

Supplementary figure legends

Fig. S1. Visual display of CanScript-bound proteins. Proteins captured by biotinylated wild-type or mutant CanScript $\times 3$ probe from AsPc1 nuclear extracts were released by boiling in loading buffer. Proteins were separated by SDS-PAGE followed by silver stain. Although “specific” protein bands were present in the C5M6c (mutant DNA)-bound sample, some of which are marked by arrowheads, excision and analysis of these bands showed them to be non-relevant. WT, wild-type.

Fig. S2. Verification of hits identified by TFM. Five arbitrarily chosen hits from Table 2 were examined by immunoblot following DNA affinity-capture. The protocol was as described in Fig. 2.

Fig. S3, Dependence of CanScript activity on expression of interactome genes. The siRNA sequences from Fig. 5 were tested for their effects using the pGL3-Can3 reporter. *TEAD1* siRNA provided a positive control. The activity of the irrelevant *FANCD2 (Irr)* siRNA-transfected sample was defined as “1”. The average activity was obtained from triplicate wells in each experiment. RLA, relative luciferase activity, as defined in Methods, was the measure of activity. Two independent experiments performed on different days had high agreement; a representative experiment is presented.

Fig. S4. No effects of *NAB1* and *RFX1* siRNAs on *TEAD1* expression. siRNA treatments of (A) *NAB1* and (B) *RFX1* were examined for effects on *TEAD1* expression in HeLa cells. Each gene was tested with the same siRNA sets in Fig. 5. Irrelevant target *FANCD2 (Irr)* and *TEAD1* siRNA served as negative and positive controls, respectively. GAPDH was used as a comparison for sample loading.

Figure S1

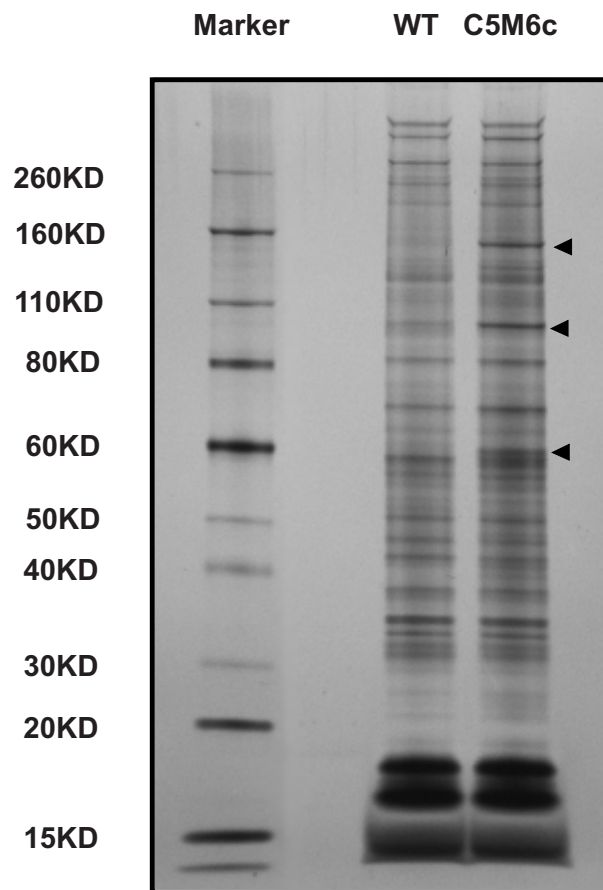


Figure S2

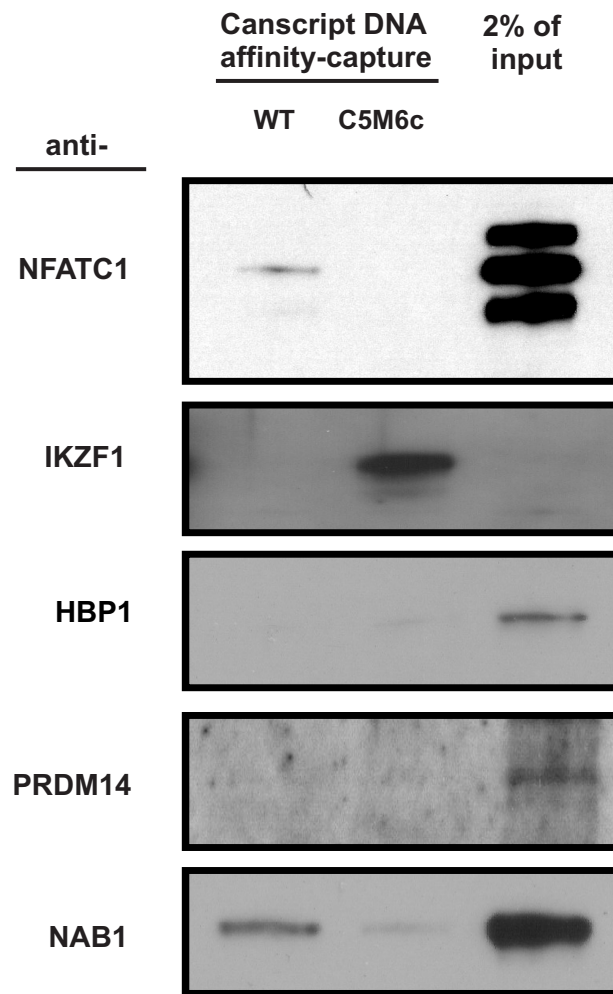


Figure S3

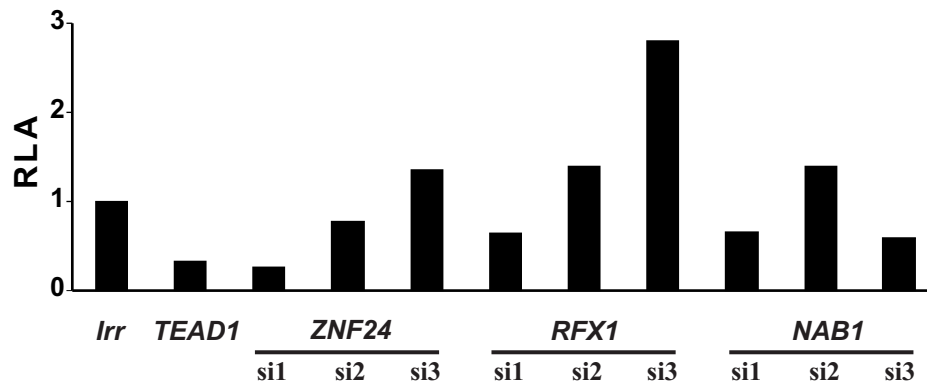
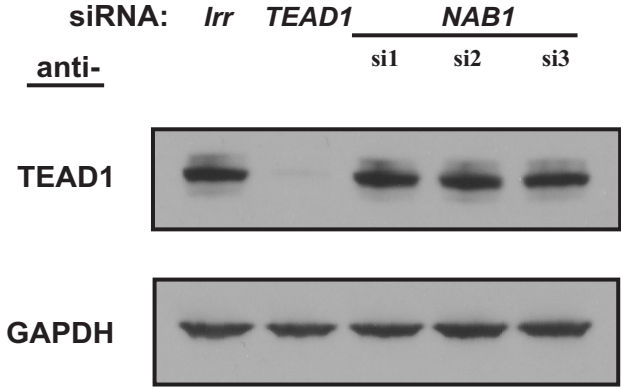


Figure S4

A



B

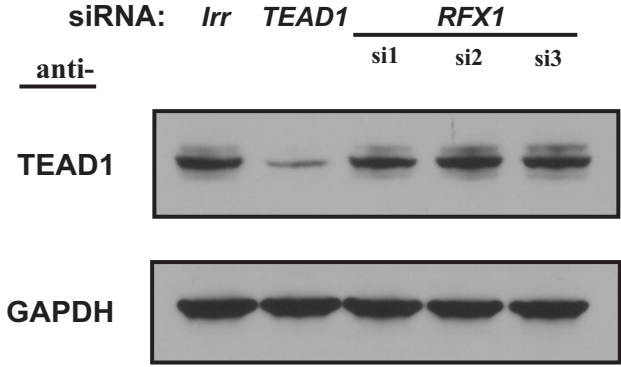


Table S1**List of proteins binding Canscript sequence as identified by SD-MS***

Accession	GeneSymbol	ΣCoverage	Σ# Peptides	Σ# PSMs	Area	Heavy/Light*	Identified protein full name
NP_003205.2	TEAD3 [§]	29.43	10	24	1.43E+07	100	transcriptional enhancer factor TEF-5
NP_003204.2	TEAD4 [§]	26.73	9	21	1.39E+07	100	transcriptional enhancer factor TEF-3 isoform 1
NP_004354.2	CEACAM5	3.13	2	4	1.58E+06	100	carcinoembryonic antigen-related cell adhesion molecule 5 preproprotein
NP_005550.2	LAMA1	0.42	1	1	5.60E+06	100	laminin subunit alpha-1 precursor
NP_068780.2	TEAD1 [§]	33.8	12	47	2.79E+07	93.142	transcriptional enhancer factor TEF-1
NP_001078936.1	CTNND1	1.8	1	1	5.18E+05	55.402	catenin delta-1 isoform 3A
NP_002909.4	RFX1 [§]	2.45	2	8	1.12E+06	54.289	MHC class II regulatory factor RFX1
NP_001129493.1	NFATC2 [§]	4.66	3	5	7.55E+05	49.42	nuclear factor of activated T-cells, cytoplasmic 2 isoform D
NP_008896.2	ZNF24 [§]	26.36	7	17	5.50E+06	40.886	zinc finger protein 24
NP_005957.2	NAB1 [§]	3.7	2	4	7.44E+05	40.246	NGFI-A-binding protein 1
NP_056276.1	ZNF521 [§]	0.92	1	2	2.40E+05	35.324	zinc finger protein 521
NP_001035514.1	PRDM15 [§]	1.95	2	2	7.82E+05	28.537	PR domain zinc finger protein 15 isoform 2
NP_001107019.1	LIMA1	1.83	1	1	2.02E+05	26.581	LIM domain and actin-binding protein 1 isoform c
NP_056009.1	MYO1D	2.39	2	3	4.18E+05	23.695	myosin-1d
NP_001091947.1	GRSF1	10.38	2	3	2.69E+06	14.797	G-rich sequence factor 1 isoform 2
NP_060365.7	DDX27	2.64	2	4	5.33E+05	8.429	probable ATP-dependent RNA helicase DDX27
NP_000440.1	RFX5	9.09	4	7	1.07E+06	8.319	DNA-binding protein RFX5
NP_001182461.1	SPTAN1	5.02	11	15	1.43E+06	7.445	spectrin alpha chain, brain isoform 3
NP_002351.1	MAFK	33.97	4	9	1.88E+06	6.522	transcription factor MafK
NP_003119.2	SPTBN1	6.68	12	22	1.46E+06	5.774	spectrin beta chain, brain 1 isoform 1
NP_001091.1	ACTA1	23.08	8	22	1.51E+07	5.254	actin, alpha skeletal muscle
NP_955458.1	ZFP64	9.13	6	14	3.88E+06	4.893	zinc finger protein 64 isoform c
NP_116100.2	MAFG	22.22	3	8	1.69E+06	4.607	transcription factor MafG
NP_002625.1	PHB	3.68	1	2	4.35E+05	4.295	prohibitin
NP_002474.3	CEACAM6	9.88	2	7	8.19E+06	4.257	carcinoembryonic antigen-related cell adhesion molecule 6 precursor
NP_003365.1	VDAC1	3.89	1	1	1.29E+06	4.097	voltage-dependent anion-selective channel protein 1
NP_036455.1	MAFF	27.44	3	6	2.11E+06	4.086	transcription factor MafF isoform a
NP_203693.3	MYO1C	1.56	1	1	6.87E+05	3.895	myosin-1c isoform c
NP_002061.1	GNAI2	7.32	2	5	5.36E+05	3.601	guanine nucleotide-binding protein G(i) subunit alpha-2 isoform 1
NP_001092.1	ACTB	43.47	12	37	2.29E+07	3.47	actin, cytoplasmic 1
NP_005002.3	NRF1	1.59	1	1	7.90E+05	3.435	nuclear respiratory factor 1
NP_057615.3	KLF3	24.93	5	10	8.38E+06	3.432	Kruppel-like factor 3
NP_001077007.1	POTEE	7.35	6	15	1.51E+07	3.126	POTE ankyrin domain family member E
NP_872589.1	BAZ1A	2.76	3	4	6.67E+05	2.889	bromodomain adjacent to zinc finger domain protein 1A isoform b
NP_001120668.1	CNBP	47.06	7	20	3.05E+06	2.748	cellular nucleic acid-binding protein isoform 6
NP_000215.1	KRT18	24.65	8	32	1.69E+07	2.745	keratin, type I cytoskeletal 18
NP_001017371.3	SP3	10.66	6	42	1.36E+07	2.692	transcription factor Sp3 isoform 2
NP_003357.2	UQCRC2	8.61	2	4	6.83E+05	2.563	cytochrome b-c1 complex subunit 2, mitochondrial precursor
NP_068799.2	ZNF148	6.05	4	11	9.99E+05	2.546	zinc finger protein 148
NP_665906.1	HMG1	25.23	3	14	9.55E+06	2.54	high mobility group protein HMG-I/HMG-Y isoform a
NP_003371.2	VIM	7.08	3	5	5.85E+05	2.523	vimentin
NP_001137307.1	NFRKB	0.92	1	3	1.20E+06	2.428	nuclear factor related to kappa-B-binding protein isoform 1
NP_004740.2	TBRG4	1.58	1	2	1.36E+05	2.153	protein TBRG4 isoform 1
NP_006507.2	SLC2A1	1.63	1	1	2.82E+05	2.119	solute carrier family 2, facilitated glucose transporter member 1
NP_002464.1	MYH9	6.53	9	20	1.61E+06	2.066	myosin-9
NP_066289.2	UBC	32.85	2	7	1.69E+06	2.057	polyubiquitin-C
NP_005309.1	H1FO	18.56	3	5	1.11E+06	2.054	histone H1.0
NP_001128181.1	DEK	3.52	1	1	9.57E+05	2.036	protein DEK isoform 2
NP_005313.1	HIST1H1B	23.89	8	42	2.14E+07	2.027	histone H1.5

* Protein candidates with WT/MT (heavy/light) ratio greater than 2.0 are listed.

§ Known transcription factors with WT/MT (heavy/light) ratio greater than 10

Table S2**List of proteins binding Canscript sequence as identified by SD-MS***

Accession	GeneSymbol	ΣCoverage	Σ# Peptides	Σ# PSMs	Area	Heavy/Light*	Identified protein full name
NP_006734.1	RBM3	55.41	5	17	1.37E+07	0.096	putative RNA-binding protein 3
NP_001271.1	CIRBP	44.77	7	24	1.18E+07	0.089	cold-inducible RNA-binding protein
NP_005841.1	SF3B4	17.45	4	10	3.70E+06	0.085	splicing factor 3B subunit 4
NP_002898.2	RECQL	61.94	37	267	1.14E+08	0.08	ATP-dependent DNA helicase Q1
NP_001138898.1	CSDA [§]	51.16	10	55	3.27E+07	0.075	DNA-binding protein A isoform b
NP_006187.2	PCBP1	70.79	13	98	6.72E+07	0.071	poly(rC)-binding protein 1
NP_004550.2	YBX1	54.32	15	82	9.96E+07	0.07	nuclease-sensitive element-binding protein 1
NP_005692.1	SNUPN	14.17	1	1	1.53E+06	0.068	snurportin-1
NP_006833.2	SF3B2	21.34	16	28	1.03E+07	0.067	splicing factor 3B subunit 2
NP_002902.2	UPF1	9.93	9	14	2.56E+06	0.06	regulator of nonsense transcripts 1
NP_001256.2	CDX2 [§]	7.99	2	4	1.01E+06	0.052	homeobox protein CDX-2
NP_004490.2	HNRNPAB	38.25	10	21	8.93E+06	0.05	heterogeneous nuclear ribonucleoprotein A/B isoform b
NP_068811.1	RXRβ [§]	5.07	2	5	1.12E+06	0.048	retinoic acid receptor RXR-beta
NP_006749.1	U2AF1	16.67	3	7	9.89E+05	0.042	splicing factor U2AF 35 kDa subunit isoform a
NP_061824.3	HOXA10 [§]	5.85	2	2	8.47E+05	0.041	homeobox protein Hox-A10 isoform a
NP_001020402.1	KPRP	1.55	1	2	2.36E+05	0.04	keratinocyte proline-rich protein
NP_002129.2	HNRNPD	45.42	14	33	1.41E+07	0.038	heterogeneous nuclear ribonucleoprotein D0 isoform c
NP_001129248.1	ZBTB43 [§]	5.57	2	3	1.33E+06	0.027	zinc finger and BTB domain-containing protein 43
NP_733829.1	DAZAP1	4.23	1	2	3.28E+05	0.027	DAZ-associated protein 1 isoform a
NP_056290.3	FAM98A	5.02	3	5	9.71E+05	0.027	hypothetical protein LOC25940
NP_004390.3	DDX11	1.64	1	2	4.24E+05	0.025	probable ATP-dependent RNA helicase DDX11 isoform 2
NP_148934.1	NRI12 [§]	3.02	1	1	4.31E+05	0.022	nuclear receptor subfamily 1 group I member 2 isoform 3
NP_002037.2	GAPDH	7.16	1	1	8.78E+05	0.021	glyceraldehyde-3-phosphate dehydrogenase
NP_001524.2	HNRNPL	67.74	22	144	4.02E+07	0.019	heterogeneous nuclear ribonucleoprotein L isoform a
NP_006796.1	HNRNPA0	29.84	7	16	1.60E+06	0.019	heterogeneous nuclear ribonucleoprotein A0
NP_001140171.1	RBM19	25.31	16	30	5.82E+06	0.018	probable RNA-binding protein 19
NP_003289.2	NR2C2 [§]	3.74	2	3	6.20E+05	0.017	nuclear receptor subfamily 2 group C member 2
NP_005546.2	KRT6B	24.47	15	159	2.27E+08	0.017	keratin, type II cytoskeletal 6B
NP_002937.1	RPA2	30	5	8	1.35E+06	0.015	replication protein A 32 kDa subunit
NP_055323.2	APOBEC3C	12.63	2	4	7.83E+05	0.014	probable DNA dC-dU-editing enzyme APOBEC-3C
NP_114366.1	PCBP2	67.4	13	96	9.75E+07	0.014	poly(rC)-binding protein 2 isoform b
NP_001009931.1	HRNR	15.44	11	46	4.82E+06	0.014	hornerin
NP_071320.2	TIA1	3.47	1	1	6.90E+05	0.014	nucleolysin TIA-1 isoform p40 isoform 1
NP_055427.2	FAM120A	5.37	4	6	1.37E+06	0.013	constitutive coactivator of PPAR-gamma-like protein 1
NP_005545.1	KRT6A	24.29	15	128	2.12E+08	0.013	keratin, type II cytoskeletal 6A
NP_060101.3	DDX60	0.76	1	1	7.04E+05	0.013	probable ATP-dependent RNA helicase DDX60
NP_444513.1	DCD	10	1	2	8.74E+05	0.013	dermcidin preproprotein
NP_003015.2	ITSN1	1.05	1	1	1.40E+06	0.012	intersectin-1 isoform ITSN-I
NP_067064.2	WIZ	5.29	3	5	1.87E+06	0.012	protein Wiz
NP_000415.2	KRT5	30	23	150	1.59E+08	0.011	keratin, type II cytoskeletal 5
NP_596869.4	TTN	0.04	1	2	6.78E+05	0.011	titin isoform N2-A
NP_001007226.1	IGF2BP2	10.07	4	17	8.08E+06	0.011	insulin-like growth factor 2 mRNA-binding protein 2 isoform b
NP_005548.2	KRT16	31.08	15	138	8.98E+07	0.011	keratin, type I cytoskeletal 16
NP_149022.3	KRT82	1.75	1	6	1.01E+08	0.011	keratin, type II cuticular Hb2
NP_002760.1	PRSS1	4.05	1	1	8.18E+05	0.01	trypsin-1 preproprotein
NP_000468.1	ALB	2.46	1	21	4.51E+06	0.01	serum albumin preproprotein
NP_001014364.1	FLG2	6.98	6	16	2.21E+06	0.01	filaggrin-2
NP_976308.1	ZNF784 [§]	16.72	4	5	1.75E+06	0.01	zinc finger protein 784
NP_002193.2	ISL1 [§]	6.59	2	2	1.15E+06	0.01	insulin gene enhancer protein ISL-1
NP_000517.2	KRT14	29.03	15	157	8.98E+07	0.01	keratin, type I cytoskeletal 14
NP_001122385.1	PCBP2	78.51	13	93	9.75E+07	0.01	poly(rC)-binding protein 2 isoform f
NP_000217.2	KRT9	56.02	25	418	1.04E+08	0.01	keratin, type I cytoskeletal 9
NP_006112.3	KRT1	65.84	39	743	3.26E+08	0.01	keratin, type II cytoskeletal 1
NP_001008844.1	DSP	1.63	4	5	1.50E+06	0.01	desmoplakin isoform II
NP_006538.2	IGF2BP3	36.79	16	58	1.26E+07	0.01	insulin-like growth factor 2 mRNA-binding protein 3
NP_000412.3	KRT10	62.84	34	780	3.66E+08	0.01	keratin, type I cytoskeletal 10
NP_000414.2	KRT2	88.42	45	658	2.93E+08	0.01	keratin, type II cytoskeletal 2 epidermal
NP_573566.2	LRPPRC	75.68	93	476	1.50E+08	0.01	leucine-rich PPR motif-containing protein, mitochondrial precursor
NP_004478.3	GOLGB1	0.49	1	1	4.55E+06	0.01	Golgin subfamily B member 1
NP_775487.2	KRT78	12.12	6	11	1.05E+07	0.01	keratin, type II cytoskeletal 78
NP_003642.3	CSDA [§]	41.94	10	53	3.27E+07	0.01	DNA-binding protein A isoform a
NP_001167566.1	ZEB1 [§]	6.24	5	10	1.41E+06	0.01	zinc finger E-box-binding homeobox 1 isoform e
NP_114368.1	PTBP1	72.88	23	195	1.53E+08	0.01	polypyrimidine tract-binding protein 1 isoform c
NP_853515.2	KRT27	5.88	3	67	1.08E+08	0.01	keratin, type I cytoskeletal 27
NP_004939.1	DSC1	1.79	1	2	2.48E+06	0.01	desmocollin-1 isoform Dsc1b preproprotein
NP_001136122.1	HNRPLL	64.06	23	82	1.93E+07	0.01	heterogeneous nuclear ribonucleoprotein L-like isoform 2
NP_001157260.1	ROD1	10.11	4	13	2.23E+07	0.01	regulator of differentiation 1 isoform 2
NP_057726.3	SPTBN5	0.44	1	1	1.54E+06	0.01	spectrin beta chain, brain 4
NP_002889.1	RBMS2	6.14	2	5	1.44E+06	0.01	RNA-binding motif, single-stranded-interacting protein 2

NP_112487.1	SLIRP	60.55	7	23	1.22E+07	0.01	SRA stem-loop-interacting RNA-binding protein, mitochondrial precursor
NP_001159506.1	SBSN	17.63	3	10	1.03E+06	0.01	suprabasin isoform 1 precursor
NP_001933.2	DSG1	3.05	2	3	4.46E+06	0.01	desmoglein-1 preproprotein
NP_689824.2	LRRN4	2.7	1	6	1.54E+08	0.01	leucine-rich repeat neuronal protein 4 precursor
NP_078843.2	HMBOX1 [§]	16.9	6	21	5.65E+06	0.01	homeobox-containing protein 1
NP_002888.1	RBMS1	9.93	3	6	1.64E+06	0.01	RNA-binding motif, single-stranded-interacting protein 1 isoform c
NP_002936.1	RPA1	36.53	15	38	5.44E+06	0.01	replication protein A 70 kDa DNA-binding subunit
NP_002732.3	PKN1	1.27	1	1	5.32E+06	0.01	serine/threonine-protein kinase N1 isoform 2
NP_112583.2	PMFBP1	0.6	1	2	3.14E+06	0.01	polyamine-modulated factor 1-binding protein 1 isoform a
NP_005800.3	PRDX2	5.56	1	2	1.53E+06	0.01	peroxiredoxin-2 isoform a
NP_001036024.3	EIF4G2	0.69	1	1	8.63E+05	0.01	eukaryotic translation initiation factor 4 gamma 2 isoform 2
NP_659489.1	UPRT	2.27	1	4	5.65E+06	0.01	uracil phosphoribosyltransferase homolog
NP_065850.1	STAMBPL1	1.61	1	1	1.84E+06	0.01	AMSH-like protease
NP_001157855.1	STAU2	2.76	1	1	4.39E+06	0.01	double-stranded RNA-binding protein Staufen homolog 2 isoform d

* Protein candidates with WT/MT (heavy/light) ratio of less than 0.1 are listed.

§ Known transcription factors