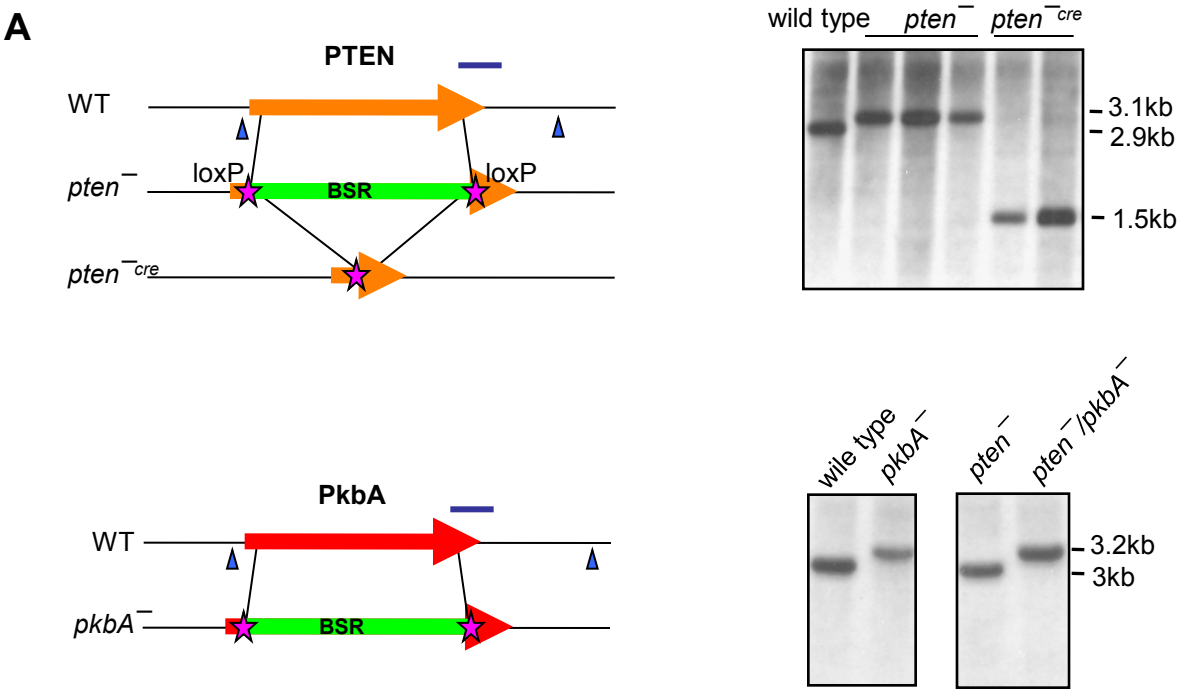

A
PTEN
WT
pten⁻
loxP
BSR
loxP
pten⁻cre
wild type
pten⁻
pten⁻cre
3.1kb
2.9kb
1.5kb
PkbA
WT
pkbA⁻
BSR
wile type
pkbA⁻
pten⁻
pten⁻/pkbA⁻
3.2kb
3kb

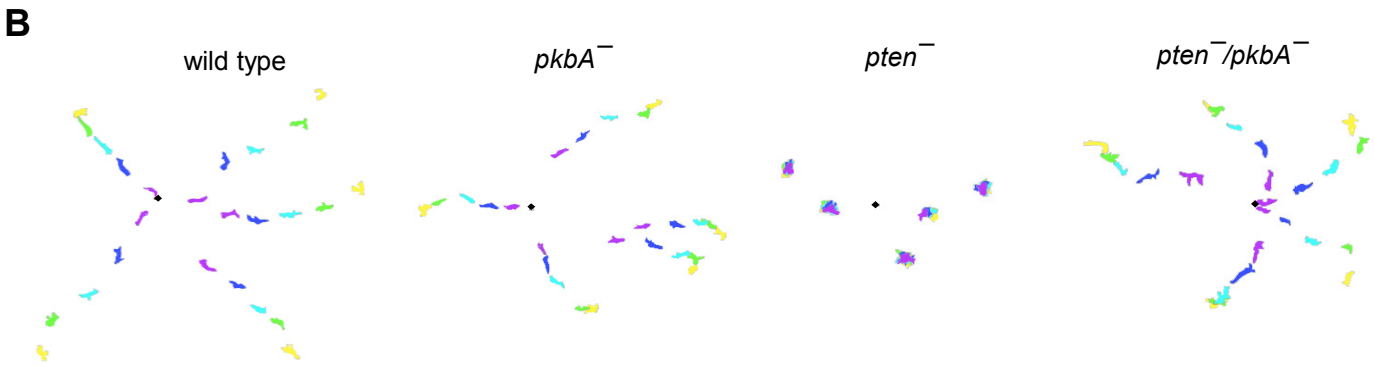

B
wild type
pkbA⁻
pten⁻
pten⁻/pkbA⁻

Figure S1

Figure S1: (A) Disruption of PTEN and PKBA. Purple stars represent loxP sites. Blue triangles indicate the restriction digest sites of EcoRV and EcoRI. Blue bar shows the location of southern probe. Results of the southern blots are shown on the right.

(B) Chemotaxis assays of wild type, *pkbA*$^{-}$, *pten*$^{-}$ and *pten*$^{-}$/*pkbA*$^{-}$ cells. Outlines of five representative cells moving toward micropipette were prepared at 7.5 min intervals and overlaid as different colors.

.

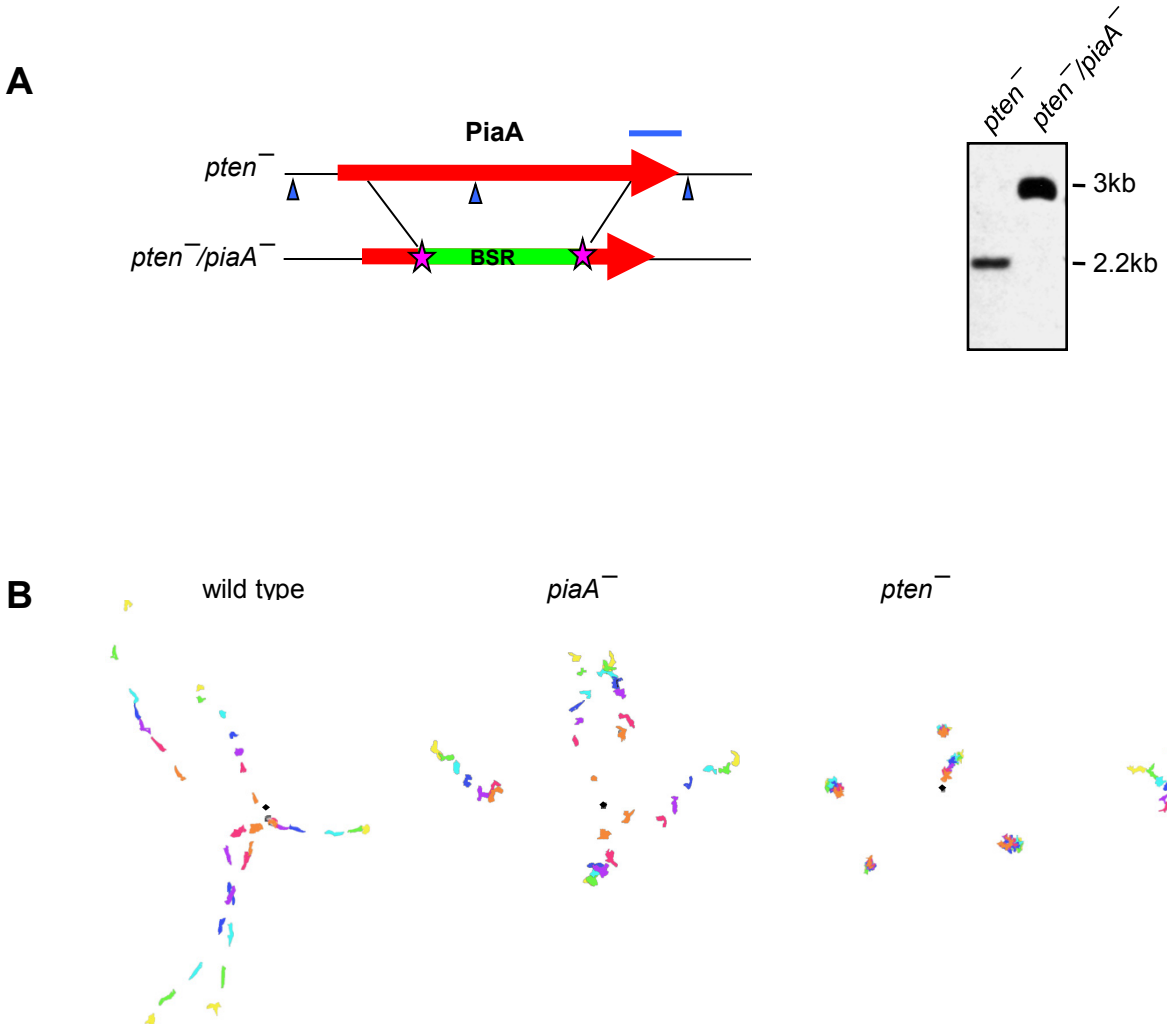

A
PiaA
pten⁻
pten⁻/piaA⁻
BSR
pten⁻
pten⁻/piaA⁻
3kb
2.2kb
B
wild type
piaA⁻
pten⁻
pten⁻/piaA⁻

Figure S2

Figure S2: (A) Disruption of PiaA in *pten*$^{-}$ cells. Purple stars represent loxP sites. Blue triangle and bar indicate the BclI digestion sites and location of the southern probe, respectively. Results of the southern blot are shown on the right.
(B) Chemotaxis assay of wild type, *piaA*$^{-}$, *pten*$^{-}$, and *pten*$^{-}$/*piaA*$^{-}$ cells. Outlines of five representative cells moving toward micropipette were prepared at 7.5 min intervals and overlaid as different colors

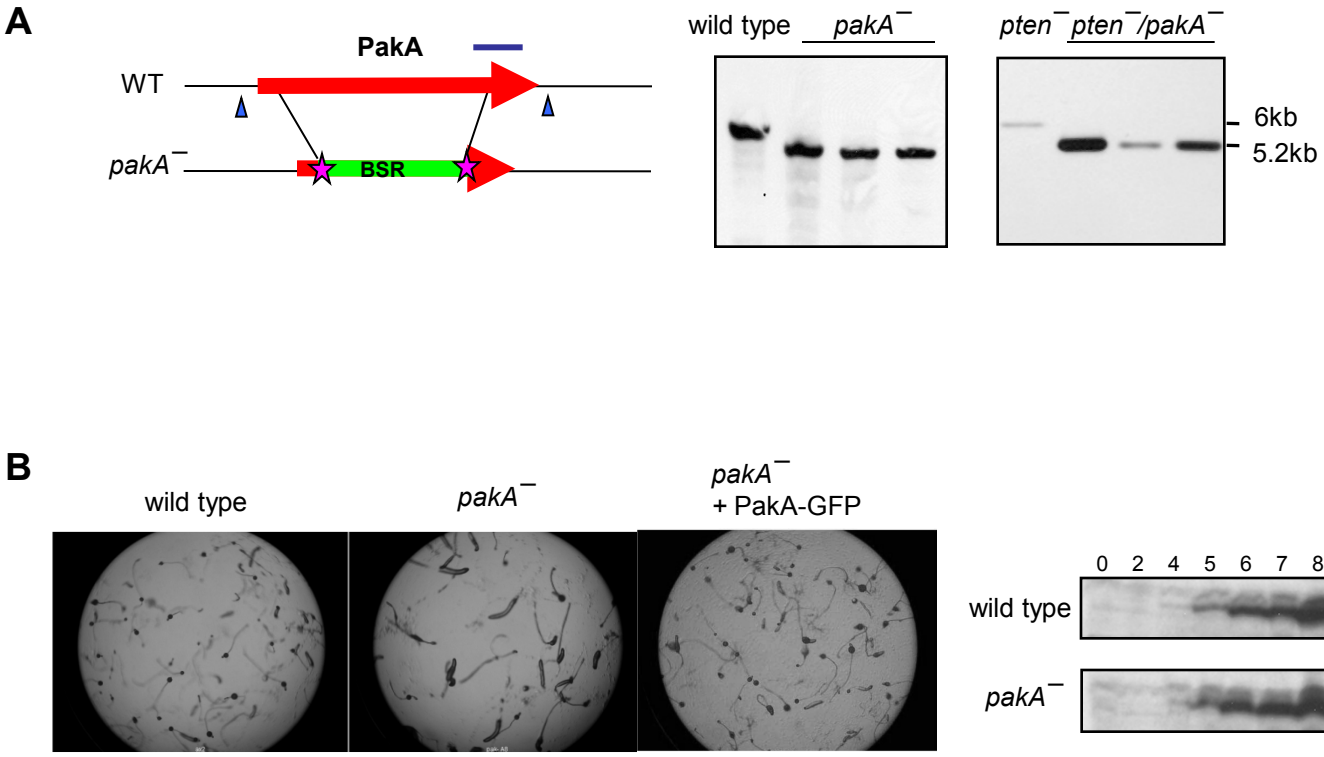

A
PakA
WT
pakA−
BSR
wild type
pakA−
pten−
pten−/pakA−
6kb
5.2kb
B
wild type
pakA−
pakA−
+ PakA-GFP
0 2 4 5 6 7 8 9 (hr)
wild type
pakA−

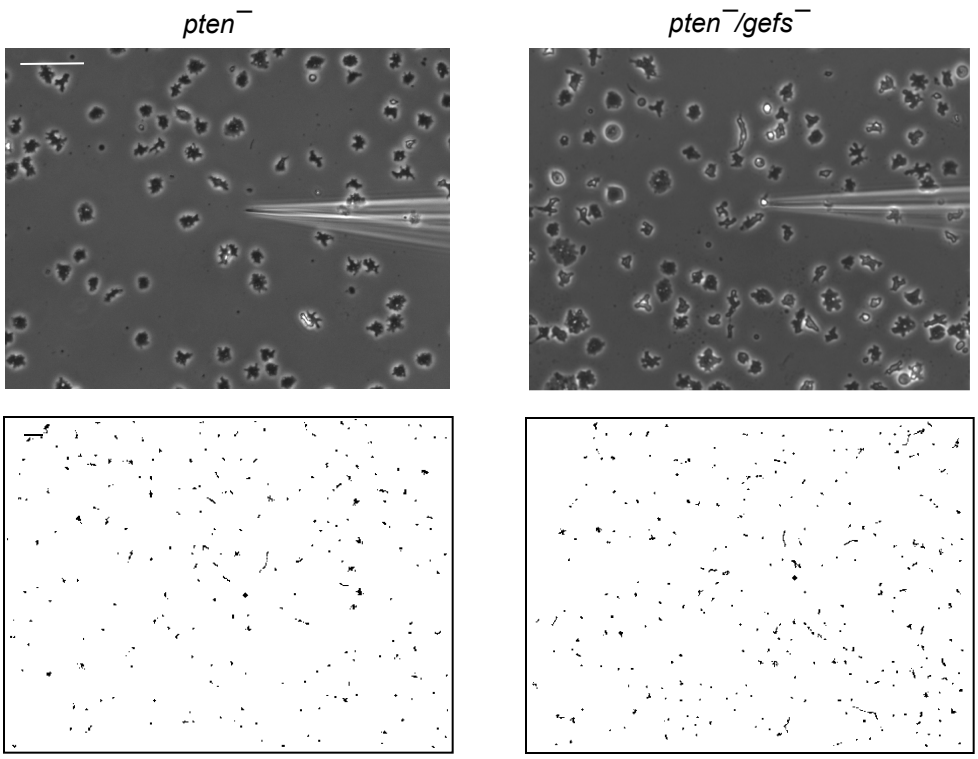
Figure S3

Figure S3: (A) Disruption of PakA in wild type or *pten*$^{-}$ cells. Blue triangle and bar indicate the BclI digestion sites and location of the southern probe, respectively. Results of the southern blots are shown on the right.

(B) Disruption of PakA in wild type causes delayed fruiting body formation on non-nutrient agar. $1X10^7$ cells/ml of each indicated cell line were plated on non-nutrient agar and images were taken after 18 hours of starvation. Expression of PakA-GFP in *pakA*$^{-}$ restores the wild type phenotype. Western blot of cAR1 expression was shown on the right.

A

B



	pten^{-}	*pten*$^{-}$/*gefs*$^{-}$
motility speed (μm/min)	1.63 ±0.29	1.66 ±0.23
chemotaxis speed (μm/min)	0.10 ±0.14	0.04 ±0.01
chemotaxis index	0.09 ±0.11	0.03 ±0.01
persistence	0.16 ±0.06	0.12 ±0.03

Average and standard deviation of at least three movies for each cell line are calculated using software provided by Y.Xiong and P.A. Iglesias. Please see Material and methods for the definition of these parameters.

Figure S4

Figure S4: (A) representative images of *pten*$^{-}$ and *pten*$^{-}$/*gefs*$^{-}$ cells growing on glass substrates. . Bar, 50 μm.

(B) Chemotaxis assay of *pten*$^{-}$ and *pten*$^{-}$/*gefs*$^{-}$ cells. Upper panels show images at 45 min after chemotaxis was initiated. Lower panels show trajectories of the entire recording field. The black diamond indicates the location of micropipette tip. Bar, 50 μm.

Table S1: Mass Spectrometry Identification of PKB substrates

Gene name & DDB number	PKB phosphorylation site	Peptide identified in mass spec	Expect
GacG DDB 0233879	RRRTSS^{365} RERSSS^{480}	FLSEVSQSAYTNK	3e-09
		TNTIFGVDPNTIVSK	6.9e-08
		NNGNEVIQSSSSTSSPR	1.8e-07
		LNLTENNLNNSSSTSSSPR	3.7e-10
PakA DDB 0191313	RSRSHT^{585}	IGEGGAGEVFEAINSR	1.4e-06
		LADFGYAAQLTQIR	0.015

Table S2: Primer List

	name	sequences
PTEN disruption	Ppten1	GCGGCCGCTCACATTGCAATTTTAAGAGCACAG
	Ppten2	CCCGGGTACATGCTAAATCTAAATCGTAACC
	Ppten3	CCCGGGATCCACAATCCTCAAATAATGTAGC
	Ppten4	GTCGACTGGAATTCACTTTCAAAATGGACACG
PkbA disruption	Ppkba1	GTCGCACTTTGCAAACCAAAGACAATAACTGC
	Ppkba2	CCCGGGACCACCTTTGAGAATGAACCATCTC
	Ppkba3	CCCGGGCTTTAACTATGGCTGGTGAATGTGC
	Ppkba4	GCGGCGCCTTCTGAAAATGGGTCTAATGGATC
PkbR1 Disruption	Ppkbr1	GAAAGTCGACACAATTTAACCTATTACATCTCCG
	Ppkbr2	GAAACCCGGGTTTGGCGGGTTTACCTGATCCTCC
	Ppkbr3	GAAACCCGGGTCTTCTCTATGAGATGTTAACTGG
	Ppkbr4	GAAAGCGGCCGCTTAATCCTTTAAGATTGAATCAGC
PiaA disruption	YK118	GACAAGCTTTGATAGTAGTGTAAATACTACATCG
	YK119	CTCATTACTCTTAACCCGGGTGTTCTAATTG
	YK120	CACTATCATTGAATGTCCCGGGTGAGGCATG
	YK121	ATCATAAATGAAGCTTCTAGCACCTAAACG
PakA disruption	MT60	GAAAAGAAATCTCATTCATATTTTAAAG
	MT61	TATTGCCCGGGTTTGTACCTCATTGAATTGACC
	MT62	ACAAACCCGGGCAATATCGTGATATCCAATTG
	MT63	CAATTCTTGCAGCATCAACAGCTT
PakA cloning	MT64	GGGATCCATGGAAGAGAAACCAAAAAGTACAACTCC
	MT47	CTTCTTGTCTTCTACGCGATGCCAATTCTCTC
	MT43	GGCATCGCGTAGAAGACAAGAAGAAG
	MT41	GCTCGAGGGAATTGCAGTTAAATTAGC
PakA T585A	MT72	GGTCACGTAGTCATGCTTTGGCTGGTG
	MT73	CACCAGCCAAAGCATGACTACGTGACC
PakA T585E	MT74	GGTCACGTAGTCATGCTTTGGCTGGTG
	MT75	CACCAGCCAAAGCATGACTACGTGACC
GacG cloning	MT65	GGGATCCATGGCGTCAATATTTTTAAATAAAAAAACAATAG
	MT53	GCTCGAGGGCTCTTCAACAATATCAGTTAAAG

Video 1: Chemotaxis assay of wild type cells. Chemotactically competent cells were allowed to attach to the glass in a one-well Lab-Tek chamber. Micropipette was filled with 10μM cAMP and the cell movement was recorded every 30 sec for 45 min. The video was played at 8fps. Related to Figure 1C, 2C and 5D.

Video 2: Chemotaxis assay of *pkbA*$^{-}$ cells. All conditions were exactly the same as for video 1 except total recording time was 30 min. Related to Figure 1C.

Video 3: Chemotaxis assay of *pten*$^{-}$ cells. All conditions were exactly the same as for video 1. Related to Figure 1C, 2C and 5D.

Video 4: Chemotaxis assay of *pten*$^{-}$/*pkbA*$^{-}$ cells. All conditions were exactly the same as for video 1 except total recording time was 30 min. Related to Figure 1C.

Video 5: Chemotaxis assay of *piaA*$^{-}$ cells. All conditions were exactly the same as for video 1. Related to Figure 2C.

Video 6: Chemotaxis assay of *pten*$^{-}$/*piaA*$^{-}$ cells. All conditions were exactly the same as for video 1. Related to Figure 2C.

Video 7: PHcrac-GFP and LimEΔcoil-RFP in wild type cells. Cells transformed with PHcrac-GFP were developed to the chemotactically competent stage and cell movement was recorded at every 5 sec for 2.5min. The video was played at 5fps. Related to Figure 3

Video 8: PHcrac-GFP and LimEΔcoil-RFP in *pten*$^{-}$ cells . All conditions were exactly the same as for video 7. Related to Figure 3

Video 9: PHcrac-GFP and LimEΔcoil-RFP in *pten*$^{-}$/*pkbA*$^{-}$ cells. All conditions were exactly the same as for video 7. Related to Figure 3

Video 10: PHcrac-GFP and LimEΔcoil-RFP in *pten*$^{-}$/*piaA*$^{-}$ cells. All conditions were exactly the same as for video 7. Related to Figure 3

Video 11: Chemotaxis assay of *pakA*$^{-}$ cells. All conditions were exactly the same as for video 1. Related to Figure 5D.

Video 12: Chemotaxis assay of *pten*$^{-}$/*pakA*$^{-}$ cells. All conditions were exactly the same as for video 1. Related to Figure 5D.

Video 13: Chemotaxis assay of *pten*$^{-}$/*pakA*$^{-}$ cells expressing PakA^{T585A}-GFP. All conditions were exactly the same as for video 1. Related to Figure 6B.

Video 14: Chemotaxis assay of *pten*$^{-}$/*pakA*$^{-}$ cells expressing PakA^{T585E}-GFP. All conditions were exactly the same as for video 1. Related to Figure 6B.