Supplementary Information for

Optimized filopodia formation requires myosin tail domain cooperation

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Materials and Methods

Plasmid design: Expression constructs were generated using a combination of standard ligation cloning, PCR cloning (StrataClone, Agilent), Q5 mutagenesis (New England Biolabs) and Gibson Assembly (New England Biolabs) (see Supp. Table 1). DNA modification and restriction enzymes were obtained from New England Biolabs and all PCR-generated DNAs were verified by Sanger sequencing (Biomedical Genomics Center, UMN). The integrating GFP-DdMyo7 expression plasmid pDTi74, KKAA autoinhibition mutant plasmid pDTi321 and ΔPro2-MF2 mutant plasmid pDTi357 have been described (1, 2). Extrachromosomal *Dictyostelium* expression plasmids were made with pTX-GFP (3). Fragments for expression in bacteria were cloned into either pET-23a (Novagen) or a custom modified pET-14 plasmid (Novagen) that lacks the thrombin cleavage site and instead has a TEV protease site.

Cell culture and transformations: Cells were cultured in HL5 (Formedium) at 22°C on bacteriological plastic. Media was supplemented with 60U/mL penicillin and 60μg/mL streptomycin sulfate (Sigma). Cells were transformed with expression plasmids as described (4) and transgenic cells were selected with either Neomycin sulfate (10 μg/mL) or Hygromycin (35 μg/mL) (Gold Biotechnology). Transformation plates were screened for fluorescence, individual colonies picked, grown to confluency, cell lysates prepared as previously described (5) and protein expression verified by western blotting. Blots were probed with either a mouse monoclonal anti-GFP antibody (MMS-118P clone B34; BioLegend Inc. catalog no. 902605) or a rabbit polyclonal antibody specific for the DdMyo7 heavy chain directed against aa 809 - 901 (UMN87 (6)), and the heavy chain of MyoB (7), a class I myosin, as a loading control. Detection was performed with either Alexa Fluor 680- or 800-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies (Invitrogen) using an Odyssey infrared imaging system (LI-COR Biosciences).

Live Cell Imaging: Confocal microscopy was performed on an AxioObserver Z.1 stand (ZEISS) with Plan-Apochromat 63×/1.4 NA oil objective (ZEISS), a Yokogawa CSU-X1 spinning disk, and Photometrics Evolve EMCCD camera, and laser stack with two 50 mW solid-state lasers (488 nm and 561 nm). The microscope is run by SlideBook software (3i - intelligent imaging innovations). Z stack, time-lapse images were captured 1 - 2 μm from the cover glass to image

the substrate adhered region with the most filopodia. Images are collected for 30 sec - 1 min to avoid photo-toxicity at a frame rate of 1 or 4 Hz.

Fixed cells: Cells were fixed with picric acid (8) and stained with Alexa647 fluorescent phalloidin and DAPI (Thermo Fisher). Slides were mounted in prolong diamond (Invitrogen) and imaged on Eclipse Ni-E microscope with a 63×/1.40 NA oil immersion objective (Nikon), a SOLA solid state white-light excitation subsystem (Lumencor), and a CoolSNAP ES2 CCD (Photometrics) run by Nikon Elements software.

Protein Expression and Purification: The 6xHis fusion proteins were expressed in either BL21-AI, BL21 (DE3) Rosetta (Invitrogen) or BL21-Gold (DE3) (Agilent) *Escherichia coli* at 20°C after induction with 0.2 mM IPTG with or without 0.2% L-arabinose, accordingly. Cells were collected by centrifugation, frozen in liquid N_2 and stored at -80°C. Frozen cells were thawed and lysed using a TS cell disruptor (Celld). The soluble fraction of the lysate was applied to a Histrap FF crude column (GE Healthcare) and the His fusion proteins were eluted with 20 mM Tris, pH 7.5, 300 mM NaCl, 200 mM imidazole or 20 mM HEPES, pH 7.5, 300 mM NaCl, 1 mM DTT, 200 mM Imidazole, pH 7.5. The proteins were further purified by ion exchange chromatography on a HiScreen CaptoQ column (GE Healthcare) and gel filtration on a Superdex 200 or Superdex 75 column (GE Healthcare) in a final buffer containing 10mM Tris pH 7.5; 50 mM NaCl or 20 mM Na-Phosphate, pH 7.5, 100 mM NaCl, 1 mM DTT. The final pool was concentrated, flash-frozen in liquid N2 and stored in small aliquots at -80°C. Samples for analytical gel filtration and MALS were digested with Tobacco Etch Virus protease and the tag removed by incubation with Histrap beads prior to use.

Analytical ultracentrifugation: Analytical ultracentrifugation was performed in an Optima AUC analytical ultracentrifuge (Beckman Coulter) either through the Beckman testing program at the Center for Analytical Ultracentrifugation of Macromolecular Assemblies at the University of Texas Health Science Center at San Antonio, by Dr. Borries Demeler, Director or at the Beckman Coulter Colorado R&D Center.

The fragments were dialyzed into 10mM phosphate, 50mM sodium chloride (pH 7.4) and spun at 45,000 X g to eliminate aggregates. The fragments were diluted to a range of concentrations using E280 5,120 OD $mol^{-1} * cm^{-1}$ extinction coefficient. AUC samples were then loaded into 2-sector charcoal-epon centerpieces with quartz windows and spun at

45,000 rpm in an An-50 Ti 8 hole rotor. Sedimentation velocity scan data was acquired at both 230 nm and at 280 nm simultaneously with a linear resolution of 10 microns and at 180 second intervals. Up to 250 scans were collected. This data was fit to provide solutions of the Lamm equation by the software package Ultrascan III (v4, rev 2475) using a 2-dimensional gridsearch over Sedimentation Coefficient space ($0.5 < S < 5$) and frictional ratio ($1 < f/f0 < 4$). The resulting sedimentation coefficient population distributions were converted to molecular weight population.

Literature Cited

1. K.J. Petersen, H.V. Goodson, A.L. Arthur, G.W. Luxton, A. Houdusse, M.A. Titus, MyTH4-FERM myosins have an ancient and conserved role in filopod formation. *PNAS* **113,** E8059–E8068 (2016).

2. R.I. Tuxworth, I. Weber, D. Wessels, G.C. Addicks, D.R. Soll, G. Gerisch, M.A. Titus, A role for myosin VII in dynamic cell adhesion. *Curr Biol* **11,** 318–329 (2001).

3. S. Levi, M. Polyakov, T.T. Egelhoff, Green fluorescent protein and epitope tag fusion vectors for *Dictyostelium discoideum*. *Plasmid* **44,** 231–238 (2000).

4. P. Gaudet, K.E. Pilcher, P. Fey, R.L. Chisholm, Transformation of *Dictyostelium discoideum* with plasmid DNA. *Nat Protoc* **2,** 1317–1324 (2007).

5. S.A. Galdeen, S. Stephens, D.D. Thomas, M.A. Titus, Talin influences the dynamics of the myosin VII-membrane interaction. *Mol Biol Cell* **18,** 4074–4084 (2007).

6. R.I. Tuxworth, S. Stephens, Z.C. Ryan, M.A. Titus, Identification of a myosin VII/talin complex. *J Biol Chem* **280,** 26557–26564 (2005).

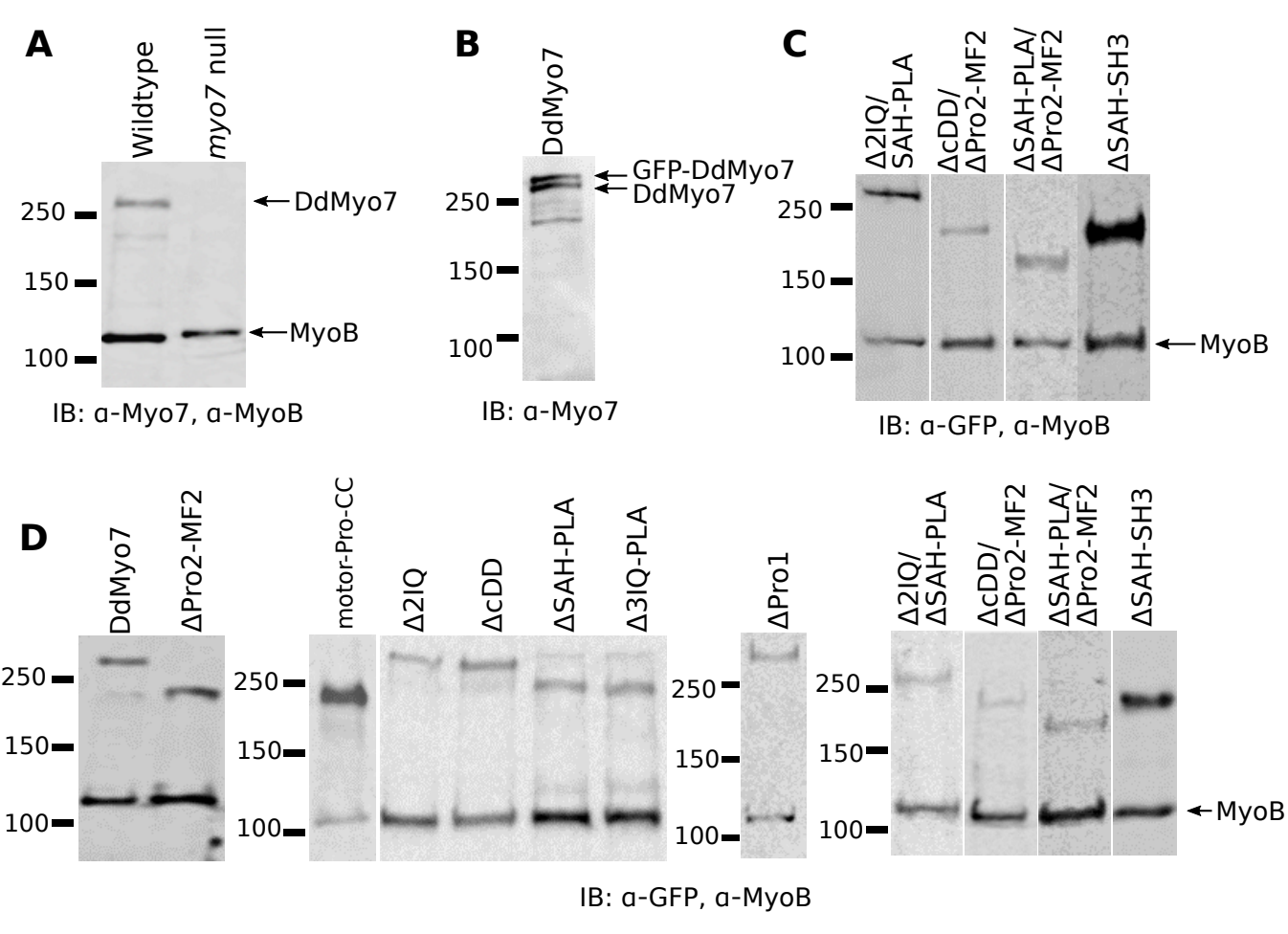
7. K.D. Novak, M.D. Peterson, M.C. Reedy, M.A. Titus, *Dictyostelium* myosin I double mutants exhibit conditional defects in pinocytosis. *J Cell Biol* **131,** 1205–1221 (1995).

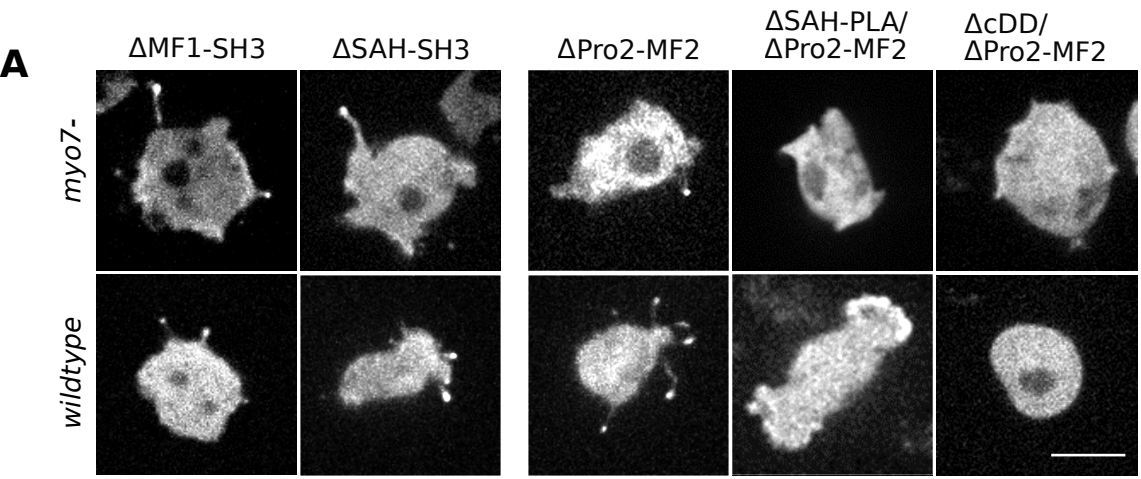
8. B.M. Humbel, E. Biegelmann, A preparation protocol for postembedding immunoelecron microscopy of *Dictyostelium discoideum* cells with monoclonal antibodies. *Scanning Micrsoc* **6,** 817–825 (1992).

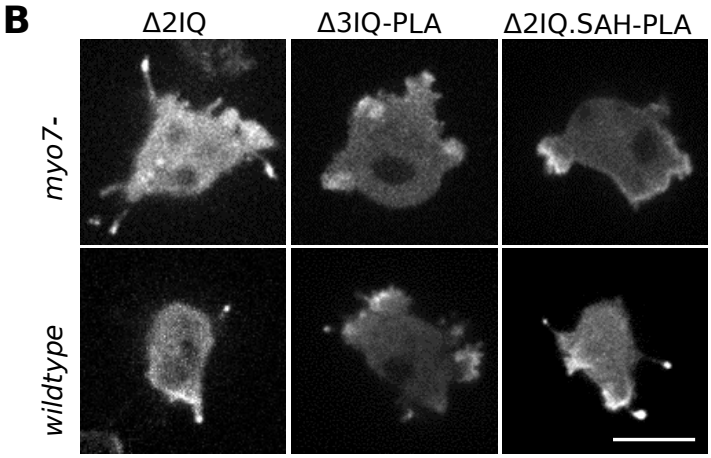
Supplementary Figures

Figure S1. Western blots of whole cell lysates of *Dictyostellium* cell lines expressing GFP-tagged DdMyo7 mutants. MyoB is used as a loading control (124kDa). **A.** Whole cell lysates of wildtype (Ax2) and *myo7* null cells (primary Ab: αMyo7, αMyoB). **B.** Whole cell lysate of GFP-DdMyo7 in wildtype cells (primary Ab: αMyo7). **C.** Whole cell lysates of wildtype cell lines expressing LA/PLA mutants (primary Ab: αGFP, αMyoB). Upper bands represent mutants, lower band representing MyoB loading control. **D.** Whole cell lysates of *myo7* null cell lines expressing motor-Pro-CC, PLA, LA/PLA, and MF/PLA mutants (primary Ab: αGFP, αMyoB). Upper bands represent mutants, lower band is the MyoB loading control.

Figure S2. Localization of various deletion mutants. Representative images of *myo7* null cells (top) or wildtype (bottom) expressing GFP tagged constructs with noted deletions, scale bar represents 10μm.


A
Wildtype
myo7 null
250
150
100
DdMyo7
MyoB
IB: α-Myo7, α-MyoB
B
DdMyo7
GFP-DdMyo7
DdMyo7
250
150
100
IB: α-Myo7
C
Δ2IQ/ ΔSAH-PLA
ΔcDD/ ΔPro2-MF2
ΔSAH-PLA/ ΔPro2-MF2
ΔSAH-SH3
250
150
100
MyoB
IB: α-GFP, α-MyoB
D
DdMyo7
ΔPro2-MF2
motor-Pro-CC
Δ2IQ
ΔcDD
ΔSAH-PLA
Δ3IQ-PLA
ΔPro1
Δ2IQ/ ΔSAH-PLA
ΔcDD/ ΔPro2-MF2
ΔSAH-PLA/ ΔPro2-MF2
ΔSAH-SH3
250
150
100
MyoB
IB: α-GFP, α-MyoB


A
ΔMF1-SH3
ΔSAH-SH3
ΔPro2-MF2
ΔSAH-PLA/
ΔPro2-MF2
ΔcDD/
ΔPro2-MF2
myo7-
wildtype


B
Δ2IQ
Δ3IQ-PLA
Δ2IQ.SAH-PLA
myo7-
wildtype

Supplementary Table 1. Plasmids and oligonucleotides used for the generation of mutant DdMyo7 expression plasmids.

Plasmid	Expressed Protein	Mutation	Template or Backbone	Primers/Ligations
pDTi74[1]	GFP-DdMyo7	full length wild type, aa 1 - 2357		
pDTi321[2]	KKAA	KK2333,2336AA		
pDTi20	n/a	cDNA clone for aa 577 - end of the *myoi* gene in pBluescript		
pDTi73	n/a	precursor clone for pDTi74 *gfp-myoi* gene fusion expression cassette driven by the Act6 promoter with the Act15 terminator		add drug resistance cassette (NEO) to generate expression plasmid
Internal PLA deletion mutant				
pDTi351	ΔcDD	PCR Δ clone for aa 855-952	pDTi20	myi229 TTTATCTTGTTGTTCTTTGAGC myi228 AAATTTGAATTACCACCAGG
pDTi359	ΔcDD	deletion of aa 712-750 in GFP fusion base plasmid	pDTi73	ligation of pDTi351 to pDTi73
pDTi361	ΔcDD	GFP-DdMyo7 Δ aa 855-952		
pDTi406	ΔPLA (SAH cDD Pro1)	PCR Δ clone for aa 791-1084	pDTi20	myi277 TCTATCTCTTTTTAGTAAAACTGCATTTC myi276 TCAGCAACAGCAACAGGT
pDTi410	ΔPLA (SAH cDD Pro1)	deletion of aa 791-1084 in GFP fusion base plasmid	pDTi73	ligation of pDTi406 to pDTi73
pDTi415	ΔPLA	GFP-DdMyo7 Δ aa 791-1084		
PLA & single MF deletion				
pDTi353	PCR clone	coding region for aa 1188-1690 (stop before Pro2-MF2)		myi28 GAATAGCAATCCATTGG myi232 ggatcc**tta**AGCAACTGGATGAACTGG
pDTi357[2]	ΔPro2-MF2	GFP-DdMyo7 Δ aa 1691-2357		
pDTi445	ΔSAH-SH3	deletion of aa 791-1684 in GFP fusion base plasmid	pDTi73	myi277 TCTATCTCTTTTTAGTAAAACTGCATTTC myi230 CCAGTTCATCCAGTTGCTAC

pDTi446	ΔSAH-SH3	GFP-DdMyo7 Δ aa 791-1684		
pDTi356	ΔPro2-MF2	aa 1-1690 in GFP fusion base plasmid	pDTi73	ligation of pDTi353 to pDTi73
pDTi357[2]	ΔPro2-MF2	GFP-DdMyo7 Δ aa 1691-2357		
pDTi394	Δ2IQ	deletion of aa 712-750 in GFP fusion base plasmid	pDTi73	myi227 ATAACGTTTCTTACAACGATAC myi226 CTTACCTATCAAAAACAATTTAAAATC
pDTi395	Δ2IQ	GFP-DdMyo7 Δ aa 712-750		
pDTi404	Δ3IQ-SAH	PCR Δ clone for aa 712-880	pDTi20	myi227 ATAACGTTTCTTACAACGATAC myi274 CAACAAGATAAAAATATTAACGAAC
pDTi408	Δ3IQ-SAH	deletion of aa 712-880 in GFP fusion base plasmid	pDTi73	ligation of pDTi404 to pDTi73
pDTi413	Δ3IQ-SAH	GFP-DdMyo7 Δ aa 712-880		
pDTi405	Δ3IQ-PLA	PCR Δ clone for aa 712-1084	pDTi20	myi275 ATAACGTTTCTTACAACGATACATTC myi276 TCAGCAACAGCAACAGGT
pDTi409	Δ3IQ-PLA	deletion of aa 712-1084 in GFP fusion base plasmid	pDTi73	ligation of pDTi405 to pDTi73
pDTi414	Δ3IQ-PLA	GFP-DdMyo7 Δ aa 791-1084		
Double domain deletions				
pDTi441	Δ2IQ; ΔPLA	deletion of aa 712-750 in GFP fusion base plasmid for Δ 791-1084	pDTi410	myi227 ATAACGTTTCTTACAACGATAC myi226 CTTACCTATCAAAAACAATTTAAAATC
pDTi442	Δ2IQ; ΔPLA	GFP-DdMyo7 Δ aa 712-750; 791-1084		
pDTi440	ΔcDD; ΔPro2-MF2	deletion of aa 855-952 in GFP fusion base plasmid for Δ 1691-2357	pDTi356	myi229 TTTATCTTGTTGTTCTTTGAGC myi228 AAATTTGAATTACCACCAGG
pDTi443	ΔcDD; ΔPro2-MF2	GFP-DdMyo7 Δ 885-952; aa 1691-2357		
pDTi423	ΔPLA; ΔPro2-MF2	deletion of aa 791-1684 in GFP fusion base plasmid for Δ 1691-2357		ligation of pDTi353 to pDTi410
pDTi425	ΔPLA; ΔPro2-MF2	GFP-DdMyo7 Δ aa 791-1084; 1691-2357		

Motor-PLA				
pDTi350[2]	motor-Pro1	motor+PLA (1-1115)	pDXA-GFP[2]	
pDTi490	Motor -Pro1-CC	motor+PLA (1-1115) fused to mouse Myo5A coiled coil region (905 - 1105; UNIPROT acc no Q99104) followed by GCN4 sequence	CC template = XXV in pBiEX3BS[5]	CC+ F cacaaccaactgtttcatCcCTCAAGAAGCTGAAGATAGAGG CC+ R tttgttctaatgcatctcgaGCTAGCTTACTCCCCGAC
Extrachromosomal expression				
pDTi201[3]	PLA-Pro1	GFP-DdMyo7 PLA-Pro1 region aa 809-1154	pTX-GFP[4]	
pDTi383	PLA	PCR clone of coding region for aa 848-1000	pSC-A	myi253 ggatcccatatgGAAGAAGAAGAATTGAAG myi254 ctcgag**tta**ATCATCATCAGCTTCATCAAC
pDTi385	PLA	GFP-DdMyo7 PLA region aa 848-1000	pTX-GFP[4]	
Bacterial expression				
pDTi397	long PLA	PCR clone of coding region for aa 830-1000	pSC-A	myi266 gctagcAGACAACTCCAAGAAGAAC myi267 ctcgagATCATCATCAGCTTCATCAAC
pDTi400	long PLA-6xHis		pET23a	
pDTi447	cDD-FERM1	PCR clone of coding region for aa 829-1690	pSC-A	myi266 gctagcAGACAACTCCAAGAAGAAC myi150 ctcgagAGCATTGTTTCTTAAATATAATG
pDTi449	cDD-FERM1-6xHis		pET23a	
pDTi426	short PLA-6xHis	aa 850-1000	pET14 rTEV	generated by Gibson assembly myi294 gcgaaaacctgtattttcagggcGAAGAATTGAAGAAATTGGAAG myi295 tcgggctttgttagcagccg**TTA**ATCATCATCAGCTTCATC
pDTi454	6xHis-rTEV-cDD	aa 879-1000	pET14 rTEV	deletion mutagenesis - pDTi426 template TEV AS GCCCTGAAAATACAGGTTTTC myi309 AAAGAACAACAAGATAAAAATATTAACG

Generation of *myoi* expression plasmids based on the cloned *myoi* gene (Titus, 1999, Curr Biol). The full protein and coding sequence can be found at dictybase.org (dictyBase:DDB_G0274455). Oligos are 5' to 3' with the 5' most oligo listed first. Lowercase letters indicate added restriction enzyme sites or overlapping sequences for Gibson assembly, bold nucleotides indicate the position of the introduced stop codon. In most cases the final expression plasmids were generated by ligating the NEO cassette carrying resistance to G418 into the GFP fusion base plasmid.

[1] Tuxworth et al (2001); Integrating plasmid carrying resistance to G418
[2] Petersen et al (2016)
[3] Galdeen et al (2012); Extrachromosomal expression plasmid carrying resistance to G418.
[4] Levi et al (2000); Base extrachromosomal expression plasmid carrying resistance to G418.
[5] based on Myo10-M5CC plasmid described by Vavra et al (2016).