Supplementary Information for

The Junction Usage Model (JUM): A method for comprehensive annotation-free analysis of alternative pre-mRNA splicing patterns

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Supplementary Information Text

Supplemental Methods

Statistical cutoffs applied for AS analysis software tools

For colon cancer patient tumor versus matched normal tissue RNA-seq data analysis, the following statistical cutoffs are applied:

MISO: at least 10 unique reads mapped to each isoform, $\Delta\Psi \geq 10\%$, Bayes factor ≥ 5.
rMATS: qvalue ≤ 0.05, $\Delta\Psi \geq 10\%$.
JUM: qvalue ≤ 0.05, $\Delta\Psi \geq 10\%$.
IRFinder: pvalue ≤ 0.05, $\Delta\Psi \geq 10\%$.

For SRSF2 mutation carrying K562 cell line versus wildtype analysis, the following statistical cutoffs are applied:

rMATS: qvalue ≤ 0.1, $\Delta\Psi \geq 10\%$
JUM: qvalue ≤ 0.1, $\Delta\Psi \geq 10\%$.

For Drosophila male head samples that carry a PSI mutation versus wildtype analysis, the following statistical cutoffs are applied:

MISO: at least 5 unique reads mapped to each isoform, $\Delta\Psi \geq 5\%$, Bayes factor 5.
rMATS: qvalue <= 0.1, $\Delta\Psi \geq 5\%$.
JUM: qvalue <= 0.1, $\Delta\Psi \geq 5\%$.

RNA extraction and qRT-PCR validation of JUM-predicted AS events

Drosophila heads were isolated from 10-20 manually sorted and snap-frozen males from the PSI mutant and wildtype PSI strains as previously described (1). RNA were extracted using the Trizol reagent. qRT-PCR primers were designed with the software Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) and qRT-PCR experiments performed by using the SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (Thermo Fisher Scientific) on a LightCycler 480 instrument (Roche). All AS events were validated as described above except for Fmr1 and lig gene AS events, as no designed primers produced one single product for qRT-PCR experiments. Thus, for these two genes RNA (0.5 μg) were first reverse-transcribed by SuperScript First-Strand Synthesis System (Invitrogen) and the splicing isoforms were analyzed by RT-PCR followed by gel electrophoresis on 6% TBE gel (Thermo Fisher Scientific), stained with SYBR Gold (Invitrogen) and quantified with ImageJ software (NIH).

Supplemental Figures
Figure S1

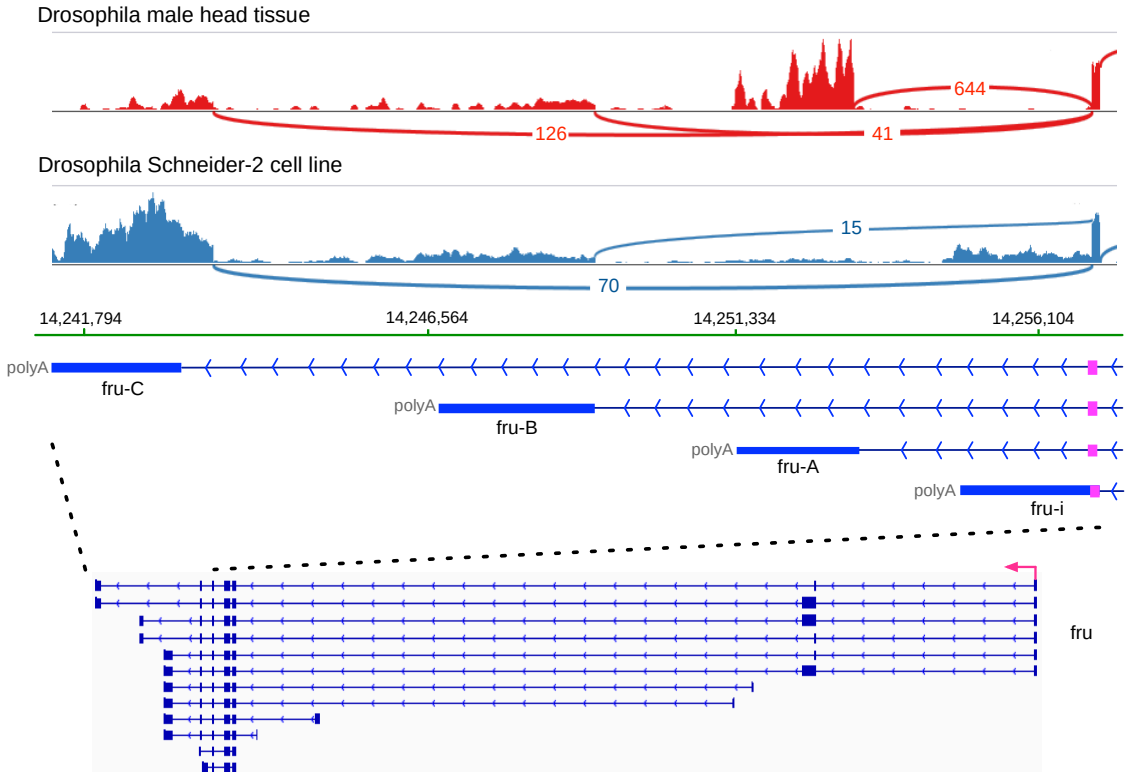

Figure S1. Drosophila Schneider-2 cells and Drosophila male head exhibit distinct, tissue-specific AS patterns. Two distinct AS patterns of the Drosophila *fruitless* gene mRNAs expressed in male Drosophila head tissue and the Drosophila Schineider-2 (S2) cell line (1). The orientation of the transcript is shown at the bottom: the red arrow indicates the direction of the promoter. The dotted lines indicate the region of transcript that is enlarged to highlight the alternatively spliced region. RNA-seq data read density tracks derived from both tissue types are shown, with arcs representing splice junctions that link a common 5' exon to the three alternative last exons, each corresponding to the *fruitless* isoform fru-A, fru-B and fru-C, respectively. The number of unique-mapped RNA-seq reads mapped to the junction is shown across the arc. The relative levels of the fru-A, fru-B and fru-C isoforms determine normal male fly courtship behavior (46). In Drosophila male heads, all three isoforms are present. However, in the Drosophila S2 tissue culture cell line only fru-B and fru-C mRNA isoforms are expressed, together with an additional isoform (fru-i) that uses an alternative polyadenylation signal downstream of the common 5' exon present in the fru-B and fru-C mRNA isoforms. AS analysis software tools that rely on fixed AS event annotation libraries cannot detect and accurately quantitate the distinct fruitless mRNA isoform distributions present in these two different types of Drosophila RNA samples.

Figure S2

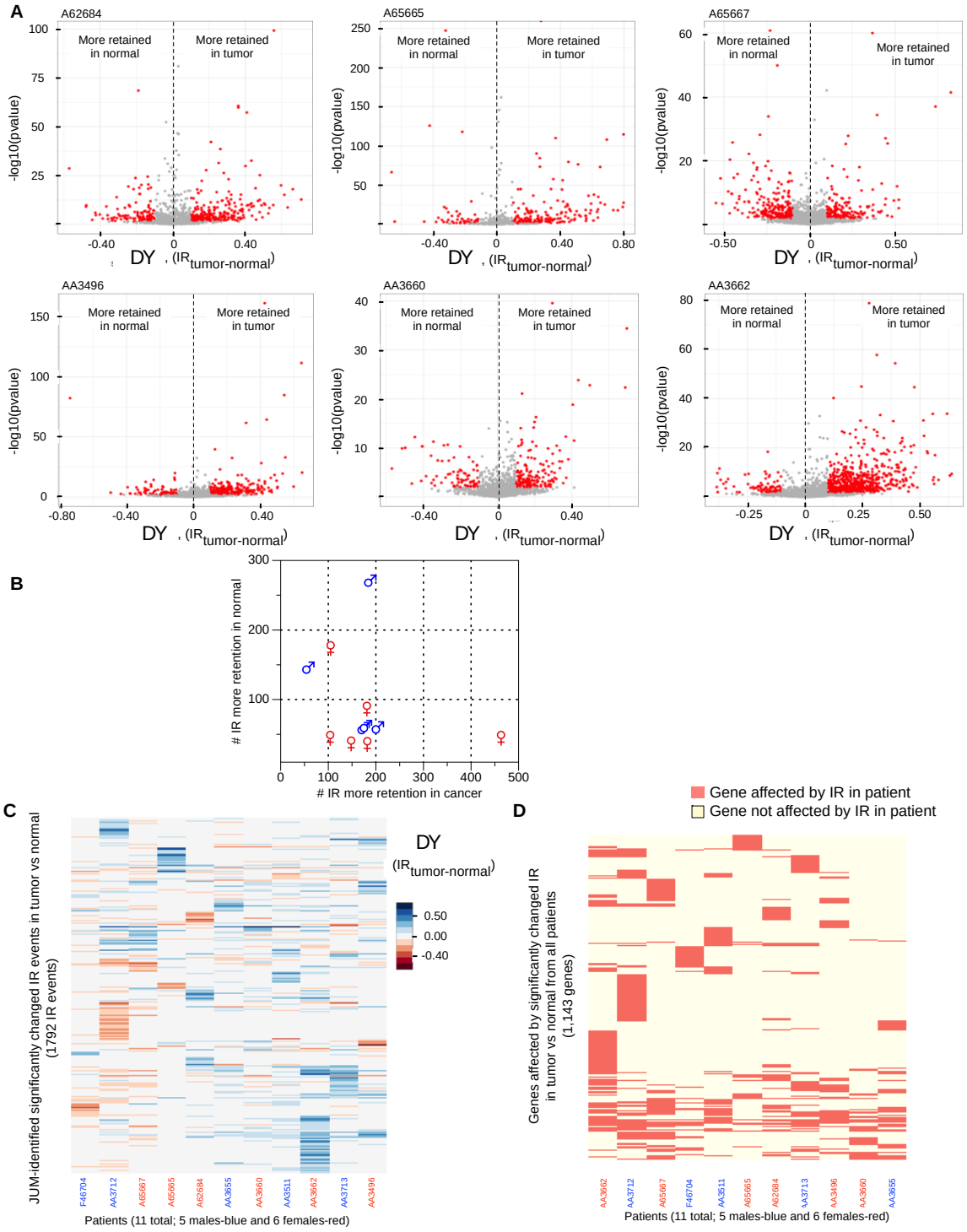

Figure S2. Striking IR heterogeneity revealed by JUM in colon cancer patients' tumor versus matched normal tissues. (A) Volcano plots showing the magnitude and direction of retained intron isoform level changes ($\Delta\Psi$, $IR_{tumor\text{-}normal}$) between tumor and

matched normal tissues on the X-axis and the statistical significance of change (-log10(pvalue)) on the Y-axis for every JUM-profiled IR event in each of the six female patients A62684, A65665, A65667, AA3496, AA3660 and AA3662, respectively. Format is as explained in Figure 5A. (B) A summary of the number of JUM-identified, significantly changed IR events that have more retained intron in tumor (# of IR shown on the X axis) versus the ones that have more retained intron in normal tissues (# of IR shown on the Y axis) for each patient. Male patients are marked by the male symbol (♂) and female patients by the female symbol (♀). (C) Heatmap plot showing the magnitude and direction of retained intron isoform level changes ($\Delta\Psi$, $IR_{tumor\text{-}normal}$) between tumor and matched normal tissues for the 1,792 IR events that are significantly changed in at least one patient's tumor versus matched normal tissues identified by JUM across all 11 patients. Format as explained in Figure 5B. (D) Heatmap showing whether genes affected by changed IR in one patient's tumor versus matched normal tissues are also affected by IR in other patients. Every row shows a gene from the set of total 1,143 genes that are affected by changed IR in at least one patient's tumor versus matched normal tissues and every column shows a patient's samples. If the gene is affected by changed IR in tumor versus normal tissues in the corresponding patient, the grid is marked in red; otherwise the grid is marked in light yellow.

Figure S3

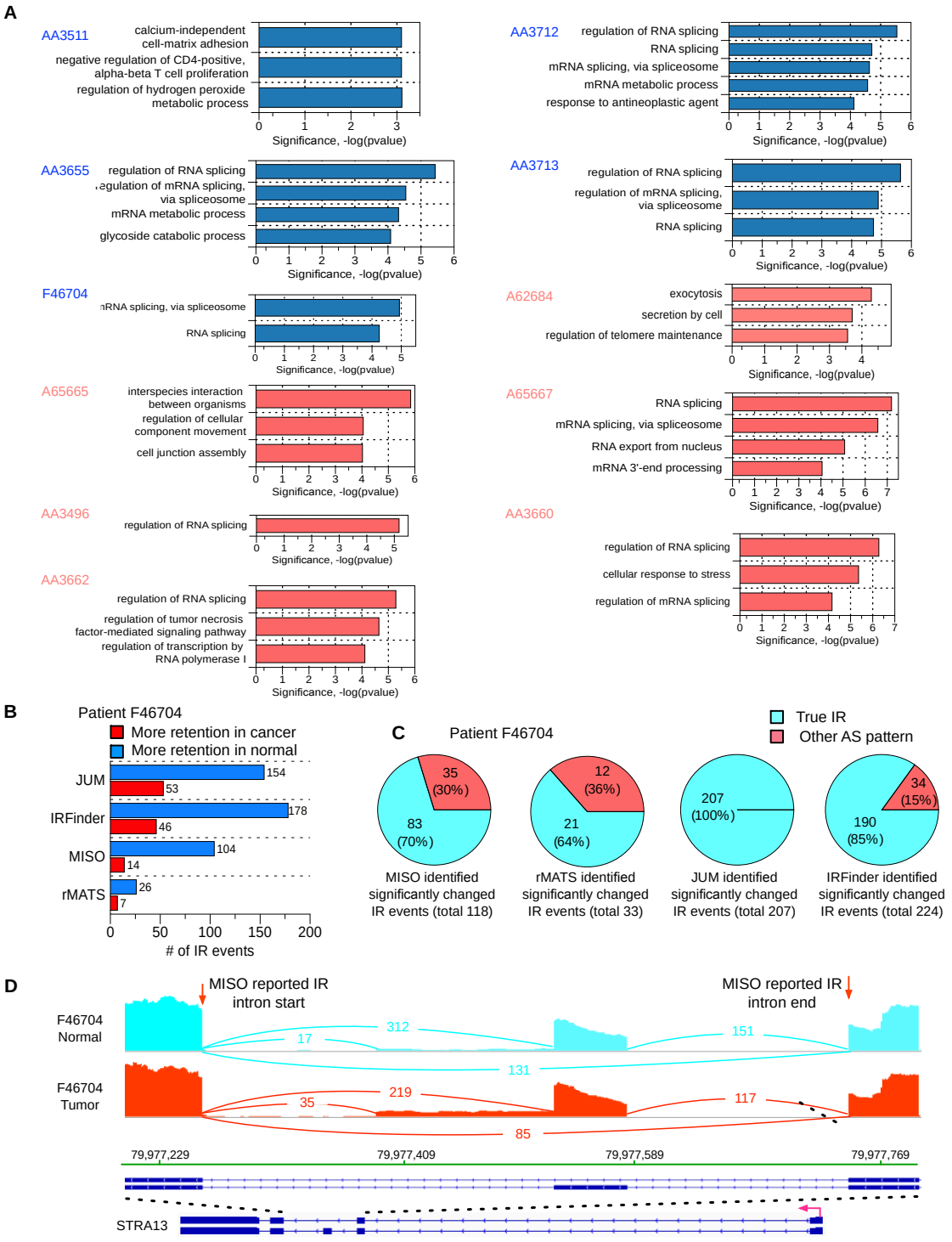

Figure S3. IR changes in colon tumor versus match normal tissues affect genes with distinct functions in different patients and comparison of JUM, rMATS, MISO and IRFinder in analyzing IR in colon cancer patients' tumor versus match normal

tissue samples. (A) Gene ontology analyses of those gene transcripts that undergo differential IR changes between tumor and matched normal tissues in each patient, respectively. (B) The number of significantly differentially spliced IR events reported by four methods, both number of IR events that are more retained in cancer and in normal tissues are shown for male patient F46704, respectively. Patient F46704 is randomly chosen here to show as an example. MISO reported a total of 118 differentially spliced IR events, among which only 14 introns were more retained in tumor samples and 104 introns were more retained in the normal tissue; rMATS identified a total of 33 differentially spliced IR events (qvalue 0.05 without restriction on $\Delta\Psi$ values due to the small number), among which 7 are more retained in the tumor sample; JUM identified a total of 207 differentially spliced IR events, among which 53 are more retained in the tumor sample; IRFinder identified a total of 224 differentially spliced IR events, among which 46 are more retained in the tumor sample. (C) Considering that IR is an intricate AS pattern that can be easily misclassified, every predicted differentially spliced IR event by MISO, rMATS, IRFinder and JUM for patient F46704 were visually examined using the genome browser viewer tool igv (45), respectively. 30% (35 out of 118) of MISO-reported differentially spliced IR events are not IR events, with an example shown in (D); The ratio for rMATS is also high, with 36% (12 out of 33) rMATS-reported IR events not real IR events, with an example shown in Figue S4A; IRFinder has much lower false positive rate than MISO and rMATS, with 15% (34 out of 224) of the reported IR events are not real IR events, with an example shown in Figure S4B. All of the JUM reported IR events are true IR events. (D) An example of an incorrectly classified IR event reported by MISO in the gene STRA13 in male patient F46704. The start and end coordinates of MISO-reported retained introns are specified by red arrows. Arcs represent splice junctions identified from the RNA-seq datasets in normal tissue (blue) and paired tumor samples (red) and the number of uniquely mapped RNA-seq reads mapped to the junctions are shown across the arc. Exon coverage from RNA-seq data is also shown. This MISO-reported "IR" event is in fact a combination of an SE and A5SS event.

Figure S4

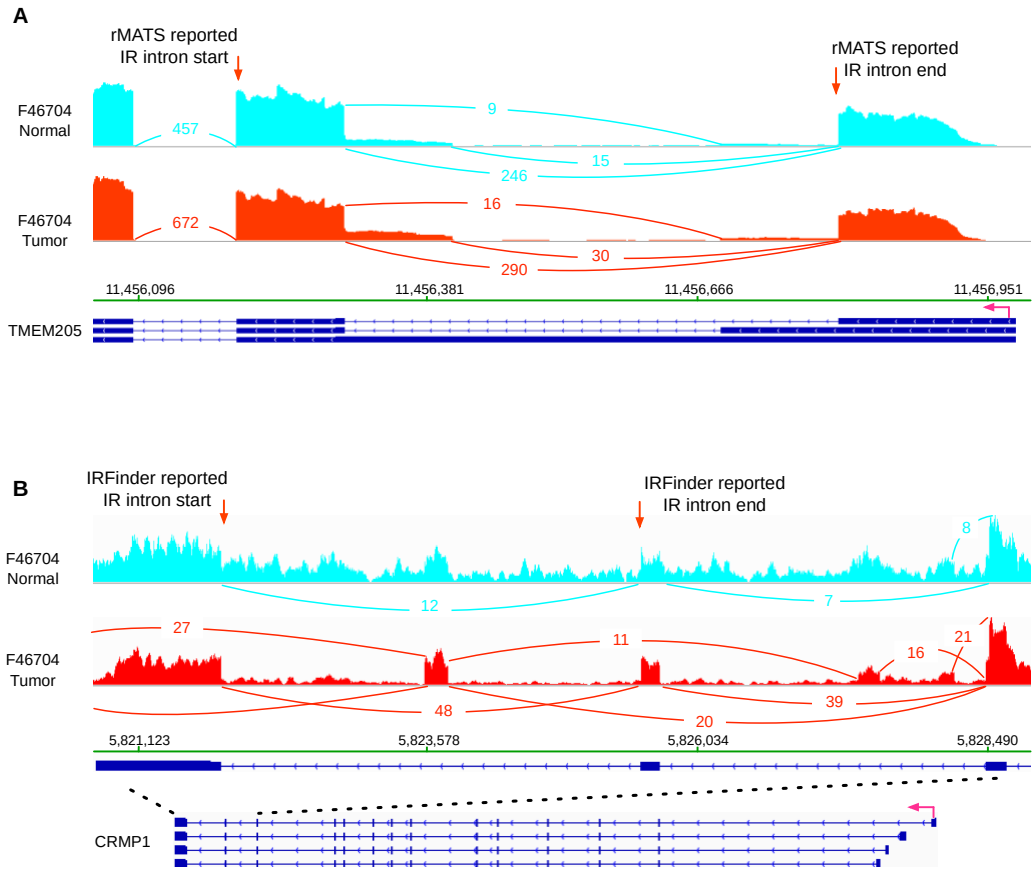

Figure S4. Examples of incorrectly classified IR events reported by other computational software. Examples of incorrectly classified IR events reported by rMATS (A) and IRFinder (B) in genes TMEM205 and CRMP1 in male patient F46704, respectively. The start and end coordinates of reported retained introns are specified by red arrows. Arcs represent splice junctions identified from the RNA-seq datasets in normal tissue (blue) and paired tumor samples (red) and the number of uniquely mapped RNA-seq reads mapped to the junctions are shown across the arc. Exon coverage from RNA-seq data is also shown. In (A), rMATS reported a retained intron that in fact covers an exon and is in combination with an A5SS event. In (B), IRFinder reported a retained intron that covers an exon that can be alternatively included or excluded in multiple ways with upstream and downstream exons.

Figure S5

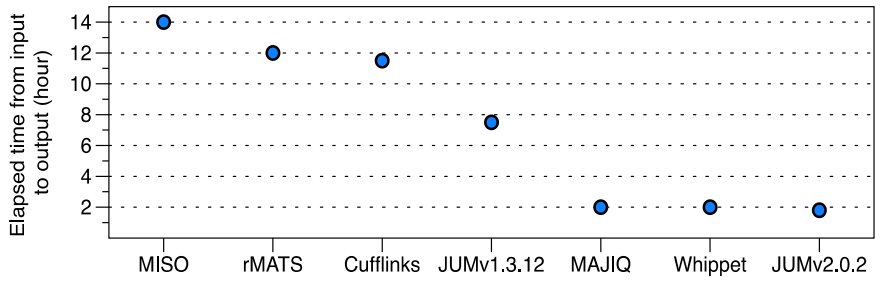

Figure S5. Computation time of the six software tools in analyzing the simulated RNA-seq datasets. For analyzing two sets of triplicates of ~80 million 100bp reads on a standard computing cluster, Whippet, MAJIQ and JUMv2.0.2 are the fastest, taking about 2 hours (for Whippet the specified time includes read alignment time while for other software tools read alignment time is excluded), followed by JUMv1.3.12 that takes about 7.5 hours, Cufflinks 11 hours, rMATS 12 hours and MISO is the slowest that takes about 14 hours (Figure 4D). JUMv2.0.2 is the most updated JUM version that optimized running time compared to JUMv1.3.12, the previous version.

Figure S6

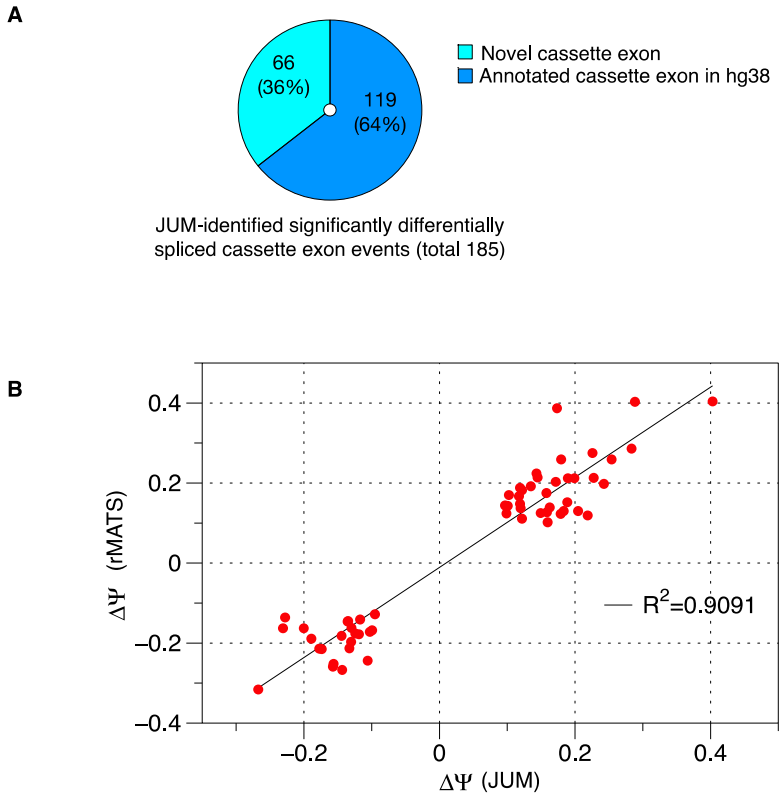

Figure S6. JUM is capable of identifying previously known AS events and novel events and quantification values of splicing changes reported by JUM and rMATS are highly correlated. (A) The distribution of previously annotated and novel SE exons from the 185 JUM-reported significantly changed SE AS events in human K562 cell lines bearing a cancer-associated SRSF2 point mutation versus wildtype**.** A further comparison of the 185 JUM-reported significantly differentially spliced SE events to current human transcriptome annotation revealed that 119 (64%) of these SE were previously known and annotated and 66 (36%) correspond to novel cassette exons that are supported by strong evidence from both RNA-seq exon coverage track signals and adjacent splice junctions, through visual examinations of the RNA-seq datasets using igv (2) (Figure 6B). This observation shows that JUM, although annotation-independent, is capable of accurately profiling AS events that are either previously known or novel to the specific tissue under study. In fact, a significant percentage of the JUM output results cover previously annotated AS events. (B) Scatter plots showing the splicing changes ($\Delta\Psi$) in the exon exclusion isoform levels reported by JUM (X axis) and rMATS (y axis) for the 58 cassette exons that are identified by both JUM and rMATS as significantly changed in the human K562 cells bearing a cancer-associated SRSF2 point mutation versus wildtype. Each dot is a cassette exon and a fitting line for these dots correlating the JUM $\Delta\Psi$ and rMATS $\Delta\Psi$ is plotted, with the square of the Pearson correlation coefficient (R^2) calculated and shown as 0.9091.

Figure S7

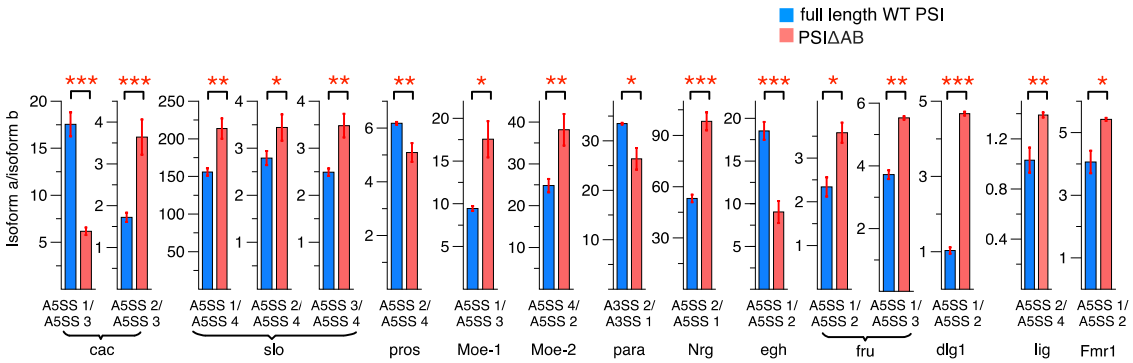

Figure S7. qRT–PCR validation of 12 significantly alternatively spliced AS events in genes associated with male courtship regulation that are only identified by JUM in the mutant male fly head. The Y-axis depicts the ratio between AS isoform a and isoform b, as indicated in the label below each bar graph. The detailed AS event structure and genome browser views of each AS event are provided in SI Appendix, Fig. S8-S11. The means of three independent measurements +/- standard deviation are shown. The differences between full-length WT PSI (blue) and PSI truncation mutation (PSIΔAB, red) male fly head samples were analyzed by one-way ANOVA test. (*) Statistically significant with P-value < 0.05; (**) statistically significant with P-value < 0.01; (***) Statistically significant with P-value < 0.001.

Figure S8-S11. Genome browser charts of the experimentally verified significantly alternatively spliced AS events associated with male courtship behavior regulation that are only identified by JUM in the PSI truncation mutant male fly heads versus wildtype. RNA-seq data tracks derived from the full-length wildtype PSI (blue) and PSI truncation mutation (PSIΔAB, red) male fly head samples are shown, with arcs representing splice junctions and the number of uniquely mapped RNA-seq reads mapped to the junctions shown across the arc. Distinct A5SS or A3SS sites corresponding to alternatively spliced junctions/isoforms are shown. The orientation of the transcript is shown at the bottom: the red arrow indicates the direction of the promoter. The dotted lines indicate the region of transcript that is enlarged to highlight the alternatively spliced region. The qRT-PCR results reflecting the ratio of the two indicated alternatively spliced isoforms are shown on the right of each chart. The Y-axis depicts the ratio between AS isoform a and isoform b, as indicated in the label below each bar graph. The means of three independent measurements +/- standard deviation are shown. See also SI Appendix, Fig. S7.

It is also worth noting that although JUM relies exclusively on splice junction reads from the specific sample and profiles tissue-specific AS patterns that are novel, these JUM-identified AS events are not necessarily in poorly annotated or lowly expressed genes in the tissue sample. For example, the male courtship behavior-associated differentially spliced AS events that are only identified by JUM in PSI mutant male fly heads are in genes are that abundantly expressed, with FPKM ranges from 20 to 200 (Fig. 7A; SI Appendix, Fig. S8-S11).

Figure S8

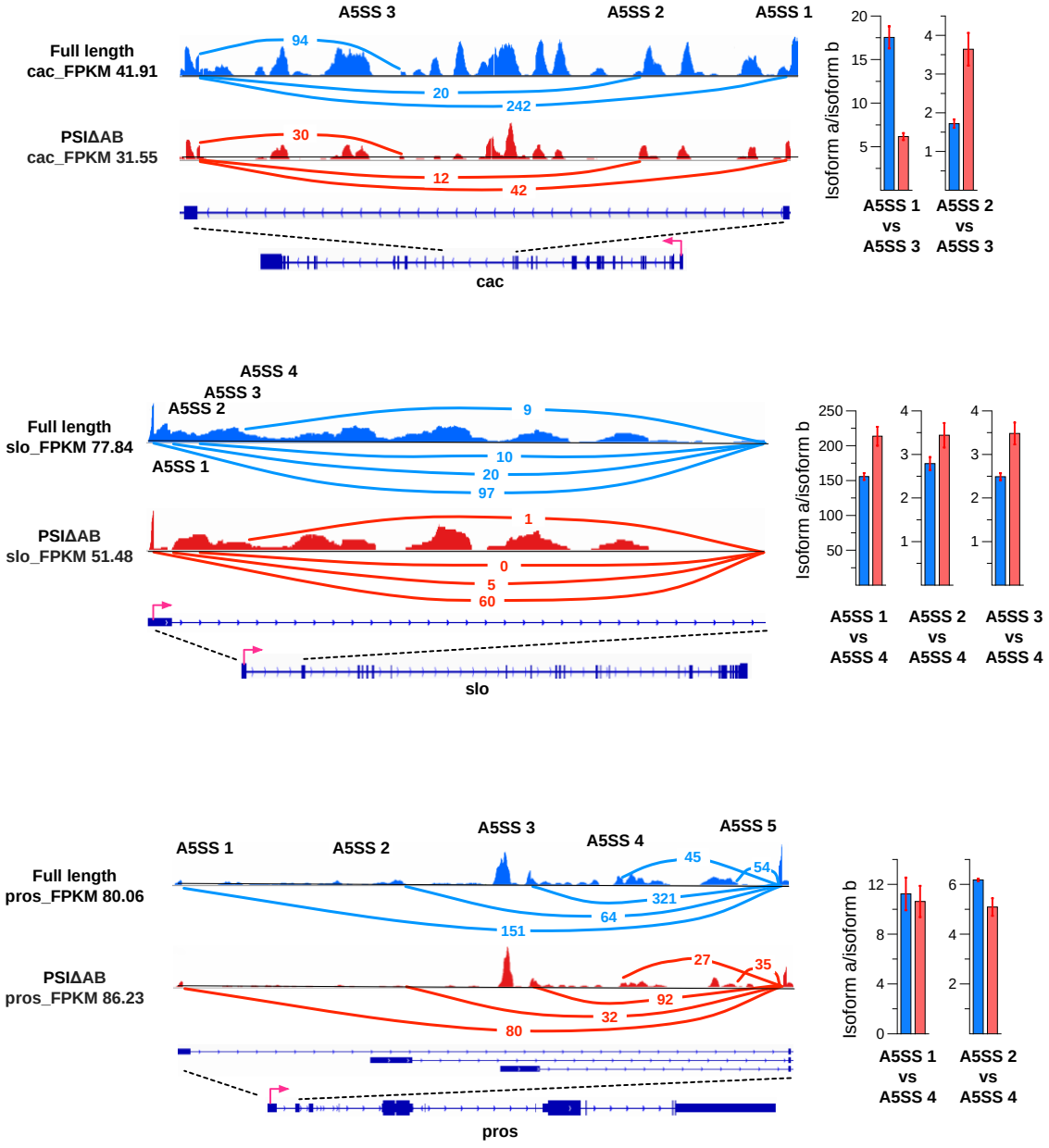
A5SS 3
A5SS 2
A5SS 1
Full length
cac_FPKM 41.91
94
20
242
PSIΔAB
cac_FPKM 31.55
30
12
42
cac
Isoform a/isoform b
A5SS 1 vs A5SS 3
A5SS 2 vs A5SS 3
A5SS 4
A5SS 3
A5SS 2
A5SS 1
Full length
slo_FPKM 77.84
9
10
20
97
PSIΔAB
slo_FPKM 51.48
1
0
5
60
slo
Isoform a/isoform b
A5SS 1 vs A5SS 4
A5SS 2 vs A5SS 4
A5SS 3 vs A5SS 4
A5SS 1
A5SS 2
A5SS 3
A5SS 4
A5SS 5
Full length
pros_FPKM 80.06
45
54
321
64
151
PSIΔAB
pros_FPKM 86.23
27
35
92
32
80
pros
Isoform a/isoform b
A5SS 1 vs A5SS 4
A5SS 2 vs A5SS 4

Figure S9

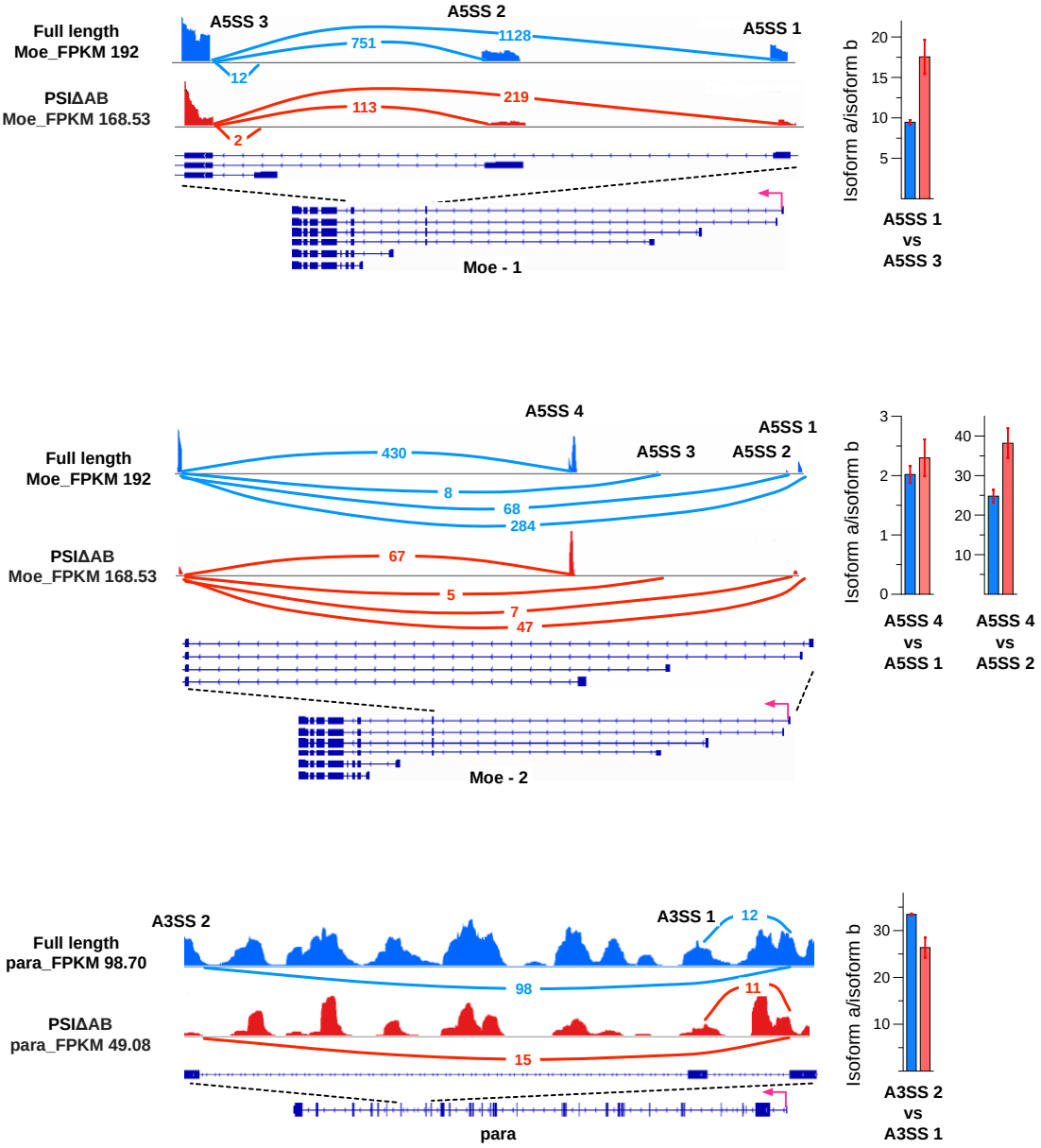
Full length
Moe_FPKM 192
PSIΔAB
Moe_FPKM 168.53
A5SS 3
A5SS 2
A5SS 1
751
1128
12
113
219
2
Moe - 1
Isoform a/isoform b
A5SS 1
vs
A5SS 3
A5SS 4
A5SS 1
A5SS 3
A5SS 2
430
8
68
284
67
5
7
47
Moe - 2
A5SS 4
vs
A5SS 1
A5SS 4
vs
A5SS 2
A3SS 2
A3SS 1
12
98
11
15
para
A3SS 2
vs
A3SS 1

Figure S10

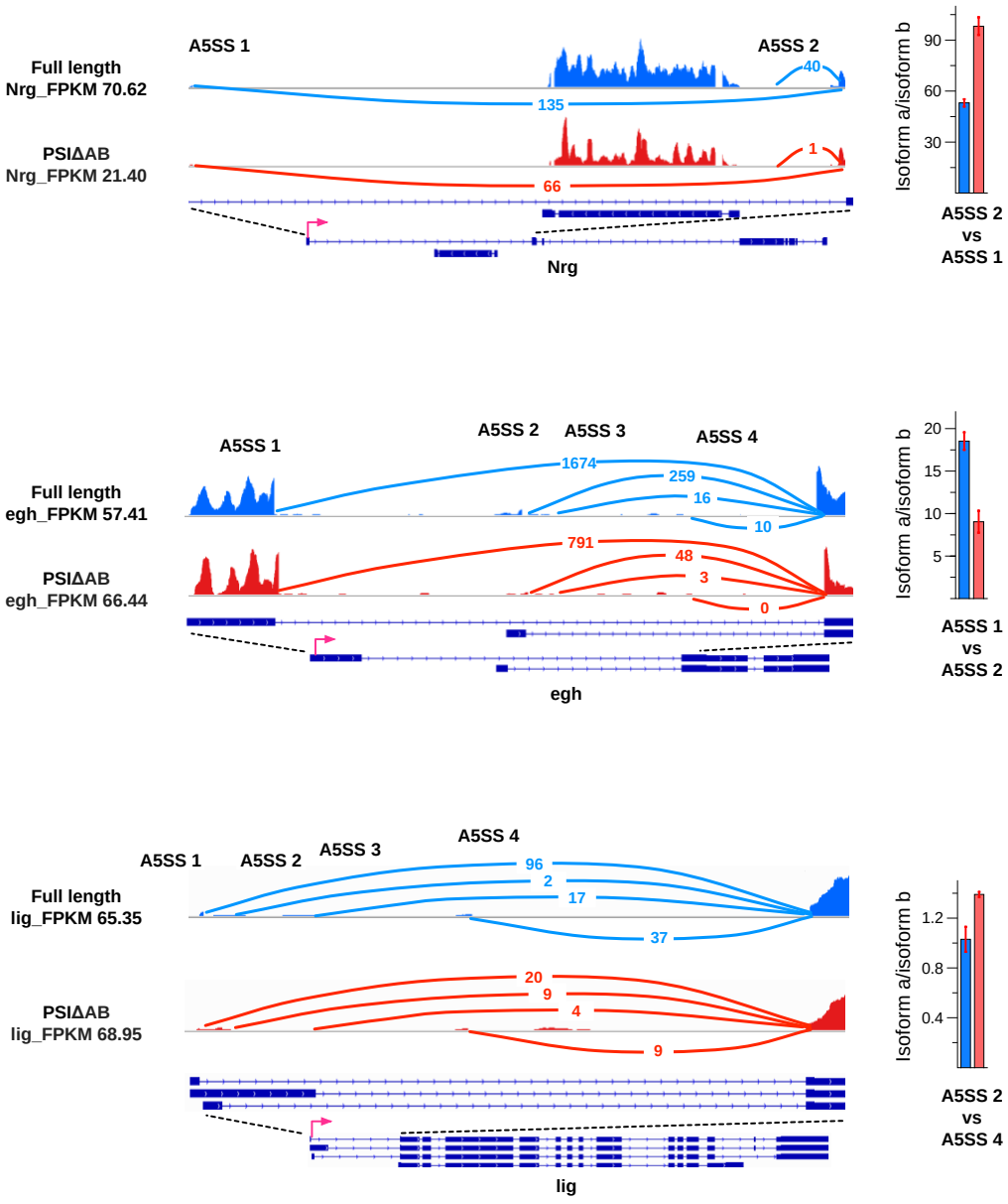
A5SS 1
A5SS 2
Full length
Nrg_FPKM 70.62
135
40
PSIΔAB
Nrg_FPKM 21.40
66
1
Nrg
Isoform a/isoform b
90
60
30
A5SS 2
vs
A5SS 1
A5SS 1
A5SS 2
A5SS 3
A5SS 4
Full length
egh_FPKM 57.41
1674
259
16
10
PSIΔAB
egh_FPKM 66.44
791
48
3
0
egh
Isoform a/isoform b
20
15
10
5
A5SS 1
vs
A5SS 2
A5SS 1
A5SS 2
A5SS 3
A5SS 4
Full length
lig_FPKM 65.35
96
2
17
37
PSIΔAB
lig_FPKM 68.95
20
9
4
9
lig
Isoform a/isoform b
1.2
0.8
0.4
A5SS 2
vs
A5SS 4

Figure S11

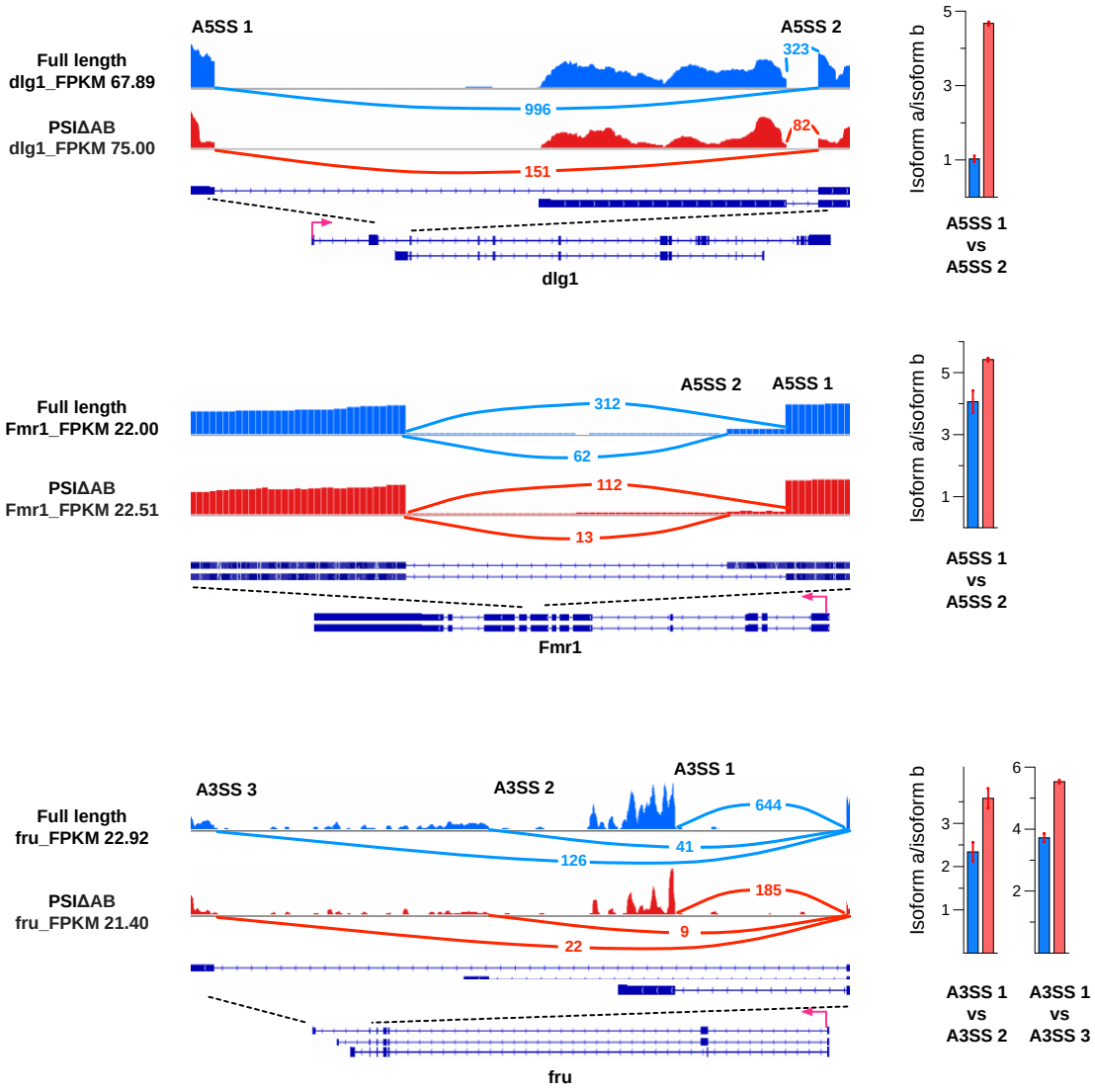
A5SS 1
A5SS 2
Full length
dlg1_FPKM 67.89
323
996
PSIΔAB
dlg1_FPKM 75.00
82
151
dlg1
Isoform a/isoform b
5
3
1
A5SS 1
vs
A5SS 2
A5SS 2
A5SS 1
Full length
Fmr1_FPKM 22.00
312
62
PSIΔAB
Fmr1_FPKM 22.51
112
13
Fmr1
Isoform a/isoform b
5
3
1
A5SS 1
vs
A5SS 2
A3SS 3
A3SS 2
A3SS 1
Full length
fru_FPKM 22.92
644
41
126
PSIΔAB
fru_FPKM 21.40
185
9
22
fru
Isoform a/isoform b
3
2
1
6
4
2
A3SS 1
vs
A3SS 2
A3SS 1
vs
A3SS 3

Figure S12

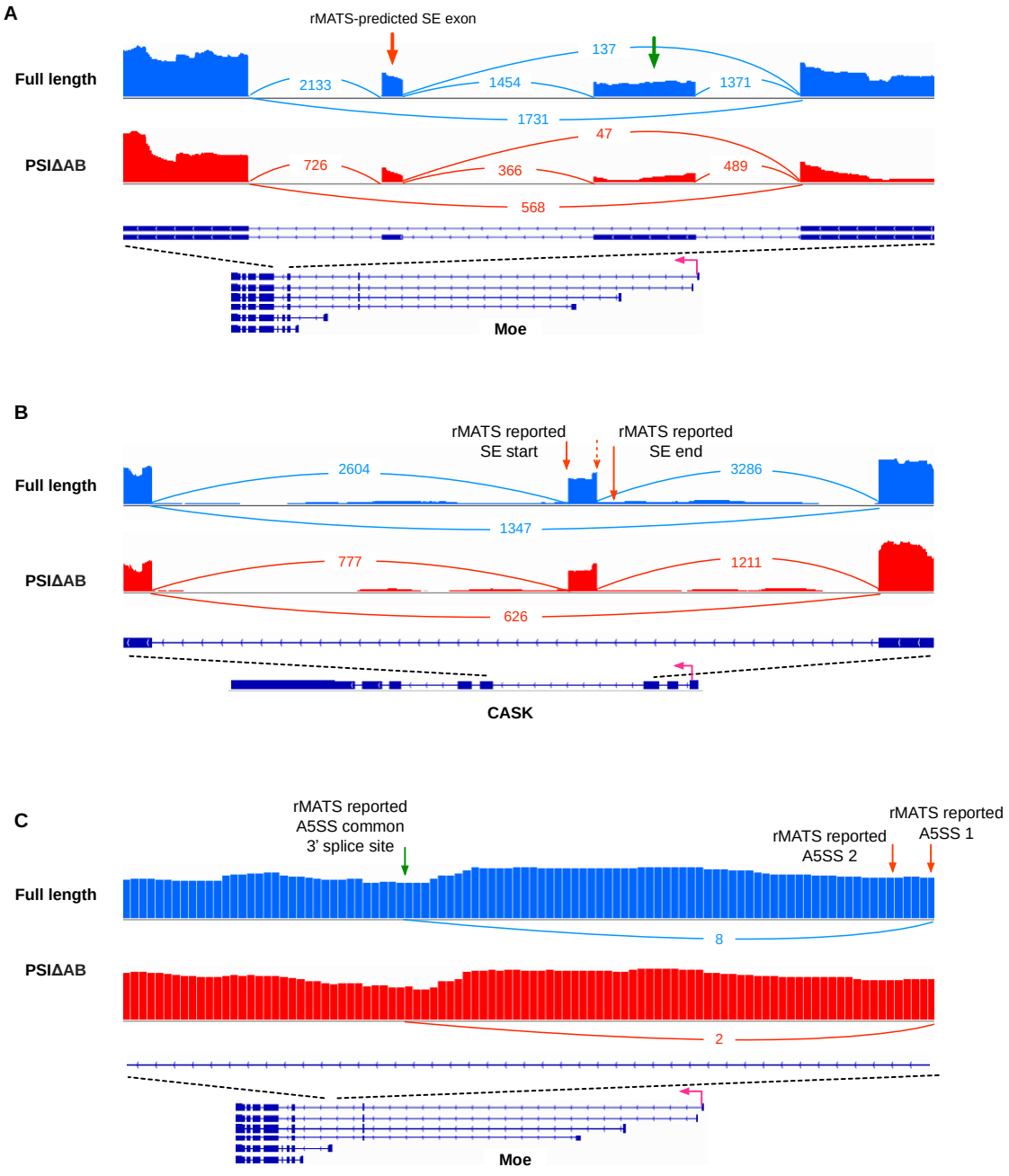

Figure S12. Examples of rMATS-predicted significantly changed AS events in genes associated with male courtship regulation that are either identified by JUM as composite AS events, or are incorrectly annotated AS events by rMATS in the first place, or for which rMATS-reported annotated AS isoforms poorly expressed in the specific fly head tissue under study. (A) An example of an rMATS-identified SE event that actually represents more complicated AS patterns in the male fly head and was re-

classified correctly by JUM as a composite AS event is shown. Exon coverage from RNA-seq data is shown in blue (full-length PSI male fly head) and red (PSI truncation mutation male fly head, PSIΔAB); arcs represent splice junctions identified from the RNA-seq data and the number of uniquely mapped RNA-seq reads mapped to the junctions are shown across the arc; Drosophila annotation (dm3) of the transcripts is shown at the bottom. The red arrow indicates the direction of the promoter. The dotted lines indicate the region of transcript that is enlarged to highlight the alternatively spliced region. rMATS-predicted SE exon is specified with a red arrow. This SE exon is in fact alternatively spliced, either as a cassette exon or together with a downstream exon (green arrow). (B) An example of an rMATS-identified SE event that is incorrectly annotated as a SE event. The start and end coordinates of the rMATS-identified SE exon are marked by solid red arrows, which is not supported by either splice junctions nor exon coverage from the RNA-seq data. There is however a real cassette exon in the region specified, but the end coordinate of the real SE exon is 45 bp upstream of the rMATS-identified SE exon end coordinate. The real SE exon is identified by JUM as a mildly changed AS event (pvalue ≤ 0.05, $\Delta\Psi = 4\%$), but not discovered by rMATS as a significantly changed SE event. (C) An example of an rMATS-identified A5SS event for which the rMATS-reported second A5SS isoform is not or poorly expressed in the fly head tissue, resulting in an invalid rMATS-identified A5SS event for the tissue under study. The two rMATS-predicted A5SS sites are shown by red arrows, while the common 3' splice site for both A5SS marked by a green arrow. No splice junctions are identified from the RNA-seq sample to support the existence of the second A5SS site, which is only 4 bp downstream of the first A5SS site. The isoform corresponding to the second A5SS site thus is either not expressed in the corresponding RNA sample or the expression is too low to be detected by the RNA-seq experiments reported here.

Supplemental Tables

Table S1. Commands for the computational simulations of RNA-seq experiments.

python cal_NB_counts.py genes.gff3 -g1 DRQW1A_2nd_passAligned.out.sam
DRQW1B_2nd_passAligned.out.sam -g2 DRQW1C_2nd_passAligned.out.sam
DRQW1D_2nd_passAligned.out.sam -n 3 -l 2000 -m AS-genes

python generate_rnaseq.py group2.nbcounts AS_genes_list.txt myPara.par testgroup2_0.4 -p 0.4 -c 500

python generate_rnaseq.py group2.nbcounts AS_genes_list.txt myPara.par testgroup2_0.6 -p 0.6 -c 500

python generate_rnaseq.py group2.nbcounts AS_genes_list.txt myPara.par testgroup2_0.8 -p 0.8 -c 500

Table S2. Commands for the five annotation-based AS analysis software in analyzing the simulated RNA-seq datasets.

MISO (version 0.5.4):

miso --run index_for_MISO/indexed_RI testgroup2_out.bam --output-dir testgroup2_output_RI/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_SE_filtered testgroup2_out.bam --output-dir
miso --run index_for_MISO/indexed_A3SS_filtered testgroup2_out.bam --output-dir testgroup2_output_A3SS/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_MXE testgroup2_out.bam --output-dir testgroup2_output_MXE/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_A5SS testgroup2_out.bam --output-dir testgroup2_output_A5SS/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_SE_filtered testgroup1_out.bam --output-dir testgroup1_output_SE/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_MXE testgroup1_out.bam --output-dir testgroup1_output_MXE/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_A3SS_filtered testgroup1_out.bam --output-dir testgroup1_output_A3SS/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_A5SS testgroup1_out.bam --output-dir testgroup1_output_A5SS/ --read-len 100 --paired-end 200 10

miso --run index_for_MISO/indexed_RI testgroup1_out.bam --output-dir testgroup1_output_RI/ --read-len 100 --paired-end 200 10

summarize_miso --summarize-samples testgroup1_output_A5SS/ testgroup1_output_A5SS/

summarize_miso --summarize-samples testgroup1_output_A3SS/ testgroup1_output_A3SS/

summarize_miso --summarize-samples testgroup1_output_SE/ testgroup1_output_SE/

summarize_miso --summarize-samples testgroup1_output_RI/ testgroup1_output_RI/

summarize_miso --summarize-samples testgroup1_output_MXE/ testgroup1_output_MXE/

summarize_miso --summarize-samples testgroup2_output_A5SS/ testgroup2_output_A5SS/

summarize_miso --summarize-samples testgroup2_output_A3SS/ testgroup2_output_A3SS/

summarize_miso --summarize-samples testgroup2_output_SE/ testgroup2_output_SE/

summarize_miso --summarize-samples testgroup2_output_RI/ testgroup2_output_RI/

summarize_miso --summarize-samples testgroup2_output_MXE/ testgroup2_output_MXE/

compare_miso --compare-samples testgroup1_output_RI/ testgroup2_output_RI/ RI_comparisons/

compare_miso --compare-samples testgroup1_output_MXE/ testgroup2_output_MXE/ MXE_comparisons/

compare_miso --compare-samples testgroup1_output_A5SS/ testgroup2_output_A5SS/ A5SS_comparisons/

compare_miso --compare-samples testgroup1_output_A3SS/ testgroup2_output_A3SS/ A3SS_comparisons/

compare_miso --compare-samples testgroup1_output_SE/ testgroup2_output_SE/ SE_comparisons/

Cufflinks (version 2.2.1):

cufflinks -p 3 -o test1_1 -L t1_1 testgroup1_1Aligned.out_sorted.bam

cuffmerge -o merged.gtf -g genes.gtf -p 3 -s genome.fa assembly_GTF_list.txt

cuffdiff -o diff_output -L testgroup1,testgroup2 -p 4 -b genome.fa -FDR 1 merged.gtf/merged.gtf testgroup1_1Aligned.out_sorted.bam,testgroup1_2Aligned.out_sorted.bam,testgroup1_3Aligned.out_sorted.bam testgroup2_0.8_1Aligned.out_sorted.bam,testgroup2_0.8_2Aligned.out_sorted.bam,testgroup2_0.8_3Aligned.out_sorted.bam

MAJIQ (version 1.0.6a):

majiq build cleaned_genes.gff3 -conf configuration.txt --nthreads 6 --output output

majiq psi output/testgroup1_1Aligned.out_sorted.majiq.hdf5 output/testgroup1_2Aligned.out_sorted.majiq.hdf5 output/testgroup1_3Aligned.out_sorted.majiq.hdf5 --nthreads 3 --output psi_out_1 --name testgroup1

majiq psi output/testgroup2_0.8_1Aligned.out_sorted.majiq.hdf5 output/testgroup2_0.8_2Aligned.out_sorted.majiq.hdf5 output/testgroup2_0.8_3Aligned.out_sorted.majiq.hdf5 --nthreads 3 --output psi_out_2 --name testgroup2

majiq deltapsi -grp1 output/testgroup1_1Aligned.out_sorted.majiq.hdf5 output/testgroup1_2Aligned.out_sorted.majiq.hdf5 output/testgroup1_3Aligned.out_sorted.majiq.hdf5 -grp2 output/testgroup2_0.8_1Aligned.out_sorted.majiq.hdf5 output/testgroup2_0.8_2Aligned.out_sorted.majiq.hdf5 output/testgroup2_0.8_3Aligned.out_sorted.majiq.hdf5 --nthreads 3 --output deltapsi_output --names testgroup1 testgroup2

voila psi --no-html psi_out_1/testgroup1.psi.voila -o viola_testgroup_1

voila psi --no-html psi_out_2/testgroup2.psi.voila -o viola_testgroup_2

voila deltapsi --no-html deltapsi_output/testgroup1_testgroup2.deltapsi.voila --threshold 0.1 --show-all -o voila_deltapsi_output_threshold_0.1

rMATS (version 3.2.5):

RNASeq-MATS.py -b1 testgroup1_1Aligned.out_sorted.bam,testgroup1_2Aligned.out_sorted.bam,testgroup1_3Aligned.out_sorted.bam -b2 testgroup2_0.8_1Aligned.out_sorted.bam,testgroup2_0.8_2Aligned.out_sorted.bam,testgroup2_0.8_3Aligned.out_sorted.bam -gtf genes.gtf -o output -t paired -len 100 -novelSS 1

Whippet (version 0.10.4):

julia ~/.julia/v0.6/Whippet/bin/whippet-quant.jl testgroup2_0.8_1_1.fq testgroup2_0.8_1_2.fq -o testgroup2_0.8_1

julia ~/.julia/v0.6/Whippet/bin/whippet-quant.jl testgroup2_0.8_2_1.fq testgroup2_0.8_2_2.fq -o testgroup2_0.8_2

julia ~/.julia/v0.6/Whippet/bin/whippet-quant.jl testgroup2_0.8_3_1.fq testgroup2_0.8_3_2.fq -o testgroup2_0.8_3

julia ~/.julia/v0.6/Whippet/bin/whippet-quant.jl testgroup1_1_1.fq testgroup1_1_2.fq -o testgroup1_1

julia ~/.julia/v0.6/Whippet/bin/whippet-quant.jl testgroup1_2_1.fq testgroup1_2_2.fq -o testgroup1_2

julia ~/.julia/v0.6/Whippet/bin/whippet-quant.jl testgroup1_3_1.fq testgroup1_3_2.fq -o testgroup1_3

julia ~/.julia/v0.6/Whippet/bin/whippet-delta.jl -a testgroup1_1.psi.gz,testgroup1_2.psi.gz,testgroup1_3.psi.gz -b testgroup2_0.8_1.psi.gz,testgroup2_0.8_2.psi.gz,testgroup2_0.8_3.psi.gz

JUM (version 1.3.12)

bash ~/JUM_1.3.12/JUM_2-1.sh

bash ~/JUM_1.3.12/JUM_2-2.sh ~/JUM_1.3.12 5 3 testgroup1

bash ~/JUM_1.3.12/JUM_2-2.sh ~/JUM_1.3.12 5 3 testgroup2

bash ~/JUM_1.3.12/JUM_2-3.sh ~/JUM_1.3.12 5 3 5 100

Rscript ~/JUM_1.3.12/R_script_JUM.R ~/JUM_1.3.12 experiment_design.txt > outputFile.Rout 2> errorFile.Rout

bash ~/JUM_1.3.12/JUM_3.sh ~/JUM_1.3.12 pvalue 1 6 3

bash ~/JUM_1.3.12/JUM_4.sh ~/JUM_1.3.12 pvalue 1 3 3 refFlat.txt

Table S3. Commands for running IRFinder (version 1.2.4) to analyze the male patient F46704 colon cancer and matched normal tissue datasets

IRFinder -m BuildRef -r REF/Human-hg38-release91 ftp://ftp.ensembl.org/pub/ release-91/gtf/homo_sapiens/Homo_sapiens.GRCh38.91.gtf.gz

IRFinder -r ~/software/IRFinder-1.2.4/REF/Human-hg38-release91 -d F46704_TMirfinder F46704TM_1.fastq F46704TM_2.fastq

IRFinder -r ~/software/IRFinder-1.2.4/REF/Human-hg38-release91 -d F46704_NTirfinder F46704NT_1.fastq F46704NT_2.fastq

analysisWithNoReplicates.pl -A F46704_TMirfinder/IRFinder-IR-nondir.txt -B F46704_NTirfinder/IRFinder-IR-nondir.txt > TM-vs-NT.txt

Table S4. Summary of the TCGA colon cancer patient samples analyzed by JUM and other software

Patient ID	Vital status	Tumor type	Sex	Age	Primary tumor RNA-seq sample uuid	Paired-end sequencing length	Paired normal tissue RNA-seq sample uuid	Paired-end sequencing length
TCGA-AA-3511	Alive	Primary tumor	Male	64	24dc3d9d-9011-4752-bf86-7308f89fd27d	48	c77b629b-ae40-450e-8154-f4fc0b6bb6e3	48
TCGA-AA-3655	Alive	Primary tumor	Male	68	ef7c0e3b-66ac-43c8-a220-abdffbdd2f24	48	6e1add3f-e334-413f-a32c-3302f80db15f	48
TCGA-AA-3712	Alive	Primary tumor	Male	65	a4299481-edf7-4286-892c-d6d4a35e061d	48	56ec9cb6-c34f-413f-b04a-ea2e5e429cd3	48
TCGA-AA-3713	Alive	Primary tumor	Male	68	29c6bd2a-d4db-40db-9249-bb2b7f24c7bf	48	34df9d7b-935c-4298-bee1-cd2d03cd7950	48
TCGA-F4-6704	Alive	Primary tumor	Male	60	f815284b-74a2-4ad8-9f1b-aa1ec54fe579	48	f2e8d1e1-001b-4a31-a4ee-23936f5f3022	48
TCGA-A6-2684	Alive	Primary tumor	Female	76	52864b61-728b-451a-a4f9-f922ca6234c2	48	18625fe4-3c19-45d9-9d7c-a295fbf83f2e	48
TCGA-A6-5665	Alive	Primary tumor	Female	85	85ab069a-51ec-42ed-8e01-3f41791dc3b1	48	fde66458-b58b-40e6-89cc-97970b566b85	48
TCGA-A6-5667	Alive	Primary tumor	Female	40	7d10f16e-737a-4351-ab41-9e9794b92785	48	d1f1002d-525b-4b8b-b52f-376bf792d74e	48
TCGA-AA-3496	Alive	Primary tumor	Female	83	bf18b4eb-ff17-4632-a0ca-9a1c0f002156	48	e3adceb2-6d55-4812-b972-e46d843cb261	48
TCGA-AA-3660	Alive	Primary tumor	Female	51	42c5c88c-1abd-456b-be8c-612219c1439f	48	dbe71479-abcf-43aa-b32a-3ffab1ed1af4	48
TCGA-AA-3662	Alive	Primary tumor	Female	81	c3269758-60aa-4c03-9085-ef9cc5862242	48	f54f934c-181b-4ab7-9837-99c6d6d21875	48

Table S5. Significantly differentially spliced AS events reported by JUM, MISO and rMATS comparing tumor and matched normal tissues in male colon cancer patients (statistical cutoff for each software tool listed in Methods).

JUM

AS pattern	F46704	AA3511	AA3655	AA3712	AA3713
A5SS	693	574	612	730	695
A3SS	460	373	449	498	623
SE	372	345	365	451	330
MXE	6	8	10	9	8
Composite	766	503	588	694	710
IR	207	252	234	488	273

rMATS

AS pattern	F46704	AA3511	AA3655	AA3712	AA3713
A5SS	13	16	20	22	35
A3SS	18	8	19	35	40
SE	267	172	167	293	132
MXE	86	35	45	81	74
IR	17	21	30	35	50

MISO

AS pattern	F46704	AA3511	AA3655	AA3712	AA3713
A5SS	51	27	34	41	70
A3SS	56	60	47	80	59
SE	256	161	172	275	190
MXE	109	59	80	89	102
IR	118	128	155	206	258

Table S6. Significantly differentially spliced AS events reported by JUM, MISO and rMATS comparing tumor and matched normal tissues in female colon cancer patients (statistical cutoff for each software tool listed in Methods).

JUM

AS pattern	A62684	AA3496	A65667	AA3662	A65665	AA3660
A5SS	1254	655	725	701	1401	532
A3SS	1051	508	565	558	1597	391
SE	571	422	481	417	625	313
MXE	10	13	10	13	13	9
Composite	930	614	773	602	770	503
IR	279	237	315	544	183	168

rMATS

AS pattern	A62684	AA3496	A65667	AA3662	A65665	AA3660
A5SS	101	33	37	33	282	20
A3SS	134	16	34	41	307	9
SE	395	329	490	169	736	131
MXE	269	66	95	50	461	22
IR	69	58	31	61	99	11

MISO

AS pattern	A62684	AA3496	A65667	AA3662	A65665	AA3660
A5SS	116	56	68	59	162	19
A3SS	144	48	73	72	176	42
SE	343	274	296	162	411	148
MXE	301	123	103	67	502	76
IR	351	252	175	356	585	111

Table S7. Summary of the sequencing depth and read genome mapping results for tumor and normal colon tissue samples in the colon cancer patient datasets from TCGA.

Patient Sample	Sex	# Input reads	# Uniquely mapped read	% Unique mapping
AA3511-Normal	Male	58,669,541	50,311,513	85.75%
AA3511-Tumor	Male	65,542,491	56,312,516	85.92%
AA3655-Normal	Male	55,633,014	46,755,733	84.04%
AA3655-Tumor	Male	51,719,615	44,977,155	86.96%
AA3712-Normal	Male	58,387,107	47,991,007	82.19%
AA3712-Tumor	Male	61,135,422	52,350,015	85.63%
AA3713-Normal	Male	50,238,563	41,950,025	83.50%
AA3713-Tumor	Male	65,100,348	55,663,103	85.50%
F46704-Normal	Male	68,523,456	60,229,537	87.90%
F46704-Tumor	Male	105,993,154	87,417,927	82.48%
A62684-Normal	Female	61,592,972	52,835,140	85.78%
A62684-Tumor	Female	78,210,700	57,466,097	73.48%
A65665-Normal	Female	54,269,419	46,377,687	85.46%
A65665-Tumor	Female	76,057,421	65,877,389	86.62%
A65667-Normal	Female	57,034,366	50,248,336	88.10%
A65667-Tumor	Female	56,642,649	49,641,446	87.64%
AA3496-Normal	Female	37,158,143	30,861,719	83.06%
AA3496-Tumor	Female	63,603,047	55,535,760	87.32%
AA3660-Normal	Female	44,240,295	37,908,252	85.69%
AA3660-Tumor	Female	52,884,618	45,521,931	86.08%
AA3662-Normal	Female	48,553,863	41,206,944	84.87%
AA3662-Tumor	Female	56,215,719	48,707,955	86.64%

Table S8. The test of rMATS and MISO in identifying JUM-predicted, experimentally-validated, significantly changed AS events in mutant PSI Drosophila male head that are functionally associated with the aberrant courtship behavior phenotype. The AS pattern type, associated gene name, and the coordinates of the JUM-identified significantly differentially spliced AS events are listed in columns 1-3. The analysis of rMATS or MISO on these events are listed in column 4 and 5, respectively. If an AS event is identified or partially recognized, the coordinates of the AS events are listed for rMATS and MISO. If the AS event is not identified by either of the two tools, the reason is specified, almost exclusively because the AS event is not included in the AS annotation table used by MISO or not recognized by rMATS even with the aided novel splicing junction detection mode.

Gene	AS event type	JUM-identified and RNA-seq experiment-validated AS event coordinate (qvalue <= 0.1; dpsi >= 5%)	rMATS output (novel junction detection mode; qvalue <= 0.1; dpsi >= 5%)	MISO output (Bayes factor 10; fold change 5%; at least 5 read support for each isoform)
fne	A5SS	chrX:12811843|12811847|12812046-12812608 (+)	Not recognized	chrX:12811847|12812046-12812608 (+) (partial; 2 A5SS sites out of 3)
	A5SS	chrX:12836880|12836889-12837117 (+)	Not recognized	chrX:12836880|12836889-12837117 (+)
	Composite	See supplemental table		
fru	A3SS	chr3R:14243022|14248904|14252888-14256552 (-)	Not recognized	Not annotated
	Composite	See supplemental table		
cac	IR	chrX:11829763-11829831 (-)	Not recognized	chrX: 11829763-11829831 (-)
	A5SS	chrX:11848314-11850953|11854106|11856051 (-)	Not recognized	Not annotated
	Composite 3X	See supplemental table		
dlg1	A5SS	chrX:11268543|11270751-11270873 (+)	Not recognized	Not annotated
	Composite 2X			
slo	A5SS	chr3R:20488244|20488285|20488293–20490084 (+)	Not recognized	Not annotated
	MXE	chr3R: 20517655-20517925:20518038-20519493:20519606-20520677	chr3R: 20517655-20517925:20518038-20519493:20519606-20520677	Not annotated

	MXE	chr3R:20513019-20513521:20513627-20515043:20515149-20517180 (+)	chr3R:20513019-20513521:20513627-20515043:20515149-20517180 (+)	Not annotated
	Composite 3X			
CASK	SE	chr3R:17615482-17616430:17616460-17617251 (-)	chr3R:17615482-17616430:17616460-17617251 (-)	Not annotated
	Composite			
pros	IR	chr3R:7201560-7202042 (+)	Not recognized	chr3R:7201560-7202044 (+)
	A5SS	chr3R:7155678|7159275|7161344|7162787|7164714-7165378 (+)	Not recognized	Not annotated
Fmr1	A5SS	chr3R:5930732-5930780|5930789 (-)	Not recognized	Not annotated
Moe	A5SS	chrX:8770785-8771045|8772500|8774323 (-)	Not recognized	Not annotated
	A5SS	chrX:8774433-8785593|8788090|8791870|8792215 (-)	Not recognized	Not annotated
qtc	SE	chr2L:5068394-5069126:5069219-5070095 (+)	chr2L:5068394-5069126:5069219-5070095 (+)	chr2L:5068394-5069126:5069219-5070095 (+)
lig	A5SS	chr2R:3955516|3955585|3955871|3956339-3957374 (+)	Not recognized	Not annotated
	Composite			
eag	SE	chrX:14884465-14885378:14884464-14886751	chrX:14884465-14885378:14884464-14886751	Not annotated
	Composite			
para	A3SS	chrX:16371604|16374835-16375357	Not recognized	Not annotated
	Composite X3			
Nrg	A5SS	chrX:8412431|8424347-8425556 (+)	Not recognized	Not annotated
	Composite			
egh	A5SS	chrX:2483539 | 2486381|2486695|2488233-2489757 (+)	Not recognized	Not annotated

Table S9. The test of JUM in detecting rMATS-predicted significantly differentially spliced AS events that are within genes associated with male courtship behavior regulation in mutant PSI Drosophila male head transcriptome versus wildtype. The AS event associated gene name, AS pattern type, and the coordinates of the rMATS-identified significantly differentially spliced AS events in male courtship regulatory genes are listed in columns 1-3. A visual genome browser verification of each AS event was performed and the results presented in column 4. The performance of JUM analysis on these AS events are listed in column 5.

rMATS identified AS event gene	rMATS identified AS event type	rMATS identified AS events coordinate	**Genome browser verification**	**JUM prediction**
fru	IR	chr3R:14332050-14332284 (-)	Incorrect annotation; not an IR event	N/A
	SE	chr3R:14261512-14275956:14276104-14332215 (+)	Composite	Detected by JUM as a Composite AS event
dlg1	A5SS	chrX:11283888|11283730|11283814-11284263 (+)	Annotated alternative isoforms not/poorly expressed in fly head tissue	N/A
	SE	chrX:11290136-11293774:11293849-11299306 (+)	Composite event 1	Detected by JUM as a Composite AS event
	SE	chrX:11294865-11296739:11296820-11299306 (+)	Composite event 1	Detected by JUM as a Composite AS event
	SE	chrX:11290136-11293774:11293849-11294820 (+)	Composite event 1	Detected by JUM as a Composite AS event
	SE	chrX:11276013-11276968:11277145-11281804 (+)	Composite event 2	Detected by JUM as a Composite AS event
slo	SE	chr3R:20490517-20490618:20490807-20495262 (+)	Composite event 1	Detected by JUM as a Composite AS event
	SE	chr3R:20501522-20505207:20505299-20508029 (+)	Composite event 2	Detected by JUM as a Composite AS events
	SE	chr3R:20501522-20502965:20503057-20508029 (+)	Composite event 2	Detected by JUM as a Composite AS event

	SE	chr3R:20505299-20508029:20508121-20512908 (+)	Composite event 2	Detected by JUM as a Composite AS event
	SE	chr3R:20517655-20519493:20519533-20520678 (+)	Composite event 2	Detected by JUM as a Composite AS event
	SE	chr3R:20521848-20525097:20525148-20526427 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20521848-20527809:20527884-20528742 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20525148-20526427:20526451-20527809 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20525148-20526430:20526451-20528742 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20521139-20523919:20523991-20528839 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20521139-20521812:20521848-20528839 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20523991-20525097:20525148-20528742 (+)	Composite event 3	Detected by JUM as a Composite AS event
	SE	chr3R:20521848-20525097:20525148-20528742 (+)	Composite event 3	Detected by JUM as a Composite AS event
	MXE	chr3R: 20517655-20517925:20518038-20519493:20519606-20520677	True MXE event	Detected by JUM as an MXE AS event
	MXE	chr3R:20513019-20513521:20513627-20515043:20515149-20517180 (+)	True MXE event	Detected by JUM as an MXE AS event
CASK	A5SS	chrX:17633456-17634210|17634338 (-)	Composite event	Detected by JUM as a Composite AS event
	SE	chr3R:17613824-17614595:17614694-17615170 (-)	Incorrect annotation; not SE event	N/A

	SE	chr3R:17615482-17616430:17616460-17617252 (-)	True SE event	Detected by JUM as an SE AS event
Moe	A5SS	chrX:8772638-8772692|8772696 (-)	Annotated alternative isoforms not/poorly expressed in fly head tissue	N/A
	SE	chr3R:8774433-8791871:8791960-8792216 (-)	Annotated alternative isoforms not/poorly expressed in fly head tissue	N/A
	SE	chr3R:8769893-8770065:8770092-8770605 (-)	Composite event	Detected by JUM as a Composite AS event
qtc	SE	chr2L:5068394-5069133:5069219-5070096 (-)	Incorrect annotation; not SE event	N/A
	SE	chr2L:5068394-5069126:5069219-5070096 (-)	True SE event	Detected by JUM as an SE AS event
lig	A5SS	chr2R:3955585|3955516|3955871-3957375 (+)	Annotated alternative isoforms not/poorly expressed in fly head tissue	N/A
eag	SE	chrX:14884465-14885378:14884464-14886751 (+)	True SE event	Detected by JUM as an SE AS event
para	SE	chrX:16392929-16394539:16394561-16398022 (-)	Composite event 1	Detected by JUM as a Composite AS event
	SE	chrX:16392929-16394539:16394602-16398022 (-)	Composite event 1	Detected by JUM as a Composite AS event
	A5SS	chrX:16377922-16380373|16380412 (-)	Composite event 2	Detected by JUM as a Composite AS event
	A5SS	chrX:16377892-16380373|16380412 (-)	Composite event 2	Detected by JUM as a Composite AS event
	A3SS	chrX:16377922|16377892-16380412 (-)	Composite event 2	Detected by JUM as a Composite AS event

	A3SS	chrX:16377922|16377892-16380373 (-)	Composite event 2	Detected by JUM as a Composite AS event
	A5SS	chrX:16394602-16398022|16398094 (-)	Composite event 3	Detected by JUM as a Composite AS event
Nrg	A5SS	chrX:8411457|8411542|8411607|8411609|8411611|8411623|8411635|8411639|8411667|8411703-8425729	Annotated alternative isoforms not/poorly expressed in fly head tissue	N/A

Supplemental Datasets.

Dataset S1-S34: Differential AS analysis results using four different software tools (JUM, MISO, rMATS and IRFinder) on the TCGA colon cancer tumor versus matched normal tissue samples.
Dataset S1: AA3511_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S2: AA3511_MISO_differential_AS_filtered.xlsx
Dataset S3: AA3511_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S4: AA3655_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S5: AA3655_MISO_differential_AS_filtered.xlsx
Dataset S6: AA3655_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S7: AA3712_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S8: AA3712_MISO_differential_AS_filtered.xlsx
Dataset S9: AA3712_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S10: AA3713_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S11: AA3713_MISO_differential_AS_filtered.xlsx
Dataset S12: AA3713_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S13: F46704_JUM_AS_differential_qvalue_0.05_dpsi_10%.xlsx
Dataset S14: F46704_MISO_AS_differential_filtered.xlsx
Dataset S15: F46704_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S16: F46704_IRFinder_differential_IR_pvalue_0_05_dpsi_10%.xlsx
Dataset S17: A62684_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S18: A62684_MISO_differential_AS_filtered.xlsx
Dataset S19: A62684_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S20: A65665_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S21: A65665_MISO_differential_AS_filtered.xlsx
Dataset S22: A65665_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S23: A65667_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S24: A65667_MISO_differential_AS_filtered.xlsx
Dataset S25: A65667_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S26: AA3496_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S27: AA3496_MISO_differential_AS_filtered.xlsx
Dataset S28: AA3496_rMATS_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S29: AA3660_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S30: AA3660_MISO_differential_AS_filtered.xlsx
Dataset S31: AA3660_rMATS_differential_AS_qvalue_0_05_dpsi_10%.xlsx
Dataset S32: AA3662_JUM_differential_AS_qvalue_0.05_dpsi_10%.xlsx
Dataset S33: AA3662_MISO_differential_AS_filtered.xlsx
Dataset S34: AA3662_rMATS_differential_AS_qvalue_0_05_dpsi_10%.xlsx
Dataset S35: Significant GO term enrichment for genes affected by IR in each patient.
Dataset S36: $\Delta\Psi$ values in IR events that are identified as significantly changed in at least one patient by JUM and also annotated in the MISO library (deltaPSI_value_in_IR_sig_change_in_at_least_one_patient_and_annotated_in_MISO_library.xlsx).

Dataset S37: ΔΨ values in IR events that are identified as significantly changed in at least one patient by JUM (deltaPSI_value_in_IR_sig_change_in_at_least_one_patient.xlsx).
Dataset S38: ΔΨ values in IR events that affect splicing factors and are identified as significantly changed in at least one patient by JUM (deltaPSI_value_in_IR_affecting_splicing_factor_in at_least_1_patients.xlsx).
Dataset S39: ΔΨ values in IR events that affect splicing factors and are identified as significantly changed in at least three patients by JUM (deltaPSI_value_in_IR_affecting_splicing_factor_in at_least_3_patients.xlsx).
Dataset S40: Differential AS analysis results using JUM on the SRSF2 single mutation carrying K562 cell lines versus wildtype (SRSF2mut_JUM_differential_AS_qvalue_0.1_dpsi_10%.xlsx).
Dataset S41-S43: Differential AS analysis results using three different software tools (JUM, MISO and rMATS) on the male fruit fly heads carrying the PSIΔAB mutation versus wildtype.
Dataset S41: PSIdeltaAB_JUM_differential_AS_qvalue_0.1_dpsi_5%.xlsx
Dataset S42: PSIdeltaAB_MISO_differential_AS_filtered.xlsx
Dataset S43: PSIdeltaAB_rMATS_differential_AS_qvalue_0_1_dpsi_5%.xlsx

References

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2. Robinson JT, *et al.* (2011) Integrative genomics viewer. *Nat Biotechnol* 29(1):24-26.