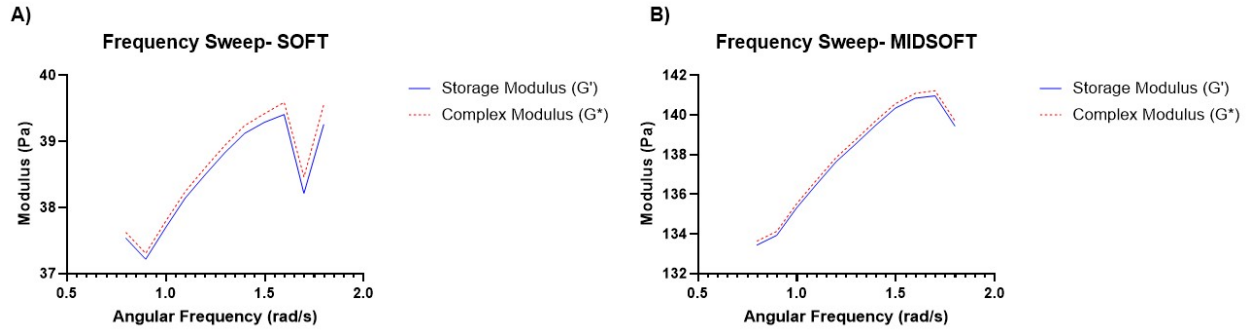
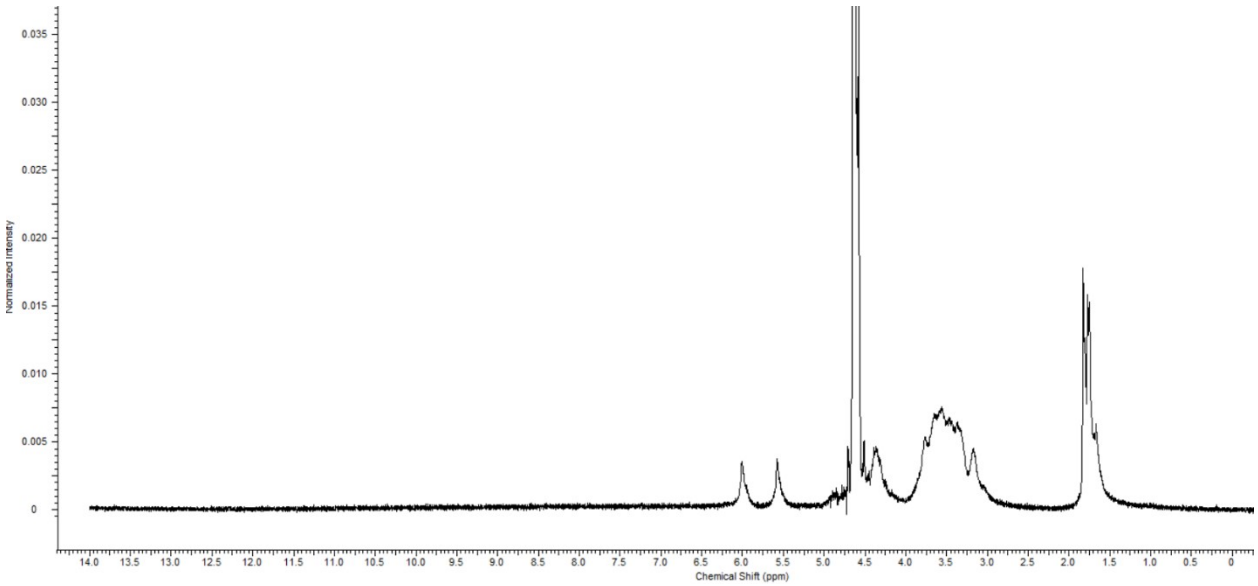
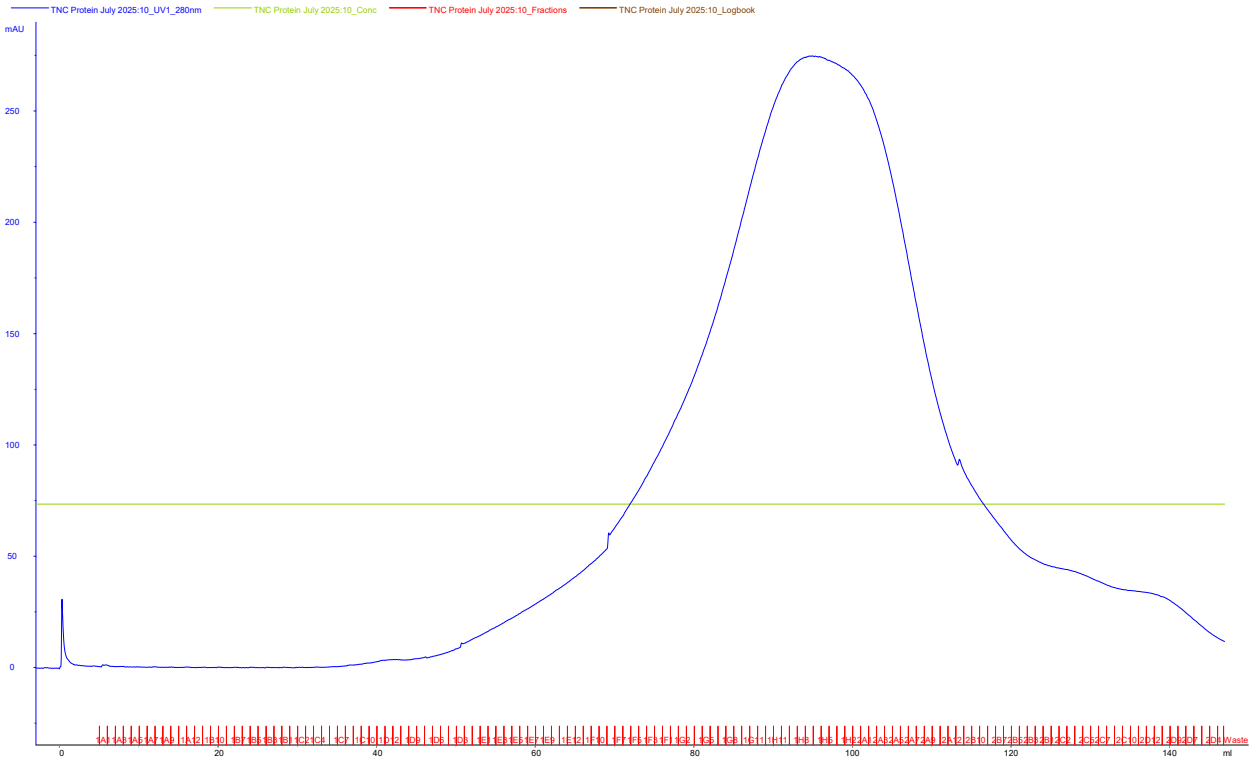
SUPPLEMENTARY FIGURES:



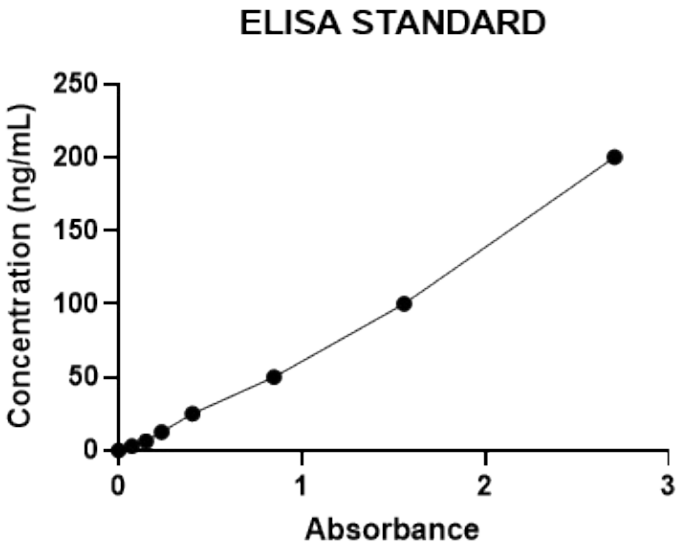
Supplementary Figure S1. Frequency-dependent rheological characterization of MeHA–PAA hydrogels. (A) Frequency sweep of *soft* MeHA–PAA hydrogels and (B) *mid-soft* MeHA–PAA hydrogels showing storage modulus (G′, solid line) and complex modulus (G*, dashed line) as a function of angular frequency (rad/s). Both formulations exhibit minimal frequency dependence and closely overlapping G′ and G* values across the tested range, indicating predominantly elastic behavior and stable network formation. Minor deviations at higher frequencies for the soft formulation are attributed to instrument sensitivity limits for low-modulus hydrogels. Data shown are representative measurements.



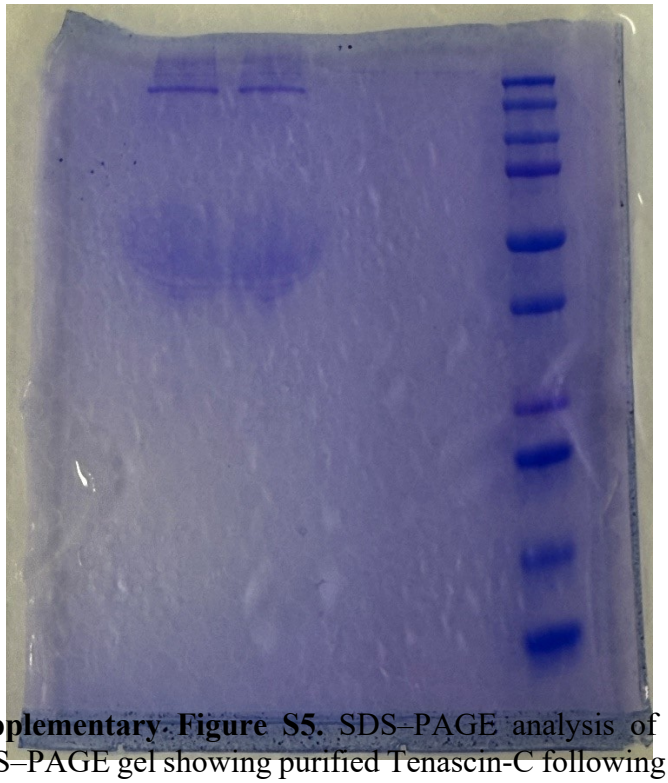
Supplementary Figure S2. Proton Nuclear Magnetic Resonance (1H NMR spectrum of methacrylated hyaluronic acid (MeHA). Characteristic vinyl proton peaks corresponding to methacrylate groups are observed at ~5.6 and ~6.1 ppm, confirming successful methacrylation of the hyaluronic acid backbone. DoM (%) = [A(δ 5.60–6.20 ppm) / 2] / [A(δ ~2.0 ppm) / 3] × 100, where A denotes integrated peak area



Supplementary Figure S3. Representative AKTA FPLC chromatogram showing the elution profile of purified Tenascin-C (TNC). UV absorbance at 280 nm (blue trace) is plotted as a function of elution volume.

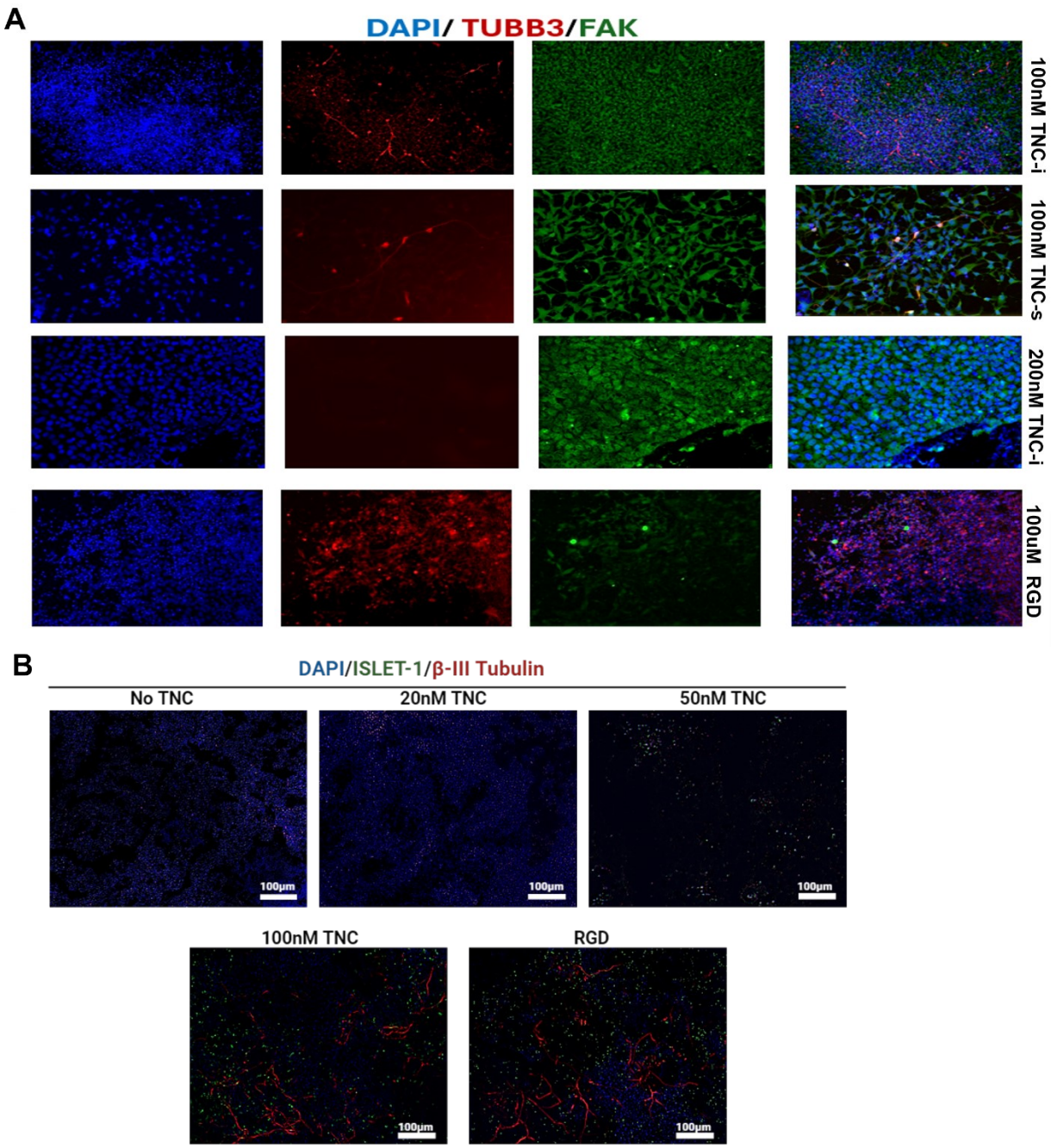


Supplementary Figure S4. ELISA standard curve used for Tenascin-C quantification. A standard curve was generated using serial dilutions of purified Tenascin-C standards and measured by absorbance according to the manufacturer's protocol. The resulting curve was used to interpolate TNC concentrations in conditioned media, purified protein fractions, and release samples. All sample values fell within the linear range of the standard curve.

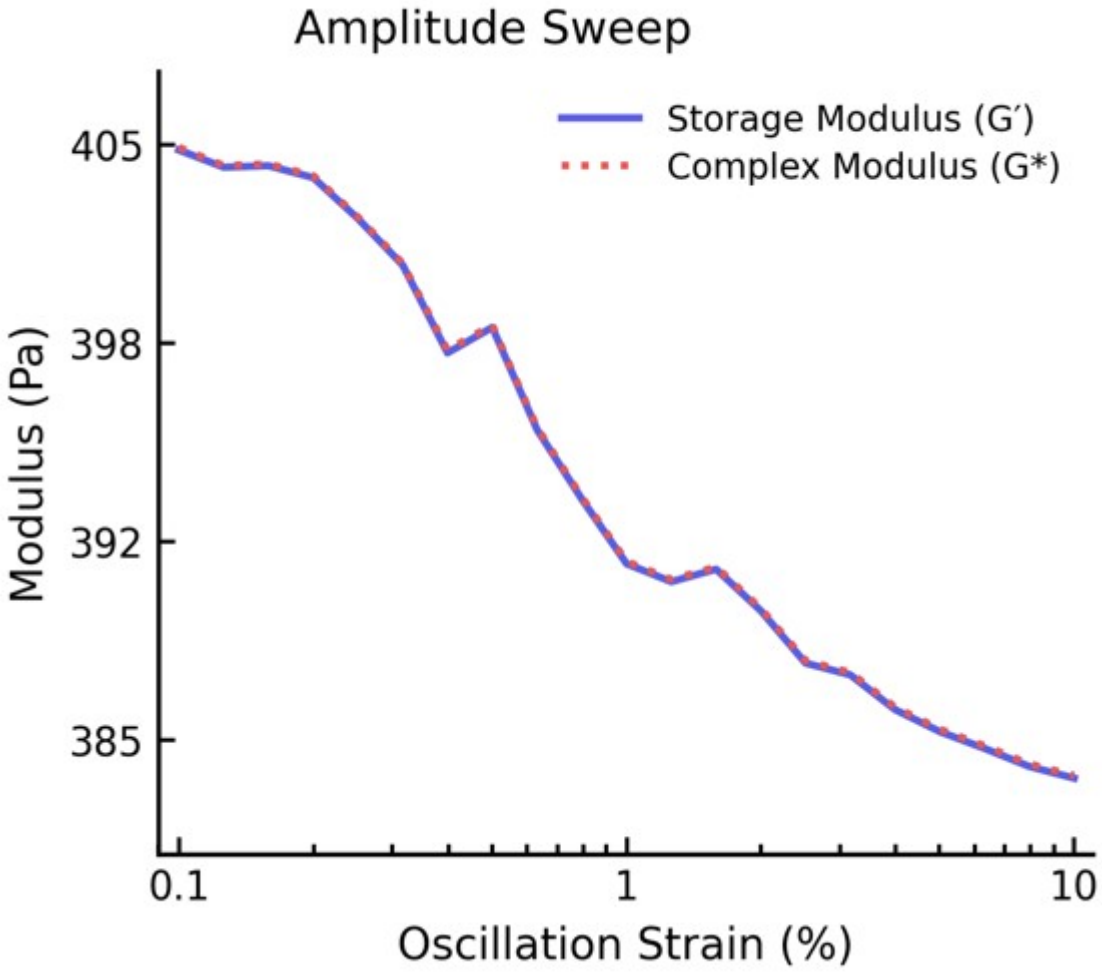


Supplementary Figure S5. SDS–PAGE analysis of purified Tenascin-C. Coomassie-stained SDS–PAGE gel showing purified Tenascin-C following ammonium sulfate precipitation and size-

exclusion chromatography. A dominant high-molecular-weight band corresponding to full-length Tenascin-C is observed with minimal lower-molecular-weight species, indicating limited degradation. Molecular weight ladder is shown on the right.



Supplementary Figure S6. Matrix-bound Tenascin-C modulates focal adhesion signaling and neuronal differentiation in 2D. (A) Representative immunofluorescence images of induced spinal cord progenitors cultured on MeHA–PAA substrates functionalized with immobilized Tenascin-C (TNC; 100 immobilized and soluble and 200 nM immobilized) or RGD (100 μM), stained for nuclei (blue), βIII-tubulin (red), and focal adhesion kinase (FAK, green). (B) Immunofluorescence images showing βIII-tubulin (red) and ISLET-1 (green) expression in cells cultured under no-ligand control, TNC (20, 50 and 100 nM), or RGD (100 μM) conditions. Scale bars: 100 μm.



Supplementary Figure S7. Amplitude sweep of 1% MeHA hydrogels showing storage modulus (G′, solid line) and complex modulus (G*, dashed line).

SUPPLEMENTARY TABLES:

Target	Antibody type	Host	Supplier	Dilution
β-III Tubulin	Primary	Rabbit polyclonal	Abcam (Cat. No. AB18207)	1:100
GFAP	Primary	Chicken polyclonal	Millipore (Cat. No. AB5541)	1:100
FAK	Primary	Sheep polyclonal	R&D Systems (Cat. No. AF4467)	1:400
ISL1	Primary	Goat polyclonal	R&D Systems (Cat. No. AF1837-SP)	1:100
Alexa Fluor (anti-rabbit)	Secondary	Donkey	Invitrogen (Cat. No. A31573)	1:500
Alexa Fluor (anti-chicken)	Secondary	Goat	Invitrogen (Cat. No. A21449)	1:500
Alexa Fluor (anti-sheep)	Secondary	Donkey	Invitrogen (Cat. No. A21098)	1:500
Alexa Fluor (anti-goat)	Secondary	Rabbit	Invitrogen (Cat. No. A21085)	1:500
Hoechst 33342	Nuclear stain	—	Invitrogen (Cat. No. H1399)	10 μM

Supplementary Table S1: Antibodies and stains used for *in vitro* immunocytochemistry

Gene	Forward (5′–3′)	Reverse (5′–3′)
GAPDH (rat)	CCAGCTCGTCCTGTAGACAA	GCCTTGACTGTGCCGTTGA
TNC (rat)	AAGAGTCGCTACAAGCTGAAG	CTGAGTCTGTGTCCTTGTCATAG
GAPDH (human)	GCCCAATACGACCAAATCC	AGCCACATCGCTCAGACAC
TUBB3 (human)	GGCCCTTTGGACATCTCTTCAG	CCTCCGTGTAGTGACCCTT
ISL1 (human)	AAACAGGGAGCTCCAGCAAAA	AAAGGACTCTTTCAGCCAAG
OLIG2 (human)	AGCTCCTCAAATCGCATCC	ATAGTCTGCAGCTTTCG
NKx6.1 (human)	TCGTTTGGCCTATTCGTTGG	TCCGAGTCCTCTTCT
Nkx6.2 (human)	TGGACAAGGACGGGAAGA	GGTACTTGGTCTGCTCGAAG
MNX1 (human)	CACCTCGCTCATGCTCAC	CTTCTGTTTCTCCGCTTCCT

Supplementary Table S2. Forward and reverse primer sequences are used for quantitative PCR (qPCR) analysis.