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Supplementary Materials for

The Homeobox Transcription Factor Cut Coordinates Patterning and Growth During *Drosophila* Airway Remodeling

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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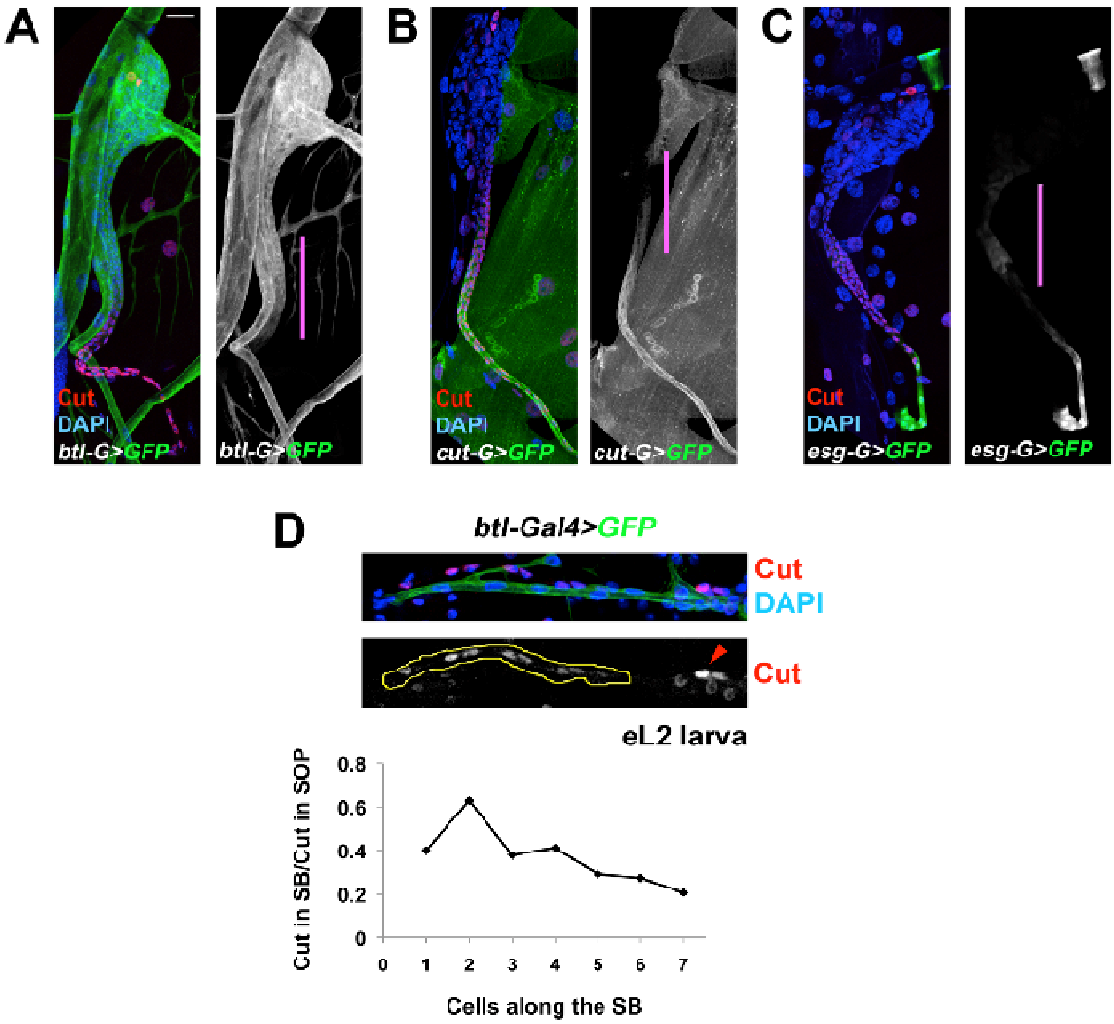

Fig. S1. The Cut gradient in flies of different genotypes and stages. (**A**) *btl-Gal4 UAS-srcGFP* (**B**) *cut-Gal4; UAS-srcGFP* and (**C**) *esg-Gal4 UAS-GFP* wild-type SBs of late L3 larvae stained for GFP (green), Cut (red), and DAPI (blue). Purple line indicates zone 2 defined on the basis of its position at the junction of TC and SB. Gray images show the indicated reporter only. (**D**) Wild-type SB of an early L2 *btl-Gal4 UAS-srcGFP* larva stained for Cut (red) and DAPI (blue). Yellow line outlines the entire SB and red arrowhead indicates the Cut-positive SOP close to the DT. The graph shows normalized Cut abundance along the length of the SB.

Fig. S2. Signaling pathway genes are differentially expressed in zone 1 and zone 3 SB cells. Heat map of differentially expressed signaling pathway genes between wild-type zone 1 and zone 3. Duplicate microarrays (1, 2) are shown for each condition.

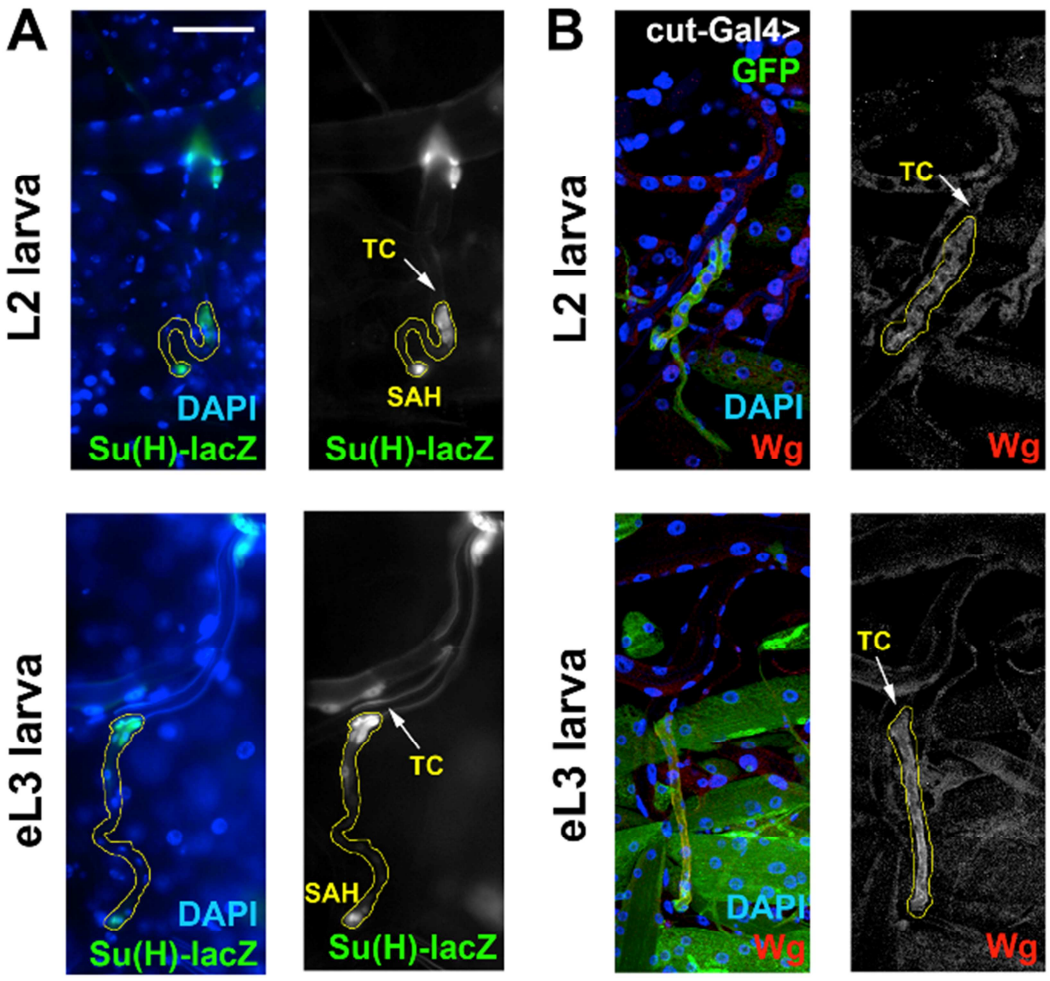

Fig. S3. The Notch and Wg pathways are activated during early SB development. (**A**) *Su(H)-GBE-lacZ*, a reporter of Notch activity, L2 and early L3 (eL3) larval SBs stained for β-galactosidase (green) and DAPI (blue). The reporter is active in 2 to 3 cells at the junction with the transverse connective (TC) and in the spiracular abdominal histoblasts (SAH). (**B**) *cut-Gal4; UAS-srcGFP*, a reporter for *cut* promoter activity, L2 and early L3 larval SBs stained for Wg (red) and DAPI (blue). Wg is present in all Cut-positive SB cells in eL3. Scale bar: 20 μm.

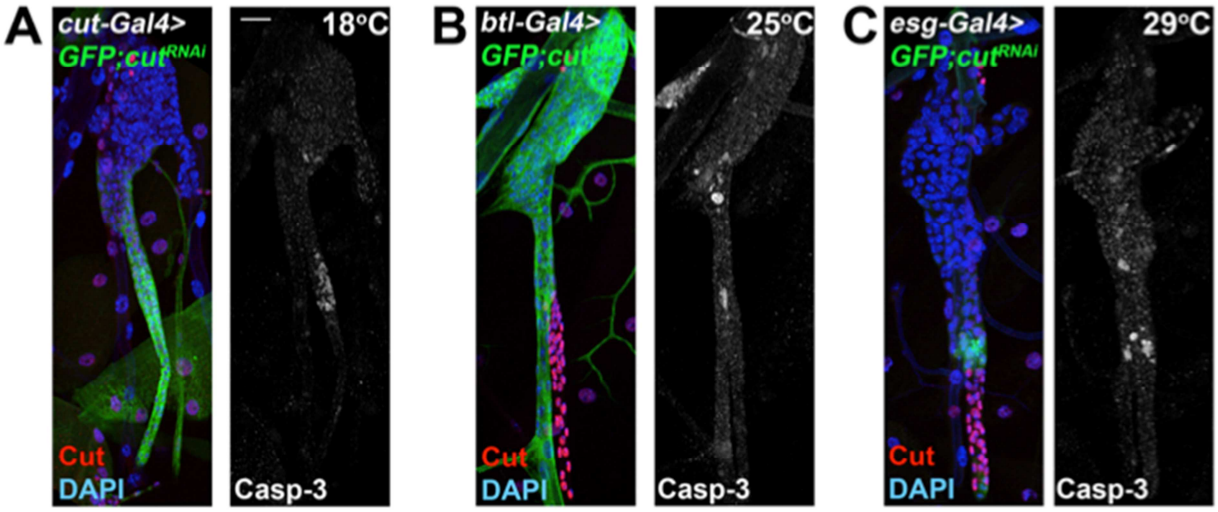

Fig. S4. A temperature-sensitive *UAS-cut*^{RNAi} line recapitulates the loss of *cut*. (**A**) *cut-Gal4; UAS-srcGFP; UAS-cut*^{RNAi} reared at 18^0C, (**B**) *btl-Gal4 UAS-srcGFP; UAS-cut*^{RNAi} reared at 25^0C, and (**C**) *esg-Gal4 UAS-GFP; UAS-cut*^{RNAi} reared at 29^0C. L3 SBs stained for Cut (red), DAPI (blue), and Caspase-3 (gray). Scale bar: 20 μm. Three independent RNAi lines targeting *cut* gave the same phenotype.

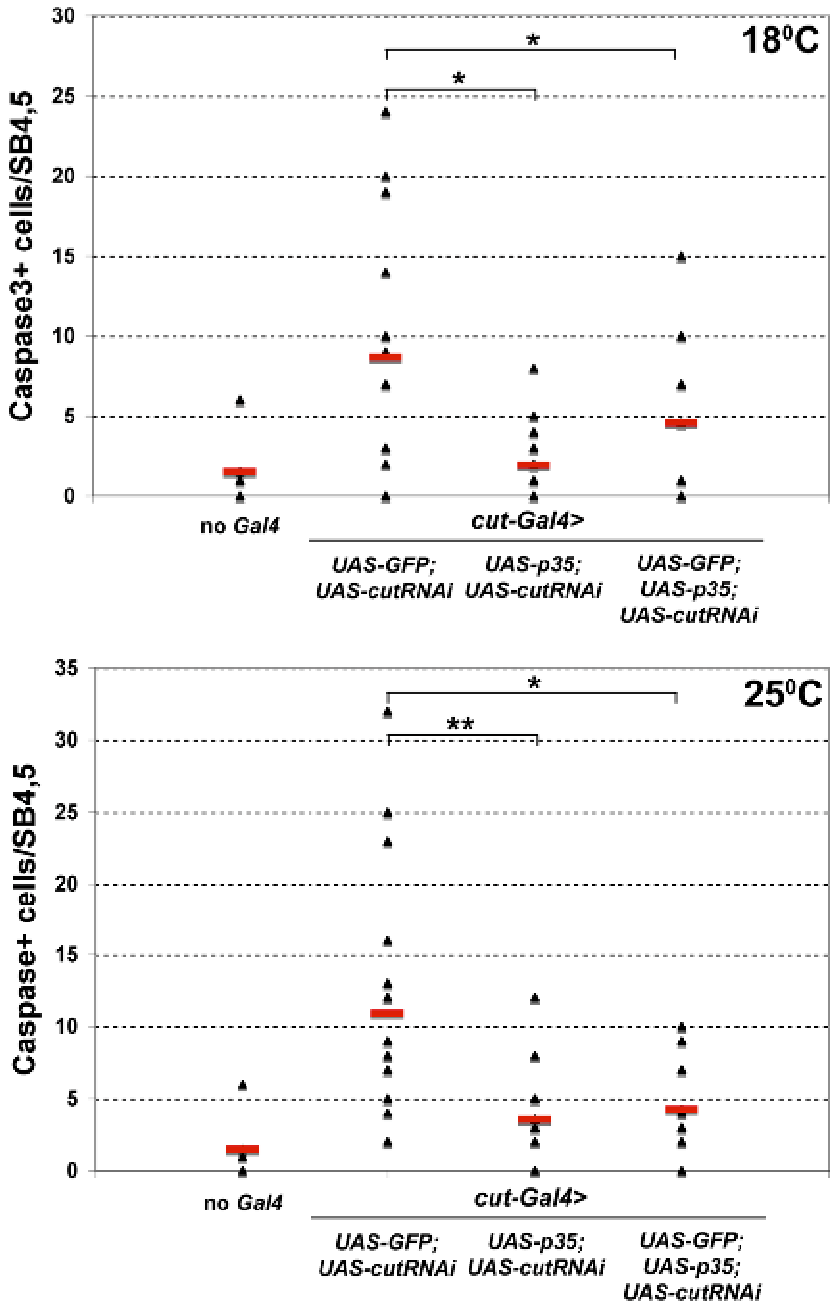

Fig. S5. p35 prevents the apoptosis induced by expression of *UAS-cut^{RNAi}* at 18^{0}C and 25^{0}C. L3 larvae of the indicated genotypes were dissected and stained for Caspase-3 to measure apoptotic cells. The graphs show the distribution of Caspase-3-positive cells in the SBs of the tracheal metameres Tr4 and Tr5 (SB4,5) and the red bar indicates the average (n > 10 SBs; *, $p < 0.05$; ** $p < 0.001$ by student's t-test). Inhibition of apoptosis is not dependent on the number of UAS transgenes expressed.

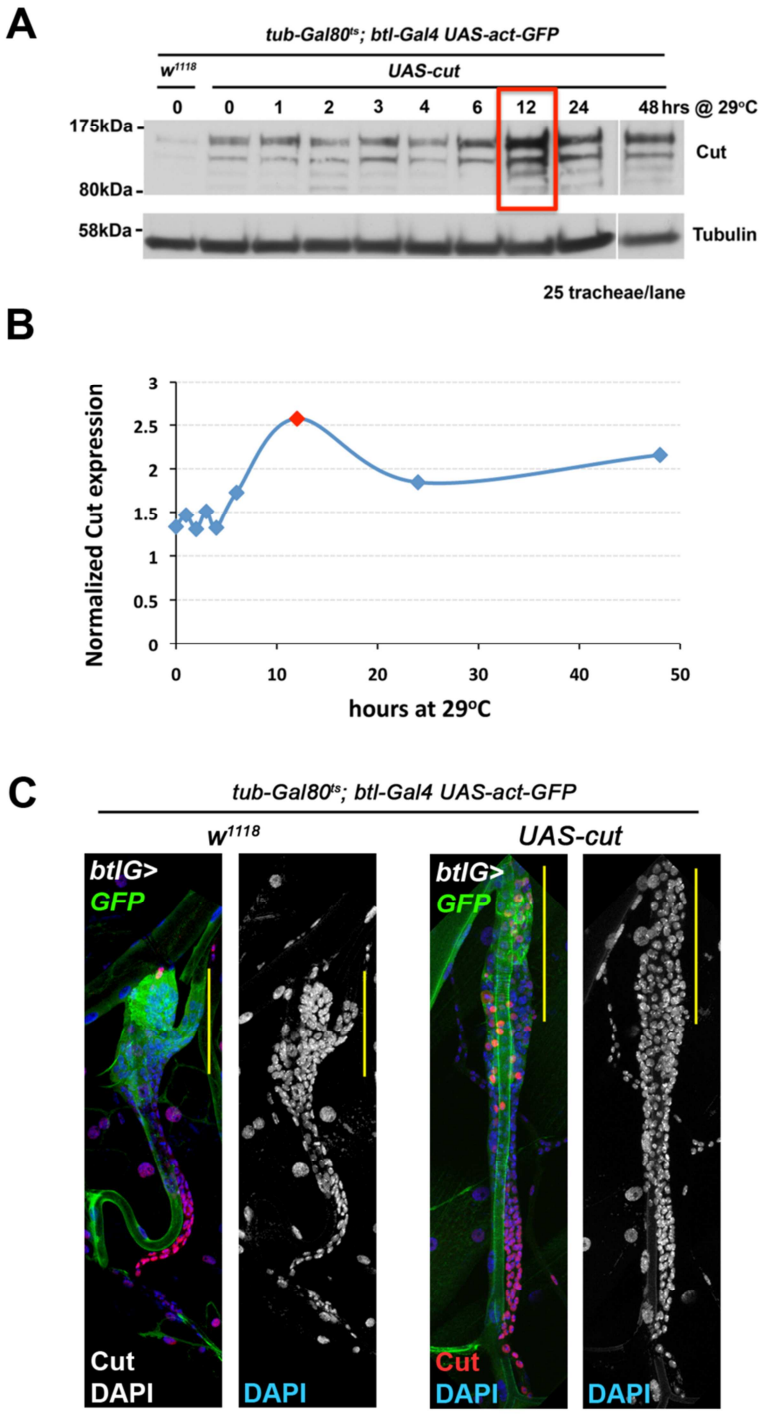

Fig. S6. Scheme for ectopic expression of *cut* in zone 1 cells. (**A**) Western blot for Cut in protein extracts from L3 tracheae indicating that the peak of Cut abundance occurred 12 hrs after induction of the transgene at 29^0C. (**B**) Quantification of Cut amounts from *UAS-cut* samples in A normalized to the tubulin loading control. Data are representative of 2 experiments, both of which showed a peak of Cut at 12 hrs. (**C**) Cut abundance in SBs of wild-type (*tub-Gal80^{ts}/ w^{1118};btl-Gal4 UAS-actGFP*) and *cut*-overexpressing (*tub-Gal80^{ts}/w^{1118};btl-Gal4 UAS-actGFP UAS-cut*) larvae reared at 29^0C for 12 hrs. Yellow line indicates zone 1.

Fig. S7. Transcriptional effects of *cut* knockdown on signaling pathway genes in zone 3. Heat map of differentially expressed signaling pathway genes between wild-type zone 3 and zone 3 in which Cut was knocked down with RNAi. Duplicate microarrays (1, 2) are shown for each condition. This experiment shows more variability between duplicates (1, 2) compared to the zone 1 and zone 3 experiment (fig. S2) and the wild-type and cutOE samples shown in (Fig. 7B), leading to the identification of fewer clear clusters of differentially expressed genes in wild-type and cutRNAi samples.

Table S1: Enrichment of GO categories in zone 1 compared to zone 3. Significantly enriched GO categories in 482 annotated genes that were differentially expressed by at least 1.5-fold in zone 1 compared to zone 3 of the *Drosophila* L3 SB. GO categories are organized alphabetically in rows with their p-values indicated in parentheses. Differentially expressed members of each GO category are listed. Columns are as follows: B: probe set (Affymetrix ID), C: gene (gene name), D: Accession (accession number), E: EntrezGene (Entrez Gene ID), F: baseline mean, G: experiment mean, H: fold change (FC), I: lower bound of FC, J: upper bound of FC, K: difference of means, L: t-statistic, M: P value, N: filtered (asterisk indicates that the gene is included in the filtered gene list).

Table S2: Signaling pathway gene list in Affymetrix *Drosophila* Genome 2.0 arrays. The signaling pathway gene list analyzed in this study was compiled from the literature to include all the known *Drosophila* components of developmental signaling pathways. Columns represent: A: Affymetrix ID, B: FlyBase ID, C: Species (*Drosophila melanogaster*), D: gene name.

Table S3: Enrichment of GO categories in wild-type zone 1 compared to cutOE zone 1. Significantly enriched GO categories in 413 annotated genes differentially expressed by at least 1.5-fold in wt zone 1 compared to cutOE zone 1 of the *Drosophila* L3 SB. Columns are organized as in Table S1.

Table S4: qPCR primer sequences. Oligonucleotides with names ending in L represent forward primers; those ending in R represent reverse primers. Sequences are shown 5′ to 3′.