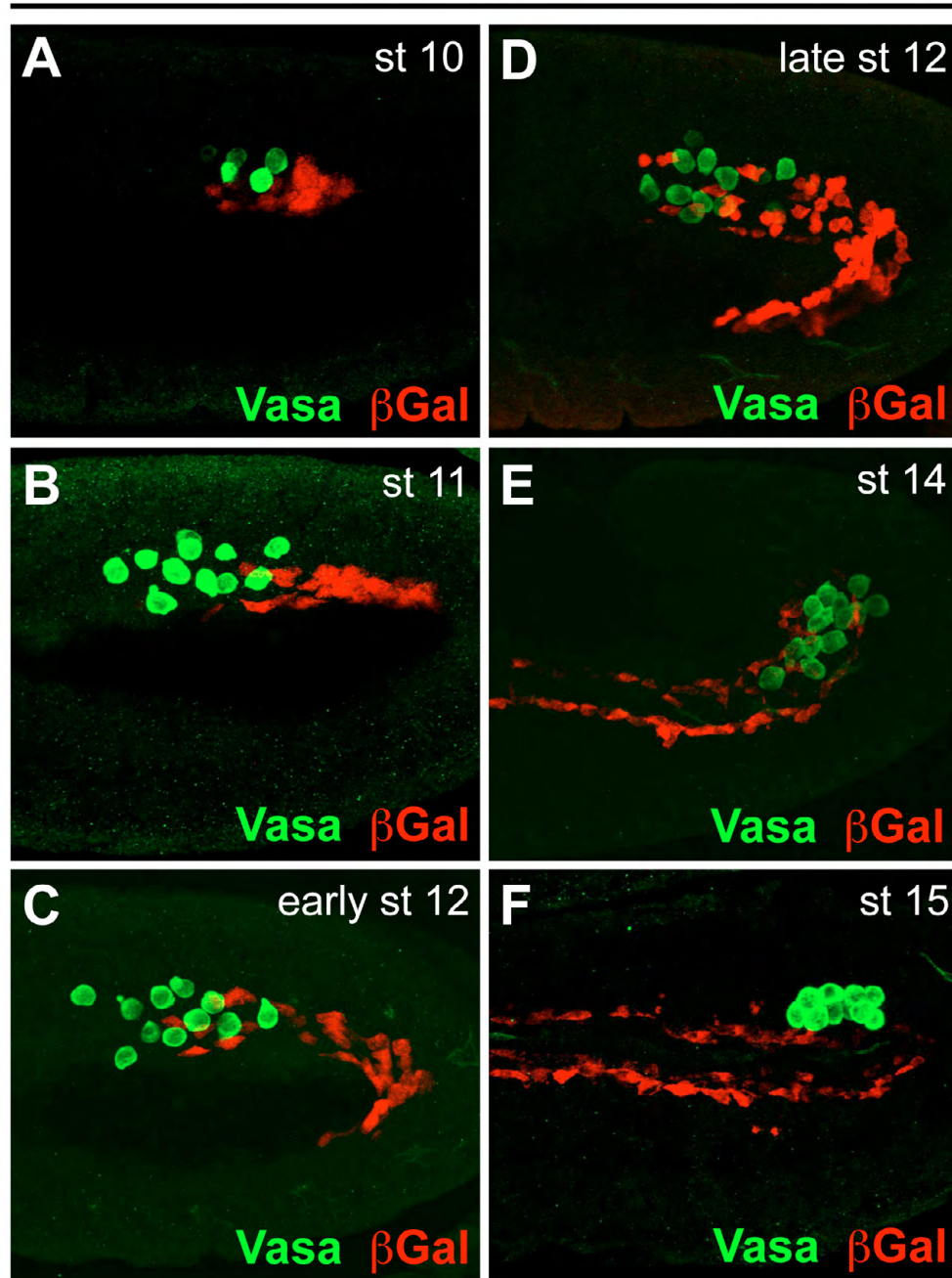


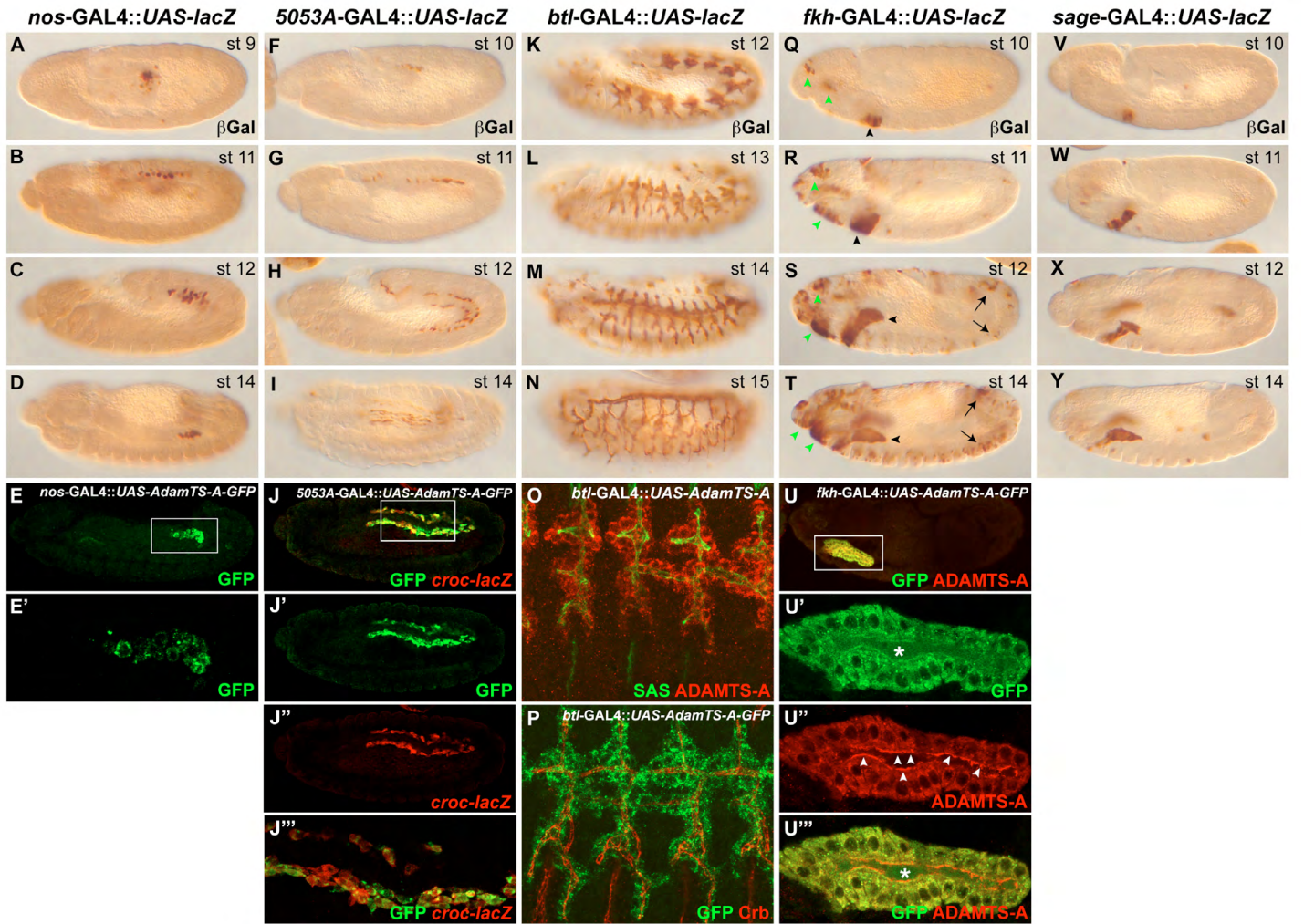
**Fig. S1. ADAMTS-A is homologous to human ADAMTS9 and ADAMTS20, and *C. elegans* GON-1.** Sequence line-up of *Drosophila* ADAMTS-A (Dm ADAMTS-A), *C. elegans* GON-1 (Ce Gon-1), human ADAMTS9 (Hs ADAMTS9) and human ADAMTS20 (Hs ADAMTS20). Residues in magenta are identical among all four proteins. Residues in green are the same in two or three of the proteins and residues in yellow are conservative changes.



## *croc-lacZ*

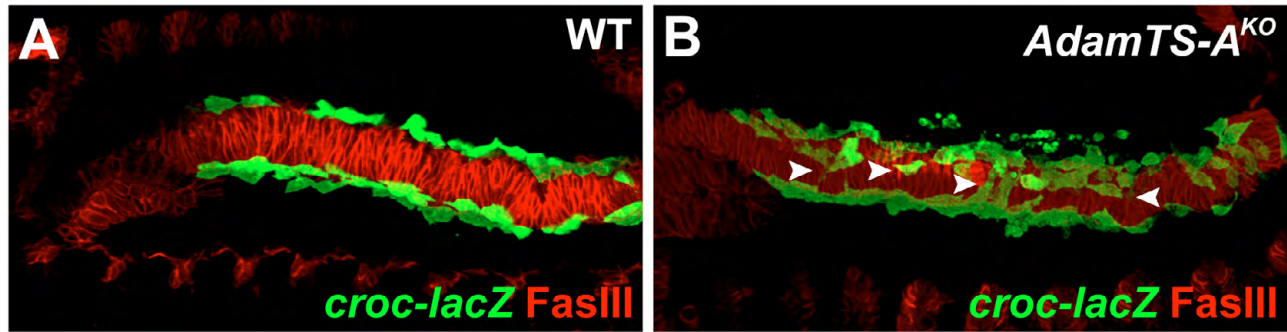


**Fig. S2. The germ cells migrate in close apposition to the migrating CVM.** (A-F) Embryos expressing the *croc-lacZ* reporter, which marks the migrating CVM cells, were immunostained with  $\alpha$ - $\beta$ Galactosidase (red) to visualize the CVM and  $\alpha$ -Vasa (green) to visualize the germ cells (GC). (A) Stage 10 embryo shows GCs just posterior to the CVM as they both begin to migrate. (B-E) Stage 11-14 embryos show the GCs following the CVM along a similar path as both groups of cells migrate around the posterior edge of the embryo (C,D) and continue anteriorly (E). (F) The germ cells (green) have started to coalesce into a tight cluster while the CVM cells (red) continue to migrate anteriorly. All panels are anterior towards the left and posterior towards the right.

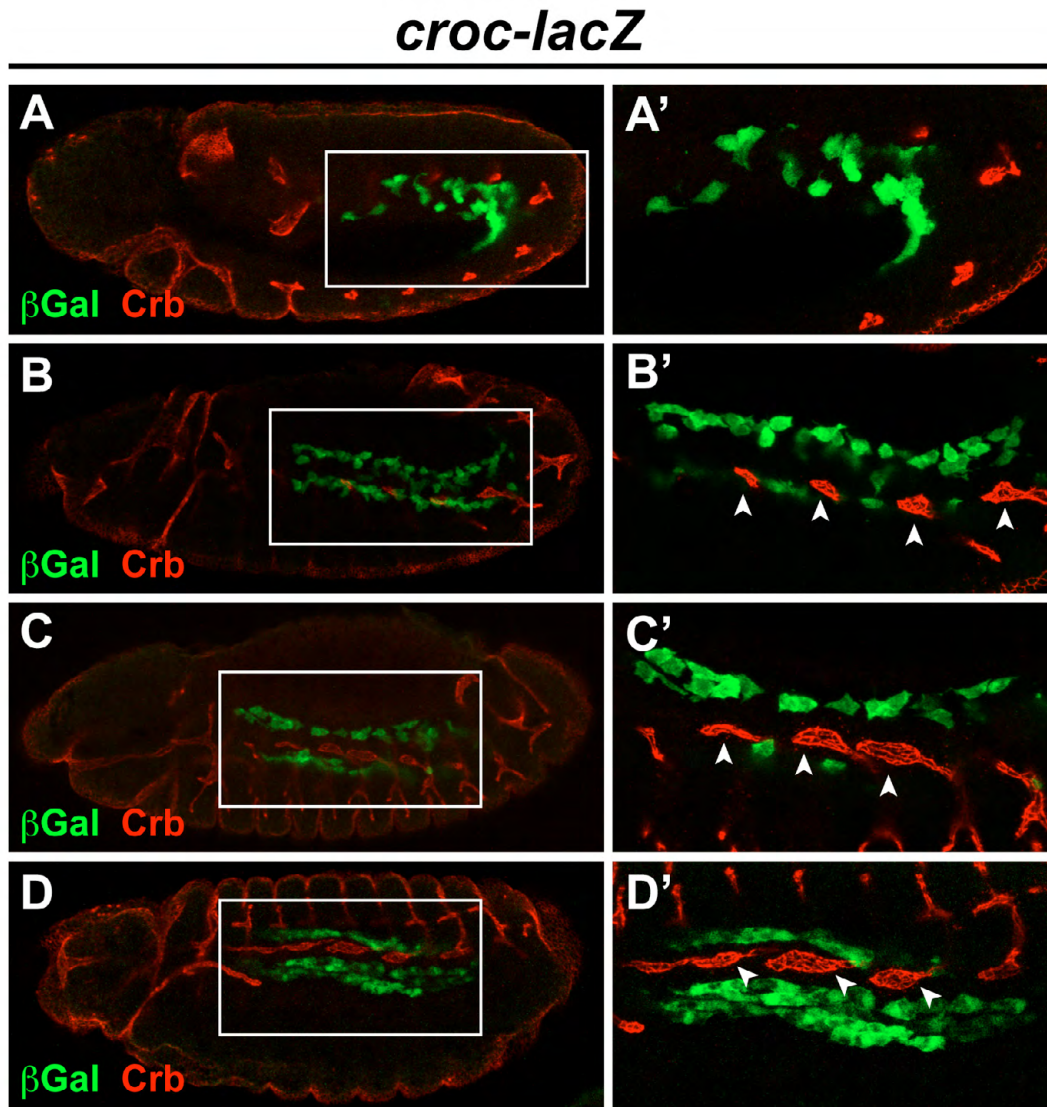


**Fig. S3. Tissue-specific expression of ADAMTS-A and ADAMTS-A-GFP.**  $\beta$ Galactosidase ( $\beta$ Gal) staining of embryos carrying *UAS-lacZ* driven by tissue-specific GAL4 drivers shows tissue-specific expression. (A-D) Embryos expressing *nos-GAL4::UAS-lacZ* show expression of  $\beta$ Gal just in the germ cells from stages 9-14. (E,E') Embryos expressing *UAS-ADAMTS-A-GFP* in only the germ cells using *nos-GAL4* show GFP expression just in the germ cells. (F-I) Embryos expressing *5053A-GAL4::UAS-lacZ* show expression of  $\beta$ Gal just in the CVM from stages 10-14. (J-J''') Embryos expressing *UAS-ADAMTS-A-GFP* just in the CVM using *5053A-GAL4* shows GFP expression in the CVM (green), colocalizing with a CVM-specific marker *croc-lacZ* (red). (K-N) Embryos expressing *btl-GAL4::UAS-lacZ* show expression of  $\beta$ Gal in the trachea from stages 12-15. (O) *UAS-ADAMTS-A* expressed in the trachea using *btl-GAL4*, and immunostained with  $\alpha$ -ADAMTS-A (red) and an apical marker for the trachea  $\alpha$ -SAS (green) shows ADAMTS-A expression in the tracheal cells, but not localized to the apical membrane of the trachea. (P) *UAS-ADAMTS-A-GFP* expressed in the trachea using *btl-GAL4*, and immunostained using antibodies against GFP (green) and an apical marker for the trachea [Crb (red)] shows ADAMTS-A-GFP expression in the tracheal cells, but not localized to the apical membrane of the trachea, similar to ADAMTS-A expression in O. (Q-T) Embryos expressing *fhk-GAL4::UAS-lacZ* show expression of  $\beta$ Gal in the SG from stages 10-14 (black arrowheads). *fhk-GAL4* also drives expression of *UAS-lacZ* in hemocytes in the head region (green arrowheads in Q-T), and other posterior structures at stages 12-14 (black arrows in S,T). (U-U''') *UAS-ADAMTS-A-GFP* expressed in the SG using *fhk-GAL4*, and immunostained using antibodies against ADAMTS-A (red) and GFP (green) show colocalization of ADAMTS-A and GFP in the SG. The intense ADAMTS-A antibody staining at the apical membrane is due to crossreaction with an unrelated protein based on using the antibody to stain *AdamTS-A* mutants and deficiency embryos (white arrowheads in U''), and therefore does not colocalize with GFP at that exact location. (V-Y) Embryos expressing *sage-GAL4::UAS-lacZ* show expression of  $\beta$ Gal in the SG from stages 10-14. All embryos are orientated anterior towards the left and posterior towards the right.



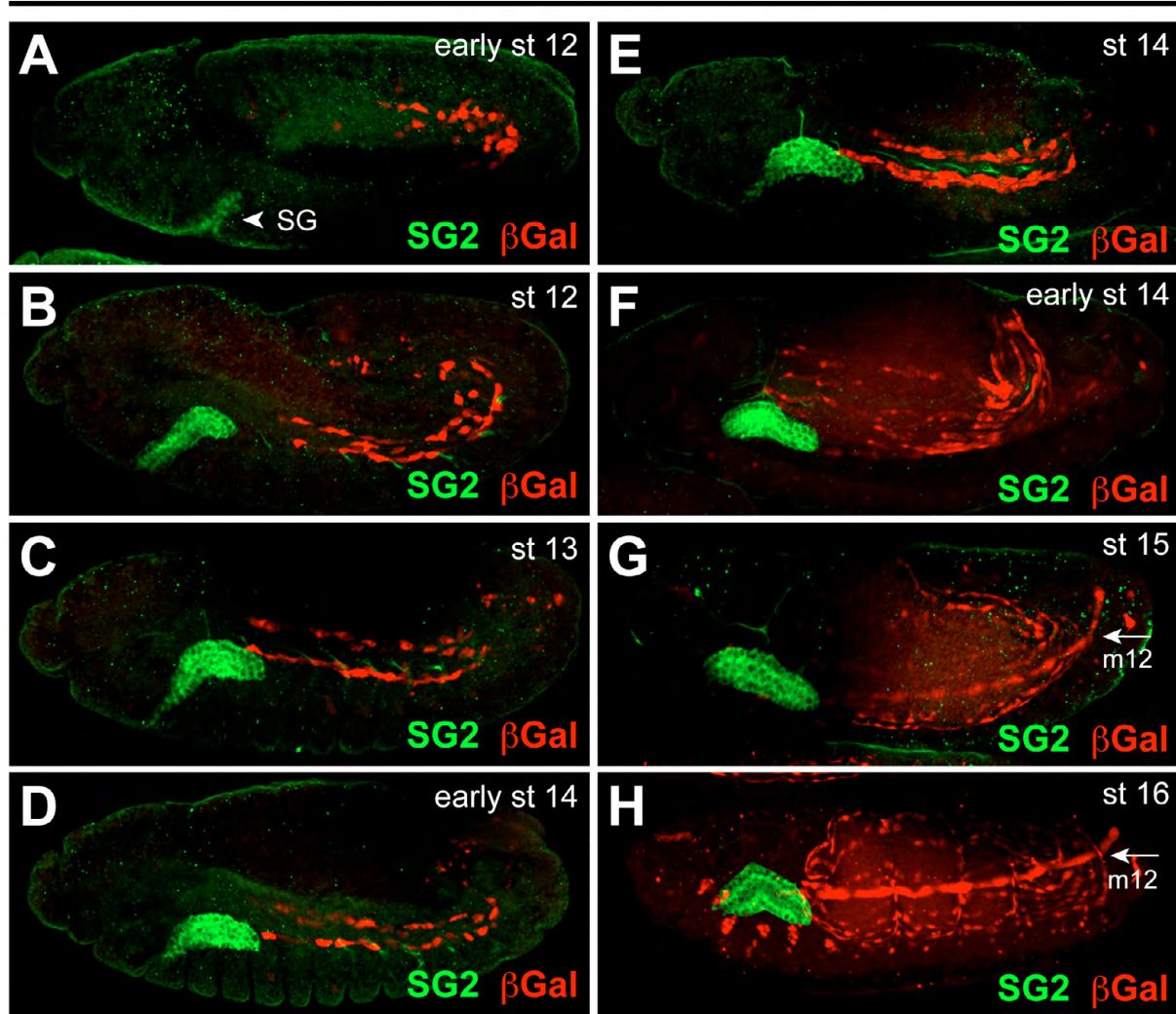


**Fig. S4. Loss of *AdamTS-A* results in mis-migration of CVM cells.** (A,B) Embryos expressing the *croc-lacZ* reporter, which marks the migrating CVM cells, were stained with  $\alpha$ - $\beta$ Galactosidase (green) to visualize the CVM and  $\alpha$ -FasIII (red) to visualize the trunk visceral mesoderm, the cells on which the CVM cells migrate. In wild type, CVM cells migrate in two distinct rows along the dorsal and ventral sides of the trunk visceral mesoderm (A), whereas in *AdamTS-A* mutants, CVM cells are migrating in all different directions (white arrowheads in B). Both panels are orientated anterior towards the left and posterior towards the right.



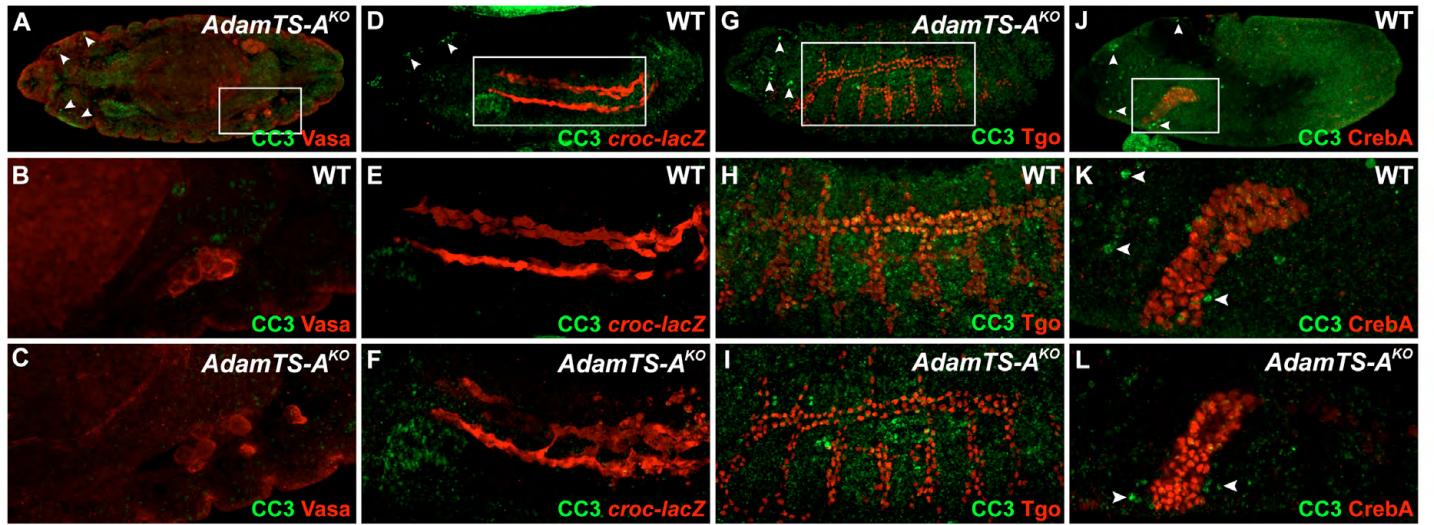
**Fig. S5. The tracheal visceral branch (VB) migrates very close to the CVM.** (A-D') Embryos containing the *croc-lacZ* reporter, which marks the migrating CVM cells, were stained using antibodies against  $\beta$ Galactosidase (green) to visualize the CVM and Crb (red) to visualize the early migrating trachea. (A-D) Low magnification images showing the entire embryo. (A'-D') Enlargements of the boxed areas in A-D with the visceral branch (VB) indicated by white arrowheads (B'-D'). All embryos are orientated anterior towards the left and posterior towards the right.

## 5053A-GAL4::*UAS-lacZ*

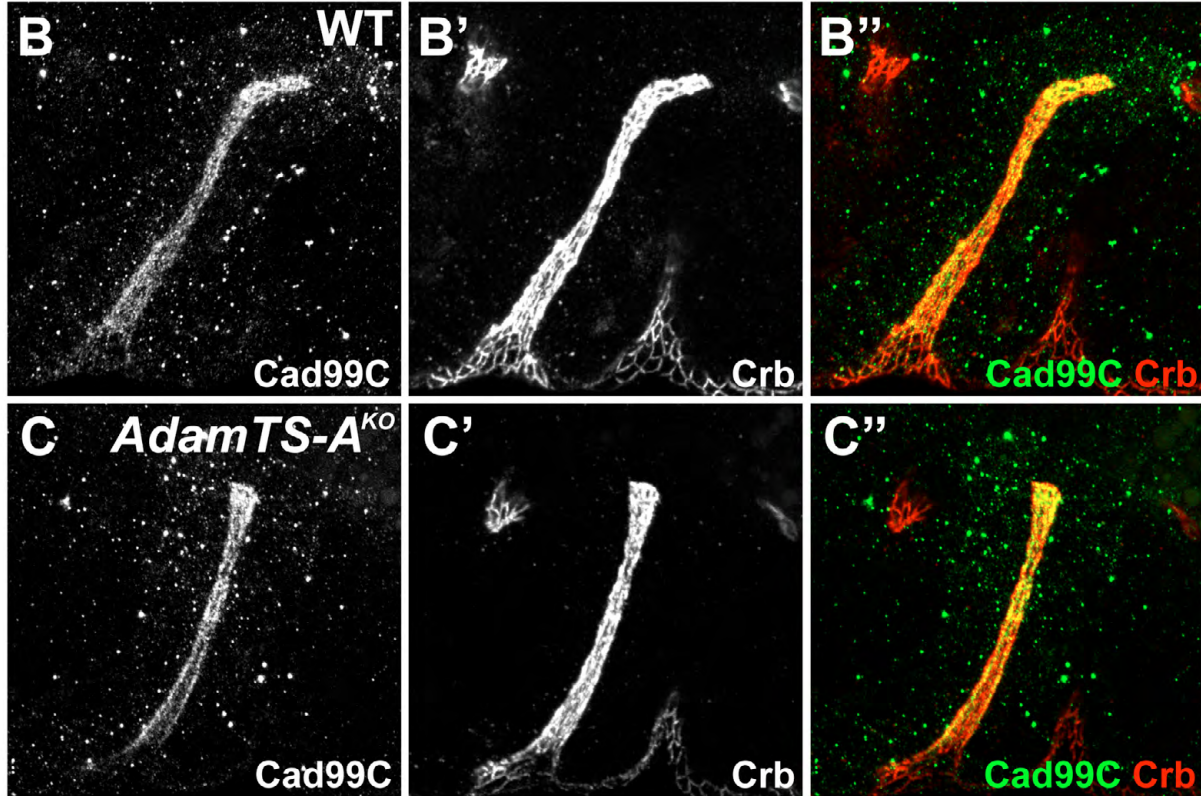
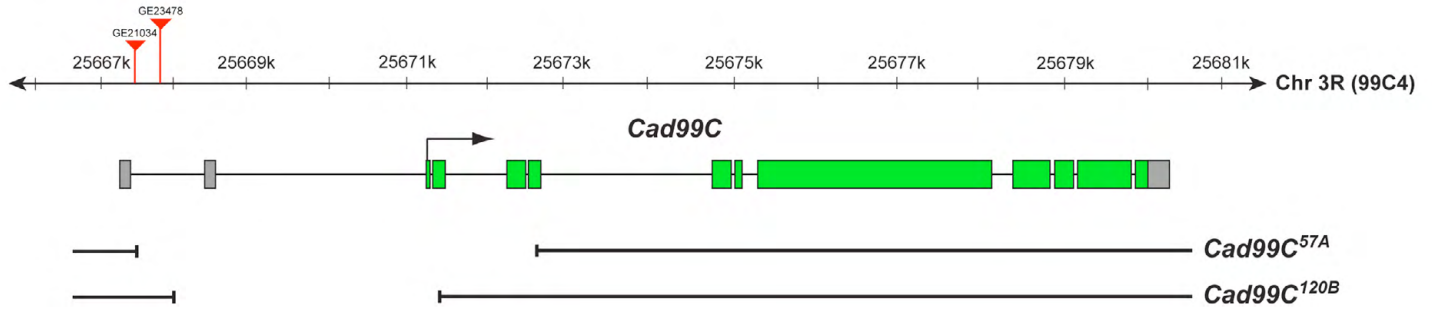


**Fig. S6. Relationship between salivary gland and CVM migration during normal embryogenesis.** (A-H) Embryos containing *UAS-lacZ* under control of the *5053A-GAL4* driver were stained using antibodies against  $\beta$ Galactosidase (red) to visualize the migrating CVM and LVMFs, and against Ph4 $\alpha$ SG2 (SG2) (green) to visualize the SG. (A) The SG and CVM are quite far apart at early stages of SG morphogenesis. (B) As the SG has completed invagination, the CVM is still quite posterior to the SG. (C) The distal region of the SG first contacts the CVM at stage 13. (D-H) The SG and CVM remain in contact at later stages. (G,H) At later stages, the *5053A-GAL4* driver is also expressed in ventral longitudinal muscle 12 (m12) (white arrow). All embryos are orientated anterior towards the left and posterior towards the right.





**Fig. S7. Loss of *AdamTS-A* does not result in increased apoptosis during embryogenesis.** (A-C) Stage 15 wild-type and *AdamTS-A*<sup>KO</sup> embryos were stained with using antibodies against an apoptosis marker CC3 (green) and a germ cell marker Vasa (red). Only cells in the anterior head region are CC3 positive (white arrowheads in A). (D-F) Stage 14 wild-type and *AdamTS-A*<sup>KO</sup> embryos containing the *croc-lacZ* reporter were stained using antibodies against  $\beta$ Galactosidase (red) to visualize the CVM, and against CC3 (green). CC3-positive cells were seen only in the anterior head region (white arrowheads in D). (G-I) Stage 14 wild-type and *AdamTS-A*<sup>KO</sup> embryos were stained using antibodies against CC3 (green) and a tracheal nuclear marker Tgo (red). CC3-positive cells were primarily seen in the anterior head region in both genotypes (white arrowheads in G and data not shown). (J-L) Stage 12 wild-type and *AdamTS-A*<sup>KO</sup> embryos were stained using antibodies against CC3 (green) and a SG nuclear marker  $\alpha$ -CrebA (red). CC3-positive cells were seen in the anterior head region (white arrowheads in J), and the same number of cells around the SG (white arrowheads in K,L).

**A.**

**Fig. S8. Cad99C proteins levels and distribution are unchanged in the *AdamTS-A* mutant SGs.** (A) *Cad99C* is located on the right arm of the third chromosome at cytological location 99C4. Two P-element excision alleles (*Cad99C*<sup>57A</sup> and *Cad99C*<sup>120B</sup>) were used in trans to each other for this study. (B-C'') Stage 12 wild-type and *AdamTS-A*<sup>KO</sup> embryos stained using embryos against *Cad99C* (green) and against an apical marker *Crb* (red). *Cad99C* localizes apically in the SG, as colocalization is seen with *Crb* (B''). No overt difference in *Cad99C* protein levels were in seen in *AdamTS-A* mutants compared with wild type (compare C,C'' with B,B'').



**A**

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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
MVLNLEPAGANLALGALALGSPVAAAPRQDCTGVCEPPLNCIEALEPGACCATCVQGGCACEGYQYDCLQGGFVR

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
GRVPAGQSYFVDFGSTECSPFPGGKISQFMLCPFLPPNCIEAVVVDSCPGQGVGVHAGNKYAAAGTVLPLPCFAC

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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FK-----DQPGDEIDKANSITDEGEFPFVREDNNVIVLTGDDICGKIENLCANIEENFDAGQKKEHPCFND
Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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STMDIEV---EDEDYIN---DRSGGPKPKQGRDG
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
HSAICSCFFGYALMADGVSC

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
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EPGAKK---EDVDECAHSHHCPSPHNDCHNRGFRCYRKTSTMLTTRTSTTVPLSLNARRSFTSRYPYPLAV

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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HPEYSQNDSTSNRRVQCPSPFNTECACTIDNECHEQ-NECGNHCRCINNGRFRGES-LDQCPGYKSTVQKSCSI
Hs_Fibulin1
Hs_Fibulin2
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Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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DDECDTGFENEGERTICRRNNGGVVSPPIGELKRSIGASTCVDTSCA-LEQVCPLNQGFNTIGALYCCNAGF
Hs_Fibulin1
Hs_Fibulin2
DDECAFFAEPCKRRHVSFGSFRCEKAGYFP---ISMVVDVNEGRVYFGLGH-KENTLGSYLSGSVGF
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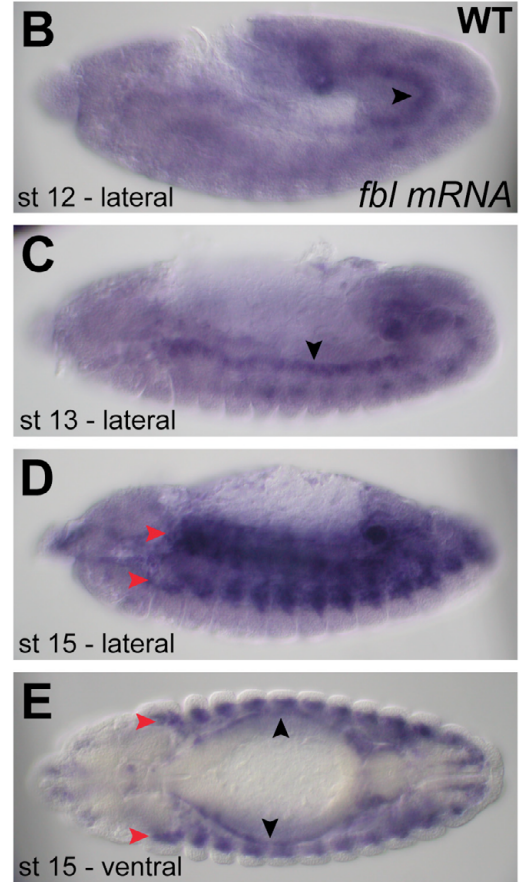
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Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
RSLVGR---SCDINCCSSS---SSGSAVYGSTQCVCRGQVLSDVDTCCEDIDECAPTE--GHISYACINTEG
LRLADGR---CEDVNECSAQR---SGGANYIGSYQCYCRGQVLA-PDRTCNDIDECAGGG---LTPFACINTEG

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin1
Hs_Fibulin2
SYGSCCP-ROHLAADMNCRDNDVDECA-DSINQVCTGRNDICTNRSSYKCTVNVPLGSLPDRNCRONLNFEG-
SYGSCCP-ROHLAADMNCRDNDVDECA-DSINQVCTGRNDICTNRSSYKCTVNVPLGSLPDRNCRONLNFEG-
SYGSCCP-ROHLAADMNCRDNDVDECA-DSINQVCTGRNDICTNRSSYKCTVNVPLGSLPDRNCRONLNFEG-

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
ECSKVPLFTYQISLARAVPSSRRPITLKVSAANNHDEEVNLEQVKTIVGAPVLPATRANLLQGEKRRSA
ECSYQSAFYVMTTVSRKTPD---RTITLRLGP-LWQDNIEQRIKVE-QATTIRKADGSDHLO--NNQV
Hs_Fibulin1
Hs_Fibulin2
ECSYQSAFYVMTTVSRKTPD---RTITLRLGP-LWQDNIEQRIKVE-QATTIRKADGSDHLO--NNQV
LQCNSARIRHEDNPGQLVVE---AHIIRIGAPAFGDTAANLIS---GHEBOYFGRRLLATG

Ce_Fibulin
Dm_Fibulin
Hs_Fibulin1
Hs_Fibulin2
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Hs_Fibulin2
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**Fig. S9. *Drosophila* Fibulin mRNA is expressed embryonically.** (A) Sequence line-up of *Drosophila* Fibulin (Dm Fibulin, CG31999), *C. elegans* Fibulin (Ce Fibulin), human Fibulin1 (Hs Fibulin1) and human Fibulin2 (Hs Fibulin2). Residues in magenta are identical among all four proteins. Residues in green are the same in two or three of the proteins and residues in yellow are conservative changes. (B-E) *fibulin* (*fbl*) mRNA is expressed during embryogenesis in the trunk visceral mesoderm (black arrowheads in B,C,E) and in the somatic mesoderm (red arrowheads in D,E).



**Table 1. Table of primers used for PCR of genomic and cDNA sequences of *AdamTS-A***

<b>AdamTS-A Knock-out (KO) primers</b>	
AdamTS-A HR primer1	5' TTGCGGCCGCCAGCGATTTC AAGGTTAGCCC 3'
AdamTS-A HR primer2	5' GGGGTACCGAGCTGAAAGATGGATAGGGAG 3'
AdamTS-A HR primer3	5' TTGGCGCGCCGCGACACACTCAGCTAACAGCAT 3'
AdamTS-A HR primer4	5' GGCGTACGGCACTTCTTGGTATTGTTCCCATTTG 3'
<b>AdamTS-A KO confirmation primers</b>	
pw25-5'	5' ACTGTGCGACAGAGTGAGAG 3'
pw25-3'	5' GGTGCGACTCTAGAGGATGAT 3'
AdamTS-A KO-F	5' CAGCTAATTGCGGACAAGCCTGTTGCAGCG 3'
AdamTS-A KO-R	5' ATGGATGGATGCCCTAGGCTTGTTCAGGG 3'
<b>UAS-AdamTS-A primers</b>	
UAS-AdamTS-A F2	5' CACCATGTCCACCCACTGGCGGCAG 3'
UAS-AdamTS-A R	5' TAACACGTCCAGGTAAAGGCC 3'
<b>ADAMTS-A MP domain primers</b>	
AdamTS-A MP 5'	5' GGGGATCCGGACGTGGACAACCAGGTG 3'
AdamTS-A MP 3'	5' GGGAATTCCTAGTGGAGCACCTTGTTGTT 3'

*AdamTS-A* Knock-out (KO) primers, primers used to generate the *AdamTS-A* knock-out construct; *AdamTS-A* KO confirmation primers, primers used to confirm the presence of the *white+* gene in place of *AdamTS-A* exons; *UAS-AdamTS-A* primers, primers used to generate both untagged and C-terminal GFP-tagged versions of *AdamTS-A*; ADAMTS-A MP domain primers, primers used to clone metalloprotease (MP) domain of *AdamTS-A* to purify a protein used to generate an antibody for ADAMTS-A.

♀ \ ♂	<i>AdamTS-A</i> <sup>KO</sup>	<i>Df(3R)88F5</i> <sup>e03525-d01653</sup>	<i>Df(3R)Exel</i> <sup>6174</sup>	<i>TM3</i>
<i>AdamTS-A</i> <sup>KO</sup>	---	0:84	0:103	0:81
<i>Df(3R)88F5</i> <sup>e03525-d01653</sup>	0:97	---	0:97	0:100
<i>Df(3R)Exel</i> <sup>6174</sup>	0:108	0:100	---	2:85
<i>TM3</i>	0:95	2:100	2:101	---

**Table S2. Complementation test of *AdamTS-A*<sup>KO</sup> with two deficiencies and TM3 allele.** All the lines were balanced over the TM6b balancer and scored for absence or presence of TM6b (TM6b<sup>-</sup>:TM6b<sup>+</sup>). The *AdamTS-A*<sup>KO</sup> completely fails to complement two different deficiencies in the region, as well as the TM3 allele. A few escapers were seen when either deficiency was in *trans* to the TM3 allele.