Supplemental Information

Supplementary Figures

Supplementary Tables

FigS1

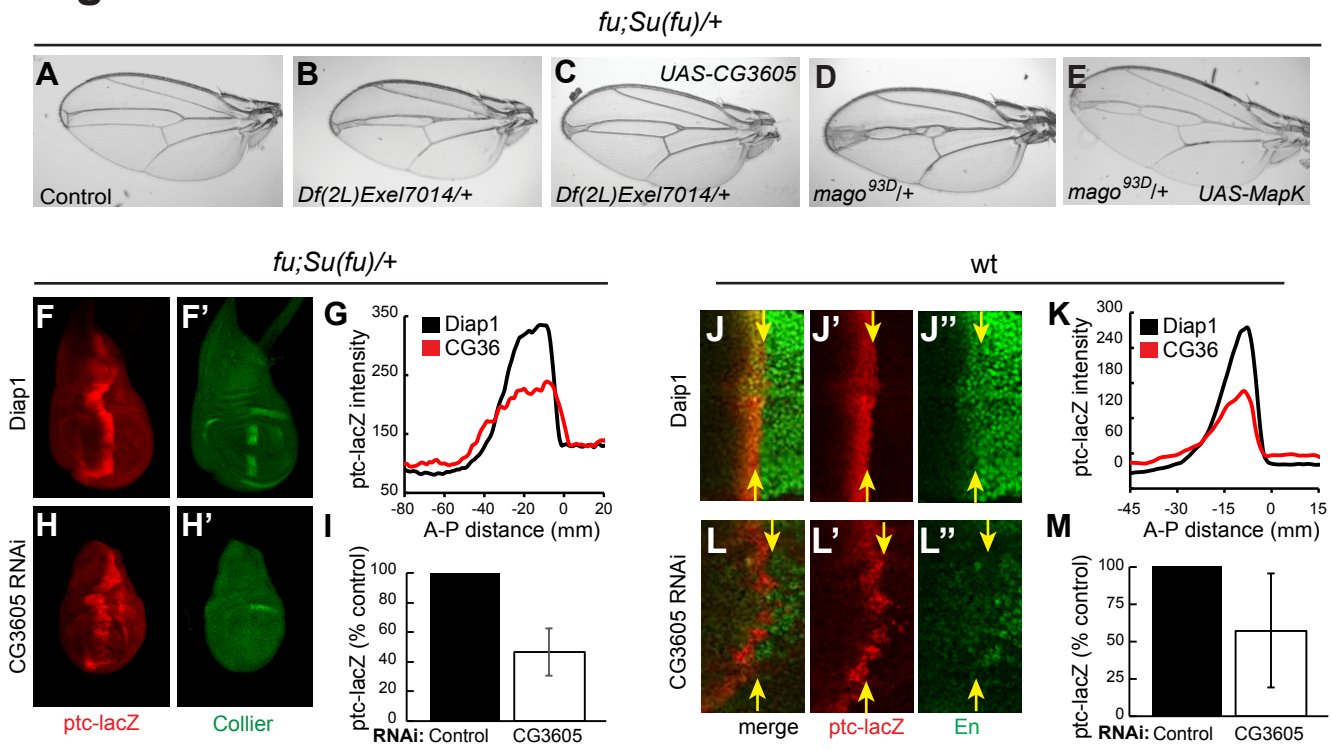

Fig. S1. CG3605, implicated in RNA splicing, modifies Hh pathway activity.

(A-C) Vein 3-4 spacing in (A) *fu; Su(fu)/+* males was (B) reduced by heterozygosity for a deficiency containing the *CG3605* gene, and (C) was partially restored by expressing a *UAS-CG3605 cDNA* transgene with *C765-GAL4*. (D, E) Narrowing of veins 3-4 in *fu; Su(fu)/+* flies due to (D) a heterozygous *mago* ^{93D} mutation was still evident when (E) *UAS-MapK* was expressed under the control of *C765-GAL4*. (F-I) Wing discs from *fu; Su(fu)/+* larvae had reduced expression of *ptc-lacZ* (red) and Collier (green) relative to wild-type (not shown). Both *ptc-lacZ* and Collier were reduced further by expressing (G-G') *UAS-CG3605 RNAi* together with *UAS-Diap1* compared to (F-F') *UAS-Diap1* controls using *C765-Gal4.* (H) Average *ptc-lacZ* intensity along the AP-axis for four wing discs in a single experiment comparing *UAS-CG3605 RNAi t*o controls (both also express *UAS-Diap1*). (I) Maximal *ptc-lacZ* intensity as a percentage of control for discs expressing *CG3605 RNAi*, showing mean and 95% CI (n=4 experiments). (J-M) Central region (around DV and AP borders) of wing discs expressing (J) *UAS-Diap1* or (L) *UAS-CG3605 RNAi* plus *UAS-Diap1* under the control of *C765-Gal4*, stained for *ptc-lacZ* (red) and En (green). *CG3605 RNAi* reduced *ptc-lacZ* expression and Hh induction of anterior En (to the left of yellow arrows indicating the AP border). (K) *ptc-lacZ* intensity profiles along the AP-axis for *CG3605 RNAi* and controls for a single experiment (4 wing discs per condition). (M) Maximal *ptc-lacZ* intensity as a percentage of controls for discs expressing *CG3605 RNAi*, showing mean and 95% CI (n=3 experiments).

FigS2

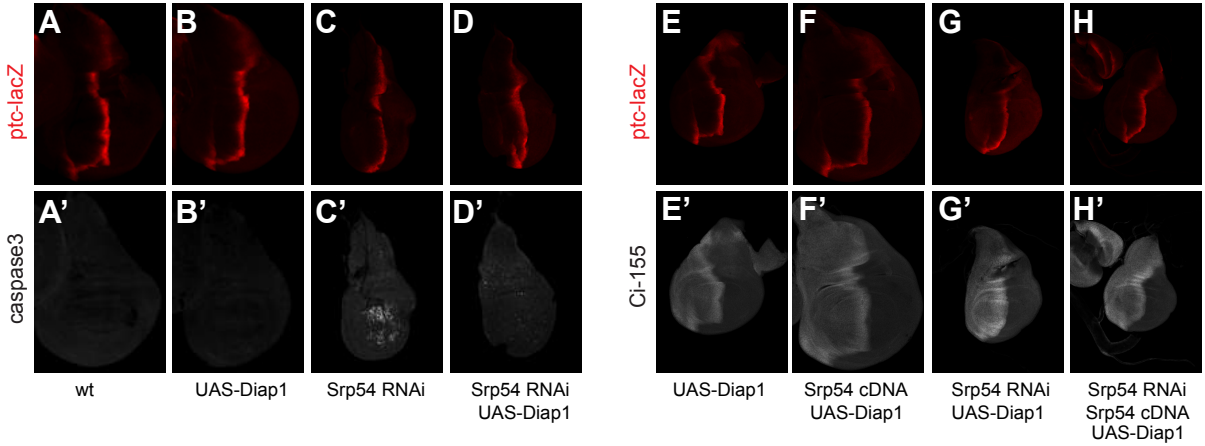

Fig. S2. Rescue of cell death and aberrant wing disc morphology induced by Srp54 RNAi.

(A-D) Wing discs (C,D) with or (A,B) without *UAS-Srp54 RNAi* expression (A, C) alone or (B, D) together with *UAS-Diap1* under the control of *C765-GAL4*, stained for *ptc-lacZ* (red) and Caspase3 (white). Rescue of apoptosis by *UAS-Diap1* expression did not restore *ptc-lacZ* levels reduced by *Srp54 RNAi*. (E-H) Wing discs expressing *UAS-Diap1* (E) alone, or together with (F) *UAS-Srp54 cDNA*, (G) *UAS-Srp54 RNAi,* or (H) *UAS-Srp54 RNAi* and *UAS-Srp54 cDNA*. The expression of *UAS-Srp54 cDNA* prevented *ptc-lacZ* (red) reduction by *Srp54 RNAi* and prevented aberrantly high Ci-155 (white) in anterior cells caused by *Srp54 RNAi*.

FigS3

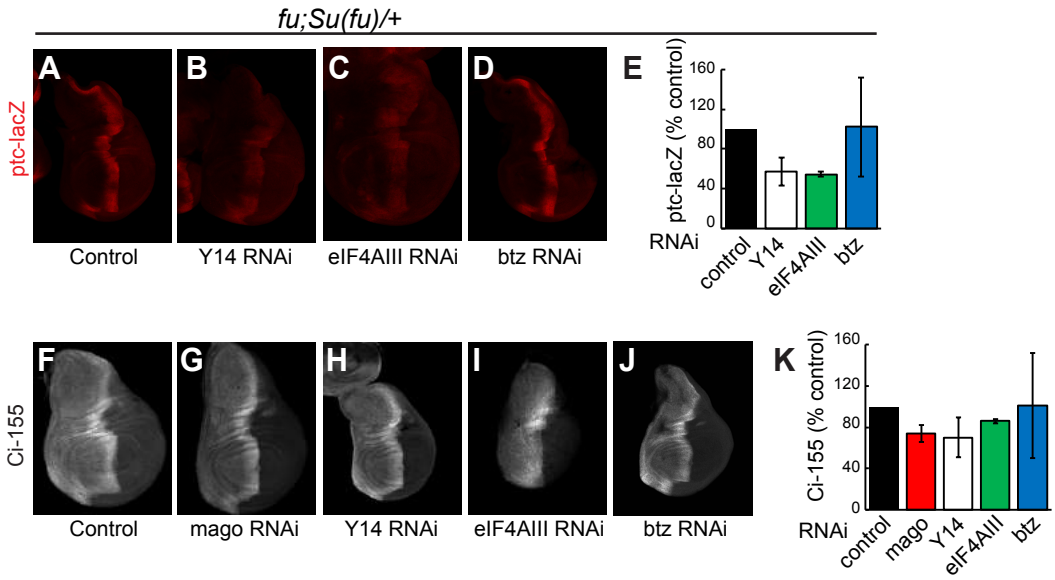

Fig. S3. Core nuclear EJC members can modify Hh pathway activity.

(A-D) Expression of *ptc-lacZ* (red) in (A) *fu; Su(fu)/+* wing discs was reduced by RNAi transgenes targeted to (B) *Y14* and (C) *eIF4AIII* but not (D) *btz* and expressed using *C765-GAL4*. (E) Maximal *ptc-lacZ* intensity as a percentage of controls for discs expressing the indicated RNAi transgenes, showing mean and 95% CI (n=3 experiments for each condition). (F-J) Ci-155 (white) in wing discs expressing RNAi to the indicated EJC components. (K) Maximum Ci-155 intensity in AP border cells as a percentage of controls for discs expressing the indicated RNAi transgenes using *C765-GAL4*, showing mean and 95% CI (n=3 experiments for each condition).

FigS4

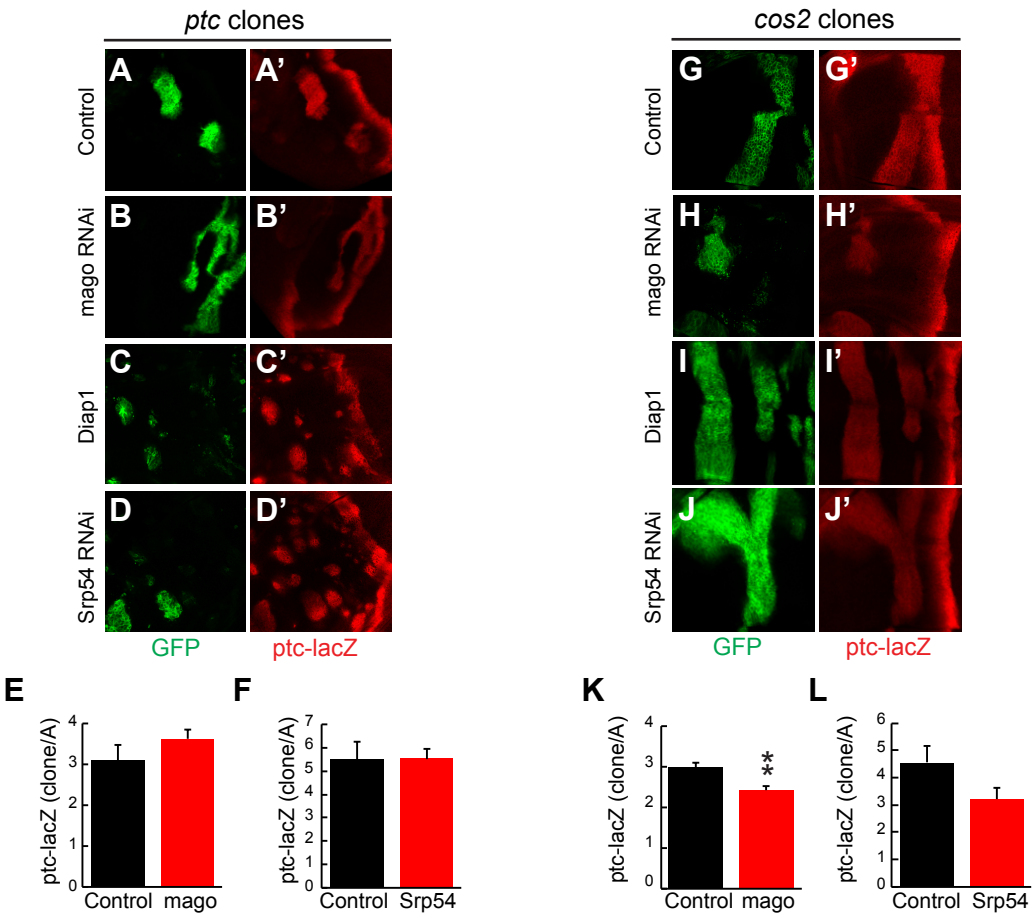

Fig. S4. Effects of mago RNAi and Srp54 RNAi on Hh pathway activity in ptc and cos2 clones.

(A-F) Ectopic *ptc-lacZ* (red) induced in anterior *ptc* clones (marked by GFP, green) was unaffected by (B) *mago RNAi* or (D) *Srp54 RNAi* compared to (A, C) controls. (E,F) *ptc-lacZ* intensity relative to controls within *ptc* clones, showing mean, SEM and significant differences calculated by Student's t-test ($p>0.05$), using (E) n=6 control and n=5 experimental clones and (F) n=8 control and n=11 experimental clones. (G-L) Ectopic *ptc-lacZ* (red) induced in anterior *cos2* clones (marked by GFP, green) was slightly reduced by (H) *mago RNAi* and (J) *Srp54 RNAi* compared to (G, I) controls. (K,L) *ptc-lacZ* intensity relative to controls within *cos2* clones, showing mean, SEM and significant differences calculated by Student's t-test (*$p<0.01$), using (K) n=11 control and n=15 experimental clones and (L) n=8 control and n=6 experimental clones.

Table S1. Deficiencies that altered v3-v4 spacing in the sensitized screen and likely genes responsible.

Cytology	Stock	Deficiency (Df)	v3-v4 spacing	Associated Gene	Phenocopy for Overlapping Df Stocks	
					Yes +	No -
23C	7784	Df(2L) Exel7014	Narrower (-)	*CG3605*	24121	23147 9713
30D-E	9715	Df(2L) BSC240	Narrower (-)	*srp54*	8042 8041 7816	7508
32E-F	9716	Df(2L) BSC241	Narrower (-)	*salr and salm*	7512 29717	7511
42E-43D	9062	Df(2R) ED1673	Wider (+)	*cos2*		
44F-45F	9063	Df(2R) ED1791	Narrower (-)	*tsu*	23167	23166
47A	25248	Df(2R) BSC595	Narrower (-)		9277	25729
66C-D	27576	Df(3L) BSC815	Narrower (-)		26830	24400 9544 24413
77B-C	27917	Df(3L) BSC839	Narrower (-)		21113	26832 24951 27369
78C-F	8101	Df(3L) ED4978	Narrower (-)		24939	24922 23669
87B-E	24990	Df(3R) BSC486	Wider (+)	*Su(fu)*		
94F-95D	24993	Df(3R) BSC489	Narrower (-)		7991	7674 7992
96B-D	24965	Df(3R) BSC461	Narrower (-)		27923	7680 24998 7994

Table S2. Primers used for molecular cloning and mutagenesis.

Name	**Sequence 5'-3'**
Forward CiF1 RsrII	CACCCGGTCCGGCCAAAAGAAAATTATGTTGGC
Reverse CiF1 PmeI	ATGTTTAAACGGGGAGGCCATTGCAATTG
Forward CiF1 ATG-B mut	GAATACAACTGTTCCA*A*GTATCCGGGAATTCTATAGGCC
Reverse CiF1 ATG-B mut	GGCCTATAGAATTCCCGGATAC*T*TGGAACAGTTGTATTC
Forward CiF1 ATG-A mut	GGACTAACTTTAATGAAA*A*GGACGCCTACGC
Reverse CiF1 ATG-A mut	GCGTAGGCGTCC*T*TTTCATTAAAGTTAGTCC
Forward ci cDNA	CACCGACGTCATTCTTGTTGTGGACTAAC
Reverse ci cDNA XbaI	ATTCTAGATTACTGCATCATTTGAAGGTATC
Forward ci 3'UTR NotI	AGCGGCCGCAAAATGTTATCTAGCTAACACTG
Reverse ci 3'UTR PacI	CACCTTAATTAAGGCGAATTGGGTACCCACG
Forward ci SV40 NotI	AGCGGCCGCGACTAGAGATCATAATCAGCC
Reverse ci 3'SV40 PacI	CACCTTAATTAAGTCGACACTAGTGGATCCAG
Forward Srp54 cDNA	CACCATAAACTGGCAAACATGGCTGG
Reverse Srp54 cDNA XhoI	ATCTCGAGCTAGGTCTAGGGCGAGTTGG
Forward CG3605 cDNA EcoRI	CACCAGAATTCTTAATTTATGGCGGACCAGTTG
Reverse CG305 cDNA KpnI	ATGGTACCGTCAAACTAAAACTTGAACTCCTTG

Underlined letters are the added restriction enzyme sites and the italic letters represent changes from original *ci* sequence. Forward primers, used for cloning, start with a CACC sequence to enable cloning into p-ENTRY-D-Topo vector.

Table S3. Pair of primers used for RNA analysis.

Name	Sequence 5'-3'
ci RNA Left	attcagcttcatcttttcacgat
ci RNA Right	tcacccaaagagctcaaacc
ci E1A Left	acgacgtcattcttgttgtg
ci E1B Left	aactgctccatctaaactaacg
ci E2 Right	tgttaccgagcttgaaacgtc
ci E2 Left	gcagtttttagcgtccagg
ci E3 Right	gcccaaaaaatcagctgatcc
ci E3 Left	atgacaccacaacaagttgc
ci E4 Right	tcggcttctacttgtgctac
ci E4 Left	tcgatgggaagattgtacacg
ci E5 Right	ttgctacatcccggatactc
ci E5 Left	accatacacttgcgagtatcc
ci E6 Right	gagagtttgcgtcatttagagg
ci E1AB Right	gttaatgtcgctcagtccac
MapK Right	tgcacatccctatttagagcaa
MapK Left	aatggcacttcagcgacag
ptc Right	caacgacgtcttcgcctac
ptc Left	gaaaatactggcgcggttc
Rpl15 Right	aagccgaagagtcatggtgt
Rpl15 Left	cgccaagtctacgaccaac
Rp49 Right	ttccttgacgtgccaaaact
Rp49 Left	aatgatctataacaaaatcccctga