

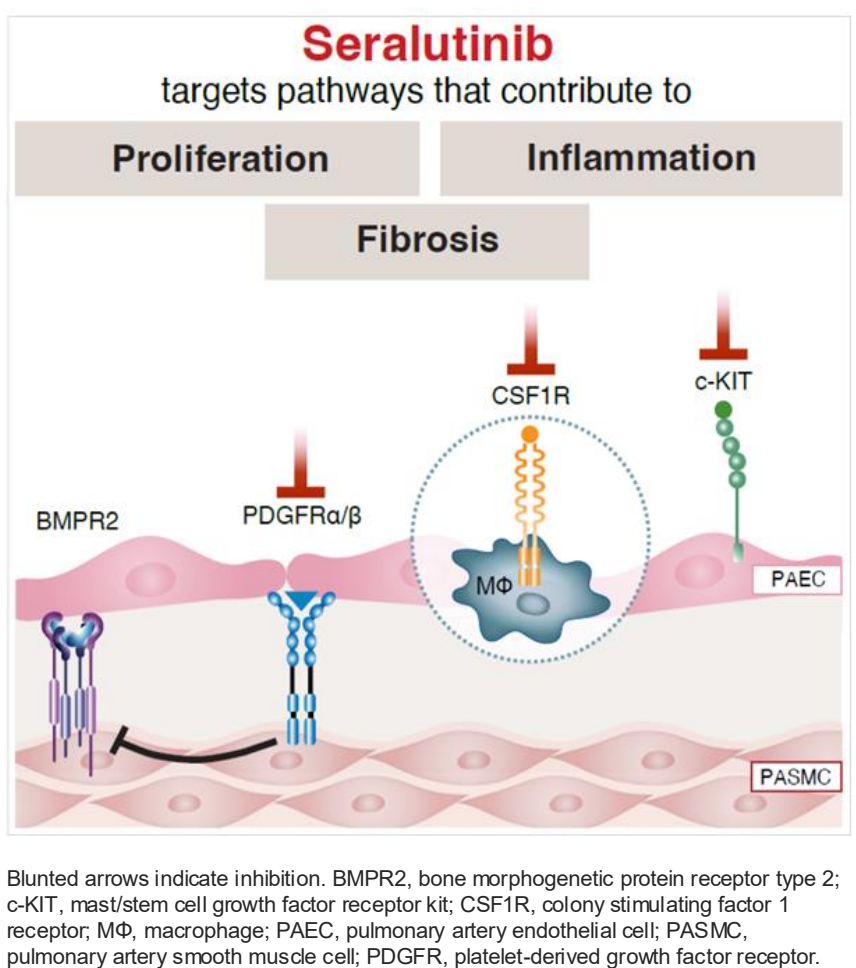
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## BACKGROUND

- Fibrosis contributes to both pulmonary vascular and parenchymal remodeling in pulmonary hypertension (PH) and interstitial lung disease (ILD)<sup>1</sup>
- Seralutinib is a potent, inhaled PDGFR $\alpha/\beta$ , CSF1R, and c-KIT kinase inhibitor in clinical development for pulmonary arterial hypertension (PAH) and PH-ILD<sup>2,3</sup> (Figure 1)
- In the phase 2 TORREY study (NCT04456998) in patients with Group 1 PH, seralutinib decreased circulating fibrotic biomarkers, including collagen I $\alpha$ 1<sup>4,5</sup>
- We examined whether seralutinib inhibits established fibrosis in idiopathic pulmonary fibrosis (IPF) patient-derived precision-cut lung slices (PCLS) and human lung fibroblasts (HLF)

Figure 1. Seralutinib mechanism of action

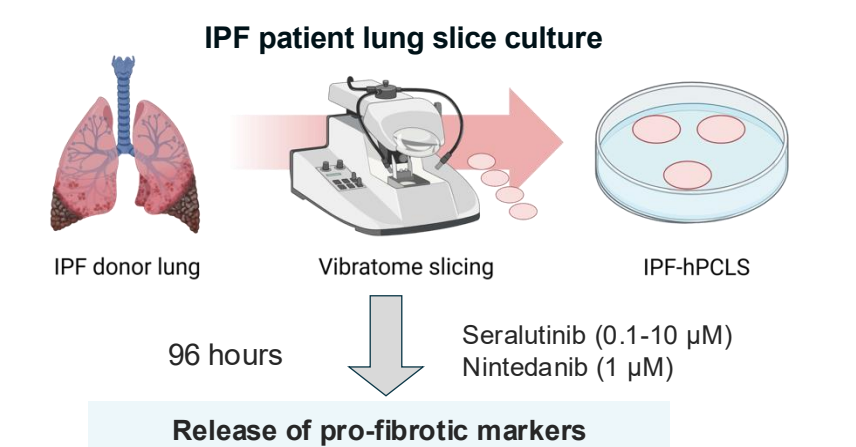


## METHODS

### Effect of seralutinib on release of pro-fibrotic markers from IPF human (h) PCLS cultures

- PCLS from two IPF donors were cultured and treated for 4 d with vehicle, seralutinib (0.1-10  $\mu$ M), or nintedanib (1  $\mu$ M)<sup>6</sup>
- Supernatants were collected at 96 h and pro-fibrotic markers evaluated using a custom Luminex panel.
- Cytotoxicity was assessed with an adenylate kinase assay

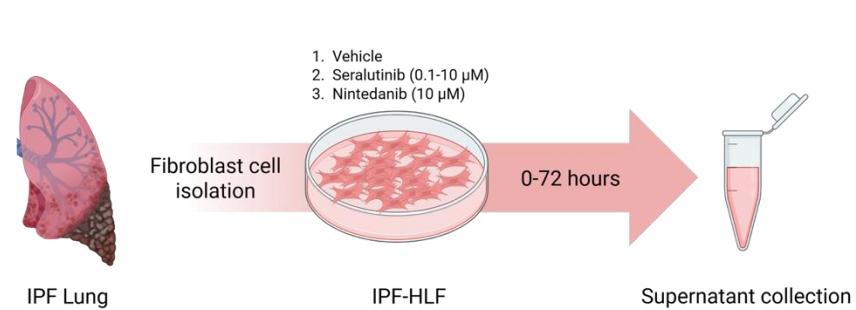
Figure 2. IPF-hPCLS experimental setup



### Effect of seralutinib on release of pro-fibrotic markers in IPF-HLFs

- IPF-HLF from three independent donors were treated with vehicle, seralutinib (0.1-10  $\mu$ M), or nintedanib (10  $\mu$ M) for 24, 48, and 72 hours
- Supernatants were collected at each timepoint and pro-fibrotic markers evaluated with a custom Luminex panel

Figure 3. IPF-HLF experimental setup



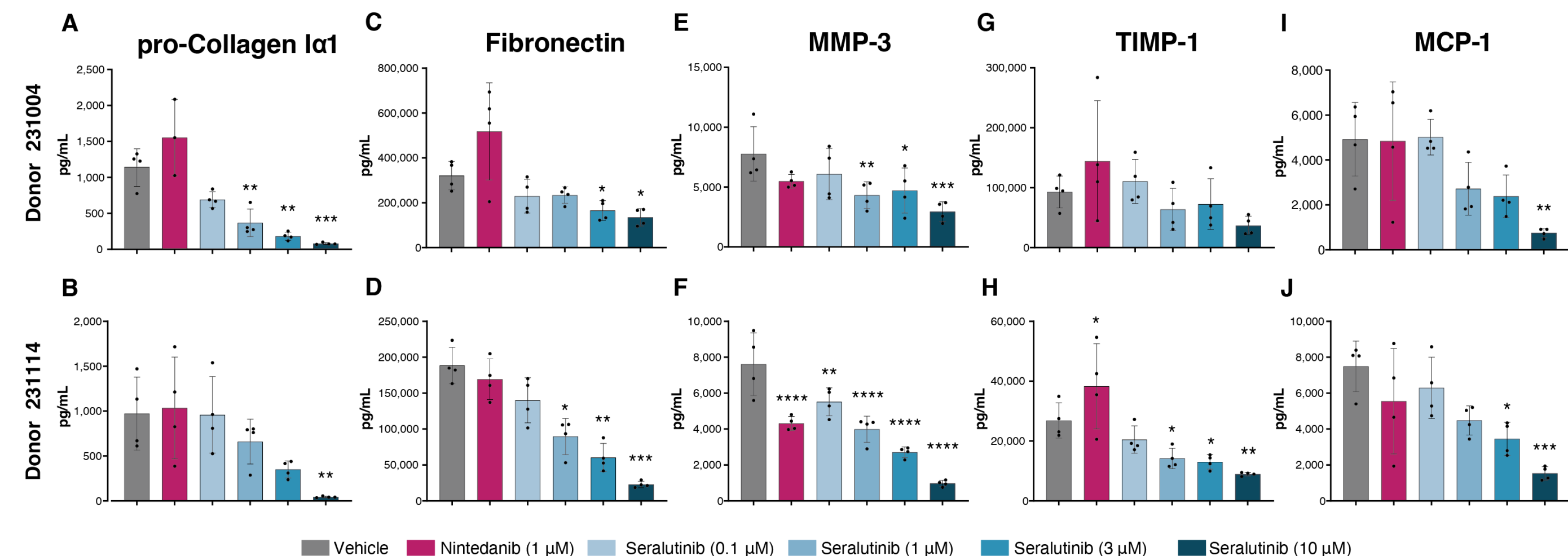
### Effect of seralutinib on fibroblast-to-myofibroblast transition in IPF-HLFs

- IPF-HLF were pre-treated with seralutinib (0.1-10  $\mu$ M) or nintedanib (3  $\mu$ M) and stimulated with 10 ng/mL TGF- $\beta$  for 72 hours
- Alpha-smooth muscle actin ( $\alpha$ -SMA) gene expression levels were determined by quantitative PCR and protein expression was assessed by immunofluorescent staining

PCR, polymerase chain reaction. Figures created with BioRender.

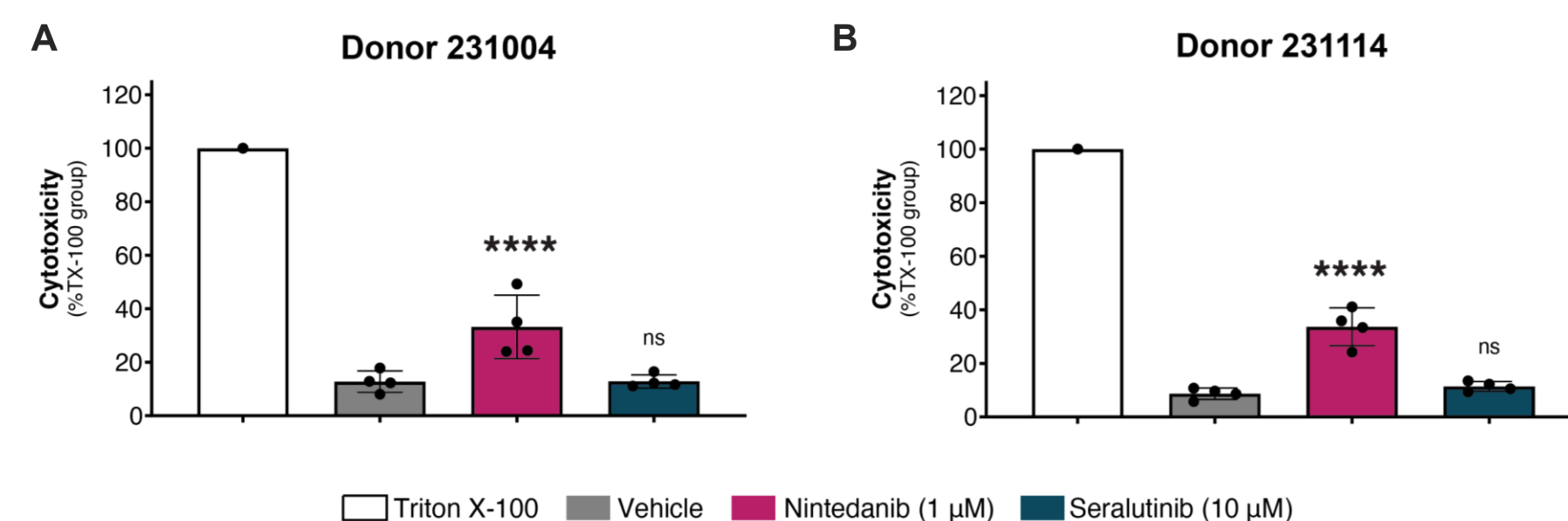
## RESULTS

Figure 4. Seralutinib inhibited release of pro-fibrotic markers in IPF-hPCLS



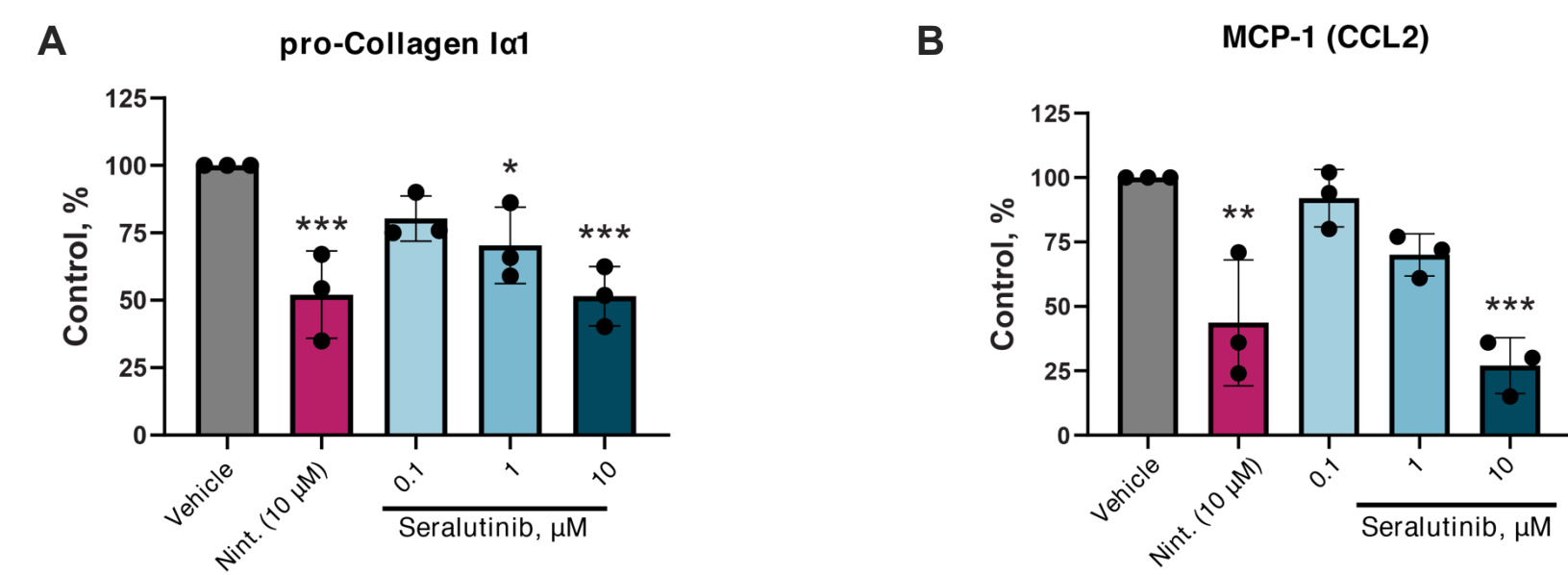
Graphs depict effect of vehicle control, seralutinib, and nintedanib on the release of pro-fibrotic markers pro-collagen I $\alpha$ 1 (A,B), fibronectin (C,D), MMP-3 (E,F), TIMP-1 (G,H), and MCP-1 (I,J) secreted by IPF-hPCLS at 96 h for both donors tested. Data are expressed as mean  $\pm$  SEM (n=4). Statistical analysis was performed using one-way ANOVA with Fisher's LSD Test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, and \*\*\*\*p<0.0001 versus vehicle.

Figure 5. Seralutinib did not induce cytotoxicity at clinically relevant concentrations in IPF-hPCLS



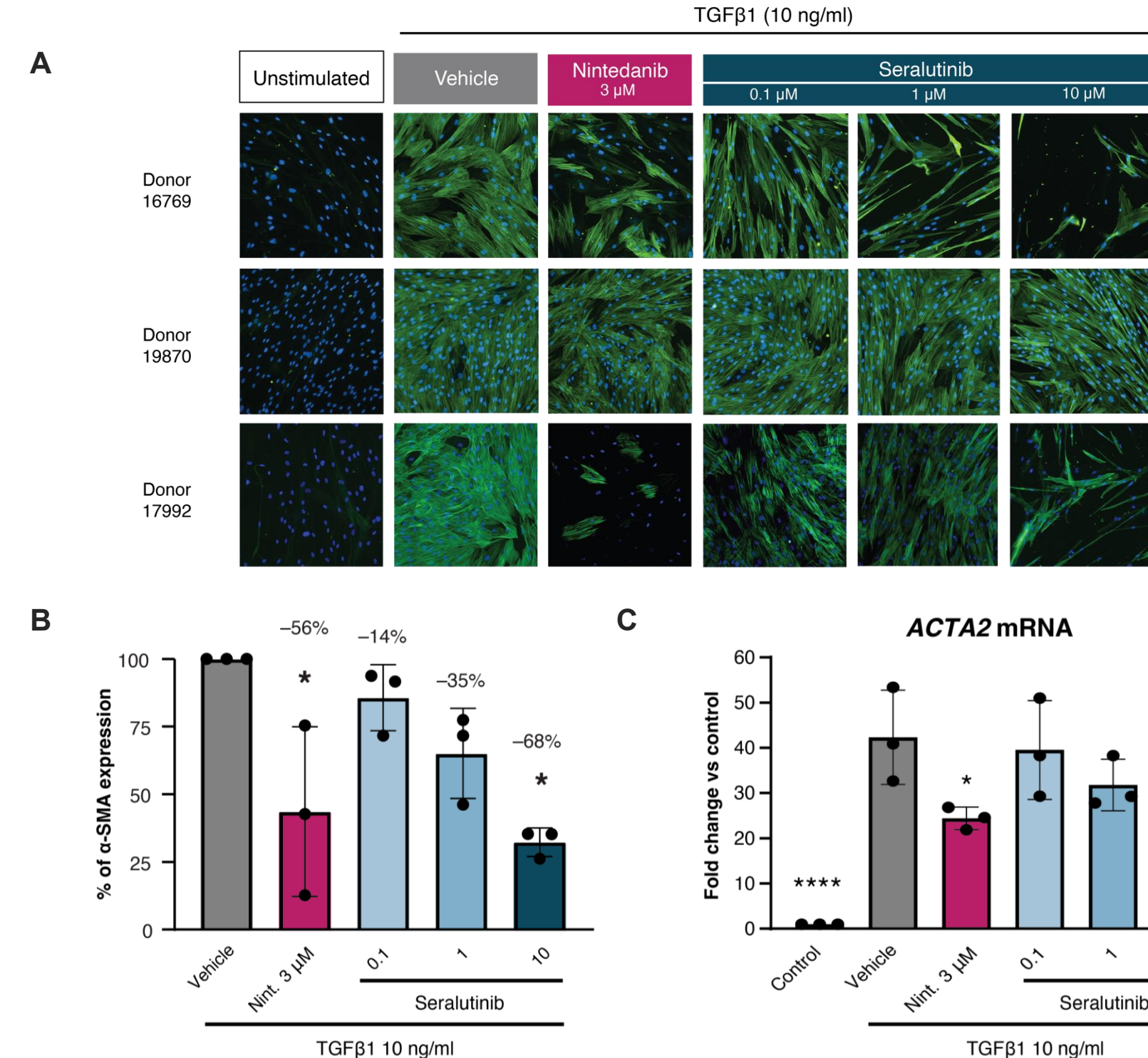
Change in adenylate kinase levels, a measurement of cytotoxicity, at 96 h for both donors (A,B). Data are expressed as mean  $\pm$  SEM (n=4). Triton X-100 was used as a positive control for cytotoxicity in this assay. Statistical analysis was performed using one-way ANOVA with Fisher's LSD Test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, and \*\*\*\*p<0.0001 versus vehicle.

Figure 6. Seralutinib dose-dependently decreased release of fibrotic markers from IPF-HLFs



Measured release of (A) pro-collagen I $\alpha$ 1 and (B) MCP-1 in supernatant of IPF-HLF cells averaged and normalized. Data are expressed as mean  $\pm$  SEM (n=3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, and \*\*\*\*p<0.0001 versus vehicle at 72 h. Nint., Nintedanib.

Figure 7. Seralutinib inhibited TGF- $\beta$ -induced  $\alpha$ -SMA in IPF-HLFs



(A) Immunofluorescence imaging and (B) quantification of  $\alpha$ -SMA levels. Seralutinib reduced amount of TGF- $\beta$ -induced  $\alpha$ -SMA expressed at clinically relevant doses in IPF-HLF cells. (C) Change in gene expression levels of *ACTA2* (smooth muscle actin gene) in IPF-HLF cells. Gene upregulated due to TGF- $\beta$  stimulation and dose-dependent inhibition by seralutinib. Data are expressed as mean  $\pm$  SEM (n=3 donors). Statistical analysis was performed using one-way ANOVA Dunnett's multiple comparisons test. \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001 versus vehicle.

## CONCLUSIONS

- Seralutinib dose-dependently decreased pro-collagen I $\alpha$ 1, fibronectin, MMP-3, TIMP-1, and MCP-1 in IPF-hPCLS, with no cytotoxicity observed
- In IPF-HLFs, seralutinib significantly inhibited the release of pro-collagen I $\alpha$ 1, MCP-1, and TGF- $\beta$ -induced  $\alpha$ -SMA expression in a dose-dependent manner
- These data support investigation of seralutinib as a potential inhaled treatment option for diseases characterized by underlying pulmonary vascular and interstitial fibrosis

References: 1 Dhont S, et al. *ERJ Open Res.* 2022;8(4):00272-2022. 2 Galkin A, et al. *Eur Respir J.* 2022;60(6):2102356. 3 Pullamsetti SS, et al. *Int J Mol Sci.* 2023;24(16):12653. 4 Ghofrani HA, et al. *Am J Respir Crit Care Med.* 2024;209:A7383. 5 Osterhout R, et al. *Eur Respir J.* 2024;64:OA1872. 6 Marimoutou M, et al. *Toxics.* 2024;12(9):637.

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