SUPPLEMENTAL DATA

Inhibition of Pulmonary Fibrosis by CXCL10 Requires Glycosaminoglycan Binding and Syndecan-4

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SUPPLEMENTAL METHODS

Primary peritoneal macrophage. To elicit the accumulation of macrophages in the peritoneal cavity, syndecan-4-deficient or control C57BL/6J mice were injected intraperitoneally with 2 ml of 3% thioglycollate broth (Sigma-Aldrich). Three days after thioglycollate injection, inflammatory cells were recovered by peritoneal lavage with HBSS. The total cells were counted and stained with a neutrophil marker Gr-1 antibody. Gr-1 positive cells were determined with flow cytometry.

Effect of FNLP on inflammation and fibrosis. Leukocyte chemoattractant *N*-formyl-neoleucyl-leucyl-phenylalanine (FNLP, Sigma-Aldrich) was simultaneously instilled with bleomycin. Three days after the treatment, total BAL and differential cell counts were performed. Some mice were sacrificed 14 days after the treatment and collagen contents in the mouse lungs were determined with a conventional hydroxypropline assay.

Effect of CXCL10 on collagen production. NIH 3T3 fibroblasts were plated onto 24-well plates. Once the cells reach 80% confluency, the fibroblasts were starved over night and then were treated with either recombinant mouse CXCL10 (10 ng/ml) or TGFβ (1 ng/ml). The 24-hour conditioned media were collected and collagen I in conditioned media was determined with a collagen I direct ELISA. A collagen I direct ELISA assay was developed with a rabbit polyclonal antibody to collagen I (Abcam). In brief, the conditioned media and collage I standards (Sigma) were coated onto 96-well microtiter plates overnight. After washes and blocking, the antibody to collagen I (1:1000) was applied to the plates, followed by secondary antibody and color development.

SUPPLEMENTAL FIGURES

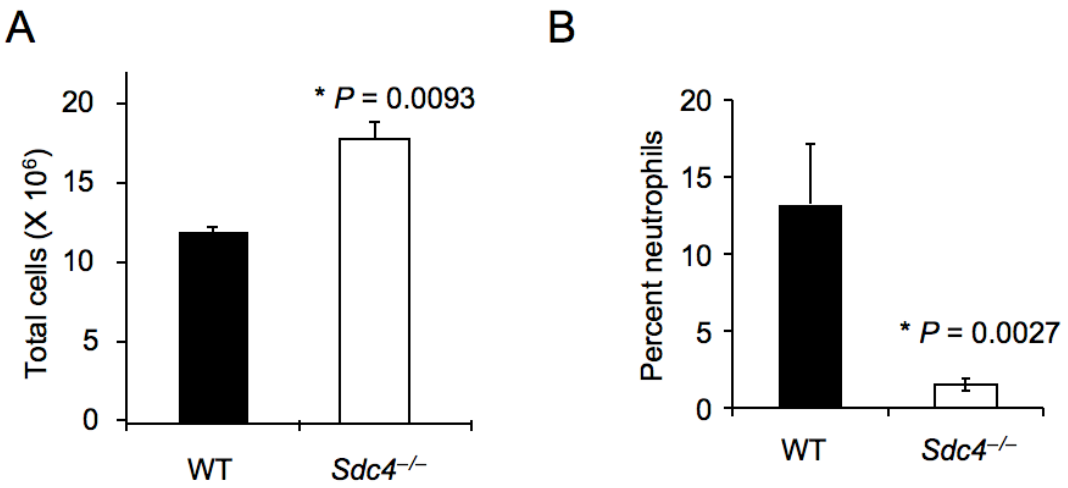

Figure S1. **Increased inflammatory cells in peritoneal cavity following thioglycollate instillation.** (A) Total inflammatory cells were lavaged from wild type and syndecan-4 deficient mice 3 days after thioglycollate injection (n = 6 - 7. P = 0.0093). (B) Percentages of neutrophils were determined by staining with Gr-1 antibody flow cytometrically (n = 6 - 7. P = 0.0027).

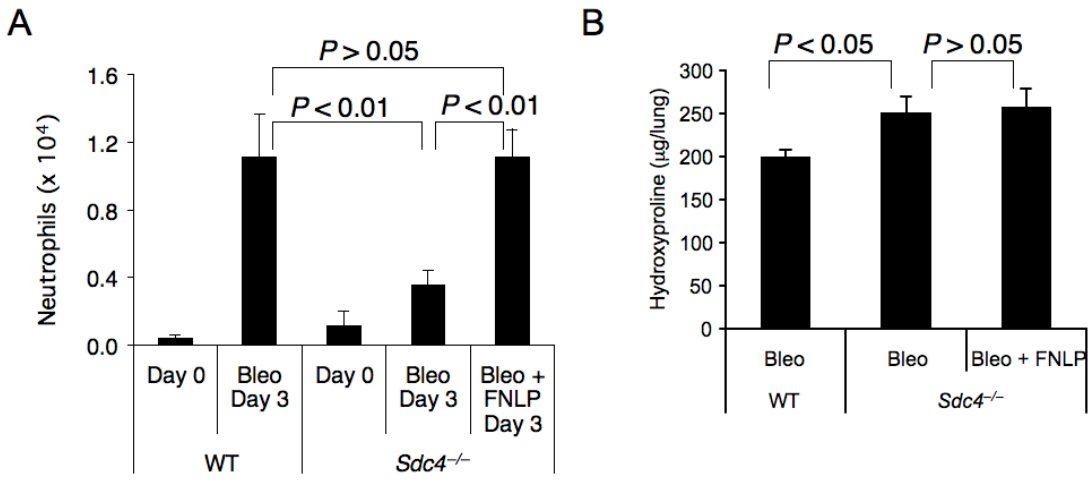

Figure S2. Restoring neutrophils with FNLP did not affect fibrogenesis in syndecan-4-deficient mice. (**A**) Bleomycin or in combination with N-formyl-neoleucyl-leucyl-phenylalanine (FNLP) were instilled to *Sdc4*$^{-/-}$ or wild type mice. Neutrophils in BAL collected from *Sdc4*$^{-/-}$ and WT mice 3 days following bleomycin-induced lung injury was counted (day 0, BAL from unchallenged mice; Bleo, bleomycin; Bleo + FNLP, bleomycin and FNLP were instilled; $n = 5$, P values are indicated). (**B**) FNLP treatment did not alter fibrotic formation of *Sdc4*$^{-/-}$ mice. Bleomycin or in combination with FNLP were instilled to *Sdc4*$^{-/-}$ or wild type mice. Hydroxyproline contents in the lung were measured 14 days post injury ($n = 3$ - 8, P values are indicated).

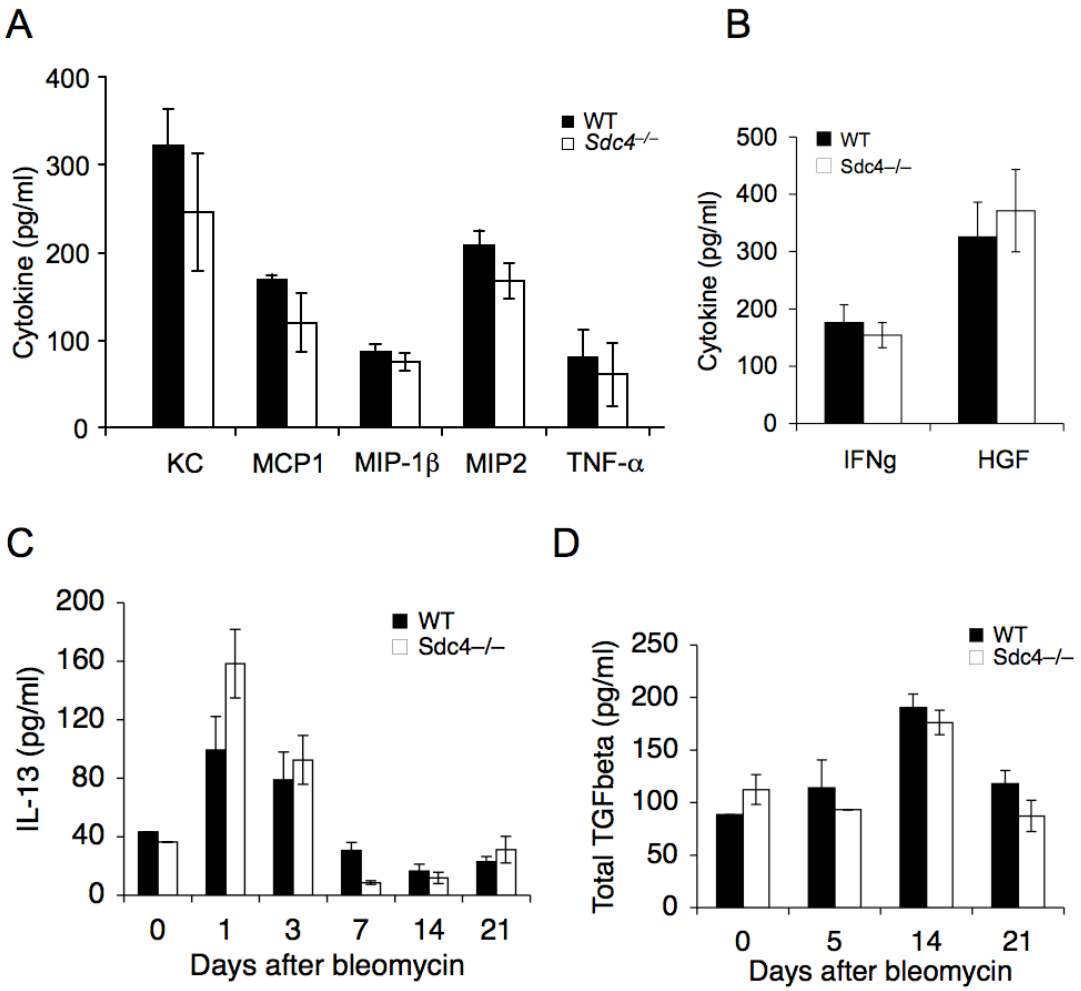

Figure S3. Cytokine expression in syndecan-4 deficient mice following bleomycin-induced lung injury. (A-B) Levels of cytokines KC, MIP-1β, MIP-2, and TNFα, IFN-γ, and HGF in BAL in syndecan-4-deficient and wild type 1 day (or 3 days for MCP-1) following bleomycin-induced lung injury were measured using ELISA (n = 4 - 5. P > 0.05 for all the chemokines measured). (C) IL-13 and (D) total TGFβ levels in BAL in syndecan-4-deficient and wild type mice after bleomycin over a 21-day period were measured using ELISA.

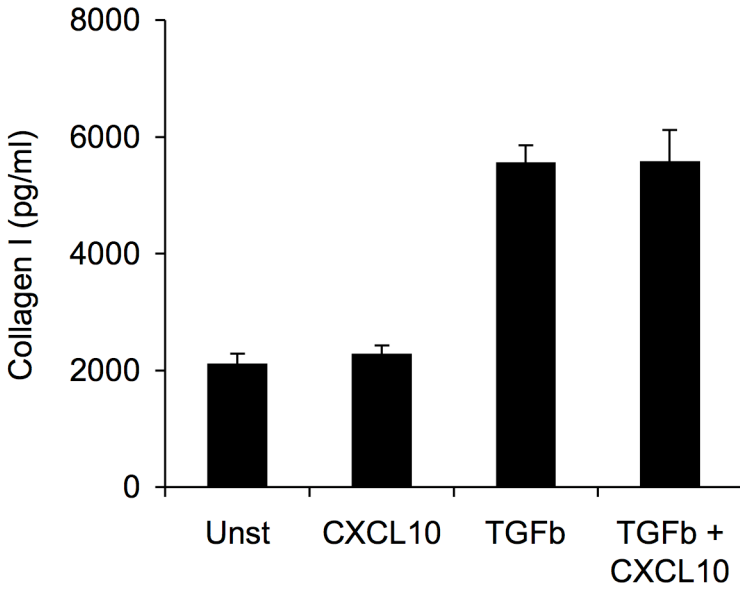

Figure S4. Effect of CXCL10 on collagen production. NIH 3T3 fibroblasts were plated onto 24-well plates. Once the cells reach 80% confluency, the fibroblasts were starved over night and then were treated with either recombinant mouse CXCL10 or TGFβ. The 24-hour conditioned media were collected and collagen I in conditioned media was determined with a collagen I direct ELISA (n = 4).

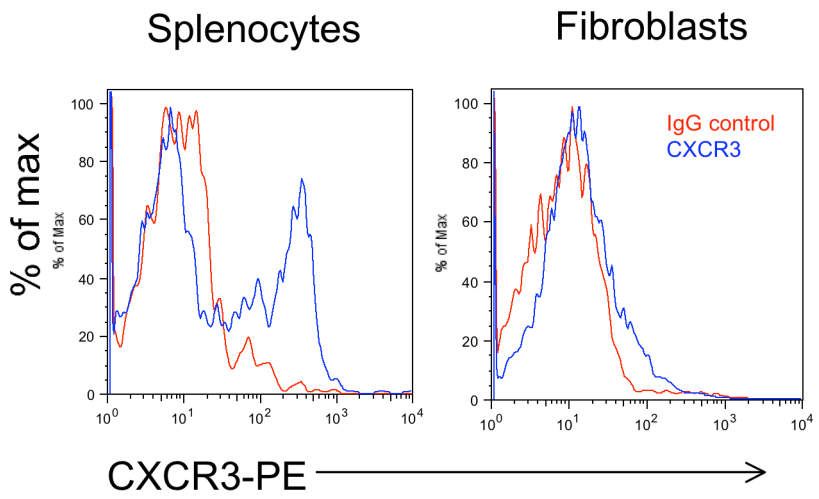

Figure S5. Fibroblasts do not express CXCR3. Primary mouse lung fibroblasts or splenocytes isolated from C57Bl/6 mice were stained with a PE conjugated CXCR3 antibody (R&D Systems) (blue lines) or isotype control IgG (red lines). Clearly, splenocytes express abundant CXCR3, while there is no CXCR3 staining on mouse lung fibroblasts.