Supporting Information

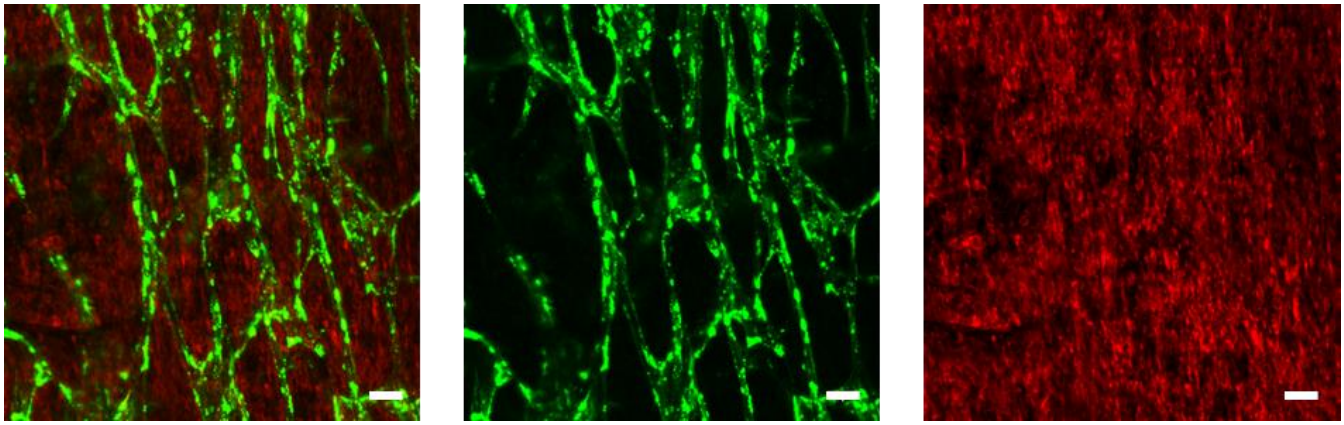

Figure S1: Orthogonal links between the well-oriented vessels. Representative images of FBs (red) and ECs (green) seeded within a Gelfoam scaffold grown under cyclic stretch, showing orthogonal links between the forming vessels. Scale bar is 50µm.

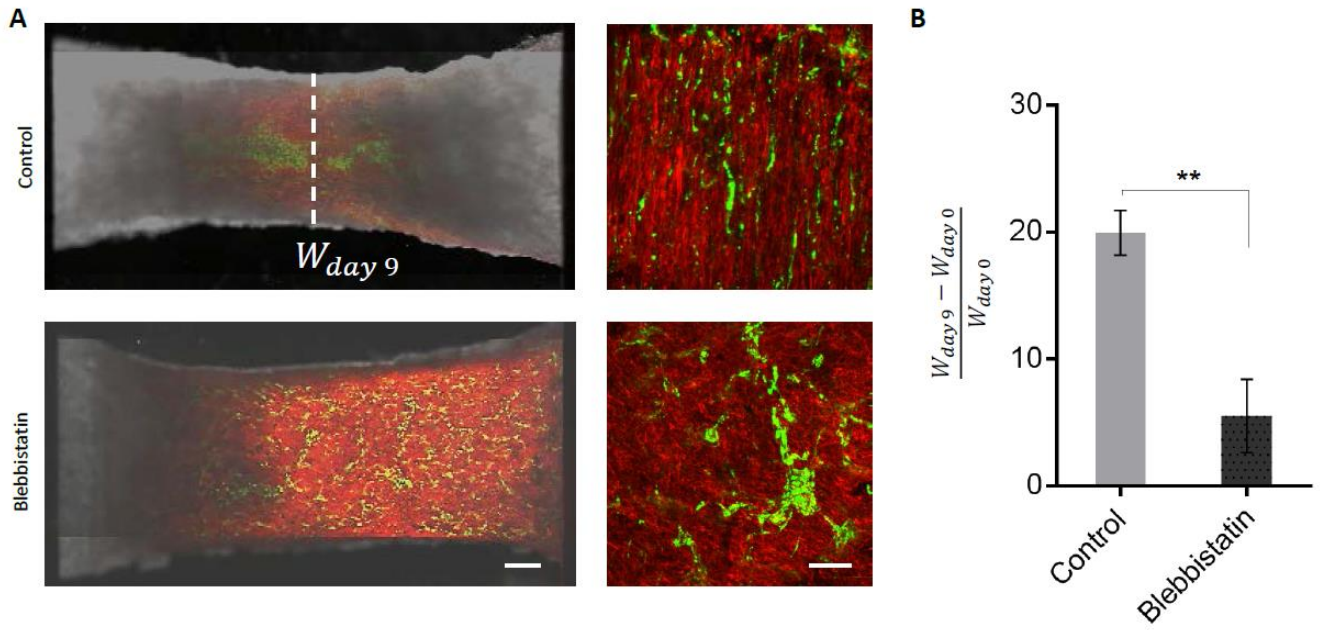

Figure S2: Lateral contraction is a result of cellular contractility. (A) A representative brightfield image of a Gelfoam scaffold seeded with FBs and ECs under cyclic stretching (top left). The image corresponds to day 9 of the cyclic stretching and the vertical dashed line represents the width of the sample at its center. A zoom-in showing the perpendicular orientation of the cells (top right). The same as the top row, but this time with blebbistatin treatment (see Methods) that significantly reduces cellular contractility (bottom row). The left scale bar is 1000µm and the right one 200µm. **(B)** The relative lateral contraction, defined as the difference between the central width of the system (cf. top left part of panel A) on days 9 and 0, normalized by the latter, for a typical cyclic stretching experiment (control) and the blebbistatin-treated sample. The results indicate that the lateral contraction is not elastic in origin, but rather a result of active cellular contractility.

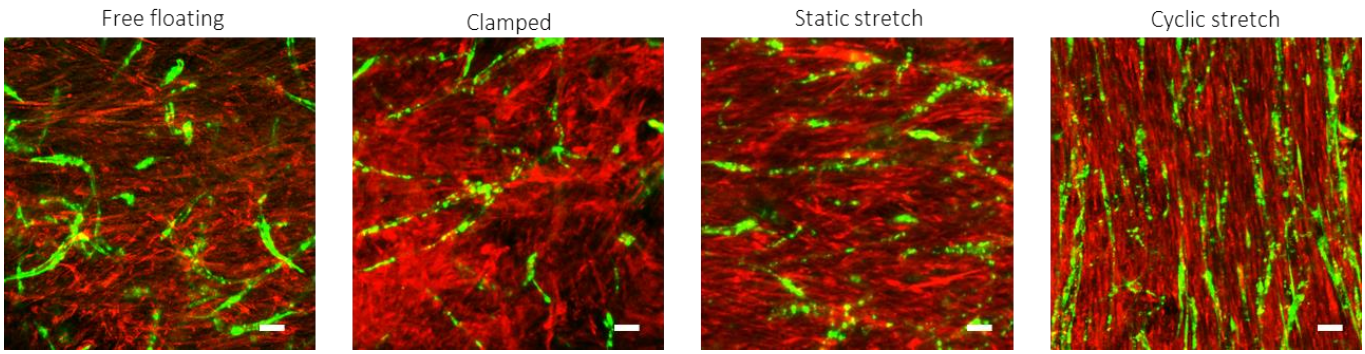

Figure S3: The effect of different external loading conditions on cells and vessels orientation. Endothelial vessels (green) and fibroblasts (red) grown within free floating, clamped, statically and cyclically stretched scaffolds for 11-14 days. In the free floating and clamped scaffolds, vessels and cells did not exhibit a distinct global orientation, whereas in the statically stretched scaffolds, vessels and cells oriented in parallel to the stretch direction and in the cyclically stretched scaffolds vessels and cells oriented perpendicularly to the stretch direction. Scale bar is 50μm.

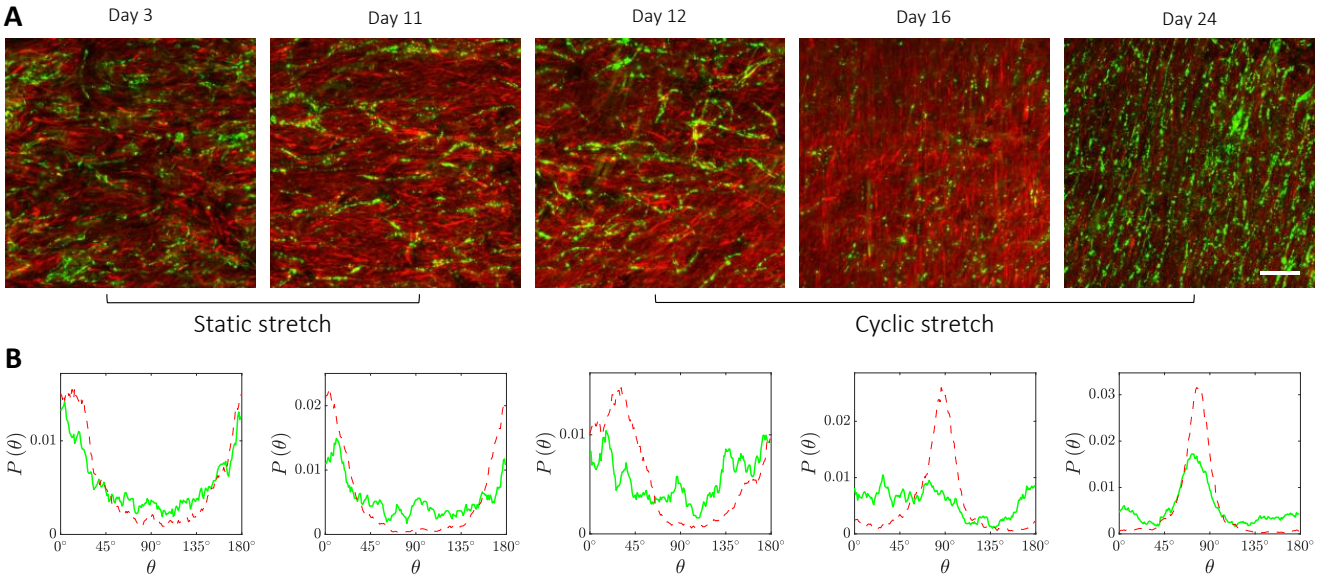

Figure S4: The orientational distribution of ECs and FBs for static stretch followed by cyclic stretch. (A) Images identical to Fig. 5A in the manuscript (see details there). **(B)** The orientational distributions, corresponding to the images in panel A, of both ECs and FBs. It is observed that under static stretch, both ECs (solid green line) and FBs (dashed red line) predominantly orient in parallel to the loading direction (hence their orientational distribution is peaked around 0° and 180°. Then, upon the application of cyclic stretch, the FBs reorient first, manifested in an orientational distribution peaked around ~ 45°. As time progresses, the orientational distribution of the FBs develops a dominant peak around 90°, while the orientational distribution of the ECs is still evolving. After 24 days both FBs and ECs are predominantly oriented around 90°, i.e. perpendicularly to the main stretch direction.