Supplementary figures and tables

Figure S1

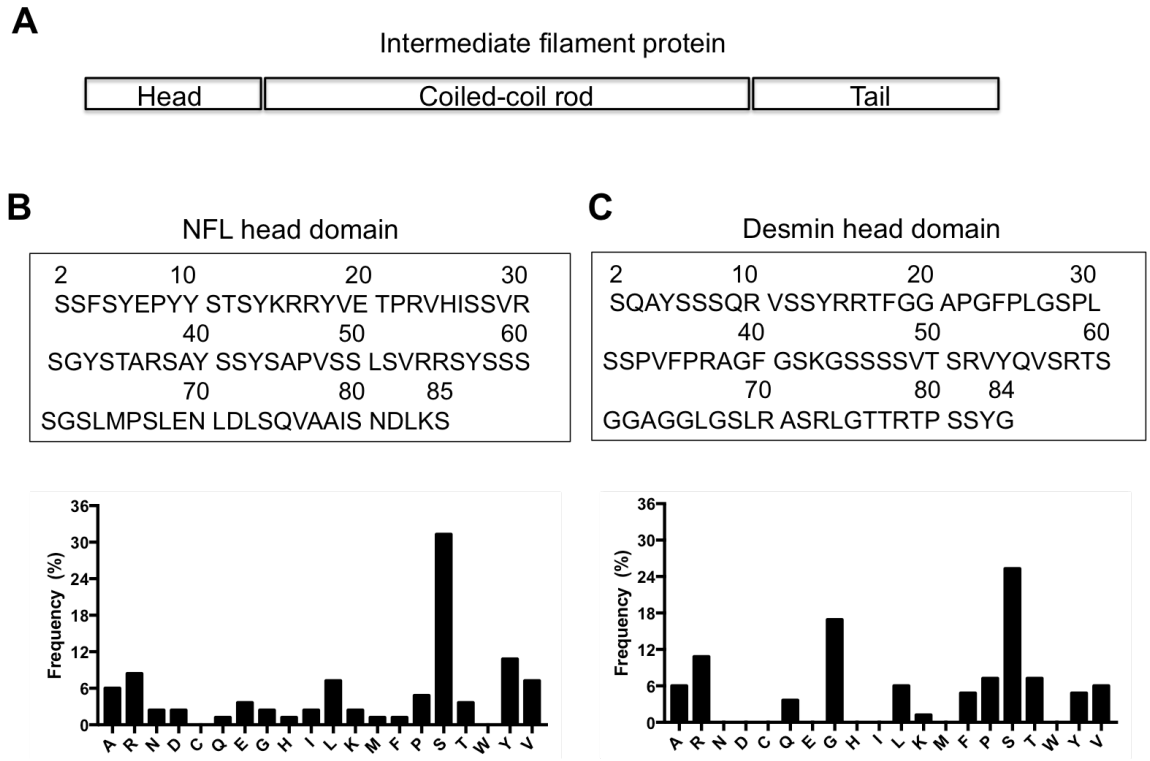

Figure S1. Amino acid sequences and composition of NFL and desmin head domains. (**A**) Schematic diagram of intermediate filament domain architecture. **(B)** Amino acid sequence (above) and composition (below) of NFL head domain. **(C)** Amino acid sequence (above) and composition (below) of desmin head domain.

Figure S2

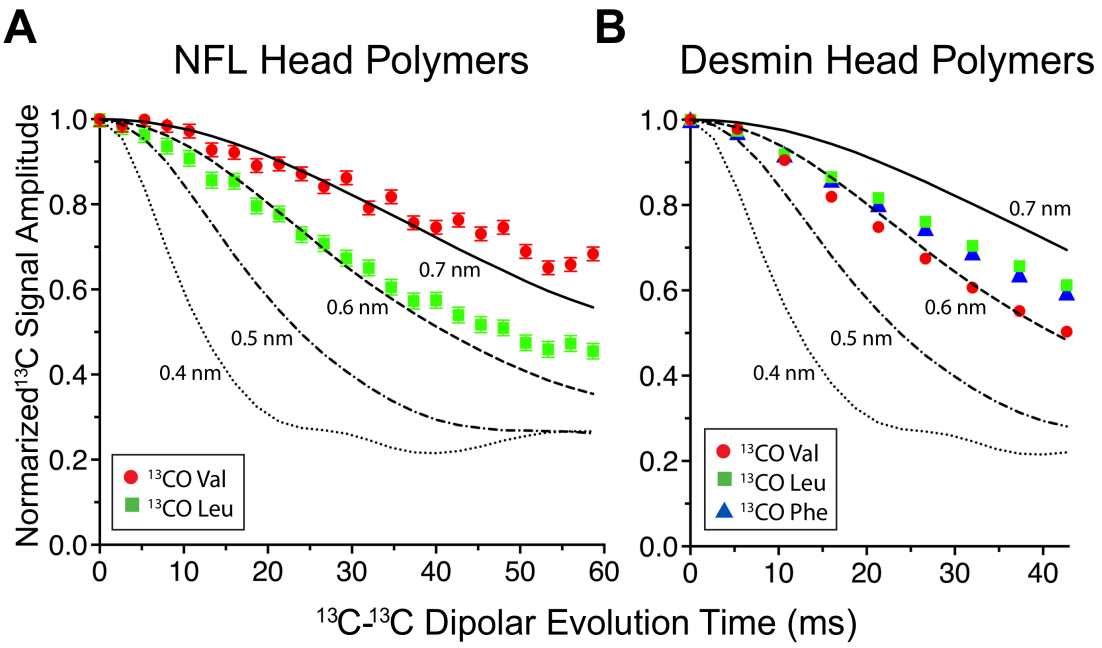

Figure S2. Quantitative intermolecular distance measurements by the ^{13}C-^{13}C dipole-dipole recoupling technique. (A) ^{13}C-^{13}C PITHIRDS-CT dipolar recoupling data for NFL head domain-only polymers that were ^{13}C-labeled only at backbone carbonyl sites of all Val or Leu residues within the head domain. Normalized peak intensities at different dipolar evolution times are plotted. **(B)** ^{13}C-^{13}C PITHIRDS-CT dipolar recoupling data for desmin head domain-only polymers that were ^{13}C-labeled only at backbone carbonyl sites of all Val, Leu or Phe residues within the head domain. Normalized peak intensities at different dipolar evolution time are plotted. Lines represent simulated data for linear chains of ^{13}C label with the indicated interatomic spacings.

Figure S3

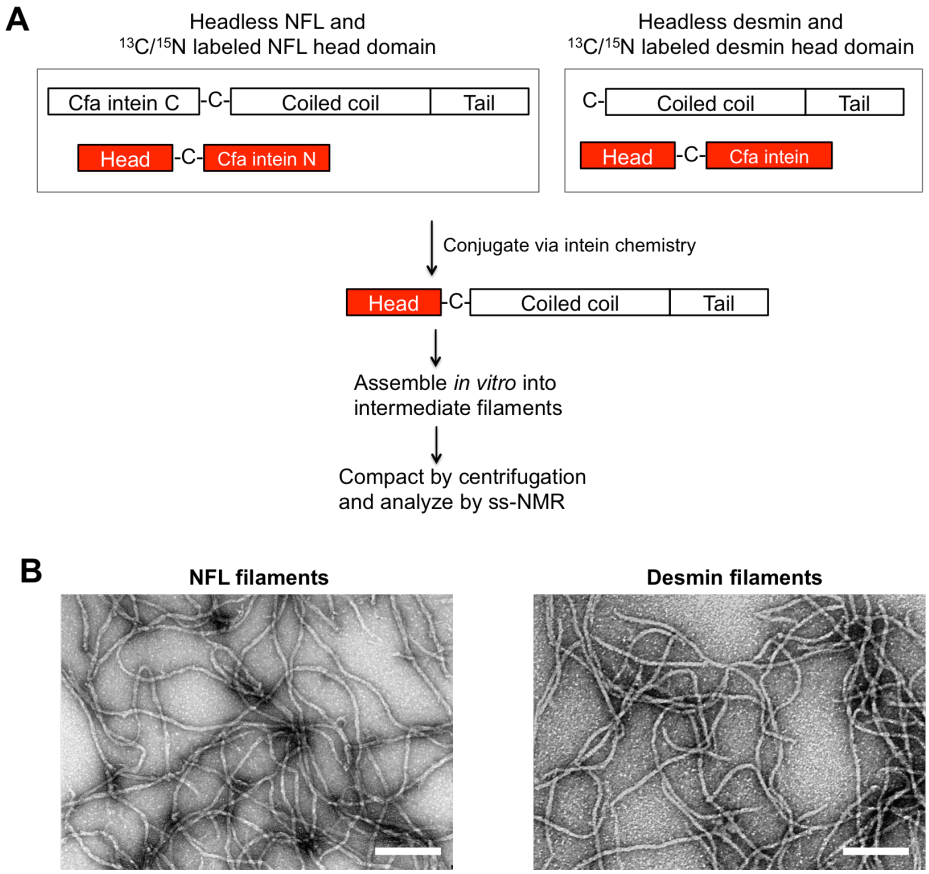

Figure S3. Segmentally labeling of NFL and desmin full-length proteins with isotopically labeled head domains. (A) Scheme of intein chemistry used for segmentally labeling of NFL and desmin full-length proteins with isotopically labeled head domains. Split intein chemistry was applied for NFL head domain labeling (left panel). Unlabeled headless NFL was N-terminally fused with C-terminal half of Cfa intein (Cfa intein C). Isotopically labeled NFL head domain was C-terminally fused with N-terminal half of Cfa intein (Cfa intein N). Unlabeled, headless NFL derivative, and isotope-labeled head domain derivative were expressed as N-terminal GFP tagged proteins and digested by caspase 3 at designed site to remove the GFP tag (Experimental Procedures) and conjugated via split intein reaction. An intact intein reaction was applied for segmental labeling of the desmin head domain (right panel). Isotopically- labeled desmin head domain was C-terminally fused to an intact Cfa intein. Both isotopically-labeled desmin head domain and unlabeled headless desmin were expressed as N-terminal His tagged proteins and digested with caspase 3 to remove the His tag (Experimental Procedures). Isotopically-labeled desmin head domain and unlabeled headless desmin were conjugated via native chemical ligation reaction. Full-length products of NFL and desmin were assembled into filaments and compacted by ultracentrifugation for analysis by ss-NMR spectroscopy. **(B)** Intermediate filaments assembled with segmentally labeled NFL (left) or desmin (right) full-length proteins were imaged by transmission electron microscopy via negative staining. Scale bars = 200 nm.

Figure S4

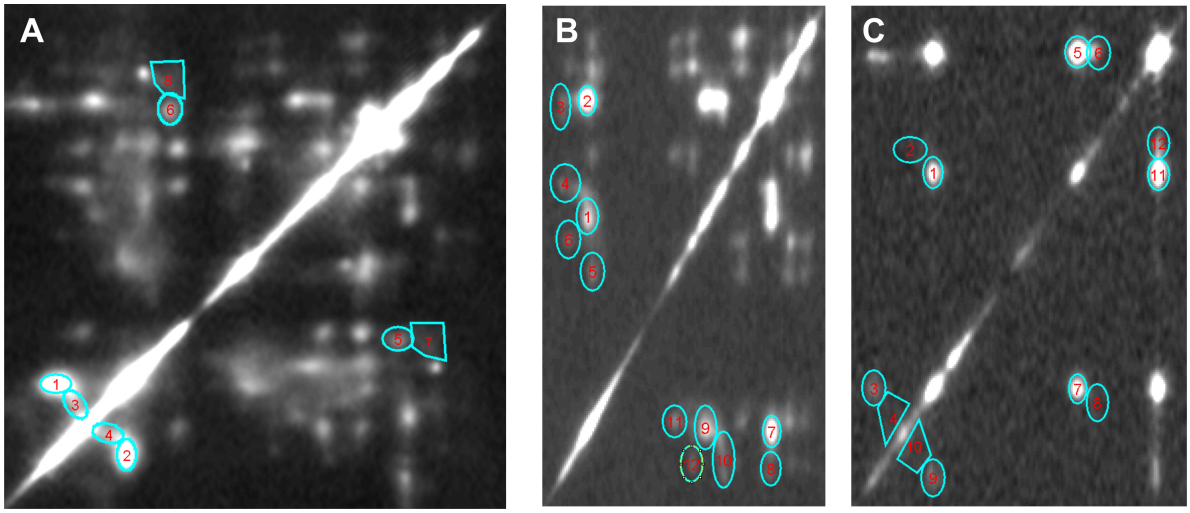

Figure S4. Estimation of residue-specific secondary structure from 2D crosspeak volumes. (A) Grey-scale representation of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of uniformly ^{15}N,^{13}C-labeled NFL head domain polymers. Volumes within regions 1 and 2 or 3 and 4 were used to estimate populations of Ser residues with β-strand or non-β-strand secondary structure, respectively. Volumes within regions 5 and 6 or 7 and 8 were used to estimate populations of Ala residues with β-strand or non-β-strand secondary structure, respectively. These regions contain C_α/C_β crosspeak signals, with conformation-dependent ^{13}C NMR chemical shifts. (B) Grey-scale representation of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of Val,Ile-labeled NFL head domain-only polymers. Regions 1 and 9 or 4 and 10 contain C_α/C_β crosspeaks of Val residues with β-strand or non-β-strand secondary structure, respectively. Regions 2 and 7 or 3 and 8 contain C_α/C_γ crosspeaks of Val residues with β-strand or non-β-strand secondary structure, respectively. Regions 5 and 11 or 6 and 12 contain C_α/C_β crosspeaks of Ile residues with β-strand or non-β-strand secondary structure, respectively. (C) Grey-scale representation of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of Val,Thr-labeled desmin head domain-only polymers. Regions 1 and 7 or 2 and 8 contain C_α/C_β crosspeaks of Val residues with β-strand or non-β-strand secondary structure, respectively. Regions 5 and 11 or 6 and 12 contain C_β/C_γ crosspeaks of Val residues with β-strand or non-β-strand secondary structure, respectively. Regions 3 and 9 or 4 and 10 contain C_α/C_β crosspeaks of Thr residues with β-strand or non-β-strand secondary structure, respectively.

Figure S5

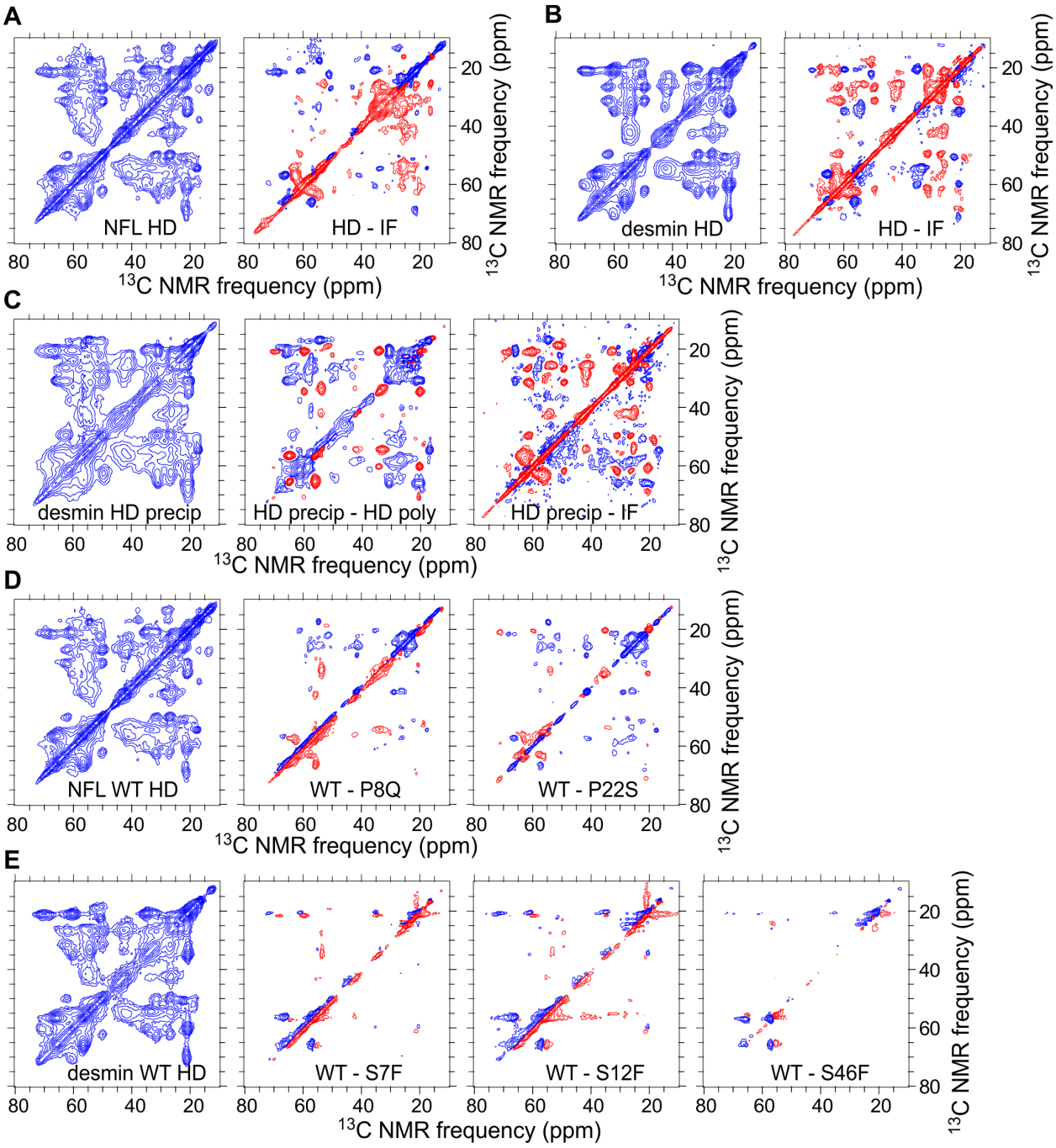

Figure S5. Assessments of structural similarities from 2D difference spectra. (A) Comparison of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of uniformly ^{15}N,^{13}C-labeled NFL head domain polymers (left) with the difference between this spectrum and the corresponding spectrum of segmentally labeled NFL IFs (right). (B) Comparison of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of uniformly ^{15}N,^{13}C-labeled desmin head domain polymers (left) with the difference between this spectrum and the corresponding spectrum of segmentally labeled desmin IFs (right). (C) 2D ^{13}C-^{13}C ss-NMR spectrum of the uniformly ^{15}N,^{13}C-labeled desmin head domain in an amorphous state,

prepared by dissolution in trifluoroacetic acid followed by precipitation in cold ether (left). Differences between this spectrum and the 2D spectra of desmin head domain polymers (middle) and segmentally labeled desmin IFs (right) are also shown. (D) Comparison of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of uniformly ^{15}N,^{13}C-labeled wild-type NFL head domain polymers with the difference between this spectrum and the corresponding spectra of P8Q and P22S mutants. (E) Comparison of the aliphatic region of the 2D ^{13}C-^{13}C ss-NMR spectrum of uniformly ^{15}N,^{13}C-labeled wild-type desmin head domain polymers with the difference between this spectrum and the corresponding spectra of S7F, S12F, and S46F mutants. In all cases, positive contours are blue, negative contours are red, and contour levels increase by successive factors of 1.5. Before calculating difference spectra, 2D spectra were rescaled to have approximately the same crosspeak volumes. 2D spectra within each row are plotted with identical intensity scales.

Figure S6

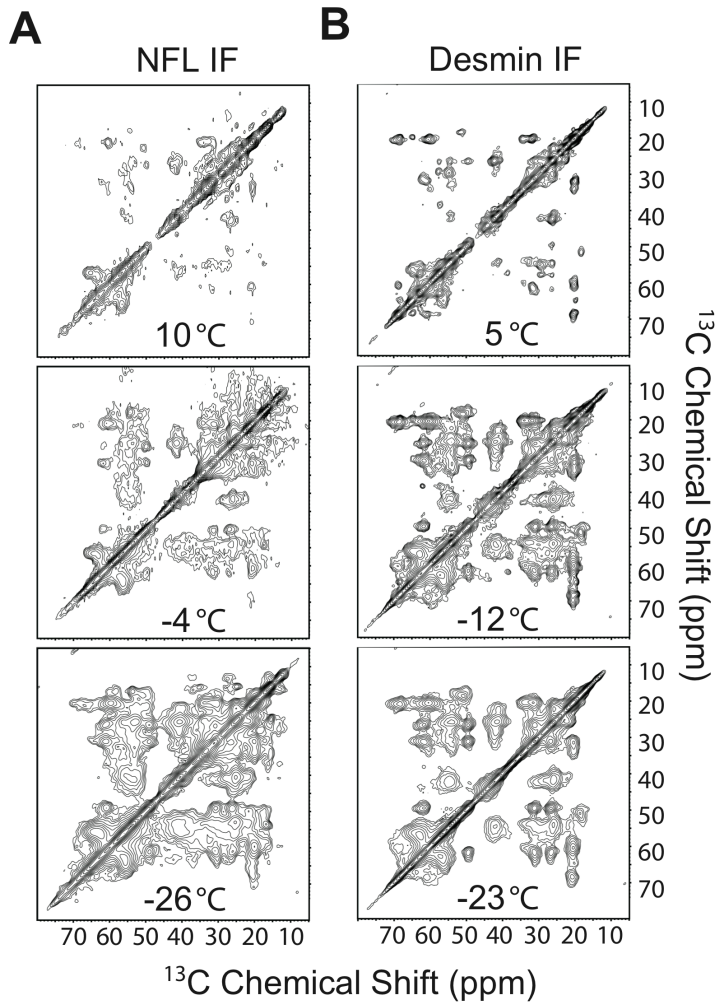

Figure S6. Temperature dependence of two dimensional ^{13}C-^{13}C ss-NMR spectra of segmentally-labeled intermediate filaments. (A) 2D ^{13}C-^{13}C spectra of the segmentally ^{15}N-^{13}C-labeled NFL head domain within assembled intermediate filaments recorded at indicated sample temperatures. **(B)** 2D ^{13}C-^{13}C spectra of the segmentally ^{15}N-^{13}C-labeled desmin head domain within assembled intermediate filaments recorded at indicated sample temperatures. Contour levels increase by factors of 1.4.

Figure S7

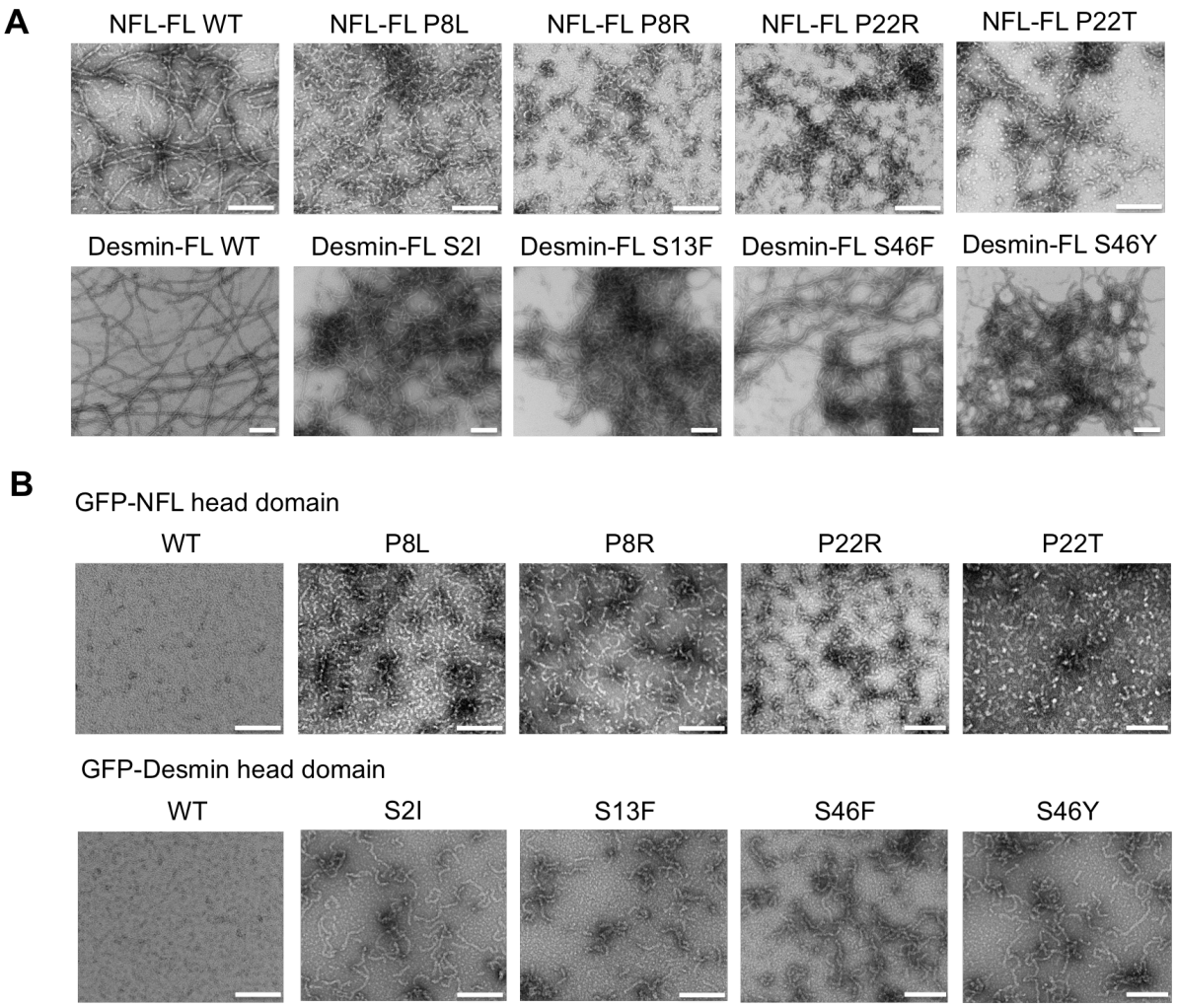

Figure S7. Assays of native and disease-causing mutations in NFL and desmin head domains for assembly into intermediate filaments and head domain-alone polymers. (A) NFL full-length (NFL-FL) wild type (WT) protein and indicated head domain mutants were subjected to conditions optimal for intermediate filament assembly (upper panel). All NFL mutants failed to form properly organized intermediate filaments. Desmin full-length (desmin-FL) wild type (WT) and indicated head domain mutants were subjected to conditions optimal for intermediate filament assembly. All desmin mutants formed tangled, aggregated intermediate filaments. (**B**) NFL head domain-only GFP fusions were prepared for the native protein (WT) and indicated head domain mutants. No evidence of polymer formation was observed for the native NFL head domain in 1 M urea. Under the same conditions of incubation, all four NFL head domain mutants formed distinct polymers (upper panel). Desmin head domain-only GFP fusions were prepared for the native protein (WT) and indicated head domain mutants. No evidence of polymer formation was observed for the native desmin head domain in 0.5 M urea. Under the same conditions of incubation, all four desmin head domain mutants formed distinct polymers (lower panel). All scale bars = 200 nm.

Table S1. List of top 20 mouse brain proteins trapped by hydrogel droplets formed from NFL head domain and low complexity domains of FUS, Nup54, Ataxin-2, Synaptophysin, and Lamin.

	NFL	FUS	Nup54	Ataxin-2	Synaptophysin	Lamin
1	NFL_MOUSE Neurofilament light polypeptide	A3KGU7_MOUSE Spectrin alpha chain, non-erythrocytic 1	A3KGU7_MOUSE Spectrin alpha chain, non-erythrocytic 1	A3KGU7_MOUSE Spectrin alpha chain, non-erythrocytic 1	A3KGU7_MOUSE Spectrin alpha chain, non-erythrocytic 1	A3KGU7_MOUSE Spectrin alpha chain, non-erythrocytic 1
2	A3KGU7_MOUSE Spectrin alpha chain, non-erythrocytic 1	DYN1_MOUSE Dynamin-1	DYHC1_MOUSE Cytoplasmic dynein 1 heavy chain 1	TBB4B_MOUSE Tubulin beta-4B chain	MTAP2_MOUSE Microtubule-associated protein 2	MTAP2_MOUSE Microtubule-associated protein 2
3	SPTB2_MOUSE Spectrin beta chain, non-erythrocytic 1	SYN1_MOUSE Synapsin-1	MTAP2_MOUSE Microtubule-associated protein 2	AT1A3_MOUSE Sodium/potassium-transporting ATPase subunit alpha-3	DYHC1_MOUSE Cytoplasmic dynein 1 heavy chain 1	DYHC1_MOUSE Cytoplasmic dynein 1 heavy chain 1
4	CLH1_MOUSE Clathrin heavy chain 1	MTAP2_MOUSE Microtubule-associated protein 2	SPTB2_MOUSE Spectrin beta chain, non-erythrocytic 1	ENOA_MOUSE Alpha-enolase	PPID_MOUSE Peptidyl-prolyl cis-trans isomerase D	SPTB2_MOUSE Spectrin beta chain, non-erythrocytic 1
5	MTAP2_MOUSE Microtubule-associated protein 2	DYHC1_MOUSE Cytoplasmic dynein 1 heavy chain 1	AT1A3_MOUSE Sodium/potassium-transporting ATPase subunit alpha-3	DYN1_MOUSE Dynamin-1	SPTB2_MOUSE Spectrin beta chain, non-erythrocytic 1	CLH1_MOUSE Clathrin heavy chain 1
6	GCSP_MOUSE Glycine dehydrogenase (decarboxylating), mitochondrial	SPTB2_MOUSE Spectrin beta chain, non-erythrocytic 1	CLH1_MOUSE Clathrin heavy chain 1	DPYL2_MOUSE Dihydropyrimidinase-related protein 2	DYN1_MOUSE Dynamin-1	AT1A3_MOUSE Sodium/potassium-transporting ATPase subunit alpha-3
7	TBA4A_MOUSE Tubulin alpha-4A chain	MAP1B_MOUSE Microtubule-associated protein 1B	MYO5A_MOUSE Unconventional myosin-Va	SPTB2_MOUSE Spectrin beta chain, non-erythrocytic 1	CLH1_MOUSE Clathrin heavy chain 1	MAP1B_MOUSE Microtubule-associated protein 1B
8	DPYL2_MOUSE Dihydropyrimidinase-related protein 2	SYNJ1_MOUSE Synaptojanin-1	MYH10_MOUSE Myosin-10	DYHC1_MOUSE Cytoplasmic dynein 1 heavy chain 1	DPYL2_MOUSE Dihydropyrimidinase-related protein 2	MAP1A_MOUSE Microtubule-associated protein 1A
9	DYHC1_MOUSE Cytoplasmic dynein 1 heavy chain 1	SYUA_MOUSE Alpha-synuclein	DYN1_MOUSE Dynamin-1	G3P_MOUSE Glyceraldehyde-3-phosphate dehydrogenase	ENOA_MOUSE Alpha-enolase	DYN1_MOUSE Dynamin-1
10	MAP1A_MOUSE Microtubule-associated protein 1A	CLH1_MOUSE Clathrin heavy chain 1	PLEC_MOUSE Plectin	CLH1_MOUSE Clathrin heavy chain 1	MAP1B_MOUSE Microtubule-associated protein 1B	HS90A_MOUSE Heat shock protein HSP 90-alpha
11	AT1A3_MOUSE Sodium/potassium-transporting ATPase subunit alpha-3	PLEC_MOUSE Plectin	MAP1B_MOUSE Microtubule-associated protein 1B	PPIA_MOUSE Peptidyl-prolyl cis-trans isomerase A	TBA4A_MOUSE Tubulin alpha-4A chain	TBA4A_MOUSE Tubulin alpha-4A chain
12	DDX17_MOUSE Probable ATP-dependent RNA helicase DDX17	AT1A3_MOUSE Sodium/potassium-transporting ATPase subunit alpha-3	TBB4B_MOUSE Tubulin beta-4B chain	SYUA_MOUSE Alpha-synuclein	ALBU_MOUSE Serum albumin	ALBU_MOUSE Serum albumin
13	PLEC_MOUSE Plectin	MAP1A_MOUSE Microtubule-associated protein 1A	MAP1A_MOUSE Microtubule-associated protein 1A	NSF_MOUSE Vesicle-fusing ATPase	AT1A3_MOUSE Sodium/potassium-transporting ATPase subunit alpha-3	DPYL2_MOUSE Dihydropyrimidinase-related protein 2
14	DYN1_MOUSE Dynamin-1	DPYL2_MOUSE Dihydropyrimidinase-related protein 2	DPYL2_MOUSE Dihydropyrimidinase-related protein 2	MTAP2_MOUSE Microtubule-associated protein 2	MAP1A_MOUSE Microtubule-associated protein 1A	ENOA_MOUSE Alpha-enolase
15	MYH10_MOUSE Myosin-10	PPIA_MOUSE Peptidyl-prolyl cis-trans isomerase A	TBA4A_MOUSE Tubulin alpha-4A chain	TBA4A_MOUSE Tubulin alpha-4A chain	HS90A_MOUSE Heat shock protein HSP 90-alpha	HSP7C_MOUSE Heat shock cognate 71 kDa protein
16	ENOA_MOUSE Alpha-enolase	ACTB_MOUSE Actin, cytoplasmic 1	NCAM1_MOUSE Neural cell adhesion molecule 1	MYH10_MOUSE Myosin-10	TBB4B_MOUSE Tubulin beta-4B chain	Q3U2G2_MOUSE Heat shock 70 kDa protein 4
17	MAP1B_MOUSE Microtubule-associated protein 1B	ENOA_MOUSE Alpha-enolase	SYUA_MOUSE Alpha-synuclein	CH60_MOUSE 60 kDa heat shock protein, mitochondrial	SYN1_MOUSE Synapsin-1	PLEC_MOUSE Plectin
18	NFM_MOUSE Neurofilament medium polypeptide	Q3U4S0_MOUSE Pantothenate kinase 2 (Hallervorden-Spatz syndrome)	HS90A_MOUSE Heat shock protein HSP 90-alpha	ACTB_MOUSE Actin, cytoplasmic 1	COF1_MOUSE Cofilin-1	MYH10_MOUSE Myosin-10
19	Q68FG2_MOUSE Protein Sptbn2	VIME_MOUSE Vimentin	SYUA_MOUSE Alpha-synuclein	PGK1_MOUSE Phosphoglycerate kinase 1	PLEC_MOUSE Plectin	SYN1_MOUSE Synapsin-1
20	NSF_MOUSE Vesicle-fusing ATPase	ENOA_MOUSE Alpha-enolase	HS90A_MOUSE Heat shock protein HSP 90-alpha	PLEC_MOUSE Plectin	NSF_MOUSE Vesicle-fusing ATPase	STXB1_MOUSE Isoform 2 of Syntaxin-binding protein 1

Table S2. NMR experiments and parameter values for the data presented in this paper.

Sample	Spectrum	NMR Parameters*	Exp. Time	Gaussian apodization
Uniform $^{13}C/^{15}N$ Desmin head Domain-Only Polymers (WT and mutants)	2D ^{13}C-^{13}C CP-DARR	B_0=14.1 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4.2 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=58 kHz; ν_{CP13C}=47 kHz; τ_{CP} =1.5 ms; ν_{dec}=68 kHz; τ_{DARR}= 25 ms; Δt_1=29.2 µs; τ_{t1}=5.84 ms; τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=64; $\tau_{recycle}$=1.5 s; T=13 ºC;	36 hr	t_1: 80 Hz t_2: 80 Hz
Uniform $^{13}C/^{15}N$ NFL head Domain-Only Polymers (WT and mutants)	2D ^{13}C-^{13}C CP-DARR	B_0=14.1 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4.86 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=53 kHz; ν_{CP13C}=39 kHz; τ_{CP} =1.5 ms; ν_{dec}=75 kHz; τ_{DARR}= 25 ms; Δt_1=26.64 µs; τ_{t1}=3.996 ms; τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=88; $\tau_{recycle}$=1.0 s; T=13 ºC;	36 hr	t_1: 80 Hz t_2: 80 Hz
Segmental $^{13}C/^{15}N$ NFL Intermediate Filaments	1D ^{13}C CP	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4 µs; ν_{CP1H}=67 kHz; ν_{CP13C}=48 kHz; τ_{CP} =1.5 ms; ν_{dec}=85 kHz; τ_{acq}=15.36 ms; τ_{dwell}=15 µs; n_{scan}=1024; $\tau_{recycle}$=1 s; T={35, 13, 0, -13, -26} ºC;	0.3 hr	t_1: 150 Hz
Segmental $^{13}C/^{15}N$ NFL Intermediate Filaments	2D ^{13}C-^{13}C CP-DARR	B_0=14.1 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4.86 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=53 kHz; ν_{CP13C}=40 kHz; τ_{CP} =0.7 ms; ν_{dec}=75 kHz; τ_{DARR}= 25 ms; Δt_1=26.64 µs; τ_{t1}=2.664 ms; τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=88; $\tau_{recycle}$=1.5 s; T={10, -4, -26} ºC;	39 hr	t_1: 80 Hz t_2: 80 Hz
Segmental $^{13}C/^{15}N$ NFL Intermediate Filaments	1D ^{13}C INEPT	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4 µs; $\tau_{\pi/213C}$=5 µs; τ_J=1.06 ms; ν_{dec}=47 kHz; τ_{acq}=15.36 ms; τ_{dwell}=15 µs; n_{scan}=2048; $\tau_{recycle}$=1.5 s; T={35, 13, 0, -13, -26} ºC;	0.6 hr	t_1: 150 Hz
Segmental $^{13}C/^{15}N$ Desmin Intermediate Filaments	1D ^{13}C CP	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4.0 µs; ν_{CP1H}=58 kHz; ν_{CP13C}=45 kHz; τ_{CP} =1.5 ms; ν_{dec}=85 kHz; τ_{acq}=15.36 ms; τ_{dwell}=15 µs; n_{scan}=128; $\tau_{recycle}$=1.5 s; T={5, 1, -7, -12, -23} ºC;	0.06 hr	t_1: 150 Hz
Segmental $^{13}C/^{15}N$ Desmin Intermediate Filaments	2D ^{13}C-^{13}C CP-DARR	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=58 kHz; ν_{CP13C}=45 kHz; τ_{CP} =1.5 ms; ν_{dec}=85 kHz; τ_{DARR}= 50 ms; Δt_1=22.4 µs; τ_{t1}=3.92 ms;	75 hr	t_1: 80 Hz t_2: 80 Hz

		τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=64; $\tau_{recycle}$=1.5 s; T={5, -12, -23} ºC;		
Segmental $^{13}C/^{15}N$ Desmin Intermediate Filaments	1D ^{13}C INEPT	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$ = 4 µs; $\tau_{\pi/213C}$=5 µs; τ_J=1.4 ms; ν_{dec}=10 kHz; τ_{acq}=30.72 ms; τ_{dwell}=15 µs; n_{scan}=2048; $\tau_{recycle}$=1.5 s; T={5, 1, -7, -12, -23} ºC;	0.9 hr	t_1: 150 Hz
Uniform $^{13}C/^{15}N$ Val and Thr-labeled Desmin head Domain (Polymer or denatured state)	2D ^{13}C-^{13}C CP-DARR	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=53 kHz; ν_{CP13C}=38 kHz; τ_{CP} =1.5 ms; ν_{dec}=85 kHz; τ_{DARR}= 50 ms; Δt_1=22.4 µs; τ_{t1}=2.91 or 2.24 ms; τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=96 or 432; $\tau_{recycle}$=1.5 s; T=5º C;	10 or 36 hr	t_1: 80 Hz t_2: 80 Hz
Uniform $^{13}C/^{15}N$ Val and Ile labeled NFL head Domain (Polymer or denatured state)	2D ^{13}C-^{13}C CP-DARR	B_0=14.1 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4.5 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=46 kHz; ν_{CP13C}=36 kHz; τ_{CP} =1.5 ms; ν_{dec}=81 kHz; τ_{DARR}= 25 ms; Δt_1=26.8 µs; τ_{t1}=4.02 ms; τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=96; $\tau_{recycle}$=1 s; T=13 ºC;	36 hr	t_1: 80 Hz t_2: 80 Hz
Uniform $^{13}C/^{15}N$ Val and Thr-segmentally labeled Desmin Intermediate Filaments	2D ^{13}C-^{13}C CP-DARR	B_0=17.5 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=53 kHz; ν_{CP13C}=38 kHz; τ_{CP} =1.5 ms; ν_{dec}=85 kHz; τ_{DARR}= 50 ms; Δt_1=22.4 µs; τ_{t1}=2.46 ms; τ_{acq}=7.68 ms; τ_{dwell}=15 µs; n_{scan}=768; $\tau_{recycle}$=1.0 s; T=-23 ºC;	47 hr	t_1: 80 Hz t_2: 80 Hz
Uniform $^{13}C/^{15}N$ Val and Ile segmentally labeled NFL Intermediate Filaments	2D ^{13}C-^{13}C CP-DARR	B_0=14.1 T; ν_{MAS}=12 kHz; $\tau_{\pi/21H}$=4 µs; $\tau_{\pi/213C}$=5 µs; ν_{CP1H}=46 kHz; ν_{CP13C}=36 kHz; τ_{CP} =1.5 ms; ν_{dec}=81 kHz; τ_{DARR}= 50 ms; Δt_1=15 µs; τ_{t1}=3.0 ms; τ_{acq}=10.24 ms; τ_{dwell}=10 µs; n_{scan}=64; $\tau_{recycle}$=1.5 s; T=-26 ºC;	36 hr	t_1: 80 Hz t_2: 80 Hz
1-^{13}C-labeled desmin head-domain polymers	PITHIRDS_CT	B_0=9.4 T; ν_{MAS}=18 kHz; $\tau_{\pi/21H}$=3.2 µs; $\tau_{\pi13C}$=18.52 µs; ν_{CP1H}=68 kHz; ν_{CP13C}=50 kHz; τ_{CP} =1.5 ms; ν_{dec}=100 kHz; τ_{dwell}=20 µs; n_{scan}=512; $\tau_{recycle}$=4.0 s; T ≈ 30 ºC;n_{PT} = 9; τ_{PT} = 58.67 ms	5 hr	

1-^{13}C-labeled NFL head-domain polymers	PITHIRDS_CT	B_0=9.4 T; ν_{MAS}=18 kHz; $\tau_{\pi/21H}$=3.5 µs; $\tau_{\pi13C}$=18.52 µs; ν_{CP1H}=73 kHz; ν_{CP13C}=53 kHz; τ_{CP} =1.5 ms; ν_{dec}=100 kHz; τ_{dwell}=20 µs; n_{scan}=16; $\tau_{recycle}$=4.0 s; T ≈ 30 ºC;n_{PT} = 23; τ_{PT} = 42.67 ms	0.4 hr	

*B_0, magnetic field; ν_{MAS}, sample spinning frequency; $\tau_{\pi/21H}$, ^{1}H π/2 pulse length; $\tau_{\pi/213C}$, ^{13}C π/2 pulse length; $\tau_{\pi13C}$, ^{13}C π pulse length; ν_{CP1H}, ^{1}H RF power during CP; ν_{CP13C}, ^{13}C RF power during CP; τ_{CP}, ^{1}H-^{13}C CP contact time; ν_{dec}, ^{1}H decoupling RF power; τ_{DARR}, ^{13}C-^{13}C DARR mixing time; ν_{DARR}, ^{13}C-^{13}C DARR RF amplitude; τ_J, J-delay for INEPT; Δt_1, t_1 increment; τ_{t1}, total t_1 evolution time; Δt_2, t_2 increment; τ_{t2}, total t_2 evolution time; τ_{acq}, acquisition time; τ_{dwell}, dwell time; n_{scan}, number of scans; $\tau_{recycle}$, recycle delay time; n_{PT}, number PITHIRDS-CT points; τ_{PT}, constant-time PITHIRDS-CT recoupling period; T, sample temperature.

Table S3. Observed ^{13}C ss-NMR chemical shifts of several amino acids in the head domain polymers. Chemical shifts are in parts per million (ppm) relative to sodium trimethylsilylpropanesulfonate. Values in parentheses are differences from random coil chemical shifts (1). Uncertainties are approximately ±0.2 ppm.

sample	amino acid	C=O	Cα	Cβ
desmin head domain polymers	Pro	*	62.1 (-1.2)	31.7 (-0.4)
	Val	174.3 (-2.0)	60.3 (-1.9)	34.9 (+2.0)
	Thr	173.0 (-1.7)	60.6 (-1.2)	70.7 (+0.9)
	Ser	173.1 (-1.5)	56.7 (-1.6)	65.7 (+1.9)
	Ala	175.0 (-2.8)	50.7 (-1.8)	22.3 (+3.2)
	Phe, Tyr	173.9 (-2.0)[a]	55.3 (-2.6)[a]	41.8 (+3.0)[a]
NFL head domain polymers	Pro	*	62.1 (-1.2)	31.8 (-0.3)
	Val	*	60.6 (-1.6)	35.0 (+2.1)
	Thr	172.4 (-2.3)	60.3 (-1.5)	70.0(+0.2)
	Ser	172.6 (-2.0)	56.6 (-1.7)	65.6 (+1.8)
	Ala (β-strand)	174.5 (-3.3)	51.2 (-1.3)	22.2 (+3.1)
	Ala (non-β-strand)	178.6 (+0.8)	54.4 (+1.9)	17.5 (-1.6)
	Phe, Tyr	173.0 (-2.9)[a]	56.5 (-1.4)[a]	41.0 (+2.2)[a]

*peak not resolved. [a]The differences are calculated from the random coil chemical shifts of Tyr.

Table S4. Amino acid-specific β-strand contents in NFL and desmin samples, estimated from conformation-dependent crosspeak volumes in 2D ^{13}C-^{13}C ss-NMR spectra.

sample	amino acid	estimated β-strand content[1]
uniformly ^{15}N,^{13}C-labeled NFL head domain polymers	Ser	56%
	Ala	49%
segmentally ^{15}N,^{13}C-labeled NFL IFs	Ser	52%
	Ala	45%
Val,Ile-labeled NFL head domain polymers	Val	76%
	Ile	65%
segmentally Val,Ile-labeled NFL IFs	Val	66%
	Ile	54%
precipitated Val,Ile-labeled NFL head domain	Val	15%
	Ile	8%
uniformly ^{15}N,^{13}C-labeled desmin head domain polymers	Ser	78%
	Ala	42%
segmentally ^{15}N,^{13}C-labeled desmin IFs	Ser	41%
	Ala	19%
Val,Thr-labeled desmin head domain polymers	Val	82%
	Thr	63%
segmentally Val,Thr-labeled desmin IFs	Val	42%
	Thr	44%
precipitated Val,Thr-labeled desmin head domain	Val	11%
	Thr	11%

[1]Relative uncertainties are estimated to be ±15% as described in the text.

Materials and Methods

Protein expression and purification:
Standard molecular cloning strategies were employed to generate expression plasmids for all recombinant protein constructs. Briefly, neurofilament light (NFL) and desmin genes were cloned from a human cDNA library. Sequences corresponding to full-length proteins and truncated variants were then amplified by PCR and cloned into pHis-parallel vectors to produce recombinant constructs harboring N-terminal fusion tags (6×His, 6×His-GFP, and 6×His-mCherry). To enable segmental isotopic labeling via protein splicing, chimeras consisting of NFL or desmin constructs fused to the Cfa split intein or intact Cfa intein (2) were assembled via the Gibson Assembly protocol (detailed construct information described below). All mutation constructs were produced via standard site-directed mutagenesis protocols. All cloning and mutagenesis was confirmed by Sanger sequencing (Eurofins Genomics).

Recombinant proteins were expressed in *E. coli* BL21 (DE3) cells grown to an OD_{600} of 0.6 in LB medium. Expression of full-length NFL, full-length desmin, 6×His-tagged NFL head domain, and 6×His-tagged desmin head domain was induced via addition of 0.8 mM IPTG at 37° C and cells were harvested 4 hours post-induction. Expression of all GFP and mCherry fusion proteins was induced with 0.6 mM IPTG at 16° C and cells were harvested 16 hours post-induction. Universal and amino acid-specific ^{13}C, ^{15}N labeling of NFL and desmin head domains was carried out as previously described (3, 4).

For purification of full-length NFL, full-length desmin, 6×His-tagged NFL head domain, and 6×His-tagged desmin head domain, cell pellets were resuspended in a lysis buffer containing 25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 10 mM β-mercaptoethanol (β-ME), and protease inhibitors (Ethylenediaminetetraacetic acid-free protease inhibitor cocktail tablet, Sigma) and disrupted via sonication for 3 minutes (Fisher Scientific, 10 seconds on/30 seconds off, 78% power). 6×His-tagged NFL head domain and 6×His-tagged desmin head domain inclusion bodies were collected by centrifugation at 3,000 × g for 30 minutes. The inclusion body pellet was resuspended in solubilization buffer containing 25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 8 M Urea, and 10 mM β-ME, sonicated for 1 minute (10 seconds on/30 seconds off, 78% power), and clarified via centrifugation at 36,000 × g for 50 minutes. Supernatant was then applied to Ni^{2+}-NTA resin (Qiagen) and the column washed with a solubilization buffer supplemented with 20 mM imidazole and eluted in solubilization buffer supplemented with 300 mM imidazole. Pure protein was concentrated by centrifugal filtration (Amicon Ultra-15) and samples were aliquoted, flash frozen, and stored at -80 °C for future use.

Full-length NFL and desmin inclusion bodies were collected by centrifugation at 36,000 ×g for 20 minutes. The inclusion body pellet was resuspended in solubilization buffer lacking NaCl and sonicated for 1 minute (10 seconds on/30 seconds off, 78% power). The solution was then clarified via centrifugation at 36,000 × g for 50 minutes and the supernatant was passed through 0.22 µm filter (Millex GV) and loaded onto Hitrap Q column (GE Healthcare). A salt gradient elution was then performed (0 – 0.5 M NaCl in solubilization buffer) and elution fractions were analyzed by SDS-PAGE. Pure protein was concentrated by centrifugal filtration (Amicon Ultra-15) and samples were aliquoted, flash frozen, and stored at -80° C for future use.

For purification of GFP- and mCherry-fused NFL and desmin proteins, cells were resuspended in a lysis buffer containing 25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 2 M urea, 10 mM β-ME, 20 mM imidazole, and protease inhibitors and disrupted by sonication for 3 minutes (10 seconds on/30

seconds off, 78% power). Cell lysates were clarified via centrifugation at 36,000 × g for 50 minutes and supernatant was applied to Ni^{2+}-NTA resin, washed with lysis buffer, and bound proteins were eluted in lysis buffer supplemented with 300 mM imidazole. Eluted protein was then diluted 5-fold with low salt ion-exchange buffer (25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 M urea, and 2 mM tris(2-carboxyethyl)phosphine (TCEP)) and applied to a HiTrap Q column. A salt gradient elution was then performed (0 – 0.5 M NaCl in ion-exchange buffer) and elution fractions were analyzed by SDS-PAGE. Pure protein was concentrated by centrifugal filtration (Amicon Ultra-15) and samples were aliquoted, flash frozen, and stored at -80° C for future use.

Preparation of NFL and desmin polymers and hydrogels:
6×His-tagged NFL head domain, 6×His-tagged desmin head domain and their respective mutants were diluted to 100 - 200 μM in gelation buffer (25 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 10 mM β-ME) supplemented with 8 M urea and dialyzed stepwise at 25° C against gelation buffer containing 4 M urea, 2 M urea, 1 M urea, 0.5 M urea, and no urea to remove denaturant and initiate polymer formation.

Preparation of GFP-fused NFL head domain hydrogel droplets was achieved by dialyzing the purified GFP-NFL head domain against a gelation buffer supplemented with 0.5 mM ethylenediaminetetraacetic acid (EDTA), and protease inhibitors for 16 hours at 25° C. Dialyzed protein was concentrated to roughly 20 mg/mL and sonicated for 3 seconds at 1% power. Following centrifugation, 20 μL droplets were deposited onto a piece of parafilm fixed inside a 10 cm cell culture dish. The dish was sealed with parafilm and incubated for 16 hours at 25° C.

The mCherry-fused NFL head domain and desmin head domain were diluted to 200 μM and dialyzed against gelation buffer for 16 hours at 25° C. Precipitates were removed by centrifugation at 10,000 × g for 1 minute. Head domain polymers, which remain in the supernatant, were analyzed by negative staining transmission electron microscopy (TEM). Hydrogel droplets were prepared as previously described (5). Briefly, 0.5 μL of the solution containing mCherry-fused head domain polymers was deposited onto a 35 mm glass bottom dish containing a piece of filter paper soaked with 35 μL of the gelation buffer. The dish was sealed with parafilm and incubated overnight at 25° C.

To compare polymer formation propensity between wild-type head domains and their mutant variants, the 6×His-tagged NFL head domain and disease mutants were diluted to 100 μM and dialyzed against gelation buffer supplemented 4 M urea for 16 hours at 25° C. Similarly, the 6×His-tagged desmin head domain and disease mutants were diluted to 100 μM and dialyzed against gelation buffer supplemented 3 M urea for 3 hours at 25° C. For the GFP-tagged NFL head domain and its mutant variants, proteins were diluted to 50 μM and dialyzed against gelation buffer supplemented 1 M urea for 2 hours at 25° C. Similarly, GFP-tagged desmin head domain and its mutant variants were diluted to 50 μM and dialyzed against gelation buffer supplemented 0.5 M urea for 16 hours at 25° C. All head domain polymers were analyzed by negative staining TEM.

Hydrogel-binding assays:
Intact mouse brain tissue was flash frozen in liquid nitrogen and subjected to cryogenic grinding (cryomill, Retsch). Resultant frozen powder was resuspended at 100 mg/mL in gelation buffer and clarified via centrifugation at 12,000 × g for 10 minutes at 4° C. 1.2 mL of supernatant (mouse brain lysate) was then incubated with ten GFP-NFL head domain hydrogel droplets for 2 hours at 4° C. Following incubation, the droplets were spun down at 800 x g for 5 minutes at 4° C, washed three times with gelation buffer supplemented with 0.5 mM EDTA and protease inhibitors, and melted in gelation buffer supplemented with 6 M guanidine-HCl. Solubilized protein solution was then applied to

Ni^{2+}-NTA resin to remove the bait protein (6×His-GFP-NFL head domain). Flow-through consisting of hydrogel-interacting proteins was collected and analyzed via shotgun mass spectrometry.

To map the domains of NFL and desmin that mediate self-association with their respective head domain hydrogel droplets, the GFP-labeled variants described in Figure 2 were purified as described above. GFP-labeled proteins were diluted to 1 μM with 1 mL of gelation buffer immediately added to the corresponding mCherry-fused head domain hydrogel droplets for 12 hours. After incubation, the hydrogel droplets were washed twice with gelation buffer to remove unbound protein prior to confocal microscopy imaging analysis (5).

Intermediate filament assembly:
To assemble NFL intermediate filaments (IFs), full-length wild-type NFL or mutant variants thereof were diluted to 10 μM with a buffer containing 25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 8 M urea, 1 mM TCEP, 0.5 mM EDTA and dialyzed against a buffer containing 50 mM MES (pH 6.25), 170 mM NaCl, 1 mM TCEP, and 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) for 16 hours at 25° C.

To assemble desmin IFs, full-length wild-type desmin or mutant variants thereof were diluted to 20 μM with a buffer containing 25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 8 M urea, 1 mM TCEP, and 0.5 mM EDTA and dialyzed stepwise against a buffer containing 5 mM Tris-HCl (pH 8.5), 1 mM EDTA, 0.1 mM EGTA, and 10 mM β-ME and supplemented with 4 M urea, 2 M urea, and no urea to remove denaturant. Dialyzed desmin proteins were then mixed with a buffer containing 45 mM Tris-HCl (pH 7.0) and 100 mM NaCl at a 1:1 ratio by volume and incubated for 1 hour at 25° C.

Negative staining transmission electron microscopy:
Head domain polymer solution (5 μL) was added to an EM grid for 10 seconds and excess sample was removed via blotting with filter paper. The grid was then washed with water for 2 seconds and stained with 5 μL uranyl acetate (2%) for 15 seconds. An identical protocol was employed to generate EM grids containing assembled IFs except the water washing step was omitted. All negative staining TEM samples were imaged on a JOEL 1400 microscope.

Intein-mediated segmental isotopic labeling of NFL and desmin proteins:
For segmental labeling of NFL, a fusion construct comprising the GFP-NFL head domain (residues 2-85) and a C-terminal CfaN split intein fragment (2) was expressed in minimal media supplemented with the desired ^{13}C, ^{15}N-labeled amino acids. A complementary fusion construct comprising headless NFL (residues 86-543, I86C) and an N-terminal GFP-CfaC split intein fragment was produced in LB medium as described above. The Cfa intein-mediated protein splicing required a cysteine (I86C) at the ligation junction (Figure S2). Purification of the fusion constructs was carried out as described above. Isotopically-labeled head domain and unlabeled headless domain fusion proteins were then mixed at a 2:1 molar ratio (100 μM : 50 μM) in the presence of 0.05 mg/mL caspase-3 enzyme to remove the GFP tag from each construct. The mixture was then dialyzed against a buffer containing 40 mM KH_2PO_4 (pH 7.2), 150 mM NaCl, and 1 mM TCEP at 25° C for 16 hours and diluted 10-fold into a buffer containing 25 mM Tris-HCl (pH 7.4), 8 M urea, and 1 mM TCEP to solubilize any precipitate that formed during dialysis. Full-length, segmentally-labeled NFL protein was then purified following the HiTrap Q chromatography protocol described above.

For segmental labeling of desmin, a fusion construct comprising the GFP-desmin head domain (residues 2-84) and the contiguous Cfa intein was expressed in minimal media supplemented with the desired ^{13}C, ^{15}N-labeled amino acids. A complementary unlabeled 6×His-headless desmin construct

(residues 85-470, A85C) was purified as described above. The 6×His tag was removed upon treatment with the caspase-3 enzyme, liberating a headless desmin construct that harbors a native chemical ligation-compatible N-terminal cysteine (Figure S2). Isotopically-labeled head domain and unlabeled headless domain fusion proteins were then mixed at a 1:1 molar ratio (200 μM : 200 μM) in a buffer containing 100 mM KH_2PO_4 (pH 7.2), 200 mM MESNa, 100 mM NaCl, 5 mM TCEP, 1 mM EDTA, 0.05 mg/mL caspase-3 enzyme, and 200 mM sodium 2-mercaptoethanesulfonate (MESNa) for 16 hours at 25° C. Following incubation, the sample was diluted 10-fold into a buffer containing 25 mM Tris-HCl (pH 7.4), 8 M urea, and 1 mM TCEP. Full-length, segmentally-labeled desmin protein was then purified following the HiTrap Q chromatography protocol described above.

Intermediate filament assembly using the full-length, segmentally-labeled proteins was carried out as described above and subsequent filament compaction was achieved by ultracentrifugation at 100,000 × g for 30 minutes.

Sample packing for ss-NMR measurement:
NFL and desmin head-domain polymers and IFs were centrifuged at 247,000 × g for 1 hour and supernatant was discarded. Pellets were then transferred to 0.7 mL ultracentrifuge tubes (Beckman Coulter, part #: 343776), from which they were packed into 3.2 mm-diameter magic-angle spinning (MAS) rotors (22 μL or 36 μL sample volume, Revolution NMR) by centrifugation (swinging bucket) at 50,000 x g for 30 minutes using a home-made device that holds the MAS rotor and the 0.7 mL tube. After centrifugation, excess buffer was blotted from the MAS rotor and the rotor caps were sealed with cyanoacrylate glue. For the ether precipitated NFL and desmin head domain samples, the head domains in 6 M guanidine-HCl buffer were dialyzed overnight against 2 L of ultrapure water. Resulting precipitates were flash frozen in liquid nitrogen and lyophilized for 3 hours. Lyophilized proteins were then dissolved in 100 μL of trifluoroacetic acid. 1 mL of ice cold tert-butyl methyl ether was then added to the solution, vortexed for 30 seconds, then centrifuged at 21,000 × g for 30 minutes in a swinging bucket rotor pre-cooled to 4° C. Supernatant was removed and the pellets were dried under a stream of nitrogen gas. Dried pellets were packed manually into MAS rotors.

PITHIRDS-CT measurements were performed on NFL or desmin head polymer samples in which backbone carbonyl sites of either all Val residues or all Leu residues (in NFL) or either all Val residues, all Leu residues, or all Phe residues (in desmin) were ^{13}C-labeled. Samples for these measurements were packed into 3.2 mm MAS rotors as fully hydrated pellets (see above) then lyophilized in the rotors. Lyophilization was performed because the transverse spin relaxation times (T_2 values) of carbonyl ^{13}C labels in the fully hydrated samples were found to be too short (<20 ms) to permit PITHIRDS-CT measurements. Such measurements require constant-time dipolar recoupling periods greater than 30 ms for ^{13}C-^{13}C distances greater than 5 Å. T_2 values of lyophilized samples were found to be ~60 ms under the PITHIRDS-CT pulse sequence.

ss-NMR measurements:
1D and 2D ss-NMR spectra were acquired at 17.5 T (746.1 MHz ^1H NMR frequency) with a Varian Infinity spectrometer console and a 3.2 mm low-E MAS probe produced by Black Fox, Inc. (Tallahassee, FL) or at 14.1 T (599.2 MHz ^1H NMR frequency) with a Varian InfinityPlus spectrometer console and a 3.2 mm Varian BioMAS probe. PITHIRDS-CT data were acquired at 9.4 T (400.8 MHz ^1H NMR frequency) with a Bruker Avance III spectrometer console and a 3.2 mm Varian T3 MAS NMR probe. Sample temperatures were controlled by cooled nitrogen gas and determined from the temperature-dependent ^1H NMR frequency of water within the NFL or desmin sample or the temperature-dependent ^{89}Br NMR frequency of an external sample of KBr powder (6). ^{13}C-^{13}C polarization transfers in 2D spectra used Dipolar-Assisted Rotational Resonance (DARR) (7). ^1H

decoupling in CP-based measurements used two-pulse phase modulation (TPPM). ^{1}H decoupling in INEPT-based measurements used 25-pulse composite π sequences (8). PITHIRDS-CT measurements used the constant-time homonuclear dipolar recoupling pulse sequence described previously (9), with pulsed spin-lock detection (10) to enhance sensitivity. Other conditions and parameters for ss-NMR measurements are given in Table S1.

1D NMR data were processed with Varian Spinsight or Bruker TopSpin software. 2D data were processed with NMRPipe software (11). Pure Gaussian apodization functions were used to process all data, with no artificial resolution enhancement to reduce apparent NMR linewidths. PITHIRDS-CT data were analyzed by comparison with numerical simulations. 2D crosspeak volumes were measured in ImageJ (available at https://imagej.nih.gov/ij/) after converting the 2D spectra to grey-scale images with the pipe2tiff function of NMRPipe.

NFL and desmin head domain phosphorylation:
As described above GFP-fused NFL head domain and GFP-fused desmin head domain monomers were to 1 μM in gelation buffer and co-incubated with respective mCherry-fused head domain hydrogel droplets for 6 hours at 25° C. Unbound monomers were removed from hydrogel droplets via gelation buffer washes and droplets were equilibrated in phosphorylation reaction buffer (40 mM Tris-HCl (pH 7.4), 20 mM $MgCl_2$). ATP (0.2 mM) and/or protein kinase A (0.1 mg/mL; PKA, Promega) were then added to hydrogel droplets for 12 hours at 25° C. Post-incubation hydrogel droplets were visualized by confocal microscopy. Phosphorylation of full-length NFL and desmin IFs was carried out by adding 20 mM $MgCl_2$ to the assembled IFs (10 μM) followed by addition of ATP (0.2 mM) and/or PKA (0.1 mg/mL) for 5 hours at 30° C. The effect of PKA activity on IF structure was analyzed by negative staining TEM.

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