

## Using Tetracysteine-Tagged TDP-43 with a Biarsenical Dye To Monitor Real-Time Trafficking in a Cell Model of Amyotrophic Lateral Sclerosis

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## Supplementary methods

### Transfection efficiency

SH-SY5Y cells expressing HA-TDP43 or HA-TDP43-TC were fixed and immunostained with anti-HA antibody and nuclear counterstain Hoechst, 24 h post-transfection (via lipofection or electroporation). These samples were then imaged using confocal microscopy at 40x magnification, and the number of green (HA-TDP43 or HA-TDP43-TC) and blue (Hoechst) stained cells present in each image were manually counted using the Cell Counter plug-in in ImageJ. The transfection efficiency was calculated as the number of green transfected cells as a percentage of the total number of blue Hoechst-stained cells counted in each image.

### Quantification and comparison of nuclear FIAsh fluorescence in cells transfected with the HA-TDP43-TC plasmid using either lipofection or electroporation

SH-SY5Y cells were transfected with HA-TDP43-TC using both lipofection and electroporation methods and imaged using confocal microscopy 24 h post-transfection. The nuclear fluorescence in transfected cells was quantified as described in the methods section. A two-tailed unpaired Student t-test was performed on the mean fluorescence for each transfection method using GraphPad Prism version 7 for Mac OS X (GraphPad Software, La Jolla California USA) to determine whether or not there was a significant difference in the intensity of nuclear fluorescence between the two different transfection methods.

### Supplementary tables and figures

**Table S1.** Oligonucleotide sequences of primers used.

Primer name	Sequence
TDP43-TC (forward)	5'-AATTGGATCCATGTACCCATACGACGTCCCAGACT ACGCTTCTGAATATATTCTGGGTAAC-3'
TDP43-TC (reverse)	5'-CTAGTCTAGACTAGGGCTCCATGCAACAGCCTGGA CAGCAGTTCAGGAACATTCCCCAGCCAGAAGACT-3'
HA-TDP43 (forward)	5'-AATTGGATCCATGTACCCATACGACGTCC-3'
HA-TDP43 (reverse)	5'-CTAGTCTAGAAGCTTCTACATTCCCCAGCCAG-3'

**Table S2.** Transfection efficiency of SH-SY5Y cells transfected with HA-TDP43 or HA-TDP43-TC plasmid using either lipofection or electroporation

	<b>Transfection method</b>	<b>Number of green cells</b>	<b>Number of blue cells</b>	<b>efficiency (%)</b>
<b>HA-TDP43</b>	Lipofection	264	2648	9.97
	Electroporation	53	374	14.17
<b>HA-TDP43-TC</b>	Lipofection	236	1947	12.12
	Electroporation	44	312	14.10

**Table S3.** Nuclear fluorescence values from SH-SY5Y cells expressing HA-TDP43-TC using both lipofection and electroporation methods 24 h post-transfection.

<b>HA-TDP43-TC lipofection</b>		
Area of measurement	Integrated density	Nuclear fluorescence
92.364	2574.874	2252.985
82.204	1430.486	1144.005
104.14	1566.261	1203.333
96.982	1213.431	875.449
63.731	1621.448	1399.346
54.957	2458.034	2266.509
60.96	881.151	668.705
78.059	1216.663	943.059
Mean background grey value	3.485	
<b>HA-TDP43-TC electroporation</b>		
Area of measurement	Integrated density	Nuclear fluorescence
78.509	1636.226	1335.327
65.347	2008.453	1757.999
94.673	2441.639	2078.789
111.76	1719.123	1290.784
99.522	4858.802	4477.367
Mean background grey value	3.833	

**A**

DNA sequence for HA-TDP43:

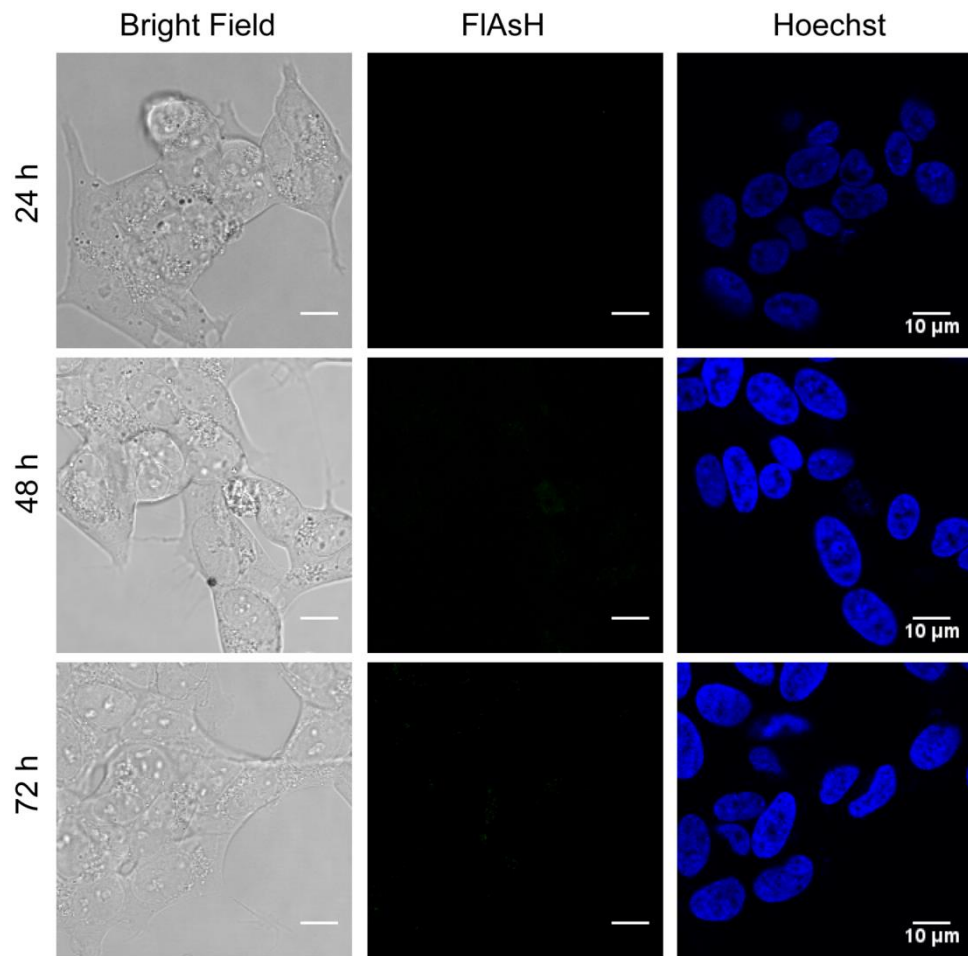
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GGACGGTGCTGCTCTCCACGGTTACAGCCCAGTTTCCAGGGCGTGTGGGCTT  
CGCTACAGGAATCCAGTGTCTCAGTGTATGAGAGGTGTCCGGCTGGTAGAAGG  
AATTCTGCATGCCCCAGATGCTGGCTGGGGAAATCTGGTGTATGTTGTCAACTA  
TCCAAAAGATAACAAAAGAAAAATGGATGAGACAGATGCTTCATCAGCAGTGAA  
AGTGAAAAGAGCAGTCCAGAAAACATCCGATTTAATAGTGTGGGTCTCCCATG  
GAAAACAACCGAACAGGACCTGAAAGAGTATTTTAGTACCTTTGGAGAAGTTCT  
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GATAGATGGACGATGGTGTGACTGCAAACCTCCTAATTCTAAGCAAAGCCAAGA  
TGAGCCTTTGAGAAGCAGAAAAGTGTGGTGGGGCGCTGTACAGAGGACATGA  
CTGAGGATGAGCTGCGGGAGTTCTTCTCTCAGTACGGGGATGTGATGGATGTC  
TTCATCCCCAAGCCATTCAAGGGCCTTTCCTTTGTTACATTTGCAGATGATCAG  
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CAGCACTACAGAGCAGTTGGGGTATGATGGGCATGTTAGCCAGCCAGCAGAAC  
CAGTCAGGCCCATCGGGTAATAACCAAACCAAGGCAACATGCAGAGGGAGCC  
AAACCAGGCCTTCGGTTCTGGAATAACTCTTATAGTGGCTCTAATTCTGGTGC  
AGCAATTGGTTGGGGATCAGCATCCAATGCAGGGTCCGGCAGTGGTTTAATG  
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CT**TCTAGACT**AG

**B**

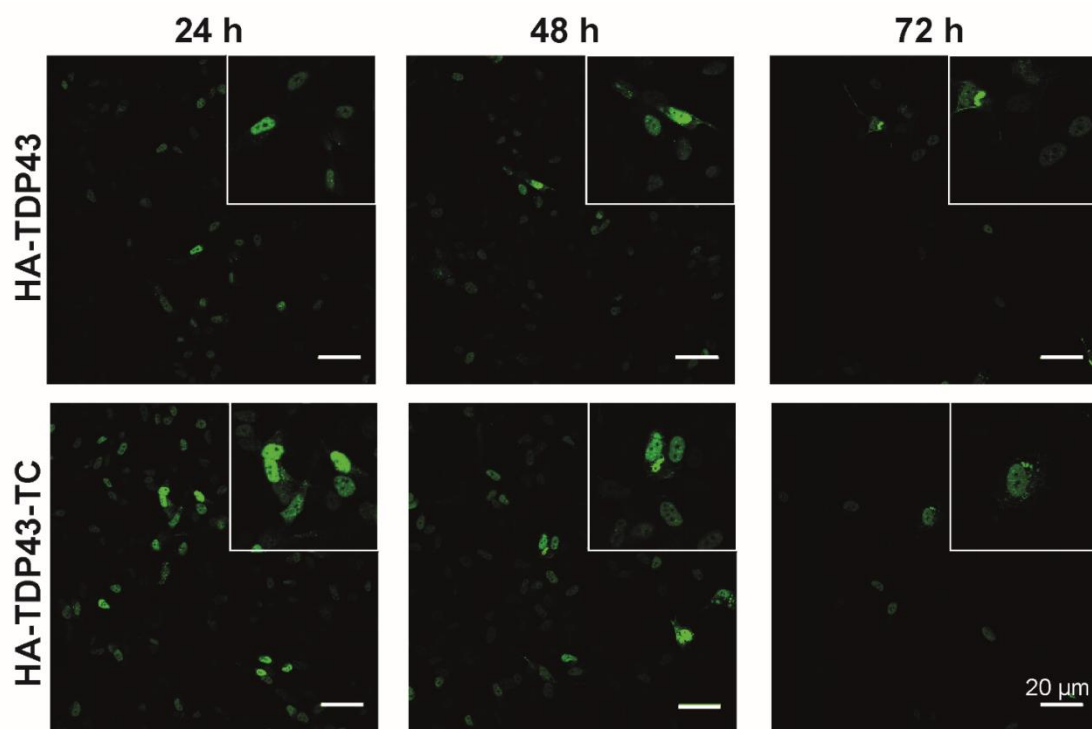
DNA sequence for HA-TDP43-TC:

AATT**GGATCC**ATG**TACCCATACGACGTCCCAGACTACGCT**TCTGAATATATTCG  
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GGACGGTGCTGCTCTCCACGGTTACAGCCCAGTTTCCAGGGCGTGTGGGCTT  
CGCTACAGGAATCCAGTGTCTCAGTGTATGAGAGGTGTCCGGCTGGTAGAAGG  
AATTCTGCATGCCCCAGATGCTGGCTGGGGAAATCTGGTGTATGTTGTCAACTA  
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AGTGAAAAGAGCAGTCCAGAAAACATCCGATTTAATAGTGTGGGTCTCCCATG  
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CAGCACTACAGAGCAGTTGGGGTATGATGGGCATGTTAGCCAGCCAGCAGAAC  
CAGTCAGGCCCATCGGGTAATAACCAAACCAAGGCAACATGCAGAGGGAGCC  
AAACCAGGCCTTCGGTTCTGGAATAACTCTTATAGTGGCTCTAATTCTGGTGC  
AGCAATTGGTTGGGGATCAGCATCCAATGCAGGGTCCGGCAGTGGTTTAATG  
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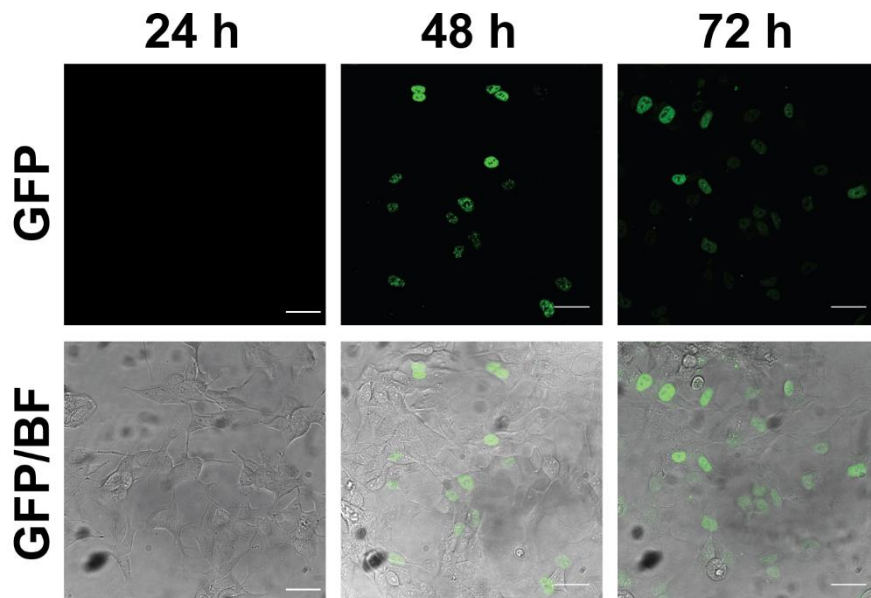
**Figure S1.** DNA sequences encoding A) HA-TDP43 and B) HA-TDP43-TC. Restriction enzyme sites (BamHI and XbaI) appear as underlined bold text. The DNA sequence encoding the HA-tag is highlighted yellow in A) and B), and the DNA sequence encoding the TC-tag in B) is highlighted in cyan.



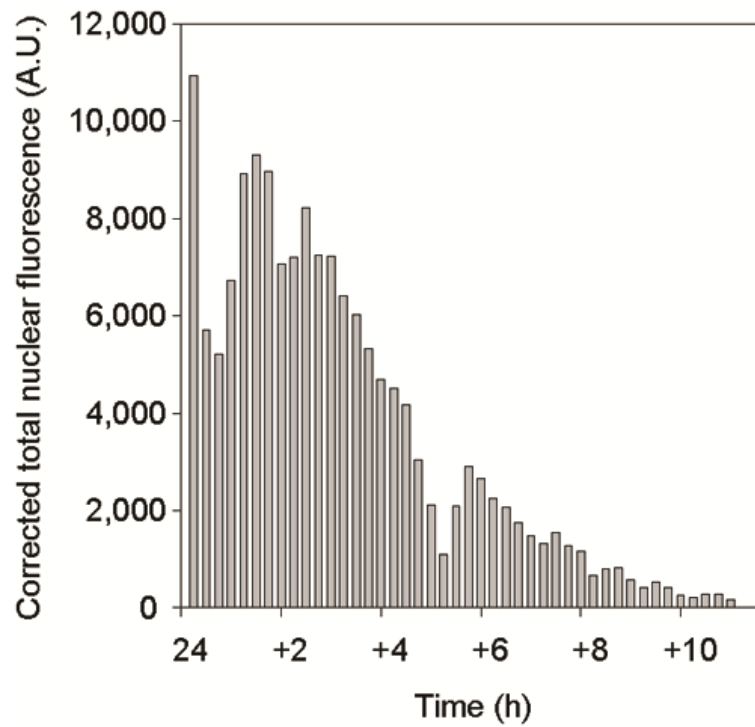
**Figure S2.** Confocal images showing HA-TDP43 (no TC-tag) in live SH-SY5Y cells at 24, 48 and 72 h post-transfection in the presence of FIAsH reagent (middle panels). No FIAsH signal is detected in the absence of the TC-tag. Bright field and Hoechst nuclear counterstain (blue) are shown for each time point.



**Figure S3.** Confocal images showing the distribution of HA-TDP43 and HA-TDP43-TC in lipofected SH-SY5Y cells at 24, 48 and 72 h post-transfection, immunostained with anti-HA antibody (green). The inset white boxes contain zoomed images of the cell nucleus (zoom factor 2 x original image).

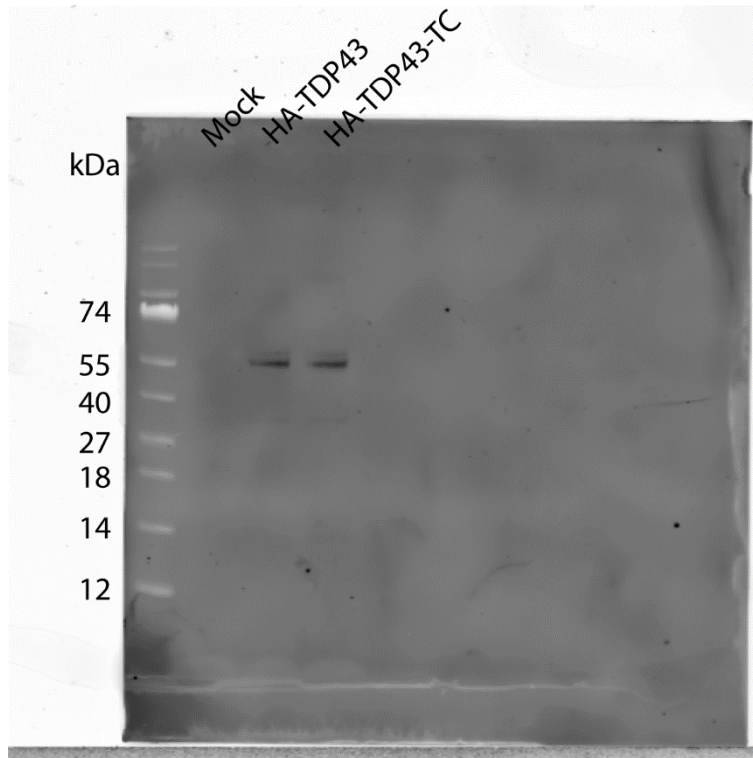


**Figure S4.** Confocal images showing the distribution of TDP43-GFP (green) in electroporated SH-SY5Y cells at 24, 48 and 72 h post-transfection. Cells were transfected with a pcDNA4/TO-TDP43-EGFP construct (GenScript, Piscataway, NJ USA) using protocols detailed in the materials and methods. Scale bars are 50 μm.



**Figure S5.** Histogram showing the loss of nuclear fluorescence over 11 h, using HA-TDP43-TC expressing SH-SY5Y cells labelled with FIAsH dye 24 h post-transfection using electroporation. Corrected total nuclear fluorescence was measured using ImageJ and calculated in Excel. The values were obtained from the representative experiment in Figure 7.





**Figure S6.** Full western blot from Figure 2, panel C. Western blot analysis following immunoprecipitation purification of HA-TDP43 and HA-TDP43-TC from SH-SY5Y cell lysates at 24 h post transfection. Mock transfection is cells transfected with buffer alone.

**Supplementary Video S1.** Time-lapse confocal microscopy video of HA-TDP43-TC expressing SH-SY5Y cells labelled with FIAsh dye. Imaging commenced at 24 h post-transfection using electroporation.