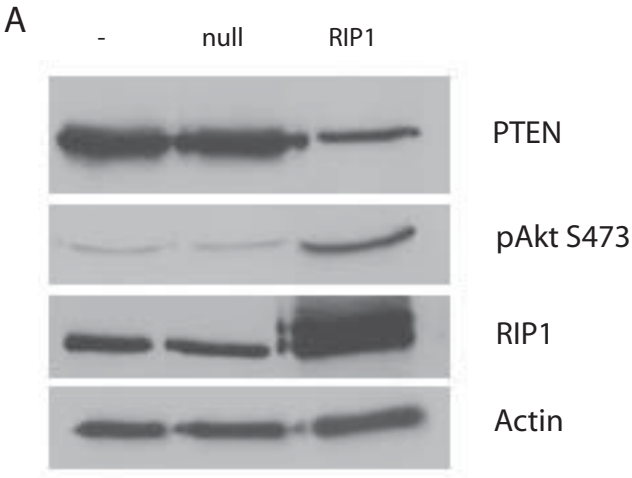
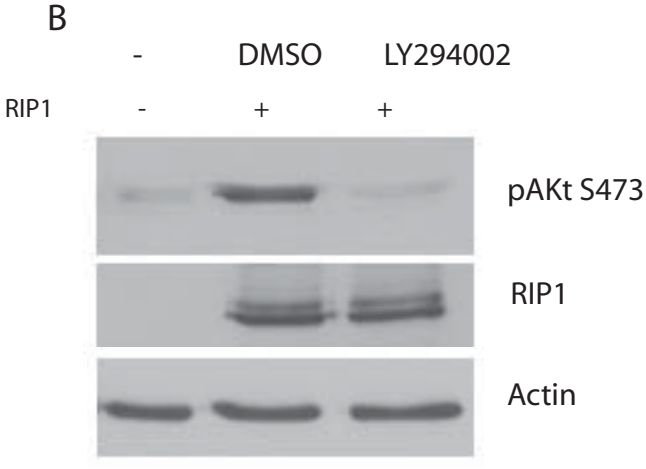
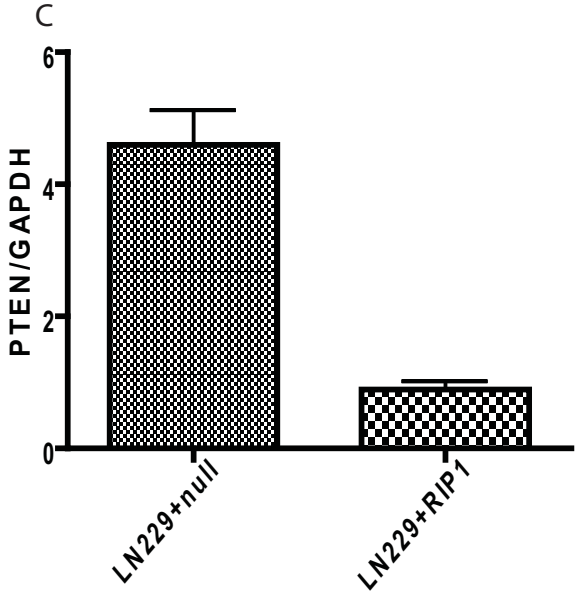
Park, Zhao et al., Supplemental information

1. Figures 1-5
2. Figure legends


A
-
null
RIP1
PTEN
pAkt S473
RIP1
Actin


B
-
DMSO
LY294002
RIP1
-
+
+
pAKt S473
RIP1
Actin


C
PTEN/GAPDH
6
4
2
0
LN229+null
LN229+RIP1

Supplemental Figure 2

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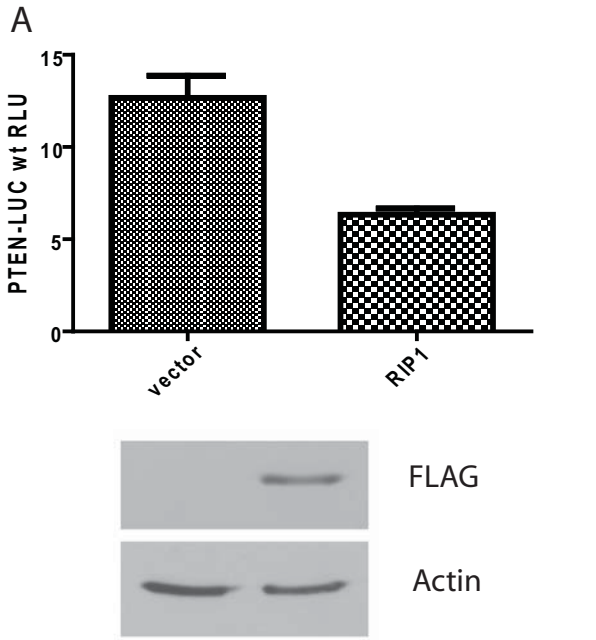
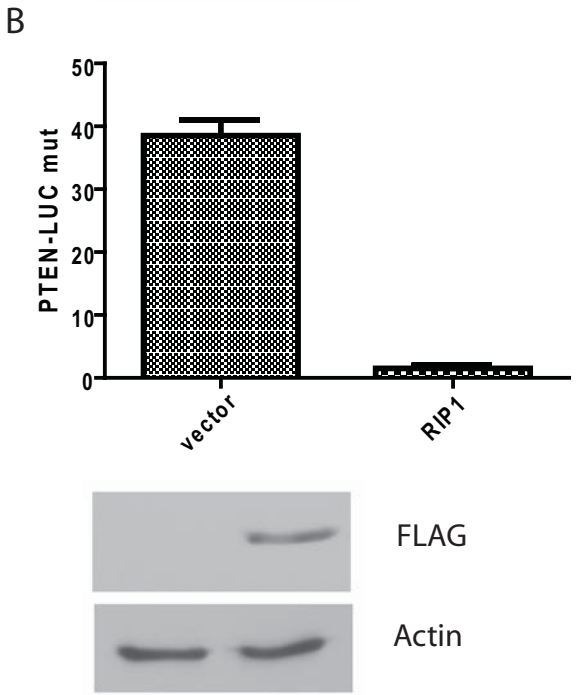
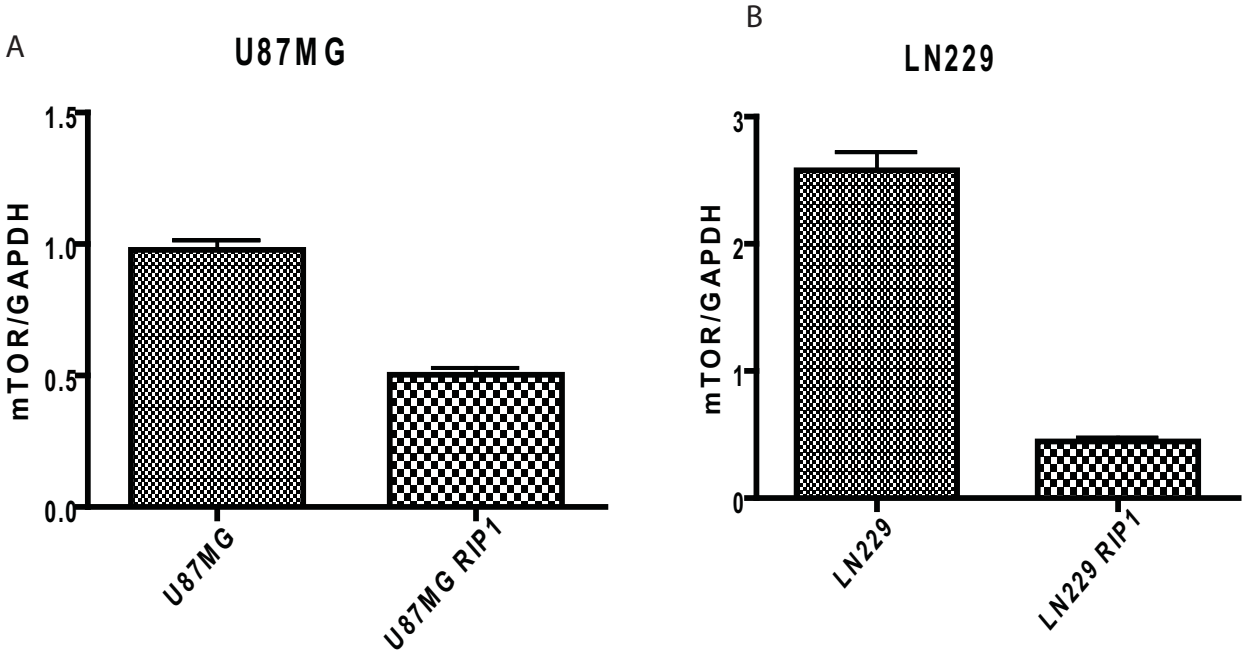




Supplemental Figure 3



Supplemental Figure 4

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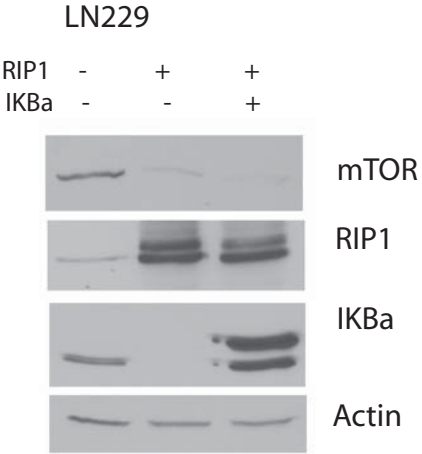

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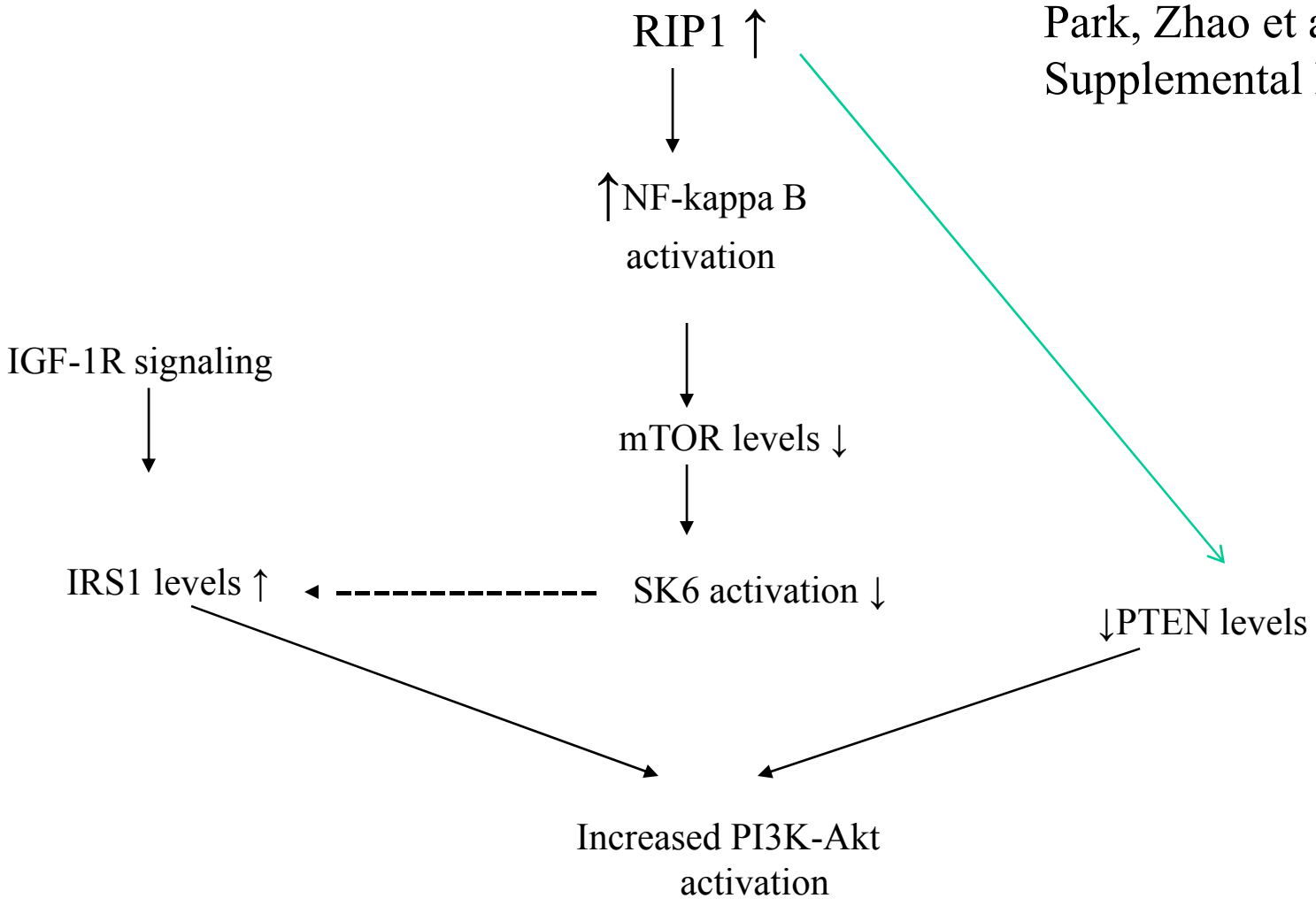
Supplemental Figure 5



Park, Zhao et al., Legends to Supplemental Figures

Supplemental Figure 1

A. LN18 cells (wild type PTEN) were infected with RIP1 adenovirus (MOI 50) followed by preparation of lysates after 24h. Adenovirus null was used as a control. Western blots were probed with a PTEN antibody. Increased RIP1 downregulates PTEN levels and activates Akt as detected by phosphorylation of Akt at S473. B. LN229 cells were infected with RIP1 adenovirus for 12h followed by exposure to DMSO or LY294002 for an additional 12h followed by Western blot. C. RIP1 regulates PTEN mRNA levels. LN229 cells were infected with adenovirus null or adenovirus RIP1 followed by RNA extraction after 24h. Quantitative real-time PCR analysis was conducted and shows that RIP1 downregulates PTEN levels.

Supplemental Figure 2

A. LN229 cells were transfected with the PTEN promoter linked to luciferase (PTEN-LUC) and co-transfected with FLAG-RIP1 plasmid or empty vector. Expression of FLAG-RIP1 was confirmed by Western blot with FLAG antibodies as shown in the lower panel. B. this experiment is similar to A except that a mutant PTEN-LUC reporter missing NF-κB binding sites was used.

Supplemental Figure 3

A and B. show real time quantitative PCR analysis data showing that RIP1 expression downregulates mTOR mRNA in U87MG and LN229 cells. mTOR/GAPDH values are shown.

Supplemental Figure 4

A dominant-negative IκBα super-repressor mutant fails to block the effect of RIP1 on mTOR in LN229 cells. LN229 cells were infected with Ad-RIP1+Ad-null or Ad RIP1+ Ad- IκBαM for 24h followed by preparation of lysates and Western blot.

Supplemental Figure 5

A schematic model of how RIP1 activates PI3K-Akt by downregulating PTEN and interrupting the mTOR-S6K-PI3K negative feedback loop.