Klingberg et al., http://www.jcb.org/cgi/content/full/jcb.201402006/DC1

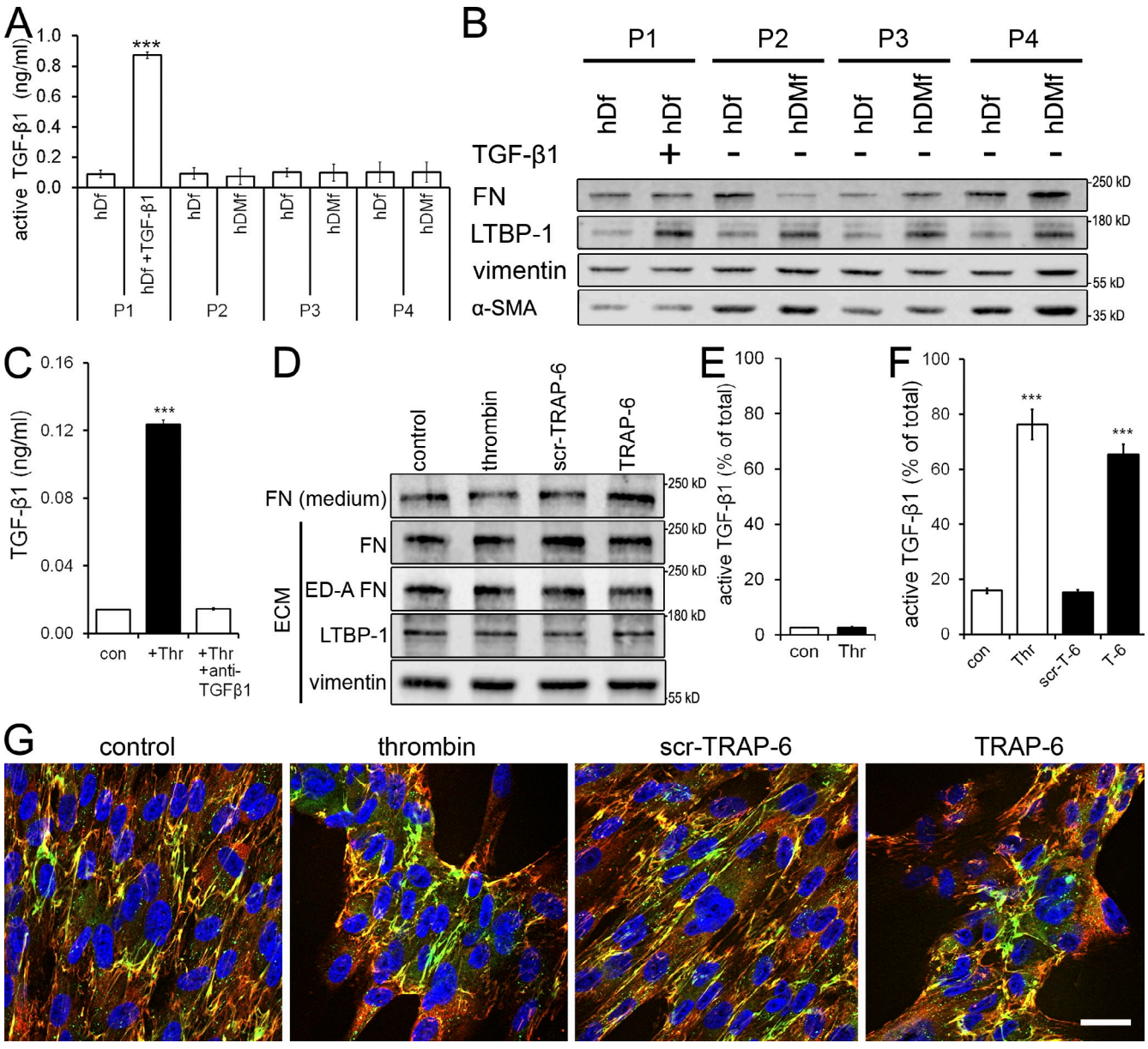


Figure S1. **TGF-β1 effect on hDf and TMLEC.** (A and B) hDfs were activated into hDMfs by adding 2 ng/ml active TGF-β1 once for 5 d to passage 1 (P1) cells. Over the following three passages, cell cultures were assessed for active TGF-β1 (A) in the supernatants and myofibroblast marker protein expression (B). (C) hDMfs under thrombin (Thr) stimulation release the TGF-β1 isoform as shown by adding the anti–TGF-β1 antibody to the TMLC/hDMf co-cultures while inducing contraction. (D) Western blotting of ECM and media from hDMf cultures in the presence of thrombin, TRAP-6 (T-6), and scrambled TRAP-6 (scr T-6). (E) hDMfs were used to produce ECM and removed using DOC; cell-free ECM was incubated with thrombin to control that no TGF-β1 is activated in the absence of contracting cells. (F) Active TGF-β1 released by hDMfs under thrombin, TRAP-6, or scrambled TRAP-6 stimulation. (G) Immunofluorescence staining of ECM and media from hDMf cultures in the presence of thrombin, TRAP-6, and scrambled TRAP-6. ED-A FN (red), LTBP-1 (green), and DAPI (blue) are shown. Graph shows mean values and SDs from at least three independent experiments (*** $P \leq 0.005$; two-tailed paired *t* test). Bar, 25 µm. con, control.

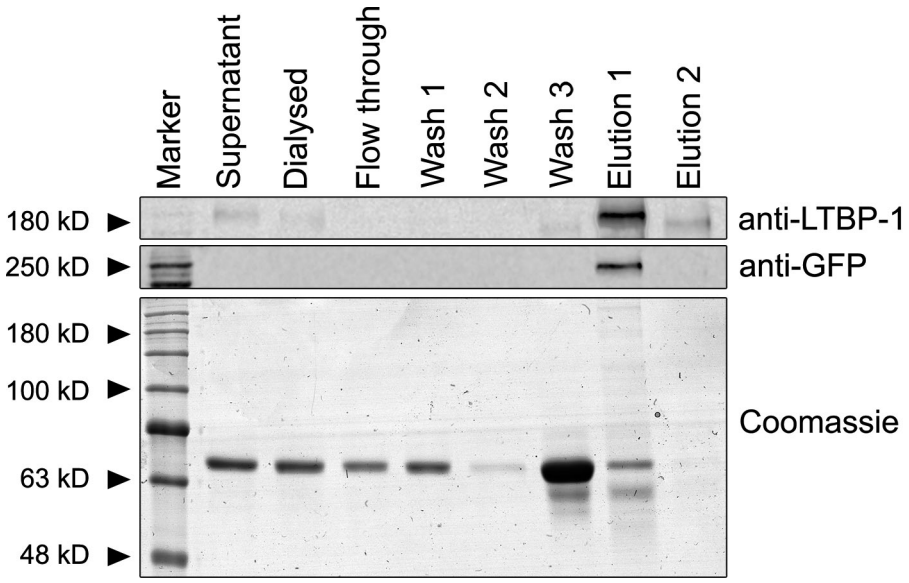


Figure S2. **Purification of LTBP-1.** LTBP-1–EGFP was purified from conditioned serum-free medium of LTBP-1–EGFP-overexpressing HEK293 cells, using the 6×His tag on LTBP-1–EGFP. Coomassie gel and Western blot assays confirmed presences of LTBP-1 in elution fraction 1.

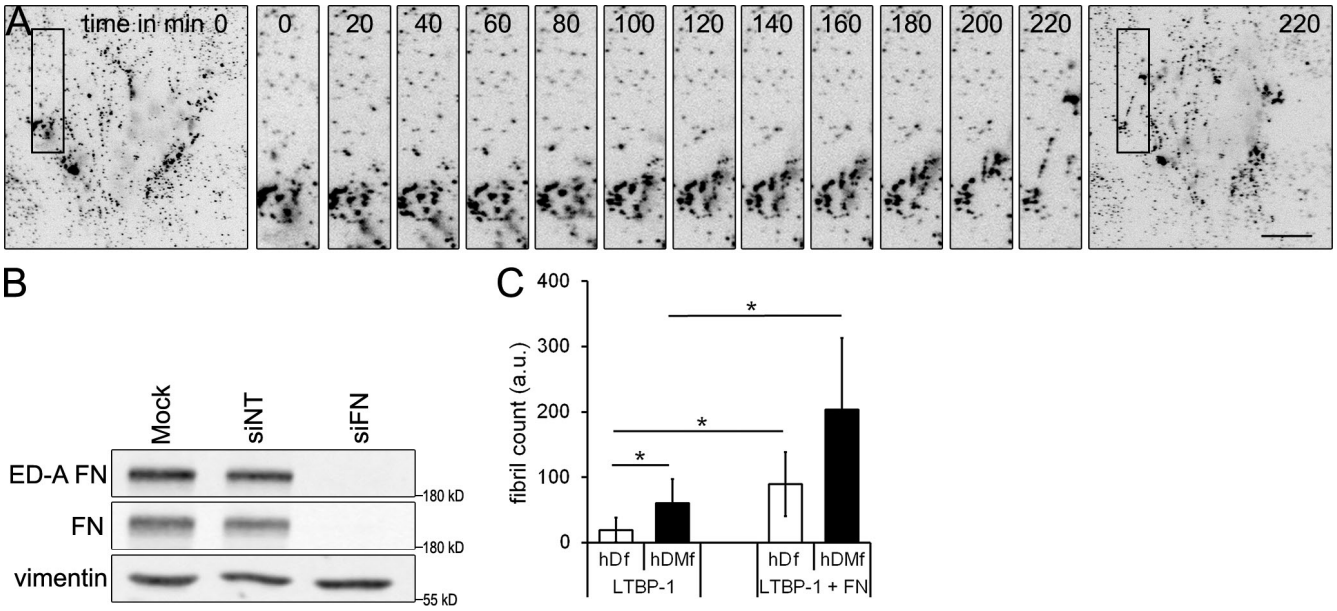


Figure S3. **LTBP-1 organization by myofibroblasts.** (A) LTBP-1–EGFP-overexpressing HEK293 cells were used to produce LTBP-1–containing ECM. After extraction of HEK293 using DOC, the remaining DOC-insoluble ECM was used as substrate for hDMfs. hDMfs were allowed to adhere for 4 h before video recording ECM organization for 220 min with image intervals of 15 s. The EGFP tag on LTBP-1 was used to visualize fibril formation at a wavelength of 488 nm. Boxes were magnified in the insets. Bar, 20 µm. (B) To knock down FN, hDMfs were transfected with siRNAs directed against the FN gene; controls were mock transfected without siRNA and nontargeting (siNT) RNA sequences. (B) hDMfs and hDfs were grown on LTBP-1–EGFP-coated substrates for 2 d in the absence and presence of excess FN (100 µg/cm^2). Fibril counts were quantified from LTBP-1–EGFP by image analysis. Graph shows mean values and SDs from at least three independent experiments (*, $P \leq 0.05$; two-tailed paired *t* test). a.u., arbitrary unit.

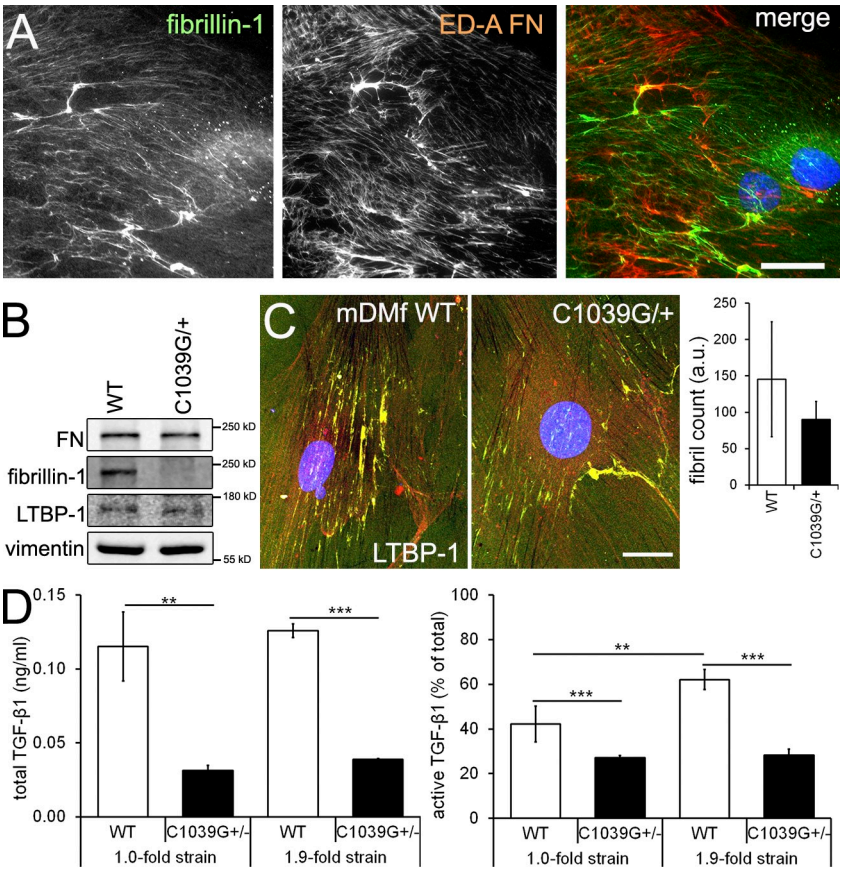


Figure S4. **Importance of fibrillin-1 in LTBP-1 fibril formation.** (A) Myofibroblasts were cultured for 6 d and coimmunostained for fibrillin-1, ED-A FN, and nuclei (blue). (B) Wild-type (WT) and fibrillin-1 C1039G/+ mutant mouse dermal myofibroblast (mDMf) cultures were grown for 7 d and analyzed by Western blotting. (C) Wild-type and fibrillin-1 C1039G/+ mutant mouse dermal myofibroblasts were grown on purified LTBP-1–EGFP for 2 d and stained for FN (red), GFP (green), and nuclei (blue) to quantify the number of LTBP-1 fibrils by image analysis from at least three images per three independent experiments. (D) Wild-type and fibrillin-1 C1039G/+ mutant mouse dermal myofibroblasts grown on relaxed highly expandable silicone membranes were removed after 6 d using DOC. The decellularized ECM was then strained in the absence of cells by 1.9-fold, and hDMfs were seeded onto nonstrained and prestrained decellularized ECM. Cell contraction was induced using thrombin, and release of active TGF-β1 was quantified as a percentage of total TGF-β1. Graph shows mean values and SDs from at least three independent experiments (**, $P \leq 0.01$; ***, $P \leq 0.005$; two-tailed paired *t* test). Bars, 20 µm. a.u., arbitrary unit.

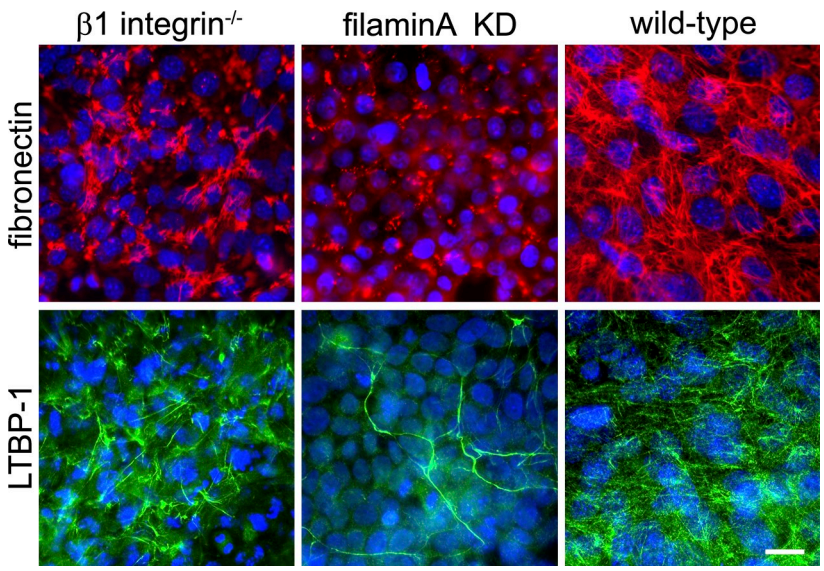
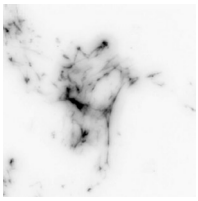
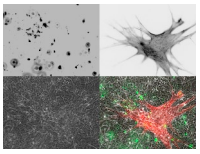


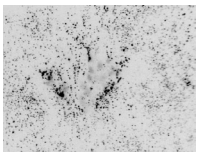
Figure S5. **ECM immunofluorescence staining of wild-type, filamin A knockdown, and integrin β1$^{-/-}$ MEFs.** Wild-type MEFs, filamin A knockdown MEFs, and integrin β1$^{-/-}$ MEFs were grown for 6 d and stained for FN (red), LTBP-1 (green), and nuclei (blue). Bar, 20 µm.



Video 1. **LTBP-1 ECM fibril stretch on highly expandable culture membranes.** hDMfs were grown on highly expandable silicone culture membranes for 6 d before cells were removed with DOC. The remaining DOC-insoluble ECM was stained for LTBP-1 (inverted, black fibrils) and subjected to a 1.0–2.6-fold change in linear strain using a uniaxial mechanical strain device. One image was taken every 0.1-fold strain increments. LTBP-1 fibrils were stained with the anti–LTBP-1 and FITC-labeled secondary antibody. Images were taken on a microscope (Axiovert 135M; Carl Zeiss) with a camera (C10600 ORCA-R2; Hamamatsu Photonics).



Video 2. **LTBP-1 organization by myofibroblasts in co-culture with HEK293 cells.** hDMfs were transfected with pLifeAct-RFP (gift from B. Geiger, Weizmann Institute, Rehovot, Israel) and seeded on top of 7-d-old cultures of LTBP-1–EGFP-expressing HEK293. hDMfs were allowed to adhere for 4 h before video recording was performed for 1 h with images taken every 60 s. GFP was detected at 488 nm (green), LifeAct-RFP was detected at 568 nm (red), and the cell layer was visualized in phase contrast. Images were taken on an upright microscope (Axio Imager) equipped with a camera (AxioCam HRm; Carl Zeiss).



Video 3. **LTBP-1 organization by myofibroblasts on DOC-insoluble ECM.** hDMfs were seeded on top of DOC-insoluble ECM produced by LTBP-1–EGFP-expressing HEK293 and allowed to adhere for 4 h. LTBP-1–EGFP (black dots) organization was recorded at 488 nm for 220 min with images taken every 15 s. Images were taken on a microscope (Axiovert 135M; Carl Zeiss) with a camera (C10600 ORCA-R2; Hamamatsu Photonics).

Supplemental material also includes a ZIP file that contains a macro showing cell contraction analyses with wrinkling assay. Images were converted to 8 bit and thresholded, and particles were analyzed with size >0 pixel and circularity between 0 and 1.