

SUPPLEMENTAL MATERIAL

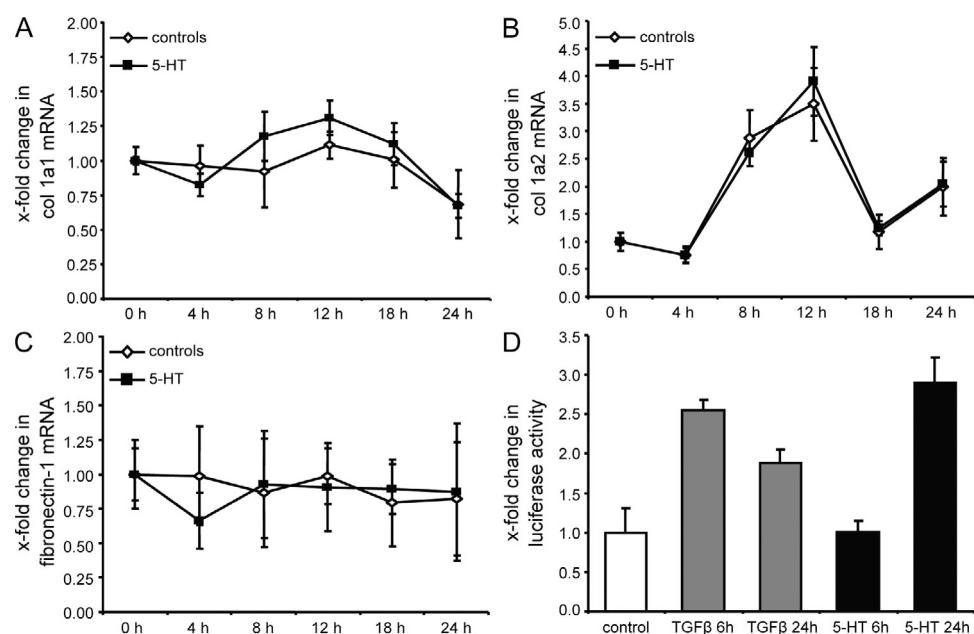
Dees et al., <http://www.jem.org/cgi/content/full/jem.20101629/DC1>

Figure S1. 5-HT stimulates the transcription of col 1a2 but does not affect the half-life of col 1a1 mRNA. (A–C) The mRNA half-life of col 1a1 (A), col 1a2 (B), and fibronectin-1 (C) upon stimulation with 5-HT ($n = 3$ each). (D) Transcription of col 1a2 in reporter assays after stimulation with 5-HT and TGF- β 1 ($n = 5$, two independent series). Error bars indicate SE.

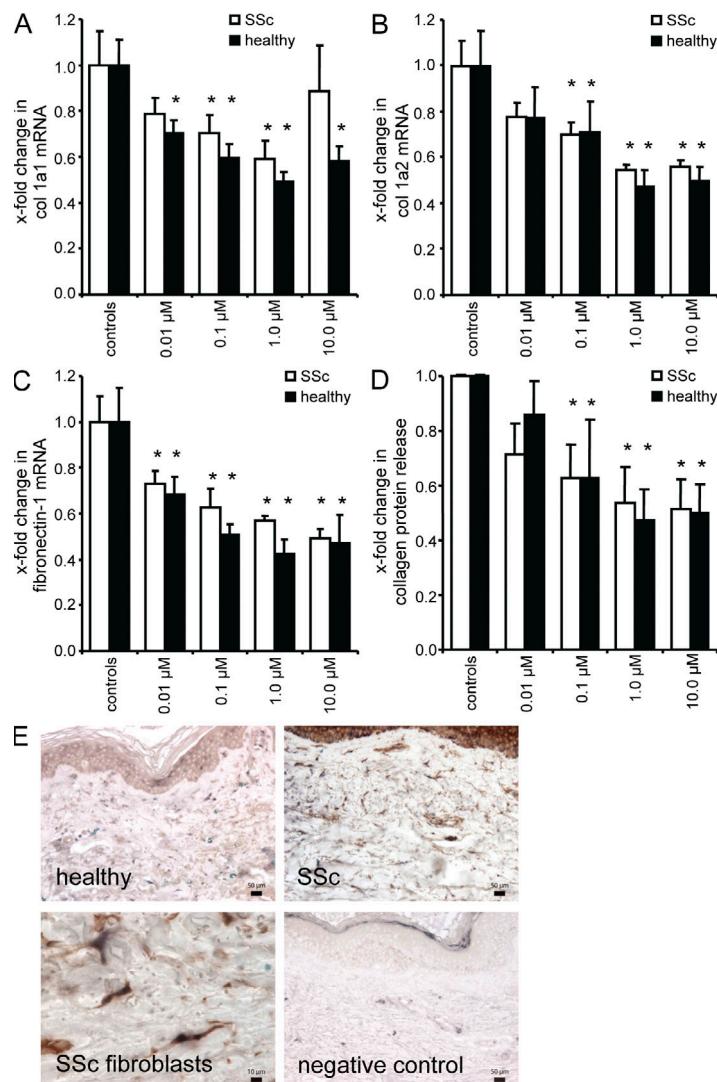


Figure S2. The 5-HT receptor antagonist terguride reduces the expression of extracellular matrix proteins. (A–C) mRNA levels of col 1a1 (A), col 1a2 (B), and fibronectin-1 (C) in 5-HT-stimulated fibroblasts incubated with terguride at concentrations from 0.01 to 10 μ M in SSC and healthy dermal fibroblasts ($n = 5$ each, two independent series). (D) Release of collagen protein from SSC and healthy dermal fibroblasts upon incubation with terguride ($n = 5$). * $P < 0.05$ compared with 5-HT-stimulated cells. Error bars indicate SE. (E) 5-HT_{2B} is overexpressed in fibroblasts in fibrotic tissue. Double staining for the fibroblast marker prolyl-4-hydroxylase β and 5-HT_{2B}. Skin sections of an SSC patient and a healthy volunteer and fibroblasts are shown. 5-HT_{2B} was stained with DAB (brown), and prolyl-4-hydroxylase β was stained with BCIP/NBT (dark blue). The stainings were performed in three independent series. Bars: (top row and bottom right) 50 μ m; (bottom left) 10 μ m.

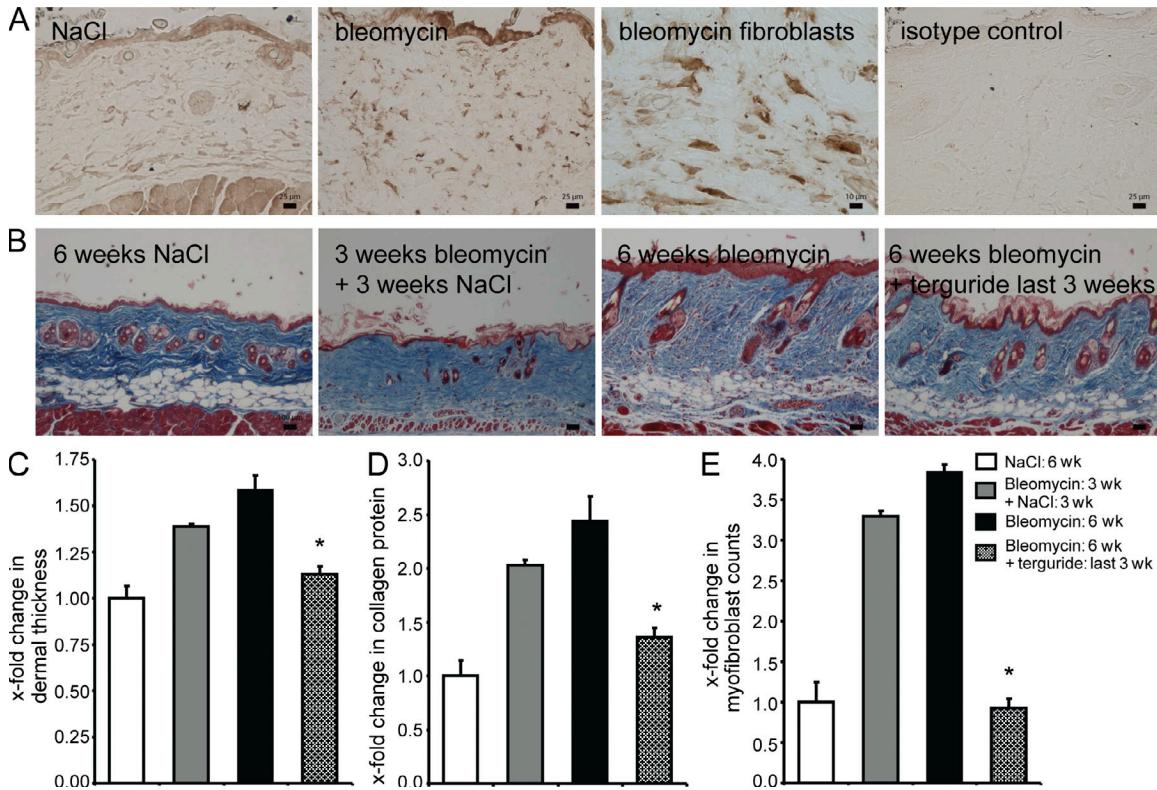


Figure S3. 5-HT_{2B} is overexpressed in bleomycin-induced skin fibrosis, and terguride induces regression of preestablished fibrosis. (A) 5-HT_{2B} is overexpressed in experimental dermal fibrosis. Representative sections and fibroblasts are shown ($n = 5$ in two independent series). (B–E) Inhibition of 5-HT₂ induces regression of preestablished fibrosis in the model of established bleomycin-induced skin fibrosis. (B) Trichrome staining of lesional skin. Representative tissue sections are shown ($n = 8$ each). (C) Dermal thickness after treatment of preestablished fibrosis with terguride at a dose of 0.6 mg/kg/bid from week 3 to 6. (D) The collagen content in lesional skin upon treatment with terguride. (E) Myofibroblast counts after treatment with terguride for the last 3 wk. *, $P < 0.05$ compared with mice challenged with bleomycin for 3 wk. The experiment was performed in two independent series. Error bars indicate SE. Bars: (A, NaCl, bleomycin, and isotype control) 25 μm; (A, bleomycin fibroblasts) 10 μm; (B) 100 μm.

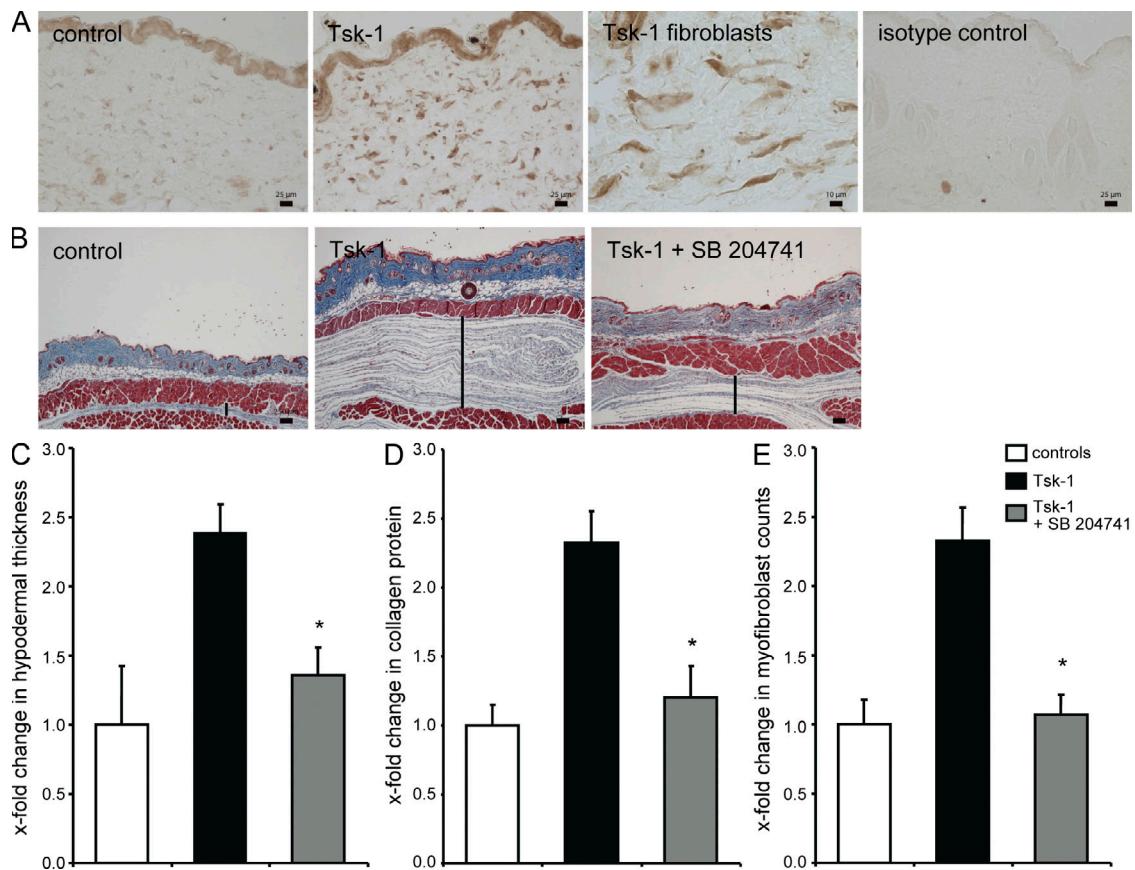


Figure S4. Increased expression of 5-HT_{2B} in Tsk-1 mice, and selective inhibition of 5-HT_{2B} ameliorates histological changes in the Tsk-1 mouse model. (A) 5-HT_{2B} is overexpressed in Tsk-1 mice. Representative sections and fibroblasts are shown ($n = 5$ in two independent series). (B-E) Pharmacologic inhibition of 5-HT_{2B} by SB 204741 ameliorates the Tsk-1 phenotype. (B) Representative trichrome-stained sections of control mice, untreated Tsk-1 mice, and Tsk-1 mice treated with SB 204741 at a dose of 5 mg/kg/d are shown ($n = 8$ each). Vertical bars indicate the hypodermal thickness. (C-E) Hypodermal thickening (C), collagen content (D), and myofibroblast counts (E) in Tsk-1 mice treated with SB 204741. *, P < 0.05 compared with untreated Tsk-1 mice. The experiment was performed in two independent series. Error bars indicate SE. Bars: (A, control, Tsk-1, and isotype control) 25 μ m; (A, Tsk-1 fibroblasts) 10 μ m; (B) 250 μ m.

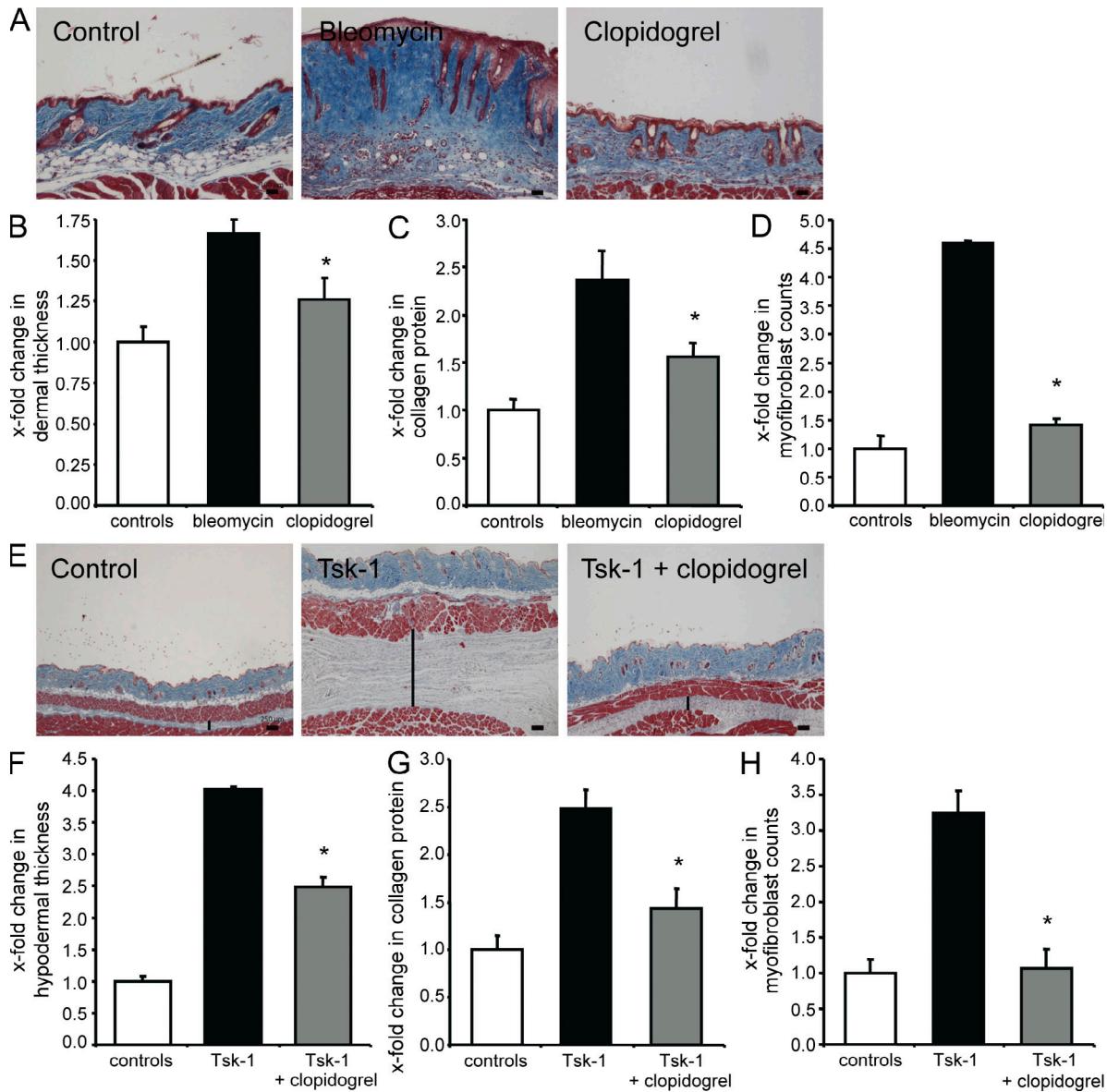


Figure S5. Inhibition of platelet aggregation ameliorates experimental bleomycin-induced dermal fibrosis and the Tsk-1 phenotype. (A) Tri-chrome-stained tissue sections of DBA/2 mice: control mice injected with NaCl ($n = 10$), bleomycin-challenged mice without treatment ($n = 10$), and mice injected with bleomycin and treated with clopidogrel at a concentration of 25 mg/kg/d ($n = 6$). (B) Dermal thickening in experimental dermal fibrosis after treatment with clopidogrel. (C) Collagen protein content in lesional skin in bleomycin-induced skin fibrosis analyzed by hydroxyproline assay. (D) Changes in α -SMA-positive myofibroblast counts upon bleomycin challenge and treatment with clopidogrel. (E) Tissue sections of Tsk-1 mice: control mice without the Tsk-1 allele ($n = 8$), untreated Tsk-1 mice ($n = 8$), and Tsk-1 mice treated with clopidogrel at 25 mg/kg/d ($n = 6$). Vertical bars indicate the hypodermal thickness. (F) Hypodermal thickness after treatment with clopidogrel in the Tsk-1 mouse model. (G) Collagen content in Tsk-1 mice after treatment with clopidogrel. (H) Effects of clopidogrel on the number of α -SMA-positive myofibroblasts in Tsk-1 mice. *, $P < 0.05$ compared with Tsk-1 or bleomycin-injected mice without clopidogrel treatment. The experiments were performed in two independent series. Error bars indicate SE. Bars: (A) 100 μ m; (E) 250 μ m.

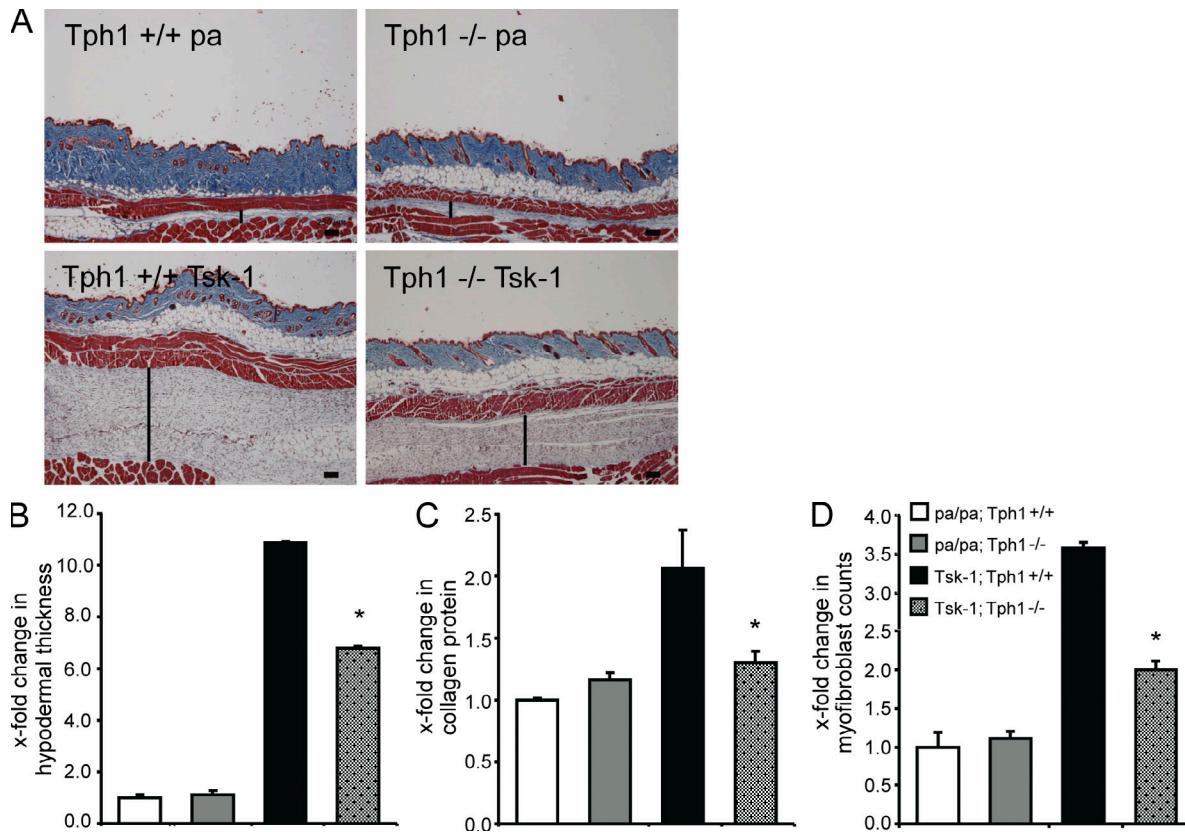


Figure S6. Deficiency for TPH1 ameliorates histological changes in Tsk-1 mice. (A) Representative tissue sections of wild-type mice without the Tsk-1 allele (TPH1^{+/+}/pa), TPH1^{-/-}/pa mice, TPH1^{+/+}/Tsk-1 mice, and TPH1^{-/-}/Tsk-1 mice are shown. Vertical bars indicate hypodermal thickness. Bars, 250 μ m. (B-D) Hypodermal thickening (B), collagen content as analyzed by hydroxyproline assay (C), and myofibroblast counts (D) in TPH1^{-/-}/Tsk-1 mice ($n = 8$ each). *, $P < 0.05$ compared with TPH1^{+/+}/Tsk-1 mice. The measurements were performed twice. Error bars indicate SE.

Table S1. Characteristics of SSc patients at date of biopsy for generation of dermal fibroblast cultures

Gender (F/M)	Age (median and range) yr	Disease subset (limited/ diffuse)	Disease duration (median and range) yr	Medications
12/2	53 (38–74)	11/3	7 (1–20)	No DMARDs, corticosteroids, or NSAIDs

DMARD, disease-modifying antirheumatic drug; F, female; M, male; NSAID, nonsteroidal antiinflammatory drug. The disease subset was determined according to the criteria proposed by LeRoy and Medsger (2001). Disease duration was measured from the onset of the first non-Raynaud symptoms attributable to SSc.

Table S2. Ki values of the chemical inhibitors according to the National Institute of Mental Health Ki Database

Inhibitor	Ki values		
	5-HT _{1B} <i>nM</i>	5-HT _{2A} <i>nM</i>	5-HT _{2B} <i>nM</i>
Cyproheptadine	ND	1.67	1.54
Ketanserin	4,350	2.25	911
SB 204741	>10,000	>10,000	94.92
SB 224289	6.91	5,012	1,349
Terguride	257	4.79	7.08

REFERENCE

LeRoy, E.C., and T.A. Medsger Jr. 2001. Criteria for the classification of early systemic sclerosis. *J. Rheumatol.* 28:1573–1576.