

**Supporting Information  
for**

**Phosphorylation of the kinase domain regulates autophosphorylation of myosin IIIA  
and its translocation in microvilli**

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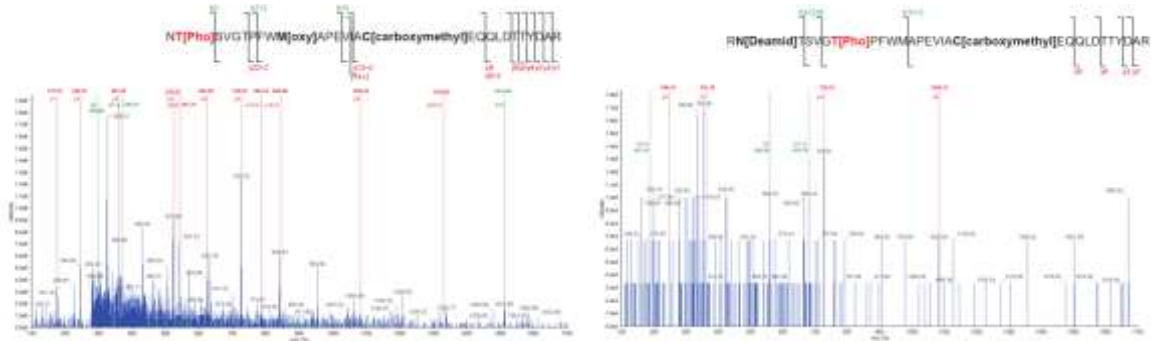
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A

hMyo3A	1	----- MFPLI GKTI I FDNFPDPSDTWEI TETI GKGT	31
hMyo3B	1	----- MKHLYGLFHYNPAMLGLESLPDPTDITWEI I ETI GKGT	37
HGK	1	----- MANDSPAKSLVDI DLSLRDPAGI FELVEVVNGT	35
M NK	1	----- MGDPAPARSLDDI DLSALRDPAGI FELVEVVNGT	35
TNI K	1	----- MASDSPARSLDEI DLSALRDPAGI FELVEVVNGT	35
NRK	1	MAGPGGWRDREVTDLGHLPDPTGI FSLDKTI GLGTYGRI YLGLHEKTGAFTAVKVMARK	60
		.....	
		73	
hMyo3A	32	YGKVFVLNKKNGQKAAVKI LDP I HDI DEEI EAEYNI LKALS DHPNVRFYGI YFKDKV	91
hMyo3B	38	YGKVKVTKNRDGS LAAVKI LDPVSDMDEEI EAEYNI LQFLPNHPNVVKFYGMFYKADHC	97
HGK	36	YQQVYKGRHVKTGQLAAI KVMVTEDEEEEEI KLEI NMLKKYS HHRNI ATYYGAFI KKSPP	95
M NK	36	YQQVYKGRHVKTGQLAAI KVMVTEDEEEEEI KQEI NMLKKYS HHRNI ATYYGAFI KKSPP	95
TNI K	36	YQQVYKGRHVKTGQLAAI KVMVTEDEEEEEI KQEI NMLKKYS HHRNI ATYYGAFI KKNPP	95
NRK	61	TPLPEI GRRVRVVKYQKSVGWRYS - DEEEDLRTTELNLLRKYS EHKNI VSFYGAFFKLSPP	119
		.....	
		177 178 184 188	
hMyo3A	92	N- GDKLWLVLELCSGGSVTDLVKGF LKRGERMSEPLI AYI LHEALMGLQHLHNNKI HRD	150
hMyo3B	98	V- GGQLWLVLELCNGGSVITELVKGLLRCCQRLDEAM S YI LYGALLGLQHLHNNRI I HRD	156
HGK	96	GHDDQLWLVMEFCGAGSI TDLVKN - TKGNLTKEDW AYI SREI LRGLAHLHI HVI HRD	153
M NK	96	GNDQLWLVMEFCGAGSVTDLVKN - TKGNALKEDECI AYI CREI LRGLAHLHAHKVI HRD	153
TNI K	96	GMDQLWLVMEFCGAGSVTDLI KN - TKGNLTKEDW AYI CREI LRGLSHLHQRKVI HRD	153
NRK	120	GQRHLWVMELCAAGSVTDVVRM - TSNQSLKEDW AYI CREI LQGLAHLHAHKVI HRD	177
		.....	
hMyo3A	151	VKGNLI LLTTEGGVKLVDFGVS AQLTS TRHRRNTS VGI PFWVAPEVI ACEQLDITTYDAR	210
hMyo3B	157	VKGNLI LLTTEGGVKLVDFGVS AQLTS TRLRRTNS VGI PFWVAPEVI ACEQQYDSYDAR	216
HGK	154	I KGQNVLLTENAEVKLVDFGVS AQLDRTVGRRTNF I GIPYWAPEVI ACENPDATYDAR	213
M NK	154	I KGQNVLLTENAEVKLVDFGVS AQLDRTVGRRTNF I GIPYWAPEVI ACENPDATYDAR	213
TNI K	154	I KGQNVLLTENAEVKLVDFGVS AQLDRTVGRRTNF I GIPYWAPEVI ACENPDATYDFK	213
NRK	178	I KGQNVLLTHNAEVKLVDFGVS AQVSRITNGRRNSF I GIPYWAPEVI DCDEDPRRSYDAR	237
		.....	
hMyo3A	211	CDTWSLGI TAI ELGDGDPPLADLHPMRALFKI PRNPPKLRQPELWSAEFNDFI SKCLTK	270
hMyo3B	217	CDVWSLGI TAI ELGDGDPPLDFDHPVKTLFKI PRNPPPTLLHPEKVEEENHFI SQCLI K	276
HGK	214	SDLWSGGI TAI EMÆGAPPLCDMPMRALFLI PRNPPRLK - SKKWSKKFFSFI EGCLVK	272
M NK	214	SDI WSLGI TAI EMÆGAPPLCDMPMRALFLI PRNPPRLK - SKKWSKKFI DFI DTCLI K	272
TNI K	214	SDLWSLGI TAI EMÆGAPPLCDMPMRALFLI PRNPPRLK - SKKWSKKFSFI ESCLVK	272
NRK	238	SDVWSVGI TAI EMÆGAPPLCNLQPLEALFVI LRESAPTVK - SSGWSRKFHFMEKCTI K	296
		.....	
		302	
hMyo3A	271	DYEKRPTVSELLQHKFI TQI EGKDWLQKQLTEFI GI HQC-----	310
hMyo3B	277	DFERRPSVTHLLDHPFI KGVHGKVLFLQKQAKVLQDQKH-----	336
HGK	273	NYMRPSTEQLLKHPFI RDQPNERQVRI QLKDHI DRTRKK-----	332
M NK	273	TYLSRPPTEQLLKHPFI RDQPNERQVRI QLKDHI DRSRKK-----	332
TNI K	273	NHSQRPATEQLMKHPFI RDQPNERQVRI QLKDHI DRTRKK-----	332
NRK	297	NFLFRPTSANLQHPFVRDI KNERHVVESLTRLHTGI I KK-----	356
		.....	

B



**Figure S1. Amino acid sequence alignment and comparison of myosin III with Msn protein kinases.** (A) Sequence homology of hMyo3-KD and human Msn kinases belong to the STE20 kinase family (HGK, Mitogen-activated protein kinase kinase kinase 4; MINK, TRAF2 and NCK-interacting protein kinase; TNIK, Misshapen-like kinase 1; NRK, Nik-related protein kinase). The activation loop is indicated with solid underline. Identified phosphorylated residues are indicated by grey boxes. The amino acid homology is indicated as follows: identical (asterisk), very similar (dot) (B) Mass spectrum showing two phosphorylation sites Thr<sup>184</sup> (left) and Thr<sup>188</sup> (right).

**A**

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Hu Myo3A      1 MFPLIGKTIIFDNFPPSDTWEIETIGKTYGKVKVFNKNGKQAAVKILDPHID-ID 59
Mu Myo3A     1 MLPLIGKTIIFDNFPPSDTWEIETIGKTYGKVKVFNKNGKQAAVKILDPHID-ID 59
Sa Myo3A     1 MLPLIGKTIIFDNFPPSDTWEIETIGKTYGKVKVFNKNGKQAAVKILDPHID-ID 56
Ga Myo3A     1 MLPLTGKTIIFDNFPPSDTWEIETIGKTYGKVKVFNKNGKQAAVKILDPHID-ID 59
Xe Myo3A     1 -----MLFDGFPDPTDNDICETIGKTYGKVKVFNKNGKQAAVKILDPHEDID 52
Mo Myo3A     1 MFPQSGKSVFDFNPDPDPTDNDICETIGKTYGKVKVFNKNGKQAAVKILDPHID-ID 59
Dr Myo3A     1 -----MMLPYAQLPDKTDFEIEEIAQGVNAKVFRAKELDNDRIVALKI-QHYDEEHQ 54
              *****
              73
Hu Myo3A     60 EEIEAEYNILKALSDHPHNVRFYGYFKKDKVNGDKLWLVLELCSGGSVTDLVKGFVKRRG 119
Mu Myo3A     60 EEIEAEYNILRTLSDHPHNVRFYGYFKKDKVNGDKLWLVLELCSGGSVTDLVKGFVKRRG 119
Sa Myo3A     57 -----LCSGGSVTDLVKGLKRRG 74
Ga Myo3A     60 EEIEAEYNILKALSDHPHNVRFYGYFKKDKVNGDKLWLVLELCSGGSVTDLVKGFVKRRG 119
Xe Myo3A     53 EEIEAEYNILKALSDHPHNVRFYGYFKKDKVNGDKLWLVLELCSGGSVTDLVKGLKRRG 112
Mo Myo3A     60 EEIEAEYNILKALSDHPHNVRFYGYFKKDKVNGDKLWLVLELCSGGSVTDLVKGLKRRG 119
Dr Myo3A     55 VSTIEEYRTLRDY(D)HNPLEFYGKYLKSPNGPDEIFWVMEYCAAGTAVDMVNNKLLKLD 114
              *****
              172-178
Hu Myo3A     120 ERMSEPLIAYLHEALMGLQHLHNNKTTHRDVKGNNLLTTEGGVKLVDFGVSAQLTSTR 179
Mu Myo3A     120 ERMSEPIAYLHEALMGLQHLHNNKTTHRDVKGNNLLTTEGGVKLVDFGVSAQLSSTR 179
Sa Myo3A     75 ERMSELLIAYLHEALMGLQHLHNNKTTHRDVKGNNLLTTEGGVKLVDFGVSAQLTSTR 134
Ga Myo3A     120 ERMNELIAYLHEALMGLQHLHNNKTTHRDVKGNNLLTTEGGVKLVDFGVSAQLTSTR 179
Xe Myo3A     113 ERMSEPIAYLHEALMGLQHLHNNKTTHRDVKGNNLLTTEGGVKLVDFGVSAQLTSTR 172
Mo Myo3A     120 DRMDEAIAYLHEALMGLQHLHNNKTTHRDVKGNNLLTTHGAVKLVDFGVSAQLTSTR 179
Dr Myo3A     115 RRMREEHIAIYLRTECRATIELNRNHVLRDRDGNILLTKNGRVKLCDFGLSROVDSTL 174
              *****
              184-188
Hu Myo3A     180 HRRNTSVGPFPMMAPEVIACEQQLDSTYDARCDWISLGTAIELGGDPPPLADLHPMRAL 239
Mu Myo3A     180 HRLNTSVGPFPMMAPEVIACEQQLDSTYDARCDWISLGTAIELGGDPPPLADLHPMRAL 239
Sa Myo3A     135 HRRNTSVGPFPMMAPEVIACEQQLDSTYDARCDWISLGTAIELADGPPPLADLHPMRAL 194
Ga Myo3A     180 LRRNTSVGPFPMMAPEVIACEQQLDSTYDARCDWISLGTAIELGGDPPPLADLHPMRAL 239
Xe Myo3A     173 LHRNTSVGPFPMMAPEVIACEQQLDSTYDARCDWISLGTAIELGGDPPPLADLHPMRAL 232
Mo Myo3A     180 LRRNTSVGPFPMMAPEVIACEQQLDSTYDARCDWISLGTAIELGGDPPPLADLHPMRAL 239
Dr Myo3A     175 KGRGTIGSPCHMAPEVVSAMEAREPDITVRADYVALLGTTIEIADGKPPFADHPTRAM 234
              *****
Hu Myo3A     240 FKIPRNPPLRQPELWSAEFNDFISKCLTKDYEKRPVSDLLQHKFITQIEGKVDMLQK 299
Mu Myo3A     240 FKIPRNPPLRQPELWSAEFNDFISKCLTKDYEKRPVSDLLQHKFITQIEGKVDMLQK 299
Sa Myo3A     195 FKIPRNPPLRQPELWTAEFNDFINKCLTKDYEKRPVSDLLQHEFITQIEGKVDMLQK 254
Ga Myo3A     240 FKIPRNPPLRQPELWSAEFNDFINKCLTKDYEKRPVSDLLQHEFITQIEGKVDMLQK 299
Xe Myo3A     233 FKIPRNPPLRQPELWSAEFNDFINKHERINTKGGHFKE SMAPNQD 279
Mo Myo3A     240 FKIPRNPPLRQPELWSDFNDFISKCLTKDFELRPVLDLQHVFTQIVGREKILQK 299
Dr Myo3A     235 FQIIRNPPLRQPELWSDFNDFISELKEAENRPMVMEVHPFITELTENEDMRSS 294
              *****
              302
Hu Myo3A     300 QLTFEIGHQC 310
Mu Myo3A     300 QLMEFIDIHQC 310
Sa Myo3A     255 QLTELIEAHLQ 265
Ga Myo3A     300 QLMEFIDVHQ 310
Xe Myo3A     280 ----- 290
Mo Myo3A     300 QLTELIDLNQ 310
Dr Myo3A     295 DIAEMLELSD 305
              *****
Hu: Homo sapiens
Mu: Mus musculus
Sa: Sarcophilus harrisii (Tasmanian devil)
Ga: Gallus gallus
Xe: Xenopus
Mo: Morone saxatilis (striped sea bass)
Dr: Drosophila

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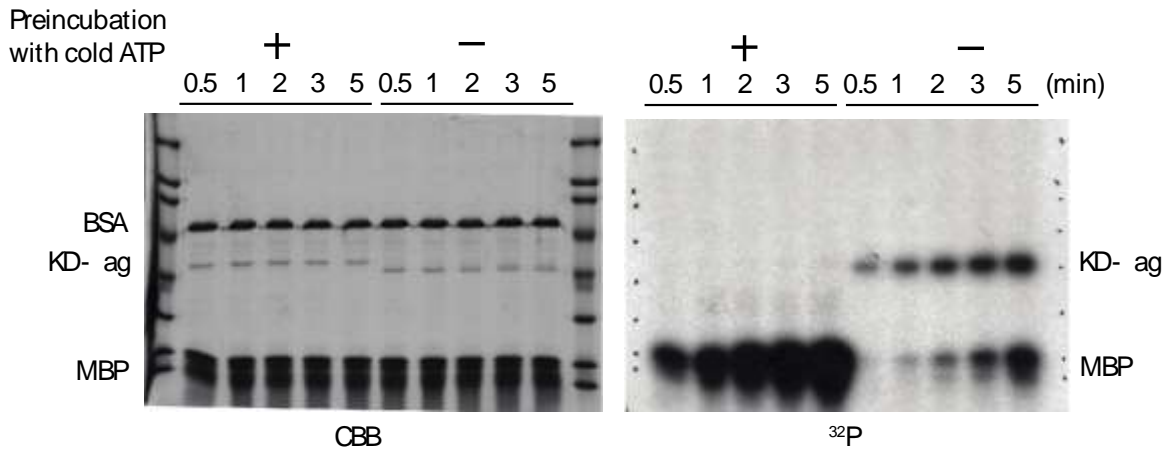
**B**

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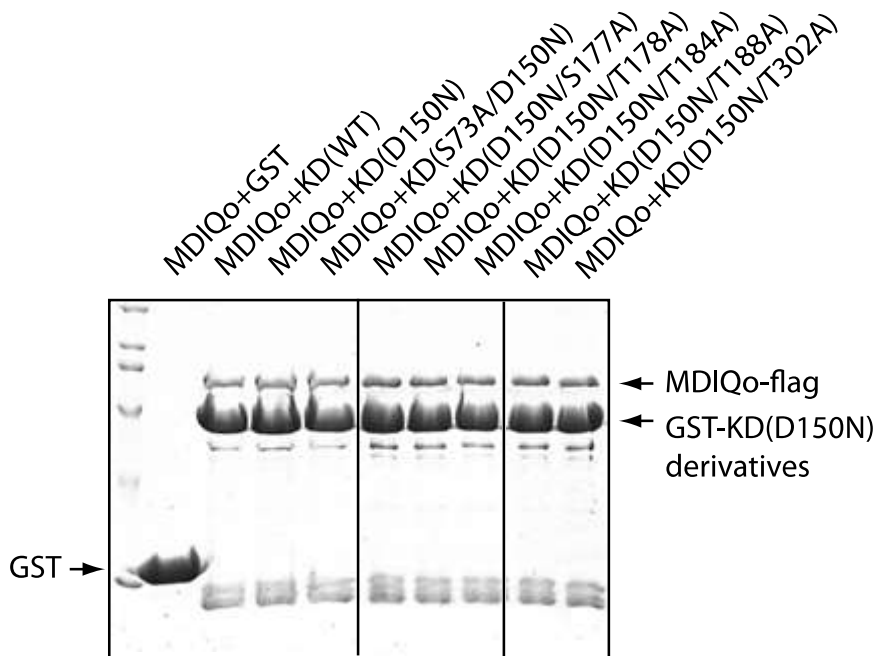
177 178 184 188
|
M3A DFGVSAQL----TSTRHR--RN--TSV---GTFPFWMAPE
M3B DFGVSAQL----TSTRLR--RN--TSV---GTFPFWMAPE
HGK DFGVSAQL----DRTVGR--RN--TFI---GTFPYWMAPE
MINK DFGVSAQL----DRTVGR--RN--TFI---GTFPYWMAPE
TNIK DFGVSAQL----DRTVGR--RN--TFI---GTFPYWMAPE
NRK DFGVSAQV----SRTVGR--RN--SFI---GTFPYWMAPE
STLK3 DFGVSAFLATGDDVTRNKVRK--TFV---GTFPCWMAPE
OSR DFGVSAFLATGDDVTRNKVRK--TFV---GTFPCWMAPE
PAK2 DFGFCAQI----TPEQSK--RS--TMV---GTFPYWMAPE
PAK1 DFGFCAQI----TPEQSK--RS--TMV---GTFPYWMAPE
PAK3 DFGFCAQI----TPEQSK--RS--TMV---GTFPYWMAPE
PAK4 DFGFCAQV----SKEVPR--RK--SLV---GTFPYWMAPE
PAK5 DFGFCAQV----SKEVPR--RK--SLV---GTFPYWMAPE
PAK6 DFGFCAQI----SKDVPK--RK--SLV---GTFPYWMAPE
MST1 DFGVAGQL----TDTMAK--RN--TVI---GTFPFWMAPE
MST2 DFGVAGQL----TDTMAK--RN--TVI---GTFPFWMAPE
MST3 DFGVAGQL----TDTQIK--RN--TFV---GTFPFWMAPE
MST4 DFGVAGQL----TDTQIK--RN--TFV---GTFPFWMAPE
YSK1/SOK1 DFGVAGQL----TDTQIK--RN--TFV---GTFPFWMAPE
TAO2/PSK DFGSASIMAPA-----N-SFV---GTFPYWMAPE
TAO1 DFGSASIM-AS-----PA--N-SFV---GTFPYWMAPE
TAO3/JIK DFGSASIM-AS-----PA--N-SFV---GTFPYWMAPE
KHS1 DFGVAAKI----TATIAK--RK--SFI---GTFPYWMAPE
KHS2 DFGVSAQI----TATIAK--RK--SFI---GTFPYWMAPE
GCK DFGVSGEL----TASVAK--RR--SFI---GTFPYWMAPE
HPK1 DFGISAQI----GATLAR--RL--SFT---GTFPYWMAPE
LOK DFGVSAKN----LTKLQK--RD--SFT---GTFPYWMAPE
SLK DFGVSAKN----TRTIAK--RD--SFT---GTFPYWMAPE
MEKK3 DFGASKRL--QTICMSGTG--MK--S-VT---GTFPYMSPE
MEKK2 DFGASKRL--QTICLSGTG--MK--S-VT---GTFPYMSPE
MAP3K8 DFGCARRLAWAGLNGTHSDMLKSMH---GTFPYWMAPE
MEKK1 DFGAAARLASKG--TGAGEFOGQLL---GTFAPMAPE
MAP3K4 DFGCSVKL----KNNAGT--MPGEVNSTLGTAAVMAPE
TAK1 DFGTACDI----QTHMTRNK-----GSAAMMAPE
ASK1 DFGTSKRL----AGINPC--TETFT---GTLQVMAPE
MEK1 DFGVSGQL----IDSM--N-SFV---GTRVMSPE
MEK2 DFGVSGQL----IDSM--N-SFV---GTRVMSPE
MEK5 DFGVSTQL----VNSTA--K-TTY---GTHAVMAPE
MKK6 DFGTSGYL----VDSVA--K-TMDA--GCKPYMAPE
MKK3 DFGTSGYL----VDSVA--K-TIDA--GCKPYMAPE
MKK4 DFGTSGQL----VDSIA--K-TRDA--GCRPYMAPE
MKK7 DFGTSGRL----VDSKA--K-TRSA--GCAVMAPE
COT DFGLSVQM----TEDVYF--PK-DR---GTEIVMSPE
NIK DFGHAVCL----OPDGLKSLTGDYIPGTEHMAPE
STLK6 GLSHLSLVKHKGRHRAVYDF--PQST--SVOVPLSPE
STLK5 GLRSNLSMISHGQRORVHDF--PKYSV--KVLPLWLSPE
PKCa DFGCMKEH----MMDGVTR--TFC---GTPDYIAPE
PKAa DFGFAKRV----KGR-----TWLTC---GTFPEYLAPE
Consensus sequence DFGxSxQL----xTxXR-Rx-TxV---GTFPFWMAPE
              A          S          S          I          Y

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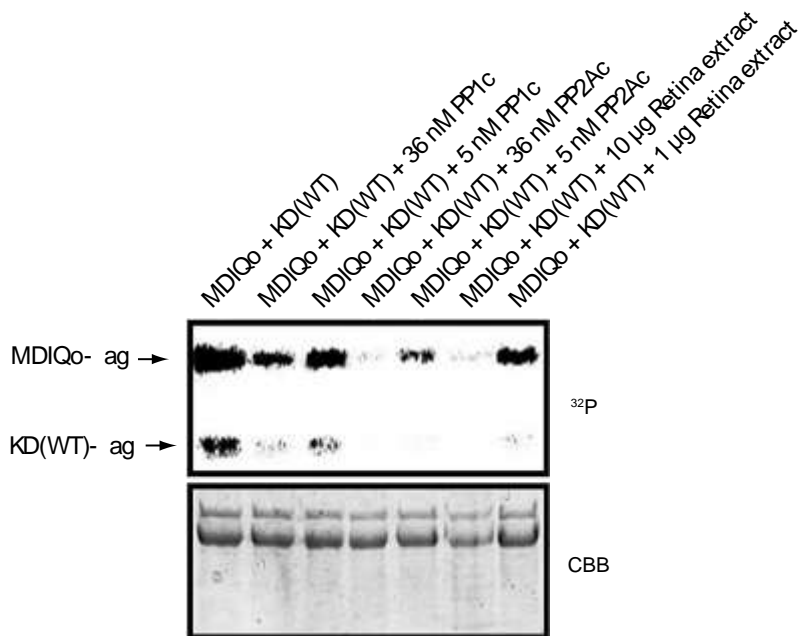
**Figure S2. Amino acid sequence alignment and comparison of myosin III with different species and various protein kinases. (A)** Amino acid sequence alignment of human myosin IIIA with other species. Hu, Homo sapiens; Mu, Mus musculus; Sa, Sarcophilus harrisii (Tasmanian devil); Ga, Gallus gallus; Xe, Xenopus; Mo, Morone saxatilis (striped sea bass); Dr, Drosophila. The activation loop is indicated with solid under line. Identified phosphorylated residues are indicated by grey boxes. The amino acid homology is indicated as follows: identical (asterisk), very similar (dot). **(B)** Sequence alignment of the activation loop of various protein kinases. Consensus sequence is shown at the bottom. Amino acid number of hMyo3A is shown at the top. STLK3, STE20/SPS1-related proline-alanine-rich protein kinase; OSR1, Serine/threonine-protein kinase OSR1; STLK6, STE20-related kinase adapter protein beta; STLK5, STE20-related kinase adapter protein alpha; PAK, p21-activated kinase PAK; MST1, Serine/threonine-protein kinase 4; MST2, Serine/threonine-protein kinase 3; MST3, Serine/threonine-protein kinase 24; MST4, Serine/threonine-protein kinase MST4; YSK1/SOK1, Serine/threonine-protein kinase 25; TAO2/PSK, Serine/threonine-protein kinase TAO2; TAO1, Serine/threonine-protein kinase TAO1; TAO3, Serine/threonine-protein kinase TAO3; KHS, Mitogen-activated protein kinase kinase kinase 5; KHS2, Mitogen-activated protein kinase kinase kinase 3; GCK, Mitogen-activated protein kinase kinase kinase 2; HPK1, Mitogen-activated protein kinase kinase kinase 1; LOK, Serine/threonine-protein kinase 10; SLK, STE20-like serine/threonine-protein kinase; MEKK3, Mitogen-activated protein kinase kinase kinase 3; MEKK2, Mitogen-activated protein kinase kinase kinase 2; MAP3K8, Mitogen-activated protein kinase kinase kinase 19; MEKK1, Mitogen-activated protein kinase kinase kinase 1; MAP3K4, Mitogen-activated protein kinase kinase kinase 4; TAK1, Mitogen-activated protein kinase kinase kinase 7; ASK1, Mitogen-activated protein kinase kinase kinase 5; MEK, ERK activator kinase; MKK; MAP kinase kinase; COT, Mitogen-activated protein kinase kinase kinase 8; NIK, Mitogen-activated protein kinase kinase kinase 14.



**Figure S3. Effect of autophosphorylation of hMyo3A-KD on its protein kinase activity.** hMyo3A-KD (10  $\mu\text{g/ml}$ ) was preincubated with or without 0.1 mM cold ATP for 90 min at 25°C, then 0.5 mM  $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$  and 0.1 mg/ml MBP were added to the reaction mixture to start the reaction. After indicated times, the reaction was stopped by boiling with 5xSDS sample buffer, and subjected to SDS-PAGE. Left; Coomassie Brilliant Blue staining, right; autoradiography of the left. Note that migration of prephosphorylated hMyo3A-KD was a little slower than the non-phosphorylated one. No radioactive  $^{32}\text{P}$  incorporation was observed for prephosphorylated hMyo3A-KD due to saturation of the phosphorylation sites with non-radioactive phosphate.



**Figure S4. Binding between the kinase domain and the motor domain of hMyo3A.** Binding experiment was done as described in Materials and Methods. All hMyo3A-KD variants have N-terminal GST module. Lane 1: MDIQo and GST, lane 2: MDIQo and GST-KD(WT), lane 3: MDIQo and GST-KD(N150D), lane 4: MDIQo and GST-KD(N150D/S73A), lane 5: MDIQo and GST-KD(N150D/S177A), lane 6: MDIQo and GST-KD(N150D/T178A), lane 7: MDIQo and GST-KD(N150D/T184A), lane 8: MDIQo and GST-KD(N150D/T188A), lane 9: MDIQo and GST-KD(N150D/T302A).



**Figure S5. Dephosphorylation of the motor domain of human myosin IIIA (hMyo3A-MDIQo) by PPases.** P-MDIQo (36 nM) and P-KD (36 nM) were dephosphorylated by PPases. Lane 1: P-MDIQo and P-KD, lane 2: P-MDIQo and P-KD with 36 nM of PP1c, lane 3: P-MDIQo and P-KD with 5 nM of PP1c, lane 4: P-MDIQo and P-KD with 36 nM of PP2Ac, lane 5: P-MDIQo and P-KD with 5 nM of PP2Ac, lane 6: P-MDIQo and P-KD with 50 µg/ml of retina tissue extract, lane 7: P-MDIQo and P-KD with 5 µg/ml of retina tissue extract. The bottom panel indicates samples stained with Coomassie Brilliant Blue R-250.