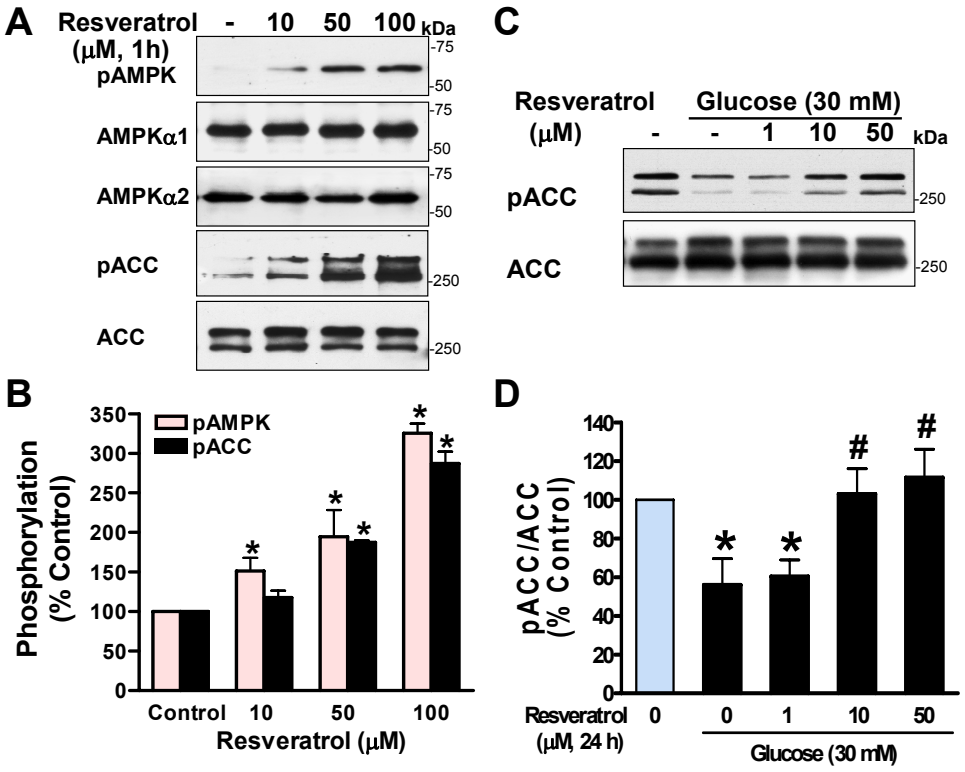
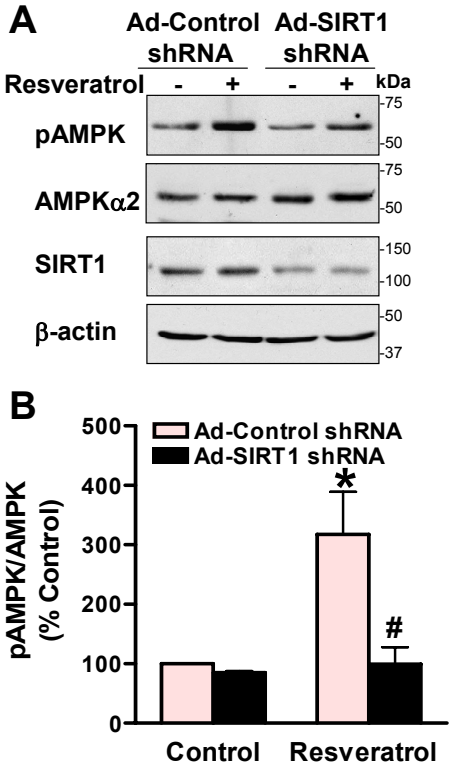
Supplemental Figure Legends

Supplemental Fig. 1. Pharmacological activation by resveratrol stimulates AMPK signaling in a dose-dependent manner in HepG2 cells. A. Activation of SIRT1 by resveratrol dose-dependently stimulates AMPK and ACC phosphorylation. Human HepG2 hepatocytes were quiesced in serum-free DMEM overnight and incubated with resveratrol (10-100 μM) for 1 h. Representative immunoblotting analysis with antibodies against AMPKα phosphorylated at Thr172 (pAMPK) and ACC phosphorylated at Ser79 in ACC1 (Ser221 in ACC2, pACC), and total AMPKα1 or α2 and ACC for loading controls, respectively, is shown. **B.** Densitometric quantification of the phosphorylation of AMPK and ACC is shown. The levels of phosphorylated AMPK or ACC (two bands) were normalized to the levels of total AMPKα or ACC (two bands), respectively, and expressed as relative phosphorylation. **C** and **D**. Resveratrol prevents the inhibition of ACC phosphorylation caused by high glucose. *P<0.05 vs normal glucose; $^{\#}$P<0.05 vs high glucose alone, mean ± S.E. (n=3).

Supplemental Fig. 2. Adenovirus-mediated knockdown of SIRT1 inhibits resveratrol-induced AMPK phosphorylation in HepG2 Cells. HepG2 cells were infected with adenovirus expressing either control shRNA or *SIRT1* shRNA, followed by treatment with resveratrol (50 μM, 1 h). **A**. Representative immunoblots for suppression of endogenous SIRT1 and phosphorylation of AMPK are shown. **B.** Knockdown of SIRT1 by Ad-SIRT1 shRNA markedly attenuates resveratrol-stimulated AMPK phosphorylation. *P<0.05 vs untreatment in cells expressing control shRNA, $^{\#}$P<0.05 vs resveratrol treatment in cells expressing control shRNA, mean ± S.E. (n=3).

Supplemental Fig. 3. Polyphenols-stimulated LKB1/AMPK signaling is independent of CaMKKβ. A-B. Pharmacological inhibition of CaMKK by STO-609 reduces the basal AMPK but does not affect LKB-mediated activation of AMPK in the absence and presence of resveratrol in HeLa cells. Cells expressing either Ad-GFP or Ad-FLAG-LKB1 were pretreated without or with STO-609 (20 μM) for 30 min and treated without or with resveratrol (50 μM) for an additional 1 h as indicated. Immunoblots show the endogenous CaMKKβ (~65 kDa) in HeLa cells and no significant change in the expression of SIRT1, CaMKKβ and AMPK throughout treatments. *P<0.05, vs Ad-GFP alone; $^{\#}$P<0.05, vs Ad-FLAG-LKB1 alone, mean ± S.E. (n=3). **C-E.** Increased phosphorylation of LKB1 and AMPK in response to polyphenols is not significantly affected by STO-609 in HepG2 cells that may express LKB1, CaMKKα and CaMKKβ. Phosphorylation of LKB1, AMPK and ACC was shown in cells preincubated with STO-609 (10 μM) for 30 min and then treated with S17834 (10 μM) for an additional 1 h as indicated. *P<0.05, vs control; $^{\#}$P<0.05, vs STO-609 alone, mean ± S.E. (n=3).


A
Resveratrol (μM, 1h)
- 10 50 100 kDa
pAMPK
AMPKα1
AMPKα2
pACC
ACC
75
50
250
B
Phosphorylation (% Control)
pAMPK
pACC
Control 10 50 100
Resveratrol (μM)
C
Resveratrol (μM)
Glucose (30 mM)
- - 1 10 50 kDa
pACC
ACC
250
D
pACC/ACC (% Control)
Resveratrol (μM, 24 h)
0 0 1 10 50
Glucose (30 mM)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Ad-SIRT1 shRNA
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AMPKα2
SIRT1
β-actin
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50
75
50
150
100
50
37
B
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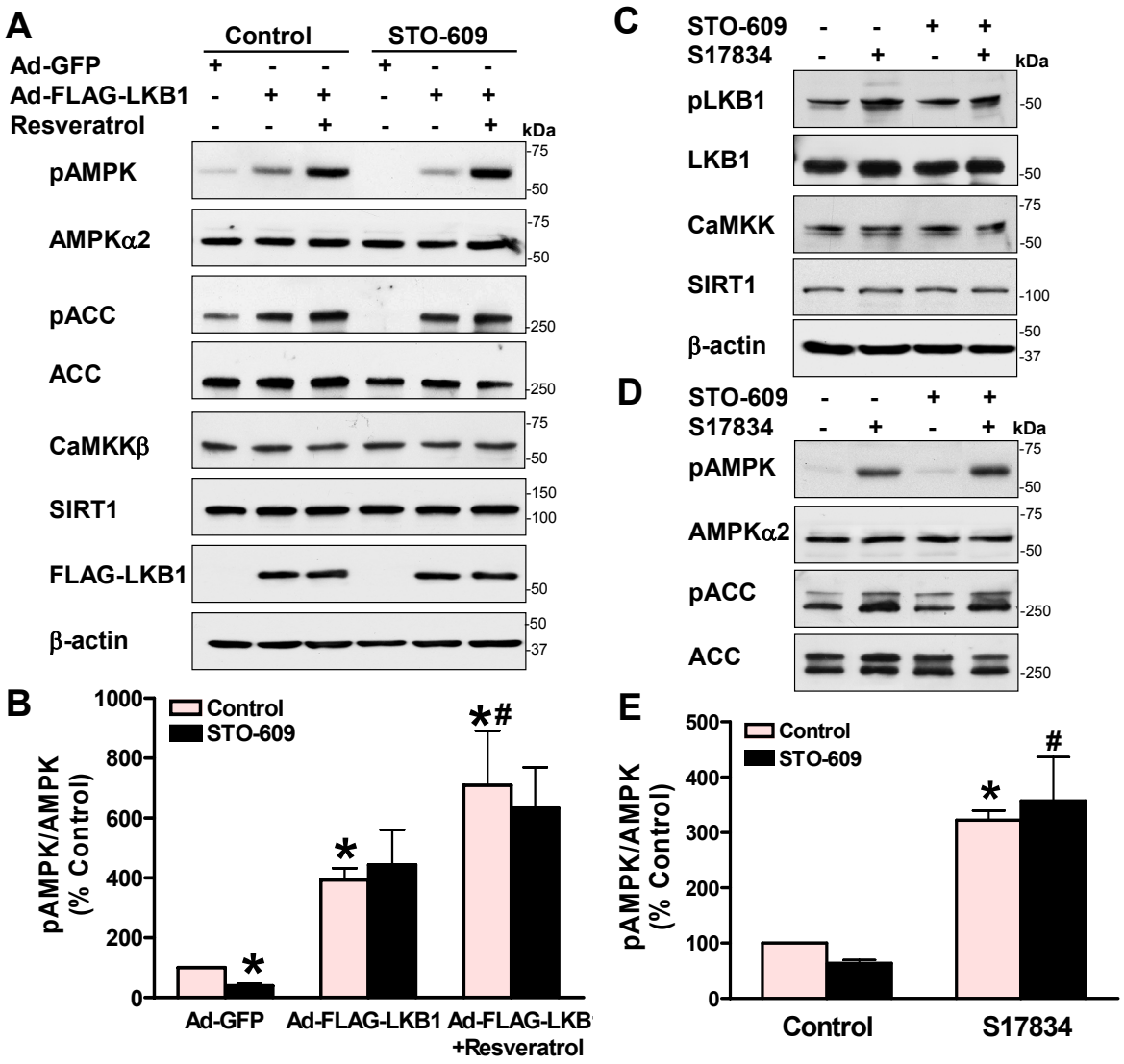
Ad-SIRT1 shRNA
pAMPK/AMPK (% Control)
500
400
300
200
100
0
*
#
Control
Resveratrol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Ad-FLAG-LKB1
Resveratrol
kDa
pAMPK
AMPKα2
pACC
ACC
CaMKKβ
SIRT1
FLAG-LKB1
β-actin
B
Control
STO-609
pAMPK/AMPK (% Control)
Ad-GFP
Ad-FLAG-LKB1
Ad-FLAG-LKB +Resveratrol
C
STO-609
S17834
pLKB1
LKB1
CaMKK
SIRT1
β-actin
D
STO-609
S17834
pAMPK
AMPKα2
pACC
ACC
E
Control
STO-609
pAMPK/AMPK (% Control)
Control
S17834