Supplementary Material

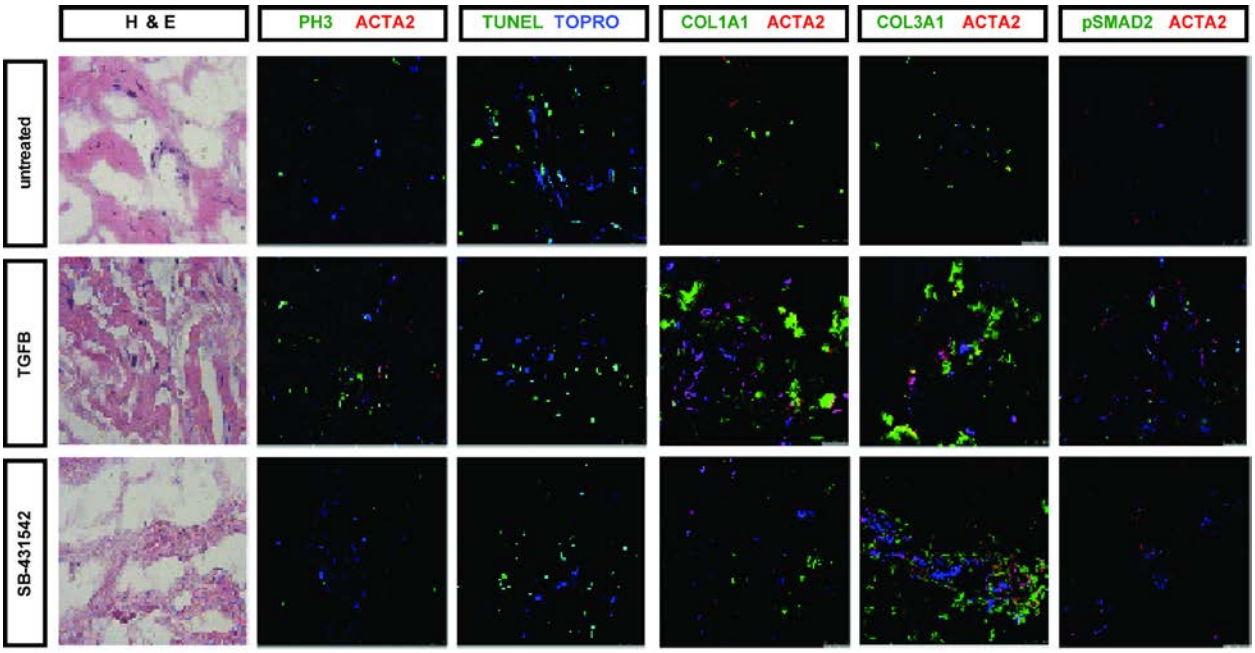

Figure S1. Normal fascia palmaris tissue cultures in the 3D *ex vivo* "clinical trial" system. Representative immunohistochemical and immunofluorescent analysis of 3D cultured normal fascia palmaris specimens (N=4). Hematoxylin and Eosin (H&E), proliferation marker phosphohistone 3 (PH3, green), apoptosis terminal deoxynucleotidyl transferase dUTP nick end labeling assay (TUNEL, green) in control normal fascia palmaris without any additional factors (untreated), after treatment with TGFB and after treatment with ALK4/5/7 kinase inhibitor SB-431542. Collagen type I (COL1A1, green), Collagen type III (COL3A1, green), phosphorylated SMAD2 (pSMAD2, green) and smooth muscle actin, alpha 2 (ACTA2, red) protein expression in control normal fascia palmaris without any additional factors (untreated), after treatment with TGFB and after treatment with ALK4/5/7 inhibitor (SB-431542). Nuclei were visualized with TO-PRO-3 (TOPRO, blue). Scale bars, 25 µm.

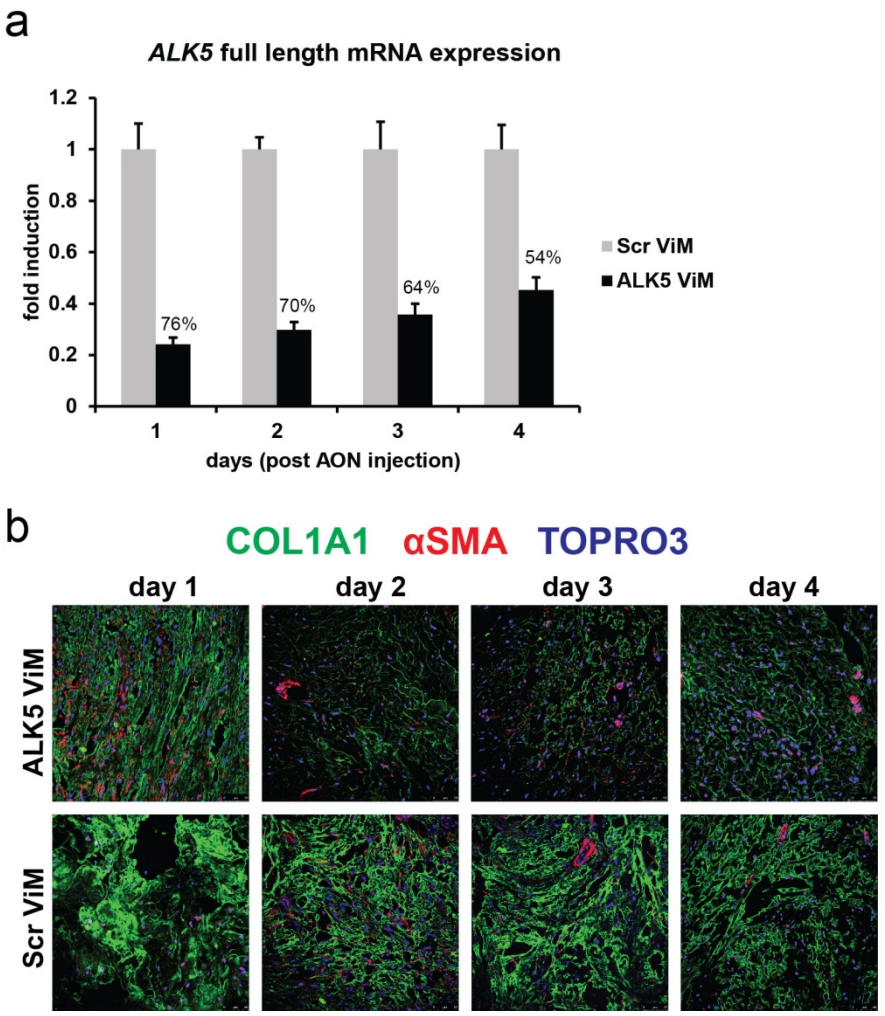

Figure S2. Time course of e*x vivo* delivery of ALK5 ViM AON. Tissues were injected with ScrViM AON (scrambled sequence) or ALK5ViM AON and cultured for 1- 4 days. **(a)** Expression of full length *ALK5* mRNA as measured by Q-PCR at different time points (day 1- day 4 after AON injection). Values were calculated as fold induction over the ScrViM (control) values for each time point. Error bars represent ±S.D. Percentages indicate the decrease of *ALK5* full length mRNA expression. **(b)** Immunofluorescent analysis of DD tissue specimens cultured in the 3D *ex vivo* system. Expression of Collagen type I (COL1A1, green), smooth muscle actin, alpha 2 (ACTA2, red) is shown. Nuclei were visualized with TO-PRO-3 (TOPRO, blue). Scale bars, 50 µm.

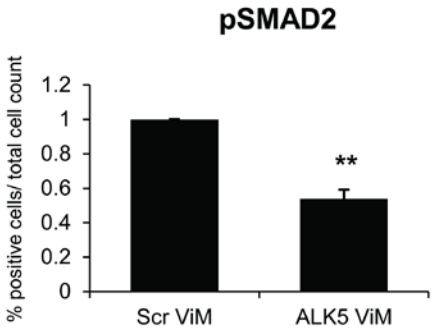

Figure S3. Quantification of pSMAD2 immunofluorescence by image analysis. Number of pSMAD2 positive nuclei over total cell count (TO-PRO3) after three-day treatment with scrambled ViM (ScrViM) and ALK5ViM. Fold induction values compared to ScrViM condition are shown. Multiple areas were quantified per sample (N=3), error bars represent ±S.E.M. Statistical significance was calculated by one-tailed paired t-test. **p<0.01

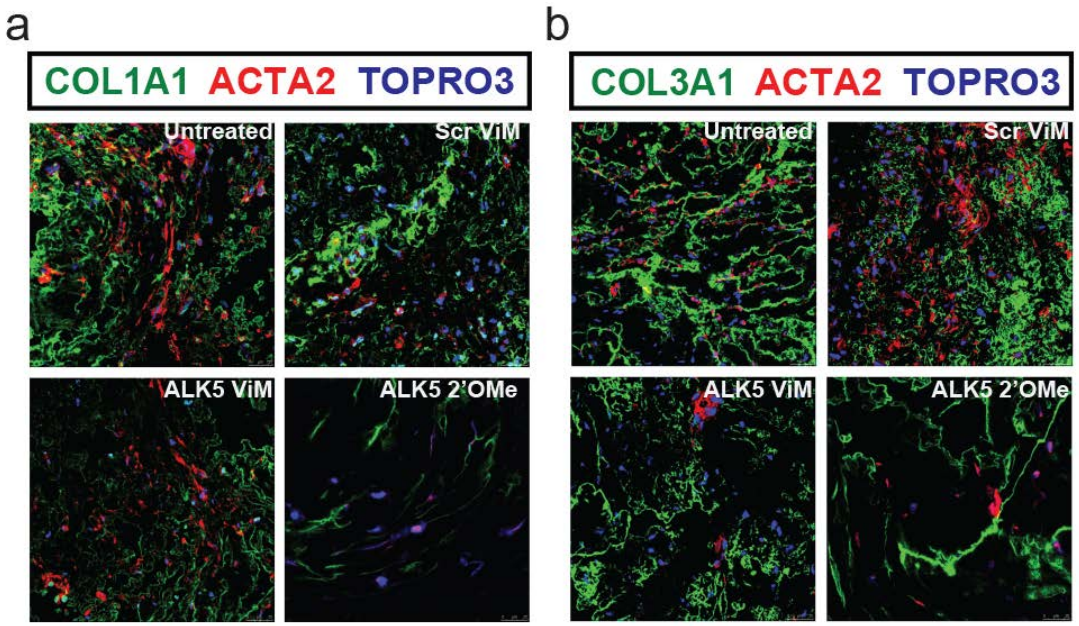

Figure S4. ***Ex vivo*** **delivery of ALK5 AON with ViM and 2'OMe chemical backbones.** Immunofluorescent analysis of DD tissue specimens cultured in the 3D *ex vivo* system. Tissues were injected with ScrViM AON (scrambled sequence), ALK5ViM AON or ALK5 2'OMe AON and cultured for 72 hours. **(a)** Expression of Collagen type I (COL1A1, green), smooth muscle actin, alpha 2 (ACTA2, red) **(b)** Collagen type III (COL3A1, green) and smooth muscle actin, alpha 2 (ACTA2, red) protein expression. Nuclei were visualized with TO-PRO-3 (TOPRO, blue). Scale bars, 25 µm.

Supplementary Materials and Methods

Generation of 3D culture system

In order to bypass the need for exogenous ECM and the derivation of fibroblasts, we have developed a patient-derived specimen culture system. This is the first tissue resection culture system that allows human tissue from DD patients to be grown *ex vivo* and functionally tested, recapitulating the *in vivo* situation. The system is based on a nitrocellulose membrane that allows contact of the tissue with the medium but not with the plastic, thus preventing the alteration of the tissue upon attachment, as previously observed when culturing DD fibroblasts. No collagen gel or other ECM protein substrate is required, since the DD tissue itself produces large amounts of these proteins. In brief: specimens from DD surgeries are equally sliced and placed in transwell plates onto nitrocellulose filters. Tissues remain continuously and statically in contact with defined culture conditions (Dulbecco's Modified Eagle's Medium (DMEM, with 1% fetal calf serum (FCS), 1% penicillin-streptomycin) but without being entirely submerged and are allowed to grow (seven days). Tissue resection specimens (N=9 DD and N=4 normal fascia palmaris) were treated with a combination of activators and inhibitors of the TGFB signaling pathway (e.g. TGFB, 5ng/ml; SB-431542, 10ng/ml, Tocris). After culture, tissues were processed for RNA isolation or they were fixed in 4% paraformaldehyde, incubated in 30% sucrose buffer, embedded in Tissue Tek-O.C.T. compound and stored at -80°C.

Immunofluorescence

Hematoxylin and Eosin (H&E) staining were performed using standard protocols on 10-µm cryosections. Immunofluorescence staining was performed on 10-µm cryosections. For antigen retrieval sections were boiled in antigen unmasking solution (Vector Labs) and were incubated in 3% H_2O_2 for endogenous peroxidase sequestering. Primary antibodies and dilutions used are as follows: anti-ACTA2 1:500 (Sigma), anti-COL1A1 1:500 (Southern Biotech), anti-COL3A1 1:500 (Southern Biotech), and anti-phospho-SMAD2 1:1000 (Cell Signaling). Sections were blocked with 1% bovine serum albumin (BSA)-PBS-0.1% v/v Tween 20) and incubated with primary antibodies diluted in the blocking solution, overnight at 4°C or room temperature. Sections then were incubated with secondary antibodies labeled with Alexa Fluor 488, 555, or 647 (Invitrogen/Molecular Probes, 1:250 in PBS-0.1% Tween 20). Detection of pSMAD2, was enhanced using tyramide amplification (Invitrogen/Molecular Probes) by incubation of slides with horseradish peroxidase (HRP)-conjugated secondary antibody (1:100 dilution) (Invitrogen/Molecular Probes), followed by incubation with tyramide-488 for 10 minutes. Sections were counterstained with TO-PRO3 (Invitrogen/Molecular Probes) at 1:1000 dilution in PBS-0.1% Tween20 for nuclei visualization, and mounted with Prolong G mounting medium (Invitrogen/Molecular Probes), which contains DAPI.

RNA isolation, RT-PCR and Quantitative PCR

Nodule parts (100 µm) were homogenized using an Ultra Turrax homogenizer (T25 basic, IKA) in TRIpure reagent (Roche) and directly processed for total RNA isolation according to the TRIpure RNA extraction protocol. Total RNA (0.5 µg) was used for first strand cDNA synthesis using RevertAid H Minus first strand cDNA synthesis kit (Fermentas). For quantitative PCR (Q-PCR) ten-fold diluted cDNA was amplified in a CFX Real Time Detection system (Bio-rad) using SYBR Green Supermix reagent (Bio-rad). To detect full length ALK5 mRNA levels, primers flanking exon 2 and exon 3 were used. Expression levels were normalized to housekeeping gene (ACTRT1 or CAPNS1) and analyzed using the linear regression method. For exon skipping test, cDNA was amplified by touchdown PCR using ALK5 specific primers, which are designed to amplify both the full length and exon 2-skipped mRNA transcripts (forward

primer in exon 1, reverse primer in exon 3). Primer sequences and detailed PCR protocol are available upon request.

Microscopy and Image analysis

Confocal microscopy of labelled specimens was performed on a Leica TC-SP5 microscope with a 40X 1.4 NA oil-immersion objective Z series were collected and reassembled in Image J software (rsbweb.nih.gov/ij). Mean area fraction fluorescence was calculated in Image J software using threshold to select the root boundary and measuring the percentage of positive surface inside the intensity defined by the threshold. Experiments were repeated three times and stained specimens in a given experiment were imaged using identical microscopic exposure and recording settings. Second-harmonic generation (SHG) was performed for imaging the organization of collagen fibers in native tissue. This type of two-photon microscopy was performed on a Zeiss 710 NLO upright confocal microscope (Jena, Germany) equipped with a femtosecond Spectra-Physics Deep See MP laser (Santa Clara, United States) using a **Plan-Apochromat 20x/1.0 NA** water-immersion objective. The images were obtained with an excitation wavelength of 750 nm and emitted light was collected between 371–467 nm. Confocal stacks were processed with the Zeiss ZEN2009 software.