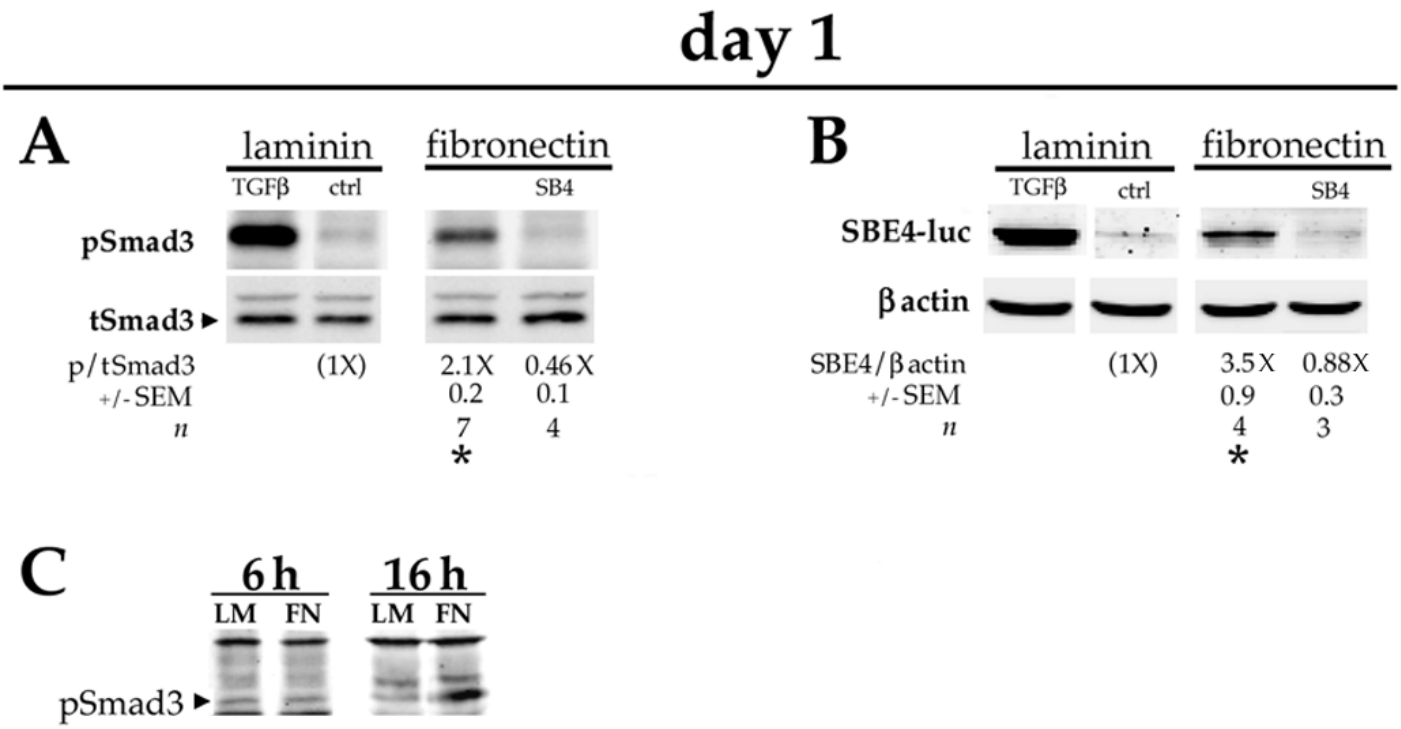

Figure S1. **Plating DCDMLs on FN increases TGFβ signaling within 24 h.** DCDMLs were plated on day 0 of culture in wells coated with either laminin or plasma-derived FN. (A) After 4 h, medium was replaced with fresh M199/BOTS with or without 4 ng/ml TGFβ1 or the TGFβR inhibitor SB431542 (SB4). Whole cell lysates were prepared 24 h after plating and probed with antibodies specific for the phosphorylated (activated) or total forms of Smad3. The p/t Smad3 ratio was quantitated as fold expression of control (ctrl; plated on laminin and cultured without TGFβ). Asterisk, $p = 0.000$. (B) DCDMLs on day 0 were transfected in suspension with the SBE4-Luc reporter construct and then immediately plated in wells coated with either laminin or pdFN. After 4 h, TGFβ or SB431542 (SB4) was added where indicated. Cells were lysed 20 h later, and luciferase assessed by Western blot. The results were normalized to β-actin in the same sample, and quantitated as fold expression of control (ctrl; plated on laminin and cultured without TGFβ). Asterisk, $p = 0.012$. (C) Activation of Smad3 is detectable 16 h after plating on pdFN, but not after 6 h. Representative of $\geq$3 experiments.

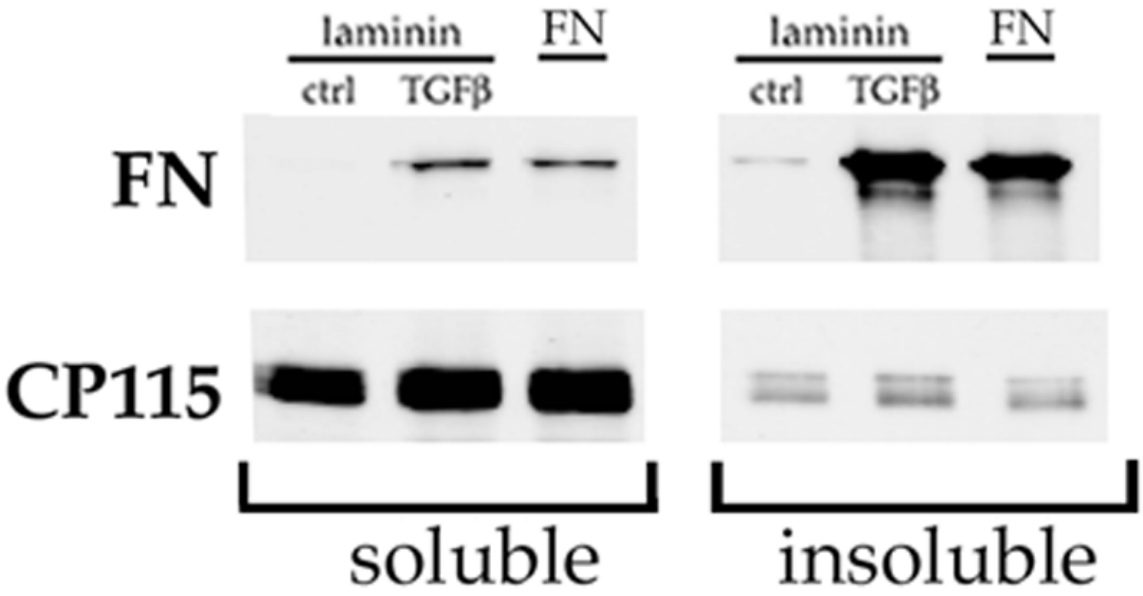

Figure S2. Cellular fibronectin produced by DCDMLs plated on pdFN is assembled into deoxycholate-insoluble fibrils. DCDMLs plated on either laminin or pdFN were cultured either with or without TGFβ. On day 7, the cells were lysed in 2% sodium deoxycholate at 4°C and subjected to centrifugation as described by Wierzbicka-Patynowski et al. 2004 prior to analysis by Western blotting. ~95% of cFN in pdFN-plated cells was recovered in the insoluble fraction, in contrast to the cytosolic protein CP115.

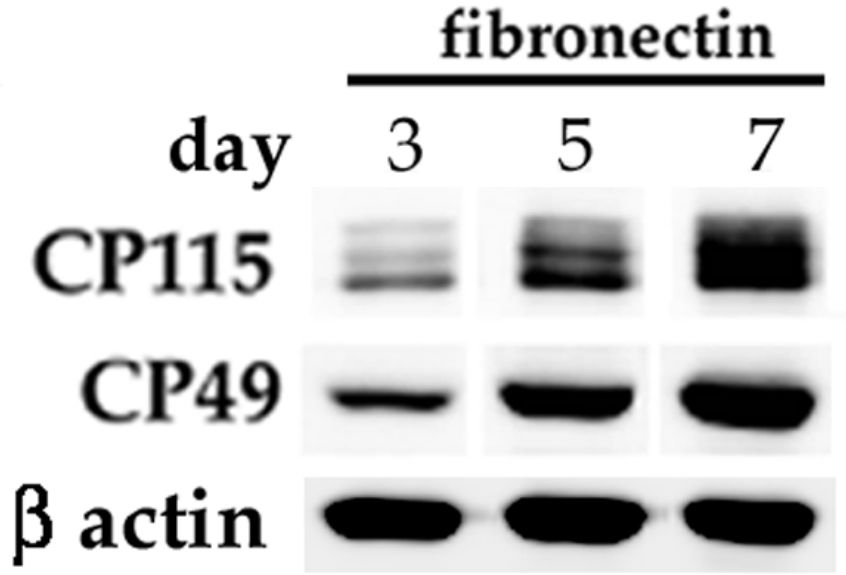

Figure S3. **Time course of induction of fiber cell proteins in pdFN-plated cells.** DCDMLs plated on pdFN on day 0 were lysed on day 3, 5, or 7 of culture. Normalized to β-actin in the same sample, levels of CP115 and CP49 increased from day 3 to day 7 by 3.5 +/- 0.4 fold and 4.6 +/- 0.5 fold, respectively (n = 4; p = 0.001). FN therefore appears to act by inducing the de novo synthesis of fiber proteins instead of by selectively promoting the adhesion of epithelial cells from the equatorial region of the lens, the region in which expression of CP115 and CP49 is first upregulated in vivo.

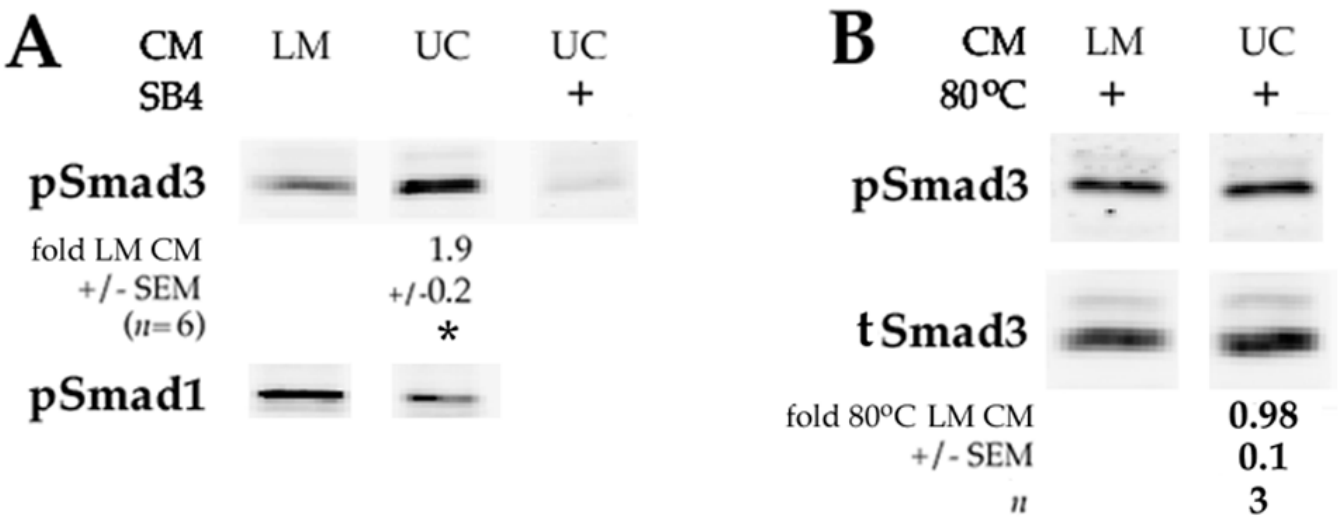

Figure S4. Plating lens cells on uncoated tissue culture plastic increases their ability to activate endogenous TGFβ. Conditioned medium donor cells were plated on laminin (LM) or uncoated TC plastic (UC) on day 0. Medium was replaced with fresh M199/BOTS on day 1, and the conditioned medium (CM) collected on day 3. The CM was then added to recipient DCDMLs plated on laminin and incubated for 1.5 h prior to lysis of recipient cells and Western blot analysis of active (phosphorylated) Smad3 and Smad1, or total Smad3. Where indicated, CM was heated to 80°C for 6 min to thermally activate endogenous latent TGFβ prior to addition to recipient cells. In some cases, recipient cells were pretreated with SB431542 for 1 h prior to addition of CM. (A) Asterisks, $p < 0.000$ compared to unheated CM from laminin-plated cells. (B) Heated CM was diluted ten-fold with fresh M199/BOTS before addition to recipient cells. The difference between the Smad3-activating activity of heated CM from donor cells plated on laminin or on uncoated TC plastic was not significant ($p = 0.775$).

Reference

Wierzbicka-Patynowski, I., Mao, Y. and Schwarzbauer, J. E. (2004). Analysis of fibronectin matrix assembly. Curr. Protoc. Cell Biol. 25, 10.12.1-10.12.10.