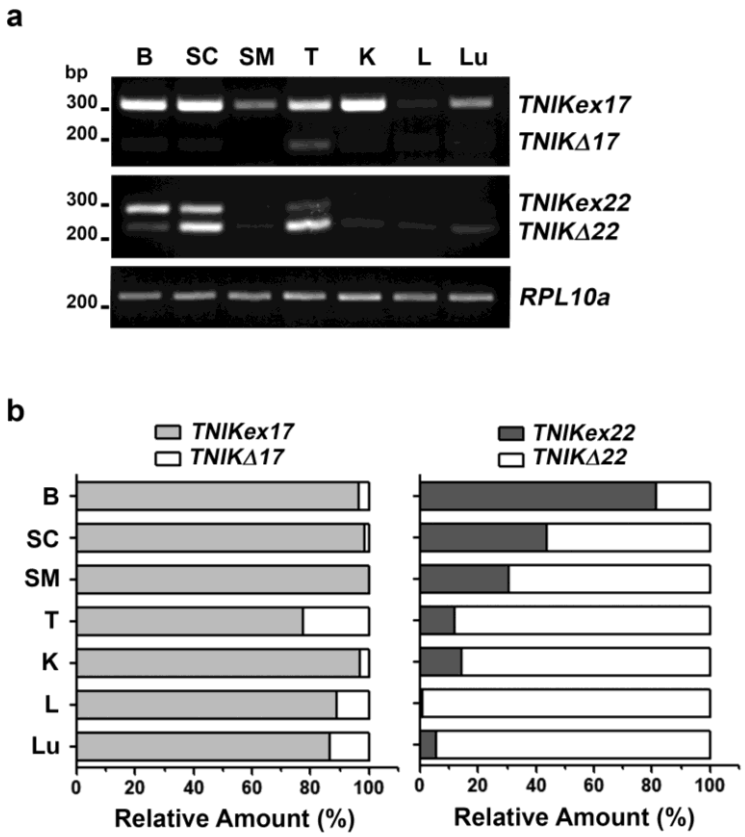
SUPPLEMENTARY MATERIAL

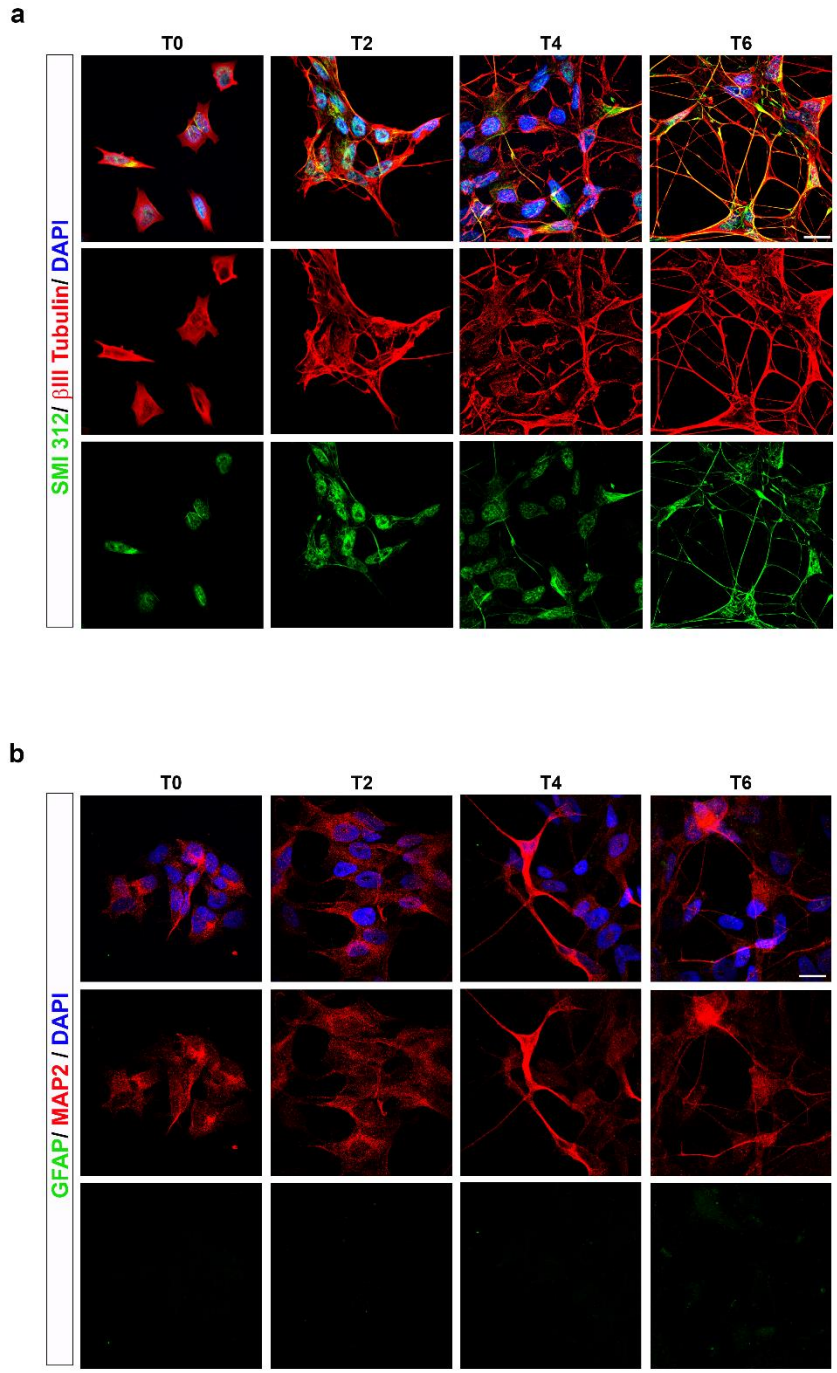
Supplementary Figure 1.



Analysis of *TNIK* exon 17 and 22 alternative splicing in human adult tissues.

a) Semi-quantitative RT-PCR analysis of the *TNIK* exon 17 (*upper panel*) and exon 22 (*middle panel*) alternative splicing in the indicated tissues (B: brain; SP: spinal cord; SM: skeletal muscle; T: testis; K: kidney; L: liver; Lu: lung). For both alternative splicing events, the upper band indicates exon inclusion (*TNIKex17* or *TNIKex22* isoforms), while the lower band represents exon skipping (*TNIKΔ17* or *TNIKΔ22* isoforms). *RPL10a* was used for sample normalization. **b)** Densitometric analysis representing the relative amount of *TNIKex17* and *TNIKΔ17* (*left panel*) and of *TNIKex22* and *TNIKΔ22* (*right panel*) isoforms in the analysed tissues.

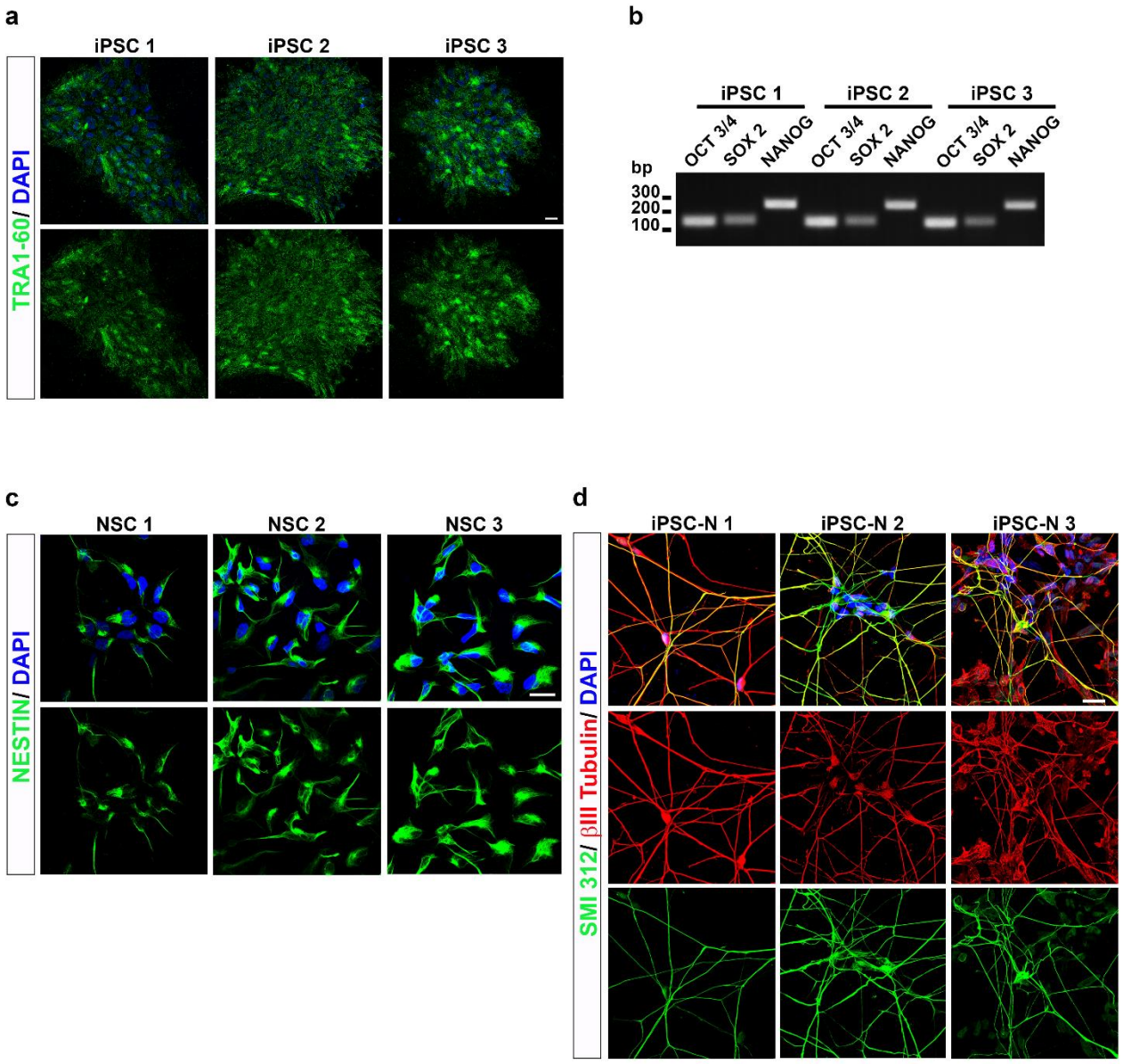
Supplementary Figure 2.



Characterization of neuronal differentiation of SK-N-BE cells with Retinoic acid.

Neuronal differentiation with Retinoic acid (10 μM) was evaluated by IF analysis of **a)** the neuronal markers βIII-Tubulin (red) and SMI 312 (green) and **b)** the neuronal MAP2 (red) and the astrocytes GFAP (green) markers. Nuclear staining is indicated in blue (DAPI) in all the merged images. Bar, 20μm.

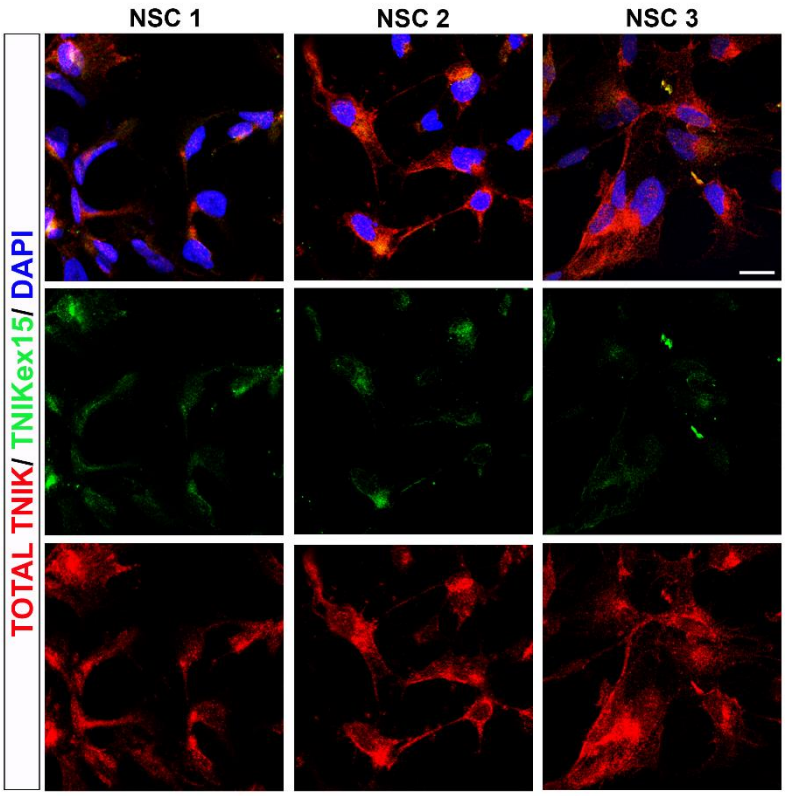
Supplementary Figure 3.



Characterization of human iPSC, NSC and iPSC-derived neurons.

Pluripotency of three human healthy iPSC lines was evaluated **a)** by IF analysis of TRA-1-60 (green) and **b)** by RT-PCR assay of *OCT 3/4*, *SOX 2* and *NANOG* stem cell markers. **c)** The three human healthy iPSC lines were differentiated into neural stem cells (NSC) and characterized for the Nestin marker (green) by IF analysis. **d)** The three iPSC lines were also differentiated into neurons (iPSC-N) and neuronal differentiation efficiency was evaluated by IF analysis with βIII-Tubulin (red) and neurofilament SMI 312 (green) markers. Nuclear staining is indicated in blue (DAPI) in all the merged images. Bar, 20μm.

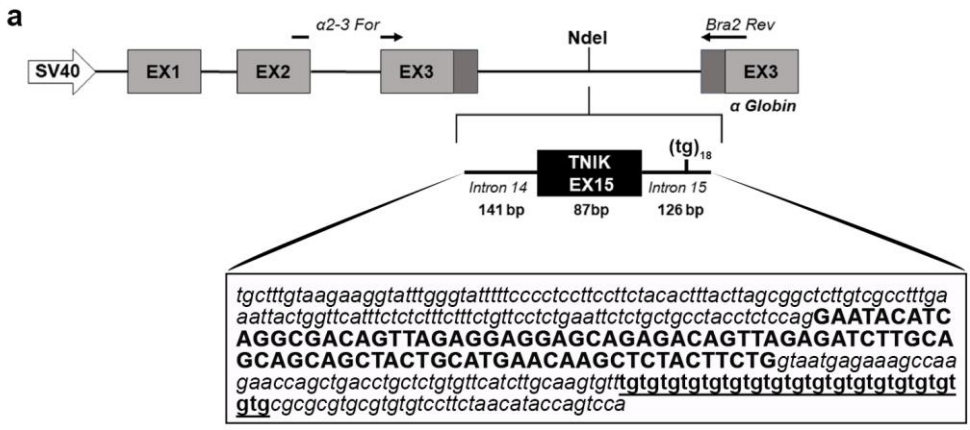
Supplementary Figure 4.

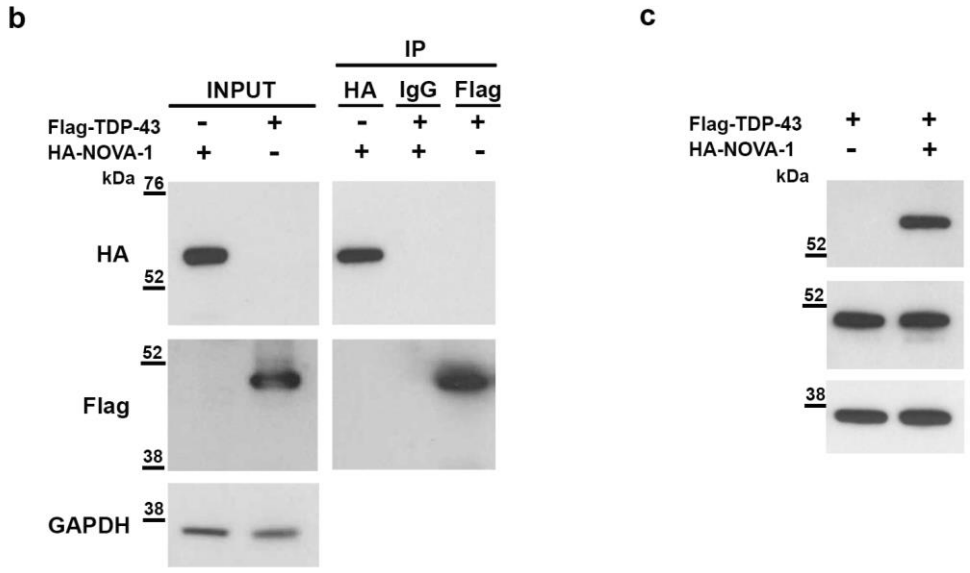


TNIKex15 protein expression in NSC.

Immunofluorescence images of TNIKex15 (green) and total TNIK proteins in the three neural stem cell lines (NSC) differentiated from three healthy iPSC lines. Nuclear staining is indicated in blue (DAPI) in the merged images. Bar, 20µm.

Supplementary Figure 5.

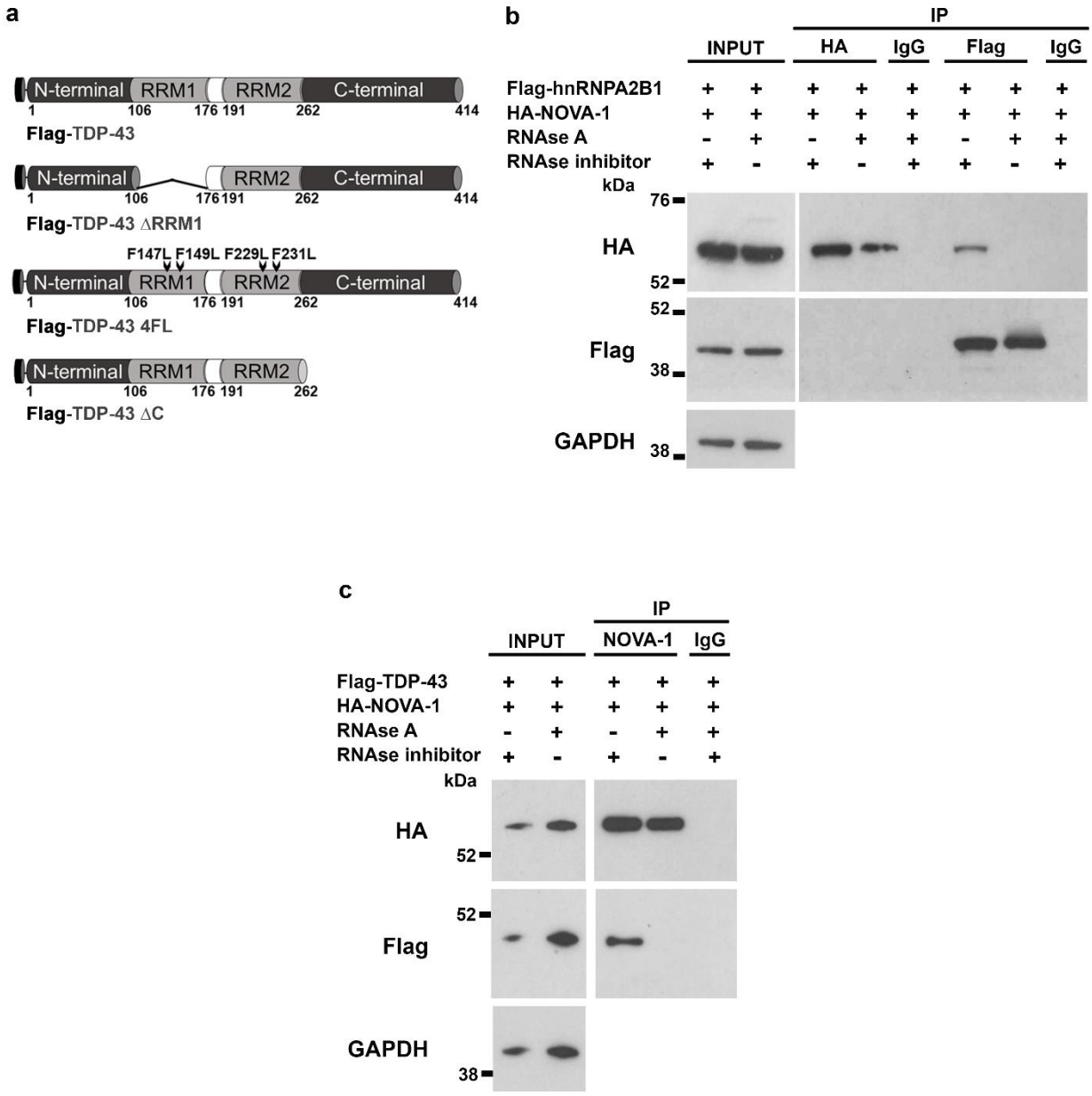




TNIK minigine splicing and UV-CLIP assays.

a) Schematic representation of the *TNIK* exon 15 minigene pTB construct as already described [12]. *TNIK* exon 15 (87bp) along with parts of 5' (141 bp) and 3' (126 bp) introns were subcloned into the NdeI restriction site of the pTB vector. The sequence of the subcloned region is reported in the box highlighting exon 15 sequence (capital letters) and the TDP-43 consensus binding motif TG_{18} (underlined). The primers used for the RT-PCR are also indicated (a2-3 For; Bra2 Rev). **b)** Representative WB images to check the transfection efficiency (INPUT) in HEK293T lysates used for UV-CLIP assays showed in Figure 3d and to assess the recombinant HA-NOVA-1 and Flag-TDP-43 recovery in IP assays. GAPDH was used for sample normalization. **c)** Representative WB images to assess the transfection efficiency of HA-NOVA-1 and Flag-TDP-43 in HEK293T lysates used for UV-CLIP assays showed in Figure 3e. GAPDH was used for sample normalization.

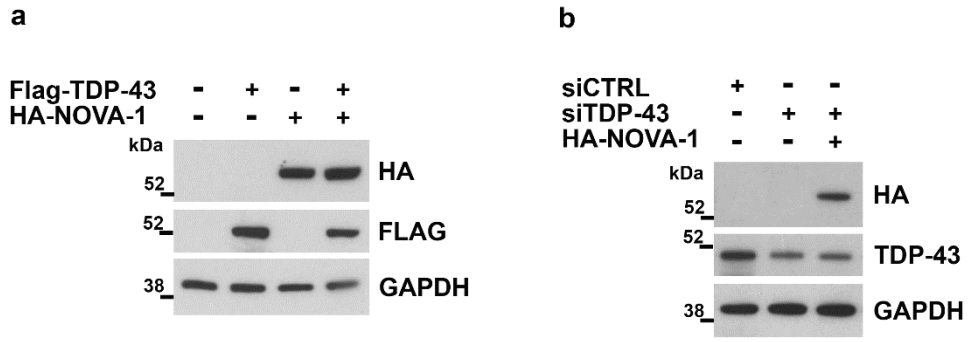
Supplementary Figure 6.



NOVA-1 protein interaction with TDP-43 and hnRNPA2B1.

a) Graphical representation of the Flag-tagged full-length and mutant TDP-43 constructs used in co-IP experiments showed in Figure 3g. **b)** Representative WB image of co-IP experiments using anti-Flag and anti-HA antibody to check for NOVA-1 binding to hnRNPA2B1. The irrelevant IgG antibody was used as negative control. HEK293T cells were co-transfected with Flag-hnRNPA2B1 and HA-NOVA-1 constructs and cell lysates treated with RNAseA or RNAse inhibitor to digest or preserve RNA respectively (n=3 independent experiments). GAPDH was used for sample normalization. **c)** Representative WB of co-IP experiments using anti-NOVA-1 antibody, recognizing a C-terminal epitope (315-537 aa) to check for NOVA-1 binding to TDP-43.

Supplementary Figure 7.



Determination of recombinant NOVA1 expression and TDP-43 knock-down in HEK293T cells.

a) Representative WB images to assess HA-NOVA-1 and Flag-TDP-43 transfection efficiency in HEK293T cells used for RT-PCR assays showed in Figure 4a (n=4). **b)** Representative WB image to assess TDP-43 knock-down and HA-NOVA-1 transfection efficiency in HEK293T cells used for assays showed in Figure 4d (n=3).

Supplementary Table S1. Description of the total RNA samples from different human adult tissues (Clontech)

Catalogue and lot numbers are reported together with the number of individuals included in each RNA set

Tissue	Cat./Lot Number	Sample features
Total brain	636530/1602002	3 male Asians, ages: 21-29
Total spinal cord	636554/1402006	male/female Asians and Caucasians, ages: 23-72
Skeletal muscle	636534/1611352A	Caucasian male, age: 20
Testis	636533/1402004	7 Asians and Caucasians, ages: 24-87
Kidney	636529/1508373A	Caucasian female; age: 40
Liver	636531/1402003	3 Asian males, ages: 24-64
Lung	636524/1508369A	3 male/female Caucasians, ages: 32-61

Supplementary Table S2. List of primer sequences used in RT-PCR and Q-PCR assays

The amplicon length is reported and also the Q-PCR efficiency calculated on 5 serial dilutions

Gene	Foward primer	Reverse primer	Ref	Assay	Size (bp)	Efficiency (%)
TNIK(ex15)	CAAAGGCGAGAGAAGGAGCTG	CTGATGCTGAAGGGAAACTAAG	12	RT-PCR	294/207	-
TNIK(ex17)	CTTCAGCATCAGCGGCAGGAGC	ACTGACTGGGAGGCGGTCAAG	-	RT-PCR	326/161	-
TNIK(ex22)	CAGAAGAATCCAGGGACATTAC	CACCTTCTTCATTGGGCGGTTTG	-	RT-PCR	137/113	-
RPL10a	CAAGAAGCTGGCCAAGAAGTATG	TCTGTCATCTTCACGTGAC	-	RT-PCR	228	-
GAPDH	TCCCCACTGCCAACGTGTCAGTG	ACCCTGTTGCTGTAGCCAAATTCG	12	RT-PCR	264	-
MINIGENE	CAACTTCAAGCTCCTAAGCCACTGC	GGTCACCAGGAAGTTGGTTAAATCA	12	RT-PCR	334/247	-
OCT3/4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC	42	RT-PCR	144	-
SOX2	TTGCGTGAGTGTGGATGGGATTGGTG	GGGAAATGGGAGGGGTGCAAAAGAGG	42	RT-PCR	151	-
NANOG	CAGCCCTGATTCTTCCACCAGTCC	GTTCTGGAACCAGGTCTTCACCTG	42	RT-PCR	244	-
POLDIP3	CCTTCATAAACCCACCCATTGGGACAG	GTGGTGGAGAAAGCCGCCTGAG	12	RT-PCR	293/155	-
STAG2	GTATGTTTACTTGGAAAAGTTCATG	TGATTCATCCATAATTGAAGCTGGA	31	RT-PCR	730/619	-
TNIK(ex15)	AATACATCAGGCGACAGTTAG	GCTTATATTCCAGAAGTAGAGCT	-	Q-PCR	96	122,38
TNIK(Total)	ACATACCATCTCATATTCAGGGC	CATTCCATCTGTTTTAGGCAAGA	-	Q-PCR	71	100,22
RPL10a	GAAGAAGGTGTTATGTCTGG	TCTGTCATCTTCACGTGAC	12	Q-PCR	51	97,95

Supplementary Table S3. List of primary antibodies used for immunofluorescence (IF), Western blot (WB) and immunoprecipitation (IP) assays.

Antibody (dilution)	Cat. Number (Company)	Assay
βIII Tubulin (1:800)	ab52623 (Abcam)	IF
TNIK exon 15 (1:500)	home-made (mouse)	IF
TRA1-60 (1:500)	14-8863-80 (Invitrogen)	IF
NESTIN (1:200)	MAB5326 (Chemicon)	IF
GFAP (1:50)	sc-33673 (Santa Cruz)	IF
MAP2 (1:200)	Ab32454 (Abcam)	IF
SMI 312 (1:700)	SMI-312R (Covance)	IF
TNIK (1:500)	GTX13141 (Gene Tex)	IF
HA (1:200)	H6908 (Sigma-Aldrich)	IF/IP
Alexa Fluor 546 Phalloidin (1:500)	A22283 (Thermo Fisher Scientific)	IF
TDP-43 (1:1000)	10782-2-AP (Protein Tech)	WB
NOVA-1 (1:1000)	PA5-21459 (Thermo Fisher Scientific)	WB/IP
FLAG (1:1000)	F3165 (Sigma-Aldrich)	WB/IP
GAPDH (1:1000)	sc-47724 (Santa Cruz)	WB
HA-Peroxidase (HRP) (1:10000)	130-091-972 (Miltenyi biotec)	WB
FLAG-Peroxidase (HRP) (1:1000)	A8592 (Sigma-Aldrich)	WB
hnRNPA2B1 (1:500)	sc-32316 (Santa Cruz)	WB
α Tubulin (1:500)	sc-8035 (Santa Cruz)	WB

Supplementary Table S4. Summary of experimental data and statistical analyses.

	Number of replicates	Mean±SEM	Statistical Test	Post *hoc* test
Figure 1				
1e	T0 : 3 T2 : 3 T4 : 3 T6 : 3	12.22%±2.42% 14.76%±1.09% 18.71%±3.25% 23.2%±4.65%	one way-ANOVA (F: 2.35 df: 11)	Tukey
1f	T0 : 4 T2 : 4 T4 : 4 T6 : 4	0.90±0.20 2.36±0.33 4.87±0.50 5.08±0.61	one way-ANOVA (F: 21.05 df: 15)	Tukey
1g	T0 : 4 T2 : 4 T4 : 4 T6 : 4	1.01±0.12 2.11±0.29 4.94±0.48 6.55±1.27	one way-ANOVA (F: 13.28 df: 15)	Tukey
1i	iPSCs : 3 NSCs : 3 iPSC-Neurons : 3	5.80%±1.30% 25.04%±3.94% 50.07%±3.90%	one way-ANOVA (F: 45.63 df: 8)	Tukey
1l	iPSCs : 3 NSCs : 3 iPSC-Neurons : 3	1.03± 0.17 0.99±0.061 2.79±0.86	one way-ANOVA (F: 4.12 df: 8)	Tukey
Figure 2				
2b	T0 : 4 T2 : 4 T4 : 4 T6 : 4	1.06±0.10 1.17±0.14 1.04±0.13 1.08±0.19	one way-ANOVA (F: 0.15 df: 15)	Tukey
2c	T0 : 4 T2 : 4 T4 : 4 T6 : 4	0.92±0.14 1.11±0.10 0.98±0.09 1.62±0.21	one way-ANOVA (F: 4.83 df: 15)	Tukey
2e	iPSCs : 3 NSCs : 3 iPSC-Neurons : 3	1.00±0.25 0.83±0.19 0.92±0.26	one way-ANOVA (F: 0.13 df: 8)	Tukey
2f	iPSCs : 3 NSCs : 3 iPSC-Neurons : 3	1.00±0.34 1.31±0.30 5.25±0.57	one way-ANOVA (F: 31.44 df: 8)	Tukey
2h	pcDNA : 3 HA-NOVA-1 : 3	4.36%±0.59% 17.42%±5.72%	One-tailed Unpaired t Test (t: 2.27 df: 4)	-
2i	pcDNA : 3 HA-NOVA-1 : 3	1.00±0.05 0.75±0.05	One-tailed Unpaired t Test (t: 3.18 df: 4)	-
2l	pcDNA : 3 HA-NOVA-1 : 3	1.00±0.11 1.02±0.09	One-tailed Unpaired t Test (t: 0,13 df: 4)	-
Figure 3				
3b	pcDNA : 3 Flag-TDP-43 : 3 HA-NOVA1 : 3 Flag-hnRNPA2B1: 3 HA-NOVA-1 + Flag-TDP-43 : 3 HA-NOVA-1 + Flag-hnRNPA2B1 : 3 Flag-TDP-43 + Flag-hnRNPA2B1 : 3 HA-NOVA-1+ Flag-TDP-43 +	88.19%±5.83% 53.47%±7.51% 92.15%±4.49% 68.82%±8.75% 90.80%±3.80% 92.77%±3.81% 32.62%±3.94% 93.54%±1.78%	one way-ANOVA (F: 17.82 df: 23)	Tukey

	Flag-hnRNPA2B1: 3			
Figure 4				
4b	pcDNA : 4 Flag-TDP-43 : 4 HA-NOVA1 : 4 HA-NOVA-1 + Flag-TDP-43 : 4	12.81%±0.66% 14.92%±1.76% 28.37%±2.30% 27.88%±4.09%	one way-ANOVA (F: 10.73 df: 15)	Tukey
4c	pcDNA : 3 Flag-TDP-43 : 3 HA-NOVA1 : 3 HA-NOVA-1 + Flag-TDP-43 : 3	26.00%±1.22% 27.10%±0.99% 53.85%±2.23% 51.61%±2.13%	one way-ANOVA (F: 76.33 df: 11)	Tukey
	pcDNA : 3 Flag-TDP-43 : 3 HA-NOVA1 : 3 HA-NOVA-1 + Flag-TDP-43 : 3	21.83%±2.74% 21.67%±2.42% 32.32%±1.82% 30.93%±1.73%	one way-ANOVA (F: 6.68 df: 11)	Tukey
4e	siCTRL : 3 siTDP-43 : 3 siTDP-43+HA-NOVA1 : 3	22.69%±1.03% 32.68%±0.21% 41.62%±1.46%	one way-ANOVA (F: 101.6 df: 8)	Tukey
4f	siCTRL : 3 siTDP-43 : 3 siTDP-43+HA-NOVA1 : 3	21.70%±4.02% 54.65%±5.71% 67.41%±0.76%	one way-ANOVA (F: 33.82 df: 8)	Tukey
	siCTRL : 3 siTDP-43 : 3 siTDP-43+HA-NOVA1 : 3	27.66%±1.30% 41.47%±4.02% 45.59%±1.84%	one way-ANOVA (F: 12.47 df: 8)	Tukey
Figure 5				
5a	pcDNA + GFP : 3 (75 cells) HA-TNIKex15 + GFP : 3 (75 cells) HATNIK Δ15 + GFP : 3 (75 cells) HA-TNIK KM + GFP : 3 (75 cells)	0.31±0.02 0.55±0.02 0.38±0.02 0.27±0.02	Kruskal-Wallis (KW stat: 81.41)	Dunn
Figure 6				
6c	pcDNA + GFP : 4 (72 cells) HA-TNIKex15 + GFP : 4 (59 cells) HATNIK Δ15 + GFP : 4 (72 cells) HA-TNIK KM + GFP : 4 (59 cells)	10.24±0.36 8.40±0.41 9.93±0.44 10.75±0.45	Kruskal-Wallis (KW stat: 14.01)	Dunn
6e	pcDNA + GFP : 4 (65 cells) HA-TNIKex15 + GFP : 4 (45 cells) HATNIK Δ15 + GFP : 4 (65 cells) HA-TNIK KM + GFP : 4 (38 cells)	1.05±0.11 0.60±0.07 0.90±0.08 0.87±0.14	Kruskal-Wallis (KW stat: 10.81)	Dunn
6f	pcDNA + GFP : 4 (65 cells) HA-TNIKex15 + GFP : 4 (45 cells) HATNIK Δ15 + GFP : 4 (65 cells) HA-TNIK KM + GFP : 4 (38 cells)	2.29±0.21 1.35±0.16 2.18±0.19 1.93±0.19	Kruskal-Wallis (KW stat: 11.31)	Dunn
6g	pcDNA + GFP : 4 (65 cells) HA-TNIKex15 + GFP : 4 (45 cells) HATNIK Δ15 + GFP : 4 (65 cells) HA-TNIK KM + GFP : 4 (38 cells)	10.78±1.12 9.27±0.7 12.04±0.95 8.49±0.81	Kruskal-Wallis (KW stat: 6.78)	Dunn
6i	pcDNA + GFP : 4 (46 cells) HA-TNIKex15 + GFP : 4 (35 cells) HATNIK Δ15 + GFP : 4 (46 cells) HA-TNIK KM + GFP : 4 (42 cells)	1.06±0.11 0.83±0.13 1.06±0.12 2.26±0.24	Kruskal-Wallis (KW stat: 27.70)	Dunn
6l	pcDNA + GFP : 4 (46 cells) HA-TNIKex15 + GFP : 4 (35 cells) HATNIK Δ15 + GFP : 4 (46 cells) HA-TNIK KM + GFP : 4 (42 cells)	283.30±17.78 200.10±9.69 238.50±11.22 240.4±12.14	Kruskal-Wallis (KW stat: 13.91)	Dunn