Modular hydrogels for organoid-based disease modelling

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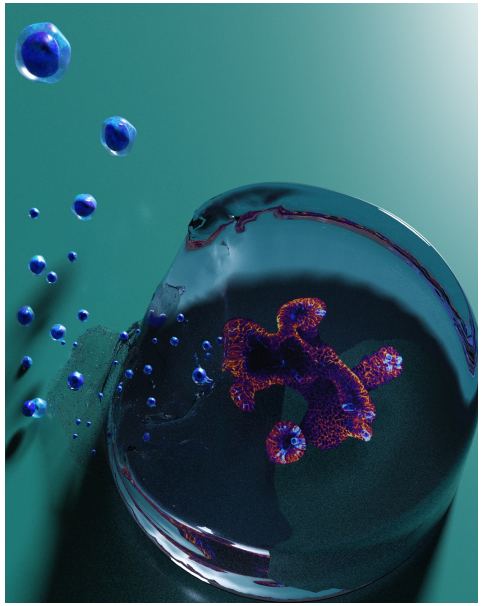
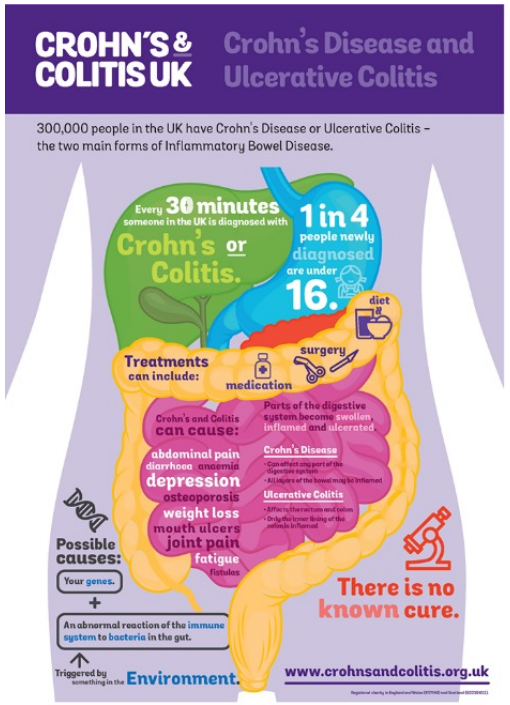
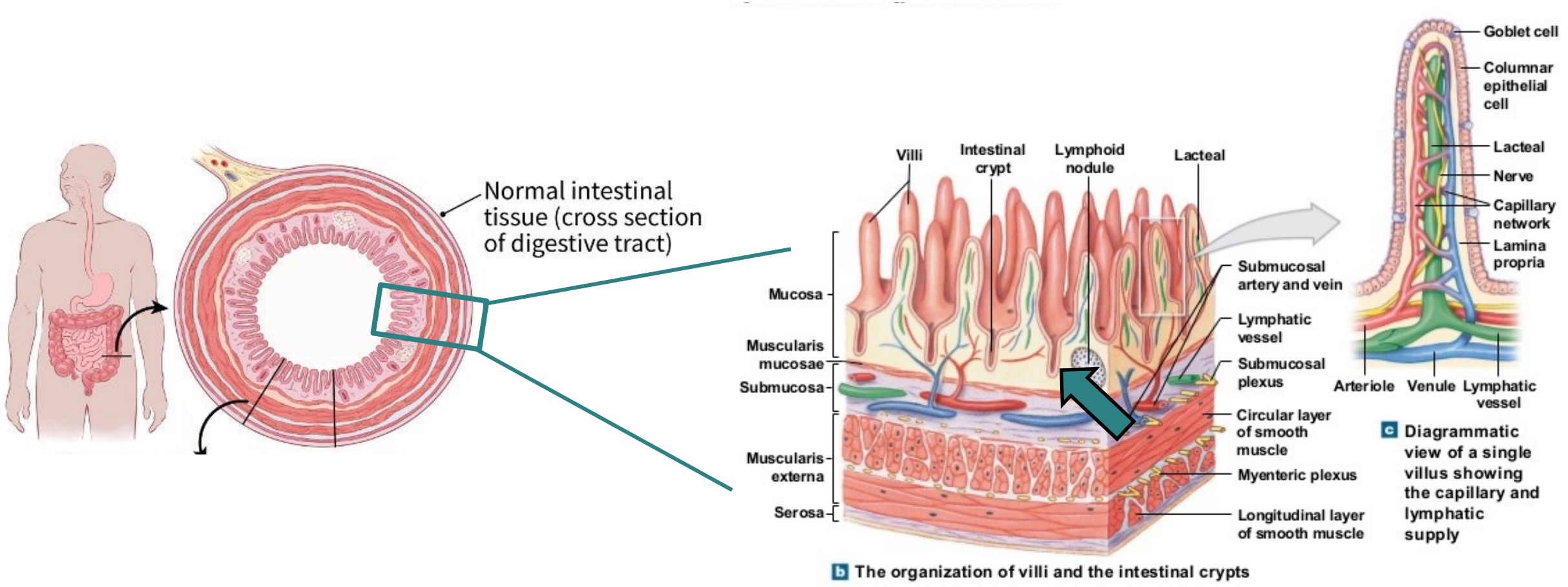


Crohn’s disease and models of the intestine

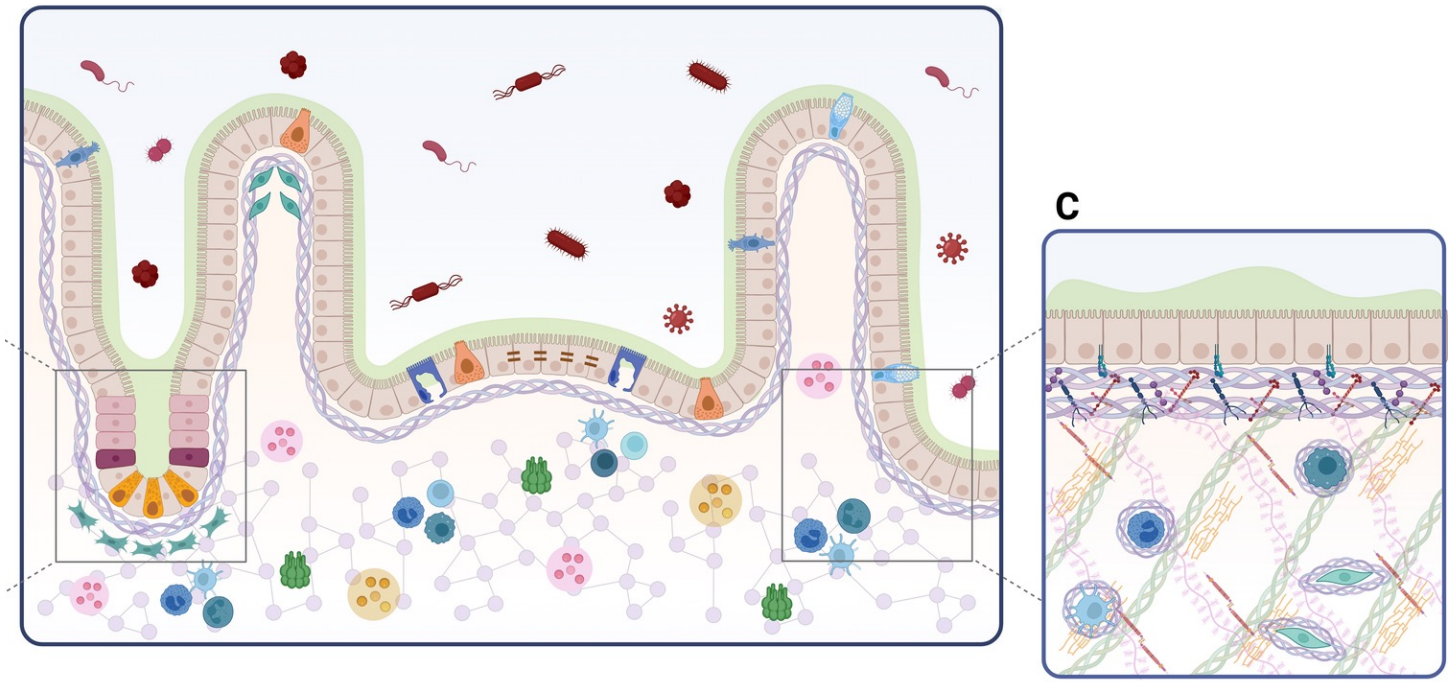


- Chronic intestinal inflammation
- Abdominal pain
- Diarrhoea
- Weight loss
- Gut resection to remove fibrotic strictures

Structure of the intestine



Cellular and matrix components of the intestine



Absorptive epithelial cells
- Enterocytes

Secretory epithelial cells
- Enteroendocrine cells
- Goblet cells
- Paneth cells
- Microfold cells
- Tuft cells

Stem cells
- Intestinal stem cells

Mesenchymal cells
- Fibroblasts
- Myofibroblasts

ECM components
- Pericellular matrix
- Interstitial matrix
- Growth factors
- Proteases

Immune cells & proteins
- Granulocytes
- Mononuclear phagocytes (MNPs)
- T and B cells
- Cytokines

- Luminal pathogens
- Mucus
- = Tight junctions

- Integrin

Basement membrane
- Non-fibrillar collagen
- Perlecan
- Laminin
- Nidogen

Interstitial matrix
- Fibrillar Collagen
- Elastin
- Fibronectin
- Proteoglycans/ Glycosaminoglycans

Epithelial cells
Absorptive and secretory cells that line the inner wall of the intestine

Mesenchymal cells
Fibroblasts and other cells that create and remodel the ECM

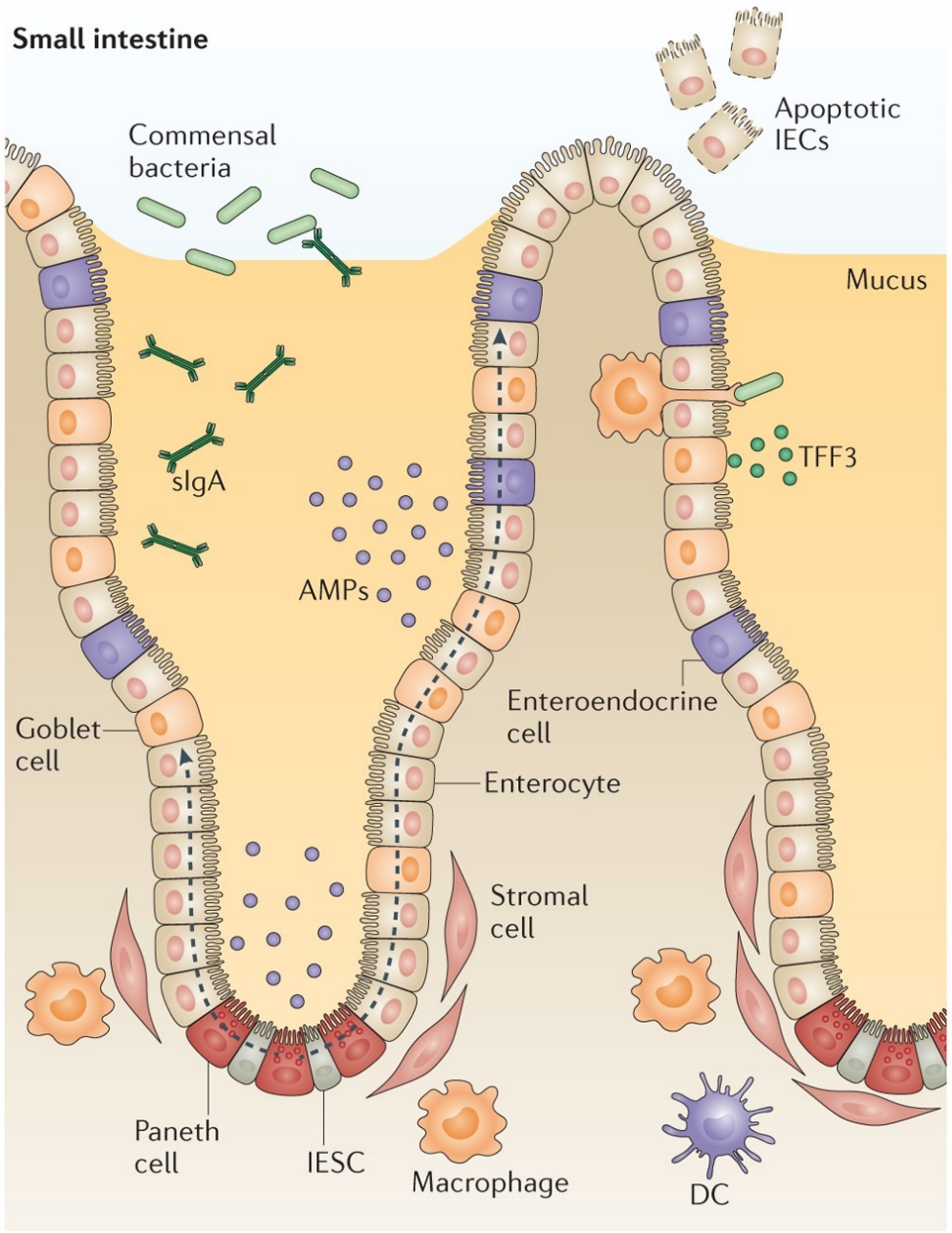
Immune cells
Innate and adaptive immune cells, including many tissue resident cells, that can secrete inflammatory cytokines

Extracellular matrix (ECM)
A large network of proteins and other molecules that surround, support, and give structure to cells and tissues in the body.

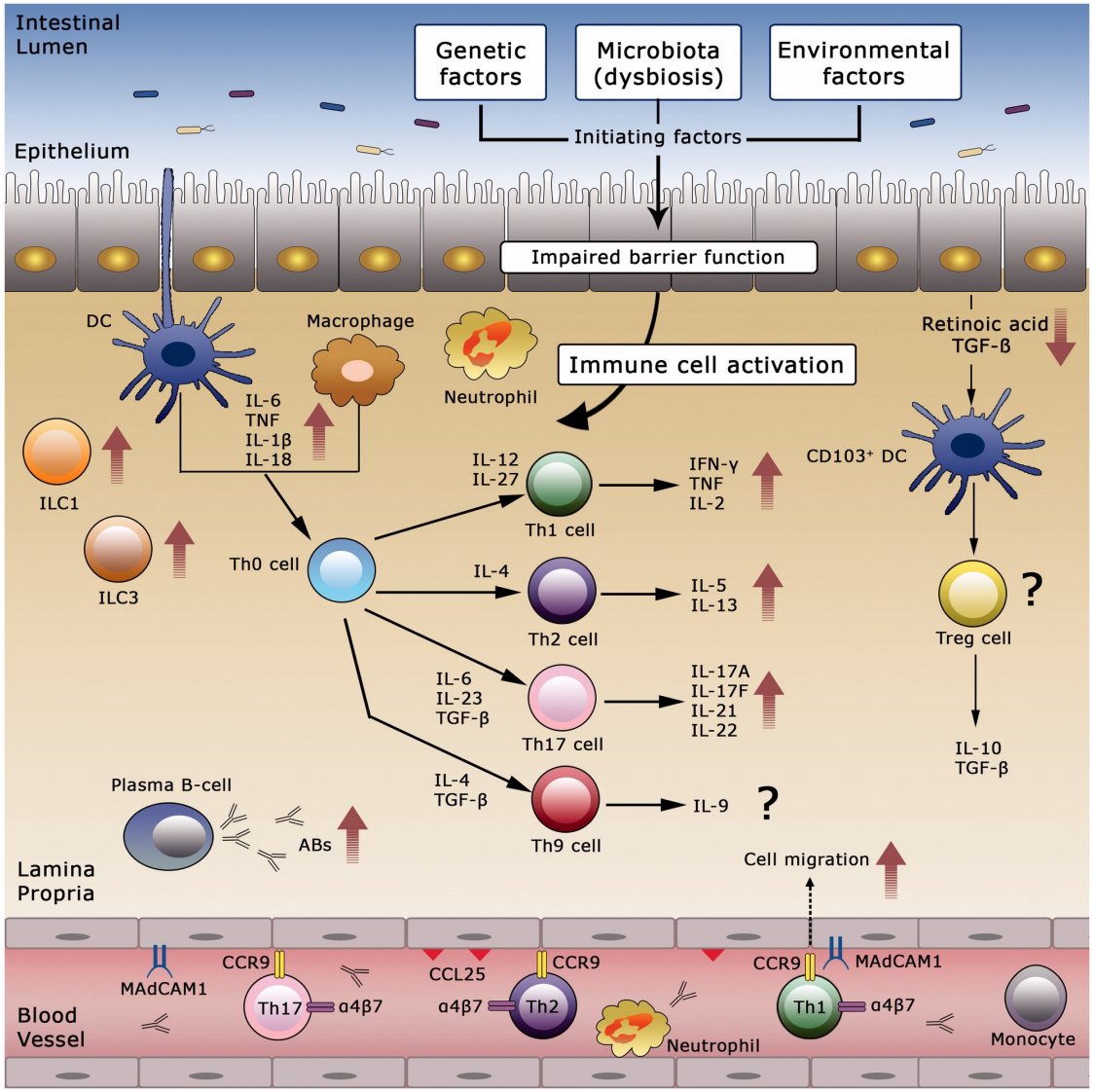
Intestinal epithelium

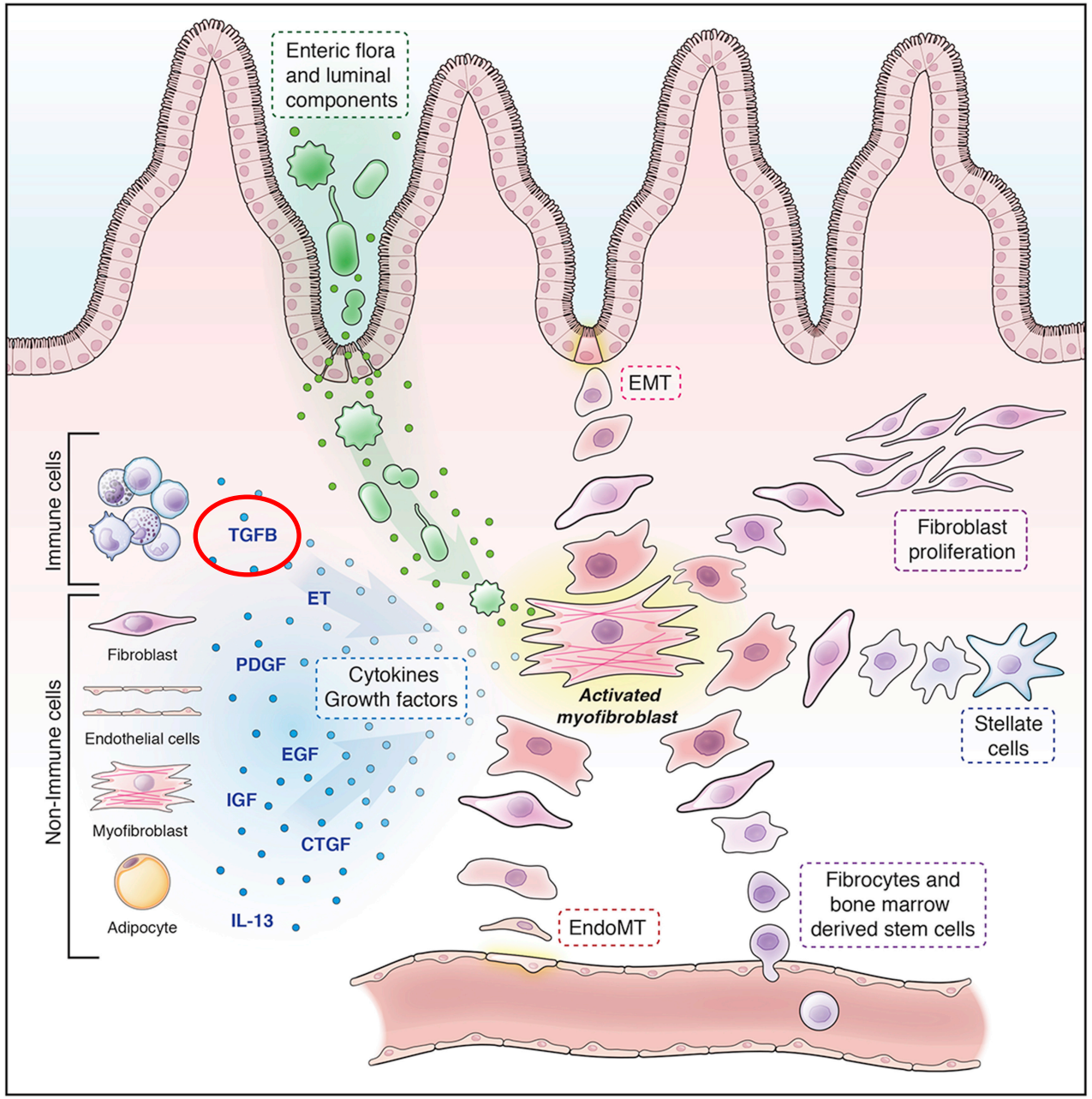
Function of the intestinal epithelium

- **Barrier protection**: Physical barrier separating gut contents (bacteria, toxins) from underlying tissue
- **Selective permeability**: Allows absorption of nutrients, electrolytes, and water but prevents entry of harmful substances
- **Immune signalling**: Interacts with immune cells and produces antimicrobial peptides to help maintain gut immune homeostasis
- **Self-renewal**: Rapidly regenerates to replace damaged or old cells
- **Wound healing**: Mobilises repair responses after injury through epithelial proliferation, migration, and differentiation

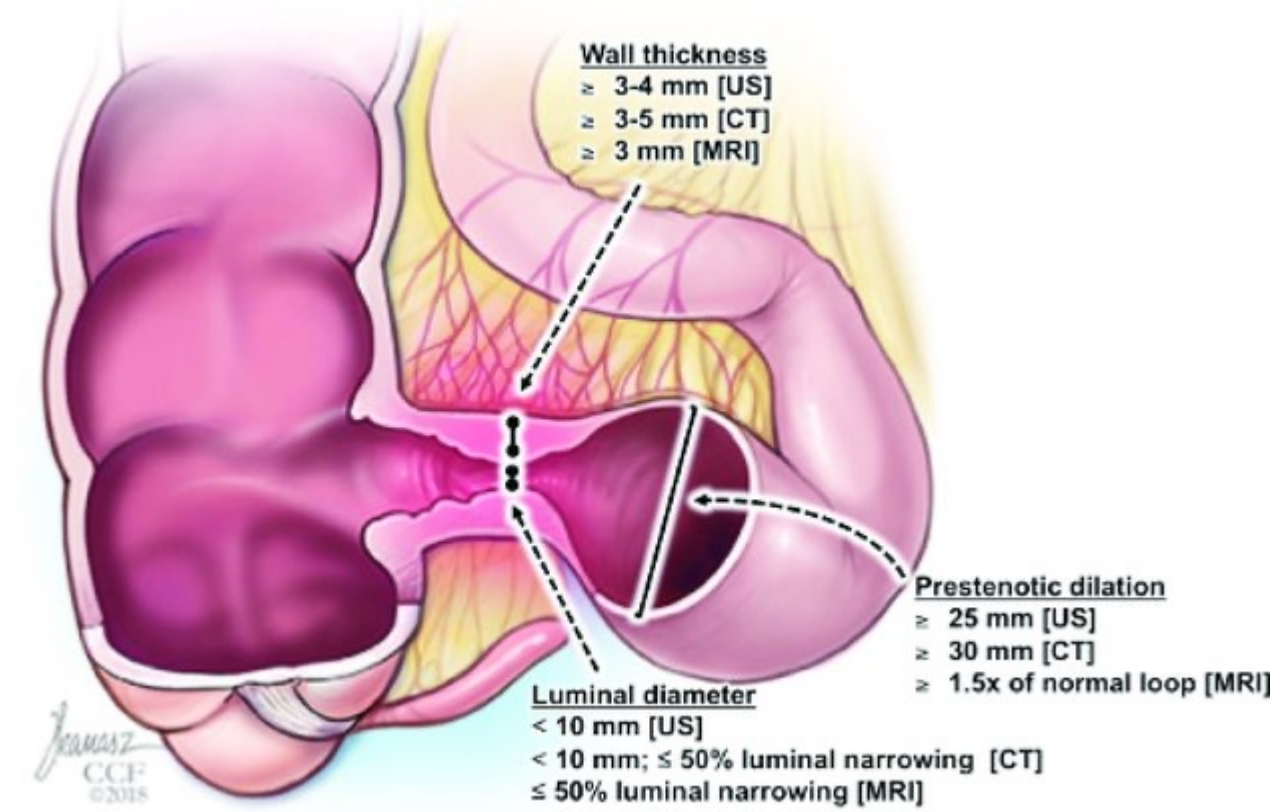


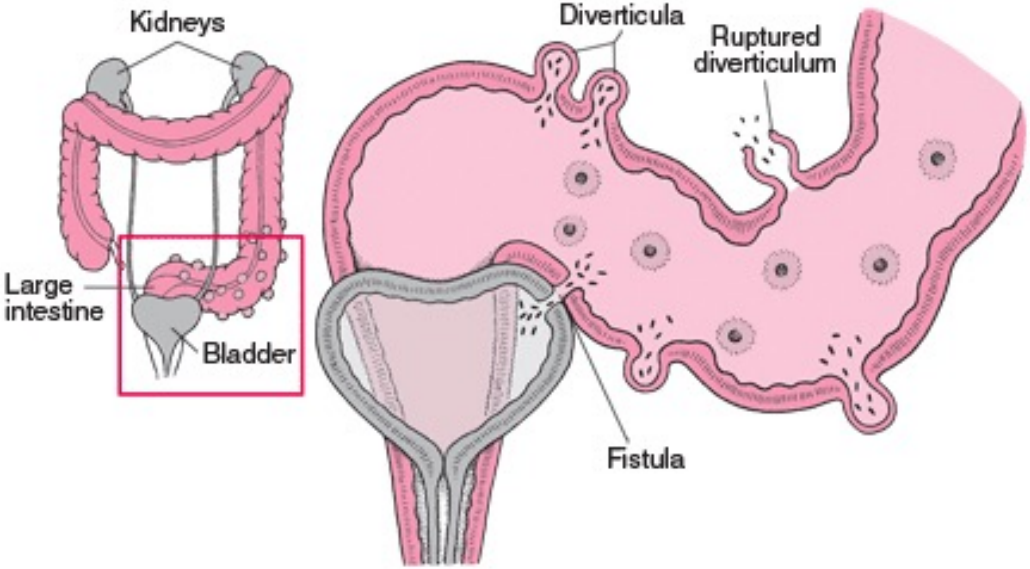
Crohn's disease and fibrosis





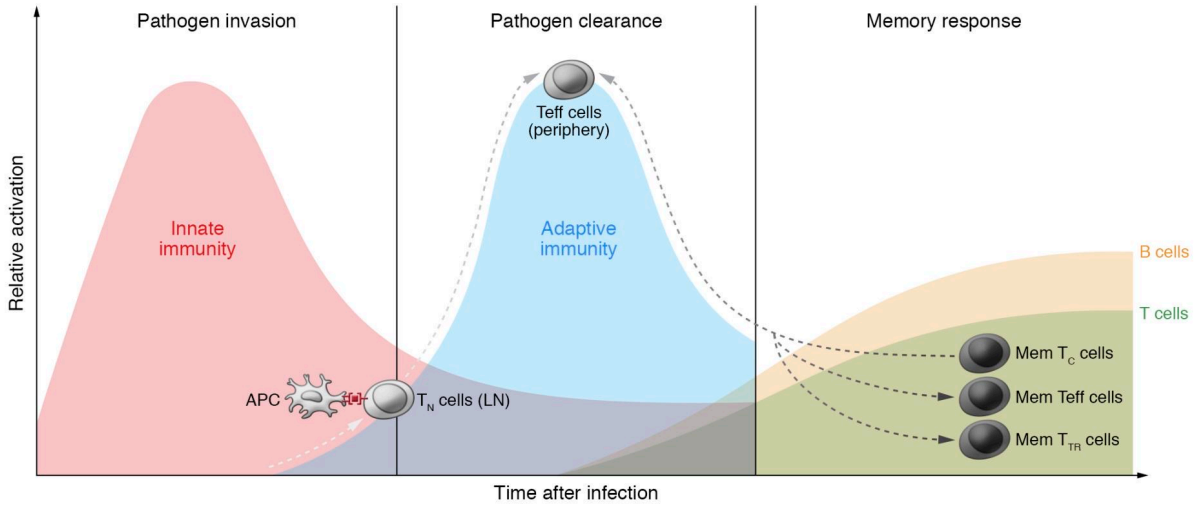
Fibrosis, stenosis and fistula in Crohn's disease

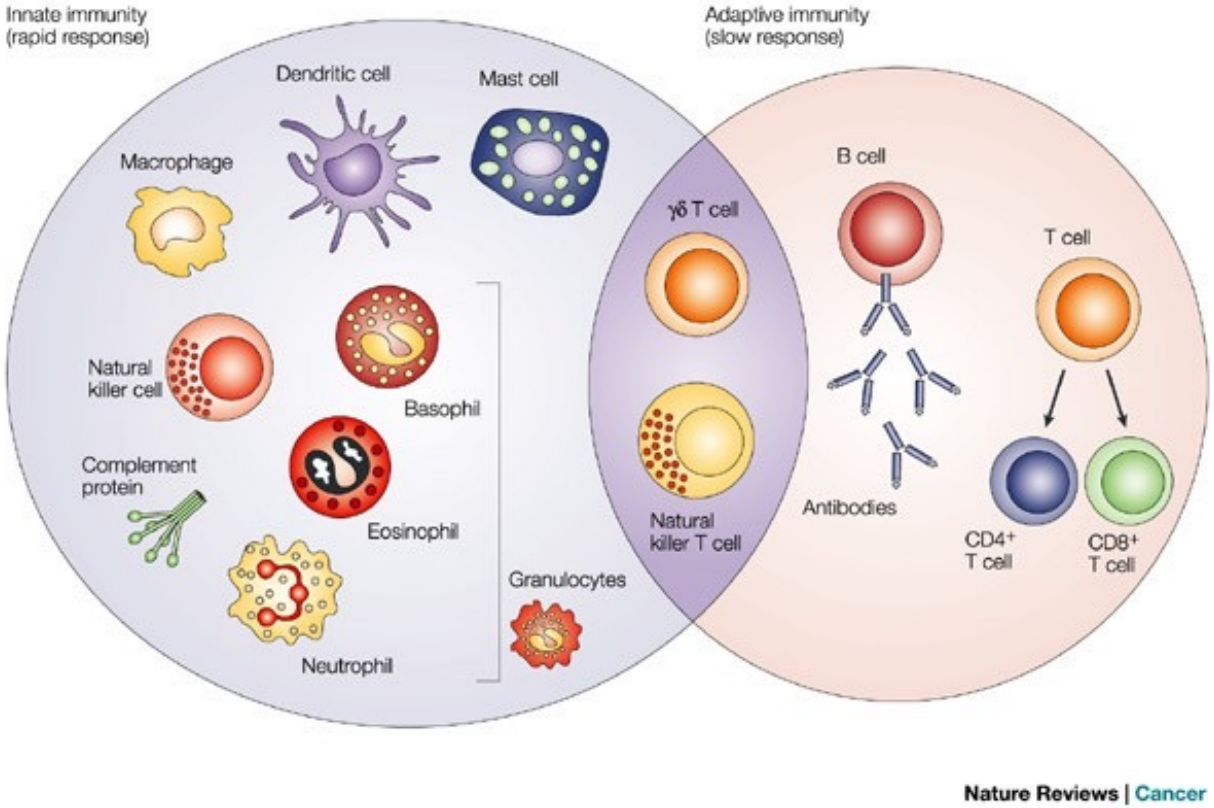




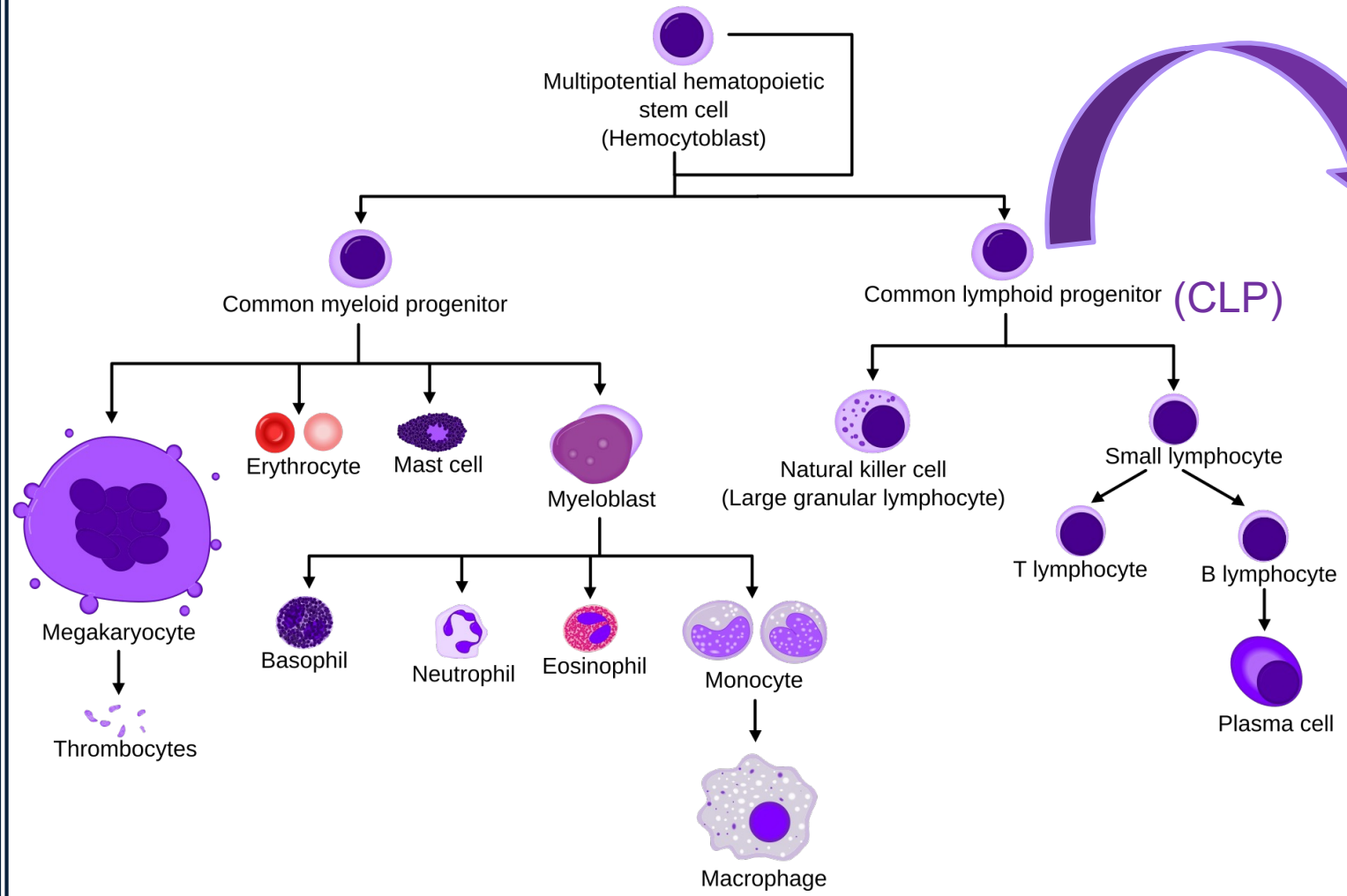
- Fibrosis/stenosis: Increase in extracellular matrix (ECM) components, like collagen
- Fistula: Breakdown of ECM components

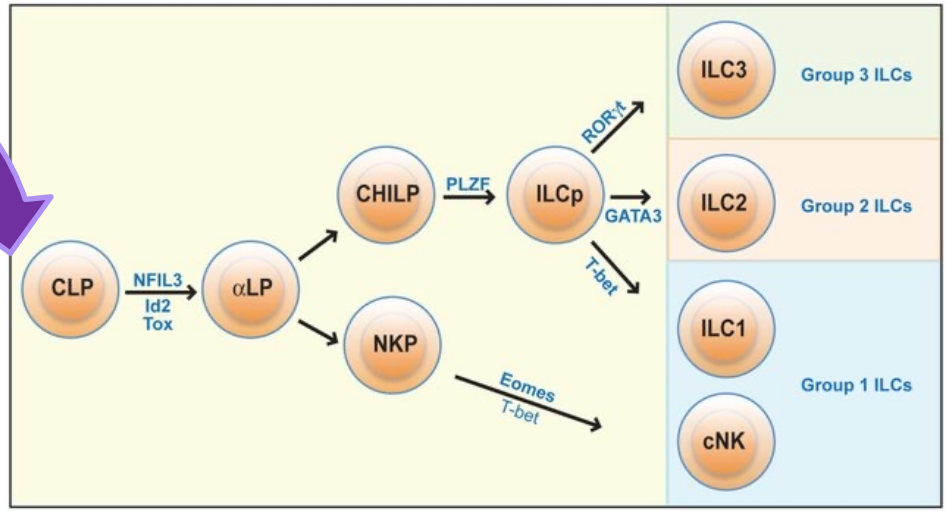
Innate and adaptive immunity





Innate lymphoid cells (ILC)

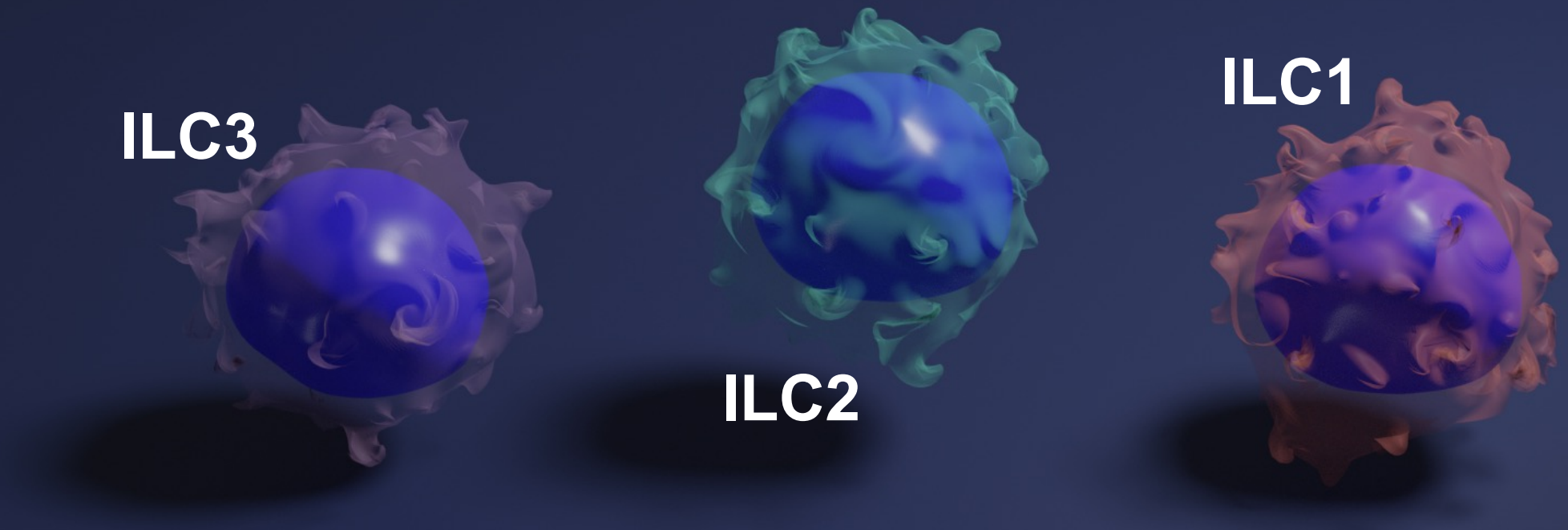




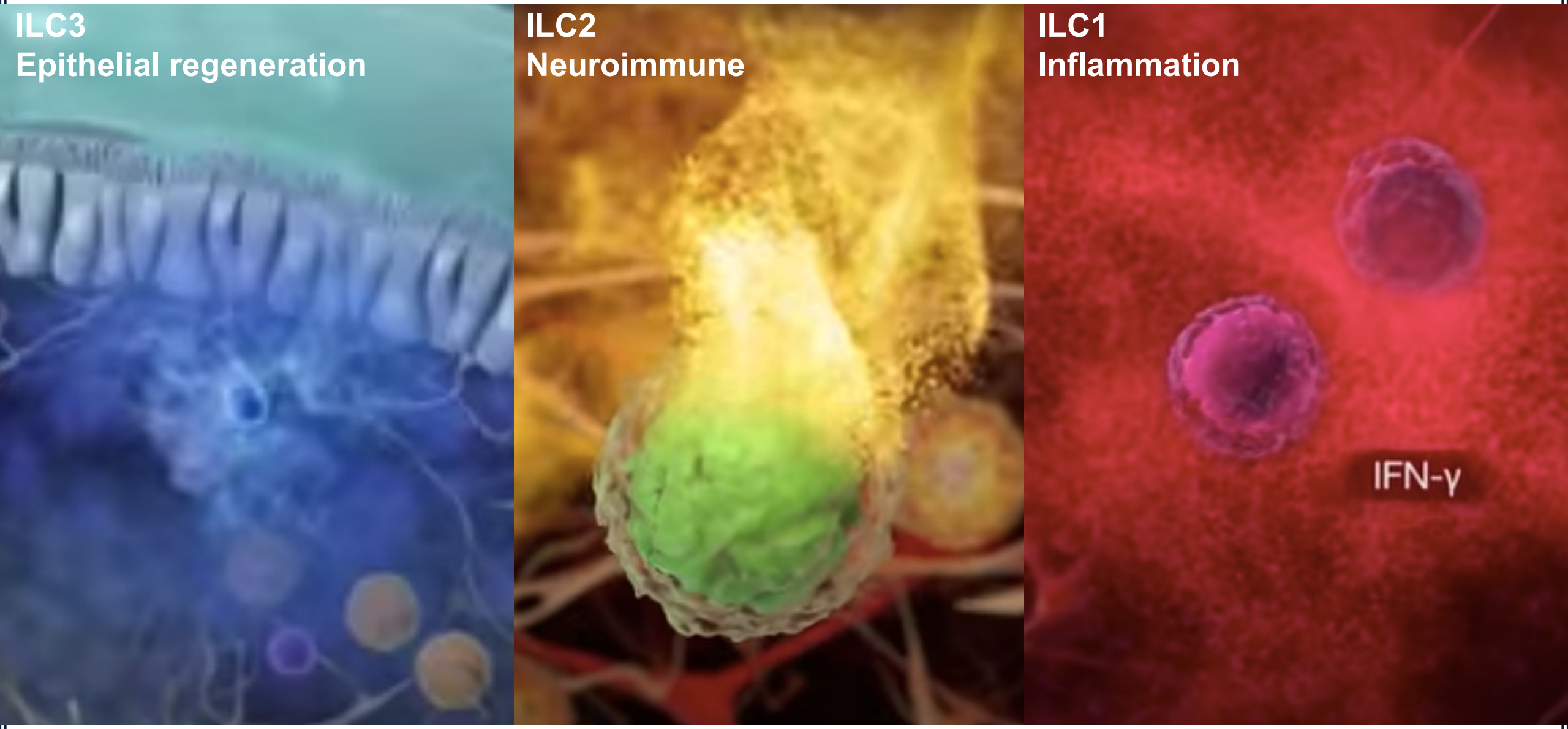
T-cell	Effector molecules		Innate cell	Activating cytokines
Th1	IFN-γ, TNF, IL-2	IFN-γ	ILC1	IL-12, IL-18
Th2	IL-4, IL-5, IL-13, L-25, AREG	IL-4, IL-5, IL-13, IL-9, AREG	ILC2	IL-25, IL-33, TSLP
Th17	IL-17, IL-21, IL-22	IL-17, IL-22, GM-CSF, lymphotoxin	ILC3	IL-1β, IL-23
CD8 T cell	Perforin granzymes IFN-γ, TNF	Perforin granzymes IFN-γ, TNF	NK cell	IL-2, IL-12, IL-18

Innate lymphoid cells (ILC)

Tissue resident - Antigen non-specific - Rapid potent cytokine responses
1st responders at mucosal barriers

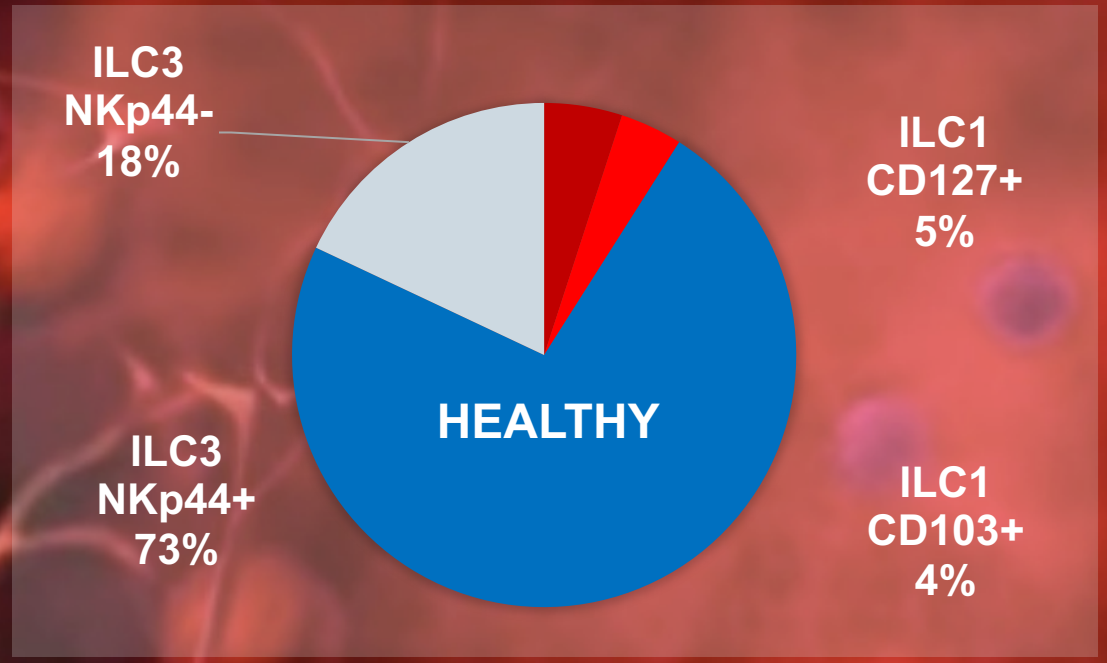


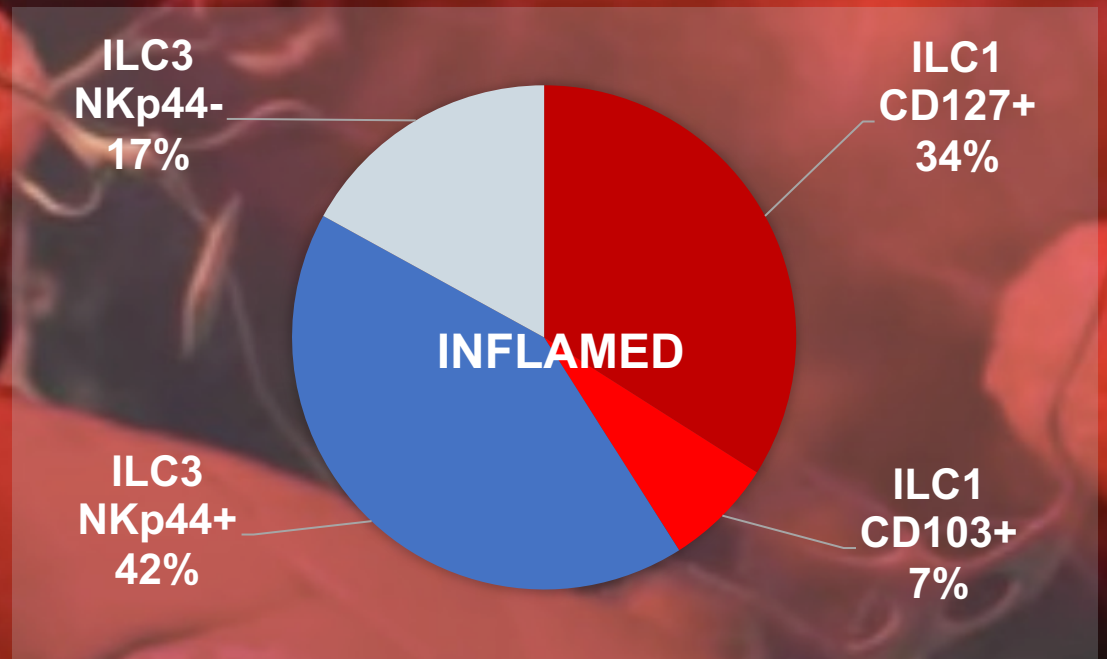
ILC subset-specific interactions with the epithelium



ILC in in Crohn's Disease

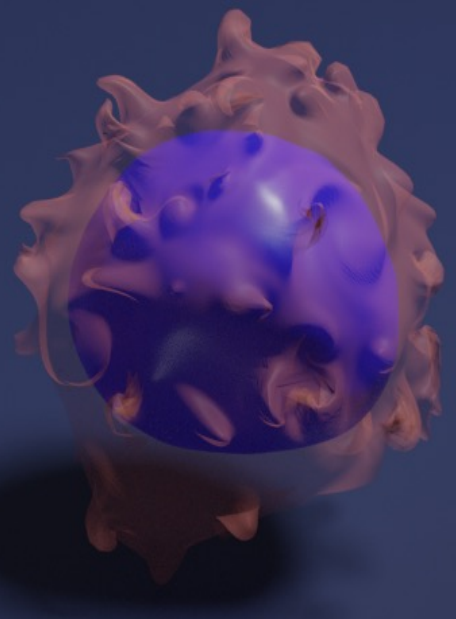
ILC1 accumulate in the inflamed intestines of patients with Crohn's Disease





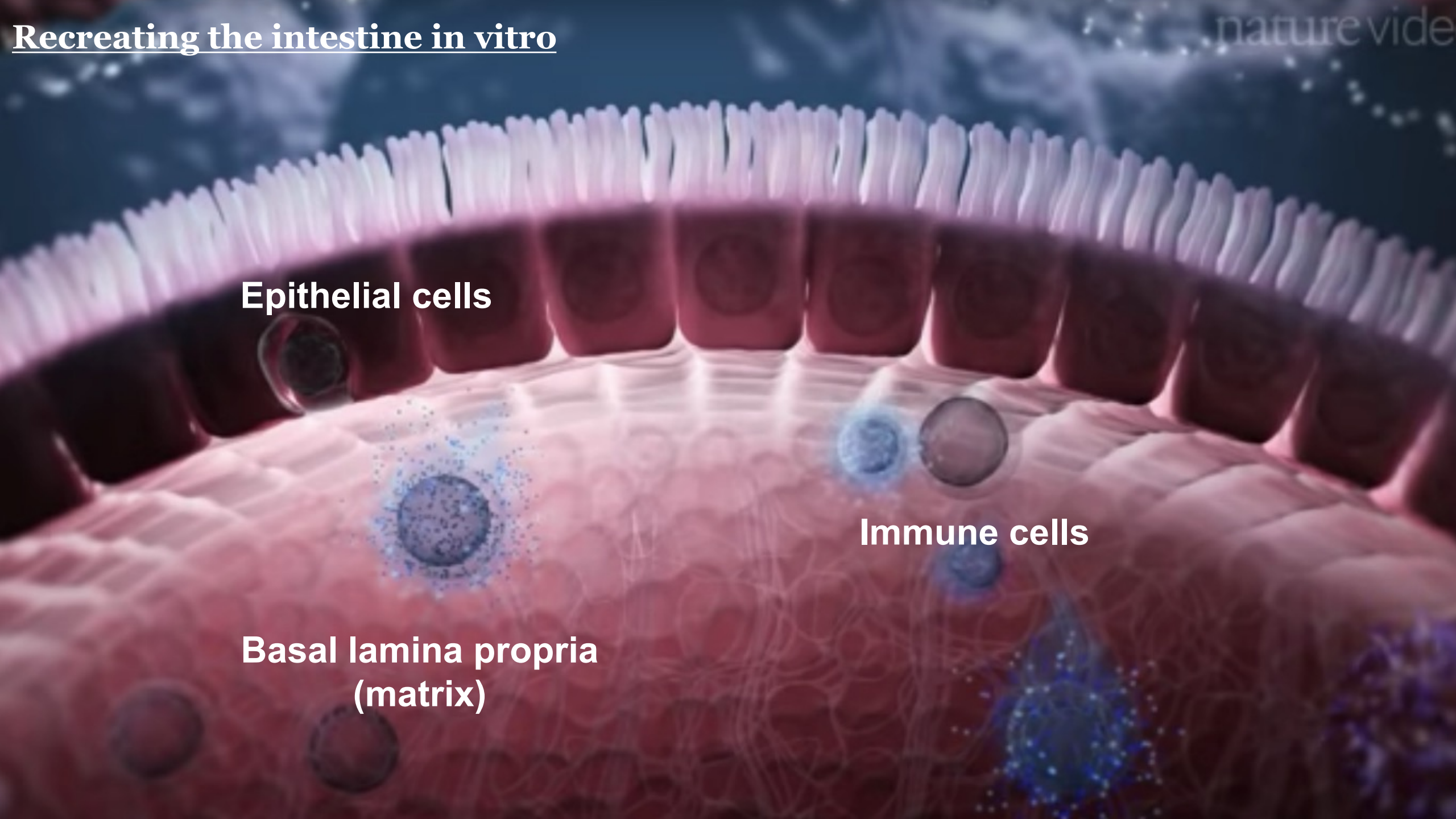
What impact do ILC1 have on their microenvironments?

Are ILC1 *responding to damage* or *driving pathology*?

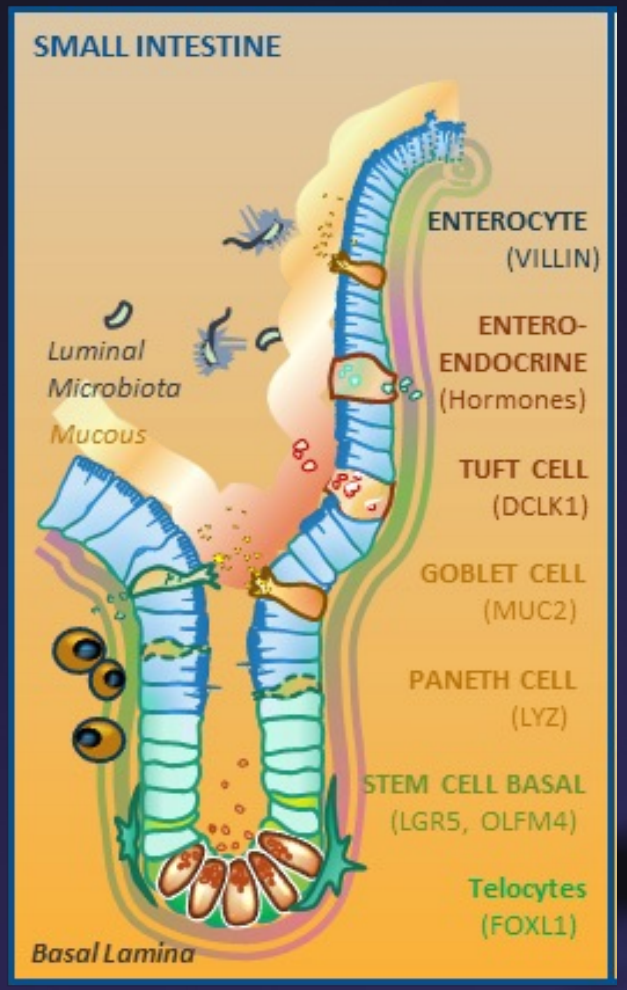


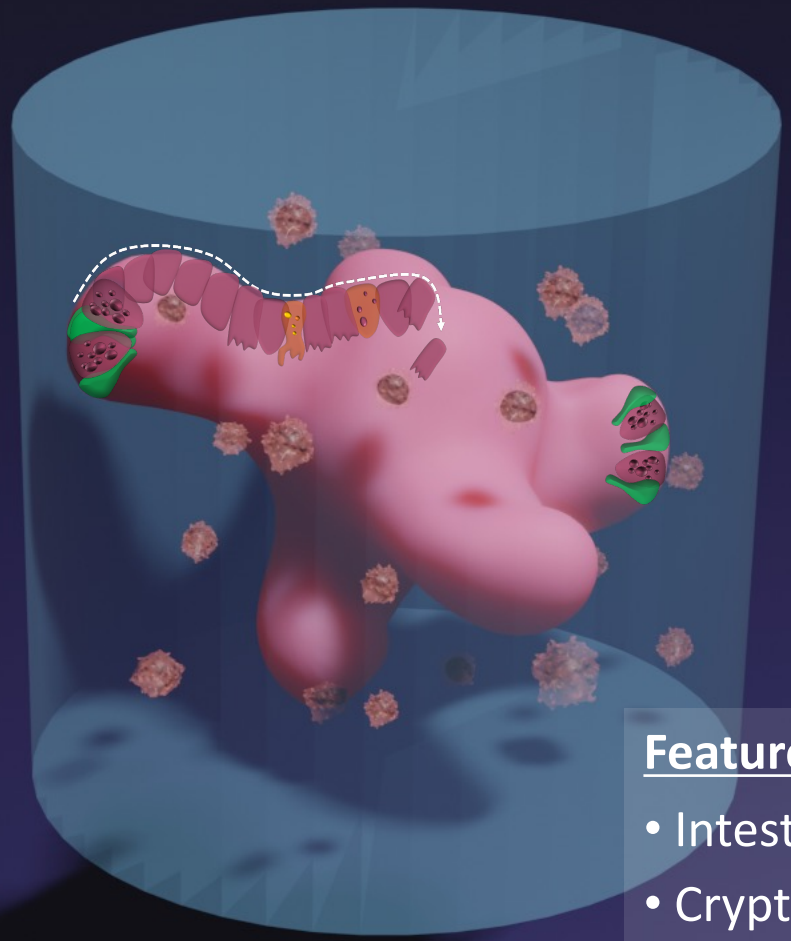
ILC1: Type I Innate lymphoid cells

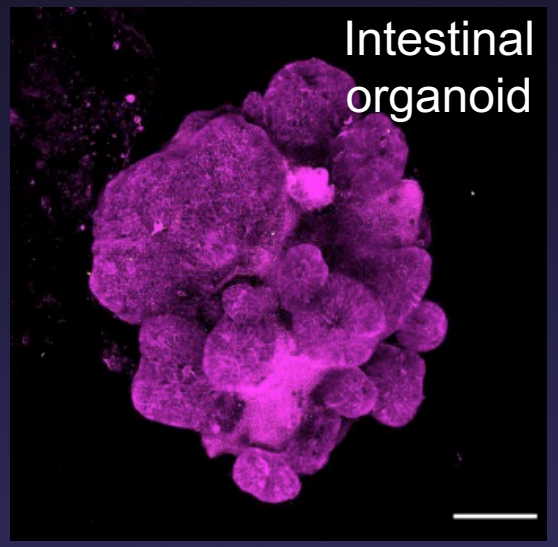
Recreating the intestine in vitro



Intestinal organoids mimic the complexity of the native epithelium



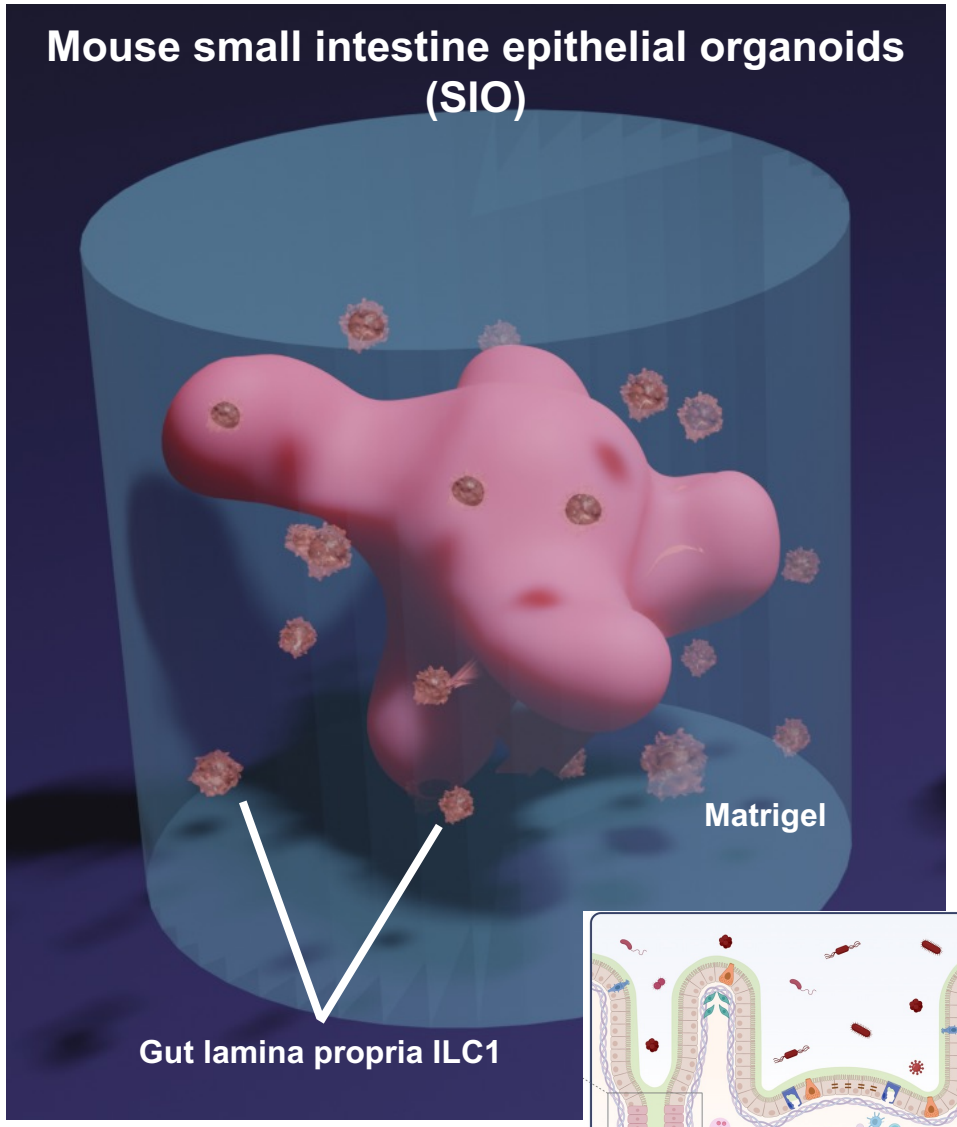


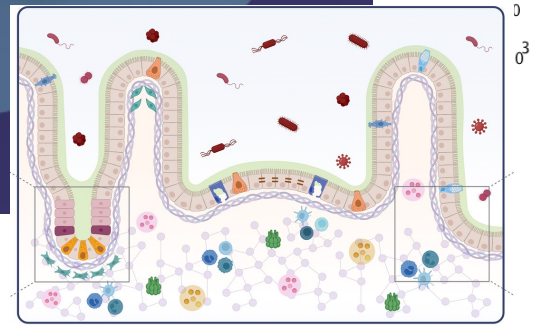


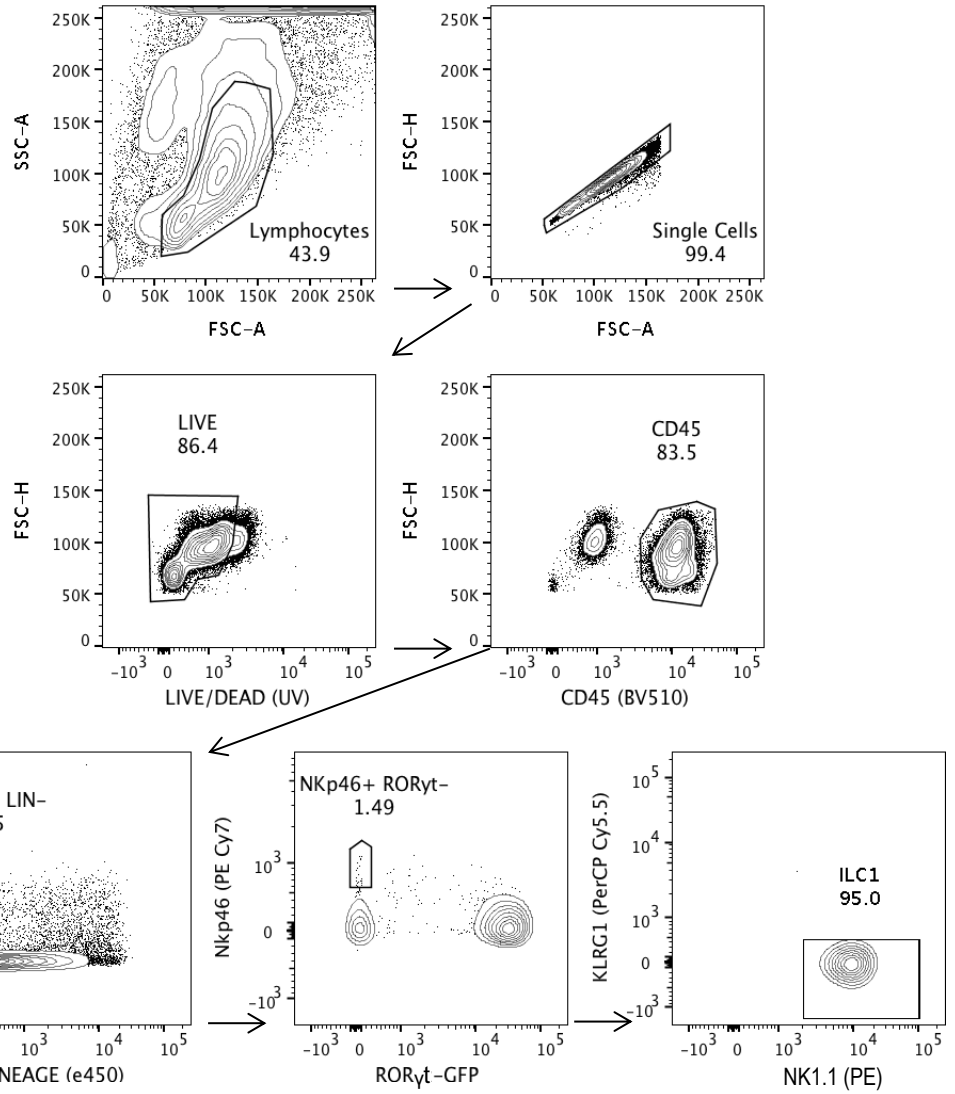
Features of intestinal organoids

- Intestinal stem cell crypts
- Crypt-villus organisation
- Absorptive and secretary enterocytes
- Apico-basal orientation

Intestinal organoid-ILC1 co-culture



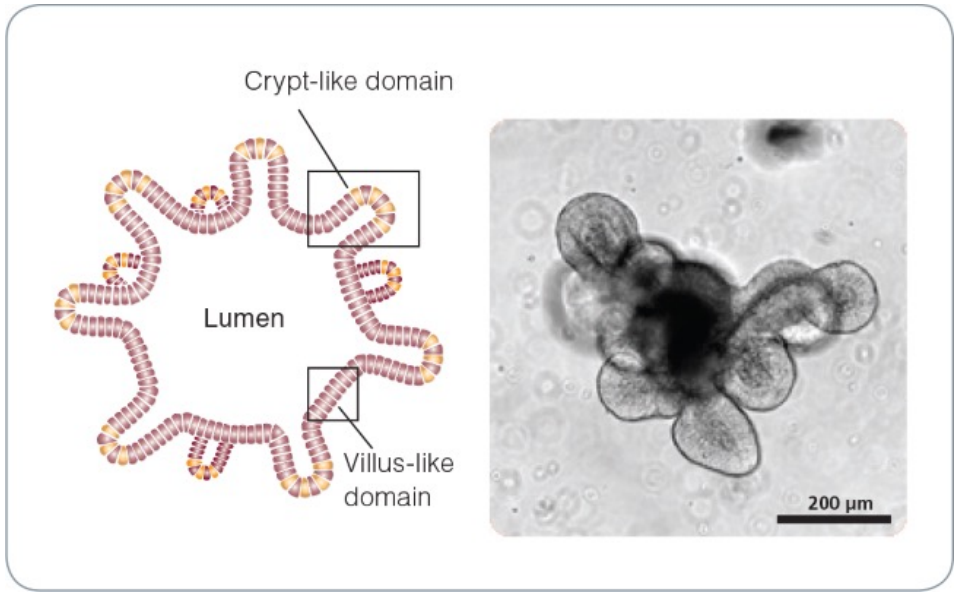


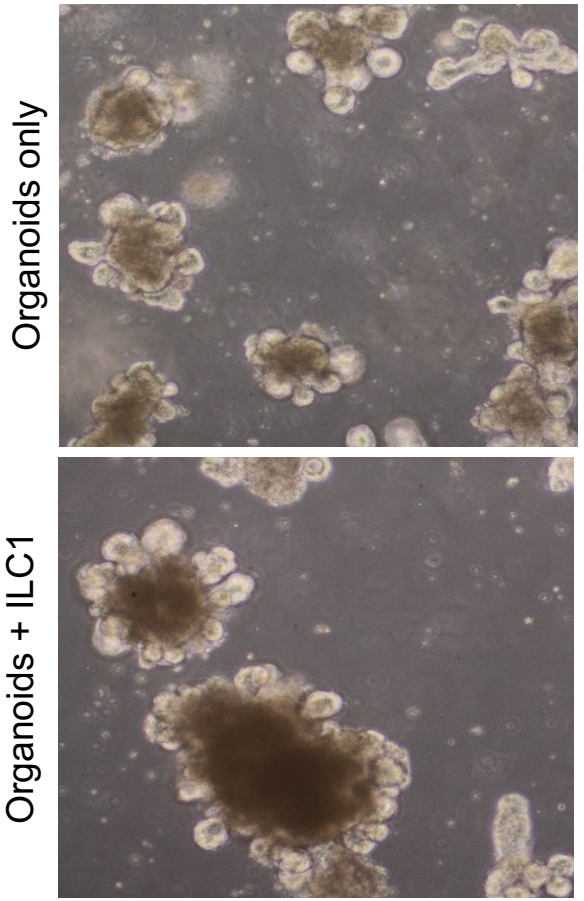


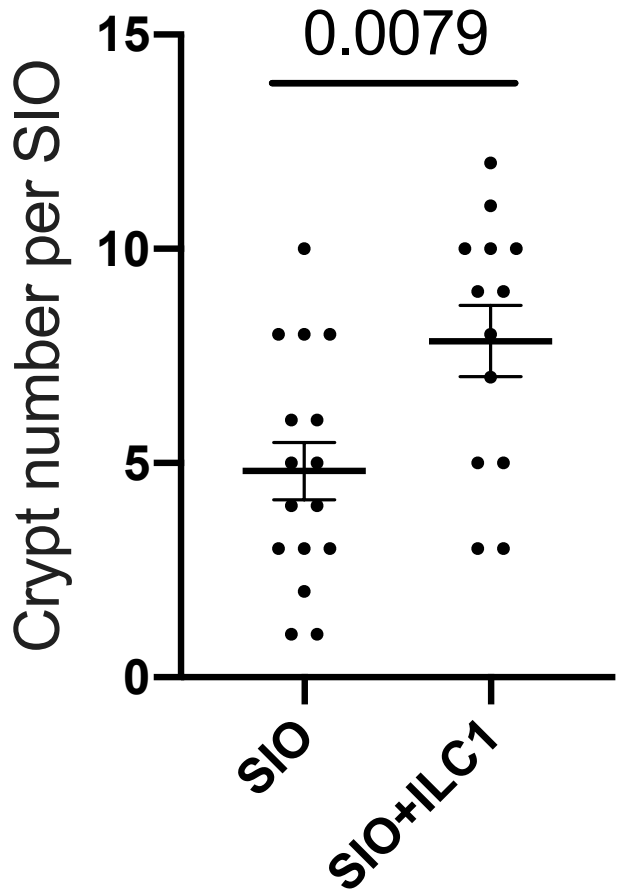
ILC1: single cells, live, CD127+, Lineage (CD3, CD5, CD19, Ly6G)- , RORγt- , NKp46+ , KLRG1- and NK1.1+

ILC1: Type I Innate lymphoid cells

ILC1-intestinal organoid co-cultures





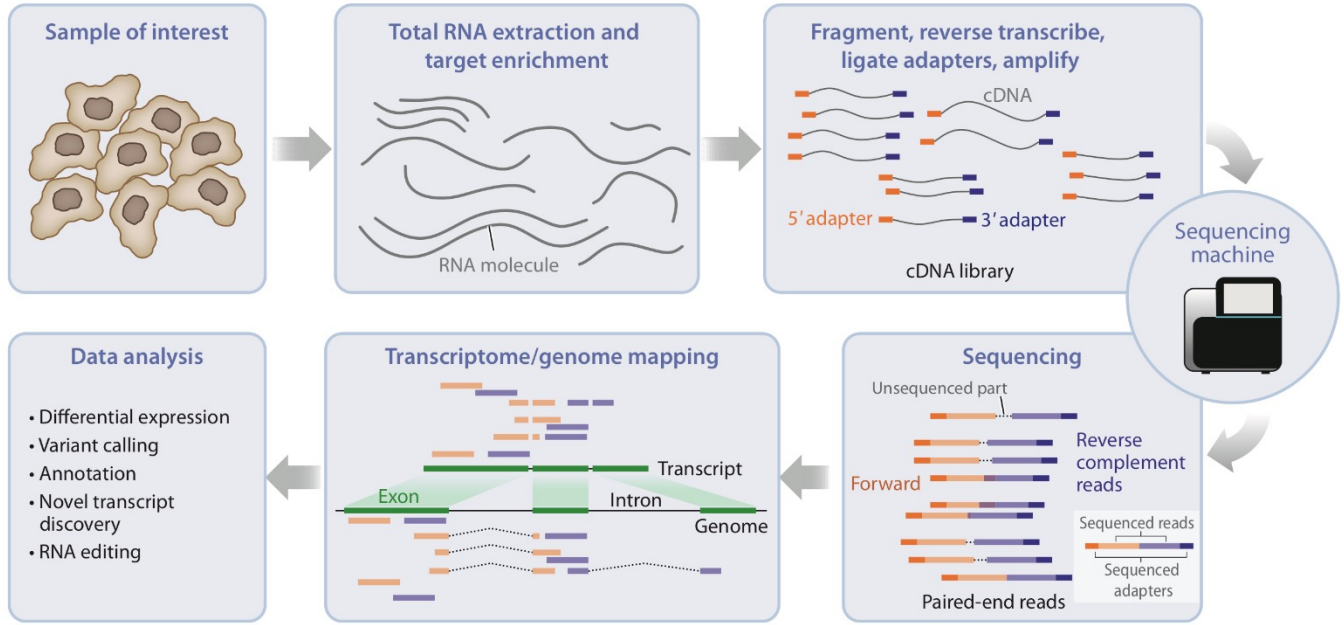


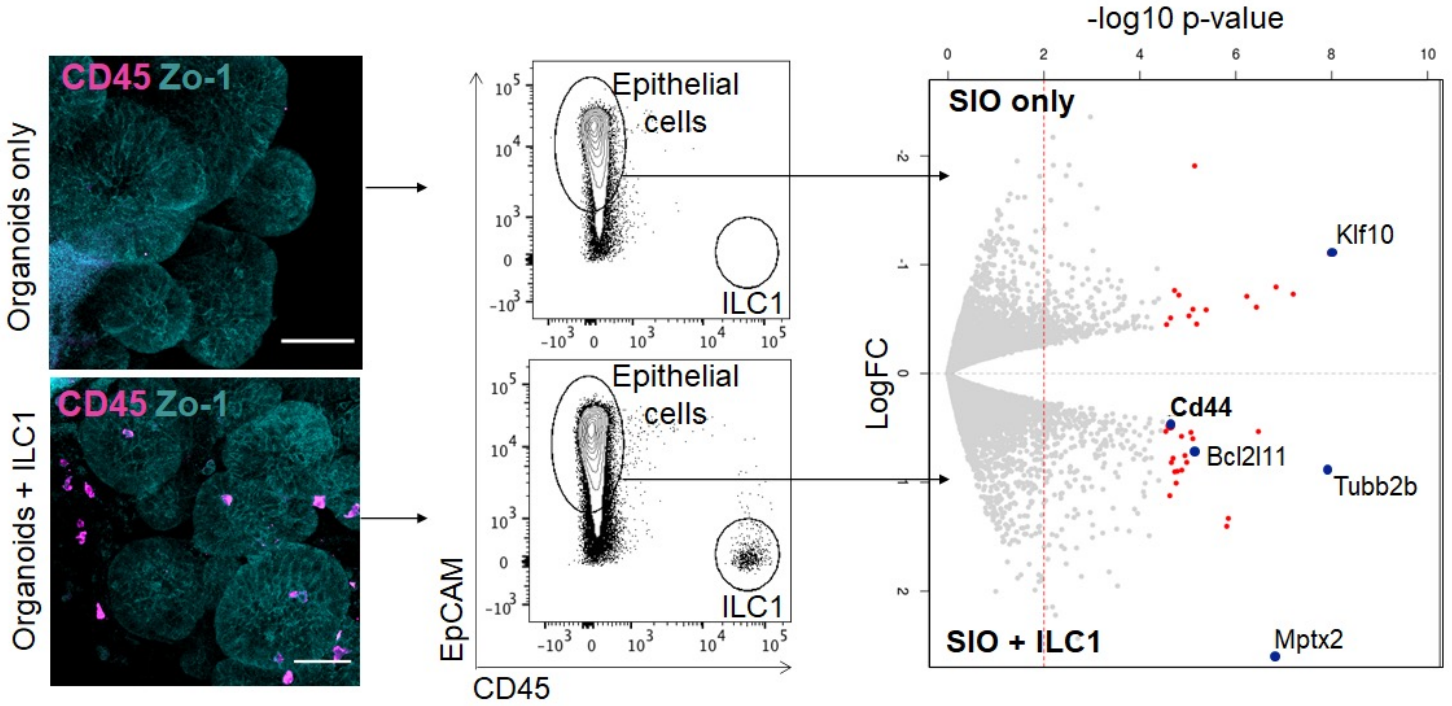
Why would organoids grow more when co-cultured with ILC1 (which secrete IFNγ)?

Jowett *et al*. ILC1 drive intestinal epithelial and matrix remodelling. *Nat Mater* 2021

ILC1: Type I Innate lymphoid cells
SIO: small intestinal organoid

RNA sequencing

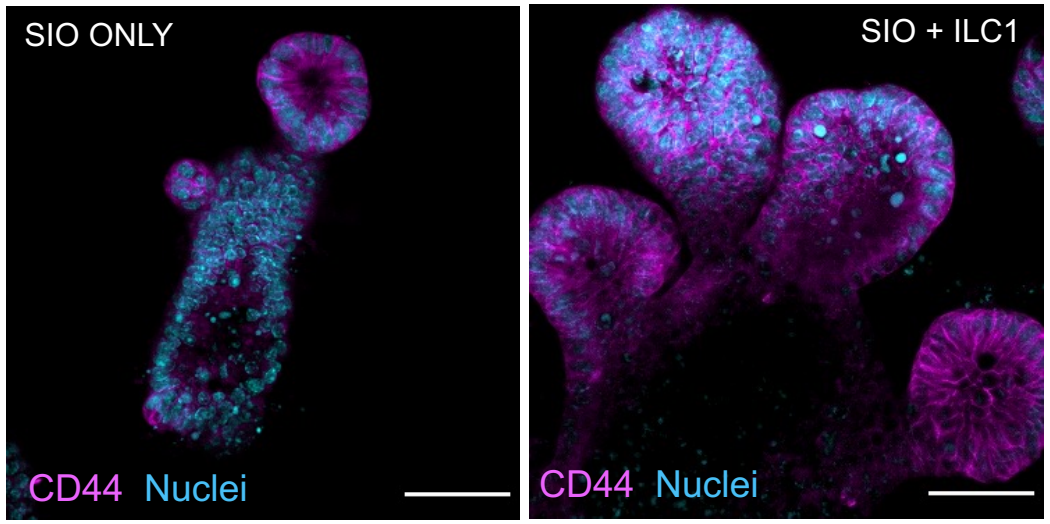


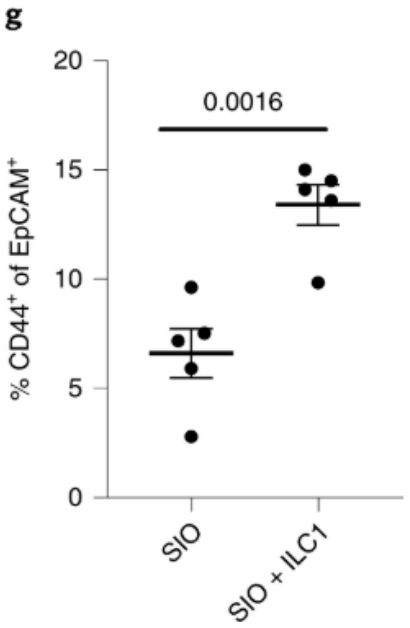


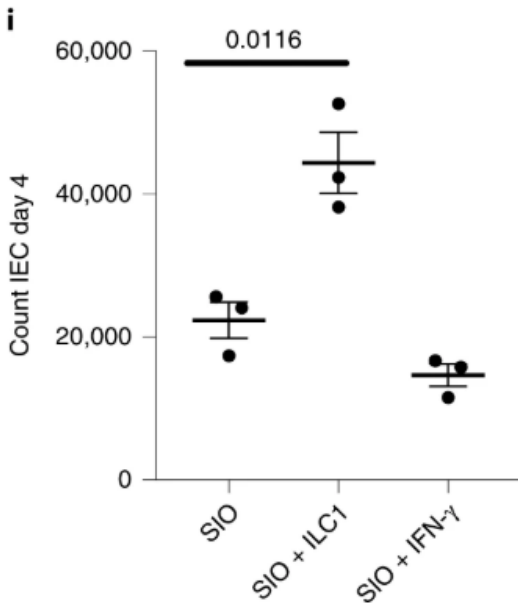
CD44

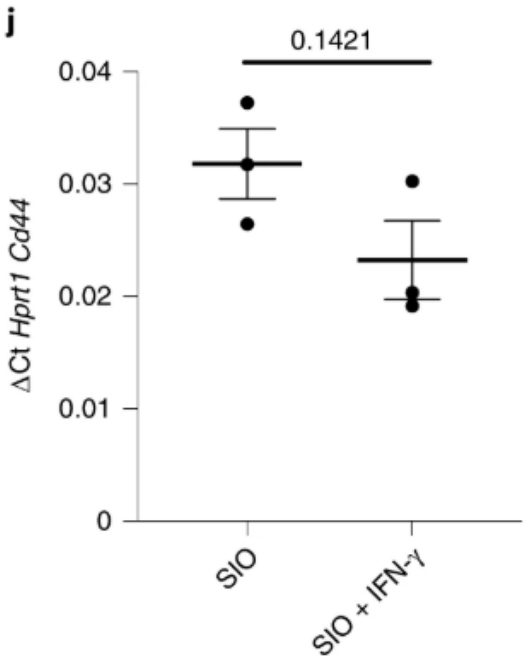
- Receptor for hyaluronic acid
- Marker of intestinal stem cells
- Roles in wound healing, regeneration and inflammation

ILC1-intestinal organoid co-cultures

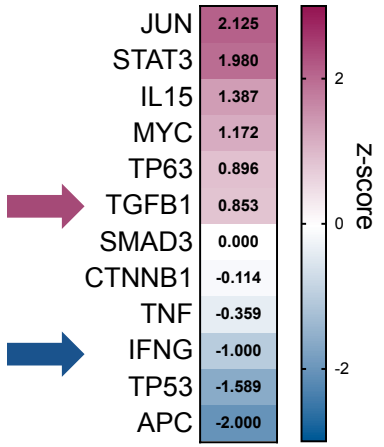






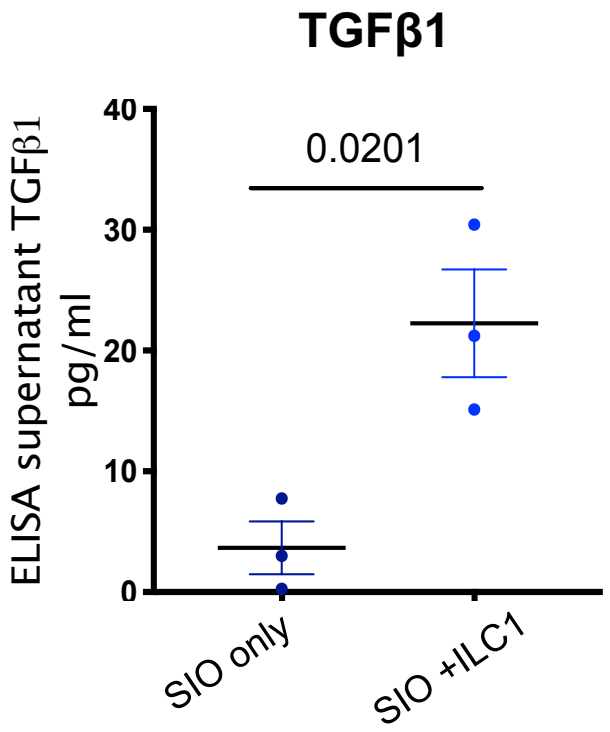


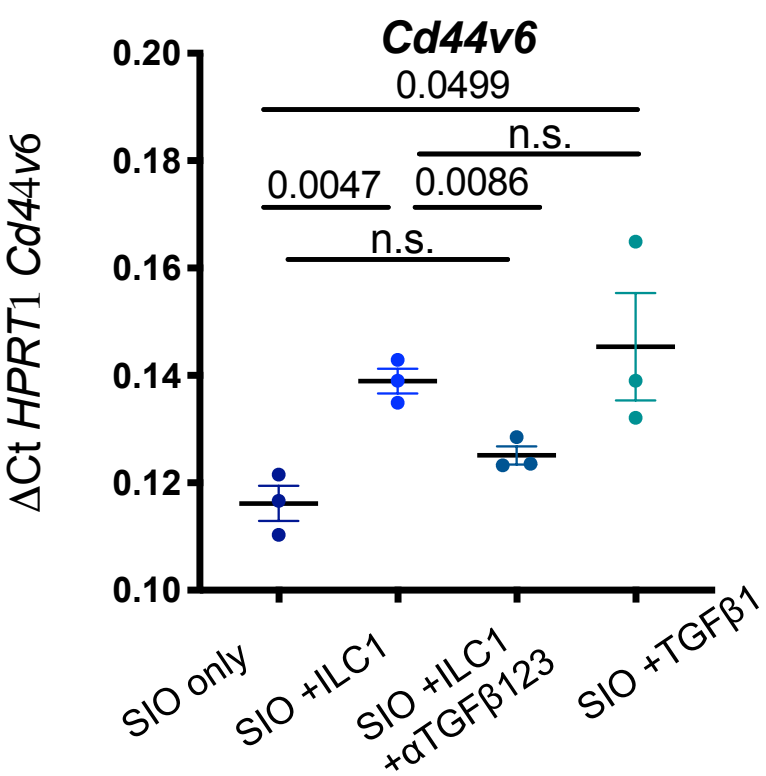
Upstream regulators

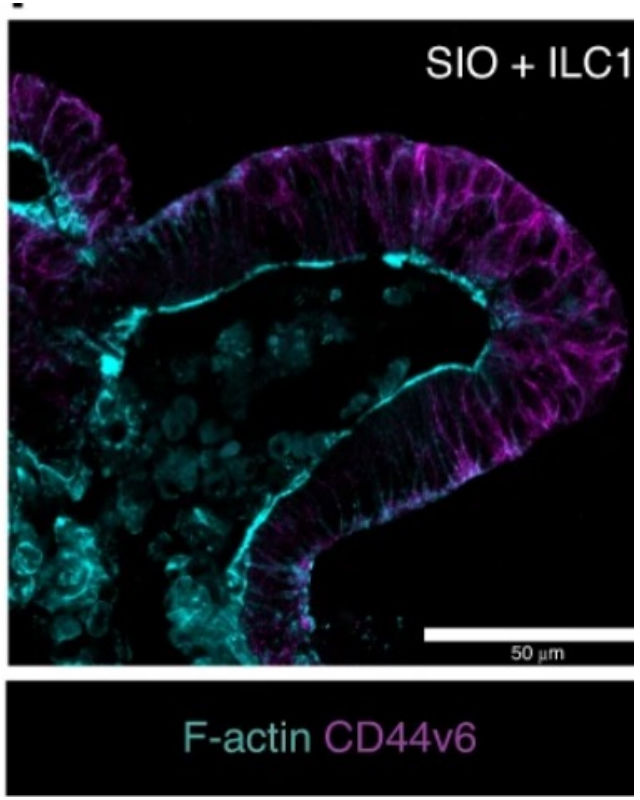


Epithelial growth and CD44 expression did not appear to be mediated by IFNγ

ILC1-derived TGFβ1 drives CD44v6



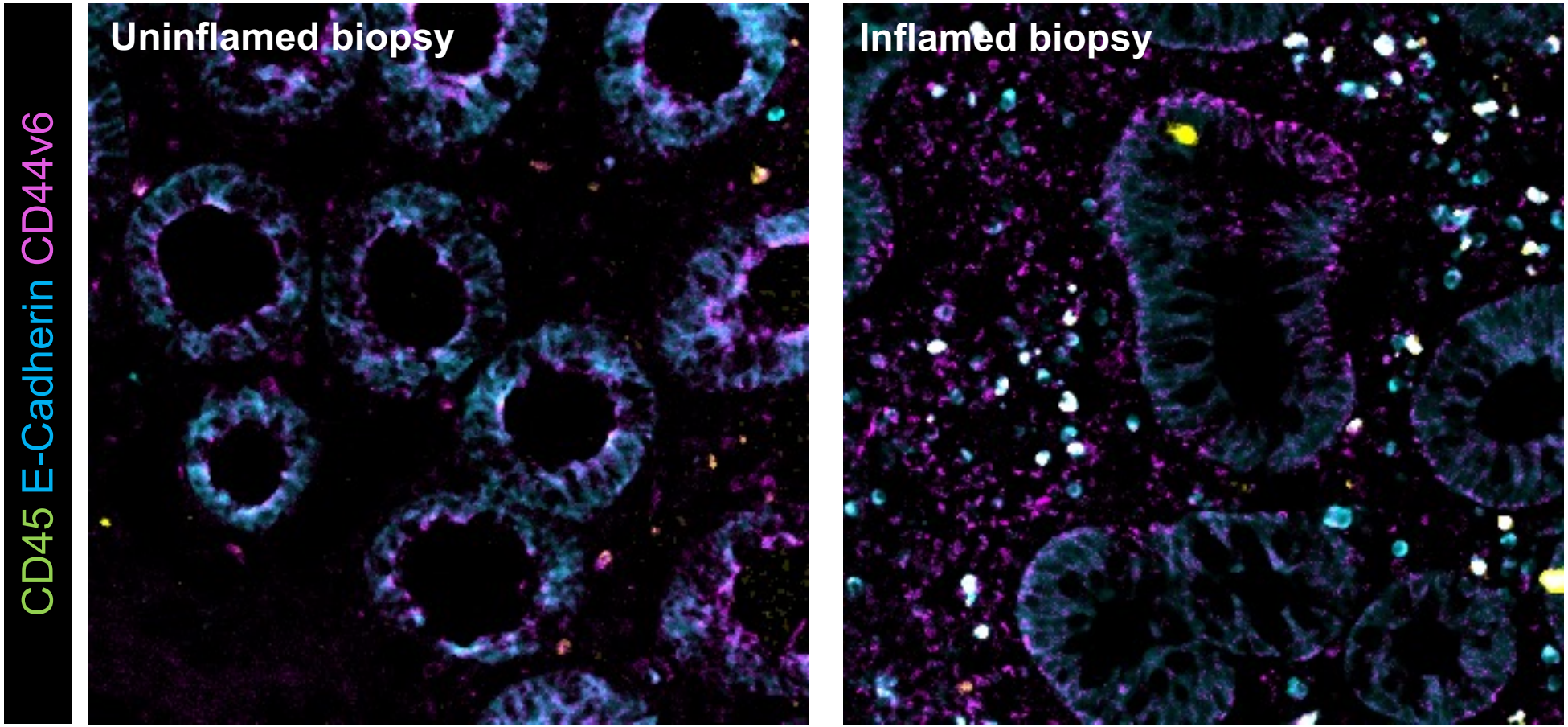




- ILC1 drive epithelial proliferation, not cytotoxicity
- ILC1 are an unexpected source of TGFβ1
- ILC1-derived TGFβ1 drives growth of CD44v6^{+} epithelial crypts
- CD44v6 is a marker of metastatic colon cancer

ILC1: Type I Innate Lymphoid Cells
SIO: mouse Small Intestinal Organoid

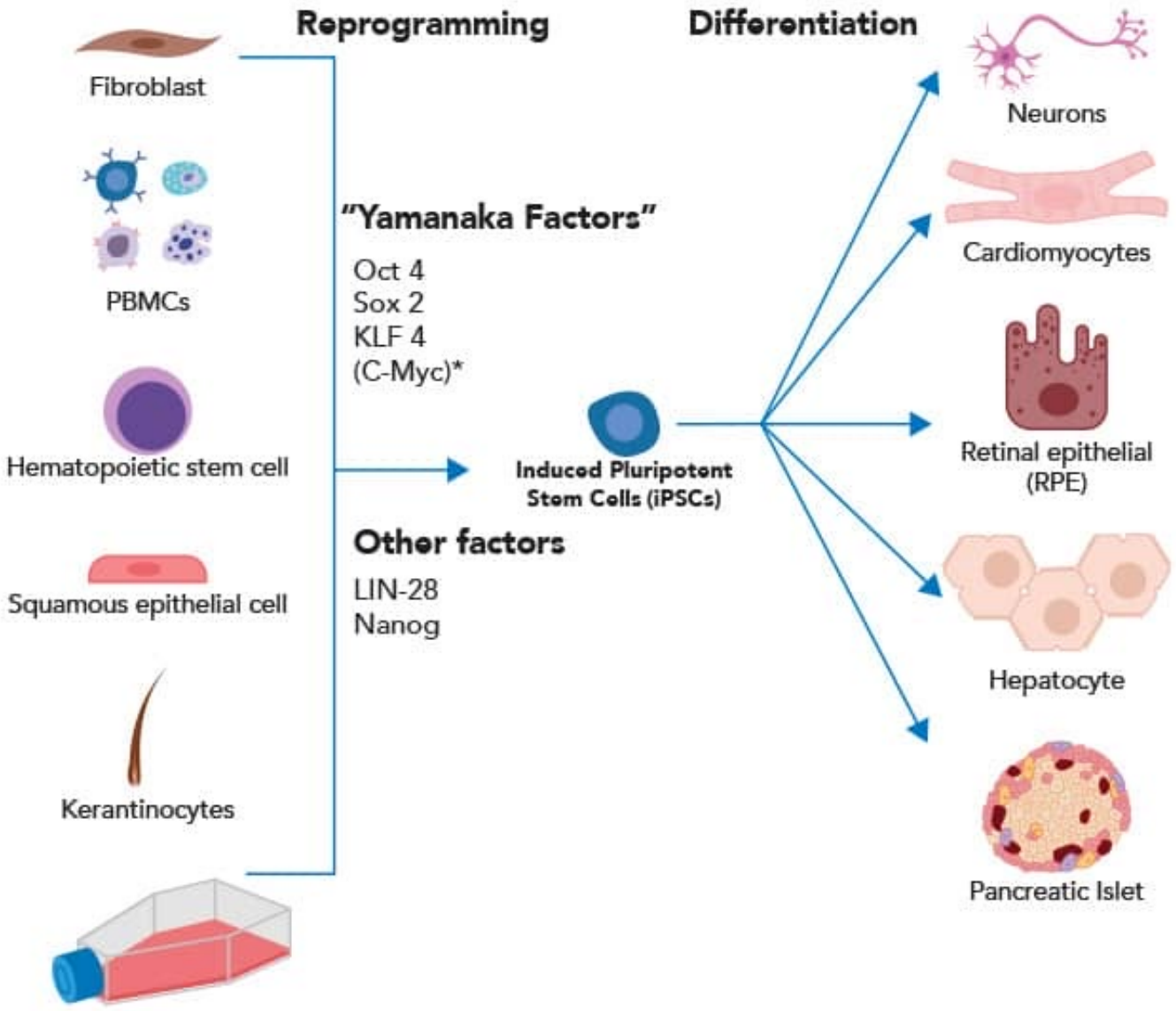
Crohn's disease patient biopsies

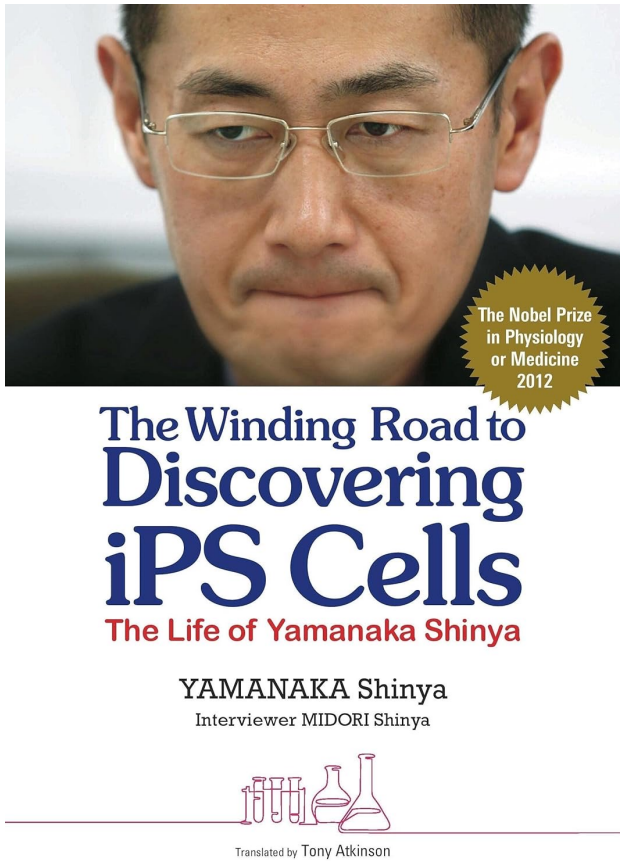


Crohn's patient tissues show positive staining for CD44v6 in the epithelium and the mesenchyme

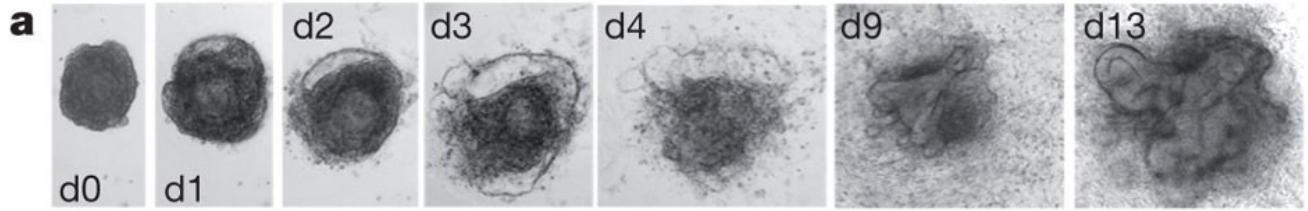
How can we model this in vitro?

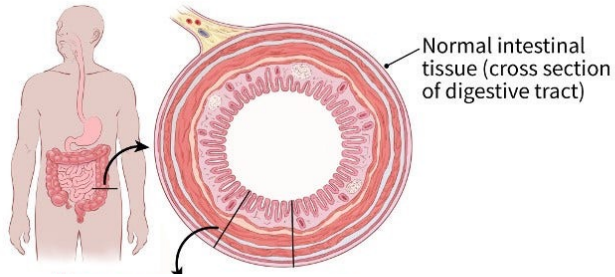
Induced pluripotent stem cells (iPSC)

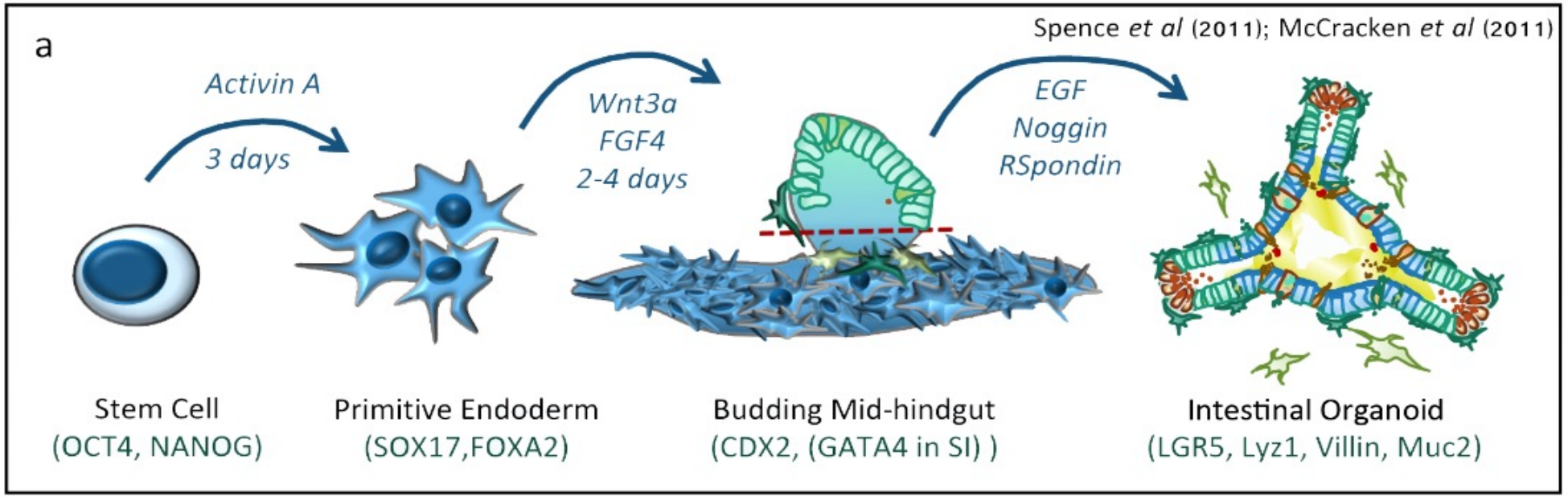




Human iPSC-derived intestinal organoids

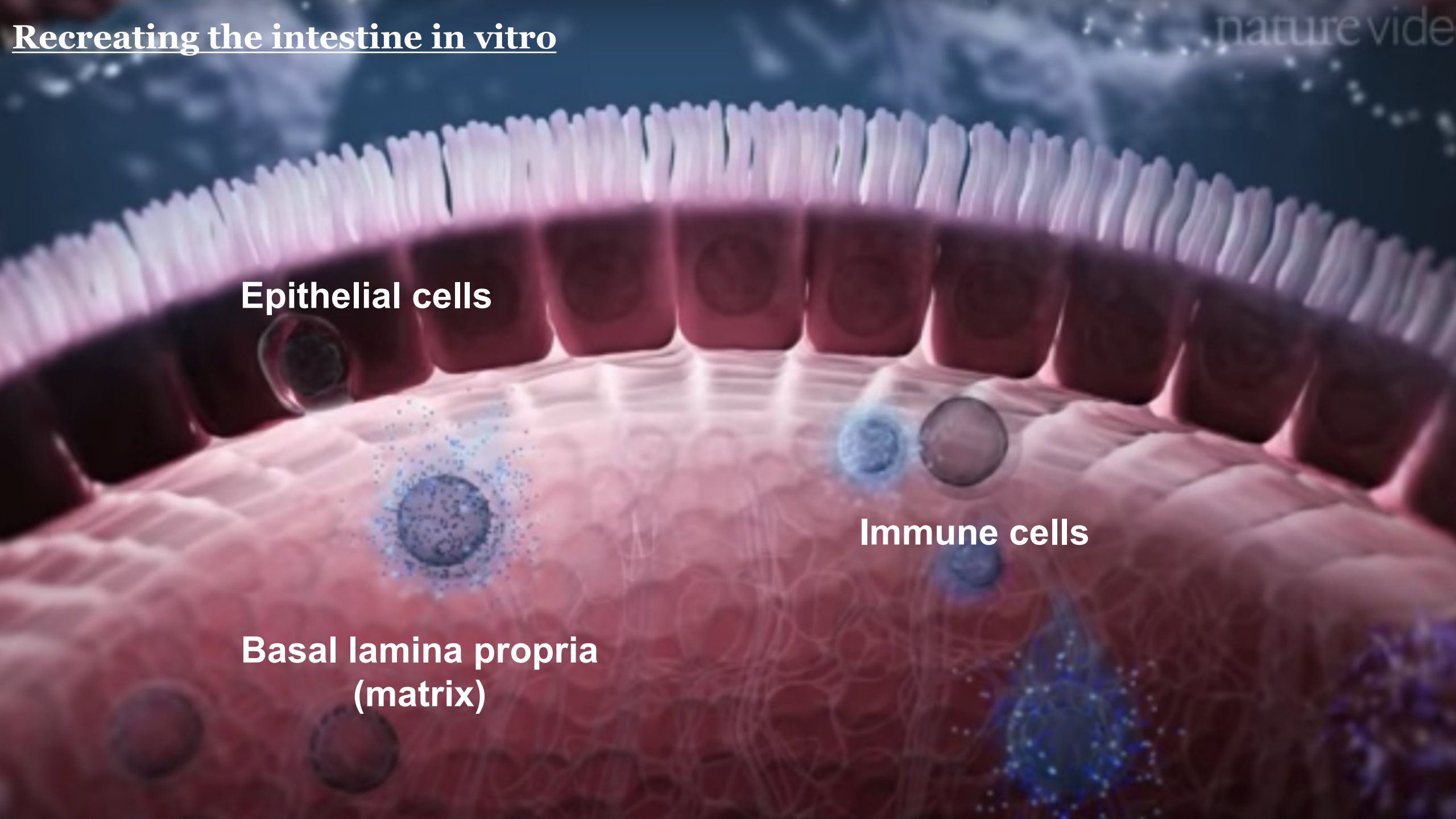




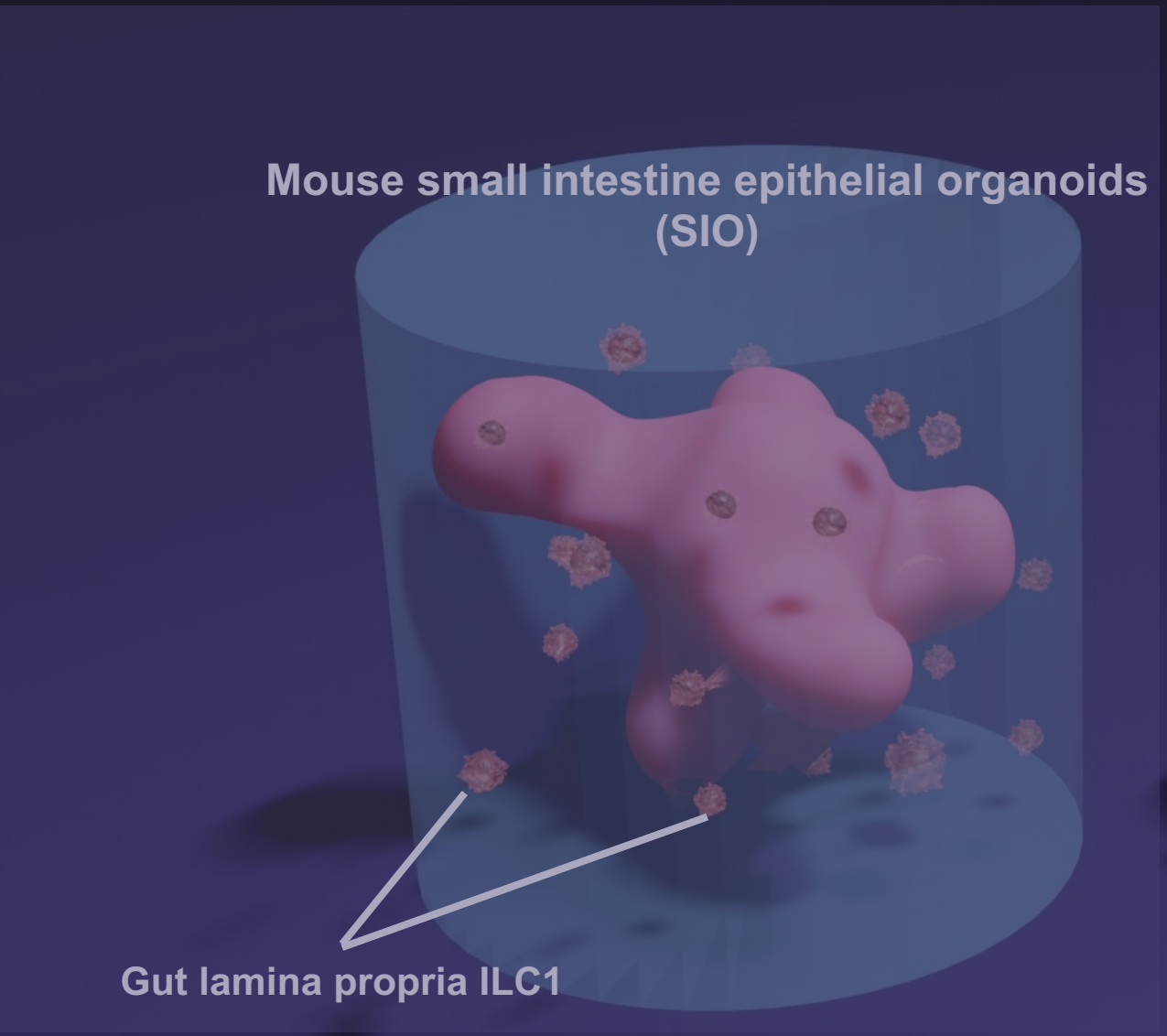


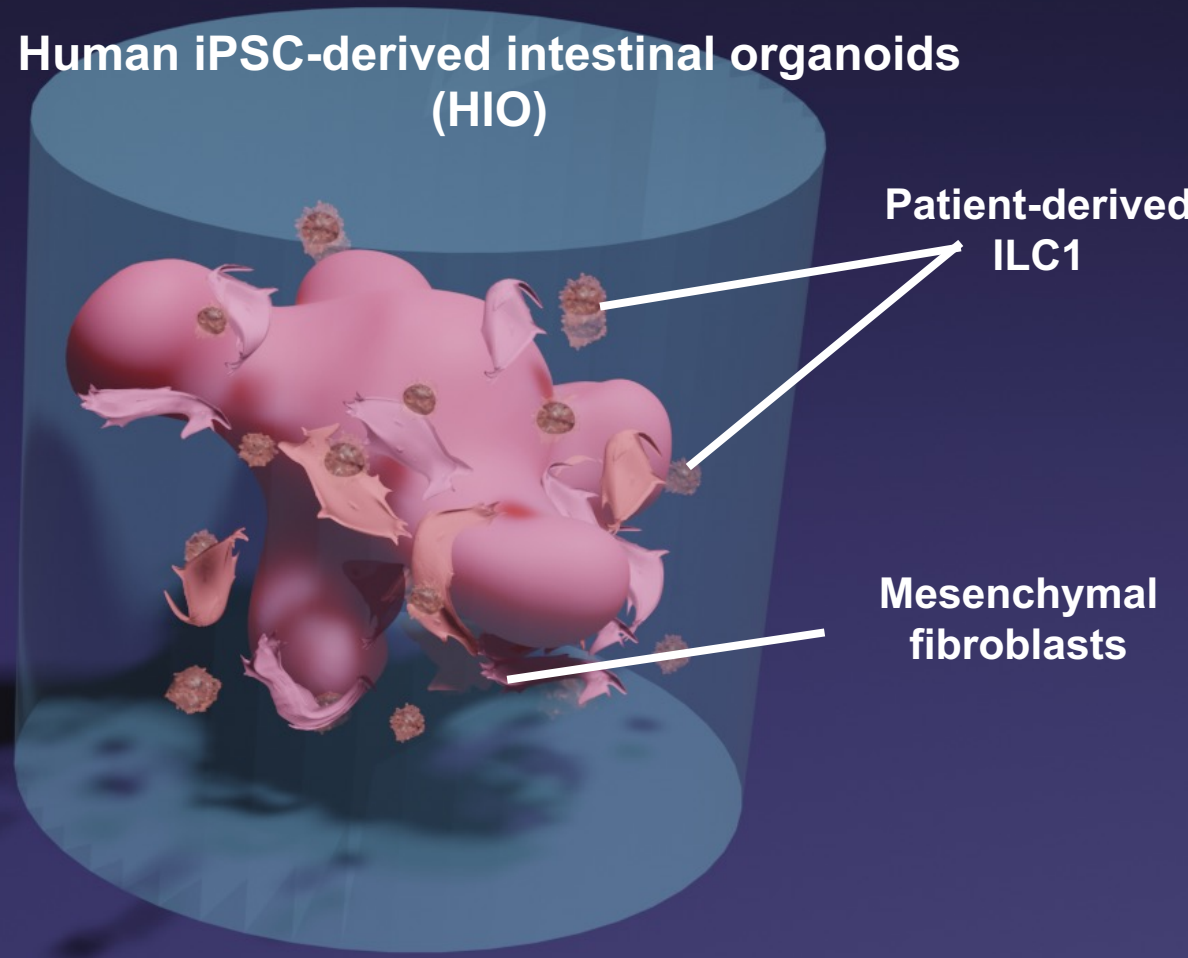
- iPSC-derived intestinal organoids created by mimicking developmental programme
- iPSC-derived intestinal organoids contain epithelial and mesenchymal cells

Recreating the intestine in vitro



Intestinal organoid-ILC1 co-cultures

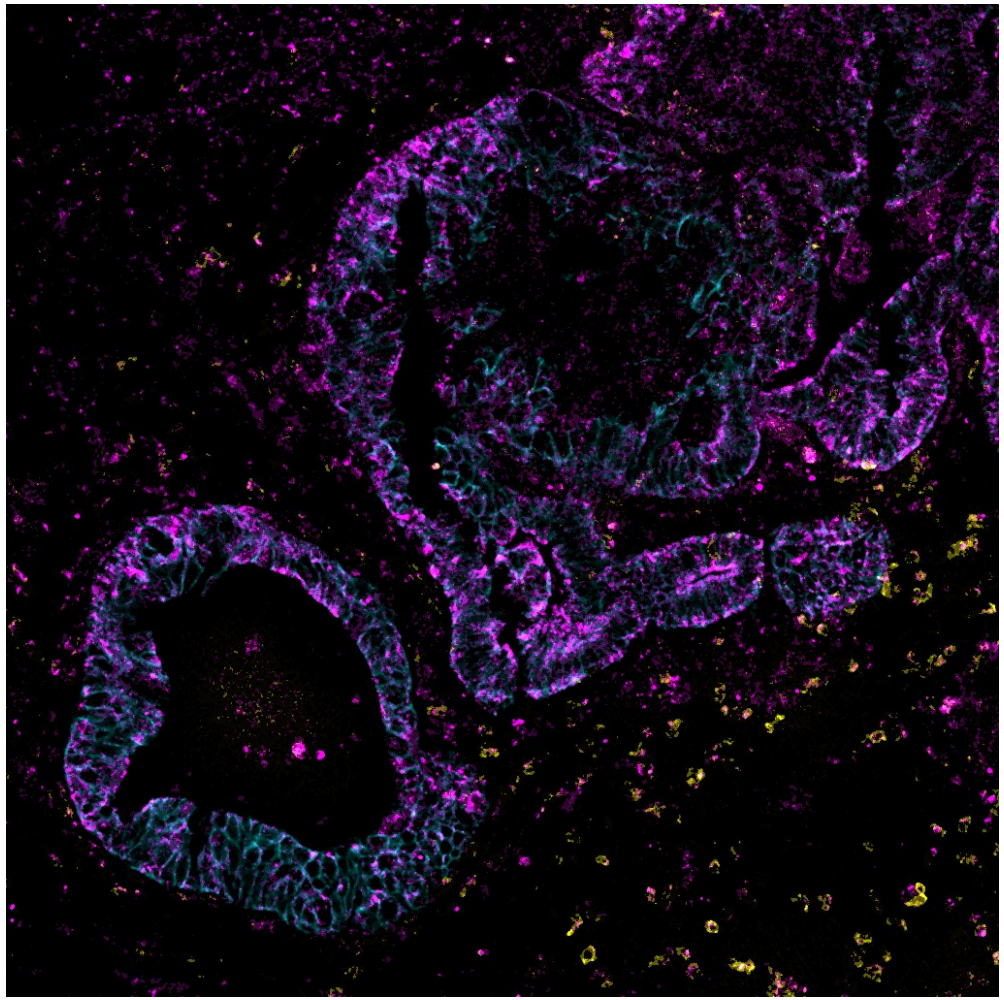




Human iPSC-derived organoids (HIO) contain epithelial and mesenchymal cells

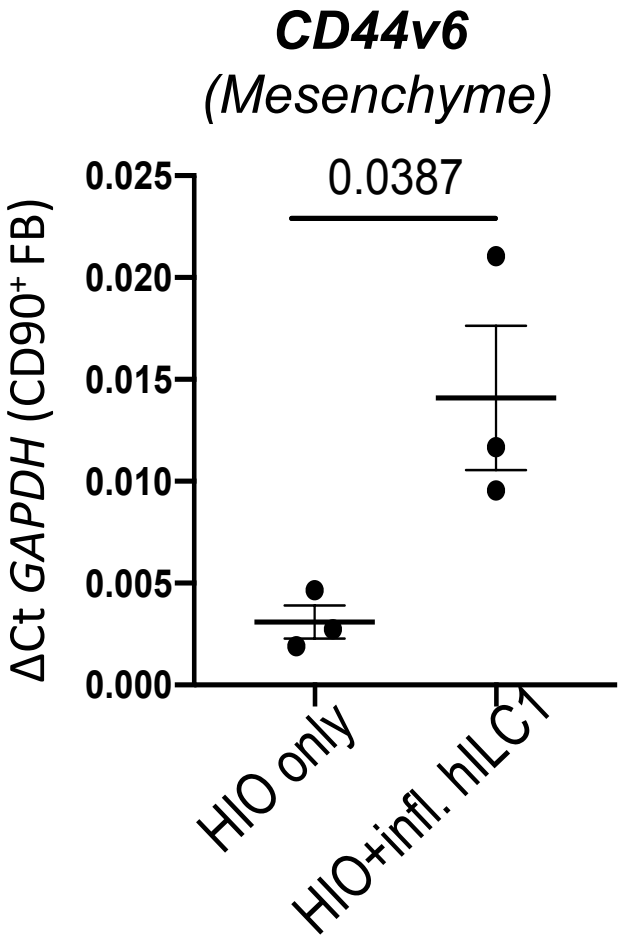
ILC1: Type I Innate lymphoid cells

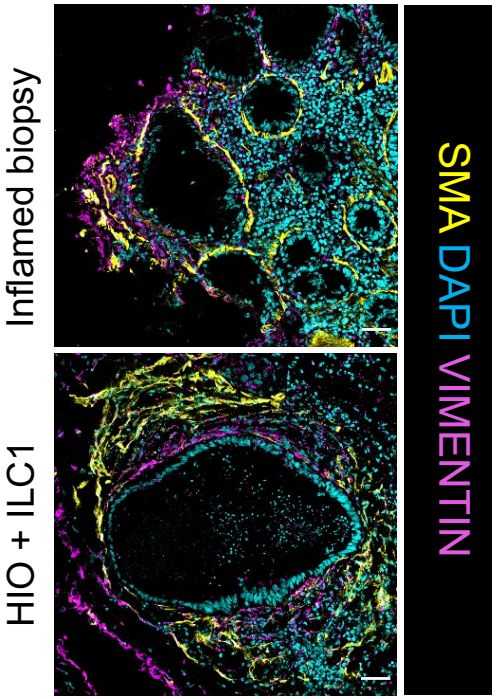
Human ILC1 drive CD44v6 expression in the mesenchyme of human intestinal organoids (HIO)



CD45 E-Cadherin CD44v6

In vitro model replicates CD44v6 expression and mesenchymal phenotype in patient biopsies

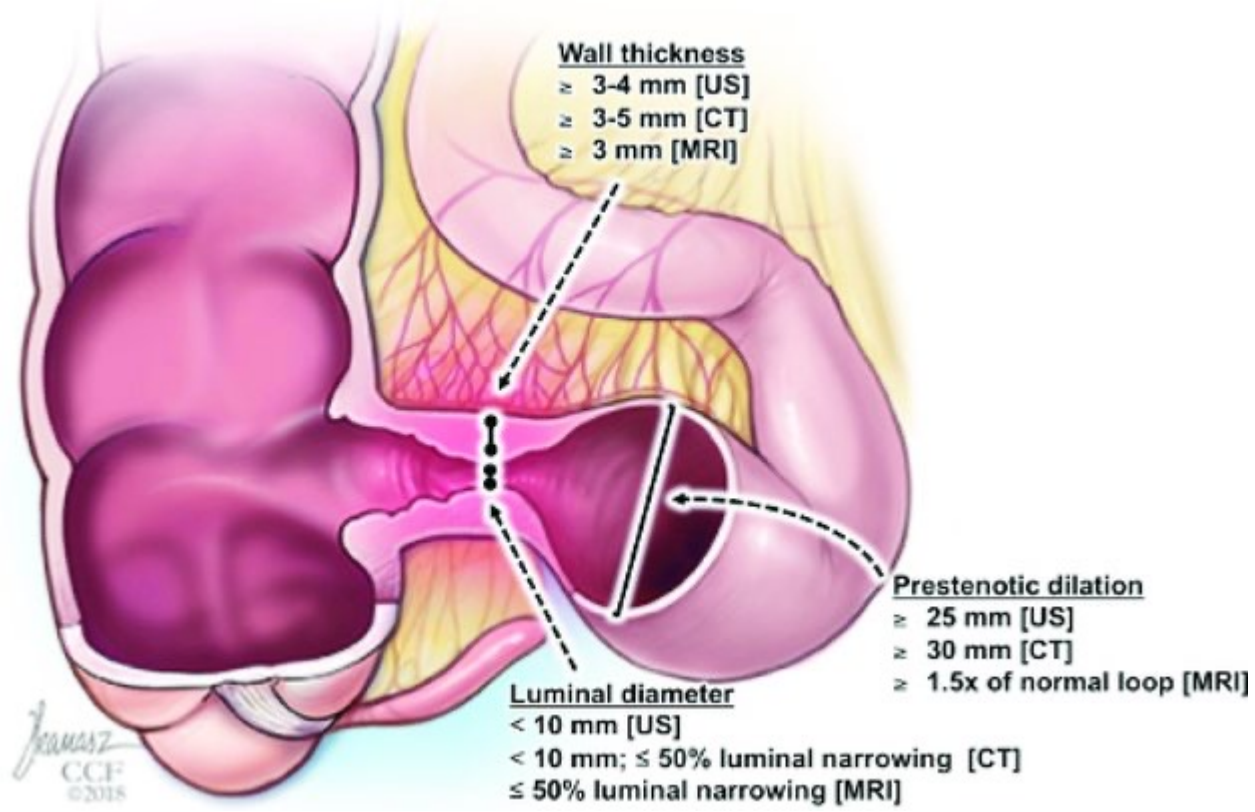




Jowett *et al*. ILC1 drive intestinal epithelial and matrix remodelling. *Nat Mater* 2021

ILC1: Type I Innate lymphoid cells

Pathological matrix remodelling in Crohn's Disease



- Up to 70% of Crohn's patients require gut resection to remove fibrotic strictures
- Almost half of patients develop penetrating disease (fistula)
- 10-15% of Crohn's patients die of cancer

- ILC1 secrete TGFβ1
- TGFβ1 is a known regulator of fibrosis

How can we study the matrix around HIO?

MATRIGEL

- Protein extract derived from a mouse tumour
- Undefined composition to which manufacturer adds unknown concentrations of matrix metalloproteinase (MMP) inhibitors
- Batch-to-batch variability

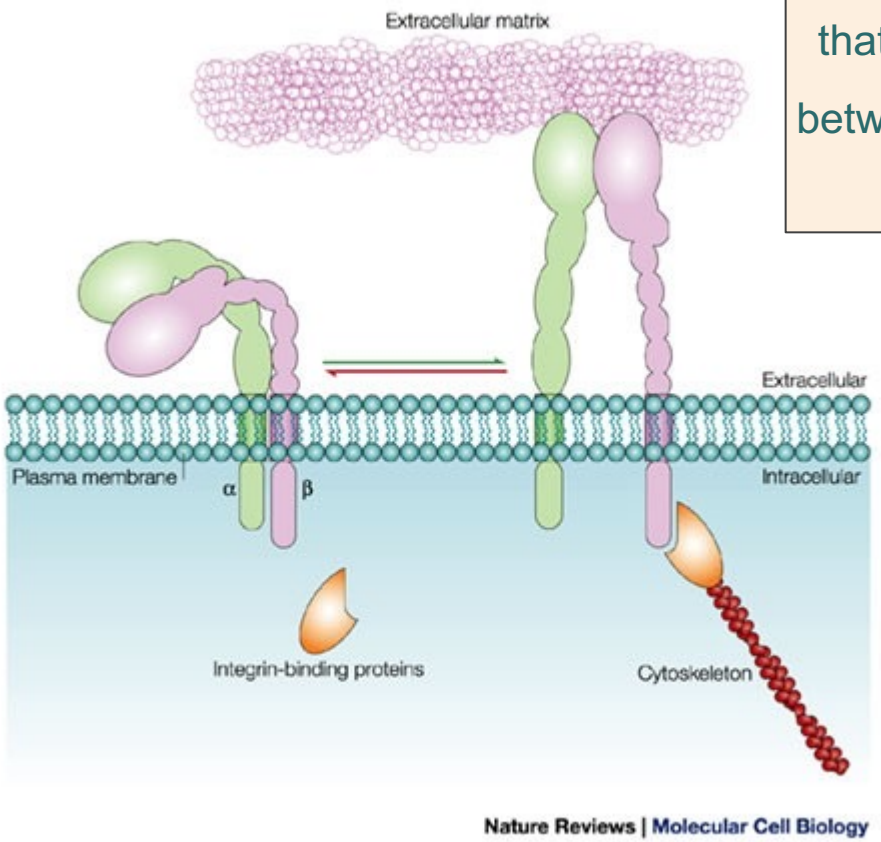
REQUIRED

- Techniques to quantify matrix remodelling around HIO
- Fully synthetic matrix suitable for organoid culture
  - Soft like the native intestine (<1kPa)
  - Independent control of mechanical and biological cues

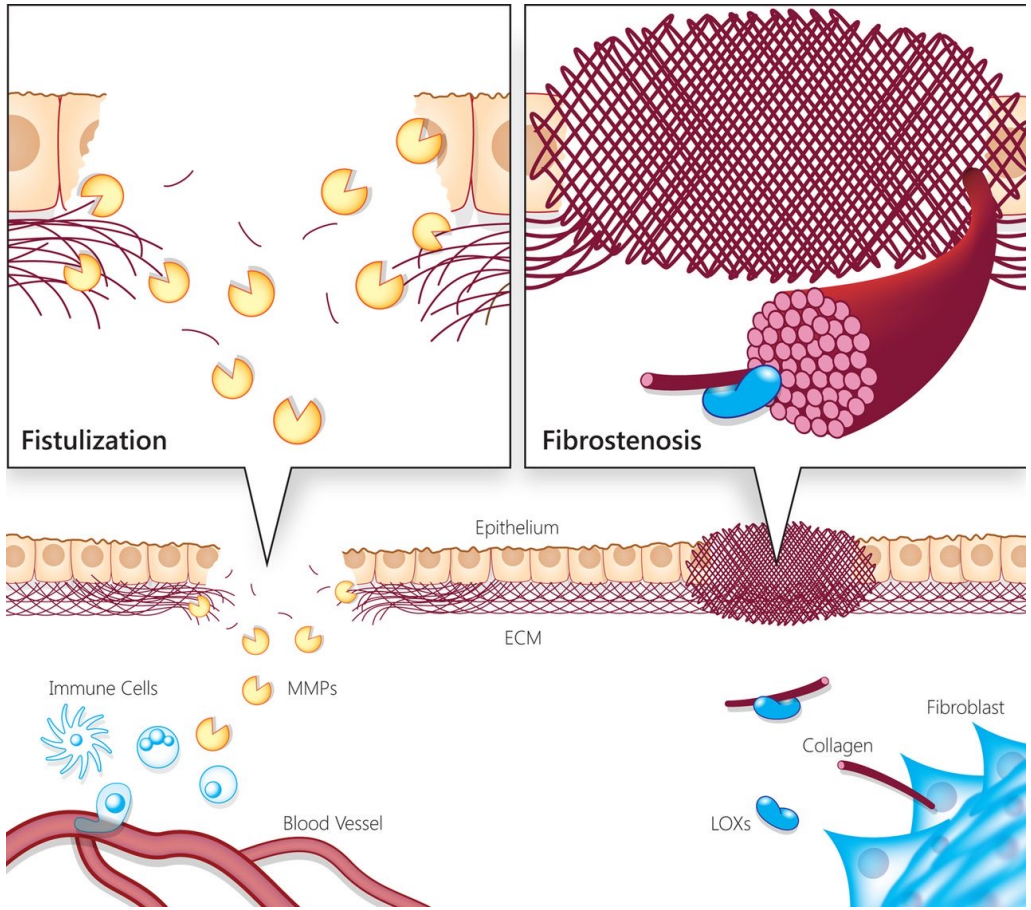
HIO: human iPSC intestinal organoid

Integrins and matrix metalloproteinases (MMPs)

Integrins – transmembrane proteins that mediate adhesion between the cell and the ECM

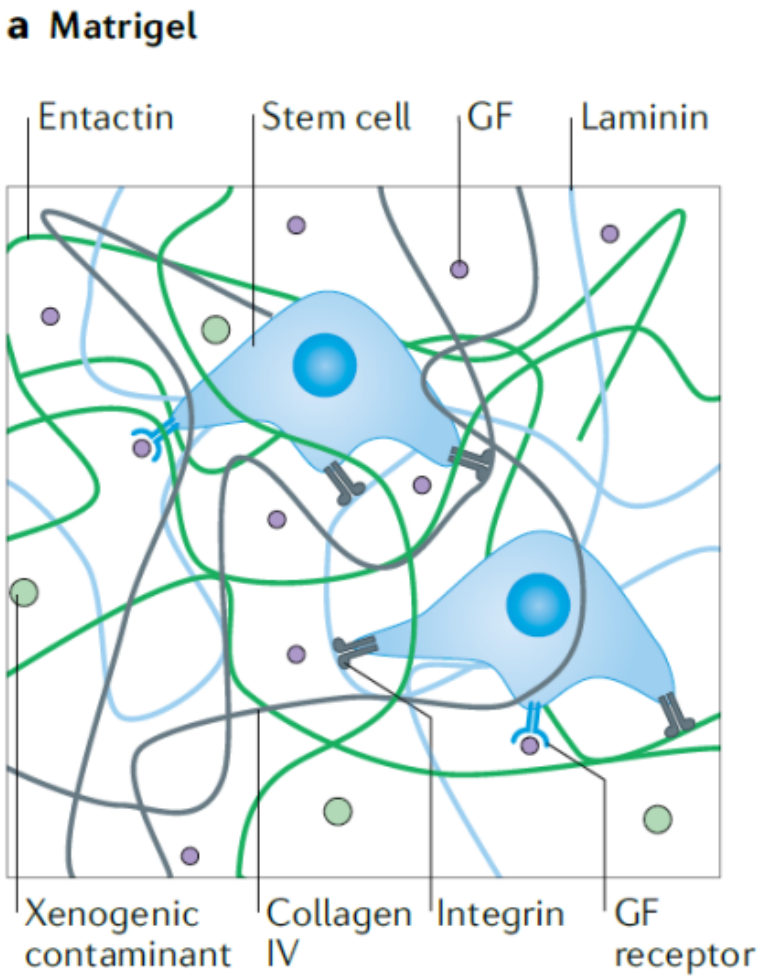


MMPs – enzymes that degrade components of the ECM



ECM: Extracellular matrix

Biologically derived versus synthetic hydrogels



Integrin-binding peptides: Avoid anoikis
Generate traction

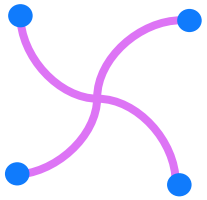
MMP degradable peptides: Allow for cell migration, changes in morphology & proliferation

Synthetic hydrogels: Fully defined, artificial mimics of the native ECM

Independently modulate stiffness and ligand concentration

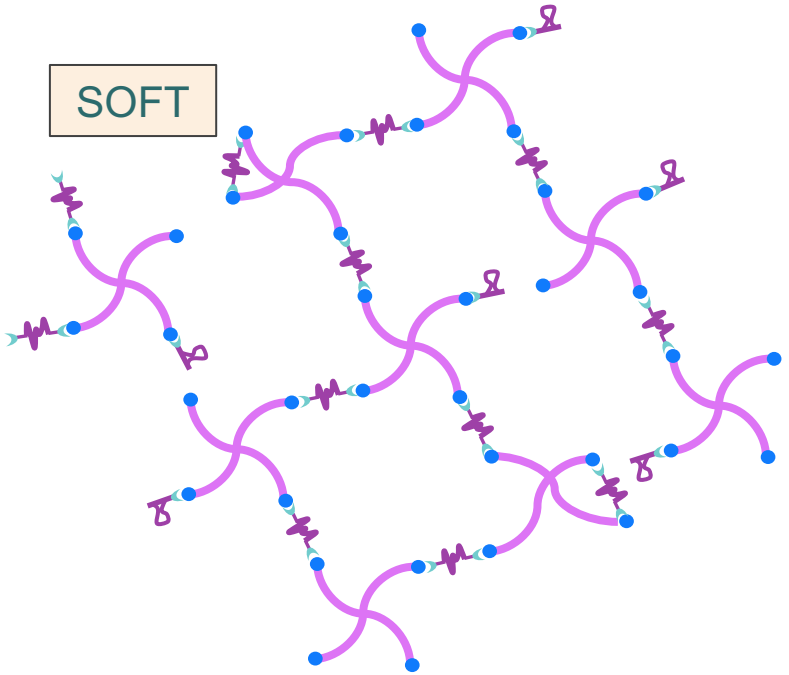
Homo-bifunctional degradable peptide

Adhesive peptide (RGD, e.g.)

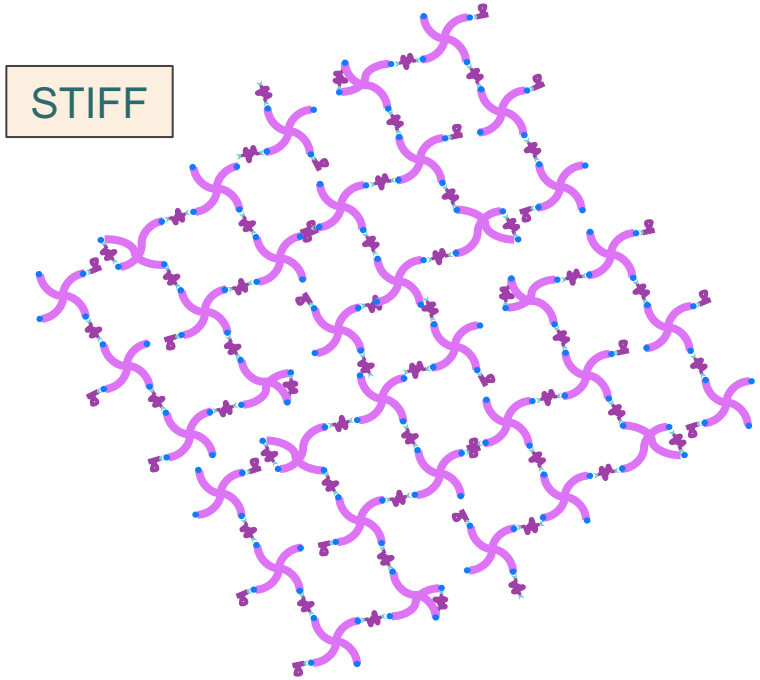


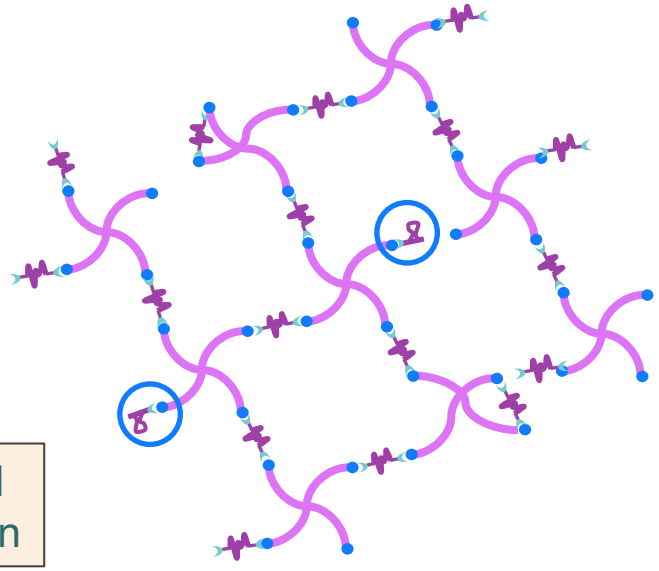
Functionalised 4-arm PEG (PEG-VS, e.g.)

SOFT

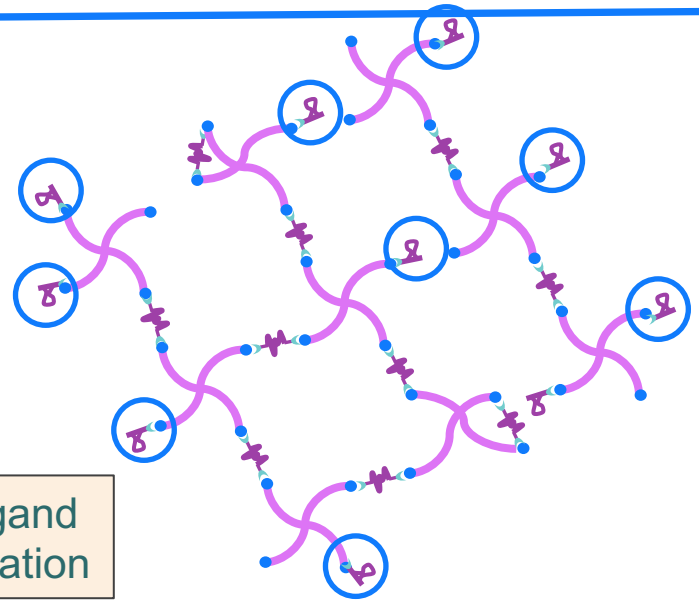


STIFF



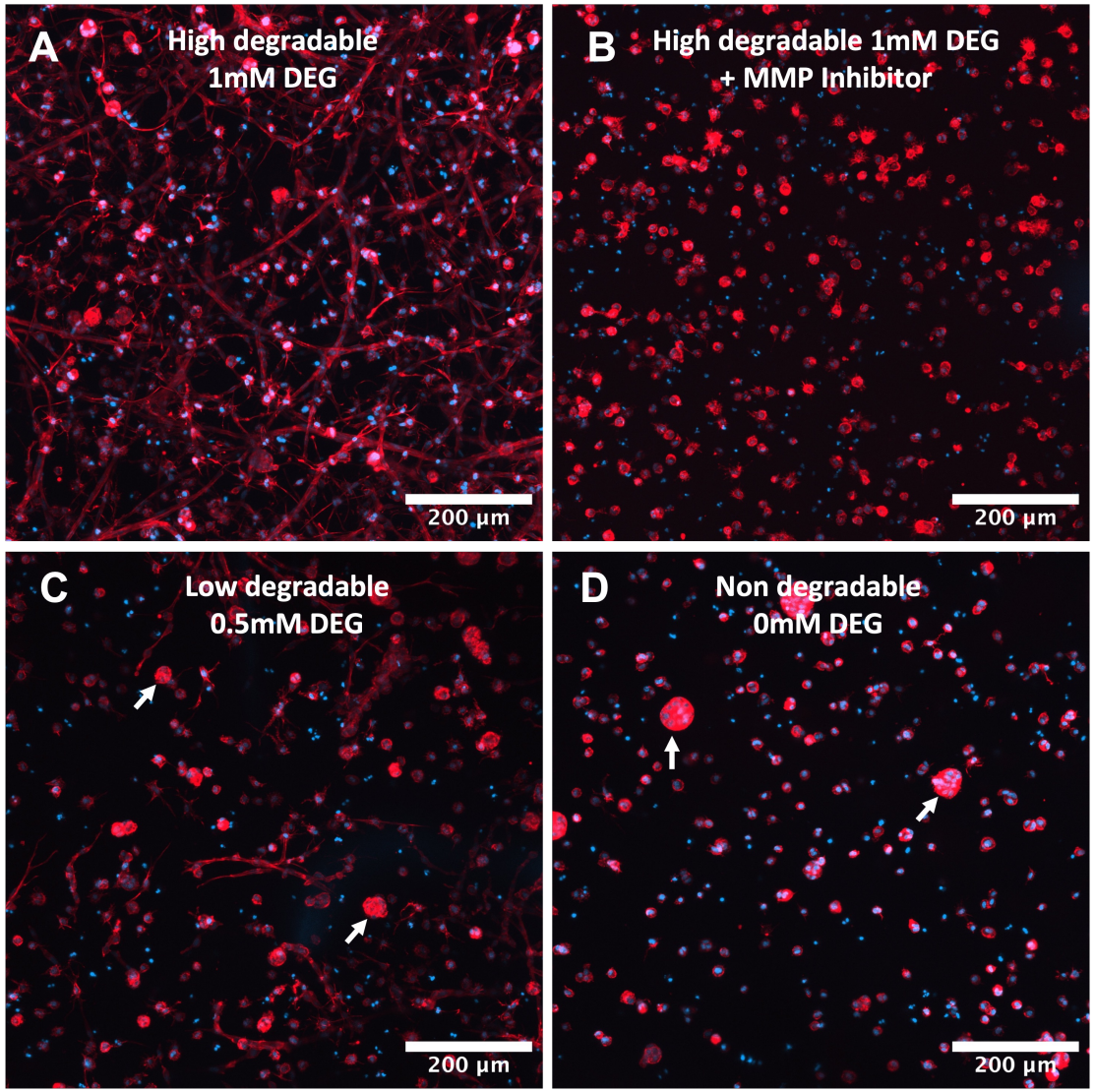


LOW ligand concentration



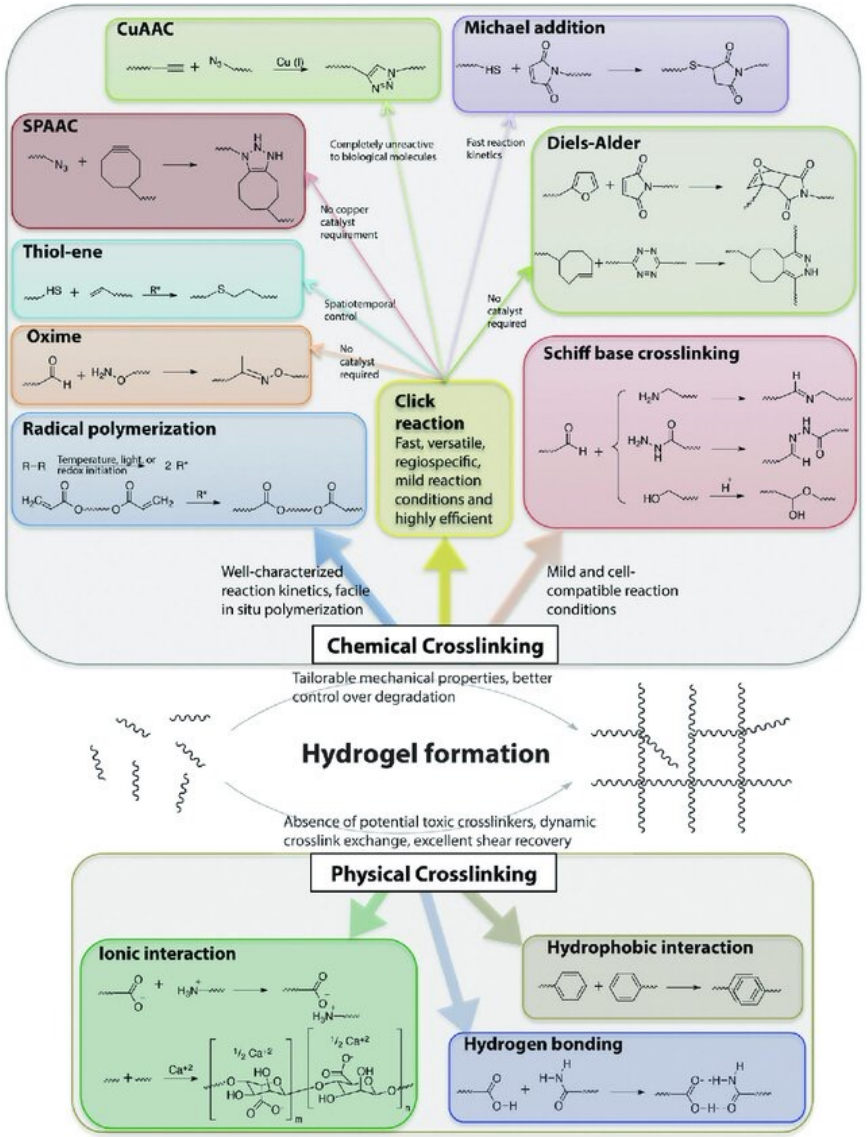
HIGH ligand concentration

Modulate hydrogel degradability

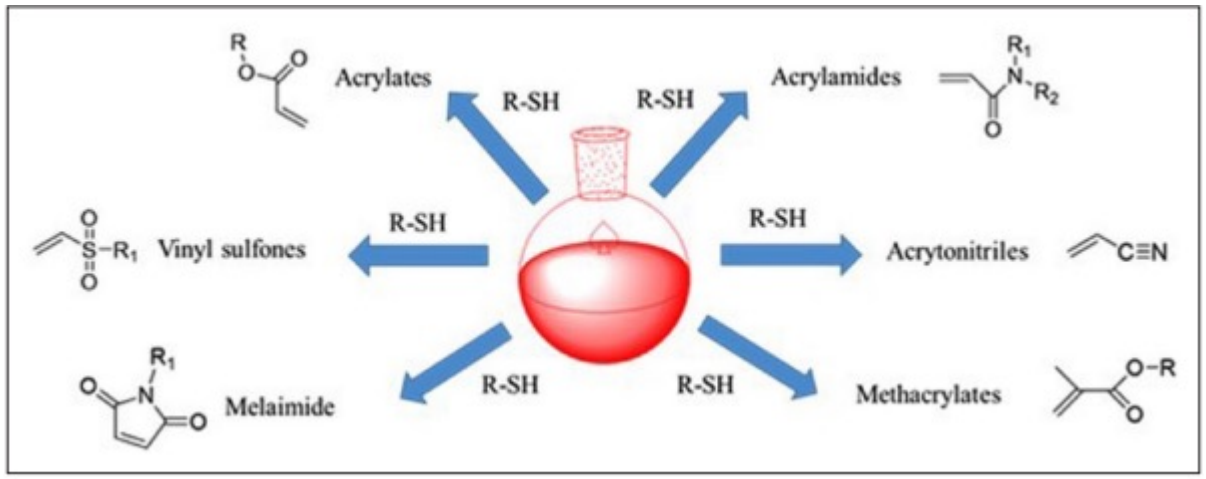


Human vascular smooth muscle cells in PEG hydrogel

Cross-linking strategies for hydrogels



Hydrogel cross-linking strategies



Thiol–Michael Addition: *Base- or nucleophile-catalysed* conjugate addition

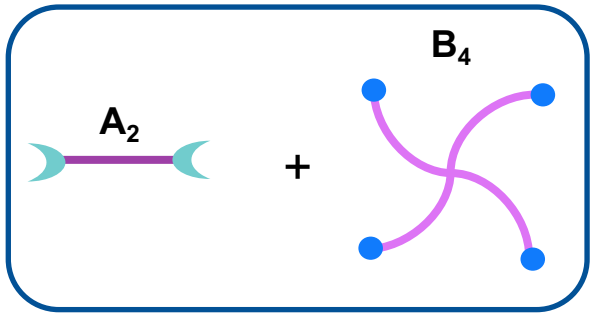
Mechanism:

- Involves a **thiol (-SH)** adding to an **electron-deficient alkene**, such as:
  - **Acrylates**, **maleimides**, **vinyl sulfones**
- A **base** deprotonates the thiol, forming a thiolate anion that attacks the β-carbon of the alkene in a **1,4-conjugate addition**

Key Features:

- **Nucleophilic**, not radical-mediated
- Mild conditions, often room temp, no light needed

Synthetic hydrogels for organoid culture



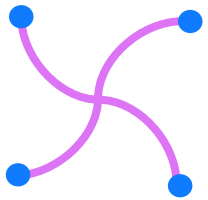
Cysteine, C (-SH)

Homo-bifunctional degradable peptide

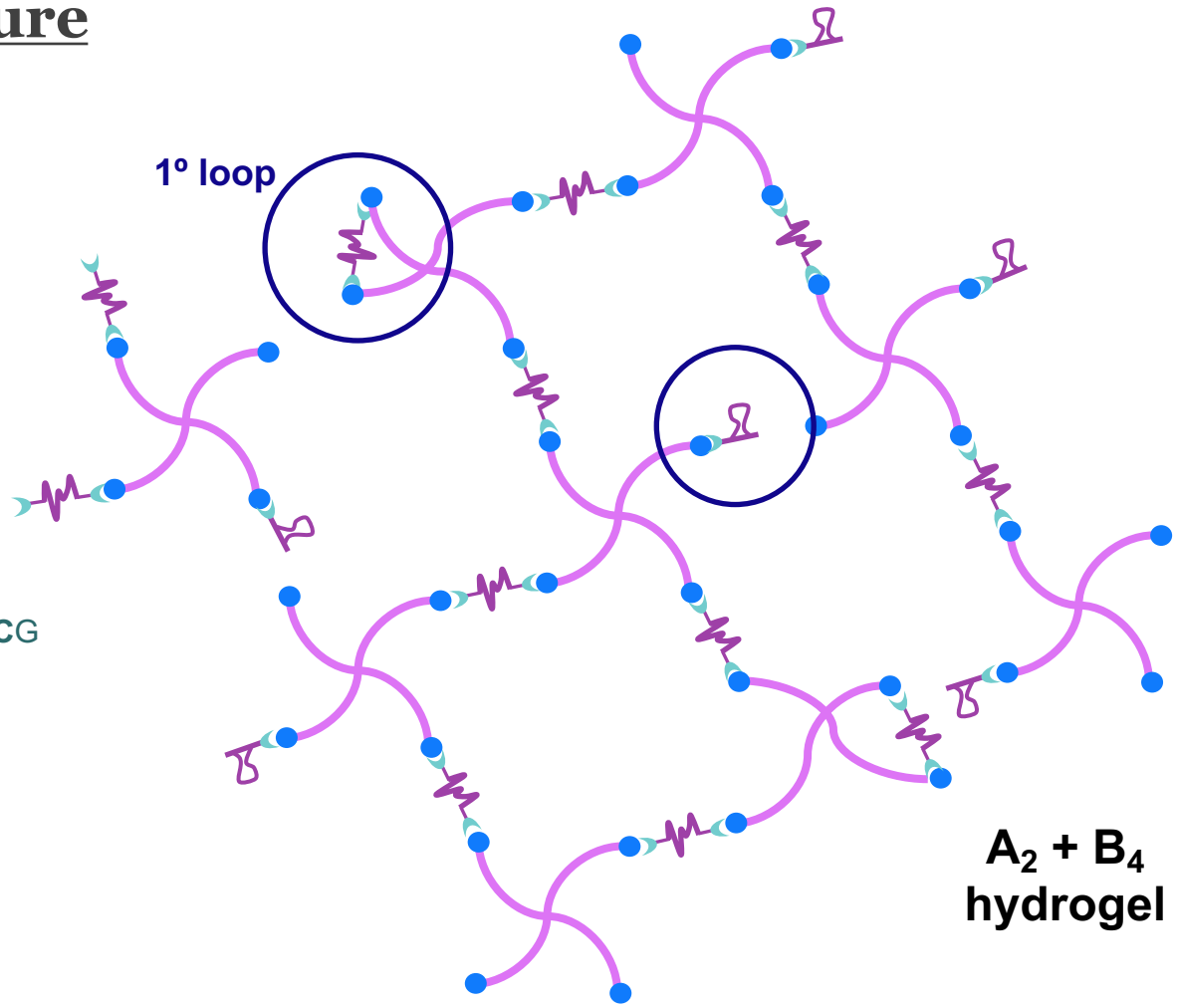
e.g. G**C**RDGPQG↓IWGQDR**C**G

Adhesive peptide (RGD, e.g.)

e.g. GRGDSP**C**



Functionalised 4-arm PEG (PEG-VS, e.g.)

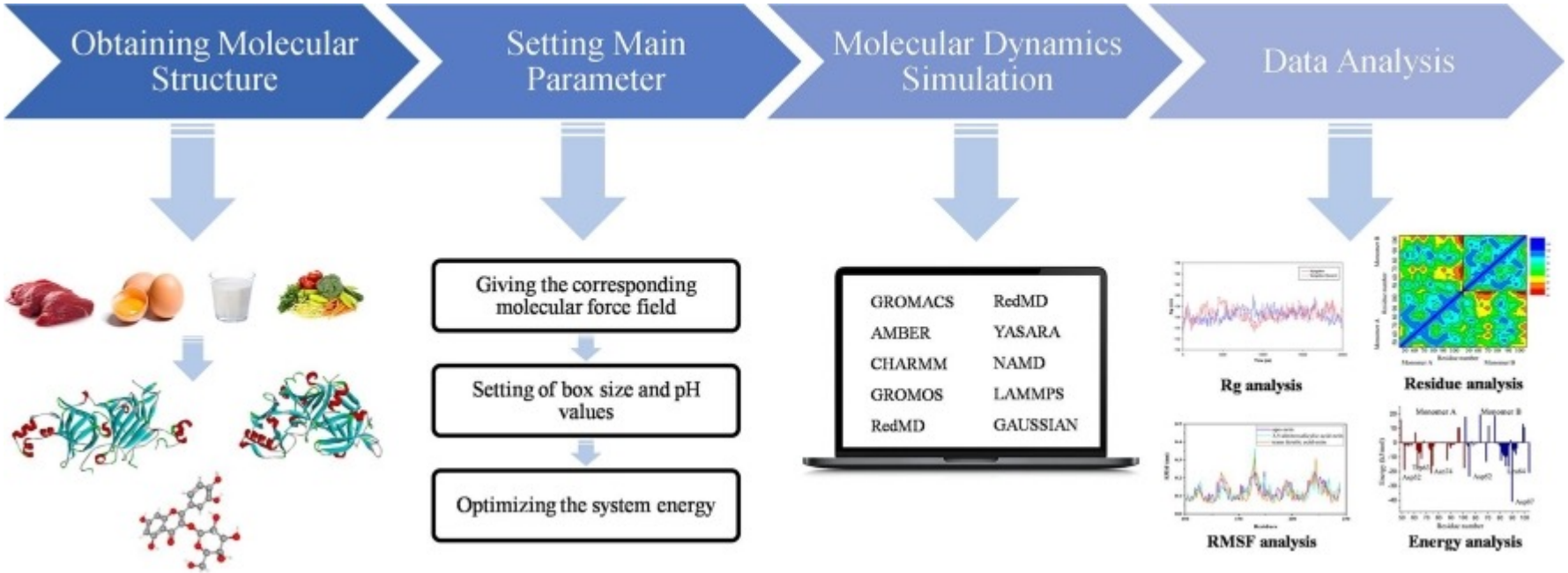


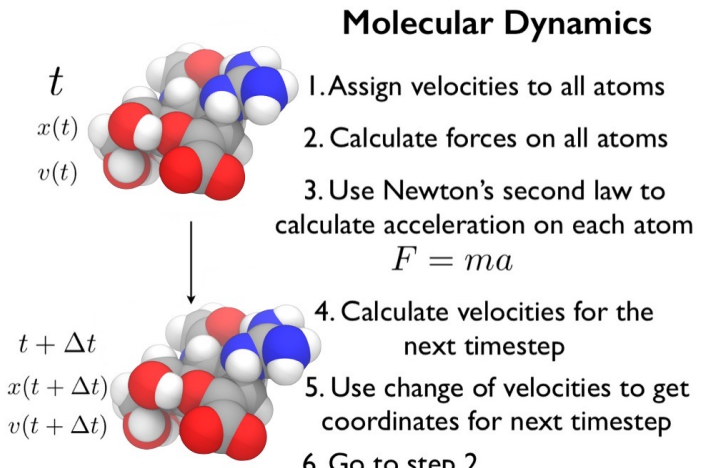
- Slow-forming, poorly cross-linked networks
- Networks do not form at low polymer concentrations (soft, native tissue-like stiffnesses)

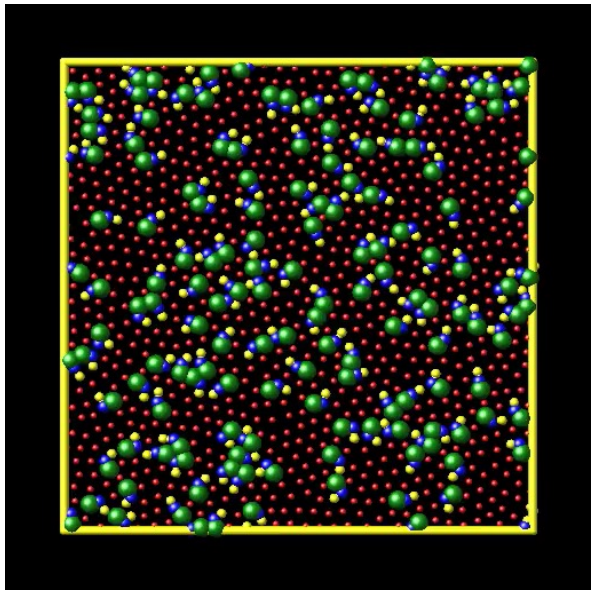
Table 1 Troubleshooting table

Spheroids sink to the bottom of the dish and fail to develop into HOs	The hydrogel is degrading too quickly or is not properly polymerized	Reduce the spheroid density per hydrogel
		Increase the frequency of passages to every 5–7 d
	Spheroids were too close to the bottom of the dish during hydrogel cross-linking	During hydrogel cross-linking, flip the plate upside-down to ensure that spheroids are not too close to the bottom of the dish

Molecular dynamics simulations

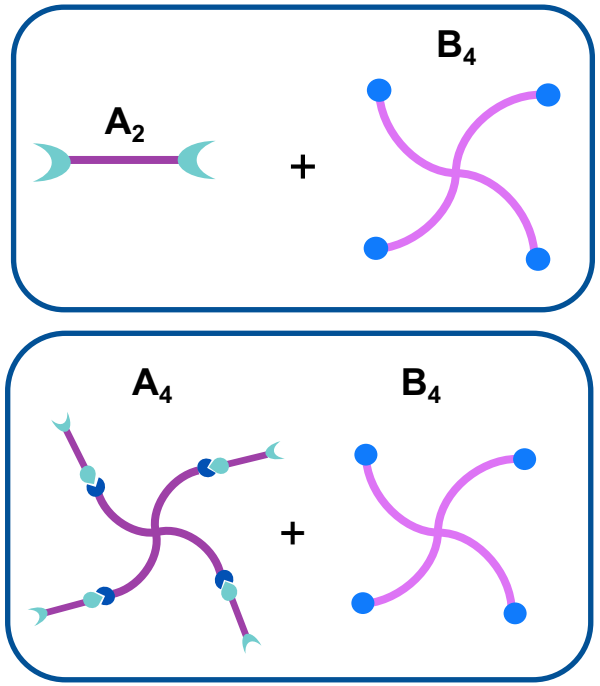


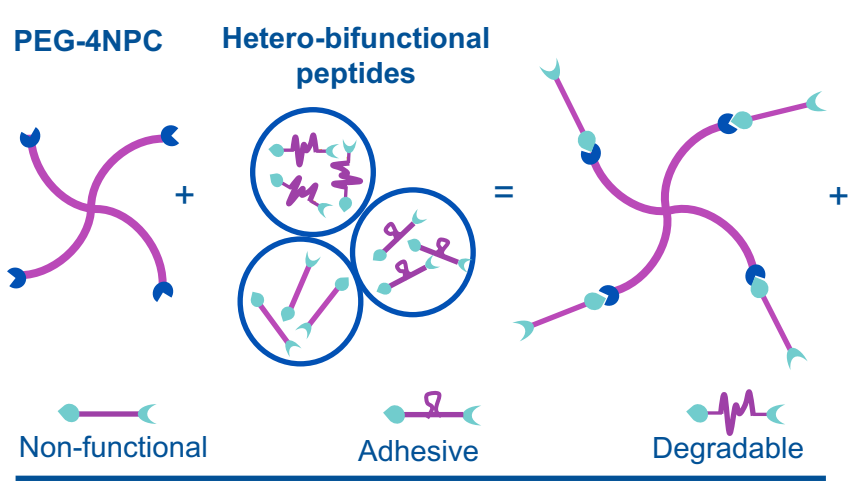




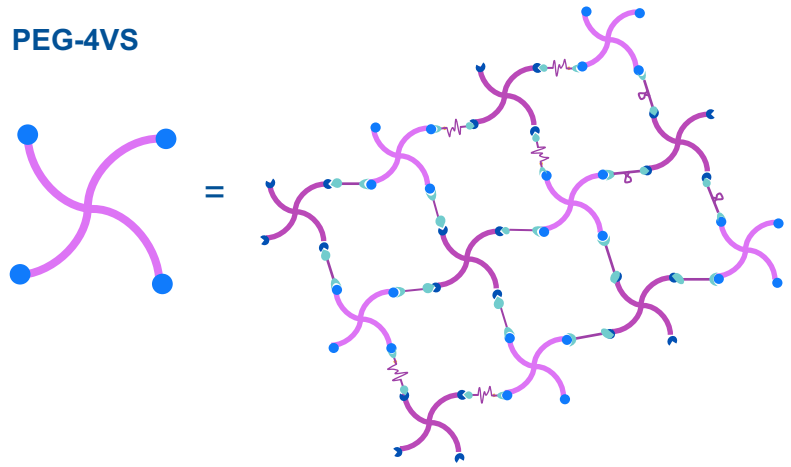
Micelle self-assembly

Molecular dynamics simulations of gelation



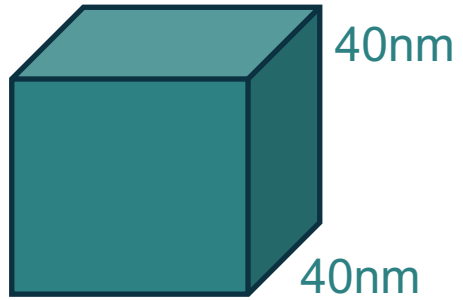


Reaction 1



Reaction 2

	System	PEG-4NPC	PEG-4VS	Peptides	Ions	Water
A_4+B_4	Ac-KDWERC-NH2	40	40	0	160 Na^+	533336
	H-SREWERC-NH2	40	40	0	0	533336
A_2+B_4	Ac-CREWERC-NH2	0	80	160	0	533336

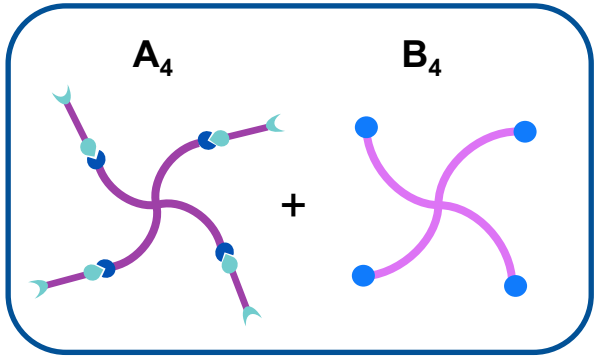


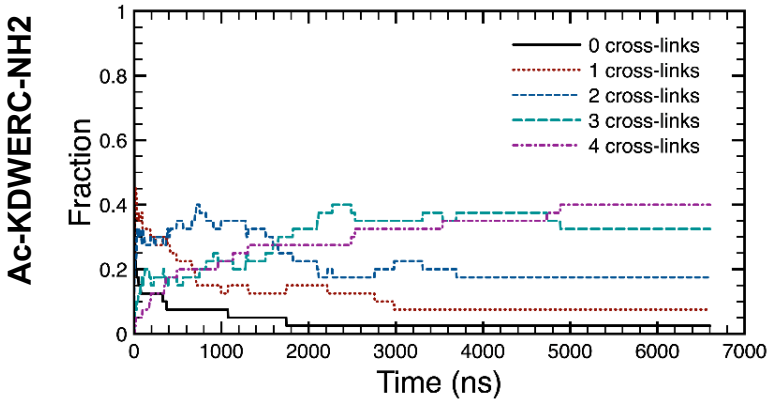
Simulation time: ~7μs

Molecular dynamics simulations of gelation

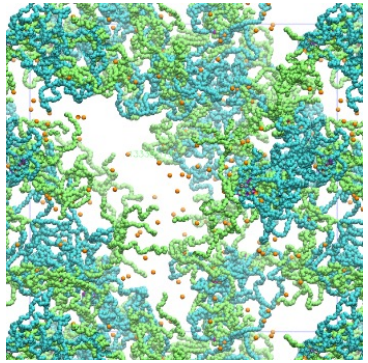
Chris Lorenz
KCL Physics

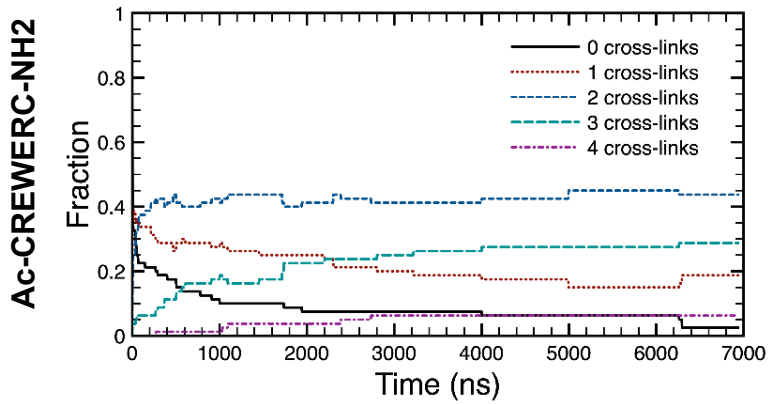
2-step hydrogel cross-linking with hetero-bifunctional peptides



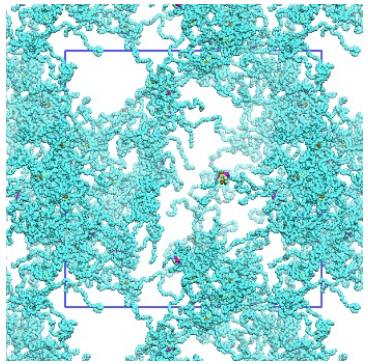


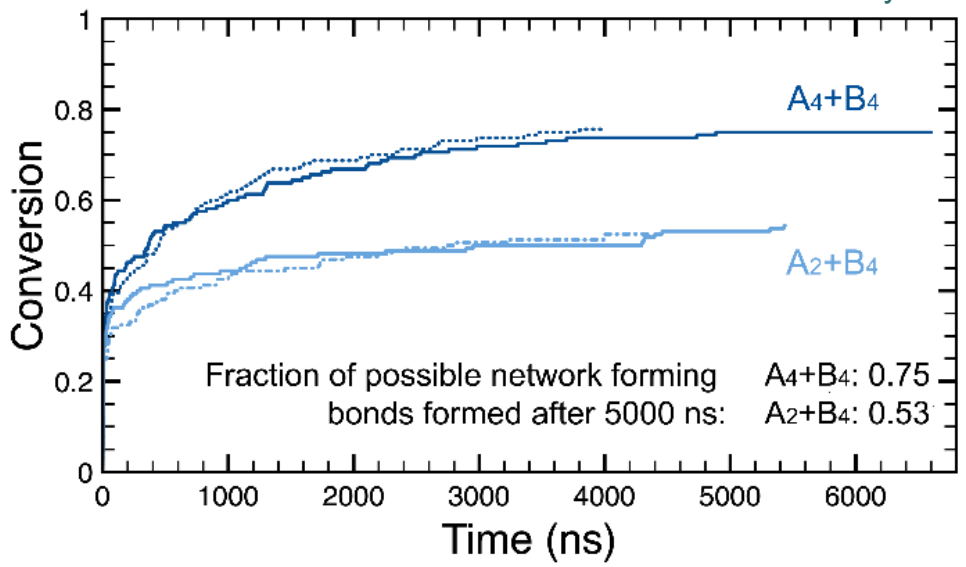
$A_4 + B_4$

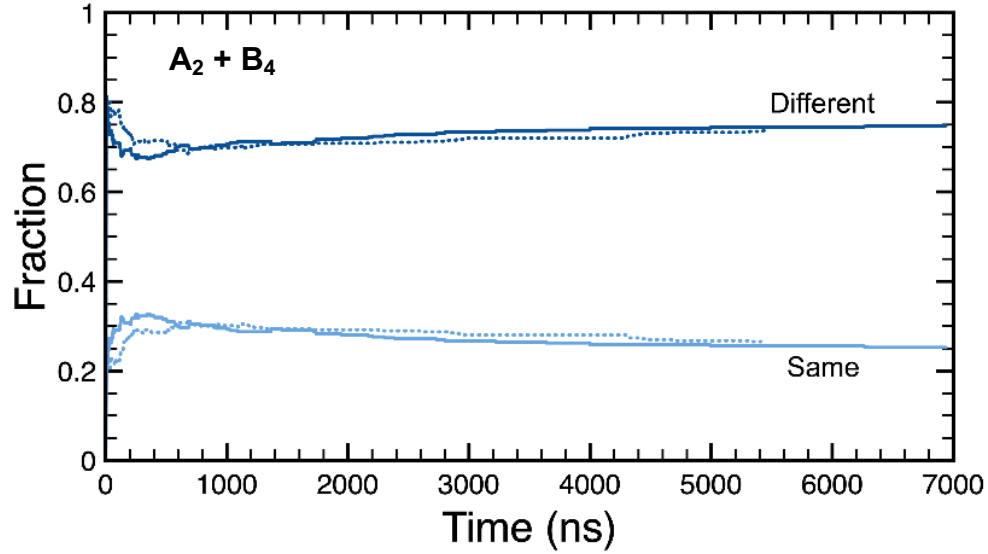




$A_2 + B_4$



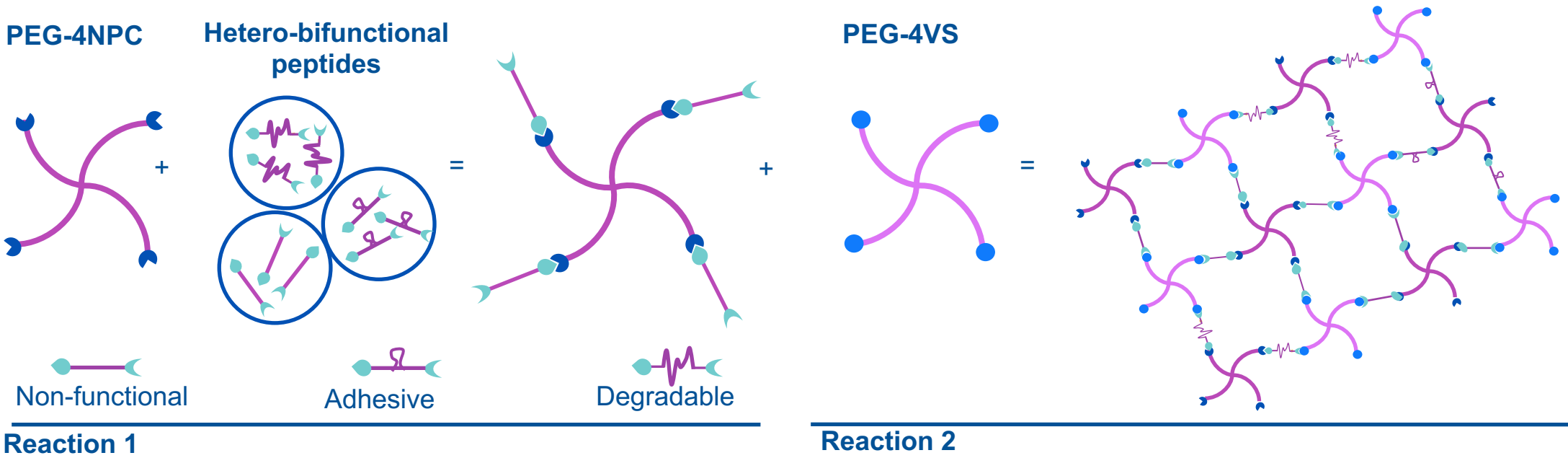


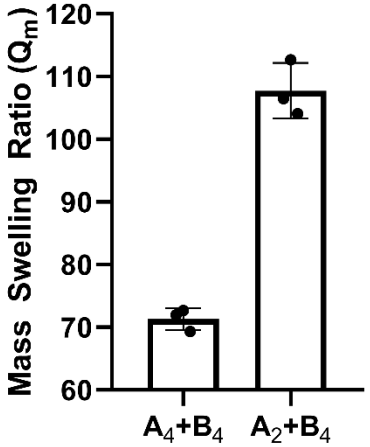


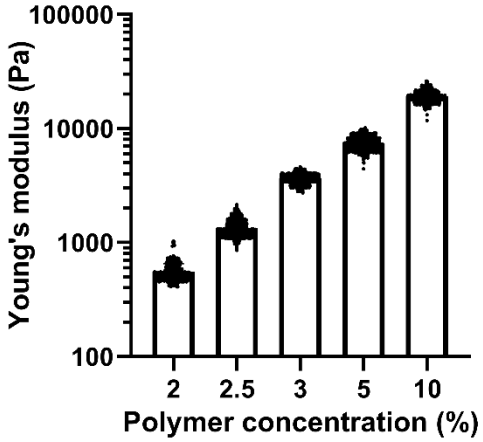
- ~25% of cross-links in $A_2 + B_4$ designs are primary loops
- $A_4 + B_4$ designs form more network-forming bonds

Jowett *et al*. *Nat Mater* 2021

Fully defined 3D matrix for organoid culture



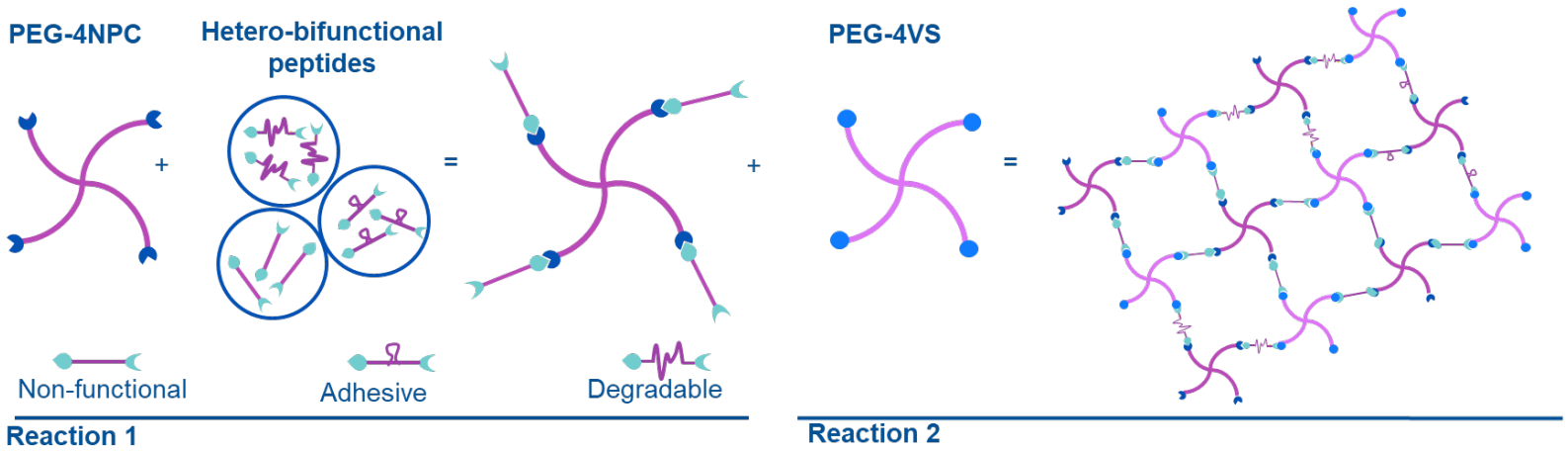


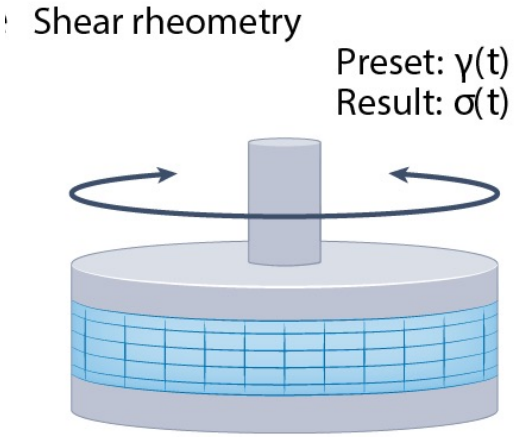


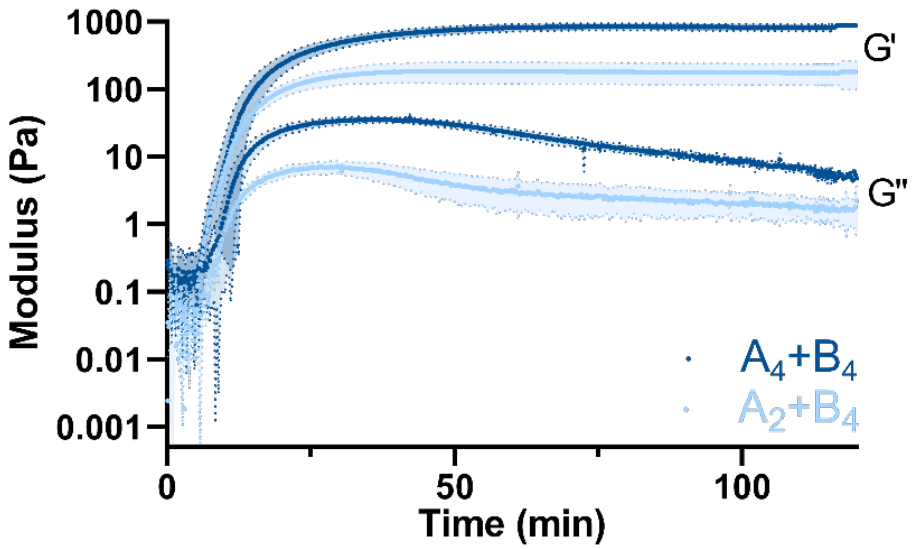
- Fully synthetic hydrogel suitable for organoid culture
- Hydrogels cross-link quickly even at low polymer concentrations
- Stiffness matches that of the native intestine

Jowett *et al*. ILC1 drive intestinal epithelial and matrix remodelling. *Nat Mater* 2021

Oscillatory shear rheometry



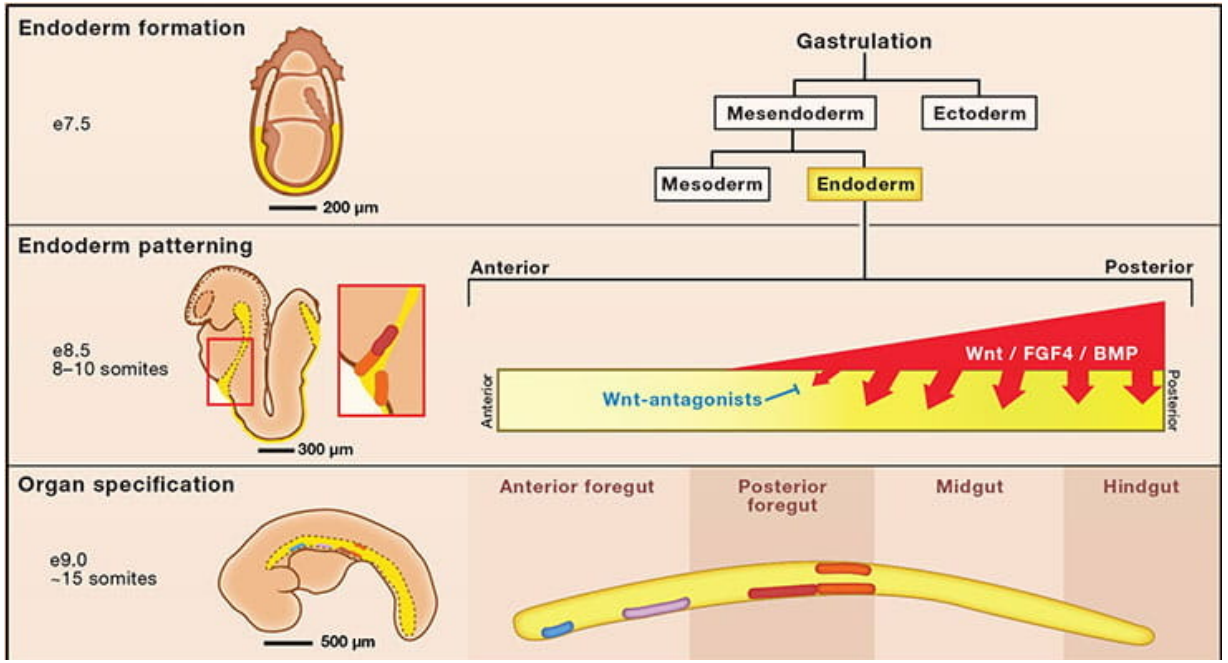


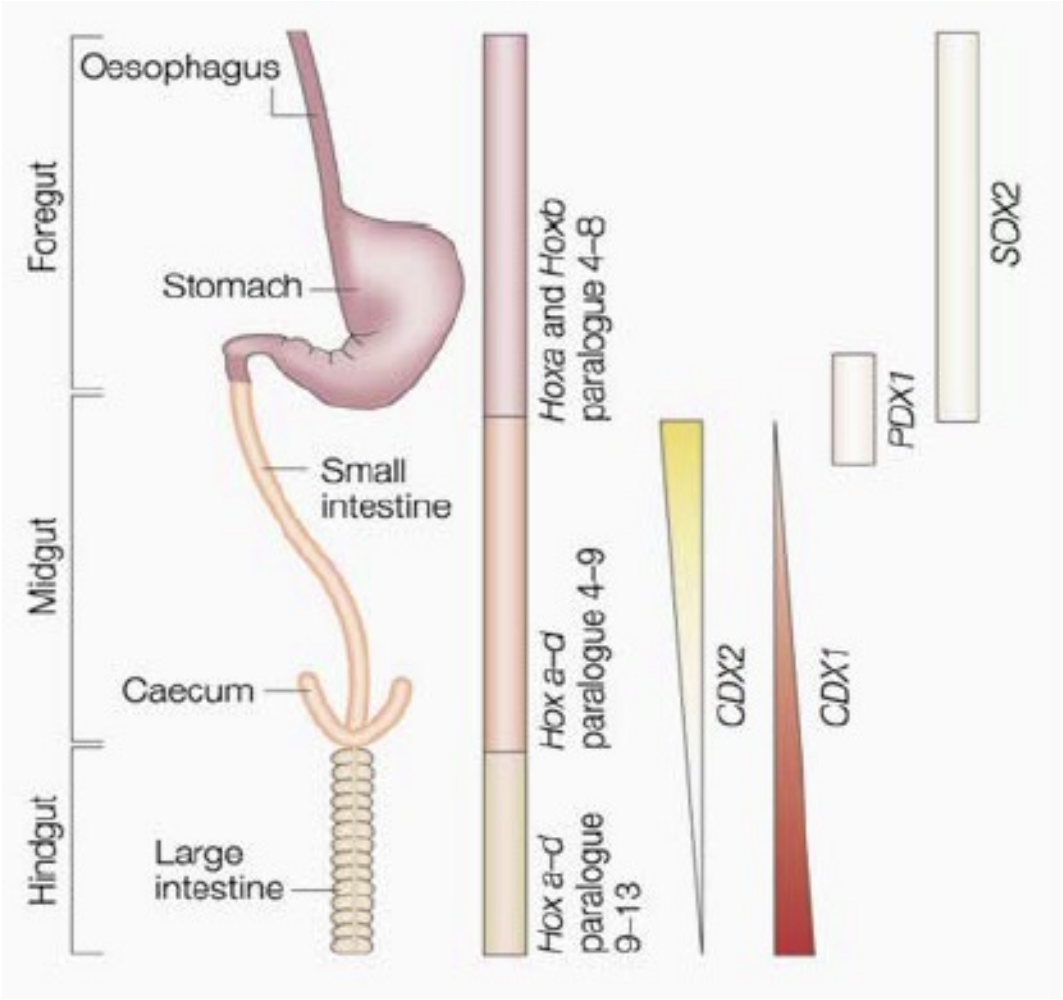


Monitor the time-dependent transition of a hydrogel from liquid-like to solid-like behaviour during gelation

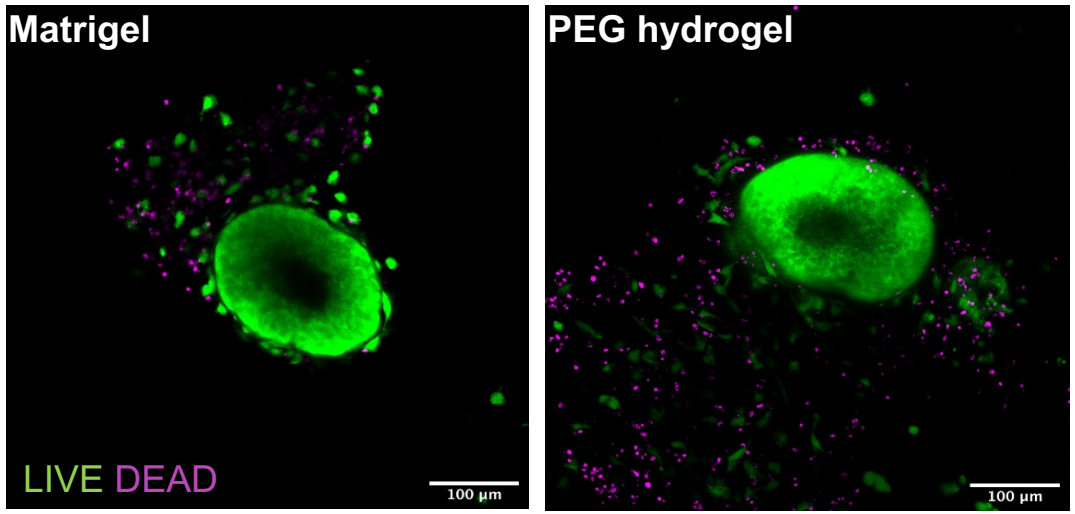
- **G′ (Storage modulus):** Elastic response
- **G″ (Loss modulus):** Viscous response
- **Gelation time**: Cross-over between G' and G"

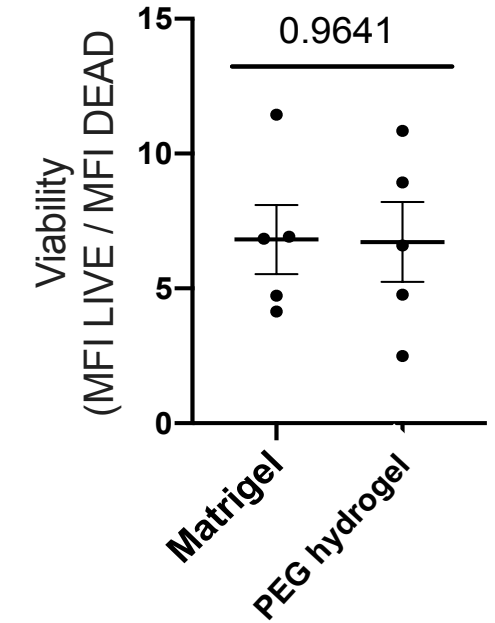
Intestinal development

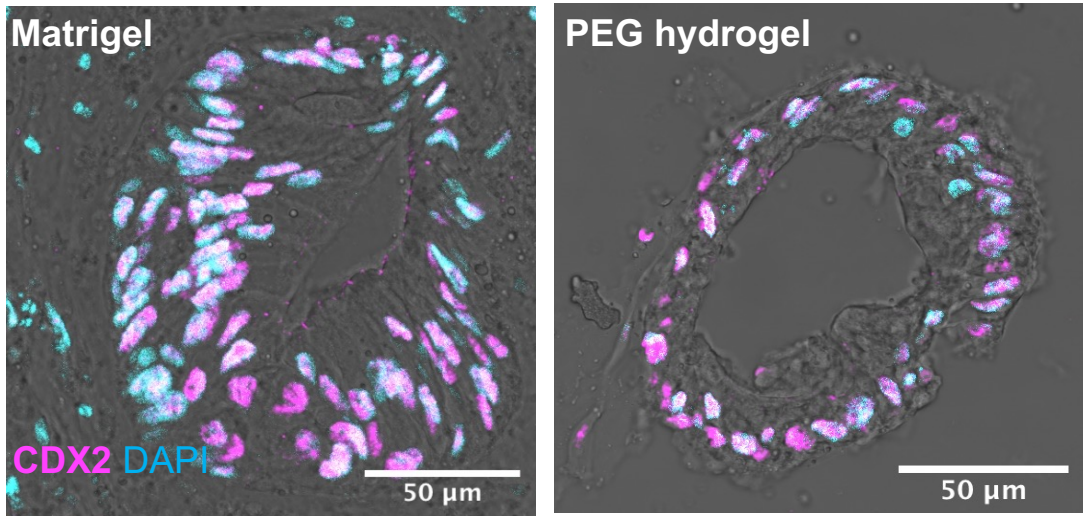


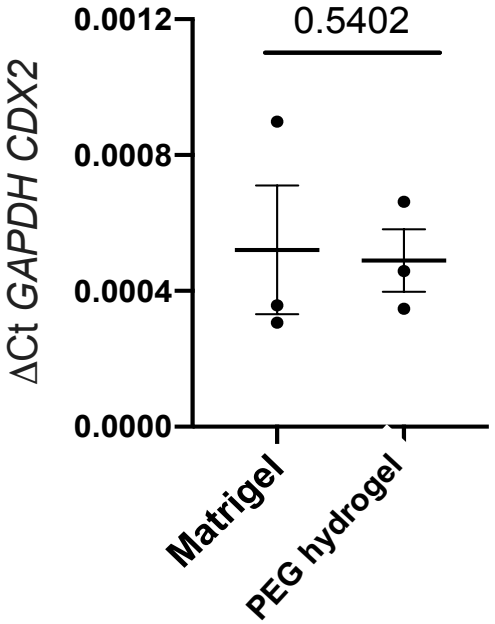


PEG hydrogels support HIO viability and phenotype



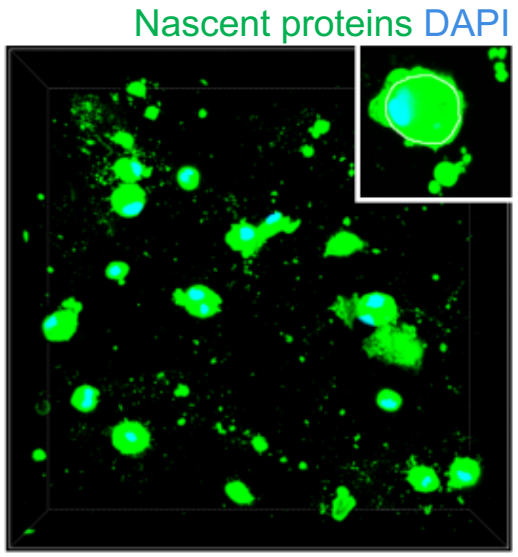


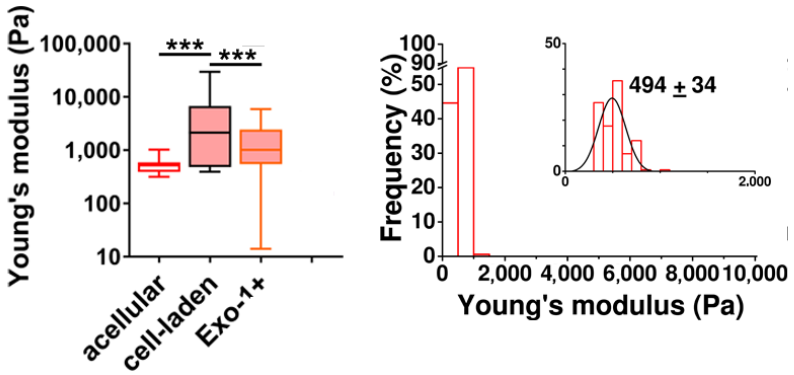


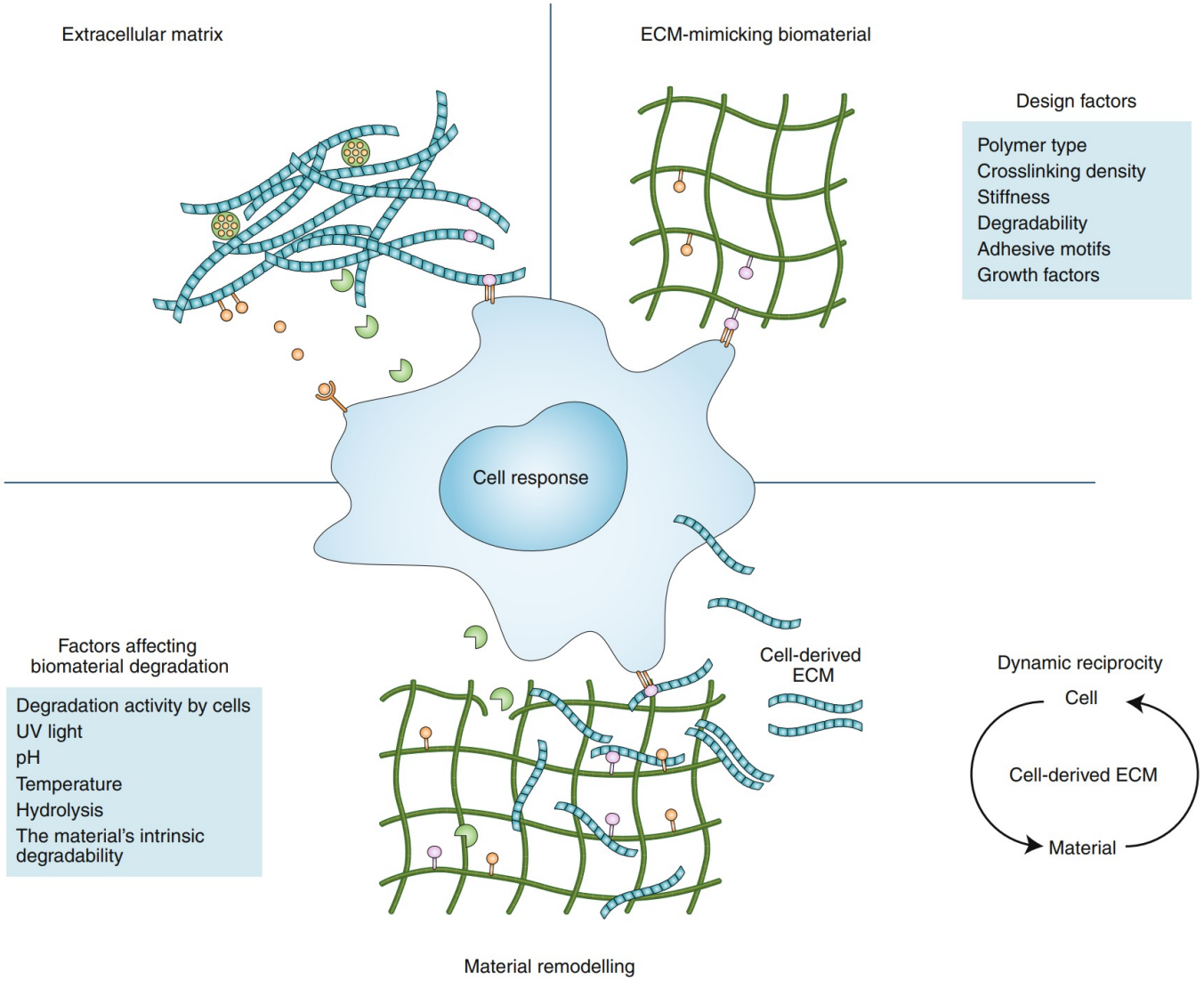


HIO: human iPSC intestinal organoid

Cells within hydrogels secrete nascent matrix and remodel their surroundings

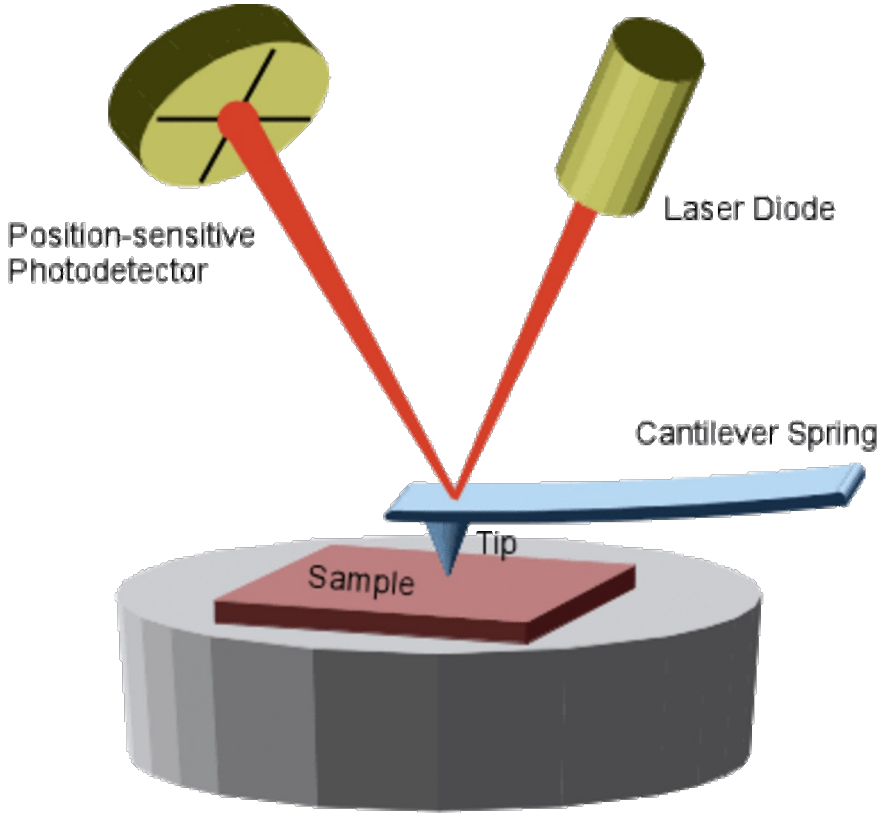


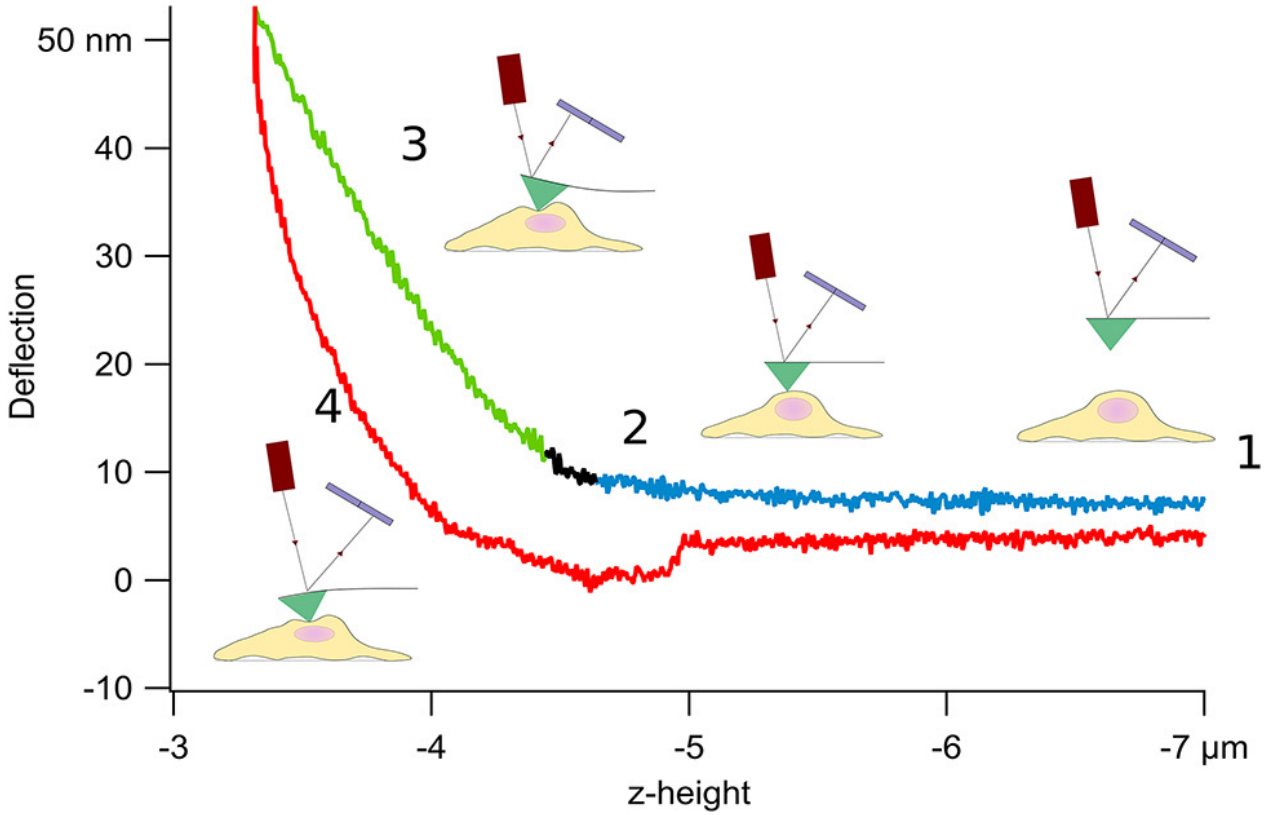




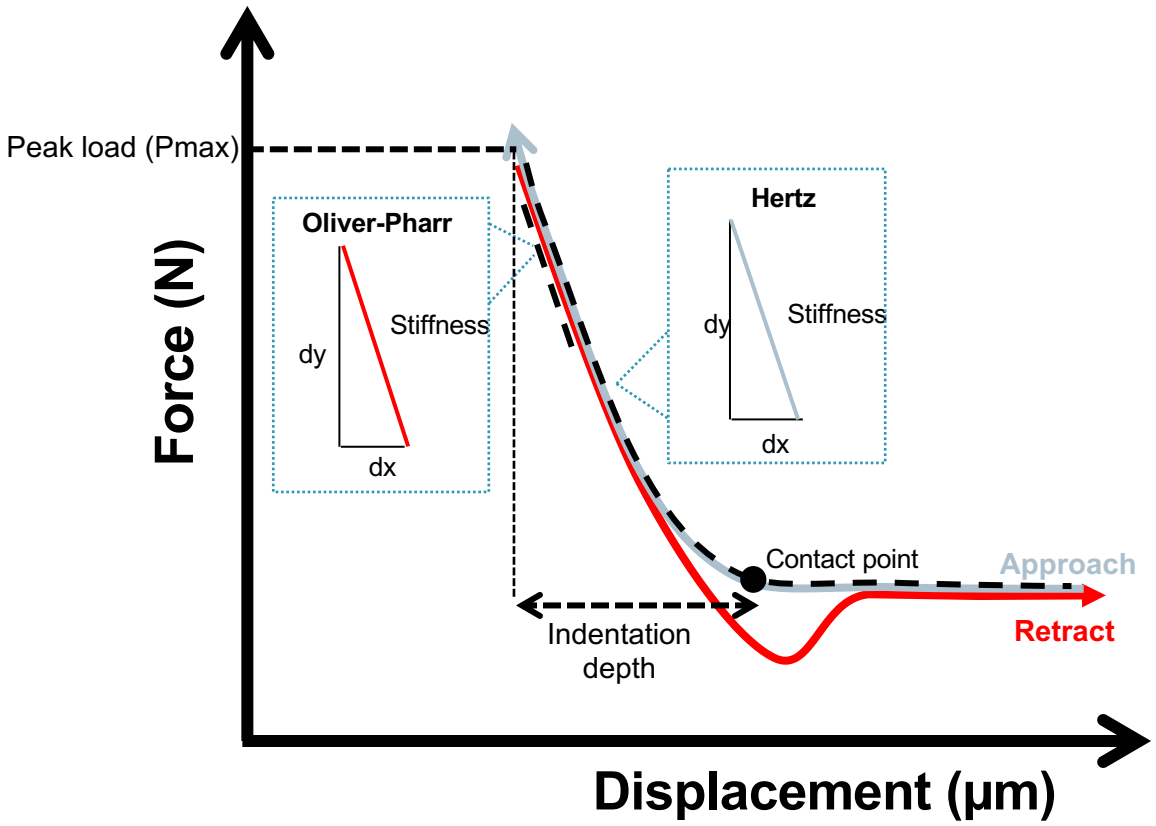
Blache U *et al. Nat Biomed Eng* 2020
Ferreira SA *et al. Nat Commun* 2018
Blache U *et al. Nat Rev Methods Primers* 2022

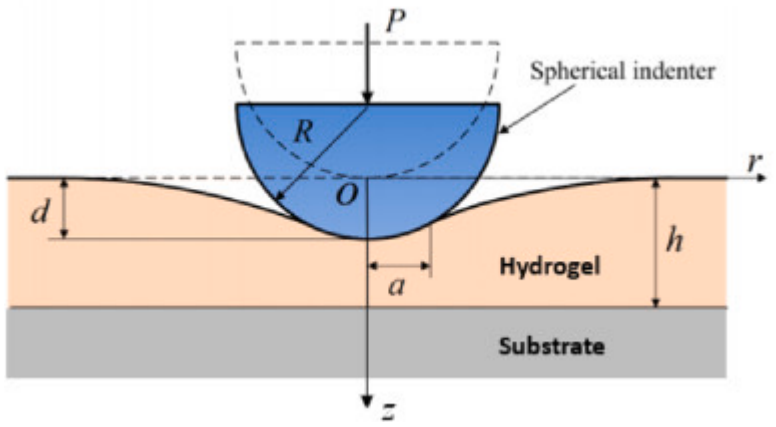
Atomic force microscope





Calculation of elastic modulus



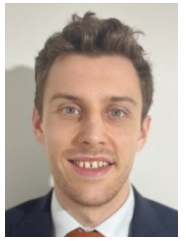


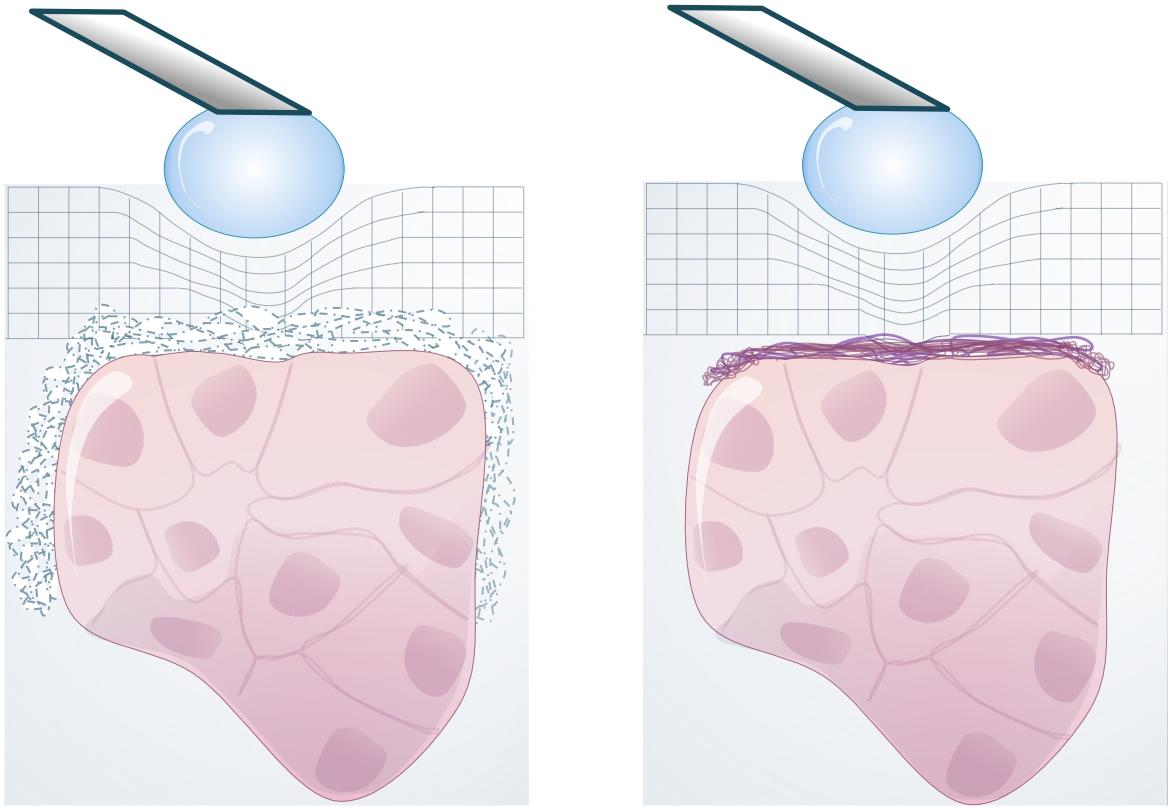
$$E = \frac{3(1-\nu^2)}{4\sqrt{R}} \cdot \frac{F}{\delta^{3/2}}$$

Where:

- F = applied force
- δ = indentation depth
- R = radius of the spherical indenter
- ν = Poisson's ratio of the soft material
- E_{eff} = effective (reduced) elastic modulus

AFM force spectroscopy on encapsulated HIO

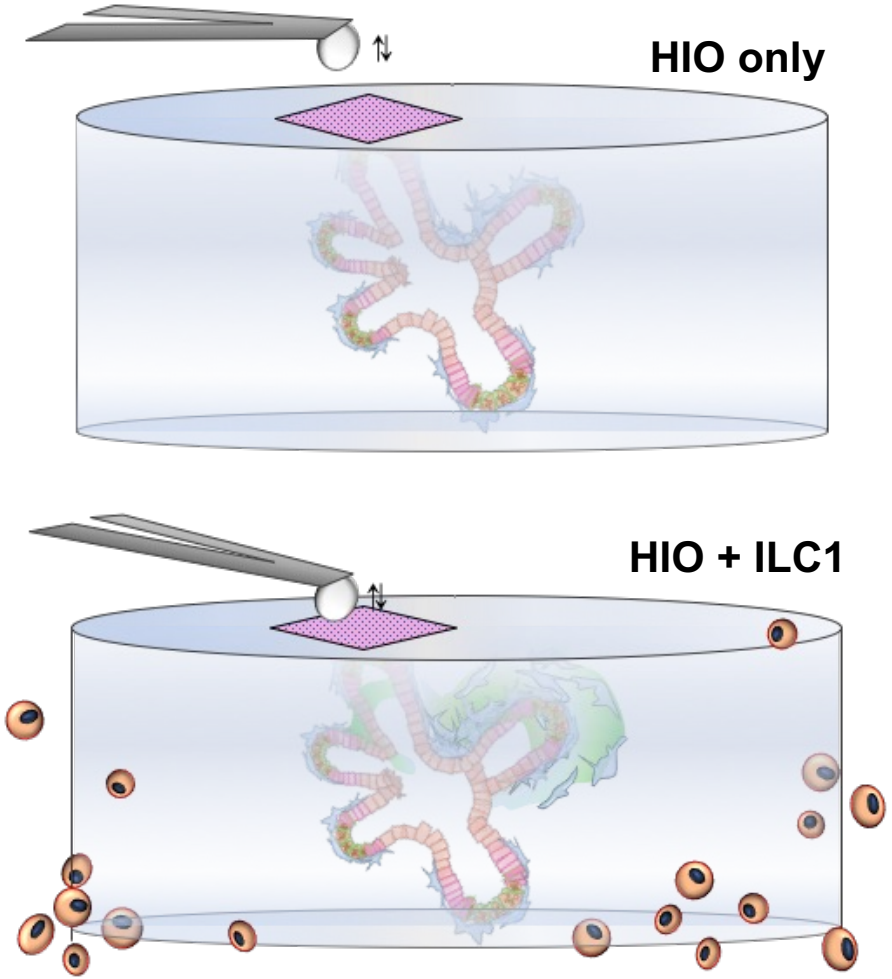




Hydrogel degradation

Matrix deposition

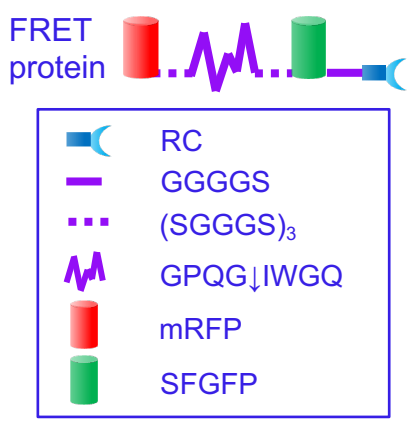
Atomic force microscopy force spectroscopy on HIO in thin hydrogels

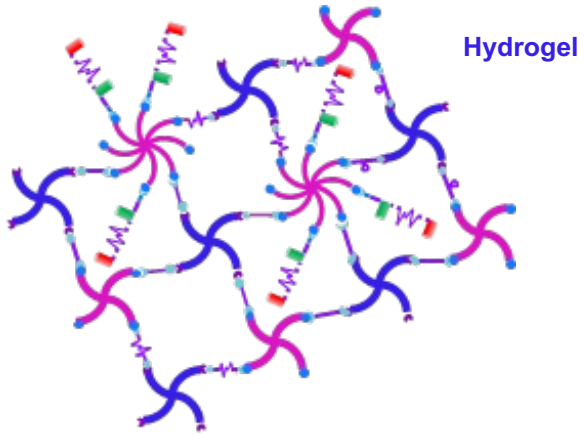


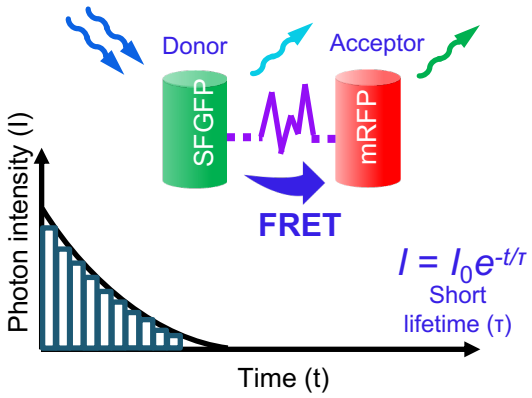
Norman *et al*. Measuring the elastic modulus of soft culture surfaces and three-dimensional hydrogels using atomic force microscopy.*Nat Protoc* 2021

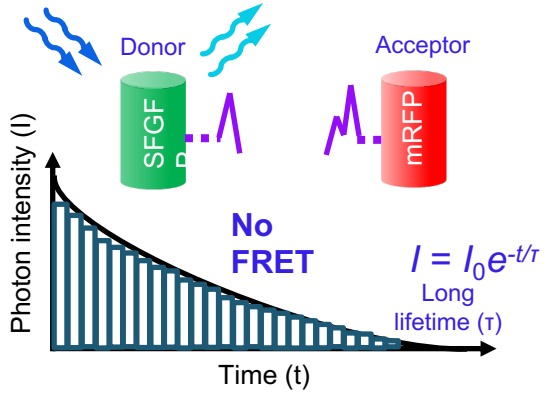
ILC1: Type I Innate lymphoid cells
HIO: human intestinal organoid

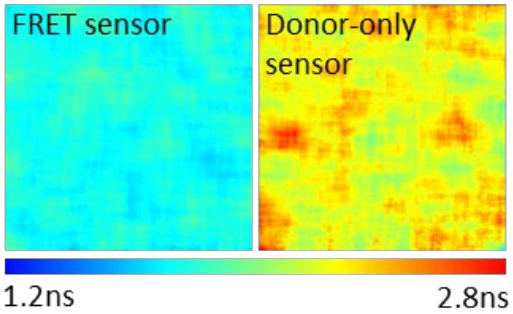
Matrix metalloproteinase (MMP)-susceptible FRET sensor

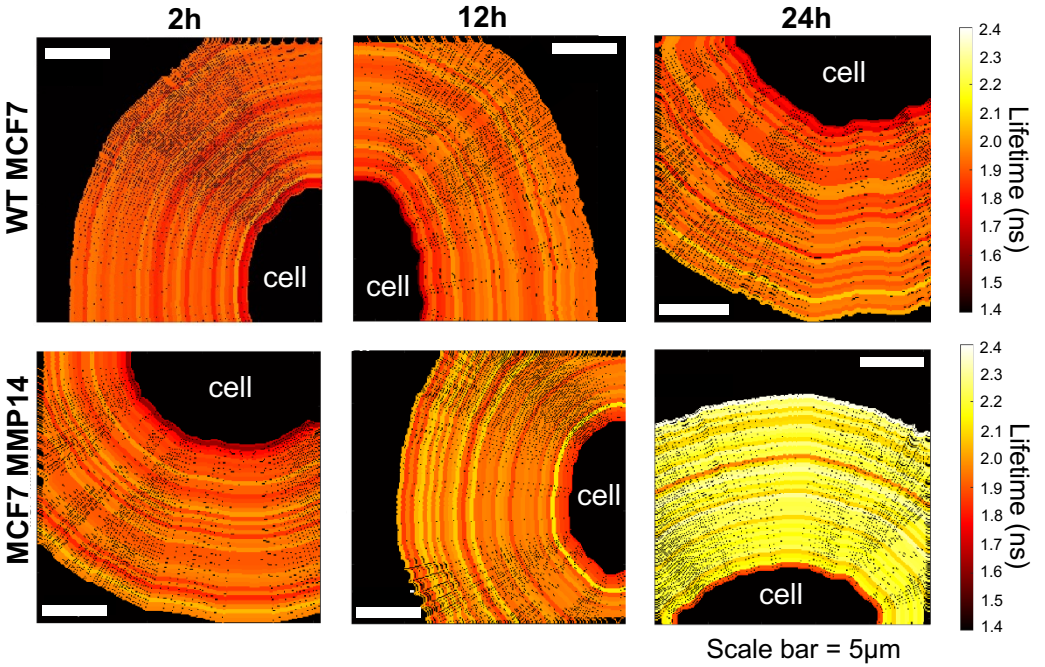








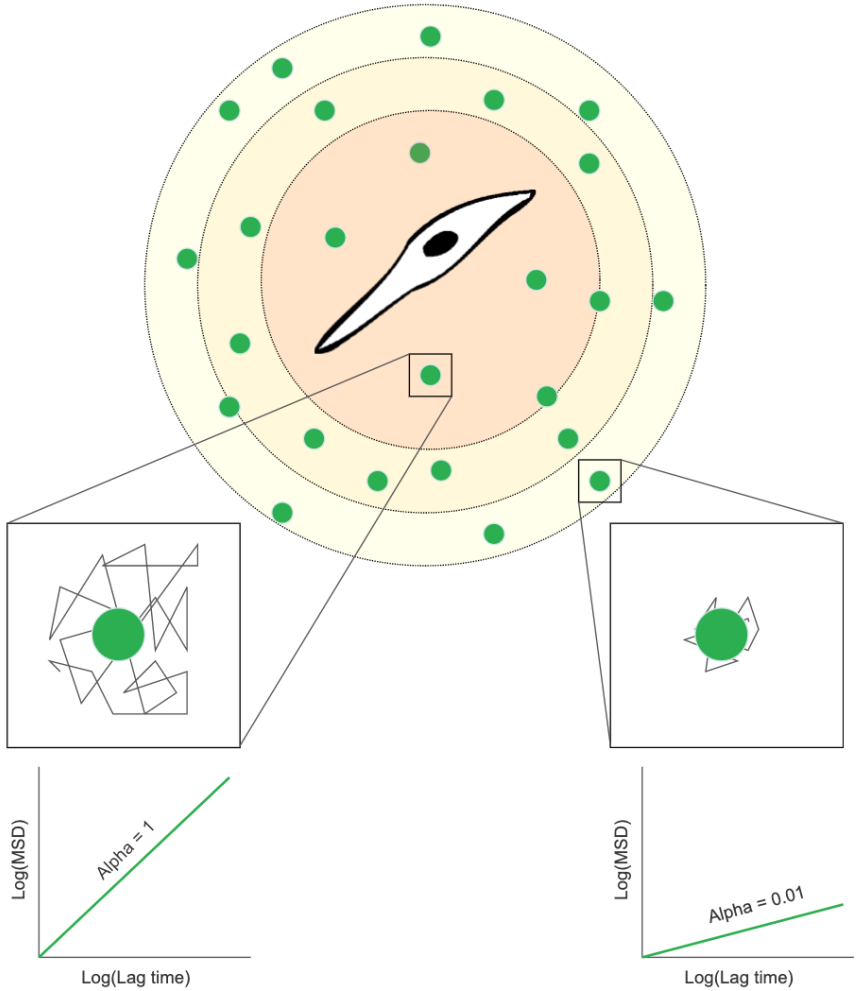


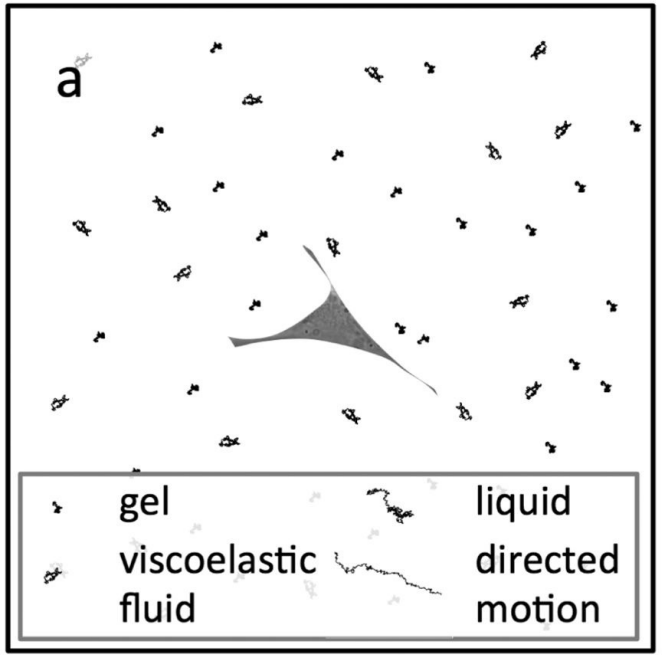


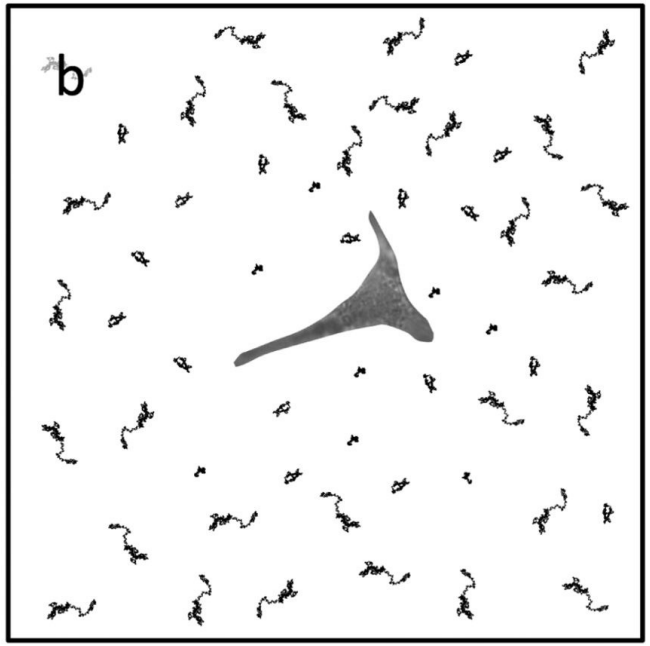
Yan *et al*. FRET sensor-modified synthetic hydrogels for real-time monitoring of cell-derived matrix metalloproteinase activity using fluorescence lifetime imaging. *Adv Func Mater* 2024

Multiple particle tracking microrheology

$$\langle \Delta r^2(t) \rangle = \frac{k_B T}{\pi a} J(t).$$



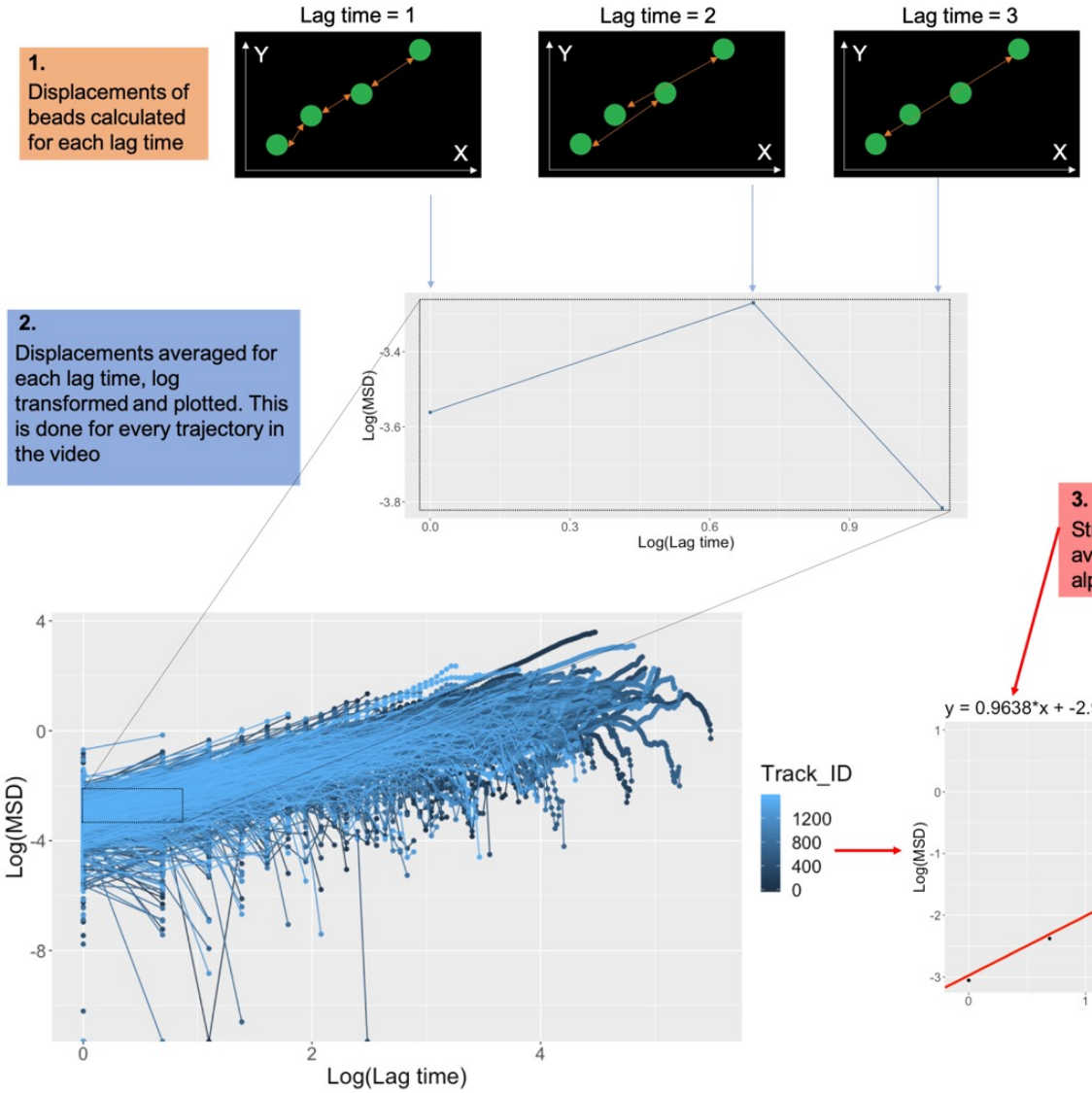


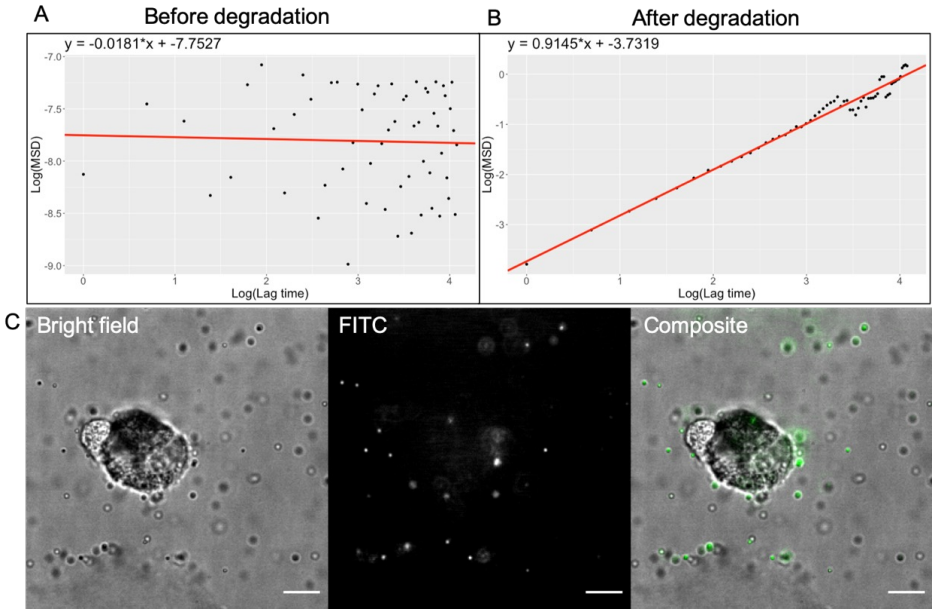


Stoke-Einstein relation relates the mean squared displacement (MSD, $\Delta r^2(t)$) of a moving particle to the material's creep compliance ($J(t)$), thermal energy ($k_B T$) and particle radius (a)

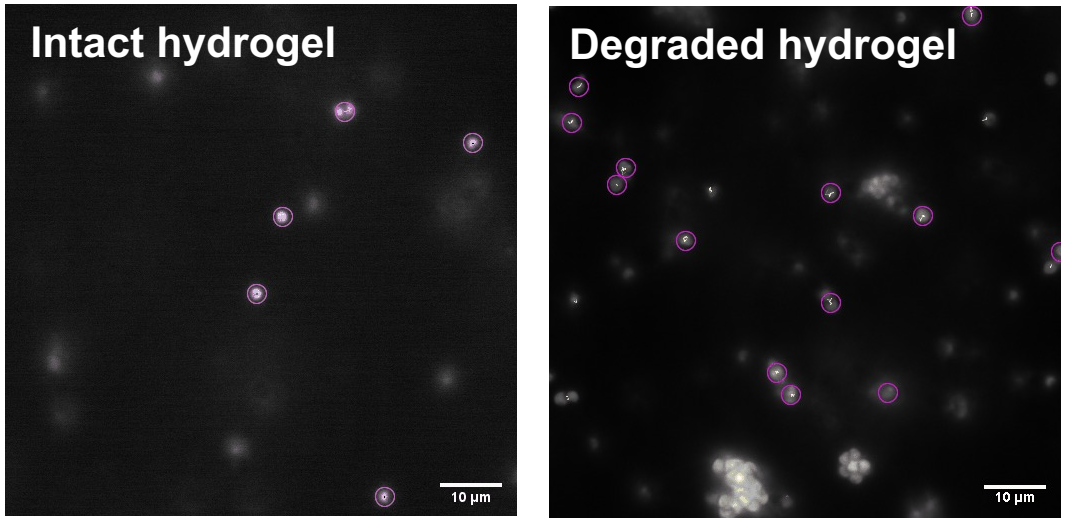
Schultz et al, PNAS 2015

Multiple particle tracking microrheology on encapsulated HIO

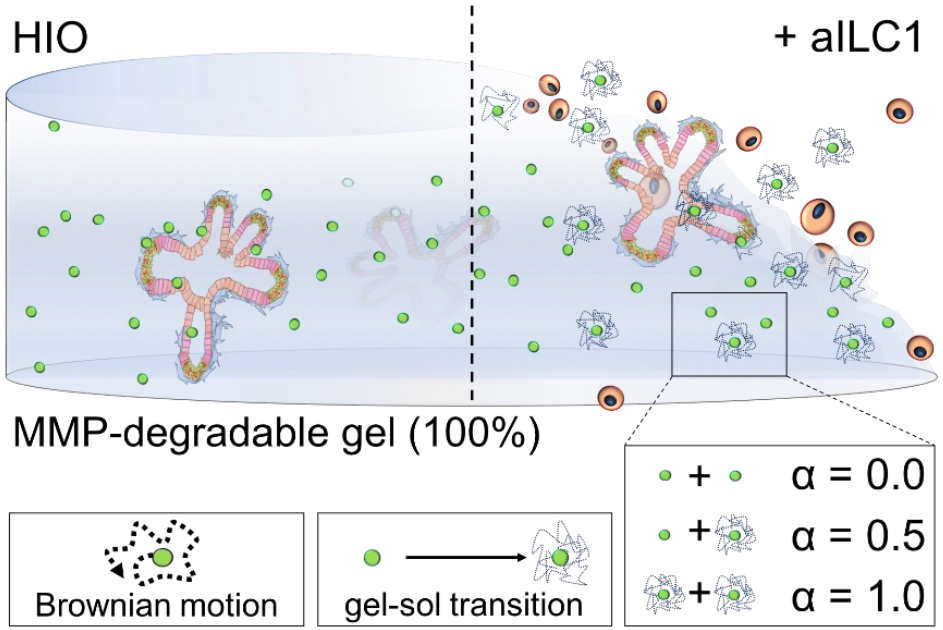


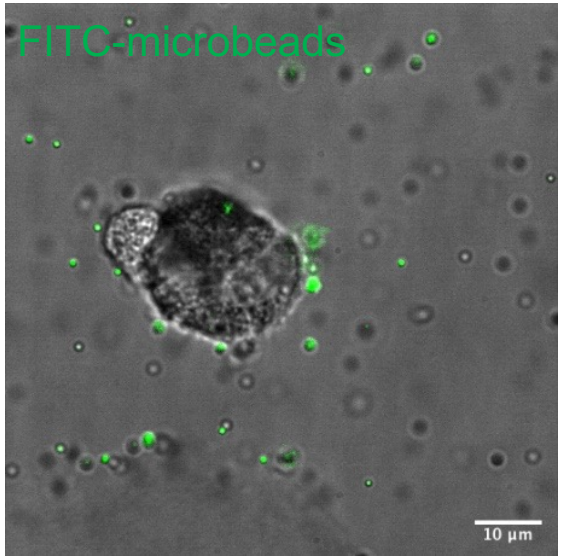


Multiple particle tracking microrheology on encapsulated HIO



Microrheology – *in situ* measurements of peri-organoid degradation

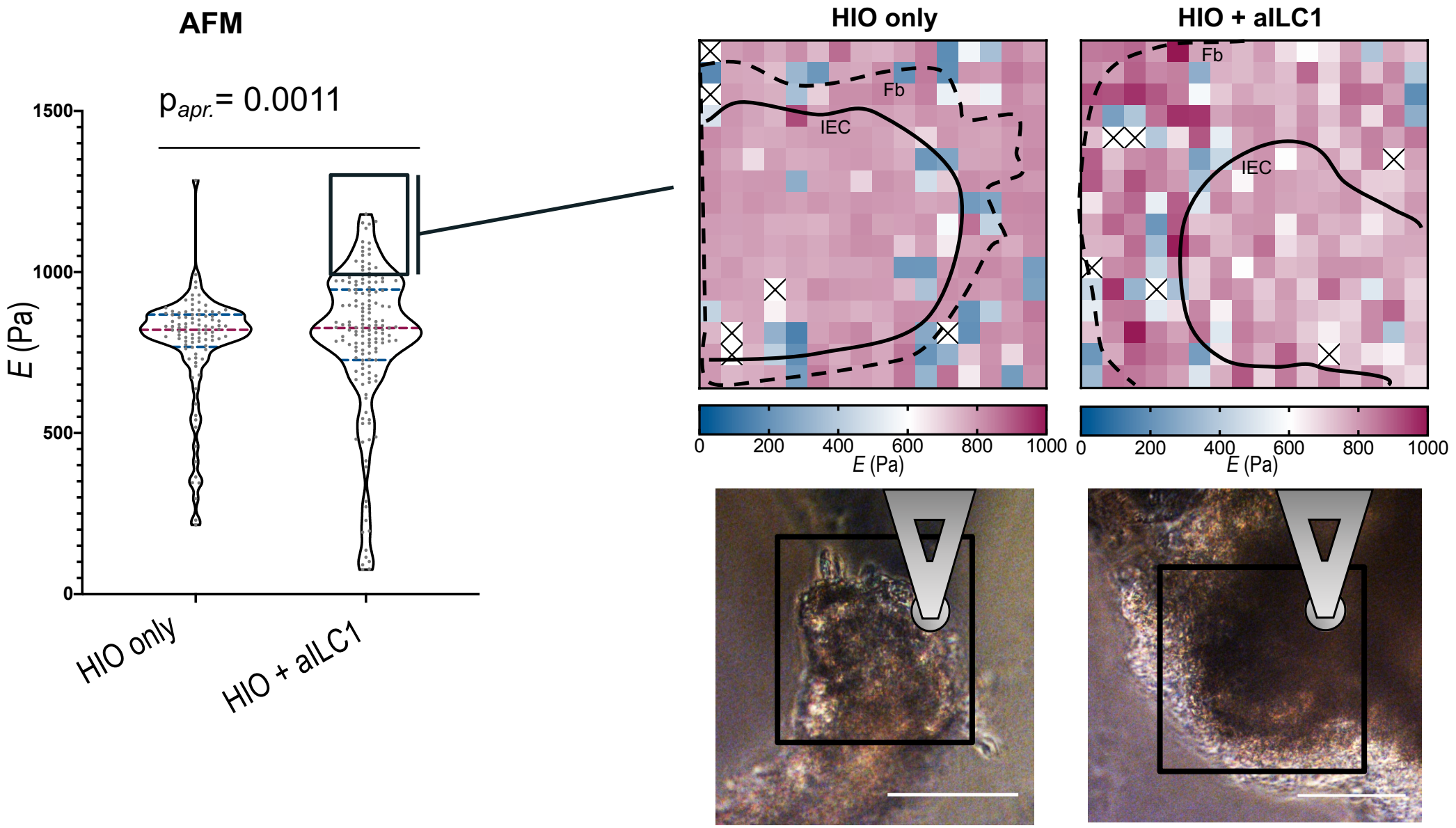




R code at https://github.com/eileengentleman/Microrheology-code

ILC1: Type I Innate lymphoid cells
HIO: human iPSC intestinal organoid

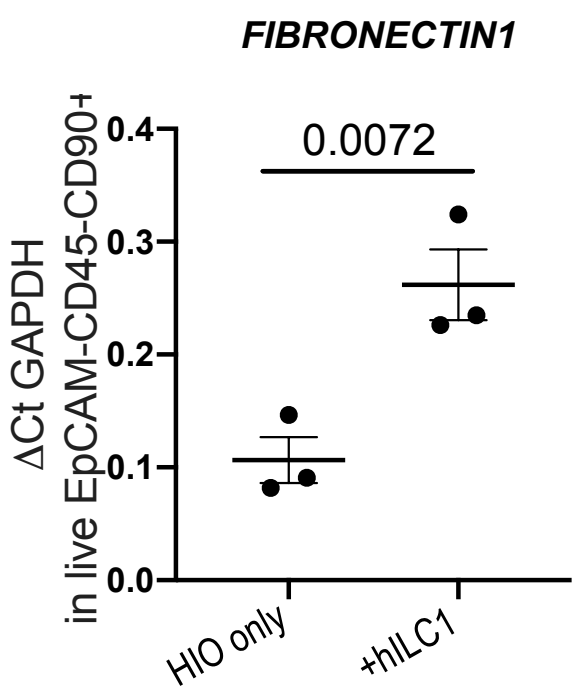
Matrix remodelling by encapsulated HIO

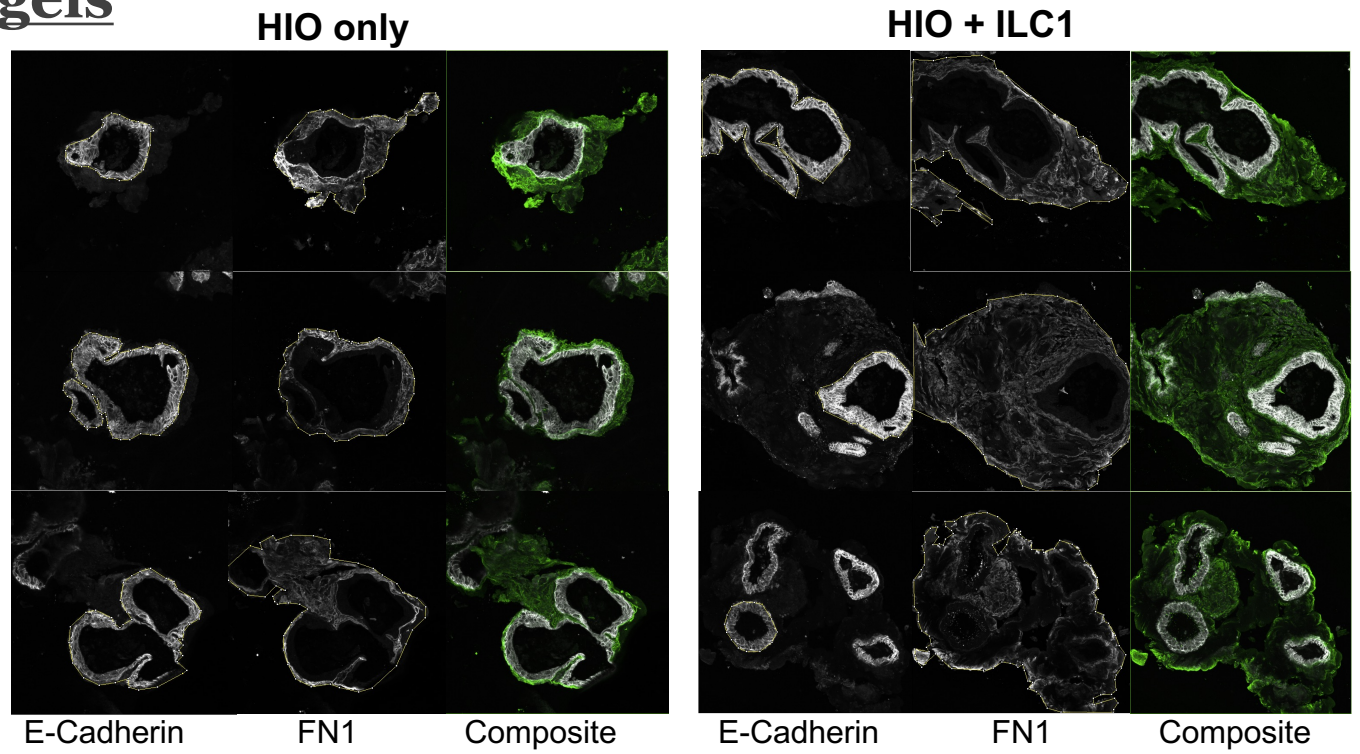


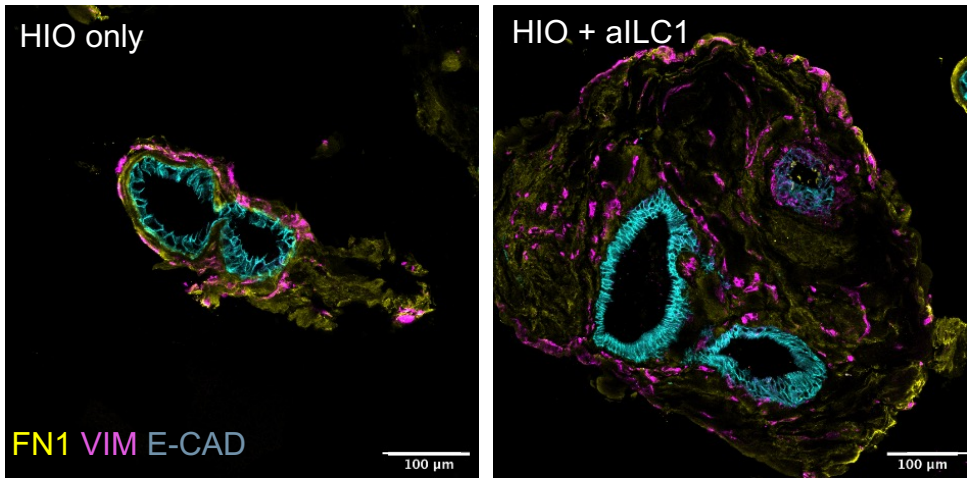
Jowett *et al. Nat Mater. 2021*

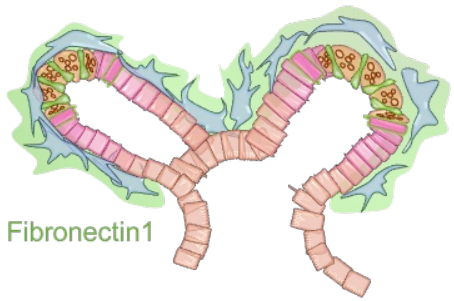
ILC1: Type I Innate lymphoid cells; HIO: human iPSC intestinal organoid

Matrix deposition by HIO in hydrogels

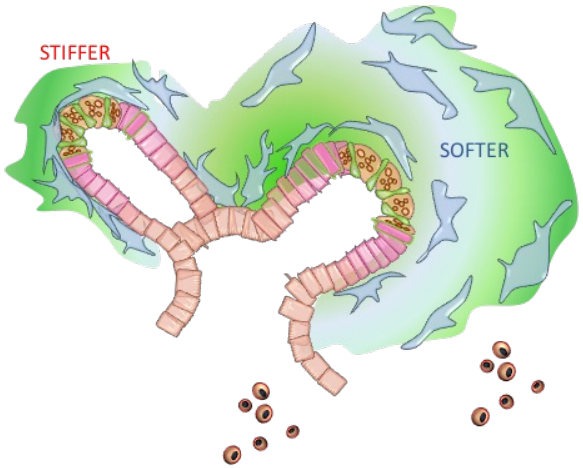








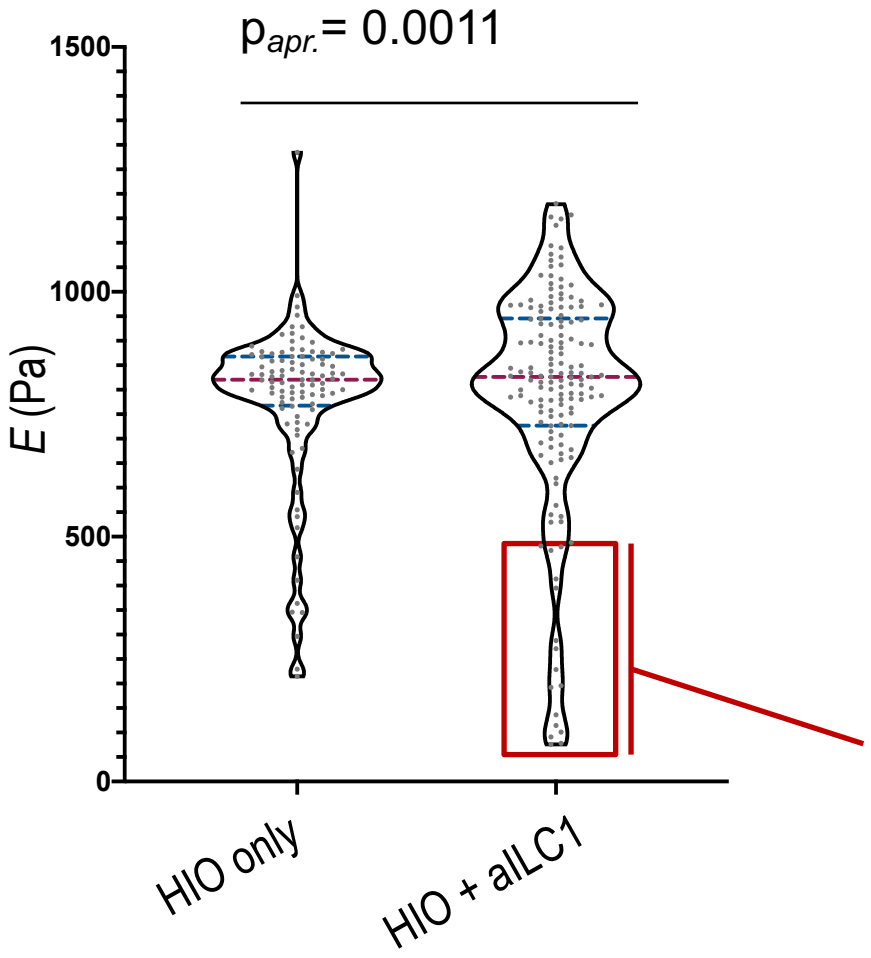
HIO only

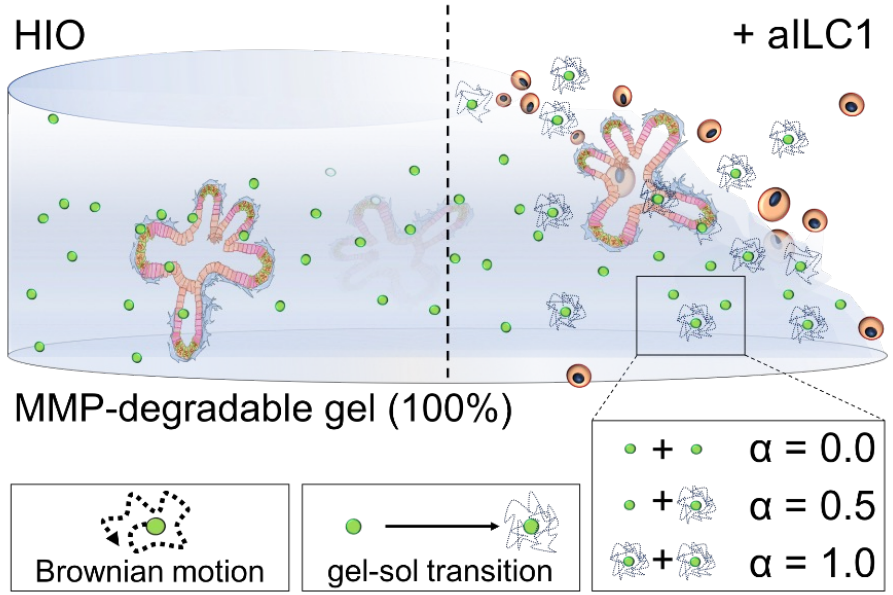


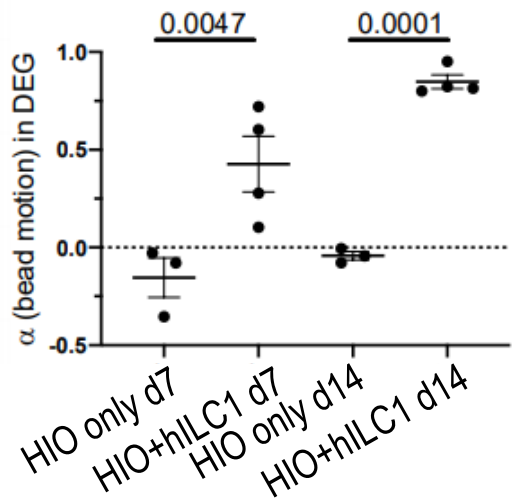
HIO+aILC1

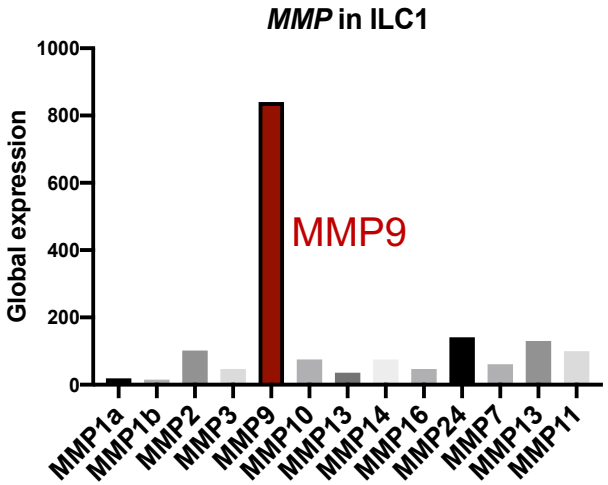
ILC1: Type I Innate lymphoid cells; HIO: human iPSC intestinal organoid

Local hydrogel degradation driven by ILC1





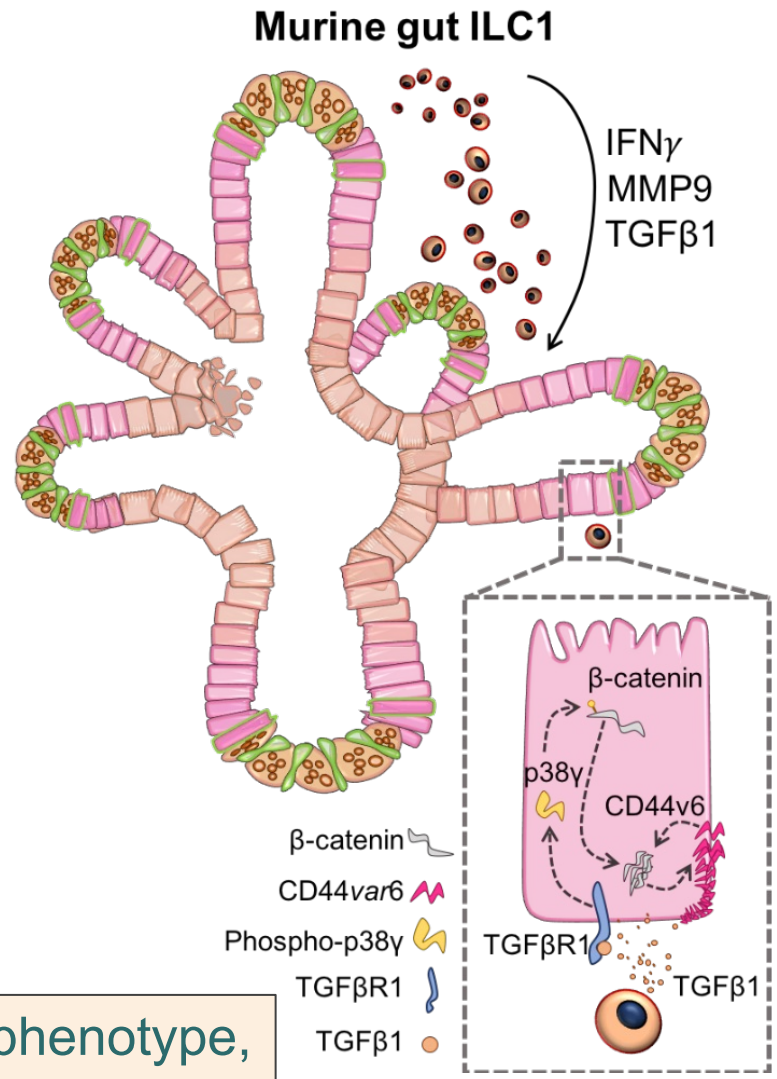




ILC1: Type I Innate lymphoid cells; HIO: human iPSC intestinal organoid

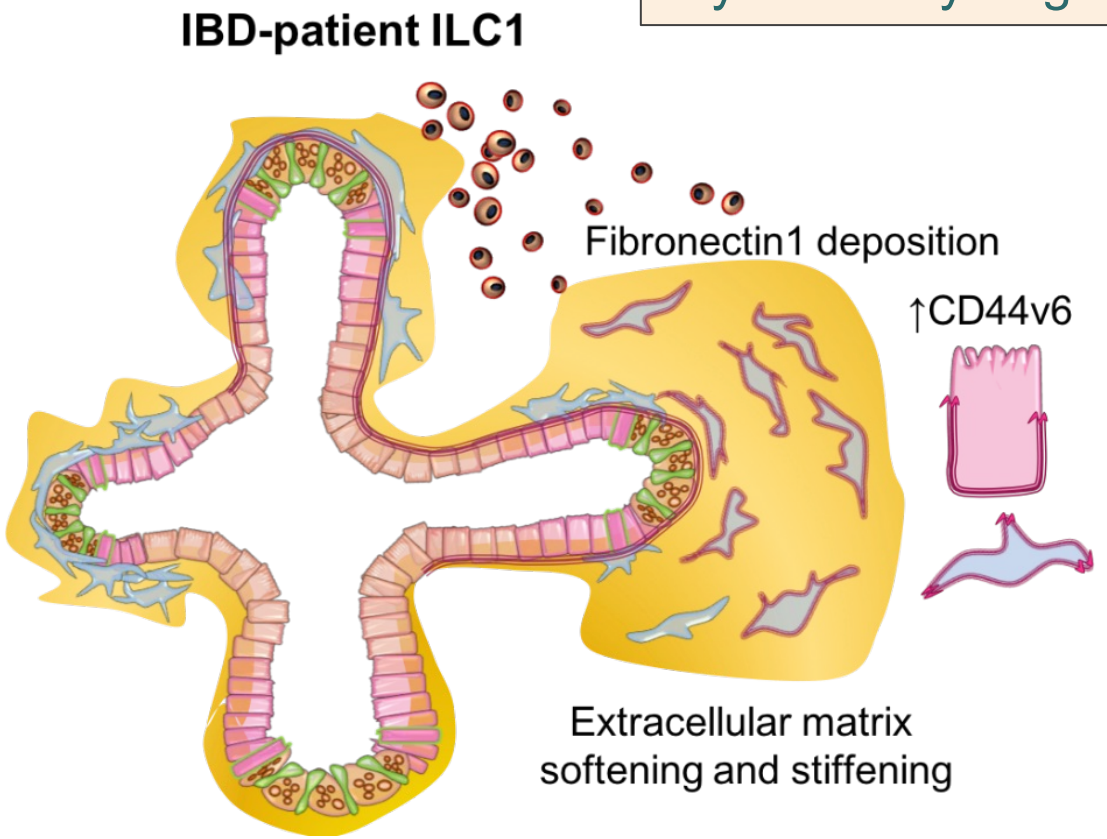
Immgen (RNAseq resource), global search

Summary of findings



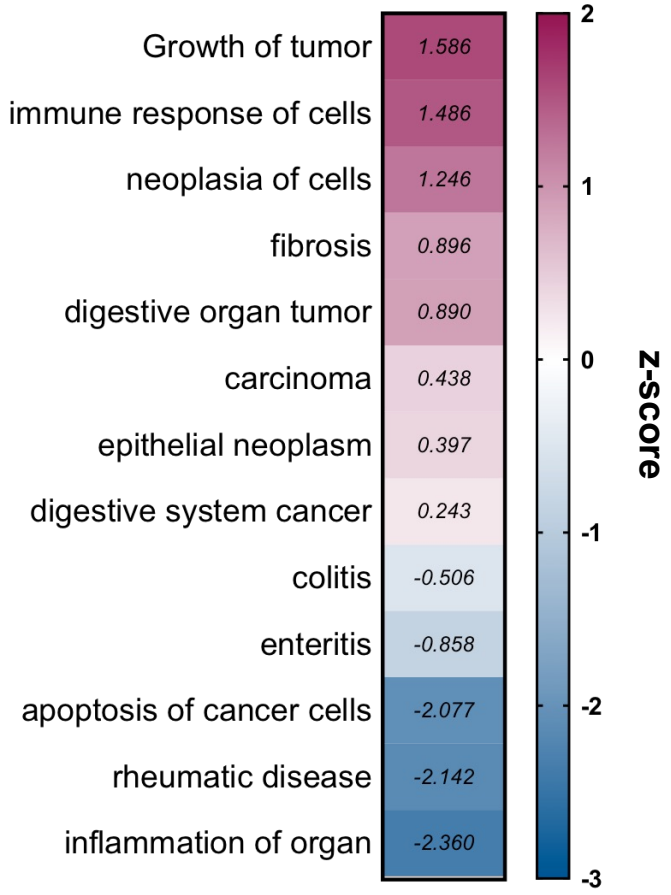
Epithelial phenotype, murine organoids-ILC1 in Matrigel

Mesenchymal phenotype, human organoids-ILC1 in synthetic hydrogels

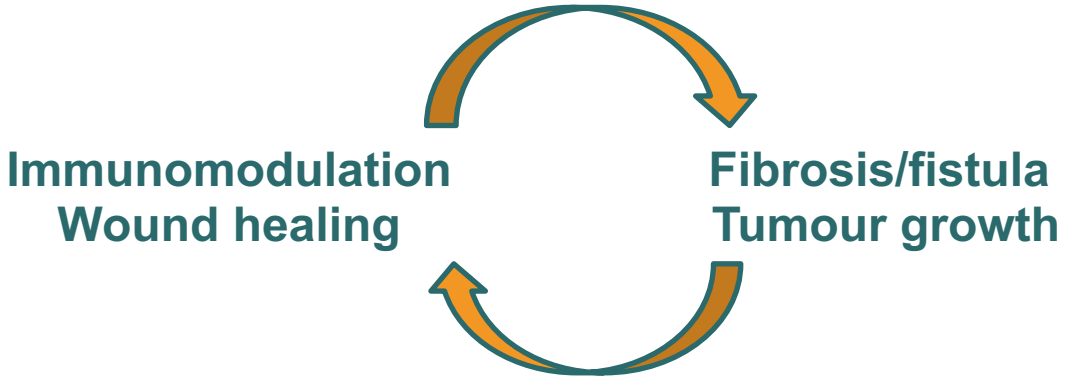


Summary of findings

Diseases and functions



- ILC1 are 1st responders at mucosal barriers, but their primary role may not be to drive inflammation (IFNγ), as previously thought.
- Instead, they may orchestrate a wound healing response.
- However, when ILC1 accumulate in inflamed tissues, this wound healing response could drive co-morbidities in Crohn's disease, like fibrosis/fistula or cancer.



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