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Supplemental Material to:

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The PTEN-Akt pathway impacts the integrity and composition of mitotic centrosomes

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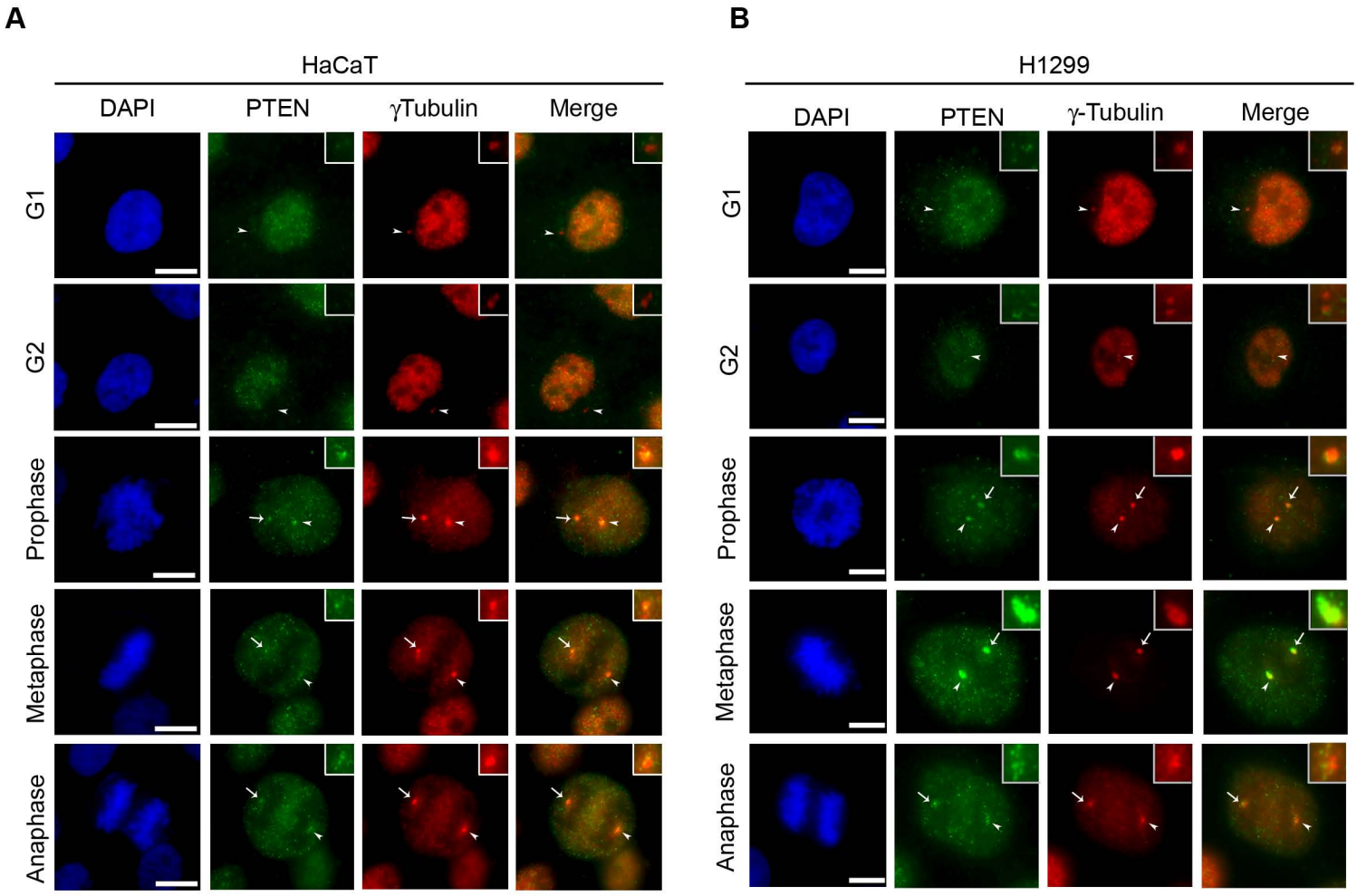
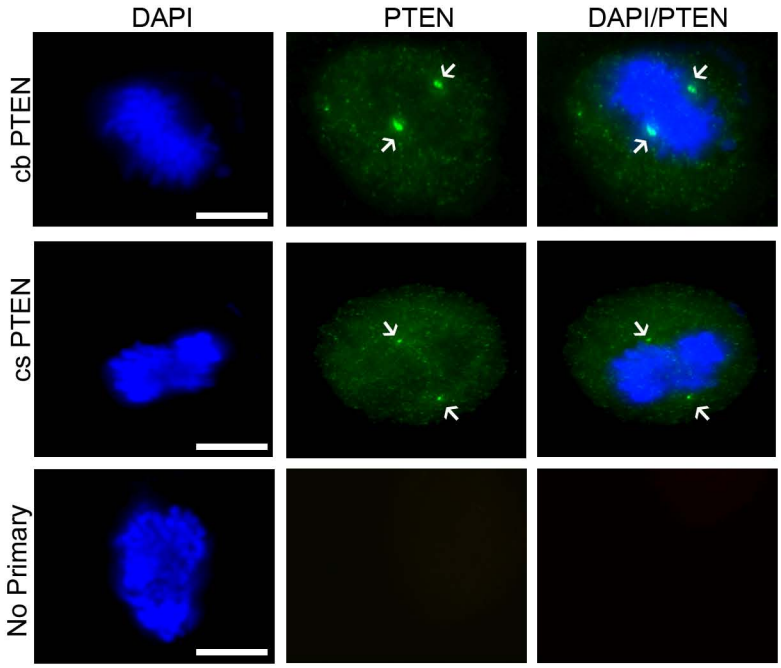
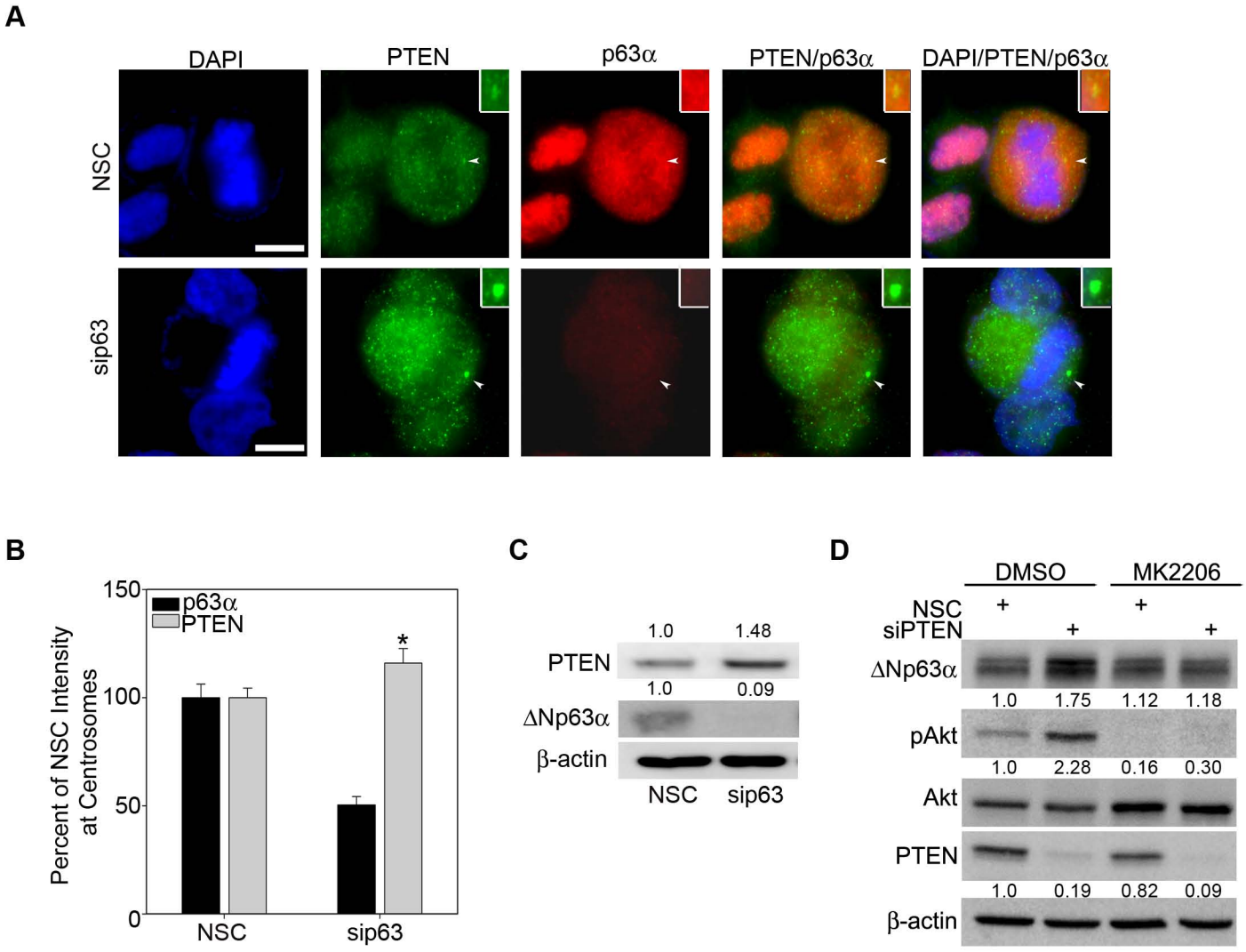
Supplementary Figure 1

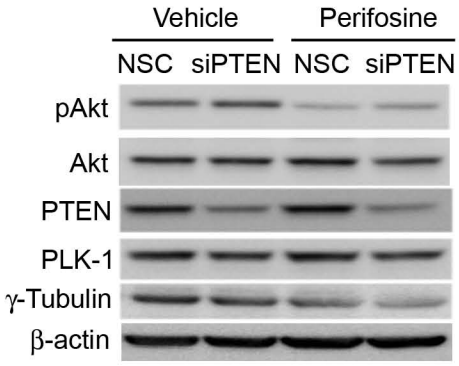


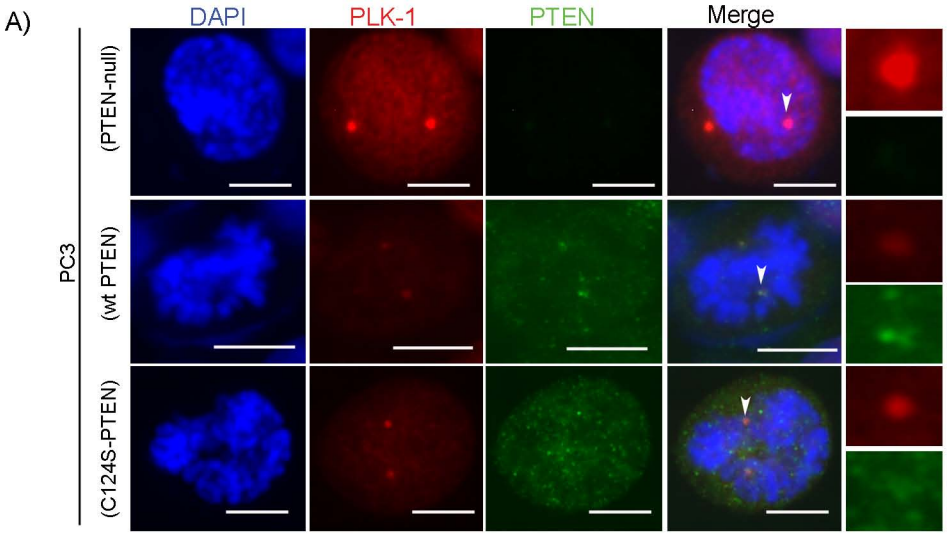
Supplementary Figure 2

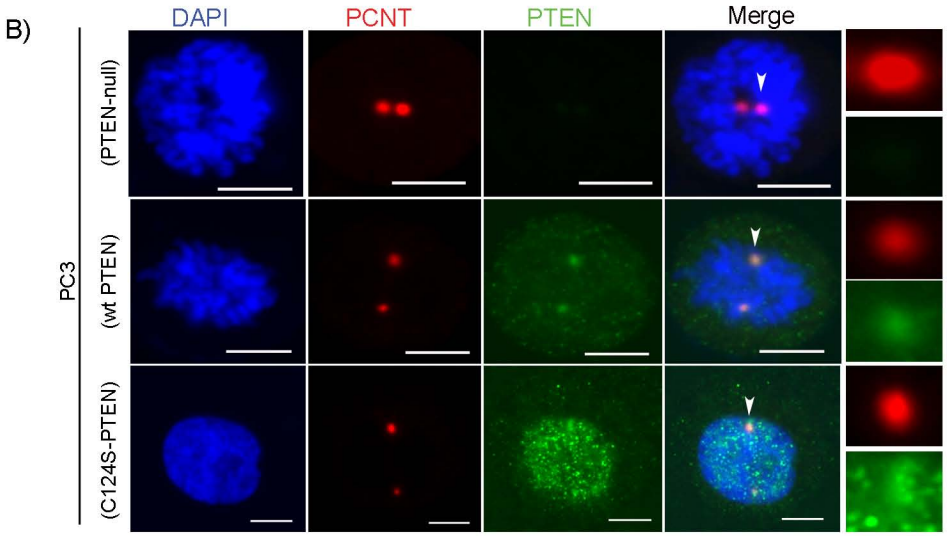


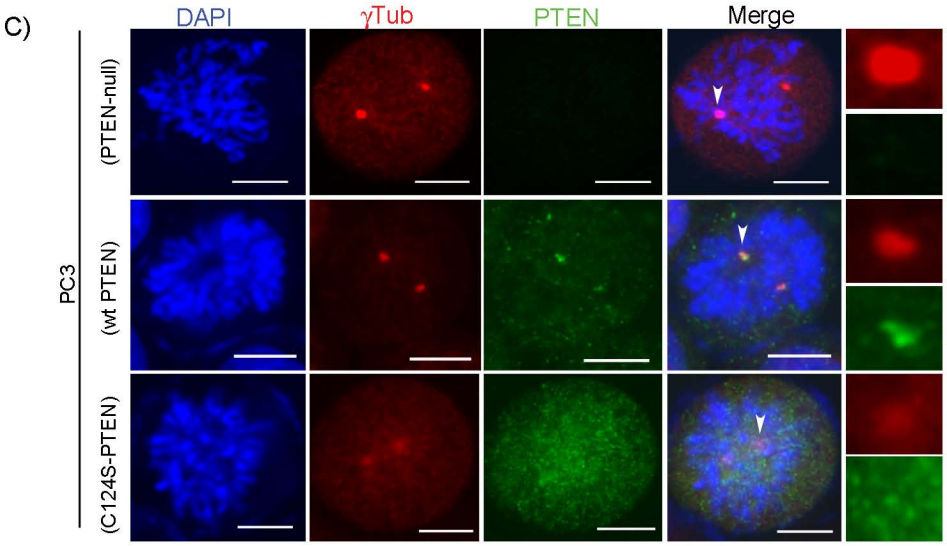
Supplementary Figure 3

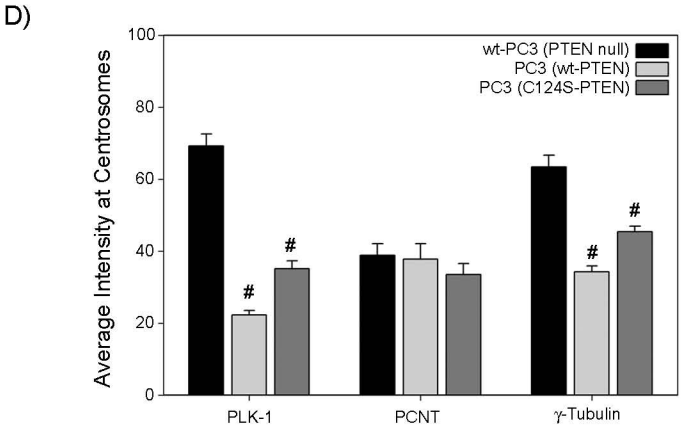



Vehicle
Perifosine
NSC
siPTEN
NSC
siPTEN
pAkt
Akt
PTEN
PLK-1
γ-Tubulin
β-actin


A)
DAPI
PLK-1
PTEN
Merge
PC3
(PTEN-null)
(wt PTEN)
(C124S-PTEN)


B)
DAPI
PCNT
PTEN
Merge
PC3
(PTEN-null)
(wt PTEN)
(C124S-PTEN)


C)
DAPI
γTub
PTEN
Merge
PC3
(PTEN-null)
(wt PTEN)
(C124S-PTEN)


D)
Average Intensity at Centrosomes
100
80
60
40
20
0
wt-PC3 (PTEN null)
PC3 (wt-PTEN)
PC3 (C124S-PTEN)
#
#
#
#
PLK-1
PCNT
γ-Tubulin

Supplementary Fig. 1: PTEN localizes to centrosomes during all stages of mitosis in multiple cell lines. (A) Actively growing asynchronous HaCaT cells and (B) H1299 cells were fixed and stained for PTEN (green), γ-tubulin (red) and DNA (DAPI, blue). Representative cells from each stage of the cell cycle are shown in each row. Arrowheads point to the centrosome enlarged within the inset box while full arrows point to the remaining centrosome. Bars = 5 μm.

Supplementary Fig. 2: Multiple antibodies detect centrosomal PTEN. H1299 cells were stained for PTEN with either Cascade Bioscience PTEN 6H2.1 (cb PTEN) or Cell Signaling rabbit monoclonal anti-PTEN (#9559, cs PTEN). Nuclei were stained with DAPI. Arrows point to centrosomes. Bars = 5 μm.

Supplementary Fig. 3: Loss of ΔNp63α, a negative regulator of PTEN, increases centrosomal PTEN. (A) HaCaT cells were transfected with non-silencing control (NSC) or p63 specific siRNA then stained for PTEN (green), p63α (red) and DNA (blue) 48 hrs later. Arrowheads point to the centrosome enlarged within the inset box. Bars = 5 μm. (B) The mean fluorescence intensities of p63 and centrosomal PTEN from (A) are plotted. Error bars represent s.e.m. from the mean, >25 centrosomes measured. *=p values ≤0.004. (C) Whole cell lysates from HaCaT cells treated NSC or p63 specific siRNA were subjected to immunoblot analysis for PTEN and p63. Fold-change in protein levels, relative

to NSC, are listed above each band. (D) NHEKs were transfected with NSC or PTEN specific siRNA followed by treatment with 10 μM MK2206 or DMSO control for 16 hrs. Immunoblot analyses for the indicated proteins were performed on whole cell lysates. Fold-change in protein levels, relative to NSC, are listed below each band.

Supplementary Fig. 4: The Akt inhibitor Perifosine blocks PTEN mediation changes in PLK-1 and γ-tubulin expression. HaCaT were transfected with NSC or PTEN specific siRNA followed by treatment with 20 μM Perifosine or vehicle control (H_2O) for 4 hrs. Immunoblot analyses for the indicated proteins were performed on whole cell lysates.

Supplementary Fig. 5: The phosphatase activity of PTEN is required to regulate centrosomal levels of PLK-1 and γ-tubulin expression. The expression of PTEN and (A) PLK-1, (B) PCNT, or (C) γ-tubulin at mitotic centrosomes was imaged by immunofluorescence in parental PC3 cells or PC3 cells stably expressing wild-type or catalytically dead (C124S) PTEN. Arrowheads point to the centrosome enlarged in the right-most panels. Bars = 5 μm. (D) The mean fluorescence intensities of each protein at mitotic centrosomes from panels (A-C) are plotted. Error bars represent s.e.m. from three separate experiments, at least 20 centrosomes measured for each protein per condition per experiment. *=p values ≤ 0.05; # = p values ≤ 0.009.