

Figure S1 Transcription of *wg* is unaffected in *Vps35* mutant cells. Wing imaginal disc with a *Vps35* mutant posterior compartment. *wg* transcripts are revealed by *in situ* hybridisation¹. The level of *wg*

transcripts in the *Vps35* mutant posterior compartment (marked by the absence of GFP in panel a) is similar to that in the control anterior compartment.

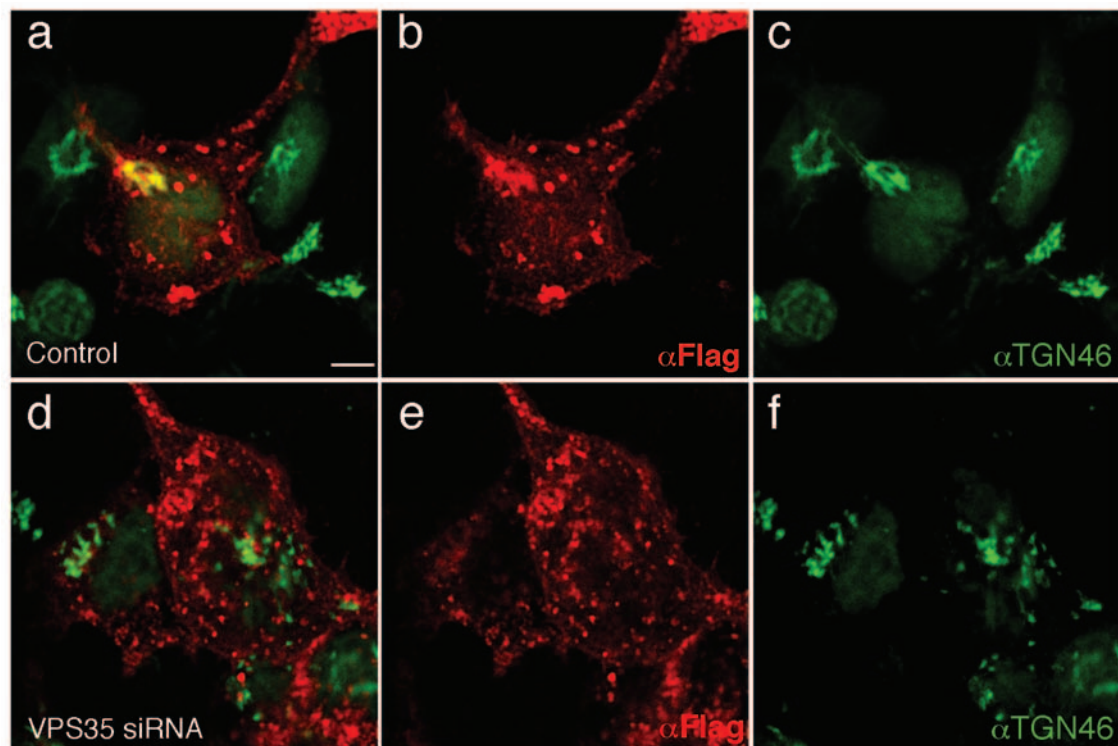


Figure S2 Vps35 knock-down reduces the steady-state level of hWls in the Golgi of HEK293T cells (**a-c**) HEK293T cells transfected with Wls-FLAG and stained with anti Flag and anti-TGN46. Significant co-localization is observed (see merge in panel **(a)**). hWls-Flag is shown in red and TGN46

in green. (**d-f**) HEK293T cells co-transfected with hWls-FLAG and siRNA against *hVps35*. Co-localization of Wls and TGN46 is strongly reduced (see merge in panel **(d)**) compared to that in control cells (shown in panel **a**). The scale bar represents 5 μ m.

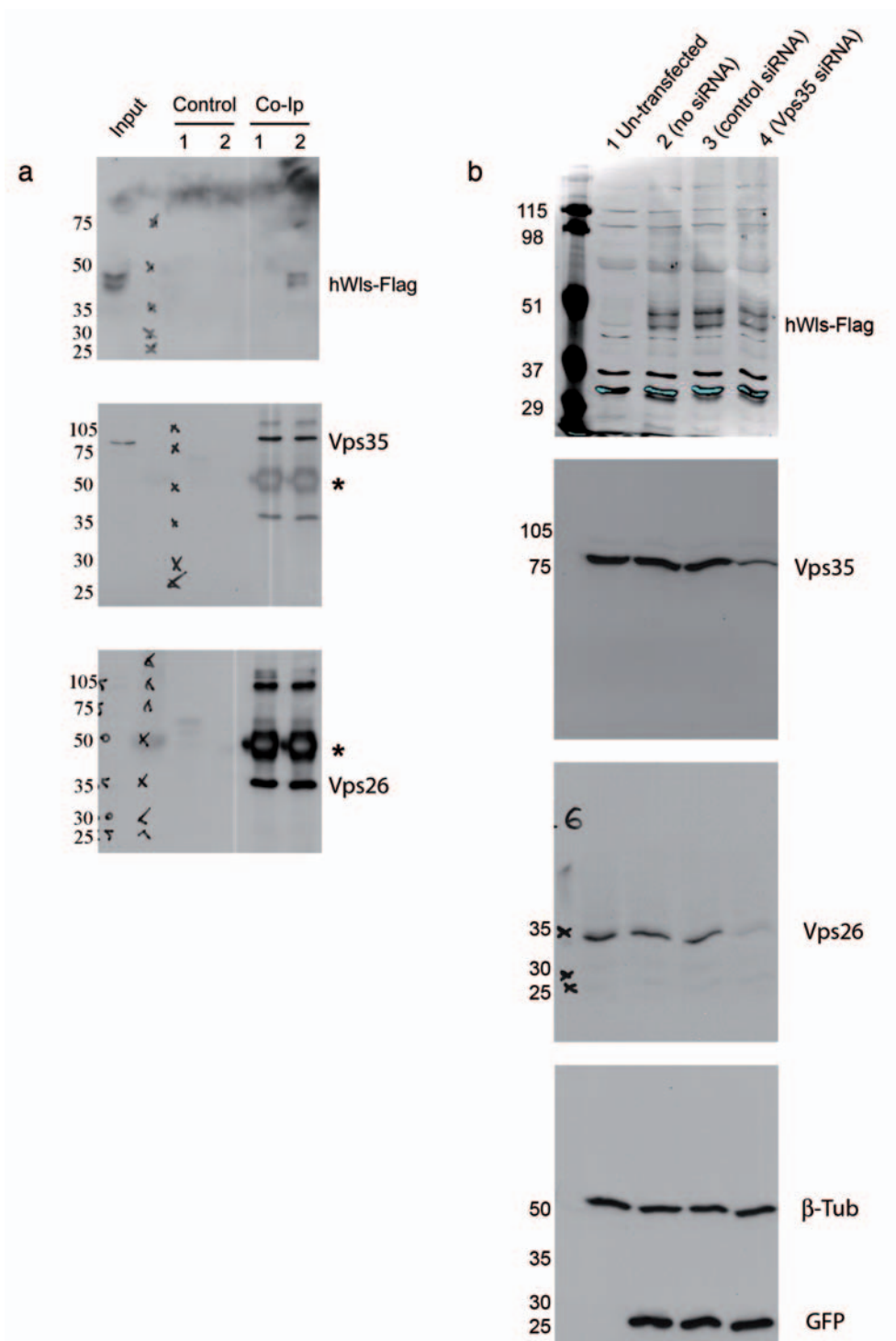


Figure S3 Unedited blots used in this paper. **(a)** Western blots of immunoprecipitated materials either in the absence (Control) or presence (Co-IP) of anti-Vps26 antibody. Immunoprecipitation was performed using extracts of HEK293 cells transfected with empty plasmid (lane 1) or with an hWls-Flag expressing plasmid (lane 2). The extract of hWls-Flag transfected cells is loaded on the left of the gel (Input). Top Panel: the blot was probed with anti-Flag to detect hWls-Flag. Middle and bottom panels: the blot was probed to detect Vps35 (middle panel) and subsequently stripped and re-probed to detect Vps26 (bottom panel). Asterisks indicate

the heavy chain of IgG. In the middle panel, apart from the VPS35 specific bands, faded bands are seen in the Co-IP due to the signals remained from the previous antibody incubation. In the bottom panel, Vps26 protein is not visible in the input but it is revealed by a longer exposure (data not shown). hWls-Flag is immunoprecipitated specifically by anti-Vps26 antibody. **(b)** Western blot of extracts from HEK293 cells. Extracts were probed with various antibodies as indicated on the right. Cells were transfected as indicated in each lane. Note the reduction in Vps35, Vps26, and Wls in lane 4. See Figures 3e and 4i, j for details.

REFERENCE

1. Alexandre, C., Lecourtois, M. & Vincent, J. Wingless and Hedgehog pattern *Drosophila* denticle belts by regulating the production of short-range signals. *Development (Cambridge, England)* **126**, 5689-5698 (1999).