Zatulovskiy et al., http://www.jcb.org/cgi/content/full/jcb.201306147/DC1

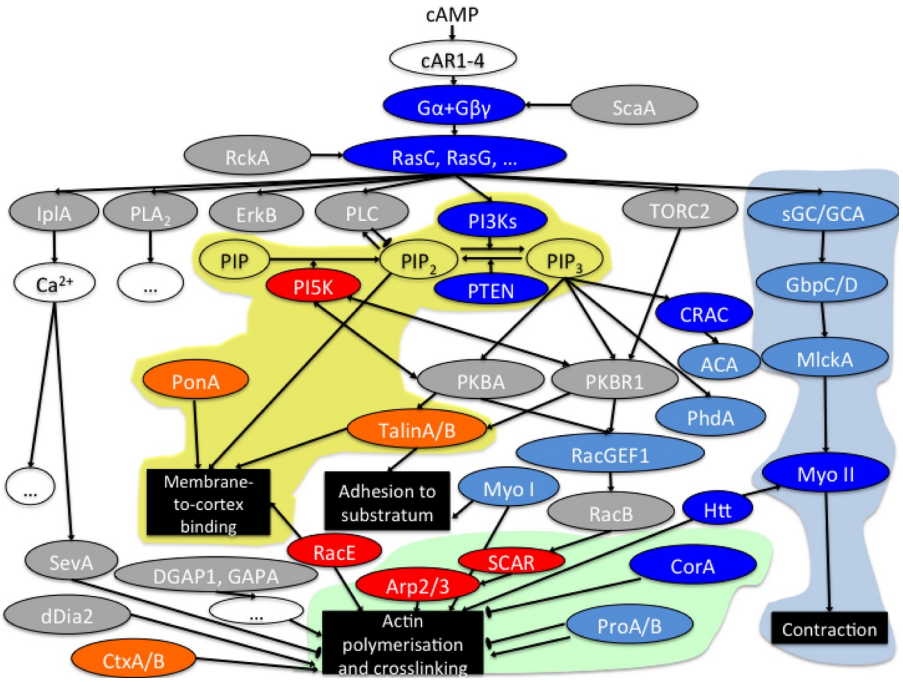
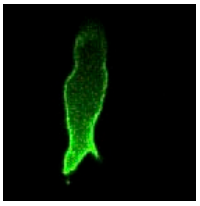
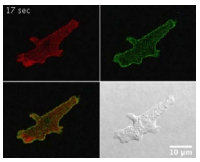


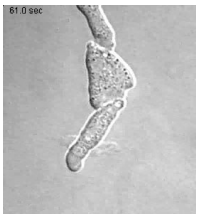
Figure S1. **Mapping of genes screened onto chemotactic network.** The genes whose mutations were tested for altered blebbing are mapped onto our current understanding of the signaling and effector network underlying chemotaxis to cyclic-AMP. Null (or hypomorphic in the case of Arp2/3) mutants were compared with their direct parents in three assays for blebbing using aggregation-competent cells: movement under buffer; movement under an agarose overlay; blebbing in response to uniform stimulation with cyclic-AMP. A combined score was given to each strain for mapping purposes. Gray, no detectable effect; blue, mutants have impaired blebbing (deep blue > light blue); red/orange, mutants have increased blebbing (red > orange); no color, not tested.



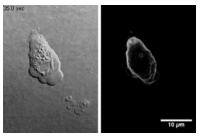
Video 1. **Cell moving with a combination of actin-driven pseudopods and blebs in standard conditions under buffer.** Aggregation-competent Ax2 cells, expressing ABD-GFP to label F-actin, were observed on a glass coverslip under KK2 buffer. Multiple blebs, marked by their F-actin scars, F-actin–driven pseudopodia, and hybrids between the two ("blebbopodia") can be seen at the leading edge. Blebs usually expand in a single frame and to observe this it is helpful to step through the movie frame-by-frame. Movie taken by DIC and laser-scanning confocal microscopy (model LSM 710; Carl Zeiss) at 1 frame per second.



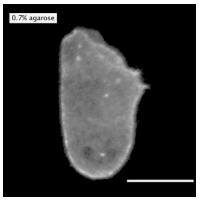
Video 2. **Transformation of blebs into a pseudopod.** An aggregation-competent Ax2 cell expressing ABD-RFP (red) as a reporter for F-actin and cAR1-GFP (green) as a marker for the plasma membrane is observed on a glass coverslip under KK2 buffer. Two successive blebs form on the flank of the cell, leaving F-actin scars behind. F-actin rapidly polymerizes (within 1 s) at the bleb membranes to reconstitute the cortex, but polymerization continues, transforming the blebs into a pseudopod. Movie taken by DIC and laser-scanning confocal microscopy (model LSM 710; Carl Zeiss) at 1 frame per second for 50 s.



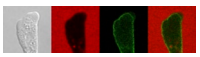
Video 3. **The leading cell of a small stream predominantly uses blebs to move.** Aggregation-competent Ax2 cells observed on a glass coverslip form small head-to-tail streams during the later stages of natural aggregation. The leading cell, which has a free leading edge, moves largely in bleb mode. Blebs are labeled with black points throughout the movie. Movie taken by DIC (model LSM 710; Carl Zeiss) at 1 frame per second.



Video 4. **A cell moving in pure bleb mode under 0.7% agarose.** An aggregation-competent Ax2 cell, expressing ABD-GFP as a reporter for F-actin, chemotaxing to cyclic-AMP under 0.7% agarose. When blebs detach from the cortex, they leave behind an F-actin scar, which gradually dissipates and sometimes moves in a retrograde direction; meanwhile, the bleb membrane becomes repopulated with F-actin in as little as one second. Left, DIC; right, ABD-GFP. Movie taken by DIC and laser-scanning confocal microscopy (model LSM 710; Carl Zeiss) at 2 frames per second.

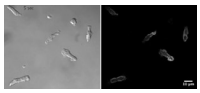


Video 5. **Three-dimensional reconstructions of cells moving under agarose overlays of different strengths, and movement of cells under agarose containing fluorescent beads.** Aggregation-competent Ax2 cells, expressing ABD-GFP as a reporter for F-actin, were attracted to move under agarose overlays of different strength by chemotaxis to cyclic-AMP. As the strength of the overlay increases, cells become more flattened, produce fewer dorsal projections, and use blebs instead of pseudopods to move (blebs and the scar left behind are very clear under 1 and 2% agarose). Cells are reconstructed from 0.38-µm sections taken by laser-scanning confocal microscopy (model LSM 780; Carl Zeiss) and reconstructed using the ImageJ 3D Viewer plug-in (National Institutes of Health). Bar, 10 µm. In the second part of the movie, similarly prepared cells are shown moving under 0.7% agarose containing red fluorescent, 0.45-µm diameter beads. The focal plane is set on the beads slightly above the cells, and when a cell moves under a bead, it dims and then brightens once the cell has passed, indicating that it is displaced out of and then back into the focal plane. This is consistent with the cells elastically deforming the agarose as they move. Taken by laser-scanning confocal microscopy (model LSM 780; Carl Zeiss) at 1 frame per second.

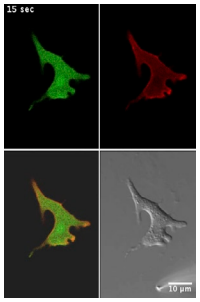


Video 6. **A cell moving in pure bleb mode under 0.7% agarose containing fluorescent dye to reveal the cell outlines.** Aggregation-competent Ax2 cells, expressing ABD-GFP (green) as a reporter for F-actin were attracted under 0.7% agarose containing 0.5 mg/ml RITC-dextran as a negative stain (shown in red) to identify the outline of the cells. The merged movie clearly shows that blebs are initially depleted in F-actin and do not have a cortex, but rapidly rebuild one. F-actin scars can be observed at the base of blebs. Taken by laser-scanning confocal microscopy (model LSM 780; Carl Zeiss) at 2 frames per second.

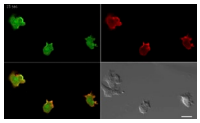
Video 7. **Four examples of cells re-orienting to a micropipette filled with 2 μM cyclic-AMP.** Aggregation-competent Ax2 cells, expressing ABD-GFP as a reporter for F-actin, were first attracted to the micropipette and when they were well polarized, the micropipette was moved rapidly to the flank (in 1–2 s). Cells can re-orientate by rapidly making blebs in the new direction of travel; these are often precursors to F-actin pseudopods and are preceded by F-actin microspikes. DIC and fluorescent images were taken by laser-scanning confocal microscopy (model LSM 710; Carl Zeiss) at 1 frame per second.



Video 8. **Blebbing assay.** In this assay, 1 μM cyclic-AMP is added to aggregation-competent Ax2 cells, expressing ABD-GFP as a reporter for F-actin. Cyclic-AMP addition is marked by a disturbance to the image (0 s), and blebbing starts 20 s later. DIC and fluorescent images were taken by laser-scanning confocal microscopy (model LSM 710; Carl Zeiss) at 1 frame per second.



Video 9. **PIP3 and F-actin dynamics during re-orientation of a cell to a micropipette releasing cyclic-AMP.** An aggregation-competent Ax2 cell expressing PH-CRAC-GFP (green) as a reporter for PIP3, and LifeAct-RFP (red) as a reporter for F-actin, is induced to change direction by moving a micropipette releasing cyclic-AMP. A PIP3 patch and F-actin microspikes are produced proximal to the needle and are followed in this case by a bleb formed from the PIP3 patch. DIC and fluorescent images were taken by laser-scanning confocal microscopy (model LSM 780; Carl Zeiss) at 1 frame per second.



Video 10. **Re-orientation of PhdA/CRAC double-null cells (strain HM1669) to a micropipette releasing cyclic-AMP.** Aggregation-competent HM1669 cells expressing PH-CRAC-GFP (green) as a reporter for PIP3, and LifeAct-RFP (red) as a reporter for F-actin, are stimulated by a micropipette releasing cyclic-AMP. In this mutant, PIP3 patches and F-actin microspikes that form proximal to the needle are not followed by blebs. DIC and fluorescent images were taken by laser-scanning confocal microscopy (model LSM 780; Carl Zeiss) at 1 frame per second.

Table S1. **Screen for mutants with altered blebbing and bleb-driven motility**

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
1	MlcE	DBS0236566	Stock Center (R. Chisholm)	Myosin essential light chain	– –	– –	– –	**−6**	Flattened, less elongated cells than WT. Do not make visible blebs.
2	MlcR	DBS0236567	Stock Center (R. Chisholm)	Myosin regulatory light chain	– –	– –	– –	**−6**	Flattened, less elongated cells than WT. Do not make visible blebs.
3	MhcA	HS2206	J. Spudich	Myosin II heavy chain	– –	– –	– –	**−6**	Flattened, less elongated cells than WT, rarely penetrate under agarose. Do not make visible blebs.
4	PI3K1–5	HM1200	Kay lab	PI3-kinases	– –	–	– –	**−5**	Smaller cells than WT, less elongated. Blebbing strongly impaired in all experimental conditions.
5	PTEN	HM1289	Kay lab	PI(3,4,5)P_3-phosphatase	– –	–	–	**−4**	In response to cAMP shock, cells produce “dendritic” or star-like shapes producing many fast-growing, branched, spike-ended protrusions
6	PI3K1–5+ PTEN	HM1295	Kay lab	See above	–	–	–	**−3**	
7	PLC	HM1308	A. Harwood	Phospholipase C	**0**	**0**	**0**	**0**	
8	PI3K1–5 + PLC	HM1475	Kay lab	See above	– –	–	–	**−4**	
9	PLA_2	HM1378	Kay lab	Phospholipase A2	**0**	**0**	**0**	**0**	
10	PI3K1–5 + PLA_2	HM1369	Kay lab	See above	– –	–	– –	**−5**	

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
11	PKB	HM1519	P. Devreotes	Protein kinase B, target of PI3Ks	0	0	0	0	
12	PKBR1	HM1520	P. Devreotes	Related to PKB	0	0	0	0	
13	PKB + PKBR1	HM1521	P. Devreotes	See above	+	0	+	+2	
14	RacB	HM1568	R. Firtel	Rho GTPase	0	0	0	0	
15	RacGEF1	HM1569	R. Firtel	Rac GEF factor A	0	0	–	−1	
16	GAPA	HM1544	J. Faix	Ras GTPase-activating protein (IQGAP-related)	0	0	0	0	
17	DGAP1	HM1143	J. Faix	Ras GTPase-activating protein (IQGAP-related)	0	0	0	0	
18	ScaA	HM1567	R. Firtel	Scaffold protein	0	0	0	0	
19	RckA	DBS0236880 (HM1564)	Stock Center (R. Firtel)	Regulator of G protein signaling, tyrosine kinase-like protein	0	0	0	0	
20	PakB + PakC	DBS0236715 (HM1563)	Stock Center (R. Firtel)	Myosin I heavy chain protein kinase, p21-activated kinase	0	0	0	0	
21	PonA	DBS0236821 (HM1565)	Stock Center (E. Luna)	Ponticulin, anchors actin cytoskeleton to plasma membrane	0	0	+ +	+2	Cells attach poorly to the substratum.
22	TalB	HM1387	K. Inouye	Talin, FERM-domain protein	0	–	+	0	Cells attach poorly to the substratum.

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
23	TalA + TalB	HM1554	M. Tsuijioka	Talins, F-actin–binding FERM domain proteins	+	ND	ND	+1	Spherical, constitutively blebbing cells, very weakly adherent to the substratum—washed off during cAMP shock; cannot penetrate under agarose.
24	CorA	DBS0236172 (HM1561, HG1569)	Stock Center (G. Gerisch)	Coronin, actin-binding protein inhibiting actin nucleation	– –	–	–	−4	Cells less polarized and less motile than WT.
25	ProA + ProB	DBS0236827 (HM1562)	Stock Center (M. Schleicher)	Profilins, G-actin binding, PIP_2-binding proteins involved in F-actin regulation	–	0	–	−2	Cells less polarized and more flattened than WT. A small subpopulation bleb constitutively, while other cells bleb less than WT.
26	SevA	DBS0236166 (HM1566, HG1132)	Stock Center (G. Gerisch)	Severin, Ca^{2+}-dependent F-actin fragmenting protein	0	0	0	0	
27	dDia2	HM1583	J. Faix	Diaphanous-related formin	0	+	–	0	
28	ArcB	HM2245	Kay lab	Actin-related protein 2/3 complex subunit 2, involved in F-actin nucleation	+ +	+	+ +	+5	Cells more elongated and more blebby than WT.
29	Arp2 (GFP KI)	HM2191	Kay lab	Actin-related protein 2, component of Arp2/3 complex	+	0	+	+2	Cells move mostly by blebbing under buffer, behaving similarly to cells under agarose but with a more elongated shape. Cells form numerous blebs in response to cAMP.
30	Nap	IR57	R. Insall	Component of SCAR complex, regulates actin polymerization	0	0	0	0	Blebs smaller but more frequent than in WT.

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
31	Pir	SB16	R. Insall	Component of SCAR complex, regulates actin polymerization	+ +	+	+	+4	Cells often produce a series of successive blebs under buffer. Under agarose cells also produce chains of blebs spreading from the front to the sides. cAMP-induced blebbing continues for a longer period than in WT.
32	Abi	AP3	R. Insall	Component of SCAR complex, regulates actin polymerization	+	0	+ +	+3	Cells produce many small blebs under buffer. Blebs induced by cAMP shock are smaller than in WT but much more numerous.
33	HSPC300	AP2	R. Insall	Component of SCAR complex, regulates actin polymerization	+	0	+	+2	Cells are more elongated than WT under buffer and make more blebs at the leading edge. Produces slightly more blebs than WT after cAMP shock. Similar to WT under agarose.
34	SCAR1	IR48	R. Insall	Component of SCAR complex, regulates actin polymerization	+	0	+	+2	Most protrusions made by cells moving under buffer are blebbopodia. After cAMP shock cells produce smaller but more numerous blebs than WT. Under agarose cells move by blebbing, and the blebs are much larger than under buffer.
35	PikI	HM1513	Kay lab	PI(4)P-5-kinase	+ +	0	+	+3	Cells produce more blebs under buffer. Blebbing continues for a long time after cAMP shock—so more blebs form than WT. Very few cells penetrate under agarose, and those that do produce less polarized, more sporadic blebs than WT.
36	IplA	HM1486	Kay lab	Inositol 1,4,5-trisphosphate receptor-like protein, Ca^{2+} channel	0	0	0	0	
37	Gca + SgcA (in DH1)	DBS0236000 (HM1581)	Stock Center (P. van Haastert)	Guanylyl cyclases	0	0	–	−1	

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
38	Gca + SgcA (in Ax3)	HM1585	P. van Haastert	Guanylyl cyclases	0	–	–	−2	
39	GbpC	HM1582	P. van Haastert	Cyclic GMP-binding protein, with RasGEF domain	0	–	–	−2	
40	GbpC + GbpD	DBS0235996 (HM1580)	Stock Center (P. van Haastert)	Cyclic GMP-binding protein, with RasGEF domain	0	0	–	−1	
41	MlckA	DBS0236386 (HM1586, HS183)	Stock Center	Myosin light chain kinase (activates myo II)	–	0	–	−2	
42	PhdA	HM1587	R. Firtel	PIP_3-binding protein, PH-domain protein	+	0	– –	−1	Cells produce nearly as many blebs as WT during migration under buffer and under agarose but do not respond to cAMP shock.
43	CRAC	DBS0235559 (HM1596)	Stock Center (P. Devreotes)	Cytosolic regulator of adenylyl cyclase, PH domain protein	–	–	–	−3	Cells are less polarized than WT and respond more weakly to cAMP shock.
44	PhdA	HM1650	Kay lab	PIP_3-binding protein, PH domain protein	0	0	– –	−2	Cells produce as many blebs as WT during migration under buffer and under agarose but do not respond to cAMP shock.
45	CRAC	HM1648	Kay lab	Cytosolic regulator of adenylyl cyclase, PH domain protein	–	–	–	−3	Cells are less polarized than WT and respond more weakly to cAMP shock
46	PhdA + CRAC	HM1669	Kay lab	See above	–	– –	– –	−5	Cells have a rounded (poorly polarized), flattened, and spiky appearance. They are much less motile than WT and produce practically no detectable blebs under any condition tested.
47	Pia	HM1461	Kay lab	Component of TOR complex 2	0	0	0	0	

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
48	Lst8	HM1415	Kay lab	Component of TOR complex 2	0	–	+	0	
49	ErkB	HS175	M. Maeda	Extracellular response kinase from MAP kinase family	+	0	–	0	
50	RasC	HM1505	Kay lab	Ras family small GTPase responsible for chemotaxis	0	–	–	−2	
51	RasG	HM1497	Kay lab	Ras family small GTPase responsible for chemotaxis	–	0	0	−1	
52	RasC + RasG	HM1429	G. Weeks	Ras family small GTPases	0	–	– –	−3	Cells less polarized than WT
53	RacE	HM1604 (originally 24EH6)	D. Robinson	Rho GTPase	+ +	+ +	+	+5	Cells produce more blebs under buffer and after cAMP shock. Cells under agarose produce numerous blebs all around their periphery, slowing down migration. Blebs larger than in WT.
54	CtxA + CtxB	DBS0235599 (HM1605)	Stock Center (J. Faix)	Cortexillins, member of the alpha-actinin/spectrin superfamily of F-actin–binding proteins	+	0	+	+2	
55	Htt	DBS0349776	M. Iijima	Huntingtin orthologue, regulates myoII	– –	–	– –	−5	Most cells are rounded (spherical) and have impaired motility; cells rarely penetrate under agarose.
56	Myo ID + Myo IE + Myo IF	myoI-D/E/F-null	M. Iijima	Class I myosins, binding with PIP_3	– –	0	– –	−4	Cells are less polarized and more rounded than WT. Under buffer they migrate by producing F-actin–driven protrusions and rarely make blebs. Very weak response to cAMP shock: cells do not cringe or retract pseudopods.

Nº	Protein knocked out	Strain*	Source	Description	Blebbing activity			Blebbing score	Comment
					Under buffer	Under agarose	cAMP shock		
57	ACA	HM1366	Kay lab	Adenylyl cyclase A, essential for streaming in early development	–	–	–	−3	Cells less elongated, less polarized, and more flattened than WT. Occasionally make blebs under buffer but usually produce filopodia and slow-growing pseudopodia. After cAMP shock cells produce fewer blebs than WT, apparently because of a weak "cringe response."
58	GpbA	HM1691	P. Devreotes	G-protein beta-subunit (Gβ)	–	–	– –	−4	Cells occasionally produce blebs during migration under buffer and slightly more under agarose (if they can get there by random motility). No detectable response to cAMP shock.

Bleb frequency per cell in each assay was scored by eye on a semi-quantitative scale. Blue colors represent reduced blebbing and yellow/red represent increased blebbing.

Strain numbers starting with "DBS" refer to the *Dictyostelium* Stock Center (http://dictybase.org) collection; numbers starting with "HM" refer to the MRC-LMB collection. ND, not determined; WT, wild type.

Parental strains tested in parallel are not shown, but all had similar blebbing activity. They are: Ax2 (Kay), Ax2 (Gerisch), Ax3 (Devreotes), Ax3 (Insall), Ax3 (van Haastert), KAx3 (Firtel), NC4A2 (Knecht), DH1 (Devreotes), and JH10 (Chisholm).

Many mutants are similarly affected in all three assays, giving robustness to the results (for example the myosin-II mutants), but because the assays potentially measure different aspects of blebbing, differences can also be informative. For example, mutants blebbing normally under buffer, but much less under agarose might be defective in sensing or responding to mechanical resistance (for example the cGMP-related mutants, Gca + SgcA and GbpC). Similarly, mutants blebbing normally under buffer, but less in response to cyclic-AMP might be defective in the signal relay from cyclic-AMP (possibly the cGMP-related mutants and PhdA). However, such interpretations need to be made with caution because (1) the scoring is only semi-quantitative, except for selected mutants; (2) many mutants are in different genetic backgrounds, which could contain specific modifiers; and (3) many mutant phenotypes are likely to be complex with blebbing affected in multiple ways. For instance, the reduced PIP2 levels in PikI might result in reduced membrane–cortex adhesion and

hence increased blebbing under buffer, but the mutant also has extensively perturbed signal transduction (Fets et al., 2014), which might impact on myosin contractility, and PIP2 is believed to be important for actin polymerization controlled through SCAR–WAVE, so that there might also be cortical defects that prevent PikI^{-} cells from producing sufficient cortical tension (and hence pressure) to move efficiently under agarose.

Reference

Fets, L., J. Nichols, and R.R. Kay. 2014. A PIP5 Kinase essential for efficient chemotactic signalling. *Curr. Biol*. In press. http://dx.doi.org/10.1016/j.cub.2013.12.052