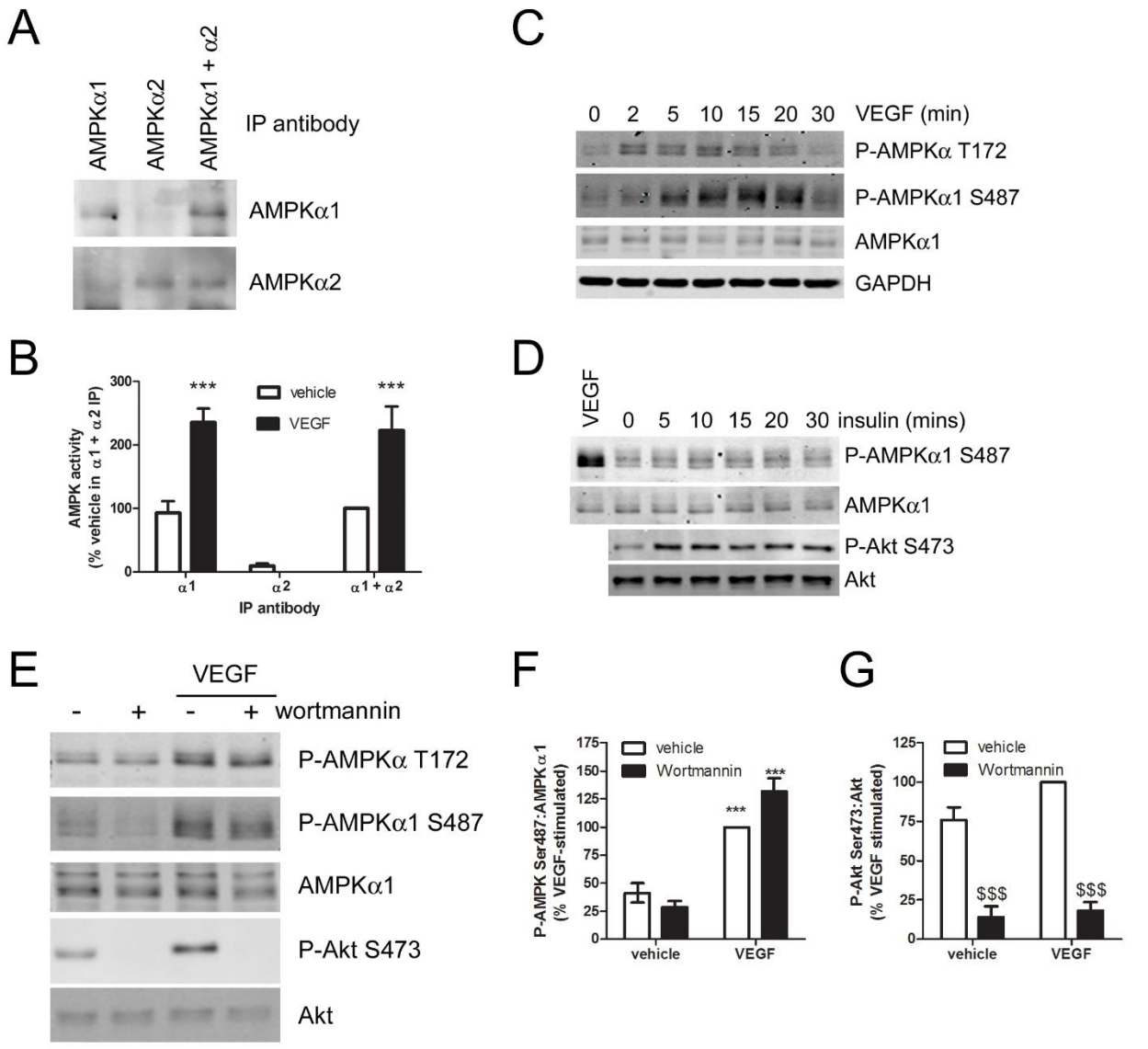
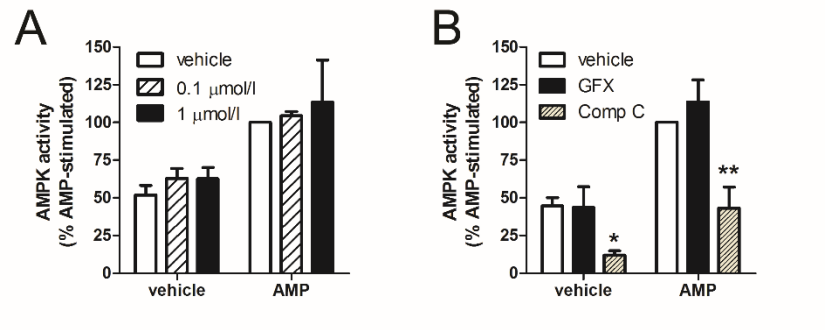


Supplemental Figure 1: VEGF-stimulated AMPKα1 Ser487 phosphorylation is not mediated by Akt.

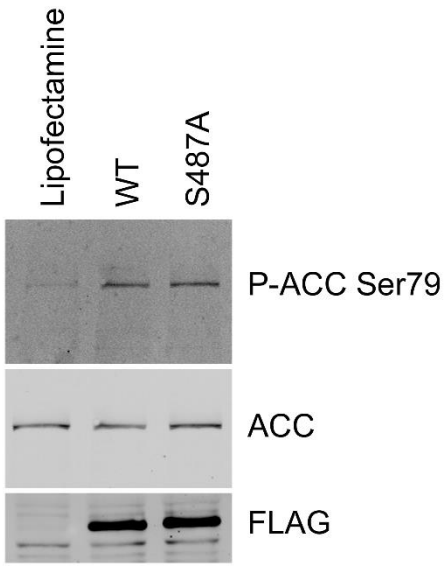
(A, B) HAECs were stimulated in the presence or absence of VEGF (10 ng/ml) for 5 min and lysates prepared. AMPK complexes were immunoprecipitated with sheep anti-AMPKα1 or anti-AMPKα2 antibodies or a mixture of both and (A) the immunoprecipitates analysed by immunoblotting with isoform-specific anti-AMPKα antibodies or (B) AMPK activity assessed in the immunoprecipitates. (A) Representative immunoblots are shown, repeated with similar results on two further occasions. (B) AMPK activity from five independent experiments (mean ± SEM). (C) HUVECs were

incubated in 10 ng/ml VEGF for the times indicated, lysates were prepared and immunoblotted with the antibodies indicated. Representative immunoblots are shown, repeated with similar results on two further occasions. (D) HAECs were incubated in 1 µmol/l insulin for the times indicated or 10 ng/ml VEGF for 5 min. Lysates were prepared and subjected to immunoblotting with the antibodies indicated. Representative immunoblots are shown, repeated on two further occasions with similar results. (E,F,G) HUVECs were pre-incubated in 100 nmol/l wortmannin for 45 min prior to stimulation in the presence or absence of 10 ng/ml VEGF for 5 min. HAEC lysates were prepared and subjected to immunoblotting with the antibodies indicated. (E) Representative immunoblots are shown, repeated on three further occasions with similar results. (F,G) Quantification of immunoblots (mean ± SEM). ***p<0.001 relative to absence of VEGF, $$$$p$<0.001 relative to absence of wortmannin.



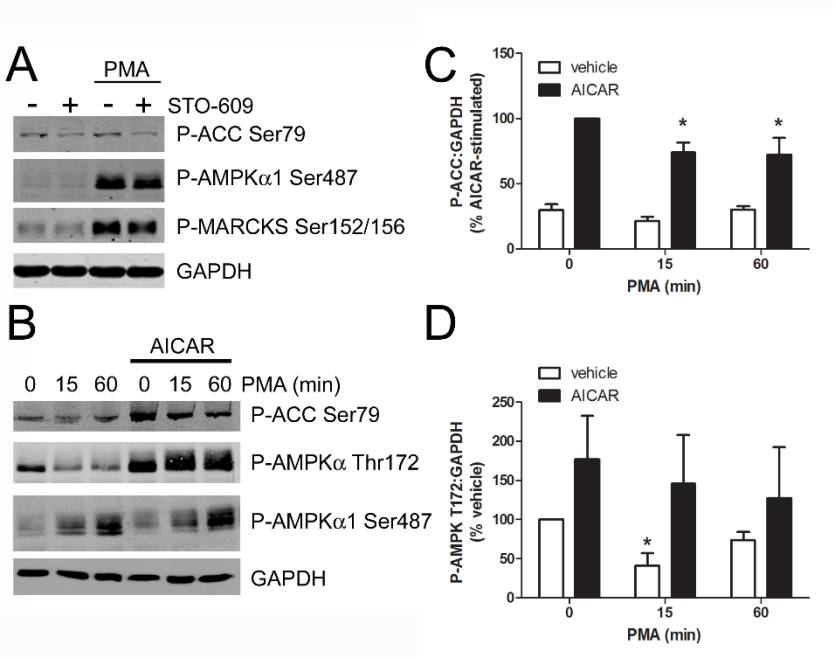
Supplemental Figure 2: LY333531 and GF109203X have no direct effect on AMPK activity.

AMPKα1 and AMPKα2 were immunoprecipitated from HUVEC lysates and incubated in the presence or absence of 0.2 mmol/l AMP and (A) the indicated concentrations of LY333531 or (B) 1 µmol/l GF109203X (GFX) or 1 µmol/l compound C prior to AMPK activity assay. Data shown is from three independent experiments (mean ± SEM). *p<0.05, **p<0.01 relative to absence of compound C.



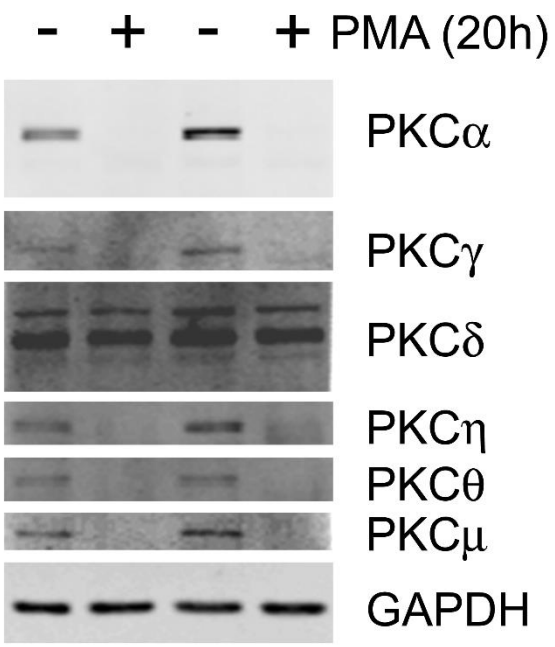
Supplemental Figure 3: Expression of AMPKα1 in AMPK KO MEFs

AMPK KO MEFs were transiently transfected with FLAG-tagged AMPKα1 (WT) or mutant AMPKα1 Ser487Ala and cell lysates prepared. Lysates were resolved by SDS-PAGE and immunoblotting with the antibodies indicated. Representative immunoblots are shown, repeated with similar results on two further occasions.



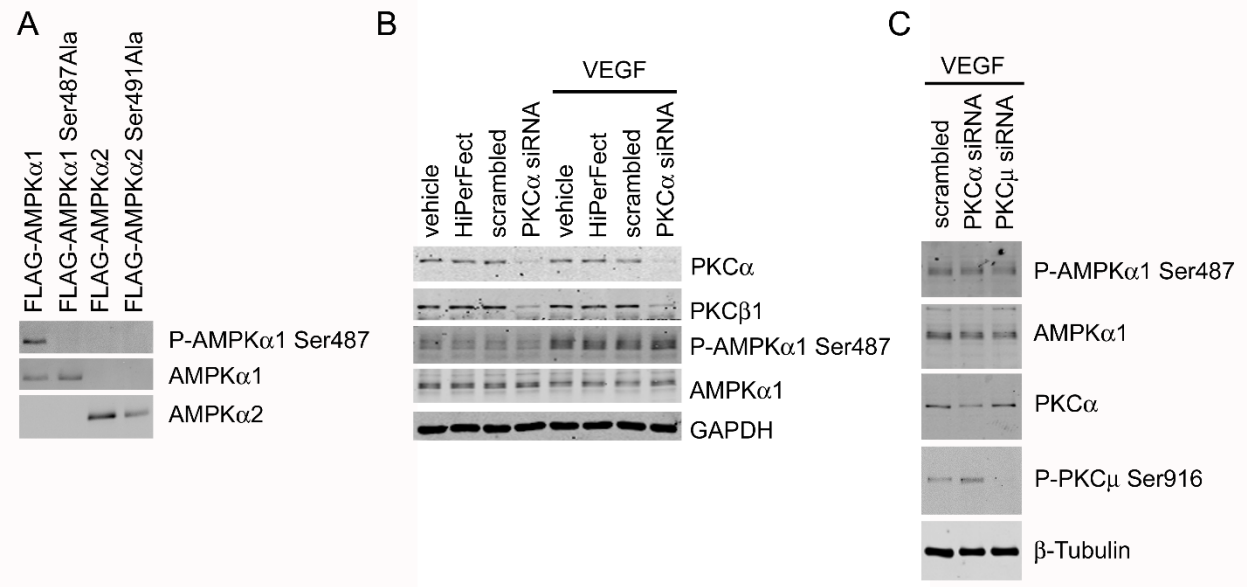
Supplemental Figure 4: PMA stimulates AMPKα1 Ser487 phosphorylation and inhibits AICAR-stimulated ACC phosphorylation in HeLa cells.

Cell lysates were prepared from (A) HeLa cells incubated in 10 µmol/l STO-609 for 1 h prior to stimulation with 1 µmol/l PMA for 20 min, (B,C,D) HeLa cells stably expressing LKB1 incubated in the presence or absence of AICAR (1 mmol/l, 45 min) and/or PMA (1 µmol/l) for 15 or 60 min. Lysates were resolved by SDS-PAGE and immunoblotting with the antibodies indicated. (A,B) Representative immunoblots are shown, repeated with similar results on four and two further occasions respectively. Quantification of (C) ACC phosphorylation or (D) AMPKα T172 phosphorylation (mean ± SEM). *p<0.05 relative to absence of PMA.



Supplemental Figure 5: Effect of chronic PMA treatment on PKC isoform levels in HUVECs and HAECs.

HUVECs or HAECs were cultured for 20 h in the presence or absence of 0.2 µmol/l PMA, cell lysates were prepared and subjected to immunoblotting with the antibodies indicated.



Supplemental Figure 6: siRNA-mediated downregulation of PKCα or PKCμ has no effect on VEGF-stimulated AMPK Ser487 phosphorylation in HUVECs.

(A) HEK-293 cells were transfected with vectors containing FLAG-tagged AMPKα1, AMPKα1 Ser487Ala, AMPKα2 or AMPKα2 Ser491Ala. FLAG-tagged AMPK was immunoprecipitated and incubated in the presence of purified rat brain PKC in the presence of Ca^{2+} and PtdSer. Proteins from washed immunoprecipitates were analysed by immunoblotting with the antibodies indicated. Representative immunoblots are shown. (B,C) HUVECs were incubated with 200 nmol/l siRNA targeting PKCα, scrambled siRNA, (C) PKCμ or HiPerFect alone for 48 h prior to stimulation with VEGF (10 ng/ml, 5 min). Cell lysates were prepared and subjected to SDS-PAGE/immunoblotting with the antibodies indicated. Blots shown are representative of three independent experiments in each case.