High throughput, high sensitivity (HTHS) lung fibroblast-to-myofibroblasts transition (FMT) assay for anti-fibrotic drug screening

The rapid screening solution for advancing drug development with more confidence

What you can achieve:

- Rapid and highly sensitive efficacy screening of anti-fibrotic drug candidates to progress all relevant ones *in vivo*
- Assessment of drug dose-dependent fibrotic marker response with high sensitivity imaging
- Comparison of drug efficacy with ALK5 inhibitors
- Evaluation of donor-to-donor variation with multiple validated human lung fibroblast donors

What forms the basis of the study:

- Primary lung fibroblasts from healthy donor or idiopathic pulmonary fibrosis patients
- High throughput format (384-well)
- Physiologically-relevant conditions for promoting extracellular matrix (ECM) protein deposition
- Laser-based imaging for faster screening and higher sensitivity

How can Newcells help?

We accelerate the development of your anti-fibrotic compounds by rapidly screening your compound library and generating accurate dataset through our high-content, high-throughput and high sensitivity FMT assay.

Why Newcells HTHS Lung FMT assay for evaluating your compounds?

Rapidly screen compounds at high sensitivity: the 384-well HTHS FMT assay enables screening of compounds for their impact on preventing lung fibrosis ensuring all relevant active compounds move forward into development.

Measuring compound efficacy: this is measured as decreasing expression of α-SMA and extracellular collagen I following TGF-*β*1 stimulation.

Detecting and quantifying sensitive signals: immunostaining and high-content imaging detect cell count, α-SMA expression, α-SMA strand perimeter and extracellular collagen I, providing all necessary data to make informed decisions **(Fig.1).**

Our **optimised assay conditions** control cell proliferation, enhancing the ability of stimuli to induce FMT, ensuring accurate data points **(Fig. 2).** The inclusion of a **macromolecular crowding agent** in our assay media mimics an in vivo environment and promotes mature collagen fibril deposition in the extracellular compartment to **deliver predictive data (Fig 3).**

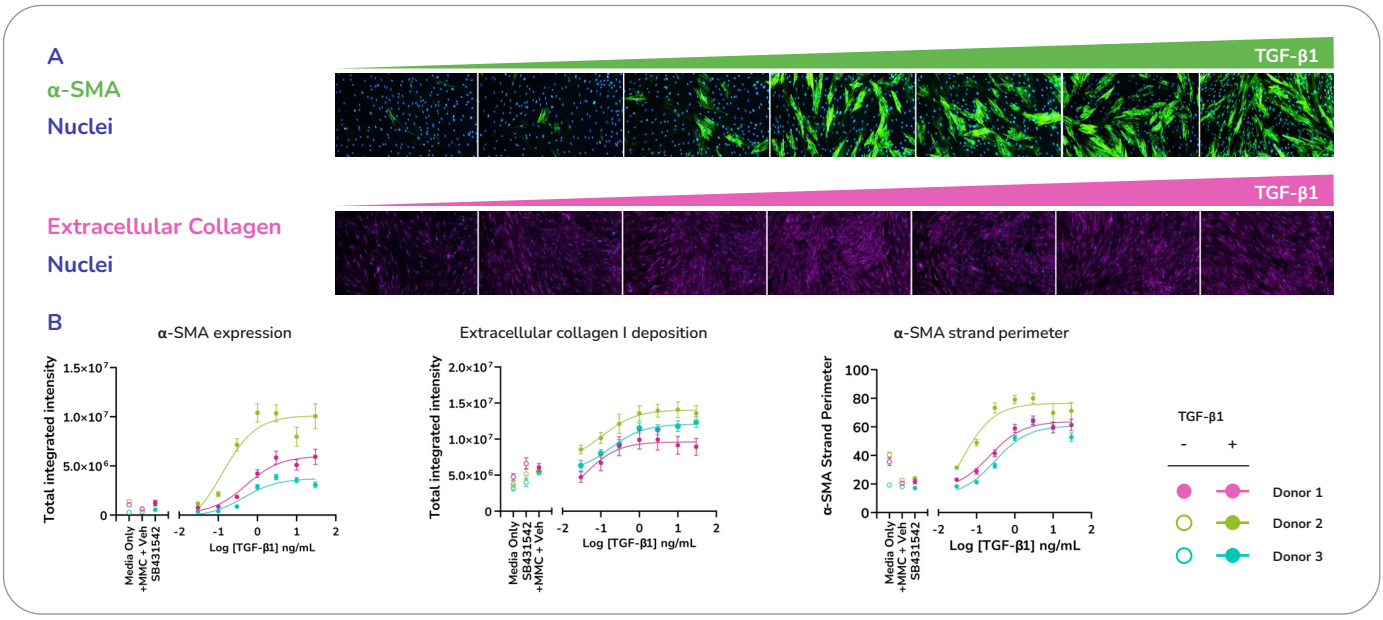

Figure 1: Assay optimised to provide 4 parameters giving ample data for efficacy screening. A) TGF-b1 dose dependent increase in expression of α-SMA and extracellular collagen I deposition. B) Graphs showing quantified expected increase in α-SMA expression, extracellular collagen I deposition and α-SMA strand perimeter. Data was derived from three human primary lung fibroblasts donors.

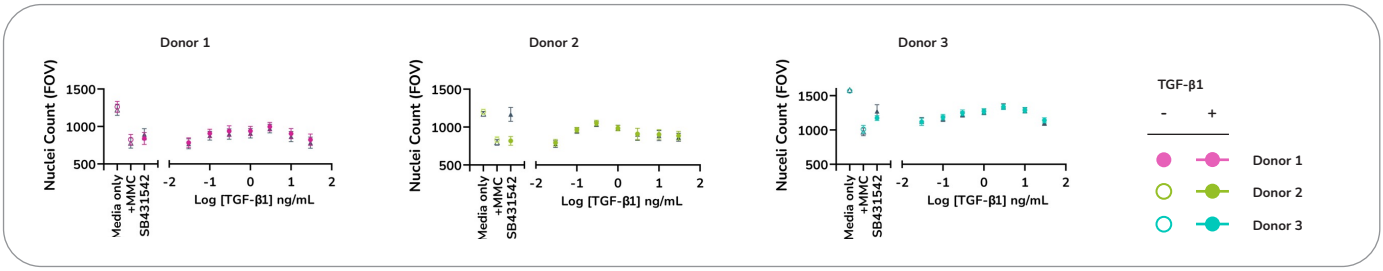

Figure 2: Optimized assay culture conditions minimize the effects of TGF-β1 on nuclei ensuring accuracy of data. Human lung fibroblasts from three healthy donors were stimulated with TGF-β1 and stained for cell nuclei.

Enhanced sensitivity with the HTHS Lung FMT assay

Avoiding false negative signals: Compared to conventional LED-based imaging assays, the HTHS Lung FMT assay employs laser-based rapid imaging with multiple channels picking up the most sensitive signals for detailed characterization of fibrotic marker expression. This helps in generating more accurate data and **avoiding false negative signals**.

Screening of multiple compounds and dilutions: Quantificatin of dose-dependent signals through image analysis allows for the calculation of EC50 values for anti-fibrotic compounds. The imaging and analysis time for the HTHS Lung FMT assay is significantly less than conventional LED-based imaging systems resulting in the ability to **screen multiple compounds and compound dilutions** along with assay controls.

The high sensitivity found in dose-response curves for α-SMA expression **(Figure 4)** and extracellular collagen I **(Figure 5)** ensure **all active compounds are moved forward**. The advanced image analysis also provides **additional data** with the quantification of α-SMA strand perimeter that has been shown to play a vital role in the contractility of myofibroblasts for driving fibrosis.

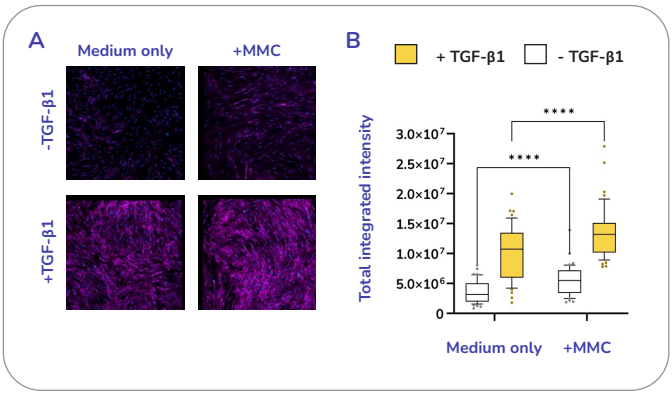

Figure 3: The addition of macromolecular crowder (MMC) promotes extracellular matrix proteins like collagen I deposition, thereby increasing sensitivity of the assay (A) Extracellular collagen I deposition images (B) quantified expression levels of extracellular collagen I deposition.

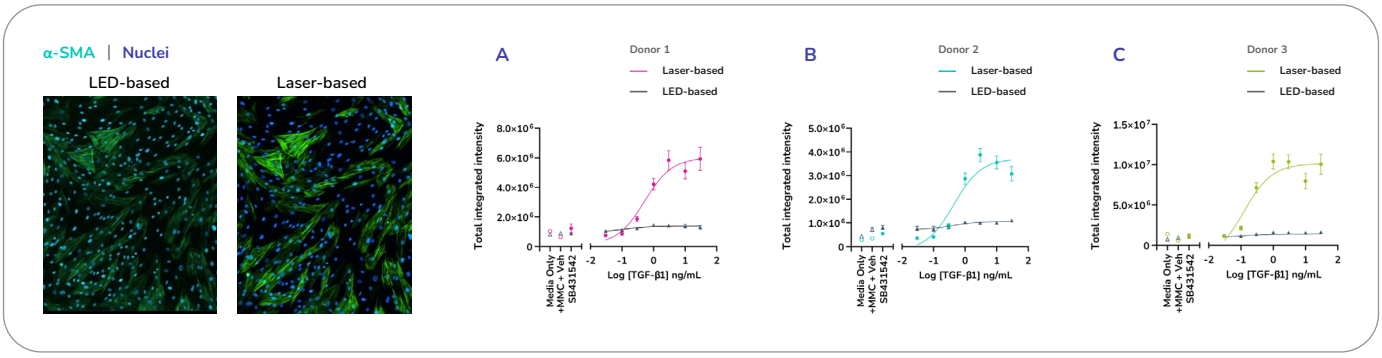

Figure 4: Increased sensitivity from laser-based imaging. α-SMA expression imaged in LED-based imaging (left) vs laser-based imaging (right). Graphs A, B and C show quantified α-SMA expression. Data was Data was derived from three human primary lung fibroblasts donors.

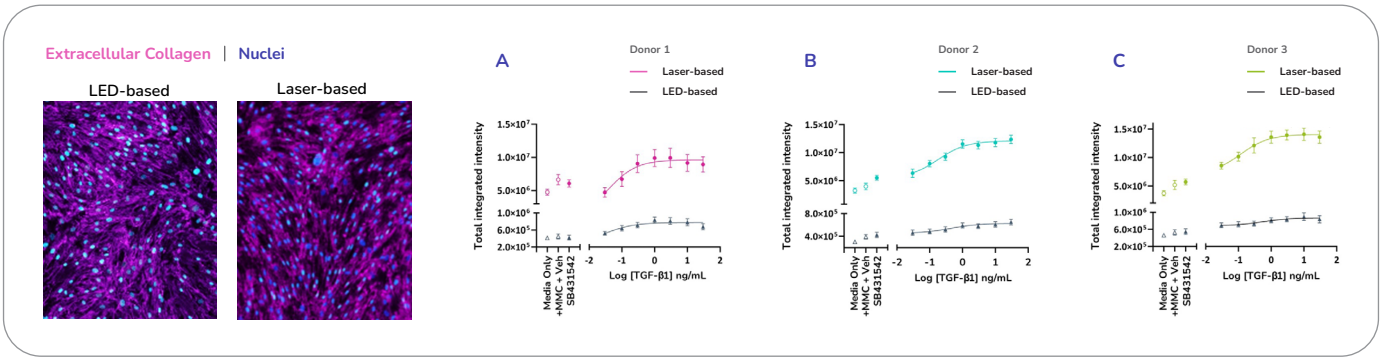

Figure 5: Increased sensitivity from laser-based imaging. Collagen I deposition in LED-based imaging (left) vs laser-based imaging (right). Graphs A, B and C show quantified extracellular collagen I deposition. Data was derived from three human primary lung fibroblasts donors.

Lung FMT Assay					
SKU No.	Offering	Format	Readouts	Time	Inclusions
LSFMT0000H	HTHS Lung FMT assay	384-wells	Cell count along with dose response curve for α-SMA expression, α-SMA strand perimeter and extracellular collagen I deposition	72 hours	1 donor, 5 compounds, 6 dilutions with internal controls and QC

For more information:

If you would like further information, please contact our experts or visit our website:

info@newcellsbiotech.co.uk
or visit: www.newcellsbiotech.co.uk/FMT

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