Therapeutic Targeting of 15-PGDH in Murine Pulmonary Fibrosis

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Running title: PGDHi therapy for pulmonary fibrosis

Supplementary Information: Four supplemental figures accompany this paper

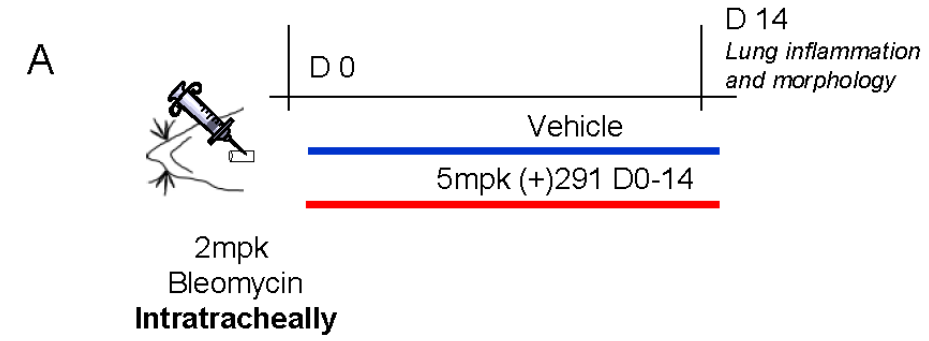
Supplemental Figure Legends:

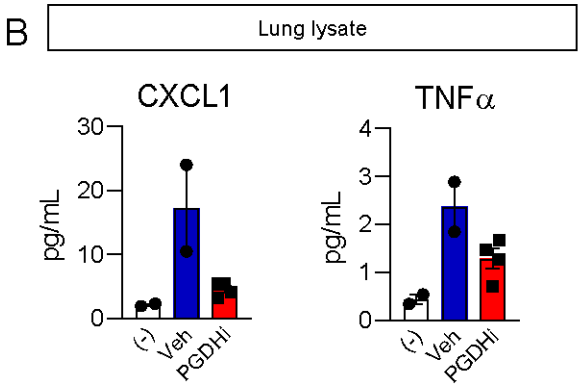
Supplementary Figure 1: PGDHi results in a trend towards mitigated pulmonary inflammation following intratracheal installation of bleomycin. (**A**) Schematic depicting intratracheal administration of 2mg/kg Bleomycin to 8wk old C57BL/6 female mice with subsequent PGDHi therapy (5mg/kg (+)SW033291, twice per day) and sacrifice at day 14. (**B**) Inflammatory factors CXCL1 and TNFα were measured in lung lysates of healthy naïve control (-), and Veh- and PGDHi-treated mice 14 days post-bleomycin administration by multiplex ELISA. Individual data points and mean±SEM are depicted; n=2-4 mice/group. (**C**) Representative images of Masson's trichrome-stained lung sections from healthy naïve control, and vehicle- and PGDHi-treated mice 14 days post-intratracheal bleomycin administration. 20X, scale bars represent 200μm.

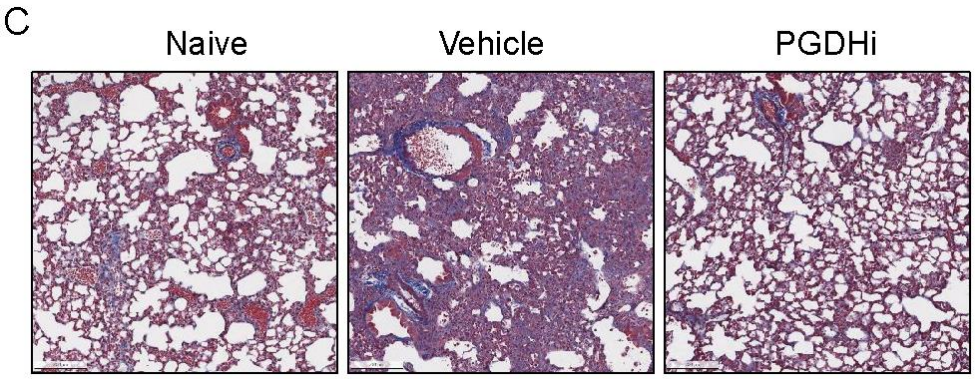
Supplementary Figure 2: PGDHi leads to improvements in overall health and survival of mice following intratracheal instillation of bleomycin. (**A**) Schematic depicting intratracheal administration of 2mg/kg Bleomycin to 8wk old C57BL/6 female mice with subsequent PGDHi therapy (5mg/kg (+)SW033291, twice per day) through day 35. (**B**) Body weight was measured for thirty-five days post-bleomycin administration as a percentage of day 0 weight for each mouse, treated as indicated. Mean±SEM is depicted. n=10 mice/group at the onset of the study, and n=5-7 mice/group at the end of the study. A two-way ANOVA with post-hoc Sidak's multiple comparisons test was used to compare vehicle- versus PGDHi-treated mice. (**C**) Kaplan-Meier survival curve following intratracheal bleomycin administration. N=25 mice per group.

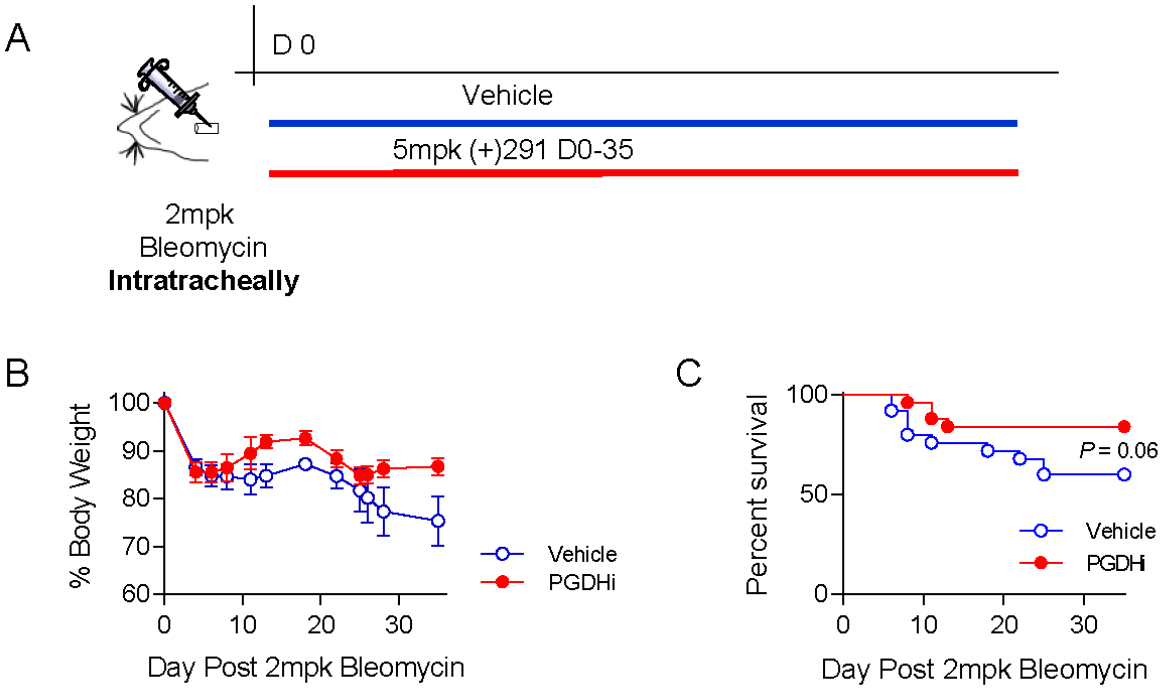
Supplementary Figure 3: Pulmonary 15-PGDH expression is maintained following bleomycin exposure. Representative images of 15-PGDH staining in lung sections from vehicle- and PGDHi-treated mice at days 7 and 35 post-bleomycin exposure.

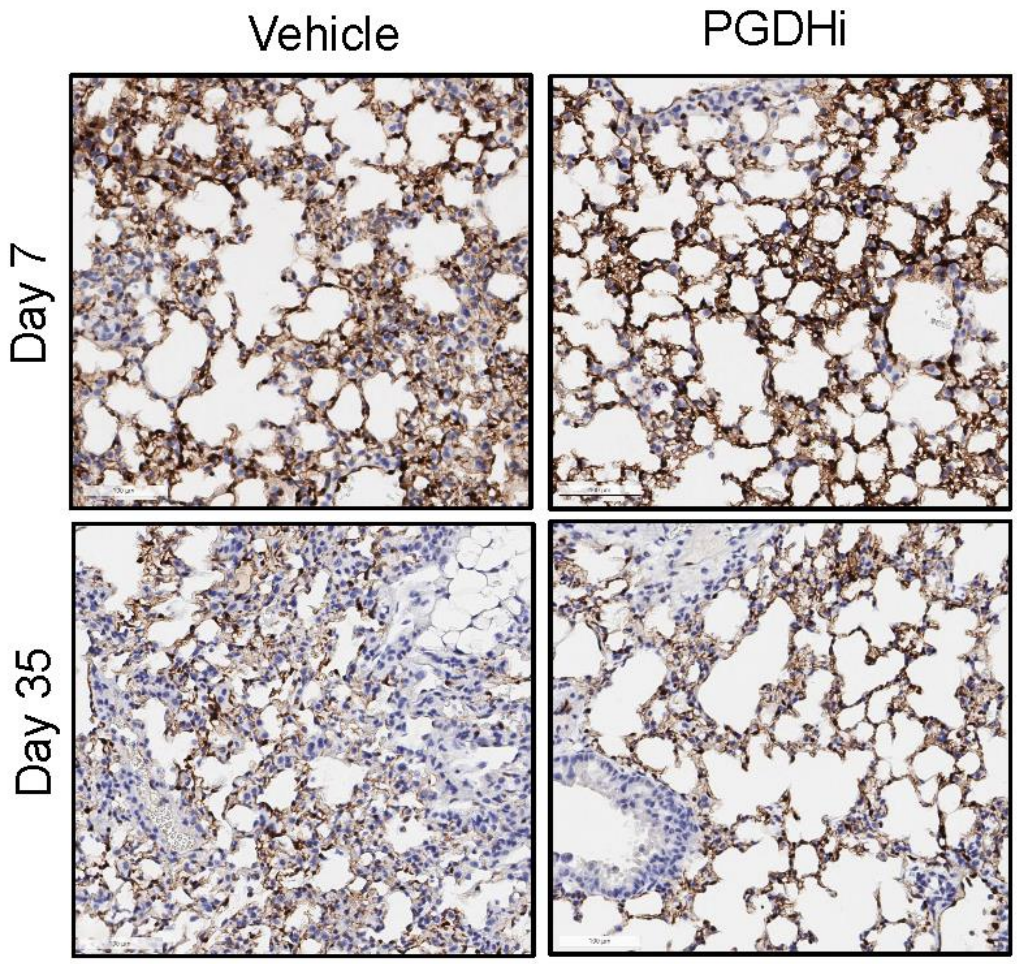
Supplementary Figure 4: PGDHi reduces fibrosis 35 days post-intratracheal instillation of bleomycin. (**A**) Schematic depicting intratracheal administration of 2mg/kg Bleomycin to 8wk old C57BL/6 female mice with subsequent PGDHi therapy (5mg/kg (+)SW033291, twice per day) through day 35. (**B**) Lung hydroxyproline levels were measured in tissue lysates from healthy naïve control (-), and Veh- and PGDHi-treated mice 35 days post-bleomycin administration. Individual data points and mean±SEM are depicted; n=15-20 experimental mice/group. An ordinary one-way ANOVA with post-hoc Tukey multiple comparisons test was used to compare groups. (**C**) Representative images of Masson's trichrome and elastin-stained lung sections from healthy naïve control, and vehicle- and PGDHi-treated mice 35 days post-intratracheal bleomycin administration. 20X, scale bars represent 200μm. *P=0.01, ***P=0.0004 for indicated comparisons.


A
D 0
D 14
Lung inflammation
and morphology
Vehicle
5mpk (+)291 D0-14
2mpk
Bleomycin
Intratracheally

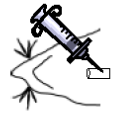

B
Lung lysate
CXCL1
TNFα
pg/mL
30
20
10
0
4
3
2
1
0
(-)
Veh
PGDHi

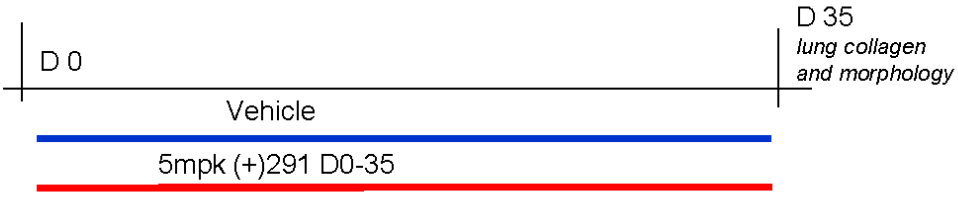

C
Naive
Vehicle
PGDHi


A
D 0
Vehicle
5mpk (+)291 D0-35
2mpk
Bleomycin
Intratracheally
B
% Body Weight
100
90
80
70
60
0
10
20
30
Day Post 2mpk Bleomycin
Vehicle
PGDHi
C
Percent survival
100
50
0
0
10
20
30
Day Post 2mpk Bleomycin
P = 0.06
Vehicle
PGDHi


Vehicle
PGDHi
Day 7
Day 35

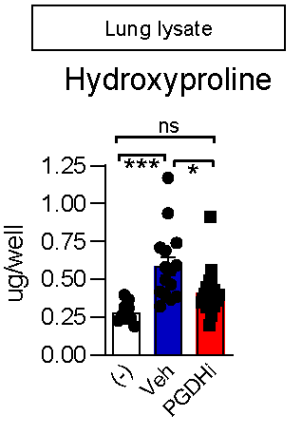
A





2mpk
Bleomycin
Intratracheally

B



C