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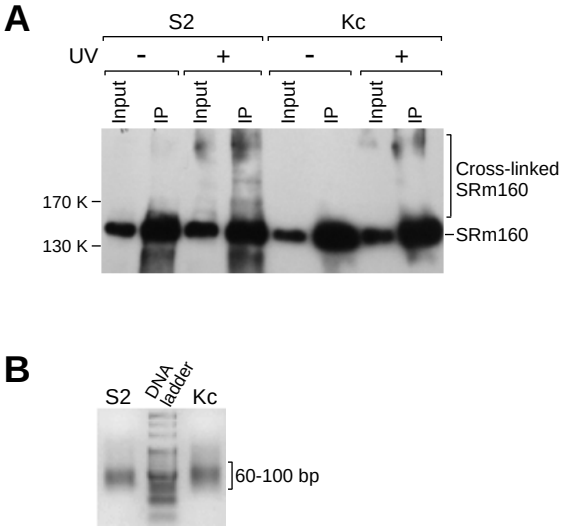
Supplementary Table S5. Significantly different alternative splicing events between Kc and S2 cells.

Supplementary Table S6. DNA primers and RNA oligonucleotides used in this study.

Qiu_Fig. S1



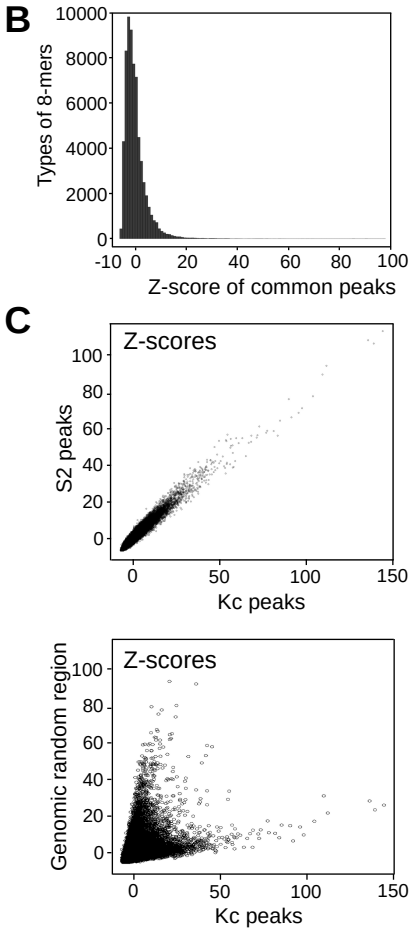
Supplemental Figure S1. HITS-CLIP of SRm160 in *Drosophila* sex-specific cell lines.

A, Slowly migrating RNA-protein complexes are visible after UV (254 nm) cross-linking and co-immunoprecipitation by SRm160 antibody. Samples without UV irradiation are used as negative controls.

B, Amplified cDNAs, from the indicated 60-100 bp regions with two RNA linkers, are used for iIlumina sequencing.

A **Z-test**

All peaks from S2			All peaks from Kc		
Top 20 Octamers	Z-score	Counts	Top 20 Octamers	Z-score	Counts
CAGCAGCA	112.5	676	CAGCAGCA	145.0	990
GCAGCAGC	107.5	648	AGCAGCAG	139.8	956
AGCAGCAG	105.7	638	GCAGCAGC	136.5	935
CAACAACA	93.8	570	CAACAACA	112.4	778
AACAACAA	89.0	543	AACAACAA	110.1	763
CAGCAACA	77.2	476	CAGCAACA	104.6	727
ACAACAAC	75.6	467	AGGAGGAG	98.2	686
AGGAGGAG	70.6	439	CAACAGCA	96.2	673
CAACAGCA	68.2	425	GAGGAGGA	92.1	646
GAGGAGGA	65.7	411	ACAACAAC	90.6	636
AGCAGCAA	63.9	401	AGCAGCAA	85.5	603
GCAACAAC	60.8	383	GGAGGAGG	84.1	594
GCAGCAAC	58.5	370	ACAGCAGC	81.8	579
ACAGCAGC	58.3	369	GCAGCAAC	78.1	555
AGCAACAA	57.4	364	AGCAACAA	77.5	551
UGUGUGUG	56.3	358	GCAACAAC	73.5	525
GGAGGAGC	56.0	356	GGAGGAGC	72.4	518
CCAGCAGC	55.8	355	AACAGCAG	71.9	515
GGAGGAGG	55.8	355	CCAGCAGC	68.9	495
AACAGCAG	54.8	349	GACGAGGA	68.7	494



Supplemental Figure S2. Analysis of RNA motif for SRm160-binding by Z-test.

A, obtained top-20 octamer sequences from the S2 and Kc samples by Z-test.

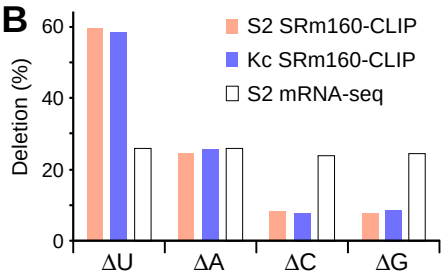
B, histogram of octamer Z-scores from the common peaks.

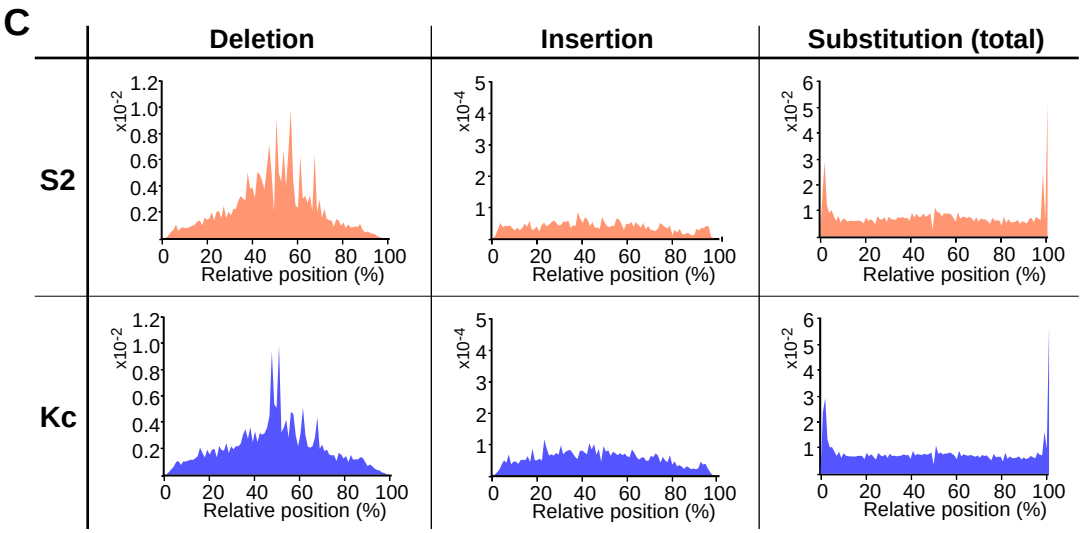
C, correlations of octamer Z-scores between the S2 and Kc peaks (upper), and between the Kc peaks and genomic random region (lower).

Qiu_Fig. S3

A

Reads	S2	Kc
Mapped	8,372,164	6,302,334
With mutations	2,132,136	1,640,813
Deletion	320,898	329,562
Insertion	3,739	8,229
Substitution	1,664,735	1,303,022



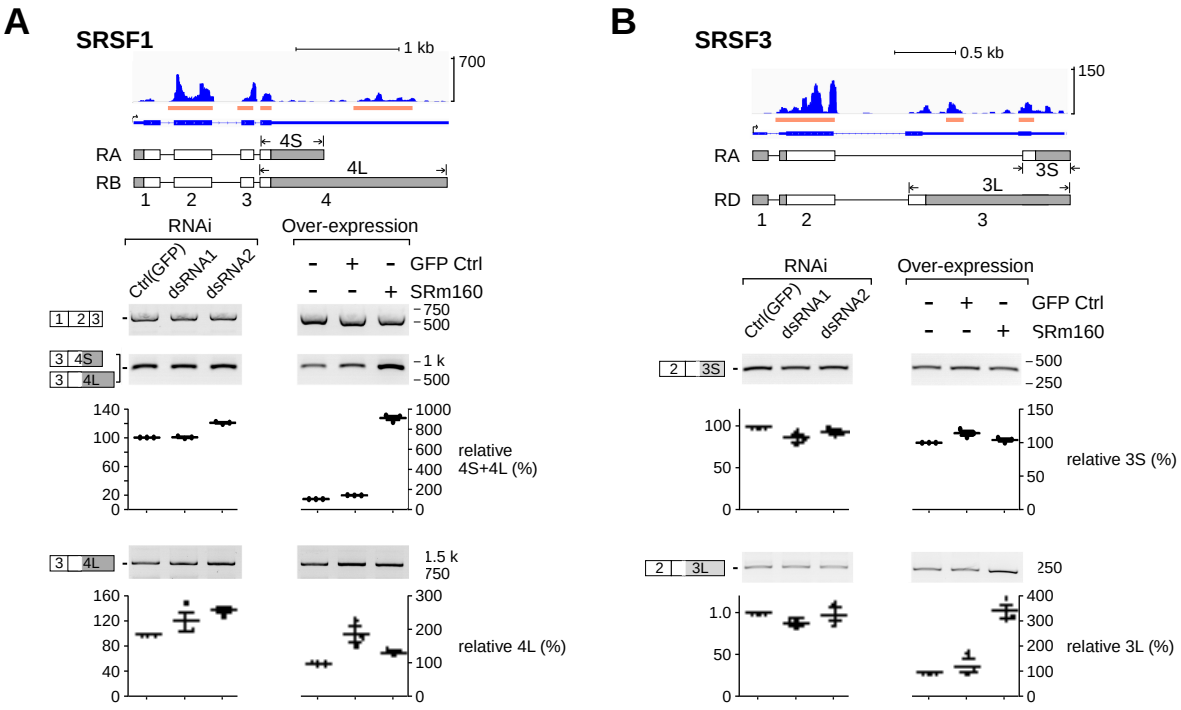


Supplemental Figure S3. Analyses of CIMS in SRm160 CLIP-seq reads.

A, analysis of SRm160 reads that contain deletion, insertion or substitution nucleotides. All reads with SNP are excluded.

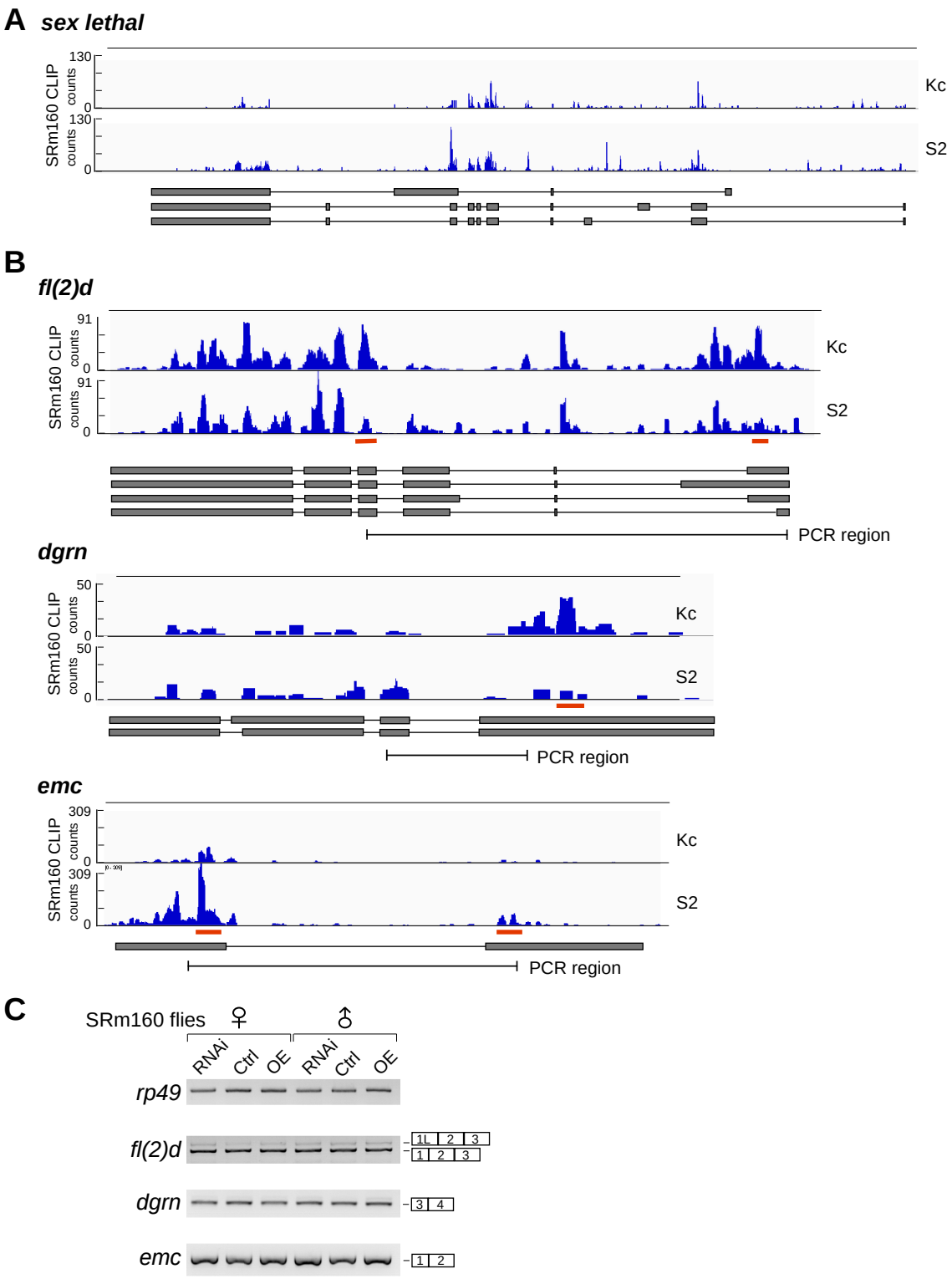
B, base frequency at the deletion sites of SRm160 CLIP-seq reads. Deletion of uracil is predominant and significantly distinguished with mRNA-seq of S2 cell.

C, positional profiles of deletion, insertion, and substitution in the SRm160 CLIP-seq reads. Relative position of each mutation is calculated by its distance to the first nucleotide in CLIP-read.



Supplemental Figure S4. SRm160 regulates alternative splicing of SRSF1 and SRSF3

Alternative exons with SRm160 binding peaks of *SRSF1* (*A*) and *SRSF3* (*B*) genes are tested in S2 cells with both down- and up-regulated SRm160. Relative (%): the value of each band is relative to control that normalized by *rp49* loading control. Semi-quantitation of RT-PCR products from three repeat analyses are presented by scatter plots.

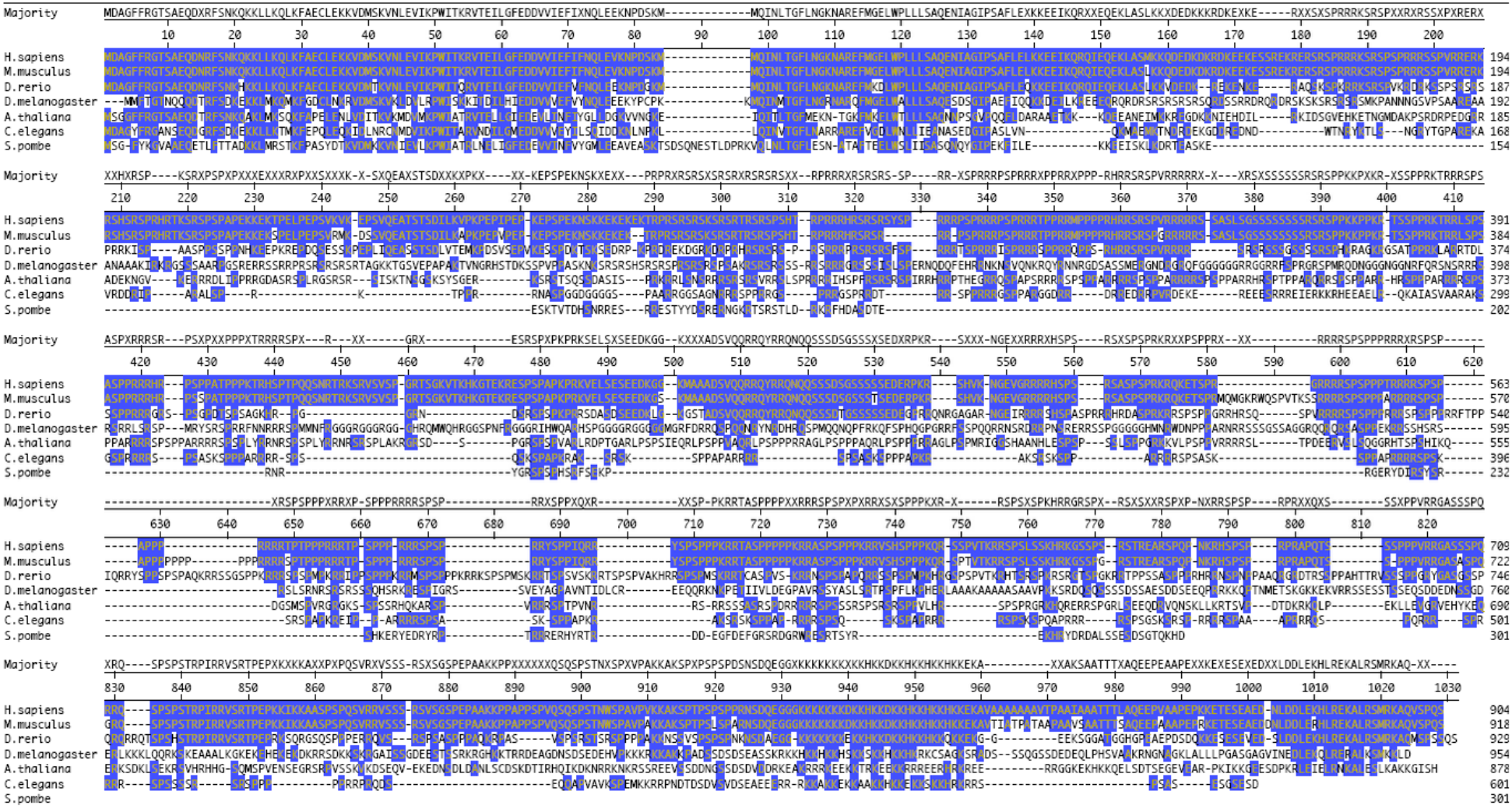


Supplemental Figure S5. Analyses of sex determination pathway related genes in the SRm160 mutant flies.

A. CLIP-peak coverage of SRm160 on *sex lethal* in Kc and S2 cell lines. Several visible different binding signals are not fit the requirement of DBS (>=25 nt).

B. CLIP-peaks coverage of three sex determination related genes. SRm160 DBSs in these genes are indicated by orange bar.

C. Alternative splicing or expression of these three sex determination related genes are not changed in the SRm160 mutant flies.



Supplemental Figure S6. Alignment of SRm160 proteins across species.

Homologous protein sequences of SRm160 from seven representative species including *H. sapiens* and *D. melanogaster*are aligned using Clustal W method within MegAlign software.

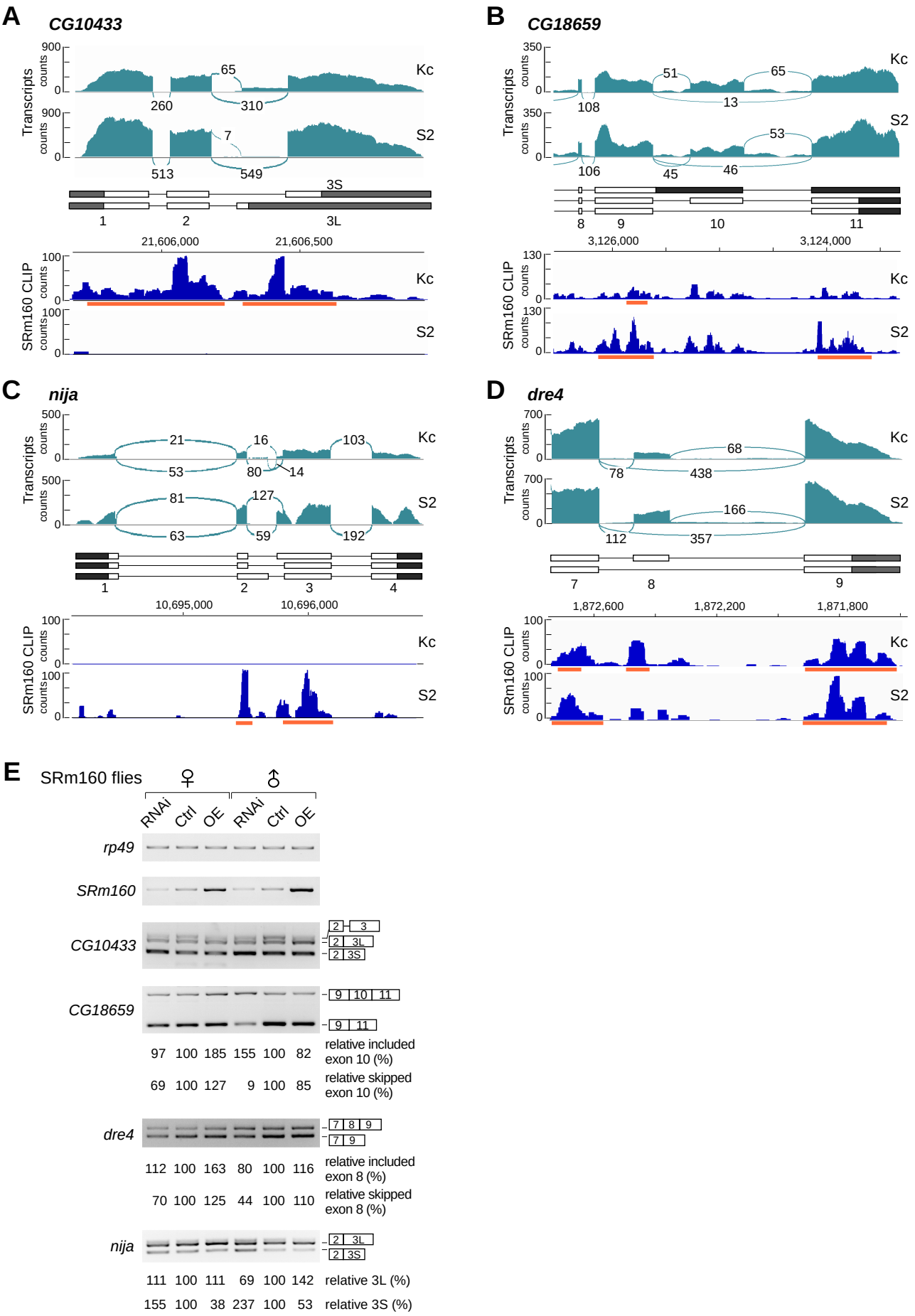


Figure S7. SRm160 regulation of non-SR protein genes.

SRm160 CLIP-peaks on A, CG10433; B, CG18659; C, nija and D, dre4 transcripts in Kc and S2 cells.

E, Alternative splicing analyese of above four genes in the SRm160 mutant flies.

Supplemental Table S1. Analysis of SRm160 CLIP-reads and peaks.

		S2		Kc	
		reads	peaks	reads	peaks
Processed reads		20,630,796		13,140,248	
Mapped / Generated		8,372,164 (100%)	15,042 (100%)	6,302,334 (100%)	17,034 (100%)
Gene	Exon	6,490,177 (77.5%)	11,979 (79.6%)	4,722,954 (74.9%)	13,956 (81.9%)
	Intron	1,186,817 (14.2%)	1,143 (7.6%)	959,589 (15.2%)	1,281 (7.5%)
	Coding genes	4,987,179 (59.6%)	11,835 (78.7%)	3,863,399 (61.3%)	14,035 (82.4%)
	5'-UTR	446,838 (5.3%)	2,761 (18.4%)	321,077 (5.1%)	3,186 (18.7%)
	3'-UTR	405,059 (4.8%)	1,585 (10.5%)	378,748 (6.0%)	2,351 (13.8%)
	CDS	2,987,042 (35.7%)	6,704 (44.6%)	2,231,828 (35.4%)	7,518 (44.1%)
	Intron	1,148,240 (13.7%)	785 (5.2%)	931,746 (14.8%)	980 (5.8%)
	Non-coding genes	2,283,443 (27.3%)	507 (3.4%)	1,547,630 (24.6%)	389 (2.3%)
	miRNA	113 (0.0%)	3 (0.0%)	121 (0.0%)	0 (0%)
	rRNA	1,839,033 (22.0%)	120 (0.8%)	1,296,946 (20.6%)	101 (0.6%)
	snoRNA	25,372 (0.3%)	11 (0.1%)	40,088 (0.6%)	12 (0.1%)
	snRNA	114,134 (1.4%)	18 (0.1%)	65,576 (1.0%)	19 (0.1%)
	tRNA	46,534 (0.6%)	208 (1.4%)	6,430 (0.1%)	88 (0.5%)
	Other ncRNA	258,257 (3.1%)	147 (1.0%)	138,469 (2.2%)	169 (1.0%)
Pseudogene		677,418 (8.1%)	87 (0.6%)	491,994 (8.3%)	71 (0.4%)
Intergenic		695,170 (8.3%)	2,613 (17.4%)	619,791 (9.8%)	2,539 (14.9%)

Splicing related reads*	S2	Kc
Mapped reads	8,260,343 (100%)	6,196,936 (100%)
Intron-less	2,978,564 (36.1%)	2,190,588 (35.3%)
Intron-containing	5,281,779 (63.9%)	4,006,348 (64.7%)
Exon	4,001,526 (48.4%)	2,842,640 (45.9%)
Intron	691,745 (8.4%)	630,887 (10.2%)
Exon-Intron Junction	114,176 (1.4%)	100,417 (1.6%)
Exon-Exon Junction	145,555 (1.8%)	108,300 (1.7%)

Notes:

1) Due to the annotation and width of peaks, small fraction of reads and peaks are counted more than once.

2) * Splicing related reads were obtained after mapping to the combined Flybase and exon-exon junction library (see methods).

Supplemental Table S6. DNA primers and RNA oligonucleotides used in this study.

DNA Primers	Sequences	Notes
dsRNA1-F	5'-TCCTAATACGACTCACTATAGGGAACAACCGTCGCCGTTCGCCGATG	The first set for SRm160 RNAi in S2 cells
dsRNA1-R	5'-TCCTAATACGACTCACTATAGGGTTCTCCGGCGGACTGGCACTGC	
dsRNA2-F	5'-TCCTAATACGACTCACTATAGGGTCGATCGAGATCCCGTTCGCG	The second set for SRm160 RNAi in S2 cells
dsRNA2-R	5'-TCCTAATACGACTCACTATAGGGACTGCGCGGCGATCGCGACCGG	
dsRNA-GFP-F	5'-TCCTAATACGACTCACTATAGGGATGGTGAGCAAGGGCGAGGAGC	For GFP RNAi in S2 cells
dsRNA-GFP-R	5'-TCCTAATACGACTCACTATAGGGTGCTTGTCGGCCATGATATAGACG	
5'-adaptor	5'-CCGCTGGAAGTGACTGACAC	DNA adaptors for HITS-CLIP sequencing
3'-adaptor	5'-AGGGAGGACGATGCGG	
1184	5'-ACTAGCTAGCATGATGTTCACGGGCACCAATCAGC	SRm160-OE in S2 cells
1185	5'-ATCCCCCGGGTCAGTCCAGCTTCTTCATCG	
1961	5'-ACTAGCTAGCATCATCCTCTCGTTATGGCCCACCG	SRSF6 minigene construction
1963	5'-ATAGTTTAGCGGCCGCTTGGATCGCGAGCGGGAGCGAGAAC	
1969	5'-CCATTGTGAGTGTGATGAGGGTGCGAATCGAATCTCCGGCTGAGACTACCGACTAGACAACCACC	
1970	5'-GGTGGTTGTCTAGTCGGTAGTCTCAGCCGGAGATTCGATTCGCACCCTCATCACACTCACAATGG	
0277	5'-AGACTATCGTGATGCCGACGATG	RT-PCR of SRSF6
0283	5'-GAGGATGATCTGGAATTTTTGTTT	
0278	5'-CCACAATCAGTCGGTACTCAGTG	
1904	5'-TTGTGGAGAATTTGTCTAGCCGC	
1960	5'-GGAATAGACGACGAATGGATGAGG	
1909	5'-GCCGTCTATGAACTGAATGGCAAAG	
1902	5'-TAACGCGGCTAGACAAATTCTCC	
0128	5'-CGAGAGCATCTGGCCAATGTG	
1716	5'-CGCGGATCCATGATGTTCACGGGCACCAATCAGC	Truncated SRm160 expression in *E. coli*
1906	5'-ATAGTTTAGCGGCCGCTCAACTGGGCACCGAACCGTTGTTG	
552	5'-CGATTGAATCTCGATCATCGT	RT-PCR of *Sxl*
553	5'-GGATGGCAGAGAATGGGACA	
503	5'-AATGGATGCCGACAGCAGTG	RT-PCR of *Tra* *sex-specific primers*
513	5'-CTGATGGACGACTGTGATCTG	
624	5'-TTTTCCGATGAAAATGGATGC	RT-PCR of *Tra* *common primers*
883	5'-CTCGATGCTGGGTTCCACTG	
RNA oligonucleotides		
3'-linker	5'-GUGUCAGUCACUUCCAGCGG-puromycin	RNA linkers for HITS-CLIP ligations
5'-linker	5' Biotin-AGGGAGGACGAUGCGG	
(GCA)7	5'-AGCAGCAGCAGCAGCAGCAGCAGC	RNA oligoes for gel-shift assays
(uCA)7	5'-AuCAuCAuCAuCAuCAuCAuCAuC	
(AAC)7	5'-ACAACAACAACAACAACAACAACA	