Distinct dystrophin and Wnt/Ror-dependent pathways establish planar-polarized membrane compartments in *C. elegans* muscles

Alice Peysson[1,*], Noura Zariohi[1,*], Marie Gendrel[2], Amandine Chambert-Loir[1], Noémie Frébault[1], Olga Andrini[1], Thomas Boulin[1,#]

[1] Université Claude Bernard Lyon 1, CNRS UMR 5284, INSERM U1314, MeLiS, Lyon 69008, France
[2] Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Ecole Normale Supérieure, CNRS, INSERM, Université Paris Sciences et Lettres Research University, Paris 75005, France
* These authors contributed equally
[#] Lead Contact

Correspondence: thomas.boulin@univ-lyon1.fr

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SUMMARY

The plasma membrane of excitable cells is highly structured and molecular scaffolds recruit proteins to specific membrane compartments. Here, we show that potassium channels and proteins belonging to the dystrophin-associated protein complex define multiple types of planar-polarized membrane compartments at the surface of *C. elegans* muscle cells. Surprisingly, conserved planar cell polarity proteins are not required for this process. However, we implicate a Wnt signaling module involving the Wnt ligand EGL-20, the Wnt receptor CAM-1, and the intracellular effector DSH-1/disheveled in the formation of this cell polarity pattern. Moreover, using time-resolved and tissue-specific protein degradation, we demonstrate that muscle cell polarity is a dynamic state, requiring continued presence of DSH-1 throughout post-embryonic life. Our results reveal the intricate, highly reproducible, and entirely unsuspected complexity of the worm's sarcolemma. This novel case of planar cell polarity in a tractable genetic model organism may provide valuable insight into the molecular and cellular mechanisms that regulate cellular organization, allowing specific functions to be compartmentalized within distinct plasma membrane domains.

INTRODUCTION

In neurons and skeletal muscle cells, molecular scaffolds concentrate ion channels in specific membrane sub compartments endowing them with specific functional properties. Distinct cellular mechanisms organize membrane domains such as the axon initial segment, nodes of Ranvier, somato-dendritic compartments and chemical synapses. Large molecular complexes centered around proteins such as ankyrin, PSD-95 or dystrophin organize membrane domains and physically anchor ion channels[1–4]. In addition to controlling their spatial distribution, it is essential that the proper number of ion channels are targeted to these compartments[5]. In epithelial cells, cortical membrane domains are organized by specialized planar cell polarity (PCP) signaling pathways[6]. These PCP cascades integrate extracellular directional cues provided by Wnt ligand gradients, with cell-cell contact based information exchange via membrane-bound protein complexes. This elaborate process allows distinct proteins to be localized asymmetrically within the cell, creating repetitive planar-polarized cellular structures within large cell layers.

The musculature of *Caenorhabditis elegans* is organized into two ventral and two dorsal quadrants that run along the length of the body. Each quadrant is composed of two bands of successive muscle cells[7]. Body wall muscles are diamond-shaped cells when observed in a dorso-ventral view (Figure 1A). They project membrane extensions, called "muscle arms", towards the neurites of presynaptic motoneurons to form post-synaptic domains that concentrate ligand-gated ion channels at chemical synapses. The molecular mechanisms that underlie the construction of these membrane nanodomains have been extensively characterized [8]. In comparison, little is known about how membrane proteins are triaged to other parts of the muscle cell. Muscle cells and sarcomeres are attached to the epidermis via a network of integrin-containing adhesive complexes that cover the outer surface of muscle cells. In addition to integrins, the Dystrophin-Associated Protein Complex (DAPC) physically connects the intracellular actin cytoskeleton to the extracellular matrix along the muscle sarcolemma[7] and have been shown to anchor the BK potassium channel SLO-1[9,10].

Recent transcriptomic analyses have revealed that a surprisingly large number of ion channels genes are co-expressed in *C. elegans* muscle cells raising interesting questions

relating to their temporal dynamics, their subcellular distribution, possible heteromeric assemblies, and individual functions[11–18]. Yet, whether and how these different channels could be addressed to different parts of the muscle cell, or even organized in different submembrane compartments has rarely been addressed.

In this study, we reveal the highly structured organization of the plasma membrane of *C. elegans* body wall muscle cells. By investigating the subcellular localization of ion channels and members of the dystrophin-associated protein complex (DAPC), we show that different proteins partition to distinctive, highly-reproducible, asymmetric membrane compartments. In particular, we found that the potassium channels TWK-28, TWK-24, and SLO-1 occupy distinct, but partially overlapping, polarized compartments at the tip, the center and the posterior part of each muscle cell, respectively. In addition, diffraction-limited microscopy analyses suggest that DAPCs with different compositions exist within a cell, highlighting an additional not previously recognized level of molecular complexity and heterogeneity. Using a forward genetic screening approach, we show that surface expression of TWK-28 is dependent on the dystrophin complex, likely via direct recruitment, but that the dystrophin complex itself is not required for TWK-28's polarized targeting. The asymmetrical distribution of ion channels and DAPC proteins at the cellular and tissue scale is highly reminiscent of canonical planar cell polarity (PCP) patterns. Intriguingly, conserved PCP genes are not involved in this process. However, we found that disheveled/DSH-1, the Wnt receptor Ror/CAM-1, and the Wnt ligand/EGL-20 are required for the asymmetric localization of ion channels and DAPC proteins along the anteroposterior axis of muscle cells. Finally, we show that disheveled is (1) continuously required to maintain cellular polarity, and (2) that post-embryonic expression can restore defective cell polarity, demonstrating that muscle polarity is a dynamic state. Taken together, these results highlight the remarkably complex organization of the worm's sarcolemma. The asymmetric membrane compartments defined by potassium channels and DAPC proteins, repeated in each muscle cell along the length of the animal, constitute the first examples of canonical planar cell polarity patterns in *C. elegans*.

RESULTS

Ion channel localization reveals a remarkably complex and planar-polarized organization of the muscle sarcolemma

Potassium-selective channels are the largest family of ion channels in the *C. elegans* genome. Transcriptomic data indicate that up to 21 distinct potassium channel subunits are co-expressed in body wall muscle cells[11,17]. Yet, little is known about the subcellular localization of most of these channels. To address this question, we used two previously published reporter strains (TWK-18[15]; SLO-1[16]) and engineered knockin lines for six two-pore domain (K2P) channels (TWK-8, TWK-12, TWK-24, TWK-28, TWK-42, TWK-43) using CRISPR/*Cas9*-based gene editing.

Careful analysis of these fluorescent reporters revealed a remarkable diversity of situations. While TWK-8 was present throughout the cell surface (Figure 2F), TWK-12 and TWK-43 were only visible in muscle arms and on the lateral sides of body wall muscle cells, but not on the outer surface that faces the hypodermis (Figure S1A). Conversely, TWK-18 and TWK-42 showed distinct distribution patterns on the outer face of each muscle cell, which were either broad (TWK-18) or punctate (TWK-42) (Figure 2F).

The localization of SLO-1, TWK-24 and TWK-28 were the most striking (Figures 1A and 1B). Indeed, TWK-28 was concentrated at the anterior tip of each muscle cell, in a singular comet-like pattern. Conversely, SLO-1 channels were only present in the posterior part of each

138 muscle cell, forming a regularly-spaced punctate pattern. Finally, while TWK-24 channels
139 appeared to be broadly distributed at first glance, outlining muscle cells revealed that TWK-24
140 was in fact absent from their extremities and enriched in the central portion of the cell.
141 Given that SLO-1, TWK-24, and TWK-28 domains partially overlap at the scale of a
142 muscle cell, we wondered whether they were also colocalized in membrane nanodomains. By
143 simultaneously labeling the three channels and using image segmentation, we observed that
144 individual ion channel clusters were clearly separable even using diffraction-limited confocal
145 microscopy, suggesting that they could be part of distinct protein complexes, even where their
146 distribution patterns overlap at the cellular scale (Figure 1C).
147 The SLO-1 channel has been shown to physically interact with the integral membrane
148 protein ISLO-1[19]. Therefore, we generated a fluorescent knockin line to assess its subcellular
149 localization. Remarkably, ISLO-1 and SLO-1 did not show identical distribution patterns. While
150 ISLO-1 colocalized with SLO-1 in the posterior part of the cell (Figure 1D), ISLO-1 was also
151 clearly enriched at the anterior tip in a comet-like pattern that coincided perfectly with TWK-28
152 (Figure 1E). Hence, the distribution pattern of ISLO-1 defines a fourth class of asymmetrical
153 subcellular localization with both anterior and posterior domains, in which ISLO-1 is organized
154 in very different subcellular patterns, i.e., a comet-shaped anterior pattern, and a punctate,
155 regularly-spaced, posterior pattern.
156 Taken together these observations reveal the remarkably complex molecular makeup
157 of the plasma membrane of *C. elegans* muscle cells. They demonstrate, for the first time, the
158 existence of asymmetric membrane sub-compartments with distinct molecular compositions.
159 Furthermore, as these asymmetric distribution patterns are repeated in each muscle cells all
160 along the length of the worm, they also represent a quintessential case of planar cell polarity
161 at the tissue scale, which has not been observed so far in *C. elegans*.
162
163 **Dystrophin and dystrophin-associated proteins are required for TWK-28 surface**
164 **expression**
165 To understand how TWK-28 is targeted to the tip of muscle cells, we performed a forward
166 genetic screen based on the rationale that disruption of factors required to address TWK-28 to
167 the cell surface would suppress the effect of a hyperactivating gain-of-function mutation. We
168 previously demonstrated that the activity of vertebrate and invertebrate two-pore domain
169 potassium channels can be tuned by mutating a single residue in the second transmembrane
170 domain, named TM2.6 [20]. After confirming that this mutation increased TWK-28 activity using
171 heterologous expression in *Xenopus* oocytes (Figure S1B), we used CRISPR/*Cas9* gene
172 editing to build a *twk-28* TM2.6 gain-of-function mutant (*bln485*, TWK-28 L210T). These
173 mutants displayed strongly reduced locomotion, as expected for a gain-of-function mutation
174 that decreases the excitability of muscle cells (Figure S1C).
175 After screening approx. 15.000 mutagenized haploid genomes, we obtained 182
176 independent suppressor lines that had regained near wild-type mobility. Among these, 102
177 were extragenic revertants based on genetic segregation, i.e., mutants that did not alter the
178 *twk-28* gene sequence. Using whole genome resequencing, we analyzed 25 of these mutants
179 and found that 14 were loss-of-function alleles of *dys-1,* the *C. elegans* ortholog of dystrophin,
180 and that one was an allele of *islo-1* (Figure S1D).
181 To measure their impact on TWK-28 surface expression, we combined *dys-1* and *islo-*
182 *1* mutants with the fluorescently-labeled TWK-28 reporter (Figures 2A and 2B). We observed
183 a drastic change in *dys-1(bln582)* and *islo-1(bln549)* mutants as the TWK-28 fluorescence was
184 reduced by 74% and 70% relative to wild type, respectively. Interestingly, *dys-1(bln582)*
185 harbors an early stop codon at W110. This mutation affects only the longest dystrophin isoform

186 that contains both calponin-homology actin-binding domains. To verify whether the remaining
187 TWK-28 fluorescence could be due to residual activity of shorter dystrophin isoforms, we
188 generated *syb2174*, a molecular null allele that deletes the entire 31 kb-long *dys-1* genomic
189 locus. This allele showed the same effect as *bln582*, arguing that the W110Stop mutation is a
190 functional null allele for TWK-28 localization (Figures 2A).
191 In addition to dystrophin, *C. elegans* muscle express the orthologs of the dystrophin-
192 associated proteins dystrobrevin (*dyb-1*), syntrophin (*stn-1*, *stn-2*), and sarcoglycan/Sgc (*sgca-*
193 *1*, *sgcb-1*, *sgn-1*). To determine the contribution of these proteins to the surface expression of
194 TWK-28, we first tested sarcoglycan mutants. Loss of individual sarcoglycans or of three Sgc
195 genes at once did not significantly modify TWK-28 fluorescence levels (Figures S1E and S1F).
196 Next, we measured the impact of *dyb-1*, *stn-1,* and *stn-2* mutants (Figures 2B and 2D). *dyb-*
197 *1(cx36), stn-1(ok292)* and *stn-2(ok2417)* each significantly reduced TWK-28 fluorescence, by
198 approx. 55%, 37%, and 76%, respectively, suggesting a predominant requirement of STN-2.
199 In all cases, residual TWK-28 channels were still restricted to the anterior tip of muscle cells,
200 indicating that targeting of TWK-28 to the comet-like domain is not solely dependent on DAPC
201 proteins.
202 To assess whether the DAPC was more broadly required for the localization of muscle-
203 expressed K2P channels, we combined four fluorescent knockin lines with a *dys-1* loss-of-
204 function allele. We observed no obvious impact for any of these channels, whether they were
205 uniformly distributed (TWK-8, TWK-18, TWK-42), or asymmetrically localized (TWK-24)
206 (Figure 2F).
207 Taken together, these results suggested a model in which TWK-28 could be stabilized
208 at the sarcolemma via interactions with the DAPC, as is the case for voltage-gated sodium
209 channels and inwardly-rectifying potassium channels in vertebrate cardiomyocytes [3,4]. Indeed,
210 a short amino acid sequence in the cytoplasmic C-terminus of Nav1.5 and Kir4.1 is recognized
211 by the PDZ domain of syntrophin, which itself binds to dystrophin and thus stabilizes these ion
212 channels at the plasma membrane. Using *in silico* prediction algorithms [21], we identified a
213 putative PDZ-binding sequence in the carboxy-terminus of TWK-28 (Figure 2C). After inserting
214 a premature stop codon that removed these amino acids by gene editing (Figure 2C) in the
215 context of the TWK-28 fluorescent reporter strain, we observed a dramatic reduction in the
216 number of TWK-28 channels (approx. 80%) at the cell surface, similar to *dys-1* and *stn-2*
217 mutants (Figures 2D and 2E). Combining this truncated TWK-28 channel with a molecular null
218 allele of *dys-1* did not further reduce TWK-28-associated fluorescence (Figure 2E), which is
219 consistent with a model in which TWK-28 is directly recognized by syntrophins and stabilized
220 at the plasma membrane by the dystrophin-associated protein complex.
221
222 **TWK-28 dynamics following post-embryonic DYS-1 degradation and recovery**
223 To examine the dynamics of the relationship between TWK-28 and the DAPC, we developed
224 a temporally-controlled dystrophin degradation strategy using the auxin-inducible degron (AID)
225 system[22]. To monitor DYS-1 levels and subcellular localization, we associated an
226 mNeonGreen coding sequence with the AID degron sequence. This knockin line was fully
227 functional as we did not observe *dys-1* loss-of-function phenotypes (e.g., exaggerated head
228 bending, reduction in TWK-28 surface expression).
229 Confocal imaging revealed a broad distribution of DYS-1 on the outer face of muscle
230 cells, facing the epidermis (Figure 2H). Consistent with the model that TWK-28 is recruited to
231 the DAPC, DYS-1 and TWK-28 were well colocalized in the anterior tip of each muscle cell
232 (Figure 2I). Hence, DYS-1 is also enriched at the tip of muscle cells, but also distributed more
233 broadly throughout the sarcolemma.

We first tested whether DYS-1 could be degraded by observing the offspring of hermaphrodites grown on auxin (Figures 2G and 2H). Although a very small fraction of the dystrophin-associated fluorescence remained detectable, mNeonGreen fluorescence was drastically diminished in these auxin-treated worms (Figure 2H and 2I). Consistently, worms exposed to auxin throughout their life displayed the exaggerated head-bending phenotype observed in dystrophin null mutants, confirming effective dystrophin degradation. Moreover, the impact of DYS-1 degradation was comparable to *dys-1* null mutants (Figure 2A), since TWK-28 fluorescence was reduced by 73% relative to untreated animals (Figures 2I and 2K).

Using this assay, we could analyze dystrophin dynamics by monitoring fluorescence recovery over 24 hours, starting at the L4 larval stage (Figure 2G). After removing animal from auxin-supplemented media, we observed a partial restoration of DYS-1-mNeonGreen fluorescence throughout the muscle cell and at the anterior tip. TWK-28 fluorescence also recovered notably over the 24-hour time period, but did not reach wild-type levels (Figures 2J, 2K). However, TWK-28 puncta remained closely associated with DYS-1, in line with a direct association of TWK-28 with the DAPC. Interestingly, the overall distribution of TWK-28 and DYS-1 in the comet-like domain was qualitatively different after recovery. It appeared broader and more dispersed than in wild type, which maintained a more condensed pattern (Figure 2J). These data indicate that functional dystrophin complexes can be reassembled post-embryonically, and that TWK-28 requires continuous dystrophin presence to be maintained at the cell surface.

Dystrophin-associated proteins localize to asymmetric membrane compartments

To further characterize the subcellular distribution of dystrophin and dystrophin-associated proteins, we used CRISPR/*Cas9* gene editing to label all members of the worm's DAPC complex[23].

In vertebrates, dystroglycan is a central component of the DAPC, linking the extracellular matrix to the intracellular cytoskeleton via dystrophin. In *C. elegans*, dystroglycan expression has been reported in different cell types, but surprisingly not in muscle[24]. However, clear muscle expression of *dgn-1*, one of three worm dystroglycans, can be detected by single-cell RNA sequencing approaches[11,17]. Consistently, DGN-1 was clearly detectable in body wall muscles in our knock-in line, and DGN-1 was colocalized with DYS-1 on the outer face of muscle cells (Figure 3A). To more precisely describe DGN-1 distribution, we used the dense body marker, PAT-2/α-integrin, as a cellular landmark [25]. Double labeling showed that both proteins occupy distinct membrane domains. Indeed, DGN-1 was excluded from dense bodies and M lines occupied by PAT-2 (Figures 3B, 3C and 3D), even using diffraction-limited light microscopy. These observations demonstrate that dystrophin and dystroglycan are present throughout the sarcolemma of worm muscle cells, and that the DAPC occupies specific membrane nanodomains, juxtaposed to integrin-containing attachment sites.

Sarcoglycans/Sgc are the other major integral membrane component of the DAPC. The Sgc complex is composed of several sarcoglycan subunits (α, β, γ, δ) in skeletal and cardiac muscles [26]. The *C. elegans* genome encodes three sarcoglycans, *sgca-1*, *sgcb-1*, and *sgn-1*, which are orthologs of α-sarcoglycan, β-sarcoglycan, and δ/γ-sarcoglycan, respectively[23]. According to previous reports[19] and transcriptomic data[11], the three sarcoglycan genes are expressed in worm muscles. Using knockin lines, we confirmed their muscular expression and found that sarcoglycans are strictly co-localized (Figures S2A and S2B). In vertebrates, assembly of individual subunits into an oligomeric protein complex is necessary for trafficking to the plasma membrane of muscle cells. Loss of individual subunits strongly decreases sarcoglycan complex formation and membrane targeting[26]. We tested the interdependence of

worm Sgc *in vivo* and found that loss of individual sarcoglycan subunits also strongly disrupted the surface localization of the other two subunits in *C. elegans* (Figure S2C). Moreover, sarcoglycan surface expression was profoundly altered by removing dystrobrevin and dystrophin, either individually or together (Figure S2D). In *dyb-1* mutants, SGCB-1 surface expression was partly reduced in the anterior domain and essentially undetectable in the posterior punctate domain. In *dys-1* mutants, we found a stronger reduction of SGCB-1 and an overall modification of the structure of the anterior domain, rendering it more diffuse. This pattern was also observed in the double mutant, suggesting a dominant role of DYS-1 over DYB-1 for sarcoglycan localization.

Interestingly, sarcoglycan distribution was visibly different from DYS-1, as it appeared more confined (Figure 3E), and strongly resembled ISLO-1 (Figures 1D and 1E). Indeed, sarcoglycans are also found at the anterior tip of the muscle cell in a comet-like pattern, and cluster into a linear pattern of regularly-spaced punctate microdomains in the posterior part of the cell. These two opposite domains are separated by a small region that appears to be mostly devoid of sarcoglycan (Figure 3F). By comparing Sgc and PAT-2 localization, we could observe that, similarly to dystroglycans, sarcoglycans occupy membrane domains that are clearly juxtaposed to integrin complexes (Figure 3F).

Consistently, by comparing DGN-1 and Sgc distributions, we could observe partial colocalization, in particular in the anterior tip of muscles cells, but also in the posterior domain (Figure 3G). In the anterior domain, sarcoglycans mostly co-localized with DGN-1 using diffraction-limited microscopy. However, some DGN-1 puncta were devoid of sarcoglycan, and vice-versa, suggesting a heterogeneous organization of dystroglycan- and sarcoglycan-containing molecular complexes within the sarcolemma (Figure 3G, *anterior domain*). In the posterior part of the cell, the clearly distinguishable sarcoglycan nanodomains co-localized with puncta that were enriched in DGN-1, as compared with the remaining DGN-1 signal. Therefore, these distinct membrane distribution patterns suggest a molecular heterogeneity within the dystrophin-associated protein complex that further illustrates the complexity of the worm's sarcolemma.

Next, we analyzed the distribution of the dystrobrevin ortholog, DYB-1, and the two worm syntrophins STN-1 and STN-2. By comparing their subcellular distributions with DGN-1 and SGCB-1, we observed find that they display two distinct and clearly recognizable localization patterns (Figures S3 and S4). Indeed, STN-2 was broadly expressed and colocalized precisely with DGN-1, while STN-1 and DYB-1 reproduced the more confined distribution pattern of sarcoglycans. Direct comparison of STN-1 and STN-2 confirmed this dichotomy. Finally, by using TWK-28 as a landmark, we confirmed that all DAPC proteins were colocalized with the channel at the anterior tip of muscle cells (Figure S5).

In conclusion, our systematic analysis has revealed that DAPC proteins define multiple membrane subcompartments at the surface of muscle cell, distinct of integrin attachment sites. The clear differences in the distribution of dystroglycans and sarcoglycans provide the first evidence for the existence of DAPCs with different protein compositions.

Dystroglycan localization is dependent on perlecan and intracellular DAPC components

In vertebrates, surface expression of dystroglycan is dependent on interactions with the extracellular matrix protein perlecan[27], and also requires dystrophin[28] and dystrobrevin[29]. First, we tested the role of perlecan/*unc-52* using a viable isoform-specific nonsense allele (*e444*) that eliminates perlecan isoforms expressed in all but the most anterior muscle cells[30]. In these mutant worms, we observed a profound disruption of DGN-1 surface expression (Figure S2E), confirming a conserved genetic link between dystroglycans and perlecans in worms. Next, we

repeated the same analysis with mutants for dystrophin and dystrobrevin (Figure S2F). We observed no obvious modification of the dystroglycan pattern in dystrobrevin/*dyb-1* mutants. However, disruption of *dys-1* using the *syb2174* null allele reduced overall surface expression, disrupted the dense anterior domain, and rendered the DGN-1 pattern more diffuse. Concomitant loss of *dys-1* and *dyb-1* had no additional effect compared to *dys-1* alone.

While the requirement of dystrobrevin may not be conserved in worms, these results confirm the links between dystroglycan/DGN-1 and dystrophin/DYS-1 in *C. elegans* muscle.

Disheveled/DSH-1 controls the asymmetric distribution of membrane proteins

While our forward genetic screen allowed us to demonstrate a requirement of DAPC proteins for the stabilization of TWK-28 channels at the cell surface, it did not reveal the mechanisms that underlie the striking asymmetric distribution of the channels or DAPC proteins within each muscle cell.

Several molecular pathways are known to generate tissue polarity in different cellular contexts. Generally, these involve Wnt ligand/receptor systems and specific proteins belonging to the core planar cell polarity (PCP) pathway[6]. We hypothesized that the polarized organization of TWK-28 could also result from the activity of such tissue polarity pathways, and undertook a candidate gene approach targeting Wnt and PCP genes that are conserved in *C. elegans*[31].

The intracellular effector disheveled is a central player in these molecular cascades as it mediates both canonical and non-canonical Wnt signaling[32]. The *C. elegans* genome encodes three disheveled orthologs: *dsh-1*, *dsh-2,* and *mig-5*. Based on transcriptomic data, *dsh-2* shows little to no detectable expression in muscle cells, contrary to *dsh-1* and *mig-5*[11]. While mutation of *mig-5* had no defect (data not shown), when we combined a *dsh-1* null mutant with the fluorescent TWK-28, SLO-1, and TWK-24 knock-in lines, we observed a striking reorganization of the channels at the muscle surface, leading to a loss of their asymmetric distribution (Figures 4A and 4B). In particular, TWK-28 channels were now found at both extremities of the cell, giving rise to a head-to-tail configuration of anterior and posterior comets in adjacent muscle cells. SLO-1 and TWK-24 distribution was also clearly modified as their domains appeared to shift to a more central position in each cell.

To precisely analyze this redistribution, we measured the position –relative to the length of a given muscle cell– of the boundaries dividing sarcolemmal domains that included or that were devoid of channels (Figure 4C).

First, we found that the anterior border was unchanged in *dsh-1(0)* mutants for each ion channel. TWK-28 channels covered on average 30% of the anterior portion of the cell, while SLO-1 and TWK-24 boundaries were more anterior (23% and 21% respectively) and therefore overlapped slightly with the TWK-28 domain.

In contrast, the posterior borders were markedly displaced. In wild type, we could rarely detect a few clusters of TWK-28 channels at the posterior end of the cell, while in *dsh-1(0)*, TWK-28 channels covered over 25% of it. Thus, anterior and posterior TWK-28 domains were now almost symmetrical. In the case of SLO-1, the posterior boundary that usually extended almost to the end of the cell, now shifted anteriorly, uncovering close to a fourth of the posterior portion of the cell. Conversely, the TWK-24 domain extended more posteriorly in *dsh-1(0)*, now closely matching the distribution of SLO-1 channels. Interestingly, image segmentation of triple-labelled cells revealed that the three channels remained optically-separable even using diffraction-limited microscopy, suggesting that they still partition into physically distinct nano-domains within the sarcolemma (Figure 4D).

377 Next, we wondered whether the redistribution of TWK-28 channels also affected their
378 surface expression levels. Based on fluorescence quantification of entire muscle cells, we
379 found that the total number of channels was unchanged in *dsh-1* mutants. Indeed, TWK-28
380 fluorescence was split between anterior and posterior comets, consistent with the respective
381 size of each domain (Figure 4E). These data thus suggest that the number of TWK-28
382 channels present at the cell surface is limited and finely regulated by mechanisms that are
383 independent of disheveled/*dsh-1*.
384 Given the requirement of dystrophin for TWK-28 surface expression, we hypothesized
385 that DYS-1 distribution must also be altered in *dsh-1* mutants. Using TWK-28 as a landmark,
386 we could clearly observe that dystrophin was indeed present at both extremities of muscle
387 cells (Figure 4F) in a *dsh-1* null mutant. The sarcoglycan SGCB-1 similarly adopted a
388 symmetrical distribution pattern, further strengthening the notion that *dsh-1* plays a major role
389 to ensure muscle cell asymmetry (Figure 4G).
390 Finally, to further investigate the interplay between processes that control TWK-28
391 surface distribution via dystrophin and DSH-1, we used the TWK-28 mutant lacking the C-
392 terminal PDZ-binding sequence (Figure 2C), as it decouples TWK-28 from the DAPC. Indeed,
393 in this context, we could still detect an accumulation of TWK-28 channels at the posterior end
394 in *dsh-1* null mutants (Figure 4H), arguing that TWK-28 channel are addressed to the
395 extremities of muscles cells independently of their interaction with the DAPC, and that the
396 DAPC likely serves to stabilize TWK-28 at the surface in a second step.
397 Taken together, these data support a model in which DSH-1 ensures that dystrophin
398 and TWK-28 are not addressed or maintained at the posterior end of muscle cells, while the
399 DAPC, subsequently and independently, stabilizes TWK-28 channels at the cell surface. The
400 process that addresses DYS-1, SGCB-1, and TWK-28 to the extremities of muscle cells
401 remains to be determined.
402
403 **The DIX domain of DSH-1 is dispensable for TWK-28 asymmetry**
404 Disheveled proteins comprise three conserved functional domains: DIX, PDZ and DEP (Figure
405 5A). Generally, DIX and PDZ domains are required for canonical Wnt β-catenin signaling, while
406 PDZ and DEP domains function in PCP signaling[32]. To investigate which pathways may act
407 downstream of DSH-1 in muscle cells, we expressed a series of protein truncations in body
408 wall muscle cells and monitored the rescue of TWK-28 localization in a *dsh-1* mutant
409 background (Figure 5B). First, we verified that a *dsh-1a* cDNA was sufficient to restore TWK-
410 28 localization (i.e., panel "+ *Pmyo-3::dsh-1a*"). Next, we expressed truncated *dsh-1a* cDNAs
411 lacking each domain using the same muscle-specific promoter, and found that only the cDNA
412 lacking the DIX domain restored TWK-28 localization in a *dsh-1* null mutant.
413 These results indicate that PDZ and DEP domains are indispensable for TWK-28
414 targeting, pointing to an involvement of PCP-type signaling downstream of DSH-1. They also
415 show that DSH-1 likely does not engage canonical β-catenin-dependent signaling in this
416 context, a conclusion that was consistent with a lack of TWK-28 redistribution in *bar-1*/β-
417 catenin mutant worms (Figure 5C).
418
419 **Sarcolemmal asymmetry requires Wnt/Ror but not PCP proteins**
420 Given the results of our DSH-1 structure-function analysis, we proceeded to test the possible
421 involvement of genes belonging to the two planar polarity pathways: the core PCP proteins,
422 VANG-1/Stan, FMI-1/Flamingo, and PRKL-1/Prickle, and the global proteins, CDH-
423 1/Dachsous and CDH-3/CDH-4/Fat. Surprisingly, none of these genes were required for
424 planar-polarized TWK-28 localization (Figure 5C).

425 Given these results, we investigated the involvement of Wnt-dependent pathways.
426 First, we tested a viable *mig-14*/Wntless hypomorphic mutant that reduces the secretion of
427 Wnt ligands and found that it led to a loss of TWK-28 polarity (Figure 5D). Next, we analyzed
428 viable mutants for four Wnt ligands and found that *egl-20* altered the localization of TWK-28
429 (Figures 5D). Finally, we tested five transmembrane proteins that bind Wnt ligands: the Wnt
430 receptors LIN-17, MIG-1, CFZ-2, LIN-18/Ryk and the receptor tyrosine kinase CAM-1/ROR.
431 *lin-17* and *lin-18*, alone, and *mig-1 cfz-2* double mutants had no effect (Figures S6A and S6B),
432 while the reference allele of *cam-1* phenocopied *egl-20* mutants (Figures 5D and 5E). To
433 confirm these results, we generated an early stop codon at position Pro21 in EGL-20 using
434 CRISPR/*Cas9* gene editing (Figure S6C), and analyzed three additional *cam-1* alleles (*ak37*,
435 *cw82*, *ks52*) (Figures 5E and 5F). These four additional mutant alleles all showed phenotypes
436 identical to the reference alleles.
437 Observing the impact of these gene mutations in more detail along the entire length of
438 the animal provided some additional information about the molecular actors upstream of
439 disheveled. Indeed, *mig-14*, *egl-20*, and *cam-1* mutants only affected the distribution of TWK-
440 28 in muscle cells that were posterior or close to the vulva (anteriorly), while *dsh-1* mutation
441 also affected muscle cells situated more anteriorly (Figure 5D).
442 This spatially-restricted effect is consistent with the known range of the EGL-20
443 gradient that is formed by secretion from cells in the tail of the worm, extending to the
444 midbody[33,34]. Furthermore, *cam-1* mutation also did not affect cells anterior to the vulva, which
445 could indicate that a different Wnt receptor/Wnt ligand combination is acting in these cells, or
446 that redundant pathways control muscle polarity in the anterior half of the worm.
447
448 **Subcellular distribution of DSH-1 in body wall muscle cells**
449 To gain further insight into how DSH-1 controls muscle membrane asymmetry, we analyzed
450 the subcellular distribution of DSH-1 in three translational knockin lines in which the
451 mNeonGreen coding sequence was inserted at the N- and C-terminus, and between the DIX
452 and PDZ domains[25] of DSH-1.
453 To ensure that these mNeonGreen-tagged DSH-1 chimeras remained functional, we
454 used TWK-28 localization as a proxy for DSH-1 function. Interestingly, although the three lines
455 showed similar fluorescence patterns, only the N- and C-terminal insertions were fully
456 functional, while TWK-28 channels were mislocalized in the intra-molecular insertion line (data
457 not shown).
458 Using the C-terminally tagged *dsh-1* alleles (*bab365*), we analyzed the distribution of
459 DSH-1 proteins within muscle cells. Remarkably, DSH-1 was enriched in the posterior third of
460 body wall muscle cells (Figures 6A and 6B), clearly distinct of TWK-28's anterior localization
461 (Figures 6C). This pattern was lost in *cam-1* and *egl-20* mutants (Figures S7A). Interestingly,
462 while *egl-20* disrupted DSH-1 localization only in muscle cells that were posterior to the vulva—
463 consistent with the EGL-20 gradient—, loss of *cam-1* affected DSH-1 distribution all along the
464 body. Yet, TWK-28 asymmetry was not affected in *cam-1* mutants anterior to the vulva.
465
466 **DSH-1 is continuously required to establish and maintain muscle membrane polarity**
467 Next, we wondered whether muscle polarity was a fixed or a dynamic state, i.e., whether DSH-
468 1 was only required to establish muscle polarity, or also to maintain it throughout the life to the
469 worm. Therefore, in addition to mNeonGreen, we inserted the AID degron sequence into our
470 DSH-1-mNeonGreen knockin line to be able to manipulate DSH-1 protein levels and study its
471 temporal window of action.

472 First, to validate this approach, we exposed fourth stage (L4) larvae to auxin and –three
473 days later– analyzed their F1 progeny at the L4 stage. Using a transgenic line expressing TIR1
474 ubiquitously, we could observe a robust degradation of DSH-1-mNeonGreen and a clear
475 redistribution of TWK-28 channels into a symmetrical localization pattern, phenocopying the
476 *dsh-1(0)* mutant (Figure S7B).
477 Next, to determine the appropriate time window for DSH-1 degradation, we observed
478 the localization pattern of TWK-28 during the first three larval stages, in wild-type and *dsh-1(0)*
479 worms (Figure 6D). While muscle polarity was fully established at the second (L2) and third
480 (L3) larval stages, we observed both polarized and non-polarized cells in freshly-hatched (L1)
481 larvae. This suggests that TWK-28 polarity is mostly established during embryogenesis, and
482 that it may derive from an initially non-polarized state.
483 We also noted that the shape of TWK-28-containing comets changed progressively.
484 Only a small number of fluorescent clusters were observed in the L1. In the L2 and L3 stages,
485 the number of clusters increased, accompanied by a widening of the comet over time. Loss of
486 *dsh-1* disrupted muscle polarity at all larval stages (Figure 6D).
487 To determine whether *dsh-1* was required to maintain muscle polarity once it was
488 established, we started degrading DSH-1-mNeonGreen at the L3 larval stage and observed
489 these worms 72 hours later. This protocol led to a clear loss of TWK-28 asymmetry,
490 demonstrating that muscle polarity is a dynamic state, and that DSH-1 is continuously required
491 to maintain it (Figure 6E).
492 Conversely, we asked whether restoring DSH-1 expression post-embryonically could
493 restore muscle polarity starting from a non-polarized state. In this case, we exposed animals
494 to auxin until the L3 stage, and monitored the recovery of DSH-1 levels and muscle polarity
495 over 72 hours (Figure 6F). First, as in our initial experiment with L4 stage animals (Figure S7B),
496 L3 animals exposed to auxin throughout their life also showed a clear loss of muscle polarity
497 and an absence of DSH-1-associated fluorescence. Next, over the course of the 72-hour
498 recovery period (i.e., in the absence of auxin) we could observe a clear restoration of the
499 polarity pattern.
500 These two complementary experiments directly demonstrate that DSH-1 is required –
501 thorough the life of the animal– to establish and to maintain the polarity of *C. elegans* muscle
502 cells, and that this state is therefore dynamic and could be regulated by extracellular signals
503 that would impact DSH-1 activity.
504
505
506 **DISCUSSION**
507 By analyzing the subcellular distribution of potassium channels and proteins linked to the
508 dystrophin-associated protein complex, we reveal here the previously unsuspected
509 compartmentalization and planar-polarized organization of the sarcolemma of *C. elegans*
510 muscle cells. Despite decades of detailed work on the structure of *C. elegans* muscle cells[7],
511 this remarkable case of cellular polarization was entirely unsuspected.
512
513 **Sarcolemmal asymmetry at the cellular scale**
514 Our initial analysis of single-cell RNAseq data and other published reports had indicated that
515 up to 21 potassium channel subunits could be co-expressed in body wall muscle cells. Apart
516 from the extensive work regarding the precise targeting of ligand-gated ion channels to the
517 ends of muscle arms[8], whether ion channels could be differentially targeted to different parts
518 of the sarcolemma has been rarely addressed specifically. By revealing the distinct subcellular
519 localization patterns of different potassium channels, we have demonstrated that clear

520 differences exist in the molecular composition of the muscle's plasma membrane. For instance,
521 while TWK-8 and TWK-18 channels are found throughout the sarcolemma, TWK-12 and TWK-
522 43 channels are only targeted to the basolateral membrane and muscle arms. Conversely,
523 TWK-28 and TWK-42 channels are only found on the outer side of the muscle cell, facing the
524 epidermis, and not on the lateral membrane or muscle arms. Importantly, the comparison of
525 TWK-28, TWK-24 and SLO-1 channels shows that even when channels are located in the
526 same region of the sarcolemma (i.e., the outer face of the cell), they can occupy distinct –yet
527 partially overlapping– regions along the antero-posterior axis. And even when they are present
528 in overlapping regions, we could show that ion channels remain confined to distinct
529 nanodomains. The high reproducibility of these patterns between different individuals and
530 within muscle cells of a single animal imply that specific molecular and cellular mechanisms
531 must target individual channels to their respective domains, likely endowing them with distinct
532 electrophysiological properties.
533 Another remarkable observation is the difference in organization of TWK-28, ISLO-1
534 and SLO-1. ISLO-1 interacts with SLO-1 and the syntrophin STN-1, allowing the recruitment
535 of the BK potassium channel to the DAPC, and mutations of *islo-1* and *dys-1* disrupt surface
536 expression of SLO-1 in muscle cells[9]. However, we have shown here that ISLO-1 and SLO-1
537 localization patterns are not identical since ISLO-1 is also present in the comet-like domain at
538 the anterior tip of each muscle cell. Similarly, we have found that *islo-1, dys-1* and syntrophins
539 are necessary for TWK-28 surface expression, but TWK-28 is nevertheless restricted to the
540 anterior domain. Notably, even when cellular polarity is disrupted by mutations in Wnt/Ror and
541 *dsh-1*, the posteriorly-localized TWK-28 does not reproduce the posterior, punctate, ISLO-1
542 pattern, but rather mirrors the comet-like distribution seen at the anterior tip in wild-type
543 animals. Therefore, there are likely specific molecular actors that allow the selective
544 recruitment of TWK-28 and SLO-1 to distinct anterior and posterior domains. Forward genetic
545 screens could directly address this question in the future by identifying gene mutations in which
546 TWK-28 or SLO-1 channels are no longer restricted to their anterior or posterior domains, but
547 rather redistributed into an ISLO-1-like pattern.
548

549 **The dystrophin-associated complex as an organizer of plasma membrane**
550 **compartments**

551 Detailed analyses of the distribution of DAPC proteins further highlight the complex
552 organization of the muscle membrane. At first glance, DAPC components can be separated
553 into two classes: one class that includes DGN-1, DYS-1 and STN-2, and the other that consists
554 of sarcoglycans, DYB-1 and STN-1. One-to-one colocalization studies however reveal that
555 there is significant overlap between both groups. For example, the posterior sarcoglycan
556 puncta coincide with areas of denser DGN-1 signal, which is surrounded by weaker DGN-1-
557 associated fluorescence. This could be explained by different diffusion kinetics with one
558 predominant population of confined sarcoglycans versus two populations of confined *versus*
559 freely diffusing dystroglycans. Fluorescence recovery or photoconversion approaches could
560 be used in the future to address this question *in vivo*.
561 Although DAPC proteins are generally thought to be part of the same molecular
562 complex, our observations challenge the notion of a singular DAPC composition. For example,
563 we observed a heterogeneous organization of dystroglycan- and sarcoglycan-containing
564 molecular complexes in the anterior tip of muscle cells where some DGN-1 puncta were devoid
565 of sarcoglycan, and vice-versa. In mammals, while costameric DAPCs are thought to contain
566 all components, the situation is also more complex at the neuromuscular junction where they
567 are all present but not colocalized and not involved in the same functions[35]. It is also interesting

568 to note the clear separation of DAPC and integrin complexes in worm muscles. Using
569 diffraction-limited microscopy, fluorescent markers could be easily distinguished suggesting
570 that they are situated far from each other, thus refining previous analyses that suggested a
571 close association with dense bodies in the I-band[36]. It thus remains to be determined which
572 molecular partners or scaffolds underpin the highly stereotyped subcellular distribution of
573 DAPC proteins.
574 To definitively clarify DAPC localization in worm muscles and determine the exact
575 molecular makeup of dystrophin-containing nanodomains, it will be essential to move beyond
576 diffraction-limited microscopy and resort to state-of-the-art super-resolution techniques. The
577 vast increase in resolution afforded by super-resolution light microscopy will clarify the
578 composition of membrane nanodomains and relative distribution of dystrophin and its
579 associated proteins. As shown here, dystrophin and sarcoglycans occupy both overlapping
580 and distinct sub-membrane domains. Yet, even when they appear co-localized using
581 conventional confocal microscopy, they could still occupy distinct nanodomains. Refining their
582 relative location will reveal the heterogeneity within DAPC complexes at the surface of muscle
583 cells. Similarly, the two worm syntrophins, STN-1 and STN-2, have distinct and partially
584 overlapping distribution patterns in muscle cells. We have also found that they contribute
585 differently to the surface expression of the potassium channel TWK-28. Fly and vertebrates
586 also co-express multiple syntrophins with different subcellular localizations and functions[37].
587 Thus, understanding the interplay between these two worm syntrophins may provide new
588 leads on the mode of action of syntrophins.
589 Interestingly, recent observations in different cellular contexts have revealed intriguing
590 asymmetric distribution patterns and functions of the DAPC. Indeed, precise *in vivo* studies of
591 dystrophin in mouse models have been greatly improved by the generation of GFP-tagged
592 mouse knockin lines. These models have confirmed the broad distribution of dystrophin along
593 the membrane of muscle fibers[38,39] but also revealed a striking enrichment at the extremities
594 of muscle fibers[40]. Asymmetric localization of dystrophin has also been described in zebrafish
595 muscles[41]. Analysis of a gene trap line that fluorescently labels the endogenous dystrophin
596 protein showed that dystrophin is highly enriched at myosepta –the contact site that separate
597 two somites– forming a repetitive chevron-like structure along the length of the body of the
598 fish. During development, more dystrophin is recruited to this interface, exacerbating the
599 asymmetric organization of dystrophin within growing muscle fibers. In activated rodent muscle
600 stem cells (i.e., satellite cells) dystrophin is located apically in close contact with the
601 extracellular matrix. This polarized distribution of dystrophin regulates the asymmetric division
602 of satellite cells via the recruitment of the serine-threonine kinase Par1b[42]. In drosophila
603 epithelial follicle cells, recent functional studies have shown that the DAPC promotes planar
604 polarization of integrin clusters and participates in the trafficking of ECM components leading
605 to the formation of polarized fibrils[43]. These observations suggest that the DAPC may be at the
606 heart of cellular mechanisms that precisely define subcellular cortical domains allowing specific
607 functions to be compartmentalized within a single cell.
608
609 **Planar cell polarity at the scale of the muscle tissue**
610 The *C. elegans* body wall muscle tissue is organized in four quadrants that are each composed
611 of two bands of successive muscle cells[7] (Figure 1A). Each muscle quadrant is essentially a
612 single layer of muscle cells that runs along the length of the animal. The asymmetrical
613 distribution of potassium channels and DAPC proteins within each muscle cell therefore
614 translates into a prototypical planar polarity pattern. Most planar cell polarity genes are
615 conserved in the *C. elegans* genome[31]. The role of VANG-1, PRKL-1, and FMI-1 in cell

migration-related processes have been well established. PCP proteins regulate cell intercalation, convergent extension movements, neuronal migration, neurite outgrowth and guidance. However, in contrast to planar cell polarity in fly epithelia, worm PCP proteins act on an individual cell basis rather than via cell-cell contact-dependent polarization processes. They have been mostly found to control asymmetry pathways that instruct antero-posterior polarity and asymmetric cell divisions during embryogenesis and larval stages. They also control the asymmetric divisions of the stem-cell-like epithelial seam cells. Their action in these contexts is transient. In contrast, the planar polarity patterns we have described here remain visible throughout the life of the cell. Consistently, continuous activity of DSH-1 is required to maintain membrane organization and muscle polarity. Indeed, by manipulating DSH-1 levels at different life stages, we could demonstrate that muscle polarity is not a fixed state, established early on in muscle cells. Continuous requirement of DSH-1 argues that it controls a dynamic machinery. It will therefore be essential to identify the molecular targets of DSH-1 in muscle cells, and to identify additional factors required to establish and maintain muscle polarity. Further work will be required to dissect the molecular and cellular signaling pathways that control this novel example of planar cell polarity in the nematode *Caenorhabditis elegans*.

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The authors declare no competing financial interests.

MATERIALS & METHODS

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Thomas Boulin (thomas.boulin@univ-lyon1.fr).

Materials Availability

Worm strains and plasmids generated in this study are available upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Strains and genetics

All *C. elegans* strains were originally derived from the wild-type Bristol N2 strain. Worm cultivation, genetic crosses, and manipulation of *C. elegans* were carried out according to standard protocols[44]. All strains were maintained at 20°C on nematode growth medium (NGM) agar plates with *Escherichia coli* OP50 as a food source. Strains and alleles used for this study are listed in Supplementary Table S1 and S2, respectively.

667
668 **METHOD DETAILS**
669 **Molecular biology**
670 Single-strand oligonucleotides, crRNA, and plasmids used in this study are described in
671 Supplementary Table 3, 4, and 5, respectively.
672
673 **Auxin-induced degradation**
674 Auxin plates were prepared by adding auxin Indole-3-Acetic Acid (Sigma) from a 400 mM stock
675 solution in ethanol into NGM at the final concentration of 1 mM[22]. DYS-1 and DSH-1
676 degradation was performed using a transgene expressing TIR1 with the ubiquitous promoter
677 *Peft-3*[45]. Protein degradation was monitored *in vivo* based on mNeonGreen fluorescence.
678
679 **Microscopy imaging and quantification**
680 ***General methods*** Freely moving worms were observed on nematode growth media (NGM)
681 plates using an AZ100 macroscope (Nikon) equipped with a Flash 4.0 CMOS camera
682 (Hamamatsu Photonics). For confocal imaging, animals were mounted on 2% dry agarose
683 pads with 2% poly-lysine beads in M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 0.25 g
684 $MgSO_4 \cdot 7\ H_2O$, and distilled water up to 1 liter). Confocal imaging was performed using an
685 inverted confocal microscope (Olympus IX83) equipped with a confocal scanner unit spinning-
686 disk scan head (Yokogawa) and an EMCDD camera (iXon Ultra 888, Andor) at the Ciqle
687 imaging facility (Centre d'Imagerie Quantitative Lyon-Est, LyMIC-CIQLE, Lyon, France).
688
689 ***TWK-28-mNeonGreen fluorescence intensity quantification*** Quantification of fluorescent
690 images were performed using ImageJ (version: 2.0.-rc-69/1.53a). Each data point represents
691 one muscle cell and data from at least three independent imaging sessions were pooled for
692 each genotype. Acquisition parameters were the same across genotypes for quantitative
693 analyses. Fluorescence intensity was measured from Figure 2A to 2E) in a region of interest
694 of 30 µm (wide) x 5 µm (high) size, and for Figure 2K in a region of interest of 50 µm (wide) x
695 5 µm (high) size. To quantify the fluorescence intensity of TWK-28 in figure 4E, we first
696 segmented individual muscle cells using TWK-12::wrmScarlet to outline the muscle
697 membrane. The total fluorescence intensity was corrected by subtracting background
698 fluorescence from the middle of the cell, where TWK-28 was undetectable.
699
700 ***Measurement of TWK-28, TWK-24 and SLO-1 domain boundaries*** To determine the
701 precise outline of individual muscle cells, we used TWK-12::wrmScarlet for cell membrane
702 segmentation. We then manually measured the length of each cell and the relative position of
703 the TWK-28, TWK-24 and SLO-1 domain boundaries along the antero-posterior axis (Figure
704 4C).
705
706 **Electrophysiology and heterologous expression of TWK-28 in *Xenopus laevis* oocytes**
707 Capped mRNAs were synthesized in vitro from linearized expression vectors using the T7
708 mMessage mMachine kit (Ambion, Austin, TX, USA). Defolliculated *X. laevis* oocytes (Ecocyte
709 Bioscience, Dortmund, Germany) were injected with 50 nL containing 1.8 ng of cRNA. Oocytes
710 were kept at 18°C in ORII Calcium solution containing (in millimolar): 82.5 NaCl, 2 KCl, 1
711 $MgCl_2$, 0.7 $CaCl_2$, 5 HEPES, gentamicin (25 µg/mL), pH 7.5 (with TRIZMA-Base).
712 Two-electrode voltage clamp (TEVC) experiments were performed 24 hours after
713 microinjection. Oocytes were mounted in a small home-made recording chamber and
714 continuously superfused with ND96 solution containing (in millimolar): 96 NaCl, 2 KCl, 1.8
715 $CaCl_2$, 2 $MgCl_2$, 5 HEPES. pH 7.4 was adjusted with Trizma base. Macroscopic currents were
716 recorded using a Warner Instrument OC-725 amplifier, filtered at 10kHz, digitized using a
717 Digidata-1322 (Axon Instrument). For current visualization and stimulation protocol application,

we used Axon pClamp 9 software (Molecular Devices, Sunnyvale, CA). Recording electrodes were pulled to 0.2-1.0 MΩ by using a horizontal puller (Sutter Instrument, Model P-97, USA) and filled with 3 M KCl. Currents were recorded in response to a voltage-step protocol consisting of a pre-pulse of -80 mV (80 ms duration) from a holding potential of -60 mV, followed by 10 mV steps (200 ms duration) from -150 mV to +50 mV, and return to a -60 mV holding potential. Current-voltage curves were obtained by plotting the steady-state currents at the end of each voltage step.

QUANTIFICATION AND STATISTICAL ANALYSIS

The statistical analysis was performed using the R software package. Normality of sample distributions was assessed using the Shapiro-Wilks normality test before choosing parametric (Student's *t* test) or a non-parametric (Mann-Whitney) tests, as indicated in relevant figure legends. Graphs were plotted using the PlotsOfData service[46].

FIGURE LEGENDS

Figure 1. Ion channel localization reveals elaborate muscle membrane compartmentalization

(A) Schematic diagram of the organization of the *C. elegans* musculature in the head and midbody region. Dorso-ventral view of two out of four muscle quadrants. Successive muscle cells are labeled in green and grey. Anterior (*Ant*) to the left, posterior (*Pos*) to the right.
(B) Asymmetric localization of TWK-28, TWK-24 and SLO-1 channels. Muscle membrane labeled by TWK-12-wrmScarlet, magenta.
(C) Image segmentation reveals little to no co-localization of ion channels in triple-labeled muscle cell using TWK-28 (cyan), TWK-24 (magenta), and SLO-1(green) fluorescent knockin lines.
(D) SLO-1 and ISLO-1 distributions overlap exclusively in the posterior domain. ISLO-1 is also found in the anterior domain of the muscle cell.
(E) TWK-28 and ISLO-1 distributions overlap in the anterior domain of muscle cells. Rightmost column, magnified view of anterior region of individual muscle cells.
Regions of interest in (D) and (E): *Anterior domain*, comet-like domain at anterior tip of muscle cells; *Posterior domain,* punctate clustered pattern in posterior part of muscle cells.
Muscle cell outlines are indicated with yellow dashed lines. Scale bars, 20 µm.

Figure 2. TWK-28 surface expression is dependent on dystrophin and DAPC-associated proteins

(A) Confocal detection and quantification of mNeonGreen-TWK-28 in a *dys-1* molecular null allele *(syb2174)* and a long-isoform-specific *dys-1* mutant *(bln582)*.
(B) Confocal detection and quantification of mNeonGreen-TWK-28 in *dyb-1(cx36)* and *islo-1(bln549)* loss-of-function mutants.
(C) C-terminal sequence of the TWK-28ΔPDZ variant engineered by gene editing of mNeonGreen-TWK-28 to eliminate a putative PDZ-binding motif (orange residues).
(D) Confocal detection and quantification of mNeonGreen-TWK-28 in *stn-1(ok292)* and *stn-2(ok2417)* loss-of-function mutants, and of an mNeonGreen-TWK-28 knockin line lacking the final four C-terminal amino acids (*twk-28ΔPDZ*).
(E) Confocal detection and quantification of mNeonGreen-TWK-28 and mNeonGreen-*twk-28ΔPDZ* in a *dys-1(syb2174)* molecular null allele.
(F) TWK-8, TWK-18, TWK-24, and TWK-42 expression and subcellular distributions are unchanged in *dys-1(bln582)* loss-of-function mutant. Representative images of head musculature.
(G) DYS-1 degradation protocol. L4 (P0) worms are transferred to auxin-containing media at day 0 (D0). F1 progeny are exposed to auxin until the L4 stage (ON Auxin) and transferred to NGM plates (OFF Auxin) for 24h. Confocal detection and quantification of fluorescence at day 3 (D3) and 24 hours later (D3 + 24h).
(H) DYS-1-AID-mNeonGreen is strongly degraded after lifelong exposure to auxin (ON Auxin, D3). Partial recovery 24 h after removal from auxin (OFF Auxin, D3 + 24h).
(I), (J) Confocal detection of wrmScarlet-TWK-28 and DYS-1-AID-mNeonGreen at D3 and D3 + 24h, in the absence of auxin (NO Auxin, age-matched controls), after auxin exposure for three days (ON Auxin), and after removal from auxin for 24h (OFF Auxin).
(K) Quantification of wrmScarlet-TWK-28 fluorescence.
Mann-Whitney test, n > 30 in all samples; n.s., not significant, * $p < 0.05$
Scale bars, 20 µm

780
781 **Figure 3. Dystrophin, dystroglycan and sarcoglycans define asymmetrical membrane**
782 **compartments at the surface of *C. elegans* muscle cells**
783 (A) DGN-1 and DYS-1 distributions overlap in body wall muscle cells.
784 (B) DGN-1 localizes to sarcolemmal domains devoid of α-integrins/PAT-2. Representative
785 images of head muscle cells of DGN-1-wrmScarlet (in magenta) and PAT-2-mNeonGreen (in
786 green) translational fusion knockin lines.
787 (C), (D) DGN-1 and PAT-2 occupy juxtaposed and optically-separable membrane domains.
788 Enlargement of images shown in panel B.
789 (E) SGCB-1 and DYS-1 distributions overlap only partially in body wall muscle cells.
790 (F) SGCB-1 partitions into distinct membrane domains along the antero-posterior axis of
791 individual muscle cells. *Anterior domain*, comet-like domain. *Gap domain,* sparse localization.
792 *Posterior domain,* clustered pattern aligned with, but optically-separable from, PAT-2-labeled
793 dense bodies.
794 (G) DGN-1 and SGCB-1 patterns only partially coincide in the anterior and posterior regions
795 of muscle cells.
796 Muscle cell outlines are indicated with yellow dashed lines. Scale bars, 20 µm.
797
798 **Figure 4. Loss of DSH-1/disheveled disrupts the planar-polarized organization of the**
799 **muscle plasma membrane**
800 (A), (B) Symmetrical distribution of TWK-28, SLO-1 and TWK-24 in *dsh-1(ok1445)* loss-of-
801 function background. Muscle membrane labeled by TWK-12-wrmScarlet, magenta.
802 (B) Magnified view of single muscle cells in wild type and *dsh-1(ok1445)*.
803 (C) Quantification of TWK-28, SLO-1 and TWK-24 domain boundary positions in wild type and
804 *dsh-1(ok1445)*. Average position of anterior (*Ant*) boundaries in wild type (black) and *dsh-*
805 *1(ok1445)* (red), respectively, as percentages of muscle cell length: TWK-28, 31% and 30%;
806 SLO-1, 24% and 23%; TWK-24, 23% and 21%. Average position of posterior (*Pos)* boundaries
807 in wild type and *dsh-1(ok1445)*, respectively, as percentages of muscle cell length: TWK-28,
808 97% and 73%; SLO-1, 90% and 78%; TWK-24, 68% and 80%. Gray and red bars indicate the
809 region containing each ion channel. Number of cells assayed in each condition: TWK-28 (WT,
810 n=29; *dsh-1*, n=25); SLO-1 (WT, n=30; *dsh-1*, n=26); TWK-24 (WT, n=33; *dsh-1*, n=26).
811 (D) Image segmentation reveals little to no co-localisation of ion channels in *dsh-1*-mutant
812 muscle cell using TWK-28 (cyan), TWK-24 (magenta), and SLO-1(green) fluorescent knockin
813 lines.
814 (E) Total TWK-28 surface expression is unchanged in *dsh-1(ok1445)* as TWK-28 is
815 redistributed between the anterior and posterior domains. Mann-Whitney test, n.s. non-
816 significant, * $p < 0.05$.
817 (F) Symmetrical distribution of DYS-1 and TWK-28 at extremities of muscle cells in *dsh-*
818 *1(ok1445)*.
819 (G) Symmetrical distribution of SGCB-1 in *dsh-1(ok1445)*.
820 (H) Symmetrical distribution TWK-28 lacking the PDZ binding-motif in *dsh-1(ok1445)*. White
821 arrowhead indicate remaining fluorescent signal.
822 Muscle cell outlines are indicated with yellow dashed lines. Scale bars, 20 µm.
823
824 **Figure 5. Loss of MIG-14/Wntless, EGL-20/Wnt and CAM-1/Ror disrupts asymmetric**
825 **localization of TWK-28**
826 (A) Schematic protein structure of DSH-1a including DIX, PDZ, DEP domains.

827 (B) Muscle-specific expression of full-length or DIX domain-truncated DSH-1a proteins
828 rescues TWK-28 localization in *dsh-1(ok1445)*. PDZ and DEP domains are necessary for
829 DSH-1 function.
830 (C) Planar-polarized distribution of TWK-28 is maintained in the absence of core planar cell
831 polarity components (*vang-1, fmi-1, prkl-1*), Cadherin/Fat/Dachsous pathway components
832 (*cdh-1, cdh-3, cdh-4*), or β-catenin (*bar-1*).
833 (D) Loss of MIG-14/Wntless, EGL-20/Wnt, and CAM-1/Ror disrupts polarized localization of
834 TWK-28. Anterior mid-body muscle cells in *mig-14*, *cam-1* and *egl-20* mutants are wild type.
835 Posterior mid-body muscle cells are affected in all mutant genotypes.
836 (E) Schematic protein structure of CAM-1/Ror receptor, with corresponding molecular lesions
837 in point mutants (*gm122* and *cw82)*, and deletion alleles (*ak37* and *ks52*).
838 (F) *cam-1(ak37), cam-1(ks52)* and *cam-1(cw82)* disrupt TWK-28 localization in posterior mid-
839 body muscles.
840 Left- or right-pointing yellow arrowheads indicate anterior or posterior extremity of muscle cells,
841 respectively. Scale bars, 20 μm.
842
843 **Figure 6. DSH-1/disheveled is required to establish and maintain muscle membrane**
844 **polarity**
845 (A) Representative micrograph of a DSH-1 mNeonGreen translational knockin line. DSH-1 is
846 enriched in the posterior portion of body wall muscle cells. DSH-1::mNeonGreen is visible in
847 ventral nerve cord motoneuron cell bodies and neurites. Ventral view.
848 (B) DSH-1::mNeonGreen distribution in a single muscle cells. Muscle membrane labeled by
849 TWK-12-wrmScarlet, magenta. Average position of DSH-1 domain boundary, 72 % of muscle
850 cell length, n=29. *Ant*, anterior. *Pos*, posterior. Gray bar indicates the region containing DSH-
851 1.
852 (C) DSH-1 and TWK-28 are found at opposite ends of muscle cells. Yellow or white
853 arrowheads and dashed lines indicate the anterior or posterior end of muscle cells,
854 respectively.
855 (D) Asymmetric localization of TWK-28 in L1, L2, and L3 larval stages. Non-polarized muscle
856 cells are found in wild-type at the L1 stage. Loss of *dsh-1* disrupts muscle cell polarity at all
857 stages. Muscle cells are labeled with cytoplasmic mCherry in the L1 stage and outlined with
858 white dotted lines.
859 (E) DSH-1 is required to maintain muscle polarity during post-embryonic development.
860 (F) Defective muscle polarity is restored by re-expression of DSH-1 during post-embryonic
861 development.
862 White arrow heads indicate the posterior end of muscle cells in (A), (B), and (C).
863 Left- or right-pointing yellow arrowheads indicate anterior or posterior extremity of muscle cells,
864 respectively. Scale bars, 20 μm.
865

866 **SUPPLEMENTARY FIGURE LEGENDS**
867
868 **Figure S1. Genetic suppression of a TWK-28 L210T gain-of-function mutant by**
869 **mutations in *dys-1* and *islo-1***
870 (A) Two-pore domain potassium channels TWK-12 and TWK-43 are enriched in muscle arms
871 and on the lateral membrane of muscle cells.
872 (B) Current–voltage relationships obtained at pH 7.4 in *X. laevis* oocytes after injection of
873 cRNA encoding TWK-28 wild-type (black squares) and TWK-28 L210T TM2.6 mutant channels
874 (red squares). Inset shows leftward shift of reversal potential in TWK-28 L210T. n=8 and n=7
875 for wild-type and TWK-28 L210T, respectively.
876 (C) Low and high magnification micrographs illustrating reduced locomotion and relaxed body
877 posture of *twk-28(L210T)* gain-of-function mutants on NGM plates.
878 (D) Loss-of-function mutations in *dys-1* and a missense mutation in *islo-1* suppress paralysis
879 of the TWK-28 L210T gain-of-function mutation.
880 (E) TWK-28 surface expression is unchanged in sarcoglycan single or triple null mutants.
881 (F) Quantification of TWK-28-associated fluorescence. Mann-Whitney test; n.s., not
882 significant.
883 Scale bar, 20 μm
884
885 **Figure S2. Dystrophin is required for sarcolemmal localization of dystroglycan and**
886 **sarcoglycans.**
887 (A), (B) Sarcoglycans SGCA-1 and SGN-1 are colocalized with SGCB-1.
888 (C) Subunit interdependence for surface expression of SGCA-1, SGCB-1 and SGN-1.
889 (D) SGBC-1 localization is disrupted differently in *dys-1* and *dyb-1* mutants.
890 (E) Reduction in surface expression and mislocalization of DGN-1 in perlecan/*unc-52* loss of
891 function mutants.
892 (F) DGN-1 localization is dependent on DYS-1, but not DYB-1.
893 Scale bars, 20 μm.
894
895 **Figure S3. Relative localization of DAPC component in *C. elegans* head muscles**
896 (A) Colocalization of DGN-1 and STN-2.
897 (B) Colocalization of SGCB-1 and DYB-1.
898 (C) Colocalization of SGCB-1 and STN-1.
899 (D) Colocalization of DGN-1 and DYB-1.
900 (E) Colocalization of STN-1 and STN-2.
901 Muscle cell outlines are indicated with yellow dashed lines. Scale bars, 20 μm.
902
903 **Figure S4. DAPC components partition to dystroglycan- or sarcoglycan-containing**
904 **membrane compartments**
905 (A) Colocalization of DGN-1 and DYS-1.
906 (B) Colocalization of DGN-1 and STN-2.
907 (C) Colocalization of SGCB-1 and DYB-1.
908 (D) Colocalization of SGCB-1 and STN-1.
909 (E) Colocalization of DGN-1 and DYB-1.
910 (F) Colocalization of SGCB-1 and DYS-1.
911 (G) Colocalization of STN-1 and STN-2.
912 *Anterior domain*, comet-like domain at anterior tip of muscle cells; *Posterior domain,* punctate
913 clustered pattern in posterior part of muscle cells.

914
915 **Figure S5. TWK-28 colocalizes with all DAPC components**
916 Colocalization of TWK-28 with SGCB-1, STN-1, DYB-1, DGN-1, DYS-1 and STN-2. Rightmost
917 column, magnified view of anterior region of individual muscle cells.
918 Scale bars, 20 µm.
919
920
921 **Figure S6. EGL-20/Wnt is required for muscle cell polarity while several Wnt ligands and**
922 **receptors are not**
923 (A), (B) Wnt ligands (*cwn-1, cwn-2, lin-44*), frizzled receptors (*lin-17, mig-1, cfz-2*) and the
924 tyrosine kinase-related receptors LIN-18/Ryk are not required for TWK-28 planar polarization.
925 (C) *egl-20(bln916)* –an early nonsense mutation at position Pro21– disrupts TWK-28
926 localization in posterior midbody muscles.
927 Scale bars, 20 µm.
928
929 **Figure S7. The localization of DSH-1 is dependent on CAM-1 and EGL-20**
930 (A) Enrichment of DSH-1 at the posterior end of muscle cells is lost in *cam-1* and *egl-20*
931 mutants. DSH-1 localization is however conserved in the anterior midbody of *egl-20* mutants,
932 consistent with the posteriorly-restricted distribution of EGL-20. White arrow heads indicate
933 DSH-1 enrichment at the posterior end of muscle cells. Ventral view.
934 (B) Life-long degradation of DSH-1-AID-mNeonGreen using an ubiquitous TIR1-expressing
935 transgene disrupts asymmetric localization of TWK-28 in muscle cells.
936 Scale bars, 20 µm.
937

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Figure 1

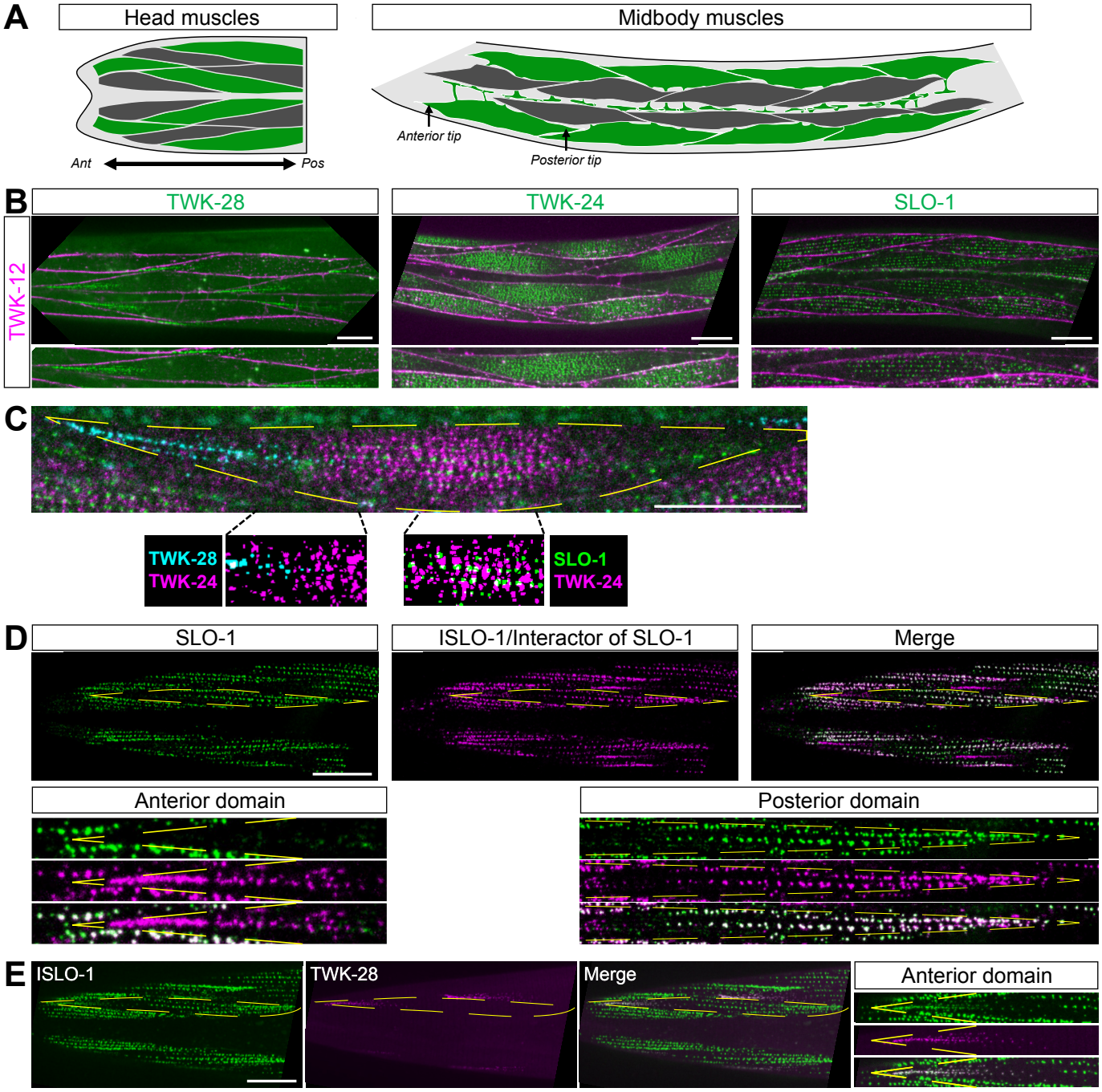

A
Head muscles
Midbody muscles
Ant
Pos
Anterior tip
Posterior tip
B
TWK-28
TWK-24
SLO-1
TWK-12
C
TWK-28
TWK-24
SLO-1
TWK-24
D
SLO-1
ISLO-1/Interactor of SLO-1
Merge
Anterior domain
Posterior domain
E
ISLO-1
TWK-28
Merge
Anterior domain

Figure 2

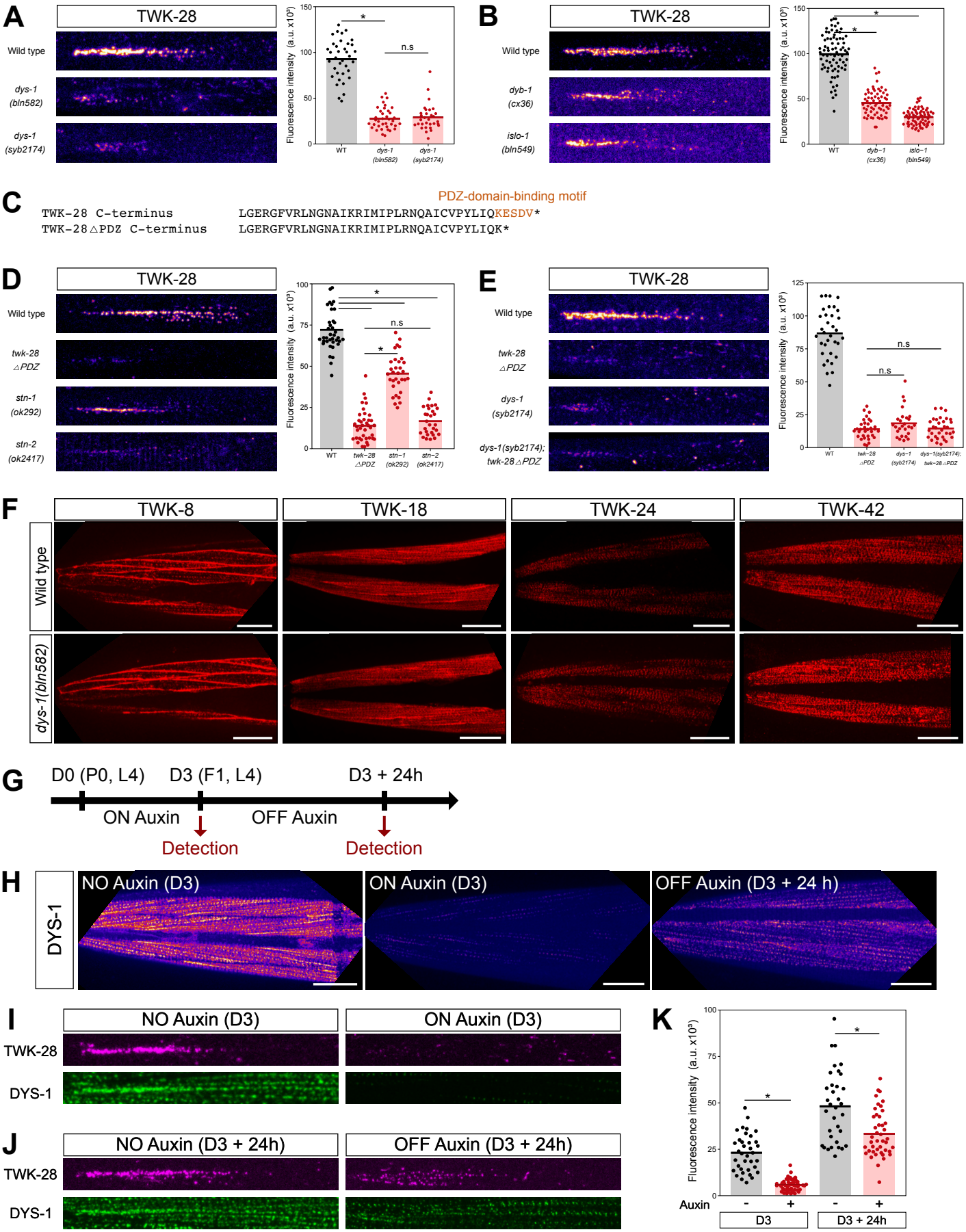

A
TWK-28
Wild type
dys-1 (bln582)
dys-1 (syb2174)
Fluorescence intensity (a.u. x10³)
*
n.s
WT
dys-1 (bln582)
dys-1 (syb2174)
B
TWK-28
Wild type
dyb-1 (cx36)
islo-1 (bln549)
Fluorescence intensity (a.u. x10³)
*
*
WT
dyb-1 (cx36)
islo-1 (bln549)
C
PDZ-domain-binding motif
TWK-28 C-terminus LGERGFVRLNGNAIKRIMIPLRNQAICVPYLIQKESDV*
TWK-28△PDZ C-terminus LGERGFVRLNGNAIKRIMIPLRNQAICVPYLIQK*
D
TWK-28
Wild type
twk-28 △PDZ
stn-1 (ok292)
stn-2 (ok2417)
Fluorescence intensity (a.u. x10³)
*
n.s
*
WT
twk-28 △PDZ
stn-1 (ok292)
stn-2 (ok2417)
E
TWK-28
Wild type
twk-28 △PDZ
dys-1 (syb2174)
dys-1(syb2174); twk-28△PDZ
Fluorescence intensity (a.u. x10³)
n.s
n.s
WT
twk-28 △PDZ
dys-1 (syb2174)
dys-1(syb2174); twk-28△PDZ
F
TWK-8
TWK-18
TWK-24
TWK-42
Wild type
dys-1(bln582)
G
D0 (P0, L4)
D3 (F1, L4)
D3 + 24h
ON Auxin
OFF Auxin
Detection
Detection
H
DYS-1
NO Auxin (D3)
ON Auxin (D3)
OFF Auxin (D3 + 24 h)
I
NO Auxin (D3)
ON Auxin (D3)
TWK-28
DYS-1
J
NO Auxin (D3 + 24h)
OFF Auxin (D3 + 24h)
TWK-28
DYS-1
K
Fluorescence intensity (a.u. x10³)
*
*
Auxin
-
+
-
+
D3
D3 + 24h

Figure 3

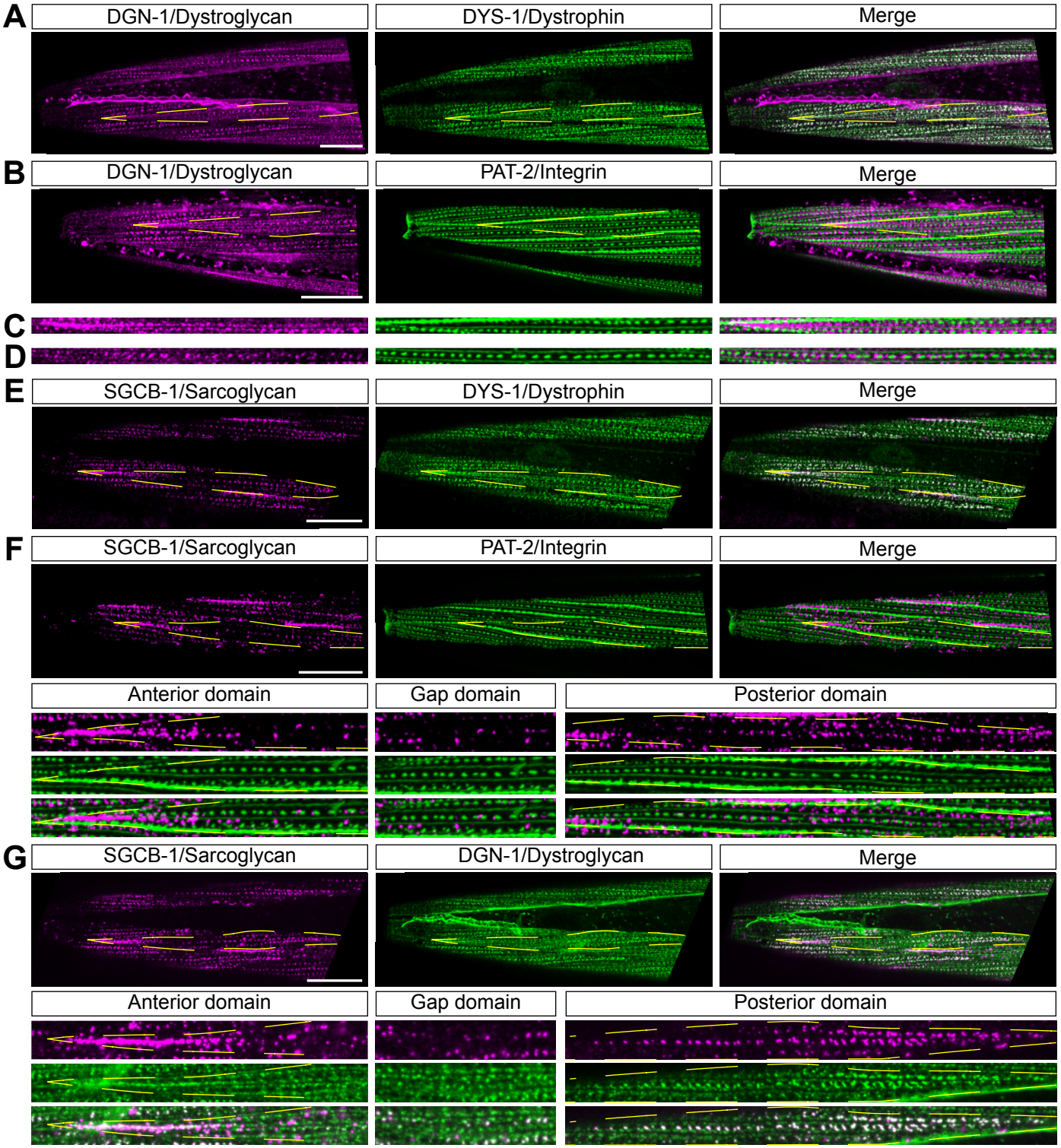
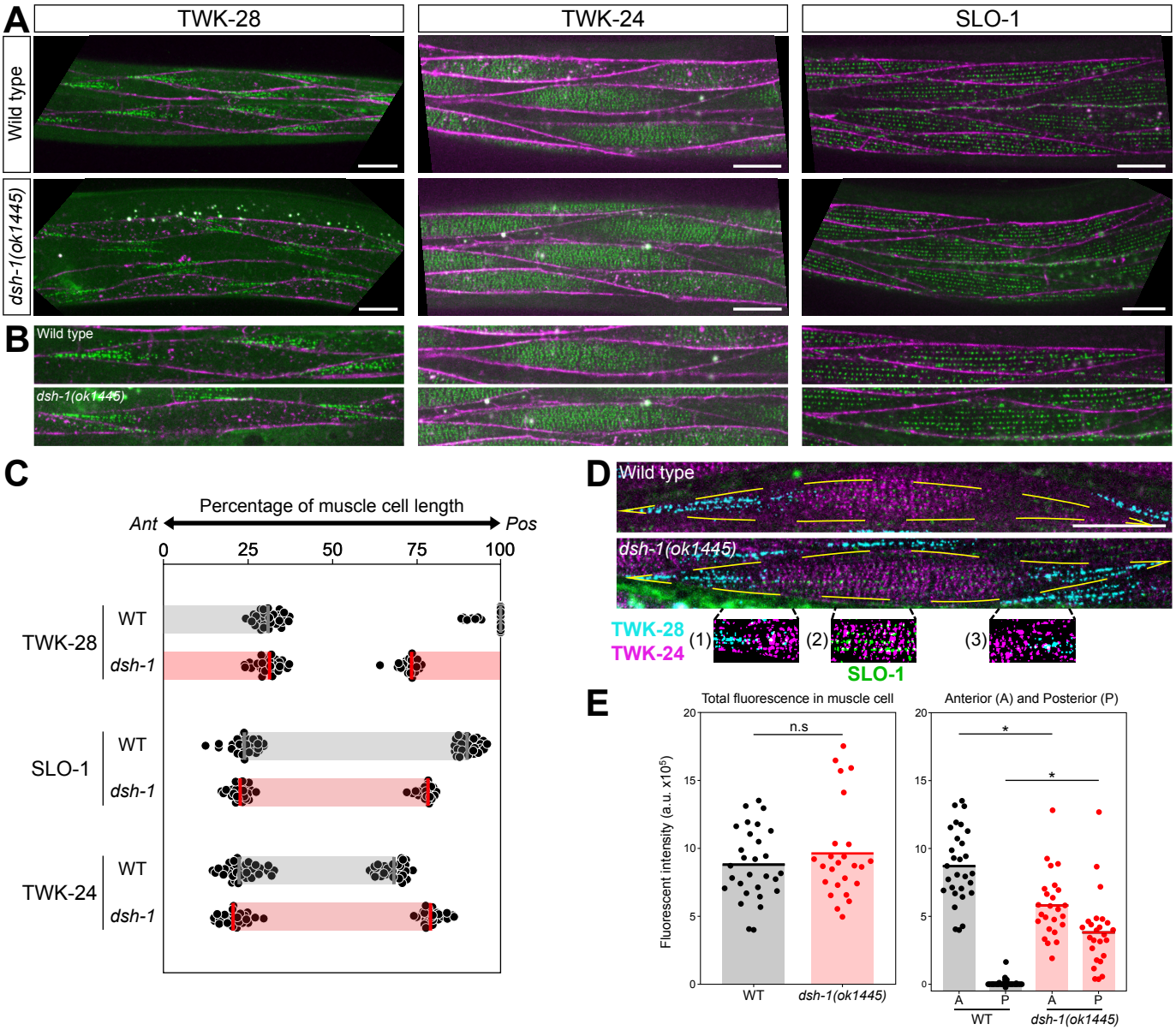
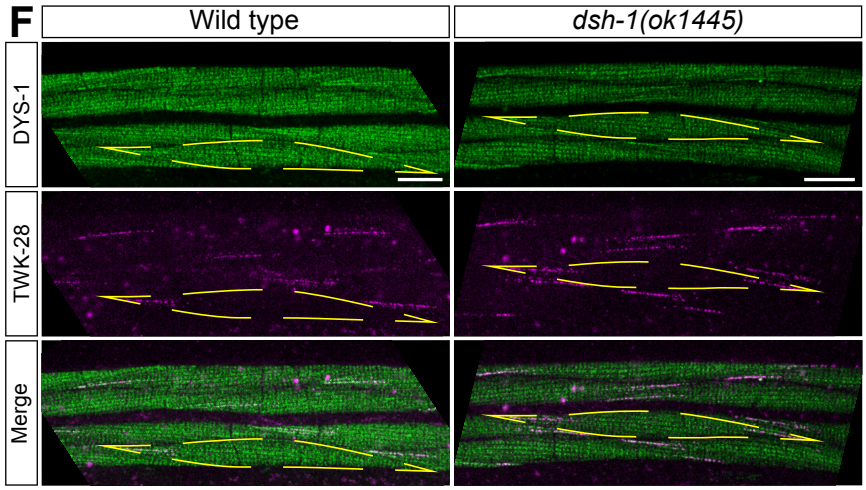
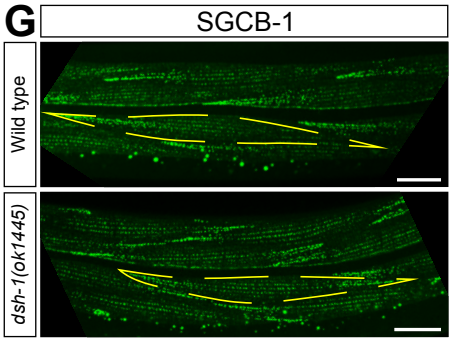

A
DGN-1/Dystroglycan
DYS-1/Dystrophin
Merge
B
DGN-1/Dystroglycan
PAT-2/Integrin
Merge
C
D
E
SGCB-1/Sarcoglycan
DYS-1/Dystrophin
Merge
F
SGCB-1/Sarcoglycan
PAT-2/Integrin
Merge
Anterior domain
Gap domain
Posterior domain
G
SGCB-1/Sarcoglycan
DGN-1/Dystroglycan
Merge
Anterior domain
Gap domain
Posterior domain

Figure 4


A
TWK-28
TWK-24
SLO-1
Wild type
dsh-1(ok1445)
B
Wild type
dsh-1(ok1445)
C
Percentage of muscle cell length
Ant
Pos
0
25
50
75
100
TWK-28
WT
dsh-1
SLO-1
WT
dsh-1
TWK-24
WT
dsh-1
D
Wild type
dsh-1(ok1445)
TWK-28
TWK-24
(1)
(2)
(3)
SLO-1
E
Total fluorescence in muscle cell
Anterior (A) and Posterior (P)
Fluorescent intensity (a.u. x10^5)
n.s
*
*
WT
dsh-1(ok1445)
A
P
A
P
WT
dsh-1(ok1445)


F
Wild type
dsh-1(ok1445)
DYS-1
TWK-28
Merge


G
SGCB-1
Wild type
dsh-1(ok1445)

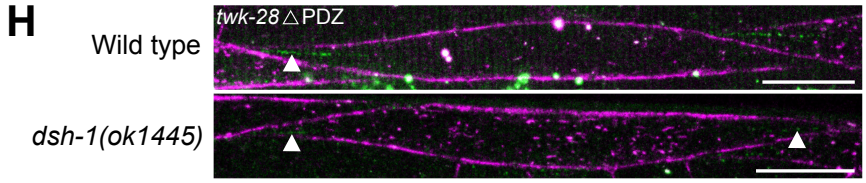

H
Wild type
dsh-1(ok1445)
twk-28△PDZ

Figure 5

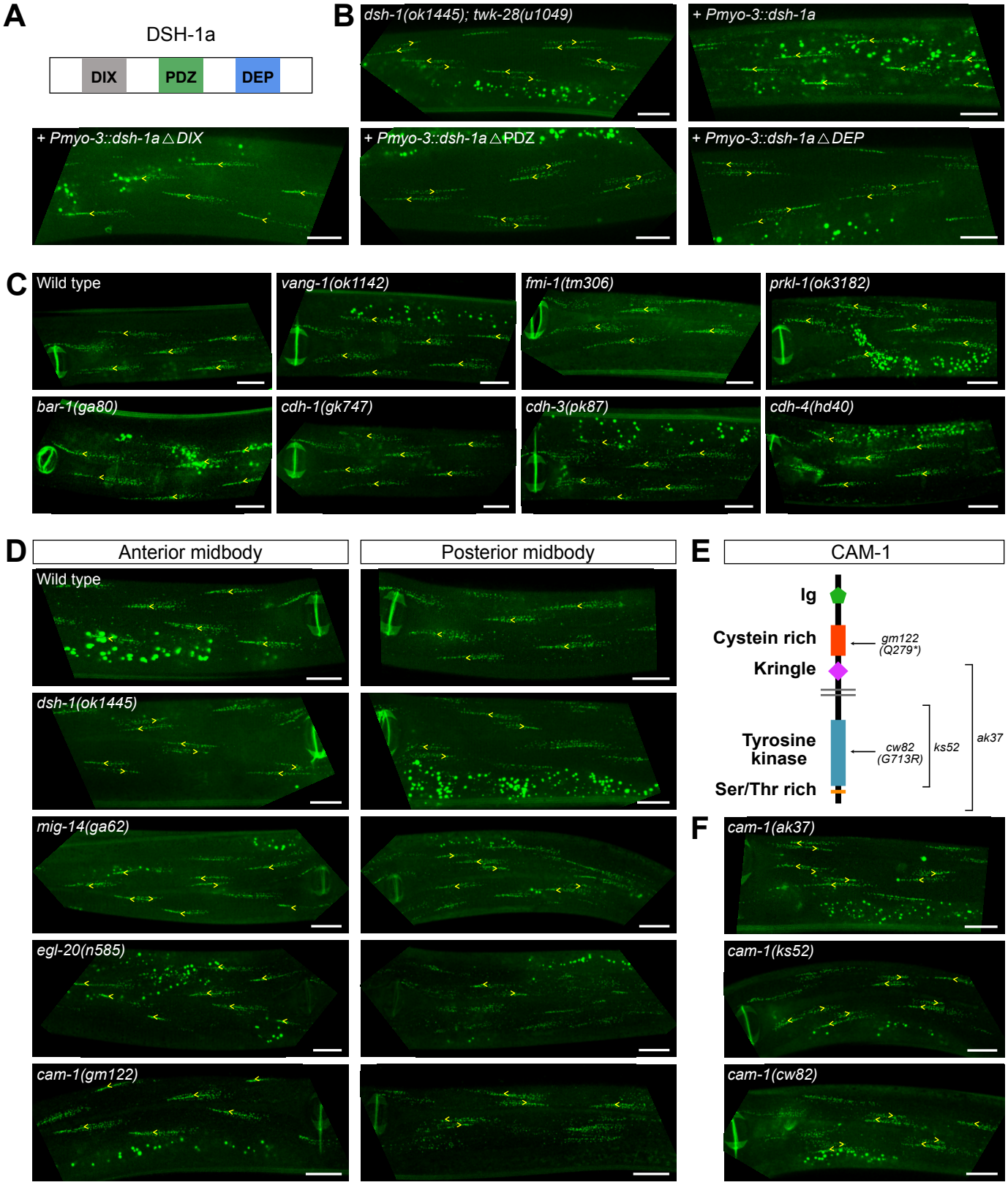

A
DSH-1a
DIX
PDZ
DEP
B
dsh-1(ok1445); twk-28(u1049)
+ Pmyo-3::dsh-1a
+ Pmyo-3::dsh-1a △DIX
+ Pmyo-3::dsh-1a △PDZ
+ Pmyo-3::dsh-1a △DEP
C
Wild type
vang-1(ok1142)
fmi-1(tm306)
prkl-1(ok3182)
bar-1(ga80)
cdh-1(gk747)
cdh-3(pk87)
cdh-4(hd40)
D
Anterior midbody
Posterior midbody
Wild type
dsh-1(ok1445)
mig-14(ga62)
egl-20(n585)
cam-1(gm122)
E
CAM-1
Ig
Cystein rich
Kringle
Tyrosine kinase
Ser/Thr rich
gm122 (Q279*)
cw82 (G713R)
ks52
ak37
F
cam-1(ak37)
cam-1(ks52)
cam-1(cw82)

Figure 6

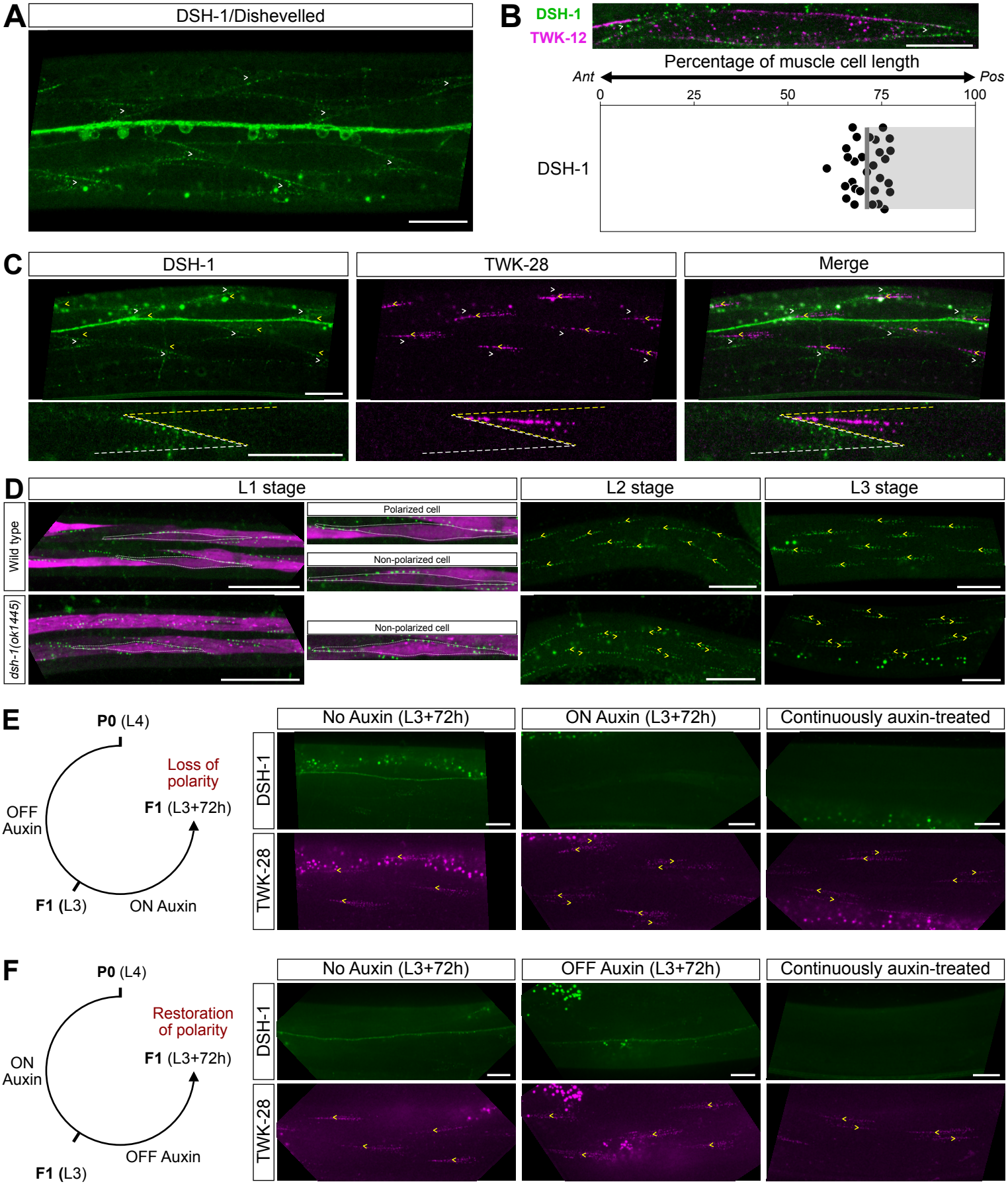
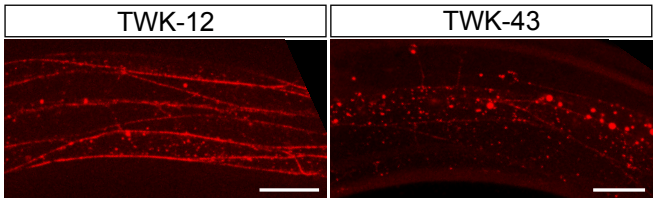
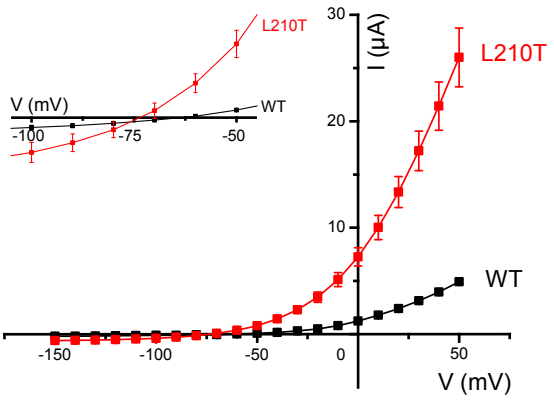

A
DSH-1/Dishevelled
B
DSH-1
TWK-12
Percentage of muscle cell length
Ant
Pos
0
25
50
75
100
DSH-1
C
DSH-1
TWK-28
Merge
D
L1 stage
L2 stage
L3 stage
Wild type
dsh-1(ok1445)
Polarized cell
Non-polarized cell
Non-polarized cell
E
P0 (L4)
Loss of polarity
F1 (L3+72h)
OFF Auxin
F1 (L3)
ON Auxin
No Auxin (L3+72h)
ON Auxin (L3+72h)
Continuously auxin-treated
DSH-1
TWK-28
F
P0 (L4)
Restoration of polarity
F1 (L3+72h)
ON Auxin
OFF Auxin
F1 (L3)
No Auxin (L3+72h)
OFF Auxin (L3+72h)
Continuously auxin-treated
DSH-1
TWK-28

Figure S1

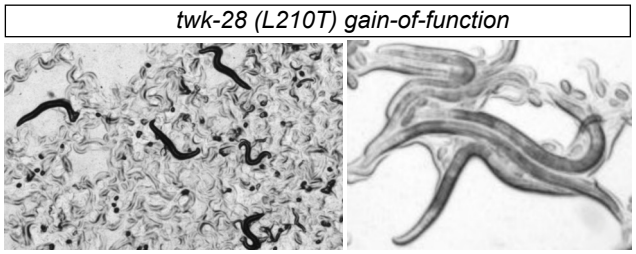
A



B



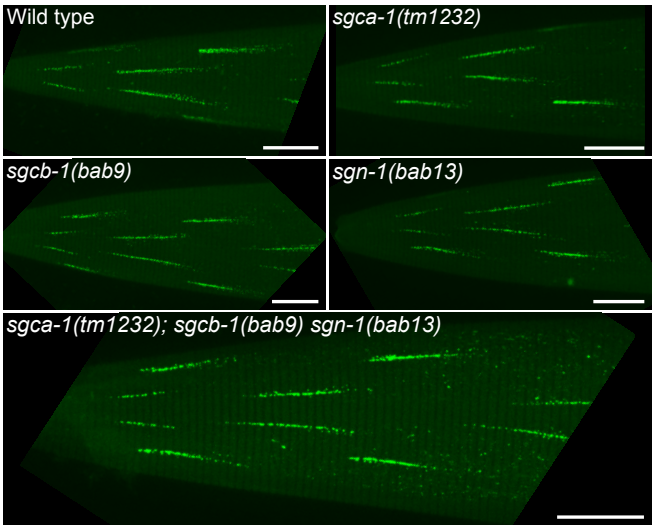
C



D

Allele	Locus	Mutation	Effect
bln557	*dys-1*	G to A ; ChrI:11556770	Q30Ochre
bln545	*dys-1*	C to T ; ChrI:11556616	W81Amber
bln582	*dys-1*	C to T ; ChrI:11556528	W110Opal
bln554	*dys-1*	C to T ; ChrI:11556280	W138Amber
bln583	*dys-1*	T to A ; ChrI:11544157	K1527Amber
bln568	*dys-1*	G to A ; ChrI:11543893	Q1615Ochre
bln552	*dys-1*	C to T ; ChrI:11540064	W2277Opal
bln571	*dys-1*	C to A ; ChrI:11538895	E2442Ochre
bln565	*dys-1*	C to T ; ChrI:11538087	W2582Opal
bln573	*dys-1*	G to A ; ChrI:11537052	Q2695Ochre
bln566	*dys-1*	C to T ; ChrI:11536816	W2773Opal
bln564	*dys-1*	C to T ; ChrI:11536387	W2807Amber
bln562	*dys-1*	G to A ; ChrI:11534654	Q2992Ochre
bln556	*dys-1*	G to A ; ChrI:11532291	loss of splice-donor
bln549	*islo-1*	G to A ; ChrIV:8137939	G165D

E



F

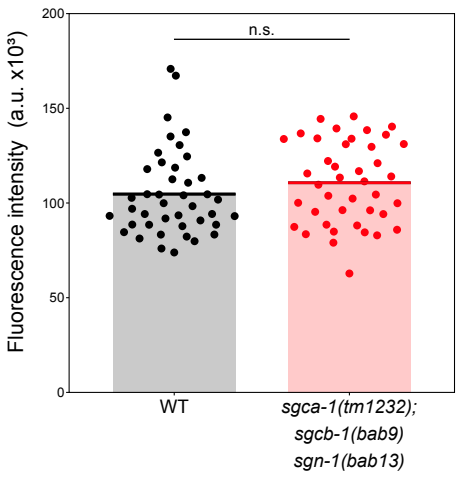


Figure S2

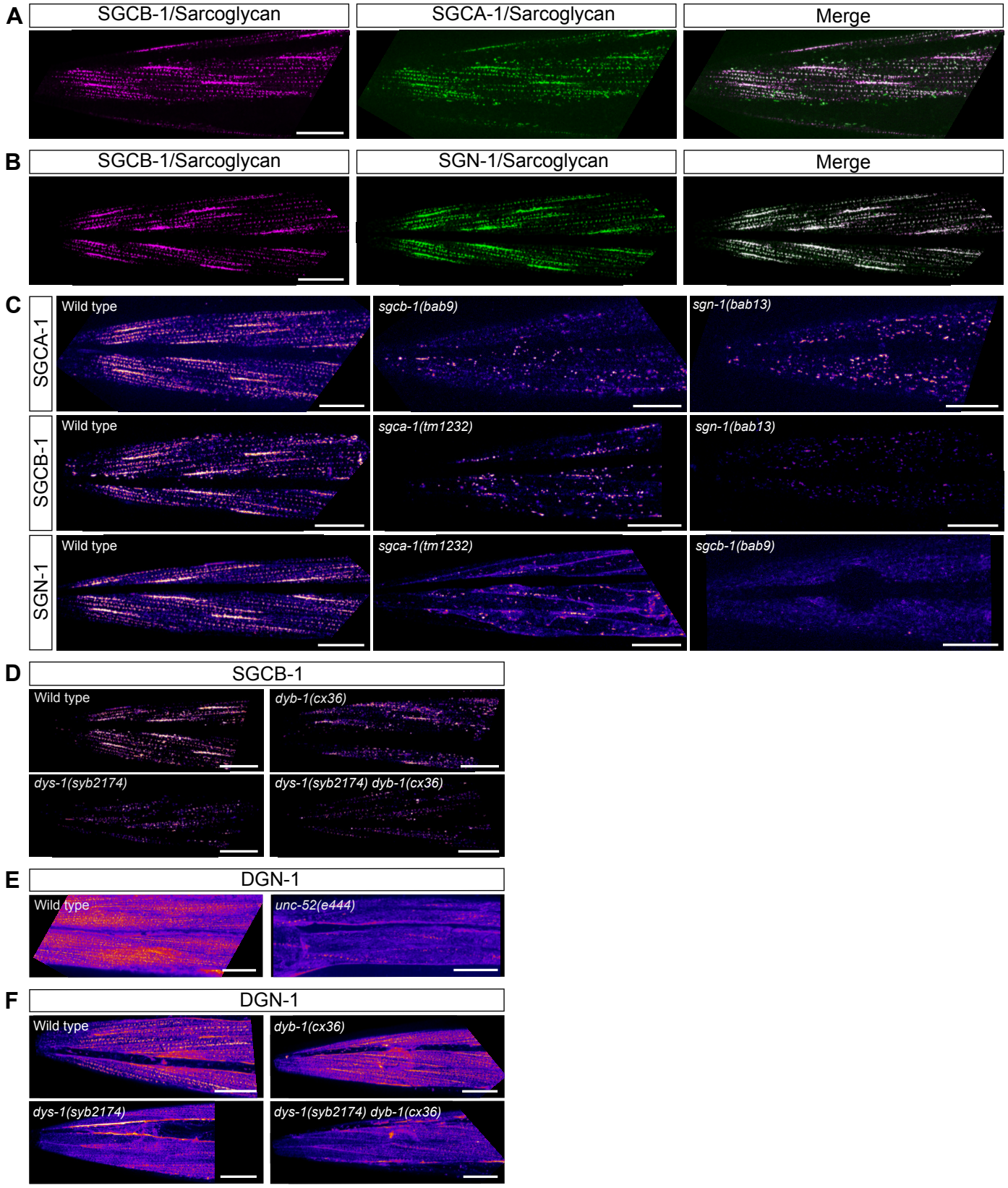

A
SGCB-1/Sarcoglycan
SGCA-1/Sarcoglycan
Merge
B
SGCB-1/Sarcoglycan
SGN-1/Sarcoglycan
Merge
C
SGCA-1
Wild type
sgcb-1(bab9)
sgn-1(bab13)
SGCB-1
Wild type
sgca-1(tm1232)
sgn-1(bab13)
SGN-1
Wild type
sgca-1(tm1232)
sgcb-1(bab9)
D
SGCB-1
Wild type
dyb-1(cx36)
dys-1(syb2174)
dys-1(syb2174) dyb-1(cx36)
E
DGN-1
Wild type
unc-52(e444)
F
DGN-1
Wild type
dyb-1(cx36)
dys-1(syb2174)
dys-1(syb2174) dyb-1(cx36)

Figure S3

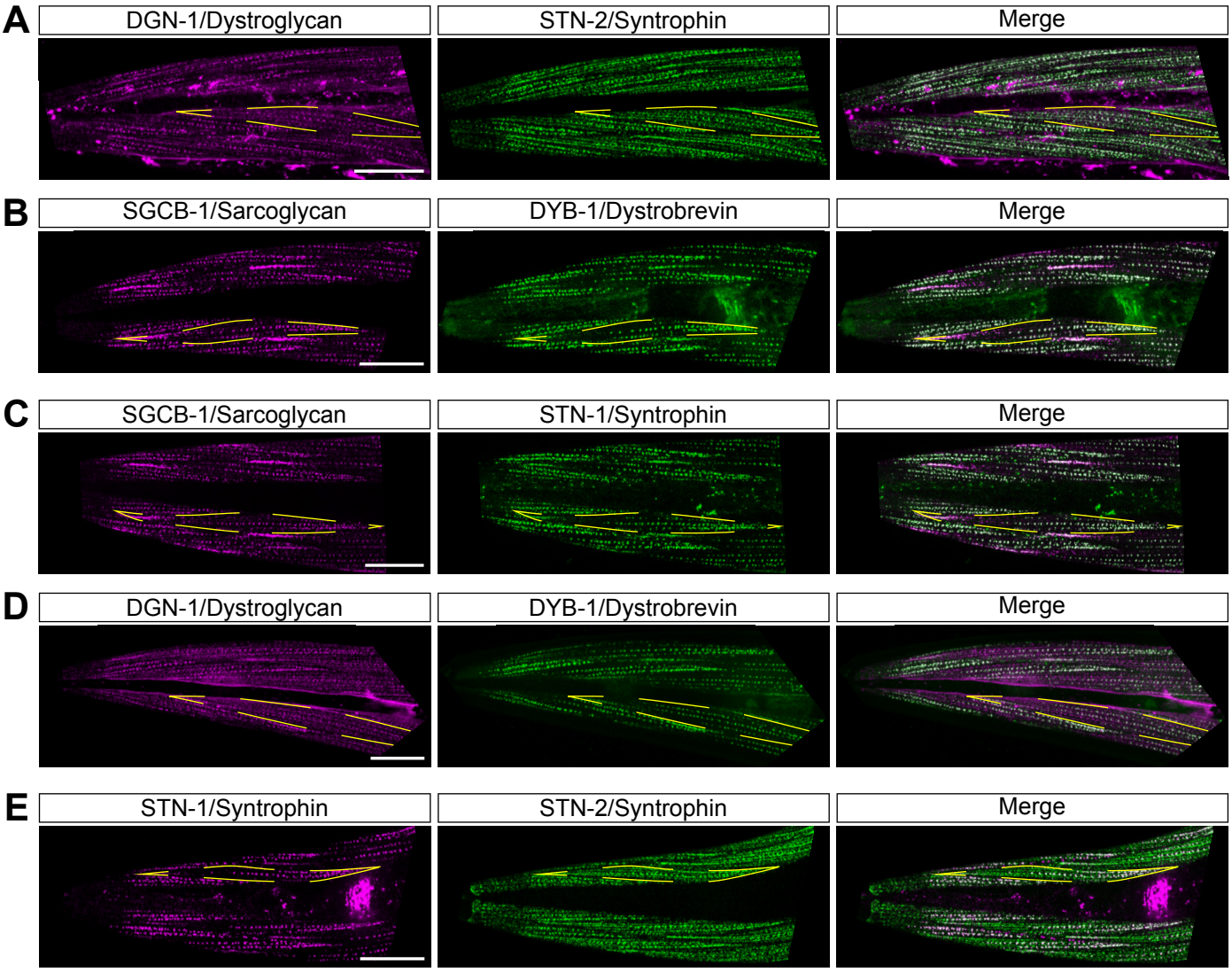

A
DGN-1/Dystroglycan
STN-2/Syntrophin
Merge
B
SGCB-1/Sarcoglycan
DYB-1/Dystrobrevin
Merge
C
SGCB-1/Sarcoglycan
STN-1/Syntrophin
Merge
D
DGN-1/Dystroglycan
DYB-1/Dystrobrevin
Merge
E
STN-1/Syntrophin
STN-2/Syntrophin
Merge

Figure S4

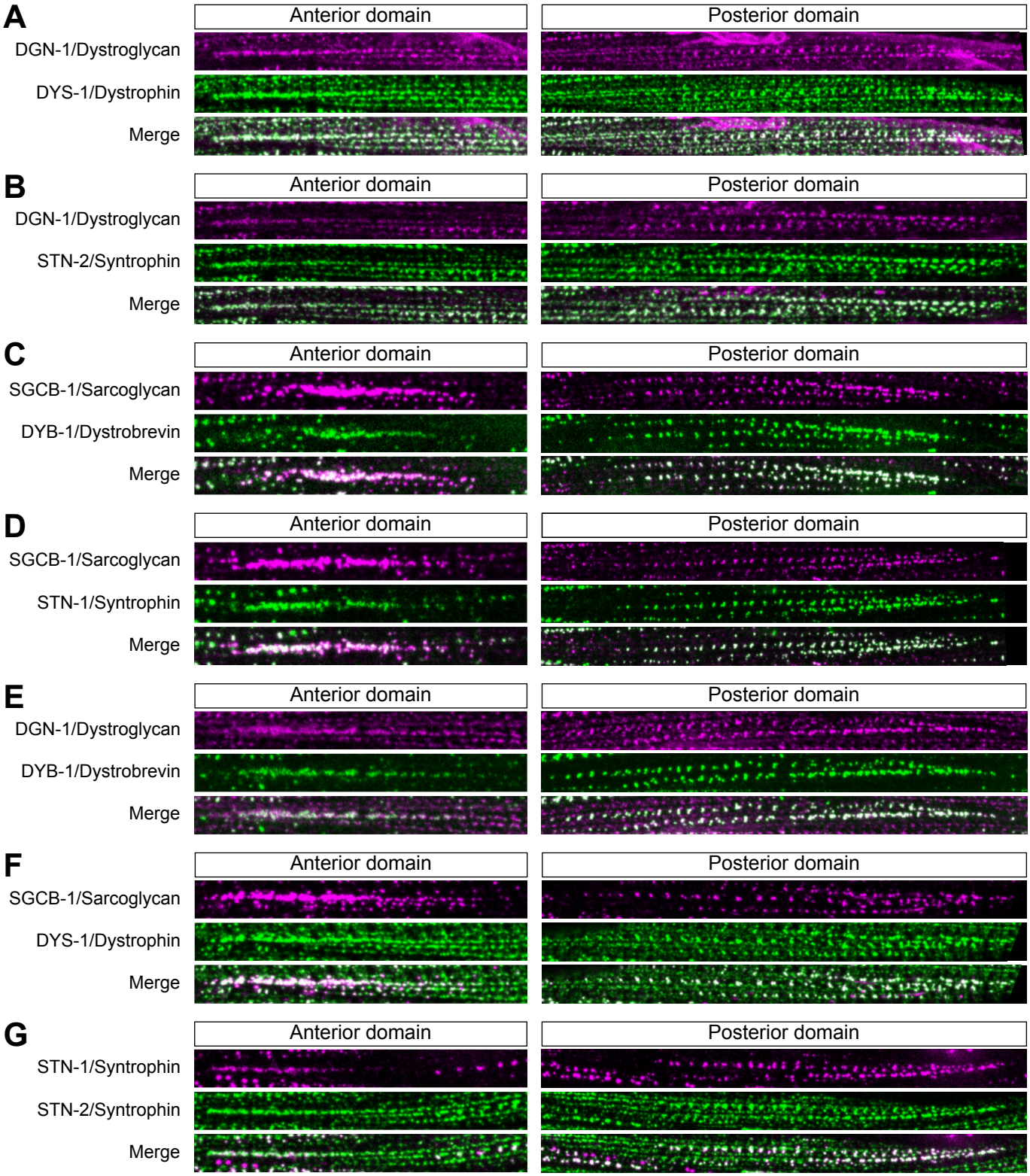

A
Anterior domain
Posterior domain
DGN-1/Dystroglycan
DYS-1/Dystrophin
Merge
B
Anterior domain
Posterior domain
DGN-1/Dystroglycan
STN-2/Syntrophin
Merge
C
Anterior domain
Posterior domain
SGCB-1/Sarcoglycan
DYB-1/Dystrobrevin
Merge
D
Anterior domain
Posterior domain
SGCB-1/Sarcoglycan
STN-1/Syntrophin
Merge
E
Anterior domain
Posterior domain
DGN-1/Dystroglycan
DYB-1/Dystrobrevin
Merge
F
Anterior domain
Posterior domain
SGCB-1/Sarcoglycan
DYS-1/Dystrophin
Merge
G
Anterior domain
Posterior domain
STN-1/Syntrophin
STN-2/Syntrophin
Merge

Figure S5

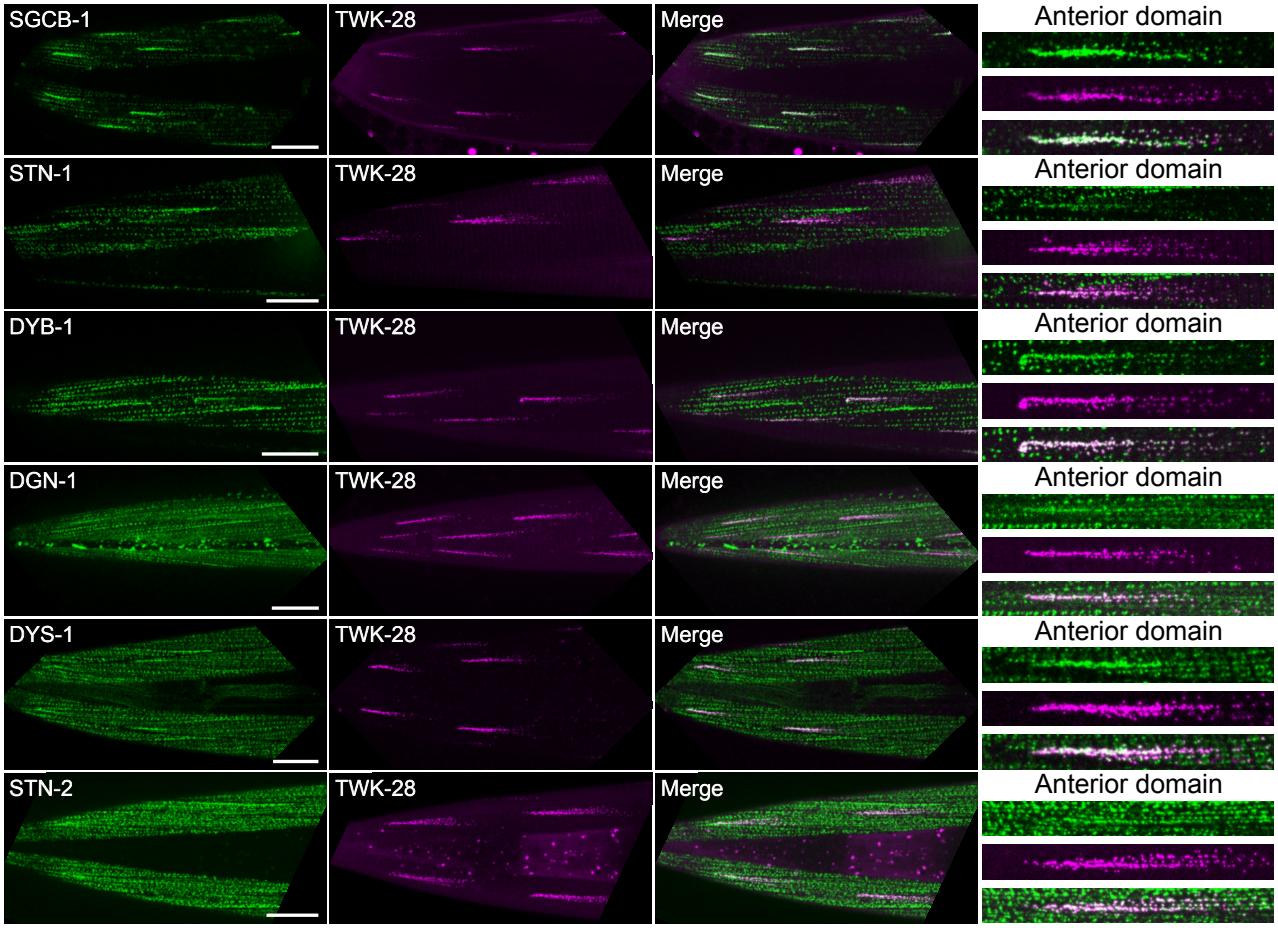

SGCB-1
TWK-28
Merge
Anterior domain
STN-1
TWK-28
Merge
Anterior domain
DYB-1
TWK-28
Merge
Anterior domain
DGN-1
TWK-28
Merge
Anterior domain
DYS-1
TWK-28
Merge
Anterior domain
STN-2
TWK-28
Merge
Anterior domain

Figure S6

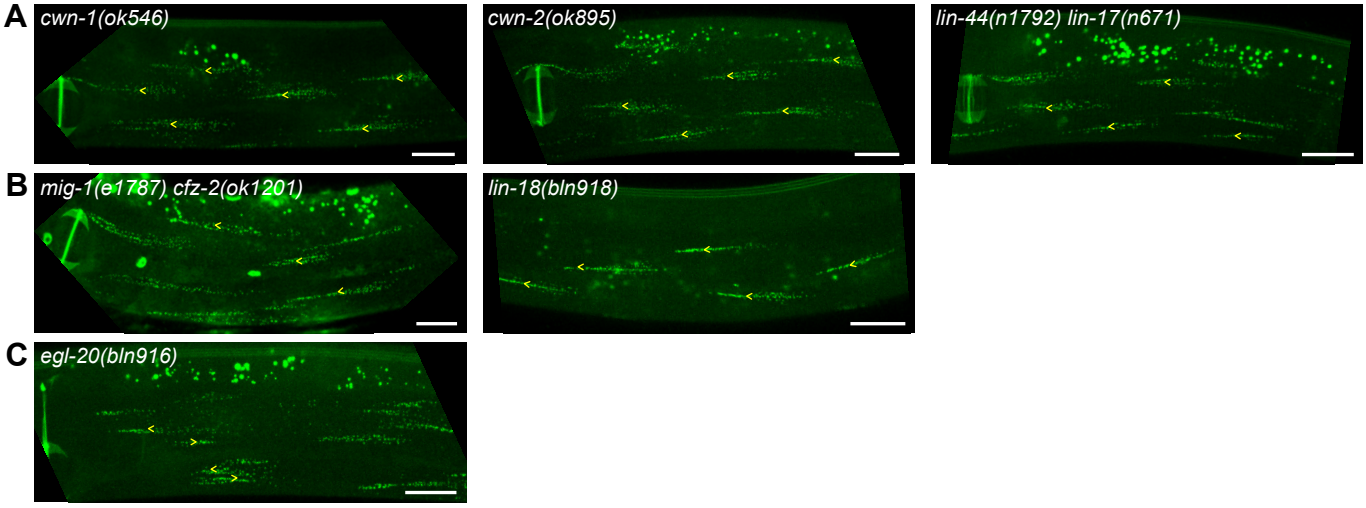
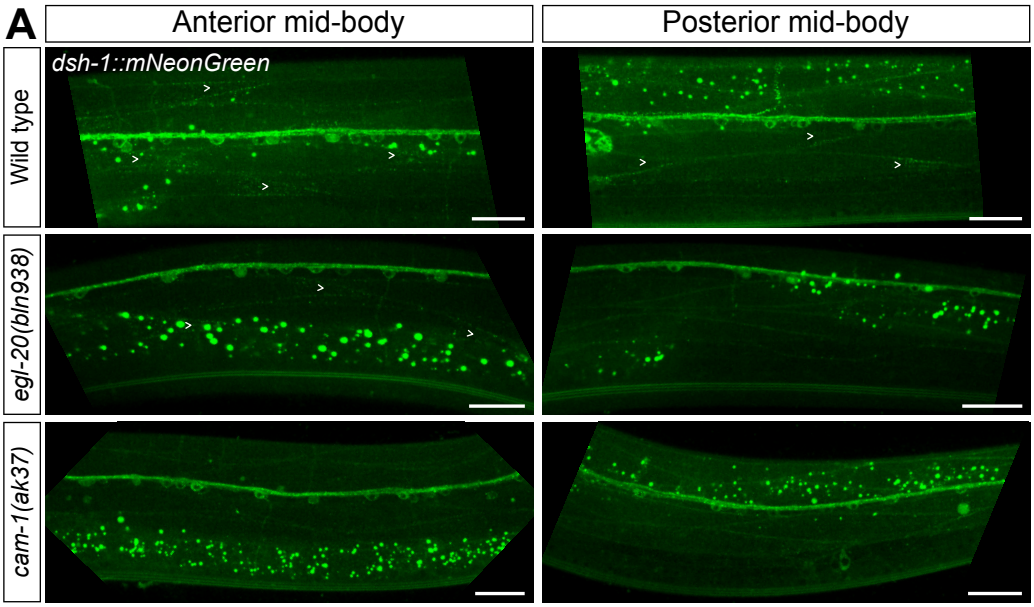
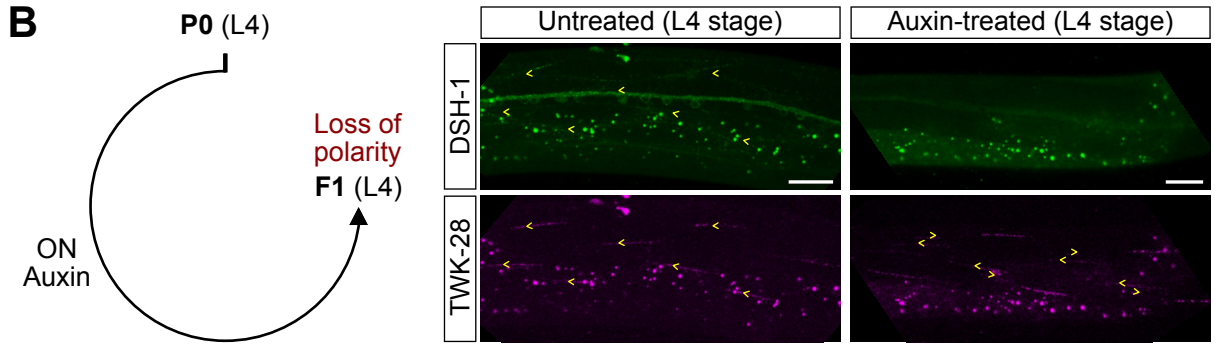

A
cwn-1(ok546)
cwn-2(ok895)
lin-44(n1792) lin-17(n671)
B
mig-1(e1787) cfz-2(ok1201)
lin-18(bln918)
C
egl-20(bln916)

Figure S7


A
Anterior mid-body
Posterior mid-body
Wild type
dsh-1::mNeonGreen
egl-20(bln938)
cam-1(ak37)


B
P0 (L4)
Loss of polarity
F1 (L4)
ON Auxin
Untreated (L4 stage)
Auxin-treated (L4 stage)
DSH-1
TWK-28