

Supplementary Materials for

Wingless Promotes Proliferative Growth in a Gradient-Independent Manner

Luis Alberto Baena-Lopez, Xavier Franch-Marro, Jean-Paul Vincent*

*To whom correspondence should be addressed. E-mail: jvincen@nimr.mrc.ac.uk

Published 6 October 2009, *Sci. Signal.* **2**, ra60 (2009) DOI: 10.1126/scisignal.2000360

This PDF file includes:

- Fig. S1. Expression area of the *nubG4* driver and mild activation of ectopic Sens protein following production of ArmS10.
- Fig. S2. Further documentation of the effects of moderate Wingless signaling on target gene expression, proliferation, and wing size.
- Fig. S3. Expression of Wingless^{S239A} in clones has an uncertain effect on proliferation.
- Fig. S4. Removal of Wingless and expression of attenuated Wingless at the early third instar stage.
- Fig. S5. Definition of the region of the discs used for area measurements.
- Fig. S6. Normal wing size was not restored by p35.

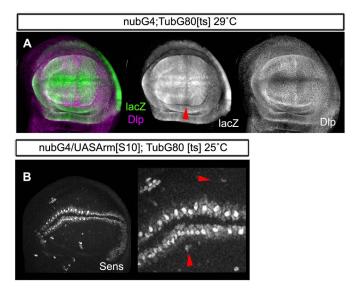


Fig. S1. Expression area of the nubG4 driver and mild activation of ectopic Sens protein following production of ArmS10. (**A**) The presence of β-galactosidase (green) and Dlp (purple) in a nubG4/UAS-lacZ; $TubG80^{ts}$ disc raised at 29°C (control). β-galactosidase acts as a reporter of nubG4 activity. The red arrowhead points to an area of low nubG4 activity in the medial region. (**B**) The abundance of Sens protein is mildly increased in a nubG4/ UAS-Arm^{S10}; $TubG80^{ts}$ disc raised at 25°C. The red arrowheads point to ectopic Sens protein, which prefigures the ectopic bristles shown in Fig. 1H.

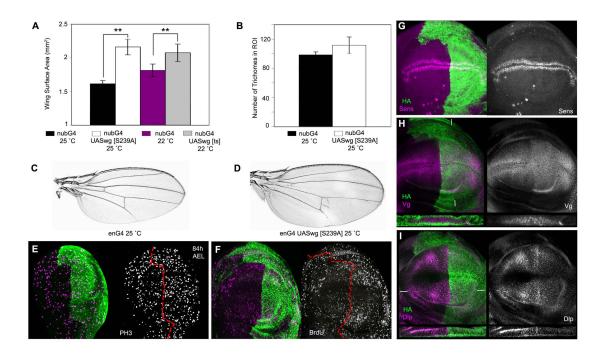


Fig. S2. Further documentation of the effects of moderate Wingless signaling on target gene expression, proliferation, and wing size. (A) Mean surface areas of wings (as defined by the shaded region in Fig. 2A) in nubG4 at 25°C (black bar), nubG4; UAS- $Wingless^{S239A}$ at 25°C (white bar), nubG4 at 22°C (purple bar), and nubG4; UAS- $Wingless^{ts}$ at 22°C (gray bar). (B) Mean numbers of trichomes per unit area (which serve as a proxy for cell size because each cell of the wing blade makes one trichome) in region C (the region between veins 2 and 3) of adult wings. Error bars (in A and B) indicate the SD. Statistical significance was assessed with a Student t-test (**, p < 0.01, n > 10). (C) Control wings carrying the enG4 driver have a normal size whereas expression of Wingless S^{239A} with the same driver (D) leads to a relative enlargement of the P compartment. (E) Phospho-histone 3 (PH3, purple) in a young third instar disc (84 hours AEL) of genotype hhG4/ UAS- $Wingless^{S239A}$. Note the increased staining in the P compartment (labeled with GFP; green). (F to I) Brdu staining, Vg and Dlp (all in purple) in third instar discs (110h AEL) of the same genotype.

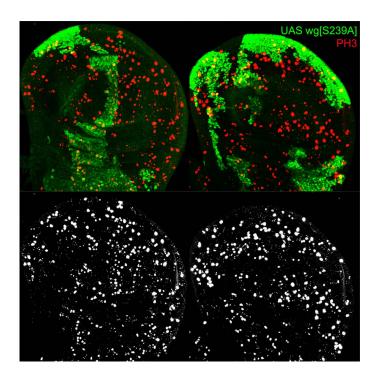


Fig. S3. Expression of Wingless^{S239A} in clones has an uncertain effect on proliferation. Two discs are shown. No unambiguous increase in proliferation (as assayed by staining with an anti-PH3 antibody) can be seen to be clearly associated with the areas in which Wingless^{S239A} is found. In the top panels, the overexpressing clones are green and PH3 is in red. The bottom panels show anti-PH3 only.

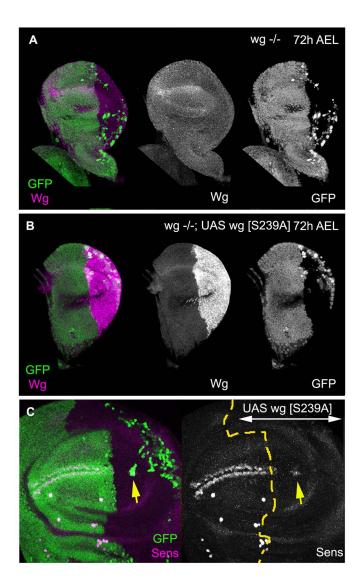


Fig. S4. Removal of Wingless and expression of attenuated Wingless at the early third instar stage. (**A**) With the hhG4 UAS-FLP system, at the end of the second instar (72 hours AEL), most of the P compartment was populated by *wingless* homozygous mutant cells (marked by the absence of GFP). Wingless protein was labeled in purple (left panel) or white (central panel). (**B**) At the same stage, expression of the *Wingless* construct in a Wingless-deficient P compartment was uniform. Staining was as for panel A. (**C**) The attenuated activity of Wingless sis apparent in the third instar where it is clear that it did not rescue the production of Sens protein in a Wingless-deficient P compartment. Imaginal disc with the P compartment lacking Wingless and expressing *UAS-Wingless* (same genotype as in panel B). As can be seen, the production of Sens protein (purple or white) was virtually abolished in the P compartment. The yellow arrowhead points to an occasional cluster of WT cells that still contained Wingless and hence contained Sens protein.

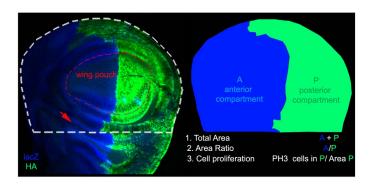
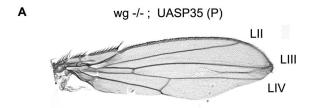


Fig. S5. Definition of the region of the discs used for area measurements. Objective landmarks were used to define the A and P compartments for the purpose of the measurement of area and cell density. The anterior edge of the area of *hhGal4* activity (marked here by the presence of Wingless sereognized with an antibody against HA) delimits the AP boundary. The bottom edge of both the A and P areas was arbitrarily defined as the tangent to the third fold at its most distal (bottom) position (red arrow). The edge of the disk defined the remainder of the boundary. Density was measured as the number of objects (PH3-positive cells) divided by the relevant surface area. The first fold encircles the area known as the pouch (dashed red line). This was used for the normalization of clone areas shown in Fig. 2I.



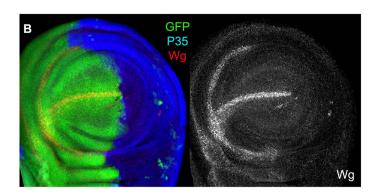


Fig. S6. Normal wing size was not restored by p35. (**A**) Wing from a fly containing the apoptosis inhibitor p35 in the P compartment, which was genetically depleted of Wingless as in Fig. 1B. The P compartment was still reduced in size. Wing veins were labeled according to standard nomenclature. (**B**) Wing imaginal disc of the same genotype as that depicted in panel A, showing the area in which Wingless was deleted and p35 was produced. The disc was analyzed by immunofluorescence staining as indicated.