Figure S1. IL-1β and TNF-α enhance NF-κB expression, at different regulatory levels, in ANF and SF

Panel A • 15 µg of nuclear extracts from ANF and SF, treated or not (control) by IL-1β or TNF-α at different concentrations, were separated on a 8 % polyacrylamide gel in denaturing conditions. Then, proteins were transferred to a PVDF membrane and immuno-blotted with polyclonal antibodies directed against p65 (1/500 dilution), and β-tubulin as a loading control (1/300 dilution). After washing with TBS-T, PVDF membranes were incubated with the appropriate secondary antibody and proteins were revealed with the super signal West-pico® detection kit.

Panel B • Total RNAs were extracted from ANF or SF incubated or not (control) with IL-1β or TNF-α (1 ng/ml), and 1 µg was reverse-transcribed into cDNA. The resulting products were diluted (1/100) and analyzed by real-time PCR using specific primers for *p50*, *p65* and *RPL13* cDNAs. Results were normalized to *RPL13* cDNA and represented graphically as relative levels of *p50* and *p65* mRNAs, expressed as % *versus* control incubated without the cytokine.

Panel C • *[i]* 15 µg of nuclear extracts from ANF treated or not (control) with TNF-α (1 ng/ml) and/or Parthenolide (PT : 5, 10 or 20 µM), were incubated in direct binding experiments with the *consensus* NF-κB probe. *[ii]* Similar incubations with IL-1β / TNF-α and/or PT were realized in order to evaluate collagen neosynthesis, as described in Fig. 1A and in "Experimental Procedures" section. The values, normalized to the amount of total protein assayed by the Bradford colorimetric method, were expressed as cpm/µg protein, and represent the means ± S.D. of triplicate dishes. The data are expressed as % *versus* control incubated without PT and cytokines.

Statistical significance was evaluated using the Student's t-test (*: $p<0.05$; ***: $p<0.001$).

NS : Nonspecific complex.

NF-κB activation by IL-1β/TNF-α was determined in Western-blotting experiments. Fig. S1A indicates that protein levels of p65 are increased in ANF and SF after cytokine activation. Particularly, the effect of TNF-α is exerted at the transcriptional level since the steady-state levels of p50/p65 mRNA were increased upon incubation with TNF-α (Fig. S1B).

To further corroborate the specific activating effect of TNF-α on NF-κB in our experimental model, we used parthenolide (PT), a post-translational inhibitor of NF-κB activation (38, 39). ANF were incubated in the presence or not of TNF-α and/or PT and nuclear extracts were submitted to EMSA. NF-κB binding activity was found to be increased by TNF-α, as previously observed, while PT abolishes in a dose-dependent manner this binding activity in basal conditions as well as after TNF-α activation (Fig. S1Ci).

The same experiments were performed in order to evaluate the resulting collagen neosynthesis in ANF in the presence of NF-κB blockade. As shown in Fig. S1Cii and as expected, IL-1β and TNF-α decreased the production of newly synthesized collagens. The concomitant addition of PT with both cytokines indicated that NF-κB, upon cytokine activation, inhibits collagen neosynthesis, since PT was able to prevent the IL-1β- and TNF-α -induced inhibition of collagen production.

Figure S2. Sp1 is transcription activator of *Sp3*, *c-Krox*, *COL1A1*, *COL1A2* and *COL3A1* genes in FF and SF.

FF and SF were transiently transfected by the calcium phosphate co-precipitation method with 10 µg of pEVR2/Sp1 or the insertless expression vector pEVR2. 15 h after transfection, the culture medium was changed and 24 h later the samples were harvested. 1µg of total RNAs extracted, was reverse-transcribed into cDNA. 2 µl of cDNAs were diluted to 1/100 and used in real time RT-PCR using specific primers for *COL1A1*, *COL1A2*, *COL3A1*, *Sp1*, *Sp3*, *c-Krox* and *18S* cDNAs. Results were normalized to *18S* cDNA and represented graphically as relative levels of *SP1*, *SP3*, and *C-KROX mRNAs* (*Panel A*)*, and COL1A1*, *COL1A2*, and *COL3A1* mRNAs (*Panel B*), expressed as % *versus* control transfected with the insertless expression vector pEVR2.

Statistical significance was evaluated using the Student's t-test (** : $p<0.01$; *** : $p<0.001$).

We previously showed that hc-Krox can physically interact with Sp1 and Sp3 transcription factors in FF (10). Attempts to elucidate potential interactions between c-Krox, Sp1 and Sp3 revealed that each factor modulates the steady-state mRNA levels of the two others in FF, ANF, and SF, and that all of them co-immunoprecipitate from FF cellular extracts when a c-Krox antibody was used (data not shown). In order to understand if Sp1 can also regulate the expression of these *trans* factors, increasing amounts of its cDNA were overexpressed in FF and SF, and *Sp1/3, c-Krox, COL1A1, COL1A2,* and *COL3A1* mRNAs were quantified by real time RT-PCR analysis. Figure S2 shows that Sp1 can increase *c-Krox* and *Sp3* mRNA steady-state levels in both FF and SF (*panel A*) and also *COL1A1, COL1A2* and *COL3A1* mRNA amounts (*panel B*) suggesting that Sp1 is a transcriptional activator. However, Sp1 is much more potent activator of Sp3 mRNA steady-state levels in SF compared to FF, which could account significantly for the fibrotic phenotype appearance.

Figure S3. Effects of Sp1-, Sp3-, c-Krox- and CBF siRNAs on the transcription of *COL1A1* in SF.
SF were transiently co-transfected by the AMAXA Nucleofector® method with 1 µg of the pSV40-β-gal expression vector, 1 µg of control siRNA, or SOX9 (another control siRNA)- or Sp1/Sp3/c-Krox/CBF-siRNA, and 2 µg of the -112/+28-bp reporter constructs. After overnight transfection, the medium was replaced and 6 h later, the samples were harvested and transcriptional activities were analyzed as described in Fig. 10. RLU are expressed as % *versus* control siRNA co-transfected cells and represent the means ± S.D. of three independent samples.
As shown in this figure, Sp1, Sp3, c-Krox and CBF are transcription activators of *COL1A1* in SF, since transfection of siRNAs against these regulators was found to decrease the expression of the reporter *COL1A1* gene. The SOX9 siRNA can be considered as another control, since this transcription factor has not been shown to modulate the transcription of *COL1A1* in dermal fibroblasts. In our experimental conditions, it does not affect transcription of the reporter gene, because the transcription of the reporter gene construct is similar to the one observed with the control siRNA.