

Figure S1. Alami and Smith et al.

**Fig. S1. TDP-43 distribution in *Drosophila* motor neurons.** Related to Figure 1. (A) TBPH null mutants show extremely poor viability ( $p=0.0027$ ), but can be rescued with ubiquitous expression of Venus-TBPH and Venus-TDP-43<sub>WT</sub>. Disease-causing mutant Venus TDP-43<sub>M337V</sub> failed to rescue the null phenotype ( $p=0.0042$ ). (B) Images from live motor neuron cell somas within the ventral ganglion in *Drosophila* third instar larvae show nuclear and cytoplasmic TDP-43 localization. pUAS<sub>t</sub>-CD8-RFP (red), pUAS<sub>t</sub>-Venus-TDP-43 (green). (C) Higher magnification of motor neuron cell bodies from panel (B). (D) Quantification of fluorescence intensity at the cell soma shows that mutant TDP-43 was significantly increased when compared to TDP-43<sub>WT</sub> ( $p<0.001$ ). (E) Live imaging of the synaptic terminal on muscle 13 reveal that Venus-TDP-43<sub>WT</sub> is present at the synapse, whereas Venus-TDP-43<sub>M337V</sub> and TDP-43<sub>A315T</sub> were less abundant. CD8-RFP was used to demarcate the synaptic terminal. (F) Quantification of fluorescence intensities at the synapse shows that Venus-TDP-43<sub>WT</sub> is significantly higher than the signal of the control ( $p<0.001$ ) and mutant TDP-43. Scale bars: 10  $\mu\text{m}$ . (G) Third instar larva ventral ganglion (cartoon for orientation) show localization of Venus-TDP-43<sub>WT</sub> in motor neuron nuclei, cell bodies, and axons, where they are punctate (arrowheads). Venus-TDP-43<sub>WT</sub> also localizes to the presynaptic terminal in third instar larvae. (H) Western blot analysis of TDP-43<sub>WT</sub>, TDP-43<sub>M337V</sub>, and TDP-43<sub>A315T</sub> protein levels in *Drosophila* motor neurons when expressed by OK371>Gal4 (top band) and levels of loading control (bottom band). Wild-type (W1118) was used as negative control. (I) Quantification of TDP-43<sub>WT</sub>, TDP-43<sub>M337V</sub>, and TDP-43<sub>A315T</sub> protein levels in *Drosophila* motor neurons when expressed by OK371>Gal4.



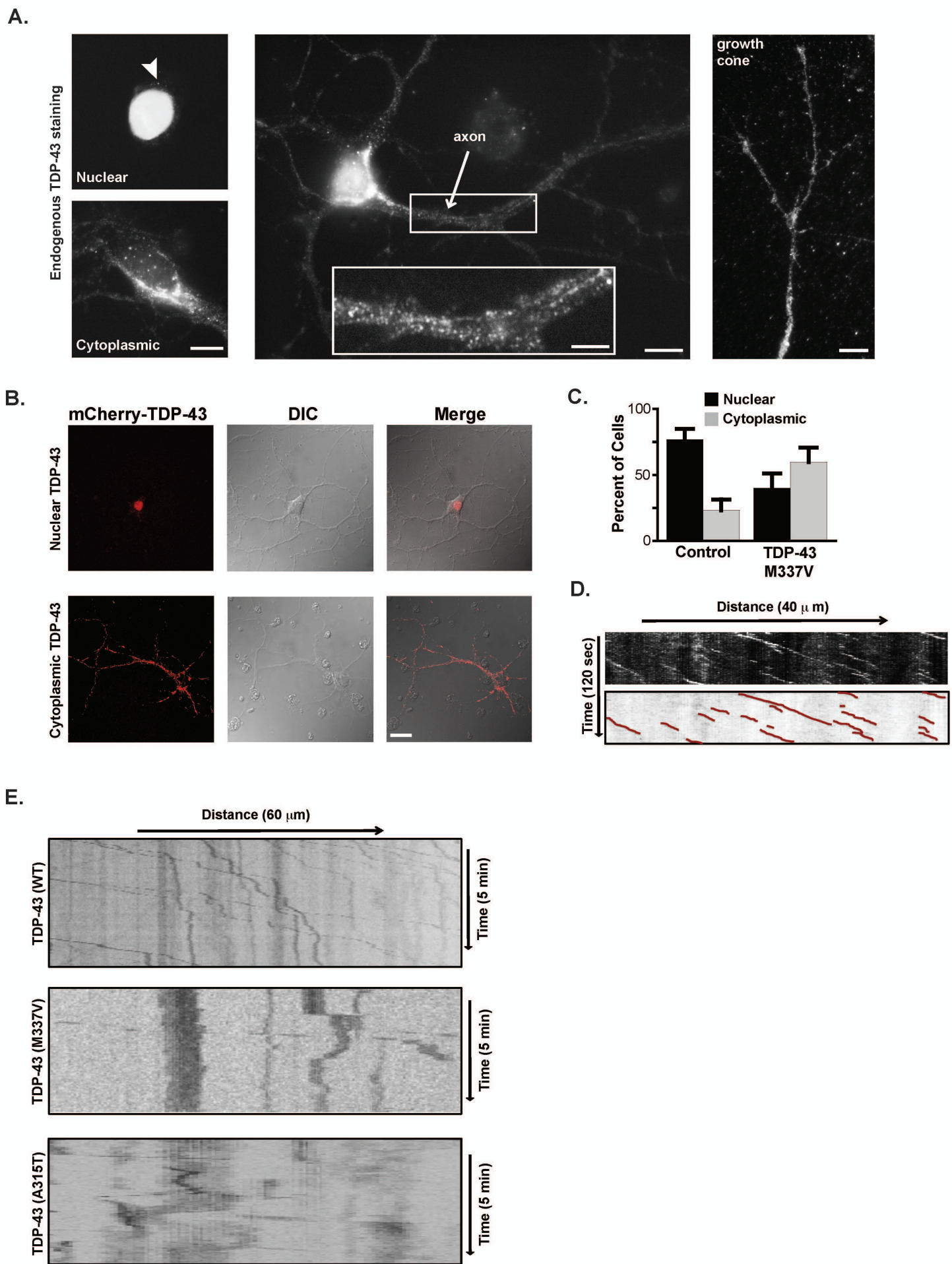


Figure S2. Alami and Smith et al.

**Fig. S2. TDP-43 in mouse cortical neurons in culture.** Related to Figure 2. (A) Fixed, non-transfected primary cortical neurons (5 DIV) showed primarily nuclear TDP-43 localization in 83% of counted cells (N=50). In some cells, TDP-43 localization was primarily cytoplasmic. The middle panel shows cytoplasmic localization of TDP-43 with TDP-43 granules present along the axon of the neuron (arrow). The panel on the right shows the presence of TDP-43 granules in the growth cone (Scale bar: right and left panels: 5  $\mu$ m, middle panel: 10  $\mu$ m, inset: 4  $\mu$ m). (B) 5 DIV cortical neurons transfected with mCherry-TDP-43<sub>WT</sub> showed primarily nuclear fluorescence similar to what we observed in our immunofluorescence experiments on non-transfected cells. Some cells expressed the fluorescent protein in the cytoplasmic and neuritic compartments. Scale bar: 10  $\mu$ m. (C) Cortical neurons transfected with mCherry-TDP-43<sub>WT</sub> show primarily nuclear localization of the protein (76.7% + 8.1 of the cells in 20 fields of view @ 63X magnification from 3 separate cultures, N=52 cells). In cells transfected with mCherry-TDP-43<sub>M337V</sub> there was a decrease in nuclear TDP-43 staining (36.3% + 10.7 of the cells in 20 fields of view @ 63X magnification from 3 separate cultures, N=44 cells). (D) To confirm that our transport analysis is performed along neuronal axons, we monitored the movements of the microtubule-capping protein EB1, which move strictly in the anterograde direction along axons, but randomly along dendrites, due to the uniform polarity of microtubules along axons, with the microtubule plus ends pointing away from the cell body. This kymograph shows that all YFP-EB1 comets are moving anterogradely along the axon of a cell transfected with mCherry-TDP-43. (E) Kymographs showing TDP-43 movement along the axons of cultured cortical neurons as line scans across the x-axis, and time over the y-axis. The vertical lines represent stationary granules while horizontal lines represent moving granules.

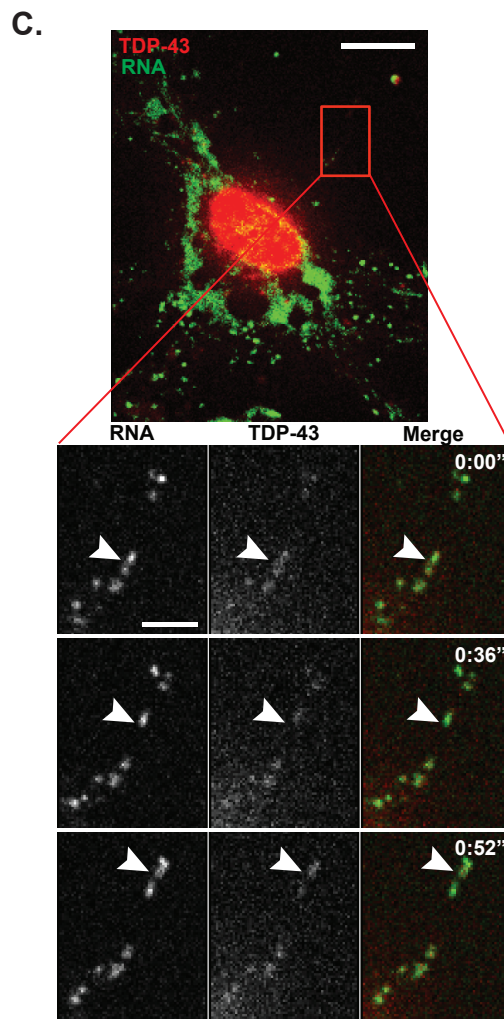
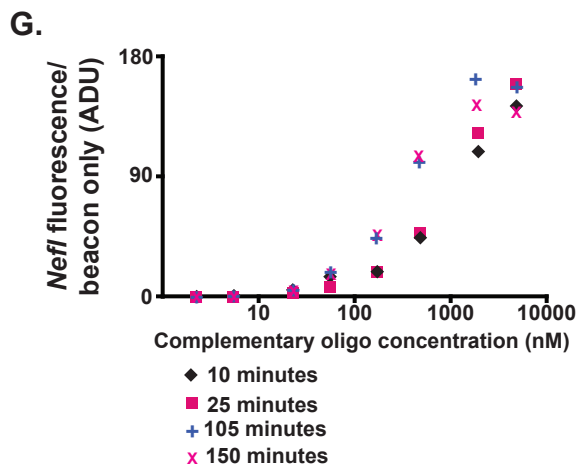
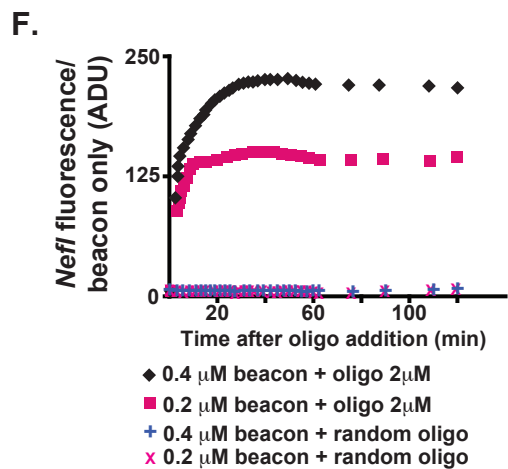
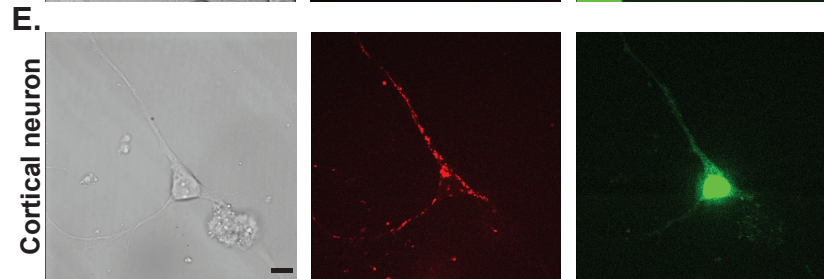
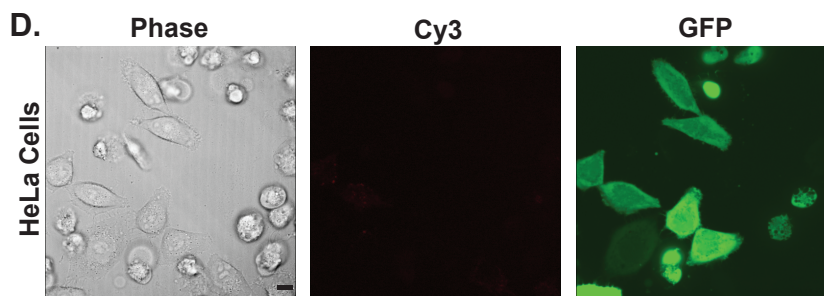
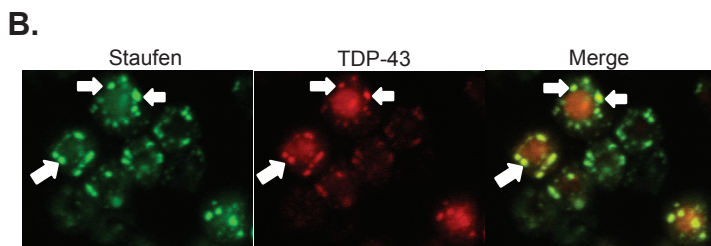
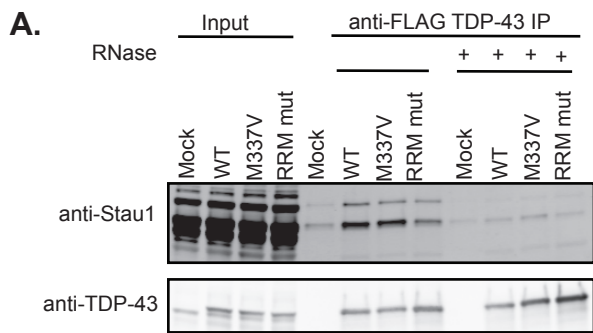


Figure S3. Alami and Smith et al.

**Fig. S3. TDP-43 is part of an RNP granule and can be detected using Nefl mRNA beacons.**

Related to Figure 3. (A) The RNA-dependence of the TDP-43/Staufen interaction is demonstrated by the inclusion of RNase A in the co-immunoprecipitation from HEK293T cells. (B) Live imaging of *Drosophila* third instar motor neurons shows colocalization of Staufen-GFP (green) and Venus-TDP-43<sup>WT</sup> (red) in the cytoplasm of these cells. (C) Live imaging of cultured mouse cortical neurons reveals co-transport of TDP-43 granules (red) with RNA transcripts that were stained using SYTO Green RNASelect dye. Scale bar: 10  $\mu\text{m}$ ; inset: 3  $\mu\text{m}$ . (D) *Nefl* mRNA beacons were tested in HeLa cells that lack Neurofilament-L mRNA. Cells were cotransfected with *Nefl* beacon and GFP construct. The fluorophore attached to the beacon (Cy3) does not emit a fluorescent signal in the absence of its target (red channel). (E) Cortical neurons cotransfected with *Nefl* beacon (red) and GFP construct (green) can be observed using fluorescent imaging. Scale bars: 10  $\mu\text{m}$ . (F) *Nefl* beacon hybridization with a complementary sequence was tested in vitro using synthetic oligonucleotide targets in a solution. *Nefl* beacon was mixed with either a complimentary or a non-complementary oligonucleotide and beacon fluorescence was measured using a microplate reader at room temperature for 1hr at 1min intervals followed by 10-min interval measurements for 2hrs. *Nefl* beacon hybridized rapidly with its complimentary oligonucleotide and a high specific fluorescence was detected. Importantly the beacon did not hybridize with a non-complimentary oligonucleotide. (G) The beacon fluorescent signal intensity increased with increasing oligonucleotide concentrations and peaked at 2000 nM.



**A.**

Patient	Sex	Diagnosis	Genotype	Age of Onset	Age at Biopsy	Reference
31	Male	FALS	<i>TARDBP</i> <sup>+M337V</sup>	Unknown	56	Bilican et al. 2012
47	Male	FALS	<i>TARDBP</i> <sup>+G298S</sup>	43	43	This study
RB20	Male	FALS	<i>TARDBP</i> <sup>+A315T</sup>	67	70	This study
33	Male	Healthy control	<i>TARDBP</i> <sup>+/+</sup>	N.A.	56	Bilican et al. 2012
11	Male	Healthy control	<i>TARDBP</i> <sup>+/+</sup>	N.A.	37	Boulting et al. 2011

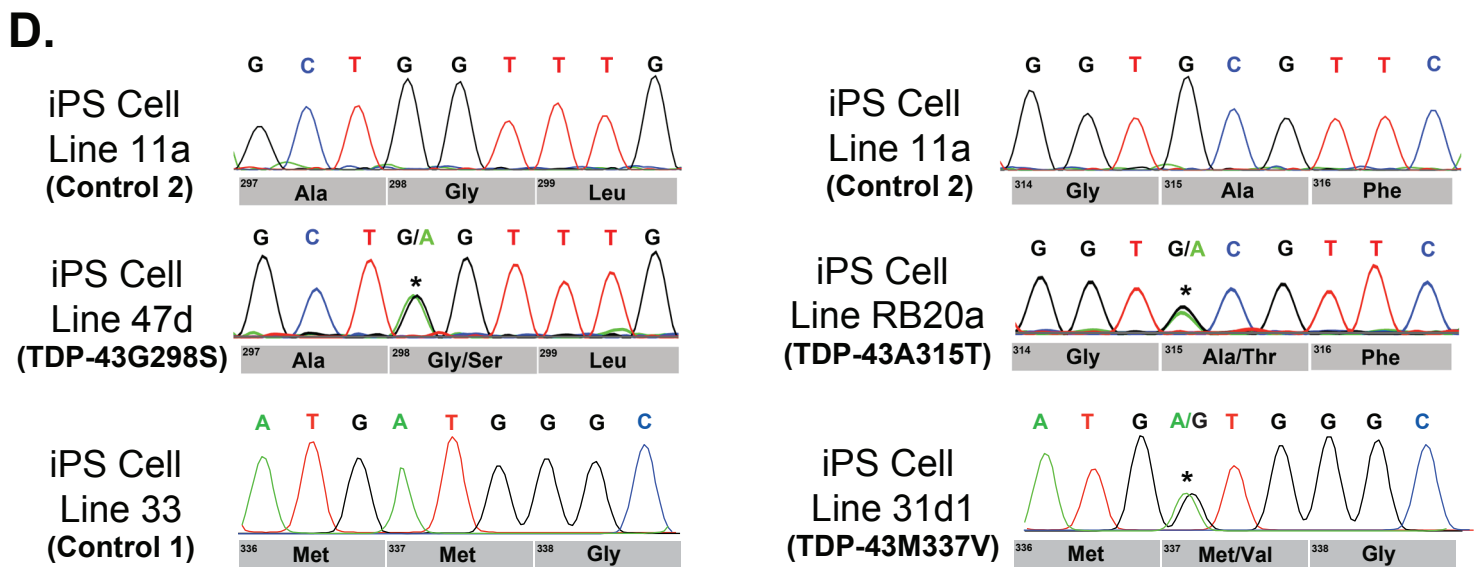
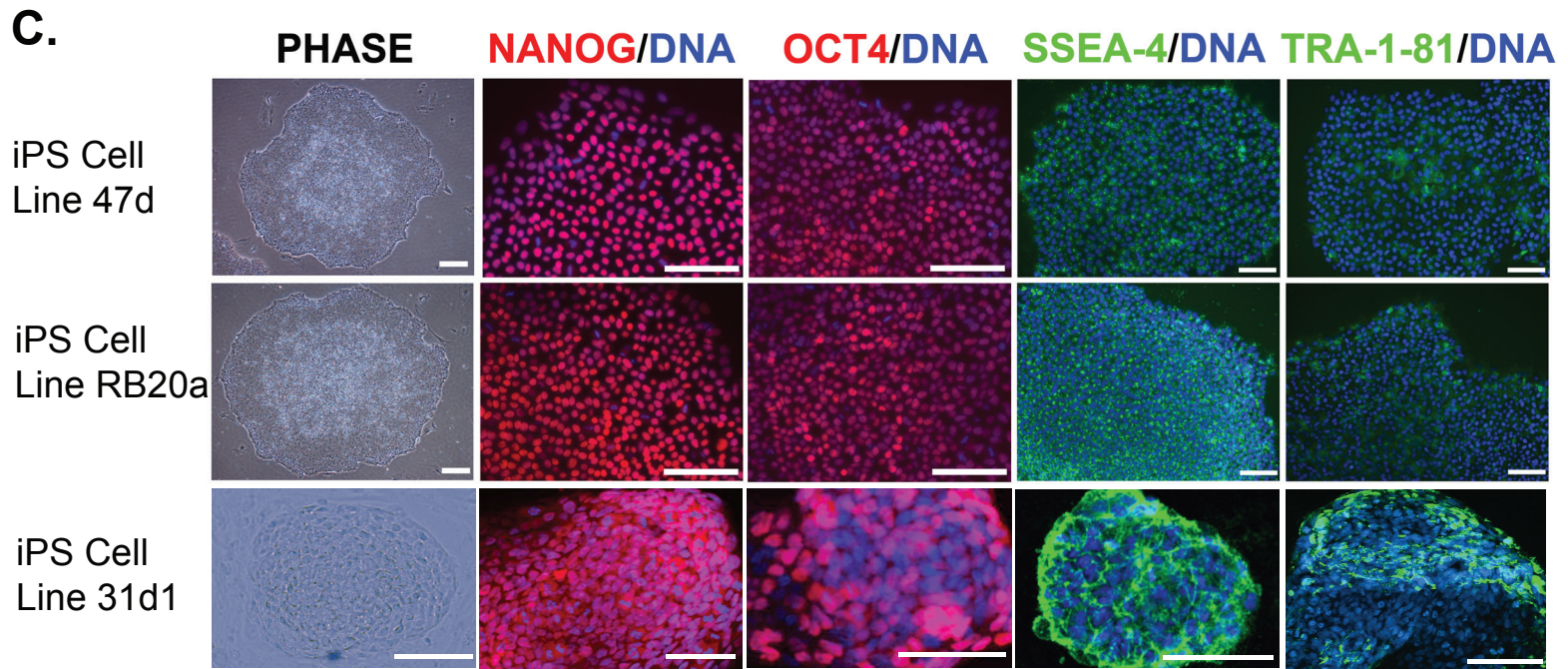
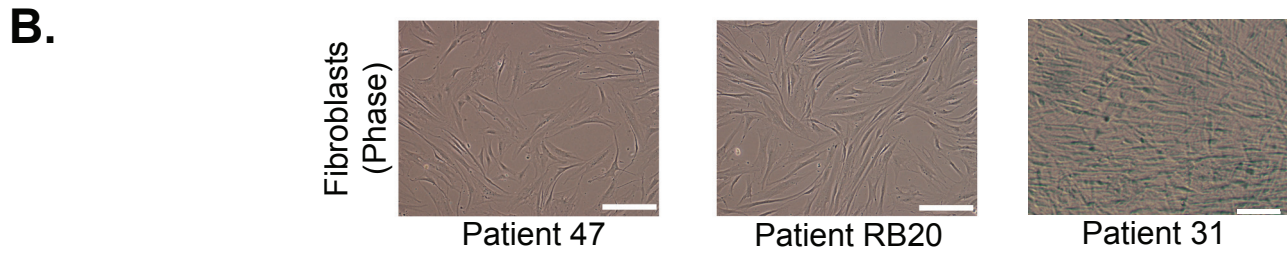


Figure S4. Alami and Smith et al.

**Fig. S4. Generation of additional mutant TDP-43 iPS cell lines.** Related to Figure 4.

(A) Patient information. (B) Cultured fibroblasts obtained from patient skin biopsies were transduced with reprogramming factors OCT4, SOX2, and KLF4 to generate iPS cells. (C) TDP-43 iPS cell lines express transcription factors and surface antigens characteristic of the pluripotent state. (D) Sequencing of *Exon 6* of the *TARDBP* locus confirmed the presence of mutations encoding ALS-linked TDP-43 variants in 47d (TDP-43<sup>G298S</sup>), RB20a (TDP-43<sup>A315T</sup>), and 31d (TDP-43<sup>M337V</sup>) iPS cell lines. Scale bars: 100  $\mu$ m.

## Supplementary Table

**Table S1.** Related to Figures 1, 2, and 4. Transport kinetics and statistical analysis of TDP-43, mitochondria, and NEFL transport in *Drosophila* motor neurons, mouse cortical neurons, and human motor neurons derived from iPS cells.

Transport kinetics of TDP-43 in <i>Drosophila</i> and mouse cortical neurons	In Vitro (mouse cortical neurons)		Total Displacement (μm)	Net Displacement (μm)	Average Velocity (μm/sec)	Max Velocity (μm/sec)
		Anterograde	TDP-43 <sub>WT</sub> (n=56)	122.3	91.8	0.24
TDP-43 <sub>M337V</sub> (n=41)	46.8 (p<0.001)		35.4 (p<0.001)	0.21 (p=0.3595)	0.53 (p=0.1100)	
TDP-43 <sub>A315T</sub> (n=43)	98.3 (p=0.0237)		53.1 (p=0.0383)	0.13 (p=0.0547)	0.68 (p=0.0885)	
Retrograde	TDP-43 <sub>WT</sub> (n=53)	107.8	75.6	0.22	0.56	
	TDP-43 <sub>M337V</sub> (n=56)	47.7 (p<0.001)	35.2 (p<0.001)	0.22 (p=0.4062)	0.55 (p=0.4899)	
	TDP-43 <sub>A315T</sub> (n=50)	101.5 (p=0.0424)	49.8 (p=0.0493)	0.14 (n=0.0833)	0.43 (p=0.0507)	
In Vivo ( <i>Drosophila</i> motor neurons)	Anterograde	TDP-43 <sub>WT</sub> (n=86)	324.9	217.7	0.31	1.01
		TDP-43 <sub>M337V</sub> (n=61)	306.0 (p=0.1007)	158.1 (p=0.0268)	0.33 (p=0.3486)	1.30 (p=0.1215)
		TDP-43 <sub>A315T</sub> (n=17)	438.6 (p=0.2328)	121.3 (p=0.0426)	0.48 (p=0.0153)	1.53 (p<0.001)
	Retrograde	TDP-43 <sub>WT</sub> (n=65)	261.2	165.7	0.34	1.07
		TDP-43 <sub>M337V</sub> (n=60)	265 (p=0.2807)	117.7 (p=0.0517)	0.41 (p=0.0566)	1.44 (p=0.0525)
		TDP-43 <sub>A315T</sub> (n=16)	433.1 (p=0.0806)	62.5 (p<0.001)	0.38 (p=0.3253)	1.56 (p<0.001)

Transport kinetics of mitochondria in mouse cortical neurons		Cells transfected with	Total Displacement (μm)	Net Displacement (μm)	Average Velocity (μm/sec)	Max Velocity (μm/sec)
		Anterograde	TDP-43 <sub>WT</sub> (n=21)	155.1	124.4	0.24
TDP-43 <sub>M337V</sub> (n=14)	167.8 (p=0.2818)		139.6 (p=0.2835)	0.21 (p=0.3266)	0.653 (p=0.4744)	
TDP-43 <sub>A315T</sub> (n=19)	146.9 (p=0.4230)		107.54 (p=0.1901)	0.18 (0.2260)	0.538 (p=0.2847)	
Retrograde	TDP-43 <sub>WT</sub> (n=17)	186.1	160.3	0.21	0.664	
	TDP-43 <sub>M337V</sub> (n=12)	197.2 (p=0.337)	148.1 (p=0.1879)	0.22 (p=0.8030)	0.505 (p=0.1390)	
	TDP-43 <sub>A315T</sub> (n=15)	169.4 (p=0.1944)	112.4 (p=0.2831)	0.19 (0.5990)	0.478 (p=0.2057)	

Transport of NEFL mRNA in human motor neurons		Net Anterograde Displacement (μm)			
		2-4 DIV	5-8 DIV	9-13 DIV	14-17 DIV
Control Cells (Line 1)	Control Cells (n=94)	315.3	612.2 (n=143)	924.7 (n=102)	N.A.
	Patient Cells (M337V) (n=116; p=0.4708)	426.3	770.6 (n=118; p=0.2132)	564.4 (n=88; p=0.0485)	N.A.
Control Cells (Line 2)	Control Cells (n=93)	551.0	556 (n=145)	1266.5 (n=408)	2090.9 (n=150)
	Patient Cells (A315T) (n=87; p=0.2127)	619.6	539.1 (n=153; p=0.1404)	451.6 (n=187; p=0.0035)	409.1 (n=42; p<0.001)
Patient Cells (G298S) (n=44; p=0.4206)	449.2	304.1 (n=117; p=0.0471)	585.7 (n=265; p=0.0022)	863.6 (n=124; p<0.001)	

## Supplemental Methods

**Mouse Cell Culture.** Timed pregnant female C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). Cells were grown in a glial/cortical sandwich culture using the Banker technique (Kaech and Banker, 2006). Glial cells were prepared from cerebral cortices of P0 pups. Cortices were dissociated and cultured in 60 mm plastic dishes coated with poly-D-lysine (PDL; Sigma) in Minimum Essential Medium (Invitrogen). Cortical neurons were prepared from the cerebral cortex of E17.5 embryos. Dissociated cells were plated onto glass coverslips coated with PDL (Sigma). Glass coverslips were suspended over the glia, using dots of paraffin as spacers and the cultures were maintained in plating medium that was replaced with FBS-free medium after 2 days. For neuronal transfection, prior to plating the neurons on glass coverslips, cells were electroporated using program O-0.05 of the Amaxa Nucleofector (Lonza Inc., Walkersville, MD) and the mouse neuron nucleofection kit provided by the manufacturer. Plasmid concentration in 100  $\mu$ l transfection solution was 5-10  $\mu$ g/construct, and the cell concentration was  $\sim 6 \times 10^6$  cells/ml. Cortices were dissociated in phosphate-buffered saline (PBS; Invitrogen, Carlsbad, CA) containing 0.25% (w/v) trypsin (Worthington Biochem. Corp., Lakewood, NJ), 1% (w/v) DNase-I (Sigma, St. Louis, MO), and 0.54 mM EDTA (Sigma), and cells were cultured in 60 mm plastic dishes previously coated with poly-D-lysine (Sigma) at 37°C/5% CO<sub>2</sub> in Minimum Essential Medium (Invitrogen) supplemented with 10 % (v/v) fetal bovine serum (Seradigm, Providence, UT), 0.7% (w/v) glucose (Sigma) and 16  $\mu$ g/ml gentamicin (Invitrogen). Cortical



neurons were prepared from the cerebral cortex of E17.5 embryos, dissociated in PBS containing 0.025% (w/v) trypsin, 0.27 mM EDTA (Sigma), and 0.5% (w/v) DNase-I. Cortical plating medium, consisted of NbActiv4 (BrainBits LLC, Springfield, IL) supplemented with 0.27% (w/v) glucose, 5% (v/v) fetal bovine serum (FBS; Seradigm), and 16 µg/ml gentamicin. The culturing medium was identical to the plating medium but lacked FBS.

**iPS and iPS-derived motor neurons: Control Line 2, G298S, and A315T.**

Spinal motor neurons were differentiated from 11a (Control 2), 47d (TDP-43<sub>G298S</sub>) and RB20a (TDP-43<sub>A315T</sub>) cell lines using a modified protocol based on adherent culture conditions in combination with dual inhibition of SMAD signaling, inhibition of NOTCH and FGF signaling, and patterning by retinoic acid and SHH signaling (42, 43). In brief, iPS cells were dissociated to single cells using accutase™ (STEMCELL Technologies) and plated at a density of 60,000 cells/cm<sup>2</sup> on matrigel-coated culture plates with mTeSR™1 medium (STEMCELL Technologies) supplemented with ROCK-inhibitor (10µM Y-27632, EMD Millipore). When cells reached ~90% confluency, medium was changed to KSR medium (KO-DMEM (Life Technologies™) supplemented with 15% Knockout Serum Replacement (Life Technologies™), 1X GIBCO® GlutaMAX™ (Life Technologies™) and 100µM non-essential amino-acids). This time point was defined as day 0 (d0) of motor neuron differentiation. Cells were fed every 24 hours, and after 4 days (on d4), increasing amounts of N2 medium (Neurobasal (Life Technologies™) supplemented with 1X N-2 (Life Technologies™), 1X GIBCO® GlutaMAX™ (Life Technologies™) and 100µM non-essential amino-acids) were used to replace KSR medium, with complete replacement by d10.

Cells were cultured in N2 medium for 4 additional days. Treatment with small molecules was carried out as follows: 10 $\mu$ M SB431542 (SIGMA) and 100nM LDN-193189 (STEMGENT) on d0-d5; 1 $\mu$ M retinoic acid (SIGMA) and 1 $\mu$ M SMOOTHEND agonist (EMD Millipore) on d2-d14; 5 $\mu$ M DAPT (EMD Millipore) and 4 $\mu$ M SU-5402 (BIOVISION) on d2-d14. On d14, differentiated cells were dissociated to single cells using accutase<sup>™</sup> (STEMCELL Technologies) and plated onto matrigel-coated cover-slips or 8-well chamber slides (BD BioCoat<sup>™</sup>), for live-fluorescence imaging or immunocytochemistry, respectively. Dissociated neuronal cells were cultured in murine glia-conditioned N2 medium supplemented with 1X B-27<sup>®</sup> (Life Technologies<sup>™</sup>), and 10 ng/mL of each of the neurotrophic factors BDNF, GDNF and CNTF (R&D). Immunofluorescence on these cells was done as reported (39), using ISLET-1 (1:2000, abcam<sup>®</sup>) and MAP2 (1:10000, abcam<sup>®</sup>) as motor neuron markers.

**iPS and derived iPS motor neurons: Control Line 1 and M337V.** iPS cells were maintained on gelatinized tissue-culture plastic on a mono-layer of neomycin selected mouse embryonic fibroblasts (MEFs; Millipore) in hiPS media consisting of Dulbecco's Modified Eagle Medium: nutrient mixture F-12 (DMEM/F:12, Invitrogen) with 20% Knockout Serum Replacer (KSR; Invitrogen), 110  $\mu$ M  $\beta$ -mercaptoethanol (BME; Sigma), L-Glutamine and nonessential amino acids (NEAA; Invitrogen), and 20 ng/ml basic fibroblast growth factor (bFGF; Invitrogen) (Bilican et al., 2012; Dimos et al., 2008). To generate motor neurons, undifferentiated iPS cells were incubated with 10  $\mu$ M Rho-associated kinase inhibitor Y27632 (Calbiochem) for 2 hours and then passaged using trypsin, triturated, and placed into ultra-low adherent culture dishes (Corning) for the

seeding of embryoid bodies (EBs). For the first 11 days, cells were kept in suspension in hiPS cell media without bFGF supplement. At day 11, EBs were switched to neural induction medium (DMEM/F:12 with L-glutamine, NEAA, N2 supplement (Invitrogen)). At day 12, EBs were incubated using retinoic acid (RA 1  $\mu$ M, Sigma) and purmorphamine (PUR 10  $\mu$ M, Calbiochem) and kept in induction for another 2 weeks. At day 28, EBs were dissociated with 0.05% trypsin (Invitrogen), transfected using Lonza Nucleofector with molecular beacons and HB9 (9Kb)-promoter-GFP, and plated onto poly-D-lysine/ laminin-coated (BD Biosciences) glass at  $50 \times 10^5$  cells/ml using a modified version of the Banker methodology (Kaech and Banker, 2006). To prepare the cells for live imaging, cells were allowed to settle on the glass coverslips that were flipped over primary glial monolayers to sustain neuronal development and maturity. Plated neurons were cultured in DMEM:F12 media supplemented with N2, B27 (Invitrogen), retinoic acid (Sigma), D-glucose and 40 ng/ml of BDNF, GDNF, CNTF and NT3. Media was changed every other day and all iPS derived motor neurons were kept for a period of 2 weeks.

**Confocal Microscope Imaging.** Glass coverslips were placed in Bioptechs FCS2 closed heated chamber that is kept at 37°C with imaging medium. The media was then changed every 2 hrs. Imaging medium consisted of Hibernate E low-fluorescence (BrainBits) supplemented with 0.3% glucose, 1mM L-glucose, 2% (v/v) B27 supplement (Invitrogen), 62.5 mM NaCl, and 2  $\mu$ g/ml gentamicin (Invitrogen). Transport was imaged with a Plan Apo 63X/1.4 NA oil-immersion objective with 2-4 s intervals for cortical neurons and 1-2 s intervals for *Drosophila* neurons. Movie length ranged from 5 to 15 min. Larval NMJ imaging

was completed with a Plan Neofluor 40X 1.3 oil objective. For Venus and mCherry single color samples, 514 and 561 laser lines were used. For GFP (or YFP)/mCherry or Venus/RFP color samples, 488 and 561 laser lines were used.

**Beacon hybridization in solution.** *Nefl* beacon hybridization with a complementary sequence was tested *in vitro* using synthetic oligonucleotide targets in a solution. Beacon (0.2  $\mu$ M) was mixed with either a complimentary or a non-complementary oligonucleotide (2  $\mu$ M) in 50 mM sodium borate, 100 mM NaCl, 0.05% tween-20, pH 8.3 (Chen et al., 2007) and beacon fluorescence was measured using a microplate reader (FLX- 800 BioTek, Winooski VT, with filter wheels  $\lambda_{Ex}$  560/ 15,  $\lambda_{Em}$  590/ 20) at room temperature throughout 1 h at 1 min intervals. *Nefl* beacon hybridized rapidly with its complimentary oligonucleotide and a high specific fluorescence was detected. Importantly the beacon did not hybridize with a non-complimentary oligonucleotide. Oligonucleotide target sequences are *Nefl* 5' CCCGGTGGCTAAGAAGAAAGATTGAGCCCC 3' and non-complementary 5' CCCATTATGACTTGACTCGATTGTGACCC 3' (Integrated DNA Technologies, Coralville, IA). We also examined *Nefl* beacon fluorescence with increasing concentrations of complementary oligonucleotide. *Nefl* beacon (0.2  $\mu$ M) was mixed with complimentary oligonucleotide (20 nM to 20  $\mu$ M) and fluorescence was measured 10 min, 25 min and 50 min later as described above. A dose-dependent increase in fluorescence was detected with increasing concentrations of complementary oligonucleotide.

**Image and movie analysis.** In live *Drosophila* larvae images of the NMJ, the synaptic area at muscle 13 was defined by motor neuron co-expression of UAS-



CD8-RFP. The synaptic terminal was outlined by an ROI in the red channel and SlideBook quantified fluorescence in the green channel. The amount of fluorescence minus the background divided by total fluorescence was recorded. In order to compare the wild-type and mutant data, synaptic fluorescence was normalized to the cell bodies' fluorescence. For the fluorescent intensity measurements at the cell body, 3<sup>rd</sup> instar larval ventral ganglion were imaged by creating a Z-stack. In 3 larvae per genotype, 10 cell bodies (2-3 cell bodies from each abdominal segment) were outlined by an ROI and quantified by SlideBook at each cell's transverse midline. To highlight the line paths of the tracked particles, we used "Display Paths" function from the "Manual Tracking" pull-down menu in SlideBook 5.5 software. The paths highlight the track of moving particles throughout the movie.

**TDP-43 immunofluorescence in mouse neurons.** Cortical neuron cultures were fixed using 4% PFA (Electron Microscopy Sciences, Hatfield, PA) for 10 min followed by permeabilization using 0.5% Triton X-100 (Sigma) in PBS (v/v, Invitrogen) for 10 min and blocking for 30 min (5% goat serum (Sigma) in PBS (v/v)). Detection of endogenous TDP-43 was performed using Rb anti-TDP-43 (ProteinTech, 1:300) and the secondary antibody AlexaFluor 566 (Gt anti-Rb, 1:300, Invitrogen).

**Kymographs.** Kymographs for the YFP-EB1 and TDP-43 granule movement were generated using ImageJ (Version 1.38x, NIH) and the plug-in "Kymograph" (<http://www.embl.de/eamnet/html/kymograph.html>).

**Co-immunoprecipitation.** Cells were lysed in ice-cold lysis buffer containing 20mM phosphate buffer, pH7.4, 150mM NaCl, 0.2% Triton X-100, 10% glycerol,

20mM N-Ethylmaleimide with EDTA-free protease inhibitor cocktail (Sigma) and PhosSTOP (Roche).

**Plasmids.** mCherry was subcloned upstream of FLAG-TDP-43 to generate mCherry-FLAG-TDP-43 in the mammalian expression vector pcDNA 3.1(+) (Invitrogen). mCherry-FLAG-TDP-43<sub>M337V</sub> and mCherry-FLAG-TDP-43<sub>A315T</sub> were generated using PCR to perform site-directed mutagenesis. IRES site was excised from the pCIG2-GFP expression plasmid (gift from Dr. David Solecki) to generate the GFP-TDP-43<sub>WT</sub> construct that was also used to generate GFP-TDP-43<sub>M337V</sub> and GFP-TDP-43<sub>A315T</sub>. YFP-EB1 construct was a gift from Dr. Chen Gu (Gu et al., 2006). pUASt-Venus-TDP-43 wild type, M337V plasmids and FLAG-STAU1 were constructed using the Gateway system. TDP-43 donor vector was obtained from PlasmID, Harvard U. The developed constructs were injected into *Drosophila* embryos by BestGene Inc., Chino Hills, CA. FLAG-TDP43 was previously described (Freibaum et al., 2010). STAU1 entry vector was obtained from the Dana Farber/Harvard Cancer Center DNA Resource Core. The destination vector was created by ligation of a Gateway vector conversion cassette (Life Technologies) into pCMV-MAT-FLAG-1 (Sigma) digested with EcoRV.

## Supplementary References

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## Supplementary Movies

**Movie S1. TDP-43 (WT) transport in *Drosophila* motor neurons.** Related to Figure 1. Venus-TDP-43 wild-type granules traffic bidirectionally along the axons of *Drosophila* motor neurons.

**Movie S2. Nefl mRNA transported along the axons of cultured cortical neurons.** Related to Figure 3. A molecular beacon targeting NEFL mRNA shows bidirectional axonal transport along the axons of cultured mouse cortical neurons.

**Movie S3. NEFL mRNA transported along the axons of human motor neurons from iPS cells.** Related to Figure 4. A molecular beacon targeting human NEFL mRNA shows that the transcript is transported bidirectionally along the axons of human motor neurons derived from iPS cells.