Alary Muscles and Thoracic Alary Related Muscles: Atypical striated muscles maintaining internal organs positions

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ABSTRACT : Alary muscles (AMs) have been described as a component of the cardiac system in various arthropods. Lineage-related thoracic muscles (TARMs), linking the exoskeleton to specific gut regions, have recently been discovered in Drosophila. Asymmetrical attachments of AMs and TARMs, to the exoskeleton on one side and internal organs on the other, suggested an architectural function in moving larvae. Here, we analyzed the shape and sarcomeric organisation of AMs and TARMs, and imaged their atypical deformability in crawling larvae. We then selectively eliminated AMs and TARMs by targeted apoptosis. Elimination of AMs revealed that AMs are required for suspending the heart in proper intra-haemocelic position and for opening of the heart lumen, and that AMs constrain the curvature of the trachea during crawling. ;ARMs are required for proper positioning of visceral organs and efficient food transit. AM/TARMcardiac versus visceral attachment depends on Hox control, with visceral attachment being the ground state. TARMs and AMs are the first example of multinucleate striated muscles connecting the skeleton to the cardiac and visceral systems in bilaterians, with multiple physiological functions.

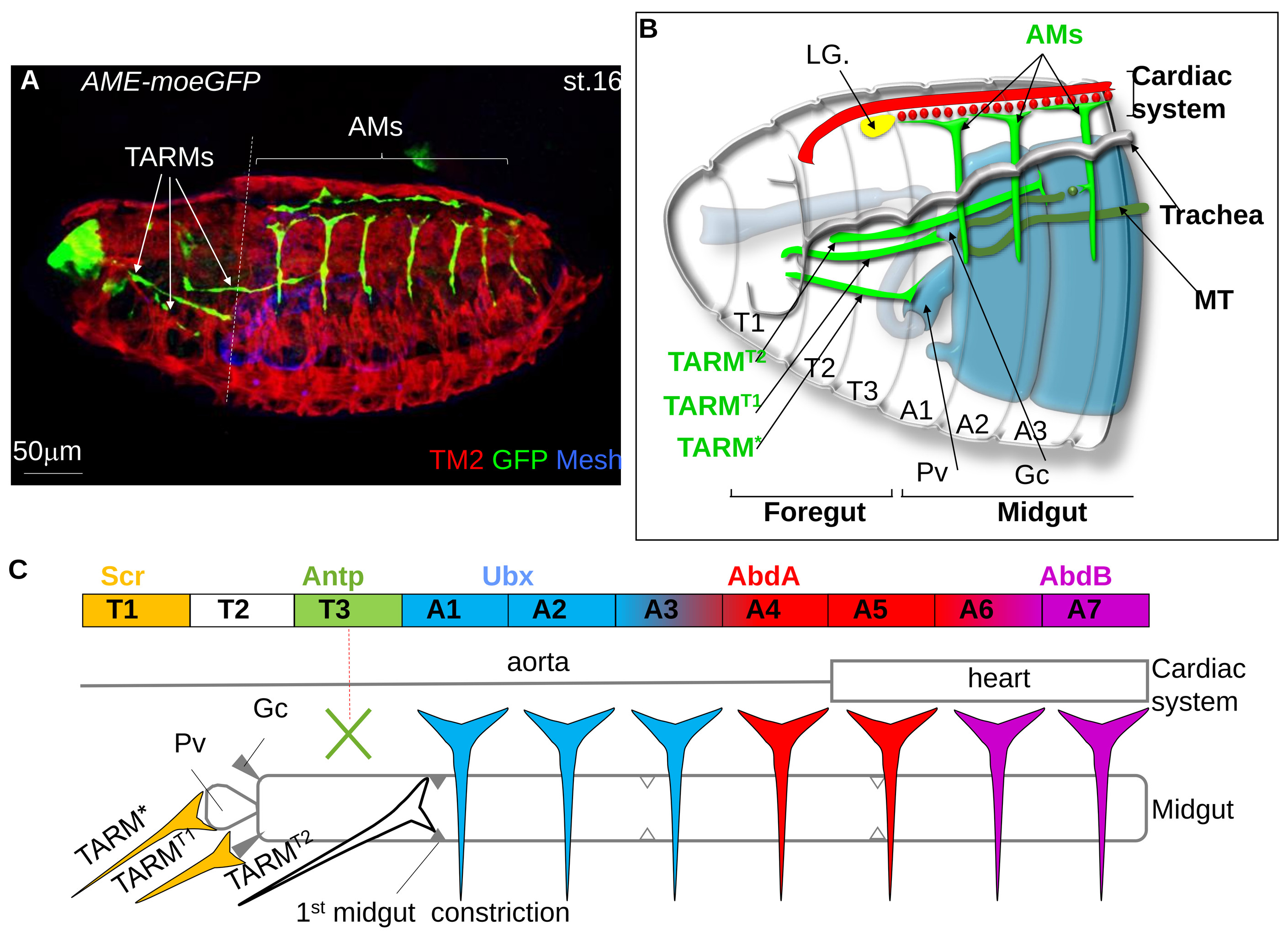


Figure 1: TARMs/AMs attachment to specific internal organs is under Hox control
(A) Stage 16 embryo showing the AMs and TARMs (green), somatic muscles (red), and gut contours, (blue)
(B) Schematized AMs connections to the cardiac system (red), lymph gland (LG, yellow) and tip cell of the Malpighian Tubule (MT, orange) and TARMs connections to the proventriculus (Pv), dorsal gastric caeca (Gc), and first midgut constriction. *Modified from Boukhatmi et al., Development, 2014.*
(C) Colored Scr, Antp, Ubx, Abd-A and Abd-B expression in TARMs/AMs. Antp expression induces TARM apoptosis and the background state is visceral attachment.

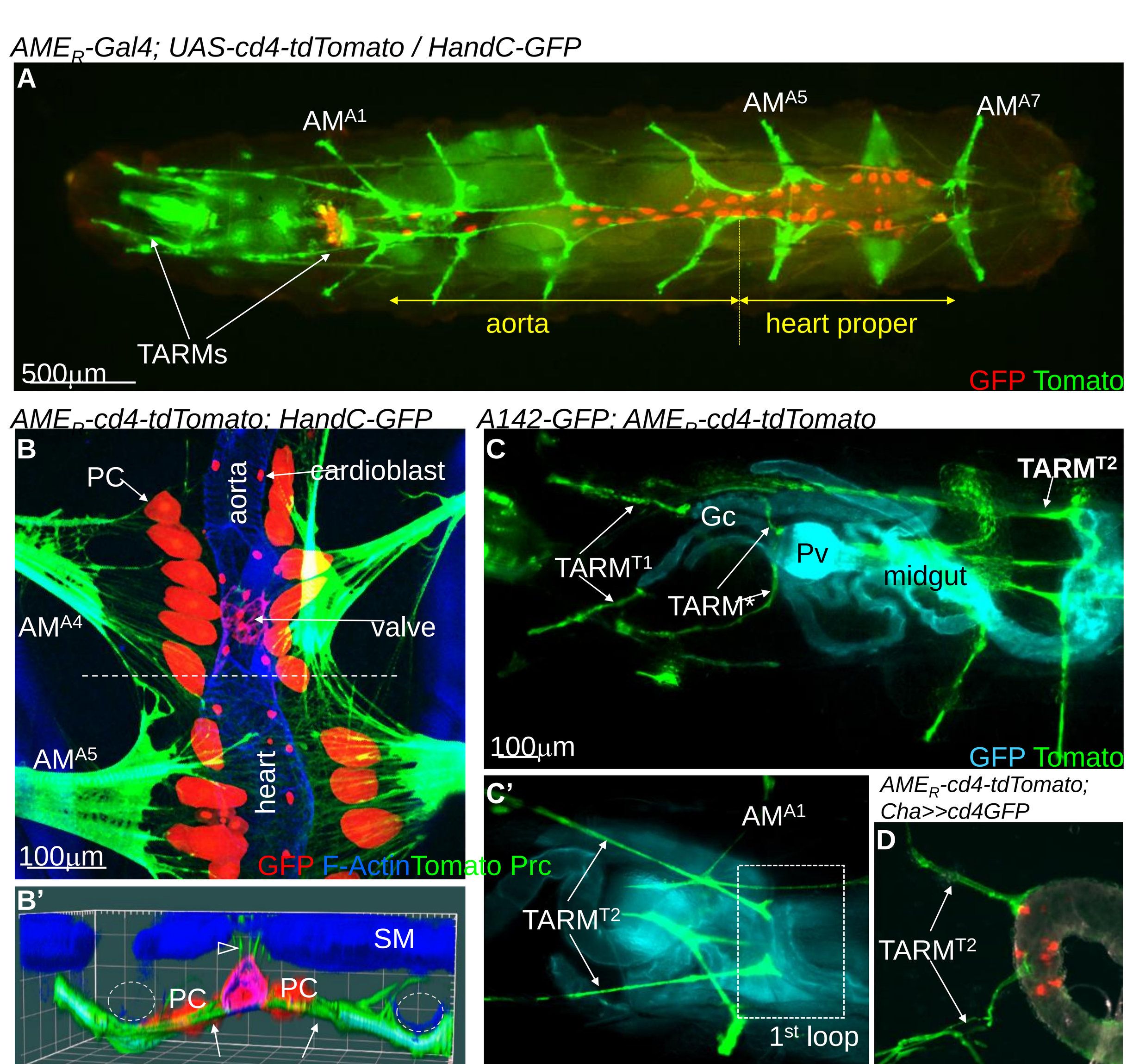


Figure 2: AMs and TARMs adopt specific shapes to connect to different larval internal organs.
(A) Dorsal view of an intact L3 larva, showing AMs and TARMs in green and pericardial cells (PCs) and cardioblasts in red. Anterior AMs connecting to the aorta display a "T" shape, posterior AMs maintain a delta-shaped connection to PCs distributed long the heart.
(B, B') Heart attachment to AMs and the dorsal wall is mediated by ECM
(C-D) TARMT2 connects to the first midgut loop, where enteroendocrine cells (red in D) expressing the diuretic hormone DH31 are located.

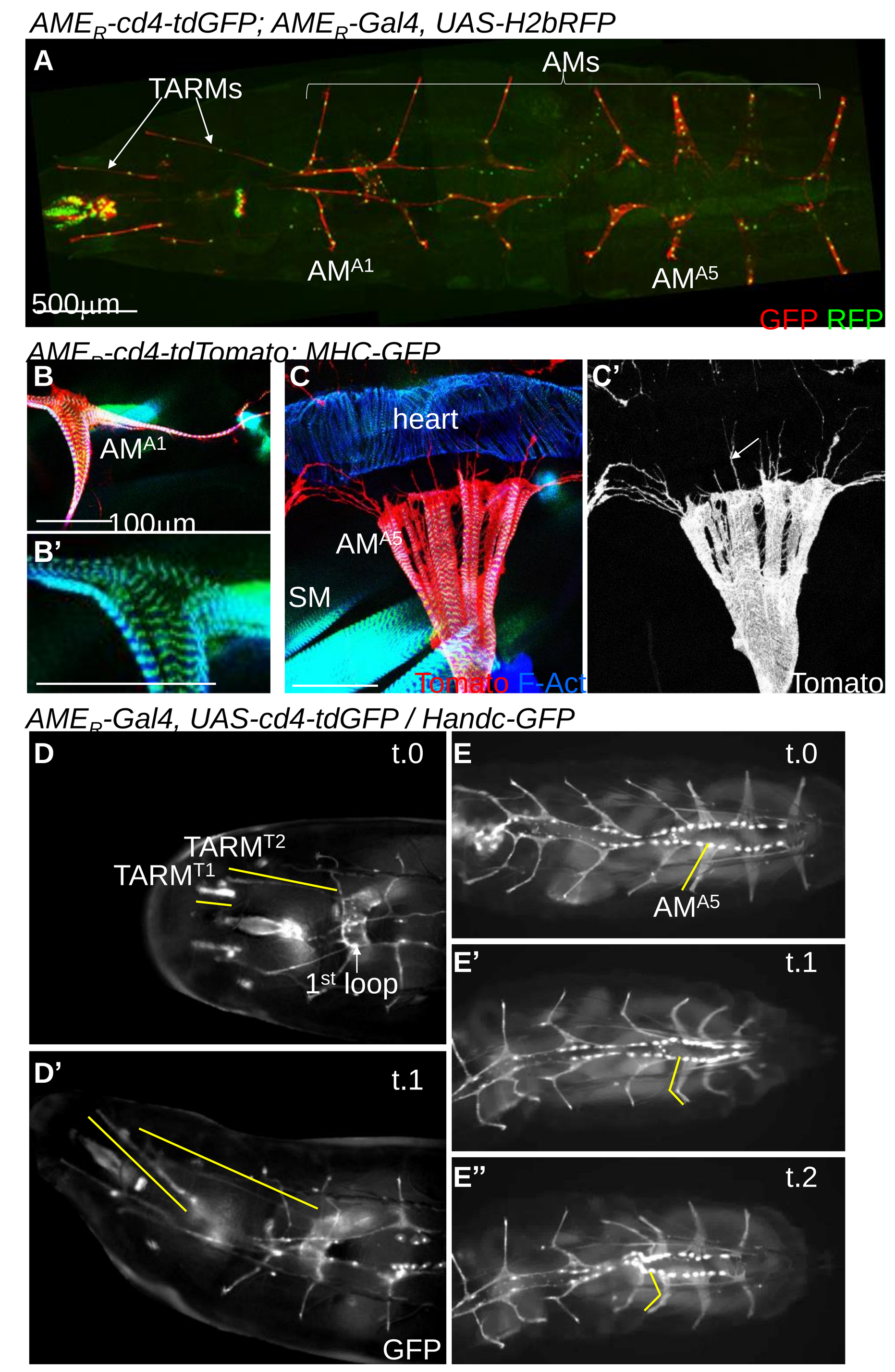


Figure 3: **AMs and TARMs are multinucleated, sarcomeric, contracting, and uniquely deformable muscles.**
(A) Dorsal view of an L3 larva showing the nuclei distribution (green) in AMs and TARMs (red).
(B-C) Dissected L3 larva showing the striated structure of AMs and multiple dorsal protrusions.
(D-E) Snapshots of a crawling larva (*see movies in Development)*, illustrating the high deformability of TARMs and AMs.

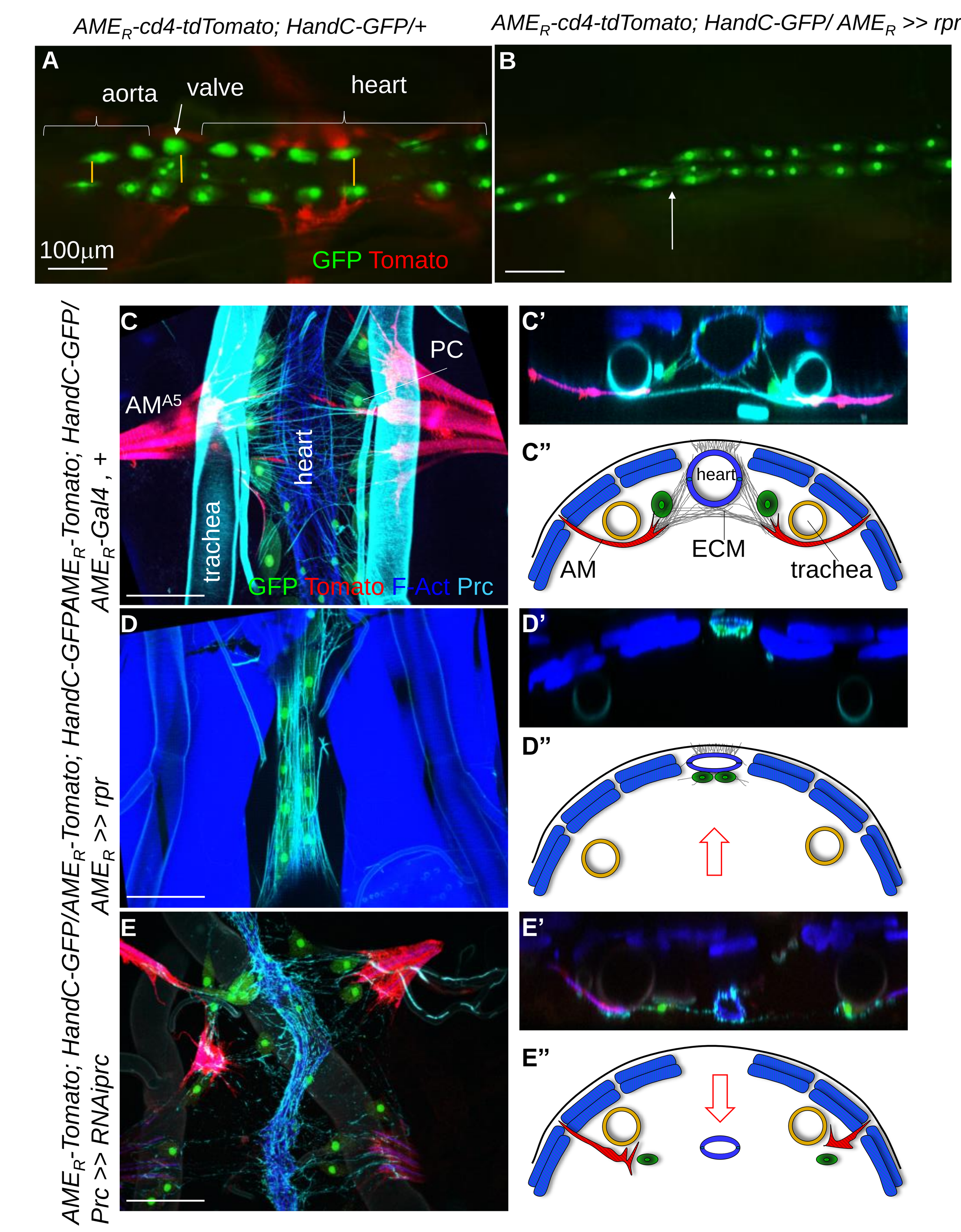


Figure 4: AMs ablation induces a collapse of the dorsal vessel
(A-B) Dorsal views of control (A) and >>*UAS-rpr* (no AMs) larvae (B); AMA4-A6 region. AMs and TARMs are in red and PCs, cardioblasts and valve cells in green. The number of PCs is unchanged following AMs ablation but the heart lumen collapses. Heart beating is normal (not shown).
(C-E") *control* (C), >>*UAS-rpr* (no AMs, D), and *UAS-RNAi-pericardin* (no ECM, F) larvae. PCs and cardioblasts are green, AMs red, somatic muscles and heart blue, the ECM and trachea, cyan.
(C', D', E') transversal views. (C", D"', E") Corresponding chemes illustrating the forces exerted by AMs and dorsal anchoring "suspending" the heart within the body.

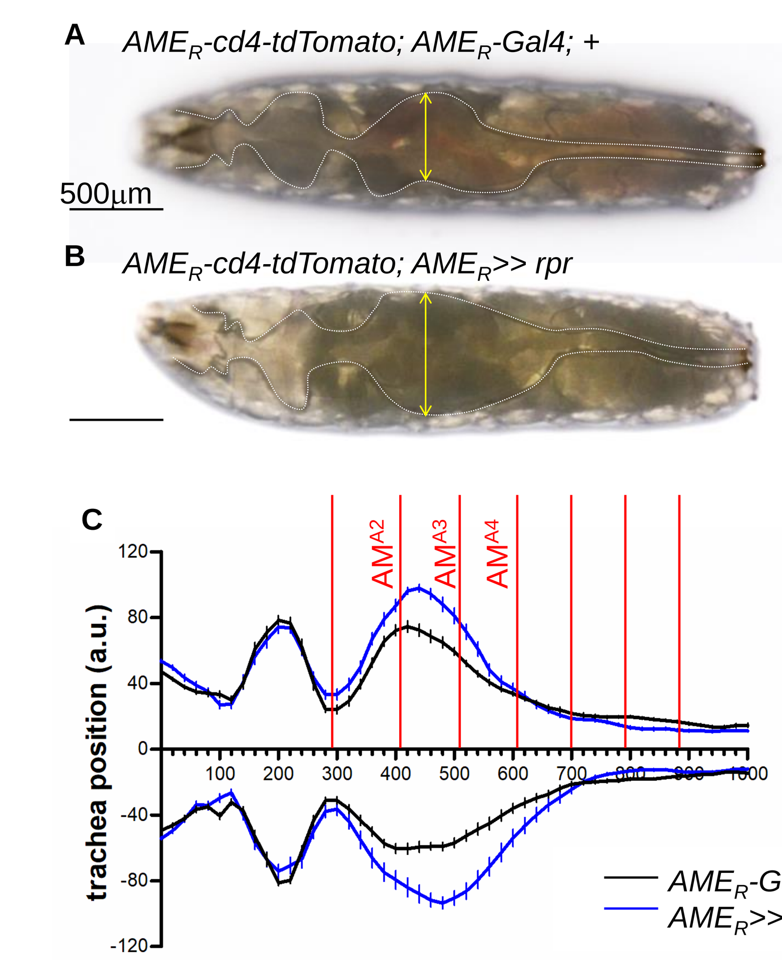


Figure 5: AMs constrain the tracheal curvature in larvae
(A, B) Dorsal views of *control* (A) and >>*rpr* (no TARMs/AMs, B) larvae. The tracheal trunk is underlined in white and spacing indicated by double arrows.
(C) Tracheal curvatures in *AMER-Gal4* (black line) and >>*rpr* (blue line) L3 larvae at the end of a peristaltic wave. The abdominal curve is larger and dorsal trachea more distant from the midline

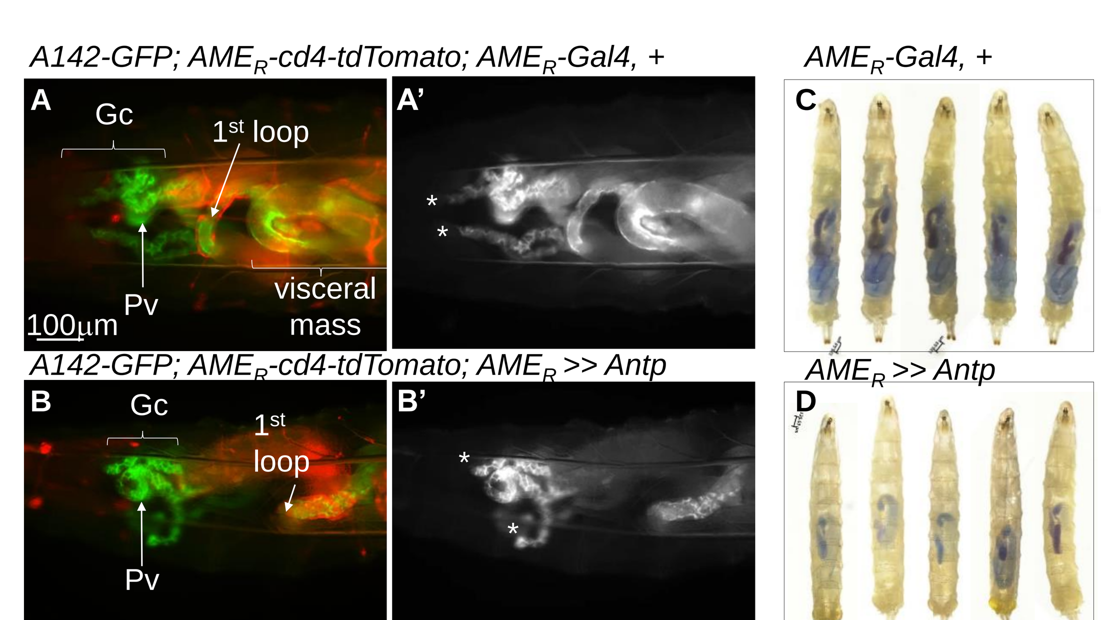
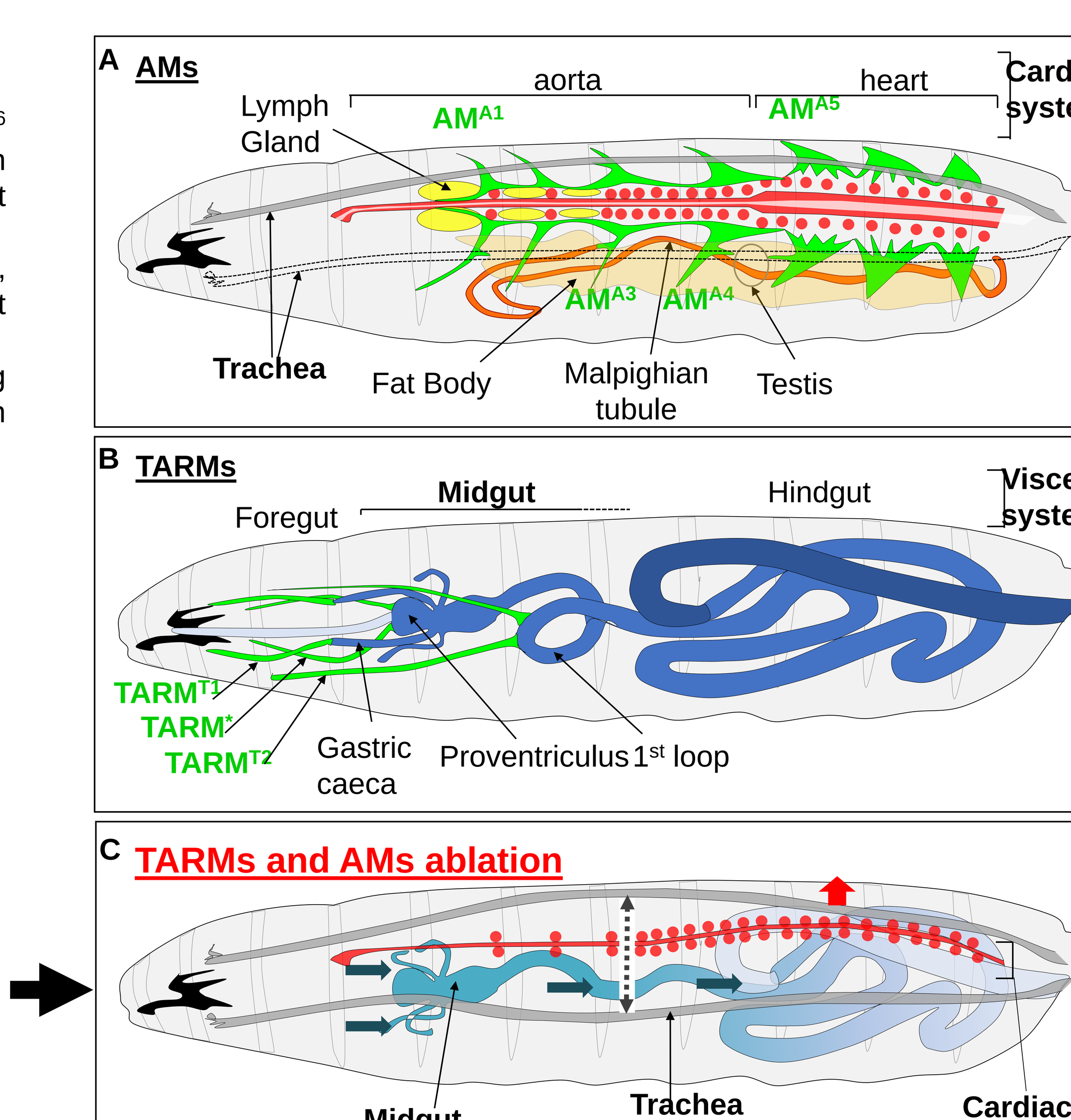


Figure 6: TARMs are required for proper topology of visceral organs and efficient food transit
(A, B) Anterior region of *control* and >>*Antp* (no TARM) larvae; AMs and TARMs are red, visceral organs green. (A', B') GFP channel only. TARMT1 maintain Gc extension (asterisk) and TARMT2 the position of the 1st loop of the gut.
C, D) ventral views of *control* and >>*Antp* (no TARM) larvae fed 45 min with blue yeast. TARMS are required for efficient food transit to the gut.



SUMMARY Figure 7: TARMs and AMs maintain internal organs positions in larva.
(A-C) Diagrammatic representations of a fully extended L3 larva (adapted from Hartenstein, 1993). (A) AMs connections to the cardiac system (red), LG (yellow), anterior MTs (brown) and Fat body (orange), and surrounding of trachea (grey).
(B) TARMs connections to specific midgut regions (blue).
(C) Mis-positioning of multiple internal organs, following elimination of TARMs and AMs, is indicated by arrows. The heart lumen is collapsed.

CONCLUSION and PERSPECTIVES : *Drosophila* organogenesis along with morphogenesis during embryonic development ends with larval hatching. Here we show that, during larval life, multiple internal organs remain physically connected to the exoskeleton by a web of thin and deformable muscles, the AMs and TARMs (Fig. 7). These atypical, asymmetrically attached muscles are required to maintain proper position of cardiac, visceral and respiratory organs within the coelomic cavity, and heart lumen opening, To the best of our knowledge, TARMs and AMs are the first example of multinucleate striated muscles connecting the (exo)skeleton to internal organs in bilaterians. It raises novel anatomical, physiological and evolutionary questions. Innervation of the heart and alary muscles in adult arthropods remains controversial. Integrating neuro-muscular connectivity into physiological roles of TARMs and AMs is a next challenge.