SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

FIGURE S1**. Specificity of PIP2 antibody tested with lipid strips.** Specificity of PIP2 antibody was tested with lipid strips. The lipids tested (SIP, sphingosine 1-phosphate; PA, phosphatidic acid) and intensity of signals are as indicated. For more details and other lipids tested with negative results see Experimental Procedures.

FIGURE S2. **BH profiles of DMIB with mutated BH-site. (**A) Original and mutated sequences of BH-sites are shown at the top of each profile. Basic residues are in bold and mutated residues are underlined. Mutations are: dBH, deletion of the BH-site; BH-Ala, all basic residues in the BH-site replaced with Ala; I810D. The I810D mutation makes the BH-site of DMIB similar to the BH-site of *Acanthamoeba* MIA (AMIA) which does not bind acidic phospholipids (9). (B) BH values and BH peak areas of DMIB and the I810D mutant. BH values above 0.6 are in bold and underlined. The areas of peaks with heights above 0.6 are shown at the bottoms of the columns.

FIGURE S3. **Localization of DMIB-N154A and F-actin in waves.** Cells expressing DMIB-N154A (green) were starved for 5 h, fixed and F-actin was visualized with rhodamine-phalloidin (red). Three subsequent Z-slices are shown. Slice thickness is 0.75 μm and slice corresponding to the bottom of the cell is on the top of the figure. Bar is 10 μm.

SUPPLEMENTAL MOVIES

MOVIE S1. **Relocation of DMIB and F-actin to cell protrusions and cell-cell contacts.** AX3 cells were cotransfected with DMIB (green) and ABD-120 (red) fused to GFP and RFP, respectively. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 2.

MOVIE S2. **DMIB locates to cell protrusions in randomly moving cell.** GFP-fused DMIB was expressed in AX2 *myoB*$^-$-cells. Cells were starved for 2 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 5.

MOVIE S3. **Tail locates uniformly on plasma membrane in randomly moving cell.** GFP-fused Tail was expressed in AX2 *myoB*$^-$-cells. Cells were starved for 2 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 5.

MOVIE S4. **DMIB missing GPQ and SH3 domains (dGPQSH3) locates to cell-cell contacts.** GFP-fused DMIB missing GPQ and SH3 domains (dGPQSH3) was expressed in AX2 *myoB*$^-$-cells. Cells were starved for 1h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 5.

MOVIE S5. **Head+IQ localizes in diffused fashion at the front of migrating cell.** GFP-fused Head+IQ was expressed in AX2 *myoB*$^-$-cells. Cells were starved for 6 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 6.

MOVIE S6. **Tail localizes on plasma membrane in migrating cells.** GFP-fused Tail was expressed in AX2 *myoB*$^-$-cells. Cells were starved for 6 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 6.

MOVIE S7. **PH-PLCδ localizes on plasma membrane in migrating cells.** GFP-fused PH-PLCδ was expressed in AX2 *myoB*$^-$-cells. Cells were starved for 6 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 6.

MOVIE S8. **Tail localizes on plasma membrane in streaming cells.** GFP-fused Tail was expressed in AX2 *myoB*$^{-}$-cells. Cells were starved for 8 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 7.

MOVIE S9. **PH-PLCδ localizes to plasma membrane in streaming cells.** GFP-fused PH-PLCδ was expressed in AX2 *myoB*$^{-}$-cells. Cells were starved for 8 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 7.

MOVIE S10. **DMIB missing GPQ and SH3 domains (dGPQSH3) locates to mouth in streaming cells.** GFP-fused DMIB missing GPQ and SH3 domains (dGPQSH3) was expressed in AX2 *myoB*$^{-}$-cells. Cells were starved for 8 h. Note that the cell on the top that does not come in contact with other cells shows intense diffuse fluorescence at the front. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 7.

MOVIE S11. **Head+IQ localizes in cytoplasm in streaming cells.** GFP-fused Head+IQ was expressed in AX2 *myoB*$^{-}$-cells. Cells were starved for 8 h. Frames were taken every 20 s and movie is speeded up 60 times. See Fig. 7.

MOVIE S12. **MIB-N154A localizes to actin waves.** GFP-fused MIB-N154A was expressed in AX2 *myoB*$^{\bar{}}$cells. Cells were starved for 5h. Frames were taken every 10 s and movie is speeded up 30 times. See Fig. 9 and Fig. S3.

MOVIE S13. **MIB-N154A localizes to actin waves.** GFP-fused MIB-N154A was expressed in AX2 myoB$^{\bar{}}$cells. Note transient wave location at the cell periphery. Movie is speeded up 30 times. See Fig. 9 and Fig. S3.

TABLE S1
Oligonucleotides used for creating the GFP-DMIB expression plasmids

Mutation	Description	Oligonucleotides (name, sequence 5' to 3')
None	DMIB wild type	myb2 ccgaattcATGtcaaaaaaagttcaagcc
dSH3	ends at aa 1058	myb60 agatctATGtcaaaaaaagttcaagcc myb22 gggggatccTTAtgcagttggtcttgatgg
dGPQ	deletes aa 922-1057	myb67/68 gttcaactgattcaactgcaaaagcactctacg
dGPQSH3	ends at aa 921	mybrmem cctctagaTTAagttgaatcagttgaac
Head + IQ	ends at aa 720	myb69 ctcgagTTAttgagctctttgttcc
Tail	aa 713 to end	myb71 ggatccCATtctttggaacaaagagc myb32 cgggatccTTAattatattgtaaataatt
Tail + IQ	aa 698 to end	myb70 ggatccACTgctaaaattcaaaaagc myb32 cgggatccTTAattatattgtaaataatt
dBH	deletes BH region: aa 801-812	myb61/62 gttgagaaatcaatagttggtttacgtgaaattaaagg
BH-Ala	KKK803AAA; RR812 AA replaces 5 basic residues in BH region with A	myb65/66 **gctgcagca**gttttggttcatactttgatt**gcagca**gttggtttacgtgaaattaaagg
I810D	mutation of BH region	myb72/73 gaaagttttggttcatactttg**gac**cgtcgtgttggtttacgtg
S332A	weakens motor activity	myb9/10 ggtaatcgtcgt**gca**acctataacg
E407K	weakens actin binding	myb78/79 gtatcaattttgtaaat**aaa**aagcttcaacaattctttattg

Note that all expression plasmids that include the motor were generated using myb60 as the forward primer, except for the wild type plasmid that was generated using a myb2 PCR product. All full-length plasmids include the native 3' end of the *myoB* gene. Nucleotides indicated in bold encode the altered amino acids, nucleotides in capital letters are either the first amino acid after the restriction site used for cloning or stop codon. Oligonucleotide names with two numbers separated by / indicate forward and reverse primer pairs.

Fig S1


SIP
46.99
PI(3.4)P2
0.23
PI(3,5)P2
13.16
PI(4,5)P2
8.23
PI(3,4,5)P3
PA
PS
Blanc

Fig S2A

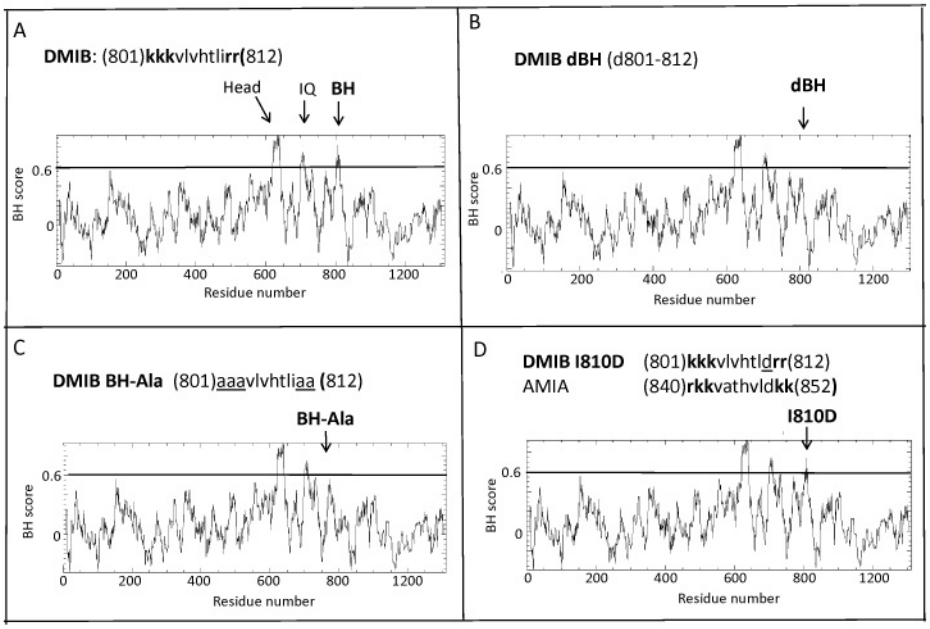
A
DMIB: (801)kkkvlvhtlirr(812)
Head
IQ
BH
BH score
0.6
0
0
200
400
600
800
1200
Residue number
B
DMIB dBH (d801-812)
dBH
BH score
0.6
0
0
200
400
600
800
1200
Residue number
C
DMIB BH-Ala (801)aaavlvhtliaa (812)
BH-Ala
BH score
0.6
0
0
200
400
600
800
1200
Residue number
D
DMIB I810D (801)kkkvlvhtldrr(812)
AMIA (840)rkkvathvldkk(852)
I810D
BH score
0.6
0
0
200
400
600
800
1200
Residue number

Fig S2B

DMIB		DMIB I810D	
aar	BH value	aar	BH value
801k	0.502	801k	0.421
802k	**0.616**	802k	0.535
803k	**0.692**	803k	0.611
804v	**0.638**	804v	0.557
805l	0.578	805l	0.497
806v	**0.612**	806v	0.531
807h	**0.823**	807h	**0.742**
808t	**0.612**	808t	0.531
809l	**0.635**	809l	0.554
810i	**0.724**	810d	**0.643**
811r	**0.618**	811r	0.537
812r	0.509	812r	0.428
Peak area (above 0.6)	**0.57**		**0.187**

Fig S3