

A comparison of compounds with claimed anti-fibrotic activity in two novel human primary cell based assays using IPF derived patient material

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1 Background and study aim

Transition of fibroblasts into myofibroblasts (FMT) and of epithelial cells into mesenchymal cells (EMT) plays an essential role in fibrotic diseases, such as idiopathic pulmonary fibrosis (IPF), leading to excessive synthesis and deposition of extracellular matrix.

In this study we aimed to establish and characterize two robust primary human cell based assays to assess the translational potential of small molecules with potential as novel therapies for IPF. Primary human bronchial fibroblasts or epithelial cells, derived from IPF donors, were isolated and used to establish FMT and EMT assay respectively using TGF- β 1 as stimulus and alpha-smooth muscle actin (α SMA) or fibronectin (FN) as markers for transdifferentiation. The disease-relevant marker procollagen I N-terminal propeptide (PINP), a measure of collagen I, was measured in the FMT assay. The assays were validated by comparing small molecules which displayed a range of efficacies in clinical trials for fibrosis, including some which failed to demonstrate clinical benefit. Several compounds showed a clear dose-dependent inhibition of TGF β -1-induced α SMA, FN and PINP in IPF donors.

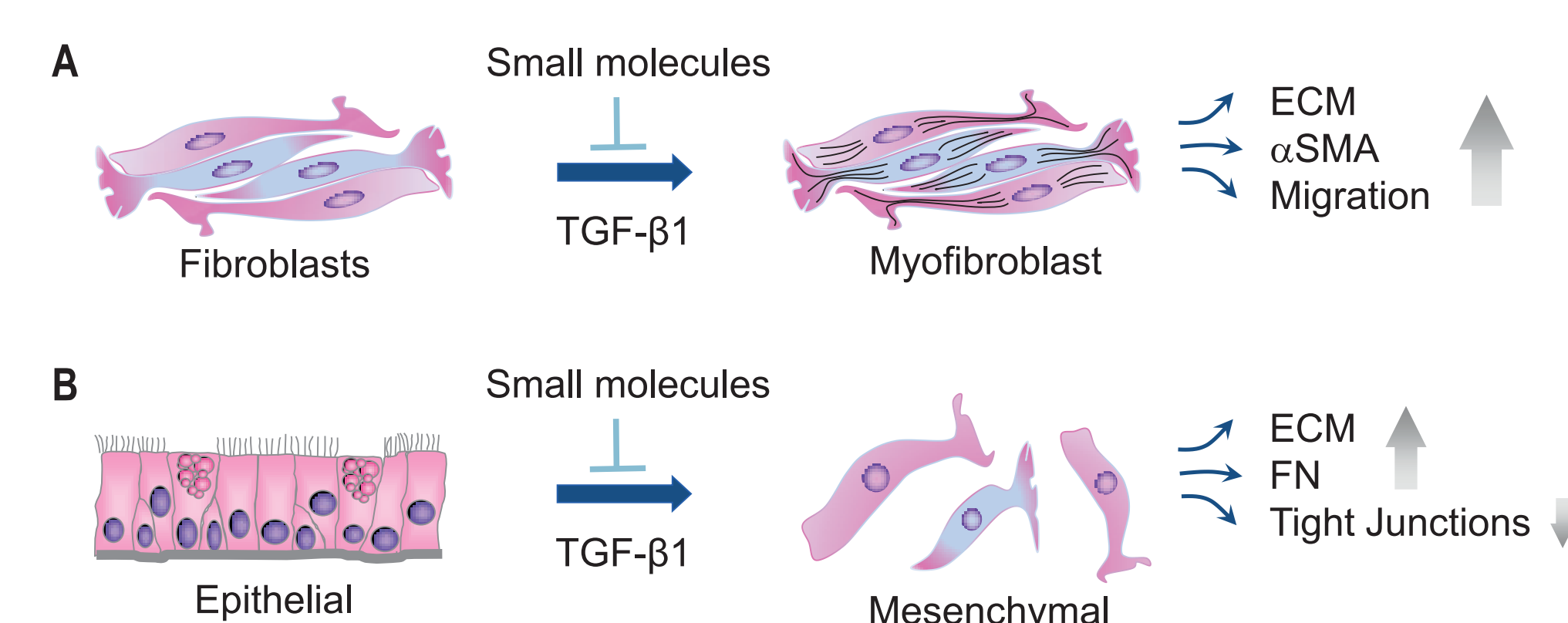


Figure 1. Transdifferentiation by TGF- β 1 and inhibition by small molecules of (A) fibroblasts (FMT) and (B) epithelial cells (EMT).

2 FMT and EMT transition assay

The FMT and EMT transition assays were established by isolation of primary human bronchial fibroblasts and lung epithelial cells derived from IPF patients (n=7). The isolated cells were plated in 96-well format and stimulated with TGF- β 1 in the presence of small molecules. In the FMT assay α SMA was assessed as a marker for transdifferentiation using high content imaging in addition to ELISA measurements of the disease-relevant collagen marker PINP. Fibronectin expression was assessed in the EMT assay as a marker for epithelial transdifferentiation. The assays were validated with a range of small molecules in concentration response curves. The tested molecules included Nintedanib, Pirfenidone, Azathioprine, Imatinib, and GSK2126458. The ALK5 (TGF- β 1 receptor kinase) inhibitor SB525334 served as an assay positive control.

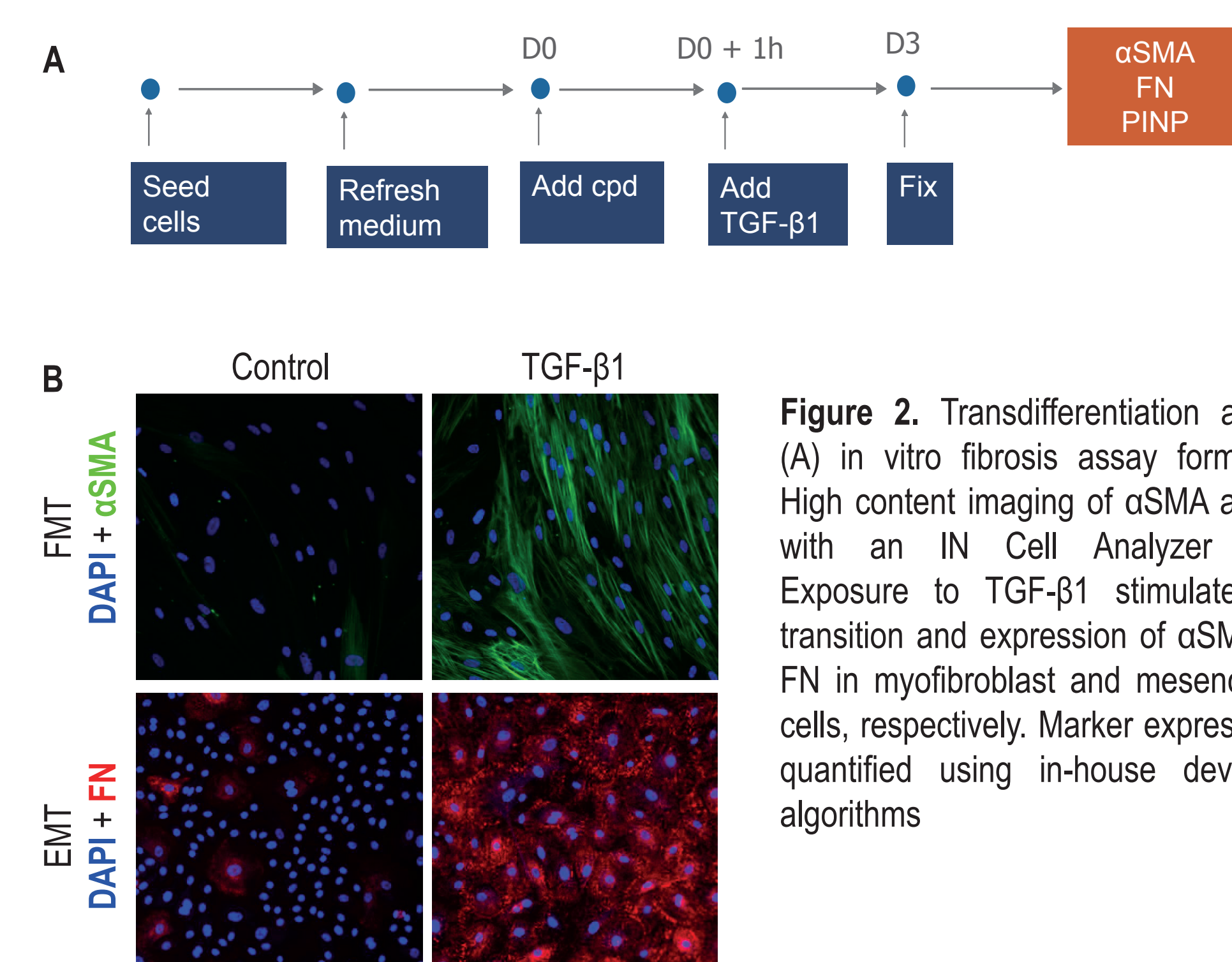


Figure 2. Transdifferentiation assays. (A) in vitro fibrosis assay format (B) High content imaging of α SMA and FN with an IN Cell Analyzer 2200. Exposure to TGF- β 1 stimulates cell transition and expression of α SMA and FN in myofibroblast and mesenchymal cells, respectively. Marker expression is quantified using in-house developed algorithms

3 Assay performance

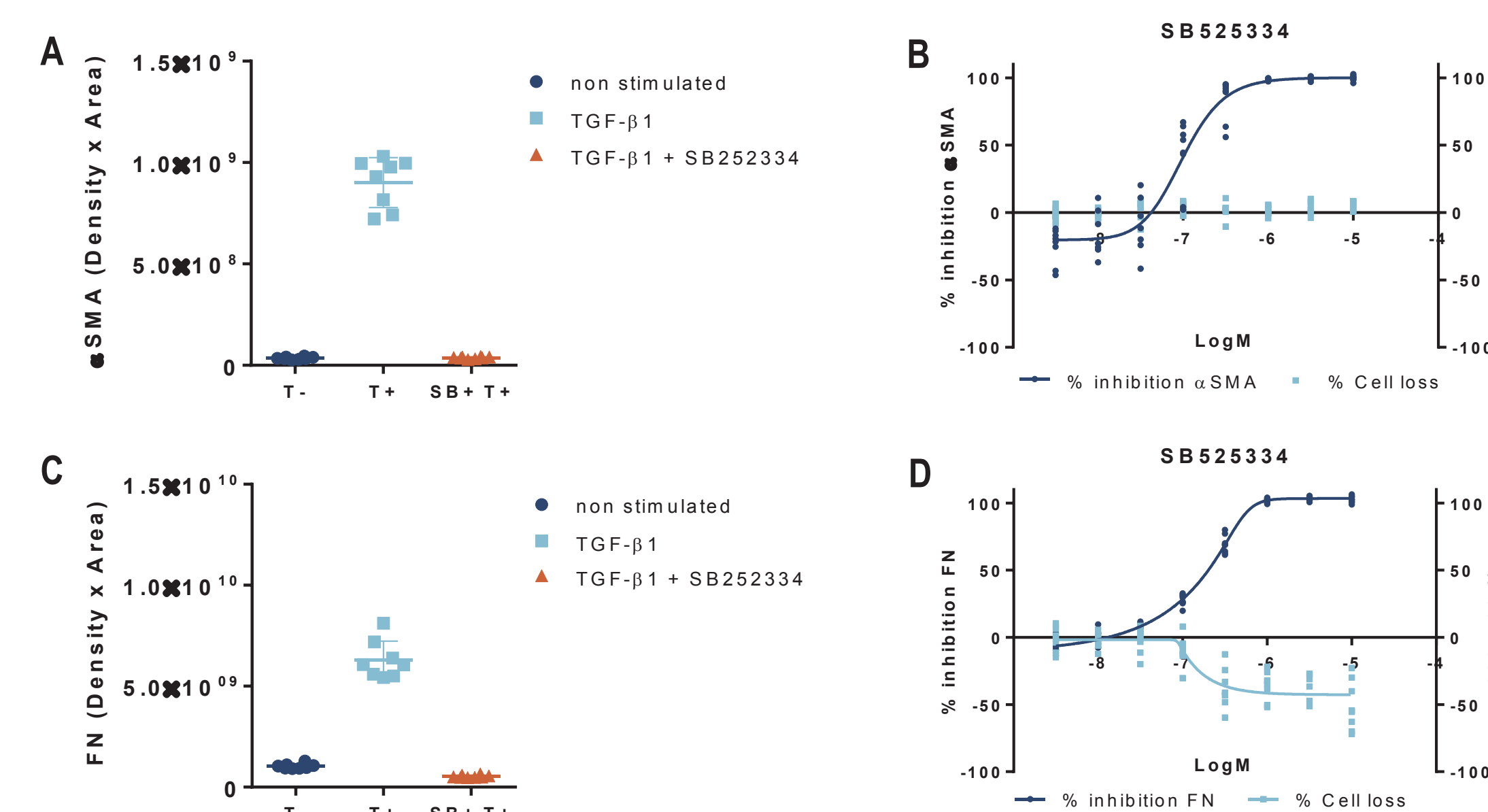


Figure 3. Performance of the FMT (A and B) and EMT (C and D) assays as measured by the reference compound SB525334. (A, C) α SMA and FN signals were quantified for non-stimulated, TGF- β 1 and TGF- β 1 in combination with 1 μ M SB525334 treated cells. Exposure to the ALK5 inhibitor SB525334 completely inhibited TGF- β 1 stimulated marker expression. An average assay window [signal TGF β 1/(signal TGF β 1 + SB525334)] of 15 for FMT and 12 for EMT was observed. (B, D) An 8-point SB525334 concentration response curve demonstrates submicromolar potency in FMT (B) and EMT (D) transition assays.

4 CRC of compounds in FMT and EMT

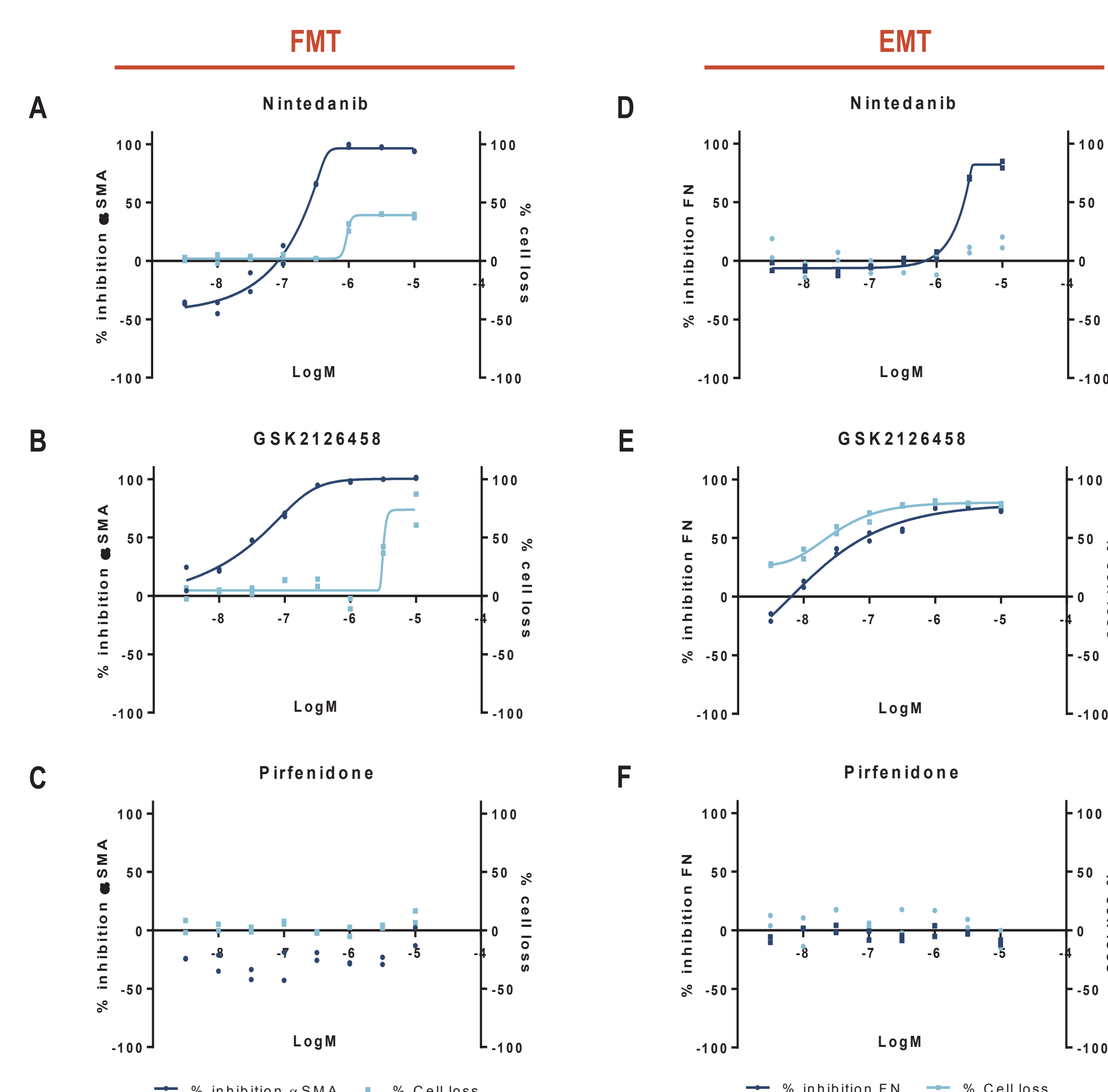


Figure 4. Example 8-point concentration response curves in FMT (A-C) and EMT (D-F) assays. Both Nintedanib and GSK2126458 demonstrated a dose-dependent reduction in TGF- β 1-induced α SMA and FN expression. Cell loss was observed in GSK2126458 and to a lesser extent in Nintedanib-treated cells. No effect on α SMA and FN expression or cell loss was observed with Pirfenidone.

5 PINP compound CRC in FMT

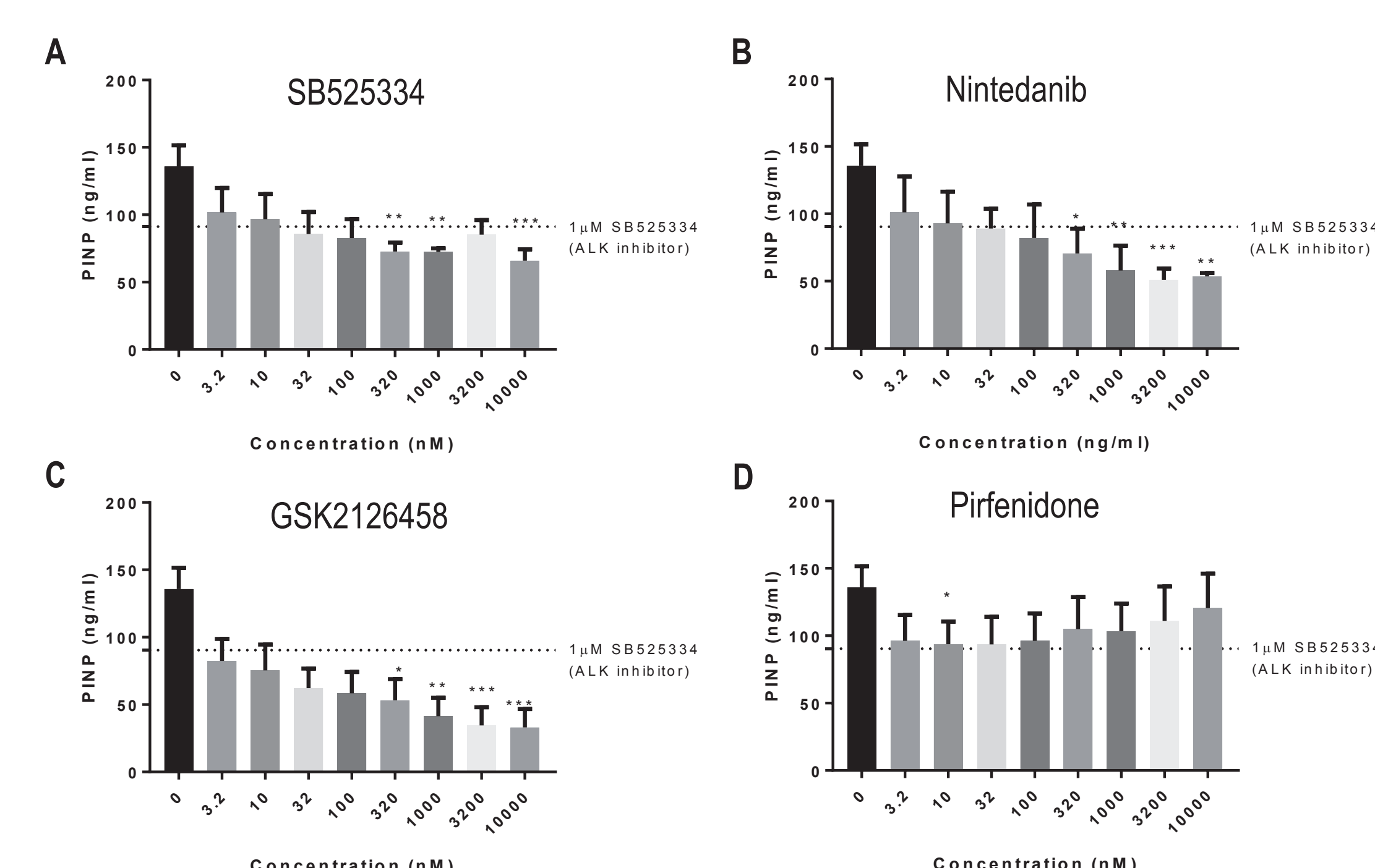


Figure 5. Example PINP concentration response curves for tested molecules. PINP was assessed by ELISA. The ALK5 inhibitor SB525334 (A) was included as positive control. Nintedanib (B) and GSK2126458 (C) provided the clearest effect resulting in significantly decreased PINP levels ($P < 0.05$) in response to 320 nM or higher dosages. By contrast, no effect of Pirfenidone (D) was detected. Changes in PINP levels were evaluated by Kruskal-Wallis test.

6 Compound potency in FMT and EMT

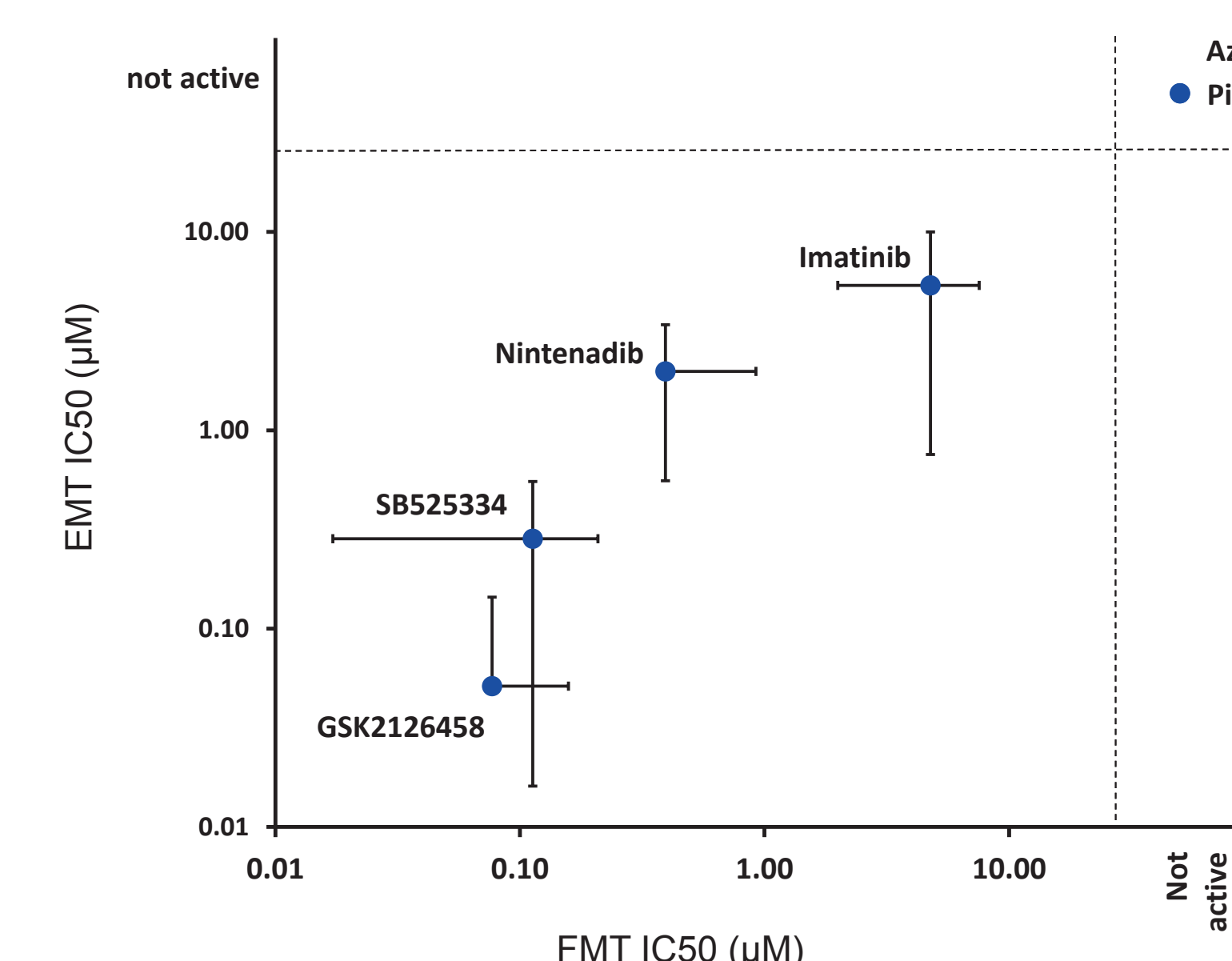


Figure 6. Correlation plot showing small molecule potencies in FMT and EMT assays as measured by α SMA and FN expression. IC50 values represent the average of 7 IPF donors. Error bars indicate the standard deviation. No IC50 values could be determined for Azathioprine and Pirfenidone in either assay.

7 Conclusions

Several compounds show clear dose-dependent inhibition of TGF- β -1-induced α SMA, FN and PINP in IPF donors. Of the compounds examined Nintedanib and GSK2126458 show the clearest efficacy, whereas Imatinib and Azathioprine showed minimal effect, matching the clinical experience. No inhibitory effect of Pirfenidone is seen. These results suggest that use of human IPF fibroblasts may be a useful translatable tool to investigate potential new drugs to treat IPF.