Concentration and localization of co-expressed ELAV/Hu proteins control specificity of mRNA processing

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Supplemental table and figure legends

Table S1. Sequence identity (similarity) in RRM1-3 among *Drosophila* ELAV RBPs and compared to human Hu RBPs and Sex lethal (Sxl).

Figure S1. ELAV, FNE and RBP9 are co-expressed in neurons.

(A and B) Expression of FNE in adult brains of trangenes harboring an HA-epitope-tagged genomic construct stained with anti-HA antibodies (top row) in the absence of endogenous FNE and ELAV stained with anti-ELAV antibodies (middle row). Note that expression of FNE completely overlaps with ELAV (bottom row), but that FNE localizes to both nucleus and cytoplasm, while ELAV is mostly nuclear. Scale bars are 100 μm in A and 30 μm in B.

(C and D) Expression of RBP9 in adult brains of trangenes harboring an myc-epitope-tagged genomic construct stained with anti-myc antibodies (top row) in the absence of endogenous RBP9 and ELAV stained with anti-ELAV antibodies (middle row). Note that expression of RBP9 completely overlaps with ELAV (bottom row), but that RBP9 localizes to the cytoplasm, while ELAV is mostly nuclear. Scale bars are 100 μm in A and 30 μm in B.

Figure S2. Generation of an *fne* null allele.

(A) Genomic organization of the *fne* locus. A deletion of the *fne* coding region was obtained by flipase mediated recombination of the FRT sites contained within PBac transposons.

(B) Genomic PCR amplifying the 5' (top) and 3'(middle) flanking region and RT-PCR (bottom) of parental transposons and two identical deletion lines.

Figure S3. Loss of FNE or RBP9 does not affect alternative splicing of *nrg* from the *UNGA* reporter.

(A-D). Alternative splicing of *nrg* from the *UNGA* reporter is not affected in photoreceptor neurons of *fne* or *Rbp9* mutants stained with anti-GFP antibodies, but dramatically reduced in $elav^{edr}$ mutants. The scale bar is 50 μm.

(E-G) Alternative splicing of *nrg* from the *UNGA* reporter is not affected in neurons of the 3rd instar larval brain in *fne* or *Rbp9* mutants visualized by GFP expression. The scale bars are 100 μm.

Figure S4. Expression and regulation of the *UNGA* reporter by Hu RBPs.

(A-O) HA-tagged ELAV and Hu proteins were expressed from *UAS* constructs in wing discs using *dppGAL4* in the presence of the *nrg* alternative splicing reporter *UNGA* and stained with anti-GFP antibodies and anti-HA antibodies. Note that HuD could not be detected although its expression results in lethality when expressed with $elavGAL4^{C155}$. The scale bar in O is 150 μm.

(P) Quantification of *UNGA* splicing showing means with the standard error from 4 wing discs.

Figure S5. Expression of *elav, fne* and *Rbp9* during development and in adults determined by RNAseq from flybase. Sexually dimorphic expression in adults is shown by dashed lines.

Supplemental Table S1

RRM1

	ELAV	FNE	RBP9
ELAV	-	81(93)	78(89)
FNE	-	-	80(90)
HuR	77(88)	81(90)	78(86)
HuB	73(90)	81(91)	78(89)
HuC	69(85)	73(86)	73(84)
HuD	75(89)	82(90)	80(87)
Sxl	49(74)	51(73)	49(73)

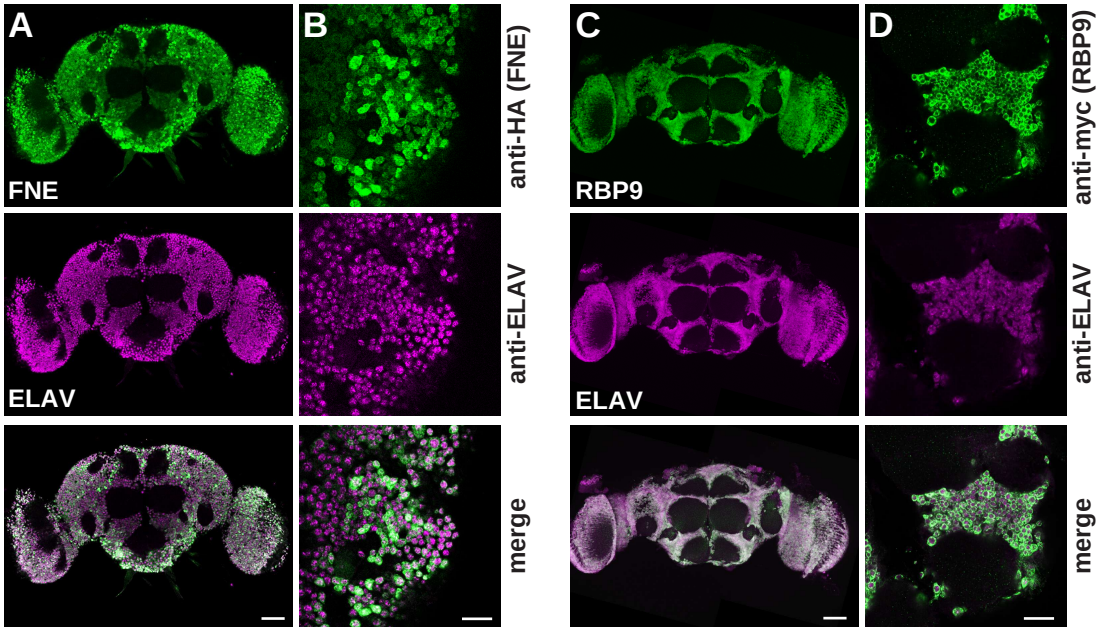
RRM2

	ELAV	FNE	RBP9
ELAV	-	63(76)	66(80)
FNE	-	-	82(90)
HuR	52(70)	63(77)	64(76)
HuB	61(73)	66(82)	73(82)
HuC	58(72)	67(84)	69(81)
HuD	63(75)	66(82)	73(82)
Sxl	41(64)	41(64)	41(65)

RRM3

	ELAV	FNE	RBP9
ELAV	-	75(90)	72(90)
FNE	-	-	80(98)
HuR	63(84)	71(86)	73(86)
HuB	67(86)	80(90)	78(89)
HuC	67(86)	78(86)	76(86)
HuD	69(89)	80(90)	80(90)

Supplemental Figure 1



Supplemental Figure 2

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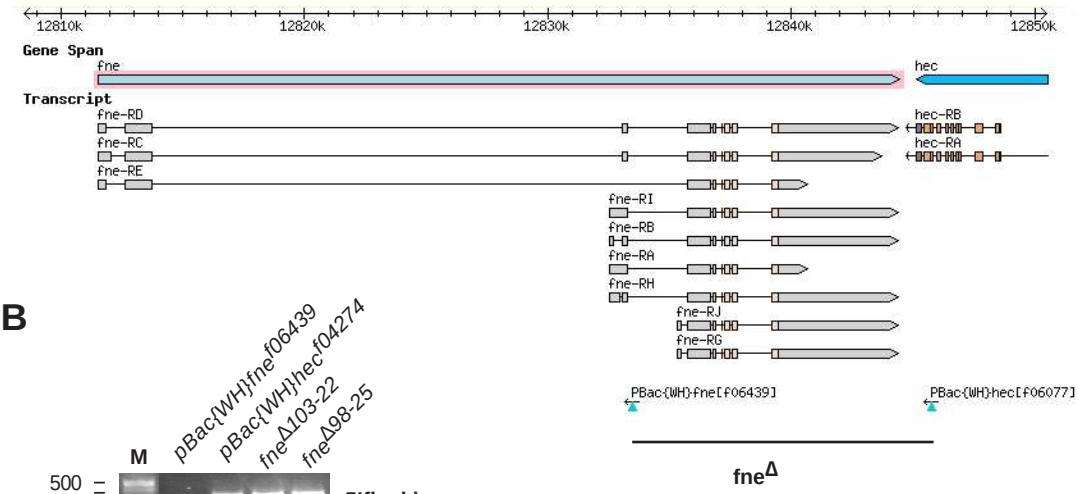
A



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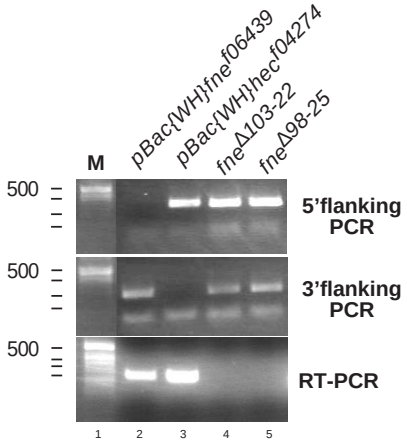
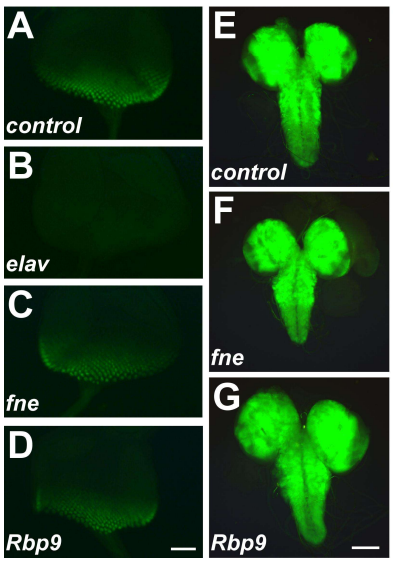
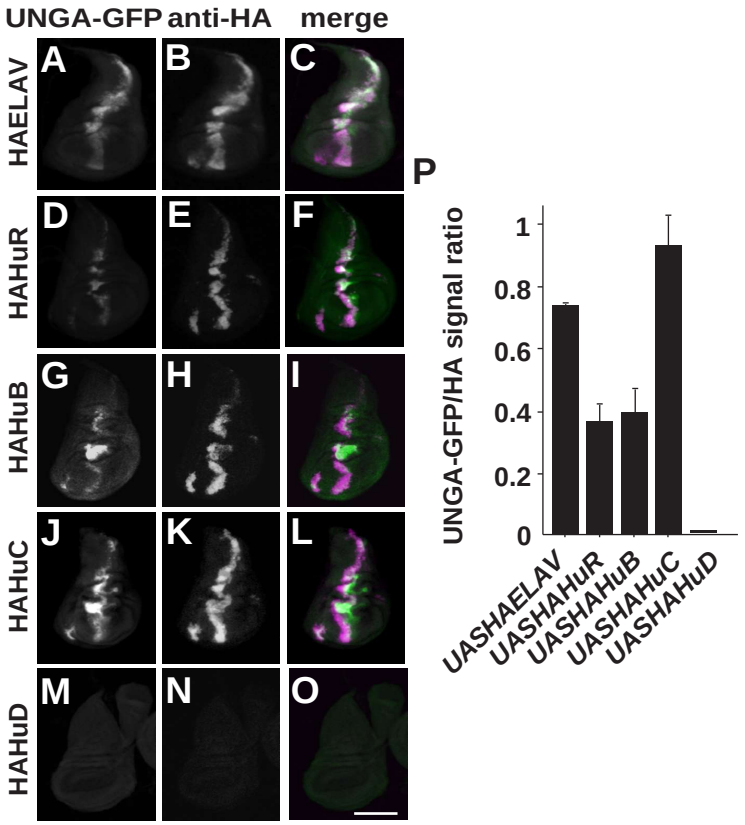


Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5