SUPPLEMENTAl INFORMATION

SUPPLEMENTAl TABLE 1. Oligonucleotides used in this study

Names	Sequences
YK151	5'-ggatccgaaaatattgttattacaaatacgagtgg-3'
YK154	5'-ggatccataaatggtcgttaccaaaaagaagatg-3'
YK155	5'-ctcgagcccttaaatgataacatatttaattcatttattaaatc-3'
YK166	5'-ctcgagccatttgaagaaccagatgataatattacac-3'
YK176	5'-ctctatgaacaatacctaatg-3'
YK177	5'-cccgggaccaccaattcaaccacctc-3'
YK178	5'-ttcccgggaccatatgcacctttcc-3'
YK179	5'-gtgcaaatactagtggtagtac-3'

SUPPLEMENTARY FIGURE AND MOVIE LEGENDS

Supplemental Fig. S1. PdkA and PdkB are the activation loop kinases of PkbA and PkbR1.
A. Wild-type cells expressing PdkA-GFP and PdkB-GFP in the presence of DMSO or 50 μM LY294002 were stimulated with 1 μM cAMP, and cell extracts were prepared at the indicated times. The phosphorylation levels of the AL, T309 for PkbR1 and T278 for PkbA, were detected by immunoblotting with α-phospho PKC (pan) antibody (the upper panel). The expression levels of PdkA-GFP and PdkB-GFP were detected by α-GFP antibody (the lower panel). *B.* Cells expressing PdkA(477-686)-GFP were treated with 10 mM DTT before cAMP stimulation. Cell extracts were prepared at the indicated time and probed with α-GFP antibody.

Supplemental Fig. S3. Cells lacking PdkA and PdkB are defective in chemotaxis.

A. pdkA^{-}*pdkB*$^{-}$ cells expressing vector (V) or PdkA-GFP were spread on a coverslip and exposed to cAMP gradients produced by a micropipette containing 10 μM cAMP. The response of cells was recorded by time-lapse video at a 30 sec for 30 min frame rate. First and last images are shown. Scale bars represent 50 μm. *B.* Tracing of the centroid of individual cells is shown. A filled circle indicates the position of needle. *C.* Speed, chemotaxis speed, chemotaxis index, and persistence were quantified by the methods described in Materials and methods.

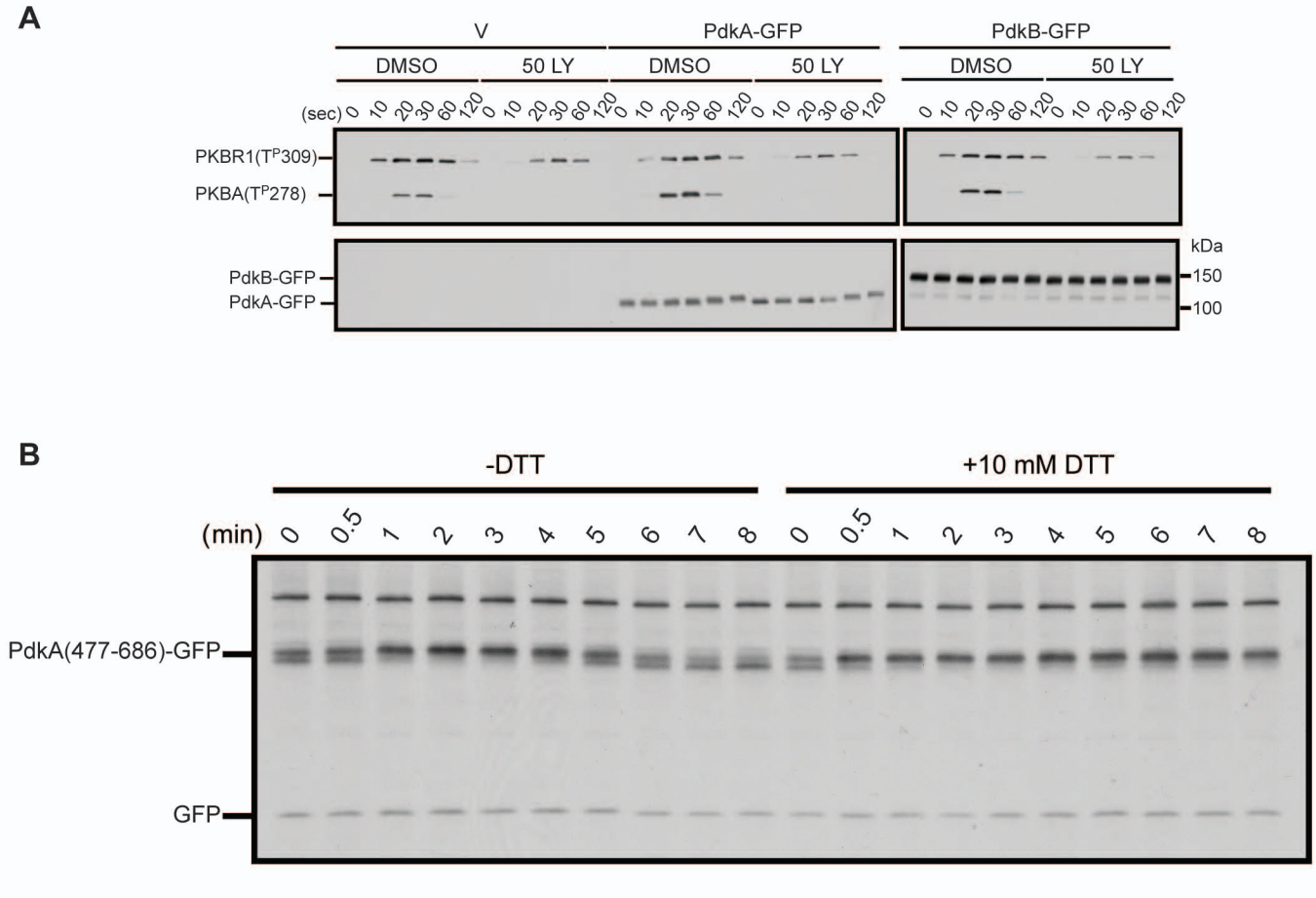
Supplemental Fig. S4. Spatial and temporal regulation of the localizaion of PdkA and PdkB.
Wild-type cells expressing PdkA(282-686)-GFP, PdkA(477-686)-GFP, or PdkA(576-686)-GFP were stimulated with 1 μM cAMP. Cell extracts taken at the indicated times were probed by α-GFP antibody.

Supplemental Fig. S5. The PH domain of PdkA is dispensable for function.

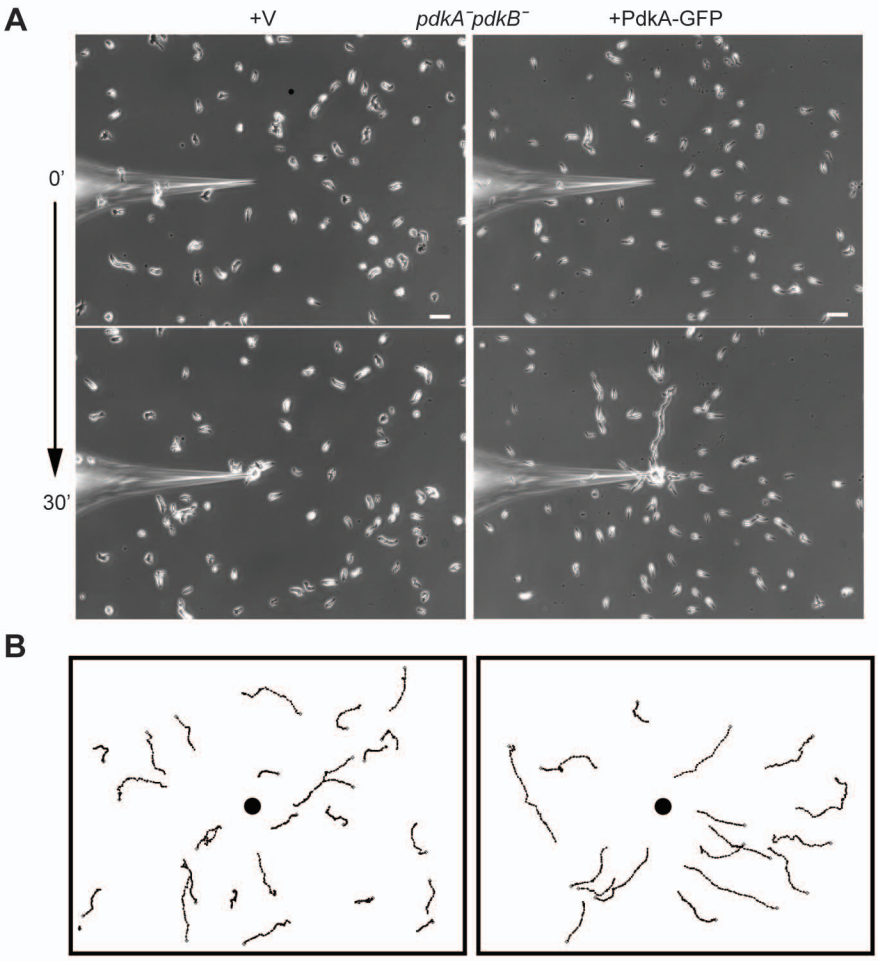
A. pdkA^{-}*pdkB*$^{-}$ cells expressing vector (V), PdkA-GFP, and PdkA(K98M)-GFP were starved on a non nutrient agar plate and allowed to form fruiting bodies. *B.* Cell extracts from *A* were prepared at the indicated times after 1 μM cAMP stimulation and probed with α-phospho PKC (pan) antibody (the upper panel) and α-GFP antibody (the lower panel).

Supplemental Movie S1. Chemotaxis of wild-type cells from Fig. 3A.

Supplemental Movie S2. Chemotaxis of *pdkA*$^{-}$*pdkB*$^{-}$ cells from Fig. 3A.



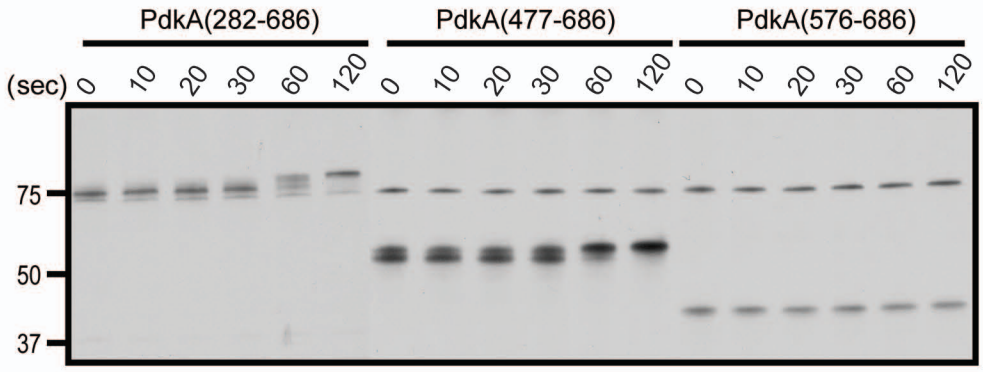
Supplemental Figure S1 Kamimura et al.



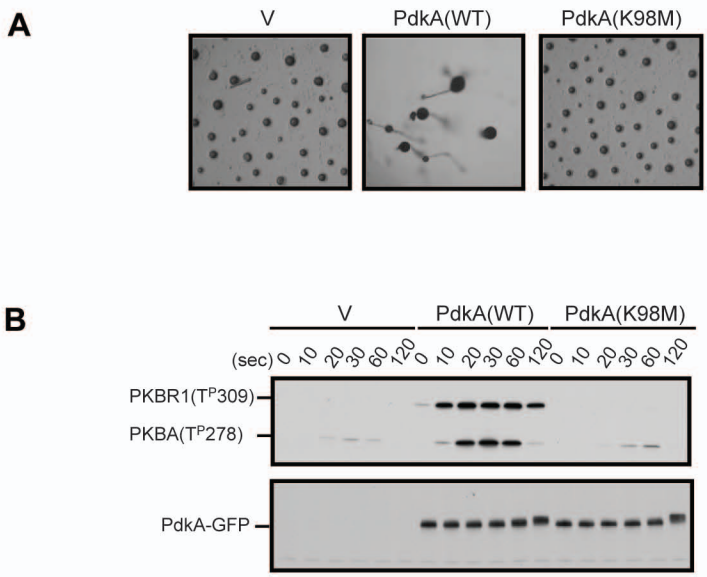
C

	speed (μm/min)	chemotactic speed (μm/min)	chemotactic index	persistence
V	6.33±2.48	2.1±3.4	0.25±0.42	0.66±0.22
PdkA-GFP	9.36±2.4	6.82±2.61	0.72±0.16	0.8±0.13

Supplemental Figure S3 Kamimura et al.



Supplemental Figure S4 Kamimura et al.



Supplemental Figure S5 Kamimura et al.