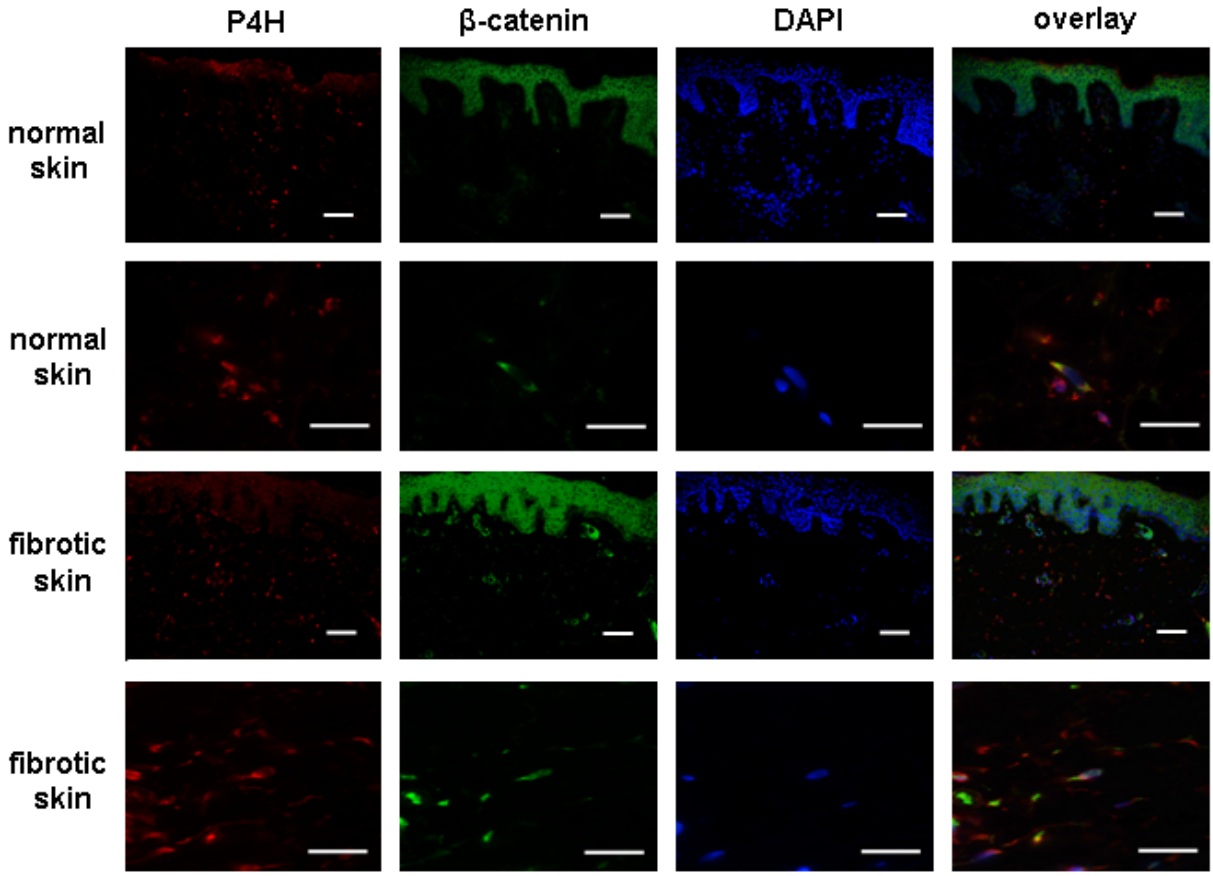
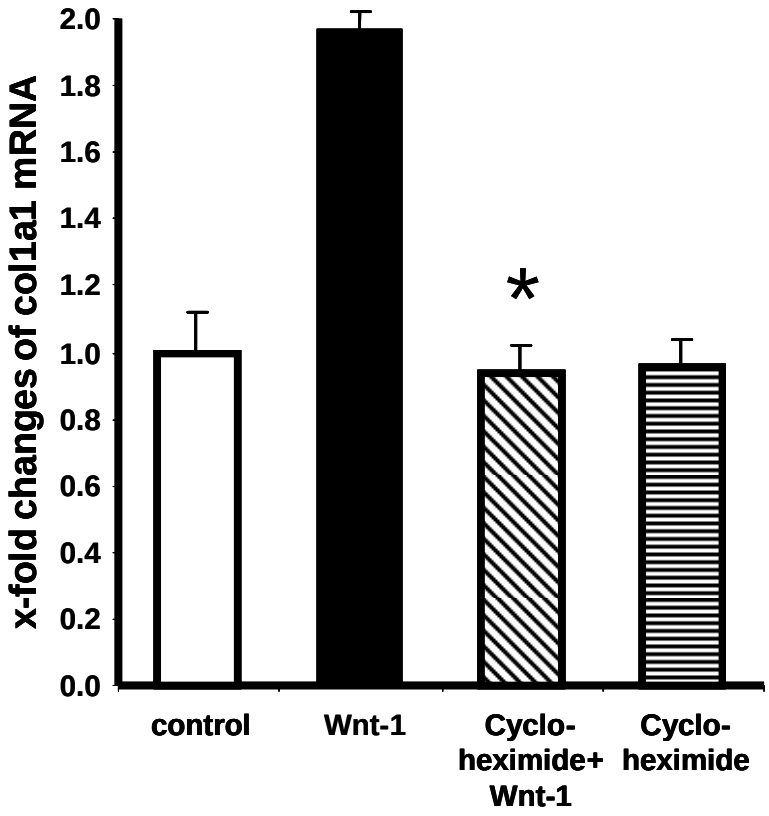
Supplementary Information

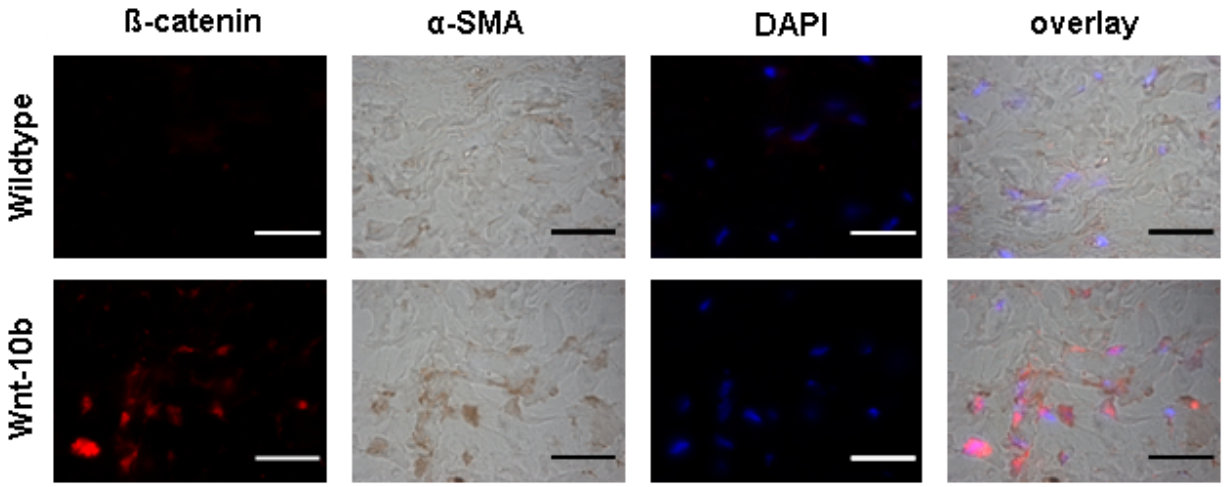
Supplementary Figures



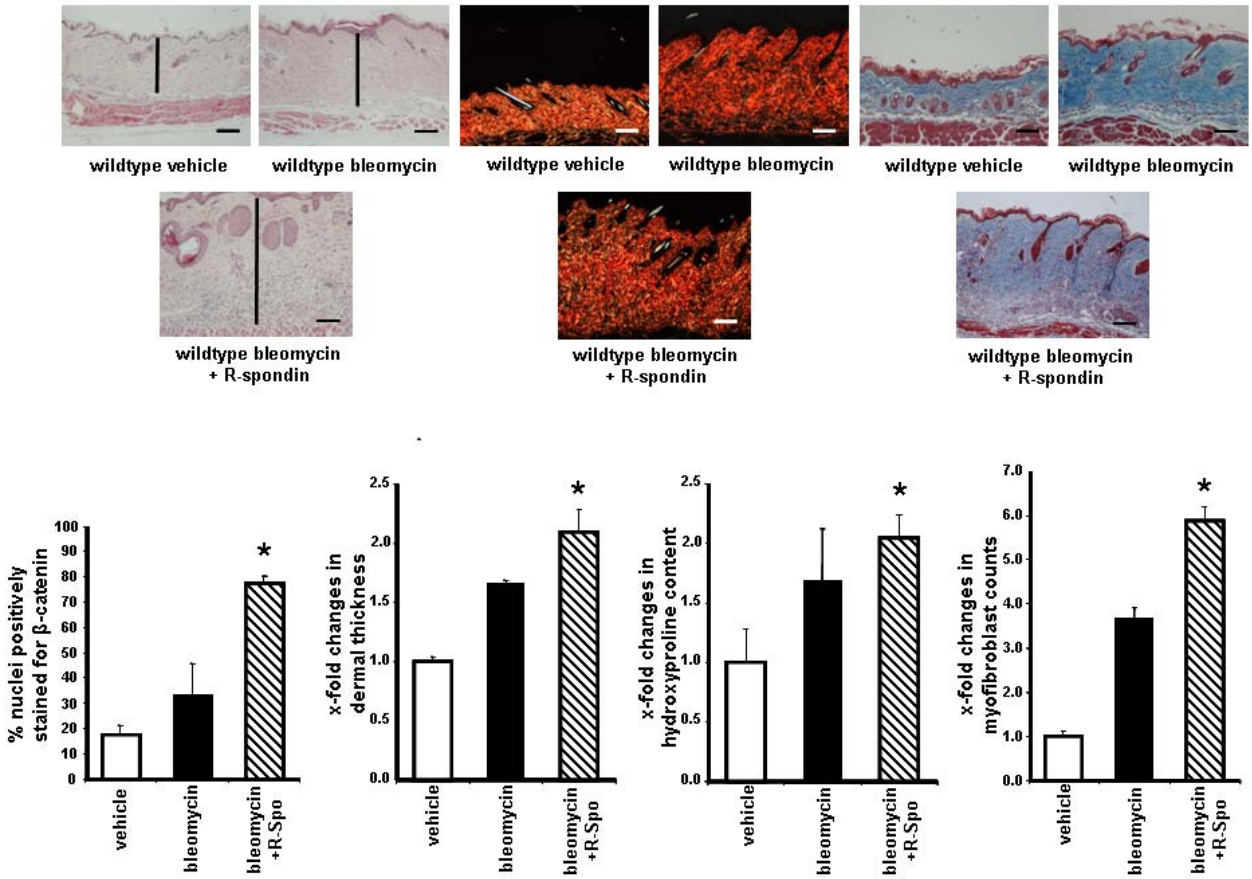
Supplementary Figure S1: β-catenin accumulates in the nuclei of fibroblasts in fibrotic skin. Representative images of fibrotic skin triple stained for β-catenin, prolyl-4-hydroxylase-β and DAPI are shown at low magnification and at high magnification as used for counting (scale bar 100 µm in all images). P4H: prolyl-4-hydroxylase-β.



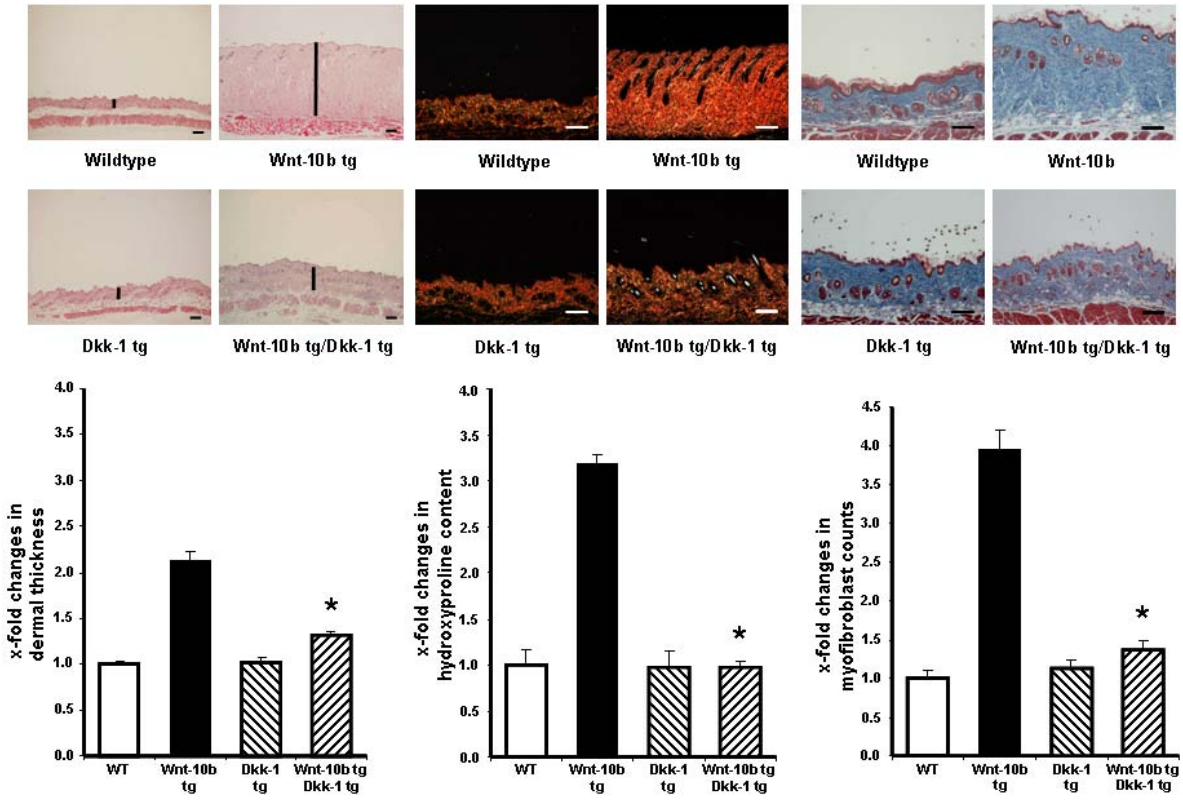
Supplementary Figure S2: The stimulatory effects of Wnts on the release of collagen require protein neosynthesis. Cycloheximide, an inhibitor of translation, completely blocked the induction of col 1a1 mRNA by recombinant Wnt-1, demonstrating that the stimulatory effects of Wnt-1 on collagen synthesis are indirect and require the synthesis of other proteins as second mediators. n = 5 for each group. * indicates p-values of less than 0.05 as analyzed using the Wilcoxon signed rank test compared to fibroblasts stimulated with Wnt-1 in the absence of cycloheximide.



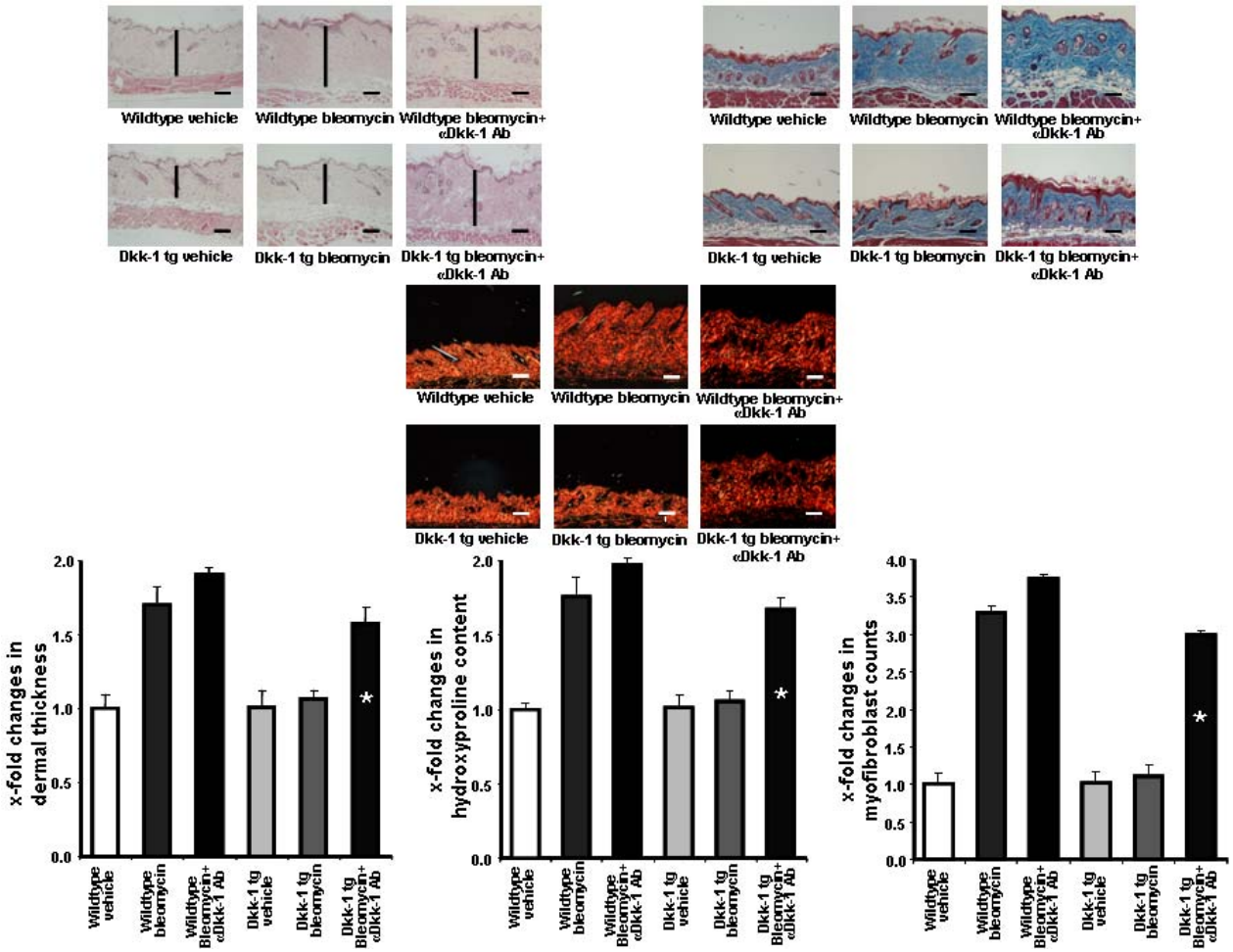
Supplementary Figure S3: Canonical Wnt signaling is activated in myofibroblasts in the skin of Wnt-10b transgenic mice. Triple staining for DAPI, β-catenin and αSMA demonstrated a prominent nuclear accumulation of β-catenin in skin sections from Wnt-10b tg mice, but not control mice. Representative images are shown at high magnification (scale bar 100 μm).



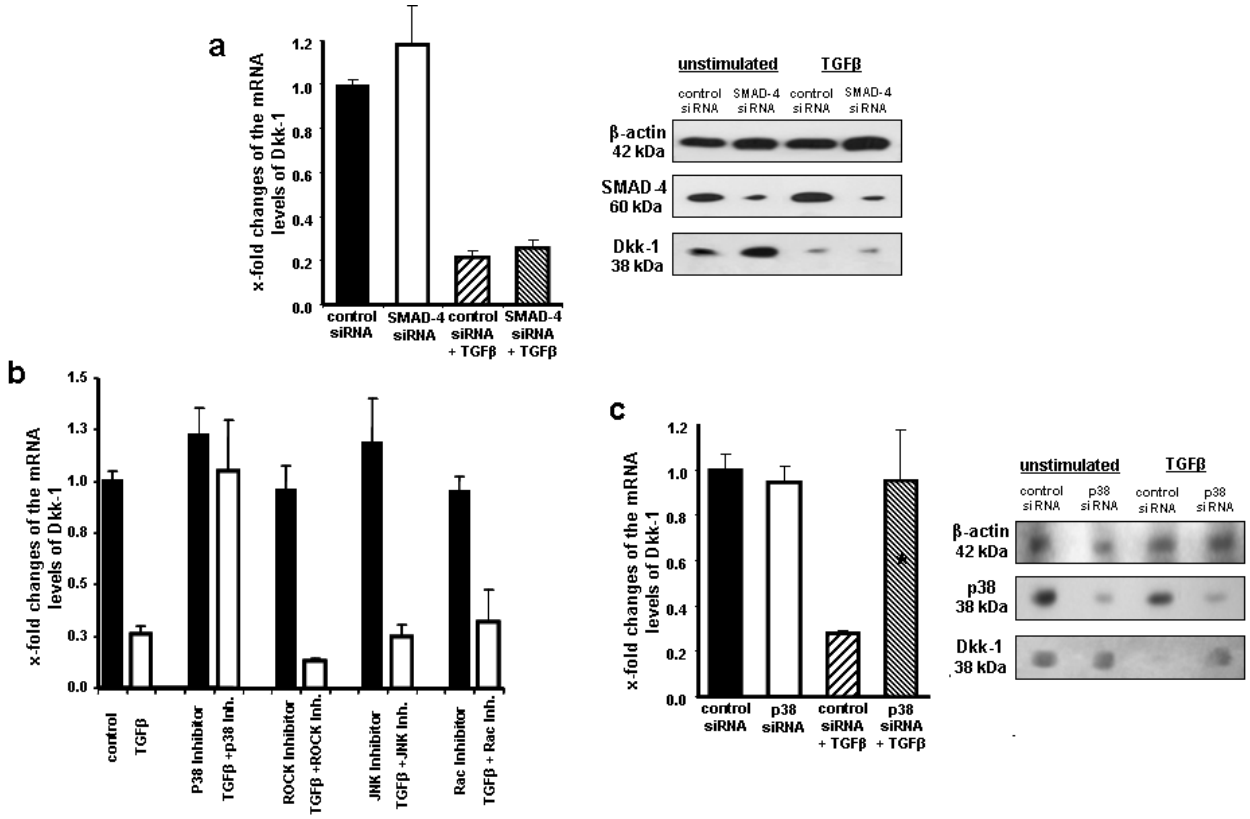
Supplementary Figure S4: R-spondin-1 exacerbates bleomycin-induced dermal fibrosis. Treatment with R-spondin-1 resulted in a more pronounced activation of the canonical Wnt pathway with increased nuclear accumulation of β-catenin in fibroblasts. R-spondin-1 enhanced bleomycin induced dermal fibrosis with more pronounced dermal thickening, increased hydroxyproline content and higher myofibroblast counts as compared to mice injected only with bleomycin. Representative sections stained with HE-, Sirius Red- and Trichrome stained sections are shown (scale bar 100 µm). $n \geq 5$ for all groups. * indicates p-values of less than 0.05 (as analyzed with the Mann-Whitney U-test) compared to bleomycin treated mice without R-spondin-1. $n = 6$ for each group.



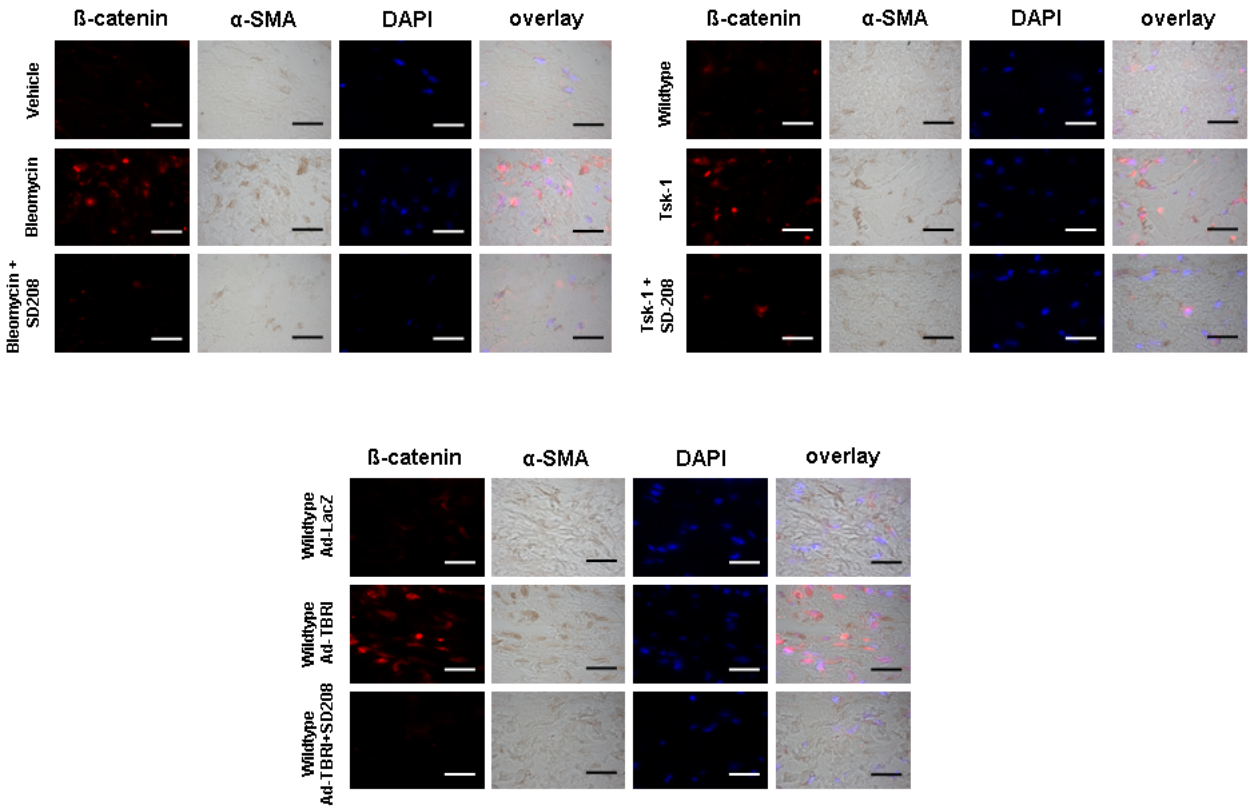
Supplementary Figure S5: Transgenic overexpression of Dkk-1 prohibited fibrosis in Wnt-10b transgenic mice. Representative sections stained with HE-, Sirius Red- and Trichrome stained sections are shown (scale bar 100). Transgenic overexpression of Dkk-1 prevented Wnt-10b induced dermal thickening, accumulation of hydroxyproline and differentiation of resting fibroblasts into myofibroblasts ($n \geq 6$ for all groups). Wnt-10b tg = Wnt-10b transgenic mice. * indicates p-values of less than 0.05 (as analyzed with the Mann-Whitney U-test) compared to Wnt-10b tg mice not overexpressing Dkk-1.



Supplementary Figure S6: Neutralization of Dkk-1 has no major effect on fibrosis in wildtype mice. Consistent with the finding that the levels of Dkk-1 are strongly decreased and almost undetectable in fibrotic diseases, treatment of wildtype mice with neutralizing antibodies against Dkk-1 resulted only in slightly increased bleomycin-induced dermal fibrosis. In contrast, treatment of Dkk-1 transgenic mice with neutralizing antibodies against Dkk-1 resulted in a significant exacerbation of fibrosis with increased dermal thickness, hydroxyproline content and myofibroblast counts (scale bar 100 µm). * indicates p-values of less than 0.05 (as analyzed with the Mann-Whitney U-test) compared to sham treated, bleomycin challenged Dkk-1 tg mice. αDkk-1: neutralizing antibodies against Dkk-1. $n \geq 5$ for each group.



Supplementary Figure S7: TGF-β - induced downregulation of Dkk-1 requires p38. (a) siRNAs against Smad4 did not prevent the decrease of Dkk-1 mRNA and protein upon incubation with TGF-β (n = 4). **(b)** Inhibition of ROCK, JNK and Rac also did not reduce the inhibitory effects of TGF-β (n = 4). However, pharmacological inhibition of p38 prevented the TGF-β induced downregulation of Dkk-1. **(c)** siRNA mediated knockdown of p38 completely prevented the TGF-β induced downregulation of Dkk-1 mRNA and protein (n = 6). * indicates p-values of less than 0.05 as compared to TGF-β stimulated fibroblasts as analyzed using the Wilcoxon signed rank test.



Supplementary Figure S8: Inhibition of TGF-β signaling reduces the nuclear accumulation of β-catenin in myofibroblasts. Triple staining for DAPI, β-catenin and αSMA demonstrated that treatment with SD-208, a selective inhibitor of TBRI, prevented the nuclear accumulation of β-catenin in myofibroblasts in bleomycin-induced fibrosis, in Tsk-1 mice and in Ad-TBRI^{act} induced fibrosis (scale bar 100 μm).