Table S1. Genotypes of *Drosophila* strains used in each figure

Figure	Panel	Genotype
1	A-G	+/+
1	D-G	*wdp^{KO}/wdp^{KO}*
1	D	*Act5C-Gal4/UAS-Chsy$^{RNAi.GD14159}$*
1	D	*wdp-HA/+*
1	D	*Act5C-Gal4/UAS-Chsy$^{RNAi.GD14159}$; wdp-HA/+*
2	A, D	+/+
2	B	*UAS-Chsy$^{RNAi.GD14159}$/+; hh-Gal4 UAS-GFP/+*
2	C, D	*wdp^{KO}/wdp^{KO}*
2	E	*hh-Gal4 UAS-GFP/UAS-wdp$^{RNAi.HM05118}$*
2	F	*UAS-C4ST (UAS-CG31743.IR.Y)/+; hh-Gal4 UAS-GFP/+*
2	G	*trol-GFP/+; wdp-HA/+*
3	A, D	*Bx^{MS1096}-Gal4/+*
3	B, D	*Bx^{MS1096}-Gal4/+; UAS-wdp/+*
3	C, D	*Bx^{MS1096}-Gal4/UAS-C4ST (UAS-CG31743.IR.Y); UAS-wdp/+*
4	A, E, H	+/+
4	B-D	*nub-Gal4/+; UAS-wdp/+*
4	F-H	*hs-flp/+; Act5C>CD2>Gal4 UAS-GFP/+; UAS-wdp/+*
5	A, E, H	+/+
5	B-D, F	*nub-Gal4/+; UAS-wdp/+*
5	G, I, J	*ap-GAL4 UAS-GFP/+; UAS-wdp/+*
5	K, L	*hs-flp/+; Act5C>CD2>Gal4 UAS-GFP/+; UAS-wdp/+*
5	M	*hs-flp/+; Act5C>CD2>Gal4 UAS-GFP/+; UAS-3xMyc-wdp $^{\Delta GAG}$/+*
6	A, J, M	+/+
6	B	*wdp^{KO}/wdp^{KO}*
6	C	*Sulf1$^{\Delta P1}$/Sulf1$^{\Delta P1}$*
6	D-I, K, L, N, O	*wdp^{KO}/wdp^{KO}; Sulf1$^{\Delta P1}$/Sulf1$^{\Delta P1}$*
7	A, B, E, J, K	+/+
7	C, D, H, I, J, L	*wdp^{KO}/wdp^{KO}; Sulf1$^{\Delta P1}$/Sulf1$^{\Delta P1}$*
7	F	*wdp^{KO}/wdp^{KO}*
7	G	*Sulf1$^{\Delta P1}$/Sulf1$^{\Delta P1}$*
8	A, C, F	+/+
8	B, E, I	*wdp^{KO}/wdp^{KO}; dally^{gem}/dally^{gem}*
8	D, H	*dally^{gem}/dally^{gem}*
8	G	*wdp^{KO}/wdp^{KO}*
8	J	*dpp^{d-ho}/dpp^{d-ho}*
8	K	*upd^{os-s}/upd^{os-s}*
8	L	*Mef2-Gal4/UAS-ttv RNAi^{GD1993}*
8	M	*Mef2-Gal4/UAS-sfl RNAi^{HMS00543}*

Table S2. Disaccharide analyses of CS from wild-type and *wdp* mutant adults.
Disaccharide composition of CS is shown for each respective genotype. The values are given as mol% of total disaccharides, and represent mean ± S.D. from three independent experiments. Graphical depiction of this result is shown in Fig. 1E and F.

	CS (unsaturated disaccharide, %)		Total amount
	ΔDi-0S	ΔDi-4S	(ng/mg)
wild-type	73.1±0.4	26.9±0.4	159.0±4.7
wdp	75.1±0.3	24.9±0.3	128.9±12.9

Table S3. Disaccharide analyses of HS from wild-type and *wdp* mutant adults.
Disaccharide composition of HS is shown for each respective genotype. The values are given as mol% of total disaccharides, and represent mean ± S.D. from three independent experiments. NAc, ΔUA-GlcNAc; NS, ΔUA-GlcNS; NAc6S, ΔUA-GlcNAc6S; NS6S, ΔUA-GlcNS6S; 2SNS, ΔUA2S-GlcNS; and 2SNS6S, ΔUA2S-GlcNS6S. A graph showing total amount of HS is shown in Fig. 1G.

	HS (unsaturated disaccharide, %)						Total amount
	NAc	NS	NAc6S	NS6S	2SNS	2SNS6S	(ng/mg)
wild-type	44.9±5.2	20.9±2.2	1.8±0.2	16.1±1.9	13.3±1.3	3.0±0.7	17.7±1.6
wdp	45.4±4.6	21.3±2.4	1.6±0.1	14.9±0.7	14.0±1.3	2.9±0.4	25.5±1.5

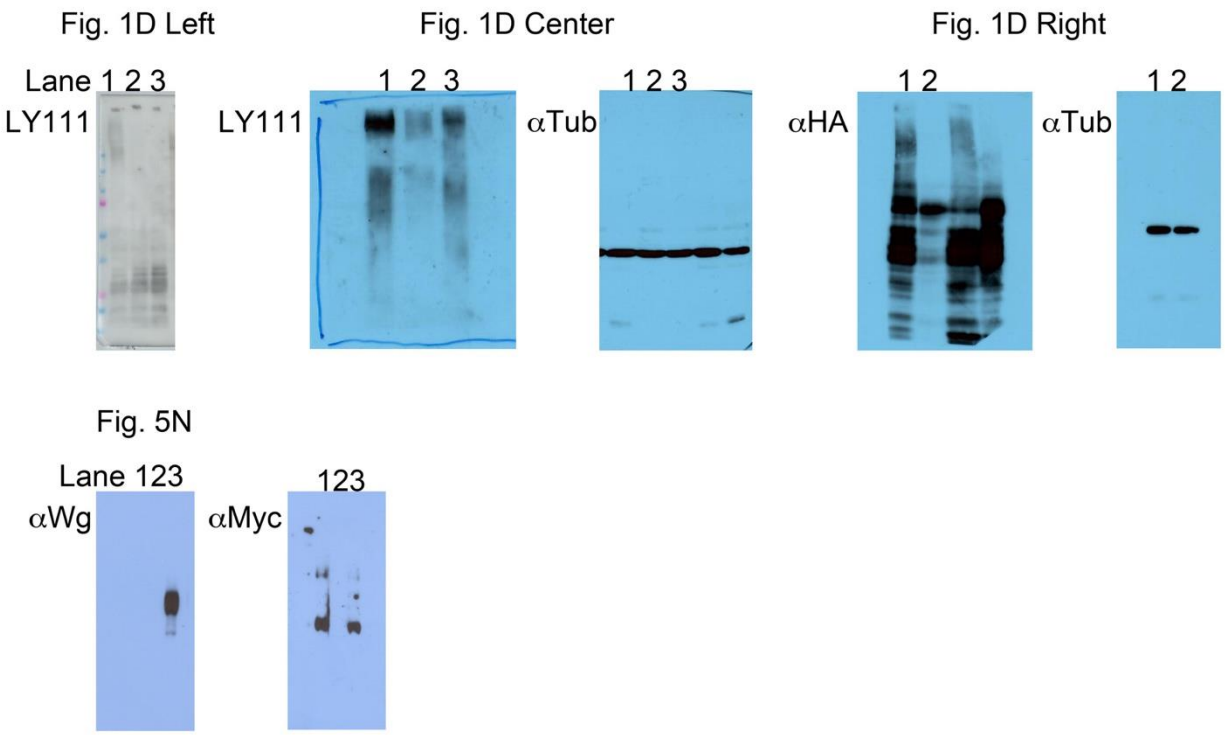

Fig. S1. Blot transparency.

Original Immunoblot data used for Fig. 1D and Fig. 5N are shown. Only the lanes with lane numbers were used in each panel. Antibodies used are indicated.