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?

The 384-well HTHS FMT assay rapidly screens compounds at high-sensitivity for their impact on preventing lung fibrosis ensuring all relevant active compounds move forward into development. The assay measures the compounds' efficacy at decreasing expression of α-SMA and extracellular collagen I following TGF-β1-stimulation.

By using immunostaining and high-content imaging, we detect and quantify sensitive signals for 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).**

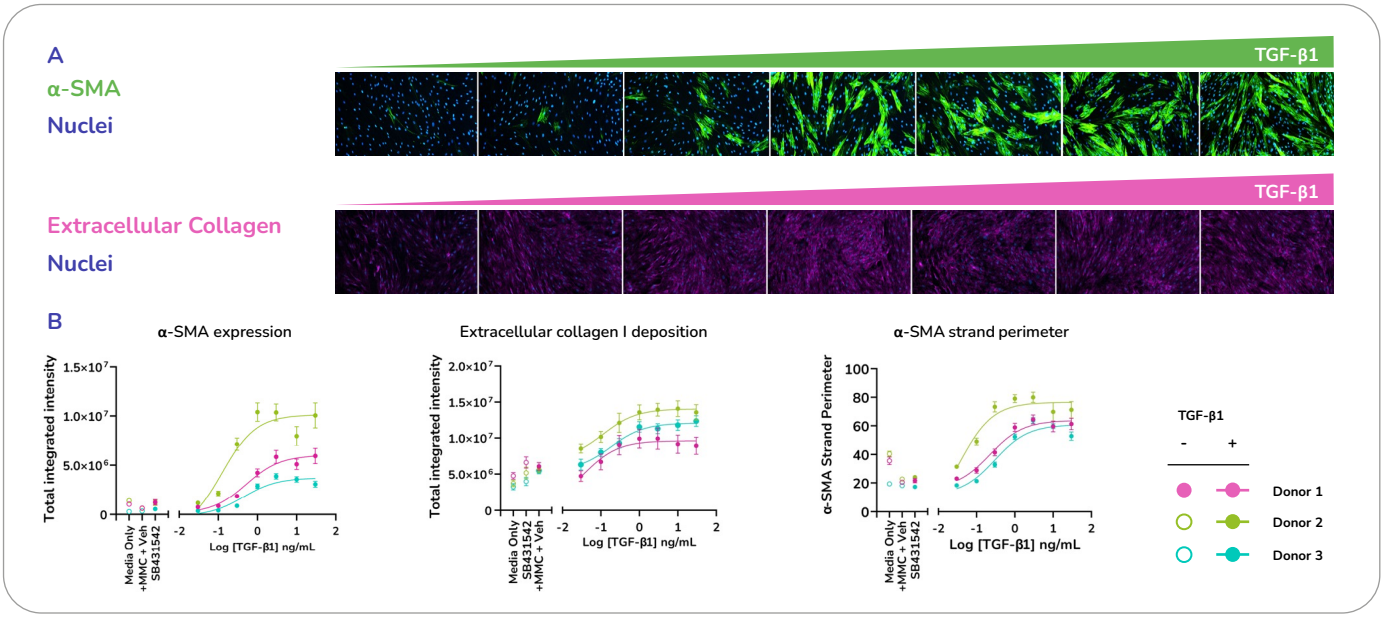

Figure 1: A) TGF-β1 dose dependent increase in expression of α-SMA and extracellular collagen I deposition. B) Graphs showing quantified a-SMA expression, extracellular collagen I deposition and a-SMA strand perimeter. Data was derived from three human primary lung fibroblast donors.

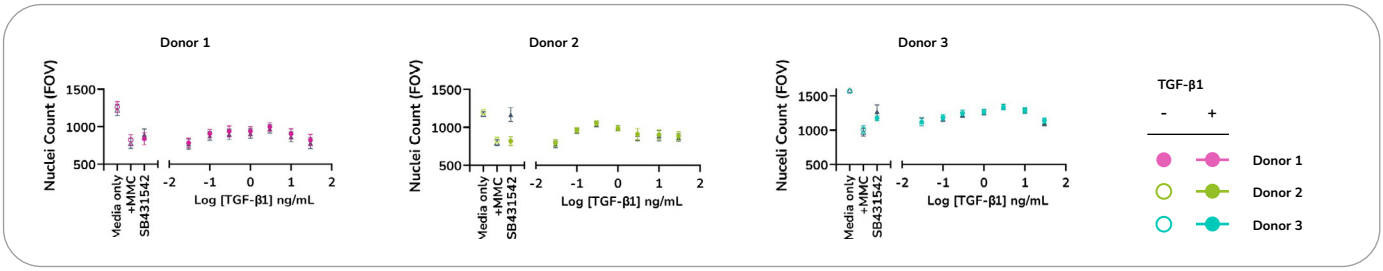

Figure 2: Optimized assay culture conditions minimize the effects of TGF-β1 on nuclei count. Human lung fibroblasts from three healthy donors were stimulated with TGF-β1 and stained for cell nuclei.

Enhanced sensitivity with the HTHS Lung FMT assay

Compared to conventional LED-based imaging, 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 avoiding false negative signals and generation of more accurate data.

Quantifying dose-dependent signals through image analysis allows for the calculation of IC50 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.

This advantage is also complemented with the high-sensitivity found in dose response curves for α-SMA expression **(Figure 4)** and extracellular collagen I **(Figure 5)** ensuring 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.

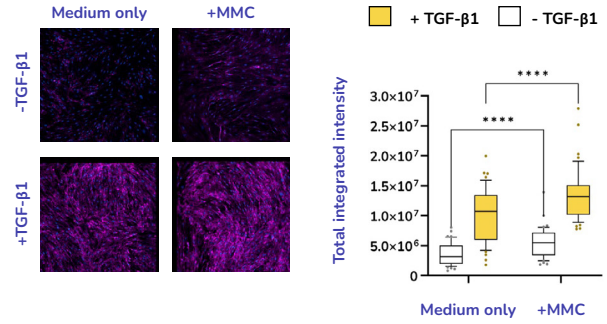

Figure 3: The addition of macromolecular crowder (MMC) promotes extracellular matrix proteins like collagen I deposition, thereby increasing sensitivity of the assay (A) Extracellular collagen I deposition images (B) quantified expression levels of extracellular collagen I deposition.

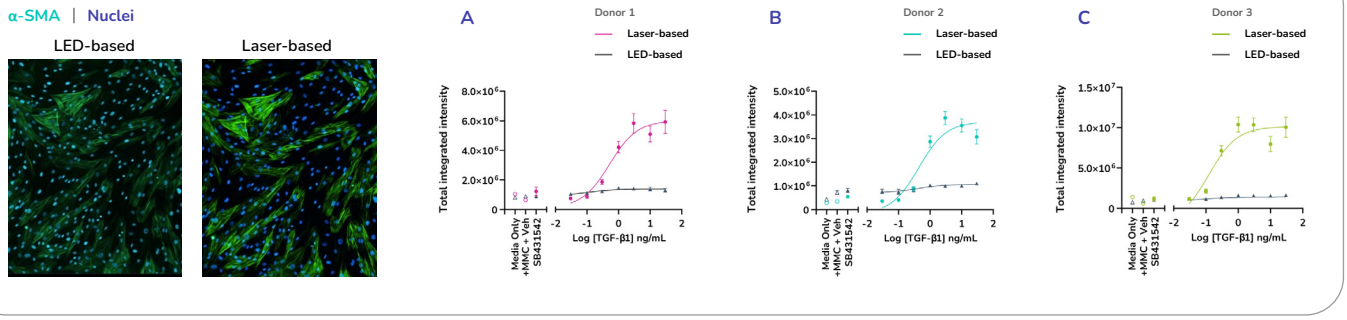

Figure 4: α-SMA expression imaged in LED-based imaging (left) vs laser-based imaging (right). Graphs A, B and C show quantified α-SMA expression. Data was derived from three human primary lung fibroblast donors.

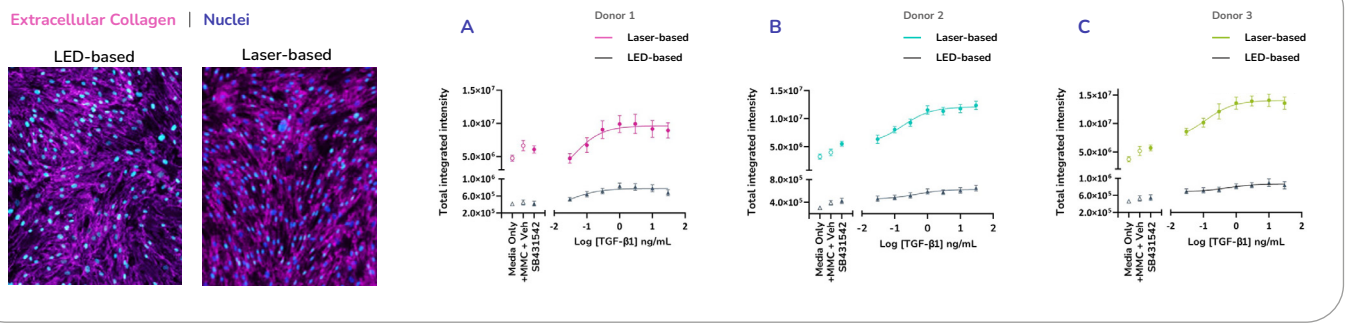

Figure 5: Extracellular collagen I deposition in LED-based imaging (left) vs laser-based imaging (right). Graphs A, B and C show quantified extracellular collagen I deposition. Data was derived from three human primary lung fibroblast donors.

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

Scan the QR code to download the factsheet