Supplemental Data

Multiple *Wnt* Genes Are Required for Segmentation in the Short-Germ Embryo of *Tribolium castaneum*

Renata Bolognesi, Laila Farzana, Tamara D. Fischer, and Susan J. Brown

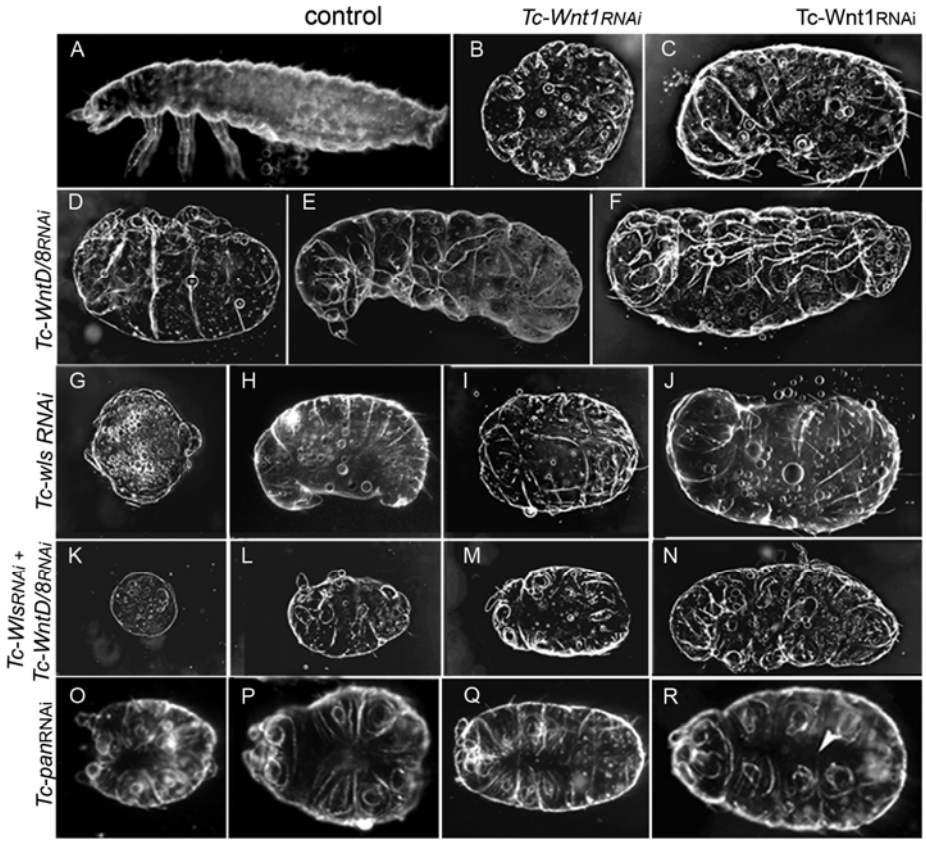

Figure S1. Hypomorphic RNAi cuticular phenotypes.

(A) Wild-type first instar larvae showing three thoracic and nine abdominal segments, as well as appendages in head and thorax.

(B-C) *Tc-Wnt1* RNAi cuticles display some signs of segmentation and defective head appendages.

(D-F) *Tc-WntD/8* RNAi cuticles with two (D) or three (E-F) well developed pairs of legs. Some of the less severely affected cuticles have fewer than normal abdominal segments (E-F).

(G-J) *Tc-wls* RNAi series of cuticles obtained one month after injection (numbers not considered in Table 1). More severely affected embryos produce small spherical cuticles (G), while less severely affected ones produce cuticles with some signs of segmentation and defective head appendages (H-J).
(K-N) *Tc-wls,Tc-WntD/8* double RNAi cuticles. The most severely affected embryos produce even smaller spheres without signs of segmentation (K), while hypomorphic cuticles are posteriorly truncated and display abnormal head and thoracic appendages (L-N).
(O-R) *Tc-pan* RNAi series of cuticles. More severely affected embryos produce small cuticles with some signs of segmentation (O), while less severely affected ones produce cuticles contain fewer abdominal segments and some signs of limb development (P-R).

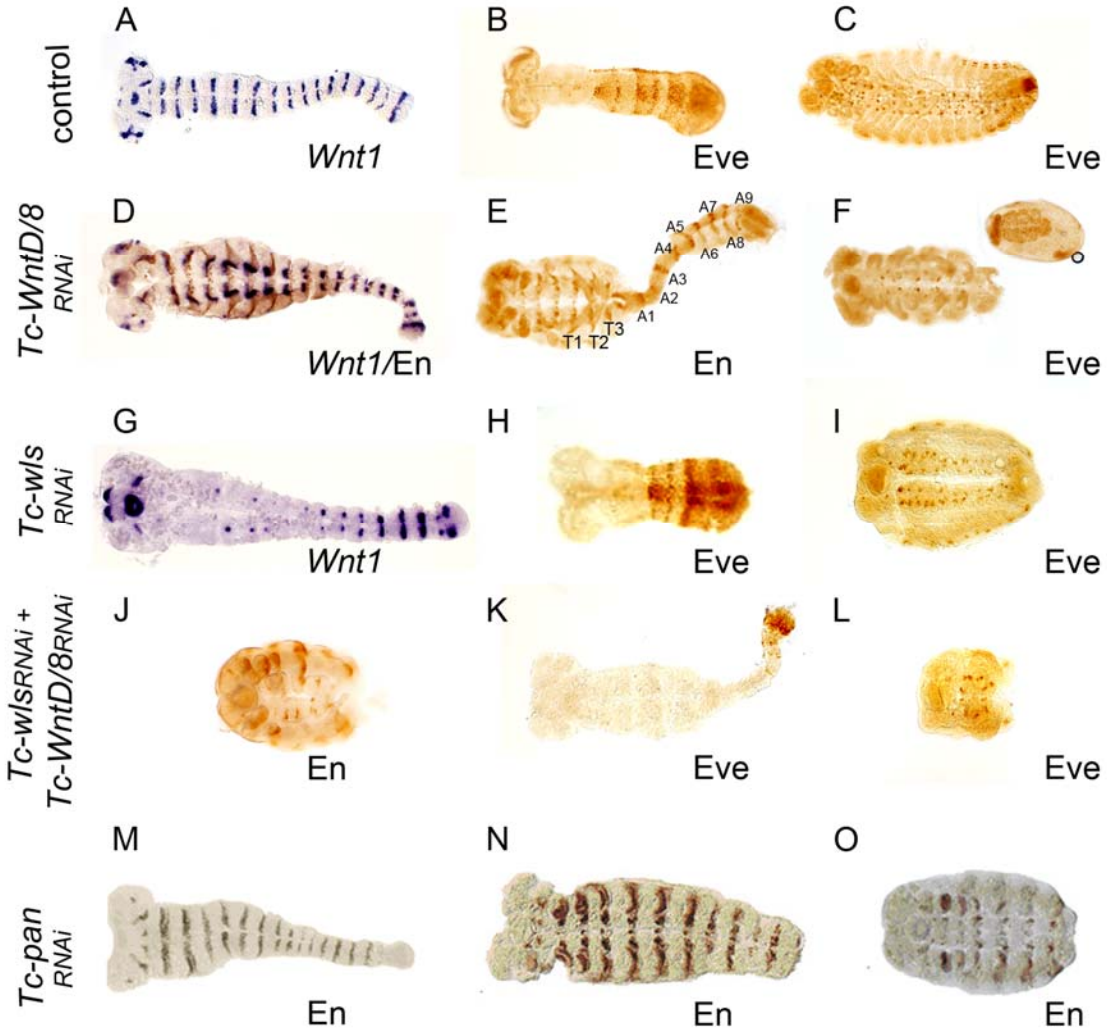

Figure S2. Segmentation in wild-type and RNAi embryos.

Tc-Eve (B-C, F, H-I, K-L) or Tc-En (D-E, J, M-O) (brown) and *Tc-Wnt1* (purple, A, D and G) were used as segmentation markers.

(A-C) Wild-type embryos during germband elongation (A-B) and retraction (C).

(D-F) Tc-En antibody and *Tc-Wnt1* riboprobe were used to detect segmental expression of these gene products and embryos during germband elongation (D-E). Tc-En expression is observed in all segments during germband elongation, including the narrow abdominal segments (D-E) in these hypomorphic RNAi embryos. During germband retraction, Tc-Eve is visible in the CNS of the remaining thoracic segments (F). Inset in (F) shows an embryo broken into two pices.

(G-I) *Tc-wls* RNAi embryos go though elongation normally but *Tc-Wnt1* expression fades (compare G with A), as in *Tc-Wnt1* RNAi embryos [1]. Tc-Eve expression in *Tc-wls* embryos is

normal during germband elongation (H) and after germband retraction Tc-Eve expression is visible in all segments of this highly compacted embryo (I).

(J-L) *Tc-wls,Tc-WntD/8* double RNAi embryos have fewer abdominal segments as assessed by Tc-En staining (J). During germband elongation Tc-Eve staining is normal (K) in the narrow abdominal segments and after germband retraction Tc-Eve is visible, although there are no boundaries between segments (L).

(M-O) *Tc-pan* RNAi embryos show narrower or do not make all abdominal segments during germband elongation, as assessed by Tc-En (M-N), and after retraction Tc-En can be visualized in the fewer remaining segments (O).

Supplementary Table 1: Cuticle phenotypes observed in combination RNAi experiments.

dsRNA (s) injected	% truncated	Other segmentation defects	Total (number examined)[†]
Tc-WntD/8	6	-	137
Tc-Wnt1	0	Range*	~300
Tc-Wnt5	0	-	~900
Tc-WntA	0	-	~900
Tc-Wnt5, Tc-WntA	0	-	~900
Tc-WntD/8, Tc-Wnt5	~6	-	~500
Tc-WntD/8, Tc-WntA	~6	-	~500
Tc-WntD/8, Tc-Wnt5, Tc-WntA	~6	-	~500
Tc-Wnt1, Tc-Wnt5	0	Range*	~100
Tc-Wnt1, Tc-WntA	0	Range*	~100
Tc-Wnt1, Tc-Wnt5, Tc-WntA	0	Range*	~100
Tc-Wnt1, Tc-WntD/8	15	Range**	300

[†] Eggs were collected 3 times for each RNAi experiment. For the *Tc-WntD/8* single RNAi and *Tc-Wnt1, Tc-WntD/8* double RNAi, the exact number of embryos were recorded. For the other experiments the total number of eggs was estimated by counting the number of eggs in the first collection and multiplying by the number of collections (3). Thus, these results are shown as estimates.

*Phenotypes ranged from wild-type to small spherical cuticles. The range of observed phenotypes is likely due to the low *Tc-Wnt1* dsRNA concentration injected into adult females to avoid sterility.

**Phenotypes ranged from wild-type to cuticles displaying random combinations of hypomorphic to severe effects of depleting both genes.