

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.

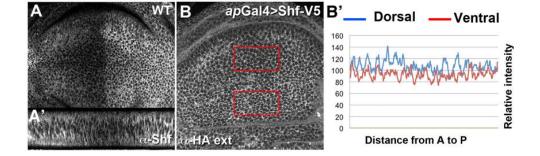


Fig. S2. Extracellular localization of Shf protein. (**A,A9**) Wild-type distribution of Shf in a wing disc. Note that Shf levels are higher in the entire P compartment and in the most anterior part of the anterior compartment, and lower at the A/P border. (**B,B9**) Extracellular staining using anti-V5 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).

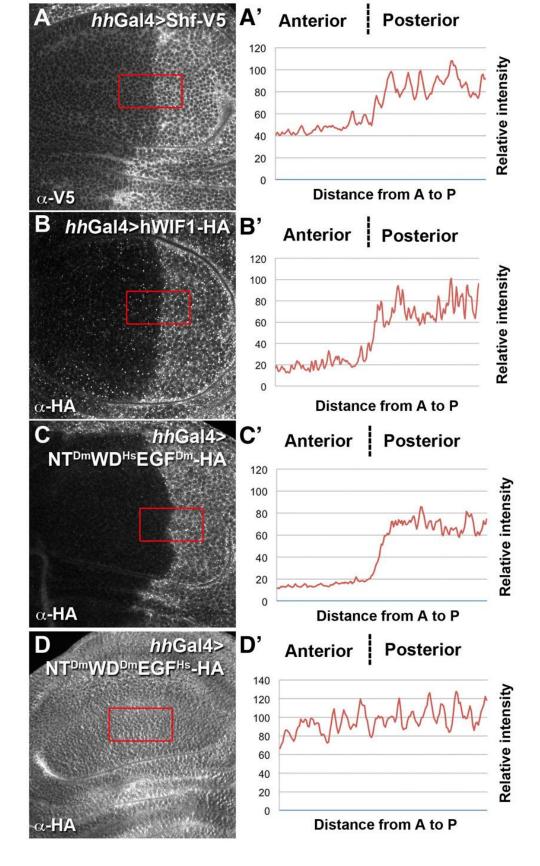


Fig. S3. Spreading properties of Shf, WIF1 and the hybrid Shf/WIF1 proteins. (A-B9) *hh*Gal4>Shf-V5 (A,A9) and *hh*Gal4>hWIF-1-HA (B,B9) wing imaginal discs stained with anti-V5 and anti-HA, respectively. Despite Shf only being induced in the P compartment, it is also found in the A compartment (A,A9). 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).

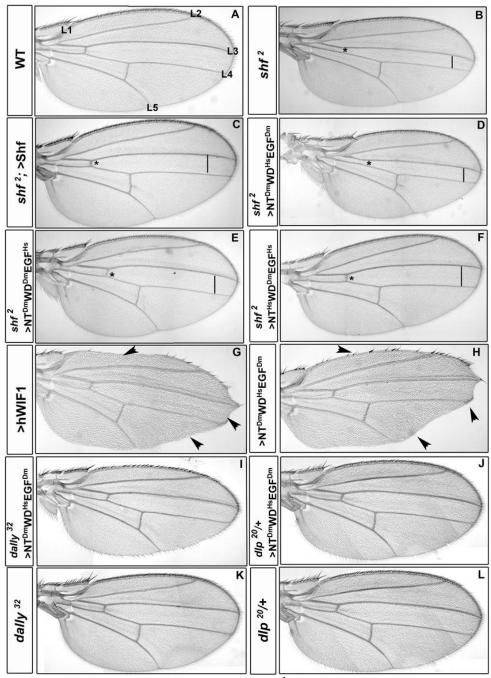


Fig. S4. Wing phenotypes. (**A**) Wild-type adult wing. (**B**) shf^2 mutant wing. Note that the distance between L3-L4 veins is reduced (bar) and the anterior crossvein is absent (asterisk). (**C-F**) shf^2 ; apGal4>UAS-Shf-V5 (**C**), shf^2 ; $apGal4>NT^{Dm}-WD^{Hs}-EGF^{Hs}$ (**D**), shf^2 ; $apGal4>UAS-NT^{Dm}-WD^{Dm}-EGF^{Hs}$ (**E**) and shf^2 ; $apGal4>UAS-NT^{Hs}-WD^{Dm}-EGF^{Hs}$ (**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**) apGal4>UAS-hWIF-1 (**G**) and $apGal4>UAS-hT^{Dm}-WD^{Hs}-EGF^{Dm}$ (**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.

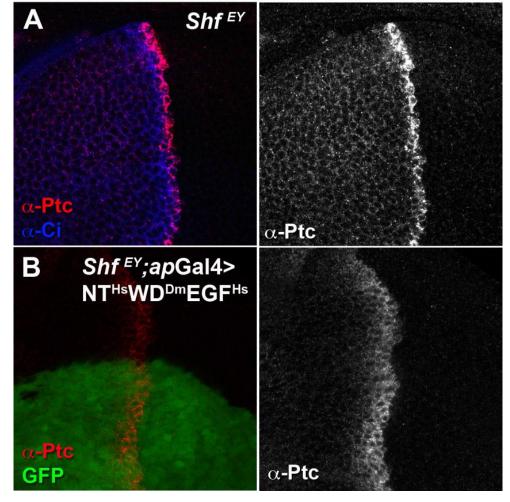


Fig. S5. Ectopic expression of NT^{Hs}-WD^{Dm}-EGF^{Hs} rescues the *shf* mutant disc phenotype. (A) Ptc expression in a *shf* mutant disc. In *shf* mutants, Ptc is expressed only in the first row of cells of the A compartment adjacent to the A/P border. (B) Normalized Ptc expression in shf^{EY} ; apGal4>NT^{Hs}WD^{Dm}-EGF^{Hs}.

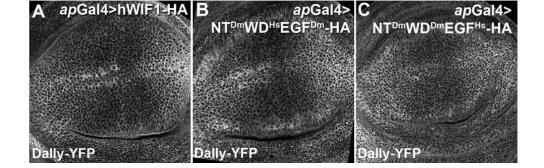


Fig. S6. Ectopic expression of WIF1 or chimeras has no effect on Dally. Overexpression of (A) WIF1, (B) NT^{Dm} -WD Hs -EGF Dm or (C) NT^{Dm} -WD Dm -EGF Hs in the dorsal compartment of the wing disc using the apGal4 driver did not have any effect on the distribution of the glypican Dally.

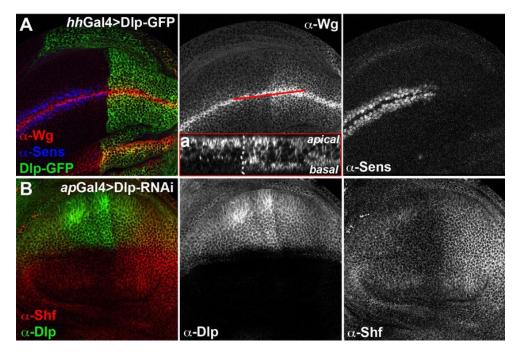


Fig. S7. Dlp attaches to Wg in the basolateral part of the disc epithelium. (A) Wg and Sens expression in hhGal4>UAS-Dlp wing disc. Note the accumulation of Wg and the repression of Sens. (B) A transverse section (red line marks the location of the section) of the same disc to show the accumulation of Dlp and Wg mainly in the basal part of the epithelium. (C) Endogenous Shf protein levels in an apGal4>UAS-Dlp-RNAi wing disc. Diminution of Dlp expression does not affect Shf protein levels.

	EGF	Aa																														_				Aa
D. rerio (NP 571304.1)	2	212	2	_	_	_	_	C	s	P	1 _R	C	L	N	G	G	L	C	М	s	P	G	v	C	I	C	P	P	G	Y	F	G	s	s	C	239
G. gallus (NP 001186536.1)	2	213	_	_	-		_	C	A	P	R	C	м	N	G	G	L	C	I	T	P	G	L	C	I	C	P	P	G	F	Y	G	I	N	C	240
H. sapiens (NP 009122.2)	2	214									200									23.	1000															
M. musculus (NP_036045.1)	2																																			241
O. anatinus (XP_001510408.2)	2										120									- 2																479
X. laevis (NP 001084220.1)	2										17									20.1	35.1															236
A. gambiae (XP_311028.4)	2	241	_	_	_	_	-	C	Y	P	l٥	C	м	N	G	G	N	C	т	A	P	G	т	C	s	C	P	P	G	Y	0	G	R	н	C	268
D. melanogaster (NP_572349.1)	2										-									200											1000					342
I. scapularis (XP_002401034.1)	2	82	-	-	-	-	-	C	Y	P	Q	C	м	N	G	G	T	C	v	s	P	G	I	C	D	C	A	v	G	Y	0	G	P	н	C	109
N. vitripennis (XP_001606619.1)	2	201	С	K	K	A	L	c	Y	P	N	C	м	N	G	G	N	C	т	A	P	G	v	C	S	C	P	P	G	F	0	G	P	Y	C	233
T. castaneum (XP_967866.1)	2	219	_	_	-	_	-	c	Y	P	Q	C	М	N	G	G	N	C	T	s	P	G	I	C	s	C	P	P	G	F	Q	G	R	Н	C	246
D. rerio (NP 571304.1)	3C	244	_	2	_	_	-	C	S	т	1 т	C	T.	N	G	G	т	C	F	н	p	G	K	C	т	C	A	v	S	F	E	G	v	R	C	271
G. gallus (NP 001186536.1)	3C	244																																		272
H. sapiens (NP_009122.2)	3C																			120 m	3653	100	100											~		273
M. musculus (NP_036045.1)	3C																																	_		273
O. anatinus (XP_001510408.2)	3C									100											- 1		100													511
X. laevis (NP_001084220.1)	3C																		22	40	25.3		200											-		268
A. gambiae (XP 311028.4)	3A	273	-	_		_	-	C	A	E	K	10	0	N	G	G	K	C	т	0	ĸ	р	K	C	E	C	т	K	G	Y	Y	G	L	R	C	300
D. melanogaster (NP_572349.1)	3A																																			374
I. scapularis (XP 002401034.1)	3A																																			141
N. vitripennis (XP_001606619.1)	3A	237																																		265
T. castaneum (XP_967866.1)	3A																																			278
D. rerio (NP 571304.1)	4	276	_	_	_	_	_	C	R	0	P	10	R	N	G	G	к	C	т	G	R	N	K	C	K	C	S	K	G	Y	н	G	D	I.	C	303
G. gallus (NP 001186536.1)	4									_																										304
H. sapiens (NP_009122.2)	4									-	100								6874		1997										_					305
M. musculus (NP_036045.1)	4																																			305
O. anatinus (XP_001510408.2)	4									-																										543
X. laevis (NP_001084220.1)	4									- 7																										300
A. gambiae (XP_311028.4)	4	304	-	-	-	-	K	c	v	I	P	c	L	н	D	G	ĸ	C	R	G	v	N	K	C	R	C	K	P	G	L	s	G	D	н	c	332
D. melanogaster (NP_572349.1)	4	378	-	-	_	_	K	c	v	I	P	c	K	N	E	G	R	C	I	G	N	N	L	C	R	C	P	N	G	L	R	G	D	н	C	406
I. scapularis (XP_002401034.1)	4																																			173
N. vitripennis (XP_001606619.1)	4	269									100								Sin	1850	100															
T. castaneum (XP_967866.1)	4	283	-	=	-	-	-	C	I	I	P	C	L	N	G	G	K	C	R	G	I	N	K	C	R	C	P	Q	G	F	R	G	D	н	C	310

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.