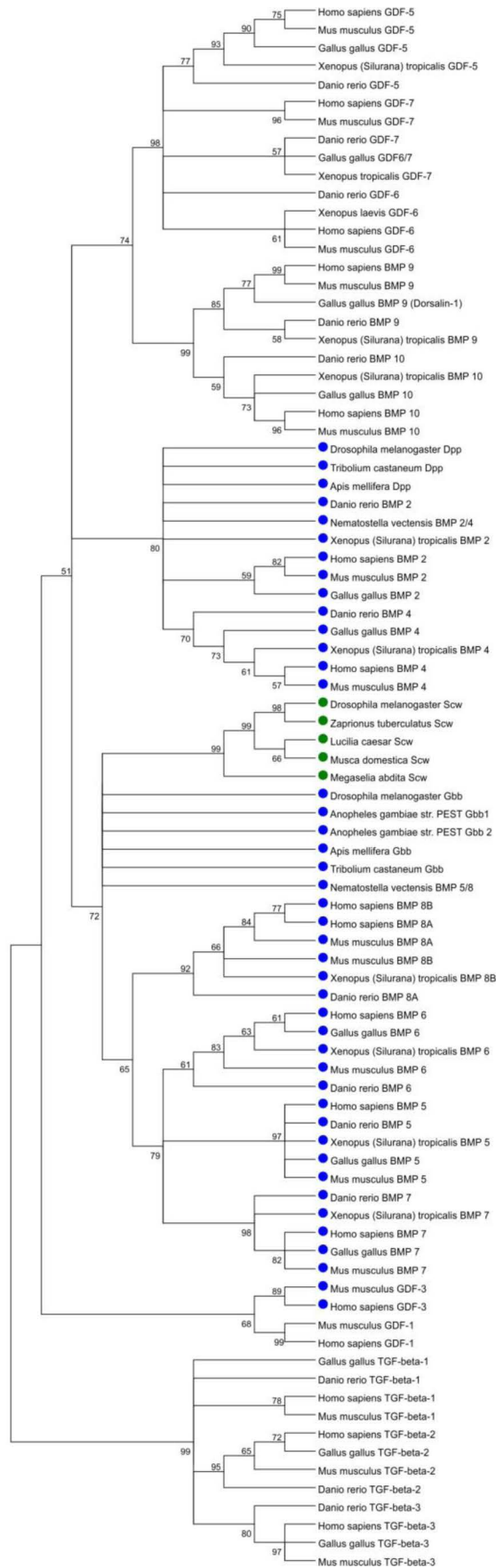


A



B

Gene	Sequence	Position
Homo sapiens_BMP_2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_BMP_2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Nematostella vectensis_BMP_2/4	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Drosophila melanogaster_Dpp	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Apis mellifera_Dpp	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Tribolium castaneum_Dpp	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_4	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_4	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_BMP_4	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_4	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_BMP_5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_BMP_6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_BMP_7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_8A	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_8A	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_8B	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_8B	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_BMP_8A	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_8B	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Nematostella vectensis_BMP_5/8	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Megastomia abditus_Scwr_Scwr	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Zaprionus tuberculosus_Scwr	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Lucilia caesar_Scwr	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Musca domestica_Scwr	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Anopheles gambiae_str_PEST_Gbb1	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Chobbi_gambiae_str_PEST_Gbb2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Drosophila melanogaster_Gbb	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Apis mellifera_Gbb	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Tribolium castaneum_Gbb	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_9	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_BMP_9	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_BMP_9 (Dorealin-1)	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_9	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_BMP_10	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_BMP_10	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_BMP_10	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_BMP_10	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_GDF-1	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_GDF-1	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_GDF-3	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_GDF-5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_GDF-5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_GDF-5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus (Silurana)_tropicalis_GDF-5	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_GDF-6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_GDF-6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_GDF-6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus laevis_GDF-6	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_GDF-7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_GDF-7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_GDF-7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Xenopus tropicalis_GDF-7	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_TGF-beta-1	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_TGF-beta-1	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_TGF-beta-1	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_TGF-beta-2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_TGF-beta-2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_TGF-beta-2	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Homo sapiens_TGF-beta-3	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Danio rerio_TGF-beta-3	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Gallus gallus_TGF-beta-3	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101
Mus musculus_TGF-beta-3	...KRRPLVDFSDVGNNDWI VAPPGYHAFYCHGCEPFLADHL	101

BMP 2/4

BMP 5/6/7/8

Scw Gbb

BMP 9/10 GDF-1/3/5/6/7

TGF-beta 1/2/3


Scw-specific  conserved 

Fig. S1. Phylogenetic analysis of the TGF- β type ligands. (A) Molecular phylogenetic analysis of BMP- and GDF-type ligands by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Jones et al., 1992). TGF- β 1/2/3 were used as outgroup. The percentage of trees in which the associated taxa clustered together is shown next to the branches and is based on 500 bootstrap trials. We used 50% as the cut-off value for condensing the tree. Branches supported by less than 50% are collapsed. The analysis involved 86 amino acid sequences (B). Blue dots indicate TGF- β type ligands comprising the conserved N-glycosylation motif. Green dots indicate Scw-type ligands including the Scw-specific N-glycosylation motif. (B) Amino acid multiple sequence alignment of various TGF- β -type ligand domains from different animal species. Amino acid sequence alignment of the conserved TGF- β ligand domain reveals a highly conserved N-glycosylation motif. N-glycosylation motifs are highlighted in red. The green arrow indicates the Scw-specific N-glycosylation motif. The blue arrow indicates the conserved, BMP-type ligand specific N-glycosylation motif. The alignment includes BMP2/4/Dpp, BMP5/6/7/8/Scw/Gbb, BMP9/10, GDF-1/3/5/6/7, and TGF- β type 1/2/3 ligand domains. Dashes are used to fill gaps. Reference numbers of the analyzed sequences can be found in Table S2.

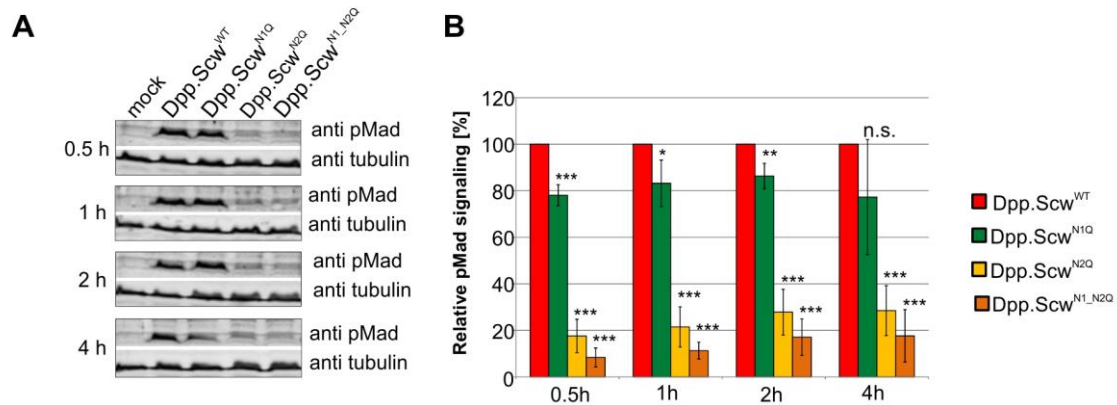


Fig. S2. Time-course of BMP signaling assay in *Drosophila* S2 cells. (A) Western blot analysis of a cell-based signaling assay in *Drosophila* S2 cells incubated with equivalent amount of either Dpp:Scw^{WT}, Dpp:Scw^{N1Q}, Dpp:Scw^{N2Q}, Dpp:Scw^{N1_N2Q}, or with PBS/0,1%BSA (mock). The pMad signal was measured at the time points 0.5, 1, 2, and 4 hours. Tubulin was used as an internal control. (B) Analysis of the Western blot shown in (A). The pMad intensity in Dpp:Scw^{WT} was calculated as 100%. Mock data were set to 0%. Data are obtained from five biological replicates, each performed in duplicates (n=5, graphs indicate means±95% CI, * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$, n.s., not significant. Significance was calculated by using the two-tailed Student's t-test.).

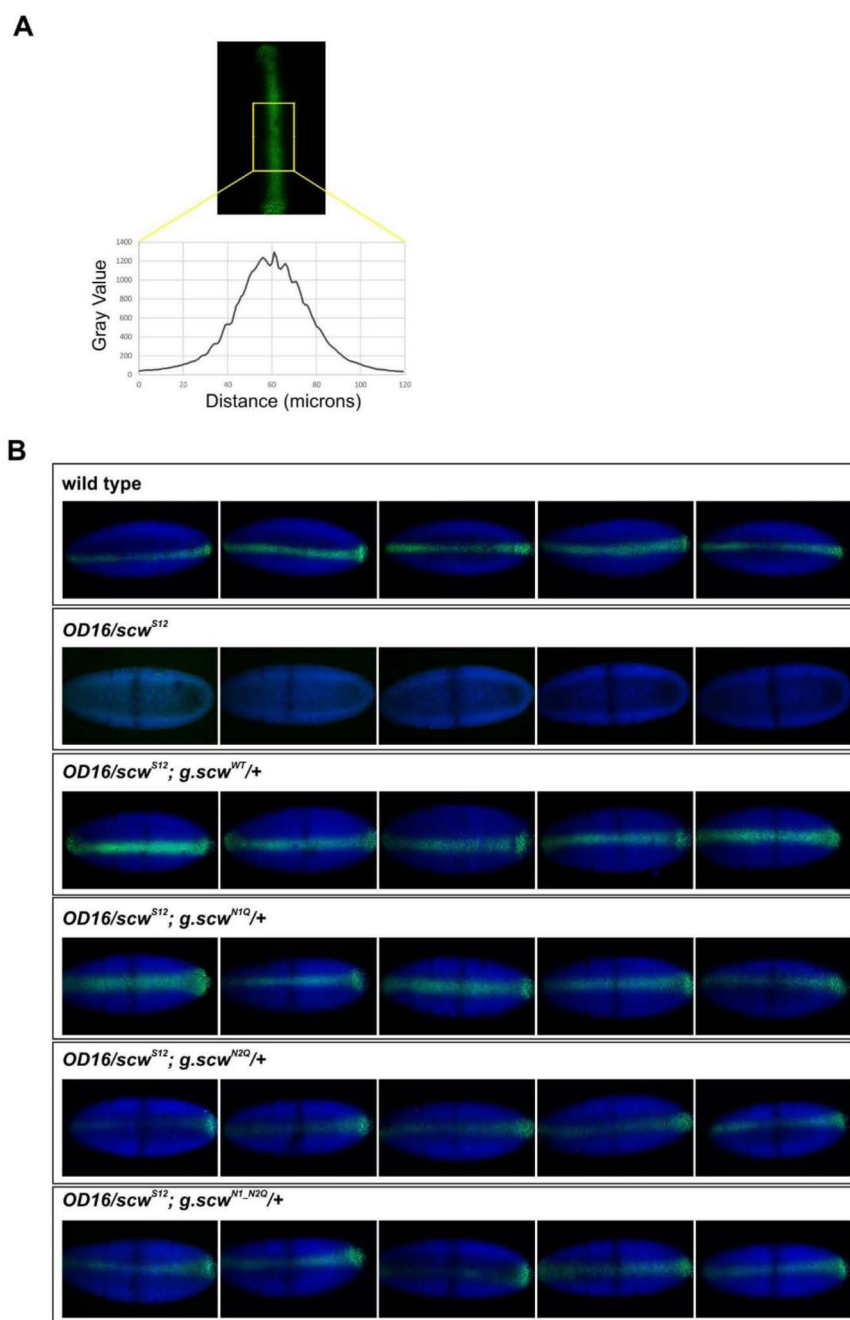


Fig. S3. Quantification of pMad signaling in *Drosophila* early embryos. (A) pMad intensity within a 120 x 200 μm rectangle was detected. The center of the rectangle was aligned with the the Kr-lacZ stripe (deriving from the *Df(2L)OD16, kr-lacZ* chromosome). (B) The pMad intensities of 5 embryos per genotype were measured for quantitative analysis (Fig. 3).

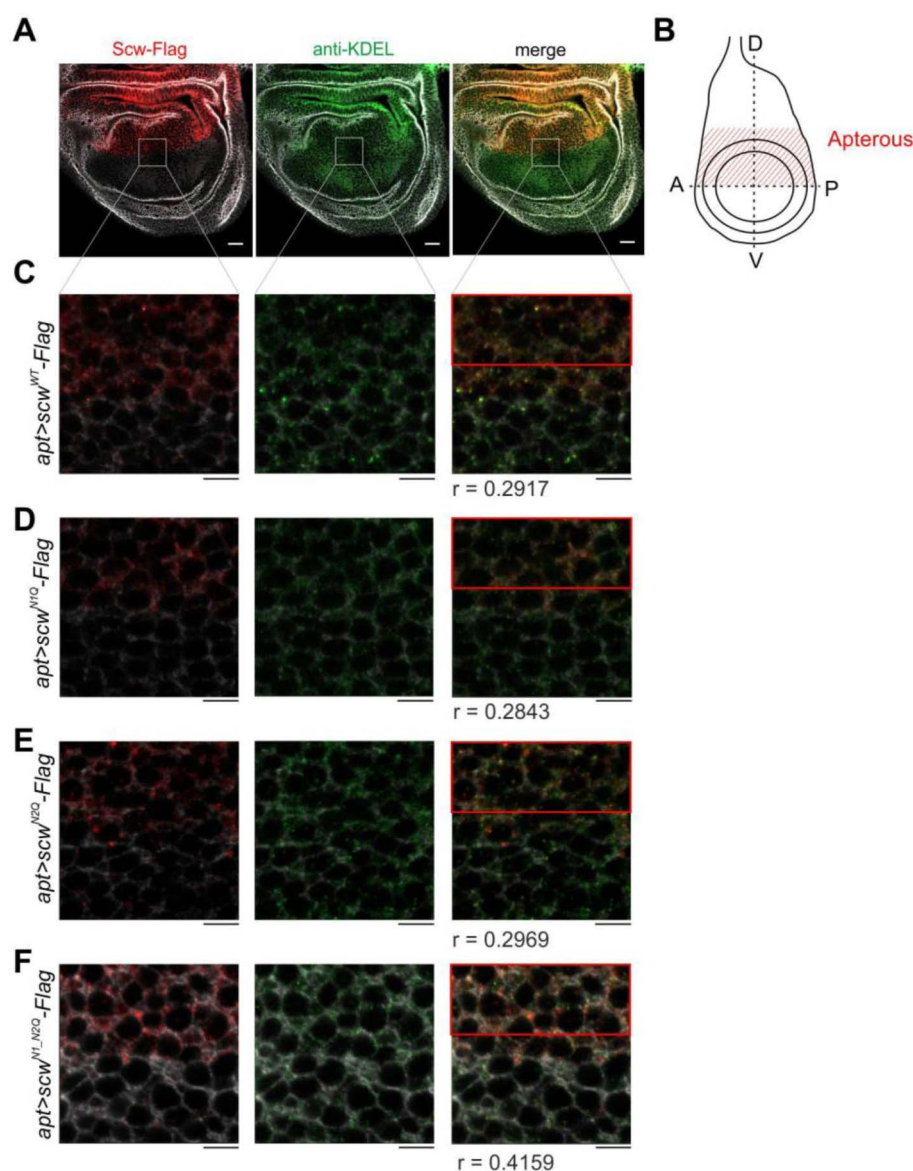


Fig. S4. Co-localization study of Scw glycosylation mutants with the endoplasmic reticulum (ER) (A) Wing imaginal disc expressing *UAS-Scw^{WT}-Flag* under control of *apterous-Gal4* in the dorsal part of the wing disc. Grey squares highlight the approximate wing disc region shown in C-F. Flag (red), KDEL (green), and Scrib (white) were used for visualizing Scw ligands, ER, and cell membrane, respectively. (B) Schematic of the wing imaginal disc indicating the expression pattern of Apterous. (D – dorsal, V – ventral, A – anterior, P – posterior) (C-F) The pixel intensity correlation of the red and the green channel was analyzed for the region highlighted in the merged images. The corresponding Pearson's coefficient (r) is listed below the image. The images shown

are one section of a Z-stack through the wing imaginal disc. Scale bars in A = 20 μm . Scale bars in C-F = 5 μm .

Reference

Jones, D. T., Taylor, W. R. and Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Computer applications in the biosciences : CABIOS* **8**, 275-282.

Table S1. Rescue experiment with transgenic flies.

	% of rescued flies carrying 1 copy of the rescue construct <i>OD16/scw^{S12};g.scw/+</i> ^a					% of rescued flies carrying 2 copies of the rescue construct <i>OD16/scw^{S12};g.scw/g.scw</i> ^a				
	<i>OD16/scw^{S12}</i>	<i>g.scw^{WT}</i>	<i>g.scw^{N1Q}</i>	<i>g.scw^{N2Q}</i>	<i>g.scw^{N1_N2Q}</i>	<i>OD16/scw^{S12}</i>	<i>g.scw^{WT}</i>	<i>g.scw^{N1Q}</i>	<i>g.scw^{N2Q}</i>	<i>g.scw^{N1_N2Q}</i>
1	0.0 (35)	112.8 (61)	11.1 (57)	0.0 (47)	0.0 (37)	0.0 (49)	88.9 (13)	71.4 (57)	0.0 (11)	6.5 (32)
2	0.0 (52)	80.0 (63)	18.2 (36)	0.0 (39)	0.0 (44)	0.0 (54)	133.3 (40)	70.8 (65)	0.0 (26)	40.0 (12)
3	0.0 (51)	61.5 (51)	36.8 (45)	0.0 (34)	0.0 (35)	0.0 (50)	50.0 (10)	153.2 (83)	22.2 (10)	0.0 (20)
4	0.0 (51)	60.6 (43)	28.6 (48)	0.0 (11)	0.0 (33)	0.0 (72)	106.7 (23)	122.2 (58)	0.0 (20)	6.3 (33)
5	0.0 (54)	88.9 (52)	48.6 (46)	0.0 (24)	0.0 (47)	0.0 (45)	105.3 (29)	94.4 (53)	0.0 (13)	0.0 (25)
6	0.0 (40)	120.9 (69)	32.3 (36)	0.0 (52)	0.0 (52)	0.0 (64)	171.4 (26)	56.0 (64)	6.7 (31)	100.0 (15)
7	0.0 (46)	133.3 (20)	47.5 (73)	0.0 (17)	0.0 (47)	0.0 (65)	271.4 (33)	66.7 (56)		13.3 (16)
8	0.0 (58)	126.3 (62)	40.0 (72)	0.0 (31)	0.0 (26)	0.0 (53)	110.0 (31)	66.7 (56)		0.0 (29)
9	0.0 (50)	87.8 (59)	43.9 (50)	0.0 (49)	0.0 (32)	0.0 (37)	123.1 (42)	54.8 (79)		0.0 (22)
10	0.0 (41)	68.4 (51)	40.0 (66)	0.0 (27)		0.0 (54)	280.0 (24)	95.5 (65)		0.0 (24)
11	0.0 (47)	53.8 (33)	71.4 (38)	0.0 (52)		0.0 (47)	158.3 (43)	128.9 (74)		0.0 (30)
12	0.0 (50)	100.0 (36)	27.3 (50)	5.1 (40)		0.0 (46)		88.5 (88)		
13	0.0 (41)	116.1 (49)	102.7 (56)	0.0 (38)		0.0 (9)		76.2 (87)		
14	0.0 (50)	90.9 (64)	15.1 (57)	4.3 (47)		0.0 (61)		113.6 (69)		
15	0.0 (44)	41.9 (52)		0.0 (37)				88.2 (49)		
16	0.0 (53)	95.2 (31)		0.0 (42)				74.5 (70)		
17	0.0 (54)	77.4 (43)		0.0 (38)				68.0 (67)		
18	0.0 (42)	109.1 (34)		0.0 (45)						
19	0.0 (38)									
Σ ^b	19	18	14	18	9	14	11	17	6	11

^a Number of counted flies is shown in parentheses. ^b Total amount of analysed crosses. 20 crosses were set for each genotype (1 virgin female/cross).

Table S2. Reference numbers of the TGF- β type ligands used in the phylogentic analysis

	Species	NCBI Reference	GenBank	UniProtKB/ Swiss-Prot
1	<i>Homo sapiens</i>	NP_001191.1		
2	<i>Mus musculus</i>	NP_031579.2		
3	<i>Gallus gallus</i>	NP_989689.1		
4	<i>Danio rerio</i>	NP_571435.1		
5	<i>Xenopus (Silurana) tropicalis</i>	NP_001015963.1		
6	<i>Nematostella vectensis</i>		AAR13362.1	
7	<i>Drosophila melanogaster</i>		AAN10431.1	
8	<i>Megaselia abdita</i>		AFK24733.1	
9	<i>Apis mellifera</i>	XP_006569849.1		
10	<i>Tribolium castaneum</i>	NP_001034540.1		
11	<i>Homo sapiens</i>	NP_001193.2		
12	<i>Mus musculus</i>	NP_031580.2		
13	<i>Gallus gallus</i>	NP_990568.3		
14	<i>Danio rerio</i>	NP_571417.1		
15	<i>Xenopus (Silurana) tropicalis</i>	NP_001017034.2		
16	<i>Homo sapiens</i>			P22003.1
17	<i>Danio rerio</i>	NP_957345.1		
18	<i>Gallus gallus</i>	NP_990479.1		
19	<i>Mus musculus</i>	NP_031581.2		
20	<i>Xenopus (Silurana) tropicalis</i>	XP_002934893.1		
21	<i>Homo sapiens</i>			P22004.1
22	<i>Danio rerio</i>	NP_001013357.1		
23	<i>Gallus gallus</i>	XP_418956.4		
24	<i>Mus musculus</i>	NP_031582.1		
25	<i>Xenopus (Silurana) tropicalis</i>	NP_001106378.1		
26	<i>Homo sapiens</i>			P18075.1
27	<i>Danio rerio</i>	NP_001070614.1		
28	<i>Mus musculus</i>	NP_031583.2		
29	<i>Gallus gallus</i>	XP_417496.4		
30	<i>Xenopus (Silurana) tropicalis</i>	NP_989197.1		
31	<i>Homo sapiens</i>	NP_861525.2		
32	<i>Mus musculus</i>	NP_001242948.1		
33	<i>Danio rerio</i>	NP_001038436.1		
34	<i>Homo sapiens</i>			P34820.2
35	<i>Mus musculus</i>	NP_031585.2		
36	<i>Xenopus (Silurana) tropicalis</i>	XP_002941697.1		
37	<i>Nematostella vectensis</i>		ABC88372.1	
38	<i>Megaselia abdita</i>		AFK24736.1	
39	<i>Drosophila melanogaster</i>	NP_001286088.1		
40	<i>Zaprionus tuberculatus</i>		ADR57149.1	
41	<i>Lucilia caesa</i>		ADR57151.1	
42	<i>Musca domestica</i>		ADR57152.1	
43	<i>Anopheles gambiae str. PEST</i>	XP_316789.3		
44	<i>Anopheles gambiae str. PEST</i>	XP_320599.3		
45	<i>Drosophila melanogaster</i>	NP_001286786.1		
46	<i>Apis mellifera</i>	XP_394252.2		
48	<i>Tribolium castaneum</i>	NP_001107813.1		
47	<i>Homo sapiens</i>			Q9UK05.1
48	<i>Danio rerio</i>	NP_001165057.1		
49	<i>Gallus gallus</i>	NP_990763.1		
50	<i>Mus musculus</i>	NP_062379.3		
51	<i>Xenopus (Silurana) tropicalis</i>	XP_002940692.2		
52	<i>Homo sapiens</i>			O95393.1
53	<i>Danio rerio</i>	NP_001124072.1		
54	<i>Gallus gallus</i>	NP_001264975.1		
55	<i>Mus musculus</i>	NP_033886.2		
56	<i>Xenopus (Silurana) tropicalis</i>	XP_002935357.1		
57	<i>Homo sapiens</i>	NP_001483		
58	<i>Mus musculus</i>	NP_032133.2		
59	<i>Homo sapiens</i>	NP_065685.1		
60	<i>Mus musculus</i>	NP_032134.2		
61	<i>Homo sapiens</i>			P43026.3
62	<i>Danio rerio</i>	XP_002662587.1		
63	<i>Gallus gallus</i>	NP_989669.1		

64	<i>Mus musculus</i>	NP_032135.2		
65	<i>Xenopus (Silurana) tropicalis</i>	NP_001128589.1		
66	<i>Homo sapiens</i>			Q6KF10.1
67	<i>Danio rerio</i>	NP_001153466.1		
68	<i>Mus musculus</i>	NP_038554.1		
69	<i>Xenopus laevis</i>			Q9W753
70	<i>Homo sapiens</i>			Q7Z4P5.2
71	<i>Danio rerio</i>	XP_694563.2		
72	<i>Mus musculus</i>	NP_038555.1		
73	<i>Gallus gallus</i>			O93573
74	<i>Xenopus tropicalis</i>			F6Z923
75	<i>Homo sapiens</i>			P01137.2
76	<i>Danio rerio</i>	NP_878293.1		
77	<i>Gallus gallus</i>		AFD30526.1	
78	<i>Mus musculus</i>	NP_035707.1		
79	<i>Homo sapiens</i>			P61812.1
80	<i>Danio rerio</i>	NP_919366.1		
81	<i>Gallus gallus</i>	NP_001026216.2		
82	<i>Mus musculus</i>	NP_033393.2		
83	<i>Homo sapiens</i>			P10600.1
84	<i>Danio rerio</i>	NP_919367.2		
85	<i>Gallus gallus</i>	NP_990785.1		
86	<i>Mus musculus</i>	NP_033394.2		