Supporting Information

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SI Materials and Methods

Mass Spectrometry. WT and R515A proteins were dissolved to 5 mg/mL in 0.1 M PBS (8 mM Na_2HPO_4, 2 mM NaH_2PO_4, 150 mM NaCl, pH 7.4), digested overnight with 0.05 mg/mL excision grade Lys-C (Calbiochem) at 25 °C, then analyzed with matrix-assisted laser desorption ionization time-of-flight mass spectrometry using a QSTAR XL mass spectrometer (Applied Biosystems). Peaks were assigned by comparison with expected monoisotopic peptide masses from a theoretical Lys-C digest using PeptideMass (http://web.expasy.org/peptide_mass) for singly charged peptides ($[M + H]^+$) allowing for a maximum of one missed cleavage.

Kallikrein Digest. WT and R515A were dissolved to 1 mg/mL in phosphate buffer (8 mM Na_2HPO_4, 2 mM NaH_2PO_4), incubated with 6×10^{-6} units/μL human plasma kallikrein at 37 °C for 2, 5, or 8 h, then analyzed on 12% SDS-PAGE with Mark12 unstained standards (Invitrogen).

Free Amine Quantification. Cross-linking in hydrogels was determined by quantifying the free amine groups using 2,4,6-trinitrobenzene sulfonic acid (TNBS). Hydrogels and uncross-linked solutions were treated with 1:1 volume ratio of 4% (wt/vol) sodium bicarbonate and 0.5% (vol/vol) TNBS at 40 °C for 2 h, then hydrolyzed with 3 vol of 8.9 M hydrochloric acid at 60 °C for 24 h. Sample absorbances at 415 nm were blanked against no protein controls and corrected according to hydrogel mass. The extent of cross-linking was estimated from the percent difference between the corrected absorbances of hydrogels and uncross-linked samples.

Far-UV Circular Dichroism. CD spectra of 0.15 mg/mL WT and R515A tropoelastin in 10 mM phosphate and 150 mM NaF were recorded on a Jasco J-815 spectrometer equipped with a Peltier-controlled sample chamber. Samples were scanned with a band width of 1.0 nm at 20 nm/min. Each spectrum was averaged from five scans, buffer-corrected, and smoothed using three-point adjacent averaging. Secondary structure composition was estimated from the CD spectrum using the CONTINLL (1) and CDSSTR (2) software packages with a reference set of 37 soluble proteins.

1. Roessle MW, et al. (2007) Upgrade of the small-angle X-ray scattering beamline X33 at the European Molecular Biology Laboratory, Hamburg. *J Appl Crystallogr* 40: S190–S194.

2. Konarev PV, Volkov VV, Sokolova AV, Koch MHJ, Svergun D (2003) PRIMUS—a Windows-PC based system for small-angle scattering data analysis. *J Appl Crystallogr* 36:1277–1282.


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Human   ------AAKSAAKVAAKAQLRAAAGLGAGIPGLGVGVGVPGLGVGAGVPGLGVGAGVPGFGA----------
Bovine  GAGVPAAAKSAAKAAAKAQFRAAAGLPAGVPGLGVGVGVPGLGVGVGVPGLGVGAGVPGLGA----------
Mouse   GAGSPAAAKSAAKAAAKAQYRAAAGLGAGVPGFGAGAGVPGFGAGAGVPGFGAGAGVPGFGAGAGVPGFGAGA
Rat     GAGTPAAAKSAAKAAAKAQYRAAAGLGAGVPGLGVGAGVPGFGAGAG--GFGAGAGVPGFGAGA---------
                ********.***** ****** **:**:*.*.****:*.*.*  *:*.******:**
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Fig. S1. Alignment of domains 25 and 26 of human, bovine, mouse, and rat tropoelastin showing identical (*), conserved (:) and semiconserved (.) residues. The amino acid sequences were obtained from the Elastin Sequence Database (www.compsysbio.org/elastin/). The degree of conservation was assigned by ClustalW based on the similarity score of all residues at each position, calculated using the Gonnet Pam250 matrix. The R515 residue in all sequences is in bold type.

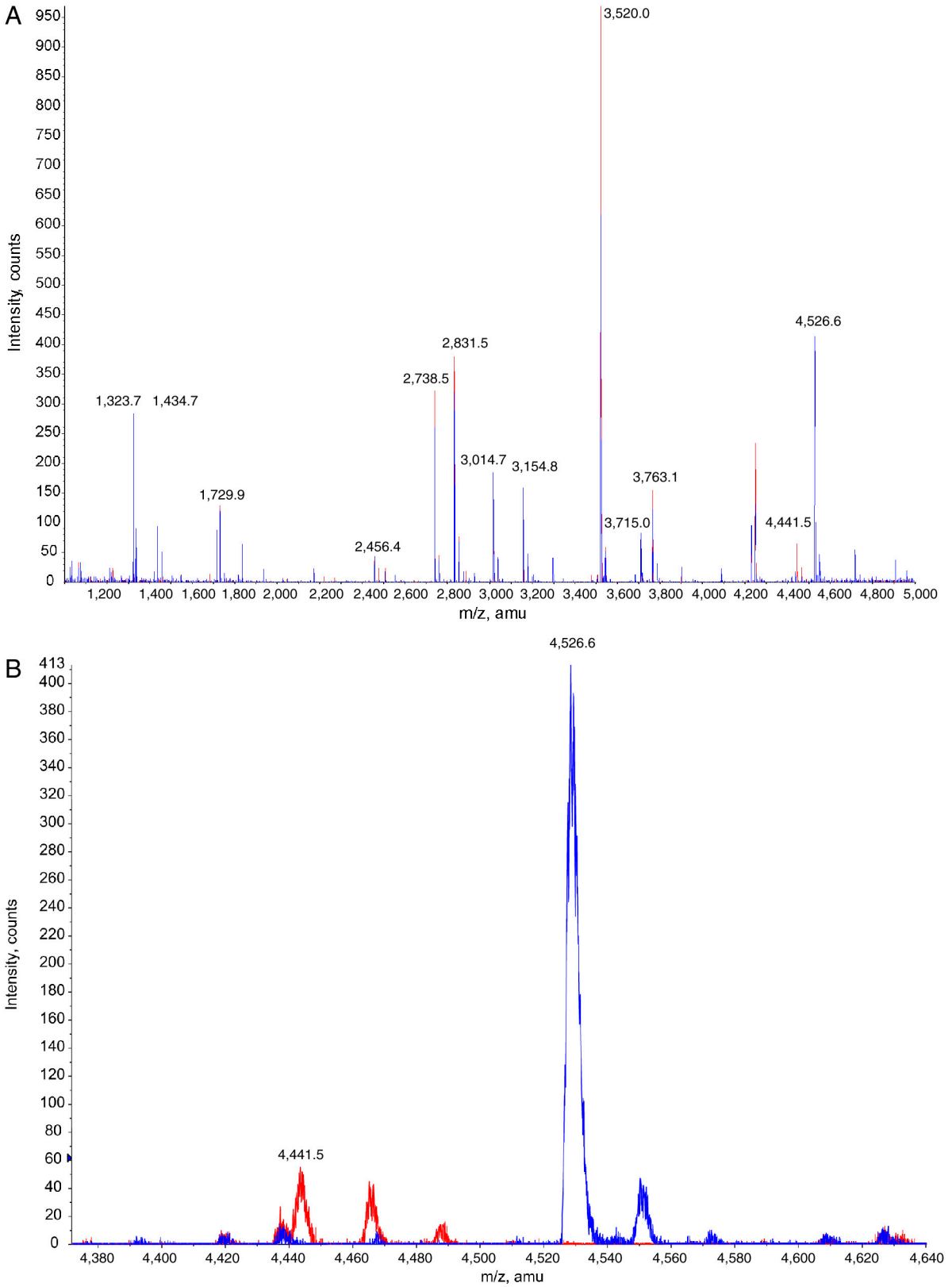

Fig. S2. Overlaid peak profiles from MALDI-TOF mass spectrometry analyses of WT (blue) and R515A (red) tropoelastin within (*A*) 0–5,000 *m/z* and (*B*) 4,400–4,600 *m/z* mass windows. All WT and R515A mass peaks overlap except for the peak assigned to tropoelastin residues 512–565. The mass shift of 85 Da from 4,526.6 *m/z* in the WT spectrum to 4,441.5 *m/z* in the R515A spectrum corresponds to the monoisotopic mass difference between arginine and alanine and is consistent with an R515A mutation in the purified R515A protein.

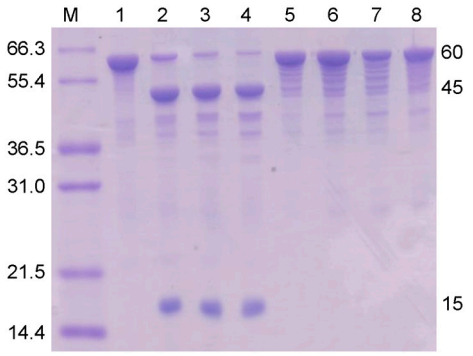

Fig. S3. WT and R515A treated with 6×10^{-6} units/μL human plasma kallikrein. The 60 kDa full-length tropoelastin and the 45 and 15 kDa cleavage products are shown. Lanes: M, protein standards in kilodaltons; 1, undigested WT; 2–4, WT digested for 2, 5, and 8 h, respectively; 5, undigested R515A; 6–8, R515A digested for 2, 5, and 8 h, respectively.

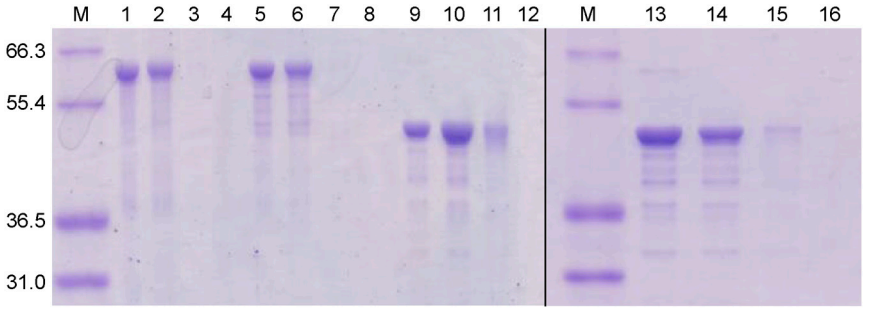

Fig. S4. SDS-PAGE of the supernatant derived after cross-linking 100 mg/mL tropoelastin with various concentrations of bis(sulfosuccinimidyl) suberate (BS3). Uncross-linked tropoelastin species (1 mg/mL) were included. Lanes: M, protein standards in kilodaltons; 1, WT; 2–4, WT with 1, 10, and 100 mM BS3, respectively, at 37 °C; 5, R515A; 6–8, R515A with 1, 10, and 100 mM BS3, respectively, at 37 °C; 9, M155n; 10–12, M155n with 1, 10, and 100 mM BS3, respectively, at 37 °C; 13, M155n; 14–16, M155n with 1, 10, and 100 mM BS3, respectively, at 45 °C.

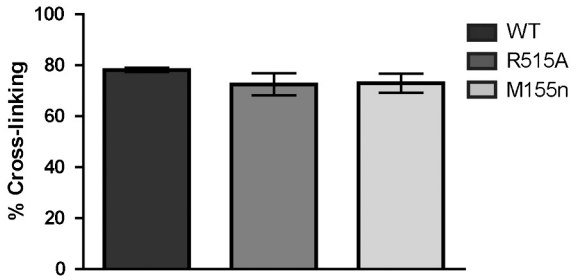

Fig. S5. Extent of cross-linking in hydrogel constructs. The amount of free amines in the hydrogels was quantified using a 2,4,6-trinitrobenzene sulfonic acid assay and compared with uncross-linked tropoelastin.

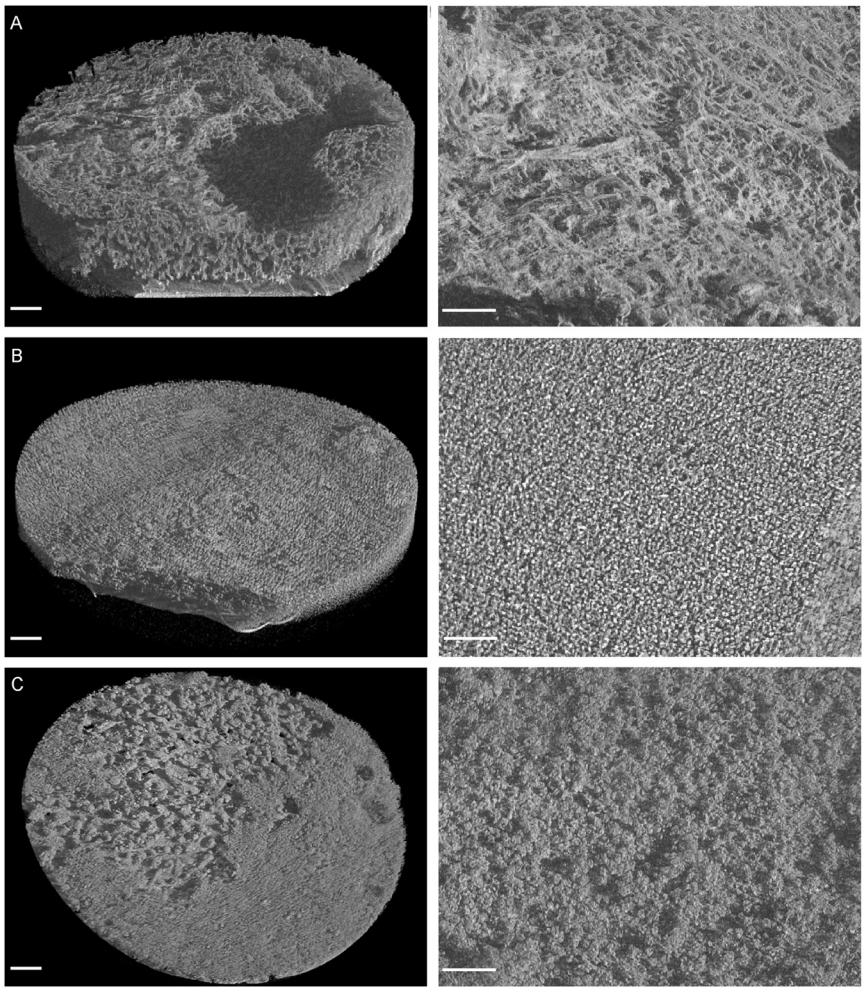

Fig. S6. Three-dimensional reconstruction of (*A*) WT, (*B*) R515A, and (*C*) M155n hydrogels by microcomputed tomography. To the right of each image is a magnified horizontal cross-section showing the structural properties of the hydrogel. (Scale bar: 0.5 mm.)

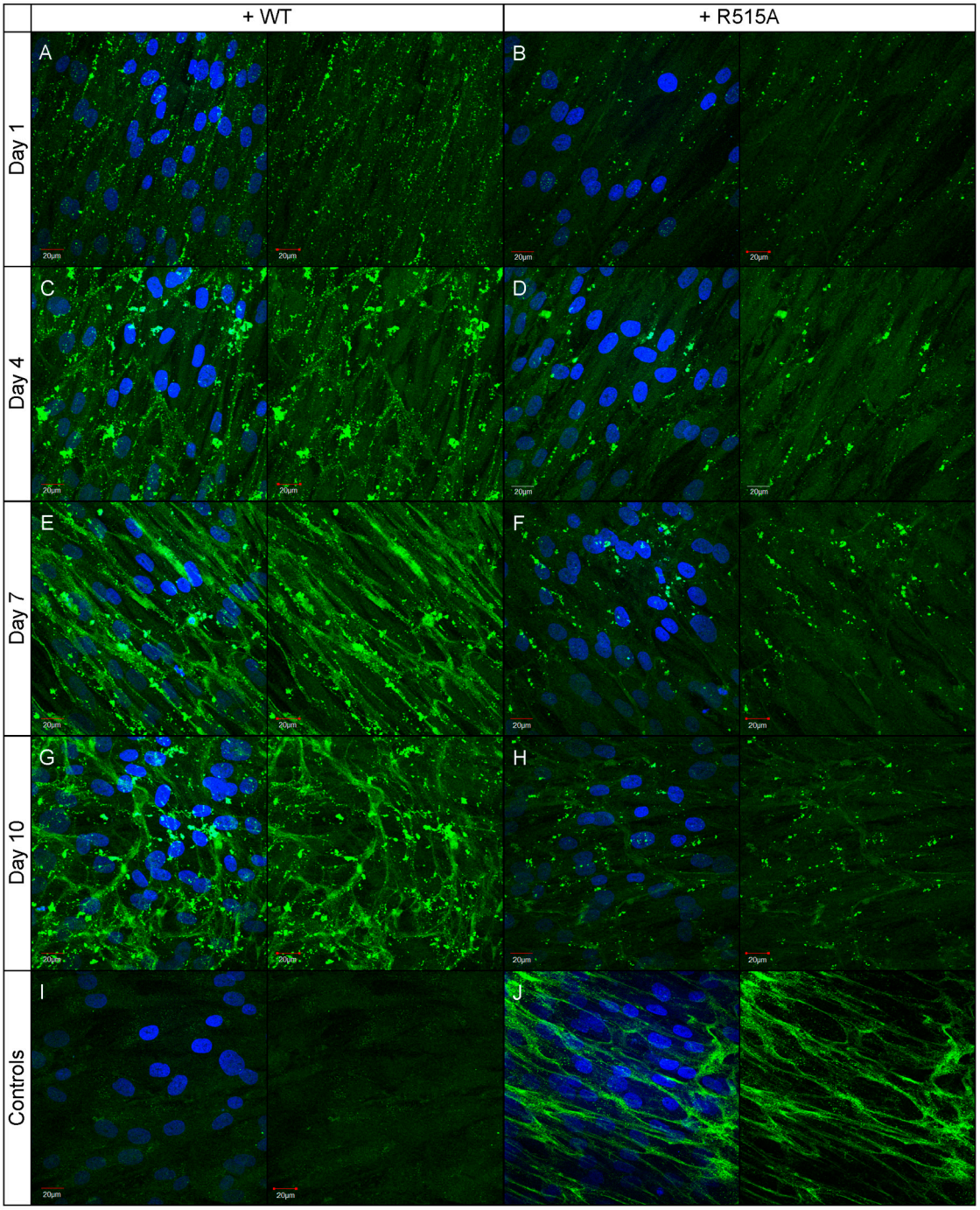

Fig. S7. (*A*–*H*) Confocal microscopy of GM3348 cells with 20 μg/mL WT or R515A tropoelastin added to the culture media 10 d after seeding. The cells were fixed at 1, 4, 7, and 10 d after tropoelastin addition. Elastin fibers were stained with mouse anti-elastin BA4 primary antibody and FITC-conjugated goat anti-mouse secondary antibody. Cell nuclei were stained with DAPI. (*I*) GM3348 cells without tropoelastin. (*J*) Fibrillin-1 fibers in GM3348 cells 10 d after seeding prior to tropoelastin addition, stained with mouse anti-fibrillin-1 primary antibody, and FITC-conjugated goat anti-mouse antibody. Each pair of images represents the same field of view; the left image shows merged signals from the DAPI and FITC channels, and the right image shows fluorescence from the FITC channel only.

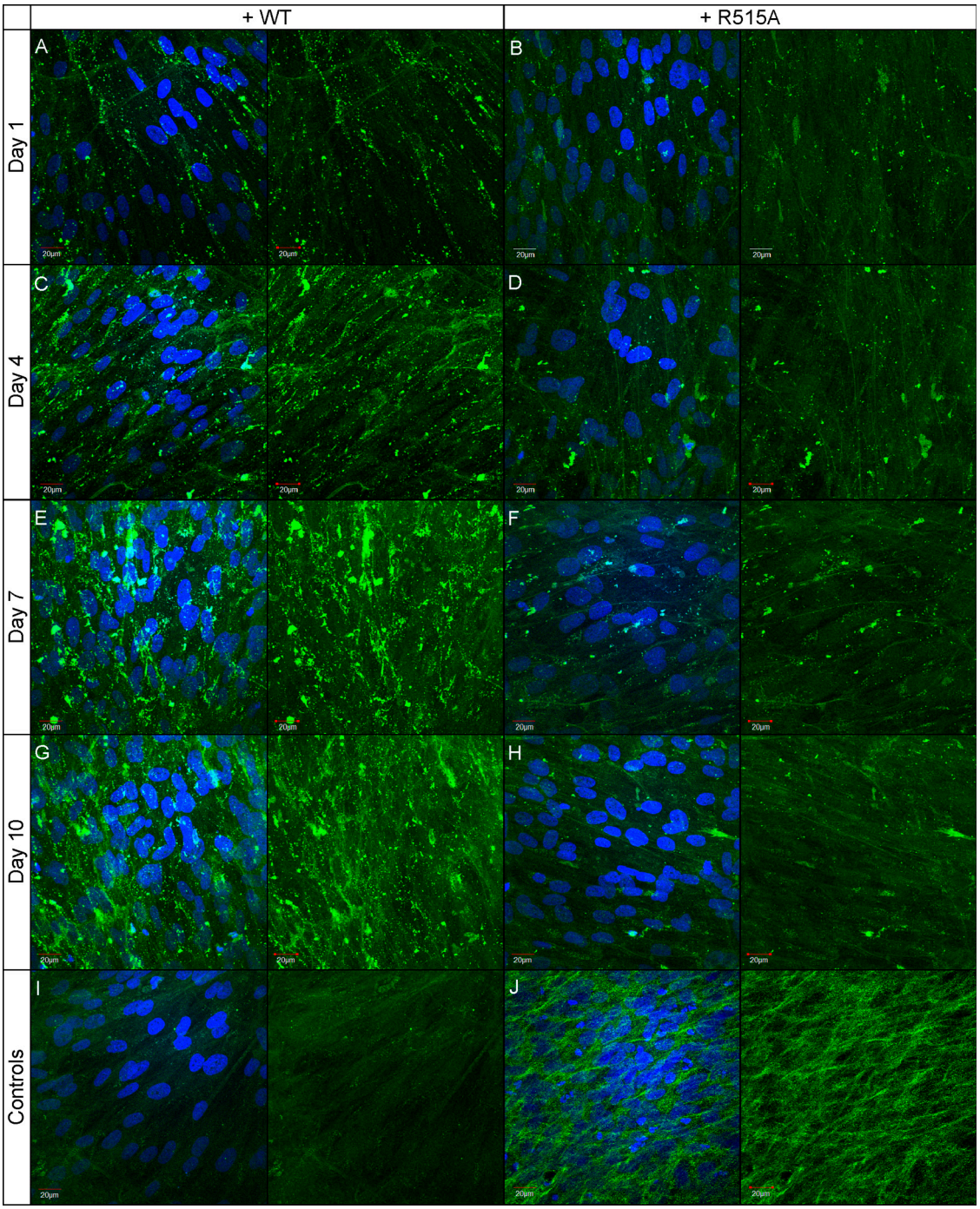

Fig. S8. (*A–H*) Confocal microscopy of NHF8909 cells with 20 μg/mL WT or R515A tropoelastin added to the culture media 10 d after seeding. The cells were fixed at 1, 4, 7, and 10 d after tropoelastin addition. Elastin fibers were stained with mouse anti-elastin BA4 primary antibody and FITC-conjugated goat anti-mouse secondary antibody. Cell nuclei were stained with DAPI. (*I*) NHF8909 cells without tropoelastin. (*J*) Fibrillin-1 fibers in NHF8909 cells 10 d after seeding prior to tropoelastin addition, stained with mouse anti-fibrillin-1 primary antibody and FITC-conjugated goat anti-mouse antibody. Each pair of images represents the same field of view; the left image shows merged signals from the DAPI and FITC channels, and the right image shows fluorescence from the FITC channel only.

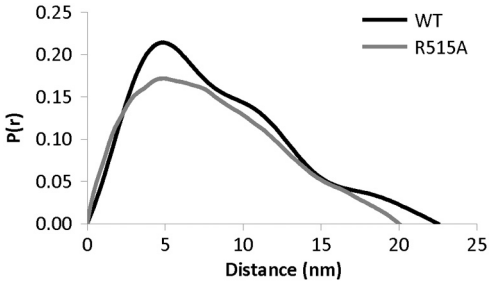

Fig. S9. Pair distribution function calculated from WT and R515A small-angle X-ray scattering datasets. The curves describe the paired set of distances between all electrons within the macromolecular structure (maxima at 5 nm).

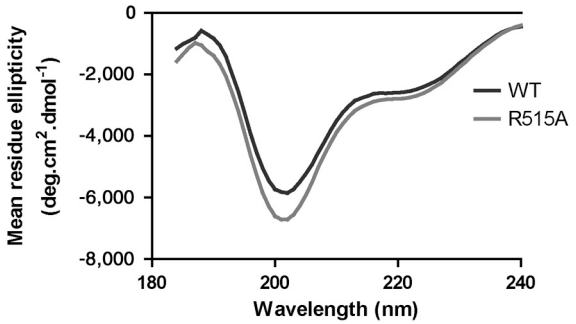

Fig. S10. Far-UV circular dichroism spectra of WT and R515A tropoelastin. The spectra show identical features, including minima at approximately 202 and 220 nm. Small differences in signal intensity likely arise from minor differences in protein concentration.

Table S1. Secondary structure composition of WT and R515A tropoelastin calculated using CONTINLL and CDSSTR software

		Ahel	BSht	Turn	PP2	UNR
WT	CONTINLL	6	26	12	11	45
	CDSSTR	3	27	15	9	46
	avg	**5 ± 1.5**	**26 ± 0.3**	**13 ± 1.4**	**10 ± 1.0**	**45 ± 0.5**
R515A	CONTINLL	7	25	12	11	46
	CDSSTR	4	25	14	10	47
	avg	**6 ± 1.4**	**25 ± 0.2**	**13 ± 1.0**	**10 ± 0.7**	**46 ± 0.5**

Ahel, α-helix; BSht, β-sheet; Turn, turn structure; PPT, poly(proline)II structure; UNR, unordered. Mean values are expressed as percentages ± standard error.