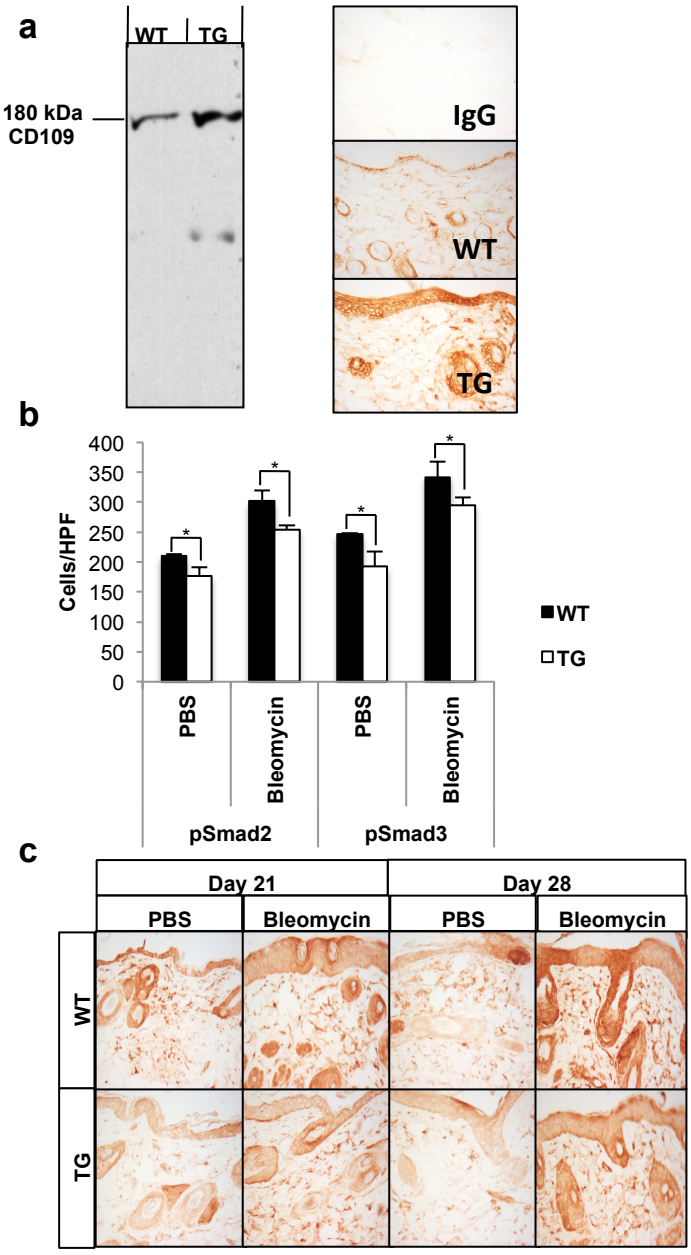
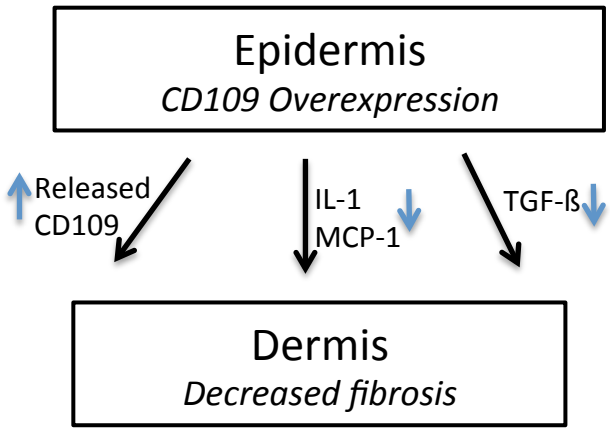


Supplementary Figure 1. (a)Confirmation of CD109 Overexpression in the Skin of transgenic mice, (b) Quantification of Smad Phosphorylation in Immunohistochemistry, and (c) determination of TGF-ß1 Expression at Injection Site. *(a):* CD109 overexpression in the skin of transgenic mice was confirmed by immunoblot analysis of CD109 transgenic and wild-type mouse skin homogenates (left panel), and by immunohistochemical analysis of CD109 transgenic and wild-type mouse skin sections (right panel). The specificity of anti-CD109 antibody used for immunostaining was confirmed by staining with IgG negative control. We consistently observe a slight increase in CD109 expression also in the dermis of the transgenic mice, which might represent exosomal delivery of CD109 released from keratinocyte cell surface due to GPI anchor cleavage known to occur in these cells [4, 11]. Exosomal delivery of proteins released by keratinocytes has been well documented by others [12]. *(b):* Quantitation of immunohistochemistry data shown in Figure 3C demonstrating decreased phosphorylation of Smad2 and Smad3 in bleomycin-induced skin fibrosis lesions of transgenic mice. Tissue sections *(n=5* each) of CD109 transgenic and wild-type mice which received PBS or bleomycin treatment for 28 days were immunostained with anti-phosphoSmad2 or anti-phosphoSmad3 antibody and quantified by taking the mean of the number of positive nuclei per high-power field *($p<0.05$). (c):* TGF-ß1 expression is decreased in bleomycin-induced skin fibrosis lesions of CD109 transgenic mice. CD109 transgenic and wild-type mice treated with PBS or bleomycin for 21 or 28 days were immunostained with an antibody against TGF-ß1 *(n=6 per group/treatment/ time-point)*. Representative images shown. *Original Magnification: 20x.*



Supplementary Figure 2: Schematic depiction of a model showing the possible mechanisms by which CD109 overexpression in the epidermis may decrease dermal fibrosis. Overexpression of CD109 in the epidermis in the transgenic mice may regulate dermal function by decreasing the levels of TGF-ß1 as seen in the present study and/or by decreasing the expression of IL-1α and MCP-1, as we have shown previously in wound healing studies [7].

Supplementary Table 1: List of Primers and Antibodies Used.*

PCR Primers:			
Method	**Forward Primer**	**Reverse Primer**	**Tm**
Genotyping	5'-GGCGAATACGATCACAAGTT-3'	5'-TCCTGGGTACGTCCGGTTACA-3'	55°C
RT-PCR	5'-CAGCCTATGCACTGCTCTCA-3'	5'-CAGGACTTGGTGAGCTAGGC-3'	55°C
Immunoblot:			
Antibody	**Cat. No.**	**Source**	
Collagen Type I	AB34710	Abcam, Cambridge MA	
Fibronectin	610078	BD Biosciences, Mississauga ON	
phospho-Smad2/3	SC-11769-R	Santa Cruz Biotechnology, Santa Cruz CA	
Smad2/3	SC-8332	Santa Cruz Biotechnology, Santa Cruz CA	
CD109	496110.111	R&D Systems, Minneapolis, MN	
ß-actin	SC-47778	Santa Cruz Biotechnology, Santa Cruz CA	
Immunohistochemistry:			
Antibody	**Cat. No.**	**Source**	
phospho-Smad2	CS-3101	Cell Signaling, Pickering ON	
phospho-Smad3	CS-9520	Cell Signaling, Pickering ON	
CD109	496910.111	R&D Systems, Minneapolis, MN	
TGF-ß1	SC-146	Santa Cruz Biotechnology, Santa Cruz CA	
normal rabbit IgG	SC-2027	Santa Cruz Biotechnology, Santa Cruz CA	
normal mouse IgG	SC-2025	Santa Cruz Biotechnology, Santa Cruz CA	

*PCR conditions were 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds.