**Drosophila** syndecan regulates tracheal cell migration by stabilizing Robo levels

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Here we identify a new role for Syndecan (Sdc), the only transmembrane heparan sulphate proteoglycan in **Drosophila**, in tracheal development. Sdc is required cell autonomously for efficient directed migration and fusion of dorsal branch cells, but not for dorsal branch formation per se. The cytoplasmic domain of Sdc is dispensable, indicating that Sdc does not transduce a signal by itself. Although the branch-specific phenotype of sdc mutants resembles those seen in the absence of Slit/Robo2 signalling, genetic interaction experiments indicate that Sdc also helps to suppress Slit/Robo2 signalling. We conclude that Sdc cell autonomously regulates Slit/Robo2 signalling in tracheal cells to guarantee ordered directional migration and branch fusion.

Keywords: cell migration; **Drosophila**; heparan sulphate; Robo; tracheal system

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**INTRODUCTION**

Directed cell migration is a core mechanism in the establishment of branched organs such as the vasculature or the **Drosophila** tracheal system (Affolter et al, 2009). In both vertebrates and invertebrates, induction and guidance of cell migration towards a target are achieved by the coordinated action of several growth factor signalling pathways. One of the main questions is how these signalling pathways are coordinated and translated into highly stereotypic movements of small groups of cells (Ghabrial et al, 2003; Uv et al, 2003; Affolter & Caussinus, 2008; Lu & Werb, 2008; De Smet et al, 2009; Phng & Gerhardt, 2009).

Heparan sulphate, a glycosaminoglycan with highly complex structure, is essential for development, as the knockout of one of the enzymes essential for heparan sulphate synthesis is lethal early in development (Lin et al, 2000). We set out to investigate the function of the sole transmembrane heparan sulphate proteoglycan, Syndecan (Sdc), in **Drosophila** (Spring et al, 1994). Our data show for the first time, to our knowledge, that Sdc is required in tracheal morphogenesis. Sdc is crucial for fine-tuning of guided migration of the subset of epithelial cells constituting the tracheal dorsal branches. Sdc stabilizes Slit/Robo signalling and functions independently of its cytoplasmic domain.

**RESULTS AND DISCUSSION**

**The Syndecan gene**

The genome of **Drosophila melanogaster** harbours a single sdc gene. Existing mutants sdc²³, sdc⁴⁸ and sdc⁶⁷ are derived from imprecise P-element excisions causing deletions including sdc exons 1 and 2 (Fig 1A; Johnson et al, 2004; Steigemann et al, 2004), but these mutants all potentially affect the function of Smad anchor for receptor activation (sara) on the complementary strand (Epstein et al, 2002). We used ends-in homologous recombination (Rong et al, 2002) to introduce a frameshift mutation in exon 3 of sdc (Fig 1B). One line (sdc²⁶⁵⁹) bearing the exon 3 mutation was further validated by genomic PCR, reverse transcription–PCR, western blotting (Fig 1C,D) and complementation analysis (Table 1). sdc²⁶⁵⁹ was semilethal in homozygosity and in transheterozygosity with sdc²³, sdc⁶⁷, sdc⁴⁸ or the larger deficiencies DfPK¹ or Df2R66D⁷⁶. Similarly, the five other sdc exon 3 allele carrying stocks that we established were semilethal in transheterozygosity with sdc²⁶⁵⁹ or DfPK¹, but not over the deficiencies DfP¹² or DfR²⁹⁰⁰, which lie outside the sdc locus.

**Syndecan tracheal branch phenotype**

The external morphology of adult escaper sdc²⁶⁵⁹ flies had no obvious phenotype (not shown). However, inspection of sdc mutant third-instar larvae showed that the 10 dorsal branches of the tracheal system often failed to establish a dorsal anastomosis at the midline (Fig 2A–C), and ganglionic branch number and length were reduced (supplementary Fig S1). Dorsal branch fusion

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Table 1 | Complementation analysis of the Syndecan locus 57E2–6

| Percentage of predicted eclosions | DfXE2900 57F2–58A1 | DfPtl12 57D8–13 | DfPext6076 57D13–57F3 | DfPK1 57C5–57F5 | sdc^{23} | sdc^{97} | sdc^{48} | sdc^{48} _{ubi > sara} | sdc^{2639} |
|-----------------------------------|------------------|----------------|----------------|----------------|--------|--------|--------|----------------|--------|
| DfPext6076                       | 71               | 107            | 0              | 1              | 40     | 47     | 0      | 0              | 29     |
| sdc^{23}                          | 109              | 88             | 40             | 23             | 9      | 30     | 35     | 48             | 51     |
| sdc^{97}                          | 104              | 114            | 35             | 14             | 30     | 20     | 39     | 50             | 32     |
| sdc^{48}, _ubi > sara_            | 108              | 77             | 0              | 0              | 48     | 50     | 0      | 0              | 40     |
| sdc^{2639}                        | 96               | 118            | 29             | 30             | 51     | 32     | 41     | 40             | 26     |
| sdc^{2451}                        | –                | –              | –              | –              | 16     | –      | –      | –              | –      |
| sdc^{2523}                        | –                | –              | –              | –              | 20     | –      | –      | –              | –      |
| sdc^{2527}                        | –                | –              | –              | –              | 16     | –      | –      | –              | –      |
| sdc^{2622}                        | –                | –              | –              | –              | 26     | –      | –      | –              | –      |
| sdc^{2626}                        | –                | –              | –              | –              | 22     | –      | –      | –              | –      |
| sdc^{2639}, _btl > sdc_           | –                | –              | –              | –              | –      | –      | –      | –              | –      |

sara, Smad anchor for receptor activation; sdc, Syndecan.
Fig 1 | The Syndecan (sdc) gene and mutants. (A) Deficiencies and mutants spanning the sdc locus. The sdc locus on chromosome 2R. sdc (green) is flanked by acox (yellow) and sara (red) on the plus strand and kbp13 (blue) on the minus strand. sdc<sup>23</sup> and sdc<sup>48</sup> delete sdc exons 1 and 2, sdc<sup>97</sup> deletes sdc exon 1, but they are not defined towards sara. D<sup>pile16079</sup> and D<sup>pile112</sup> span the sdc locus, D<sup>pile2090</sup> are outside the sdc locus. (B) sdc exon 3 sequence. Alignment of wild-type (upper) and sdc exon 3 mutant (lower) sequences. Upper alignment: Wild-type and mutant DNA sequences, with or without deletion of a guanidine residue in exon 3 that gives rise to a frameshift (FS). Lower alignment: Corresponding Sdc translation products with and without the premature stop codon with the new protein sequence after the frameshift (yellow), the first glycosaminoglycan (GAG) attachment site (green). (C) sdc<sup>2639</sup> exon 3 RNA sequence. ABI sequencing of reverse-transcribed RNA of wild-type and sdc exon 3 mutant line sdc<sup>2639</sup>. Red arrow, guanidine deleted in the mutant. (D) Effect of sdc mutants on Sdc protein level. Sdc protein levels in wild-type, sdc<sup>2639</sup>/+, sdc<sup>2639</sup> and sdc<sup>23</sup> flies, on anti-Sdc western blot, anti-tubulin as loading control. Sdc, Syndecan; Tub, tubulin; wt, wild type.

Fig 2 | Syndecan tracheal dorsal branch phenotype. (A) Segmental quantification of dorsal branch (DB) fusion failures in sdc mutants. Dorsal anastomoses (DA) missing in each of the 10 segments separately in wild-type (wt, black bar), sdc<sup>23</sup> heterozygotes (white bar), sdc<sup>23</sup> homozygotes (grey bar) or sdc<sup>2639</sup> homozygotes (blue bar; n = 35 animals per genotype). (B) Quantification of total dorsal branch fusion failures in sdc mutants. The number of DA missing per animal, in wild type (black bar), sdc<sup>23</sup>/+ (white bar), sdc<sup>23</sup> from sdc<sup>23</sup>/+ parents (‘sdc<sup>23</sup>/zyg’, light grey bar), sdc<sup>2639</sup> from sdc<sup>23</sup>/+ parents (‘sdc<sup>23</sup>/zyg’, light blue bar), sdc<sup>23</sup>/sdc<sup>2639</sup> from sdc/+ parents (‘sdc<sup>23</sup>/sdc<sup>2639</sup>zyg’, green bar), sdc<sup>23</sup> from sdc<sup>23</sup> parents (‘sdc<sup>23</sup>/zyg + mat’, dark grey bar), sdc<sup>2639</sup> from sdc<sup>2639</sup> parents (‘sdc<sup>2639</sup> zyg + mat’, dark blue bar; ± confidence interval (CI), n = 10 animals per genotype; **P < 0.01 in T-test). (C) sdc dorsal branch phenotypes. Fluorescence stereomicroscopy of living sdc<sup>2639</sup> third-instar larval fillets expressing a tracheal CD8:GFP reporter to illustrate the categories of dorsal branch phenotypes. A/P, anterior–posterior misalignment; Stub, incomplete or absent branch. The arrows indicate the exact site of the phenotype. Scale bar, 100 µm. (D) Effect of sdc on dorsal branch terminal extension number. Upper: Frequency distribution for the number of termini (n = 50 cells) of wild-type (wt, open triangles) and sdc<sup>2639</sup> (filled diamonds) terminal cells of the dorsal branch in segment 5 (DB5). Lower: Quantification of the average number of termini of wild-type (wt) and sdc<sup>2639</sup> terminal cells in segment 5 (± CI; n = 50 cells). (E) Dorsal branch cell reporter. Fluorescence stereomicroscopy of two fused wild-type dorsal branches in a living third-instar larval fillet expressing tracheal CD8:GFP and histone:RFP to label tracheal membranes and nuclei. Scale bar, 100 µm. (F) sdc dorsal branch cell numbers. Quantification of the number of dorsal branch cell types, in wild-type, sdc<sup>2639</sup>/+ or sdc<sup>2639</sup> larvae. FC, fusion cell; GFP, green fluorescent protein; RFP, red fluorescent protein; SC, stalk cell; TC, terminal cell (± CI; ***P < 0.005; ****P < 0.001 in T-test, n = 30 branches).
As the cells in a mature dorsal branch are different cell types was not random but followed a hierarchical order (Table 2). Intriguingly, the loss of a cell type affecting all three individual cell types: stalk cell (SC), terminal cells into the dorsal trunk (supplementary Video 1 online), reintegration of cells into the dorsal trunk (supplementary Video 2 online) and loss of cells from the branch (supplementary Video 3 online).

Syndecan cellular phenotypes

When a wild-type dorsal branch develops, 5–6 cells of the tracheal placode migrate dorsally and eventually intercalate. One of the tip cells develops into the terminal cell and extends fine terminal extensions, whereas the other tip cell becomes the fusion cell and forms a cell–cell contact with the fusion cell of the contralateral dorsal branch (Samakovlis et al., 1996; Tanaka-Matakatsu et al., 1996).

To understand the cellular mechanisms through which syndecan affects dorsal branch fusion, we analysed the syndecan dorsal branch phenotype at the single-cell level. We identified three dorsal branch phenotypes leading to fusion failures: dorsal branch that fail to meet at the midline because of anterior–posterior axis guidance defects (‘A/P’ phenotype), dorsal branch that reach the midline but fail to establish a dorsal anastomosis (‘mIdl’ phenotype) and, the most severe, incomplete dorsal branch or completely absent dorsal branch (‘stub’ phenotype; Fig 2C).

Although the terminal extensions were not significantly reduced in syndecan mutants in segments in which terminal cells were found (Fig 2D), the average total number of dorsal branch cells was reduced from 5.3 or 5.4 cells in wild-type or heterozygous mutant dorsal branches, affecting all three individual cell types: stalk cell (SC), terminal cell (TC) and fusion cell (FC; Fig 2E,F). Intriguingly, the loss of the different cell types was not random but followed a hierarchical order (Table 2). As the cells in a mature dorsal branch are arranged linearly, and their physical order from tip to base (TC/FC > SC > SC > SC) is different from the hierarchical order we found for cell loss in syndecan dorsal branch (SC > SC > TC > FC > SC > SC), cells cannot simply be lost from the end of the branch. More likely, Syndecan is required for dorsal branch cell migration before cell intercalation and linear alignment, and before specification, which is known to occur after the cells leave the dorsal trunk (Ghabrial & Krasnow, 2006).

Live imaging showed that many mechanisms accounted for a loss of syndecan dorsal branch cells: an insufficient number of cells leaving the dorsal trunk (supplementary Video 1 online), reintegration of cells into the dorsal trunk (supplementary Video 2 online) and loss of cells from the branch (supplementary Video 3 online).

Syndecan cell autonomy

To test whether syndecan is required in a tissue-autonomous manner, we established Syndecan Upstream Activating Sequence (UAS) knockdown lines (supplementary Fig S2 online). Dorsal branch fusion rates in pan-tracheal syndecan knockdown animals were similar to those observed in syndecan mutants (Fig 3A compared with Fig 2A), and were completely reversed using an RNA interference-rescue syndecan transgene (Schulz et al., 2009; Fig 3A). Fusion-cell-specific Syndecan knockdown was sufficient to induce dorsal branch fusion failures, and was also rescued with syndecanRESC (Fig 3B). We investigated whether Syndecan expressed in only one fusion cell was sufficient for efficient dorsal branch fusion. We found that dorsal branch fusion still occurred in 15 out of 16 unilateral syndecan fusion cell MARCM (mosaic analysis with a repressible cell marker) clones (Lee & Luo, 1999; Fig 3C, Table 3), indicating that Syndecan might also function in trans on the contralateral fusion cell. Confocal images of stage 14 embryos of the syndecan-GFP FlyTrap line CC00871, which bears an in-frame, oriented green fluorescent protein (GFP) between Syndecan exons 2 and 3, showed that Syndecan is indeed expressed in developing dorsal branches (supplementary Fig S3A and B online).

Syndecan core protein function

Syndecan is a type-I transmembrane protein with glycosaminoglycan chains attached to the ectodomain. Our RNAi Escape Strategy Construct (RESC) rescue approach allowed us to perform Syndecan structure–function analyses in Syndecan tracheal knockdown animals in surrounding wild-type tissue. The dorsal branch phenotype was completely reversed by wild-type Syndecan, and also by a truncated version of Syndecan lacking the cytoplasmic domain entirely (SyndecanC). Tracheal expression of Syndecan did not rescue the semilethality observed in the mutant (Table 1), indicating that the tracheal phenotype alone does not account for lethality and that the tracheal Syndecan is not sufficient for full viability. Syndecan alone (SyndecanC) was partly functional in dorsal branch formation. Exon 5, coding for a large ectodomain sequence lacking in Syndecan isoform B/C, was not essential in the context of dorsal branch fusion. By contrast, the Syndecan core protein lacking glycosaminoglycan chains (SyndecanAGC) did not rescue tracheal dorsal branch fusion (Fig 3D). Wild-type Syndecan or SyndecanAGC expressed in tracheal cells fully rescued the dorsal branch phenotype in a syndecan mutant background (Fig 3E). In summary, the glycosaminoglycan bearing ectodomain but not the cytoplasmic domain is essential for Syndecan function, which is in line with the findings of Chanana et al. (2009) in the nervous system.

Syndecan and Slit/Robo signalling

The combined dorsal branch and ganglionic branch phenotype we observed in syndecan mutants and under tracheal knockdown

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Table 2 | Quantification of Syndecan dorsal branch cell types

| Total | Stalk cells | Fusion cells | Terminal cells |
|-------|-------------|--------------|----------------|
| 6     | 4.0         | 1.0          | 1.0            |
| 5     | 3.07        | 0.97         | 0.97           |
| 4     | 2.13        | 1.05         | 0.82           |
| 3     | 1.83        | 0.93         | 0.23           |
| 2     | 1.81        | 0.19         | 0              |
| 1     | 0.83        | 0.17         | 0              |
| 0     | 0           | 0            | 0              |

Average contribution of stalk cells, fusion cells and terminal cells to syndecan dorsal branch with a total cell number between 0 and 6 (n = 30 branches); syndecan, Syndecan.

failures were observed only rarely in wild-type (0.5 non-fused segments per animal) or syndecan heterozygotes (0.8 per animal; Fig 2A). By contrast, almost every second dorsal anastomosis was missing in syndecan23 and syndecan2639 mutants, in all segments at much higher rates but with a similar anterior–posterior distribution. In the progeny of escaper syndecan mutant parents, which lack maternal and zygotic Syndecan (‘syndecan2337-1-mutd’), dorsal branch fusion failure rates were further elevated (5.6–6.4 per animal, Fig 2B) when compared with the zygotic mutants, whereas the dorsal trunk remained fused and primary branches were present. This result indicates that Syndecan has a branch-specific and incompletely penetrant role in tracheal development, a process that has many similarities with vertebrate angiogenesis, of which Syndecan is a well-known modulator (Chen et al., 2004; Dedkov et al., 2007; Beauvais et al., 2009).

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Fig 3 | Autonomy and rescue of sdc dorsal branch phenotype. (A) Quantification of dorsal branch fusion failures with Syndecan (Sdc) RNA interference (RNAi) and rescue. Dorsal anastomoses (DA) missing in each of the 10 segments, in wild-type (wt, black bars), btl-Gal44-1-driven tracheal Sdc RNAi alone (white bars) or tracheal Sdc knockdown with tracheal SdcRESC protein wt rescue (red bars; n = 25 animals/genotype). (B) Fusion-cell-specific Sdc RNAi and rescue. Quantification of total DA missing per third-instar larva expressing the fusion cell-specific driver p127 > Gal4 alone (‘p127’, black bar), with Sdc RNAi (‘p127 > RNAi’, white bar) or with Sdc RNAi and SdcRESC rescue (‘p127 > RNAi + rescue’, yellow bar; ± confidence interval (CI); *P < 0.05; **P < 0.01 in T-test, n = 25). (C) Dorsal branch with sdc2639 MARCM (mosaic analysis with a repressible cell marker) clone. Fluorescence stereomicroscopy of a third-instar sdc2639 fusion cell (upper panel) or stalk cell (lower panel) hs-Flippase (FLP)-induced clone, positively marked with CD8:GFP, histone:RFP (MARCM technique) and btl-Gal44-1. Scale bar, 100 μm. (D) Rescue of tracheal Sdc RNAi with Sdc protein mutants. DA missing per animal with tracheal driver btl-Gal44-1 alone (white bar), tracheal overexpression of long Sdc isoform A (‘Sdc-A’, white bar), tracheal Sdc knockdown alone (‘RNAi’, black bar) or tracheal Sdc knockdown with Sdc RESC rescue construct (red bars) bearing protein wild-type Sdc-ARESC (‘RNAi + Sdc-A’), protein wild-type Sdc-B/C lacking exon 5 coding for part of the extracellular domain (‘RNAi + Sdc-B/C’), SdcRESC lacking the cytoplasmic domain (‘RNAi + Sdc-6C’), SdcRESC ectodomain (‘RNAi + Sdc-Ecto’) or SdcRESC lacking the glycosaminoglycan-attachment sites (‘RNAi + Sdc–6GAG’; ± CI; **P < 0.01 in T-test, n ≥ 10 animals per genotype). (E) Rescue of sdc mutant dorsal branch phenotype. Dorsal branch missing per animal in sdc2639 (white), sdc2639 with tracheal protein wild-type Sdc (‘tracheal Sdc’) or Sdc lacking the cytoplasmic domain (‘tracheal Sdc-6C’) rescue (blue; ± CI; *P < 0.05; **P < 0.01 in T-test, n ≥ 10). GFP, green fluorescent protein; NS, non-significant.

Table 3 | Syndecan dorsal branch MARCM clones

| Cell type     | Number of clones | Percentage of clones | Number of clones in non-fused dorsal branch | Percentage of clones in non-fused dorsal branch |
|---------------|------------------|---------------------|---------------------------------------------|-----------------------------------------------|
| Stalk cell    | 47               | 74.6                | 4                                           | 8.5                                           |
| Fusion cell   | 16               | 25.4                | 1                                           | 6.3                                           |
| Terminal cell | 0                | 0                   | 0                                           | NA                                            |
| Total         | 63               | 100                 | 5                                           | 7.9                                           |

Clones are generated by hs > FLP-induced recombination and positively marked with GFP and RFP (MARCM technique). Indicated are the absolute number or the percentage of clones found in one of the three dorsal branch cell types, and whether these clones are part of a fused or non-fused branch. DB, dorsal branch; GFP, green fluorescent protein; MARCM, mosaic analysis with a repressible cell marker; NA, not applicable; sdc, Syndecan.
conditions was highly reminiscent of the phenotype observed in *slit* or *robo2* mutants (Englund et al., 2002). Therefore, we proposed that *sdc* interacts with *slit*/*robo2* in tracheal development. To test this prediction, we combined an intermediate Sdc knockdown with a loss-of-function *slit* or *robo2* allele in heterozygosity (Fig 4A). The sdc dorsal branch phenotype was suppressed by heterozygosity for the ligand *slit*. Heterozygous loss of the receptor *robo2* had a smaller effect, not reaching statistical significance (Fig 4A). Heterozygosity for *slit* in a homozygous sdc mutant suppressed the dorsal branch phenotype as well, whereas neither sdc and *slit* or sdc and *robo2* in transheterozygosity, nor a heterozygous *robo2* allele in combination with an sdc mutant had an effect (Fig 4B). This indicates that the sdc loss-of-function phenotype is due to a gain in Slit/Robo2 signalling that is more sensitive to *slit* than to *robo2* heterozygosity. In agreement with this hypothesis, tracheal Robo or Robo2 overexpression alone caused a dorsal branch fusion phenotype, Sdc overexpression suppressed the Robo overexpression phenotype and Sdc knockdown markedly enhanced the Robo overexpression phenotype (Fig 4C, D). Moreover, tracheal overexpression of Commiserless,
known to downregulate Robo in axons committed to cross the midline (Keleman et al., 2002, 2005; Myat et al., 2002), resulted in a dorsal branch fusion phenotype that was significantly attenuated by Sdc knockdown (Fig 4E).

As Sdc functions cell autonomously (Fig 3), it might suppress Slit/Robo signalling by affecting Robo levels. When we performed Robo stainings of stage 13/14 wild-type, sdc mutant, or tracheal Sdc overexpressing embryos, we could not detect a change in Robo2 or Robo signal intensity (supplementary Fig S4A,B online). As this might be due to the dynamic expression of Robo or to a lack of sensitivity of the staining method, we addressed this further in systems in which Robo levels are more quantifiable. In salivary glands, Sdc knockdown caused a 3.7-fold increase in ectopically expressed Robo:GFP levels, but not in the levels of a control transmembrane protein, CD8:GFP (Fig 4F,G). To confirm the effect of Sdc on endogenous Robo levels, we knocked down Sdc in human MCF7 cells, expressing Robo1, Sdc1 and Sdc4, and found a significant 1.8-fold increase in cell surface Robo localization (Fig 4H).

Our genetic interaction studies in combination with the cell culture experiments strongly indicate that disrupted Slit/Robo2 signalling accounts for the branch-specific and incompletely penetrant sdc tracheal phenotype (Englund et al., 2002). The insufficient restriction of Slit/Robo2 signalling in space and/or time in sdc mutants might generate aberrant attractive forces and disrupt spatial information. A general role for Sdc in Slit/Robo signalling is likely, as the different Robo receptors share the same extracellular domain structure.

In addition to the known role of Sdc in promoting Slit/Robo signalling, as shown for axon guidance in the nervous system (Johnson et al., 2004; Steigemann et al., 2004; Rhiner et al., 2005; Chanana et al., 2009), we now show that tracheal Sdc can also suppress Slit/Robo2 signalling. At present, such dual function is not explained, but a similar buffering role has been suggested for another heparan sulphate proteoglycan, Dally-like, in Wingless signalling (Kreuger et al., 2004). Whether signalling is enhanced or suppressed by Sdc might depend on Slit/Robo and Sdc levels, and also on the concomitant expression of other heparan sulphate proteoglycans. Alternatively, it could be related to the tissue-specific mode of action of Slit/Robo signalling, switching between attractive and repulsive depending on the tissue or the stage of development (Kramer et al., 2001; Englund et al., 2002).

How could Sdc function on Robo2 levels? We show that Sdc is required cell autonomously, in the same cells as Robo2 is expressed, and find that Sdc can downregulate Robo post-translationally. Sdc might be required for sorting a fraction of de novo synthesized Robo2 directly to a degradation pathway, similar to the function of Commissureless, transporting Robo directly from the synthetic pathway to late endosomes, thereby preventing Robo cell surface expression (Kidd et al., 1998; Keleman et al., 2005). Alternatively, Sdc might promote the removal of Robo2 from the cell surface or an endosomal recycling compartment, as Sdc is known to be important for endosomal growth factor receptor endocytosis and recycling (Tkachenko et al., 2004; Zimmermann et al., 2005). Interestingly, Arf6, a central component in epithelial cell migration and endosomal recycling (Palacios et al., 2001; Sabe, 2003), has been recently identified as a downstream target of Slit/Robo signalling in angiogenesis (Jones et al., 2009). Hence Sdc might regulate Robo transport to or from an Arf6 endosomal compartment.

In conclusion, we provide genetic, biochemical and cell biological evidence for a specific role for Sdc in tracheal development where cell autonomous Sdc is required for Slit/Robo2-mediated cell migration and midline fusion of the dorsal branch.

**METHODS**

For more details see supplementary information online.

**Constructs.** Sdc constructs were cloned into pUAST. The Sdc RNAi line target exons 6 and 7, and this sequence was replaced within the Sdc rescue lines by a RESC sequence.

SdcAC contains a stop codon after Lys 368, SdcCeto a stop after Asn 318 and SdcACAG Ser > Ala mutations of amino acids 62, 79, 81, 110 and 194. The sdc homologous recombination mutant contains a deletion of guanine at position 89 of exon 3, producing a premature stop codon.
**Standard culture conditions.** Flies were maintained on standard fly food at 25 °C. RNAi experiments were conducted at 28 °C except when indicated differently.

**Supplementary information** is available at EMBO reports online (http://www.emboreports.org).

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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