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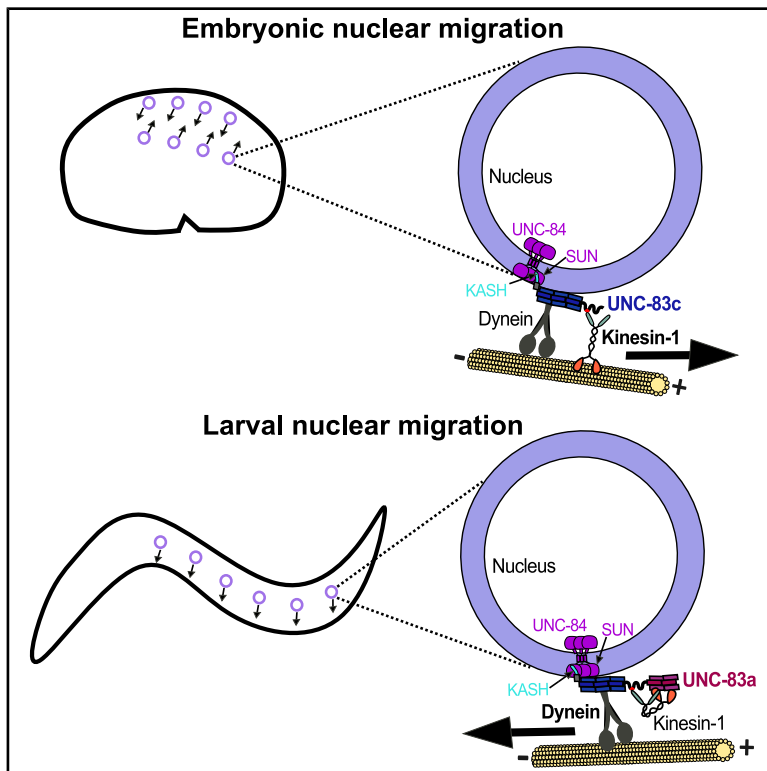
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Current Biology

The KASH protein UNC-83 differentially regulates kinesin-1 activity to control developmental stage-specific nuclear migration

Graphical abstract



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In brief

Gümüşderelioğlu et al. show how the nuclear envelope KASH protein UNC-83 uses alternative isoforms to regulate the directionality of nuclear migration. UNC-83c promotes kinesin-1-dependent movement in some tissues, while the longer UNC-83a directly binds and inhibits kinesin-1 in other tissues, favoring dynein-mediated nuclear transport.

Highlights

- UNC-83 isoforms establish motor bias through differential kinesin-1 regulation
- The short UNC-83c promotes kinesin-1 activation by binding the kinesin light chain
- UNC-83a's unique N-terminal domain directly binds and inhibits the kinesin heavy chain
- Spectrin-like repeats in UNC-83a are required for dynein-mediated migration



Article

The KASH protein UNC-83 differentially regulates kinesin-1 activity to control developmental stage-specific nuclear migration

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SUMMARY

Nuclear migration plays a fundamental role in development, requiring precise spatiotemporal control of bidirectional movement through dynein and kinesin motors. Here, we uncover a differential isoform-dependent mechanism for developmental regulation of nuclear migration directionality. The nuclear envelope Klarsicht/ANC-1/Syne homology (KASH) protein UNC-83 in *Caenorhabditis elegans* exists in multiple isoforms that differentially control motor activity to achieve tissue-specific nuclear positioning. The shorter UNC-83c isoform promotes kinesin-1-dependent nuclear movement in embryonic hyp7 precursors, while longer UNC-83a/b isoforms facilitate dynein-mediated nuclear migration in larval P cells. We demonstrate that the UNC-83a-specific N-terminal domain functions as a kinesin-1 inhibitory module by directly binding the kinesin heavy chain (UNC-116). This interaction prevents kinesin-1 activation and reduces the protein's affinity for kinesin light chain (KLC-2), allowing for dynein-mediated transport. By contrast, UNC-83c exhibits high-affinity binding to KLC-2, promoting kinesin-1 activation for plus-end-directed movement. AlphaFold structural predictions reveal that UNC-83 contains five spectrin-like repeats, with two located within the inhibitory N-terminal domain. Genetic analysis demonstrates that these spectrin-like repeats are essential for dynein-dependent P cell nuclear migration but dispensable for kinesin-1-dependent hyp7 migration. This isoform-specific inhibition, combined with differential affinity for KLC-2, establishes a mechanism for achieving directional control of nuclear positioning during development. Together, these interdisciplinary studies reveal how alternative isoforms of cargo adaptors can generate developmental stage-specific regulation of motor activity.

INTRODUCTION

Nuclear positioning orchestrates essential cellular and developmental processes, including pronuclear migration, muscle and neuronal differentiation, and cell migration.^{1–4} Defects in nuclear positioning underlie severe congenital disorders such as lissencephaly^{5,6} and centronuclear myopathies.⁷ As the largest intracellular cargo, nuclei require precise spatial and temporal control of their movement along microtubules.⁴ Nuclei move toward different directions along microtubules at different times in development. For example, the minus-end-directed motor cytoplasmic dynein (dynein from here on) drives pronuclear migration toward centrosomes during fertilization.^{8–12} Alternatively, the plus-end-directed motor kinesin powers nuclear movement in developing neuroepithelia and sensory cells.^{1,13} However, the molecular mechanisms controlling bidirectional nuclear movements remain poorly understood. We hypothesize that nuclear envelope cargo adaptors regulate bidirectional nuclear movements by directly influencing the activity of kinesin and dynein motors.

The conserved linker of the nucleoskeleton and cytoskeleton (LINC) complex bridges the nuclear envelope to enable many nuclear movements.^{14–16} LINC complexes are formed by the interaction between Sad1p/UNC-84 (SUN) proteins in the inner nuclear membrane and Klarsicht/ANC-1/Syne homology (KASH) proteins in the outer nuclear membrane.^{17,18} SUN proteins interact with lamins in the nucleoplasm,^{19,20} cross the inner nuclear membrane, span the lumen of the nuclear envelope, and recruit KASH proteins to the outer nuclear membrane.^{16,21} KASH proteins, including the mammalian nuclear envelope spectrin-repeat proteins (nesprins), extend into the cytoplasm to interact with a variety of cytoskeletal and motor proteins.^{22–24} Here, we focus on the role of KASH proteins as cargo adaptors to recruit kinesin-1 and dynein motors to nuclei.

Motor protein recruitment by KASH proteins involves complex regulatory mechanisms. Kinesins are primarily plus-end-directed motors, while cytoplasmic dynein is the major minus-end-directed motor in animal cells.^{25,26} Kinesin-1 is a heterotetramer composed of two kinesin heavy chains (KHCs) with their motor domains and two kinesin light chains (KLCs).^{25,27,28}



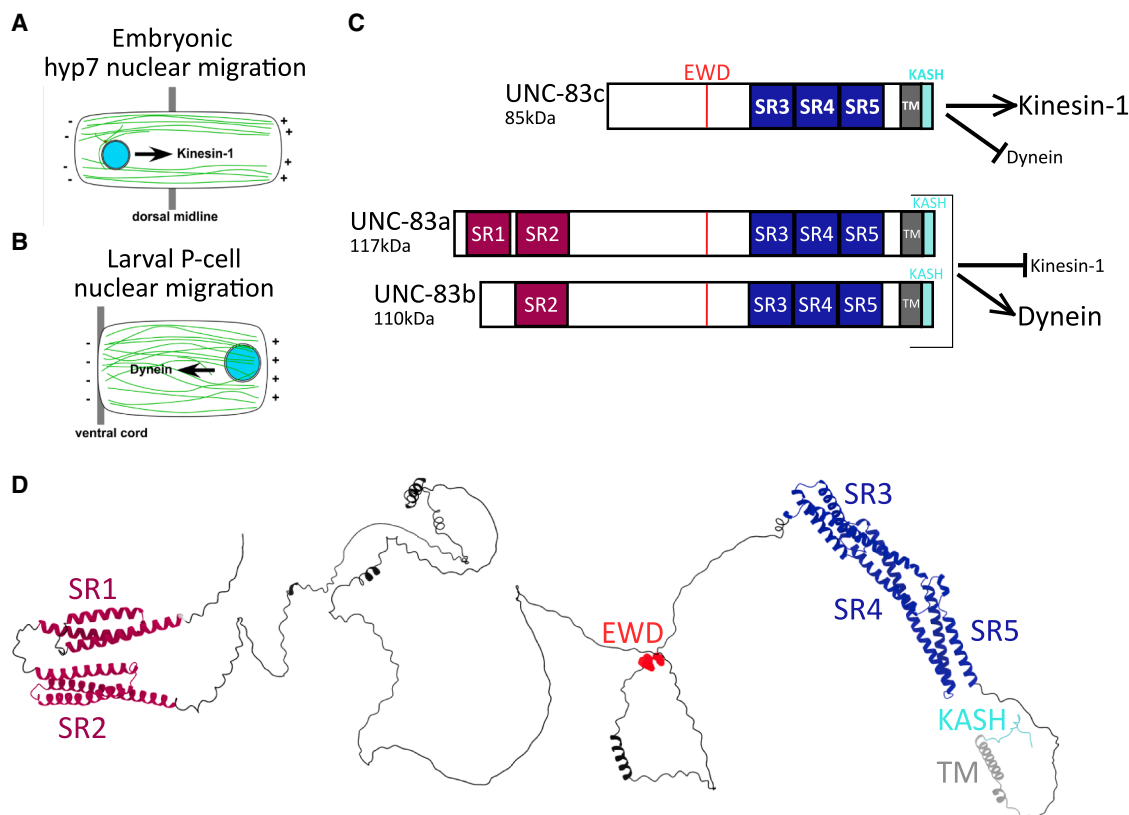


Figure 1. Different isoforms of UNC-83 mediate nuclear migration throughout development

(A) Schematic showing the kinesin-1-mediated migration of embryonic hypodermal hyp7 nuclei (cyan) across the dorsal midline (gray) toward the plus ends of microtubules (green).

(B) Schematic illustrating the dynein-driven migration of larval P-cell nuclei (cyan) toward 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 inhibits 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 the structure of the long isoform, UNC-83a.

(C and 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).

In the absence of cargo, kinesin-1 is inactive as KHCs and KLCs are folded in a conformation that inhibits microtubule binding and motility.^{29,30} Binding of cargo adaptor molecules to the KLC releases this autoinhibition and activates kinesin-1 motility.^{31–35} Mammalian Nesprin-2 and Nesprin-4 and *C. elegans* UNC-83 activate kinesin-1 through their W-acidic (LEWD/EWD) motifs that interact with the tetratricopeptide repeat (TPR) domain of KLCs.^{13,36–38} Similarly, dynein exists in an autoinhibited state until activated by co-factors and cargo adaptors.^{26,39,40} At the nuclear envelope, mammalian KASH5 and *C. elegans* ZYG-12 are meiosis-specific activators of dynein.^{41–44} Nesprin-1 and -2 also bind dynein.⁴⁵ Despite our understanding of these individual interactions, the mechanisms controlling tissue-specific activation of different motor proteins by KASH proteins remain unclear.

We studied two distinct nuclear migration events in *C. elegans* development to dissect how KASH proteins coordinate microtubule motor protein regulation. First, embryonic hypodermal hyp7

precursor nuclei migrate across the dorsal midline to the opposite lateral side of the embryo, after which the cells fuse to form the hyp7 syncytium⁴⁶ (Figure 1A). Failed migration results in nuclei mislocalized to the dorsal cord.^{15,47} By contrast, larval hypodermal P cell nuclei migrate ventrally through constricted spaces between body wall muscles and the cuticle. After migration, P cells undergo divisions to ultimately form vulval cells and GABA neurons^{48,49} (Figure 1B). Failures in P-cell nuclear migration result in P-cell death and missing lineages that lead to egg-laying and locomotion defects.^{49,50}

Both nuclear migration events require the LINC complex proteins UNC-84 (SUN) and UNC-83 (KASH).^{51–54} UNC-83 functions as a cargo adaptor that directly interacts with both kinesin-1 and dynein at the nuclear envelope.^{54–56} However, cellular and genetic analyses reveal strict tissue-specific motor requirements. Embryonic hyp7 precursor nuclei migrate toward the plus ends of microtubules (Figure 1A), while P-cell nuclei migrate toward minus ends of microtubules (Figure 1B).^{15,49} Mutations in

kinesin-1 heavy chain (*unc-116*) or light chain (*klc-2*) severely disrupt hyp7 nuclear migration, while dynein (*dhc-1*) plays only a minor role in moving nuclei backward to avoid obstacles.^{55,56} By contrast, dynein, but not kinesin-1, is necessary for P-cell nuclear migration.^{49,57} This precise tissue specificity in motor preference suggests that UNC-83 can differentially recruit and/or activate kinesin-1 for plus-end-directed hyp7 nuclear migration and dynein for minus-end-directed P-cell nuclear migration.

We hypothesized that the differential expression of *unc-83* isoforms controls tissue-specific nuclear migration directionality. There are three known *unc-83* isoforms: *unc-83a*, *unc-83b*, and *unc-83c*, encoding proteins with 1,041, 974, and 741 amino acid residues, respectively. The three isoforms have 741 residues in their C termini in common but differ in their N termini.⁵² Previous genetic analyses revealed that mutations affecting all three isoforms disrupt both migration events, while those specific to long isoforms (*unc-83a/b*) only impair P-cell nuclear migration. Therefore, the short isoform (*unc-83c*) suffices for hyp7 nuclear migration.⁵² In this model, the long UNC-83a/b isoforms favor minus-end-directed nuclear migration by inhibiting kinesin-1 and/or activating dynein, while the short UNC-83c isoform favors plus-end-directed movements by activating kinesin-1 and/or inhibiting dynein (Figure 1C). Here, we test our model with *in vivo* and *in vitro* approaches. Together, our findings reveal that the Nterminus of long UNC-83 isoforms specifically inhibits kinesin-1, enabling precise spatial and temporal control of nuclear positioning during development.

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 $\sim$ 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 modeling⁵⁹ revealed five spectrin-like repeats interspersed by large stretches of predicted disordered regions (Figure 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 (Figures 1C and 1D). The KLC-binding EWD motif was in an unstructured region (Figure 1D), similar to Nesprin-2.⁶⁰ These structural predictions show high confidence scores (Figure S1A) and low predicted error rates (Figure S1B), suggesting that UNC-83, like other KASH proteins, evolved from an ancestral spectrin-like protein.

The long UNC-83a isoform has lower affinity to KLC-2 than the short UNC-83c isoform

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 (Figure 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 interferes with the interaction between EWD and KLC-2 TPR domain.

To test our hypothesis and quantitatively assess UNC-83c isoform binding to KLC-2, we expressed and purified KLC-2 and the cytoplasmic domains of UNC-83a and UNC-83c (excluding their transmembrane domains and KASH peptides for solubility) from *E. coli* (Figure 2B). Mass photometry analysis⁶¹ confirmed that all three purified proteins were predominantly monomeric, with measured masses within 10% of their predicted molecular weights (Figures 2C and 2D). Direct binding assays revealed that UNC-83c and KLC-2 formed a stable complex (one UNC-83c monomer binding to one KLC-2 monomer) with an average mass of 174 kDa (Figure 2E). By contrast, UNC-83a did not form a robust complex with KLC-2 under identical conditions (Figure 2E). While a minor peak at $\sim$ 489 kDa was observed, this represented less than 20% of the detected particles and likely reflects transient or non-specific associations rather than a stable complex formation. These results suggest that the N-terminal domain of UNC-83a interferes with the interaction between KLC-2 and UNC-83a.

To further quantify these differences in binding affinity, we used bio-layer interferometry for real-time interaction analysis. UNC-83c exhibited robust binding to KLC-2 with a dissociation constant (K_D) of 0.15 μ M. However, UNC-83a binding to KLC-2 showed non-saturating kinetics, indicating a K_D at least 10-fold higher ($K_D > 1.0 \mu$ M) than that for UNC-83c (Figure 2F). We conclude that the N-terminal domain specific to UNC-83a disrupts the interaction between the long isoform, UNC-83a, and the TPR domain of the isolated KLC-2.

Replacing the short isoform *unc-83c* with the long *unc-83a* isoform disrupts kinesin-1-dependent nuclear migration

To test whether the strong binding of UNC-83c to KLC-2 compared with the relatively weak binding of UNC-83a to KLC-2 that we found *in vitro* extends to nuclear migration *in vivo*, we engineered *unc-83(yc108[UNC-83a])* to express the long *unc-83a* isoform under control of the endogenous short *unc-83c* promoter. This transgenic line is expected to express only the long *unc-83a* isoform in embryonic hyp7 precursor cells, where normally the short *unc-83c* isoform is expressed. The transgenic line was made by fusing cDNA encoding the 301-amino acid UNC-83a-specific N-terminal domain to the 5' end of *unc-83c* (Figure 3A). Importantly, this modification preserves all functional domains of the C terminus shared between UNC-83a and UNC-83c isoforms, thus allowing us to specifically test the effect of the N-terminal domain rather than simply eliminating UNC-83c function. This experimental design ensures that any observed phenotypes can be attributed to the presence of the UNC-83a N-terminal domain rather than the absence of UNC-83c.

We confirmed the proper localization of UNC-83a to the nuclear envelope of hyp7 precursors during the nuclear migration period in *unc-83(yc108[UNC-83a])* by immunofluorescence

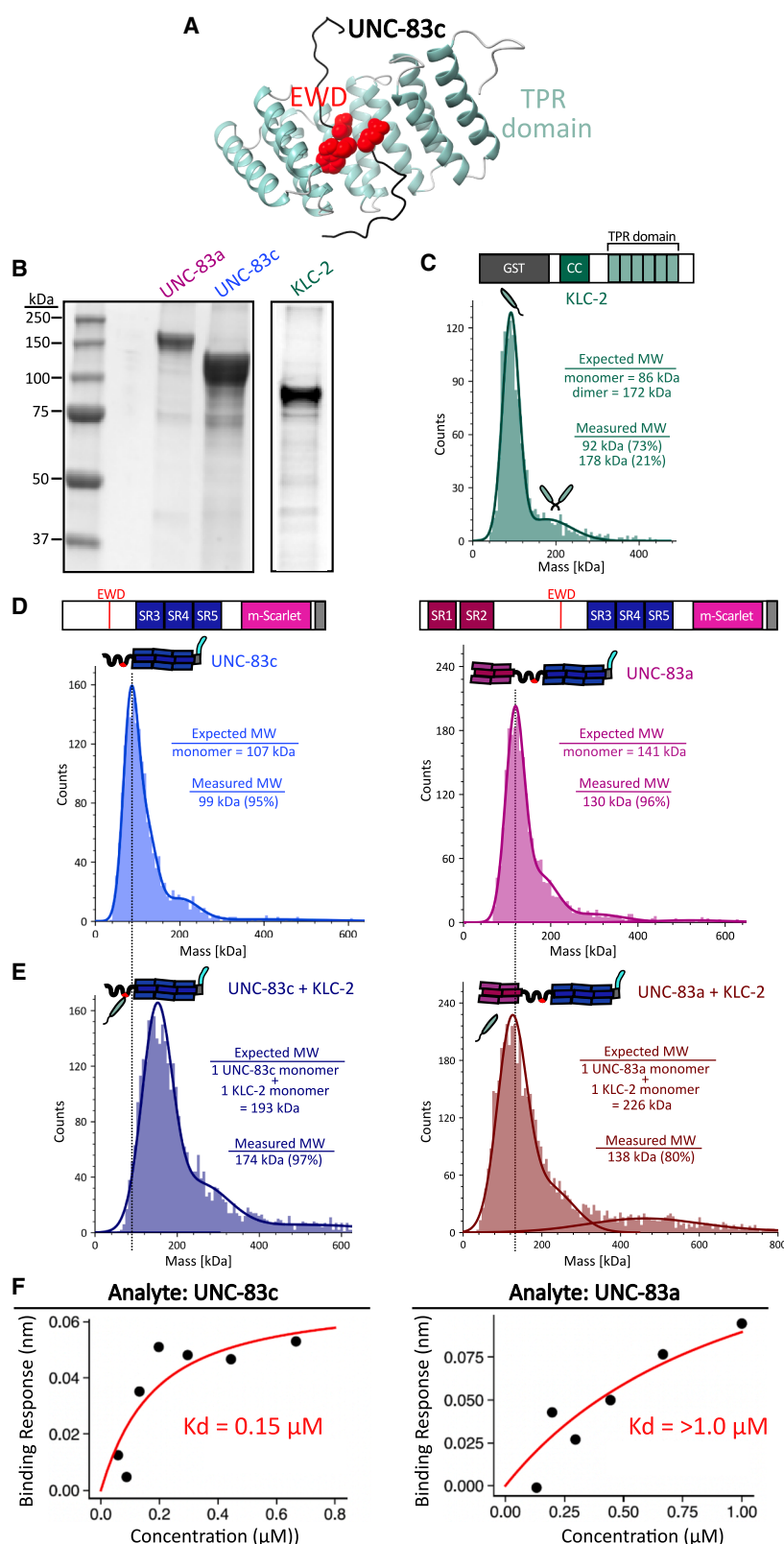


Figure 2. The long UNC-83a isoform binds KLC-2 with significantly lower affinity than the short UNC-83c isoform

(A) AlphaFold3-predicted interaction of UNC-83c's EWD motif (red) with the TPR domain (green) of the KLC-2.

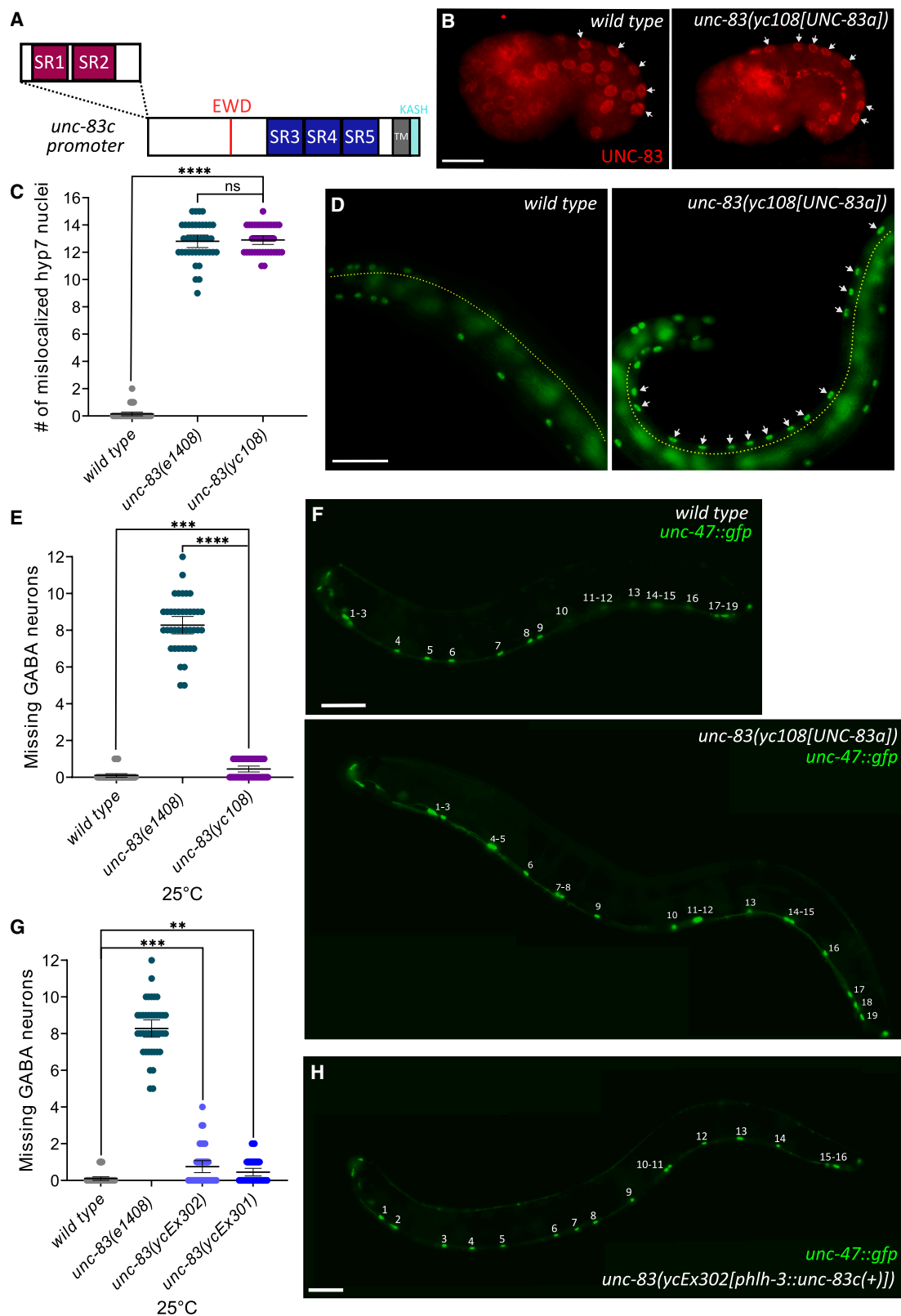
(B) Coomassie-stained protein gels of purified UNC-83a, UNC-83c, and KLC-2 recombinant proteins.

(C) Schematic of the glutathione-S-transferase (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 ± 47 kDa, 95% peak) and UNC-83a-mScarlet (30 nM; monomer = 130 ± 62 kDa, 96% peak). SR1 and SR2 (unique to UNC-83a/b) are in magenta; SR3, SR4, and SR5 (common to all isoforms) are in dark blue; mScarlet tag is in fuchsia; and Strep-II tag is in dark gray.

(E) Top, schematics showing the interaction between UNC-83c and KLC-2 (on the left) and the lack of interaction between UNC-83a and KLC-2 (on the right). Bottom, molecular weight distributions of KLC-2 pre-incubated with UNC-83c (30 nM; one UNC-83c monomer + one KLC-2 monomer = 174 ± 134 kDa, 97% peak) and UNC-83a (30 nM; one UNC-83a monomer + one KLC-2 monomer = 138 ± 62 kDa, 80% peak).

(F) Binding curves of UNC-83a and UNC-83c to KLC-2, showing an affinity of $0.15 \mu\text{M}$ for UNC-83c and $>1.0 \mu\text{M}$ for UNC-83a. The bait, GST-KLC-2, was diluted to $1 \mu\text{M}$ and immobilized onto the biosensors. Both UNC-83a and UNC-83c were tested at concentrations of 1.0, 0.667, 0.444, 0.296, 0.197, 0.132, and $0.088 \mu\text{M}$.



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(Figure 3B). To assess nuclear migration defects, we quantified hyp7 nuclei abnormally localized in the dorsal cord of larvae.⁶² Wild-type animals had almost no mislocalized hyp7 nuclei. By contrast, both *unc-83(e1408)* null and *unc-83(yc108[UNC-83a])* animals had severe defects with 12.8 ± 0.45 and 12.9 ± 0.32 (mean $\pm$ 95% confidence interval [CI]) mislocalized nuclei, respectively (Figures 3C and 3D). Importantly, *unc-83(yc108[UNC-83a])* animals had only a very mild defect in P-cell nuclear migration, with only 0.45 ± 0.16 missing GABA neurons (Figures 3E and 3F). This tissue-specific effect demonstrates that the UNC-83a N-terminal domain specifically disrupts kinesin-1-mediated nuclear migration events, confirming its selective inhibitory function *in vivo*.

Overexpressing the short *unc-83c* isoform disrupts dynein-mediated P-cell nuclear migration

Our findings suggested a model where UNC-83 isoforms establish motor protein bias through reciprocal inhibition. In this model, UNC-83a inhibits kinesin-1, while UNC-83c would be predicted to inhibit dynein. To test this prediction, we examined whether elevated UNC-83c levels in larval P cells could disrupt dynein-dependent P-cell nuclear migration. We generated transgenic animals overexpressing the short *unc-83c* isoform under control of the P-cell-specific *hlh-3* promoter.⁶³ Two independent extrachromosomal arrays caused mild but significant P-cell nuclear migration defects with 0.45 ± 0.20 and 0.75 ± 0.33 missing GABA neurons compared with 0.1 ± 0.09 missing in wild-type animals (Figures 3G and 3H). These mild defects support our model where UNC-83c spuriously activates kinesin-1 in P cells, occasionally disrupting the normally dynein-dependent nuclear migration process.

Spectrin-like repeats in the N-terminal domain of UNC-83a are necessary for dynein-dependent nuclear migration

We next sought to investigate the regulatory mechanism behind dynein-dependent P-cell nuclear migration. Previous genetic analysis revealed that mutations eliminating *unc-83a/b* while preserving *unc-83c* cause temperature-sensitive defects in P-cell nuclear migration.⁵² To determine the critical regions within the

UNC-83a/b N terminus, we engineered a series of in-frame deletions targeting the predicted spectrin-like repeats (Figure 4A). We confirmed that the deletion variants localized normally to the nuclear envelope by immunofluorescence (Figure 4B). Quantitative analysis of P-cell nuclear migration revealed distinct requirements for different parts of the N-terminal domain. Disruption of both predicted spectrin-like repeats in *unc-83a(Δ 58-233)* caused severe P-cell nuclear migration defects at 25°C (an average of 7.70 ± 0.75 missing GABA neurons), comparable to the *unc-83* null phenotype (Figures 4C and 4D). Two smaller deletions in the spectrin-like repeat region, *unc-83a(Δ 58-166)* and *unc-83a(Δ 167-233)*, also led to null-like defects (Figure 4C). All three mutations exhibited temperature sensitivity, with defects progressively worsening from 15°C to 20°C and then to 25°C (Figure 2S), matching the same temperature-sensitive phenotype of *unc-83* null animals. By contrast, a deletion just downstream of the predicted spectrin-like repeats, *unc-83a(Δ 234-305)*, caused only minor P-cell nuclear migration defects (1.25 ± 0.45 missing GABA neurons) (Figures 4C and 4D).

None of these N-terminal deletions disrupted the role of *unc-83c* in embryonic hyp7 nuclear migrations. None had a significant accumulation of hyp7 nuclei in the dorsal cord (Figures 4E and 4F). Together, these results demonstrate that the spectrin-like repeats in the UNC-83a/b N-terminal domain are required for dynein-dependent P-cell nuclear migration but dispensable for kinesin-1-dependent hyp7 nuclear migration.

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

The *unc-83* gene is hypothesized to have developmentally regulated expression of different isoforms, with *unc-83a/b* thought to be expressed in larval P cells and *unc-83c* in embryonic hyp7 precursors. We identified potential enhancer elements within the unusually long introns just upstream of the first *unc-83c* exon. Comparative genomic analysis across four *Caenorhabditis* species (*C. elegans*, *C. brenneri*, *C. remanei*, and *C. briggsae*) identified a highly conserved 870 bp sequence (Figures 3S and 5A).

To test the necessity of this predicted enhancer, we generated two independent alleles, *unc-83(yc121)* and *unc-83(yc122)*,

Figure 3. Abnormal presence of the long *unc-83a* isoform in embryonic hyp7 precursors disrupts kinesin-1-dependent nuclear migration, while expression of the short *unc-83c* isoform in larval P cells disrupts dynein-mediated nuclear migration

(A) Schematic of the *unc-83(yc108[UNC-83a])* mutant, expressing the *unc-83a* isoform under control of the *unc-83c* promoter at the endogenous *unc-83c* locus. (B) UNC-83 (shown in red) in *unc-83(yc108[UNC-83a])* is localized to the nuclear envelope in a comma-stage embryo. Dorsal is up and anterior is left. Arrows point to the hyp7 nuclei.

(C) Quantification of hyp7 nuclear migration defects in *wild type* *unc-83(yc108[UNC-83a])*, and *unc-83(e1408)* null animals via counting the number of mislocalized (at the dorsal cord) nuclei. Each point represents the total number of abnormally located hyp7 nuclei per animal; $n = 40$ for each strain.

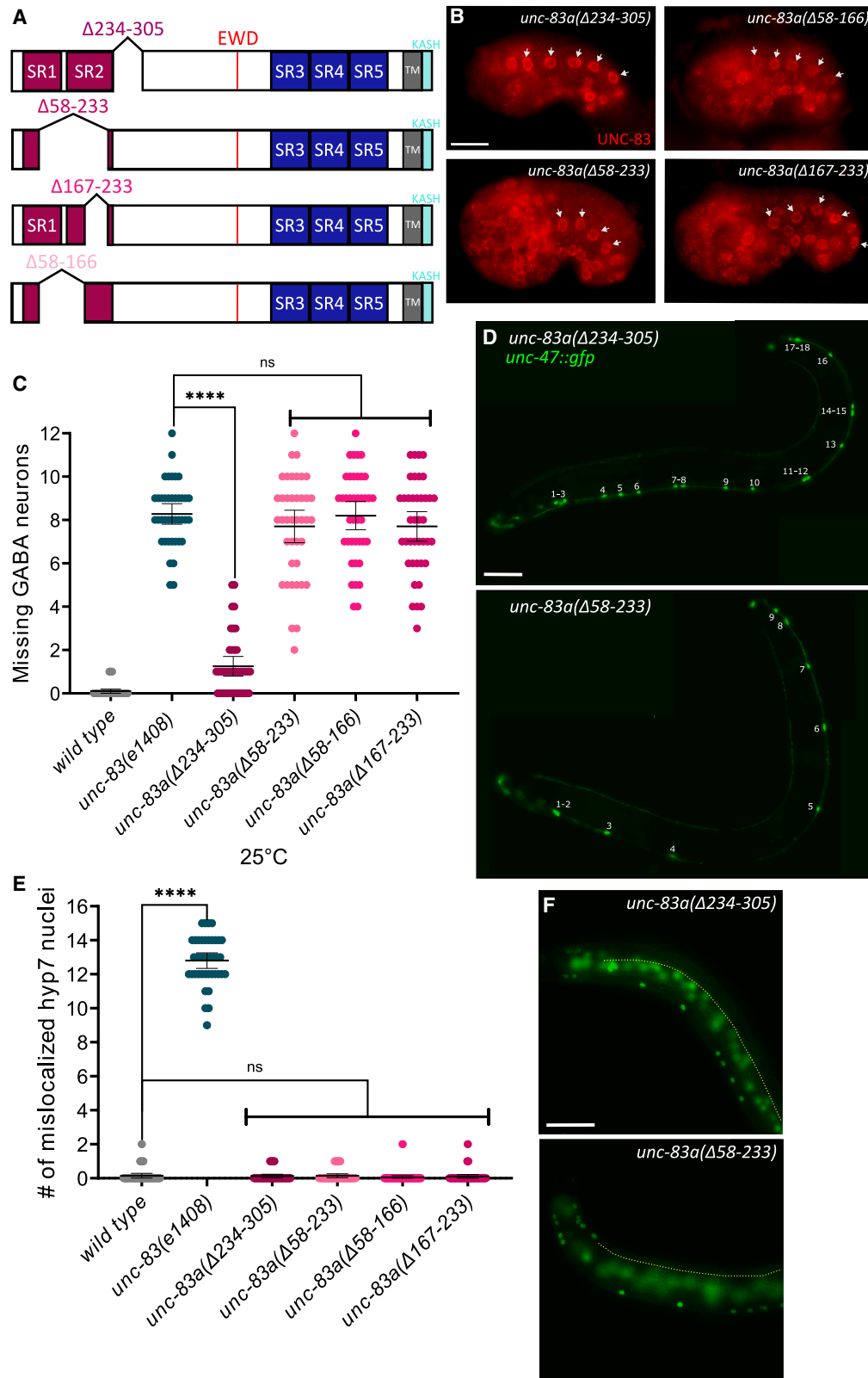
(D) Lateral views of *wild type* and *unc-83(yc108[UNC-83a])* L4 larva expressing hypodermal nuclear GFP. Dashed yellow lines mark the dorsal cord of the animal. Mislocalized nuclei (arrows) indicate migration failure.

(E) Quantification of dynein-mediated larval P-cell nuclear migration defects in *wild type*, mutant *unc-83(yc108[UNC-83a])*, and *unc-83(e1408)* null animals via counting number of missing GABA neurons at 25°C. Each point represents the number of missing GABA neurons per animal; $n = 40$ for each strain.

(F) Lateral views of *wild type* and *unc-83(yc108[UNC-83a])* L4 larva expressing a GABA neuron nuclear marker (*unc-47::gfp*). Numbers indicate a total of 19 GABA neurons.

(G) Quantification of larval P cell nuclear migration defects in *wild type*, *unc-83(e1408)* null, and *unc-83c* overexpression animals (*unc-83(ycEx301[phl3::unc-83c(+)]*) and *unc-83(ycEx302[phl3::unc-83c(+)]*) via counting number of missing GABA neurons at 25°C. Both transgenic lines show mild but statistically significant defects in cell nuclear migration, with 0.45 ± 0.20 and 0.75 ± 0.33 missing GABA neurons compared with 0.1 ± 0.09 missing GABA neurons in *wild type*. Each point represents the number of missing GABA neurons per animal; $n = 40$ for each strain.

(H) Lateral view of *unc-83(ycEx302[phl3::unc-83c(+)]* L4 larva expressing *unc-47::gfp*. Numbers indicate a total of 16 GABA neurons in larva. For all plots: means with 95% CI are shown in error bars. Unpaired Student's *t* tests were performed on the indicated comparisons; ns means not significant; $p > 0.05$, $^*p < 0.05$, $^{***}p < 0.0001$. For images: scale bars, 10 μ m for (B), and 42.2 μ m for (D), (F), and (H).



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where we deleted 750 bp of this region using CRISPR-Cas9 gene editing (Figure 5A). Quantitative analysis of embryonic hyp7 and larval P-cell nuclear migrations revealed identical phenotypes in *unc-83(yc121)* and *unc-83(yc122)* strains. An average of about two hyp7 nuclei mislocalized in the dorsal cord (Figures 5B and 5C), significantly higher than in wild type, indicating the enhancer's necessity for normal hyp7 nuclear migration. The enhancer deletion also caused subtle but significant defects in P-cell nuclear migration (Figures 5D and 5E). These results suggest that this conserved non-coding element contributes to the regulation of *unc-83c* expression in embryonic hyp7 precursors. While deleting the enhancer does not abolish nuclear migration, the significant hyp7 nuclear migration defect indicates that this enhancer plays a role in the developmental control of isoform expression. The incomplete penetrance likely reflects redundant mechanisms controlling isoform expression, including potential post-transcriptional regulation or the activity of additional enhancer elements not identified in our analysis.

To test the enhancer's sufficiency to drive the expression of *unc-83c* in embryonic hyp7 precursors, we generated a transgene by fusing the 870 bp region to a minimal promoter driving the expression of GFP fused to the SV40 nuclear localization sequence (*nls::gfp*). This construct specifically drove expression in pre-comma stage embryonic hyp7 precursors (Figure 5F). It also was expressed in unidentified tissues later in development. These results demonstrate that this conserved non-coding 870 bp element functions as a cell-type-specific enhancer driving *unc-83c* expression in embryonic hyp7 precursors. This is consistent with the earliest detected expression pattern of UNC-83 protein.⁵²

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

Building on our genetic evidence that the UNC-83a-specific N-terminal domain inhibits kinesin-1 while promoting dynein activity during P-cell nuclear migration, we investigated its direct effects on kinesin-1 activity using biochemical approaches. We expressed and purified the N-terminal domain of UNC-83a (UNC-83a^{N-term}) from *E. coli* (Figures 6A and 6B). Additionally, we purified recombinant *C. elegans* kinesin-1 heterotetramers from Sf9 insect cells by co-expressing full-length KHC (UNC-116) and KLC (KLC-2) (Figures 6A and 6B). As mammalian kinesin-1 heterotetramers require cargo adaptors for activation from their autoinhibited state,^{30,64} we first analyzed *C. elegans*

kinesin-1 recruitment to microtubules in the presence of a non-hydrolysable nucleotide analog, AMP-PNP, which locks the motor domains in a high-affinity state on microtubules⁶⁴ (Figures 6C and 6D). Kinesin-1 alone bound to microtubules as expected,⁶⁴ whereas neither UNC-83a nor UNC-83c showed any observable microtubule binding in the absence of kinesin-1 (Figure 6C). Addition of excess UNC-83c significantly enhanced kinesin-1 binding to microtubules (Figures 6C and 6D). By contrast, both full-length UNC-83a and UNC-83a^{N-term} failed to increase kinesin-1 recruitment to microtubules above baseline levels (Figures 6C and 6D). To examine whether UNC-83a^{N-term} inhibits UNC-83c from enhancing kinesin-1 binding to microtubules, we preincubated kinesin-1 with a 5-fold molar excess of each of UNC-83c and UNC-83a^{N-term}. We observed significantly less kinesin-1 bound to microtubules than when kinesin-1 was preincubated with an excess amount of UNC-83c alone (Figures 6C and 6D). We also observed it to be comparable with the amount of kinesin-1 bound to microtubules when kinesin-1 was preincubated with full-length UNC-83a (Figures 6C and 6D). These results indicate that UNC-83a^{N-term} is sufficient to inhibit UNC-83c from enhancing kinesin-1 binding to microtubules.

We next assessed the impact of UNC-83a, UNC-83c, and UNC-83a^{N-term} on kinesin-1 motor function using microtubule gliding assays (Figures 6E and 6F; Videos S1, S2, S3, and S4). Both UNC-83a and UNC-83c enhanced microtubule gliding velocity when added in excess to kinesin-1 (Videos S2 and S3). Strikingly, UNC-83a^{N-term} significantly reduced the average gliding velocity compared with kinesin-1 alone (Figures 6E and 6F; Videos S1 and S4), providing direct evidence that the N-terminal domain of UNC-83a functions as a kinesin-1 inhibitor. Importantly, we saw no evidence of UNC-83a or UNC-83a^{N-term} binding directly to microtubules (Figure 6C) or preventing kinesin-1 from binding to microtubules (Figures 6E and 6F; Videos S1, S2, S3, and S4), indicating the blockage of kinesin-1 motility by UNC-83a is a direct effect on kinesin-1 motor activity and not a secondary effect of inhibiting kinesin-1 from microtubule binding.

The UNC-83a N-terminal domain directly binds the KHC

Having established that the UNC-83a-specific N-terminal domain inhibits kinesin-1 activity both *in vivo* and *in vitro*, we investigated the molecular mechanism underlying this inhibition. We hypothesized that the UNC-83a^{N-term} domain inhibits kinesin-1 by directly binding the KHC UNC-116. To test this hypothesis, we purified full-length UNC-116 from Sf9 cells

Figure 4. Spectrin-like repeats in the N-terminal domain of UNC-83a are necessary for dynein-dependent nuclear migration

(A) Schematics of the *unc-83* N-terminal domain deletion mutants.

(B) Immunofluorescence staining shows that the deletion mutants *unc-83a(Δ234-305)*, *unc-83a(Δ58-166)*, *unc-83a(Δ58-233)*, and *unc-83a(Δ167-233)* have a normal UNC-83 (red) localization pattern at the nuclear envelope of hyp7 precursor cell nuclei in comma-stage embryos. White arrows indicate nuclei with UNC-83 localization. Dorsal is up, and anterior is left.

(C) Quantification of larval P-cell nuclear migration defects in the indicated strains at 25°C (see Figure S2 for the P-cell nuclear migration defects of the indicated strains at 20°C and 15°C). Each point represents the number of missing GABA neurons per animal; *n* = 40 for each strain. *Wild type* and *unc-83(e1408)* data are the same as shown in Figure 3.

(D) Lateral views of *unc-83a(Δ234-305)* and *unc-83a(Δ58-233)* L4 larvae expressing *unc-47::gfp*. Numbers indicate total GABA neurons.

(E) Quantification of hyp7 nuclear migration defects in the indicated strains. Each point represents the total number of abnormally located hyp7 nuclei per animal; *n* = 40 for each strain. *Wild type* and *unc-83(e1408)* data are the same as shown in Figure 3.

(F) Lateral views of *unc-83a(Δ234-305)* and *unc-83a(Δ58-233)* L4 larvae expressing hypodermal nuclear GFP. Dashed yellow lines mark the dorsal cord of the animals. For all plots: means with 95% CI are shown in error bars. Unpaired Student's *t* tests were performed on the indicated comparisons; ns means not significant; **p* > 0.05, ***p* < 0.05, *****p* < 0.0001. For images: scale bars, 10 μm for (B) and 42.2 μm for (D) and (F).

See Figure S2 for more details.

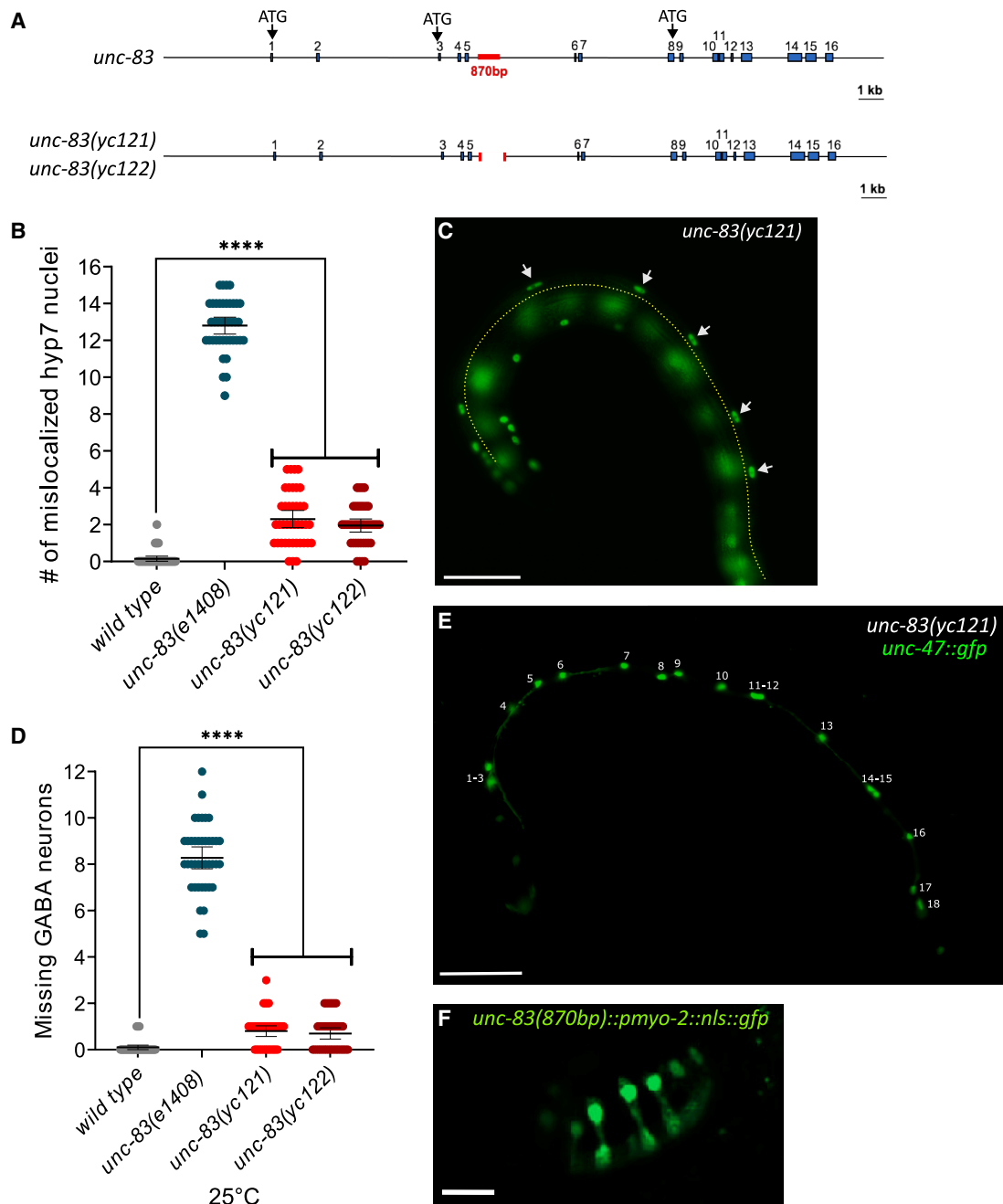


Figure 5. A conserved enhancer controls cell-type-specific expression of *unc-83c* in embryonic hypodermal precursors

(A) Schematics of the *unc-83* locus in wild type and enhancer deletion mutants (*unc-83(ya121)* and *unc-83(ya122)*). The 870 bp enhancer region (see Figure S3 for sequences) is highlighted in red. Exons are shown as blue boxes. The start codons for *unc-83a* in exon 1, *unc-83b* in exon 3, and *unc-83c* at the end of exon 8 are indicated. The sizes of introns and exons are according to the scale as indicated (scale bar, 1 kb). (See Figure S3 for the sequence alignment.)

(B) Quantification of hypodermal hyp7 nuclear migration defects in the indicated strains. Each point represents the total number of abnormally located hyp7 nuclei per animal; $n = 40$ for each strain. Wild type and *unc-83(e1408)* data are the same as shown in Figure 3.

(C) Lateral view of *unc-83(ya121)* L4 larva expressing hypodermal nuclear GFP. Dashed yellow lines mark the dorsal of the animal. Arrows indicate mislocalized hypodermal nuclei, representing failed nuclear migrations.

(D) Quantification of larval P-cell nuclear migration defects in the indicated strains at 25°C. Each point represents the number of missing GABA neurons per animal; $n = 40$ for each strain. Wildtype and *unc-83(e1408)* data are the same as shown in Figure 3.

(E) Lateral view of *unc-83(ya121)* L4 larva expressing *unc-47::gfp*. Numbers indicate a total number of 18 GABA neurons.

(F and G) *C. elegans* pre-comma stage embryos expressing *unc-83* 870 bp enhancer region driving *nls::gfp*. (F) Expression of the 870 bp enhancer region (green) in pre-comma stage embryo, showing hyp7 precursor-specific expression. For all plots: means with 95% CI are shown in error bars. Unpaired Student's *t* tests were performed on the indicated comparisons; ns means not significant; $p > 0.05$, $*p < 0.05$, $****p < 0.0001$. For images: scale bars, 42.2 μm for (C) and (E) and 10 μm for (F) and (G).

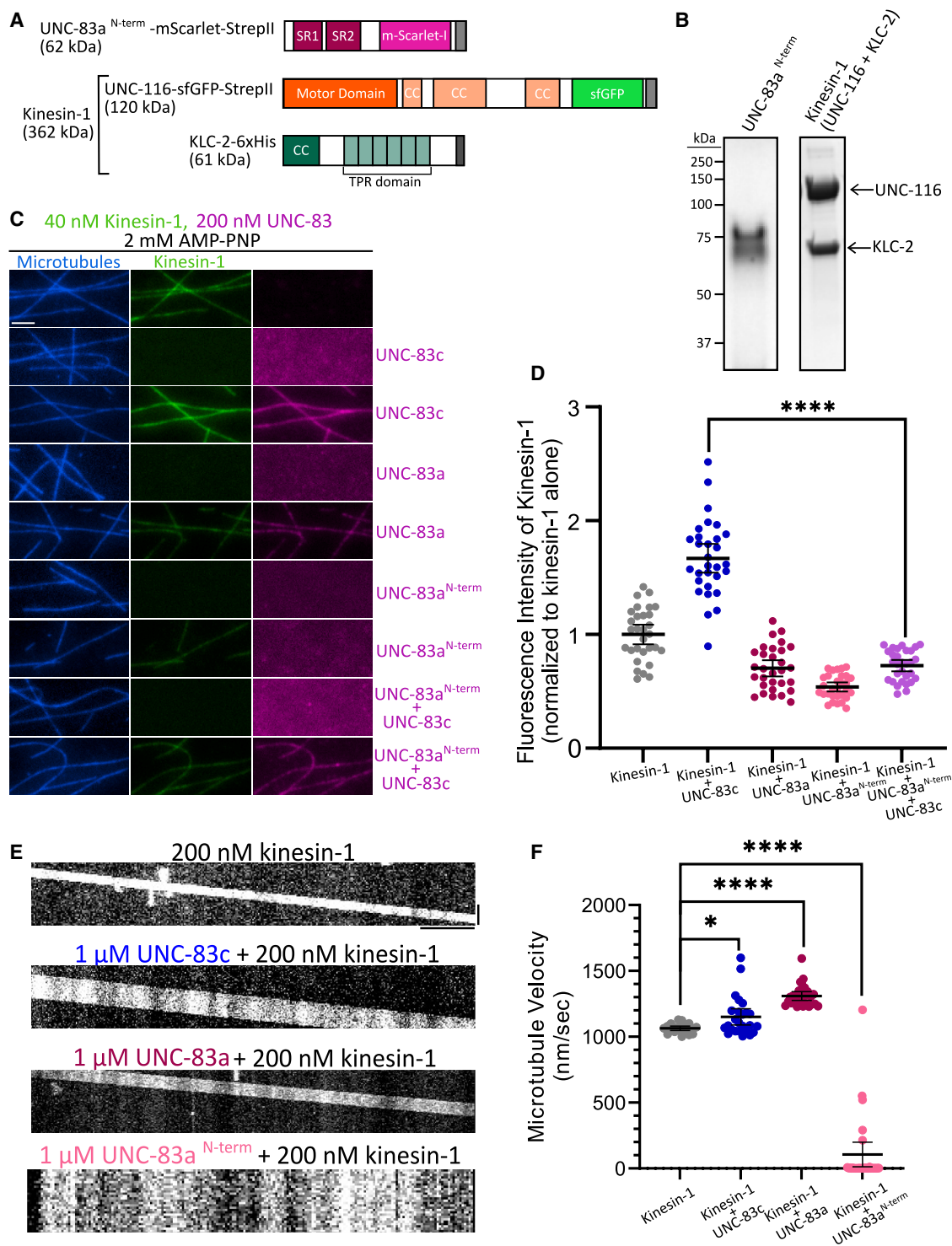


Figure 6. The UNC-83a N-terminal domain inhibits kinesin-1 activity *in vitro*

(A) Schematics of the indicated recombinant protein constructs. The coiled-coil domains of UNC-116 and KLC-2 are indicated as “CC.” UNC-116 is co-expressed with KLC-2. The co-expression leads to the formation of a kinesin-1 heterotetramer that is expected to be 362 kDa.

(B) Coomassie-stained gels of purified UNC-83a^{N-term} and UNC-116-KLC-2 (kinesin-1) recombinant proteins. Arrows indicate the bands for the different proteins.

(C) Representative TIRF images showing the recruitment of kinesin-1 (green) in the rigor state (with 2 mM AMP-PNP) onto microtubules (blue) and the effects of the indicated UNC-83 constructs (magenta). Scale bar, 2.5 μm.

(D) Quantification of microtubule-bound kinesin-1 fluorescence intensity (normalized to the kinesin-1 signal when no UNC-83 constructs were added) when kinesin-1 is preincubated with the indicated UNC-83 constructs. $n = 3$, $n = 30$ microtubules were analyzed for each condition.

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(Figure 7A), confirming that the purified protein existed predominantly (82%) in its expected dimeric state²⁸ (Figure 7B). We then examined protein binding using mass photometry by separately incubating UNC-83a^{N-term}, UNC-83a, and UNC-83c with UNC-116 (Figures 7D'–7F'). Both UNC-83a^{N-term} and UNC-83a formed stable complexes with UNC-116 (the major stable complex is likely one monomer of UNC-83a or UNC-83a^{N-term} binding one dimer of UNC-116, Figures 7D, 7D', 7E, and 7E', although the peak is broad, suggesting there may be other complexes). By contrast, UNC-83c failed to interact with UNC-116 (Figures 7F and 7F'). We then separately incubated UNC-83a, UNC-83a^{N-term}, and UNC-83c with kinesin-1 tetramer (UNC-116 + KLC-2) (Figure 7C). We observed one UNC-83a monomer and one UNC-83a^{N-term} monomer, separately, forming a predominant stable complex with one kinesin-1 tetramer, while UNC-83c did not (Figures 7D', 7E'', and 7F''). These findings demonstrate that the UNC-83a-specific N-terminal domain mediates direct binding to UNC-116, providing a molecular mechanism for the observed kinesin-1 inhibition both *in vivo* (Figures 3A–3D) and *in vitro* (Figures 6E and 6F).

While our total internal reflection fluorescence (TIRF)-based experiments showed that UNC-83c was able to bind to kinesin-1 tetramer (Figure 6), we did not observe a stable complex formation between UNC-83c and kinesin-1 tetramer in solution (Figure 7F''). We hypothesize that this is due to the presence of microtubules in TIRF-based assays and/or the significant difference between the protein concentrations used. Microtubule binding likely induces structural rearrangement of the kinesin-1 tetramer.⁶⁵ This could be sufficient to significantly increase the stability of the interaction between UNC-83c and KLC-2. For the TIRF assays, kinesin-1 was used at 40 nM for binding assays and 200 nM for gliding assays, with UNC-83 constructs at 200 nM and 1 μ M, respectively. By contrast, all proteins were used at 30 nM in the mass photometry assays, a substantially lower concentration that may reduce apparent binding affinities in solution. Furthermore, nucleotides, AMP-PNP, or ATP, were present in the TIRF-based experiments but not in the mass photometry experiments. We also note that the mass photometry method relies on single particle detection and thus precludes utilizing higher protein concentrations.

DISCUSSION

Spatial and temporal control of nuclear positioning is fundamental for development, yet the mechanisms regulating bidirectional nuclear movement have remained elusive. Our work reveals a novel paradigm where expression of alternative isoforms of a cargo adaptor creates an intrinsic molecular switch for controlling nuclear migration directionality. Previous studies identified UNC-83 as a nuclear-specific cargo adaptor that recruits both kinesin-1 and dynein to mediate bidirectional movement of nuclei.^{15,55,56} Genetic analyses suggested a model

where the short UNC-83c isoform is sufficient for kinesin-driven nuclear migration in the embryo and the long UNC-83a/b isoforms are necessary for dynein-mediated nuclear migration in larva.⁵² Here, we demonstrate that distinct UNC-83 isoforms differentially regulate motor activity to achieve precise developmental control of nuclear positioning.

Our discovery of an inhibitory N-terminal-specific domain in the long UNC-83a/b isoforms represents a previously unrecognized mechanism for motor regulation. The genetic and biochemical analyses reported here reveal this domain's dual regulatory function: direct binding and potential inhibition of the KHC UNC-116, combined with reduced affinity for the KLC-2. Based on our findings, we propose a motor regulation model where the UNC-116/UNC-83a interaction prevents kinesin-1 activation by inhibiting the conformational change induced by the KLC-2/UNC-83c interaction. This would lead to dynein-directed movement. We also propose that the N-terminal domain of UNC-83a is autoinhibitory, as we observed the addition of UNC-83a^{N-term} to UNC-83c to significantly lower the amount of kinesin-1 bound to microtubules when compared with UNC-83c alone (Figures 6C and 6D).

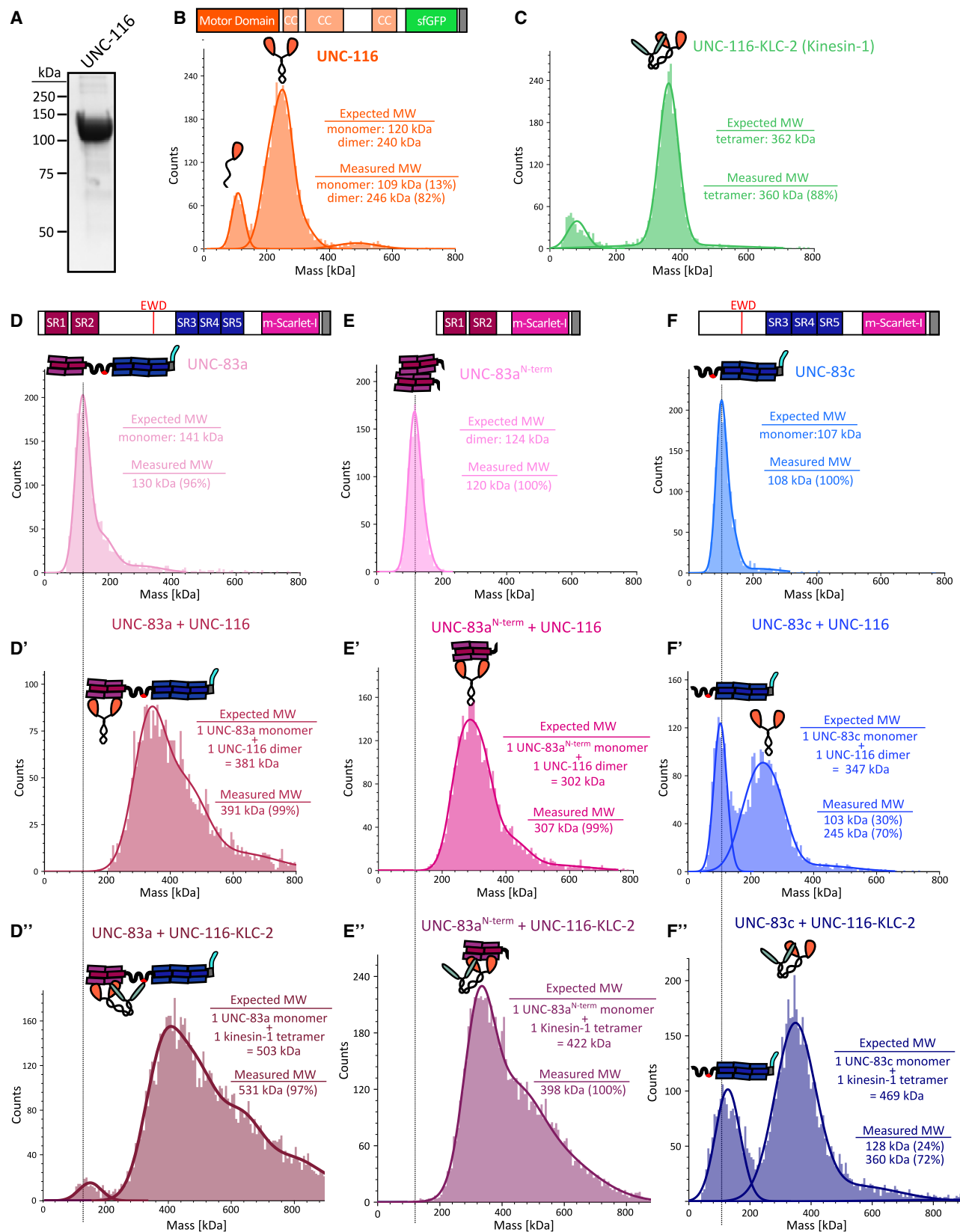
By contrast, UNC-83c's high-affinity interaction with KLC-2 through its EWD motif^{13,36} promotes kinesin-1 activation, enabling plus-end-directed nuclear movement in embryonic hyp7 precursors. This isoform-specific regulation solves a challenge in developmental biology: how to achieve opposing directional movements of the same cargo using the same molecular machinery.

The involvement of UNC-83 with multiple dynein-regulatory proteins, including the homolog of long coiled-coil dynein activating adaptor Bicaudal D, BICD-1,⁵⁶ suggests integration into a larger regulatory network. This organization parallels other bidirectional transport events.^{66,67} For example, TRAK1/2 mediates mitochondrial trafficking through the outer mitochondrial membrane receptor Miro and metaxins,^{68–70} and HOOK3 regulates endosomal trafficking with FTS and FHPIB.^{71–74} Nuclei, which are larger and stiffer than mitochondria and endosomal vesicles,⁷⁵ present unique challenges for intracellular transport. Our findings and the conservation of LINC complex-mediated nuclear positioning^{17,22,54} suggest that evolution has developed specialized mechanisms to address the challenges of moving nuclei. Mammalian KASH proteins Nesprin-2, Nesprin-4, and KASH5 each interact with microtubule motors through mechanisms similar to UNC-83.^{13,14,41,42,60,64,76}

The identification of spectrin-like repeats in UNC-83 with AlphaFold predictions reveals structural similarities to *Drosophila* Klarsicht⁷⁷ and mammalian nesprins.^{13,14,41,42,60,64,76} This structural conservation, combined with common motor-binding mechanisms, suggests that motor regulation at the nuclear envelope through alternatively spliced inhibitory domains may be more widespread than previously appreciated. The modular nature of KASH protein interactions with the cytoskeleton appears to be an evolutionarily conserved

(E) Representative kymographs of microtubule gliding with kinesin-1 alone (see Video S1), kinesin-1 + UNC-83c (see Video S2), kinesin-1 + UNC-83a (see Video S3), and kinesin-1 + UNC-83a^{N-term} (see Video S4). Scale bars: 20 s (vertical) and 5 μ m (horizontal).

(F) Quantification of microtubule gliding velocities for the indicated conditions. $n = 2$, $n = 25$ microtubules analyzed for each condition. The average velocities are $1,064 \pm 35$ nm/s (kinesin-1), $1,150 \pm 149$ nm/s (kinesin-1 + UNC-83c), $1,309 \pm 82$ nm/s (kinesin-1 + UNC-83a), and 187 ± 533 nm/s (kinesin-1 + UNC-83a^{N-term}). For all plots: means with 95% CI error bars are shown. Unpaired t-tests were performed in the indicated comparisons; * $p < 0.05$; **** $p < 0.0001$.



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feature, potentially allowing rapid adaptation to diverse cellular contexts.

Our data support a selective activation model for bidirectional transport, rather than selective recruitment or tug-of-war models.⁶⁷ The selective activation model could explain the steady but slow nature of nuclear movements.⁷⁸ Moreover, cargo stiffness has been speculated to alter motor engagement.^{79,80} Nuclei are the largest and one of the stiffer intracellular cargos and move significantly slower than small cargos. Hyp7 and P-cell nuclei migrate at a speed of $\sim 1 \mu\text{m}/\text{min}$.^{49,56} The discovery that expression of alternative isoforms can generate cargo adaptors with opposing regulatory functions provides a new framework for understanding how cells achieve precise spatiotemporal control of organelle positioning. This mechanism may be particularly crucial for nuclei, where their unique physical properties⁷⁵ require additional regulation.

These findings open several exciting directions for future investigation. Does Klarsicht employ similar inhibitory mechanisms during *Drosophila* development? Have mammalian systems evolved analogous regulatory strategies through their expanded repertoire of KASH proteins? Understanding how broadly this regulatory paradigm extends could reveal fundamental principles of developmental organelle positioning. Moreover, our work suggests that expression of alternative isoforms of cargo adaptors may represent a general mechanism for generating precise spatiotemporal control of intracellular transport during development.

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.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.G., G.W.G.L., R.J.M., and D.A.S.; data curation, S.G., N.S., D.E., E.F.G., and K.C.; formal analysis, S.G., N.S., D.E., E.F.G., and K.C.; investigation, S.G., N.S., D.E., E.F.G., and K.C.; methodology, all authors; project administration, S.G. and D.A.S.; resources, all authors; validation, all authors; visualization, S.G., G.W.G.L., R.J.M., and D.A.S.; writing – original draft, S.G. and D.A.S.; and writing – review and editing, S.G., S.N., G.W.G.L., R.J.M., and D.A.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [EXPERIMENTAL MODEL AND SUBJECT DETAILS](#)
 - *C. elegans* maintenance and genetics
 - Protein expression systems
- [METHOD DETAILS](#)
 - Protein structure prediction
 - Plasmid construction and molecular cloning
 - Protein expression and purification
 - Single-molecule mass photometry
 - Biolayer interferometry binding assays
 - *C. elegans* transgenic strains
 - Nuclear migration assays in *C. elegans*
 - Immunofluorescence in *C. elegans*
 - Microtubule preparation
 - TIRF assays
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2025.08.025>.

Figure 7. The UNC-83a N-terminal domain directly binds the KHC

(A) A Coomassie-stained gel showing purified UNC-116-sfGFP.

(B) A schematic of the UNC-116-sfGFP recombinant protein and a Gaussian fit of its measured molecular weight in solution by mass photometry (30 nM; monomer = 109 ± 19.5 kDa, 13% peak; dimer = 246 ± 44 kDa; 82% peak). Coiled-coil domains are indicated as “CC.”

(C) A Gaussian fit of UNC-116-KLC-2 (kinesin-1) recombinant protein’s measured molecular weight in solution by mass photometry (30 nM; tetramer = 360 ± 69 kDa, 88% peak).

(D–D’) A schematic of the UNC-83a-mScarlet recombinant protein and a Gaussian fit of its measured molecular weight in solution alone (30 nM; monomer = 130 ± 62 kDa; 96% peak) (D), when preincubated with UNC-116 (30 nM; one UNC-83a monomer + one UNC-116 dimer = 391 ± 153 kDa, 99% peak) (D’), and when preincubated with UNC-116-KLC-2 (kinesin-1) (30 nM; one UNC-83a monomer + one kinesin-1 tetramer = 531 ± 272 kDa, 97% peak) (D’).

(E–E’) A schematic of the UNC-83a^{N-term}-mScarlet recombinant protein and a Gaussian fit of its measured molecular weight in solution alone (30 nM; dimer = 120 ± 23 kDa, 100% peak) (E) or when preincubated with UNC-116 (30 nM; one UNC-83a^{N-term} monomer + one UNC-116 dimer = 307 ± 89 kDa, 99% peak) (E’) or when preincubated with UNC-116-KLC-2 (kinesin-1) (30 nM; 398 ± 132 kDa, 72% peak) (E’).

(F–F’) A schematic of the UNC-83c-mScarlet recombinant protein and a Gaussian fit of its measured molecular weight in solution alone (30 nM; monomer = 108 ± 41 kDa, 100% peak) (F), when preincubated with UNC-116 (30 nM; 103 ± 21 kDa, 30%; 245 ± 82 kDa, 70% peak) (F’), and when preincubated with UNC-116-KLC-2 (kinesin-1) (30 nM; 128 ± 41 kDa, 24% peak; 360 ± 110 kDa, 72% peak) (F’).

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
UNC-83 antibody	Starr et al. ⁵²	Clone 1209D7; RRID: AB_3713279
Alexa Fluor® 555 conjugated goat anti-mouse (H+L) Cross-Adsorbed IgG	ThermoFisher Scientific	Cat# A21422; RRID: AB_2535844
Bacterial and virus strains		
BL21-CodonPlus (DE3)-RIPL competent cells	Agilent	230280
NEB 5-alpha competent <i>E. coli</i>	NEB	C2987H
DH10MultiBac competent cells	Geneva Biotech	N/A
<i>E. coli</i> OP50	<i>Caenorhabditis</i> 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-carboxylic acid, 97%)	Acros	AC218940050
3,4-Dihydroxybenzoic Acid (Protocatechuic acid) (PCA)	Sigma-Aldrich	37580
Protocatechuate 3,4-Dioxygenase from <i>Pseudomonas</i> sp. (PCD)	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
Apo ferritin	Sigma-Aldrich	A3641
Thyroglobulin	Sigma-Aldrich	T9145
tetramisole	Sigma-Aldrich	Cat#L9756
Cas9 protein	QB3 UC Berkeley	N/A
Experimental models: Organisms/strains		
<i>C. elegans</i> strain: wild type	Brenner ⁸¹	N2
<i>C. elegans</i> strain: zen-4 (<i>cle10</i>)/IV	Farboud et al. ⁸²	AFS222
<i>C. elegans</i> strain: <i>oxIs12[punc-47::gfp]</i> X	McIntire et al. ⁸³	EG1285
<i>C. elegans</i> strain: <i>unc-83(e1408)</i> V	Starr et al. ⁵²	CB1408
<i>C. elegans</i> strain: <i>oxIs12; unc-83(e1408)</i> V	Starr et al. ⁵²	MH1654
<i>C. elegans</i> strain: <i>ycls3[unc-83(870bp)::min_myo-2 promoter::nls::gfp; odr-1::rfp]</i>	This study	UD338
<i>C. elegans</i> strain: <i>ycls9 [pcol-10nls::gfp::lacZ]</i> I	Fridolfsson et al. ⁶²	UD393

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc77</i> [Δ 234-305]) <i>V</i>	This study	UD646
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc77</i>) <i>V</i>	This study	UD657
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc79</i> [Δ 167-233]) <i>V</i>	This study	UD658
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc77</i>) <i>V</i>	This study	UD659
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc79</i>) <i>V</i>	This study	UD682
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc79</i>) <i>V</i>	This study	UD684
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc95</i> [Δ 58-166]) <i>V</i>	This study	UD741
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc95</i>) <i>V</i>	This study	UD742
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc95</i>) <i>V</i>	This study	UD743
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc108</i> [<i>punc-83c::unc-83a</i>]) <i>V</i>	This study	UD805
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc108</i>) <i>V</i>	This study	UD871
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc108</i>) <i>V</i>	This study	UD894
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>e1408</i>) <i>V</i>	Taiber et al. ¹³	UD900
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>ycEx301</i> [<i>phlh-3::unc-83c</i> ; <i>Pmyo-2::mRFP</i>]	This study	UD973
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>ycEx302</i> [<i>phlh-3::unc-83c</i> ; <i>Pmyo-2::mRFP</i>]	This study	UD974
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc121</i> [<i>delta enhancer</i>]) <i>V</i>	This study	UD999
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc122</i> [<i>delta enhancer</i>]) <i>V</i>	This study	UD1000
<i>C. elegans</i> strain: <i>unc-83</i> (<i>yc123</i> [Δ 58-233]) <i>V</i>	This study	UD1001
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc121</i>) <i>V</i>	This study	UD1002
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc122</i>) <i>V</i>	This study	UD1003
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc121</i>) <i>V</i>	This study	UD1004
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc122</i>) <i>V</i>	This study	UD1005
<i>C. elegans</i> strain: <i>oxls12</i> ; <i>unc-83</i> (<i>yc123</i>) <i>V</i>	This study	UD1007
<i>C. elegans</i> strain: <i>ycls9</i> <i>I</i> ; <i>unc-83</i> (<i>yc123</i>) <i>V</i>	This study	UD1010
Recombinant DNA		
<i>Podr-1::RFP</i>	L'Etoile and Bargmann ⁸⁴	N/A
pFastBac_Dual UNC-116-sfGFP-StrepII	This study	MOM842 (pSN492)
pFastBac_Dual UNC-116-sfGFP-StrepII KLC-2-6xHis	This study	MOM845 (pSN500)
pPD97.82 with <i>unc-83</i> (870bp)::min_ <i>myo-2</i> promoter::nls::gfp	This study	pSL439
pDS22 <i>phlh-3::unc-83c</i>	This study	pSL536
pET21a UNC-83c-mScarlet StrepII	This study	MOM1135
pET21a UNC-83a N-term (1-305aa)-mScarlet-StrepII	This study	pSL913
pET21a UNC-83a-mScarlet StrepII	This study	pSL912
pGEX-2T <i>klc-2</i>	Meyerzon et al. ⁵⁵	pSL409
Software and algorithms		
Fiji	Schindelin et al. ⁸⁵	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 et al. ⁸⁶	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

EXPERIMENTAL MODEL AND SUBJECT DETAILS

C. elegans maintenance and genetics

C. elegans strains were maintained at room temperature (approximately 22°C), unless otherwise indicated, on nematode growth medium plates seeded with OP50 *E. coli*.⁸¹ Some strains were obtained from the Caenorhabditis Genetics Center, funded by the National Institutes of Health Office of Research Infrastructure Programs (P40 OD010440). All the strains used in this study are listed on the [key resources table](#).

Protein expression systems

Spodoptera frugiperda Sf9 cells were cultured in suspension using Sf-900II serum-free medium (SFM) (ThermoFisher Scientific) supplemented with 10 µg/mL gentamycin. Cultures were maintained at 27°C with constant rotation at 150 rpm. The culture density was maintained between 1.0×10⁶ and 8.0×10⁶ cells/mL during routine passaging. Cells were not authenticated. The sex of the cells is unknown.

BL21-CodonPlus (DE3)-RIPL *E. coli* (Agilent) transformed with expression plasmids were cultured in LB medium at 37°C with shaking at 200 rpm.

METHOD DETAILS

Protein structure prediction

Structural models of UNC-83a and the UNC-83c/KLC-2 complex were generated using AlphaFold3⁵⁹ with default parameters, without template structures. Five models were generated for each protein or complex. The models with the highest confidence scores (for the UNC-83c/KLC-2 complex: pTM = 0.86 and ipTM = 0.74) were used for figure preparation. For visualization, some residues within the low-confidence unstructured region between the spectrin-like repeats #3 and #4 in UNC-83a were straightened by altering the torsion angles⁸⁷ (ϕ and ψ to approximately -135° and 135°, respectively) with ChimeraX.⁸⁸

Plasmid construction and molecular cloning

Expression constructs

The glutathione-S-transferase (GST)-KLC-2 construct was generated as previously described.⁵⁵ A cDNA construct encoding for UNC-83a⁵² was used to generate the UNC-83a-mScarlet-StrepII, UNC-83c-mScarlet-StrepII, and UNC-83a^{N-term}-mScarlet-StrepII constructs. Using Gibson Assembly, UNC-83a, UNC-83c and UNC-83a^{N-term} coding regions were cloned into pET21a-Novagen bacterial expression vector (Millipore Sigma) with a C-terminal mScarlet-StrepII tag. For insect cell expression, UNC-116-sfGFP-StrepII and KLC-2-6xHis-UNC-116-sfGFP-StrepII constructs were cloned into pFastBac_Dual vector (ThermoFisher Scientific).

Promoter and enhancer constructs

The pSL536 plasmid was made by cutting the 3.4 kb fragment of *hlh-3* promoter from pRD15⁶³ (using HindIII and SmaI restriction enzyme sites) and ligating this fragment 442 bp upstream of the *unc-83c* start codon into AgeI/BamHI/Klenow treated pDS22.⁵² For pSL439, the 870 bp intronic fragment from the *unc-83* region between exons 5 and 6 was cloned using StuI and XbaI, and inserted into pPD97.82 (Addgene plasmid #1512, a gift from the Fire Lab). The *odr-1::rfp* plasmid was a gift from Noelle L'Etoile (University of California, San Francisco).⁸⁴

Sequence verification

All constructs were verified by whole plasmid sequencing performed by Plasmidsaurus using Oxford Nanopore Technology with custom analysis and annotation. A complete list of plasmids used in this study is provided in the [key resources table](#).

Protein expression and purification

Bacterial expression and preparation of GST-KLC-2, UNC-83a-mScarlet-StrepII, UNC-83c-mScarlet-StrepII, and UNC-83a^{N-term}-mScarlet-StrepII were performed as described below. BL21-CodonPlus (DE3)-RIPL (Agilent) *E. coli* were transformed with each plasmid and grown at 37°C in LB medium with 100 µg/mL carbenicillin until optical density at 600 nm (OD₆₀₀) reached 0.8. The cultures were then cooled down to room temperature and protein expression was induced by adding 0.2 mM isopropyl-β-D-thiogalactoside (IPTG) and incubating overnight at 18°C. Bacterial pellets were stored at -80°C. For recombinant protein purification, frozen pellets were thawed on ice and resuspended in 50 mL lysis buffer (PB: 50 mM Tris-HCl pH=8.0, 150 mM KCH₃COO, 2 mM MgSO₄, 1 mM EGTA, and 5% glycerol) freshly supplemented with 1 mM dithiothreitol (DTT), 0.2 mM phenylmethyl-sulfonyl fluoride (PMSF), 0.1 mM ATP, NucA nuclease, and protease inhibitor mix (Promega). Bacteria were lysed using an Emulsiflex C3 high-pressure homogenizer (Avestin). For UNC-83a-mScarlet-StrepII, UNC-83c-mScarlet-StrepII, and UNC-83a^{N-term}-mScarlet-StrepII purifications, a subsequent chemical lysis was performed with 0.15% Triton X-100 for 5 minutes on ice. Lysed cells were centrifuged at 23,000xg for 20 minutes at 4°C. For affinity purification, the clarified lysates were incubated with resin as follows. UNC-83a-mScarlet-StrepII, UNC-83c-mScarlet-StrepII, and UNC-83a^{N-term}-mScarlet-StrepII lysates were incubated with StrepTactin XT resin (IBA Lifesciences) for 1 hour at 4°C and washed with PB buffer. Proteins were eluted with biotin elution buffer (100 mM biotin in PB buffer freshly supplemented with 1 mM DTT, 0.1 mM PMSF, 0.1 mM ATP, NucA nuclease, 0.013% Triton X-100 at pH=8.0). For GST-KLC-2, Glutathione Sepharose 4B resin (Cytiva) was added to the clarified lysate and incubated on a rocker for 5 hours at 4°C. The beads were then washed with 1x PBS

(Phosphate-buffered saline) and protein was eluted with glutathione elution buffer (50 mM Tris-HCl pH = 8.0, 10 mM L-Glutathione reduced; (Sigma-Aldrich).

For the UNC-116-sfGFP-StrepII and KLC-2-6xHis-UNC-116-sfGFP-StrepII constructs cloned into pFastBac_Dual vector (ThermoFisher Scientific). *E. coli* were transformed to generate bacmids. To prepare baculovirus, 1.0×10^6 Sf9 cells were transferred to each well of a tissue-culture treated 6-well plate. Once the cells were adhered to the bottom of the dish, about 3 μ g bacmid were transfected using 6 μ L of Cellfectin II reagent (ThermoFisher Scientific). 5–7 days after initial transfection, the culture media were collected and spun at 3,000xg for 3 minutes to obtain the viral supernatant (P1). Next, 50 mL of Sf9 cells (2.0×10^6 cells/mL) was infected with 30 μ L of P1 and cultured for 5–7 days to obtain P2 viral supernatant. For protein expression, 400 mL of Sf9 cells (2.0×10^6 cells/mL) were infected with 4 mL of P2 virus and cultured for 65 hours at 27°C while shaking. Cells were harvested by spinning down at 1,992xg for 20 minutes at 4°C. The pellets were stored at -80°C.

UNC-83a^{N-term}-mScarlet-StrepII, UNC-116-sfGFP-StrepII and KLC-2-6xHis-UNC-116-sfGFP-StrepII were further purified via size exclusion chromatography using Superose6 10/300 GL column (Cytiva) equilibrated in buffer GF150 (25 mM HEPES pH = 7.2, 150 mM KCl, 2 mM MgCl₂). GST-KLC-2, UNC-83a-mScarlet-StrepII and UNC-83c-mScarlet-StrepII were purified via size exclusion chromatography using Superdex 200 10/300 GL column (Cytiva) equilibrated in buffer GF150. Fractions containing the proteins were combined and concentrated on Amicon spin filters with a 50 kDa or 100 kDa cutoff. Concentrated proteins were supplemented with 10% glycerol, flash frozen in LiN₂ and stored at -80°C.

Single-molecule mass photometry

Single-molecule mass photometry was performed using a OneMP instrument (Refeyn) as previously described.^{60,89} Briefly, #1.5 24 x 50 mm Deckgläser glass coverslips (ThermoFisher Scientific) were cleaned by 1 hour sonication in isopropanol and subsequently stored in a fresh aliquot of isopropanol. Before assembling the imaging chambers, the coverslips were rinsed with fresh isopropanol followed by Milli-Q water and dried with filtered air. Then, CultureWell silicone gaskets (Grace Bio-Labs) were placed onto the freshly cleaned coverslips providing 4–6 independent sample chambers. For all the cleaning steps, 99.9% (HPLC grade) isopropanol (Fisher Scientific) was used. Samples and standard proteins (Apoferitin, Bovine serum albumin (BSA), and Thyroglobulin; Sigma-Aldrich) were diluted to 10–100 nM in freshly 0.1 μ m filtered SRP90 buffer (90 mM HEPES-KOH, 50 mM KCH₃COO, 2 mM Mg(CH₃COO)₂, 1 mM EGTA, and 10% glycerol, pH = 7.6). The focal position was identified and maintained throughout the measurement using an autofocus system based on total internal reflection. Data acquisition was performed at 1 kHz for 60 seconds using AcquireMP (Refeyn), and data analysis was conducted using DiscoverMP (Refeyn).

Biolayer interferometry binding assays

The real-time binding kinetics of UNC-83a-mScarlet-StrepII and UNC-83c-mScarlet-StrepII to GST-KLC-2 were measured using biolayer interferometry on a ForteBio Octet®-RED384 at the UC Davis Octet® Real-Time Drug and Protein Binding Kinetics Unit. Binding assays were performed in binding buffer (20 mM Tris pH = 7.8, 150 mM NaCl, 0.1 mg/mL BSA, 0.05% Tween-20) (modified from Bhattacharya et al., 2014)⁹⁰ using the Octet® Anti-Glutathione-S-Transferase (GST) Biosensors (Sartorius AG). Operating temperature was maintained at 26°C with a 1,000 rpm rotary agitation throughout the experiment. Samples or buffer were transferred into 96-well plates (Greiner) at a volume of 250 μ L per well. GST biosensor tips were rehydrated in binding buffer before loading the bait onto them. The bait, GST-KLC-2, was diluted to 1 μ M and immobilized onto the biosensors for 10 minutes to attain 1.5 – 2.5 nm response. Binding association of UNC-83a and UNC-83c with biosensor tips was monitored for 20 minutes, and subsequent disassociation in buffer was monitored for 30 minutes. Both UNC-83a and UNC-83c were tested at concentrations of 1.0, 0.667, 0.444, 0.296, 0.197, 0.132, 0.088 μ M. The average of the last 100 points (20 seconds) of the association phase response was used as an estimate for the equilibrium value of the binding reaction. The plots of concentration versus averaged plateau values were fitted using a quadratic binding equation plus a constant offset to find K_d .

C. elegans transgenic strains

To generate transgenic strains UD336 and UD338, which express nuclear GFP (*nls::GFP*) under a minimal promoter with the 870 bp predicted *unc-83* enhancer, N2 animals were injected with 5 ng/ μ L pSL439 and 100 ng/ μ L *odr-1::rfp* co-injection marker.⁸⁴ The integrated transgene *yc1s3[unc-83(870bp)::min_myo-2 promoter::nls::gfp; odr-1::rfp]* was generated with gamma irradiation from a Cs-137 source.⁹¹ For strains UD973 and UD974, *oxIs12* (EG1285) animals were injected with 5 ng/ μ L of pSL536 (*phih-3::unc-83c*), 93 ng/ μ L of pBlue-script SK, and 2 ng/ μ L of Pmyo-2::mRFP.

unc-83a deletion mutant alleles (*yc77,yc79, yc95* and *yc123*) and *unc-83* enhancer region deletion mutant alleles (*yc121* and *yc122*) were generated using the *dpy-10* co-CRISPR strategy.^{92–94} The CRISPR injection mix was made as described previously.⁹⁵ As repair templates, 100-nucleotide-long single-strand DNA oligonucleotides (ssODN) were synthesized (Integrated DNA Technologies). Deletion mutations were screened by PCR amplification of the guide target region, and products showing a smaller band size were confirmed via Sanger sequencing. The crRNA and ssODN repair template sequences are listed in Tables S1 and S2, respectively.

unc-83a^{N-term} overexpression insertion mutant allele (*yc108*) was generated by using *zen-4(+)* as a co-CRISPR marker.⁸² A single-stranded repair template containing the insertion flanked by 50-nucleotide right and left homology arms, was synthesized (Genewiz). The CRISPR injection mix contained 0.2 μ L *zen-4* crRNA (0.6 mM), 0.5 μ L target gene crRNA (0.6 mM), 2.46 μ L tracr (0.17 mM), 7.68 μ L

Cas9 (40 μ M), 0.28 μ l ssODN *zen-4(+)* repair template (500 ng/ μ l) and 2 μ l ssDNA repair template (1 μ g/ μ l). The mixture was injected into the germline of temperature-sensitive *zen-4(cle10)* mutant young adults and screened as previously described.⁸²

Nuclear migration assays in *C. elegans*

To quantify larval P-cell nuclear migration, strains were crossed into *oxIs12[punc-47::gfp]*, which labels P-cell derived GABA neurons.⁸³ The presence of 19 GABA neurons (12 derived from P cells) that are normally GFP-positive with cell bodies located in the ventral cord were scored as previously described.⁶² The assay was performed at 3 different temperatures (15°C, 20°C and 25°C) as *unc-83*-dependent P-cell nuclear migration is temperature-sensitive.⁵²

To quantify embryonic hyp7 nuclear migration, strains were crossed into *ycIs9 I*, a GFP-expressing nuclear marker specific to larval hypodermal nuclei.²⁰ Syncytial hyp7 nuclei were scored as abnormally located if they were in the dorsal cord as previously described.⁶²

Immunofluorescence in *C. elegans*

Immunofluorescence staining with the anti-UNC-83 antibody (Clone 1209D7) was done as previously described.⁵² The primary antibody 1209D7 was used undiluted. The secondary antibody, Alexa Fluor® 555 conjugated goat anti-mouse (H+L) Cross-Adsorbed IgG (ThermoFisher Scientific) was diluted 1:200 in phosphate-buffered saline containing 0.1% Triton X-100 (PBST). DNA was visualized by a 10-minute stain in 100 ng/ml of 4,6-diamidino-2-phenylindole (DAPI; Sigma) in PBST. Images were captured using a Leica DM6000 fluorescence microscope with a 63 $\times$ Plan Apo 1.40 NA objective, a Leica DC350 FX camera, and Leica LAS AF software.

Microtubule preparation

Porcine brain tubulin was isolated using the high-molarity PIPES procedure and then labeled with biotin NHS ester or Dylight-405 NHS ester (ThermoFisher Scientific), as described previously (https://mitchison.hms.harvard.edu/files/mitchisonlab/files/labeling_tubulin_and_quantifying_labeling_stoichiometry.pdf). Pig brains were obtained from a local abattoir and used within ~4 hours post-mortem. To polymerize microtubules, 50 mM of unlabeled tubulin, 10 μ M of biotin-labeled tubulin and 3.5 μ M of Dylight-405-labeled tubulin were incubated with 2 mM GTP for 20 minutes at 37°C. Polymerized microtubules were stabilized by the adding 20 μ M taxol (ThermoFisher Scientific) and incubated an additional 20 minutes at room temperature. Microtubules were pelleted at 20,000 $\times$ g by centrifugation through a 150 μ l of 25% sucrose cushion. The pellet was resuspended in 50 μ l BRB80 (80 mM Pipes (pH = 6.8), 1 mM MgCl₂ and 1 mM EGTA) containing 10 μ M taxol.

TIRF assays

For TIRF assays, imaging chambers were assembled by combining an acid-washed glass coverslip prepared as previously described (<http://labs.bio.unc.edu/Salmon/protocolscoverslip-preps.html>), a precleaned slide, and double-sided sticky tape. Microtubules were diluted in BRB80 containing 10 μ M Taxol. SRP90 assay buffer (90 mM HEPES, 50 mM KCH₃COO, 2 mM Mg(CH₃COO)₂, 1 mM EGTA, 10% glycerol, pH = 7.6), supplemented with 1 mg/mL BSA, 0.2 mg/mL K-casein, and oxygen scavenging system composed of 2.5 mM protocatechuic acid (PCA) (Sigma-Aldrich), 50 nM protocatechuate-3,4-dioxygenase (PCD) (Sigma-Aldrich), and 1 mM trolox (6-hydroxy-2,5,6,7,8-tetramethylchroman-2-carboxylic acid, 97%) (Acros) was used.^{64,89,96}

For microtubule binding assays, TIRF chambers were first coated with 0.5 mg/ml PLL-PEG-biotin (Surface Solutions Group) followed by with 0.5 mg/ml streptavidin (ThermoFisher Scientific). Diluted microtubules were then flowed into the chambers. After addition of each component, chambers were incubated for 5 minutes at room temperature. Once it was confirmed that the microtubules were adhered and polymerized sufficiently, the unbound microtubules were removed by washing the chambers with SRP90 assay buffer. To analyze kinesin-1 recruitment onto the microtubules, the purified motor protein was diluted to 40 nM in the assay buffer with 2 mM 5-adenylyl-imidodiphosphate (AMP-PNP) (Roche Diagnostics GmbH). Kinesin-1 was combined with each UNC-83 construct separately at a 1:5 molar ratio and incubated on ice for 30 minutes. Following the incubation, the protein mixes were diluted with the assay buffer to get 40 nM kinesin-1 and 200 nM UNC-83 construct in the final solution. As controls, each purified UNC-83 construct was diluted to 200 nM in the assay buffer with 2 mM of AMP-PNP. The solutions were then flowed into the TIRF chambers, one at a time, and incubated for 10 minutes at room temperature to let the proteins interact with AMP-PNP and microtubules. Sequential images of 405 nm, 488 nm and 561 nm channels were acquired. Kinesin-1 average fluorescence fold intensity on microtubules were manually measured using ImageJ (Fiji).

For gliding assays, kinesin-1 was diluted in SRP90 buffer to 200 nM and mixed separately with each UNC-83 construct separately at a 1:5 molar ratio and incubated on ice for 30 minutes. Following the incubation, the protein mixes were further diluted with the SRP90 buffer to obtain final concentrations of 200 nM kinesin-1 and 1 μ M UNC-83 construct. Controls consisted of UNC-83 constructs alone at 1 μ M. The solutions were incubated for 5-10 minutes at room temperature to allow protein adhesion to the slide, after which diluted microtubules were introduced. Following a 5-minute incubation, unbound proteins and microtubules were washed off using assay buffer containing 2 mM ATP. Microtubules were imaged for 1 minute using 405 nm laser at 10% power and images were taken at 1 frame per second. Single images of 561 nm and 488 nm channels were captured using 15% laser power. Kymographs were generated using ImageJ (Fiji), and microtubule velocities were determined using ImageJ Velocity Measurement Tool plug-in.⁵⁰

TIRF imaging was performed on a Nikon TE microscope equipped with a1.49 N.A. PlanApo 100x objective, a TIRF illuminator (LUN4), and an Andor iXon EMCCD camera. Images were acquired using NIS Elements software (Nikon).

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism version 10. Data from hyp7 and P-cell nuclear migration assays were visualized as scatter plots, with means and 95% CI represented as error bars. Sample sizes and specific statistical tests are detailed in the figure legends. Unpaired student *t*-tests were conducted for the indicated comparisons.