	HaloTag fusion protein		DUX4term	DUX4c		
Function		1-424	349-424	1-374		
Function Cytoskeletal organization (GO0006996: organelle organization)		number of identified peptides				
	Actin and actin-associated proteins					
Uniprot nb Q9BTI9	* [¶] NPM1, nucleophosmin	21 (C)				
F8W7K3	*SPTAN1 spectrin alpha-chain, subunit of fodrin	21 (C)				
D6W5C0	*SPTBN1 Spectrin, beta-chain, subunit of fodrin	17 (C)				
Q53SB5	Desmin, isoform CRA a	6 (C) 14 (C)				
Q13707	¹ Actin, ACTA2 protein	14 (C)		E (NI)		
Q13707 Q6UYC3	Lamin A/C, LMNA	E (C)		5 (N)		
QOUICS	Only peptides common to lamin A and C were identified	5 (C)				
Q1HE25	Actinin alpha 1 isoform b, ACTN1	2 (C)				
B4DM97	*highly similar to α -centractin/ARP1	2 (C)				
F8WCF6	*ARPC4 (actin-related protein 2/3 complex, subunit 4) and TTLL3 (tubulin tyrosine ligase-like family, member 3), fusion protein	2 (C)				
Q6FI97	Actin-Like 6A/ARP4	2(C)				
Q5T0I2	Gelsolin, GSN	2 (C)				
Q60FE2	[¶] MYH9 (non-muscle MHC-IIA) variant protein	9 (C)				
B7Z6Z4	MYL6		1(C)			
F2Z2D5	POF1B, non-muscle	4 (C)				
Q53R15	MYL1, myosin, light chain 1	2 (C)	3 (C)			
Q5VU72	Tropomyosin 3, TPM3, non-muscle	2 (C)				
B5BU28	CTNNB1, Catenin beta 1	4 (C)				
E7ETZO	CALM1, calmodulin	3 (C)				
Q9BRL5	CALM3, calmodulin		2 (C)			
Q5VXV3	SET	2(C)				
C9JPM4	§ADP-Ribosylation Factor 41, ARF4	2(C)		2(C)		
	Microtubule-associated and centrosomes organization					
Q9H2R7	* Nuclear Distribution Protein, NUDC	2(C)				
A4D177	Chromobox homolog 3, CBX3	2(C)				
B5MDF5	GTP-binding nuclear protein RAN	5(C)				
Q06S70	*TREX2	2(C)				
* NPM1, AF	RP1, fodrin and Catenin beta 1	see above*				
protein fold	ing (GO:0006457)					
	Actin and tubulin folding					
F5H282	[§] Chaperonin containing TCP1 subunit alpha, CCTA	5 (C)		3 (C)		
B7ZAT2	TCP1, subunit beta, isoform	2 (C)				
F8VQ14	TCP1, subunit beta, CCT2			7 (C)		
Q5SZY0	TCP1 subunit gamma, CCT3	7 (C)		5 (C)		
F5H5W3	TCP1 subunit delta, CCT4	4 (C)				

S3 Table. DUX4 partners identified by HaloTag co-purification in human TE671 and LHCN-M2

B7Z9L0	TCP1 subunit delta, CCT4			5 (C)
A8K2X8	highly similar to TCP1 subunit epsilon, CCT5			5 (C)
B4DDU6	highly similar to TCP 1 subunit epsilon	3 (C)		
Q59ET3	TCP1 subunit 6A isoform a variant	3 (C)		
Q53HV2	§TCP1 subunit eta variant	4 (C)		
Q6IBT3	TCP1 subunit eta, CCT7			3 (C)
Q7Z759	TCP1 subunit theta, CCT8	7 (C)		
Q53HU0	TCP1 subunit theta variant			7 (C)
	Others			
Q5CAQ5	HSP90B1, Heat Shock Protein 90 kDa Beta (Grp94), Member 1	18 (C)		9 (C)
Q53RC7	disulfide isomerase family A, member 6, isoform	8 (C)		
B4DQ50	highly similar to Protein-glutamine gamma-	2(C)		
	glutamyltransferase E			
RNA-associ	ated proteins			
	Transcriptional regulation			
Q86VG2	*SFPQ, Splicing factor proline/glutamine-rich**	8 (C)		3 (N)
F5GYZ3	NONO, Non-POU Domain Containing, Octamer- Binding1**	2 (C)		
B5BUE6	* ¹ DDX5, DEAD box polypeptide 5	6 (C)		4 (C)
Q59F66	*DDX17, DEAD box polypeptide 17 isoform p82 variant	2 (C)		
Q6FHQ0	RBBP4/ RbAp48**	3 (C)		
Q6FHQ0	RBBP7**	4 (C)		
F4ZW66	^{&*¶} ILF3, Interleukin enhancer-binding factor 2 variant (90 kDa NFAT subunit)	5 (C)		
Q53FG3	^{&} * [¶] ILF2 (45 kDa NFAT subunit)	2 (C)		
B3KRS5	HDAC2**	2 (C)		
Q59EB5	*CSDA protein variant	5 (C)		
	Splicing regulation			
B2R8Z8	^{&} Similar to SYNCRIP, hnRNP Q	7 (C)		
Q8TBR3	^{&} FUS, hnRNP P2	3 (C)		1 (N)
B4DZZ1	MYBBP1 nucleolar transcriptional regulator	2 (C)		
A8K651	* [¶] C1QBP, p32 subunit of splicing factor 2 (SF2)	5 (C)		2 (N)
B4DUA4	[§] SRSF5, Serine/arginine-rich-splicing factor 5	2 (C)		
Q49AN9	SNRPG, small nuclear ribonucleoprotein G (snRNP)	2 (C)		
*SFPQ, NO	NO, DDX5, DDX17, NMP1, NFAT		* See above	
	RNA stability			
Q6IQ30	[¶] PABPC4, Poly(A)-binding protein 4	14 (C)		
E7ERJ7	^{&¶} PABPC1, Poly(A)-binding protein 1		4 (C)	
B3KT93	highly similar to Poly(A)-binding protein 1	14 (C)		
Q53SS8	PCBP1, Poly(RC)-binding protein 1, hnRNP E1/X	7 (C)	1 (C)	2 (C)
Q6IPF4	^{&} PCBP2, Poly(RC)-binding protein 2, hnRNP E2	5 (C)		3 (C)

B4DVB8	^{&} *Similar to ELAV-like 1	3 (C)		
*NFAT			* See above	
	mRNA and mRNP granules export to the cytoplasm,			-
C9JT33	^{&¶} IGF2BP1, IGF2 mRNA-binding protein 1	5(C)		
D3DTW3	^{&} IGF2BP1, IGF2 mRNA-binding protein 1, isoform CRA	7 (C)		
*NFAT, DD>	(5, FUS, SFPQ, NONO, TREX2, RAN		* See above	
	Translational regulation			
A8K7F6	highly similar to EIF4A1, eukaryotic translation initiation factor 4A, isoform 1	2 (C)		6 (C)
Q53HK3	EIF2, Eukaryotic translation initiation factor 2, subunit 3 gamma, 52 kDa variant	5 (C)		
Q96G38	EIF3B, Eukaryotic translation initiation factor 3, subunit beta	4 (C)		
Q5U0F4	EIF3I, Eukaryotic translation initiation factor 3, subunit 2 beta, 36 kDa	4 (C)		
Q6IAM0	EIF3S4, Eukaryotic translation initiation factor 3 subunit G	4 (C)		
Q24JU4	EIF3A, Eukaryotic translation initiation factor 3, subunit A	3 (C)		
F5H425	EIF3J	3 (C)		
B3KXU8	*Highly similar to CAPRIN 1, Cell Cycle-Associated Protein 1	3 (C)		
E9PD14	*GNB2L1, Guanine Nucleotide-Binding Protein (G Protein),			3 (C)
	Beta Polypeptide 2-Like			
*CSDA prot	ein variant, IGF2BP1		*See above	
	Ribosome biogenesis			-
Q3KQS4	*NOP2 nucleolar protein	2 (C)		
*NPM1, DD	X5, DDX17, C1BPQ, MYBBP1A		*See above	
	Ribosomal proteins			
Q6NXR8	^{&¶} Ribosomal protein S3A	9(C)		
Q9NY85	[¶] Ribosomal protein L3 (Fragment)	8(C)		
A9C4C1	^{&¶} Ribosomal protein S9	5(C)		
Q5T8U4	[¶] Ribosomal protein L7a	7(C)		
E5RI99	[¶] Uncharacterized protein GN=RPL30	8(C)		
E7EQV9	[¶] Ribosomal protein L15	4(C)		
B7Z4K2	[¶] Ribosomal protein L31, isoform CRA_c	4(C)		
A8MUD9	¹ Uncharacterized protein GN=RPL7	3(C)		
B2R4D8	¹ 60S ribosomal protein L27	5(C)		
Q96IR1	^{&¶} RPS4X protein (Fragment)	2(C)		2(C)
E9PKZ0	¹ Uncharacterized protein GN=RPL8	3(C)		
Q59GY2	[¶] Ribosomal protein L4 variant (Fragment)	3(C)		
Q5JR94	^{&¶} 40S ribosomal protein S8	2(C)		
Q9HBB3	¹ 60S ribosomal protein L6	2(C)		
Q53G25	Ribosomal protein S5 variant (Fragment)	2(C)		
Q53G83	[¶] Ribosomal protein S3 variant (Fragment)	2(C)		5(C)
A8MUS3	¹ Ribosomal protein L23a, isoform CRA_a	2(C)		
Q5VVD0	Ribosomal protein L11	3(C)		
Q59FS3	¹ Ribosomal protein S7 variant (Fragment)	2(C)		
Q6IPH7	RPL14 protein	2(C)		

Q9BSW5	RPS2 protein (Fragment)			2(C)	
Q7Z4W8	Heparin-binding protein HBp15, Ribosomal protein RPL22	7(C)			
Q96RS2	Ribosomal Protein SA Pseudogene 151, LAMR1P15	2(C)			
Q5MK14	Leukemia-associated protein	2(C)	1(C)		
*GNB2L1			*see above		
	TP53 regulation				
*NPM1, Sin	nilar to ELAV-like 1, NOP2		*See above		
	Neuronal differentiation				
B5MCE7	BZW2, Basic leucine zipper and W2 domains 2, isoform CRA_b	3 (C)			
Q8N274	Similar to AHNAK	4 (C)			
B7Z9Y9	Weakly similar to Paraneoplastic Ma Antigen 2	11(C)			
*NFAT, simi	lar to CAPRIN1, NUDC, SFPQ		*See above	1	
	other				
A8K0N0	SRP9	2(C)			
WNT signal	ing				
*Catenin be	eta 1, NFAT, GNB2L1, DDX5		* See above		
Oxidative s					
B2R4P2	highly similar to Peroxiredoxin-1	8 (C)	3 (C)	4 (C)	
B4DF70	highly similar to Peroxiredoxin-2	8 (C)	2 (C)	4 (C)	
A8MX94	GSTP1		3(C)		
Others					
	transcription				
P52926	[§] HMGA2	2(N)		1(N)	
B1AHC7	[¶] XRCC6	3(C)			
	translation				
B4DQY1	highly similar to Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha	3(C)			
Q6ZTI3	highly similar to Lysyl-tRNA synthetase	6(C)			
B4E266	highly similar to Leucyl-tRNA synthetase, cytoplasmic	2(C)			
	proteasome				
Q6IBM2	Proteasome (Prosome, Macropain) Activator Subunit 1, PSME1	5(C)			
Q05DH1	PSMA7	2(C)		2(C)	
Q6IAT9	PSMB6	2(C)	1(C)		
Q5TZN3	UBE2C	2(C)		1	
Q5UGI3	UBC, ubiquitin C splice variant			2(C)	
E9KL27	Epididymis tissue protein Li 174	2(C)			
B4DXJ9	PSMA6	2(C)			
Q96IF9	VCP	2(C)		1	
	Extracellular(?), membrane proteins and membrane vesicle trafficking				
B4DIN1	[§] Clathrin light chain A, CLTA	3(C)			
B2R4M6	highly similar to Homo sapiens S100 calcium-binding protein A9	11(C)	2(C)		
	others			1	

B4DUU6	[¶] Pyruvate kinase, PKM	14(C)		8(C)
B7Z5E3	[¶] L-lactate dehydrogenase, LDHA	8(C)		5(C)
Q5U077	L-lactate dehydrogenase, LDHB			8(C)
Q75MT9	Malate dehydrogenase, MDH2			4(C)
Q5SZU1	Phosphoglycerate dehydrogenase, PHGDH			2(C)
B2R721	FLJ93240, highly similar to insulin-degrading enzyme (IDE)	3(C)		
A8K008	cDNA FLJ78387	4(C)		
B4DE76	highly similar to Ran-specific GTPase-activating protein	3(C)		
Q6FIG4	RAB1A	2(C)		
Q6FI42	RAB11B		3(C)	
C9JQV0	Uncharacterized Protein C7orf502	6(C)		
B7ZLF8	Uncharacterized protein, cytoplasmic	4(C)		
Q5EFE6	Anti-RhD monoclonal T125 kappa light chain	2(C)		

Scaffold Viewer settings summary: protein threshold 95%, min # peptides 2, peptide threshold 95%

GO: gene ontology

Any peptide of the proteins identified were found in the control HaloTag-EGFP co-purification.

Table data represent all the identified proteins from 7 co-purifications experiments (2 with HaloTag-DUX4, 4 with HaloTag-DUX4c and 1 with HaloTag-DUX4term realized each time in parallel with the control HaloTag-EGFP). We always found C1QBP, SFPQ and FUS in each HaloTag-DUX4 or - DUX4c co-purifications.

We found many peptides from <u>different **tubulin** forms</u> in each conditions. However, fewer peptides corresponding to conserved regions were found following HaloTag-EGFP co-immunoprecipitation. In contrast, more peptides in non-conserved regions were retrieved following HaloTag-DUX4, - DUX4term or -DUX4c co-immunoprecipitation.

* Involved in different functions: some are depicted in the table.

**The heterodimers SFPQ/NONO and RBBP4/RBBP7 are involved in transcriptional repression by association with HDAC.

[¶] Identified (or highly similar) as putative DUX4 partner using GST pull-down assays in human myoblasts or HEK293 cells (see S4 Table)

[§] Isoform (or similar function) identified in other approaches (see other tables)

& Known to be associated in IGF2BP1-dependent mRNP granules