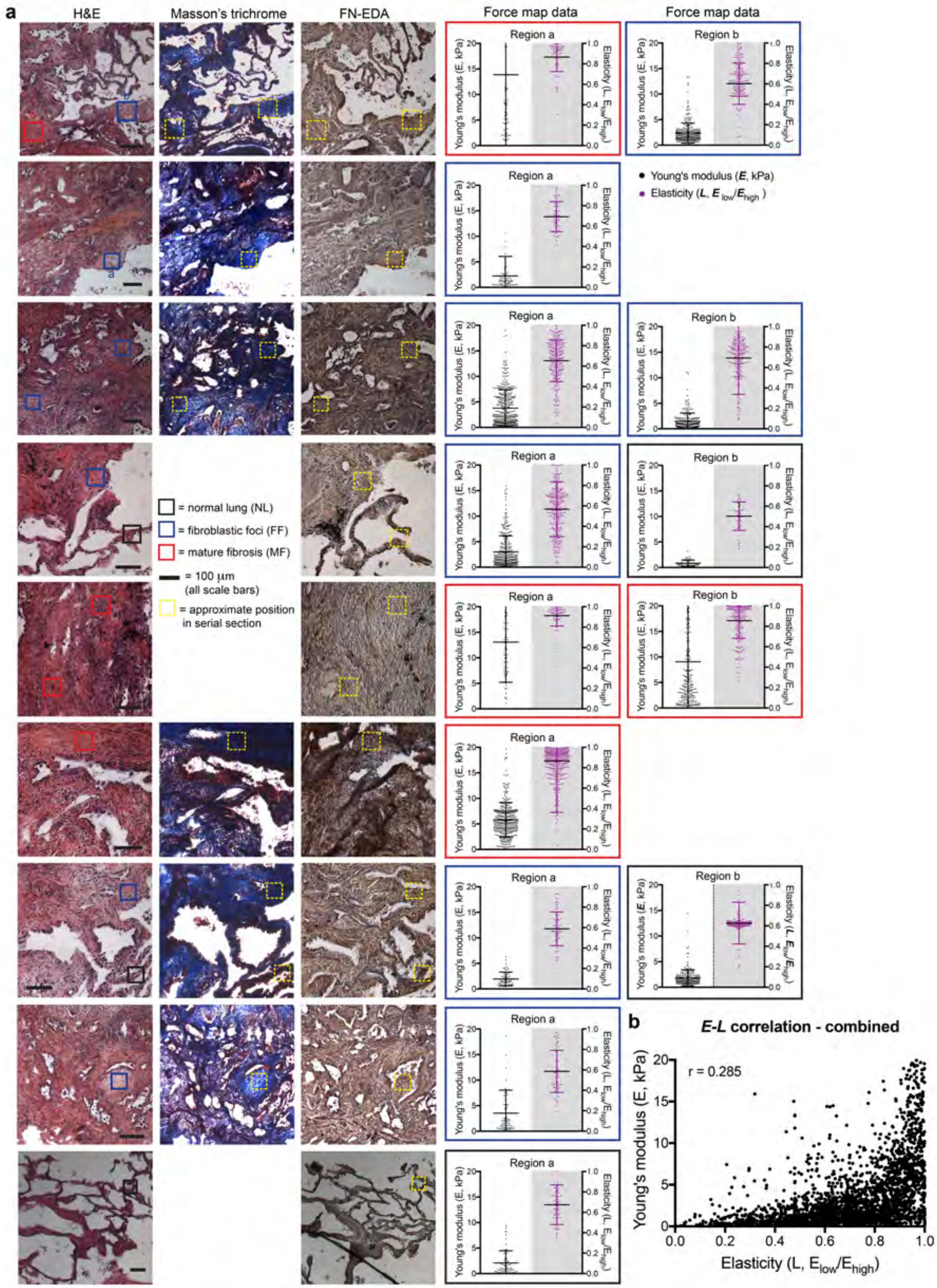
Integrin $\alpha_v\beta_3$ drives fibroblast contraction and strain stiffening of soft provisional matrix during progressive fibrosis

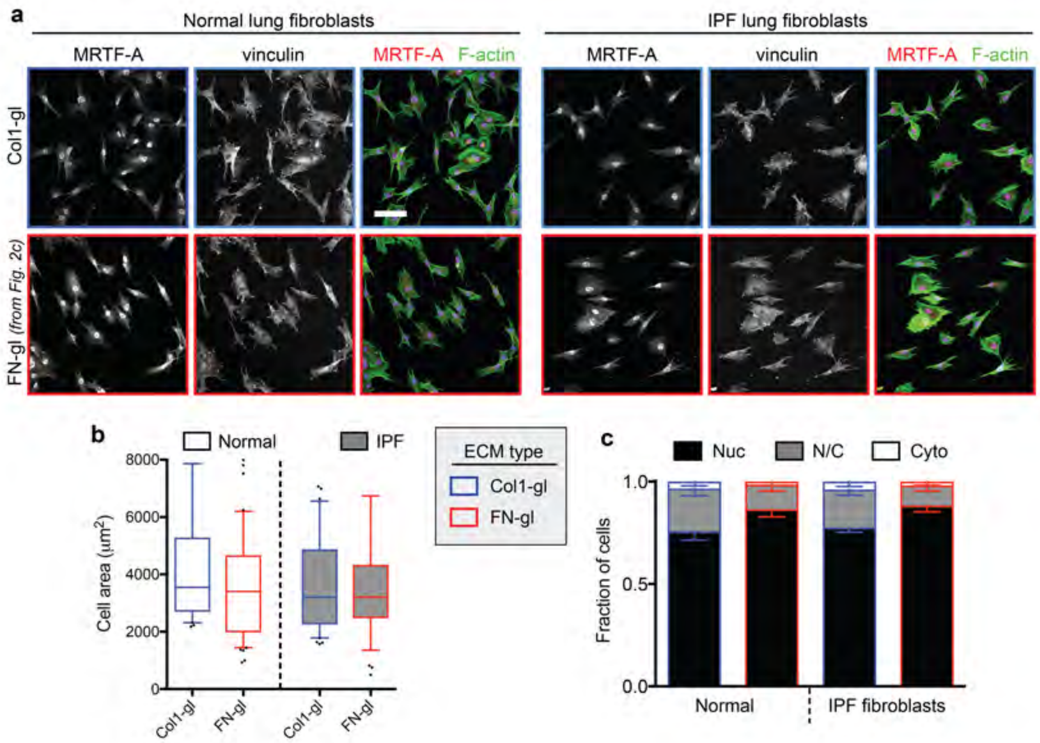
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE 1



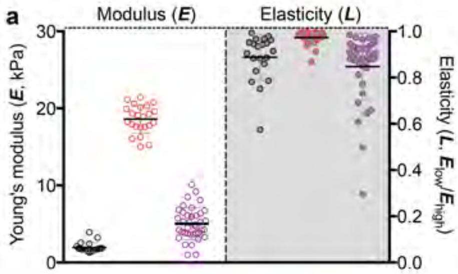
Supplementary Figure 1: Characterization of microscale IPF tissue rigidity and elasticity. **(a)** Serial sections stained for H&E (left), Masson's trichrome (middle), and FN-EDA (right) are shown. The region measured by AFM is indicated with colored boxes (NL, black; FF, blue; MF, red) overlaying H&E images, and the approximate corresponding position in serial sections stained for Masson's trichrome and FN-EDA are depicted (yellow dashed box). Scale bar, 100 µm. Dot plots of individual data points and mean ± S.D. for Young's modulus (*E*; left, black) and elasticity (*L*; right, purple) are shown for individual force maps in the rightmost columns. Ranges indicated are 0-20 kPa for *E* and 0-1 for *L* for all datasets. **(a)** Elasticity (*L*) plotted against Young's modulus (*E*) for all data points measured and presented in Fig. 1g,i. Pearson's correlation value is 0.285 and $P < 0.0001$.

SUPPLEMENTAL FIGURE 2



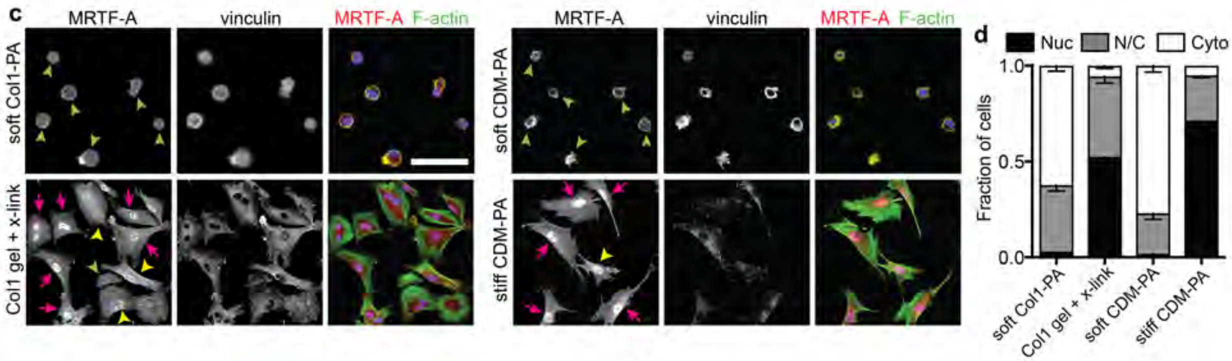
Supplementary Figure 2. Normal and IPF fibroblasts are activated on Col1- and FN-coated rigid substrates. **(a)** IF images of normal or IPF lung fibroblasts cultured on Col1-gl (top) and FN-gl (bottom) stained for MRTF-A (grayscale, left; red, overlay), vinculin (grayscale, middle), F-actin (green, overlay), and DAPI (blue, overlay). Scale bar, 100 μm. **(b)** Box-and-whisker plots (10^{th}-90^{th} percentiles with outliers) of cell area from two-independent experiments of normal (white fill, left) and IPF (gray fill, right) lung fibroblasts cultured on Col1-gl (blue outline) or FN-gl (red outline) matrices. **(c)** Fraction of cells with nuclear (Nuc, black fill), nuclear and cytoplasmic (N/C, gray fill), and cytoplasmic (Cyto, white fill) MRTF-A localization (mean ± S.E.M.) in conditions same as (b). Scale bars, 100 μm.

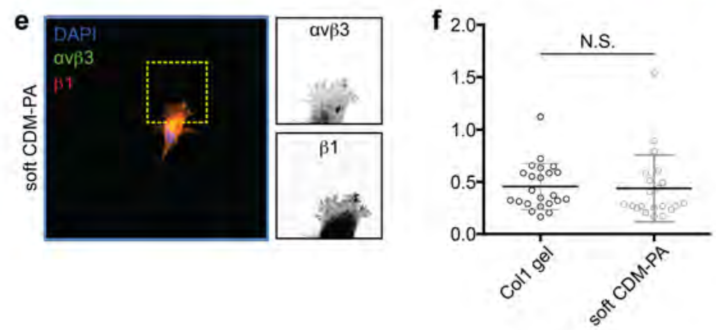
SUPPLEMENTAL FIGURE 3



b

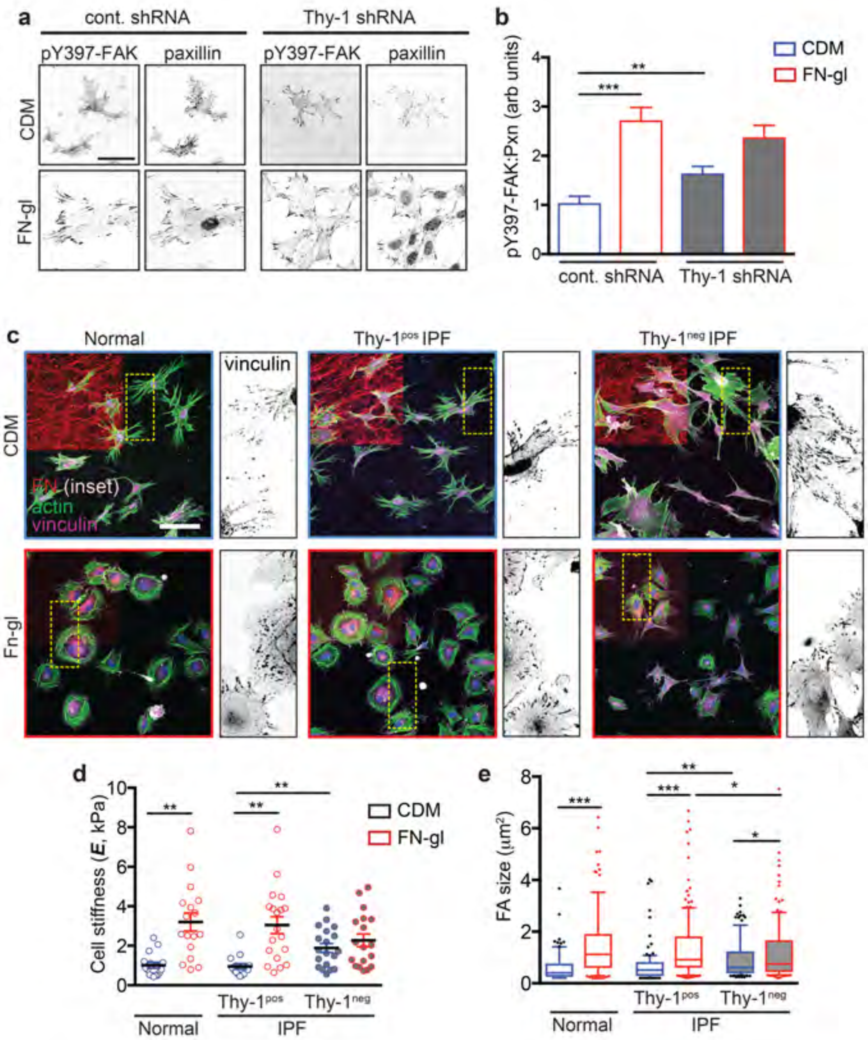
Matrix type	E (avg. ± S.D.)	L (avg. ± S.D.)	n
○ soft CDM-PA (1.7 kPa)	1.73 ± 0.29	0.88 ± 0.10	25
○ stiff CDM-PA (18.7 kPa)	18.62 ± 1.83	0.97 ± 0.04	23
○ Col1 gel + x-link (3.3 mg/mL)	5.04 ± 2.08	0.86 ± 0.15	38





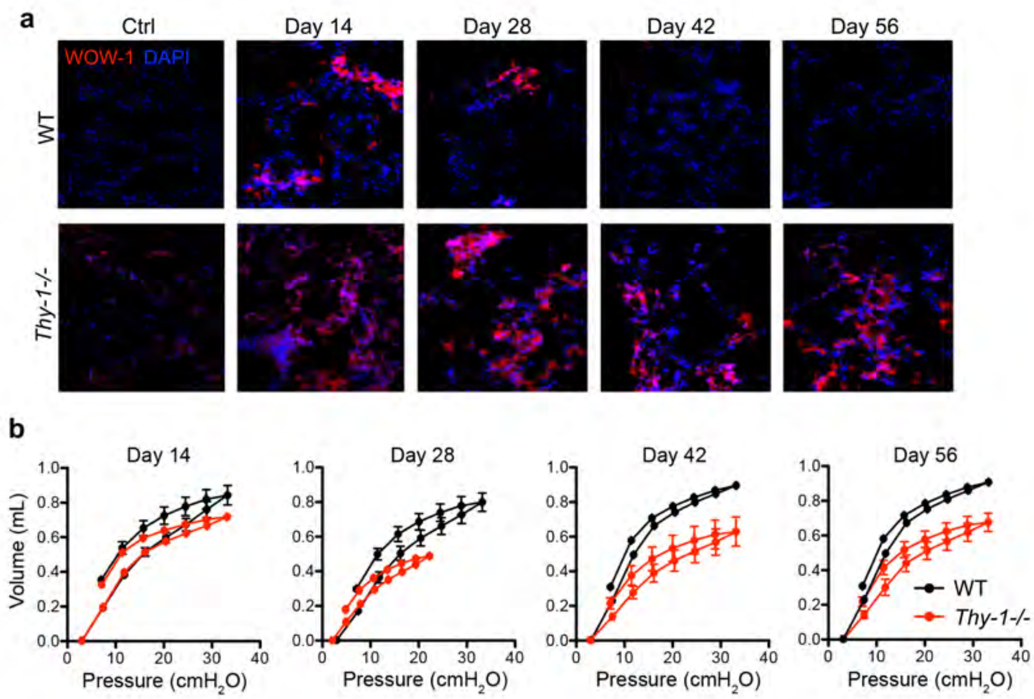
Supplemental Figure 3: Fibroblasts are not activated and do not engage $\alpha_v\beta_3$ integrin on soft, linearly elastic Col1 or FN-rich ECMs. **(a)** ECM Young's modulus (E, left) and elasticity (L, right) of soft Col1/CDM-PA gels (1.7 kPa, black outline), stiff Col1/CDM-PA gels (18.7 kPa, red outline), or crosslinked Col1 gels (Col1 gel + x-link, purple outline) as measured by AFM. Individual data points and mean ± S.D. are shown. **(b)** Data table of E and L mean ± S.D. for the number of measurements, n. **(c)** IF images of normal or IPF lung fibroblasts cultured on the indicated substrates stained for MRTF-A (grayscale, left; red, overlay), vinculin (grayscale, middle), F-actin (green, overlay), and nuclei (blue, overlay). Nuclear (pink arrows), nuclear and cytoplasmic (yellow arrowheads), and cytoplasmic (green arrowheads) MRTF-A staining is denoted. Scale bar, 100 μm. **(d)** Fraction of cells with nuclear (Nuc, black fill), nuclear and cytoplasmic (N/C, gray fill), and cytoplasmic (Cyto, white fill) MRTF-A localization (mean ± S.E.M.) in conditions same as (c). **(e)** IF images of normal lung fibroblasts cultured on soft CDM-PA gels stained for $\alpha_v\beta_3$ (green, overlay), β_1 integrin, and DAPI (blue, overlay). Zoom insets of the yellow-boxed region are shown (inverted). FN staining (purple) is shown for CDM. **(b)** Integrin engagement was quantified for $\alpha_v\beta_3$ and β_1 integrins by segmenting individual FAs and calculating the ratiometric pixel intensity between these two signals. All identified FAs were averaged for a single cell. One-way ANOVA and Newman-Keuls multiple comparisons tests were used to calculate statistical significance. Scale bars, 100 μm.

SUPPLEMENTAL FIGURE 4



Supplemental Figure 4: Thy-1 loss potentiates focal adhesion signaling and contractility of soft, nonlinearly elastic provisional ECM. **(a)** IF micrographs of Tyr397 phosphorylation of FAK (pY397-FAK, left) and paxillin (pxn, right) in shRNA-treated NLFs on CDMs versus FN-gl. **(b)** FAK activity was quantified in individual FAs using pxn to mask and normalize pY397-FAK signal (pY397-FAK:pxn); data is shown for a minimum of n = 10 cells from two-independent experiments. **(c)** IF images of NLFs or FACS-sorted Thy-1^{pos} and Thy-1^{neg} IPFLFs cultured on CDMs or FN-gl. F-actin (green), vinculin (purple) and nuclei (blue) are overlaid for the entire viewing field; FN (red) is overlaid for the corresponding area (inset, upper left), and a magnified view (yellow box) of vinculin is shown (inverted, right). Scale bar, 100 µm. **(d)** Dot plots and the mean ± S.E.M. of single-cell cortical stiffness measurements for NLF (white fill), Thy-1^{pos} IPFLF (white fill), or Thy-1^{neg} IPFLF (gray fill) cultured on CDMs (blue outline) and FN-gl (red outline). Data shown is pooled from three independent experiments. One-way ANOVA and Newman-Keuls multiple comparisons tests were used to calculate statistical significance. **(e)** Box-and-whisker plots (10^{th}-90^{th} percentiles with outlier points shown) of FA size for a minimum of n = 10 cells from two-independent experiments are shown. Statistical significance was calculated using Kruskal-Wallis non-parametric test with Dunn's multiple comparison. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ between indicated groups.

SUPPLEMENTAL FIGURE 5



Supplemental Figure 5: *Thy-1* loss elevates $\alpha_v\beta_3$ integrin activity and causes progressive fibrosis in a model of lung fibrosis. **(a)** IF images of active $\alpha_v\beta_3$ (WOW-1 Ab, red) and nuclei (blue) in lung tissue sections in WT and *Thy-1-/-* mice at 0 (Ctrl), 12, 28, 42, and 56 days after bleomycin treatment. **(b)** Pressure-volume (P-V) loops from whole-lung forced oscillation maneuvers in WT and *Thy-1-/-* mice at 14, 28, 42, and 56 days after bleomycin treatment.