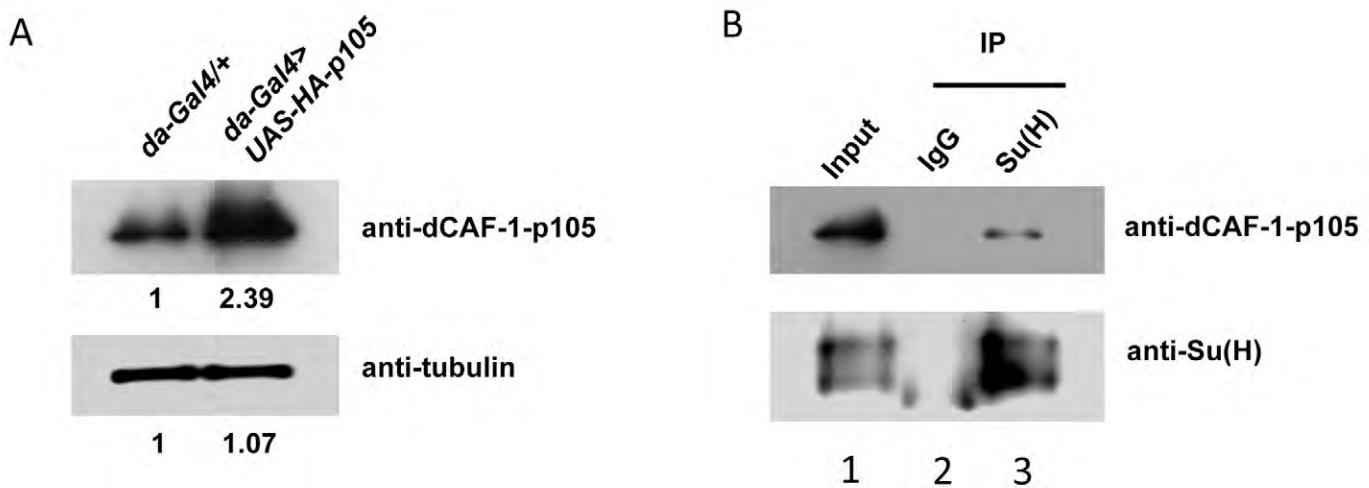
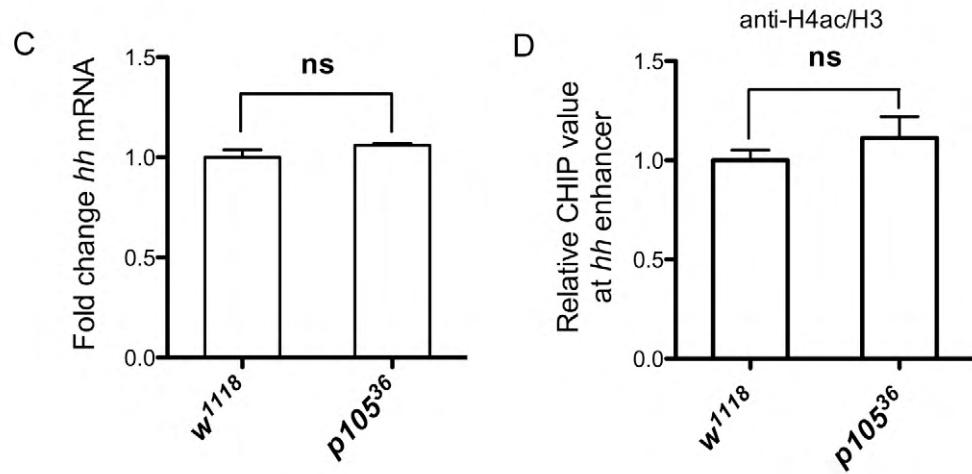
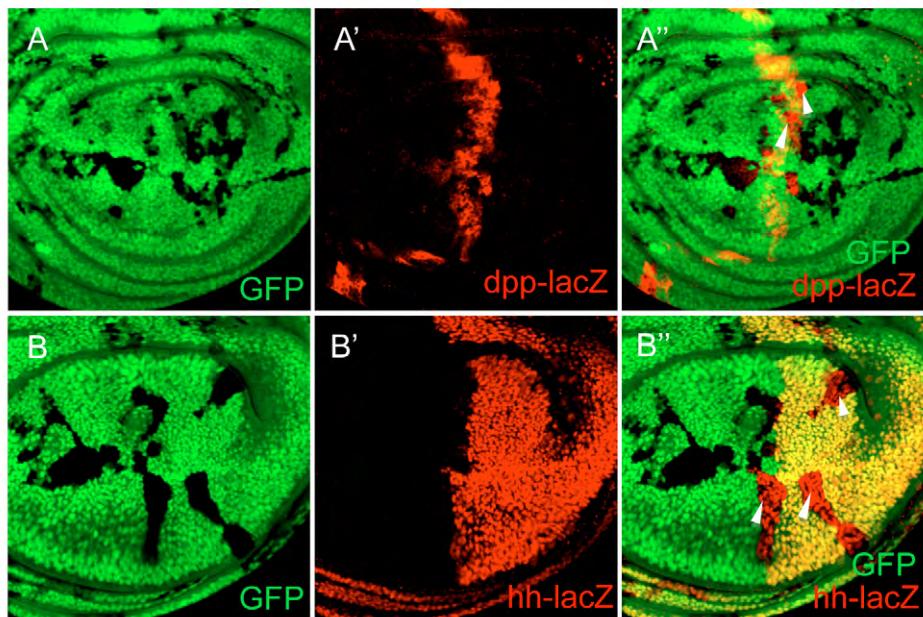


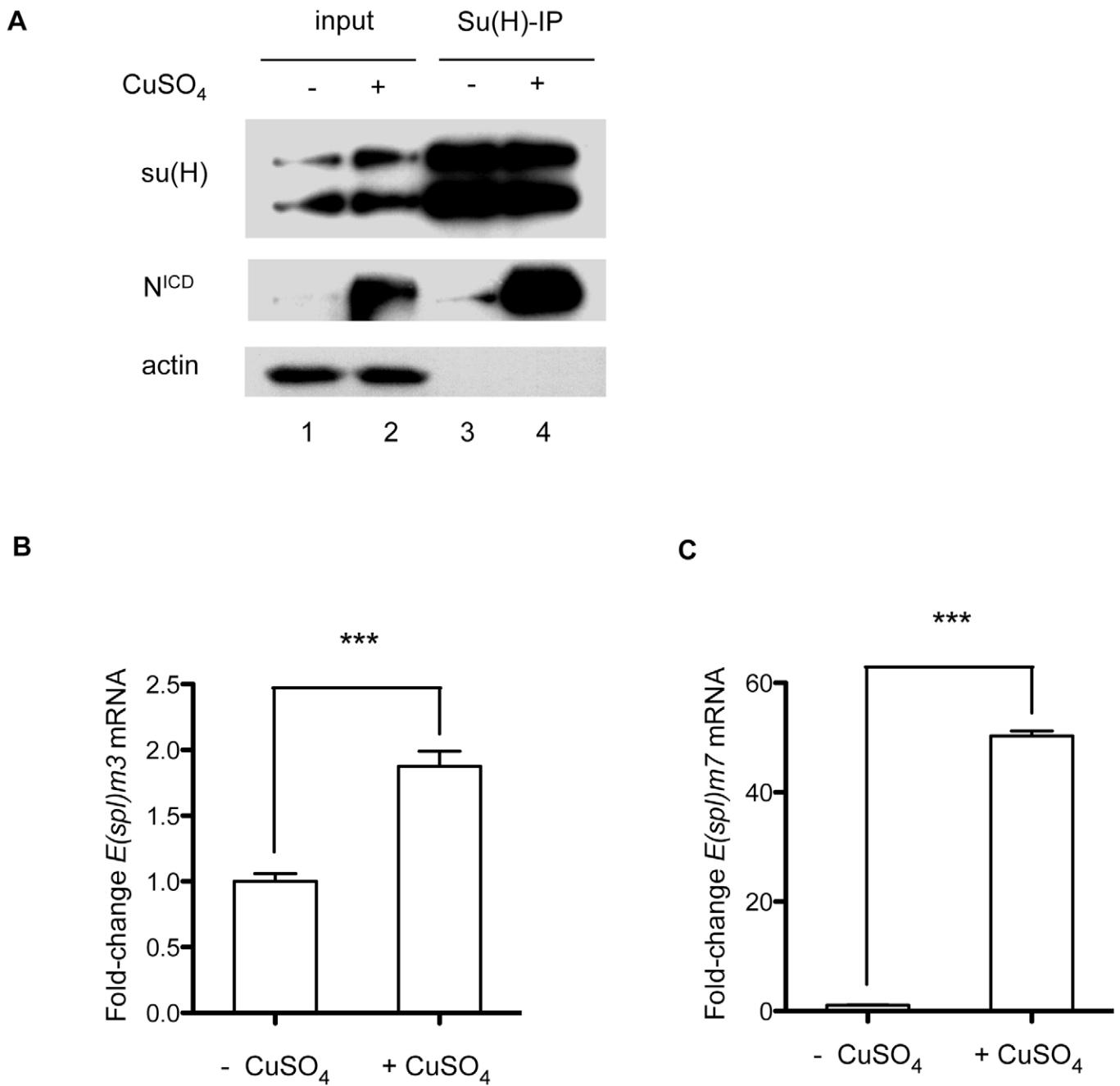
**Fig. S1. Knockdown efficiency of *dCAF-1-p105<sup>IR</sup>* and the genetic interaction between *N<sup>l</sup>* and *p105<sup>36</sup>*.** (A) RT-PCR shows that the *dCAF-1-p105* mRNA level is significantly reduced in *ey>dCAF-1-p105<sup>IR</sup>* animals. Lane 1, wild type; lane 2, *p105<sup>36</sup>* mutant. *Rp49* was used as the internal loading control. (B-D) The *p105<sup>36</sup>* mutation enhances the notched wing phenotype of *N<sup>l</sup>*. (D) Statistics of B and C. Total wings scored: (B)  $n=110$ ; (C)  $n=134$ .



**Fig. S2. Expression of the transgene *UAS-HA-p105* and the physical interaction of dCAF-1-p105 and Su(H).** (A) Quantification of the dCAF-1-p105 protein level in *da-Gal4/+* and *da-Gal4/UAS-HA-dCAF-1-p105* embryos. Quantifications were performed using ImageJ software. (B) Physical interaction between Su(H) and endogenous dCAF-1-p105 (non-tagged).



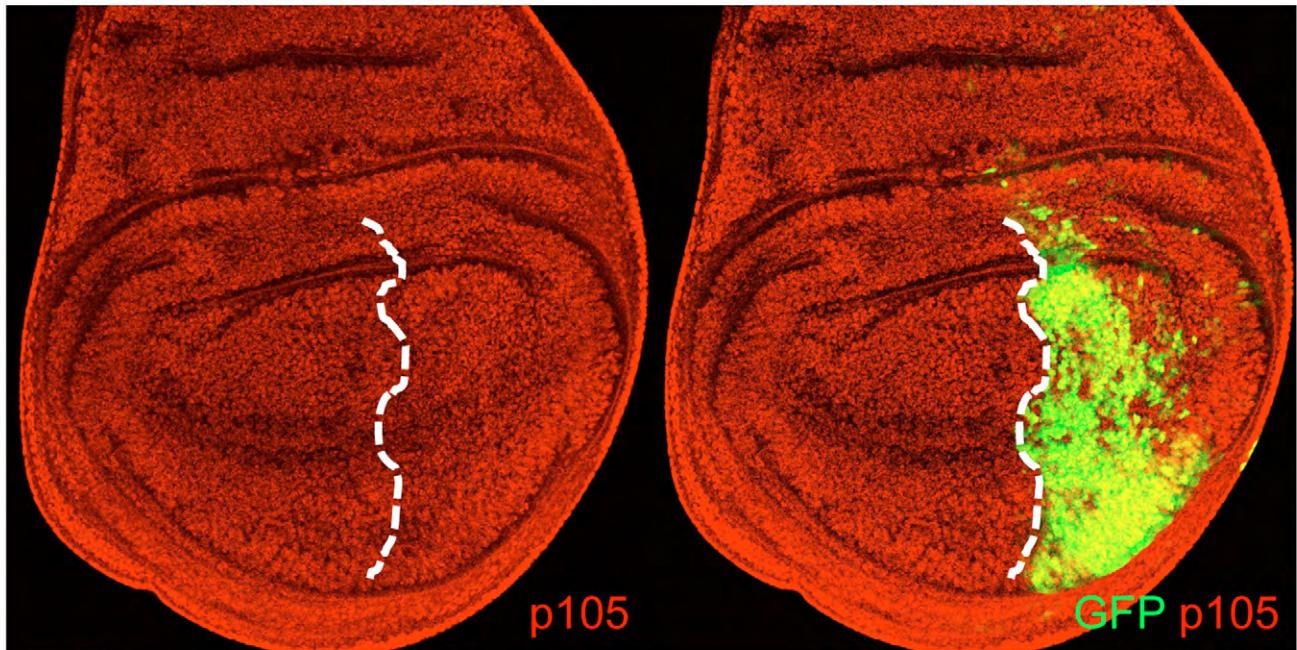
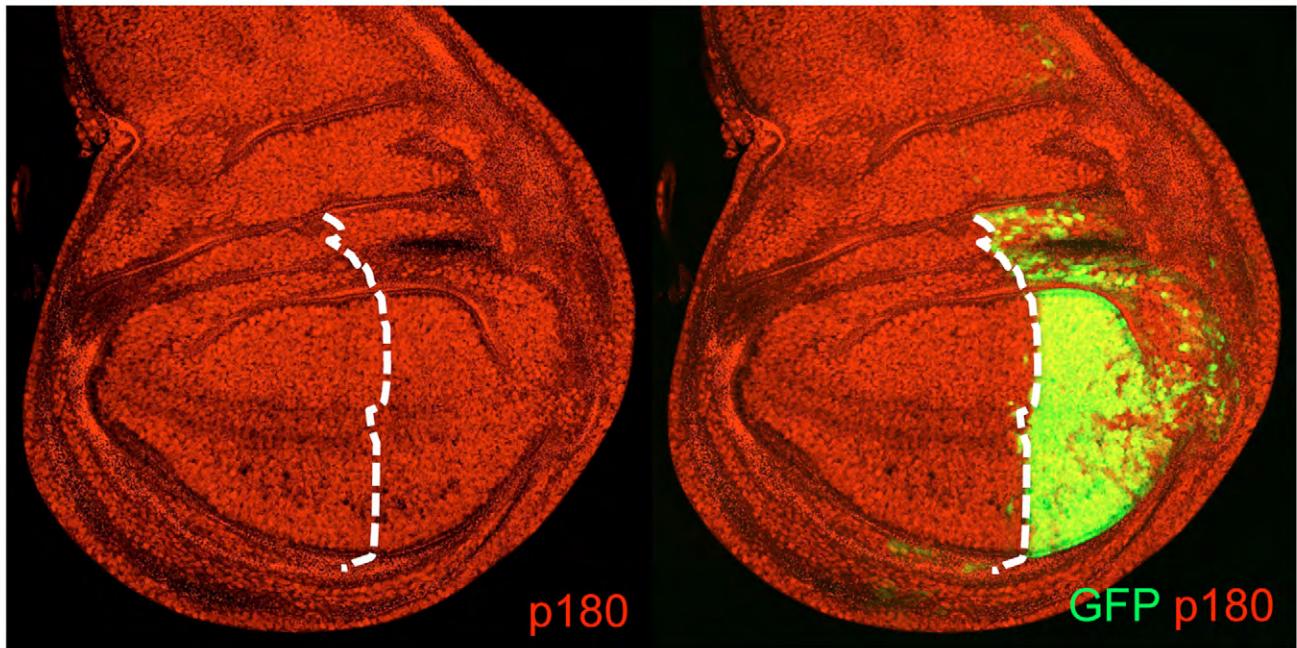
**Fig. S3. Mutation of *dCAF-1-p105* does not affect Hh signaling.** (A-B'') Expression of *dpp-lacZ* and *hh-lacZ* is unaffected in *p105<sup>36</sup>* mutant clones. *p105<sup>36</sup>* mutant clones are indicated at GFP-negative regions. *lacZ* expression is red, GFP is green. (C) *p105<sup>36</sup>* mutation did not affect *hh* transcription. (D) The H4ac level (normalized) was unchanged in the *hh* enhancer region in *p105<sup>36</sup>* mutant larvae as compared with that of the wild-type control.



**Fig. S4. N<sup>ICD</sup> associates with Su(H) upon Notch induction in cultured S2 cells, activating the expression of Notch target genes *E(spl)m3* and *E(spl)m7*. (A)** Physical interaction between N<sup>ICD</sup> and Su(H). The CuSO<sub>4</sub> induction of full-length Notch is indicated by (+), as opposed to no induction indicated by (-). Actin was used as the loading control. **(B,C)** Induced expression of the Notch target genes *E(spl)m3* and *E(spl)m7*. \*\*\*P<0.0001, Student's t-test.

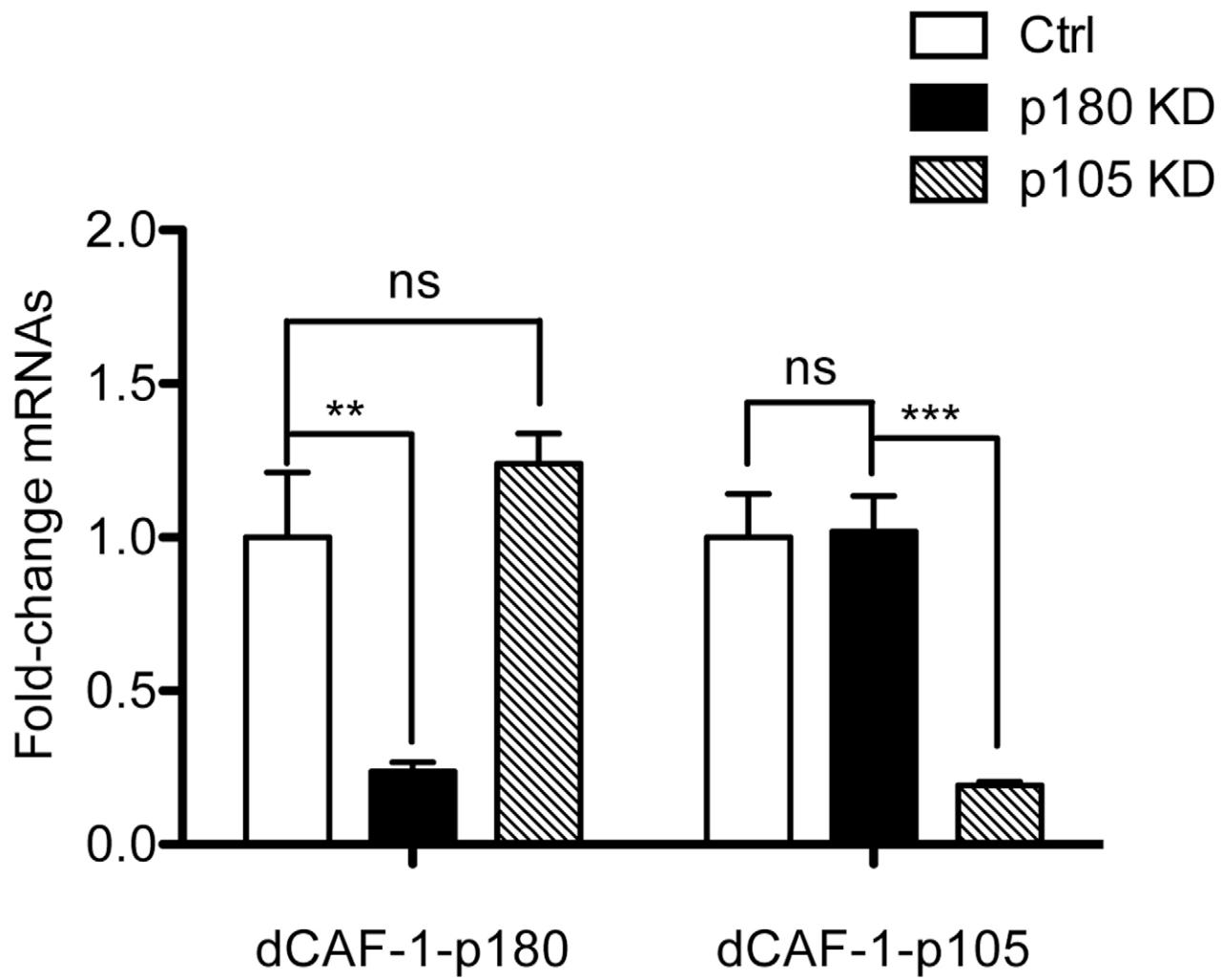
*en>dCAF-1-p55<sup>IR</sup>*

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**Fig. S5. Knockdown of dCAF-1-p55 does not lead to a reduction in the protein level of dCAF-1-p180 or dCAF-1-p105.**

Knocking down dCAF-1-p55 at the wing disc posterior (as indicated in green) does not apparently affect the level of dCAF-1-p180 or dCAF-1-p105 (red). The full genotype of *en>dCAF-1-p55<sup>IR</sup>* is *en-Gal4, UAS-GFP/+; UAS-dCAF-1-p55<sup>IR</sup>/+*.



**Fig. S6. Knockdown of *dCAF-1-p180* does not affect the transcription of *dCAF-1-p105* and vice versa.** Quantitative RT-PCR was used to monitor the transcription of *dCAF-1-p180* and *dCAF-1-p105* with or without RNAi to one of them. Ctrl, second instar larvae of *da-Gal4/+*; p180KD, second instar larvae of *da-Gal4/dCAF-1-p180<sup>IR</sup>*; p105KD, second instar larvae of *da-Gal4/dCAF-1-p105<sup>IR</sup>*.

**Table S1. Statistics of the fly eyes that were categorized in Fig. 1**

Genotype	(-)	(+)	(++)	(+++)	n (total)
Ctrl	39	18	2	0	59
<i>smo</i>	34	20	12	0	66
<i>yki</i>	34	16	0	0	50
<i>Egfr</i>	25	15	6	0	46
<i>tkv</i>	24	15	2	0	41
<i>N</i> *	0	0	0	2	2
<i>H</i>	36	0	0	0	36
<i>Dl</i>	0	4	18	18	40
<i>mam</i>	0	14	15	16	45
<i>eyg</i>	0	5	15	13	33
Rescue <sup>‡</sup>	45	0	0	0	0

\*Most *N*<sup>l/+</sup>; *ey-Gal4,UAS-dCAF-1-p105*<sup>IR/+</sup> flies are lethal when cultured in 25°C.

<sup>‡</sup>*ey-Gal4,UAS-dCAF-1-p105*<sup>IR/UAS-HA-dCAF-1-p105</sup>.

**Table S2. Primers used to generate constructs for S2 cell transfections**

Primer	Sequence (5'-3')
NICD-F	ATGGTCTTGAGTACGCAAAG
NICD-R	TCAAATGTAGATGGCCTCGGAAC
dCAF-1-p105-F	ATGAAGTGCAGATAACCGAGATTTCGT
dCAF-1-p105-R	GTAAAGTCTAATCTATTGCATTGTCTACTC
dCAF-1-p55-F	ATGGTGGATCGCAGCGATAATG
dCAF-1-p55-R	TTAACCGGTATTGGTTCTAACTCG

pAc5.1-Myc, pAc5.1-HA and pAc5.1-Flag vectors were modified from pAc5.1 before being used for the construction of pAc5.1-HA-N<sup>ICD</sup>, pAc5.1-Flag-dCAF-1-p105, pAc5.1-Flag-dCAF-1-p55 and pAc5.1-Myc-dCAF-1-p105 using the primers above. pAc5.1-Flag-dCAF-1-p180 coding sequence was cut from UAS-dCAF-1-p180 (Song et al., 2007) and cloned into the pAc5.1-Flag vector.

**Table S3. Primers used for quantitative PCR**

Primer	Sequence (5'-3')
Rp49-F	ACGTTGTGCACCAGGAACCTT
Rp49-R	TACAGGCCAAGATCGTGAA
dCAF-1-p105-F	GATGGTAAGCTGCTGTTAAC
dCAF-1-p105-R	TTCTTCTCCGCATTGTACGG
E(spl)m $\beta$ -F	GTGACCATAGCTTGATCCTC
E(spl)m $\beta$ -R	CGTTCATGCTGCCAATGAAG
E(spl)m3-F	CGAGCCAGGATCAACAAGTG
E(spl)m3-R	CGGTCAACTCCAGGATATCC
E(spl)m7-F	GTTCCTCGTGGATACATC
E(spl)m7-R	CTGCTCCAGTTGGTTGAGAC
GAPDH-F	GTTCAAGTTCGATTGACCC
GAPDH-R	GTGGACTCCACGATGTATT
Notch-F	CAGTCGCGCACCAAACACTT
Notch-R	CAGTTCCCGCGAACCGGTGTT
dCAF-1-p180-F	ACGAGGAGGATGATGACGAT
dCAF-1-p180-R	CGAGATGCTGCTGCTCTTT
hh-F	GGAAAAGGATGTCCCTGTTG
hh-R	TCTTGCCGATGGTCTTAGC
E(spl)m $\beta$ enhancer-F	CACATGGCAAGACTTGAGAC
E(spl)m $\beta$ enhancer-R	TTGGGATCCCAGATAACGATG
hh enhancer-F	ACGCACACTATCACACATCC
hh enhancer-R	CAGCATACTCACAGACTGG