

Supplementary Materials: The Roles of the Wnt-Antagonists Axin and Lrp4 during Embryogenesis of the Red Flour Beetle *Tribolium castaneum*

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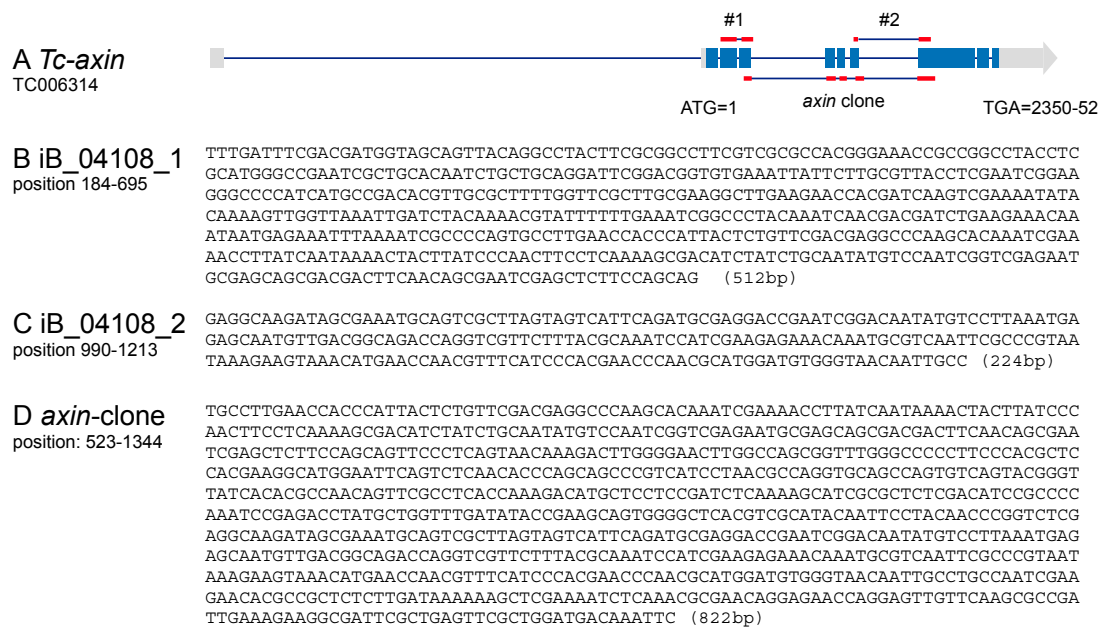


Figure S1. Structure of *Tc-axin* gene. The position (A) and sequences (B–D) of the non-overlapping iBeetle constructs #1 and #2 and the clone used for the generation of dsRNA.

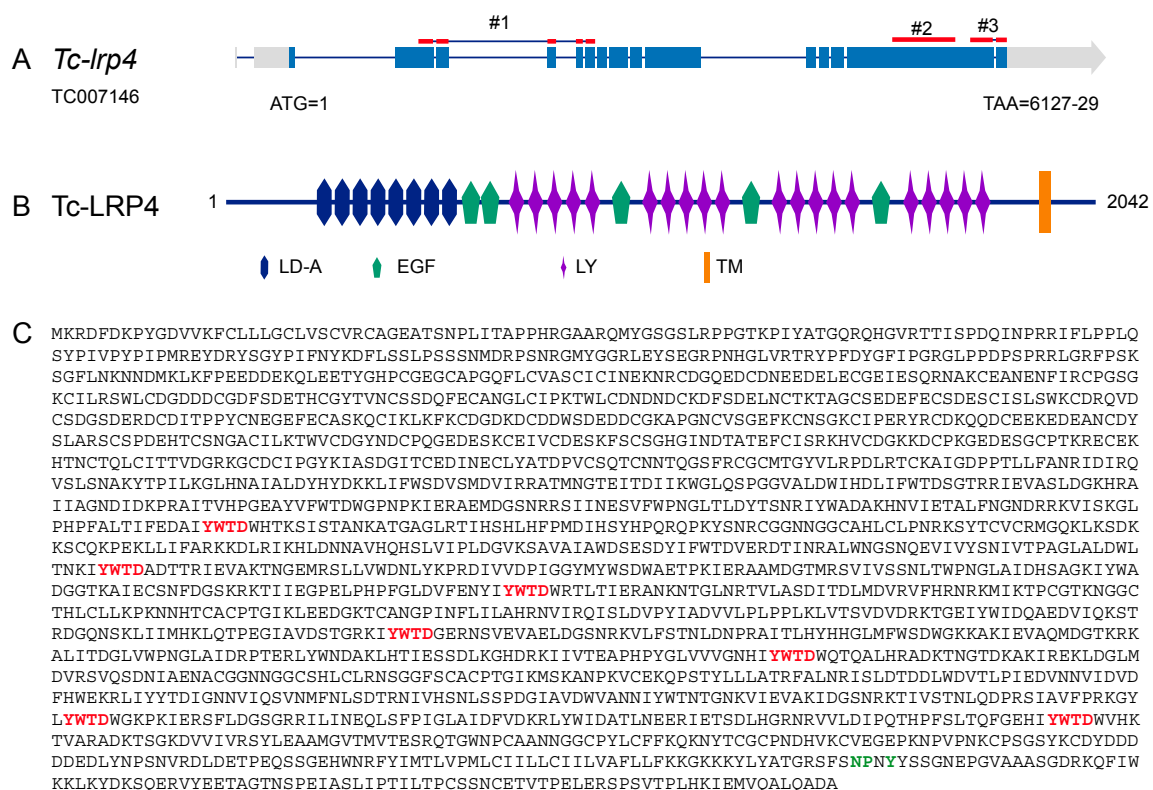


Figure S2. Structure of the *Tc-Lrp4* gene and protein. (A) Exon–Intron structure and position of the non-overlapping PCR fragments used for RNAi: 16 Exons, red-bars show the position of the PCR-

fragments. (B) Structure of the protein domains predicted with SMART (EMBL). LDA: low-density lipoprotein receptor domain class A; EGF: epidermal growth factor-like domain; LY low-density lipoprotein-receptor YWTD domain; TM: transmembrane domain. (C) Predicted protein sequence highlighting the YMTD repeats.

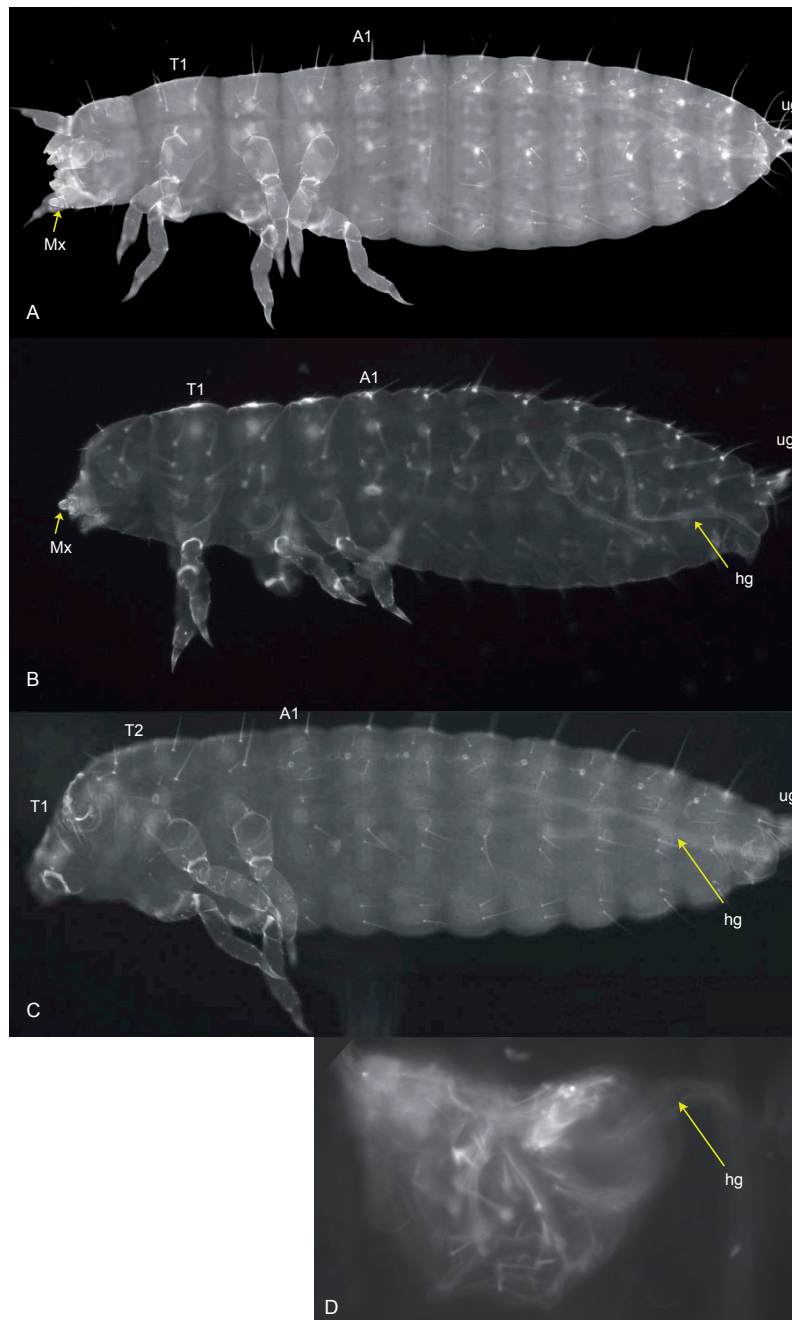


Figure S3. Loss of anterior structures in *Tc-axin*^{RNAi} embryos – weak phenotypes. Cuticle preparation, autofluorescence viewed with ultraviolet (UV) illumination. (A) Wild-type cuticle (as in Figure 2A). (B) Head structures anterior to the maxilla (mx) are missing. (C) Head completely absent; appendages of thoracic segment 1 shortened. (D) Strongly reduced abdomen consisting of few segments with the hindgut (hg) sticking out Mx: maxilla; T1: thoracic segment1; A1: abdominal segment 1; ug: urogomphi; hg: hindgut; anterior points to the left, dorsal is up.

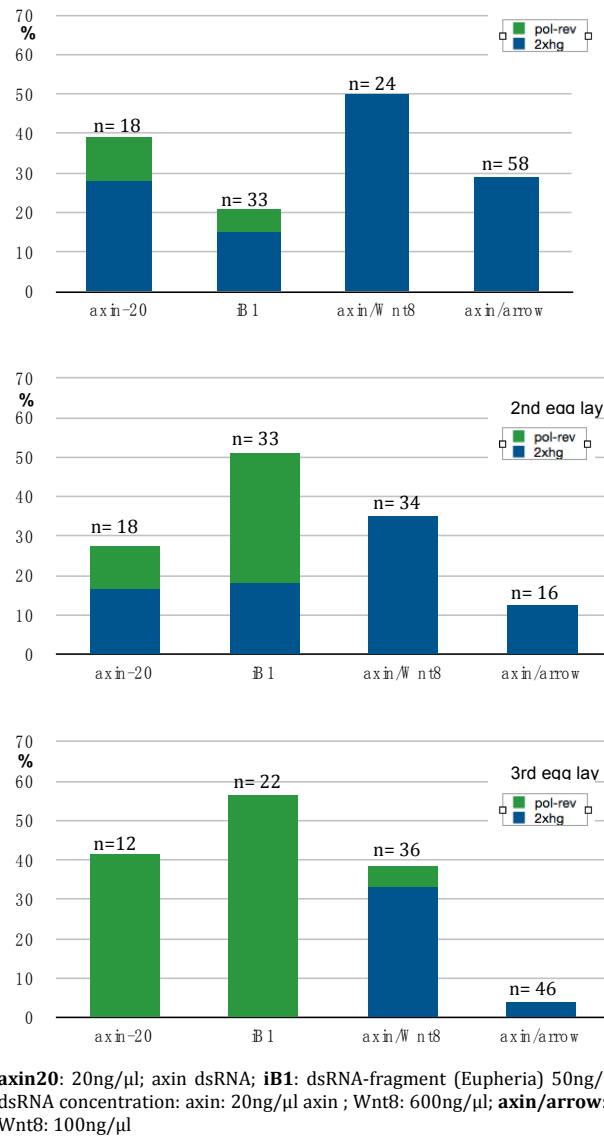


Figure S4. Larval cuticles showing anterior polarity reversal. Quantitative representation of cuticles showed a mild or more severe double abdomen phenotype or “anterior reversal” (green bars), and the development of a second anteriorly positioned hindgut (blue bars). Discrimination was done in relation to the number of egg-lays, concentration of dsRNA applied, the fragment used for dsRNA production and single or double RNAi experiments.

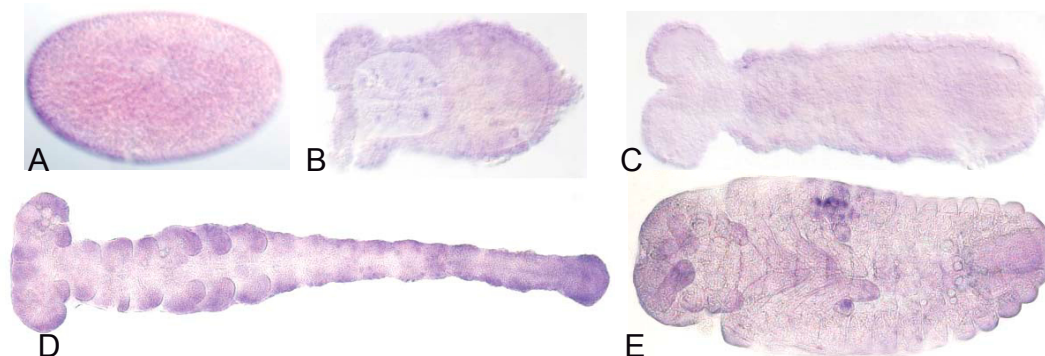


Figure S5. *Tc-lrp4* is ubiquitously expressed during embryogenesis. (A) Blastoderm egg, (B) Germ anlage, some dots likely representing yolk particles are stained, (C) elongating germband, (D) nearly fully elongated embryo, (E) Retracted germband pseudopodia and yolk particles show unspecific staining.

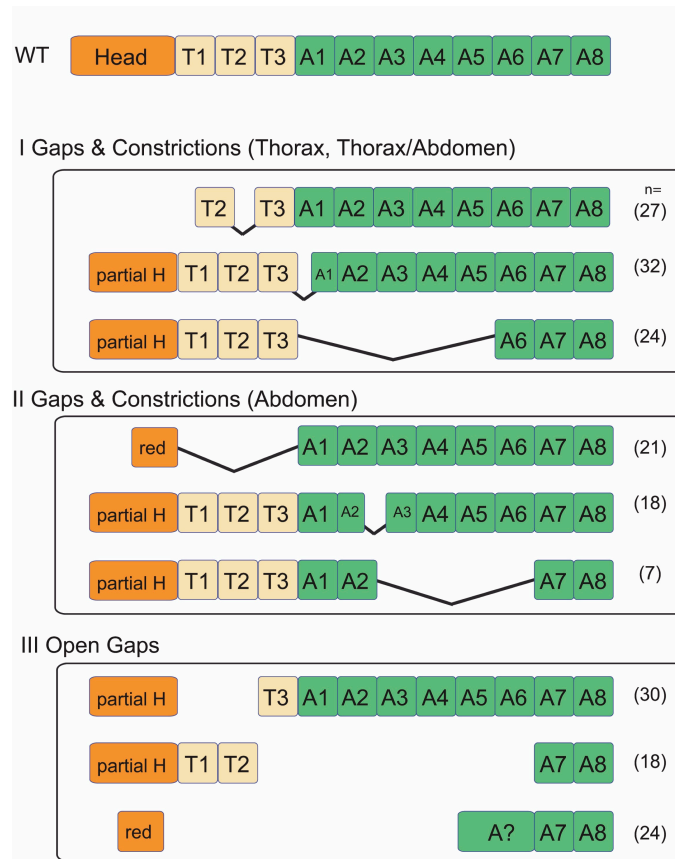


Figure S6. Gap-phenotype in *lrp4*-RNAi cuticles. Schematic representation of the cuticles showing constrictions and/or gaps in the thorax or between thorax and abdomen (Class I), in the abdomen (Class II) or consisting of two pieces (Class III). Number (n =) of observed cases are given in parentheses; red: head strongly reduced to a ball-shaped sphere.

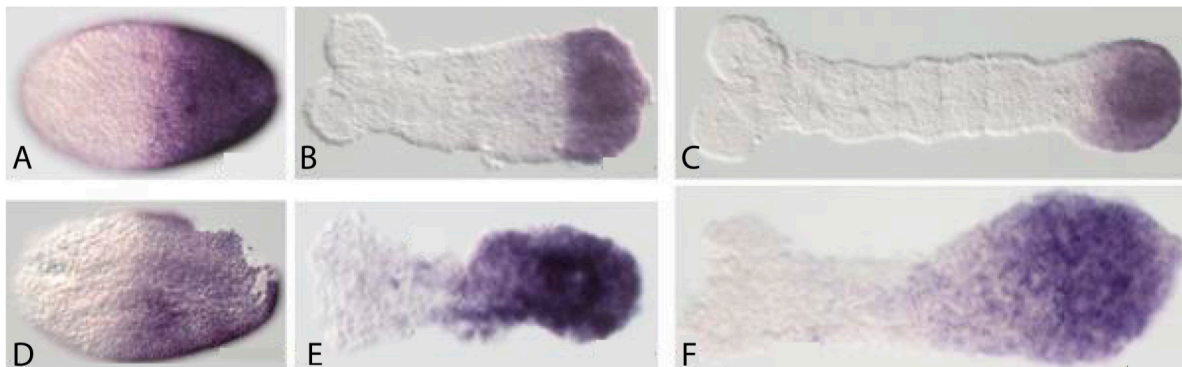


Figure S7. Upregulation of the posterior marker gene *caudal Tc-lrp4*^{RNAi} embryos. In blastoderm-stage *Tc-lrp4*^{RNAi} embryos, the *Tc-cad* domain was found to be slightly expanded towards the anterior (D) when compared to the same stage in the wild-type (A). During germband extension, *cad* expression that in the wild-type was strictly confined to a region within the growth-zone (B,C) was found enlarged up to about 50% embryonic length in *Tc-lrp4*^{RNAi} embryos (E,F).

***Tc-lrp4* RNAi injection series**

Table S1. *Tc-lrp4*-dsRNA-injections (without 800 ng, s. Table S2).

Conc. dsRNA	340 ng/μL	1500 ng/μL	1600 ng/μL *	2036 ng/μL	2500 ng/μL	3000 ng/μL
wildtype	2% (6)	0.7% (4)	2.8% (13)	8% (24)	0.7% (1)	2% (18)
empty egg	78% (201)	75.5% (428)	77.8% (362)	69.2% (207)	88.1% (126)	63.6% (548)
phenotype	20% (52)	23.8% (135)	19.4% (90)	22.8% (68)	11.2% (16)	34.4% (296)

* Injection with RNAi-Template #2, s. Figure S1A.

Table S2. *Tc-lrp4*-dsRNA-injections (800 ng/μL), 6 independent experiments.

	800 ng/μL	800 ng/μL 2*	800 ng/μL	800 ng/μL	800 ng/μL	800 ng/μL
wildtype	2.8% (2)	4.6% (18)	1.4% (8)	1.5% (10)	0.4% (3)	5.1% (14)
empty egg	61.9% (44)	70.8% (276)	69.4% (391)	82.1% (550)	83% (568)	58.8% (161)
phenotype	35.3% (25)	24.6% (96)	29.1% (164)	16.4% (110)	16.6% (115)	36.1% (99)

2*: iB-fragment (= fragment #3) synthesised by Eupheria.

Table S3. *Tc-lrp4*-dsRNA-injections: Classification of observed phenotypes.

	340 ng/μL	800 ng/μL	1500 ng/μL	1600 ng/μL	2036 ng/μL	2500 ng/μL	3000 ng/μL	Σ
I	3.8% (2)	11.7% (71)	6% (8)	13.3% (12)	25% (17)	6.3% (1)	22% (65)	13.5% (191)
Ia	23.1% (12)	21% (128)	20.7% (28)	22.2% (20)	36.8% (25)	18.7% (3)	26% (77)	23.4% (331)
II	9.6% (5)	15.6% (95)	23.6% (32)	12.2% (11)	5.9% (4)	0	18.6% (55)	15.8% (223)
III	63.5% (33)	51.7% (315)	49.7% (67)	52.3% (47)	32.4% (22)	75% (12)	33.4% (99)	47.3% (669)

Class Ia: head defects only; Class Ib: missing head + part of the thorax or head + complete thorax;
 Class II showing constriction, deletion of segments or completely separated pieces of cuticles; Class
 III: unstructured pieces of cuticles.