Supporting Information

Amyloidogenic Oligomers Derived from TDP-43 LCD Promote the Condensation and Phosphorylation of TDP-43

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Materials

List of peptides

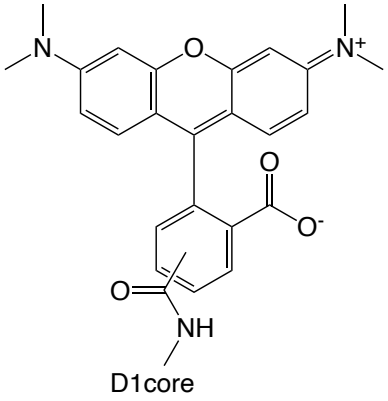
Name	Sequence	Calc'd m/z	Observed m/z
D1core	MGGGMNFGAFSINPAM	1599.7	1622.9 ($M+Na^+$)
ScD1core	GMAFSNGGNPAMFIGM	1599.7	1622.8 ($M+Na^+$)
TAMRA-D1core	(TAMRA)-MGGGMNFGAFSINPAM 	2013.2	2014.0 ($M+H^+$)

Table S1. The list of peptides.

Supplementary Figures

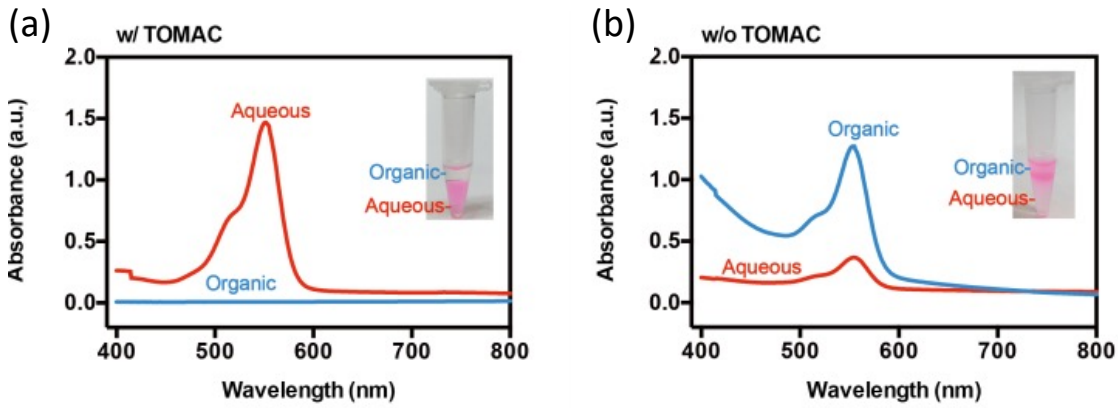


Figure S1. The partitioning of TAMRA-D1core (pinkish solute) between isooctane/water during the back extraction. The back extraction of TAMRA-D1core with (a) or without TOMAC (b). TAMRA-D1core in different phases were collected, lyophilized and re-dissolved in water for UV-VIS absorbance measurements.

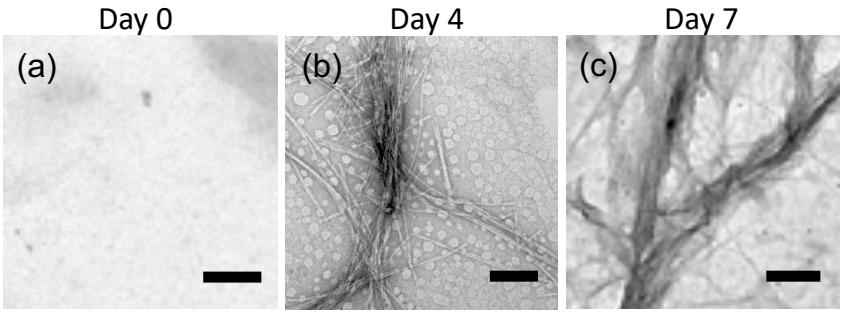


Figure S2. TEM images of D1core peptides incubated from the monomeric state in Tris buffer, without prior incubation in reverse micelles. (a) Freshly prepared monomeric D1core. Monomeric D1core peptides were incubated for 4 (b) and 7 days (c), respectively.

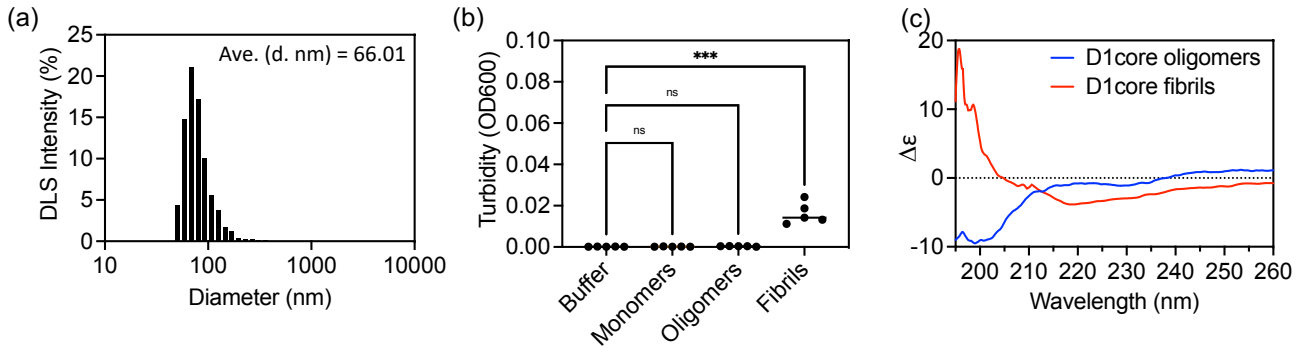


Figure S3. Characterizations of D1core oligomers. (a) DLS analysis of D1core oligomers. (b) Turbidity measurement of D1core peptides in various aggregation states. (c) Circular dichroism of D1core oligomers and fibrils.

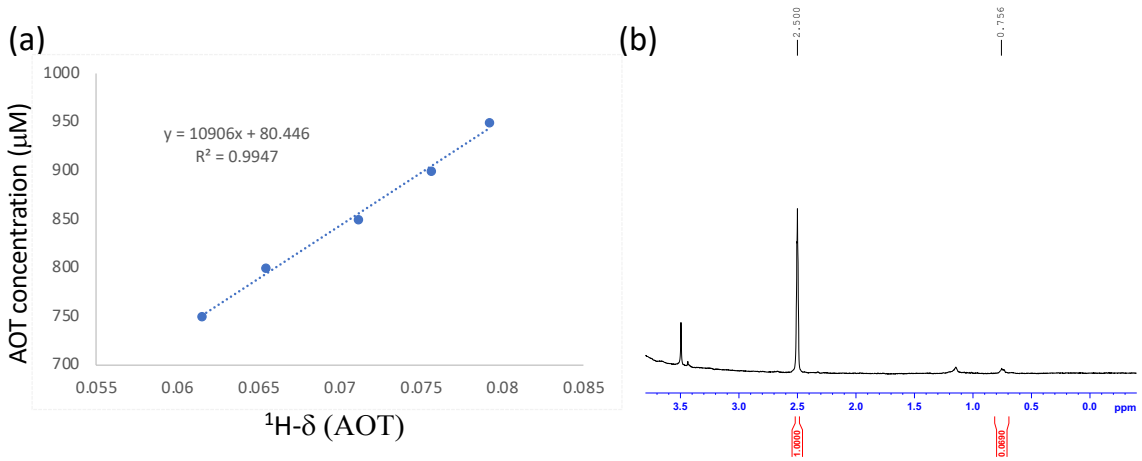


Figure S4. The quantification of residual AOT in back extracted D1core oligomer with liquid state NMR. 1 mL of standard AOT solutions and samples were lyophilized and re-dissolved in 500 μL d-DMSO for measurements. (a) The calibration curve for AOT concentration vs. ^{1}H signal integration obtained from liquid state NMR. (b) The NMR spectrum of residual AOT in 50 μM peptide.

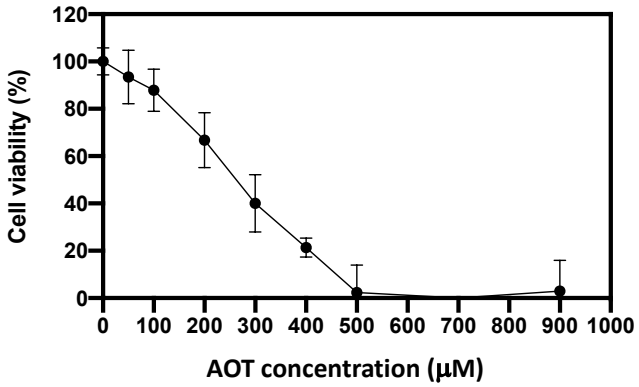


Figure S5. Cell viability of U2OS cells in various concentration of AOT. The statistic results were shown as mean ± SD of 5 independents replicates.

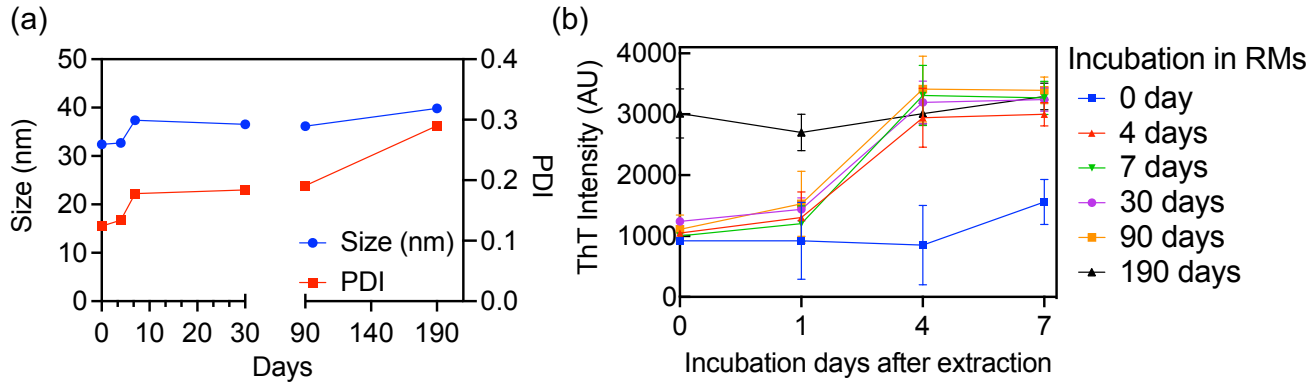


Figure S6. Shelf life evaluation of D1core oligomers encapsulated in RMs. (a) Time-dependent DLS analyses of RM solution encapsulating D1core peptides. (b) ThT kinetic measurements of the back extracted D1core peptides from RMs incubated for different period of time. The statistic results were shown as mean ± SD of 3 independents replicates.

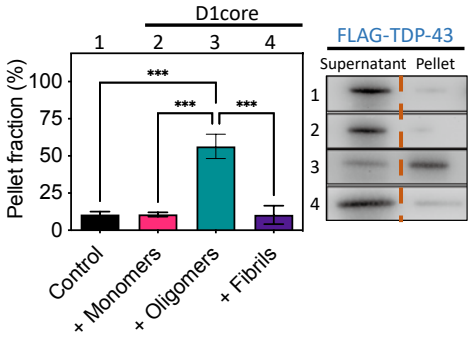


Figure S7. Seeding experiment in cell-free system expressing TDP-43. The cell-free cocktail was expressed with TDP-43 protein, followed by the addition of D1core peptides. After incubation, the mixture was fractionated by centrifugation and stained with TDP-43 antibodies using a chemiluminescent HRP substrate. The blotting signals were analyzed using ImageJ, and the fraction of TDP-43 protein in the supernatant was calculated using the equation S/(S+P), where S and P represent the signals of TDP-43 in the supernatant and pellet fractions, respectively. Each experiment was performed in triplicate for all samples.

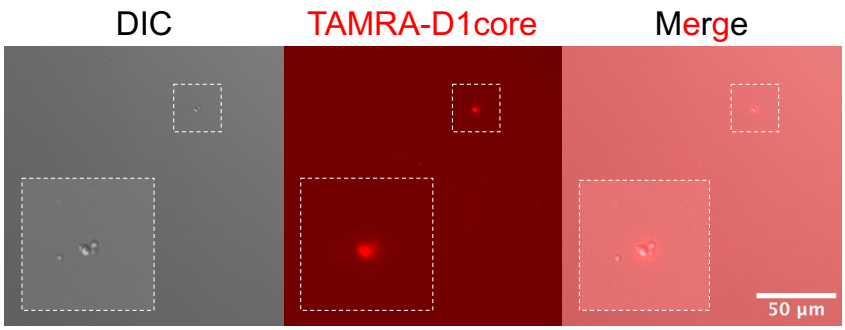


Figure S8. Microscopy image of TDP-CTD incubated with D1core fibrils in the presence of 20% 1,6-HD.

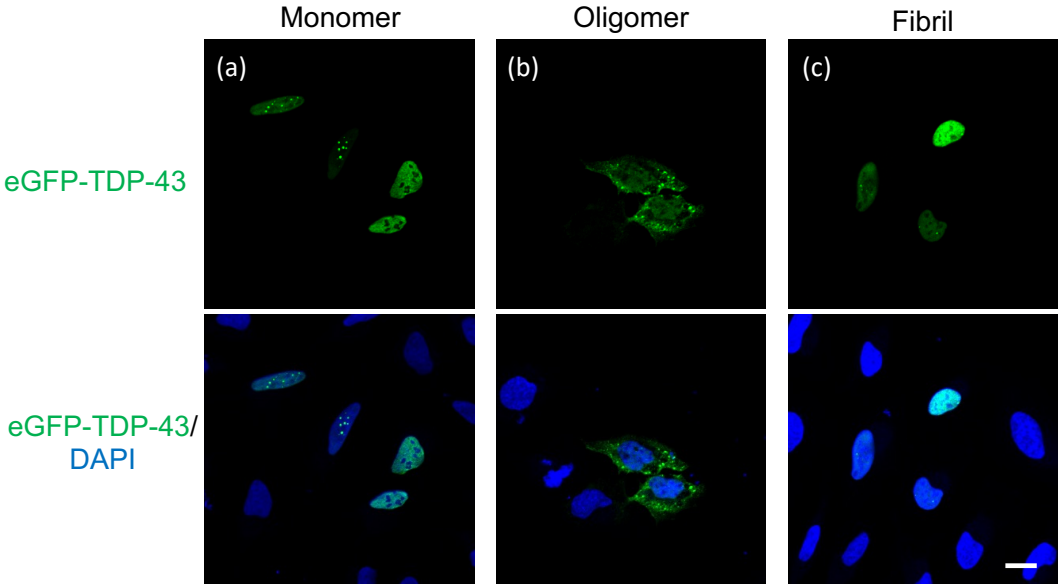


Figure S9. Low-magnification fluorescence images of U2OS cells transfected with eGFP-TDP-43 in the presence of monomeric (a), oligomeric (b) and fibrillar (c) D1core peptides, respectively. (Scale bar = 20 μm)

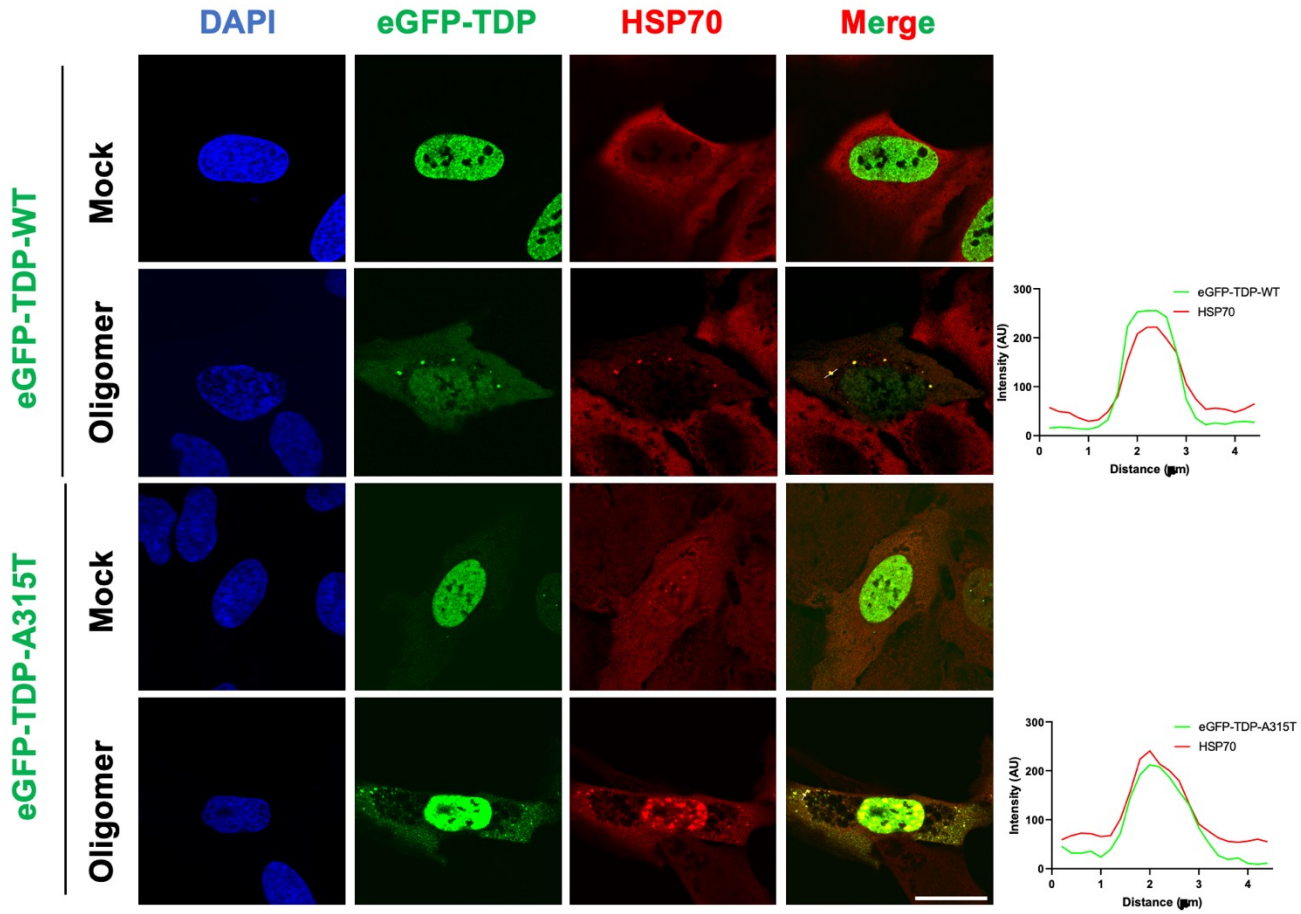


Figure S10. Cells transfected with eGFP-TDP-43 and eGFP-A315T were treated with D1core oligomers and immunostained for HSP70. (Scale bar = 10 μm)

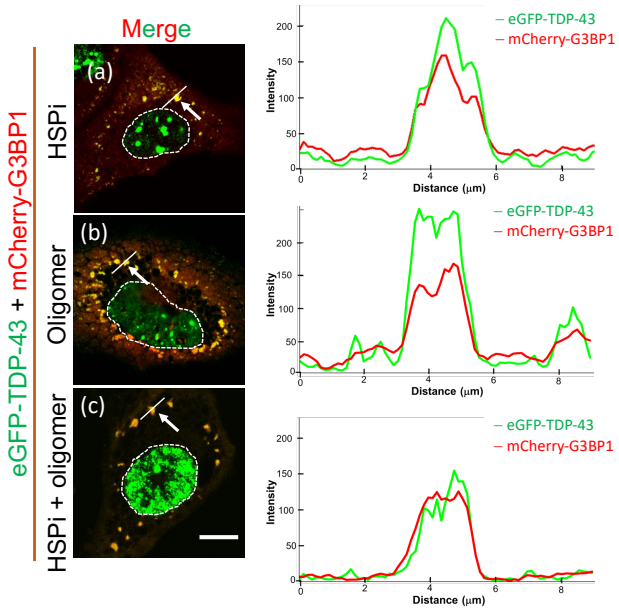


Figure S11. Fluorescence intensity profiling of eGFP-TDP-43 and mCherry-G3BP1 after treatments to U2OS cells for 3 hours. (a) HSPi (b) oligomer (c) HSPi and oligomer (Scale bar = 2 μm; profiling cross sections are indicated in A, B and C).

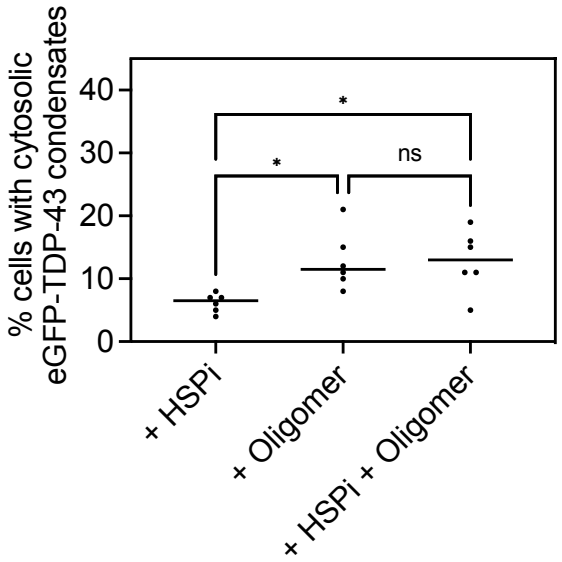


Figure S12. Percentage of cells with cytosolic eGFP–TDP-43 condensates treated with HSPi, D1core oligomers, or both for 3 hours. Data were analyzed by one-way ANOVA using Tukey post-hoc test with a 95% confidence interval, with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Results are presented as mean ± SD from five independent replicates, with 150 cells counted per replicate.

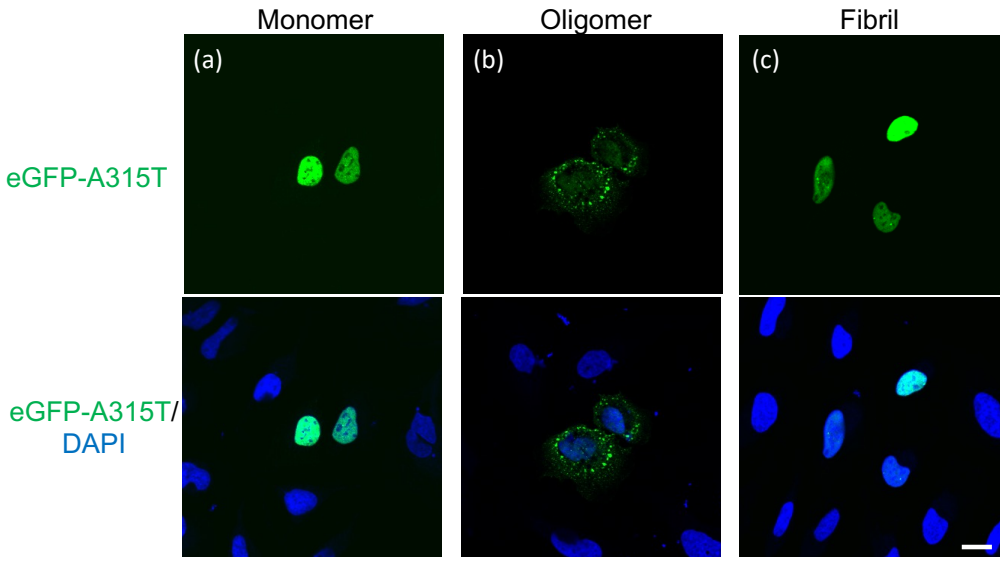


Figure S13. Low-magnification fluorescence images of U2OS cells transfected with eGFP-A315T in the presence of monomeric (a), oligomeric (b) and fibrillar (c) D1core peptides, respectively. (Scale bar = 20 μm)

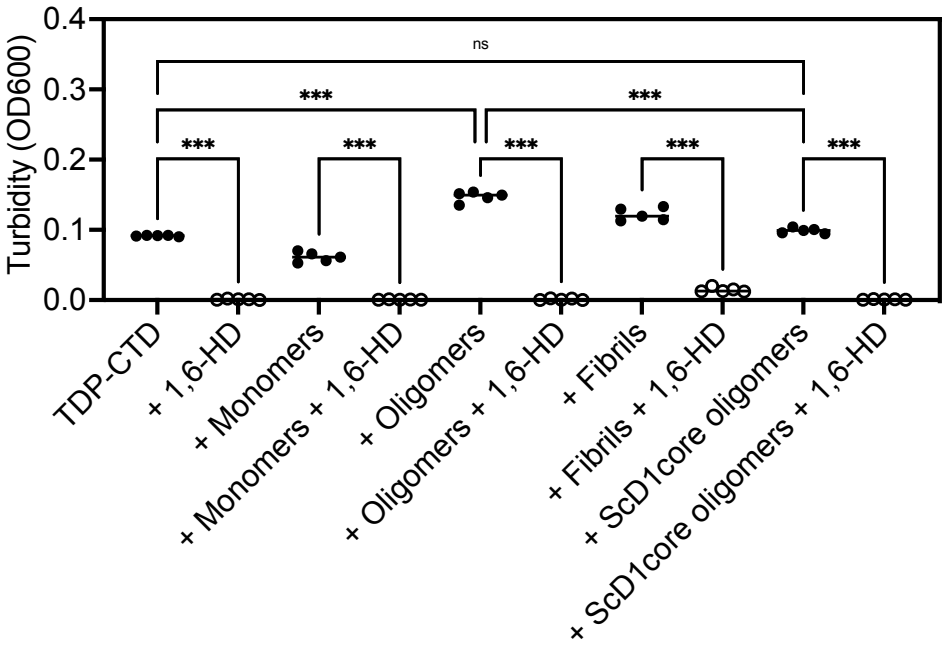


Figure S14. Optical density at 600 nm (OD600) of TDP-CTD alone and with D1core and ScD1core peptides, without and with 20% of 1,6-hexanediol. Data were analyzed by one-way ANOVA using Tukey post-hoc test with a 95% confidence interval, with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. The statistic results were shown as mean ± SD of 5 independents replicates.