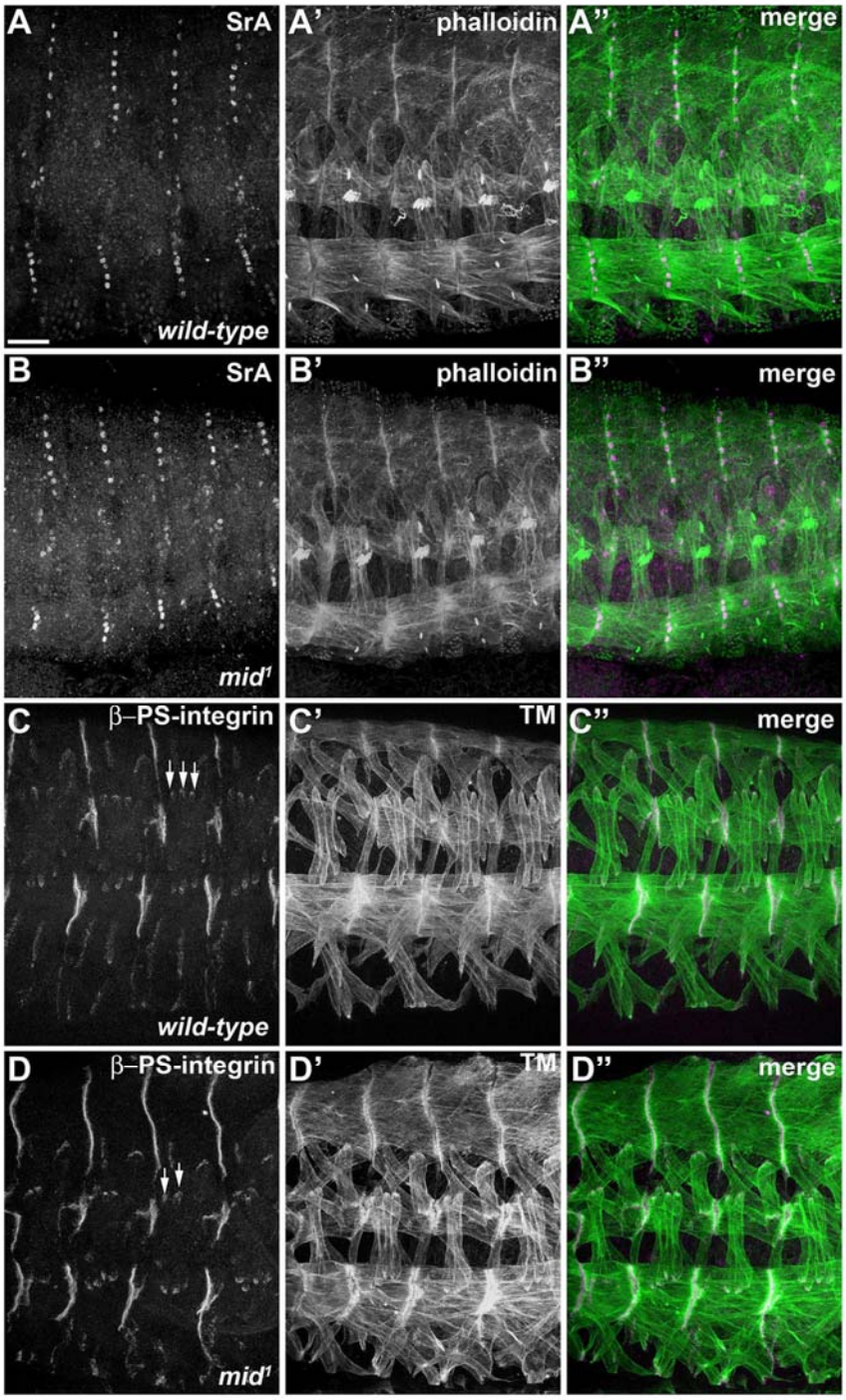

Figure S1 Tendon cells are specified and β-PS-Integrin marks attachment sites in *mid* mutants. Lateral views of fixed stage 16 embryos. (A-B") Embryos immunostained for StripeA (SrA - white in single channel, magenta in merge) and phalloidin (white in single channel, green in merge), to mark the actin cytoskeleton. (C-D") Embryos immunostained for β-PS-Integrin (white in single channel, magenta in merge) and tropomyosin (tropo - white in single channel, green in merge). (A-A", C-C'') wild type embryo. (B-B", D-D'') *mid*^{1} homozygote. Arrows point to LT muscle attachment. Scale bar: 25µm.

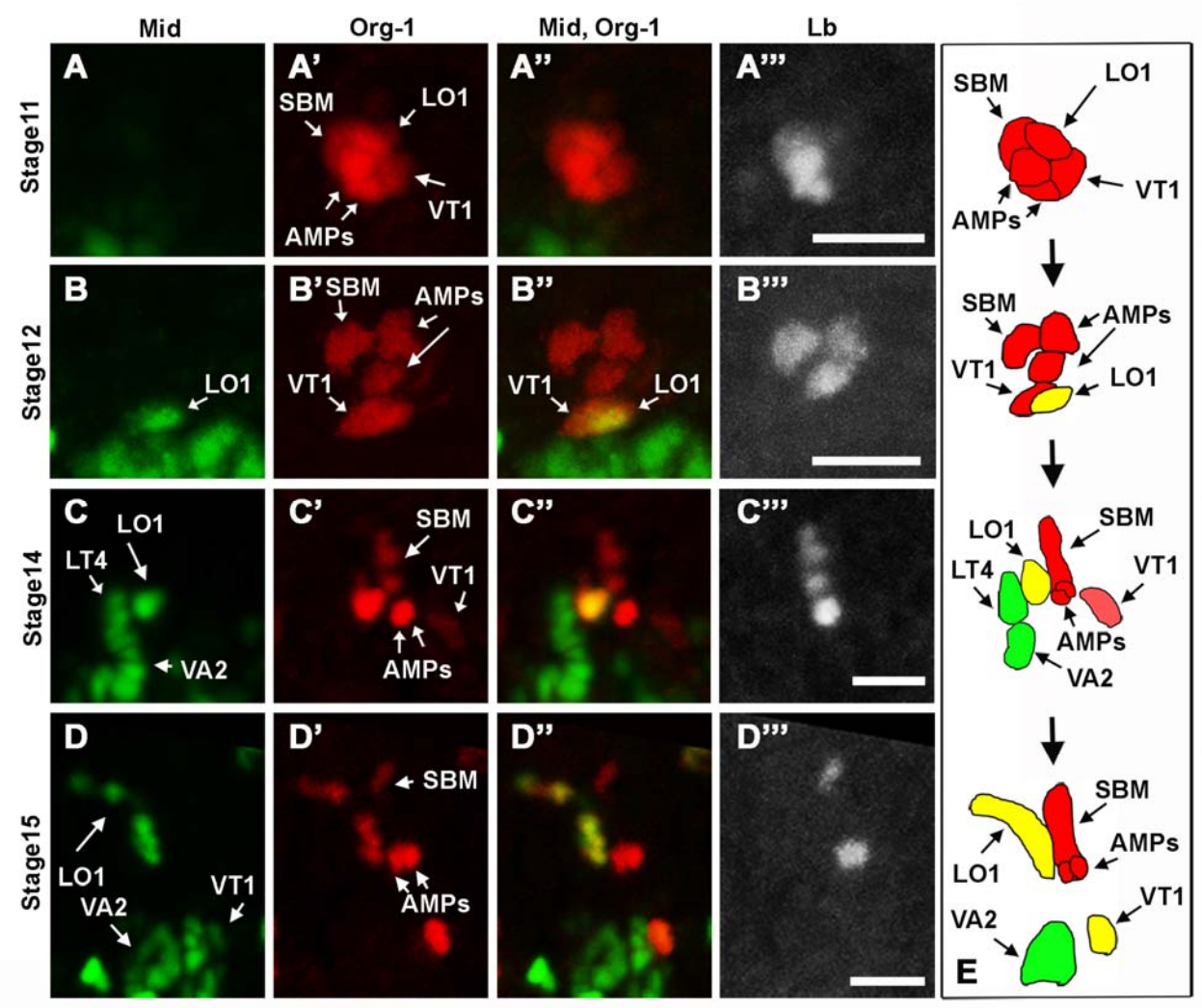

Figure S2 Mid expression is detected in the LO1/VT1 lineage but is absent from the SBM/AMP lineage. Lateral view of a single abdominal hemisegment in embryos immunostained for Mid, Org-1 and Lb. (A) At stage 11, Org-1 and Lb expressing cells are clearly detectable. (B-D) Mid is visible with Org-1 in the FC and growing myotube for LO1 but is not detected in the sibling VT1 muscle until stage 15. Note that Mid is never detected in the SBM, which is marked by expression of both Org-1 and Lb, despite robust Mid in other FCs and myotubes. Nevertheless, as discussed in the text, the SBM is lost in *mid* and *mid, H15* mutants and ectopic Lb-expressing cells are detected upon pan-mesodermal expression of Mid. Scale bar: 10µm. (E) Schematic representation of Mid, Org-1 and Lb expression during LO1, VT1 SBM and AMPs development.

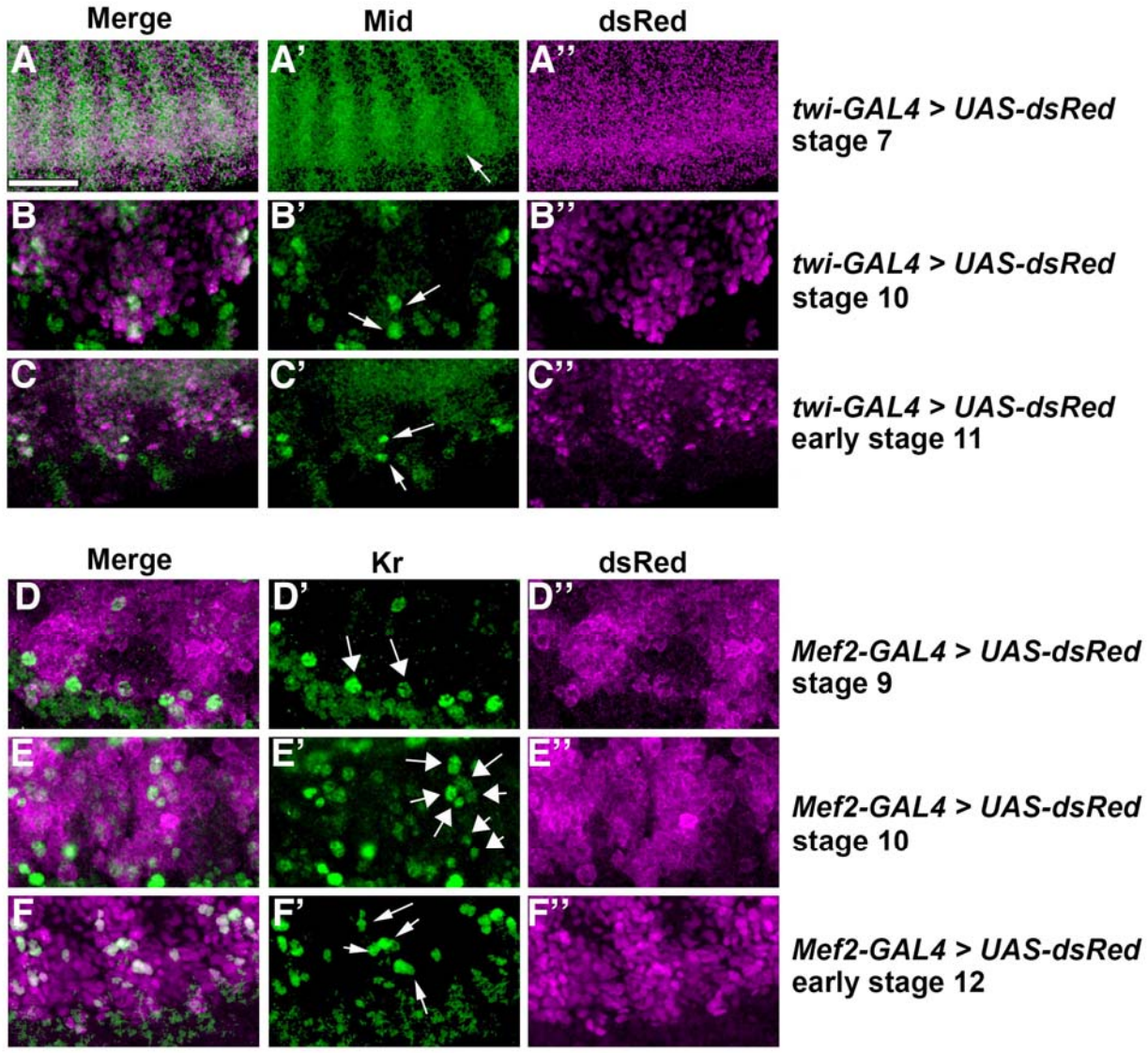

Figure S3 Expression of GAL4 lines used in this study. *twi-GAL4* and *Mef2-GAL4* are expressed during LT formation and development, and their expression overlaps with Mid. (A-A''') Panels show embryos in which *twi-GAL4* drove a *UAS-nuc::dsRed* construct. Embryos were stained with anti-dsRed (magenta) and anti-Mid (green). Arrow points to co-expression at stage 7. (B-B''') *twi-GAL4 > UAS-nuc::dsRed* embryos at stage 10. Arrows point to the Mid-expressing progenitors. (C-C''') *twi-GAL4 > UAS-nuc::dsRed* embryos at early stage 11. Arrows point to the Mid-expressing progenitors. (D-D''') *Mef2-GAL4 > UAS-nuc::dsRed* embryos stained with anti-Kr (green) and anti-dsRed (magenta). Arrows point to Kr-expressing founders at late stage 11.

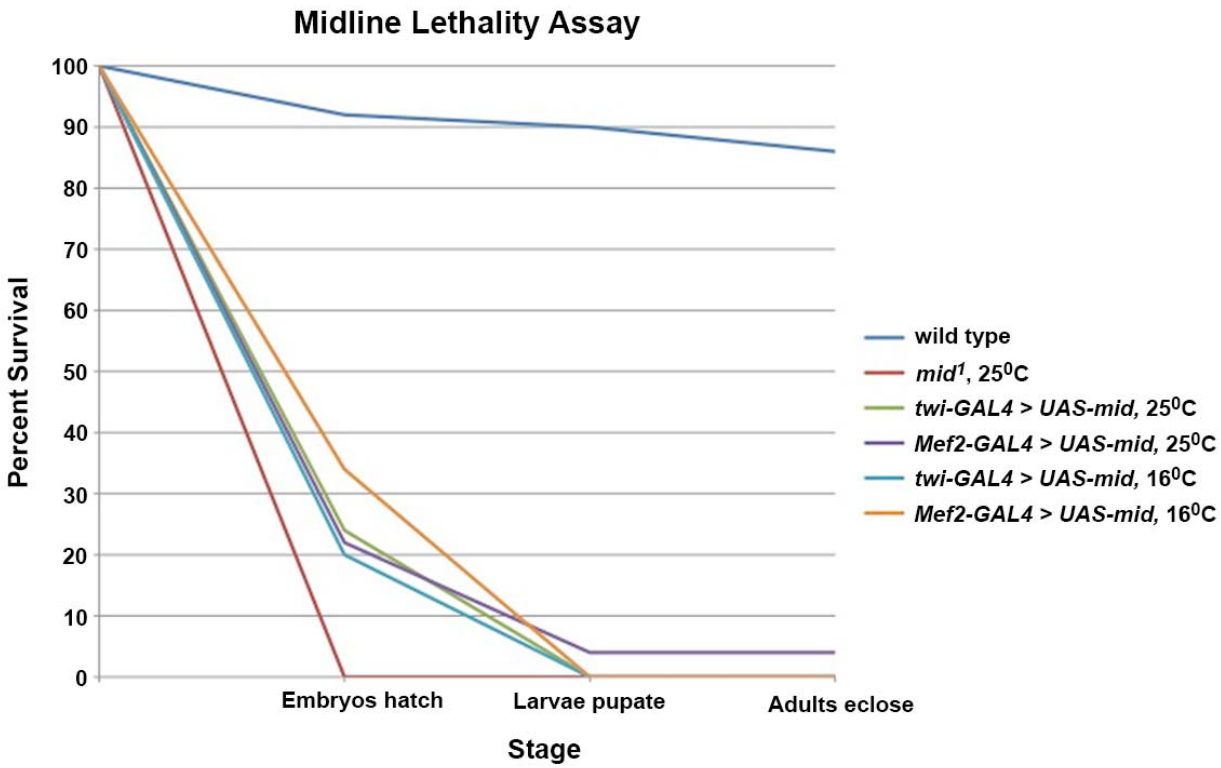

Figure S4 Viability of Midline Loss and Gain. Graph depicting viability of embryos, larvae, pupae and adults of the indicated genotypes, measured as a percent of survivors/total number of embryos selected. Fertilized embryos of the appropriate genotype were hand-selected using fluorescently-marked balancer chromosomes to identify homozygotes. Embryos were then transferred to a plate and monitored for hatching. First instar larvae were then transferred to a vial containing standard cornmeal media for the remainder of the experiment. For all genotypes, n=50.

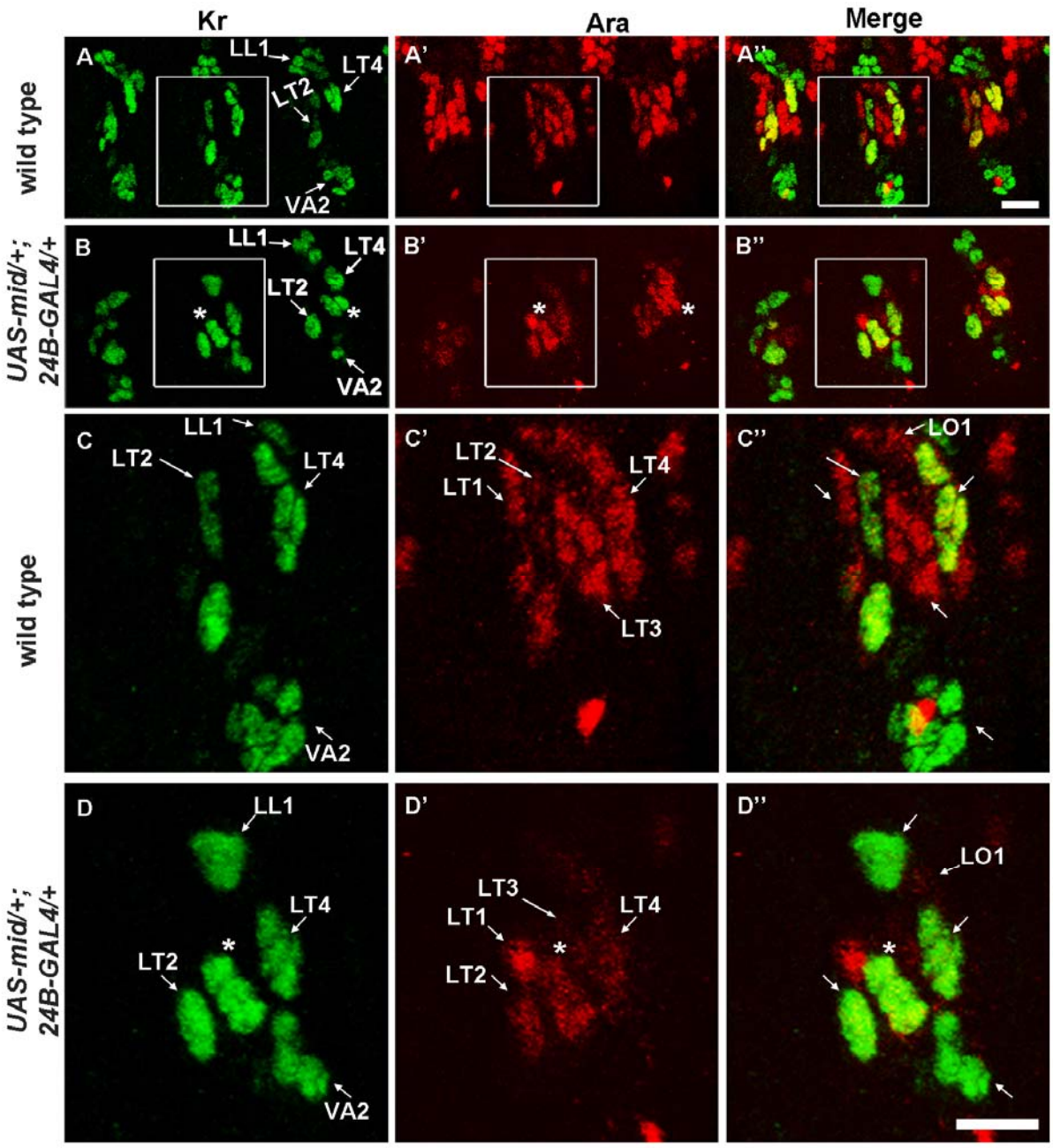

Figure S5 Extra Kr-expressing cells generated by pan-mesodermal expression of Mid also express Ara. Lateral views of stage 15 embryos immunostained for Kr and Ara. (A) wild type and (B) *UAS-mid/+; 24B-GAL4/+*. Extra Kr positive cells observed upon pan-mesodermal expression of Mid also expressed the LT marker Ara (asterisks). (C-C''') Enlarged view from A (white box). (D-D''') Enlarged view from B (white box). These data indicated the LT-like identity of the ectopic Kr-expressing cell generated by ectopic Mid. Scale bar: 10µm.

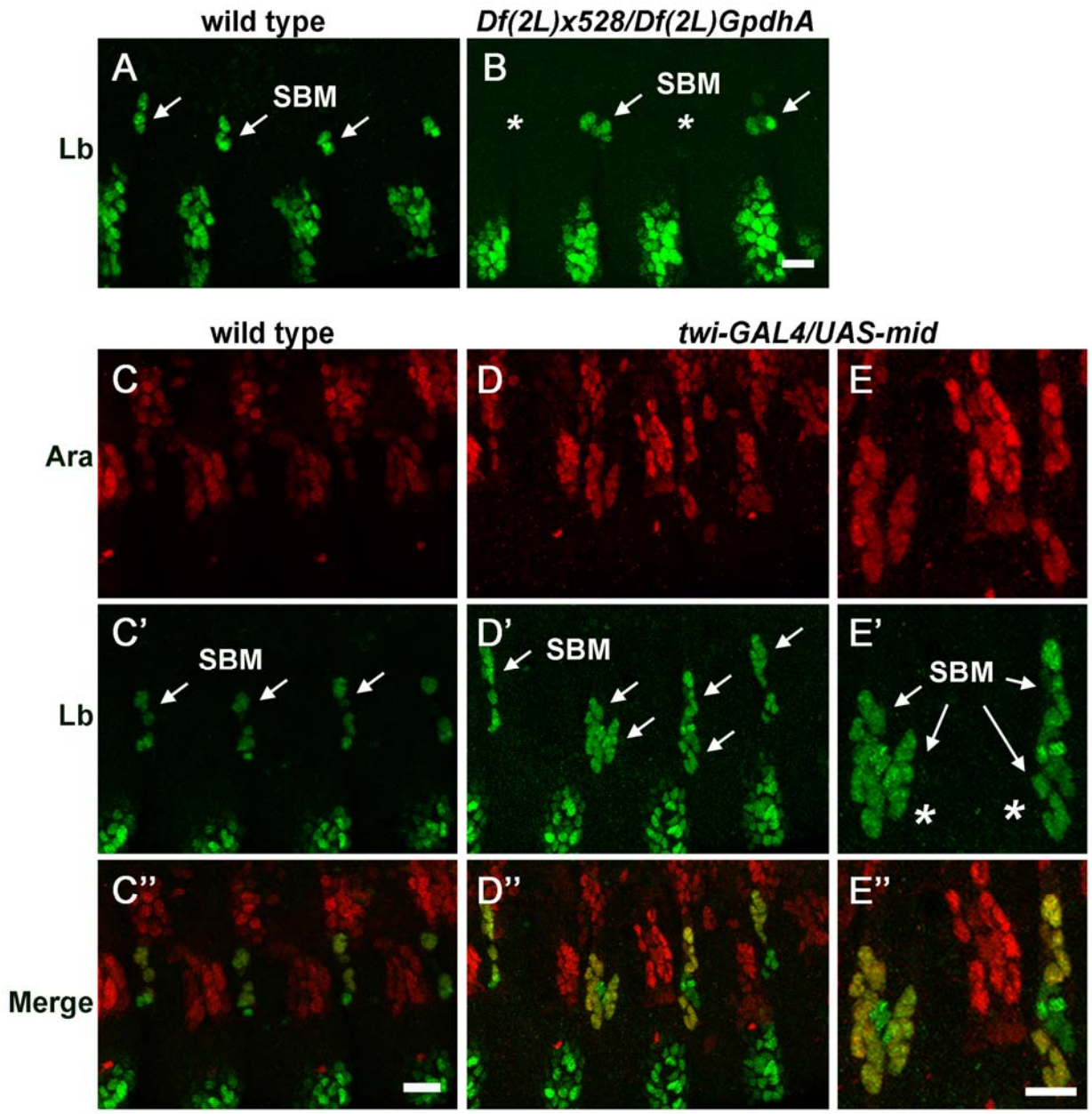

Figure S6 Founder cells for the SBM is impacted by the loss of *mid* and pan-mesodermal expression of Mid generates extra Lb- expressing cells. Lateral views of abdominal hemisegments from stage 14 (A-B) and stage 15 (C-E) embryos immunostained for Lb (green, A-B, and C'-E'), Ara (red, C-E). (A, C-C'') wild type. (B) *Df(2L)x528/Df(2L)GpdhA* which lacks both *mid* and *H15* and (D-E) *twi-GAL4/UAS-mid.* Asterisks indicate missing FCs for SBM (B). The presence of ectopic Lb-expressing muscles upon pan-mesodermal expression of Mid are clearly visible in some segments. (E-E") Enlarged view from D-D" (white box). Note that additional Lb and Ara expressing syncytia are apparent (asterisks). Scale bar: 10µm.

Table S1 Quantification of Somatic Muscle Defects in *mid* Mutants

	% Affected Hemisegments	% Affected Embryos
mid^{1}	100	100
mid^{los1}	100	100
mid^{2}	100	100
mid^{1}*/Df(2L)BSC810*	100	100
wild type	13	3

Stage 16 embryos of the indicated genotypes were stained with an antibody against myosin heavy chain and scored for muscle defects. For all genotypes, five abdominal hemisegments A3-A7 were scored in 20 embryos, for a total of 100 hemisegments quantified.