Figure S4. Expression and purification of recombinant TDP-43 proteins for the \textit{in vitro} assays. \textbf{a} Domains of full-length TDP-43 (TDP-43-FL), TDP-43\textsuperscript{1-274} and TDP-43\textsuperscript{274-414}. TDP-43-FL contains two RNA recognition motifs (RRM1/2) and a glycine-rich (GRD) domain. \textbf{b-e} Recombinant TDP-43-FL proteins expressed in the \textit{Escherichia coli} are separated in the SDS-PAGE gel and stained with Coomassie Blue. T, total lysates; S, soluble in the lysis buffer (50 mM NaH\textsubscript{2}PO\textsubscript{4}, 500 mM NaCl, pH 8.0, 1 mM DTT and 1 mM PMSF); P, pellets re-suspended in 9 M of urea. TDP-43 proteins of predicated size are indicated with arrows. The solubility of TDP-43-FL protein is too low for the subsequent purification steps. This is regardless of the expression vectors used, including pET28a-6*his (b-c) and pCold-6*his employed by Elden et al. previously\textsuperscript{66} (d), and the induction temperatures tested, such as 16 °C (b) and 22 °C (c). As an attempt to increase the solubility of TDP-43-FL, the pET32M.3C-MBP-6*his was also tried. However, it did not improve its solubility, while the total expression level is much lower than the other two vectors (e). \textbf{f} Given that a significant portion of the recombinant His-tagged TDP-43\textsuperscript{1-274} truncation protein is in the soluble fraction (left), it is subject to Ni affinity purification and HPLC (right), which is used in the \textit{in vitro} PARylation and LLPS assays in the current study. \textbf{g} Considering that the his-tagged TDP-43\textsuperscript{274-414} fragment is unstructured and extremely insoluble (left), it is purified using a denaturing lysis buffer containing 6 M of guanidine hydrochloride and subsequently purified using the Ni column and HPLC (right). The purified TDP-43\textsuperscript{274-414} protein is employed in the \textit{in vitro} assays in the present study.