# Supporting Information
for

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

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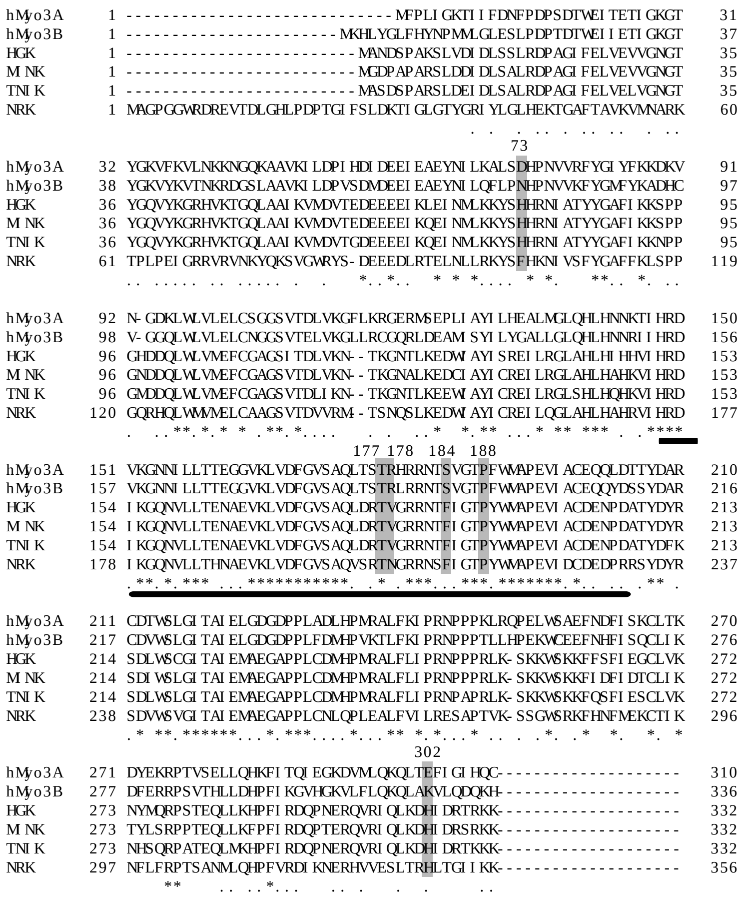
A



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                                73
hMyo3A     1 -----------------------------MFPLIGKTIIFDNFPDPSDTWEITETIGKGT  31
hMyo3B     1 -----------------------MKHLYGLFHYNPMMLGLESLPDPTDTWEIIETIGKGT  37
HGK        1 -------------------------MANDSPAKSLVDIDLSSLRDPAGIFELVEVVGNGT  35
MINK       1 -------------------------MGDPAPARSLDDIDLSALRDPAGIFELVEVVGNGT  35
TNIK       1 -------------------------MASDSPARSLDEIDLSALRDPAGIFELVELVGNGT  35
NRK        1 MAGPGGWRDREVTDLGHLPDPTGIFSLDKTIGLGTYGRIYLGLHEKTGAFTAVKVMNARK  60
                                                 . .  . .. . .. ..  . ..

hMyo3A    32 YGKVFKVLNKKNGQKAAVKILDPIHDIDEEIEAEYNILKALSDHPNVVRFYGIYFKKDKV  91
hMyo3B    38 YGKVYKVTNKRDGSLAAVKILDPVSDMDEEIEAEYNILQFLPNHPNVVKFYGMFYKADHC  97
HGK       36 YGQVYKGRHVKTGQLAAIKVMDVTEDEEEEIKLEINMLKKYSHHRNIATYYGAFIKKSPP  95
MINK      36 YGQVYKGRHVKTGQLAAIKVMDVTEDEEEEIKQEINMLKKYSHHRNIATYYGAFIKKSPP  95
TNIK      36 YGQVYKGRHVKTGQLAAIKVMDVTGDEEEEIKQEINMLKKYSHHRNIATYYGAFIKKNPP  95
NRK       61 TPLPEIGRRVRVNKYQKSVGWRYS-DEEEDLRTELNLLRKYSFHKNIVSFYGAFFKLSPP 119
             .. ..... .. ..... .   .     *..*..   * * *....  * *.   **..  *.  ..

hMyo3A    92 N-GDKLWLVLELCSGGSVTDLVKGFLKRGERMSEPLIAYILHEALMGLQHLHNNKTIHRD 150
hMyo3B    98 V-GGQLWLVLELCNGGSVTELVKGLLRCGQRLDEAMSYILYGALLGLQHLHNNRIIHRD 156
HGK       96 GHDDQLWLVMEFCGAGSITDLVKN--TKGNTLKEDWIAYISREILRGLAHLHIHHVIHRD 153
MINK      96 GNDDQLWLVMEFCGAGSVTDLVKN--TKGNALKEDCIAYICREILRGLAHLHAHKVIHRD 153
TNIK      96 GMDDQLWLVMEFCGAGSVTDLIKN--TKGNTLKEEWIAYICREILRGLSHLHQHKVIHRD 153
NRK      120 GQRHQLWMVMELCAAGSVTDVVRM--TSNQSLKEDWIAYICREILQGLAHLHAHRVIHRD 177
             .  ..**.*.* *  .**.*....     .  .  ..*   *.**  ...* ** ***  .  .****
                                      177 178   184  188
hMyo3A   151 VKGNNILLTTEGGVKLVDFGVSAQLTSTRHRRNTSVGTPFWMAPEVIACEQQLDTTYDAR 210
hMyo3B   157 VKGNNILLTTEGGVKLVDFGVSAQLTSTRLRRNTSVGTPFWMAPEVIACEQQYDSSYDAR 216
HGK      154 IKGQNVLLTENAEVKLVDFGVSAQLDRTVGRRNTFIGTPYWMAPEVIACDENPDATYDYR 213
MINK     154 IKGQNVLLTENAEVKLVDFGVSAQLDRTVGRRNTFIGTPYWMAPEVIACDENPDATYDYR 213
TNIK     154 IKGQNVLLTENAEVKLVDFGVSAQLDRTVGRRNTFIGTPYWMAPEVIACDENPDATYDFK 213
NRK      178 IKGQNVLLTHNAEVKLVDFGVSAQVSRTNGRRNSFIGTPYWMAPEVIDCDEDPRRSYDYR 237
             .**.*.***  ...***********.  .*  .***...***.*******.*..  ..  .** .

hMyo3A   211 CDTWSLGITAIELGDGDPPLADLHPMRALFKIPRNPPPKLRQPELWSAEFNDFISKCLTK 270
hMyo3B   217 CDVWSLGITAIELGDGDPPLFDMHPVKTLFKIPRNPPPTLLHPEKWCEEFNHFISQCLIK 276
HGK      214 SDLWSCGITAIEMAEGAPPLCDMHPMRALFLIPRNPPPRLK-SKKWSKKFFSFIEGCLVK 272
MINK     214 SDIWSLGITAIEMAEGAPPLCDMHPMRALFLIPRNPPPRLK-SKKWSKKFIDFIDTCLIK 272
TNIK     214 SDLWSLGITAIEMAEGAPPLCDMHPMRALFLIPRNPAPRLK-SKKWSKKFQSFIESCLVK 272
NRK      238 SDVWSVGITAIEMAEGAPPLCNLQPLEALFVILRESAPTVK-SSGWSRKFHNFMEKCTIK 296
             .*  **.******...*.***....*...**  *.*...*   ..   .  .*.  .*   *.   *.  *
                                            302
hMyo3A   271 DYEKRPTVSELLQHKFITQIEGKDVMLQKQLTEFIGIHQC-------------------- 310
hMyo3B   277 DFERRPSVTHLLDHPFIKGVHGKVLFLQKQLAKVLQDQKH-------------------- 336
HGK      273 NYMQRPSTEQLLKHPFIRDQPNERQVRIQLKDHIDRTRKK-------------------- 332
MINK     273 TYLSRPPTEQLLKFPFIRDQPTERQVRIQLKDHIDRSRKK-------------------- 332
TNIK     273 NHSQRPATEQLMKHPFIRDQPNERQVRIQLKDHIDRTKKK-------------------- 332
NRK      297 NFLFRPTSANMLQHPFVRDIKNERHVVESLTRHLTGIIKK-------------------- 356
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B

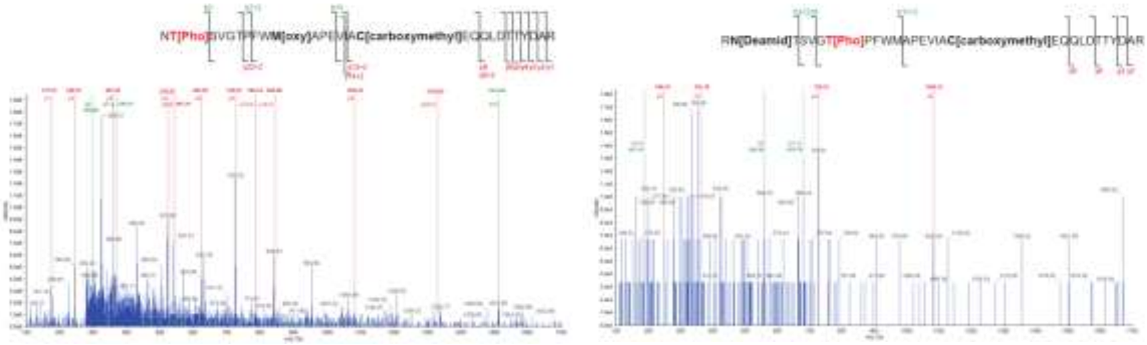

**Figure S1. Amino acid sequence alignment and comparison of myosin III with Msn protein kinases.** (**A**) Sequence homology of hMyo3-KD and human Msn kinases belong to the STE20 kinase family (HGK, Mitogen-activated protein kinase kinase kinase kinase 4; MINK, TRAF2 and NCK-interacting protein kinase; 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$^{184}$ (left) and Thr$^{188}$ (right).

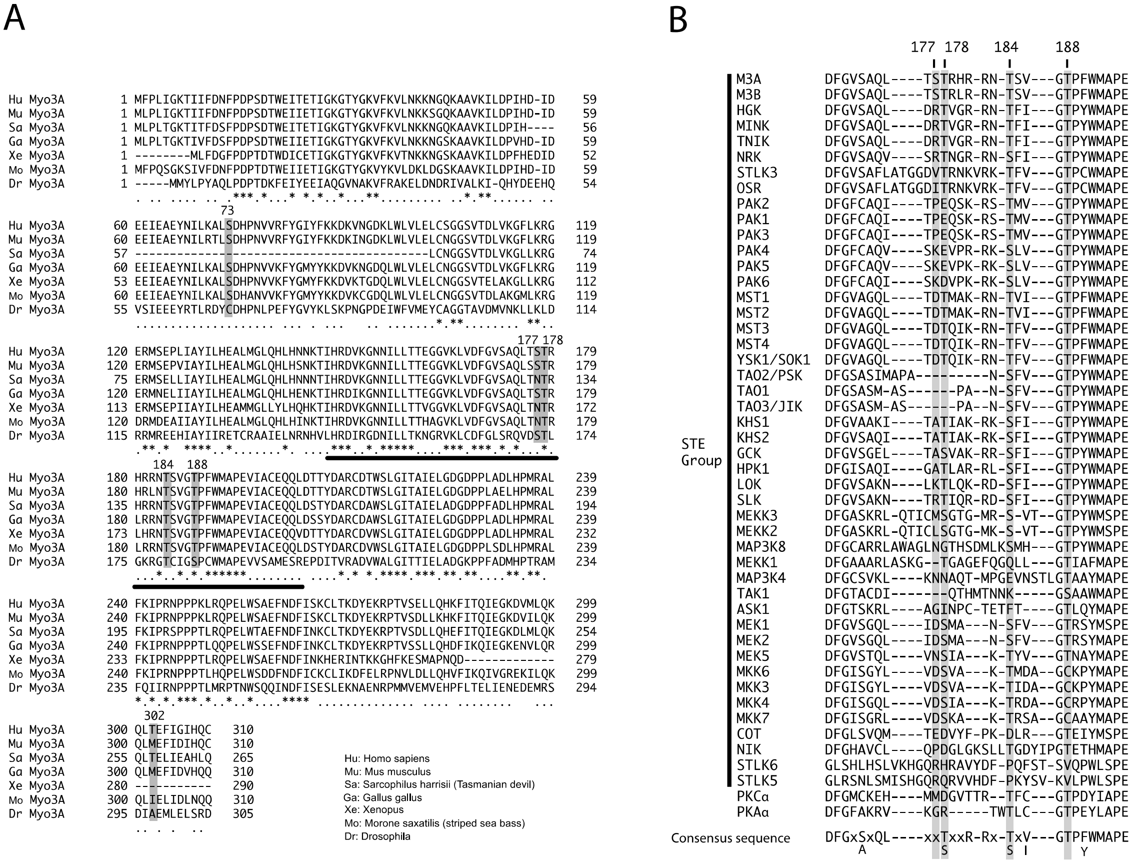

**Figure S2. Amino acid sequence alignment and comparison of myosin III with different species and various protein kinases. (A)** Amino acid sequence alignment of human myosin IIIA with other species. Hu, Homo sapiens; Mu, Mus musculus; Sa, Sarcophilus harrisii (Tasmanian devil); Ga, Gallus gallus; Xe, 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 kinase 5; KHS2, Mitogen-activated protein kinase kinase kinase kinase 3; GCK, Mitogen-activated protein kinase kinase kinase kinase 2; HPK1, Mitogen-activated protein kinase 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.

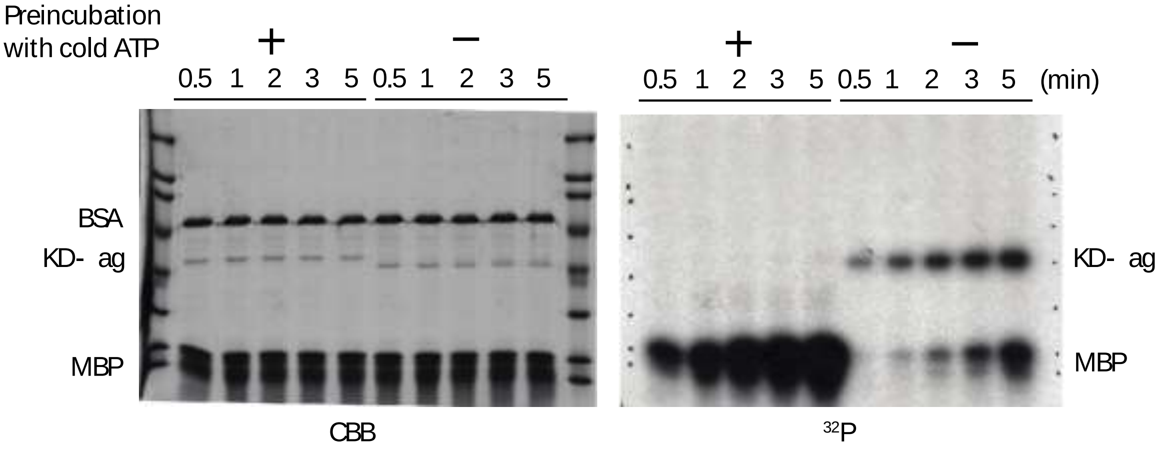

**Figure S3. Effect of autophosphorylation of hMyo3A-KD on its protein kinase activity.** hMyo3A-KD (10 µg/ml) was preincubated with or without 0.1 mM cold ATP for 90 min at 25°C, then 0.5 mM [γ-$^{32}$P]-ATP and 0.1 mg/ml MBP were added to the reaction mixture to start the reaction. After indicated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

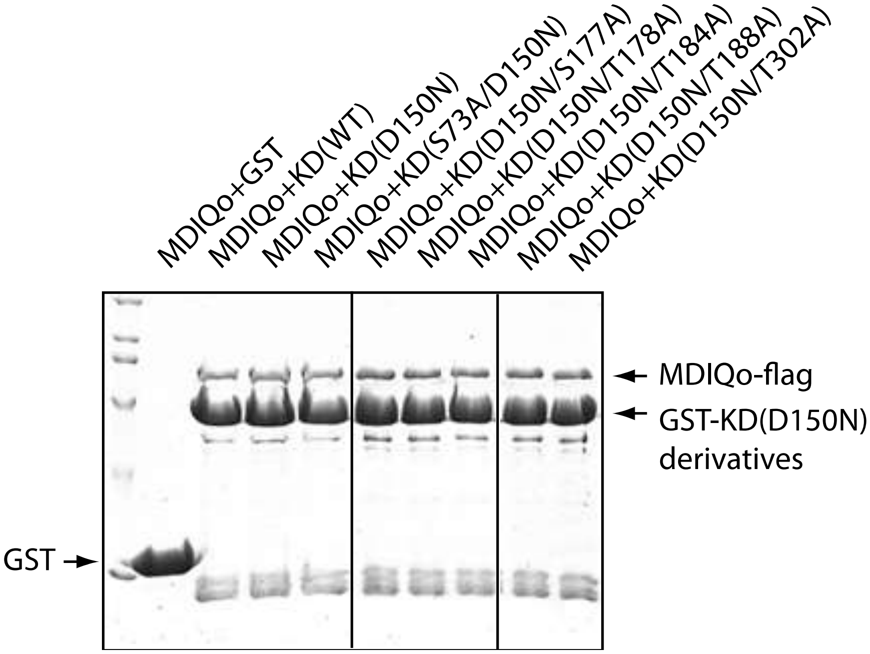

**Figure S4. Binding between the kinase domain and the motor domain of hMyo3A**. Binding experiment was done as described in Materials and Methods. All hMyo3A-KD variants have N-terminal GST module. Lane 1: MDIQo and GST, lane 2: MDIQo and GST-KD(WT), lane 3: MDIQo and GST-KD(N150D), lane 4: MDIQo and 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).

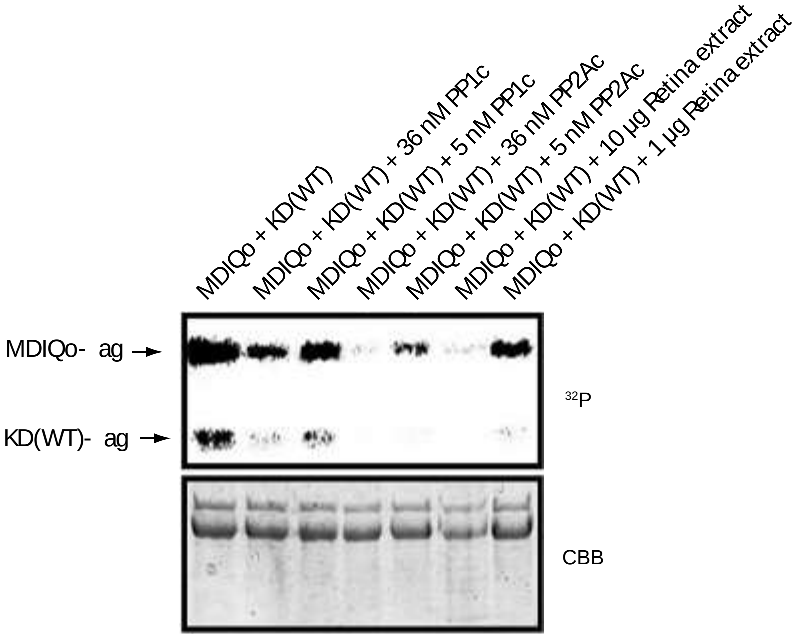

**Figure S5. Dephopshorylation** of the **motor domain of human myosin IIIA (hMyo3A-MDIQo) by PPases**. P-MDIQo (36 nM) and P-KD (36 nM) were dephopshorylated by PPases. Lane 1: P-MDIQo and P-KD, lane 2: P-MDIQo and P-KD with 36 nM of PP1c, lane 3: P-MDIQo and P-KD with 5 nM of PP1c, lane 4: P-MDIQo 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.