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Supplemental Data

Oncogenic B-RAF Negatively Regulates the Tumor Suppressor LKB1 to Promote Melanoma Cell Proliferation

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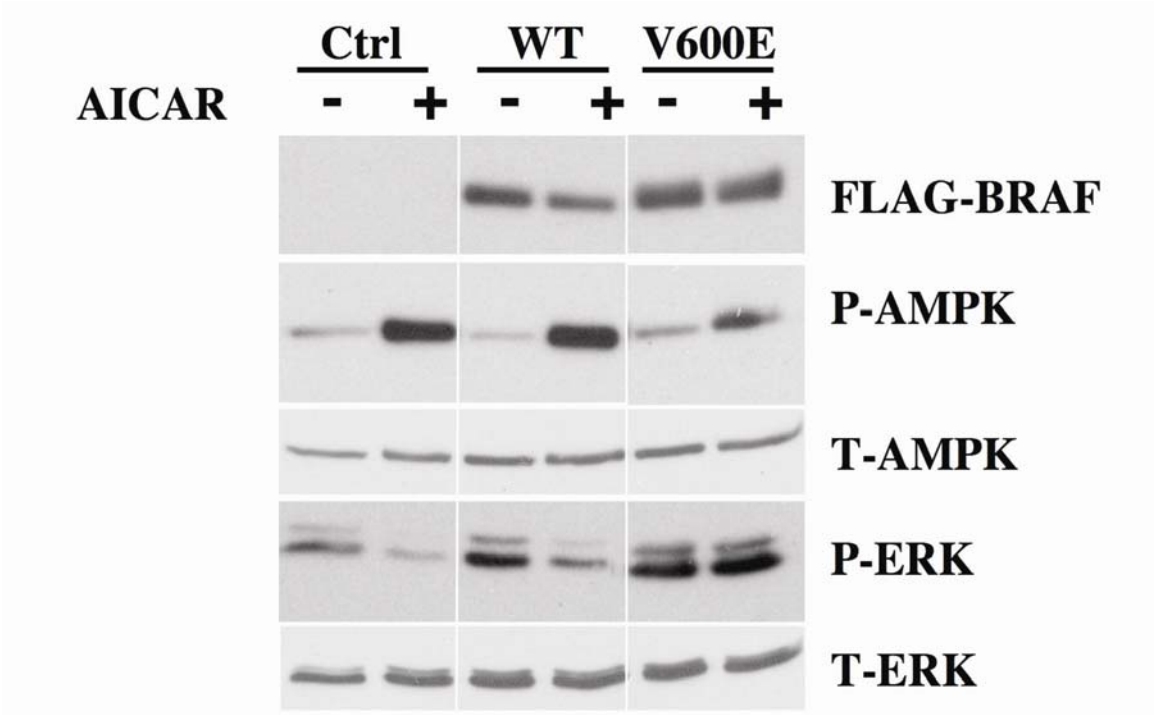
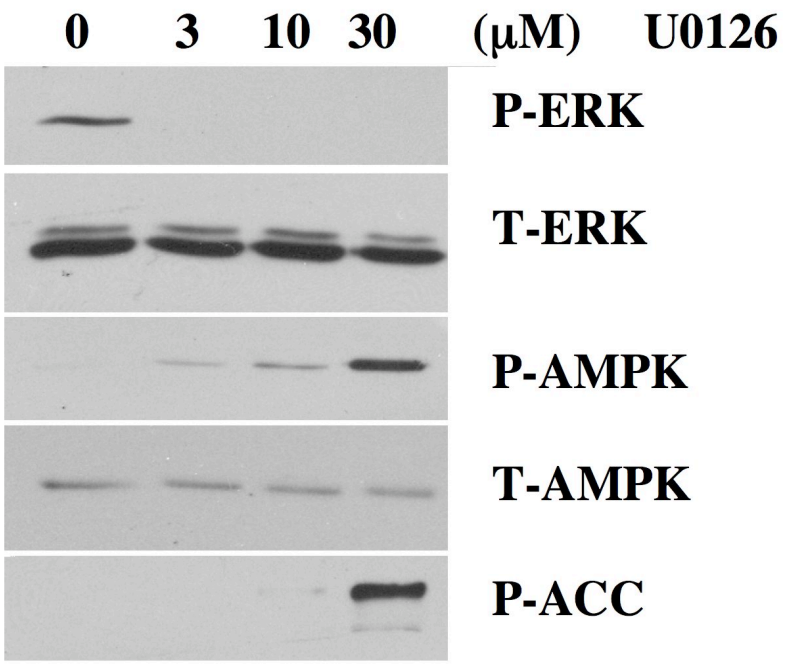
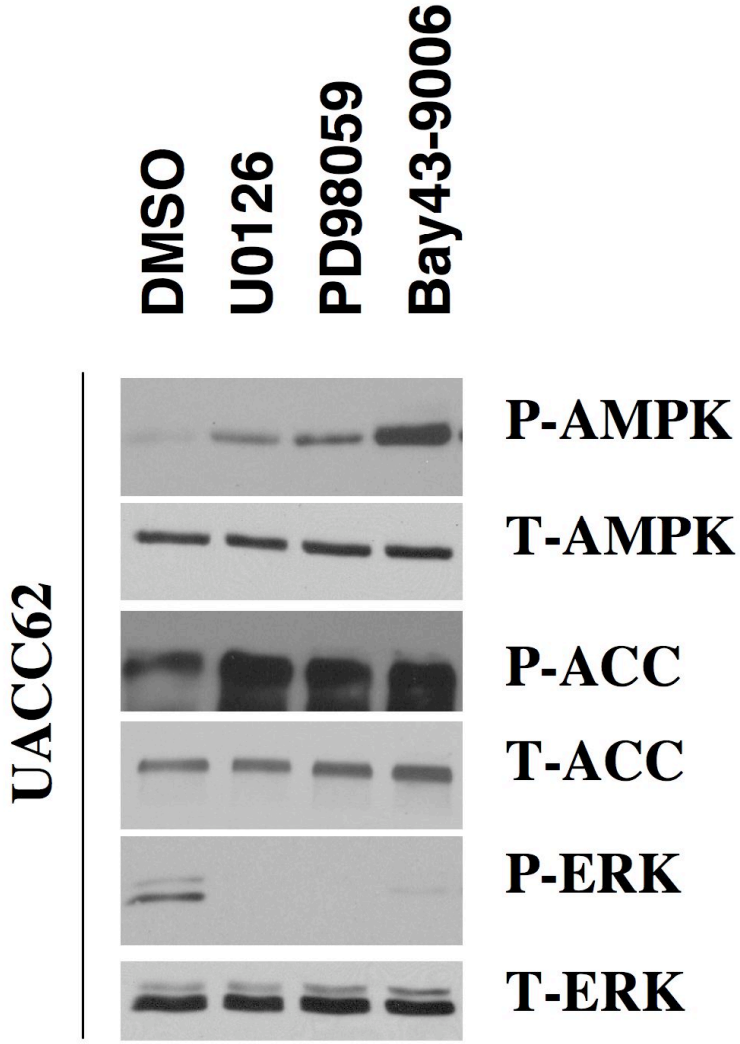


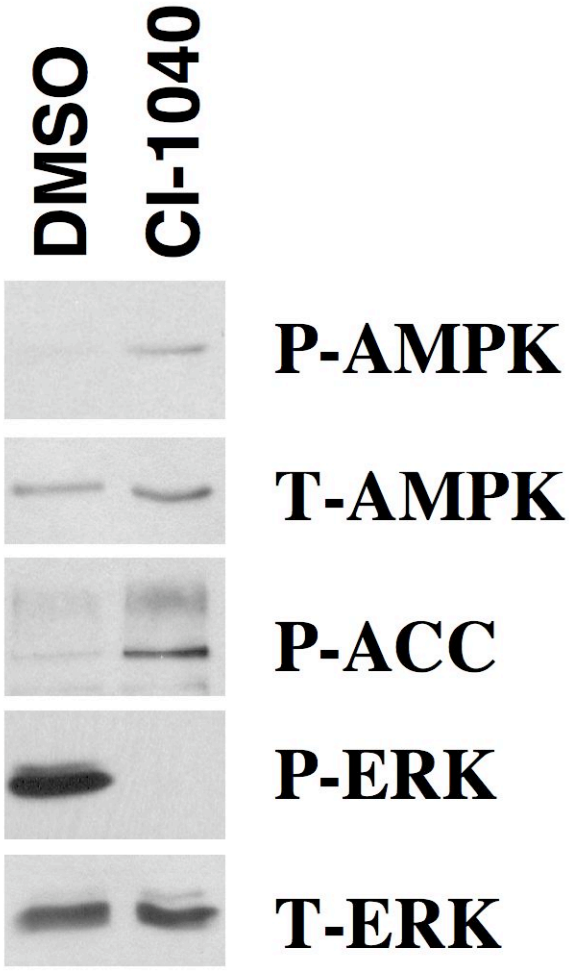
Supplemental Figure 1. Expression of B-RAF V600E attenuates AMPK activation in Cos-7 cells. Cos-7 cells were transiently transfected with FLAG-B-RAF WT or V600E mutant and treated with or without 1 mM AICAR for 1 hr. Cell lysates were used for western blotting with indicated antibodies.



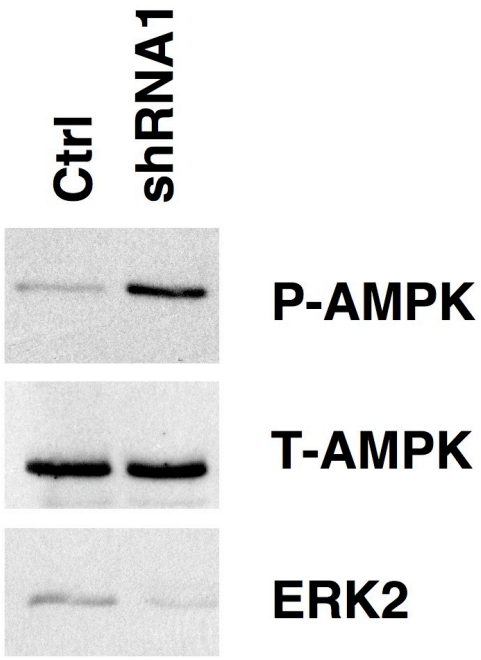
Supplemental Figure 2. U0126 induces AMPK activation in a concentration-dependent manner. SK-MEL-28 cells were treated with the indicated concentration of U0126 for 1 hr. Cell lysates were used for western blotting with indicated antibodies.



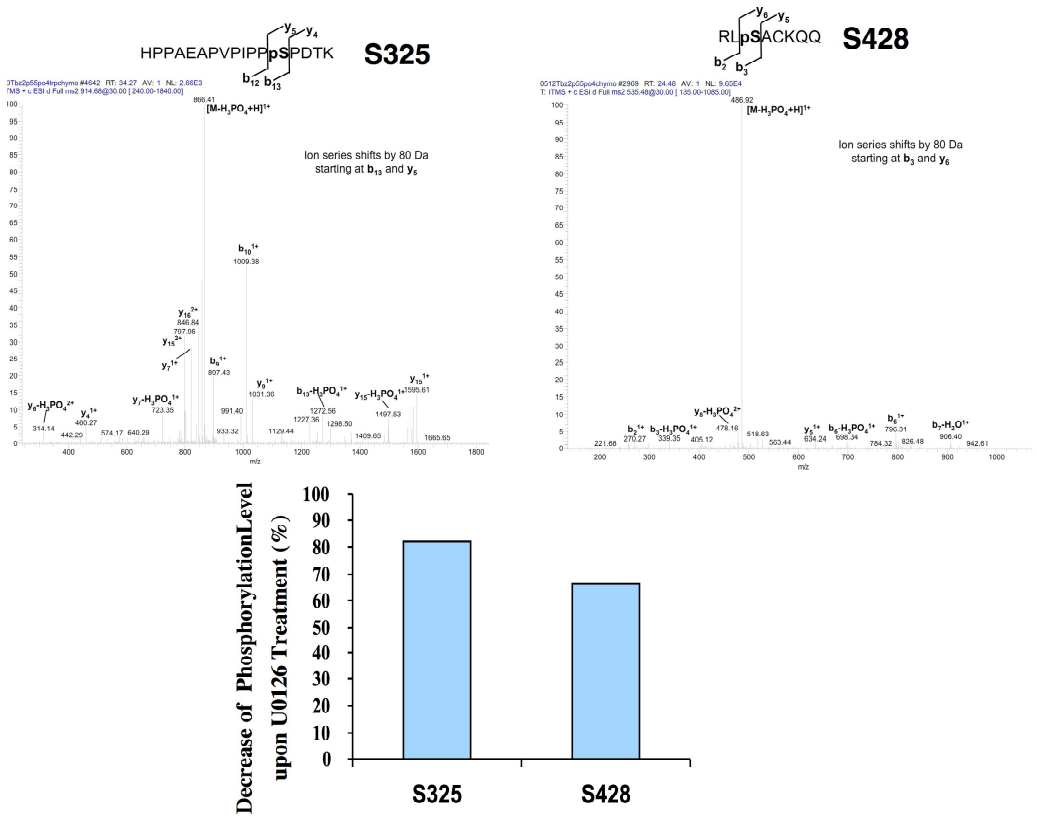
Supplemental Figure 3. Induction of AMPK phosphorylation by various inhibitors against the RAF-MEK-ERK signaling cascade in UACC62 cells. Cells were treated with DMSO, 20 μM U0126, 20 μM BAY43-9006, or 50 μM PD98059 for 1 hr. Cell lysates were used for western blotting with indicated antibodies.



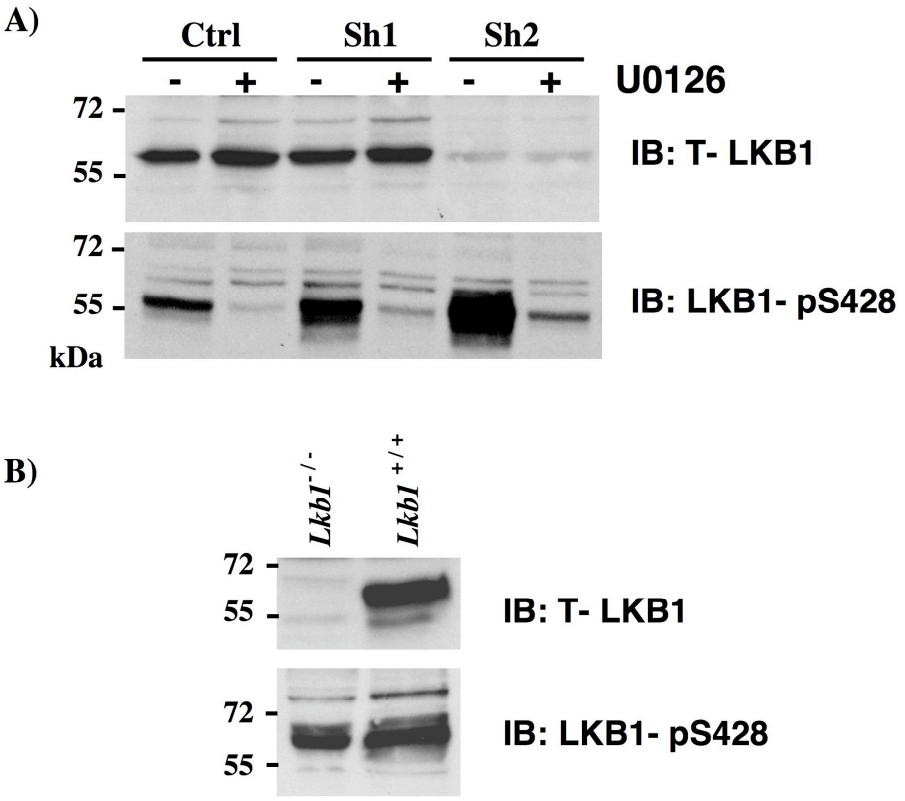
Supplemental Figure 4. Induction of AMPK phosphorylation by MEK inhibitor CI-1040. SK-Mel-28 cells were treated with 5 μM of CI-1040 for 2 hr. Cell lysates were used for western blotting with indicated antibodies.



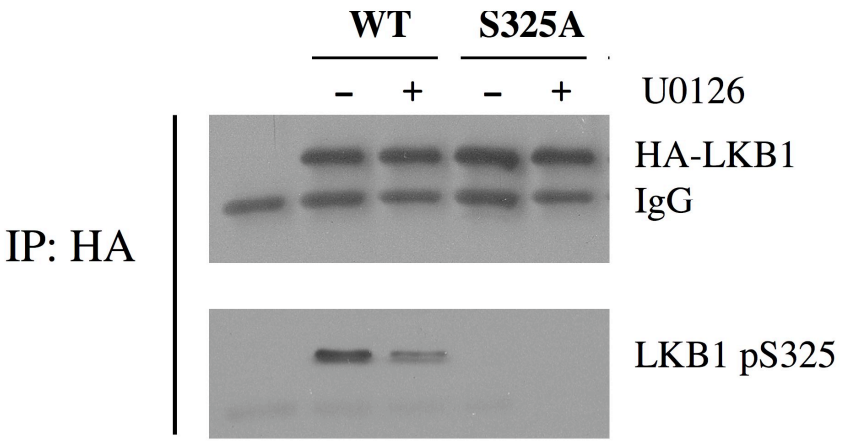
Supplemental Figure 5. Knockdown of ERK2 expression by RNA interference activates AMPK. SK-Mel-28 cells were infected with retrovirus containing shRNA construct in pSM2C against ERK2 or control empty vector.



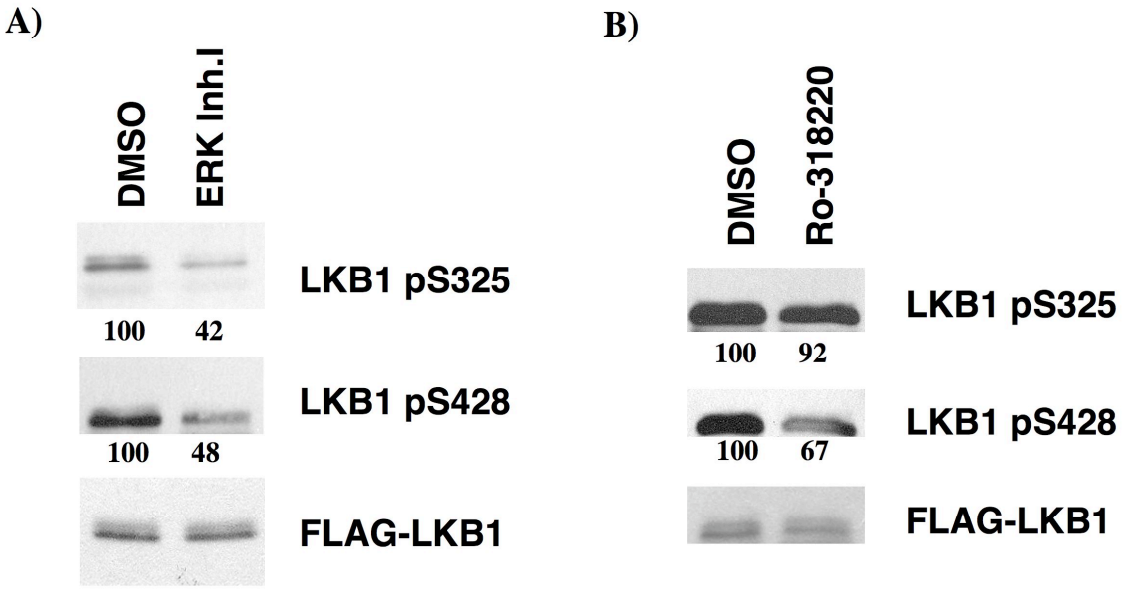
Supplemental Figure 6. MS/MS spectra of LKB1 peptides containing phosphorylated Ser325 and Ser428, and relative quantitative ratio analysis on the phosphorylation signal levels of Ser325 and Ser428 based on the total ion counts (TIC) from all sequencing events for the phosphorylated and non-phosphorylated forms of each peptide.



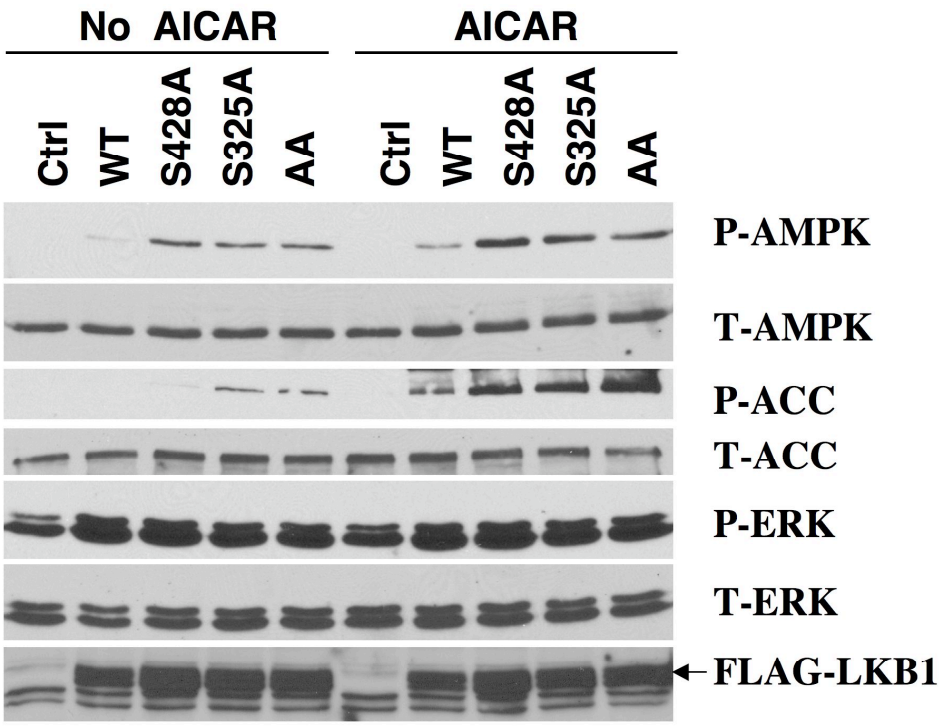
Supplemental Figure 7. Anti-phospho-LKB1 S428 antibodies do not recognize endogenous LKB1 proteins in SK-Mel-28 cells (A) and MEFs (B). For SK-Mel-28, cells were infected with lentivirus encoding shRNA against LKB1 (sh1 and sh2) or control shRNA (ctrl), serum-starved and treated with 20 μM of U0126 for 2 hr. Cell lysates were used for western blotting with indicated antibodies.



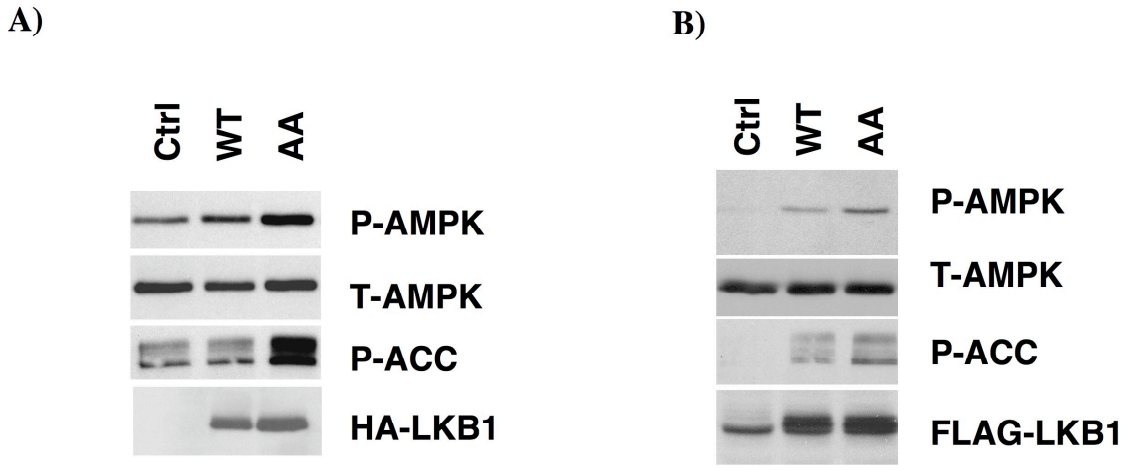
Supplemental Figure 8. Characterization of phospho-LKB1 S325 antibodies. HEK293 cells were transfected with HA-LKB1 WT or S325A mutant, and treated with 20 μM U0126 for 1 hr. Cell lysates were immunoprecipitated with anti-HA antibodies followed by western blotting using indicated antibodies.



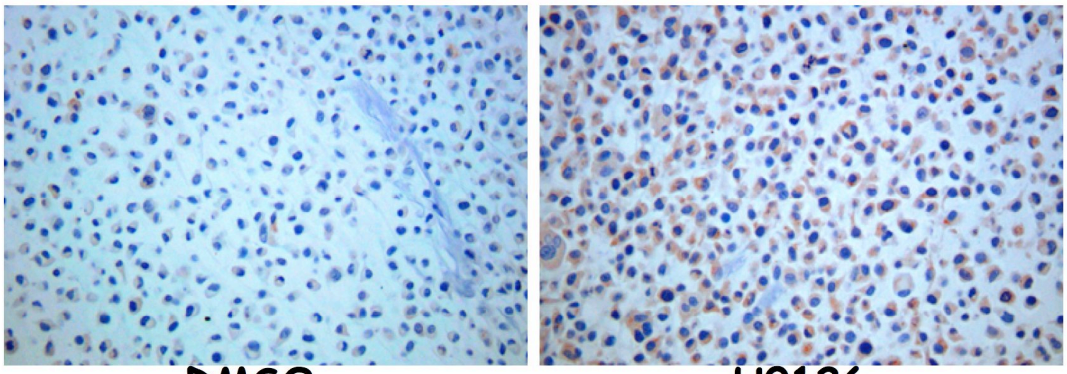
Supplemental Figure 9. Inhibition of LKB1 Ser325 and Ser428 phosphorylation by ERK inhibitor I and Ro-318220. SK-Mel-28 stably expressing FLAG-LKB1 were treated with DMSO, ERK inhibitor I (25 μM, Calbiochem) or Ro-318220 (20 μM) for 2 hr. Cell lysates were immunoprecipitated with anti-FLAG M2 agarose beads followed by western blotting using indicated antibodies. Numbers indicate relative intensity as quantified by image J analysis.



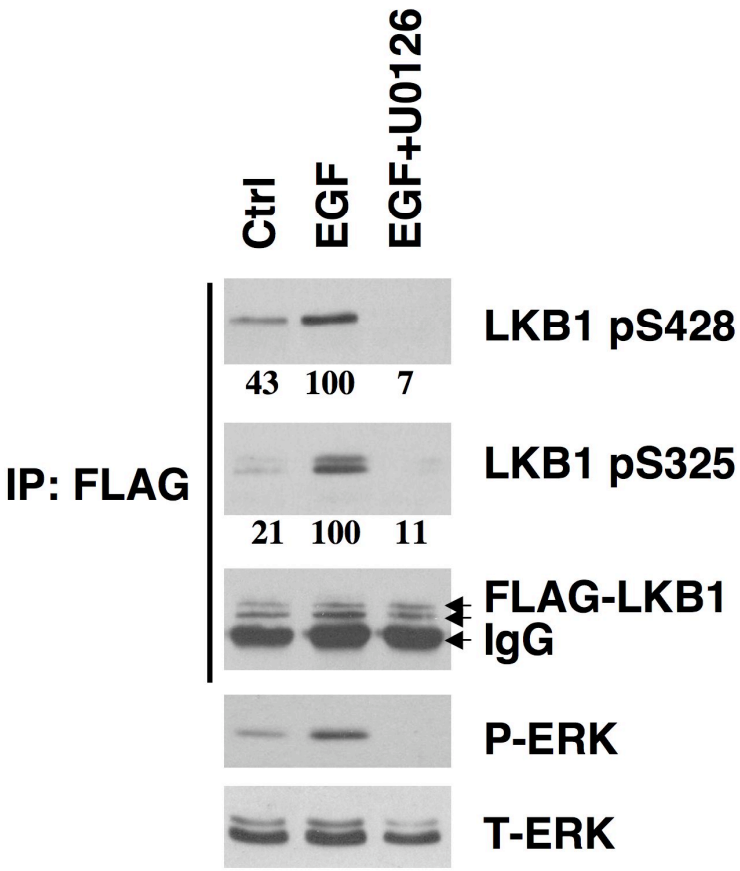
Supplemental Figure 10. Mutation of Ser325 or Ser428 of LKB1 to Ala enhances its activity on AMPK activation in HeLa cells. HeLa cells were infected with retrovirus containing vector control, WT LKB1, S325A, S428A or S325A/S428A LKB1, and treated with 1 mM AICAR for 1 hr. Cell lysates were used for western blotting with indicated antibodies.



Supplemental Figure 11. Expression of LKB1 S325A/S428A mutant stimulates AMPK in CHO (A) and UACC257 (B) cells. CHO cells were transfected with HA-tagged LKB1 WT, AA mutant or control vector. UACC257 melanoma cells were infected with retrovirus containing LKB1 WT, AA mutant or control vector. Cell lysates were used for western blotting analysis with indicated antibodies.



Supplemental Figure 12. U0126 treatment enhances phospho-AMPK staining in SK-Mel-28 cells. SK-Mel-28 cells were treated with DMSO or U0126 for 2 hr, embedded in parafin and stained with phospho-AMPK antibody. To prepare for a cell-block, cells were trypsinized, fixed with 10% formalin for 20 minutes, washed with human plasma, and mixed with thrombin (1:1 ratio) for gel formation.



Supplemental Figure 13. Regulation of LKB1 Ser325 and Ser428 phosphorylation by EGF stimulation. Cos-7 cells stably expressing FLAG-LKB1 were treated with DMSO, U0126 (20 μM) for 2 hr before stimulated with 100 ng/ml EGF for 10 min. Cell lysates were immunoprecipitated with anti-FLAG M2 agarose beads followed by western blotting using indicated antibodies. Numbers indicate relative intensity as quantified by image J analysis.