Notch modulates Wnt signalling by associating with Armadillo/β-catenin and regulating its transcriptional activity

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Supplementary Material

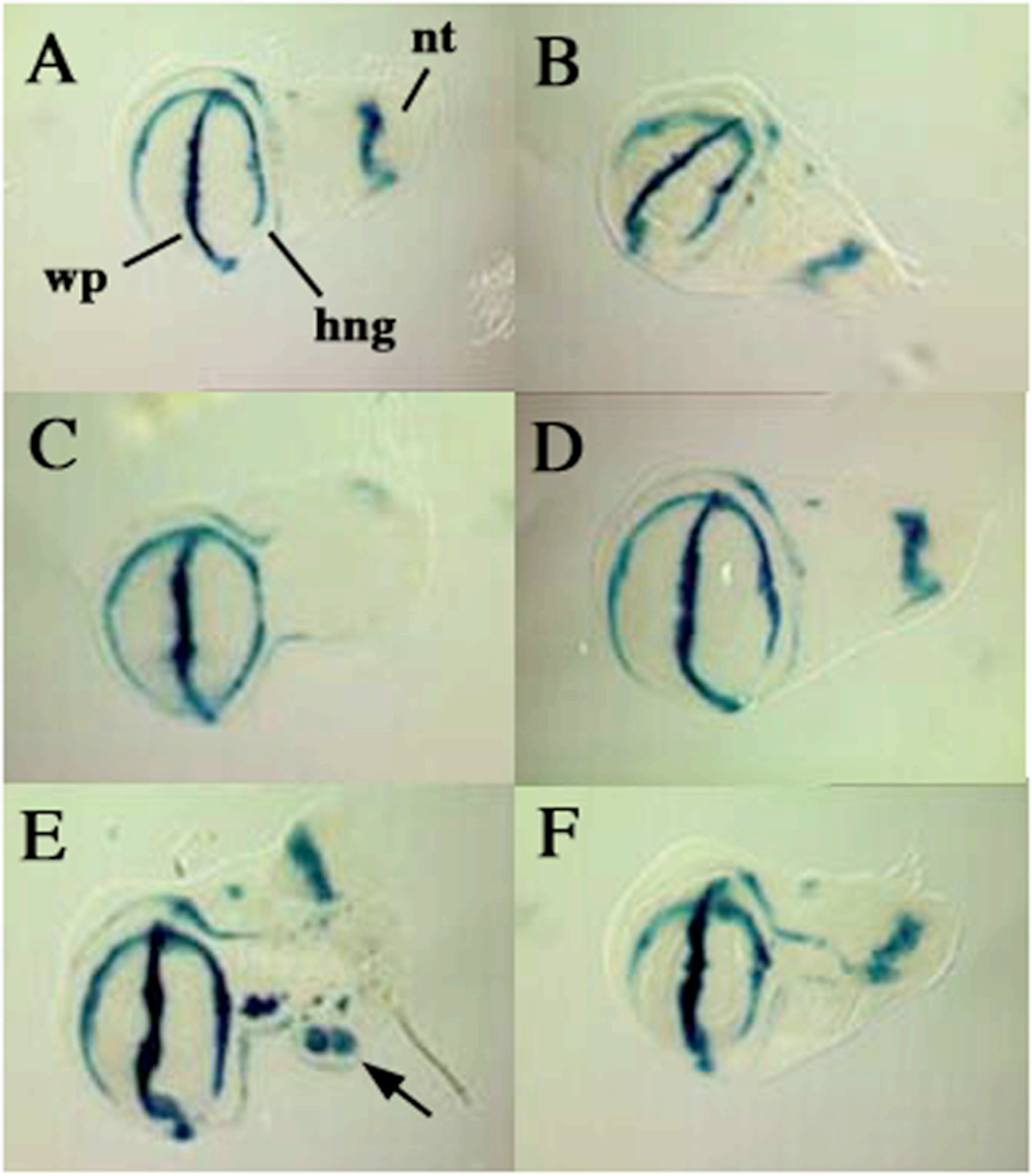
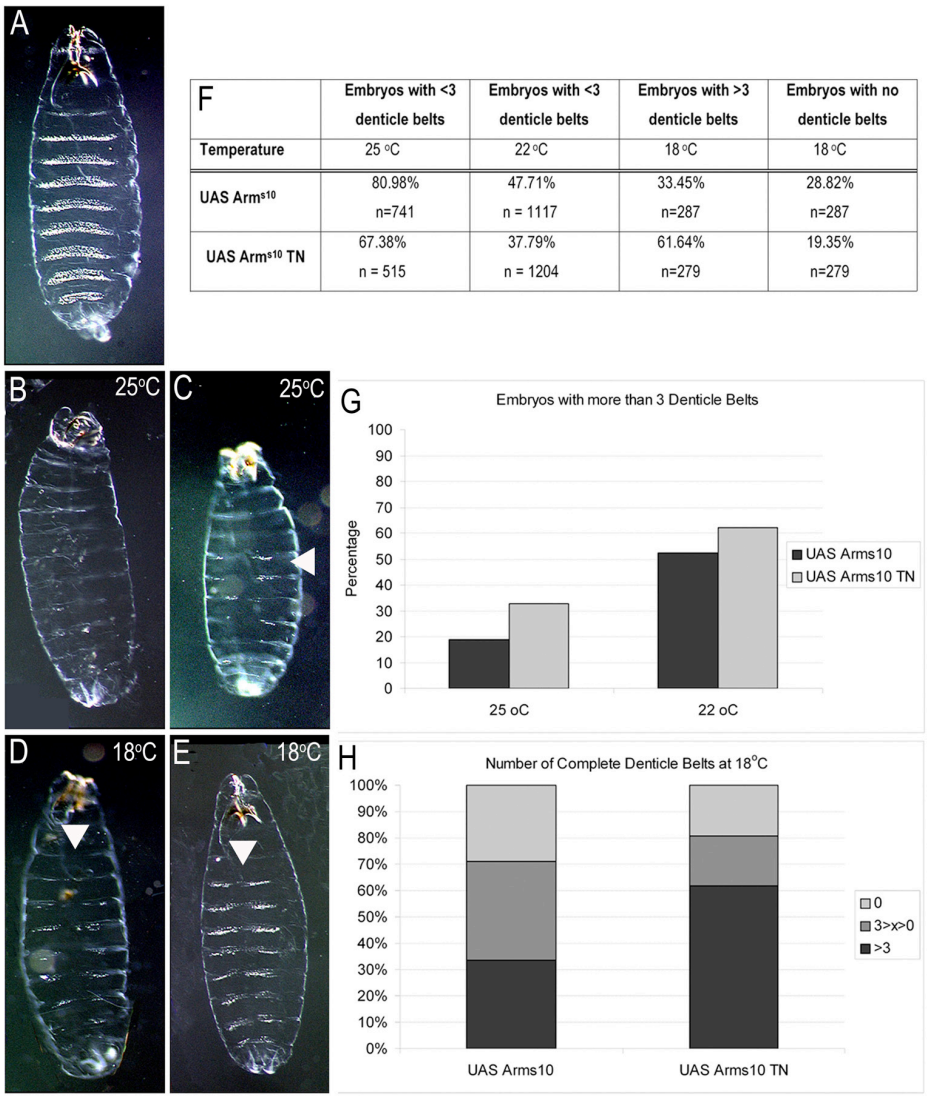


Fig. S1. Effects of wingless and Notch signalling on wing development. Third instar larval discs carrying a *lacZ* reporter for the expression of Wingless, expressing different transgenes. (A) Wild-type disc showing the wing pouch (inner circle, wp), the hinge region (outer circle, hng) and the notum (nt). The stripe bisecting the wing pouch is the wing margin. (B) Disc expressing *dpp*GAL4 UASTNotch. Notice that the wing pouch region is smaller (see text for details). (C) Disc expressing *dpp*GAL4 UASWingless. Notice the enlargement of the hinge region, as has been described before. (D) Disc expressing *dpp*GAL4 UASWingless UASTNotch. Notice that TNotch completely suppresses the effects of UASWingless. (E) Disc expressing *dpp*GAL4 UASArmadillo^{S10}. In addition to the expansion of the hinge, there are several winglets developing in the region of the notum (arrow). (F) Disc expressing *dpp*GAL4 UASArmadillo^{S10} UASTNotch. Notice the very effective, albeit not complete in this example, suppression of the effects of UASArmadillo^{S10}.



F	Embryos with <3 denticle belts	Embryos with <3 denticle belts	Embryos with >3 denticle belts	Embryos with no denticle belts
Temperature	25 °C	22 °C	18 °C	18 °C
UAS Arm^{s10}	80.98% n=741	47.71% n = 1117	33.45% n=287	28.82% n=287
UAS Arm^{s10} TN	67.38% n = 515	37.79% n = 1204	61.64% n=279	19.35% n=279

Fig. S2. Modulation of Wnt signalling by Notch during the patterning of the larval cuticle. (A) Wild-type pattern of the larval cuticle at the end of embryogenesis. The cuticle exhibits reiterated sets of denticles that demarcate the anterior of each segment; the posterior part of each segment is devoid of denticles and this phenotype requires Wingless signalling. (B-E) Ventral cuticles of larvae expressing UASArmadillo^{s10} (B,D), or UASArmadillo^{s10} together with UASTNotch (C,E), under the control of *arm*GAL4 at different temperatures. The activity of GAL4 is temperature dependent and elicits more expression of the genes under GAL4 UAS control at the higher temperature; this effect can be seen in the penetrance of the phenotypes (F-H). (B-D) Cuticles representative of the phenotypes caused by expression of Arm^{s10} (B) or Arm^{s10} together with TNotch (C) at 25C. At this temperature Arm^{s10} consistently elicits the naked cuticle fate (see also F). TNotch suppresses the effects of Arm^{s10} and allows the development of denticles (arrow). The effect of TNotch is more pronounced at 18C (D,E), when the effects of Arm^{s10} are weaker. At 18C most embryos expressing Arm^{s10} develop some denticles, however they tend not to cross the midline (see arrowhead in D). At this temperature the effects of TNotch on Arm^{s10} are manifested in the appearance of denticles across the midline (see arrowhead in E). (F-H) Quantitative analysis of the effects of TNotch on Arm^{s10} at different temperatures (25C, 22C and 18C). (F,G) At 25C and 22C the effects of Arm^{s10} were assessed by the number of embryos with three or less segments with denticles, regardless of whether they crossed the midline or not. Notice that at both temperatures (25C, 22C) embryos that express TNotch have more segments with denticles. (F,H) At 18C most embryos expressing Arm^{s10} have segments with denticles because of the low expression of Arm^{s10}. In this case we evaluated the number of embryos with denticle belts, i.e. embryos in which the denticles cross the midline. As in G, we chose three as an arbitrary threshold for our quantification. In this instance we also counted the number of embryos with naked cuticle. TNotch can suppress the effects of Arm^{s10}.

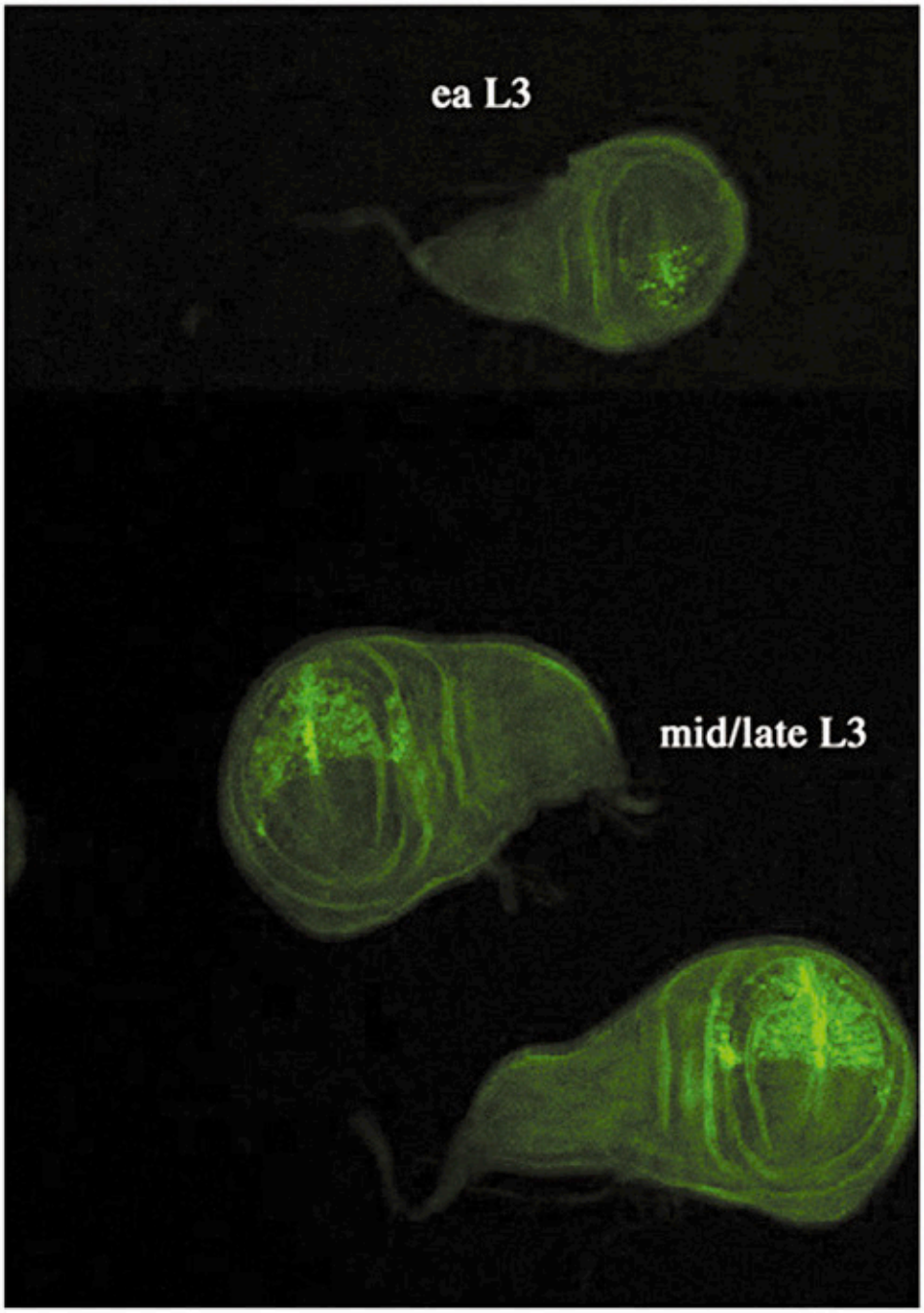


Fig. S3. Third instar larval wing discs expressing full length Armadillo under the control of enGAL4. At the beginning of the third larval instar (ea L3), although *engrailed* is expressed throughout the posterior compartment (indicated by region of intense green) Armadillo protein is only visible in a very small domain near the dorsoventral compartment boundary which coincides with the expression of Wingless at this time (Klein et al., 2000). Later on (mid/late L3) Armadillo is visible throughout the wing pouch but still its highest levels are at the dorsoventral boundary where the levels of Wingless are highest.

Reference

Klein, T., Seugnet, L., Haenlin, M. and Martinez Arias, A. (2000). Two different activities of Suppressor of Hairless during wing development in *Drosophila*. *Development***127**, 3553-3566.

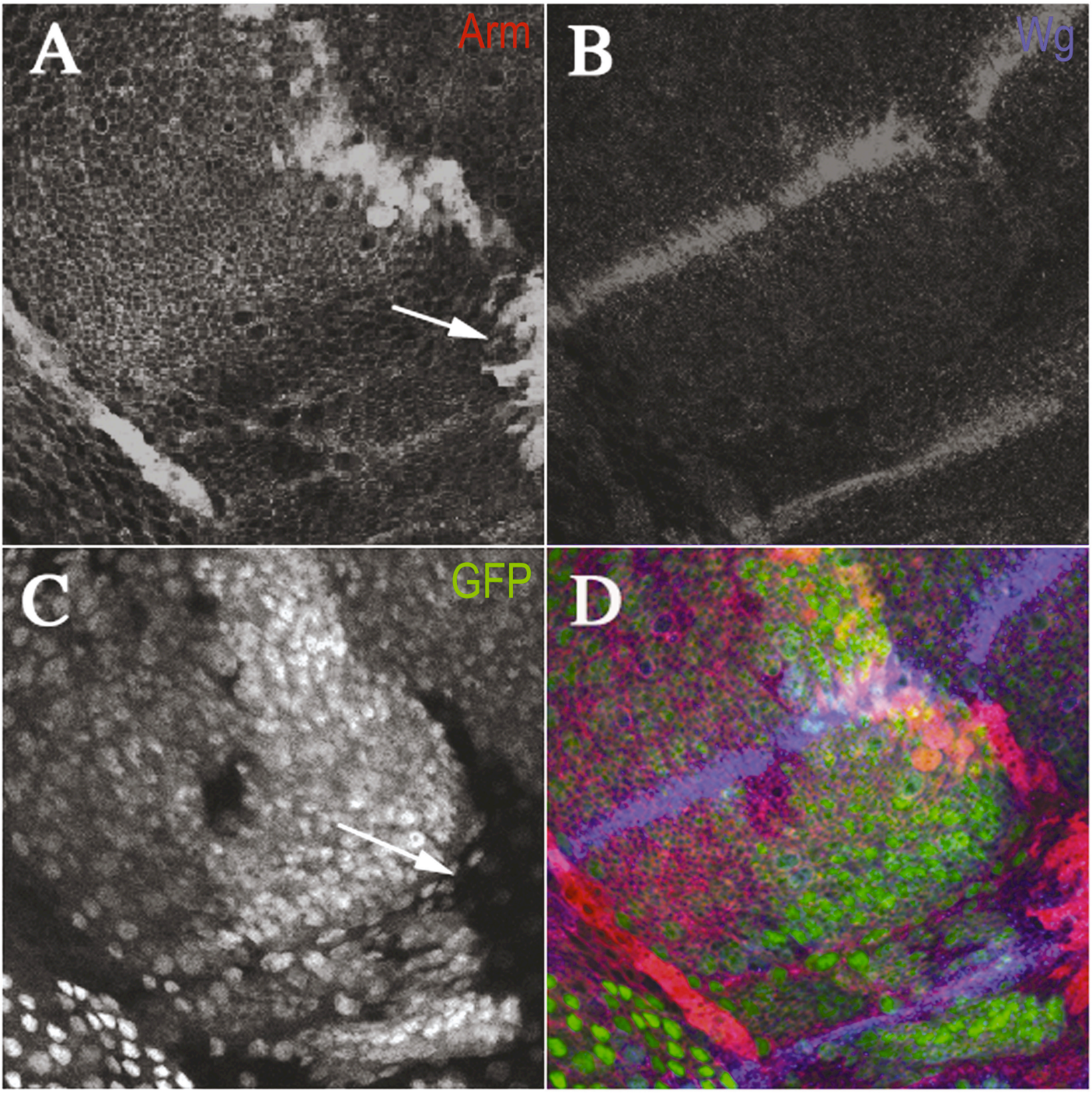


Fig. S4. In the absence of Notch the stabilisation of Armadillo does not require Wingless signalling. Confocal section through the wing pouch of a third instar larval wing disc expressing UASArmadillo under the control of ptcGAL4 (see text for details). (A) Armadillo expression. (B) Wingless expression. (C) GFP indicating the position of *Notch* mutant cells through the absence of GFP. (D) Merged image of A, B and C. GFP is green; Armadillo protein is red. The stripe of Wingless expression (blue) indicates the position of the dorsoventral boundary. Notice that there is a gap, probably produced by the absence of Notch function early in wing development and that a clone of Notch mutant cells (arrow) which lies in the path of the disc maintains high levels of expression of Armadillo (red).

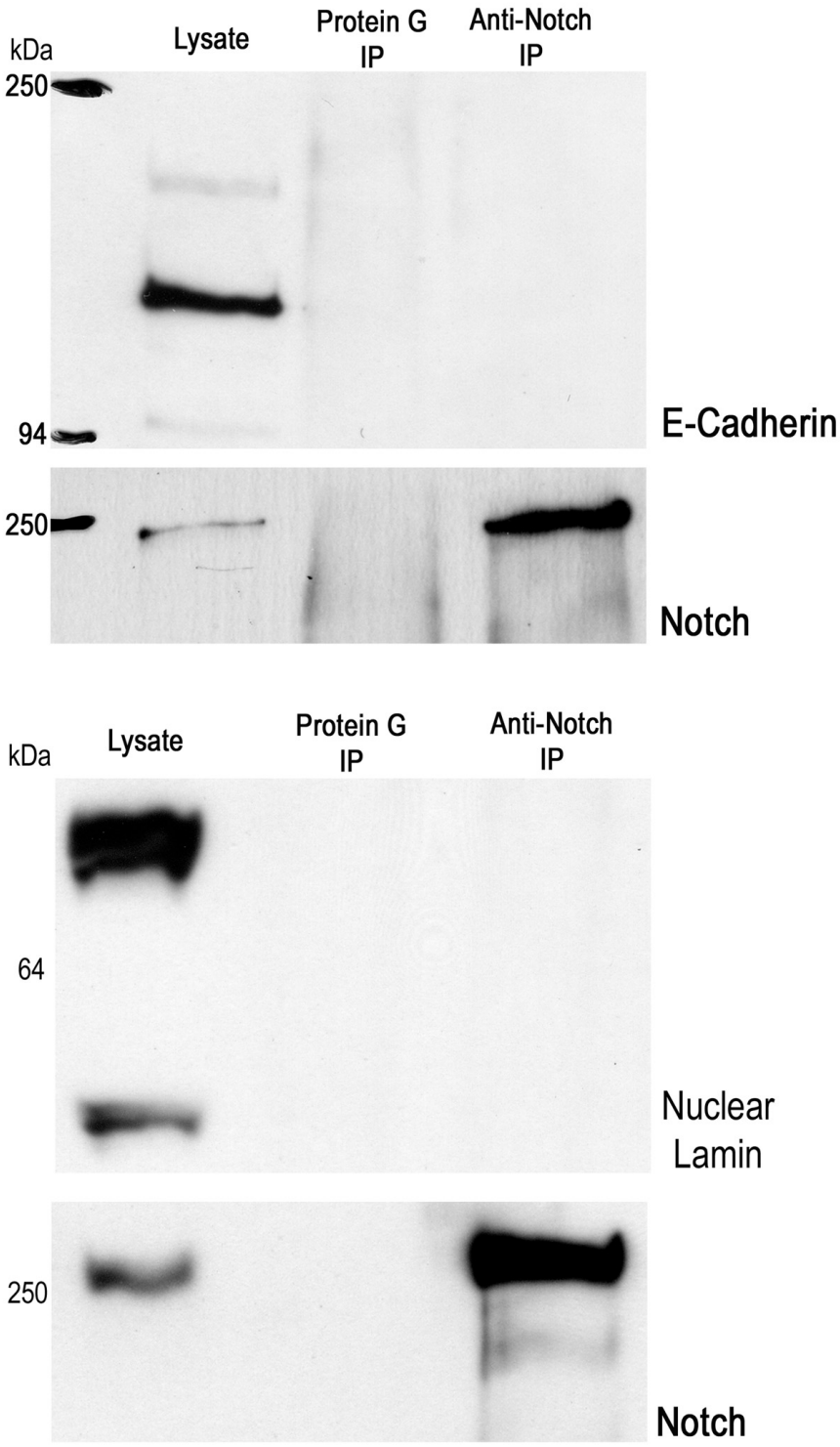


Fig. S5. Notch protein was immunoprecipitated from wild-type embryos and the presence of associated proteins was assessed by western blot. In Notch immunoprecipitate that contained Armadillo (data not shown) no detectable association of E-cadherin (rat monoclonal DCAD2) or nuclear lamin (mouse monoclonal T47) proteins with Notch was observed.