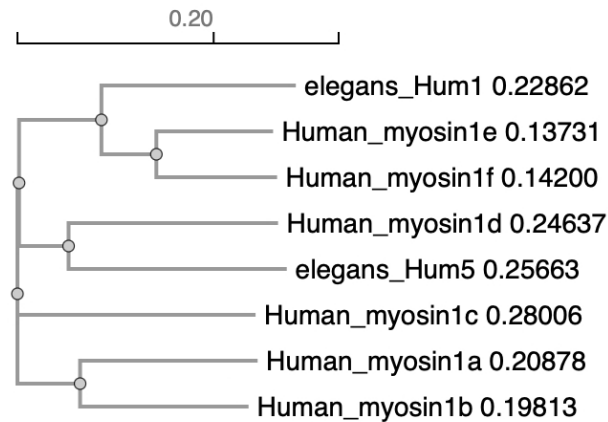


Supplementary Figure 1: (a) Phylogenetic tree from Clustal Omega alignments of Human and *C. elegans* class 1 myosins. (b) Clustal Omega alignments of class 1d & 1e myosins from *S. pombe*, *C. elegans*, and humans.

(a)



(b)

Myo1e TH1 domain highlighted in grey. Conserved TH1 phosphoserine bold red text.

	Motor domain	
<i>C.elegans</i> Hum-5	-----MSYG-GHDPNGYGVEDLVLLSTIDLKSVVQN	30
<i>H.sapiens</i> Myold	-----MA-EQESLEFGKADFVLMDFVSMPEFMAN	28
<i>S.pombe</i> Myo1	MAILKRTNRAKAATAAAPNSTGKSNIGKAVYT-STRKKTGVDDLTLLSKITDEEINKN	59
<i>C.elegans</i> Hum-1	-----MAFHWSKVNQHVGVDDMVLLPKLTEQSIVEN	33
<i>H.sapiens</i> Myole	-----MGSKGVYQYHWQSH-NVKHSGVDDMVLLSKITENSIVEN	38
	* * : * : : : : * *	
	Motor domain	
<i>C.elegans</i> Hum-5	LQLRFQKGRIYTYIGEVLVAVNPYRQLGIYEKSTVDQYKGREIYERAPHVFADAAAYRS	90
<i>H.sapiens</i> Myold	LRLRFQKGRIYTYIGEVLVAVNPYRQLGIYEKSTVDQYKGREIYERAPHVFADAAAYRS	88
<i>S.pombe</i> Myo1	LELRFQKGRIYTYIGEVLVAVNPYRQLGIYEKSTVDQYKGREIYERAPHVFADAAAYRS	119
<i>C.elegans</i> Hum-1	LKKRLQANSIFTYIGPVLISVNPFPKQMPYFTEKEMLLYQGAQYENAPHIYALADNMYRN	93
<i>H.sapiens</i> Myole	LKKRYMDDYIFTYIGSVLISVNPFPKQMPYFGEKEIEMYYQGAQYENAPHIYALADNMYRN	98
	* . * . * : * : * : * : * : * : * : * : * : * : *	
	Motor domain	
<i>C.elegans</i> Hum-5	MKRFRGDSICIVISGESGAGKTETSKIIMKYLAATNVRQQGEIERVKNVLLRSNCILEAF	150
<i>H.sapiens</i> Myold	MKRFRGDSICIVISGESGAGKTETSKIIMKYLAATNVRQQGEIERVKNVLLRSNCILEAF	148
<i>S.pombe</i> Myo1	MKSYHENQCIIISGESGAGKTEAAKRIMQYITHVSKS-VGTEIERVSEIILATNPLLESF	178
<i>C.elegans</i> Hum-1	MLIDNESQCVIISGESGAGKTVNAKFIMNYISRVSG--GGQKVQHIKDVILQSNPLLEAF	151
<i>H.sapiens</i> Myole	MIIDRENQCVIISGESGAGKTVAAKYIMSISRVSG--GGTKVQHVKDIILQSNPLLEAF	156
	* . . * : * : * : * : * : * : * : * : * : * : *	
	Motor domain	
<i>C.elegans</i> Hum-5	GCAKTTRNDNSSRFGKYMHNFDYDQGVGGNINNYLLEKSRVVRQQEGERNFHFVYQLV	210
<i>H.sapiens</i> Myold	GNAKTNRNDNSSRFGKYMHNFDYDQGVGGNINNYLLEKSRVVRQQEGERNFHFVYQLV	208
<i>S.pombe</i> Myo1	GCAKTNRNDNSSRFGKYMHNFDYDQGVGGNINNYLLEKSRVVRQQEGERNFHFVYQLV	238
<i>C.elegans</i> Hum-1	GNSATVRNWNSSRFGKYVEIVFSRGGEPIGGKLSNFFLEKSRVVRQNEGDRNFHFVYQLC	211
<i>H.sapiens</i> Myole	GNAKTNRNNDNSSRFGKYFEIQFSPGGEPIGGKLSNFFLEKSRVVRQNEGDRNFHFVYQLI	216
	* : * * * * * : * : * * * : * : * : * : * : *	
	Motor domain	
<i>C.elegans</i> Hum-5	NGGDDGLLRQFGLTKDAKQYFFLNQGGSHKVASINDSRDFAEVQTALRSIHTFDKQDVES	270
<i>H.sapiens</i> Myold	QGGSEQMLRSLHLQKSLSSNYIYHVAQLK-SSINDAAEFVRVADAMK-VIGFKPEEIQT	266
<i>S.pombe</i> Myo1	KSAPQKYRDTYGIQ-GPENYVYTSACQCLSDGKDFQGTMMAMK-VIGITEPEQDE	296
<i>C.elegans</i> Hum-1	AGADKNLRSTFGIG-ELQYYNYLNMGSVFKADDTDDGKEFESTLHAMK-VVGVDNDQDQLE	269
<i>H.sapiens</i> Myole	EGASAEQKHSGLGIT-SMDYYYLSSGSYKVDIDDRREFQETLHAMN-VIGIFAEQTL	274
	. . : . * : . . . * : * . * : : . :	
	Motor domain	
<i>C.elegans</i> Hum-5	MWSVIAGLIHLGNVRFIDGENSSGAVHIAEKAAALQNAARCLNVTPELAKSLSSQVVA-	329
<i>H.sapiens</i> Myold	VYKILAAIHLGNLKFVVDGDTPL---IENGKVVSIIAELLSTKTMVEKALLYRTVAT-	322
<i>S.pombe</i> Myo1	IFRMLSIIILWLGNIQFQEGQDGS--VISDKSITEFLGYLIGVPVAAIERALTIRIMQTQ	354
<i>C.elegans</i> Hum-1	VLRIVATVLIHGNITFTEENNFAA---VSGKDYLEYPALLGLTSADIEAKLTGRKMESK	326
<i>H.sapiens</i> Myole	VLQIVAGILHLGNISFKEVGNVYAA---VESEEFLLFPAYLLGINQDRLKEKLTSRQMSDK	331

	: : : : : * : : : *	
	Motor domain	
<i>C.elegans</i> Hum-5	----HGDIVKKQHDVNAAYYTRDALAKALYERLFSWVMVSKVNEAISVQNSS--RYSKSHV	383
<i>H.sapiens</i> Myold	----GRDIIDKQHTEQEASYGRDAFAKAIYERLFCWIVTRINDIEVKNYDTTIHGKNTV	378
<i>S.pombe</i> Myol	HGARRGSVYEVPLNPTQALAVRDALSMAIYNCLFDWIVERVKNALVTSDN-----SVSNS	409
<i>C.elegans</i> Hum-1	WGTQKEE-IDMKLNVEQASYTRDAWVKAIYARLFDYLVKKVNDAMNITSQS---TSDNFS	382
<i>H.sapiens</i> Myole	WGGKSES-IHVTLNVEQACYTRDALAKALHARVDFDLVDSINKAMEK-----DHEEYN	383
	. . * *** * : : * : * : * . : .	
	Motor domain	
<i>C.elegans</i> Hum-5	IGVLDIYGFEIFGTNSFEQLCINYCNEKLQQLFIELVLKQEQEYEREGIKWVKIEYFNN	443
<i>H.sapiens</i> Myold	IGVLDIYGFEIFDNNSEFQFCINYCNEKLQQLFIQLVLKQEQEYQREGIPWKHIDYFNN	438
<i>S.pombe</i> Myol	IGILDIYGFEIFENNSFEQLCINYNVEKLQQIFIELTLKTEQEEYVREQIAWTPIKYFNN	469
<i>C.elegans</i> Hum-1	VGILDIYGFEIFNNNGFEQFCINFVNEKLQQIFIELTLKAEQEEYVREGIKWTEIDYFDN	442
<i>H.sapiens</i> Myole	IGVLDIYGFEIFQKNGFEQFCINFVNEKLQQIFIELTLKAEQEEYVQEGIRWTPIEYFNN	443
	:*:***** .*.***:***:*****:*.** ***** :* * * *.***:*	
	Motor domain	
<i>C.elegans</i> Hum-5	KVICDLVEIP--RTGILSILDEACASI---GNVTDKVFLGELDKKLKSHKHYTSRNLKQS	498
<i>H.sapiens</i> Myold	QIIVDLVEQQ--HKGIIAILDDACMNV---GKVTDMEFLEALNSKLGKHAHFSSRKLCS	493
<i>S.pombe</i> Myol	KVVCDLIESK-RPPGLFAAMNDAIATAHADSAAADSAFAQRLN-FLSSNPHEQRRQ----	523
<i>C.elegans</i> Hum-1	KIVCDLIETK-RPPGIMSLDDTCAQNHGQREGVDRQLLTLSKSFAGHPHFGPGS----	497
<i>H.sapiens</i> Myole	KIVCDLIENKVNPPGIMSLDDVCATMHAVGEGADQTLQLQKLMQIGSHEHFNNSWN----	499
	:*:***: * : : : . . * : * . : : * : .	
	Motor domain	
<i>C.elegans</i> Hum-5	DKSMGFE-EFKITHYAGDVTYSVMGFMDKNKDTLQDLKRLLYHSHKNRLVKSFLPDGSKS	557
<i>H.sapiens</i> Myold	DKILEFDRDFRIRHYAGDVVYSVIGFIDKNKDTLQDFKRLMYNSSNPVLKNMWPEGKLS	553
<i>S.pombe</i> Myol	-----NQFIVKHYAGDVTYSITGMTDKNKQDLATDILNLIHSSNNEFMKSIFPVAEES	576
<i>C.elegans</i> Hum-1	-----DSFVIKHYAGDVTYNVDGFCDRNRDVLYPDLILLMQSSRPFIQALFPENVA	550
<i>H.sapiens</i> Myole	-----QGFIIHHYAGKVSYDMDGFCERNRDVLFMDLIELMQSSELFPKISLFPENLQA	552
	* : ****.* *: * : :*: * * * : * : * . . : : * : :	
	Motor domain	
<i>C.elegans</i> Hum-5	MAEVNRRPPTAGFLFKNSMSELVKQLAQKEPHYIRCIKPNEEKNSNVFDLERVEHQVRYL	617
<i>H.sapiens</i> Myold	ITEVTKRPLTAATLFKNSMIALVDNLASKEPYVRCIKPNDKKSQIFDDERCRHQVEYL	613
<i>S.pombe</i> Myol	--NSRRRPPTAGDRIKTSANDLVETLMKCQPSYIRTIKPNQTKSPNDYDQQMVLHQIKYL	634
<i>C.elegans</i> Hum-1	--SAGKRPTTFSTKIRTQANTLVESLMKCSPHYVRCIKPNETKRPNDWEESRVKHQVEYL	608
<i>H.sapiens</i> Myole	--DKKGRPTTAGSKIKKQANDLVSTLMKCTPHYIRCIKPNETKKPRDWEESRVKHQVEYL	610
	. ** * . : : . ** . * * * : * * : : . ** : **	
	Motor domain	
<i>C.elegans</i> Hum-5	GLLENVRVRRAGFAHRMPYDRFVNRYKLICAST----WPNPRRGQQLKDSCMQILESAGL	673
<i>H.sapiens</i> Myold	GLLENVRVRRAGFAFRQTYEKLHRYKMISEFT----WPNHDLPSD-KEAVKKLIERCGF	668
<i>S.pombe</i> Myol	GLQENIRIRRAGFAYRQAFDTFAQRFAVLSGKTSYAGEYTWQ--GDDKSACEQILKDTNI	692
<i>C.elegans</i> Hum-1	GLRENIRVRRAGFAYRRADFKAQRYAIVSPQT----WPCFQ--GDQQRACEIICDSVHM	662
<i>H.sapiens</i> Myole	GLKENIRVRRAGYAYRRIFQKFLQRYAILTKAT----WPSWQ--GEEKQGVLLHLLQSVNM	664
	** **:***:***.* * : : * : : : * : : . : . : :	
	Motor domain Neck region	
<i>C.elegans</i> Hum-5	AQD-CVQGRTKIFIRSPQTVFRLEELRTEQLPNVITFLQKMVRGVQQRERYRMLAVRKI	732
<i>H.sapiens</i> Myold	QDD-VAYGKTKIFIRTPRTLFTLEELRAQMLIRIVLFLQKVWRGTLARMRYKRTKAALTI	727
<i>S.pombe</i> Myol	PSSEYQMGTSKVFIKNPETLFALEDMDRKFDWDTMATRIQRAWRSYVRRRSEAAA-----CI	748
<i>C.elegans</i> Hum-1	EKNQYQMGKTKIFVKNPESLFLLEETRETRKFDGYARVIQKAWRQFSARKQHI-----	714
<i>H.sapiens</i> Myole	DSDQFQLGRSKVFIKAPESLFLLEEMRERKYDGYARVIQKSWRKVFVARKKYV-----	716
	. . * :*:***:***.* * : : * : : : * : : * *	
	Neck region	
<i>C.elegans</i> Hum-5	IGAYRRYKLKSYIWQLINAFRDVRRMRDLGKSIRWPAPPLVLAQFVSRLRVMHQWRRAAT	792
<i>H.sapiens</i> Myold	IRYRRYKVKSYIHEVARRFHGVKTMRDYGKHVKWPSPPKVLRRFEEALQTIENRWRSQ	787
<i>S.pombe</i> Myol	QKLWNRNKNV-----MELERV-----	765
<i>C.elegans</i> Hum-1	-----KQK-----	717
<i>H.sapiens</i> Myole	-----QMR-----	719
	:	
<i>C.elegans</i> Hum-5	ILARMPPHLRASLPQKIAAFEVLNNKKENWG--Y--TRMWRGDYLSQEELELPTTVSTY	848
<i>H.sapiens</i> Myold	LIKSI PASDLPQVRAKVA AVEMLKGQRADLG--L--QRAWEGNYLASKPD--TPQTSGETF	841
<i>S.pombe</i> Myol	-----NEGTKLLQGGKQRRRY SI LGSRKFYGDYLSASKPNG--- T ----	802
<i>C.elegans</i> Hum-1	-----EQAADLMYGKKERRRY SI --NRNFVGDYIGLEHHPT-----	751
<i>H.sapiens</i> Myole	-----EEASDLLLNKKERRR SI --NRNFIGDYIGMEEHPE-----	753
	. : : : : * : * : * . .	
<i>C.elegans</i> Hum-5	HDGIQALRQSHPFQKVLFS----TYVQKFNKFNKSSRLVLIVTRDFVAKLENKKFK----	900
<i>H.sapiens</i> Myold	VPVANELKRKDKYMNVLFS----CHVRKVNRFKSKVEDRAIFVTRHLYKMDPTKQY----	893
<i>S.pombe</i> Myol	---LWNTCGLSQNDHVFISMRCEVLVHKLGRTSKPSRQLVLTKKNLYLVITKIVDQ---	856

<i>C.elegans</i> Hum-1	---LQSLVGK--RQRVLFA-----CTANKYDRKFRVTKLDLLLTVNHLTLIGKEKVKNQPE	802
<i>H.sapiens</i> Myole	---LQQFVGK--REKIDFA-----DTVTKYDRRFKGVKRDLLLTTPKCLYLIGREKVQGGPD	804
	.: *: . * .: : .:* . : :	
<i>C.elegans</i> Hum-5	-----LLKEPIPLQISIRISVCAESNGLFVIHVGDN---D-IVGC--AKNTKNEERVGE	948
<i>H.sapiens</i> Myold	-----KVMKTIPLYNLTLGLSVSNGKDQLVVFHTKDN---KDLIVCLFSKQPTHESTRIGE	944
<i>S.pombe</i> Myol	---KLTQQVEKKFAVSSIDSVGLTNLQDDWVAIRNKSSQNGDMFLRCFFK-----TE	905
<i>C.elegans</i> Hum-1	KGKIVEVIKRQFDLPQIKSIGLSPYQDDFVILYLG-NDDYSSLLETPEK-----TE	852
<i>H.sapiens</i> Myole	KGLVKEVLKRKIEIERILSVSLSTMQDDIFI--LH-EQEYDSLLESVFK-----TE	852
	: . : : : :.: . . . : : *	
<i>C.elegans</i> Hum-5	MIGTLLAHYDKITMRRSPVLIQSAVVCTLG-----GKTRTIRVFDAENNNVPPVFK	999
<i>H.sapiens</i> Myold	LVGVLVNHFKEKSEK-RHLQVNVNPNVQCSLH-----GKKCTVSVETRLNQP-QPDFT	993
<i>S.pombe</i> Myol	FITTLKRINRNI-----QVIVGPTIQYCRK-----PGKVQTVKTAKDETTKDYYD--	950
<i>C.elegans</i> Hum-1	FCTALSKAYKERTNGTLHLDFRSSHVVSYKKMKFDF--SDGKRTVQFGNDGTSSAEKTLK	910
<i>H.sapiens</i> Myole	FLSLLAKRYEEKTQKQLPLKFSNTLELKLKKENWGPWSAGGSRQVQFHQ--GFGDLAVLK	910
	: * : . . : :	
<i>C.elegans</i> Hum-5	KNG-NDVDLICHQLTAQVA-----	1017
<i>H.sapiens</i> Myold	KNR-SGFILSVPGN-----	1006
<i>S.pombe</i> Myol	--YKSGTIHVGTGLPPTSKSKPFPRLATGGSTAAARG---PRPVVQNKAATKPVSMQA	1004
<i>C.elegans</i> Hum-1	PNGKVLNVSIGTGLPNTTRPSTERPQ--GGYTPRRDQLRTSTRTKQNNQSYGQ-NGQSQ	967
<i>H.sapiens</i> Myole	PSNKVLQVSIQGPLPKNSRPTRRNTTQNTGYSSGTQNNANY-PVRAAPPPPGYHQ-NGVIR	968
<i>C.elegans</i> Hum-5	-----	1017
<i>H.sapiens</i> Myold	-----	1006
<i>S.pombe</i> Myol	AKSKPAPMANPV-----STAQQTQNRPPAPAMQARPNTTQAAAPVTSTTTTIKQATTVSA	1059
<i>C.elegans</i> Hum-1	AMRAPVPAHGMNNN-----Y---NQTPAPVS---TNHQYSQEPA-----	1000
<i>H.sapiens</i> Myole	NQYVPYPHAPGSQRSNQKSLYTSMARPLPRQQSTSSDRVSQTPE-----	1013
	<u>SH3 domain</u>	
<i>C.elegans</i> Hum-5	-----	1017
<i>H.sapiens</i> Myold	-----	1006
<i>S.pombe</i> Myol	SKPAPSTVTSAAASSPSNISKPSAPVANNVS-----KPSAVPPPPPPPPAEVEKKDLYLAL	1114
<i>C.elegans</i> Hum-1	RIPVMGNVIN-----Q--LNNMNLSGNGNSPAGRGPPPARGPKPPPAKPKLNPVVIAY	1052
<i>H.sapiens</i> Myole	SLDFL-----K--VPDQGAAGVRRQTTSRPPAGGRPKPQPKPKP-QVPQCKAL	1059
	<u>SH3 domain</u>	
<i>C.elegans</i> Hum-5	-----	1017
<i>H.sapiens</i> Myold	-----	1006
<i>S.pombe</i> Myol	YDFAGRSPNEMTIKKDEIIIEIVQKEPSGWLALKNAGWVPATYVTEYKGSTPQTASS	1174
<i>C.elegans</i> Hum-1	YPYEAQDVDELSFEAGAEIELMNKDASGWWQGVNNRVGLFPGNYVKE-----	1100
<i>H.sapiens</i> Myole	YAYDAQDTDELSFNANDIIDIIKEDPSGWWTGRLRGKQGLFPNNYVTKI-----	1108
	<u>Acidic tail region</u>	
<i>C.elegans</i> Hum-5	-----	1017
<i>H.sapiens</i> Myold	-----	1006
<i>S.pombe</i> Myol	TNVAQAQANNNASPAEVNNLAGSLADALRMRAVAVRGSDEEEDW	1217
<i>C.elegans</i> Hum-1	-----	1100
<i>H.sapiens</i> Myole	-----	1108

Percentage identity matrix of myosins 1d & 1e from *C. elegans*, *H. sapiens* & *S. pombe*

	1.	2.	3.	4.	5.
1: <i>C.elegans</i> Hum5	100.00	49.70	37.36	37.46	39.55
2: <i>H.sapiens</i> Myold	49.70	100.00	36.95	37.16	39.04
3: <i>S.pombe</i> Myol	37.36	36.95	100.00	40.56	41.81
4: <i>C.elegans</i> Hum1	37.46	37.16	40.56	100.00	57.84
5: <i>H.sapiens</i> Myole	39.55	39.04	41.81	57.84	100.00

Percentage identity matrix of *C. elegans*, *H. sapiens* & *S. pombe* myosin 1e TH1 domains

	1.	2.	3.
1: <i>S.pombe</i> Myol	100.00	28.81	31.21
2: <i>C.elegans</i> Hum1	28.81	100.00	51.30
3: <i>H.sapiens</i> Myole	31.21	51.30	100.00

Supplementary Figure 2: Densitometric analysis of anti-mNeongreen western blots of PIP strips incubated with either wild type (white) or S782D phosphomimetic (grey) mNeongreen-HUM1-TH1 domain fusion proteins (shown in Figure 4F).

