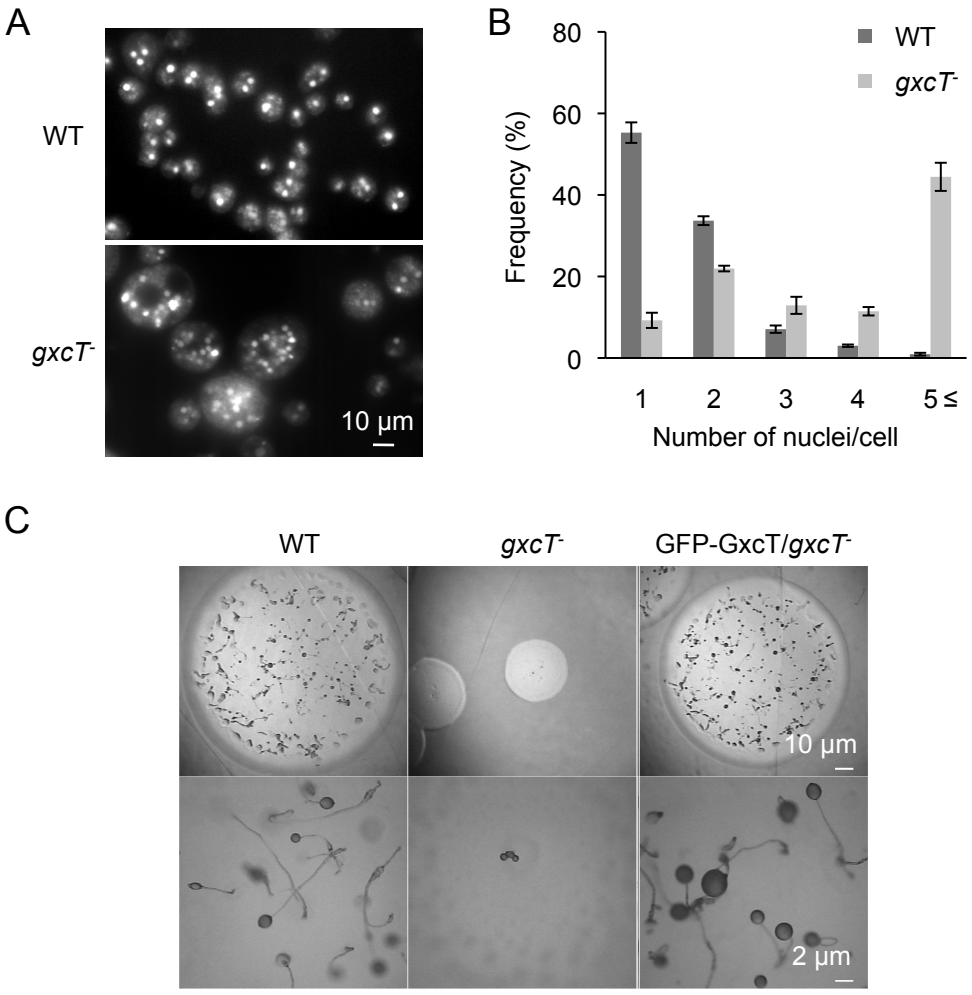
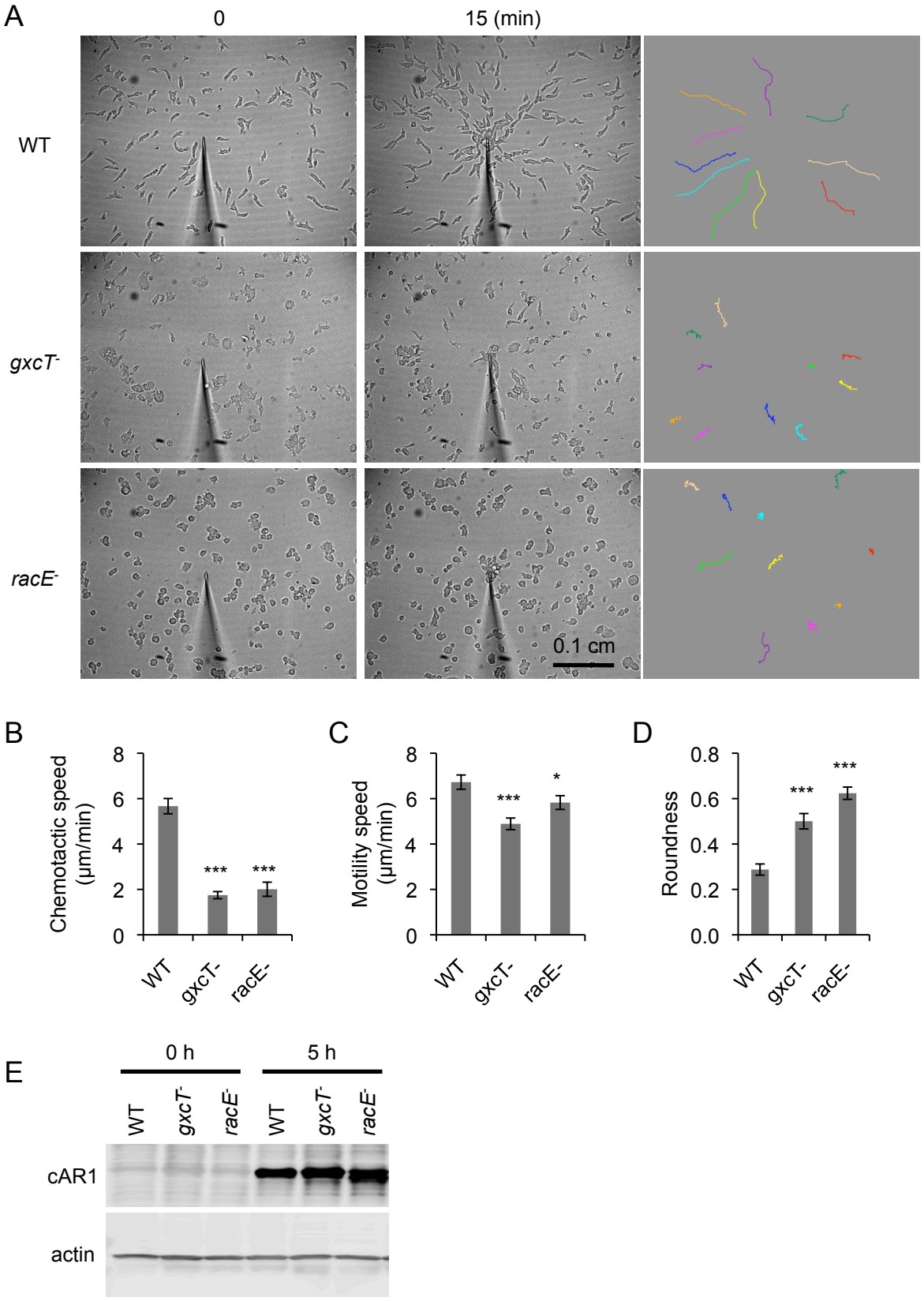
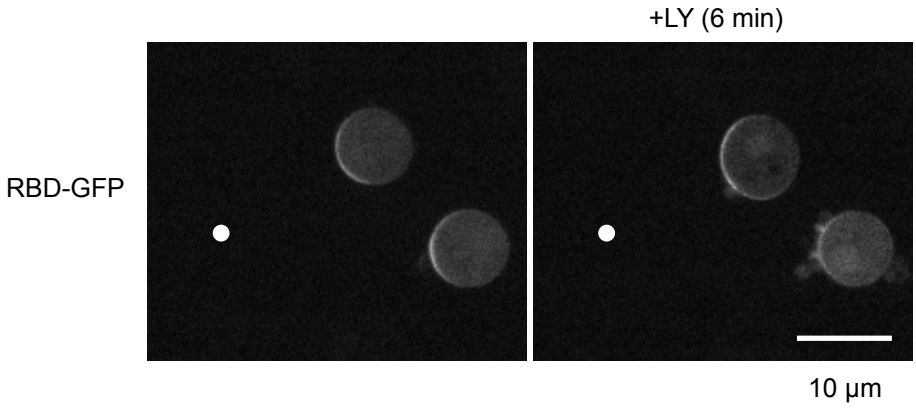


Wang et al. Figure S1

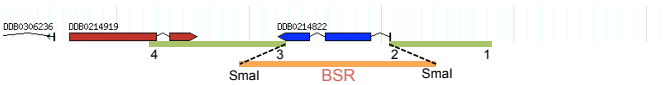


Wang et al. Figure S2

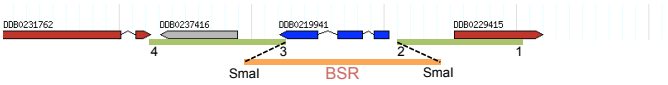


Wang et al. Figure S3

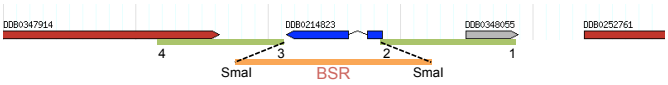
rac1A: Chr. 3 coordinates 441220 to 442067



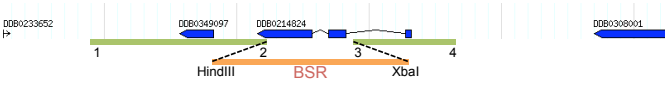
rac1B: Chr. 1 coordinates 2364212 to 2365034



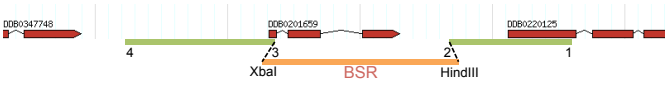
rac1C: Chr. 3 coordinates 6045140 to 6045859



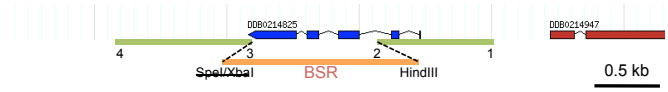
racB: Chr. 3 coordinates 2370154 to 2371319



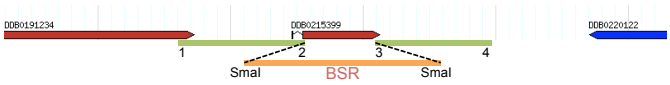
racC: Chr. 6 coordinates 3000314 to 3001296



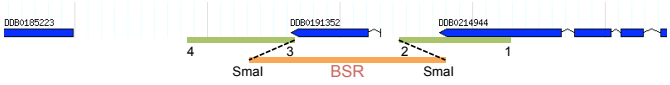
racE: Chr. 3 coordinates 4354163 to 4355462



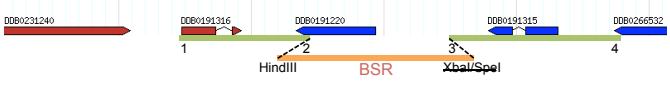
racF1: Chr. 1 coordinates 3007465 to 3008125



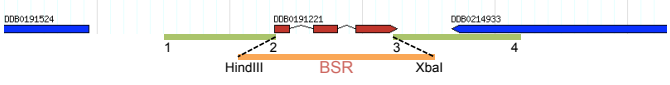
racF2: Chr. 2 coordinates 7215630 to 7216308



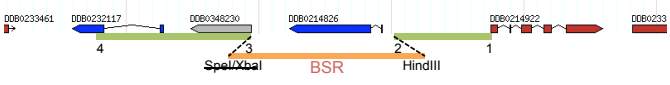
racG: Chr. 1 coordinates 3735334 to 3735939



racH; Chr. 1 coordinates 3360340 to 3361265

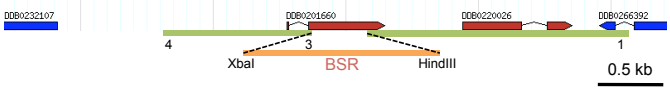


racI: Chr. 3 coordinates 93233 to 93933

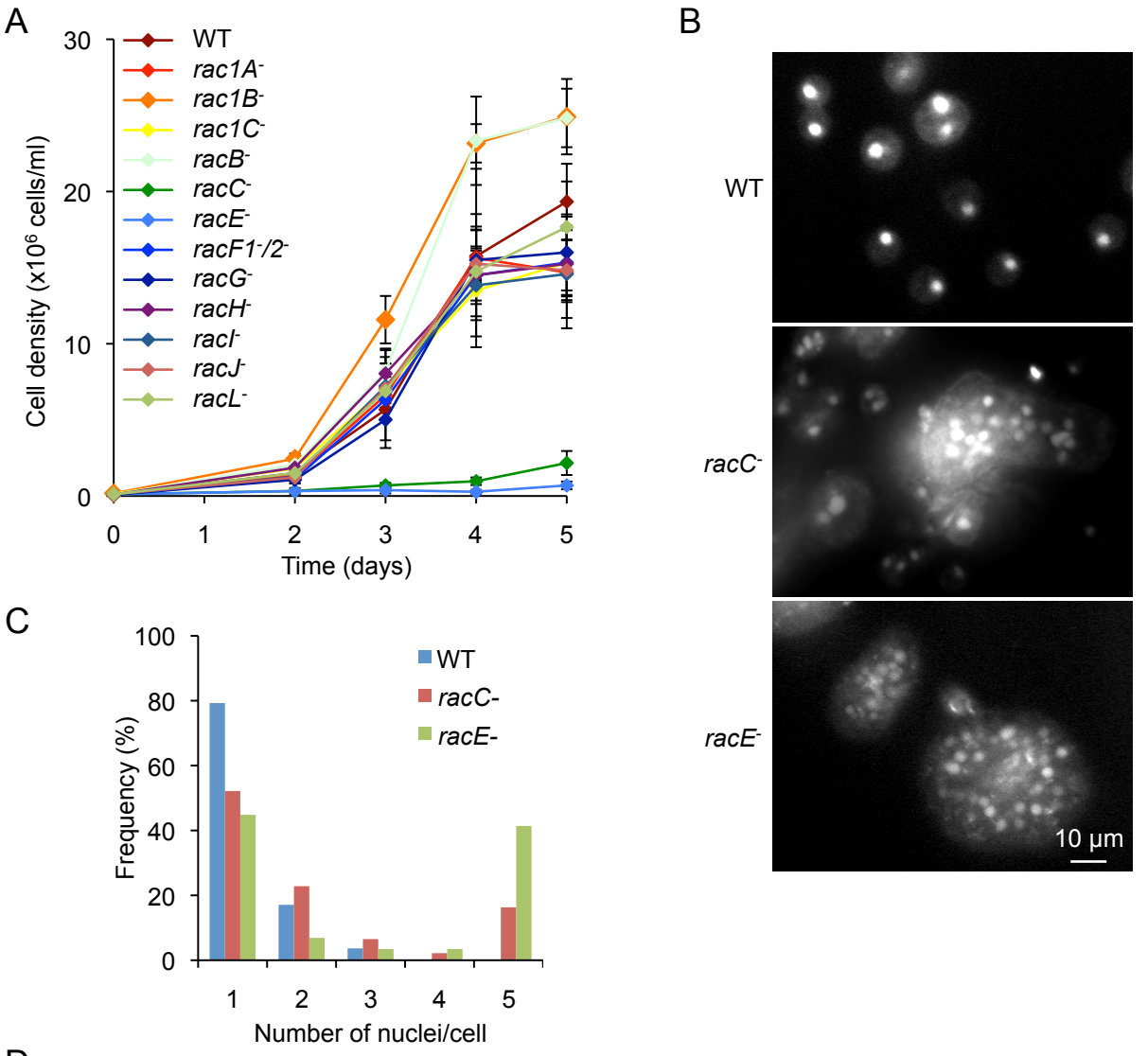


racJ: Chr. 6 coordinates 1884330 to 1885070

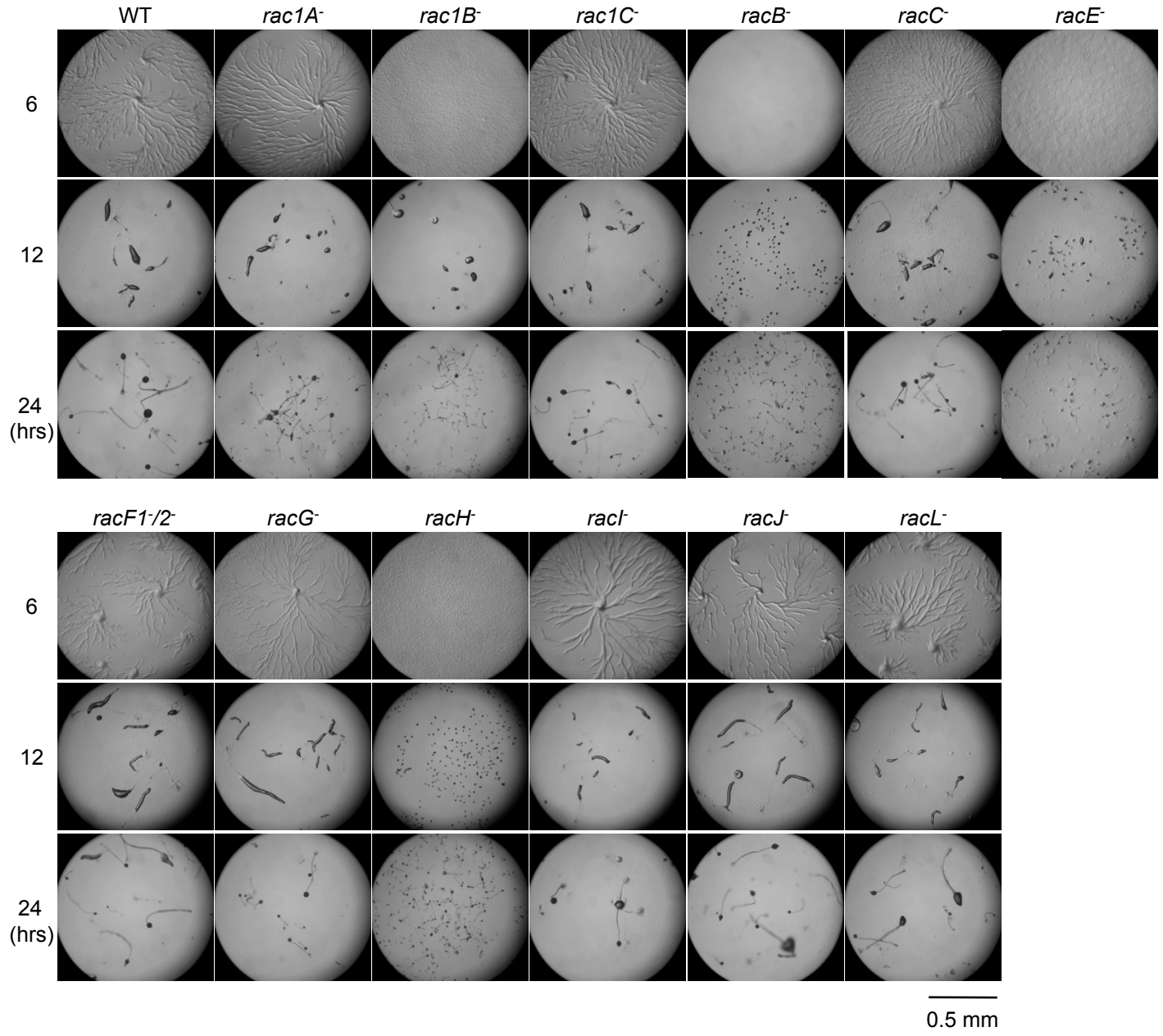
racL: Chr. 6 coordinates 2181555 to 2182295



Wang et al. Figure S4

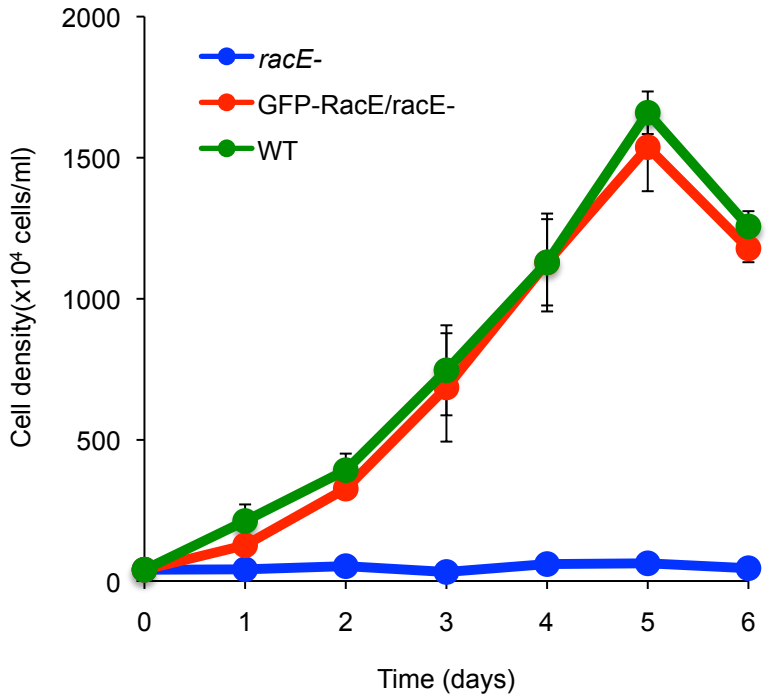


Wang et al. Figure S5

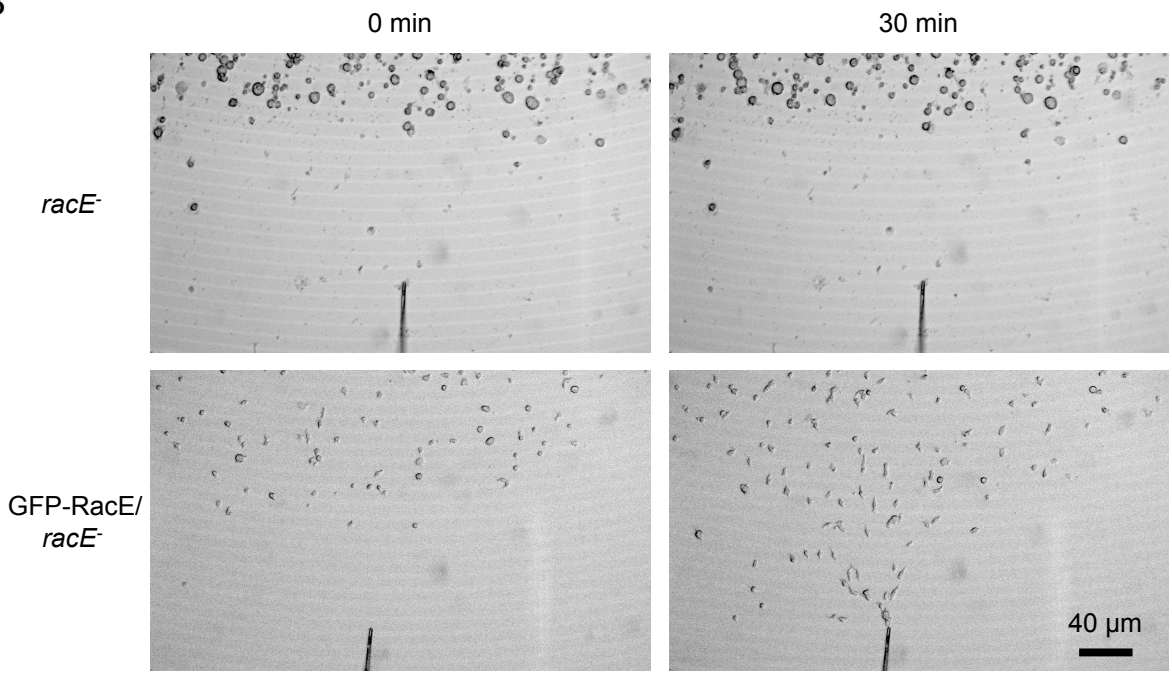


Wang et al. Figure S6

A



B



Wang et al. Figure S7

A

Average Φ (degrees)

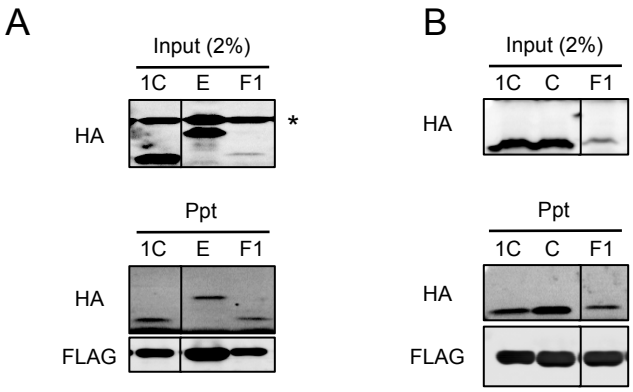
	PHcrac
WT	20.8 ± 1.9 (*n*=22)
racE^{-}	35.9 ± 5.1 (*n*=6) **
RacE/*racE*$^{-}$	26.0 ± 2.3 (*n*=31)
RacE(G20V)/*racE*$^{-}$	35.6 ± 3.8 (*n*=33) **

B

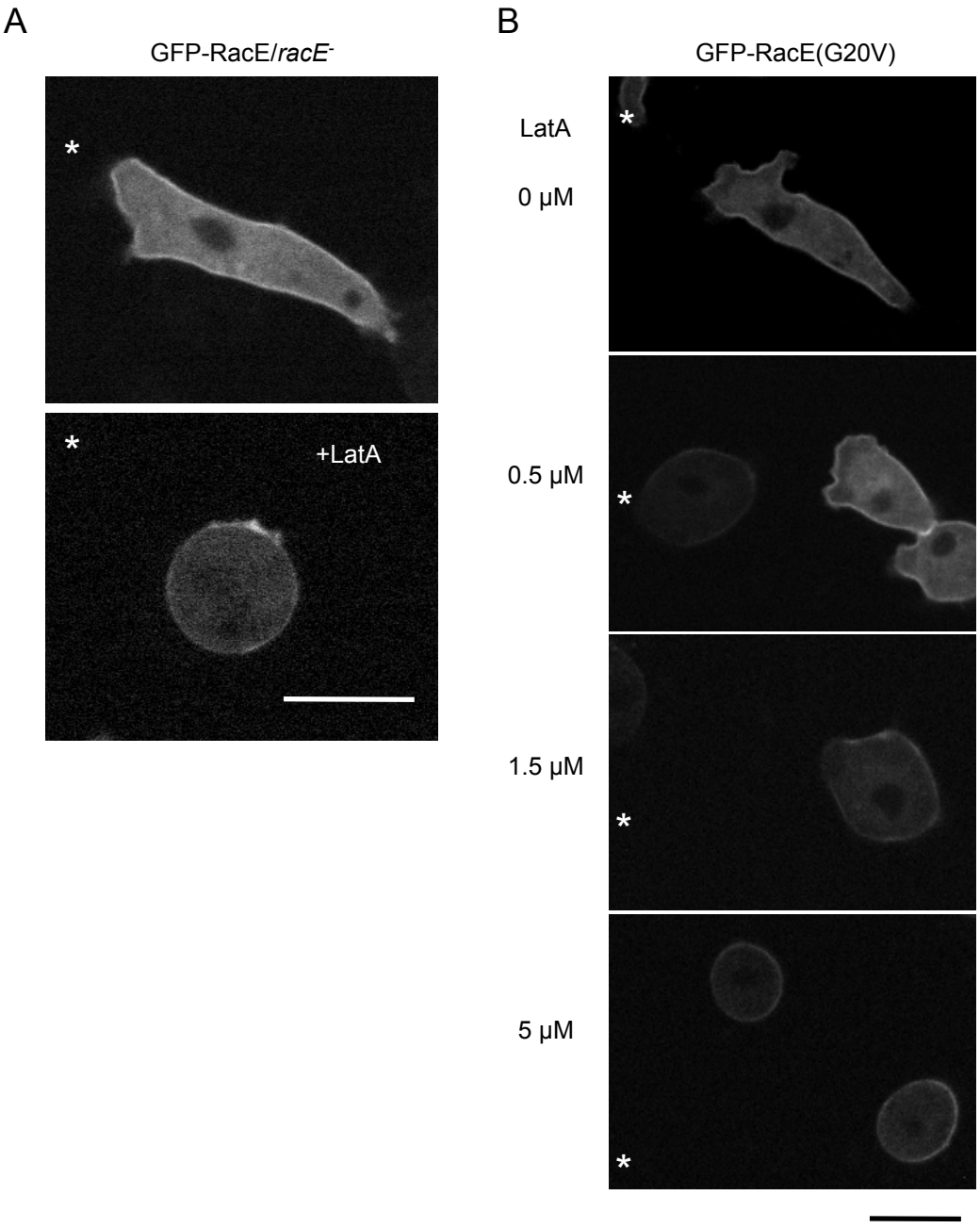
Average Φ (degrees)

	PHcrac	
WT	21.4 ± 1.6 (*n*=17)	
gxcT^{-}	36.3 ± 5.7 (*n*=17) *	*ns*
RacE(G20V)/*gxcT*$^{-}$	45.3 ± 5.8 (*n*=22) **	

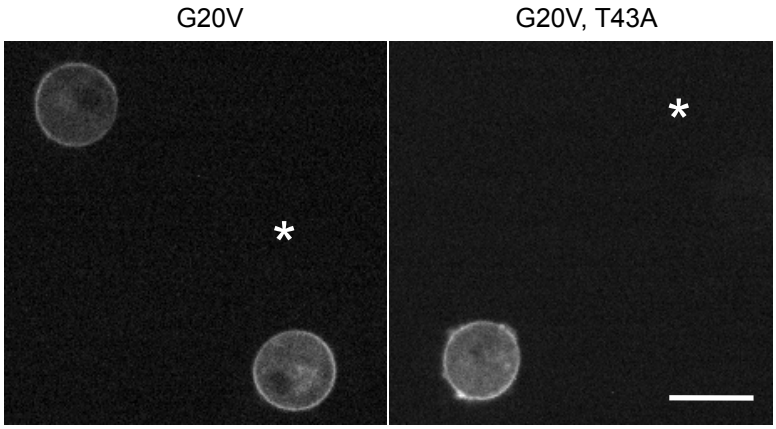
Wang et al. Figure S8



Wang et al. Figure S9



Wang et al. Figure S10



Wang et al. Figure S11

Table S1. RhoGEF genes containing DH domain in the *Dictyostelium* genome

Gene Name	Gene ID	dictyBase ID	PH domain	KO in this study	Publication
DDB_G0269934	DDB_G0269934	DDB0233497			Zhang, et. al. (2008)
gefC	DDB_G0282381	DDB0215004			Wilkins, et. al. (2005)
gxcA	DDB_G0277987	DDB0231342	PH-like		Park, et.al. (2004)
gxcAA	DDB_G0282717	DDB0233317	PH		
gxcB	DDB_G0269424	DDB0233173	PH		Strehle, et.al. (2006)
gxcBB	DDB_G0277131	DDB0233182	PH		
gxcC	DDB_G0284845	DDB0191188	PH		
gxcCC	DDB_G0279123	DDB0232239	PH-like		Plak, et.al (2013)
gxcD	DDB_G0267854	DDB0231995	PH-like		
gxcDD	DDB_G0279733	DDB0348652	PH	X	Mondal, et.al. (2007)
gxcE	DDB_G0291085	DDB0233475	PH		
gxcEE	DDB_G0281047	DDB0233446			
gxcF	DDB_G0282475	DDB0233357	PH		
gxcFF	DDB_G0284739	DDB0233467	PH	X	
gxcG	DDB_G0282073	DDB0233311	PH		
gxcGG	DDB_G0268354	DDB0233491	PH	X	
gxcH	DDB_G0288377	DDB0233496	PH	X	
gxcHH	DDB_G0290493	DDB0233466	PH	X	
gxcI	DDB_G0288383	DDB0304693	PH-like	X	
gxcII	DDB_G0278703	DDB0233473	PH	X	
gxcJ	DDB_G0293978	DDB0233315	PH-like		
gxcJJ	DDB_G0275679	DDB0233356	PH	X	Sawai, et.al. (2007)
gxcK	DDB_G0291007	DDB0233470	PH		
gxcKK	DDB_G0293340	DDB0233318	PH	X	
gxcL	DDB_G0290023	DDB0233472	PH		
gxcM	DDB_G0272372	DDB0233314	PH		Wilkins, et.al. (2005)
gxcN	DDB_G0277017	DDB0233358	PH		
gxcO	DDB_G0293396	DDB0233490	PH	X	
gxcP	DDB_G0285859	DDB0233468	PH	X	
gxcQ	DDB_G0284501	DDB0233494	PH	X	
gxcR	DDB_G0285303	DDB0233469	PH	X	
gxcS	DDB_G0280087	DDB0233355	PH		
gxcT	DDB_G0269610	DDB0233444	PH	X	
gxcU	DDB_G0291996	DDB0233310	PH	X	
gxcV	DDB_G0282271	DDB0233445	PH	X	
gxcW	DDB_G0278147	DDB0233316	PH		
gxcX	DDB_G0274889	DDB0233493	PH		
gxcY	DDB_G0293266	DDB0233471	PH	X	
gxcZ	DDB_G0293928	DDB0233312	PH-like		
kxcA	DDB_G0289859	DDB0229867	PH	X	
kxcB	DDB_G0293124	DDB0229973	PH		
myoM	DDB_G0292262	DDB0191100	PH		Geissler, et.al. (2000)
roco5	DDB_G0294533	DDB0232931	PH		Sawai, et.al. (2007)
xacA	DDB_G0291978	DDB0216228	PH		
xacB	DDB_G0278417	DDB0233495	PH	X	
xacC	DDB_G0285391	DDB0231560	PH	X	Ruchira, et.al. (2004)

Table S2. Rho family GTPase in the *Dictyostelium* genome

A

Gene	Gene ID	dictyBase ID	CAAX	KO in this study	Previous publication on KO
rac1A	DDB_G0277869	DDB0214822		X	
rac1B	DDB_G0268622	DDB0219941		X	
rac1C	DDB_G0282365	DDB0214823		X	
racA	DDB_G0286555	DDB0191173	No (RhoBTB)		
racB	DDB_G0279605	DDB0214824		X	Park et.al. (2004)
racC	DDB_G0293526	DDB0201659		X	Han et.al. (2006)
racD	DDB_G0291976	DDB0216194	No		
racE	DDB_G0280975	DDB0214825		X	Larochelle et.al. (1996)
racF1	DDB_G0269176	DDB0215399		X	
racF2	DDB_G0276967	DDB0191352		X	Muramoto and Urushihara (2006)
racG	DDB_G0269178	DDB0191220		X	Somesh et.al. (2006)
racH	DDB_G0269240	DDB0191221		X	Somesh et.al. (2006)
racI	DDB_G0277897	DDB0214826		X	
racJ	DDB_G0292560	DDB0201669		X	
racL	DDB_G0292816	DDB0201660		X	
racM	DDB_G0289103	DDB0230015			
racN	DDB_G0278009	DDB0230036			
racO	DDB_G0277791	DDB0230035			
racP	DDB_G0285453	DDB0230013	No		
racQ	DDB_G0278011	DDB0233216			

B

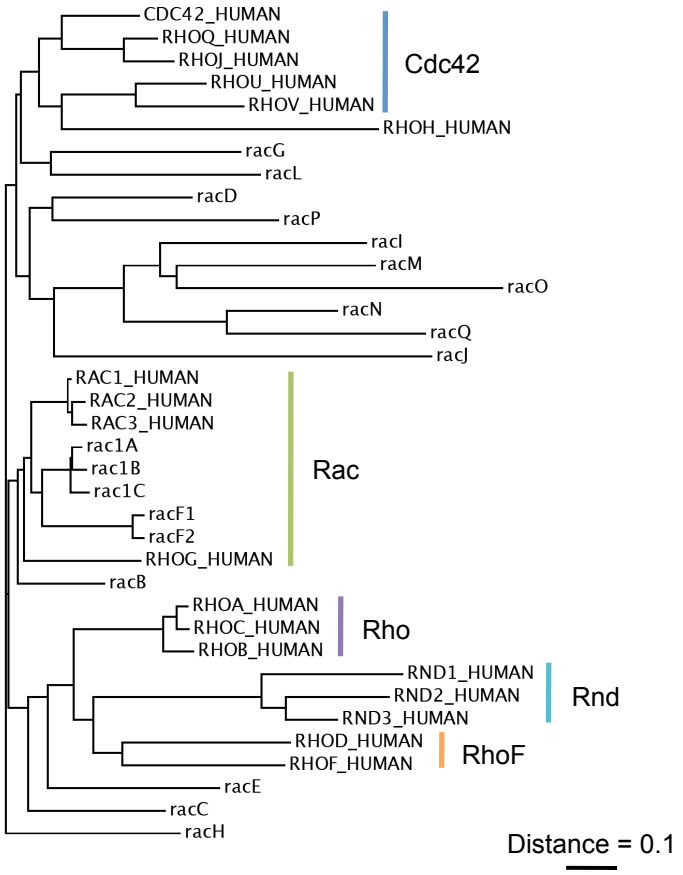


Table S2. Phylogenetic analysis of the Rho GTPases in *Dictyostelium* and human. (A) A list of *Dictyostelium* Rho family GTPases. The genes that have been disrupted in this and previous studies are indicated. (B) The amino acid sequences of Dictyostelium and human Rho family proteins were aligned using Clustal Omega - Multiple Sequence Alignment (www.ebi.ac.uk). RhoBTB proteins were excluded. A phylogenetic tree based on this alignment was created using ClustalW2 - Phylogeny (www.ebi.ac.uk). True distance correction, true gap exclusion, and the neighbor-joining method were used to create the phylogenetic tree.

Table S3. Primers for gene disruption of Rho family GTPase in the *Dictyostelium* genome

Name	Sequence	Position in genomic DNA	Length of gene (bp)
rac1A-1	GCGGCCGCAAATGTTGATATTTTTCTATTTTCC	(-774)-(-750)	848
rac1A-2	CCCGGGTATACAAAACTAAAATTTACCTTGC	3-27	
rac1A-3	CCCGGGAAAGAAGAAAAGTTCAGGTGGTTGC	812-836	
rac1A-4	GTCGACTCACTTATTTCAATCATTTGGTTCG	1803-1827	
rac1B-1	GCGGCCGCGATTTTGAATCACAAAATTGTTTAG	(-1000)-(-976)	823
rac1B-2	CCCGGGAATTTTTGTTTGTTTATGCCATTTG	(-96)-(-72)	
rac1B-3	CCCGGGAAAAACCAAAATCTTCAAAAGGTTG	786-810	
rac1B-4	GTCGACATGGGTTCAAGAATTCAATAAAGTC	1794-1818	
rac1C-1	GCGGCCGCTCAGATTTTAAATGATCTAAAATGG	(-981)-(-957)	720
rac1C-2	CCCGGGTACAACTACACATTTAATTGCTTGC	3-27	
rac1C-3	CCCGGGCTAACCAACCATACTTAATGAAATG	739-763	
rac1C-4	GTCGACTAAAATTAGAAGATATCGAATATCC	1632-1656	
racB-1	GTCGACTCAATAATAAACTATGCACTATCTG	2373-2397	1166
racB-2	AAGCTTCAAAAAGGTCTCAAACAAGTTTTCG	1062-1086	
racB-3	TCTAGAATTAGTAATCAATCAGATAACATAG	442-466	
racB-4	GCGGCCGCCATTAAACTAACCTACAATTGCTCG	(-331)-(307)	
racC-1	GTCGACGAAAAACATTATCAAACTCCATCGG	2203-2227	983
racC-2	TTTGATCTTAAAACAGTCTCAAGC	1383-1406	
racC-3	TCTAGAACCCTACAGCACCATCACCAATAACG	30-55	
racC-4	GCGGCCGCTAAAGCCTTTAGAGATAATTATGGC	(-1162)-(-1138)	
racE-1	GTCGACTTCTAAATATAGCCAACCAATTTGG	(-608)-(-584)	1300
racE-2	AAGCTTTGAAATATAGAATCCATTTGATTTG	326-350	
racE-3	ACTAGTGAAAAGCAGGTAAAAAGAAATCTGG	1260-1284	
racE-4	GCGGCCGCTGTTGTTTTTCTTAGCTTGATCG	2249-2271	
racF1-1	GCGGCCGCATACTTGTCAAAACTACACTAGTGC	(-988)-(-964)	661
racF1-2	CCCGGGACAACAACACATTTAATATTTTGCC	81-105	
racF1-3	CCCGGGAAAAACCAAAGAAGAAGAGTTGTAC	627-651	
racF1-4	GTCGACTTATGAAATTGAAGTTACTAATTGG	1474-1498	
racF2-1	GCGGCCGCTTTACAAATCATATGATCCACGTGC	(-995)-(-971)	679
racF2-2	CCCGGGTTTTTTTGTCCAAAAATTTTCATCG	(-172)-(-148)	
racF2-3	CCCGGGAAAAACCAAAGAAGAAGACCTGTAC	645-669	
racF2-4	GTCGACAGGTATTTTGGATTCAATGATTGCG	1429-1453	
racG-1	GGGGTCGACTAGTTTAAGAAGAGCACCTGATTATCATGG	1357-1386	606
racG-2	CCCAAGCTTACATTTAGTATTTGAAAAAGCAATTGACGC	495-524	
racG-3	GGGACTAGTTTCCTTTAGCAAACACATAACTGAGTAGTC	(-595)-(-566)	
racG-4	CCCGCGGCCGCATGAAATGGTTGATCCACCAATTTCCCATG	(-1869)-(-1840)	
racH-1	GTCGACATAGTAATAGCAACAATCATAATGG	(-837)-(-813)	926
racH-2	AAGCTTAACCATTACTTTAATATCTTTTACC	3-27	
racH-3	TCTAGAAAAAAAGGTGATAAAGACTCAAAGG	885-909	
racH-4	GCGGCCGCACCAAGTAATGATAGTATAGAATCC	1812-1836	
racI-1	GGGGTCGACAAATAGCCATAATAAATAAGTAACGCACAC	(-862)-(-833)	701
racI-2	CCCAAGCTTAAAAATCTTCTTTGAAAATATCCAACTCAC	(-143)-(-114)	
racI-3	GGGACTAGTTCTAAAACCTTATTGGGTTTATCATAATGC	1000-1029	
racI-4	CCCGCGGCCGCCATGTAGCATTCAAAAGCAGTACGAAGAGC	2192-2221	
racJ-1	GTCGACACCATGTTATTATTATAAGCAAACG	(-788)-(-764)	741
racJ-2	AAGCTTTTACCAACTCCATCATCTCCAAGAC	41-65	
racJ-3	TCTAGATACAAACAAAAAATCCTGTAAAACC	711-735	
racJ-4	GCGGCCGCAAGGTTTAAATGATAACATTTTACC	1707-1731	
racL-1	GTCGACTTGGAATGGTCATTGAAATCTTTGG	2640-2664	741
racL-3	TCTAGAATAAACTTGTTTTACCAACTGCTCC	184-208	
racL-4	GCGGCCGCTTATACAATAAATAAACTGGGTCAC	(-923)-(-899)	

Table S4. Primers used in this study

	***Dictyostelium* GxcT KO construct**	
1	SLB367-5S	AAATAATGGTGGTAGTGGTTCATC
2	SLB367-5A	AAGATGAAGAGTTTTGGACCATTG
	***Dictyostelium* expression plasmids**	
3	gxcT-FS2	GAAGATCTATGCAAGGACAGGGACAACAATTTTTATCCCAACAACAAAAATTATCACAGC
4	gxcT-IA	GGGTTGTTTGGGAAGATAC
5	gxcT-IS	TCAACTACAATATCATCTGC
6	gxcT-FA stop BamHI	CCGGATCCTTATTTTGGAAGTAAATTTAATAAAGC
7	5' SLB367	CCAGATCTAATCTCCTCCAAGAAGGTAG
8	3' SLB367	CCCTCGAGTTTGGAAGTAAATTTAATAAAGC
9	racE-start	AGATCTATGTCAGAAGATCAAGGTTCAGGAGC
10	racE-stop	AGATCTTTAAAGTATAATACAACCAGATTTC
11	racE(G20V)-1	GTGATGTTGCTGTTGGTAAAACATGTC
12	racE(G20V)-2	ACAGCAACATCACCGACAACTAC
13	racE(T25N)-1	GCTGTTGGTAAAAACTGTC
14	racE(T25N)-2	GACAGTTTTTACCAACAGC
15	racE(T43A)-1	GTACCAGCTGTTTTTG
16	racE(T43A)-1	CAAAAACAGCTGGTAC
17	rhoA-start	GGAAGATCTTCCATGGCTGCCATCC
18	rhoA-stop	GGAAGATCTTCCTCACAAGACAAGGC
	Bacterial expression plasmids	
15	GxcT-BamHI-3721	CCCGGATCCCAATTGCGTTACAAAGC
16	GxcT-SmaI-4725	CCCCCCGGGTTATTTTGGAAGTAAATTTAATAAAGC

Table S5. Plasmids used in this study

***Dictyostelium* expression**	**Primers used for construction**				**Drug for selection**	**References**
pJK1-GFP: pIS1					G418 (20 µg/ml)	Zhang et. al. (2011)
pJK1-GFP-GxcT	3	4	5	6	G418 (20 µg/ml)	This study
pJK1-PHgxcT-GFP:pMSG-PHgxcT	7	8			G418 (20 µg/ml)	This study
pJK1-PHcrac-GFP: pWF38					G418 (20 µg/ml)	Dormann et. al. (2002)
pDM181-PHcrac mCherry					G418 (20 µg/ml)	Chen et. al. (2012)
pDRH-LimEΔcoli-mRFP					Hygromycin (50 µg/ml)	Gift from Dr. Devreotes (JHMI)
pJK1-GFP-RacE	9	10			G418 (20 µg/ml)	This study
pJK1-GFP-RacE(G20V)	9	10	11	12	G418 (20 µg/ml)	This study
pJK1-GFP-RacE(T25N)	9	10	13	14	G418 (20 µg/ml)	This study
pJK1-GFP-RacE(G20V,T43A)	9	10	15	16	G418 (20 µg/ml)	This study
pDM323-RBD(Raf)–GFP					G418 (20 µg/ml)	Xiong et.al. (2010)
pDRH-PHcrac-RFP					Hygromycin (50 µg/ml)	Gift from Dr. Devreotes (JHMI)
pJK1-GFP-RhoA	17	18			G418 (20 µg/ml)	This study
pKJ1-GFP-RhoA(Q63L)	17	18			G418 (20 µg/ml)	This study
Bacterial expression						
pGEX-2T						Amersham
pGEX-GxcT	15	16				This study

SUPPLEMENTARY IINFORMATION

Supplementary Experimental Procedures

Lipid dot blot assay

A lipid dot blot assay was performed as described (1, 2). Cells were cultured in HL5 medium to 3-5 $\times 10^6$ cells/ml and starved for 2 hours in DB. After washing, cells were resuspended to 5×10^7 cells/ml in 10 mM sodium phosphate (pH 7.0) containing 1% protein inhibitor cocktail (P8340, Sigma), and filter-lysed by passing through polycarbonate membranes with 5-μm pores (110613, Whatman) on ice. Cell lysates were clarified by centrifugation and mixed with equal volumes of 2× binding buffer (10 mM sodium phosphate [pH 7.0], 0.5% NP40, 300 mM NaCl). Membranes spotted with different phospholipids (PIP membrane P-6001; Echelon) were blocked in PBS containing 3% fatty acid-free bovine serum albumin and then mixed with the lysates for at least 3 hours. After washing, the membranes were probed with anti-GFP antibodies followed by Alexa488-labeled anti-rabbit IgG antibodies (Invitrogen). The membranes were scanned with a PharosFX Plus molecular imager and analyzed with Quantity One software (Bio-Rad).

Actin polymerization assay

Differentiated cells were pretreated with 3 mM caffeine for 20 min and stimulated with 1 μM cAMP (1, 3, 4). At various time points after stimulation, 5×10^6 cells were harvested and lysed in Triton X-100 buffer (1% Triton X-100, 10 mM KCl, 10 mM imidazole, 10 mM EGTA, 50 μg/ml NaN_3). Samples were vortexed, held on ice for 10 min, and then incubated at room temperature for 10 min. After centrifugation at 8000 × g for 4 min, the pellet fractions were washed and resuspended in 2× SDS-PAGE sample buffer. Proteins were resolved by SDS-PAGE and visualized by Coomassie Brilliant Blue staining. Actin was quantified by densitometric analysis using ImageJ software.

GST-pull down

A GST pull-down assay was performed as described with some modification (5). GST fused to the RhoGEF and PH domains of GxcT (GST-GxcT) was expressed from pGEX-2T (GE Healthcare) in the *E. coli* BL21 strain with 0.5 mM IPTG at 16°C overnight. Cells were frozen in PBS containing 1 mM DTT and 1 mM PMSF at -80°C for 1-2 hours and thawed in the presence of 100 μg/ml of lysozyme on ice for 20 min. Cells were sonicated ten times (30 seconds each) on ice. After clarification by centrifugation, cell lysates mixed with glutathione Sepharose 4B (GE Healthcare) at 4°C for 1 hour. After washing, GST-GxcT beads were kept at 4°C. To prepare *Dictyostelium* cell lysates, cells expressing GFP or GFP-RacE were starved for 3 hours and lysed in lysis buffer (25 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-100, 1 mM NaF, 0.5 mM Na_3VO_4, 1 mM DTT, 10% glycerol, and protease-inhibitor cocktail [Roche]) with or without 20 mM EDTA on ice for 10 min. After clarification by centrifugation, cell lysates were diluted with 4x volumes of dilution buffer (25 mM Tris-HCl, 15 mM NaCl, 1 mM NaF, 1 mM DTT, 10% glycerol, and protease-inhibitor cocktail) with or without 20 mM EDTA. GST-GxcT or GST beads were added to the cell lysates and incubated at 4°C overnight. After washing with dilution buffer, bound proteins were eluted with 2x SDS-PAGE sample buffer and analyzed by SDS-PAGE and immunoblotting using anti-GFP antibodies.

Supplementary Figure Legends

Fig. S1. Cytokinesis defects in *gxcT*⁻ cells and localization of the PH domain of GxcT. (A) DAPI staining showed that *gxcT*⁻ cells, grown as described in Fig. S6A, were multinucleated, similar to *racE*⁻ cells. This result suggests that *gxcT*- cells have cytokinesis defects. To avoid multinucleation in *gxcT*⁻ cells, we grew this mutant strain as adherent cells on Petri-dishes for all other experiments described in this study. (B) The number of nuclei per cell was quantified using the DAPI-stained cells described in (A). Values represent the mean ± SEM (n = 3). (C) WT cells, *gxcT*⁻ cells, and *gxcT*⁻ cells expressing GFP-GxcT were plated on bacterial lawn and examined for the formation of fruiting bodies. (D) WT cells expressing GFP fused to the PH domain of GxcT or Crac were observed by fluorescence microscopy.

Fig. S2. *gxcT*⁻ and *racE*⁻ cells are defective in chemotaxis after longer starvation and normally express cAR1. (A) WT, *gxcT*⁻ and *racE*⁻ cells were developed for 8 hours and placed in a chemoattractant gradient, established by a micropipette releasing cAMP, and observed for 15 min by phase contrast microscopy. The trajectories of cell migration are shown. These chemotaxis assays were then quantified (B-D). Values represent the mean ± SEM (n = 3). At least 10 cells were analyzed for each experiment. *$p < 0.05$; ***$p < 0.001$. (E) Whole cell lysates were prepared from WT, *gxcT*⁻ and *racE*⁻ cells and analyzed by immunoblotting using antibodies to cAR1 and actin.

Fig. S3. The localization of Ras activation is independent of PIP3. WT cells expressing RBD-GFP were exposed to a cAMP gradient in the presence of Latrunculin A (5 μM) and LY294002 (20 μM). Images were taken 1 min after the cAMP gradient was formed. White dots indicate the position of the micropipette tip that was releasing cAMP.

Fig. S4. Disruption of the genes encoding the Rho family of GTPases. 13 Rho family genes were deleted using homologous recombination in *Dictyostelium*. The blasticidin resistance cassette (BSR) was inserted into each Rho gene in the indicated regions. The PCR primers used to make the disruption constructs are listed in Table S2, and their position within the genome relative to the gene sequence is indicated by the numbers (1-4) after the oligo name. The restriction enzyme sites used for cloning are also shown. The depictions shown for each genomic region were modified from www.dictybase.org.

Fig. S5. The growth phenotypes of Rho family GTPase knockout strains. (A) Cells were cultured in suspension, shaking at 180 rpm on a rotary shaker, in HL5 medium at 22°C. Each mutant strain was counted daily with a hemocytometer. Values represent the mean ± SEM ($n \geqq 3$). (B) WT, *racC*⁻ and *racE*⁻ cells were stained with DAPI to visualize nuclei after cultured in the HL5 medium for 3 days. Unlike the majority of WT cells, *racC*⁻ and *racE*⁻ cells were multinucleated, suggesting impaired cytokinesis, consistent with previous reports (6, 7). It is known that *Dictyostelium* cells that have cytokinesis defects become multinucleated when grown in suspension culture (8). To avoid multinucleation in *racE*⁻ cells, we grew this mutant strain as adherent cells on Petri-dishes for all other experiments described in this study. (C) The number of nuclei per cell was quantified using the DAPI-stained cells. (D and E) Cells were cultured as in (A) until they reached the exponential growth phase, at which point they were plated clonally on bacterial lawns and incubated at 22°C for 5 days to monitor growth and plaque formation.

Fig. S6. The developmental phenotypes of Rho family GTPase knockout strains. To examine developmental phenotypes, cells were plated at a density of 5×10^5 cells/cm^2 on 1% non-nutrient DB agar. Images were taken at various time points, as indicated, after the cells were plated on DB agar.

Fig. S7. GFP-RacE rescues growth and chemotaxis defects in *racE*$^-$ cells. (A) WT cells, *racE*$^-$ cells, and *racE*$^-$ cells expressing GFP-RacE were cultured in HL5 medium and counted daily with a hemocytometer. Values represent the mean ± SEM ($n = 3$). (B) Developed *racE*$^-$ cells and *racE*$^-$ cells expressing GFP-RacE were placed in a cAMP gradient and observed for 30 min by phase contrast microscopy.

Fig. S8. GFP-RacE rescues gradient sensing defects in *racE*$^-$ cells. The average of the absolute value of Φ for PHcrac-RFP crescents was calculated from the time-lapse analyses in *racE*$^-$ cells (A) and *gxcT*$^-$ cells (B). Values represent the mean ± SEM. *n* indicates the number of cells analyzed. $^*p < 0.05$; $^{**}p < 0.01$.

Fig. S9. GxcT binds to Rac1C, RacC, RacE and RacF1. *Dictyostelium* cell lysates were prepared from cells expressing HA-tagged Rac1C, RacE or RacF1 (A) and HA-tagged Rac1C, RacC or RacF1 (B). These lysates were incubated with cell extracts expressing FLAG-tagged RhoGEF and PH domains of GxcT (FLAG-GxcT). FLAG-GxcT was immunoprecipitated and the bound fractions were analyzed by Western blotting with antibodies to the HA and FLAG epitopes. The input and bound (Ppt) fractions are shown. An asterisk indicates non-specific bands.

Fig. S10. Analyses of GFP-RacE localization. (A) *racE*$^-$ cells expressing GFP-RacE were examined by fluorescence microscopy in the presence or absence of Latrunculin A (5 μM). Asterisks indicate higher concentrations of cAMP in a gradient. (B) WT cells expressing GFP-RacE(G20V) were examined by fluorescence microscopy at the indicated concentrations of Latrunculin A.

Fig. S11. The localization of RacE(G20V) and GFP-RacE(G20V, T43A) in a cAMP gradient in the presence of Latrunculin A. WT cells expressing GFP-RacE(G20V) or GFP-RacE(G20V, T43A) were observed in the presence of Latrunculin. White asterisks indicate the position of the cAMP-releasing micropipette tips

Supplementary References

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