An *in-vitro* high-content imaging assay for the study of fibroblast activation and matrix deposition

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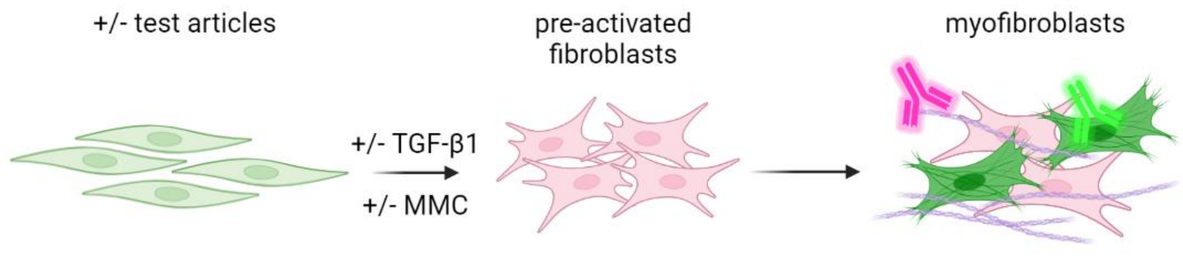
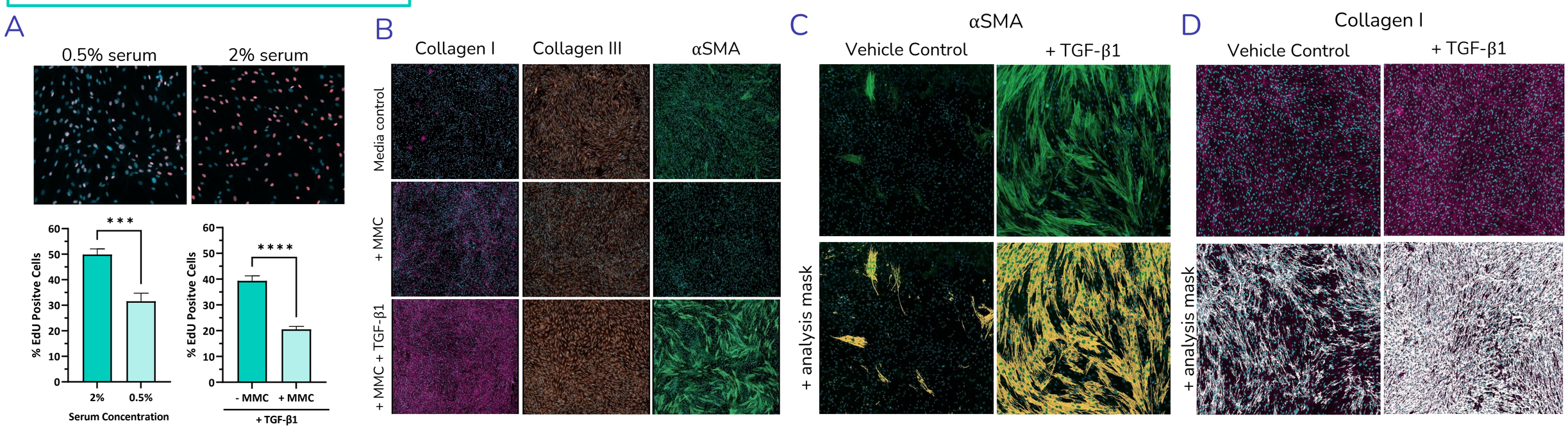
Modelling Fibroblast-to-Myofibroblast Transition (FMT)

- Idiopathic Pulmonary Fibrosis (IPF) is a chronic and progressive interstitial lung disease, whereby damage to the lung architecture leads to an irreversible loss of function.
- The aetiology of IPF is unknown, but is thought to occur due to epithelial damage, abnormal epithelial-fibroblast communication, and subsequent dysregulated tissue repair response.
- TGF-β1 mediated activation of fibroblasts, their transition to α-SMA expressing myofibroblasts, and associated increase in the expression and deposition of extracellular matrix proteins represents one of the underlying pathologic mechanisms of fibrosis.
- Modelling this process *in-vitro* allows the testing of potential anti-fibrotic therapeutics for their ability to reduce or reverse FMT.



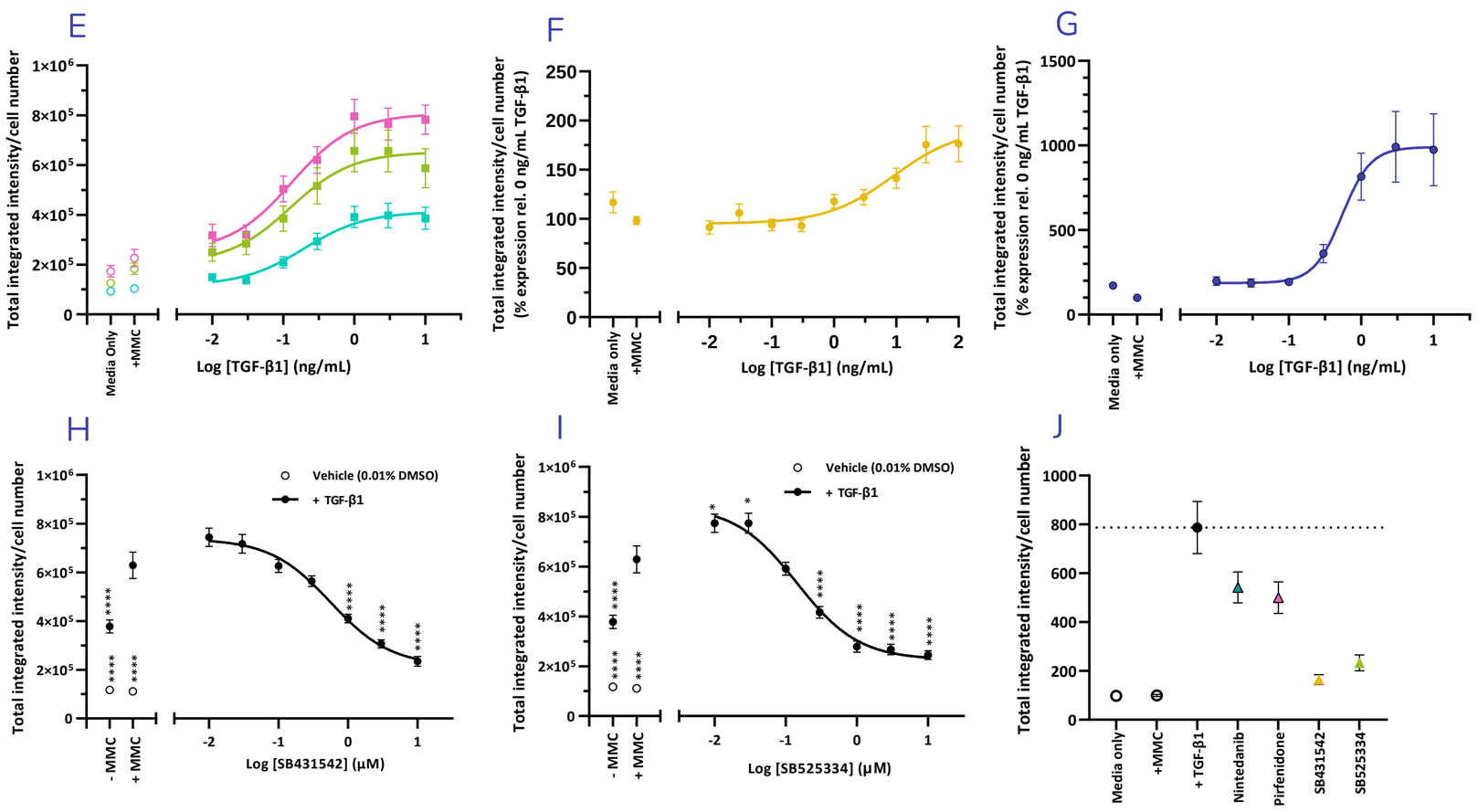
- ✓ 3 primary human lung fibroblast (HLF) donors
- ✓ High-throughput, 384-well format
- ✓ Reduced serum concentrations to control for cellular proliferation
- ✓ Test article pre-treatment
- ✓ Physiologically relevant stimulus
- ✓ Macromolecular crowder to induce matrix deposition
- ✓ Internal assay/imaging controls
- ✓ ICC protein detection with high-content imaging
  - Cell Number
  - Collagen I
  - αSMA
  - Collagen III

Assay Characterisation



Assay characterisation showing the effects of serum concentration and macromolecular crowding agents on cell proliferation and the expression of fibrosis related proteins collagen I, collagen III, and αSMA expression in primary HLF cells with examples of imaging analysis by application of segmentation masks.

Lung fibroblasts upregulate fibrotic markers in response to TGF-β1



Effects of increasing TGF-β1 concentration on the expression of collagen I **(E)**, collagen III **(F)** and αSMA **(G)** expression in normal HLFs. TGF-β1 induced collagen I expression is dose-dependently reduced by ALK5 inhibitors SB431512 and SB525334 **(H & I)**. Physiologically relevant concentrations of Nintedanib (100 nM) and Pirfenidone (100 μM) reduce collagen I expression **(J)**.

Newcells Biotech's FMT- assay

Newcells' FMT assay utilises high-content imaging to determine the effects of potential therapeutics on fibroblast activation and collagen expression and deposition in high-throughput format.

Assay Features:

- 3 validated primary HLF donors
- High-throughput 384-well format
- 6-point dose response for upto 6 TAs per plate
- 6 technical replicates per condition
- Plus validated experimental assay controls

Available Assay Readouts:

- ✓ Cell Number
- ✓ Collagen I
- ✓ αSMA
- ✓ Collagen III

Further details available online or contact us at
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