Supplementary Methods

Haematoxylin and eosin staining

1. Deparaffinise and rehydrate in serial xylene-ethanol wash
2. Incubate in Mayer's Haematoxylin for 8 minutes followed by running tap water for 10 minutes
3. Wash in demi-water for 1 minute
4. Incubate in eosin for 30 seconds
5. Wash in demi-water for 1 minute
6. Dry and mount coverslip

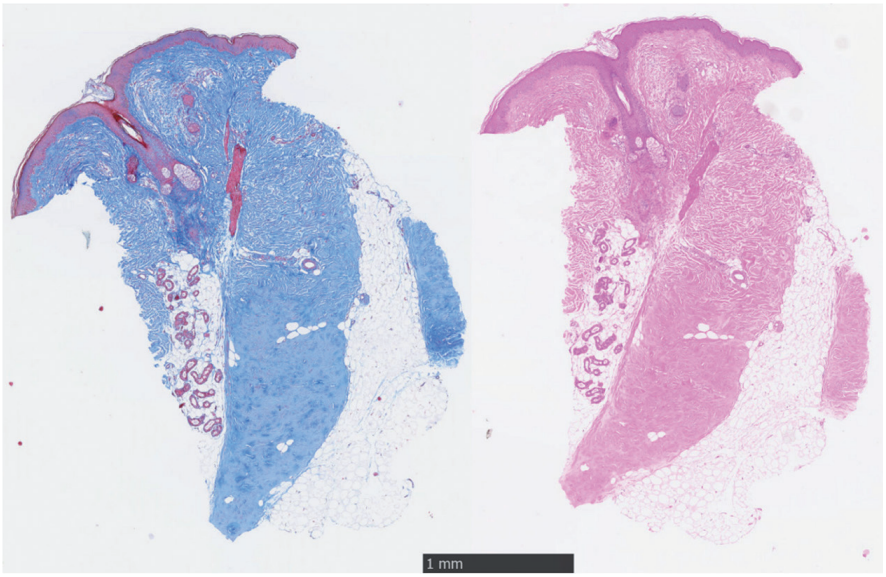
Immunohistochemistry (IHC) and immunofluorescence (IF) staining

1. Deparaffinise and rehydrate slides in serial xylene-ethanol wash
2. Block endogenous peroxidase with incubating in 1.5% H_2O_2 for 30 minutes (if proceed with peroxidase-reacted substrate, ex. Nova red and DAB)
3. Wash with demi water for 5 minutes
4. Antigen retrieval in boiled citrate (10 mM, pH 6) or EDTA (1 mM, pH 9) buffer for 20 minutes and then cool to room temperature
5. Incubate with primary antibody for 1 hour at room temperature
6. Wash with PBS for 5 minutes 3 times
7. Incubate in secondary antibody (peroxidase, alkaline phosphatase or fluorescence labelled) for 45 minutes
8. Wash with PBS for 5 minutes 3 times (For IF staining, skip step 9 to 13)
9. Incubate with substrate (peroxidase or alkaline phosphatase reacted) for 10 minutes
10. Wash with demi water for 1 minute twice

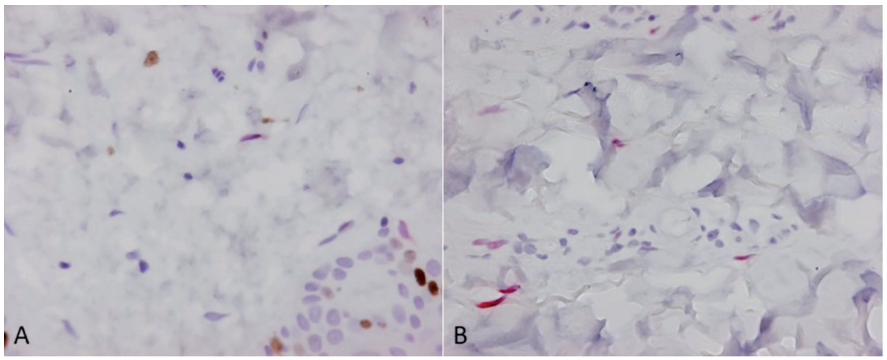
(Repeat step 5 – 10 for double staining)

11. Counterstain with Mayer's Haematoxylin (1:3 diluted) for 10 seconds
12. Wash with running tap water for 10 minutes
13. Dehydrate and mount coverslip (Counterstain in IF staining)
14. Counterstain with DAPI for 5 minutes
15. Wash with BPS for 5 minutes twice
16. Mount with VECTASHIELD® Antifade Mounting Medium
17. Seal/fasten coverslips with nail polish

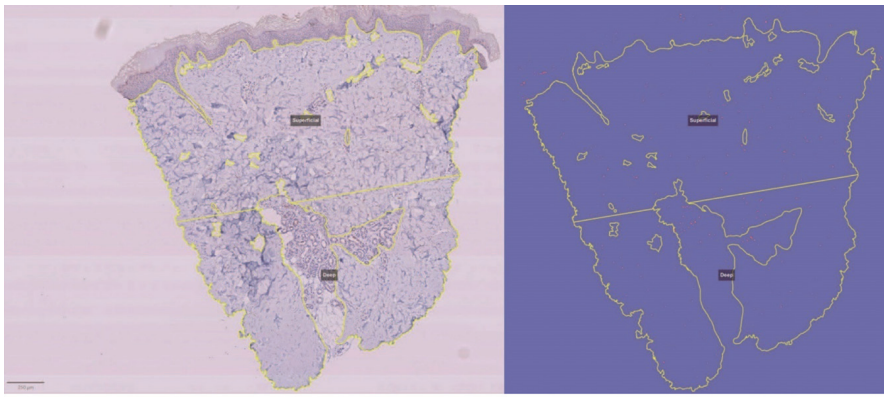
Masson's Trichrome staining was performed automatically with Dako Artisan Link Pro according to the manufacturer's instructions.



Supplementary Fig. S1. Full view of a Masson's trichrome stained (left) and haematoxylin and eosin stained (right) skin biopsy. This biopsy had fibrosis score 1 in superficial dermis and 3 in deep dermis.



Supplementary Fig. S2. Senescence markers, P16 (**A**) and P21 (**B**) was stained in bright red. The presence of senescence was observed from P21 or P16 positive without co-localised Ki-67 (brown).



Supplementary Fig. S3. A CCN2 stained skin biopsy with annotation on superficial and deep dermis by the yellow line (left). The result of pixel classification on the CCN2 staining with red for positive pixels and blue for negative pixels (right).

Supplementary Table S1. Summary of staining markers.

Markers	All	SSc	Healthy controls
Dermal fibrotic score, n			
1		5	
2		5	
3		8	
Tissue inflammation score, n			
Superficial dermis			
0	7	3	4
1	13	13	0
2	2	2	0
Deep dermis			
0	15	11	4
1	6	6	0
2	1	1	0
Senescence at endothelia, n			
P16, superficial dermis			
0	21	17	4
1	1	1	0
P16, deep dermis			
0	22	18	4
P21, superficial dermis			
0	8	6	2
1	13	11	2
2	1	1	0
P21, deep dermis			
0	16	12	4
1	6	6	0
Senescence at fibroblasts			
$P16^+$ percentage, median (IQR)	1.1 (0.5-6.3)	1.9 (0.5-7.7)	0.7 (0.4-1.0)
P16, superficial dermis			
0	9	7	2
1	8	6	2
2	5	5	0
P16, deep dermis			
0	13	9	4
1	4	4	0
2	5	5	0
$P21^+$ percentage, median (IQR)	15.7 (9.5-38.1)	20.8 (10.4-43.9)	3.3 (0.6-7.6)*
P21, superficial dermis			
0	2	1	1
1	9	7	2
2	11	10	1
P21, deep dermis			
0	8	5	3
1	9	8	1
2	5	5	0
EndMT, median (IQR)%			
αSMA/CD31	6.2 (3.1-7.7)	6.4 (4.2-8.3)	0.4 (0.0-1.4)*
αSMA/ERG	7.8 (4.8-12.8)	9.1 (6.7-14.5)	2.8 (1.7-3.9)*
CCN2 on endothelia, superficial dermis			
0	2	2	0
1	15	13	2
2	5	3	2
CCN2 on endothelia, deep dermis			
0	11	9	2
1	11	9	2
CCN2 on fibroblasts, superficial dermis			
0	7	5	2
1	10	8	2
2	5	5	0
CCN2 on fibroblasts, deep dermis			
0	11	7	4
1	4	4	0
2	7	7	0
Lymph density, median (IQR) (/mm^2)	6.9 (4.5-9.4)	7.2 (4.5-9.6)	5.5 (4.4-7.0)

SSc: systemic sclerosis; EndMT: endothelial to mesenchymal transition; IQR: interquartile range.
A significant difference between SSc and healthy control skin were labelled with *.