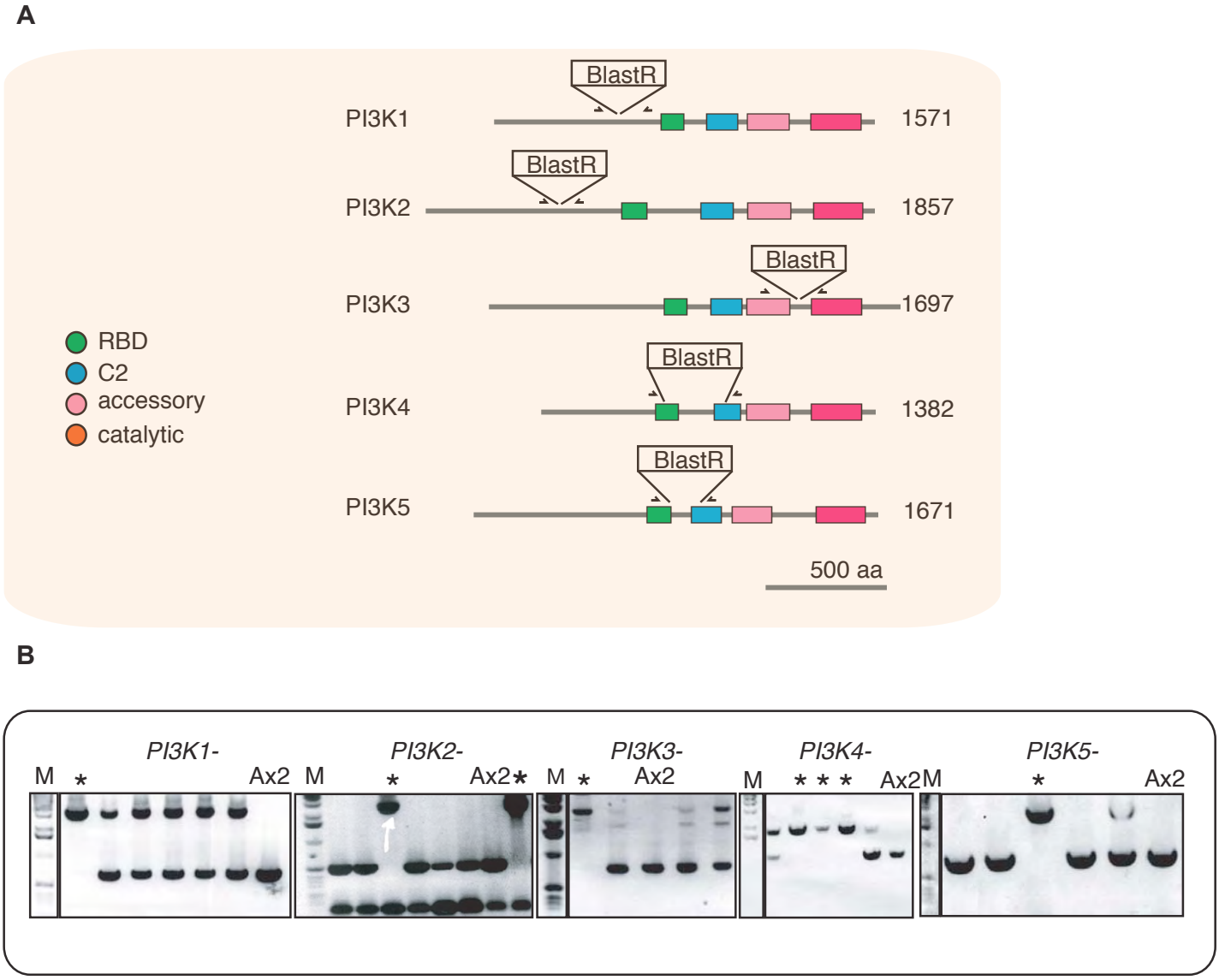

Fig. S1. Genome wide disruption of class 1 PI3-kinase genes. (A) Schematic presentation of the position of a gene disruption cassette (BlastR) with respect to the gene locus. Position of primers for screening of homologous integration events are indicated by arrows. (B) We used a PCR strategy that amplifies a new, longer fragment at the expense of the short, wild-type fragment. Homologous and non-homologous integration events can be distinguished in a single PCR reaction. Representative PCRs identifying homologous integration events at each PI3-kinase locus (lanes marked by an asterisk and a white arrow for PI3K2-). Lanes marked Ax2 show wild-type PCR product. M indicates 1 kb ladder (Invitrogen).

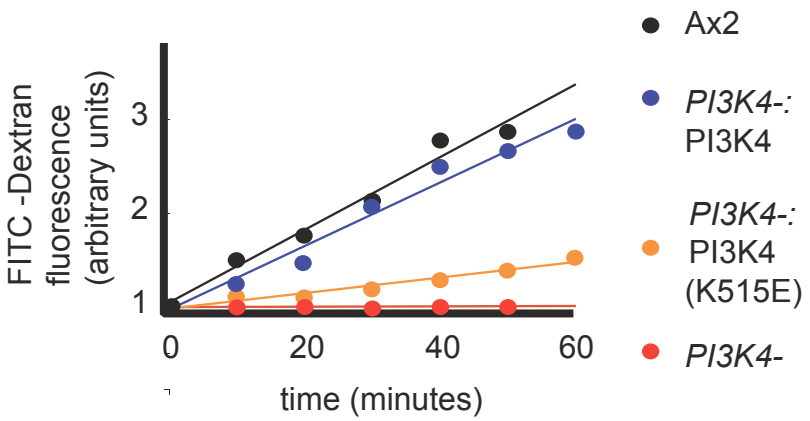

Fig. S2. Rescue of fluid phase uptake in PI3K4- mutants. A representative time course of FITC-dextran uptake of PI3K4- cells, PI3K4- cells expressing PI3K4, or PI3K4- cells expressing PI3K4 with a point mutation that abolishes Ras binding (*K515E*).

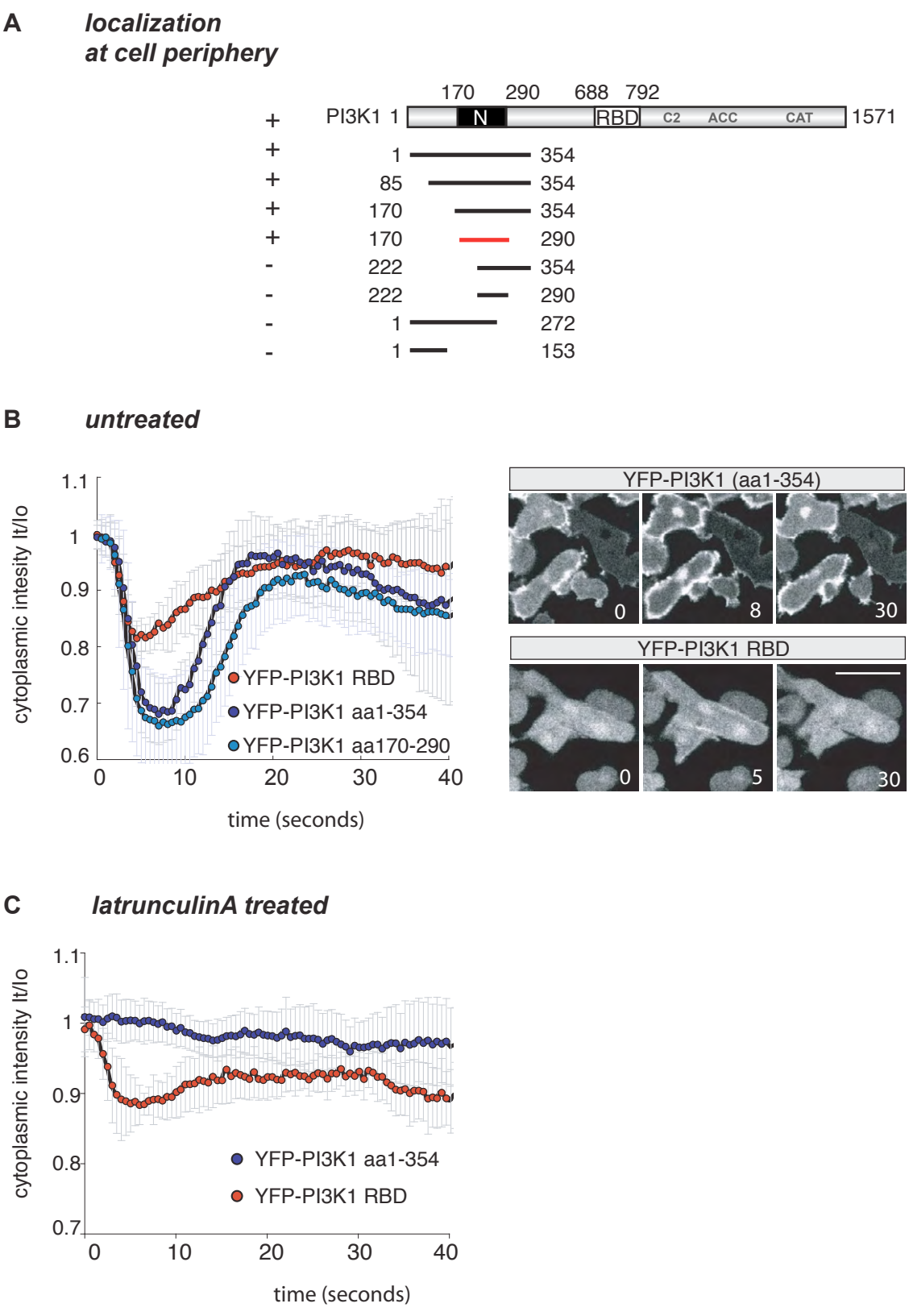

Fig. S3. Recruitment domains of PI3K1. Aggregation competent cells were prepared and localization behaviour of fragments of PI3K1 was observed. (A) YFP-tagged fragments were expressed in *Dictyostelium* cells and localization at the cell periphery was determined. A domain map, similar to Fig. 7B, is shown aligned with PI3K1 truncations tested. Numbers indicate amino acid position in the full-length protein. (B) Time course and extent of cytoplasmic depletion of indicated fragments after uniform stimulation with 1 μM cAMP. A 120 aa minimal N-terminal fragment behaves similar to a larger, previously described, 354 aa fragment. The Ras binding domain of PI3K1 also binds to the plasma membrane upon stimulation. Images of representative cells are shown. (C) Time course and extent of cytoplasmic depletion of indicated fragments after uniform stimulation with 1 μM cAMP in the presence of 20 μM latrunculin A. While the N-terminal domain is sensitive to treatment, the Ras binding domain is not. Graphs display means ± s.d. of responses of multiple cells recorded on several days. Numbers indicate time in seconds. Scale bar: 10 μm.

Table S1. Mutant PI3-kinase strains used in this study

strain	genotype	Protein affected	notes
Ax2			wildtype
HM1135	*pikA-*	PI3K1	
HM1128	*pikB-*	PI3K2	
HM1161	*pikC-*	PI3K3	
HM1149	*pikF-*	PI3K4	insertion
HM1241	*pikF-*	PI3K4	null
HM1151	*pikG-*	PI3K5	
HM1141	*pikA-,B-*	PI3K1, PI3K2	(Hoeller and Kay, 2007)
HM1200	*pikA-,B-,C-,F-,G-*	PI3K1-5	(Hoeller and Kay, 2007)

Table S1. Mutant PI3-kinase strains used in this study

Table S2. Motility: analysis of random motility and chemotactic properties of PI3-kinase mutant cells in a spatial folate gradient

	random motility		chemotaxis to folate		
strain	No. of cells	Velocity (mm/min) (mean ± sd)	No. of cells	Velocity (mm/min) (mean ± sd)	Chemotactic index
Ax2	1425	3.55 ± 1.82	20	2.89 ± 0.64	0.69 ± 0.2
PI3K1-	1316	4.42 ± 2.18	20	2.98 ± 0.64	0.64 ± 0.1
PI3K2-	1085	4.26 ± 2.75	20	2.79 ± 0.68	0.76 ± 0.1
PI3K3-	1253	4.16 ± 2.11	20	3.2 ± 0.74	0.71 ± 0.1
PI3K4-	869	4.03 ± 2.27	20	3 ± 0.45	0.76 ± 0.1
PI3K5-	1328	5.25 ± 3.46	20	2.96 ± 0.69	0.7 ± 0.1
PI3K(1-2)-			20	2.79 ± 0.61	0.75 ± 0.1
PI3K(1-5)-			20	2.8 ± 0.85	0.74 ± 0.2

Random motility data were extracted from at least three independent experiments, while chemotaxis data are from one representative experiment.

Chemotactic index: net distance travelled towards the source of folate divided by the total distance travelled in that time period.

Table S2. Motility: analysis of random motility and chemotactic properties of PI3-kinase mutant cells in a spatial folate gradient

Table S3. Phagocytosis: growth of mutants in bacterial suspension and rates of uptake of 1 μm FITC-labelled latex beads

strain	MGT [a] (hours) (mean ± sd)	Uptake/ cell volume (arbitrary units) (mean ± sd)
Ax2	3.36 ± 0.52	13.3 ± 1.3
PI3K1-	3.03 ± 1.01	13 ± 3.5
PI3K2-	3.78 ± 0.59	11.3 ± 3.4
PI3K3-	3.78 ± 1.16	12.7 ± 1.7
PI3K4-	3 ± 0.21	20.1 ± 4.6
PI3K5-	2.98 ± 0.76	10.3 ± 1.1
PI3K(1-2)-	3.82 ± 1.19	19.1 ± 8
PI3K(1-5)-	3.96 ± 1.01	13.7 ± 6.7

Data were extracted from at least three independent experiments.

[a]MGT: mean generation time

Table S3. Phagocytosis: growth of mutants in bacterial suspension and rates of uptake of 1 μm FITC-labelled latex beads

Table S4. Rates of fluid-phase uptake in *PI3K*- and *ras*- cell lines

strain	Initial rate of FITC-Dextran uptake/cell volume (% of parent; mean ± sd)
Ax2	100
PI3K1-	109 ± 22
PI3K2-	87 ± 9
PI3K3-	74 ± 8
PI3K4-	12 ± 6
PI3K5-	78 ± 16
PI3K(1-2)-	13 ± 5
PI3K(1-5)-	10 ± 2
JH10	100
rasC-	118 ± 23
rasG-	39 ± 12
Ax2	100
rasS-	54 ± 6
rasG-	51 ± 9

Data were extracted from at least three independent experiments.

Table S4. Rates of fluid-phase uptake in *PI3K*- and *ras*- cell lines