Identifying collagen VI as a target of fibrotic diseases regulated by CREBBP/EP300

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Content

1. SI Appendix, Supplementary Materials and Methods
2. SI Appendix, References
3. SI Appendix, Tables 5
4. SI Appendix, Figures 10

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1. SI Appendix, Supplementary Materials and Methods

Dupuytren’s Tissue Collection

Nodular tissue was obtained from patients with Dupuytren’s disease undergoing dermofasciectomy as previously described (1). Informed written consent was obtained through approval of the regional ethics committee (REC 07/H0706/81), in accordance with the Declaration of Helsinki.

Cell culture

Tissue samples were dissected into small pieces and digested in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, UK) with type I collagenase 4mg/ml (Worthington Biochemical Corporation) + DNase I (Roche Diagnostics) for up to 3 hours at 37 °C. Digested tissue fragments were filtered through a 70-μm cell strainer, and the cell suspension was centrifuged at 1500 rpm for 10mins. Isolated cells were cultured in DMEM with 5% FBS and 1% penicillin–streptomycin and used until passage 2.

Compound screening

Using a focused library of small-molecule inhibitors targeting epigenetic modifications, provided by Structural Genomics Consortium, Oxford (Table S2), we assessed their effect on steady state mRNA expression levels of ACTA2, COL1A1, and TGFB1 in passage 2 DD myofibroblasts. All experiments were harvested after 3 or 7 days in culture and performed in cells from 5 donors, using compound doses between 0.1-10μM. Cell viability was assessed after 3 or 7 days using the MTS assay (Promega).
**Transfections for siRNA**

Cells were seeded at 2 x 10^4/24 well plate and the following day transfected with siRNA against BRD4 (s23901), CREBBP (s3595), EP300 (s4695) or non-targeting control oligo at 1-20nM (Life Technologies). All transfections were performed with Dharmafect 1 (GE Healthcare) and Optimem™ (Life Technologies) according to the manufacturer's instructions. Media was replaced 2 hrs later with 5% FBS phenol red free DMEM (Gibco). Experiments were performed either 3-6 days post transfection.

**Antibodies**

Antibodies used in immunoblotting; anti-αSMA (Sigma A5228), anti-BRD4 (Abcam 128874), anti-CREBBP (Cell Signalling 7425), anti-EP300 (Millipore 05-257), anti-collagen alpha-1(I) chain (Millipore ABT257), anti-collagen alpha-1(VI) chain (Abcam 182744), anti-collagen alpha-3(VI) chain (Millipore MAB1944), Fibronectin (ED-A, Abcam 632800), Fibroectin-ED-B (Abcam 154210), anti-Lamin B1 (Santa Cruz 20662) and anti-Lamin A/C (BD Biosciences 612163). Antibodies used in ChIP experiments; rabbit Ig (Diagenode C15410206), anti-BRD4 (Cell Signallin E2A7X), anti-EP300 (Millipore 05-257), anti-CREBBP (Abcam ab2832) (Diagednoe C15200211), anti-H3K27ac (Abcam ab177178) and anti-H3K4me1 (Cell Signaling D1A9).
**Measurement of mRNA**

RNA was extracted using Quick-RNA MiniPrep kit (Zymo) and cDNA was generated using the RNA-Ct kit (Life Technologies). Gene expression was determined by quantitative PCR on a ViiA™7 using Taqman™ Fast Advanced Mastermix (Life Technologies) and Assay-On-Demand premixed Taqman™ probe master mixes (Life Technologies, Table S4). The relative gene expression was calculated using the ΔΔCt method with the GAPDH gene for normalization of mRNA levels.

**RNA isolation and RNA-seq library preparation**

RNA was isolated from myofibroblasts using the Quick-RNA MiniPrep kit (Zymo) according to the manufacturer's protocol. The quality of the RNA samples was verified by electrophoresis on 2200 TapeStation (Agilent). The RIN scores for all samples were in the range of 7.5–9.5. RNA-seq libraries were prepared using the NEBNext® Ultra™ RNA library prep kit for Illumina® using NEB indexes, according to the manufacturer's protocol. The concentration of each library was determined using Agilent TapeStation. The resulting libraries were sequenced on a NextSeq 500 platform (Illumina) using a paired-end run 2 × 80 bp, to an average depth of 20 × 10^6 paired-end reads/sample. The concentration of each library was determined using Agilent 2200 TapeStation.

**Chromatin immunoprecipitation (ChIP) assay/ChIP-Seq**

For ChIP experiments nuclear lysates from 2 x 10^6 myofibroblasts were fixed for 10 minutes in 1% formaldehyde and were isolated as described previously (2) and subjected to 6 cycles of sonication using the Biorupter Pico system (Diagenode). Each lysate was immunoprecipitated using 2.5-5 μg of the relevant antibodies. Real-time PCR was performed on fragmented DNA using specific primers for the ACTA2 or COL1A1 (ACTA2-F 5’- gaagcttggcgtttatc, ACTA2-
R, 5’- ctggtcctcacaaggggt-3’, COL1A1-F, 5’- gccagtcgagcaga-3’, COL1A1-R, 5’-
c tgacttgcatctctc-3) using SYBER green Power Master mix (Life Technologies). ChiP-Seq was performed using 2 biological replicates from 2 donors. Nuclear lysates from 10 x 10⁶ myofibroblasts were fixed in 1% formaldehyde for 15mins prior to quenching. For histone marks H3K27ac, H3K4me1 and BRD4 chromatin was precleared with protein G Dynabeads (Life Technologies) prior to an overnight incubation at 4°C with 5-10ug of protein G pre-coupled antibody. DNA was purified using Qiagen PCR purification kit. For EP300 ChIP was performed with 25 x 10⁶ myofibroblasts using iDeal transcription factor kit (Diagenode) as per manufacturer’s protocol, with an additional 30 minute fixation step using ChIP cross-ling Gold (Diagenode), followed by a further conventional fixation step of 10 minutes with 1 % formaldehyde. Purified DNA was used for library preparation using a NEBNext ® Ultra ™ II sample preparation kit (NEB) according to the manufacturer's recommendations. The samples were multiplexed, quantified using a Bioanalyser (Aglient), and sequenced on a NextSeq 500 (Illumina) platform (paired-end, 2 x 41 bp). Sequencing depth was in excess of 20 million reads/sample, suggesting sufficient coverage.

Processing of next-generation sequencing data
A computational pipeline was written calling scripts from the CGAT toolkit to analyse the next generation sequencing data https://github.com/cgat-developers/cgat-flow (3). For RNA-seq experiments, sequencing reads were mapped to the reference human genome sequence (GRCh37 (hg38) assembly) using HISAT v0.1.6 (4). To count the reads mapped to individual genes, the program featureCounts v1.4.6 (5) was used. Only uniquely mapped reads were used in the counting step. The counts table that was generated was then used for differential expression analysis. Differential gene expression analysis was performed with DESeq2 v1.12.3 within the R
To define differentially expressed genes, a threshold of >2-fold change, and a false discovery rate of <0.05 were used. For analysis of ChIP-seq, Bowtie2 software v0.12.5 was used to align the reads to the human hg19 reference genome (7). Reads were only considered that were uniquely aligned to the genome with up to two mismatches. The peak calling step used MACS2 using the broad option and for each condition the corresponding input was used. Homer tag directories were then produced using the raw read function, and coverage plots around the TSS were plotted in R. MACS software (v1.4.2) or SICER (v1.1) was used to identify enrichment of intervals of EP300, BRD4 H3K4me3 and H3K27ac following ChIP-seq. Sequencing of the whole cell extract was performed to determine the background model when analysing ChIP-seq. Enrichment of reads around the TSS and enhancer sites was calculated and plotted across the genome using the ngs.plot.r package (8). For visualization as a University of California Santa Cruz Genome Browser track, the bam files generated from Bowtie were converted to BigWig files using bam2wiggle (9). Pathway enrichment analysis was performed using XGR (10).

**Data Availability**

RNA-seq, and ChIP-seq datasets are deposited within the SRA database under accession numbers PRJNA625874, PRJNA624331 and PRJNA624119 respectively.

**Immunofluorescence and confocal microscopy**

Myofibroblasts were grown on poly-L lysine (Sigma) coated glass coverslips and stained for F-actin as previously described (1). Fluorescent images were obtained with a LSM710 confocal microscope (Leica Microsystems). Circularity: $4\pi \times ([\text{Area}] / [\text{Perimeter}]^2)$, with a value
of 1.0 indicating a perfect circle), Feret and Perimeter calculations were analyzed and processed with ImageJ.

Mass spectrometry analysis and protein identification

DD myofibroblasts were treated with either DMSO, JQ1 (0.5µM) or CBP30 (2.5µM) for 3 days. Cell pellets were lysed in 25 µl of lysis buffer, consisting of radioimmunoprecipitation assay (RIPA) buffer (Sigma) with 4% NP-40 (IPEGAL, Sigma). After thawing, sample preparation involved an in-solution digestion protocol followed by a purification step. Briefly, after reduction and alkylation of cysteine residues, protein precipitation was undertaken via methanol/chloroform extraction (REF: https://www.ncbi.nlm.nih.gov/pubmed/6731838). After this, we carried out an overnight digestion in 0.2µg/ml Trypsin (Promega) at 37°C and purified the resulting peptides by reversed-phase extraction. Peptides were analyzed with a nLC-MS/MS platform consisting of a Dionex Ultimate 3000 and Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific). Label-free quantification of proteins was performed using Progenesis QI for Proteomics (version 4.1, Waters) and proteins were identified using MASCOT (Matrix Science) by searching against the Swissprot human database (Feb. 2018). The Benjamini–Hochberg multiple testing correction was used to estimate the FDR.
**Traction force microscopy**

Polyacrylamide (PAA) (Bio-Rad, CA) hydrogels used in traction force microscopy were prepared as previously described (4). The Young’s modulus of the PAA gels were 2.55±0.5 KPa. Bead displacement was tracked with an ImageJ PIV plugin (11) (12) and cellular forces reconstructed using a FTTC algorithm also implemented in ImageJ. Plots were generated in MATLAB (Mathworks).

**Chemotaxis Assay**

5 x 10^5 THP-1 cells (ATCC) were allowed to adhere for 2 hrs to a Corning Transwell® plate with 8µM pore in 1% FBS DMEM, the reservoir plate was then replaced with conditioned media generated from DD myofibroblasts treated with either control non-targeting Silencer Select™ siRNA (20nM) or a combination of COL6A1/2/3 siRNA (8.3nM each) (Life Technologies, UK). For the last 3 days of the assay, the media was replaced with 1% FBS, DMEM. Supernatants harvested, filtered and used neat, alongside CCL2 (2 ng/ml) (Peprotec h, London UK) as a positive control. Cells were allowed to migrate for 6 hrs, the number of migrated cells were counted in the reservoir plate following the addition of Hoechst 3342 Fluorescent Stain (Life Technologies) at 0.1µg/ml for 1 hr using the Celigo Imaging Cytometer, Nexelom Bioscience (Lawrence, MA).
**Scratch Wound Assay**

5 x 10³ DD myofibroblasts were allowed to adhere for 24 hrs to a collagen coated (0.1µg/ml) 96 well tissue culture plate. Cells were visualised by treating with Calcein-AM for 1 hr prior making a scratch using an IncuCyte® woundmaker. Cells were imaged using a Celigo Imaging Cytometer, Nexcelom Bioscience (Lawrence, MA), and then imaged 24 hrs later and whole-well images were acquired using the Celigo Wound Healing application which reports the wound healing confluence and cell count for each well over time within the defined analysis area.

**Immunofluorescent Microscopy**

Multiplex-immunofluorescent (IF) staining of COL1A1/αSMA/COL6A3 was performed on Leica Bond-Rx Autostainer (Leica Microsystems) with Opal 7-Color Automation IHC Kit (PerkinElmer, NEL821001KT). FFPE tissue sections (4 μm) were antigen-unmasked with epitope retrieval solution 1 (Leica, AR9961) for 20 minutes at 100°C, blocked with PKI blocking buffer for 5 minutes. Anti-Collagen I α1 antibody (Abcam, ab138492, 5ug/ml) was applied for 30 minutes at room temperature, followed by Opal Polymer HRP for 10 minutes, and Opal 540 dye for 10 minutes. The tissue sections were then heated again in epitope retrieval solution 1 for 20 minutes at 95°C to remove excessive unbounded antibody before they were stained for the next antibody. The similar staining cycle was repeated for anti-αSMA antibody (Sigma A5228, 1:15K) with Opal 620 dye, and anti-Collagen α3 antibody (Sigma, HPA010080, 2.8ug/ml) with Opal 690 dye sequentially. Finally the slides were cover-slipped with Prolong gold anti-fade reagent with DAPI (Invitrogen, P36935), and images were taken with Vectra Polaris (PerkinElmer) using Phenochart (version 1.0.10) and inForm (version 2.4.4) softwares.
ELISA

The concentration of collagen 6 α3, CCL2 and CCL7 in cell supernatants was determined by ELISA (BD Biosciences, Oxford UK and R&D systems, Oxford, UK respectively) according to the manufacturer’s instructions.

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3. **SI Appendix, Tables**

**SI Appendix, Table S1 Pi pathway genes**

| Name                      | nAnno<sup>a</sup> | nOverlap<sup>b</sup> | Z score | P value      | Adjusted P value | Overlapped genes<sup>c</sup>                                                                 |
|---------------------------|-------------------|----------------------|---------|--------------|------------------|------------------------------------------------------------------------------------------------|
| FoxO signaling pathway    | 129               | 15                   | 7.57    | 2.70E-08     | 4.40E-07         | MAPK3, AKT1, EGFR, GRB2, CREBBP, EP300, SIRT1, FOXO3, CDK2, GABARAP, MDM2, PLK1, GABARAPL1, GABARAPL2, RBL2 |
| MAPK signalling pathway   | 247               | 16                   | 4.91    | 2.90E-05     | 0.00023          | PRKACA, HSPA1A, HSPA1B, TP53, HSPA8, ATF2, MAPK3, AKT1, TRAF2, RELA, CDC42, EGFR, JUN, MYC, GRB2, NTRK1 |
| ErbB signalling pathway   | 84                | 8                    | 4.69    | 0.00035      | 0.0018           | MAPK3, AKT1, EGFR, JUN, MYC, GRB2, SRC, ERBB2                                                                 |
| PI3K-Akt signalling pathway| 333              | 16                   | 3.57    | 0.0011       | 0.0043           | TP53, ATK2, MAPK3, AKT1, RELA, EGFR, MYC, GRB2, FOXO3, CDK2, MDM2, RBL2, YWHAE, YWHAZ, HSP90AA1, HSP90AB1 |
| cAMP signalling pathway   | 194               | 11                   | 3.48    | 0.0023       | 0.0072           | PRKACA, MAPK3, AKT1, RELA, JUN, PPP1CB, PPP1CC, PPP1CA, CREBBP, CFTR, EP300                                                                 |
| Wnt signalling pathway    | 141               | 9                    | 3.53    | 0.0027       | 0.0073           | PRKACA, TP53, JUN, MYC, CTNNB1, CREBBP, EP300, RUVBL1, CSNK1A1                                                                 |
| HIF-1 signalling pathway  | 96                | 7                    | 3.5     | 0.0041       | 0.0093           | MAPK3, AKT1, RELA, EGFR, ERBB2, CREBBP, EP300                                                                 |
| TGF-beta signalling pathway| 82               | 6                    | 3.24    | 0.0078       | 0.016            | MAPK3, MYC, CREBBP, EP300, E2F4, SP1                                                                 |
| Hippo signalling pathway  | 150               | 8                    | 2.73    | 0.014        | 0.025            | MYC, CTNNB1, ACTB, PPP1CB, PPP1CC, PPP1CA, YWHAE, YWHAZ                                                                 |
| TNF signalling pathway    | 108               | 6                    | 2.46    | 0.028        | 0.045            | ATF2, MAPK3, AKT1, TRAF2, RELA, JUN                                                                 |

<sup>a</sup>Number of genes annotated to a pathway; <sup>b</sup>Number of genes in a pathway that overlap with the crosstalk gene network; <sup>c</sup>List of overlapped genes.
SI Appendix, Table S2. Binding of EP300 at genomic locations of SNPs significantly associated with Dupuytren’s Disease

| Chromosome | Position / rsID          | SNP discovery P value | Maximal EP300 binding location | Selected nearby genes       |
|------------|--------------------------|----------------------|--------------------------------|-----------------------------|
| 1          | 162672011 rs17433710     | 9.13 x 10^-7         | 162508632                      | DDR2, HSD17B7               |
| 7          | 37973014 rs2598107       | 1.10 x 10^-30        | 37970400                       | SFRP4, EPDR1                |
| 7          | 37989095 rs16879765      | 7.15 x 10^-41        | 37970400                       | SFRP4, EPDR1                |
| 7          | 116892846 rs38904        | 1.02 x 10^-11        | 116899830                      | WNT2                        |
| 13         | 44842503 rs9525927       | 5.8 x 10^-6          | 4487194                        | SMIM2, SERP2                |
| 14         | 23312594 rs1042704       | 8.72 x 10^-13        | 23320747                       | MMP14                       |
| 14         | 51074461 rs1032466       | 4.90 x 10^-9          | 51142673                       | ATL1, MAP4K5, SAV1          |
| 15         | 56229760 rs1509406       | 4.03 x 10^-6          | 56200240                       | NEDD4                       |
| 15         | 68628163 rs2306022       | 7.59 x 10^-6          | 68644962                       | ITGA11                      |
### SI Appendix, Table S3. Epigenetic probes used in this study

| Probe   | Target Protein family         | Specific target     | Conc. µM | Duration (days) |
|---------|-------------------------------|---------------------|----------|-----------------|
| A-366   | Histone lysine methyltransferases | G9a/GLP             | 0.1      | 7               |
| UNC0638 | Histone lysine methyltransferases | G9a/GLP             | 0.1      | 7               |
| UNC0642 | Histone lysine methyltransferases | G9a/GLP             | 0.1      | 7               |
| R-PFI-2 | Histone lysine methyltransferases | SETD7               | 0.1      | 7               |
| GSK343  | Histone lysine methyltransferases | EZH2               | 0.1      | 7               |
| UNC1999 | Histone lysine methyltransferases | EZH2               | 0.1      | 7               |
| LLY-507 | Histone lysine methyltransferases | SMYD2              | 1        | 7               |
| BAY-598 | Histone lysine methyltransferases | SMYD2              | 0.1      | 7               |
| A-196   | Histone lysine methyltransferases | SUV420H2           | 0.1      | 7               |
| SGC0946 | Histone lysine methyltransferases | DOT1L            | 5        | 7               |
| MS023   | Protein lysine methyltransferase | PRMT1,3,4,6,8      | 2.5      | 7               |
| SGC707  | Protein lysine methyltransferase | PRMT3              | 2.5      | 7               |
| TP-064  | Protein lysine methyltransferase | PRMT4,6            | 2.5      | 7               |
| MS049   | Protein lysine methyltransferase | PRMT4,6            | 2.5      | 7               |
| GSK591  | Protein lysine methyltransferase | PRMT5              | 2.5      | 7               |
| OICR-9429 | Methyl lysine binder          | WDR5               | 0.1      | 3               |
| A-395   | Methyl lysine binder          | EED                | 0.1      | 3               |
| UNC1215 | Methyl lysine binder          | L3MBTL3            | 0.1      | 3               |
| NVS-CER2-1 | Bromodomains         | CERC2              | 1        | 3               |
| LP99    | Bromodomains               | BRD7, 9            | 10       | 3               |
| BI-9564 | Bromodomains               | BRD7, 9            | 1        | 3               |
| TP472   | Bromodomains               | BRD7, 9            | 0.5      | 3               |
| I-BRD9  | Bromodomains               | BRD 9              | 1        | 3               |
| BAZ2-1CR | Bromodomains            | BAZ2A/B            | 10       | 3               |
| GSK2801 | Bromodomains               | BAZ2A/B            | 10       | 3               |
| Bromosporine BrSp | Bromodomains | BRD2, BRD3, BRD4, BRDT | 5 | 3 |
| JQ1     | Bromodomains               | BRD2, BRD3, BRD4, BRDT | 5 | 3 |
| PFI-1   | Bromodomains               | BRD2, BRD3, BRD4, BRDT | 10 | 3 |
| PFI-3   | Bromodomains               | SMARCA2/4          | 10       | 3               |
| I-CBP112 | Bromodomains          | CREBBP/EP300 BRD  | 10       | 3               |
| SGC-CBP30 | Bromodomains         | CREBBP/EP300 BRD  | 10       | 3               |
| NI-57   | Bromodomains               | BRPF1              | 10       | 3               |
| PFI-4   | Bromodomains               | BRFP1              | 10       | 3               |
| BAY-299 | Bromodomains               | BRFP1B/2           | 10       | 3               |
| OF-1    | Bromodomains               | BRFP1B/2           | 10       | 3               |
| L-Moses | Bromodomains               | PCAF,GCN5         | 1        | 3               |
| GSK-J4  | Histone demethylase        | JMJD3/UTX          | 5        | 3               |
| GSK-LSD1 | Histone demethylase       | Lysine specific demethylase 1 | 10 | 3 |
| IOX2    | 2OG                         | 2-oxoglutarate oxygenases | 10 | 3 |
**SI Appendix, Table S4. Taqman primers and siRNA oligos used in this study**

| Target     | Taqman primer/probe   | siRNA  | Target     | Taqman primer/probe   | siRNA  |
|------------|-----------------------|--------|------------|-----------------------|--------|
| ACTA2      | Hs00426835_g1         |        | GAPDH      | 4352665               |        |
| ADAMTS-4   | Hs00192708_m1         |        | HAS2       | Hs00193435_m1         |        |
| ADAMTS-8   | Hs00199836_m1         |        | HGF        | Hs00300159_m1         |        |
| BMP1       | Hs00241807_m1         | S2017  | IL6        | Hs99999932_m1         |        |
| BCL2A1     | Hs0018745_m1          |        | IL8        | Hs00174103_m1         |        |
| BRD4       | Hs00293232-m1         | s23901 | ITGA10     | Hs01006910_m1         |        |
| CCL2       | Hs00234140_m1         |        | JAM2       | Hs01022006_m1         |        |
| CCL7       | Hs00171147_m1         |        | MMP1       | Hs00899658_m1         |        |
| CCL26      | Hs00171145_m1         |        | PCSK1      | Hs01026107_m1         | S10147 |
| CCNB1      | Hs01030099-m1         |        | PCSK5      | Hs01964400_m1         | n32338 |
| Col1A1     | Hs00943009_m1         |        | PCSK6      | Hs01060079_m1         | S224155|
| Col6A1     | Hs00355783-m1         | s3312  | PCSK7      | Hs00237114_m1         | S17509 |
| Col6A2     | Hs01540400_m1         | s3314  | POSTN      | Hs01566734_m1         |        |
| Col6A3     | Hs00914223_m1         | s3317  | RUNX1      | Hs02558380_m1         | s2469  |
| CTGF       | Hs00170014_m1         |        | SHROOM2    | Hs01113636_m1         |        |
| CREBBP     | Hs00231733-m1         | s3595  | SDC1       | Hs00896424_g1         |        |
| ELN        | Hs00355783-m1         |        | SFRP4      | Hs00180066_m1         |        |
| ENPP1      | Hs00355783-m1         |        | TEAD4      | Hs01125032_m1         | s13964 |
| EP300      | Hs00914223_m1         | s4695  | TNC        | Hs0115665_m1          |        |
| FAIM       | Hs00202349_m1         |        | TGFB1      | Hs00998133_m1         |        |
| FN1        | Hs01549976_m1         |        | VCAM1      | Hs01003372_m1         |        |
| FOSL1      | Hs00759776_s1         |        | Non-targeting |                  | 4390847|
| FOXM1      | Hs01073586_m1         |        |            |                       |        |
**SI Appendix, Table S5. Proteomic analysis of CBP30 treated cells**

| Accession | Gene Name | Peptide count | Unique peptides | Confidence Score | Anova (p value) | Max fold change |
|-----------|-----------|---------------|-----------------|------------------|-----------------|-----------------|
| **Upregulated** | | | | | | |
| O95793;Q9NUL3 | STAU1 | 4 | 1 | 298 | 3.23E-02 | 4.52 |
| Q6ZVM7 | TOM1L2 | 1 | 1 | 50 | 3.61E-03 | 3.90 |
| Q9UK45 | LSM7 | 3 | 3 | 114 | 3.85E-02 | 3.15 |
| Q15397 | FUM1 | 1 | 1 | 55 | 3.03E-02 | 2.66 |
| Q9N7Q9;Q9RQ0 | FAM49B | 2 | 2 | 118 | 3.88E-03 | 2.33 |
| Q43148 | RNMT | 1 | 1 | 45 | 2.13E-02 | 2.33 |
| Q1TBIF2 | FAM213H | 1 | 1 | 69 | 6.66E-03 | 2.24 |
| P62140 | PPP1CB | 14 | 4 | 834 | 1.78E-02 | 2.03 |
| O33567 | RNFL13 | 1 | 1 | 106 | 3.30E-02 | 1.88 |
| Q8WW22 | DNAJA4 | 2 | 1 | 80 | 2.94E-02 | 1.86 |
| O60488 | ACGL4 | 4 | 2 | 217 | 5.01E-02 | 1.86 |
| Q9JZG9 | FCHO2 | 1 | 1 | 65 | 1.94E-03 | 1.85 |
| Q14980 | NUMA1 | 6 | 5 | 259 | 2.21E-02 | 1.75 |
| P61018 | RAB4B | 2 | 1 | 91 | 4.18E-02 | 1.72 |
| Q9Y5Y8 | SEC23P | 8 | 7 | 367 | 4.52E-04 | 1.68 |
| Q10713 | FMPCA | 2 | 2 | 96 | 2.97E-02 | 1.65 |
| Q01105;P6DME0 | SET | 11 | 10 | 559 | 1.93E-02 | 1.64 |
| Q9UBG0 | MRC2 | 13 | 13 | 595 | 3.63E-03 | 1.61 |
| O95479 | H6PD | 3 | 3 | 138 | 3.03E-02 | 1.61 |
| Q9BWS9 | CHID1 | 2 | 2 | 63 | 1.41E-02 | 1.57 |
| P35222 | CTNNB1 | 15 | 10 | 831 | 3.97E-02 | 1.55 |
| O60716 | CTNND1 | 9 | 9 | 487 | 1.12E-03 | 1.54 |
| Q9BRF8 | CPPED1 | 2 | 2 | 173 | 5.22E-04 | 1.54 |
| Q7LS11 | ARMCX2 | 3 | 1 | 121 | 3.24E-03 | 1.53 |
| A3YKK6 | CNOT1 | 3 | 1 | 179 | 3.71E-02 | 1.51 |
| **Downregulated** | | | | | | |
| P42276 | STAT6 | 2 | 1 | 75 | 1.58E-02 | -1.50 |
| P12109 | COL6A1 | 29 | 28 | 1756 | 3.47E-03 | -1.52 |
| Q15582 | TGFBI | 3 | 3 | 126 | 4.84E-02 | -1.59 |
| Q9Q7X4 | MYO5C | 1 | 1 | 33 | 3.81E-03 | -1.62 |
| Q7KZ85 | SUPT6H | 2 | 1 | 68 | 3.71E-02 | -1.62 |
| P12111 | COL6A3 | 104 | 92 | 6341 | 1.58E-02 | -1.62 |
| Q14495 | PLPP3 | 2 | 2 | 78 | 3.64E-03 | -1.66 |
| P17096 | HMGA1 | 1 | 1 | 72 | 3.74E-02 | -1.70 |
| P12110 | COL6A2 | 20 | 18 | 1058 | 1.70E-03 | -1.75 |
| P62751 | FN1 | 18 | 17 | 993 | 3.54E-03 | -1.77 |
| P23800 | LOX | 1 | 1 | 35 | 3.69E-05 | -1.88 |
| P32722 | PVR | 2 | 1 | 102 | 1.77E-02 | -1.95 |
| CG8 | CTHRC1 | 1 | 1 | 23 | 2.00E-02 | -2.53 |
| P29373;P29762 | CRABP2 | 6 | 6 | 266 | 4.16E-04 | -2.95 |
**SI Appendix Figure S1.**

Dose dependent inhibition of *ACTA2* and *COL1A1* gene expression by bromodomain inhibitors. PCR analysis following 3 days exposure of myofibroblasts to either CBP30 (A-C), I-CBP112 (D-F), A485 (G-H), JQ1 (J-L) and PF1 (M-O). Gene expression was analysed by Taqman qPCR using the ΔΔ Ct method, normalised GAPDH, mean ± SEM of 4-6 donors. *P* value was determined by one sample *t* test to normalised DMSO of 1, *P* ≤ 0.5, **P** ≤ 0.01, ***P* ≤ 0.001, ****P* ≤ 0.0001.
**SI Appendix Figure S2.**

Pharmacological targeting of CBP/EP300 alters myofibroblast morphology. (A) Myofibroblast were treated with DMSO, JQ1 (0.5µM) and SGC-CBP30 (2.5µM) for 3 days. The effects of the drugs on cell morphology was determined following phalloidin staining of actin filaments was quantified (B-D) from 5 donors using a minimum 50 cells per donor with ImageJ and analysed with a Mixed Linear Model with Tukey Post-hoc Test *** $P = <0.001$ (*Circularity: $4\pi \times ([Area]/[Perimeter]^2)$ with a value of 1.0 indicating a perfect circle.). Viability assays were performed on 4 donors using Hoechst and Calcein stained cells, quantified using the Celigo Image cytometer. Mean ± SEM of 4 donors $P$ value was determined by one sample $t$ test to normalised DMSO of 1. * $P \leq 0.5$, ** $P \leq 0.01$, *** $P \leq 0.001$. 
SI Appendix, Figure S3.

Binding of EP300 at genomic locations of FoxO and WNT pathway genes.

Visualisation in IGV of ChIP-Seq signal at the loci of FOXO3, EGFR, JUNB and CSK1A1, autoscaling was used, and all files normalised to input.
SI Appendix, Figure S4. Binding of EP300 at genomic locations of SNPs significantly associated with Dupuytren’s Disease. Visualisation in IGV of EP300 ChIP-Seq signal at the loci of DDR2, MMP14, SFRP4 and ITGA11, autoscaling was used and all files normalised to input.
**SI Appendix, Figure S5.**

**FOSL1, TEAD4 and RUNX1 depletion highlight differential regulation of specific subsets of genes.** PCR analysis of 3 day siRNA (20nM) mediated depletion studies show Fra1 regulates A CXCL8 (A), IL-6 (B), CTGF (C), FOSL1 (D), CCL26 (E), SFRPF4 (F) and COL6A3 (G). Gene expression was analysed by Taqman qPCR using the ∆∆ Ct method, normalised GAPDH. TEAD4 depletion regulates the gene expression of the chemokines CXCL8 (H) and CCL2 (I) and TEAD4. (J). RUNX1 depletion regulates the gene expression of ECM components FN1 (K) and HAS2 (L) and RUNX1 (M). Mean ± SEM of 4 donors P value was determined by one sample t test to normalised control non-targeting oligo value of 1. * P ≤ 0.5, ** P ≤ 0.01, *** P ≤ 0.001. Western
blot analysis confirms knockdown at the protein level of FOSL1/Fra1 (N), TEAD4 (O) RUNX1 (P) representative of 3 experiments.

**SI Appendix, Figure S6.**

CREBBP/CBP, EP300 and BRD4 siRNA mediated validation of RNA-Seq data. PCR analysis following 4 day depletion of either CBP (20nM), EP300 (20nM) or a combination of CBP/EP300 (10nM each), BRD4 or a non-targeting control oligo siRNA (20nM) (A) CREBBP, (B) EP300, (C) BRD4, and confirms regulation of FABP3 (D), HAS2 (E), FN1 (F), MMP1 (G), TNC (H),
POSTN (I), COL1A1 (J) and COL6A3 (K). Gene expression was analysed by Taqman qPCR using the ΔΔ Ct method, normalised GAPDH, mean ± SEM of 6 donors, $P$ value was determined by one sample t test to normalised control non-targeting oligo value of 1. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Western blot analysis confirms knockdown at the protein level of EP300, CBP and BRD4 (L) representative of 3 donors.
**SI Appendix, Figure S7.**

Immunofluorescence staining of 3 further DD and IPF patient samples. Confocal microscopy after immunostaining using anti-αSMA, anti-Collagen VI α3 or anti-Collagen 1 α1 specific antibodies in either (A) DD nodule or IPF (B) sections, shown are 3 (D1-3) donors, scale bar 25 or 50 μM for higher magnification slides.
SI Appendix, Figure S8.

PCR validation of COL6A1/2/3 siRNA mediated validation of RNA-Seq data. PCR analysis following 6 day depletion of COL6A1/2/3, COL6A3 or a non-targeting control oligo siRNA
(20nM) effects on (A) HGF (B) SDC1 (C) FOXM1(D), CCNB1(E), CCL2. (F) CCL7, (G) CCL26, (H) ITG10, (I) TNC, (J) SHROOM2, (K) VCAM1, (L) JAM2, (M) HAS2, (N) ADAMTS4, (O) ADAMTS8, (P) COL6A1, (Q) COL6A2 and (R) COL6A3. Gene expression was analysed by Taqman qPCR using the ΔΔ Ct method, normalised GAPDH. Mean ± SEM of 6 donors, P value was determined by one sample t test to normalised control non-targeting oligo value of 1. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

**SI Appendix Figure S9.**

COL6A1/2/3 siRNA depletion inhibits CCL2 and CCL7 secretion in DD myofibroblasts. Cells were treated with either COL6A1/2/3 or a non-targeting control oligo siRNA (20nM) for 6 days and effects on CCL2 and CCL7 secretion were determined by ELISA. P value was determined by paired sample t test in 8 donors. (B) Western blot analysis confirms knockdown at the protein level of Col6A1 and Col6A3 in 3 donors, β-actin confirms equal loading.
**SI Appendix, Figure S10.**

PCR validation of *PCSK* depletion. PCR analysis following 6 day depletion of PCSKs or a non-targeting control oligo siRNA (20nM) effects on (A) *ADAMTS8*, (B) *HAS2* (C) *CCL26*, (D) *BMP1*, (E) *PCSK1*, (F) *PCSK5*, (G) *PCSK6* and (H) *COL6A*. Gene expression was analysed by Taqman qPCR using the ∆∆ Ct method, normalised to *GAPDH*. Mean ± SEM of 6 donors, *P* value was determined by one sample *t* test to normalised control non-targeting oligo value of 1. *P* ≤ 0.05, **P** ≤ 0.01, ***P** ≤ 0.001, ****P** ≤ 0.0001.