Supplementary methods

All subjects were subject to endoscopic bronchial biopsy taken from lobar and segmental carinae of the right lung. Biopsies were immediately moved to dry-ice and within 1 hour stored in liquid nitrogen for later analysis.

After samples were removed from the liquid nitrogen, they were immediately immersed in freshly prepared fixative solution, 2% paraformaldehyde and 2,5% glutaraldehyde in 0.1M cacodylic buffer (pH 7.4) for 24 hours at 4°C. The fixated tissue was post-fixed in 1% osmium tetroxide in water for 1 hour at 4°C. The specimens were dehydrated in graded ethanol series and embedded in epoxy resin (Eponate 12 Pelco) via acetone. Semi-thin sections (1.5 μm) were made using a Leica EM UC7 ultramicrotome with a glass knife and stained with Richardson's solution to examine the orientation of the tissue in the trimmed block. Ultra-thin sections (50 nm) were made using a Leica EM UC7 ultratome with a diamond knife. The sections were mounted on single slot cupper grids and stained with uranyl acetate (2%, 30 min) and lead citrate (4 min) and examined using a JEOL JEM 1400 Plus transmission electron microscope at 100kV.

From all subjects, bronchial biopsies encompassing the subepithelial basement membrane was examined by TEM. Firstly, the basal lamina of the basement membrane was identified (Figure 1A). Thereafter, the lamina reticularis was analysed. From a region extending 2-10 um from the basal lamina, transversally cut collagen fibrils were identified at 50 000 times magnification. Photographs were analysed in Image J by blinded assessor (MW) and collagen fibril diameter measured for all available fibrils (n>300 for every subject) within the photograph.

A histogram containing all measurements was made for all subjects. Each SSc subject was compared to the pooled data from the 5 healthy controls. All statistics were made with JMP Student Edition.

The study was conducted in accordance with the declaration of Helsinki and was approved by the local ethics committee, Etikprövningsnämnden Lund 193-01.

Supplementary Table S1. Study participants characteristics.

	Pat 1	Pat 2	Pat 3	Ctrl A	Ctrl B	Ctrl C	Ctrl D	Ctrl E
Sex	F	M	F	F	M	F	F	M
Age	69	39	68	56	31	56	30	30
Smoking	none	none	none	none	none	none	none	none
SSc subtype	lcSSc	dcSSc	lcSSc					
HRCT	Subpleural fibrosis	Fibrosis	Extensive fibrosis					
Vital capacity (% predicted)	>100	68	57					
DLCO (% predicted)	99	30	45					
Oesophageal disease	yes	yes	yes					
PAH	none	none	none					
mRSS	3	9	9					
Disease duration, skin (Mo)	5	15	23					
Disease duration, Raynaud (Mo)	13	21	48					
Immunology	ANA+, speckled	ANA+, Pm-scl-75+, Pm-scl-100+	ANA+, Scl-70+					
Mean (SD) collagen diameter, nm	31 (3.3)	38 (5.2)	34 (4.1)	31 (4.7)	29 (4.4)	34 (4.5)	31 (4.3)	31 (3.5)

ANA: anti-nuclear antibodies; DLCO: diffusing capacity of the lungs; HRCT: high resolution computed tomography; Mo: months; mRSS: modified Rodnan Skin Score: PAH: pulmonary arterial hypertension.