Supplemental Materials

Molecular Biology of the Cell

Senoo et al.

A

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271 YGGETIAVVDDSGTTPRDYRRSRSISCEIIPKINGVITTSPQRVTTTTTTTTPSTGGVVVADEESDSSEEESDSSEEESDEYTDEESETE 360
361 LQVVSNATPRRSDDFTPTIVESPPLTSVNSNDNTSSGTVVAPIDLNSSTGGNTGSQQPPQPSSQQQKPDQASENTAVAASSISATTNVTS 450
451 AASTTTVAPDSIINTKDVTVVSSTLTTTTSATSSTTSATTQSIPAPPSPSQQRAAQSISTSSVTPAAITKPTKDAKDKKDPAKKSIGATL 540
541 TRTVTKTFIRDSKENNKVPTGTSPPVSSSTSISSSTGIKKDKVKLSKEEKDRIKKEKSAKKKKEKDEKKQQKTNKKALTKNNSSTDVKKG 630
631 FVSPQQQQILDSPYRIYGVRLTQLVLSNDGDLPAILTQTITVLSNSNKLDVNSVFVGAENEPAVREIRKRSDLERIDFSIIGDPRVVAGL 720
721 LILFFAELPQPLFNSKFFGDLVEINDITNPQVKLNDLKQLINSLSQLRRSLLQILVTFFTSKYINGNSVTTRAIAIQSIAQSFGPQFFRG 810
811 TSSSDSDIQVGIETLKLIIDNYVFLFEKTNEPDVKYKNIDGKMIISEGSIDKLIDKATDQYYPYNEKYFSLTFFITHLFFIQPHELADKL 900
901 ITLYRENLDTLETKKKWKKHRRSKKASFINEAVKLWVDYCYKEMREDKELSKKILKGFPHLEAQLASRLSHRTTINDFLKLPKRVHSRTR 990
991 SASFSDTLLSTGGIGSTSGGIGGGVNNCLLSAMEIAEQCTLVDYDLFTNVRLSDWVRLVQGSVDPQTAPSLSLALKRSTIWAQWAMGEIL 1080
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1441 DSSNVEKEKLSSSQEQQEQQEQKQQEQQQQQQEPQPLFIRHYKKQRSRSAQSKDFFEFEWFIQLNLDADCKNILSFDLKISNLVFSTDTS 1530
1531 PNIRDQLLESFKSYLVSQECVQYINFNENKQPVAAPVTPATTIVTTKEESTTVTSSTTTVVQESVPSTNAE 1601
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Asparagine rich region (72-126)

Region require for plasma membrane localization (361-644)

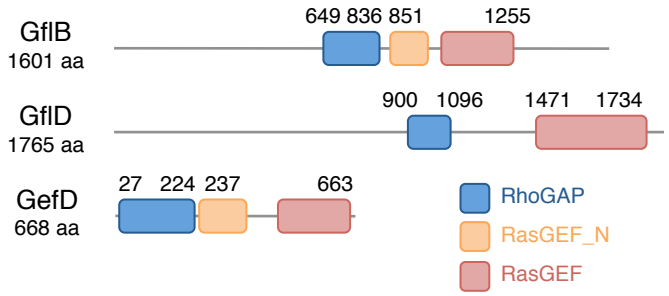
Lysine rich region (520-534), (579-616)

RhoGAP (649-836)

RasGEF_N (851-983)

RasGEF (1021-1255)

B



C

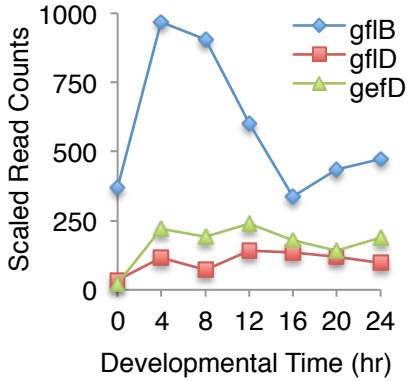


Figure S1. Amino acid sequence, domain structure, and expression profile of GflB. (A) Amino acid sequence of GflB. (B) Domain structures of GflB, GflD, and GefD. (C) Expression profiles of GflB, GflD, and GefD during development. The values are obtained from dictyExpress in the *Dictyostelium* database (www.dictyexpress.org) as described elsewhere (Parikh et al., 2010).

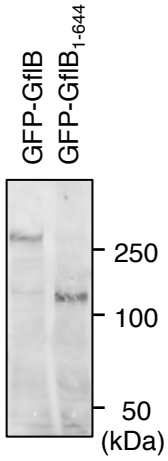


Figure S2. Whole-cell lysates analyzed using immunoblotting with anti-GFP antibodies to detect the indicated PTEN-GFP fusions.

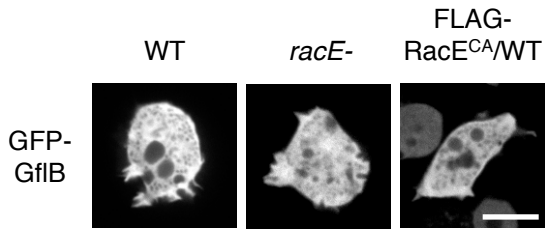


Figure S3. GFP-GflB was examined in WT cells, *racE*$^{-}$ cells, and WT cells expressing constitutively active $RacE_{G20V}$ using fluorescence microscopy. Bar, 10 μm.

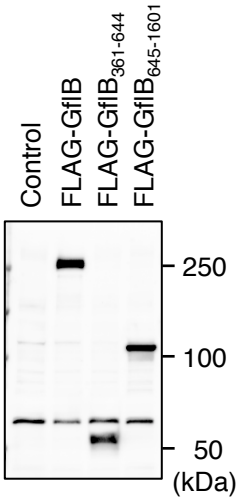


Figure S4. Whole-cell lysates analyzed using immunoblotting with anti-FLAG antibodies.

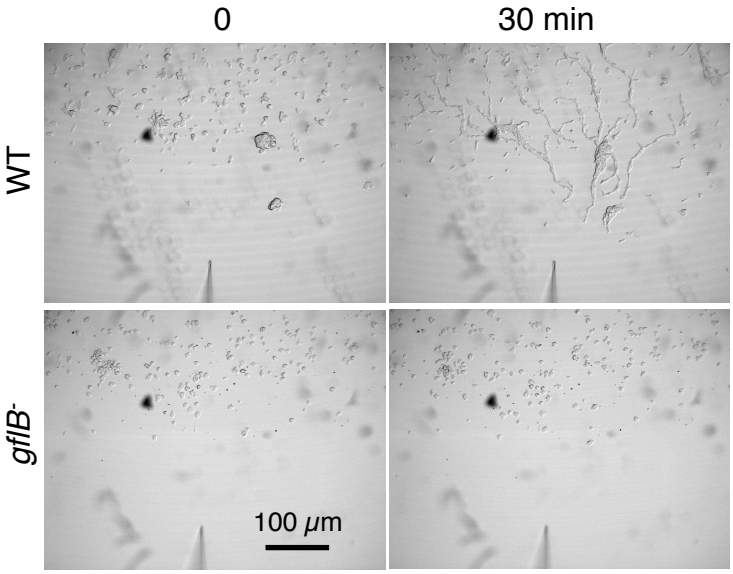


Figure S5. After 8 hours starvation, WT and *gflB*$^{-}$ cells were placed in a chemoattractant gradient generated by a micropipette that released cAMP and observed for 20 min using phase contrast microscopy.

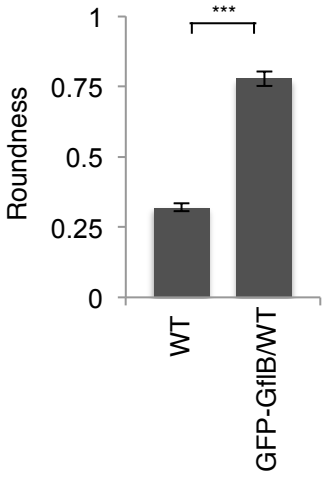


Figure S6. Roundness was determined by calculating the ratio between the short axis (As) and long axis (Al) of cells (As/Al) in WT cells in the presence or absence of GFP-GflB expression. Values represent mean ± SEM. At least 30 cells were analyzed for each group.

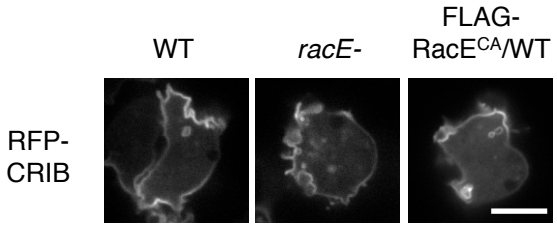


Figure S7. CRIB-RFP was examined in WT cells, *racE*$^{-}$ cells, and WT cells expressing constitutively active $RacE_{G20V}$ using fluorescence microscopy. Bar, 10 μm.

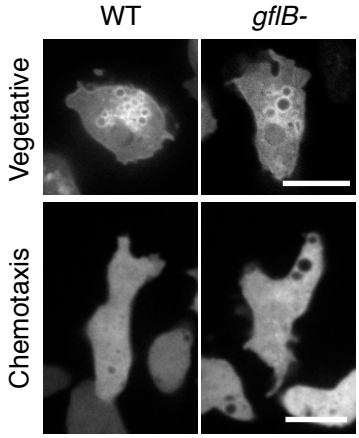


Figure S8. The PS biosensor GFP-LactC2 was observed in WT and *gflB*$^{-}$ cells during growth (vegetative) and after differentiation (chemotaxis).

Table S1. Plasmids used in this study

Plasmids	Primers used	Drug for selection	References
Dictyostelium expression			
pJK1-GFP: pIS1		G418 (20 µg/ml)	Zhang et.al. (2011)
pJK1-GFP-racIA	10 11	G418 (20 µg/ml)	This Study
pJK1-GFP-racB	12 13	G418 (20 µg/ml)	This Study
pJK1-GFP-racE		G418 (20 µg/ml)	Wang et.al. (2013)
pJK1-GFP-racE (G20V)		G418 (20 µg/ml)	Wang et.al. (2013)
pJK1-GFP-racE (T25N)		G418 (20 µg/ml)	Wang et.al. (2013)
pDRH-FLAG-RasC		Hygromysin (50 µg/ml)	Gift from Dr. Devreotes (JHMI)
pDRH-FLAG-RasG		Hygromysin (50 µg/ml)	Gift from Dr. Devreotes (JHMI)
pDRH-LimEΔcoli-mRFP		Hygromysin (50 µg/ml)	Gift from Dr. Devreotes (JHMI)
pDM323-RBD(Raf)-GFP		G418 (20 µg/ml)	Xiong et.al. (2010)
pDRH-PHcrac-RFP		Hygromysin (50 µg/ml)	Gift from Dr. Devreotes (JHMI)
pTX-GFP		G418 (20 µg/ml)	Levi et.al. (2000)
pTX-GFP-GflB	14 15	G418 (20 µg/ml)	This Study
pTX-GFP-GflB 1-644	14 16	G418 (20 µg/ml)	This Study
pTX-GFP-GflB 361-644	16 17	G418 (20 µg/ml)	This Study
pTX-GFP-GflB 645-1601	15 18	G418 (20 µg/ml)	This Study
pTX-FLAG		G418 (20 µg/ml)	Levi et.al. (2000)
pTX-FLAG-GflB	14 15	G418 (20 µg/ml)	This Study
pTX-FLAG-GflB 361-644	16 17	G418 (20 µg/ml)	This Study
pTX-FLAG-GflB 645-1601	15 18	G418 (20 µg/ml)	This Study
pDRH-CRIB-RFP	19 20	Hygromysin (50 µg/ml)	This Study
Bacterial expression			
pGEX 4T-1			Amersham
pGEX-Byr2-RBD			Kae et.al. (2004)

Table S2. Primers used in this study

Primers for gene disruption in Dictyostelium cells

1	A15P	CCAACCCAAGTTTTTTTAAACC
2	gflB-2	GACTCGAGTTATTCGGCATTTGTTGAAGGAAC
3	gflB-3	GTGTACCAAATTTCTCTATACTTCG
4	gflB-4	CACCACCACTACCTCAACTACAACC
5	gflB-5	GAGCGGCCGCGTTGTAGTACTACTAGTTAC
6	gflB salI 1-19	GAGTCGACATGACAGATTTAAATTCAG
7	gflB smaI 800-781	GACCCGGGAATTGATTAGCCTGTAAAAC
8	gflB smaI 4116-4134	GACCCGGGAATCATCAGAATTGGAGAC
9	gflB notI 4875-4856	GAGCGGCCGCGTTGTAGTACTACTAGTTAC

Primers for expression plasmids in Dictyostelium cells

10	rac1A-1	GGAAGATCTTCCATGCAAGCAATTAAATG
11	rac1A-2	GGAAGATCTTCCTTATAAAATGTTGC
12	racB-1	GGAAGATCTTCCATGCAATCAATTAAATTGG
13	racB-2	GGAAGATCTTCCTTATAAAATTGAACATTTTG
14	gflB-Start	CCCGAGCTCATGACAGATTTAAATTC
15	gflB-Stop	CCCCTCGAGTTATTCGGCATTTGTTGAAGGAAC
16	gflB 1932-1909	GACTCGAGTTAATATGGTGAATCTAAAATTTGTTG
17	gflB 1081-1101	GAGAGCTCTTACAAGTAGTTTCCAATGCC
18	gflB 2042-2066	GAGAGCTCGGAATTTATGGTGTTAGATTAACAC
19	CRIB 943	CCCGAGCTCACTACATCACCACCATCC
20	CRIB 1233	CCCCTCGAGTTAATGGAAATCTAAAACATC