

Thioredoxin 1 regulates TDP-43 proteinopathy

Ji Min Lee^{1,2,†}, Hyeonjin Jeon^{1,†}, Eugene Bok¹, Seonhyeok Hwang³, Chuna Kim³, Byung-Hoon Lee⁴, Sang Ryong Kim², Sung Bae Lee^{5,*}, Minyeop Nahm^{1,*}, Jaekwang Kim^{1,*}

¹Dementia Research Group, Korea Brain Research Institute (KBRI), Daegu 41062, South Korea,

²School of Life Sciences, Kyungpook National University, Daegu 41566, South Korea

³Aging Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology, 125 Gwahak-ro Yuseong-gu Daejeon 34141, South Korea

⁴Department of New Biology, Daegu-Gyeongbuk Institute of Science and Technology (DGIST), Daegu 42988, South Korea

⁵Department of Brain Sciences, Daegu Gyeongbuk Institute of science and Technology(DGIST), Daegu 42988, South Kore

*Corresponding author: kim_jaekwang@kbri.re.kr; [†]These authors contributed equally.



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ABSTRACT

Transactive response (TAR) DNA-binding protein 43 (TDP-43) is widely expressed nuclear protein which regulates the expression of a variety of genes via regulating RNA splicing, trafficking, and/or stabilization. Under pathological context, TDP-43 is mislocalized to cytoplasm, and aggregated into insoluble inclusions. The aggregation of TDP-43 induces oxidative stress and neurodegeneration in various neurodegenerative diseases, such as Amyotrophic lateral sclerosis (ALS), Frontotemporal dementia (FTD), and Alzheimer's disease (AD). However, our understanding of the pathological mechanisms of TDP-43 proteinopathies remains elusive. Therefore, it is critical to elucidate the molecular mechanisms of TDP-43 pathology to develop novel therapeutic interventions. We developed a cellular model of TDP-43 proteinopathy using mouse neuronal N2a cells. Through proteomic profiling, we identified that the levels of Thioredoxin 1 (TRX1), a master regulator of oxidative stress, are markedly decreased in N2a cells overexpressing wild-type (WT) TDP-43. We demonstrated that overexpression of TRX1 strongly alleviates TDP-43 pathology evidenced by a marked reduction of cytosolic aggregation of TDP-43 and ROS(reactive oxygen species) in N2a cells and neurite deficits and ROS in primary cortical neurons. Importantly, we also validated the therapeutic effect of TRX1 overexpression against TDP-43 pathology in human iPSC-derived motor neurons harboring pathogenic M337V TDP-43 mutant. Taken together, Our data suggest that TRX1 may represent an effective therapeutic target for TDP-43 proteinopathy.

RESULTS

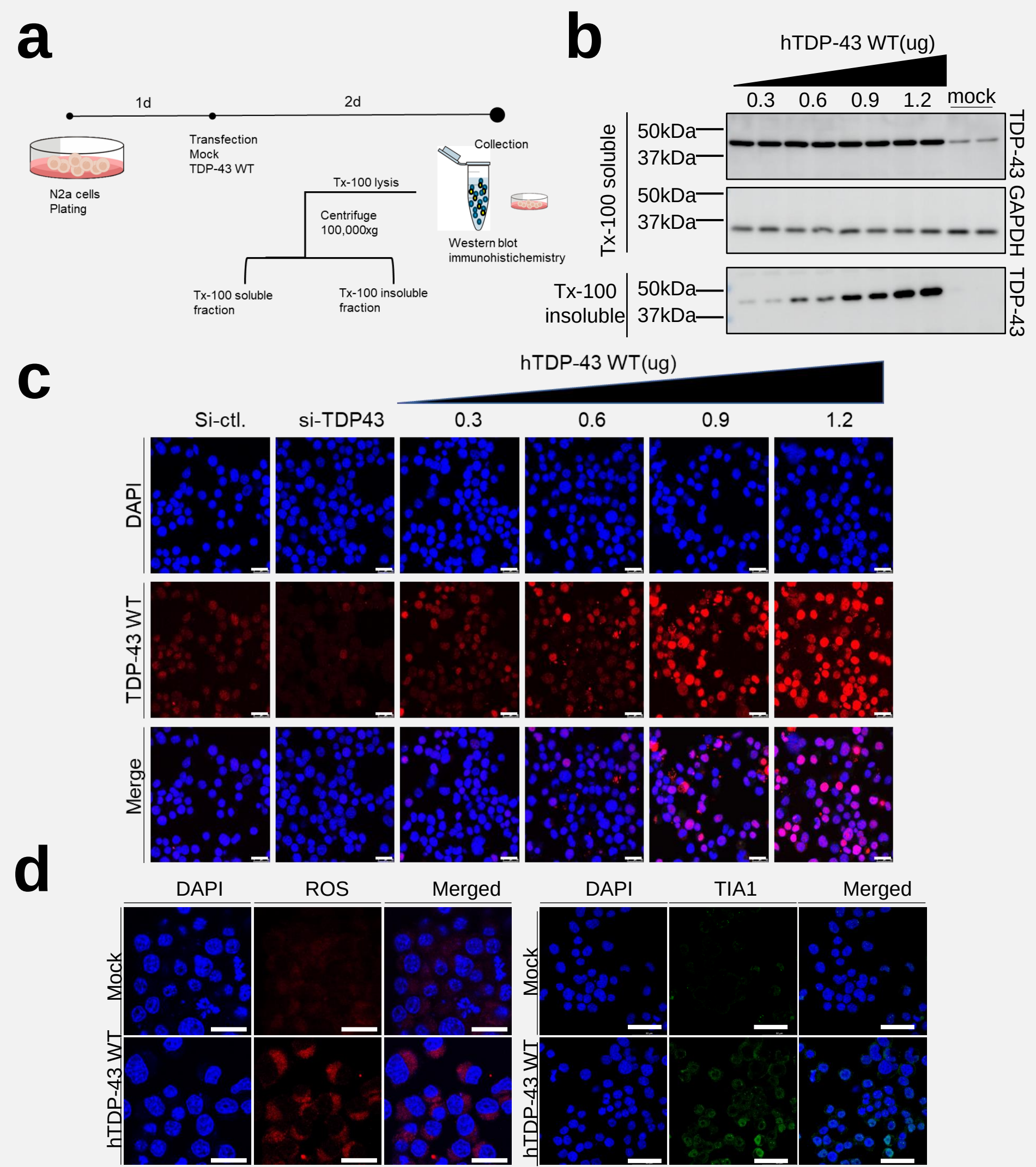


Figure 1. Establishment of a cellular model of TDP43 proteinopathy.

a. A experimental scheme **b-c.** Triton X-100 insoluble TDP43 proteins (b) and cytosolic mislocalization of TDP-43 (c) are increased in N2a cells expressing human WT TDP-43 in a dose-dependent manner. **d.** TDP-43 overexpression dramatically increased ROS, stress granule(TIA1) in N2a cells. (scale bar 25 μ m).

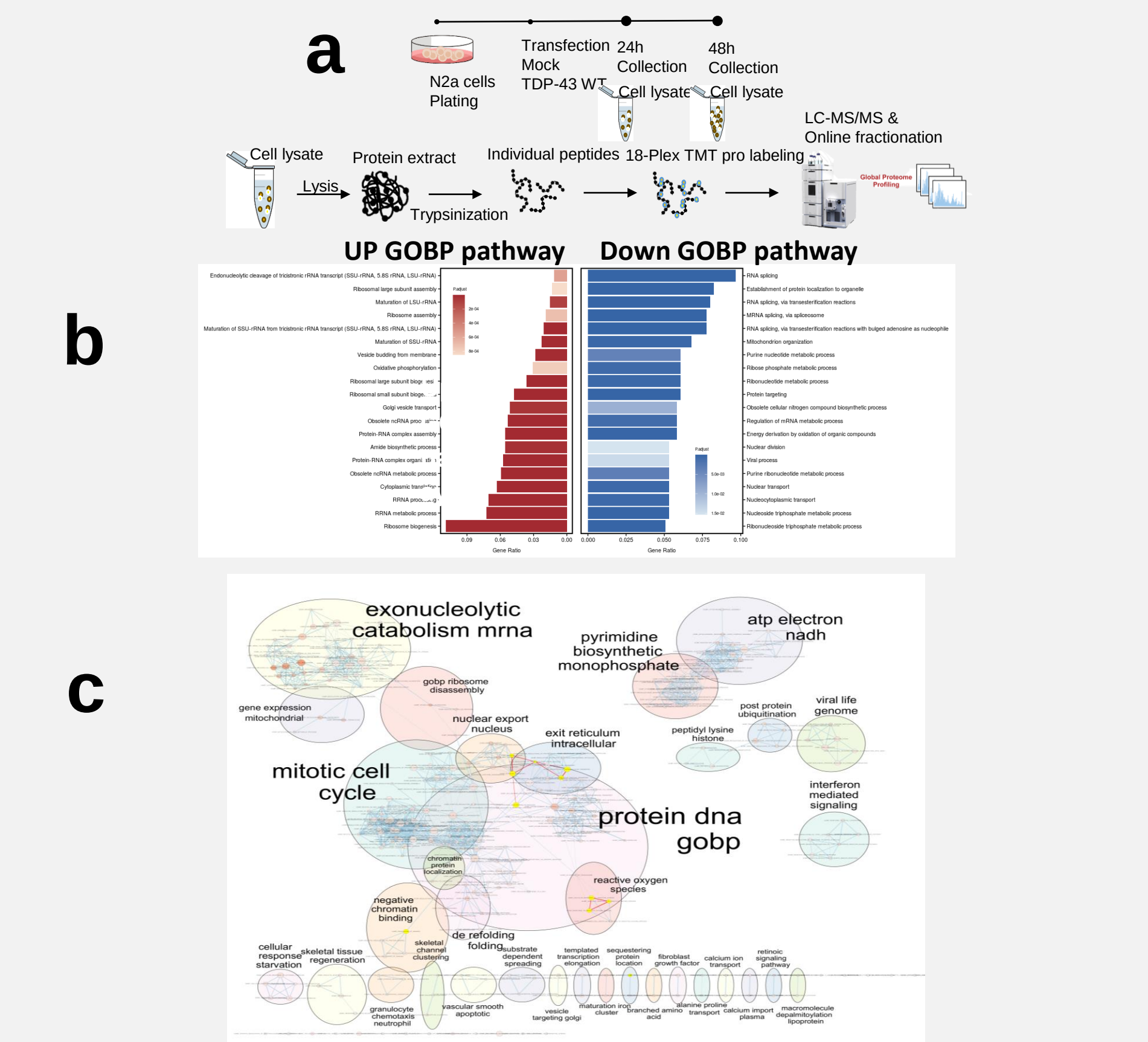


Figure 2. Comprehensive proteomics analysis of a cellular model of TDP43 proteinopathy.

a. Experimental scheme. The extracted proteins were digested, labeled with TMT reagents, and analyzed by DO-RP/RP-MS/MS. Cellular processes represented at 24 h and 48 h. **b.** Over-representation analysis of Gene Ontology Biological Process terms was performed on proteins differentially expressed in N2a cells overexpressing wild-type TDP-43. **c.** Enriched Biological Process terms identified in Fig. 2b were visualised as a Cytoscape network. TRX1 (yellow circle) resides within the 'oxidative stress' cluster, linking this pathway to TDP-43 pathology.

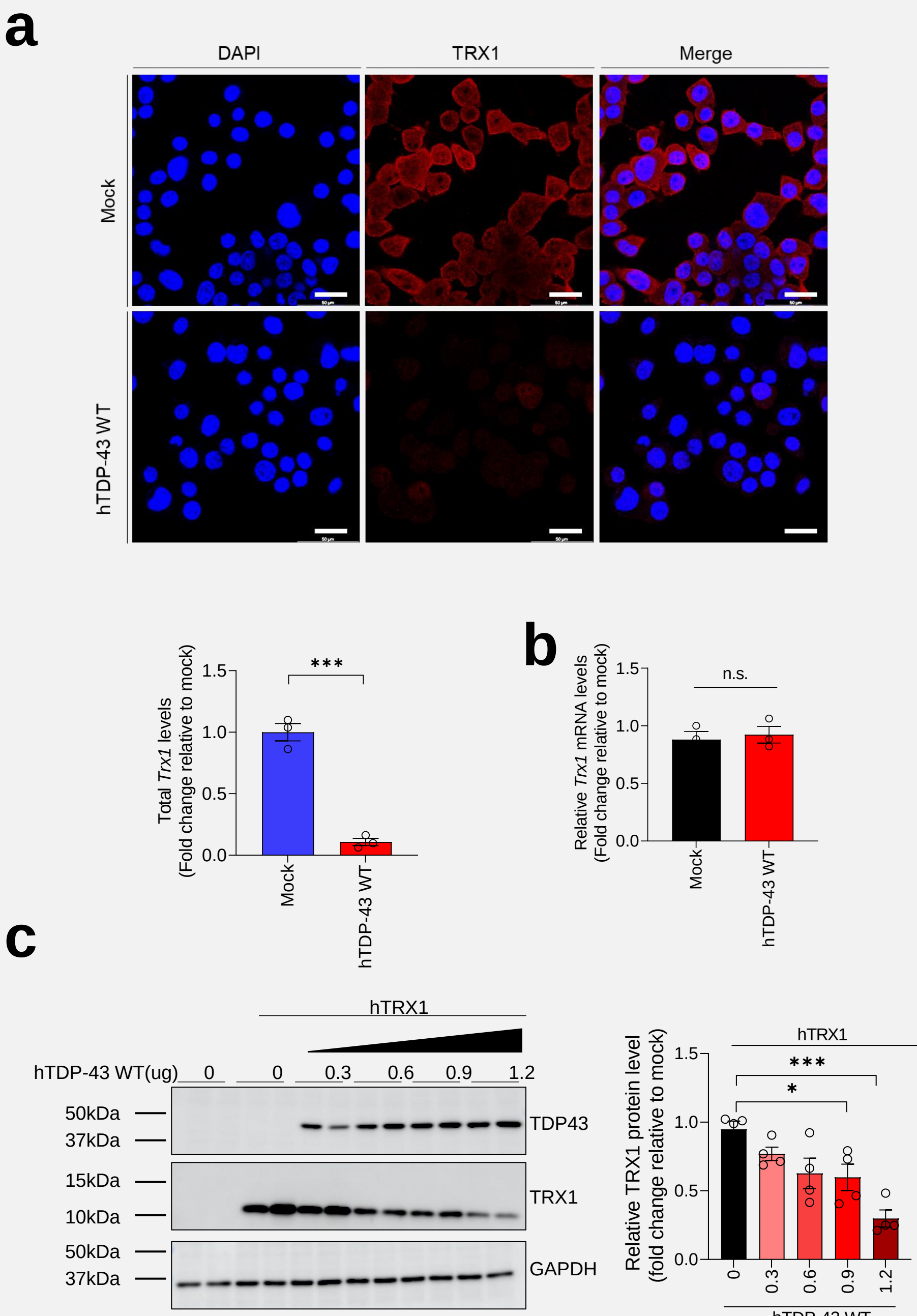


Figure 3. The levels of TRX1 protein are downregulated by TDP-43 overexpression in neuronal cells.

a-b. TDP-43 overexpression dramatically decreases the protein levels of endogenous Trx1 in N2a cells (a) without altering its mRNA levels (b). 48h post-transfection, N2a cells were stained with anti-TRX1 antibody(red) and nuclear dye (DAPI) as indicated. (scale bar 25 μ m n =3, t-test). Each intensity of Trx1 was normalized to the corresponding cell number. *Trx1* mRNA levels were normalized by Gapdh levels. (n=3 one-way ANOVA) **c.**TDP-43 overexpression reduces the protein levels of human TRX1 ectopically expression in N2a cells in a dose-dependent (n=4 one-way ANOVA). Values are mean $\pm$ SEM (n.s. = non-significant, *p <0.1, **p <0.01, ***p <0.001).

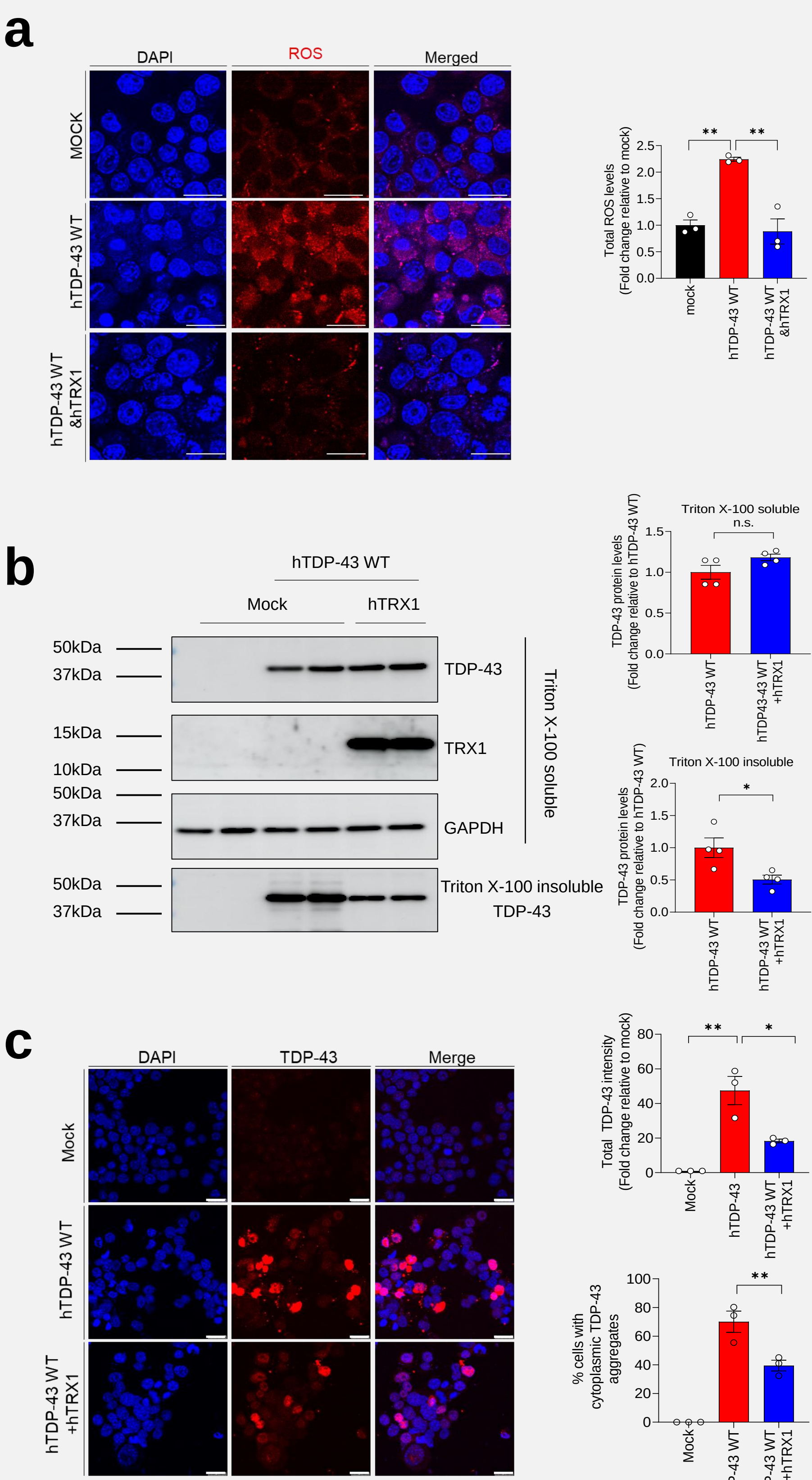


Figure 4. Overexpression of TRX1 alleviates TDP-43 proteinopathy in neuronal cells.

a. TRX1 overexpression dramatically suppresses ROS induction by TDP-43 overexpression in N2a cells (scale bar 25 μ m , n=3 one-way ANOVA). Cells were stained with CellROX Deep Red dye to visualize ROS. **b.** TRX1 overexpression significantly reduces Triton X-100 insoluble TDP-43 aggregates in N2a cells. Each TDP-43 level was normalized by the corresponding Gapdh level (n =4, t-test) **c.** The cytosolic TDP-43 aggregates are markedly decreased by TRX1 overexpression in N2a cells. Cells were stained with anti-TDP-43 antibody (red) and nuclear dye (DAPI) as indicated. (scale bar 25 μ m n =3 , one-way ANOVA). Values are mean $\pm$ SEM (n.s. = non-significant, *p <0.1, **p <0.01).

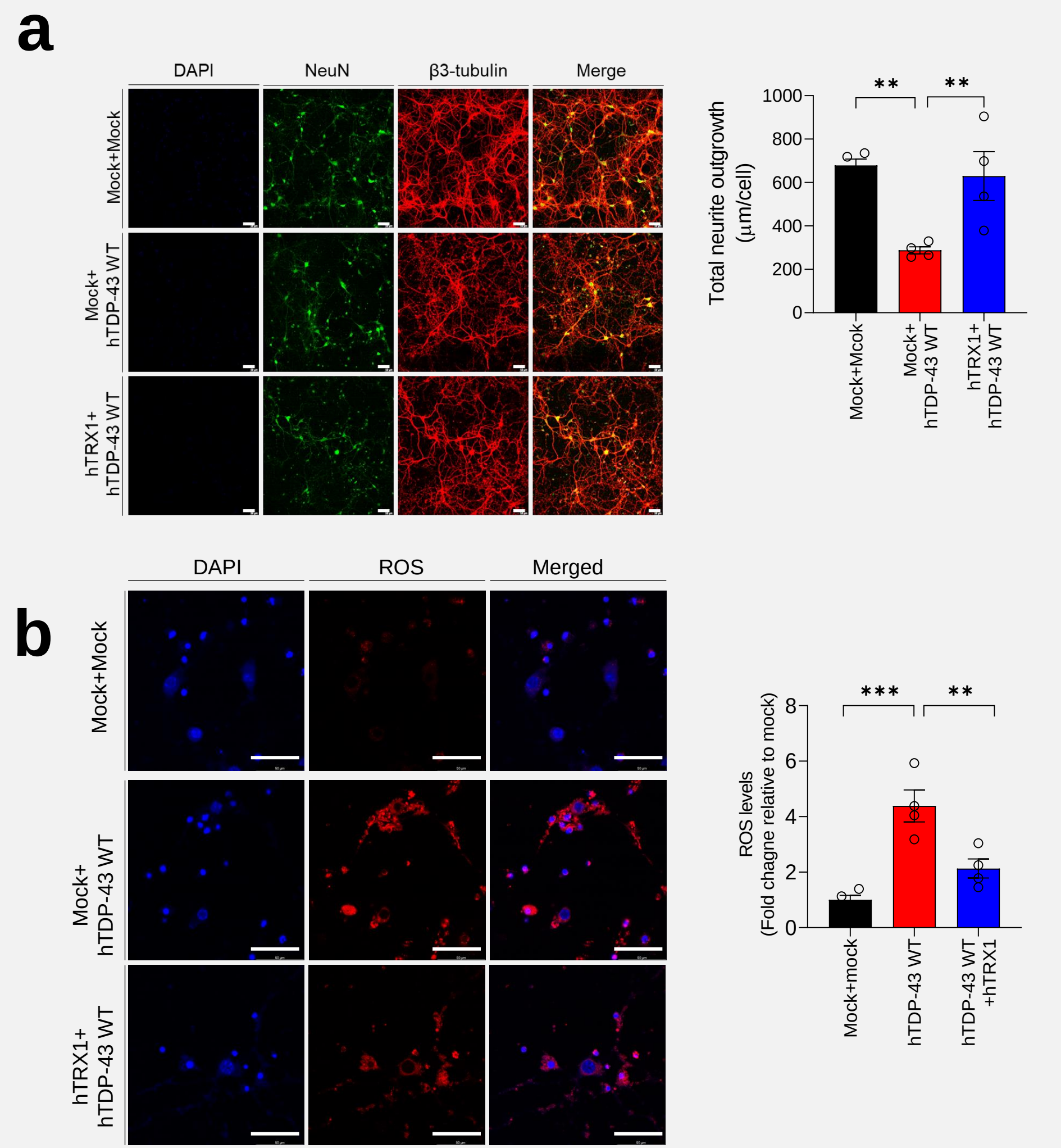


Figure 5. TRX1 overexpression attenuates TDP-43-induced neurotoxicity and ROS in primary cortical neuron.

Primary cortical neurons were transduced with AAV viruses expressing hTDP-43 WT and hTRX1 as indicated at DIV7. Neurons were stained with anti- β -tubulin (red) and NeuN (green) antibodies at DIV14. **a.** The length of neurite outgrowth was measured using metamorph. The neurite outgrowth was normalized with corresponding cell number. **b.** TRX1 overexpression suppresses TDP-43-induced ROS in primary cortical neuron.(n =4, one-way ANOVA ; scale bar 50 μ m). Values are mean $\pm$ SEM (**p <0.01, *** p < 0.001).

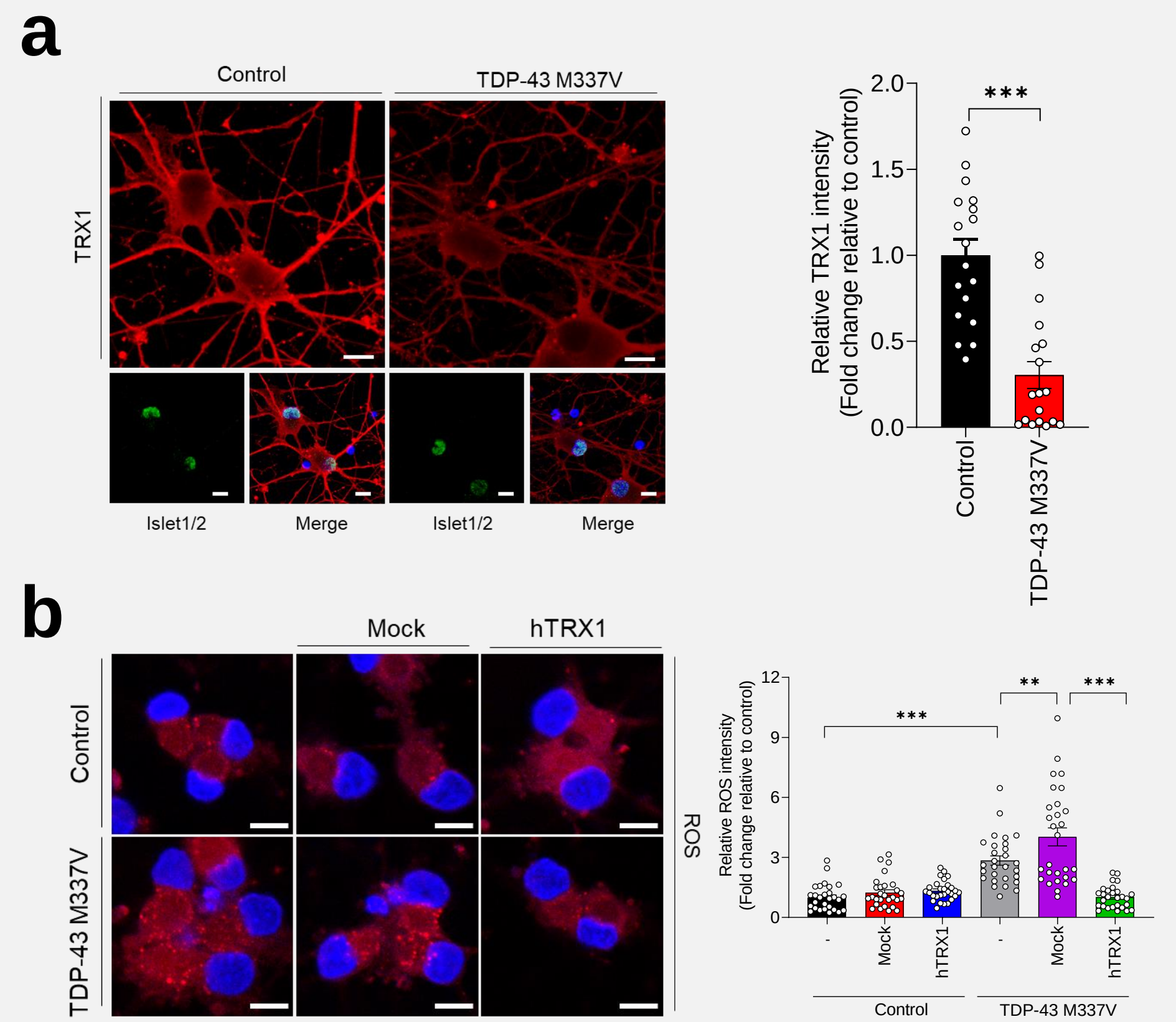


Figure 6. Endogenous hTRX1 was decreased in human iPSC-derived motor neurons expressing TDP43 M337V

a. Immunofluorescent analysis of endogenous hTRX1 expression in control and TDP43 M337V iPSC-derived motor neurons. Motor neurons were stained with anti-TRX1 (red), anti-Islet 1/2 (green) as a motor neuronal marker, and DAPI (blue). (scale bar 10 μ m n = 18 Bar, t-test). Data are shown as fold change relative to endogenous hTRX1 intensity. **b.** Neurons were transduced with AAV virus expressing hTRX1 or mock as indicated. Representative florescence image of intracellular ROS in control and TDP-43 M337V iPSC-derived motor neurons dependent on expressing of hTRX1. (Scale bar 10 μ m, n = 28, two-way ANOVA) Data are shown as fold change relative to endogenous ROS intensity. Values are mean $\pm$ SEM (** p < 0.01, *** p < 0.001).

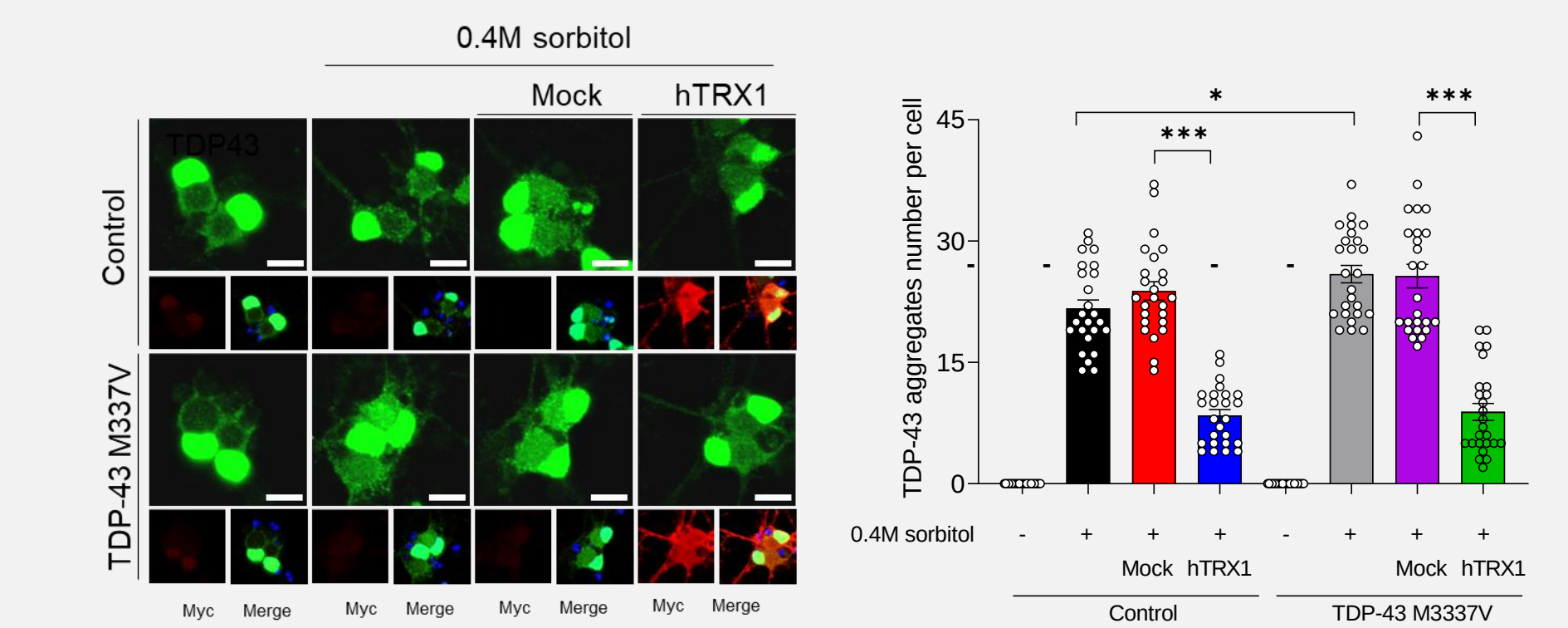


Figure 7. hTRX1 overexpression attenuates the accumulation of cytosolic TDP43 aggregates induced by osmotic stress in motor neurons expressing TDP43 M337V mutant.

Representative florescence images of TDP43 in control and TDP43 M337V mutant iPSC-derived motor neurons upon expression of hTRX1 under stress conditions. Motor neurons were stained with anti-TDP43 (green), anti-myc (red; AAV-hTRX1-myc), and DAPI (blue). The stress reagent is 0.4 M Sorbitol which treated to iPSC for 1.5 h. (Scale bar, 20 μ m, n = 28, two-way ANOVA) TDP-43 aggregates were normalized by TDP43-positive cell number. Values are mean $\pm$ SEM (* p < 0.05, *** p < 0.001.).

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