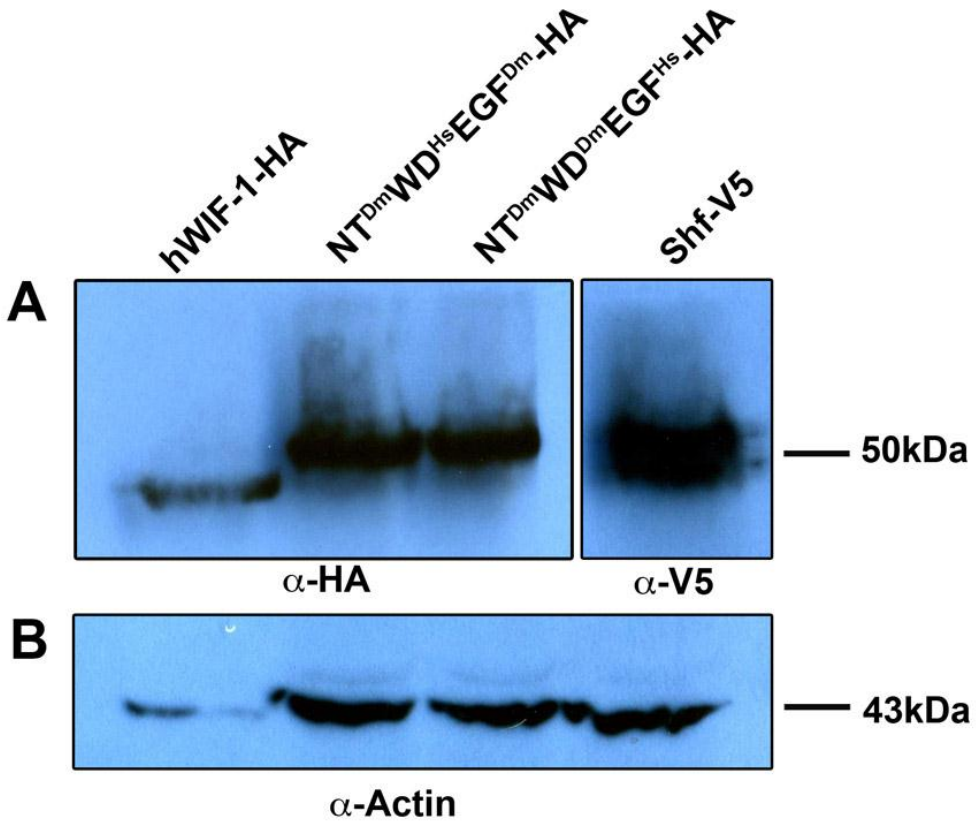

Fig. S1. Expression levels of the UAS constructs analyzed by western blotting. (**A**) hWIF-1-HA, NT^{Dm}-WD^{Hs}-EGF^{Dm}-HA, NT^{Dm}-WD^{Dm}-EGF^{Hs}-HA and Shf-V5 third instar larvae extracts stained with anti-HA or anti-V5 antibodies after their induction by the tub-gal4/tub-$gal80^{ts}$ system for 24 hours. (**B**) Endogenous actin protein levels were used as a control in all extracts.

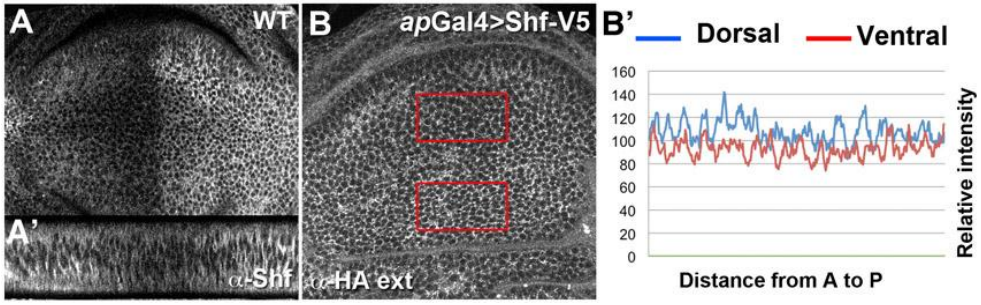

Fig. S2. Extracellular localization of Shf protein. (**A**,**A9**) Wild-type distribution of Shf in a wing disc. Note that Shf levels are higher in the entire P compartment and in the most anterior part of the anterior compartment, and lower at the A/P border. (**B**,**B9**) Extracellular staining using anti-V5 antibody of a wing disc expressing Shf-V5 in the dorsal compartment (*ap*Gal4>UAS-Shf-V5 wing imaginal disc). The extracellular Shf protein is homogenously distributed in both D and V compartments (B9). Quantification of proteins in dorsal and ventral compartments was performed using 12 discs for each genotype (B9).

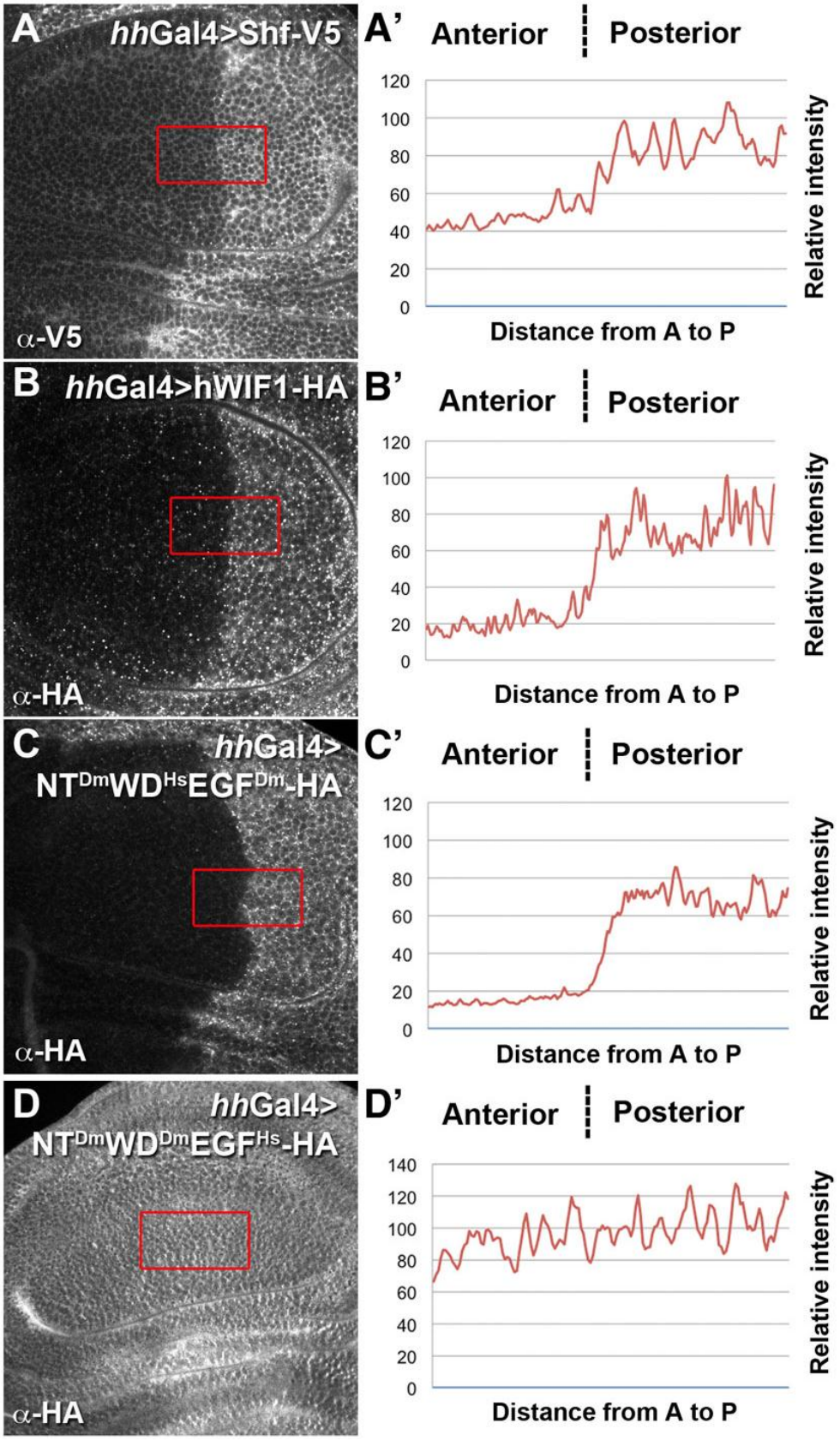

Fig. S3. Spreading properties of Shf, WIF1 and the hybrid Shf/WIF1 proteins. (**A-B9**) *hh*Gal4>Shf-V5 (A,A9) and *hh*Gal4>hWIF-1-HA (B,B9) wing imaginal discs stained with anti-V5 and anti-HA, respectively. Despite Shf only being induced in the P compartment, it is also found in the A compartment (A,A9). However, WIF1 is mostly restricted to its expression domain (B,B9). **(C-D9)** *hh*Gal4>NT^{Dm}-WD^{Hs}-EGF^{Dm}-HA (C,C9) and *hh*Gal4>NT^{Dm}-WD^{Dm}-EGF^{Hs}-HA (D,D9) wing discs. Note that NT^{Dm}-WD^{Hs}-EGF^{Dm}-HA protein is restricted to its expression domain (C,C9), while the distribution of NT^{Dm}-

WD^{Dm}-EGF^{Hs}-HA is similar to that of Shf (D,D9). Quantification of proteins in the A and P compartments was performed using an average of 13 discs for each genotype (A9-D9).

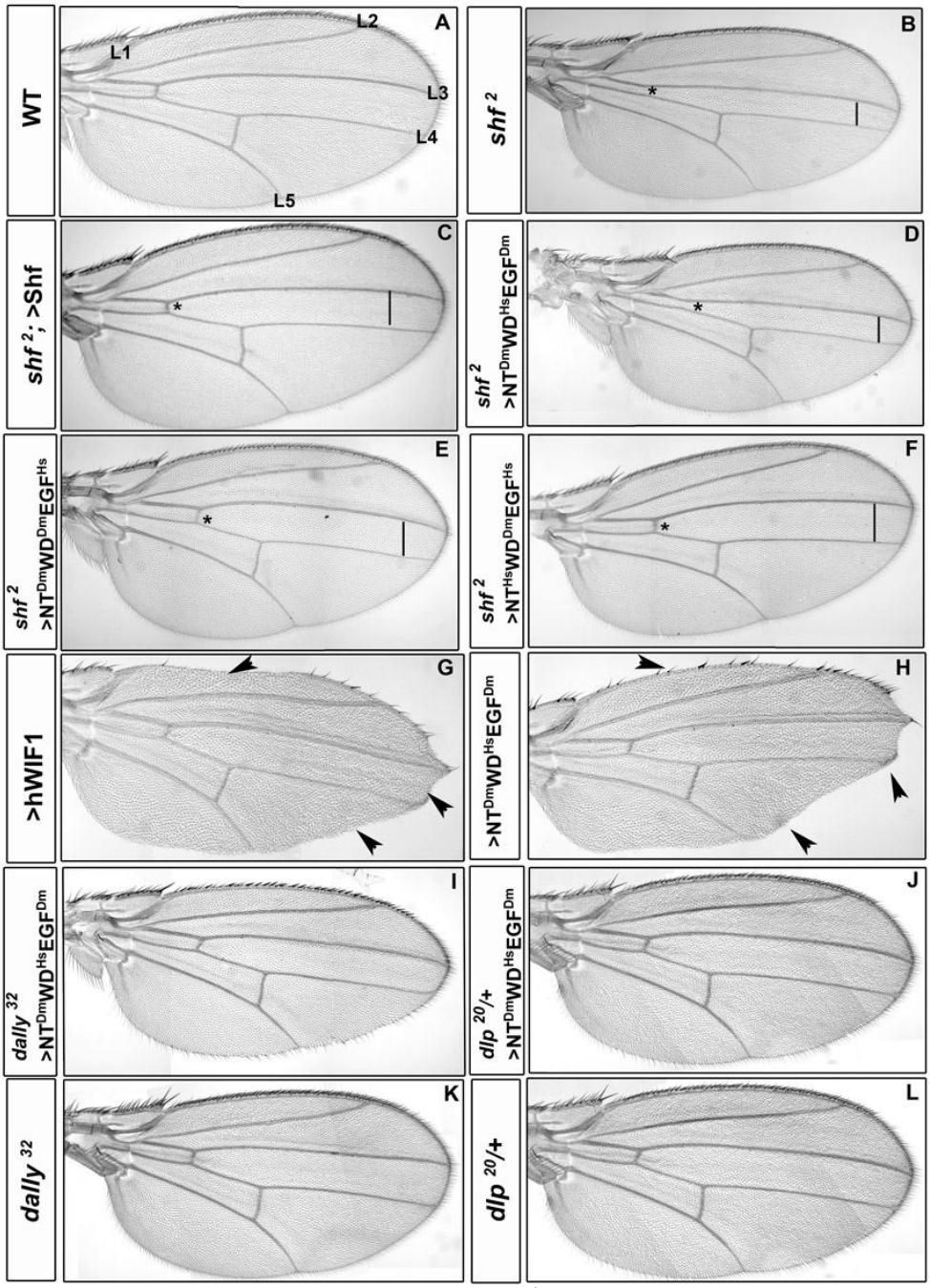

Fig. S4. Wing phenotypes. (**A**) Wild-type adult wing. (**B**) *shf*^{2} mutant wing. Note that the distance between L3-L4 veins is reduced (bar) and the anterior crossvein is absent (asterisk). (**C-F**) *shf*^{2}; *ap*Gal4>UAS-Shf-V5 (C), *shf*^{2};*ap*Gal4>NT^{Dm}-WD^{Hs}-EGF^{Hs} (D), *shf*^{2}; *ap*Gal4>UAS-NT^{Dm}-WD^{Dm}-EGF^{Hs} (E) and *shf*^{2}; *ap*Gal4>UAS-NT^{Hs}-WD^{Dm}-EGF^{Hs} (F) adult wings. Note that the L3-L4 distance reduction and the anterior crossvein of *shf* wings are fully rescued in C, E and F but not in D. At least ten flies were analyzed for each genotype. (**G**,**H**) *ap*Gal4>UAS-hWIF-1 (G) and apGal4>UAS-NT^{Dm}-WD^{Hs}-EGF^{Dm} (H) adult wings. Note the characteristic *wg* mutant phenotype with nicks in the wing margin (arrows). (**I**,**J**) Overexpression of NT^{Dm}-WD^{Hs}-EGF^{Dm} in a *dally* (I) or a *dlp* (J) mutant background. The *wg* mutant adult phenotype is partially rescued in both mutant backgrounds (compare with H). (**K**,**L**) *dally*^{32} and *dlp*^{20} mutant wings.

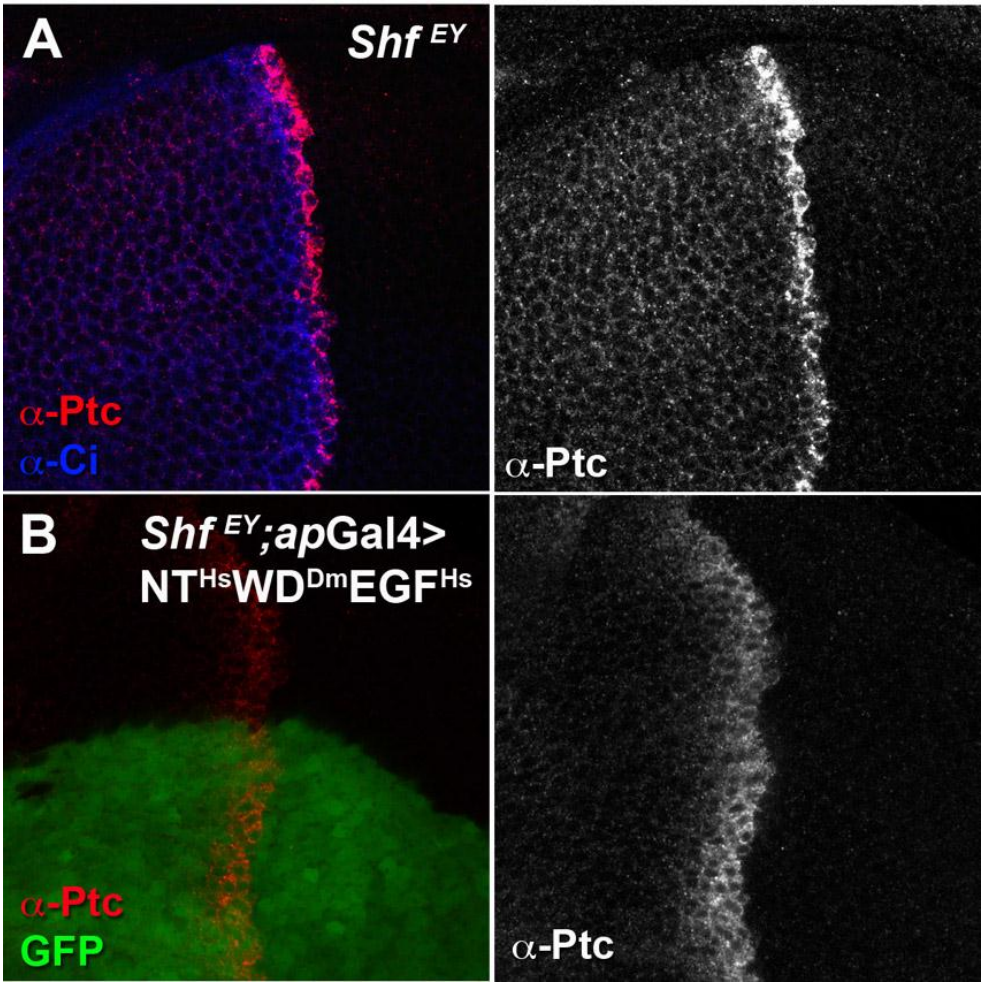

Fig. S5. Ectopic expression of NT^{Hs}-WD^{Dm}-EGF^{Hs} rescues the *shf* mutant disc phenotype. (**A**) Ptc expression in a *shf* mutant disc. In *shf* mutants, Ptc is expressed only in the first row of cells of the A compartment adjacent to the A/P border. (**B**) Normalized Ptc expression in shf^{EY}; *ap*Gal4>$NT^{Hs}WD^{Dm}$-EGF^{Hs}.

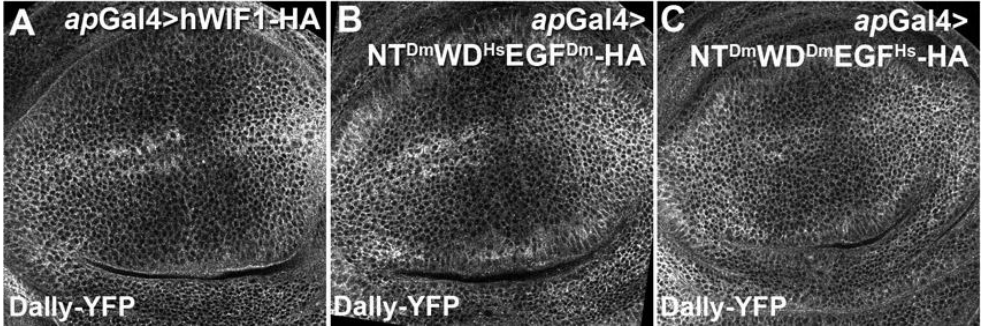

Fig. S6. Ectopic expression of WIF1 or chimeras has no effect on Dally. Overexpression of (**A**) WIF1, (**B**) NT^{Dm}-WD^{Hs}-EGF^{Dm} or (**C**) NT^{Dm}-WD^{Dm}-EGF^{Hs} in the dorsal compartment of the wing disc using the *ap*Gal4 driver did not have any effect on the distribution of the glypican Dally.

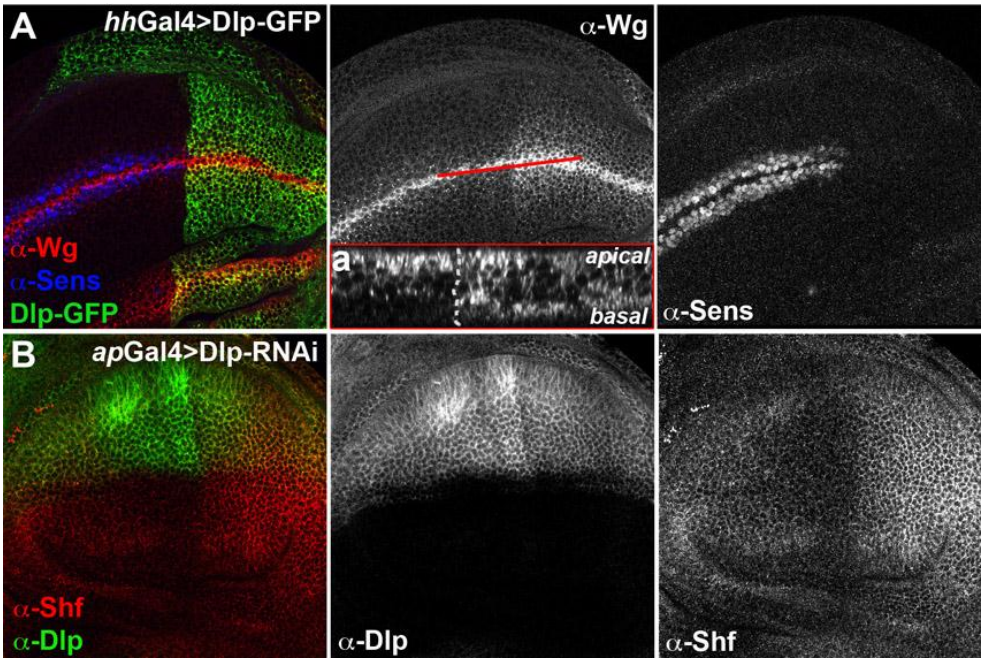

Fig. S7. Dlp attaches to Wg in the basolateral part of the disc epithelium. (**A**) Wg and Sens expression in *hh*Gal4>UAS-Dlp wing disc. Note the accumulation of Wg and the repression of Sens. (**B**) A transverse section (red line marks the location of the section) of the same disc to show the accumulation of Dlp and Wg mainly in the basal part of the epithelium. (**C**) Endogenous Shf protein levels in an *ap*Gal4>UAS-Dlp-RNAi wing disc. Diminution of Dlp expression does not affect Shf protein levels.

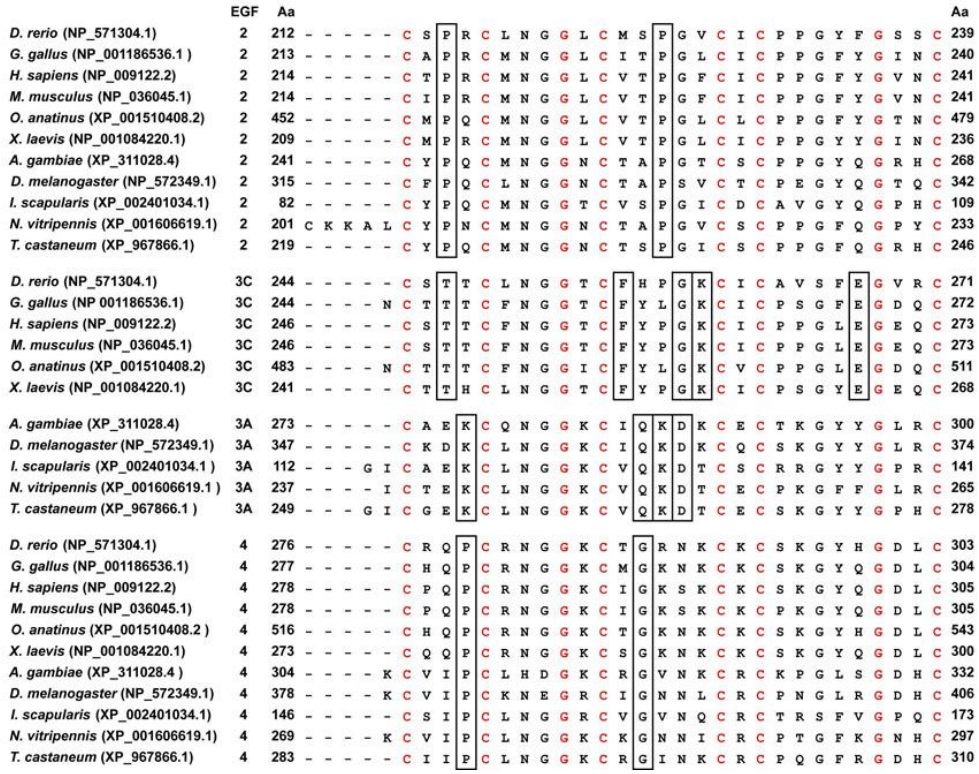

Fig. S8. Alignment of EGF domains 2, 3 and 4, extracted from sequences of Wif-1 and Shf. EGF 3 of chordates (3C) does not share conserved positions with EGF 3 of arthropods (3A), unlike EGFs 2 and 4. Positions conserved in all EGFs are marked in red and amino acids conserved in each EGF are boxed.