A Critical SUMO1 Modification of LKB1 Regulates AMPK Activity during Energy Stress

Graphical Abstract

Highlights
- SUMO1 conjugation of LKB1 increases as intracellular ATP levels decline
- During energy stress, lysine 178 of LKB1 is a critical SUMO1 conjugation site
- SUMO1 modification of LKB1 enables the recognition and activation of AMPK via a SIM
- LKB1 K178R SUMO mutant is defective in AMPK activation and downstream signaling

Authors
Joan Ritho, Stefan T. Arold, Edward T.H. Yeh

Correspondence
etyeh@mdanderson.org

In Brief
SUMOylation is important in cellular stress adaptation. However, its role in regulating LKB1, one of the key regulators of cellular energy balance, is unknown. Ritho et al. illustrate that energy stress triggers the SUMO1 modification of LKB1, consequently enhancing the recognition and activation of AMPK via a SUMO-interacting motif.
A Critical SUMO1 Modification of LKB1 Regulates AMPK Activity during Energy Stress

Joan Ritho,1,2 Stefan T. Arold,3 and Edward T.H. Yeh1,*
1Department of Cardiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
2Cancer Biology Program, The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA
3Computational Bioscience Research Center, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia
*Correspondence: etyeh@mdanderson.org
http://dx.doi.org/10.1016/j.celrep.2015.07.002
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

SUMMARY

SUMOylation has been implicated in cellular stress adaptation, but its role in regulating liver kinase B1 (LKB1), a major upstream kinase of the energy sensor AMP-activated protein kinase (AMPK), is unknown. Here, we show that energy stress triggers an increase in SUMO1 modification of LKB1, despite a global reduction in both SUMO1 and SUMO2/3 conjugates. During metabolic stress, SUMO1 modification of LKB1 lysine 178 is essential in promoting its interaction with AMPK via a SUMO-interacting motif (SIM) essential for AMPK activation. The LKB1 K178R SUMO mutant had defective AMPK signaling and mitochondrial function, inducing death in energy-deprived cells. These results provide additional insight into how LKB1-AMPK signaling is regulated during energy stress, and they highlight the critical role of SUMOylation in maintaining the cell’s energy equilibrium.

INTRODUCTION

Energy homeostasis in a cell is critical for its survival during metabolic stress. Liver kinase B1 (LKB1), one of the key regulators of cellular energy balance, was initially discovered as a tumor suppressor mutated in patients with Peutz-Jeghers syndrome (Hemminki et al., 1998). Germline mutations in a tumor suppressor mutated in patients with Peutz-Jeghers syndrome (Hemminki et al., 1998). Germline mutations in

This process has been implicated in important processes such as transcription, protein stability, and protein subcellular localization (Yeh, 2009). SUMOylation can (1) inhibit the interaction between the target and its interacting partner; (2) enhance this interaction through the creation of a binding surface where the target would recognize the partner via a SUMO-interacting motif (SIM); or (3) change the conformation of the target, thereby altering its function (Wilkinson and Henley, 2010). Given the diverse roles SUMOylation plays in the eukaryotic cell, we hypothesized that, during energy stress, SUMOylation regulates the LKB1-AMPK interaction and that this accordingly affects the kinase activity of AMPK.

Our findings here demonstrate that energy stress triggers an increase in the modification of LKB1 by SUMO1 despite a global reduction in both SUMO1 and SUMO2/3 conjugates. During metabolic stress, LKB1 is specifically modified by SUMO1 at lysine 178 (K178), but not by SUMO2/3, acetylation, or ubiquitination. This modification is essential in promoting LKB1-AMPK interaction. On the basis of the crystal structure depicting the non-covalent recognition of SUMO1 by RanBP2, we identified a SIM in the N-terminal region of AMPK. Mutation of the hydrophobic residues necessary for SUMO1 interaction prevented LKB1 from recognizing and activating AMPK. Finally, we observed that cells with the LKB1 K178R SUMO mutant had defective AMPK signaling and mitochondrial function, inducing apoptosis in energy-deprived cells. Thus, we propose a model in which energy stress upregulates the modification of LKB1 by SUMO1, thereby facilitating its interaction with AMPK. This enhances the rate at which AMPK can respond to the metabolic needs of the cell.
Figure 1. SUMO1 Modification of LKB1 Is Upregulated during Energy Stress

(A) Endogenous SUMO1 modification of LKB1 in HEK293 cells in which SENP1 expression level was knocked down by siRNA. Immunoprecipitates were subjected to western blot using the indicated antibodies. Messenger RNA levels of SENP1 were measured by quantitative real-time PCR. Data are presented as the mean ± SD (error bars).

(B) SUMOylation of LKB1 via co-expression of HA-SUMO1 and LKB1 cDNA with or without FLAG SENP1 in HeLa cells (LKB1 null) is shown. See also Figures S1A and S1B.

(legend continued on next page)
SUMO1 Modification of LKB1 Is Upregulated during Energy Stress

To our knowledge, the SUMOylation status of LKB1 has not been established. We therefore made use of Sentrin/SUMO-specific protease 1 (SENP1) (Yeh et al., 2000) to first characterize LKB1 SUMOylation. We noted an increase in modification of LKB1 by SUMO1 in HEK293 cells in which endogenous SENP1 was knocked down by SENP1-specific small interfering RNA (siRNA) (Figure 1A). To further verify the SUMOylation of LKB1, we over-expressed either HA-tagged SUMO1 or HA-tagged SUMO2/3 together with LKB1 cDNA in LKB1-null HeLa cells, and we demonstrated that LKB1 was modified by both SUMO1 monomeric and SUMO2/3 polymeric chains (Figures 1B, S1A, and S1B). In vitro SUMOylation experiments corroborated that LKB1 is indeed SUMOylated (Figure S1C). These findings establish that both endogenous and exogenous LKB1 are modified by SUMOylation.

Since LKB1 is a critical protein involved in maintaining energy homeostasis in a cell, we investigated whether there were any LKB1 SUMOylation changes that occur during metabolic stress. We induced energy stress in HEK293 (expressing empty vector or FLAG SENP1) by subjecting the cells to low-glucose conditions, low glucose together with glycolysis inhibitor 2-DG, or treatment with phenformin (a biguanide compound that inhibits complex 1 of the mitochondria). CAMKK inhibitor STO-609 was included in the treatment regimen since CAMKK mediate AMPK activation, thereby allowing the appropriate cellular response during energy stress. All things considered, our data propose that LKB1 SUMOylation is essential in maintaining the cell’s energy balance.

LKB1 K178 Is Modified by SUMO1 during Energy Stress

The covalent conjugation of a SUMO protein to a target’s lysine residue is dictated by a consensus motif (VVxEx, in which $\Psi$ is an aliphatic branched amino acid and x is any amino acid) (Yeh et al., 2000). In some cases, this motif is not observed, and a lysine residue can be modified by SUMO in a non-consensus mode (Wilkinson and Henley, 2010). To identify the possible LKB1 lysine residues that may be SUMO1 modified during energy stress, we used SUMOylation site prediction software (Ren et al., 2009) that took into consideration the different types (i.e., through consensus and non-consensus motifs) of SUMO conjugation. The software generated a number of lysine targets and we chose the top three candidates with the best cutoff scores: K96, K122, and K178. We generated LKB1 K96R, K122R, and K178R mutants and transfected them or their wild-type counterpart into HeLa cells (LKB1 null) together with HA-SUMO1. The cells were subjected to energy stress by

(C) HEK293 cells (expressing empty vector or FLAG SENP1) were subjected to energy stress for 6 hr though low-glucose conditions, low glucose together with glycolysis inhibitor 20 mM 2-DG, or treatment with 5 mM phenformin; 10 $\mu$g/ml CAMKK inhibitor STO-609 was included in the treatment regimen. LKB1 was immunoprecipitated and its SUMO1 conjugation levels were assessed. Whole-cell lysates were subjected to western blot using the indicated antibodies. In addition, intracellular ATP levels were evaluated in these cells. Data are presented as the mean ± SD (error bars).

(E) HEK293 cells were transiently transfected with either empty vector (pcDNA3) or FLAG SENP1 followed by treatment for 20 hr with vehicle (CAMKK inhibitor) alone or with 5 mM phenformin. Whole-cell lysates were subjected to western blot using the indicated antibodies. (F) Endogenous SENP1 or SENP2 levels were detected in HEK293 cells treated with vehicle (CAMKK inhibitor) alone or with 5 mM phenformin for 15 hr.

(G) HEK293 cells transfected with FLAG SENP1 or SENP2 were treated with vehicle (CAMKK inhibitor) alone or with 5 mM phenformin for 15 hr. Cell lysates were subjected to western blot using the indicated antibodies. (H) LKB1 was immunoprecipitated from HEK293 cells expressing either vector control or FLAG SENP1. The precipitates were equally divided and one set was incubated with recombinant human His6 SENP1 catalytic domain. LKB1 autophosphorylation status was assessed. Cell lysates were subjected to western blot using the indicated antibodies.
phenformin for 6 hr, and then LKB1 was immunoprecipitated and the lysates blotted for HA. We observed that, upon metabolic stress, SUMO1 modification of the LKB1 K178R mutant was markedly lower than that of wild-type LKB1 (Figure 2A). However, SUMO1 modification of the LKB1 K96R mutant remained unaltered (Figure S2A). The LKB1 K122R mutant was unstable and thus not tested further (data not shown). Notably, when the cells were not subjected to energy stress, levels of

Figure 2. LKB1 K178 Is Modified by SUMO1 and Is Critical in Activating AMPK

(A–C) Post-translational modification of LKB1 wild-type and K178R mutant by (A) SUMO1 and SUMO2, (B) acetylation, and (C) ubiquitin in HeLa cells during metabolic stress (2 mM phenformin and 10 μg/ml STO-609) is shown. See also Figure S2B.

(D) Wild-type LKB1, LKB1 K178R mutant, and the various Peutz-Jeghers syndrome LKB1 mutants (Q170P, I177N, N181E, and L182P) were transfected and immunoprecipitated from HeLa cells, and their interactions with STRADα and MO25 were assessed.

(E and F) LKB1 was immunoprecipitated from HeLa cells transiently expressing empty vector (pcDNA3), wild-type LKB1, or LKB1 K178R mutant. (E) An in vitro kinase assay using GST-AMPKα1 recombinant protein was performed. (F) Autophosphorylation of LKB1 and the LKB1 K178R mutant was analyzed.

(G) A549 cells (LKB1 null) stably expressing empty vector (pcDNA3), wild-type LKB1, or LKB1 mutant K178R were treated with 2 mM phenformin and/or 10 μg/ml STO-609 as indicated. Cell lysates were subjected to western blot using the indicated antibodies. See also Figure S2C.
SUMO1-modified LKB1 remained the same for both the wild-type and K178R mutant LKB1 (Figure S2B). Thus, the SUMOylation status of the K178R mutant only changed upon metabolic stress. These data suggest that, during energy stress, K178 is a critical SUMO1 conjugation site. Since lysine residues also can be modified by SUMO2/3, acetylation, and ubiquitination, we examined whether these post-translational modifications occurred in the LKB1 K178R mutant during energy stress. We found that the modification of K178 by SUMO2/3, acetylation, or ubiquitination was not altered in these conditions (Figures 2A–2C). These results led to the conclusion that, during energy stress, conjugation of K178 with SUMO1 may be important in regulating LKB1 function in maintaining the energy equilibrium in a cell.

Owing to the proximity of lysine 178 to the active site, we had to ascertain that mutating the lysine to arginine did not alter LKB1 catalytic activity. While the Peutz-Jeghers syndrome LKB1 mutations (Q170P, I177N, N181E, and L182P) impeded the formation of the LKB1-STRADα-MO25 heterotrimeric complex, which is important in the allosteric activation of LKB1 (Alessi et al., 2006), the LKB1 K178R mutant did not (Figure 2D). Thus, mutation of K178 to arginine did not change the ability of LKB1 to form this active complex. Moreover, the autophosphorylation status and kinase activity of the LKB1 K178R mutant remained unaltered (Figures 2E and 2F). This further demonstrates that LKB1 K178R is not a kinase-dead mutant.

To further evaluate the physiological relevance of the specific LKB1 K178R mutant in the maintenance of the cell’s energy balance, we transfected empty vector (pcDNA3), wild-type LKB1, or LKB1 K178R into LKB1-null HeLa cells and treated the cell cultures with phenformin and STO-609 at the indicated times to induce metabolic stress. Interestingly, the phosphorylation of endogenous AMPK at T172 was markedly reduced in cells expressing empty vector (pBABE) or the LKB1 K178R mutant (Figures 2G and S2C). Notably, in these cells in which the phosphorylation of AMPK at T172 was markedly reduced in cells expressing empty vector (pBABE) or the LKB1 K178R mutant (Figure 2D). This, mutation of K178 to arginine did not change the ability of LKB1 to form this active complex. Moreover, the autophosphorylation status and kinase activity of the LKB1 K178R mutant remained unaltered (Figures 2E and 2F). This further demonstrates that LKB1 K178R is not a kinase-dead mutant.

To further evaluate the physiological relevance of the specific LKB1 K178R mutant in the maintenance of the cell’s energy balance, we transfected empty vector (pcDNA3), wild-type LKB1, or LKB1 K178R into LKB1-null HeLa cells and treated the cell cultures with phenformin and STO-609 at the indicated times to induce metabolic stress. Interestingly, the phosphorylation of endogenous AMPK at T172 was markedly reduced in cells expressing empty vector (pBABE) or the LKB1 K178R mutant (Figures 2G and S2C). Notably, in these cells in which the phosphorylation of AMPK at T172 was markedly reduced in cells expressing empty vector (pBABE) or the LKB1 K178R mutant (Figure 2D). This, mutation of K178 to arginine did not change the ability of LKB1 to form this active complex. Moreover, the autophosphorylation status and kinase activity of the LKB1 K178R mutant remained unaltered (Figures 2E and 2F). This further demonstrates that LKB1 K178R is not a kinase-dead mutant.

SUMOylation Promotes LKB1-AMPK Interaction

Previous studies have demonstrated that proteins can non-covalently interact with SUMO via their SIM and that this may promote the interaction between the protein and its substrate or partner (Kerscher, 2007). Given that SUMOylation is important in regulating protein-protein interaction, we examined whether the LKB1 K178R mutant had lower affinity to AMPK than the wild-type LKB1. We observed that, during energy stress, wild-type LKB1 bound AMPK more than the LKB1 K178R mutant (Figure 3A). Furthermore, overexpression of the deSUMOylating enzyme SENP1 caused a marked decrease in LKB1-AMPK interaction in stable LKB1-expressing A549 cells (Figure 3B) as well as in HEK293 cells that endogenously expressed wild-type LKB1 (Figure S3A). To establish that SUMO1 was responsible for the increase in LKB1-AMPK interaction during energy stress, we assessed whether SUMO1 interacted with AMPK. We over-expressed vector control or HA-SUMO1 in A549 cells and conducted a co-immunoprecipitation study. As expected, endogenous AMPK co-precipitated with HA-SUMO1 (Figure 3C) and also with endogenous SUMO1 in HEK293 cells (Figure S3B). We then determined whether this interaction was mediated via a SIM by conducting a competition assay using peptides containing the SUMO E3 ligase PIASy SIM sequence. We observed that the SIM peptides reduced the affinity of the SUMO1 beads to the GST-AMPK recombinant protein (Figure 3D). As a control, we also demonstrated that boiled SUMO1 beads have a lower affinity to AMPK. This affirmed that AMPK does interact with SUMO1 via a SIM.

To further confirm this model, we sought to locate a possible SIM using the consensus motifs predicted by previous studies (Hecker et al., 2006). Various SIMs have been proposed, but the common theme in all the paradigms is the presence of a hydrophobic core that is often flanked by a cluster of negatively charged amino acids (Geiss-Friedlander and Melchior, 2007; Kerscher, 2007). Using the crystal structure depicting the non-covalent recognition of SUMO1 by RanBP2 through β strand interactions (taken from Protein Data Bank [PDB]: 3UIP) as a structural basis, we then searched for potential SIMs in AMPK (Figure 3E). We identified a conserved region in the N-terminal region of AMPK that conforms to the specifications of a SIM proposed by Minty et al. (2000; Figure 3F). Biotinylated wild-type and mutant AMPK SIM peptides were designed and generated (Figure 3G). We then performed a competition assay and observed that the wild-type AMPK SIM peptides competed with GST-AMPK recombinant protein, thereby decreasing the affinity of GST-AMPK to SUMO1 (Figure 3G). Finally, the peptides were conjugated to avidin beads to generate resin for a peptide pull-down assay. HEK293 cell lysates were incubated with the peptide-bound resin and their affinity to LKB1 was assessed. As expected, the binding affinity of the mutant AMPK SIM peptides to endogenous LKB1 was markedly lower than that of the wild-type SIM peptide (Figure 3H). These results confirmed that the predicted AMPK SIM was indeed vital in recognizing SUMO1 and may eventually be important in enhancing LKB1-AMPK interaction.

We further verified this model by mutating both hydrophobic residues (V93 and I94) to alanines in AMPK cDNA and checked their binding affinities to SUMO1 and LKB1. V93 and I94 are mostly solvent exposed, suggesting that their mutation to alanines is unlikely to compromise the 3D fold or stability of the AMPK domain (Figure 3E). We found that the double SIM AMPK mutant (V93I94AA) had a lower affinity to both SUMO1 and LKB1 in AMPKα−/− mouse embryo fibroblast (MEF) cells than wild-type AMPK (Figures 3I and 3J). To prove that this finding is physiologically relevant, we then checked whether this SIM mutant affected AMPK phosphorylation. We noted a consistent decrease in phosphorylation of AMPK when the cells transfected with the AMPK SIM mutant were subjected to energy depletion (Figures 3J and S3D). The single SIM AMPK mutant
(I94A), which we found was important in the activation of AMPK during stress (Figure S3D), decreased the SUMO1-AMPK interaction (Figure S3C), but not with LKB1 (Figure 3J). This indicates that, while I94 is the key hydrophobic residue in the SIM, both V93 and I94 are important in regulating LKB1-AMPK interaction and activation during stress. Together, these data support the hypothesis that LKB1 is modified by SUMO1 at K178 during energy stress, thereby enabling the recognition of AMPK through its SIM, leading to AMPK activation.

**LKB1 K178 SUMOylation Is Essential in the AMPK Signaling Pathway**

We then examined whether AMPK signaling is defective in LKB1-null cells expressing the LKB1 K178R mutant, resulting in...
impaired mitochondrial function, and the induction of apoptosis (Shackelford et al., 2013). LKB1-null A549 cells stably transfected with empty vector (pBABE), wild-type LKB1, LKB1 K178R, or kinase-dead LKB1 (K78I) were treated with vehicle control (DMEM) or with 10 μg/ml CAMKK inhibitor STO-609 alone or with 2 mM phenformin for 48 hr to induce metabolic stress. Cell lysates were subjected to western blot using the indicated antibodies.

(B) Cells stably expressing the indicated plasmids stained with annexinV and 7AAD staining (Figure 4B), showed that the cells expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A).

Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A). Furthermore, through the measurement of intracellular ATP levels, we confirmed that A549 cells stably expressing empty vector, kinase-dead mutant, and the LKB1 K178R mutant were defective in AMPK signaling (Figure 4A).

Expression of the empty vector, kinase-dead LKB1, or LKB1 K178R mutant (Figure 4C). These findings illustrate that LKB1 K178 SUMOylation is essential in the clearance of defective mitochondria, consequently enabling the efficient production of energy during metabolic stress (Melser et al., 2013). To assess the mitochondrial health of these different cells, we also checked the oxygen consumption rates as a measure of mitochondrial respiration. As expected, A549-vector, A549-LKB1 dead-kinase, and A549-LKB1 K178R mutant cells had lower basal oxygen consumption rates than A549 cells transfected with wild-type LKB1 (Figures 4D and S4B). Taken together, these data confirm that SUMO1 modification of LKB1 K178 is indeed important in maintaining cellular energy balance during metabolic stress.

**DISCUSSION**

SUMOylation has been implicated in diverse biological processes, including protein stability (Yeh, 2009), DNA damage response (Dou et al., 2010), and immune system development (Van Nguyen et al., 2012). However, insights into its fundamental role in regulating metabolism are still in the nascent stages.
SUMOylation influences this cellular process and defines the responses to ensure cell survival. Anabolic processes are inhibited, while processes that generate energy are activated (Hardie and Alessi, 2013). AMPK also is involved in regulating mitophagy (Egan et al., 2011). This process facilitates the effective elimination of defective mitochondria, consequently enabling the cell to maintain full mitochondrial fitness and efficient respiration (Melser et al., 2013). Accumulation of malfunctioning mitochondria leads to an increase in levels of reactive oxygen species, thereby initiating apoptosis (Murphy, 2009). This therefore highlights the significance of LKB1-AMPK activation in calibrating the cell’s energy balance and regulating cell survival.

Here we demonstrate that LKB1 is increasingly conjugated by SUMO1 during metabolic stress. The importance of SUMO1 modification of LKB1 was validated when we saw that SENP1 hampers the ability of LKB1 to bind, phosphorylate, and activate AMPK during energy stress. We further illustrated that lysine 178 is the site that is important for SUMO1 conjugation (and not SUMO2/3, acetylation, or ubiquitylation) during metabolic stress. In normal conditions, there is no difference in SUMO1 conjugation of either wild-type LKB1 or the LKB1 K178R mutant. However, during metabolic stress, SUMO1 conjugation markedly increases in the wild-type LKB1, but not in the K178 mutant. These results suggest that LKB1 is modified by SUMO1 at lysine residues other than K178 under normal conditions. In addition, we note that the SUMO1 conjugation of LKB1 at K178 leads to increased binding and activation of AMPK.

A number of studies have characterized the importance of SIMs in dictating protein interactions, thereby influencing their downstream functions (Kerscher, 2007). Our study provides an additional example that illustrates the importance of a SIM in aiding the recognition and activation of AMPK by LKB1 during stress. Using the crystal structure depicting the non-covalent recognition of SUMO1 by RanBP2 as a structural basis, we identified a SIM in the N-terminal region of AMPK. Mutation of the hydrophobic residues necessary for SUMO1 interaction prevents LKB1 from recognizing and activating AMPK. Interestingly, in the AMPK crystal structure produced with a truncation in the carbohydrate-binding module, the C-interacting helix (residues 161–170) of the β subunit (PDB: 4CFH), the kinase N-lobe surface contacting the catalytically important z helix of the kinase domain. In this structure, the z helix is in a cataclysmically inactive conformation, supposedly promoted by the C-interacting helix (Xiao et al., 2013). The C-interacting helix has above-average β factors in the crystal structure, suggesting increased mobility and hence a loose interaction. We therefore speculate that binding of SUMO1 to the SIM also promotes AMPK activation by displacing the C-interacting helix, and, hence, may allosterically activate AMPK by rearranging the z helix, as seen in many other kinases (Endicott et al., 2012). LKB1 also can phosphorylate and activate 12 other AMPK-related kinases (Alessi et al., 2006). Examining whether SIMs are involved in facilitating these interactions is beyond the scope of this study, but will be further investigated.

Taken together, our results support a model in which energy stress triggers SUMO1 modification of LKB1 at K178, leading to activation of its downstream target and energy sensor, AMPK (Figure 4E).

**EXPERIMENTAL PROCEDURES**

**Antibodies and siRNAs**

Antibodies used for Immunoblotting, including LKB1 (D60C5; 3047), phospho-AMPK Thr172 (2531), total AMPKα 1/2 (2532), phospho-Raptor Ser792 (2083), total Raptor (2280), phosphoULK1 (5555; 5869), total ULK1 (8054), and SUMO1 (4930), were obtained from Cell Signaling Technology. Actin (sc-47778) and AMPKα1 (ab32047) antibodies were obtained from Santa Cruz Biotechnology and Abcam, respectively. Nontargeting siRNA (ON-TARGETplus 2, catalog number D-001810-02-20) and SENP1 siRNA (ON-TARGETplus SMARTpool Human SENP1, catalog number L-006357-00-0010) were purchased from Dharmacon.

**Cell Culture and Transfections**

Non-small-cell lung carcinoma A549 cells, cervical cancer HeLa cells, HEK293 cells, Phoenix cells, and AMPKα1/2 MEFs were maintained in DMEM (Thermo Fisher Scientific) containing 10% fetal bovine serum (Thermo Fisher Scientific), penicillin, and streptomycin. The cells were incubated at 37°C at 5% CO2 levels. The AMPKα1/2 MEF cell line was a gift from Dr. Hui-Kuan Lin. Myc-AMPK and catalytic mutant Myc-AMPK (D157A) were gifts from Dr. David Carling, and pBabe empty vector was a gift from Dr. Hui-Kuan Lin. pBabe-FLAG-LKB1 (8592), pBabe-FLAG-LKB1-KD (8593), and pcDNA3-FLAG-LKB1 (8590) were obtained from Addgene.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.07.002.

**AUTHOR CONTRIBUTIONS**

J.R. performed the experiments. S.T.A. conducted the protein structure analysis. J.R. and E.T.H.Y. designed the study and wrote the manuscript.

**ACKNOWLEDGMENTS**

We are grateful to all the members of the E.T.H.Y. laboratory for their support and valuable discussion. We thank Kathryn L. Hale (MD Anderson Cancer Center) for editing the manuscript, Dr. Umadevi Thirumurthi (University of Texas Graduate School of Biological Sciences) for the discussion, and Craig Smith (Seahorse Bioscience) and Dr. Pradip K. Saha (Mouse Metabolism Core, Baylor College of Medicine) for the use of the Seahorse XF24 extracellular flux experimentation.
analyzer supported by BCM Diabetes and Endocrinology Research Center (DERC) grant (P30 DK079638). We also thank Wendy Schober and Nalini Patel at MD Anderson’s Flow Cytometry & Cellular Imaging core facility for their assistance with the data acquisition. (This core is supported in part by the NIH through MD Anderson’s Cancer Center support grant CA016672). This work was supported in part by NIH R01 CA239520 (E.T.H.Y.) and by KAUST (S.T.A.).

Received: February 2, 2015
Revised: May 12, 2015
Accepted: July 1, 2015
Published: July 23, 2015

REFERENCES


