Supplemental Materials

Molecular Biology of the Cell

Brzeska et al.

<u>Supplemental Materials</u> for Brzeska et. al. "Basic-hydrophobic (BH) sites are localized in conserved positions inside and outside of PH domains and affect localization of *Dictyostelium* myosin 1s"

Plasmid	Expressed Protein	Base Clone	Mutation	Template	Primers
pDTc25	GFP-Myo1C	pDTc24	none	AX2 genomic DNA	myoC26 gccgaattcatgagatctGCACAACAAAAACCAGAATGG myoC27 ctcgagTTAAATTTGTTGAACATAATTTG
pDTc31	GFP-Myo1C-BH-N-Ala	pDTc28	R776A, R783A, K784K	pDTc24 base clone	myoC30n TAGCGTTgcagcaTACTTTGGTGACTATTTAGAC myoC31 ATCATAGAGAAtgcATTTCTCTCTTTACGACC
pDTc27	GFP-Myo1C-BH-Ala	pDTc26	K838A, K845A, K847A	pDTc24 base clone	myoC28 TTAATTgcaTTGgcaCAAAAGAAGAATTTAGCTACC myoC29 ATAGATGGCTTGtgcAGTTACAATGAGGAATCTT
pDTc32	GFP-Myo1C-2BH-Ala	pDTc29	R776A, R783A, K784K, K838A, K845A, K847A	pDTc26 base clone	myoC30n TAGCGTTgcagcaTACTTTGGTGACTATTTAGAC myoC31 ATCATAGAGAAtgcATTTCTCTCTTTACGACC
pDTd6	GFP-Myo1D	pDTd5	none	AX2 genomic DNA	myd1 ggatccGCATATAAAAGTCAACATGGTG myd2 gctagctcgagTTAAACTCTTGGTGCCATTCC
pDTd8	GFP-Myo1D-BH-Ala	pDTd7	KR804/805AA, KR812/813AA	pDTd5	myd8 TACGTTCAAgcagcaAGACTTTTATTGGCTGGTATC myd9 AATCCAAGGtgctgcTTTACGATCTTCTTTATCCTTATTC
pDTe10	GFP-Myo1E	pDTe7	none	AX2 genomic DNA	mye5 ggatccATTCCAAAGACAAAAGCAG mye6 ctcgagTAATCTTTAAATTGGATTGTTGCTTG
pDTe17	GFP-Myo1E-BH-N-Ala	pDTe12	KKK808/809/810AAA	pDTe7	mye12 agcaTGGGACTTCCGTCGTCAT mye13 gcagcACCATGGAAAATATCGTAAGC
pDTf21*	GFP-Myo1F	pDTf20*	none	AX2 genomic DNA	
pDTf28	GFP-MyoF-BH-N-Ala	pDTf25	RKK871/872/873AAA , RRK878/879/880AAA	pDTf20	myf15 GATTGTgctgcagctTTCCAAGCTGATTATCTATCTG myf16 CCATTCtgcagctgcAGAATAACCCATACCAAACAAATC
pDTf29	GFP-MyoF-BH-Ala	pDTf26	KK946/947AA, KK951/952AA	pDTf20	myf17 TCAagcagcAGTGGGTCTTAAACTTCAC myf18 GTATAtgctgcAGTATCGTATTTGTAGATATGTTG
pDTb151	GFP-Myo1B-BH-Myo1B	pDTb92#	KKKVLVHTLIRR/RK KRPWIYVQKRR	pDTb2&	myb18 ggctctagaccatggtcaatcgatagaaagttcact myb81 TCTTCTCTTTTGAACGTAAATCCAAGGTCTCTTTTTAC Gtattgatttctcaacaaagtagag myb80 CGTAAAAAGAGACCTTGGATTTACGTTCAAAAGAGAA GAgttggtttacgtgaaattaaagg mybRMEM cctctagattaagttgaatcagttgaac

Table S1. Plasmids and oligonucleotides used for cloning of wild type and mutant myosin 1s. The base clone for each construct carries the full myosin 1 gene (including introns) in a standard cloning vector. Alterations to the BH sequences were made by mutagenizing a starting base clone using the listed primers and then cloning the final mutant full-length gene into a GFP-expression plasmid. In all cases except GFP-Myo1B-BH-Myo1D lowercase letters indicate added restriction enzyme sites or mutagenic sequences, underlined nucleotides indicate the stop codon. In case of GFP-Myo1B-BH-Myo1D the lower case letters indicate myo1B sequence and capital letters indicate myo1D sequence and two pairs of oligos that were used for overlapping PCR done to make the plasmid are shown. Several of the plasmids have been described previously: * (1), # (2) and & (3)

Figure legends

Figure S1. BH plots of full length *Dictyostelium* myosin 1s. BH plots were created by BH search (4) of *Dictyostelium* myosin 1s sequences from Cymobase (5,6) with window 19 (default settings). The arrows point to BH peaks located within PH domains (BH), N-terminal to PH domain (BH-N) and within neck (Neck). BH peaks within neck overlap with light chain binding sites.

Figure S2. BH plots of full length human myosin 1s. BH plots were created by BH search (4) of human myosin 1s sequences from Cymobase (5,6) with window 19 (default settings). The arrows point to BH peaks located within PH domains (BH), N-terminal or C-terminal to PH domain (BH-N and BH-C) and within neck (Neck).

Figure S3. Myosin 1s with BH sites at the C-terminus of neck region. BH sites identified in diverse myosin 1s sequences from Cymobase (5,6) as defined by BH search (4) are boxed. Secondary structures are marked based on the crystal structure of HsMyo1C (7) the sequence of which is included in the alignment for reference. BH sites in positions homologous to BH sites of HsMyo1A and HsMyo1B lie in the vicinity of the α3 helix that is located at the C-terminus of neck region. Some of the BH sites in positions homologous to the BH site of DdMyo1E that lie at the N-terminus of the TH1 domain are also shown for comparison (see Fig. 2B). Alignment was done with MUSCLE (8). Sequences from the following species, abbreviated as in Cymobase (5,6) are presented: mosquito (Aea – *Aedes aegypti*), bumble bee (Boi - *Bombus impatiens*), single-celled eukaryote (Co - *Capsaspora owczarzaki*), water flea (Dap - *Daphnia pulex*), tsetse fly (Gom - *Glossina morsitans morsitans*), human (Hs – *homo sapiens*), human body louse (Pdc - *Pediculus humanus corporis*), tilapia (Orn - *Oreochromis niloticus*), fire ant (Siu - *Solenopsis invicta*) and smooth cauliflower coral (Syp - *Stylophora pistillata*).

Figure S4. Positions of β3 and β4 in *Dictyostelium* myosin 1s according to Phyre2 sequence alignments. Positions of β3 and β4 in Phyre2 (9) alignments used for creating 3D models shown in Fig. 3 are marked as red arrows. Positions of β3 and β4 in HsMyo1C are marked as blue arrows. BH sites are boxed.

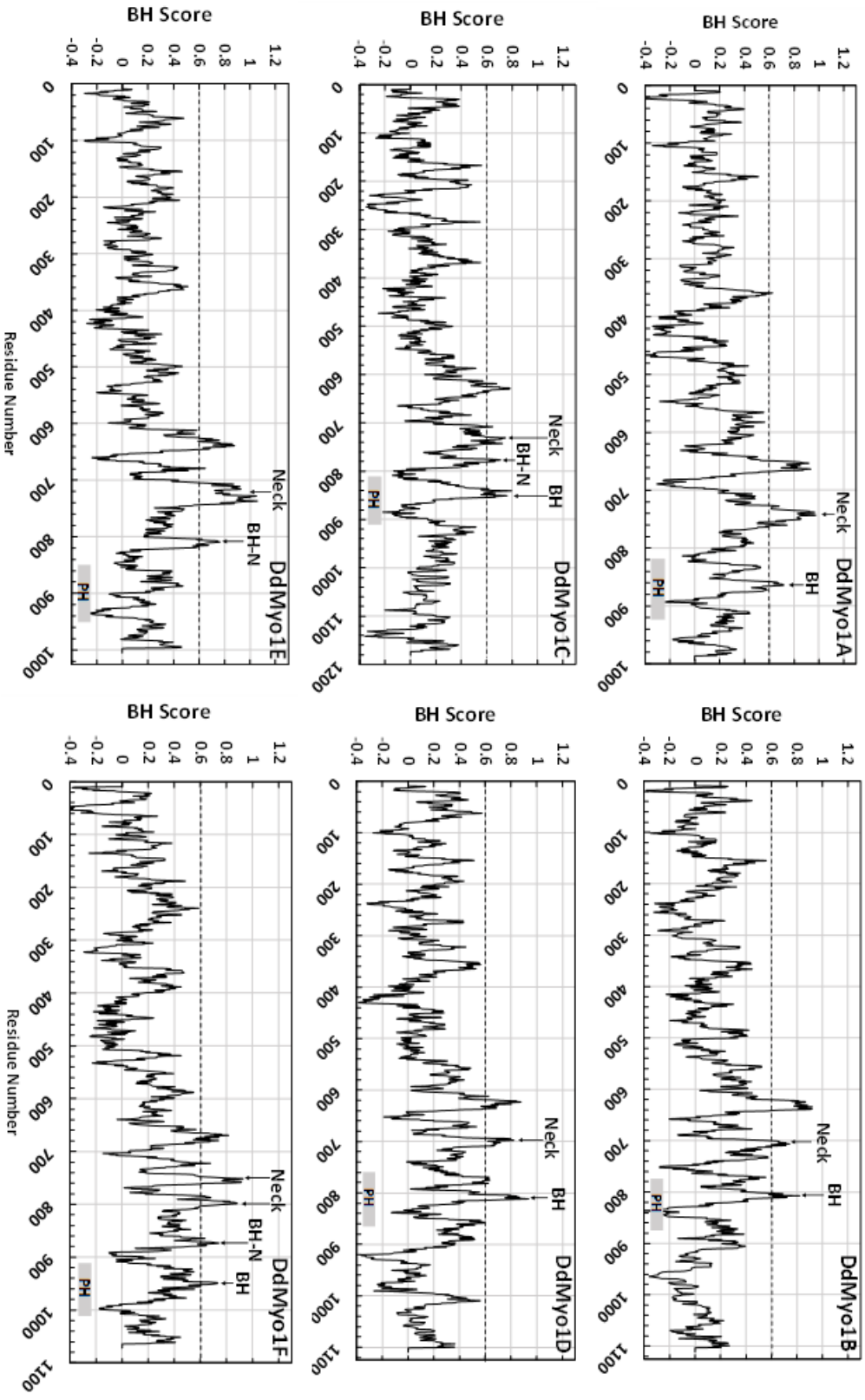
Fig S5. Immunoblotting GFP-myosin 1s strains. Immunoblot of total cell lysates from each of the strains expressing GFP-myosin 1 was probed with anti-GFP and anti-actin antibodies. The positions of molecular weight standards (kD) are indicated on the left of each blot panel. Myo1D line was blotted twice.

Fig S6. GFP fluorescence intensities in individual cells expressing *Dictyostelium* GFP-myosin 1s and their mutants. The average fluorescence intensity of individual cells expressing either wild type or mutant Myo1s was measured from confocal microscopy images. Fluorescence intensities of all cells in randomly chosen fields were measured. Each panel compares the intensities of GFP fluorescence of cells expressing mutant protein with that of cells expressing wild type myosin that were observed in the same experiment. The data are reported as histogram. The fluorescence is in relative units where value "1" corresponds to the average fluorescence intensity of the wild type myosin 1 measured in the same experiment. Note that although the fluorescence of individual cells varies, in each experiment there was always a population of cells expressing mutant and wild type proteins that show similar fluorescence intensity (for example look at the 0.5-1.0 and 1.0-1.5 columns). This allowed for comparison of

localization of wild type and mutant proteins in cells with similar expression levels. The number of cells scanned for each protein (N) varied between 69 and 225 and is indicated in the figure.

Figure S7. Myosin 1s localization during closure of macropinocytic vesicles. (A) Myo1C localizes sharply to the plasma membrane at the site of previous vesicle closure where a new cup is forming. (B) Time course of vesicle closure monitored with Myo1F and F-actin. Myo1F is present at the site of vesicle closure prior to the localization of actin. (6 s, 12 s). Note that Myo1F does not localize to actin-enriched cup edges (0 s). Arrows point to regions of interest. Experiments were done in AX2 cells (A) and Myo1B null cells (B). Bars are 10 μm.

Fig. S1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DdMyo1C
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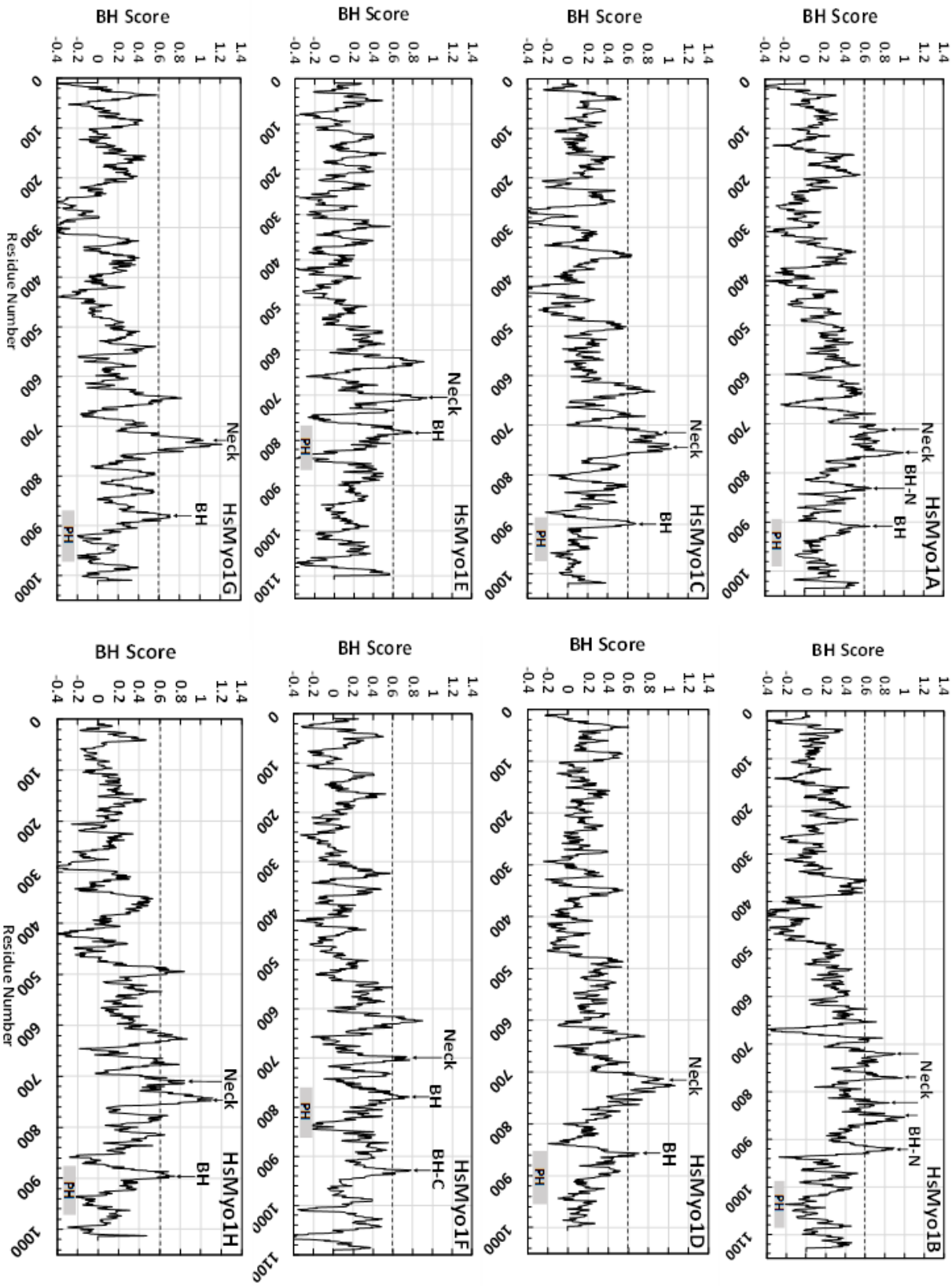
Neck
BH
BH-N
PH
BH Score
Residue Number

Fig. S2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Residue Number
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HsMyo1C
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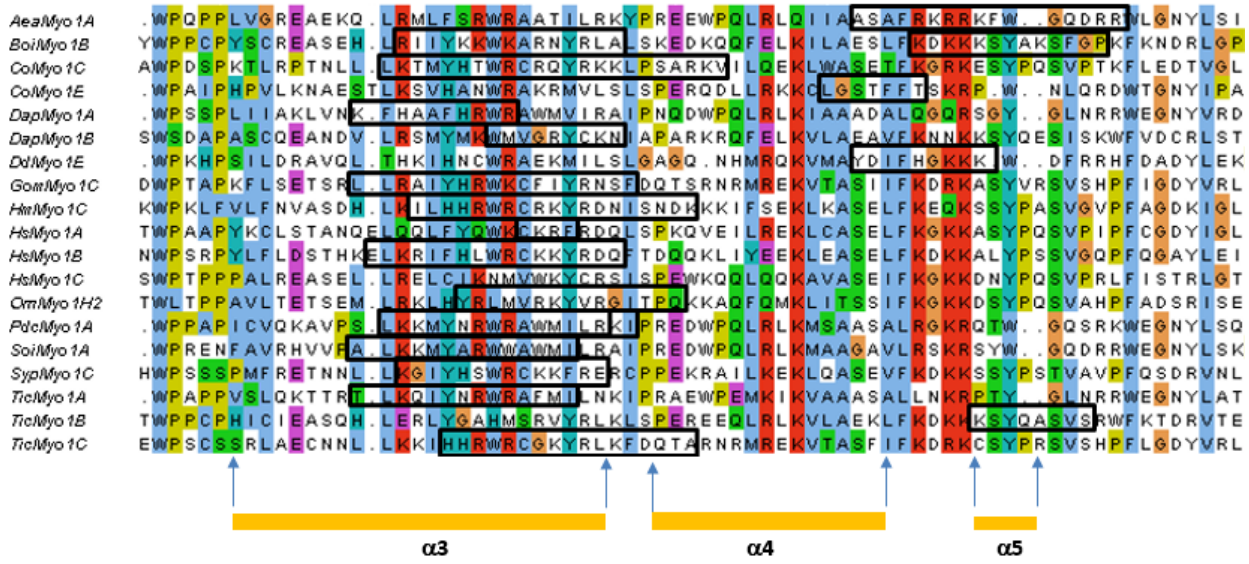
Neck
BH-N
BH
BH-C
PH

Fig. S3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HmMyo 1C
HsMyo 1A
HsMyo 1B
HsMyo 1C
OmMyo 1H2
PdcMyo 1A
SoiMyo 1A
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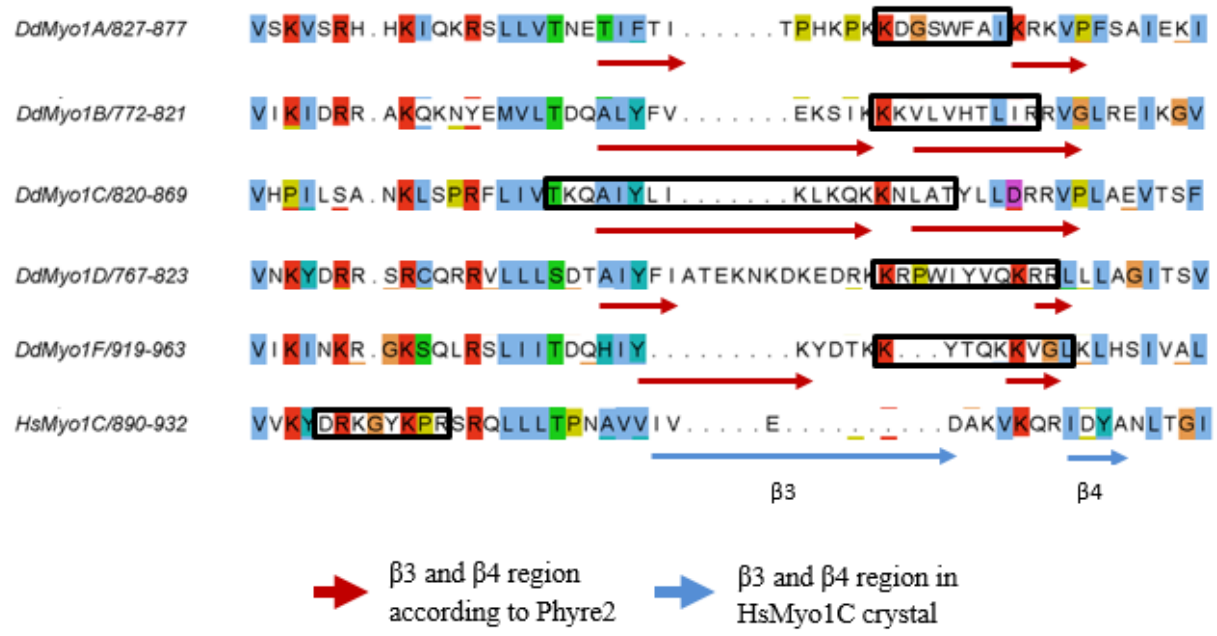
TicMyo 1B
TicMyo 1C
α3
α4
α5

Fig. S4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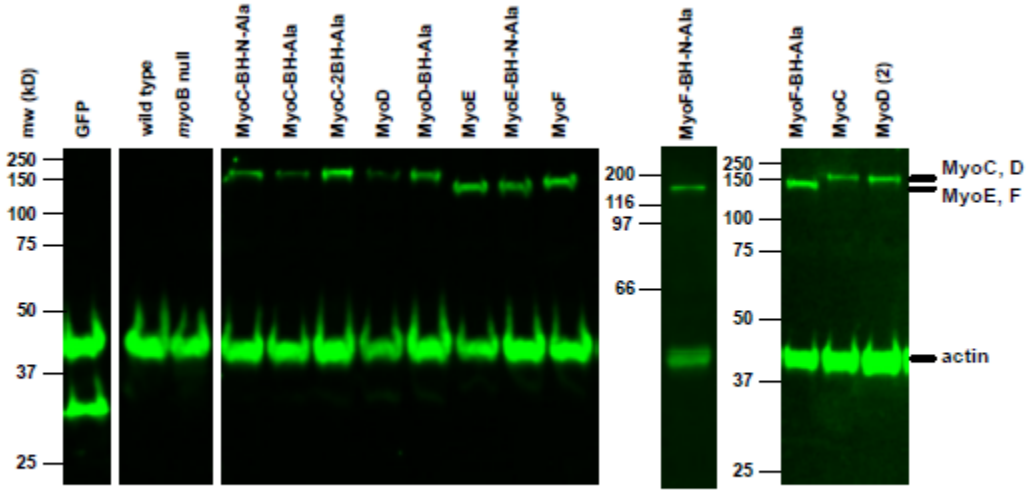
DdMyo1D/767-823
DdMyo1F/919-963
HsMyo1C/890-932
β3
β4
β3 and β4 region according to Phyre2
β3 and β4 region in HsMyo1C crystal

Fig S5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myoB null
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MyoD
MyoD-BH-Ala
MyoE
MyoE-BH-N-Ala
MyoF
MyoF-BH-N-Ala
MyoF-BH-Ala
MyoC
MyoD (2)
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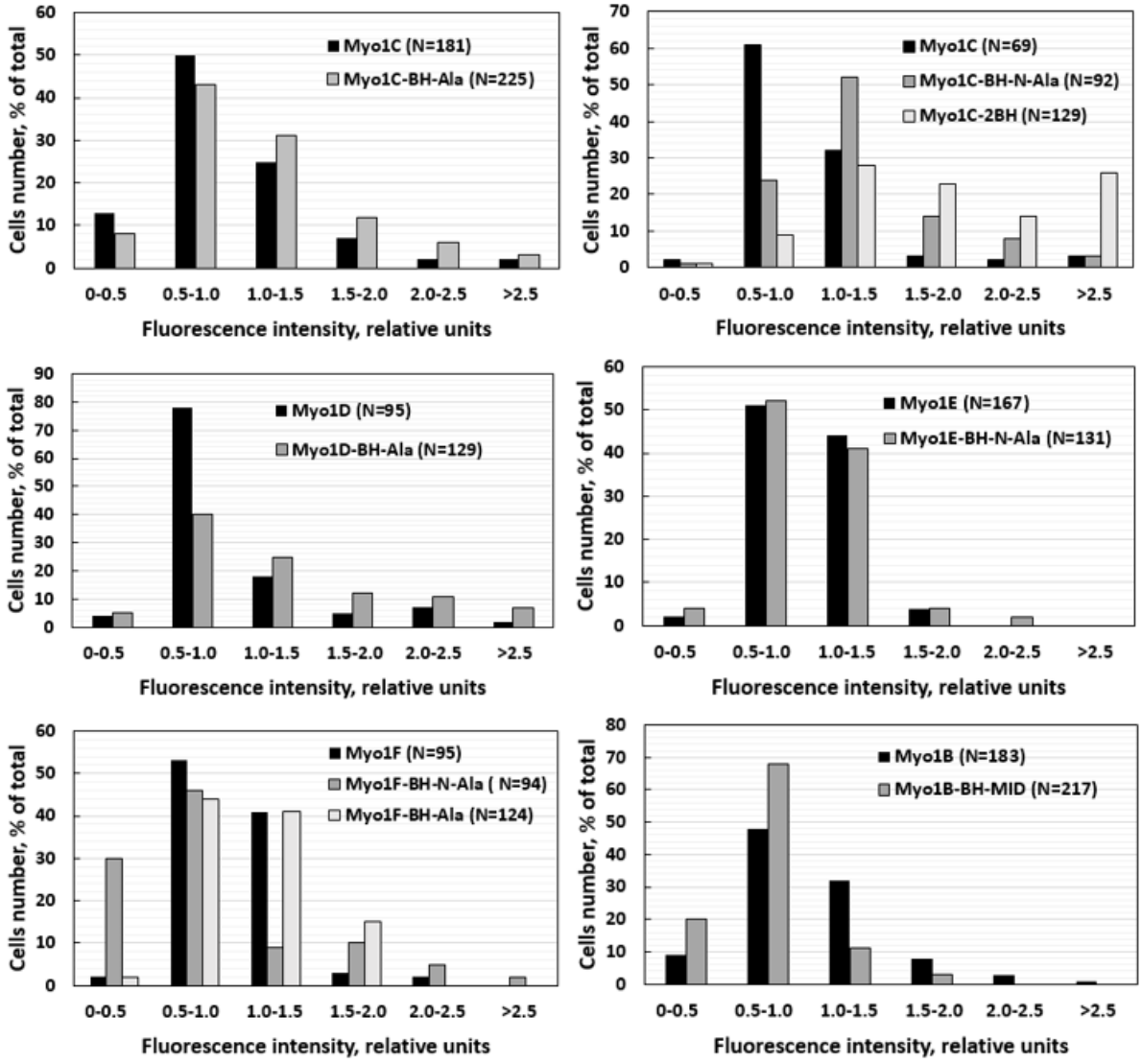
150
100
75
50
37
25
200
116
97
66
MyoC, D
MyoE, F
actin

Fig S6


Cells number, % of total
Fluorescence intensity, relative units
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0.5-1.0
1.0-1.5
1.5-2.0
2.0-2.5
>2.5
Myo1C (N=181)
Myo1C-BH-Ala (N=225)
Myo1C (N=69)
Myo1C-BH-N-Ala (N=92)
Myo1C-2BH (N=129)
Myo1D (N=95)
Myo1D-BH-Ala (N=129)
Myo1E (N=167)
Myo1E-BH-N-Ala (N=131)
Myo1F (N=95)
Myo1F-BH-N-Ala (N=94)
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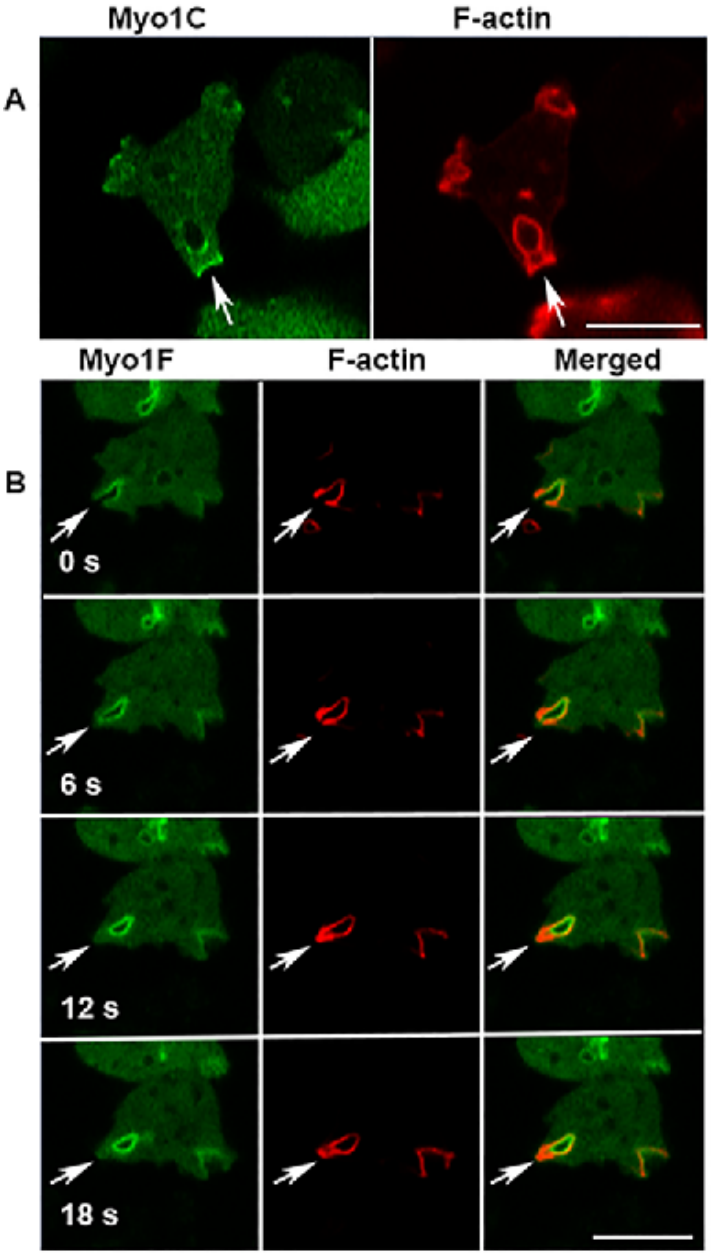
Fig. S7



References

1. Brzeska, H., Koech, H., Pridham, K. J., Korn, E. D., and Titus, M. A. (2016) Selective localization of myosin-I proteins in macropinosomes and actin waves. *Cytoskeleton (Hoboken, N.J.)* **73**, 68-82
2. Brzeska, H., Guag, J., Preston, G. M., Titus, M. A., and Korn, E. D. (2012) Molecular basis of dynamic relocalization of Dictyostelium myosin IB. *J Biol Chem* **287**, 14923-14936
3. Novak, K. D., Peterson, M. D., Reedy, M. C., and Titus, M. A. (1995) Dictyostelium myosin I double mutants exhibit conditional defects in pinocytosis. *J Cell Biol* **131**, 1205-1221
4. Brzeska, H., Guag, J., Remmert, K., Chacko, S., and Korn, E. D. (2010) An experimentally based computer search identifies unstructured membrane-binding sites in proteins: application to class I myosins, PAKS, and CARMIL. *J Biol Chem* **285**, 5738-5747
5. Kollmar, M., and Muhlhausen, S. (2017) Myosin repertoire expansion coincides with eukaryotic diversification in the Mesoproterozoic era. *BMC evolutionary biology* **17**, 211
6. Odronitz, F., and Kollmar, M. (2006) Pfarao: a web application for protein family analysis customized for cytoskeletal and motor proteins (CyMoBase). *BMC genomics* **7**, 300
7. Lu, Q., Li, J., Ye, F., and Zhang, M. (2015) Structure of myosin-1c tail bound to calmodulin provides insights into calcium-mediated conformational coupling. *Nature structural & molecular biology* **22**, 81-88
8. Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-1797
9. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. E. (2015) The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols* **10**, 845