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# Supplementary Materials for

## Epigenetic activation and memory at a *TGFB2* enhancer in systemic sclerosis

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Published 19 June 2019, *Sci. Transl. Med.* **11**, eaaw0790 (2019)
DOI: 10.1126/scitranslmed.aaw0790

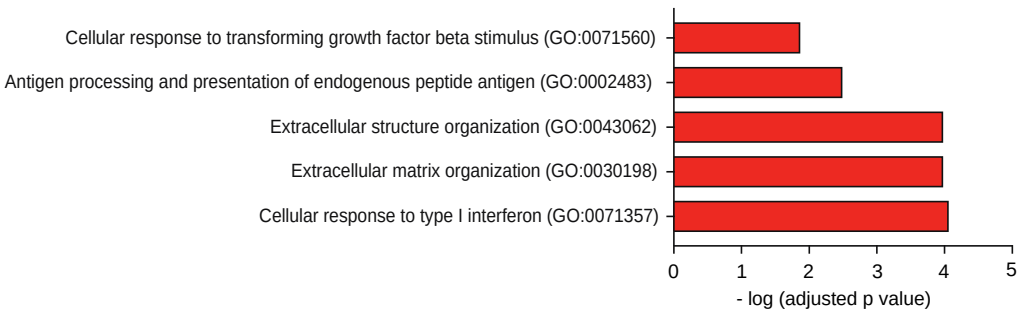
**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

(available at stm.sciencemag.org/cgi/content/full/11/497/eaaw0790/DC1)

**A**




| GO Term | Gene members |
|---|---|
| Cellular response to transforming growth factor beta stimulus (GO:0071560) | *KLF10, TGFB2, CLEC3B, COL4A2, BAMBI, SMURF1, MYC, UBC, ANKRD1, PDGFA, PPP1CA* |
| Antigen processing and presentation of endogenous peptide antigen (GO:0002483) | *ERAP1, TAP2, HLA-B, HLA-E* |
| Extracellular structure organization (GO:0043062) | *COL18A1, TGFB2, OLFML2B, ITGA3, ITGB3, ACTN1, NTN4, ILK, PDGFA, LOXL2, MMP11, MMP14, ADAMTS2, COL4A2, COL4A1, COL7A1, COL5A3, COL6A2, ABI3BP, COL21A1, EMILIN1, A2M* |
| Extracellular matrix organization (GO:0030198) | *COL18A1, TGFB2, OLFML2B, ITGA3, ITGB3, ACTN1, NTN4, ILK, PDGFA, LOXL2, MMP11, MMP14, ADAMTS2, COL4A2, COL4A1, COL7A1, COL5A3, COL6A2, ABI3BP, COL21A1, EMILIN1, A2M* |
| Cellular response to type I interferon (GO:0071357) | *IFITM1, SP100, IFITM2, STAT1, HLA-B, ISG15, IFI35, GBP2, IFIT3, HLA-E* |

**B**

| Ingenuity Pathway Analysis | Gene members |
|---|---|
| TGF-b Signaling (adjusted P = 2.79E-04) | *TGFB2, HRAS, MAP2K2, MAPK8, MAPK9, RAP1B, RRAS, SMAD4, SMAD5, SMURF1, SOS2, TGFBR1* |

**Fig. S1. SSc fibroblasts maintain profibrotic gene expression pathways.** (**A**) Top Gene-Ontology (GO) categories of differentially expressed genes assessed by RNA-seq. All genes showed increased expression in SSc fibroblasts. Adjusted P value < 0.05. Table shows genes included in each GO term. (**B**) Ingenuity pathway analysis revealed enrichment of TGFβ signaling genes among genes differentially expressed between control and SSc fibroblasts.

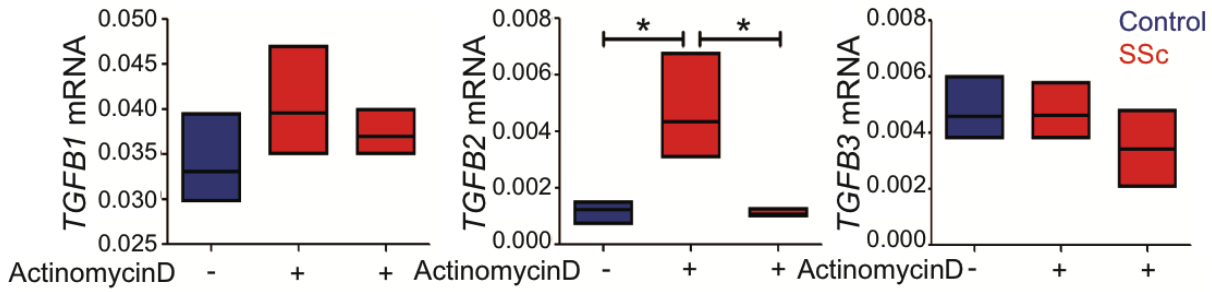



**Fig. S2. SSc fibroblasts actively transcribe *TGFB2* mRNA.** Active transcription of *TGFB2* mRNA was demonstrated in SSc fibroblasts through actinomycin D treatment. *P < 0.05. $n_{ontrol}$ = 3 and $n_{SSc}$ = 3. Blue and red bars represent control and SSc fibroblasts, respectively. Lower and upper margins of each box indicate range; the internal line indicates the mean. All mRNA expression was normalized by *GAPDH*.

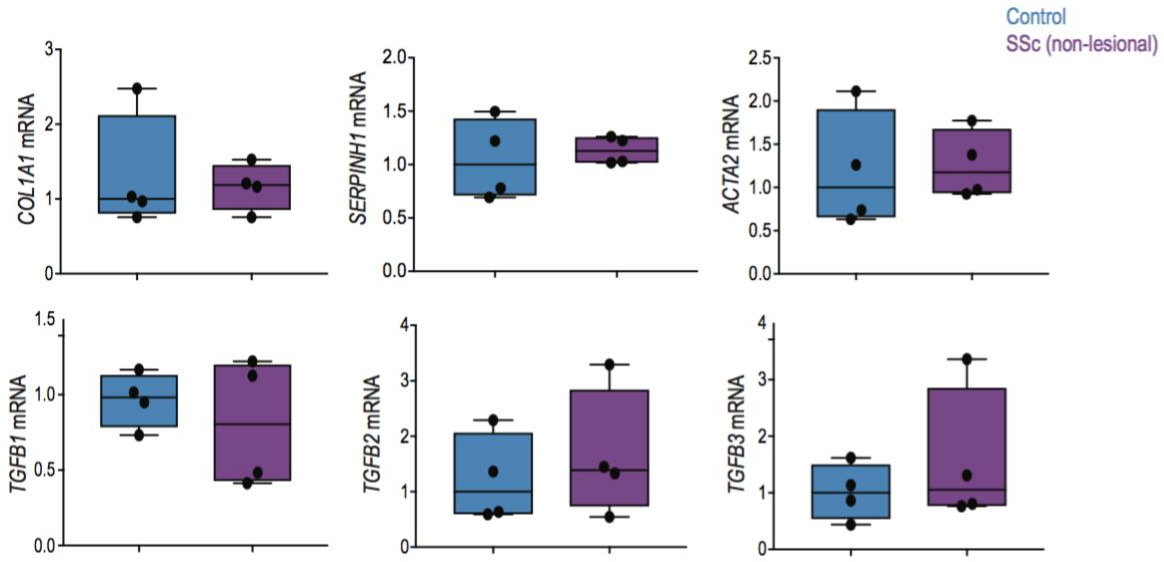



**Fig. S3. Nonlesional SSc fibroblasts exhibit profibrotic gene expression.** mRNA quantification of fibroblast lines established from healthy control donors or non-lesional skin biopsies of diffuse SSc patients (blue and purple bars, respectively). *P < 0.05; **P < 0.01; ***P < 0.001. N = 4. Blue and purple bars represent control and non-lesional SSc fibroblasts, respectively. Lower and upper margins of each bar indicate 25$^{th}$ and 75$^{th}$ percentiles, respectively; the internal line indicates the mean, and whiskers indicate the range. Black points represent individual biological replicates. All mRNA expression values were normalized by *GAPDH*.

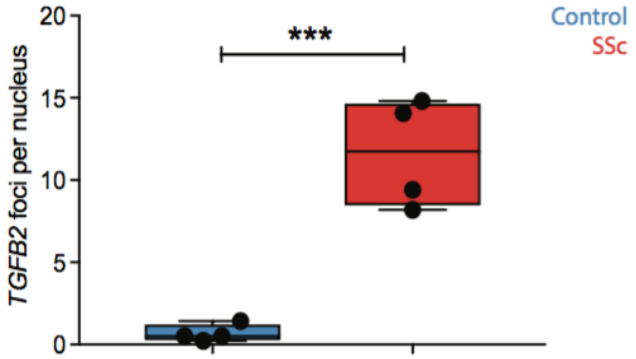



**Fig. S4. Quantification of *TGFB2* mRNA by in situ hybridization.** Confocal images of in situ hybridization of *TGFB2* mRNA in Fig. 1E were quantified by an algorithm in Imaris software. Number of *TGFB2* foci was normalized to nuclei count. ***P < 0.001, two-way Student's t-test. $n_{control} = 4$ and $n_{SSc} = 4$. Blue and red bars represent control and SSc fibroblasts, respectively. The lower and upper margins of each box indicate the range; the internal line indicates the mean.

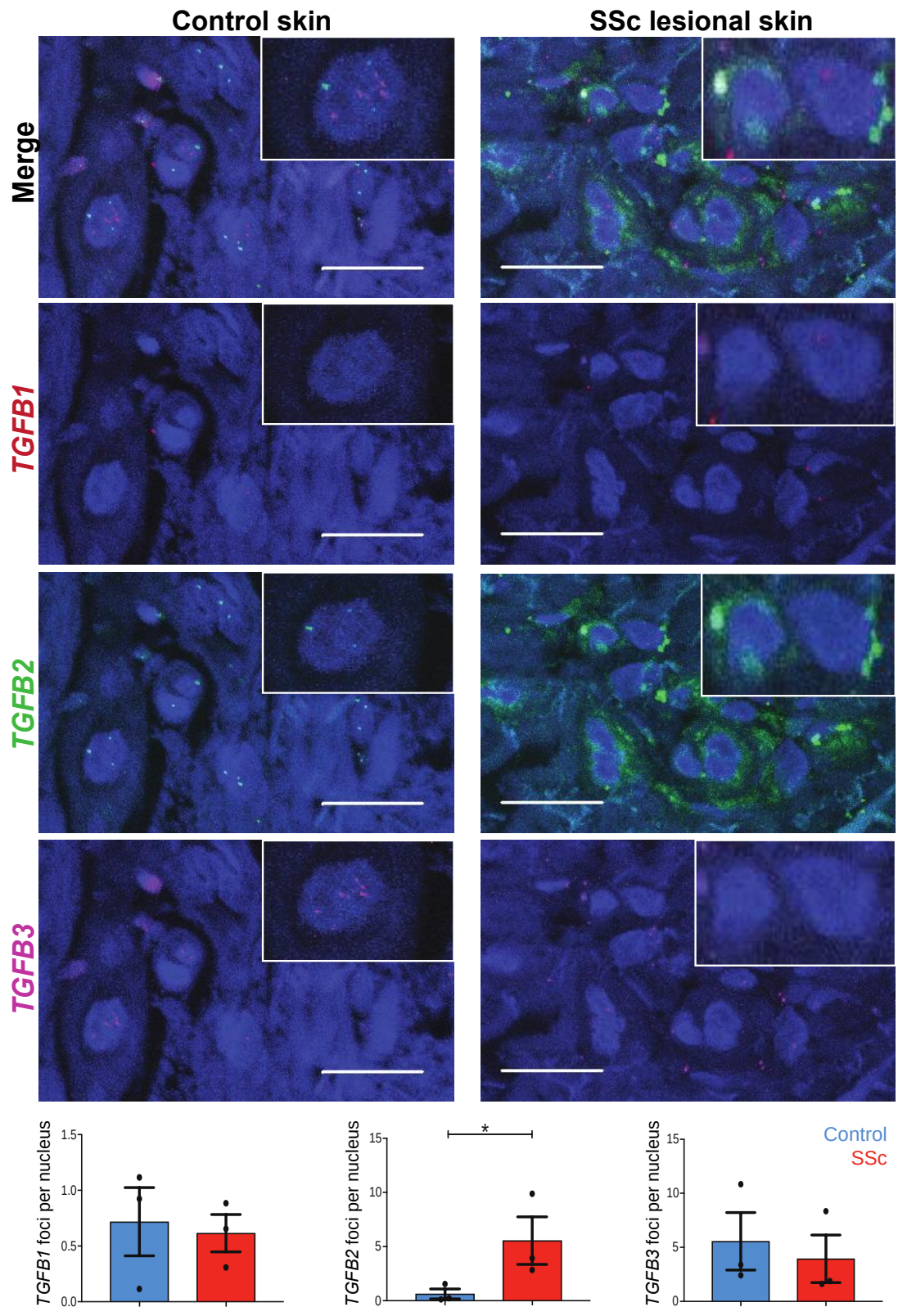

Control skin
SSc lesional skin
Merge
TGFB1
TGFB2
TGFB3
TGFB1 foci per nucleus
TGFB2 foci per nucleus
TGFB3 foci per nucleus
*
Control
SSc

**Fig. S5. mRNA in situ hybridization for *TGFB* isoform expression in SSc lesional skin.**

*TGFB1* (red), *TGFB2* mRNA (green), and *TGFB3* (magenta) expression were measured by mRNA in situ hybridization of healthy control skin and lesional SSc skin sections. Scale bars, 20 um. mRNA foci were counted using Imaris and normalized by nuclei count. *P < 0.05, two-tailed Student's t-test. n = 3. Blue and red bars represent control and SSc fibroblasts, respectively. The lower and upper margins of each box indicate the range; the internal line indicates the mean.

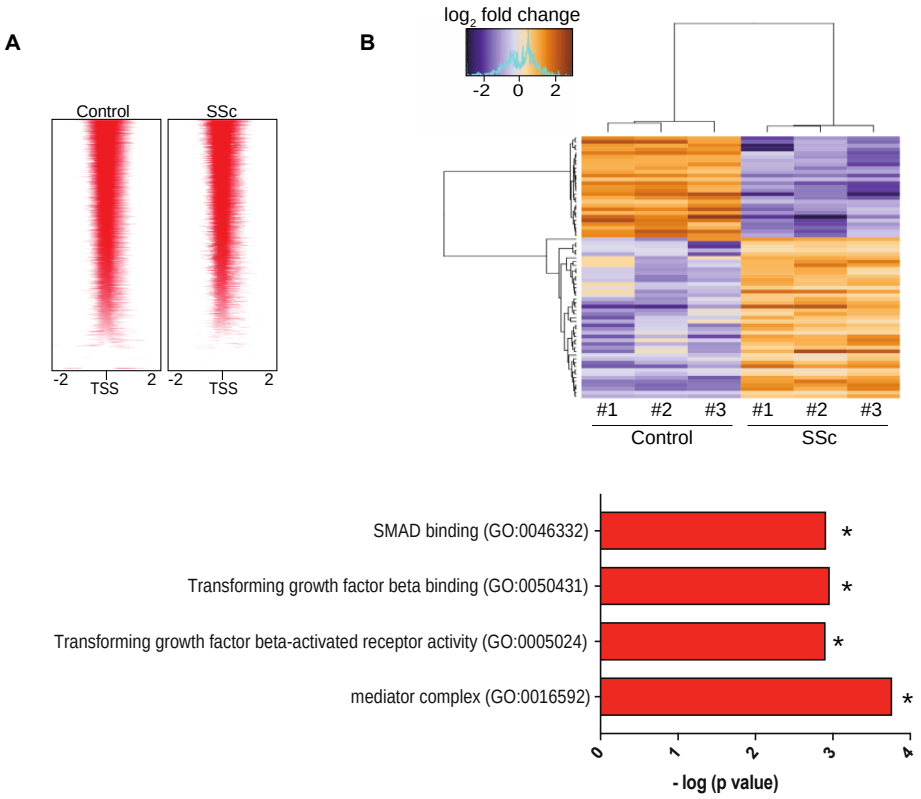



**Fig. S6. SSc fibroblasts exhibit increased chromatin accessibility in genes related to profibrotic pathways.** (**A**) Normalized read density heat map of ATAC-seq signals at transcription start site (TSS) regions revealed no differences between cultured control and SSc fibroblasts. Each row shows $\pm$ 2 kilobase pairs centered on the TSS of all sequenced genes, and rows are ordered by maximum ATAC-seq signal. (**B**) Heatmap of genes with differential chromatin accessibility (rows) at genome-wide significant accessibility peaks ($P \leq 5E\text{-}8$) between three healthy control fibroblasts and primary lesional SSc fibroblasts from three patients by ATAC-seq (n = 3, average FDR < 0.05). Shown are top GO term categories of genes with heightened chromatin accessibility in SSc fibroblasts versus control fibroblasts. * adjusted P value < 0.05.

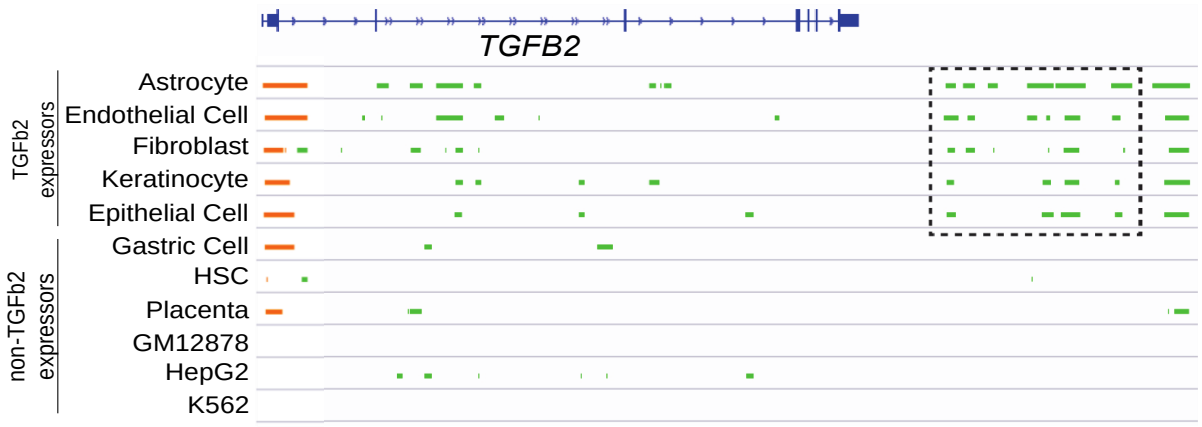
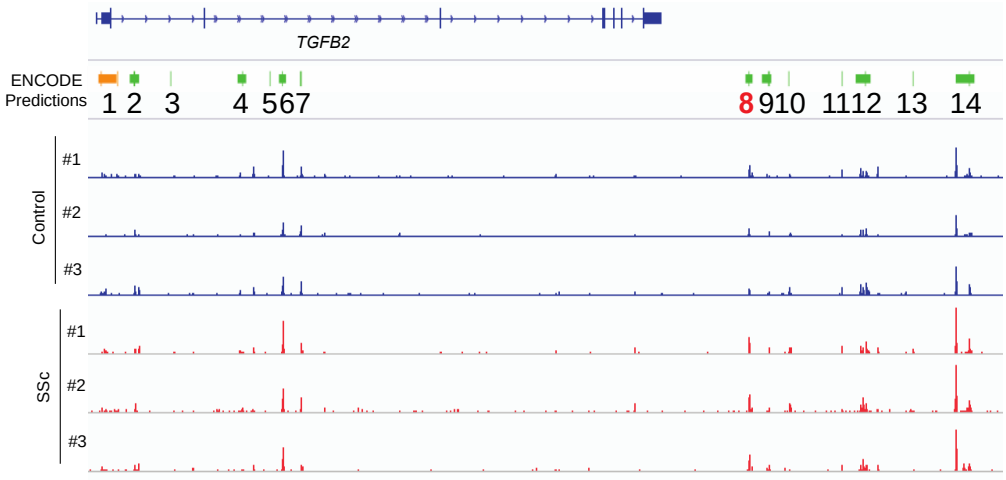



**Fig. S7. TGFβ2-expressing cell lines contain unique predicted enhancers at the *TGFB2* locus.** A comparison of proximal promoter (H3K4me3$^{+}$ DNAse hypersensitivity$^{+}$; orange) and candidate enhancer (H3K27ac$^{+}$, DNAse hypersensitivity$^{+}$; green) predictions in *TGFB2*-expressing and *TGFB2* non-expressing cell lines revealed a distal cluster of predicted enhancers uniquely accessible in *TGFB2*-expressing cell lines (highlighted with dashed lines).




| Legend | Prediction | R-squared | P value |
|---|---|---|---|
| 1 | Proximal Promoter | 0.1737 | 0.41 |
| 2 | Enhancer 4378 | 0.0000 | 0.99 |
| 3 | Enhancer 17697 | 0.0190 | 0.79 |
| 4 | Enhancer 3988 | 0.2841 | 0.28 |
| 5 | Enhancer 1233 | 0.0000 | N/A |
| 6 | Enhancer 2049 | 0.0400 | 0.70 |
| 7 | Enhancer 7144 | 0.3300 | 0.23 |
| **8** | **Enhancer 4514** | **0.7375** | **0.029** |
| 9 | Enhancer 9058 | 0.2297 | 0.34 |
| 10 | Enhancer 11247 | 0.1299 | 0.48 |
| 11 | Enhancer 7333 | 0.0148 | 0.82 |
| 12 | Enhancer 513 | 0.1694 | 0.42 |
| 13 | Enhancer 12430 | 0.0000 | N/A |
| 14 | Enhancer 787 | 0.4888 | 0.12 |

**Fig. S8. TGFβ2 enhancer accessibility correlates with *TGFB2* mRNA expression.** The table displays $R^2$ and P values for linear regression between *TGFB2* mRNA expression and the chromatin accessibility of each predicted enhancer. The putative *TGFB2* enhancer under consideration of this study (Enhancer 4514) is highlighted in red. A two-tailed Student's t-test was performed for significance for linear regression.

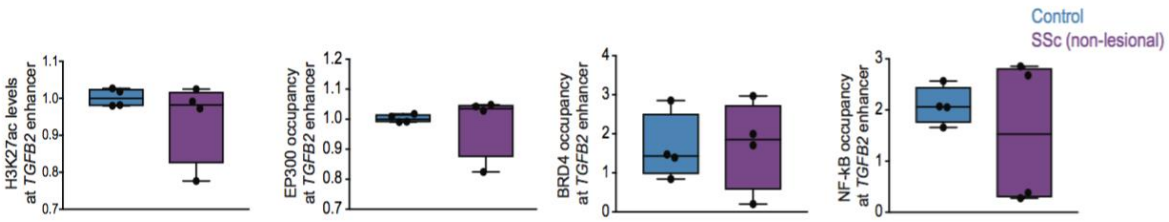



**Fig. S9. *TGFB2* enhancer is inactive in nonlesional SSc fibroblasts.** ChIP-qPCR analysis for epigenetic modifications at the *TGFB2* enhancers of fibroblast lines established from healthy control or non-lesional skin biopsies from diffuse SSc patients (blue and purple bars, respectively). n = 4. Lower and upper margins of each bar indicate 25$^{th}$ and 75$^{th}$ percentiles, respectively; the internal line indicates the mean, and whiskers indicate the range. Black points represent individual biological replicates. Values for ChIP-qPCR are represented as percent input normalized to IgG controls.

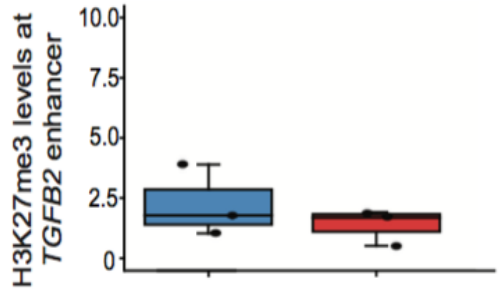



**Fig. S10. The *TGFB2* enhancer exhibits minimal H3K27me3 modification in lesional SSc fibroblasts.** ChIP-qPCR analysis for H3K27me3 at the *TGFB2* enhancer in control and SSc fibroblasts established from lesional skin of diffuse SSc patients ($n_{ontrol}$ = 3, $n_{ssc}$ = 3). Blue and red bars represent control and lesional SSc fibroblasts, respectively. Lower and upper margins of each bar indicate 25$^{th}$ and 75$^{th}$ percentiles, respectively; the internal line indicates the mean, and whiskers indicate the range. Black points represent individual biological replicates. Values for ChIP-qPCR are represented as percent input normalized to IgG controls.

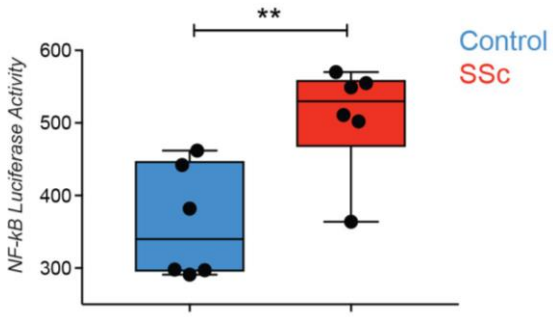



**Fig. S11. SSc fibroblasts exhibit high NF-κB signaling activity.** Control or SSc fibroblasts were co-cultured with NF-kB luciferase reporter cell lines for 48 hours. Luciferase activity was measured with firefly luciferase substrate, and quantified using a spectrophotometer. **P < 0.0, two-way Student's t-test. $n_{ontrol} = 5$ and $n_{SSc} = 6$.

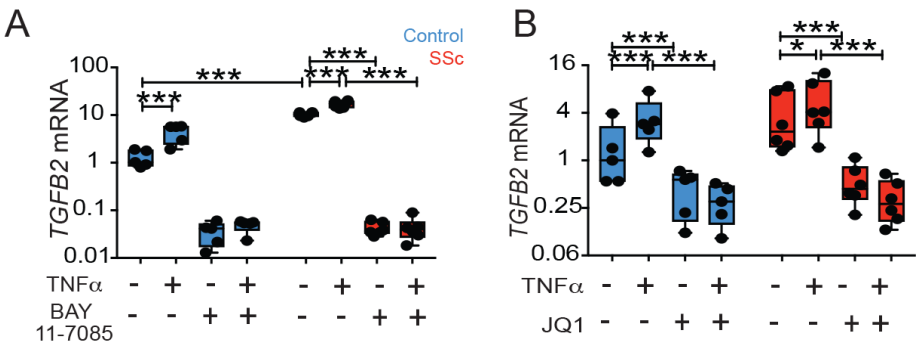



**Fig. S12. TNFα induces *TGFB2* enhancer activity in a NF-κB– and BRD4-dependent manner.** (**A** and **B**) *TGFB2* expression in response to TNFα treatment in fibroblasts pre-conditioned with vehicle, NF-kB inhibitor (BAY 11-7082) or BET inhibitor (JQ1). *P < 0.05; ***P < 0.001, one-factor ANOVA with corrected false discovery rate of multiple hypotheses. $n_{control} = 5$ and $n_{SSc} = 6$.

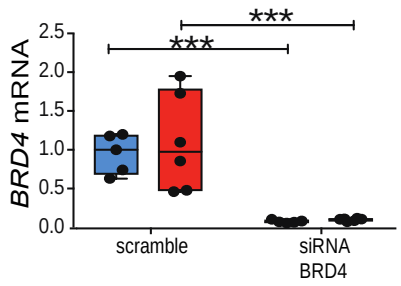



**Fig. S13. siRNA knockdown of *BRD4* abrogates *BRD4* mRNA expression.** *BRD4* mRNA expression at day 5 post-transfection with siRNA against *BRD4* mRNA in control and SSc fibroblasts. ***P < 0.001, one-factor ANOVA with corrected false discovery rate of multiple hypotheses. $n_{ontrol} = 5$ and $n_{SSc} = 6$.

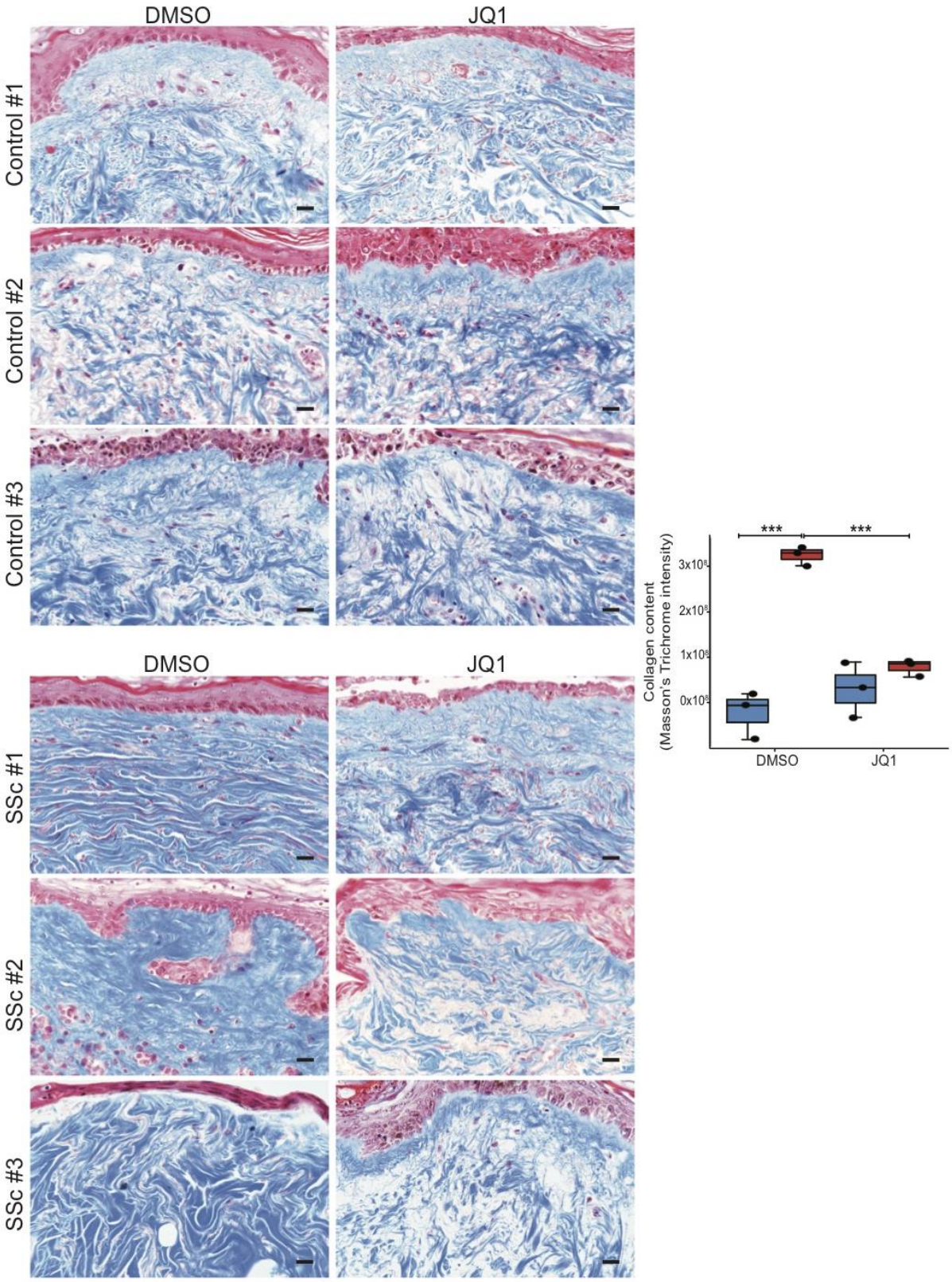

DMSO
JQ1
Control #1
Control #2
Control #3
DMSO
JQ1
SSc #1
SSc #2
SSc #3
***
***
Collagen content
(Masson's Trichrome intensity)
3x10⁹
2x10⁹
1x10⁹
0x10⁹
DMSO
JQ1

**Fig. S14. JQ1 treatment reduces collagen content in SSc skin explants.** Masson's Trichrome staining revealed reduction in total collagen (blue) in the superficial dermis of SSc skin maintained in organ-culture with JQ1 treatment. Staining intensity for collagen was quantified from two different sections per biological replicate. ***P < 0.001, one-factor ANOVA with corrected false discovery rate of multiple hypotheses. Blue and red bars represent control and patient-derived SSc fibroblasts, respectively. Lower and upper margins of each bar indicate 25$^{th}$ and 75$^{th}$ percentiles, respectively; the internal line indicates the mean, and whiskers indicate the range. Scale bar, 50 microns. Black points represent individual biological replicates.

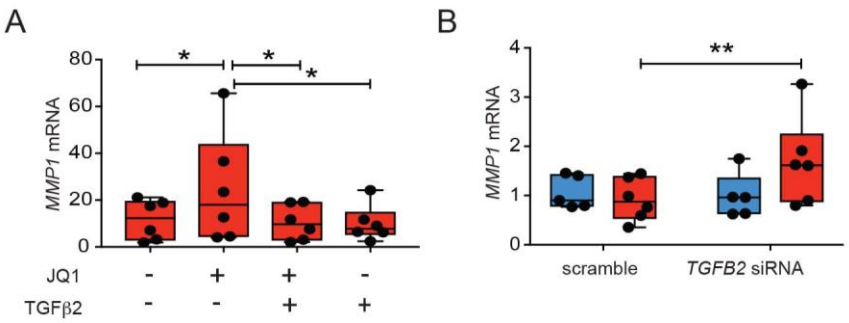



**Fig. S15. TGFβ2 inhibition by JQ1 or siRNA induces *MMP1* expression in SSc fibroblasts.** (**A**) *MMP1* mRNA expression after treatment with JQ1, TGFβ2 ligand, or both in SSc fibroblasts. (**B**) *MMP1* mRNA expression quantified at day five post-siRNA knockdown of *BRD4* mRNA in control and SSc fibroblasts. *P < 0.05; **P < 0.01. Parametric tests were used (Fig. S15: paired analysis by one-factor ANOVA with corrected false discovery rate of multiple hypotheses, Fig. S15: Two-factor ANOVA with corrected false discovery rate of multiple hypotheses). $n_{ontrol} = 5$ and $n_{SSc} = 6$.

**Table S1. Patient data on SSc skin biopsy donors and healthy volunteers.** Age and anti-nuclear antibody status of each patient were recorded at time of biopsy procurement. Topo, anti-topoisomerase I autoantibody. Pol III, anti-RNA polymerase III autoantibody.

| ID | Diagnosis | Age | Gender | Anti-nuclear antibody profile | Purpose in study |
|---|---|---|---|---|---|
| SSc1 | Diffuse SSc | 54 | M | Topo | Dermal fibroblast generation |
| SSc2 | Diffuse SSc | 41 | F | Topo | Dermal fibroblast generation |
| SSc3 | Diffuse SSc | 60 | F | Pol III | Dermal fibroblast generation |
| SSc4 | Diffuse SSc | 29 | F | Pol III | Dermal fibroblast generation |
| SSc5 | Diffuse SSc | 65 | M | Pol III | Dermal fibroblast generation |
| SSc6 | Diffuse SSc | 48 | F | Pol III | Dermal fibroblast generation |
| SSc7 | Diffuse SSc | 58 | F | Topo | Skin Explant |
| SSc8 | Diffuse SSc | 54 | F | Pol III | Skin Explant |
| SSc9 | Diffuse SSc | 63 | F | Topo | Skin Explant |
| SSc10 | Diffuse SSc | 46 | F | Topo | Skin Explant |
| Control | Healthy volunteer | 50 | M | - | Dermal fibroblast generation |
| Control | Healthy volunteer | 55 | M | - | Dermal fibroblast generation |
| Control | Healthy volunteer | 43 | M | - | Dermal fibroblast generation |
| Control | Healthy volunteer | 60 | F | - | Dermal fibroblast generation |
| Control | Healthy volunteer | 55 | F | - | Dermal fibroblast generation |
| Control | Healthy volunteer | 46 | M | - | Skin Explant |
| Control | Healthy volunteer | 55 | F | - | Skin Explant |
| Control | Healthy volunteer | 74 | M | - | Skin Explant |
| Control | Healthy volunteer | 48 | M | - | Skin Explant |