**Supporting information**

**SQSTM1/p62 activates NFE2L2/NRF2 via ULK1-mediated autophagic KEAP1 degradation and protects mouse liver from lipotoxicity**

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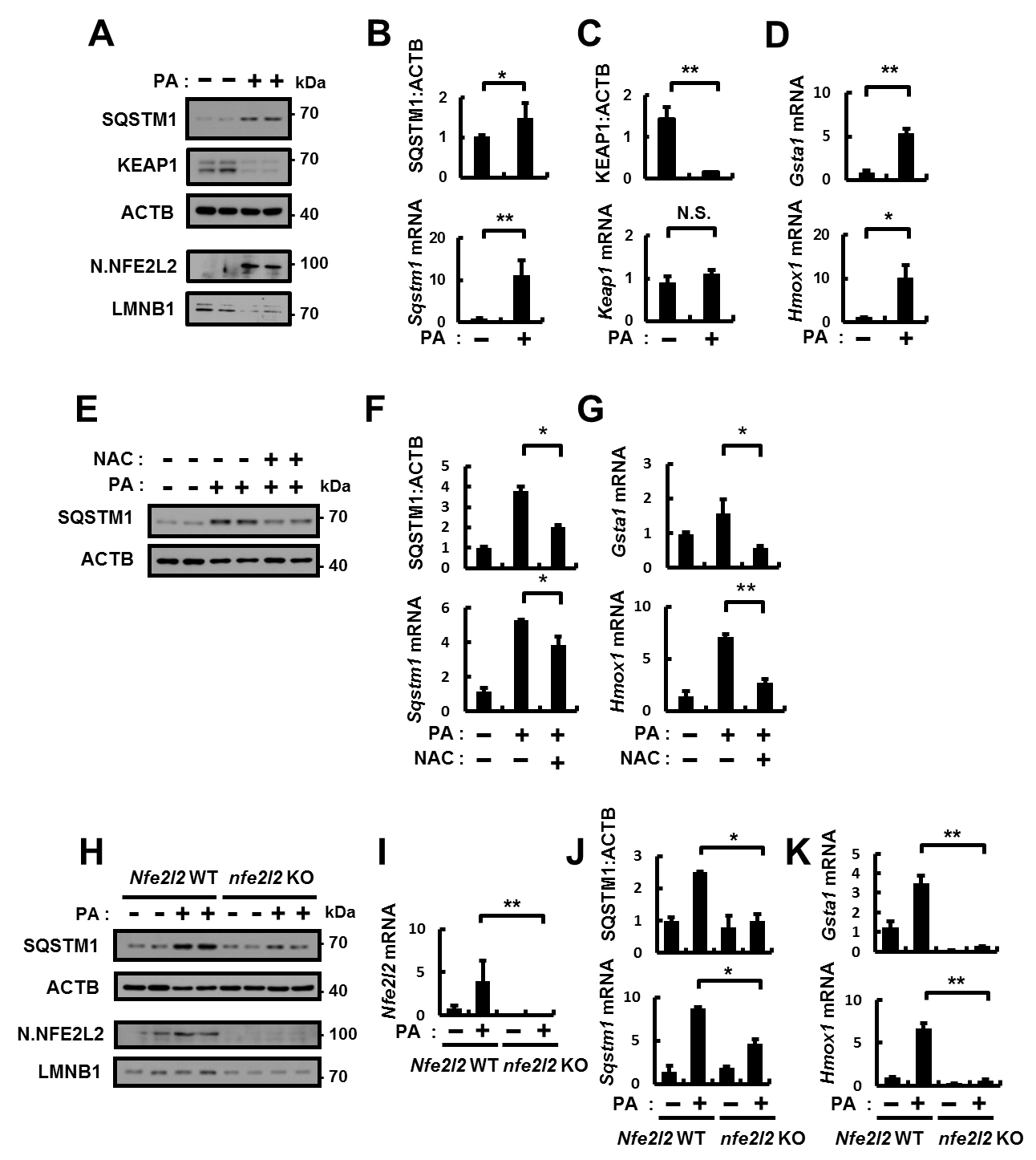
#These authors contributed equally to this work.

**Corresponding author:**

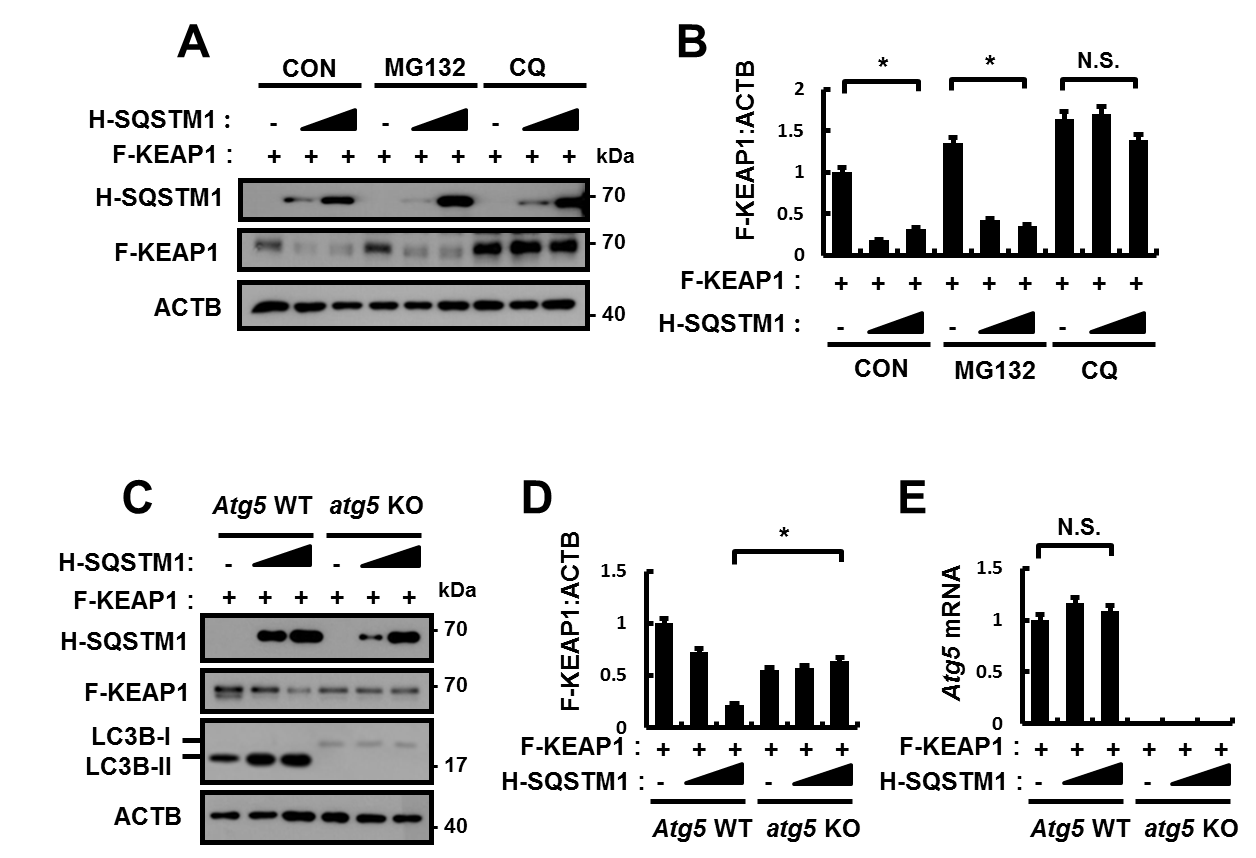
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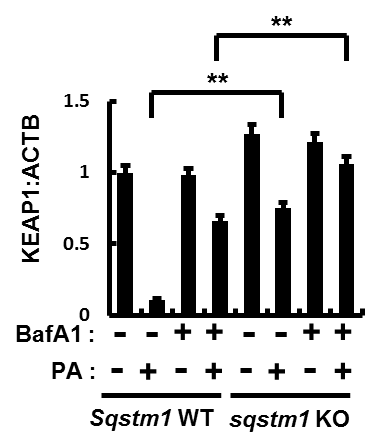
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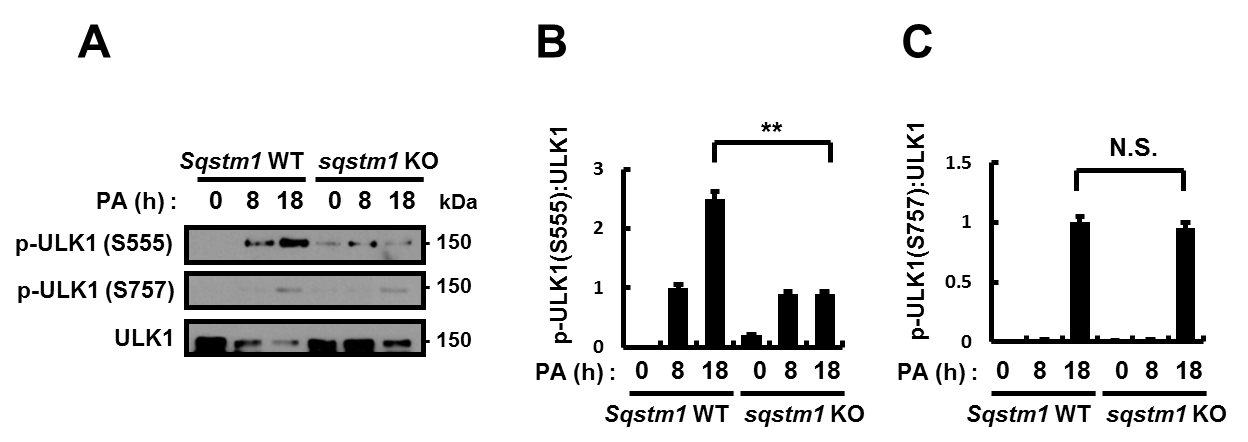
**Figure S1.** SQSTM1 induction is mainly dependent on ROS-NFE2L2 axis under lipotoxic stress. (**A**) Hepa1c1c7 cells were incubated with PA (500 μM) for 18 h. Immunoblot analysis with antibodies against SQSTM1, KEAP1, and ACTB (loading control), nuclear NFE2L2, and LMNB1 (nuclear marker). (**B-D**) The densitometric analysis of SQSTM1 (B) and KEAP1 (C) immunoblots. Total mRNA isolation from cells were treated as described in (A) and subjected to qRT-PCR analysis for relative mRNAexpression of *Sqstm1* (B), *Keap1* (C)*,* and *Gsta1, Hmox1* (D). (**E**) Hepa1c1c7 cells were incubated with PA (500 μM) and NAC (5 mM) for 18 h. Immunoblot analysis with antibodies against SQSTM1 and ACTB (loading control). (**F**) The densitometric analysis of SQSTM1 immunoblots and relative mRNAexpression of *Sqstm1*. (**G**) qRT-PCR analysis for relative mRNAexpression of *Gsta1* and *Hmox1*. (**H**) *Nfe2l2* WT or *nfe2l2*KO MEFs were incubated with PA (500 μM) for 18 h. Immunoblot analysis with antibodies against SQSTM1, ACTB (loading control), nuclear (N) NFE2L2, and LMNB1 (nuclear marker). (**I**) qRT-PCR analysis for relative mRNAexpression of *Nfe2l2*. (**J**) The densitometric analysis of SQSTM1 immunoblots and relative mRNAexpression of *Sqstm1*. (**K**) qRT-PCR analysis for relative mRNAexpression of *Gsta1* and *Hmox1*. Data are mean ± SD from 3 independent experiments. *\*p* < 0.05 and *\*\*p* < 0.01. Related to Figure 1.

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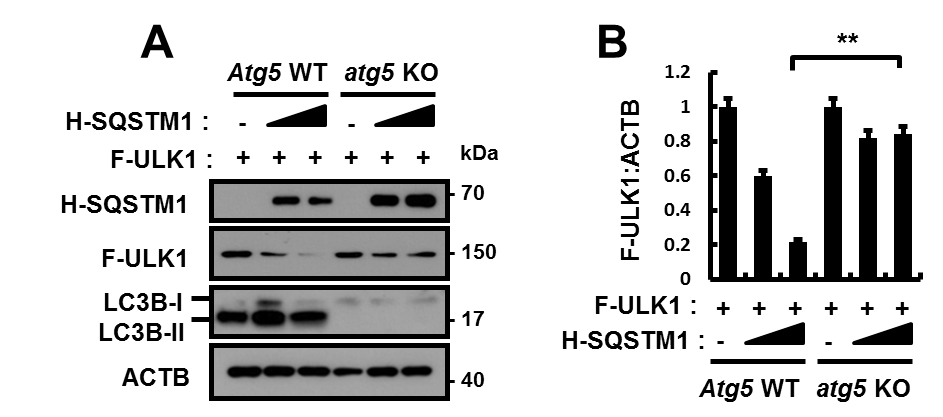
**Figure S2.** SQSTM1-mediated KEAP1 degradation is mainly dependent on autophagy. (**A**) HEK293 cells were transfected with vectors of HA (H)-SQSTM1 and FLAG (F)-KEAP1, and incubated with or without DMSO, MG132, and CQ. Lysates of the cells were subjected to immunoblot analysis with antibodies against HA, FLAG, and ACTB (loading control). (**B**) Densitometric analysis was obtained. (**C**) *Atg5* WT or *atg5* KO MEFs were transfected with vectors of HA-SQSTM1 and FLAG-KEAP1. Lysates of the cells were subjected to immunoblot analysis with antibodies against HA, FLAG, LC3B, and ACTB (loading control). (**D**) Densitometric analysis was obtained. (**E**) qRT-PCR analysis for relative mRNAexpression of *Atg5.* Data are means ± SDs from 3 independent experiments. *\*p <* 0.05 and N.S., notsignificant. Related to Figure 1.



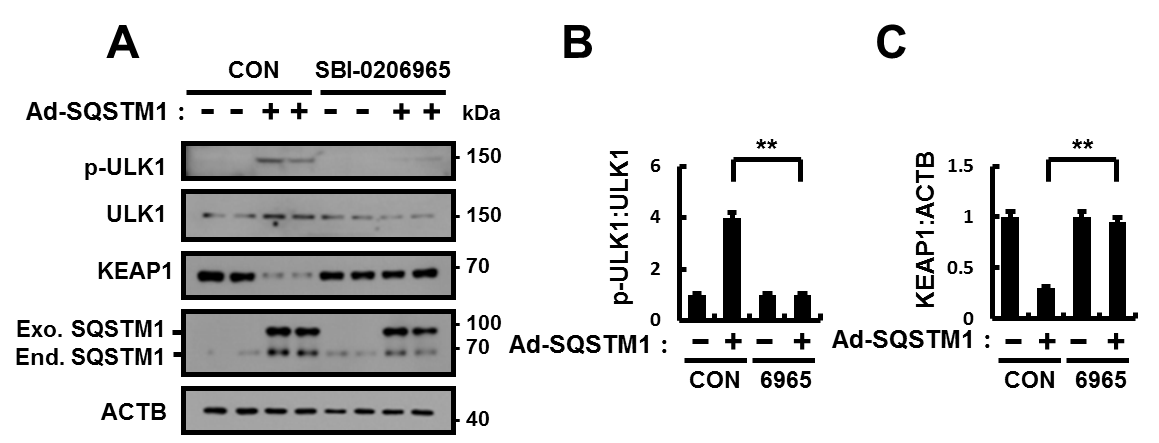
**Figure S3.** SQSTM1 is required for autophagic KEAP1 degradation. The densitometric analysis of KEAP1 immunoblot. Data are means ± SDs from 3 independent experiments. *\*\*p <* 0.01. Related to Figure 1D.

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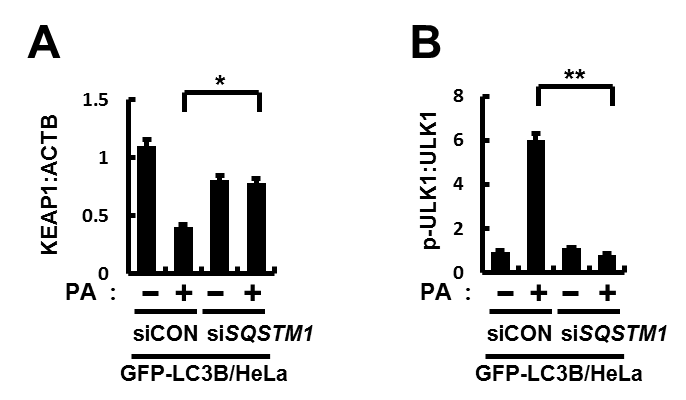
**Figure S4.** The phosphorylation sites of ULK1 in response to PA.(**A**)*Sqstm1* WT or *sqstm1* KOMEFs were incubated with PA (500 μM) for indicated times and subjected to immunoblot analysis with antibodies against p-ULK1 (S555), p-ULK1 (S757), and ULK1. (**B-C**) Densitometric analysis of p-ULK1 (S555):ULK1 (B), and p-ULK1 (S757):ULK1 (C). Data are means ± SDs from 3 independent experiments. *\*\*p <* 0.01 and N.S., notsignificant. Related to Figure 2.

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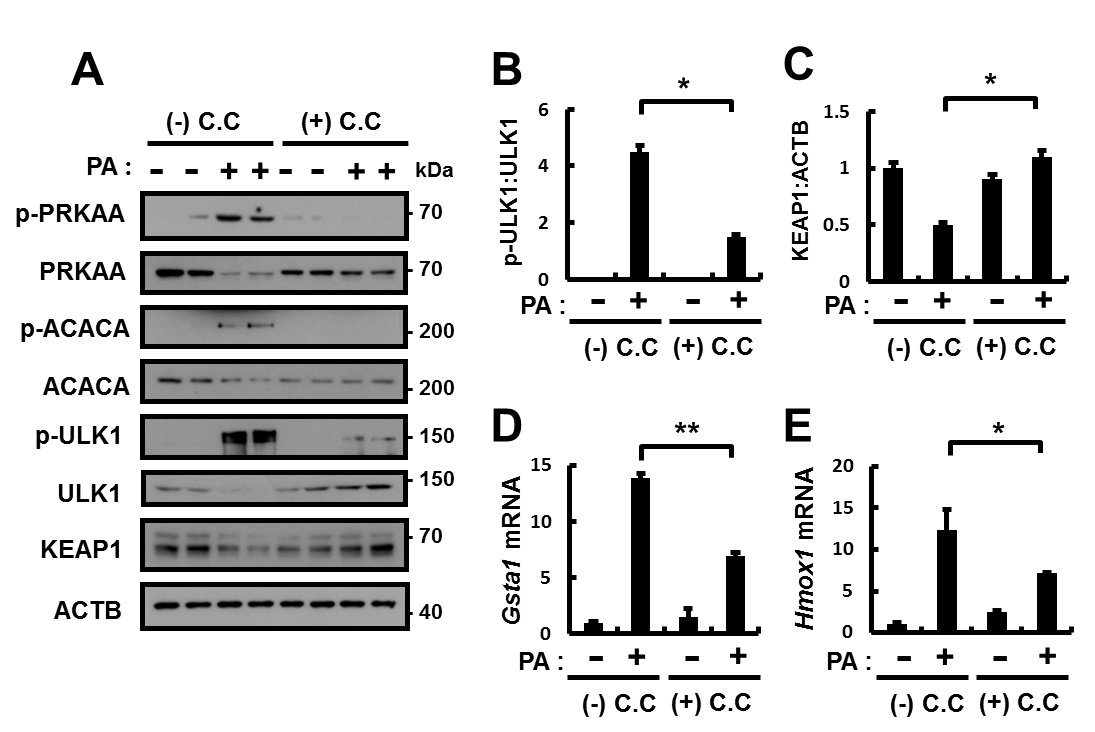
**Figure S5.** SQSTM1-mediated ULK1 degradation is mainly dependent on autophagy.(**A**)*Atg5* WT or *atg5* KO MEFs were transfected with vectors of HA (H)-SQSTM1 and FLAG (F)-ULK1. Lysates of the cells were subjected to immunoblot analysis with antibodies against HA, FLAG, LC3B, and ACTB (loading control). (**B**) Densitometric analysis was obtained. Data are means ± SDs from 3 independent experiments. *\*\*p <* 0.01. Related to Figure 2.

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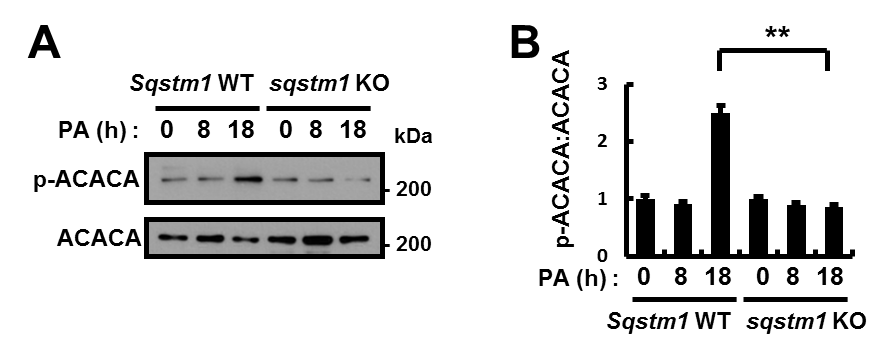
**Figure S6.** ULK1 is required for SQSTM1-induced autophagic KEAP1 degradation. (**A**)Hepa1c1c7 cells were treated with SBI-0206965 (ULK1 inhibitor), and infected with Ad-SQSTM1 for 18 h. The lysates from cells subjected to immunoblot analysis with antibodies against p-ULK1 (S317), ULK1, KEAP1, SQSTM1, and ACTB (loading control). (**B-C**) Densitometric analysis of p-ULK1:ULK1 (B) and KEAP1 (C) immunoblots. Data are means ± SDs from 3 independent experiments. *\*\*p <* 0.01. Related to Figure 2.



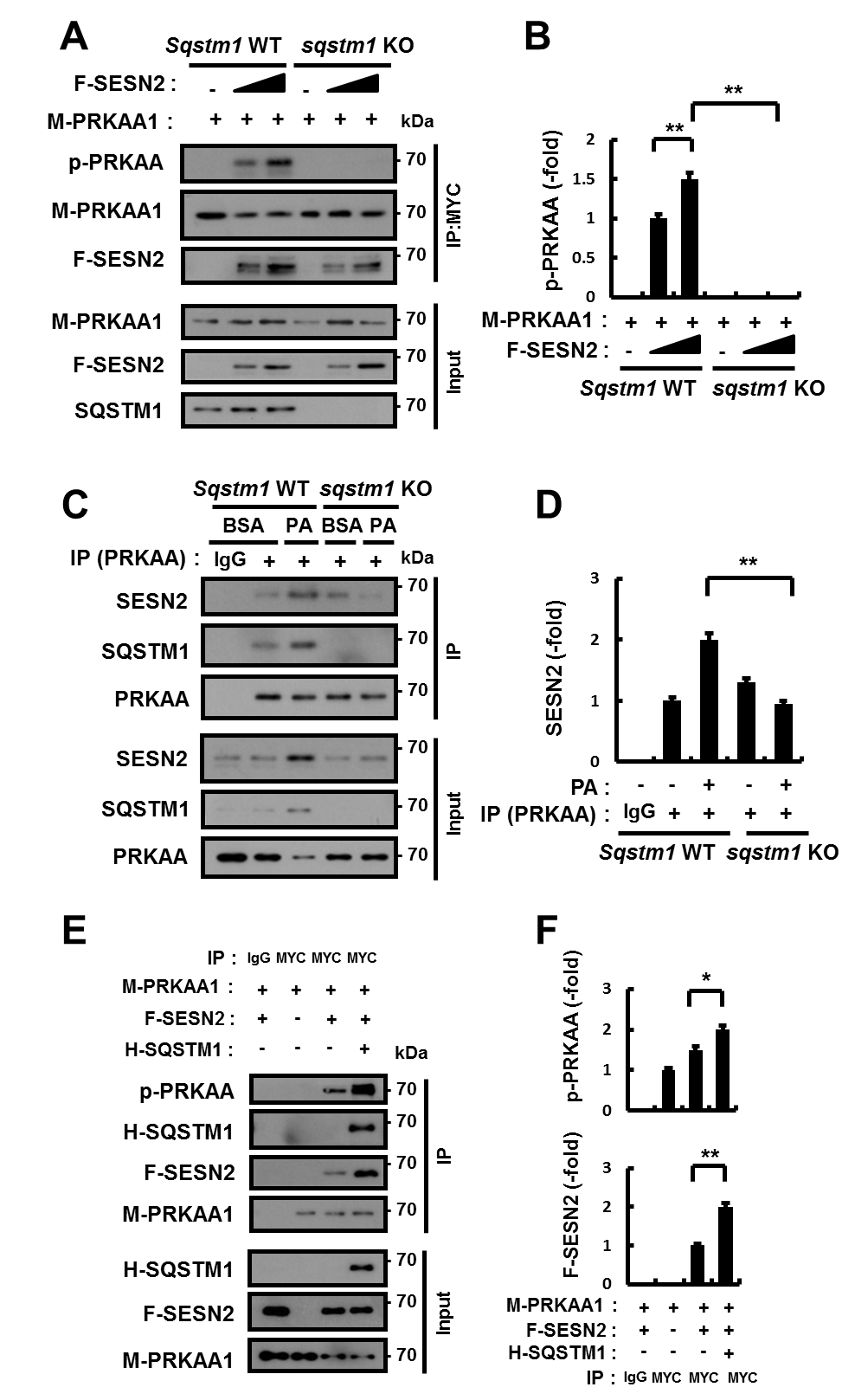
**Figure S7.** SQSTM1 activates autophagy in response to lipotoxicity. (**A-B**) Densitometric analysis of KEAP1 (A) and p-ULK1:ULK1 (B) immunoblots. Data are means ± SDs from 3 independent experiments. *\*p <* 0.05 and *\*\*p <* 0.01. Related to Figure 2L.

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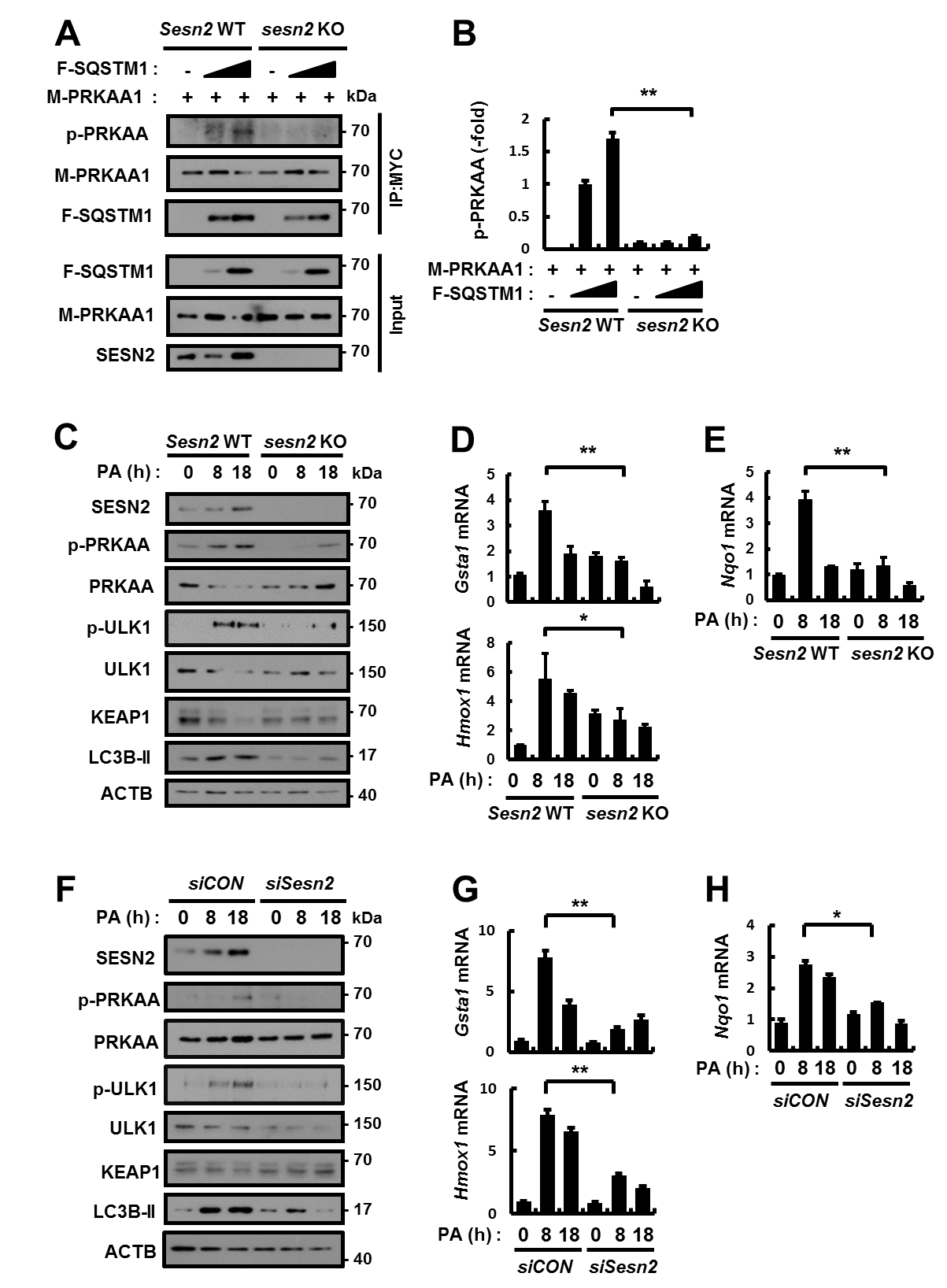
**Figure S8.** Palmitica acid (PA)-mediated AMPK-ULK1 axis induces autophagic KEAP1 degradation and NFE2L2 activation. Hepa1c1c7 cells were treated with compound C (C.C, AMPK inhibitor), and incubated with PA (500 μM) for 18 h. (**A**)The lysates from cells subjected to immunoblot analysis with antibodies against p-PRKAA (T172), PRKAA, p-ACACA (S79), ACACA, p-ULK1 (S317), ULK1, KEAP1, and ACTB (loading control). (**B-C**) Densitometric analysis of p-ULK1:ULK1 (B) and KEAP1 (C) immunoblots. (**D-E**) qRT-PCR analysis for relative mRNAexpression of *Gsta1* (D) and *Hmox1* (E). Data are means ± SDs from 3 independent experiments. *\*p <* 0.05 and *\*\*p <* 0.01. Related to Figure 5.



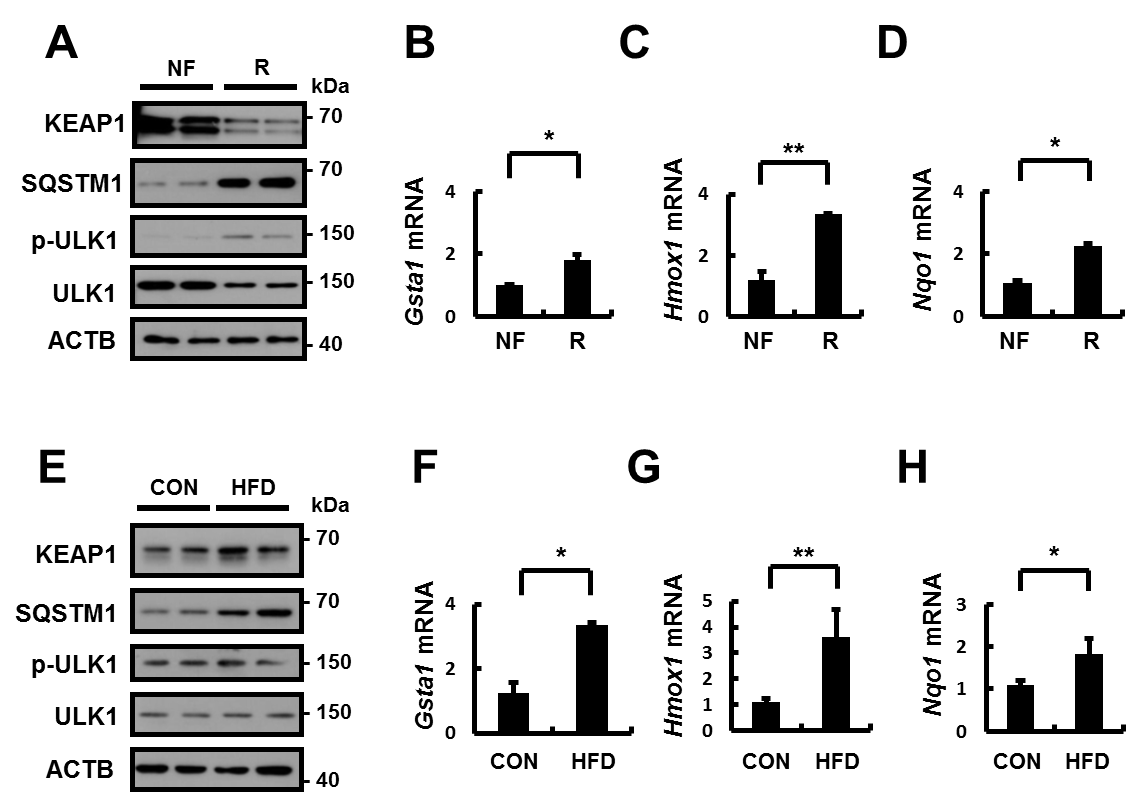
**Figure S9.** SQSTM1 is required for AMPK activation. (**A**) Immunoblot analysis with antibodies against p-ACACA (S79) and ACACA. (**B**) The densitometric analysis of p-ACACA:ACACA immunoblot. Data are means ± SDs from 3 independent experiments. *\*\*p <* 0.01. Related to Figure 2A.



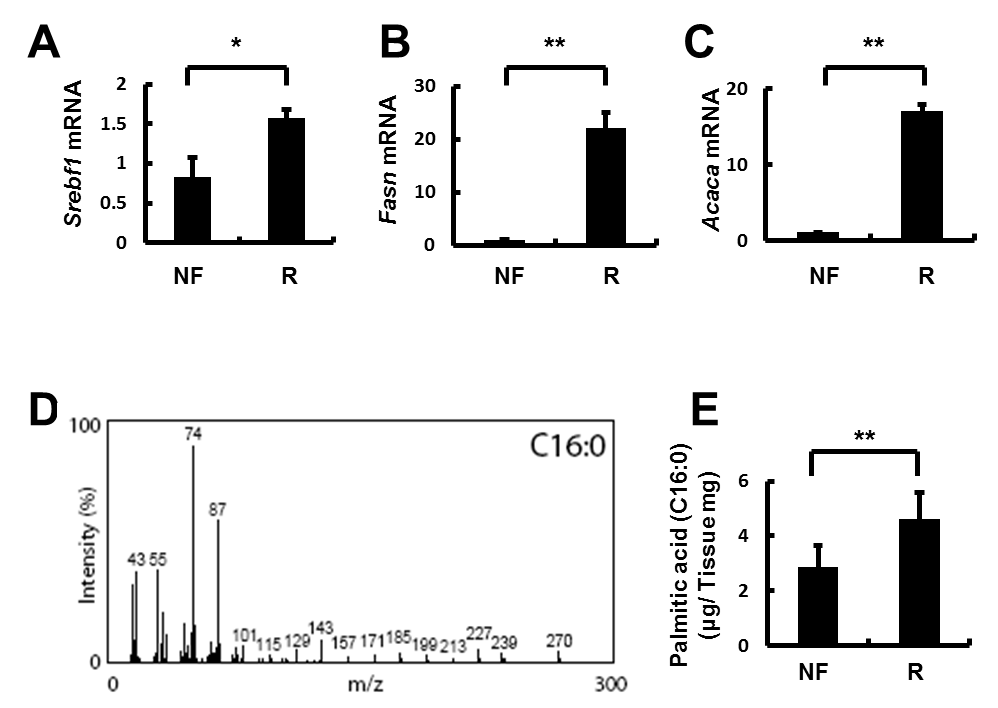
**Figure S10.** SQSTM1 mediated AMPK activation dependent on SESN2 (sestrin 2). (**A**) *Sqstm1* WT or *sqstm1* KO MEF cells were transfected with vectors of FLAG (F)-SESN2 and MYC-PRKAA1/AMPKα1 (M-PRKAA1). Lysates of the cells were subjected to immunoblot analysis with antibodies against p-PRKAA (T172), MYC, FLAG, and SQSTM1. (**B**) Densitometric analysis was obtained. (**C**) *Sqstm1* WT or *sqstm1* KO MEFs were treated with PA (500 μM) for 18 h, and subjected to immunoprecipitation with antibodies against PRKAA, and the resulting precipitates (IPs) were subjected to immunoblot analysis with antibodies against SESN2, SQSTM1, and PRKAA. (**D**) Densitometric analysis was obtained. (**E**) Lysates from HEK293 cells transfected with vectors encoding MYC-PRKAA1/AMPKα1 and FLAG-SESN2 together with expressing HA (H)-SQSTM1 were subjected to immunoprecipitation with antibodies against MYC, and the resulting precipitates (IPs) as well as whole cell lysates (WCLs) were subjected to immunoblot analysis with antibodies against p-PRKAA (T172), HA, FLAG, and MYC. (**F**) Densitometric analysis was obtained. Data are means ± SDs from 3 independent experiments. *\*p <* 0.05 and *\*\*p <* 0.01. Related to Figure 5 and Figure 6.

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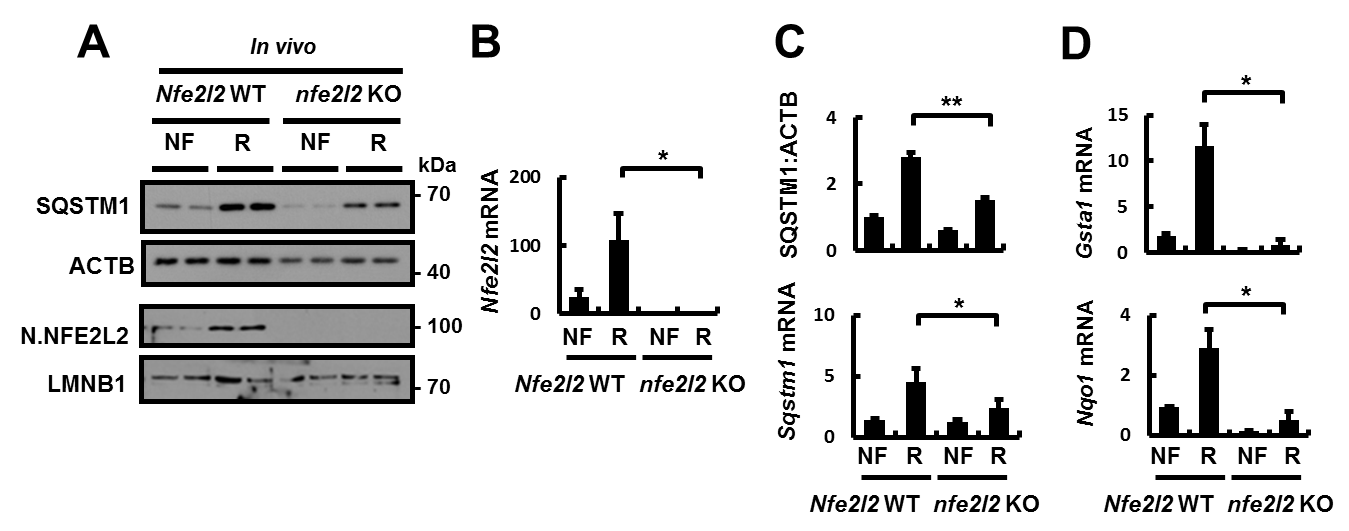
**Figure S11.** SQSTM1 mediated AMPK activation dependent on SESN2 (sestrin 2) in response to palmitic acid (PA). (**A**) *Sesn2* WT or *sesn2* KOMEFs transfected with vectors encoding FLAG (F)-SQSTM1 and MYC-PRKAA1/AMPKα1 (M-PRKAA1) were subjected cell lysates to co-immunoprecipitation with antibodies against MYC, and the resulting precipitates (IPs) as well as whole cell lysates (WCLs) were subjected to immunoblot analysis with antibodies against p-PRKAA (T172), MYC, FLAG, and SESN2. (**B**) Densitometric analysis was obtained. (**C**) *Sesn2* WT or *sesn2* KOMEFs were incubated with PA (500 μM) for indicated times and subjected to immunoblot analysis with antibodies against SESN2, p-PRKAA (T172), PRKAA, p-ULK1 (S317), ULK1, KEAP1, LC3B, and ACTB (loading control). (**D-E**) Total mRNA isolation from cells were treated as described in (C) and subjected to qRT-PCR analysis for relative mRNAexpression of *Gsta1, Hmox1* (D), and *Nqo1* (E). (**F**) Hepa1c1c7 cells transfected with control siRNA (siCON) or *Sesn2* siRNA and treated with PA for 18 h and subjected to immunoblot analysis with antibodies against SESN2, p-PRKAA (T172), PRKAA, p-ULK1 (S317), ULK1, KEAP1, LC3B, and ACTB (loading control). (**G-H**) Total mRNA isolation from cells were treated as described in (F) and subjected to qRT-PCR analysis for relative mRNAexpression of *Gsta1, Hmox1* (G), and *Nqo1* (H). Data are means ± SDs from 3 independent experiments. *\*p <* 0.05 and *\*\*p <* 0.01. Related to Figure 5 and Figure 6.



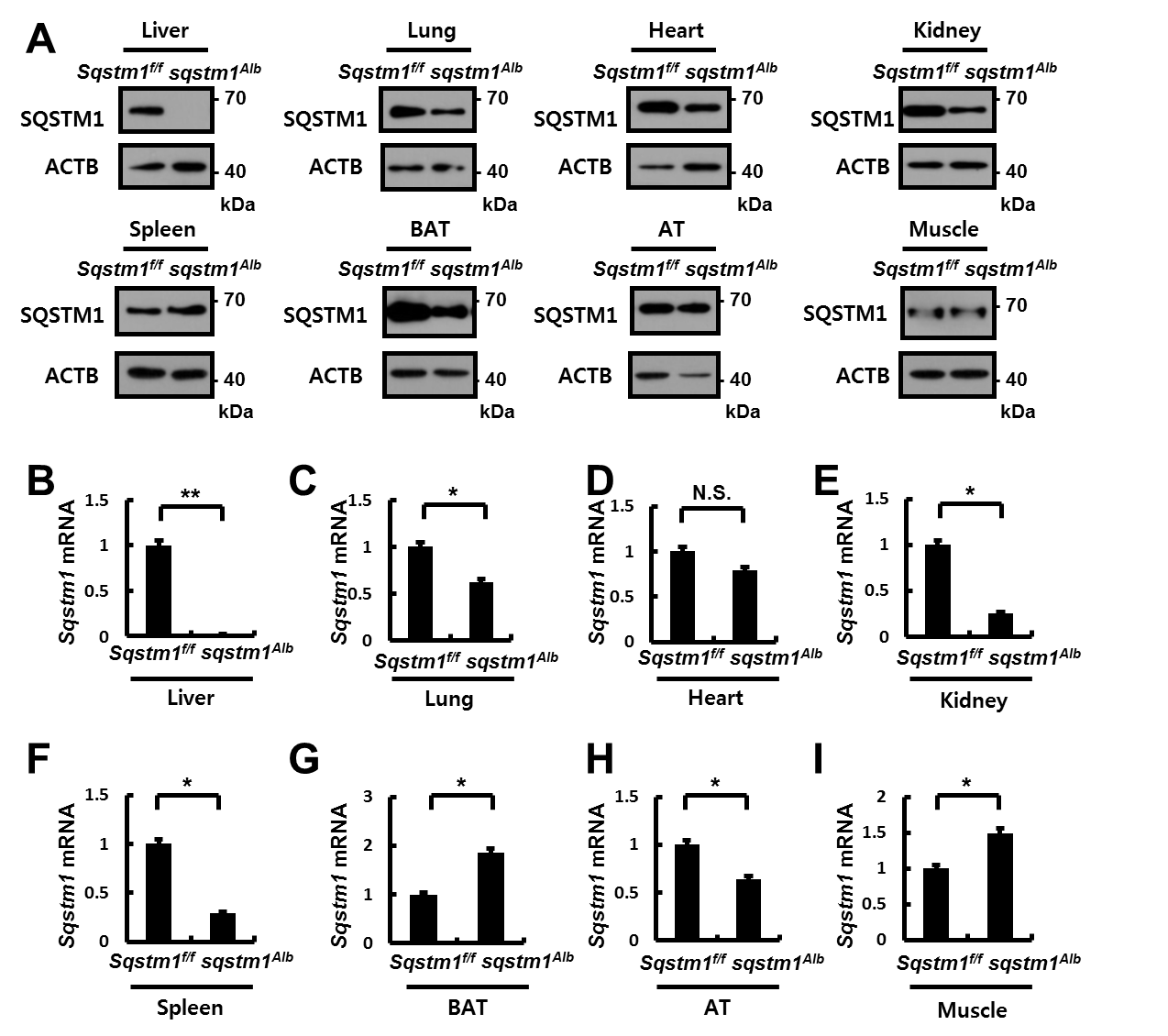
**Figure S12.** The regulation of SQSTM1-KEAP1-NFE2L2 pathway under acute and chronic lipotoxic stress in mouse liver. C57BL/6Jmice were maintained in a non-fasted state (NF) or fasted overnight and then re-fed a high-carbohydrate, fat-free diet (R). These animals were randomly assigned to 2 groups (8-9 mice in each group). (**A**) Immunoblot analysis of KEAP1, SQSTM1, p-ULK1 (S317), ULK1, and ACTB (loading control). (**B-D**) qRT-PCR analysis for relative mRNAexpression of *Gsta1* (B), *Hmox1* (C), and *Nqo1* (D). C57BL/6Jmice were maintained in chow diet (CON) or high-fat diet (HFD). These animals were randomly assigned to 2 groups (8-9 mice in each group). (**E**) Immunoblot analysis of KEAP1, SQSTM1, p-ULK1 (S317), ULK1, and ACTB (loading control). (**F-H**) qRT-PCR analysis for relative mRNAexpression of *Gsta1* (F), *Hmox1* (G), and *Nqo1* (H). Data are means ± standard errors for 8 or 9 mice per group. \*p < 0.05 and \*\*p < 0.01. Related to Figure 8.

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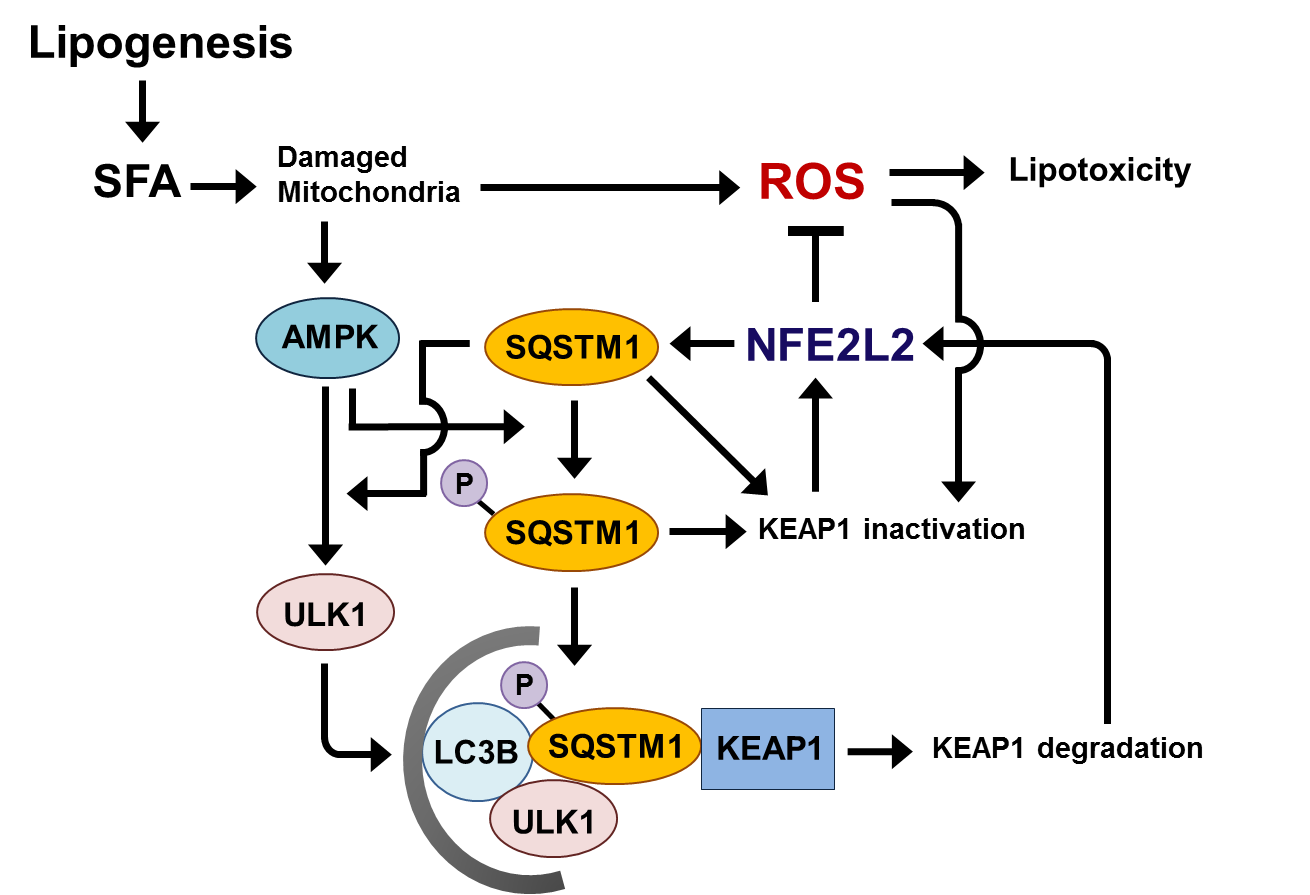
**Figure S13.** The increase of palmitic acid under acute lipotoxic stress in mouse liver. C57BL/6Jmice were maintained in a non-fasted state (NF) or fasted overnight and then re-fed a high-carbohydrate, fat-free diet (R). These animals were randomly assigned to 2 groups (8-9 mice in each group). (**A-C**) qRT-PCR analysis for relative mRNAexpression of mRNAs of *Srebf1* (A), *Fasn* (B), and *Acaca* (C). (**D**) Retention index (RI) was measured using alkane mixture (C7-C40, Sigma-Aldrich) and NIST mass spectral library (NIST08) was compared with the spectrum of each peak. Peak area of fatty acids was integrated using total ion chromatogram and normalized based on that of the internal standard. (**E**) The livers of 8 or 9 mice of each group were homogenized, respectively. Lipid of the homogenates were subjected to hydrolysis and acidic transmethylation, and then detected by GC-MS. The Amount of fatty acids containing palmitic acid is expressed per mg of liver. Data are means ± Standard error for 8 or 9 mice of each group. *\*p <* 0.05 and *\*\*p <* 0.01. Related to Figure 8.



**Figure S14.** SQSTM1 induction is mainly dependent on ROS-NFE2L2 axis under lipotoxic stress in the mouse liver. *Nfe2l2* WT or *nfe2l2*KO mice were maintained in a non-fasted state (NF) or fasted overnight and then re-fed a high-carbohydrate, fat-free diet (R). These animals were randomly assigned to 4 groups (3-4 mice in each group). (**A**) Immunoblot analysis with antibodies against SQSTM1, ACTB (loading control), nuclear NFE2L2, and LMNB1 (nuclear marker). (**B**) qRT-PCR analysis for relative mRNAexpression of *Nfe2l2*. (**C**) The densitometric analysis of SQSTM1 immunoblot and relative mRNA expression of *Sqstm1.* (**D**) qRT-PCR analysis for relative mRNAexpression of *Gsta1* and *Nqo1*. Data are means ± standard errors for 3 or 4 mice per group. *\*p* < 0.05 and *\*\*p* < 0.01. Related to Figure 1.



**Figure S15.** Generation of liver-specific *sqstm1* KO mice. Liver-specific *sqstm1* KO mice (*sqstm1Alb* mice) were generated by crossing *Sqstm1*-floxed mice (*Sqstm1f/f*mice) with *Alb-Cre* transgenic mice. These animals were randomly assigned to 2 groups (3 mice in each group). (**A**) Immunoblot analysis of SQSTM1 and ACTB (loading control) from various organs. (**B-I**) qRT-PCR analysis for relative mRNAexpression of *Sqstm1* in liver (B), lung (C) , heart (D) , kidney (E) , spleen (F) , brown adipose tissue (BAT) (G) , adipose tissue (AT) (H), and muscle (I). Data are means ± standard errors for 3 mice per group. *\*p* < 0.05, *\*\*p* < 0.01, and N.S., notsignificant. Related to Figure 12.



**Figure S16.** Underlying mechanism of hepatoprotective role of SQSTM1 against lipotoxicity through AMPK-ULK1-mediated activation of noncanonical KEAP1-NFE2L2 pathway.See the text for details.