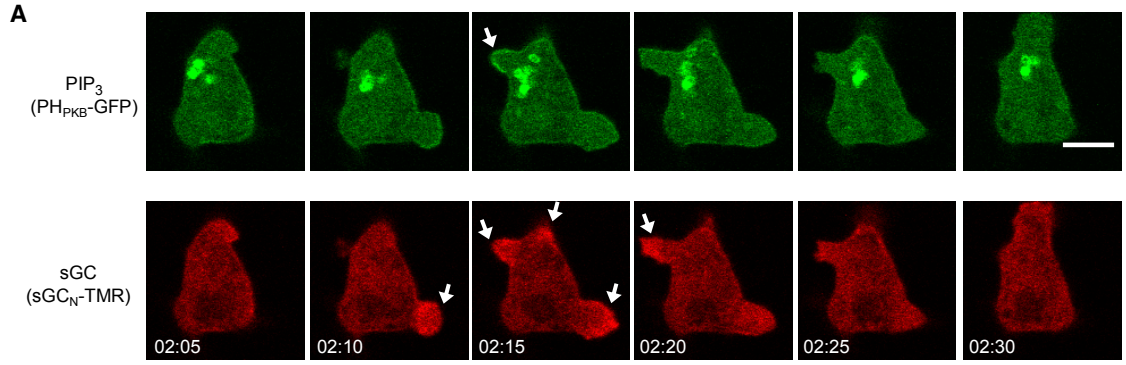
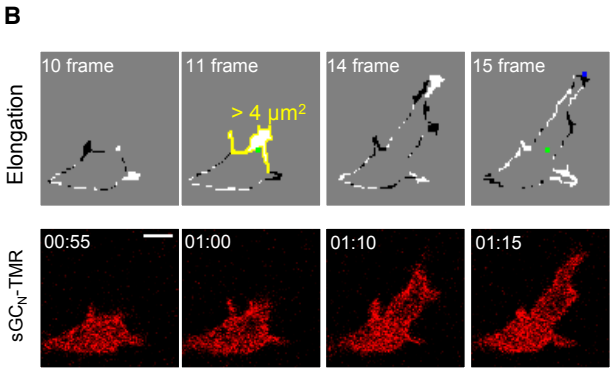
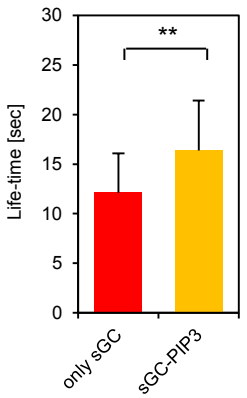
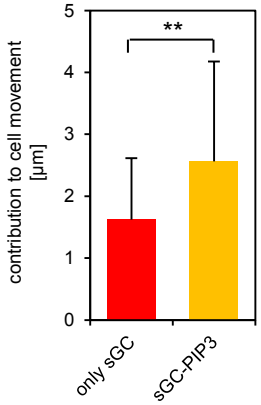
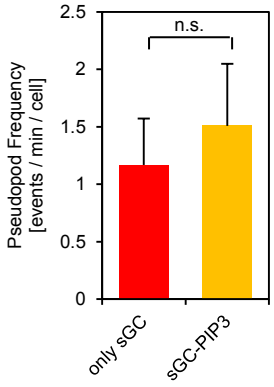
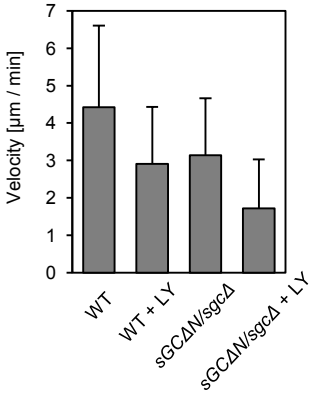


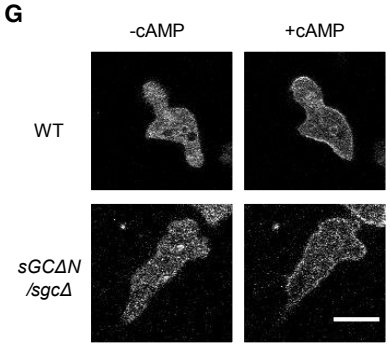


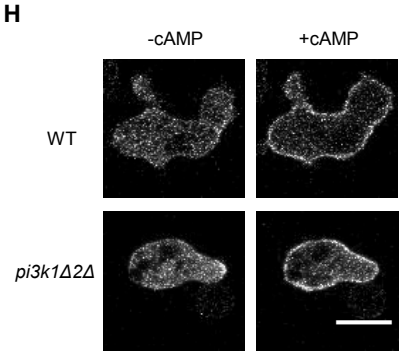












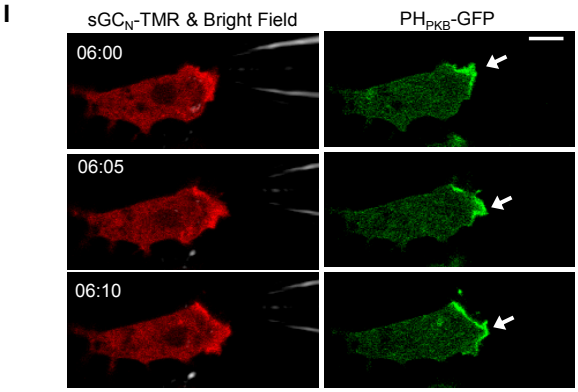


Fig. S1. Independent function of parallel pathways
(A) The time evolution of pseudopod dynamics shown in Figure 1A. sGC and PIP3 localizations are indicated by white arrows. (B) A representative pseudopod analysis. The fluorescence image of a cell with sGC_N-TMR, shown in the bottom, was binarized and subtracted in two consecutive frames. The white and black colors in the upper panel show the positive and negative changes, respectively. An area more than 4 μm^2 (shown by the yellow ROI) was defined as a pseudopod and was pursued when it stopped expanding. The green and blue dots show the start and end points of the pseudopod, respectively. (C) The life-time of pseudopods with sGC alone or with the co-localization of sGC and PIP3 (mean + s.d. for n = 147 and 189 pseudopods, respectively). (D) Contribution to cell movement for pseudopods with sGC alone or with the co-localization of sGC and PIP3 (mean + s.d. for n = 147 and 189 pseudopods, respectively). (E) Frequencies of pseudopod formation with sGC alone or with the co-localization of sGC and PIP3. The number of pseudopods was counted if the elongation area was over 4 μm^2 (mean + s.d. for n = 13 cells). (F) Migration velocity of wild-type AX2 and *sgcΔ* cells expressing sGCΔN-Halo was analyzed in the presence or absence of 50 μM LY294002 (see Methods). (G and H) PIP3 and sGC responses of the indicated cell lines at 1 μM cAMP. PIP3 production of wild-type AX2 and sGCΔN / *sgcΔ* cells (G) and sGC localization of wild-type AX2 and *pi3k1Δ2Δ* cells (H) were observed by the expression of PH_{PKB}-GFP and sGC-GFP, respectively. (I) Image galleries of a cell with sGC (red) and PIP3 (green) signals taken from supplementary Movie 1. The elongating pseudopod is shown by white arrows. Time format is “mm:ss”. Scale bars are 5 and 10 μm in (A)-(C) and (H)-(I), respectively (** $P < 0.01$, t-test).

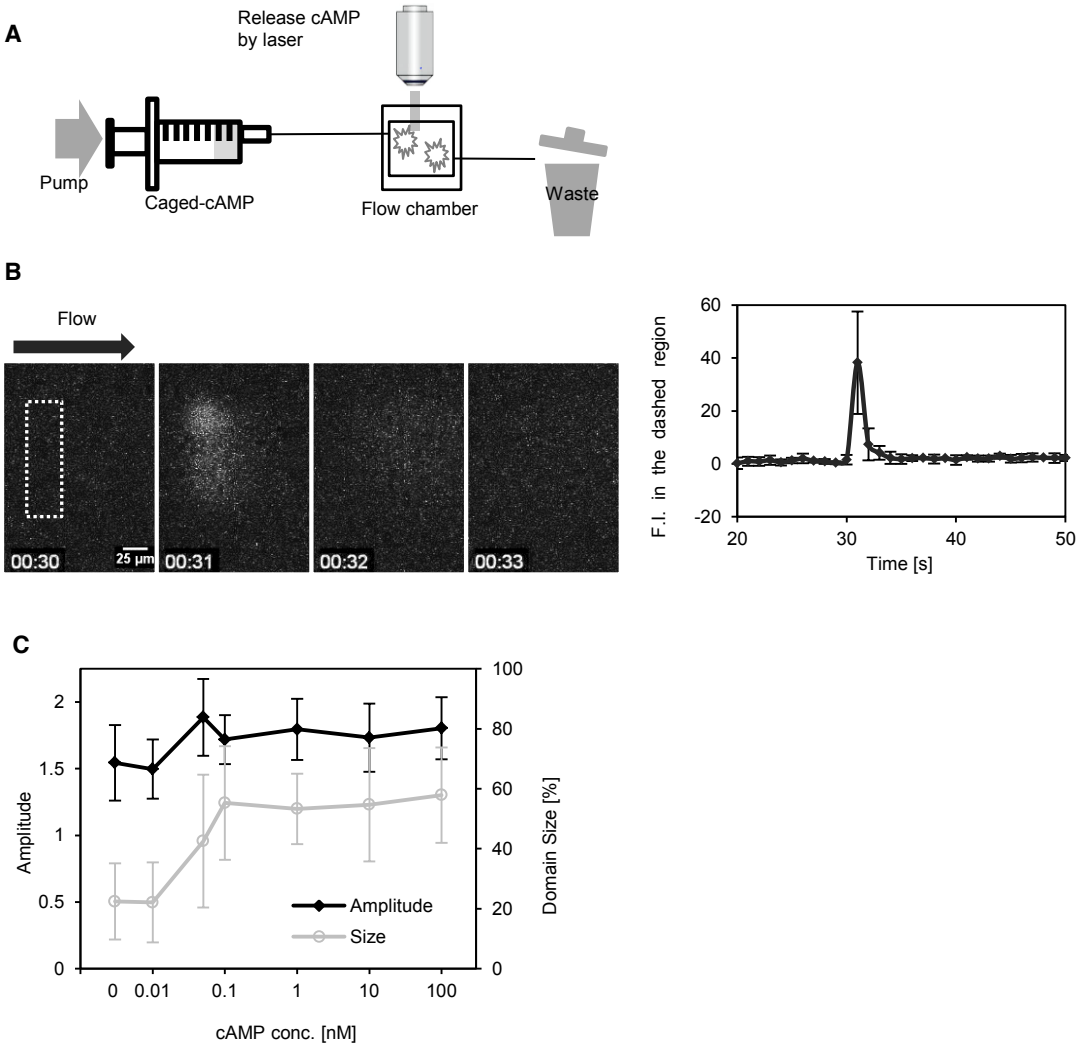


Fig. S2. Construction of the cAMP pulse stimulation system

(A) A schematic drawing of the pulse stimulation system. (B) Time course of the transient release of caged compound. Photolysis of 100 µM caged fluorescein in solution was carried out at the dashed region by UV flash (left). Fluorescence intensity of the region where UV was irradiated (right) (mean ± s.d. for n = 3). (C) *gcΔ* cells expressing sGC-GFP were stimulated with various cAMP concentrations. Response amplitude and domain size of the sGC-enriched region are shown as functions of cAMP concentrations. (mean ± s.d. for at least 8 cells).

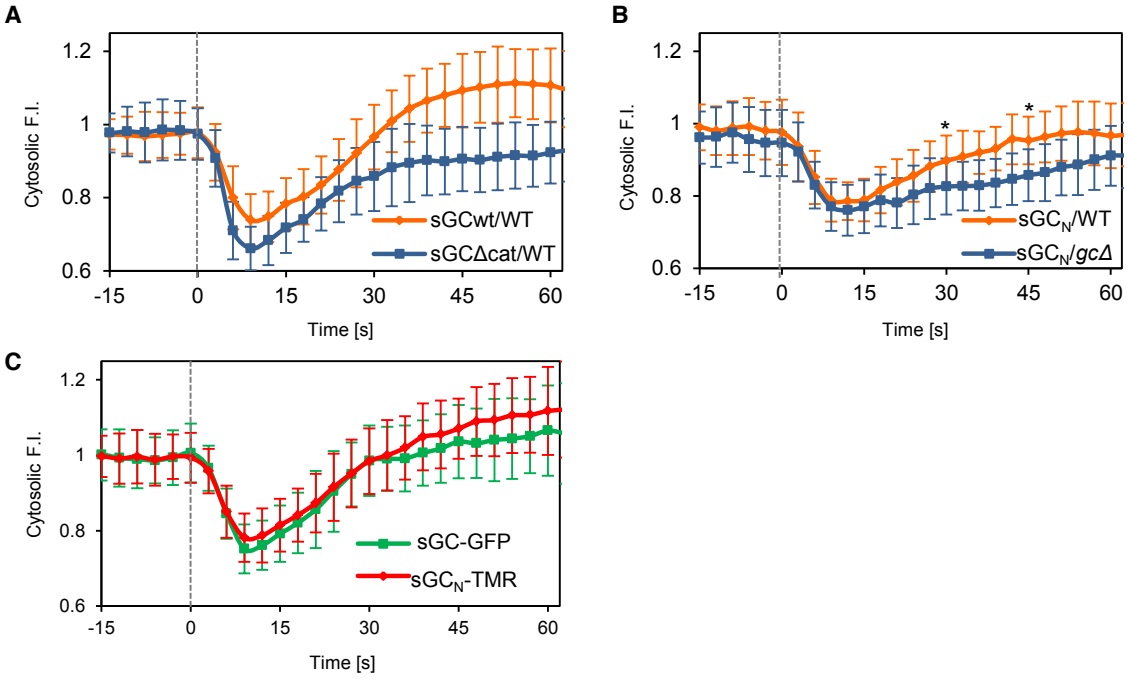


Fig. S3. Evaluation of various sGC constructs

(A) sGC responses of the indicated cell lines upon cAMP stimulation. Wild-type AX3 cells expressing sGC$_{WT}$-GFP or sGC$_{\Delta cat}$-GFP were stimulated with 100 nM cAMP. Cytosolic intensity was normalized to the pre-stimulus level (mean ± s.d. for at least n = 19 cells). For sGC$_{WT}$-GFP, the same data is shown in Figure 4C. (B) The responses of the N terminus of sGC (sGC$_N$) in wild-type or *gcΔ* cells were observed as in (A) (mean ± s.d. for n = 29 and 28 cells, respectively; * $P < 0.01$ versus wild-type cell at 30 and 45 sec, t-test). (C) The responses of the full-length (sGC) and the N-terminal sGC (sGC$_N$) in the same wild-type cell were observed as in (A) (mean ± s.d. for n = 19 cells).

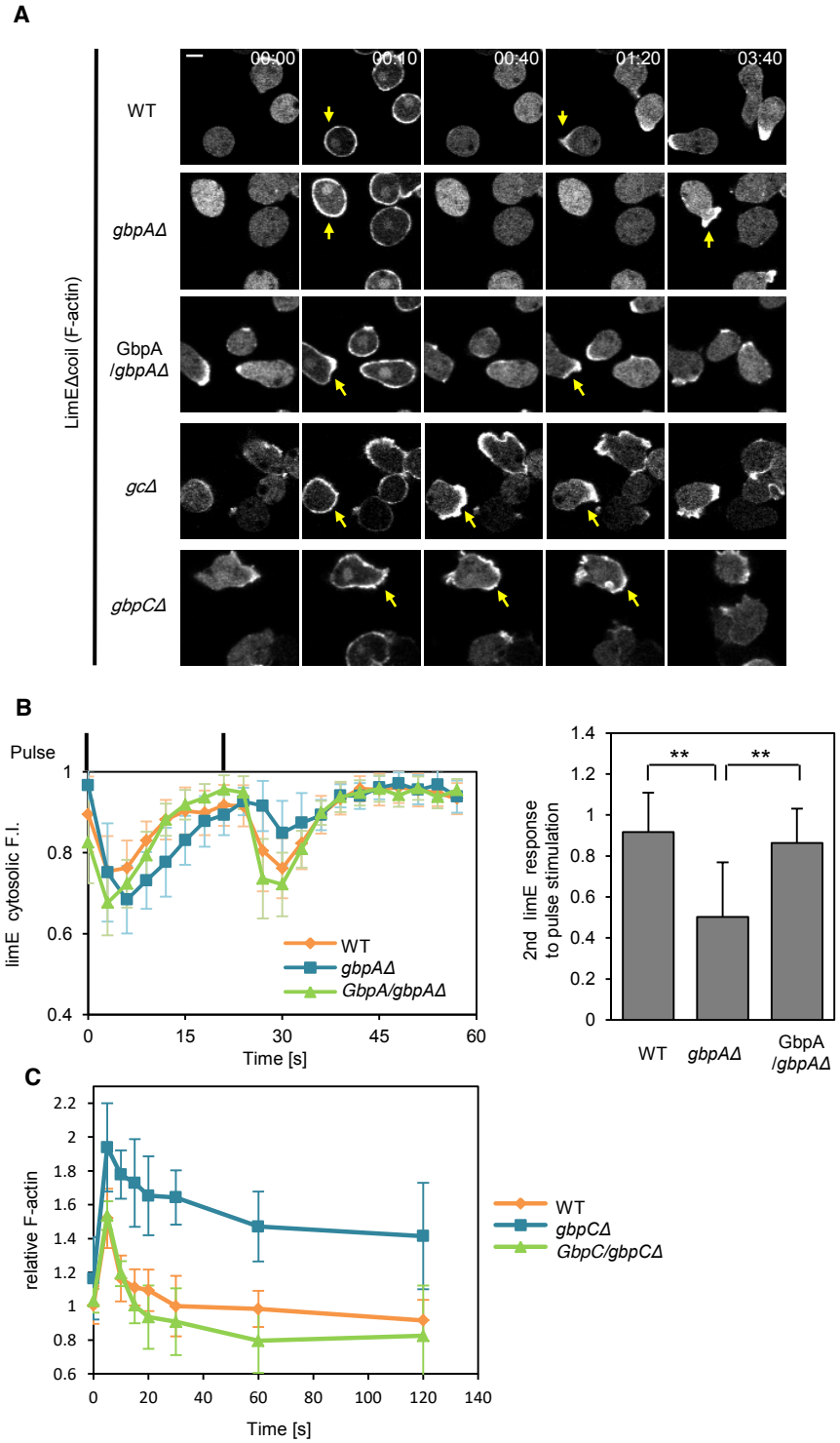


Fig. S4. The recovery time of F-actin response depends on cGMP concentration
(A) Wild-type AX3 and mutant cell lines expressing mRFP-LimEΔcoil were stimulated with 10 nM cAMP. Yellow arrows show the pseudopods where mRFP-LimEΔcoil localized. These pictures were clipped from Movies 3 and 4. (B) The refractoriness of F-actin depends on the amount of intracellular cGMP. Wild-type AX3, *gbpAΔ* and *gbpAΔ* rescued by GbpA cells expressing mRFP-LimEΔcoil were repetitively stimulated with 21-sec interval pulses (left) (mean ± s.d. for n = 23 cells). Cytosolic responses to 21-sec interval stimuli were normalized by the maximum fluorescence value in the time course. Black bars on the abscissa represent the pulse timing. The second responses normalized by the first responses of each cell line are shown (right) (mean + s.d.; ** $P < 0.001$, t-test). (C) Cytoskeletal F-actin amounts upon 10 nM cAMP shown in Figure 5D were normalized to the value of wild-type cells at 0 sec (mean ± s.d. for at least 3 experiments).

Table S1. Plasmid list.

Plasmid name	Protein expressed	Tag	Backbone	Source or reference
sGC-GFP	SgcA	GFP	pMB74	Veltman et al., 2005
sGCΔcat-GFP	SgcA(D1106A)	GFP	pMB74	Veltman et al., 2006
sGCN-Halo7	SgcA(1-1019)	Halo7	pHK12	this study
sGCΔN-Halo7	SgcA(877-2843)	Halo7	pHK12	this study
PH_{PKB}-eGFP	PkbA(1-113)	eGFP	pBIG	Meili et al., 1999
mRFP-LimEΔcoil	LimE(1-145)	mRFP	pHK12	this study
GbpA-eGFP	GbpA	eGFP	pDM358	this study

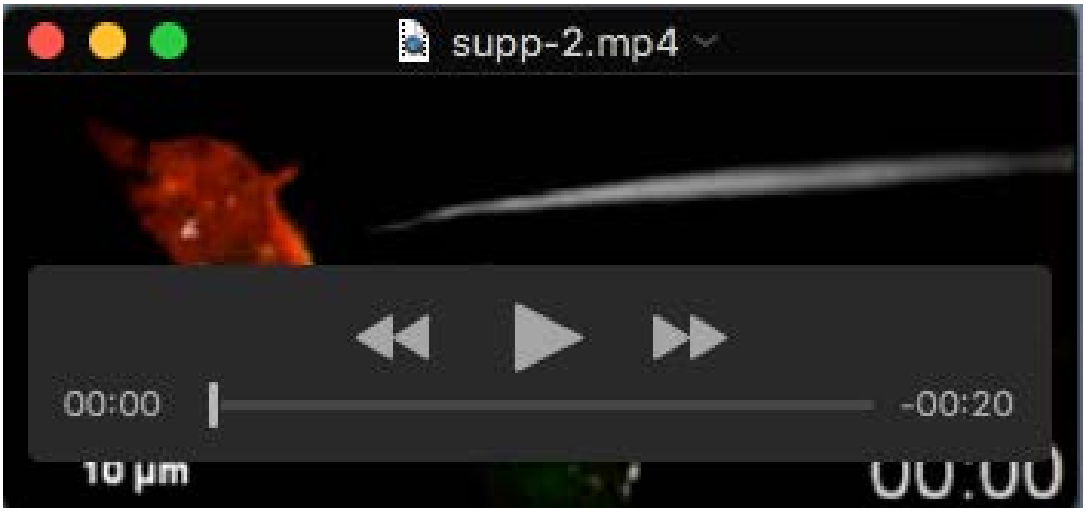
Numbers in parentheses refer to a mutation or regions of amino acid residues.

Table S2. Strain list.

Strain name	Genotype	Background	Source or reference
AX2		AX2	Lab stock
AX3		AX3	NBRP
sgcΔ	*sgcAΔ*	AX2	this study
gcΔ	*sgcAΔ, gcAΔ*	AX3	Veltman et al., 2006
pi3k1Δ/pi3k2Δ	*pikAΔ, pikBΔ*	AX2	Kamimura et al., 2016
gbpAΔ	*pdeDΔ*	AX3	Bosgraaf et al., 2002
gbpABΔ	*pdeDΔ, pdeEΔ*	AX3	Bosgraaf et al., 2002
gbpCΔ	*gbpCΔ*	AX3	Bosgraaf et al., 2002
gbpDΔ	*gbpDΔ*	AX3	Bosgraaf et al., 2002
mhcAΔ	*mhcAΔ*	AX3	Ruppel et al., 1994
arcB	arcB(I191L/D197Y/K206V/A213V/F223L/P224S/E232G/I237T/H245L/S250S)	AX2	Langridge and Kay, 2007
PH_{PKB}-eGFP, sGC_N-Halo7/AX2		AX2	this study
sGC-GFP/mRFP-LimEΔcoil /AX2		AX2	this study
sGC-GFP/AX2		AX2	this study
mRFP-LimEΔcoil/AX3		AX3	this study
sGCΔN-Halo7/*sgcΔ*	*sgcAΔ*	*sgcΔ*	this study
PH_{PKB}-eGFP, sGCΔN-Halo7 /*sgcΔ*	*sgcAΔ*	*sgcΔ*	this study
sGC-GFP/*gcΔ*	*sgcAΔ, gcAΔ*	*gcΔ*	Sato et al., 2009

NBRP, National BioResource Project in Japan.

Strain name	Genotype	Background	Source or reference
sGCΔcat-GFP/*gc Δ*	*sgcAΔ, gcAΔ*	*gcΔ*	Sato et al., 2009
mRFP-LimEΔcoil/*gcΔ*	*sgcAΔ, gcAΔ*	*gcΔ*	this study
sGC-GFP/*pi3k1Δ2Δ*	*pikAΔ, pikBΔ*	*pi3k1Δ /pi3k2Δ*	this study
sGC-GFP/*gbpAΔ*	*pdeDΔ*	*gbpAΔ*	this study
mRFP-LimEΔcoil/*gbpAΔ*	*pdeDΔ*	*gbpAΔ*	this study
mRFP-LimEΔcoil/GbpA-eGFP /*gbpAΔ*	*pdeDΔ*	*gbpAΔ*	this study
sGC-GFP/*gbpABΔ*	*pdeDΔ, pdeEΔ*	*gbpABΔ*	this study
sGC-GFP/*gbpCΔ*	*gbpCΔ*	*gbpCΔ*	this study
GbpC/*gbpCΔ*	*gbpCΔ*	*gbpCΔ*	van Egmond et al., 2008
GbpCΔcGMP/*gbpCΔ*	*gbpCΔ*	*gbpCΔ*	van Egmond et al., 2008
GbpCΔkinase/*gbpCΔ*	*gbpCΔ*	*gbpCΔ*	van Egmond et al., 2008
mRFP-LimEΔcoil/*gbpCΔ*	*gbpCΔ*	*gbpCΔ*	this study
sGC-GFP/*gbpDΔ*	*gbpDΔ*	*gbpDΔ*	this study
sGC-GFP/*mhcAΔ*	*mhcAΔ*	*mhcAΔ*	this study
sGC-GFP/*arcB*	arcB(I191L/D197Y/K206V/A213V/F223L/P224S/E232G/I237T/H245L/S250S)	*arcB*	this study

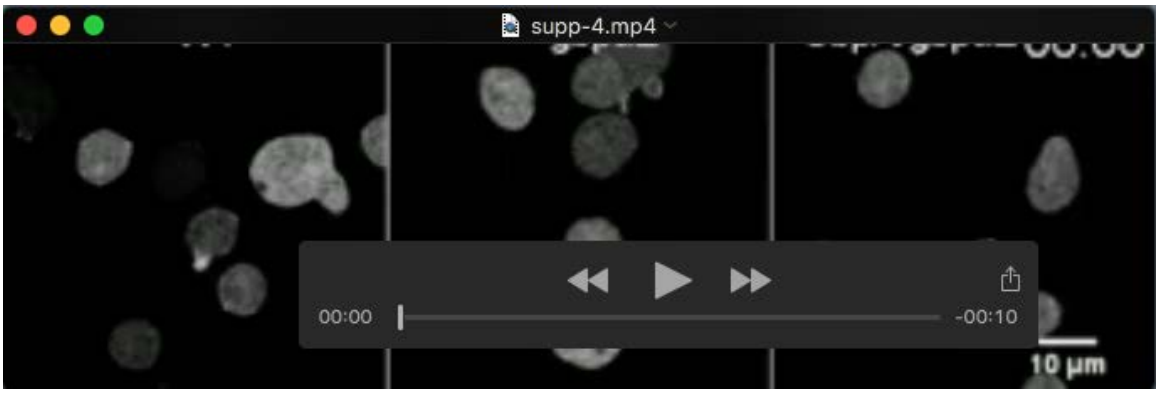


Movie 1. Chemotactic sGC and PIP3 responses under a cAMP gradient.

Wild-type AX2 cells expressing PH_{PKB}-GFP (green) and sGC_N-TMR (red) were stimulated with a pipette containing 40 nM cAMP. The position of the pipette (grey) was controlled by hand manipulation. The video was captured every 5 sec and is shown at 10 frame/sec. Time format is "mm:ss".

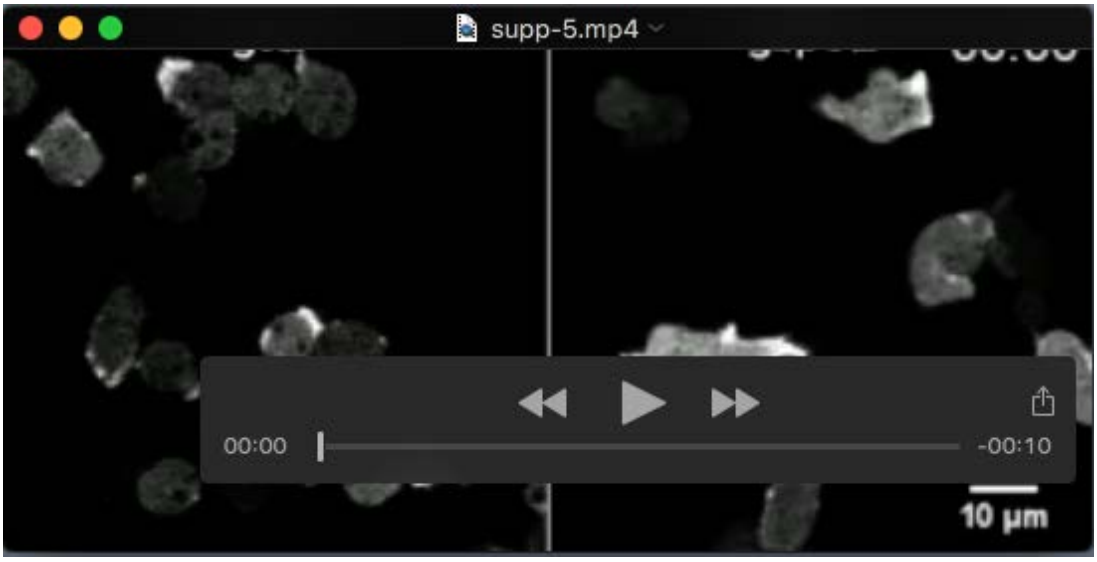
Movie 2. Wavelike pattern of sGC and F-actin localization.

A wild-type AX2 cell expressing sGC-GFP and mRFP-LimEΔcoil was pretreated with 1 µM LatA for 30 min. The bottom layer of the cell was observed by confocal microscopy at 5-sec intervals. Time format is "mm:ss".



Movie 3. F-actin responses to 10 nM cAMP, related to Fig. 5.

mRFP-LimEΔcoil was observed in wild-type AX3, *gbpAΔ*, and GbpA-eGFP-expressing *gbpAΔ* cells. cAMP was added at 30 sec. The video was captured every 5 sec and is shown at 12 frame/sec. Time format is "mm:ss".



Movie 4. F-actin response in *gcΔ and gbpCΔ* cells.

mRFP-LimEΔcoil-expressing *gcΔ and gbpCΔ* cells were stimulated with 10 nM cAMP. The video was captured every 5 sec and is shown at 12 frame/sec. Time format is "mm:ss".