Supplemental figures

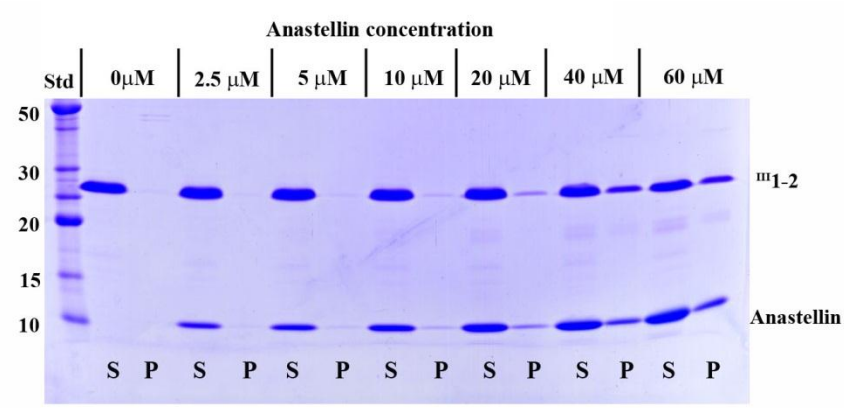

FIGURE S1. Concentration of anastellin required for aggregation with ^{III}1-2. Increasing concentrations of anastellin were mixed with 10 μM ^{III}1-2 for ~16 hr, and centrifuged at 20,000x*g* for 10 min. To efficiently form the precipitate, concentrations at or above 40 μM anastellin were needed. S. supernatant. P, pellet.

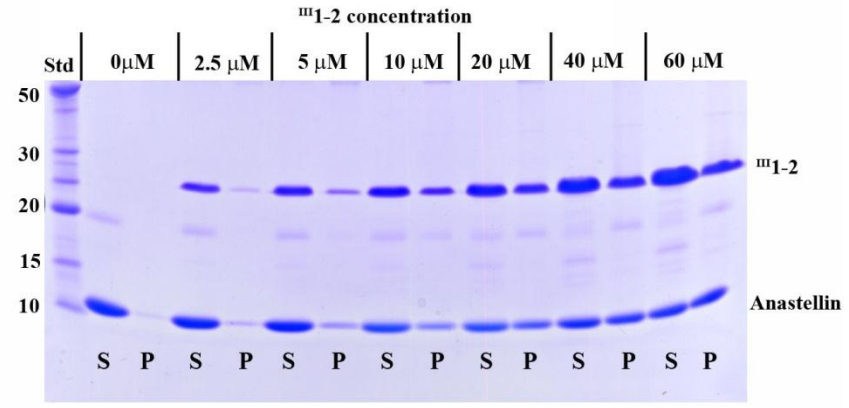

FIGURE S2. Concentration of ^{III}1-2 required for aggregation with anastellin. Increasing concentrations of ^{III}1-2 were mixed with 40 μM anastellin for ~16 hr, and centrifuged at 20,000x*g* for 10 min. To efficiently form the precipitate, concentrations at or above 10 μM ^{III}1-2 were needed. S. supernatant. P, pellet

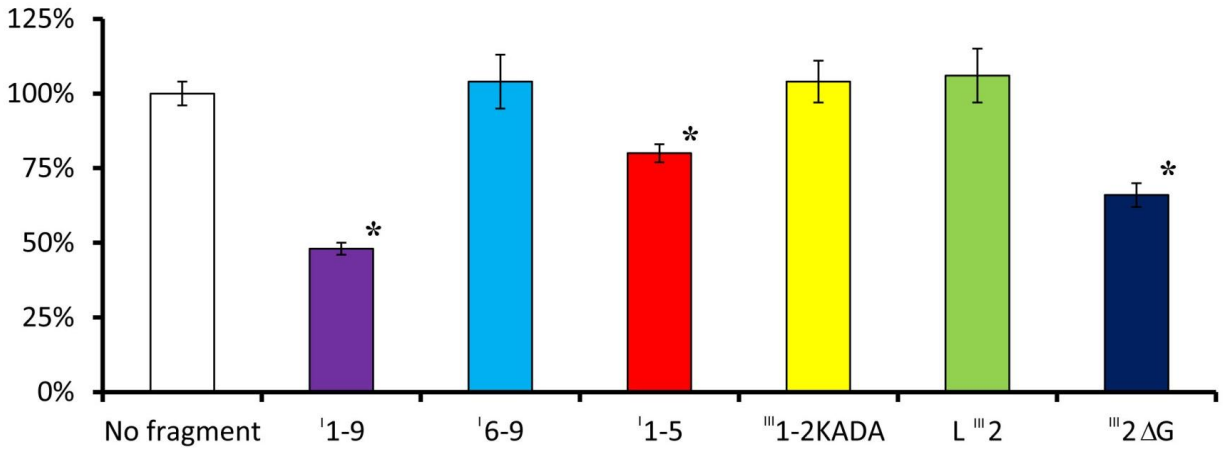

FIGURE S3. Inhibition of FN matrix formation by FN fragments. Various FN fragments (1 μM) were added to FN(-/-) cell culture with FN-YPet (30 nM) and cultured for ~6 h. The amount of matrix FN was estimated by measuring YPet fluorescence after solubilizing YPet from cell culture by trypsin treatment. The emission intensity was normalized to that of a control sample (no fragment). ^{I}1-9, ^{I}1-5 and ^{III}2ΔG reduced the FN matrix by 50%, 20% and 35%, respectively. The inhibitory effects of these fragments were very similar to the results from 16 h culture (Fig. 7C). Error bars indicate standard deviation. *, P<0.01.

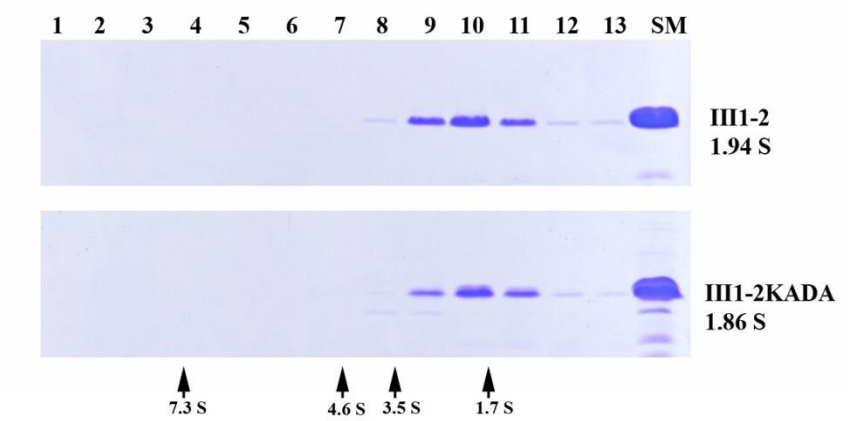

FIGURE S4. Glycerol gradient sedimentation of purified ^{III}1-2 and ^{III}1-2KADA. Fractions from the gradients were analyzed by SDS-PAGE (15%) under reducing conditions. Lane numbers correspond to gradient fraction numbers. The positions of external standard proteins (from the left, aldolase (7.3 S), bovine serum albumin (4.6 S), ovalbumin (3.5 S) and cytochrome C (1.7 S)) are indicated by the arrows. The KADA mutant sedimented identically to wild type ^{III}1-2.

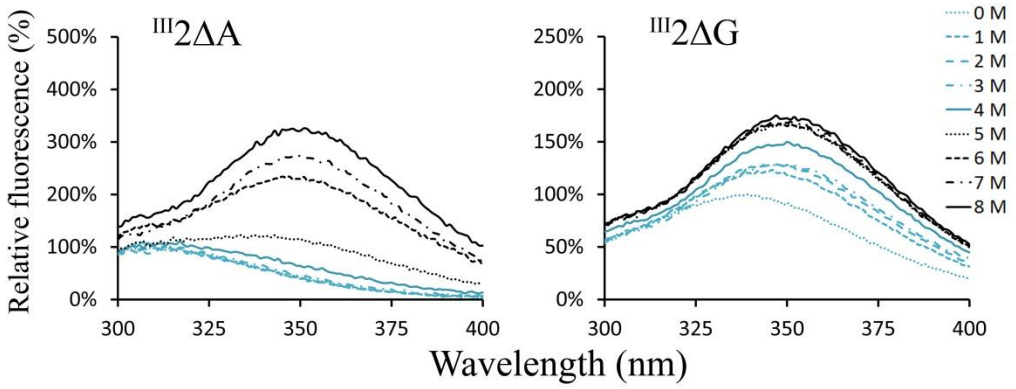

FIGURE S5. Stability of ^{III}2ΔA and ^{III}2ΔG. Purified proteins were excited at 280 nm and tryptophan fluorescence was recorded at wavelengths from 300 to 400 nm. Urea was used for denaturation. The emission intensity was normalized to that of the emission peak in 0 M urea. The strand G deletion destabilized this domain.

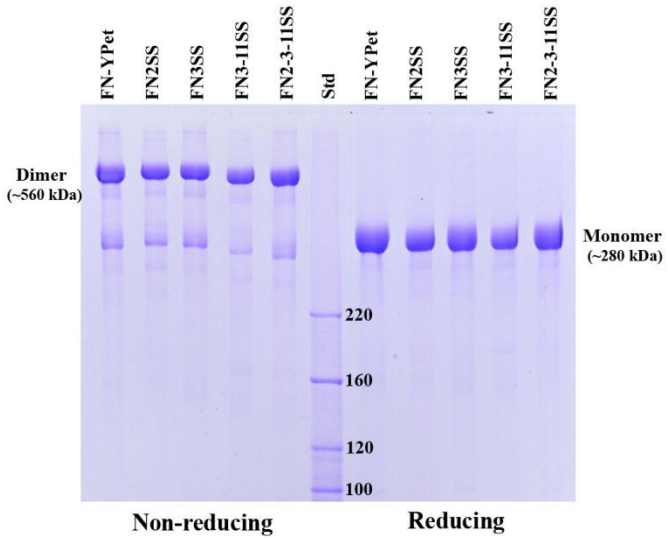

FIGURE S6. Quality of purified FN-YPet and the disulfide mutants. Gelatin affinity purified proteins (~1 μM) were analyzed by SDS-PAGE (5%) under non-reducing and reducing conditions. The majority of the proteins migrated as dimers. Minor monomeric forms were also seen under non-reducing conditions. ~1.5 mg of proteins was obtained from ~100 ml culture medium (obtained from three 150 cm^2 tissue culture flasks).

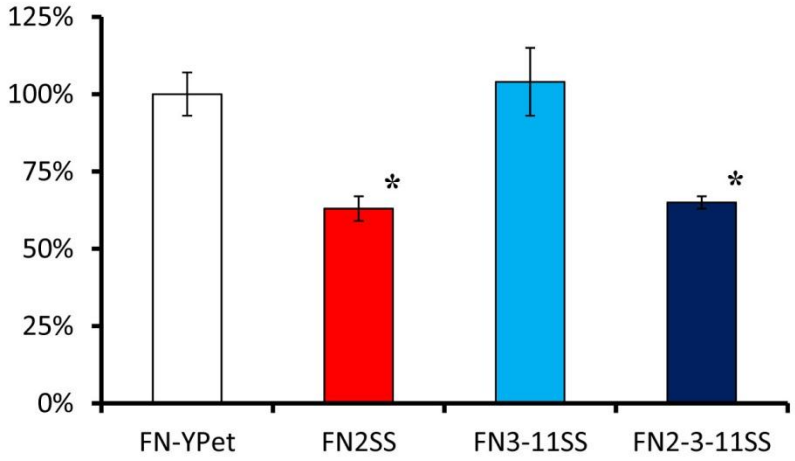

FIGURE S7. Effects of engineered disulfide bonds in full-length FN on matrix assembly. Purified 30 nM FN-YPet, FN2SS, FN3-11SS and FN2-3-11SS were added to FN(-/-) cell culture and cultured for ~6 h. The amount of matrix FN was estimated by measuring YPet fluorescence after solubilizing YPet from cell culture by trypsin treatment. FN2SS and FN2-3-11SS reduced the FN matrix by 30% relative to wild type and FN3-11SS. These results were similar to the results from 16 h culture (Fig. 9C). Error bars indicate standard deviation. *, $P<0.01$.