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Supplementary Information for

Spatiotemporal regulation of PEDF signaling by type I collagen remodeling

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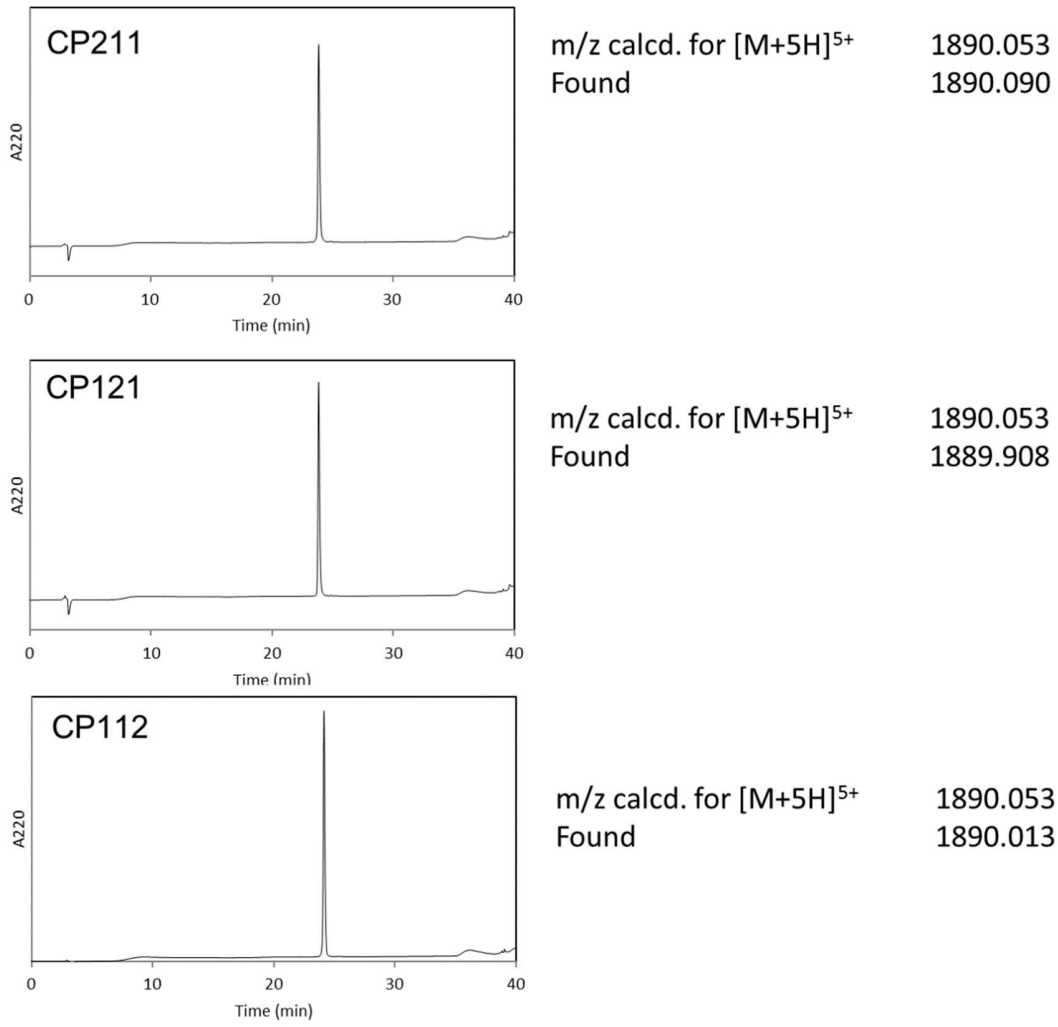

Fig. S1. Reverse phase-high performance liquid chromatography (RP-HPLC) profiles and electrospray ionization-time of flight mass spectrometry (ESI-TOF MS) analysis data of the collagen peptides for CP211, CP121, and CP112 in the top, middle, and bottom panels, respectively. Column: Cosmosil 5C18-ARII (4.6 i.d. × 250 mm). Gradient: 10%–30% CH_3CN in 0.05% TFAaq. over 30 min at 60 °C. Flow rate: 1.0 mL/min. For each collagen peptide, the result of the mass analysis is shown to the right of the graph. Mass spectra were recorded with a Bruker micrOTOF-2focus spectrometer in the positive ion detection mode.

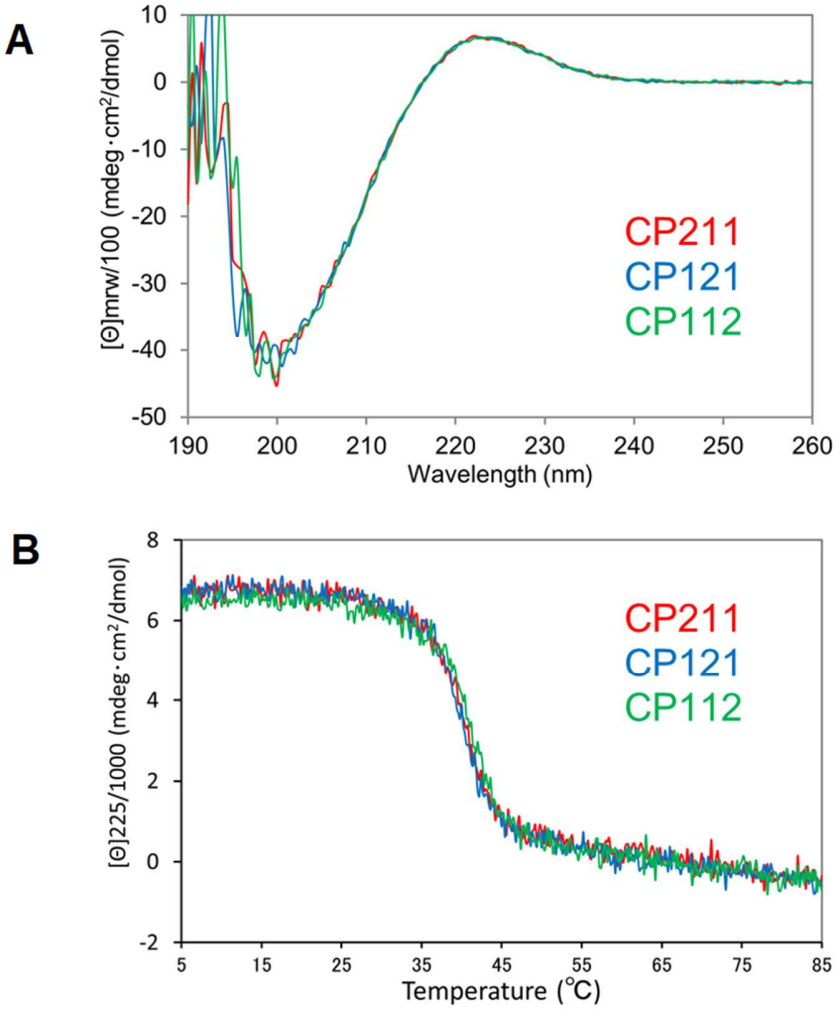

Fig. S2. Circular dichroism (CD) analysis of the collagen peptides, CP211, CP121, and CP112. (A) CD spectra measured at 4°C and (B) thermal denaturation profiles of CP211 (red), CP121 (blue), and CP112 (green).

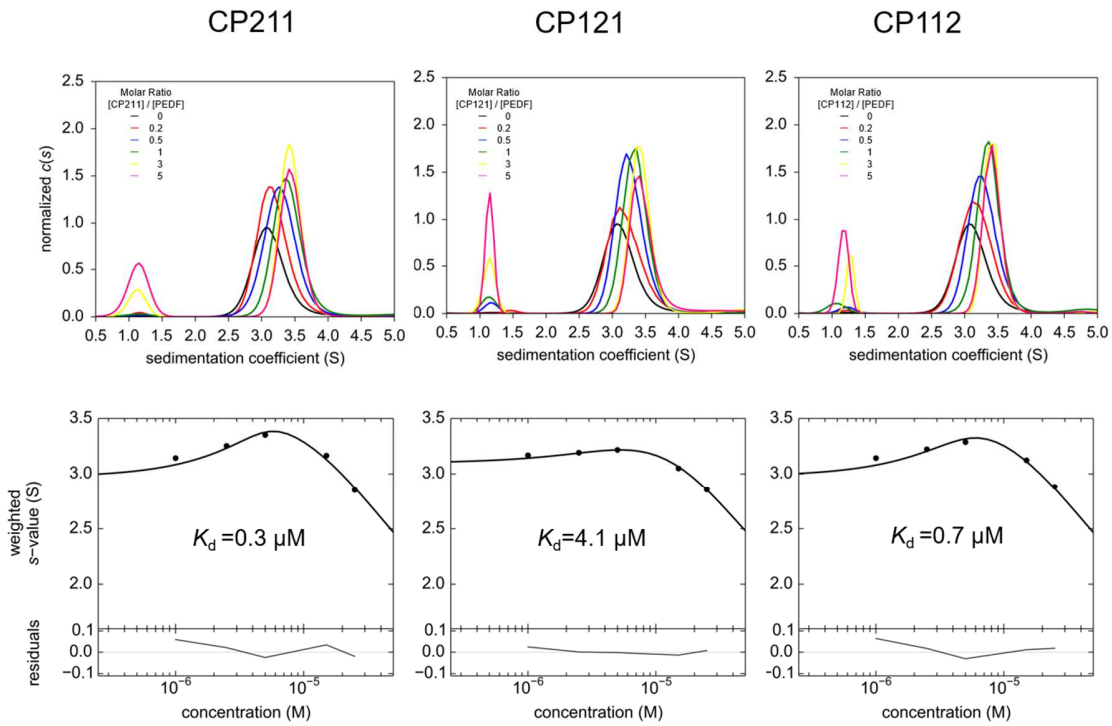

Fig. S3. Interactions between PEDF and collagen peptides, CP211, CP121, and CP112, analyzed by sedimentation velocity analytical ultracentrifugation (SV-AUC). Upper panels show the overlay of the distributions of the sedimentation coefficients (S) of collagen peptide/PEDF mixtures measured at molar ratios of 0, 0.2, 0.5, 1, 3, and 5. Lower panels show the concentration dependence of weight-average sedimentation coefficients (S) of collagen peptide/PEDF mixtures (black circles). For each case, a non-linear fitting (black line) was performed to determine the dissociation constant (K_d) value. The K_d value for each collagen peptide is shown inside each lower panel.

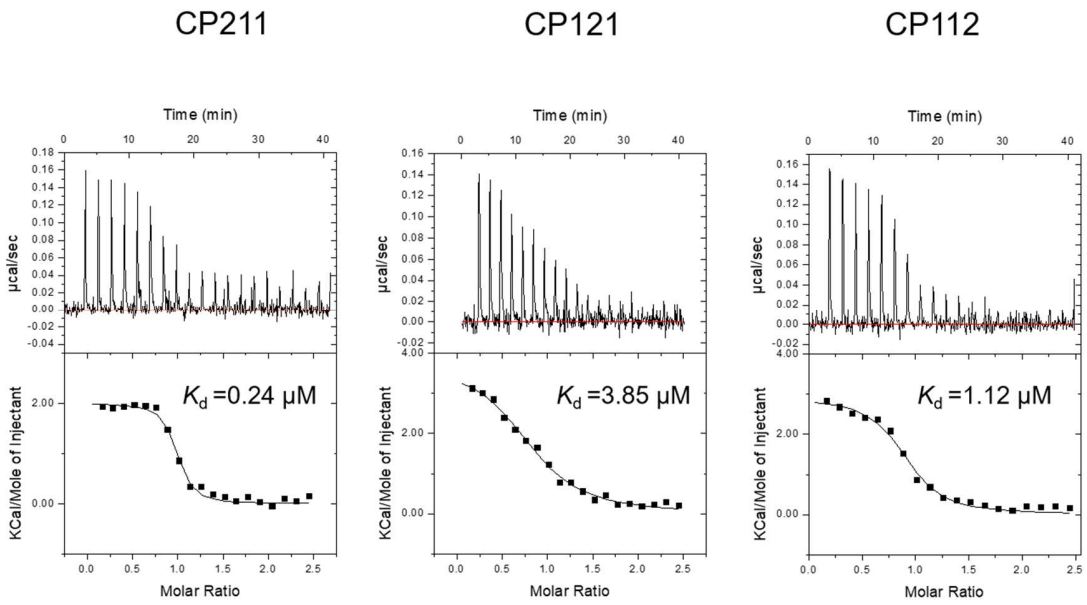

Fig. S4. Interactions between PEDF and collagen peptides, CP211, CP121, and CP112, analyzed by isothermal titration calorimetry (ITC). Left, middle, and right, ITC profiles of the titration of the collagen peptides CP211, CP121, and CP112, respectively, with mouse PEDF. For all experiments: top panel, raw titration data for collagen peptide injected into the mouse PEDF; bottom panel, integrated heat measurements for each titration. Thermograms were analyzed using Origin 7.0 software (MicroCal Inc., USA). The K_d value for each collagen peptide is shown inside each lower panel.

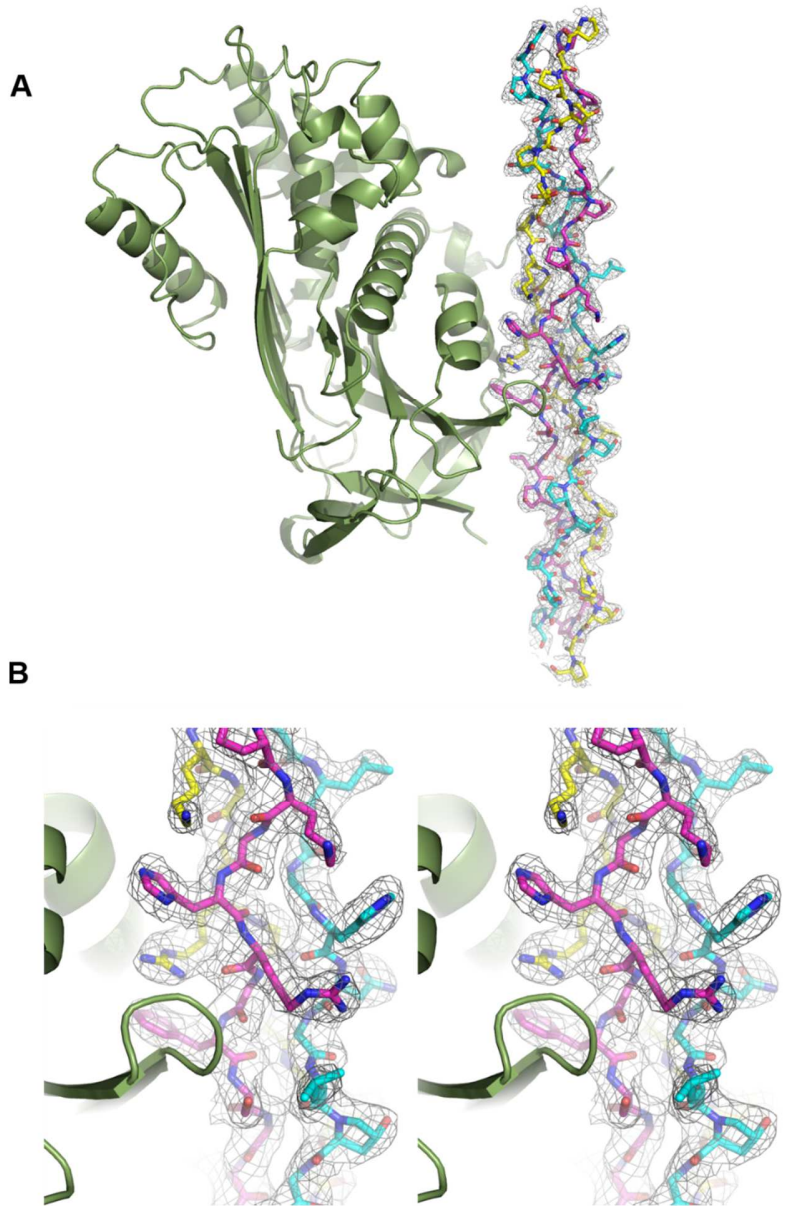

Fig. S5. Electron density map for the collagen peptide CP211 in the PEDF-CP211 complex structure. (A) The 2Fo-Fc electron density map (gray) is shown for CP211 at a contour level of 1.0 σ. (B) Stereo view of the 2Fo-Fc electron density map at the PEDF-CP211 binding interface. In the triple helix, the leading, middle, and trailing chains are shown as stick models and colored light blue, yellow, and purple, respectively.

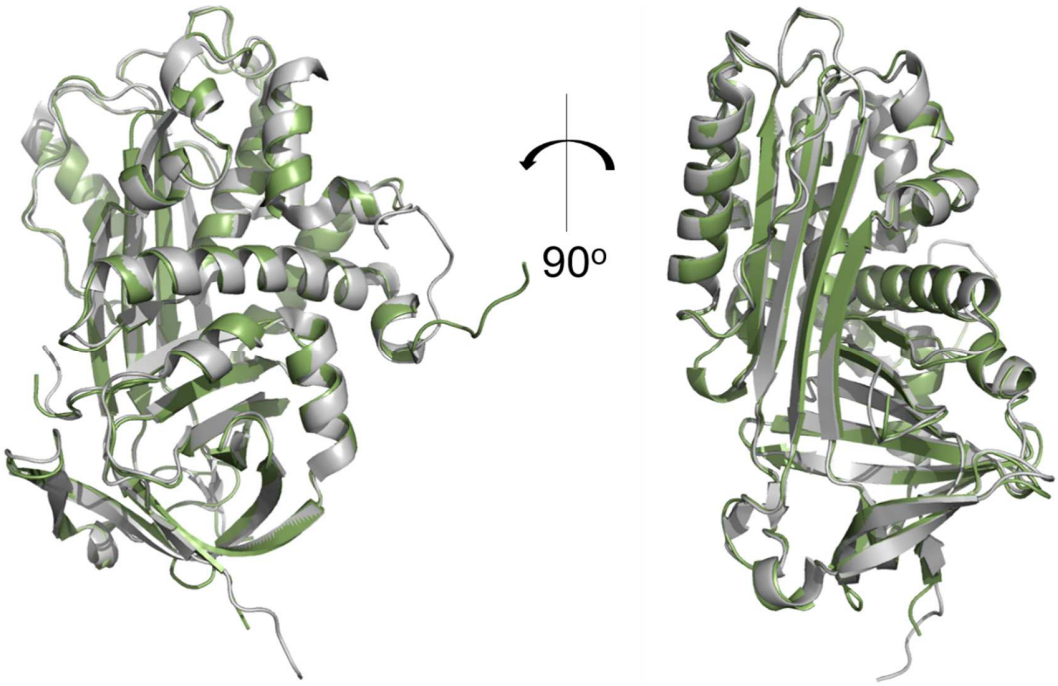

Fig. S6. Structural comparison between apo and collagen-bound PEDF. Superposition of the apo human PEDF structure (PDB ID code 1IMV) (gray) on the mouse PEDF structure (green) complexed with CP211.

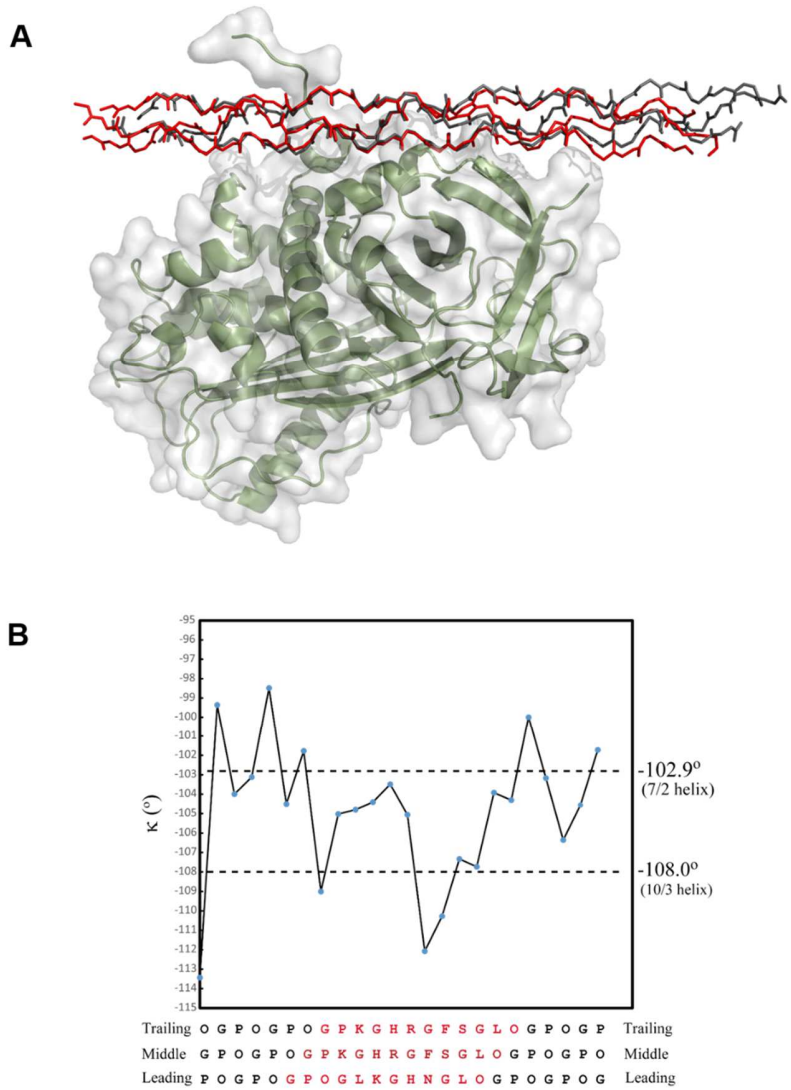

Fig. S7. Structure of CP211 in the PEDF-CP211 complex. Top panel, superposition of CP211 in the complex and collagen model peptide (Pro-Hyp-Gly)$_9$ (PDB ID code 3b0s). Backbone structures of CP211 and (Pro-Hyp-Gly)$_9$ are shown and colored red and gray, respectively. Bottom panel, internal triple helical twist (κ) diagram for collagen peptide CP211 in complex with PEDF. Each κ value was calculated as reported previously (1). The two dashed horizontal lines show the internal twist values of ideal helices with 7/2 (-102.9 °) and 10/3 (-108.0 °) screw symmetry.

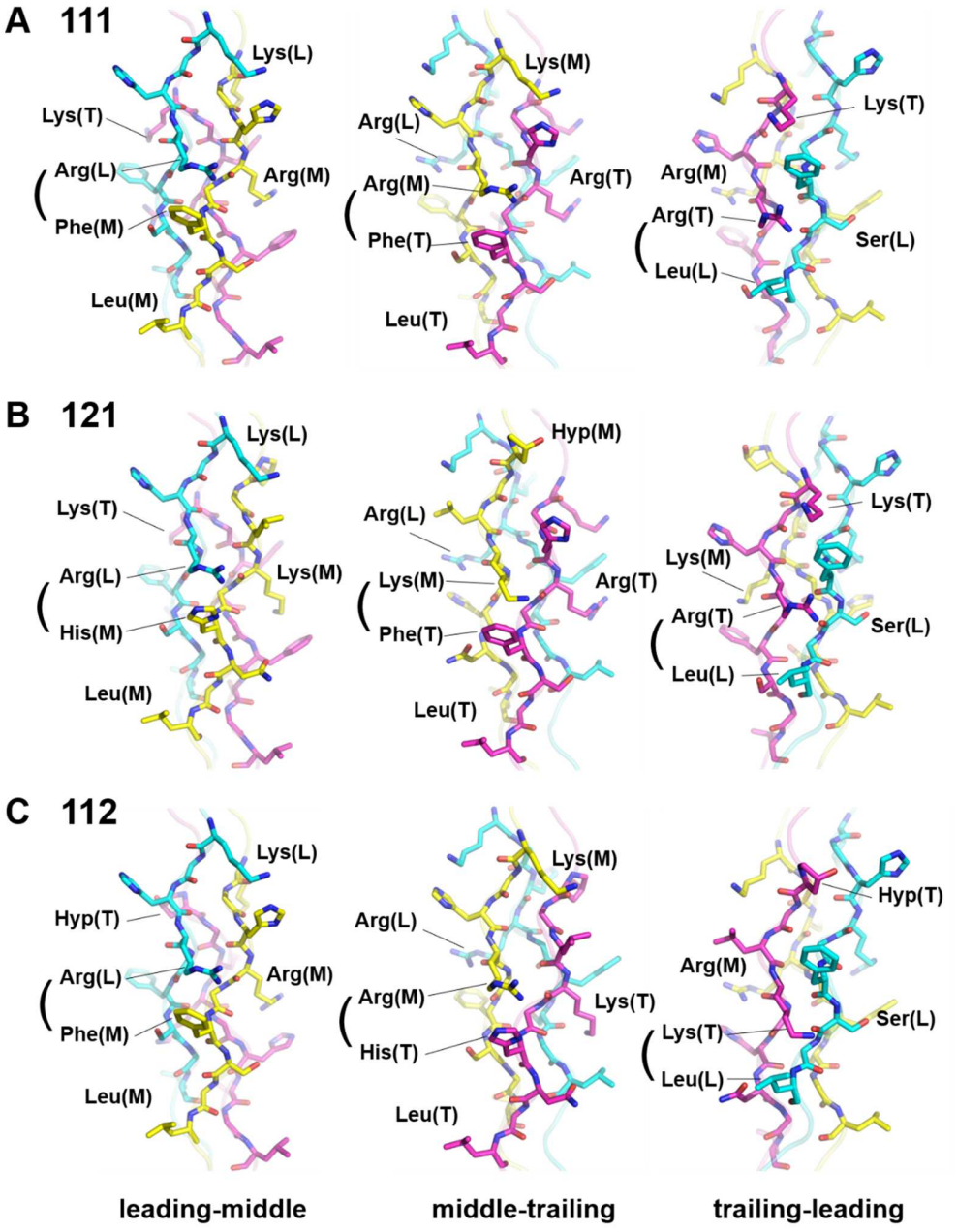

Fig. S8. Molecular models for the PEDF-binding regions of triple helices with chain compositions α1α1α1 (111), α1α2α1 (121), and α1α1α2 (112). For each peptide, three different orientations, each rotated by 120° about the vertical axis and highlighted leading-middle chains (left panel), middle-trailing chains (middle panel), and trailing-leading chains (right panel) are shown. For each orientation, surface residues are labeled. The leading, middle, and trailing chains are colored light blue, yellow, and magenta, respectively.

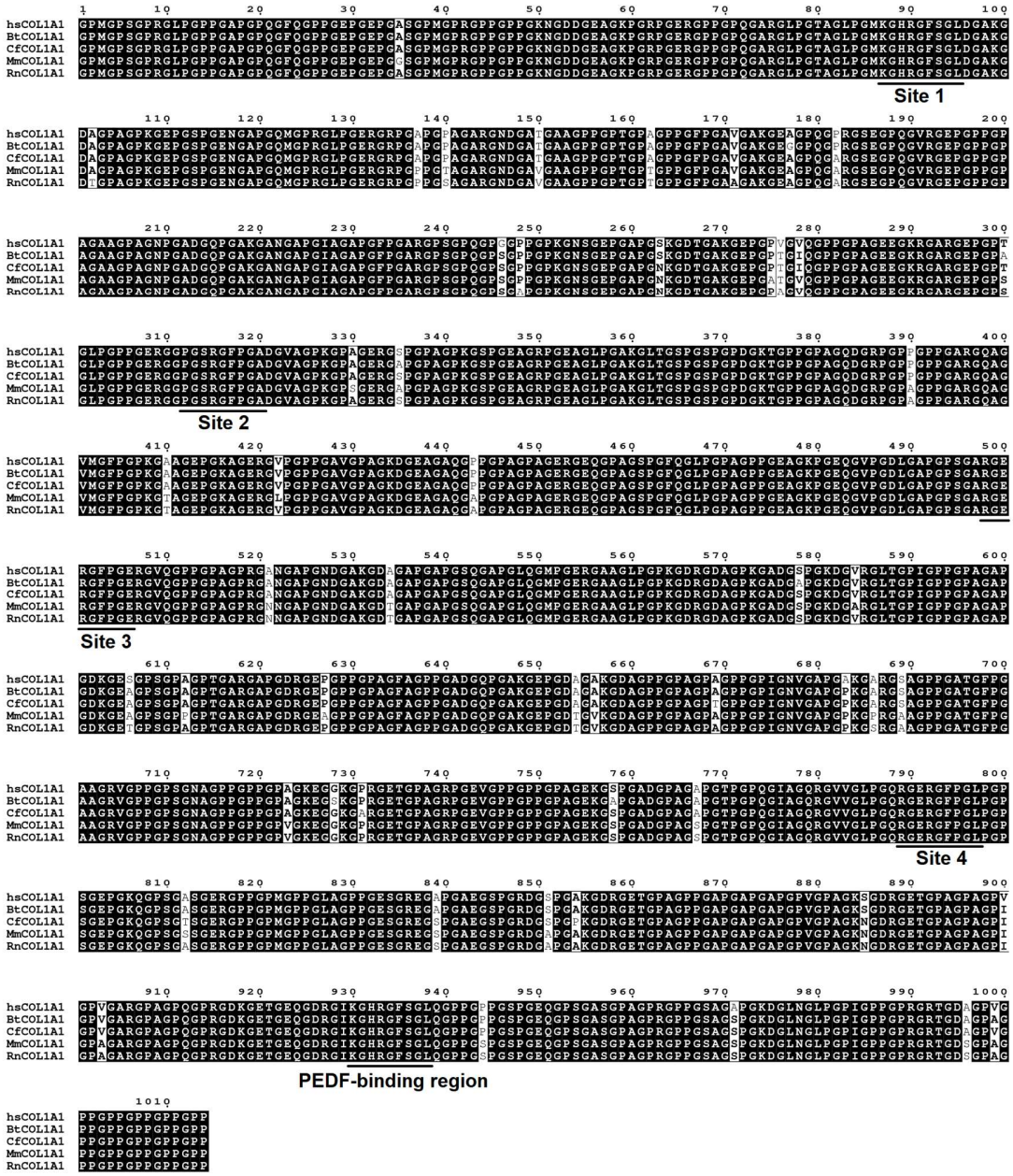

Fig. S9. Sequence alignment of type I collagen α1 chains: *Homo sapiens* (hs) AAH36531.1, *Bos taurus* (Bt) AAI05185.1, *Canis lupus familiaris* (Cf) NP_001003090.1, *Mus musculus* (Mm) AAH50014.1, and *Rattus norvegicus* (Rn) NP_445756.1.

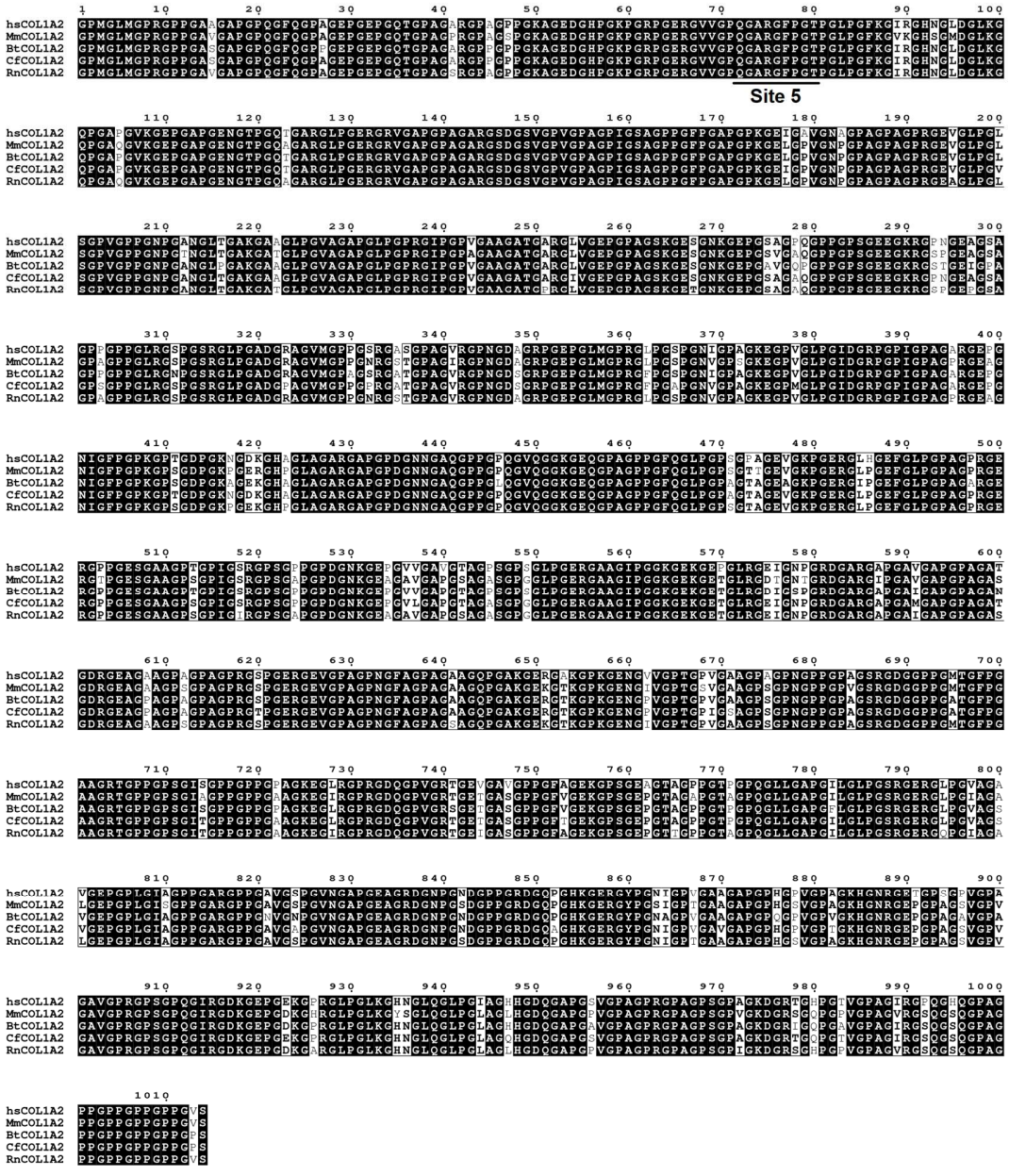

Fig. S10. Sequence alignment of type I collagen α2 chain: *Homo sapiens* (hs) AAH54498.1, *Bos taurus* (Bt) AAI49096.1, *Canis lupus familiaris* (Cf) NP_001003187.1, *Mus musculus* (Mm) AAH42503.2, and *Rattus norvegicus* (Rn) NP_445808.1.

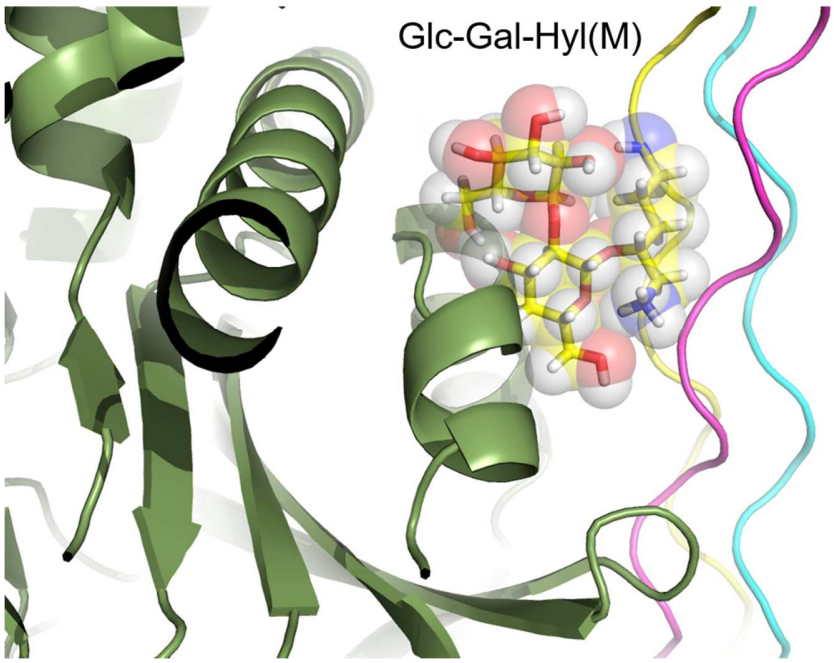

Fig. S11. Steric clash between glycosylated CP211 and mouse PEDF. The lysyl residue at the middle chain of CP211, which is glucosylgalactosylated, in the PEDF-CP211 complex crystal structure is shown as a stick-and-sphere model. The glycosyl moiety was modeled with the topology of α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl (Glc-Gal) moiety, composed by the Glycam Carbohydrate Builder (http://glycam.org/, Univ. of Georgia). The Glc-Gal moiety was introduced on the δ-carbon atom of the lysyl residue of the middle chain of CP211.

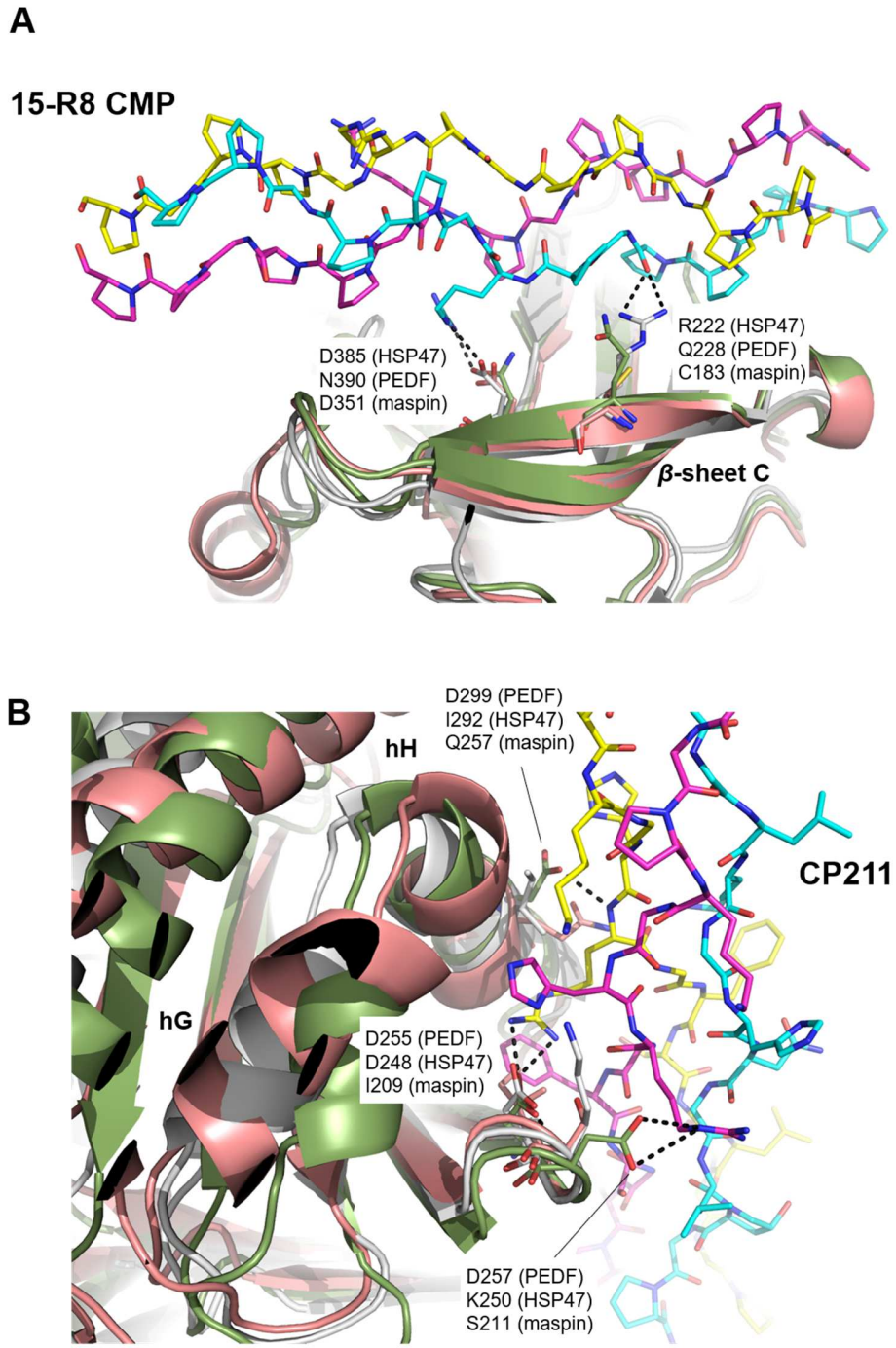

Fig. S12. Comparison of collagen-binding modes among collagen-binding serpins. (A) Superposition of the collagen-binding region of HSP47 in complex with the homotrimeric collagen peptide, Ac-$(PPG)_2$-PRG-$(PPG)_2$-NH_2 (15-R8 CMP) (PDB ID code 4AU2) and the corresponding region of PEDF in complex with collagen peptide CP211 (this study) and maspin in an apo state (PDB ID code 1XU8). The residues Arg222 and Asp385 of HSP47 (corresponding to Gln228 and

Asn390 of PEDF, and Cys183 and Asp351 of maspin), previously shown to be critical for collagen recognition (2), are shown as sticks. (B) Superposition of collagen-binding regions of PEDF in complex with CP211 and the corresponding region of HSP47 in complex with 15-R8 CMP and maspin in an apo state. The amino acid residues Asp255, Asp257, and Asp299 of PEDF (corresponding to Asp248, Lys250, and Ile292 of HSP47, and Ile209, Ser211 and Gln257 of maspin), previously shown to be critical for collagen recognition (3) and also confirmed in the current complex structure of PEDF-CP211, are shown as sticks. HSP47, PEDF, and maspin are colored white, green, and magenta, respectively. The leading (L), middle (M), and trailing (T) chains of the collagen peptides 15-R8 CMP and CP211 are colored light blue, yellow, and magenta, respectively.

Table S1. Data collection and refinement statistics.

	mouse PEDF-CP211 complex
Data collection	
Space group	$C222_1$
Cell dimensions	
a, *b*, *c* (Å)	95.38, 147.77, 91.55
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	50.00-2.47 (2.51-2.47) *
R_{merge}	0.12 (0.86)
$I / \sigma I$	20.94 (1.59)
Completeness (%)	95.6 (75.3)
Redundancy	7.0 (5.7)
Refinement	
Resolution (Å)	47.69-2.48
No. reflections	22,268
R_{work} / R_{free}	0.223/0.282
No. atoms	
Protein	3,439
Ligand/ion (SO_4)	15
Water	82
B-factors (Å^2)	
Protein	66.91
Ligand/ion (SO_4)	85.15
Water	63.75
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.037

*Values in parentheses are for highest-resolution shell.

SI References

1. J. Bella, A new method for describing the helical conformation of collagen: dependence of the triple helical twist on amino acid sequence. *J. Struct. Biol*. **170**, 377-391 (2010).
2. C. Widmer et al., Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 13243-13247 (2012).
3. N. Yasui et al., Dual-site recognition of different extracellular matrix components by anti-angiogenic/neurotrophic serpin, PEDF. *Biochemistry*. **42**, 3160-3167 (2003).