Supporting Information

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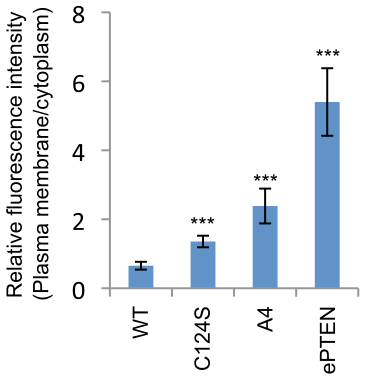

Fig. S1. HEK293T cells expressing the indicated forms of PTEN-GFP were observed by fluorescence microscopy. Intensity of GFP at the plasma membrane was quantified relative to that at the cytosol. Values represent the mean ± SD ($n \geq 10$).

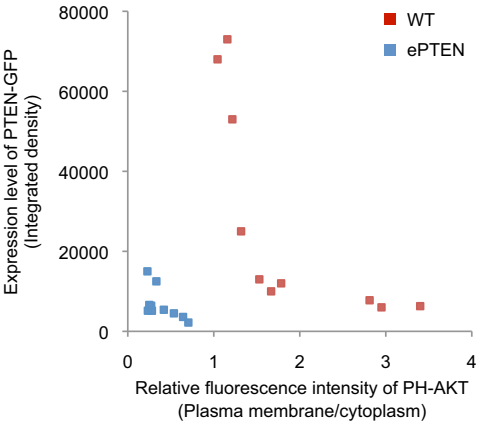

Fig. S2. HEK293T cells expressing PH_{AKT}-RFP along with PTEN-GFP (WT) or enhanced PTEN (ePTEN)-GFP were observed by fluorescence microscopy. The *x* axis indicates the relative fluorescence intensity of PH_{AKT}-RFP at the plasma membrane and the *y* axis shows the total fluorescence intensity of GFP. Each square represents single cells.

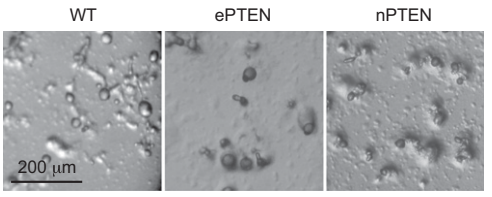

Fig. S3. PTEN-null *Dictyostelium* cells expressing different PTEN-GFP constructs were starved for 36 h to induce differentiation into fruiting bodies.

Table S1. Primer sequences

Primer	Sequence
HuPTEN5BamHI2	ctgggatccaaataaaaATGACAGCCATC
HuPTEN3XhoI2	ctcctcgagccGACTTTTGTAATTTG
PTEN-Q17R-f	GATATCgAGAGGATGGATTCGACTTAGAC
PTEN-Q17R-r	GTCTAAGTCGAATCCATCCTCTcGATATC
PTEN-R41G-f	CTGCAGAAgGACTTGAAGGCGTATAC
PTEN-R41G-r	GTATACGCCTTCAAGTCcTTCTGCAG
PTEN-E73D-f	GTGCTGATAGACATTATGACAC
PTEN-E73D-r	GTGTCATAATGTCTATCAGCAC
PTEN-Q17E-f	GAGATATgAAGAGGATGGATTCGACTTAGAC
PTEN-Q17E-r	GTCTAAGTCGAATCCATCCTCTTcATATCTC
PTEN-R11G-f	ATGACAGCCATCATCAAAGAGATCGTTAGCgGAAAC
PTEN-K13A,R14A,R15A-f	ATGACAGCCATCATCAAAGAGATCGTTAGCAGAAACgcAgcGgcATATCA
PTEN-TOPO-f	caccATGA CAGCCATCAT CAAAG
GFP-TOPO-r	TTACTTGTACAGCTCGTCCATG
PTEN-BamHI-Kozak-f	GTTGGATCCGCCACCATGACAGCCATC
GFP-NotI-r	CTGCGGCCGCTTACTTGTACAGCTC

f, forward; r, reverse.

Table S2. Plasmids

Plasmids	Description	Source
pKF3		(1)
pKF3-hPTEN	hPTEN amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/XhoI pKF3	(1)
pKF3-A4	A4 amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/XhoI pKF3	(1)
pKF3-Q17R	hPTEN mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-Q17R-f, and PTEN-Q17R-r and inserted into BglII/XhoI pKF3	This study
pKF3-R41G	hPTEN mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-R41G-f, and PTEN-R41G-r and inserted into BglII/XhoI pKF3	This study
pKF3-E73D	hPTEN mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-E73D-f, and PTEN-E73D-r and inserted into BglII/XhoI pKF3	This study
pKF3-Q17R,R41G	R41G mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-Q17R-f, and PTEN-Q17R-r and inserted into BglII/XhoI pKF3	This study
pKF3-Q17R,E73D	E73D mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-Q17R-f, and PTEN-Q17R-r and inserted into BglII/XhoI pKF3	This study
pKF3-R41G,E73D	E73D mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-R41G-f, and PTEN-R41G-r and inserted into BglII/XhoI pKF3	This study
pKF3-Q17R,R41G,E73D	R41G,E73D mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-Q17R-f, and PTEN-Q17R-r and inserted into BglII/XhoI pKF3	This study
pKF3-Q17R,A4	Q17R amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-R41G,E73D,A4	R41G,E73D amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-Q17R,R41G,E73D,A4	Q17R,R41G,E73D amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-Q17E	hPTEN mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-Q17E-f, and PTEN-Q17E-r and inserted into BglII/XhoI pKF3	This study
pKF3-Q17E,A4	Q17E amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-Q17E,R41G,E73D	R41G,E73D mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-Q17E-f, and PTEN-Q17E-r and inserted into BglII/XhoI pKF3	This study
pKF3-R11G	hPTEN mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, and PTEN-R11G-f and inserted into BglII/XhoI pKF3	This study
pKF3-R11G,A4	R11G amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-K13A,A4		Gift from D. Bolduc*
pKF3-R14A,A4		Gift from D. Bolduc
pKF3-R15A,A4		Gift from D. Bolduc
pKF3-K13A,R14A,R15A,A4	hPTEN mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, and PTEN-K13A,R14A,R15A-f and inserted into BglII/XhoI pKF3	This study
pKF3-N262Y,N329H		(1)
pKF3-N262Y,N329H,A4		(1)
pKF3-R41G,E73D,N262Y,N329H	N262Y,N329H amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/XhoI pKF3-R41G,E73D	This study
pKF3-R41G,E73D,N262Y,N329,A4	R41G,E73D,N262Y,N329H amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-Q17R,R41G,E73D,N262Y,N329H	N262Y,N329H amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/XhoI pKF3-Q17R,R41G,E73D	This study
pKF3-Q17R,R41G,E73D,N262Y,N329,A4	Q17R,R41G,E73D,N262Y,N329H amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/NheI pKF3-A4	This study
pKF3-Q17E,R41G,E73D,N262Y,N329H	N262Y,N329H amplified using primers HuPTEN5BamHI2 and HuPTEN3XhoI2 and inserted into BglII/XhoI pKF3-Q17E,R41G,E73D	This study
pKF3-Q17R,R41G,E73D,C124S, N262Y, N329H	Q17R,R41G,E73D,N262Y,N329H mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-C124Sf, and PTEN-C124Sr and inserted into BglII/XhoI pKF3	This study
pKF3- Q17E,R41G,E73D,C124S, N262Y, N329H	Q17E,R41G,E73D,N262Y,N329H mutated using primers HuPTEN5BamHI2, HuPTEN3XhoI2, PTEN-C124Sf, and PTEN-C124Sr and inserted into BglII/XhoI pKF3	This study
pcDNA3.1		Invitrogen
pcDNA3.1-hPTEN352-403-YFP-FLAG		(2)
pcDNA3.1-hPTEN	hPTEN-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	(1)

Table S2. Cont.

Plasmids	Description	Source
pcDNA3.1-A4	A4-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	(1)
pcDNA3.1-C124S,A4	C124S,A4-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	(1)
pcDNA3.1-C124S	C124S-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	(1)
pcDNA3.1-R41G,E73D	R41G,E73D-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-Q17R,R41G,E73D	Q17E,R41G,E73D-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-N262Y,N329H	N262Y,N329H-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	(1)
pcDNA3.1-R41G,E73D,N262Y,N329H	R41G,E73D,N262Y,N329H-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-Q17R,R41G,E73D,N262Y, N329H	Q17R,R41G,E73D,N262Y,N329H-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-R41G,E73D,N262Y, N329H,A4	R41G,E73D,N262Y,N329H,A4-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-Q17R,R41G,E73D,N262Y, N329H,A4	Q17R,R41G,E73D,N262Y,N329H,A4-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-Q17E,R41G,E73D,N262Y, N329H	Q17E,R41G,E73D,N262Y,N329H-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-Q17R,R41G,E73D, C124S, N262Y,N329H	Q17R,R41G,E73D,C124S,N262Y,N329H-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pcDNA3.1-Q17E,R41G,E73D, C124S, N262Y,N329H	Q17E,R41G,E73D,C124S,N262Y,N329H-GFP amplified using primers PTEN-TOPO-f and GFP-TOPO-r and inserted into pcDNA3.1	This study
pmCherry-PH_{AKT}		Gift from T. Inoue*
peGFP-$PH_{PLC\delta}$		Gift from T. Inoue
$peGFP_{LactC2}$		Gift from T. Inoue
pHR-SIN-CSGW		Gift from T. Waldman†
pHR-SIN-hPTEN	hPTEN-GFP amplified using primers PTEN-BamHI-Kozak-f and GFP-NotI-r and inserted into BamHI/NotI pHR-SIN-CSGW	This study
pHR-SIN-A4	A4-GFP amplified using primers PTEN-BamHI-Kozak-f and GFP-NotI-r and inserted into BamHI/NotI pHR-SIN-CSGW	This study
pHR-SIN-C124S	C124S-GFP amplified using primers PTEN-BamHI-Kozak-f and GFP-NotI-r and inserted into BamHI/NotI pHR-SIN-CSGW	This study
pHR-SIN-Q17R,R41G,E73D,N262Y, N329H	Q17R,R41G,E73D,N262Y,N329H-GFP amplified using primers PTEN-BamHI-Kozak-f and GFP-NotI-r and inserted into BamHI/NotI pHR-SIN-CSGW	This study
pHR-SIN-Q17R,R41G, E73D, C124S, N262Y,N329H	Q17R,R41G, E73D,C124S,N262Y,N329H amplified using primers PTEN-BamHI-Kozak-f and GFP-NotI-r and inserted into BamHI/NotI pHR-SIN-CSGW	This study
pHR-SIN-Q17E,R41G, E73D, C124S, N262Y,N329H	Q17E,R41G, E73D,C124S,N262Y,N329H amplified using primers PTEN-BamHI-Kozak-f and GFP-NotI-r and inserted into BamHI/NotI pHR-SIN-CSGW	This study
pHRCMV8.2ΔR		Gift from T. Waldman
pCMV-VSVG		Gift from T. Waldman

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1. Nguyen HN, et al. (2013) Mechanism of human PTEN localization revealed by heterologous expression in Dictyostelium. *Oncogene*, 10.1038/onc.2013.507.
2. Rahdar M, et al. (2009) A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN. *Proc Natl Acad Sci USA* 106(2):480–485.