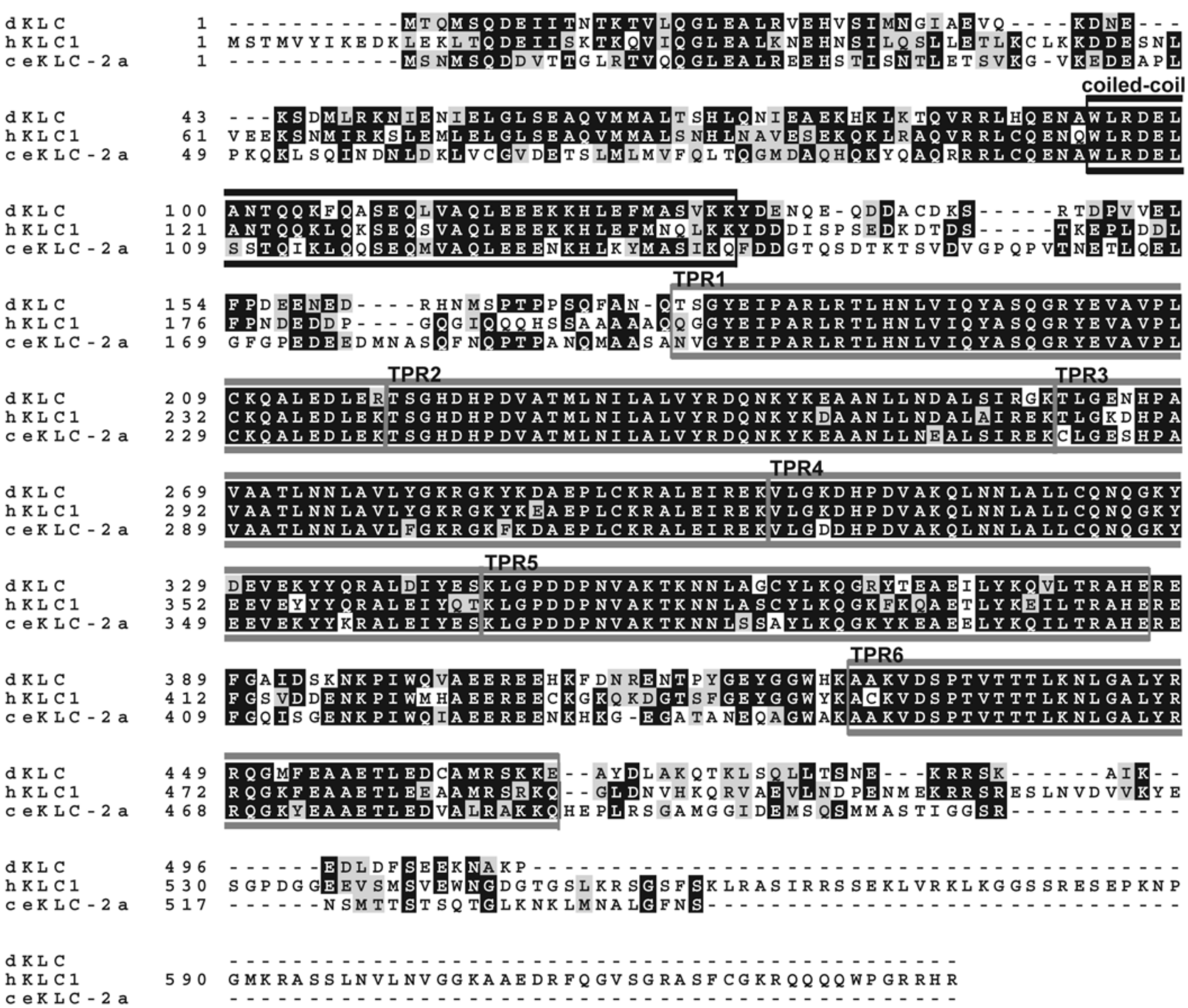

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hKLC1 1 MSTMVYIKEDKLEKLTQDEIISKTKQVIQGLEALKNEHNSILQSLLETLKCLKKDDESNL
ceKLC-2a 1 -----------MSNMSQDDVTTGLRTVQQGLEALREEHSTISNTLETSVKG-VKEDEAPL
coiled-coil
dKLC 43 ---KSDMLRKNIENIELGLSEAQVMMALTSHLQNIEAEKHKLKTQVRRLHQENAWLRDEL
hKLC1 61 VEEKSNMIRKSLEMLELGLSEAQVMMALSNHLNAVESEKQKLRAQVRRLCQENQWLRDEL
ceKLC-2a 49 PKQKLSQINDNLDKLVCGVDETSLMLMVFQLTQGMDAQHQKYQAQRRLCQENAWLRDEL
dKLC 100 ANTQQKFQASEQLVAQLEEEKKHLEFMASVKKYDENQE-QDDACDKS-----RTDPVVEL
hKLC1 121 ANTQQKLQKSEQSVAQLEEEKKHLEFMNQLKKYDDDISPSEDKDTDS-----TKEPLDDL
ceKLC-2a 109 SSTQIKLQQSEQMVAQLEEENKHLKYMASIKQFDDGTQSDTKTSVDVGPQPVTNETLQEL
TPR1
dKLC 154 FPDEENED----RHNMSPTPPSQFAN-QTSGYEIPARLRTLHNLVIQYASQGRYEVAVPL
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TPR2
TPR3
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ceKLC-2a 229 CKQALEDLEKTSGHDHPDVATMLNILALVYRDQNKYKEAANLLNEALSIREKCLGESHPA
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ceKLC-2a 409 FGQISGENKPIWQIAEEREENKHKG-EGATANEQAGWAKAAKVDSPTVTTTLKNLGALYR
dKLC 449 RQGMFEAAETLEDCAMRSKKE--AYDLAKQTKLSQLLTSNE---KRRSK------AIK--
hKLC1 472 RQGKFEAAETLEEAAMRSRKQ--GLDNVHKQRVAEVLNDPENMEKRRSRESLNVDVVKYE
ceKLC-2a 468 RQGKYEAAETLEDVALRAKKQHEPLRSGAMGGIDEMSQSMMASTIGGSR-----------
dKLC 496 ------EDLDFSEEKNAKP---
hKLC1 530 SGPDGGEEVSMSVEWNGDGTGSLKRSGSFSKLRASIRRSSEKLVRKLKGGSSRESEPKNP
ceKLC-2a 517 ------NSMTTSTSQTGLKNKLMNALGFNS------------------------------
dKLC ---
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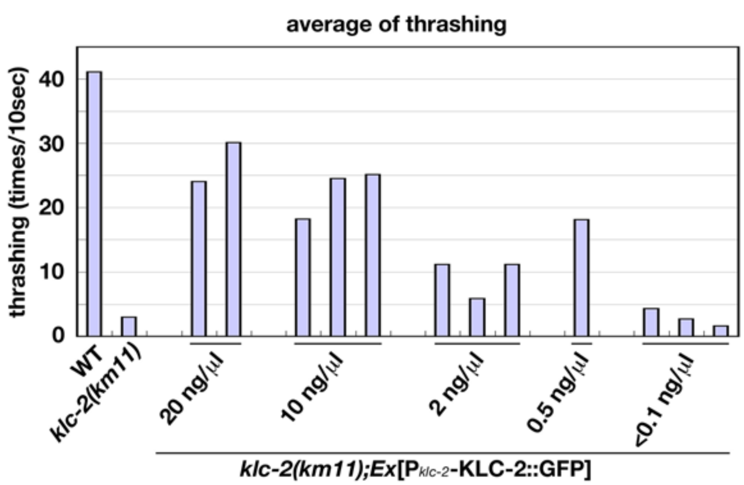
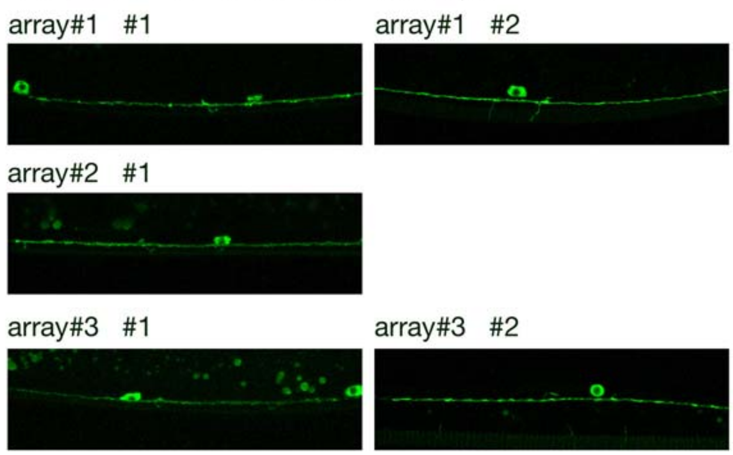
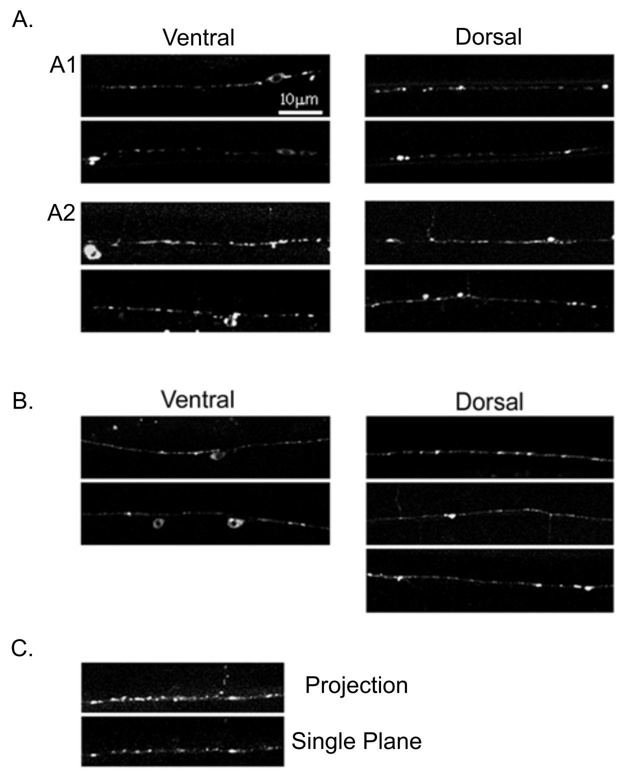
ceKLC-2a ---


average of thrashing
thrashing (times/10sec)
40
30
20
10
0
WT
klc-2(km11)
20 ng/µl
10 ng/µl
2 ng/µl
0.5 ng/µl
<0.1 ng/µl
klc-2(km11);Ex[Pklc-2-KLC-2::GFP]

P*unc-25*-KLC-2::GFP (2ng/μl)




A.
Ventral
Dorsal
A1
10μm
A2
B.
Ventral
Dorsal
C.
Projection
Single Plane

Supplementary Table 1. Transgene rescue of *klc-2(km11)* at different concentrations

Genotype	*Ex*[P$_{klc-2}$-KLC-2-GFP] transgene (conc. ng/μl)	Thrashing (times/10sec)			n
		Average	Highest[1]	Lowest[2]	
N2	N/A	41.1	48	33	30
klc-2(km11)	N/A	3.0	20	0	30
klc-2(km11)	*kmEx 807 (20)*	24.0	38	2	22
	kmEx 808 (20)	30.1	44	16	25
	kmEx 850 (10)	18.2	37	0	30
	kmEx 851 (10)	24.5	36	6	30
	kmEx 852 (10)	25.1	34	6	30
	kmEx 853 (2)	11.2	38	0	30
	kmEx 854 (2)	5.9	17	0	30
	kmEx 855 (2)	11.2	40	0	30
	kmEx 856 (0.5)	18.1	40	0	30
	kmEx 857 (<0.1)	4.3	30	0	30
	kmEx 858 (<0.1)	2.7	14	0	30
	kmEx 859 (<0.1)	1.6	7	0	10

klc-2(*km11*) mutants exhibited uncoordinated movement compared with the wild type in a thrashing assay (Miller et al, 1996). The movement defects of *klc-2*(*km11*) animals were rescued by expressing P$_{klc-2}$-KLC-2-GFP at the concentrations as specified.
1) Highest means the highest frequency of thrashing in each strain.
2) Lowest means the lowest frequency of thrashing in each strain.
n= number of animals scored

Supplementary Figure legends

Supp. Figure 1. Sequence alignment KLC2 with KLC from Drosophila and human.

Supp. Figure 2. Dosage-dependent rescue of *klc-2* by P $_{klc\text{-}2}$ KLC-2::GFP transgenes.

Supp. Figure 3. Images of Punc-25 KLC-2::GFP at 2ng/µl from independent lines. The localization is indistinguishable from those at 20ng/µl as in Figure 4B. No GFP can be seen below 2ng /µl. Array#1 is *km844;* array#2 is *km845;* array#3 is *km846.*

Supp. Figure 4. Images of UNC-16::XFP. A. *Punc*-25UNC-16::CFP localization at low (A1, *juEx834*) and moderate (A2, *juEx836*) expression levels. B. *Punc*-25UNC-16::GFP localization (*juEx841).* Images in A and B are shown as projections of confocal images. No difference for CFP, YFP or GFP (A, B and Figure 5). C. *Punc*-25UNC-16::CFP (*juEx836)* images shown by focal plane. GFP pattern may vary slightly depending on the concentration and image projection.