

## SUPPLEMENTARY MATERIAL

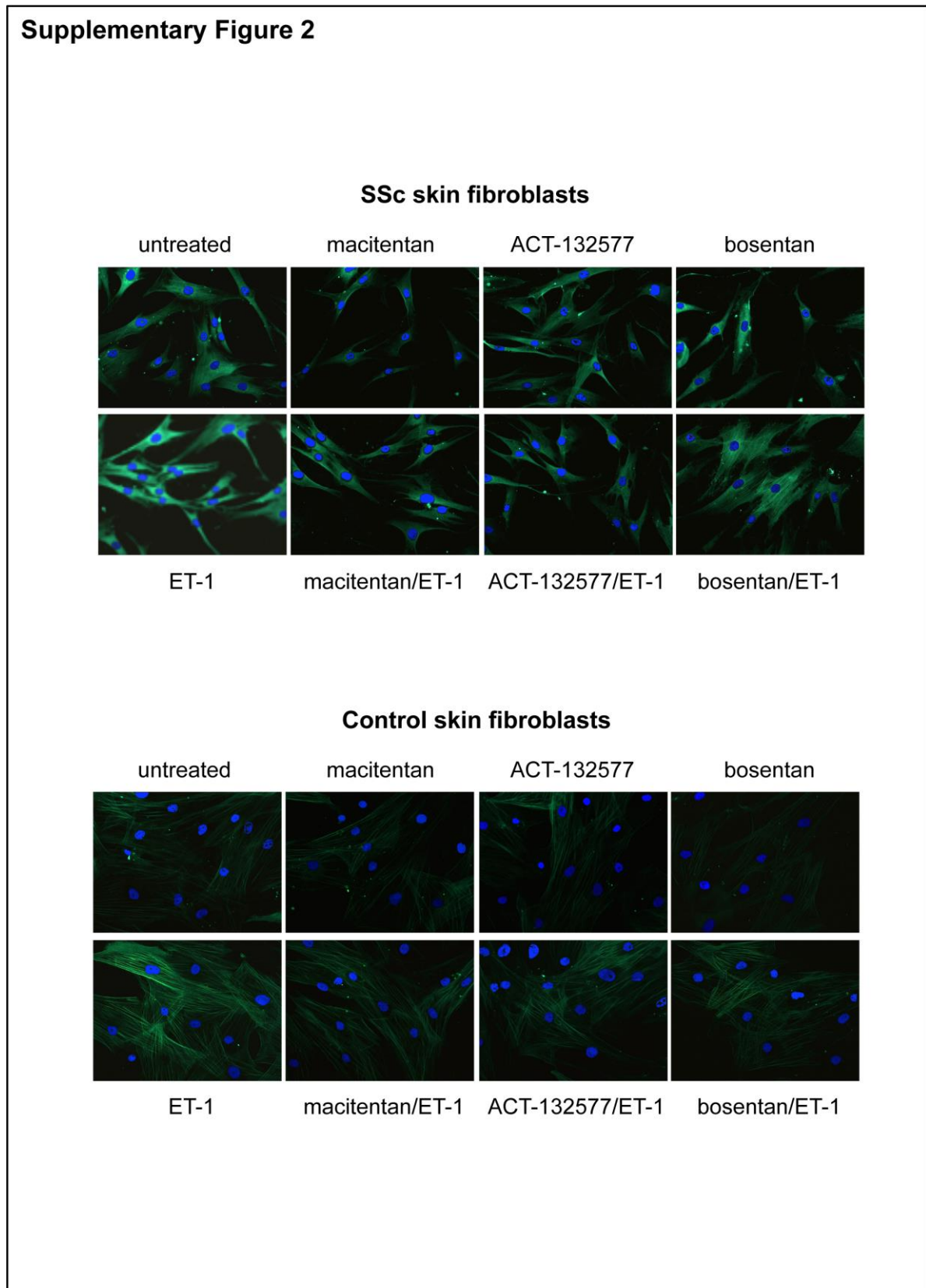
*Supplementary Figure 1.* Clinical and demographic data of patients with SSc.

**Supplementary Table 1. Clinical and demographic data of SSc patients**

Mean Age (years $\pm$ SD)	Sex (F/M)	Mean Disease duration (years $\pm$ SD)	Skin involvement (ISSc/dSSc)	NVC pattern (E/A/L)
65 $\pm$ 7	4/2	4.9 $\pm$ 3.5	4/2	1/3/2

ISSc: limited cutaneous skin involvement; dSSc: diffuse cutaneous skin involvement; NVC: nailfold videocapillaroscopy; E: early pattern of microangiopathy; A: active pattern of microangiopathy; L: late pattern of microangiopathy.

*Supplementary Figure 2.* Evaluation of  $\alpha$ -SMA expression in cultured control skin fibroblasts by immunofluorescence.

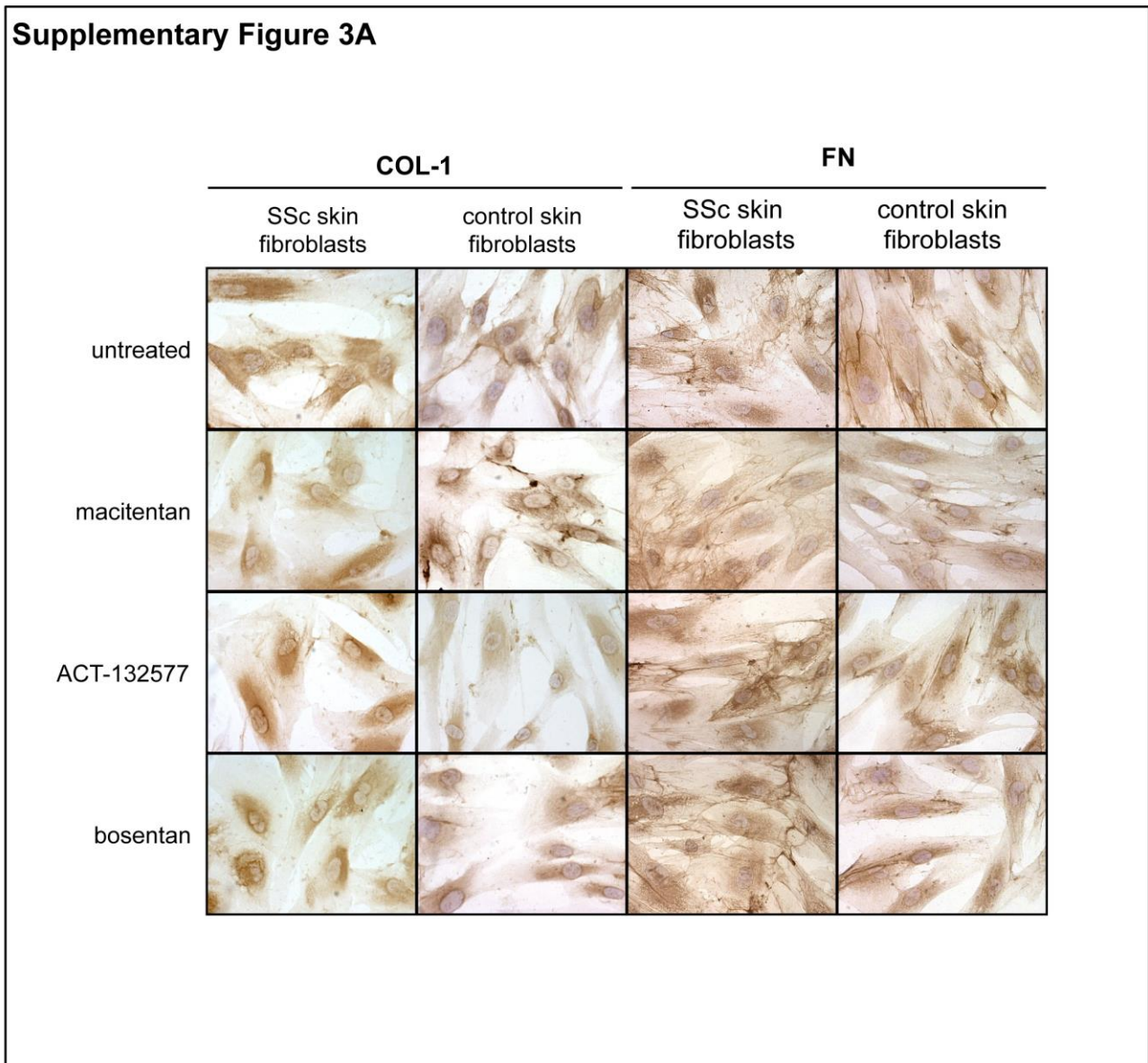


Immunofluorescence of  $\alpha$ -SMA expression in cultured human skin fibroblasts from patients with SSc (n = 6) and healthy subjects (n = 5) after 48 h of treatment. Cultured SSc and control skin

fibroblasts were either untreated or treated with macitentan (10  $\mu\text{m}$ ), or ACT-132577 (10  $\mu\text{m}$ ), or bosentan (10  $\mu\text{m}$ ) alone; treated with ET-1 alone (100 nm); treated with macitentan, or ACT-132577, or bosentan (all 10  $\mu\text{m}$ ) and after 1 h also stimulated with ET-1 (image on top).

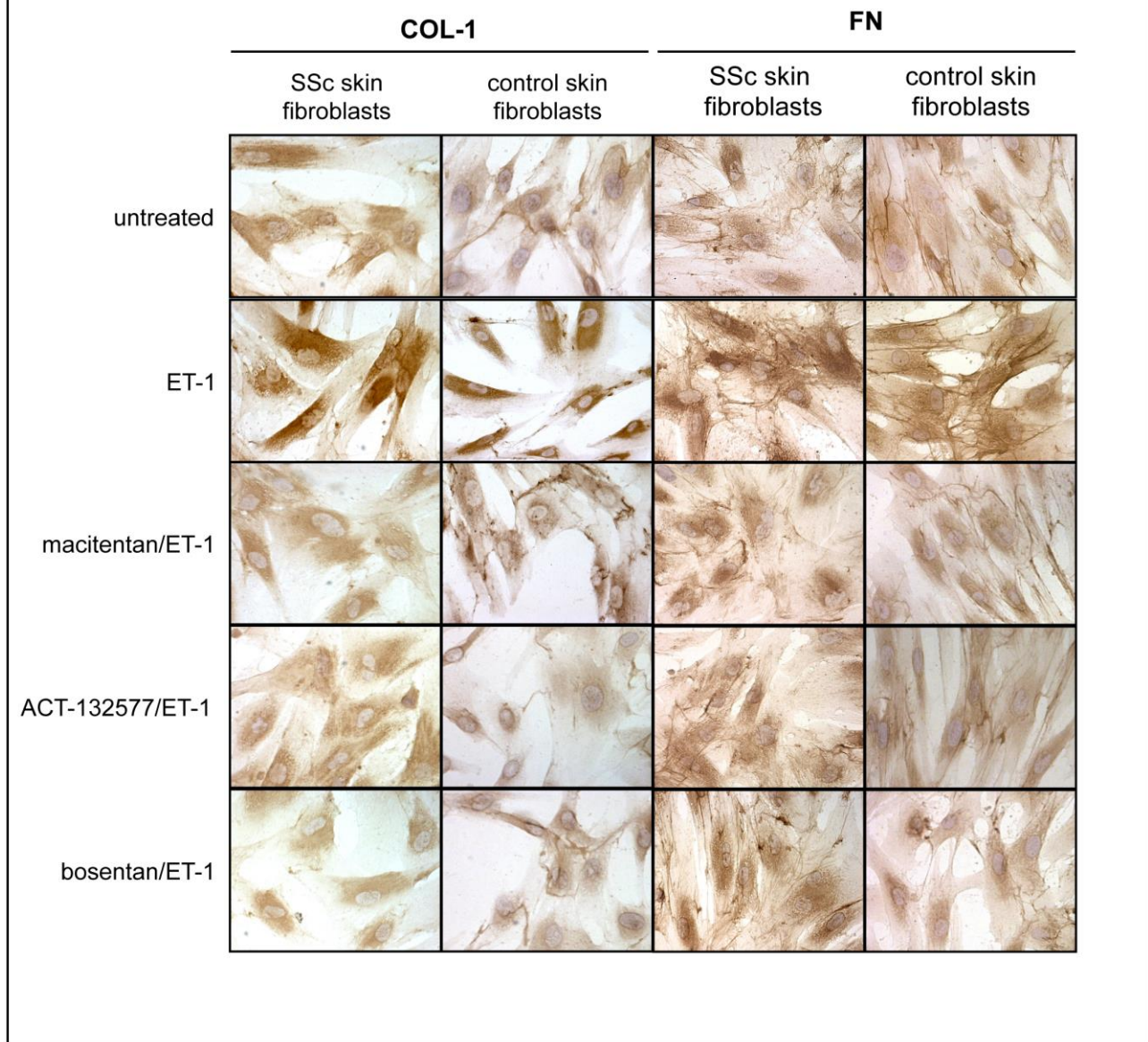
$\alpha$ -SMA:  $\alpha$ -smooth muscle actin; SSc: systemic sclerosis; ET-1: endothelin 1.

*Supplementary Figure 3.* Evaluation of ECM protein synthesis in cultured SSc and control skin fibroblasts by immunocytochemistry. Immunocytochemistry of COL-1 and FN synthesis in cultured skin fibroblasts from patients with SSc (n = 6) and healthy subjects (n = 5) after 48 h of treatment.



(A) Cultured SSc and control skin fibroblasts were either untreated or treated with macitentan (10  $\mu$ m), ACT-132577 (10  $\mu$ m), or bosentan (10  $\mu$ m) alone.

### Supplementary Figure 3B

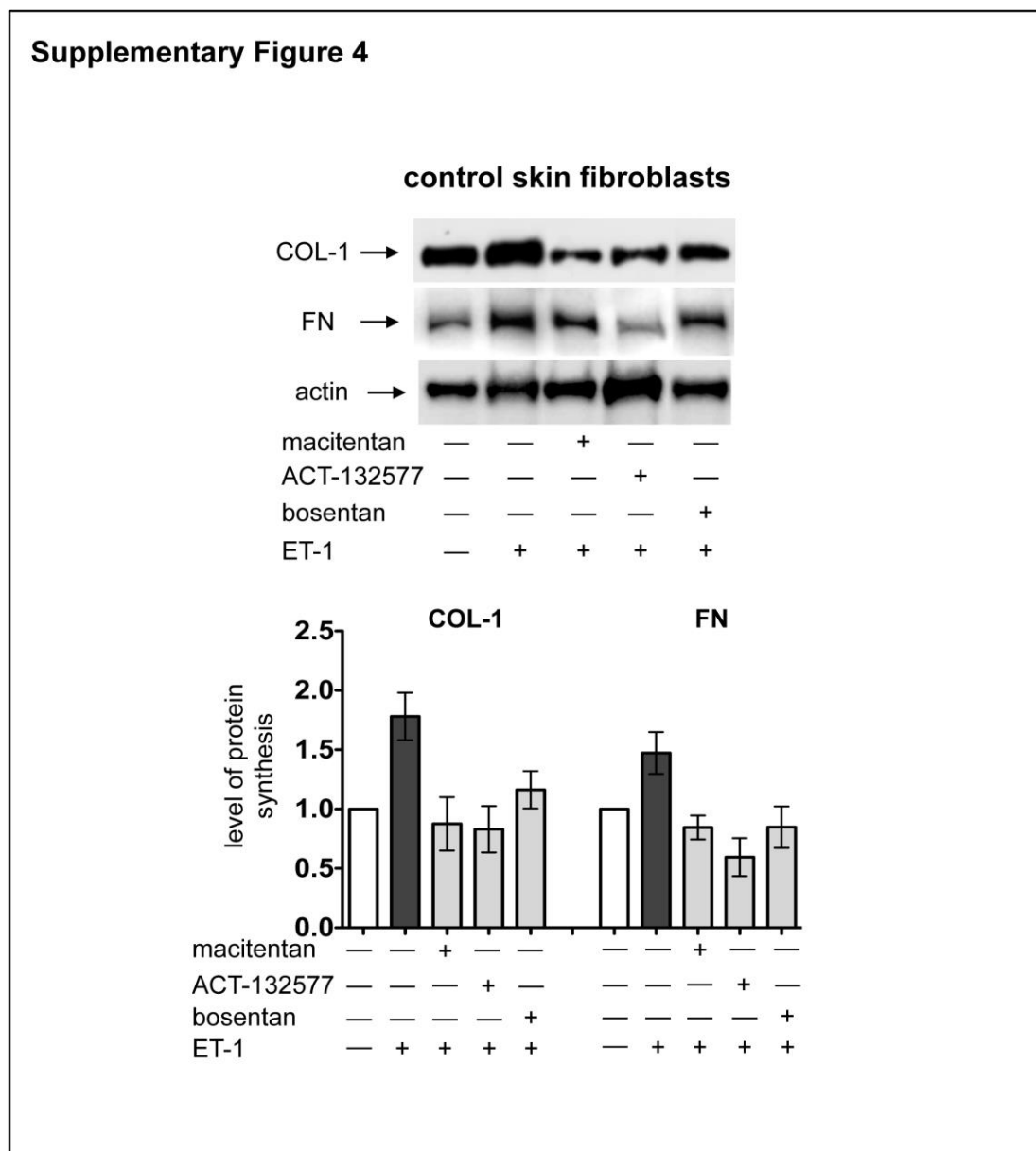


(B) Cultured SSc and control skin fibroblasts were untreated, treated with ET-1 alone (100 nm), treated with macitentan, or ACT-132577, or bosentan (all 10  $\mu$ m) and after 1 h also stimulated with ET-1.

ECM: extracellular matrix; SSc: systemic sclerosis; COL-1: type I collagen; FN: fibronectin; ET-1: endothelin 1.



*Supplementary Figure 4.* Evaluation of ECM protein synthesis in cultured control skin fibroblasts by Western blotting.



Western blotting and relative densitometric analysis of COL-1 and FN synthesis in cultured skin fibroblasts from healthy subjects ( $n = 4$ ) after 48 h of treatment. Cultured control skin fibroblasts were untreated, treated with ET-1 (100 nm), treated with macitentan, or ACT-132577, or bosentan (all 10  $\mu\text{m}$ ) and after 1 h also stimulated with ET-1. For each experimental condition, the values of COL-1 and FN synthesis were normalized to that of the corresponding actin. The values of protein synthesis obtained for each treatment were normalized to that of the untreated cells taken as unit value by definition. The data of COL-1 and FN synthesis were shown as mean  $\pm$  SD and indicated as level of protein synthesis.

ECM: extracellular matrix; COL-1: type I collagen; FN: fibronectin; ET-1: endothelin 1.