

Figure S1

Fig. S1. Images of wild-type or *gtaC*⁻ cell chemotaxis to folate. Representative images of wild-type (WT) and *gtaC*⁻ cells after 3 h in above agar chemotaxis assays to folate. In the appropriate panels the source of folate is above the cell droplet (upper side of panel). Circles indicate initial cell droplet perimeters and scars in the agar were used to align images before and after the assay. All images are the same magnification.

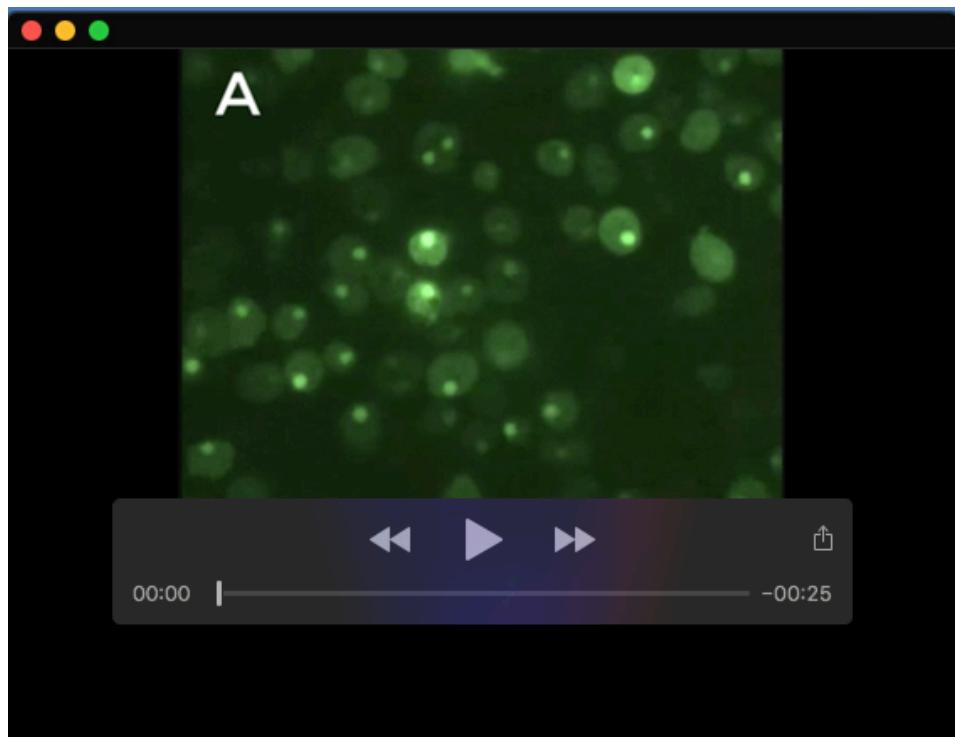
<i>CfGtaC</i>	ASTTPRGQAQDHLLLNNSTSNLSSAPNTPRNTSSTTSTFTTTTTNNNNQNQEDTNNN
<i>HaGtaC</i>	SRTPQKSMAEASPTILSPSSCSSLPESPRNTGSGCSSYPSSPSTPCSTPRG----ATN
<i>DpGtaC</i>	QIPIINTPEIHQRNSPPSATNSPRYYIPSQQQESSVENSPFTTPLSSPRGPISPRAASN
<i>TlGtaC</i>	QSPRNTNNIISQGVNNSLNSTPSSQLSSPNTPQSLSLN---TPLSSPRG-----
<i>DdGtaC</i>	GIPLINTTEIHQ-RSNPSSATNSPRALYYG--NESSVENSPFTTPLSSPRGPISPRAATIN
<i>AsGtaC</i>	PSTPCTHRDGASSYSLPNSARGADSQRCPPSSPTSSSTSTTPGSTPRLTHSS-----SS
<i>CfGtaC</i>	NNNQSTDYLKKAEEQWKKLEYFSNDFNHVFEIINKEFHQ-LSALNSKLGEITSTVQEI
<i>HaGtaC</i>	DRNGEPQYPKQAEQQWKKLEYYANEFNHFIFETLKTFESQ-IELTNAKLSEITNTVKEI
<i>DpGtaC</i>	SSLLNNQSNIRVEEQWKKIEYYVNDLSHFIYDCIRSKDFSN-LSELKDKEEVVNTSKEI
<i>TlGtaC</i>	---GNQPIDKVEEHWRKLEYYTTSNLNNYVYESIKSKDFSN-LSELNEKVEEIKNSIKEI
<i>DdGtaC</i>	SSMSNLQSNIRVEEQWKKIEYYVNDLSHFIYESIRNKDYSN-LSELKEKIDEVVNTSKEI
<i>AsGtaC</i>	ESIVDEEYLKRAEVQWKKLEYYTNEFNHFIFDTFKSRDFGNGIASLKSKELEDLTNTVKEI
<i>CfGtaC</i>	EITNNIFKTLPPQTRARKKRATKAELKKSSGGVLGVKRTYVTTPKSKGNYCVFGCGTMET
<i>HaGtaC</i>	EITNNIYKSLPPQTRARKKRATKAELQKD---LLGIKRTYVTTPKSKGNYCFFCGTMET
<i>DpGtaC</i>	EIIHSIAKSLPPQTRARKKRSTKAELQKD---LIGIKRTYVTTPKSKGTYCIFCGTMET
<i>TlGtaC</i>	EIINSIAKTLPPQTRARKKRSTKAELQRD---AISVKRTYVTTPKSKGNHCAFQGTLET
<i>DdGtaC</i>	EIIHSIAKSLPPQTRARKKRSTKAELQKD---LIGIKRSYVTTPKSKGTYCIFCGTMET
<i>AsGtaC</i>	EIANNVYKLIPQTRSRRKKRSTKAEKYHKE---LLGIKRTYVSTPKSKGLYCAFQGTLET
<i>CfGtaC</i>	PEWRKGPGGHKTLCNAACGLHYAKNLKREGANKSKTNNDNIATPSNTTNKGDNSNSASSST
<i>HaGtaC</i>	PEWRKGPGGHKTLCNAACGLHYAKNIKKESVKNSQQN-PSESTSCQSMNRKTTTTIRDVL
<i>DpGtaC</i>	PEWRKGPGGHKTLCNAACGLHYAKNIKENQNNNNSPNPQSSPNSNIGNNNNNNSNSNN---
<i>TlGtaC</i>	PEWRKGPGGMKSLCNACGLHYAKNLKRENKLKSQS-----TATTDGN---
<i>DdGtaC</i>	PEWRKGPGGHKTLCNAACGLHYAKNIKENQNNNGSPNPQQNNVTTTTTSTSTNSPNS
<i>AsGtaC</i>	PEWRKGPGGHKTLCNAACGHYSKNIKKEKLNSNGGASANNPSANANEPTTQLQGLQS--

Fig. S2. Alignment of Dictyostelid GtaC homologs. GtaC homologs from *Dictyostelium discoideum* (*Dd*) (residue 336), *Cavenderia fasciculata* (*Cf*) (residue 321), *Heterostelium album* (*Ha*) (residue 280), *Dictyostelium purpureum* (*Dp*) (residue 306), *Tieghemostelium lacteum* (*Tl*) (residue 226), and *Acytostelium subglobosum* (*As*) (residue 290) alignment using ClustalW and entire protein sequence (amino terminal portion not show prior to residue indicated). Erk2 preferred phosphorylation motifs (yellow), zinc finger conserved cysteines (green), and highly conserved region (line).

Table S1. Oligonucleotides

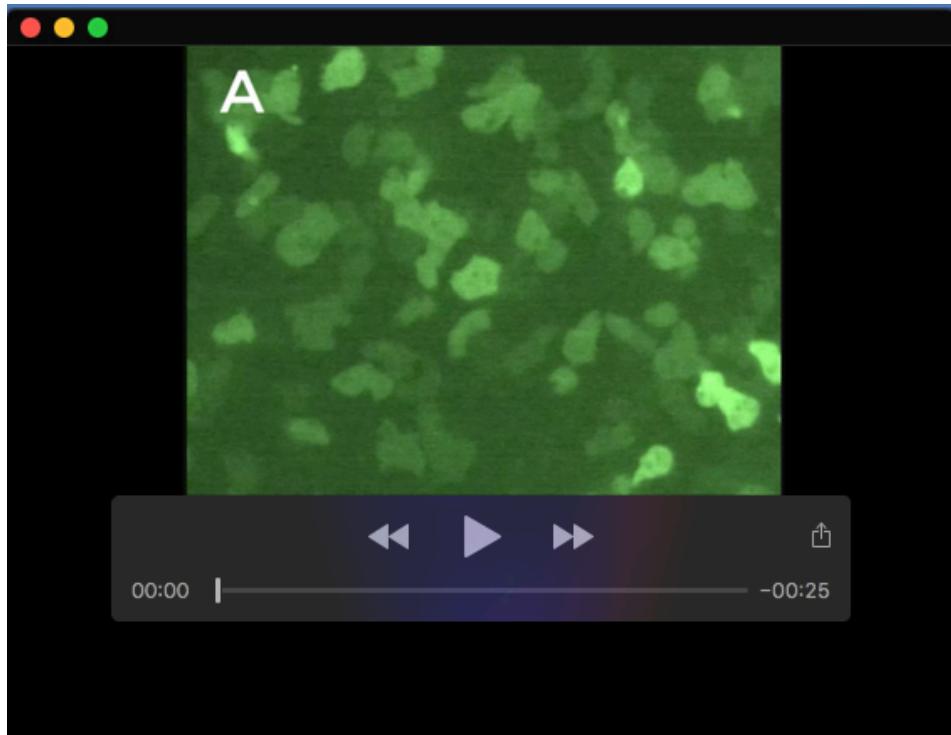
Oligonucleotides used to modify *gtaC* (intron removal, alteration of phosphorylation sites, and reversal of zinc finger alteration) and isolate *thyA* sequence for gene disruption.

Oligonucleotides used to modify <i>gtaC</i>		
<i>gtaC</i> intron removal		
1.	GatCl1Up	TGGTGGTAATGATTAGCAATACTATGAATAATTCAATTTC
2.	GatCl1Do	TTGCTAAATCATTACCACCAAAACAAGAGC
3.	GatCl2Up	ATTACATAATGTTTATGCCACCTGGAC
4.	GatCl2Do	GCCATAAAACATTATGTAATGCATGTGGTTACATTATGC
<i>gtaC</i> phosphorylation site alteration		
5.	GtaCS357AUp	TGCATTGGTTGCACTTGATGGATTGATC
6.	GtaCS357ADo	TCAAGTGCAACCAATGCACCACCGT
7.	GtaCS380AUp	CACGTGGTGTGCTGAAAGTGGTGTGAAATG
8.	GtaCS380ADo	ACTTCAGCACCGTGGTCCAATTTC
9.	GtaCS386AUp	CTTGGTGCATTGGACCACGTGG
10.	GtaCS386ADo	GGTCCAATTGCACCAAGAGCAACAATTAAATTC
11.	GtaCS380/6AUp	CAATTGGACCACGTGGTGTGAAAGTGGTG
12.	GtaCS380/6ADo	CACCACTGGTCCAATTGCACCAAGAGCAAC
13.	GtaCT492AUp	CTCTTGGAGCAGTTACATATGATCTTTAACCC
14.	GtaCT492ADo	TATGTAACTGCTCCAAAGAGTAAAGGAACCTATTG
<i>gtaC</i> reversal of zinc finger alteration		
15.	GtaCs-csens	CTTATTGTATATTCTGTGGTACCATGGAACTCCAGAATGG
16.	GtaCs-canti	ACCACAGAATATACAATAAGTCCCTTACTCTTGGAGTAG
Isolation of <i>thyA</i> sequence for <i>gtaC</i> gene disruption		
17.	ThyAUpH3	CAAGCTTGGAACATTGGTGTATGTGTTTCCCAC
18.	ThyADownH3	CAAGCTTCCTCAACAACAAATACCAAGATGATTCTG



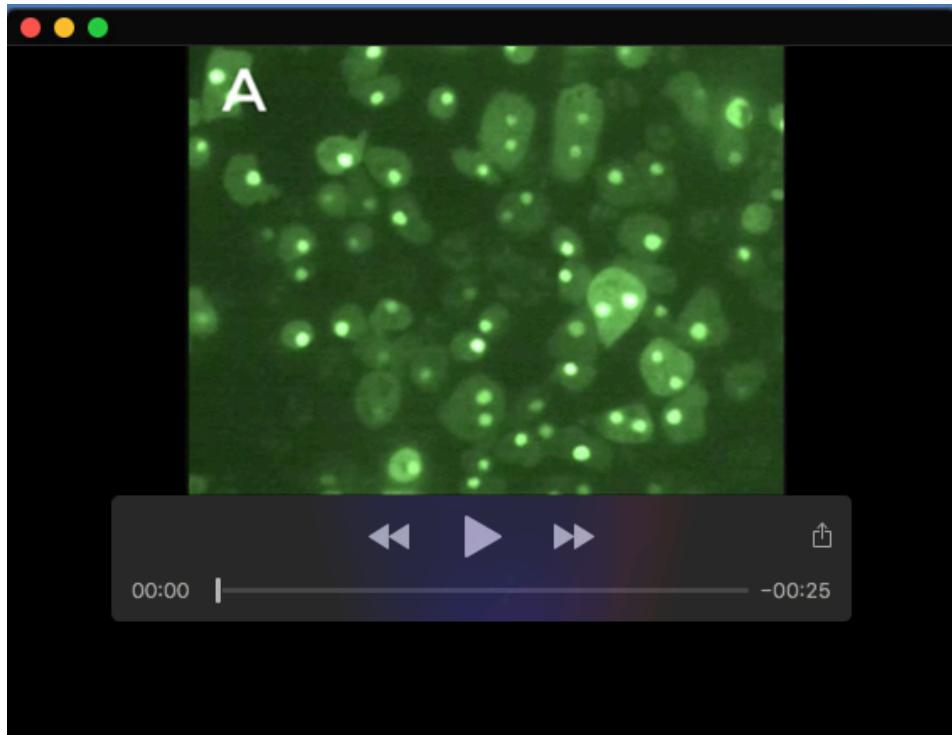
Movie 1. Movies of GFP-GtaC^{c-s} translocation in strains stimulated with 10 nM cAMP. Time-lapse video with images every 30 sec. Stimulant added at time 0 min (image 2, contains red dot). Order of movies:

- 1A: wild-type cells
- 1B: *erk2*⁻ cells
- 1C: *erk2*⁻(Erk2) cells
- 1D: *erk1*⁻ cells



Movie 2. Movies of GFP-GtaC^{c-s} translocation in strains stimulated with 1 μ M folate. Time-lapse video with images every 30 sec. Stimulant added at time 0 min (image 2, contains red dot). Order of movies:

- 2A: wild-type cells
- 2B: *erk2*⁻ cells
- 2C: *erk2*⁻(Erk2) cells
- 2D: *erk1*⁻ cells

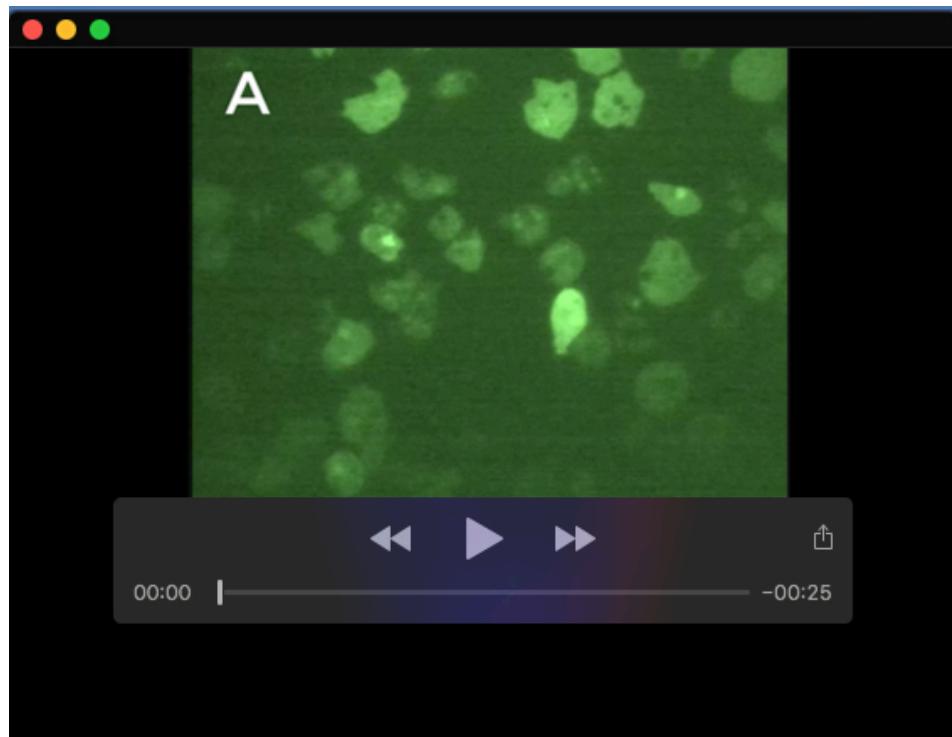


Movie 3. Movies of GFP-GtaC^{C-s} translocation in receptor and G protein mutant strains.

Cells stimulated with 1 μ M folate (S3A-C) or 10 nM cAMP (S3D-F). Time-lapse video with images every 30 sec. Stimulant added at time 0 min (image 2, contains red dot).

Order of movies:

- 3A: *far1*⁻ cells
- 3B: *gα4*⁻ cells
- 3C: *gα5*⁻ cells
- 3D: *far1*⁻ cells
- 3E: *gα4*⁻ cells
- 3F: *gα5*⁻ cells



Movie 4. Movies of GFP-GtaC^{c-s} mutants with altered phosphorylation sites. Wild-type cells expressing translocation in folate strains stimulated with 1 μM folate. Timelapse video with images every 30 sec. Stimulant added at time 0 min (image 2, contains red dot). Order of movies:

- 4A: GtaC^{S357A} (p911)
- 4B: GtaC^{T492A} (p913)
- 4C: GtaC^{S380A} (p926)
- 4D: GtaC^{S386A} (p925)
- 4E: GtaC^{S357A,T492A} (p937)
- 4F: GtaC^{S380A,S386A} (p929)
- 4G: GtaC^{S357A,S380A,S386A} (p931)
- 4H: GtaC^{S380A,S386A,T492A} (p932)
- 4I: GtaC^{S357A,S380A,S386A,T492A} (p935)