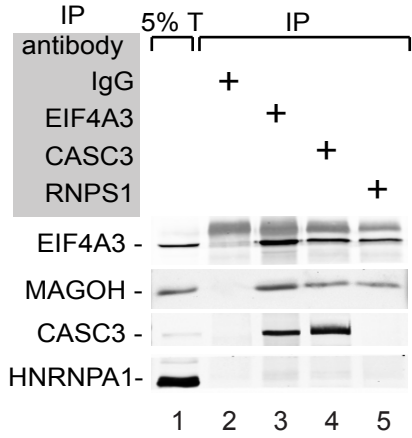
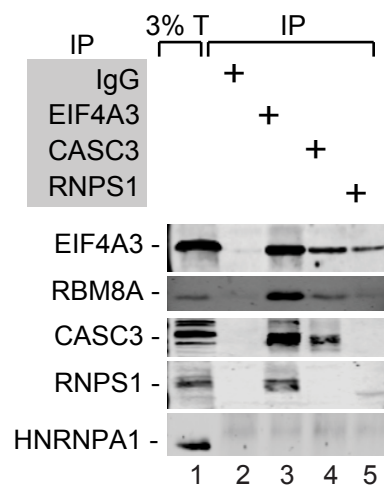


Figure S1

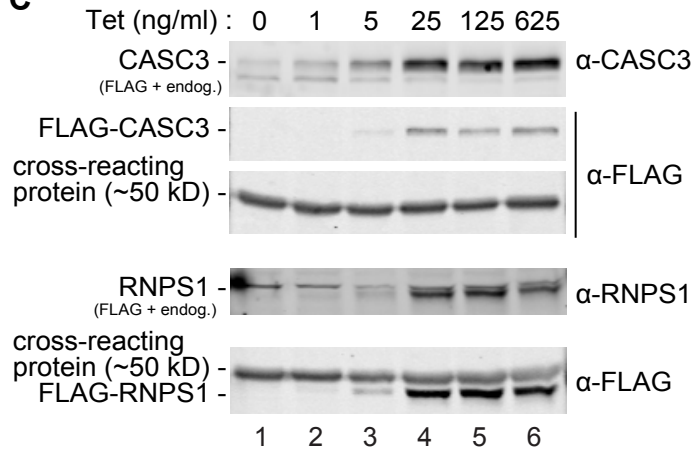
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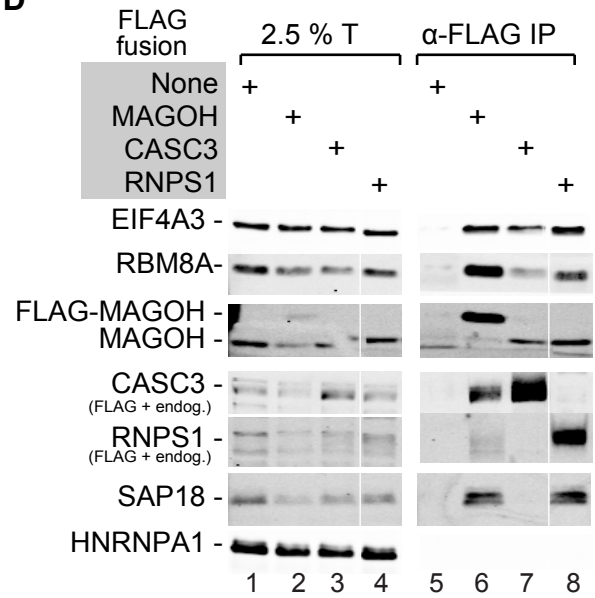
B



C



D



E

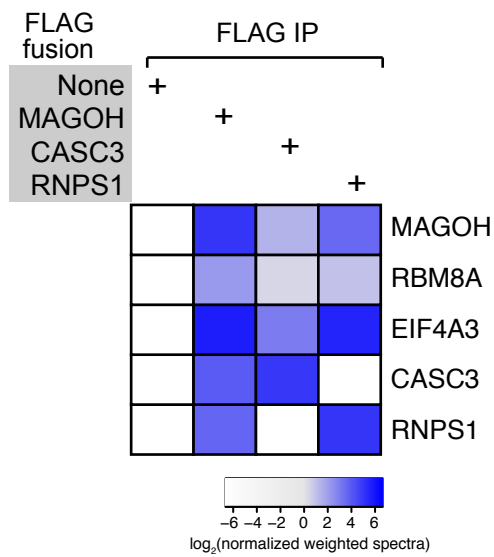
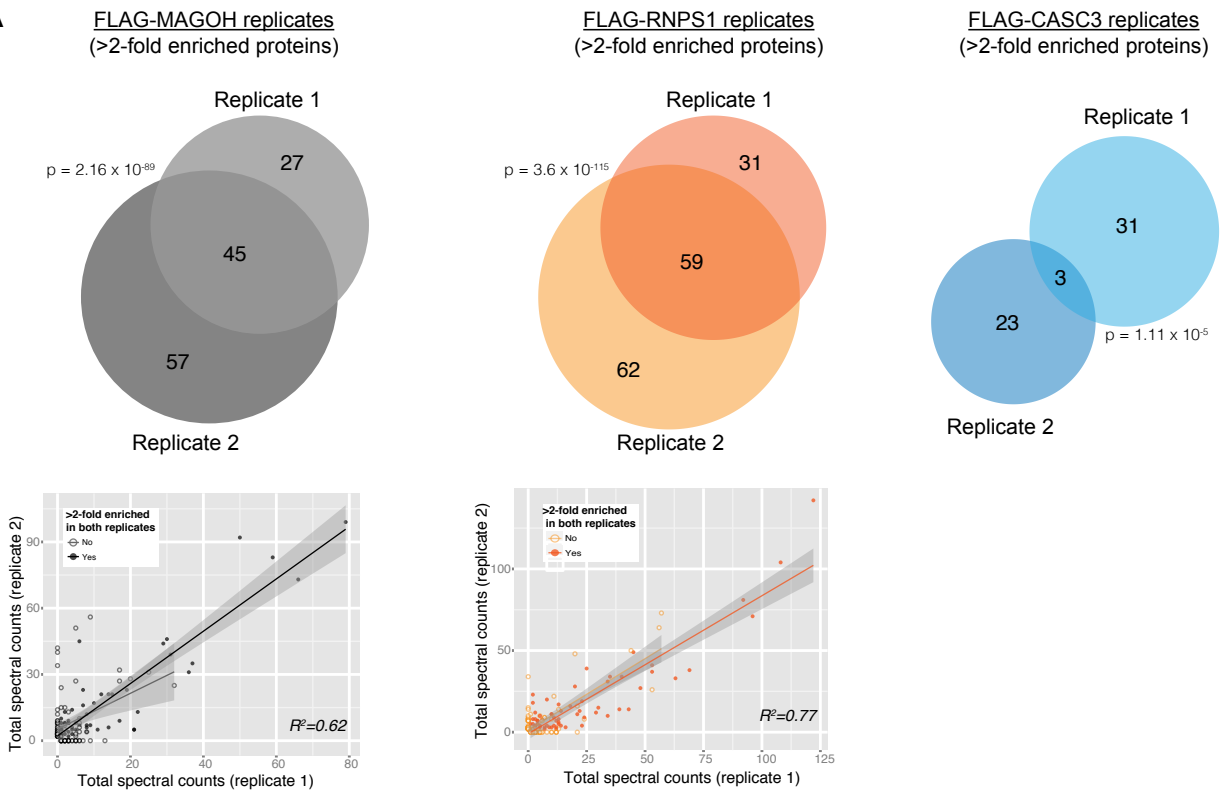


Figure S1. (related to Figure 1)

- (A) Alternate EJCs in P19 cells. Western blots showing proteins on the left in P19 total cell extract (T, lane 1) or the immuno-precipitates (IP, lanes 2-5) using the antibodies listed on the top.
- (B) Alternate EJCs in HeLa cells as in (A).
- (C) Tetracycline (Tet)-induction of FLAG-tagged CASC3 and RNPS1. Western blots showing Tet-induced expression of FLAG-fusion proteins in FLP-In TREx Tet-on HEK293 cell lines. Tet concentrations are on the top, proteins detected are on the left and antibodies used for westerns are on the right.
- (D) Tagged proteins assemble into alternate EJCs. Western blots showing proteins on the left in TE or FLAG IPs of FLAG-fusion proteins expressed in HEK293 cells at near-endogenous levels.
- (E) Alternate EJC proteomic analysis (replicate 2). Heatmap showing enrichment of proteins on the right in FLAG-EJC protein IP proteomic analysis (indicated on the top).

Figure S2

A



B

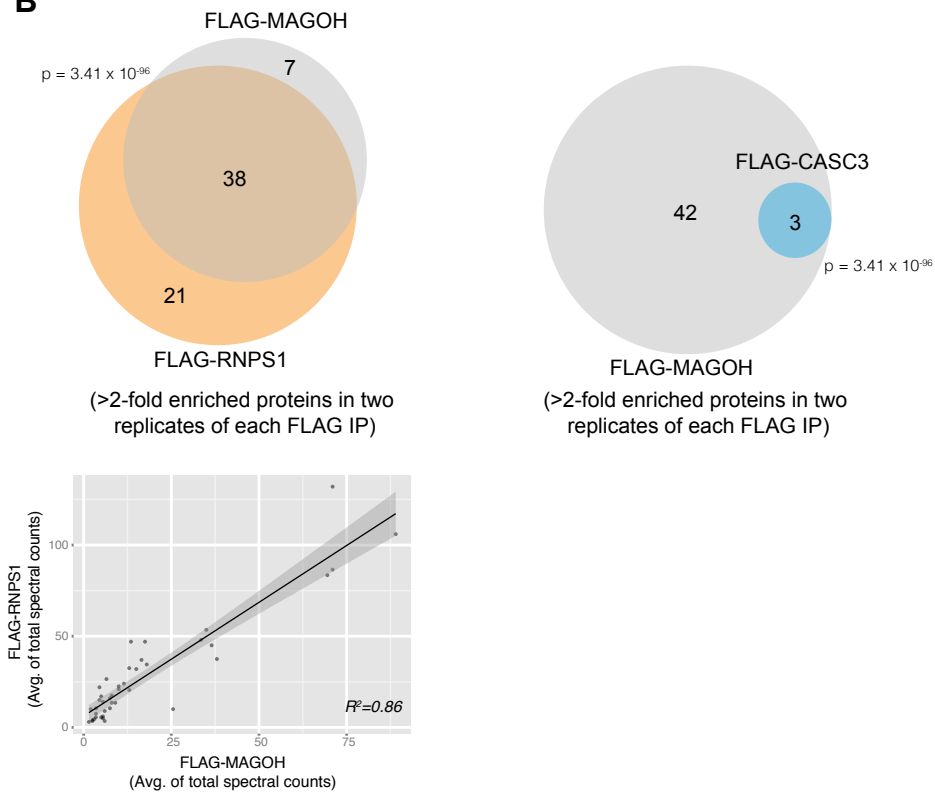


Figure S2. (related to Figure 2)

- (A) Comparison between the two biological replicates of each FLAG-EJC mass spec samples. Overlap between >2-fold enriched proteins in the two biological replicates of proteomics samples of FLAG-EJC proteins indicated on the top. Hypergeometric test p-values are shown next to each venn diagram. For FLAG-MAGOH and FLAG-RNPS1 replicates, scatter plots compare total spectral counts for proteins detected in the two biological replicates. The linear fits for proteins enriched less or more than 2-fold are shown as indicated in the legend. Spearman correlation coefficient for >2-fold enriched proteins in each sample is shown.
- (B) Comparisons as in (A) between the reproducibly enriched proteins in FLAG-MAGOH with those enriched in both replicates of FLAG-RNPS1 (left) and FLAG-CASC3 (right).

Figure S3

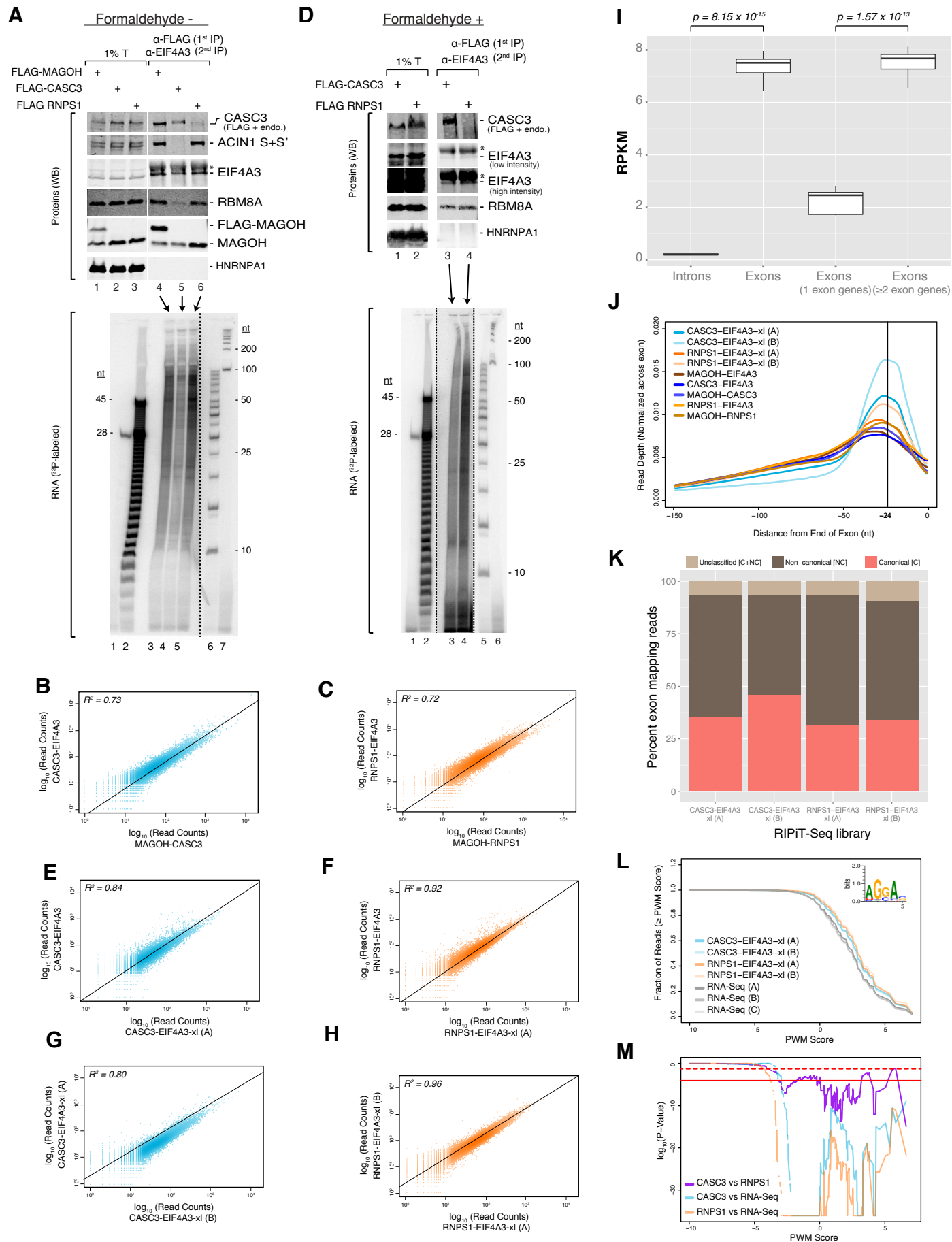


Figure S3. (related to Figure 3)

- (A) Proteins and RNAs enriched in native alternate EJC RIPiTs. Top: Western blots with proteins indicated on the right in TE or RIPiT elutions. Bottom: Autoradiogram of end labeled RNAs from RIPiT elutions as well as size-markers (indicated on either side). The asterisk (*) indicates detection of IgG heavy chain.
- (B) Comparison between native CASC3-EJC RIPiT-Seq samples [FLAG-MAGOH:CASC3 (x-axis) versus FLAG-CASC3:EIF4A3 (y-axis)]. Scatter plot comparing read counts for canonical transcripts for each known gene in GRChg38 (represented by each dot). A linear fit and Spearman correlation coefficient are also shown.
- (C) Comparison between native RNPS1-EJC RIPiT-Seq samples [FLAG-MAGOH:RNPS1 (x-axis) versus FLAG-RNPS1:EIF4A3 (y-axis)] as in (C).
- (D) Proteins and RNAs enriched in formaldehyde-crosslinked alternate EJC RIPiTs as in (A).
- (E) Comparison as in (C) between native and formaldehyde-crosslinked CASC3-EJC RIPiT-Seq samples [FLAG-CASC3:EIF4A3, formaldehyde-crosslinked (xl), replicate A (x-axis) versus native FLAG-CASC3:EIF4A3 (y-axis)].
- (F) Comparison as in (C) between native and formaldehyde-crosslinked RNPS1-EJC RIPiT-Seq samples [FLAG-RNPS1:EIF4A3, formaldehyde-crosslinked (xl), replicate A (x-axis) versus native FLAG-RNPS1:EIF4A3 (y-axis)].
- (G) Comparison as in (C) between two replicates of the formaldehyde-crosslinked CASC3-EJC RIPiT-Seq samples [FLAG-CASC3:EIF4A3-xl, replicate B (x-axis) versus FLAG-CASC3:EIF4A3-xl, replicate A (y-axis)].
- (H) Comparison as in (C) between two replicates of the formaldehyde-crosslinked RNPS1-EJC RIPiT-Seq samples [FLAG-RNPS1:EIF4A3-xl, replicate B (x-axis) versus FLAG-RNPS1:EIF4A3-xl, replicate A (y-axis)].
- (I) Normalized read densities in alternate EJC footprint libraries in the genomic region indicated at the bottom. Each box plot comprises values from all eight RIPiT-Seq datasets listed in Table S2. Top: p-values (Wilcoxon rank sum test).
- (J) Meta-exon plots showing read depth in different RIPiT-Seq or RNA-Seq libraries (top left corner) in the 150 nucleotides (nt) from the exon 3' end.

- (K) Distribution of exon mapping reads among the exonic regions indicated on top in the RIPiT-Seq libraries (bottom).
- (L) Cumulative distribution function plots showing frequency of reads in the indicated samples with the highest score for match to PWM for SRSF9 motif (inset, top right). Bottom left: sample identity. The SRSF9 motif shown in the inset is from (Paradis et al., 2007).
- (M) A negative binomial based assessment of significance of differences in SRSF9 motif PWM scores in (L) between reads from samples in the legend on the bottom left. Horizontal dotted red line: $p=0.05$; Horizontal solid red line: Bonferroni adjusted p-value.

Figure S4

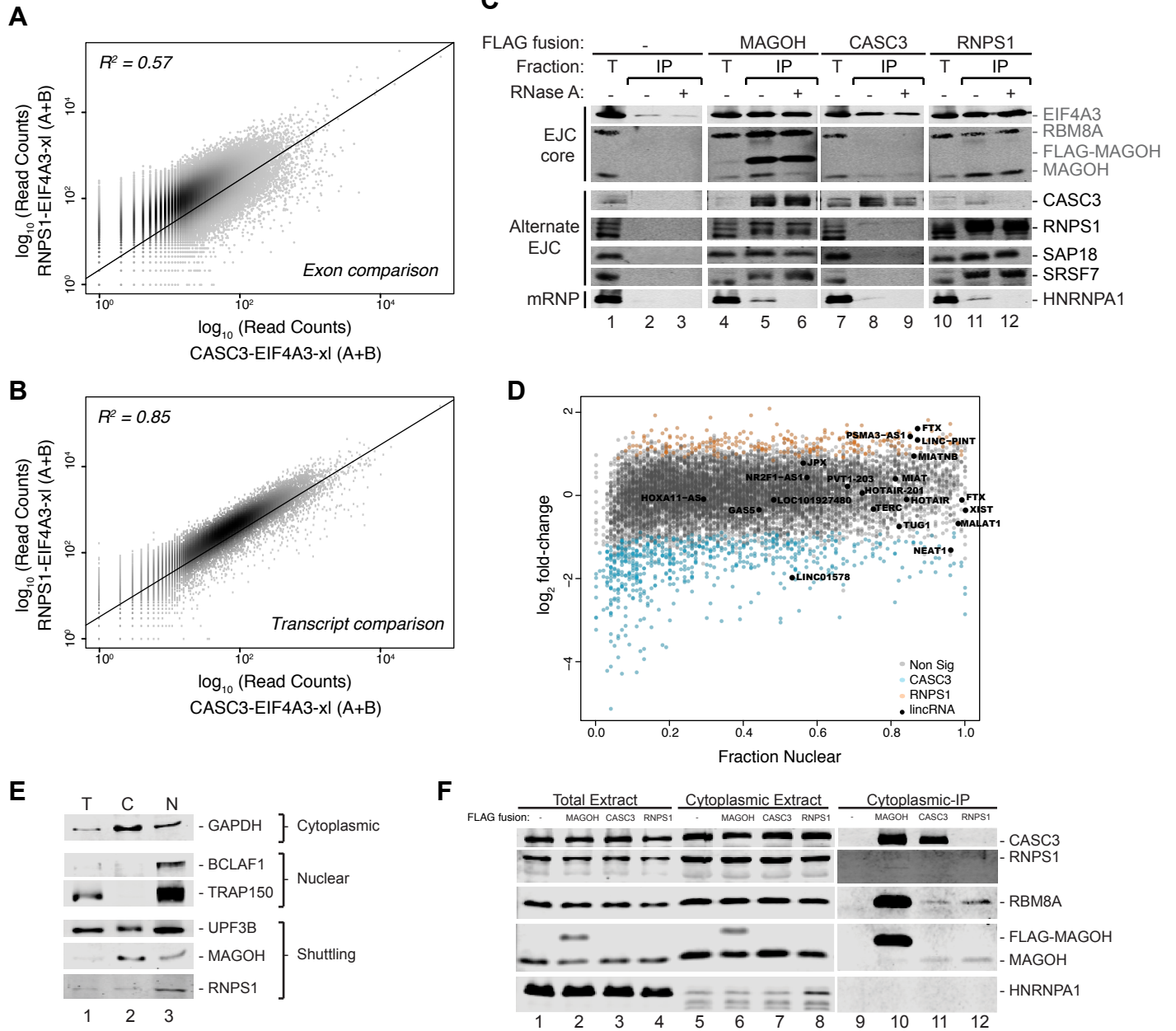


Figure S4. (related to Figure 4)

- (A) Comparison of read counts from both cross-linked replicates of CASC3-EJC (x-axis) versus RNPS1-EJC (y-axis). Each dot on the scatter plot represents each exon in the knownCanonical transcript annotation for UCSC known genes. A linear fit and coefficient of determination (R^2) are shown.
- (B) Comparison of read counts from both cross-linked replicates of CASC3-EJC (x-axis) versus RNPS1-EJC (y-axis). Each dot on the scatter plot represents knownCanonical transcript for UCSC known genes. A linear fit and coefficient of determination (R^2) are shown.
- (C) RNA dependent and independent interactions between EJC proteins. Western blots of proteins on the right in total extracts (T) or FLAG immunoprecipitates (IP) from HEK293 cells expressing FLAG-tagged proteins on the top. Inclusion of RNase A in extracts during IP is indicated on top of each lane.
- (D) Relative alternate EJC levels versus nuclear abundance of long non-coding RNAs (lncRNAs). Each dot on the scatter plot represents knownCanonical transcript with spliced lncRNAs labeled in black. CASC3 or RNPS1-enriched transcripts are colored as in the legend (bottom right). x-axis: -ve fold-change= CASC3-enrichment, +ve fold-change= RNPS1-enrichment.
- (E) Subcellular fractionation. Western blots for proteins on the right in total extracts (T), cytoplasmic (C), or nuclear (N) fractions of HEK293 cells.
- (F) Presence of alternate EJCs in cytoplasmic extracts. Western blots for proteins on the right in the fractions of HEK293 cells (top). FLAG-tagged proteins expressed in cells are indicated above each lane.

Figure S5

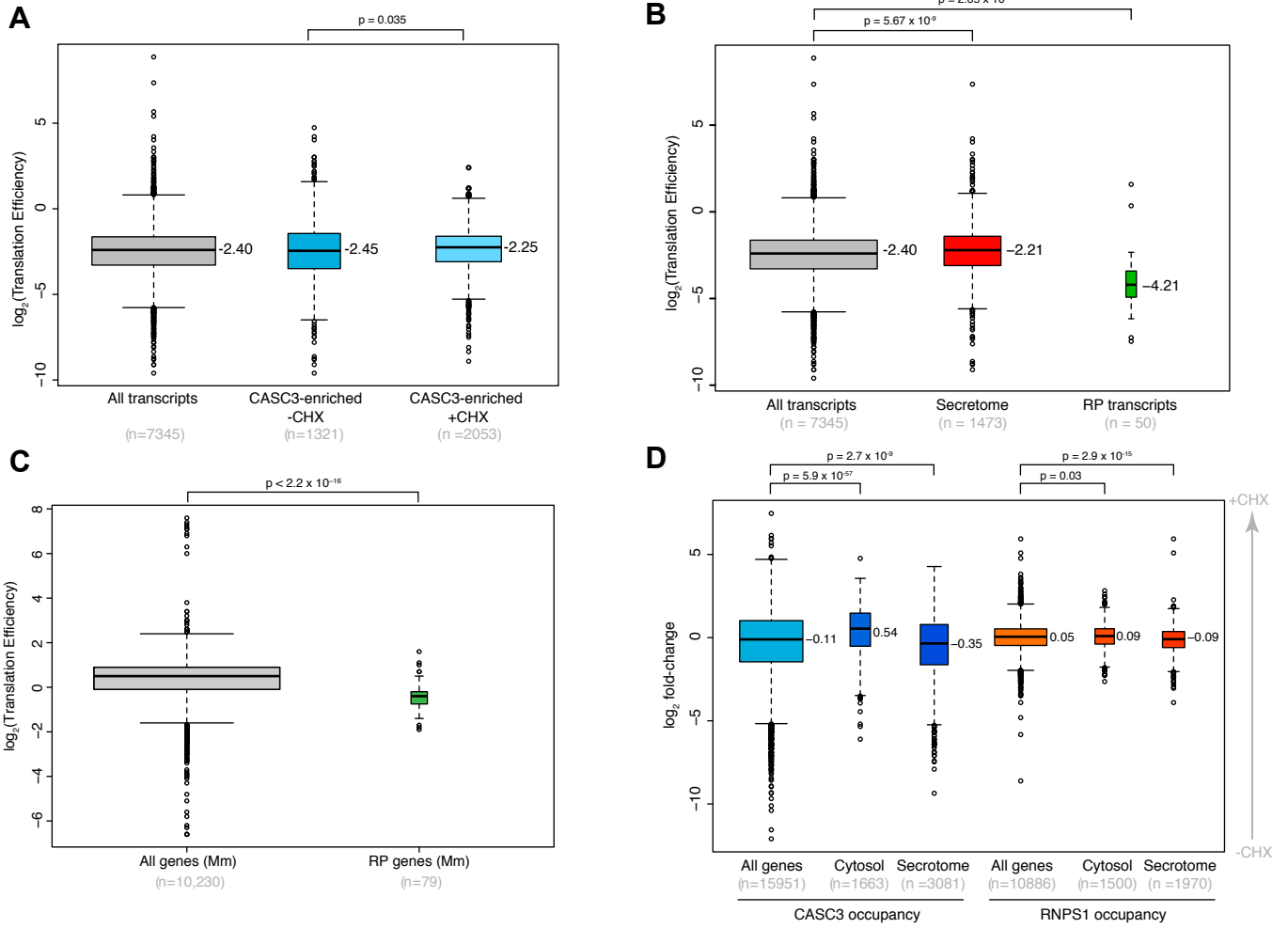
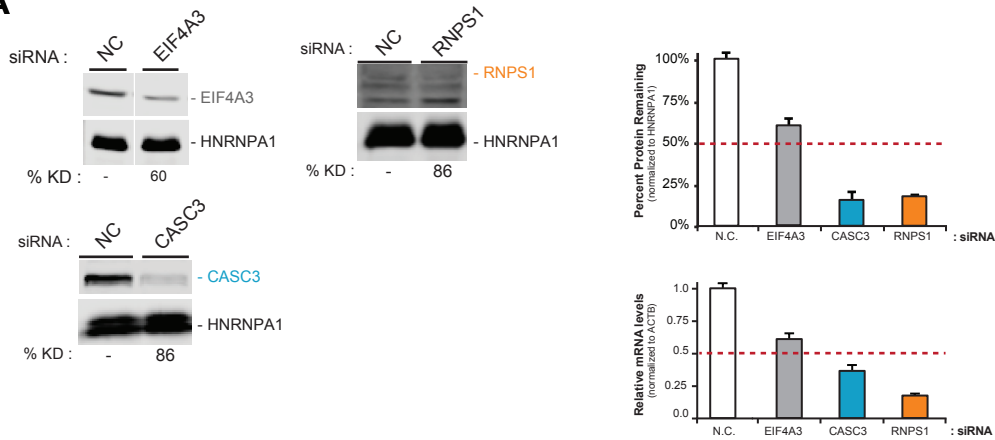


Figure S5. (related to Figure 5)

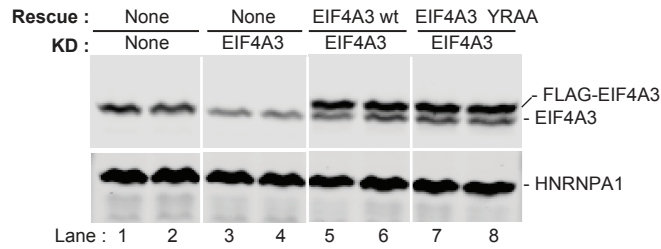
- (A) Box plots showing distribution of translation efficiency estimates (y-axis) of transcript groups on y-axis. The median values are given to the right of each box plot. Top: p-values (Wilcoxon rank sum test). Bottom: the number of transcripts in each group.
- (B) Comparison of translation efficiency (TE, y-axis) of secretome (red) and ribosomal protein (RP) transcripts (green) to all transcripts (grey). Number of genes in group are at the bottom. TE data from (Kiss et al., 2017).
- (C) Translation efficiency of RP genes in mouse embryonic stem cells. TE data from (Ingolia et al., 2011).
- (D) Box plots showing fold-change (y-axis) in alternate EJC occupancy (x-axis, at the bottom) for transcript groups shown at the bottom. x-axis: -ve fold-change= enrichment in -CHX condition, +ve fold-change= enrichment in +CHX condition. The median fold-change values are given to the right of each box plot. Top: p-values (Wilcoxon rank sum test). Bottom: the number of transcripts in each group.

Figure S6

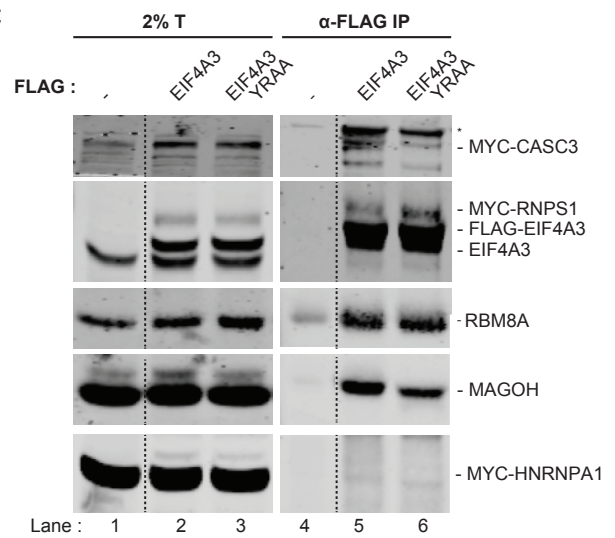
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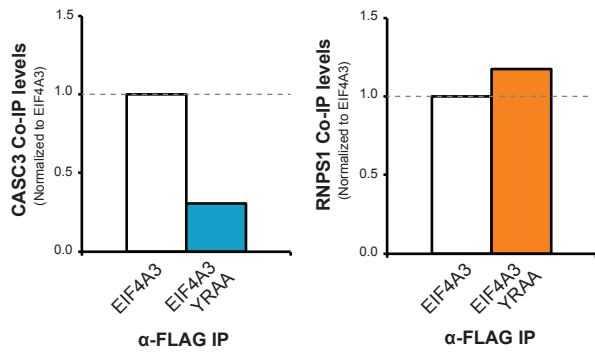
B



C



D



E

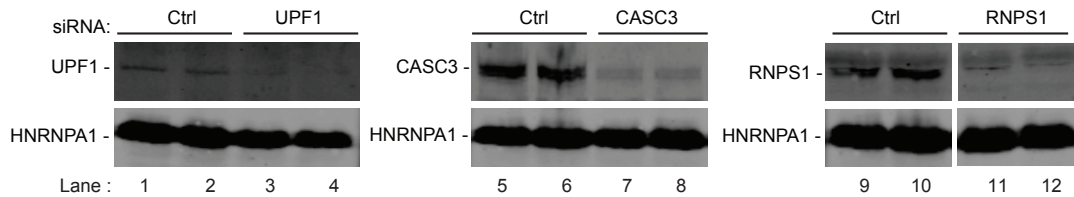


Figure S6 (related to Figure 6)

- (A) Knockdowns of EJC proteins (related to Figure 6A). Western blots of EJC core and alternate factor siRNA-mediated knockdowns (left). Right, top: Quantification of knockdown efficiency of EJC protein remaining relative to HNRNPA1 protein levels. Right, bottom: Knockdown efficiency as determined by qPCR of EJC factor mRNA levels relative to ACTB mRNA levels .
- (B) Rescue of EIF4A3-depleted cells. Western blots for proteins on the right from HEK293 cells depleted of EIF4A3 and expressing either wild-type EIF4A3 or a mutant EIF4A3 lacking CASC3 interaction as indicated on the top.
- (C) Interaction of EIF4A3-YRAA with CASC3. Western blots showing proteins on the right in total extract or FLAG IPs from HEK293 cells expressing FLAG-tagged proteins on the top along with MYC-CASC3, -RNPS1, and -HNRNPA1. The asterisk indicates cross-reacting band likely to be unreduced FLAG-IgG heavy chain.
- (D) Quantification of western blots in (C) showing relative MYC-CASC3 (left, blue) and MYC-RNPS1 (right, orange) co-IP with FLAG-EIF4A3 proteins on the bottom.
- (E) Knockdown of EJC/UPF1 proteins (Figure 6C). Western blots showing proteins indicated on the left from HeLa Tet-off cells treated with siRNAs indicated on the top. Two technical replicates of each knockdown are shown. HNRNPA1 panel shown in lanes 5-6 is shown again in lanes 9-10.

Figure S7

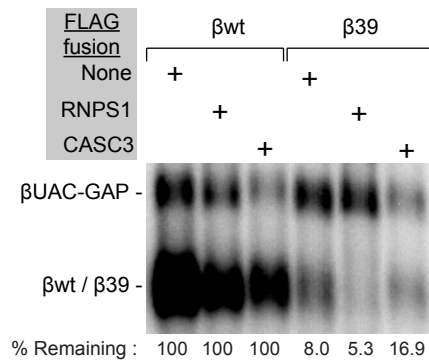
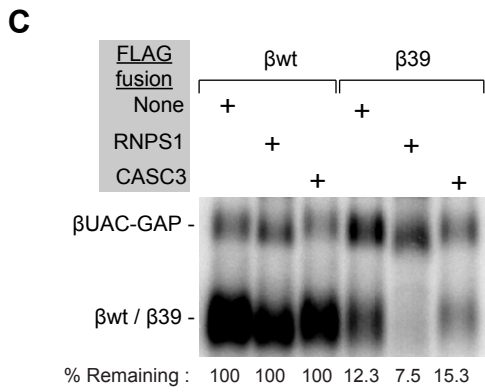
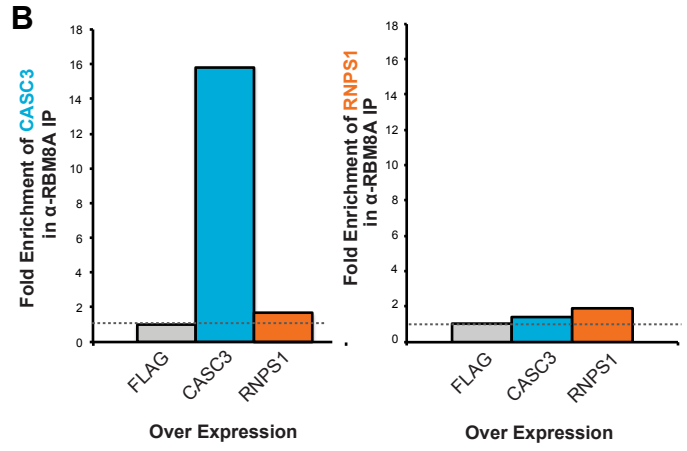
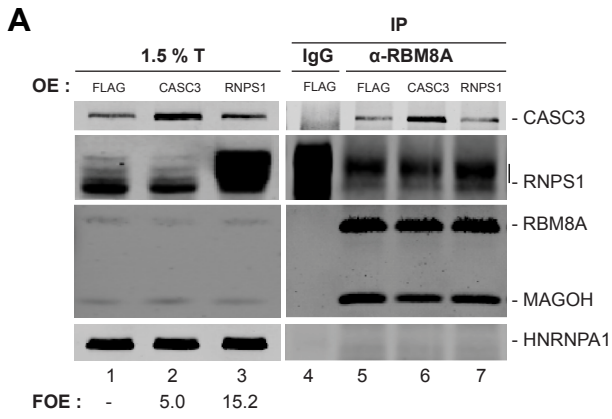


Figure S7 (related to Figure 7)

- (A) Effect of alternate EJC factor overexpression on EJC composition. Western blots for proteins on the right in total extract (lanes 1-3), control IgG IP (lane 4) or RBM8A IPs (lanes 5-7) from HEK293 cells overexpressing (OE) FLAG-fusion proteins at top. Fold overexpression (FOE) on bottom is relative to protein levels in lane 1 after normalizing to HNRNPA1 protein levels in particular lane.
- (B) Quantification of (A) for co-IP of CASC3 (left) or RNPS1 (right) upon RBM8A IP from cell lines overexpressing the proteins on the bottom. Fold enrichment was normalized by co-purifying MAGOH levels.
- (C) Effect of alternate EJC overexpression on β -globin NMD. Northern blots showing levels of wild-type β -globin RNA (β wt) and an NMD-targeted β -globin RNA (β 39) (as indicated on the top) along with co-transfected β UAC-GAP RNA from HeLa cells expressing alternate EJC factors (top left). % Remaining: β wt or β 39 levels in each sample were normalized to β UAC-GAP RNA. Numbers below each lane show normalized β 39 RNA remaining as % of normalized β wt RNA for the corresponding treatment (set to 100). Two independent replicates are shown.

Table S1. Summary of proteins identified by LC-MS/MS in the two replicates of FLAG-EJC and FLAG-only IPs.

#Alternate EJC_replicate1			
341 proteins at 99% minimum, 2 minimum peptides, 0.0% decoy FDR;; 9540 spectra at 95% minimum, 0.00% decoy FDR			
From proteins clusters, only member proteins with a non-zero value in at least one sample were retained			
Normalized spectral abundance factor (NSAF) values from Scaffold were used for quantitation; a psuedo count of 0.000001 was added to each NSAF value before determining enrichment in EJC IPs as compared to FLAG-only			
Proteins enriched >2-fold in any of the three EJC IPs over FLAG-only IP are shown			
	log2(NSAF)		
Accession Number	MAGOH	CASC3	RNPS1
SAP18_HUMAN	12.12344246	9.600730633	13.95255926
LC7L2_HUMAN	10.76661172	0	12.67785175
SRSF1_HUMAN	9.42840205	8.905056005	11.86905398
H4_HUMAN	12.27926234	12.49192823	11.75826486
SRSF6_HUMAN	11.27676417	0	11.69657638
PR40A_HUMAN	11.06379952	0	11.54245153
SCML1_HUMAN	0	0	11.46142829
PRP4B_HUMAN	11.4926043	0	11.23463783
RBM25_HUMAN	10.46954007	0	11.04753294
GPTC8_HUMAN	8.417008587	0	10.96275144
SNIP1_HUMAN	0	0	10.95325069
SRSF7_HUMAN	9.487699374	0	10.81338039
TRA2A_HUMAN	10.24210228	0	10.79100005
PPIG_HUMAN	8.82527683	0	10.73479428
DDX41_HUMAN	10.10105646	0	10.64988486
CWC22_HUMAN	10.55487659	0	10.10446771
SRS10_HUMAN	9.349303347	0	10.09037711
CRNL1_HUMAN	9.654385415	0	9.980853606
MGN_HUMAN	12.77585716	12.25168951	9.934059395
CATIN_HUMAN	9.81605565	0	9.879828273
CSK21_HUMAN	0	0	9.834897633
SRSF3_HUMAN	0	0	9.766512345
RBM8A_HUMAN	12.93774162	11.73564064	9.681238412
ANM1_HUMAN	8.887708033	0	9.628390857
SRSF5_HUMAN	9.295332732	0	9.621740545
CDK12_HUMAN	9.840982435	0	9.583195621
RPB1_HUMAN	10.1382718	0	9.572643389
RL6_HUMAN	10.21176665	12.0086037	9.539391485
PGAM5_HUMAN	9.208014828	0	9.534400161
CCNK_HUMAN	0	0	9.529430554
SRSF4_HUMAN	8.43629512	0	9.497971558
PLRG1_HUMAN	9.961290512	0	9.440786132
RBM22_HUMAN	9.668034004	0	9.410281737
ZC3HE_HUMAN	9.443979543	0	9.408329741

SRSF9_HUMAN	0	0	9.336752009
DHX8_HUMAN	10.1291542	0	9.331432034
H2AY_HUMAN	9.842915995	8.321612471	9.170726276
RU2A_HUMAN	0	0	9.130647779
H2A1A_HUMAN	0	0	9.091646948
RT29_HUMAN	0	0	9.073445375
ZCH18_HUMAN	9.807709112	0	9.036201092
SRSF8_HUMAN	0	0	8.985699652
SPT6H_HUMAN	7.634593268	0	8.957073013
NKTR_HUMAN	9.675904121	0	8.933572638
CD11A_HUMAN	0	0	8.834629058
CWC27_HUMAN	8.501837185	0	8.82791427
DGC14_HUMAN	0	0	8.815767536
AKP8L_HUMAN	8.050556113	0	8.790283326
RBBP6_HUMAN	6.588219912	0	8.77778133
FXR2_HUMAN	7.991748578	0	8.731352976
CSK22_HUMAN	0	0	8.674721924
HP1B3_HUMAN	9.271719795	7.75181182	8.599950025
LUC7L_HUMAN	0	0	8.590886411
SNUT2_HUMAN	0	0	8.569058109
FIP1_HUMAN	8.171226847	0	8.497053445
CMBL_HUMAN	0	12.08988078	8.190713617
SMU1_HUMAN	9.964095343	0	8.124793379
RS4X_HUMAN	0	9.818933622	8.088788239
NCBP1_HUMAN	0	0	8.0869854
PHRF1_HUMAN	9.017838416	0	8.025527183
SRRT_HUMAN	8.609437329	0	7.938462236
PAXB1_HUMAN	7.547434405	0	7.87276721
RBMX_HUMAN	0	0	7.519243094
GPTC1_HUMAN	7.525755693	0	7.269126679
RECQ5_HUMAN	9.015526561	0	7.179610571
PPWD1_HUMAN	10.04644195	0	6.799993888
PININ_HUMAN	6.8673278	-6.910133013	6.25017964
IF4A3_HUMAN	6.771047915	6.220329511	6.19106795
DDB1_HUMAN	0	8.704906538	5.99047842
SON_HUMAN	6.126682474	-5.179948888	5.676165346
TCOF_HUMAN	0	7.326069892	5.61303063
SFR19_HUMAN	5.367405791	-6.048388784	4.575401278
SRRM1_HUMAN	4.360907368	0.470829819	4.257178256
RNPS1_HUMAN	3.31613842	-0.526593813	4.227919599
ACINU_HUMAN	4.051882067	-1.108281217	4.080682378
LC7L3_HUMAN	3.797146519	-7.636334602	4.053576036
RBM39_HUMAN	3.799733114	-8.33860269	3.82174148
TRA2B_HUMAN	3.801634108	-9.216479117	3.594192925
PUF60_HUMAN	1.989468646	1.466254652	3.314884359
RPB2_HUMAN	3.56347561	-6.206428335	3.305719523
PCBP1_HUMAN	1.992068594	2.052717845	3.192553494
SRRM2_HUMAN	3.974038446	-2.821225465	3.167279714
SNW1_HUMAN	1.989809969	0.46980974	3.052499512

PRP17_HUMAN	2.573419515	-7.216260837	2.899953146
RL3_HUMAN	2.5786637	1.470114407	2.735523603
TR150_HUMAN	2.749709192	-9.486633935	2.409244244
BCLF1_HUMAN	2.802239395	-9.54041868	2.26048122
SYF1_HUMAN	2.453011539	-1.520974484	2.259183547
U2AF2_HUMAN	0.994555861	-8.496174702	2.057358601
GNL3_HUMAN	0.991971421	0.46977667	2.053387389
HNRPF_HUMAN	2.575736378	2.466527005	1.733568579
AQR_HUMAN	1.408956925	-8.436878077	1.674127144
XRCC6_HUMAN	0.581108117	-0.941562133	1.322646441
PRP8_HUMAN	1.132917086	-3.823514146	1.143372415
CLK3_HUMAN	0.993848117	-8.071944968	1.057569892
OAT_HUMAN	-9.929302799	2.734055165	1.000769209
CDC5L_HUMAN	2.073375358	-9.907942177	0.960108676
H2A1J_HUMAN	0.58130639	1.056951881	0.738216988
ODB2_HUMAN	0.994496198	2.638771496	0.736795312
TBA1A_HUMAN	-7.574555686	2.466145001	0.735420807
NOP56_HUMAN	0.991616276	0.469501869	0.734475527
HS71L_HUMAN	0.991118205	0.469252722	0.734107857
SSRP1_HUMAN	0.99505304	-1.522078189	0.544862646
PPM1B_HUMAN	0.20003204	1.577484201	0.308705987
U5S1_HUMAN	1.298784908	-2.846251758	0.290901674
SP16H_HUMAN	1.315840207	-1.519385898	0.060224306
CASC3_HUMAN	11.73089465	13.48645796	0
WIBG_HUMAN	11.70852352	10.18512321	0
CCDC9_HUMAN	9.914400082	0	0
RL5_HUMAN	9.168697186	10.22809716	0
REN3B_HUMAN	8.468705491	10.74852832	0
BOLA2_HUMAN	0	12.01531051	0
RL13_HUMAN	0	10.13647852	0
RO52_HUMAN	0	9.551362204	0
P53_HUMAN	0	9.24026662	0
PRDX2_HUMAN	0	9.229299509	0
GSHR_HUMAN	0	8.831528936	0
C1TM_HUMAN	0	8.511475946	0
TBB2A_HUMAN	0	8.063988217	0
LAP2A_HUMAN	0	7.425845306	0
EF1A1_HUMAN (+1)	-0.22569663	1.249191701	-0.068927127
TBB5_HUMAN	-0.00348044	0.986567489	-0.161984954
IRS4_HUMAN	0.986035447	2.776196272	-0.257407108
DJC10_HUMAN	-6.766065051	1.463811228	-0.258872981
RS8_HUMAN	2.578589362	1.470068567	-0.26084481
EFTU_HUMAN	0.410946763	1.692939326	-0.261061789
STK38_HUMAN	-0.003433705	1.056732389	-0.261408701
REST_HUMAN	-1.3198622	1.848156873	-0.581625339
CK084_HUMAN	-11.39580187	1.346444398	-0.676241922
RL4_HUMAN	1.731826571	2.344806224	-0.844616109
TBA4A_HUMAN	-0.003471556	1.055272517	-1.256318815

ILF2_HUMAN	-8.779916297	1.055549917	-1.256981736
SERA_HUMAN	-0.225705018	0.986138055	-1.481694665
CLAP2_HUMAN	-0.58481771	2.051714144	-1.827854863
HNRPL_HUMAN	-8.770135276	1.470179053	-1.837979591
E2F7_HUMAN	-9.140190703	1.055919581	-1.839883907
SERPH_HUMAN	-7.683626283	1.467796235	-7.683626283
HDAC6_HUMAN	-0.584962501	1.689961089	-7.72900887
AIFM1_HUMAN	-8.129386063	1.054571811	-8.129386063

#Alternate EJC_replicate2			
175 proteins at 99% minimum, 2 minimum peptides, 0.0% decoy FDR;; 7282 spectra at 95% minimum, 0.00% decoy FDR			
From proteins clusters, only member proteins with a non-zero value in at least one sample were retained			
Normalized spectral abundance factor (NSAF) values from Scaffold were used for quantitation; a psuedo count of 0.000001 was added to each NSAF value before determining enrichment in EJC IPs as compared to FLAG-only			
Proteins enriched >2-fold in any of the three EJC IPs over FLAG-only IP are shown			
		log2(NSAF)	
Accession Number	Magoh	MLN51	RNPS1
IF4A3_HUMAN	13.7638348	11.03967305	13.42245903
RNPS1_HUMAN	12.09199728	0	13.31783654
MGN_HUMAN	14.55895875	10.9479291	12.93590168
SMD1_HUMAN	11.36260139	0	12.86834029
PININ_HUMAN	13.14623453	0	12.80982841
H2A1D_HUMAN (+6)	12.04217235	0	12.74081375
ACINU_HUMAN	12.39159267	0	12.71647653
H4_HUMAN	0	0	12.5912652
RU2A_HUMAN	10.8482317	12.3128546	12.48507456
SRSF7_HUMAN	11.68439798	0	12.23101112
SRSF1_HUMAN	11.30320995	0	12.08921873
SAP18_HUMAN	11.32198445	0	12.02049387
LC7L3_HUMAN	11.50248377	0	12.00842862
RBM39_HUMAN	11.5997734	0	11.9935753
SF3B1_HUMAN	11.7159191	11.37378957	11.79709406
SMD3_HUMAN	0	0	11.78610623
TRA2B_HUMAN	10.6727789	0	11.69300772
U5S1_HUMAN	11.46183752	0	11.67511904
RBM25_HUMAN	11.0617087	0	11.52214012
PRP19_HUMAN	12.03479896	0	11.43827206
RUXE_HUMAN	0	0	11.4325941
SRRM1_HUMAN	11.72250872	0	11.38392025
TR150_HUMAN	11.78418496	0	11.26631806
SRRM2_HUMAN	11.4604559	0	11.23038076
H13_HUMAN	0	12.51929225	11.16835873
SRSF9_HUMAN	0	0	11.16835873

LC7L2_HUMAN	10.64304445	0	11.1489214
PR40A_HUMAN	10.74760559	0	11.14134082
RSMB_HUMAN (+1)	9.352175878	11.55252501	11.04937211
RU2B_HUMAN	0	11.32378662	10.94989965
PRP4B_HUMAN	9.453003062	0	10.93472289
SRSF3_HUMAN	10.48522554	0	10.92072502
U520_HUMAN	9.841595796	0	10.89565101
BCLF1_HUMAN	11.90358063	0	10.86581058
SRSF6_HUMAN	9.832177982	0	10.70017959
SMD2_HUMAN	0	0	10.65883628
SNR40_HUMAN	10.7779128	0	10.64664871
SF3B6_HUMAN	0	0	10.57572832
H2B1C_HUMAN (+8)	0	10.16100588	10.56424478
SF3A2_HUMAN	11.20683123	10.28019079	10.55803798
U2AF2_HUMAN	9.688757349	0	10.52424786
PPIG_HUMAN	9.700179593	0	10.39820926
DDX41_HUMAN	10.78422586	0	10.37634248
SNW1_HUMAN	10.89193556	0	10.35005518
FIP1_HUMAN	0	0	10.32732808
CDC5L_HUMAN	11.13359178	0	10.30924896
PCBP1_HUMAN	9.782768932	0	10.28840471
SRS10_HUMAN	10.54631572	0	10.24531501
RS4X_HUMAN	10.21916852	0	10.23983717
SCML1_HUMAN	0	0	10.17990909
RBMX_HUMAN	10.45419646	0	10.15317199
PRP8_HUMAN	8.771390425	0	10.12528431
CH60_HUMAN	11.00204143	0	10.11647393
RBM22_HUMAN	10.54361188	0	10.05012073
XRCC6_HUMAN	0	0	9.876777836
H33_HUMAN	0	0	9.86968637
TRA2A_HUMAN	0	0	9.817655232
RIOK1_HUMAN	10.27821712	10.79547151	9.807483728
BUD31_HUMAN	0	0	9.787314917
CCNK_HUMAN	9.66370041	0	9.777353958
SON_HUMAN	9.968090752	0	9.757306662
GRP75_HUMAN	10.5510353	0	9.71998768
RS18_HUMAN	0	0	9.709411269
AQR_HUMAN	9.307861087	0	9.669062209
RL7_HUMAN	0	0	9.588246152
RL7A_HUMAN	0	0	9.487297414
XRCC5_HUMAN	0	0	9.441989675
ISY1_HUMAN	9.688757349	0	9.387909618
PGAM5_HUMAN	9.08459582	0	9.367829698
RS2_HUMAN	9.064796646	10.52816105	9.348042047
CSK21_HUMAN	0	0	9.3468018
SNIP1_HUMAN	8.631140669	0	9.328495421
SMU1_HUMAN	10.42500604	0	9.277054876

SR140_HUMAN	9.421854445	10.59039992	9.272839701
PR38A_HUMAN	0	0	9.257529276
RLA0_HUMAN	9.535430915	8.831845581	9.234625857
GPTC8_HUMAN	0	0	9.21273955
SFR19_HUMAN	8.226074963	0	9.185494924
SNUT1_HUMAN	7.620366471	0	9.121714814
PAXB1_HUMAN	9.003855181	0	9.117435318
H2A1A_HUMAN (+2)	0	0	8.925168681
RL3_HUMAN	0	0	8.888986721
HNRH1_HUMAN	9.034001065	0	8.733388238
TBA1B_HUMAN	9.027601948	0	8.727001465
PPIE_HUMAN	0	0	8.725400344
HNRPC_HUMAN	10.00084508	10.4655664	8.701687664
GRP78_HUMAN	9.490690457	0	8.6061831
PLRG1_HUMAN	8.255972742	0	8.538848521
RED_HUMAN	8.72369507	0	8.423241996
CDK12_HUMAN	7.722602637	0	8.418738108
PRP17_HUMAN	10.08081753	0	8.3675458
DDX21_HUMAN	8.647602329	0	8.347178416
RBBP6_HUMAN	7.04537744	0	8.322694323
SPF45_HUMAN	10.61028666	10.07588021	8.312701473
NKAP_HUMAN	0	0	8.263363107
RL4_HUMAN	9.842271763	0	8.222360218
SYF1_HUMAN	8.842161743	0	8.220668531
SRRT_HUMAN	8.072427412	0	8.185767456
MFAP1_HUMAN	0	0	8.182493675
OAT_HUMAN	0	0	8.182493675
CWC22_HUMAN	8.434503133	0	8.13422094
EF1A1_HUMAN (+1)	8.409348503	0	8.109099329
SRS11_HUMAN	0	0	8.04220656
HNRPM_HUMAN	9.33230514	0	8.034358798
DHX8_HUMAN	0	0	8.03039126
ZC3HE_HUMAN	9.734997453	0	8.022589749
SPTN1_HUMAN	8.574139362	6.877866923	8.011674533
TAB1_HUMAN	8.867618521	9.162265434	7.984019633
MATR3_HUMAN	0	0	7.820753373
CRNL1_HUMAN	8.119096751	0	7.819029455
CHERP_HUMAN	8.008204497	0	7.708256223
CDK13_HUMAN	0	0	7.401903472
MYH9_HUMAN	9.229515825	9.372473325	7.34978987
TBB4B_HUMAN	9.046878442	8.343807528	7.168020189
ZCH18_HUMAN	0	0	7.070067262
NKTR_HUMAN	0	0	7.037821465
IPO8_HUMAN	8.564416278	8.124017889	6.949184722
CAMP3_HUMAN	0	0	6.683134985
MAP1B_HUMAN	0	0	6.29000041
SPTB2_HUMAN	6.649256178	0	5.775182264

FLNA_HUMAN	7.218393711	0	5.615210284
IF4B_HUMAN	2.319331128	3.873106719	1.625925187
RBM8A_HUMAN	11.39943816	0	0
CASC3_HUMAN	11.19241612	12.26488238	0
CCDC9_HUMAN	10.20554891	0	0
COR1C_HUMAN	9.691795853	10.24946904	0
REN3B_HUMAN	9.664678382	0	0
PYM1_HUMAN	9.586314394	0	0
HNRPK_HUMAN	8.989791843	0	0
HNRPR_HUMAN	8.953701754	0	0
SRSF4_HUMAN	8.896483781	0	0
SLU7_HUMAN	8.65065687	0	0
FL2D_HUMAN	8.631140669	0	0
HNRPF_HUMAN	8.563692024	0	0
OTUD4_HUMAN	7.727103604	0	0
LIMA1_HUMAN	7.695854658	8.572851782	0
TBA1C_HUMAN	7.454504938	0	0
K2C8_HUMAN	7.349878311	0	0
TMOD3_HUMAN	0	11.26315181	0
PRDX1_HUMAN	0	11.0860035	0

Table S2. Summary of all described RIPit-Seq and RNA-Seq libraries.

*All numbers represent unique counts
 **Tables A-D show counts in sequential order of processing

Table A. Multi-mapping and PCR duplicate read removal

<i>Library</i>	<i>TopHat Input Total</i>	<i>Mapped</i>	<i>(%)</i>	<i>Multi-Mapped</i>	<i>(%)</i>	<i>Input Reads</i>	<i>Multi-Mappers</i>	<i>(%)</i>	<i>PCR Duplicates</i>	<i>(%)</i>
MAGOH-EIF4A3	12289132	11788001	95.92	919103	7.8	11788001	919103	7.8	38395	0.33
CASC3-EIF4A3	7918724	7552965	95.38	501748	6.64	7552965	501748	6.64	14235	0.19
RNPS1-EIF4A3	9353236	8964991	95.85	921854	10.28	8964991	921854	10.28	16528	0.18
MAGOH-CASC3	6792045	6423758	94.58	408153	6.35	6423758	408153	6.35	31314	0.49
MAGOH-RNPS1	11996168	11169040	93.11	617910	5.53	11169040	617910	5.53	35527	0.32
CASC3-EIF4A3-XL-A	5718072	5309270	92.85	370143	6.97	5309270	370143	6.97	10068	0.19
CASC3-EIF4A3-XL-B	21898851	20055735	91.58	3554071	17.72	20055735	3554071	17.72	2013494	10.04
RNPS1-EIF4A3-XL-A	8370005	7838852	93.65	651639	8.31	7838852	651639	8.31	23133	0.3
RNPS1-EIF4A3-XL-B	24859586	23434622	94.27	4666719	19.91	23434622	4666719	19.91	715018	3.05
CASC3-EIF4A3-XL-A (+CHX)	15795694	15084794	95.5	684318	4.54	15084794	684318	4.54	182916	1.21
CASC3-EIF4A3-XL-B (+CHX)	13937900	13413713	96.24	523380	3.9	13413713	523380	3.9	75455	0.56
RNPS1-EIF4A3-XL-A (+CHX)	27687914	26580410	96	2258708	8.5	26580410	2258708	8.5	187891	0.71
RNPS1-EIF4A3-XL-B (+CHX)	2532100	2274851	89.84	95329	4.19	2274851	95329	4.19	26077	1.15
RNA-Seq-A	18740947	12180518	64.99	4873419	40.01	12180518	4873419	40.01	58947	0.48
RNA-Seq-B	19476485	12592943	64.66	4811993	38.21	12592943	4811993	38.21	46021	0.37

Table B. Removal of miscellaneous RNAs

<i>Library</i>	<i>Mitochondrial</i>	<i>(%)</i>	<i>miRNA</i>	<i>(%)</i>	<i>(%)</i>	<i>rRNA (+ChrUn)</i>	<i>(%)</i>	<i>scaRNA</i>	<i>(%)</i>	<i>snoRNA</i>	<i>(%)</i>	<i>snRNA</i>	<i>(%)</i>	<i>tRNA</i>	<i>(%)</i>	<i>Final Count</i>
MAGOH-EIF4A3	144700	1.23	1384	0.01	0	3524726	29.9	396	0	20182	0.17	677876	5.75	2223	0.02	6459016
CASC3-EIF4A3	124075	1.64	787	0.01	0	4946036	65.48	102	0	4582	0.06	462939	6.13	3095	0.04	1495366
RNPS1-EIF4A3	108423	1.21	879	0.01	0	3215430	35.87	193	0	7039	0.08	1275182	14.22	3955	0.04	3415508
MAGOH-CASC3	35630	0.55	936	0.01	0	3663566	57.03	105	0	3038	0.05	163479	2.54	4305	0.07	2113232
MAGOH-RNPS1	60106	0.54	1511	0.01	0	5766613	51.63	133	0	4938	0.04	385709	3.45	4516	0.04	4292077
CASC3-EIF4A3-XL-A	22902	0.43	1264	0.02	0	3382695	63.71	322	0.01	7479	0.14	140262	2.64	3892	0.07	1370243
CASC3-EIF4A3-XL-B	98373	0.49	3261	0.02	0	11708237	58.38	1866	0.01	6384	0.03	1297149	6.47	10183	0.05	1362717
RNPS1-EIF4A3-XL-A	28638	0.37	1455	0.02	0	3253080	41.5	896	0.01	16125	0.21	506409	6.46	4484	0.06	3352993
RNPS1-EIF4A3-XL-B	50751	0.22	6254	0.03	0	9460234	40.37	3923	0.02	13060	0.06	2295813	9.8	8177	0.03	6214673
CASC3-EIF4A3-XL-A (+CHX)	47903	0.32	14652	0.1	0	4289703	28.44	334	0	7472	0.05	76118	0.5	8924	0.06	9772454
CASC3-EIF4A3-XL-B (+CHX)	60192	0.45	6725	0.05	0	4702167	35.05	243	0	4686	0.03	71546	0.53	6357	0.05	7962962
RNPS1-EIF4A3-XL-A (+CHX)	132946	0.5	2653	0.01	0	12002630	45.16	1679	0.01	36309	0.14	1267031	4.77	21014	0.08	10669549
RNPS1-EIF4A3-XL-B (+CHX)	3478	0.15	110	0	0	1526002	67.08	49	0	235	0.01	9165	0.4	93	0	614313
RNA-Seq-A	507860	4.17	11319	0.09	0	693781	5.7	5463	0.04	183690	1.51	481999	3.96	1883466	15.46	3480574
RNA-Seq-B	535924	4.26	13299	0.11	0	506876	4.03	6870	0.05	298611	2.37	673413	5.35	1945855	15.45	3754081

Table C. Genomic read distribution (51% overlap)

<i>Library</i>	<i>Exonic</i>	<i>(%)</i>	<i>RPKM</i>	<i>Intronic</i>	<i>(%)</i>	<i>RPKM</i>	<i>Intergenic</i>	<i>(%)</i>	<i>1 Exon Genes</i>	<i>RPKM</i>	<i>2+ Exon Genes</i>	<i>RPKM</i>
MAGOH-EIF4A3	4384496.00	67.88	7.40	1797361.00	27.83	0.19	277159.00	4.29	30122.00	1.70	4473662.00	7.78
CASC3-EIF4A3	906277.00	60.61	6.61	504613.00	33.75	0.23	84476.00	5.65	11554.00	2.82	924113.00	6.94
RNPS1-EIF4A3	2162371.00	63.31	6.90	1098461.00	32.16	0.22	154676.00	4.53	23011.00	2.46	2209957.00	7.27
MAGOH-CASC3	1476411.00	69.87	7.61	531464.00	25.15	0.17	105357.00	4.99	15495.00	2.68	1505597.00	8.00
MAGOH-RNPS1	3046675.00	70.98	7.74	1026440.00	23.91	0.16	218962.00	5.10	30176.00	2.57	3105684.00	8.13
CASC3-EIF4A3-XL-A	914747.00	66.76	7.28	379830.00	27.72	0.19	75666.00	5.52	7480.00	1.99	936445.00	7.68
CASC3-EIF4A3-XL-B	1193285.00	87.57	9.54	692445.00	50.81	0.34	523013.00	38.38	16125.00	4.32	1231702.00	10.15
RNPS1-EIF4A3-XL-A	2274827.00	67.84	7.39	908998.00	27.11	0.18	169168.00	5.05	15559.00	1.70	2336427.00	7.83
RNPS1-EIF4A3-XL-B	7357943.00	118.40	12.90	3281206.00	52.80	0.36	4424476.00	71.19	53784.00	3.16	7573547.00	13.69
CASC3-EIF4A3-XL-A (+CHX)	7860415.00	80.43	8.77	1741791.00	17.82	0.12	170248.00	1.74	47235.00	1.77	8010464.00	9.21
CASC3-EIF4A3-XL-B (+CHX)	6528217.00	81.98	8.94	1331364.00	16.72	0.11	103381.00	1.30	26773.00	1.23	6674856.00	9.42
RNPS1-EIF4A3-XL-A (+CHX)	7057878.00	66.15	7.21	3096590.00	29.02	0.20	515081.00	4.83	46214.00	1.58	7205508.00	7.59
RNPS1-EIF4A3-XL-B (+CHX)	454507.00	73.99	8.06	117397.00	19.11	0.13	42409.00	6.90	4588.00	2.73	463153.00	8.47
RNA-Seq-A	1059522.00	30.44	3.32	1761478.00	50.61	0.34	659574.00	18.95	81172.00	8.52	1064409.00	3.44
RNA-Seq-B	1080657.00	28.79	3.14	1785669.00	47.57	0.32	887755.00	23.65	110593.00	10.76	1072926.00	3.21

Table D. Canonical/Non-canonical read classification

<i>Library</i>	<i>Canonical</i>	<i>(%ofExonic)</i>	<i>Non-Canonical</i>	<i>(%ofExonic)</i>	<i>Uncounted</i>	<i>(%ofExonic)</i>
MAGOH-EIF4A3	1546096.00	35.26	2722620.00	62.10	115780.00	2.64
CASC3-EIF4A3	289135.00	31.90	579485.00	63.94	37657.00	4.16
RNPS1-EIF4A3	811486.00	37.53	1267130.00	58.60	83755.00	3.87
MAGOH-CASC3	476638.00	32.28	951547.00	64.45	48226.00	3.27
MAGOH-RNPS1	898705.00	29.50	2006039.00	65.84	141931.00	4.66
CASC3-EIF4A3-XL-A	326669.00	35.71	530307.00	57.97	57771.00	6.32
CASC3-EIF4A3-XL-B	587174.00	49.21	543975.00	45.59	62136.00	5.21
RNPS1-EIF4A3-XL-A	723741.00	31.82	1407464.00	61.87	143622.00	6.31
RNPS1-EIF4A3-XL-B	2555021.00	34.72	4178245.00	56.79	624677.00	8.49
CASC3-EIF4A3-XL-A (+CHX)	2386087.00	30.36	4692151.00	59.69	782177.00	9.95
CASC3-EIF4A3-XL-B (+CHX)	3145982.00	48.19	2672452.00	40.94	709783.00	10.87
RNPS1-EIF4A3-XL-A (+CHX)	1593969.00	22.58	4853509.00	68.77	610400.00	8.65
RNPS1-EIF4A3-XL-B (+CHX)	46447.00	10.22	358889.00	78.96	49171.00	10.82

Table S3. List of reagents (siRNA sequences, qRT-PCR primers, antibodies, plasmids)

siRNAs

Gene	siRNA sequence	Reference
<i>AllStars Negative Control</i>	<i>Qiagen proprietary sequence</i>	
<i>EIF4A3_187</i>	CGA GCA AUC AAG CAG AUC AUU	This Study
<i>CASC3_534</i>	CCA GCA UAC AUA CCU CGG AUU	This Study
<i>RNPS1_2</i>	GCA UCC AGC CGC UCA GGA AUU	This Study

qRT-PCR primers

Gene	Primer (Forward)	Primer (Reverse)	Reference
<i>GADD45A</i>	GAGCTCCTGCTCTTGGAGAC	GCAGGATCCTTCCATTGAGA	This Study
<i>UPP1</i>	CCAGCCTTGTTTGGAGATGT	ACATGGCATAGCGGTCAATT	This Study
<i>ARC</i>	CTGAGATGCTGGAGCACGTA	GCCTTGATGGACTTCTTCCA	This Study
<i>DNAJB2</i>	TGGCATCTACTACGAGATCC	GTTTTTGTCTGGGTGCCACT	This Study
<i>eIF4A2</i>	TGTGGAGACGGTGACAGATTC	TTCCTGCTTTACCCACCAGTAC	This Study
<i>TMEM33</i>	AGGGTCAAGTCGTGTTCTGATC	ACCAACTGCTGCTATCGACTC	This Study
<i>SF3B1</i>	CACGGCAATGTGGCTTTCTC	ACTCACCAAGAAGAGGCAGAAC	This Study
<i>CAPN7</i>	AATTTCCCCAGAGCGTCTTG	TTCGTGCCTTTGTCTCCATC	This Study
<i>TPT1</i>	CAACGGGAAGGCGAGCTCTC	GGAAGGAGCGGCAAAGTTTAC	This Study
<i>C1orf37</i>	TCATCAGCCATGGTCAGTAGG	TGCAGGTGATGGTTCATGAC	This Study
<i>SRSF2</i>	TTGCTGCTCGAATCTCCAAG	ACTTCTGCTGCCATCACAAC	Lareau et al., 2007
<i>SRSF4</i>	CCTCTTAAGAAAATGCTGCGGTCTC	ATCAGCCAAATCAGTTAAAATCTGC	Lareau et al., 2007
<i>SRSF6</i>	GGATCTGAAGAACGGTCTGTTATGT	TCACTCGCTTTTGGTCCCATTAG	Lareau et al., 2007
<i>RPL3</i>	TGGTGGAAAAGGTCCTTAG	TCAGGAGCAGAGCAGAGTTG	This Study
<i>RPL12</i>	CTGGGCCTTAGCTTCTTCAC	AAGTGGCACCGACTTCACCT	This Study
<i>ACTB</i>	CGCGAGAAGATGACCCAGAT	TCACCGGAGTCCATCACGAT	This Study

Antibodies

Protein	Source	Catalog#
CASC3	Bethyl Laboratories	A302-472
RNPS1	Sigma Aldrich	HPA044014
RNPS1	Akila Mayeda	
EIF4A3	Bethyl Laboratories	A302-980A
HNRNPA1	Santa Cruz Biotechnology	SC-32301
ACIN1	Bethyl Laboratories	A300-999A
SAP18	Santa Cruz Biotechnology	SC-25377
RBM8A	Sigma Aldrich	Y1253 (4C4)
MAGOH	Santa Cruz Biotechnology	SC-271365
SRSF1	Santa Cruz Biotechnology	SC-33652
FLAG	Sigma Aldrich	F1804

Reagents

Reagent	Source	Catalog#
Protein A Dynabeads	Life Technologies	10002D
Protein G Dynabeads	Life Technologies	10003D
FLAG-Agarose Beads	Sigma Aldrich	A2220
FLAG peptide	Sigma Aldrich	F3290
MWCO 7,000 Da Dialysis Column	Pierce	88242
4X Lammelli SDS load buffer	Bio-Rad	161-0737
4%–15% Mini-PROTEAN TGX	Bio-Rad	4561086
Trypsin Gold	Promega	V5280
ProteaseMAX™ Surfactant	Promega	V2071
TransIT-X2	Mirus	MIR 6003
RNAiMAX	Thermo Fisher	13778030
JetPrime	PolyPlus	114-01
oligo-(dT)15 Primer	Promega	C1101
Superscript III	Invitrogen	18080044
Ribonuclease H	Promega	M4281
2X SYBR Green Master Mix	Applied Biosystems	4309155
RNase I	Promega	M4261
Ribo-Zero rRNA Removal Kit (H/M/R)	Illumina	MRZH116

Plasmids

Plasmid	Reference
pc β wt β	Lykke-Andersen, 2000
pc β 39 β	Lykke-Andersen, 2000
pcTET2- β wt β	Singh et al., 2007
pcTET2- β 39 β	Singh et al., 2007
pc β wtGAP3UAC	Lykke-Andersen, 2000
pcDNA3	Singh et al., 2012
pcDNA3 YFP	This study
pcDNA3 FLAG	Singh et al., 2012
pcDNA3 FLAG-EIF4AIII	Singh et al., 2012
pcDNA3 FLAG-EIF4AIII YRAA	This study
pcDNA3 FLAG-MAGOH	Singh et al., 2012
pcDNA3 FLAG-RNPS1	This study
pcDNA3 FLAG-CASC3	This study
pcDNA3 FLAG-HNRNPA1	This study
pcDNA5 FLAG-MAGOH	Singh et al., 2012
pcDNA5 FLAG-RNPS1	This study
pcDNA5 FLAG-CASC3	This study