1 **The KASH protein UNC-83 differentially regulates kinesin-1 activity to**

2 **control developmental stage-specific nuclear migration**

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13 **SUMMARY**

14 Nuclear migration plays a fundamental role in development, requiring precise

15 spatiotemporal control of bidirectional movement through dynein and kinesin motors. Here, we

16 uncover a mechanism for developmental regulation of nuclear migration directionality. The

17 nuclear envelope KASH protein UNC-83 in *Caenorhabditis elegans* exists in multiple isoforms

18 that differentially control motor activity. The shorter UNC-83c isoform promotes kinesin-1-

19 dependent nuclear movement in embryonic hyp7 precursors, while longer UNC-83a/b isoforms

20 facilitate dynein-mediated nuclear migration in larval P cells. We demonstrate that UNC-83a's

21 N-terminal domain functions as a kinesin-1 inhibitory module by directly binding kinesin heavy

22 chain (UNC-116). This isoform-specific inhibition, combined with differential affinity for kinesin

23 light chain (KLC-2), establishes a molecular switch for directional control. Together, these

24 interdisciplinary studies reveal how alternative isoforms of cargo adaptors can generate

25 developmental stage-specific regulation of motor activity during development.

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28

29   **INTRODUCTION**

30   Nuclear positioning orchestrates essential cellular and developmental processes

31   including pronuclear migration, muscle and neuronal differentiation, and cell migration[1,2,3,4].

32   Defects in nuclear positioning underlie severe congenital disorders such as lissencephaly[5,6] and

33   centronuclear myopathies[7]. As the largest intracellular cargo, nuclei require precise spatial and

34   temporal control of their movement along microtubules[4]. Nuclei move towards different

35   directions along microtubules at different times in development. For example, the minus-end

36   directed motor cytoplasmic dynein (dynein from here on) drives pronuclear migration toward

37   centrosomes during fertilization [8,9,10,11,12]. Alternatively, the plus-end directed motor kinesin

38   powers nuclear movement in developing neuroepithelia and sensory cells[1,13]. However, the

39   molecular mechanisms controlling bidirectional nuclear movements remain poorly understood.

40   We hypothesize that nuclear envelope cargo adaptors regulate bidirectional nuclear movements

41   by directly influencing the activity of kinesin and dynein motors.

42   The conserved **li**nker of the **n**ucleoskeleton and **c**ytoskeleton (LINC) complex bridges

43   the nuclear envelope to enable many nuclear movements[14,15,16]. LINC complexes are formed by

44   the interaction between **S**ad1p/**UN**C-84 (SUN) proteins in the inner nuclear membrane and

45   **K**larsicht/**A**NC-1/**S**yne **h**omology (KASH) proteins in the outer nuclear membrane[17,18]. SUN

46   proteins interact with lamins in the nucleoplasm[19,20], cross the inner nuclear membrane, span

47   the lumen of the nuclear envelope, and recruit KASH proteins to the outer nuclear

48   membrane[16,21]. KASH proteins, including the mammalian nuclear envelope spectrin repeat

49   proteins (nesprins), extend into the cytoplasm to interact with a variety of cytoskeletal and motor

50   proteins [22,23,24]. Here we focus on the role of KASH proteins as cargo adaptors to recruit kinesin-

51   1 and dynein motors to nuclei.

52   Motor protein recruitment by KASH proteins involves complex regulatory mechanisms.

53   Kinesins are primarily plus-end directed motors while cytoplasmic dynein is the major minus-end

54   directed motor in animal cells[25,26]. Kinesin-1 is a heterotetramer composed of two heavy chains

55   (KHCs) with their motor domains and two kinesin light chains (KLCs)[25,27,28]. In the absence of

56   cargo, kinesin-1 is inactive as KHCs and KLCs are folded in a confirmation that inhibits

57   microtubule binding and motility[29,30]. Binding of cargo adaptor molecules to the KLC releases

58   this autoinhibition and activates kinesin-1 motility[31,32,33,34,35]. Mammalian nesprin-2 and nesprin-

59   4, and *C. elegans* UNC-83 activate kinesin-1 through their W-acidic (LEWD/EWD) motifs that

60   interact with the tetratricopeptide repeat (TPR) domain of KLCs[13,36,37,38]. Similarly, dynein exists

61   in an autoinhibited state until activated by co-factors and cargo adaptors[26,39,40]. At the nuclear

62   envelope, mammalian KASH5, and *C. elegans* ZYG-12 are meiosis-specific activators of

63   dynein[41,42,43,44]. Nesprin-1 and -2 also bind dynein[45]. Despite our understanding of these

64   individual interactions, the mechanisms controlling tissue-specific activation of different motor

65   proteins by KASH proteins remains unclear.

66   We studied two distinct nuclear migration events in *C. elegans* development to dissect

67   how KASH proteins coordinate microtubule motor protein regulation. First, embryonic

68   hypodermal hyp7 precursor nuclei migrate across the dorsal midline to the opposite lateral side

69   of the embryo, after which the cells fuse to form the hyp7 syncytium[46] (Fig. 1A). Failed migration

70   results in nuclei mislocalized to the dorsal cord[15,47]. In contrast, larval hypodermal P-cell nuclei

71   migrate ventrally through constricted spaces between body wall muscles and the cuticle. After

72   migration, P cells undergo divisions to ultimately form vulval cells and GABA neurons[48,49] (Fig.

73   1B). Failures in P-cell nuclear migration result in P-cell death and missing lineages that lead to

74   egg-laying and locomotion defects[49,50].

75   Both nuclear migration events require the LINC complex proteins UNC-84 (SUN) and

76   UNC-83 (KASH) [51,52,53,54]. UNC-83 functions as a cargo adaptor that directly interacts with both

77   kinesin-1 and dynein at the nuclear envelope[54,55,56]. However, cellular and genetic analyses

78   reveal strict tissue-specific motor requirements. Embryonic hyp7 precursor nuclei migrate

79   toward the plus ends of microtubules (Fig. 1A), while P-cell nuclei migrate toward minus ends of

80   microtubules (Fig. 1B)[15,49]. Mutations in kinesin-1 heavy chain (*unc-116*) or light chain (*klc-2*)

81   severely disrupt hyp7 nuclear migration, while dynein (*dhc-1*) plays only a minor role to move

82   nuclei backward to avoid obstacles [55,56]. In contrast, dynein, but not kinesin-1, is necessary for

83   P-cell nuclear migration[49,57]. This precise tissue specificity in motor preference suggests that

84   UNC-83 can differentially recruit and/or activate kinesin-1 for plus-end-directed hyp7 nuclear

85   migration and dynein for minus-end-directed P-cell nuclear migration.

86   We hypothesized that the differential expression of *unc-83* isoforms controls tissue-

87   specific nuclear migration directionality. There are three known *unc-83* isoforms; *unc-83a*, *unc-*

88   *83b*, and *unc-83c*, encoding proteins with 1041, 974 and 741 amino acid residues, respectively.

89   The three isoforms have 741 residues in their C-termini in common, but differ in their N-

90   termini[52]. Previous genetic analyses revealed that mutations affecting all three isoforms disrupt

91   both migration events, while those specific to long isoforms (*unc-83a/b*) only impair P-cell

92   nuclear migration. Therefore, the short isoform (*unc-83c*) suffices for hyp7 nuclear migration[52].

93   In this model, the long UNC-83a/b isoforms favor minus-end-directed nuclear migration by

94   inhibiting kinesin-1 and/or activating dynein while the short UNC-83c isoform favors plus-end-

95   directed movements by activating kinesin-1 and/or inhibiting dynein (Fig. 1C). Here, we test our

96   model with *in vivo* and *in vitro* approaches. Together, our findings reveal that the N-terminus of

97   long UNC-83 isoforms specifically inhibits kinesin-1, enabling precise spatial and temporal

98   control of nuclear positioning during development.

99

100   **RESULTS**

AlphaFold predicts a modular spectrin-repeat architecture for UNC-83.

The molecular architecture of UNC-83's cytoplasmic domain remains unexplored. Given that UNC-83 is predicted to be highly α-helical and that other KASH proteins contain characteristic ~100 residue long spectrin repeats composed of triple α-helical bundles[56,58], we hypothesized that UNC-83 might harbor previously unidentified spectrin-like domains. AlphaFold3-mediated structural modeling[59] revealed five spectrin-like repeats interspersed by large stretches of predicted disordered regions (Fig. 1D). Two spectrin-like repeats were located within the N-terminal domain specific to the longer UNC-83a/b isoforms while the other three were located within the C-terminal region shared by all isoforms (Fig. 1C-D). The KLC-binding EWD motif was in an unstructured region (Fig.1D), similar to nepsrin-2[60]. These structural predictions show high confidence scores (Fig. S1A) and low predicted error rates (Fig. S1B), suggesting that UNC-83, like other KASH proteins, evolved from an ancestral spectrin-like protein.

The N-terminal domain of the long UNC-83a isoform inhibits KLC-2 binding

Previous studies established that the short UNC-83c isoform directly binds KLC-2 via yeast two hybrid and *in vitro* pull-down assays[55], and that both proteins are required for plus-end directed nuclear migration in embryonic hyp7 precursors[15]. The conserved EWD motif of UNC-83 is predicted to interact with the TPR repeats of KLCs as modeled by AlphaFold3 (Fig. 2A) and as demonstrated for other kinesin-1 cargo adaptors[36]. The importance of this motif is supported by the *unc-83(yc74)* allele, where mutation of EWD to GSA residues abolishes hyp7 nuclear migration[13]. While this EWD motif is present in all three UNC-83 isoforms and is necessary for kinesin-1-mediated hyp7 nuclear migration, we hypothesized that the 301-residue N-terminal portion unique to UNC-83a/b isoforms inhibits binding to KLC-2.

125   To test our hypothesis and quantitatively assess UNC-83c isoform binding to KLC-2, we

126   expressed and purified KLC-2 and the cytoplasmic domains of UNC-83a and UNC-83c

127   (excluding their transmembrane domains and KASH peptides for solubility) from *E. coli* (Fig.

128   2B). Mass photometry analysis[61] confirmed that all three purified proteins were predominantly

129   monomeric, with measured masses within 10% of their predicted molecular weights (Fig. 2C-D).

130   Direct binding assays revealed that UNC-83c and KLC-2 formed a stable complex (one UNC-

131   83c monomer binding to one KLC-2 monomer) with an average mass of 174 kDa (Fig. 2E). In

132   contrast, UNC-83a did not form a robust complex with KLC-2 under identical conditions (Fig.

133   2E). While a minor peak at ~489 kDa was observed, this represented less than 20% of the

134   detected particles and likely reflects transient or non-specific associations rather than a stable

135   complex formation. These results suggest the N-terminal domain of UNC-83a modulates the

136   ability of the EWD motif to interact with KLC-2.

137   To further quantify these differences in binding affinity, we used bio-layer interferometry

138   for real-time interaction analysis. UNC-83c exhibited robust binding to KLC-2 with a dissociation

139   constant (K_d) of 0.15 µM. However, UNC-83a binding to KLC-2 showed non-saturating kinetics

140   indicating a K_d at least 10-fold higher ($K_d > 1.0$) than that for UNC-83c (Fig. 2F). We conclude

141   that the N-terminal domain specific to UNC-83a disrupts the interaction between its EWD motif

142   and the TPR domain of KLC-2.

143

144   **Ectopic expression of *unc-83a* disrupts kinesin-1-dependent nuclear migration.**

145   Our biochemical data showed that the N-terminal domain of UNC-83a disrupts KLC-2

146   binding *in vitro*. To test whether this inhibitory effect extends to nuclear migration *in vivo*, we

147   engineered *unc-83(yc108[UNC-83a])* using CRISPR/Cas9 to express the long UNC-83a isoform

148   under control of the endogenous *unc-83c* promoter. This was achieved by fusing the 301-amino

149   acid UNC-83a-specific N-terminal domain to the 5' end of *unc-83c* (Fig. 3A), effectively

150   replacing the short isoform with UNC-83a in hyp7 precursor cells*.* We confirmed the proper

151   localization of UNC-83a to the nuclear envelope of hyp7 precursors during the nuclear migration

152   period in *unc-83(yc108[UNC-83a])* by immunofluorescence (Fig. 3B). To asses nuclear

153   migration defects, we quantified hyp7 nuclei abnormally localized in the dorsal cord of larvae[62].

154   Wild-type animals had almost no mislocalized hyp7 nuclei. In contrast, both *unc-83(e1408)* null

155   and *unc-83(yc108[UNC-83a])* animals had severe defects with 12.8 ± 0.45 and 12.9 ± 0.32

156   (mean $\pm$ 95% confidence interval (CI)) mislocalized nuclei, respectively (Fig. 3C-D). Importantly,

157   *unc-83(yc108[UNC-83a])* animals had normal P-cell nuclear migrations, with only 0.45 ± 0.16

158   missing GABA neurons (Fig. 3E-F). This tissue-specific effect demonstrates that the UNC-83a

159   N-terminal domain specifically disrupts kinesin-1-mediated nuclear migration events, confirming

160   its selective inhibitory function *in* vivo.

161

162   **Ectopic expression of *unc-83c* disrupts dynein-mediated P-cell nuclear migration.**

163   Our findings suggested a model where UNC-83 isoforms establish motor protein bias

164   through reciprocal inhibition. In this model, UNC-83a inhibits kinesin-1 while UNC-83c would be

165   predicted to inhibit dynein. To test this prediction, we examined whether elevated UNC-83c

166   levels in larval P cells could disrupt dynein-dependent P-cell nuclear migration. We generated

167   transgenic animals ectopically overexpressing the short *unc-83c* isoform under control of the P-

168   cell specific *hlh-3* promoter[63]. Two independent extrachromosomal arrays caused mild but

169   significant P-cell nuclear migration defects with 0.45 ± 0.20 and 0.75 ± 0.33 missing GABA

170   neurons compared to 0.1 ± 0.09 missing in wild-type animals (Fig. 3G, H). These mild defects

171   support our model where ectopic UNC-83c spuriously activates kinesin-1 in P-cells, occasionally

172   disrupting the normally dynein-dependent nuclear migration process.

173

174   **Spectrin-like repeats in the N-terminal domain of UNC-83a are necessary for dynein-**

175   **dependent nuclear migration.**

176   We next sought to investigate the regulatory mechanism behind dynein-dependent P-cell

177   nuclear migration. Previous genetic analysis revealed that mutations eliminating *unc-83a/b* while

178   preserving *unc-83c* cause temperature-sensitive defects in P-cell nuclear migration[52]. To

179   determine the critical regions within the UNC-83a/b N-terminus, we engineered a series of in-

180   frame deletions targeting the predicted spectrin-like repeats (Fig. 4A). We confirmed that the

181   deletion variants localized normally to the nuclear envelope by immunofluorescence (Fig. 4B).

182   Quantitative analysis of P-cell nuclear migration revealed distinct requirements for different parts

183   of the N-terminal domain. Disruption of both predicted spectrin-like repeats in *unc-83a(Δ58-*

184   *233)* caused severe P-cell nuclear migration defects at 25°C (an average of 7.70 ± 0.75 missing

185   GABA neurons), comparable to the *unc-83* null phenotype (Fig. 4C-D). Two smaller deletions in

186   the spectrin-like repeat region, *unc-83a(Δ58-166)* and *unc-83a(Δ167-233)*, also led to null-like

187   defects (Fig 4C). All three mutations exhibited temperature sensitivity, with defects progressively

188   worsening from 15°C to 20°C and then to 25°C (Fig. 2S), matching the same temperature-

189   sensitive phenotype of *unc-83* null animals. In contrast, a deletion just downstream of the

190   predicted spectrin-like repeats, *unc-83a(Δ234-305)*, caused only minor P-cell nuclear migration

191   defects (1.25 ± 0.45 missing GABA neurons) (Fig. 4C-D).

192   None of these N-terminal deletions disrupted the role of *unc-83c* in embryonic hyp7

193   nuclear migrations. None had a significant accumulation of hyp7 nuclei in the dorsal cord (Fig.

194   4E-F). Together, these results demonstrate that the spectrin-like repeats in the UNC-83a/b N-

195   terminal domain are required for dynein-dependent P-cell nuclear migration, but dispensable for

196   kinesin-1-dependent hyp7 nuclear migration.

197

198   **A conserved enhancer controls cell-type specific expression of *unc-83c* in embryonic**

199   **hypodermal precursors.**

200   The *unc-83* gene is hypothesized to have developmentally regulated expression of

201   different isoforms, with *unc-83a/b* thought to be expressed in larval P cells and *unc-83c* in

202 embryonic hyp7 precursors. We identified potential enhancer elements within the unusually long

203 introns just upstream of the first *unc-83c* exon. Comparative genomic analysis across four

204 *Caenorhabditis* species (*C. elegans*, *C. brenneri*, *C. remanei* and *C. briggsae*) identified a highly

205 conserved 870bp sequence (Fig. 3S; Fig. 5A).

206   To test the necessity of this predicted enhancer, we generated two independent alleles,

207 *unc-83(yc121)* and *unc-83(yc122)*, where we deleted 750bp of this region using CRISPR/Cas9

208 gene editing (Fig. 5A). Quantitative analysis of embryonic hyp7 and larval P-cell nuclear

209 migrations revealed identical phenotypes in *unc-83(yc121)* and *unc-83(yc122)* strains. An

210 average of about two hyp7 nuclei mislocalized in the dorsal cord (Fig. 5B-C), significantly higher

211 than in wild type, indicating the enhancer's necessity for hyp7 nuclear migration. The enhancer

212 deletion also caused subtle but significant defects in P-cell nuclear migration (Fig. 5D-E).

213   To test the enhancer's sufficiency to drive the expression of *unc-83c* in embryonic hyp7

214 precursors, we generated a transgene by fusing the 870bp region to a minimal promoter driving

215 the expression of GFP fused to the SV40 nuclear localization sequence (*nls::gfp*). This construct

216 specifically drove expression in pre-comma stage embryonic hyp7 precursors (Fig. 5F). These

217 results demonstrate that this conserved non-coding 870bp element functions as a cell-type

218 specific enhancer driving *unc-83c* expression in embryonic hyp7 precursors. This is consistent

219 with the earliest detected expression pattern of UNC-83 protein[52].

220

221 **The UNC-83a N-terminal domain inhibits kinesin-1 activity *in vitro*.**

222   Building on our genetic evidence that the UNC-83a-specific N-terminal domain inhibits

223 kinesin-1 while promoting dynein activity during P-cell nuclear migration, we investigated its

224 direct effects on kinesin-1 activity using biochemical approaches. We expressed and purified the

225 N-terminal domain of UNC-83a (UNC-83a[N-term]) from *E. coli* (Fig. 6A-B). Additionally, we purified

226 recombinant *C. elegans* kinesin-1 heterotetramers from Sf9 insect cells co-expressing full-length

227 KHC (UNC-116) and KLC (KLC-2) (Fig. 6A-B).   As mammalian kinesin-1 heterotetramers

228   require cargo adaptors for activation from their autoinhibited state[30,64], we first analyzed *C.*

229   *elegans* kinesin-1 recruitment to microtubules in the presence of a non-hydrolysable nucleotide

230   analog, AMP-PNP, which locks the motor domains in a high-affinity state on microtubules[64] (Fig.

231   6C-D). Kinesin-1 alone bound to microtubules as expected[64], whereas neither UNC-83a nor

232   UNC-83c showed any observable microtubule binding in the absence of kinesin (Fig. 6C).

233   Addition of excess UNC-83a or UNC-83c significantly enhanced kinesin-1 binding to

234   microtubules, with UNC-83c demonstrating greater efficiency (Fig. 6C-D). In contrast, UNC-

235   83a[N-term] failed to increase kinesin-1 recruitment to microtubules above baseline levels (Fig. 6C-

236   D).

237   We next assessed the impact of UNC-83a, UNC-83c and UNC-83a[N-term], on kinesin-1

238   motor function using microtubule gliding assays (Fig. 6E-F, Movie S1-4). Both UNC-83a and

239   UNC-83c enhanced microtubule gliding velocity when added in excess to kinesin-1 (Movie S2-

240   3). Strikingly, UNC-83a[N-term] significantly reduced the average gliding velocity compared to

241   kinesin-1 alone (Figs. 6E-F and Movies S1 and S4), providing direct evidence that the N-

242   terminal domain of UNC-83a functions as a kinesin-1 inhibitor. Importantly, we saw no evidence

243   of UNC-83a or UNC-83a[N-term] binding directly to microtubules (Fig. 6C), indicating the blockage

244   of kinesin-1 motility is a direct effect on kinesin and not a secondary effect of microtubule-

245   binding.

246

247   The UNC-83a N-terminal domain directly binds kinesin heavy chain.

248   Having established that UNC-83a inhibits kinesin-1 activity both *in vivo* and *in vitro*, we

249   investigated the molecular mechanism underlying this inhibition. We hypothesized that the

250   UNC-83a[N-term] domain inhibits kinesin-1 by directly binding the KHC UNC-116. To test this

251   hypothesis, we purified full-length UNC-116 from Sf9 cells (Fig. 7A), confirming that the purified

252   protein existed predominantly (82%) in its expected dimeric state[28] (Fig. 7B). We then

253   performed binding studies by separately incubating UNC-83a[N-term], UNC-83a, and UNC-83c with

254   UNC-116 (Fig. 7D'-F'). Both UNC-83a^{N-term} and UNC-83a formed stable complexes with UNC-

255   116 (one monomer of UNC-83a or UNC-83a^{N-term} binding one dimer of UNC-116) as detected by

256   mass photometry (Fig. 7D-D', E-E'). In contrast, UNC-83c failed to interact with UNC-116 (Fig.

257   7F-F'). We then performed binding studies by separately incubating UNC-83a and UNC-83c

258   with kinesin-1 tetramer (UNC-116 + KLC-2) (Fig. 7C). We observed one UNC-83a monomer

259   forming a stable complex with one kinesin-1 tetramer while UNC-83c did not (Fig. 7D'', F'').

260   These findings demonstrate that the UNC-83a-specific N-terminal domain mediates direct

261   binding to UNC-116, providing a molecular mechanism for the observed kinesin-1 inhibition both

262   *in vivo* (Fig. 3A-D) and *in vitro* (Fig. 6E-F).

263

264   **DISCUSSION**

265   Spatial and temporal control of nuclear positioning is fundamental for development, yet

266   the mechanisms regulating bidirectional nuclear movement have remained elusive. Our work

267   reveals a novel paradigm where expression of alternative isoforms of a cargo adaptor creates

268   an intrinsic molecular switch for controlling nuclear migration directionality. Previous studies

269   identified UNC-83 as a nuclear-specific cargo adaptor that recruits both kinesin-1 and dynein to

270   mediate bidirectional movement of nuclei[15,55,56]. Genetic analyses suggested a model where the

271   short UNC-83c isoform is sufficient for kinesin-driven nuclear migration in the embryo and the

272   long UNC-83a/b isoforms are necessary for dynein-mediated nuclear migration in larva[52]. Here

273   we demonstrate that distinct UNC-83 isoforms differentially regulate motor activity to achieve

274   precise developmental control of nuclear positioning.

275   Our discovery of an inhibitory N-terminal-specific domain in the long UNC-83a/b

276   isoforms represents a previously unrecognized mechanism for motor regulation. The genetic

277   and biochemical analyses reported here reveal this domain's dual regulatory function: direct

278   binding and inhibition of the kinesin heavy chain UNC-116, combined with reduced affinity for

279   the kinesin light chain KLC-2. This regulation allows UNC-83a/b to suppress kinesin-1 activity

280   while permitting dynein-directed movement. In contrast, UNC-83c's high-affinity interaction with

281   KLC-2 through its EWD motif[13,36] promotes kinesin-1 activation, enabling plus-end-directed

282   nuclear movement in embryonic hyp7 precursors. This isoform-specific regulation solves a

283   challenge in developmental biology: how to achieve opposing directional movements of the

284   same cargo using the same molecular machinery.

285   The involvement of UNC-83 with multiple dynein-regulatory proteins, including the

286   homolog of long coiled-coil dynein activating adaptor Bicaudal D, BICD-1[56], suggests integration

287   into a larger regulatory network. This organization parallels other bidirectional transport

288   events[65,66]. For example, TRAK1/2 mediates mitochondrial trafficking through the outer

289   mitochondrial membrane receptor Miro and metaxins[67,68,69], and HOOK3 regulates endosomal

290   trafficking with FTS and FHIP1B[70,71,72,73]. Nuclei, which are larger and stiffer than mitochondria

291   and endosomal vesicles[74], present unique challenges for intracellular transport. Our findings

292   and the conservation of LINC complex-mediated nuclear positioning[17,22,54] suggest that

293   evolution has developed specialized mechanisms to address the challenges of moving nuclei.

294   Mammalian KASH proteins Nesprin-2, Nesprin-4, and KASH5 each interact with microtubule

295   motors through mechanisms similar to UNC-83[13,14,60,41,42,64,75].

296   The identification of spectrin-like repeats in UNC-83 with AlphaFold predictions reveals

297   structural similarities to *Drosophila* Klarsicht[76] and mammalian nesprins[13,14,60,41,42,64,75]. This

298   structural conservation, combined with common motor-binding mechanisms, suggests that

299   motor regulation at the nuclear envelope through alternatively spliced inhibitory domains may be

300   more widespread than previously appreciated. The modular nature of KASH protein interactions

301   with the cytoskeleton appears to be an evolutionarily conserved feature, potentially allowing

302   rapid adaptation to diverse cellular contexts.

303   Our data support a selective activation model for bi-directional transport, rather than

304   selective recruitment or tug-of-war models[66]. The selective activation model could explain the

305   steady, but slow nature of nuclear movements[77]. Moreover, cargo stiffness has been speculated

306   to alter motor engagement[78,79]. Nuclei are the largest and one of the stiffer intracellular cargos

307   and move significantly slower than small cargos. Hyp7 and P-cell nuclei migrate at a speed of

308   ~1μm per minute[49,56]. The discovery that expression of alternative isoforms can generate cargo

309   adaptors with opposing regulatory functions provides a new framework for understanding how

310   cells achieve precise spatiotemporal control of organelle positioning. This mechanism may be

311   particularly crucial for nuclei, where their unique physical properties[74] require additional

312   regulation.

313   These findings open several exciting directions for future investigation. Does Klarsicht

314   employ similar inhibitory mechanisms during *Drosophila* development? Have mammalian

315   systems evolved analogous regulatory strategies through their expanded repertoire of KASH

316   proteins? Understanding how broadly this regulatory paradigm extends could reveal

317   fundamental principles of developmental organelle positioning. Moreover, our work suggests

318   that expression of alternative isoforms of cargo adaptors may represent a general mechanism

319   for generating precise spatiotemporal control of intracellular transport during development.

320

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be

fulfilled by the lead contact, Daniel A. Starr (dastarr@ucdavis.edu)

Materials availability

All unique reagents generated in this study are available from the lead contact without

restriction.

Data and code availability

Data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

AUTHOR CONTRIBUTIONS

Conceptualization: SG, GWGL, RJM, DAS. Data curation: SG, NS, DE, EFG, KC. Formal analysis: SG, NS, DE, EFG, KC. Investigation: SG, NS, DE, EFG, KC. Methodology: all authors. Project Administration: SG, DAS. Resources: all authors. Validation: all authors. Visualization: SG, GWGL, RJM, DAS. Writing-original draft: SG, DAS. Writing-review and editing: SG, SN, GWGL, RJM, DAS.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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FIGURE LEGENDS

Figure 1: Different isoforms of UNC-83 mediate nuclear migration throughout development. A) Schematic showing the kinesin-1-mediated migration of embryonic hypodermal hyp7 nuclei (cyan) across the dorsal midline (gray) towards the plus-ends of microtubules (green). B) Schematic illustrating the dynein-driven migration of larval P-cell nuclei (cyan) towards the ventral cord (gray), where the minus-ends of microtubules are positioned. C) Model of the hypothesis tested in this study. The short isoform UNC-83c activates kinesin-1 and/or inhibit dynein, making kinesin-1 the main motor driving embryonic hyp7 nuclear migration. The longer isoforms UNC-83a/b activate dynein and/or inhibit kinesin-1, making dynein the main driver of larval P-cell nuclear migration. D) AlphaFold3 predicted structure of the long isoform, UNC-83a. C-D) Spectrin-like repeats (SRs) SR1 and SR2 (unique to UNC-83a/b) are in magenta; SR3, SR4 and SR5 (common to all isoforms) are in dark blue. The transmembrane domain (TM) is shown in dark gray. The KASH peptide is shown in cyan. The EWD motif is highlighted in red (see Figure S1 for confidence and error scores of the predicted structure).

Figure 2: The N-terminal domain of the long UNC-83a isoform inhibits KLC-2 binding. A) AlphaFold3-predicted interaction of UNC-83c's EWD motif (red) with the TPR domain (green) of the kinesin light chain, KLC-2. B) Coomassie-stained protein gels of purified UNC-83a, UNC-83c and KLC-2 recombinant proteins. C) Schematic of the GST-KLC-2 construct (GST tag in gray, coiled-coil domain in dark green, and TPR domain in light green) and a Gaussian fit of its measured molecular weight in solution (30 nM; monomer = 92 ± 19.8 kDa, 72% peak population; dimer = 177 ± 59 kDa, 21% peak) as determined by mass photometry. D) Schematics and molecular weight distributions of UNC-83c-mScarlet (30 nM; monomer = 99 ±

380   47 kDa, 95% peak) and UNC-83a-mScarlet (30 nM; monomer = 130 ± 62 kDa, 96% peak). SR1

381   and SR2 (unique to UNC-83a/b) are in magenta; SR3, SR4 and SR5 (common to all isoforms)

382   are in dark blue; mScarlet-I tag in fuchsia, and Strep-II tag in dark gray. E) Molecular weight

383   distributions of KLC-2 preincubated with UNC-83c (30 nM; one UNC-83c monomer + one KLC-2

384   monomer = 174 ± 134 kDa, 97% peak) and UNC-83a (30 nM; one UNC-83a monomer = 138 ±

385   62 kDa, 80% peak). F) Binding curves of UNC-83a and UNC-83c to KLC-2, showing an affinity

386   of 0.15 µM for UNC-83c and >1.0 µM for UNC-83a. The bait, GST-KLC-2, was diluted to 1 µM

387   and immobilized onto the biosensors. Both UNC-83a and UNC-83c were tested at

388   concentrations of 1.0, 0.667, 0.444, 0.296, 0.197, 0.132, 0.088 µM.

389   **Figure 3: Ectopic expression of *unc-83a* and *unc-83c* disrupts kinesin-1-dependent hyp7**

390   **nuclear migration and dynein-mediated P-cell nuclear migration, respectively.**

391   A) Schematic of the *unc-83(yc108[UNC-83a])* mutant, expressing the UNC-83a isoform under

392   control of the *unc-83c* promoter. B) UNC-83 (shown in red) in *unc-83(yc108[UNC-83a])* is

393   localized to the nuclear envelope in a comma-stage embryo. Dorsal is up and anterior is left.

394   Arrows point to the hyp7 nuclei. C) Quantification of hyp7 nuclear migration defects in wild type,

395   *unc-83(yc108[UNC-83a])*, and *unc-83(e1408)* null animals via counting the number of

396   mislocalized (at the dorsal cord) nuclei. Each point represents the total number of abnormally

397   located hyp7 nuclei per animal; n = 40 for each strain. D) Lateral views of *wild type* and *unc-*

398   *83(yc108[UNC-83a])* L4 larva expressing hypodermal nuclear GFP. Dashed yellow lines mark

399   the dorsal cord of the animal. Mislocalized nuclei (arrows) indicate migration failure. E)

400   Quantification of dynein-mediated larval P-cell nuclear migration defects in *wild type*, mutant

401   *unc-83(yc108[UNC-83a])*, and *unc-83(e1408)* null animals via counting number of missing

402   GABA neurons at 25°C. Each point represents the number of missing GABA neurons per

403   animal; n = 40 for each strain. F) Lateral views of *wild type* and *unc-83(yc108[UNC-83a])* L4

404   larva expressing a GABA neuron nuclear marker (*unc-47::gfp*). Numbers indicate a total of 19

405   GABA neurons. G) Quantification of larval P-cell nuclear migration defects in wild type, *unc-*

406 *83(e1408)* null, and *unc-83c* overexpression animals (*unc-83(ycEx301[phlh3::unc-83c(+)])* and

407 *unc-83(ycEx302[phlh3::unc-83c(+)])*) via counting number of missing GABA neurons at 25°C.

408 Each point represents the number of missing GABA neurons per animal; n = 40 for each strain.

409 H) Lateral view of *unc-83(ycEx302)* L4 larvae expressing *unc-47::gfp*. Numbers indicate a total

410 of 16 GABA neurons in larva. For all plots: Means with 95% CI are shown in error bars.

411 Unpaired student t-tests were performed on the indicated comparisons; ns means not

412 significant, p>0.05; * p<0.05; **** p<0.0001. For images: Scale bars = 10 µm for B), and 42.2

413 µm for D), F) and H).

414

415 **Figure 4: Spectrin-like repeats in the N-terminal domain of UNC-83a are necessary for**

416 **dynein-dependent nuclear migration.** A) Schematics of the *unc-83* N-terminal domain

417 deletion mutants. B) Immunofluorescence staining shows that the deletion mutants *unc-*

418 *83a(Δ234-305), unc-83a(Δ58-166), unc-83a(Δ58-233)*, and *unc-83a(Δ167-233)* have a normal

419 UNC-83 (red) localization pattern at the nuclear envelope of hyp7 precursor cell nuclei in

420 comma-stage embryos. White arrows indicate nuclei with UNC-83 localization. Dorsal is up and

421 anterior is left. C) Quantification of larval P-cell nuclear migration defects in the indicated strains

422 at 25°C (See Figure S2 for the P-cell nuclear migration defects of the indicated strains at 20°C

423 and 15°C.). Each point represents the number of missing GABA neurons per animal; n = 40 for

424 each strain. D) Lateral views of   *unc-83a(Δ234-305)* and *unc-83a(Δ58-233)* L4 larvae

425 expressing *unc-47::gfp.* Numbers indicate total GABA neurons. E) Quantification of hyp7

426 nuclear migration defects in the indicated strains. Each point represents the total number of

427 abnormally located hyp7 nuclei per animal; n = 40 for each strain. F) Lateral views of *unc-*

428 *83a(Δ234-305)* and *unc-83a(Δ58-233)* L4 larvae expressing hypodermal nuclear GFP. Dashed

429 yellow lines mark the dorsal cord of the animals. Arrows indicate mislocalized hypodermal

430 nuclei, representing failed nuclear migrations. For all plots: Means with 95% CI are shown in

431 error bars. Unpaired student t-tests were performed on the indicated comparisons; ns means

432   not significant, p>0.05; * p<0.05; **** p<0.0001. For images: Scale bars = 10 µm for B) and 42.2

433   µm for D) and F).

434

435   **Figure 5: A conserved enhancer controls cell-type specific expression of *unc-83c* in**

436   **embryonic hypodermal precursors.** A) Schematics of the *unc-83* locus in *wild type,* and

437   enhancer deletion mutants (*unc-83(yc121)* and *unc-83(yc122)*). The 870bp enhancer region is

438   highlighted in red. Exons are shown as blue boxes. The start codon for *unc-83a* in exon 1, *unc-*

439   *83b* in exon 3, and *unc-83c* at the end of exon 8 are indicated. The sizes of introns and exons

440   are according to the scale as indicated (scale bar=1 kb). (See Figure S3 for the sequence

441   alignment). B) Quantification of hypodermal hyp7 nuclear migration defects in the indicated

442   strains. Each point represents the total number of abnormally located hyp7 nuclei per animal; n

443   = 40 for each strain. C) Lateral view of *unc-83(yc121)* L4 larva expressing hypodermal nuclear

444   GFP. Dashed yellow line mark the dorsal of the animal. Arrows indicate mislocalized

445   hypodermal nuclei, representing failed nuclear migrations. D) Quantification of larval P-cell

446   nuclear migration defects in the indicated strains at 25°C. Each point represents the number of

447   missing GABA neurons per animal; n = 40 for each strain. E) Lateral view of *unc-83(yc121)* L4

448   larva expressing *unc-47::gfp.* Numbers indicate a total number of 18 GABA neurons. F-G) *C.*

449   *elegans* pre-comma stage embryos expressing *unc-83* 870bp enhancer region driving *nls::gfp.*

450   F) Expression of the 870 bp enhancer region (green) in pre-comma stage embryo, showing

451   hyp7 precursor-specific expression. For all plots: Means with 95% CI are shown in error bars.

452   Unpaired student t-tests were performed on the indicated comparisons; ns means not

453   significant, p>0.05; * p<0.05; **** p<0.0001. For images: Scale bars = 42.2 µm for C) and E) and

454   10 µm for F) and G).

455

456   **Figure 6: The UNC-83a N-terminal domain inhibits kinesin-1 activity *in vitro.*** A)

457   Coomassie- stained gels of purified UNC-83a[N-term] and UNC-116-KLC-2 (kinesin-1) recombinant

458   proteins. Arrows indicate the bands for the different proteins. B) Schematics of the indicated

459   recombinant protein constructs. The coiled-coil domains of UNC-116 and KLC-2 are indicated

460   as 'CC'. UNC-116 is co-expressed with KLC-2. The co-expression leads to the formation of

461   kinesin-1 heterotetramer that is expected to be 362 kDa. C) Representative TIRF images

462   showing the recruitment of kinesin-1 (green) in the rigor state (with 2 mM AMP-PNP) onto

463   microtubules (blue) and the effects of the indicated UNC-83 constructs (magenta). Scale bar = 5

464   µm. D) Quantification of fluorescence intensity the indicated constructs, n = 30 microtubules for

465   each condition. E) Representative kymographs of microtubule gliding with kinesin-1 alone (See

466   Movie S1), kinesin-1 + UNC-83c (See Movie S2), kinesin-1 + UNC-83a (See Movie S3), and

467   kinesin-1 + UNC-83a$^{\text{N-term}}$ (See Movie S4). Scale bars: 20 s (vertical) and 5 µm (horizontal). F)

468   Quantification of microtubule gliding velocities for the indicated conditions, n = 25 microtubules

469   for each condition. The average velocities are: 1,064 ± 35 nm/s (kinesin-1), 1,150 ± 149 nm/s

470   (kinesin-1 + UNC-83c), 1,309 ± 82 nm/s (kinesin-1 + UNC-83a), 187 ± 533 nm/s (kinesin-1 +

471   UNC-83a$^{\text{N-term}}$).

472

473   **Figure 7: The UNC-83a N-terminal domain directly binds kinesin heavy chain.** A) A

474   Coomassie-stained gel showing purified UNC-116-sfGFP. B) A schematic of the UNC-116-

475   sfGFP recombinant protein and a Gaussian fit of its measured molecular weight in solution by

476   mass photometry (30 nM; monomer = 109 ± 19.5 kDa, 13% peak; dimer = 246 ± 44 kDa; 82%

477   peak). Coiled-coil domains indicated as 'CC'. C) A Gaussian fit of UNC-116-KLC-2 (kinesin-1)

478   recombinant protein's measured molecular weight in solution by mass photometry (30 nM;

479   tetramer = 360 ± 69 kDa, 88% peak).   D-D'') A schematic of the UNC-83a-mScarlet

480   recombinant protein and a Gaussian fit of its measured molecular weight in solution alone (30

481   nM; monomer = 130 ± 62 kDa; 96% peak) (D), when preincubated with UNC-116 (30 nM; one

482   UNC-83a monomer + one UNC-116 dimer = 391 ± 153 kDa, 99% peak) (D'), and when

483   preincubated with UNC-116-KLC-2 (kinesin-1) (30 nM; one UNC-83a monomer + one kinesin-1

484   tetramer = 531 $\pm$ 272 kDa, 97% peak) (D''). E-E') A schematic of the UNC-83a$^{N\text{-term}}$-mScarlet

485   recombinant protein and a Gaussian fit of its measured molecular weight in solution alone (30

486   nM; dimer = 120 $\pm$ 23 kDa, 100% peak) (E) or when preincubated with UNC-116 (30 nM; one

487   UNC-83a$^{N\text{-term}}$ monomer + one UNC-116 dimer = 307 $\pm$ 89 kDa, 99% peak) (E'). F-F'') A

488   schematic of the UNC-83c-mScarlet recombinant protein and a Gaussian fit of its measured

489   molecular weight in solution alone (30 nM; monomer = 108 $\pm$ 41 kDa, 100% peak) (F), when

490   preincubated with UNC-116 (30 nM; 103 $\pm$ 21 kDa, 30%; 245 $\pm$ 82 kDa, 70% peak) (F'), and

491   when preincubated with UNC-116-KLC-2 (kinesin-1) (30 nM; 128 $\pm$ 41 kDa, 24% peak; 360 $\pm$

492   110 kDa, 72% peak) (F'').

493

494

495   **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

496   *C. elegans* **maintenance and genetics**

497   *C. elegans* strains were maintained at room temperature (approximately 22°C), unless

498   otherwise indicated, on nematode growth medium plates seeded with OP50 *E. coli* [80]. Some

499   strains were obtained from the Caenorhabditis Genetics Center, funded by the National

500   Institutes of Health Office of Research Infrastructure Programs (P40 OD010440). All the strains

501   used in this study are listed on the Key Resources Table.

502

503   **Protein expression systems**

504   *Spodoptera frugiperda* Sf9 cells were cultured in suspension using Sf-900II serum-free

505   medium (SFM) (ThermoFisher Scientific) supplemented with 10 µg/mL gentamycin. Cultures

506   were maintained at 27°C with constant rotation at 150 rpm. The culture density was maintained

507 between 1.0×10^6 and 8.0×10^6 cells/mL during routine passaging. Cells were not authenticated.

508 The sex of the cells is unknown.

509 BL21-CodonPlus (DE3)-RIPL *E. coli* (Agilent) transformed with expression plasmids

510 were cultured in LB medium at 37°C with shaking at 200 rpm.

511

512 **METHOD DETAILS**

513 **Protein structure prediction**

514 Structural models of UNC-83a and the UNC-83c/KLC-2 complex were generated using

515 AlphaFold3[59] with default parameters, without template structures. Five models were generated

516 for each protein or complex. The models with the highest confidence scores (for the UNC-

517 83c/KLC-2 complex: pTM = 0.44 and ipTM = 0.39) were used for figure preparation. For

518 visualization, some residues within the low-confidence unstructured region between the

519 spectrin-like repeats #3 and #4 in UNC-83a were straightened by altering the torsion angles[81] (ϕ

520 and ψ to approximately -135° and 135°, respectively) with ChimeraX[82].

521

522 **Plasmid construction and molecular cloning**

523 *Expression constructs*

524 The glutathione-S-transferase (GST)-KLC-2 construct was generated as previously

525 described[55]. A cDNA construct encoding for UNC-83a[52] was used to generate the UNC-83a-

526 mScarlet-StrepII, UNC-83c-mScarlet-StrepII, and UNC-83a^N-term-mScarlet-StrepII constructs.

527 Using Gibson Assembly, UNC-83a, UNC-83c and UNC-83a^N-term coding regions were cloned

528 into pET21a-Novagen bacterial expression vector (Millipore Sigma) with a C-terminal mScarlet-

529 StrepII tag. For insect cell expression, UNC-116-sfGFP-StrepII and KLC-2-6xHis-UNC-116-

530 sfGFP-StrepII constructs were cloned into pACEBac1 vector (Geneva Biotech).

531 *Promoter and enhancer constructs*

532   The pSL536 plasmid was made by cutting the 3.4 kb fragment of *hlh-3* promoter from

533 pRD15[63] (using HindIII and SmaI restriction enzyme sites) and ligating this fragment 442 bp

534 upstream of the *unc-83c* start codon into AgeI/BamHI/Klenow treated pDS22[52]. For pSL439, the

535 870bp intronic fragment from the *unc-83* region between exons 5 and 6 was cloned using StuI

536 and XbaI, and inserted into pPD97.82 (Addgene plasmid #1512, a gift from the Fire Lab). The

537 *odr-1::rfp* plasmid was a gift from Noelle L'Etoile (University of California, San Francisco)[83].

538 *Sequence verification*

539   All constructs were verified by whole plasmid sequencing performed by Plasmidsaurus

540 using Oxford Nanopore Technology with custom analysis and annotation. A complete list of

541 plasmids used in this study is provided in the Key Resources Table.

542

543 **Protein expression and purification**

544   Bacterial expression and preparation of GST-KLC-2, UNC-83a-mScarlet-StrepII, UNC-

545 83c-mScarlet-StrepII, and UNC-83a[N-term]-mScarlet-StrepII were performed as described below.

546 BL21-CodonPlus (DE3)-RIPL (Agilent) *E. coli* were transformed with each plasmid and grown at

547 37°C in LB medium with 100 µg/mL carbenicillin until optical density at 600 nm (OD_{600}) reached

548 0.8. The cultures were then cooled down to room temperature and protein expression was

549 induced by adding 0.2 mM isopropyl-b-D-thiogalactoside (IPTG) and incubating overnight at

550 18°C. Bacterial pellets were stored at -80°C. For recombinant protein purification, frozen pellets

551 were thawed on ice and resuspended in 50 mL lysis buffer (PB: 50 mM Tris-HCl pH 8.0, 150

552 mM KCH3COO, 2 mM MgSO4, 1 mM EGTA, and 5% glycerol) freshly supplemented with 1 mM

553 dithiothreitol (DTT), 0.2 mM phenylmethyl- sulfonyl fluoride (PMSF), 0.1 mM ATP, NucA

554 nuclease, and protease inhibitor mix (Promega). Bacteria were lysed using an Emulsiflex C3

555 high-pressure homogenizer (Avestin). For UNC-83a-mScarlet-StrepII, UNC-83c-mScarlet-

556 StrepII, and UNC-83a[N-term]-mScarlet-StrepII purifications, a subsequent chemical lysis was

557 performed with 0.15% Triton X-100 for 5 minutes on ice. Lysed cells were centrifuged at

558   23,000xg for 20 minutes at 4°C. For affinity purification, the clarified lysates were incubated

559   with resin as follows. UNC-83a-mScarlet-StrepII, UNC-83c-mScarlet-StrepII, and UNC-83a^{N-term}-

560   mScarlet-StrepII lysates were incubated with Streptactin XT resin (IBA Lifesciences) for 1 hour

561   at 4°C and washed with PB buffer. Proteins were eluted with biotin elution buffer (100mM biotin

562   in PB buffer freshly supplemented with 1 mM DTT, 0.1 mM PMSF, 0.1 mM ATP, NucA

563   nuclease, 0.013% Triton X-100 at pH 8.0). For GST-KLC-2, Glutathione Sepharose 4B resin

564   (Cytiva) was added to the clarified lysate and incubated on a rocker for 5 hours at 4°C. The

565   beads were then washed with 1x PBS (Phosphate-buffered saline) and protein was eluted with

566   glutathione elution buffer (50 mM Tris-HCl pH8.0, 10 mM L-Glutathione reduced; (Sigma-

567   Aldrich).

568   For the UNC-116-sfGFP-StrepII and KLC-2-6xHis-UNC-116-sfGFP-StrepII constructs

569   cloned into pACEBac1 vector, DH10MultiBac (Geneva Biotech) *E. coli* were transformed to

570   generate bacmids. To prepare baculovirus, $1.0x10^6$ Sf9 cells were transferred to each well of a

571   tissue-culture treated 6-well plate. Once the cells were adhered to the bottom of the dish, about

572   3 µg bacmid were transfected using 6 µL of Cellfectin II reagent (ThermoFisher Scientific). 5-7

573   days after initial transfection, the culture media were collected and spun at 3,000xg for 3

574   minutes to obtain the viral supernatant (P1). Next, 50 mL of Sf9 cells ($2.0 x10^6$ cells/mL) was

575   infected with 30 µL of P1 and cultured for 5-7 days to obtain P2 viral supernatant. For protein

576   expression, 400 mL of Sf9 cells ($2.0x10^6$ cells/mL) were infected with 4 mL of P2 virus and

577   cultured for 65 hours at 27°C while shaking. Cells were harvested by spinning down at 1,992xg

578   for 20 minutes at 4°C. The pellets were stored at -80°C.

579   UNC-83a^{N-term}-mScarlet-StrepII, UNC-116-sfGFP-StrepII and KLC-2-6xHis-UNC-116-

580   sfGFP-StrepII were further purified via size exclusion chromatography using Superose6 10/300

581   GL column (Cytiva) equilibrated in buffer GF150 (25 mM HEPES pH 7.2, 150 mM KCl, 2 mM

582   MgCl$_2$). GST-KLC-2, UNC-83a-mScarlet-StrepII and UNC-83c-mScarlet-StrepII were purified via

583   size exclusion chromatography using Superdex 200 10/300 GL column (Cytiva) equilibrated in

584   buffer GF150. Fractions containing the proteins were combined and concentrated on Amicon

585   spin filters with a 50 kDa or 100 kDa cutoff. Concentrated proteins were supplemented with 10%

586   glycerol, flash frozen in LiN2 and stored at □-80°C.

587

588   **Single-molecule mass photometry**

589   Single-molecule mass photometry was performed using a OneMP instrument (Refeyn)

590   as previously described[60, 84]. Briefly, #1.5 24 x 50 mm Deckgläser glass coverslips

591   (ThermoFisher Scientific) were cleaned by 1 hour sonication in isopropanol and subsequently

592   stored in a fresh aliquot of isopropanol. Before assembling the imaging chambers, the

593   coverslips were rinsed with fresh isopropanol followed by Milli-Q water and dried with filtered air.

594   Then, CultureWell silicone gaskets (Grace Bio-Labs) were placed onto the freshly cleaned

595   coverslips providing 4-6 independent sample chambers. For all the cleaning steps, 99.9%

596   (HPLC grade) isopropanol (Fisher Scientific) was used. Samples and standard proteins

597   (Apoferritin, Bovine serum albumin (BSA), and Thyroglobulin; Sigma-Aldrich) were diluted to 10-

598   100 nM in freshly 0.22 µm filtered SRP90 buffer (90 mM HEPES-KOH, 50 mM KCH3COO, 2

599   mM Mg(CH3COO)2, 1 mM EGTA, and 10% glycerol, pH = 7.6). The focal position was identified

600   and maintained throughout the measurement using an autofocus system based on total internal

601   reflection. Data acquisition was performed at 1 kHz for 60 seconds using AcquireMP (Refeyn),

602   and data analysis was conducted using DiscoverMP (Refeyn).

603

604   **Biolayer interferometry binding assays**

605   The real-time binding kinetics of UNC-83a-mScarlet-StrepII and UNC-83c-mScarlet-

606   StrepII to GST-KLC-2 were measured using biolayer interferometry on a ForteBio Octet®-

607   RED384 at the UC Davis Octet® Real-Time Drug and Protein Binding Kinetics Unit. Binding

608   assays were performed in binding buffer (20 mM Tris pH7.8, 150 mM NaCl, 0.1 mg/mL BSA,

609   0.05% Tween-20) (modified from Bhattacharya et al., 2014)[85] using the Octet® Anti-Glutathione-

610   S-Transferase (GST) Biosensors (Sartorius AG). Operating temperature was maintained at

611   26°C with a 1,000 rpm rotary agitation throughout the experiment. Samples or buffer were

612   transferred into 96-well plates (Greiner) at a volume of 250 µL per well. GST biosensor tips were

613   rehydrated in binding buffer before loading the bait onto them. The bait, GST-KLC-2, was

614   diluted to 1 µM and immobilized onto the biosensors for 10 minutes to attain 1.5 - 2.5 nm

615   response. Binding association of UNC-83a and UNC-83c with biosensor tips was monitored for

616   20 minutes, and subsequent disassociation in buffer was monitored for 30 minutes. Both UNC-

617   83a and UNC-83c were tested at concentrations of 1.0, 0.667, 0.444, 0.296, 0.197, 0.132, 0.088

618   µM. The average of the last 100 points (20 seconds) of the of the association phase response

619   was used as an estimate for the equilibrium value of the binding reaction. The plots of

620   concentration versus averaged plateau values were fitted using a quadratic binding equation

621   plus a constant offset to find K_d.

622

623   ***C. elegans* transgenic strains**

624   To generate transgenic strains UD336 and UD338, which express nuclear GFP

625   (*nls::*GFP) under a minimal promoter with the 870 bp predicted *unc-83* enhancer, N2 animals

626   were injected with 5 ng/µL pSL439 and 100 ng/µL *odr-1::rfp* co-injection marker[83]. The

627   integrated transgene *ycIs3[unc-83(870bp)::min_myo-2 promoter::nls::gfp; odr-1::rfp]* was

628   generated with gamma irradiation from a Cs-137 source[86]. For strains UD973 and UD974,

629   *oxIs12* (EG1285) animals were injected with 5 ng/µl of pSL536 (*phlh-3::unc-83c*), 93 ng/µl of

630   pBlue-script SK, and 2 ng/µl of Pmyo-2::mRFP.

631   *unc-83a* deletion mutant alleles (*yc77,yc79, yc95* and *yc123*) and *unc-83* enhancer

632   region deletion mutant alleles (*yc121* and *yc122*) were generated using the *dpy-10* co-CRISPR

633   strategy[87, 88, 89]. The CRISPR injection mix was made as described previously[90]. As repair

634   templates, 100-nucleotide-long single-strand DNA oligonucleotides (ssODN) were synthesized

635   (Integrated DNA Technologies). Deletion mutations were screened by PCR amplification of the

636   guide target region, and products showing a smaller band size were confirmed via Sanger

637   sequencing. The crRNA and ssODN repair template sequences are listed in the Key Resources

638   Table .

639   *unc-83a*[N-term] overexpression insertion mutant allele (yc108) was generated by using

640   *zen-4(+)* as a co-CRISPR marker[91]. A single-stranded repair template containing the insertion

641   flanked by 50-nucleotide right and left homology arms, was synthesized (Genewiz). The

642   CRISPR injection mix contained 0.2 µl *zen-4* crRNA (0.6 mM), 0.5 µl target gene crRNA (0.6

643   mM), 2.46 µl tracr (0.17 mM), 7.68 µl Cas9 (40 µM), 0.28 µl ssODN *zen-4(+)* repair template

644   (500 ng/µl) and 2 µl ssDNA repair template (1µg/µl). The mixture was injected into the germline

645   of temperature-sensitive *zen-4(cle10)* mutant young adults and screened as previously

646   described[91].

647

648   **Nuclear migration assays in *C. elegans***

649   To quantify larval P-cell nuclear migration, strains were crossed into *oxIs12[punc-*

650   *47::gfp],* which labels P-cell derived GABA neurons[92]. The presence of 19 GABA neurons (12

651   derived from P cells) that are normally GFP-positive with cell bodies located in the ventral cord

652   were scored for as previously described[62]. The assay was performed at 3 different temperatures

653   (15°C, 20°C and 25°C) as *unc-83*-dependent P-cell nuclear migration is temperature-sensitive[52].

654   To quantify embryonic hyp7 nuclear migration, strains were crossed into *ycIs9 I,* a GFP-

655   expressing nuclear marker specific to larval hypodermal nuclei[20]. Syncytial hyp7 nuclei were

656   scored as abnormally located if they were in the dorsal cord as previously described [62].

657

658   **Immunofluorescence in *C. elegans***

659   Immunofluorescence staining with the anti-UNC-83 antibody (1209D7) was done as

660   previously described[52]. The primary antibody 1209D7 was used undiluted. The secondary

661   antibody, Alexa Fluor® 555 conjugated goat anti-mouse (H+L) Cross-Adsorbed IgG

662   (ThermoFisher Scientific) was diluted 1:200 in phosphate-buffered saline containing 0.1% Triton

663   X-100 (PBST). DNA was visualized by a 10-minute stain in 100 ng/ml of 4,6-diamidino-2-

664   phenylindole (DAPI; Sigma) in PBST. Images were captured using a Leica DM6000

665   fluorescence microscope with a 63 Å~ Plan Apo 1.40 NA objective, a Leica DC350 FX camera,

666   and Leica LAS AF software.

667

668   **Microtubule preparation**

669   Porcine brain tubulin was isolated using the high-molarity PIPES procedure and then

670   labeled with biotin NHS ester or Dylight-405 NHS ester (ThermoFisher Scientific), as described

671   previously

672   (https://mitchison.hms.harvard.edu/files/mitchisonlab/files/labeling_tubulin_and_quantifying_lab

673   eling_stoichiometry.pdf). Pig brains were obtained from a local abattoir and used within ~4

674   hours. post-mortem. To polymerize microtubules, 50 mM of unlabeled tubulin, 10 µM of biotin-

675   labeled tubulin and 3.5 µM of Dylight-405-labeled tubulin were incubated with 2 mM GTP for 20

676   minutes at 37°C. Polymerized microtubules were stabilized by the adding 20 µM taxol

677   (ThermoFisher Scientific) and incubated an additional 20 minutes at room temperature.

678   Microtubules were pelleted at 20,000 x g by centrifugation through a 150 µl of 25% sucrose

679   cushion. The pellet was resuspended in 50 µl BRB80 (80 mM Pipes (pH 6.8), 1 mM MgCl2 and

680   1 mM EGTA) containing 10 µM taxol.

681

682   **TIRF assays**

683   For TIRF assays, imaging chambers were assembled by combining an acid-washed

684   glass coverslip prepared as previously described

685   (http://labs.bio.unc.edu/Salmon/protocolscoverslip-preps.html), a precleaned slide, and double-

686   sided sticky tape. Microtubules were diluted in BRB80 containing 10 µM taxol. SRP90 assay

687   buffer (90 mM HEPES, 50 mM KCH3COO, 2 mM Mg(CH3COO)2, 1 mM EGTA, 10% glycerol,

688   pH = 7.6), supplemented with 1 mg/mL BSA, 0.2 mg/mL K-casein, and oxygen scavenging

689   system composed of protocatechuic acid(PCA)/protocatechuate-3,4-dioxygenase(PCD)/Trolox)

690   was used.

691   For microtubule binding assays, TIRF chambers were first coated with 0.5 mg/ml PLL-

692   PEG-biotin (Surface Solutions Group) followed by with 0.5mg/ml streptavidin (ThermoFisher

693   Scientific). Diluted microtubules were then flowed into the chambers. After addition of each

694   component, chambers were incubated for 5 minutes at room temperature. Once it was

695   confirmed that the microtubules were adhered and polymerized sufficiently, the unbound

696   microtubules were removed by washing the chambers with SRP90 assay buffer. To analyze

697   kinesin-1 recruitment onto the microtubules, the purified motor protein was diluted to 40 nM in

698   the assay buffer with 2 mM 50-adenylylimidodiphosphate (AMP-PNP) (Roche Diagnostics

699   GmbH). Kinesin-1 was combined with each UNC-83 construct separately at a 1:5 molar ratio

700   and incubated on ice for 30 minutes. Following the incubation, the protein mixes were diluted

701   with the assay buffer to get 40 nM kinesin-1 and 200 nM UNC-83 construct in the final solution.

702   As controls, each purified UNC-83 construct was diluted to 200 nM in the assay buffer with 2

703   mM of AMP-PNP. The solutions were then flowed into the TIRF chambers, one at a time, and

704   incubated for 10 minutes at room temperature to let the proteins interact with AMP-PNP and

705   microtubules. Sequential images of 405 nm, 488 nm and 561 nm channels were acquired.

706   Kinesin-1 average fluorescence fold intensity on microtubules were manually measured using

707   ImageJ (Fiji).

708   For gliding assays, kinesin-1 was diluted in assay buffer to 200 nM and mixed separately

709   with each UNC-83 construct separately at a 1:5 molar ratio and incubated on ice for 30 minutes.

710   Following the incubation, the protein mixes were further diluted with the assay buffer to obtain

711   final concentrations of 200 nM kinesin-1 and 1 µM UNC-83 construct. Controls consisted of

712   UNC-83 constructs alone at 1 µM. The solutions were incubated for 5-10 minutes at room

713   temperature to allow protein adhesion to the slide, after which diluted microtubules were

714   introduced. Following a 5-minute incubation, unbound proteins and microtubules were washed

715   off using assay buffer containing 2 mM ATP. Microtubules were imaged for 1 minute using 405

716   nm laser at 10% power and images were taken at 1 frame per second. Single images of 561 nm

717   and 488 nm channels were captured using 15% laser power. Kymographs were generated

718   using ImageJ (Fiji), and microtubule velocities were determined using ImageJ Velocity

719   Measurement Tool plug-in[50].

720   TIRF imaging was performed on a Nikon TE microscope equipped with a1.49 N.A. PlanApo

721   100x objective, a TIRF illuminator (LUN4), and an Andor iXon EMCCD camera. Images were

722   acquired using NIS Elements software (Nikon).

723

724   **QUANTIFICATION AND STATISTICAL ANALYSIS**

725   All statistical analyses were performed using GraphPad Prism version 10. Data from hyp7 and

726   P-cell nuclear migration assays were visualized as scatter plots, with means and 95% CI

727   represented as error bars. Sample sizes and specific statistical tests are detailed in the figure

728   legends. Unpaired student *t*-tests were conducted for the indicated comparisons.

729

730   **Supplemental Figure Legends**

731   **Figure S1: Alphafold3-predicted structure of UNC-83a shows high confidence for**

732   **spectrin-like repeats, related to Figure 1.** A) Plot of the predicted local distance difference

733   test (pLDDT) scores per residue; high confidence in dark blue, low confidence in orange/yellow.

734   B) Plot of the predicted aligned error (PAE) per residue; higher error in light green.

735

736   **Figure S2: Deleting spectrin-like repeats in the UNC-83a-specific N-terminal domain**

737   **causes temperature-sensitive P-cell nuclear migration defects, related to Figure 4.**

738   Quantification of larval P-cell nuclear migration in *wild type*, *unc-83(e1408)* null, and *unc-83a* N-

739 terminal domain deletion mutant animals (A) at 20°C and (B) at 15°C. Each point represents the

740 number of missing GABA neurons per animal and n = 40 for each strain.

741

742 **Figure S3: The *unc-83* enhancer region shows high conservation across *Caenorhabditis***

743 **species, related to Figure 5.** Multiple sequence alignment comparing the long intron region of

744 *C. elegans unc-83* with corresponding sequences from *C. brenneri*, *C. remanei* and *C. briggsae*.

745 Black asterisks indicate nucleotides conserved across all four species while magenta asterisks

746 indicate that the *C. elegans* nucleotides conserved in two other species.

747

748 **Movie S1: *C. elegans* kinesin-1 heterotetramer demonstrates microtubule gliding activity,**

749 **related to Figure 6.** Representative time-lapse showing microtubules (blue) gliding across a

750 glass surface. Scale bar (= 5 μm) and time stamp are indicated in the movie.

751

752 **Movie S2: The *C. elegans* kinesin-1 heterotetramer preincubated with UNC-83c increases**

753 **the gliding velocity of microtubules, related to Figure 6.** Representative time-lapse showing

754 microtubules (blue) gliding across a glass surface when kinesin-1 heterotetramer is

755 preincubated with UNC-83c. Scale bar (= 5 μm) and time stamp are indicated in the movie.

756

757 **Movie S3: The *C. elegans* kinesin-1 heterotetramer preincubated with UNC-83a increases**

758 **the gliding velocity of microtubules, related to Figure 6.** Representative time-lapse showing

759 microtubules (blue) gliding across a glass surface when kinesin-1 heterotetramer is

760 preincubated with UNC-83a. Scale bar (= 5 μm) and time stamp are indicated in the movie.

761

762 **Movie S4: UNC-83a N-terminus inhibits *C. elegans* kinesin-1 heterotetramer motor**

763 **activity, related to Figure 6.** Representative time-lapse showing reduced microtubule (blue)

764   gliding across a glass surface when kinesin-1 heterotetramer is preincubated with UNC-83a[N-term]

765   . Scale bar (= 5 μm) and time stamp are indicated in the movie.

766

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1085   sensitivity of progressive multiple sequence alignment through sequence weighting,
1086   position-specific gap penalties and weight matrix choice.

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1088 **Key Resources Table**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
UNC-83 antibody	Starr et al.[52]	1209D7
Alexa Fluor® 555 conjugated goat anti-mouse (H+L) Cross-Adsorbed IgG	ThermoFisher Scientific	A21422

Bacterial and virus strains		
BL21-CodonPlus (DE3)-RIPL competent cells	Agilent	230280
NEB 5-alpha competent *E.coli*	NEB	C2987H
DH10MultiBac competent cells	Geneva Biotech	N/A
E. coli OP50	*Caenorhabditis* Genetics Center	N/A
Chemicals, peptides, and recombinant proteins		
Dylight 405 NHS Ester	ThermoFisher Scientific	46401
Biotin NHS ester	ThermoFisher Scientific	439302500
Biotinylated poly(L-lysine)-[g]-poly(ethylene-glycol) (PLL-PEG Biotin)	SuSoS AG	PLL(20)-G[3.5]-PEG(2)/PEG(3.4)-biotin(50%)
Streptavidin	ThermoFisher Scientific	21135
Trolox (6-hydroxy-2,5,6,7,8-tetramethylchroman-2-carbonsaure, 97%)	Acros	AC218940050
3,4-Dihydroxybenzoic Acid (Protocatechuic acid)	Sigma-Aldrich	37580
Protocatechuate 3,4-Dioxygenase from Pseudomonas sp.	Sigma-Aldrich	P8279
k-casein from bovine milk	Sigma-Aldrich	C0406
SuperFrost Microscope slides	ThermoFisher Scientific	12-550-143
Glass cover slides (18x18-1.5)	ThermoFisher Scientific	12-541A
Adenosine 50-triphosphate disodium salt hydrate	Sigma-Aldrich	A2383
DNase I	NEB	M0303L
Bovine Serum Albumin	Sigma-Aldrich	A2058
DTT (dithiothreitol)	ThermoFisher Scientific	R0862
PMSF (Phenylmethylsulfonyl fluoride)	ThermoFisher Scientific	36978
Streptactin XT Superflow resin	IBA	2-4010-025
D-Biotin	CHEM-IMPEX	00033
Glutathione Sepharose 4B	Cytiva	17075601
L-glutathione reduced	Sigma-Aldrich	G4251-5G
Cellfectin II Reagent	ThermoFisher Scientific	10362100
Sf-900 II SFM	ThermoFisher Scientific	10902104
Apoferritin	Sigma-Aldrich	A3641
Thyroglobulin	Sigma-Aldrich	T9145
tetramisole	Sigma-Aldrich	Cat#L9756

Cas9 protein	QB3 UC Berkeley	N/A
Experimental models: Organisms/strains		
C. elegans strain: *wild type*	Brenner[80]	N2
C. elegans strain: *zen-4 (cle10)IV*	Farboud et al.[91]	AFS222
C. elegans strain: *oxIs12[punc-47::gfp] X*	McIntire et al.[92]	EG1285
C. elegans strain: *unc-83(e1408) V*	Starr et al.[52]	CB1408
C. elegans strain: *oxIs12; unc-83(e1408)V*	Starr et al.[52]	MH1654
C. elegans strain: *ycIs3[unc-83(870bp)::min_myo-2 promoter::nls::gfp; odr-1::rfp]*	This study	UD338
C. elegans strain: *ycIs9 [pcol-10nls::gfp::lacZ]) I*	Fridolfsson et al.[62]	UD393
C. elegans strain: *unc-83 (yc77[Δ234-305]) V*	This study	UD646
C. elegans strain: *oxIs12; unc-83 (yc77) V*	This study	UD657
C. elegans strain: *unc-83(yc79[Δ167-233]) V*	This study	UD658
C. elegans strain: *ycIs9 I; unc-83(yc77) V*	This study	UD659
C. elegans strain: *oxIs12; unc-83(yc79) V*	This study	UD682
C. elegans strain: *ycIs9 I; unc-83(yc79) V*	This study	UD684
C. elegans strain: *unc-83 (yc95[Δ58-166])V*	This study	UD741
C. elegans strain: *ycIs9 I; unc-83(yc95) V*	This study	UD742
C. elegans strain: *oxIs12; unc-83(yc95) V*	This study	UD743
C. elegans strain: *unc-83 (yc108 [punc-83c::unc-83a]) V*	This study	UD805
C. elegans strain: *ycIs9 I; unc-83 (yc108) V*	This study	UD871
C. elegans strain: *oxIs12; unc-83(yc108)V*	This study	UD894
C. elegans strain: *ycIs9 I; unc-83(e1408) V*	Taiber et al.[13]	UD900
C. elegans strain: *oxIs12; ycEx301[phlh-3::unc-83c; Pmyo-2::mRFP]*	This study	UD973
C. elegans strain: *oxIs12; ycEx302[phlh-3::unc-83c; Pmyo-2::mRFP]*	This study	UD974
C. elegans strain: *unc-83 (yc121[delta enhancer]) V*	This study	UD999
C. elegans strain: *unc-83 (yc122[delta enhancer]) V*	This study	UD1000
C. elegans strain: *unc-83 (yc123[Δ58-233]) V*	This study	UD1001
C. elegans strain: *ycIs9 I; unc-83 (yc121) V*	This study	UD1002
C. elegans strain: *ycIs9 I; unc-83 (yc122) V*	This study	UD1003
C. elegans strain: *oxIs12; unc-83 (yc121) V*	This study	UD1004
C. elegans strain: *oxIs12; unc-83 (yc122) V*	This study	UD1005
C. elegans strain: *oxIs12; unc-83 (yc123) V*	This study	UD1007
C. elegans strain: *ycIs9 I; unc-83 (yc123) V*	This study	UD1010
Recombinant DNA		
Podr-1::RFP	L'Etoile and Bargmann[83]	N/A
pACEBac1 UNC-116-sfGFP-StrepII	This study	MOM842 (pSN492)

pACEBac1 UNC-116-sfGFP-StrepII KLC-2-6xHis	This study	MOM845 (pSN500)
pPD97.82 with *unc-83 (870bp)*::min_*myo-2* promoter::nls::gfp	This study	pSL439
pDS22 phlh-3::unc-83c	This study	pSL536
pET21a UNC-83c-mScarlet-I StrepII	This study	MOM1135
pET21a UNC-83a N-term (1-305aa)-mScarlet-StrepII	This study	pSL913
pET21a UNC-83a-mScarlet-I StrepII	This study	pSL912
pGEX-2T klc-2	Meyerzon et al.[55]	pSL409
Software and algorithms		
FIJI	Schindelin et al.[93]	https://Fiji.sc/
Prism Version 10	GraphPad Software	https://www.graphpad.com/scientificsoftware/prism/
AlphaFold3	Abramson et al.[59]	https://golgi.sandbox.google.com
Clustal W	Thompson, Higgins and Gibson[94]	http://www.clustal.org/
Other		
Superose 6 10/300 GL	Cytiva	17517201
Superdex 200 increase 10/300 GL	Cytiva	28990944
Octet® Anti-Glutathione-S-Transferase (GST) Biosensors	Sartorius AG	18-5096

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1095 **Table S1. guideRNAs used in this study. Related to STAR Methods.**
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guideRNAs used for CRISPR/Cas9 gene editing in *C. elegans*				
Identifier	Target Gene	RNA Target Sequence	Strains	Reference
crRNA #42	*unc-83*	agaagacucggaaagugauc	UD646	This study
crRNA #43	*unc-83*	gaguacgaaaguauuaugga	UD657 UD659	This study
crRNA #50	*unc-83*	caacuugaaauacaacacga	UD658 UD682 UD684	This study

crRNA #51	*unc-83*	ugaagagcuuauuaaccauc	UD1001 UD1007 UD1010	This study
crRNA #56	*unc-83*	caaagaaaauucucgccgaa	UD741	This study
crRNA #57	*unc-83*	gagagcaacagcugcaaguu	UD742 UD743	This study
crRNA #84	*unc-83*	agauuuugucucacagucgg	UD805 UD871 UD894	This study
crRNA #103	*unc-83*	aaaauugauggacuuuugga	UD999 UD1000 UD1002	This study
crRNA #104	*unc-83*	uugacugaugaagacagaug	UD1003 UD1004 UD1005	This study
dpy-10(cn64) crRNA	*dpy-10*	gcuaccauaggcaccacgag	Co-CRISPR	Arribere et al.[87]
zen-4(cle10ts) crRNA	*zen-4*	ugauggaagcuaacuaccag	Co-CRISPR	Farboud et al.[91]

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1111 **Table S2. CRISPR/Cas-9 repair templates used in this study. Related to STAR**
1112 **Methods.**
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oligonucleotides used as CRISPR/Cas9 repair templates

Repair Target	ssODN Sequence	Strain	Reference
unc-83(yc77)	atcaacaaaattttgaggtaaagcctctgccagactcaccttccatccat cgagtcttctgatgcttgctgaaaaatttaaaattacatagaatcgaaca	UD646 UD657 UD659	This study
unc-83(yc79)	caaaaactggtactttatctttcgatttacaggctctacaacttgaaatag agcttattaaccatcaagtgagaattcattttgtgtaacttgaagaatt	UD658 UD682 UD684	This study

unc-83(yc95)	atatcatgtgaatgttctcgacgaaatgacatctacacttgaaaaatcgg cttgctctctgatcaacaggttagtcttttggaactcttagattgttcttt	UD741 UD742 UD743	This study
unc-83(yc108)	tgcatcggttgcctcctcctcatcctccaataaccccagagccacataa attatcgcccgcctagattttgtctctttcagtatggacgtaatggactcatt ctcggaggtcgagatgcccaatgatatatcatcggaggaccatcttttga aggtcatcgaatcgtcggctgaggaagtggatatctttttggagaactgc tcctcgctctacaatttaatcctcgactcgttgcacaacctcacctcaaaa acgatatcatgtgaatgtctcgacgaaatgacatctacacttgaaaaat cggcaaagaaaattctcgccgaacggcccgaggccgagaattcggt ccttctccgccttaacacaatatgctgcgcaatggatcaactacgtgtcc agcacaattcacgaatgatgagcggtgccgattcggacacagcaagc tcagcacgtagttccacgtcatcatcgacaggcgaaatgcgtttgtggtt acacgaggtcgagagaagactcgaaatacatgagaaacggattcga gtggagccaaacttgcagctgttgctctctgatcaacaggctctacaact tgaaatacaacacgaaggtcaactattggttaatcgactcaacaaaca aattaaagacgatcacgacagcgactcgtcagaagaagagaaacg gaaaacttgtgtcgatgcgatcagaaaacggtggcatactatctacttg aatagtttgtctctagtctgcagaattgaagagcttattaaccatcagcaa gcatcagaagactcggaaagtgatccggacctcgtcggaccaccgat caaacgggctcgcattcgaactgttggtcacctgacggcttctgatacg gaagagtctgaagctgacgaggaagacagacatagtcagactgaaa ctgtggtcactgaagacgataacgttcttccatttgcggagaacgagtac gaaagtattatggatggaaggtgagtcggaggataatattttttaatttcaa ttcatatttcattcgtttaataaatcttcatttcagagtaactgtcgatt	UD805 UD871 UD894	This study
unc-83(yc121)	caattcttttctgcttcttcttcttttccttcatctttcccttcaatccgtctgtactt gagtcaatttgttggtttgtcagattttaattgaaatcac	UD999 UD1002 UD1004	This study
unc-83(yc122)	caattcttttctgcttcttcttcttttccttcatctttcccttcaatccgtctgtactt gagtcaatttgttggtttgtcagattttaattgaaatcac	UD1000 UD1003 UD1005	This study
unc-83(yc123)	caaaaactggtactttatctttcgatttacaggctctacaacttgaaatag agcttattaaccatcaagtgagaattcattttgtgtaacttgaagaatt	UD1001 UD1007 UD1010	This study
dpy-10(cn64)	cacttgaacttcaatacggcaagatgagaatgactggaaaccgtaccg catgcggtgcctatggtagcggagcttcacatggcttcagaccaacag cctat	Co-CRISPR	Arribere et al.[87]
zen-4(cle10ts)	gaccatcttcaactcttcttactatgattcgccaatacatgatggaagcag actaccagcgagtagagattgcacgtctcaaagattctctaaacgaca aggatg	Co-CRISPR	Farboud et al.[91]

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A Embryonic hyp7 nuclear migration

Kinesin-1

dorsal midline

B Larval P-cell nuclear migration

Dynein

ventral cord

C

UNC-83c
85kDa
EWD | SR3 | SR4 | SR5 | TM | KASH → Kinesin-1
⊣ Dynein

UNC-83a
117kDa
SR1 | SR2 | SR3 | SR4 | SR5 | TM | KASH

UNC-83b
110kDa
SR2 | SR3 | SR4 | SR5 | TM | KASH

⊣ Kinesin-1
→ Dynein

D

SR1

SR2

EWD

SR3

SR4

SR5

KASH

TM

A unc-83c promoter — SR1 SR2 — EWD — SR3 SR4 SR5 — TM — KASH

B wild type | unc-83(yc108[UNC-83a]) — UNC-83

C # of mislocalized hyp7 nuclei — wild type, unc-83(e1408), unc-83(yc108) — ns

D wild type | unc-83(yc108[UNC-83a])

E Missing GABA neurons — wild type, unc-83(e1408), unc-83(yc108) — *** — 25°C

F wild type unc-47::gfp (1-3, 4, 5, 6, 7, 8, 9, 10, 11-12, 13, 14-15, 16, 17-19); unc-83(yc108[UNC-83a]) unc-47::gfp (1-3, 4-5, 6, 7-8, 9, 10, 11-12, 13, 14-15, 16, 17, 18, 19)

G Missing GABA neurons — wild type, unc-83(e1408), unc-83(ycEx302), unc-83(ycEx301) — *** ** — 25°C

H unc-47::gfp unc-83(ycEx302[phlh-3::unc-83c(+)]) (1, 2, 3, 4, 5, 6, 7, 8, 9, 10-11, 12, 13, 14, 15-16)

A

ATG ATG ATG

unc-83

1   2   3 4 5   6 7   8 9   10 11 12 13   14 15 16
870bp

1 kb

unc-83(yc121)
unc-83(yc122)

1   2   3 4 5   6 7   8 9   10 11 12 13   14 15 16

1 kb

B

# of mislocalized hyp7 nuclei

****

wild type   unc-83(e1408)   unc-83(yc121)   unc-83(yc122)

C

unc-83(yc121)

D

Missing GABA neurons

****

wild type   unc-83(e1408)   unc-83(yc121)   unc-83(yc122)

25°C

E

unc-83(yc121)
unc-47::gfp

1-3 4 5 6 7 8 9 10 11-12 13 14-15 16 17 18

F

unc-83(870bp)::pmyo-2::nls::gfp

A UNC-116

kDa	
250	
150	
100	
75	
50	

B Motor Domain | CC | CC | CC | sfGFP

UNC-116-sfGFP

Expected MW
monomer: 120 kDa
dimer: 240 kDa

Measured MW
monomer: 109 kDa (13%)
dimer: 246 kDa (82%)

C **UNC-116-sfGFP-KLC-2 (Kinesin-1)**

Expected MW
tetramer: 362 kDa

Measured MW
tetramer: 360 kDa (88%)

D SR1 | SR2 | EWD | SR3 | SR4 | SR5 | m-Scarlet-I

UNC-83a-mScarlet

Expected MW
monomer: 141 kDa

Measured MW
130 kDa (96%)

E SR1 | SR2 | m-Scarlet-I

UNC-83a^{N-term}-mScarlet

Expected MW
dimer: 124 kDa

Measured MW
120 kDa (100%)

F EWD | SR3 | SR4 | SR5 | m-Scarlet-I

UNC-83c-mScarlet

Expected MW
monomer: 107 kDa

Measured MW
108 kDa (100%)

D' **UNC-83a-mScarlet + UNC-116-sfGFP**

Expected MW
1 UNC-83a monomer
+
1 UNC-116 dimer = 381 kDa

Measured MW
391 kDa (99%)

E' **UNC-83a^{N-term}-mScarlet + UNC-116-sfGFP**

Expected MW
1 UNC-83a^{N-term} monomer
+
1 UNC-116 dimer = 302 kDa

Measured MW
307 kDa (99%)

F' **UNC-83c-mScarlet + UNC-116-sfGFP**

Expected MW
1 UNC-83c monomer
+
1 UNC-116 dimer = 347 kDa

Measured MW
103 kDa (30%)
245 kDa (70%)

D'' **UNC-83a-mScarlet + UNC-116-KLC-2 (Kinesin-1)**

Expected MW
1 UNC-83a monomer
+
1 kinesin-1 tetramer = 503 kDa

Measured MW
531 kDa (97%)

F'' **UNC-83c-mScarlet + UNC-116-KLC-2 (Kinesin-1)**

Expected MW
1 UNC-83c monomer
+
1 kinesin-1 tetramer = 469 kDa

Measured MW
128 kDa (24%)
360 kDa (72%)

B

A

Missing GABA neurons (y-axis, 0–12)

x-axis categories: wild type, unc-83(e1408), unc-83a(Δ234-305), unc-83a(Δ58-233), unc-83a(Δ58-166), unc-83a(Δ167-233)

20°C

B

Missing GABA neurons (y-axis, 0–12)

x-axis categories: wild type, unc-83(e1408), unc-83a(Δ234-305), unc-83a(Δ58-233), unc-83a(Δ58-166), unc-83a(Δ167-233)
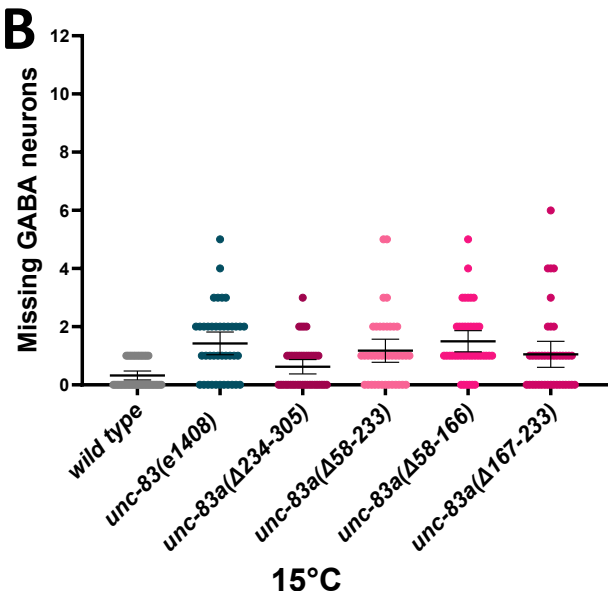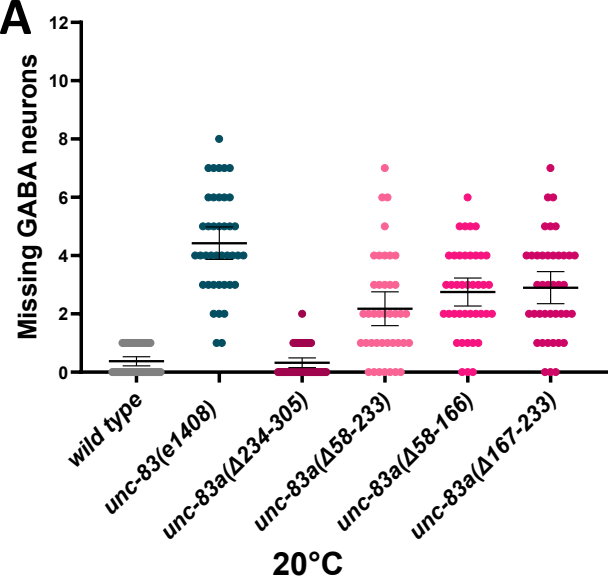
15°C

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C.elegans  ---------AGTGACCAGTCCCCTCCTTTTTCGTGTCGCCGTGCATTTTCAGATGACGGA
C.brenneri ---------AGTGACTCTCTTTTCCTTTTTTCCATGTCGCCGTGCATTTTCAGATGACGGA
C.briggsae GAGTGACCAGTTGCTCTTTTTCCTTCTTTTCCATGTCGCCGTGCATTTTCAGATGACGGA
C.remanei  ---------TCGAGTGACCAGTTGCTCTTTTCCCGTCGCCGTGCATTTTCAGATGACGGA
             *       *          ****  *  ***************************

C.elegans  TTGCCGACATGAGACAGGCGGTCGCAGCAGTCCATTTCTCGTTTGACATCCGTGAGAACC
C.brenneri TTGCCGACATGAGACGGCCGGTCCCAGTCCATTTCTCGTT--TTGACATCCGTGAGAACC
C.briggsae TTGCCGACATGAGACGGGCGGTCCCAAGTCCATTTGACATCCTTGAGA---------ACA
C.remanei  TTGCCGACATGAGACGGGCGGTCCCATTTCCATTTCTCGT----TTGACATTCGTGAGAACC
            ************** ****** ***** **   *   ***** **  ***********

C.elegans  TTTTGGGATTTTCCGC----TC-------TTGGGTCACTTTTGACCGCTTCTTT-------
C.brenneri TCTTCTGCGGTTTTCTTGTTT-------TCTTTGACCGCTTGACCGCTTCTTC-------
C.briggsae TTCTTCTTCTACATTCTTTTT------TCTTCTTCCAACCGACCGCTCCTCT-------
C.remanei  TTTTTATTTTTCCTTTTTCTTGCCTTCTTCTTCTTCTTCTTGACCGCTCGTTGGCTATTC
               * *   *** **       *     *   **  *  ********** * *

C.elegans  -CGTATTCCAAAAATAGAAAGAAGC----TACTGGACGCGGCGGCAGGTGCGGATTGAAAG
C.brenneri -CATAT-------TCTAAAAGAAGG----ACGCGGCGGCGGCAGCAGGTGCGGATTGAAAG
C.briggsae -CGTATTCCAAAAAGAA-----------GGACGCGGCGGCGGCCAGGTGCAGATTGAAA-
C.remanei  CCGTATTCCAAAAAGAAGCGGTTGGGACGCGGCGGCGCTGTGGCGGCAGGTGCGGATTGAAA-
            *****  ****  ****            **    ********** **** ***********

C.elegans  GGTCCGTCGTGTGTTTGCGTGTGCGCTCAGCAATTCCACGCACTTTTGTCCTCTTCTTCTC
C.brenneri GGTCCGTGTCCG--TTGCGTGTCGCTCAGCCATTCCACGCACTTTTCTCTTGTTTCCATT
C.briggsae --------GGGTCCGTTGCGTGTCGCTCAGCGATTCCACGACTTTTGTCTTGTTTGTTTGC
C.remanei  -----AGGGTCCCGTTGCGTGTCGCTCAGCGATTCCACGACTTTTGTTTCCTCTCTCGACC
                    *        ********  ****** ********* ******* ** *

C.elegans  GACCTTC----------ATCTGCCTCGTTATACTATACCTCCAAACAACAATTAACATTT
C.brenneri CCACTCGACATTCTTCTTTCTTTTCTCCGTGATATACTATACCTCTGAACAACAATTAGCACTT
C.briggsae CTCTCGG-----CCTTCTCTCCGCTCATTTTACTATACCCCTGAACAACAATTAACACTT
C.remanei  -----------TTCTCTTCGCCCTCGTTATACTATACCTCTAAACAACAATTAACACTT
                          **  ** ***********  ***********  **
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C.elegans  TTCTTCCCTCACTTTTCATCCTTCCTGTTCCTCAACAAAACCTCACGCGCCCTGT-CGTT
C.brenneri TTCATCACATCCACACTCTGTCATTTTGCCATTTTGTCCGGTTCTTTTC----TT-CTTC
C.briggsae TTTCTTCGTCTTCTCATTCAACCGAAAACCACGCACTCTTTCA-TCATCCTCAATCCTCC
C.remanei  TTTCGTCCTCCTACACCATGTCATTTTGTCATTTTCGTCTTCCTTCCCTCCCAATTTTTG
              *  * **** *     *   *  *   **        *  ** * ** *  *  *  *

C.elegans  TCGGCCT---TCTTACCTGTTGTCCGGTTCTTACGTGCATATCAGATTTCCATTA------
C.brenneri CTTCTT--TACTTCTTCTTCTTCCTGACGTCCGTGCATATCACATTTCCATGTCATTCT
C.briggsae TCGGGTAATTTTGTCTCCGGTTTTTCTTCGTCCGTGGAATATCACATTTCCACGTACT---
C.remanei  TCCGGT---TCTTTTCCATTCTTCCTGACGTCCGACAATCCGTGAATATCA-----TGTT
             **  *    *   *  *    ** **  * **  ***  ***** *****  ****

C.elegans  --------------------CCTTCAGCAGTTATTCTAATTCCTTCCGCGCGCATATTATCTT-
C.brenneri TCTTCTTCTTGTTCAGTTCATTCAGTAGTTGTT-CTTAGTTCGTCCGCGCATATTATCTT-
C.briggsae ----------------TGCCTTT---CAGTACAGTCTTCTTCTTCCGCGCATATTATCTCC
C.remanei  TCATA--------CTCTAACTTCCACAGTT----CTTAGTCTGCCGCGCATATTATCTTC
                             * ***    * ** *  **  ***************

C.elegans  ------GTAGATATCCC---TTGCCGATACAATAATGTTTATATATCAATCAAATAATACT
C.brenneri ------GTAGATATCCCTCACTCCGATACAATAATGTTTATATATCAATCAAATACTCTT
C.briggsae T----CTTGTAGATATCCTCGTCCGATACAATAATGTTTATATATCAATCAAATACTCTT
C.remanei  TTTGTAGATATCCACCTCTTTCCCGATACAATAATGTTTATATATCAATCAAATACTCTT
               *   ***              ************************** *   *

C.elegans  CTTGAAGATGGACGAAGACTCTGCGCCCTCCGATTATTC--CGTGTTCTTGTTTCACGTG
C.brenneri GAAGATTGACGACTCTGCGCCT----CTGATGATTTAGTGCTCTTATTCAATTTTCACGTG
C.briggsae GAAGATTGACAACTCTGCACTCTCTATTAATTCCGTGCTT---TTCTTCT---------TGTA
C.remanei  GAAGATTGACGACTCTGCACTCTCCACTA------ATTCC--GTGCTTTTTCTTCACGTG
             **     ***   ***       *   *             *   ***  *******

C.elegans  TGTTTGTATA----TGTTAGGGAGGTTGTTGTACTGTGCAGTGTCACTAGAAACTTACTA
C.brenneri TATTTGTACATTTCTCAGAGGAGGTTTGTTGTACTGTGCCGTGTGCAACTTTC--CAATTTC
C.briggsae TAT----------------ACAAAAGGTTGTACTGTGCAGTGACACGGAGA------AGA
C.remanei  TTTGTA---------------TATATGTTGTACTGTGCAGTGACACAAGAAAAACCCT--
                 * * *            *  **************  **  *   *  * *
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