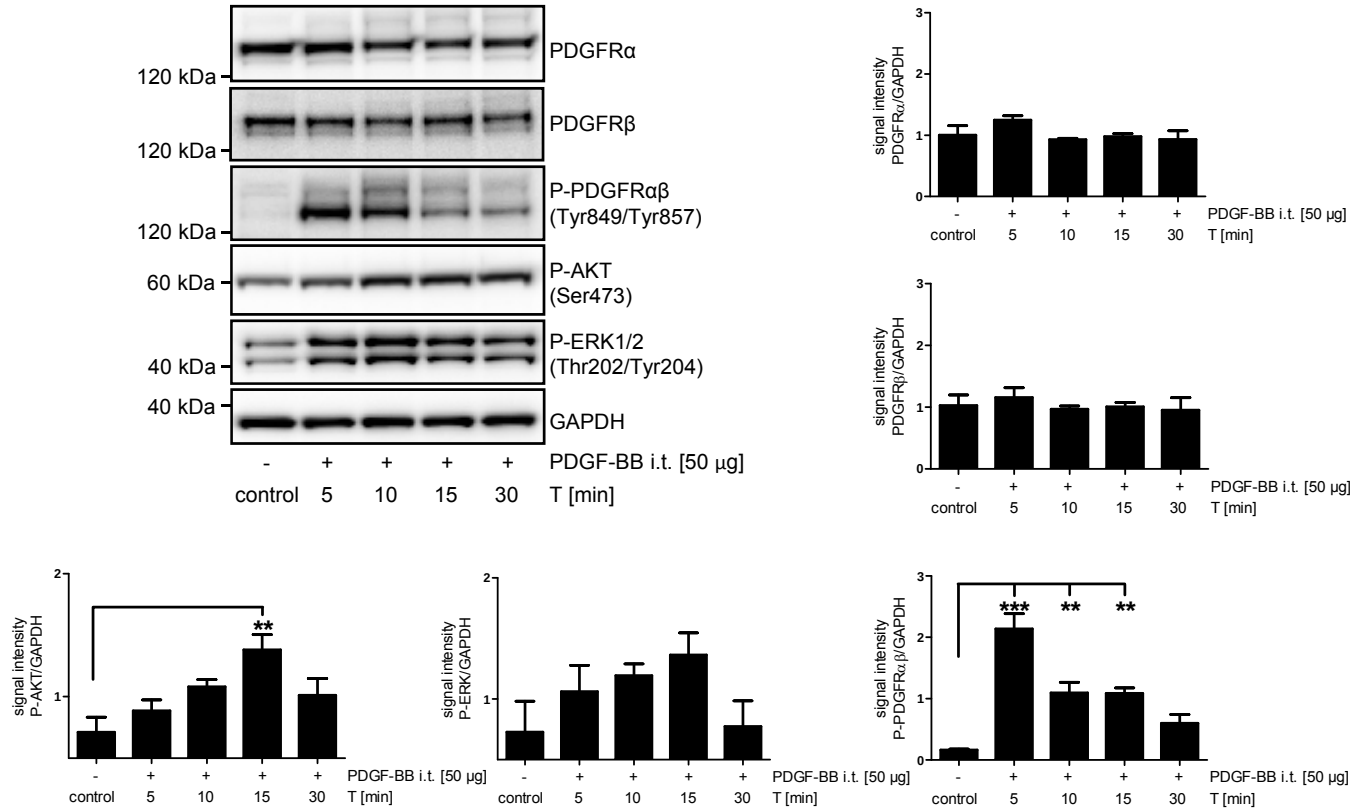
Anti-fibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor, nintedanib, in experimental models of lung fibrosis
Lutz Wollin, Isabelle Maillet, Valérie Quesniaux, Alexander Holweg, Bernhard Ryffel
Target Journal: The Journal of Pharmacology and Experimental Therapeutics



Supplementary figure 1 Time course of PDGF-BB-stimulated expression of PDGFRα and β, phosphorylated PDGFRs and downstream P-AKT and P-ERK1/2.

To identify the time of maximum activation of the PDGF receptors upon stimulation with exogenous PDGF-BB *in vivo*, C57BL/6 mice were stimulated with vehicle or 50 µg PDGF-BB by intratracheal instillation to induce the phosphorylation of the PDGF receptors (n = 4 per group). Mice were euthanized at different times (5, 10, 15 and 30 min) after PDGF-BB application and total lungs were lysed and used for Western blot analysis to detect total and phosphorylated PDGF receptors, as well as the phosphorylation of the downstream signaling molecules AKT and ERK1/2 by specific antibodies. An antibody directed against GAPDH was used as a control. One representative Western blot showing 1 animal from each group is depicted for each antibody analyzed. The relative signal intensity of the bands was evaluated by densitometry and corrected by the signal intensity of the control. Data are presented as mean ± S.E.M of 4 animals per group; Statistical analysis was performed in Prism Graph Pad using one-way ANOVA combined with a Dunnett's multiple comparison test, ** $P < 0.01$, *** $P < 0.001$.

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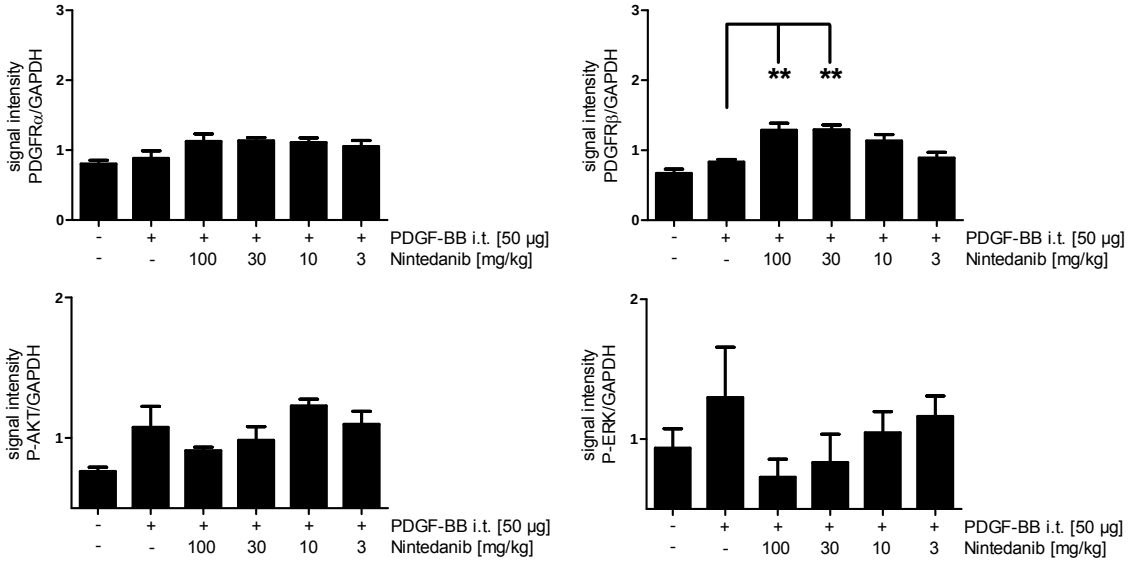

Supplementary figure 2 Effect of nintedanib on PDGF-BB-stimulated expression of PDGFRα and β and downstream P-AKT and P-ERK-1/2.

To address the pharmacological inhibition of the PDGF receptors by nintedanib *in vivo*, C57BL/6 mice received vehicle or nintedanib at 3, 10, 30 and 100 mg/kg by gavage (n=4 per group). Two hours after dosing, the mice were stimulated with vehicle or 50 µg PDGF-BB by intratracheal instillation to induce the phosphorylation of the PDGF receptors. Mice were euthanized 5 minutes after PDGF-BB application and total lungs were lysed and used for Western blot analysis to detect total PDGF receptors, as well as the phosphorylation of the downstream signaling molecules AKT and ERK1/2 by specific antibodies. The relative signal intensity of the bands was evaluated by densitometry and corrected by the signal intensity of the control. Data are presented as mean ± S.E.M of 3 animals per group. Statistical analysis was performed in Prism Graph Pad using one-way ANOVA combined with a Dunnett's multiple comparison test * $P < 0.05$.

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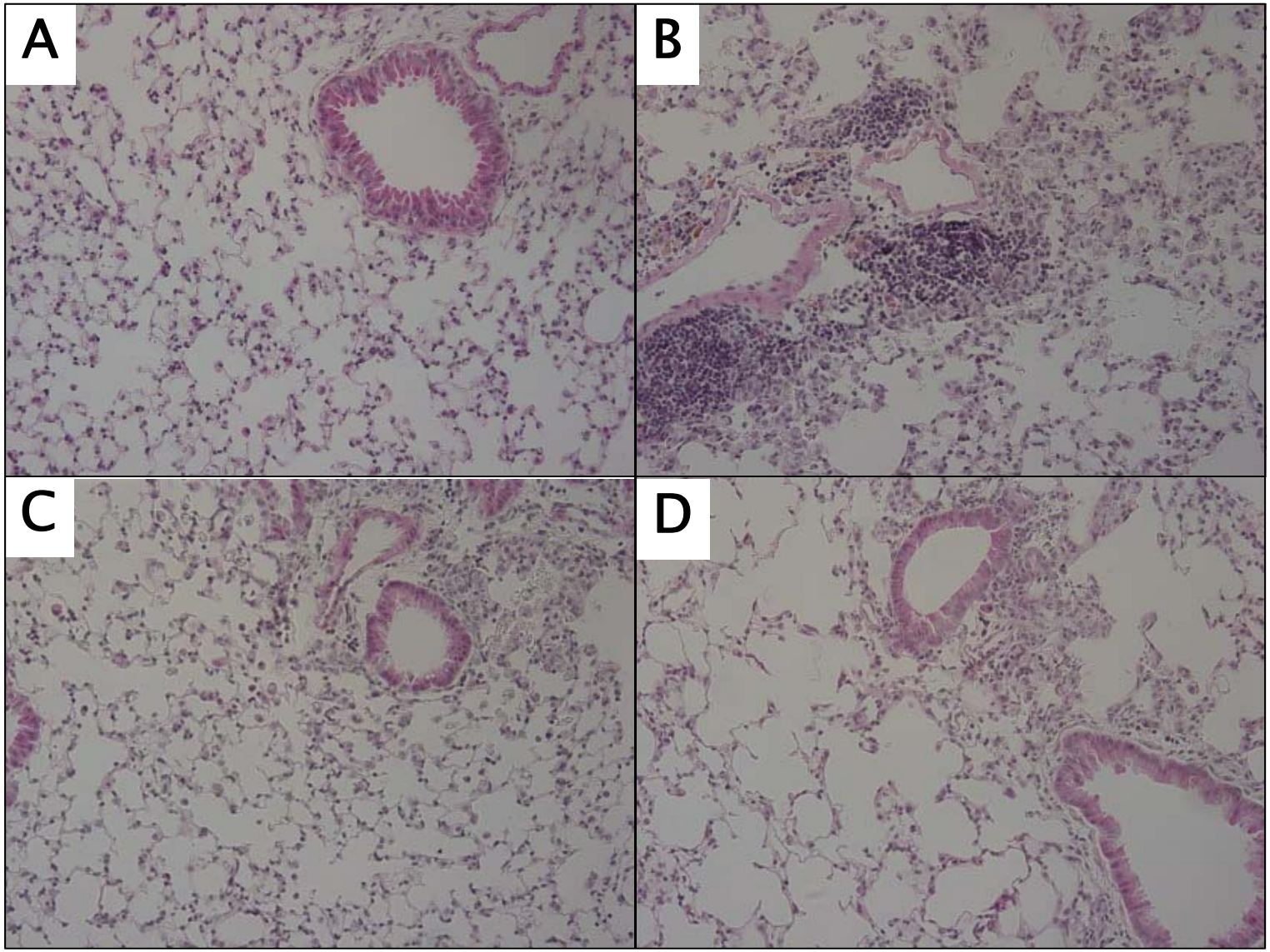

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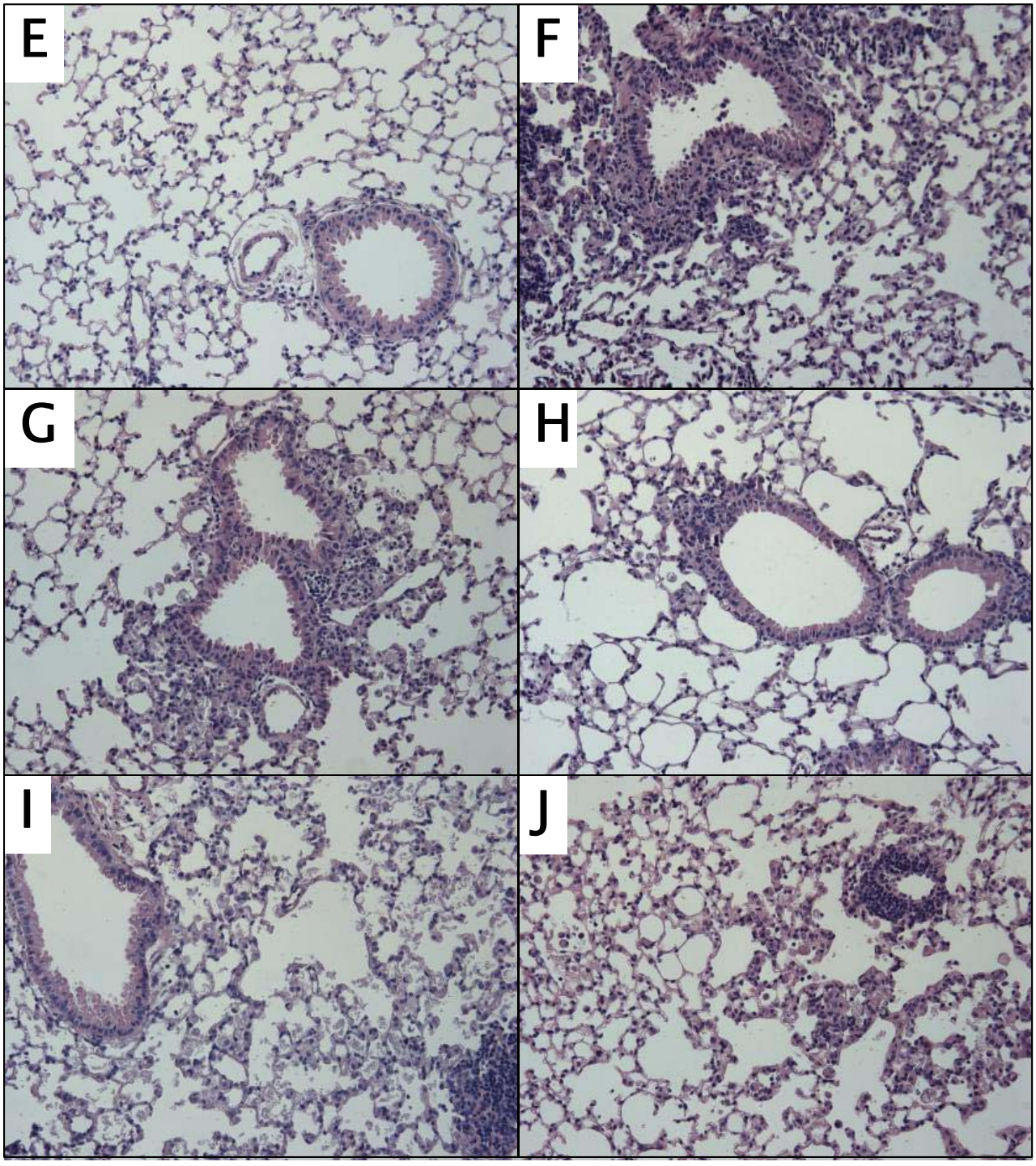

Supplementary figure 3. Nintedanib treatment reduces silica-induced lung inflammation and fibrosis. Representative micrographs of hematoxylin and eosin-stained lung sections from mice of different treatment groups are shown. C57Bl/6 mice who received an intranasal instillation of silica crystals 2.5 mg/mouse (B, F) showed prominent areas of inflammation and granuloma formation that were absent in control animals (A, E). Nintedanib was administered qd by gavage at 30 mg/Kg (C, G, I) and 100 mg/kg (D, H, J). Daily nintedanib treatment from day 0 to day 30 in the preventive study (C-D) and from day 10 till day 30 (G-H) in the therapeutic study reduced silica-induced lung pathology. If nintedanib treatment was administered from day 20 to 30, lung pathology was not significantly reduced (I, J). Analyses were performed at the end of the studies (day 30).

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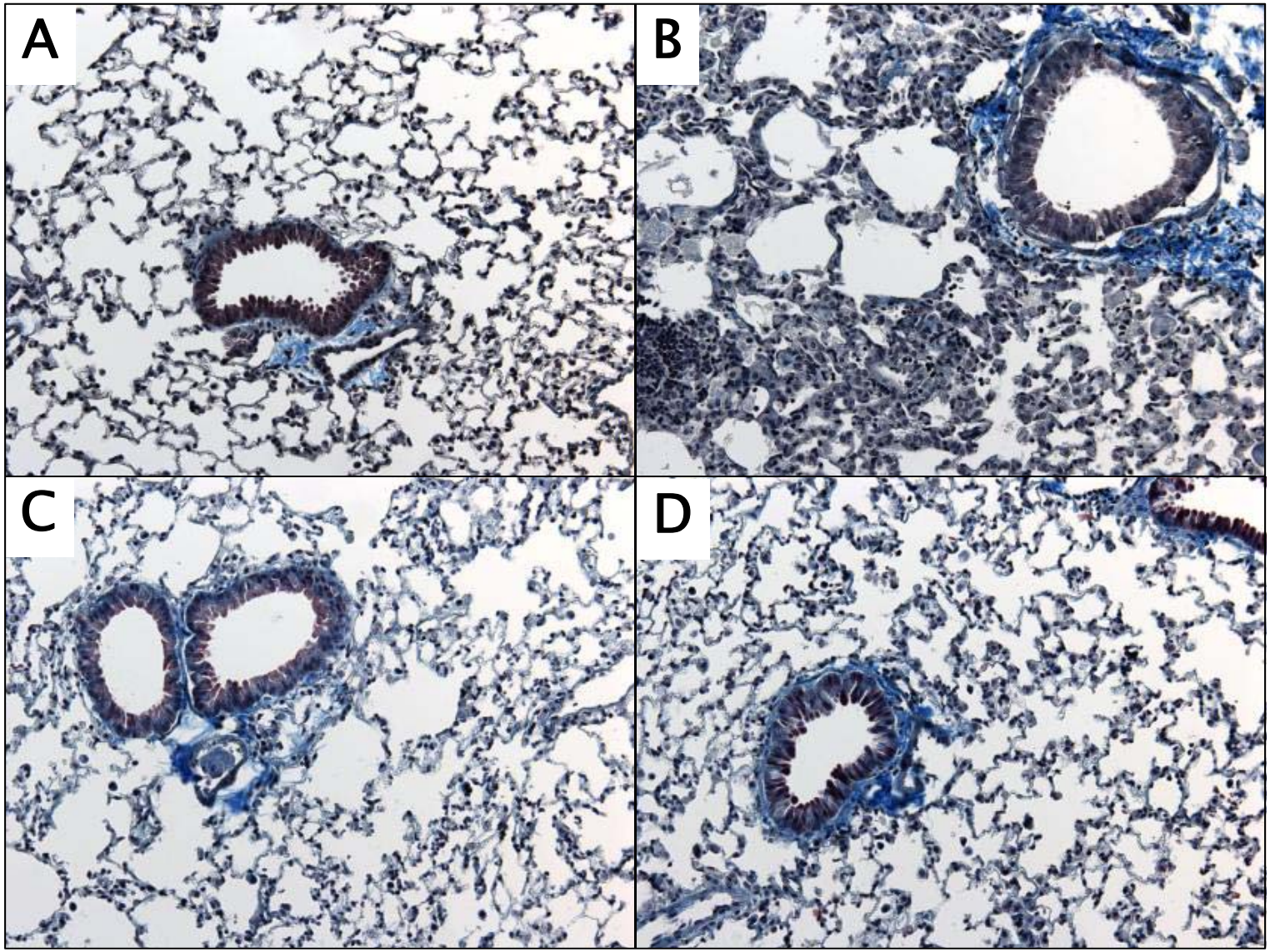

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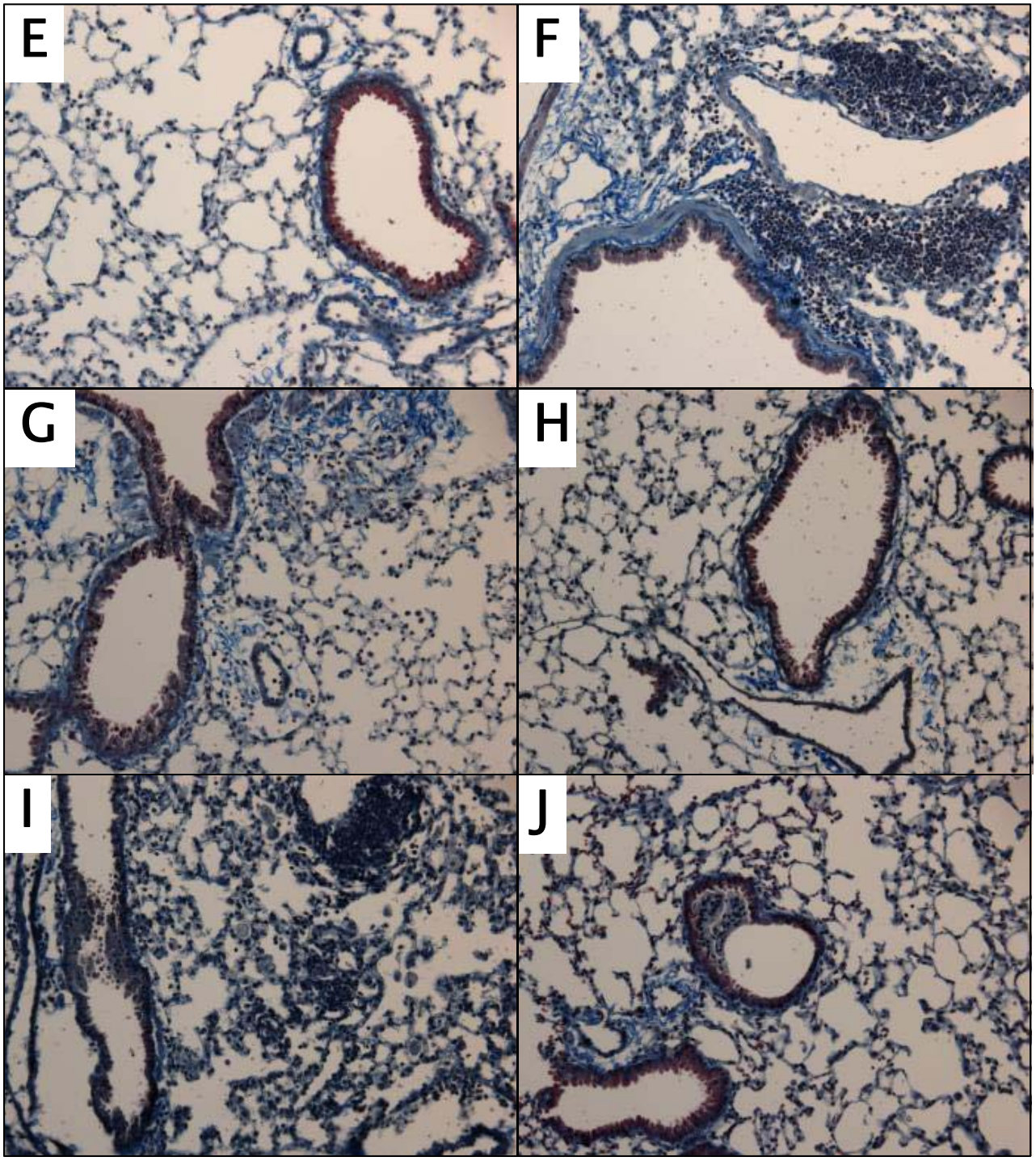

Supplementary figure 4 Nintedanib treatment reduces silica-induced lung inflammation and fibrosis. Representative micrographs of Chromotrope Aniline Blue-stained lung sections from mice of different treatment groups are shown. C57Bl/6 mice who received an intranasal instillation of silica crystals 2.5 mg/mouse (B, F) showed prominent areas of peribronchial and perivascular collagen accumulation, inflammation and granuloma formation that were absent in control animals (A, E). Nintedanib was administered qd by gavage at 30 mg/Kg (C, G, I) and 100 mg/kg (D, H, J). Daily nintedanib treatment from day 0 to day 30 in the preventive study (C-D) and from day 10 till day 30 (G-H) in the therapeutic study reduced silica-induced lung pathology. If nintedanib treatment was administered from day 20 to 30, lung pathology was not significantly reduced (I, J). Analyses were performed at the end of the studies (day 30).