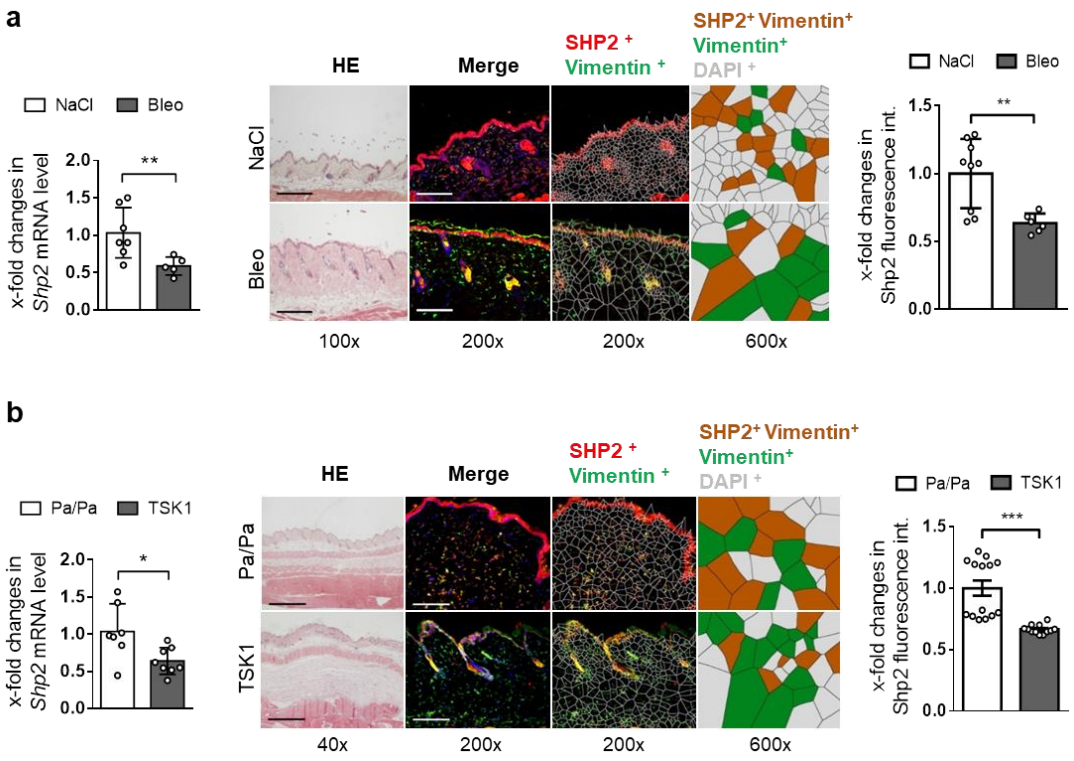
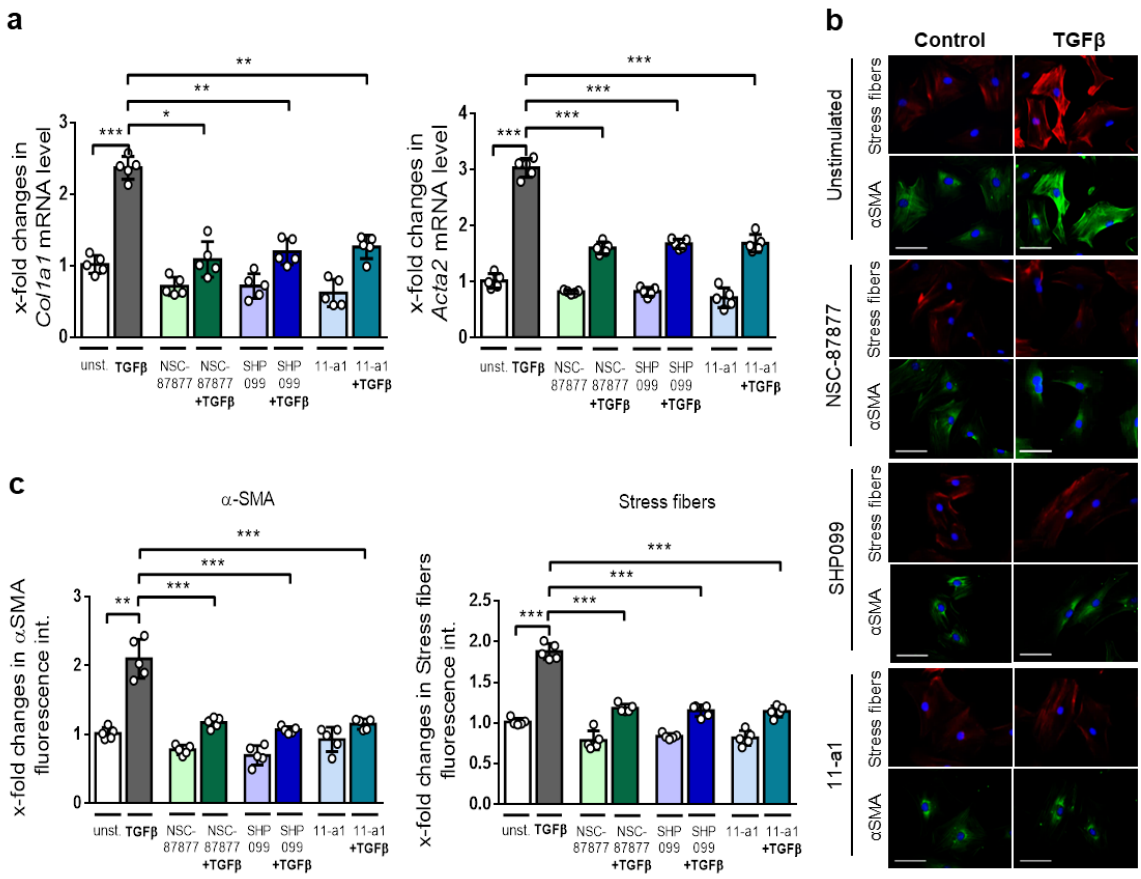
Supplementary Information:

The tyrosine phosphatase SHP2 controls TGFβ-induced STAT3 signaling to regulate fibroblast activation and fibrosis

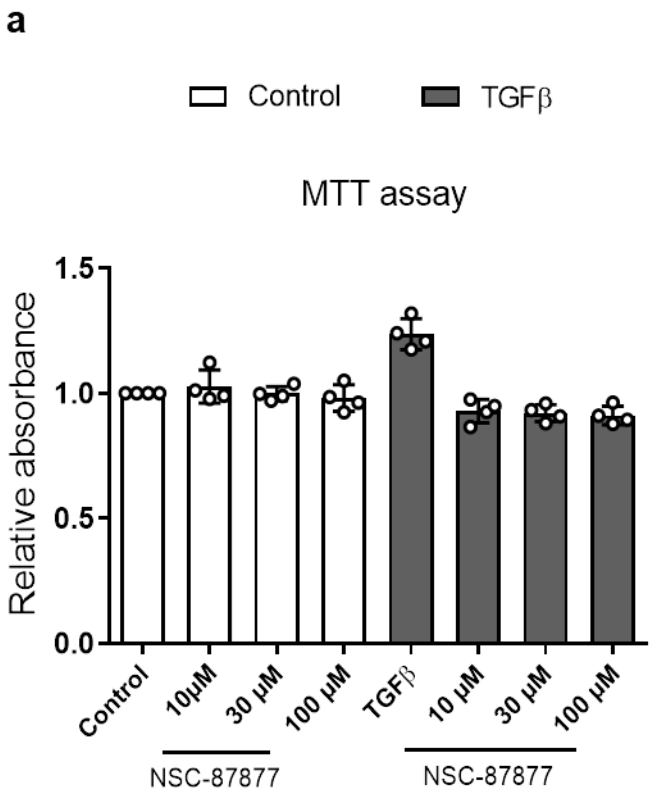
Zehender *et al.*



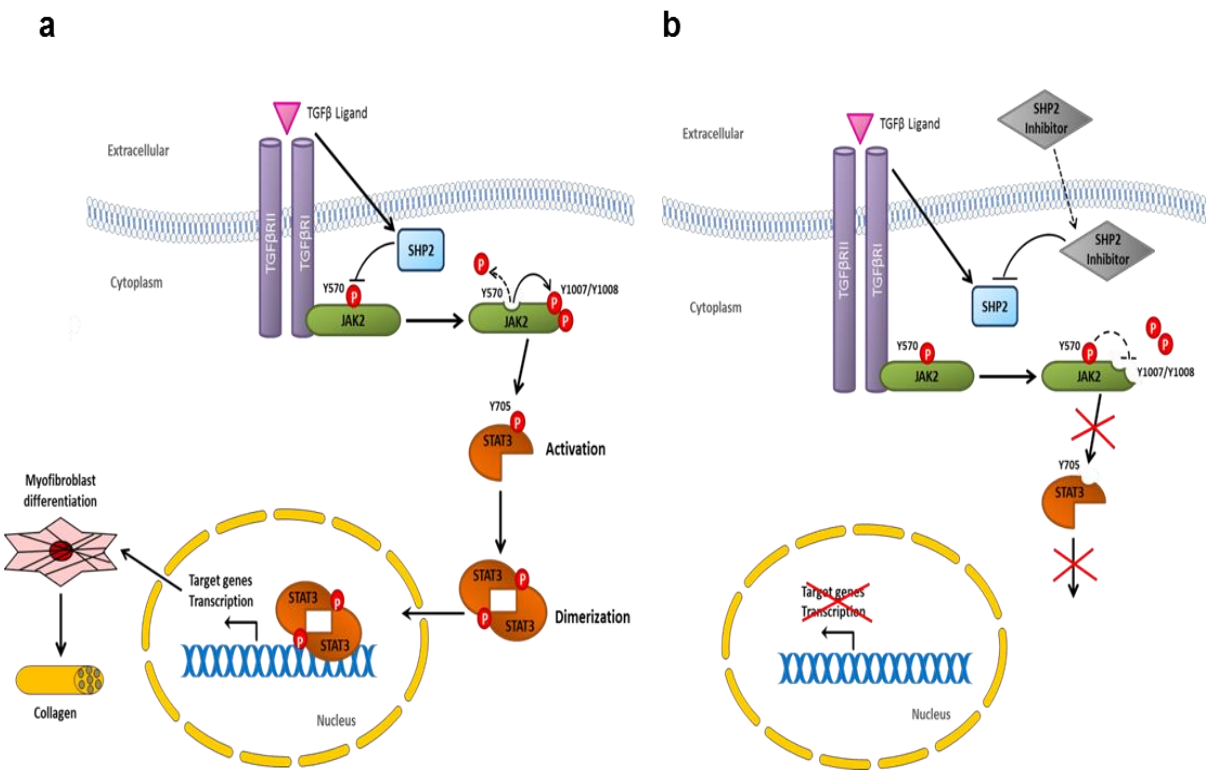
Supplementary Figure 1: Decreased expression of SHP2 in experimental skin fibrosis. a: Decreased expression of Shp2 and reduced mRNA levels of *Shp2* in the skin of mice challenged with bleomycin (n ≥ 5). Representative HE images and immunofluorescence staining of SHP2, vimentin and DAPI shown at 200- and 600-fold magnification. Horizontal scale bar, 500 µm. Voronoi tessellated pictures and histograms of respective Immunofluorescence signals are included. **b:** Reduced mRNA levels of *Shp2* in TSK1 mice (2 mg tamoxifen over 5 days) (n ≥ 7). Representative images stained for SHP2, vimentin and DAPI shown at 200- and 600-fold magnification. Results shown are representative of a minimum of two independent experiments per model. All data are presented as median ± s.e.m. The p-values are expressed as follows: 0.05 > p > 0.01 as *; 0.01 > p > 0.001 as **; p < 0.001 as ***; ns= not significant. Significance was determined by Mann–Whitney test. NSC-87877= SHP1/SHP2 inhibitor; TSK1= Tight skin; Pa/Pa= Tight skin control; Bleo= Bleomycin.



Supplementary Figure 2: Inhibition of Shp2 by NSC-87877 (100 µM), SHP099 (1.4 µM) and 11-a1 (0.2 µM) inhibitors in cultured dermal fibroblasts. a: mRNA levels of *Col1a1* and *Acta2* (n = 5). **b-c:** Representative immunofluorescence staining of αSMA, Stress fibers and DAPI shown at 400-fold magnification (**b**) with quantification (**c**). Horizontal scale bar, 500 µm. Results shown are representative of three independent experiments. All data are presented as median ± s.e.m. The p-values are expressed as follows: 0.05 > p > 0.01 as *; 0.01 > p > 0.001 as **; p < 0.001 as ***; ns= not significant. Significance was determined by Mann–Whitney test. NSC-87877, SHP099 and 11-a1 are SHP1/SHP2 inhibitor; unst.= unstimulated.



Supplementary Figure 3: a: Treatment with NSC-87877 does not modify the metabolic activity in resting fibroblasts, but prevents the increase induced by TGFβ. Results shown are representative of two independent experiments (n = 4). All data are presented as median ± s.e.m. The p-values are expressed as follows: 0.05 > p > 0.01 as *; 0.01 > p > 0.001 as **; p < 0.001 as ***; ns= not significant. Significance was determined by Mann–Whitney test. NSC-87877= SHP1/SHP2 inhibitor; MTT= tetrazolium dye.



Supplementary Figure 4: Schematic summary of the proposed role of SHP2 in TGFβ-dependent fibroblast activation **(a)** and of the effects of SHP2-inhibitors **(b).** The Figure was created by the authors using in part images from Servier Medical Art, licensed under a Creative Common Attribution 3.0 Generic License. http://smart.servier.com/. P= Phosphorylation

Figure 1e

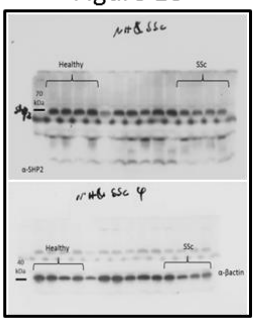


Figure 2b

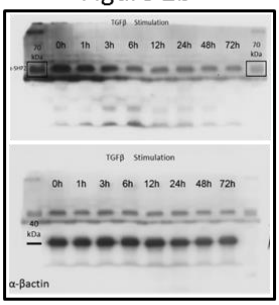


Figure 2h

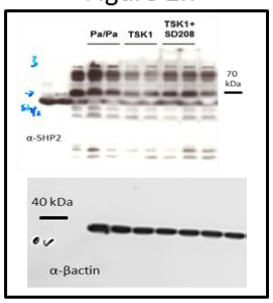


Figure 2f

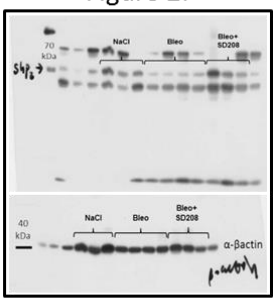


Figure 3a

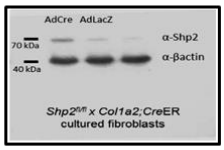


Figure 5 a, b, c

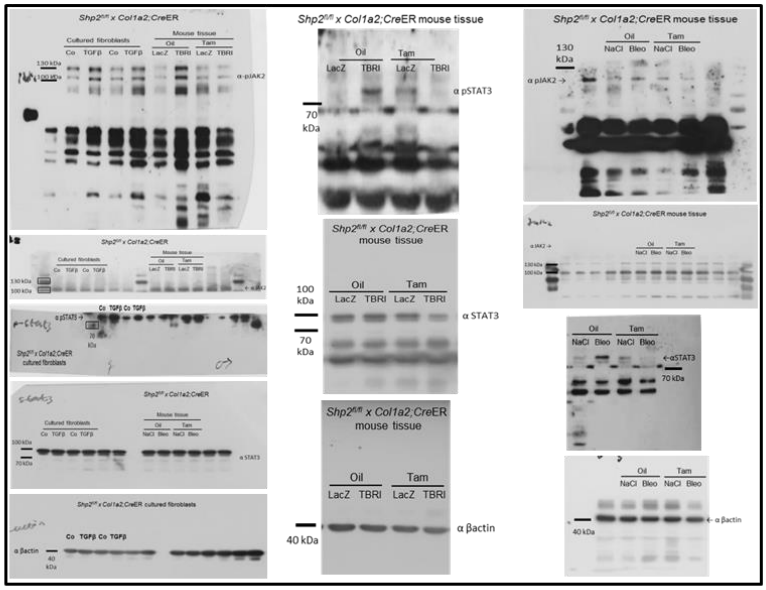


Figure 5 d

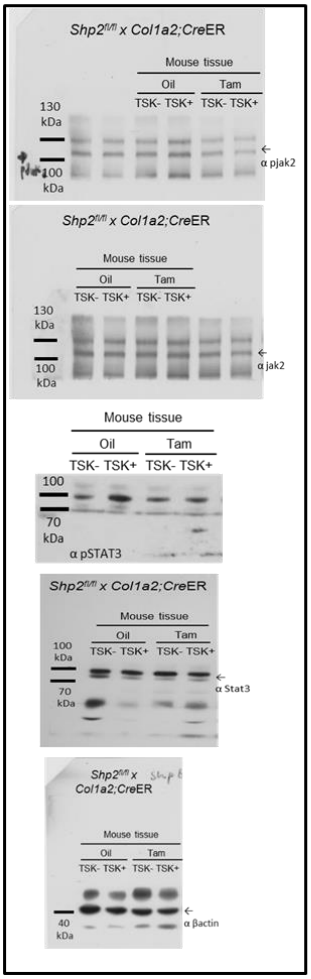
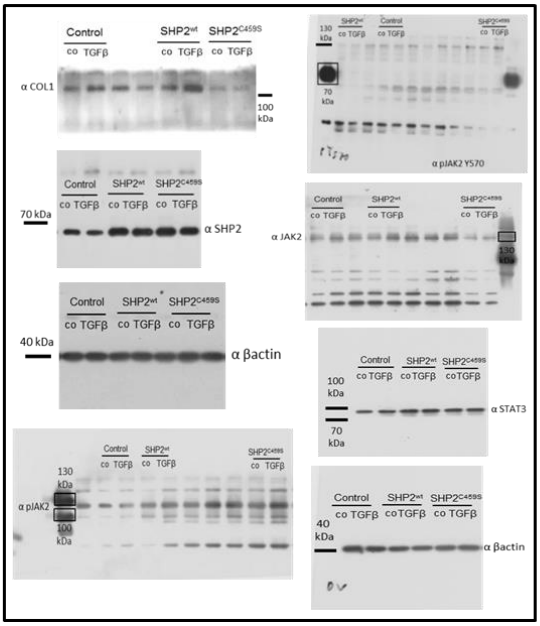


Figure 6b



Supplementary Figure 5: Uncropped scan of Original Western blots.

Figure 7e

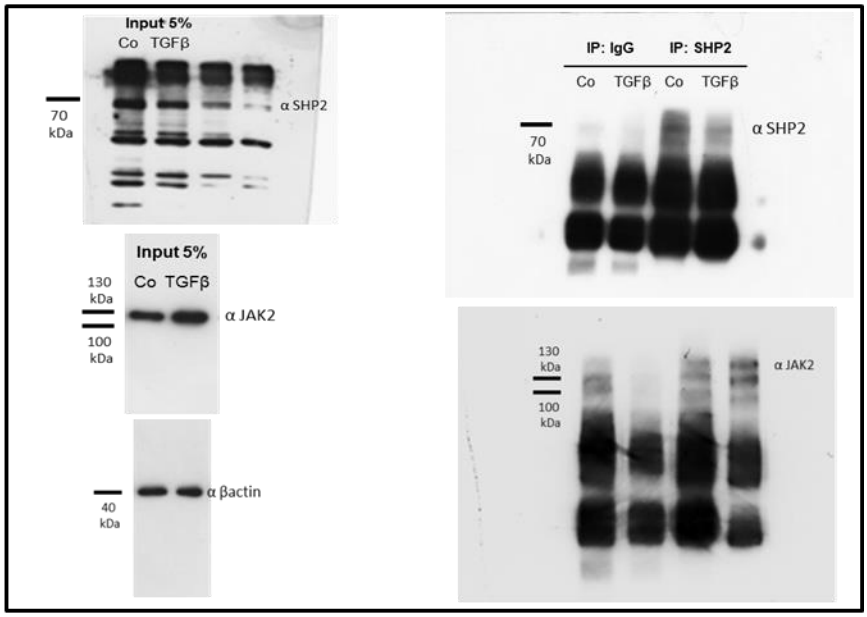
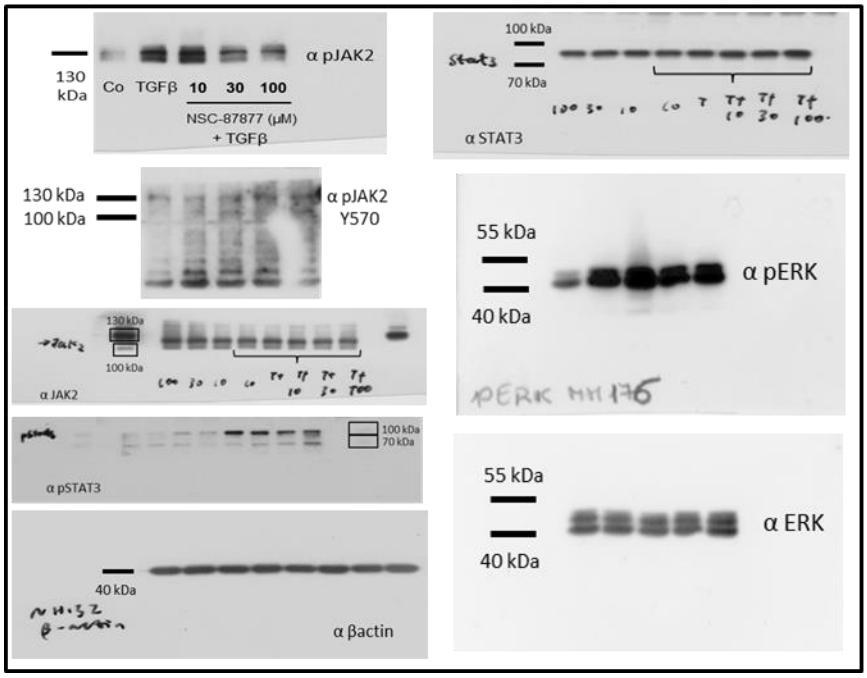


Figure 8d



Supplementary Figure 6: Uncropped scan of Original Western blots.

Supplementary Table 1: primers

human β-ACTIN forward	5'-AGA AAA TCT GGC ACC ACA CC-3'
human β-ACTIN reverse	5'-TAG CAC AGC CTG GAT AGC AA-3'
human SHP2 forward	5'-TAT CCT CTG AAC TGT GCA GAT CC-3'
human SHP2 reverse	5'-TCT GGC TCT CTC GTA CAA GAA AA-3'
human COL1A1 forward	5'-ACG AAG ACA TCC CAC CAA TC-3'
human COL1A1 reverse	5'- ATG GTA CCT GAG GCC GTT C-3'
human ACTA2 forward	5'-TGG GCT GAA GCG CAC TGA CC-3'
human ACTA2 reverse	5'- CCG CGG CTC TTG CCC ACA T-3'
murine β-actin forward	5'-TCT TTG ATG TCA CGC ACG AT-3'
murine β-actin reverse	5'-TAC AGC TTC ACC ACC ACA-3'
murine shp2 forward	5'-GGA GAG CAT CGT GGA TGC-3'
murine shp2 reverse	5'-TCC CAG CGC TGC AGT GAA-3'
murine Acta2 forward	5'-ATG CCT CTG GAC GTA CAA CTG-3'
murine Acta2 reverse	5'-CAC ACC ATC TCC AGA GTC CA-3'