Decoding composition and biology of extracellular matrix (ECM) to identify novel therapeutic targets in fibrosis and solid tumors.

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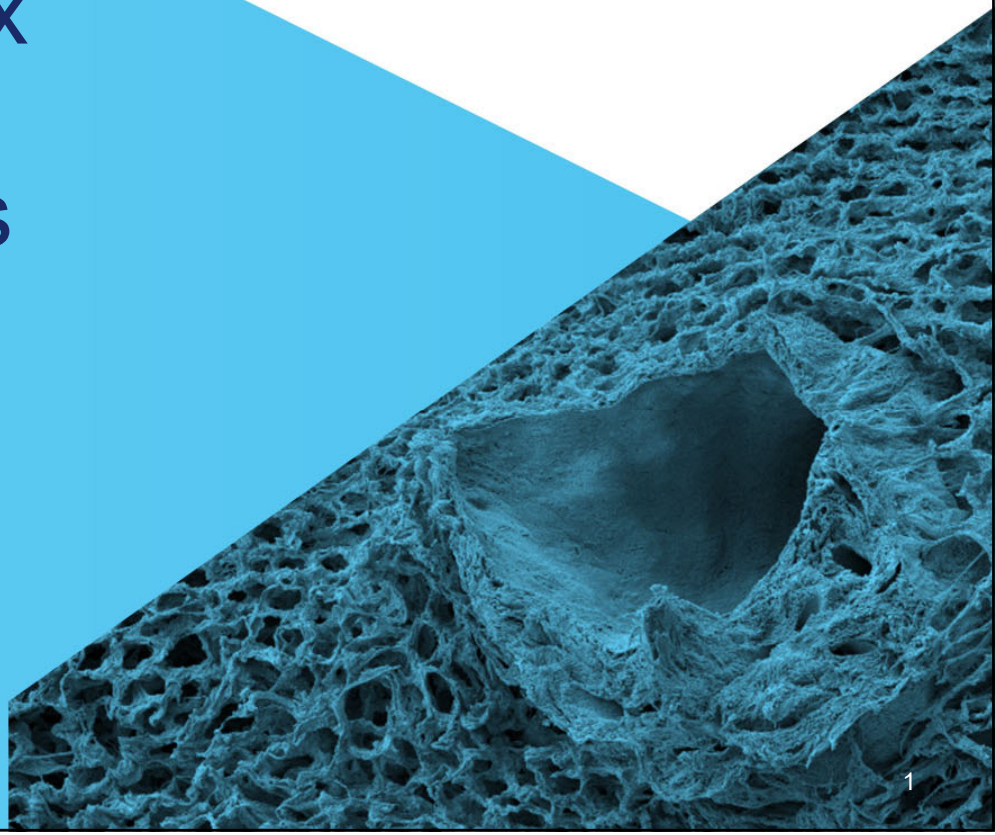
Pavia 20th June 2025



Topics

- Intro to Engitix
- Intro to ECM, its development and applications
- Matrisome
- ECM assays development
- ECM targets' classes

Engitix aims to transform the therapeutic landscape for fibro-inflammatory diseases and solid tumors by decoding the ECM

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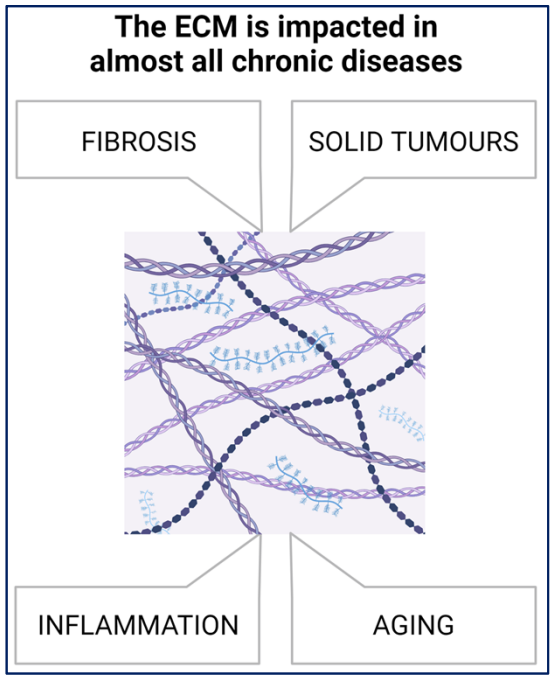
>35% of deaths are due to fibrosis and solid tumours[1]

The ECM is impacted in almost all chronic diseases



ECM biology is a novel therapeutic opportunity

- The ECM is a dynamic, bioactive environment[2]
- ECM is comprised of ~300 proteins (matrisome)
- ECM environment modified by >1000 secreted proteins
- Changes to ECM alter cell function & organ physiology
- ECM is a largely untapped source of innovative targets

The ECM company

Founded 2016

- **World's largest human diseased-ECM dataset from decell'ed tissues**
- **Patient-centric approach**
  >2000's of samples with data
- **Proprietary pipeline**
  3x assets H2L/LO stage
- **Partnerships worth > $800m biodollars**
- **Multidisciplinary team**
  55 people in 13,000 sqft London-based facility
- **>$30MM raised to-date from seed & Series A**
  Access to $28MM work-for-equity with Dompe

(1) Mutsaers et al., Journal of Translational Medicine (2023; (2) Frantz C et al., J Cell Sci (2010)

Track record of successfully identifying and validating novel therapeutic targets using state-of-the-art ECM discovery platform

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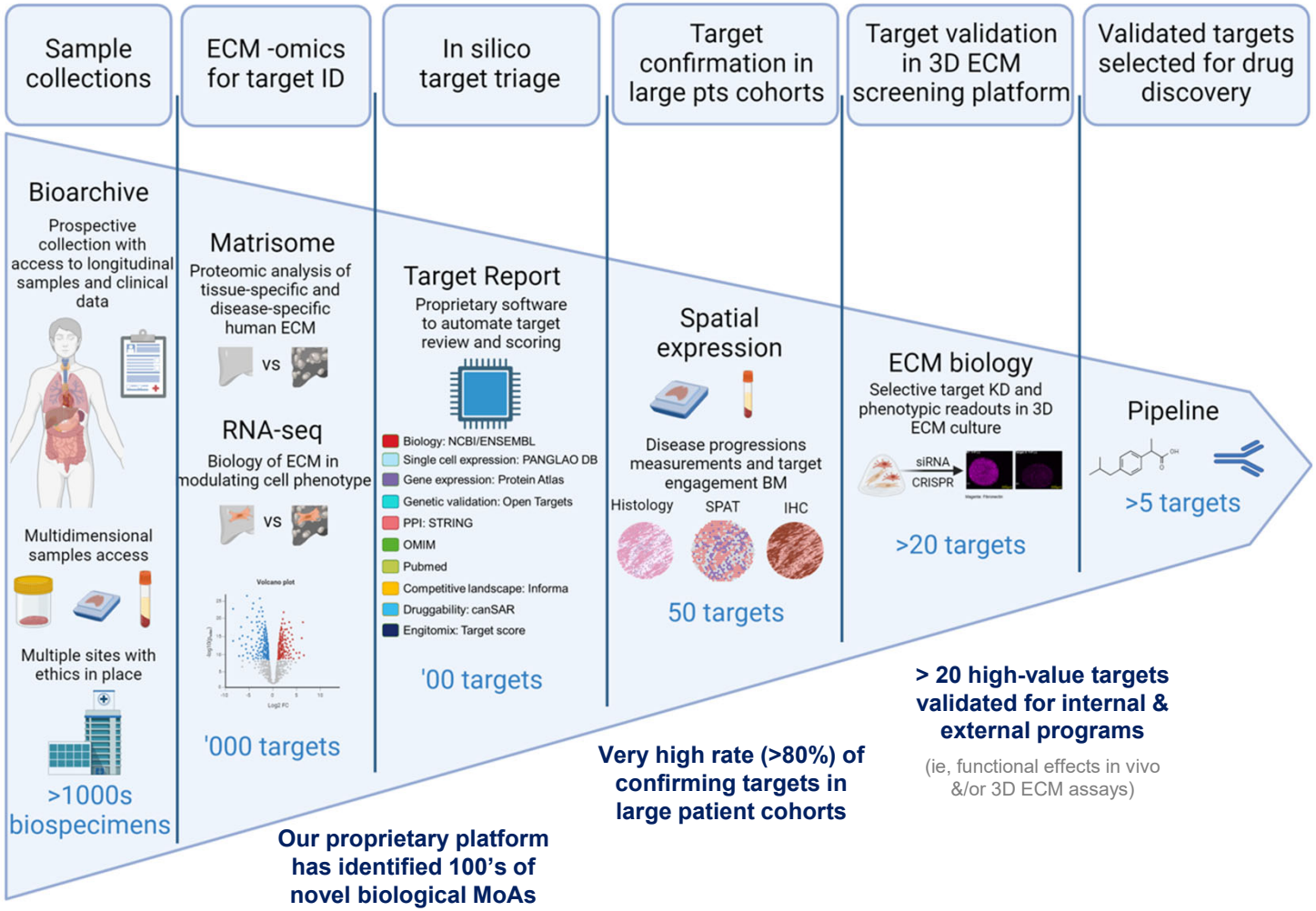
All discovery efforts start with well characterized patient samples

Deep clinical and pathological evaluation



Our proprietary platform has identified 100's of novel biological MoAs

Very high rate (>80%) of confirming targets in large patient cohorts

> 20 high-value targets validated for internal & external programs

(ie, functional effects in vivo &/or 3D ECM assays)

Several validated target programs are progressing within external partner & internal pipelines

Engitix aims to **transform the therapeutic landscape** for multiple diseases **by decoding the ECM**

Multiple assets in our discovery pipeline targeting fibro-inflammation, fibrosis and solid tumors

Programs	MoA	Indication(s)	Target Validation	Discovery	Upcoming milestones	Commercial rights
EGTX001	Inhibition of matricellular target driving fibrosis	Pan-anti fibrotic			DC 1Q26	ENGITIX THERAPEUTICS
EGTX003	Inhibition of inflammatory intra- cellular target	Inflammation / fibrosis			DC 2Q26	ENGITIX THERAPEUTICS
EGTX004	Targeted-delivery by anchoring on ECM protein	Solid tumours and advanced fibrosis			DC 3Q26	ENGITIX THERAPEUTICS
Confidential	Confidential	Advanced MASH fibrosis			Confidential	Takeda
Confidential	Confidential	FSCD			Confidential	Takeda

DC: Development Candidate
MASH: Metabolic Associated SteatoHepatitis
FCSD: Fibrostenotic Crohns' Disease

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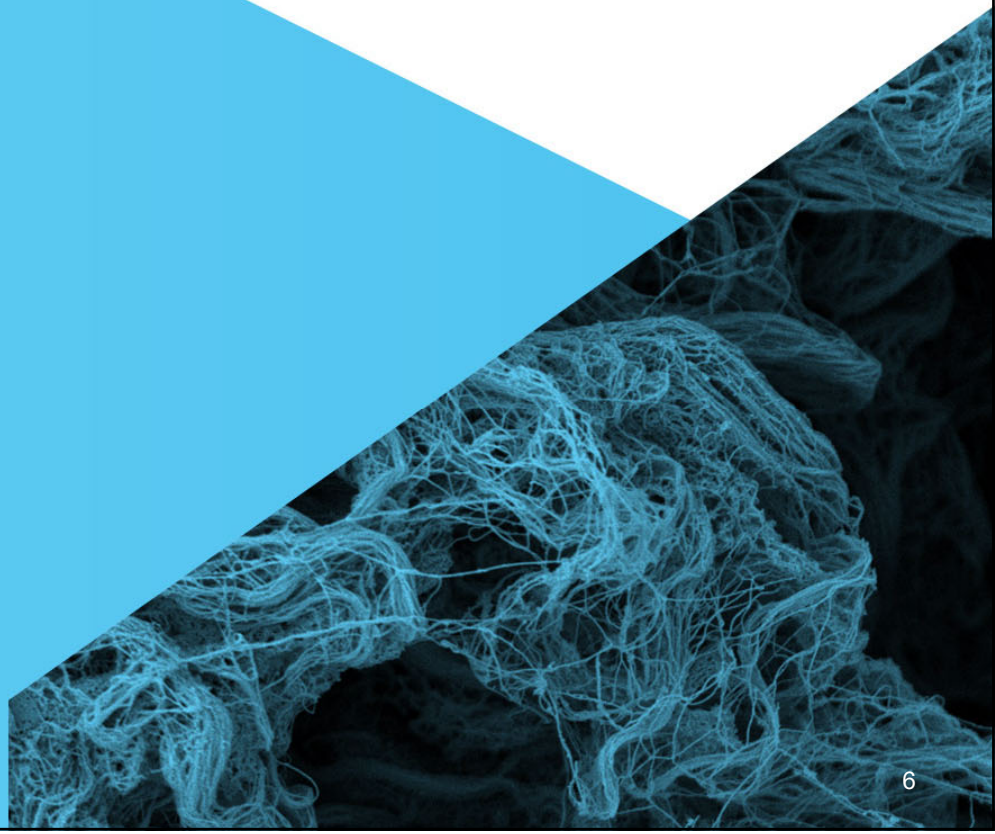
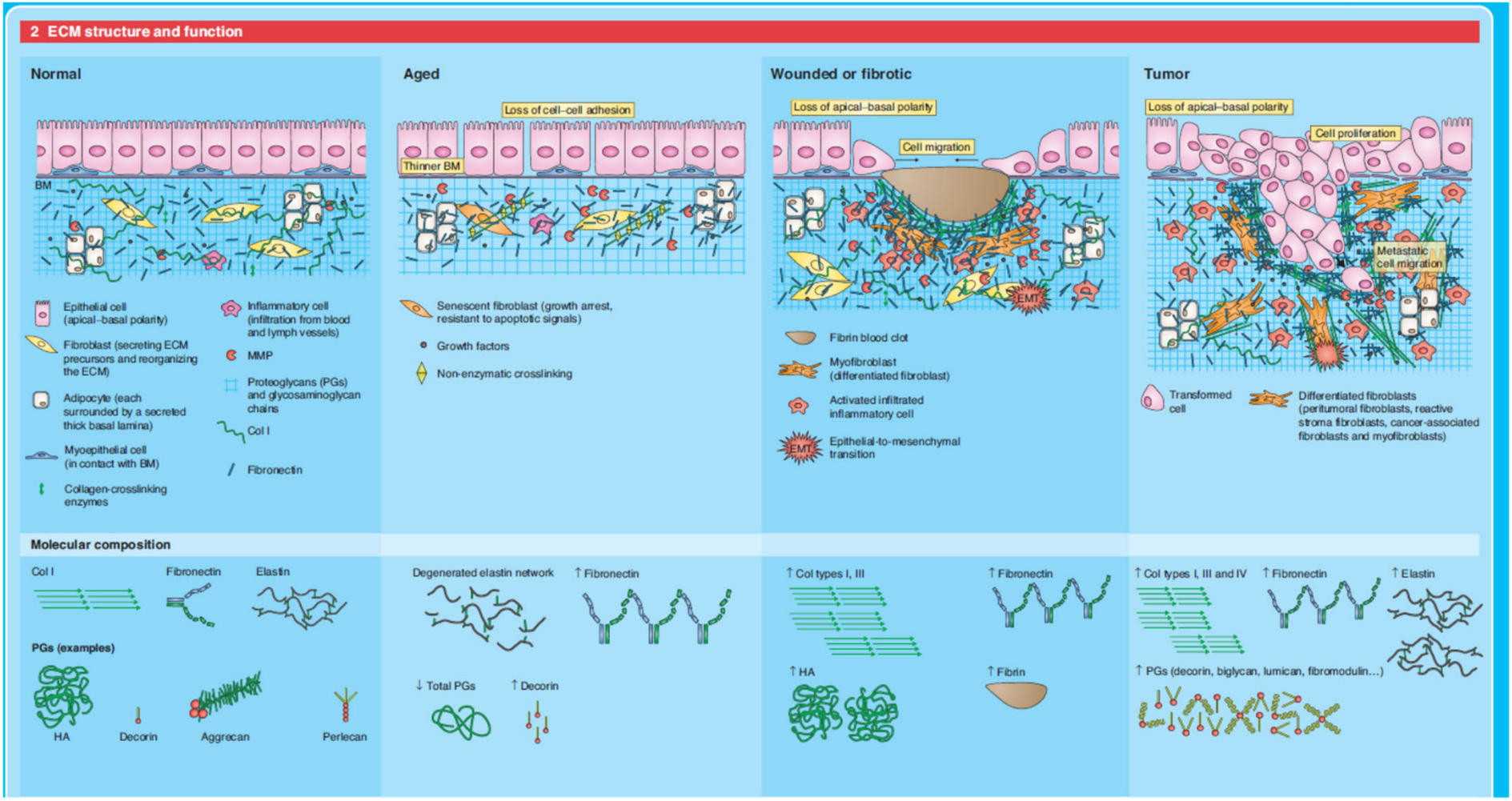
ECM: development and applications



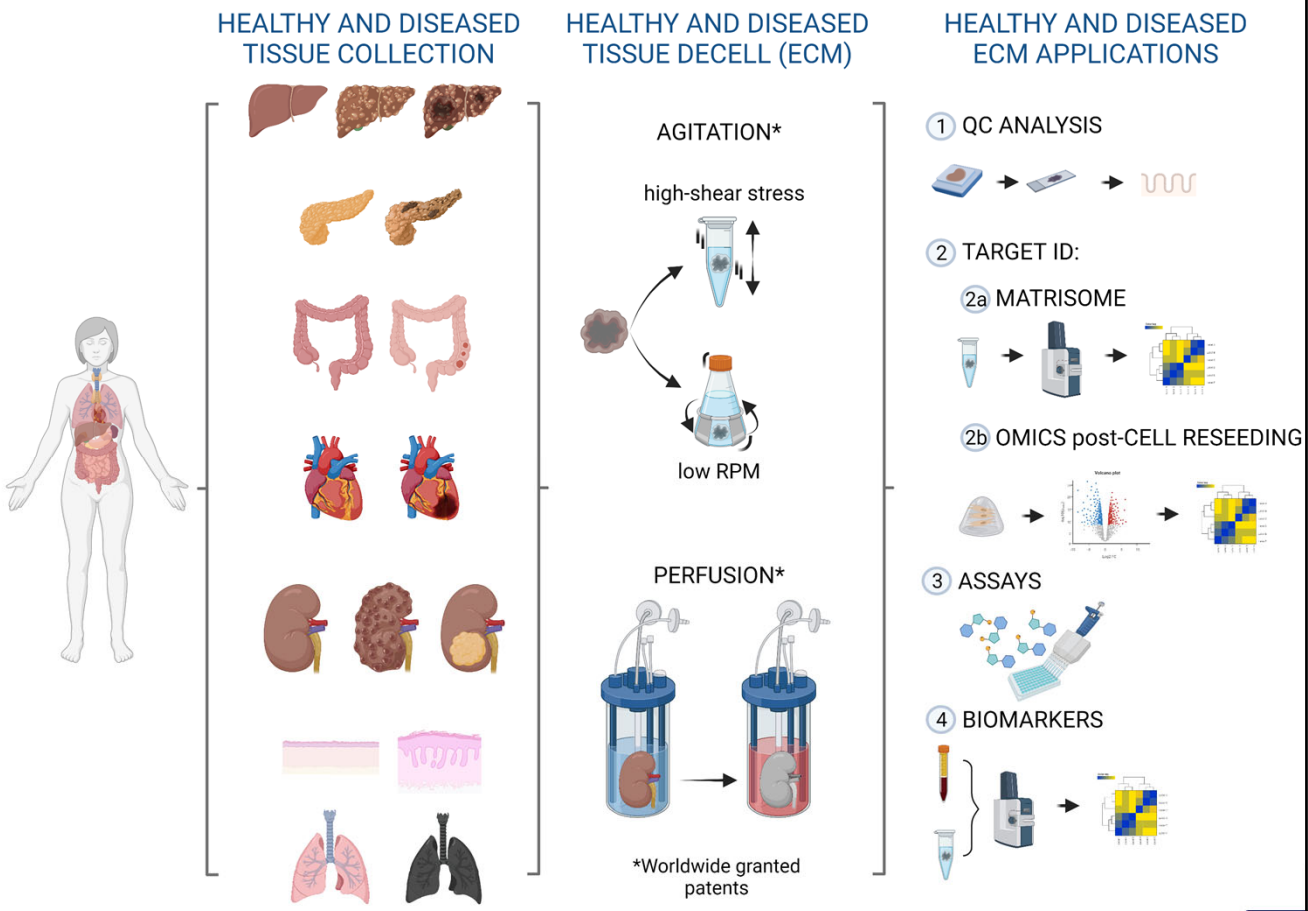
The extracellular matrix at a glance



Frentz C et al. J of Cell Sci (2010)

End-to-end platform established to decode composition and biology of ECM

- Established tissue collection for both healthy and diseased tissues
- Proprietary decellularization protocols
  - Agitation: wedge tissue resections, biospies
  - Perfusion: whole organs
- Established QC panel for confirming tissue decell efficiency by assessing cellular material removal and ECM preservation
- Multiple application of resultant ECMs
  - Target ID: i) **ECM composition** by matrisome analysis, and ii) **ECM biology** by mics analysis of reseeded cells in different ECMs
  - **ECM-based assay** to study ECM lifecycle
  - Biomarkers: i) ECM-enhanced biomarkers discovery in serum, and ii) targeted-mass spec



Mazza et al, Sci Rep (2015)
Mazza et al, Sci Rep (2017)
Mangione, et al, J Proteomic (2017)
Giuffrida et al, IBD (2019)
Mazza et al, Cells (2020)
Al-Akkad et al, Cells (2022)

Human whole organ decellularisation

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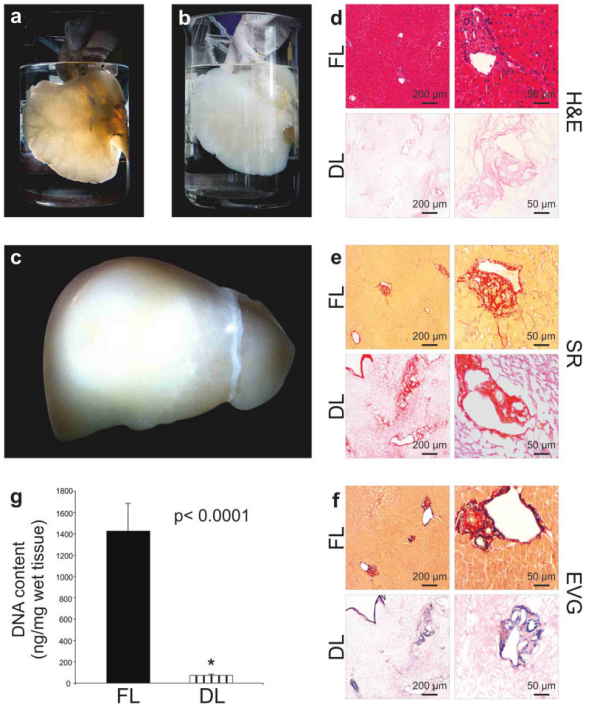
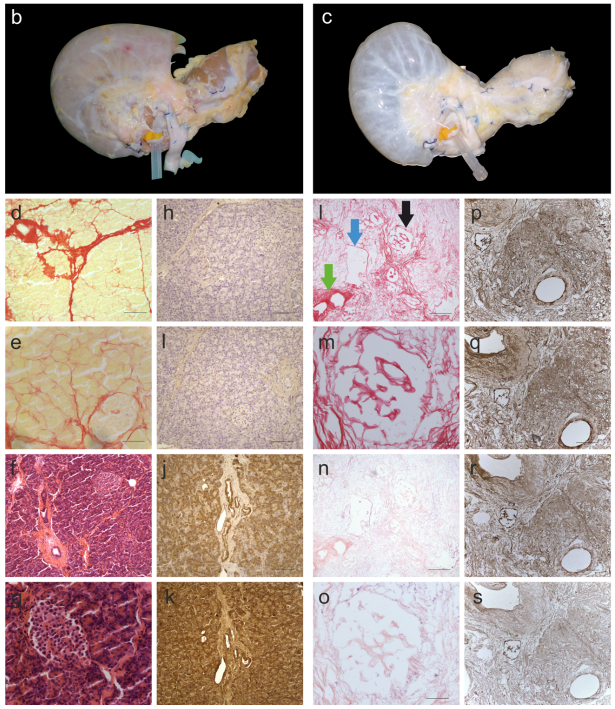
Healthy whole human liver



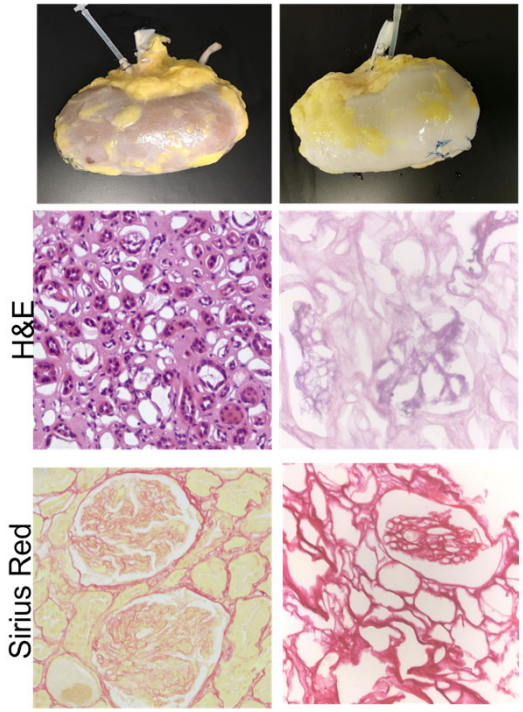
Mazza et al, Sci Rep (2015)*
* First ever human liver ECM publication

Healthy whole human pancreas



Al Akkad et al, Cells (2022)

Healthy whole human kidney



Unpublished

Human tissue agitation-decellularisation

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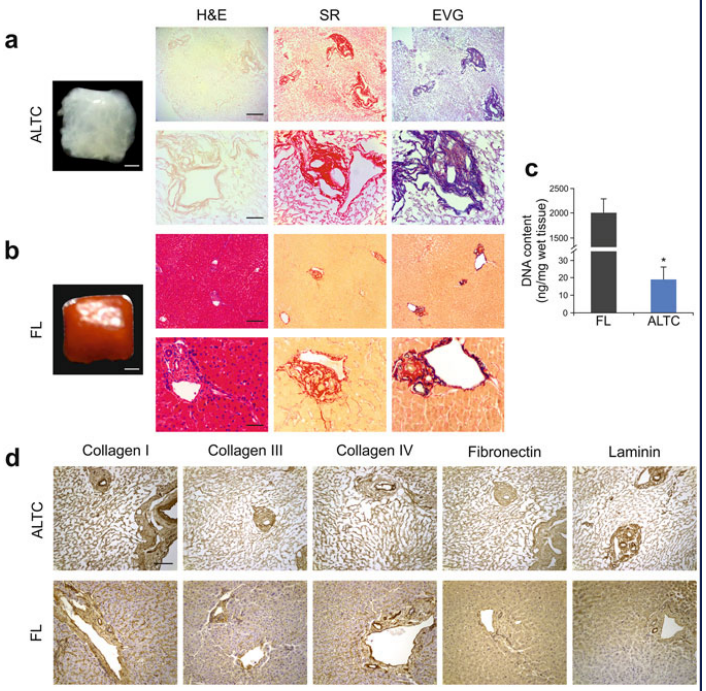
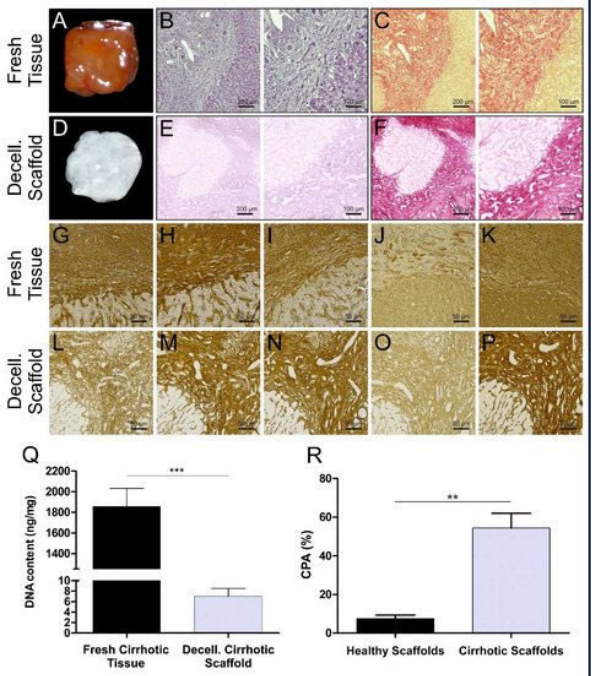
Healthy human liver



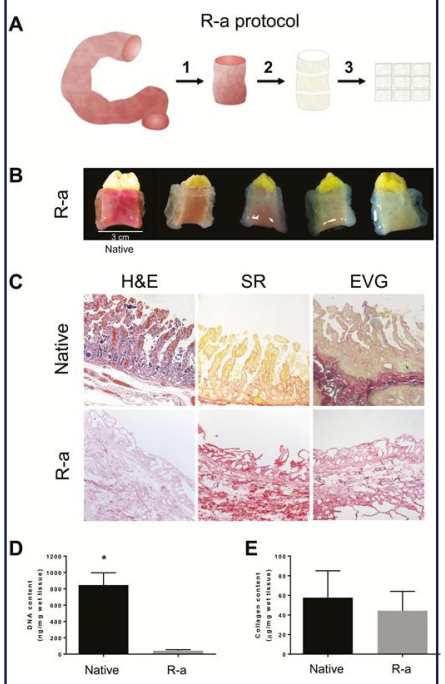
Mazza et al, Sci Rep (2017)

Cirrhotic human liver



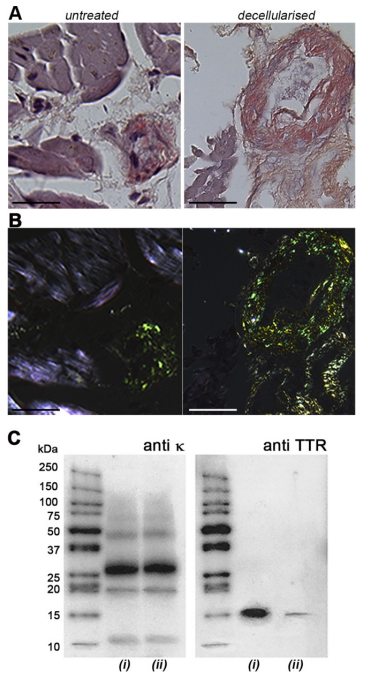
Mazza et al, Cells (2020)

Healthy human intestine



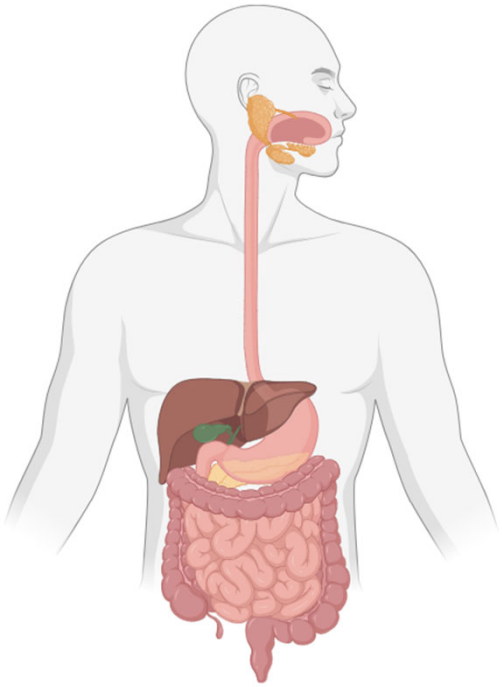
Giuffrida et al, Cells (2019)

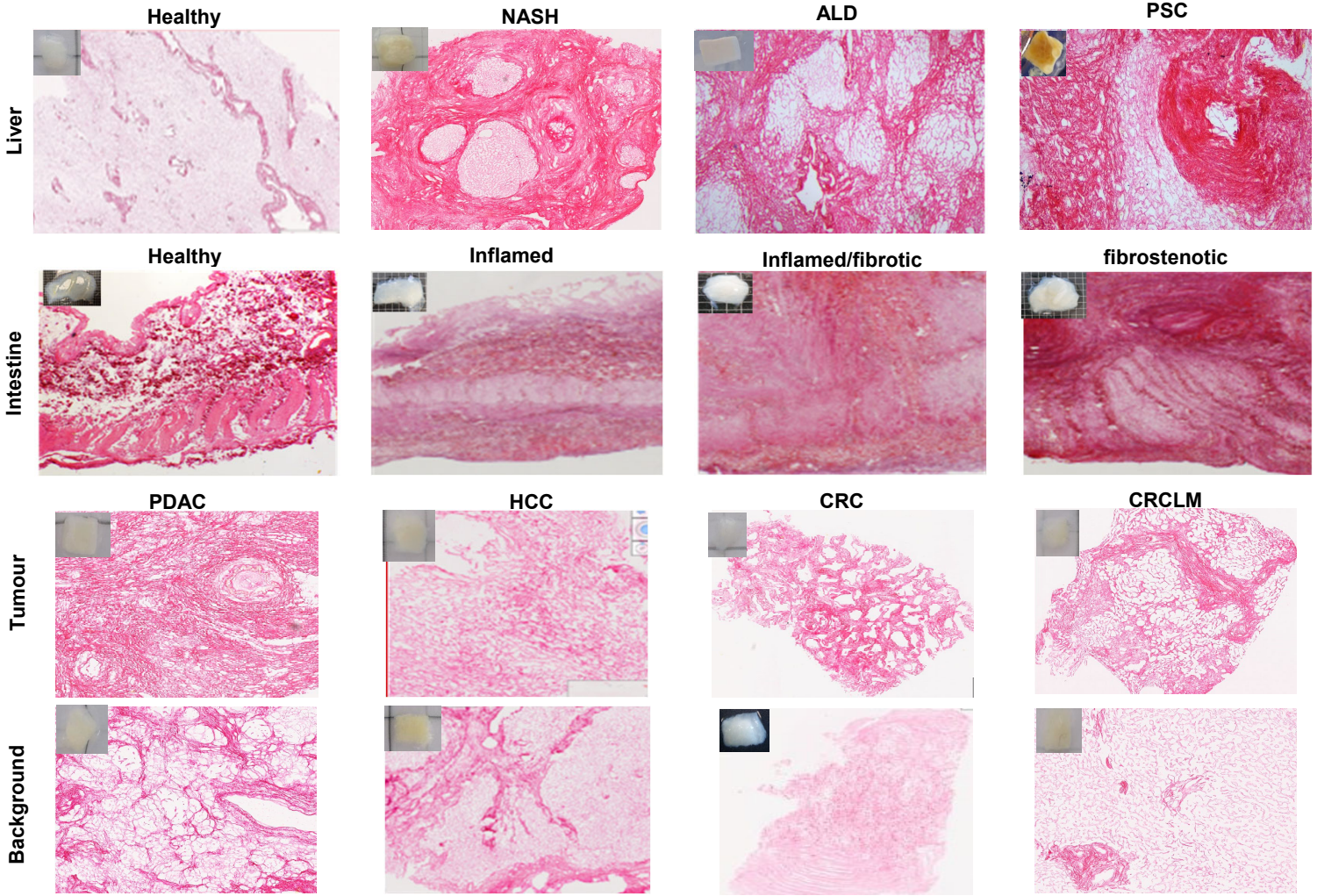
Amyloid human heart



Mangione, et al, J Proteomic (2017)

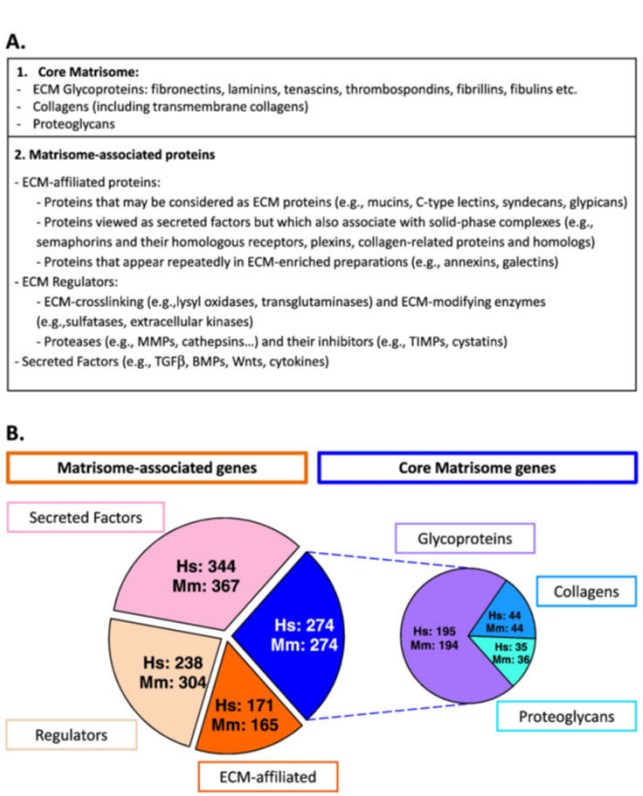
Established one of the world's largest gastrointestinal and hepatopancreatic biliary human matrisome dataset

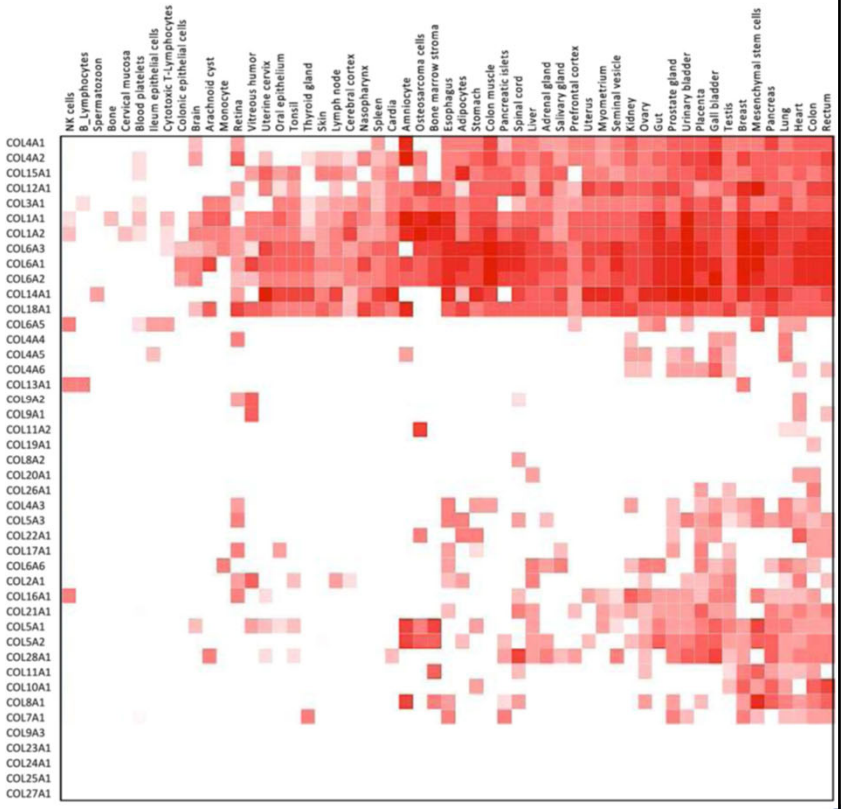




Matrisome

- The extracellular matrix has long been considered as a structural component of tissue organization
- In 1984, Martin and colleagues coined the term “matrisome” in the context of basement membranes to define “supramolecular complexes of matrix components which are the functional units of the forming extracellular matrix”
- In 2016, Naba and colleagues curated manually the lists obtained computationally and, based on structural or functional features, distinguished core matrisome proteins from matrisome-associated proteins.
- Recent development of experimental techniques (e.g tissue decell) have triggered a thorough characterization of ECM composition by proteomic and other “omic” approaches to provide new insights into understanding of the enduring fascinating mysteries of ECM biology

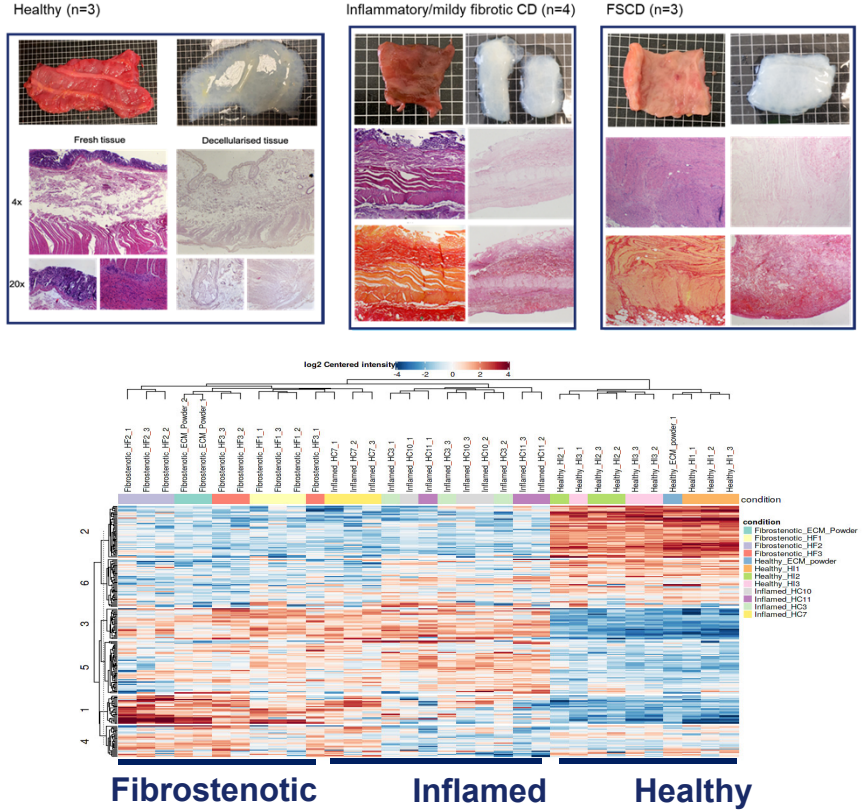




The Pathogenic Matrisome – Unveiling a Key Disease Driver

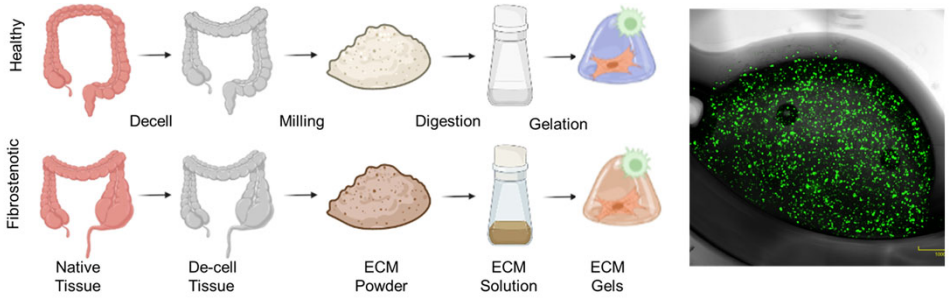
ECM COMPOSITION

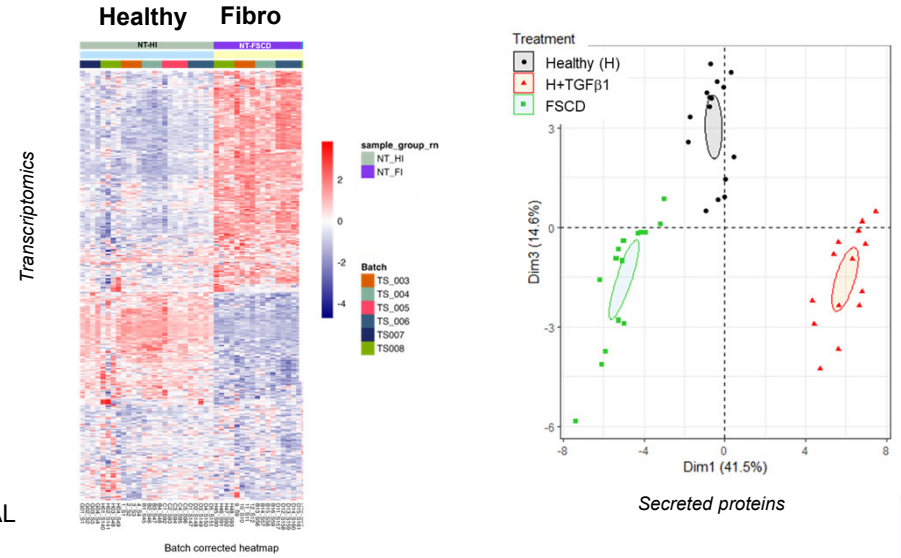
We use proprietary tissue decellularization techniques to study the matrisome (ECM & associated proteins)



ECM BIOLOGY

Incorporation of FSCD ECM in our 3D assay reveals how changes in the matrisome affect cell behaviour and drive fibrosis





CONFIDENTIAL

Engitix unpublished data

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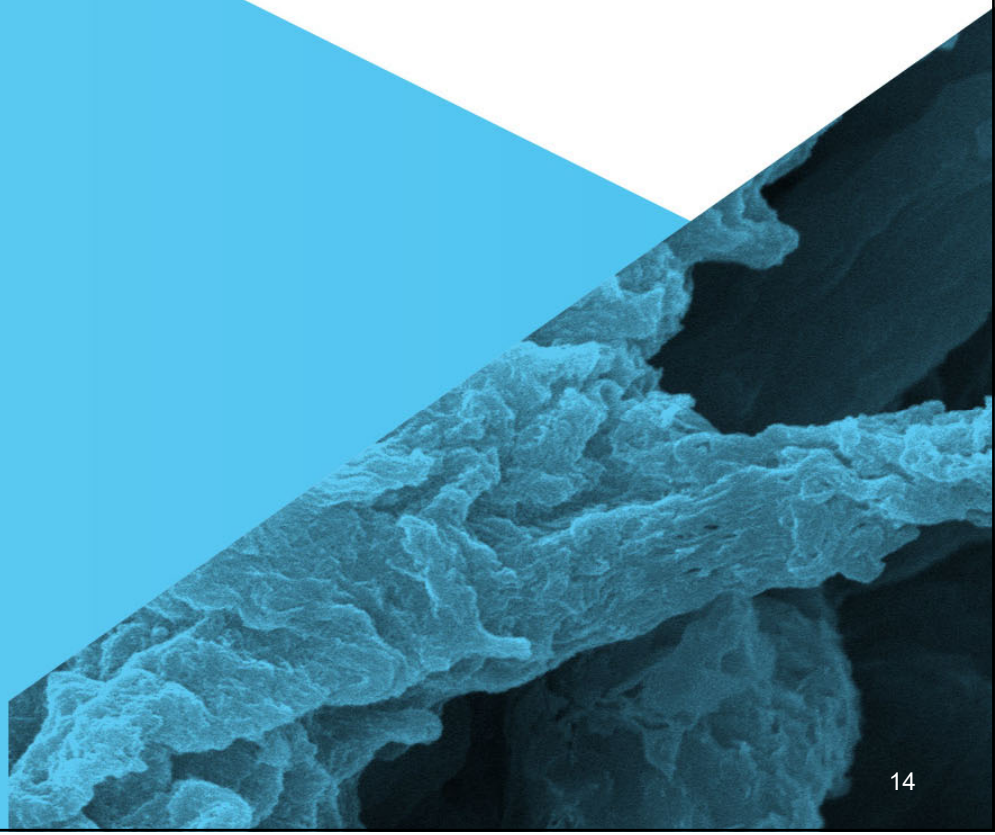
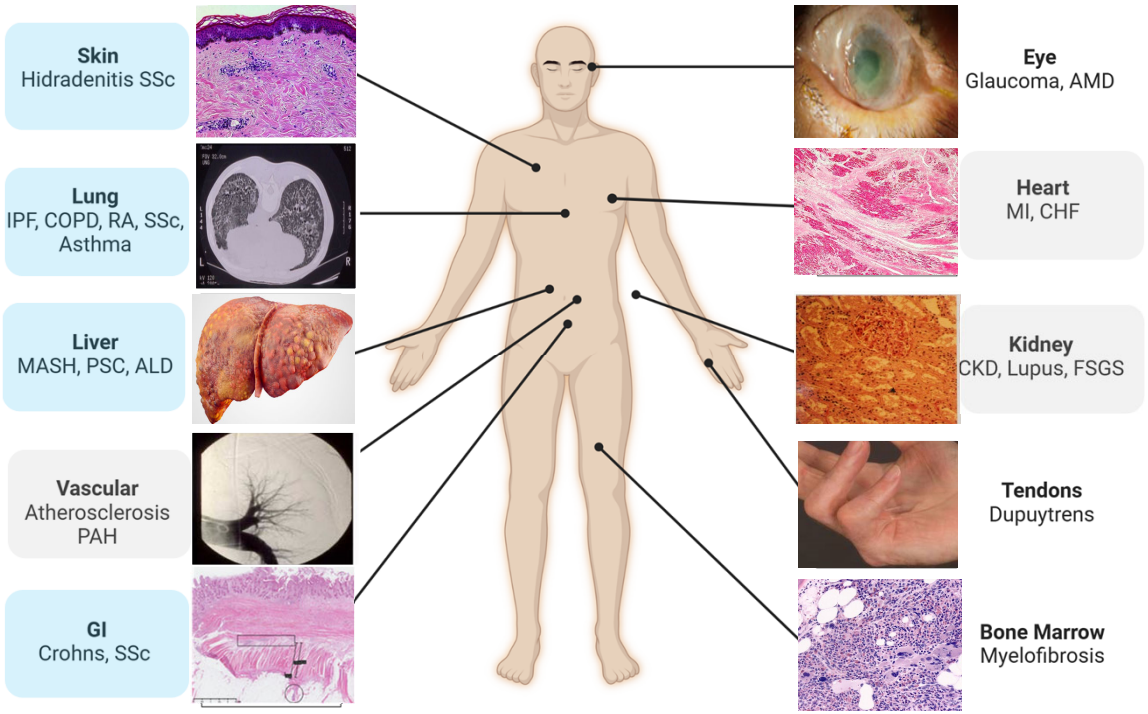
ECM-centric assay to functionally validate targets



Chronic tissue damage promotes pathogenic changes in ECM driving loss of organ function



Established indications at Engitix

Early development activities (currently not active)

Chronic tissue damage drives fibroblast activation resulting in ECM remodelling and altered deposition. This forms a pathogenic matrisome that sustains a chronic fibro-inflammatory process and organ dysfunction.

The pathogenic ECM/matrisome that fuels fibrosis progression is characterized by differential:

- **Composition**
  - Quality and Quantity: Enriched with growth factors, chemokines, and cytokines as well as increased ECM
  - Architecture: Modified fibre structures (fibre multiplicity)
- **Biology**:
  - Activation and differentiation of mesenchymal and immune cells (e.g., myofibroblasts & macrophages)

Tissue homeostasis can be restored in fibrotic tissues by activating fibrolytic pathways

Established novel end-to-end ECM life cycle assays to decode fibrogenesis and fibrolysis

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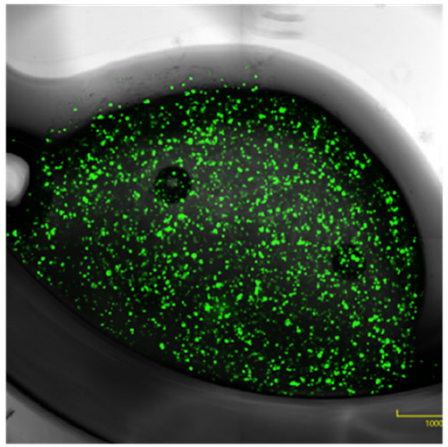
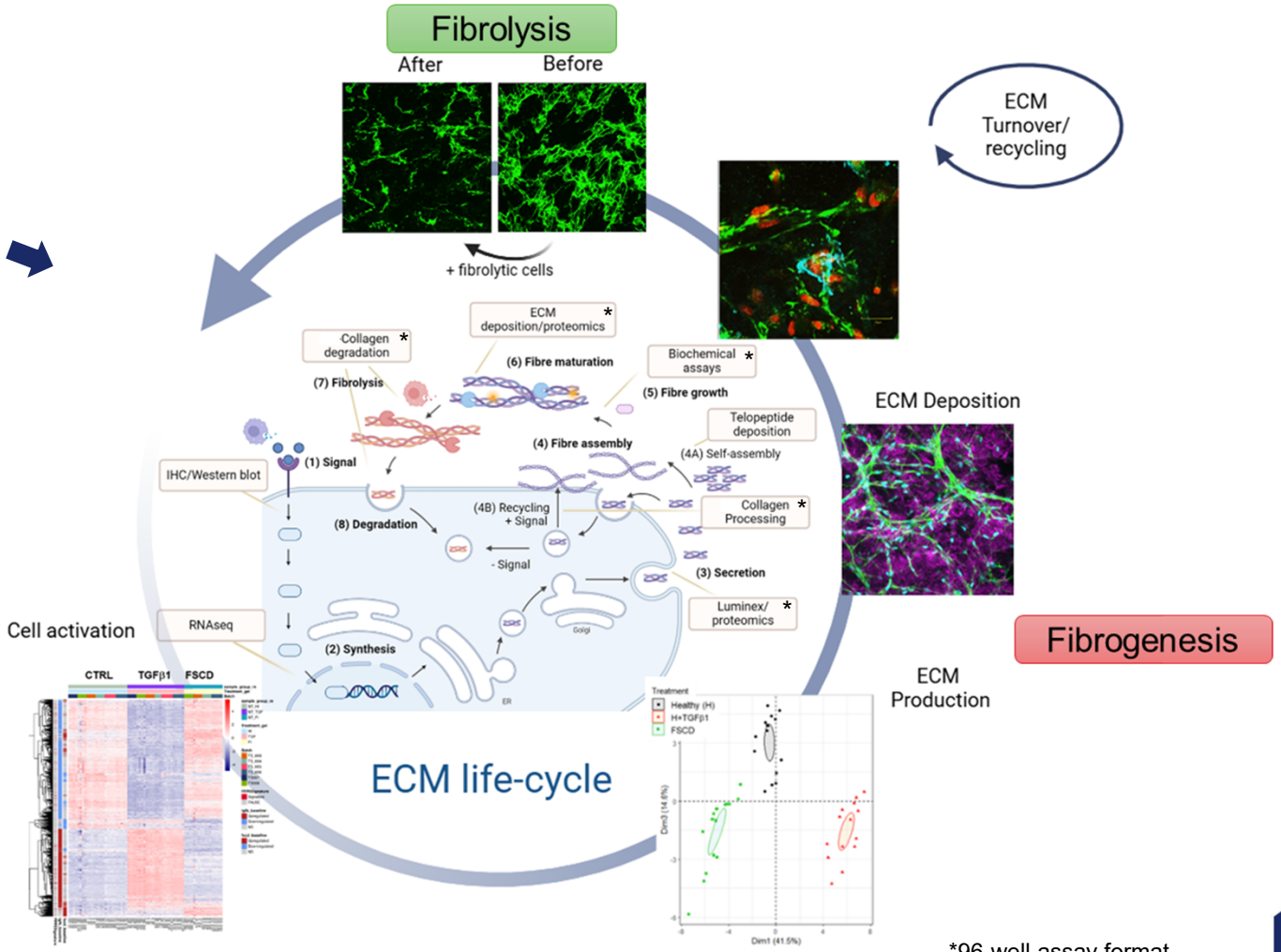
2D and 3D mono and co-culture myofibroblast macrophage ECM Disease Platform

+/- Healthy or diseased ECM

+/- Pro-fibrotic &/or inflammatory stimuli

+/- Target modulation (siRNA/shRNA/ antibodies)





*96-well assay format

Proprietary assays and readouts to study ECM life cycle to decode fibrogenesis and fibrolysis

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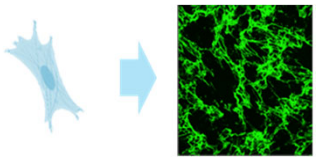
Fibrogenesis assays



Fibrogenesis assay format (10 days):

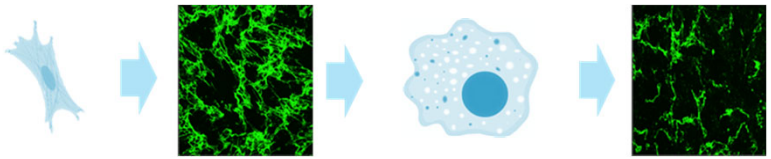
- 3D ECM co-culture (fibroblasts + macrophages)
- Stimuli: disease ECM (vs healthy) and/or pro-fibrogenic stimuli (vs healthy)

Assay	Readouts
ECM synthesis	Gene expression (e.g RNAseq, scRNAseq)
ECM secretion	ELISA, Luminex, proteomic
ECM deposition	High content imaging of ECM fibers, proteomic

Fibrolysis assays

1- ECM-acellular: assessing protease activity in pathophysiological ECM

2- ECM-cells: assessing macrophages activity in clearing pathogenic ECM

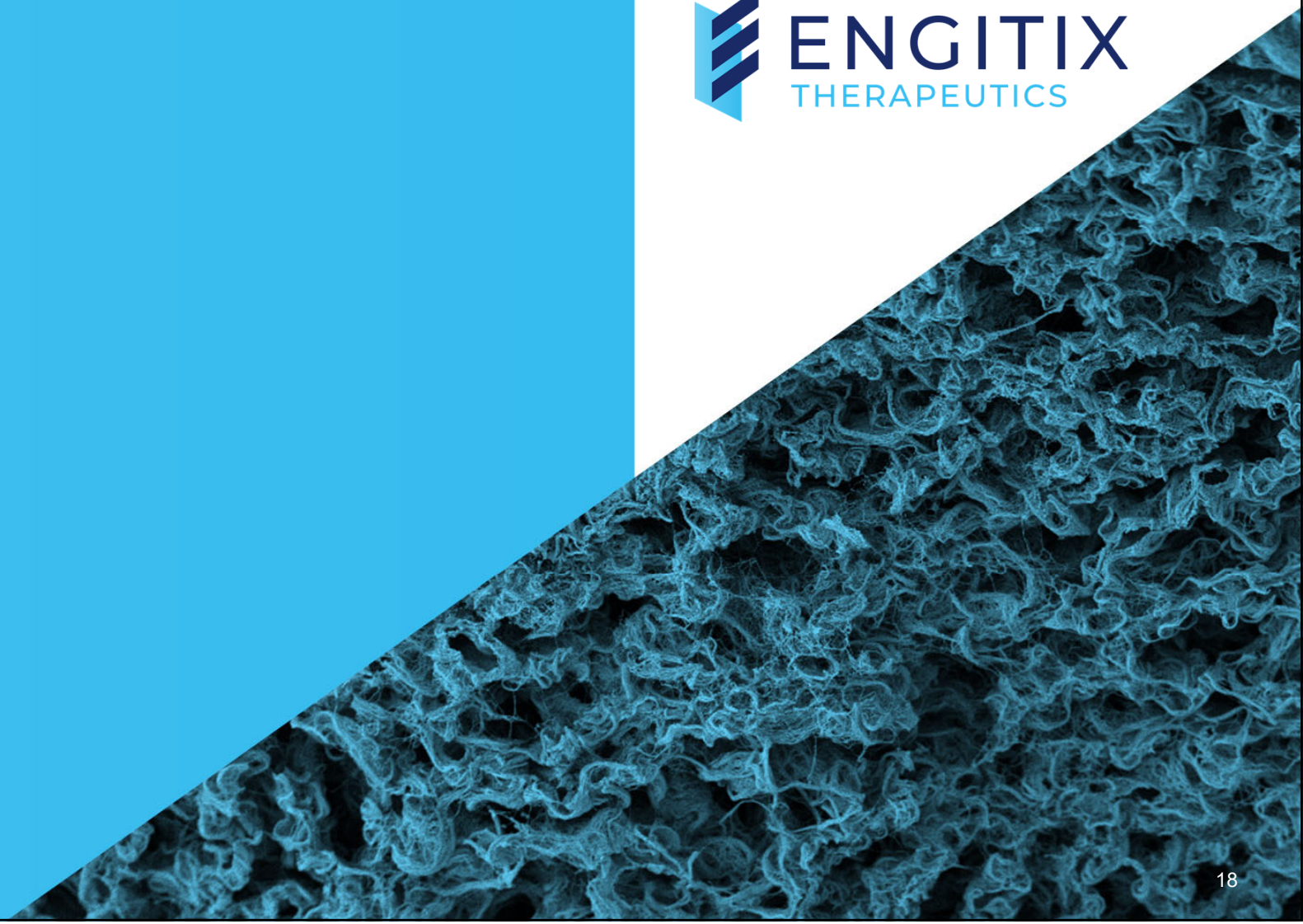


Multi targets perturbation by siRNA/CRISPR coupled with following readouts:

i) High content imaging of ECM fibers

ii) scRNASeq to ID drivers of fibroblast/macrophages reprogramming

IBD platform



Extensive Biobank and Clinical Network Offering Access to Well-Characterized Matched Tissues and Serum for Longitudinal Studies on Fibrosis Progression in IBD

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IBD Bioarchive

Biospecimen type	Number of cases
Crohn's Disease - Surgical resections	> 50
Serum samples	> 80
FFPE – FSCD/Inflamed and matched controls	>100

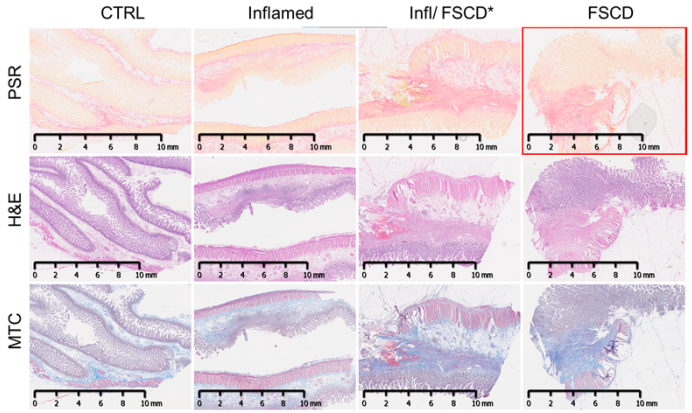
- Prospective collections established in 2023
- Longitudinal samples access
- Matched tissue available
- Clinical data available including lifestyle medications, medical treatment (e.g. incretins), etc.
- Expanding network of clinical sites

Prof Antonio Di Sabatino

Prof Marco Lenti

IBD tissue characterization

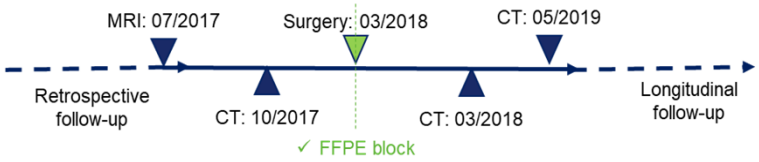
Matched patient full-thickness surgical resections and serum samples available to study the progression of intestinal fibrosis.



IBD clinical data

Access to medical, histology, and imaging reports, allowing for longitudinal follow-up.

Age: 46y Gender: Male Ethnicity: White-British	Disease behavior: stricturing	Date of last CRP: 30/10/2018 CRP reading: 7
Diagnosis: CD	Date of resection: 11/07/2019	Biologics/immunomodulators/steroids at collection: Infliximab and Azathioprine
Age at diagnosis: 17y	MRE/MRI available: 08/01/2019	Previous medications: Pentasa, Amlodipine, Ramipril, Bisoprolol
Disease duration: 29y	Imaging data summary: active disease in distal and terminal ileum with skipped lesions and strictures.	Refractory to TNFα therapy: Symptoms unchanged despite maximal therapy
Site of disease: terminal ileum and caecum	Previous resection: No Previous balloon dilation: No	



Engitix's ECM-Centric Strategy Integrates Matrisome, Transcriptomics, Spatial Profiling, Digital Pathology, and a Novel 3D FSCD ECM *In Vitro* Platform for Target and Biomarker Discovery

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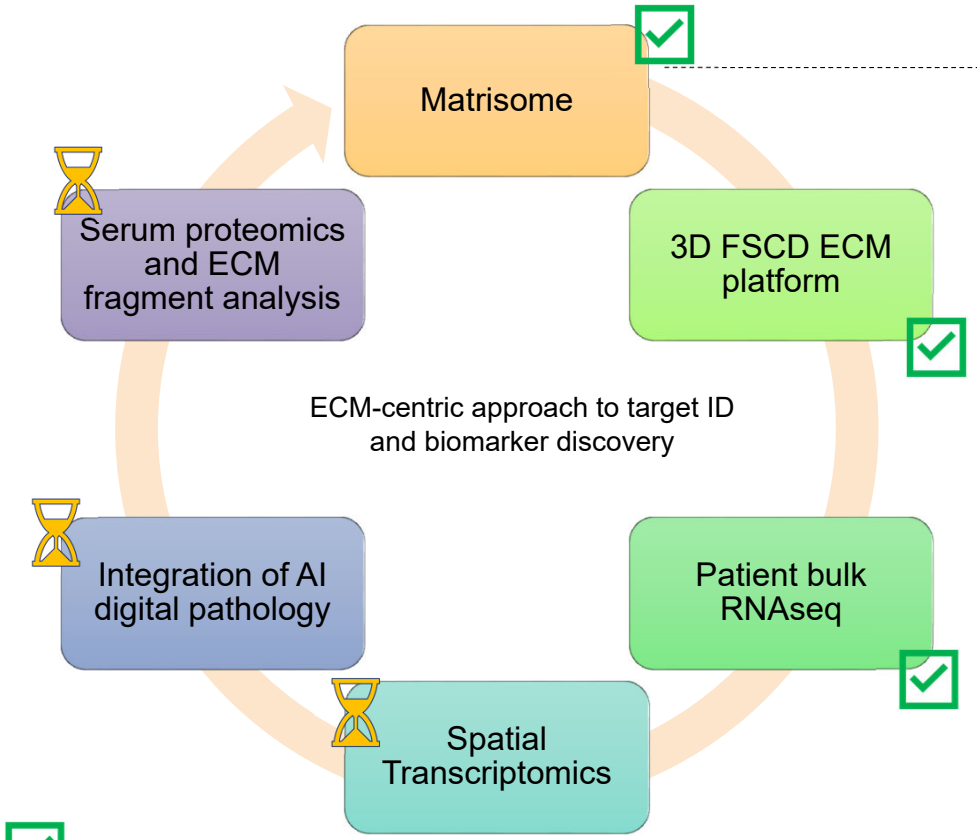
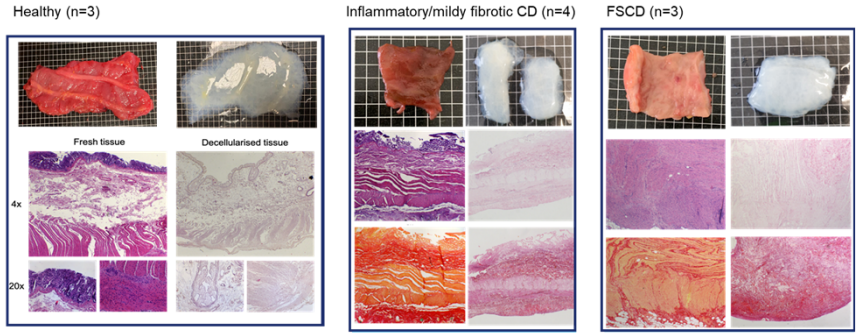
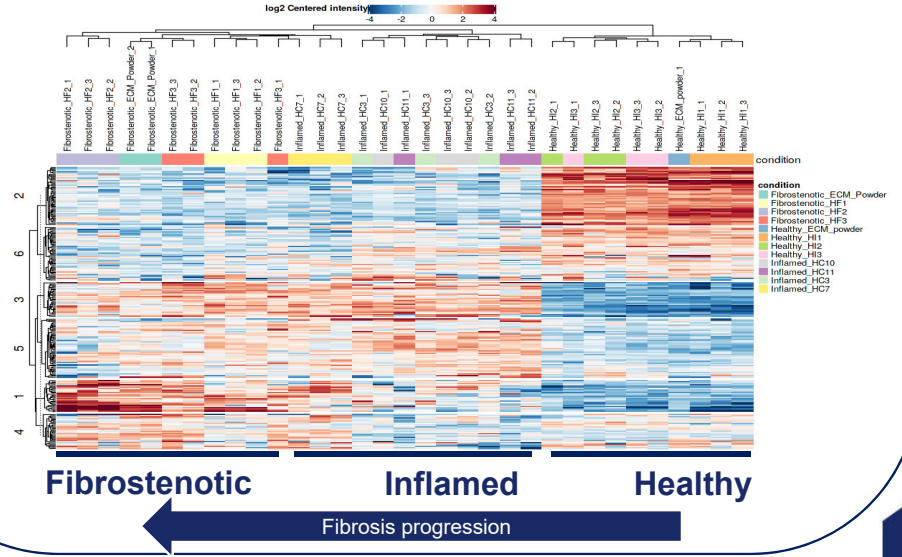


Dataset acquired

Dataset acquisition on-going; delivery September 2025

Matrisome reveals progressive changes in ECM composition in IBD





Loss of Tissue Homeostasis: The Pathogenic FSCD Matrisome — Unveiling a Novel Central Disease Driver

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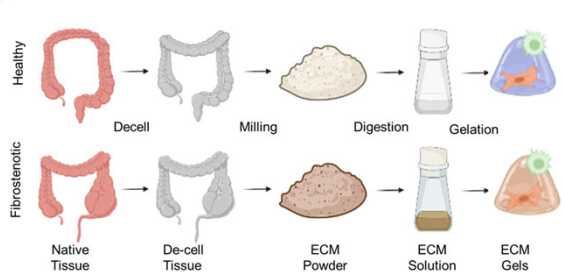
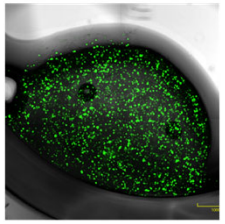
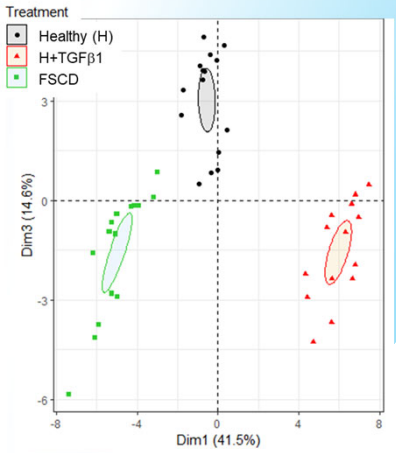
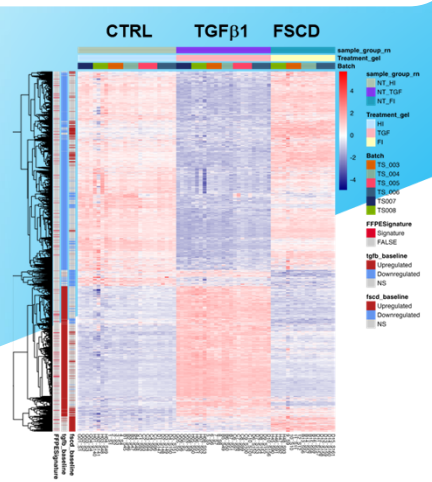
Incorporation of FSCD ECM in our 3D assay reveals how changes in the matrisome affect cell behaviour and drive fibrosis





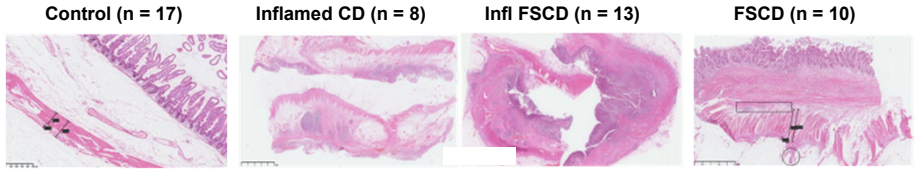


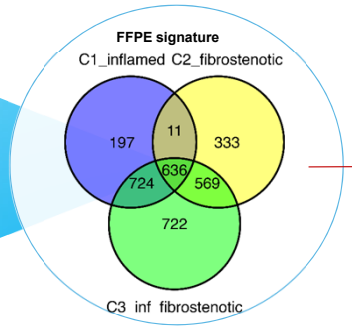
Secreted proteins

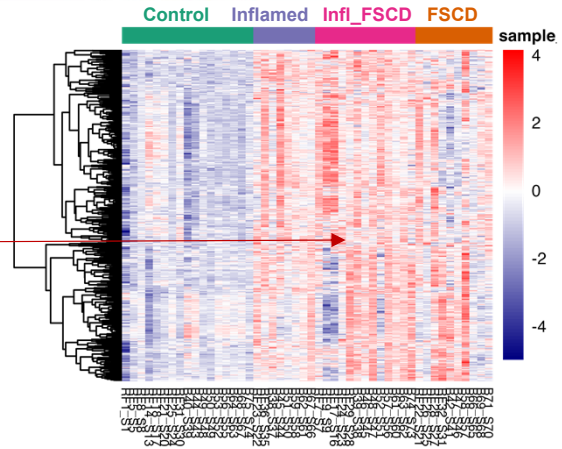


Transcriptomics

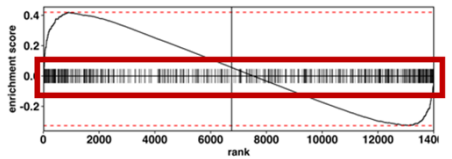
In Vitro FSCD ECM Induces a Patient-Derived Disease Signature Validated by Bulk RNA-Seq of FFPE Tissue, Highlighting Its Clinical Relevance





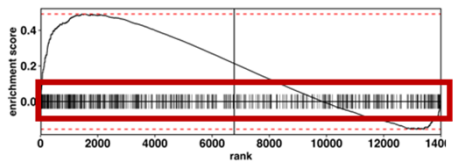


FSCD disease signature genes upregulated in TGFβ1 stimulated ECM assay



TGFβ – Healthy ECM; Enrichment Score = +0.42 ($p = 4.06e^{-2}$)

FSCD disease signature genes upregulated in FSCD ECM assay



FSCD ECM – Healthy ECM; Enrichment Score = +0.49 ($p = 1.88e^{-9}$)

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ECM and solid tumors

Antibody drug conjugate development strategy

Tumors with high ECM content are a significant unmet need and offer unique therapeutic opportunities

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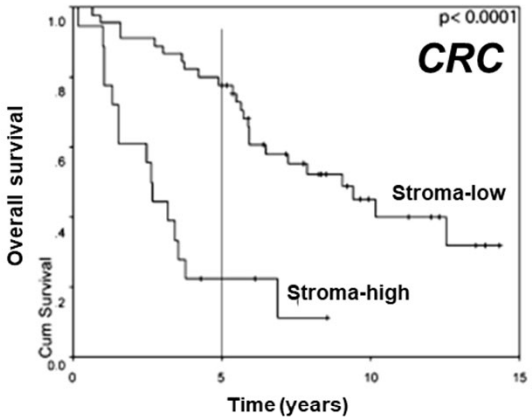
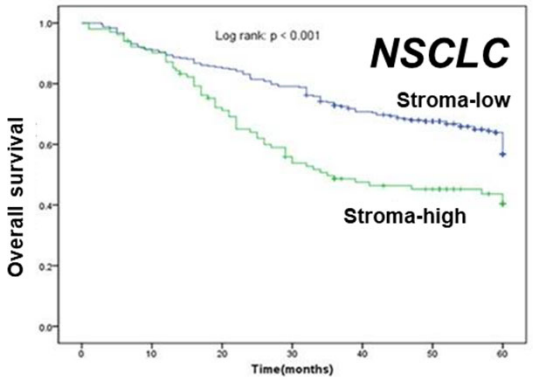
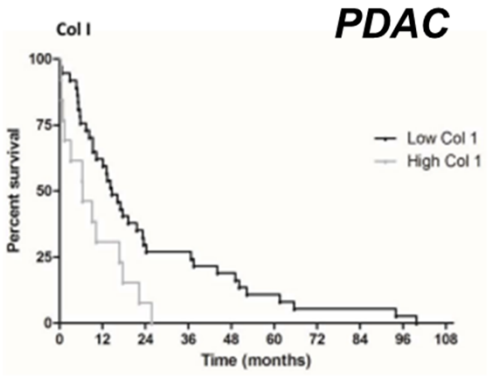
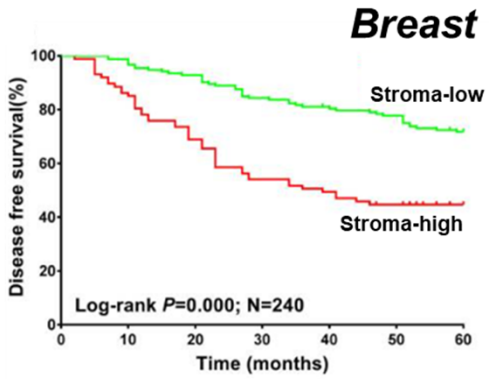
High tumor stromal and ECM content is associated with poor survival across multiple solid tumors

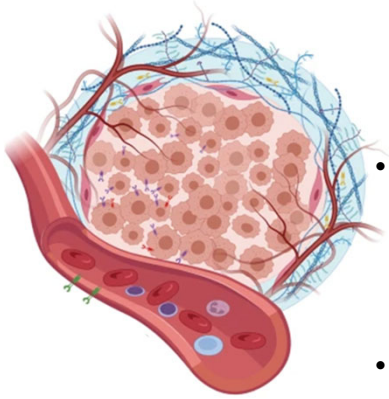




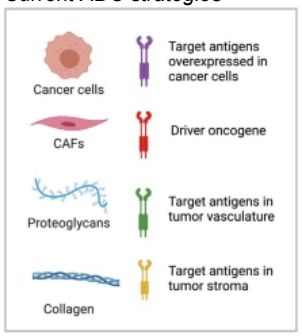




Targeting tumor ECM can offer advantages over other ADC strategies



Current ADC strategies



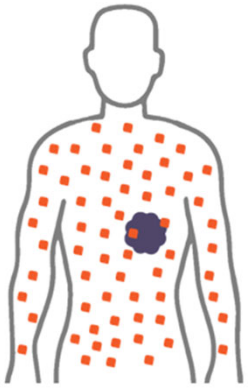
- Anchoring to the tumor ECM as opposed to tumor cell surface antigen will reduce incidence of resistance caused by antigen depletion due to tumor cell death
- Human malignancies have common ECM responses, which would enable a broader target population than conventional ADCs
- Extracellular release of ADCs' cytotoxic payloads is clinically effective

References: Mesker et al Cell Oncol 2007; Huijbers et al Ann Ocol 2013; Zhang et al Int J Clin Exp Pathol 2015; Whatcott et al Clin Cancer Res 2015; Yan et al BMC Cancer 2022; Pearce et al Cancer Disc 2018; Mascharak et al Cell Rep Med 2023; Fu et al Sig Trans Targ Ther 2022; Tsao et al Nat Comm 2025;

Strategy for identifying ECM-anchoring targets

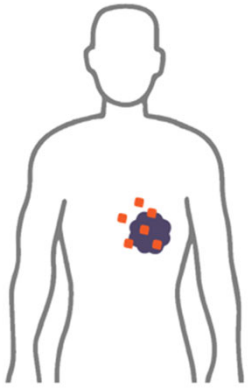
EGTX004 may provide a novel therapeutic platform addressing multiple unmet clinical needs

PROBLEM



High rates of AEs associated with anti-fibrosis & anti-cancer therapies are due to systemic distribution of potent agents

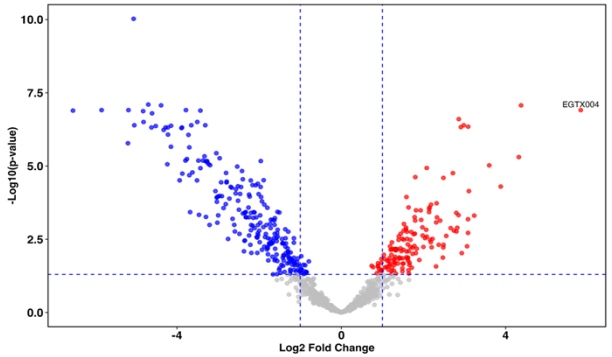
SOLUTION



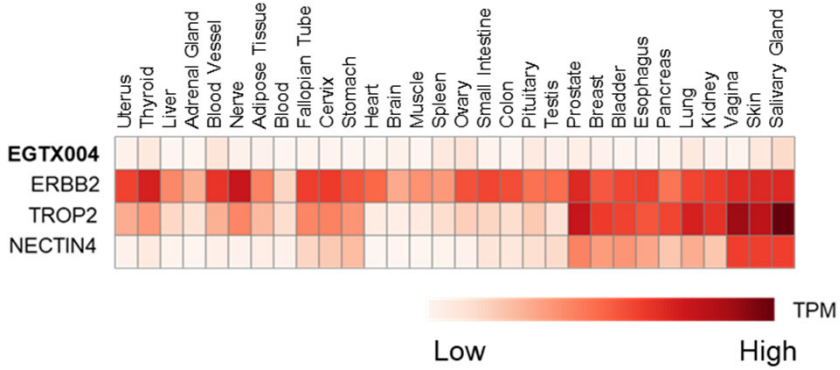
Localize therapies in diseased tissue by conjugating to antibodies targeting disease-specific ECM proteins

Identify ECM proteins that are highly and specifically expressed in tumors, and not in histopathologically normal adjacent tissues

Tumor vs Matched Background Tissue Matrisome



Select targets that have no significant expression in healthy organs



Established biospecimens' collection, ECM platform and matrisome dataset in multiple solid tumors

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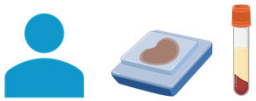
Extensive biospecimens available across indications and sampling modalities

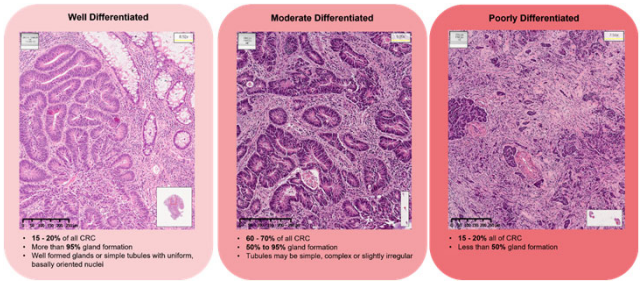
	CRC	CRCLM	PDAC
FFPE	>215	>300	>70
Frozen	>80	>40	>25
Serum	>145	>75	>70

Samples linked to longitudinal EMR data extracted and curated by clinicians

- Demographics
- Medications
- Labs
- Comorbidities
- Imaging Reports
- Dx and Outcomes

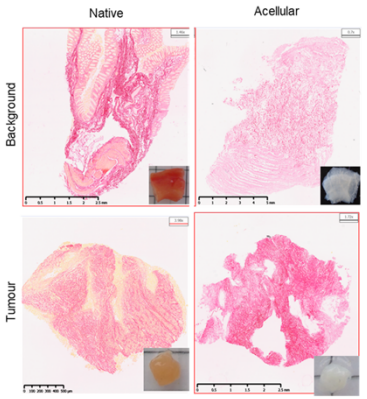


Pathologist QC/annotation of histopathology to characterize tumor grade and phenotype

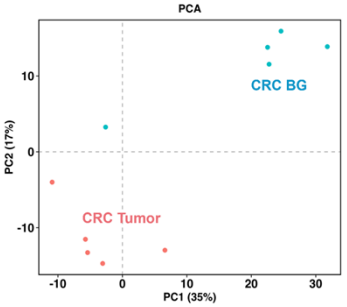


Matrisome reveals unique protein signatures in tumor ECM from CRC, CRC-LM, and PDAC vs background adjacent non-cancerous tissues

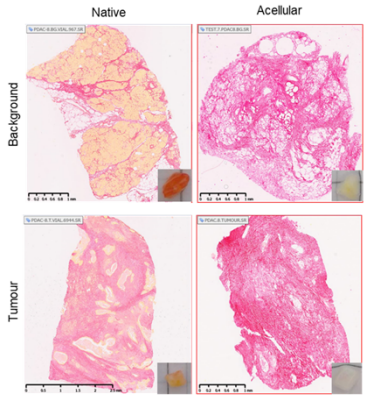
Sirius Red Staining of Decellularized CRC Primary Tumours vs Background Tissue



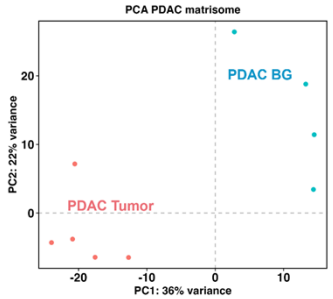
CRC tumours and matched background colon have distinct matrisome profiles



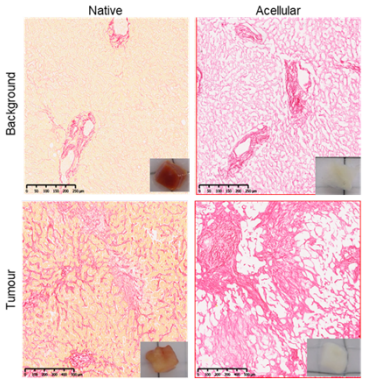
Sirius Red Staining of Decellularized PDAC Tumours vs Background Pancreas Tissue



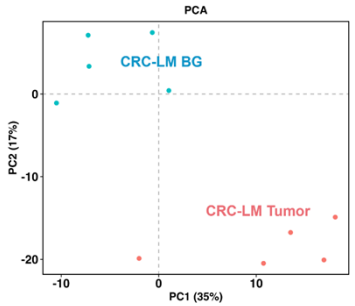
PDAC tumors and background pancreas tissues have distinct matrisome profiles



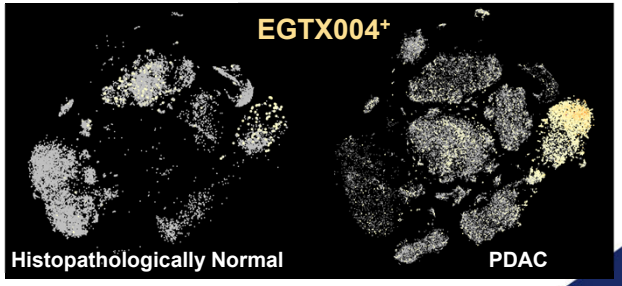
Sirius Red Staining of Decellularized CRC Liver Metastasis vs Liver Background Tissue



CRC liver metastasis and background liver tissues have distinct matrisome profiles



Normal pancreas and PDAC scRNA have different cell type composition and matrisome expression



Proprietary matrisome data ID'ed a highly tumor-specific stromal protein (EGTX004) that may provide alternative ADC strategy

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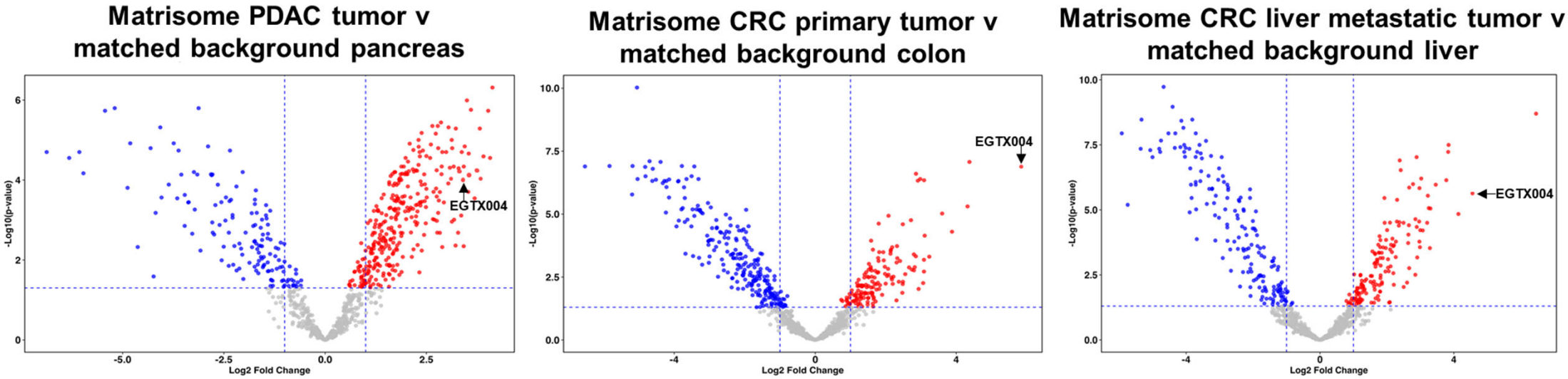
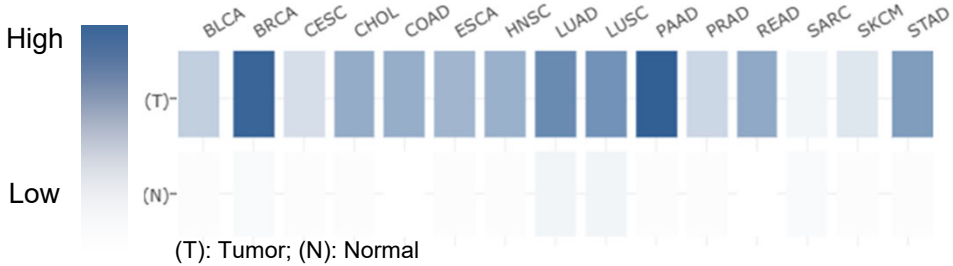


- Up-regulated
- Down-regulated
- Unchanged



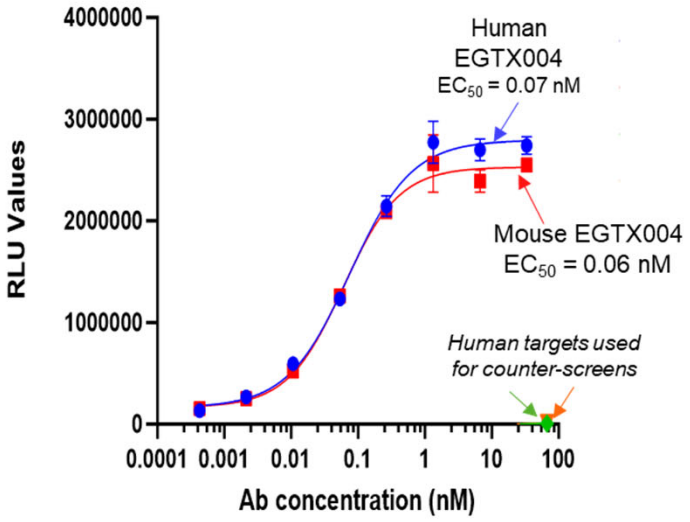
(T): Tumor; (N): Normal

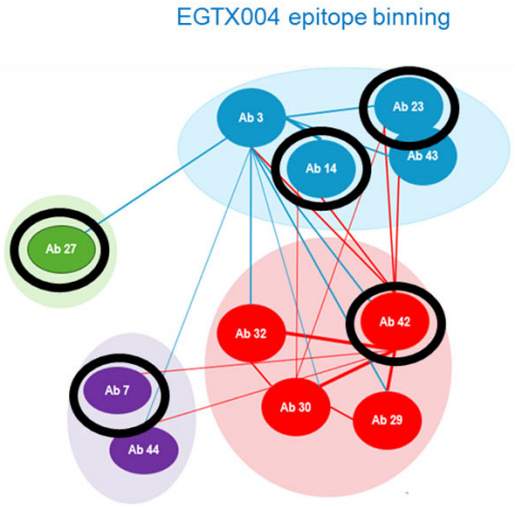
Gene expression analysis show increased EGTX004 expression across multiple solid tumors

Confirming EGTX004 protein expression by IHC

This represents a unique dataset using EGTX004 specific mAbs

EGTX004 specific mAbs identified to enable confirmation of tumor expression

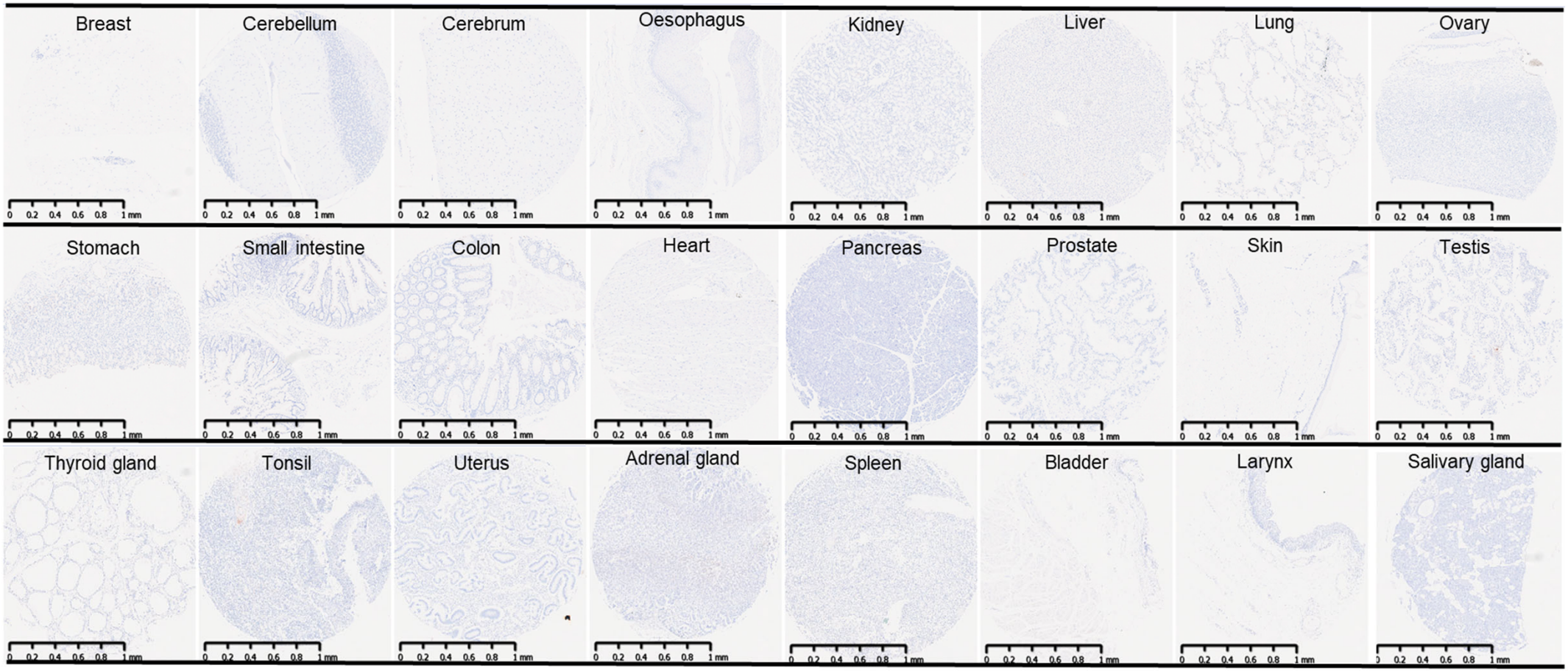




- All commercial Mabs to EGTX004 found to be non-selective
- Novel α-EGTX004 tool antibodies were developed and IHC methodology optimised to allow assessment of EGTX004 protein levels in different oncology indications
- Different protocols were required for antigen retrieval in PDAC and CRC suggesting differential structure of the EGTX004 in the ECM in various organs or indications

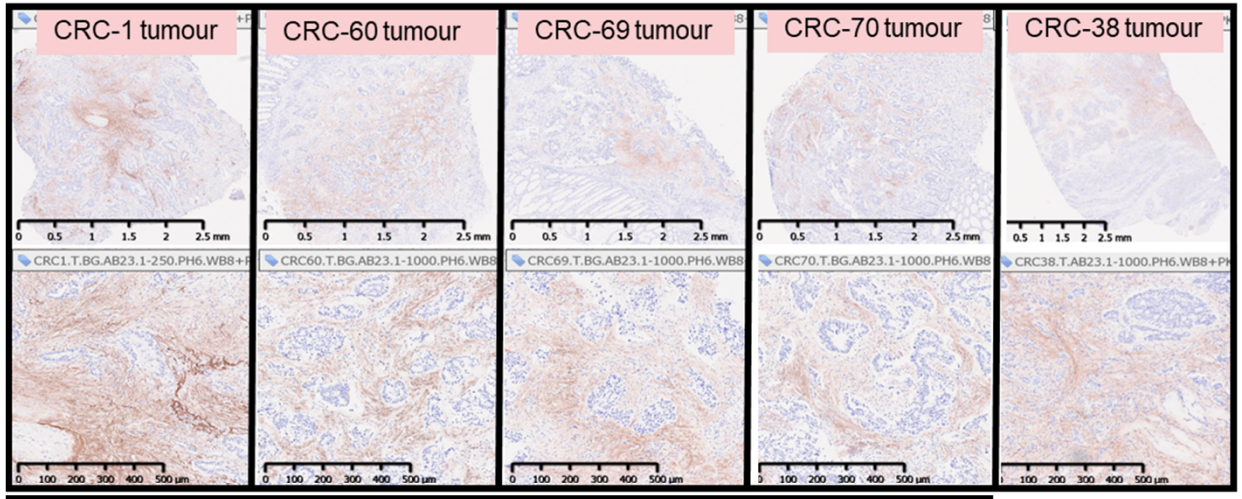
- 45 Rabbit mAbs screened for selectivity, potency, cross reactivity, & epitope diversity assessed
- **5 Abs selected for IHC/Characterisation**

EGTX004 expression has a very clean profile with no staining seen in healthy tissue arrays

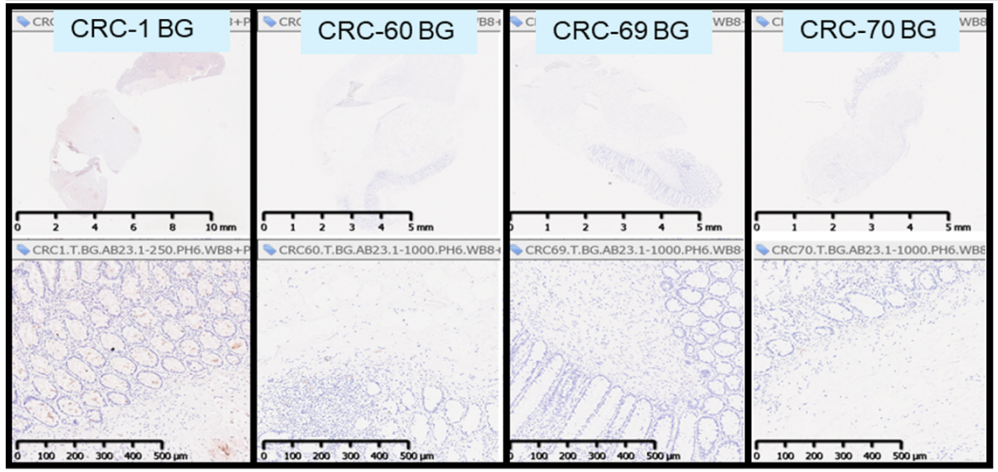


EGTX004 is expressed in stromal areas of CRC 1ry tumor

CRC Primary Tumor



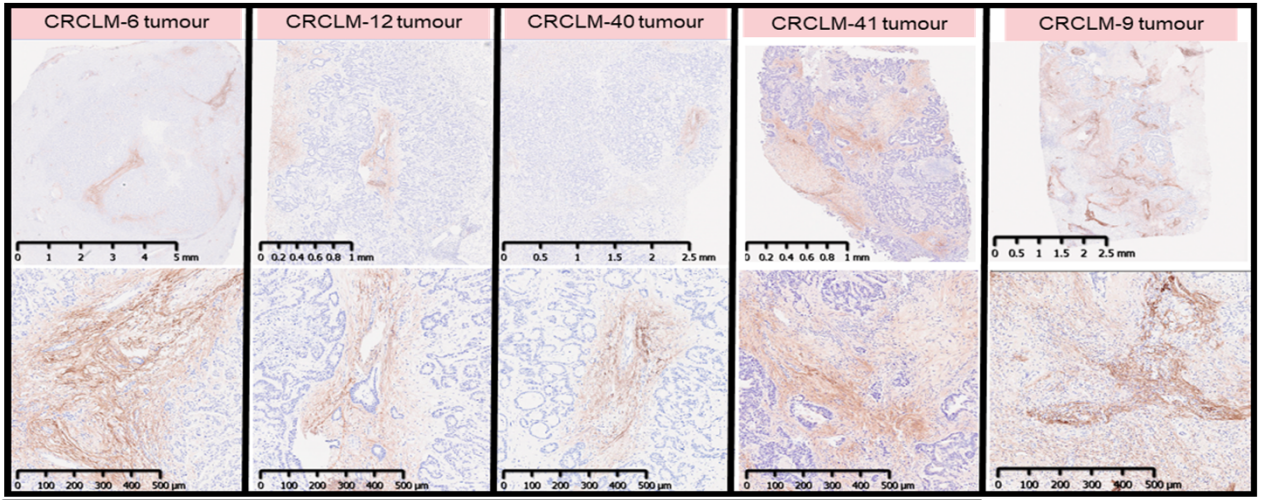
Background tissue (tumour adjacent)



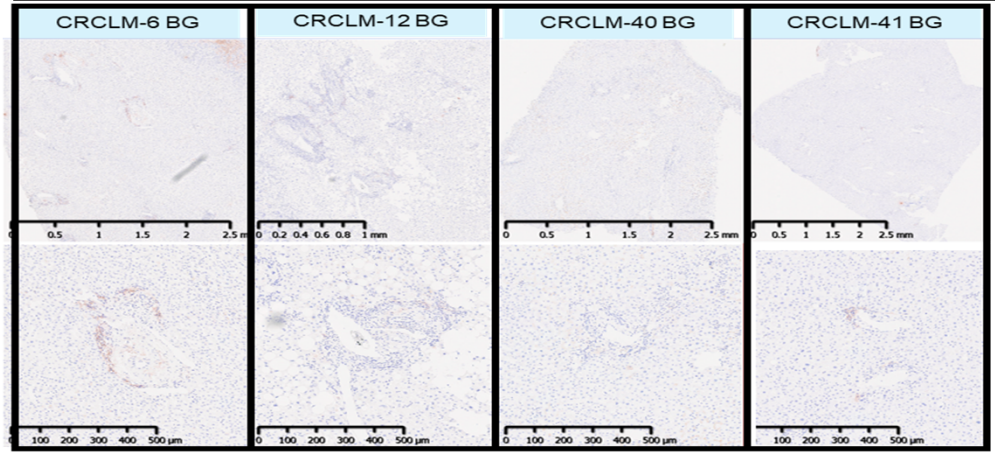
- 6/6 CRC samples have stained positive for EGTX004 expression
- No staining in tumour adjacent tissue

EGTX004 is expressed in stromal areas of CRC Liver Mets

CRC Liver mets

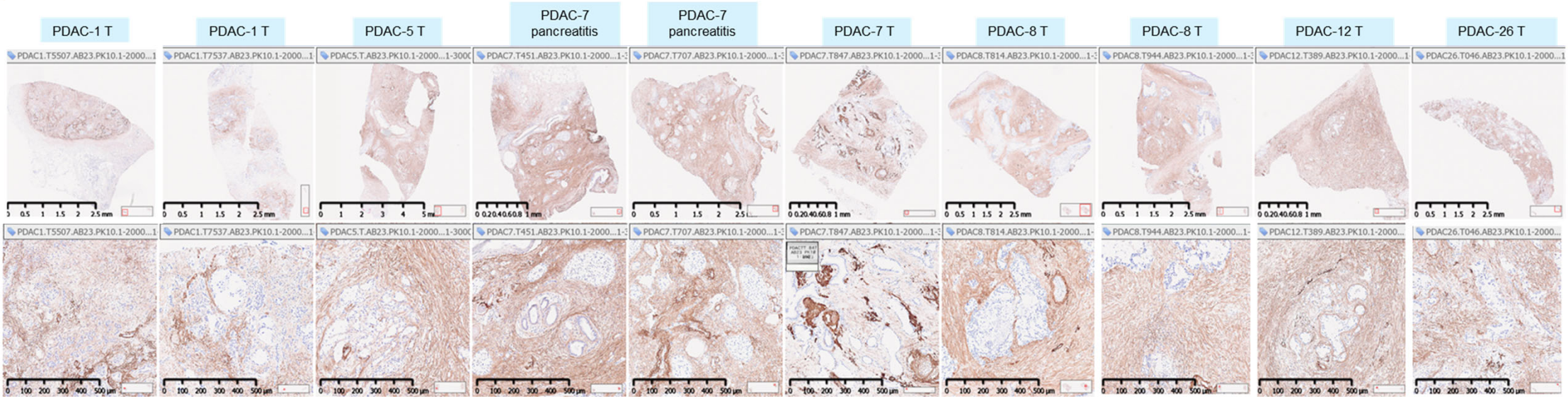


Background tissue (tumour adjacent)



- 5/5 CRC-LM samples have stained positive for EGTX004 expression
- No staining in tumour adjacent tissue.

Strong positivity of EGTX004 in PDAC tumour cohort

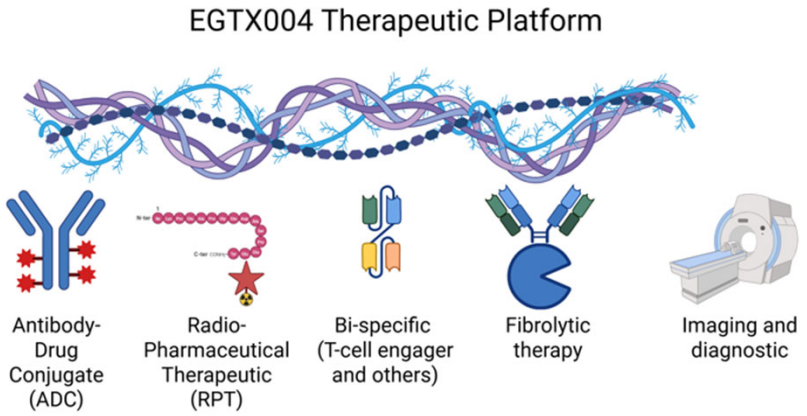


- EGTX004 shows strong immunopositive staining in desmoplastic stroma of PDAC tumours
- 7/7 donors positive for EGTX004
- Findings consistent with transcriptomics and proteomics data, indicating EGTX004 expression increased in PDAC
- Histopathologically normal background tissue shows no staining

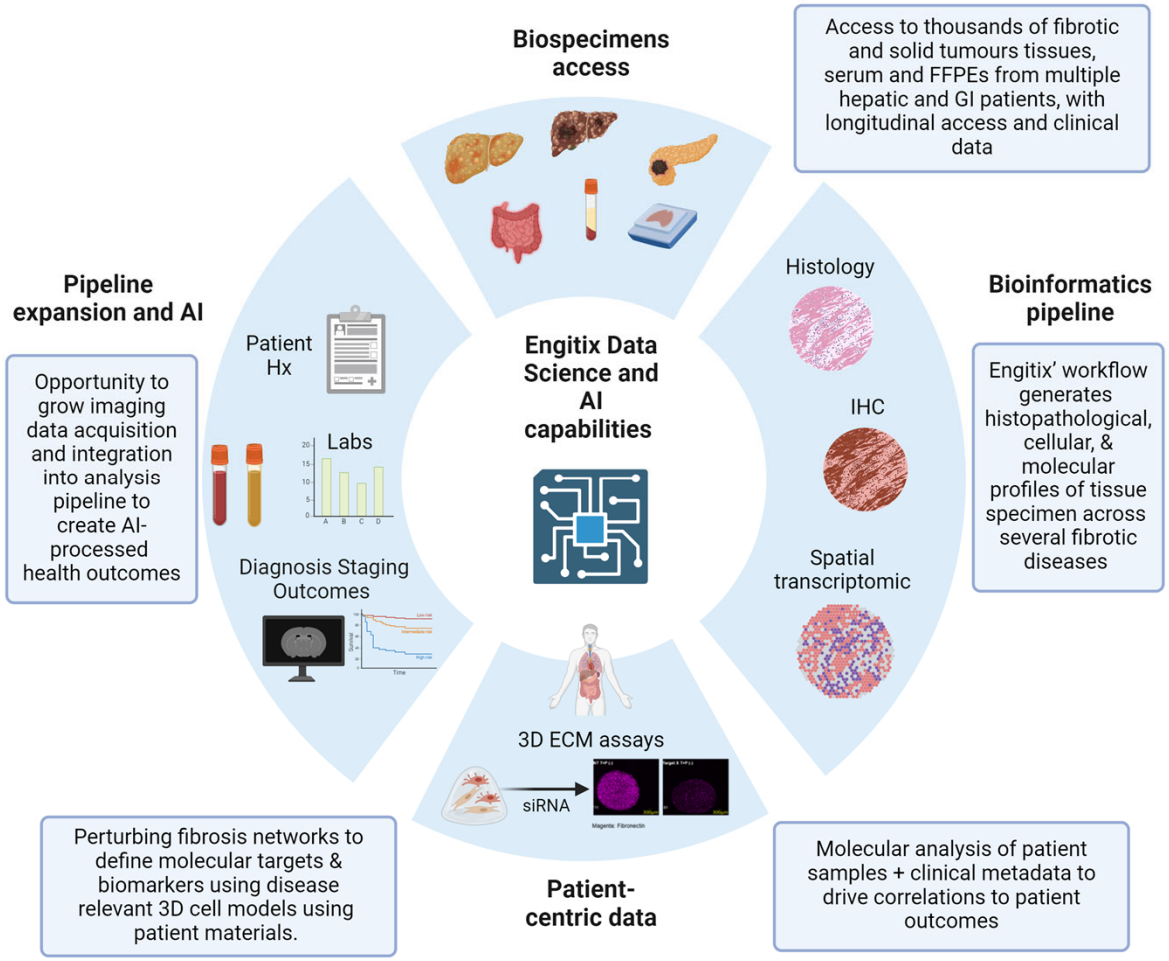
Summary and opportunities:

- EGTX004 represents a first-in-class tumor ECM targeted cytotoxic therapy
- EGTX004 is highly and specifically expressed within the ECM of many solid tumors, including CRC (1ry & mets)
- EGTX004 has a more restrictive tissue expression profile compared to other tumor stroma-targeted cytotoxic therapies
- Engitix has developed mAbs with high affinity and selectivity for EGTX004 across different protein domains
- EGTX004 is a platform opportunity that can enable the development of other oncology and anti-fibrotic therapies

EGTX004 may provide a novel therapeutic platform addressing multiple unmet clinical needs



Building towards an AI-powered analysis of multidimensional data from patients & 3D patient-derived tissue assays



Summary

- The ECM plays a central role in the progression from inflammation to fibrosis to solid tumours, but remains an untapped area of biology in drug discovery
- Engitix 1st and best-in-class human ECM platform aims at decoding biology and composition of healthy and diseased human ECM
  - Growing biorepository with longitudinal samples & metadata from N > 2000 patients (indications: fibro-inflammation and solid tumors)
  - Integrated analysis of multidimensional patient-level data to drive target selection and confirmation
  - Patient-derived 3D ECM screening systems to validate target function and therapeutic potential
  - Validated platform by two exclusive license agreements with Takeda in liver fibrosis and fibrostenotic IBD
- >$30MM raised between Seed and Series A + access to up to $28MM work-for-equity + > $45MM revenues to date
- Proprietary pipeline of 3 assets (LO stage) in fibro-inflammation (EGTX003), fibrosis (EGTX001) and solid tumors (EGTX004) progressing through drug discovery campaign
- Next inflection points: EGTX001 DC (1Q26), EGTX003 DC (2Q26), EGTX004 DC (3Q26) with FIH studies in 2027
- Partnership opportunities: platform target ID/validation deals (liver, gut, skin, lung) and asset deals

Acknowledgements

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Prof Antonio Di Sabatino

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