Berg, et al Supplementary Information

Supplemental Figure Legends

Figure S1. Anti-EJC monoclonal antibodies immunoprecipitate spliced mRNA

Denaturing PAGE analysis for splicing of ^{32}P-labeled Ad2ΔIVS pre-mRNA (Input) followed by immunoprecipitations with Control (SP2/0), Y14 (4C4), eIF4AIII (3F1) and hnRNP C1/C2 (4F4) mouse monoclonal antibodies. Identities of splicing intermediates are depicted to the right of the gel.

Figure S2. Optimization of the EJIPT assay parameters

(A) Splicing reactions with ATP (0.5 mM), without ATP, or with 5 mM AMP-PNP were immunoprecipitated with an anti-EJC antibody. SP2/0 is a control anti-mouse antibody substituted for the anti-EJC antibody. Ad2 mRNA produced without splicing was immunoprecipitated with an anti-EJC antibody. Parameters for the EJIPT assay were examined using increasing amounts of splicing extract (B) and increasing amounts of biotin-labeled Ad2ΔIVS pre-mRNA (C) in a 10 μl reaction volume.

Figure S3. NSC663284 and BN82685 are more selective splicing inhibitors compared to NSC95397

In vitro snRNP assembly was performed in the presence of increasing amounts of compounds. Chemiluminescence signals are the averages of quadruplicate measurements. The red dashed line indicates the average for DMSO controls ($6.0 \pm 1.1 \times 10^4$ RLU).

Supplemental Table S1. Association of individual proteins with drug-treated spliceosomes.

Peptide counts for mass spectrometry of DMSO and BN82685 streptavidin pull downs are shown for three independent experiments, along with a technical repeat of experiment 3. The ratio of BN82685 to DMSO is shown in dark grey columns and the average and standard deviation of the four data points is listed on the right in light grey columns. Data points not used in the calculation are in grey type with italics.

Top 200 Identified Proteins	Accession Number	Molecular Weight	Exp 1 DMSO	Exp 1 BN82685	ratio	Exp 2 DMSO	Exp 2 BN82685	ratio	Exp 3A DMSO	Exp 3A BN82685	ratio	Exp 3B DMSO	Exp 3B BN82685	ratio	Average	Std Dev
Isoform 1 of Acetyl-CoA carboxylase 1	IPI00011569	266 kDa	51	73	1.43	11	2	0.18	376	401	1.07	910	984	1.08	0.94	0.53
Splicing factor 3B subunit 1	IPI00026089	146 kDa	188	175	0.93	305	231	0.76	137	114	0.83	307	204	0.66	0.80	0.11
Dihydrolipoyllysine-residue acetyltransferase component	IPI00021338	69 kDa	109	110	1.01	251	260	1.04	120	135	1.13	312	337	1.08	1.06	0.05
Pre-mRNA-processing-splicing factor 8	IPI00007928	274 kDa	170	143	0.84	232	106	0.46	174	188	1.08	200	213	1.07	0.86	0.29
Isoform 1 of Splicing factor 3B subunit 3	IPI00300371	136 kDa	104	105	1.01	199	169	0.85	118	96	0.81	246	196	0.80	0.87	0.10
116 kDa U5 small nuclear ribonucleoprotein component	IPI00003519	109 kDa	132	99	0.75	203	122	0.60	94	98	1.04	196	218	1.11	0.88	0.24
Lipoamide acyltransferase component	IPI00003944	53 kDa	80	61	0.76	136	168	1.24	54	69	1.28	128	147	1.15	1.11	0.24
Splicing factor 3A subunit 1	IPI00017451	89 kDa	106	77	0.73	163	108	0.66	64	60	0.94	130	107	0.82	0.79	0.12
Splicing factor 3B subunit 2	IPI00221106	100 kDa	82	70	0.85	95	98	1.03	78	60	0.77	162	108	0.67	0.83	0.15
Isoform 1 of U5 small nuclear ribonucleoprotein 200 kDa helicase	IPI00420014	245 kDa	78	92	1.18	77	39	0.51	94	83	0.88	137	190	1.39	0.99	0.38
U2 small nuclear ribonucleoprotein A'	IPI00297477	28 kDa	58	48	0.83	92	82	0.89	48	40	0.83	149	129	0.87	0.85	0.03
Isoform 2 of U5 small nuclear ribonucleoprotein 200 kDa helicase	IPI00168235	71 kDa	80	56	0.70	73	38	0.52	55	60	1.09	74	91	1.23	0.89	0.33
Putative uncharacterized protein ALB	IPI00022434	72 kDa	40	34	0.85	62	76	1.23	43	46	1.07	109	107	0.98	1.03	0.16
Isoform 2 of Splicing factor 3B subunit 3	IPI00179138	30 kDa	46	31	0.67	69	65	0.94	28	30	1.07	85	85	1.00	0.92	0.17
Isoform 1 of Polyadenylate-binding protein 1	IPI00008524	71 kDa	48	45	0.94	25	40	1.60	46	62	1.35	124	102	0.82	1.18	0.36
Cell division cycle 5-like protein	IPI00465294	92 kDa	51	58	1.14	61	34	0.56	40	50	1.25	70	90	1.29	1.06	0.34
Intron-binding protein aquarius	IPI00297572	171 kDa	53	51	0.96	42	23	0.55	54	52	0.96	40	69	1.73	1.05	0.49
Isoform Long of Splicing factor, proline- and glutamine-rich	IPI00010740	76 kDa	5	8	1.60	0	1	n/a	74	64	0.86	237	80	0.34	0.93	0.63
Keratin, type II cytoskeletal 1	IPI00220327	66 kDa	52	95	1.83	39	57	1.46	37	42	1.14	49	75	1.53	1.49	0.28
Splicing factor 3A subunit 3	IPI00029764	59 kDa	47	45	0.96	80	50	0.63	39	22	0.56	73	47	0.64	0.70	0.18
Isoform 1 of Heterogeneous nuclear ribonucleoprotein M	IPI00171903	78 kDa	53	49	0.92	30	34	1.13	77	53	0.69	72	73	1.01	0.94	0.19
Pre-mRNA-processing factor 19	IPI00004968	55 kDa	35	43	1.23	80	53	0.66	32	42	1.31	71	96	1.35	1.14	0.32
Pyruvate carboxylase, mitochondrial	IPI00299402	130 kDa	18	27	1.50	5	3	0.60	78	75	0.96	109	89	0.82	0.97	0.38
Pre-mRNA-splicing factor SYF1	IPI00163084	100 kDa	62	63	1.02	50	23	0.46	39	63	1.62	45	59	1.31	1.10	0.49
Pyruvate dehydrogenase protein X component, mitochondrial	IPI00298423	54 kDa	51	31	0.61	43	72	1.67	34	58	1.71	61	52	0.85	1.21	0.56
Isoform 1 of Crooked neck-like protein 1	IPI00177437	100 kDa	55	39	0.71	48	24	0.50	30	49	1.63	41	63	1.54	1.09	0.57
Keratin, type I cytoskeletal 10	IPI00009865	59 kDa	66	74	1.12	23	40	1.74	44	39	0.89	22	26	1.18	1.23	0.36
Insulin-like growth factor 2 mRNA-binding protein 1	IPI00008557	63 kDa	33	43	1.30	22	28	1.27	44	32	0.73	81	60	0.74	1.01	0.32
Propionyl-CoA carboxylase beta chain, mitochondrial	IPI00007247	58 kDa	25	13	0.52	17	10	0.59	48	47	0.98	95	89	0.94	0.76	0.24
Non-POU domain-containing octamer-binding protein	IPI00304596	54 kDa	0	1	n/a	0	0	0.00	66	50	0.76	147	48	0.33	0.36	0.38
propionyl-CoA carboxylase alpha chain	IPI00552419	75 kDa	22	13	0.59	11	9	0.82	47	58	1.23	71	80	1.13	0.94	0.29
Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	IPI00024580	80 kDa	4	2	0.50	0	0	0.00	69	78	1.13	68	82	1.21	0.71	0.57
SM-B' of Small nuclear ribonucleoprotein-associated proteins B and B'	IPI00027285	25 kDa	28	5	0.18	19	23	1.21	31	23	0.74	81	51	0.63	0.69	0.42
Heterogeneous nuclear ribonucleoprotein U-like protein 1	IPI00013070	96 kDa	21	12	0.57	8	2	0.25	40	36	0.90	80	32	0.40	0.53	0.28
Keratin, type II cytoskeletal 2 epidermal	IPI00021304	66 kDa	36	49	1.36	24	38	1.58	21	16	0.76	31	33	1.06	1.19	0.36
Eukaryotic initiation factor 4A-III	IPI00009328	47 kDa	22	4	0.18	20	1	0.05	21	42	2.00	17	99	5.82	2.01	2.69
SNW domain-containing protein 1	IPI00013830	61 kDa	25	10	0.40	22	18	0.82	25	30	1.20	30	33	1.10	0.88	0.36
Isoform 1 of Terminal uridylyltransferase 4	IPI00289861	185 kDa	8	6	0.75	28	21	0.75	5	9	1.80	31	76	2.45	1.44	0.84
cDNA FLJ56825, highly similar to WD repeat protein 57	IPI00006723	45 kDa	17	12	0.71	11	5	0.45	17	30	1.76	56	56	1.00	0.98	0.57
Isoform 1 of Pleiotropic regulator 1	IPI00002624	57 kDa	40	16	0.40	14	8	0.57	29	34	1.17	22	32	1.45	0.90	0.50
Pre-mRNA-processing factor 17	IPI00026307	66 kDa	28	22	0.79	10	7	0.70	28	33	1.18	22	30	1.36	1.01	0.32
Tubulin alpha-4A chain	IPI00007750	50 kDa	7	6	0.86	4	9	2.25	19	27	1.42	44	58	1.32	1.46	0.58
Actin, cytoplasmic 1	IPI00021439	42 kDa	17	4	0.24	19	5	0.26	7	20	2.86	21	32	1.52	1.22	1.25
Matrin-3	IPI00017297	95 kDa	4	15	3.75	0	0	0.00	42	35	0.83	52	28	0.54	1.28	1.68
Tubulin beta-2C chain	IPI00007752	50 kDa	5	4	0.80	1	0	0.00	33	53	1.61	25	48	1.92	1.08	0.86
Isoform 1 of RNA-binding protein 14	IPI00013174	69 kDa	11	4	0.36	3	0	0.00	27	27	1.00	51	25	0.49	0.46	0.41
Heterogeneous nuclear ribonucleoprotein C-like 1	IPI00027569	32 kDa	17	13	0.76	10	10	1.00	26	23	0.88	28	29	1.04	0.92	0.12
Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2	IPI00216592	32 kDa	13	5	0.38	10	11	1.10	20	22	1.10	40	19	0.48	0.76	0.39
pyruvate dehydrogenase E1 alpha 1 isoform 2 precursor	IPI00306301	48 kDa	13	17	1.31	26	32	1.23	8	9	1.13	29	22	0.76	1.11	0.24
Dihydrolipoyl dehydrogenase, mitochondrial	IPI00015911	54 kDa	27	17	0.63	14	20	1.43	12	14	1.17	21	28	1.33	1.14	0.36
Isoform 1 of Pre-mRNA-splicing factor ISY1 homolog	IPI00063673	35 kDa	17	5	0.29	11	10	0.91	15	29	1.93	25	29	1.16	1.07	0.68
Keratin, type I cytoskeletal 9	IPI00019359	62 kDa	18	46	2.56	14	20	1.43	8	12	1.50	2	18	9.00	3.62	3.62
Ribonuclease inhibitor	IPI00550069	50 kDa	24	15	0.63	14	16	1.14	12	12	1.00	29	11	0.38	0.79	0.35
PHD finger-like domain-containing protein 5A	IPI00005511	12 kDa	16	12	0.75	34	25	0.74	9	10	1.11	11	9	0.82	0.85	0.18
Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	IPI00003925	39 kDa	23	13	0.57	12	16	1.33	9	16	1.78	8	7	0.88	1.14	0.53
TUBA1C protein	IPI00166768	37 kDa	6	11	1.83	13	12	0.92	6	13	2.17	33	35	1.06	1.50	0.60
Isoform 1 of Pre-mRNA-splicing factor RBM22	IPI00019046	47 kDa	11	6	0.55	6	4	0.67	12	22	1.83	6	20	3.33	1.59	1.30
WD40 repeat-containing protein SMU1	IPI00305833	58 kDa	25	8	0.32	5	3	0.60	11	21	1.91	19	19	1.00	0.96	0.69
Beta-actin-like protein 2	IPI00003269	42 kDa	14	4	0.29	20	9	0.45	6	15	2.50	20	22	1.10	1.08	1.01
Putative uncharacterized protein FUBP3	IPI00063245	35 kDa	14	9	0.64	5	13	2.60	20	18	0.90	18	7	0.39	1.13	1.00
Vimentin	IPI00418471	54 kDa	18	13	0.72	3	6	2.00	15	21	1.40	8	19	2.38	1.62	0.72
Actin, aortic smooth muscle	IPI00008603	42 kDa	5	3	0.60	19	8	0.42	6	15	2.50	19	30	1.58	1.28	0.96
cDNA FLJ55034	IPI00384122	40 kDa	15	18	1.20	24	25	1.04	7	1	0.14	10	6	0.60	0.75	0.48
U2 small nuclear ribonucleoprotein B''	IPI00029267	25 kDa	12	9	0.75	1	7	7.00	14	11	0.79	27	28	1.04	0.86	0.16
Isoform A of Peptidyl-prolyl cis-trans isomerase E	IPI00009316	33 kDa	11	2	0.18	6	4	0.67	19	16	0.84	29	23	0.79	0.62	0.30
KH-type splicing regulatory protein	IPI00479786	73 kDa	14	23	1.64	1	11	11.00	23	8	0.35	11	1	0.09	0.69	0.83
Isoform 1 of Paraspeckle component 1	IPI00103525	59 kDa	1	0	0.00	0	0	0.00	31	19	0.61	42	13	0.31	0.46	0.21
SF3A2 protein (Fragment)	IPI00017341	51 kDa	12	11	0.92	5	13	2.60	12	13	1.08	15	19	1.27	1.47	0.77
cDNA FLJ60076, highly similar to ELAV-like protein 1	IPI00301936	39 kDa	9	9	1.00	3	5	1.67	22	10	0.45	28	22	0.79	0.98	0.51

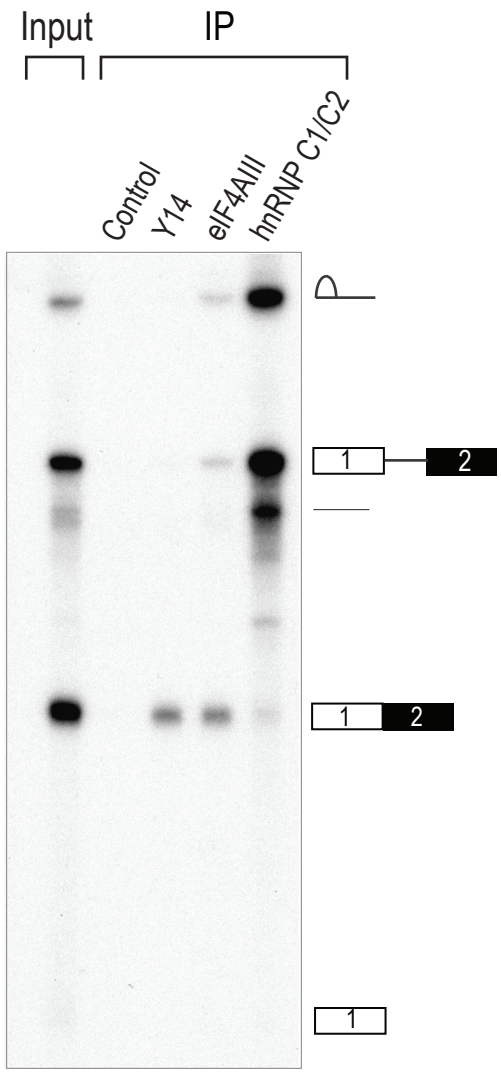
Table S1. Peptide counts for top proteins (1-69) detected by mass spectrometry of DMSO and BN82685 streptavidin pull downs.

Top 200 Identified Proteins	Accession Number	Molecular Weight	Exp 1 DMSO	Exp 1 BN82685	ratio	Exp 2 DMSO	Exp 2 BN82685	ratio	Exp 3A DMSO	Exp 3A BN82685	ratio	Exp 3B DMSO	Exp 3B BN82685	ratio	Average	Std Dev
Pre-mRNA branch site protein p14	IPI00032827	15 kDa	14	21	1.50	26	17	0.65	6	7	1.17	3	2	0.67	1.00	0.41
Pre-mRNA-splicing factor SPF27	IPI00025178	26 kDa	7	9	1.29	9	7	0.78	10	12	1.20	22	16	0.73	1.00	0.29
Coiled-coil domain-containing protein 12	IPI00453463	19 kDa	11	14	1.27	10	7	0.70	14	11	0.79	13	10	0.77	0.88	0.26
Tubulin, beta	IPI00645452	48 kDa	3	4	1.33	1	1	1.00	6	12	2.00	33	42	1.27	1.40	0.42
U1 small nuclear ribonucleoprotein A	IPI00012382	31 kDa	9	6	0.67	12	11	0.92	8	12	1.50	18	17	0.94	1.01	0.35
Pre-mRNA-processing factor 6	IPI00305068	107 kDa	19	9	0.47	21	2	0.10	6	8	1.33	11	4	0.36	0.31	0.19
Isoform 2 of RNA-binding motif, single-stranded-interacting protein 1	IPI00033611	44 kDa	5	3	0.60	10	9	0.90	12	12	1.00	25	20	0.80	0.83	0.17
Isoform 1 of RNA-binding protein 4	IPI00003704	40 kDa	12	4	0.33	1	0	0.00	7	12	1.71	30	23	0.77	0.94	0.71
Isoform 1 of Insulin-like growth factor 2 mRNA-binding protein 3	IPI00658000	64 kDa	6	7	1.17	0	0	0.00	16	18	1.13	22	18	0.82	1.04	0.19
Isoform 2 of Double-stranded RNA-specific adenosine deaminase	IPI00025057	133 kDa	5	8	1.60	0	0	0.00	24	25	1.04	8	15	1.88	1.51	0.42
Peptidyl-prolyl cis-trans isomerase-like 1	IPI00007019	18 kDa	19	10	0.53	12	3	0.25	14	4	0.29	7	2	0.29	0.34	0.13
Heterogeneous nuclear ribonucleoprotein H	IPI00013881	49 kDa	10	14	1.40	3	4	1.33	13	12	0.92	18	8	0.44	1.03	0.44
Trypsin-1	IPI00011694	27 kDa	10	12	1.20	10	13	1.30	3	3	1.00	3	24	8.00	1.17	0.15
Isoform 1 of Polyadenylate-binding protein 4	IPI00012726	71 kDa	13	6	0.46	4	3	0.75	16	8	0.50	14	8	0.57	0.57	0.13
60S acidic ribosomal protein P0	IPI00008530	34 kDa	3	3	1.00	1	0	0.00	18	15	0.83	28	12	0.43	0.75	0.29
Small nuclear ribonucleoprotein Sm D3	IPI00017964	14 kDa	7	5	0.71	14	16	1.14	4	3	0.75	8	9	1.13	0.93	0.23
Small nuclear ribonucleoprotein Sm D1	IPI00302850	13 kDa	16	16	1.00	7	8	1.14	5	4	0.80	13	11	0.85	0.95	0.16
Protein CWC15 homolog	IPI00009009	27 kDa	9	4	0.44	2	3	1.50	11	12	1.09	12	8	0.67	0.93	0.47
cDNA FLJ77422	IPI00011268	33 kDa	4	2	0.50	0	1	0.00	10	7	0.70	13	10	0.77	0.66	0.14
Isoform 1 of Protein lin-28 homolog B	IPI00398131	27 kDa	2	1	0.50	1	0	0.00	16	9	0.56	27	12	0.44	0.50	0.06
Small nuclear ribonucleoprotein Sm D2	IPI00017963	14 kDa	11	9	0.82	12	8	0.67	7	7	1.00	8	9	1.13	0.90	0.20
Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	IPI00216049	51 kDa	5	11	2.20	0	3	n/a	15	8	0.53	21	6	0.29	1.01	1.04
Heterogeneous nuclear ribonucleoprotein F	IPI00003881	46 kDa	6	13	2.17	0	3	n/a	12	9	0.75	11	10	0.91	1.28	0.78
Keratin, type II cytoskeletal 75	IPI00005859	60 kDa	8	8	1.00	5	9	1.80	8	5	0.63	10	12	1.20	1.16	0.49
DEAD (Asp-Glu-Ala-Asp) box polypeptide 39 transcript variant	IPI00062206	35 kDa	1	4	4.00	0	2	n/a	7	3	0.43	25	16	0.64	1.69	2.00
Splicing factor 3B subunit 4	IPI00017339	44 kDa	7	12	1.71	8	10	1.25	4	4	1.00	4	9	2.25	1.55	0.55
ubiquitin and ribosomal protein S27a precursor	IPI00179330	18 kDa	5	8	1.60	2	9	4.50	5	12	2.40	9	7	0.78	2.32	1.60
Small nuclear ribonucleoprotein E	IPI00029266	11 kDa	13	13	1.00	9	7	0.78	6	7	1.17	2	1	0.50	0.86	0.29
60S ribosomal protein L7	IPI00030179	29 kDa	2	1	0.50	0	2	n/a	17	13	0.76	8	8	1.00	0.75	0.25
Tubulin beta-1 chain	IPI00006510	50 kDa	2	0	0.00	0	0	0.00	12	12	1.00	11	17	1.55	1.27	0.39
Propionyl Coenzyme A carboxylase, alpha polypeptide	IPI00552081	17 kDa	0	3	n/a	8	6	0.75	1	4	4.00	17	18	1.06	1.94	1.79
Isoform 1 of Polypyrimidine tract-binding protein 1	IPI00179964	57 kDa	2	4	2.00	0	0	0.00	7	7	1.00	12	1	0.08	0.77	0.94
poly(rC)-binding protein 2 isoform b	IPI00012066	38 kDa	0	1	n/a	0	2	n/a	14	8	0.57	17	11	0.65	0.61	0.05
Full-length cDNA 5-PRIME end of clone CS0DJ009YL13	IPI00384016	30 kDa	8	9	1.13	4	16	4.00	5	5	1.00	6	1	0.17	1.57	1.67
pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	IPI00396435	91 kDa	3	1	0.33	0	0	0.00	16	5	0.31	9	6	0.67	0.44	0.20
Splicing factor 3B subunit 5	IPI00010404	10 kDa	9	12	1.33	17	6	0.35	5	3	0.60	0	0	0.00	0.76	0.51
Protein BUD31 homolog	IPI00013180	17 kDa	13	6	0.46	9	7	0.78	5	2	0.40	1	1	1.00	0.66	0.28
Isoform 1 of Putative helicase MOV-10	IPI00444452	114 kDa	3	8	2.67	0	1	n/a	18	13	0.72	2	1	0.50	1.30	1.19
Protein Red	IPI00011875	66 kDa	7	3	0.43	5	2	0.40	5	8	1.60	6	8	1.33	0.94	0.62
Complement C4-A	IPI00032258	193 kDa	5	2	0.40	2	2	1.00	6	8	1.33	9	14	1.56	1.07	0.50
Isoform 1 of GTP-binding protein 8	IPI00107246	32 kDa	8	3	0.38	5	4	0.80	10	7	0.70	4	4	1.00	0.72	0.26
Isoform 1 of Heterogeneous nuclear ribonucleoprotein D-like	IPI00011274	46 kDa	4	3	0.75	1	2	2.00	6	6	1.00	15	8	0.53	1.07	0.65
Isoform 1 of U4/U6 small nuclear ribonucleoprotein Prp4	IPI00150269	58 kDa	16	2	0.13	10	5	0.50	6	7	1.17	1	0	0.00	0.31	0.27
Guanine nucleotide-binding protein subunit beta-2-like 1	IPI00848226	35 kDa	0	0	0.00	0	0	0.00	14	12	0.86	14	6	0.43	0.64	0.30
Heterogeneous nuclear ribonucleoprotein L	IPI00027834	64 kDa	0	1	n/a	3	1	0.33	6	5	0.83	3	1	0.33	0.50	0.29
RNA binding motif protein, X-linked-like 1	IPI00061178	42 kDa	3	3	1.00	0	2	n/a	6	2	0.33	19	5	0.26	0.53	0.41
Isoform 1 of DNA-binding protein A	IPI00031801	40 kDa	4	6	1.50	3	2	0.67	5	8	1.60	9	5	0.56	1.08	0.55
Isoform 2 of Spliceosome RNA helicase BAT1	IPI00641829	51 kDa	4	6	1.50	1	1	1.00	4	5	1.25	12	10	0.83	1.15	0.29
Keratin, type I cytoskeletal 24	IPI00004550	55 kDa	2	6	3.00	8	10	1.25	1	2	2.00	5	8	1.60	1.96	0.76
Isoform 2 of RISC-loading complex subunit TARBP2	IPI00472583	37 kDa	3	3	1.00	0	2	n/a	7	0	0.00	15	10	0.67	0.83	0.24
Pre-mRNA-splicing factor CWC22 homolog	IPI00177381	105 kDa	6	4	0.67	0	0	0.00	8	5	0.63	6	7	1.17	0.82	0.30
40S ribosomal protein S3	IPI00011253	27 kDa	1	0	0.00	0	0	0.00	10	6	0.60	16	7	0.44	0.52	0.11
40S ribosomal protein S4, X isoform	IPI00217030	30 kDa	1	1	1.00	0	0	0.00	13	6	0.46	13	3	0.23	0.56	0.39
Alpha-ketoglutarate dehydrogenase complex dihydrolipoyl succinyltransferase	IPI00033034	49 kDa	6	8	1.33	3	8	2.67	1	3	3.00	2	2	1.00	2.00	0.98
Isoform 1 of Insulin-like growth factor 2 mRNA-binding protein 2	IPI00179713	66 kDa	5	10	2.00	1	0	0.00	19	14	0.74	15	11	0.73	1.16	0.73
Isoform 1 of ELAV-like protein 2	IPI00030250	40 kDa	4	2	0.50	0	0	0.00	6	5	0.83	12	6	0.50	0.61	0.19
Poly(rC)-binding protein 1	IPI00016610	37 kDa	0	1	n/a	1	2	2.00	0	7	n/a	13	14	1.08	1.54	0.65
Protein mago nashi homolog 2	IPI00059292	17 kDa	10	1	0.10	11	0	0.00	2	7	3.50	0	5	n/a	0.05	0.07
ADP/ATP translocase 2	IPI00007188	33 kDa	2	1	0.50	0	0	0.00	6	6	1.00	11	7	0.64	0.71	0.26
Isoform Long of Acetyl-CoA carboxylase 2	IPI00013003	277 kDa	0	0	0.00	1	0	0.00	12	10	0.83	3	5	1.67	1.25	0.59
Iso 1 of Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	IPI00784044	61 kDa	12	0	0.00	0	0	0.00	7	12	1.71	1	1	1.00	0.90	0.86
Probable ATP-dependent RNA helicase DDX5	IPI00017617	69 kDa	0	3	n/a	1	2	2.00	8	3	0.38	13	3	0.23	0.87	0.98
Heterogeneous nuclear ribonucleoprotein G	IPI00304692	42 kDa	0	1	n/a	0	0	0.00	7	1	0.14	19	5	0.26	0.20	0.09
Pre-mRNA-splicing factor SYF2	IPI00022963	29 kDa	6	3	0.50	0	5	n/a	4	6	1.50	3	3	1.00	1.00	0.50
ATP-dependent RNA helicase A	IPI00844578	141 kDa	3	2	0.67	0	0	0.00	10	5	0.50	9	1	0.11	0.43	0.29
RNA-binding motif protein, X-linked-like-2	IPI00004450	43 kDa	0	3	n/a	0	0	0.00	5	4	0.80	16	6	0.38	0.59	0.30

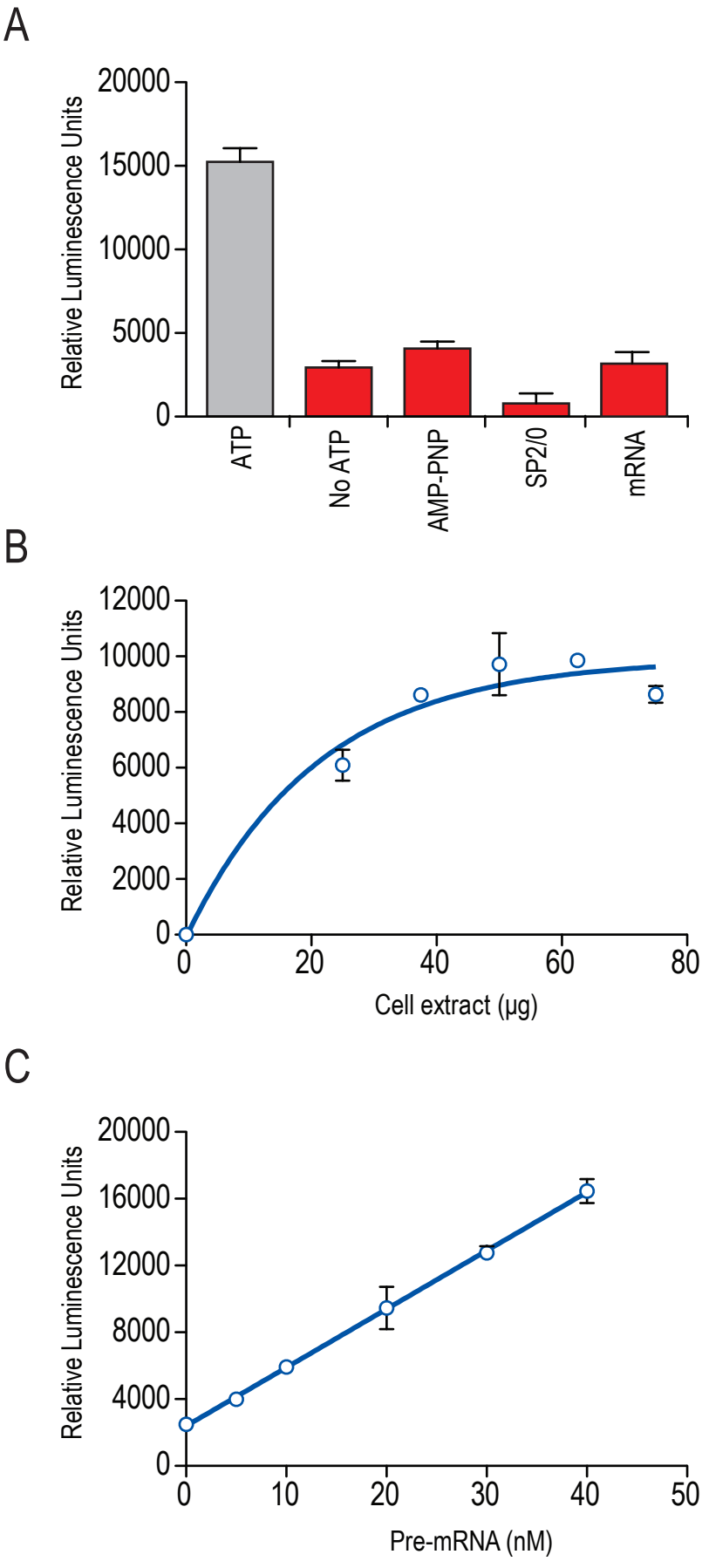
Table S1. Peptide counts for top proteins (70-137) detected by mass spectrometry of DMSO and BN82685 streptavidin pull downs.

Top 200 Identified Proteins	Accession Number	Molecular Weight	Exp 1 DMSO	Exp 1 BN82685	ratio	Exp 2 DMSO	Exp 2 BN82685	ratio	Exp 3A DMSO	Exp 3A BN82685	ratio	Exp 3B DMSO	Exp 3B BN82685	ratio	Average	Std Dev
Isoform 1 of Far upstream element-binding protein 3	IPI00377261	62 kDa	1	1	1.00	2	4	2.00	7	4	0.57	6	2	0.33	0.98	0.74
Survival of motor neuron-related-splicing factor 30	IPI00025176	27 kDa	4	1	0.25	0	3	n/a	7	8	1.14	5	1	0.20	0.53	0.53
Nuclear cap-binding protein subunit 1	IPI00019380	92 kDa	3	1	0.33	0	0	0.00	11	10	0.91	2	1	0.50	0.58	0.30
Keratin, type II cytoskeletal 5	IPI00009867	62 kDa	8	7	0.88	1	2	2.00	2	2	1.00	2	2	1.00	1.22	0.52
Isoform 2 of Insulin-like growth factor 2 mRNA-binding protein 3	IPI00165467	22 kDa	3	0	0.00	0	0	0.00	6	3	0.50	10	7	0.70	0.60	0.14
Isoform F of Protein SON	IPI00000192	264 kDa	4	3	0.75	0	0	0.00	9	6	0.67	2	2	1.00	0.81	0.17
Small nuclear ribonucleoprotein F	IPI00220528	10 kDa	3	3	1.00	9	7	0.78	2	1	0.50	1	2	2.00	1.07	0.65
Nucleolysin TIAR	IPI00005615	42 kDa	8	0	0.00	0	0	0.00	2	2	1.00	7	4	0.57	0.52	0.50
Isoform 1 of DNA-dependent protein kinase catalytic subunit	IPI00296337	469 kDa	3	1	0.33	2	2	1.00	2	3	1.50	4	2	0.50	0.83	0.53
Microfibrillar-associated protein 1	IPI00022790	52 kDa	4	0	0.00	0	0	0.00	8	8	1.00	4	1	0.25	0.63	0.53
Peptidyl-prolyl cis-trans isomerase H	IPI00007346	19 kDa	9	5	0.56	5	1	0.20	2	0	0.00	0	0	0.00	0.38	0.25
Small nuclear ribonucleoprotein G	IPI00016572	8 kDa	7	7	1.00	4	1	0.25	0	1	n/a	0	1	n/a	0.63	0.53
Isoform 1 of 60S ribosomal protein L12	IPI00024933	18 kDa	1	2	2.00	0	0	0.00	10	4	0.40	8	0	0.00	0.80	1.06
Interferon-inducible dsRNA-dependent protein kinase activator A	IPI00021167	34 kDa	4	0	0.00	0	0	0.00	7	6	0.86	5	2	0.40	0.63	0.32
Isoform 1 of U4/U6 small nuclear ribonucleoprotein Prp3	IPI00005861	78 kDa	14	1	0.07	3	0	0.00	1	5	5.00	0	0	0.00	0.04	0.05
Isoform 2 of NLR family member X1	IPI00377214	102 kDa	0	1	n/a	5	0	0.00	0	0	0.00	3	2	0.67	0.33	0.47
Isoform 4 of Heterogeneous nuclear ribonucleoprotein A/B	IPI00106509	31 kDa	0	0	0.00	0	0	0.00	3	1	0.33	10	8	0.80	0.57	0.33
A-kinase anchor protein 8	IPI00014474	76 kDa	0	1	0.00	0	0	0.00	5	6	1.20	2	6	3.00	2.10	1.27
Isoform Short of Heterogeneous nuclear ribonucleoprotein U	IPI00479217	89 kDa	0	2	n/a	0	0	0.00	6	7	1.17	1	0	0.00	1.17	0.00
Ribosomal protein L14 variant	IPI00555744	24 kDa	1	0	0.00	0	0	0.00	9	8	0.89	2	2	1.00	0.94	0.08
Tubulin beta-2A chain	IPI00013475	50 kDa	0	2	n/a	0	0	0.00	9	10	1.11	4	0	0.00	0.56	0.79
Endoribonuclease Dicer	IPI00219036	219 kDa	0	1	n/a	0	0	0.00	19	0	0.00	2	0	0.00	0.00	0.00
60S ribosomal protein L24	IPI00306332	18 kDa	5	1	0.20	0	0	0.00	4	5	1.25	7	0	0.00	0.48	0.67
Nuclease-sensitive element-binding protein 1	IPI00031812	36 kDa	1	0	0.00	0	0	0.00	5	5	1.00	1	3	3.00	2.00	1.41
Isoform 1 of Serine/arginine repetitive matrix protein 2	IPI00782992	300 kDa	7	7	1.00	0	0	0.00	4	2	0.50	0	0	0.00	0.75	0.35
Isoform 1 of Bcl-2-associated transcription factor 1	IPI00006079	106 kDa	0	0	0.00	0	0	0.00	7	12	1.71	0	0	0.00	1.71	0.00
Zinc finger matrin-type protein 2	IPI00069734	24 kDa	0	0	0.00	1	0	0.00	6	8	1.33	1	1	1.00	1.17	0.24
Interleukin enhancer-binding factor 2	IPI00005198	43 kDa	1	1	1.00	0	0	0.00	9	0	0.00	7	1	0.14	0.38	0.54
Superkiller viralicidic activity 2-like 2	IPI00647217	118 kDa	0	0	0.00	0	0	0.00	6	6	1.00	3	3	1.00	1.00	0.00
NHP2-like protein 1	IPI00026167	14 kDa	7	3	0.43	7	0	0.00	0	0	0.00	0	0	0.00	0.21	0.30
60S ribosomal protein L13a	IPI00304612	24 kDa	0	0	0.00	0	0	0.00	10	6	0.60	0	0	0.00	0.60	0.00
Pyruvate dehydrogenase E1 component subunit alpha, testis-specific	IPI00024087	43 kDa	2	0	0.00	1	6	6.00	0	2	n/a	1	5	5.00	5.50	0.71
Isoform ASF-1 of Splicing factor, arginine/serine-rich 1	IPI00215884	28 kDa	0	0	0.00	0	0	0.00	6	6	1.00	3	1	0.33	0.67	0.47
Iso 2 of Serine/threonine-protein phosphatase PGAM5, mitochondrial	IPI00063242	28 kDa	0	0	0.00	0	0	0.00	10	4	0.40	5	0	0.00	0.20	0.28
Isoform 1 of Clathrin heavy chain 1	IPI00024067	192 kDa	0	0	0.00	0	0	0.00	4	4	1.00	0	0	0.00	1.00	0.00
DEAH (Asp-Glu-Ala-His) box polypeptide 16	IPI00292510	63 kDa	4	2	0.50	0	0	0.00	7	1	0.14	2	1	0.50	0.38	0.21
Propionyl Coenzyme A carboxylase, alpha polypeptide	IPI00553241	13 kDa	0	0	0.00	1	3	3.00	1	2	2.00	5	5	1.00	2.00	1.00
40S ribosomal protein S11	IPI00025091	18 kDa	1	1	1.00	0	0	0.00	3	2	0.67	6	0	0.00	0.56	0.51
Isoform 2 of Heterogeneous nuclear ribonucleoprotein A/B	IPI00334587	36 kDa	0	0	0.00	0	0	0.00	5	3	0.60	5	5	1.00	0.40	0.49
60S ribosomal protein L21	IPI00247583	19 kDa	1	0	0.00	0	0	0.00	2	2	1.00	6	2	0.33	0.67	0.47
Isoform 1 of RNA-binding protein 8A	IPI00001757	20 kDa	4	0	0.00	1	0	0.00	2	3	1.50	0	2	n/a	0.75	1.06
Polymerase delta interacting protein 46	IPI00429180	20 kDa	0	0	0.00	0	0	0.00	4	3	0.75	2	5	2.50	1.62	1.24
40S ribosomal protein S8	IPI00216587	24 kDa	0	0	0.00	0	0	0.00	5	6	1.20	4	0	0.00	0.60	0.85
Elongation factor 1-alpha 2	IPI00014424	50 kDa	4	5	1.25	1	1	1.00	2	2	1.00	0	0	0.00	1.08	0.14
40S ribosomal protein S15a	IPI00221091	15 kDa	1	1	1.00	1	0	0.00	8	3	0.38	1	0	0.00	0.69	0.44
Isoform 1 of Peptidyl-prolyl cis-trans isomerase CWC27 homolog	IPI00025174	54 kDa	4	3	0.75	2	0	0.00	2	5	2.50	0	0	0.00	1.62	1.24
Isoform 4 of Dynein heavy chain 6, axonemal	IPI00884959	290 kDa	0	0	0.00	5	5	1.00	1	2	2.00	0	0	0.00	1.50	0.71
cDNA FLJ54030, highly similar to Polymerase delta-interacting protein 3	IPI00440688	48 kDa	0	0	0.00	0	0	0.00	5	11	2.20	0	0	0.00	2.20	0.00
Isoform 3 of Probable ATP-dependent RNA helicase DDX17	IPI00651653	73 kDa	1	1	1.00	0	0	0.00	6	4	0.67	2	1	0.50	0.72	0.25
Isoform 1 of Peptidyl-prolyl cis-trans isomerase-like 2	IPI00003824	59 kDa	2	0	0.00	0	0	0.00	3	5	1.67	1	1	1.00	1.33	0.47
Protein DGCR14	IPI00165171	53 kDa	0	0	0.00	0	0	0.00	3	9	3.00	0	0	0.00	3.00	0.00
Putative uncharacterized protein ENSP00000382160	IPI00180956	49 kDa	1	3	3.00	1	1	1.00	2	1	0.50	0	4	n/a	1.50	1.32
Targeting protein for Xklp2	IPI00008477	86 kDa	2	1	0.50	0	0	0.00	4	3	0.75	2	1	0.50	0.58	0.14
WD repeat domain 57 (U5 snRNP specific), isoform CRA_b	IPI00385642	14 kDa	3	0	0.00	0	0	0.00	2	5	2.50	1	0	0.00	1.25	1.77
HLA-B associated transcript 1	IPI00641634	26 kDa	0	0	0.00	0	0	0.00	2	2	1.00	5	5	1.00	1.00	0.00
TDP43	IPI00025815	45 kDa	1	1	1.00	0	0	0.00	2	4	2.00	2	2	1.00	1.33	0.58
U6 snRNA-associated Sm-like protein LSm3	IPI00219229	12 kDa	3	0	0.00	5	2	0.40	1	1	1.00	0	0	0.00	0.46	0.50
U4/U6.U5 tri-snRNP-associated protein 1	IPI00021417	90 kDa	8	1	0.13	1	1	1.00	0	1	n/a	0	0	0.00	0.56	0.62
Isoform 1 of Heterogeneous nuclear ribonucleoprotein A3	IPI00419373	40 kDa	0	2	n/a	0	0	0.00	3	1	0.33	6	0	0.00	0.17	0.24
ATP-dependent RNA helicase DHX8	IPI00031508	139 kDa	0	1	n/a	1	0	0.00	0	5	n/a	1	3	3.00	3.00	0.00
Isoform 3 of PCI domain-containing protein 2	IPI00072541	43 kDa	3	1	0.33	0	0	0.00	1	3	3.00	2	0	0.00	1.66	1.89
32 kDa protein	IPI00399077	32 kDa	0	0	0.00	0	0	0.00	3	3	1.00	5	1	0.20	0.60	0.57
HSPA5 protein	IPI00003362	72 kDa	5	3	0.60	1	1	1.00	0	0	0.00	0	0	0.00	0.80	0.28
33 kDa protein	IPI00413108	33 kDa	0	0	0.00	0	0	0.00	5	2	0.40	5	0	0.00	0.20	0.28
Protein CASC3	IPI00289491	76 kDa	0	0	0.00	0	0	0.00	0	8	n/a	0	3	n/a	0.00	0.00
Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0	IPI00028888	38 kDa	0	0	0.00	0	0	0.00	4	5	1.25	9	5	0.56	0.90	0.49
Splicing factor, arginine/serine-rich 9	IPI00012340	26 kDa	0	0	0.00	0	0	0.00	6	1	0.17	2	0	0.00	0.17	0.00
Isoform 1 of Splicing factor U2AF 65 kDa subunit	IPI00031556	54 kDa	2	0	0.00	0	0	0.00	7	1	0.14	0	0	0.00	0.14	0.00

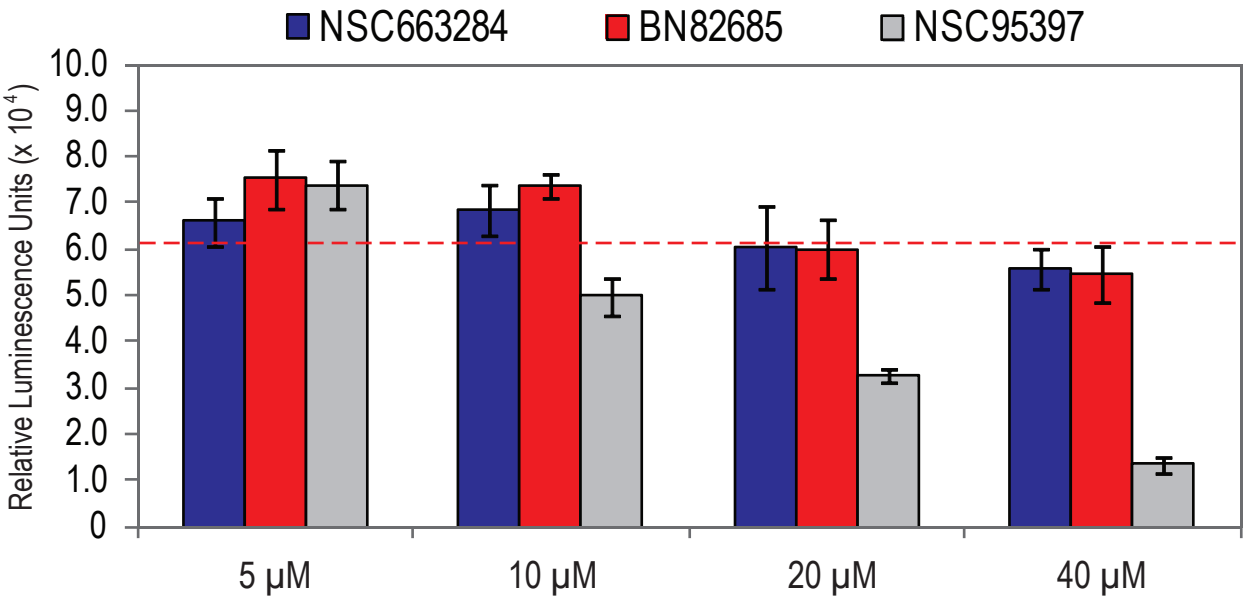
Table S1. Peptide counts for top proteins (138-203) detected by mass spectrometry of DMSO and BN82685 streptavidin pull downs.



Berg et al. Figure S1



Berg et al. Figure S2



Berg et al. Figure S3