SUPPLEMENTAL DATA

Fig. S1. AMPK activity in different cancer cell lines and in primary keratinocytes. GAPDH served as a gel loading control. P-AMPKα and P-ACC levels were normalized to GAPDH levels (bottom). The graphs show the calculated averages and S.D. from two independent experiments.

Fig. S2. c-Src regulates AMPK through a PKC isoform. Cells were starved for serum and after 2h were treated with BIS1 **(A, B)** or PP1 **(C, D).** After 4 h cells were lysed, and analyzed by western blot. **(A, C)** A431 cells **(B, D)** OVCAR3 cells. Representative western analysis using the indicated antibodies. GAPDH served as a gel loading control. P-AMPKα and P-ACC levels were normalized to GAPDH levels (bottom) **(A, B)**. The graphs show the calculated averages and S.D. from three independent experiments. **(C, D)** The graphs show the calculated averages and S.D. from two independent experiments.

Fig. S3. c-Src regulates AMPK through PKCα. **(A, B)** Cells were starved for serum and after 2h were treated with PP1. After 4 h cells were lysed, and analyzed by western blot. **(C, D)** Cells were transfected with siRNA against c-Src. After 96h cells were lysed, and analyzed by western blot. **(A, C)** A431 cells **(B, D)** OVCAR3 cells. Representative western analysis using the indicated antibodies. GAPDH served as a gel loading control. V-vehicle; siCtrl-non-targeting siRNA; siSrc- siRNA against c-Src. P-PKCα levels were normalized to GAPDH levels (bottom) **(A, B)**. The graphs show the calculated averages and S.D. from four independent experiments. **(C, D)** The graphs show the calculated averages and S.D. from three independent experiments.

Fig. S4. Depletion of PDK1 had no effect on the activity of PKCα. Cells were transfected with siRNA against PDK1. After 96h, cells were lysed, and analyzed by western blot. **(A)** OVCAR3 cells **(B)** A431 cells. Representative.western analysis using the indicated antibodies. GAPDH served as a gel loading control. V-vehicle, siCtrl-non-targeting siRNA; siPDK1- siRNA against PDK1. **(A, B)** P-PKCα levels were normalized to GAPDH levels (bottom). The graphs show the calculated averages and S.D. from two independent experiments.

Fig. S5. Immunoprecipitation with PLCγ1 specific antibody. Protein lysates from A431 **(A)** or OVCAR3 cells **(B)** were immunoprecipitated with PLCγ1-specific antibody (2822, which recognizes an epitope in the C-terminus) or control IgG. The blots, which are part of the same gel, were cut for ease of presentation.

Fig. S6. c-Src regulates PKCα through PLCγ1. (A, B) Cells were starved for serum and after 2h were treated with PP1. After 4 h cells were lysed, and analyzed by western blot. **(C, D)** Cells were transfected with siRNA against c-Src. After 96h cells were lysed, and analyzed by western blot. **(A, C)** A431 cells **(B, D)** OVCAR3 cells. Representative western analysis using the indicated antibodies. GAPDH served as a gel loading control. V-vehicle; siCtrl-non-targeting siRNA; siSrc- siRNA against c-Src. **(A-D)** P-PLCγ levels were normalized to GAPDH levels (bottom). The graphs show the calculated averages and S.D. from two independent experiments.

Fig. S7. c-Src regulates PLCγ1. NIH 3T3 and SrcNIH cells were starved for serum and after 2h were treated with PP1. After 4 h cells were lysed, and analyzed by western blot. P-PLCγ levels were normalized to GAPDH levels (bottom). The graphs show the calculated averages and S.D. from two independent experiments.

Fig. S8. Activation of AMPK by c-Src is mediated through PKCα and LKB1. (A, B) Cells were starved for serum and after 2h were treated with PP1. After 4 h cells were lysed, and analyzed by western blot. **(C, D)** Cells were transfected with siRNA against c-Src. After 96h cells were lysed, and analyzed by western blot. **(A, C)** A431 cells **(B, D)** OVCAR3 cells. Representative western analysis using the indicated antibodies. GAPDH served as a gel loading control. V-vehicle; siCtrl-non-targeting siRNA; siSrc- siRNA against c-Src. **(A-D)** P-LKB1 levels were normalized to GAPDH levels (bottom). The graphs show the calculated averages and S.D. from two independent experiments. **(E). OVCAR** cells were transfected

with siRNA against LKB1. After 72 h cells were lysed and analyzed by western blot. A representative blot is shown.

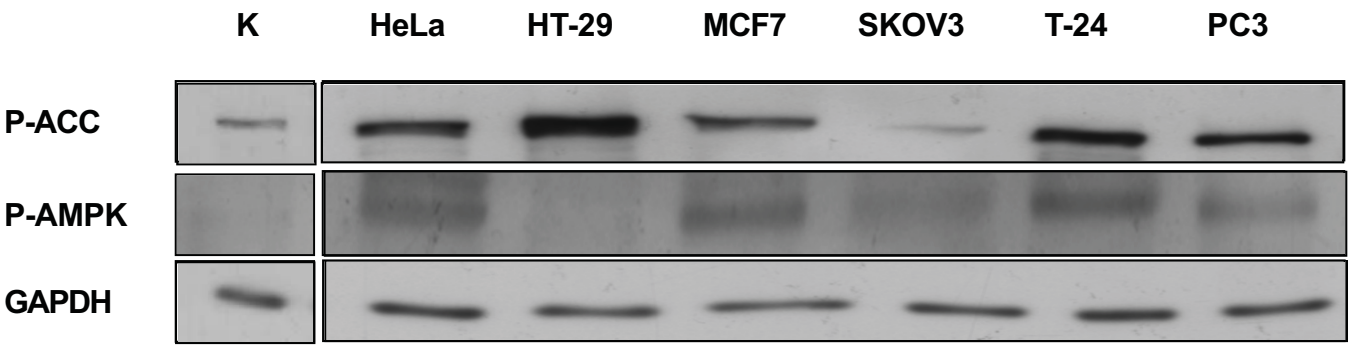
Fig. S9. (A). GST-LKB1 is a direct substrate of PKCα in a cell free assay. GST-tagged LKB1 (lanes 4-7), was expressed in HEK-293T cells, purified on glutathione-Sepharose and added in the indicated amounts to test for the ability to undergo phosphorylation by PKCα. Recombinant GST-PKCα was tested for its ability to phosphorylate GST-LKB1 using [γ-^{32}P] ATP as described in Materials and Methods. Lanes 1-3 represent controls. The reaction was analyzed by SDS-PAGE and autoradiography (UPPER PANEL). Arrows show the migration of GST-PKCα (which autophosphorylates) and GST-LKB1. The membrane was also analyzed by western blotting and probed with anti PKCα, anti-P-LKB1 and anti-LKB1 antibodies (LOWER PANEL). The experiment was performed two times (radioactive assay and non-radioactive assay). **(B). Inhibition of c-Src leads to inhibition of PLCγ, PKCα, LKB1 and ACC.** OVCAR3 and A431 cells were starved for 2 h and then treated with PP1 (10μM) for 4 hours longer. Cells were lyzed and analyzed by western blot. Representative blots are shown. The graph shows average values from two independent experiments, where values for the PP1-treated cells (10 μM) were normalized to values for vehicle-treated cells (V).

Fig. S10. IRES vs. cap-dependent translation. A431 and OVCAR3 cells were transfected with the bicistronic reporter construct. 48 h after transfection the cells were treated for 4 h with AICAR and Compound C (CC). After 52 h, cell lysates were prepared and assayed for firefly luciferase activity (FLuc) **(A,C,F,H)** and renilla activity (RLuc) **(B,D,E,G)**. **(A-D)** A431 cells, **(E-H)** OVCAR3 cells. IRES-dependent translation **(A,B,E,F)** and cap-dependent translation **(C,D,G,H)** were measured as described in (138). Values are expressed as a percentage of the control (V-vehicle). Error bars represent the SD of duplicate samples. The experiment was performed three times. **(I)** Cells were transfected with pRF, pRHifF and pStemRHifF. After 48 hours cells were treated with AICAR for 4 hours. Cell lysates were prepared and assayed for firefly luciferase activity (FLuc) and renilla activity (RLuc). Cap-dependent translation of renilla luciferase is repressed in the pStemRHifF plasmid. The increased ratio of firefly luciferase to renilla luciferase activity indicates bona fide IRES translation from the Hif 5' UTR.

Fig. S11. AMPK is not regulated by c-Src in HeLa cells and MCF7 cells. Cells were starved for serum and after 2h were treated with PP1 **(A, B)** or BIS1 **(C, D)**. After 4 h cells were lysed, and analyzed by western blot **(A, C)** HeLa **(B, D)** MCF7. Representative western analysis using the indicated antibodies was shown. Tubulin served as a gel loading control.

Fig. S12. AMPK is not regulated by c-Src in primary keratinocytes. Cells were starved for serum and after 2h were treated with PP1 to inhibit c-Src. After 4 h cells were lysed, and analyzed by western blot. The graph shows the calculated averages and S.D. from two independent experiments.

Fig. S1


K
HeLa
HT-29
MCF7
SKOV3
T-24
PC3
P-ACC
P-AMPK
GAPDH

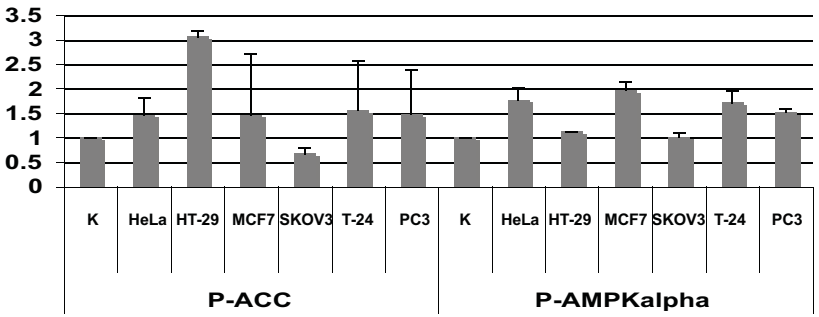
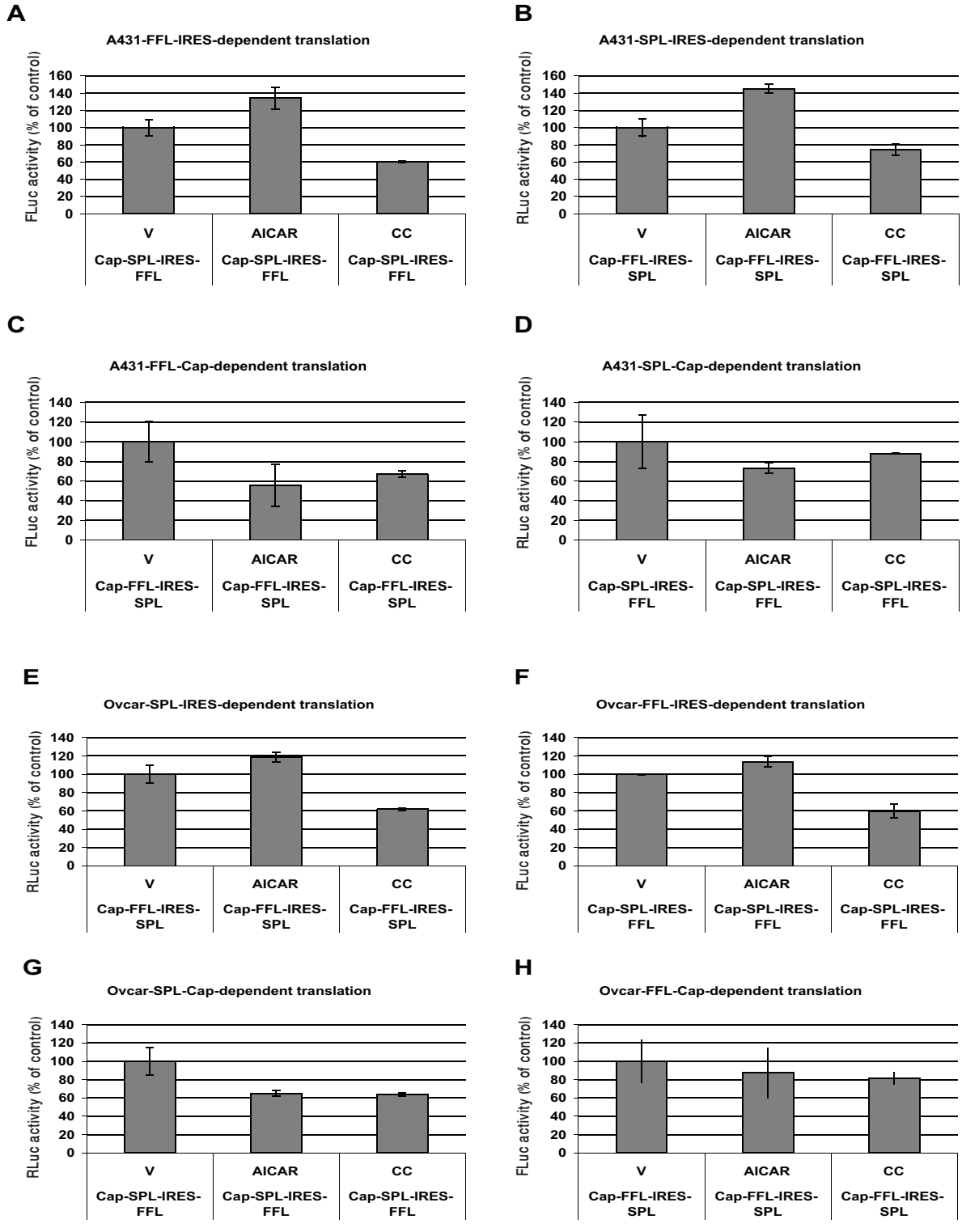

3.5
3
2.5
2
1.5
1
0.5
0
K
HeLa
HT-29
MCF7
SKOV3
T-24
PC3
K
HeLa
HT-29
MCF7
SKOV3
T-24
PC3
P-ACC
P-AMPKalpha

Fig. S10


A
A431-FFL-IRES-dependent translation
FLuc activity (% of control)
V
AICAR
CC
Cap-SPL-IRES-FFL
B
A431-SPL-IRES-dependent translation
RLuc activity (% of control)
Cap-FFL-IRES-SPL
C
A431-FFL-Cap-dependent translation
D
A431-SPL-Cap-dependent translation
E
Ovcar-SPL-IRES-dependent translation
F
Ovcar-FFL-IRES-dependent translation
G
Ovcar-SPL-Cap-dependent translation
H
Ovcar-FFL-Cap-dependent translation

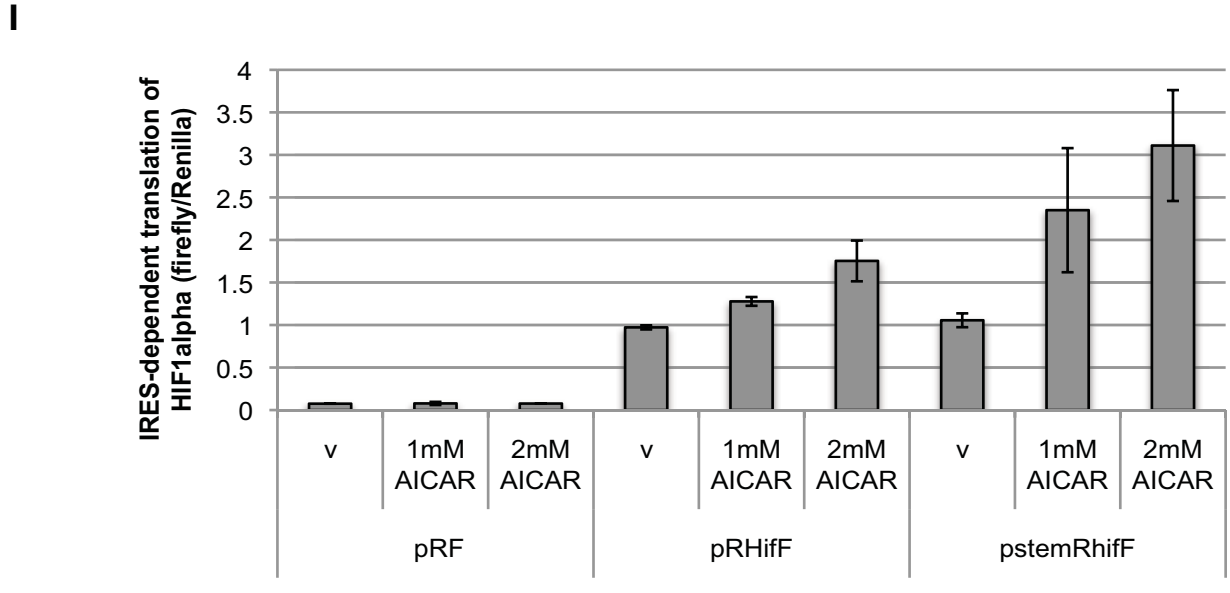

I
IRES-dependent translation of HIF1alpha (firefly/Renilla)
v
1mM AICAR
2mM AICAR
pRF
pRHifF
pstemRhifF

Fig. S11

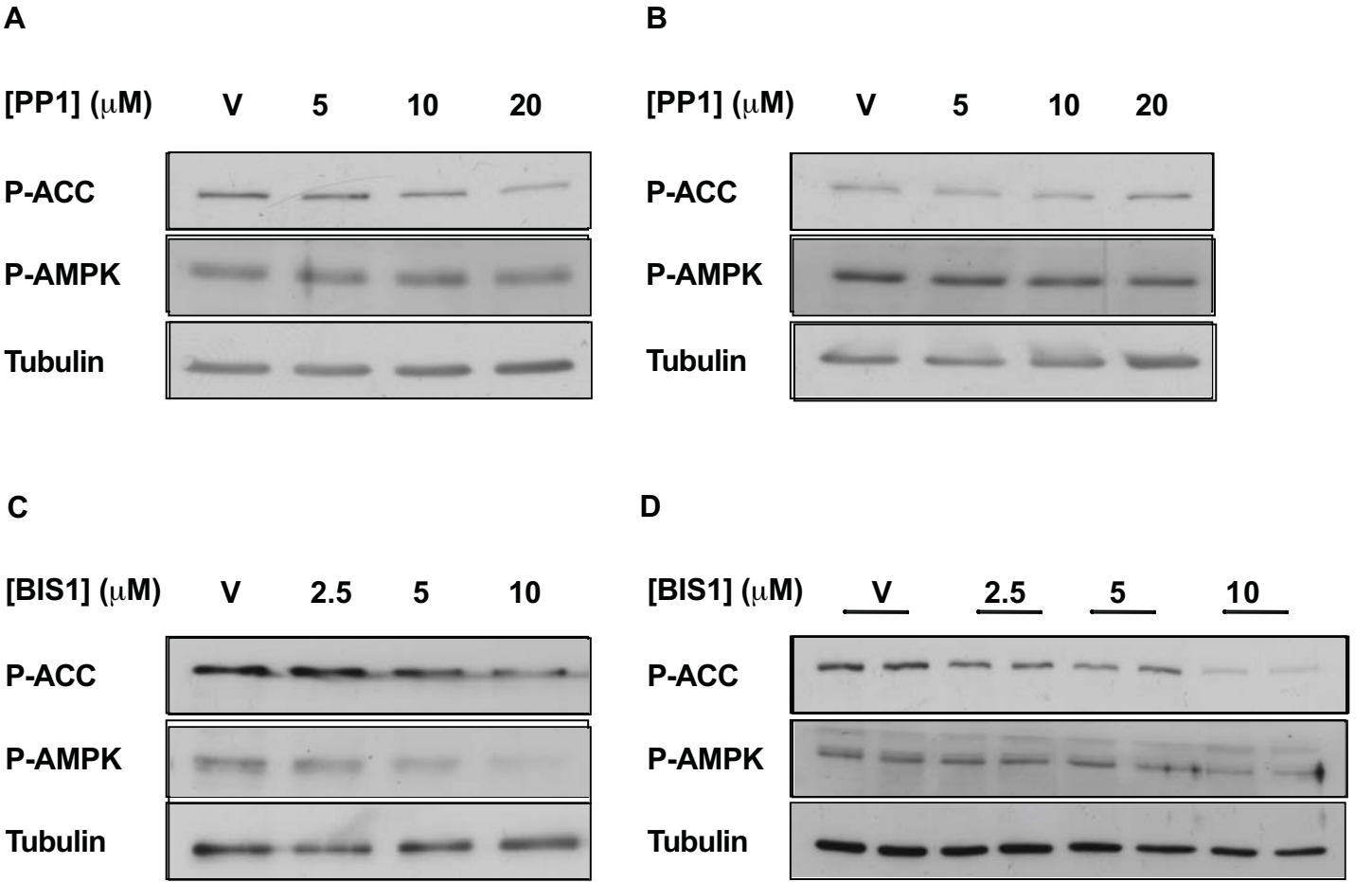
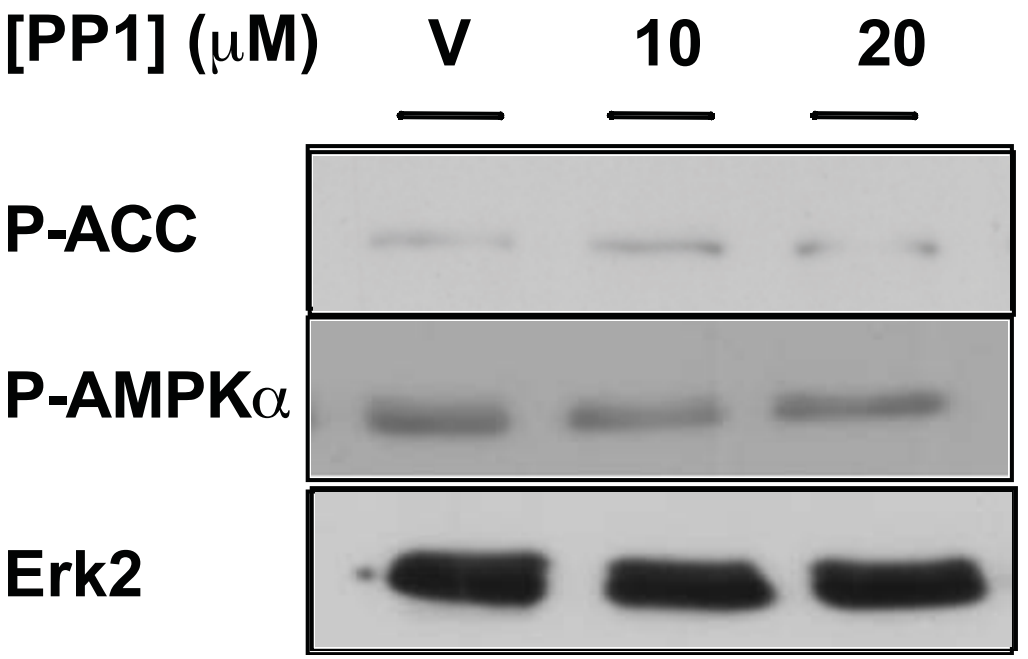

A
[PP1] (μM)
V
5
10
20
P-ACC
P-AMPK
Tubulin
B
[PP1] (μM)
V
5
10
20
P-ACC
P-AMPK
Tubulin
C
[BIS1] (μM)
V
2.5
5
10
P-ACC
P-AMPK
Tubulin
D
[BIS1] (μM)
V
2.5
5
10
P-ACC
P-AMPK
Tubulin

Fig. S12


[PP1] (μM)
V
10
20
P-ACC
P-AMPKα
Erk2

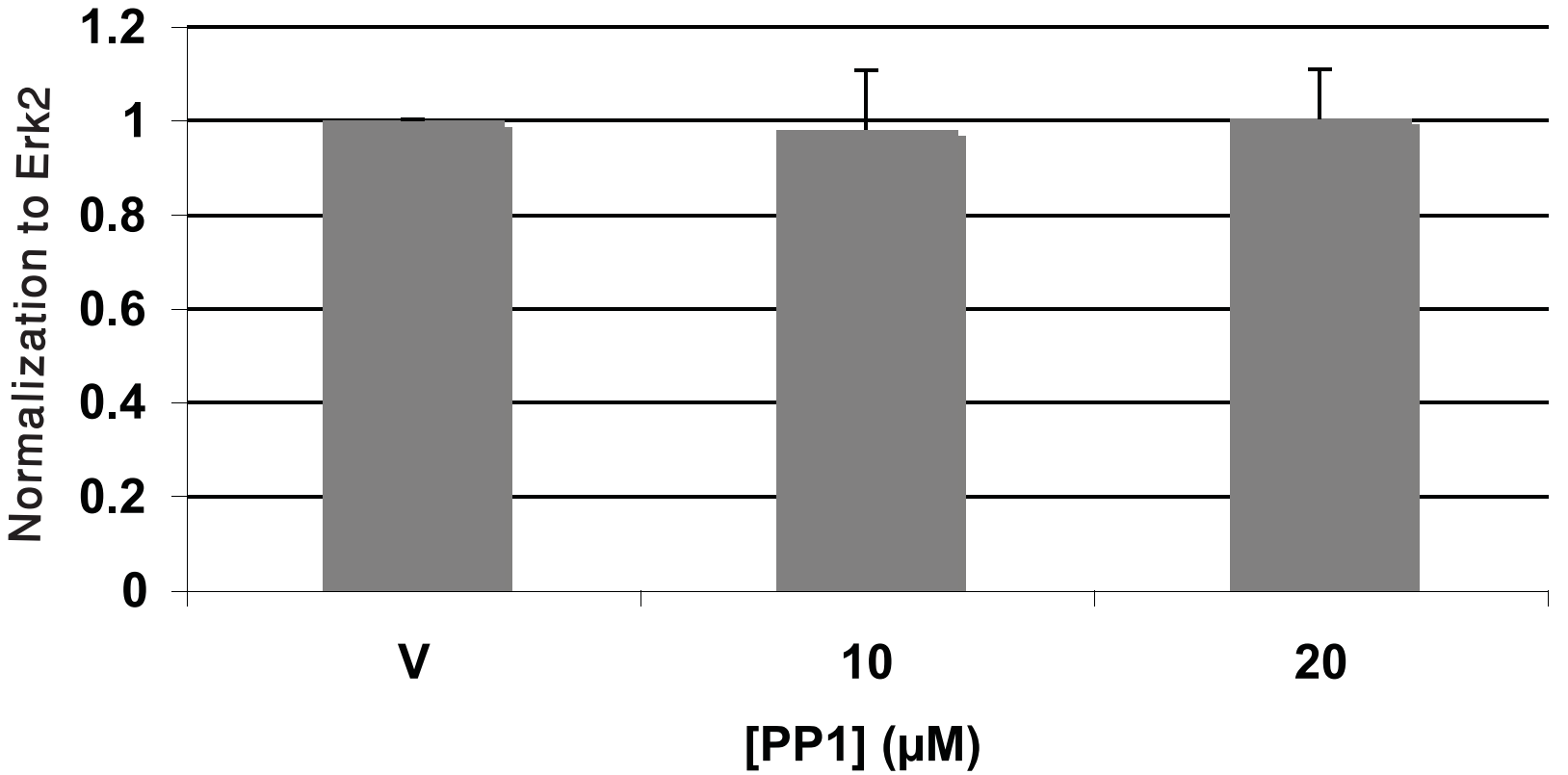

1.2
1
0.8
0.6
0.4
0.2
0
Normalization to Erk2
V
10
20
[PP1] (μM)

Fig. S2

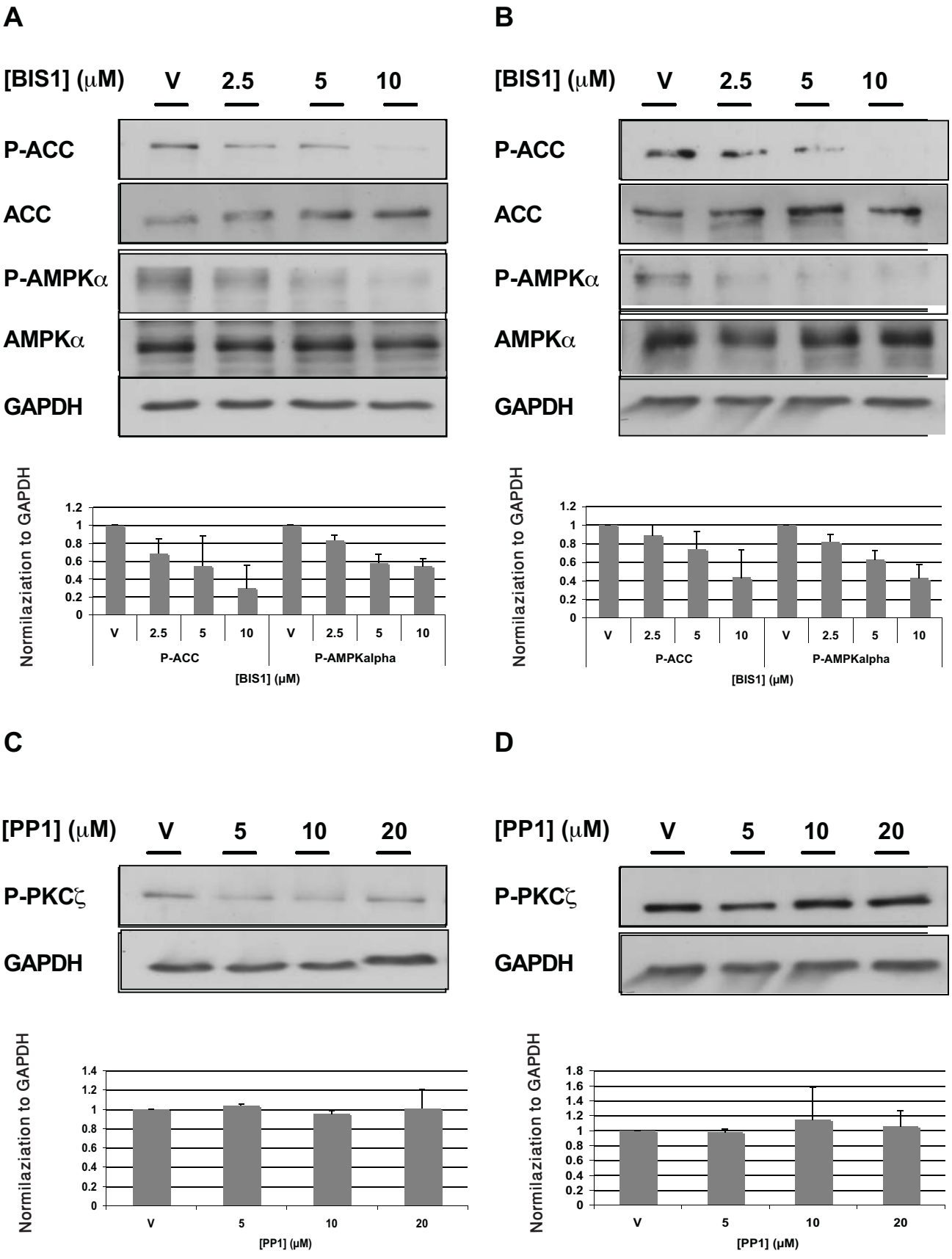

A
[BIS1] (μM)
V
2.5
5
10
P-ACC
ACC
P-AMPKα
AMPKα
GAPDH
Normilaziation to GAPDH
P-ACC
P-AMPKalpha
[BIS1] (μM)
B
[BIS1] (μM)
V
2.5
5
10
P-ACC
ACC
P-AMPKα
AMPKα
GAPDH
Normilaziation to GAPDH
P-ACC
P-AMPKalpha
[BIS1] (μM)
C
[PP1] (μM)
V
5
10
20
P-PKCζ
GAPDH
Normilaziation to GAPDH
[PP1] (μM)
D
[PP1] (μM)
V
5
10
20
P-PKCζ
GAPDH
Normilaziation to GAPDH
[PP1] (μM)

Fig. S3

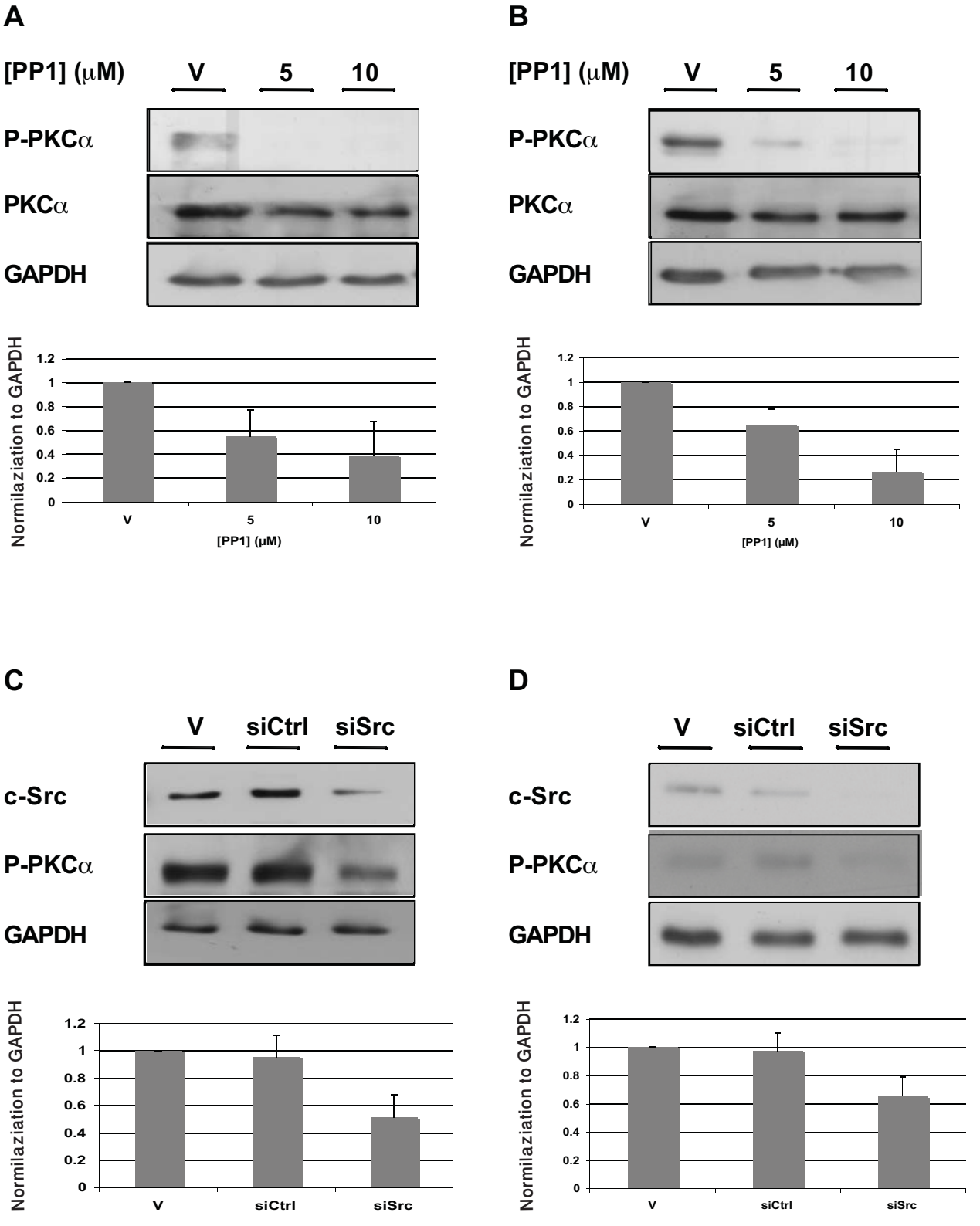

A
[PP1] (μM)
V
5
10
P-PKCα
PKCα
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
5
10
[PP1] (μM)
B
[PP1] (μM)
V
5
10
P-PKCα
PKCα
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
5
10
[PP1] (μM)
C
V
siCtrl
siSrc
c-Src
P-PKCα
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
siCtrl
siSrc
D
V
siCtrl
siSrc
c-Src
P-PKCα
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
siCtrl
siSrc

Fig. S4

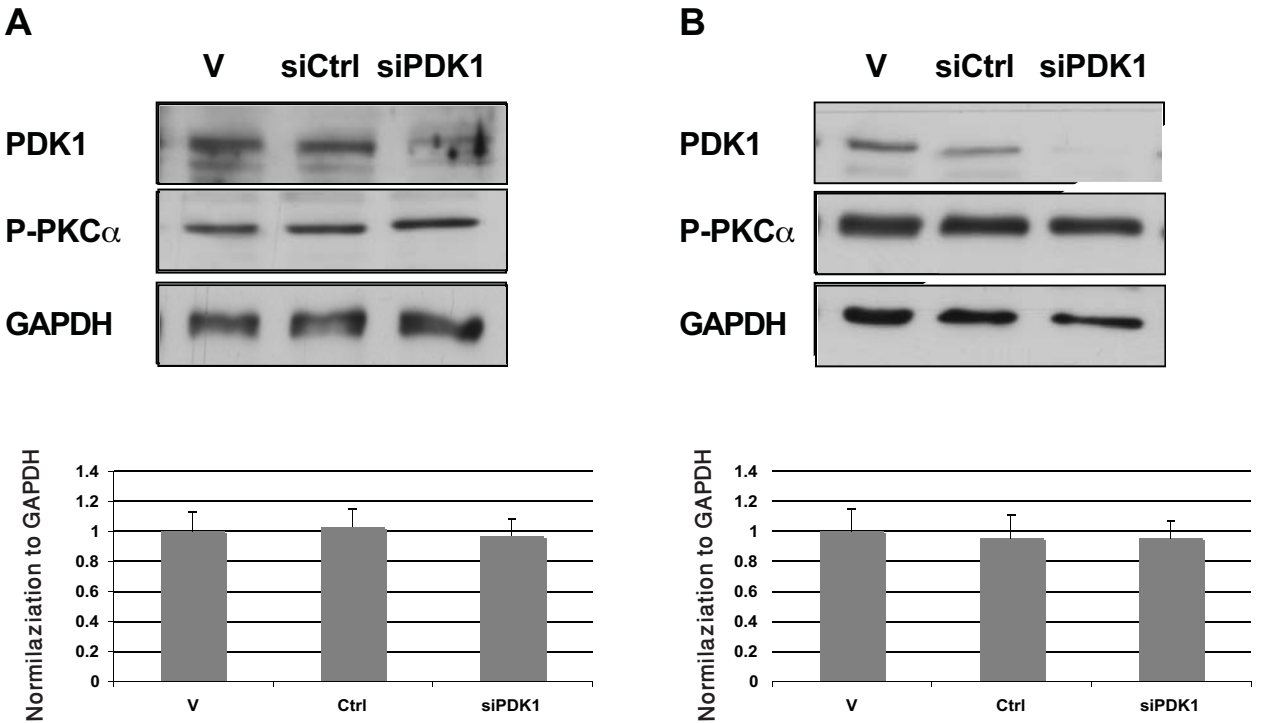

A
V
siCtrl
siPDK1
PDK1
P-PKCα
GAPDH
B
V
siCtrl
siPDK1
PDK1
P-PKCα
GAPDH
Normilaziation to GAPDH
1.4
1.2
1
0.8
0.6
0.4
0.2
0
V
Ctrl
siPDK1
Normilaziation to GAPDH
1.4
1.2
1
0.8
0.6
0.4
0.2
0
V
Ctrl
siPDK1

Fig. S5

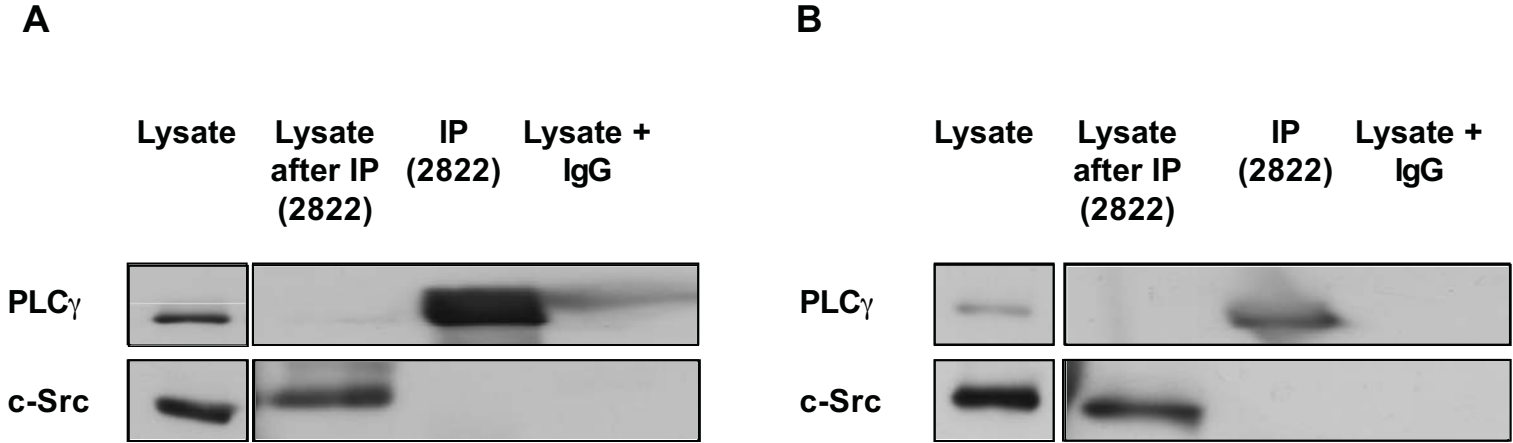

A
Lysate
Lysate after IP (2822)
IP (2822)
Lysate + IgG
PLCγ
c-Src
B
Lysate
Lysate after IP (2822)
IP (2822)
Lysate + IgG
PLCγ
c-Src

Fig. S6

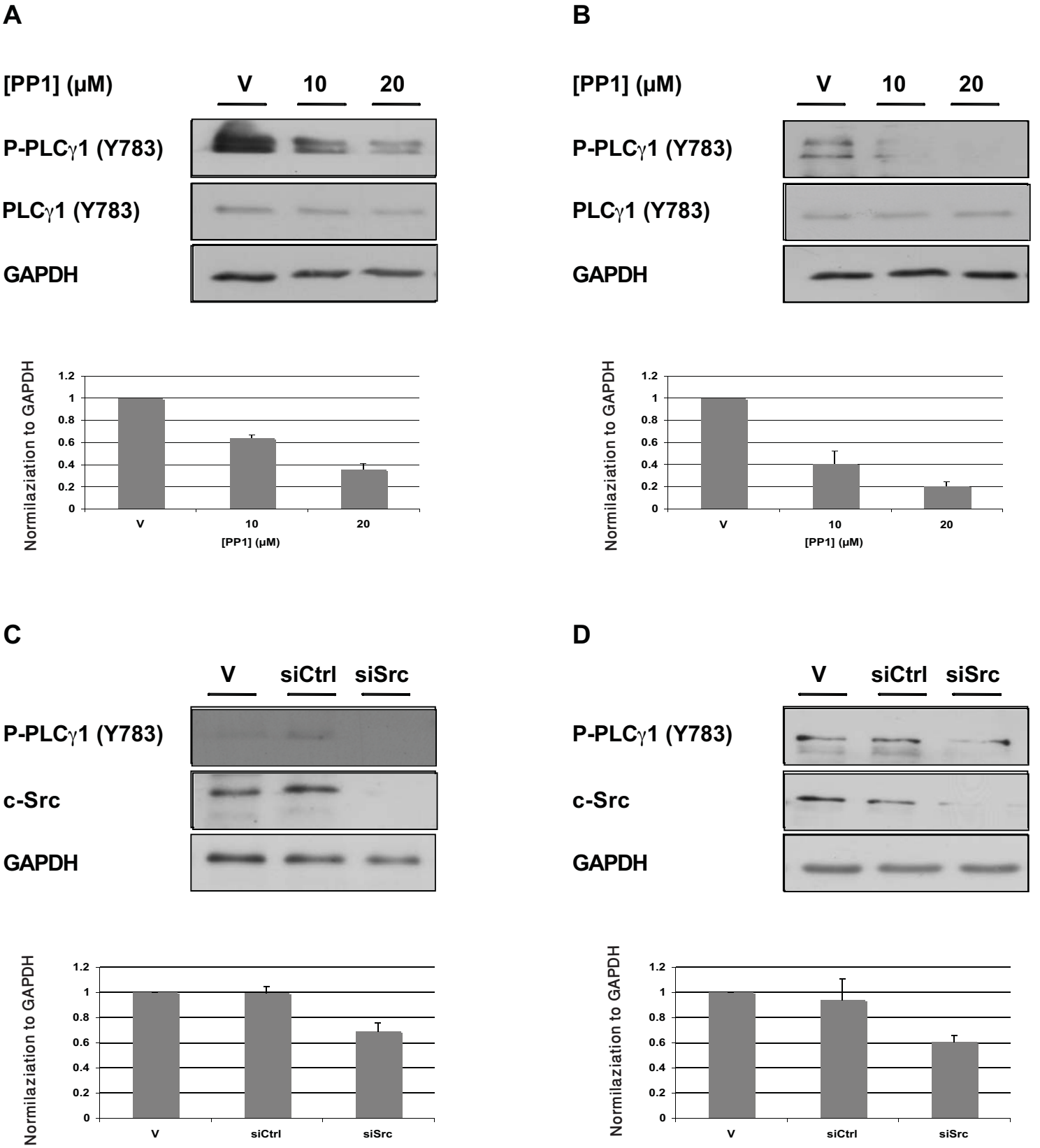
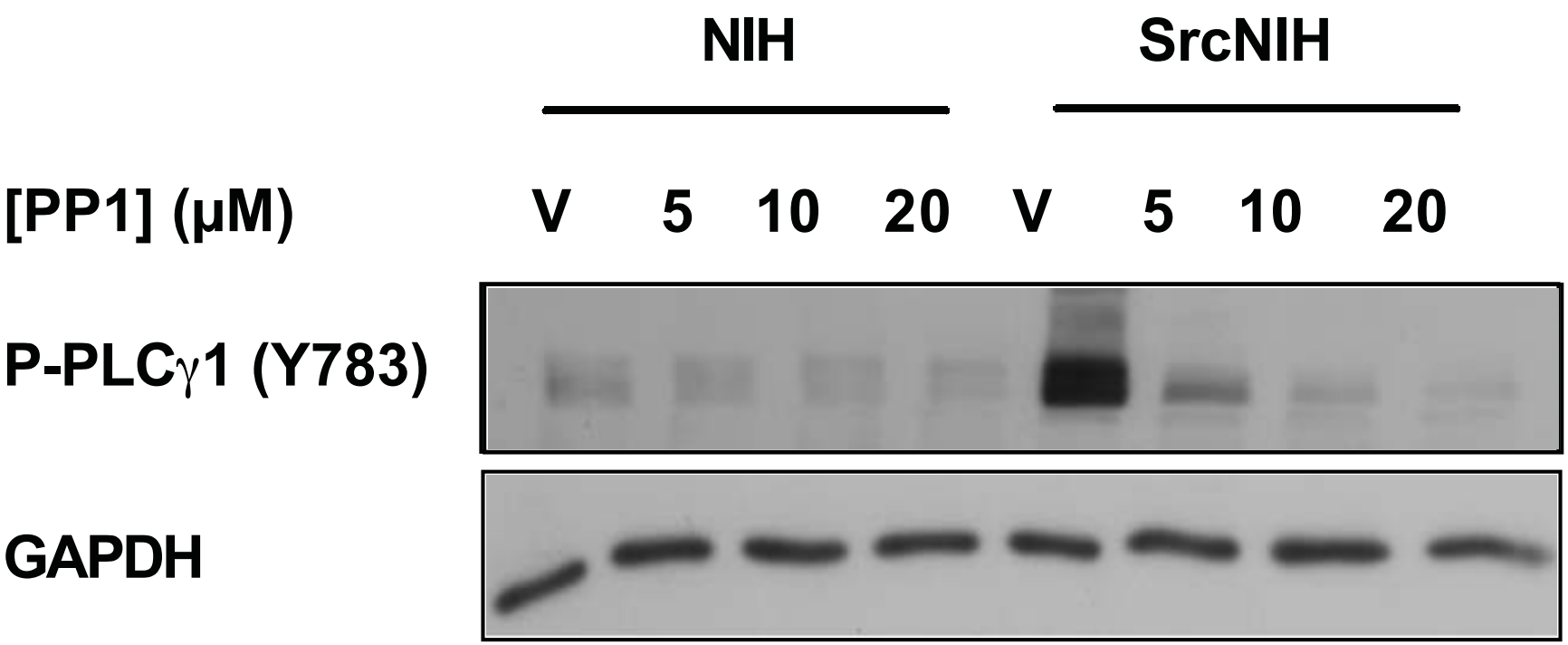

A
[PP1] (μM)
V
10
20
P-PLCγ1 (Y783)
PLCγ1 (Y783)
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
10
20
[PP1] (μM)
B
[PP1] (μM)
V
10
20
P-PLCγ1 (Y783)
PLCγ1 (Y783)
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
10
20
[PP1] (μM)
C
V
siCtrl
siSrc
P-PLCγ1 (Y783)
c-Src
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
siCtrl
siSrc
D
V
siCtrl
siSrc
P-PLCγ1 (Y783)
c-Src
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
siCtrl
siSrc

Fig. S7


NIH
SrcNIH
[PP1] (μM)
V 5 10 20 V 5 10 20
P-PLCγ1 (Y783)
GAPDH

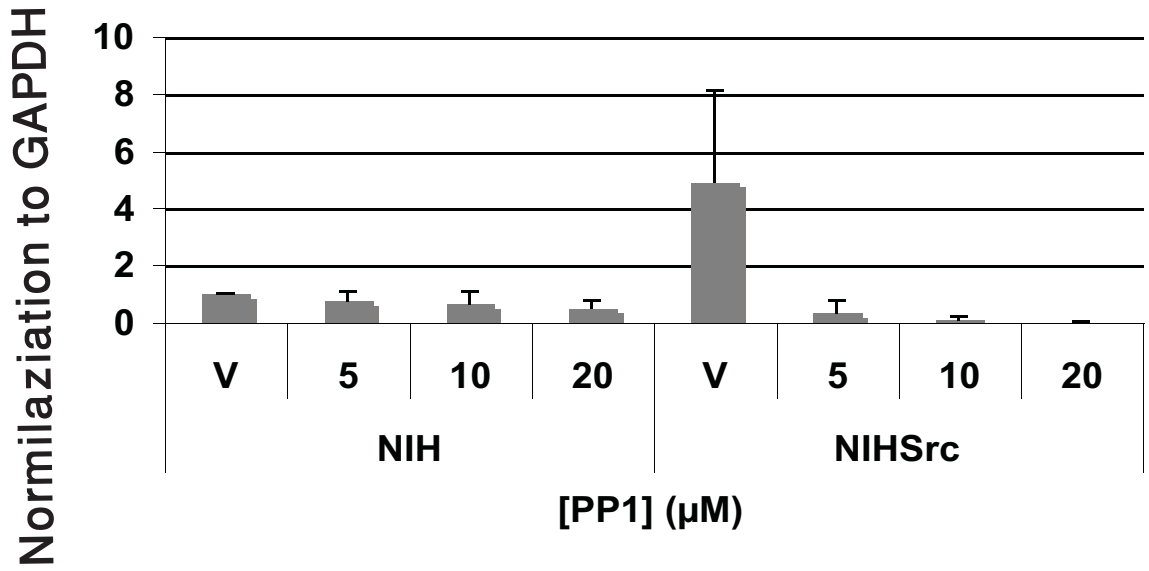

Normilaziation to GAPDH
10
8
6
4
2
0
V 5 10 20 V 5 10 20
NIH
NIHSrc
[PP1] (μM)

Fig. S8

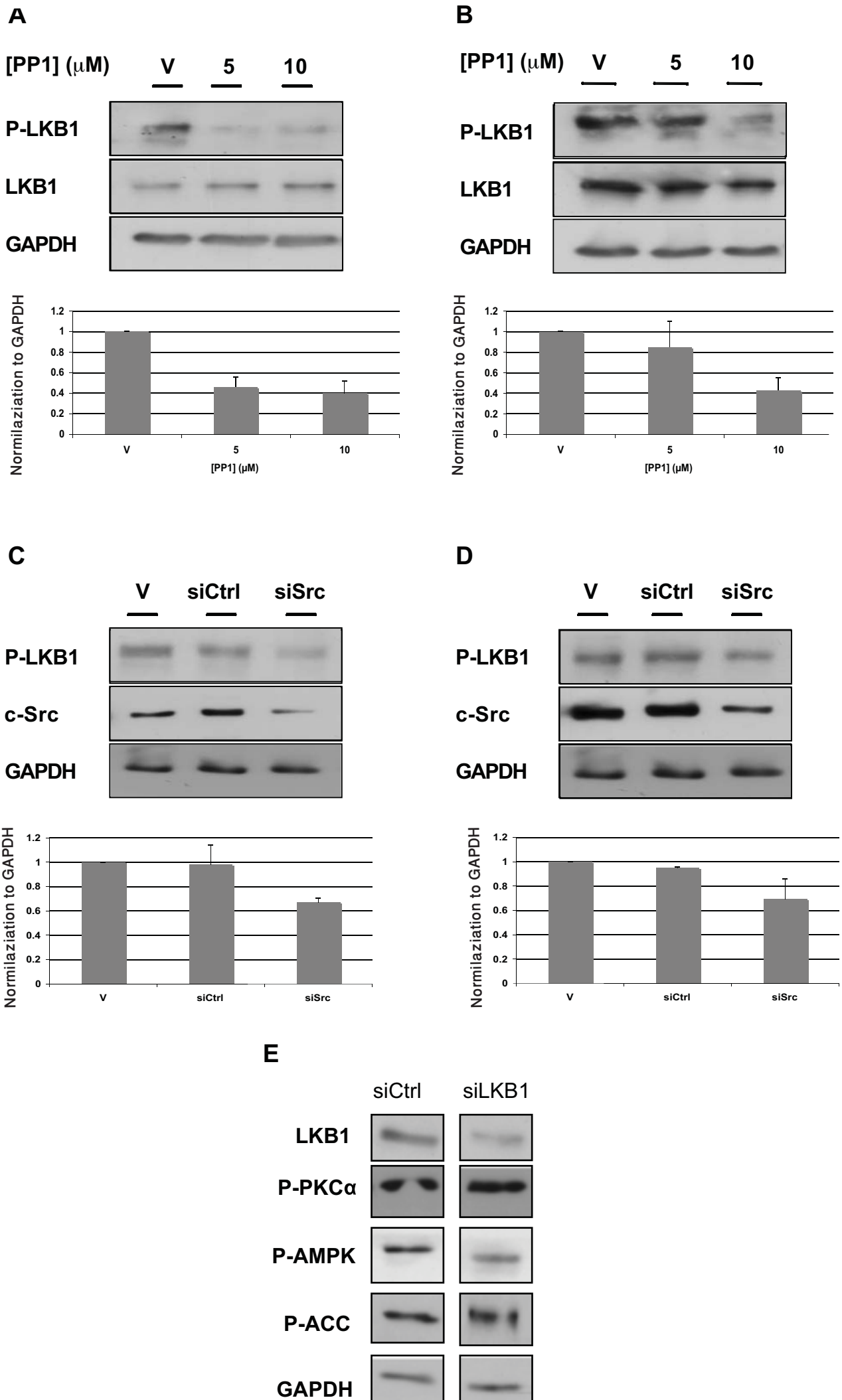
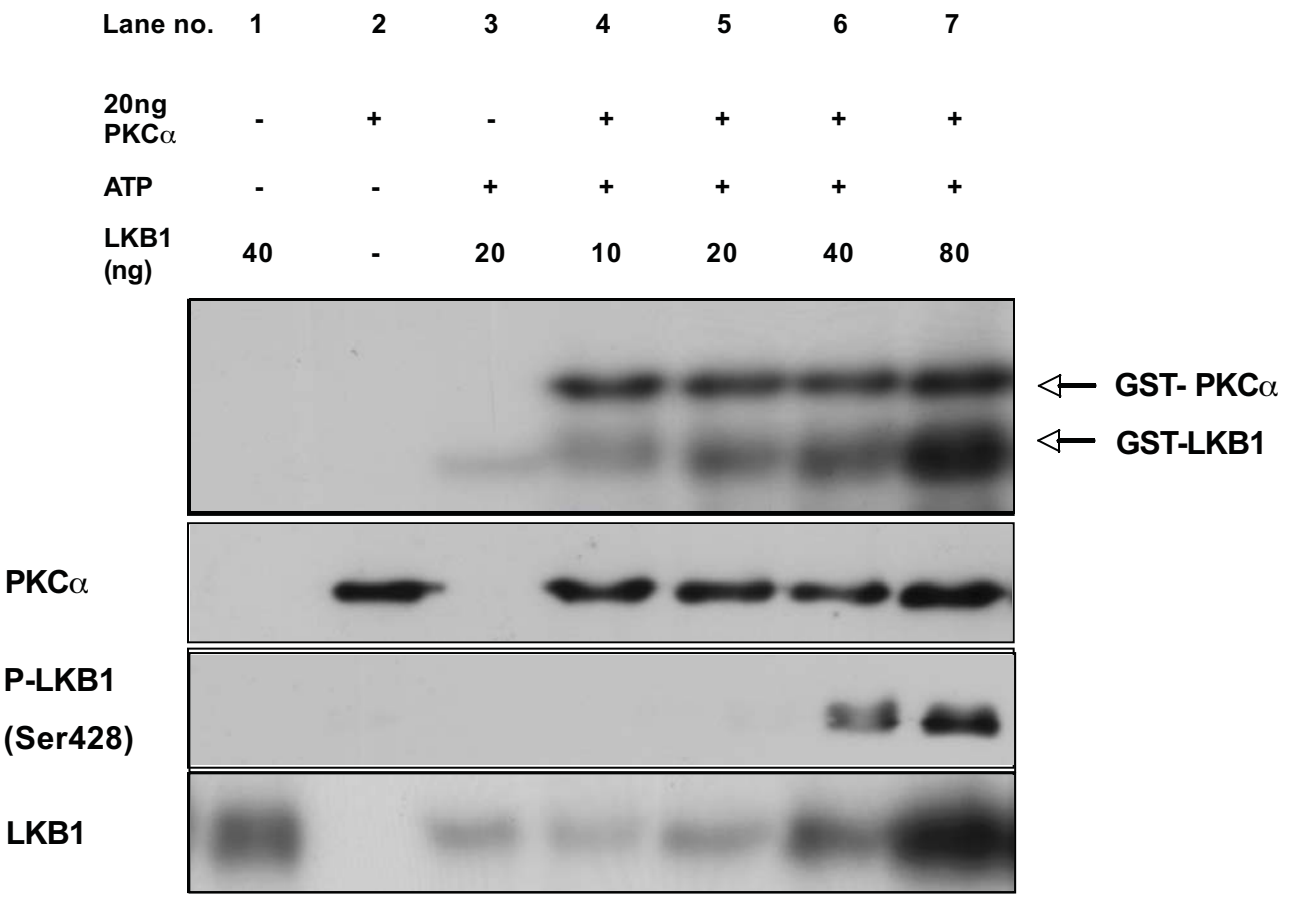
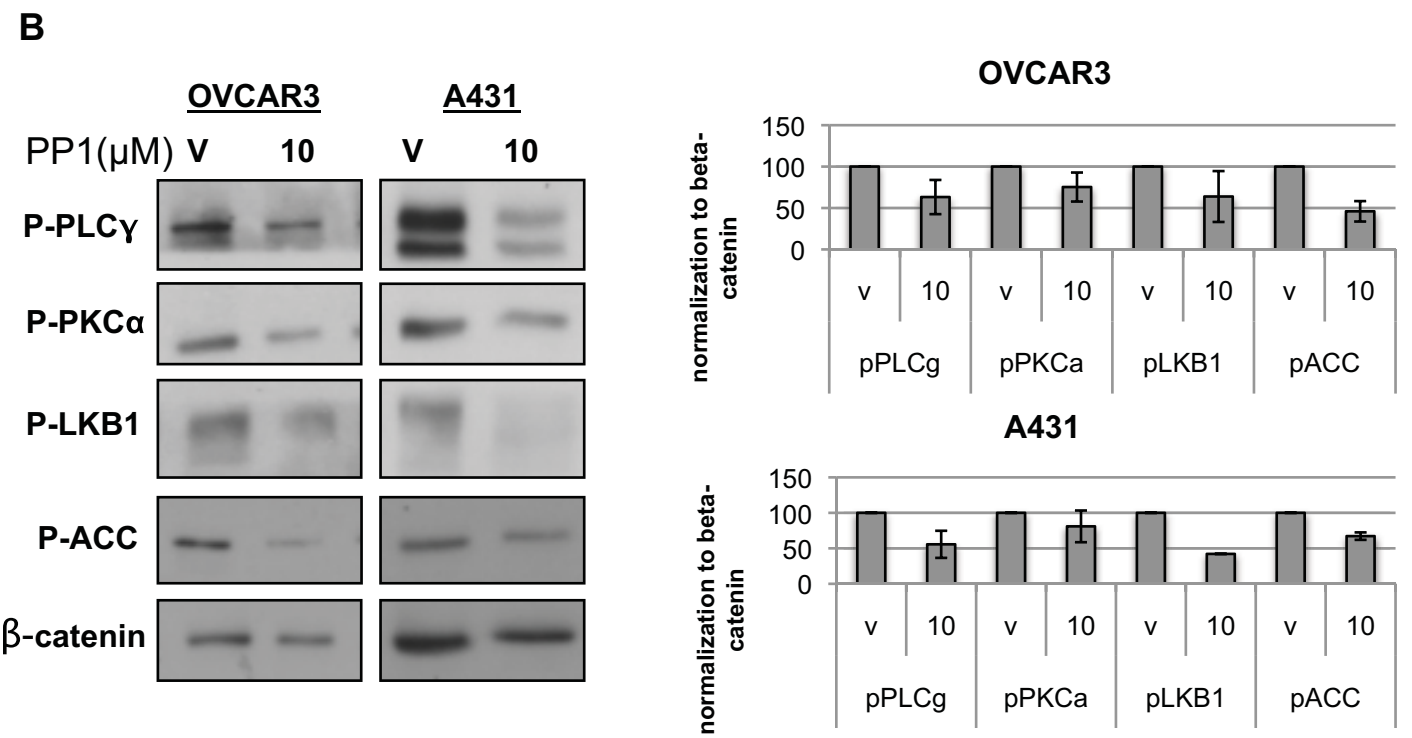

A
[PP1] (μM)
V
5
10
P-LKB1
LKB1
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
5
10
[PP1] (μM)
B
[PP1] (μM)
V
5
10
P-LKB1
LKB1
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
5
10
[PP1] (μM)
C
V
siCtrl
siSrc
P-LKB1
c-Src
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
siCtrl
siSrc
D
V
siCtrl
siSrc
P-LKB1
c-Src
GAPDH
Normilaziation to GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
V
siCtrl
siSrc
E
siCtrl
siLKB1
LKB1
P-PKCα
P-AMPK
P-ACC
GAPDH

Fig. S9


Lane no. 1 2 3 4 5 6 7
20ng PKCα - + - + + + +
ATP - - + + + + +
LKB1 (ng) 40 - 20 10 20 40 80
GST- PKCα
GST-LKB1
PKCα
P-LKB1 (Ser428)
LKB1

B


OVCAR3
A431
PP1(μM) V 10 V 10
P-PLCγ
P-PKCα
P-LKB1
P-ACC
β-catenin
OVCAR3
normalization to beta-catenin
150
100
50
0
v 10 v 10 v 10 v 10
pPLCg pPKCa pLKB1 pACC
A431
normalization to beta-catenin
150
100
50
0
v 10 v 10 v 10 v 10
pPLCg pPKCa pLKB1 pACC