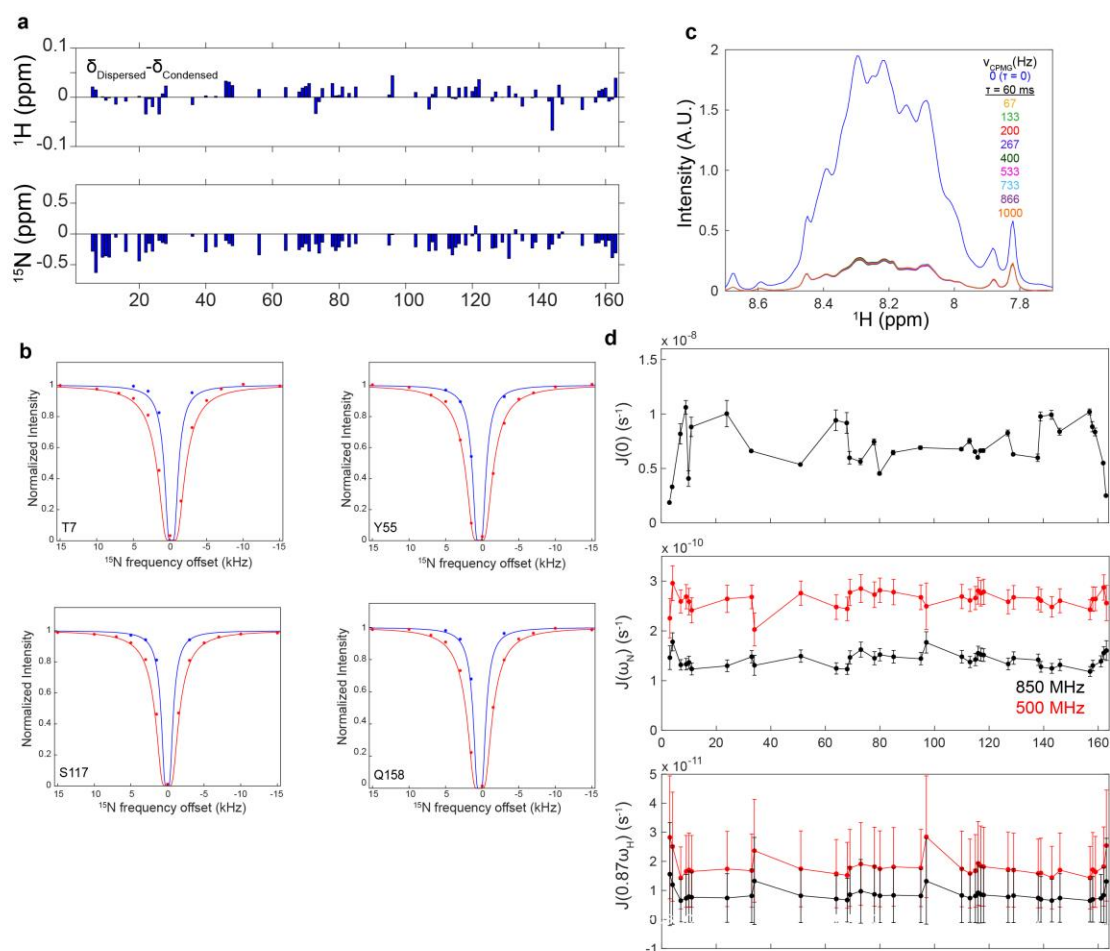


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Molecular interactions underlying liquid–liquid phase separation of the FUS low-complexity domain

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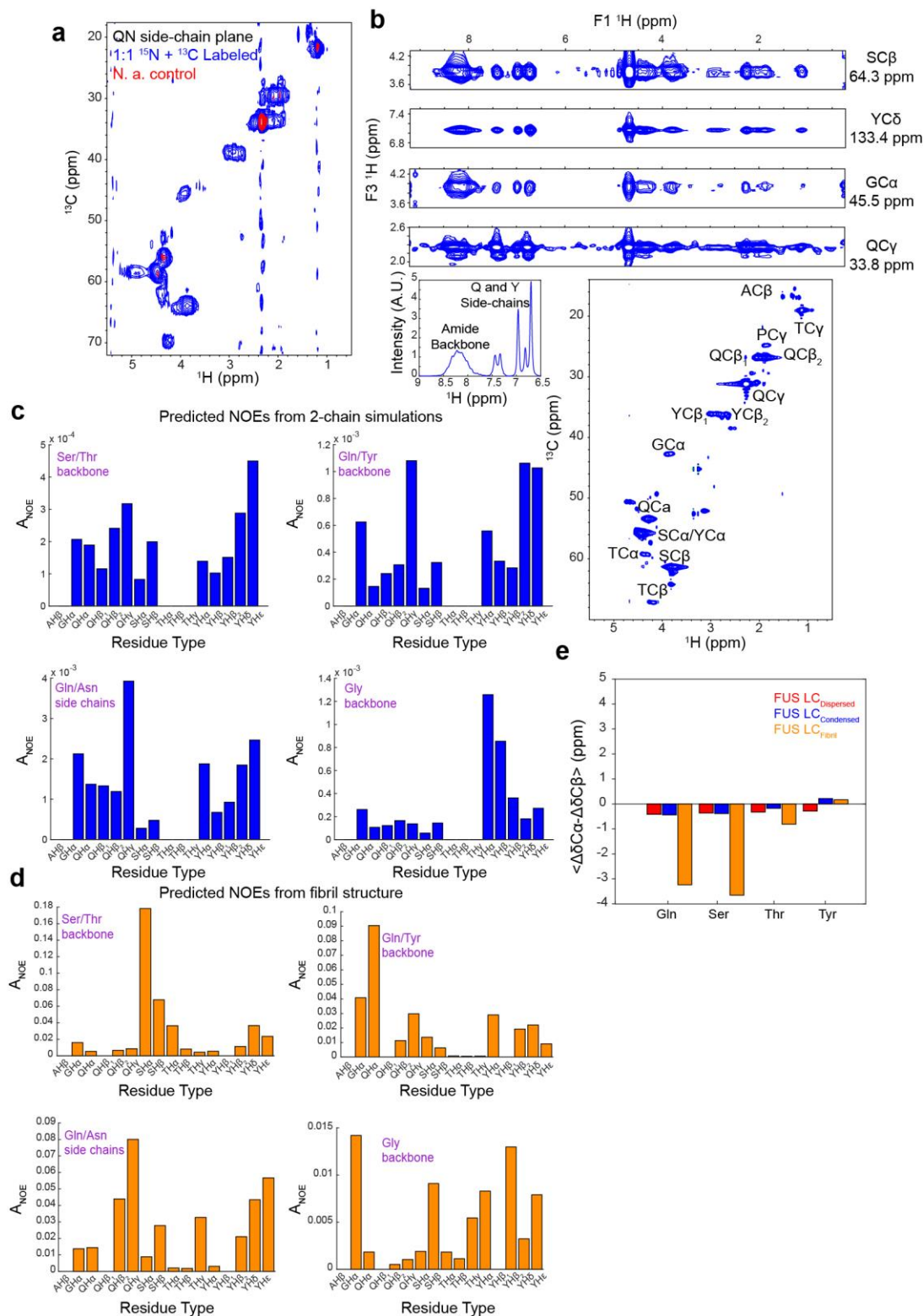
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Supplementary Figure 1

NMR characterization of conformational exchange processes within the condensed phase.

A) Chemical shift differences between the dispersed and condensed phase of FUS LC are small. B) Additional dark-state exchange saturation transfer profiles of condensed FUS LC as a function of the offsets from the ^{15}N carrier frequency and applied saturation field. The differences between profiles can be explained by the differences in value of the transverse relaxation rate constant, ^{15}N R_2 , of an individual residue. Data are plotted as mean $\pm$ s.d. (approximately the size of the circles) propagated from best-fit parameter confidence interval equal to 1 s.d. in the representative data set of two independent experiments. C) ^{15}N CPMG 1D signal intensity profiles of the amide backbone for the reference experiment ($\tau=0$, no relaxation) and as a function of refocusing pulses field ($\tau=60$ ms, V_{CPMG} as listed) indicate that there is no evidence for exchange on the μs -ms timescale for all backbone positions. D) Reduced spectral density mapping for ^{15}N relaxation parameters measured at 850 MHz (black) and 500 MHz (red) ^1H Larmor frequencies. Data represent values of $J(\omega)$ at frequencies of 0, 86.2, 740 and 0, 50.7, 435 MHz. Data are plotted as mean $\pm$ propagated best-fit parameter confidence interval equal to 1 s.d. in the representative data set of two independent experiments.

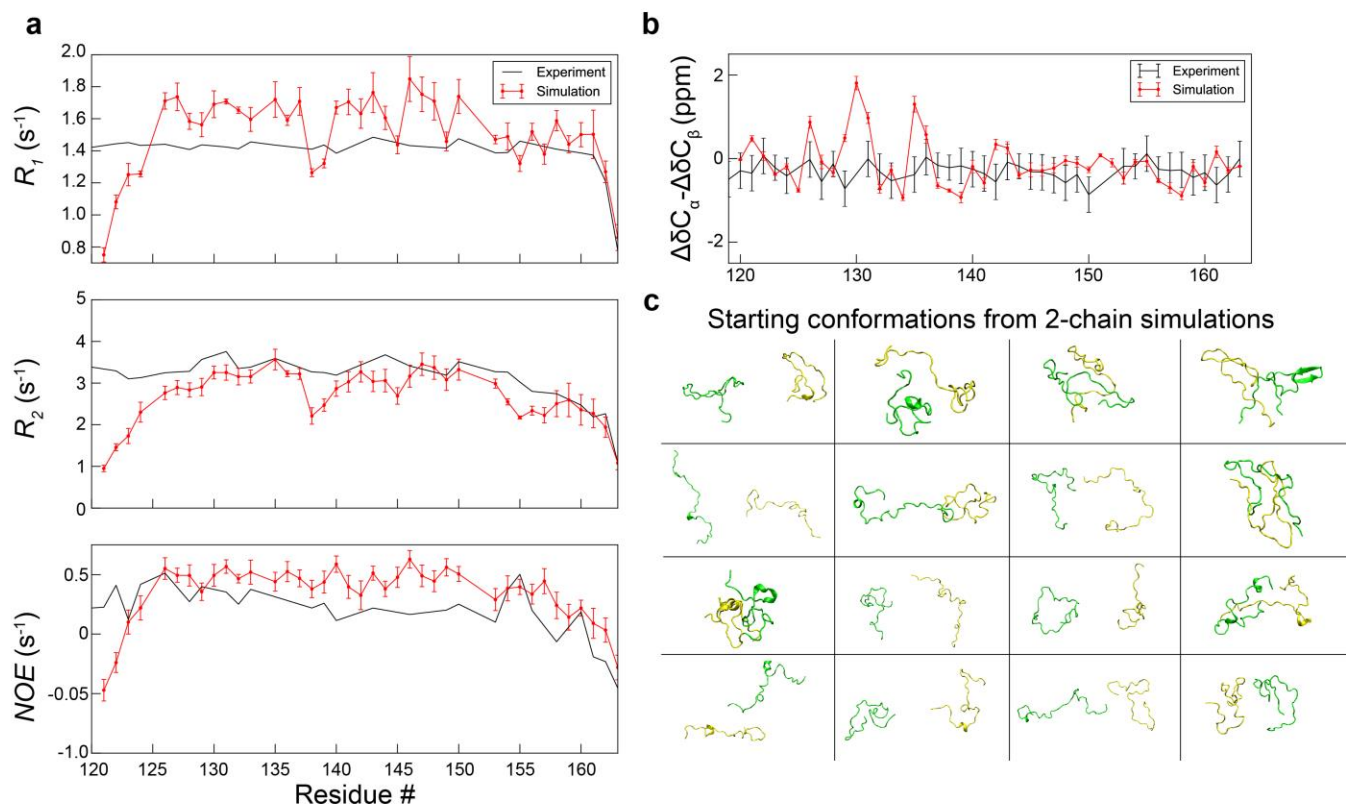


Supplementary Figure 2

Intermolecular contacts in the condensed phase.

A) Comparison of the glutamine/asparagine side chain plane of the 3D $(^1\text{H})^{15}\text{N}$ -HSQC-NOESY- $^1\text{H}^{13}\text{C}$ -

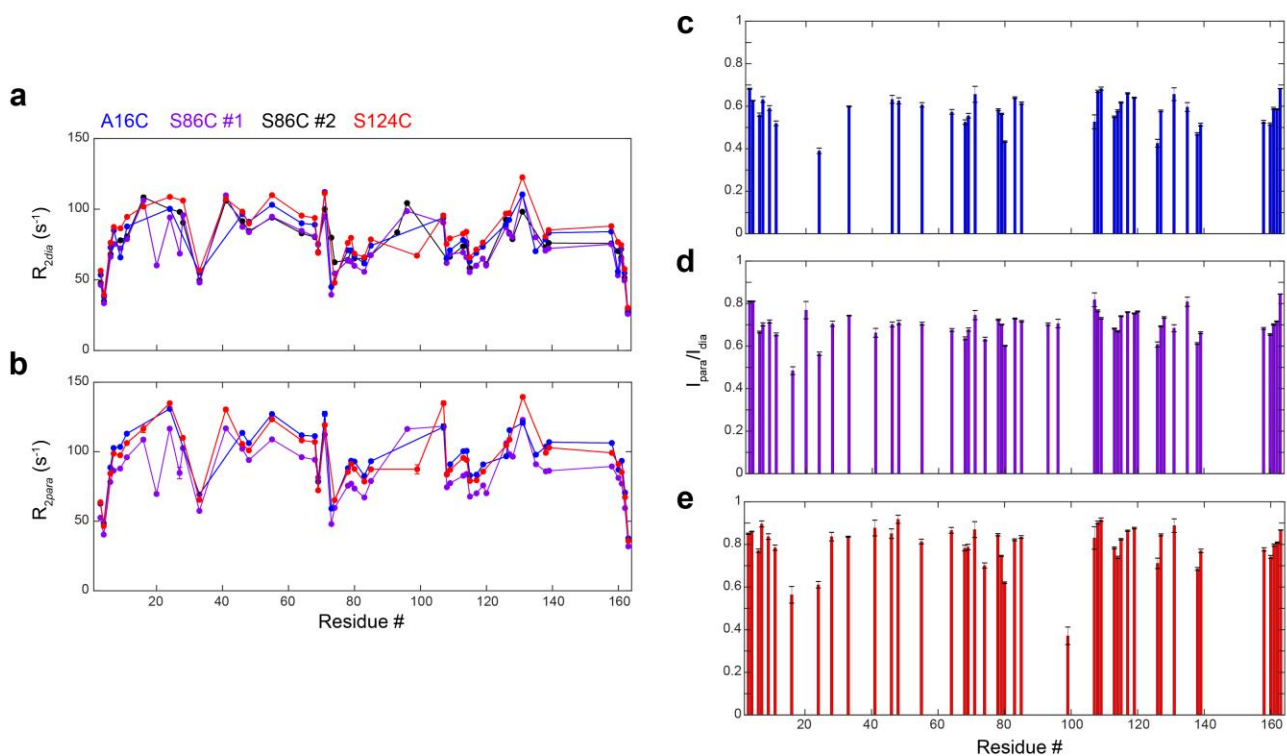
HSQC between a 1:1 $^{15}\text{N}/^{12}\text{C}$ and $^{14}\text{N}/^{13}\text{C}$ sample (blue) and a 1:1 $^{15}\text{N}/^{12}\text{C}$ + $^{14}\text{N}/^{12}\text{C}$ sample (red). Decreased intensity of the natural abundance control (red) suggests a low contribution of intramolecular artifact to the NOEs. B) $^{13}\text{C}/^{12}\text{C}$ filtered-edited NOESY-HSQC on a 1:1 $^{15}\text{N}/^{12}\text{C}$ and $^{15}\text{N}/^{13}\text{C}$ sample. This experiment shows NOEs to all the different residue types. Effective NOE amplitudes calculated from a two-chain simulation of FUS₁₂₀₋₁₆₃ for serine/threonine backbone (C), glutamine/tyrosine backbone (D), glutamine/asparagine side chains (E), and glycine backbone (F). The overall trend of interaction is similar to experimental NOE profiles, but slight differences in the amplitudes of the NOEs exist, possibly due to the absence of dynamical information in the simulation. Predicted intermolecular NOEs from the FUS 37-97 fibril structure (PDB ID: 5W3N) for serine/threonine backbone (G), glutamine/tyrosine backbone (H), glutamine/asparagine side chains (I), and glycine backbone (J). The overall pattern of NOEs in the fibril structure differs from the condensed phase. The difference between the observed and predicted random coil C_α and C_β chemical shift values for condensed FUS LC and fibrillar FUS 37-97. Condensed FUS LC values are between -1 and 1, indicating that disorder is preserved in the condensed phase, while chemical shifts in the fibrillar form are closer to values associated with β -sheet structure.



Supplementary Figure 3

Validation of all-atom simulations of FUS₁₂₀₋₁₆₃.

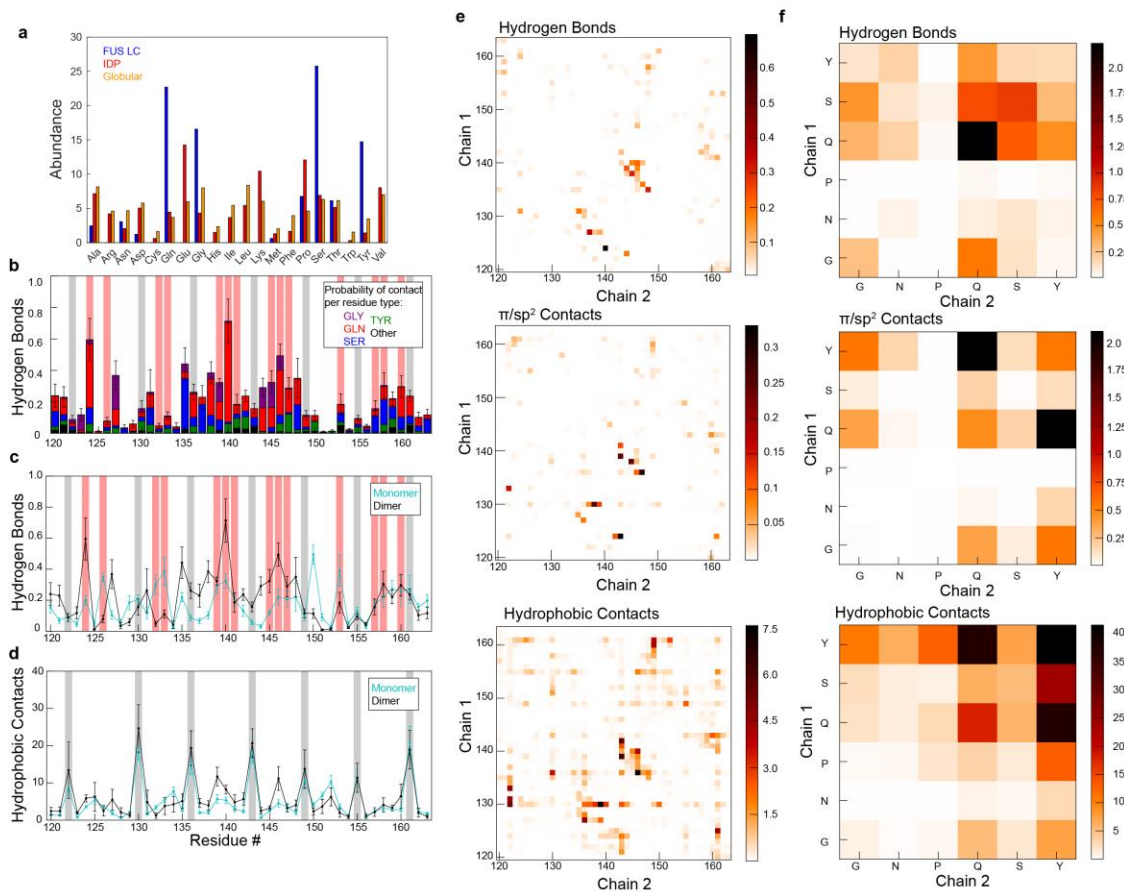
A) Comparison of experimental dispersed FUS LC and simulated NMR parameters. The simulated dataset is qualitatively similar to the experimental NMR observables, indicating that the all-atom simulation is capturing the molecular details of the 120-163 region. Differences in the N-terminal region between the simulation and experimental dataset arise from the use of a short 44 residue fragment in the simulation. As a result, the N-terminus of the simulated fragment is free to move, while the same region in the experimental dataset is constrained due to being in the middle of the chain. Simulation data are plotted as mean $\pm$ s.e.m of $n=5$ equal divisions of the total data. B) Comparison of secondary shifts between experimental dispersed FUS LC and simulated datasets. Simulation data are plotted as mean $\pm$ s.e.m of $n=5$ equal divisions of the total data. Experimental data are plotted as mean $\pm$ estimated uncertainty in peak position. C) Starting configurations of the two-chain simulation of the 120-163 region. This diverse array of configurations was used to avoid bias in the resulting conformations.



Supplementary Figure 4

Paramagnetic relaxation enhancement within the condensed phase.

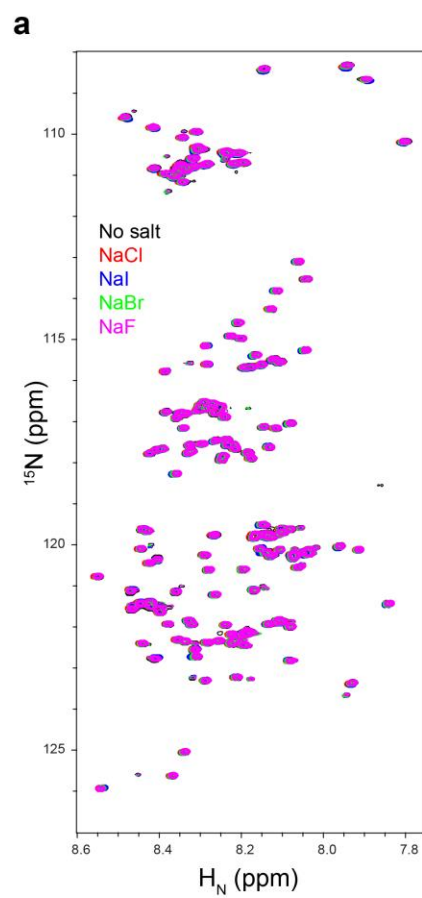
A) ^1H transverse relaxation rates of condensed FUS LC containing ~1% Ac-MTSL labeled A16C, S86C, and S142C. Labeling at position S86C was repeated (S86C #2) and shows a similar relaxation rate as the original experiment (S86C #1). Data are plotted as mean $\pm$ best-fit parameter confidence interval equal to 1 s.d. in one data set, with two data sets plotted for S86C. B) ^1H transverse relaxation rates of condensed FUS LC containing ~1% MTSL labeled A16C, S86C, and S142C. C-E) Signal intensity ratios of the condensed FUS LC diamagnetic MTSL and paramagnetic MTSL. The global decrease in signal intensity indicates labeling with paramagnetic MTSL. Data are plotted as peak height ratio mean $\pm$ propagated uncertainty equal to r.m.s.d. of baseline noise in the representative data set of two experiments.



Supplementary Figure 5

Hydrogen bonding, π/sp^2 and hydrophobic interactions calculated from two-chain simulations of FUS₁₂₀₋₁₆₃.

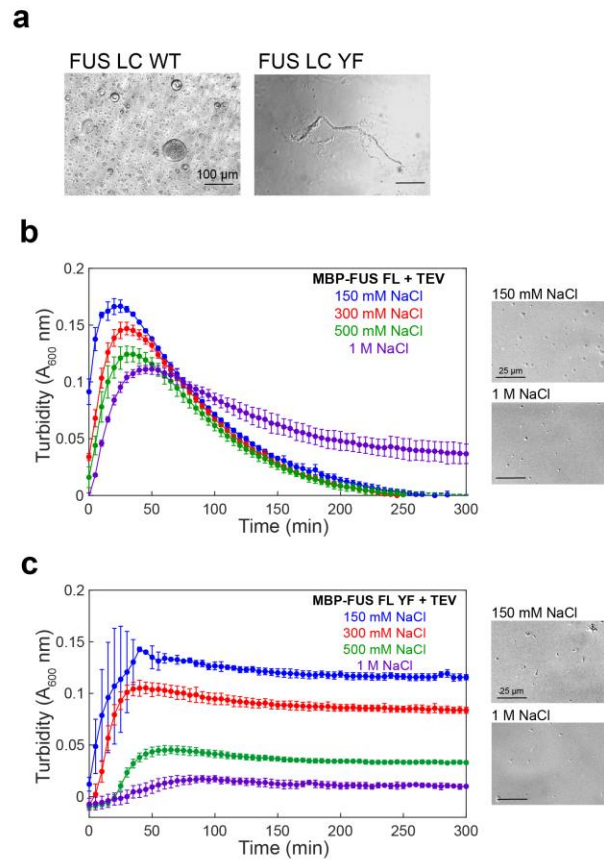
A) Comparison of the amino acid frequencies found within the low complexity domain of FUS and average disordered and globular proteins²⁴. B) Per-residue hydrogen bonds between each residue of the sequence and different residue types in the other chain. Error bars represent the standard error of the mean for five equal blocks. Tyrosine and glutamine residues are highlighted with grey and red bars respectively. C) Average number of hydrogen bonds formed by each residue of the FUS₁₂₀₋₁₆₃ sequence in simulation. For the two-chain simulation (dimer) case, only intermolecular hydrogen bonds are considered, and for the single chain simulation (monomer), neighbors of sequence separation of 5 residues or fewer are not counted. D) Average number of hydrophobic contacts formed by each residue of the FUS₁₂₀₋₁₆₃ sequence in simulation. For the two-chain (dimer) case, only intermolecular hydrogen bonds are considered, and for single chain (monomer), neighbors of sequence separation of 5 residues or fewer are not counted. E) Average number of intermolecular hydrogen bonds, π/sp^2 or hydrophobic contacts between all residues of chain 1 with each residue of chain 2. F) Average total number of hydrogen bonds, π/sp^2 or hydrophobic contacts between residues of type X in chain 1 with type Y in chain 2. Simulation data are plotted as mean $\pm$ s.e.m of $n=5$ equal divisions of the total data.



Supplementary Figure 6

Hofmeister salts do not change the structure of dispersed FUS LC.

A) ^1H - ^{15}N HSQC spectral overlay of dispersed FUS LC in the presence of the Hofmeister salts.



Supplementary Figure 7

Substitution of tyrosines with phenylalanine in FUS LC leads to aggregation.

A) DIC microscopy of a FUS LC variant in which all 24 tyrosines are mutated to phenylalanine (FUS LC YF). This variant forms aggregates instead of spherical droplets. Phase separation as monitored by turbidity of MBP-FUS FL WT (B) or YF (C). The increased turbidity and light scattering and long incubation time in the YF variant indicates the presence of stable aggregates. Data are plotted as mean $\pm$ s.d. of $n=3$ technical replicates.

Supplementary Information Table 1. FUS LC constructs used.

FUS LC WT	GMASNDYTQQATQSYGAYPTQPGQGYSSQSSQPYGQQSYSGYSQSTDTSQGYGQSSYSSYG QSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQS SSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNS
FUS LC QQ4xSS #1	GMASNDYTQQATQSYGAYPTQPGQGYSSSSSQPYGQQSYSGYSQSTDTSQGYGQSSYSSYG QSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGSSSSYPGYGQQPAPSSTSGSYGSSSQS SSYGQPQSGSYSSSPSYGGQQQSYGQQQSYNPPQGYGSSNQYNS
FUS LC QQ4xSS #2	GMASNDYTSSATQSYGAYPTQPGQGYSSQSSQPYGQQSYSGYSQSTDTSQGYGQSSYSSYG SSSNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGSSPAPSSTSGSYGSSSQS SSYGQPQSGSYSQQPSYGGSSQSYGQQQSYNPPQGYGQQNQYNS
FUS LC S12xQ	GMASNDYTQQATQSYGAYPTQPGQGYSSQSSQPYGQQSYSGYSQQTDTQGYGQQSYSSYG QSQNTGYGTQQTPQGYGSTGGYGSQQSSQQSYGQQQSYPGYGQQPAPSQTSGSYGSSQQQS SYGQPQQGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNS
FUS LC Y24xF	GMASNDFTQQATQSFGAFPTQPGQGFSQSSQPFQQQSFGFSQSTDTSFGFGQSSFSSFG QSQNTGFGTQSTPQGFGSTGGFGSSQSSQSSFQQQSFPFGGQQPAPSSTSGSFGSSSQS SSFQQPQSGSFSQQPSFGGQQQSFQQQQSFNPPQGFQQQNQFNS