

**Supplementary Table 1(a): A) Profile of 200 sequenced ALS cases (80 FALS, 120 SALS).**

**(b) Primer sequences for TDP-43 amplifications.**

**a**

Sex <sup>a</sup>	Age <sup>a</sup>	Duration <sup>a</sup>	Onset site <sup>a</sup>	Origin <sup>a</sup>
M = 111	Avg. = 55.3	Avg = 3.4	Spinal = 103	France = 99
F = 88	St. dev = 12.9	St. dev. = 2.8	Bulbar = 25	Quebec = 100

<sup>a</sup> - Information is not available for each patient.

**b**

TDP-43 exon	Forward Primer	Reverse Primer
2	AGAACTCTGACATGGTTTGGG	TGCCACCAAAGAGGCTAAG
3	TCACTTCTTGCCAAGTTTTCAG	AGTATGGGCCAGGAGTTTG
4	CACTGCATCCAGTTGAAACC	GGATTTTCATGAACACACCCTG
5	ATCCAAGGCGAATGATTTTG	GAGAAATTACCTAATTACCTGGGAC
6A	TGCTTGTAATCTAAGTTTTGTTGC	GCTGGCTGGCTAACATGC
6B	TGGTGGGATGAACTTTGGTG	TGCTGAATATACTCCACACTGAAC

**Supplementary Table 2:** Haplotype comparison for two FALS patients with A382T mutations.

Marker	Patient 1 allele 1	Patient 1 allele 2	Patient 2 allele 1	Patient 2 allele 2	shared allele	CEPH shared allele freq
D1S503	3	3	5	2	none	
D1S1634	6	1	2	1	1	1 = 13%
TDP-43 Ex.6	A	T	A	T	T	
D1S2667	9	2	3	2	2	2 = 29%
D1S489	6	1	1	1	1	1 = 50%
D1S228	2	6	2	6	6 and 2	6 = 8%; 2 = 33%
D1S507	6	1	1	1	1	1 = 27%
D1S2728	1	1	1	1	1	1 = 33%

**Supplementary Table 3:** Predicted protein conformational changes due to TDP-43 missense variants using the Polyphen, SIFT, and SNAP programs.

Status	protein change	PolyPhen		SIFT		SNAP	expected accuracy <sup>c</sup>
		Prediction	Score <sup>a</sup>	prediction	Score <sup>b</sup>	prediction	
Affected	D169G	Benign	1.053	Tolerated	0.07	Non-neutral	58%
Affected	G287S	Benign	1.098	Tolerated	0.34	Neutral	60%
Affected	A315T	Benign	0.994	Tolerated	0.42	Neutral	69%
Affected	G348C	probably damaging	2.256	Affect protein function	0.01	Non-neutral	78%
Affected	R361S	possibly damaging	1.807	Tolerated	0.36	Non-neutral	70%
Affected	A382T	Benign	0.9	Tolerated	0.27	Neutral	60%
Affected	N390D	Benign	1.125	Affect protein function	0	Non-neutral	63%
Affected	N390S	Benign	1.125	Affect protein function	0	Neutral	69%
Control	A90V	Benign	0.346	Tolerated	0.36	Neutral	89%

<sup>a</sup>The lower the score, the more benign the substitution. <sup>b</sup>The higher the score for SIFT, the more tolerated the change is. <sup>c</sup> The higher the percentage, the greater the confidence for the prediction.

**Supplementary Table 4:** Amino acid changes and phosphorylation site prediction scores [S2] corresponding to identified sequence variants. Phosphorylation scores range from 0.0 (minimal probability) to 1.0 (high probability). Variant scores for high-probability phosphorylation sites due to mutation of N390 are shown in bold.

Variant	Amino acid change	Putative effects	Putative site of phosphorylation	Context	Wildtype Score	Variant Score
1	D169G	unknown		n/a	n/a	n/a
2	G287S	possible increased phosphorylation	S287	FGNQ <b>S</b> GFGN	0.000	0.026
			S292	GFGN <b>S</b> RGGG	0.179	0.288
3*	A315T	possible increased phosphorylation	T315	MNFG <b>T</b> FSIN	0.000	0.011
3 <sup>†</sup>	A315T	possible increased phosphorylation	T315	MNFG <b>T</b> FSIN	0.000	0.011
4	G348C	increased inter-molecular aggregation	n/a	QNQ <b>S</b> CPSGN	n/a	n/a
5	R361S	possible increased phosphorylation	S361	GNMQ <b>S</b> EPNQ	0.000	0.007
6	A382T	possible increased phosphorylation	S379	SGSN <b>S</b> GATI	0.086	0.031
			T382	NSGA <b>T</b> IGWG	0.000	0.111
7	N390D	probable increased phosphorylation	S387	IGWGS <b>S</b> ASDA	0.028	<b>0.959</b>
			S389	WGSAS <b>S</b> DAGS	0.012	0.041
			S393	SDAG <b>S</b> GSGF	0.074	0.323
			S395	AGSG <b>S</b> GFNG	0.043	0.207
8	N390S	probable increased phosphorylation	S387	IGWGS <b>S</b> ASSA	0.028	0.102
			S389	WGSAS <b>S</b> SAGS	0.012	0.031
			S390	GSAS <b>S</b> AGSG	0.000	0.169
			S393	SSAG <b>S</b> GSGF	0.074	<b>0.583</b>
			S395	AGSG <b>S</b> GFNG	0.043	0.268

**Supplementary Figure 1:** Multi-species alignment for *TDP-43*. Sequence cluster alignment was done by the Clustal W method. Mutated residues are highlighted (yellow when they are conserved between humans and other species; green when they differ). Two regions in grey, corresponding to residues 319-341 and 397-414 in human *TDP-43*, are 100% conserved. Residues 320-341 are predicted to form an alpha helix using the PROF prediction program<sup>3</sup>. Animal sequences used include: *Danio rerio* (NP\_958884.1), *Drosophila melanogaster* (BAA34422.1), *Gallus gallus* (NP\_001026049.1), *Macaca mulatta* (XP\_001102660), *Mus musculus* (BAE21557.1), *Pongo pygmaeus* (CAH92854.1), *Rattus norvegicus* (EDL81132.1), and *Xenopus laevis* (Q8JJ42).

```

- M S E Y I R V T E D E N D E P I E I P S E D D G T V L L S Majority
-----+-----+-----
              10              20
-----+-----+-----
1 - M S E Y I R V T E D E N D E P I E I P S E D D G T V L L S TDP43 Homo sapiens
1 - M S E Y I R V T E D E S D E P I E I P S E D D G T V L L S TDP43 Pongo pygmaeus
1 - M S E Y I R V T E D E N D E P I E I P S E D D G T V L L S TDP43 Macaca mulatta
1 - M S E Y I R V T E D E N D E P I E I P S E D D G T V L L S TDP43 Gallus gallus
1 - M S E Y I R V T E D E N D E P I E I P S E D D G T V L L S TDP43 Mus musculus
1 - M S E Y I R V T E D E N D E P I E I P S E D D G T V L L S TDP43 Rattus norvegicus
1 M E M E Y I R V A E D E N E E P M E I P S E D D G T V L L S TDP43 Xenopus tropicalis
1 - - M E Y I R V A E D E N E E P M E I P S E D D G T V L L S TDP43 Xenopus laevis
1 M A E M Y I R V A E E E N E E P M E I P S E D D G T V L L S TDP43 Danio rerio

T V T A Q F P G A C G L R Y R N P V S Q C M R G V R L V E G Majority
-----+-----+-----
              30              40              50
-----+-----+-----+-----
30 T V T A Q F P G A C G L R Y R N P V S Q C M R G V R L V E G TDP43 Homo sapiens
30 T V T A Q F P G A C G L R Y R N P V S Q C M R G V R L V E G TDP43 Pongo pygmaeus
30 T V T A Q F P G A C G L R Y R N P V S Q C M R G V R L V E G TDP43 Macaca mulatta
30 T V T A Q F P G A C G L R Y R N P V S Q C M W G V R L V E G TDP43 Gallus gallus
30 T V T A Q F P G A C G L R Y R N P V S Q C M R G V R L V E G TDP43 Mus musculus
30 T V T A Q F P G A C G L R Y R N P V S Q C M R G V R L V E G TDP43 Rattus norvegicus
31 T V T A Q F P G A C G L R Y R N P V N Q C M R G V R L V E G TDP43 Xenopus tropicalis
29 T V T A Q F P G A C G L R Y R N P V N Q C M R G V R L V E G TDP43 Xenopus laevis
31 T V S A Q F P G A C G L R F R S P V S Q C M R G V R L V D G TDP43 Danio rerio

I L H A P D A G W G N L V Y V V N Y P K D - - - - N K R K Majority
-----+-----+-----
              60              70              80
-----+-----+-----
60 I L H A P D A G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Homo sapiens
60 I L H A P D A G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Pongo pygmaeus
60 I L H A P D A G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Macaca mulatta
60 I L H A P E A G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Gallus gallus
60 I L H A P D A G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Mus musculus
60 I L H A P D A G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Rattus norvegicus
61 I L H A P E N G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Xenopus tropicalis
59 I L H A P E N G W G N L V Y V V N Y P K D - - - - N K R K TDP43 Xenopus laevis
61 I L H A P E N G W G N L V Y V V N Y P K E T V L P D N K R K TDP43 Danio rerio

- M D E T D A S S A V K V K R A V Q K T S D L I V L G L P W Majority
-----+-----+-----
              90              100              110
-----+-----+-----
85 - M D E T D A S S A V K V K R A V Q K T S D L I V L G L P W TDP43 Homo sapiens
85 - M D E T D A S S A V K V K R A V Q K T S D L I V L G L P W TDP43 Pongo pygmaeus
85 - M D E T D A S S A V K V K R A V Q K T S D L I V L G L P W TDP43 Macaca mulatta
85 - M D E T D A S S A V K V K R A V Q K T S D L I V L G L P W TDP43 Gallus gallus
85 - M D E T D A S S A V K V K R A V Q K T S D L I V L G L P W TDP43 Mus musculus
85 - M D E A D A S S A V K V K R A V Q K T S D L I V L G L P W TDP43 Rattus norvegicus
86 - M D E T D A S S A V K I K R A V T K T S D L I V L G L P W TDP43 Xenopus tropicalis

```

84	D G M K T D A S S A V K L K R A V Q K H Q D L I V L G L P W	TDP43	Xenopus laevis
91	- M D E I D A S S A T K I K R G D Q K T S D L I V L G L P W	TDP43	Danio rerio
K T T E Q D L K D Y F S T F G E V L M V Q V K K D L K T G H		Majority	
-----+-----+-----			
120                  130                  140			
-----+-----+-----			
114	K T T E Q D L K E Y F S T F G E V L M V Q V K K D L K T G H	TDP43	Homo sapiens
114	K T T E Q D L K E Y F S T F G E V L M V Q V K K D L K T G H	TDP43	Pongo pygmaeus
114	K T T E Q D L K E Y F S T F G E V L M V Q V K K D L K T G H	TDP43	Macaca mulatta
114	K T T E Q D L K E Y F S T F G E V L M V Q V K K D I K T G H	TDP43	Gallus gallus
114	K T T E Q D L K D Y F S T F G E V L M V Q V K K D L K T G H	TDP43	Mus musculus
114	K T T E Q D L K D Y F S T F G E V L M V Q V K K D L K T G H	TDP43	Rattus norvegicus
115	K T T E Q D L K D Y F S T F G E V I M V Q V K K D A K T G H	TDP43	Xenopus tropicalis
114	K T T E Q D L K D Y F S T F G E V I M V Q V K K D A K T G H	TDP43	Xenopus laevis
120	K T S E Q D L K D Y F G T F G E V I M V Q V K R D V K T G N	TDP43	Danio rerio
S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C		Majority	
-----+-----+-----			
150                  160                  170			
-----+-----+-----			
144	S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C	TDP43	Homo sapiens
144	S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C	TDP43	Pongo pygmaeus
144	S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C	TDP43	Macaca mulatta
144	S K G F G F V R F T D Y E T Q V K V M S Q R H M I D G R W C	TDP43	Gallus gallus
144	S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C	TDP43	Mus musculus
144	S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C	TDP43	Rattus norvegicus
145	S K G F G F V R F A D Y E T Q V K V M S Q R H M I D G R W C	TDP43	Xenopus tropicalis
144	S K G F G F V R F T E Y E T Q V K V M S Q R H M I D G R W C	TDP43	Xenopus laevis
150	S K G F G F V R F G D W E T Q S K V M T Q R H M I D G R W C	TDP43	Danio rerio
D C K L P N S K Q S P D E P L R S R K V F V G R C T E D M T		Majority	
-----+-----+-----			
180                  190                  200			
-----+-----+-----			
174	D C K L P N S K Q S Q D E P L R S R K V F V G R C T E D M T	TDP43	Homo sapiens
174	D C K L P N S K Q S Q D E P L R S R K V F V G R C T E D M T	TDP43	Pongo pygmaeus
174	D C K L P N S K Q S Q D E P L R S R K V F V G R C T E D M T	TDP43	Macaca mulatta
174	D C K L P N S K Q S P D E P L R S R K V F V G R C T E D M T	TDP43	Gallus gallus
174	D C K L P N S K Q S P D E P L R S R K V F V G R C T E D M T	TDP43	Mus musculus
174	D C K L P N S K Q S P D E P L R S R K V F V G R C T E D M T	TDP43	Rattus norvegicus
175	D C K L P N S K - S P D E P M R S R K V F V G R C T E D M S	TDP43	Xenopus tropicalis
174	D C K L P N S K - S P D E P M R S R K V F V G R C T E D M S	TDP43	Xenopus laevis
180	D C K L P N S K Q G I D E P M R S R K V F V G R C T E D M T	TDP43	Danio rerio
A D E L R Q F F S Q Y G E V V D V F I P K P F R A F A F V T		Majority	
-----+-----+-----			
210                  220                  230			
-----+-----+-----			
204	E D E L R E F F S Q Y G D V M D V F I P K P F R A F A F V T	TDP43	Homo sapiens
204	E D E L R E F F S Q Y G D V M D V F I P K P F R A F A F V T	TDP43	Pongo pygmaeus
204	E D E L R E F F S Q Y G D V M D V F I P K P F R A F A F V T	TDP43	Macaca mulatta
204	A D E L R Q F F A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Gallus gallus
204	A E E L Q Q F F C Q Y G E V V D V F I P K P F R A F A F V T	TDP43	Mus musculus
204	A E E L Q Q F F C Q Y G E V V D V F I P K P F R A F A F V T	TDP43	Rattus norvegicus
204	A E E L R Q F F S Q Y G E V V D V F I P K P F R A F A F V T	TDP43	Xenopus tropicalis
203	A E E L R Q F F S Q Y G E V V D V F I P K P F R A F S F V T	TDP43	Xenopus laevis
210	A D E L R Q F F M Q Y G E V T D V F I P K P F R A F A F V T	TDP43	Danio rerio
F A D D Q V A Q S L C G E D L I I K G I S V H I S N A E P K		Majority	
-----+-----+-----			
240                  250                  260			
-----+-----+-----			
234	F A D D Q I A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Homo sapiens
234	F A D D Q I A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Pongo pygmaeus
234	F A D D Q I A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Macaca mulatta
234	F A D D Q V A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Gallus gallus
234	F A D D K V A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Mus musculus
234	F A D D K V A Q S L C G E D L I I K G I S V H I S N A E P K	TDP43	Rattus norvegicus
234	F A D D Q V A Q S L C G E D L I I K G V S V H V S T A E P K	TDP43	Xenopus tropicalis
233	F A D D Q V A Q S L C G E D L I I K G V S V H V S T A E P K	TDP43	Xenopus laevis
240	F A D D Q V A A A L C G E D L I I K G V S V H I S N A E P K	TDP43	Danio rerio
H N S N R Q - L E R S G R F G G N P G G F G N Q G G F G N S		Majority	
-----+-----+-----			
270                  280                  290			
-----+-----+-----			
264	H N S N R Q - L E R S G R F G G N P G G F G N Q G G F G N S	TDP43	Homo sapiens
264	H N S N R Q - L E R S G R F G G N P G G F G N Q G G F G N S	TDP43	Pongo pygmaeus
264	H N S N R Q - L E R S G R F G G N P G G F G N Q G G F G N S	TDP43	Macaca mulatta
264	H N S N R Q - L E R G G R F G G N P G G F G N Q G G F G N S	TDP43	Gallus gallus

264 H N S N R Q - L E R S G R F G G N P G G F G N Q G G F G N S TDP43 Mus musculus  
 264 H N S N R Q - L E R S G R F G G N P G G F G N Q G G F G N S TDP43 Rattus norvegicus  
 264 H N N N R Q - L E R G G R F P G P S - - F G N Q G - Y P N S TDP43 Xenopus tropicalis  
 263 H N N N R Q - L E R E G R F P G P S - - F G S Q G - Y P S N TDP43 Xenopus laevis  
 270 H N N T R Q M M E R A G R F G N G - - - F G G Q G - F A G S TDP43 Danio rerio

R G G G A G L G N N Q G S N M G - - - - - G G M N F G A F Majority  
 -----+-----  
 300 310

293 R G G G A G L G N N Q G S N M G - - - - - G G M N F G A F TDP43 Homo sapiens  
 293 R G G G A G L G N N Q G S N M G - - - - - G G M N F G A F TDP43 Pongo pygmaeus  
 293 R G G G A G L G N N Q G S N M G - - - - - G G M N F G A F TDP43 Macaca mulatta  
 293 R G G G G L G N N Q G S N M G - - - - - G G M N F G A F TDP43 Gallus gallus  
 293 R G G G A C L G N N Q G G N M G - - - - - G G M N F G A F TDP43 Mus musculus  
 293 R G G G A G L G N N Q G G N M G - - - - - G G M N F G A F TDP43 Rattus norvegicus  
 290 R P S S G A L G N N Q G G N M G - - - - - G G G G M N F G A F TDP43 Xenopus tropicalis  
 289 R P S G G T L G N N Q G G N M G S G G G G G G M N F G A F TDP43 Xenopus laevis  
 296 R S N M G G G G G S S S L G - - - - - N F G F TDP43 Danio rerio

S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q Majority  
 -----+-----+-----  
 320 330 340

317 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Homo sapiens  
 317 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Pongo pygmaeus  
 317 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q H N Q TDP43 Macaca mulatta  
 317 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Gallus gallus  
 317 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Mus musculus  
 317 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Rattus norvegicus  
 316 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Xenopus tropicalis  
 319 S I N P A M M A A A Q A A L Q S S W G M M G M L A S Q Q N Q TDP43 Xenopus laevis  
 317 N L N P A M M A A A Q A A L Q S S W G M M G M L A - Q Q N Q TDP43 Danio rerio

S G P S G N N Q S Q G N M Q R E P N Q A F G S G N N S Y S G Majority  
 -----+-----+-----  
 350 360 370

347 S G P S G N N Q N Q G N M Q R E P N Q A F G S G N N S Y S G TDP43 Homo sapiens  
 347 S G P S G N N Q N Q G N M Q R E P N Q A F G S G N N S Y S G TDP43 Pongo pygmaeus  
 347 S G P S G N N Q S Q G N M Q R E P N Q A F G S G N N S Y S G TDP43 Macaca mulatta  
 347 S G P S G N N Q P Q G N M Q R E Q N Q G F S S G N N S Y G G TDP43 Gallus gallus  
 347 S G P S G N N Q S Q G S M Q R E P N Q A F G S G N N S Y S G TDP43 Mus musculus  
 347 S G P S G N N Q S Q G S M Q R E P N Q A F G S G N N S Y S G TDP43 Rattus norvegicus  
 346 S G P Q G S N Q G Q G N Q Q R D Q P Q S F G S - N N S Y - G TDP43 Xenopus tropicalis  
 349 S G P Q G S N P G Q G N Q Q R E Q P Q S F G S - N N S Y - G TDP43 Xenopus laevis  
 346 S G T S G T S T S G T S S S R D Q A Q T Y S S A N S N Y - G TDP43 Danio rerio

S N S G A A I G W G S A S N A G S G S - G F N G G F G S S M Majority  
 -----+-----+-----  
 380 390 400

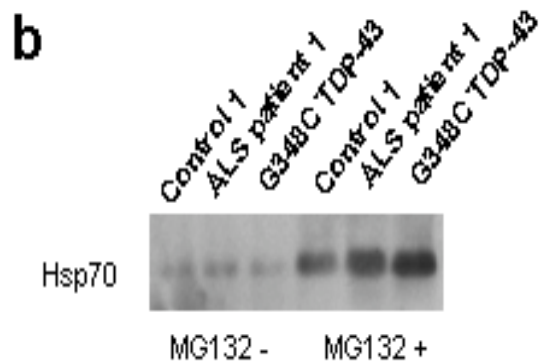
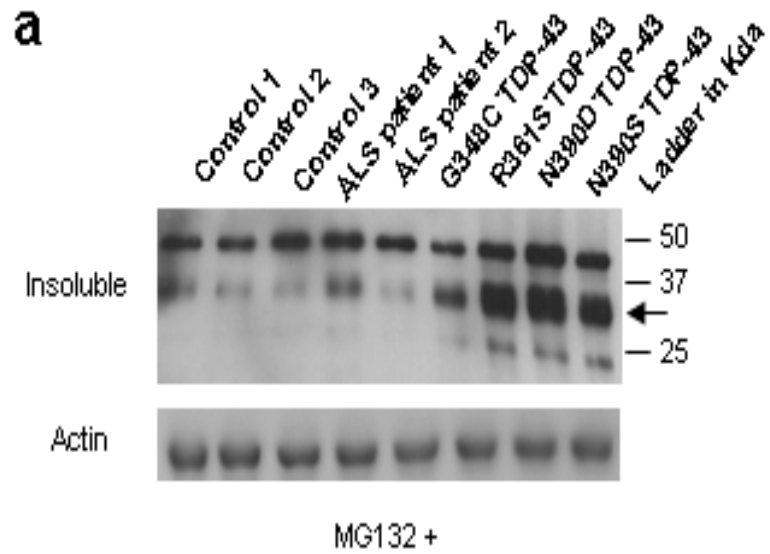
377 S N S G A A I G W G S A S N A G S G S - G F N G G F G S S M TDP43 Homo sapiens  
 377 S N S G A A I G W G S A S N A G S G S - G F N G G F G S S M TDP43 Pongo pygmaeus  
 377 S N S G A A I G W G S A S N A G S G S - G F N G G F G S S M TDP43 Macaca mulatta  
 377 S N S G A A I G W G S A S N A G S S S - G F N G G F G S S M TDP43 Gallus gallus  
 377 S N S G A A I G W G S A S N A G S G S - G F N G G F G S S M TDP43 Mus musculus  
 377 S N S G A A I G W G S A S N A G S G S - G F N G G F G S S M TDP43 Rattus norvegicus  
 374 S N S G - A I G W G S P - N A G S G S - G F N G G F S S S M TDP43 Xenopus tropicalis  
 377 S N S G - A I G W G S P - N A G S G S - G F N G G F S S S M TDP43 Xenopus laevis  
 375 S S S A - A I G W G T G S N S G A A S A G F N S S F G S S M TDP43 Danio rerio

D S K S S G W G M - Majority  
 -----+-----  
 410

406 D S K S S G W G M TDP43 Homo sapiens  
 406 D S K S S G W G M TDP43 Pongo pygmaeus  
 406 D S K S S G W G M TDP43 Macaca mulatta  
 406 D S K S S G W G M TDP43 Gallus gallus  
 406 D S K S S G W G M TDP43 Mus musculus  
 406 D S K S S G W G M TDP43 Rattus norvegicus  
 401 E S K S S G W G M TDP43 Xenopus tropicalis  
 404 E S K S S G W G M TDP43 Xenopus laevis  
 404 E S K S S G W G M TDP43 Danio rerio

**Supplementary Figure 2(a):** Additional unaffected controls and ALS patients with and without TDP-43 mutations confirm the accumulation of a truncated TDP-43 protein in lymphoblastoids derived from ALS patients with TDP-43 mutations. Protein lysates from lymphoblastoid cell lines derived from four ALS patients expressing TDP-43 mutants show the accumulation of a smaller isoform of TDP-43 protein (~28 kDa), mainly in a detergent-insoluble fraction. The expression of this shorter isoform was substantially lower in three unaffected individuals, controls 1, 2 and 3 and in two ALS patients where no TDP-43 mutation was identified. **(b)** Proteasome inhibition leads to rapid elevation of Hsp70, a substrate that is degraded by the proteasome catalytic machinery<sup>18</sup>. Treatment with MG132 (see Supplemental methods) leads to increased levels of Hsp70 in lymphoblastoid cell lines derived from two ALS patients with TDP-43 mutations and one





unaffected control.

**Supplementary Note**

All available patients affected from ALS and unaffected individuals underwent a standardized neurological examination and were ascertained independently by at least two neurologists from the French and Quebec clinics. All procedures were in accordance with the ethical standards of all of the institutional ethic committees involved in this project, and the Declaration of Helsinki protocols were followed. After participants gave informed consent, blood samples were obtained from affected and unaffected individuals. DNA was extracted from peripheral blood by standard methods.

## Supplementary Methods

### *Patient and control sample collection*

Informed written consent was obtained from each individual and blood samples were collected from which DNA was extracted. Neurologists specialized in ALS collected and diagnosed all ALS cases as probable or definite as per El Escorial criteria<sup>5</sup>. All FALS patients were initially screened for mutations in the SOD1 gene prior to inclusion in this study. In total, 80 FALS and 120 SALS patients were collected (Supplemental Table 3). Unrelated, ethnically matched control individuals were used in this study. Cases were negative for *SOD1*<sup>6</sup>, *VAPB*<sup>7</sup> and *ANG*<sup>8</sup> gene mutations prior to their inclusion in this study.

### *Gene screening and variation analysis*

The coding region of all five coding exons of *TARDBP* (accession number NM\_007375) was sequenced in each patient plus at least 50bp into introns before and after each exon. Primers were designed using the Exon Primer program from the UCSC genome browser (Supplemental Table 4). PCR products were sequenced at the Genome Quebec Centre for Innovation. In each case, the forward primer was used for sequencing and variations were confirmed by reverse-sequencing.

### *Bioinformatics*

Default conditions were used for each of the programs that were used. The PolyPhen program<sup>9</sup>, SIFT<sup>10</sup> and SNAP<sup>11</sup> were used to predict the overall severity of the mutations. The NetPhos 2.0 program was used to predict potential phosphorylation sites<sup>1</sup>. Further, IUPred, DISOPRED2<sup>4</sup> and several algorithms were used to predict the disordered structure of the C-terminus of TDP-43 (Supplemental Figure 2).

### *3D protein modeling*

The c-terminal portion of TDP-43 (amino acids 250-414) was entered in the LOOPP (version 3.0) program<sup>12-14</sup>. The backbone model with the greatest predictive score, 2DBU\_B, was used as a template for 3D-modelling. The structure of TDP-43 in its wildtype form and its mutated form, incorporating the six mutations found in the C-terminal region of the protein, was visualized using the SwissProt database viewer program (version 3.7)<sup>15</sup>.

### *Protein detection*

Western blot<sup>16</sup> analysis were performed using methods previously described by our group. Lymphoblastoid cells were lysed with a 0.2% Triton X in PBS solution, homogenized and sonicated. Following centrifugation of 12000 rpm for 15 minutes, the supernatant was recovered as the soluble fraction, whereas the pellet was resuspended in a buffer containing SDS, urea and -mercaptoethanol as previously described<sup>16</sup>. The following antibodies were used to probe the Western blots: Mouse monoclonal to TDP-43, rabbit polyclonal to TDP-43 (Proteintech Group Inc.), monoclonal to Hsp70 (Stressgen) and monoclonal to actin (Chemicon). The specific proteasome inhibitor, MG132 (Calbiochem) was added to the cell culture media (20 M) for a period of six hours at a concentration that inhibits more than 80% of the degradatory activity of the proteasomal core<sup>17</sup>.

### Supplemental References

1. Blom, N., Gammeltoft, S. & Brunak, S. *J Mol Biol* **294**, 1351-62 (1999).
2. Buratti, E. et al. *J Biol Chem* **280**, 37572-84 (2005).
3. Rost, B. & Sander, C. *J Mol Biol* **232**, 584-99 (1993).
4. Ward, J.J., Sodhi, J.S., McGuffin, L.J., Buxton, B.F. & Jones, D.T. *J Mol Biol* **337**, 635-45 (2004).
5. Brooks, B.R. *J Neurol Sci* **124 Suppl**, 96-107 (1994).
6. Rosen, D.R. et al. *Nature* **362**, 59-62 (1993).
7. Nishimura, A.L. et al. *Am J Hum Genet* **75**, 822-31 (2004).
8. Greenway, M.J. et al. *Nat Genet* **38**, 411-3 (2006).
9. Sunyaev, S. et al. *Hum Mol Genet* **10**, 591-7 (2001).
10. Ng, P.C. & Henikoff, S. *Nucleic Acids Res* **31**, 3812-4 (2003).
11. Li, S. et al. *Nucleic Acids Res* **35**, D707-10 (2007).
12. Meller, J. & Elber, R. *Proteins* **45**, 241-61 (2001).
13. Teodorescu, O., Galor, T., Pillardy, J. & Elber, R. *Proteins* **54**, 41-8 (2004).
14. Tobi, D. & Elber, R. *Proteins* **41**, 40-6 (2000).
15. Guex, N. & Peitsch, M.C. *Electrophoresis* **18**, 2714-23 (1997).
16. Meijer, I.A. et al. *Ann Neurol* **61**, 599-603 (2007).
17. Kabashi, E. et al. *J Neurochem* **89**, 1325-1335 (2004).
18. Bush, K.T. et al. *J Biol Chem* **272**, 9086-9092 (1997).