Nintedanib does not improve lung function and fibrosis in a spirometry-confirmed and bleomycin-induced mouse model of IPF

Authors

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Background & Aim

Idiopathic pulmonary fibrosis (IPF) is a chronic and fatal interstitial lung disease, characterized by progressive lung fibrosis and declining pulmonary function. Nintedanib is currently one of only two drugs that are clinically approved for treating IPF patients.

The present study aimed to characterize Nintedanib in a bleomycin-induced (BLEO) and spirometry-confirmed mouse model of IPF.

Methods

Male C57BL/6JRj mice (16-19 weeks old) received a single intratracheal instillation of BLEO (2 mg/kg, 50 µL) or saline (CTRL). BLEO-IPF animals were randomized and stratified to treatment based on whole-body plethysmography (WBP; enhanced pause, PenH) and body weight on day 6 after BLEO administration. Nintedanib (50 mg/kg, PO or BID) or vehicle (PO or BID) was administered for 14 days (study 1) or 21 days (study 2), starting on day 7. See study outline in Figure 1.

Terminal pulmonary endpoints included spirometry (flexiVent), hydroxyproline (HP), Ashcroft score using Gubra Histopathological Objective Scoring Technique (GHOST), and quantitative histological markers of fibrosis (PSR, Col1a1, Col3), fibrogenesis (α-SMA), and inflammation (Gal-3). Terminal plasma exposure of Nintedanib and liver RNA sequencing was determined in study 2.

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1 Study outline

Figure 1. Study outline and group overview

2 Randomization

Figure 2. Baseline randomization. (A) WBP (PenH). **(B)** Body weight. One-way ANOVA with Dunnett's test for multiple comparisons or Kruskal-Wallis test with Dunn's test for multiple comparisons. ***p<0.001 vs. BLEO-IPF Vehicle

3 Metabolic and biochemical parameters
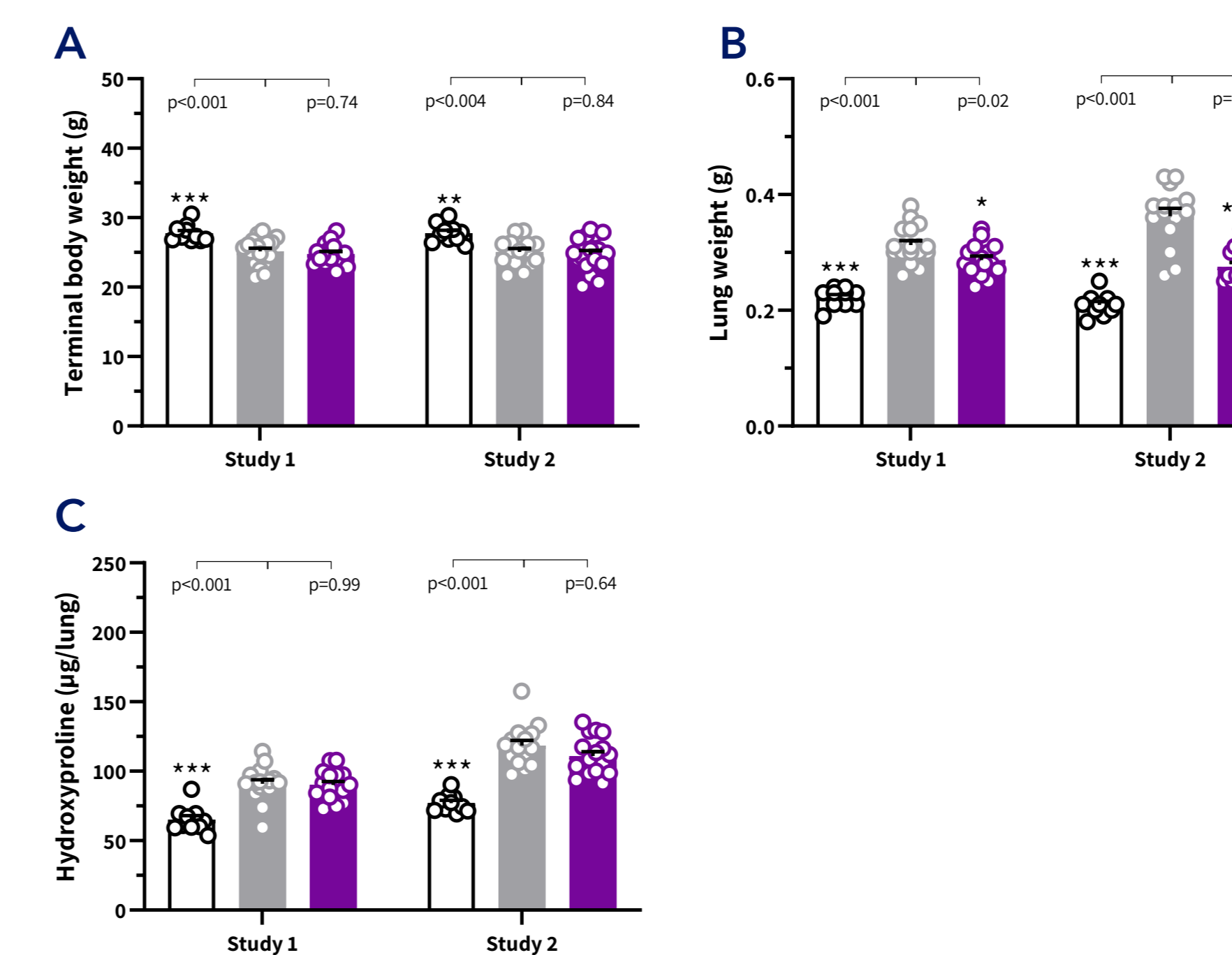
Figure 3. Terminal metabolic and biochemical parameters. (A) Body weight (g). **(B)** Lung weight (g). **(C)** Lung total hydroxyproline (HP). One-way ANOVA with Dunnett's test for multiple comparisons or Kruskal-Wallis test with Dunn's test for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001 vs. BLEO-IPF Vehicle.

4 Spirometry

Figure 4. Spirometry. (A) Forced expiratory volume in 0.1 seconds (FEV0.1). **(B)** Forced vital capacity (FVC). **(C)** Inspiratory capacity (IC). **(D)** Static compliance. One-way ANOVA with Dunnett's test for multiple comparisons or Kruskal-Wallis test with Dunn's test for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001 vs. BLEO-IPF Vehicle
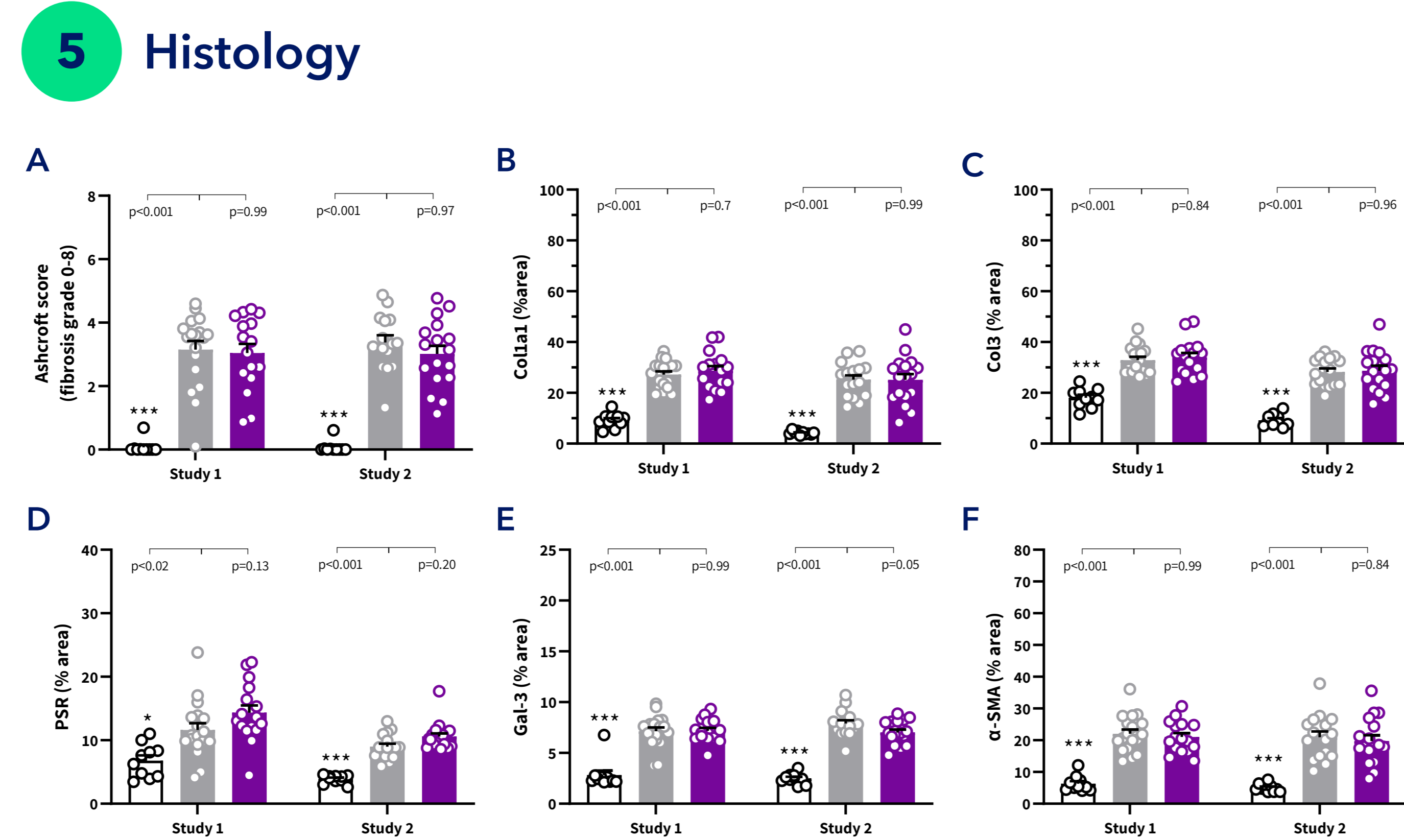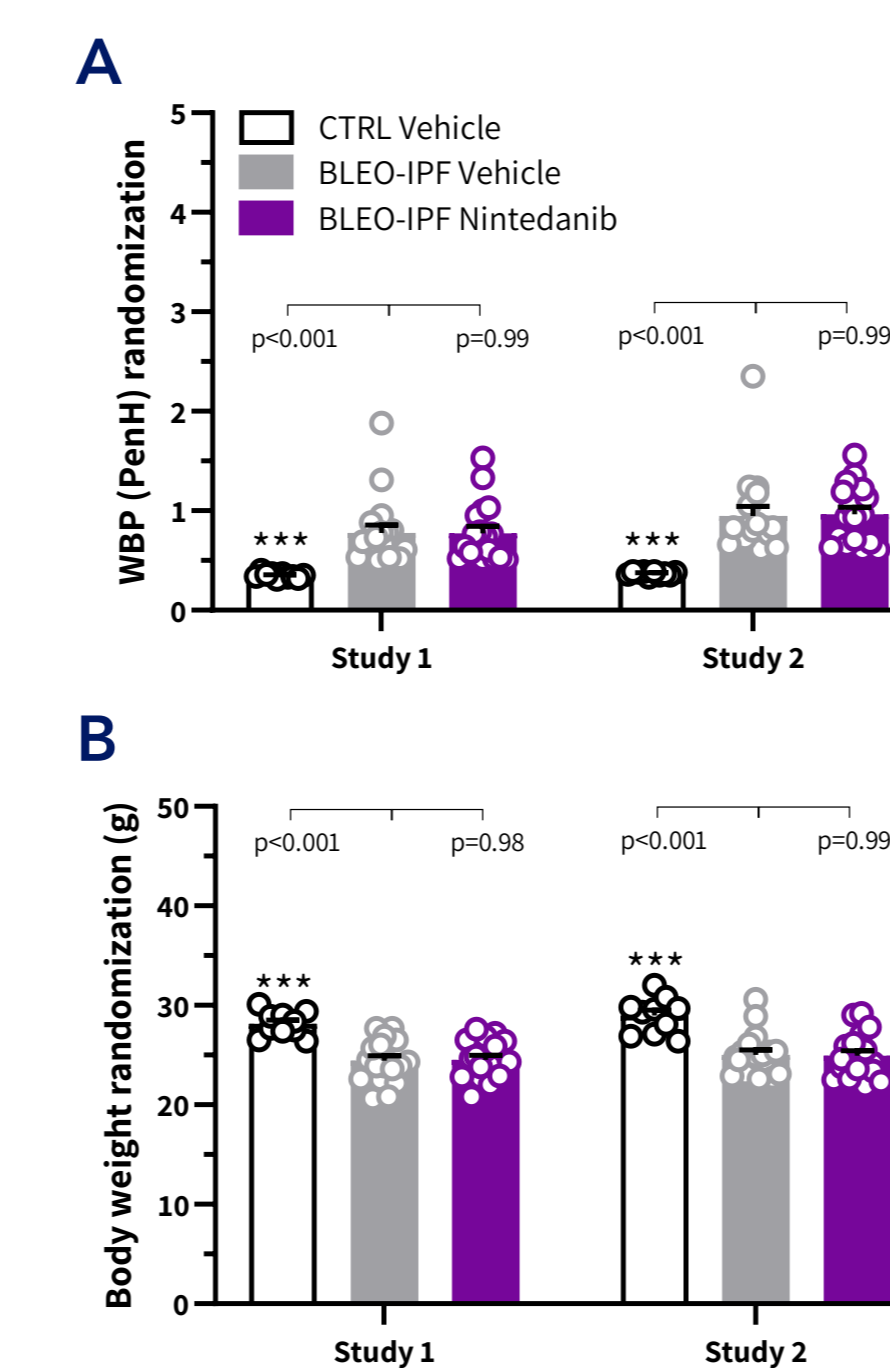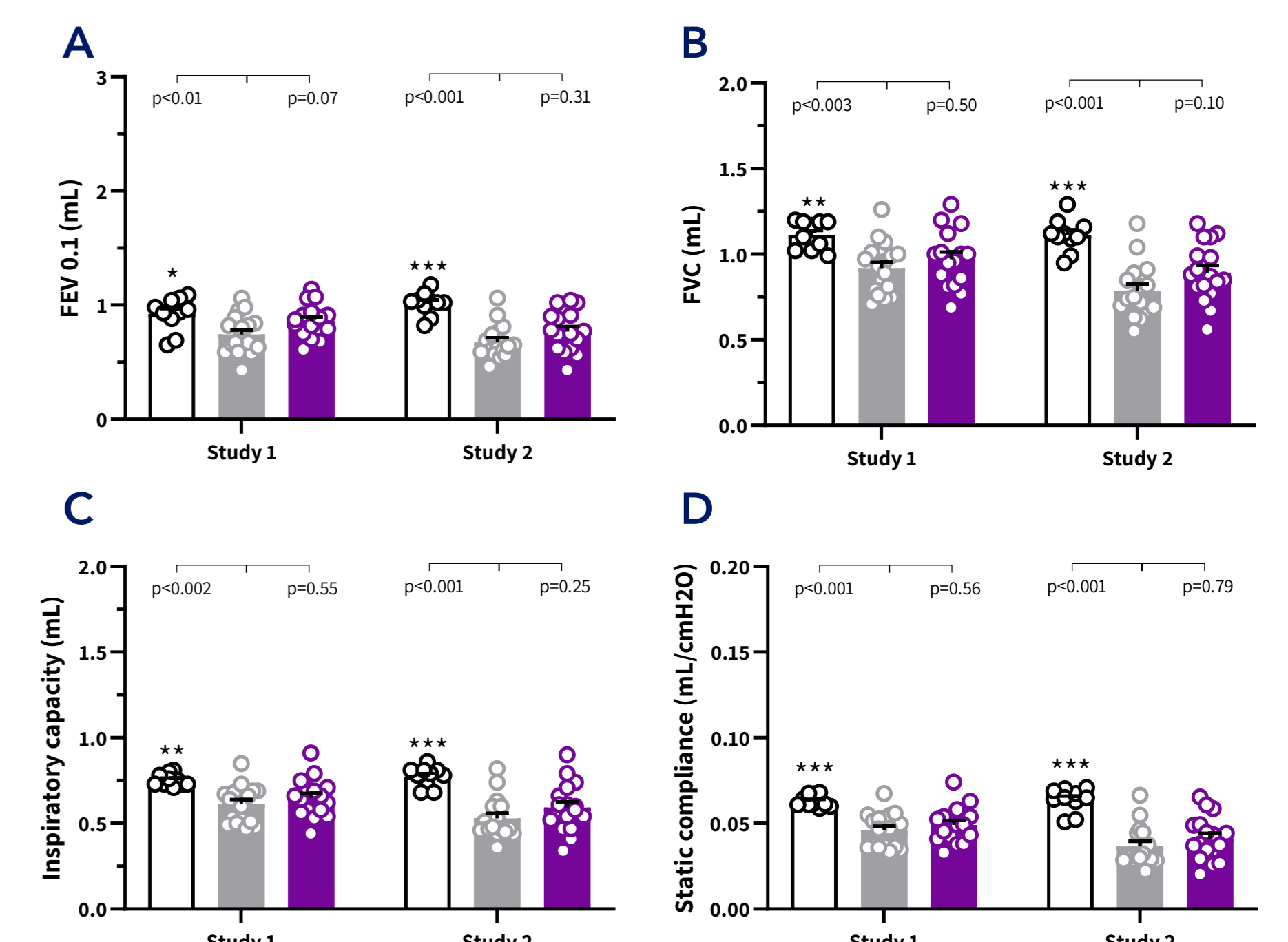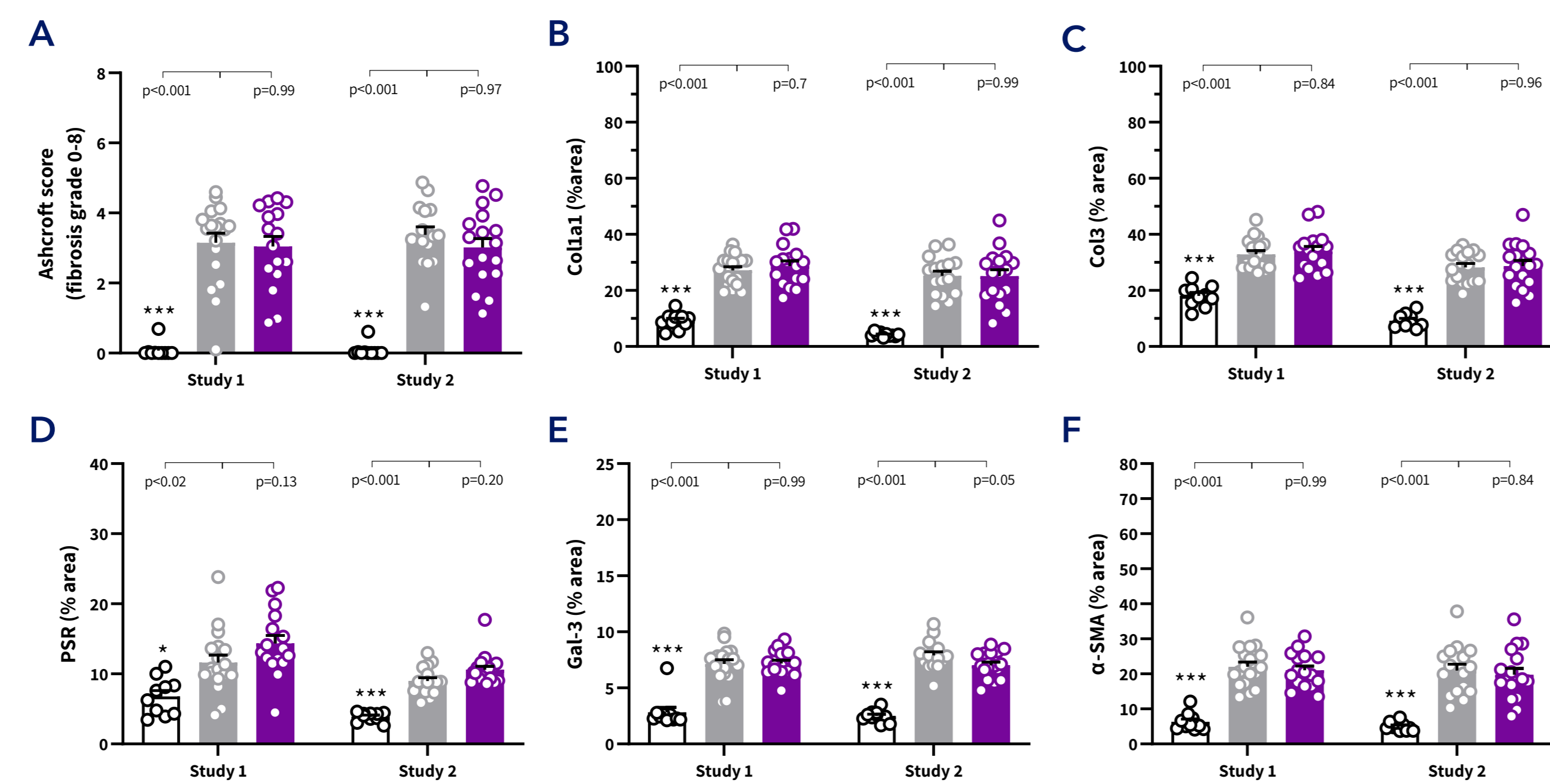
5 Histology

Figure 5. Lung histology. Histomorphometric assessments were performed by conventional image analysis. Quantitative data were calculated as proportionate (%) area of histological staining (mean ± SEM). **(A)** GHOST-based Ashcroft score. **(B)** Collagen-1α1. **(C)** Collagen-3. **(D)** PSR-stained fibers. **(E)** Galectin-3. **(F)** α-smooth muscle actin (α-SMA). One-way ANOVA with Dunnett's test for multiple comparisons or Kruskal-Wallis test with Dunn's test for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001 vs. corresponding BLEO-IPF Vehicle group.
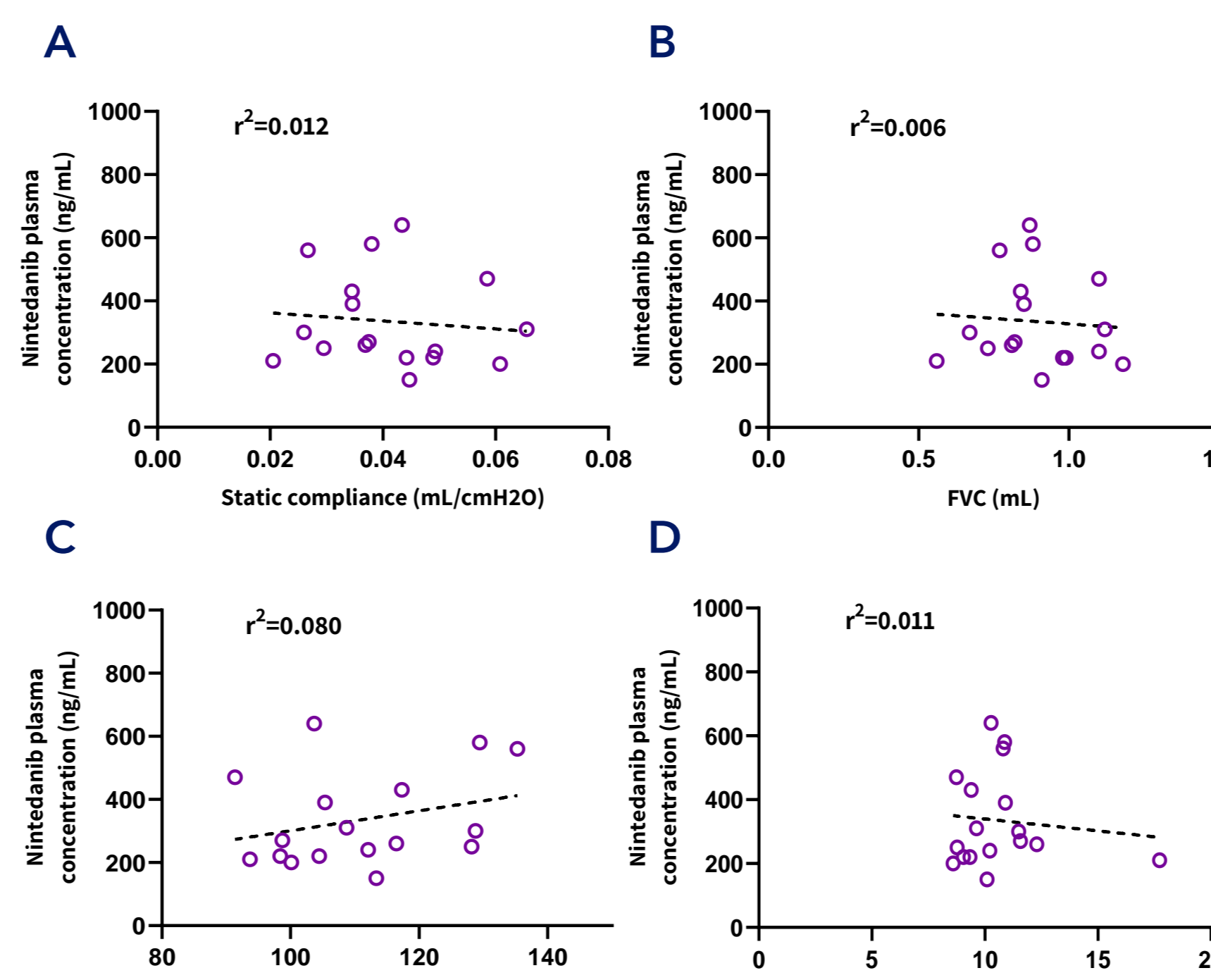
6 Nintedanib exposure

Figure 6. Nintedanib exposure. Terminal plasma concentrations of Nintedanib (measured 2.5 hours after last dosing) vs. **(A)** Static compliance, **(B)** FVC, **(C)** Total lung HP levels, and **(D)** %-area of PSR staining. Simple linear regression analysis.
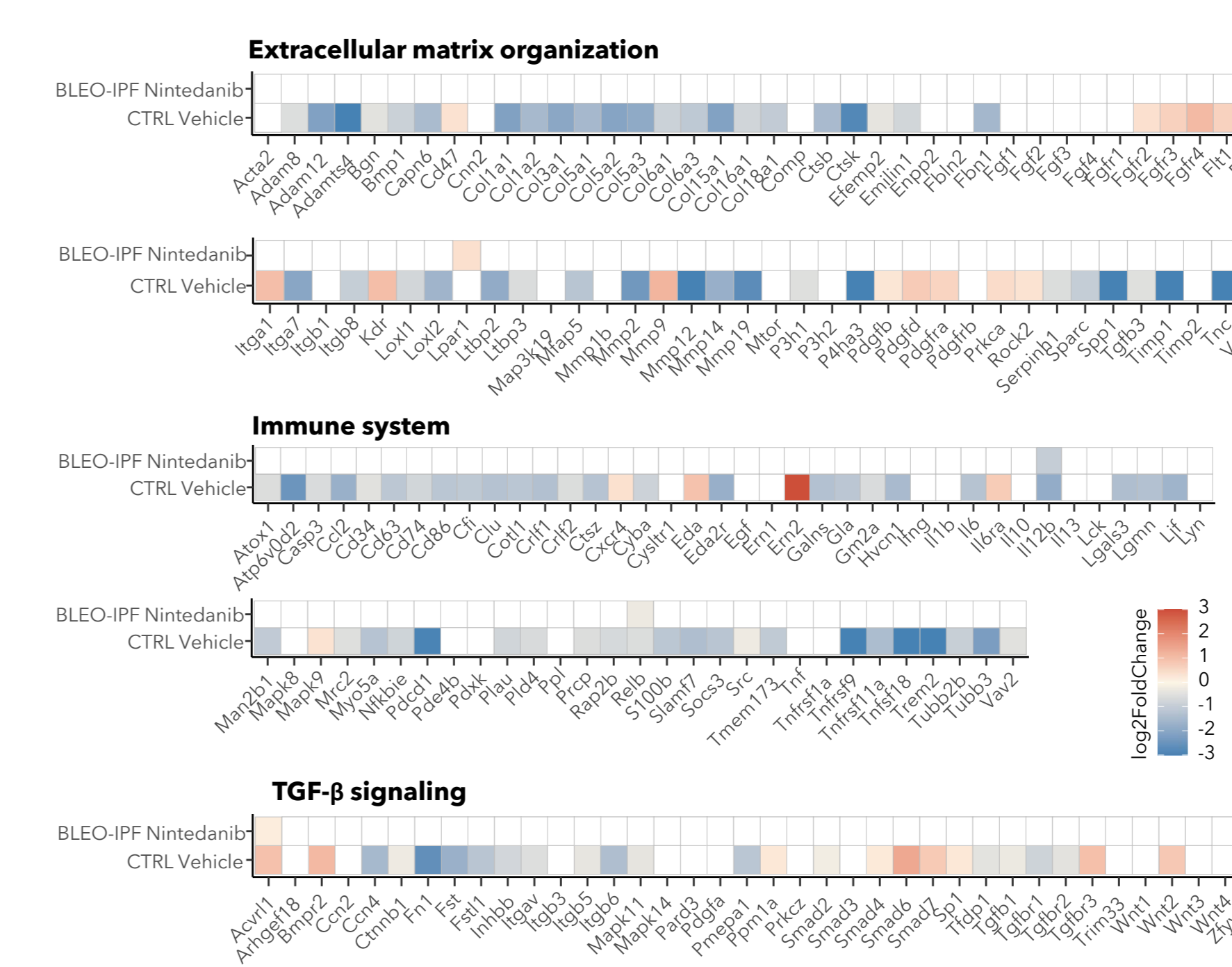
7 Lung transcriptome profiling

Figure 7. Lung transcriptome signatures. Heatmap on gene candidate markers of Extracellular matrix organization, Immune system and TGF-β signalling (log2-fold change vs. BLEO-IPF Vehicle, p_{adj}<0.05 after correcting for multiple testing. CTRL Vehicle, n=6; BLEO-IPF Vehicle, n=10; BLEO-IPF Nintedanib, n=12). Red and blue colours indicate up- and down-regulation as compared to BLEO-IPF Vehicle. Unregulated genes are indicated in white. Compared to BLEO-IPF Vehicle mice, the total number of differentially expressed genes was 6,294 (CTRL Vehicle mice) and 305 (BLEO-IPF Nintedanib mice).

Conclusion

+ Nintedanib significantly reduces lung weight in BLEO-IPF mice

+ Nintedanib has no effect of lung functional outcomes in BLEO-IPF mice

+ Nintedanib has no effect on lung HP levels in BLEO-IPF mice

+ Nintedanib has no effect on histological and transcriptional markers of fibrosis, fibrogenesis and inflammation in BLEO-IPF mice

+ Nintedanib plasma exposure shows no correlation to key endpoints in BLEO-IPF mice, including static compliance, FVC, lung HP and %-area of PSR

+ Nintedanib is not an applicable reference drug in BLEO-IPF mouse studies

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