Supplemental information

Attenuation of lung fibrosis in mice with clinically-relevant inhibitor of glutathione S-transferase pi

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Methods:

RT-qPCR:

Total RNA was extracted from mouse lungs with a QIAshredder and a RNeasy Mini kit according to the manufacturer's instructions (79656 and 74106; Qiagen, Valencia, CA). Total RNA concentrations were determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). cDNA was generated with the M-MLV reverse transcriptase system (M1701; Promega) and quantified via real-time PCR with SYBR® Select master mix (4472908; Applied Biosystems, Foster City, CA) and a C1000™ Thermal Cycler with a CFX96™ real-time PCR detection system (Bio-Rad, Hercules, CA). Primers were obtained from Integrated DNA Technologies (GSTP1 Fw: AGCAGGCATGCCACCATA, *Gstp1* Rv: GCTGCCCATACAGACAAGTG, *Col1a1* Fw: CACCCTCAAGAGCCTGAGTC, *Col1a1* Rv: AGACGGCTGAGTAGGGAACA, *Col5a1* Fw: GGTCCCTGACACACCTCAGT, *Col5a1* Rv: TGCTCCTCAGGAACCTCTGT, *Fn1* Fw: AATGGAAAAGGGGAATGGAC, *Fn1* Rv: CTCGGTTGTCCTTCTTGCTC, *Fsp1* Fw:AGGAGCTACTGACCAGGGAGCT, *Fsp1* Rv: TCATTGTCCCTGTTGCTGTCC).

Supplemental Figure Legends:

Figure S1 – Confirmation of GSTP absence in *Gstp*$^{-/-}$ mouse lungs. A) Detection of GSTP expression in untreated wild-type and *Gstp*$^{-/-}$ mouse lung homogenates by Western blot (3 per group). B) Detection of *Gstp1* mRNA expression by RT-qPCR. *$p<0.05$ by two-tailed unpaired Student's t-test, n = 4 per group. GSTP: Glutathione S Transferase P.

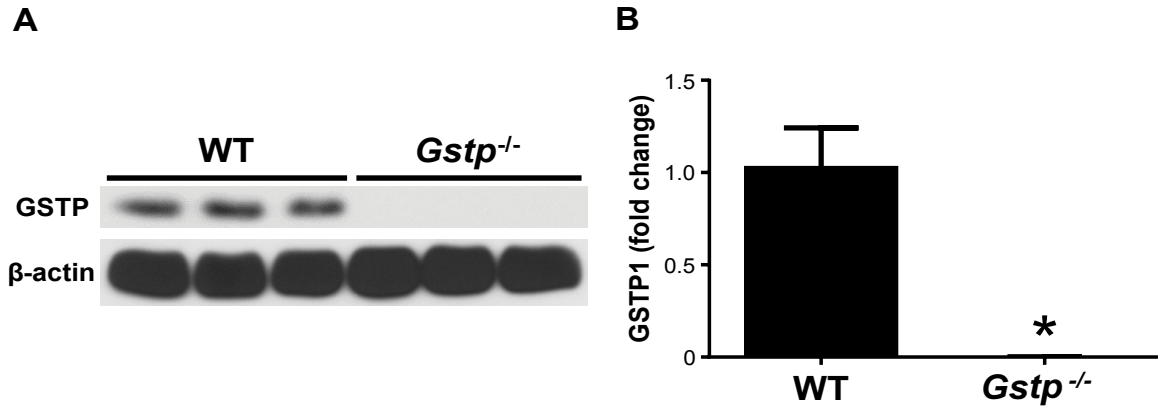
Figure S2 – *Gstp* deficiency attenuates collagen content and fibrotic remodeling 28 days post-bleomycin. Wild-type and *Gstp*$^{-/-}$ mice were treated with bleomycin for 28 days as previously indicated. A-B) Assessment of hydroxyproline and soluble collagen content. *$p<0.05$ relative to PBS group, †$p<0.05$ relative to respective wild-type group by two-way ANOVA with a Tukey posttest (n = 4-6 per group). C) Assessment of fibrotic remodeling by Masson's trichrome staining 15 days post-bleomycin administration. Scale bar = 100 μm. GSTP: Glutathione S Transferase P.

Figure S3 – GSTP deficiency attenuates bleomycin- and AdTGFβ-induced fibrotic marker expression in mouse lungs. Wild-type and *Gstp*$^{-/-}$ mice were treated with either bleomycin for 15 days (A) or AdTGFβ for 21 days (B). A) Measurement of bleomycin-induced *Col1a1*, *Col5a1*, *Fsp1* and *Fn1* mRNA expression in wild-type and *Gstp*$^{-/-}$ mouse lungs by RT-qPCR. B) Measurement of AdTGFβ-induced *Col1a1* mRNA expression in wild-type and *Gstp*$^{-/-}$ mouse

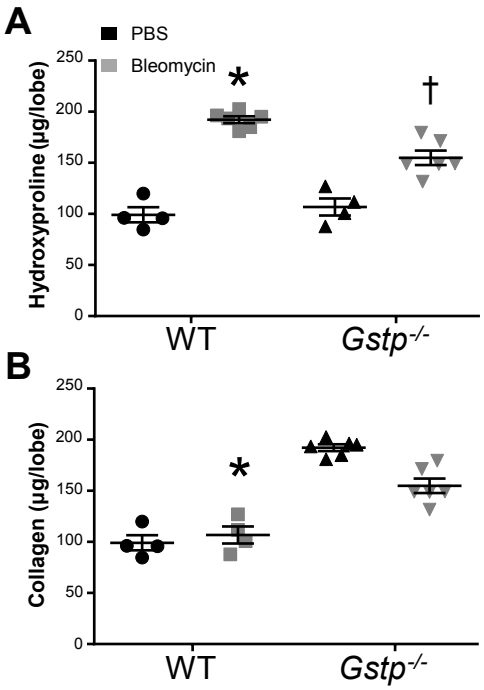
lungs by RT-qPCR. Shown are pooled data from two independent experiments. *p<0.05 relative to PBS/AdCtrl group, †p<0.05 relative to respective wild-type group by two-way ANOVA with a Tukey posttest (n = 6-11 per group). GSTP: glutathione S-transferase P, AdCtrl: control adenovirus, AdTGFβ: Adenovirus expressing recombinant active transforming growth factor beta-1, *Col1a1*: collagen type 1A1, *Col5a1*: collagen type 5A1, *Fsp1*: fibroblast-specific protein 1, *Fn1*: fibronectin.

Figure S4 – Confirmation of FAS reconstitution in *lpr* fibroblasts. Detection of FAS in *lpr* fibroblasts following transfection with either wild-type (WT) or C294A FAS by Western blot. Lpr: *Fas*-deficient, Fib: fibroblasts.

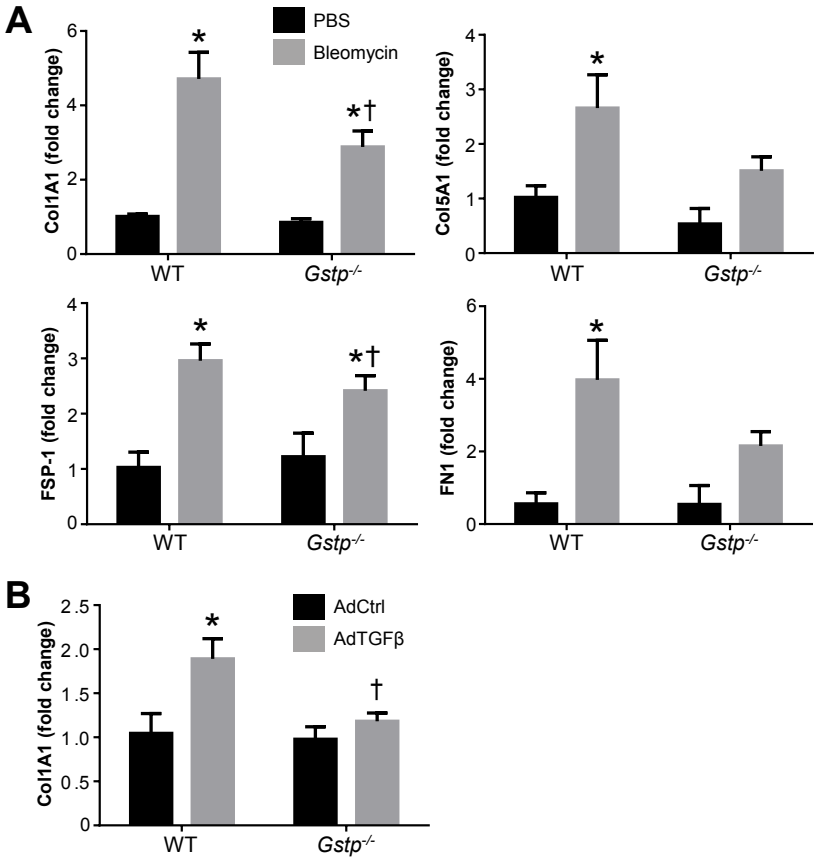
Supplemental figure 1



Supplemental figure 2



Supplemental figure 3



Supplemental figure 4