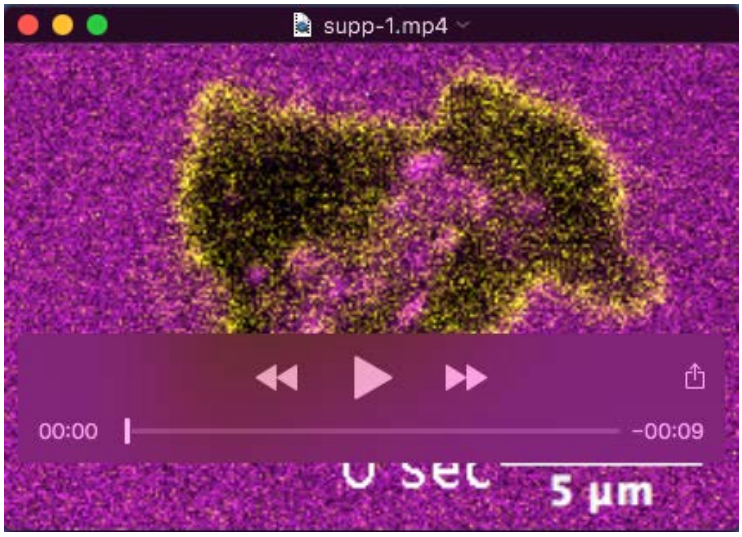
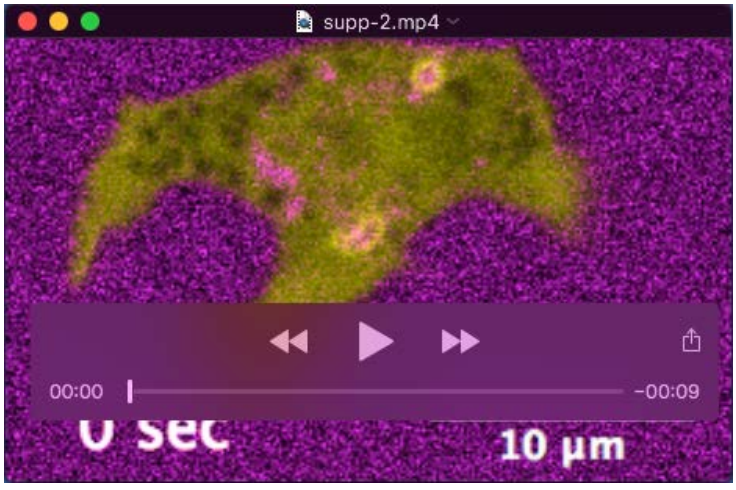


Movie 1: PkbR1-GFP is present on macropinosomes
PkbA-/PkbR1- cell overexpressing PkbR1-GFP (yellow) in TRITC-dextran containing media (magenta). PkbR1-GFP is present all over the cell membrane, including the macropinocytic cup, which is internalised and PkbR1-GFP remains on the endosome for a time after internalisation. Movie was taken at one frame per second on a Zeiss 710 microscope.



Movie 2: GFP-PkbA labels macropinosomes
PkbA-/PkbR1- cell overexpressing GFP-PkbA (yellow) in TRITC-dextran containing media (magenta). GFP-PkbA is recruited to macropinocytic cups and remains on endosomes for a time after internalisation. Movie was taken at one frame per second on a Zeiss 710 microscope.

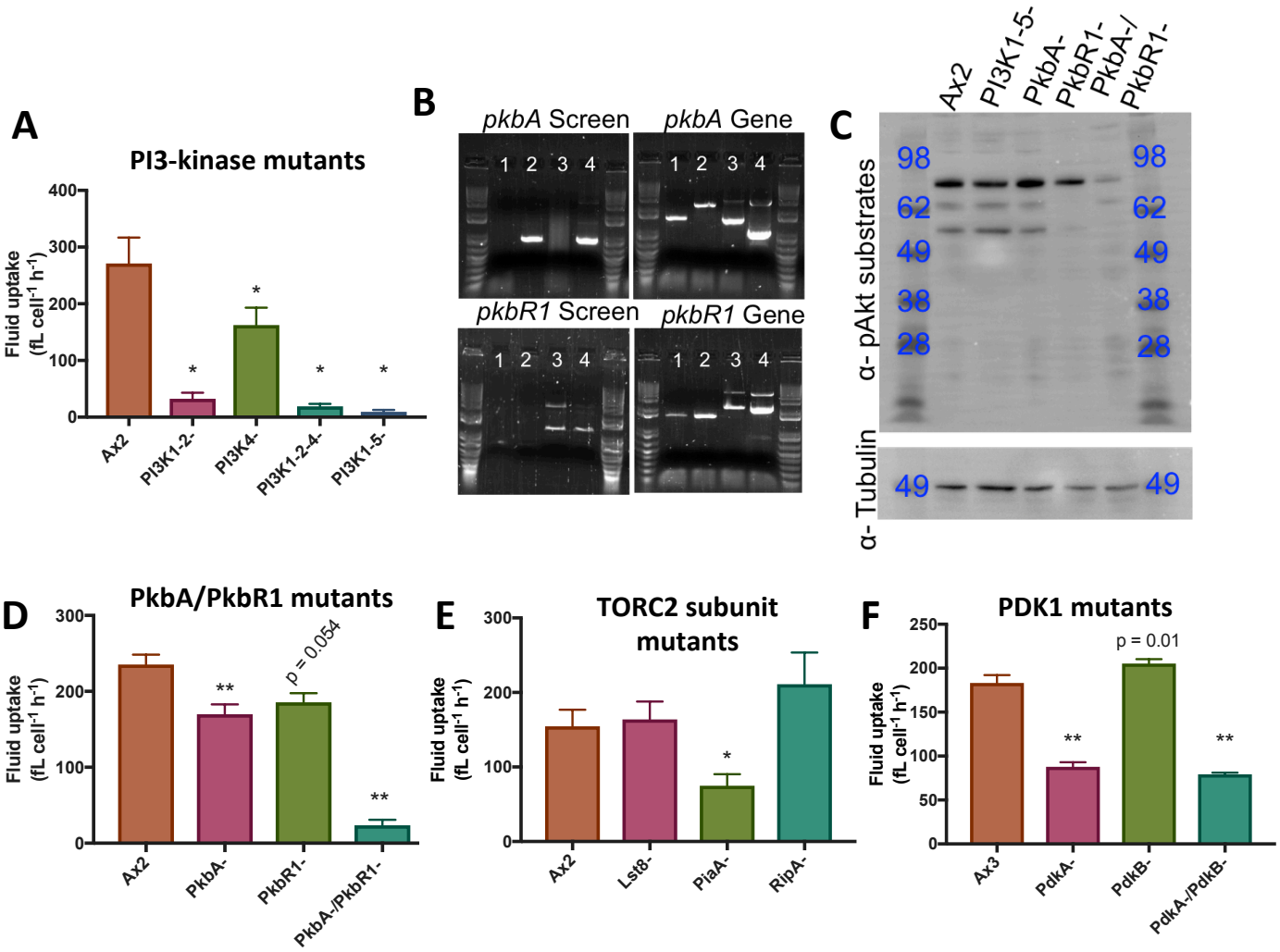


Figure S1; related to figure 1: Isolation and characterisation of PkbA/PkbR1 mutants and upstream activators

A) PI3-kinase mutants had similar fluid uptake defects after 24 h incubation in HL5 medium + 10% FBS compared to HL5 alone (Figure 1A). **B)** PCRs confirming the knockout of PkbA and PkbR1. The "gene" PCRs go across the full length of the gene, and are altered in length in the mutants while the "screen" PCRs are only visible in the mutants. Ax2 is shown in lane 1, PkbA- in lane 2, PkbR1- in lane 3 and PkbA-/PkbR1- in lane 4. Genomic DNA was prepared using a Zymogen gDNA miniprep kit according to the manufacturers instructions. **C)** PkbA-/PkbR1- mutants have negligible Akt substrate phosphorylations, while PI3K1-5- and single mutants are largely unaffected at a global level. **D)** Fluid uptake of PkbA/PkbR1 mutants was measured by TRITC-dextran addition for 1 h after 24 h incubation in HL5 medium + 10% FBS following harvesting from bacteria for PkbA/PkbR1 mutants, with similar fluid uptake defects observed as when they were incubated in HL5 alone (Figure 1B). Fluid uptake was generally improved, compared to HL5 medium alone (Figures 1C&1E) by addition of 10% FBS for **E)** TORC2 subunit mutants and **F)** PDK1 mutants.

For fluid uptake determination, cells were harvested from bacteria and inoculated in HL5 medium + 10% FBS for 24 h, after which TRITC-Dextran was added for 1 h. Cells were washed in ice-cold KK_2 buffer, detached and analysed by flow cytometry. Graphs show mean ± SEM, n = 3. $p \leq 0.1$ is specified, * $p < 0.05$, ** $p < 0.01$ compared to parent strain. For western blots, cells were incubated in HL5 medium + 10% FBS for 24 h before protein was harvested and lysate from approximately $2x10^5$ cells run out. The size of the protein markers are shown in kDa in blue text.

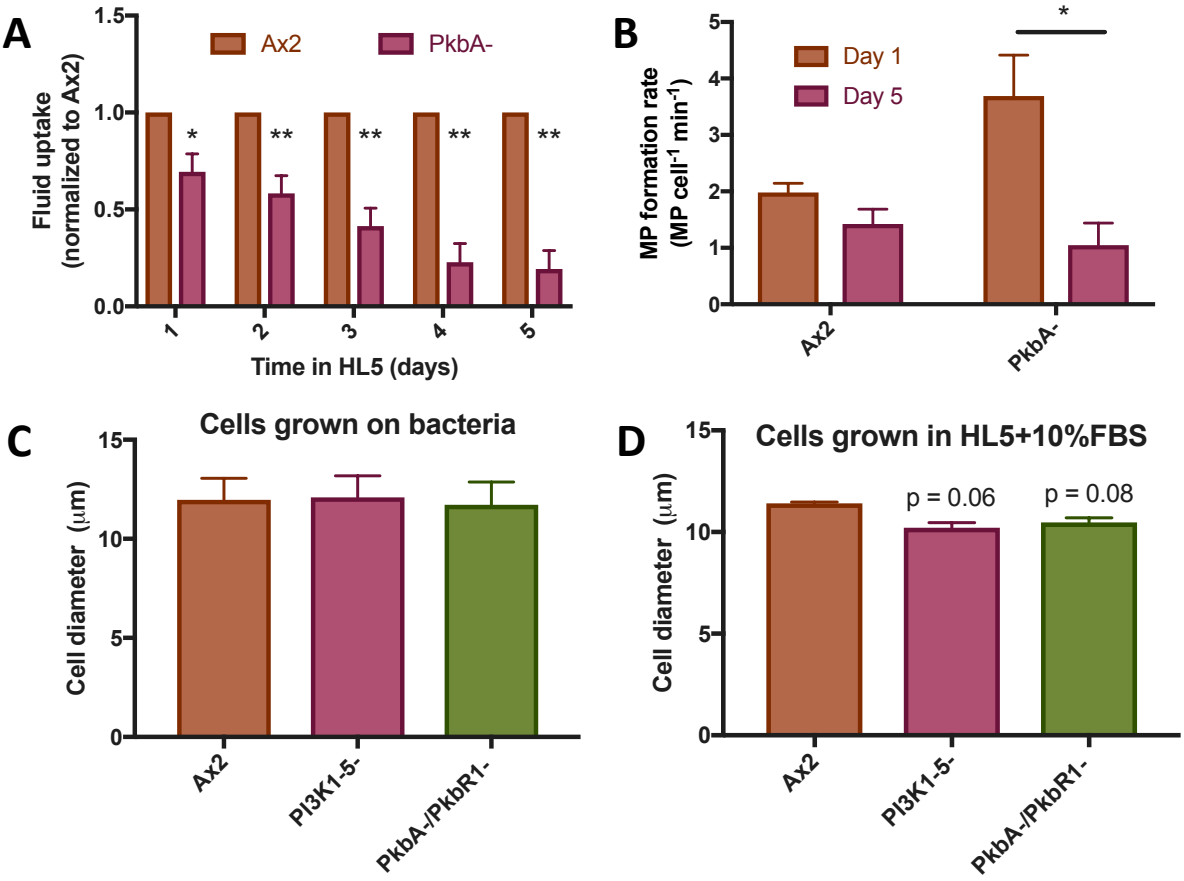


Figure S2; related to figure 2: PkbA- macropinocytosis over time and PkbA-/PkbR1- cell size
A) Fluid uptake decreases over time in PkbA- cells, accounting for the unexpected growth defect (Figure 2A). Ax2 and PkbA- cells were harvested from bacteria and inoculated into HL5 in 96-well plates at various densities, to account for cell proliferation, and fluid uptake measured every day for 5 days. **B)** The decrease in fluid uptake over time in PkbA- cells is due to a decrease in macropinosome formation. Ax2 and PkbA- cells were harvested from bacteria into HL5 for 1 or 5 days, after which their macropinosome formation rate was measured by pulsing cells for 1 min with FITC-dextran, fixing them and counting the number of macropinosomes by microscopy. **C)** PI3K1-5- and PkbA-/PkbR1- cells are the same size as Ax2 cells when cultivated on bacteria, where there is no proliferation defect (n = 4). Cells were grown on SM agar, washed and inoculated into KK_2MC + *Ka* bacteria for 24 h before being washed, resuspended in KK_2MC and size measured using an Eclipse (Sony iCyt) **D)** The PI3K1-5- and PkbA-/PkbR1- cell diameter is slightly decreased after they have been incubated in HL5 medium + 10% FBS for 24 h. Cells were prepared as in C.
Graphs show mean ± SEM, n = 3 unless stated, $p < 0.1$ is stated, * $p < 0.05$, ** $p < 0.01$ compared to Ax2.

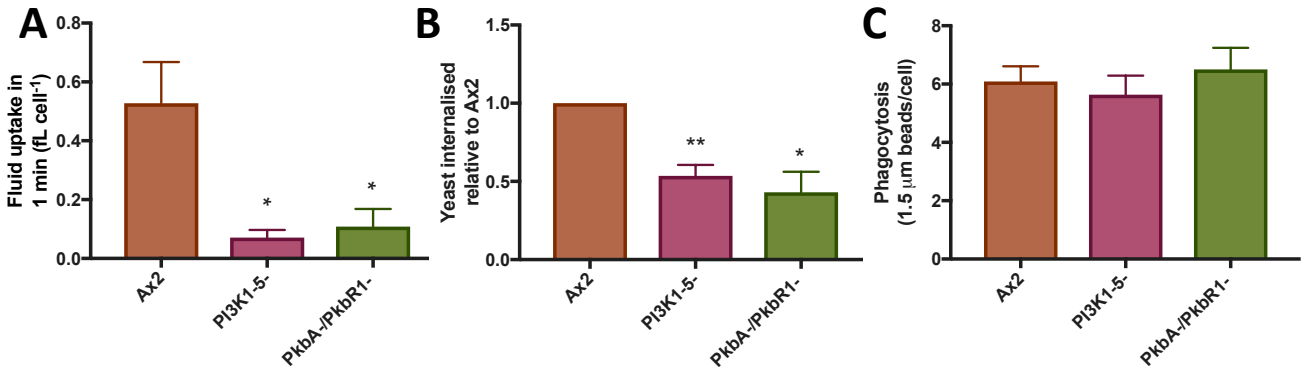


Figure S3; related to figure 4: PI3K1-5- and Akt- cells do not have increased endosome recycling, but do have reduced large particle uptake

A) The fluid uptake decrease observed in PI3K1-5- and PkbA-/PkbR1- cells is apparent after 1 min. Cells were harvested from bacteria and incubated in HL5 medium + 10% FBS for 24 h. The medium was then swapped to SUM for ≥1.5 h to minimise background fluorescence. Fluid uptake was then measured using 5 mg/ml TRITC-Dextran (n = 4). As this decrease is greater than for the macropinosome formation rate (Figure 4A), increased endosome recycling cannot account for the fluid uptake defects. **B)** Yeast endocytosis is reduced in PI3K1-5- and PkbA-/PkbR1- cells, consistent with these mutants having smaller macropinocytic patches (Figure 4C). Cells were harvested from bacterial SM agar plates, washed and incubated with fluorescent yeast for 1 h, after which the non-phagocytosed yeast fluorescence was quenched with trypan blue. Internalised fluorescence was measured on a fluorimeter and set relative to Ax2. **C)** Phagocytosis of 1.5 µm beads is unaffected in the PI3K1-5- and PkbA-/PkbR1- mutants, indicating there is no general phagocytosis defect in these cells. Cells were harvested as in B then distributed into 96-well plates in KK_2MC and allowed to settle. 1.5 µm beads were added to $1x10^8$ beads/ml for 20 min, after which the cells were washed, detached and analysed by flow cytometry.
Graphs show mean ± SEM, n = 3 unless otherwise stated, * $p < 0.05$, ** $p < 0.01$ compared to Ax2.

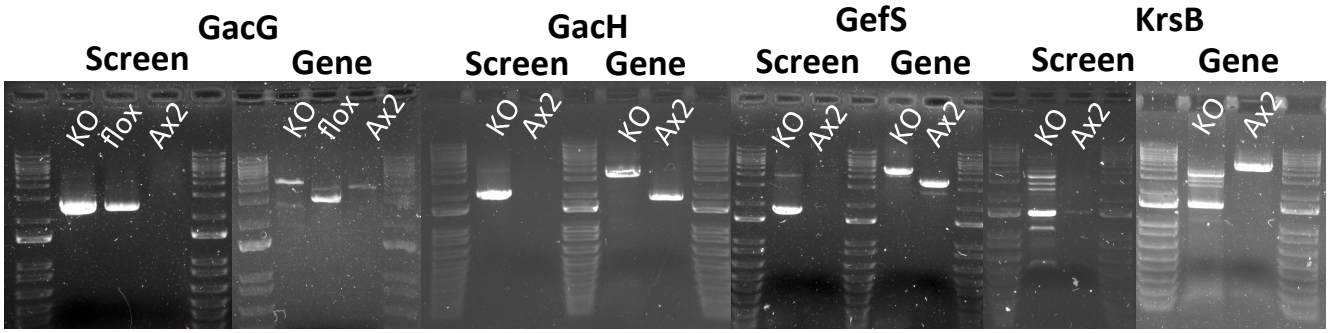
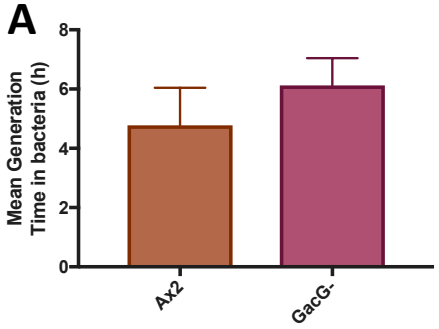


Figure S4; related to figure 5: Knockout of PkbA/PkbR1 targets

PCRs confirming the knockout of proteins identified in the phosphoproteomic screen. "Screen" PCRs only have a product when the gene is knocked out, while the "Gene" PCRs are across the full-length gene and have altered size in the mutants. Genomic DNA was prepared using a Zymogen gDNA miniprep kit according to the manufacturers instructions.

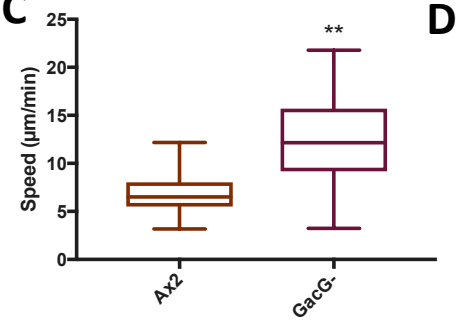
A



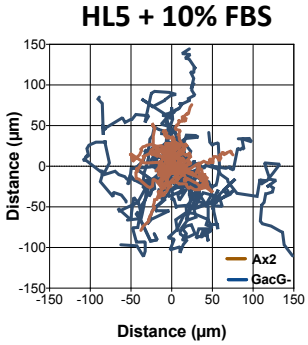
B

Strain	Macropinosome "diameter" (%)	Macropinosome radius (%)	Macropinosome volume (%)
Ax2	100 (1.30 µm)	100 (0.75 µm)	100 (1.78 µm^3)
GacG-	75	75	41

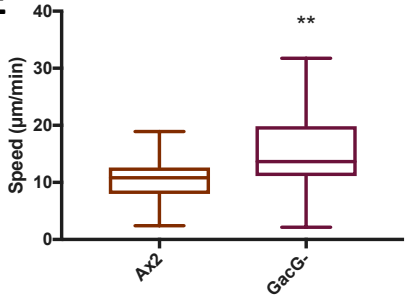
C



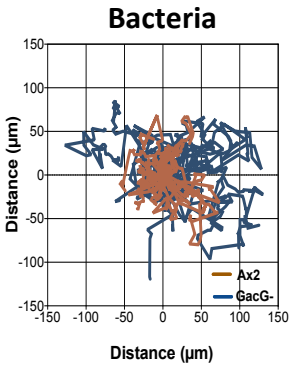
D



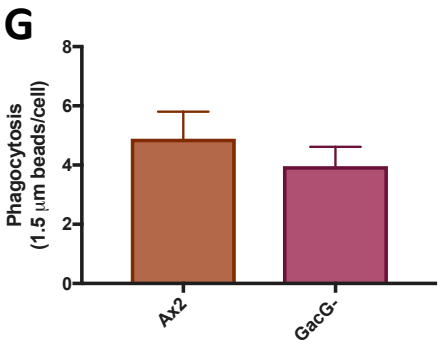
E



F



G



H

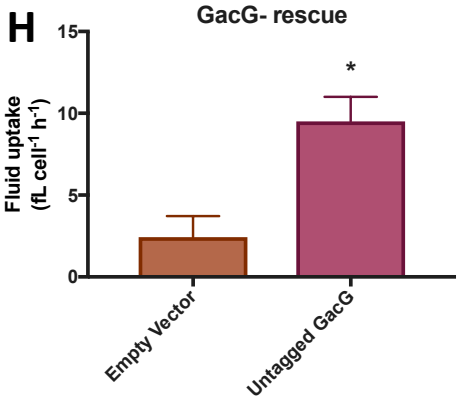


Figure S5; related to figure 6: GacG- cell proliferation, endocytosis and movement (previous page)
A) GacG- cells proliferate normally on bacteria. Cells were harvested from growth on SM bacterial plates and incubated in 20 $OD_{600\ nm}$ *Ka* in KK_2MC at 22 °C, 220 rpm. Cell density was determined at regular intervals using a hemacytometer. **B)** Summary table of Figure 6C showing the average values as a percentage of the parent control. The measured diameter of the macropinosomes tends to halfway between the equator and the poles of the macropinosome (assuming the observed diameters were obtained from random sections of perfectly spherical macropinosomes). The average radius is calculated by multiplying the median observed diameter by tan(30°), and the volume calculated from that. As macropinosomes are often not perfect spheres when they are formed, and sampling at the periphery of macropinosomes is technically challenging, these values are approximate. **C)** GacG- cells move faster than Ax2 cells when grown axenically in HL5 medium + 10% FBS. The speed of 120 randomly moving cells was measured over 20 minutes using a Zeiss 700 series microscope. **D)** The tracks of 20 cells incubated in HL5 medium + 10% FBS for 24 h, then filmed randomly moving using a Zeiss 700 series microscope from Figure 6G are shown. **E)** GacG- cells move faster than Ax2 cells when grown on bacteria. The speed of 120 randomly moving cells was measured over 20 minutes using a Zeiss 700 series microscope. **F)** The tracks of 20 cells from E are shown. **G)** Phagocytosis of 1.5 µm beads is unaffected in the GacG- mutant, like the PkbA-/ PkbR1- and PI3K1-5- mutants, indicating there is no general phagocytosis defect in these cells. Cells were harvested from SM plates, distributed into 96-well plates in KK_2MC and allowed to settle. 1.5 µm beads were added to $1x10^8$ beads/ml for 20 min, after which the cells were washed, detached and analysed by flow cytometry. **H)** The fluid uptake defect of GacG- cells can be rescued by overexpression of untagged GacG, albeit to far below the levels of Ax2. GacG- cells were transformed with an empty expression vector, or the vector containing GacG. Transformants were selected for on bacteria, then transferred to HL5 medium + 10% FBS and fluid uptake was assessed 24-72h after transfer.
Bar graphs show mean ± SEM, box plots show the minimum and maximum values, the box is the 25^{th} to 75^{th} percentile and the middle line shows the median. n = 3 unless specified. * $p < 0.05$, ** $p < 0.01$ compared to Ax2 or the control.

Table S1: Phosphopeptide abundance in Akt mutant cells

The phosphopeptides identified by TMT mass spectrometry and their abundance in PkbA/ PkbR1 mutants relative to Ax2. Strains were harvested from bacterial plates, washed free of bacteria and incubated in HL5 medium + 10% FBS for 24 h at 22 ^{o}C, 220 rpm at 1-2x10^{6} cells/ ml. Cells were then washed free of medium, resuspended in KK_2MC buffer and treated for 30 minutes with DMSO or 100 µM LY294002 before protein was harvested, TMT tagged, phosphopeptides purified and analysed by mass spectrometry. The abundance of each phosphopeptide in each condition was measured relative to Ax2. Condition 1- Ax2, condition 2- PkbA-, condition 3- PkbR1-, condition 4- PkbR1- + LY294002, condition 5- PkbA-/PkbR1-.

Click here to Download Table S1

Uniprot ID	Dictybase ID	Phosphosite(s)	Most likely kinase	Predicted function
P0CD60	FrmC	S306	PKA	Cytoplasm/membrane linker
P28178	PkbR1	T432 ; S449 ; T470	PKC ; GSK3 ; GSK3	Protein Kinase
Q00766	GlpV	T10 ; T13	Cdc2 ; PKC	Glycogen phosphorylase
Q54B11	DDB_G0293990	S230	PKA	Unknown
Q54DP0	DDB_G0292106	S212	PKA	Endosomal transport
Q54ET0	GrlE	T765 ; S770	PKC ; CKII	GPCR
Q54GL1	GDE1	S271	PKA	Phosphodiesterase
Q54H00	DDB_G0289807	S531	PKA	Protein/Lipid phosphatase
Q54HC8	DDB_G0289551	S123	DNAPK	Membrane protein
Q54IA6	DDB_G0288895	T395	PKG	Unknown
Q54LB8	Vps13A	S1685	PKA	Vacuolar sorting
Q54P47	NdrC	S12 ; S529	RSK ; GSK3	Protein Kinase
Q54QE5	DDB_G0283919	S465	GSK3	Protein Phosphatase
Q54QI2	DDB_G0283821	S750	PKA	Proten Kinase
Q54R82	MkkA	S488	CAM-II	Proten Kinase
Q54RZ3	DDB_G0282823	S694	RSK	Unknown
Q54SC3	DDB_G0282779	S495	PKC	Transmembrane protein
Q54SL6	GacQ	T272	Cdc2	RhoGAP
Q54U31	ShkD	S267	PKA	Protein Kinase
Q54VX2	GxcS	S317	RSK	RhoGEF
Q54WH7	DDB_G0279653	S60	PKA	Unknown
Q552C1	DDB_G0276181	S268	PKA	Protein Kinase
Q553Q6	DDB_G0275401	S513	DNAPK	Unknown
Q555M9	DDB_G0274847	S989	Cdc2	Nucleotidyltransferase
Q55DM1	LvsA	S93	PKA	Contractile Vacuole protein
Q6XHA6	Roco10	S1671	Cdc2	LRRK family protein kinase
Q86C65	Tor	S454	PKA	Protein Kinase
Q86KF9	SgkA	S424	Cdc2	Sphingosine Kinase
Q54TT8	DDB_G0281503	S938	PKA	Unknown

Table S2: Candidate proteins involved in macropinocytosis not phosphorylated by PkbA/PkbR1
Phosphorylated proteins that were less abundant in PkbA/PkbR1 mutants (>4-fold decreased in PkbA-/PkbR1- cells, >2-fold in PkbR1- + LY294002) compared to Ax2 which did not have an Akt consensus motif. Cells were incubated for 24 h in HL5 medium + 10% FBS at 22 ^{o}C, 220 rpm to allow macropinocytosis upregulation, then washed and resuspended in KK_2MC and treated with 100 µM LY294002 inhibitor or DMSO for 30 min. Proteins were then precipitated, labelled with TMT tags, enriched for phosphopeptides and the abundance of each compared using mass spectrometry.

GO molecular function term	Proteins matching terms (Total proteome)	Proteins matching terms (Akt targets)	Expected proteins	Fold Enrichment	Raw P-value	False Discovery Rate
clathrin light chain binding (GO:0032051)	2	1	0	> 100	0.0035	1.56
clathrin adaptor activity (GO:0035615)	2	1	0	> 100	0.0035	1.30
endocytic adaptor activity (GO:0098748)	2	1	0	> 100	0.0035	1.12
phosphatidylinositol-3,4-bisphosphate binding (GO:0043325)	3	1	0	> 100	0.0047	1.30
phosphatidylinositol-3,5-bisphosphate binding (GO:0080025)	3	1	0	> 100	0.0047	1.16
cargo receptor activity (GO:0038024)	4	1	0	> 100	0.0059	1.30
protein-containing complex scaffold activity (GO:0032947)	8	1	0.01	> 100	0.0105	1.46
clathrin binding (GO:0030276)	10	1	0.01	84.9	0.0129	1.68
molecular adaptor activity (GO:0060090)	14	1	0.02	60.64	0.0175	1.76
protein binding, bridging (GO:0030674)	14	1	0.02	60.64	0.0175	1.69
Rac GTPase binding (GO:0048365)	15	1	0.02	56.6	0.0187	1.66
phosphatidylinositol bisphosphate binding (GO:1902936)	15	1	0.02	56.6	0.0187	1.59
microtubule motor activity (GO:0003777)	17	1	0.02	49.94	0.0210	1.72
phosphatidylinositol phosphate binding (GO: 1901981)	31	1	0.04	27.39	0.0370	1.91
Rho GTPase binding (GO:0017048)	68	2	0.08	24.97	0.0030	1.65
motor activity (GO:0003774)	34	1	0.04	24.97	0.0404	1.99
microtubule binding (GO:0008017)	44	1	0.05	19.3	0.0517	2.44
Rho guanyl-nucleotide exchange factor activity (GO:0005089)	46	1	0.05	18.46	0.0539	2.39
tubulin binding (GO:0015631)	53	1	0.06	16.02	0.0617	2.68
phosphatidylinositol binding (GO:0035091)	54	1	0.06	15.72	0.0628	2.68
guanyl-nucleotide exchange factor activity (GO:0005085)	114	2	0.13	14.89	0.0080	1.47
GTPase activator activity (GO:0005096)	116	2	0.14	14.64	0.0082	1.41
GTPase regulator activity (GO:0030695)	117	2	0.14	14.51	0.0084	1.33
nucleoside-triphosphatase regulator activity (GO:0060589)	124	2	0.15	13.69	0.0094	1.38
Ras guanyl-nucleotide exchange factor activity (GO:0005088)	64	1	0.08	13.27	0.0738	2.92
GTPase binding (GO:0051020)	195	3	0.23	13.06	0.0015	1.08
phospholipid binding (GO:0005543)	74	1	0.09	11.47	0.0847	3.13
Ras GTPase binding (GO:0017016)	149	2	0.18	11.4	0.0132	1.63
small GTPase binding (GO:0031267)	149	2	0.18	11.4	0.0132	1.54
phosphoprotein phosphatase activity (GO: 0004721)	76	1	0.09	11.17	0.0869	3.11
enzyme activator activity (GO:0008047)	155	2	0.18	10.95	0.0142	1.58
molecular function regulator (GO:0098772)	326	4	0.38	10.42	0.0005	1.06

Table S3; related to table 1 and figure 5: Terms enriched by GO analysis in the Akt target proteins
The 16 proteins identified as being phosphorylated at an Akt consensus sequence with a reduced abundance by mass spectrometry (Table 1) were subject to GO analysis against the *Dictyostelium discoideum* proteome. The terms with a >10-fold enrichment are shown here. Statistical analysis was performed using fishers exact test.

	Uniprot ID	Dictybase ID	Phosphosite(s)	Most likely kinase	Predicted function
PkbA dependent	Q55FS2	KrsB	S505	Akt	Protein Kinase
	Q6S004	Kif6	S1016	Akt	Kinesin
PkbR1 dependent	Q54YK2	DDB_G0278629	S679	Akt	Unknown
	Q8SSW7	GefS	S623	Akt	RasGEF

Table S4 related to table 1 and figure S4: PkbA and PkbR1 dependent phosphorylations
The proteins with phospho-sites which appear to be dependent on either PkbA or PkbR1 specifically. Phosphopeptides that had an Akt recognition motif were identified which were >4-fold reduced compared to the Ax2 control in either PkbA- cells (PkbA-dependent phosphorylations) or PkbR1- cells (PkbR1-dependent phosphorylations).

Parent	Mutation(s)	Resistance	Strain reference(s)	Source
Ax2 (Ka)	-	-	Ax2 (Ka)	Rob Kay
Ax2 (Ka)	*pi3k1-/2-*	Blasticidin	HM1141	(Hoeller and Kay, 2007)
Ax2 (Ka)	*pi3k4-*	Blasticidin	HM1148	(Hoeller and Kay, 2007)
Ax2 (Ka)	*pi3k1-/2-/4-*	Blasticidin	HM1159	(Hoeller and Kay, 2007)
Ax2 (Ka)	*pi3k1-5-*	Blasticidin	HM1200	(Hoeller and Kay, 2007)
Ax2 (Ka)	*pkbA-*	G418	HM1815-7	This work
Ax2 (Ka)	*pkbA-*	-	HM1818	This work
Ax2 (Ka)	*pkbR1-*	G418	HM1832-4	This work
Ax2 (Ka)	*pkbR1-*	-	HM1835	This work
Ax2 (Ka)	*pkbA-/pkbR1-*	G418	HM1850-2	This work
Ax2 (Ka)	*pkbA-/pkbR1-*	-	HM1853	This work
Ax2 (Ka)	*lst8-*	Blasticidin	HM1415	Louise Fets
Ax2 (Ka)	*piaA-*	Blasticidin	HM1461	Louise Fets
Ax2 (Ka)	*ripA-*	Blasticidin	HM1364	Oliver Hoeller
Ax3 (Devreotes)	-	-	Ax3 (Devreotes)	Peter Devreotes
Ax3 (Devreotes)	*pdkA-*	Blasticidin	HM1950	(Kamimura and Devreotes, 2010)
Ax3 (Devreotes)	*pdkB-*	Blasticidin	HM1951	(Kamimura and Devreotes, 2010)
Ax3 (Devreotes)	*pdkA-/B-*	Blasticidin	HM1952	(Kamimura and Devreotes, 2010)
Ax2 (Ka)	*gacG-*	G418	HM1945-9	This work
Ax2 (Ka)	*gacG-*	-	HM1956	This work
Ax2 (Ka)	*gacH-*	G418	HM1942-4	This work
Ax2 (Ka)	*krsB-*	G418	HM1970-3	This work
Ax2 (Ka)	*gefS-*	G418	HM1953-5	This work
Ax4 (Kuspa)	-	-	Ax4 (Kuspa)	Adam Kuspa
Ax4 (Kuspa)	*scaA-*	Blasticidin	V10285	(Sawai et al., 2008)

Table S5: Strains used in this study
The strains used in this study. Where there is more than one strain of a particular mutant, the phenotype presented in the text is representative of all the strains. Where strains have been cured of their resistance by cre-lox recombination, the data represented in this work is of the strain without a resistance cassette.

Hoeller, O. and Kay, R. R. (2007). Chemotaxis in the absence of PIP3 gradients. *Curr. Biol.* **17**, 813-817.
Kamimura, Y. and Devreotes, P. N. (2010). Phosphoinositide-dependent protein kinase (PDK) activity regulates phosphatidylinositol 3,4,5-trisphosphate-dependent and -independent protein kinase B activation and chemotaxis. *J. Biol. Chem.* **285**, 7938-46.
Sawai, S., Guan, X. J., Kuspa, A. and Cox, E. C. (2008). High-throughput analysis of spatio-temporal dynamics in *Dictyostelium*. *Genome Biol.* **8**, R144 (15 pages).

Vector	Markers	Selection
pDM1140	PakB CRIB-GFP (active Rac), Raf1 RBD-mCherry (active Ras)	G418
pDM1383	HSPC300-GFP (SCAR), Raf1 RBD-mCherry (active Ras)	G418
pDM1489	Cre Recombinase	Hygromycin
pPI157	Raf1 RBD-GFP (active Ras)	G418
pPI321	Raf1 RBD-GFP (active Ras), Lifeact-mCherry (F-actin)	G418
pTW001	Raf1 RBD-GFP (active Ras), PTEN-mCherry	G418
pTW002	Raf1 RBD-GFP(active Ras), PH PkgE-mCherry (PIP3)	G418
pDM1207	Empty Vector control	G418
pTW003	GFP-PkbA	G418
pTW044	GFP-PkbA T435A	G418
pTW046	GFP-PkbA T278A	G418
pDM1209	Empty Vector control	G418
pTW008	PkbR1-GFP	G418
pTW063	PkbR1-GFP T470A	G418
pTW065	PkbR1-GFP T309A	G418
pTW032	GacG-GFP	G418
pPI582	GFP-GacG	G418
pDM1203	Empty Vector control	G418
pTW058	GacG	G418

Table S6: Over-expression vectors used in this study
The overexpression vectors used in this study. These were made based in the pDM expression system (Veltman et al., 2009).

Veltman, D. M., Akar, G., Bosgraaf, L. and Van Haastert, P. J. (2009). A new set of small, extrachromosomal expression vectors for *Dictyostelium discoideum*. *Plasmid* **61**, 110-118.

Vector name	Target gene	Linearising digest	Selection marker	Construction details
pPkbAKO	*pkbA*	SmaI	G418	G418, 5' and 3' KO arms amplified and then joined by PCR. Product was cloned into pJet1.2 (Thermo-Fisher)
pPkbR1KO	*pkbR1*	SmaI	G418	G418, 5' and 3' KO arms amplified and then joined by PCR. Product was cloned into pJet1.2 (Thermo-Fisher)
pGacGKO	*gacG*	BamHI & SpeI	G418	Gene amplified from Ax2 DNA and cloned into pJet1.2 (Thermo-Fisher). Digested with EcoRV and a G418 EcoRV digest inserted.
pGacHKO	*gacH*	BamHI & NheI (partial digest)	G418	Gene amplified from Ax2 DNA and cloned into pJet1.2 (Thermo-Fisher). Digested with EcoRV and a G418 EcoRV digest inserted.
pGefSKO	*gefS*	BglII/SpeI	G418	Gene amplified from Ax2 DNA and cloned into pJet1.2 (Thermo-Fisher). Digested with EcoRV and a G418 EcoRV digest inserted.
pKrsBKO	*krsB*	PvuI	G418	Gene amplified from Ax2 DNA and cloned into pJet1.2 (Thermo-Fisher). Digested with EcoRV and a G418 EcoRV digest inserted.

Table S7: Knockout vectors used in this study
The knockout vectors used in this study and how they were made. The resistance cassette was obtained from pDM vectors.

Table S8: Primers used in this study (overleaf)
The primers used in this study, and what they were used to amplify. Restriction enzyme sites incorporated into the sequences are shown in lower case, while overlapping primer sequences are shown in lower case and italicised. Introduced point mutations are shown in bold.

PCR product	Primer 1 name	Primer 2 name	Primer 1 sequence	Primer 2 sequence
pkbA gene	pkbA_F	pkbA_R	agatctATGTCAACAGCACCAATTAAA	actagtTTATCTTAAATGTTCAGATTC
pkbR1 gene	pkbR1_F	pkbR1_R	agatctATGGGAAAAGGACAAAGTAAA	actagtATCCTTTAAGATTGAATCAGC
gacG gene	gacG_F	gacG_R	ggatccATGGCGTCAATATTTTTAAATAAAAAAC	actagtCTCTTCAACAATATCAGTTAAAGAAGG
gacH gene	gacH_F	gacH_R	agatctATGAGTGGTGTAGGAGGTGAATCAGTAC	gctagcATTATCATAAAATAAATTATAATTTTCAATTAATAAAG
krsB gene	krsB_F	krsB_R	ggatccATGGAGGAAAGTGGTCAAC	aagctagcATTATTATAAAAATCTAAATCAAATTCTGAATAAAT
gefS gene	gefS_F	gefS_R	agatctATGGGAGAAGTAAGTTTAATCGG	actagtCTTTGGTTCGAGAGCAATTGATA
pDM resistance cassette	oDM1015	oDM1016	CTTCAGTAGGCAGAGCTATC	CAGCTTGGATACTCCAGTAG
pkbA KO 5' arm	pkbA_5'F	pkbA_5'R	cccgggCCAAACCACAACAATATTCGCATAA	*gatagctctgcctactgaag*TCCATTAACTCTTTTCTTACGATCAGA
pkbA KO 3' arm	pkbA_3'F	pkbA_3'R	*ctactggagtatccaagctg*TTGACACCAACCGACAAAACTG	cccgggTCTTAAATGTTCAGATTCAGCGACA
pkbR1 KO 5' arm	pkbR1_5'F	pkbR1_5'R	cccgggTTTAATTACCACACTGTCACTATTTCCTATAAG	*gatagctctgcctactgaag*TTGATAAAAGACTTGGAACTAAAG
pkbR1 KO 3' arm	pkbR1_3'F	pkbR1_3'R	*ctactggagtatccaagctg*GTCCTTCATCTTCATCATCATT	cccgggACTCTCTTTCTCACACCAATAACAC
pkbA screen	pkbA_ExtR	oDM1014	TTTTACCAAAGACGGAAACTTATTCTAAC	CTACTGGAGTATCCAAGCTG
pkbR1 screen	pkbR1_ExtR	oDM1014	cccgggCACAAACCAAATAAAAGTATTCATCATGTC	CTACTGGAGTATCCAAGCTG
gacG screen	gacG_5F4	oPI236	ATTACACATATATACATAAAATATTCAAAACCACAC	atctcgAGAGTAGTATAACTTCGTATAGCATAC
gacH screen	gacH_5F1	oPI236	TTAAAAGAGGGTGTTAATTGAAATTTC	atctcgAGAGTAGTATAACTTCGTATAGCATAC
krsB screen	krsB_Ext3'	oPI236	GGTAATTTCCTCTTTTGAAGCCATT	atctcgAGAGTAGTATAACTTCGTATAGCATAC
gefS screen	gefS_Ext5'	oPI236	TTTAAATATACTTCACTCTCTCCCAAGT	atctcgAGAGTAGTATAACTTCGTATAGCATAC
pkbA T278A point mutation	pkbA_T278A_F	pkbA_T278_R	**GCT**TTCTGTGGTACTCCTGAATATTTAGC	ACCAGTTTTGTCGGTTGGTGTCAATAGACCCTC
pkbA T435A point mutation	pkbA_T435A_F	pkbA_T435_R	**GCA**TATGTCGCTGAATCTGAACATTTAAG	AAATCCTTCAAAATCTTTTTGTTGTTGCGG
pkbR1 T309A point mutation	pkbA_T309A_F	pkbA_T309_R	**GCT**TTCTGTGGTACACCAGAGTATTTAGC	GAATGTACCATCGGTTGTTTCAAT
pkbR1 T470A point mutation	pkbA_T470A_F	pkbA_T470_R	**GCT**TATGTAGCTGATTCAATCTTAAAGG	GAAACCTTCAAAGCTTGTATC