

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1. Clinical and demographic information of patient AL-59.

Variable	N (%) – median (IQR)
Age, years	56
Echocardiographic features:	
Interventricular septum (mm)	17.5
Posterior wall (mm)	15
Ejection fraction (%)	56
Laboratory data	
Serum immunofixation	Free light chain lambda
Urine immunofixation	Bence Jones protein lambda
Free light chain lambda (mg/L)	292
Free light chain kappa (mg/L)	11.6
Free light chain ratio / dFLC (mg/L)	0.04 / 280
serum creatinine (mg/dL)	0.86
eGFR (mL/min per 1.73 mq)	76
24h proteinuria (g/24h)	0.16
ALP (U/L, u.r.l: 150 U/L)	78
ALT (U/L, u.r.l: 34 U/L)	35
NT-proBNP (ng/L)	6,043
cTnI (ng/L)	127
Hb (g/dL)	13.6
WBC (mcL)	7,290
PLT (mcL)	253,000
Bone marrow plasma cells (%)	12

IQR: interquartile range; dFLC: difference between involved and uninvolved FLCs; eGFR: estimated glomerular filtration rate; u.r.l. upper reference limit; ALP: alkaline phosphatase; ALT: alanine aminotransferase; NT-proBNP: N-terminal fragment of B-type natriuretic peptide; cTnI: cardiac troponin I; Hb: hemoglobin; WBC: white blood cell count; PLT: platelet count.

Supplementary Table 2. Cryo-EM data overview**Data acquisition**

Microscope (Company)	TALOS Arctica (Thermo Fischer Scientific)
Voltage (kV)	200
Camera	Falcon 3EC
Data collection software	EPU-2.8
Magnification	120,000
Defocus range (μm)	-0.5 and -2.5
Pixel size (Å)	0.889
Micrographs (no.)	1,366
total dose (e ⁻ /Å ²)	40
number of frames	40

Data processing

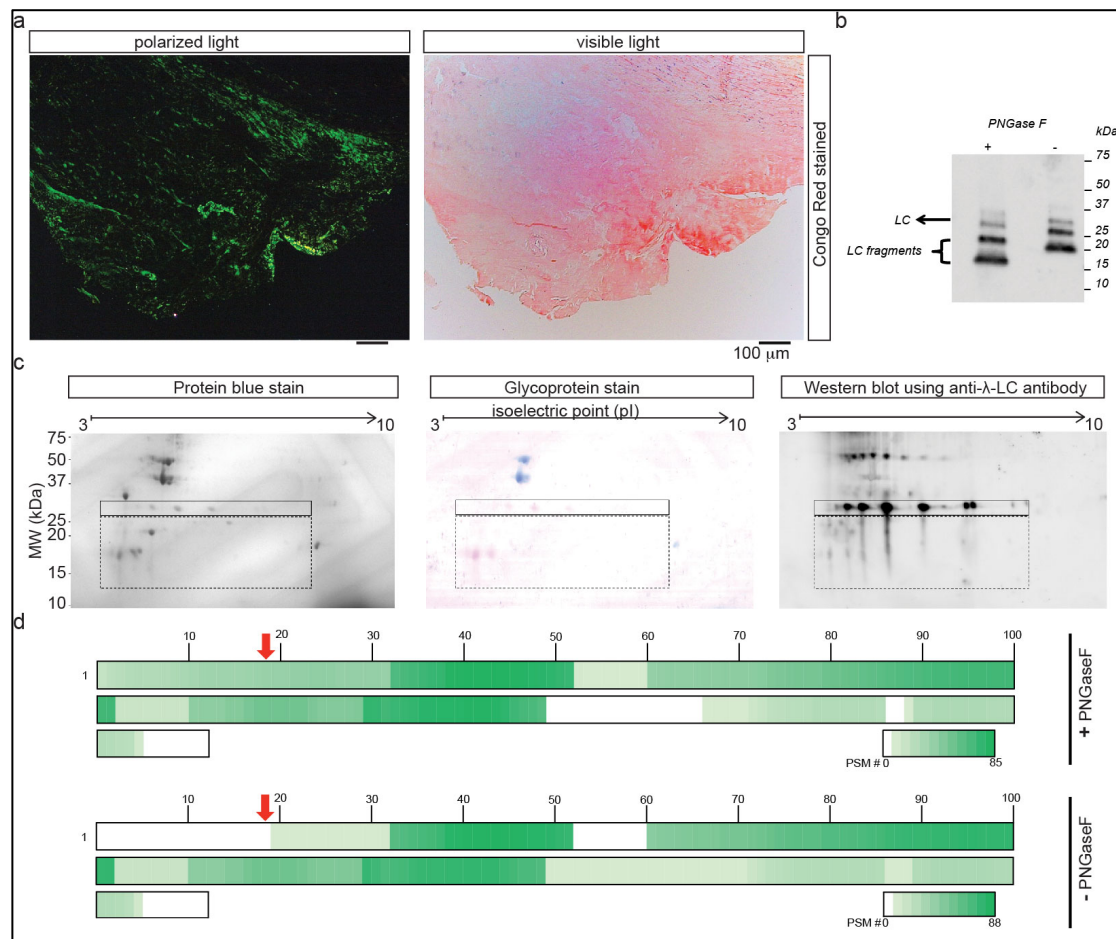
Number of picked tubes	5,112	
Initial segments	121,533	
Structure	bent	straight
Helical reconstruction		
Imposed symmetry	C1	C1
Helical symmetry - Twist / rise	-0.74° / 4.93 Å	-0.72° / 4.92 Å
Final number of segments	10,594	13,119
Post-processing B-factor (Å ²)	-101	-107
Resolution (Å)	4.1	4.0
Map-model parameters		
r.m.s. deviations		
Bond lengths (Å)	0.005	0.003
Bond angles (°)	1.109	0.869
Ramachandran plot		
Favored (%)	85.7	92.3
Allowed (%)	14.3	7.69
Outliers (%)	0.00	0.00
Validation		
Molprobity score	2.22	1.53
Clashscore	11.56	2.72
Poor rotamers (%)	0.00	0.00
EM-Ringer Score	3.2	5.3
Map-model correlation		
CC mask/box/peaks/volume	0.74 / 0.77 / 0.62 / 0.73	0.78/0.78/0.8/0.76
Atom inclusion/Q-score (0.02 σ)	0.75 / 0.46	0.75 / 0.48
Deposition codes	bent	straight
PDB	8CDI	8CDH
EMDB	EMD-16573	EMD-16574
EMPIAR	EMPIAR-11408	EMPIAR-11408

Supplementary Table 3. Top 20 protein hits with at least two unique peptides as identified by LC-MS/MS (total identified proteins = 221). For each protein the number of amino acids in the fasta sequence (#AAs), the sequence coverage in percentage and the number of peptide-spectrum matches (#PSMs) are indicated. Collagen subunits are highlighted in red.

Accession	Description	# AAs
AL59	AL59	212
A0A0B4J231	Immunoglobulin lambda-like polypeptide 5 OS=Homo sapiens OX=9606 GN=IGLL5 PE=1 SV=1	215
P06727	Apolipoprotein A-IV OS=Homo sapiens OX=9606 GN=APOA4 PE=1 SV=3	396
P12883	Myosin-7 OS=Homo sapiens OX=9606 GN=MYH7 PE=1 SV=5	1,935
P12111	Collagen alpha-3(VI) chain OS=Homo sapiens OX=9606 GN=COL6A3 PE=1 SV=5	3,177
A0A0A0MTS7	Titin OS=Homo sapiens OX=9606 GN=TTN PE=1 SV=1	3,5991
P12109	Collagen alpha-1(VI) chain OS=Homo sapiens OX=9606 GN=COL6A1 PE=1 SV=3	1,028
P02649	Apolipoprotein E OS=Homo sapiens OX=9606 GN=APOE PE=1 SV=1	317
P35555	Fibrillin-1 OS=Homo sapiens OX=9606 GN=FBN1 PE=1 SV=4	2,871
P12110	Collagen alpha-2(VI) chain OS=Homo sapiens OX=9606 GN=COL6A2 PE=1 SV=4	1,019
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein OS=Homo sapiens OX=9606 GN=HSPG2 PE=1 SV=4	4,391
P04004	Vitronectin OS=Homo sapiens OX=9606 GN=VTN PE=1 SV=1	478
P35609	Alpha-actinin-2 OS=Homo sapiens OX=9606 GN=ACTN2 PE=1 SV=1	894
Q9UKZ9	Procollagen C-endopeptidase enhancer 2 OS=Homo sapiens OX=9606 GN=PCOLCE2 PE=1 SV=1	415
P01024	Complement C3 OS=Homo sapiens OX=9606 GN=C3 PE=1 SV=2	1,663
P78539	Sushi repeat-containing protein SRPX OS=Homo sapiens OX=9606 GN=SRPX PE=1 SV=1	464
P06576	ATP synthase subunit beta, mitochondrial OS=Homo sapiens OX=9606 GN=ATP5F1B PE=1 SV=3	529
P02647	Apolipoprotein A-I OS=Homo sapiens OX=9606 GN=APOA1 PE=1 SV=1	267
P07093	Glia-derived nexin OS=Homo sapiens OX=9606 GN=SERPINE2 PE=1 SV=1	398
Q6ZN40	Tropomyosin 1 (Alpha), isoform CRA_f OS=Homo sapiens OX=9606 GN=TPM1 PE=1 SV=1	326
P00747	Plasminogen OS=Homo sapiens OX=9606 GN=PLG PE=1 SV=2	810

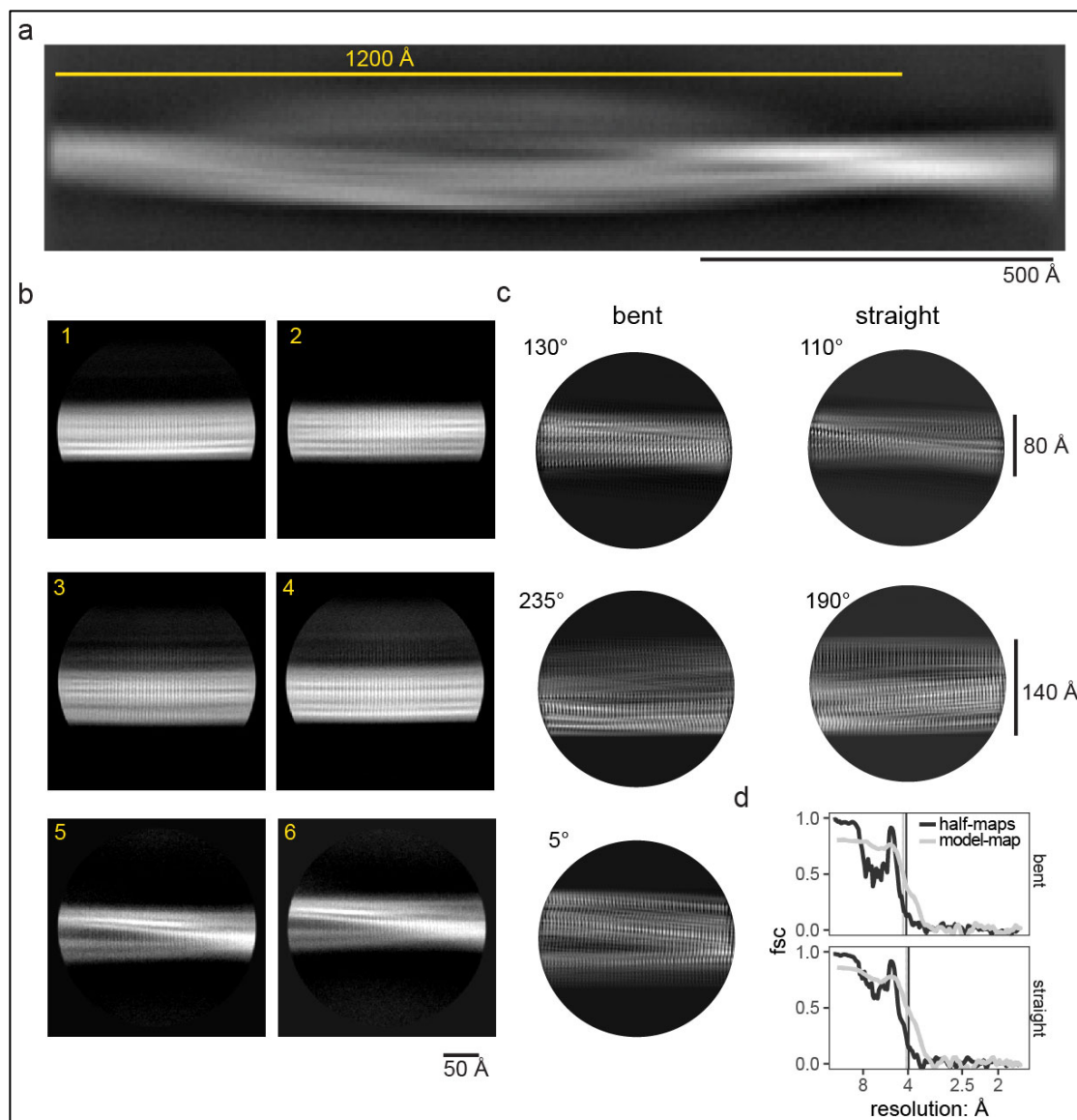
A0A0S2Z4G6	Tropomyosin 1 (Alpha), isoform CRA_o (Fragment) OS=Homo sapiens OX=9606 GN=TPM1 PE=1 SV=1	284
P10909	Clusterin OS=Homo sapiens OX=9606 GN=CLU PE=1 SV=1	449
A0A0G2JPR0	Complement C4-A OS=Homo sapiens OX=9606 GN=C4A PE=1 SV=1	1,744
P02768	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	609
P0C0L4	Complement C4-A OS=Homo sapiens OX=9606 GN=C4A PE=1 SV=2	1,744
P0C0L5	Complement C4-B OS=Homo sapiens OX=9606 GN=C4B PE=1 SV=2	1,744
P15814	Immunoglobulin lambda-like polypeptide 1 OS=Homo sapiens OX=9606 GN=IGLL1 PE=1 SV=1	213
Q15582	Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens OX=9606 GN=TGFBI PE=1 SV=1	683
P02743	Serum amyloid P-component OS=Homo sapiens OX=9606 GN=APCS PE=1 SV=2	223
P68032	Actin, alpha cardiac muscle 1 OS=Homo sapiens OX=9606 GN=ACTC1 PE=1 SV=1	377
P51888	Prolargin OS=Homo sapiens OX=9606 GN=PRELP PE=1 SV=1	382
P12814	Alpha-actinin-1 OS=Homo sapiens OX=9606 GN=ACTN1 PE=1 SV=2	892
P01031	Complement C5 OS=Homo sapiens GN=C5 PE=1 SV=4	1,676
O14958	Calsequestrin-2 OS=Homo sapiens OX=9606 GN=CASQ2 PE=1 SV=2	399
P07585	Decorin OS=Homo sapiens OX=9606 GN=DCN PE=1 SV=1	359
H0YFD6	Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens OX=9606 GN=HADHA PE=1 SV=2	792
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens OX=9606 GN=ATP5F1A PE=1 SV=1	553
B7ZKJ8	ITIH4 protein OS=Homo sapiens OX=9606 GN=ITIH4 PE=1 SV=1	935
P12235	ADP/ATP translocase 1 OS=Homo sapiens OX=9606 GN=SLC25A4 PE=1 SV=4	298
H0YNC7	Tropomyosin alpha-1 chain (Fragment) OS=Homo sapiens OX=9606 GN=TPM1 PE=1 SV=1	223
Q14315	Filamin-C OS=Homo sapiens OX=9606 GN=FLNC PE=1 SV=3	2,725
P48735	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens OX=9606 GN=IDH2 PE=1 SV=2	452
P02748	Complement component C9 OS=Homo sapiens OX=9606 GN=C9 PE=1 SV=2	559
Q15063	Periostin OS=Homo sapiens OX=9606 GN=POSTN PE=1 SV=2	836
P68871	Hemoglobin subunit beta OS=Homo sapiens OX=9606 GN=HBB PE=1 SV=2	147
P01008	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	464
Q92954	Proteoglycan 4 OS=Homo sapiens OX=9606 GN=PRG4 PE=1	1,404

	SV=3	
Q76M96	Coiled-coil domain-containing protein 80 OS=Homo sapiens OX=9606 GN=CCDC80 PE=1 SV=1	950

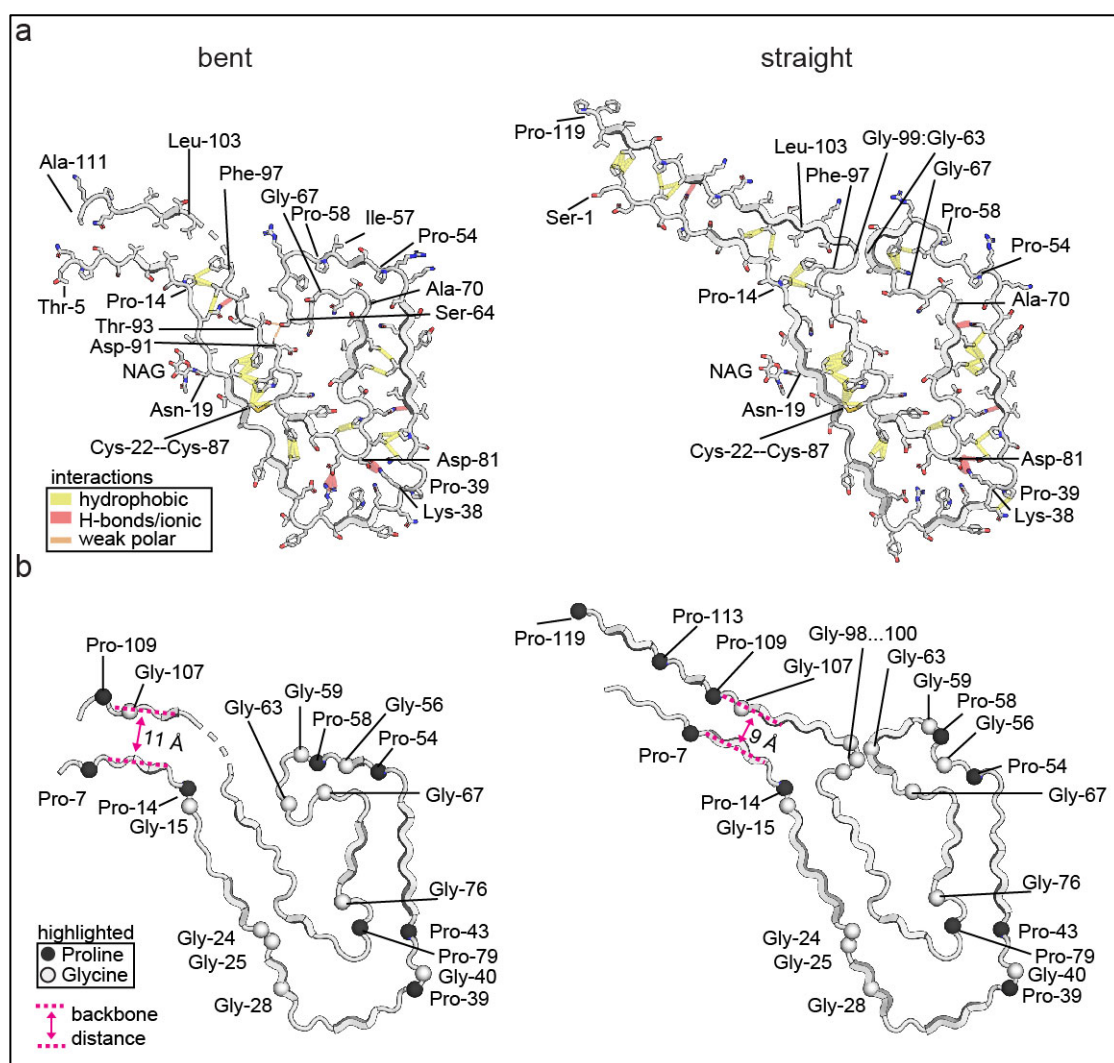


Supplementary Figure 1. Characterization of AL59 amyloid by tissue staining, Western blot, 2D SDS-PAGE and LC-MS/MS analyses (a) Congo red-stained aortic heart tissue from patient AL59. *left*: The apple-green birefringence under polarized light indicates amyloid deposits. *right*: The same slide recorded with visible light. (b) Western blot analysis of AL59 extract #3 from heart tissue after overnight-digestion with and without PNGase F (peptide-N-glycosidase F, New England Biolabs). Polyclonal anti-human λ-LC antibodies (Dako) were used for detection. Full-length LCs and fragments show a molecular weight shift due to N-linked oligosaccharides removal after PNGase F digestion. (c) 2D-PAGE analysis of enriched fibrils from AL59 heart tissue. The whole pellet, obtained after tissue homogenization and extensive washing in Tris-EDTA buffer, was analyzed. The same gel was treated (*left*) with GelCode™ Blue Stain Reagent (Pierce) to detect protein, and (*middle*) subsequently with Glycoprotein Staining Kit (Pierce) to detect

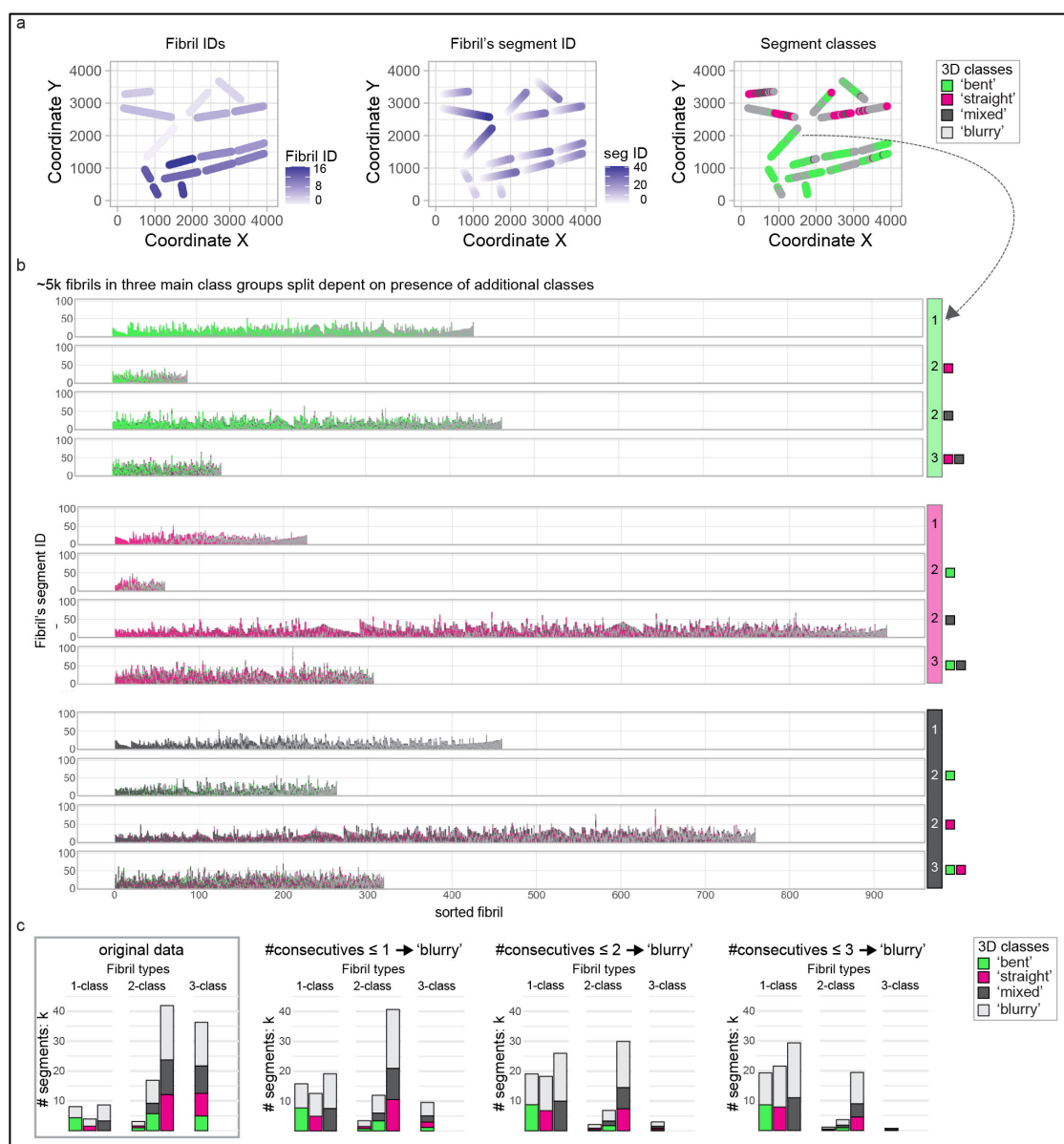
glycoproteins. Boxes with continuous and dotted lines highlight molecular weight (MW) regions corresponding to glycosylated full-length and fragmented light chains, respectively. Additional spots at higher molecular weight indicate other tissue proteins in the sample. LCs were identified by (*right*) Western blot using polyclonal anti- λ -LC antibody (Dako). Boxes highlight regions as described for the gel shown to the left (d) AL59 sequence coverage detected in water extract #3. Sample was treated with and without PNGase F (Glycerol-free, New England Biolabs). The number of peptide spectrum matches (PSMs) identifying each sequence position in the samples with and without PNGase F-treatment is shown on a white to green gradient for counts from 0 to 85 and 0 to 88, respectively. Red arrows indicate the N-glycosylated Asn-19. Glycosylated peptides are not detected due to glycan interference.



Supplementary Figure 2. 2D class averages of AL59 obtained by helical reconstruction. (a) 2D class average obtained from particles extracted with a box size of $\sim 1,600$ Å. This class with an estimated crossover length of $\sim 1,200$ Å was used to generate the initial model following established protocols ¹. (b) 2D class averages with visible β -spines were obtained for particles extracted with a box size of ~ 300 Å. These classes are compared to (c) 2D projections of the straight and bent structures. (d) Half-map and model-map based FSC curve are shown in black and grey, respectively. The half-map based FSC drops below the value of 0.143 at resolutions of 4.0 and 4.1 Å for the straight and bent structures, respectively. The model-map based FSC threshold of 0.5 is reached at resolutions of 4.0 and 4.2 Å.



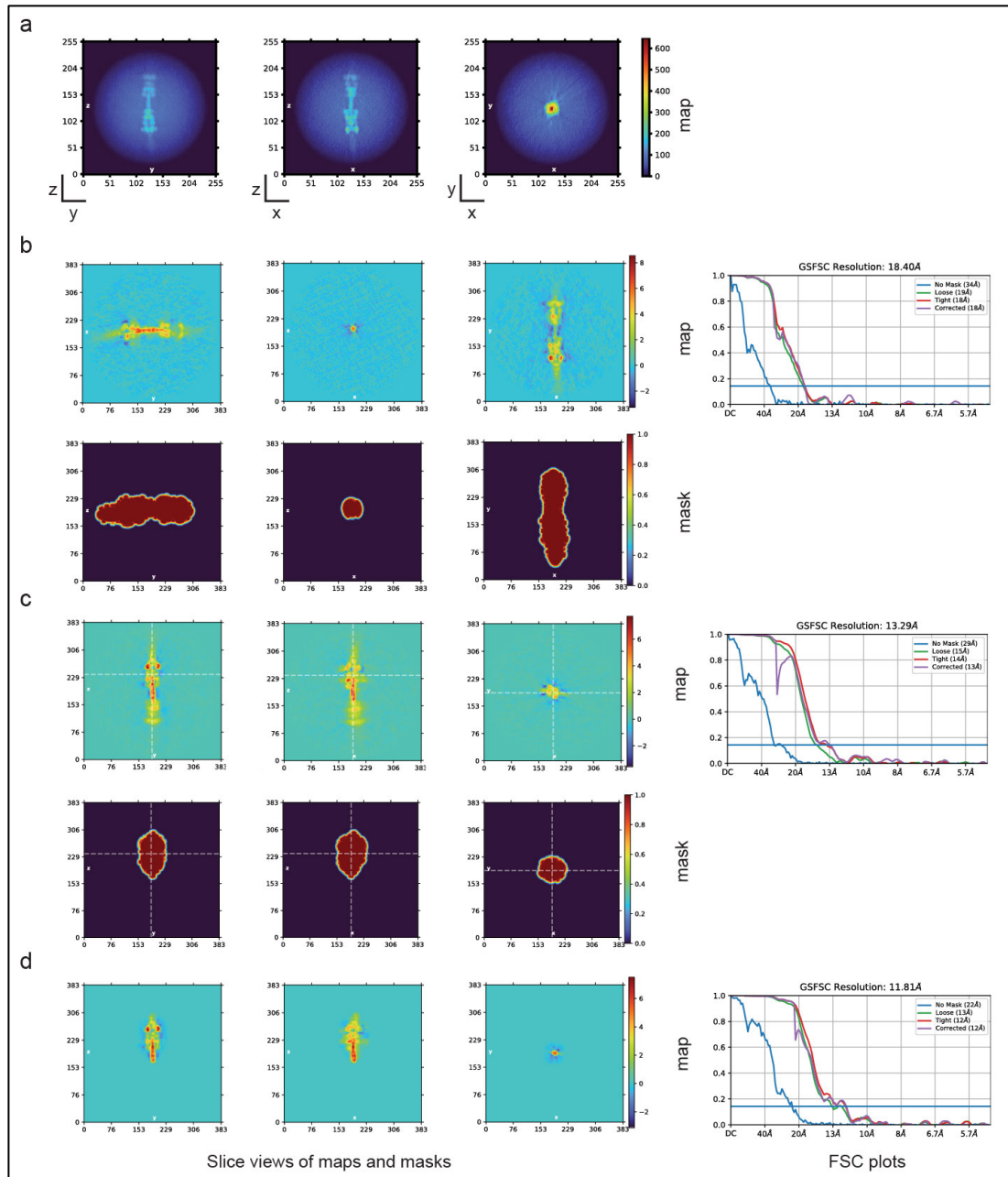
Supplementary Figure 3. High number of Glycine and Proline residues renders AL59 prone to adopt altered conformations. (a) Backbone and side chain atoms of the opposing strands are represented in mixed cartoon/stick format in white with black outlines. Hydrophobic and hydrogen (H) bond as well as ionic contacts between opposing side-chains, classified by Arpeggio ², are shown as yellow and pink semi-transparent lines, respectively. For clarity, weak polar interactions are highlighted only for the interactions between Ser-64 and Thr-93 as well as Asp-91, as orange, thinner semi-transparent lines. Residues are labeled as in Fig. 2b. (b) Proline and Glycine residues are labeled and highlighted as black and white C α spheres, respectively. The backbone-to-backbone C α distances of the terminal tails are indicated in pink. In the straight structure, hydrophobic interactions keep the backbones of the opposing N- and C-terminal tails within a distance of 9 Å. The loose association between the N- and C-terminal tails is recognized as lacking stabilizing interactions and the increased backbone distance of 11 Å.

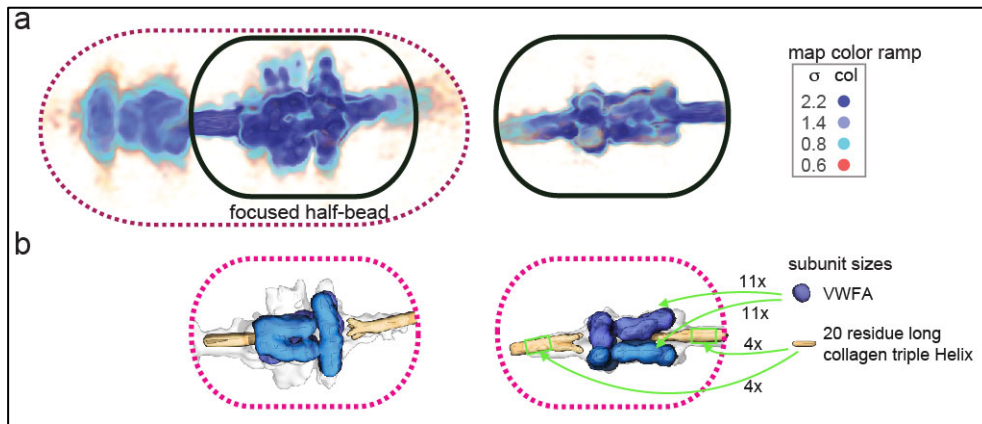


Supplementary Figure 4. The bent and straight structures are on separate fibrils.

(a) In Relion, the metadata of each extracted segment, or particle, is stored as STAR file. Each particle is listed with its associated micrograph name, fibril ID (helical tube ID in Relion), segment ID and assigned structural class, as visualized from left to right. The fibril length, and thus the number of segments per fibril, was set during manual picking. Coordinates are plotted on pixel scale with a pixel-size of $0.889 \text{ \AA}$. Segments were extracted with a box-size and inter-box distance of 300 and 30 $\text{\AA}$, respectively. (b) To visualize the distribution of structural classes across fibrils, the ~5k fibrils were sorted into panels according to their dominant structural class (bent, straight or mixed). These main panels were sub-divided into 1-class, as well as 2- and

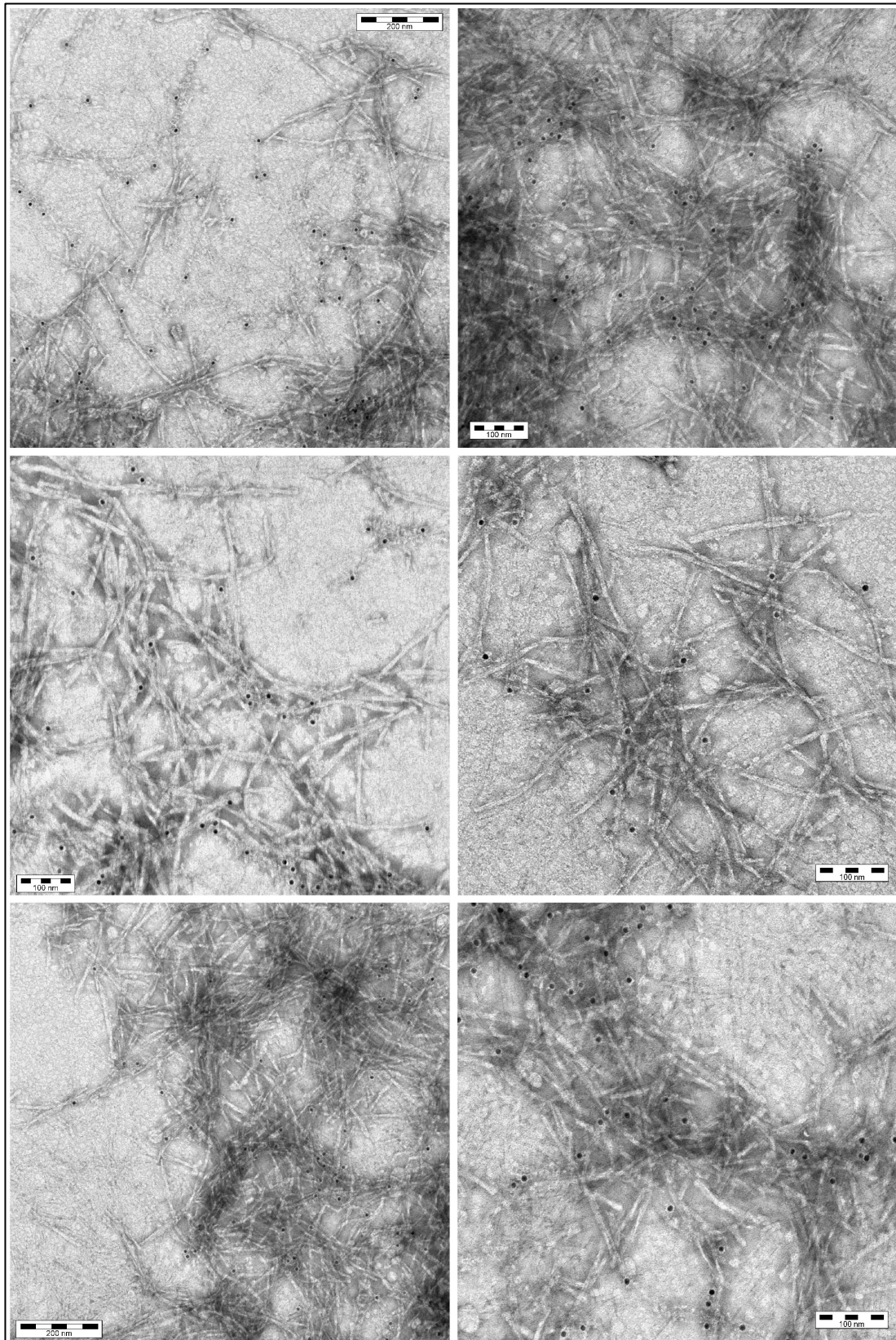
3-class fibril type groups depending on the presence of one or two additional classes, respectively. The identity of the additional classes is indicated by its respective color. The majority of fibrils comprise long consecutive regions of the same class, interspersed by short regions of the other classes. The value of the fibril's segment ID (y-axis) indicates its position along the fibril, and its value correlates to fibril length. The dotted arrow (from panel a to b) exemplifies the classification of a 1-class dominant-bent fibril. (c) To analyze the effect of short interspersing regions on fibril type counts, the analysis was repeated for three cases in which short regions of different lengths (≤ 1 , ≤ 2 , ≤ 3) were re-defined as blurry.



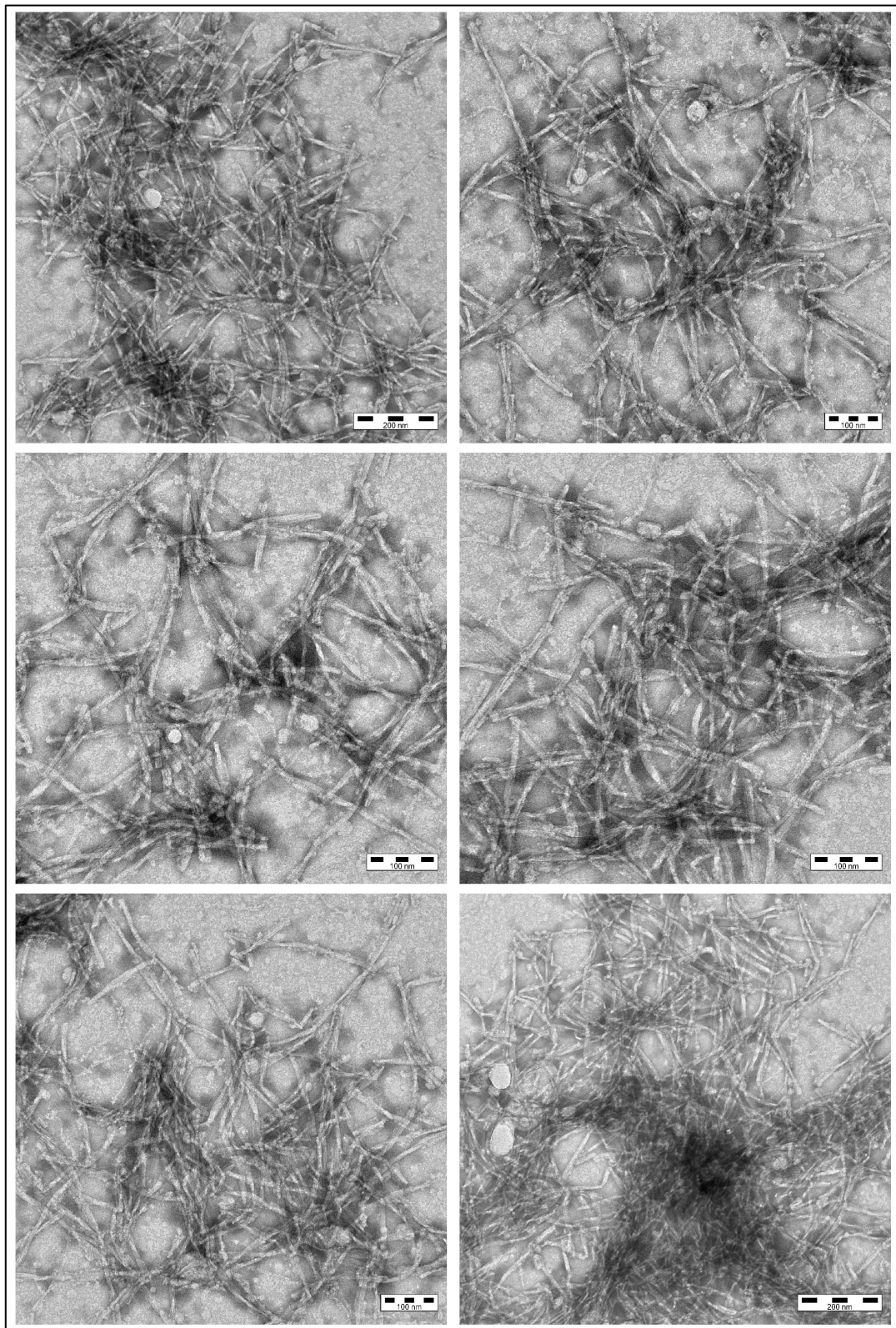


Supplementary Figure 6. Domain assembly in reconstructed COLVI beads.

(a) The reconstructed map is shown as in Fig. 5c, but including the non-focused half-bead in the view on the left. (b) Assemblies of VWFA domains and collagen are represented as blue and orange surfaces corresponding to a resolution of 12 Å. To estimate the available space of the quarter-bead volume, we placed 11 copies of an $\alpha 2$ -VWFA2 AI-model [<https://www.uniprot.org/uniprotkb/P12110/entry>]. The surfaces of the two quarter-beads are colored light and dark blue. The crystal structure of a collagen triple helix [<https://www.rcsb.org/structure/1k6f>]⁴ was assembled into elongated parallel tetramers, as the molecular structure of the predicted nanoscale rope is not known⁴⁻⁷. Arrows indicate the placement of subunits to generate the shown surfaces. Subunit sizes are indicated. Domains were placed solely for the purpose to estimate the available space of the known components.



Supplementary Figure 7. IEM of AL59 extracted stained with anti-COLVI IgG and gold-conjugated anti-IgG antibodies. Six images were taken of different sample regions with varying magnifications. The image shown on the top left is also shown in Fig. 6a. Scale bars are shown for each image.



Supplementary Figure 8. IEM of AL59 extract incubated with only the gold-conjugated anti-IgG antibodies, applied as negative control. Six images were taken of different sample regions with varying magnifications. The image shown on the top left is also shown in Fig. 6a. Scale bars are shown for each image.

References

1. Scheres, S. H. W. Amyloid structure determination in RELION-3.1. *Acta Crystallogr. Sect. Struct. Biol.* **76**, 94–101 (2020).
2. Jubb, H. C. *et al.* Arpeggio: A Web Server for Calculating and Visualising Interatomic Interactions in Protein Structures. *J. Mol. Biol.* **429**, 365–371 (2017).
3. Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290–296 (2017).
4. Berisio, R., Vitagliano, L., Mazzarella, L. & Zagari, A. Crystal structure of the collagen triple helix model [(Pro-Pro-Gly)₁₀]₃. *Protein Sci. Publ. Protein Soc.* **11**, 262–270 (2002).
5. Shoulders, M. D. & Raines, R. T. Collagen structure and stability. *Annu. Rev. Biochem.* **78**, 929–958 (2009).
6. Bozec, L., van der Heijden, G. & Horton, M. Collagen Fibrils: Nanoscale Ropes. *Biophys. J.* **92**, 70–75 (2007).
7. Bella, J., Eaton, M., Brodsky, B. & Berman, H. M. Crystal and Molecular Structure of a Collagen-Like Peptide at 1.9 Å Resolution. *Science* **266**, 75–81 (1994).