Supplementary Materials for

Title: An ALS-mutant TDP-43 neurotoxic peptide adopts an anti-parallel β-structure and induces TDP-43 redistribution

Authors:

Li Zhu[1a, †], Meng Xu[1b, †], Mengxue Yang[1a,2,3], Yanlian Yang[1b], Yang Li[3,4], Jianwen Deng[1a,2], Linhao Ruan[1a,2], Jianghong Liu[1a], Sidan Du[3,4], Xuehui Liu[1a], Feng Wei[1a], Kazuo Fushimi[3], Eileen H. Bigio[5], Marsel Mesulam[5], Wang Chen[1b, *] and Jane Y. Wu[1a,3, *]

Corresponding author. E-mail: wangch@nanoctr.cn (C.W.); jane-wu@northwestern.edu (J.W.)

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Supplementary Table S1

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Supplementary Table S1.

	Wt	A315T	pA315T	A315E
α-helix	29.1%	16.3%	9.2%	13.7%
β-sheet	39.7%	0	14.6%	37.7%
β-turn	11.2%	34.0%	19.6%	18.1%
random-coil	20.0%	49.7%	56.6%	30.5%

Table S1 The ratio of four secondary structures (α-helix, β-sheet, β-turn and random-coil) for synthetic TDP-43 peptides: Wt-, A315T-, pA315T- and A315E-. These data are obtained by using Young's algorithm (23).

Supplementary Figures

Figure S1. Molecular dynamic simulation of the TDP-43 peptides. The molecular dynamic simulation was carried out as previously described (4).

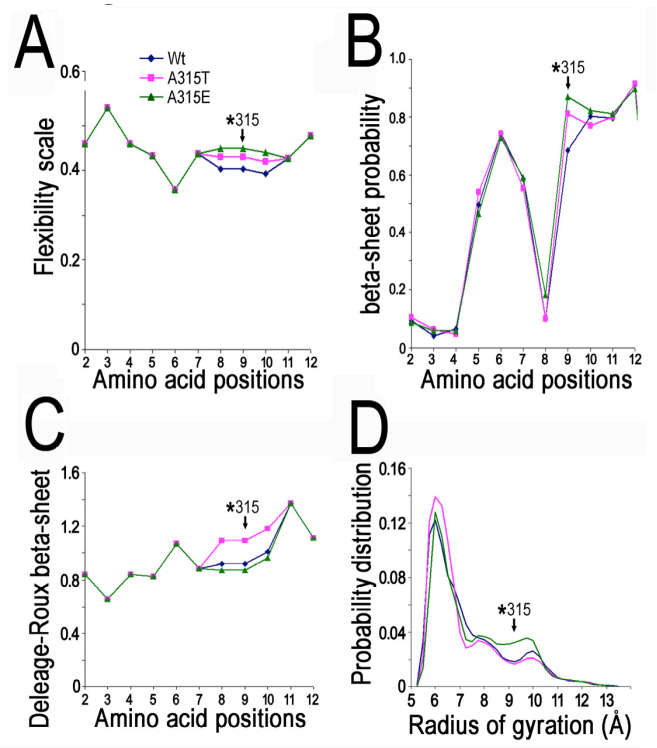


Figure S2. AFM reveals that the G314V mutation eliminates the amyloid fibril formation in Wt- or A315E-mutant TDP-43 peptides.

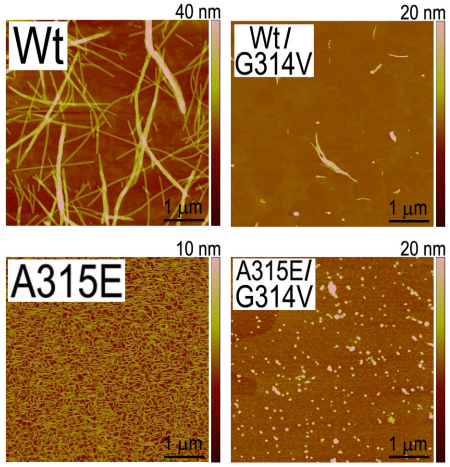


Figure S3. The A315E-mutant TDP-43 peptides applied in the culture media induces cytoplasmic redistribution of the endogenous TDP-43 protein. The A315E-mutant TDP-43 peptide was added to HEK293 cells at 30uM and cells were fixed at different time points for TDP-43 immunostaining. Nuclei (Nu) were revealed by Hoechst dye staining. The confocal images were obtained in Z-stacks to ensure the entire cells were included and projected into single images. The images were intentionally overexposed to detect weak signals. The arrowheads and arrows respectively mark the cells in which nuclear TDP-43 staining was partially and almost completely redistributed into the cytoplasm.

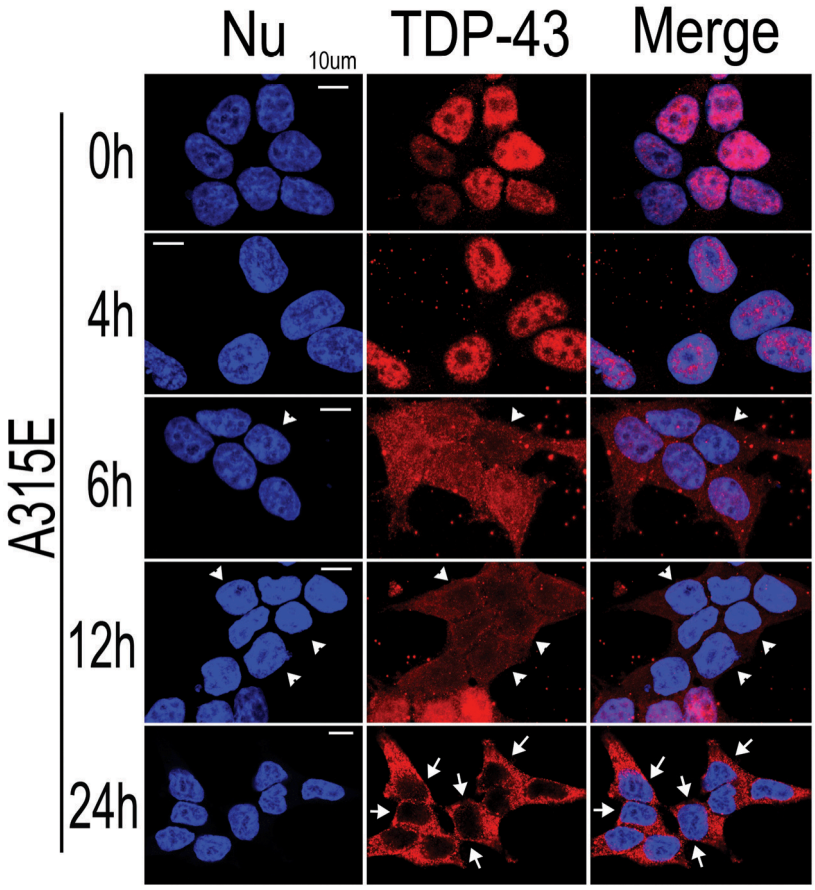
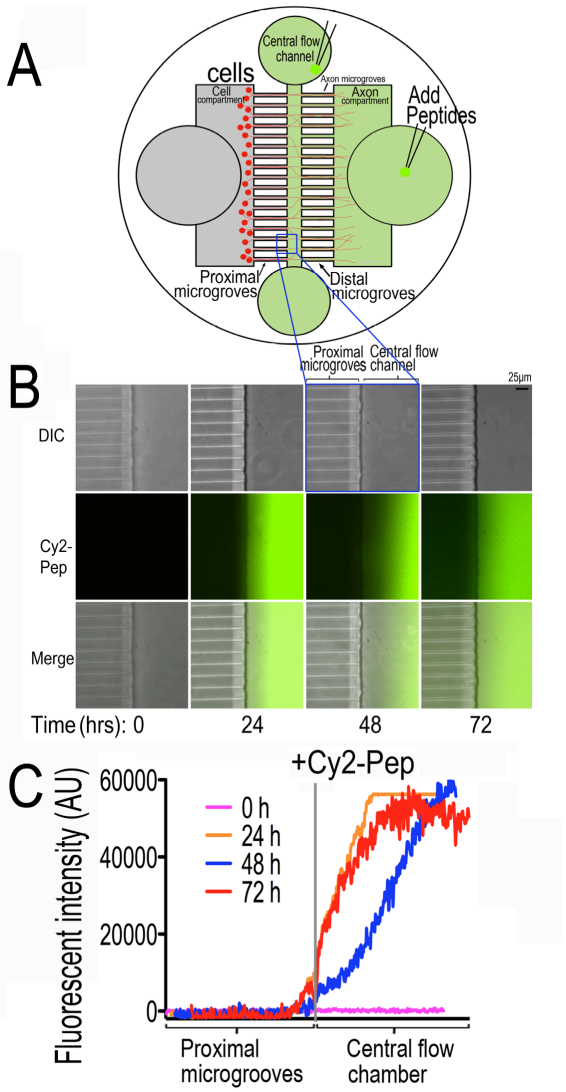


Figure S4. The microfluidic chamber allows compartmentalized application of peptides.

A. A diagram illustrating the application of fluorescently labeled peptides. **B.** Fluorescent microscopy reveals that the fluorescently labeled peptide does not diffuse to cell bodies following 72-hr incubation after application of the peptide to the axonal compartment and the central flow channel. **C.** Quantification of the fluorescence intensity (in arbitrary units, AU) along a horizontal line through proximal microgrooves and central flow chamber shown in (**B**) shows that the fluorescence intensity in proximal microgrooves is nearly undetectable, indicating that the amount of Cy2-labeled TDP-43 peptides diffused into the proximal microgrooves was extremely small.



Reference:

4 Guo, W., Chen, Y., Zhou, X., Kar, A., Ray, P., Chen, X., Rao, E.J., Yang, M., Ye, H., Zhu, L. *et al.* (2011) An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. *Nat. Struct. Mol. Biol*, **18**, 822-830.

23 Chen, Y.H., Yang, J.T. and Martinez, H.M. (1972) Determination of the secondary structures of proteins by circular dichroism and optical rotatory dispersion. *Biochemistry*, **11**, 4120-4131.