Electronic Supplementary Information (ESI)

Hierarchical strain-stiffening of semiflexible wormlike bundles

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Supplementary Figures

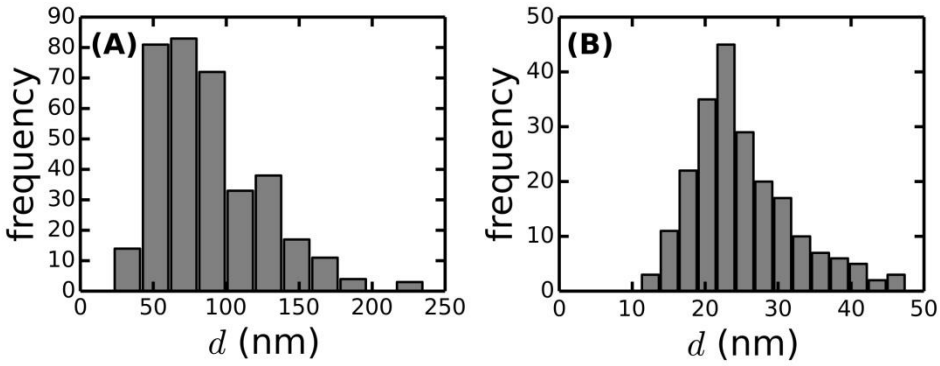

Figure S1. Histogram of fiber diameters determined from TEM images of fibers prepared under (A) coarse network conditions, which promote protofibril bundling, and (B) fine network conditions, which suppress protofibril bundling. In both cases, more than 200 fibers were taken into account, and data from networks polymerized at concentrations between 0.5 and 2 mg/ml were combined.

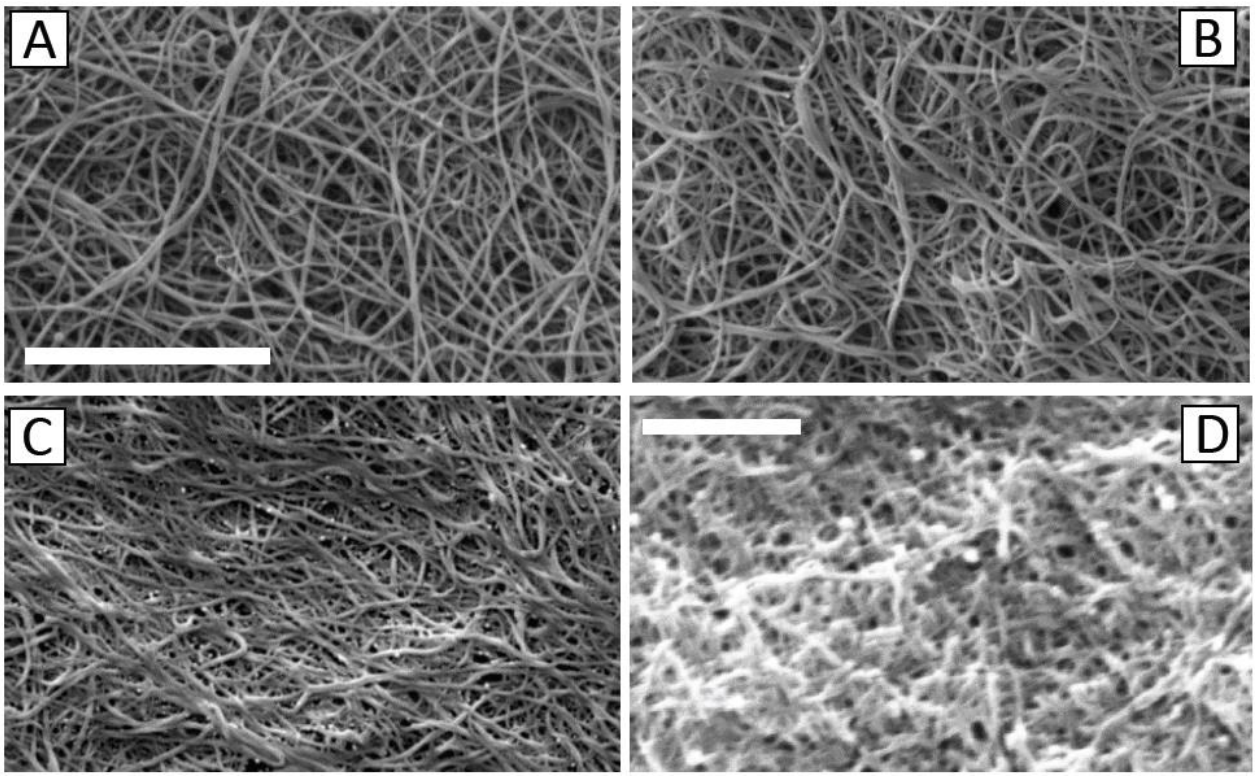

Figure S2. SEM images of fibrin networks. (A-C) Coarse fibrin networks polymerized at concentrations of 1, 3 and 7 mg/ml respectively. Scale bar represents 5 µm for (A-C). (D) Fine fibrin network polymerized at a concentration of 1 mg/ml. Scale bar denotes 400 nm.

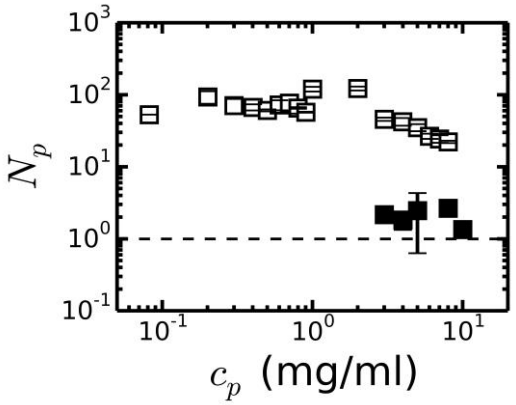

Figure S3. Number of protofibrils per fiber for fibrin fibers polymerized under coarse (open squares) and fine (filled squares) network conditions, based on turbidity measurements. The protofibril limit (i.e. N_p = 1) is indicated.

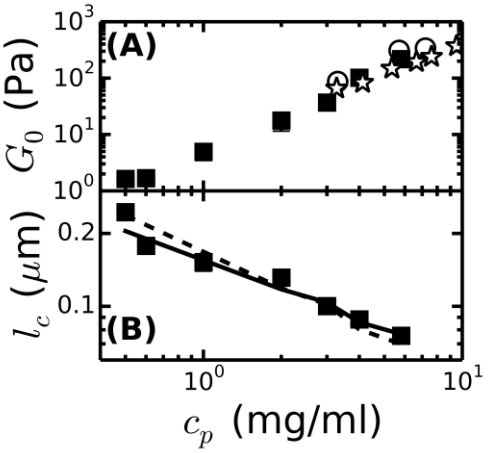

Figure S4. (A) The linear elastic modulus for fibrin clots polymerized under fine clot conditions, which show minimal bundling (black squares), compared with previous measurements (open circles: Ref. (1) and open stars: Ref. (2)). (B) Cross-link distance inferred by fitting the rheology data for fine clots to the affine model for wormlike chains to theoretical predictions according to $l_c \sim l_e = l_p^{1/5} (\rho^F)^{-2/5}$ (solid line) or $l_c \propto (\rho^F)^{-1/2}$ (dashed line), using a prefactor of 0.75 in both cases.

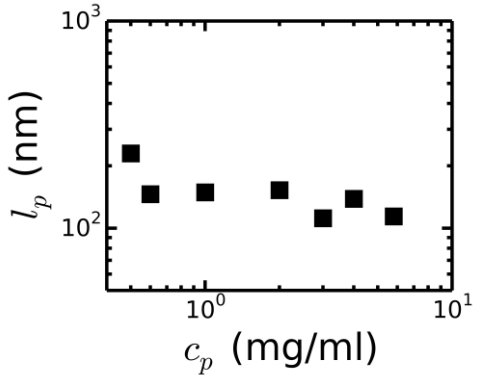

Figure S5.The persistence length of fine fibrin fibers, obtained by fitting the full theoretical prediction for the stress-stiffening response of extensible wormlike chains to the fine fibrin rheology data. The persistence length does not vary significantly with concentration.

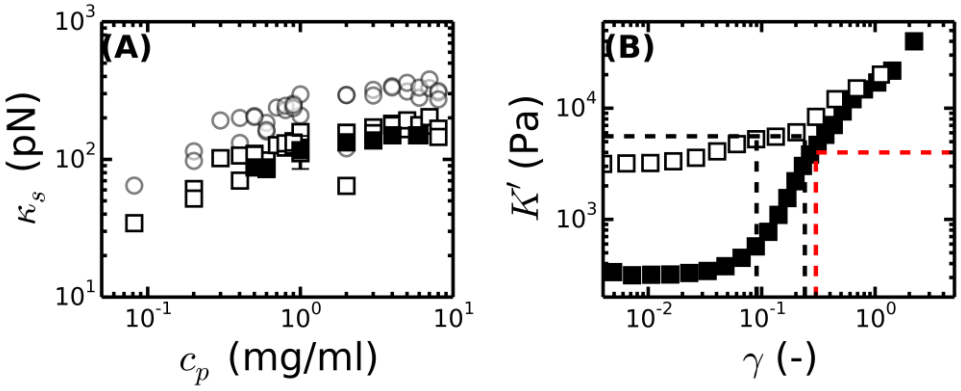

Figure S6. (A) The protofibril stretch modulus extracted from coarse and fine fibrin network rheology data. Solid squares represent data for fine fibrin, open squares represent data for coarse fibrin in the aligned limit ($K_s = 1/15\rho\kappa_s$), and open gray circles represent coarse clots in the isotropic limit ($K_s = 1/8\rho\kappa_s$). (B) The differential elastic modulus for 8 mg/ml fine (closed black squares) and coarse fibrin networks (open black squares) plotted against shear strain. For coarse fibrin, the region where κ_s is determined is indicated in black dashed lines. For fine fibrin, the inflection point is indicated by red dashed lines, indicating the beginning of the enthalpic stretching regime. The stretch modulus is determined by fitting the non-linear mechanical properties by the full theoretical prediction.

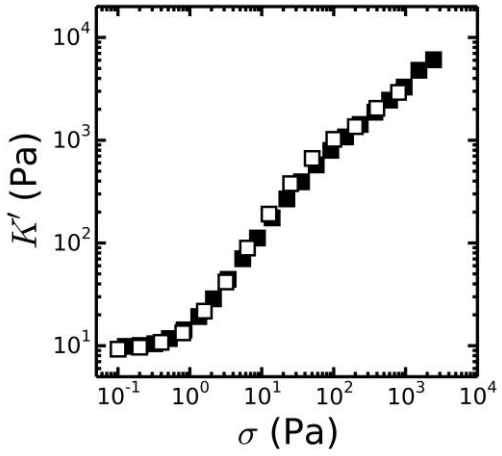

Figure S7. Nonlinear rheology of fine fibrin networks (2 mg/ml), in the presence (open squares) and absence (closed squares) of 200 µM D004.

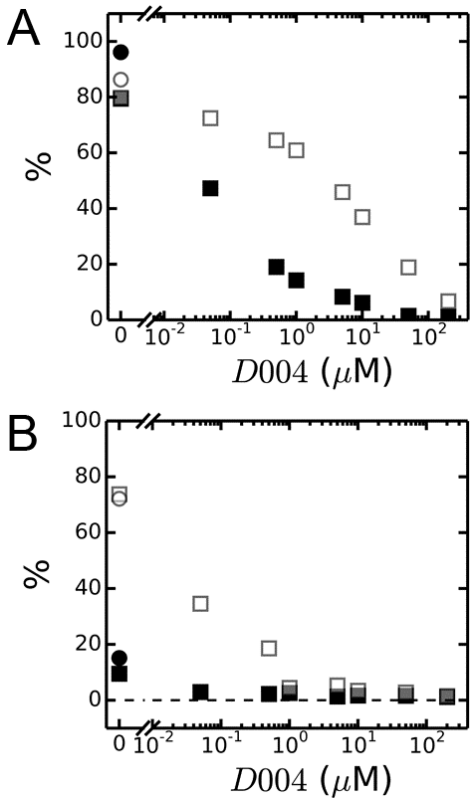

Figure S8. Percentage of crosslinked α-chains (closed squares) and crosslinked γ-chains (open grey squares) in 2 mg/ml (A) coarse and (B) fine fibrin networks in the presence of varying amounts of FXIII inhibitor D004, determined by densitometric analysis of SDS-PAGE gel. Circles correspond to the 2 mg/ml fibrin control with no DMSO present. Gray regions indicate where the open symbols and closed symbols overlap.

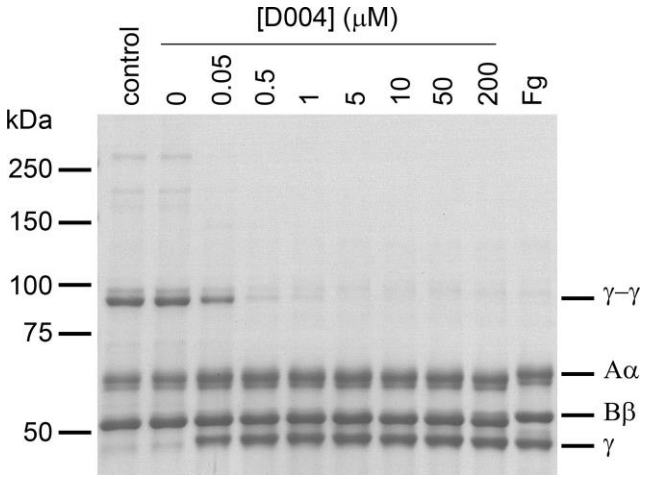

Figure S9. Reducing SDS-PAGE gel for 2 mg/ml fine fibrin networks formed in the presence of different concentrations of the FXIII inhibitor D004, as indicated. The control consists of fine fibrin without the presence of DMSO. Fg is fibrinogen in fine fibrin buffer without thrombin and calcium.

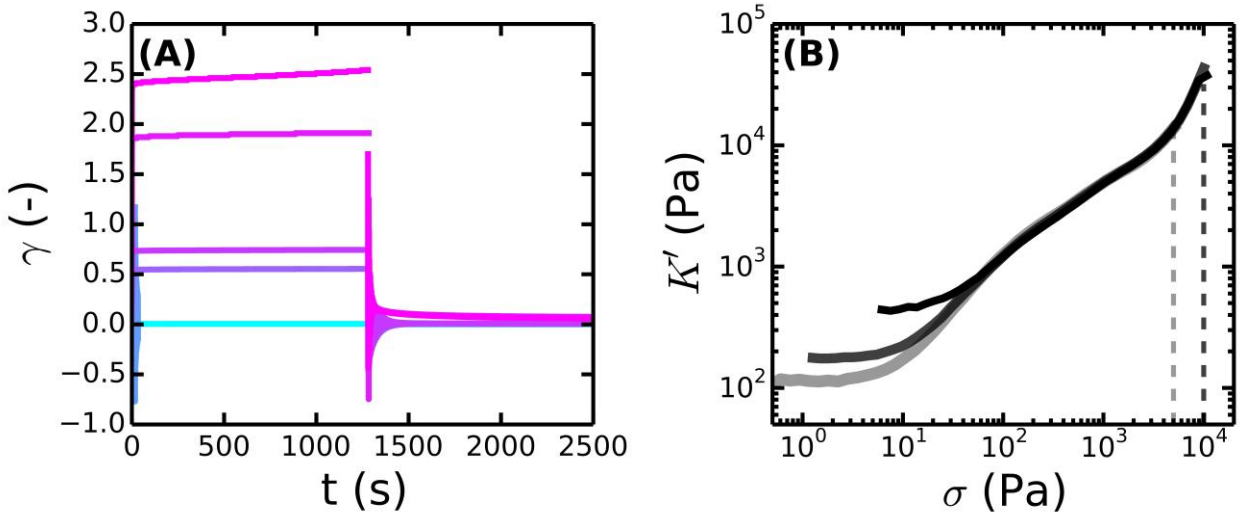

Figure S10. (A) Creep response of a 3 mg/ml fine fibrin network at increasing constant shear stress levels: from light blue to pink (bottom to top) the shear stress is 0.2, 4, 500, 1000, 4000 and 6000 Pa, respectively. The last stress level was just before sample breakage. (B) Repeatability of the stress-stiffening curves of a 4 mg/ml fine fibrin network. Subsequent stress sweeps are shown in increasing shades of gray, where the maximum stress level is indicated by vertical dashed lines. The stress-stiffening curves are repeatable in the nonlinear regime, while some work hardening can be seen in the linear regime. This is similar to what has been published previously for coarse fibrin gels [3].

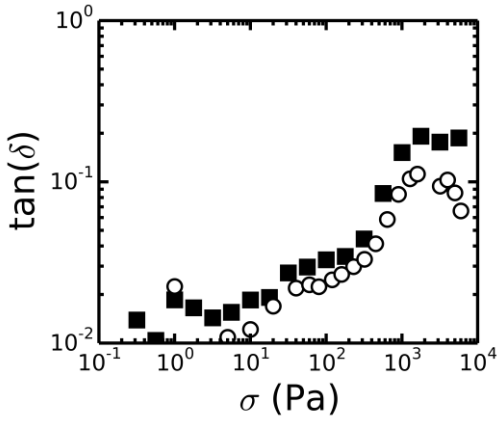

Figure S11. Viscoelastic behavior of 3 mg/ml coarse (open circles) and fine (closed squares) fibrin networks. The loss tangent, tan(δ) = *K''/K'*, is always much less than 1, indicating that fibrin is mainly elastic over the entire stress range.

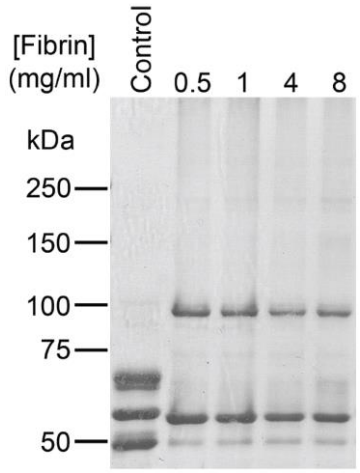

Figure S12. SDS-PAGE analysis of crosslinking of coarse fibrin networks with increasing protein concentration in mg/ml, as indicated. Control consists of fibrinogen without thrombin and calcium.

References

1. Bale, M. D., and J. D. Ferry. 1988. Strain enhancement of elastic modulus in fine fibrin clots. *Thromb. Res.* 52:565-572.
2. Nelb, G. W., C. Gerth, and J. D. Ferry. 1976. Rheology of fibrin clots. III. Shear creep and creep recovery of fine ligated and coarse unligated closts. *Biophys. Chem.* 5:377-387.
3 I. K. Piechocka, R. G. Bacabac, M. Potters, F. C. MacKintosh and G. H. Koenderink, *Biophys. J.*, 2010, **98**, 2281-2289.