Supporting Information

Jensen et al. 10.1073/pnas.1401697111

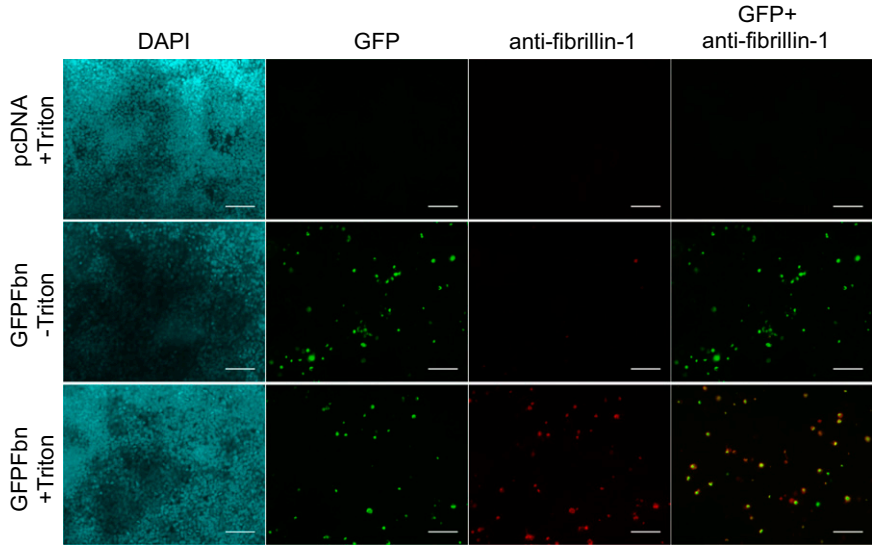

Fig. S1. Single cultures of HEK293T transfected with either empty pcDNA vector or pcDNA-GFPFbn were grown in eight-well chambered slides and processed for immunofluorescence as described in *Materials and Methods*, except that the cultures were not stained with the anti-GFP antibody. In addition, a permeabilization step was included (0.5% Triton X-100 in PBS for 10 min after fixation with 4% PFA) in some cultures (GFPFbn + Triton) to determine whether the green fluorescence observed without staining was due to cell surface or intracellular deposits of GFP-Fbn. (Scale bars: 100 μm.) The results show that anti–fibrillin-1 antibody cannot bind the green deposits without permeabilization (GFPFbn − Triton), indicating that they are contained within the cells. The use of stable versus transiently transfect cells may explain the differences in fibrillin expression patterns in the single HEK293T cell cultures seen here compared with those previously observed by Hubmacher et al. (1). In the stable expression system, a punctate pattern of extracellular fibrillin-1 deposition is seen in contrast to the green fluorescent material observed intracellularly in our transient system. The bright fluorescent cells we see, showing very high levels of GFP-Fbn expression, would presumably not be viable long term, which would explain the lack of intracellular staining previously seen on permeabilization of stable clones (1).

1. Hubmacher D, Bergeron E, Fagotto-Kaufmann C, Sakai LY, Reinhardt DP (2014) Early fibrillin-1 assembly monitored through a modifiable recombinant cell approach. *Biomacromolecules* 15(4):1456–1468.

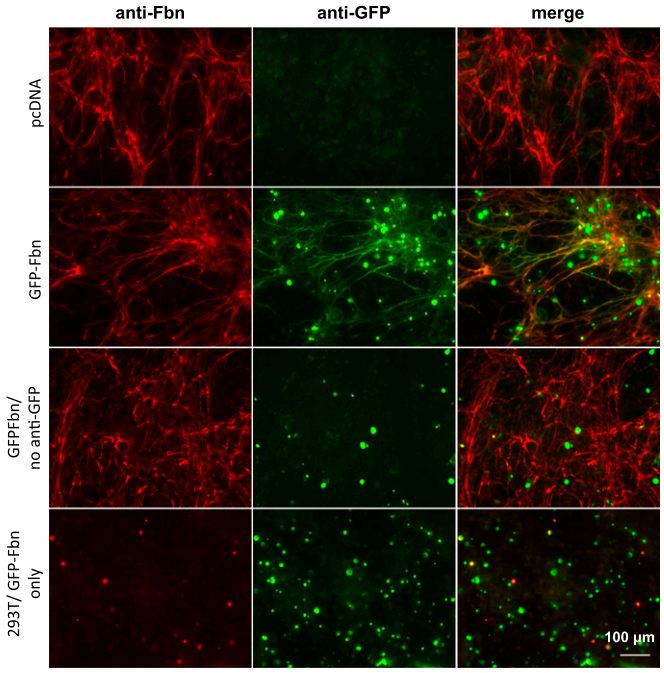

Fig. S2. FS2 fibroblasts were cocultured with HEK293T cells transiently transfected with either empty vector (pcDNA) or pcDNA-GFPFbn (GFP-Fbn). Cells were fixed and stained for fibrillin and GFP, or for fibrillin alone ("no anti-GPF"), as described in *Materials and Methods*. Comparison of the GFP-Fbn and GFP-Fbn/no-anti-GFP data shows that although cells transfected with GFP-Fbn are fluorescent, the concentration of GFP-labeled material deposited in the matrix is relatively low and requires antibody detection. HEK293T cells expressing GFP-Fbn in the absence of fibroblasts (293T/GFP-Fbn only) are unable to produce a fibrillin microfibril network.

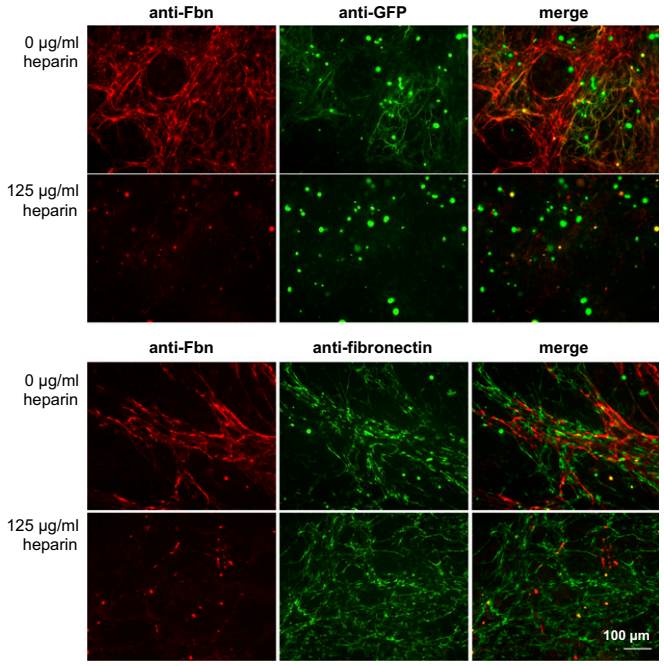

Fig. S3. Cocultures of FS2 fibroblasts with HEK293T cells expressing GFP-Fbn were grown in the presence or absence of 125 μg/mL heparin (from porcine intestinal mucosa; Sigma), then stained for either fibrillin and GFP (*Upper*, as described in *Materials and Methods*) or fibrillin and fibronectin (Sigma, F7387; *Lower*). The lack of a GFP-positive network in the presence of heparin shows that the incorporation of GFP-Fbn is dependent on the assembly of the endogenous fibrillin-1 produced by the fibroblasts. Fibronectin staining was similar in both cases, indicating that heparin was not disrupting the general architecture of the matrix.

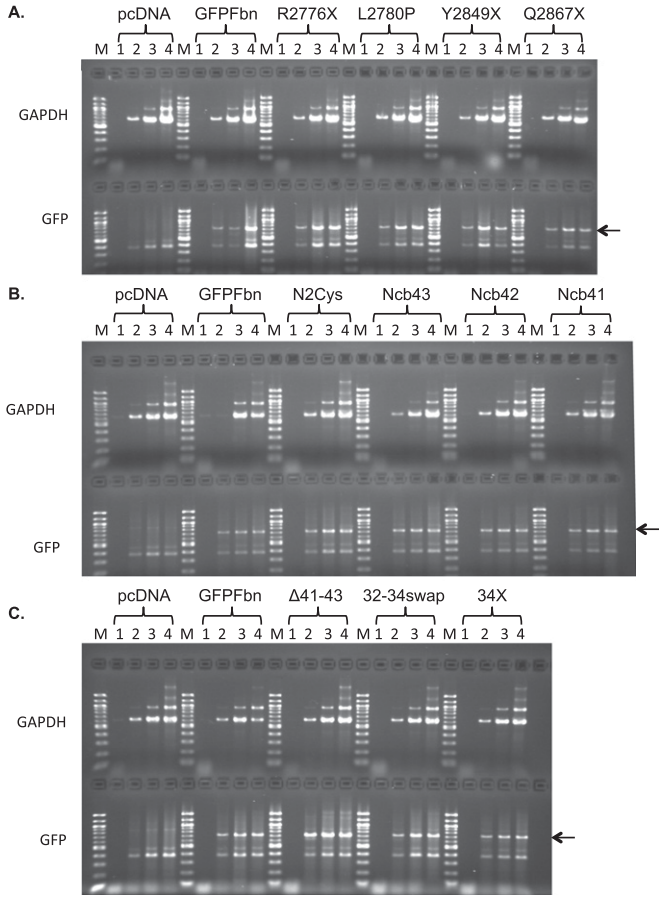

Fig. S4. Semiquantitative RT-PCR analysis of transiently transfected HEK293T cells expressing variants of GFP-Fbn that display intracellular retention show that the levels of transcript for these constructs is not markedly different to the wild-type form. (*A*) MFS-associated mutants. (*B*) C-terminal domain deletions. (*C*) Domain-swap constructs. Total RNA was extracted from transiently transfected cells, grown to 80% confluence in 25-cm^2 tissue culture flasks, using the RNeasy Mini kit (Qiagen). Reverse transcriptase reactions were carried out as described (1) using primers GAPDH_1002r (5′-CTTGGAGGCCATGTGGGCC-3′) and GFPrev (5′-CTTGTACAGCTCGTCCATGC-3′) for first strand synthesis of GAPDH and GFP-Fbn transcripts, respectively. A GAPDH fragment (629 bp) was then amplified using Taq DNA polymerase using primers GAPDH_303f (5′-CTTCACCACCATGGAGAAGG-3′) and GAPDH_932r (5′-ATGAGCTTGACAAAGTGGTCG-3′), whereas a fragment specific for the GFP-Fbn transcript (717 bp; arrows) was amplified using primers GFPfor (5′-ATGGTGAGCAAGGGCGAGC-3′) and GFPrev, which bind to the sequence encoding the GFP tag. Samples were run against 100-bp ladders (M; New England Biolabs N3231S). In each case, lane 1 contains the control reaction set up without reverse transcriptase and amplified over 30 cycles. Lanes 2, 3, and 4 contain samples from reactions amplified over 20, 25, and 30 cycles, respectively.

1. Schneider R, et al. (2010) Biophysical characterisation of fibulin-5 proteins associated with disease. *J Mol Biol* 401(4):605–617.

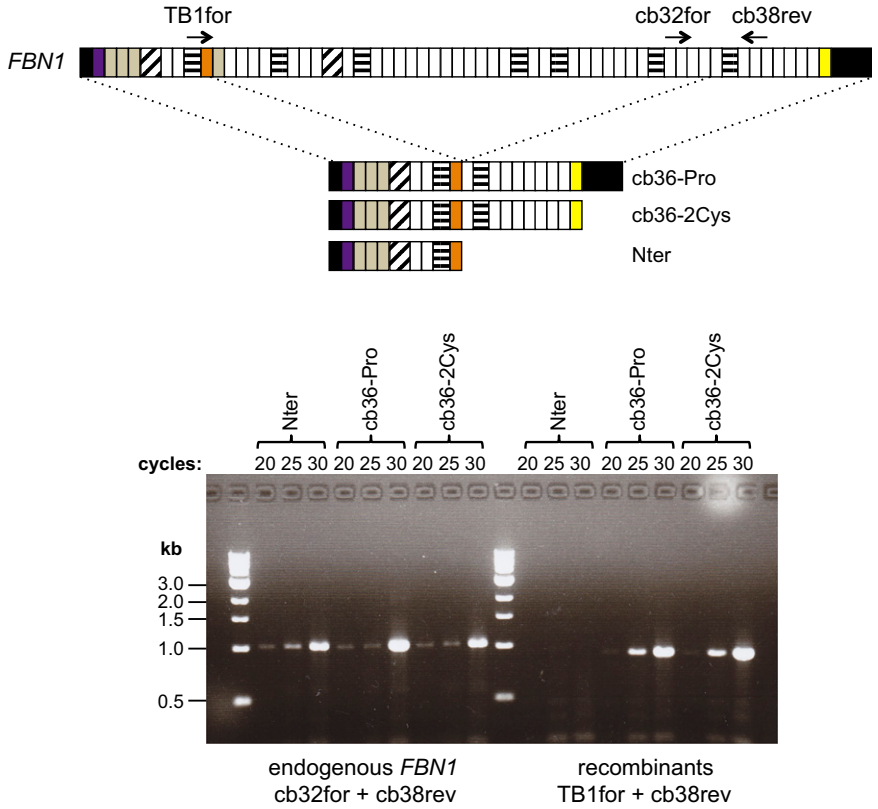

Fig. S5. Semiquantitative RT-PCR analysis of pools of MSU-1.1 fibroblast clones. Total RNA was extracted from pools of clones grown to 80% confluence in 25 cm^2 tissue culture flasks using the RNeasy Mini kit (Qiagen). RT-PCR reactions as described (1) using primer cb39rev_Xba (5′-TAGTAGTCTAGATTA-AATGCAGGACGTATGGTGTTG-3′) for first strand synthesis. Primers cb32for (5′- CAGATGGTTCCTATCGCTGC-3′) and cb38rev_Xba (5′-TAGTAGTCTAGATTA-TTTGCAGCTCCTTCCATCCTC-3′) were used to selectively amplify material from endogenous *FBN1* and primers TB1for (5′- CTCTAACCAGCTGCCACAG-3′) and cb38rev_Xba were used to amplify material derived from recombinant transcripts. 20, 25 or 30 cycles of denaturation at 95 °C (1 min), annealing at 60 °C (1 min), and extension at 72 °C (1 min), with a final extension step of 10 min were carried out. Comparable levels of recombinant transcript were seen in each case. Endogenous *FBN1* transcript levels were assessed as an internal control for variations in the amounts of starting material used in each of the reactions.

1. Schneider R, et al. (2010) Biophysical characterisation of fibulin-5 proteins associated with disease. *J Mol Biol* 401(4):605–617.