Supplemental information

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

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The authors have stated that no conflicts of interest exist
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: CTCGGTTGTCTCTTTTGCTC, Fsp1 Fw:AGGAGCTACTGACCAGGGAGCT, Fsp1 Rv: TCATTGTCCCTGTTGCTGCC).

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

A

WT | Gstp⁻/⁻
---|---
GSTP | 
β-actin | 

B

GSTP1 (fold change)

WT | Gstp⁻/⁻
---|---
0.0 | 1.5
0.5 | 1.0
1.0 | 0.5
1.5 | 0.0

*
Supplemental figure 2

A

Bleomycin PBS

WT

0

100

200

Hydroxyproline (μg/lobe)

50

150

250

Gstp-/-

B

Gstp-/-

Supplemental figure 2
Supplemental figure 3

A

Col1A1 (fold change)

WT   GSTP-1 (fold change)

PBS   Bleomycin

WT   GSTP-1 (fold change)

WT   GSTP-1 (fold change)

WT   GSTP-1 (fold change)

WT   GSTP-1 (fold change)

B

Col1A1 (fold change)

AdCtrl   AdTGFβ

WT   GSTP-1 (fold change)

WT   GSTP-1 (fold change)

WT   GSTP-1 (fold change)

WT   GSTP-1 (fold change)
### Supplemental figure 4

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[Image of Western blot showing Fas and β-actin]