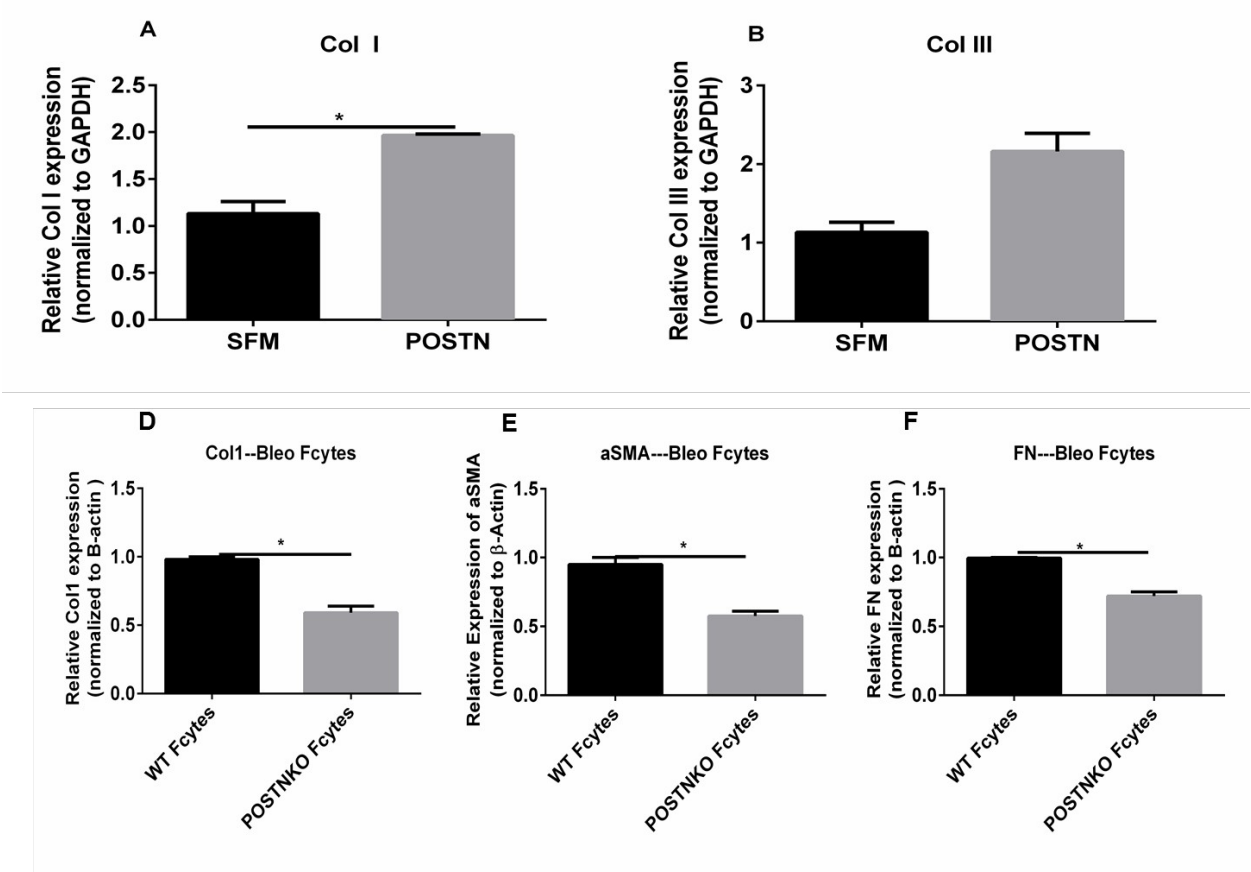
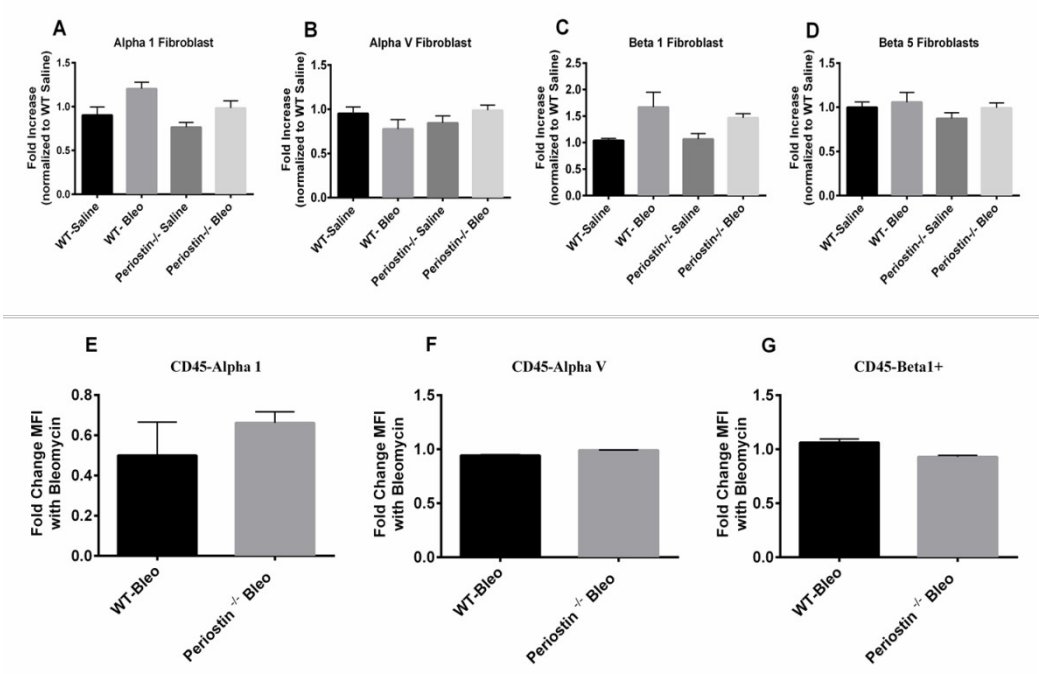
| Supplemental Table I. Primers used for qRT-PCR | | |
|---|---|---|
| Gene Name | | Primer Sequence |
| β-Actin | Forward | 5'CCGTGAAAGATGACCCAGATC-3 |
| | Reverse | 5'-CACAGCCTGGATGGCTACGT-3' |
| | Probe | 5'-TTTGAGACCTTCAACACCCCCAGCCA-3' |
| Alpha 1 | Forward | 5'-ATTTATCATGGCAGTGGCAA-3' |
| | Reverse | 5'-AATTTCAGCGTCTTCCCATC-3' |
| | Probe | 5'-ATGCGCAACGCATTCCCTCA-3' |
| Alpha V | Forward | 5'-TGAGAATCAAGACACCCGAA-3' |
| | Reverse | 5'-TGATTCCTTTCTCCCTGTCC-3' |
| | Probe | 5'-CGCCGCCGCTGTGTCATT-3' |
| Beta 1 | Forward | 5'-CTCCAGAAGGTGGCTTTGAT-3' |
| | Reverse | 5'-TGTTACATTCCTCCAGCCAA-3' |
| | Probe | 5'-TCAGCGATCCACAAACCGCA-3' |
| Beta 5 | Forward | 5'-TACAGTAGCATCCGGGCTAA-3' |
| | Reverse | 5'-CAGGTGGCAGTGAAGAAGAG-3' |
| | Probe | 5'-TGGCTGATCCCACACTGACAGC-3' |
| CTGF | Forward | 5'-GAGTGTGCACTGCCAAAGAT-3' |
| | Reverse | 5'-GGCAAGTGCATTGGTATTTG-3' |
| | Probe | 5'-CGCAGCGGTGAGTCCTTCCA-3' |
| PDGFα | Forward | 5'-CGAAGTCAGATCCACAGCAT-3' |
| | Reverse | 5'-GGGCTCTCAGACTTGTCTCC-3' |
| | Probe | 5'-CCGGGACCTCCAGCGACTCT-3' |
| GAPDH | Forward | 5'-AACCTGCCAAGTACGATGACATC-3' |
| | Reverse | 5'- GTAGCCCAGGATGCCCTTGA-3' |
| | Probe | 5'-TCCGACGCCTGCTTCACCACCTTCT-3' |
| Col III | Forward | 5'-GGATCTGTCCTTTGCGATGAC-3' |
| | Reverse | 5'-GCTGTGGGCATATTGCACAA-3' |
| | Probe | 5'-TGCCCCAACCCAGAGATCCCATTT-3' |
| Col I | Forward | 5'-GGGGTTGTCACTGTGAACTG-3' |
| | Reverse | 5'-CGGCTGCTCTAAATGATGAA-3' |
| | Probe | 5'-CGTGTCCTGACACAAATTGG-3' |
| POSTN | Forward | 5'-TGACTGGAAGAGCGGAGAGTACT-3' |
| | Reverse | 5'-GGTCTGACCTGTCTCCATGTTG-3' |
| | Probe | 5'-CTGCAACCTGGACGCCATCAAGG-3' |



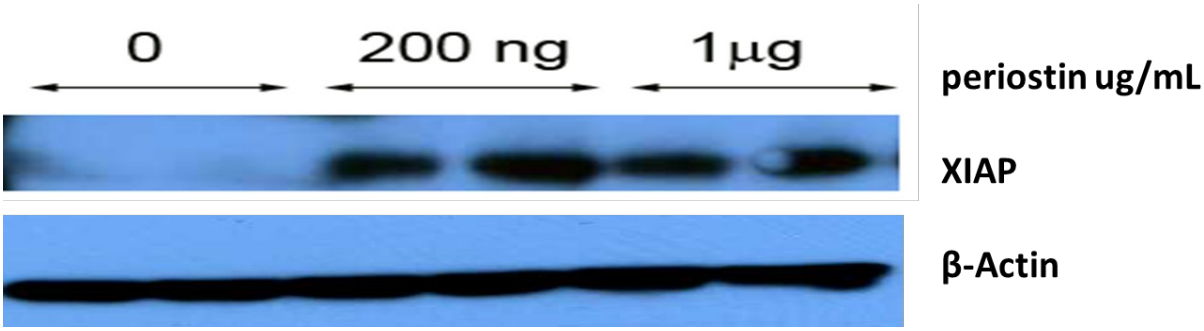

**Supplemental Figure 1- Exogenous periostin increase collagen expression in WT fibrocytes but these genes were not upregulated by bleomycin in the absence of periostin**

(A-B)WT mesenchymal cells were cultured in complete media for 14 days and fibrocytes (CD45+) were treated with 500ng/mL of mouse recombinant periostin. Total RNA was isolated and the mRNA expressions of Col I and III were assessed by real time RT-PCR. (C-E)Wild type or periostin$^{-/-}$ mice were given bleomycin intratracheally on day 0. On day 14 lungs were harvested and mesenchymal cells were cultured and sorted as previously described. Total RNA was isolated and by real time RT-PCR we measured the expression of Col I, α-SMA and Fibronectin (FN) in bleomycin treated fibrocytes. Values are expressed as mean ± SEM, and represent n=3 animals/group from two independent experiments *$p<0.05$.



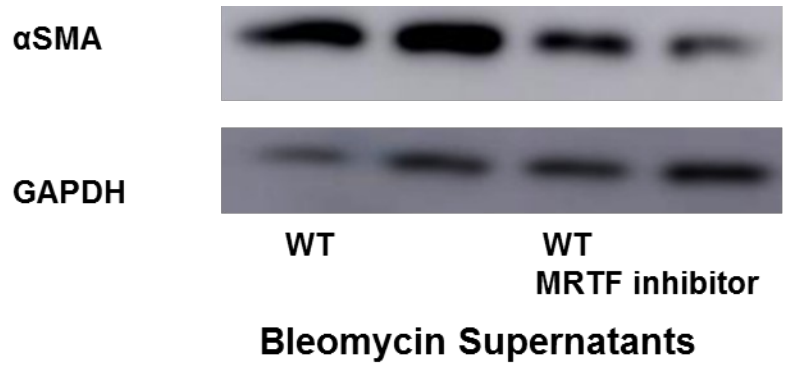

**Supplemental Figure 2- Integrin expression in fibroblasts post-bleomycin treatment**

(A-D) Wild type or periostin$^{-/-}$ mice were given bleomycin or saline intratracheally on day 0. On day 14 lungs were harvested and mesenchymal cells were cultured and sorted for CD45 negative fibroblasts as previously described. Total RNA was isolated and by real time RT-PCR we measured the expression of alpha1, alphaV, beta 1 and beta 5 expression and (E-H) surface expressions of alpha1, alphaV and beta 1 by flow cytometry assessing mean fluorescence intensity (MFI) of each integrin, comparing the change in intensity to cells from saline treated mice. Values are expressed as mean ± SEM, and represent n=3 animals/group from two independent experiments.

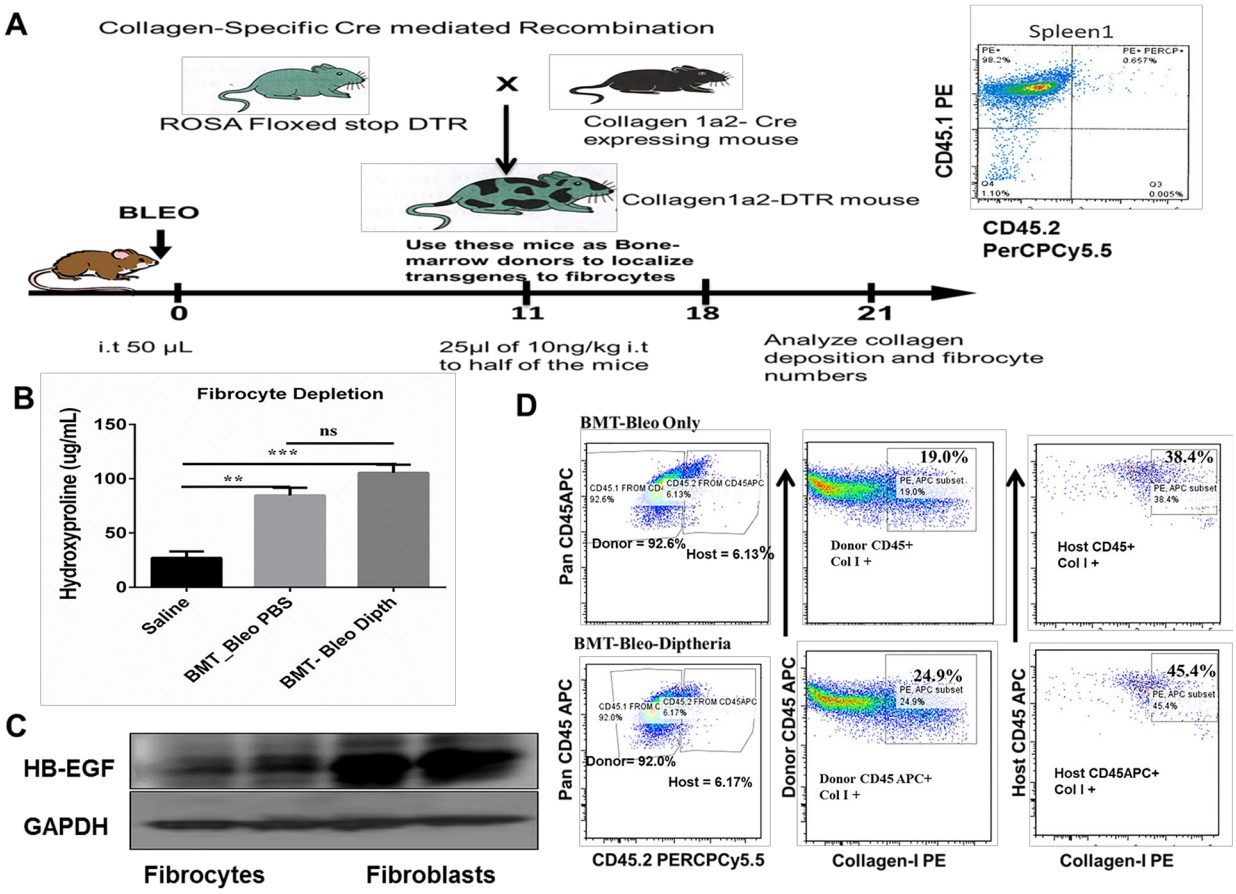



**Supplemental Figure 3 - Exogenous periostin treatment increased protein expression of XIAP in lung mesenchymal cells**

WT mesenchymal cells were cultured in complete media for 14 days. Cells were then plated in serum-free media where they were treated with different concentrations of mouse recombinant periostin. Cells were then lysed in RIPA buffer with protease inhibitor and immunoblotted for X-linked inhibitor of apoptosis (XIAP) and β-actin




**Supplemental Figure 4- Inhibition of CTGF expression in fibroblasts limits the upregulation of αSMA by WT fibrocyte supernatants**
WT mesenchymal cells were cultured in complete media for 14 days. Cells were then sorted in fibroblasts (CD45-) and fibrocytes (CD45+). Fibroblasts were plated in serum-free media overnight. Cell-free supernatants from fibrocytes from bleomycin- treated WT mice were added 1:1 onto WT untreated fibroblasts (CD45 negative) with or without 10μM MRTF inhibitor for 24h. Cells were lysed in RIPA buffer with protease inhibitor and we assessed the expression of αSMA and GAPDH by western blot. Data shown represent two different cultures for each condition.




**Supplemental Figure 5- Generation of a Col1a2-Cre DTR mouse to attempt to deplete fibrocytes**

**(A)**Rosa- DTR mice (Jackson labs) were crossed with Col1a2-Cre mice (a gift from Paul Noble's laboratory) to generate Col1a2-Cre-DTR-expressing mice. Cells from the spleen were assessed by flow cytometry to confirm that these mice were CD45.1 positive **(B)** Bone-marrow chimeras were generated using these Col1a2-Cre-DTR mice as donors into WT-C57BL/6 (CD45.2) mice. After 5 weeks, the bone-marrow transplantation mice were given intratracheal bleomycin 0.025U. 11 days post-bleomycin treatment, half of the mice were given 10 µg/kg diphtheria toxin in PBS intratracheally to deplete the bone-marrow derived DTR expressing cells that were recruited to the lungs. This dose was repeated on day 18. Lungs were harvested from these mice on day 21 post-bleomycin treatment and lung collagen content was assessed by hydroxyproline assay. **(C)** Lung mesenchymal cells were cultured for 14 days from the lung minces from the Col1a2-Cre-DTR-expressing mice (donor mice). Cells were then sorted in fibroblasts (CD45-) and fibrocytes (CD45+). Cells were lysed in RIPA buffer with protease inhibitor and we assessed the expression of for DTR expression using human HB-EGF antibody (BAF259 R&D systems) and GAPDH by western blot. **(D)** Lungs were harvested from the mice used in (B) on day 21 post-bleomycin treatment. Three lungs from each group were collagenase digested to generate single cell suspension of leukocytes. Cells were stained with a pan-CD45APC, CD45.2-PERCPCy5.5 and collagen-I- PE to assess the percentages of

donor derived vs host fibrocytes. For the flow cytometry analyses: Left most panels) cells were gated on pan CD45APC+, then from the CD45 + gate we looked for CD45.2PERCPCy5.5+ cells to indicate host-derived leukocytes. Cells that were pan CD45+, but negative for CD45.2 were considered donor-derived leukocytes. Gates show percentages of donor and host leukocytes present in each lung. Mice treated with bleomycin alone are the top dot plot in each panel while mice treated with bleomycin + diphtheria toxin are shown on bottom. Middle panels) To identify fibrocytes specifically, cells gated on donor or host CD45 expression were also analyzed for intracellular collagen 1 expression. Note that the donor-derived fibrocytes shown in the middle panels did not show a reduction in percentages with diphtheria toxin injection. We attribute this lack of DT-mediated fibrocyte depletion to the low level of DTR expression (HBEGF receptor) driven by the collagen 1 promoter in fibrocytes (panel C). Additionally, the right hand panels demonstrate that a significant percentage of fibrocytes present in the lung on day 21 post-bleomycin are derived from radio-resistant host cells. It should be noted that we also tried to deliver diphtheria toxin at doses of 50µg/kg and 10µg/kg intraperitoneally every 4 or 7 days to deplete fibrocytes. However, these doses caused significant mortality. The10 µg/kg dose delivered intratracheally as analyzed above was the only dose that did not kill the mice. However, as demonstrated, it was also not effective in depleting fibrocytes. Our conclusion is that this strategy to deplete fibrocytes in vivo is not viable for two reasons: 1) Low level expression of DTR on circulating donor-derived fibrocytes and 2) radio-resistant host-derived fibrocytes remain present and may expand.