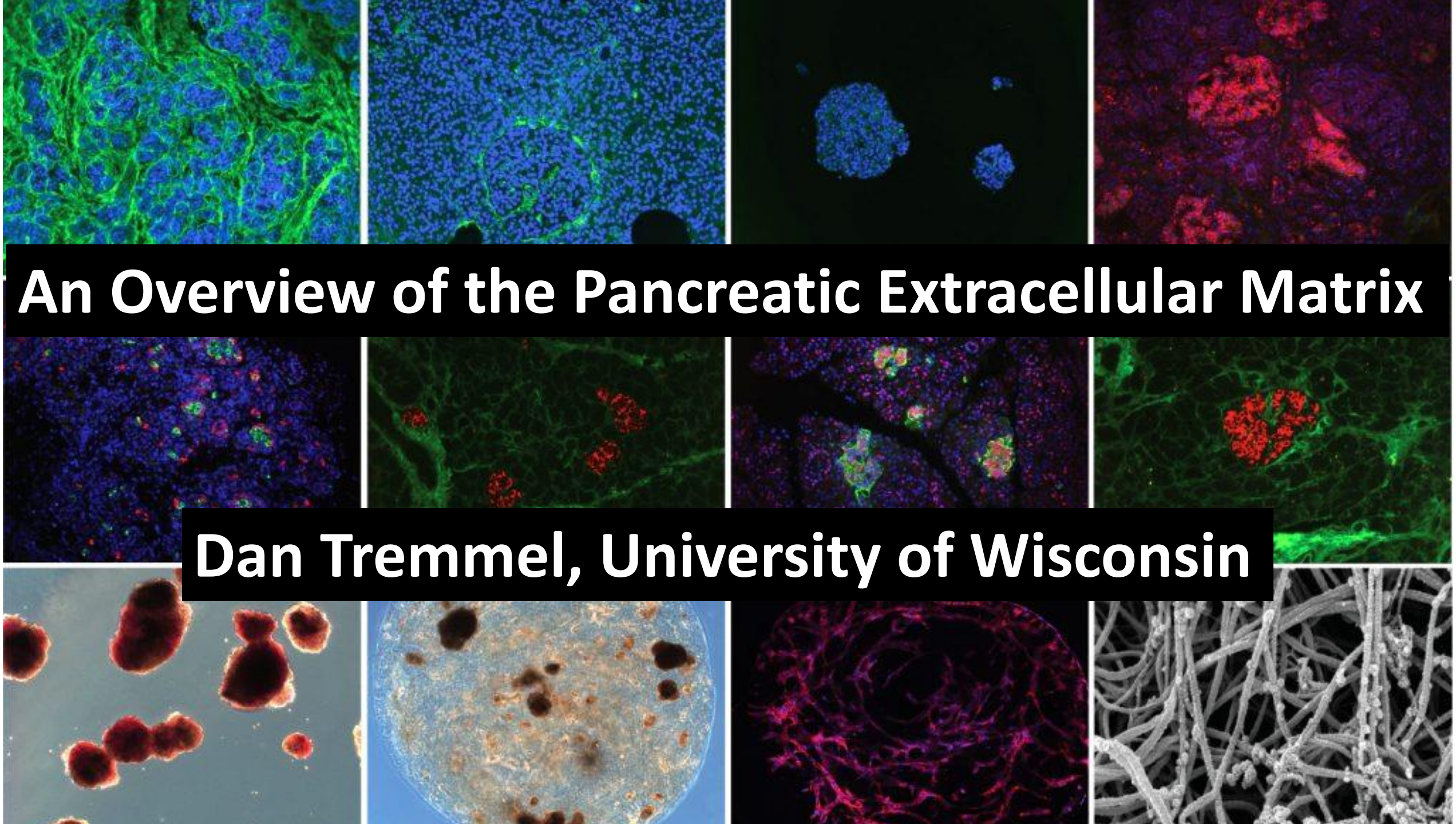
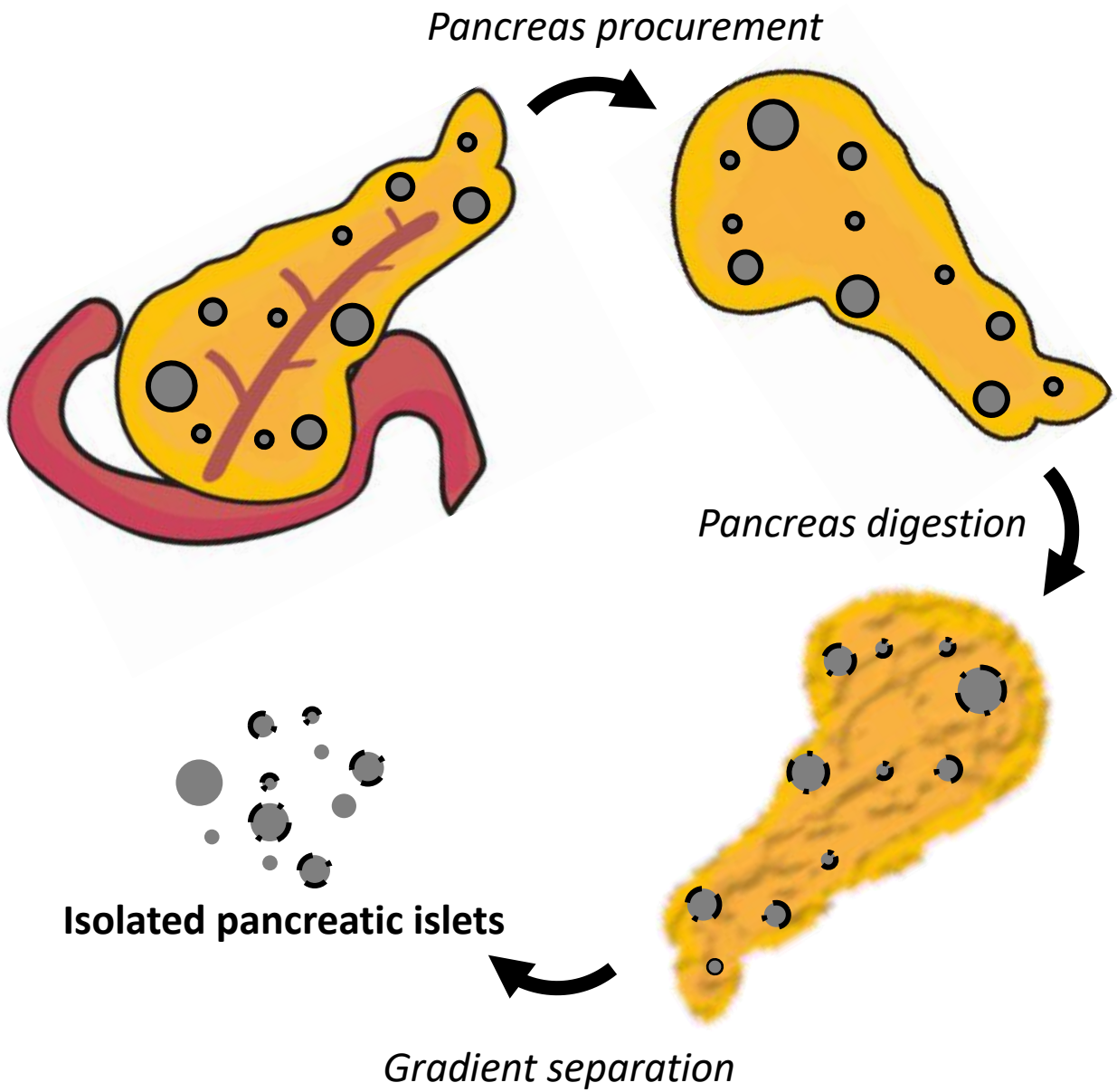


An Overview of the Pancreatic Extracellular Matrix

Dan Tremmel, University of Wisconsin



Islet isolation:

In order to recover islets from the pancreas, collagenases and proteases are used to digest the ECM

≥60% collagen digestion leads to higher islet yield and increased odds of being transplanted[1]

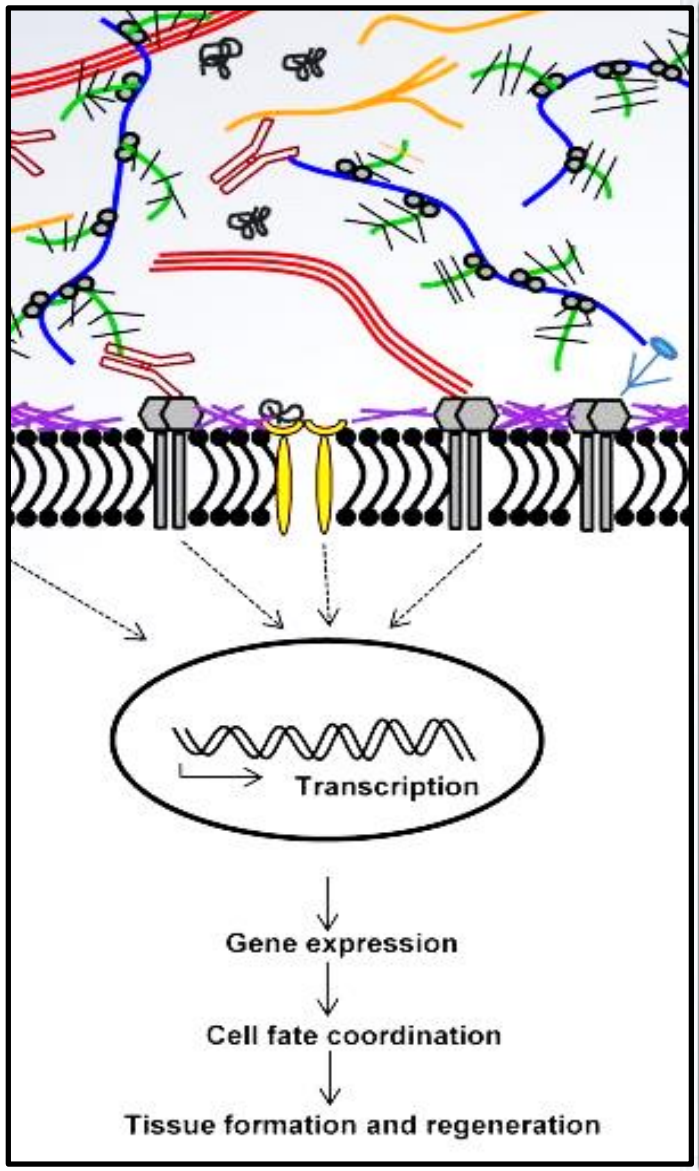
Human islet basement membrane is substantially disrupted during the islet isolation procedure[2]

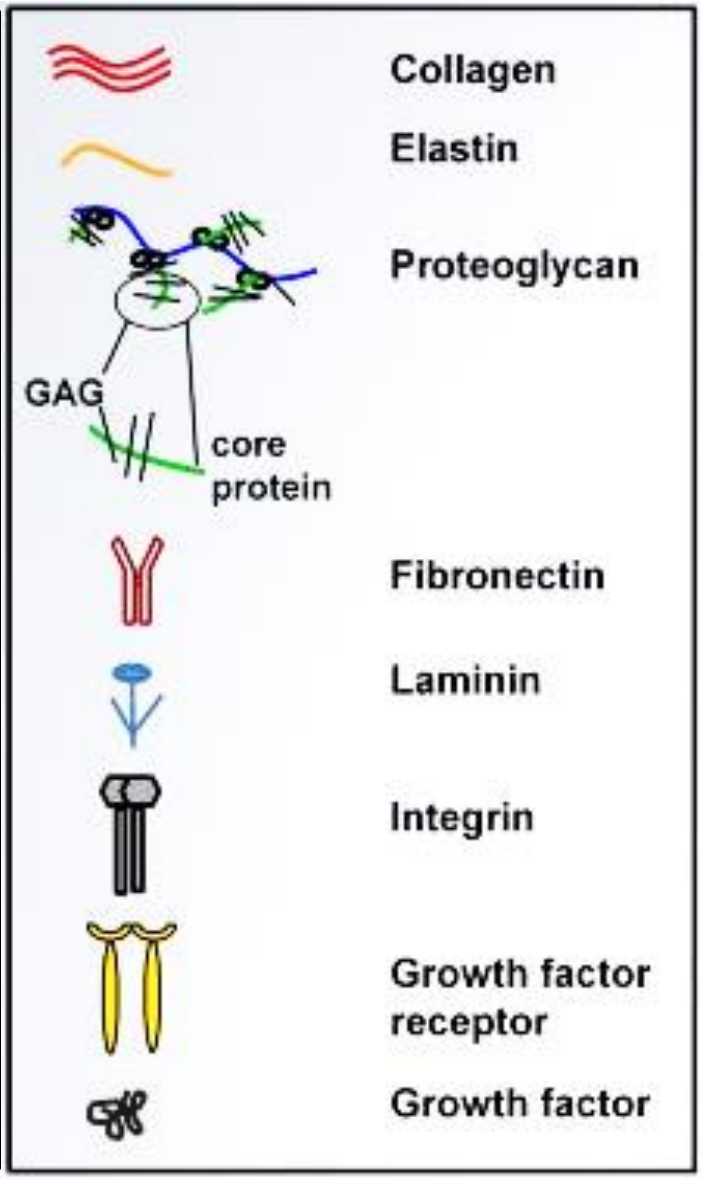
(1) Meier et al. 2020 Transplant International *(2) Cross et al. 2017 AJT*

Biologic Properties of Extracellular Matrix

- ECM proteins can directly **bind membrane receptors**, such as **integrins**
- The ECM can **bind and regulate growth factors**, influencing and modulating cell signaling
- Provides **structural and mechanical support**

ECM composition is **unique** within each tissue

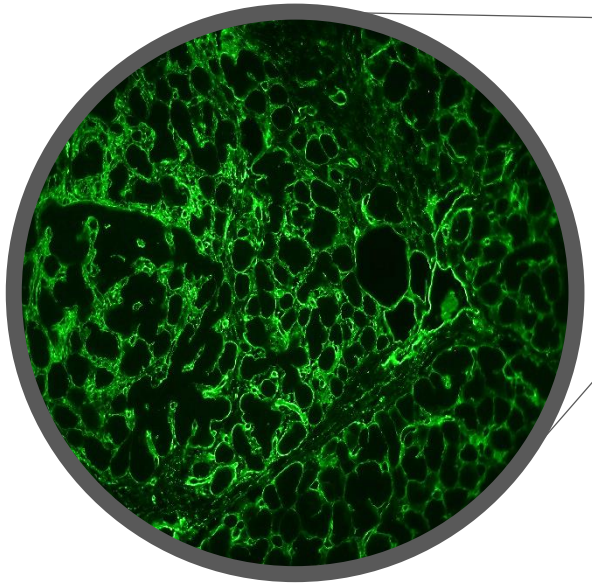


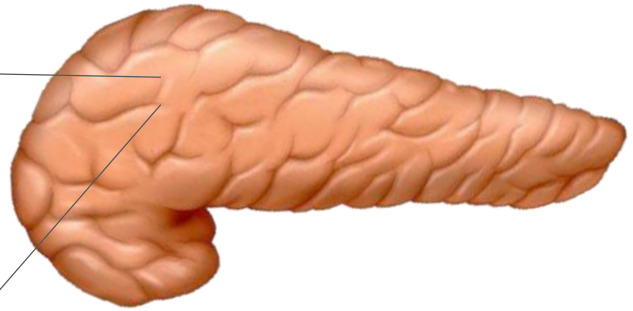


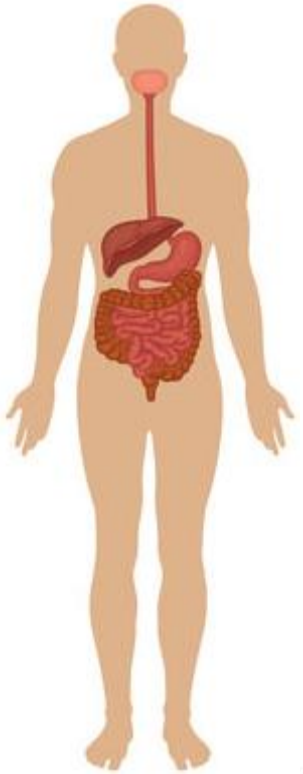
Biochemistry, Genetics and Molecular Biology » "Biotechnology - Molecular Studies and Novel Applications for Improved Quality of Human Life", *book edited by Reda Helmy Sammour, ISBN 978-953-51-0151-2, Published: March 14, 2012*

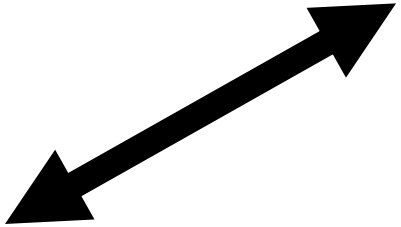

The matrisome is a jungle

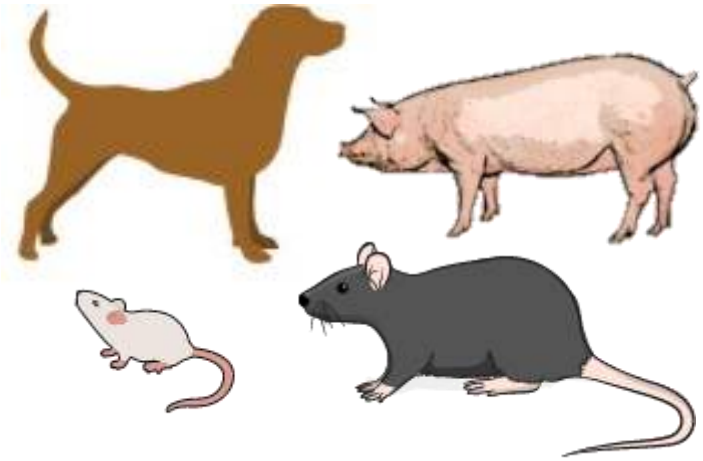
Characterizing the Pancreas ECM









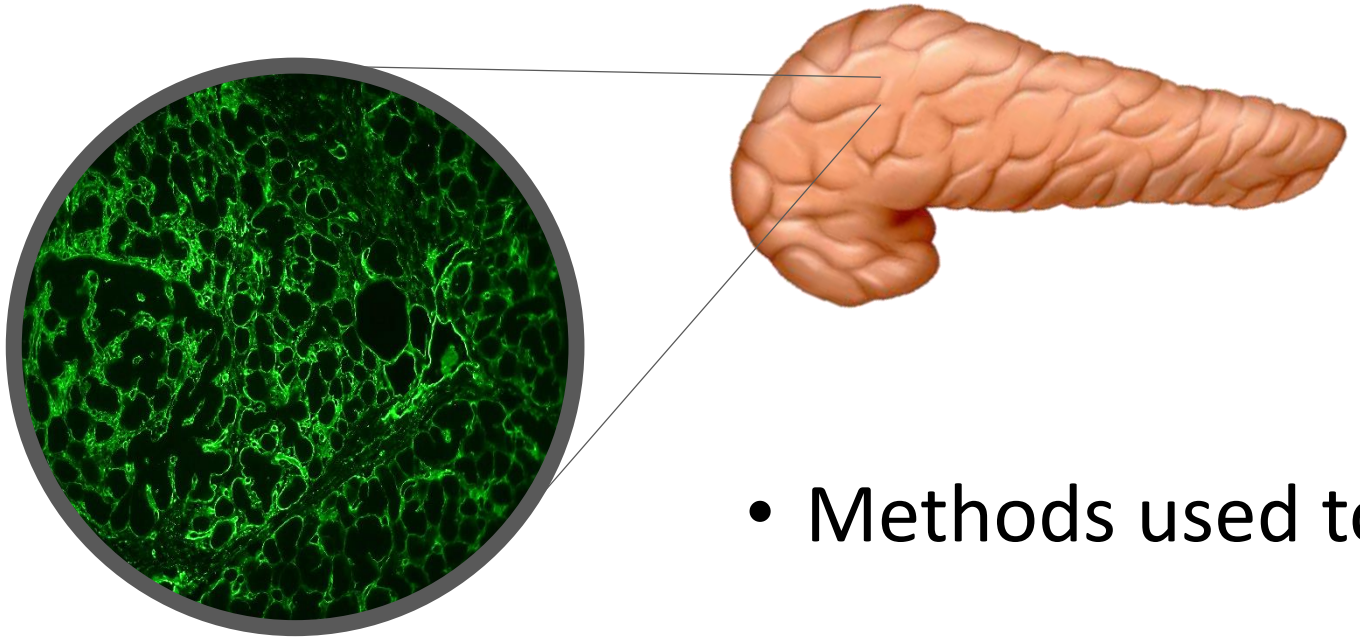


Understanding Pancreas ECM:

- Improve islet isolation technology
- Support islet health and function for culture and transplantation
- Understand roles of ECM in disease (pancreatitis, diabetes, etc.)

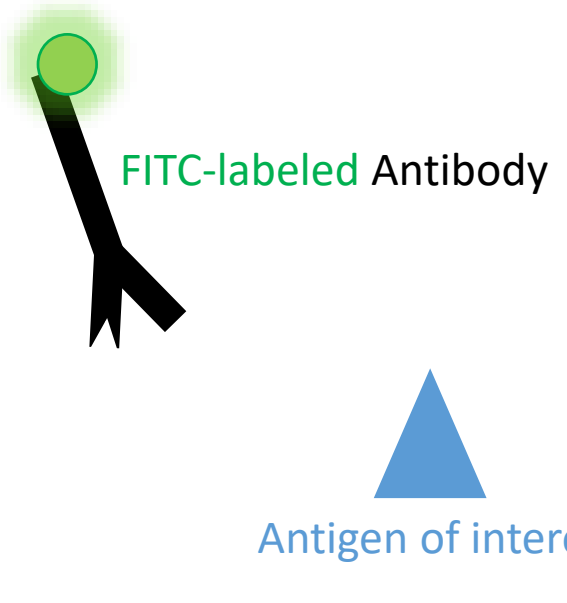
Potential organ donors

Outline

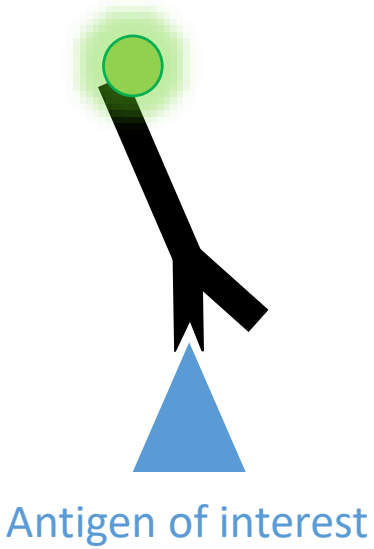


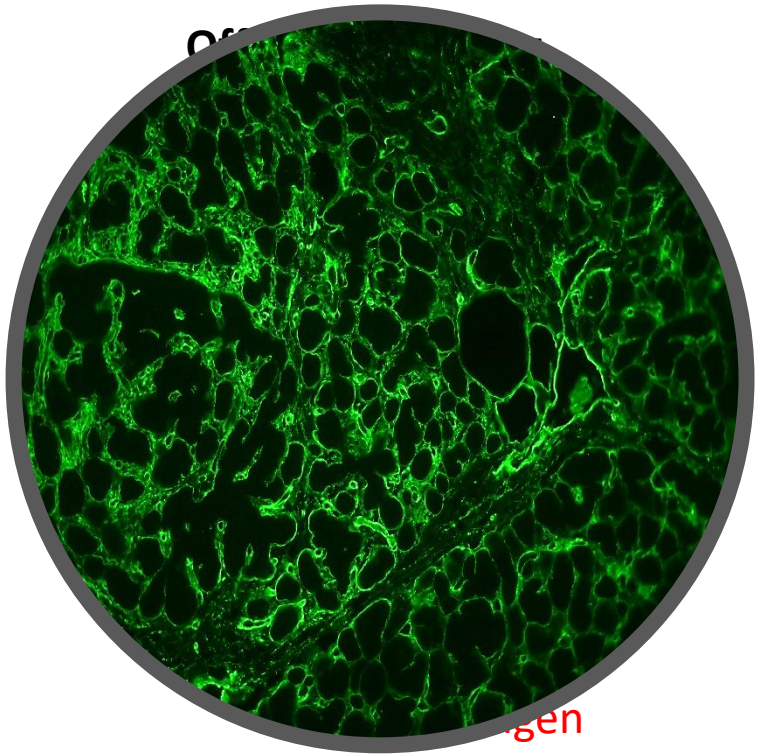
- Methods used to characterize the matrisome
- Quantitative studies of human pancreas ECM protein composition
- Collagenase/protease digestion studies on human pancreas tissue

Immunostaining



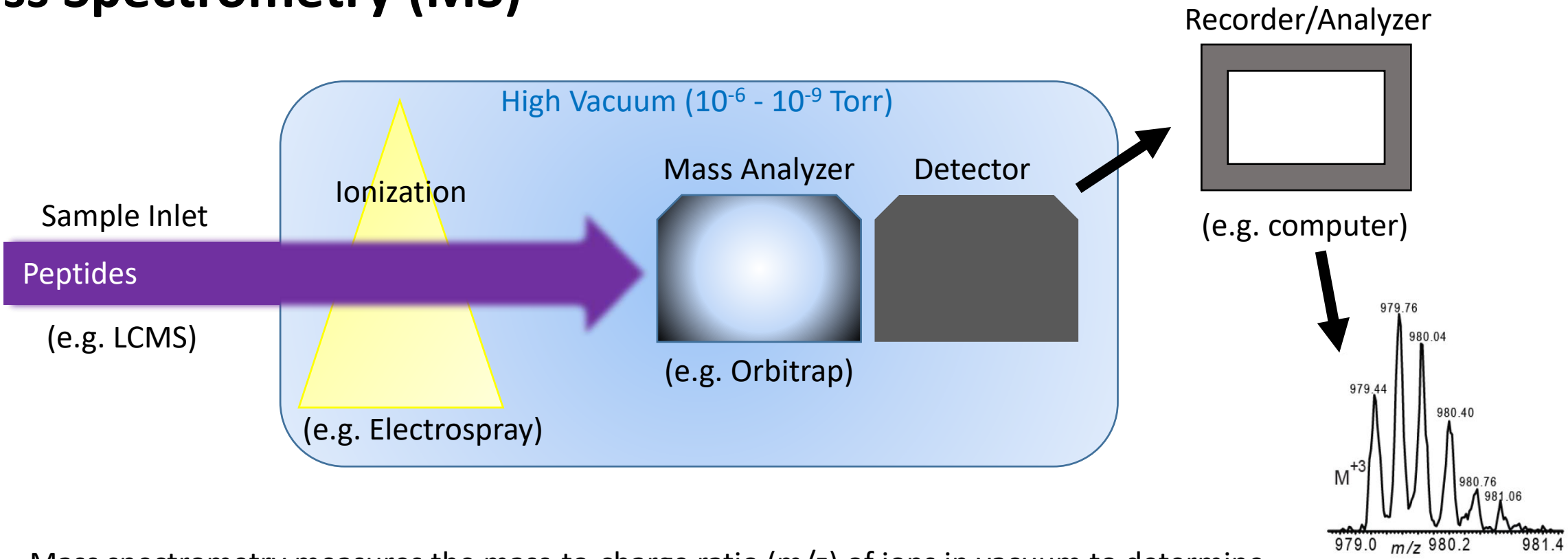
Antigen-specific binding





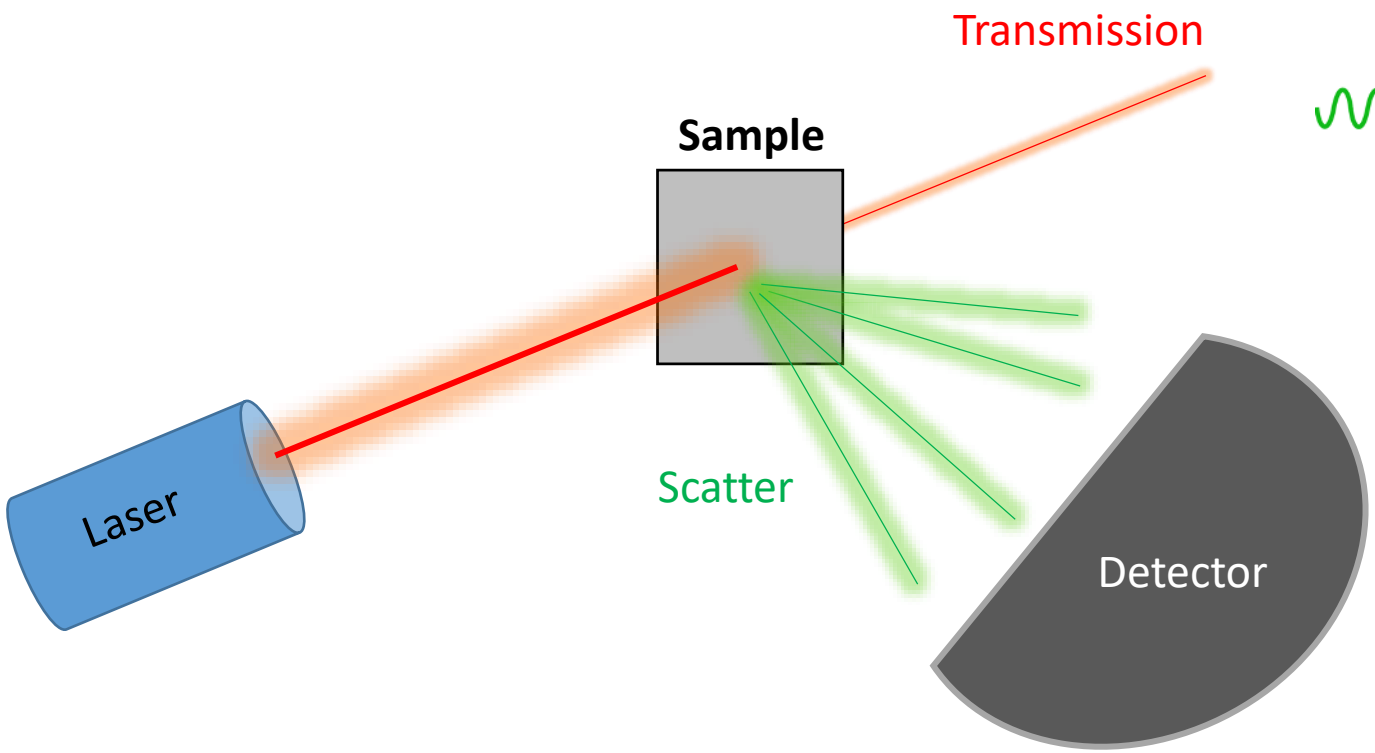
- Antibodies can be used to detect and image protein abundance and localization in tissue sections
- The use of immunostaining is limited by antibody availability and specificity, which often only work on select target species
- Some proteins (such as ECM proteins) have highly similar sequence homology and require specific and well-characterized antibodies to faithfully detect
- Off-target and background antibody binding may create false-positive results

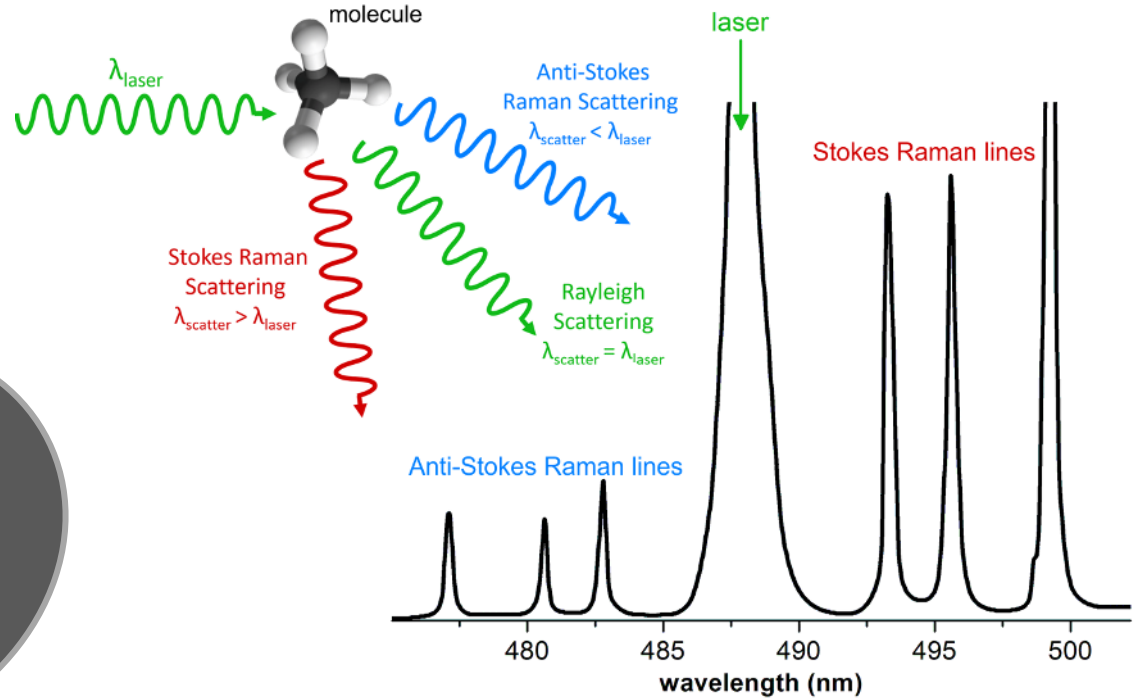
Mass Spectrometry (MS)



- Mass spectrometry measures the mass-to-charge ratio (m/z) of ions in vacuum to determine their molecular weights.
- In practice: charged molecules of interest, measure the trajectories of the resulting ions in vacuum response to various combinations of electric and magnetic fields.
- Sample digestion with known proteases (e.g. Trypsin) combined with a library of m/z ratios for known tryptic peptides, complex samples can be analyzed to determine both the identity and abundance of the proteins that make up the sample

Raman microspectroscopy (RMS)





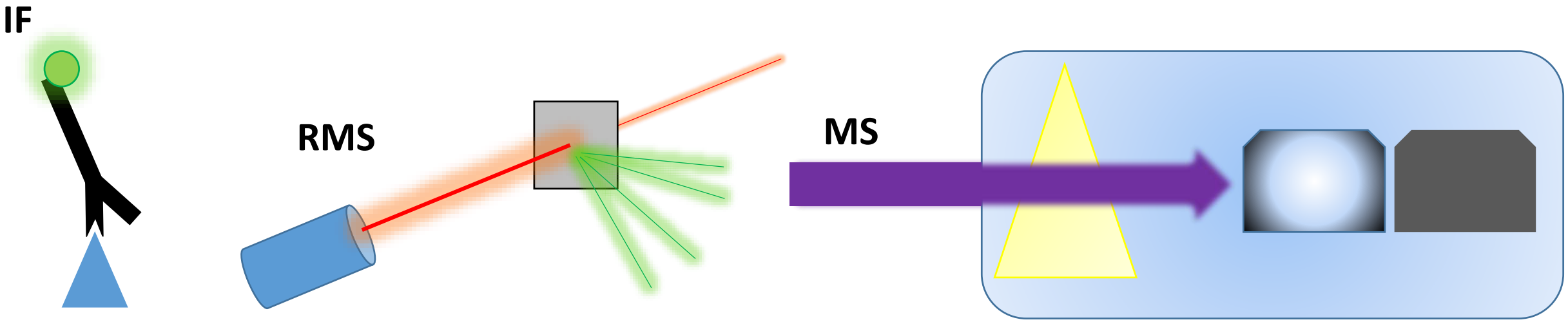
- Molecular vibrations due to excitement of the sample by the light allow for chemical identification
- The **Raman shift** is the energy difference between the incident (laser) light and the scattered (detected) light
- Identification of the composition of the studied material is often based on the comparison of its Raman spectrum with a spectral library of reference materials

Sisu@UT (https://sisu.ut.ee/heritage-analysis/book/export/html/19022)

Comparison of Methodologies

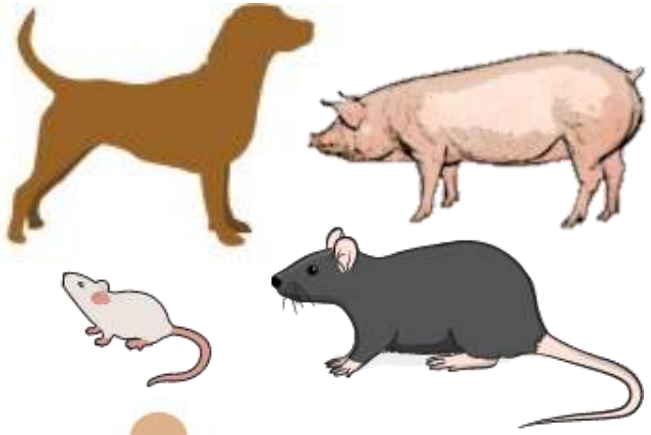
Method	High Throughput	Protein Specificity	Quantifiable	Ease	Tissue Visualization
Immunostaining (IF)	+ /-	+/+++	+	+++	+++
Raman (RMS)	+	-	++	+	+++
Mass Spec (MS)	++	+++	++/+++	+	+ / -

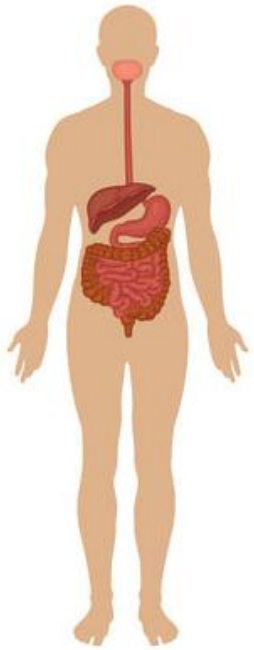
* Variations of each method may be better for different desired outcomes



Immunostaining established initial pancreas matrisome data

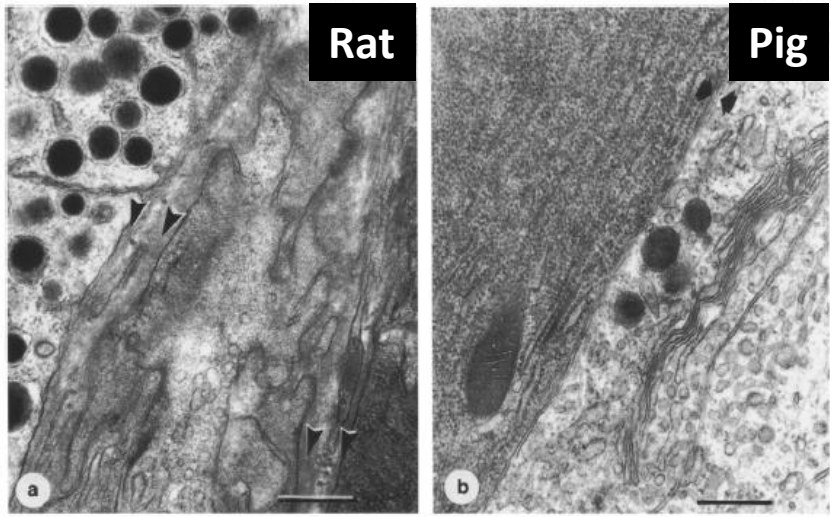
Composition, continuity and thickness of ECM vary among species[1]

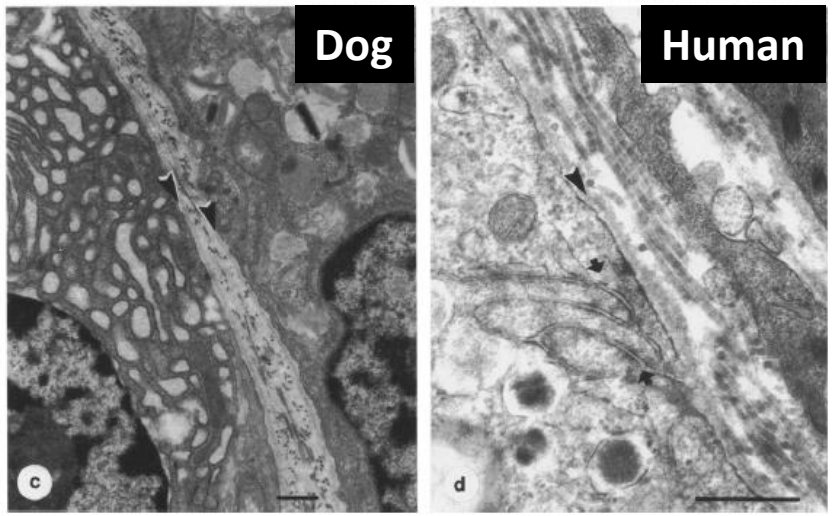




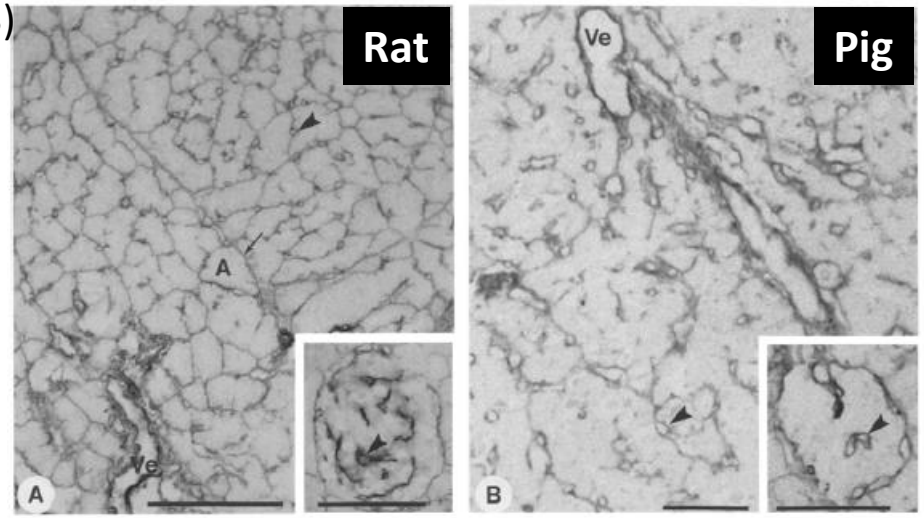
Laminins,
Col1, Col3,
Col4, Col5,
Col6, FN1[1]

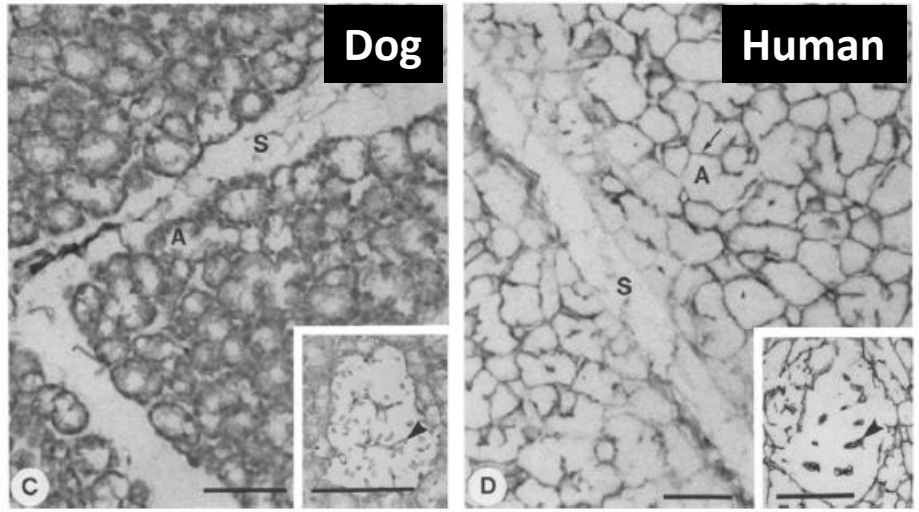
(2)





(3)





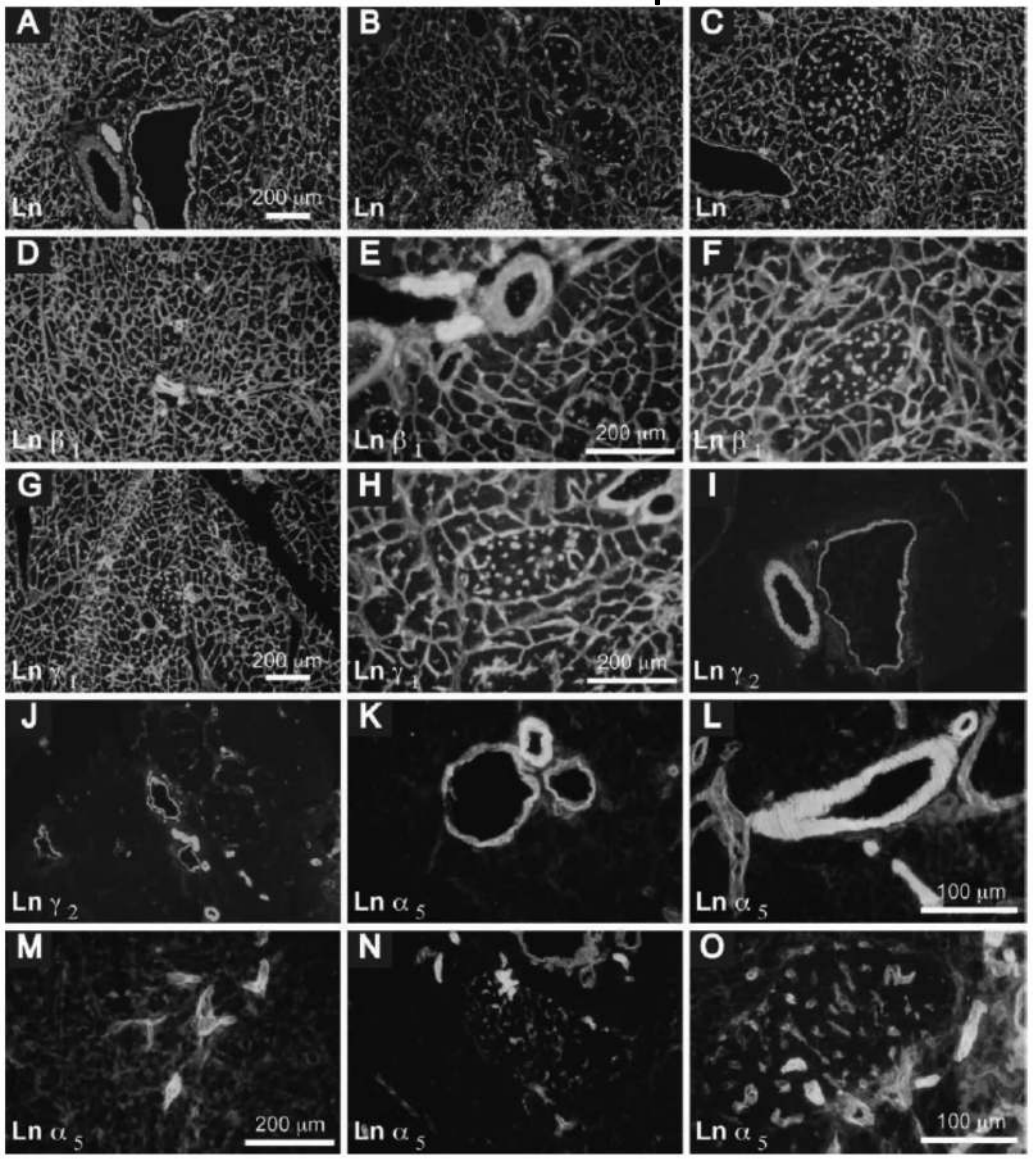
(1) Stendahl et al. 2010 Cell Transplant.

(2) Deijnen et al. 1992 Cell Tissue Res..

(3) Deijnen et al. 1994 Cell Tissue Res..

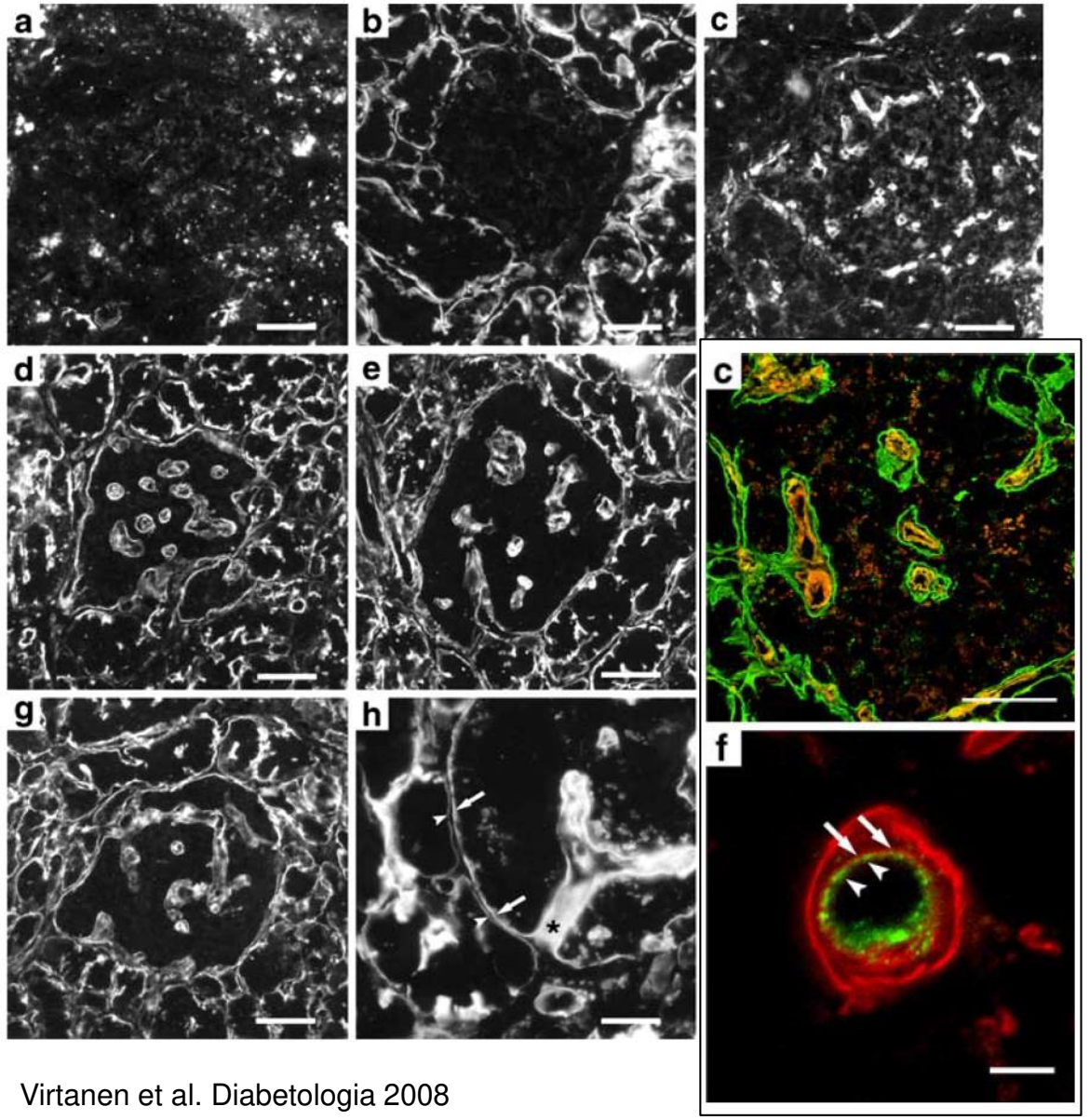
Human pancreas/islet ECM is structurally and chemically different

Laminins in mouse pancreas



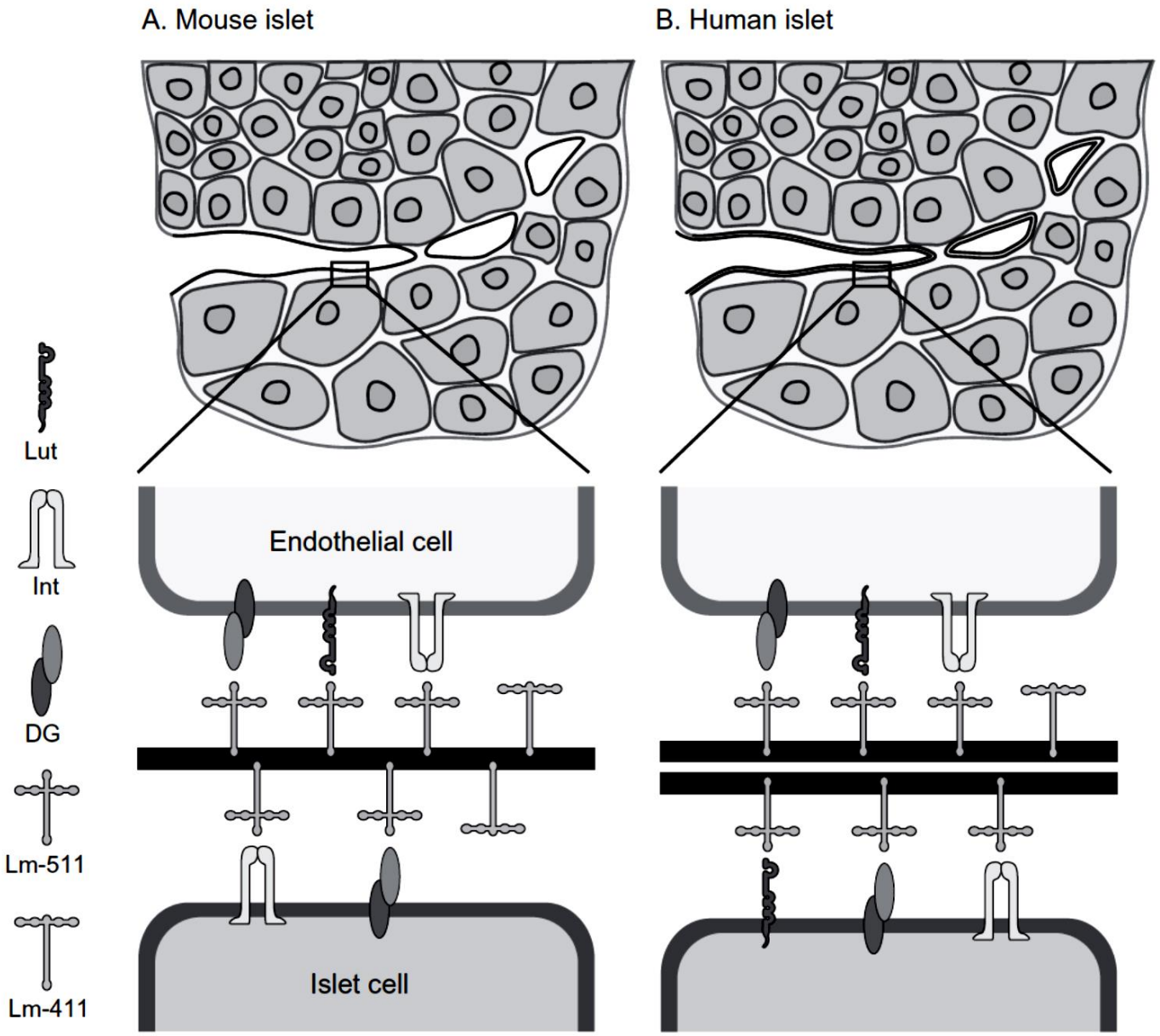
Jiang et al. The Journal of Histochemistry & Cytochemistry 2002

Laminins in human pancreas



Virtanen et al. Diabetologia 2008

Human pancreas/islet ECM is structurally and chemically different

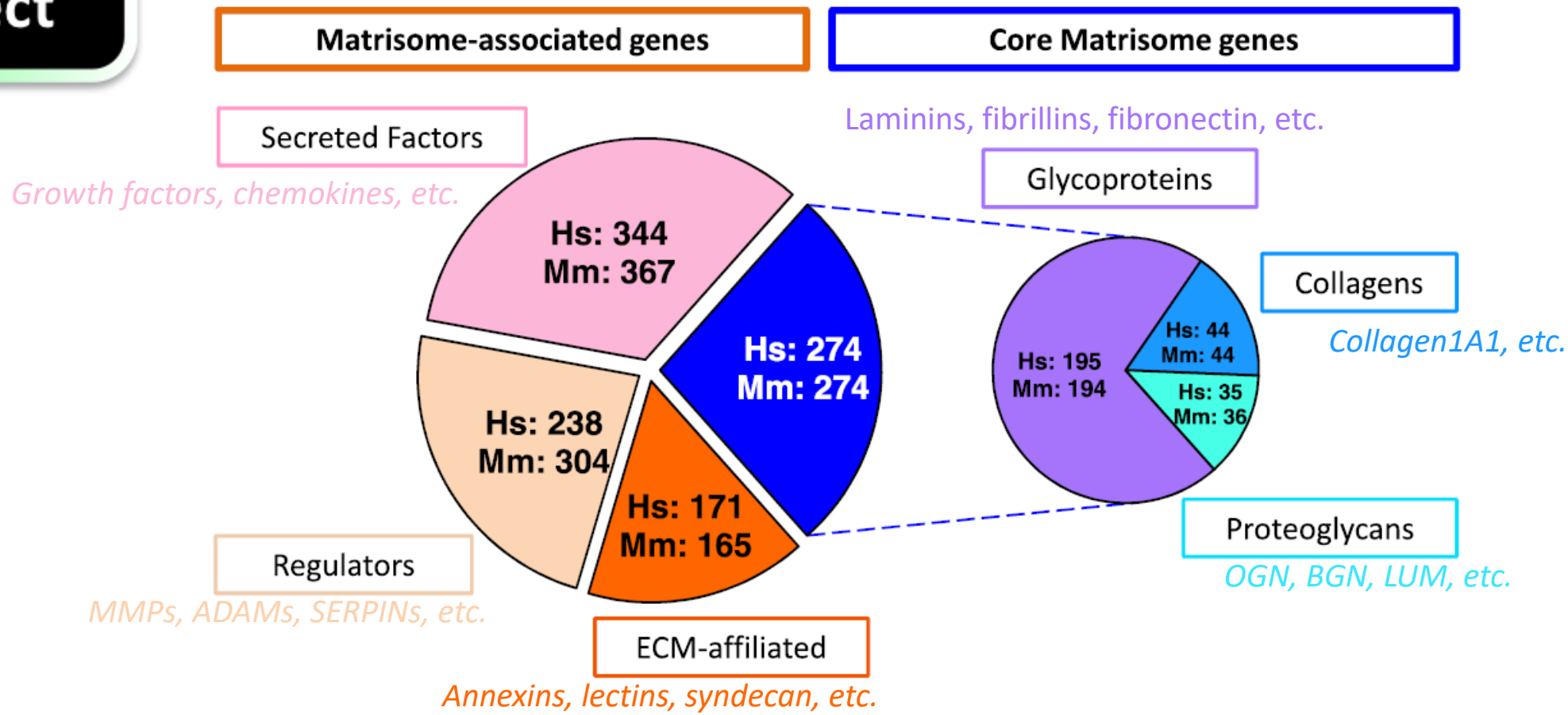


Define the composition of the human pancreas matrisome (by protein identification and quantification)

Otonkoski et al. Diabetes, Obesity and Metabolism 2008

Matrisome Project: Identification and Classification of ECM proteins

Matrisome Project



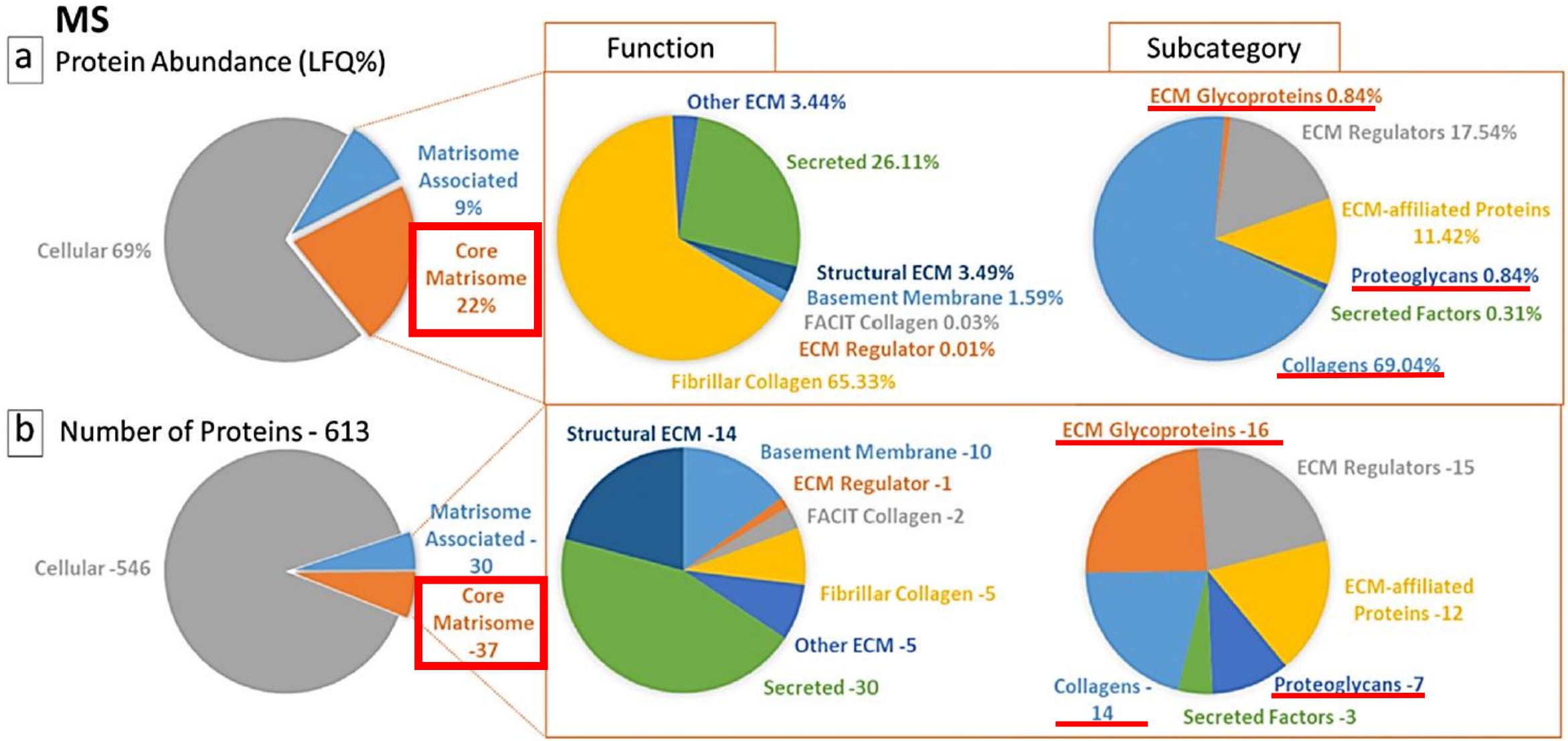
MatrixDB

Chautard et al. 2009 Bioinformatics

Naba et al. 2015 Matrix Biology

Mass spectrometry enables comprehensive matrisome characterization

15 donors, age 16-58



37 structural ECM proteins

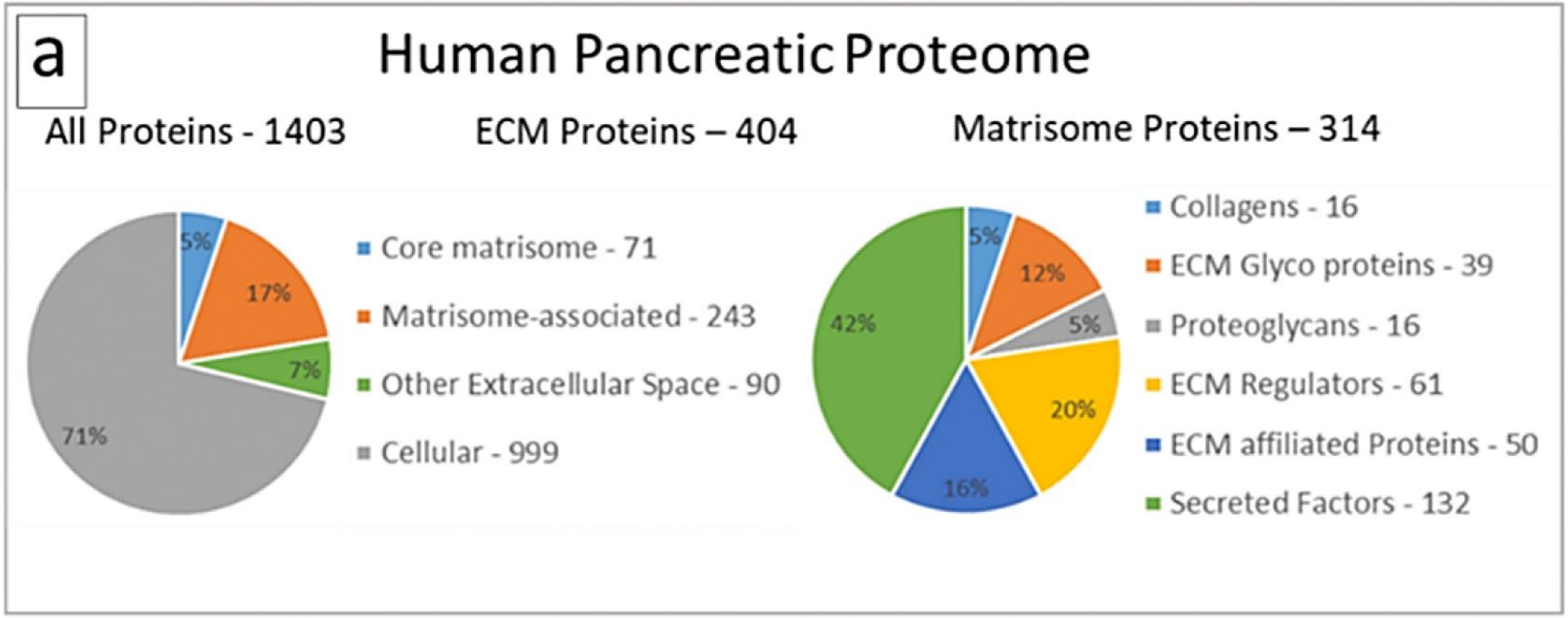
Asthana et al. Biomaterials 2021

Mass spectrometry enables comprehensive matrisome characterization

15 donors, age 16-58

Data as presented isn't useful for exploring abundance of different ECM components
No analysis of donors by age

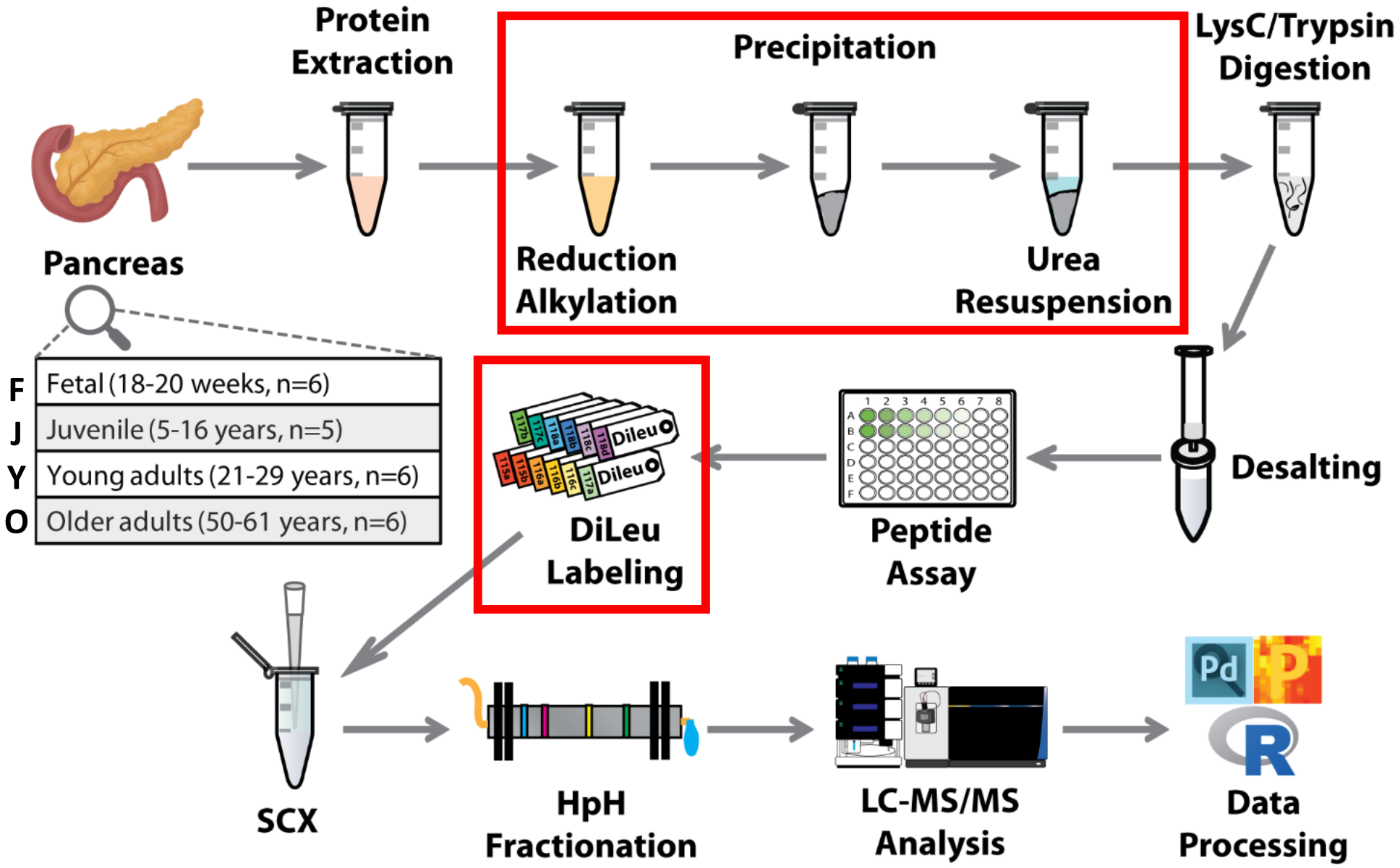
Combining all results from native pancreas and decellularized pancreas, using Mass Spec and ELISA:



314 ECM proteins: 71 structural ECM proteins
Core matrisome = ~5% of the pancreas proteome (by number of proteins)

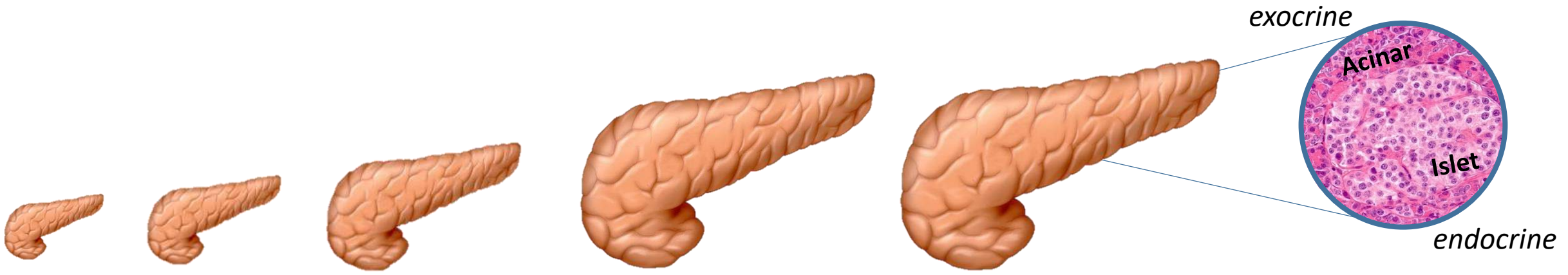
Asthana et al. Biomaterials 2021

Quantitative characterization using multiplex mass spectrometry



Li and Tremmel et al., *Nature Communications* 2021

Does ECM Composition Change Throughout Development?



Fetal	Infant	Juvenile	Younger Adult	Older Adult
18-20 gest. wks	< 1 year	5-16 years	21-30 years	51-60 years

Are there differences between **Islet** and **Acinar** ECM?

Previous studies have identified that differences in islet ECM of younger and older donors affect islet isolation efficiency[2]

Glucose responsiveness and expression of some mature genes increases until around puberty[1]

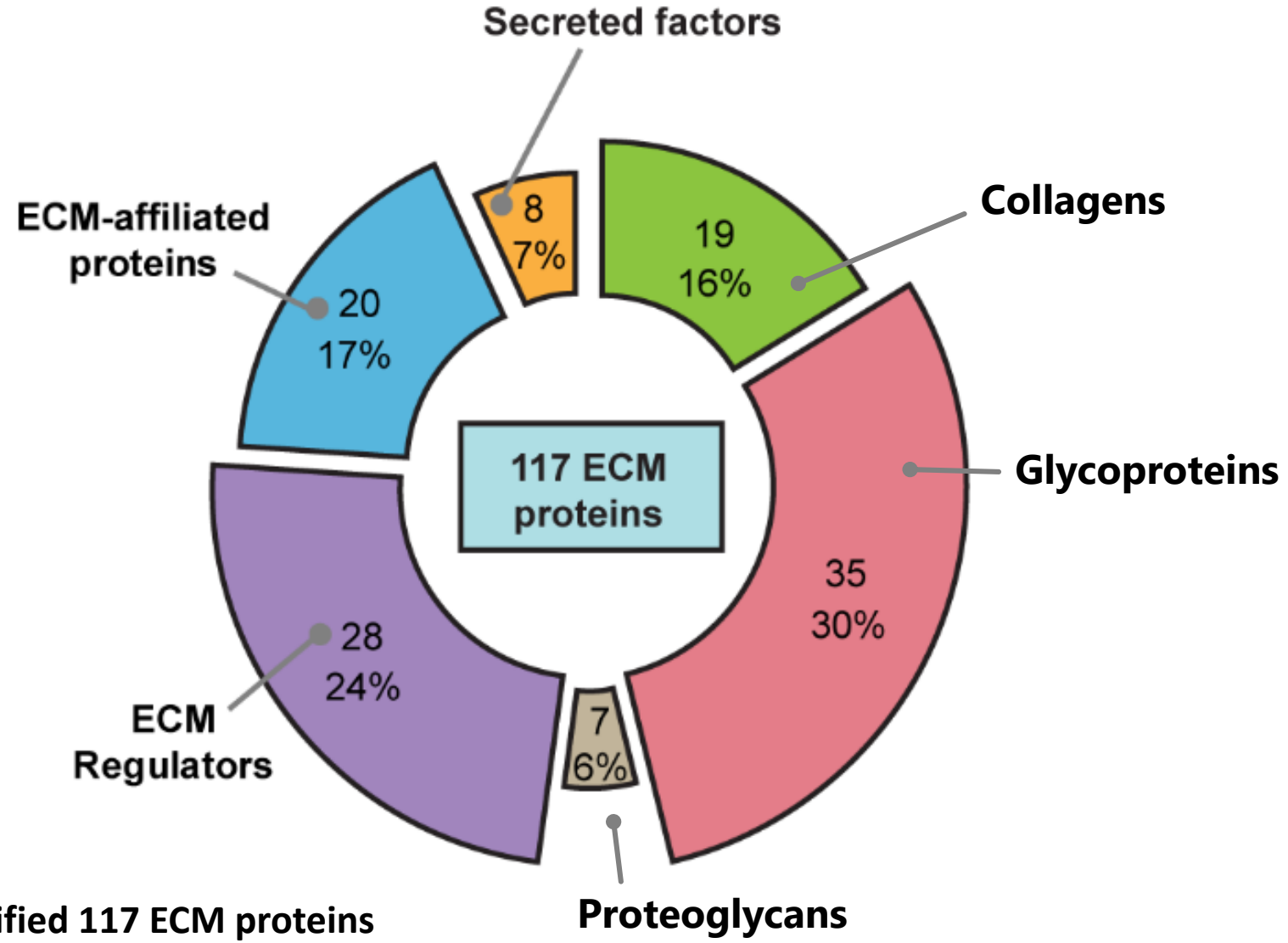
Functional maturation, in which islets become glucose-responsive

Immature islets lack response to high glucose levels

(1) Arda et al. 2016 Cell Metab

(2) Spiers et al. 2019 Acta Biomaterialia

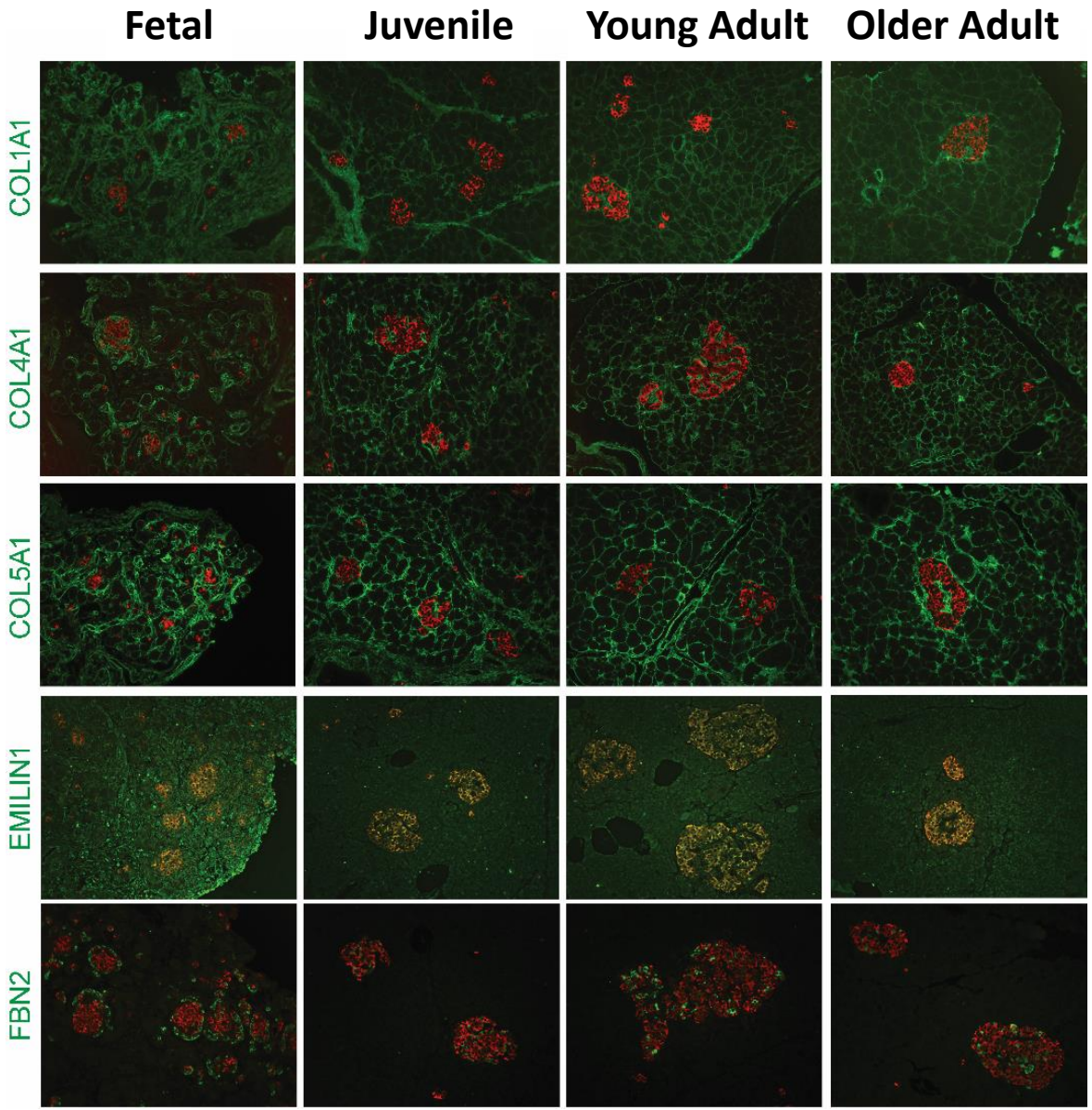
ECM remodeling during fetal and post-natal stages of human pancreas development

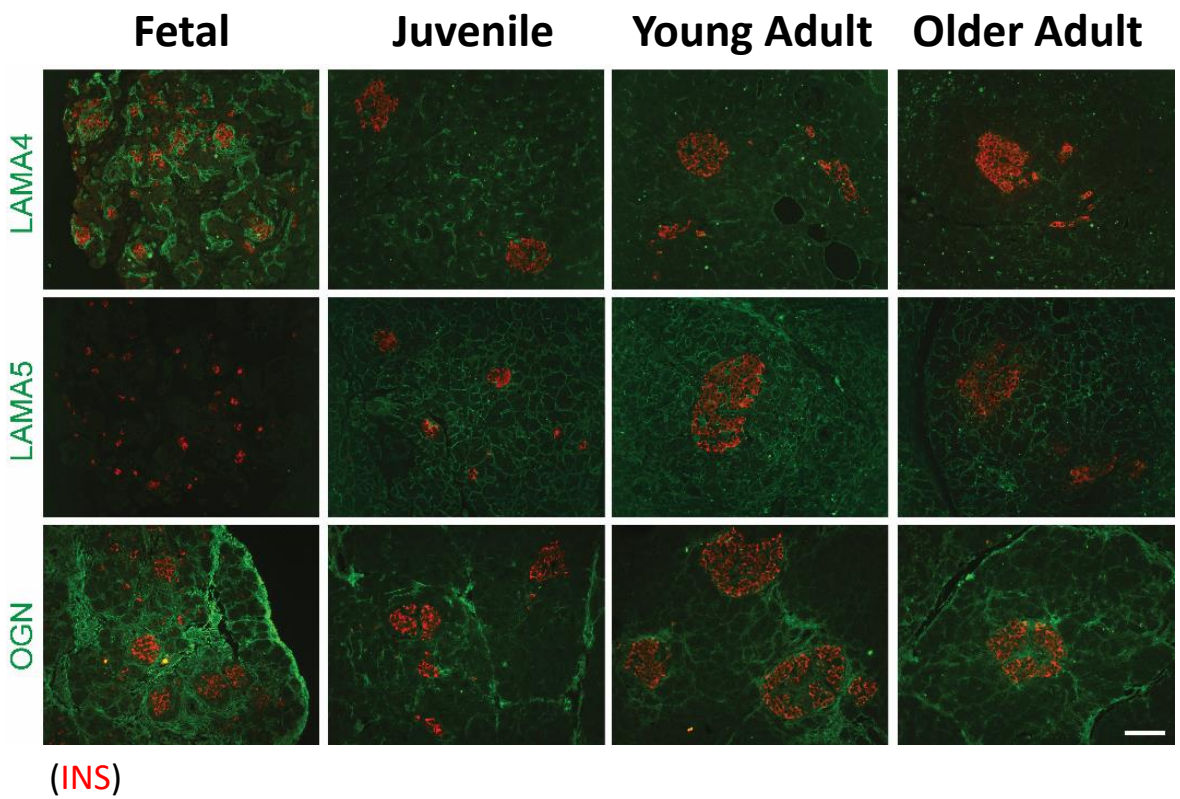


Identified 185 ECM proteins / Quantified 117 ECM proteins
84 ECM proteins significantly changed (72%)

Li and Tremmel et al., *Nature Communications* 2021

Visualizing ECM proteins across multiple developmental stages



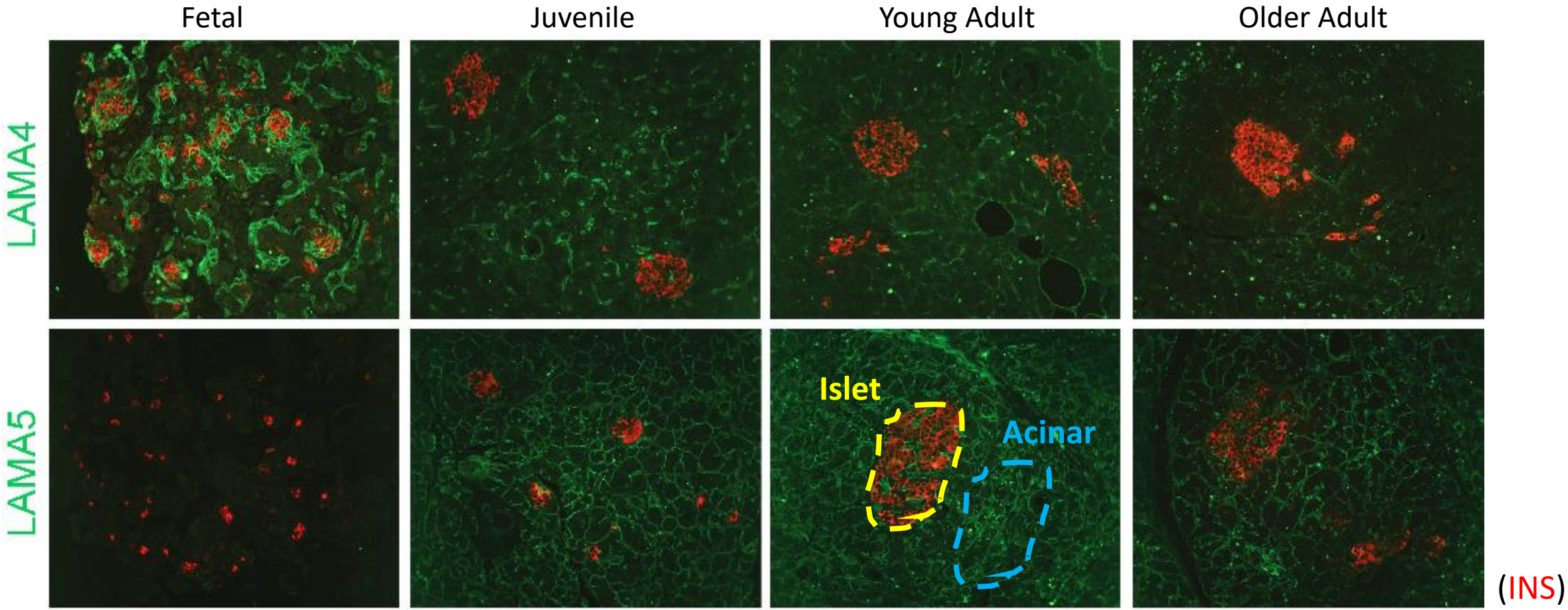


(INS)

Li and Tremmel et al., *Nature Communications* 2021

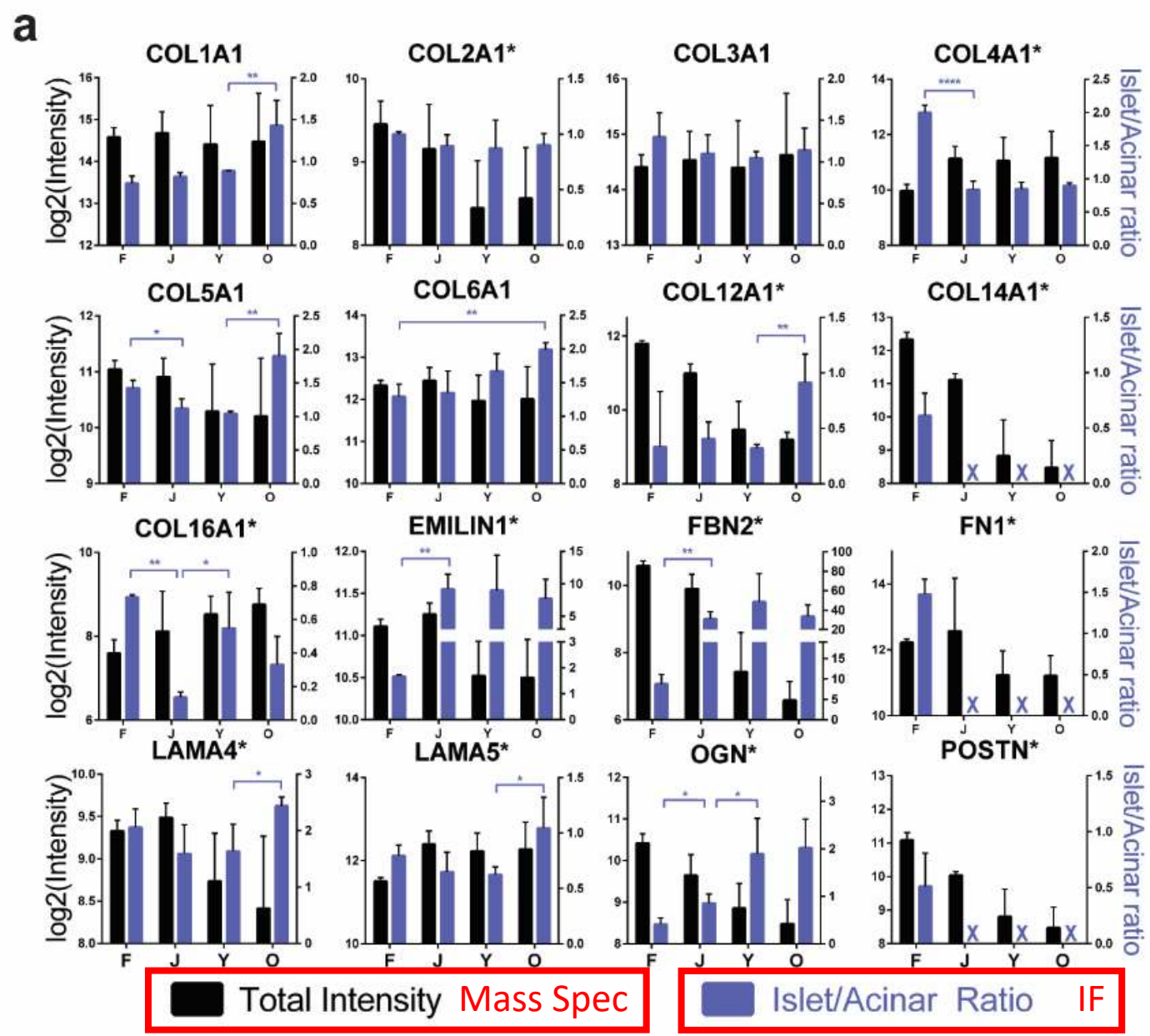
Visualizing ECM proteins across multiple developmental stages

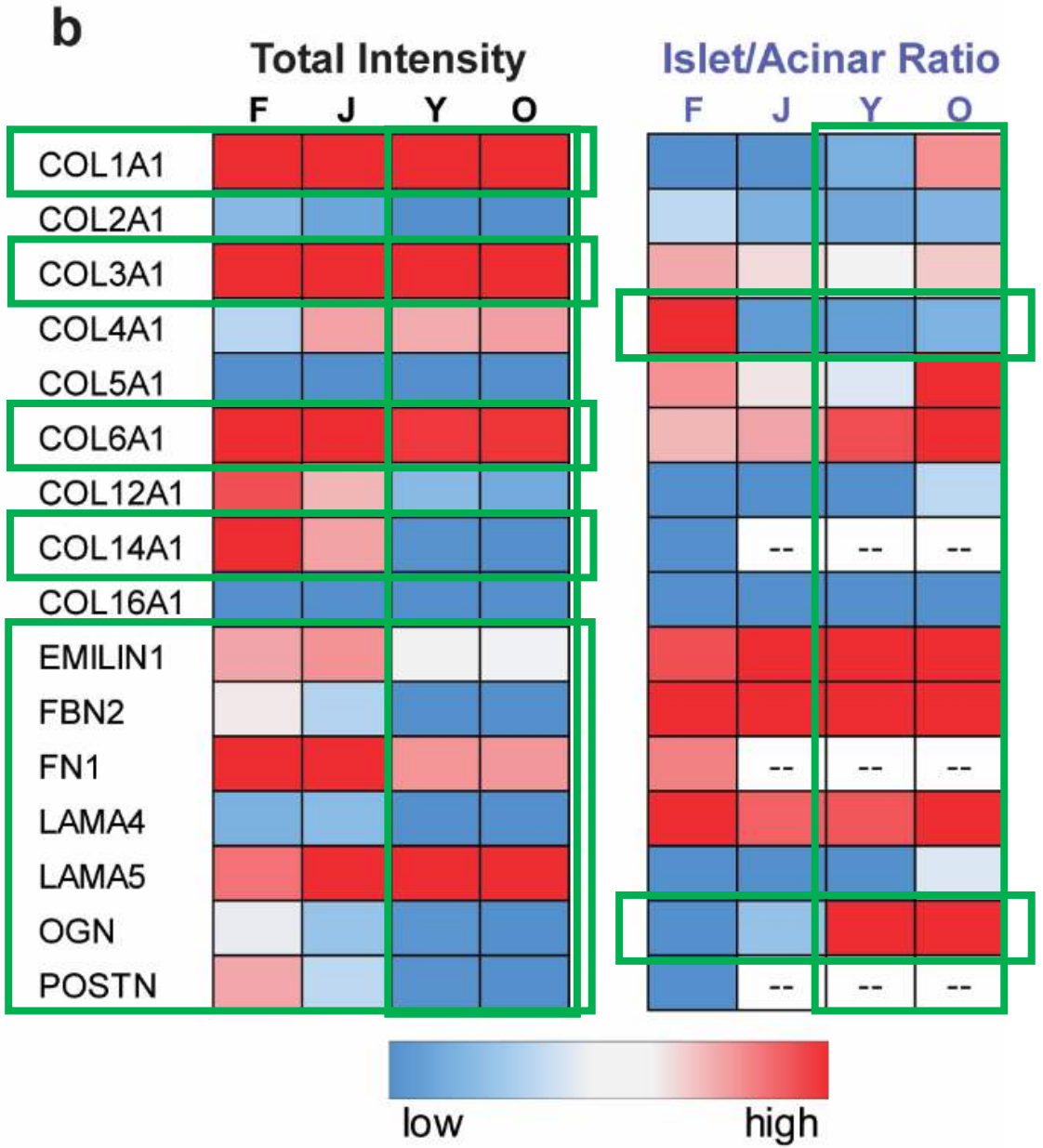
Different developmental trends with different ECM proteins



Li and Tremmel et al., *Nature Communications* 2021

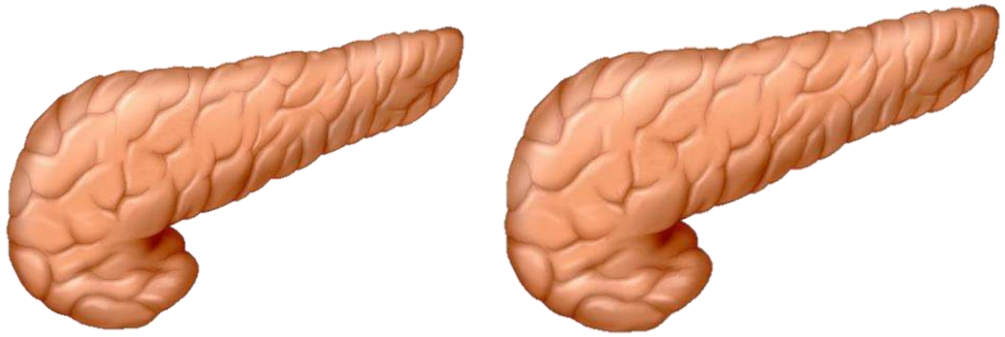
ECM proteins change in abundance and localization across developmental time points





Li and Tremmel et al., *Nature Communications* 2021

Differences between Younger and Older Adult Pancreas



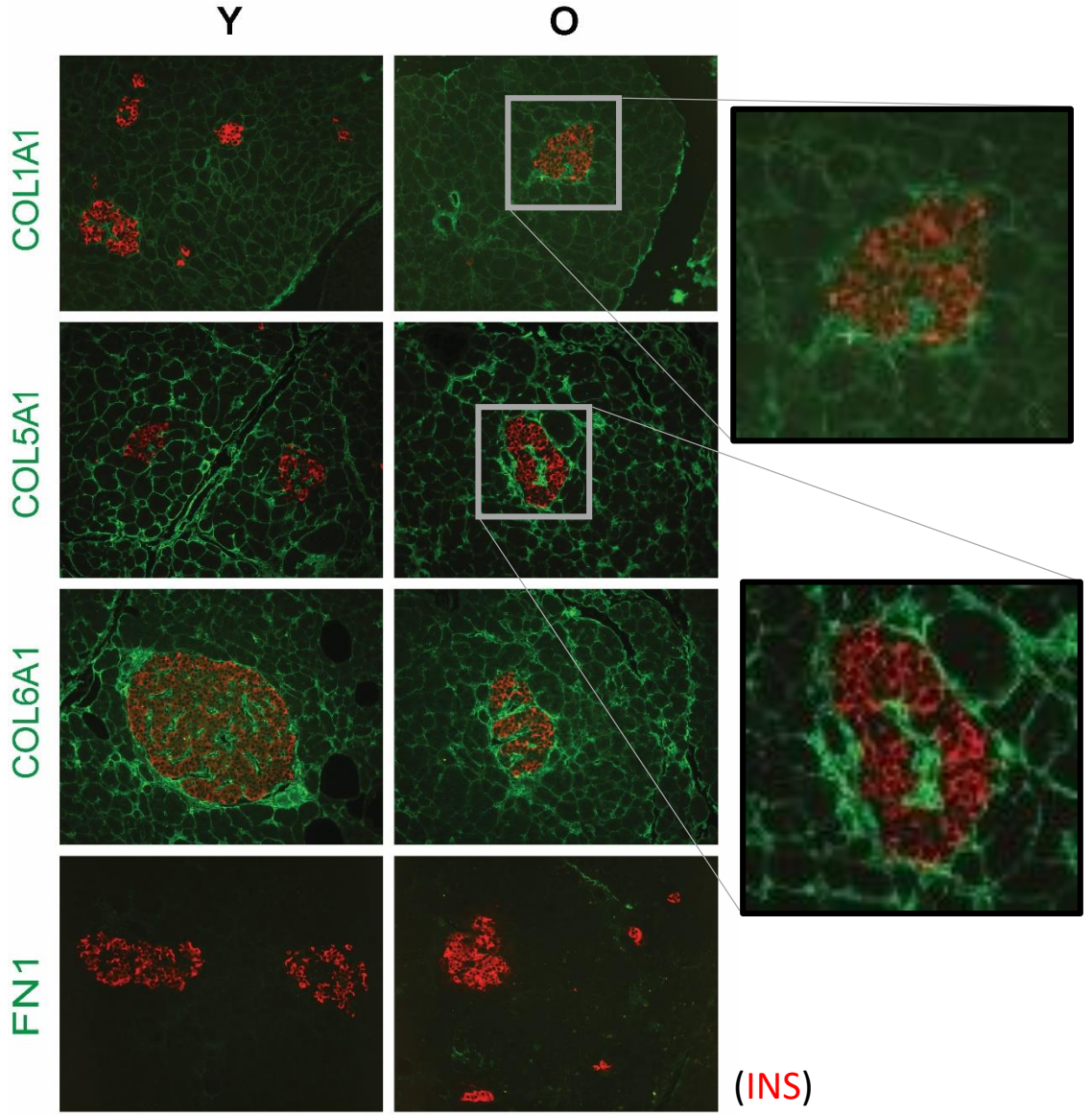
Younger Adult (**Y**)

21-30 years

Older Adult (**O**)

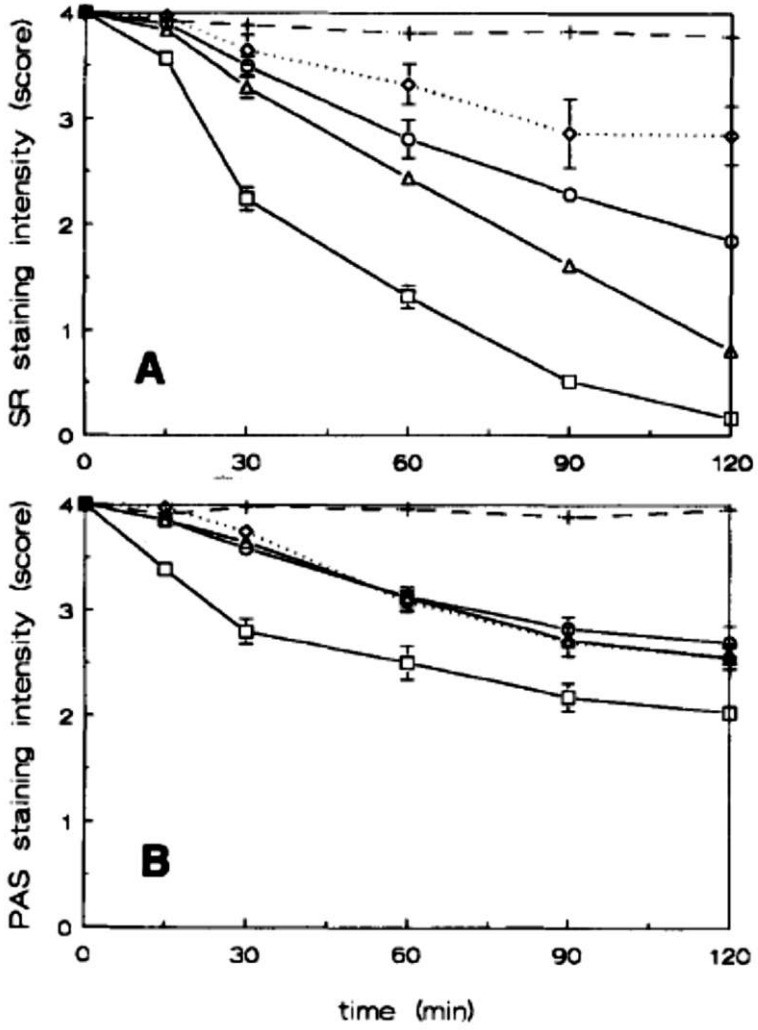
51-60 years

- **No differences in whole pancreas ECM protein content**
- **Differences in islet-enriched ECM proteins with age:**
  - Col1, Col5, Col12 LamA4, LamA5 are more enriched in/around islets in older adults
  - Col6 is enriched around both Y and O islets
  - Fibronectin is not present in adult islets



Li and Tremmel et al., *Nature Communications* 2021

An assay for measuring ECM digestion in rat pancreas



2 mg collagenase class I (o)

2 mg collagenase class II (Δ)

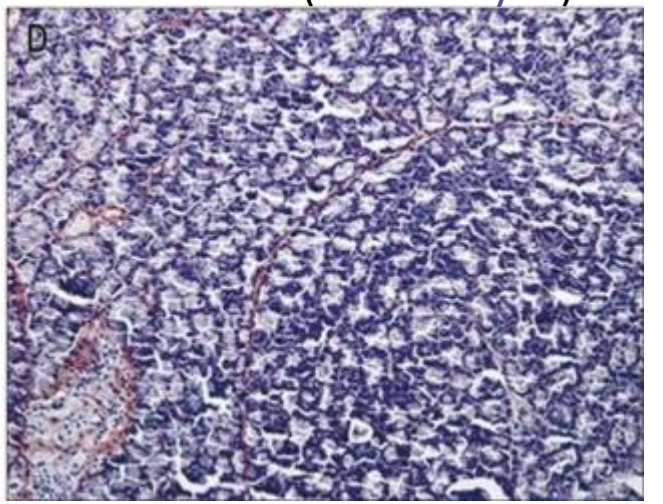
1 mg class I + 1 mg class II (•)

0.11 mg neutral protease (0, dotted line)

No enzyme (+ , broken line)

Vos-Scheperkeuter et al. Cell Transplantation 1997

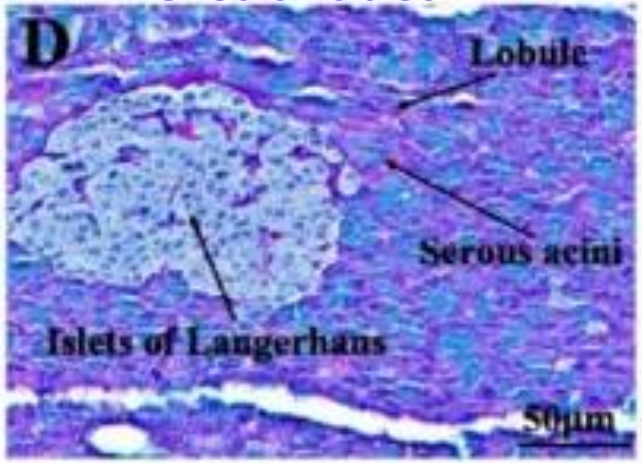
Sirius Red (Hematoxylin)



Yan and Ren Gut Liver 2012; 6(3): 381-387

Stains fibrillar collagens

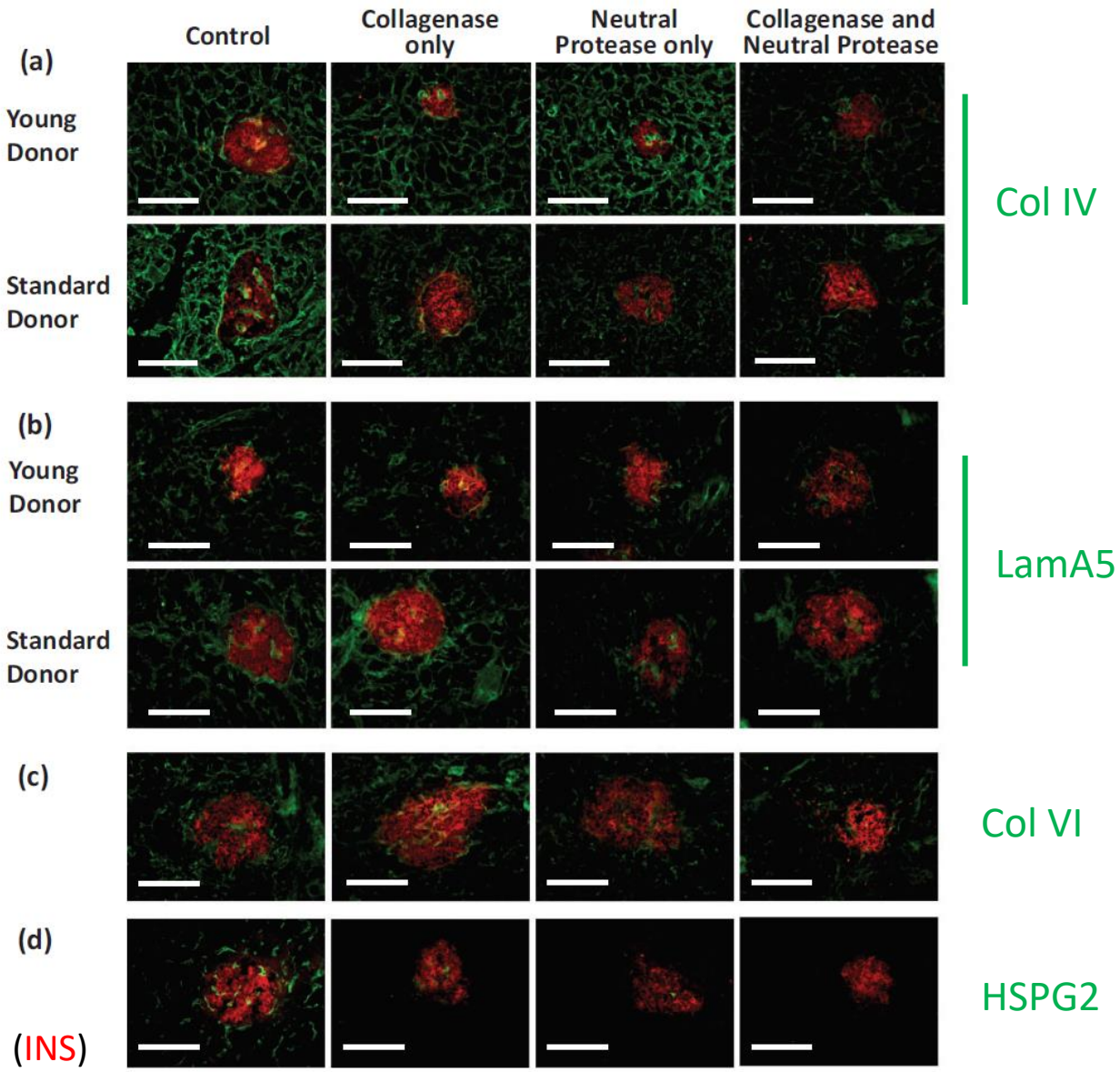
Periodic Acid Schiff



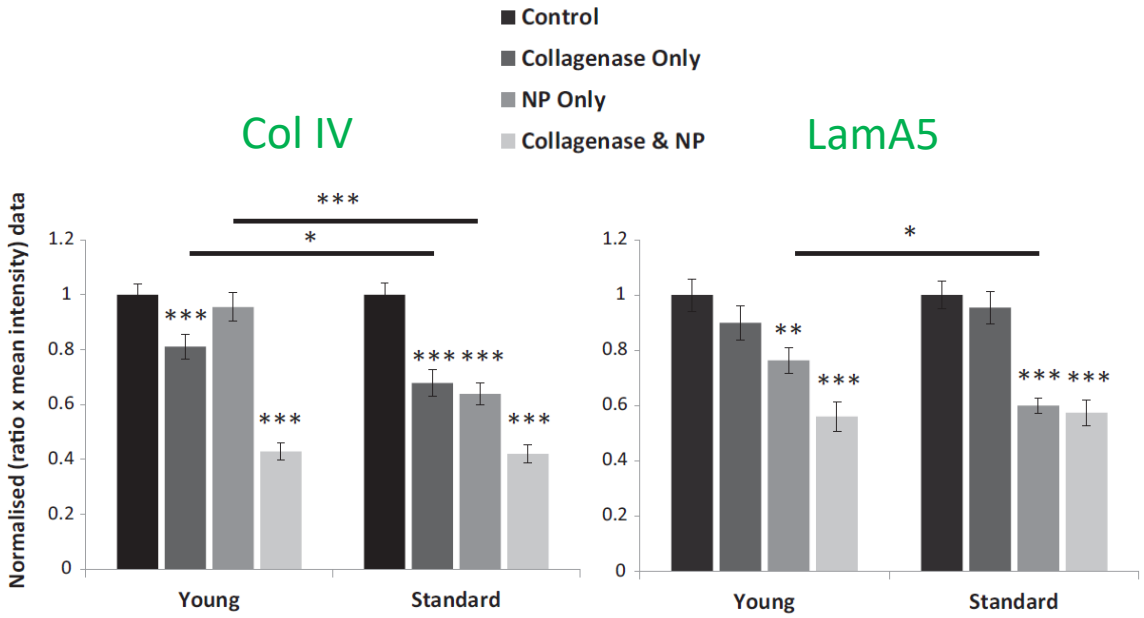
Aamir et al. 2019 PeerJ

Stains glycoproteins

An assay for measuring ECM digestion in human pancreas



Standard (age >45)
Young (age <35)

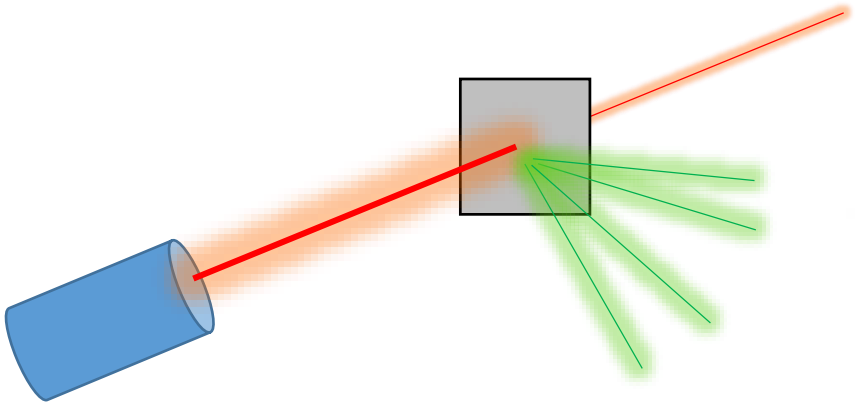


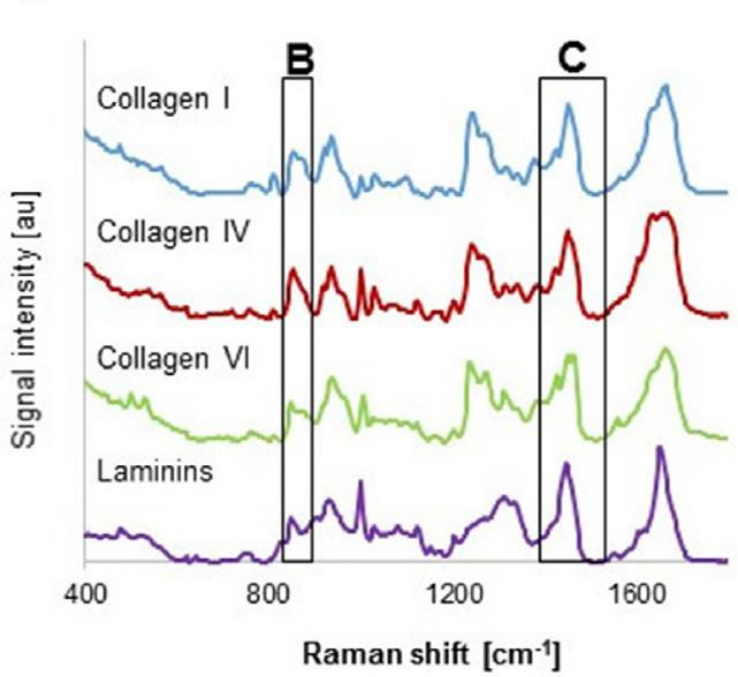
Protein digestion significantly influenced by donor age.

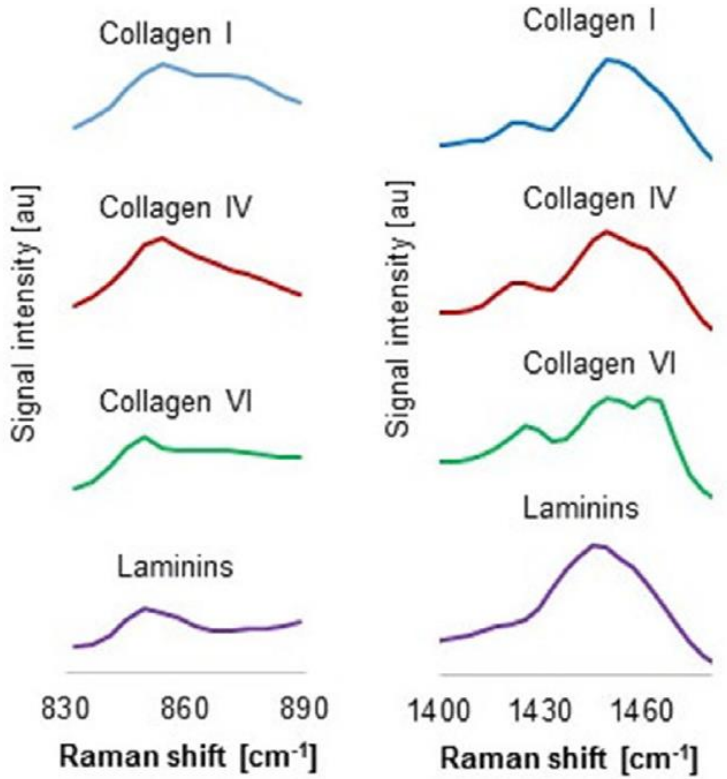
Combining the two enzymes ameliorated the age-related differences in the digestion of both proteins.

Spiers et al. 2018 Cell Transplantation

Raman microspectroscopy (RMS)





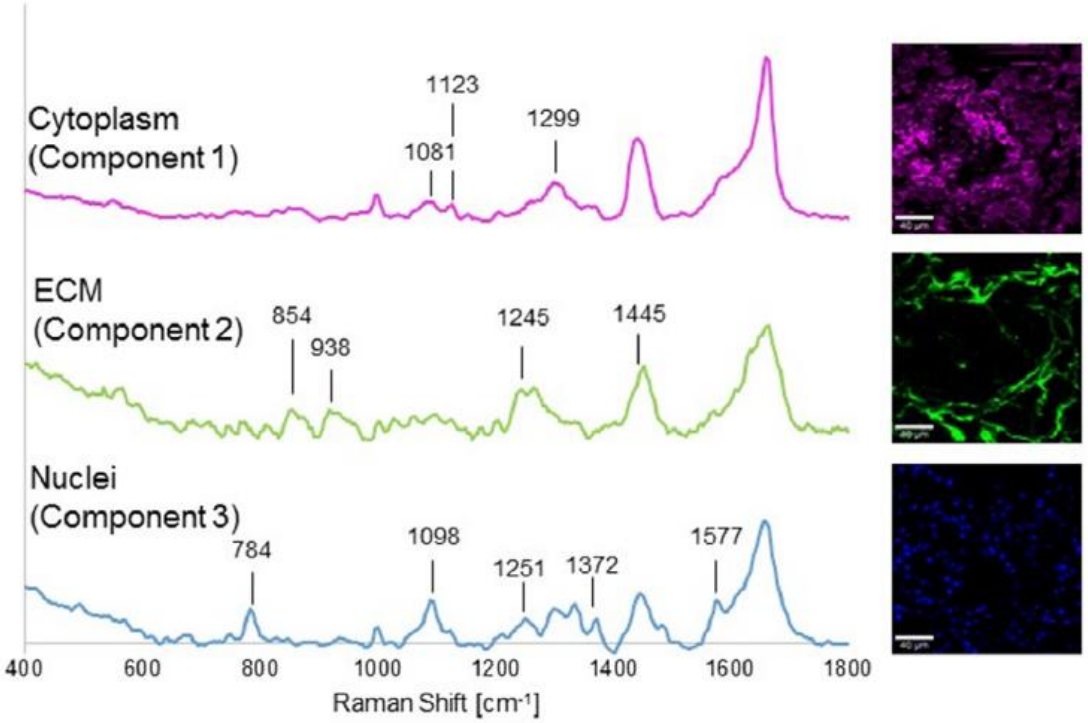


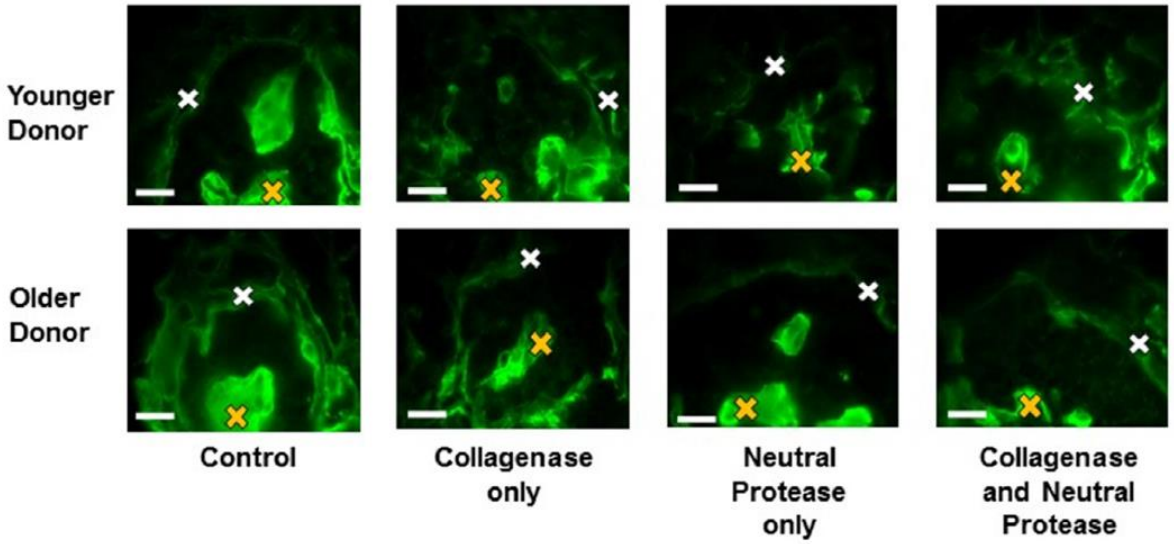
	Raman Shift [cm^{-1}]			
Molecular assignment	**Collagen I**	**Collagen IV**	**Collagen VI**	**Laminins**
Proline	858	858	854	854
C-C backbone stretch	938	938	938	938
Phenylalanine	1005	1005	1005	1005
Amide III	1242	1242	1242	-
Unassigned peak	-	-	-	1307
CH_2 deformation	1453	1449	1453, 1465	1449
Amide I	1666	1666	1666	1666

Used purified ECM proteins to establish a signature Raman shift profile

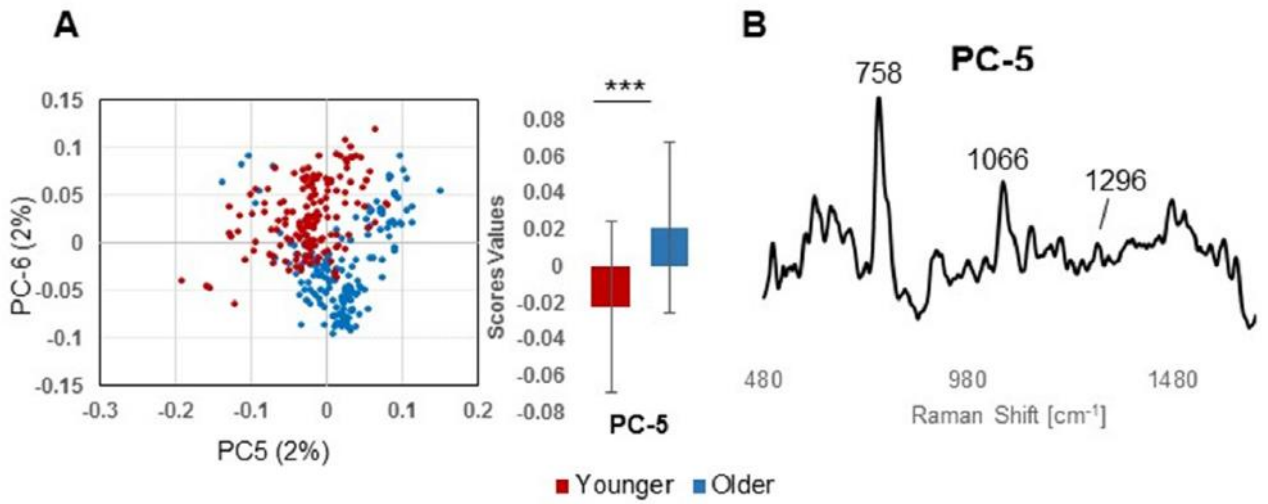
Spiers et al. 2019 Acta Biomaterialia

RMS reveals differences in ECM digestion by donor age



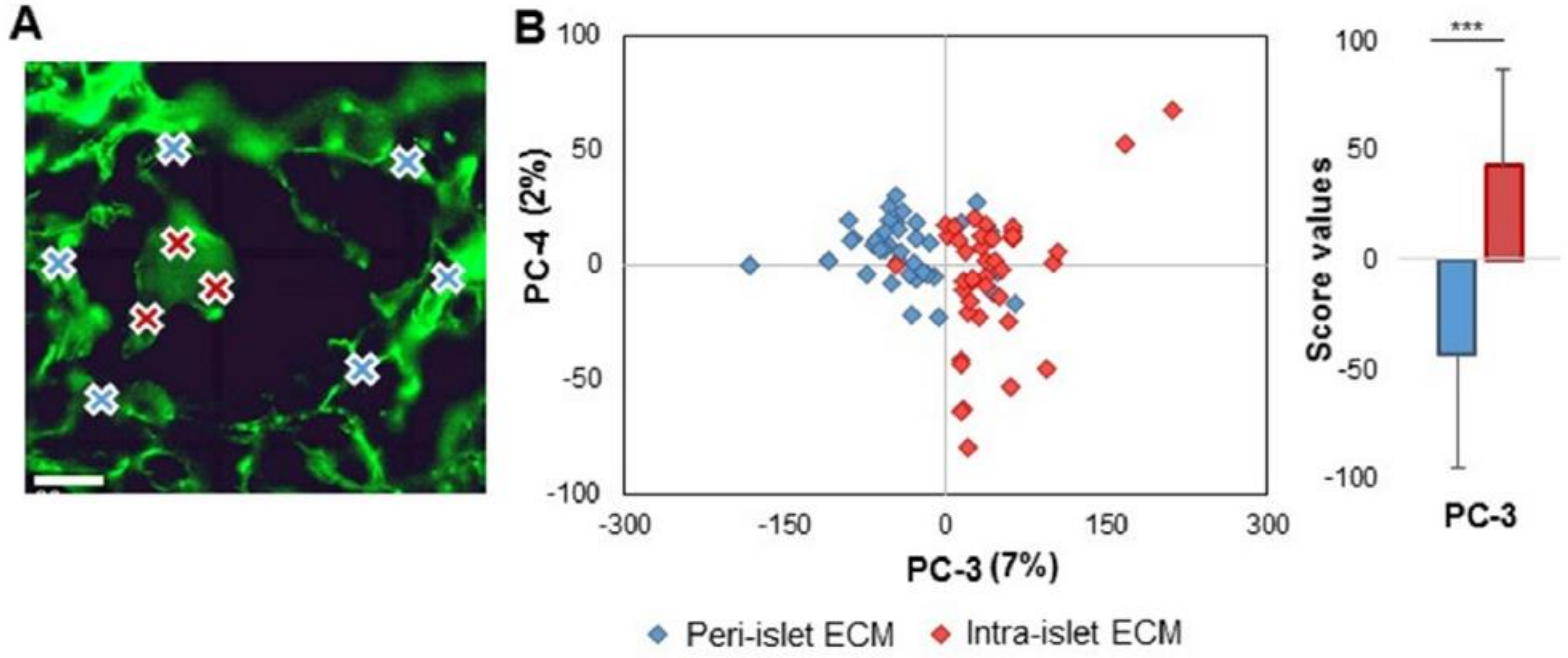


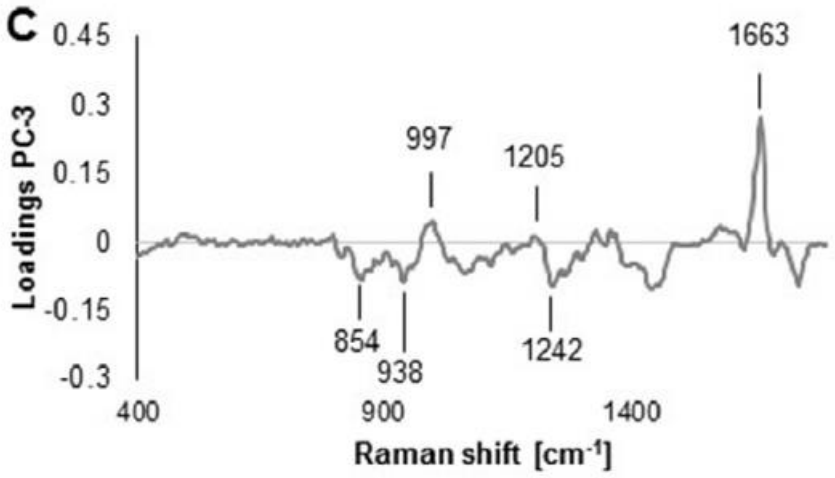
- Younger (30 years) and older (>55 years) BMI-matched donors
- RMS demonstrated that the ECM at the islet-exocrine interface was differentially digested with respect to donor age.



Spiers et al. 2019 Acta Biomaterialia

RMS detects differences in peri- and intra-islet ECM profiles





D

Raman shift [cm^{-1}]	Assignment
854	C-C proline/hydroxyproline
938	C-C backbone stretch
997	C-C, C-O
1205	amide II, hydroxy-/proline
1242	amide III (collagen)
1663	amide I (proteins, collagen)

- RMS spectra collected from within (intra-islet) and at the islet perimeter (peri-islet) cluster into different groups which are highly significantly different by PCA analysis (B)
- Collagen-related peaks are the main influencing differences between the data sets (C and D)

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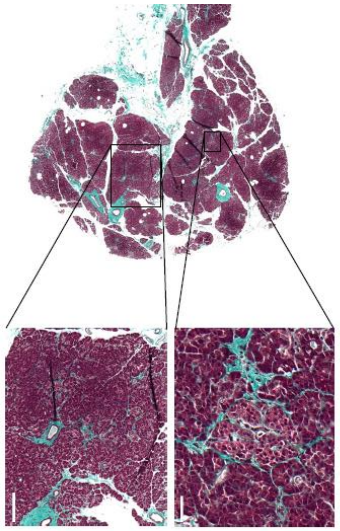
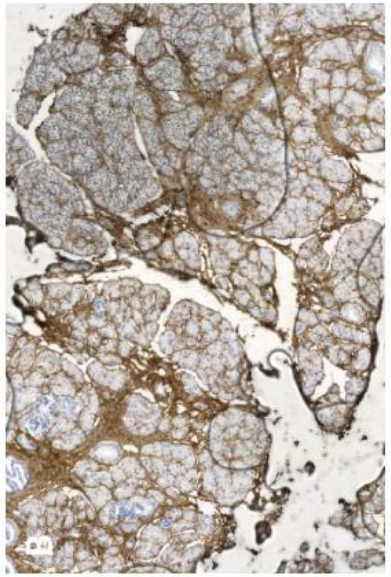
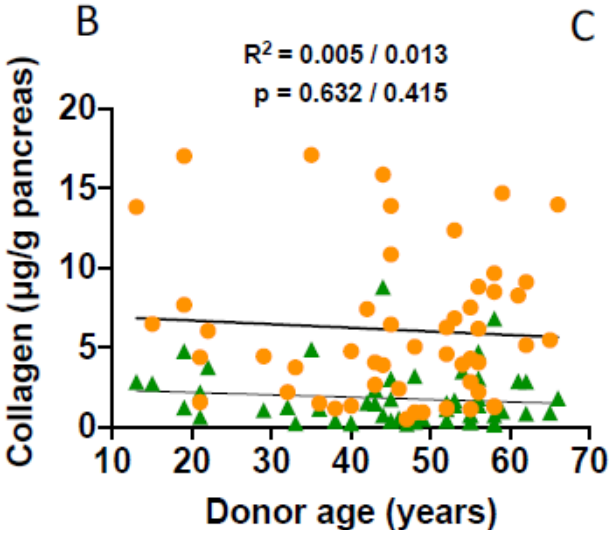
Collagen digestion is necessary for successful islet isolation

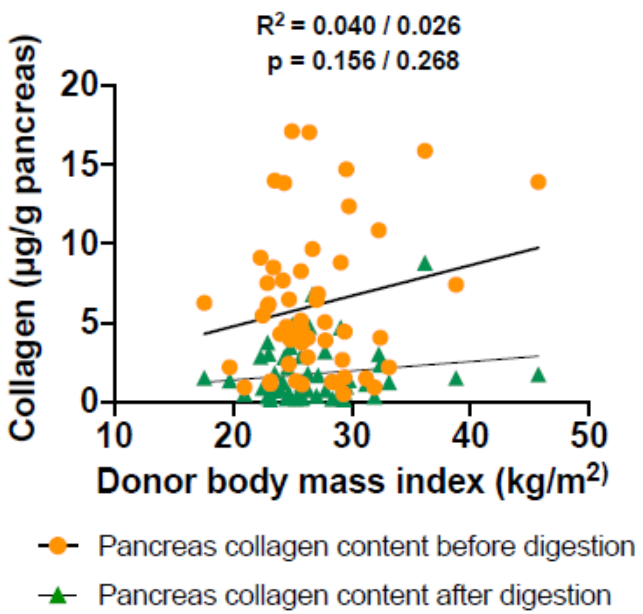
Goldner Stain

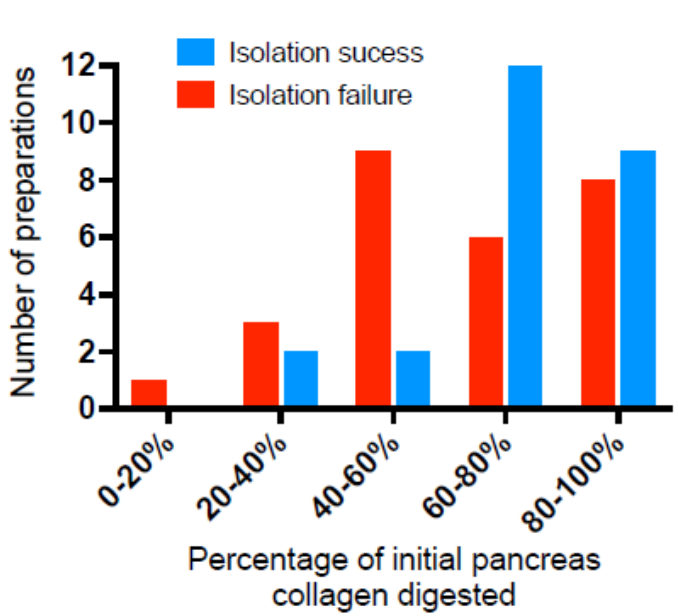


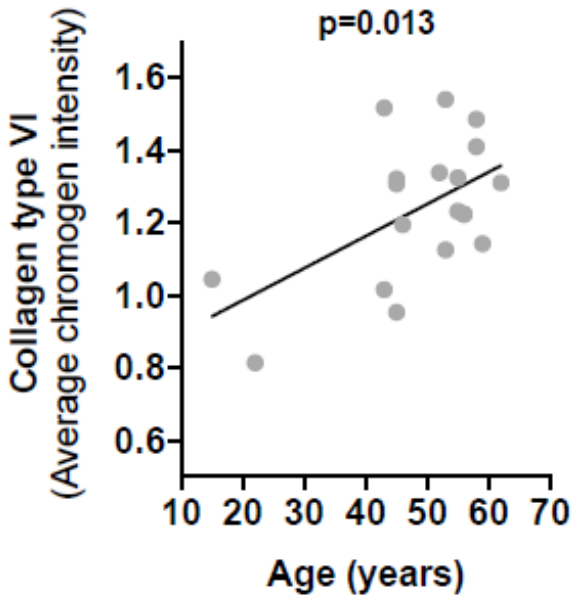
COL6





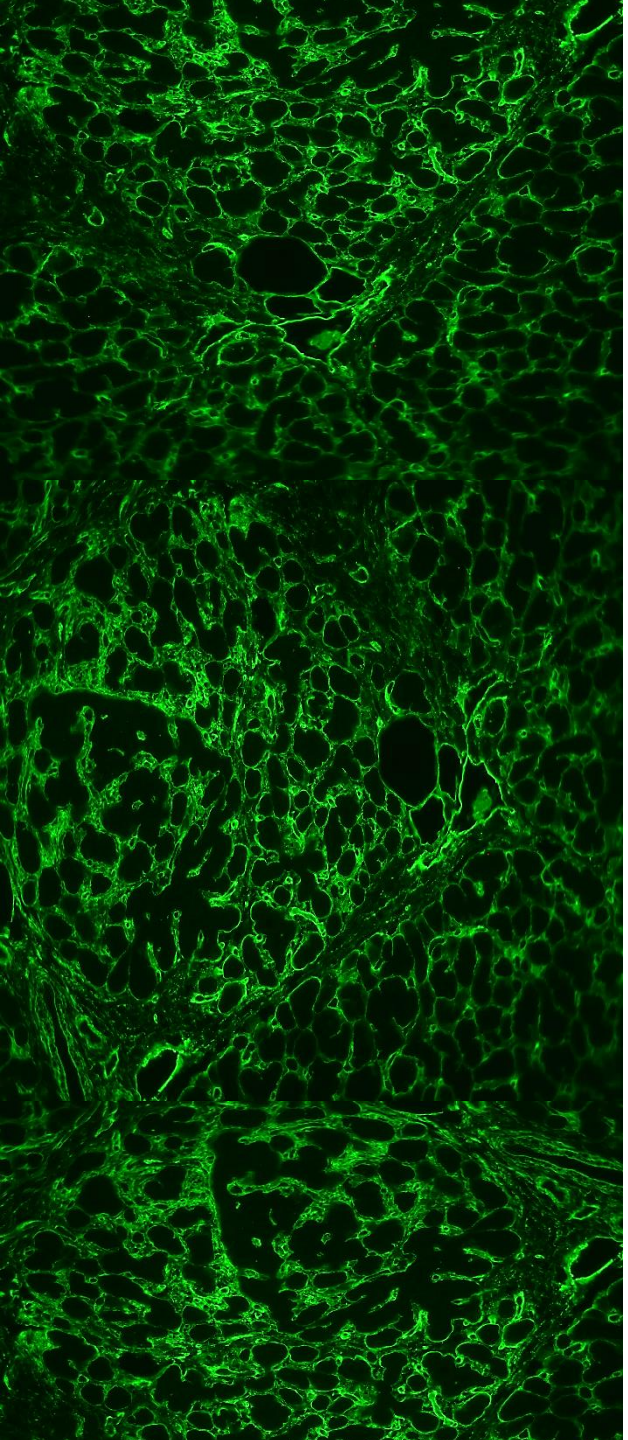






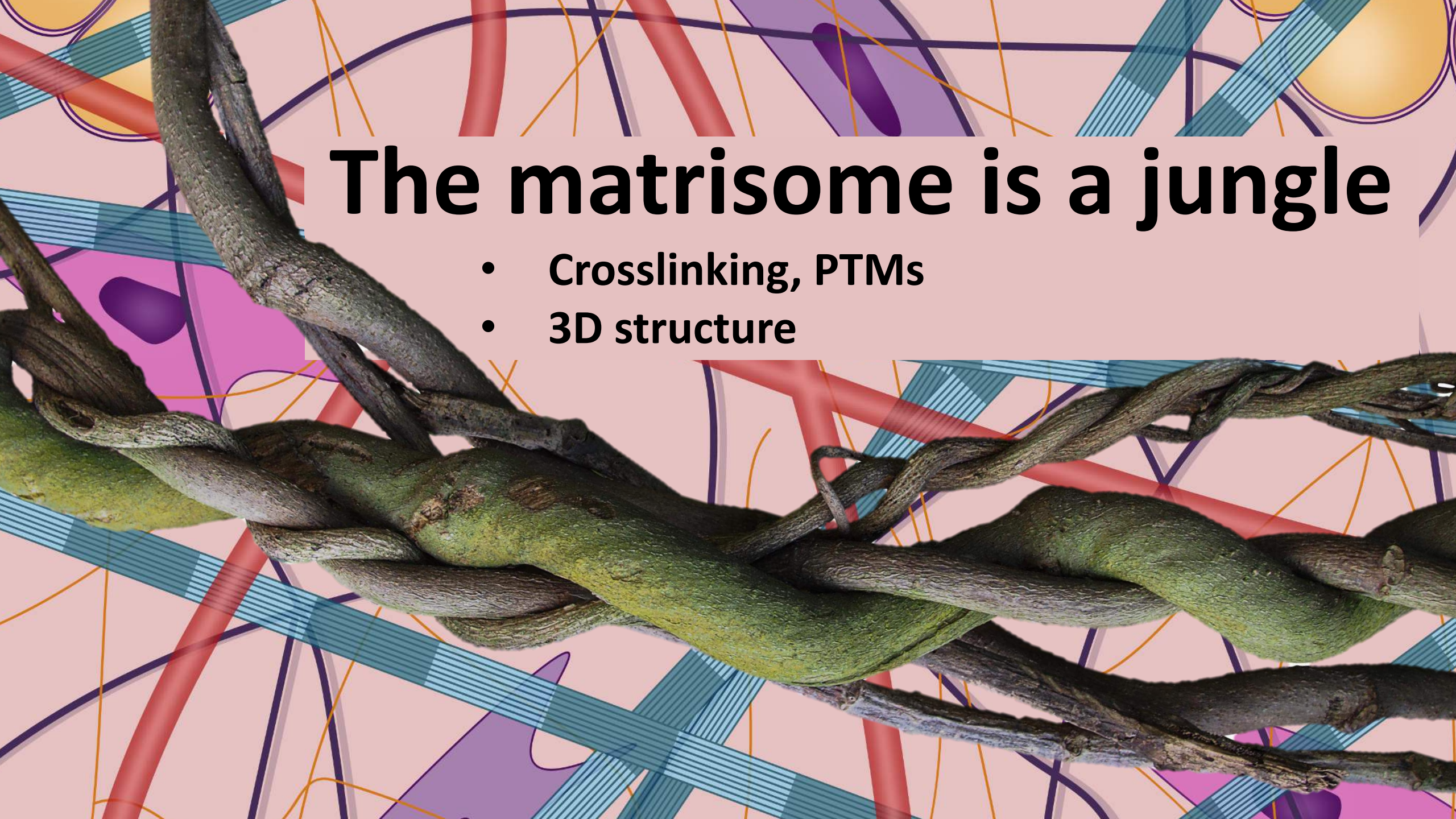
- **Collagen (I-V) content does not vary with age or BMI**
- **Successful islet isolations are more common when at least 60% of collagen is digested**
- **Collagen VI content may increase in older adults (low N at young ages though)**

Meier et al. 2020 Transplant International



Summary and Future Directions

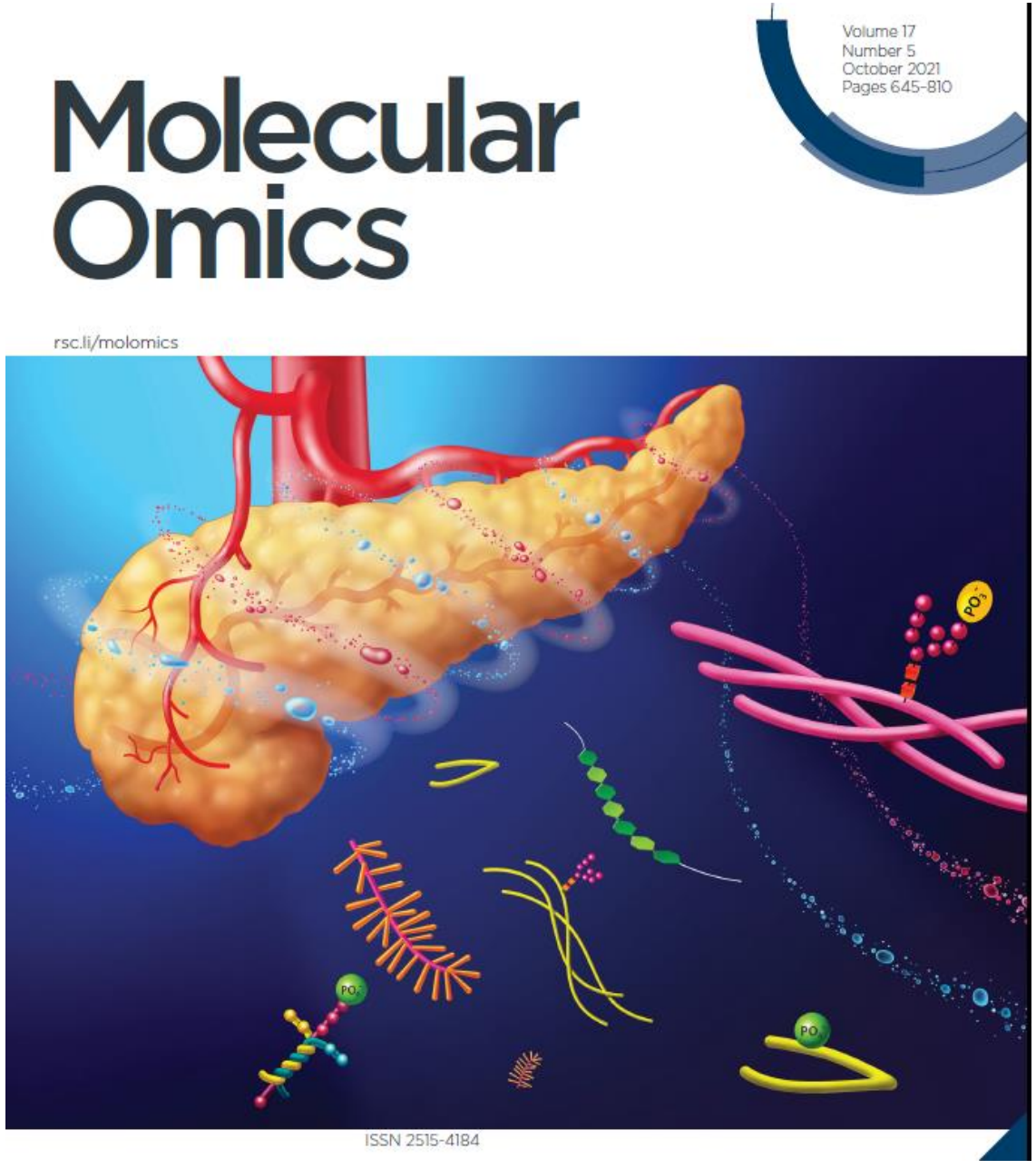
- The ECM composition of the human pancreas has only recently been explored in depth
- ECM changes dramatically throughout pancreas development, but in adulthood no single ECM protein has significantly different abundance in older or younger adults
- Based on immunofluorescent staining and enzyme-digestion assays, the ECM at the islet-acinar interface appears to differ between older and younger donors
  - Col6 abundant in both, but Col1 and Col5 are enriched in older adult
- Further studies are needed to quantify these localized differences in islet ECM proteins based on age and disease-state
  - What other donor variables may affect islet ECM? Deeper studies may be necessary to define donor parameters for improved islet isolation

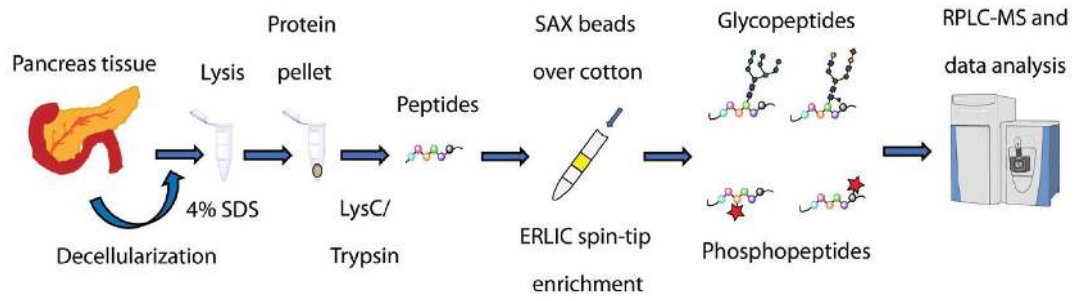


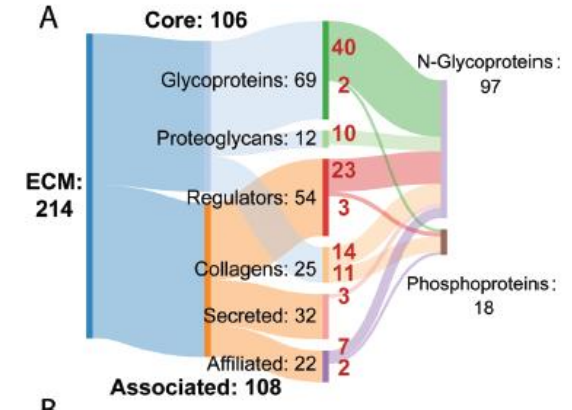
The matrisome is a jungle

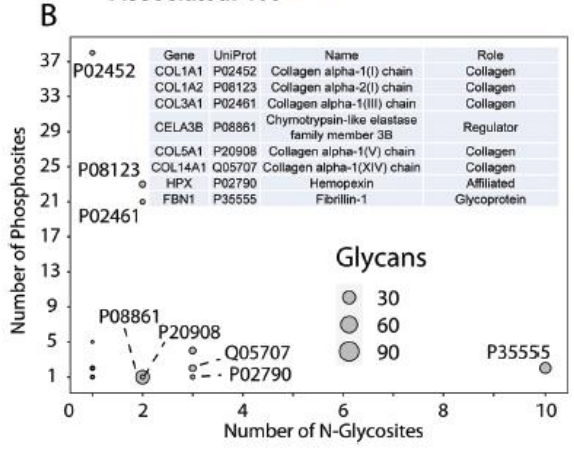
- Crosslinking, PTMs
- 3D structure

Human Pancreas ECM Post-Translational Modifications (PTMs)









Tabang *et al.*, Mol. Omics, 2021, 17, 652–664