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Supplementary Materials for

Clots reveal anomalous elastic behavior of fiber networks

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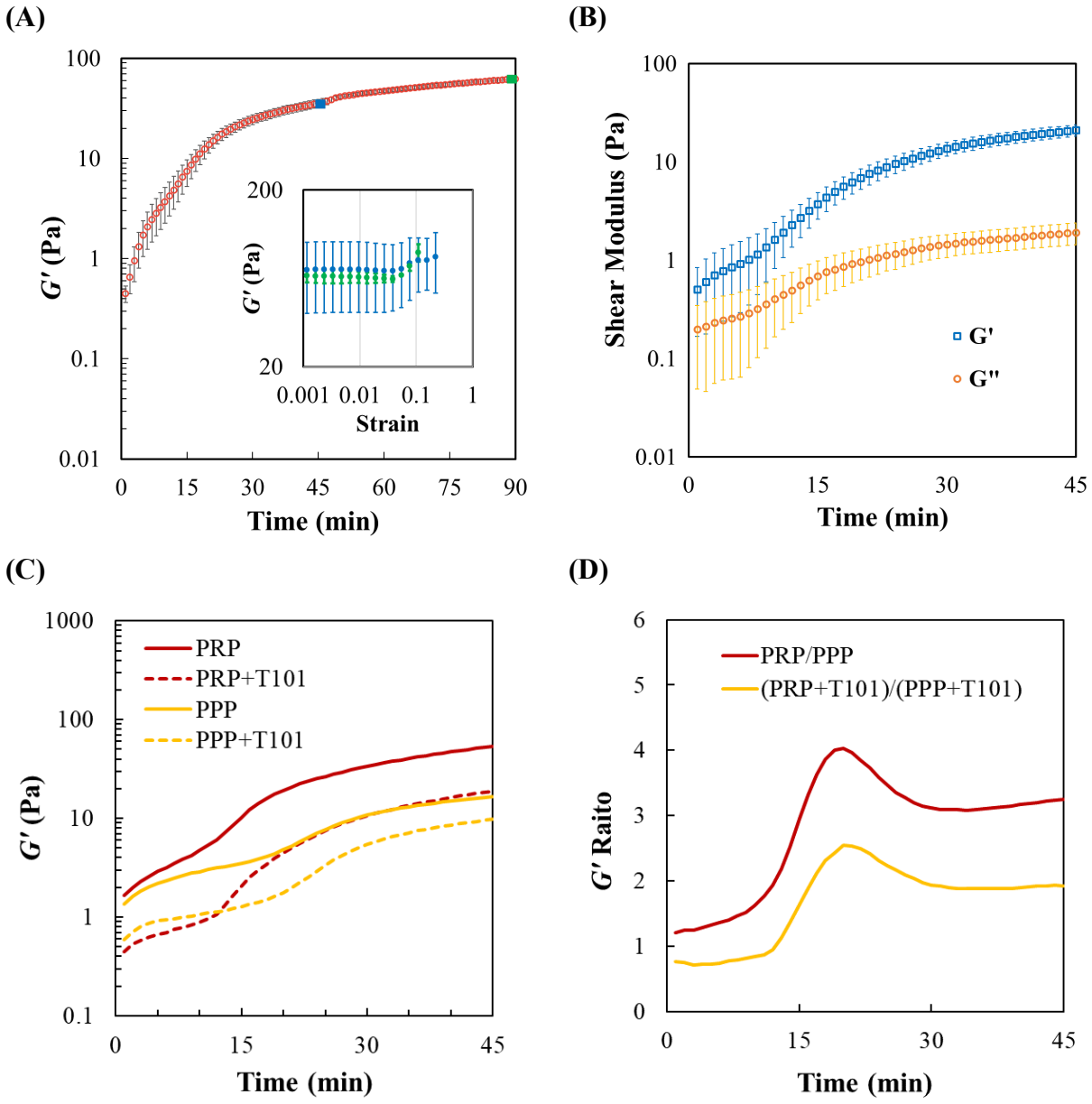

Fig. S1. Gelation of PPP and PRP clots

(A) Representative gelation profile of platelet poor plasma (PPP). The gelation plateaus by 45 min and increases slowly afterward. The response of the clots gelled for 45 min and 90 min (inset) were nearly identical (3 donors). **(B)** At 45 min, *G"* attains a saturation value that is more than one-log lower than *G'*, indicating complete gelation (5 donors). **(C)** Representative gelation profiles for platelet poor plasma (PPP) and platelet rich plasma (PRP) clots, showing elastic storage moduli (*G'*) vs. time for clots prepared with and without T101 as a crosslinking inhibitor. **(D)** After ~30 min, the average relative increase in *G'* associated with the presence of platelets (i.e., ratio of PRP/PPP) was approximately threefold for crosslinked fibrin clots and approximately twofold for the uncrosslinked (T101-treated) case.

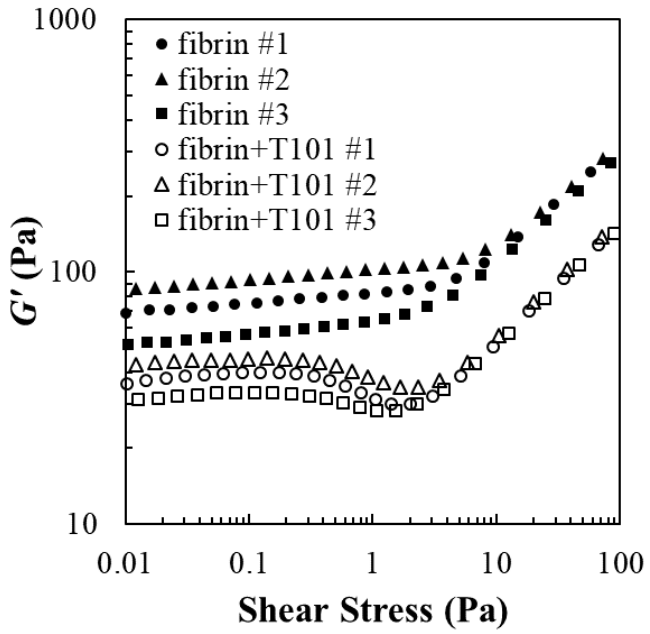

Fig. S2. Shear rheology of pure fibrin networks

Elastic storage modulus G' vs. shear stress for fibrin gels, comparing crosslinked and uncrosslinked fibrin, showing $n = 3$ identically prepared replicates for each case. Fibrin gels were formed using 3 mg/mL fibrinogen with 1 U/mL thrombin and 20 mM $CaCl_2$. For the uncrosslinked gels, 100 μM T101 was added as an inhibitor. Both types of fibrin gels are stress-stiffening. However the uncrosslinked case exhibits a dip near 1 Pa, which is similar to the model-predicted behavior and T101-treated PPP data in the main text (**Fig. 2**).

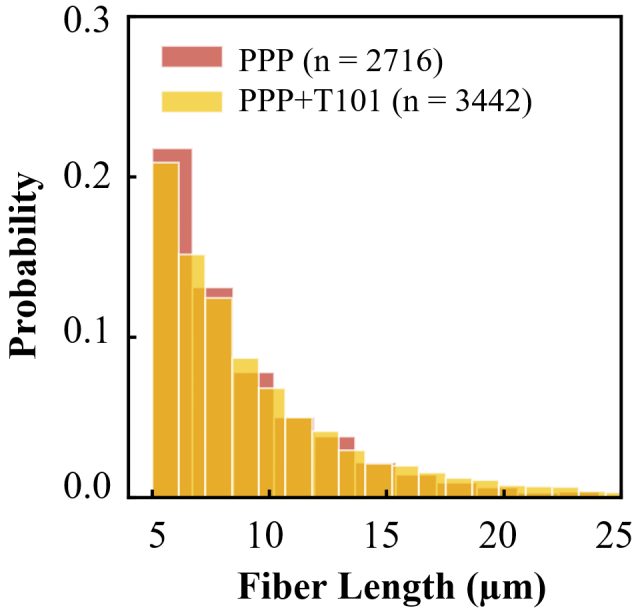

Fig. S3. Distribution of fiber lengths in crosslinked and uncrosslinked PPP clots.
Fiber lengths for PPP clots with and without T101 were determined from a set of fluorescence microscopy images at equal magnification (3 images for each case). A threshold of 5 µm was used to filter artifactually short segments and image fragments. The histograms reveal that there is no substantial difference in the distribution of fiber lengths between crosslinked and uncrosslinked fibrin.

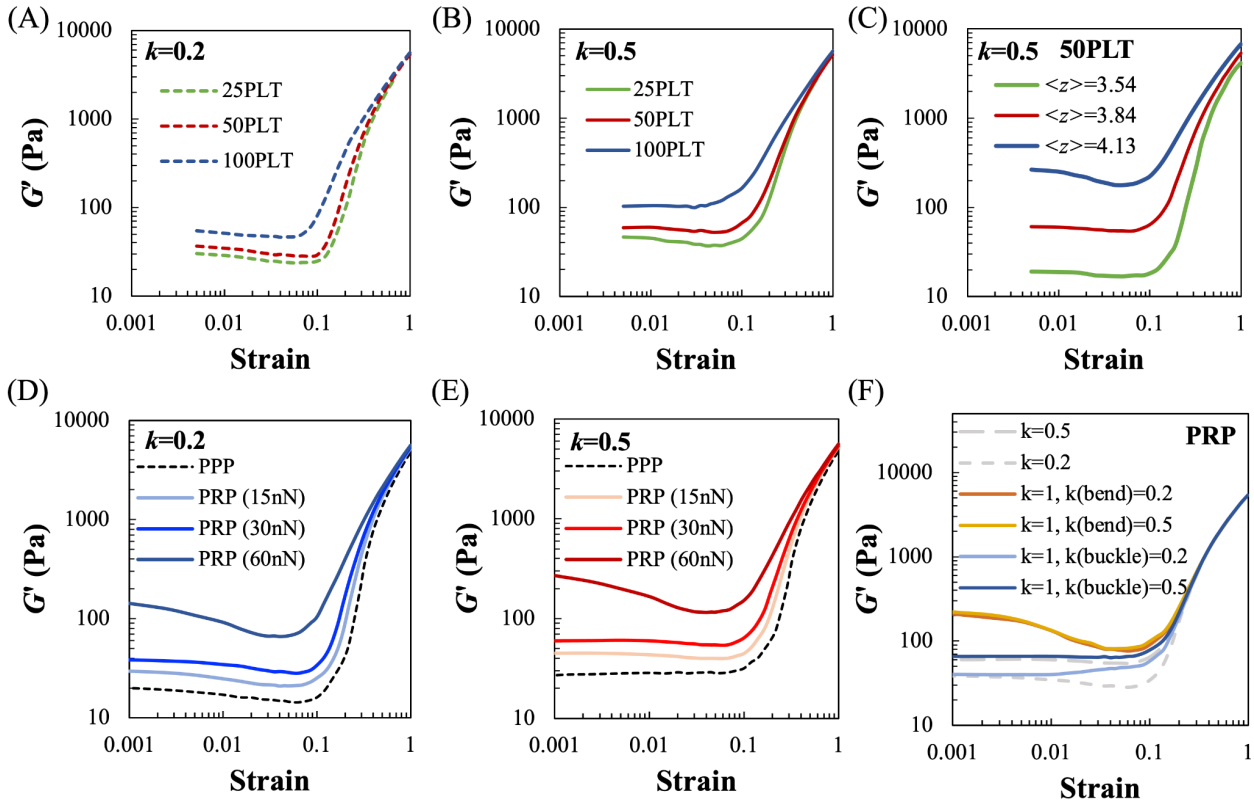

Fig. S4. Effect of number of platelet aggregates, connectivity, platelet force and fiber stiffness on the response of active networks.

The critical strain at which stiffening transition takes place strongly depends on the number of platelets. Simulations reveal that increasing platelet density leads to lowering critical strain in both uncrosslinked **(A)** and crosslinked **(B)** clots. Contracting platelet aggregates pull out available soft bending and buckling modes, and thus the transition to the stiff stretching dominated mode occurs at smaller strains. Platelet aggregates prestress the network and create additional buckling, which results in softening of the network, and this effect is more pronounced in uncrosslinked clots at lower k, or in over-coordinated networks at larger $\langle z \rangle$ **(C)**, because bending is limited in networks at larger $\langle z \rangle$ and platelet aggregates cause more fibers to buckle. Increasing platelet contractile force stiffen the network in a non-linear manner **(D, E)**. Reducing exclusively bending or buckling stiffness **(F)** shows that platelets stiffen the networks with smaller bending resistance at small strains (orange and red lines in **(F)**). Since bending is energetically cheaper (easy to change the angle between fibers at branch points), platelets can efficiently form force chains without fiber buckling, along which the network is reinforced. With strain, buckling becomes unavoidable, this results in network softening until transition to stiff stretching mode. Platelets in networks with rigid branch points (blue and light blue lines in **(F)**), conversely, pull out available energetically cheap buckling modes and demonstrate only bending dominated regime (the plateau in G'). Since bending is energetically unfavorable, platelets deform the network more uniformly and at small ranges, and thus the network is weakly stiffened by platelets.

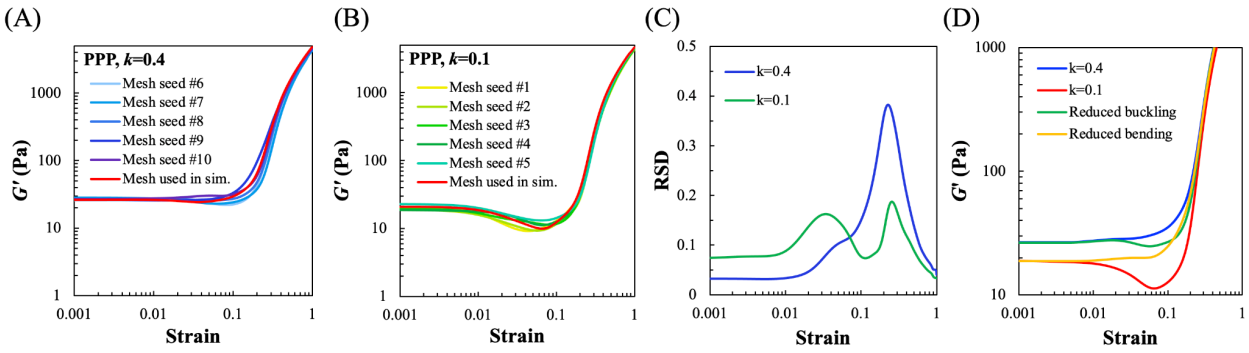

Fig. S5. Statistical validation of the numerical model and decoupling bending and buckling stiffness.

(A-C) Dependence of shear modulus (G') on shear strain in PPP clots of different mesh seeding at constant average coordination number ($\langle z \rangle = 3.84$). Different curves correspond to different randomly generated networks in both crosslinked ($k = 0.4$, panel A) and uncrosslinked ($k = 0.1$, panel **B**) clots. The results for the mesh used in simulations presented in the main text are colored in red. **(C)** Relative standard deviation (RSD) for crosslinked ($k = 0.4$) and uncrosslinked ($k = 0.1$) clots is increased at strains of large softening and stiffening, and appears only due to a slight shift in the softening/stiffening transitions between different mesh seeds. **(D)** Reducing exclusively bending stiffness of fibrin fibers (from $k_{bend} = 0.4$ to $k_{bend} = 0.1$) does not lead to appearance of softening dip, whereas reduced buckling stiffness (from $k_{buckle} = 0.4$ to $k_{buckle} = 0.1$) causes a small narrow region of decreased G'. The combined effect of reduced buckling and bending stiffness not only increases the magnitude of the dip but also makes it wider.

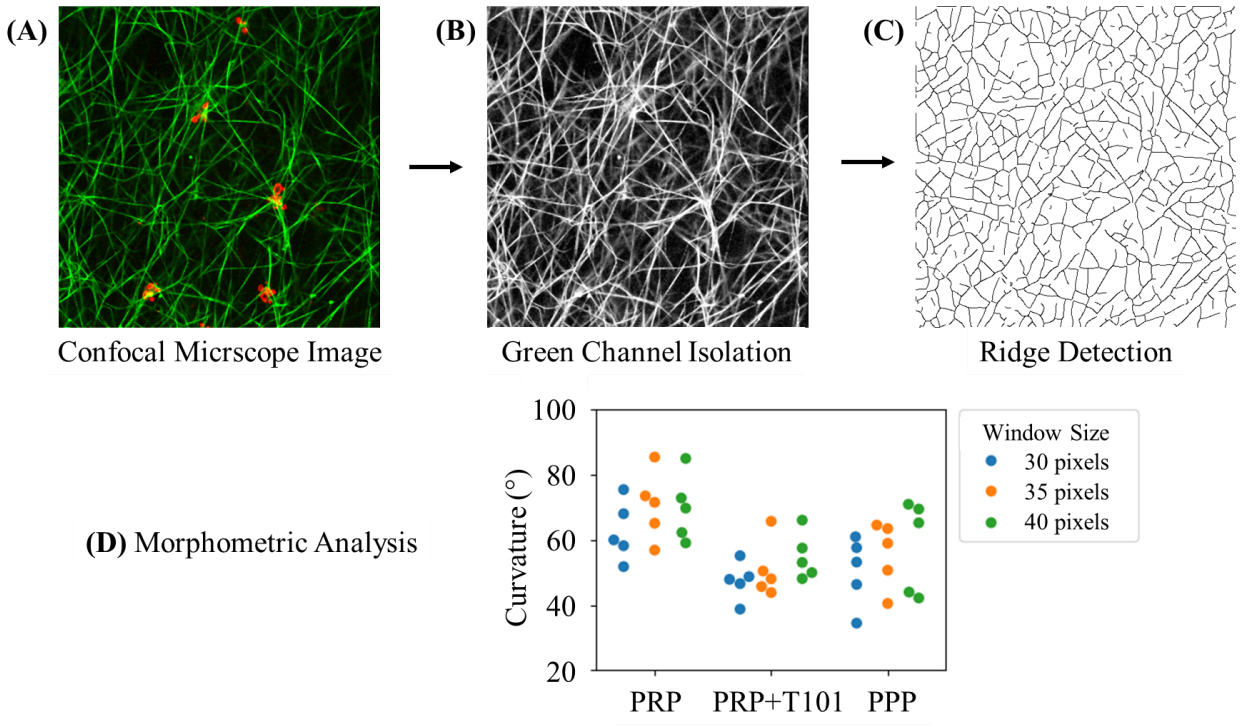

Fig. S6. Curvature analysis of fibers in PRP clots.

Confocal fluorescence microscope images **(A)** of PRP, PRP+T101, and PPP clots (n = 5 images for each) were compared using the TWOMBLI macro for ImageJ, whereby curvature is defined as "the mean change in angle moving incrementally along individual mask fibres by user-specified windows" (*59*). The green channel was isolated to examine fibrin without platelets **(B).** The Ridge Detection plugin (*97*) was used for identifying fibers **(C)**, with pre-calibration as guided within TWOMBLI. Different combinations of parameter values were screened, and the combination of 10 pixels for line width and 5 pixels for minimum branch length produced networks that were most visually consistent with the density and coordination number seen in the network model. Curvature windows of 30, 35, and 40 pixels were examined. Although there is some sensitivity to the particular choice of curvature window **(D)**, morphometric analysis using AnaMorf (*98*), integrated within TWOMBLI, showed that curvature is characteristically higher for PRP than for PRP+T101 and PPP. Using the 35-pixel window size as representative, PRP had an average curvature value of 70.5° ± 10.6° (mean ± standard deviation), compared to 50.8° ± 8.73° for PRP+T101 and 55.6° ± 10.1° for PPP. These experimental observations are consistent with model behavior, whereby applied strain induces buckling within the network (**Fig. 3A**) and a higher occurrence of buckled bonds is expected for crosslinked networks (**Fig. 4C**).

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