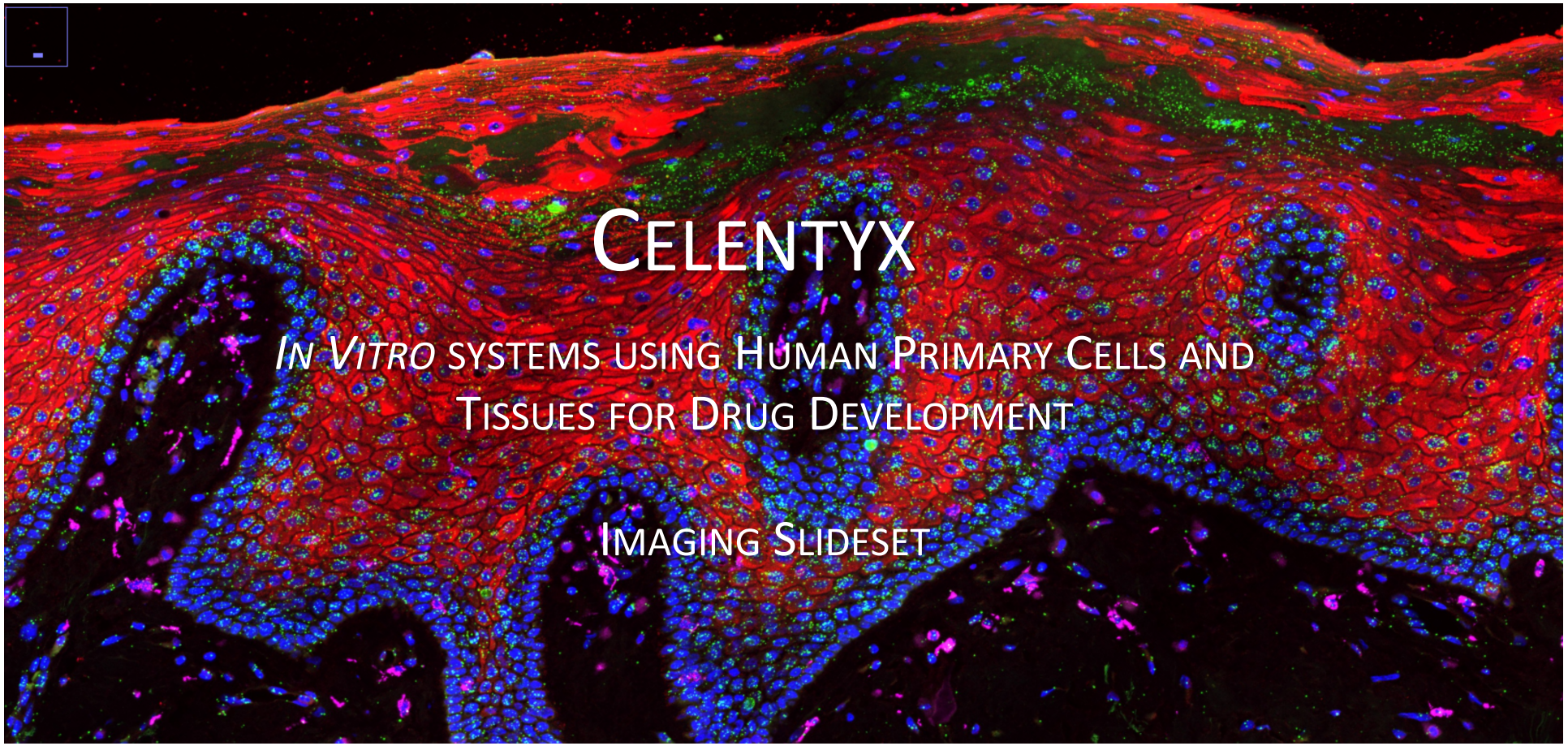

# CELENTYX

## *IN VITRO* SYSTEMS USING HUMAN PRIMARY CELLS AND TISSUES FOR DRUG DEVELOPMENT

IMAGING SLIDESET

Celentyx

www.celentyx.com

# Company Background; Celentyx Ltd – a specialist CRO

Founded in 2007 by Professors John Gordon and Nicholas Barnes
Provide translational human primary cell and tissue assays for drug development

**Professor John Gordon**

Internationally recognised authority on immune cell biology and one of the world's most highly cited researchers

**Professor Nicholas Barnes**

Applied neuropharmacologist with a proven track record in the investigation of potential drug targets and the delivery of candidate therapeutics

Institute of Immunology and Immunotherapy

Institute of Translational Medicine

Celentyx

QE Hospital

NHS Blood Service

University of Birmingham

Corporate Headquarters
Birmingham, UK

Celentyx Innovation Lab
Oxford, UK

Celentyx

# Company Background II

## Established links to hospitals and clinicians within the Midlands area of the UK

- QE Hospital is the largest single site hospital in UK
- Largest critical care in Europe
- Serving a large and diverse community covering a large range of ethnic groups


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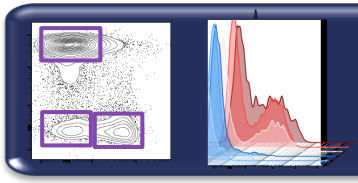
Collection of cells, tissues and/or fluids from patients and 'controls' with subsequent processing for client service projects

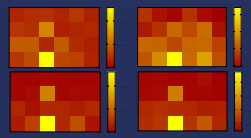
All material is consented, collected and stored according to statutory codes of practice

Samples provided with the appropriate demographic and clinical data and opportunity for retrospective data mining of clinical records

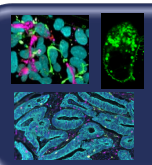
## Broad scientific and technical expertise applied across the drug discovery pipeline



**Multiparameter flow-cytometry**
Immune subsets, activation markers, proliferation, target expression



**Luminex analysis of fluids**
Cytokines, chemokines, other soluble factors



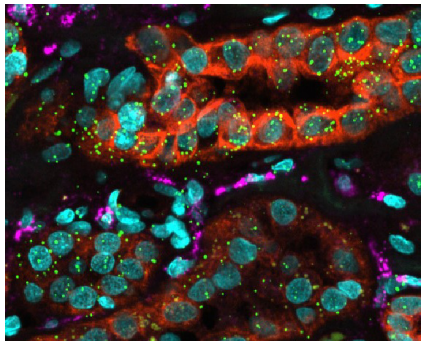
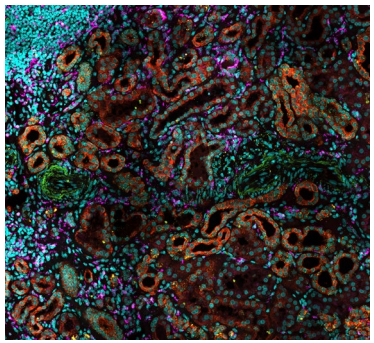
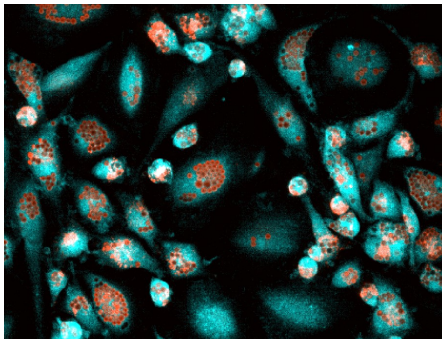
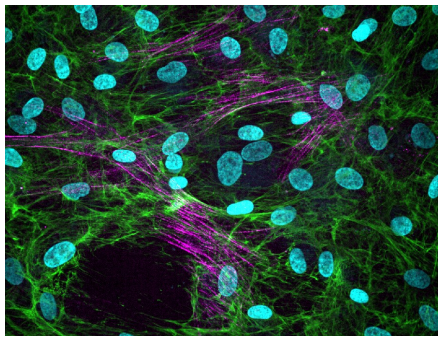
**High-throughput confocal microscopy**
Plate based high-content screening with automated analysis, tissue immunofluorescence, IHC, RNAScope

Broad range of other assay techniques available e.g. ELISAs, Western-blotting, calcium imaging, luminescence/ fluorescence assays, radioligand binding

# Imaging Assays

**Celentyx Imaging Platform**

- Range of cutting-edge microscopy platforms available
- Assays can be performed in 96- or 384-well format
- Kinetic, live cell readouts available (including high-speed imaging)
- Multi-colour (4-colour imaging performed routinely; increased colours available)
- Labelling using antibodies, dyes, probes and reporters
- Automated image analysis performed in 2D or 3D
- Applied to a broad range of tissues and cells from patients and healthy donors

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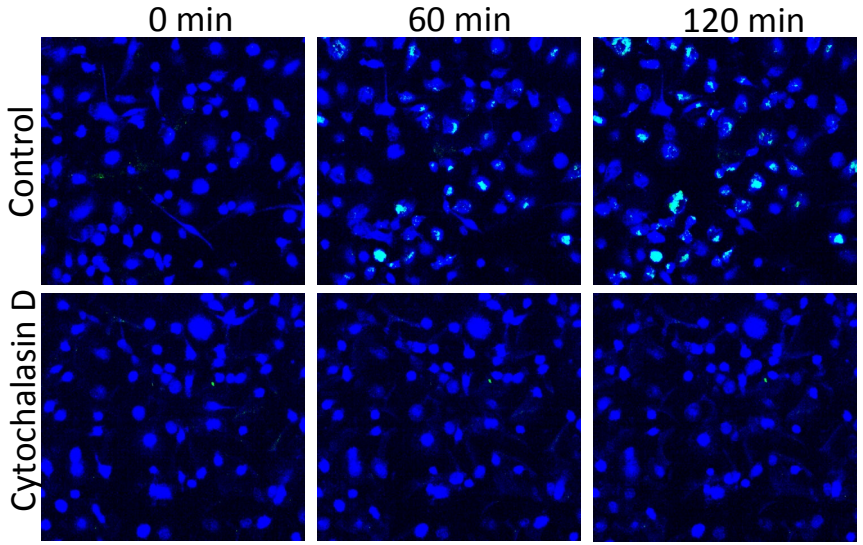
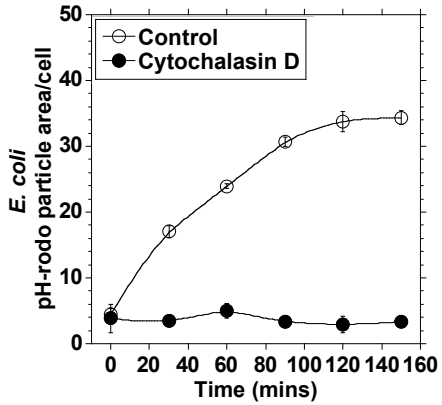
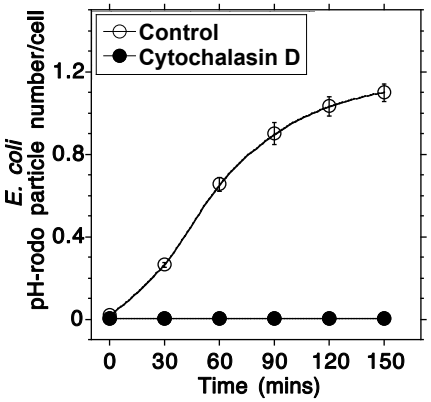
# PHAGOCYTOSIS ASSAYS: *E. COLI*

- Human monocyte-derived macrophages phagocytosing pH-rodo labelled *E.coli*
- Assay performed with or without opsonization of target particle
- Phagocytosis imaged over time by high-throughput spinning disk confocal microscopy
- Time-dependent uptake of *E.coli* by macrophages that is abolished in the presence of cytochalasin D

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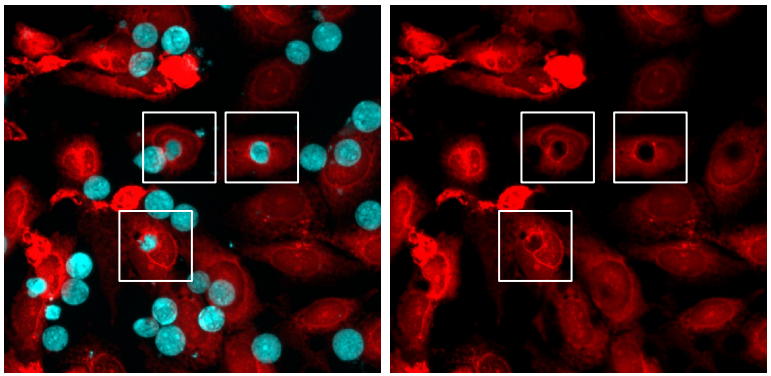
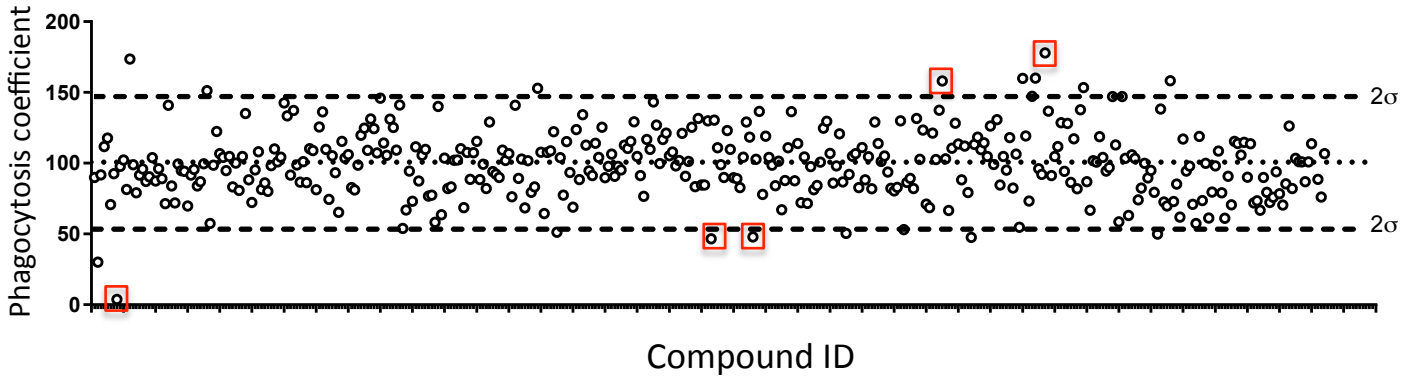
# Screening for Phagocytosis Modulators

- High content-imaging screen of a small molecule library for modulators of tumour cell phagocytosis
- Identification of novel modulators that were subsequently validated in orthogonal assays



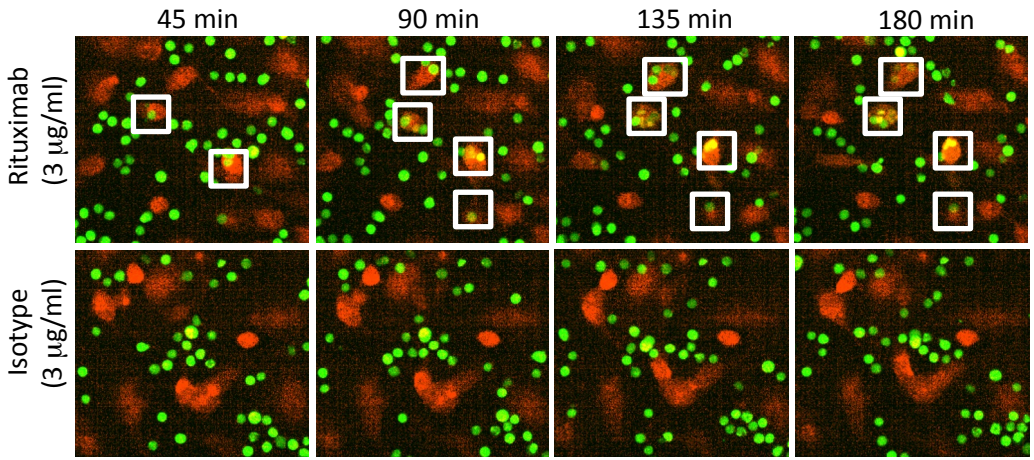
Phagocytic cell
Target cell

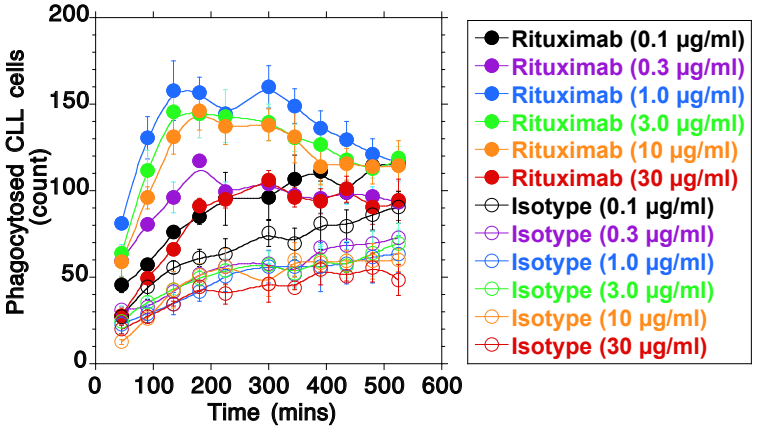
Example screen results shown below. Phagocytosis co-efficient indicates the extent of phagocytosis. Red boxes highlight compounds that were subsequently tested and validated in orthogonal assays.

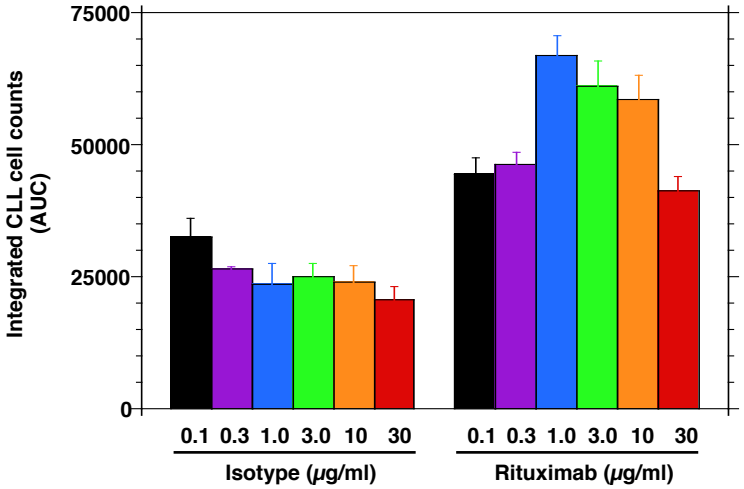


# PHAGOCYTOSIS ASSAYS: TUMOUR CELLS

- Phagocytosis of patient CLL cells by human monocyte-derived macrophages
- Rituximab enhances capture of CLL cells
- Identifies bell-shaped concentration-response

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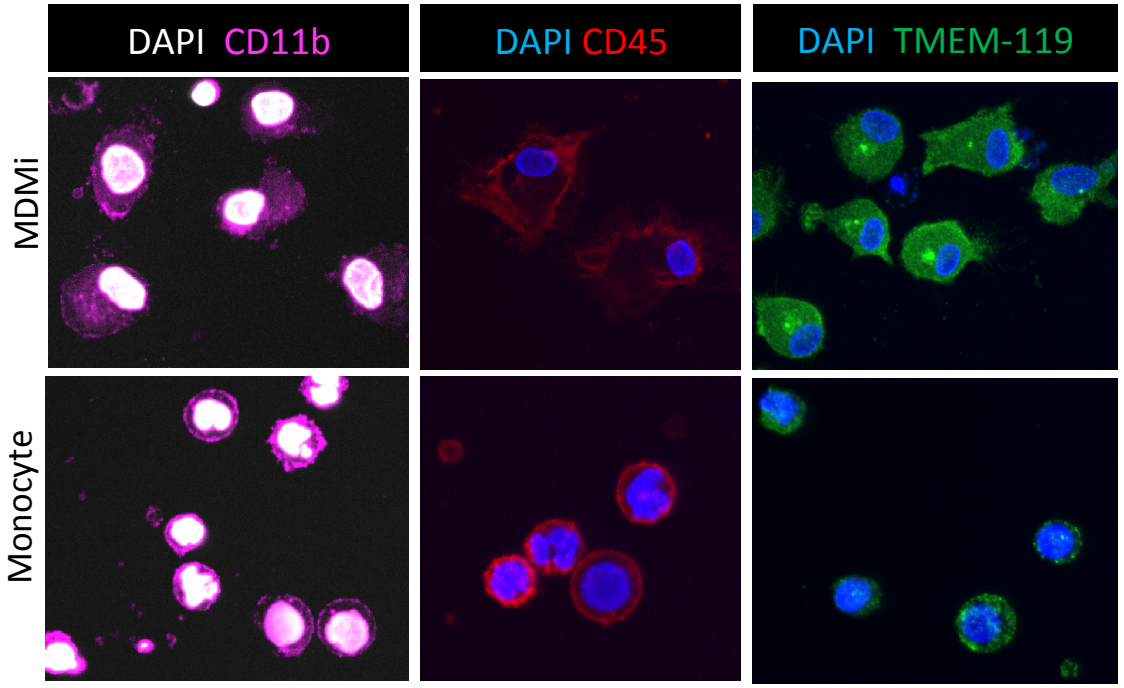
# Induced Microglia from Monocytes

- Human peripheral blood monocytes cultured with a CNS cytokine cocktail to induce differentiation to a microglial phenotype

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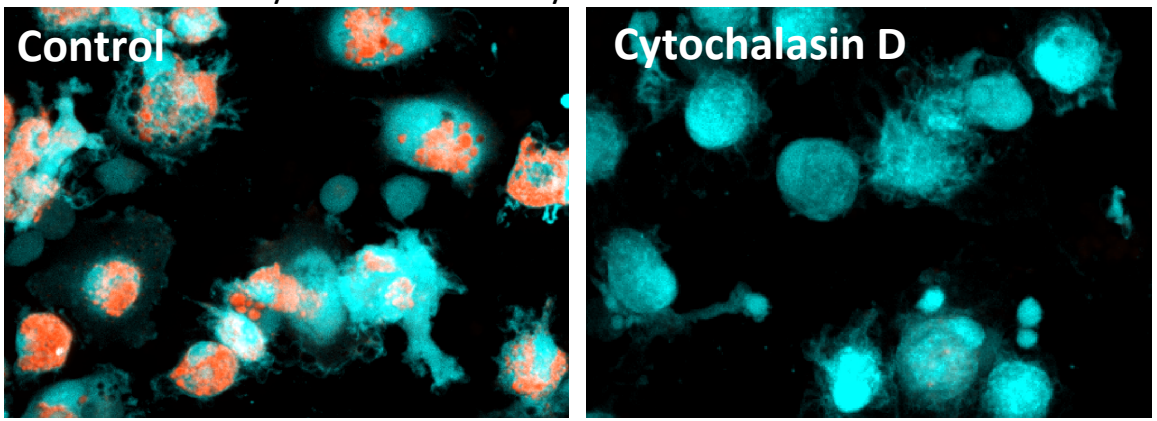
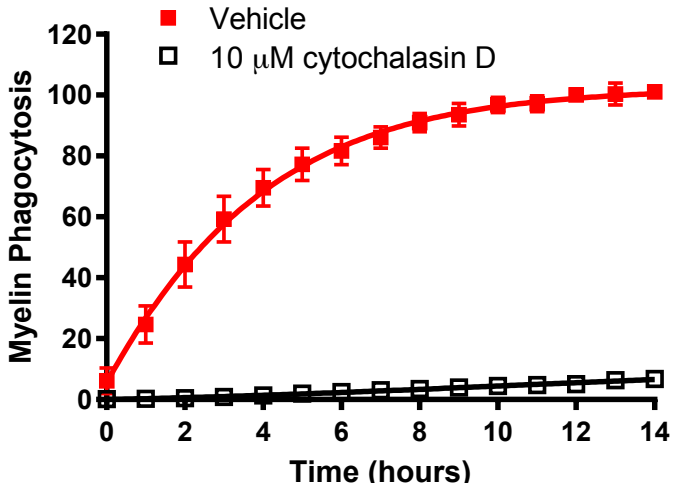
# Phagocytosis of a Myelin Basic Protein Membrane Preparation

- The phagocytosis of myelin is important during neuronal restructuring and the persistence of myelin debris may contribute to neuroinflammation in a range of diseases
- We have generated human pH-rodo-labelled myelin basic protein (pH-rodo-MBP) for use as a model cargo in phagocytosis assays
- Uptake monitored over time by high-content confocal microscopy in a 96-well plate format
- Time-dependent uptake of pH-rodo-MBP by human monocyte-derived microglia that was sensitive to disruption of the actin-cytoskeleton with cytochalasin D.




MDMi
pHrodo-MBP

pH-rodo-MBP uptake after 12 hours




Video of myelin phagocytosis assay available at:
http://celentyx.com/imaging_cro_induced_microglia_phagocytosis_assays.html

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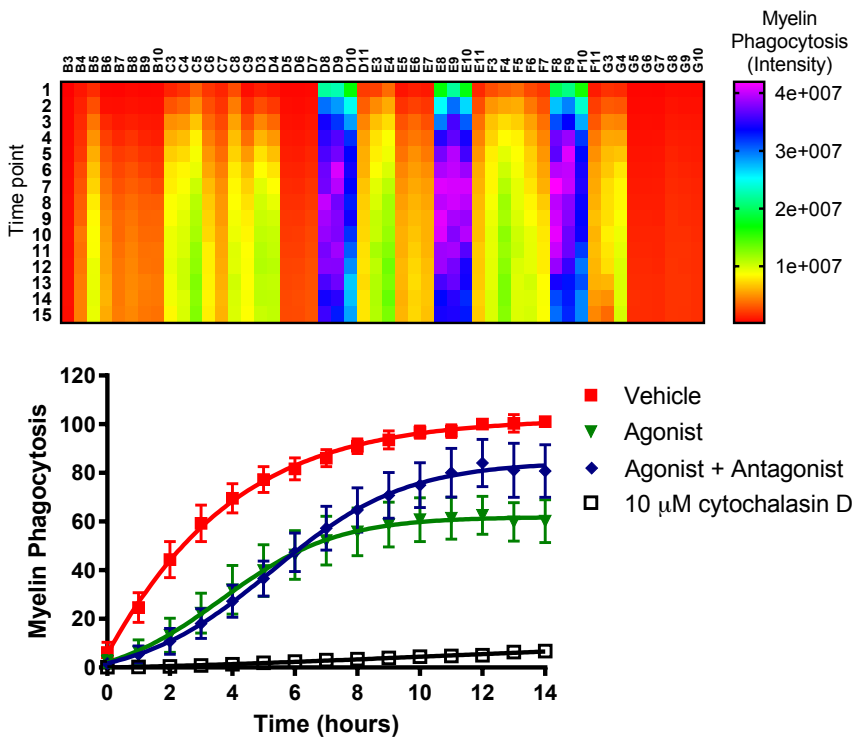
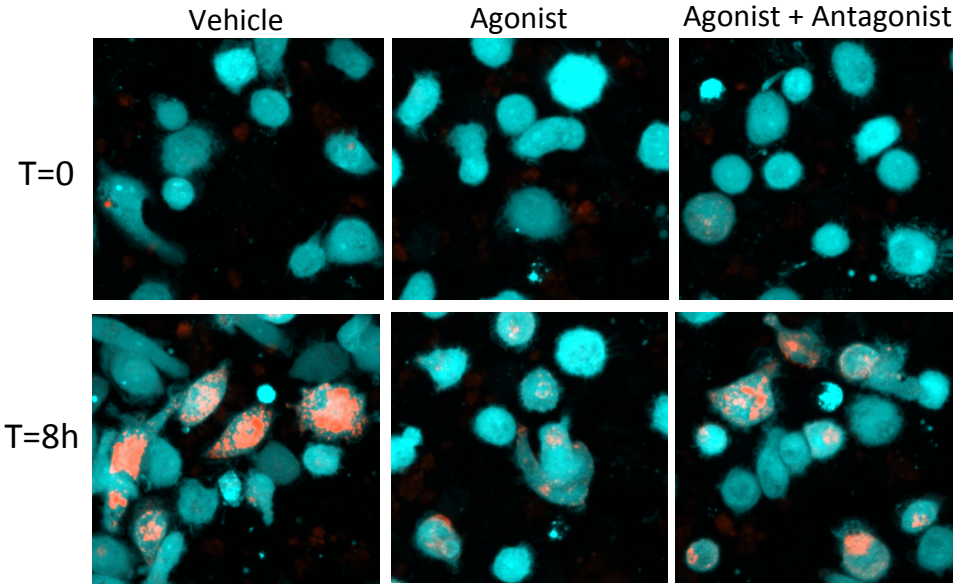
# Modulation of Myelin Basic Protein uptake by Small Molecules

Plate-based format allows testing the impact of multiple modulators of phagocytosis

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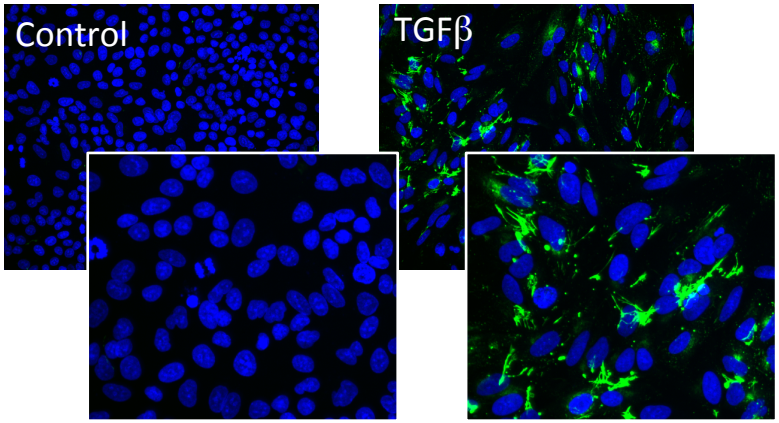
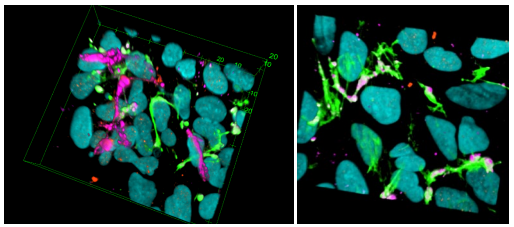
# CELL-BASED READOUTS I

## Quantification of fibrotic end-points by high-content imaging



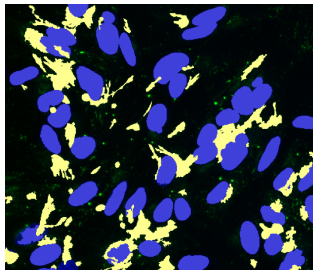

- A human lung epithelial cell line stimulated with TGFβ and **nuclei** and **fibronectin** stained.
- Confocal Z-stacks from multiple fields of view from 96- or 384-well plate acquired and analysed.
- Thickness and overlap between matrix components can also be analysed (Images below).

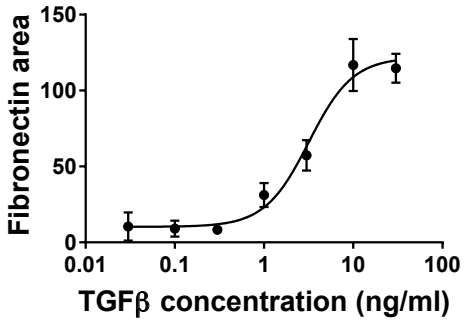
3D projection

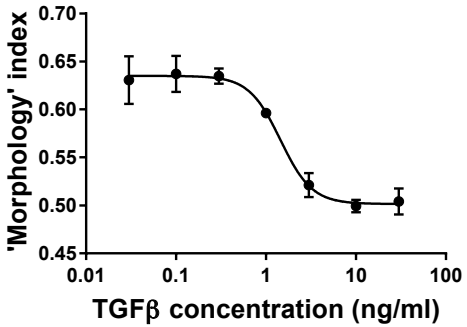


Collagen
Fibronectin
Nuclei

Automated quantitative image analysis:



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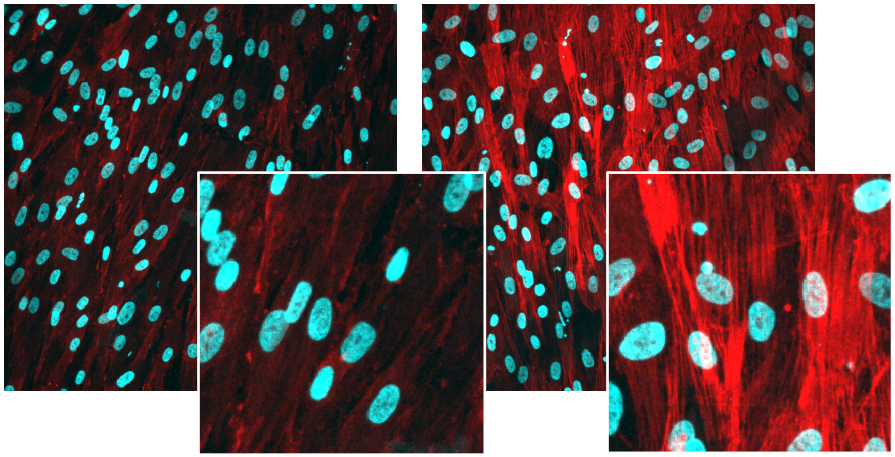
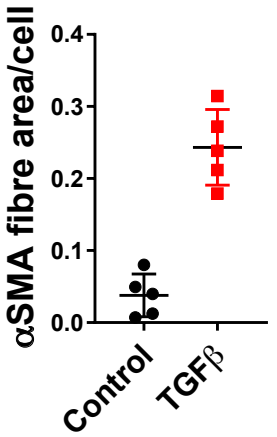
# CELL-BASED READOUTS II

Quantification of α-smooth muscle actin labelling by high-content imaging

- Human primary corneal fibroblasts stimulated with TGFβ and **nuclei** and **α-smooth muscle actin** stained
- Confocal Z-stacks from multiple fields of view from 96- or 384-well plate acquired and analysed.

**Control** **TGFβ**



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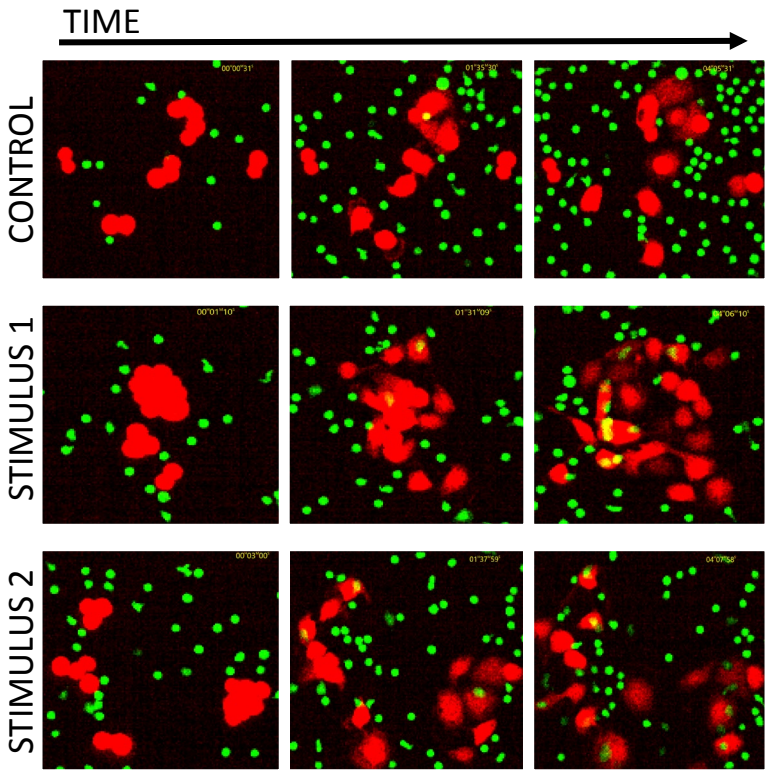
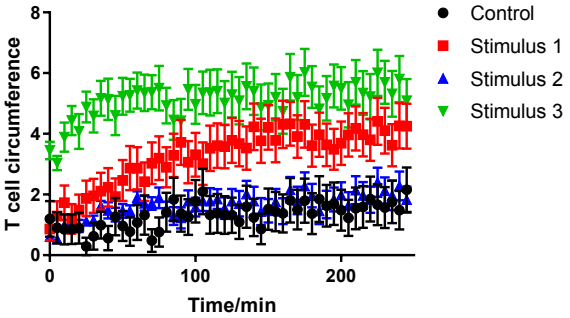
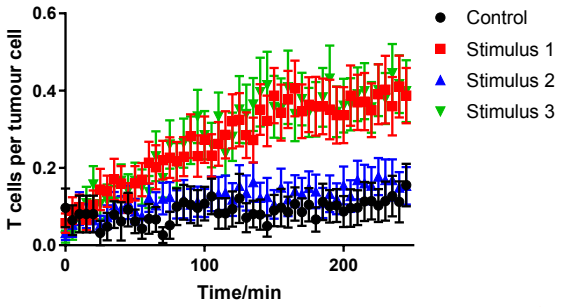
# ENGAGEMENT OF TARGET CELLS BY T CELLS

Labeled **human CD8+ T cells** were incubated with **a labelled cell line**
Z-stacks were acquired by spinning-disk confocal microscopy

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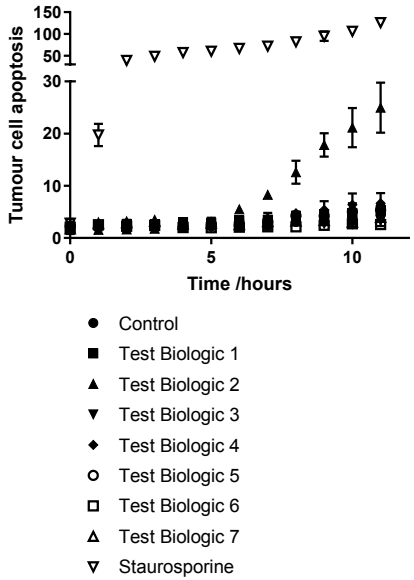
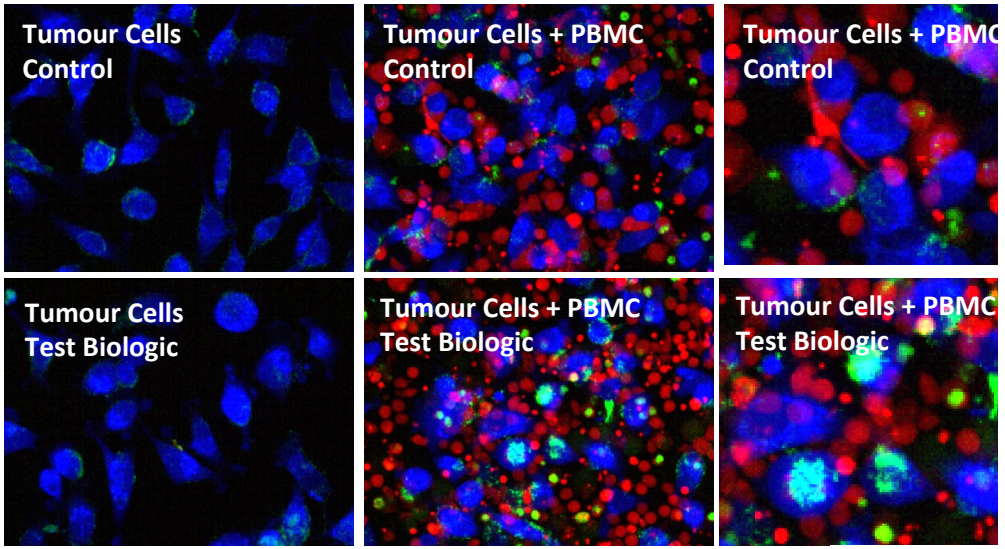
# KILLING OF TUMOUR CELLS BY IMMUNE CELLS

Labeled human **PBMC** were incubated with human labelled **tumour cells**
Apoptosis measured by **caspase activity**
Z-stacks were acquired by spinning-disk confocal microscopy
Time-dependent killing of tumour cells by PBMC promoted by the test biologic




Tumour (A549 cells) PBMCs Apoptosis (Caspase 3/7 activity)




Video of immune cell killing of tumour cells available at:
http://celentyx.com/imaging_cro_t_cell_killing.html

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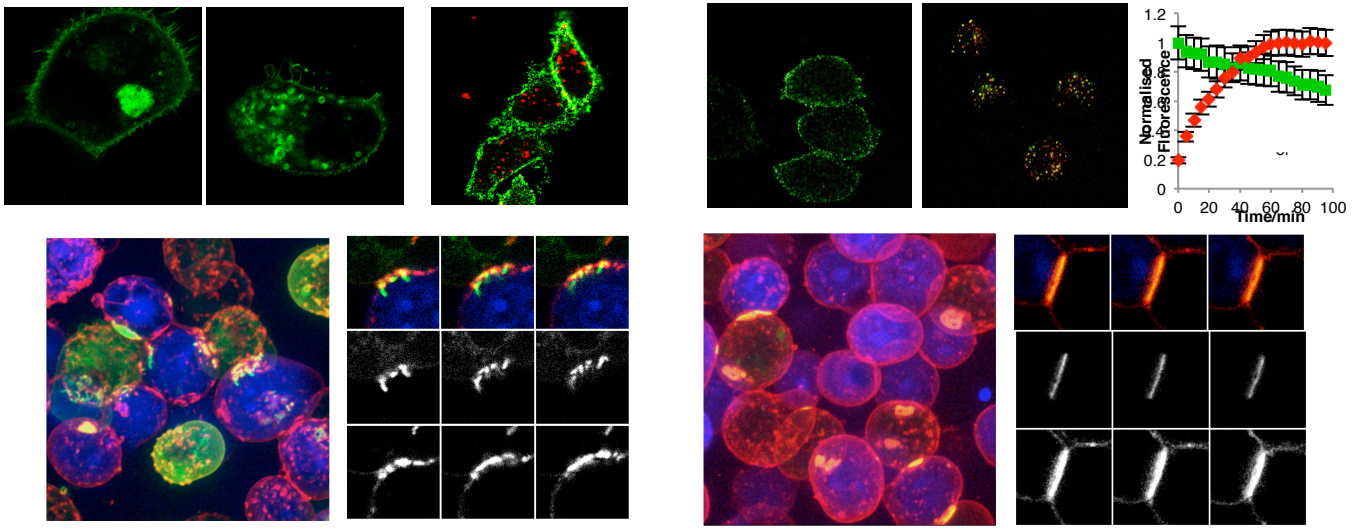
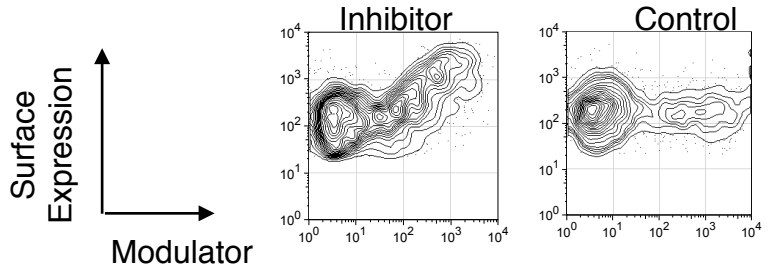
# RECEPTOR TRAFFICKING

A range of fluorescence microscopy techniques applied to track and quantify the movement of therapeutics or receptors within cells

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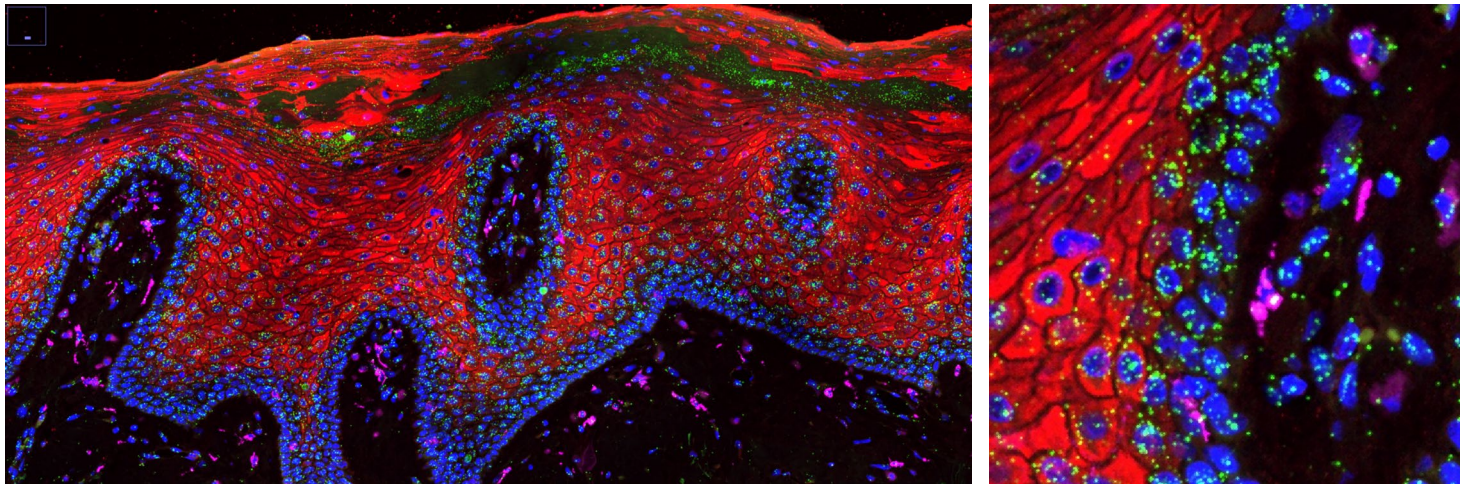
# VALIDATION OF TARGET EXPRESSION IN PATIENT TISSUE

- Detection of either target protein by immunofluorescence or when suitable antibodies not available, mRNA-based detection methods such as RNAScope
- Access to comprehensive human tissue bank covering a broad range of indications with associated clinical information - offering investigation of comparable pathological specimens from hundreds of patients for target validation
- Multiplex immunofluorescence analysis (+RNAScope) by confocal microscopy

Tissue, immune cell and target mRNA (RNAScope detection) in patient tissue



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# SUMMARY

- Celentyx - a responsive and knowledgeable CRO, with highest level scientific and technical expertise applied across the drug discovery pipeline including target validation, screening (both primary screens and secondary assays), benchmarking/differentiation studies, safety and mechanism of activation studies
- The internationally recognised leadership team and scientists have a deep knowledge of therapeutic areas involving the immune system including:
  - Immuno-oncology
  - Autoimmunity & inflammation
  - Fibrosis
  - Neuro-inflammation
- An excellent track record designing bespoke research experiments and programmes to provide solutions to client questions
- Extensive links to hospitals and clinicians, providing outstanding access to healthy donor and patient blood and tissue samples (fresh and fixed) with extensive mining of clinical notes
- Utilization of state of the art technologies to deliver high quality research findings
- Flexible and competitive business models from standard fee-for-service to project partnerships
- Contact Dr Catherine Brady at enquiries@celentyx.com to arrange a no obligation discussion with Celentyx's senior scientists to see how the we can help your project

Celentyx

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