TDP-43 is more toxic in respiring than in non-respiring cells, but respiration is not absolutely required for TDP-43 toxicity.

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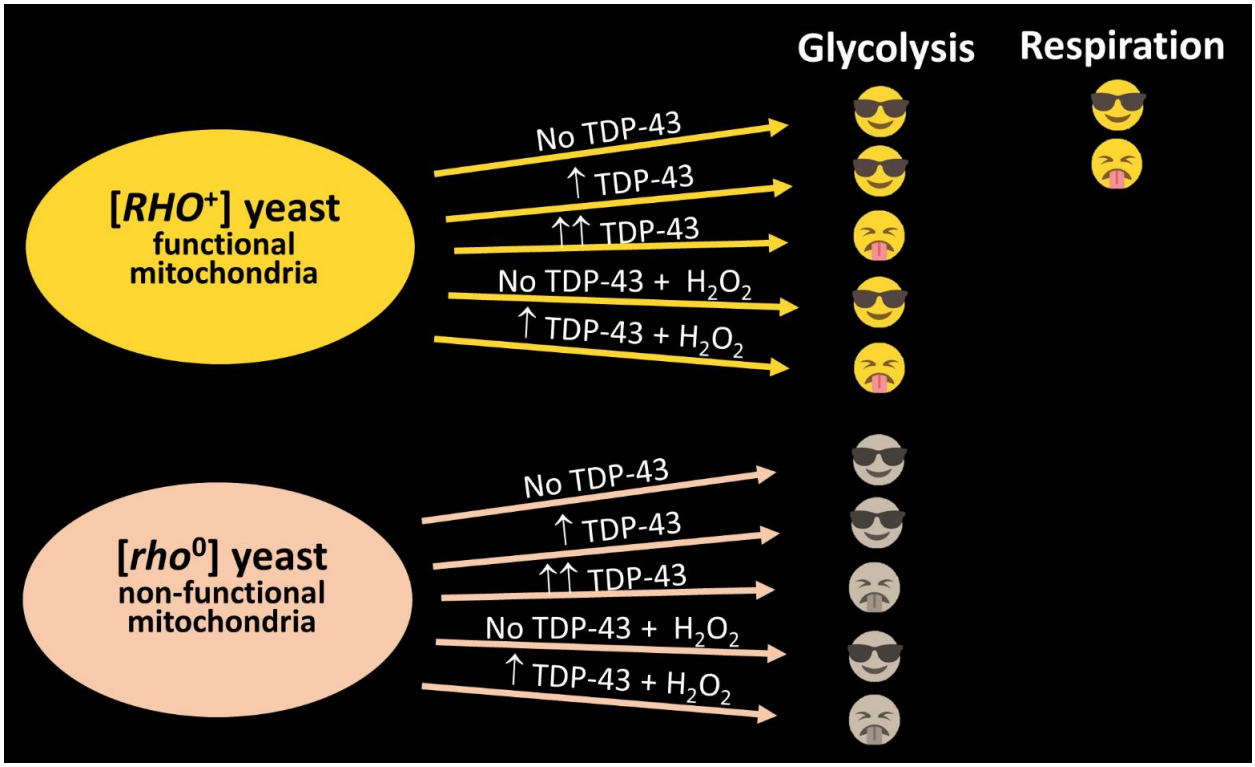
Highlights

- TDP-43 toxicity and aggregation is enhanced when yeast are grown in media that requires respiration.
- Respiration is not the sole target of TDP-43 toxicity because TDP-43 still aggregates and is toxic in cells that are not respiring.
- Hydrogen peroxide enhances TDP-43 toxicity in the absence of respiration suggesting that reactive oxygen species (ROS) produced by respiration may likewise enhance TDP-43 toxicity.
- ROS could activate TDP-43 to become more toxic or make TDP-43 targets more vulnerable.

Graphical Abstract



Abstract

The trans-activating response DNA-binding protein 43 (TDP-43) is a transcriptional repressor and splicing factor. TDP-43 is normally mostly in the nucleus, although it shuttles to the cytoplasm. Mutations in TDP-43 are one cause of familial amyotrophic lateral sclerosis (ALS). In neurons of these patients, TDP-43 forms cytoplasmic aggregates. In addition, wild-type TDP-43 is also frequently found in neuronal cytoplasmic aggregates in patients with neurodegenerative diseases not caused by TDP-43 mutations. TDP-43 expressed in yeast causes toxicity and forms cytoplasmic aggregates. This disease model has been validated because genetic modifiers of TDP-43 toxicity in yeast have led to the discovery that conserved genes in humans are ALS genetic risk factors. While it is still unknown how TDP-43 is associated with toxicity, several studies find that TDP-43 alters mitochondrial function. We now report that TDP-43 is much more toxic when yeast is grown in non-fermentable media requiring respiration than when grown on fermentable carbon sources. However, we also establish that TDP-43 remains toxic in the absence of respiration. Thus, there is a TDP-43 toxicity target in yeast distinct from respiration and respiration is not required for this toxicity. Since we find that H_2O_2 increases the toxicity of TDP-43, the free oxygen radicals associated with respiration could likewise enhance the toxicity of TDP-43. In this case, the TDP-43 toxicity targets in the presence or absence of respiration could be identical, with the free radical oxygen species produced by respiration activating TDP-43 to become more toxic or making TDP-43 targets more vulnerable.

Abbreviations**:** trans-activating response DNA-binding protein 43 (TDP-43); amyotrophic lateral sclerosis (ALS); dextrose (Dex); galactose (Gal); doxycycline (Dox); copper sulfate ($CuSO_4$); hydrogen peroxide (H_2O_2)

Key Words: TDP-43, amyotrophic lateral sclerosis, yeast, respiration, mitochondria

Communication

The trans-activating response DNA-binding protein 43 (TDP-43) is a nucleic acid binding protein that functions as a transcriptional repressor, splicing factor and in translational regulation. TDP-43 is normally found mostly in the nucleus, although it shuttles between the nucleus and the cytoplasm. Mutations in *TARDBP,* the gene encoding TDP-43, are one cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In neurons of these patients, TDP-43 is no longer found in the nucleus, but instead forms cytoplasmic aggregates. In addition, wild-type TDP-43 is also frequently found in cytoplasmic aggregates in neurons of patients with other neurodegenerative diseases or with ALS/FTD not caused by TDP-43 mutations[1; 2; 3; 4].

How TDP-43 aggregates are associated with toxicity is the subject of intense research. Evidence links TDP-43 toxicity with both inhibition of the ubiquitin proteasome system[5], and inhibition of lysosome and endosomal activity[6; 7]. Also, several studies find that TDP-43 alters mitochondrial function.

Expression of overexpressed TDP-43 in a number of model organisms causes neurodegeneration similar to that seen in ALS patients. In motor neurons of TDP-43 transgenic mice, mitochondria were found in cytoplasmic inclusions or in abnormal juxtanuclear aggregates and were missing in motor axon termini[8; 9; 10]. In mammalian neuron-like cell culture, TDP-43 localized to mitochondria and caused mitophagy[11]. Likewise, in a mouse model, TDP-43 co-localization with motor neuron mitochondria was enhanced by TDP-43 ALS mutations and this co-localization was associated with inhibited mitochondrial function[12; 13]. Also, overexpressed TDP-43 in human cell culture binds to, and inhibits maturation of, mitochondrial RNA causing a phenotype similar to that seen in cells deficient in mitochondrial RNase P[14]. Finally, mutations in the mitochondrial intermembrane protein, CHCHD10 (yeast homolog MIX17) have recently been associated with sporadic and familial ALS and FDT [15; 16; 17; 18; 19; 20; 21] and functional CHCHD10 appears to help keep TDP-43 in the nucleus away from mitochondria[22].

There is also evidence that TDP-43 expression in yeast causes mitochondrion-dependent apoptosis and that TDP-43 toxicity requires respiratory capacity[23]. TDP-43 expressed in yeast causes toxicity and forms cytoplasmic aggregates[24]. Furthermore, conserved genetic modifiers of this toxicity have been validated in higher organisms and have led to the discovery of human ALS genetic risk factors[25]. This confirms the usefulness of the yeast model.

To test the idea that the primary source of TDP-43 toxicity in yeast is mitochondria, we used the fact that yeast turns off respiration in fermentable media where it instead uses glycolysis to grow. We found that TDP-43-GFP expressed with a *TET* promoter aggregated in yeast cells whether grown on fermentable, e.g. glucose or non-fermentable, e.g. galactose media. However, toxicity was only detected on non-fermentable media requiring respiration for growth and cell elongation and TDP-43 aggregation was much more pronounced on non-fermentable vs. fermentable media (Fig. 1ab). Furthermore, this difference is not due to increased TDP-43 expression on the non-fermentable media (Fig. 1c). One explanation is that TDP-43 inhibits respiration which only has an effect when cells use respiration to grow. Alternatively, respiration itself could enhance TDP-43 toxicity.

By expressing TDP-43 from integrated vectors with the *CUP1* promoter which caused higher levels of TDP-43, we were able to detect TDP-43 toxicity in the absence of respiration on glucose (Fig.2). Since yeast lacking functional mitochondria are viable we overexpressed TDP-43 in isogenic cells containing ([*RHO*$^{+}$]) or lacking ([*rho*^{0}]) functional mitochondria. We found similar levels of TDP-43 toxicity, expression and aggregation in the [*RHO*$^{+}$] and the [*rho*^{0}] cells (Fig. 2). Also as we reported previously[5] TDP-43 is more toxic in the presence of the [*PIN*$^{+}$] prion (Fig. 2a). Since the [*rho*^{0}] cells can never respire and the [*RHO*$^{+}$] cells do not respire on glucose, this clearly establishes that there is a TDP-43 toxicity target in yeast distinct from respiration and that respiration is not required for this TDP-43 toxicity.

To test the idea that increased levels of free oxygen radicals associated with respiration could be the cause of the observed increase in TDP-43 toxicity in the presence of respiration, we looked at the effect of H_2O_2, a source of oxygen derived free radicals, on TDP-43 toxicity. We saw no toxicity either for 2mM H_2O_2 without

TDP-43 (Fig. 3 left) or for expression of TDP-43 with 250 μM $CuSO_4$ in glucose medium without H_2O_2 expression (Fig. 3 right rows marked 0). However, the combination of 2mM H_2O_2 and induction of TDP-43 with 250 μM $CuSO_4$ was toxic (Fig. 3 right row marked 2). This was true in both [*RHO*$^+$] or [*rho*^{0}] cells. Since H_2O_2 increases the toxicity of TDP-43, the free oxygen radicals associated with respiration could likewise enhance the toxicity of TDP-43. In this case, the TDP-43 toxicity targets in the presence or absence of respiration could be identical with the radical oxygen species produced by respiration activating TDP-43 to become more toxic or making TDP-43 targets more vulnerable. For example, free radical oxygen stress causes an increase in protein aggregation[26; 27] which could cause an increased burden on the proteasome. However, it is unclear if the lower level of protein aggregation associated with normal respiration would likewise enhance protein aggregation thereby increasing the toxicity of TDP-43.

Figure Legends

Fig. 1. TDP-43 overexpression causes more toxicity and cell elongation in cells grown in non-fermentable media than in cells grown in fermentable media. (a) Yeast cells (L2910 [*pin*$^-$] 74D-694) transformed with *TET*-regulated *CEN TDP-43-YFP TRP1* (p2223), or vector control (p1576) plasmid were obtained on plasmid selective glucose plates with 10 µg/ml of doxycycline (Dox) to inhibit expression of TDP-43-YFP. Normalized suspensions of cells were 10X serially diluted in water and were spotted on plasmid selective 2% dextrose (Dex), 2% galactose (Gal), 2% glycerol (Gly), and 2% ethanol (EtOH) plates without doxycycline (0) and with 10 µg/ml of doxycycline to respectively express or not express TDP-43-YFP. The plates were photographed after 4 (dextrose) or 6 (galactose) d of incubation at 30^{o}C. (b) TDP-43 aggregates in both galactose (Gal) and glucose (Dex) but causes more aggregation and cell elongation in galactose vs. glucose media. Cells from TDP-43-YFP inducing plates in (a) were imaged with a fluorescence microscope.. Bright field images show control cells with no TDP-43-YFP expression. (c) Expression of *TET* controlled TDP-43-YFP is not enhanced on galactose medium. The level of TDP-43-YFP was compared in L2910 transformants with *TET*-regulated *TDP-43-YFP* (p2223) grown in 2% glucose (Dex) vs. 2% raffinose + 2% galactose (SRGal) plasmid selective media. Cells grown overnight in medium containing 8 µg/ml of doxycycline were diluted to OD_{600} =0.5 in plasmid selective Dex or SRGal without doxycycline, grown for 24 or 48 h, harvested, and lysed for immunoblotting[29]. Normalized cell lysates boiled for 5 mins with 2% SDS in 80 mM DTT sample buffer were resolved on 10% SDS-PAGE followed by immunoblotting with rabbit polyclonal α-TDP-43 antibody (1:3000, Proteintech Group). Also, PGK, yeast 3-Phosphoglycerate Kinase, was detected with anti PGK antibodies (1:10,000, Novex) as an internal control (3c upper). Immunoblot signals for TDP-43-YFP and PGK were quantified and converted into % ratios of TDP43-YFP to PGK (3c lower). Gateway destination vector p1576 was made by dropping reading frame B gateway cassette (http://www.lifetechnologies.com) into pCM184[28]. TDP-43-YFP from entry vector pDONR TDP-43-YFP (Addgene plasmid # 27470) was then cloned into p1576 using an LR reaction, creating *TET*-regulated *CEN TDP-43-YFP TRP1* (p2223).

Fig. 2. TDP-43-YFP is still toxic in cells lacking functional mitochondria. (a) Spot test measure of TDP-43-YFP toxicity. *CUP1-TDP-43-YFP* in p2261 (pAG305 *CUP1::TDP43-EYFP*) was integrated at the *LEU2* locus in [*RHO*$^+$] [*PIN*$^+$] and [*RHO*$^+$] [*pin*$^-$] 74D-694 cells. Isogenic [*rho*^{0}] versions of the *CUP1-TDP-43-YFP* integrants were acquired by growing [*RHO*$^+$] *CUP1-TDP-43-YFP* integrants on YPD with 0.4 mg/ml ethidium bromide. The lack of functional mitochondria was verified as cells failed to grow on glycerol medium. To assay for TDP-43-YFP toxicity, three independent [*RHO*$^+$] and [*rho*^{0}] *CUP1-TDP-43-YFP* integrants and control empty integrants grown on glucose medium without $CuSO_4$ were normalized, 1/10 serially diluted and spotted on dextrose (Dex) and galactose (Gal) plates including 0, 100, and 250 µM $CuSO_4$. Plates were scanned after 4 d for Dex and 6 d for Gal. Reduced growth indicated toxicity. As expected [*rho*^{0}] cells failed to grow on Gal. The integrants were constructed by transforming cells with purified linear integrating vector p*CUP1-TDP-43-YFP* (p2261) that had been cut in the *LEU2* gene with BstxI and control integration vector without TDP-43-YFP, pAG305 GAL1-ccdB (Addgene plasmid #14137) that was cut similarly, and selecting for transformants on integrant selective glucose plates. Integration was verified by observing TDP-43-YFP aggregation in transformants in glucose medium with 50 µM $CuSO_4$. To generate p2261, the HpaI-PmeI fragment containing the *TET* promoter from pAG305 *TET-TDP-43-EYFP* (p2237) was switched with the *CUP1* promoter from p1988 (*pCUP1-SOD1-GFP*) cut with the same restriction site. Plasmid pAG305 *TET-TDP-43-EYFP* (p2237) was made by replacing the *GAL* promoter in pAG305 *GAL-ccdB* (Addgene plasmid # 14137) with PCR amplified HpaI-XbaI *TET* promotor from pCM184. (b) Clonogenicity measure of TDP-43-YFP toxicity. Cytotoxicity of the *CUP1* driven TDP-43-YFP in both [*RHO*$^+$] and [*rho*^{0}] cells was determined by colony forming units[23]. Overnight cultures of TDP-43-YFP integrant or vector controls grown without $CuSO_4$ were inoculated into synthetic glucose media with 0, 50 or 100 µM $CuSO_4$ to a final OD_{600} of 0.5. Samples were taken thereafter at 8, 24, 48, and 72 h and colony forming units determined on synthetic glucose plates lacking $CuSO_4$. The number of colonies grown after 3 d of incubation at 30°C were counted and converted into clonogenicity (%) calculated as ratio of cells grown in TDP-43-YFP inducing media (with 50 or 100 µM $CuSO_4$) over cells grown in non-inducing media (0 µM $CuSO_4$). Error bars present the standard error

calculated from 3 independent integrants. *(*c) No significant difference in levels of TDP43-YFP expression in [*RHO*$^{+}$] vs. [*rho*^{0}] *CUP1-TDP-43-YFP* integrated strains. Fresh cells taken from glucose plates were inoculated into 50 ml glucose containing 0, 50, or 100 μM $CuSO_4$ to an OD_{600} of 0.5. Cells were harvested and lysed at 24 and 48 h. Normalized cell lysates were boiled for 5 min with 2% SDS in 80 mM DTT sample buffer, and resolved on 10% SDS-PAGE followed by immunoblotting with rabbit polyclonal α-TDP-43 antibody (1:3000, Proteintech Group) and anti-PGK (1:10,000, Novex) as an internal loading control (upper). The intensity of each protein band was scanned and the ratio between TDP-43-YFP and PGK was calculated (lower). (d) TDP-43-YFP aggregates similarly in [*RHO*$^{+}$] and [*rho*^{0}] *CUP1-TDP-43-YFP* integrated strains. Samples from 2b were visualized with fluorescent microscope.

Fig. 3. H_2O_2 enhances TDP-43 toxicity. [*RHO*$^{+}$] *and* [*rho*^{0}] versions of strain 74D-694 with integrated *CUP1-TDP-43-YFP* (↑TDP-43), or *CUP1-YFP* (No TDP-43), were grown to OD_{600}=0.5 in synthetic glucose non-inducing medium and then for an additional 24 h after the addition of $CuSO_4$ to 250 μM to induce TDP-43-YFP or YFP expression and H_2O_2 to the concentrations listed. Serial dilutions of cells were then spotted on plates lacking $CuSO_4$ and H_2O_2.

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References

1. Dormann, D. & Haass, C. (2011). TDP-43 and FUS: a nuclear affair. *Trends Neurosci* **34**, 339-48.
2. Baloh, R. H. (2011). TDP-43: the relationship between protein aggregation and neurodegeneration in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *FEBS J* **278**, 3539-49.
3. McKee, A. C., Gavett, B. E., Stern, R. A., Nowinski, C. J., Cantu, R. C., Kowall, N. W., Perl, D. P., Hedley-Whyte, E. T., Price, B., Sullivan, C., Morin, P., Lee, H. S., Kubilus, C. A., Daneshvar, D. H., Wulff, M. & Budson, A. E. (2010). TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. *J Neuropathol Exp Neurol* **69**, 918-29.
4. Robinson, J. L., Geser, F., Stieber, A., Umoh, M., Kwong, L. K., Van Deerlin, V. M., Lee, V. M. & Trojanowski, J. Q. (2013). TDP-43 skeins show properties of amyloid in a subset of ALS cases. *Acta Neuropathol* **125**, 121-31.
5. Park, S. K., Hong, J. Y., Arslan, F., Kanneganti, V., Patel, B., Tietsort, A., Tank, E. M. H., Li, X., Barmada, S. J. & Liebman, S. W. (2017). Overexpression of the essential Sis1 chaperone reduces TDP-43 effects on toxicity and proteolysis. *PLoS Genet* **13**, e1006805.
6. Leibiger, C., Deisel, J., Aufschnaiter, A., Ambros, S., Tereshchenko, M., Verheijen, B. M., Buttner, S. & Braun, R. J. (2018). Endolysosomal pathway activity protects cells from neurotoxic TDP-43. *Microb Cell* **5**, 212-214.
7. Leibiger, C., Deisel, J., Aufschnaiter, A., Ambros, S., Tereshchenko, M., Verheijen, B. M., Buttner, S. & Braun, R. J. (2018). TDP-43 controls lysosomal pathways thereby determining its own clearance and cytotoxicity. *Hum Mol Genet* **27**, 1593-1607.
8. Shan, X., Chiang, P. M., Price, D. L. & Wong, P. C. (2010). Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. *Proc Natl Acad Sci U S A* **107**, 16325-30.

9. Magrane, J., Cortez, C., Gan, W. B. & Manfredi, G. (2014). Abnormal mitochondrial transport and morphology are common pathological denominators in SOD1 and TDP43 ALS mouse models. *Hum Mol Genet* **23**, 1413-24.

10. Xu, Y. F., Gendron, T. F., Zhang, Y. J., Lin, W. L., D'Alton, S., Sheng, H., Casey, M. C., Tong, J., Knight, J., Yu, X., Rademakers, R., Boylan, K., Hutton, M., McGowan, E., Dickson, D. W., Lewis, J. & Petrucelli, L. (2010). Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J Neurosci* **30**, 10851-9.

11. Hong, K., Li, Y., Duan, W., Guo, Y., Jiang, H., Li, W. & Li, C. (2012). Full-length TDP-43 and its C-terminal fragments activate mitophagy in NSC34 cell line. *Neurosci Lett* **530**, 144-9.

12. Wang, W., Li, L., Lin, W. L., Dickson, D. W., Petrucelli, L., Zhang, T. & Wang, X. (2013). The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. *Hum Mol Genet* **22**, 4706-19.

13. Wang, W., Wang, L., Lu, J., Siedlak, S. L., Fujioka, H., Liang, J., Jiang, S., Ma, X., Jiang, Z., da Rocha, E. L., Sheng, M., Choi, H., Lerou, P. H., Li, H. & Wang, X. (2016). The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. *Nat Med* **22**, 869-78.

14. Izumikawa, K., Nobe, Y., Yoshikawa, H., Ishikawa, H., Miura, Y., Nakayama, H., Nonaka, T., Hasegawa, M., Egawa, N., Inoue, H., Nishikawa, K., Yamano, K., Simpson, R. J., Taoka, M., Yamauchi, Y., Isobe, T. & Takahashi, N. (2017). TDP-43 stabilises the processing intermediates of mitochondrial transcripts. *Sci Rep* **7**, 7709.

15. Dols-Icardo, O., Nebot, I., Gorostidi, A., Ortega-Cubero, S., Hernandez, I., Rojas-Garcia, R., Garcia-Redondo, A., Povedano, M., Llado, A., Alvarez, V., Sanchez-Juan, P., Pardo, J., Jerico, I., Vazquez-Costa, J., Sevilla, T., Cardona, F., Indakoechea, B., Moreno, F., Fernandez-Torron, R., Munoz-Llahuna, L., Moreno-Grau, S., Rosende-Roca, M., Vela, A., Munoz-Blanco, J. L., Combarros, O., Coto, E., Alcolea, D., Fortea, J., Lleo, A., Sanchez-Valle, R., Esteban-Perez, J., Ruiz, A., Pastor, P., Lopez De

Munain, A., Perez-Tur, J. & Clarimon, J. (2015). Analysis of the CHCHD10 gene in patients with frontotemporal dementia and amyotrophic lateral sclerosis from Spain. *Brain* **138**, e400.

16. Johnson, J. O., Glynn, S. M., Gibbs, J. R., Nalls, M. A., Sabatelli, M., Restagno, G., Drory, V. E., Chio, A., Rogaeva, E. & Traynor, B. J. (2014). Mutations in the CHCHD10 gene are a common cause of familial amyotrophic lateral sclerosis. *Brain* **137**, e311.

17. Muller, K., Andersen, P. M., Hubers, A., Marroquin, N., Volk, A. E., Danzer, K. M., Meitinger, T., Ludolph, A. C., Strom, T. M. & Weishaupt, J. H. (2014). Two novel mutations in conserved codons indicate that CHCHD10 is a gene associated with motor neuron disease. *Brain* **137**, e309.

18. Zhang, M., Xi, Z., Zinman, L., Bruni, A. C., Maletta, R. G., Curcio, S. A., Rainero, I., Rubino, E., Pinessi, L., Nacmias, B., Sorbi, S., Galimberti, D., Lang, A. E., Fox, S., Surace, E. I., Ghani, M., Guo, J., Sato, C., Moreno, D., Liang, Y., Keith, J., Traynor, B. J., St George-Hyslop, P. & Rogaeva, E. (2015). Mutation analysis of CHCHD10 in different neurodegenerative diseases. *Brain* **138**, e380.

19. Chio, A., Mora, G., Sabatelli, M., Caponnetto, C., Traynor, B. J., Johnson, J. O., Nalls, M. A., Calvo, A., Moglia, C., Borghero, G., Monsurro, M. R., La Bella, V., Volanti, P., Simone, I., Salvi, F., Logullo, F. O., Nilo, R., Battistini, S., Mandrioli, J., Tanel, R., Murru, M. R., Mandich, P., Zollino, M., Conforti, F. L., Brunetti, M., Barberis, M., Restagno, G., Penco, S. & Lunetta, C. (2015). CHCH10 mutations in an Italian cohort of familial and sporadic amyotrophic lateral sclerosis patients. *Neurobiol Aging* **36**, 1767 e3-1767 e6.

20. Ronchi, D., Riboldi, G., Del Bo, R., Ticozzi, N., Scarlato, M., Galimberti, D., Corti, S., Silani, V., Bresolin, N. & Comi, G. P. (2015). CHCHD10 mutations in Italian patients with sporadic amyotrophic lateral sclerosis. *Brain* **138**, e372.

21. Jiao, B., Xiao, T., Hou, L., Gu, X., Zhou, Y., Zhou, L., Tang, B., Xu, J. & Shen, L. (2016). High prevalence of CHCHD10 mutation in patients with frontotemporal dementia from China. *Brain* **139**, e21.

22. Woo, J. A., Liu, T., Trotter, C., Fang, C. C., De Narvaez, E., LePochat, P., Maslar, D., Bukhari, A., Zhao, X., Deonarine, A., Westerheide, S. D. & Kang, D. E. (2017). Loss of function CHCHD10 mutations in cytoplasmic TDP-43 accumulation and synaptic integrity. *Nat Commun* **8**, 15558.

23. Braun, R. J., Sommer, C., Carmona-Gutierrez, D., Khoury, C. M., Ring, J., Buttner, S. & Madeo, F. (2011). Neurotoxic 43-kDa TAR DNA-binding protein (TDP-43) triggers mitochondrion-dependent programmed cell death in yeast. *J Biol Chem* **286**, 19958-72.

24. Johnson, B. S., McCaffery, J. M., Lindquist, S. & Gitler, A. D. (2008). A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. *Proc Natl Acad Sci U S A* **105**, 6439-44.

25. Elden, A. C., Kim, H. J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M. M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P. J., Rub, U., Auburger, G., Trojanowski, J. Q., Lee, V. M., Van Deerlin, V. M., Bonini, N. M. & Gitler, A. D. (2010). Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* **466**, 1069-75.

26. David, D. C., Ollikainen, N., Trinidad, J. C., Cary, M. P., Burlingame, A. L. & Kenyon, C. (2010). Widespread protein aggregation as an inherent part of aging in C. elegans. *PLoS Biol* **8**, e1000450.

27. Weids, A. J., Ibstedt, S., Tamas, M. J. & Grant, C. M. (2016). Distinct stress conditions result in aggregation of proteins with similar properties. *Sci Rep* **6**, 24554.

28. Gari, E., Piedrafita, L., Aldea, M. & Herrero, E. (1997). A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in Saccharomyces cerevisiae. *Yeast* **13**, 837-48.

29. Bagriantsev, S. N., Gracheva, E. O., Richmond, J. E. & Liebman, S. W. (2008). Variant-specific [PSI+] infection is transmitted by Sup35 polymers within [PSI+] aggregates with heterogeneous protein composition. *Mol Biol Cell* **19**, 2433-43.

Figure 1

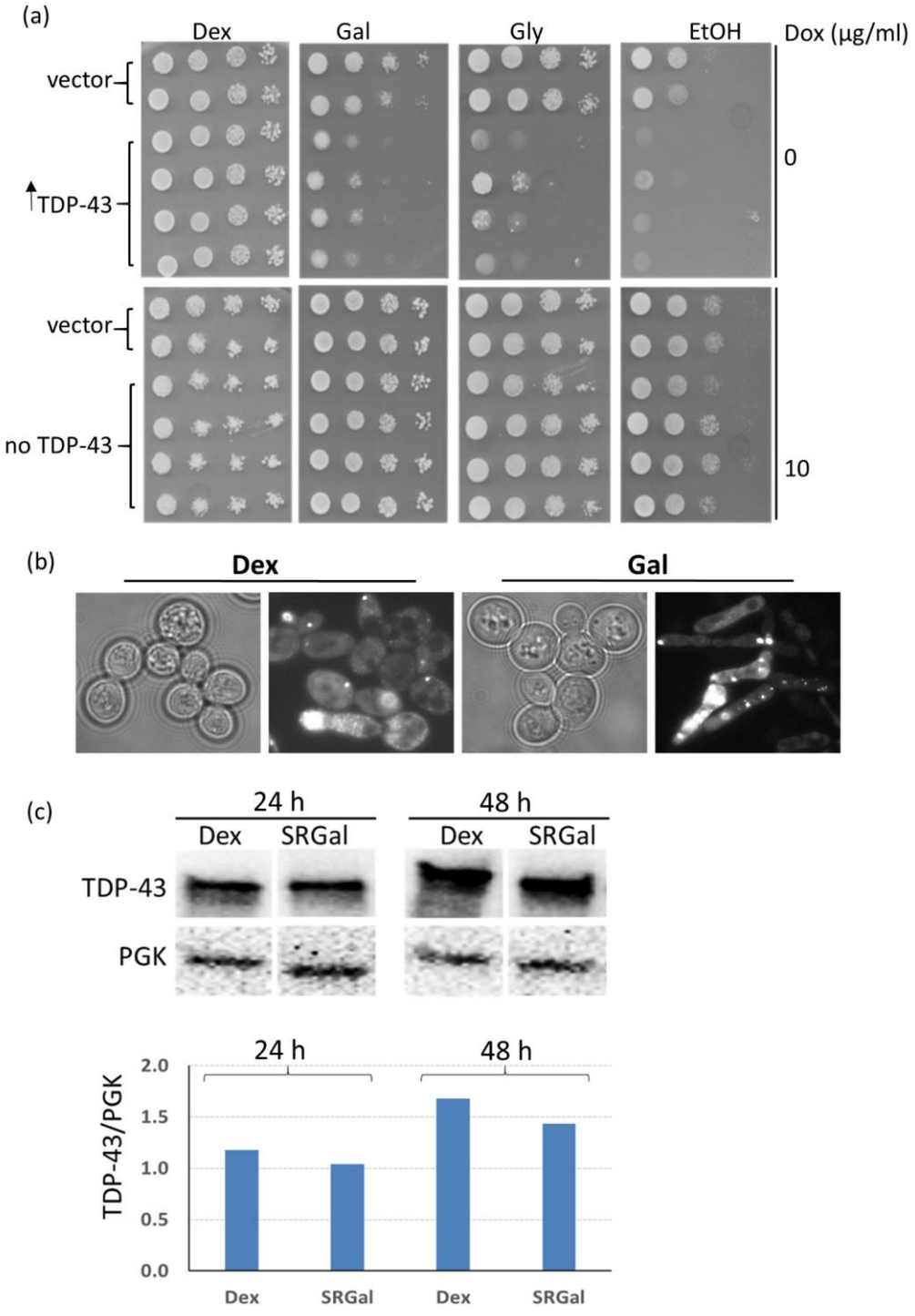
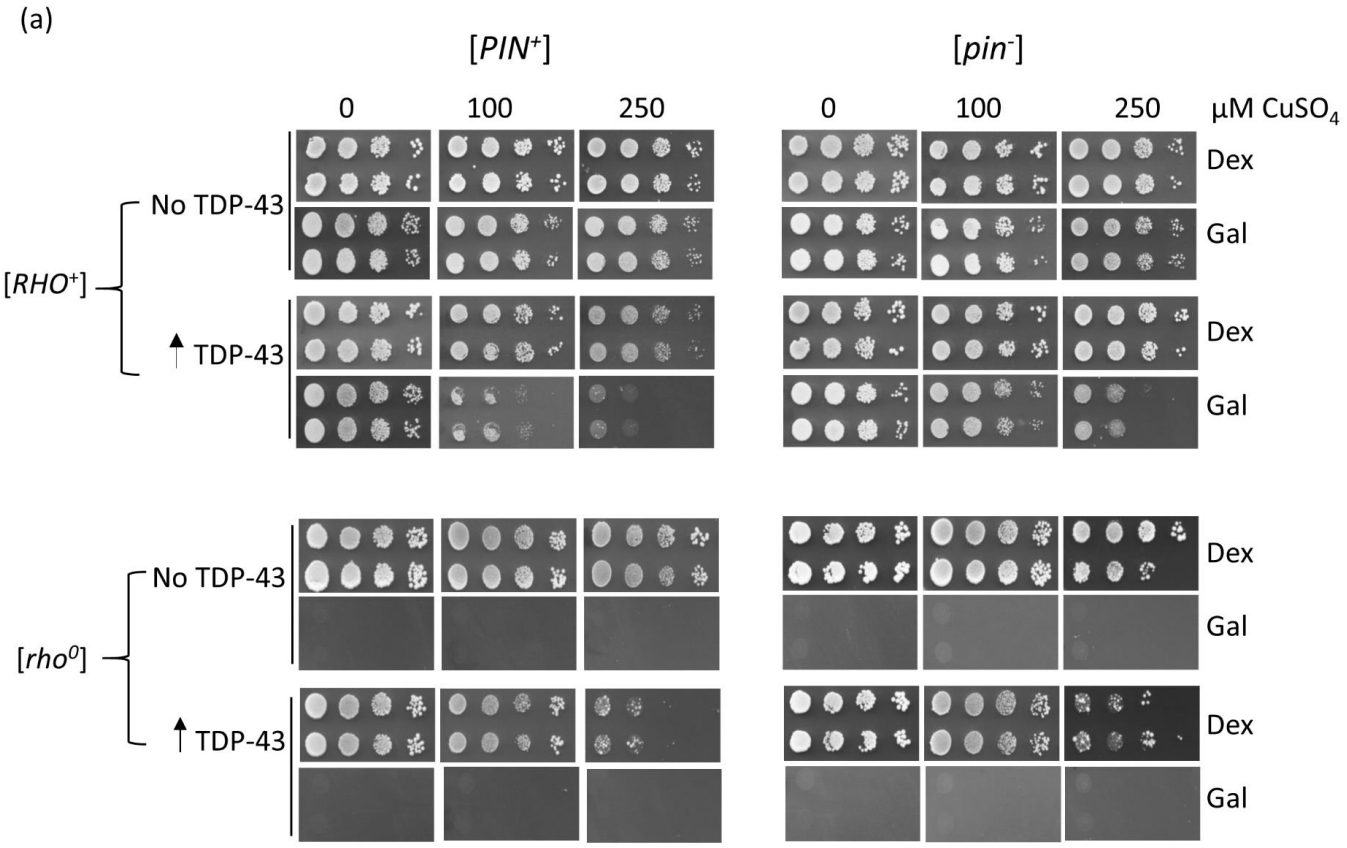


Figure 2



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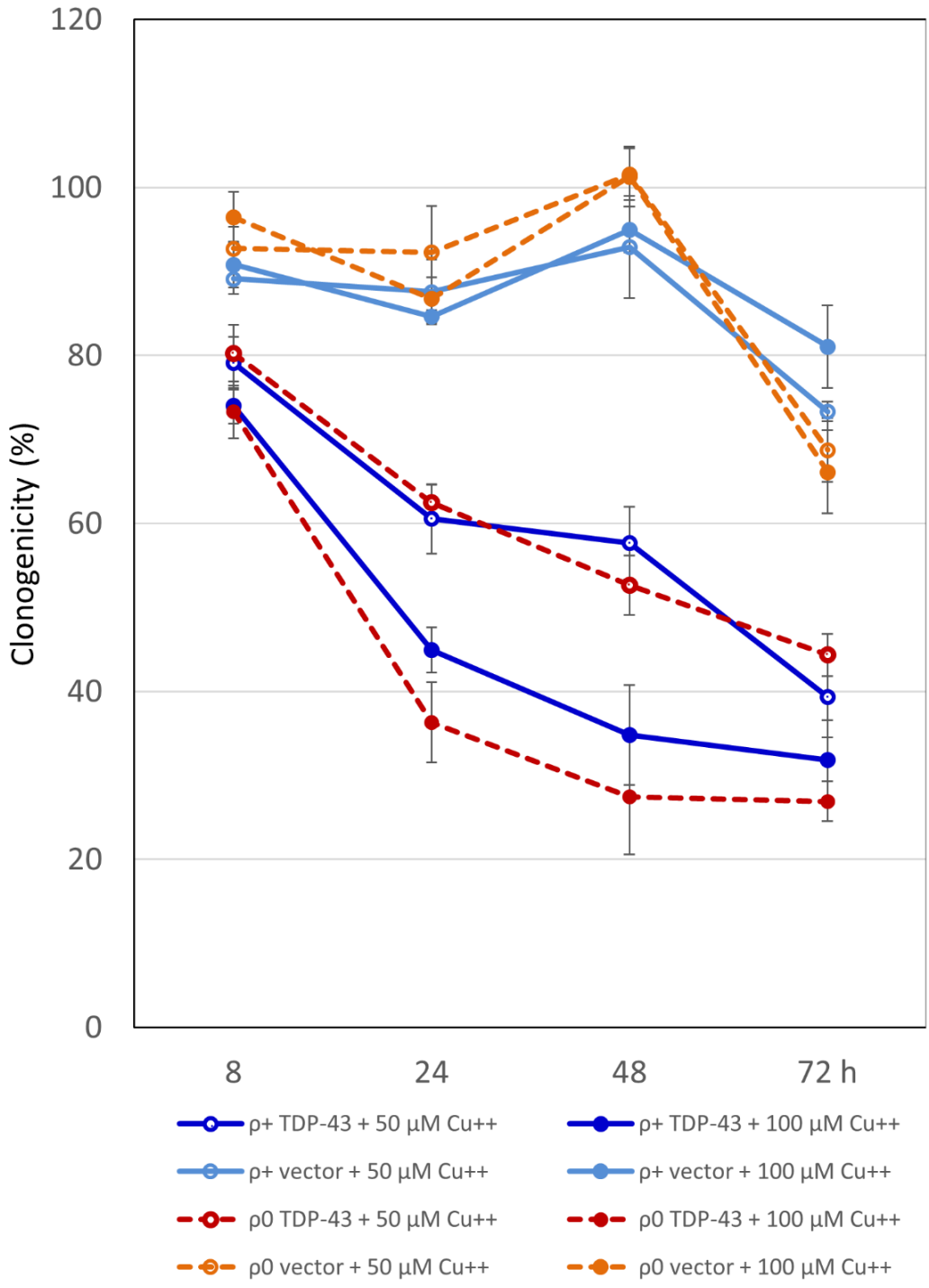

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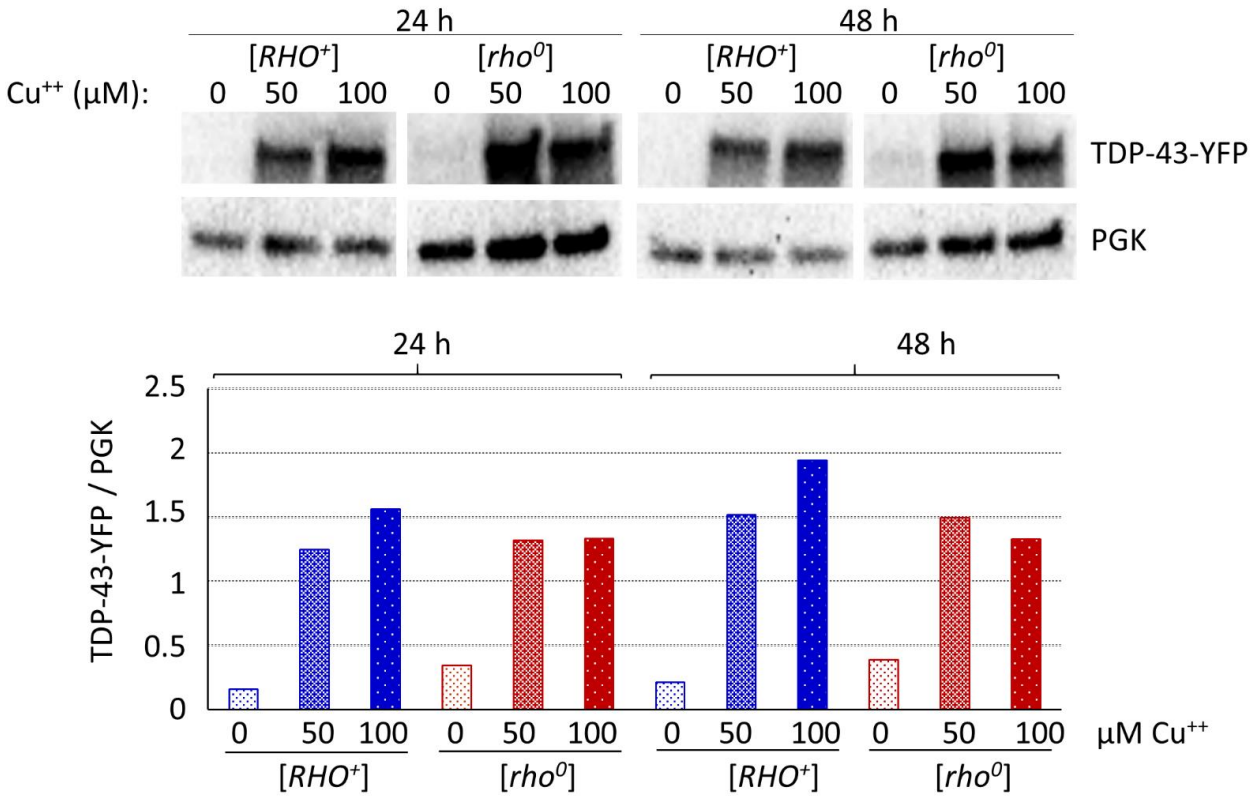

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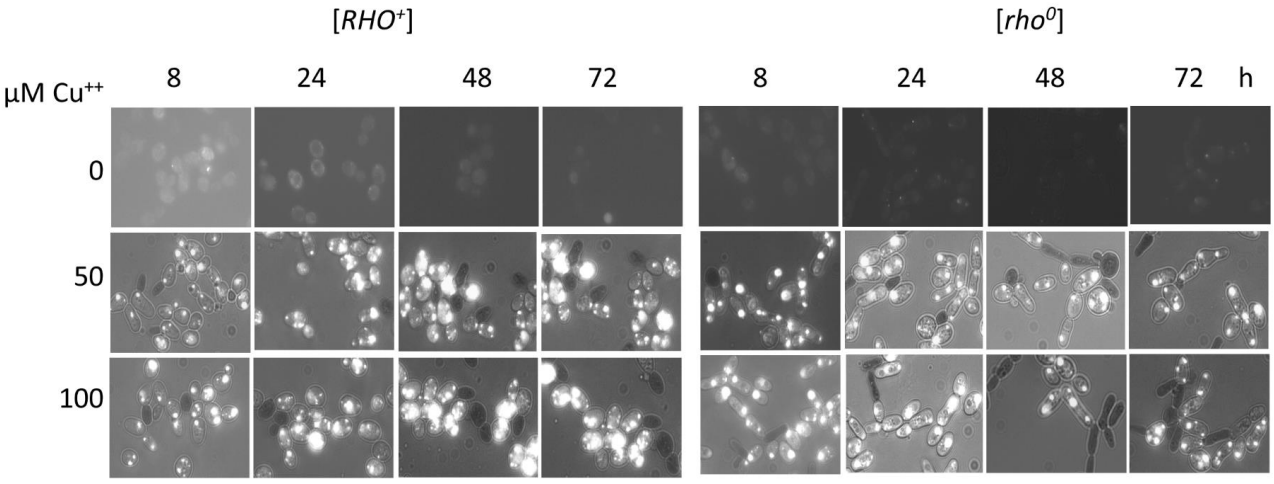

Figure 3