Supporting Information

Supplementary Figures

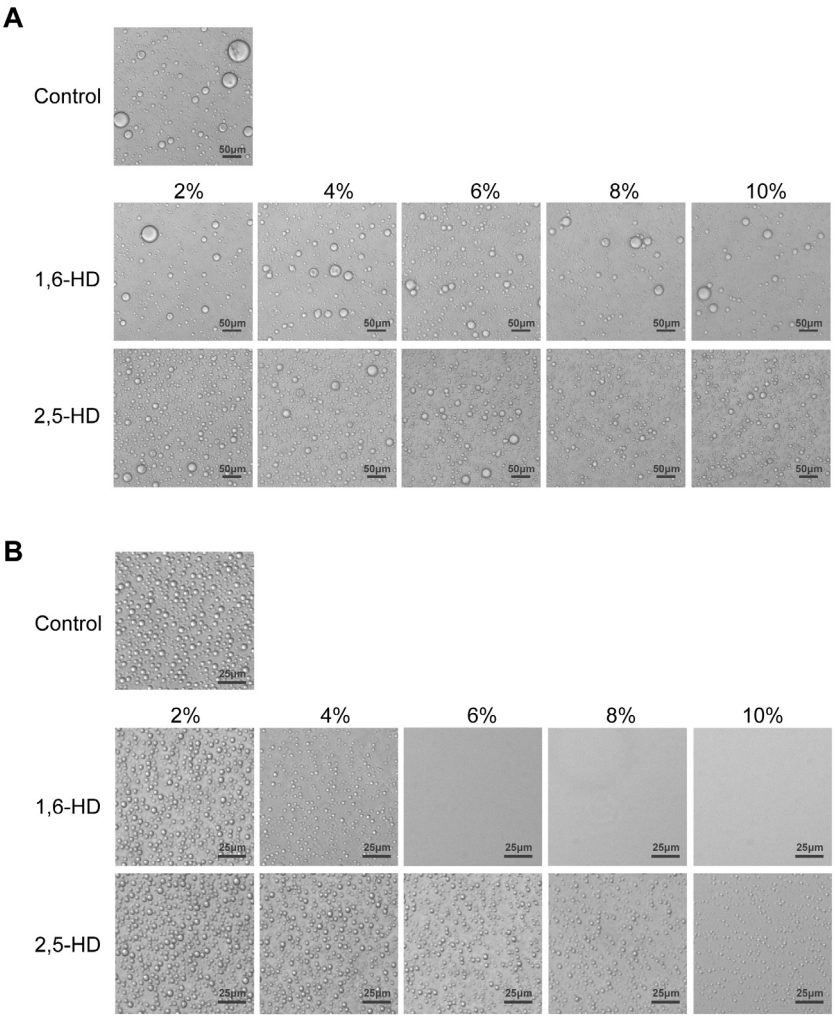

Fig. S1. Effects of aliphatic alcohols on phase-separated liquid-like droplet formation by the structured and unstructured halves of TDP-43.

(A) Liquid-like droplet formation by the structured N-terminal half of TDP-43 was induced by diluting the protein into the buffer in the presence 4% PEG-8000 and graded increases in concentration (w/v) of aliphatic alcohols as indicated. **(B)** Liquid-like droplet formation by the disordered C-terminal half of TDP-43 in buffer containing graded increases in concentration (w/v) of aliphatic alcohols as indicated. Scale bars = 25 µm.

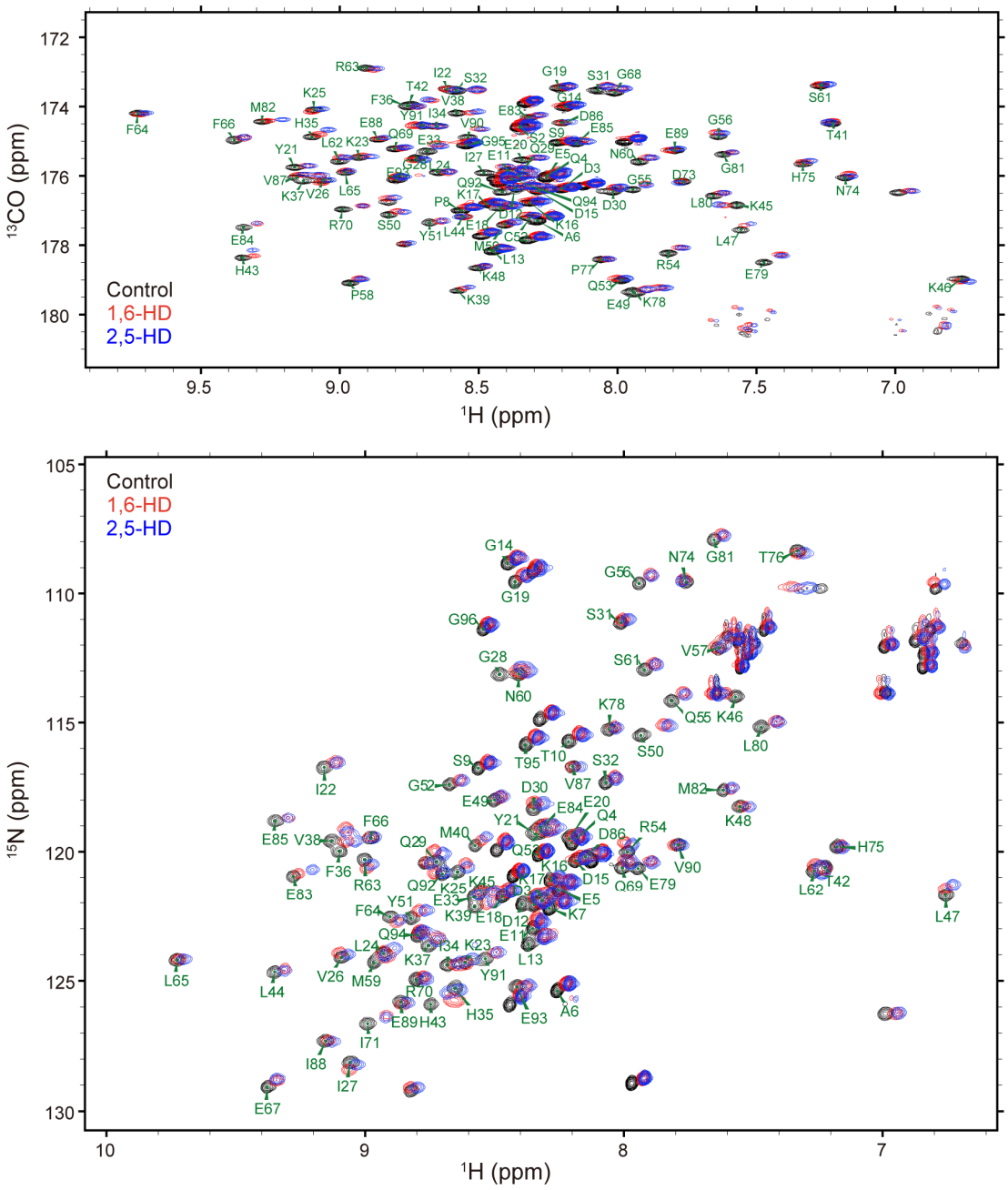

Fig. S2. Chemical shift perturbation (CSP) analysis of the SUMO1 polypeptide backbone in the absence and presence of aliphatic alcohols.

Diagrams show overlays of ^{1}H-^{13}C HNCO spectra (upper panel) or ^{1}H-^{15}N HSQC spectra (lower panel) of 150 µM SUMO1 obtained in NMR buffer alone (control, black), 10% (w/v) 1,6-HD (red), and 10% (w/v) 2,5-HD (blue). The NMR buffer consisted of 10 mM potassium phosphate, pH 6.5, 100 mM KCl, and 2 mM DTT (*1*).

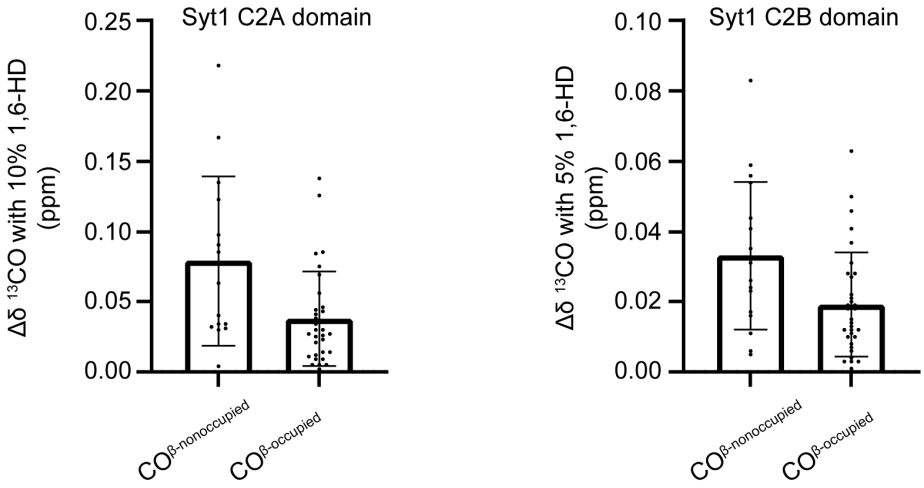

Fig. S3. Effects of 1,6-HD on chemical shift perturbation of hydrogen bond-occupied or non-occupied carbonyl groups residing in β-sheets of the well-folded synaptotagmin1 C2A and C2B domains.

Graphs show comparisons of absolute chemical shift perturbation (CSP) values of non-occupied and occupied peptide backbone hydrogen bonds as visualized by ^{13}CO in the presence of 1,6-HD. The CSP values are derived from all assigned residues from β-sheet structures of synaptotagmin1 C2A (left) and C2B (right) domains. Spectra were analyzed at 150 μM protein concentration in a buffer containing 50 mM HEPES, pH6.8, 100 mM NaCl, 2 mM DTT, and 1 mM EDTA (*2, 3*).

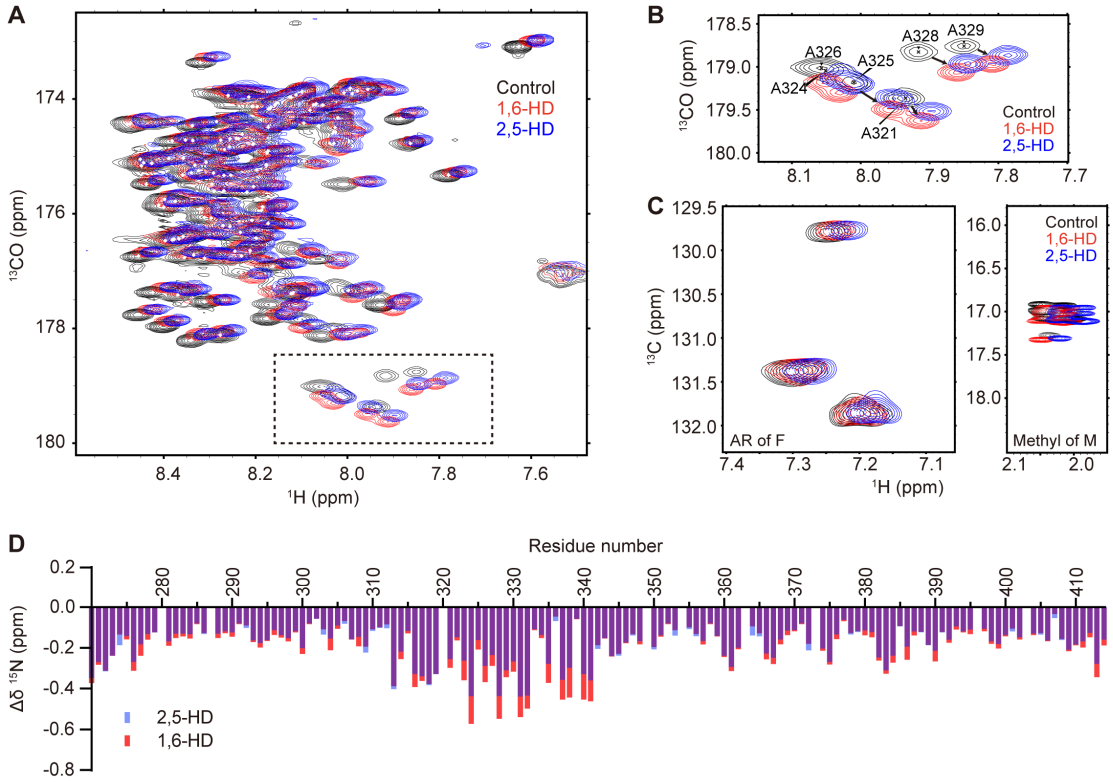

Fig. S4. Chemical shift perturbation (CSP) analysis of TDP-43 low complexity domain (LCD) backbone and amino acid sidechains in the absence and presence of aliphatic alcohols.

(A) Graph shows the overlay of 2D HNCO spectra of 100 μM ^{15}N-/^{13}C-double-labelled TDP-43 LCD in a buffer containing 25 mM MES pH6.1 (black), or supplement with 10% (w/v) 1,6-HD (red) or 10% (w/v) 2,5-HD (blue). **(B)** Enlarged view of the area boxed with dash line in panel A. This area illustrates the CSP of Ala within TDP-43 LCD evolutionarily conserved domain. Black arrows indicate changes of cross-peaks of the indicated residues in the presence of aliphatic alcohols. **(C)** CSP of constant time ^{1}H-^{13}C HSQC spectra of 100 μM Phe-[aromatic ring (AR), ^{13}C6]-labelled TDP-43 LCD in the presence of 10% (w/v) aliphatic alcohols (left). The ^{1}H-^{13}C HSQC spectra of 100 μM Met-[$^{13}CH_3$]-labelled TDP-43 LCD with/without 10% (w/v) aliphatic alcohols (right). NMR buffer: 25 mM MES pH6.1. **(D)** CSP of ^{15}N atoms from the backbone of TDP-43 LCD with 10% (w/v) aliphatic alcohols at pH 6.1.

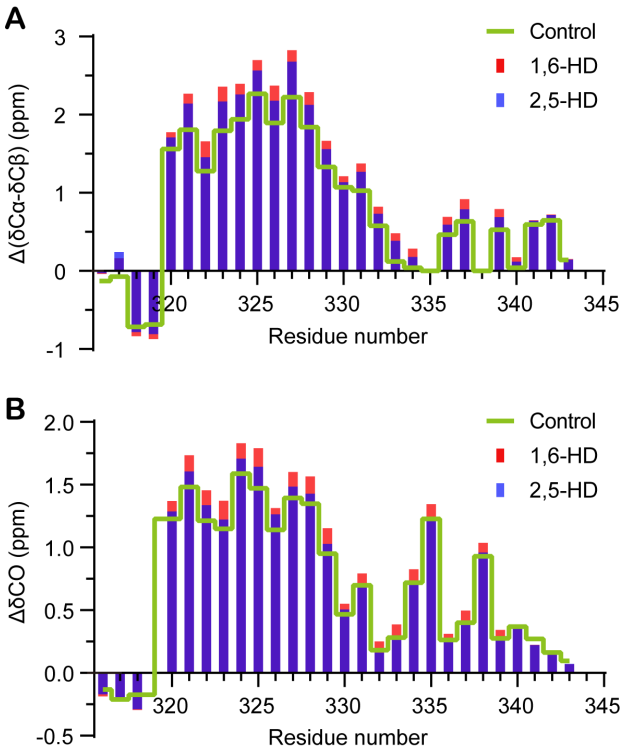

Fig. S5. Aliphatic alcohols increase α-helical conformation of the evolutionarily conserved region (ECR) of the TDP-43 low complexity domain (LCD).

(A) Secondary shifts (ΔδCα-ΔδCβ) of TDP-43 LCD (residue 316-343) in the absence or presence of 10% aliphatic alcohols in 25 mM MES buffer at pH6.1. The ECR refers to residues from 320 to 339. **(B)** Chemical shift deviations of backbone carbonyls of the TDP-43 LCD (residue 316-343) with respect to random coil. The ΔδCO represents the CO chemical shifts with or without 10% aliphatic alcohol treatment minus CO chemical shifts of the random coil in 25 mM MES buffer at pH6.1 (*4*).

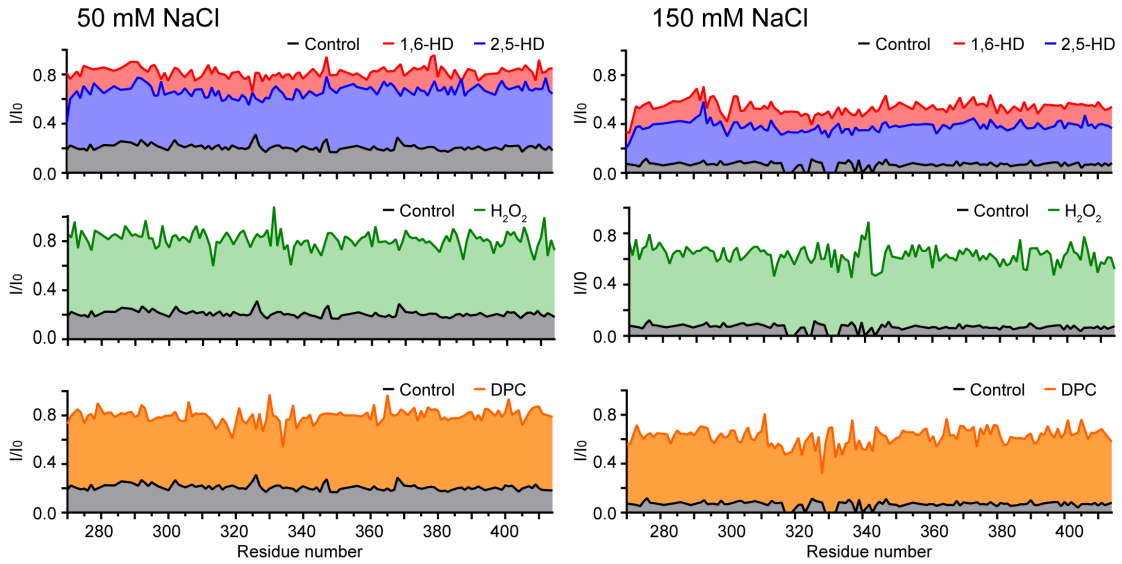

Fig. S6. The effects of aliphatic alcohols, H_2O_2-mediated methionine oxidation, and exposure to the DPC lipid mimic on maintaining TDP-43 LCD ^{1}H-^{15}N HSQC signals in the presence of monovalent salt ions.

^{1}H-^{15}N HSQC signals (grey) of TDP-43 LCD drastically dropped due to self-association in buffer supplemented with 50 mM NaCl (left) or 150 mM NaCl-mediated phase separation (right). Exposure to 10% (w/v) of either 1,6-HD or 2,5-HD (top panel, red = 1,6-HD, blue = 2,5-HD) significantly enhanced NMR signals, as did H_2O_2-mediated methionine oxidation (middle panel), as did exposure to the lipid-mimic DPC (bottom panel). The I_0 represents the cross-peak intensity of native TDP-43 LCD, fully-oxidized TDP-43 LCD or native TDP-43 LCD with 200-fold DPC for each titration, under 40 μM ^{15}N-labeled proteins, 25 mM MES (pH 5.5) without salts.

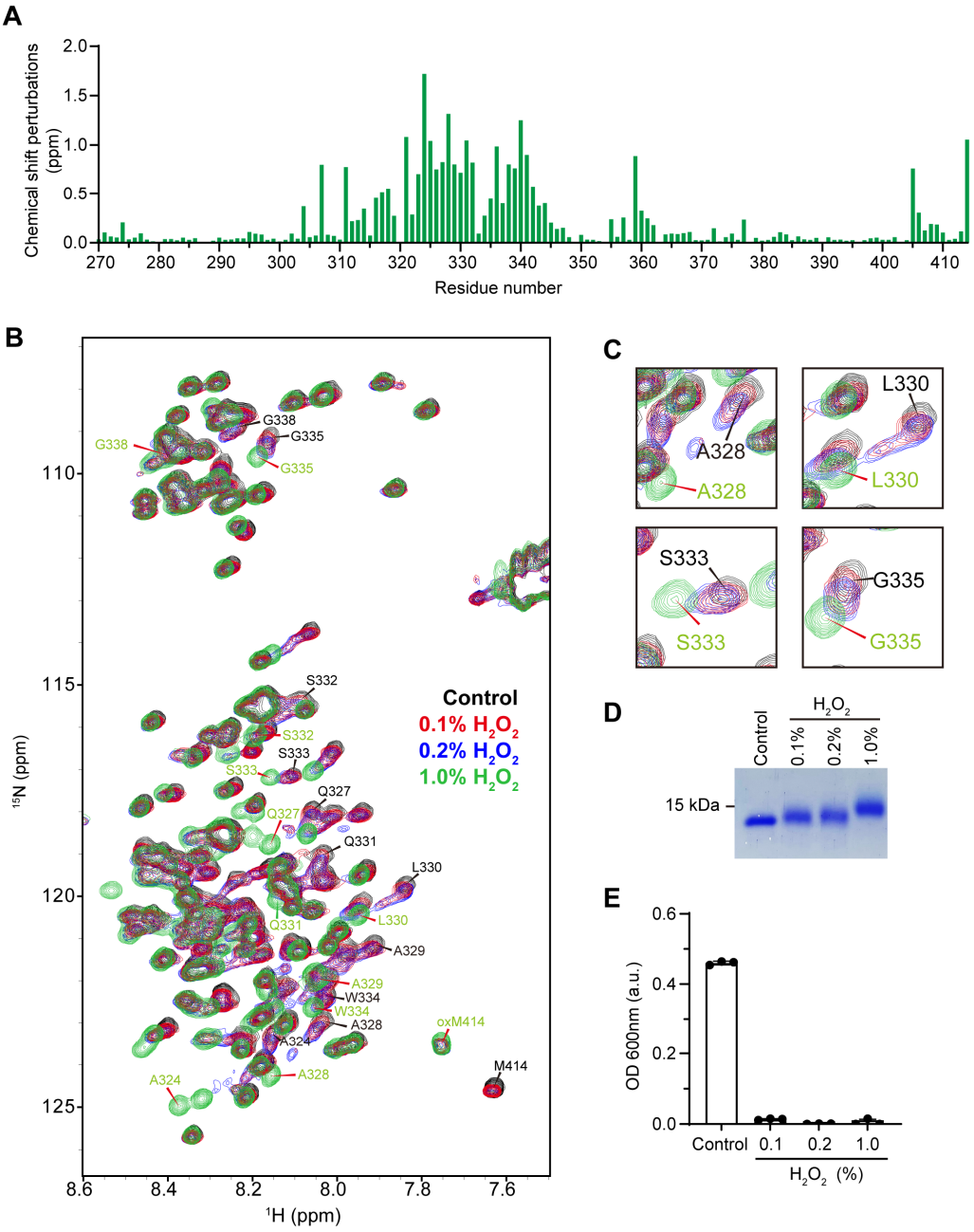

Fig. S7. Partial oxidation retains α-helical structure of evolutionarily conserved region (ECR) of TDP-43 low complexity domain (LCD) while fully impeding phase separation.

(A) The chemical shift perturbation (CSP) of fully-oxidized TDP-43 LCD on methionine (1% H_2O_2 for 30 min), compared to native TDP-43 LCD as assayed in buffer composed of 25 mM MES, pH6.1. The CSP was calculated by the following formula: $\sqrt{((\Delta\delta N)^2+(\Delta\delta HN)^2)}$. The graph shows significant CSP in TDP43-LCD ECR, representing the change of α-helix structures after full oxidation. **(B)** Overlay of ^{1}H-^{15}N HSQC spectra of native TDP-43 LCD (control, black), partially-oxidized TDP-43 LCD (0.1% H_2O_2 red; 0.2% H_2O_2 blue), and fully-oxidized TDP-43 LCD (1% H_2O_2, green) in buffer composed of 25 mM MES, pH6.1. Representative residues are labelled in black (for control) or green (for 1% H_2O_2). For partially-oxidized samples,

the majority of signals from the TDP-43 LCD ECR residues were retained near the signals of the native TDP-43 LCD, indicating that the partially-oxidized TDP-43 LCD retained higher population of α-helical structure compared with the fully-oxidized sample. The CSP of M414 represents an indicator of oxidation level. **(C)** Enlarged views show the CSP of A328, L330, S333, and G335 located within the ECR. **(D)** Coomassie stained SDS-PAGE showing the band shifts of TDP-43 LCD after partial and full oxidation. **(E)** Turbidity measurement of the samples in (B) at 50 μM protein concentration in buffer composed of 100 mM NaCl, 25 mM MES, pH 6.1. Fully reduced protein (control) was phase separated into liquid-like droplets. Exposure to 0.1%, 0.2% or 1% levels of H_2O_2 fully melted all liquid-like droplets.

Reference

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