Supplementary Figures

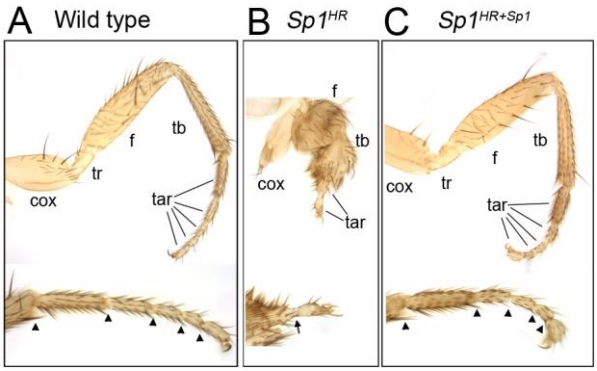

Figure S1: Rescue of *Sp1*^{HR} mutant phenotype by the reintegration of the third exon of Sp1. Adult leg phenotypes of wild type (A), *Sp1*^{HR} (B) and *Sp1*$^{HR+Sp1}$ (C). The inset below displays an amplification of the tarsal region of each genotype. Correct joint formation is indicated with arrowheads .

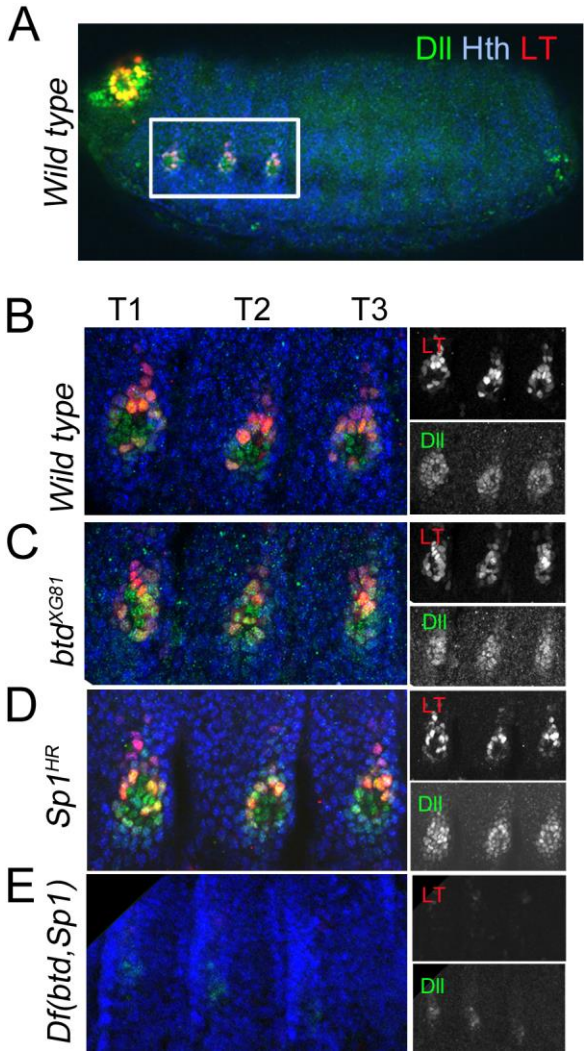

Figure S2: Individual contribution of *btd* and *Sp1* to *Dll* regulation during embryonic development. (A) Wild type embryo (stage 15), stained with antibodies against Dll, Hth, and βgal for the *Dll*-LT-*lacZ* enhancer (green, blue and red, respectively and in all panels). The thoracic region, where the three leg primordia are located, is framed in a white box. (B-E) Magnification of the leg primordia in wild type (B), btd^{XG81} (C), $Sp1^{HR}$ (D) and *Df(btd,Sp1)* (E) mutant embryos. Only when both *Sp1* and *btd* are eliminated, *Dll* and *Dll*-LT fails to be properly activated at the presumptive leg region. T1, T2 and T3 designate first, second and third thoracic segments, respectively. At the right side of each panel is shown in grey the individual signal for Dll and *Dll*-LT for each genotype. All embryos are oriented anterior left and dorsal up.

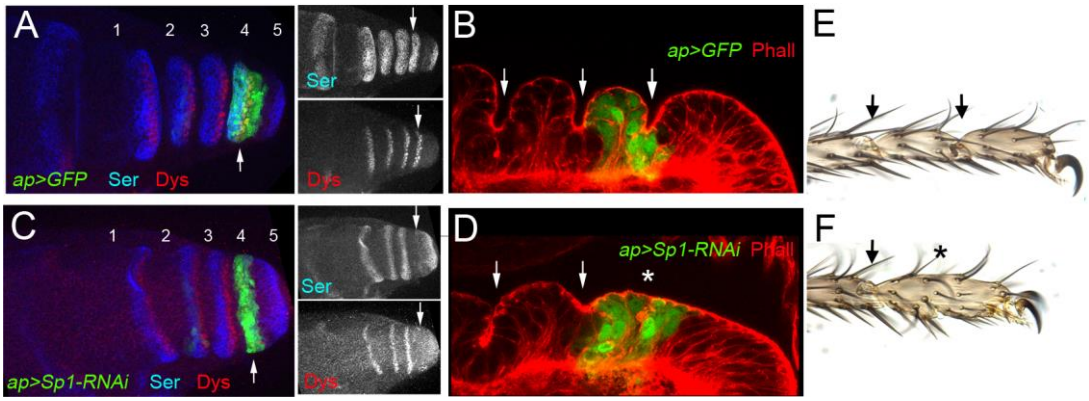

Figure S3: Sp1 loss of function alters Ser positioning causing defects in joint formation. (A) *ap-Gal4*; UAS-*GFP* (green) control prepupal leg disc stained for Ser (blue) and Dys (red). The individual signal for Ser and Dys is displayed in grey at the right of each panel. Arrows indicate the *ap-Gal4* domain. (B) Sagittal view of the leg epithelium (same genotype as in A) stained with Phallodin (Phal, red) and GFP (green). The joints between tarsal segments are marked with arrows. Close view of the adult tarsal joints is shown in (E). (C) Knock-down of Sp1 levels by the expression of UAS-*Sp1* RNAi in the *ap-Gal4* domain (green) cause the loss of Ser (blue) and Dys (red) staining. Reduction of the levels of Sp1 in the *ap-Gal4* domain cause defects in joint formation that can be assessed both in prepupal leg discs and in adult legs (D and F, respectively), as compared with Wt prepupal leg discs and adult leg discs (B and E, respectively). Arrows indicate correct fold or joint morphology, while asterisks indicate defects in these processes. Cell actin cytoskeleton in B and D is stained with Phal (red).

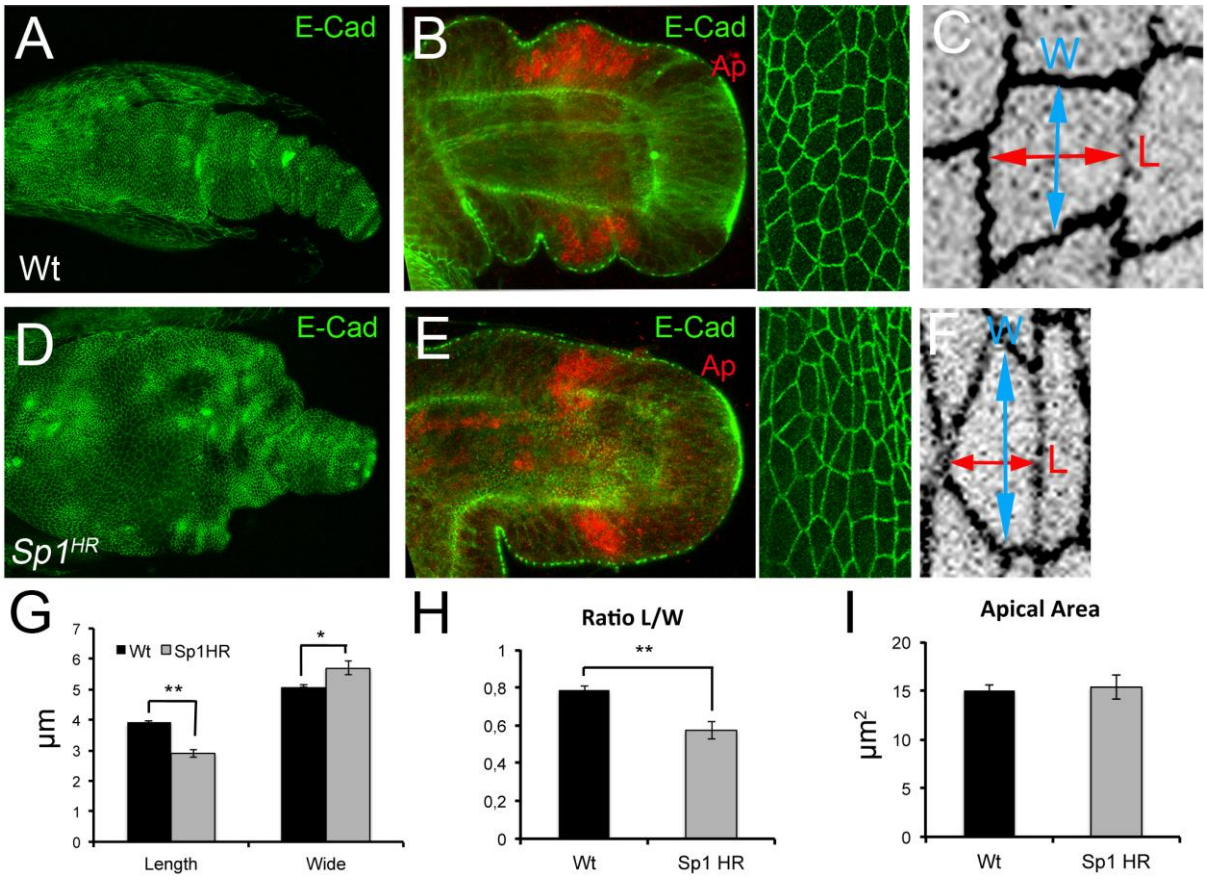

Figure S4: Cell shape changes during leg eversion are impaired in *Sp1^{HR}* mutants. Study of cell shape in control (*FM7-GFP*, referred from now onwards as Wt) (A, B, C) and *Sp1^{HR}* (D, E, F) prepupal leg discs (4hrs APF). E-cadherin-GFP is marked in green in all panels and black in C and F, and Ap protein is shown in red in B and E. (A and D) View of prepupal Wt and *Sp1^{HR}* leg discs, respectively. (B and E) Amplified view of the *ap* domain of the same discs as in A and D, and magnification of the apical region of the Ap domain in the right panels. Note that *Sp1^{HR}* prepupal leg discs are shorter and wider compared to Wt and the defects in fold formation in the *Sp1^{HR}* mutant condition. Length (L, red) and width (W, blue) of control cells (n=77) and *Sp1^{HR}* mutant cells (n=80) (C and F, respectively) within the *ap* domain of prepupal leg discs was measured (G), and L/W ratio is shown in H. Note that cells in *Sp1^{HR}* mutants tend to be shorter and wider along the PD axis than control cells. (I) Apical area was also measured, and it did not differ between control (n=50 cells) and *Sp1^{HR}* (n=62 cells). In G and H (*) indicates significative difference with $p \leq 0{,}05$ and (**) for $p \leq 0{,}005$ with Student's t-test.

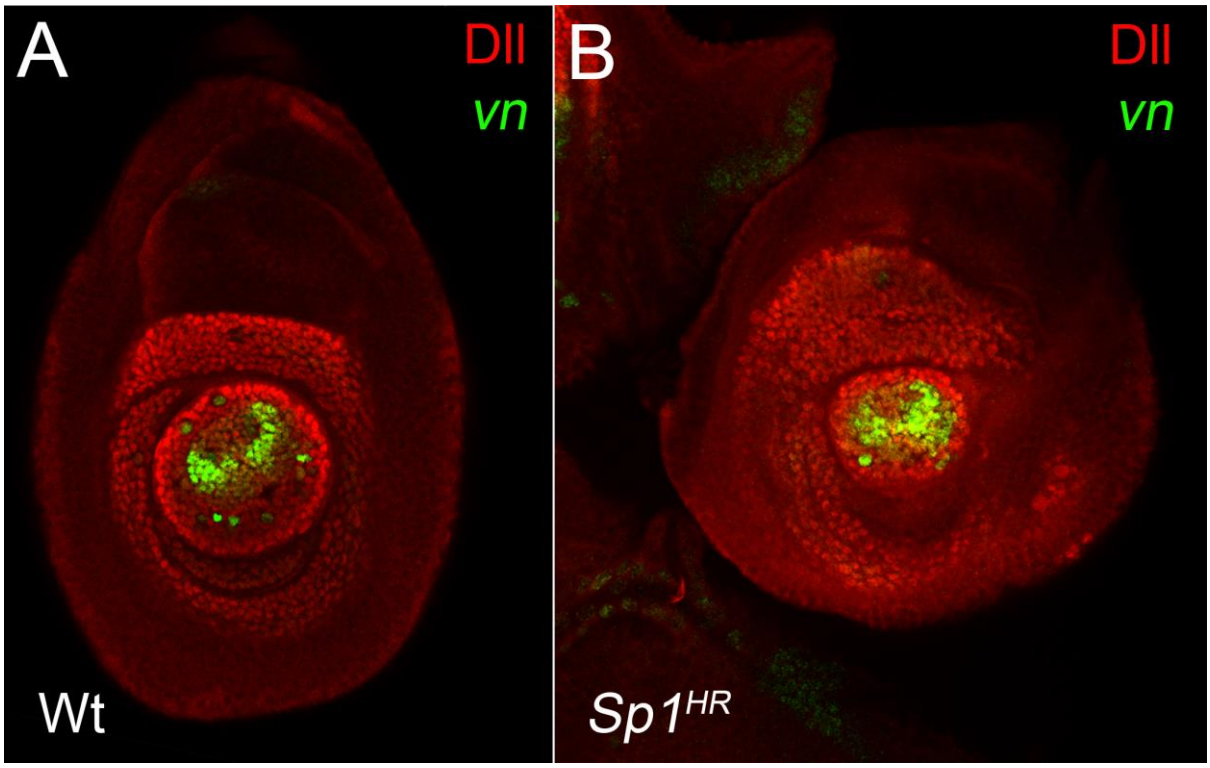

Figure S5: Expression of the EGFR ligand *vn* is maintained in *Sp1^{HR}* mutants. Dll (red) and *vn-lacZ* (green) expression in Wt (A) and *Sp1^{HR}* (B) L3 imaginal leg discs. Note that the expression of the EGFR ligand *vn* in the distal tip of the leg disc is not altered in the absence of *Sp1*.

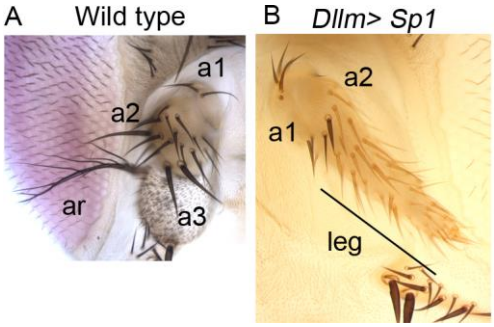

Figure S6: Misexpression of Sp1 in the antenna cause transformation to leg tissue. (A) Wild type antenna, where antennal segments 1, 2 and 3, and arista (ar) are indicated. (B) Misexpression of *Sp1* using the *Dllm-Gal4* driver causes the transformation of a3 and ar to leg-like structures (marked by *yellow*).

Table S1: List of the 337 genes identified (179 up-regulated and 158 down-regulated) that were significantly differentially expressed between $Sp1^{HR}$ and control leg discs.

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Table S2: From the total of genes identified, we decided to select those with a log2 fold change higher than one (absolute value) and that have a minimal expression level (see Materials and Methods). Following these criteria, a total of 30 up-regulated and 53 down-regulated genes were selected.

Click here to Download Table S2

Table S3: Primers used in this study.

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Table S4: List of RNAi lines used in this study.

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