Expanded View Figures

Figure EV1. The MS/MS spectrum of the peptide of endogenous PTENε in Hela cells that matches the N-terminal sequence of PTENε.

A The MS/MS spectrum of the peptide (MAAEEKQAQSLQPSSSR) of endogenous PTENε in Hela cells that matches the most N-terminal sequence of PTENε. Protein lysates of Hela cells were subjected to sequential immunoprecipitation with the anti-PTENα antibody. The bound proteins were separated with SDS–PAGE, and gel slices of the band ranked fifth by molecular weight were analyzed by mass spectrometry.

B, C Detection of the relative expression of PTEN isoforms vs. canonical PTEN in *Pten*^{FLAG} knock-in mice. Various tissues from *Pten*^{FLAG} knock-in mice or control wild-type mice were lysed for immunoprecipitation with the anti-FLAG M2 magnetic beads before immunoblotting with the anti-PTEN antibody (B). The protein level of canonical PTEN and PTENε was measured by evaluating the gray value of corresponding protein bands in each panel (C).

Source data are available online for this figure.

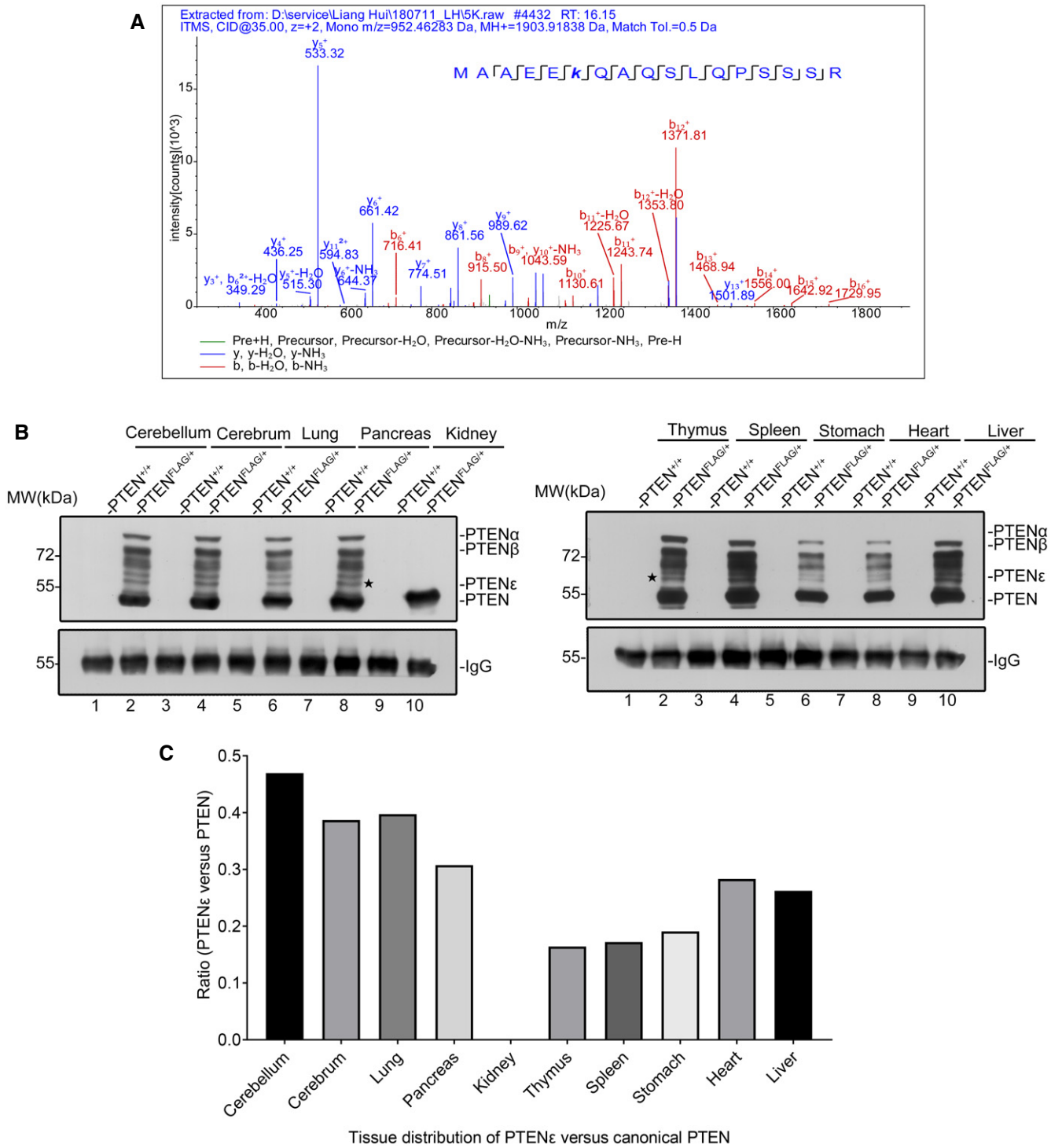

Figure EV1.

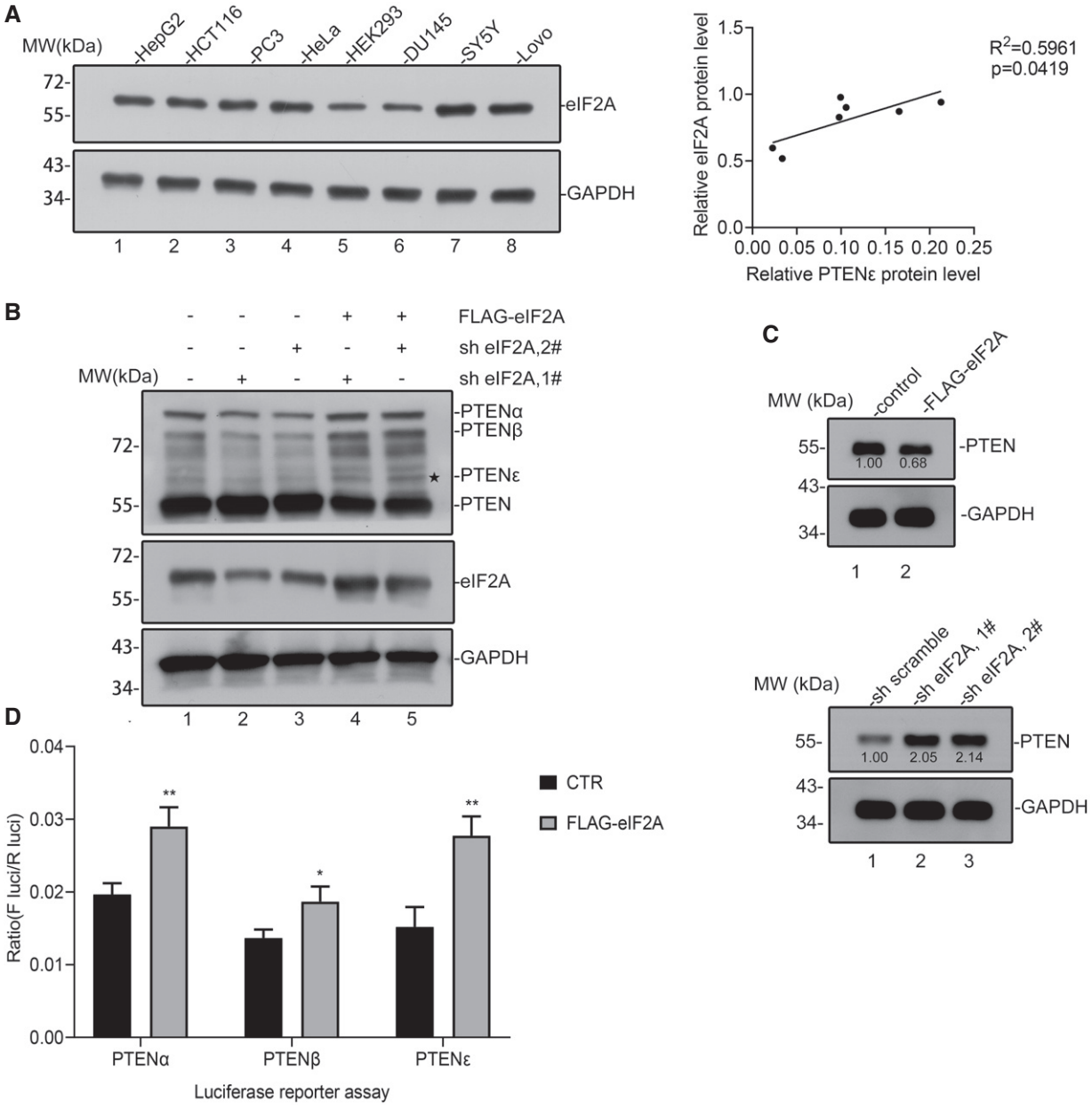

Figure EV2. The endogenous expression of PTENε is regulated by eIF2A.

A Correlation analysis of PTENε protein with endogenous eIF2A protein. The eIF2A protein level in cell lines as listed in Fig 1B was measured by evaluating the gray value of relevant protein bands. Pearson's correlation test was utilized to perform the correlation analysis between endogenous eIF2A and PTENε through the GraphPad Prism 8 software.

B Reduced expression of PTENε in eIF2A knockdown Hela cells was rescued by eIF2A reintroduction. Wild-type FLAG-eIF2A was reintroduced into eIF2A knockdown cells used in Fig 4B, and the lysates of these Hela cells were collected and subjected to Western blot analysis with antibodies against PTEN, eIF2A, and GAPDH.

C The influence of eIF2A alteration on canonical PTEN expression. The relative expression of canonical PTEN in eIF2A overexpression or knockdown cells was quantified by Western blot. PTEN protein level in the aforementioned cells was measured by evaluating the gray value of corresponding protein bands. GAPDH was used as the negative control.

D Luciferase reporter activity driven by PTEN variants after eIF2A overexpression. HEK293 cells were co-transfected with pCMV-tag2b-eIF2A or pCMV-basic plasmid and Luc2-IRES-Report plasmids (GS0112, GENESEED Biotech Co.) carrying the specific N-terminal extended nucleotide of corresponding PTEN isoforms compared with canonical PTEN. Luciferase activity was measured 24 h after transfection. All data are presented as mean ± SD of three independent experiments and were analyzed with the unpaired *t*-test. $*P < 0.05$; $**P < 0.01$.

Source data are available online for this figure.

Figure EV3. PTENε, but not other known PTEN isoforms, dephosphorylates ACTR2 and VASP by its protein phosphatase activity.

A PTENε, but not other known PTEN isoforms, interacts with filopodia formation-related proteins. HEK293 cells were transfected with C-terminal HA and S-tagged PTEN, PTENα, PTENβ, PTENε, or mock plasmid separately, followed by immunoprecipitation with S-protein beads and Western blotting with antibodies against CDC42, VASP, ACTR2, or FSCN1, and HA, respectively.

B Images of immunofluorescence staining for PTENε and endogenous interacting proteins (VASP, FSCN1, CDC42, and ACTR2). C-terminal GFP-tagged PTENε was introduced into Hela *PTEN*$^{-/-}$ cells. Then, these transfected cells were seeded on coverslips and sequentially stained with monoclonal antibodies (Santa Cruz, sc-8401 (CDC42), sc-21743(FSCN1), sc-166103(ACTR2), and Cell Signaling Technology, 3132(VASP)) and DAPI separately. Then, all the coverslips were imaged by confocal microscopy. The scale bars represent 5 μm.

C Overexpression of PTENε, but not other PTEN variants, reduces the endogenous phosphorylation of ACTR2 (lower panel) and VASP (upper panel). HA and S-tagged canonical PTEN, PTENα, PTENβ, PTENε, and PTENε Y210L were introduced into Hela *PTEN*$^{-/-}$ cells, followed by immunoblotting with antibodies against p-ACTR2 (Thr237 and Thr238), p-VASP(Ser157), p-VASP (Ser239), p-VASP (Thr278), VASP, ACTR2, GAPDH, and HA tag.

D Purified ACTR2 or VASP was phosphorylated *in vitro* by NIK or PKA and subsequently used in a phosphatase assay with wild-type His-PTENε, protein phosphatase-deficient His-PTENε Y210L or without purified PTENε protein as a control, followed by immunoblotting with antibodies against p-ACTR2 (Thr237 and Thr238, lower panel) and p-VASP (Ser157, upper panel). Purified proteins (PTENε, PTENε Y210L, PKA, NIK, ACTR2, and VASP) were detected by anti-His antibody (ZSGB-BIO, TA-02).

Source data are available online for this figure.

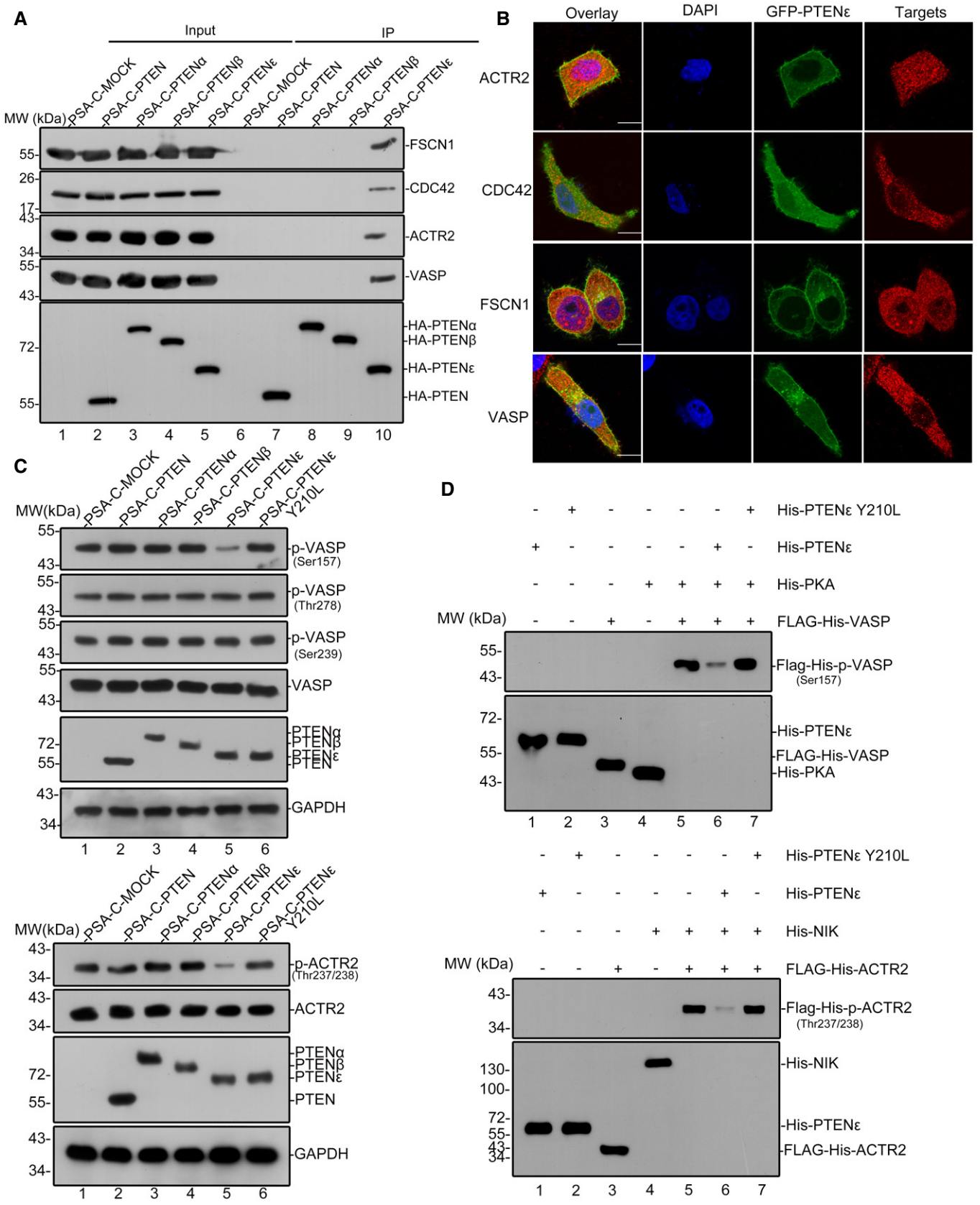
A
Input
IP
PSA-C-MOCK
PSA-C-PTEN
PSA-C-PTENα
PSA-C-PTENβ
PSA-C-PTENε
MW (kDa)
FSCN1
CDC42
ACTR2
VASP
HA-PTENα
HA-PTENβ
HA-PTENε
HA-PTEN
B
Overlay
DAPI
GFP-PTENε
Targets
ACTR2
CDC42
FSCN1
VASP
C
PSA-C-PTENε Y210L
p-VASP (Ser157)
p-VASP (Thr278)
p-VASP (Ser239)
VASP
PTENα
PTENβ
PTENε
PTEN
GAPDH
p-ACTR2 (Thr237/238)
ACTR2
D
His-PTENε Y210L
His-PTENε
His-PKA
FLAG-His-VASP
Flag-His-p-VASP (Ser157)
His-NIK
FLAG-His-ACTR2
Flag-His-p-ACTR2 (Thr237/238)

Figure EV3.

Figure EV4. PTENε plays a critical role in suppressing filopodia formation.

A Immunofluorescence staining of F-actin in wild-type PTENε or mutant PTENε overexpressed Hela $PTEN^{-/-}$ cells and control cells. PTENε-GFP, PTENε 69–81aa delete-GFP, or mock construct was introduced into Hela $PTEN^{-/-}$ cells. The transfected cells were stained with Phalloidin and DAPI before being imaged with confocal microscopy. The scale bars represent 5 μm (left panel). White arrows shown in the magnified immunofluorescence images indicate the filopodia in the cell membrane.

B Quantification of the number of filopodia per cell by FiloQuant software. The data are presented as mean ± SD of three independent experiments and were analyzed with the unpaired *t*-test. ****$P < 0.0001$.

C The phosphorylation of VASP and ACTR2 in PTENε depleted Hela cells. Hele WT and Hela $PTEN\varepsilon^{+/-}$ cell lysates were immunoblotted with antibodies against p-ACTR2 (Thr237 and Thr238), p-VASP (Ser157), p-VASP (Ser239), p-VASP (Thr278), VASP, ACTR2, and GAPDH.

D, E The influence of endogenous PTENε depletion on filopodia formation. (D) Immunofluorescence staining of F-actin in Hela $PTEN\varepsilon^{+/-}$ cells and wild-type cells. Hela $PTEN\varepsilon^{+/-}$ cells and wild-type cells were stained with Phalloidin and DAPI before being imaged with confocal microscopy. The scale bars represent 5 μm. White arrows shown in the magnified immunofluorescence images indicate the filopodia in the cell membrane. (E) Quantification of the number of filopodia per cell by FiloQuant software and the data are presented as mean ± SD of three independent experiments and were analyzed with the unpaired *t*-test. ****$P < 0.0001$.

F The influence of PTENε deficiency on Hela cell morphology. The morphology of Hela $PTEN\varepsilon^{+/-}$ cells was observed with a Nikon microscope. Representative images of each group were shown at a higher magnification. The scale bars represent 80 μm.

Source data are available online for this figure.

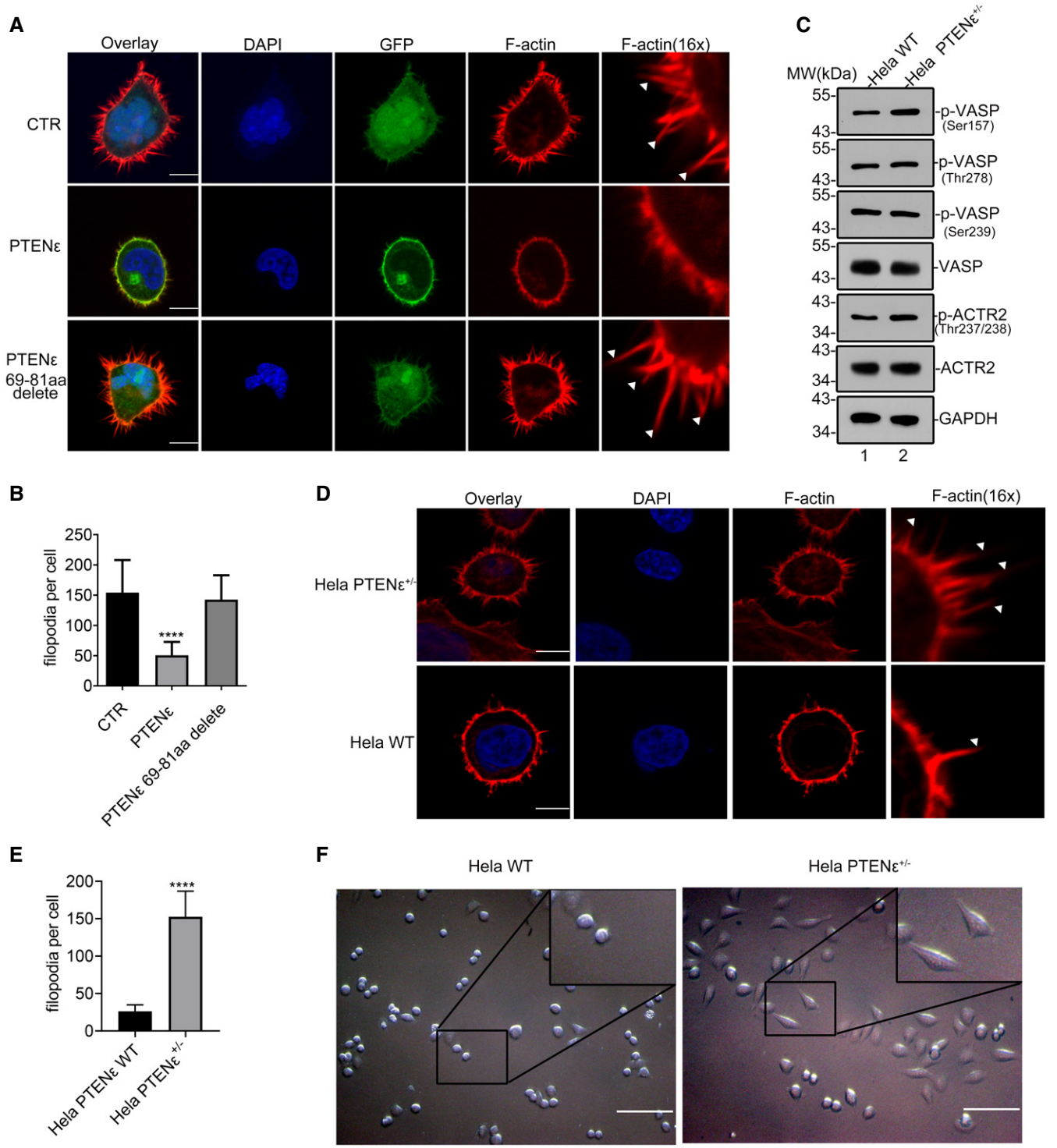
A
Overlay
DAPI
GFP
F-actin
F-actin(16x)
CTR
PTENε
PTENε 69-81aa delete
C
MW(kDa)
Hela WT
Hela PTENε+/-
55-
43-
p-VASP (Ser157)
p-VASP (Thr278)
p-VASP (Ser239)
VASP
34-
p-ACTR2 (Thr237/238)
ACTR2
GAPDH
1
2
B
filopodia per cell
250
200
150
100
50
0
****
CTR
PTENε
PTENε 69-81aa delete
D
Overlay
DAPI
F-actin
F-actin(16x)
Hela PTENε+/-
Hela WT
E
filopodia per cell
200
150
100
50
0
****
Hela PTENε WT
Hela PTENε +/-
F
Hela WT
Hela PTENε+/-

Figure EV4.