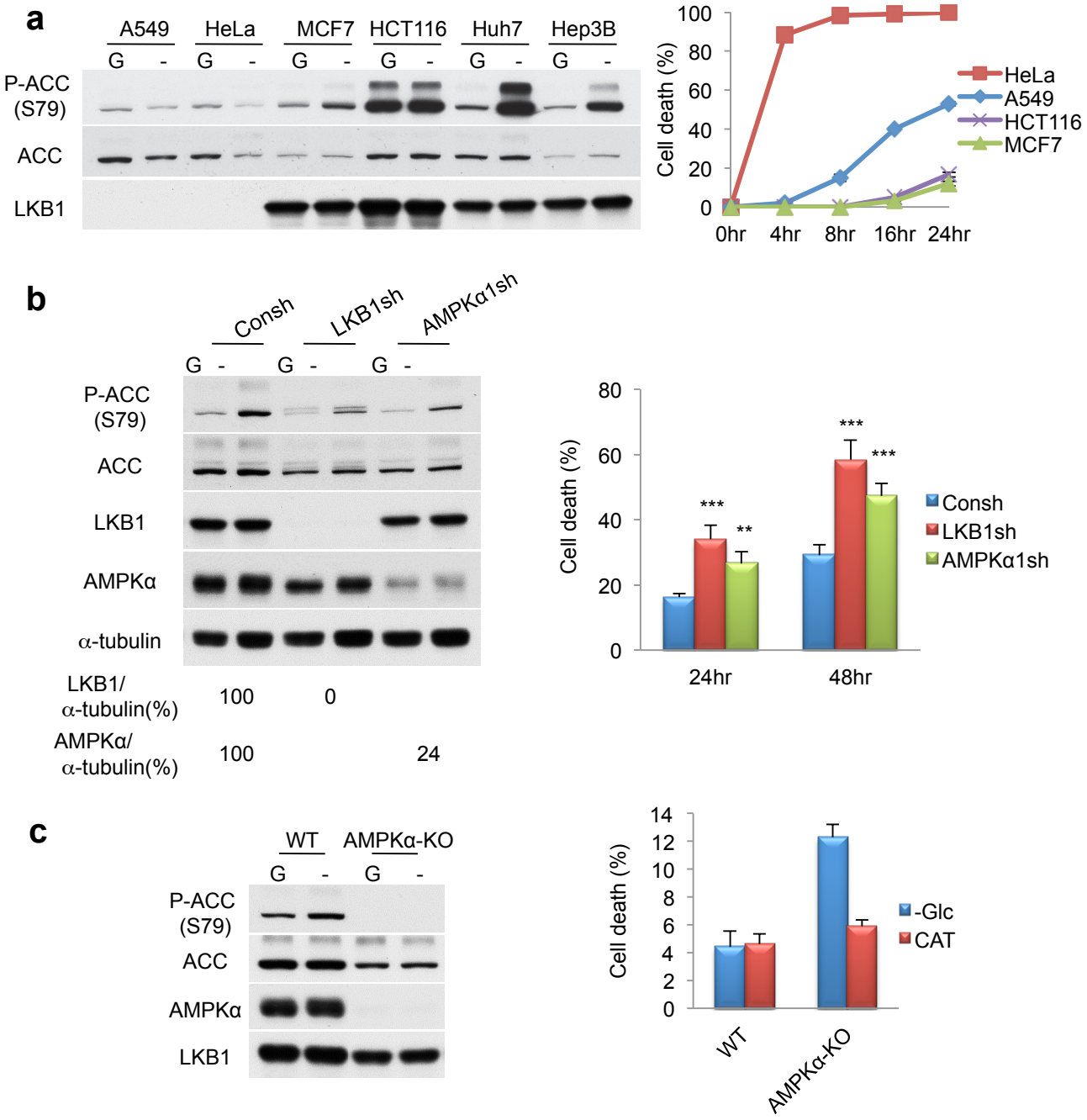
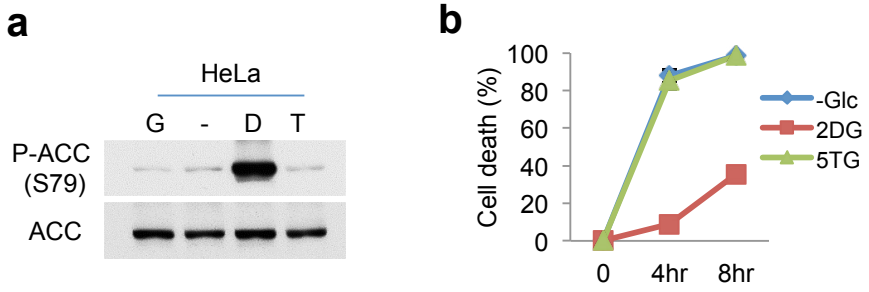
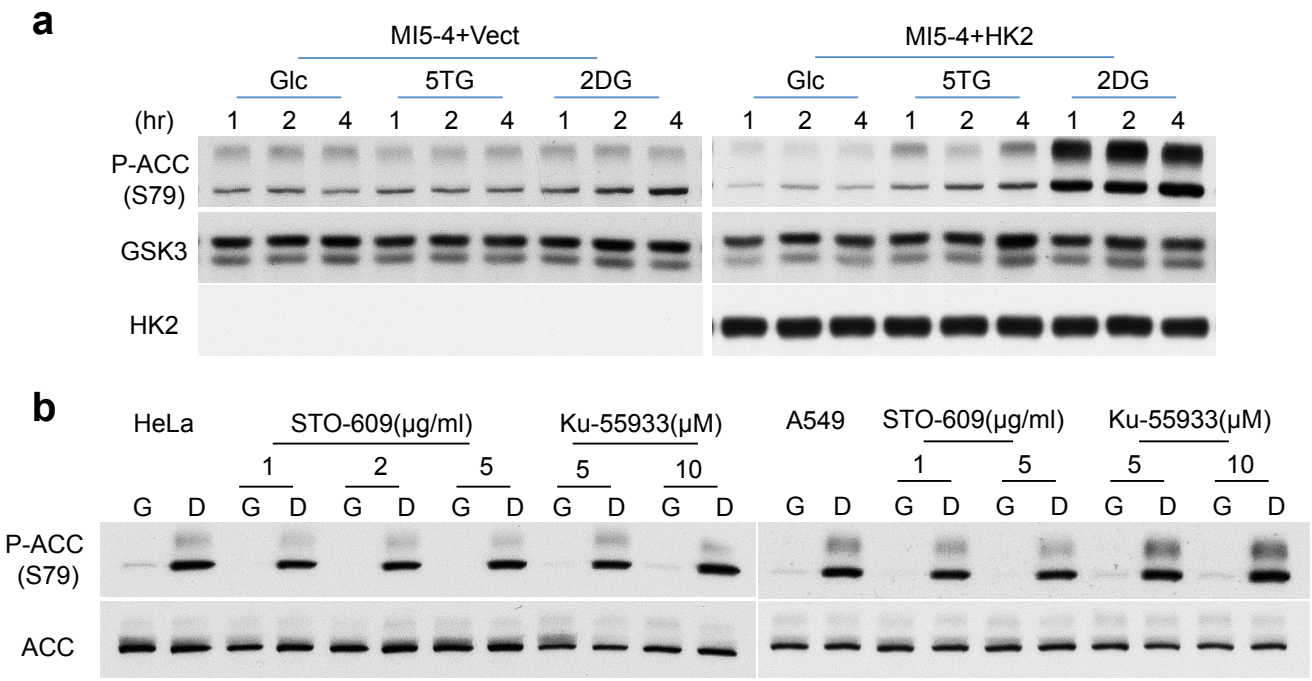
doi:10.1038/nature11066



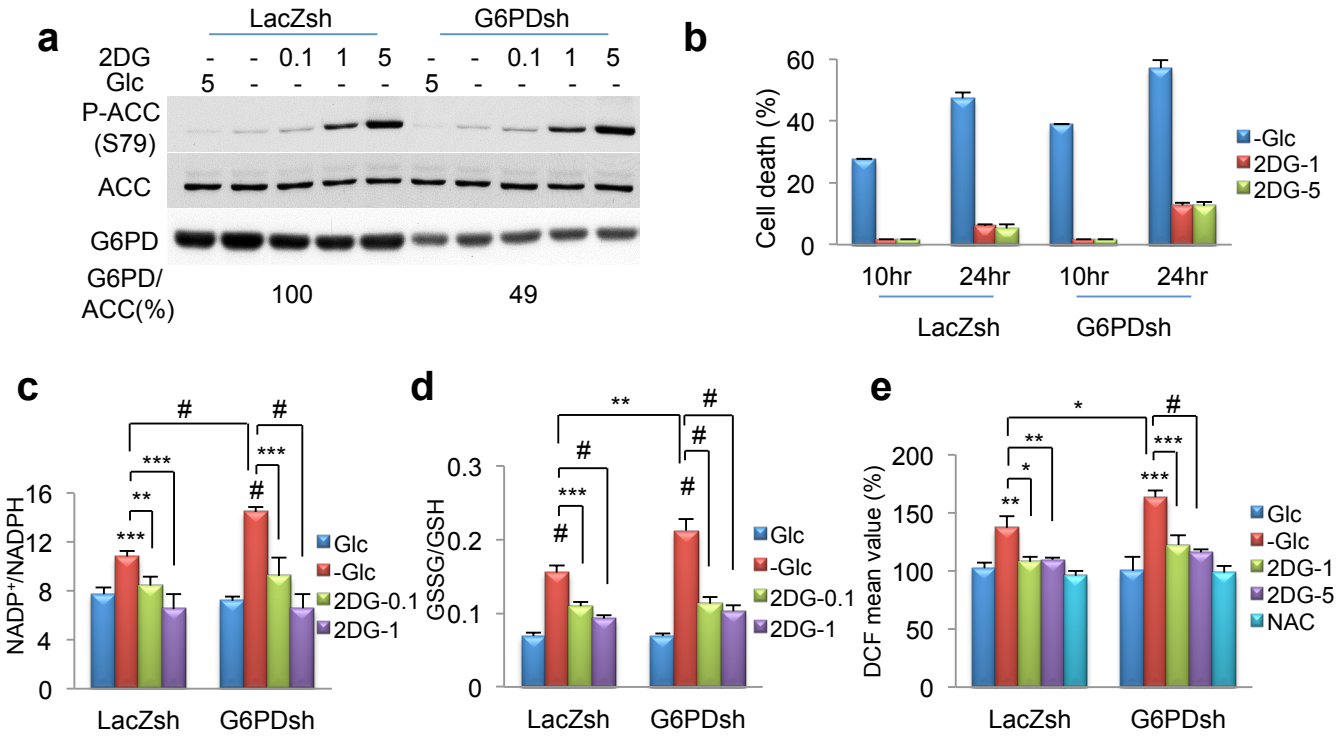
Supplementary Figure 1. The LKB1-AMPK pathway regulates metabolic adaptation in response to glucose deprivation. **a,** Cancer cell lines were incubated in glucose free medium in the absence (-) or presence of 5 mM glucose (G) for 2 hr to extract proteins for immunoblotting (left panel) or incubated in glucose free medium for the indicated time point to quantify cell death (right panel). **b,** MCF7 cells expressing control-shRNA (Consh), LKB1-shRNA (LKB1sh) or AMPKα1-shRNA (AMPKα1sh) were incubated in glucose free medium in the absence (-) or presence of 5 mM glucose (G) for 6 hr to extract proteins for immunoblotting (left panel-kockdown levels are indicated), or incubated in glucose free medium for the indicated time point to quantify cell death (right panel). **c,** Wild-type or AMPKα-KO MEFs were incubated in glucose free medium in the absence (-) or presence of 5 mM glucose (G) for 2 hr to extract proteins for immunoblotting (left panel) or incubated in glucose free medium in the absence (-Glc) or presence of soluble catalase (CAT, 400 unit/ml) for 8 hr to quantify cell death (right panel). Data represent mean ± s.e. of 3 independent experiments. ** p<0.01, *** p<0.005, versus Consh at each time point (**b**).



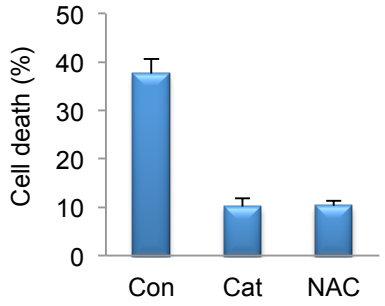
Supplementary Figure 2. 2DG activate AMPK and protect from cell death during glucose deprivation. a, HeLa cells were incubated in glucose free medium in the absence (-) or presence of 5 mM glucose (G), 2DG (D) or 5 TG (T) for 2 hr to extract proteins for immunoblotting. **b,** HeLa cells were subjected to glucose deprivation in the absence (-Glc) or presence of 5 mM 2DG or 5TG to quantify cell death at the indicated time points. Data represent mean ± s.e. of 3 independent experiments.



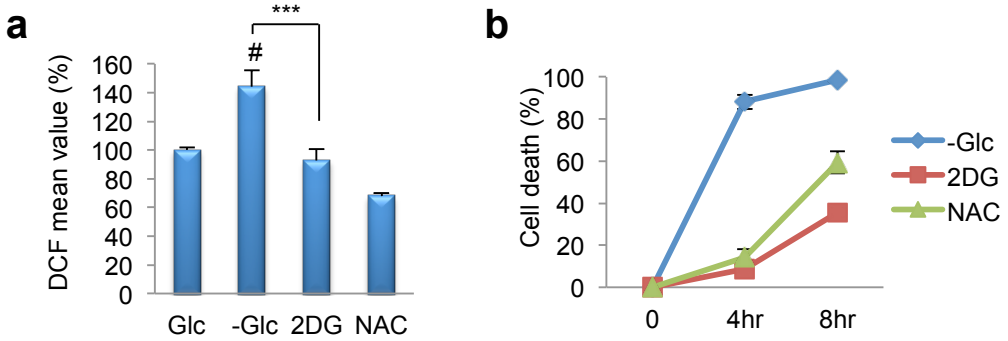
Supplementary Figure 3. The effect of 2DG on AMPK activation is dependent on hexokinase but independent on CaMKK or ATM. **a,** Hexokinase deficient MI5-4 CHO cells expressing empty vector or hexokinase 2 were incubated in glucose free medium containing 5 mM of glucose, 5TG or 2DG. After incubation for each of the indicated time points, protein extracts were isolated for immunoblotting. **b,** HeLa (left panel) and A549 (right panel) cells were incubated in glucose free medium containing 5 mM of glucose (G) or 2DG (D) in the absence or presence of STO-609 or Ku-55933 for 2 hr, then protein extracts were isolated for immunoblotting.



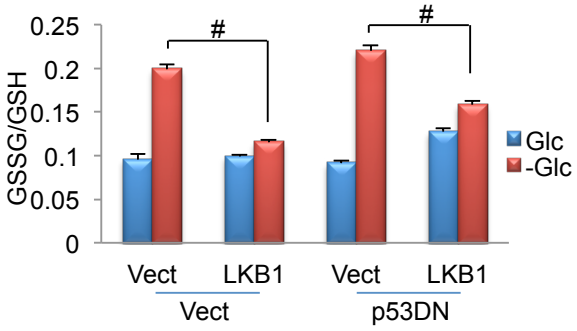
Supplementary Figure 4. The knockdown of G6PD sensitizes to cell death in the absence of glucose but cannot completely overcome the protective effect of 2DG. **a,** A549 cells stably expressing LacZ-shRNA or G6PD-shRNA were incubated in glucose free medium in the absence (-) or presence of 5 mM of glucose (G), or the indicated concentrations of 2DG(D), for 2 hr, then cell extracts were subjected to immunoblotting (the level of the knockdown is indicated). **b**, Quantification of cell death at different time points after incubation of A549 cells expressing LacZ-shRNA or G6PD-shRNA in glucose free medium in the absence (-Glc) or presence of 1 mM or 5 mM of 2DG. **c-d,** A549 cells expressing LacZ-shRNA or G6PD-shRNA were incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc), or 0.1 mM and 1 mM 2DG. After 5 hr of incubation, cells were harvested for the quantification of $NADP^+$/NADPH ratio (**c**) and GSH/GSSG ratio (**d**). **e,** A549 cells expressing LacZ-shRNA or G6PD-shRNA were incubated in glucose-free medium in the absence (-Glc) or presence of 5 mM glucose (Glc), or 1 mM and 5 mM 2DG or 2 mM NAC. After 5 hr of incubation, the intracellular level of H_2O_2 was measured. Data are expressed as % change of DCF mean values over the values of glucose treated LacZsh control. Data represent mean ± s.e. of 3 (**b-d**) or 4 (**e**) independent experiments. * p<0.05, ** p<0.01, *** p<0.005, # p<0.001 versus glucose treated control in each group.



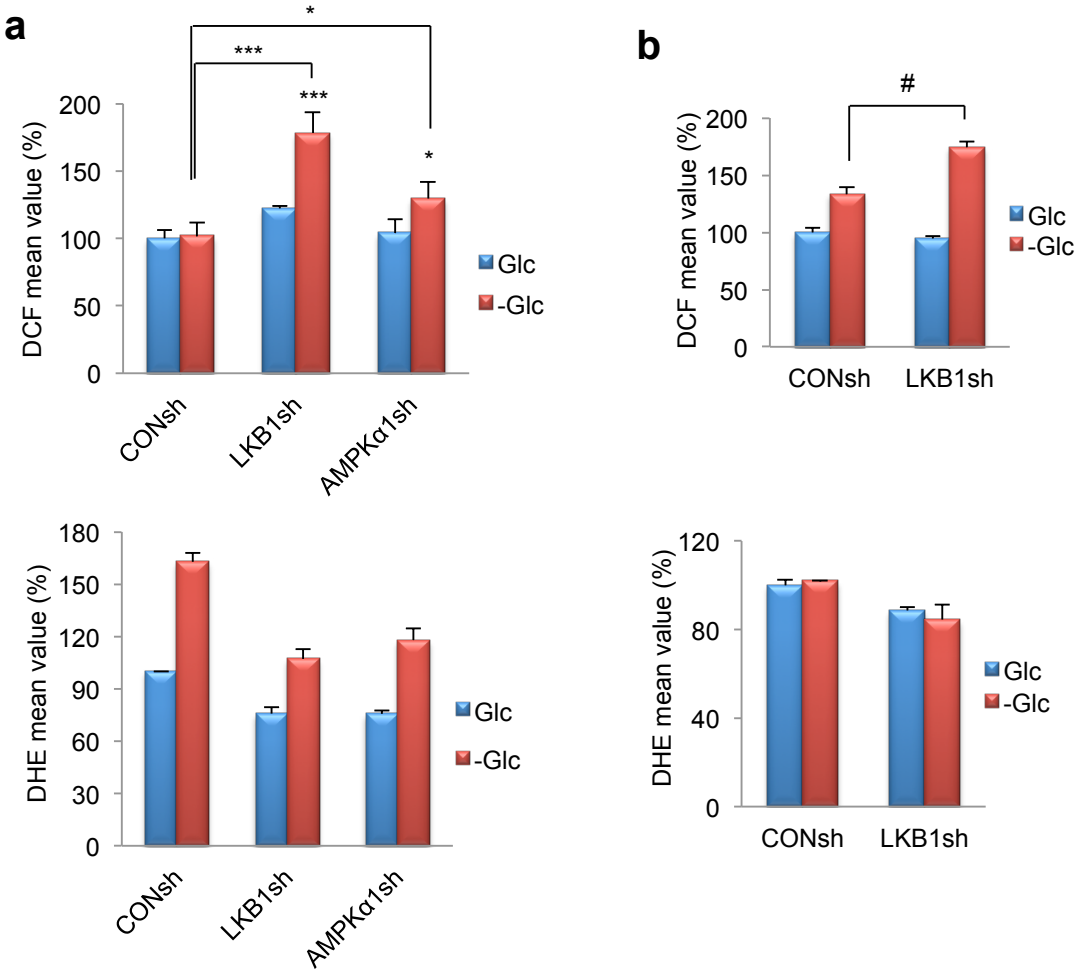
Supplementary Figure 5. Antioxidants protect A549 cells from glucose deprivation-induced cell death. Quantification of cell death after incubation of A549 cells in glucose-free medium in the absence (Con) or presence of 400 unit/ml catalase (Cat), or 2 mM NAC for 16 hr. Data represent mean ± s.e. of 3 independent experiments.



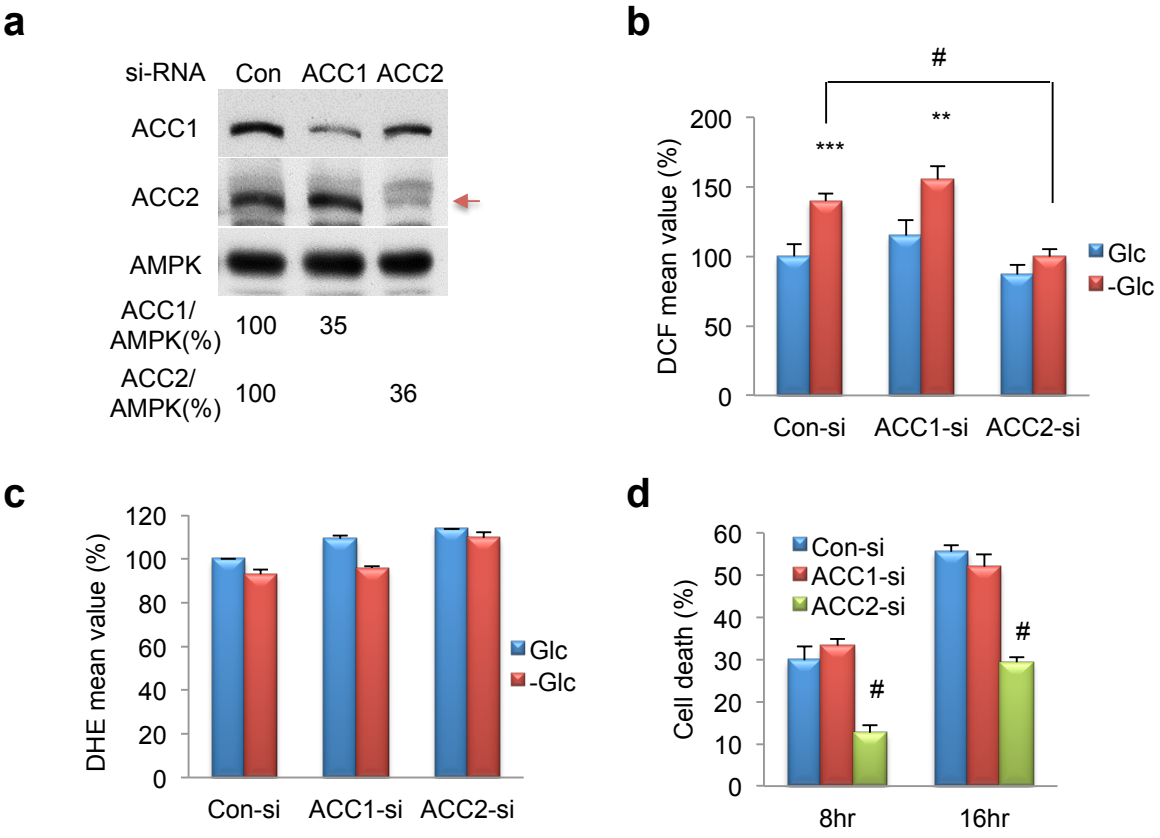
Supplementary Figure 6. 2DG and NAC protects from cell death induced by glucose deprivation by reducing H_2O_2 level in HeLa cells. **a,** H_2O_2 levels were measured after medium was replaced with glucose free medium in the absence (-Glc) or presence of 5 mM of glucose (Glc), 2DG, or 2 mM of NAC for 2hr. Data are expressed as % change of DCF mean values over the values of Glc control. **b,** Cell death was quantified after incubation at the same conditions described in (**a**). Data represent mean ± s.e. of 3 independent experiments. *** p<0.005, # p<0.001 versus Glc control.



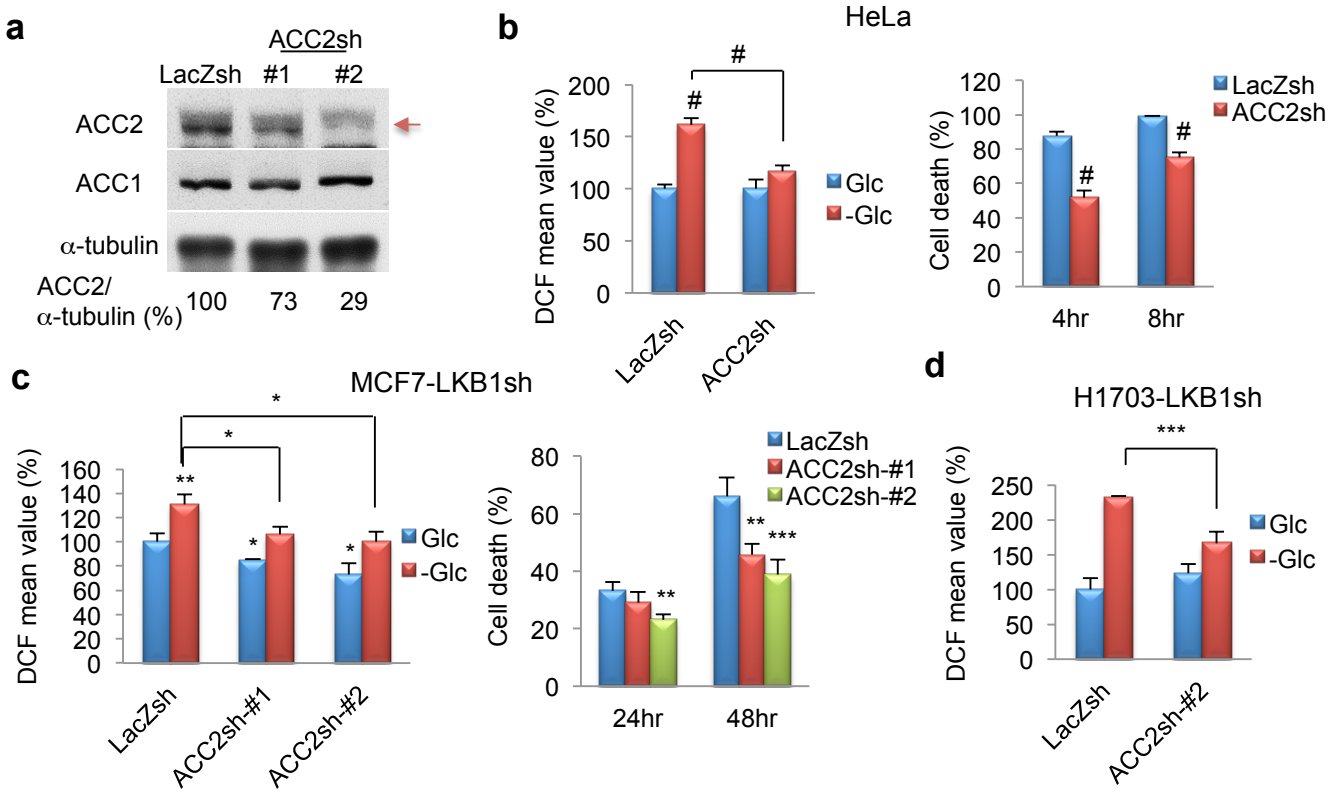
Supplementary Figure 7. Re-expression of LKB1 in A549 cells maintains GSH levels under glucose deprivation. Cells were incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) for 6 hr and then lysed to measure GSSG and GSH. Data represent mean ± s.e. of 3 independent experiments. # p<0.001.



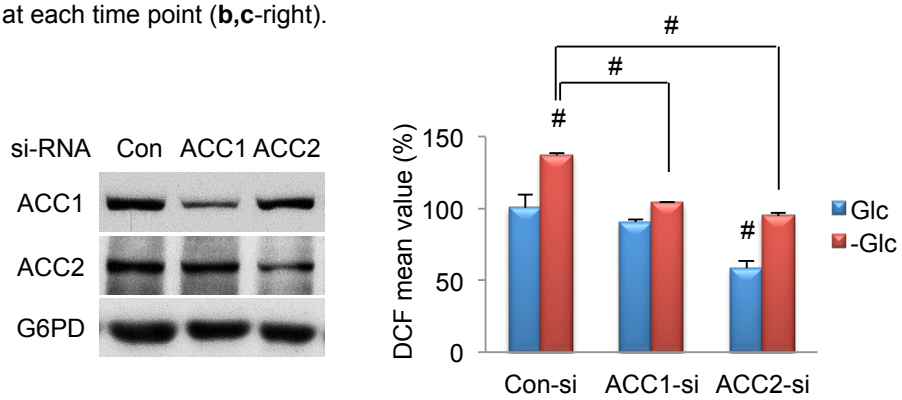
Supplementary Figure 8. The knockdown of LKB1 or AMPK increases hydrogen peroxide but not superoxide levels under glucose deprivation. **a,** MCF7 cells expressing control-shRNA, LKB1-shRNA, or AMPKa1-shRNA were incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) for 16 hr. **b,** H1703 cells expressing control-shRNA or LKB1-shRNA were incubated in the same conditions for 2 hr. The incubation was followed by measurement of H_2O_2 levels (top) and superoxide levels (bottom). Data are expressed as % change of DCF or DHE mean values over the values of glucose treated CONsh control. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, *** p<0.005, # p<0.001 versus Glc control of each group.



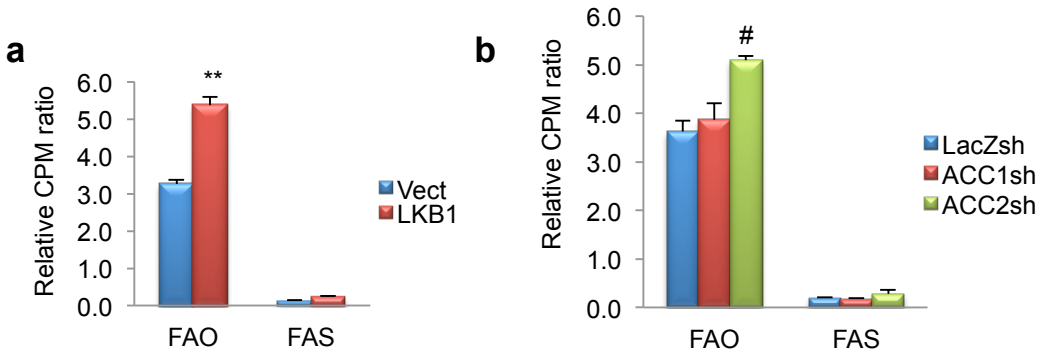
Supplementary Figure 9. The knockdown of ACC2 but not ACC1 recapitulates the LKB1 reconstitution in A549 cells. A549 cells were transiently transfected with control, ACC1 or ACC2 siRNA. After 72 hr, cells were lysed for immunoblot (**a**) or incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) to measure H_2O_2 (**b**) or O_2^- level (**c**) after 6hr, or cell death (**d**). Data(**b**, **c**) are expressed as % change of DCF/DHE mean values over the values of glucose treated Con-si control. Data represent mean ± s.e. of 3 independent experiments. ** p<0.01, *** p<0.005, # p<0.001



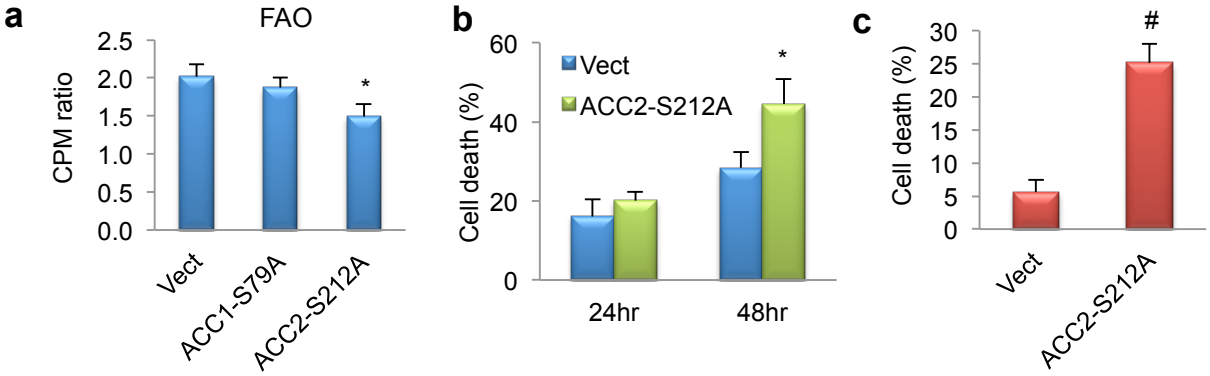
Supplementary Figure 10. The knockdown of ACC2 inhibits the elevation of H_2O_2 and cell death in LKB1 deficient cells during glucose deprivation. **a,** Immunoblots showing the level of knockdown in A549 cells stably expressing ACC2-shRNA-#1 or #2. **b,** HeLa cells stably expressing LacZ-shRNA or ACC2-shRNA-#2 were incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) for 2 hr to measure H_2O_2 levels (left panel), or incubated in glucose free medium for the indicated time points to quantify cell death (right panel). **c,** MCF7-LKB1sh cells stably expressing LacZ-shRNA or ACC2-shRNA-#1 or #2 were incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) for 16 hr to measure H_2O_2 level (right panel), or incubated in glucose free medium for the indicated time points to quantify cell death (left panel). **d,** H1703-LKB1sh cells stably expressing LacZ-shRNA or ACC2-shRNA-#2 were incubated in glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) for 2 hr to measure H_2O_2 level. All ROS data are expressed as % change of DCF mean values over the values of glucose treated LacZsh control. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, ** p<0.01, *** p<0.005, # p<0.001 versus glucose treated LacZsh (**b,c**-left) or versus LacZsh at each time point (**b,c**-right).



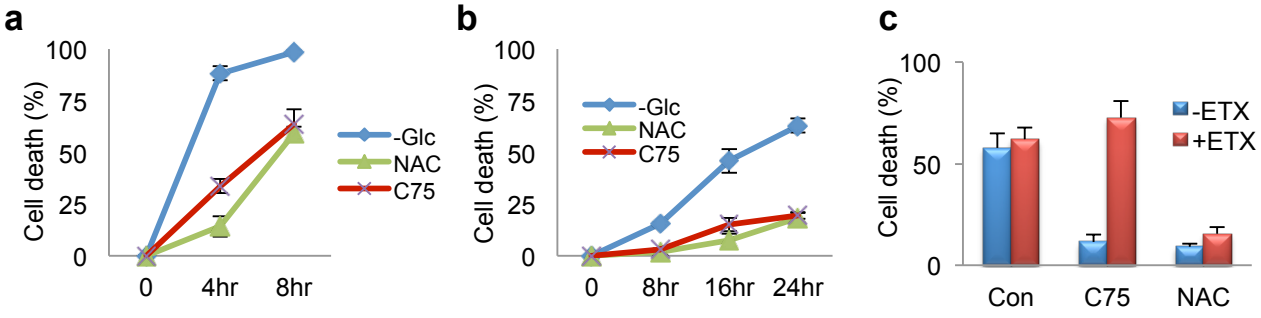
Supplementary Figure 11. The knockdown of either ACC1 or ACC2 inhibits the elevation of H_2O_2 in AMPKα-KO MEFs during glucose deprivation. AMPKα-KO MEFs were transiently transfected with control, ACC1 or ACC2 siRNA. After 72 hr, cell were lysed for immunoblotting (left panel) or medium was replaced with glucose free medium in the absence (-Glc) or presence of 5 mM glucose (Glc) for 5 hr, followed by H_2O_2 levels measurement (right panel). Data are expressed as % change of DCF mean values over the values of glucose treated Con-si control. Data represent mean ± s.e. of 3 independent experiments. . # p<0.001 versus glucose treated Con-si control.



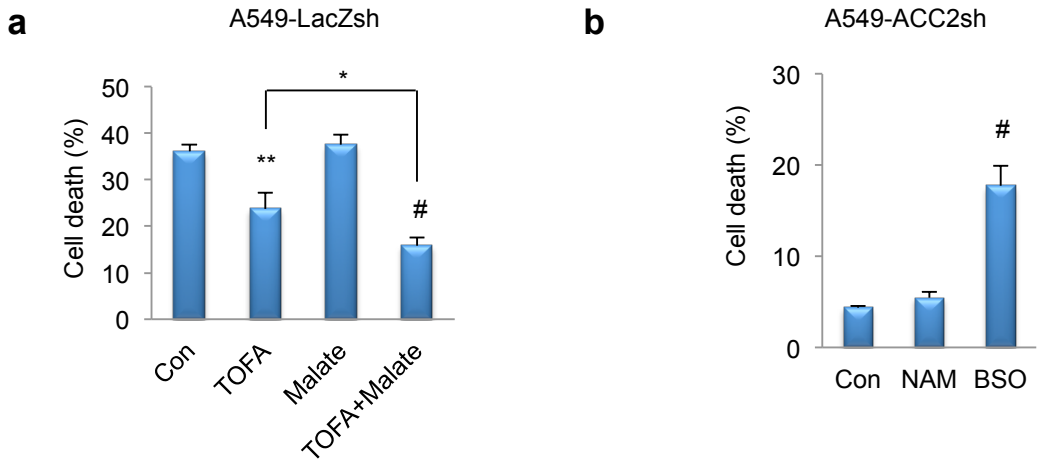
Supplementary Figure 12. The regulation of fatty acid metabolism by LKB1, ACC1 or ACC2 in A549 cells during glucose starvation. FAO and FAS were measured after glucose starvation in A549 cells expressing empty vector (Vect) or LKB1 (**a**) and expressing LacZsh, ACC1sh, or ACC2sh (**b**). The values are expressed as a ratio of CPM in no glucose versus 5 mM glucose conditions. Data represent mean ± s.e. of 3 independent experiments. ** p<0.01, # p<0.001 versus corresponding vector (**a**) or LacZsh (**b**) control. The measurements of FAO and FAS are described in details in Methods.



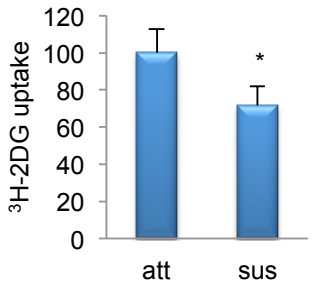
Supplementary Figure 13. Constitutively active mutant of ACC2 sensitizes to cell death and abrogated the protective effect of 2DG during glucose starvation. **a,** FAO was measured after glucose starvation for 6hr in MCF7 cells expressing empty vector (Vect), ACC1-S79A or ACC2-S212A. The values are expressed as a ratio of CPM in no glucose versus 5 mM glucose conditions. **b,** Quantification of cell death at different time points after incubation of MCF7 cells expressing either empty vector (Vect) or ACC2-S212A in glucose free medium. **c,** HeLa cells expressing either empty vector (Vect) or ACC2-S212A were incubated in glucose free medium containing 5 mM of 2DG for 5 hr to quantify cell death. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, # p<0.001 versus vector control.



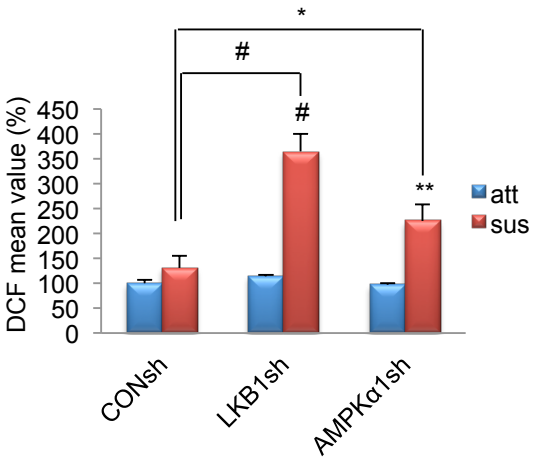
Supplementary Figure 14. C75 protects LKB1 deficient cells from cell death during glucose deprivation via activation of CPT1. HeLa cells (**a**) and A549 cells (**b**) were incubated in glucose free medium in the absence (-Glc) or presence of 20 μg/ml of C75 or 2 mM of NAC. Cell death was quantified at the indicated time points. **c,** A549 cells were incubated in glucose free medium in the absence of glucose (Con) or presence of 20μg/ml C75 or 2mM of NAC with or without 500μM etomoxir (ETX) for 16hr. Data represent mean ± s.e. of 3 independent experiments.



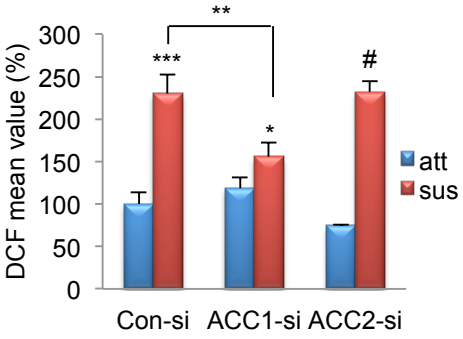
Supplementary Figure 15. The elevation of the NADPH-GSH axis confers resistance to glucose deprivation induced cell death. a, A549 cells were incubated in glucose free medium in the absence (Con) or presence of 5 μM TOFA, 2 mM malate or both for 16 hr, then cell death was quantified. **b,** Quantification of cell death after incubation of glucose-free medium in the absence (Con) or presence of 5 mM nicotinamide (NAM), or 1 mM buthionine sulfoximine (BSO) in A549 expressing ACC2-shRNA for 24 hr. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, ** p<0.01, # p<0.001 versus Con.



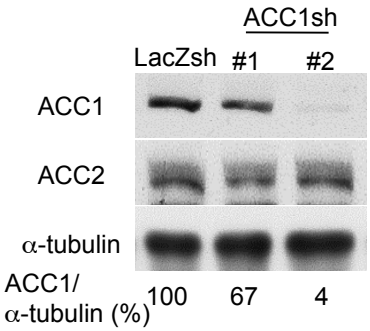
Supplementary Figure 16. Matrix detachment inhibits glucose uptake in A549 cells. A549 cells were allowed to grow in attached condition (att) or in suspension (sus) for 6 hr to measure glucose uptake. Data represent mean ± s.e. of 3 independent experiments. * p<0.05 versus attached condition. Glucose uptake measurement is described in Methods.



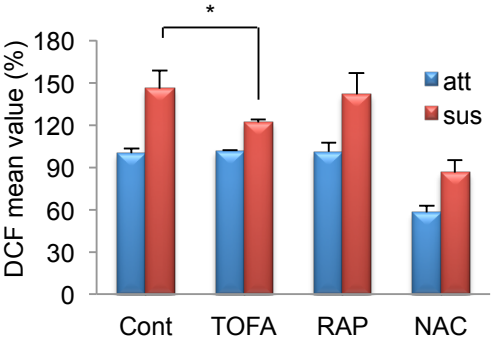
Supplementary Figure 17. The LKB1-AMPK pathway regulates H_2O_2 levels during matrix detachment in MCF7 cells. MCF7 cells expressing control-shRNA, LKB1-shRNA or AMPKα1-shRNA were allowed to grow in attached condition (att) or in suspension (sus) for 24 hr, followed by H_2O_2 level measurement. Data are expressed as % change of DCF mean values over the values of attached CONsh control. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, ** p<0.01, # p<0.001 versus each attached control.



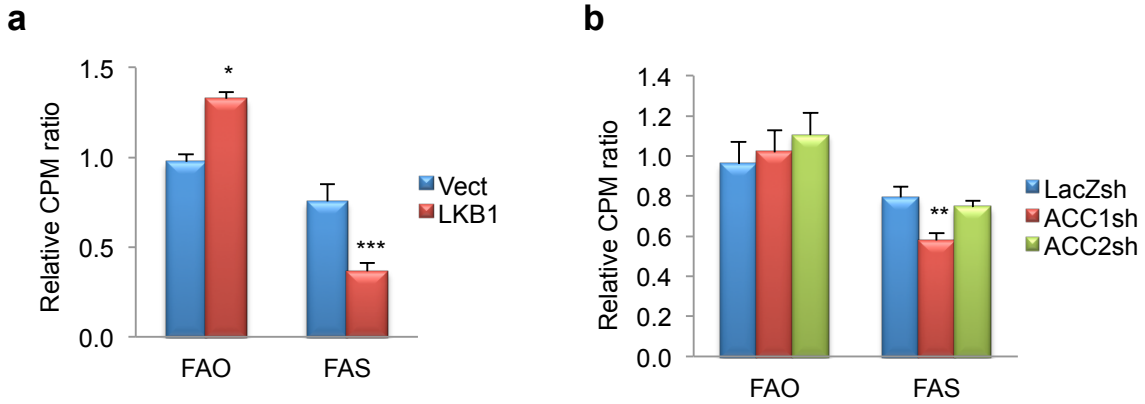
Supplementary Figure 18. The knockdown of ACC1 but not ACC2 inhibits the elevation of H_2O_2 during matrix detachment in A549 cells. After 48hr of siRNA transfection, cell were cultured in suspension for 24hr and then H2O2 level was measured. Data are expressed as % change of DCF mean values over the values of attached Cont. Data represent mean ± s.e. of 3 independent experiments. *p<0.05, ** p<0.01, *** p<0.005, # p<0.001 versus each attached control.



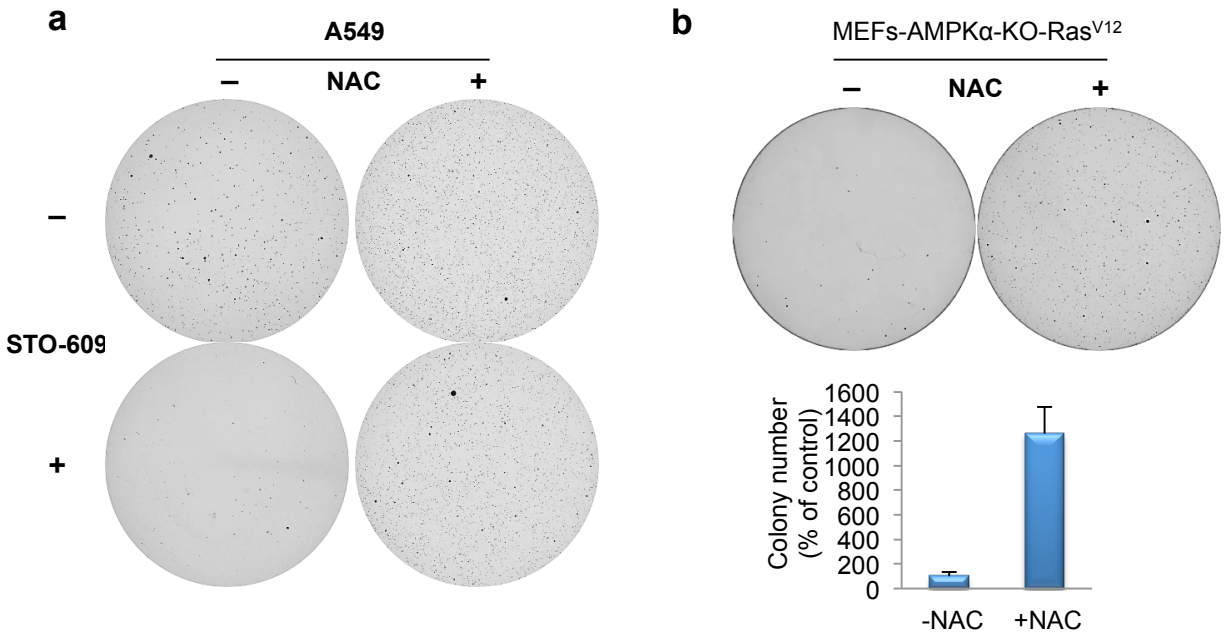
Supplementary Figure 19. The efficiency of ACC1-shRNA in A549 cells. Immunoblots showing the efficiency of ACC1 knockdown in A549 cells stably expressing ACC1-shRNA-#1 or -#2. Based on the knockdown efficiency, A549 cells expressing ACC1sh-#2 were used in other experiments.



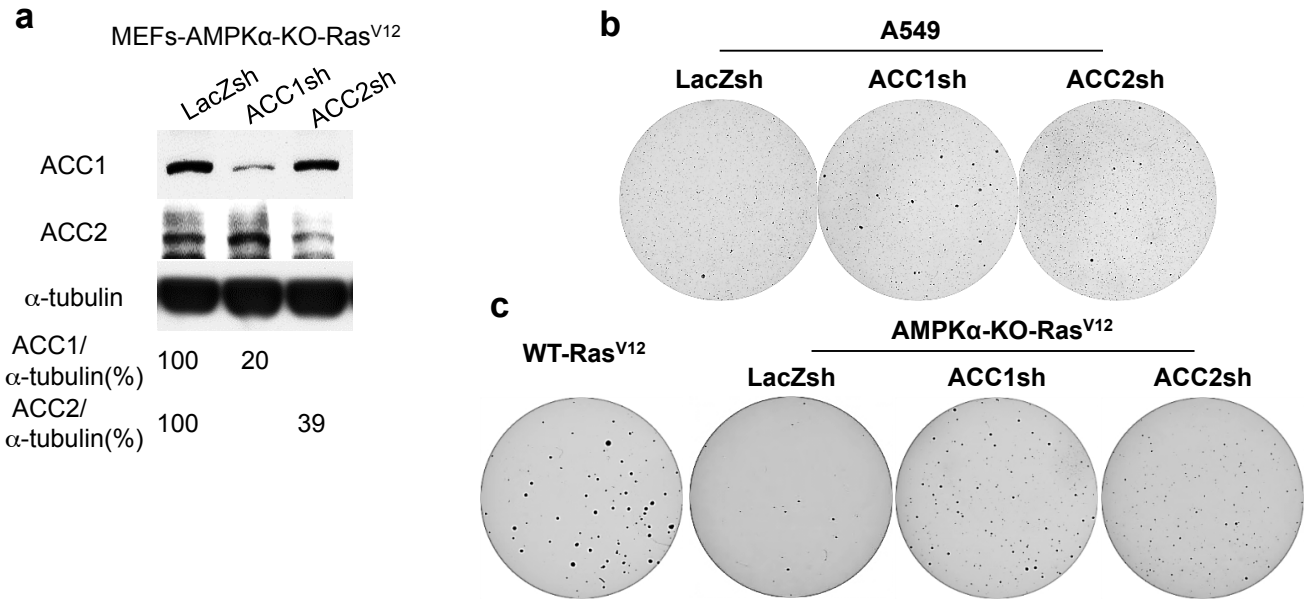
Supplementary Figure 20. Inhibition of ACC but not mTORC1 reduces H_2O_2 levels during matrix detachment. A549 cells were allowed to grow in attached condition (att) or in suspension (sus) in the absence (Cont) or presence of 5 μM TOFA, 100 nM rapamycin (RAP) or 2 mM NAC for 12 hr, followed by H_2O_2 levels measurement. Data are expressed as % change of DCF mean values over the values of attached Cont. Data represent mean ± s.e. of 3 independent experiments. * p<0.05.



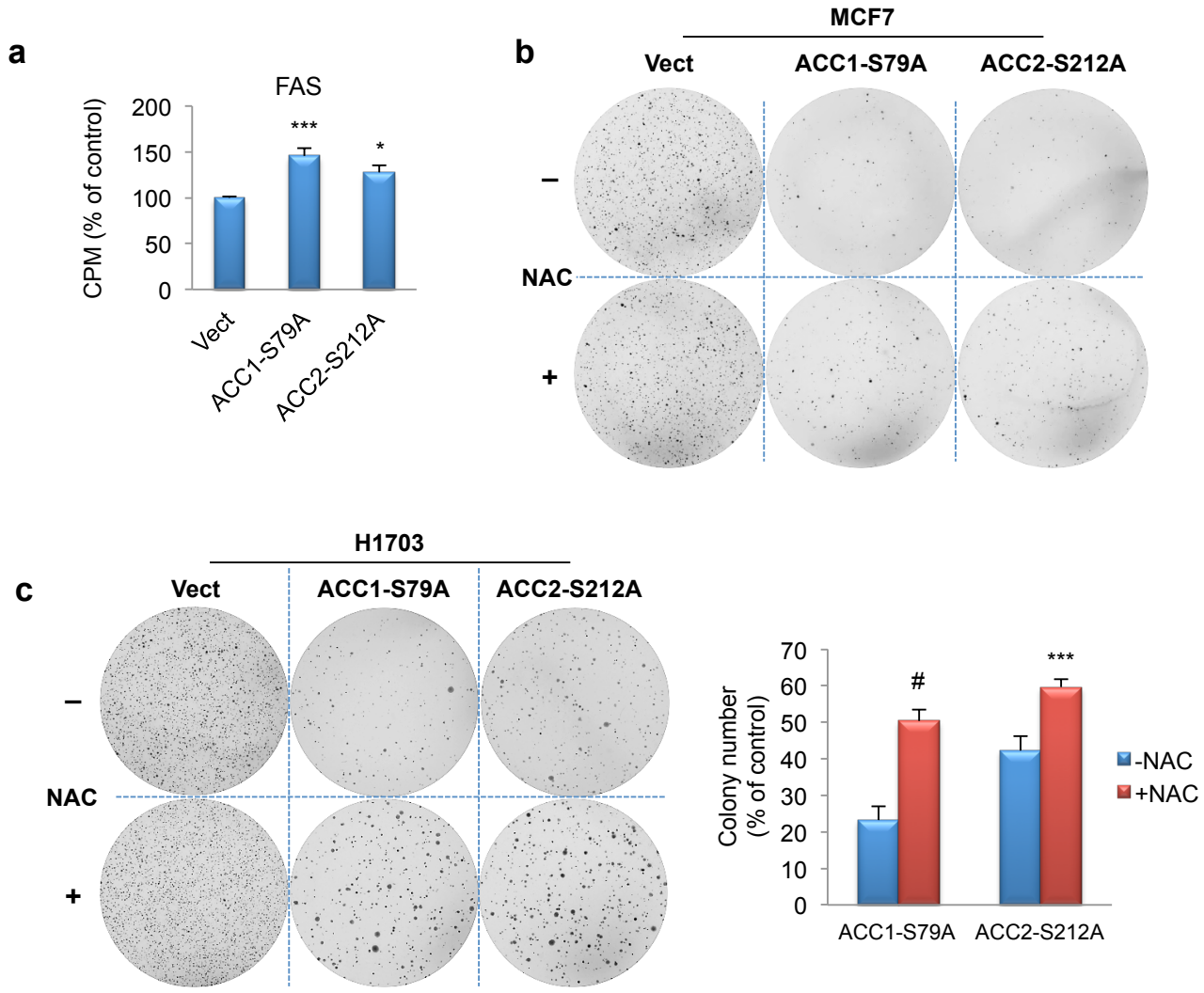
Supplementary Figure 21. The regulation of fatty acid metabolism by LKB1, ACC1 or ACC2 during matrix detachment in A549. FAO and FAS were measured after matrix detachment of A549 cells expressing empty vector (Vect) or LKB1 (**a**) and expressing LacZsh, ACC1sh or ACC2sh (**b**). Data are expressed as CPM ratio of matrix detached versus attached condition in each group. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, ** p<0.01, *** p<0.005 versus corresponding vector (**a**) or LacZsh (**b**) control.



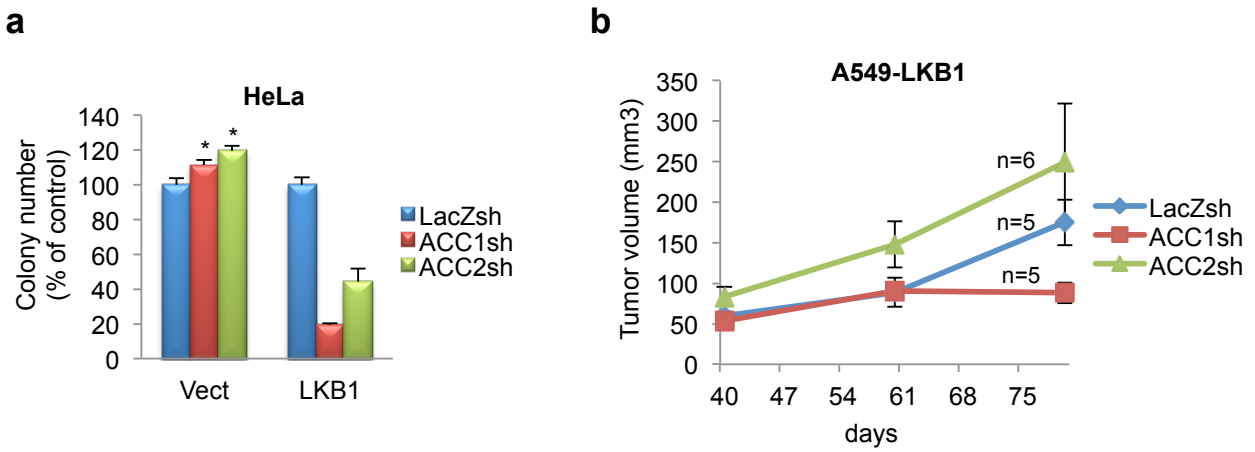
Supplementary Figure 22. Antioxidant promotes anchorage-independent growth after the inhibition of CaMKK and in the absence of LKB1 or AMPK. a, Representative images of soft agar colonies of Fig. 4a. **b,** MEFs-AMPKα-KO-RAS^{V12} were plated on soft agar in the absence or presence of 2 mM NAC. After 3-4 weeks the images were taken and the colony number were analyzed using imageJ software. Data are expressed as % change of colony number over the values of -NAC control. Data represent mean ± s.e. of 3 independent experiments.



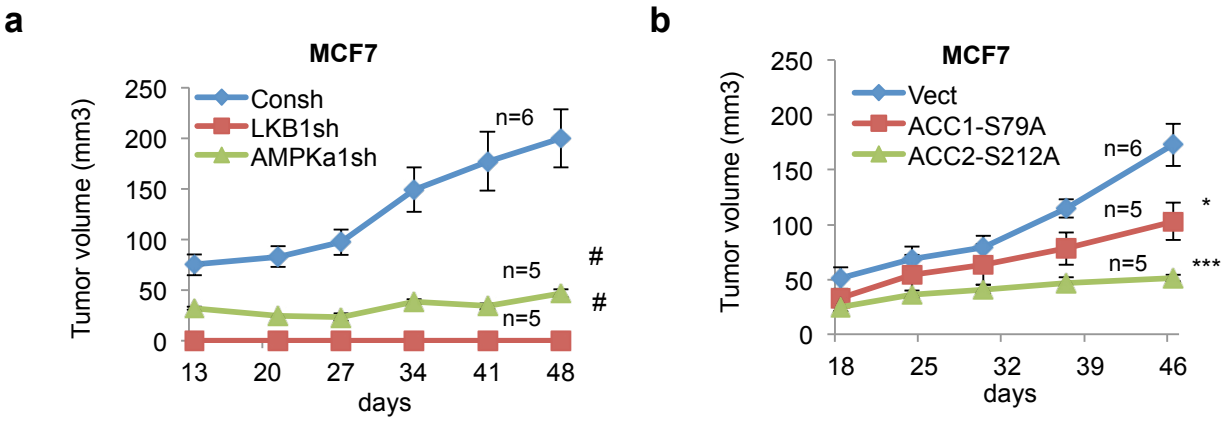
Supplementary Figure 23. The knockdown efficiency of ACC1-shRNA and ACC2-shRNA in AMPKα-KO-RasV12 MEFs. a, Proteins were extracted from AMPKα-KO-Ras^{V12} MEFs stably expressing LacZ-shRNA, ACC1-shRNA, or ACC2-shRNA to analyze the knockdown efficiency by Western blotting. The levels of the knockdowns are indicated. **b-c,** Representative images of soft agar colonies of Fig. 4b-c respectively.



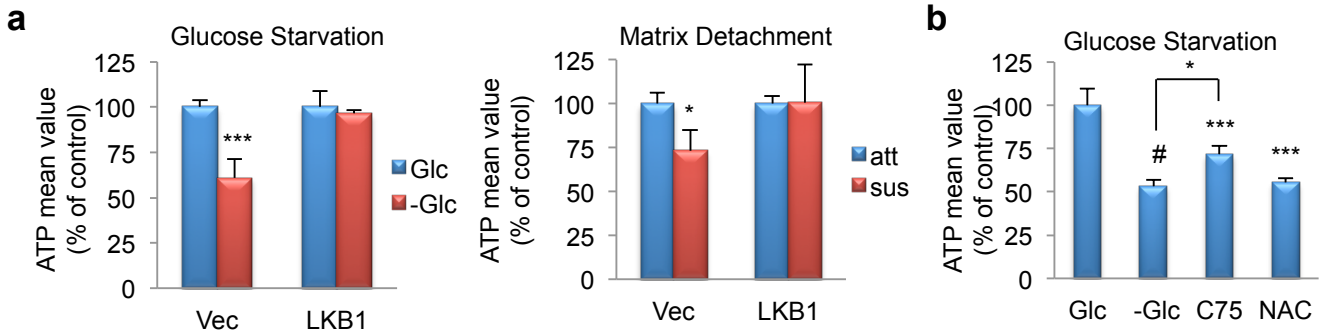
Supplementary Figure 24. Constitutively active mutants of ACC1 and ACC2 inhibit anchorage-independent growth. **a,** FAS was measured in MCF7 cells expressing empty vector (Vect), ACC1-S79A or ACC2-S212A. Data are expressed as % change of CPM mean values over the values of Vect control. **b,** Representative images of soft agar colonies of Fig. 4e. **c,** H1703 cells were infected with lentiviruses encoding constitutively active mutant of ACC1 (S79A) or ACC2 (S212A). Immediately after selection, the cells were subjected to anchorage-independent growth on soft agar in the absence or presence of 2 mM NAC. After 3 weeks on soft agar, colonies were analyzed using imageJ software. Data are expressed as % change of colony number over the values of Vect control. Data represent mean ± s.e. of 3 independent experiments. * $p<0.05$, *** $p<0.005$, # $p<0.001$ versus Vect control (**a**) or -NAC control in each group (**c**).



Supplementary Figure 25. The knockdown of ACC1 or ACC2 in the presence of LKB1 does not increase solid tumor growth or anchorage independent growth. a, HeLa cells stably expressing LacZ-shRNA, ACC1-shRNA-#2, or ACC2-shRNA-#2 in the absence (Vect) or presence of LKB1 expression (LKB1) were plated on soft agar as described in Methods. After 3 weeks on soft agar, number of colonies were analyzed using imageJ software. Data are expressed as % change of colony number over the values of each LacZsh control. Data represent mean ± s.e. of 3 independent experiments. * p<0.05 versus LacZsh control. **b,** A549-LKB1 cells expressing LacZ-shRNA, ACC1shRNA or ACC2-shRNA were injected subcutaneously in athymic nude mice. The growth of the tumors was monitored and measured at weekly intervals. Data represent mean ± s.e. of the indicated numbers in each group.



Supplementary Figure 26. The knockdown of LKB1 or AMPKa1, or the expression of constitutively active mutants of ACC1 or ACC2 inhibit tumor growth in vivo. MCF7 cells expressing Con-shRNA, LKB1-shRNA or AMPKα1-shRNA (**a**), or expressing empty vector (Vect), ACC1-S79A or ACC2-S212A (**b**) were orthotopically injected into the mammary fat pads of female athymic nude mice (for details see Methods). The growth of the tumors was monitored and measured weekly. Data represent mean ± s.e. of the indicated numbers in each group. * p<0.05, *** p<0.005, # p<0.001 versus the Con-shRNA group (**a**) or Vect group (**b**).



Supplementary Figure 27. LKB1 reconstitution maintains ATP level during glucose starvation or matrix detachment but NAC does not restore ATP level during glucose starvation in A549. **a,** ATP level was measured in A549-Vect and A549-LKB1 cells after incubation in glucose free media in the absence (-Glc) or presence of 5mM glucose (Glc) for 6hr (left panel) or after cells were grown in suspension for 16hr (right panel). **b,** ATP level was measured in A549 cells after incubation in glucose free media in the absence (-Glc) or presence of 5mM glucose (Glc), 2ug/ml C75 or 2mM NAC for 6hr. Data are normalized with cell numbers and expressed as % change of ATP mean values over the values of each control. Data represent mean ± s.e. of 3 independent experiments. * p<0.05, ** p<0.01, *** p<0.005, # p<0.001 versus glucose treated control (**a**-left**, b**) or attached control (**a**-right) in each group.