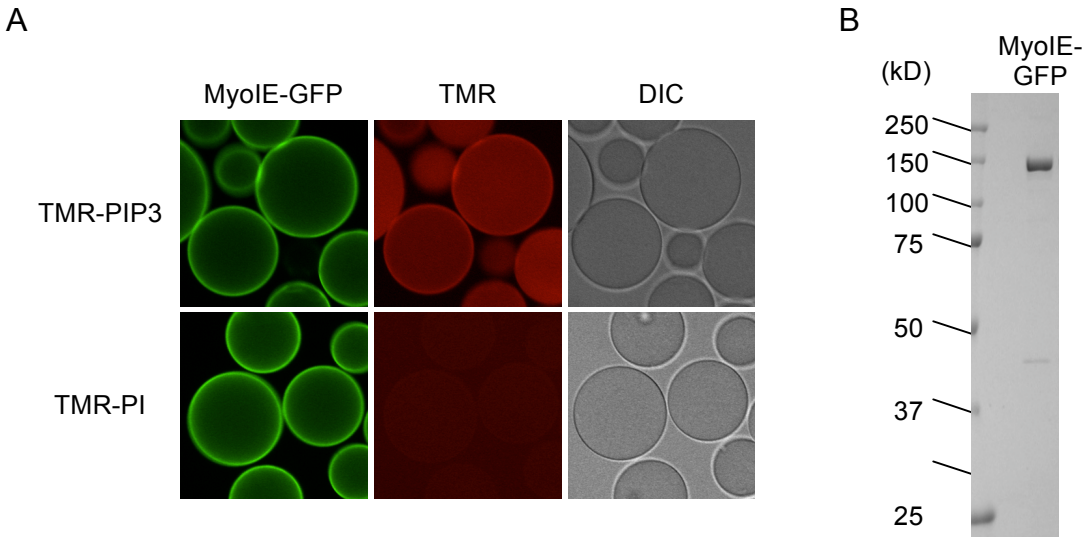

Figure S1. Immunopurified myosin IE-GFP binds to PIP_3. 2 x 10^8 *Dictyostelium* cells expressing myosin IE-GFP were lysed in 2 ml of 0.5% Triton X-100, 150 mM NaCl, and 10 mM sodium phosphate (pH 7.0). The whole cell extract was incubated with beads coupled to anti-GFP antibodies (GFP-trap; Allele Biotechnology) for 1 hour at 4°C. After extensive washing, myosin IE-GFP-beads were incubated with BODIPY tetramethylrhodamine-labeled $PtdInsP_3$ (1 μM, C-39M6; Echelon) or BODIPY tetramethylrhodamine-labeled phosphatidylinositol (1 μM, C-00M6; Echelon) for 1 hour at 4°C. After washing, beads were observed using fluorescence and differential interference contrast microscopy. Immunopurified myosin IE-GFP was resolved by SDS-PAGE and stained with Coomassie Brilliant Blue.

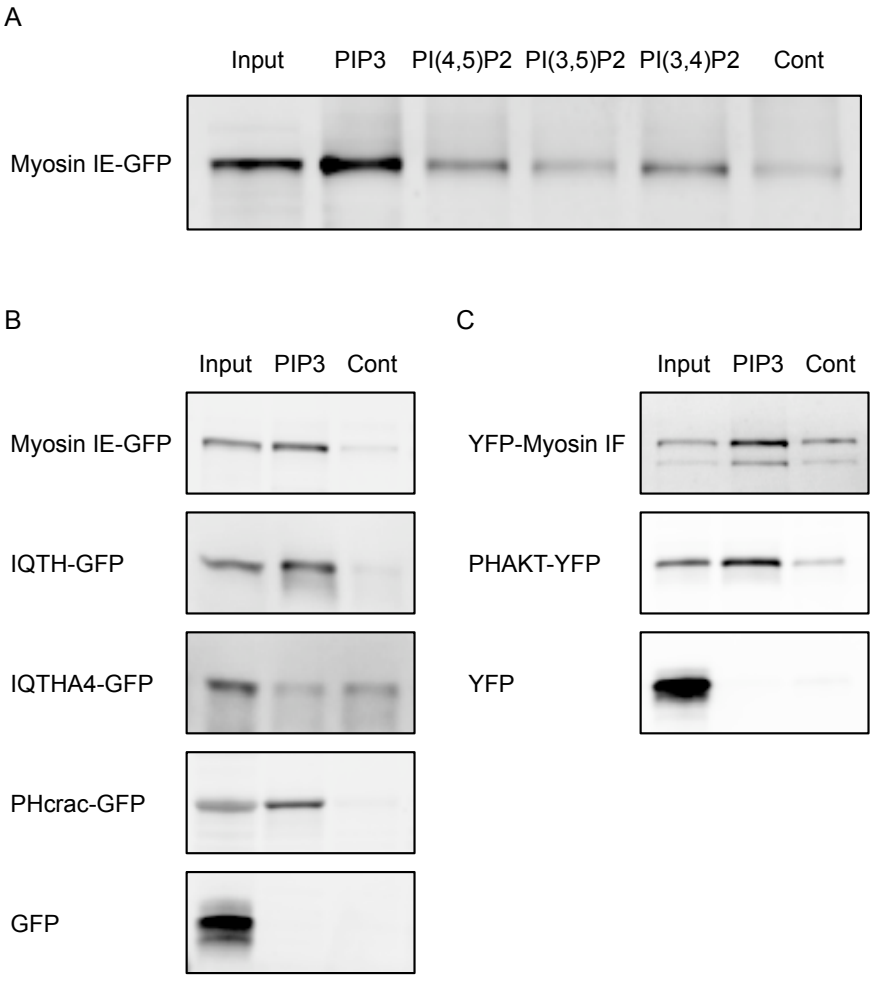

Figure S2. Liposome binding assays. (A) Liposomes containing the indicated phosphoinositides were incubated with *Dictyostelium* cell lysates expressing myosin IE-GFP. Input indicates five percent of lysates. Images are representative of at least two independent experiments. (B) *T*he indicated GFP fusion proteins were incubated with liposomes containing 0% (control) or 5% PIP_3. Bound fractions were analyzed by SDS-PAGE followed by immunoblotting using anti-GFP antibodies. Input indicates five percent of lysates. Images are representative of at least two independent experiments. (C) HEK293T cell lysates expressing the indicated YFP fusion proteins were mixed with PIP_3-containing or control liposomes. Input indicates five percent of lysates. Images are representative of three independent experiments.

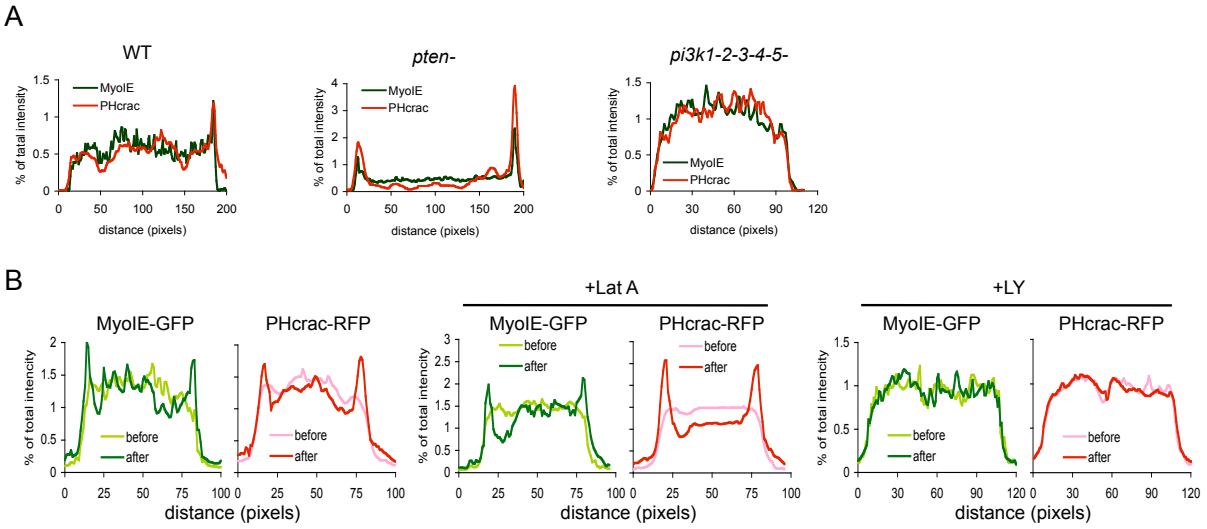

Figure S3. Quantification of myosin IE-GFP localization. (A and B) Fluorescence intensity was quantified along the lines shown in Fig. 1D (A) and E (B) using NIH Image J. In (A), protein localization was examined in 5 independent experiments. In each experiment, more than 10 cells were analyzed. 100% of cells showed the presented distribution for wild-type, *pten-* and *pi3k1-2-3-4-5-* cells. In (B), protein localization was examined in 2 independent experiments. In each experiment, more than 25 cells were analyzed. 100%, 96% and 100% of cells showed the presented distribution for no treatment, Lat A treatment, and LY treatment, respectively.

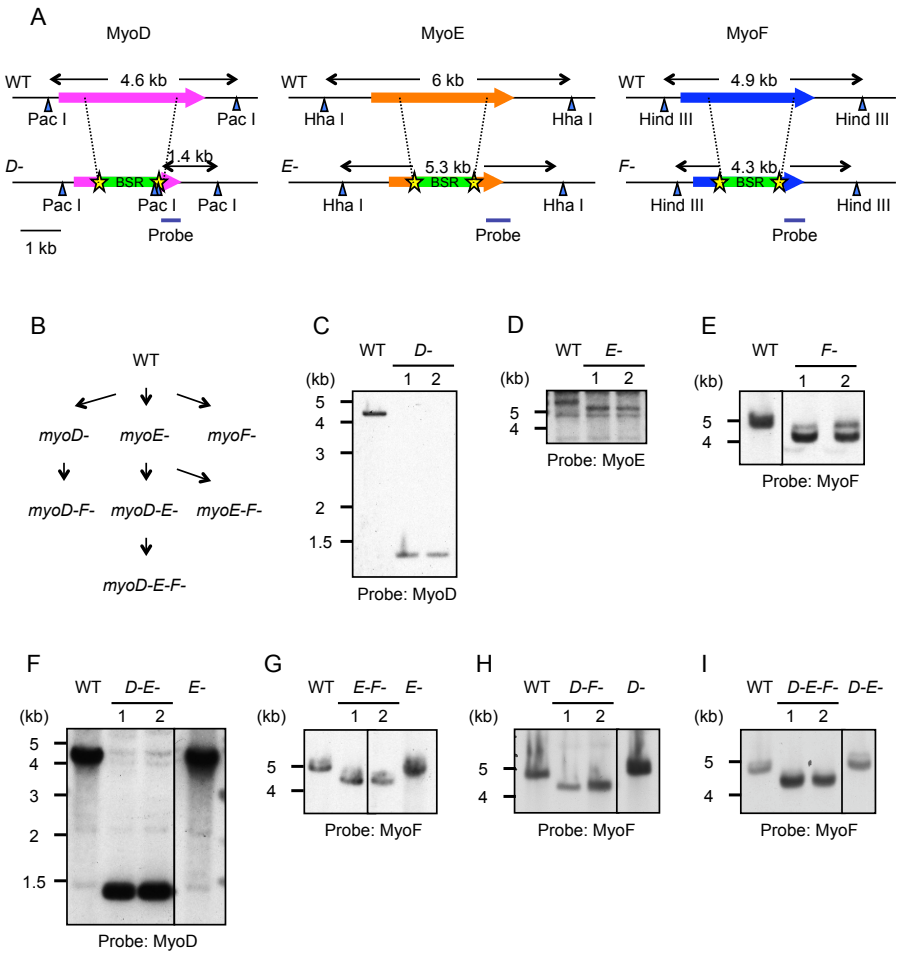

Figure S4. Generation of myosin I-null cells. (A) The blasticidin-S-resistance cassette, which carries the loxP site (indicated by stars), replaced the genes encoding myosin ID (*myoD*), IE (*myoE*), and IF (*myoF*). (B) For simultaneous deletion of these genes, the blasticidin-S-resistance cassette was removed by transforming cells with pDEX-NLS-cre, a plasmid that carries Cre recombinase fused to a nuclear localization signal (*1*). (C to I) Each gene disruption was confirmed by Southern blot analysis.

1. J. Faix, L. Kreppel, G. Shaulsky, M. Schleicher, A. R. Kimmel, A rapid and efficient method to generate multiple gene disruptions in Dictyostelium discoideum using a single selectable marker and the Cre-loxP system. *Nucleic acids research* **32**, e143 (2004).

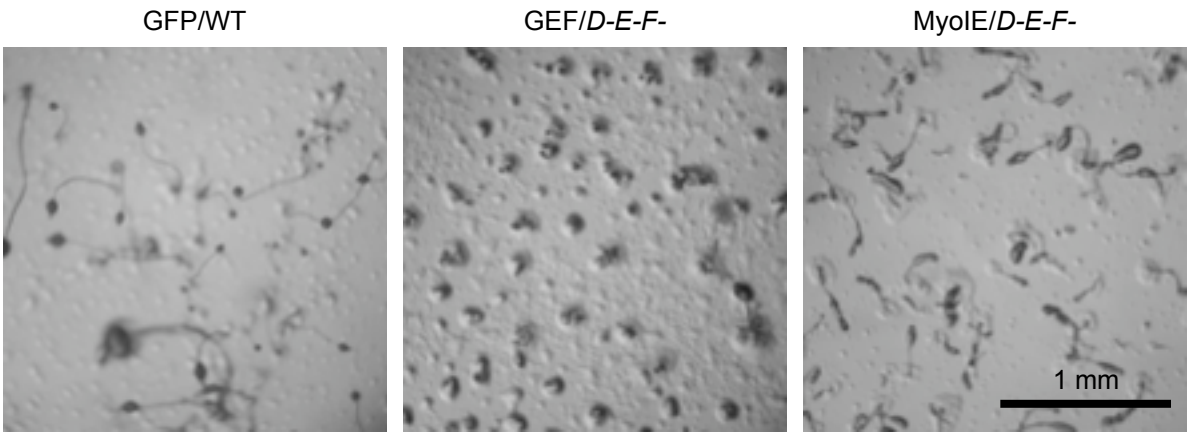

Figure S5. Myosin IE-GFP restores the formation of fruiting bodies in triple knockout cells. Wild-type cells expressing GFP, triple knockout cells expressing GFP, and triple knockout cells expressing myosin IE-GFP were cultured on non-nutrient agar for 24 hours to induce differentiation into fruiting bodies.

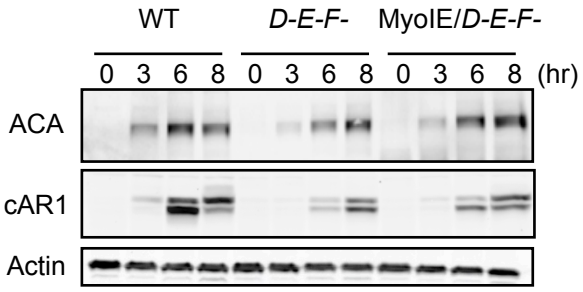

Figure S6. Expression of adenylate cyclase and cAMP receptor 1. Immunoblot analysis of the cAMP receptor (cAR1) and adenylate cyclase A (ACA) in wild-type and triple knockout cells differentiated for 5 hours. Actin was used as a loading control. Images are representative from five independent experiments.

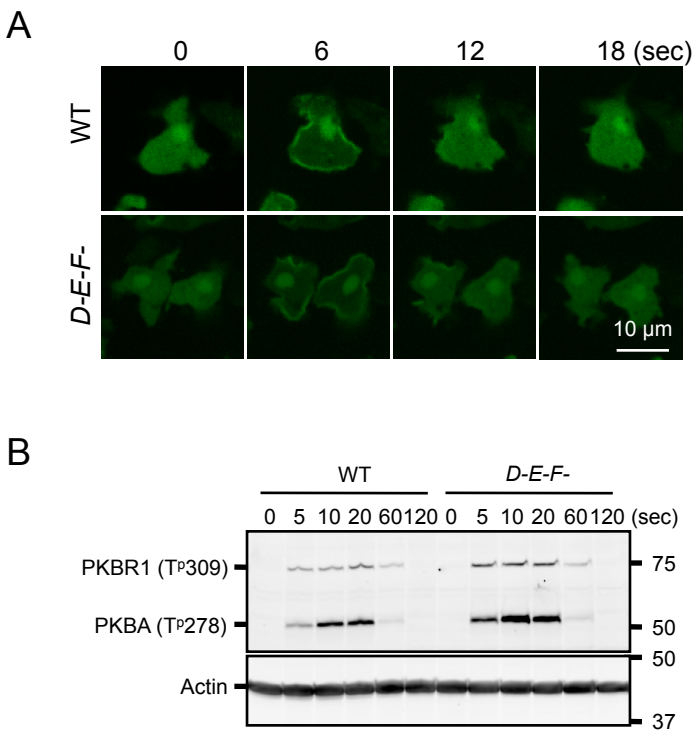

Figure S7. PIP_3 production and responses in triple knockout cells. (A) Wild-type and triple knockout cells expressing PHcrac-GFP were observed at the indicated time points after cAMP stimulation (1 μM). More than 50 cells analyzed in each experiment ($n = 4$). (B) Wild-type and triple knockout cells were developed for 5 hours. Cells were collected and whole cell lysates were analyzed by immunoblotting using antibodies against phosphorylated forms of PKBR1 and PKBA (2, 3) at the indicated time points after cAMP stimulation. Images are representative of four independent experiments.

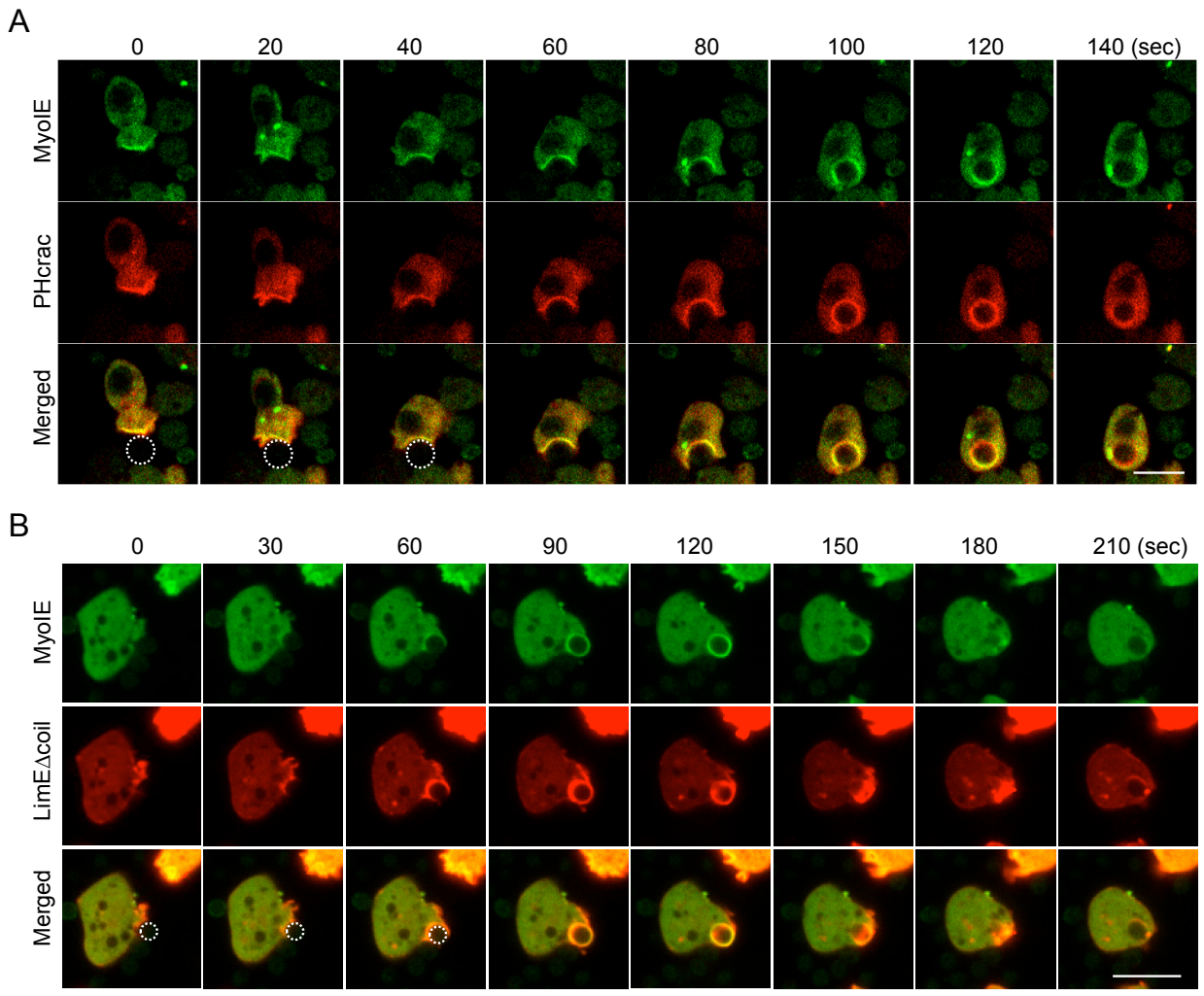

Figure S8. Myosin IE-GFP localization during phagocytosis. (A) Merged images of cells shown in Fig. 3E. (B) Wild-type cells expressing myosin IE-GFP and LimEΔcoil-RFP were examined during phagocytosis of yeast cells. Dotted circles indicate yeast cells in the first three time points.

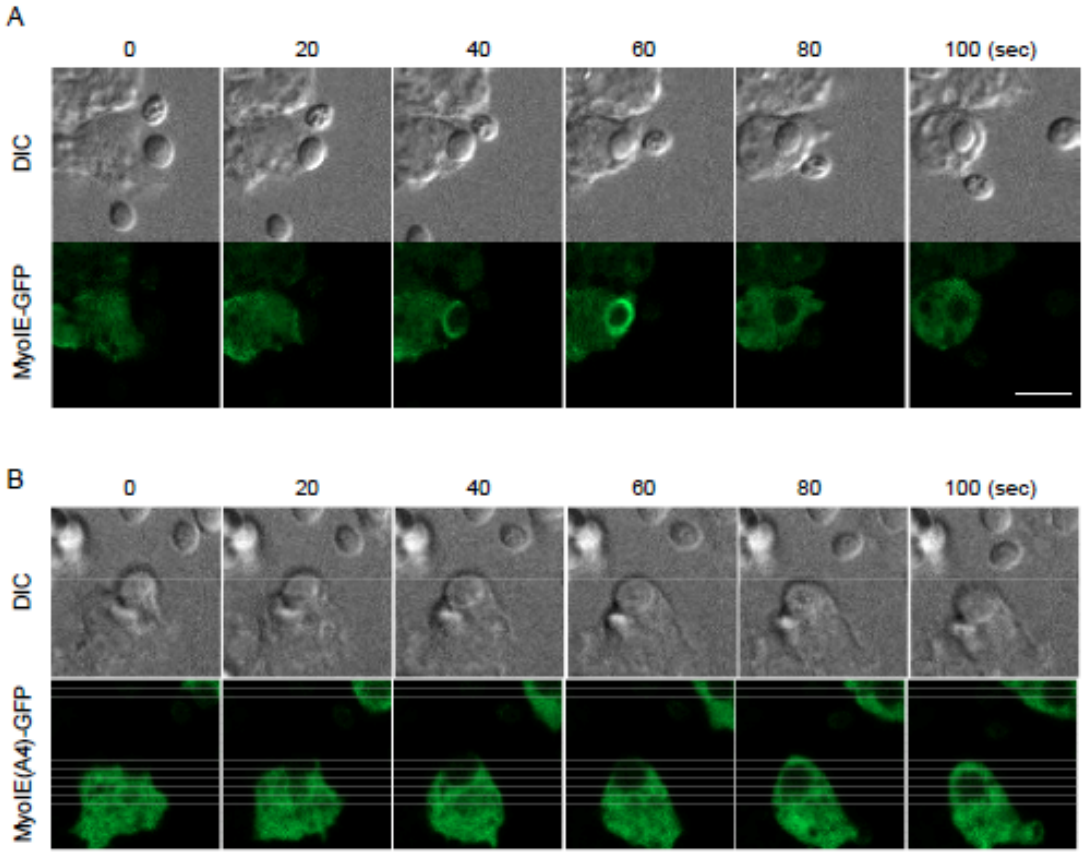

Figure S9. Myosin IE(A4)-GFP localization during phagocytosis. Triple knockout cells expressing wild-type myosin IE-GFP (A) or myosin IE(A4)-GFP were observed during phagocytosis of yeast cells. More than 10 cells were examined in each experiment ($n = 4$). Wild-type myosin IE-GFP, but not myosin IE(A4)-GFP, was transiently recruited to phagocytic cups.

Table S1. Primers used in this study

Primers for expression plasmids in *Dictyostelium* cells

MyoA5	cccagatctATGGCAACATTTAAAAGAGATTTAACTAAAAATGTTGG
MyoA2	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCTTTTTCAATTAAAATAGATTTTGATTCAGAAGAAAG ACCAGG
MyoB1	cccagatctATGTCAAAAAAAGTTCAAGCC
MyoB2	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCATTATATTGTAAATAATTTGTTGGAGCCCAACC
MyoC1	cccagatctATGGCACAACAAAAACCAGAATGG
MyoC2	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCAATTTGTTGAACATAATTTGAAGG
MyoD1	cccggatccATGGCATATAAAAGTCAACATGG
MyoD11	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCAACTCTTGGTGCCATTCCACCTCTTGGTGCC
MyoE1	cccagatctATGATTCCAAAGACAAAAGCAGAAGG
MyoE8	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCATCTTTAAATTGGATTGTTGCTTGG
MyoE9	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCTCTCATATGATTTTGACCGGCACCC
MyoE10	cccagatctatgCAAAAGGTTATGGCTTACGATATTTTCC
MyoE11	cccagatctatgCTTGAAATGCCAAGAATTGTAAC
MyoE12	cccagatctatgGAGTTACATCGTGCTTTTAAAGATG
MyoE21	GCAGTCAATCCAGCGGGTGTGCCACAAGCTGCTGG
MyoE22	CCAGCAGCTTGTGGCACACCCGCTGGATTGACTGC
MyoE23	CTATGGTTTTGCGGTTTTTCAAAATAATAGTTTCG
MyoE24	CGAAACTATTATTTTGAAAAACCGCAAAACCATAG
MyoF1	cccagatctATGGAACCACTTCCTTTAG
MyoF2	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCATCTAAATTATAATAAACAATTGC
MyoK1	cccggatccATGTTTCGTTTATTTTCATCAGG
MyoK2	cccctcgagACCACTACCACCACTACCACCACTACCACCACTACCTTGAATAAGACATTTTTGTTTCATTGAGTGTTTACC
PHcracRFP1	cccagatctATGGGGAAAACAGAGAGAAAGAAAGAGC
PHcracRFP2	CACCATACCACCACCTCTAATTCTTGCGATGAG
PHcracRFP3	ATTAGAGGTGGTGGTATGGTGAGCAAGGGCGAGG
PHcracRFP4	cccctcagaTTACTTGTACAGCTCGTCCATGC

Primers for expression plasmids in mammalian cells

HuMyoIF1	ccctgatcaATGGGCAGCAAGGAGCGCTTCC
HuMyoIF2	cccgtcgacACCACTACCACCACTACCACCACTACCACCACTACCGATCTTCTCCACGTAGTTTCCTGG
HuMyoIF3	CGGTCACCGCGTACGACCGCCGCTTCAAGCCCATCAAGGCGGACTTGATCC
HuMyoIF4	GGATCAAGTCCGCCTTGATGGGCTTGAAGCGGCGGTCGTACGCGGTGACCG

Primers for gene disruption in *Dictyostelium* cells

MyoDd1	cccgcggccgcAAATGGCATATAAAAGTCAACATGG
MyoDd2	ccccccgggTGCTTCCAACAATGGATTTGATTCC
MyoDd3	ccccccgggACACCACCAGAATCATTACCAGTCG
MyoDd4	cccgtcgacTTGGAGCAATACCACCTCTTGGAGC
MyoDd5	TTCTTTAAACTCTTGGTGCCATTCC
MyoE1	cccagatctATGATTCCAAAGACAAAAGCAGAAGG
MyoE2	cccgcggccgcATCACAGAAAATGCATTCATAGAG
MyoE3	ccccccgggGGTGAACCAACTGCATTGAATTGC
MyoE4	ccccccgggTATGGCTTACGATATTTTCCATGG
MyoE5	cccctcgagATCTTTAAATTGGATTGTTGCTTGG
MyoFd1	cccgcggccgcATGGAACCACTTCCTTTAGAAAATG
MyoFd2	ccccccgggTGTTTTAGCGTTACCAAATGATTCC
MyoFd3	ccccccgggCTAGAAAGAAAGAATGGGATTGTCG
MyoFd4	cccgtcgacTGGATATTGAGAGAAACTTACAACG
MyoFd5	AAACAATTGCAGTATTACCTTTACC
L-A15P-1	CCAACCCAAGTTTTTTTAAACC

Table S2. Plasmids used in this study

Plasmids	Primers used for construction				References
***Dictyostelium* plasmids**					
pJK1-GFP: pIS1					Zhang et al. (2011)
pIS1-MyoA	MyoA5	MyoA2			This study
pIS1-MyoB	MyoB1	MyoB2			This study
pIS1-MyoC	MyoC1	MyoC2			This study
pIS1-MyoD	MyoD1	MyoD11			This study
pIS1-MyoE	MyoE1	MyoE8			This study
pIS1-MyoE (1-798)	MyoE1	MyoE9			This study
pIS1-MyoE (694-1005)	MyoE11	MyoE8			This study
pIS1-MyoE (740-1005)	MyoE12	MyoE8			This study
pIS1-MyoE (799-1005)	MyoE10	MyoE8			This study
pIS1-MyoF	MyoF1	MyoF2			This study
pIS1-MyoK	MyoK1	MyoK2			This study
pIS1-MyoE (E391A)	MyoE1	MyoE8	MyoE23	MyoE24	This study
pIS1-MyoE (A4)	MyoE1	MyoE8	MyoE21	MyoE22	This study
pDM181-PHcrac mCherry	PHcracRFP1	PHcracRFP2	PHcracRFP3	PHcracRFP4	This study
pDM181-PHcrac-mCherry/MyoE-GFP					This study
pDEX-NLS-cre					Faix et al. (2004)
pJK1-PHcrac-GFP: pWF38					Dormann et al. (2002)
pDRH-LimEΔcoli-mRFP					A gift from Drs. P. Devreotes and J. Franca-Koh (JHMI)

Mammalian plasmids

pEYFP					Clontech
pEYFP-PHakt					A gift from Dr. T. Inoue (JHMI)
pEYFP-MyoIF	HuMyoIF1	HuMyoIF2			This study
pEYFP-MyoIF (K770A, R780A)	HuMyoIF1	HuMyoIF2	HuMyoIF3	HuMyoIF4	This study

Movie S1. Localization of myosin IE-GFP after cAMP stimulation. Wild-type cells expressing myosin IE-GFP and PHcrac-RFP were stimulated by cAMP (1 μM) at 6 s. The same cells are shown in Fig. 1E.

Movie S2. Localization of myosin IE-GFP after cAMP stimulation in the presence of latrunculin A. Wild-type cells expressing myosin IE-GFP and PHcrac-RFP were stimulated by cAMP (1 μM) at 6 s in the presence of 5 μM latrunculin A. The same cells are shown in Fig. 1E.

Movie S3. Localization of myosin IE-GFP after cAMP stimulation in the presence of LY294002. Wild-type cells expressing myosin IE-GFP and PHcrac-RFP were stimulated by cAMP (1 μM) at 6 s in presence of 20 μM LY294002. The same cells are shown in Fig. 1E.

Movie S4. Localization of myosin IE (E391A)-GFP and myosin IE (E391A)-GFP. Differentiated triple knockout cells expressing different myosin IE-GFP constructs were observed after cAMP stimulation. The same cells are shown in Fig. 2F.

Movie S5. Localization of PHcrac-GFP in wild-type and triple knockout cells after cAMP stimulation. Wild-type (WT) and triple knockout (D-E-F) cells expressing PHcrac-GFP were observed at the indicated time points after cAMP stimulation (1 μM).

Movie S6. Localization of myosin IE-GFP and PHcrac-RFP during phagocytosis. The same cell is shown in Fig. 3E and fig. S7.

Movie S7. Localization of myosin IE-GFP and LimEΔcoil-RFP during phagocytosis. The same cell is shown in Fig. S7.

Movie S8. Localization of LimEΔcoil-RFP during phagocytosis. Wild-type and triple knockout cells expressing LimEΔcoil-RFP were observed during phagocytosis of yeast cells. The same cell is shown in Fig. 3G.

Movie S9. Localization of myosin IE-GFP or myosin IE(A4)-GFP during phagocytosis. Triple knockout cells expressing wild-type myosin IE-GFP or myosin IE(A4)-GFP were observed during phagocytosis of yeast cells. The same cell is shown in fig. S8.

Movie S10. Localization of YFP-human myosin IF, PH_{AKT}-YFP, and YFP-human myosin IF (K770A, R780A) in COS-7 cells upon EGF treatment. EGF was added at 45 s. The same cells are shown in Fig. 4D.

Movie S11. Localization of YFP-human myosin IF and PH_{AKT}-YFP during EGF treatment in the presence of LY294002. EGF was added at 45 s. The same cells are shown in Fig. 4D.