Syndecan defines precise spindle orientation by modulating Wnt signaling in *C. elegans*

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ABSTRACT

Wnt signals orient mitotic spindles in development, but it remains unclear how Wnt signaling is spatially controlled to achieve precise spindle orientation. Here, we show that *C. elegans* syndecan (SDN-1) is required for precise orientation of a mitotic spindle in response to a Wnt cue. We find that SDN-1 is the predominant heparan sulfate (HS) proteoglycan in the early *C. elegans* embryo, and that loss of HS biosynthesis or of SDN-1 core protein results in misorientation of the spindle of the ABar blastomere. The ABar and EMS spindles both reorient in response to Wnt signals, but only ABar spindle reorientation is dependent on a new cell contact and on HS and SDN-1. SDN-1 transiently accumulates on the ABar surface as it contacts C, and is required for local concentration of Dishevelled (MIG-5) in the ABar cortex adjacent to C. These findings establish a new role for syndecan in Wnt-dependent spindle orientation.

KEY WORDS: Heparan sulfate, Proteoglycan, Embryo, Endocytosis, Dishevelled, *C. elegans*

INTRODUCTION

Mitotic spindle orientation defines cell division orientation and plays crucial roles in animal development and tissue homeostasis (Gillies and Cabernub, 2011; Inaba and Yamashita, 2012; Lu and Johnston, 2013; Morin and Bellaiche, 2011; Siller and Doe, 2009). Aberrant cell division orientation is associated with neurological diseases and cancer (Noatynska et al., 2012; Pease and Timplauer, 2011). Cells can orient their spindles according to default intrinsic rules, or in response to external cues. Wnt/β-catenin signaling is a widespread extrinsic cue for mitotic spindle orientation (Segalen and Bellaiche, 2009). In the *C. elegans* early embryo, mitotic spindle orientation in the EMS and ABar blastomeres is regulated by directional Wnt/MOM-2 signals, acting in parallel to a Src-dependent pathway (Hardin and King, 2008; Park and Priest, 2003). EMS cell fate determination is also dependent on these pathways, in which a Wnt signal pathway regulates transcription in a β-catenin/WRM-1-dependent manner (Sawa and Korswagen, 2009). Interaction of HSPGs with Frizzled (Fz) receptors and Wnt ligands is thought to promote internalization of receptor-ligand complexes, which in turn either positively or negatively regulate extracellular Wnt ligand distribution and Wnt signaling (Gagliardi et al., 2008; Okhawara et al., 2011). This biphasic activity of HSPGs is influenced by the ratio of ligand to receptor and co-receptors (Yan et al., 2009), the stability of free ligand (Kleinschmit et al., 2013) and possibly by specific HS structures (Ai et al., 2003). Additionally, the HSPG core protein can modulate signaling independently of or in conjunction with its HS side chains. In *C. elegans*, HS synthesis is essential for embryonic morphogenesis (Katagawa et al., 2007). However, the cellular role of HSPGs in embryonic morphogenesis has remained unclear. The membrane-spanning HSPG syndecan (SDN-1) has been identified as a negative regulator of Wnt/eegl-20 in distal tip cell migration (Schwabik et al., 2009), but it is not known whether HSPGs are involved in other Wnt-dependent processes. Here, we show that *C. elegans* embryos express the HSPG syndecan/SDN-1 from the one-cell stage onwards. We show that SDN-1 is required for a specific Wnt-dependent spindle orientation signal in the context of a newly formed cell-cell contact. Our results indicate that HSPGs can regulate precise spindle orientation by modulating Wnt signaling.

RESULTS

SDN-1 is the predominant HSPG core protein in the early embryo

To understand the roles of HSPGs in early *C. elegans* embryogenesis, we first examined the expression of total HSPGs by immunostaining. The antibody 3G10 (David et al., 1992; Minniti et al., 2004) recognizes stubs of HS chains formed by heparitinase cleavage, allowing the detection of all HS-modified proteins. We detected total HS (3G10) in one-cell stage embryos, which displayed patchy but specific 3G10 staining on the cell surface (Fig. 1Aa); at later stages, HS was concentrated at cell contacts (Fig. 1Ab-f,B; supplementary material Fig. S1A). We did not detect 3G10 staining in the absence of heparitinase treatment (Fig. 1B; data not shown). We noted that in two- and four-cell stage embryos, expression of total HS was higher in anterior cells (AB and ABA/
ABp) than in posterior cells (P₁ and P₂/EMS) (Fig. 1Ab,c). At the eight-cell stage (∼30 min post first cleavage, pfc), we detected HS on the surfaces of all blastomeres. HS was distributed at most cell-cell interfaces, but was highly concentrated at the contact site between ABar and C (Figs 1Ad and 2). We noticed that during ABar mitosis, HS localized to an intracellular punctum in ABar, close to the ABar-C cell contact site (Fig. 1Ae). Expression of HS gradually increased during embryogenesis, and by comma stage (395 min pfc) was visible on the surface of almost all cells (supplementary material Fig. S1B).

C. elegans encodes multiple HSPG core proteins, of which syndecan/SDN-1 and glypican/GPN-1 are HS modified (Hudson et al., 2006; Minniti et al., 2004). To determine which core proteins are expressed in early embryos, we examined total HS expression in embryos lacking known HSPG core proteins using null or strong loss-of-function mutants. UNC-52/perlecan was not expressed in early embryos, as judged by MH3 antibody staining (not shown), confirming previous results (Mullen et al., 1999). 3G10 staining of gpn-1(ok377), lon-2(e678) or agrin/agr-1(tm2051) embryos was indistinguishable from that of wild type (not shown). By contrast, 3G10 staining was undetectable in sdn-1(zh20) or sdn-1(ok449) embryos prior to the AB₁₆ (28-cell) stage (Fig. 1C,D). In sdn-1(zh20) embryos, we detected 3G10 staining in a few posterior cells at the AB₃₂ stage, suggesting that expression of additional HSPGs begins between the AB₁₆ and AB₃₂ stages. We generated a functional SDN-1::GFP translational reporter under the control of its own promoter and 3′UTR ([sdn-1;juSi119[Psdn-1-SDN-1::GFP-sdn-1 3′UTR]]a at the eight-cell stage; anti-GFP immunostaining is shown on the right. Scale bars: 10 μm. All images, except in E, are of embryos treated with heparitinase.

Fig. 1. Syndecan/SDN-1 is the predominant heparan sulfate proteoglycan in the early C. elegans embryo. (A) Expression of total HS in the wild-type early C. elegans embryo, as detected by staining with the anti-HS stub antibody 3G10; DAPI counterstain for nuclei. HS staining (green) is detectable at the one-cell stage (a; surrounded by a yellow dashed line); at the two-cell stage (b), HS is significantly enriched in AB compared with P₁. At the early eight-cell stage (d), HS accumulates on the contact site between ABar and C (white arrowhead); by the late eight-cell stage (e,e′), HS internalizes as a ∼0.5 μm diameter particle in ABar (red arrowhead). At the AB₁₆-cell stage (f), HS is widespread in the embryo. (B) Quantification of cell surface 3G10 staining intensity in wild type, with (black or red bars) or without (gray bars) heparitinase treatment, and enrichment of HS immunostaining at the AB-P₁ cell contact and the ABar-C contact; data are arbitrary units (mean±s.e.m.). Red bars represent data obtained from cell-cell interfaces; black and gray bars represent data from regions away from cell contacts. Numbers in the bars represent the number of embryos. Student’s t-test was used to analyze data: *P<0.05; ***P<0.001. (C,D) Anti-HS (3G10) immunostaining is absent from sdn-1(zh20) embryos prior to the AB₁₆-cell stage. Confocal projections of 3G10 staining (C) and quantification (D) of cell surface staining on the contact-free cell surface of ABar in wild type and sdn-1 mutants, and in embryos untreated with heparitinase (gray bar). One-way ANOVA and Tukey’s test were used to analyze data.

(E) Expression of SDN-1::GFP under the control of the sdn-1 promoter and 3′ UTR [sdn-1(zh20); juSi119[Psdn-1-SDN-1::GFP-sdn-1 3′UTR]]a at the eight-cell stage; anti-GFP immunostaining is shown on the right. Scale bars: 10 μm. All images, except in E, are of embryos treated with heparitinase.
protein expressed in early embryos, and that SDN-1::GFP reflects endogenous SDN-1/HS expression.

**HS synthesis and SDN-1 are required for proper ABar division orientation**

SDN-1 is required for numerous aspects of post-embryonic development and morphology (Rhiner et al., 2005), and in ventral cleft closure during mid-embryogenesis (Hudson et al., 2006); however, it had not previously been implicated in early embryonic development. To assess the role of SDN-1 expression in the early embryo, we examined *sdn-1(zh20)* null mutant embryos using time-lapse DIC microscopy. *sdn-1* mutants developed normally until the eight-cell stage, and displayed normal orientation of the EMS division (Figs 2 and 3A,B; supplementary material Movies 1 and 2). However, the division axis of ABar was consistently misoriented in *sdn-1(zh20)*. We observed similar ABar division orientation defects in the HS synthesis mutants *rib-1(tm516), rib-2(tm710)* and *hst-1(ok1068)* (Fig. 3C, supplementary material Movie 3; data not shown). In wild-type embryos, the ABar spindle undergoes a distinctive rotation so that ABar divides orthogonally to the axes of division of the other AB granddaughters: ABal, ABpl and ABpr. In HS synthesis and *sdn-1* mutants, ABar typically divided parallel to ABpr [five out of five embryos each for *rib-1(tm516), hst-1(ok1068)* and *rib-2(tm710)*; eight out of 10 for *sdn-1(zh20)*]. These observations used visual estimation of the division axis or of the positions of daughter cells. To quantitate ABar division orientation more precisely, we used the NucleiTracker4D software (Giumurcescu et al., 2012) to track histone-GFP labeled nuclei in 4D movies (Fig. 3D,E). This approach allowed us to plot 3D angles between the ABar and ABpr division axes, based on the positions of the GFP-labeled daughter nuclei immediately after chromosome segregation. This analysis revealed that, in wild-type embryos, the ABar-ABpr division axes were oriented at an angle of 91.5 ± 12.9° (mean ± s.d., n=21) 1 min after chromosome segregation (Fig. 3F; supplementary material Fig. S2A,B), consistent with our DIC microscopy observations and with previous studies (Walston et al., 2004). In the HS synthesis and *sdn-1* mutants, the angle between ABar and ABpr division was significantly decreased (Fig. 3F,G; supplementary material Fig. S2B), as was the ABar-ABal division angle (supplementary material Fig. S2C). By contrast, the angle between ABal and ABpl division axes was normal (Fig. 3F,G; supplementary material Fig. S2D). *sdn-1(RNAi)* embryos displayed a weaker ABar orientation defect (Fig. 3G). The division orientation defects of *sdn-1(zh20)* were fully rescued by SDN-1::GFP expressed under its endogenous control elements (91.0° ± 12.0, n=18, not shown). A mutant with a deletion in another HSPG core protein gene, *gpm-1*, showed normal ABar cell division orientation. *sdn-1 gpm-1* double mutants showed a slightly more severe ABar orientation defect than did *sdn-1* single mutants, although this was not statistically significant (Fig. 3G). As the ABar orientation defect of HS synthesis mutants is significantly more severe than that of *sdn-1*, additional HSPGs may contribute to ABar orientation. Taking our expression and mutant results together, we conclude that SDN-1 is the predominant HSPG required for normal orientation of the ABar cell division.

Previous embryological and genetic studies established that the mitotic spindle of ABar rotates in response to contact with and signaling from the C blastomere (Fig. 2) (Walston et al., 2004). The ABar-C contact is partly dependent on Wnt/MOM-2 (Pohl and Bao, 2010) and on the cell adhesion molecules L1CAM/SAX-7 and cadherin/HMR-1 (Grana et al., 2010). Using DIC microscopy, we observed that the ABar and C blastomeres form normal contacts in *sdn-1* mutant embryos, starting 5.6±0.4 min (mean±s.d., n=10) before the onset of anaphase in ABar, similar to the wild type (6.1±0.3 min, n=10). Likewise the ABar-C cell contact appeared normal in *rib-1* mutant embryos (supplementary material Movie 3). We conclude that neither SDN-1 nor HS are required for ABar to contact C, suggesting that the spindle orientation defects in these mutants arise from a failure in signaling.

**SDN-1 is required for proper orientation of the mitotic spindle in ABar**

To analyze ABar spindle orientation directly in these mutants, we labeled microtubules using GFP::TBB-2 (*ofy-1*) (Strome et al., 2001) and quantitated spindle dynamics, using NucleiTracker 4D software to track centrosomal asters. Previous studies indicated that the wild-type ABar spindle initially aligns parallel to the ABpr spindle, after which the aster closest to the ABar-C contact rotates to adopt the proper orientation before mitosis (Fig. 2) (Walston...
et al., 2004). Under our imaging conditions, in which we use bead mounting to reduce embryo compression during imaging (Giurumescu et al., 2012), we found that in wild-type embryos ABar astral arrays were initially set up in variable orientations, and by metaphase become oriented towards the ABar-C contact independent of their initial orientation (Fig. 4A,B; supplementary material Movie 4).

To assess this observation quantitatively, we measured ABar spindle orientation relative to the AP axis of the embryo. In the wild type, the ABar spindle becomes oriented with the posterior aster dorsal and the anterior ventral. The ABar spindle axis also has a large left-right component, in that the posterior pole lies on the left-hand side of the embryo. In \textit{sdn-1} mutants, the ABar spindle is initially variable in the AP, DV and LR planes, and rotates to an orientation that is consistent with respect to the AP-DV axis, but variable in the LR axis. Thus, ABar angles measured relative to the AP axis are initially variable but converge on an angle that is smaller than in the wild type (Fig. 4C-F). We also measured ABar orientation relative to the other AB granddaughters; ABar angles measured relative to the ABpr spindles were more variable in \textit{sdn-1} mutants because of the larger LR component of these angles (data not shown). The mean orientation of the ABar spindle in \textit{sdn-1} mutants was affected throughout the ABar cell cycle (Fig. 4E; supplementary material Movie 5). The abnormal spindle dynamics in \textit{sdn-1(zh20)} were rescued by overexpression of SDN-1::GFP driven by the germline-specific \textit{mex-5} promoter and the germline-permissive \textit{tbb-2} 3′UTR (Merritt et al., 2008) (Fig. 4E). We also observed that overexpression of SDN-1::GFP resulted in significantly lower variance in the orientation of ABar astral arrays throughout mitosis without affecting mean orientation (Fig. 4F). These observations suggest SDN-1 is not required for spindle rotation per se, but modulates a cue that orients the astral microtubule array.

SDN-1 acts in a Wnt-dependent spindle orientation pathway

ABar spindle orientation requires two partly redundant signaling pathways: a Wnt pathway involving Wnt/MOM-2, Fz/MOM-5 and the Dishevelled proteins DSH-2 and MIG-5; and a receptor tyrosine kinase MES-1 that acts via Src/SRC-1 (Fig. 5A) (Walston et al., 2004). HSPGs, including syndecans, regulate Wnt signaling in many contexts (Lin, 2004; Munoz et al., 2006; Ohkawara et al., 2011), yet the involvement of HSPGs in Wnt-dependent spindle orientation has not been reported. Syndecan has also been implicated in Src-dependent stabilization of focal adhesions in fibroblasts (Morgan et al., 2013). To determine whether SDN-1
acts in the Wnt or Src spindle orientation pathways, we inhibited dsh-2 and mig-5 or src-1 by RNAi in the wild-type and sdn-1(zh20) mutant backgrounds. When Wnt signaling was inhibited by dsh-2 mig-5 double RNAi, the chromosome segregation angle between ABar and ABpr (Fig. 5B,C) but not between ABal and ABpl (not shown), was strongly reduced, consistent with previous reports (Walston et al., 2004). src-1 RNAi had a similar, although weaker, effect (Fig. 5D). In sdn-1(zh20) src-1(RNAi) embryos, the angle between ABar and ABpr division was significantly reduced compared with src-1(RNAi) alone (Fig. 5D). By contrast, sdn-1(zh20) did not further enhance the ABar-ABpr division angle defect in dsh-2 mig-5 double RNAi (Fig. 5C). The HS synthesis mutant rib-1(m516) also displayed synergistic effects with src-1(RNAi), but not with dsh-2 mig-5 double RNAi (Fig. 5C). This pattern of synergism suggests HS-modified SDN-1 acts in parallel to the Src-dependent spindle orientation pathway, likely in the Wnt-dependent pathway. In addition, ABar spindle dynamics in sdn-1(zh20) dsh-2 mig-5 double RNAi resembled those of dsh-2 mig-5 double RNAi (Fig. 5E,F). These results suggest that the variable spindle rotation in wild type and sdn-1 mutant depends on Dishevelled activity.

**SDN-1 accumulates on the ABar protrusion and then at the ABar-C contact, and is then bi-directionally internalized into ABar or C**

To address how SDN-1 modulates Wnt-dependent spindle orientation, we analyzed the dynamics of SDN-1 subcellular localization. The SDN-1::GFP transgene expressed under endogenous control elements (Fig. 1E) rescued the ABar division orientation defects of sdn-1(zh20) (supplementary material Fig. S2C,D), but its fluorescence level was too low for live imaging. For live imaging, we overexpressed SDN-1::GFP using the germline-specific mex-5 promoter. *p*<0.01. Pmex-5-SDN-1::GFP accumulated around the ABar protrusion and then at the ABar-C contact (Fig. 5D). This pattern of localization with cell division dynamics, we also expressed SDN-1::GFP with HIS-48::mCherry (supplementary material Movie 6). To correlate SDN-1::GFP localization with cell division dynamics, we also expressed SDN-1::GFP and an integrated single-copy transgene juSi98[mex-5-SDN-1::GFP-tbb-2 3’UTR], which overexpresses SDN-1::GFP in the early embryo, respectively. (F) Bar graphs showing SDN-1::GFP standard deviation of indicated genotypes at t=−5 min (early prophase) and 0 min (onset of anaphase). The variance of ABar spindle orientation in the AP/DV plane is significantly higher in sdn-1 than in wild type at the onset of anaphase, whereas it is smaller in sdn-1(++) (F test: *P*<0.05). The ABar spindle angle is calculated by tracking centrosomal asters (TBB-2::GFP) from −5.5 min prior to onset of ABar anaphase.
that SDN-1 accumulation on ABar has ABpr and/or ABpl immediately underneath it (Fig. 6A, right). Subsequently, SDN-1::GFP formed a ∼0.5 µm diameter punctum either in ABar (13 out of 18, Fig. 6B) or in C (four out of 18, Fig. 6C, supplementary material Movie 7). These puncta may reflect internalization of the entire SDN-1 protein, as we observed similar structures using 3G10 immunostaining, which reflects endogenous SDN-1 HS chains (Fig. 1Ae). As the behavior of SDN-1::GFP-enriched puncta on ABar resembled that of midbody remnants (Singh and Pohl, 2014), we tested whether endogenous HS localizes to midbody remnants using the midbody marker ZEN-4::GFP. At the eight-cell stage, although the midbody remnant in P2 (originating from P1) did not contain HS, the midbody remnant localizing to the ABar-C interface showed strong HS expression (Fig. 6E), suggesting SDN-1 associates with a subset of midbody remnants.

How is SDN-1 enriched in the protrusion of the ABar blastomere? Syndecans are clustered by extracellular ligand stimulation through their HS side chains (Tkachenko and Simons, 2002). SDN-1 contains three potential glycosaminoglycan (GAG) attachment sites, i.e. Ser-Gly motifs; based on sequence context, only the first two Ser-Gly motifs are likely to be modified (Minniti et al., 2004). We expressed mutant forms of SDN-1, in which the first two putative GAG attachment sites were mutated (S71A, S86A or 2×S>A). When expressed under the control of the mex-5 promoter, SDN-1::GFP induced strong expression of total HS in sdn-1(zh20) early embryos (supplementary material Fig. S4C). SDN-1(2×S>A)::GFP resulted in much weaker but detectable HS immunoreactivity in sdn-1(zh20) early embryos (supplementary material Fig. S4D), suggesting that although these GAG attachment sites are predominant, they may not account for all early embryonic HS. As the third potential GAG attachment site (S214) does not appear to contribute to early embryonic HS (supplementary material Fig. S4E), we conclude that S71 and S86 are the major HS modified sites in SDN-1, and that other sources of HS may account for the residual HS detected in these embryos (see Discussion). SDN-1(2×S>A)::GFP enrichment on the ABar protrusion before cell contact was delayed relative to wild type (−0.7±0.2 min before cell contact, n=10, supplementary material Fig. S5B). The SDN-1(2×S>A)::GFP accumulation remained 12.2±1.7 min after contact with C (n=10, supplementary material Fig. S5B).

We next addressed whether the intracellular domain of SDN-1 is required for SDN-1 dynamics. To test this, we examined GFP tagged-
SDN-1 lacking its cytoplasmic domain (SDN-1ΔC). Although SDN-1ΔC::GFP accumulated on the tip of ABar before cell contact (supplementary material Fig. S5B), endocytosis of this mutant form was delayed and less frequent compared with wild-type SDN-1::GFP (supplementary material Fig. S5B). We attempted to express SDN-1 lacking both its cytoplasmic domain and GAG attachment sites (S71A, S86A). However, this mutant form of SDN-1::GFP was not correctly localized on the cell surface (supplementary material Fig. S5A).

To address the importance of GAG modification and cytoplasmic domain of SDN-1, we next examined whether SDN-1ΔC::GFP and SDN-1(2×S>A)::GFP expressed under the control of the sdn-1 promoter and 3′ UTR could rescue abnormal ABar spindle dynamics in sdn-1(zh20). SDN-1::GFP (wild type) rescued both the variable initial spindle orientation and the continuously misoriented ABar spindle phenotypes of the sdn-1(zh20) mutant (supplementary material Fig. S5A). SDN-1ΔC::GFP rescued the variable initial spindle orientation, but failed to fully rescue the continuous misorientation of the ABar spindle, suggesting the SDN-1 cytoplasmic domain is required for precise spindle orientation regulated by Wnt. Correlating with its ability to restore low levels of HS expression, SDN-1(2×S>A)::GFP rescued both sdn-1 phenotypes.

**Wnt/MOM-2 defines the site of SDN-1 accumulation, which in turn is required for local accumulation of MIG-5/Dsh**

To examine the effect of Wnt signaling on SDN-1 accumulation, we tested SDN-1::GFP dynamics in mom-2 RNAi and dsh-2 mig-5 double RNAi-treated embryos. Depletion of mom-2 by RNAi eliminated SDN-1::GFP accumulation on ABar; instead, we observed premature SDN-1::GFP accumulation on C or ABpl during early prophase of ABar (Fig. 7B,C). We did not observe premature accumulation of SDN-1::GFP in dsh-2 mig-5 double RNAi embryos (Fig. 7A,C), suggesting that MOM-2 engagement rather than downstream Wnt signal transduction defines the location of the site of SDN-1::GFP accumulation.
observed before cell contact. MIG-5::GFP accumulation on the ABar-C contact site was reduced in embryos treated with mom-2 RNAi, even in embryos where ABar-C contact occurred normally, suggesting that local accumulation of MIG-5::GFP might reflect activation of Wnt signaling rather than contact itself (Fig. 7E,G). Importantly, MIG-5::GFP accumulation on the ABar-C contact site was significantly reduced in sdn-1(zh20) (Fig. 7F,G), indicating that SDN-1 is required for MIG-5 accumulation. Dishevelled overexpression has been shown to rescue defective convergent extension caused by loss of glypicans 4 and syndecan 4 in Xenopus (Munoz et al., 2006; Ohkawara et al., 2003). However, overexpression of MIG-5::GFP (4- to 5-fold overexpression; data not shown) did not rescue the ABar misorientation phenotype in sdn-1(zh20) (Fig. 7H), suggesting that SDN-1 localization does not simply enhance Wnt signaling but provides a positional cue.

**DISCUSSION**

This study reveals a highly specific requirement for syndecan in Wnt-dependent mitotic spindle regulation. Our results support a model in which SDN-1 functions in the Wnt signaling pathway, either at the level of Wnt/MOM-2 or Fz/MOM-5, to promote ABar spindle reorientation. Localization of SDN-1 on ABar requires MOM-2, and SDN-1 is required for localization of Dsh/MIG-5. Moreover, the difference between the sdn-1 phenotype and the dsh-2 mig-5 phenotype can be explained if SDN-1 restricts Wnt signaling. Speculatively, syndecan/SDN-1 on the ABar surface might concentrate either Wnt/MOM-2 or its receptor Fz/MOM-5. The HS side chains of SDN-1 might allow this process to begin prior to physical contact with the C blastomere, triggering SDN-1 clustering (Fig. 8). SDN-1 accumulation could concentrate Wnt/MOM-2 onto the ABar protrusion, which in turn weakly orients the mitotic spindle in ABar towards C. In addition, we have analyzed ABar spindle dynamics in sdn-1(zh20) dsh-2 mig-5 double RNAi, and find that they resemble dsh-2 mig-5 double RNAi, i.e. no spindle rotation. This epistasis test shows that the variable rotation in sdn-1 mutants requires Dishevelled activity, consistent with our model. At present, reagents to visualize Wnts or their receptors in the early embryo are not available. Our attempts to generate MOM-2 transgenic animals have so far been unsuccessful, and antibodies to MOM-2 have not been generated. MOM-5::GFP expression is not detectable in the early embryo (Park et al., 2004). Wnt ligands and receptors may be expressed transiently or at low levels in the early embryo, necessitating the involvement of accessory proteins such as SDN-1. We do not yet know whether SDN-1 function is required in the Wnt-sending or Wnt-receiving cell, or both, and we cannot yet exclude models in which HSPG/MOM-2 signaling but provides a positional cue.

Finally, to examine the relevance of SDN-1 accumulation to downstream Wnt signaling, we followed the dynamics of a functional MIG-5::GFP (Wu and Herman, 2007). MIG-5::GFP expressed under the control of the mec-5 promoter was enriched at the cortex in all cells of the early embryo, consistent with previous studies (Walston et al., 2006). In ABar, MIG-5::GFP accumulated similarly to SDN-1::GFP at the contact site with C (Fig. 7D; supplementary material Movie 8); however, unlike SDN-1::GFP, MIG-5::GFP accumulation was not

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**Fig. 7. Accumulation of SDN-1::GFP on the ABar-C contact site requires MOM-2/Wnt; SDN-1 is required for accumulation of MIG-5::GFP at the contact site.** (A,B) In dsh-2 mig-5 double RNAi embryos SDN-1::GFP accumulates on ABar (red arrowhead) as in the wild type (compare with Fig. 6A). mom-2 RNAi resulted in failure in SDN-1::GFP accumulation on the ABar contact site; instead, premature SDN-1::GFP accumulated on the ABl-pl contact site before ABar contacts C, quantified in C. (D-F) 3D projections of embryos expressing MIG-5::GFP in wild-type (D), mom-2 RNAi (E) and sdn-1(zh20) (F) are shown. The ABar-C contact site is indicated by magenta arrowheads. In wild-type embryos, MIG-5::GFP accumulated after formation of the ABar-C contact (see also supplementary material Movie 8); mom-2 RNAi and sdn-1(zh20) mutant embryos showed normal ABar-C contacts but did not accumulate MIG-5::GFP. (G) Quantification of MIG-5::GFP on the ABar-C contact site, normalized to PH::mCherry and expressed as a ratio of GFP intensity in the ABar-C-ABpl contact to the ABar-ABpl-ABal contact. Data are means ± s.e.m.; **P<0.01. (H) Overexpression of MIG-5 (juSi138[Pmex-5-MIG-5::GFP-tbb-2 3’ UTR]) did not suppress the sdn-1 division orientation defect. The ABar division angle is defined by the orientation of daughter nuclei (HIS-72::GFP) 1 min after the onset of ABar anaphase.
more able to orient its spindle along its long axis (Hertwig’s rule) even in the absence of positional cues (Goldstein, 1995).

Cytokinetic midbody remnants have been recently shown to contribute to orientation of mitotic spindles in the early embryo (Singh and Pohl, 2014). We found that total HS colocalized with midbody remnants on the ABar-C contact site. Some, but not all, midbody remnants contain total HS, suggesting that HSPGs may be selectively recruited into midbodies. Based on its location, the midbody remnant containing HS at the 8-cell stage seems to be that generated from the division of ABp. A previous study demonstrated that the midbody remnant from ABp is inherited by MS (Singh and Pohl, 2014), different from the dynamics of SDN-1. Possibly, SDN-1 is dissociated from the midbody remnant and is internalized into ABar or C during or after ABar mitosis.

An unexpected finding in this study is that SDN-1::GFP is apparently endocytosed into the signaling or receiving cells after spindle reorientation is complete. Endocytosis can either positively or negatively regulate Wnt signaling (Gagliardi et al., 2008). In Xenopus, syndecan 4 promotes Wnt/PCP signaling by inducing clathrin-mediated endocytosis of Rsport, a positive Wnt modulator (Ohkawara et al., 2011). SDN-1 endocytosis in ABar might promote establishment or maintenance of the external cue for mitotic spindle orientation by recruiting the signaling complex to the acidic environment where the signal is activated (Niehrs and Boutros, 2010) or by sequestering unidentified negative regulators (Gagliardi et al., 2008). Alternatively, SDN-1 endocytosis by C may be involved in signal termination, as it was observed after ABar was in anaphase (Fig. 6C and supplementary material Fig. S5B). As SDN-1 is expressed at high levels in ABar and at lower levels in C, it is unclear whether the endocytosis of SDN-1 into C reflects endocytosis of SDN-1 on the C cell surface or bidirectional endocytosis of ABarp-expressed SDN-1. In any case, the variable SDN-1 dynamics after accumulation may reflect a flexible or a context-sensitive signal modulation by SDN-1, which provides robustness in oriented cell division. Our experiments demonstrated the requirement of the SDN-1 cytoplasmic domain for regulation of ABar spindle orientation and SDN-1 internalization during mitosis. However, overexpression of SDN-1 lacking its cytoplasmic domain can rescue the ABar spindle orientation phenotype (supplementary material Fig. S5C, D). This suggests that the cytoplasmic domain modulates Wnt signaling rather than playing an essential role in signal transduction. Despite our findings that HS synthesis mutants display strong ABar spindle orientation defects, overexpression of a mutant form of SDN-1 predicted to lack GAG attachments was able to rescue sdn-1 spindle orientation defects. This is reminiscent of previous findings where HSPG core proteins have been shown to...
function independently of their HS side chains (Chanana et al., 2009; Kirkpatrick et al., 2006; Williams et al., 2010; Yan et al., 2009). However, we observed weak restoration of HS expression by overexpression of these SDN-1 mutants. There are several possible explanations for this unexpected result: SDN-1 itself might be modified at additional non-canonical sites; SDN-1 overexpression may induce the expression of other HSPG core-protein(s); or overexpression of an unmodifiable SDN-1 may result in inappropriate modification of other proteins not normally HS modified. The relationship of the GAG-dependent and core-protein functions of syndecans is complex (Eriksson and Spillmann, 2012) and an important avenue for future investigation.

Syndecans might be involved in mitotic spindle orientation in other situations when the signal is transiently transmitted from nascent cell contacts, e.g. in stem cell competition for niche occupancy (Johnston, 2009; Zhao and Xi, 2010). Syndecan 1 has been shown to promote proliferation of neural progenitor cells via canonical Wnt signaling (Wang et al., 2012). Because syndecans play major roles in wound healing and cancer progression (Alexander et al., 2000; Echtermeyer et al., 2001), such a context-specific mechanism may be involved in mitotic regulation of pathologies in mammals, in addition to the well-established role of syndecans in cell migration.

**MATERIALS AND METHODS**

**C. elegans strains**

Strains used are summarized in supplementary material Table S1.

**Plasmid construction and transgene generation**

Plasmids were made by Gibson isothermal assembly (Gibson et al., 2009). To make the mutated nucleotides, site-directed mutagenesis was performed with Phusion polymerase (NEB). Mos-SCI was performed as described, using strains EG4322 and EG6699 (Frøkjær-Jensen et al., 2008). Plasmids used are summarized in supplementary material Table S2.

**Immunofluorescence**

Embryos and gonads dissected from gravid adult worms were put on slides coated with poly-L-Lysine (Sigma-Aldrich), fixed in −20°C chilled methanol for 3 min, and treated with Heparin lyase II (Sigma-Aldrich) in buffer A [50 mM sodium acetate, 5 mM CaCl₂, 0.05% Tween-20 (pH 6.0)] for 2-3 h at 37°C, then blocked with TBS containing 0.2% Tween-20 and 5% BSA. After blocking, primary antibody was added and incubated overnight at 4°C. After washing twice with TBST, secondary antibody was added and incubated at room temperature (23-25°C) for 2 h. 3G10 antibody (US Biological) and anti-mouse IgG conjugated with Alexa 488 or 594 (Invitrogen) were used at 1:1000 dilution. 3D projections were made by Zen software (Zeiss) and images processed with Adobe Photoshop.

**Measurement of total HS, SDN-1::GFP and MIG-5::GFP**

For all fluorescence intensity measurement, we used maximum intensity projections of three z-slices. The first peak obtained by a line scan (perpendicular to the cell edge) was defined as a cell border and was used to select an ROI (3×3 pixels) on the cell border, then the mean intensity was acquired. To measure the intensity of SDN-1::GFP and MIG-5::GFP, fluorescence intensity ratios were calculated by dividing the mean intensity of GFP by that of PH::mCherry. Three-dimensional projections and measurements used ImageJ.

**Confocal microscopy**

Imaging was performed as previously reported (Giurumescu et al., 2012). Briefly, living embryos were observed on LSM510 or LSM710 confocal microscopes with 100× NA 1.46 oil immersion objectives. One to three embryos obtained from four or five gravid adults were imaged in each experiment. Each data set is derived from at least six experiments. Three-dimensional stacks were acquired every minute (for HIS-72::GFP) or every 30 s (other backgrounds). Thirty-five z-sections were collected at 0.85 μm intervals for HIS-72::GFP and TBB-2::GFP imaging. To avoid photobleaching and phototoxicity, only the dorsal one-third of the embryo (10 slices, 0.85 μm intervals for SDN-1::GFP and MIG-5::GFP) was scanned. Each embryo was imaged for ~5-30 min, and we confirmed that this was not toxic to wild-type embryos. When calculating the ABx division angle from HIS-72::GFP tracking, we compared cells 1 min after the chromosomes segregate, as this was the first time point when daughter nuclei positions are automatically determined by NucleiTracker4D.

**RNAi**

PCR was performed with primer containing T7 promoter sequence on the 5’ end using N2 (Bristol) total cDNA as a template. Primers used are listed below. Double stranded RNA (dsRNA) was synthesized using Megascript kit (Ambion), and then purified through a PCR Purification kit (QIAGEN). The purified dsRNA was injected into 1 mg/ml into young adult worms 22-28 h before analysis. Primers used for synthesis are summarized in supplementary material Table S3.

**Data analysis**

Semi-automated tracking of histone-labeled nuclei (transgene zulu178) using NucleiTracker4D was performed as previously described (Giurumescu et al., 2012). We found that the NucleiTracker4D program could also track TBB-2::GFP-labeled centrosomal asters without further modifications. The angle between two vectors in 3D space is given by the inner product of two vectors:

\[
\cos \theta = \frac{\mathbf{A} \cdot \mathbf{B}}{||\mathbf{A}|| ||\mathbf{B}||}
\]

After selecting four nuclei to generate two vectors, the formula is used to measure the angle between two selected vectors. The resulting angle θ ranges from 0 to 180°. We also want to measure the relative angle between the vector of two sister nuclei and the entire embryonic axis. The anterior-posterior axis (AP vector) was manually estimated from two projection images in xy plane and zc plane. The x and y points of the AP vector were decided by selecting two points in xy projection image. z points of the AP vector are decided by an identical method in an zc projection image. The angle between the AP vector and the vector of two sister nuclei is computed using the equation above.

To visualize the angles on a circular plot, the MATLAB toolbox CircStat (http://www.jstatsoft.org/v31/i10) is used. Codes are available upon request. As the range of angles used in this study was 0-180°, the angles were statistically treated as linear data. Statistical analysis used GraphPad Prism. We used the F-test for comparison of variance, Student’s t-test and Fisher’s exact test for comparison of two independent data sets. For multiple comparisons, we used one-way analysis of variance (ANOVA) followed by a Tukey or Dunnett post-hoc test for multiple comparison.

**Acknowledgements**

We thank all the members of the Jin and Chisholm laboratories for comments, advice and encouragement. We thank Jeffrey Esko, Yishi Jin and Hiroshi Nakato for comments, Kazuya Nomura for initial work on 3G10 immunostaining, Shaohe Wang for introducing us to Gibson cloning and plasmids, Claudiu Giurumescu for initial help with NucleiTracker4D, and the Caenorhabditis Genetics Center (CGC) for strains.

**Competing interests**

The authors declare no competing financial interests.

**Author contributions**

K.D. and A.D.C. designed the experiments, analyzed data and wrote the manuscript. K.D. performed the experiments. S.K. contributed analytical tools. A.D.C., S.M. and P.C.C. supervised the project.

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Figure S1. SDN-1 and additional HSPGs contribute to total HS at mid- to late embryonic stages

(A) Montage of confocal images of anti-HS (3G10) immunostaining in 8-cell stage embryo with DAPI (blue) counter staining. Total HS is present in the cell-cell interface, which is not visible in 3D projection image in Fig. 1Ae. (B, C) Single focal plane images of 3G10 immunostaining of wild type (B) and sdn-1(zh20) embryos (C), at comma-1.5 fold stage and 2-3 fold stage. In wild type embryos, HS is expressed on cell surfaces of most cells at the comma stage. In sdn-1(zh20) HS staining is absent from most cells apart from the basement membrane of the intestine and pharynx, indicating the existence of additional HSPGs at these stages. Scale bars: 10 μm.
Figure S2. Loss of SDN-1 or HS synthesis affects ABar division orientation throughout the cell cycle

(A) Representative 3D reconstructed confocal images of HIS-72::GFP in ABar and ABpr during their divisions in wild-type embryos, color depth code. (B) Graphs of 3D division angle (y-axis) over time (x-axis, min relative to onset of anaphase). The angles (θ) of ABar division axes relative to the ABpr division axis (left panels) or to the ABal division axis (middle panels) are affected in the sdn-1(zh20) and rib-1(tm516) mutant throughout the cell cycle. The angles between ABal and ABpl division axes are unaffected (right panels). Mean angles are plotted as lines, shaded areas denote s.d.
Figure S3. Pattern of SDN-1::GFP localization during the ABar cell cycle.

Projections of confocal z-stacks of a single embryo during ABar mitosis. SDN-1::GFP initially accumulates on the ABar cell surface (red arrowhead) during interphase (8.5 min before anaphase). SDN-1::GFP remains localized as ABar and C contact in prophase (-5 min) and is internalized during metaphase (-1 min) and anaphase (0 min). Chromosomes are labeled with HIS-48::mCherry; nuclei can be seen in outline from the exclusion of cytosolic SDN-1::GFP. Because we scanned only the dorsal 1/3 of each embryo, ABAr is not visible at the onset of anaphase. Embryos expressing the tjIs57 transgene often display cytosolic mCherry aggregates in ABxx cells (arrows). Genotype: sdn-1(zh20); juSi99[Pmex-5-SDN-1::GFP-sdn-1 3'UTR]; tjIs57[Pie-1-mCherry::HIS-48]). Scale bar = 10 µm.
Figure S4. Overexpression of exogenous SDN-1::GFP, SDN-1(2xS>A)::GFP and SDN-1(3xS>A)::GFP can induce 3G10 immunoreactivity in sdn-1(zh20).

Shown are 3D projection images of 6–8-cell stage embryos from (A) wild type, (B) sdn-1(zh20), (C) sdn-1(zh20); juSi99[Pmex-5-SDN-1::GFP-sdn-1 3’UTR], (D) sdn-1(zh20); juSi117[Pmex-5-SDN-1(2xS>A)::GFP-tbb-2 3’UTR] and (E) sdn-1(zh20); juSi175[Pmex-5-SDN-1(3xS>A)::GFP-tbb-2 3’UTR] stained with anti-GFP (green) and 3G10 (red) after heparitinase treatment. Embryos are counter-stained with DAPI (blue). Scale bar = 10 µm.
Figure S5. The cytoplasmic domain of SDN-1 is required for SDN-1 internalization during mitosis and for ABar spindle orientation

(A) 3D projection of GFP and PH::mCherry from embryos expressing wild-type SDN-1::GFP (juSi99) or mutant forms of SDN-1::GFP (ΔC; juSi105, 2xSA; juSi117 and 2xSA ΔC; juSi144[Pmex-5::SDN-1 2xSA, ΔC::GFP::sdn-1 3’UTR]) at metaphase (-1 min from the onset of anaphase). SDN-1 2xSA, ΔC::GFP does not show plasma membrane localization.
SDN-1::GFP accumulation is not clearly visible in these panels because the projections were created using a sum of slices to better visualize cytoplasmic GFP. (B) Timing of SDN-1::GFP accumulation and internalization relative to ABar mitosis. Wild type SDN-1::GFP (mex-5 promoter) begins to accumulate in the 5 minutes preceding contact of ABar and C cells (filled circles). Deletion of the SDN-1 cytoplasmic domain (∆C) did not affect accumulation dynamics (filled squares); mutation of the SDN-1 GAG attachment sites (2xS>A) significantly delayed accumulation (filled triangles). Wild type SDN-1::GFP was either internalized into ABar during metaphase-anaphase, or less often into C, after cytokinesis. SDN-1∆C::GFP and SDN-1 2xS>A::GFP both showed delayed internalization. Statistics: Fisher’s exact test (before vs after onset of anaphase); *, P<0.05, ***, P<0.001. (C) Wild type SDN-1::GFP (black line) and SDN-1 2xS>A::GFP (blue line) driven by sdn-1 own promoter and 3’UTR fully rescued the misoriented ABar spindle in sdn-1(zh20) mutants. However, SDN-1∆C::GFP driven by sdn-1 promoter and 3’UTR did not fully rescue ABar centrosome/spindle orientation relative to the AP axis (dashed pink line). Overexpression of SDN-1∆C::GFP by mex-5 promoter and tbb-2 3’UTR fully rescued abnormal ABar spindle dynamics in sdn-1(zh20) mutants (purple line). sdn-1(-), sdn-1(+), sdn-1(C+) and sdn-1(GAG+) indicate sdn-1(zh20), juSi119[Psdn-1::SDN-1::GFP::sdn-1 3’UTR], juSi151[Psdn-1-SDN-1∆C::GFP-sdn-1 3’UTR] and juSi170[Psdn-1-SDN-1 2xS>A::GFP-sdn-1 3’UTR], respectively. **, P<0.01, ANOVA, followed by Dunnett test comparing orientation at onset of anaphase. (D) Bar graphs showing standard deviation at early prophase and at onset of anaphase. Variance of spindle orientation at early prophase is not statistically different between these transgenic strains.

Figure S6. Effects of sdn-1 loss of function on EMS spindle orientation.

Three dimensional angles between the EMS centrosome axes and AP axis are plotted over time relative to anaphase. Although sdn-1(zh20) and sdn-1 RNAi displayed slight delays in EMS spindle rotation, the mean EMS division angles at -1 min relative to the onset of anaphase are not significantly different.
Movies 1-3. *sdn-1* mutants and mutants defective in HS synthesis display aberrant ABar cell division orientation.

Differential interference contrast (DIC) imaging of wild-type (Movie 1), *sdn-1(zh20)* (movie 2) and *rib-1(tm516)* (Movie 3) embryos during ABar division. Each movie shows two focal planes from the same 4D movie to display ABar division (left) and ABar-C contact (right). The movie starts in the 6-cell stage and ends at 12-cell stage. Straight lines indicate the orientations of the EMS, ABar, and ABpr divisions. Freehand lines indicate the cell surfaces of ABar (white) and C (green) before and during contact. The cell contact and onset of anaphase in AB granddaughters occur at 06:30~7:00 and 14:00~14:30, respectively. Anterior is left and dorsal is up. Time stamps, min:sec.
Movies 4, 5. ABar spindle orientation is initially variable in wild-type and in sdn-1 embryos

Movies of wild type (Movie 4) and of sdn-1(zh20) (Movie 5). Each movie shows two representative embryos in which the ABar spindle (marked using TBB-2::GFP) is initially unrotated (i.e. far from its final orientation) left) or is initially in its final orientation (right). Time stamps, min:sec.
Movie 6. Dynamics of SDN-1::GFP accumulation and colocalization with membrane marker PH::mCherry.

Single focal planes from confocal z-stack movie are shown, moving from 3 µm to 0 µm focal planes (fp) to follow SDN-1::GFP (green) and PH::mCherry (purple). SDN-1::GFP accumulation in ABar is indicated by white arrowheads. Time stamp, min:sec.

Movie 7. Dynamics of SDN-1::GFP internalization into ABar or into C

3D projection movies of two embryos, one showing that SDN-1::GFP accumulation on the contact site is endocytosed in ABar (left), and one showing internalization into C (right). Red arrows indicate accumulation on the contact while yellow arrows indicate internalization. Time stamp, min:sec.
Movie 8. Comparison of the localization dynamics of SDN-1::GFP and MIG-5::GFP. In the left hand embryo SDN-1::GFP (*juSi98*) accumulation is visible by 8:00 and has begun to internalize by 12:00; ABar anaphase is at 13:00 (double arrow). In the right hand embryo MIG-5::GFP (*juSi138*) accumulation is visible at 9:00. ABar anaphase is at 13:00. Time stamps, min: sec.
Table S1. Strains and Genotypes

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Table S2. Plasmids generated for this study

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| pCZ839       | Pmex-5-SDN-1::GFP-tbb-2 3’UTR | PCR #1 (mex-5 promoter): AC3273 (5’-gacctcaagtgggccagatcaaatatcagttttttaaa -3’) and AC3274 (5’-tcctgtctgaaaccattca -3’)
|              |          | PCR #2 (sdn-1 cDNA): AC3275 (5’-gaatgtttcaagagagatgagttttc -3’) and AC3276 (5’-ccaccacactctccgaggcaegtagaatcttttttctg -3’)
|              |          | PCR #3 (gfp::tbb-2 3’UTR): AC3277 (5’-tgcccgggaggaggtgttg -3’) and AC3276 (5’-ccaccacactctccgaggcaegtagaatcttttttctg -3’)
|              |          | PCR #4 (vector backbone): AC3278 (5’-iggaggaattccggtg -3’) and AC3279 (5’-gtgtgacagtaatctccggtgagtc -3’)
| pCZ840       | Pmex-5-SDN-1::GFP-sdn-1 3’UTR | PCR #1 (sdn-1 3’UTR): AC3288 (5’-tggatgctgactaataataatcctctgtcatttgttca -3’)
|              |          | AC3280 (5’-atcctgcaggaattcctcgataatgtaatattttatatat -3’)
|              |          | PCR #2 (Pmex-5-SDN-1::GFP): AC3273 and AC3281 (5’-ttatttgtatagttcatcca -3’)
|              |          | PCR #3 (vector backbone): AC3278 and AC3279
| pCZ841       | Pmex-5-SDN-1::GFP::tbb-2 3’UTR | PCR #1 (mex-5 promoter): AC3273 and AC3274
|              |          | PCR #2 (sdn-1 ∆C cDNA): AC3275 and AC3282 (5’-gtctctctctctactatgtgactacacaagagagtagaatctctgtc -3’)
|              |          | PCR #3 (gfp::tbb-2 3’UTR): AC3277 and AC3276
|              |          | PCR #4 (vector backbone): AC3278 and AC3279
| pCZ843       | Pmex-5-SDN-1(S71A, S86A)::GFP-sdn-1 3’UTR | AC3259 (5’-tgatgttcagggggcagcaagccccc-3’) and AC3260 (5’-ggcggctttgctcgggctgttagacatctctgtc -3’)
|              |          | AC3272 (5’-cagacatcagaatggagccccgtaccaaa-3’) and AC3263 (5’-tggtagtcgtaacagtcttcttctcggga -3’)
|              |          | for Ser86Ala
|              |          | AC3272 (5’-cagacatcagaatggagccccgtaccaaa-3’) and AC3273 (5’-tggtagtacagttctccttcttctcggga -3’)
|              |          | for Ser71Ala
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|              |          | for Ser214Ala
|              |          | PCR#2 (vector backbone): AC3278 and AC3279
| pCZ838       | Psdn-1-SDN-1::GFP-sdn-1 3’UTR | PCR #1 (sdn-1 promoter): AC3283 (5’-actcaggtggccagatcagegtgaatacagaaactatt -3’) and AC3284 (5’-ttgtgctgacatcagatc -3’)
|              |          | PCR #2 (vector): AC3287 (5’-gggtagttccagctgagatggttgatgtgac -3’) and AC3279 (5’-gtgtgacagatcagatc -3’)
| pCZ861       | Pmex-5_sdn1_deltaGAG3x_sdn-1 3’UTR | AC3259 (5’-tgatgttcagggggcagcaagccccc-3’) and AC3260 (5’-ggcggctttgctcgggctgttagacatctctgtc -3’)
|              |          | for Ser214Ala
### Table S3. Primer list for dsRNA synthesis and real-time qPCR

<table>
<thead>
<tr>
<th>Template for</th>
<th>Primers (T7 promoter sequence is underlined.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>dsh-2</em> dsRNA</td>
<td>AC3289 5’-taatacgactcactatagggagcatgattcccttcacc-3’&lt;br&gt;AC3290 5’-taatacgactcactatagggagcatgattcccttcacc-3’</td>
</tr>
<tr>
<td><em>mig-5</em> dsRNA</td>
<td>AC3291 5’-taatacgactcactatagggagcatgattcccttcacc-3’&lt;br&gt;AC3292 5’-taatacgactcactatagggagcatgattcccttcacc-3’</td>
</tr>
<tr>
<td><em>mom-2</em> dsRNA</td>
<td>AC3295 5’-taatacgactcactatagggagcatgattcccttcacc-3’&lt;br&gt;AC3296 5’-taatacgactcactatagggagcatgattcccttcacc-3’</td>
</tr>
<tr>
<td><em>src-1</em> dsRNA</td>
<td>AC3293 5’-taatacgactcactatagggagcatgattcccttcacc-3’&lt;br&gt;AC3294 5’-taatacgactcactatagggagcatgattcccttcacc-3’</td>
</tr>
<tr>
<td><em>sdn-1</em> dsRNA</td>
<td>AC3297 5’-taatacgactcactatagggagcatgattcccttcacc-3’&lt;br&gt;AC3298 5’-taatacgactcactatagggagcatgattcccttcacc-3’</td>
</tr>
<tr>
<td><em>wrm-1</em> dsRNA</td>
<td>kpr303 5’-taatacgactcactatagggagcatgattcccttcacc-3’&lt;br&gt;kpr304 5’-taatacgactcactatagggagcatgattcccttcacc-3’</td>
</tr>
</tbody>
</table>

References for Supplementary Materials


