     |       |
| FAS   |     |       |
| C/EBPα|     |       |
| PPARγ|     |       |
| α-Tubulin | |       |

![Graphs showing expression levels of various adipogenesis markers](image)

C

**Adipogenesis markers**

3T3-L1 differentiated adipocytes (day 10)

|       | WT  | F121A |
|-------|-----|-------|
| PLIN1 |     |       |
| ADIPOQ|     |       |
| FABP4 |     |       |
| FAS   |     |       |
| PPARγ|     |       |
| α-Tubulin | |       |

![Graphs showing expression levels of various adipogenesis markers](image)

D

**Primary adipocyte**

|       | Low magnification | High magnification |
|-------|-------------------|--------------------|
| Adiponectin | GFP-LC3 | Merge |
| Merge | | |

![Images showing Adiponectin and GFP-LC3 expression in primary adipocytes](image)

E

IP: GFP-LC3 in adipocytes

- **GFP-LC3**
- **Adiponectin**
- **GAPDH**
Figure S4. Beclin1$^{F121A}$ expression leads to increased autophagy, but not accelerated adipogenesis or adiponectin incorporation by autophagosomes in adipocytes. Related to Figure 4.

(A) Western blot analysis and quantification of LC3-II and SQSTM1/p62 in the inguinal white adipose tissue (iWAT) of RD-fed WT and Beclin1$^{F121A}$ mice intraperitoneally treated with vehicle or chloroquine (CQ) 4 hr prior to tissue collection. n=4 mice. One-way ANOVA with Tukey-Kramer test.

(B-C) Adipogenesis is not affected by the Beclin1$^{F121A}$ mutation in vitro in primary adipocytes or adipocyte cell lines.

(B) Western blot analysis of adipogenesis markers in SVF-differentiated primary adipocytes from WT and Beclin1$^{F121A}$ mice. n=3. T-test.

(C) Adipogenesis markers were analyzed by Western blot in 3T3-L1 differentiated adipocytes stably expressing HA-tagged WT Beclin1 or Beclin1$^{F121A}$ on the last day of differentiation (day 10). n=3. One-way ANOVA with Tukey-Kramer test. Data represent mean±SEM. *, P<0.05; **, P<0.01; ***, P<0.001; NS, not significant.

(D) Primary adipocytes (SVF) isolated from WT GFP-LC3 and Beclin1$^{F121A}$ GFP-LC3 reporter mice were immunostained with anti-adiponectin antibody, and visualized by confocal fluorescence microscopy. Scale bar: low magnification, 10 μm; high magnification, 5 μm.

(E) No detection of adiponectin by Western blot analysis inside autophagosomes immunoisolated by a GFP antibody in 3T3-L1-differentiated adipocytes stably expressing the autophagosome marker GFP-LC3.
Figure S5

A

B

3T3-L1-differentiated adipocyte

WT Becn1

Becn1[121A]

Input

IP

IgG

HA

HA-Becn1

Sec6

GAPDH

C

3T3-L1 differentiated adipocyte

shSec6/Exoc3

shRNA:

Expt:

Scr #1 #2 #3

Adiponectin

Leptin

Ponceau S

Sec6

Actin

Medium

Cell

Adiponectin

Leptin

Sec6

D

3T3-L1 differentiated adipocyte

shRNA:

Scr Sec5

Sec5 #1 #2 #1 #2

Adiponectin (medium)

Actin

E

3T3-L1 differentiated adipocyte

shRNA:

Scr Sec8

Sec8 #1 #2 #1 #2

Adiponectin (medium)

Actin

3T3-L1-differentiated adipocyte

WT

Becn1[121A]

Leptin

BECN1

Merge

High-magnification

Pearson correlation coefficient

WT

F121A

0.75

0.5

0.25

NS
Figure S5. The exocyst components Sec6 and Sec8 are affinity-purified by Becn1 and are important for adiponectin secretion. Related to Figure 5.

(A) Tandem affinity purification of exocyst components (including Sec5, Sec6 and Sec8) by Becn1 in tetracycline-inducible Flp-In T-REx-293 cells expressing cleavable zz tag-hBecn1-3XFlag or the control zz tag-3XFlag. Pulldown of the Becn1-PtdIns3K complex subunits Vps34 and UVRAG is used as positive control. CBB, Coomassie Brilliant Blue.

(B) Stronger binding of the exocyst component Sec6 with Becn1^{F121A}, than with WT Becn1, in 3T3-L1 preadipocyte-differentiated adipocytes. Coimmunoprecipitation of Sec6 with the Becn1 protein in 3T3-L1-differentiated adipocytes stably expressing HA-tagged WT Becn1 or Becn1^{F121A}.

(C) Adiponectin and leptin secreted into the conditioned media by 3T3-L1 differentiated adipocytes stably expressing scrambled (Scr) or Sec6 shRNAs during 24-h culture were analyzed and quantified by Western blot assays. Sec6 knockdown efficiency was also analyzed and quantified by Western blot. n=3. One-way ANOVA with Dunnett’s test.

(D) Adiponectin secreted into the conditioned media by 3T3-L1 differentiated adipocytes stably expressing scrambled (Scr) or Sec5 or Sec8 shRNAs during 24-h culture was analyzed by Western blot assays. Knockdown of Sec8 also reduces Sec6 stability.

(E) Leptin has limited colocalization with Becn1 in adipocytes. Immunofluorescence staining of leptin and Becn1 in primary adipocytes (SVF) of WT and Becn1^{F121A} mice. Low magnification, bar: 10 µm; high magnification, bar: 5 µm. n=16-17 regions. T-test. Pearson correlation coefficient is used to quantify colocalization. Data represent mean±SEM. *, P<0.05; **, P<0.01; ***, P<0.001; NS, not significant.
Figure S6

(A) Western blots of gWAT and Liver tissues from Adipoq-Cre WT and +AAV-FLEX-Becn1F121A mice

(B) Body weight of mice on RD and HFD

(C) GTT and ITT of mice on RD and HFD

(D) Weights of gWAT and iWAT

(E) Insulinogenic index

(F) Western blots of skeletal muscle

(G) Western blots of Liver with and without insulin

(H) qPCR analysis of liver gene expression

* p<0.05
** p<0.01
*** p<0.001
NS = non-significant
Figure S6. Metabolic parameters of adipose tissue-specific Becn1_F121A mice generated via AAV2/8 delivery, including elevated AMPK signaling in the muscle and liver after HFD feeding. Related to Figure 6.

(A) Becn1_F121A specifically expresses in gWAT and iWAT of Adipoq-Cre, but not WT, mice injected with AAV2/8-FLEX-3XFlag-Becn1_F121A. Becn1_F121A does not express in the liver of Adipoq-Cre mice injected with AAV2/8-FLEX-3XFlag-Becn1_F121A either.

(B) Body weight of WT or Adipoq-Cre mice injected with AAV-FLEX-Becn1_F121A fed with RD or HFD for 12 weeks. RD, n=4-7; HFD, n=9-11.

(C) GTT and ITT of WT or Adipoq-Cre mice injected with AAV-FLEX-Becn1_F121A fed with RD. n=4-7.

(D) gWAT and iWAT weight (g and % of body weight) of WT or Adipoq-Cre mice injected with AAV-FLEX-Becn1_F121A fed with HFD for 12 weeks. WT+AAV-Becn1_F121A, n=11; Adipoq-Cre+AAV-Becn1_F121A, n=9.

(E) Insulinogenic index of WT or Adipoq-Cre mice injected with AAV-FLEX-Becn1_F121A fed with HFD for 12 weeks. WT+AAV-Becn1_F121A, n=11; Adipoq-Cre+AAV-Becn1_F121A, n=9.

(F) Western blot analysis and quantification of AMPK activation in the skeletal muscle of Adipoq-Cre or control (WT) mice injected with AAV2/8-FLEX-Becn1_F121A and then fed with HFD for 12 weeks. n=4.

(G) Western blot analysis and quantification of insulin-induced IRS-1 tyrosine phosphorylation in the liver of Adipoq-Cre or control (WT) mice injected with AAV2/8-FLEX-3XFlag-Becn1_F121A and fed with HFD for 12 weeks. n=3.

(H) qPCR analysis of Acox1, Cpt1a, Ucp2 and Fabp1 in the liver of Adipoq-Cre or WT mice injected with AAV2/8-FLEX-Becn1_F121A and fed with HFD for 12 weeks. WT+AAV-Becn1_F121A, n=7; Adipoq-Cre+AAV-Becn1_F121A; n=5.

T-test. Data represent mean±SEM. *, P<0.05; ***, P<0.001; NS, not significant.
Figure S7. Regulation of adiponectin secretion by genetic (Becn1F121A) and physiological (exercise) autophagy inducers. Related to Figure 7.

(A) Increased circulating adiponectin after RD feeding in adipose-specific Becn1F121A mice. Western blot analysis of serum adiponectin in Adipoq-Cre or WT mice injected with AAV2/8-FLEX-Becn1F121A and then fed with RD for 8 weeks. n=4. T-test.

(B-C) The serum adiponectin level in Adiponectin (Adipoq) heterozygous KO mice is half of the WT level. Western blot analysis of serum adiponectin levels in WT and Adipoq+/- KO mice after RD (B) or HFD (C) feeding for 8 weeks. n=3-4. One-way ANOVA with Dunnett’s test.

(D-E) Comparable body weight (D), glucose tolerance and insulin tolerance (E) of WT and adipose-specific Becn1F121A mice expressing both or one copy of Adiponectin after 8 weeks of RD feeding. One-way ANOVA with Dunnett’s test.

(F) Comparable body weight of WT and adipose-specific Becn1F121A mice expressing both or one copy of Adiponectin after 8 weeks of HFD feeding. WT+AAV-Becn1F121A, n=9; Adipoq-Cre+AAV-Becn1F121A, n=9; Adipoq+/- KO+AAV-Becn1F121A, n=7; Adipoq+/- KO Adipoq-Cre+AAV-Becn1F121A, n=6. One-way ANOVA with Dunnett’s test.

(G) Exercise, a physiological autophagy inducer, increases circulating adiponectin in vivo. Western blot and quantification of adiponectin in the blood of RD-fed male and female WT mice at resting conditions or after 90 min exercise. n=4-5. T-test.

(H) Co-IP of endogenous Becn1 by endogenous Bcl-2 in iWAT (inguinal white adipose tissue) of WT mice after a time course of treadmill exercise.

(I) Co-IP of endogenous TLR9 by endogenous Becn1 in WAT and skeletal muscle of WT mice before (0 min) and after (15 min) treadmill exercise. Data represent mean±SEM. *, P<0.05; **, P<0.01; NS, not significant.