

Supplemental Table 4

| Uniprot ID | Function | DUX4 | DUX4 | Gene ontology function | Gene ontology ID |
|------------|--|-------------------------------|--------|---|--------------------------|
| | | human myoblasts | HEK293 | | |
| Uniprot ID | Actin-associated protein | number of identified peptides | | Gene ontology function | Gene ontology ID |
| Q13885 | Tubulin beta-2A chain | 2 | 0 | microtubule family cytoskeletal protein | PC00157 |
| P07437 | Tubulin beta chain | 18 | 16 | microtubule family cytoskeletal protein | PC00157 |
| P35580 | Myosin-10 | 0 | 16 | actin-binding motor protein | PC00040 |
| P63261 | [¶] Actin, cytoplasmic 2 | 9 | 0 | actin and actin related protein | PC00039 |
| P21333 | Filamin-A | 7 | 0 | actin cytoskeleton | GO:0015629 |
| Q13509 | Tubulin beta-3 chain | 2 | 1 | microtubule family cytoskeletal protein | PC00157 |
| P14649 | Myosin light chain 6B | 4 | 1 | actin cytoskeleton | GO:0015629 |
| Q9BUF5 | Tubulin beta-6 chain | 2 | 0 | microtubule family cytoskeletal protein | PC00157 |
| P08670 | Vimentin | 11 | 3 | intermediate filament | PC00129 |
| Q14204 | Cytoplasmic dynein 1 heavy chain 1 | 5 | 3 | microtubule family cytoskeletal activity | PC00157 |
| P35579 | [¶] Myosin heavy chain 9 | 41 | 10 | actin-binding motor protein | PC00040 |
| P06748 | [¶] Nucleophosmin | 2 | 5 | Organelle organization RNA metabolic process | GO:0006996 GO:0016070 |
| P84077 | [§] ADP-ribosylation factor 1 | 2 | 0 | Organelle organization hydrolase activity | GO:0006996 GO:0016787 |
| | RNP complex | | | | |
| P46781 | ^{&¶} 40S ribosomal protein S9 | 2 | 0 | ribonucleoprotein complex | GO:0030529 |
| P62753 | 40S ribosomal protein S6 | 2 | 0 | ribonucleoprotein complex | GO:0030529 |
| P62847 | 40S ribosomal protein S24 | 2 | 1 | ribonucleoprotein complex | GO:0030529 |
| P62917 | [¶] 60S ribosomal protein L8 | 2 | 1 | ribonucleoprotein complex | GO:0030529 |
| P62750 | [¶] 60S ribosomal protein L23a | 2 | 1 | ribonucleoprotein complex | GO:0030529 |

| | | | | | |
|---------|-------------------------------------|---|---|---------------------------|------------|
| P62081 | 40S ribosomal protein S7 | 0 | 2 | ribonucleoprotein complex | GO:0030529 |
| P61247 | 40S ribosomal protein S3a | 4 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62906 | 60S ribosomal protein L10a | 3 | 2 | ribonucleoprotein complex | GO:0030529 |
| P32969 | 60S ribosomal protein L9 | 3 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62244 | 40S ribosomal protein S15a | 3 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62841 | 40S ribosomal protein S15 | 2 | 2 | ribonucleoprotein complex | GO:0030529 |
| P84098 | 60S ribosomal protein L19 | 2 | 2 | ribonucleoprotein complex | GO:0030529 |
| P46776 | 60S ribosomal protein L27a | 1 | 2 | ribonucleoprotein complex | GO:0030529 |
| P46783 | 40S ribosomal protein S10 | 1 | 2 | ribonucleoprotein complex | GO:0030529 |
| P60866 | 40S ribosomal protein S20 | 1 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62249 | 40S ribosomal protein S16 | 0 | 2 | ribonucleoprotein complex | GO:0030529 |
| P15880 | 40S ribosomal protein S2 | 2 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62899 | 60S ribosomal protein L31 | 2 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62829 | 60S ribosomal protein L23 | 2 | 2 | ribonucleoprotein complex | GO:0030529 |
| P62269 | 40S ribosomal protein S18 | 4 | 4 | ribonucleoprotein complex | GO:0030529 |
| P26373 | 60S ribosomal protein L13 | 4 | 4 | ribonucleoprotein complex | GO:0030529 |
| P62241 | 40S ribosomal protein S8 | 4 | 4 | ribonucleoprotein complex | GO:0030529 |
| P62888 | 60S ribosomal protein L30 | 3 | 4 | ribonucleoprotein complex | GO:0030529 |
| P62263 | 40S ribosomal protein S14 | 3 | 4 | ribonucleoprotein complex | GO:0030529 |
| P62277 | 40S ribosomal protein S13 | 3 | 4 | ribonucleoprotein complex | GO:0030529 |
| P18621 | 60S ribosomal protein L17 | 2 | 4 | ribonucleoprotein complex | GO:0030529 |
| P36578 | 60S ribosomal protein L4 | 1 | 4 | ribonucleoprotein complex | GO:0030529 |
| P62851 | 40S ribosomal protein S25 | 3 | 3 | ribonucleoprotein complex | GO:0030529 |
| Q9Y3U8 | 60S ribosomal protein L36 | 3 | 3 | ribonucleoprotein complex | GO:0030529 |
| P62424 | 60S ribosomal protein L7a | 2 | 3 | ribonucleoprotein complex | GO:0030529 |
| P18124 | 60S ribosomal protein L7 | 6 | 5 | ribonucleoprotein complex | GO:0030529 |
| P39023 | 60S ribosomal protein L3 | 6 | 4 | ribonucleoprotein complex | GO:0030529 |
| P62701 | 40S ribosomal protein S4, X isoform | 5 | 3 | ribonucleoprotein complex | GO:0030529 |
| P300050 | 60S ribosomal protein L12 | 4 | 3 | ribonucleoprotein complex | GO:0030529 |

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|--------|--|---|----|---|---|
| P27635 | 60S ribosomal protein L10 | 4 | 3 | ribonucleoprotein complex | GO:0030529 |
| Q07020 | 60S ribosomal protein L18 | 1 | 3 | ribonucleoprotein complex | GO:0030529 |
| P61313 | [¶] 60S ribosomal protein L15 | 1 | 2 | ribonucleoprotein complex | GO:0030529 |
| P46778 | 60S ribosomal protein L21 | 1 | 2 | ribonucleoprotein complex | GO:0030529 |
| P42766 | [¶] 60S ribosomal protein L35 | 2 | 3 | ribonucleoprotein complex | GO:0030529 |
| P35268 | [¶] 60S ribosomal protein L22 | 2 | 3 | ribonucleoprotein complex | GO:0030529 |
| P23396 | ^{&¶} 40S ribosomal protein S3 | 6 | 4 | ribonucleoprotein complex | GO:0030529 |
| POCW22 | 40S ribosomal protein S17-like | 5 | 3 | ribonucleoprotein complex | GO:0030529 |
| Q02878 | [¶] 60S ribosomal protein L6 | 1 | 3 | ribonucleoprotein complex | GO:0030529 |
| | Transcription-related protein | | | | |
| Q6NZI2 | Polymerase I and transcript release factor | 4 | 0 | transcription cofactor | PC00218 |
| P09429 | High mobility group protein B1 | 2 | 0 | transcription cofactor | PC00218 |
| Q9BQG0 | [¶] Myb-binding protein 1A | 0 | 2 | transcription factor | PC00218 |
| Q86VP6 | Cullin-associated NEDD8-dissociated protein 1 | 0 | 3 | transcription factor | PC00218 |
| P67809 | Nuclease-sensitive element-binding protein 1 | 4 | 5 | regulation of transcription | GO:0006355 |
| Q12906 | ^{&¶} Interleukin enhancer-binding factor 3, ILF3 | 0 | 3 | transcription factor immune system process | PC00218 GO:0002376 |
| | RNA processing | | | | |
| A8K651 | ^{*#¶} Complement component 1 Q subcomponent-binding protein | 7 | 11 | apoptotic process, mRNA processing, immune response, ribosome biogenesis | GO:0006915, GO:0006397, GO:0006955, GO:0042254 |
| Q00839 | ^{&} Heterogeneous nuclear ribonucleoprotein U | 4 | 8 | mRNA processing | GO:0006397 |

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|--------|---|---|----|--|--------------------------|
| O75643 | U5 small nuclear ribonucleoprotein 200 kDa helicase | 0 | 6 | mRNA processing via spliceosome | GO:0000398 |
| P52272 | Heterogeneous nuclear ribonucleoprotein M | 2 | 5 | mRNA processing | GO:0006397 |
| Q9NZI8 | ^{&¶} Insulin-like growth factor 2 mRNA-binding protein 1 | 0 | 4 | mRNA processing | GO:0006397 |
| Q6P2Q9 | Pre-mRNA-processing-splicing factor 8 | 5 | 3 | mRNA processing | GO:0006397 |
| P52597 | Heterogeneous nuclear ribonucleoprotein F | 1 | 3 | mRNA processing | GO:0006397 |
| O00425 | ^{&¶} Insulin-like growth factor 2 mRNA-binding protein 3 | 0 | 2 | mRNA processing | GO:0006397 |
| P61978 | Heterogeneous nuclear ribonucleoprotein K | 0 | 2 | mRNA processing | GO:0006397 |
| P84103 | [§] Serine/arginine-rich splicing factor 3 | 1 | 3 | mRNA processing via spliceosome | GO:0000398 |
| Q32P51 | Heterogeneous nuclear ribonucleoprotein A1-like 2 | 0 | 3 | mRNA processing | GO:0006397 |
| O75533 | Splicing factor 3B subunit 1 | 0 | 3 | mRNA processing | GO:0006397 |
| Q13310 | [¶] Polyadenylate-binding protein 4 | 0 | 4 | RNA processing | GO:0006396 |
| P19338 | ^{&} Nucleolin | 4 | 14 | developmental process ribosome biogenesis | GO:0032502 GO:0042254 |
| | Helicase | | | | |
| P33993 | DNA replication licensing factor MCM7 | 0 | 2 | helicase activity | GO:0004386 |
| Q9H2U1 | Probable ATP-dependent RNA helicase DHX36 | 0 | 2 | helicase activity | GO:0004386 |
| Q08211 | ^{&} ATP-dependent RNA helicase A DHX9 | 9 | 10 | helicase activity | GO:0004386 |
| Q7L2E3 | Putative ATP-dependent RNA helicase DHX30 | 3 | 5 | helicase activity | GO:0004386 |

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|--------|---|---|---|-------------------|------------|
| Q9NR30 | Nucleolar RNA helicase 2 | 1 | 4 | helicase activity | GO:0004386 |
| P12956 | [¶] X-ray repair cross-complementing protein 6 | 3 | 3 | helicase activity | GO:0004386 |
| O00571 | ATP-dependent RNA helicase DDX3X | 2 | 3 | helicase activity | GO:0004386 |
| P17844 | [¶] Probable ATP-dependent RNA helicase DDX5 | 1 | 3 | helicase activity | GO:0004386 |

| | | | | | |
|--------|--|---|----|-----------------------------|------------|
| | Translation | | | | |
| P13639 | Elongation factor 2 | 0 | 2 | translation factor activity | GO:0008135 |
| | Metabolic processes | | | | |
| P49327 | Fatty acid synthase | 2 | 10 | metabolic process | GO:0008152 |
| P11586 | C-1-tetrahydrofolate synthase, cytoplasmic | 0 | 4 | metabolic process | GO:0008152 |
| P36542 | ATP synthase subunit gamma, mitochondrial | 1 | 3 | metabolic process | GO:0008152 |
| P07814 | [§] Bifunctional glutamate/proline--tRNA ligase | 0 | 3 | metabolic process | GO:0008152 |
| P05141 | ADP/ATP translocase 2 | 0 | 3 | metabolic process | GO:0008152 |
| Q15029 | 116 kDa U5 small nuclear ribonucleoprotein component | 2 | 2 | metabolic process | GO:0008152 |
| P31943 | ^{&} Heterogeneous nuclear ribonucleoprotein H | 1 | 2 | metabolic process | GO:0008152 |
| O15067 | Phosphoribosylformylglycinamide synthase | 0 | 2 | metabolic process | GO:0008152 |
| P00338 | [¶] L-lactate dehydrogenase A chain | 4 | 1 | metabolic process | GO:0008152 |
| P06733 | Alpha-enolase | 0 | 6 | metabolic process | GO:0008152 |
| | Endocytosis | | | | |
| Q00610 | [¶] Clathrin heavy chain 1 | 2 | 3 | vesicle mediated transport | GO:0006897 |
| P53621 | Coatamer subunit alpha | 4 | 1 | vesicle mediated transport | GO:0006897 |

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|--------|--|---|---|--|------------|
| O95782 | AP-2 complex subunit alpha-1 | 2 | 0 | vesicle mediated transport | GO:0006897 |
| | Molecule transport | | | | |
| P21796 | Voltage-dependent anion-selective channel protein 1 | 0 | 3 | voltage-gated ion channel | PC00241 |
| P45880 | [¶] Voltage-dependent anion-selective channel protein 2 | 0 | 2 | transporter activity | GO:0005215 |
| Q00325 | Phosphate carrier protein, mitochondrial | 2 | 2 | transporter | PC00227 |
| P12236 | ADP/ATP translocase 3 | 2 | 0 | transporter activity | GO:0005215 |
| | Protein transport | | | | |
| O14617 | AP-3 complex subunit delta-1 | 1 | 2 | intracellular protein transport | GO:0006886 |
| P55060 | Exportin-2 | 0 | 2 | transporter activity | GO:0005215 |
| | Additional enzymatic function | | | | |
| P13010 | X-ray repair cross-complementing protein 5 | 2 | 6 | double-strand break repair via nonhomologous end joining | GO:0006303 |
| P09874 | Poly [ADP-ribose] polymerase 1 | 0 | 5 | ligase activity | GO:0003909 |
| P14618 | [¶] Pyruvate kinase PKM | 6 | 4 | glycolytic process | GO:0006096 |
| P41250 | Glycine--tRNA ligase | 0 | 4 | aminoacyl-tRNA synthetase | PC00047 |
| P17987 | [§] T-complex protein 1 subunit alpha | 0 | 3 | chaperone | PC00072 |
| P41252 | [§] Isoleucine--tRNA ligase, cytoplasmic | 0 | 3 | aminoacyl-tRNA ligase activity | GO:0004812 |
| P11940 | ^{&¶} Polyadenylate-binding protein 1 | 4 | 2 | developmental process | GO:0032502 |
| P40227 | [§] T-complex protein 1 subunit zeta | 0 | 2 | chaperone | PC00072 |
| P56192 | [§] Methionine--tRNA ligase, cytoplasmic | 0 | 2 | aminoacyl-tRNA synthetase | PC00047 |
| P34897 | Serine hydroxymethyltransferase, mitochondrial | 0 | 2 | transferase activity | GO:0016749 |
| P30153 | [¶] Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform | 0 | 2 | phosphoprotein phosphatase activity | GO:0004721 |

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|--------|--|----|---|---------------------------------|------------|
| P46940 | Ras GTPase-activating-like protein IQGAP1 | 12 | 0 | small GTPase regulator activity | GO:0005083 |
| P62826 | [§] GTP-binding nuclear protein Ran | 2 | 0 | hydrolase activity | GO:0016787 |
| P04843 | Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 | 2 | 0 | transferase activity | GO:0016740 |
| P17655 | Calpain-2 catalytic subunit | 2 | 0 | hydrolase activity | GO:0016787 |
| P04406 | Glyceraldehyde-3-phosphate dehydrogenase | 2 | 0 | oxidoreductase activity | GO:0016491 |
| P10809 | 60 kDa heat shock protein, mitochondrial | 0 | 7 | protein binding | GO:0005515 |
| P78527 | DNA-dependent protein kinase catalytic subunit | 2 | 7 | protein kinase activity | GO:0004672 |

Display options: Exclusive Unique Peptide Count, No filter selected for Req Mods

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

Note: desmin, Interleukin enhancer binding factor 2 (ILF2), DDX17 and peroxiredoxin are not mentioned as only one peptide was identified.

*Identified as a putative DUX4-binding partner using yeast two-hybrid (see table 3)

[¶]Identified (or highly similar) as a putative DUX4 binding partner using HaloTag co-purification (see S3 Table)

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

Validated interaction by co-immunoprecipitation in HEK293 cells

&Known to be associated in an IGF2BP1-dependent mRNP complex

Any peptide of the proteins identified were found in the different controls made in parallel (non-specific binding peptides purified following incubation of Glutathione Sepharose 4B with total protein lysate without addition of GST-DUX4), except for tubulin beta-chain (8 peptides found with HEK293 lysate but no peptide found with myoblast lysate); actin 2 (8 peptides found with myoblast lysate but no peptide found with HEK293 lysate); C1QBP, alpha-enolase and 60 kDa heat shock protein (1 peptide found with HEK293 lysate alone but no peptide found with myoblast lysate); and myosin 9, vimentin and Ribosomal Protein P2 (1 peptide found with myoblast lysate but no peptide found with HEK293 lysate).