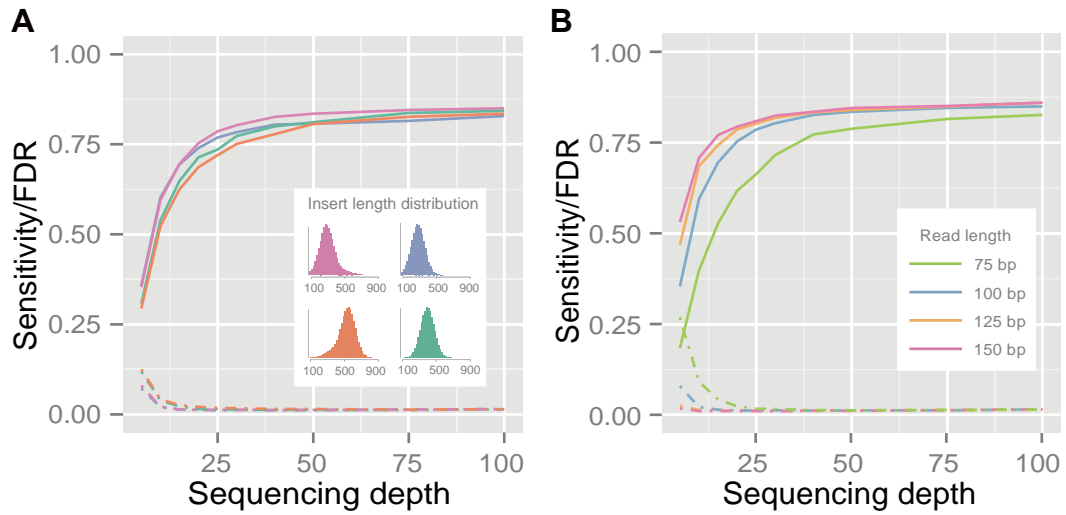
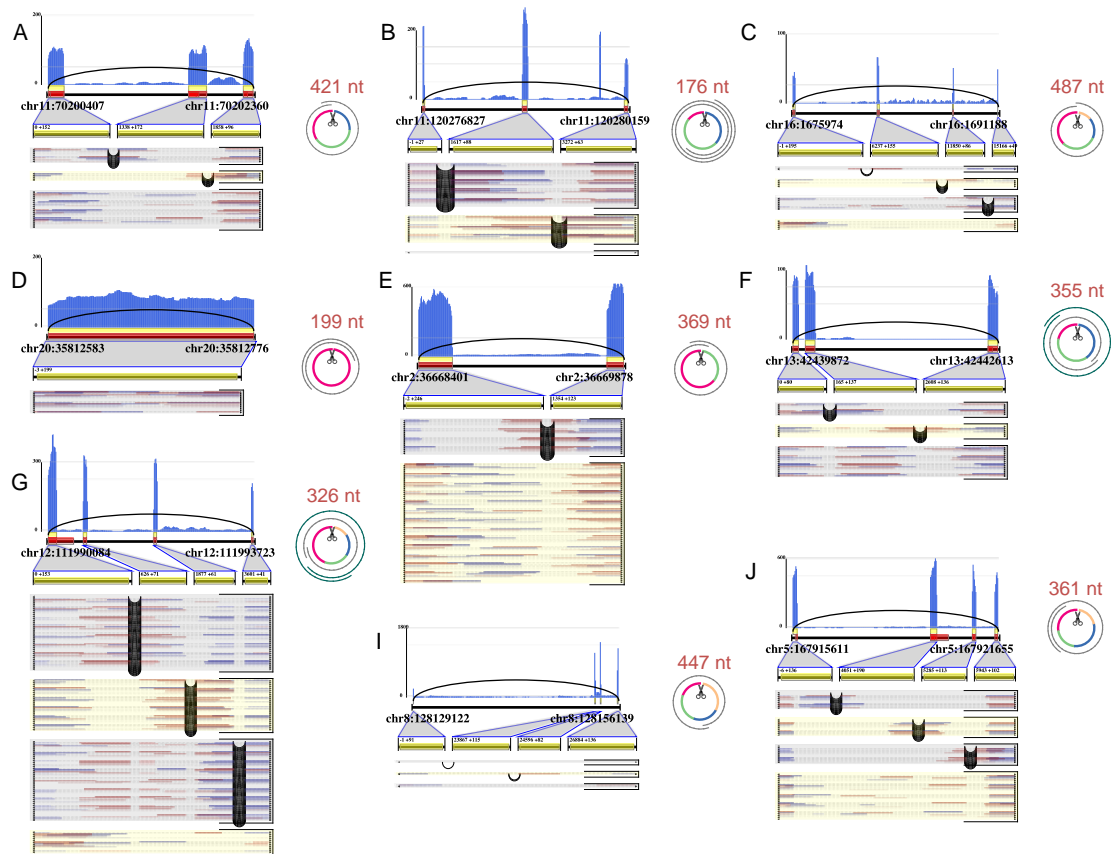


Supplementary Figure 1. Alternative splicing and circlexon detection in CIRI-AS. (A) Split-alignment of BSJ read pairs in detecting exon skipping, alternative 5'/3' splicing site and intron retention events. (B) Sequencing depth variation and non-BSJ read pairs are used to detect FSJs and circlexons missed by BSJ-read pairs. (C) Impact of library insert sizes and variances as well as sequencing depth on FSJ detection by BSJ read pairs.

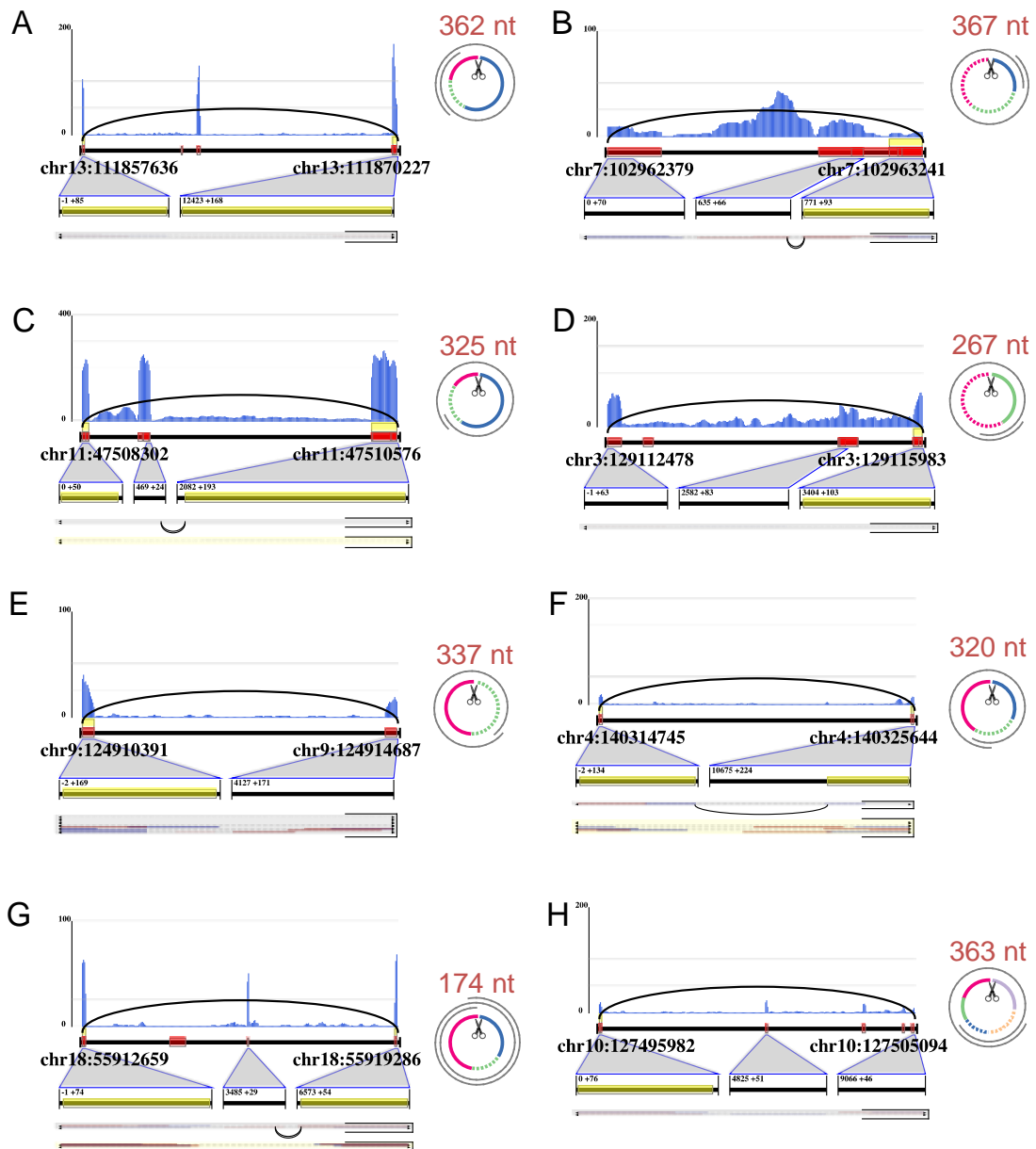


Supplementary Figure 2. Performances of CIRI-AS for cirexon detection on simulated data sets.

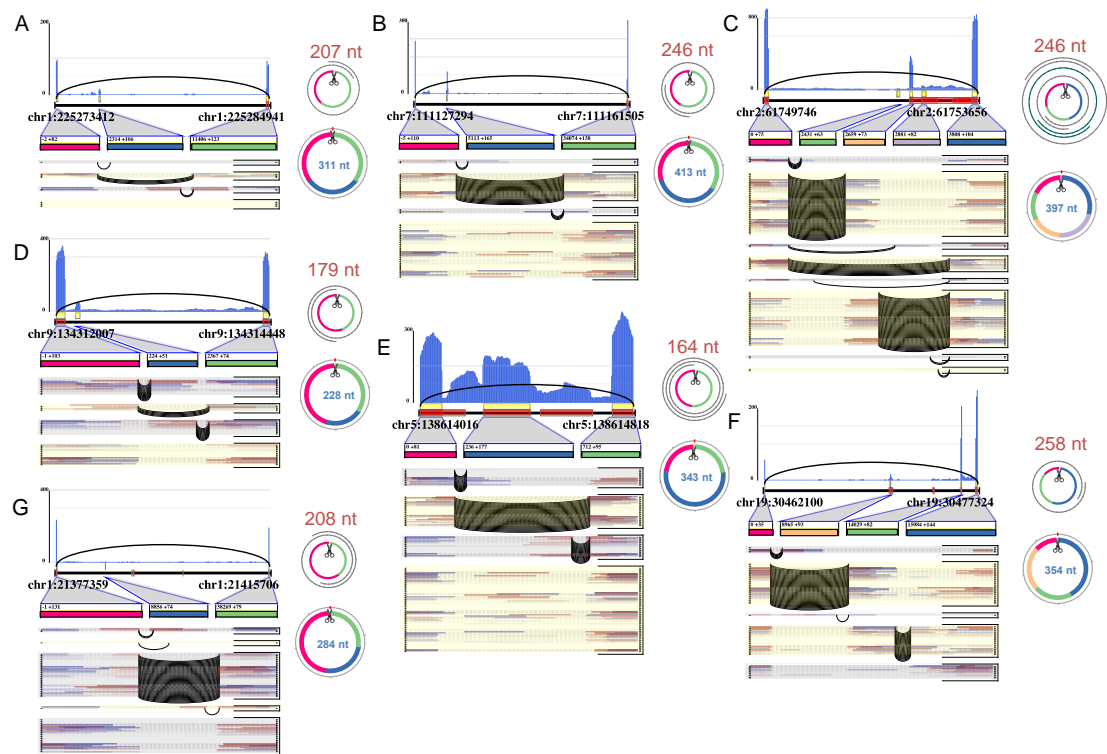
(A) Sensitivity and FDR for cirexon detection with varying sequencing depths in simulated data sets (PE100 bp) for four distributions of insert lengths. (B) Sensitivity and FDR for cirexon detection with varying sequencing depths in simulated data sets (read length distribution parameter: $\mu_1=280$ bp, $\sigma_1=80$ bp, $\mu_2=370$ bp, $\sigma_2=100$ bp, $p=0.9$) for four sequencing read lengths.



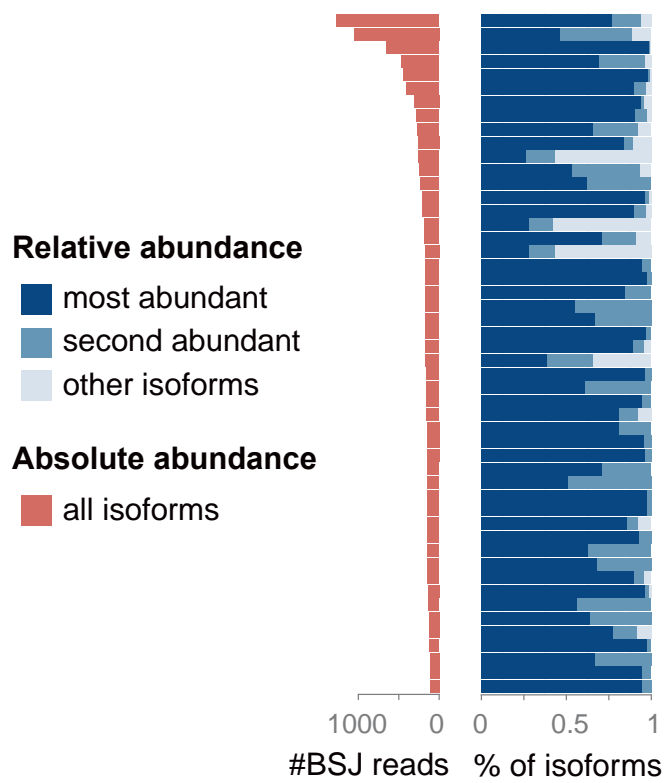
Supplementary Figure 3. Examples of validated circexons detected by CIRI-AS using long-read data. Detected circexons by CIRI-AS were shown on left and the corresponding components identified in long-read data were shown on right.



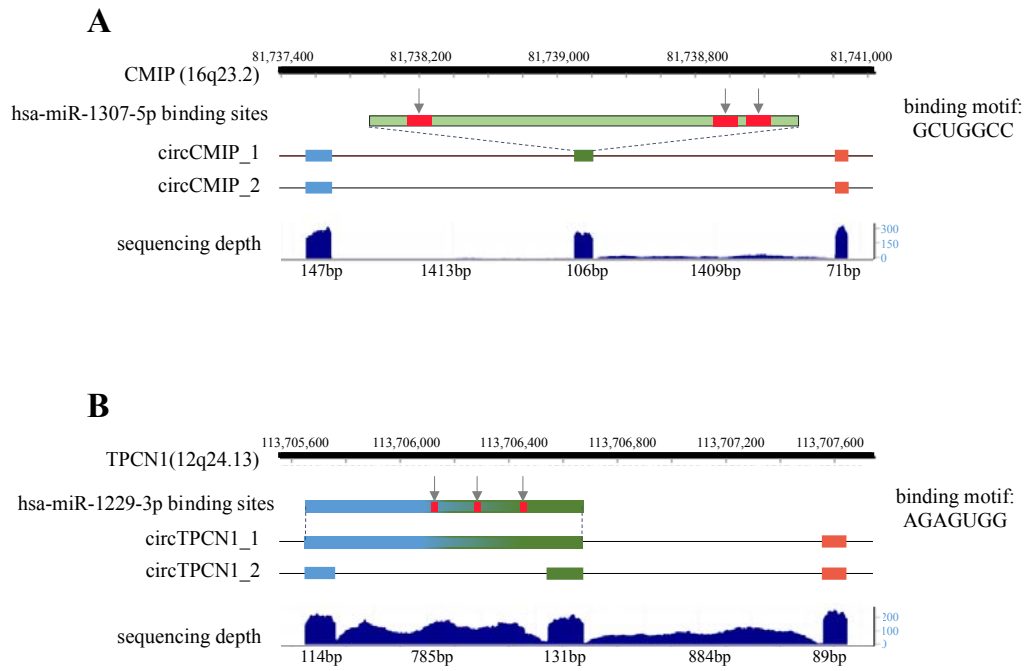
Supplementary Figure 4. Examples of circexons identified using long-read data but not detected by CIRI-AS using 2×100 bp paired reads. Detected circexons by CIRI-AS were shown on left and the corresponding components identified in long-read data were shown on right. Inner colored solid-line curves indicate the circexons detected by CIRI-AS, whereas the dashed-line curves represent the circexons missed by CIRI-AS.



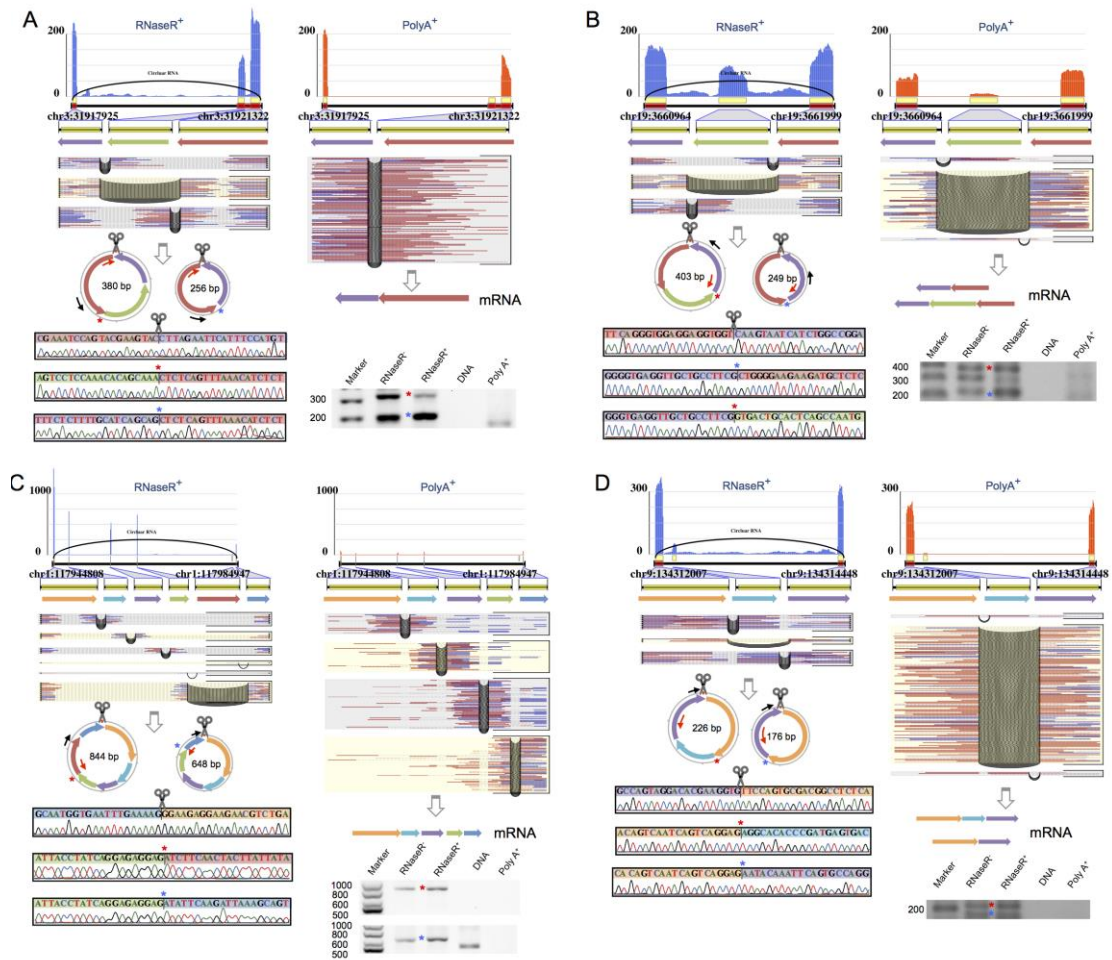
Supplementary Figure 5. Application of CIRI-AS on 2×100 bp paired reads can detect more isoforms than low-coverage long-read based identification. Detected cirexons by CIRI-AS were shown on left and the corresponding components identified in long-read data were shown on right. The internal compositions of alternatively splice transcripts not identified in long-read data were shown in the left bottom.



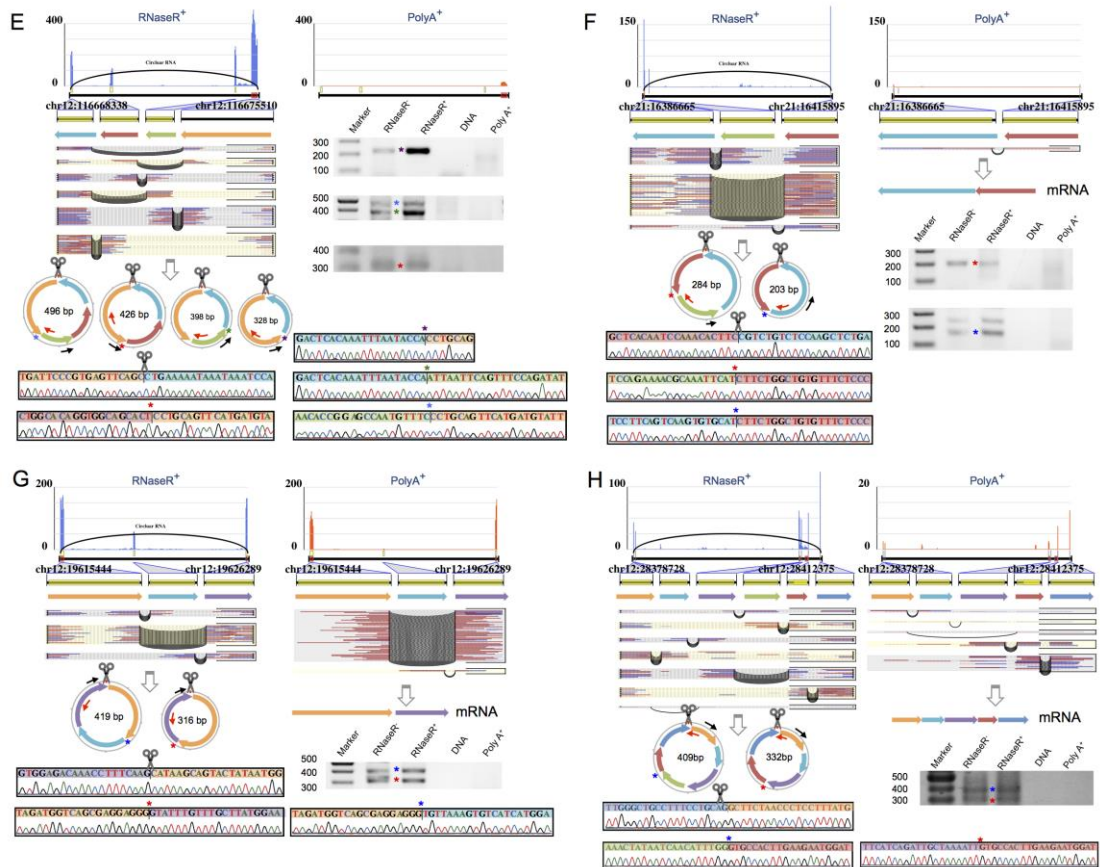
Supplementary Figure 6. The absolute abundances of top 50 most abundant alternatively spliced circRNAs as well as relative abundances of their AS transcripts detected in RNase R+ sample of HeLa cells.



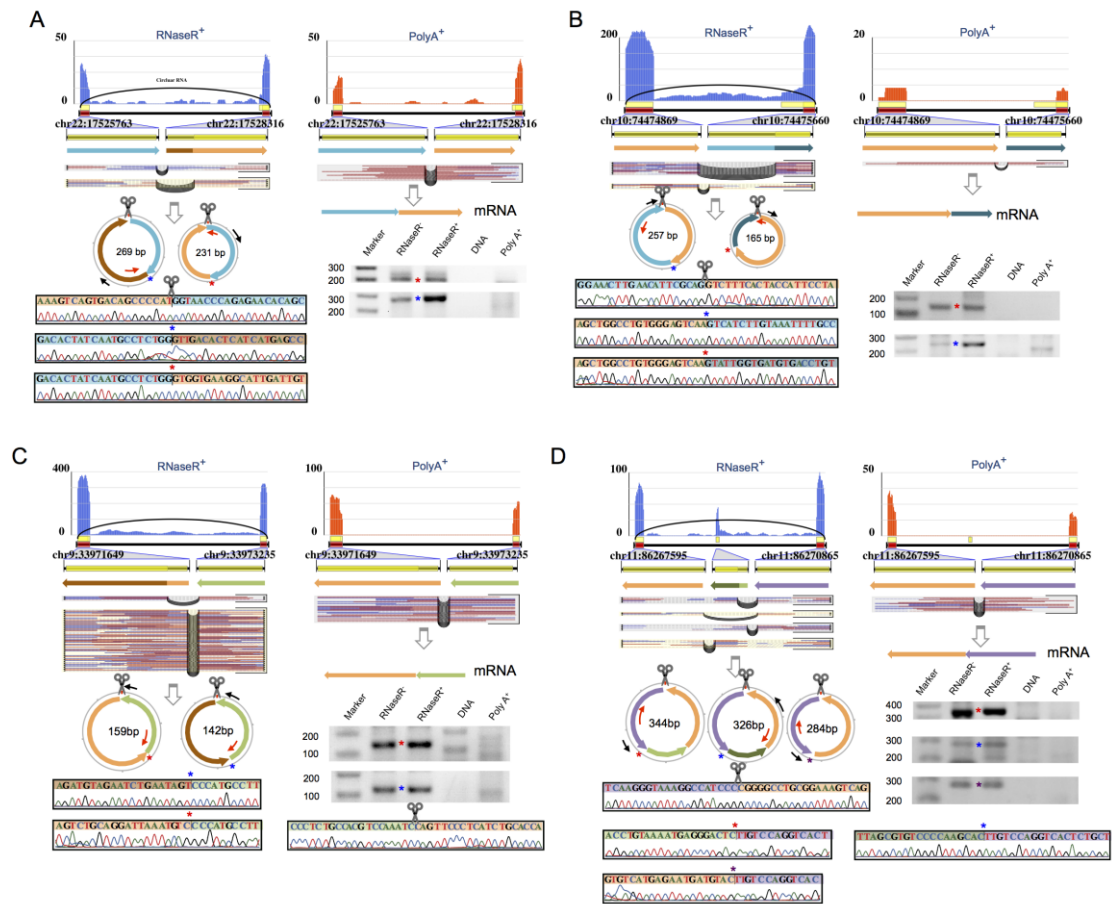
Supplementary Figure 7. Examples of the density of miRNA binding sites within circRNAs altered by AS events.



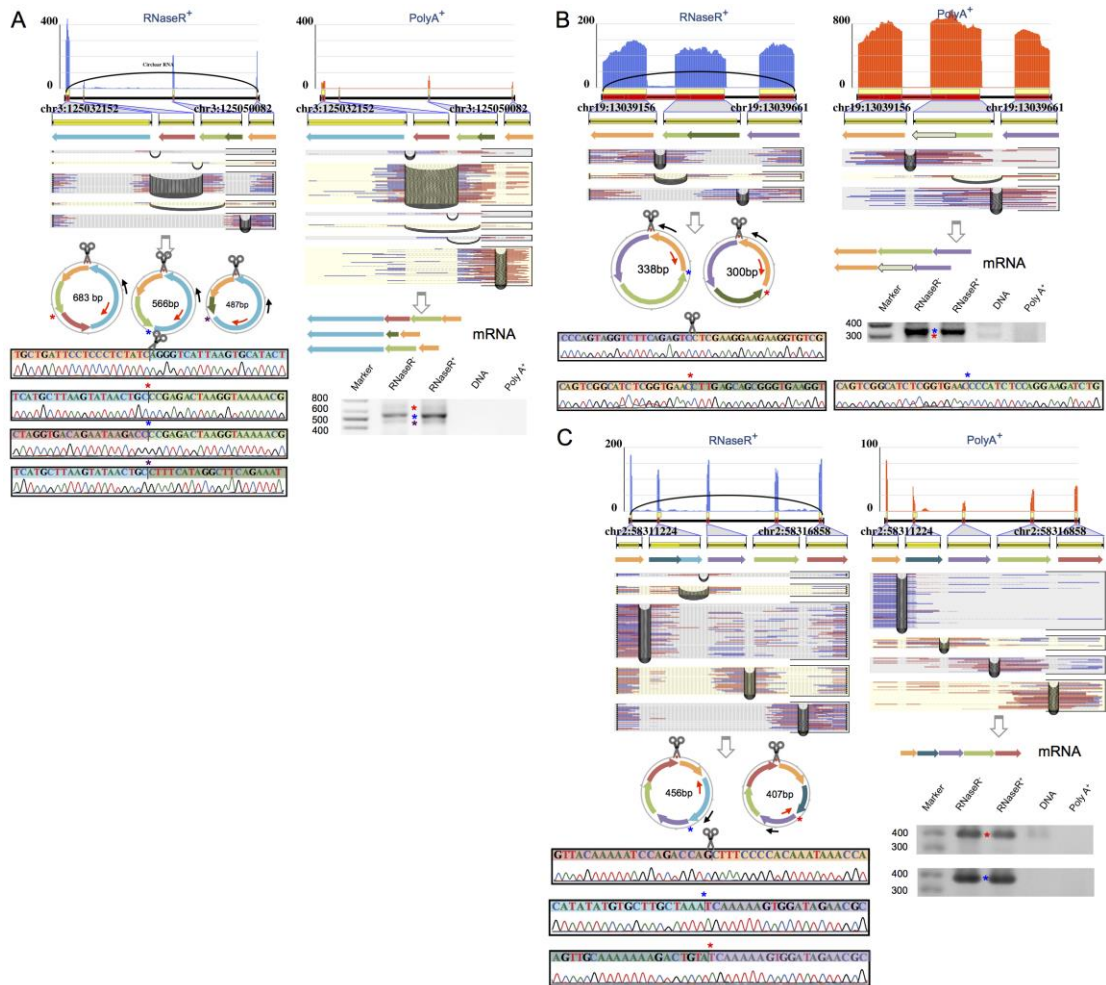
Supplementary Figure 8. Circular transcripts with exon skipping validated by RT-PCR and Sanger sequencing in HeLa cells. For the FSJs in transcripts with exon skipping, only related BSJ read pairs were shown. Reads in poly(A) selected sample of HeLa cells that indicate the corresponding splice junctions were shown in the right column.



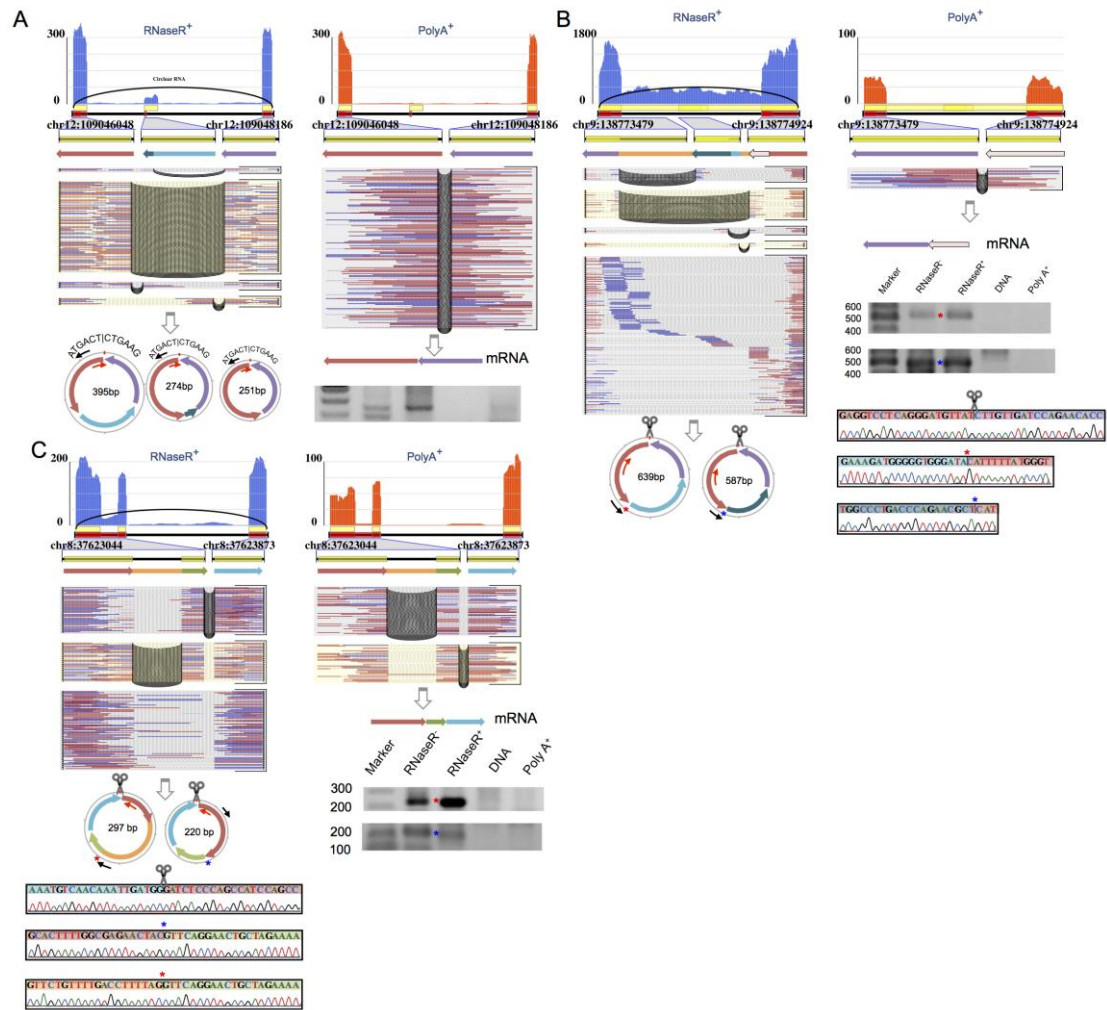
Supplementary Figure 8. (continued)



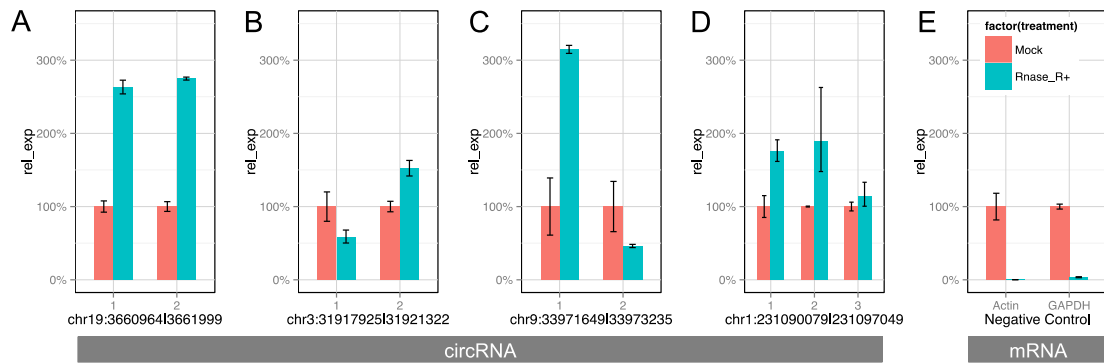
Supplementary Figure 9. Circular transcripts with alternative 3' splicing site validated by RT-PCR and Sanger sequencing in HeLa cells. For the FSJs in transcripts with alternative 3' splicing site, only related BSJ read pairs were shown. Reads in poly(A) selected sample of HeLa cells that indicate the corresponding splice junctions were shown in the right column.



Supplementary Figure 10. Circular transcripts with alternative 5' splicing site validated by RT-PCR and Sanger sequencing in HeLa cells. For the FSJs in transcripts with alternative 5' splicing site, only related BSJ read pairs were shown. Reads in poly(A) selected sample of HeLa cells that indicate the corresponding splice junctions were shown in the right column.

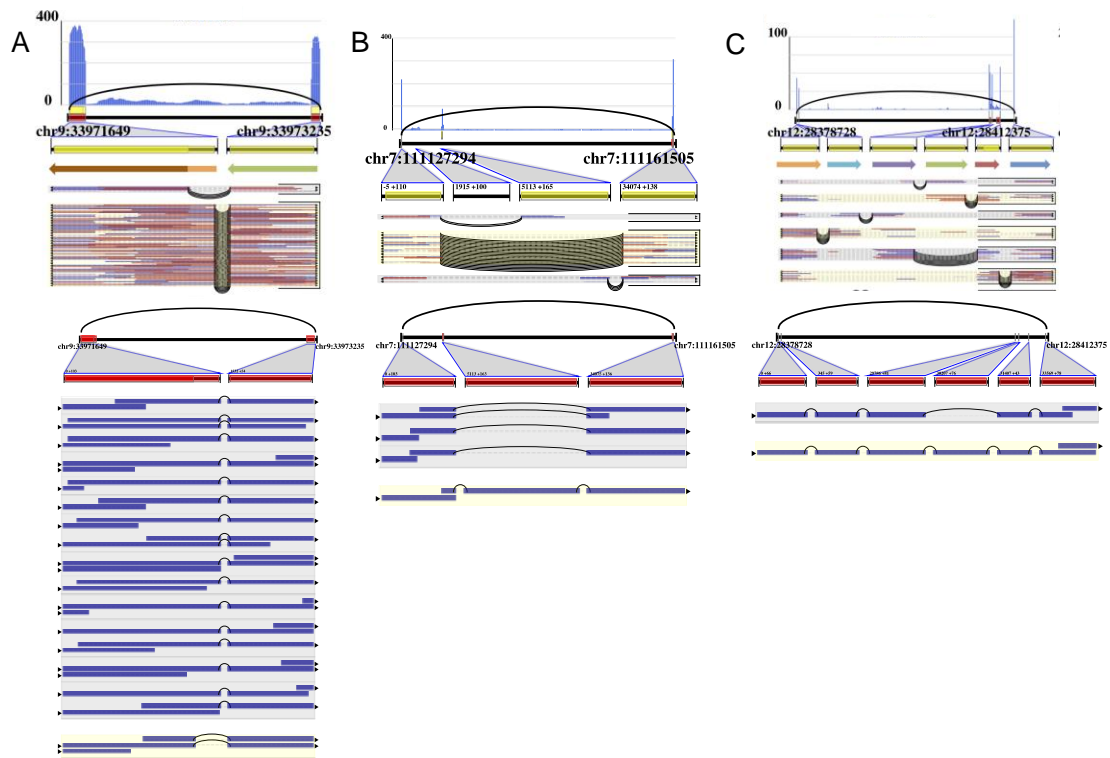


Supplementary Figure 11. Circular transcripts with intron retention validated by RT-PCR and Sanger sequencing in HeLa cells. For the FSJs and retained introns in the transcripts, only related BSJ read pairs were shown. Reads in poly(A) selected sample of HeLa cells that indicate the corresponding splice junctions were shown in the right column.

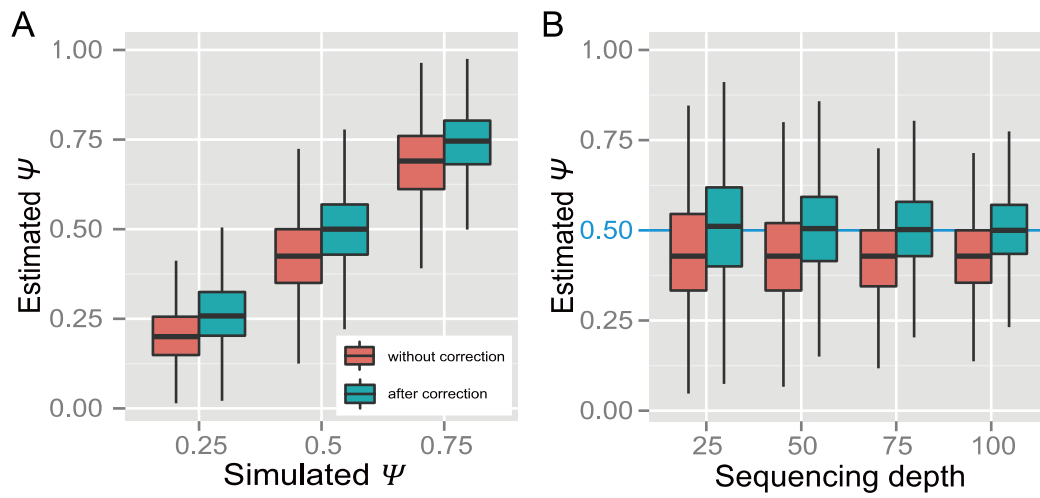


Supplementary Figure 12. Resistance of circular AS isoforms to RNase R quantified by qRT-PCR.

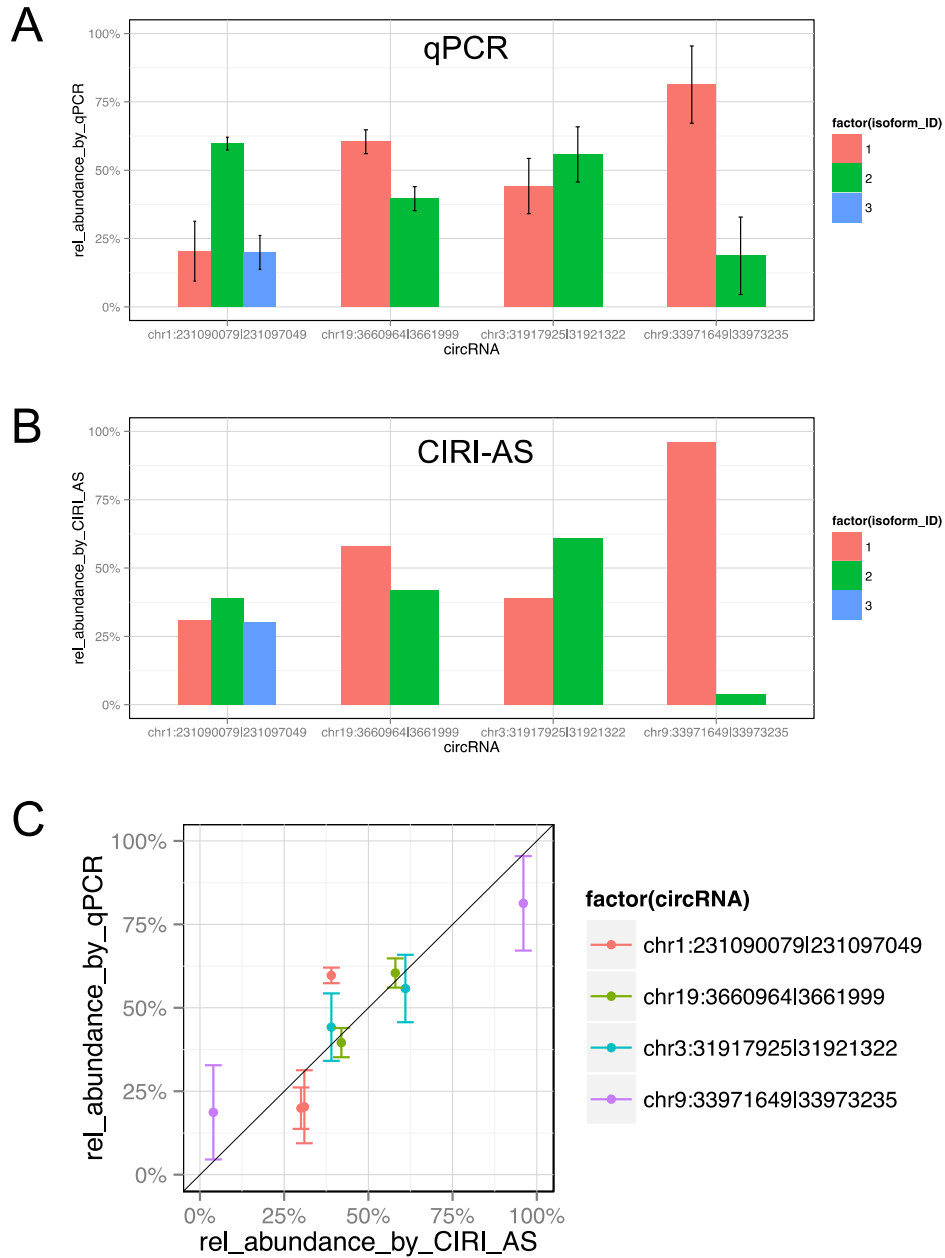
(A-D) Nine circular AS isoforms of four circRNA loci (n = 3; mean ± SE). (E) Linear control of GAPDH and Actin (n = 3; mean ± SE). Red bars represent total RNA samples, whereas blue bars represent RNase R-treated RNA samples.



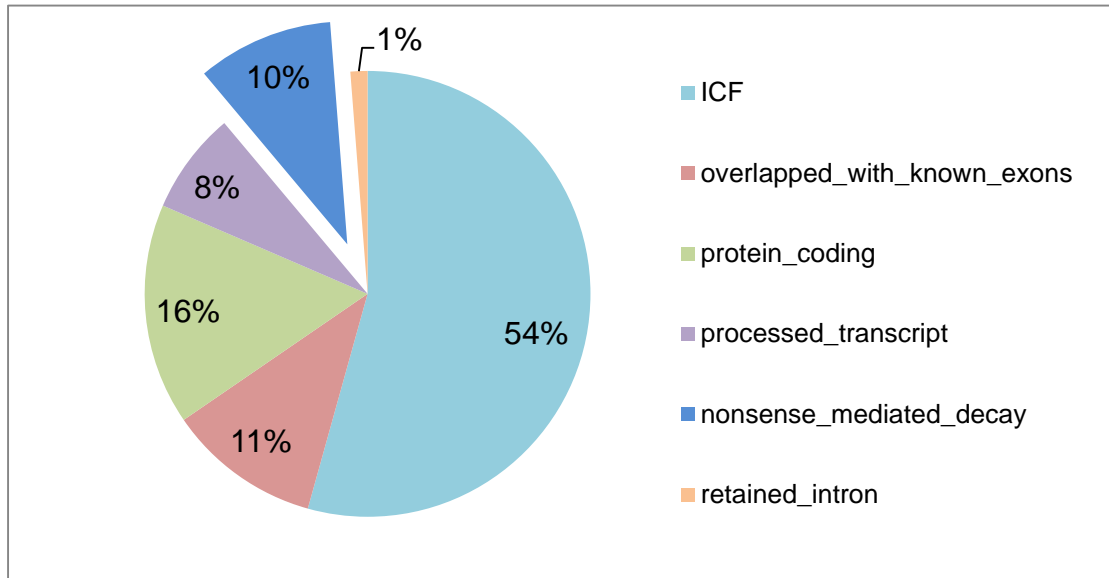
Supplementary Figure 13. Three examples of circular AS isoforms detected by both CIRI-AS (upper panel) and long-read sequencing (lower panel). In the lower panel, blue lines indicate the long reads that can cover the full length of circRNAs. Curved lines indicate the splicing junctions. Black triangles indicate BSJs located in the long reads.



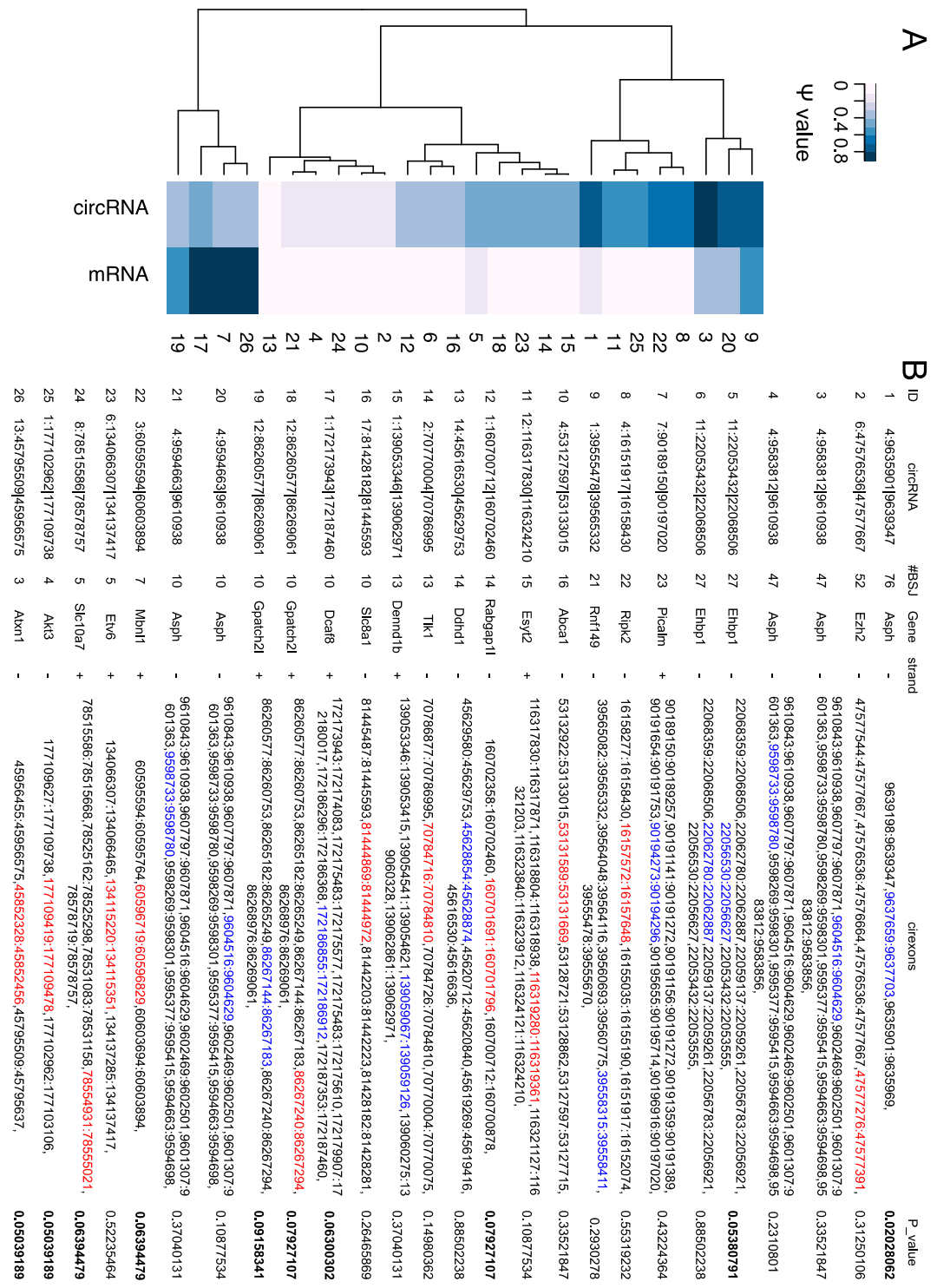
Supplementary Figure 14. Performances of CIRI-AS for relative abundance estimation on simulated data sets. (A) Estimated Ψ values without correction or after correction in CIRI-AS for exon skipping within simulated circRNAs with three simulated Ψ values. (B) Estimated Ψ values without correction or after correction in CIRI-AS for exon skipping within simulated circRNAs with four sequencing depths.



Supplementary Figure 15. Relative abundance of nine AS isoforms in four circRNA loci estimated by qRT-PCR and CIRI-AS. (A) Relative abundance of nine AS isoforms in four circRNA loci estimated by qRT-PCR (n = 4; mean \pm SE); (B) Relative abundance of nine AS isoforms in four circRNA loci estimated by CIRI-AS; (C) Comparison of relative abundances estimated by CIRI-AS and qRT-PCR (n = 4; mean \pm SE).

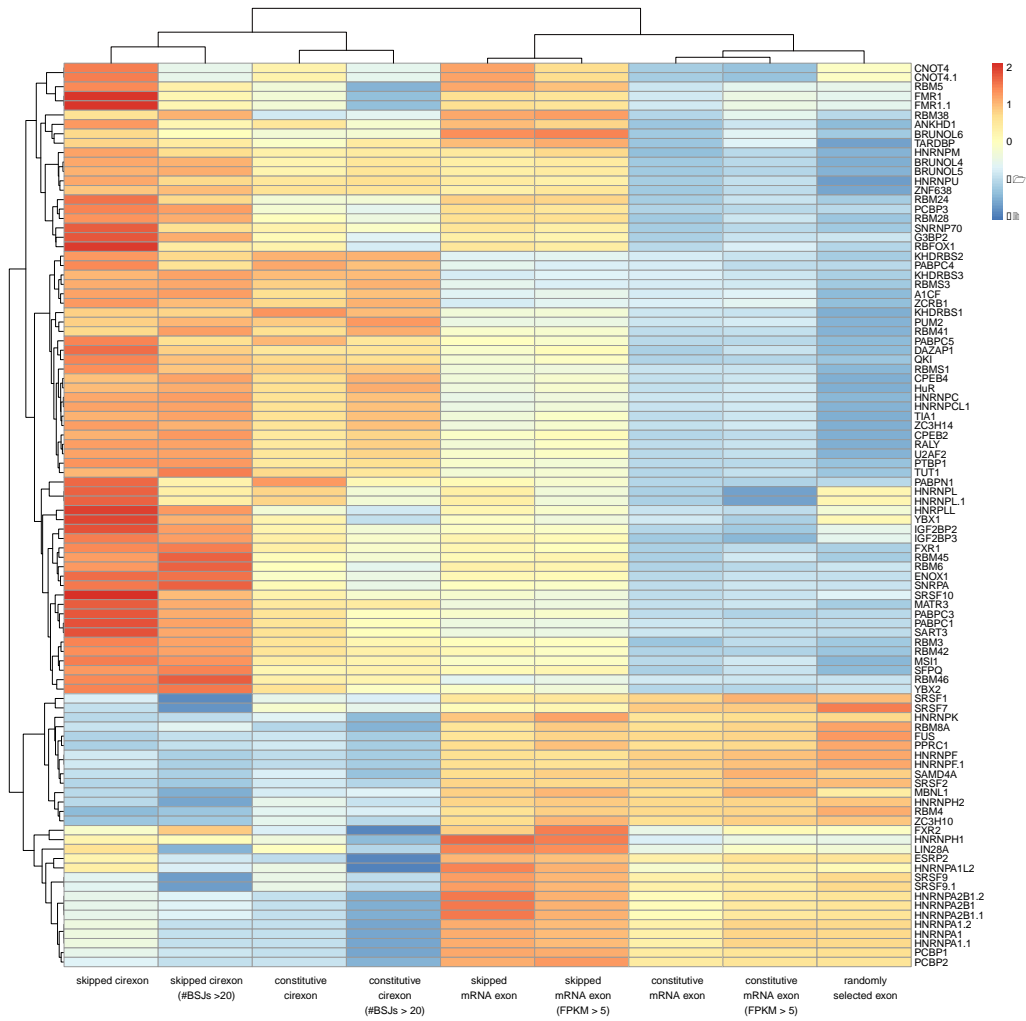


Supplementary Figure 16. Compositions of circexons with higher relative abundance in circRNAs compared to mRNAs in HeLa and HEK293. Circexon classification is based on the Gencode annotation.

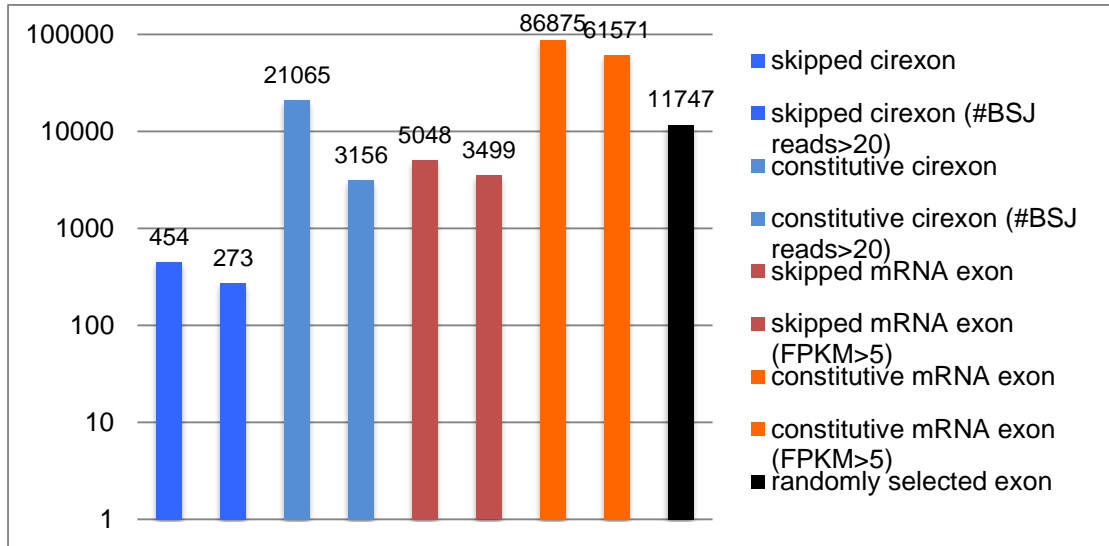


Supplementary Figure 17. Exon skipping events detected in mouse immune dendritic cells with 10 min labeling pulse of 4-thiouridine. (A) Comparison of Ψ value for exon skipping events between circRNA and mRNA. (B) Information about skipped circexons including read counts, parent gene names of circRNAs, position of circexons as well as P values after correction for multiple testing (corrected P values <0.10 are shown in bold). Skipped circexons classified as ICFs are shown in red, and other skipped circexons in blue.

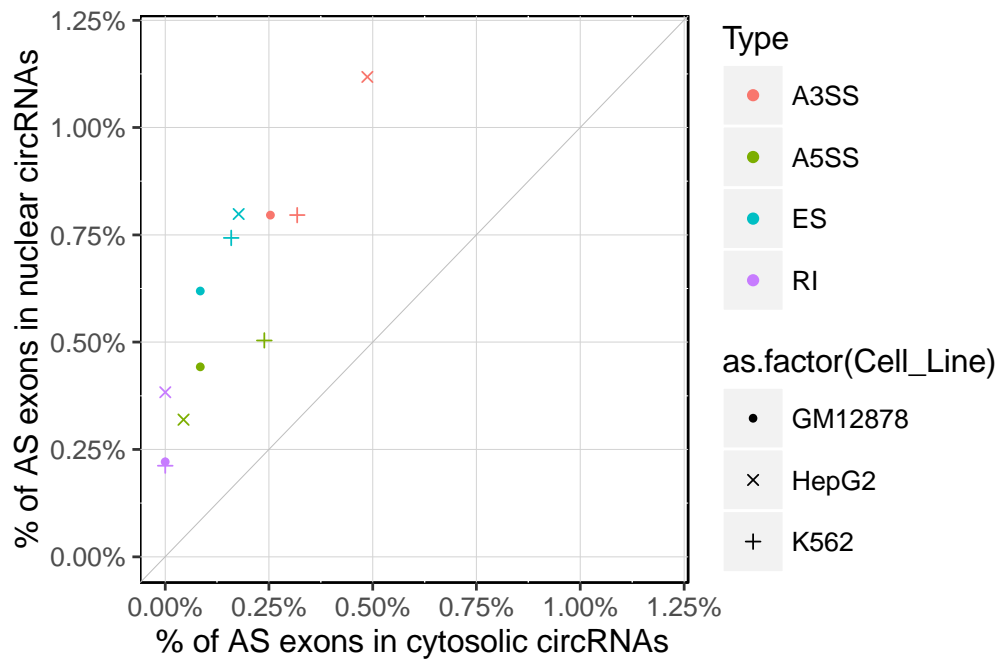
RNA Binding Protein Binding Sites



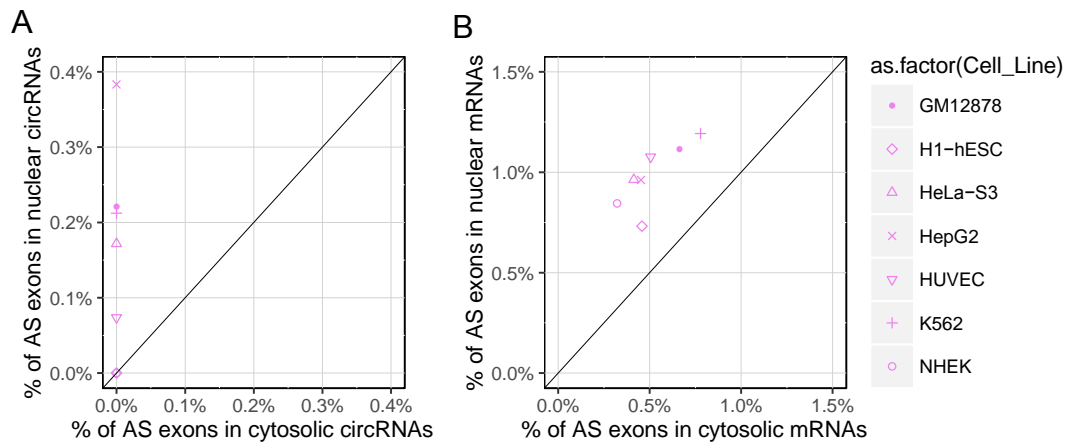
Supplementary Figure 18. Comparison of binding site density of RNA binding proteins on skipped cirexons, constitutive cirexons, skipped mRNA exons in HeLa cells and randomly selected annotated exons. The binding numbers were normalized by corresponding exon length to calculate average binding density, which was shown in the heatmap after Z-score normalization.



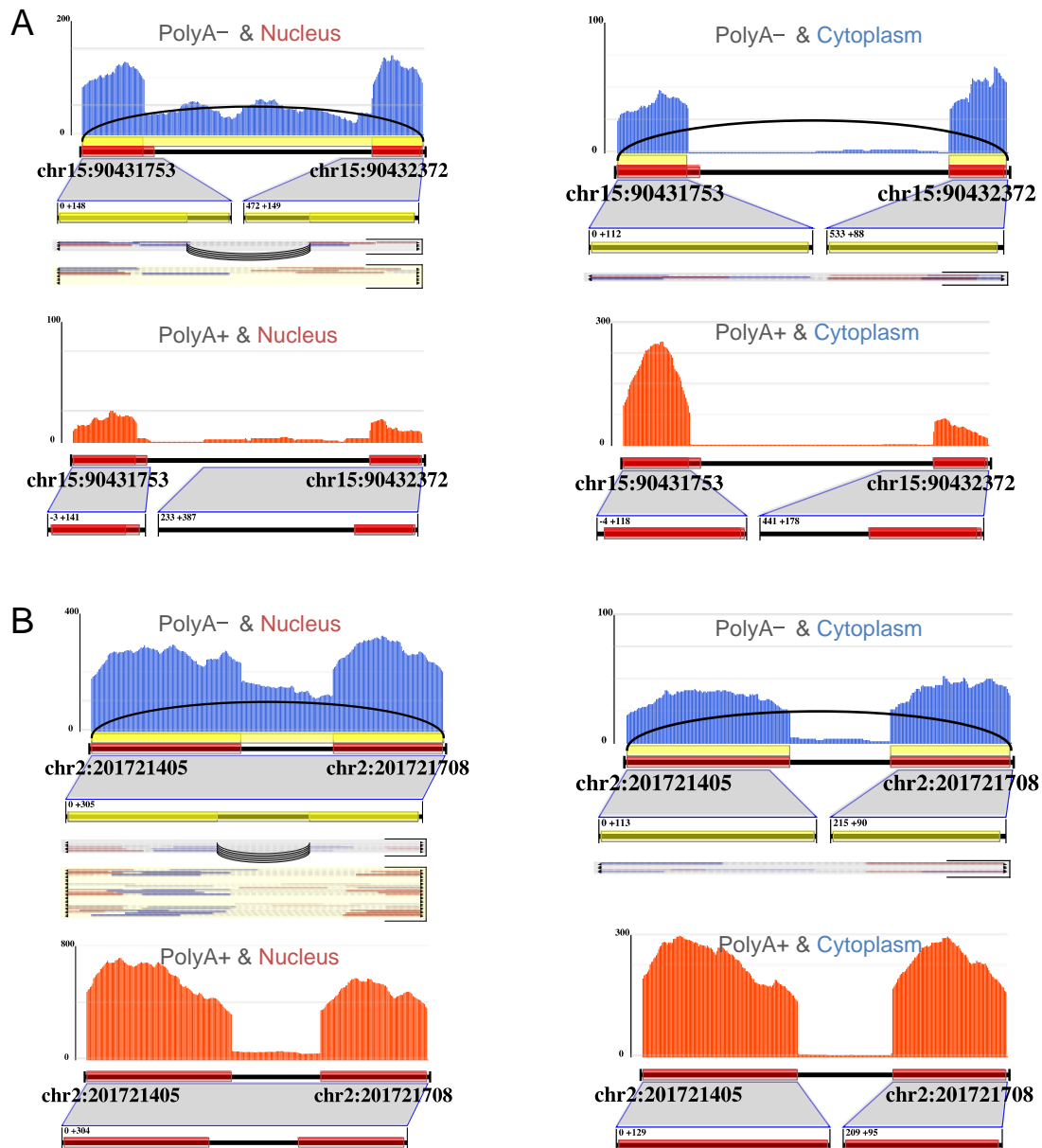
Supplementary Figure 19. Total number of exons in each group used for splicing factor binding site prediction. Colors of each group correspond to those in Fig 3A.



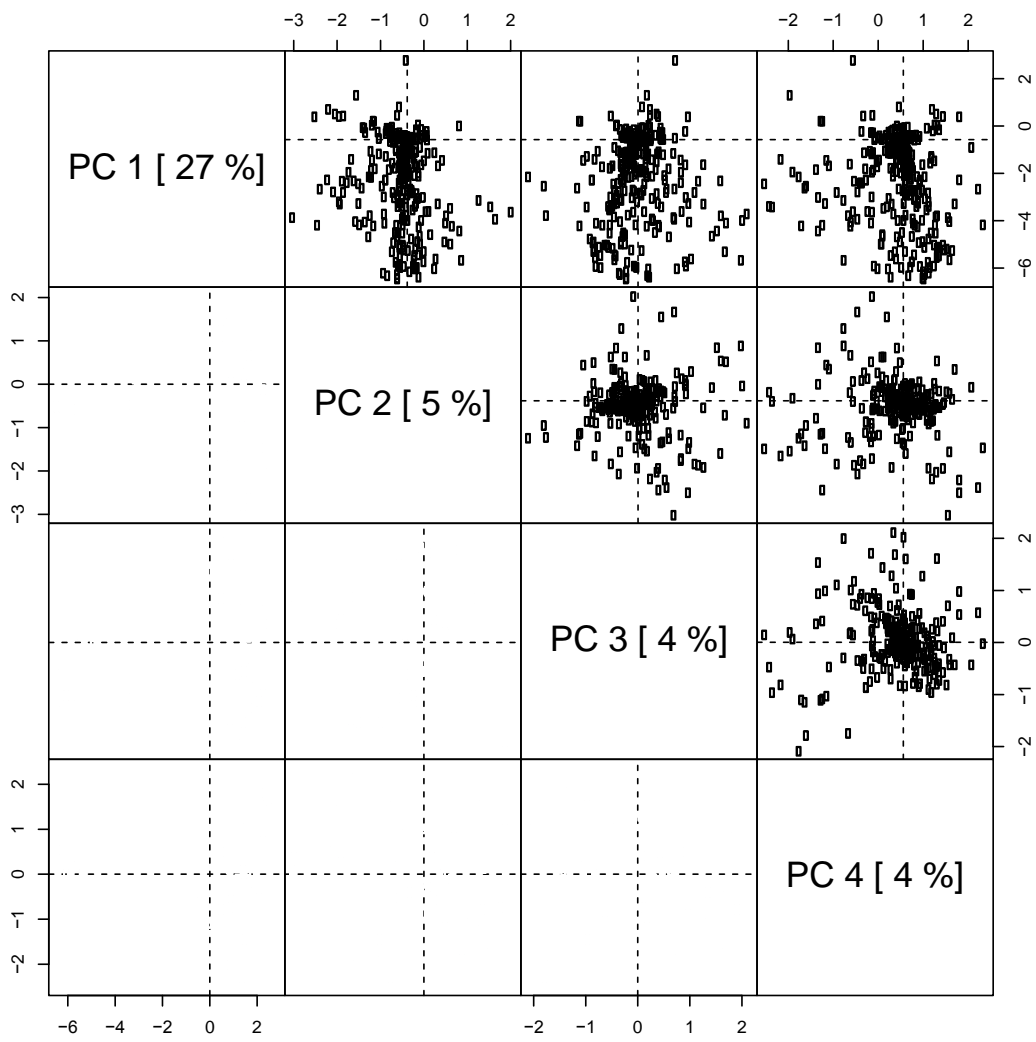
Supplementary Figure 20. A comparison between nucleus and cytoplasm for percentage of four types of alternatively spliced circexons in HepG2, K562 and GM12878 cell lines. All of the three cell lines have comparable sequencing data size in nucleus and cytoplasm.



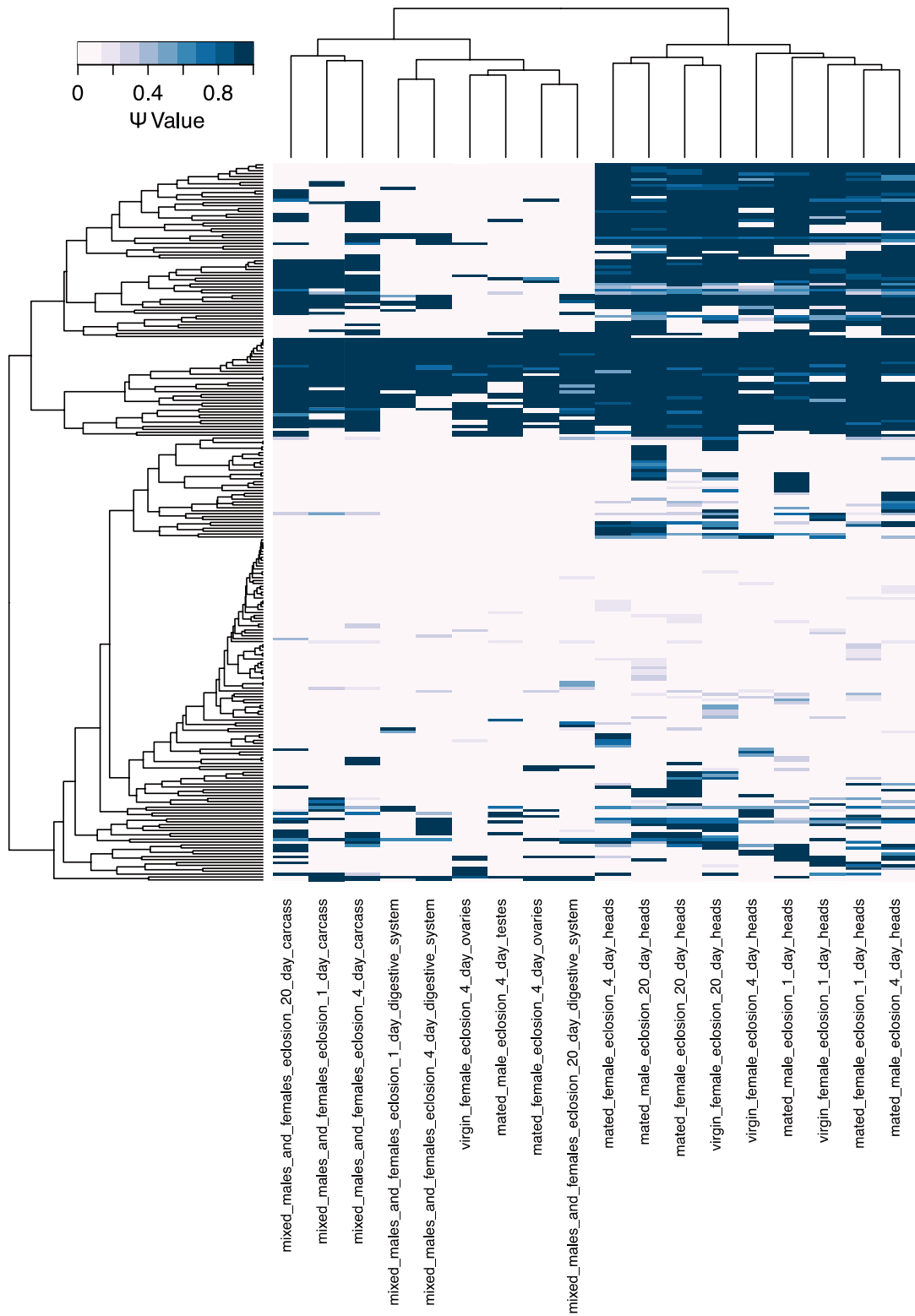
Supplementary Figure 21. A comparison between nucleus and cytoplasm for percentage of retained intron in seven cell lines. (A) percentage of retained intron in circRNAs; (B) percentage of retained intron in mRNAs.



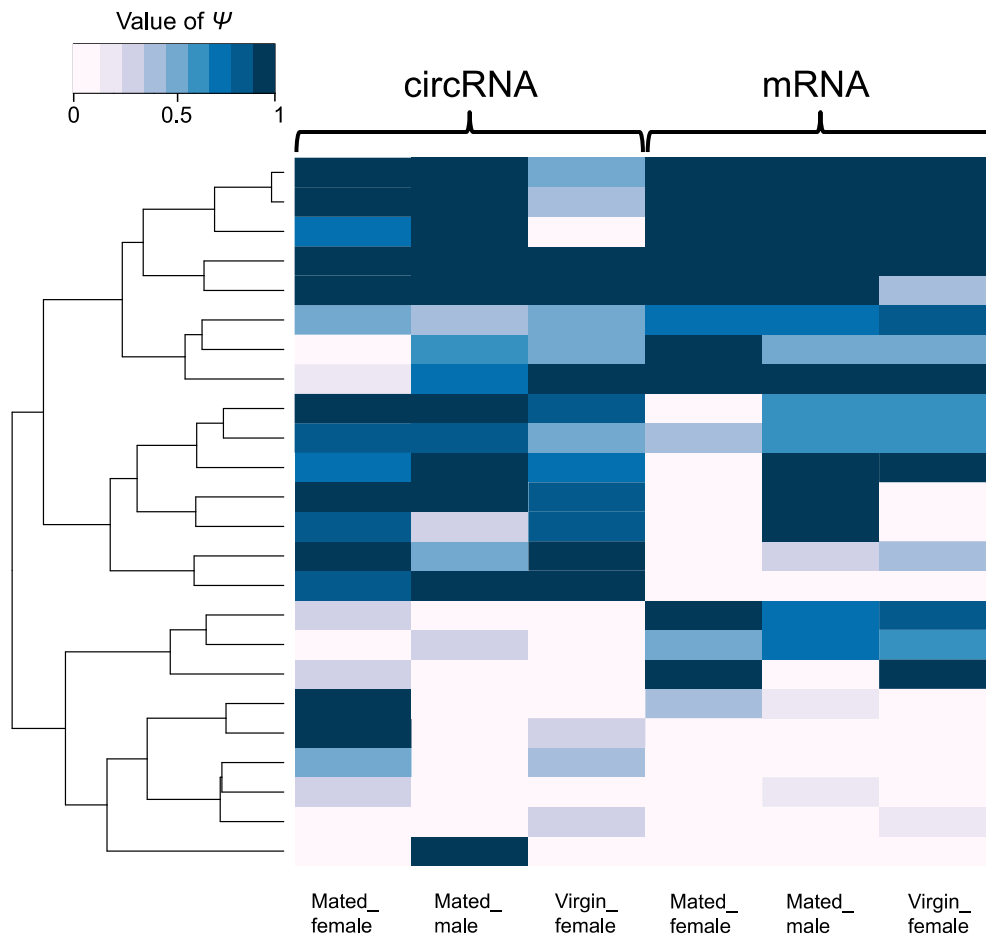
Supplementary Figure 22. Examples of intron retention within circRNA observed in poly(A)⁻ sequencing data set of nucleus but not in cytoplasm. Sequencing depths of mRNA observed in corresponding poly(A) selected sequencing data sets of both nucleus and cytoplasm are shown. (A) Retained intron 2 in circAP3S2; (B) Retained intron 8 in circCLK1.



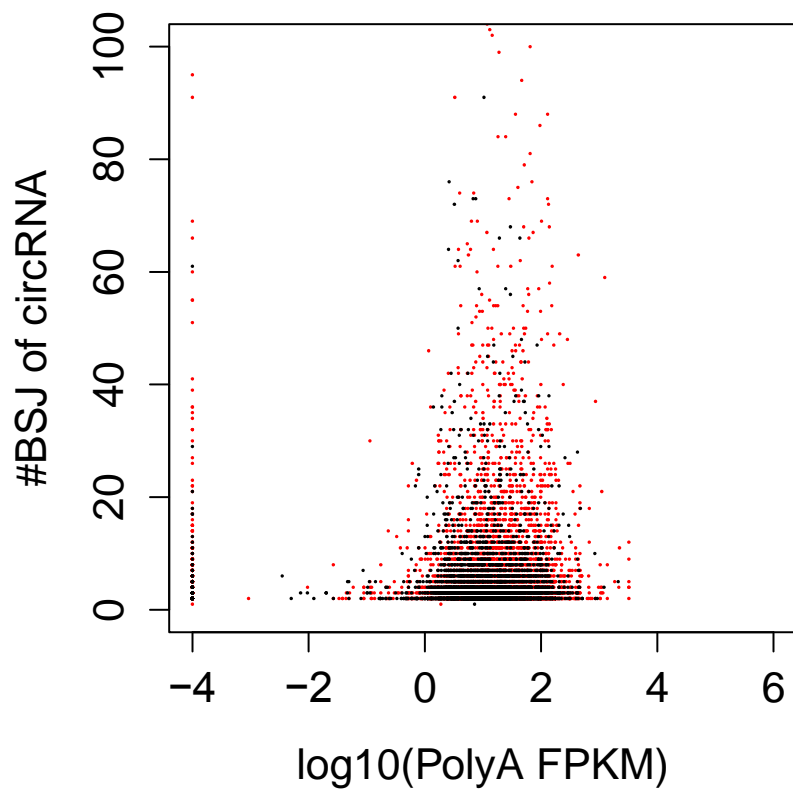
Supplementary Figure 23. Principal component analysis based on the Ψ values of the AS cirexons in fruit fly. Each dot represents a sample and its color corresponds to the sample type on the top of Figure 4A. The first four principal components were displayed.



Supplementary Figure 24. Relative abundance of alternatively spliced circexons in 18 fruit fly eclosion samples.



Supplementary Figure 25. Relative abundance of skipped circexons detected in circRNAs compared with corresponding mRNAs in three fruit fly samples (eclosion + 1 day, heads).



Supplementary Figure 26. Number of BSJ reads for circRNAs and FPKM of the corresponding linear RNAs in HEK293 and HeLa cell lines. Red dot: HeLa cell line; Black dot: HEK293 cell line.

Supplementary Table 1. Data sets of human samples used in this study for circexon/exon detection

Cell type	Treatment	Read	Insert size (bp)	Data (Gb)	# BSJs	# circexons	# ICFs	Data source (SRA accession #)
HeLa	RiboMinus	PE100	148.8±48.6	21.5	6,606	11,325	921	This study (SRR1637089, SRR1637090 & SRR3476958)
HeLa	RiboMinus & RNase R+	PE100	224.6±97.2	17.6	15,631	33,241	2,755	This study (SRR1636985, SRR1636986 & SRR3476956)
HeLa	PolyA+	PE100	216.9±109.2	2.9	n/a	n/a	n/a	This study (SRR3479116)
HEK293	RiboMinus	PE150	236±86.7	9.3	3,916	8,847	499	This study (SRR3479243)
HEK293	RiboMinus & RNase R+	PE150	250.3±103.2	12.7	13,465	33,650	2,453	This study (SRR3479244)
HEK293	PolyA+	PE150	268.7±103.5	3.9	n/a	n/a	n/a	This study (SRR3479143)
Hs68	RiboMinus	PE100	103.3±20.3	31.4	2,532	3,916	659	Jeck et al., 2013 (SRR444655)
Hs68	RiboMinus	PE100	174.6±65.7	41.3	4,387	7,475	1,089	Jeck et al., 2013 (SRR444975)
Hs68	RiboMinus & RNase R+	PE100	181.89±52.7	40.0	26,685	54,785	6,735	Jeck et al., 2013 (SRR445016)
Hs68	RiboMinus & RNase R+	PE100	179.2±66.3	31.7	26,537	53,974	5,702	Jeck et al., 2013 (SRR444974)

Supplementary Table 2. Exon skipping in top 50 most abundant circRNAs of HeLa cells.

circRNA	circexon	Psi value	#BSJ reads
chr9:138773479 138774924	138774061:138774274	0.032	1275
chr9:138773479 138774924	138774061:138774222	0.04	1275
chr2:61749746 61761038	61752627:61752656	0.012	468
chr2:61749746 61761038	61752405:61752464	0.012	468
chr2:61749746 61761038	61756201:61756294	0.005	468
chr2:61749746 61761038	61752405:61752468	0.279	468
chr2:61749746 61761038	61752177:61752238	0.012	468
chr21:46548317 46554747	46552130:46552221	0.008	441
chr21:46548317 46554747	46553474:46553580	0.011	441
chr3:196118684 196129890	196127500:196127603	0.018	311
chr3:196118684 196129890	196120421:196120490	0.019	311
chr3:196118684 196129890	196127272:196127603	0.018	311
chr3:196118684 196129890	196120425:196120490	0.975	311
chr7:99621042 99621930	99621175:99621555	0.975	285
chr15:25650608 25657118	25652214:25652379	0.019	271
chr15:25650608 25657118	25653719:25653831	0.019	271
chr15:25650608 25657118	25652214:25652284	0.556	271
chr15:25650608 25657118	25653767:25653831	0.481	271
chr15:25650608 25657118	25652214:25652409	0.111	271
chr21:17135210 17138460	17136557:17136616	0.206	260
chr21:17135210 17138460	17137101:17137203	0.4	260
chr21:17135210 17138460	17137124:17137203	0.257	260
chr21:17135210 17138460	17137093:17137203	0.029	260
chr3:149563798 149639014	149619859:149619949	0.895	243
chr5:32135678 32143986	32139658:32139818	0.02	209
chr12:109046048 109048186	109046814:109046957	0.069	207
chr12:109046048 109048186	109046814:109046836	0.03	207
chr8:128152989 128186609	128176683:128176771	0.029	184
chr8:128152989 128186609	128174167:128174329	0.81	184
chr8:128152989 128186609	128153719:128153816	0.951	184
chr8:128152989 128186609	128161042:128161159	0.286	184
chr8:128152989 128186609	128161042:128161163	0.143	184
chr8:128152989 128186609	128181143:128181362	0.171	184
chr2:61749746 61753656	61752177:61752201	0.015	180
chr2:61749746 61753656	61752405:61752468	0.776	180
chr2:61749746 61753656	61752627:61752694	0.03	180
chr2:61749746 61753656	61752177:61752238	0.015	180
chr2:61749746 61753656	61752627:61752709	0.03	180
chr8:128152989 128161917	128161042:128161163	0.2	174
chr8:128152989 128161917	128161042:128161159	0.52	174
chr8:128152989 128161917	128153719:128153816	0.855	174
chr8:141874411 141900868	141889570:141889736	0.973	172
chr4:186168448 186188309	186176837:186176896	0.449	169
chr3:142455221 142467302	142462327:142462428	0.667	167
chr9:96233423 96238620	96236515:96236612	0.029	166
chr12:116668338 116675510	116674626:116674695	0.66	166
chr12:116668338 116675510	116669864:116669961	0.586	166
chr10:7285520 7327916	7318854:7318951	0.931	166
chr21:38792601 38794168	38793237:38793297	0.038	160
chr3:31917925 31921322	31920922:31921045	0.612	158
chr3:125032152 125050082	125033781:125033897	0.08	154

chr21:16386665 16415895	16387466:16387546	0.187	152
chr1:117944808 117984947	117983937:117984132	0.026	148
chr5:138614016 138614818	138614254:138614429	0.509	148
chr22:46125305 46136418	46132848:46132963	0.137	146
chr20:57014001 57016139	57014202:57014364	0.37	145
chr9:114148657 114154104	114152264:114152381	0.68	144
chr17:80858527 80869665	80861281:80861354	0.937	141
chr17:80858527 80869665	80865637:80865697	0.958	141
chr5:179688684 179707608	179706796:179707036	0.64	126
chrX:24190832 24197887	24191013:24191050	0.03	124
chrX:24190832 24197887	24193506:24193560	0.135	124
chrX:24190832 24197887	24191724:24191840	0.059	124
chr5:176370336 176385155	176378475:176378578	0.949	115
chr7:24663285 24708279	24690104:24690331	0.333	115
chr7:24663285 24708279	24689231:24689383	0.333	115
chr19:3660964 3661999	3661371:3661524	0.424	112
chr11:128993341 129034322	128993783:128993824	0.903	111
chr11:128993341 129034322	128997107:128997200	0.235	111
chr20:30954187 30959677	30957627:30957686	0.046	110
chr13:61013822 61041513	61018817:61018882	0.871	109
chr13:61013822 61041513	61034514:61034674	0.935	109
chr13:61013822 61041513	61018821:61018882	0.097	109
chr10:93185038 93221941	93221011:93221113	0.85	106
chr9:33953283 33963789	33956077:33956144	0.644	106
chr6:159004986 159010814	159007791:159007882	0.048	105
chr2:240929491 240946787	240942466:240942557	0.086	101
chr2:240929491 240946787	240942466:240942553	0.029	101
chr2:240929491 240946787	240939600:240939804	0.296	101
chr2:240929491 240946787	240945165:240945258	0.024	101
chr9:98740343 98766983	98759528:98759695	0.078	101
chr21:42598193 42629253	42599114:42599149	0.02	97
chr21:42598193 42629253	42622679:42622828	0.882	97
chr12:19615444 19626289	19619682:19619784	0.355	97
chr14:97299804 97327072	97322467:97322587	0.5	96
chr9:126519982 126641300	126554866:126554909	0.872	95
chr11:46098305 46113774	46112006:46112117	0.3	93
chr21:46538479 46554747	46540709:46540874	0.889	93
chrX:53672263 53681075	53675516:53675593	0.119	93
chr8:141856359 141900868	141889570:141889736	0.455	91
chr8:141856359 141900868	141874411:141874498	0.889	91

Supplementary Table 3. Alternative 5'/3' splicing site events in top 50 most abundant circRNAs of HeLa cells.

circRNA	cirexon	Psi value	#BSJ reads
chr9:138773479 138774924	138774061:138774274	0.032	1275
chr9:138773479 138774924	138774061:138774222	0.04	1275
chr9:138773479 138774924	138774854:138774924	0.008	1275
chr9:138773479 138774924	138774662:138774924	0.992	1275
chr1:247318868 247323115	247318868:247318997	0.958	1055
chr1:247318868 247323115	247318868:247318927	0.042	1055
chr10:7318854 7327916	7318854:7318951	0.995	656
chr10:7318854 7327916	7325866:7325921	0.99	656
chr10:7318854 7327916	7318854:7318934	0.005	656
chr10:7318854 7327916	7325866:7326112	0.01	656
chr2:61749746 61761038	61752405:61752468	0.279	468
chr2:61749746 61761038	61753555:61753656	0.988	468
chr2:61749746 61761038	61752177:61752238	0.012	468
chr2:61749746 61761038	61753597:61753656	0.012	468
chr2:61749746 61761038	61752405:61752464	0.012	468
chr5:619105 620376	619105:619185	0.007	405
chr5:619105 620376	620184:620376	0.932	405
chr5:619105 620376	619105:619232	0.993	405
chr5:619105 620376	620251:620376	0.068	405
chr3:196118684 196129890	196127500:196127603	0.018	311
chr3:196118684 196129890	196127272:196127603	0.018	311
chr3:196118684 196129890	196120421:196120490	0.019	311
chr3:196118684 196129890	196120425:196120490	0.975	311
chr15:25650608 25657118	25653767:25653831	0.481	271
chr15:25650608 25657118	25652214:25652409	0.111	271
chr15:25650608 25657118	25652214:25652379	0.019	271
chr15:25650608 25657118	25652214:25652284	0.556	271
chr15:25650608 25657118	25653719:25653831	0.019	271
chr5:167915607 167921655	167919664:167919826	0.056	264
chr5:167915607 167921655	167920924:167921007	0.056	264
chr5:167915607 167921655	167919664:167919852	0.889	264
chr5:167915607 167921655	167919664:167919796	0.056	264
chr5:167915607 167921655	167920899:167921007	0.944	264
chr21:17135210 17138460	17137124:17137203	0.257	260
chr21:17135210 17138460	17138344:17138460	0.029	260
chr21:17135210 17138460	17137101:17137203	0.4	260
chr21:17135210 17138460	17138316:17138460	0.829	260
chr21:17135210 17138460	17137093:17137203	0.029	260
chr21:17135210 17138460	17138321:17138460	0.143	260
chr3:149563798 149639014	149613260:149613357	0.4	243
chr3:149563798 149639014	149613260:149613347	0.6	243
chr5:32135678 32143986	32143907:32143986	0.014	209
chr5:32135678 32143986	32143855:32143986	0.986	209
chr12:109046048 109048186	109046814:109046957	0.069	207
chr12:109046048 109048186	109048082:109048186	0.99	207
chr12:109046048 109048186	109046814:109046836	0.03	207
chr12:109046048 109048186	109048047:109048186	0.01	207
chr8:128152989 128186609	128156008:128156164	0.2	184
chr8:128152989 128186609	128156008:128156104	0.8	184
chr8:128152989 128186609	128161042:128161159	0.286	184
chr8:128152989 128186609	128161042:128161163	0.143	184
chr2:61749746 61753656	61752627:61752694	0.03	180
chr2:61749746 61753656	61752627:61752709	0.03	180
chr2:61749746 61753656	61752177:61752238	0.015	180
chr2:61749746 61753656	61752177:61752201	0.015	180
chr8:128152989 128161917	128161042:128161163	0.2	174

chr8:128152989 128161917	128161042:128161159	0.52	174
chr8:128152989 128161917	128156008:128156164	0.182	174
chr8:128152989 128161917	128156008:128156104	0.636	174
chr8:128152989 128161917	128156008:128156139	0.182	174
chr12:69644909 69656342	69653824:69653977	0.947	172
chr12:69644909 69656342	69653824:69653852	0.053	172
chr18:19345733 19359646	19358064:19358130	0.848	170
chr18:19345733 19359646	19358007:19358130	0.152	170
chr10:7285520 7327916	7325866:7326112	0.04	166
chr10:7285520 7327916	7325866:7325921	0.96	166
chr9:140705913 140712590	140707481:140707625	0.053	155
chr9:140705913 140712590	140707458:140707625	0.947	155
chr3:125032152 125050082	125042232:125042288	0.12	154
chr3:125032152 125050082	125042153:125042288	0.88	154
chr9:33971649 33973235	33971649:33971752	0.956	152
chr9:33971649 33973235	33971649:33971735	0.044	152
chr12:46622936 46637097	46622936:46623044	0.037	151
chr12:46622936 46637097	46622936:46623051	0.963	151
chr2:58449077 58459247	58453863:58453910	0.025	148
chr2:58449077 58459247	58453863:58453919	0.975	148
chr22:46125305 46136418	46134641:46134719	0.043	146
chr22:46125305 46136418	46134611:46134719	0.957	146
chr22:46125305 46136418	46125305:46125445	0.036	146
chr22:46125305 46136418	46125305:46125470	0.964	146
chr22:46125305 46136418	46136249:46136418	0.929	146
chr22:46125305 46136418	46136273:46136418	0.071	146
chr9:74838036 74846097	74842863:74842956	0.071	145
chr9:74838036 74846097	74842859:74842956	0.929	145
chr9:114148657 114154104	114152264:114152381	0.68	144
chr2:72958136 72960247	72958136:72958175	0.013	140
chr2:72958136 72960247	72958136:72958181	0.987	140
chr2:72958136 72960247	72960112:72960247	0.024	140
chr2:72958136 72960247	72960200:72960247	0.976	140
chr2:58311224 58316858	58312016:58312085	0.565	135
chr2:58311224 58316858	58312016:58312134	0.435	135
chr7:24663285 24708279	24681335:24681457	0.5	115
chr7:24663285 24708279	24681335:24681487	0.5	115
chr15:41988273 41991357	41988273:41988388	0.056	113
chr15:41988273 41991357	41988273:41989221	0.944	113
chr19:3660964 3661999	3660964:3661106	0.029	112
chr19:3660964 3661999	3660964:3661044	0.029	112
chr19:3660964 3661999	3660964:3661081	0.941	112
chr7:148543562 148544397	148543562:148543690	0.958	111
chr7:148543562 148544397	148543562:148543679	0.042	111
chr11:128993341 129034322	128997107:128997200	0.235	111
chr13:61013822 61041513	61018821:61018882	0.097	109
chr13:61013822 61041513	61018817:61018882	0.871	109
chr6:159004986 159010814	159010680:159010814	0.048	105
chr6:159004986 159010814	159010704:159010814	0.952	105
chr1:9991949 9994918	9994830:9994918	0.06	102
chr1:9991949 9994918	9994820:9994918	0.94	102
chr14:102506573 102507010	102506573:102506747	0.615	101
chr14:102506573 102507010	102506573:102506776	0.385	101
chr2:240929491 240946787	240942466:240942557	0.086	101
chr2:240929491 240946787	240942466:240942553	0.029	101
chr14:97299804 97327072	97326894:97327072	0.7	96
chr14:97299804 97327072	97326984:97327072	0.3	96
chr9:126519982 126641300	126531793:126531842	0.944	95
chr9:126519982 126641300	126531793:126531838	0.056	95
chr21:46538479 46554747	46540709:46540874	0.889	93
chr8:141856359 141900868	141900642:141900868	0.875	91

chr8:141856359 141900868	141900764:141900868	0.125	91
chr16:69404386 69406258	69404386:69404647	0.13	85
chr16:69404386 69406258	69404386:69404532	0.87	85
chr8:18656805 18662408	18662032:18662132	0.397	85
chr8:18656805 18662408	18662032:18662112	0.603	85
chr1:59180504 59184420	59181166:59181313	0.917	83
chr1:59180504 59184420	59181199:59181313	0.083	83
chr1:59180504 59184420	59183429:59183592	0.75	83
chr1:59180504 59184420	59183429:59183596	0.125	83
chr10:74468041 74475660	74468041:74468128	0.111	82
chr10:74468041 74475660	74475520:74475660	0.067	82
chr10:74468041 74475660	74475612:74475660	0.933	82
chr10:74468041 74475660	74468041:74468114	0.889	82
chr17:1746097 1756483	1747214:1747321	0.062	80
chr17:1746097 1756483	1747214:1747292	0.938	80
chr6:159001972 159010814	159010680:159010814	0.056	80
chr6:159001972 159010814	159010704:159010814	0.944	80
chr1:21377359 21415706	21386239:21386287	0.061	79
chr1:21377359 21415706	21386218:21386287	0.156	79
chr4:77055328 77065626	77057339:77057559	0.5	78
chr4:77055328 77065626	77057339:77057565	0.5	78

Supplementary Table 4. Intron retention in circRNAs of HeLa cells.

circRNA	circexon	Psi value	#BSJ reads
chr9:138773479 138774924	138773479:138774924	0.168	1275
chr5:619105 620376	619105:620376	0.035	405
chr7:99621042 99621930	99621042:99621555	0.931	285
chr12:120592774 120593523	120592774:120593234	0.624	231
chr8:37623044 37623873	37623044:37623264	0.288	150
chr15:59204762 59209198	59204762:59205895	0.023	122
chr19:3660964 3661999	3660964:3661999	0.086	112
chr10:105197772 105198565	105197772:105198565	0.07	64
chr10:74474869 74475660	74474869:74475660	0.231	58
chr11:128993341 128994789	128993783:128994789	0.077	46
chr1:47761437 47767420	47765577:47765824	0.482	31
chr19:4941540 4941943	4941540:4941943	0.559	20
chr2:201721405 201721708	201721405:201721708	0.531	17
chr1:1192372 1192690	1192372:1192690	0.383	15
chr19:8987045 8987334	8987045:8987334	0.466	11
chr2:39564061 39564722	39564061:39564722	0.467	9

Supplementary Table 5. Increased miRNA binding sites within skipped circexons in Hs68 cell line.

miRNA	circexon(start:end) within circRNA(chr:start end)	Length_miRNA	Length_circexon	Postions_binding_sites
hsa-miR-7851-3p	245185471:245185598 chr1:245165423 245222760	22	128	86 54 1
hsa-miR-7114-5p	122279976:122280258 chr10:122273423 122280607	21	283	173 72 213
hsa-miR-6765-5p	120277289:120277546 chr11:120276827 120278532	25	258	205 77 48
hsa-miR-3186-3p	114173850:114174116 chr13:114164553 114175048	21	267	94 167 240
hsa-miR-3614-3p	114173850:114174116 chr13:114164553 114175048	23	267	83 156 227
hsa-miR-4462	114173850:114174116 chr13:114164553 114175048	23	267	237 91 164
hsa-miR-4802-3p	114173850:114174116 chr13:114164553 114175048	23	267	91 164 237
hsa-miR-6514-5p	114173850:114174116 chr13:114164553 114175048	23	267	89 162 235
hsa-miR-4530	27250927:27251320 chr17:27248706 27254081	18	394	218 304 272
hsa-miR-15a-5p	42232117:42232306 chr17:42231924 42232779	22	190	158 44 118
hsa-miR-15b-5p	42232117:42232306 chr17:42231924 42232779	22	190	158 120 44
hsa-miR-497-5p	42232117:42232306 chr17:42231924 42232779	21	190	119 163 45
hsa-miR-4725-3p	234298495:234298687 chr2:234296903 234299129	22	193	89 58 160
hsa-miR-4725-3p	234298541:234298687 chr2:234296903 234299129	22	147	43 12 114
hsa-miR-6860	372617:372746 chr5:353845 376831	22	130	49 8 78
hsa-miR-330-5p	136791245:136791449 chr9:136790965 136804341	22	205	47 159 178
hsa-miR-326	136791245:136791449 chr9:136790965 136804341	20	205	46 164 180

Supplementary Table 6. circRNA producing loci with AS events validated in RT-PCR of this study and corresponding outward-facing primers.

chr	start position	end position	outward-facing primer		isoform length (bp)	PCR & Sanger
			P1	P2		
chr1	117944808	117984947	GTGCTACTTTTCCTAATAGAAC	TCTCTTATATGCATCAAGTCTA	844	y
chr1	117944808	117984947	TTGGGCAATGGTGAATTTGAA	AGGTGTGTTAAAGGCAGGAA	648	y
chr1	231090079	231097049	GTTTCGAGTCTGAGTCATGGAA	ACCCTGTGACTCCTTGCTTT	382	y
chr1	231090079	231097049	GTTTCGAGTCTGAGTCATGGAA	ACCCTGTGACTCCTTGCTTT	304	y
chr1	231090079	231097049	GTTTCGAGTCTGAGTCATGGAA	ACCCTGTGACTCCTTGCTTT	242	y
chr1	1192372	1192690	GAGCTTCATGGTGGAGAAGGG	CGCAGCCAGAAAGCCTCC	319	n
chr1	1192372	1192690	GCAATATAGAGACGTCGGACT	TGTCCGGGTGAAATCCGTGA	242	y
chr10	74474869	74475660	CAGTGACATCTTAGCTGCTA	CGGTATCTTGCCAATCACTGTA	165	y
chr10	74474869	74475660	CAGGGGAAACTTGAACATTCCG	CACCAATACCTAAGACAGAGCAG	257	y
chr11	86267595	86270865	AGTGACCTGGACAAGAGT	TGCTGCCGCTCGTAATATCT	344	y
chr11	86267595	86270865	GACGTGGAGAAGTTCATGCCAA	TCGTTCCGGTCTTGGAGTGTCA	326	y
chr11	86267595	86270865	GTGACCTGGACAAGTACATCATT	TGCTGCCGCTCGTAATATCT	284	y
chr12	19615444	19626289	CGTTCAACTCCAGCAATGATGAAT	ACTGGAAATTGTGCTGTCTACAT	419	y
chr12	19615444	19626289	CGTTCAACTCCAGCAATGATGAAT	ACTGGAAATTGTGCTGTCTACAT	316	y
chr12	28378728	28412375	CCAAGTTCAGGATTAATTCTG	GTCACATAAAGGAGGGTTAGAA	409	y
chr12	28378728	28412375	CCAAGTTCAGGATTAATTCTG	GTCACATAAAGGAGGGTTAGAA	332	y
chr12	28378728	28412375	CCAAGTTCAGGATTAATTCTG	GTCACATAAAGGAGGGTTAGAA	272	n
chr12	109046048	109048186	AGTGGACGTACGGGGATTTCAT	ACACATTCAGCAGCAAGGAG	251	y
chr12	109046048	109048186	TGAACAATGACGGCAATCTTGT	GAGGGGACTTGATGTTAACAAA	395	n
chr12	109046048	109048186	AGTGGACGTACGGGGATTTCAT	ACACATTCAGCAGCAAGGAG	274	y
chr12	116668338	116675510	TTGCAGACAGCGGATGAAACTTA	ACTGCAGGTGGTATTAATTTGTG	328	y
chr12	116668338	116675510	AAGCTAAAAATCAGGCAACACC	GCATCTGATATCTGGAAACTGAA	398	y
chr12	116668338	116675510	AAGCTAAAAATCAGGCAACACC	GCATCTGATATCTGGAAACTGAA	496	y
chr12	116668338	116675510	TTGCAGACAGCGGATGAAACTTA	ACATCATGAACTGCAGGAGTG	426	y
chr12	116668238	116675510	CTGCTGCAAAATGGTATCATT	ATTGGTATCACAGTGCACCT	338	y
chr12	116668238	116675510	CTGCTGCAAAATGGTATCATT	ATTGGTATCACAGTGCACCT	408	y
chr12	116668238	116675510	CTGCTGCAAAATGGTATCATT	ATTGGTATCACAGTGCACCT	436	y
chr13	33091994	33101669	TCTGTATCCTTTTCCTATGGC	TGGTGAAATTTGATCCTGA	438	y
chr13	33091994	33101669	TCTGTATCCTTTTCCTATGGC	TGGTGAAATTTGATCCTGA	388	y
chr14	102506573	102507010	GTGCTGGAATTCTGCATCGTAGGT	TGAGATTGCCTGAGTGTGGCT	280	n
chr14	102506573	102507010	GTGCTGGAATTCTGCATCGTAGGT	TGAGATTGCCTGAGTGTGGCT	251	n
chr19	3660964	3661999	CAGGTGCATAGGTCTTGAAGCG	TTCCGGGAGCTCTTTGGGAT	249	y
chr19	3660964	3661999	CAGGTGCATAGGTCTTGAAGCG	TTCCGGGAGCTCTTTGGGAT	403	y
chr19	13039156	13039661	GCTCTCAATGAAGTTATCAGTCGGC	TCTGGAACCTTTGACGCCCTCTT	300	y
chr19	13039156	13039661	GCTCTCAATGAAGTTATCAGTCGGC	TCTGGAACCTTTGACGCCCTCTT	338	y
chr2	58311224	58316858	TAACGGGCCATTTTCTTGA	GCTACTTCCATATATGTGCTT	456	y
chr2	58311224	58316858	GACCAGATCCATAAAACAGA	GACTGAATTCAGGGGAAGAA	407	y
chr21	16386665	16415895	TGTCATCCGGAGTCTTCAAGATT	AGAGCTACTTTTCAACAGCCTT	203	y
chr21	16386665	16415895	ACATTCCAGAAAACGCCAAATTC	GTCTGGCTTCTTGCACCTCAG	284	y
chr22	17525763	17528316	GCAACTGTGGATGAAGAGGGCT	TTCGGCTGTGTTCTCTGGGTTA	231	y
chr22	17525763	17528316	GGTGAAGGCATTGATTGTCCTGA	AACGAGGGCAGAGGCTCATGAT	269	y
chr3	31917925	31921322	CGAGGCTTCTGGTGTGTTGCTTT	TGCTGGTGGTGTACTCTGCTAAT	380	y
chr3	31917925	31921322	CGAGGCTTCTGGTGTGTTGCTTT	TGCTGGTGGTGTACTCTGCTAAT	256	y

chr3	125032152	125050082	TGGTGAGGCATACTTCGATCTT	TTGCTGCAGATGAAGTGTTACA	683	y
chr3	125032152	125050082	TGGTGAGGCATACTTCGATCTT	TTGCTGCAGATGAAGTGTTACA	566	y
chr3	125032152	125050082	TGGTGAGGCATACTTCGATCTT	TTGCTGCAGATGAAGTGTTACA	487	y
chr3	142455221	142467302	ACGATGAGCAGCTAAAATGACA	CCCATGCAGTTGGCTGTGAAT	460	n
chr3	142455221	142467302	ACGATGAGCAGCTAAAATGACA	CCCATGCAGTTGGCTGTGAAT	358	n
chr7	99621042	99621930	GCATCTCAGGTCCATGAACTTG	AGTCCTCGAGCTTTGACCTTCAT	578	n
chr7	99621042	99621930	GCATCTCAGGTCCATGAACTTG	AGTCCTCGAGCTTTGACCTTCAT	197	n
chr8	37623044	37623873	CAGGTTTGGTTTTGCTGACCGC	GGTGATCGAGGCCTATGGACAT	220	y
chr8	37623044	37623873	TGGTTTTGCTGACCGCCACTAG	CCTGAAGCCCTTTGGAAGCAT	297	y
chr8	37623044	37623873	CAGGTTTGGTTTTGCTGACCGC	GGTGATCGAGGCCTATGGACAT	220	y
chr8	37623044	37623873	TGGTTTTGCTGACCGCCACTAG	CCTGAAGCCCTTTGGAAGCAT	297	y
chr9	33953283	33963789	GAGTTGGCTTCTGAGGCTTGACT	ATTCGCAACACAACAATCAGATGG	377	y
chr9	33953283	33963789	GAGTTGGCTTCTGAGGCTTGACT	ATTCGCAACACAACAATCAGATGG	309	y
chr9	134312007	134314448	AGCCAGTAGGACACGAAGGT	TCAGCTGTGCCCATGACTTTG	226	y
chr9	134312007	134314448	AGCCAGTAGGACACGAAGGT	TCAGCTGTGCCCATGACTTTG	176	y
chr9	33971649	33973235	TCTCCCTCTGCCACGTCCAAAT	AAGGCATGGGGACATTTAATCCT	159	y
chr9	33971649	33973235	TCTCCCTCTGCCACGTCCAAAT	AAGGCATGGGACTATTCAGATTCT	142	y
chr9	138773479	138774924	ATGAGCGTTCTGGGTCAGG	TGGTGTCCCTGTAAGGCGG	587	y
chr9	138773479	138774924	ACCCATAAAAATGTATCCACCC	TGGTCCCCTTTCAGGATGAGG	639	y

Supplementary Table 7. circRNAs AS isoforms validated and quantified in qRT-PCR of this study and the corresponding primers used.

chr	start position	end position	outward-facing primer		isoform length (bp)
			P1	P2	
chr19	3660964	3661999	GCCAGATGATTACTTGACCACCTCCT	TGAGGTTGCTGCCTTCGCTGGGGAA	249
chr19	3660964	3661999	AGGAGGTGGTCAAGTAATCATCTGGC	TTCTTCCCAGCTCTGTGACCAGT	403
chr3	31917925	31921322	GCAGCTCTCAGTTTAAACATCT	GGAAATGAATTCTAAGTACTTCGT	256
chr3	31917925	31921322	CTCCAAACACAGCAAACCTCTCA	GGAAATGAATTCTAAGTACTTCGT	380
chr9	33971649	33973235	AAGGCATGGGACTATTCAGATTCT	CGTCCAAATCCAGTTCCTCAT	142
chr9	33971649	33973235	AAGGCATGGGGACATTTAATCCTG	CGTCCAAATCCAGTTCCTCAT	158
chr1	231090079	231097049	AGTAAATATCTAACTCTTCATTTGTGC	TTTTCAACAATGCTTCAGAGTCATC	382
chr1	231090079	231097049	TTCAAAAAGGATGACTCTGAAGCATT	CAGCACAAATGAAGAGTTAGGCTTA	304
chr1	231090079	231097049	CAAGACATAAGCAATAGCTAACTCTTC	TTTTCAACAATGCTTCAGAGTCATCC	242

Supplementary Table 8. Significance of Mann–Whitney U tests after FDR-correction for comparison of splicing factor binding density between groups of exons.

	cirES~ mES	cirES20 ~mES5	cirES~c irConst	cirES20 ~cirCon st20	mES~ mConst	mES5~ mConst 5	cirES~r and	cirES20 ~rand	mES~r and	mES5~ rand
FOX1	4.3E-01	4.3E-01	8.5E-02	1.6E-01	7.1E-64	4.1E-35	2.0E-04	1.6E-02	6.6E-45	5.1E-31
Tra2alpha	2.4E-01	1.5E-01	8.1E-01	5.6E-01	5.2E-08	1.4E-04	7.8E-01	5.4E-01	1.5E-03	2.0E-03
SRp55	1.5E-01	4.5E-01	1.4E-02	9.4E-03	1.6E-17	3.0E-07	2.6E-04	1.7E-02	1.6E-13	6.4E-10
Tra2beta	5.9E-04	4.4E-03	7.1E-03	5.0E-01	6.7E-50	9.1E-32	1.8E-18	4.1E-12	9.0E-48	5.9E-36
9G8	4.5E-04	8.8E-04	7.8E-01	7.7E-01	4.8E-04	1.2E-01	2.9E-05	3.3E-05	3.4E-01	5.5E-02
SC35	3.4E-13	2.0E-10	7.5E-01	7.3E-01	2.4E-08	3.7E-04	4.2E-13	2.1E-10	3.4E-01	3.5E-01
QK1	8.8E-04	4.4E-03	2.0E-03	1.2E-02	8.4E-54	2.9E-38	3.5E-16	2.7E-11	4.9E-38	2.1E-29
MBNL	4.4E-02	1.1E-02	2.3E-02	5.6E-02	1.2E-21	2.7E-12	8.2E-01	4.2E-01	6.2E-09	8.6E-09
NOVA1	5.0E-01	7.6E-01	2.0E-03	1.6E-03	1.9E-37	1.4E-15	1.9E-01	2.4E-01	5.2E-11	7.3E-04
hnRNPM	1.4E-01	5.0E-01	7.1E-04	2.2E-02	8.4E-96	2.2E-53	4.4E-15	2.6E-08	1.9E-75	6.0E-59
SF2ASF	3.1E-06	2.0E-05	8.1E-01	7.3E-01	1.2E-07	1.7E-04	2.9E-05	2.3E-04	3.0E-02	6.6E-03
SRp40	6.5E-02	4.5E-02	2.0E-03	5.2E-03	1.4E-45	7.2E-21	5.4E-01	8.2E-01	3.2E-20	8.2E-12
YB1	2.7E-01	7.6E-01	1.2E-02	4.2E-02	1.9E-16	6.8E-10	4.2E-02	5.4E-01	2.6E-03	1.1E-01
hnRNPA1	6.8E-02	2.7E-01	1.5E-02	7.1E-03	7.3E-16	9.4E-09	7.7E-01	9.7E-01	4.1E-06	2.6E-05
hnRNPU	2.0E-10	1.4E-06	2.7E-06	4.2E-02	1.1E-78	1.9E-55	1.8E-42	1.7E-27	3.0E-88	7.6E-73
hnRNPA2B	1.2E-02	1.8E-02	8.5E-02	3.5E-01	5.4E-30	4.7E-14	8.7E-01	5.7E-01	1.4E-13	7.3E-10
1										
PTB	4.4E-03	7.6E-03	2.6E-03	1.4E-02	1.0E-97	4.7E-51	1.6E-01	7.7E-01	3.7E-48	3.5E-31
hnRNPH1	3.4E-04	2.0E-03	7.7E-01	5.8E-01	1.4E-01	8.5E-01	2.3E-06	9.6E-05	2.7E-02	5.4E-02
hnRNPF	1.1E-05	8.8E-04	5.5E-01	5.6E-01	4.3E-30	1.2E-14	2.6E-02	1.3E-01	2.3E-13	1.2E-10
SRp20	1.2E-04	5.9E-04	2.1E-02	1.5E-02	7.5E-26	3.1E-13	4.9E-03	2.0E-03	4.3E-05	3.7E-02

Supplementary Table 9. Intron retention events detected in ENCODE nucleus transcriptomic data and their Ψ values.

circRNA	cirexon	GM12878	HUVEC	HeLa-S3	HepG2	K562
chr10:105197772 105198565	105197772:105198565	0.167	n/a	n/a	0.495	0.167
chr11:66006275 66006748	66006275:66006748	0.077	n/a	n/a	n/a	n/a
chr12:122218778 122219098	122218778:122219098	0.451	n/a	n/a	n/a	n/a
chr19:13039156 13039661	13039156:13039477	0.25	n/a	n/a	n/a	0.091
chr5:619105 620376	619105:620376	0.125	n/a	0.121	n/a	n/a
chr19:3660964 3661999	3660964:3661999	n/a	0.419	n/a	n/a	n/a
chr16:29917109 29917448	29917109:29917448	n/a	n/a	0.316	0.667	n/a
chr3:50145503 50145737	50145503:50145737	n/a	n/a	0.312	n/a	n/a
chr5:138994171 138994551	138994171:138994359	n/a	n/a	0.25	n/a	n/a
chr12:42604157 42604482	42604157:42604482	n/a	n/a	n/a	0.1	n/a
chr15:90431753 90432372	90431753:90432372	n/a	n/a	n/a	0.346	n/a
chr16:1859239 1859834	1859239:1859834	n/a	n/a	n/a	0.333	n/a
chr16:14720963 14721193	14720963:14721193	n/a	n/a	n/a	0.111	n/a
chr19:2137726 2138713	2137726:2138713	n/a	n/a	n/a	0.408	0.238
chr19:17387304 17387718	17387304:17387718	n/a	n/a	n/a	0.849	n/a
chr2:61749746 61753656	61752405:61753656	n/a	n/a	n/a	0.385	n/a
chr2:201721405 201721708	201721405:201721708	n/a	n/a	n/a	0.625	n/a
chr9:134312007 134314448	134312007:134312282	n/a	n/a	n/a	0.049	n/a
chr9:134381501 134381840	134381501:134381840	n/a	n/a	n/a	0.385	0.035
chr18:691068 691276	691068:691276	n/a	n/a	n/a	n/a	0.009
chr22:26853825 26854543	26853825:26854543	n/a	n/a	n/a	n/a	0.118
chr22:38208894 38209616	38208894:38209616	n/a	n/a	n/a	n/a	0.5
chr8:37623044 37623873	37623044:37623264	n/a	n/a	n/a	n/a	0.215

Supplementary Table 10. Samples of fruit fly used in this study for cirexon/exon detection.

Sample	Data_amount	#BSJs	#Cirexons
virgin_female_eclosion_1_day_heads	17.6G	1034	1670
mated_female_eclosion_1_day_heads	18.2G	1139	1845
mated_male_eclosion_1_day_heads	16.9G	1196	1984
virgin_female_eclosion_4_day_heads	23.0G	1297	2071
mated_female_eclosion_4_day_heads	17.7G	1446	2340
mated_male_eclosion_4_day_heads	21.2G	1460	2399
virgin_female_eclosion_20_day_heads	16.0G	1835	3091
mated_female_eclosion_20_day_heads	16.9G	1354	2215
mated_male_eclosion_20_day_heads	19.3G	1995	3206
virgin_female_eclosion_4_day_ovaries	19.6G	323	451
mated_female_eclosion_4_day_ovaries	18.4G	379	515
mated_male_eclosion_4_day_testes	11.6G	353	500
mixed_males_and_females_eclosion_1_day_carcass	15.7G	464	689
mixed_males_and_females_eclosion_4_day_carcass	16.4G	759	1167
mixed_males_and_females_eclosion_20_day_carcass	15.2G	844	1298
mixed_males_and_females_eclosion_1_day_digestive_system	15.9G	532	706
mixed_males_and_females_eclosion_4_day_digestive_system	16.7G	805	1031
mixed_males_and_females_eclosion_20_day_digestive_system	19.3G	1004	1244
adult_midgut	22.8G	2447	2938
embryos_0-2_hr_after_egg_laying	23.2G	387	497
embryos_2-4_hr_after_egg_laying	14.1G	132	167
embryos_4-6_hr_after_egg_laying	14.5G	144	185
embryos_6-8_hr_after_egg_laying	13.0G	178	237
embryos_8-10_hr_after_egg_laying	10.0G	190	273
embryos_10-12_hr_after_egg_laying	11.2G	185	255
embryos_12-14_hr_after_egg_laying	19.9G	300	414
embryos_14-16_hr_after_egg_laying	12.4G	308	427
embryos_16-18_hr_after_egg_laying	13.1G	352	506
embryos_18-20_hr_after_egg_laying	23.6G	554	815
embryos_20-22_hr_after_egg_laying	12.7G	336	460
embryos_22-24_hr_after_egg_laying	21.1G	348	471
L3_stage_larvae_dark_blue_gut_PS1-2	36.1G	482	702
L3_stage_larvae_light_blue_gut_PS3-6	18.7G	278	391
L3_stage_larvae_clear_gut_PS_7-9	32.8G	637	940
third_instar_larvae_wandering_stage_digestive_system	16.6G	860	1096
third_instar_larvae_wandering_stage_CNS	30.2G	1020	1625
third_instar_larvae_wandering_stage_imaginal_discs	29.4G	446	664
third_instar_larvae_wandering_stage_salivary_glands	9.3G	190	275
WPP_salivary_glands	21.0G	498	718
larval_midgut	41.1G	6394	8387
Pupae_WPP_2d_CNS	13.9G	768	1240
S2-DRSC	9.6G	583	810
S2R	9.0G	401	532
S1	8.8G	268	377

ML-DmBG3-c2	9.3G	319	431
BG3-C2-R2-untreated	12.2G	438	598
BG3-C2-R2-5h_20E	11.4G	361	488
BG3-C2-R2-24h_20E	11.6G	398	540
BG3-C2-R3-5h_20E	12.5G	389	542
BG3C2_0hr_20E	19.9G	682	932
BG3C2_1hr_20E	19.8G	679	930
BG3C2_3hr_20E	9.2G	391	546
BG3C2_5hr_20E	10.0G	576	772
BG3C2_7hr_20E	19.3G	705	978
Kc167-R1-untreated	9.4G	427	634
Kc167-R2-untreated	11.6G	492	714
Kc167-R1-5h_20E	9.4G	378	554
Kc167-R2-5h_20E	9.2G	359	529
Kc167-R1-24h_20E	12.2G	644	943
Kc167-R2-24h_20E	9.9G	482	705
CME_W2	10.9G	415	575
CME_L1	10.8G	568	851