Single-cell multiomics: from reference atlases to human diseases

Human Cell Atlas Latin America

October, 7th 2022

Elisabetta Mereu, PhD
Group Leader @ Cellular Systems Genomics Lab
Josep Carreras Leukemia Research Institute (IJC)
Badalona, Spain
Email:emereu@carrerasresearch.org
Web: https://mereulab.org

Josep Carreras
LEUKAEMIA
Research Institute

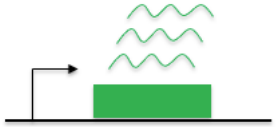
@eli_mereu

https://github.com/mereulab

Characterizing complex tissues at molecular and cellular level

Molecular and spatial reference maps of normal tissues

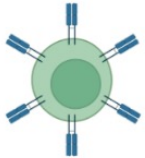
scRNA-seq



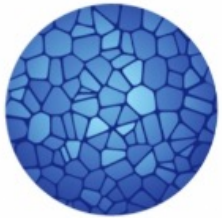
scATAC-seq

Spatial Transcriptomics

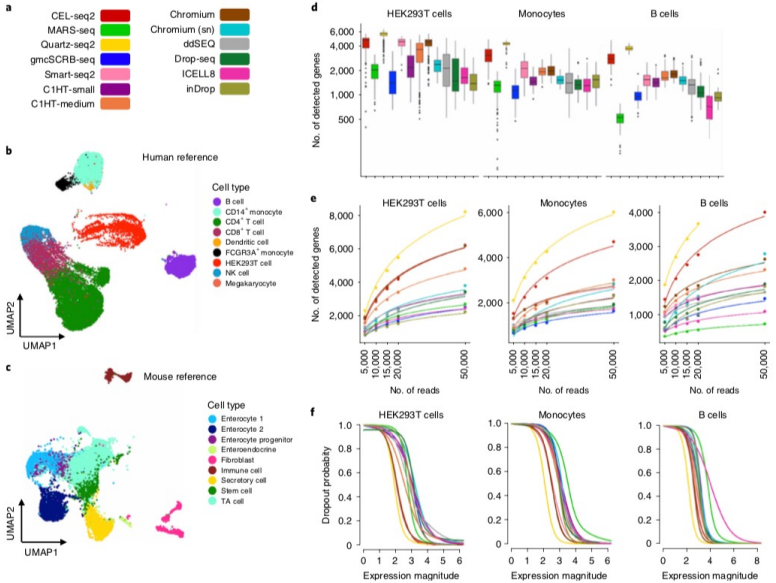
T Cell Receptors enrichment



HUMAN CELL ATLAS



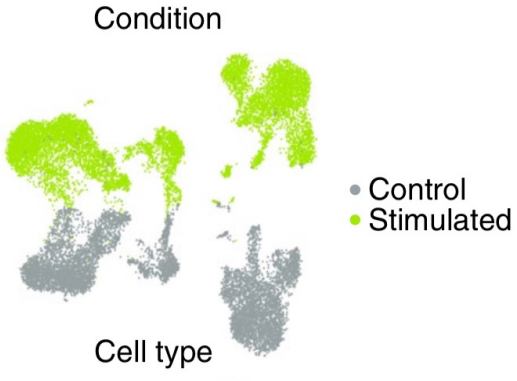
Technology Benchmarking



Mereu, Nat. Biotech (2020)

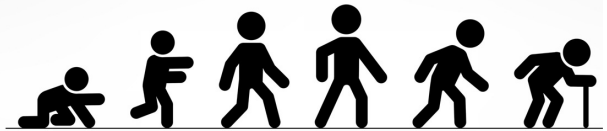
Targeting inflammation in ageing and human diseases

Perturbed/Diseased states

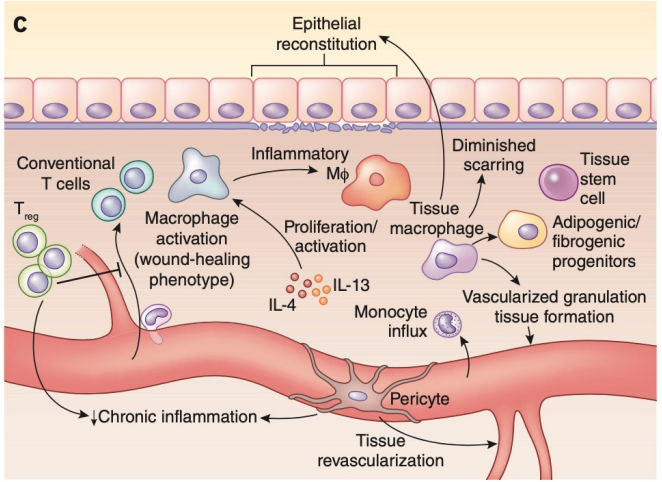


Adapted from scGEN, Lotfollahi et al, Nat. Methods. 2020

Ageing

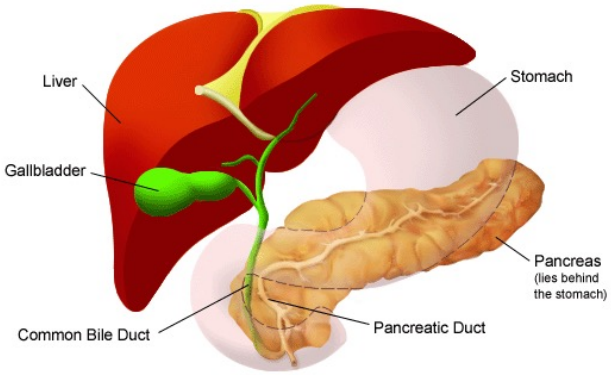


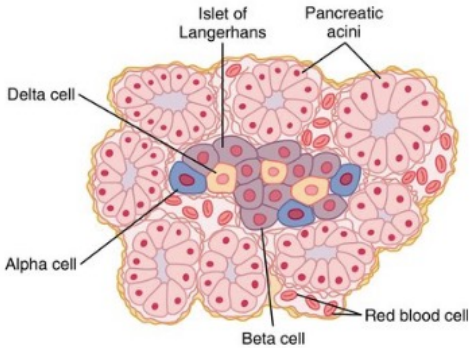
Tissue-level inflammation and regeneration



Forbes & Rosenthal, Nat. Medicine (2014)

The Human Pancreas Atlas: why is it important?





- The pancreas is a vital organ consisting of:
  - *Exocrine: 95% of cells*
  - *Endocrine: 5% of cells*

- Dual function:
  - the secretion of enzymes for the digestive system.
  - the regulation of several hormones (e.g. insulin)

- Several human diseases are associated with the pancreas, including:
  - *Pancreatic Adenocarcinoma*
  - *Diabetes Mellitus*

- Difficult to study due to its high autolytic activity, resulting in the rapid degradation of cells upon pancreatic resection.

1. The ESPACE consortium is the European HCA Pancreas initiative

cnag

CRG Centre for Genomic Regulation

Steinbeis-Transferzentrum Health Data

LINQ. management GmbH

Technical University of Munich

I.R.C.C.S. Ospedale San Raffaele
Gruppo San Donato

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

HADASSAH UNIVERSITY MEDICAL CENTER
Founded by Hadassah, the Women's Zionist Organization of America

Leiden University Medical Center

Josep Carreras LEUKAEMIA Research Institute

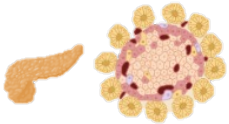
espace
THE HCA PANCREAS INITIATIVE

Diabetes Research Institute

Hubrecht Institute
Developmental Biology and Stem Cell Research

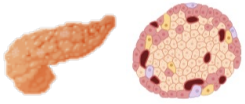
A single-cell multiomics atlas of the Human Pancreas

ESPACE- Comprehensive molecular characterization of the Human Pancreas



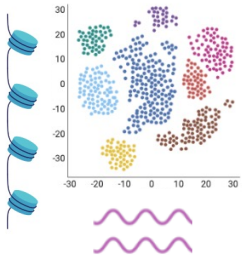
Fetal samples
(4-13 wpc)

Adult samples
Focus on Exocrine cells

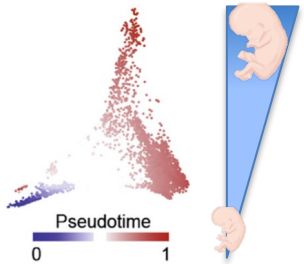


Diseased samples
Focus on endocrine cells

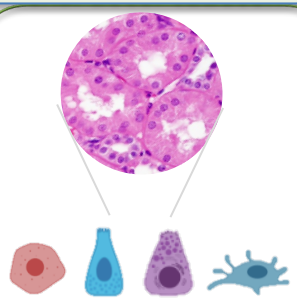
Transcriptomics/Chromatin accessibility



Cell-type Development



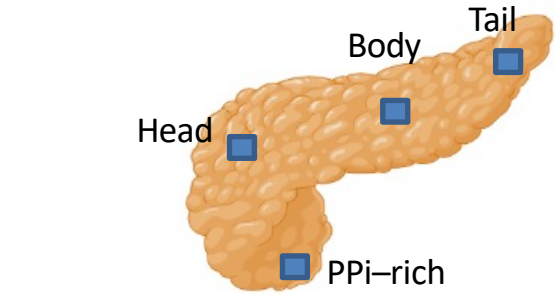
Micropathology characterization



Our deep characterization includes:

- snRNA-seq
- snATAC-seq
- VASA-seq – full length
- Spatial proteomics by CODEX and ISS

The transcriptomic and epigenomic human adult pancreas atlas

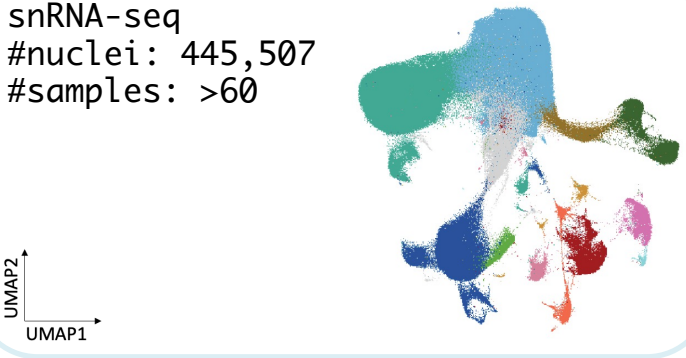


snRNA-seq snATAC-seq

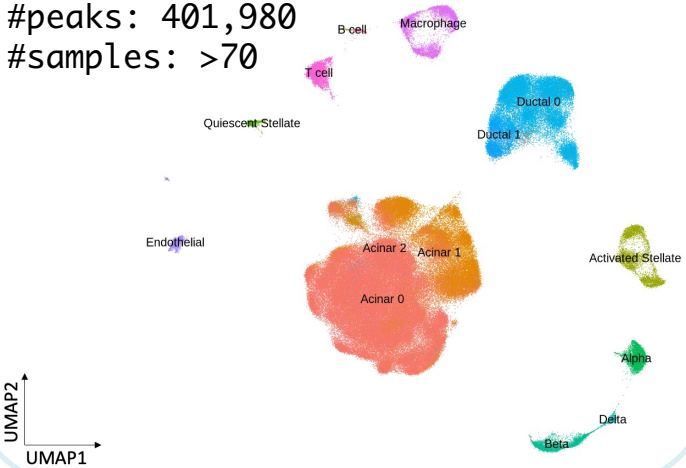
Cell types

- Acinar–s
- Acinar–i
- Ductal
- Undefined
- Fibroblasts
- Alpha
- Beta
- Endothelial
- Macrophage
- Immune
- Ductal_MUC5+
- Lymphocytes
- Lymphatic Endothelium
- Schwann
- Delta

snRNA-seq
#nuclei: 445,507
#samples: >60



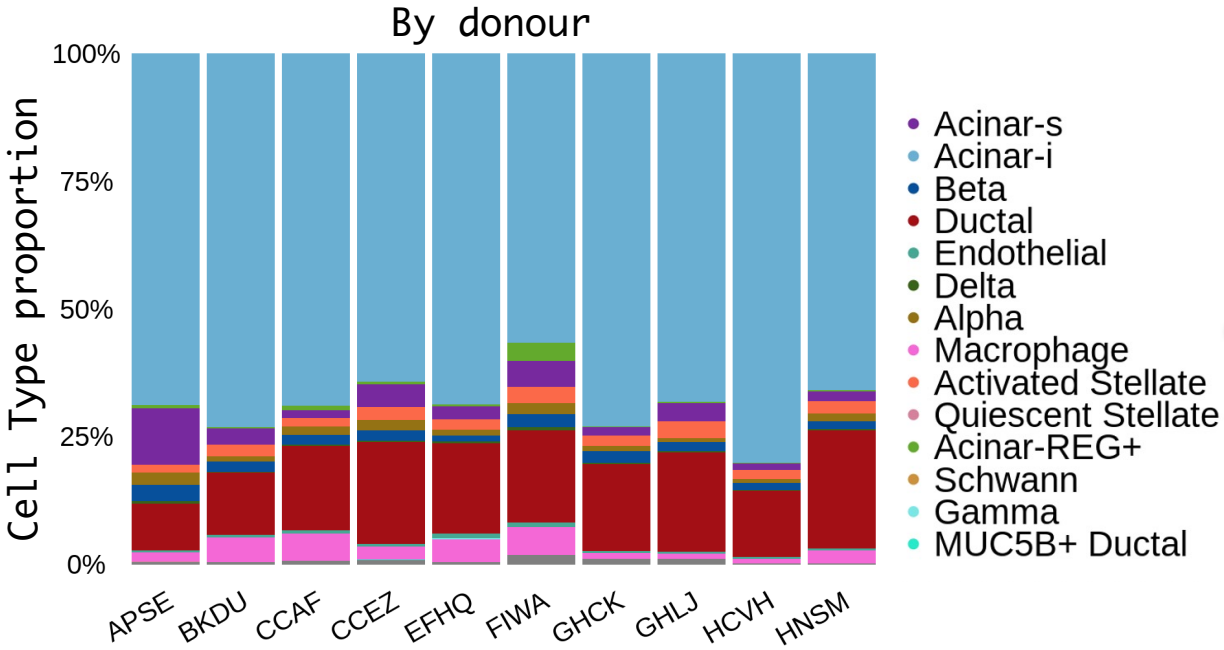
snATAC-seq:
#nuclei: 211,481
#peaks: 401,980
#samples: >70

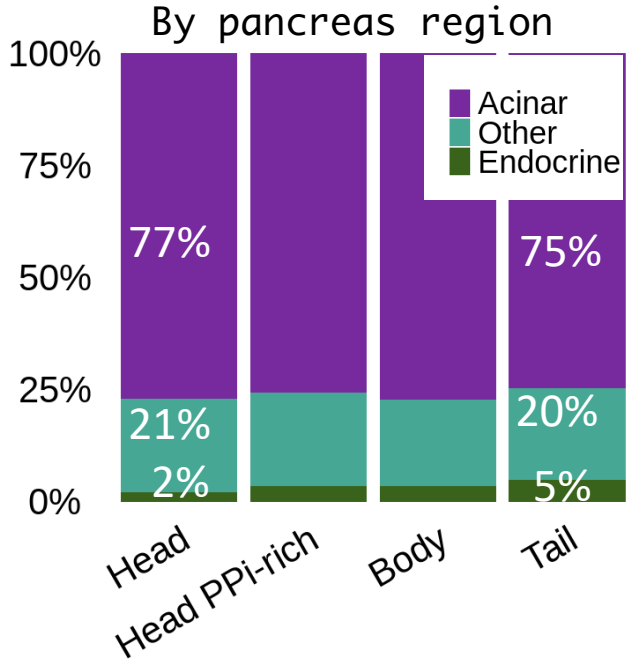


Josep Carreras LEUKAEMIA Research Institute

esoace THE HCA PANCREAS INITIATIVE

Cell-type composition of the healthy human pancreas (snATAC data)





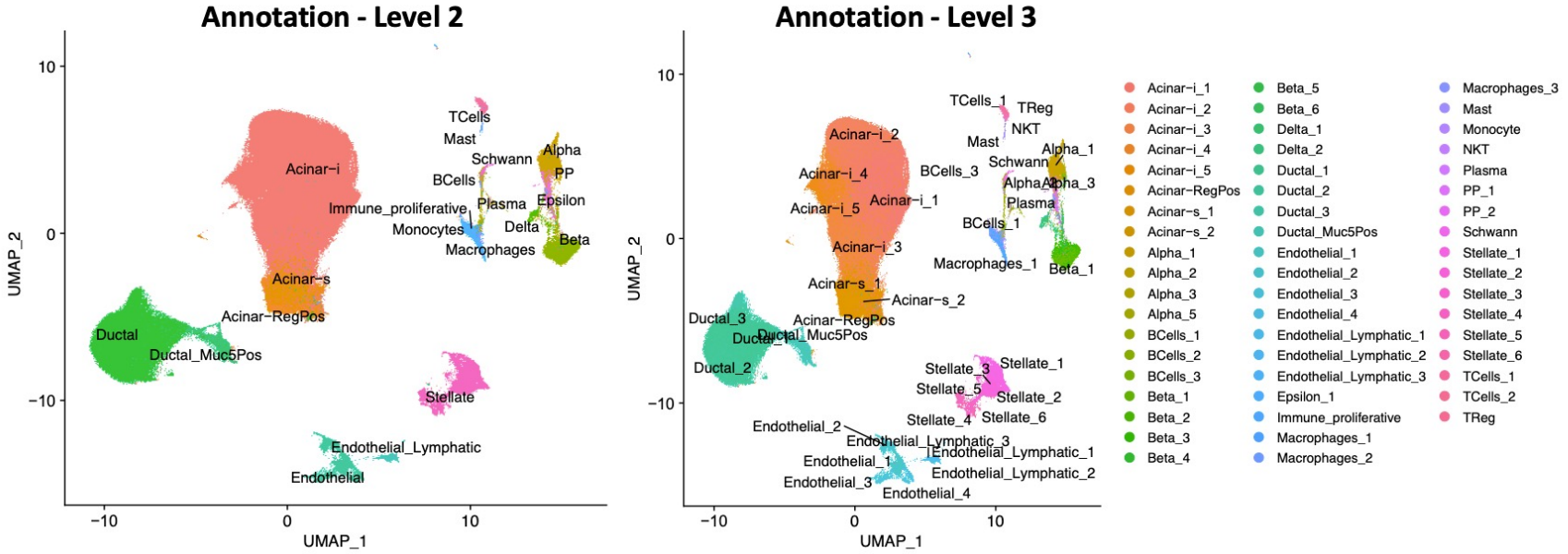
Josep Carreras
LEUKAEMIA
Research Institute

Roadmap towards a Human Pancreas Atlas

1. Reads quality checks and mapping to generate the gene/peak count matrix
2. Characterize, merge and filter peaks in snATAC-seq
3. Filtering of low-quality cells
4. Integrating samples
5. Ambient RNA removal
6. Technical doublet removal in both RNA/ATAC
7. Consensus cell-type annotations
8. …..Downstream analysis ..

1. Generating a comprehensive cell-type reference dataset

Total: 445.507 cells – 16 donors – 64 samples



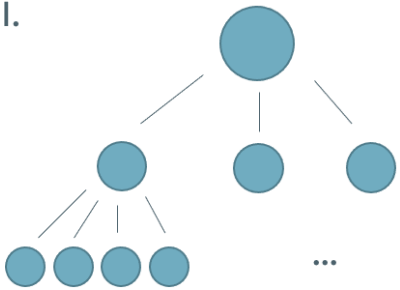
Josep Carreras
LEUKAEMIA
Research Institute

Identification and characterization of pancreatic subpopulations

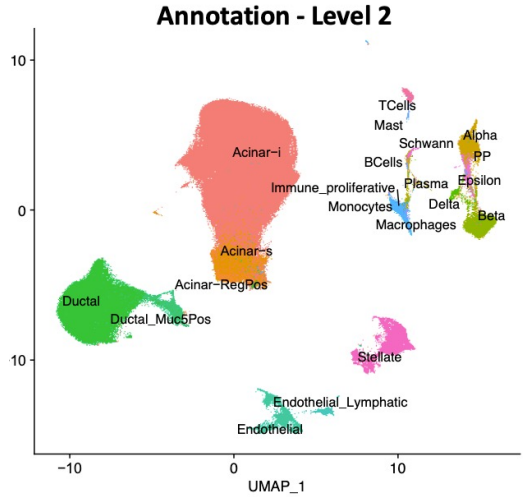
Total: 445.507 cells – 16 donors – 64 samples

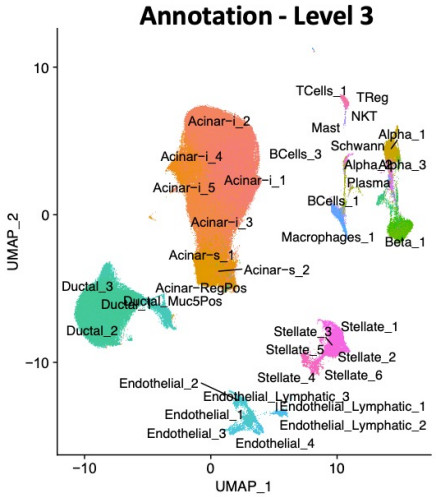
Clustering Strategy

I.



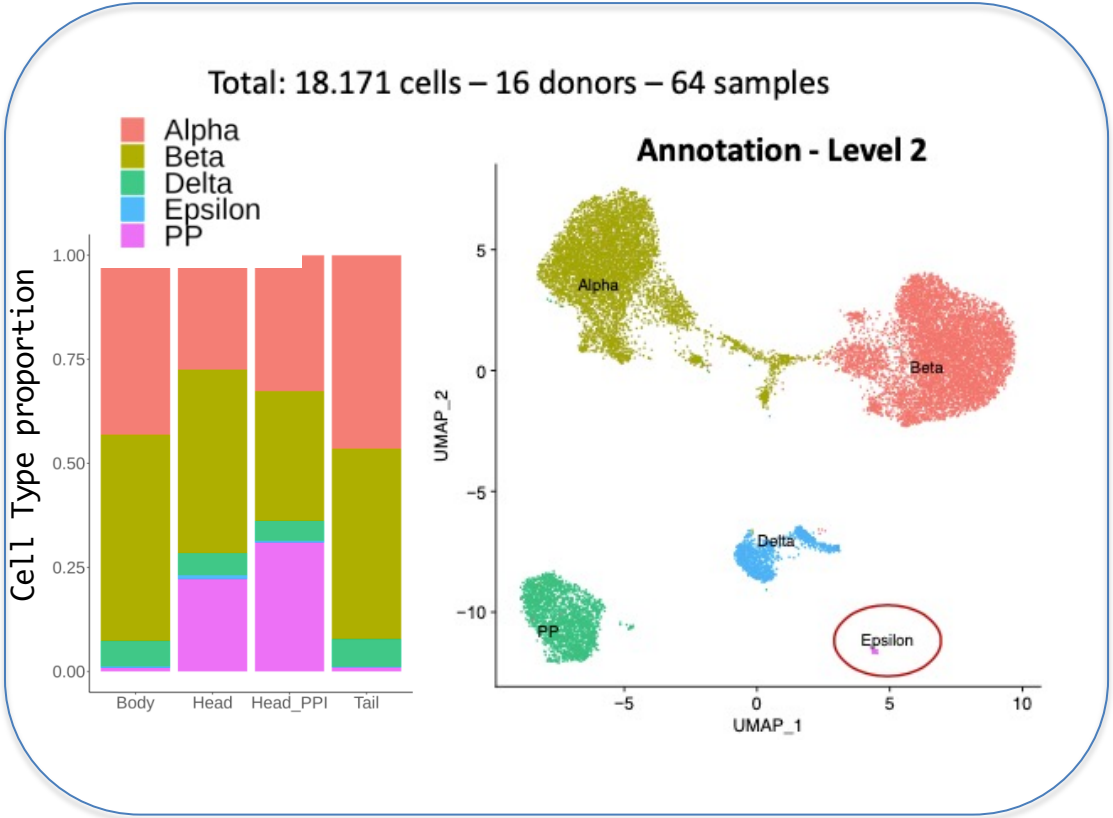
- Integrate once
- Subset and recalculate clusters



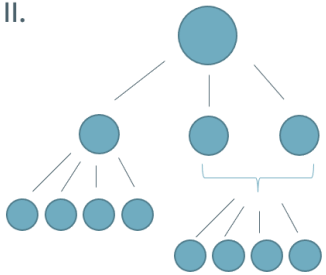


Josep Carreras LEUKAEMIA Research Institute

Identification and characterization of pancreatic subpopulations – Endocrine cell types



II.

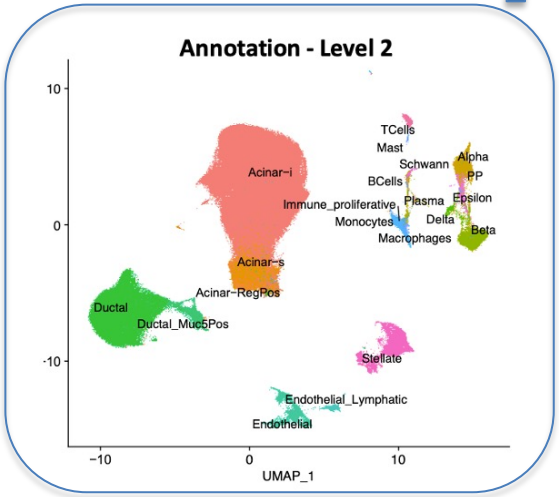


- Integrate at each step
- Subset and recalculate integration and clusters

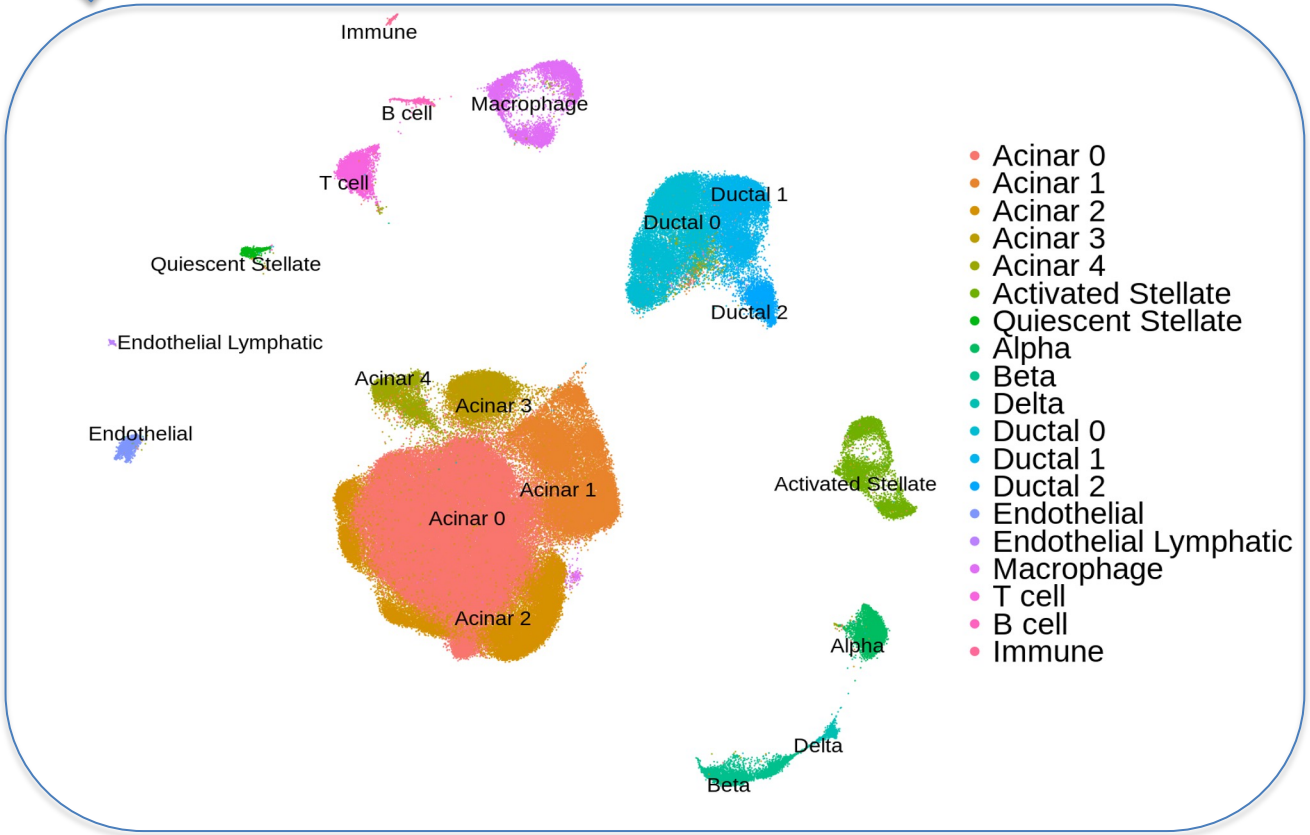
Josep Carreras LEUKAEMIA Research Institute

2. Integration with scATAC-data throught label transferring

snRNA



snATAC



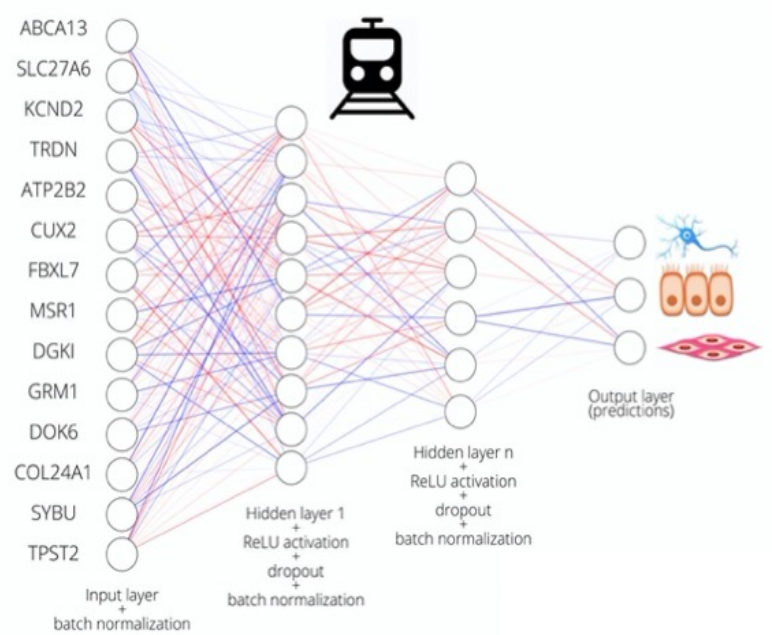
Josep Carreras LEUKAEMIA Research Institute

Harmonizing cell annotations across samples, regions and modalities

deepSCore

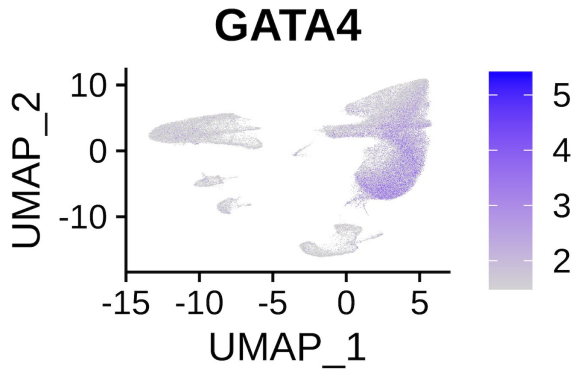
- User-friendly and fast
- Multi-language (R/Python)
- Multi-modal count data

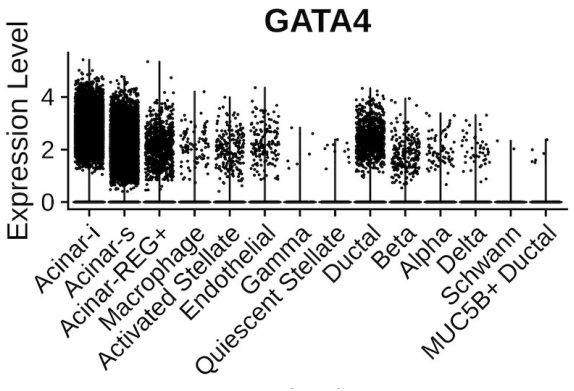
the_model



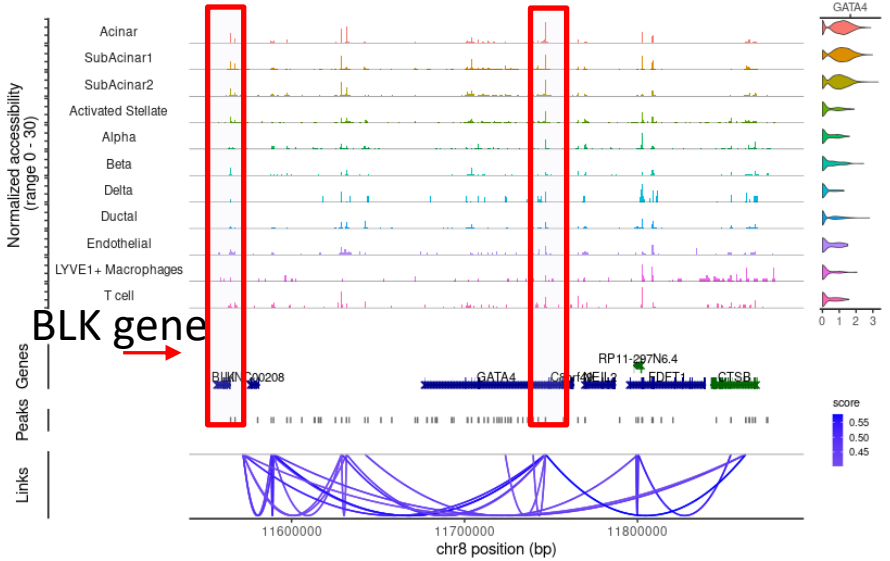
/Fig4. **Multi-language metro map of the main different platforms and packages that DeepScore integrates.**

Linking regulatory DNA elements to their target genes by co-accessibility networks



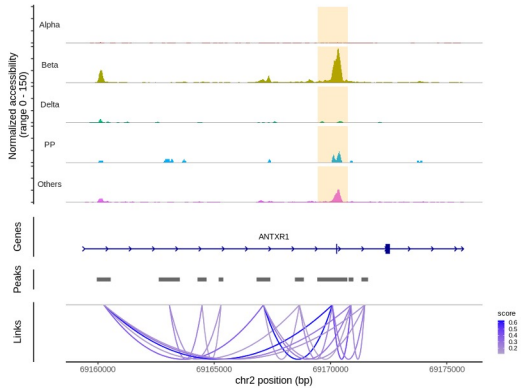


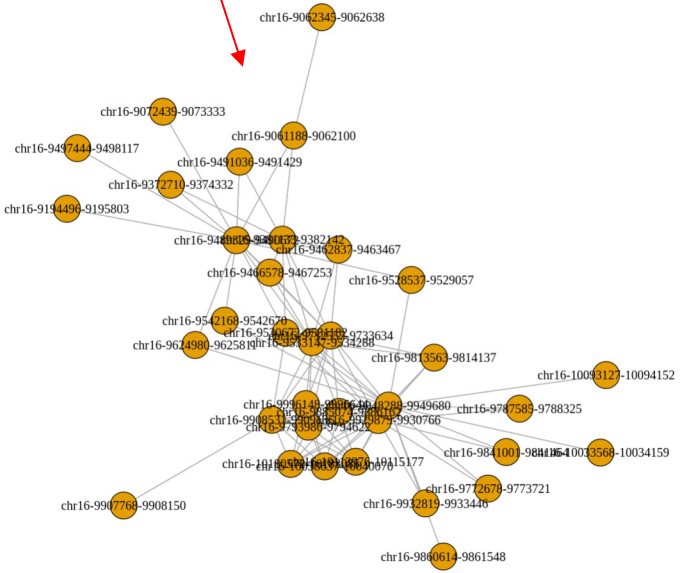
GATA4 (Acinar development)

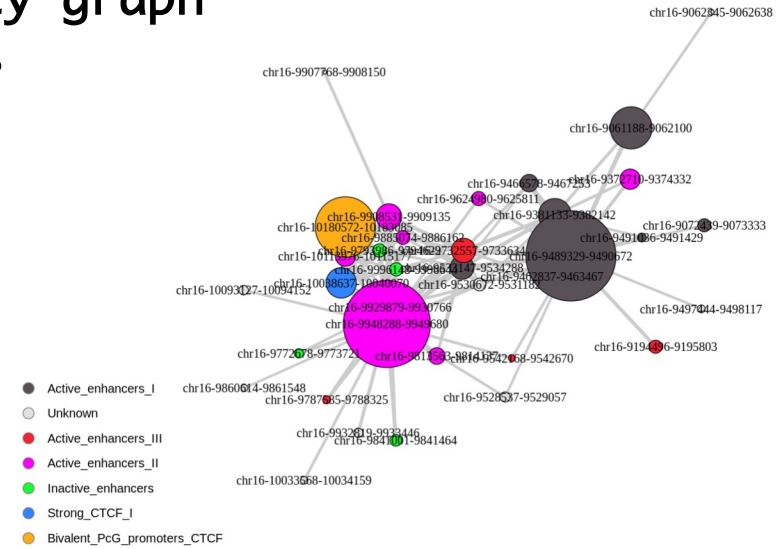


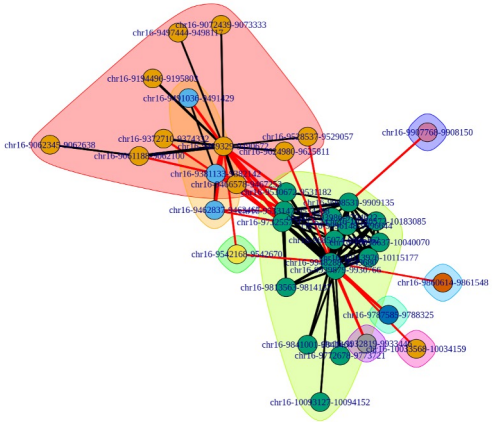
BLK: The gene encodes a protein that stimulates insulin synthesis and secretion in response to glucose and enhances the expression of several beta-cell transcription factors.

Co-accessibility graph networks





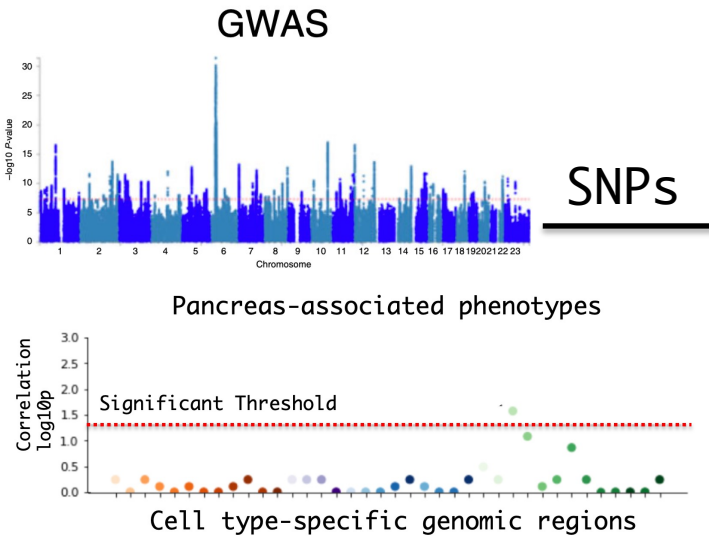


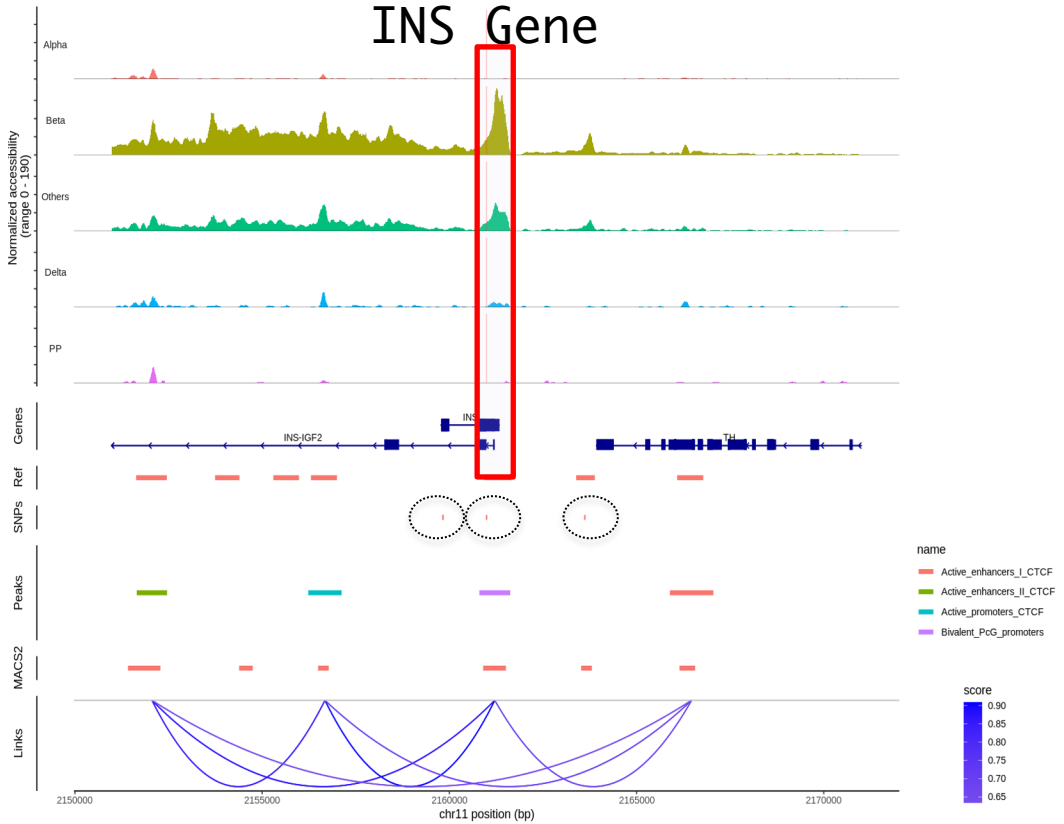


- Identification of clusters of co-accessible peaks
- Definition of graph metrics (e.g. centrality, betweenness, closenness, etc..)

Integrating GWAS signals with peaks to obtain genetic mapping of cell-type specificity for complex traits and diseases

Many risk variants for common diseases fall within accessible genomic regions in disease-relevant tissues or cell types

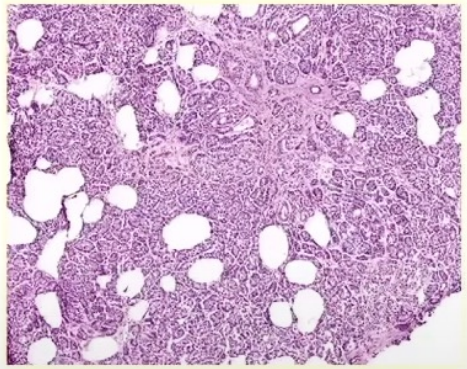




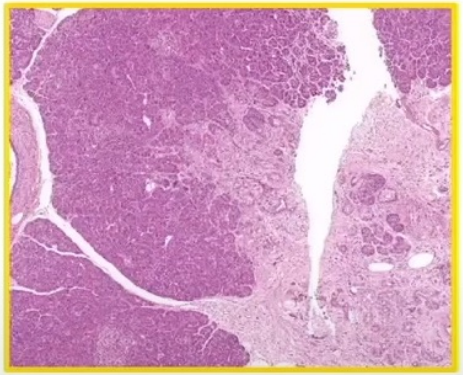
Josep Carreras LEUKAEMIA Research Institute

3. Pancreatic lesions affects healthy pancreas

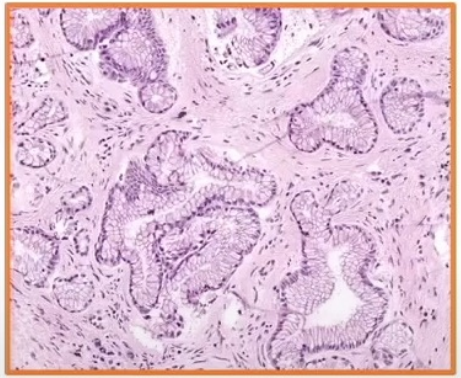
Lipomatosis



Fibrosis

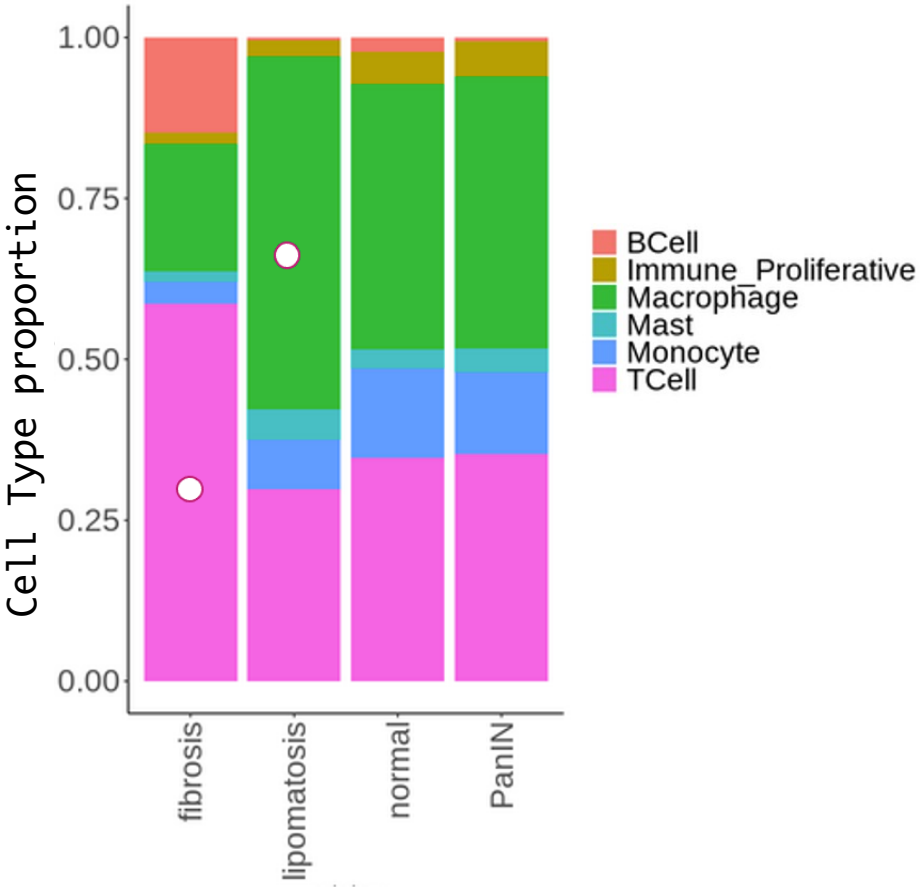


PanIN (Pancreatic Intraepithelial Neoplasia)



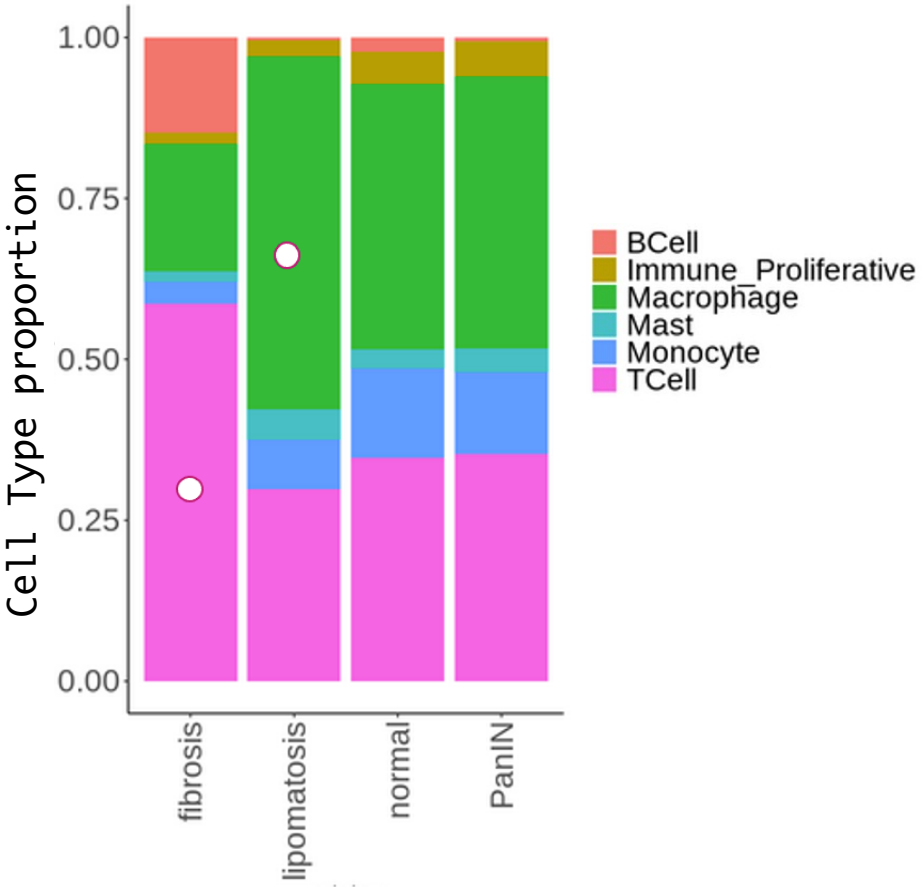
Donor	Normal	Lipomatosis	Fibrosis	PanIN
Healthy	36	17	17	6
Pancreatectomy	5		1	
T2D	3		2	

Cell-type composition changes in micropathologies



- Higher abundance of macrophages in lipomatosis

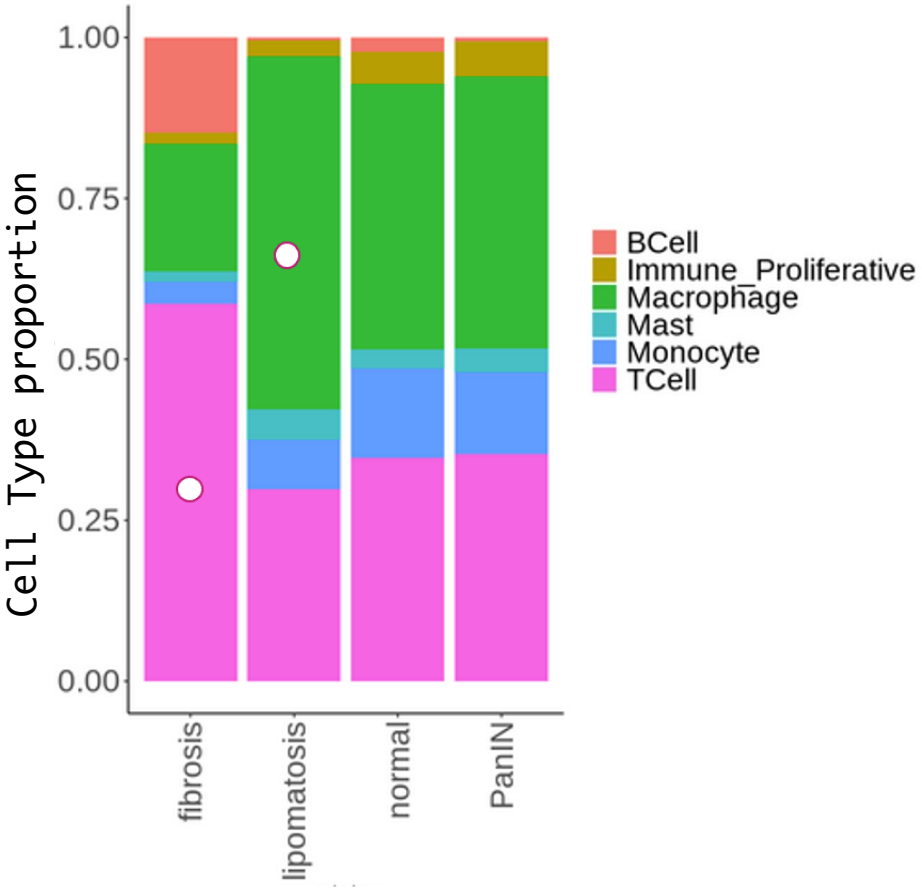
Cell-type composition changes in micropathologies



- Higher abundance of macrophages in lipomatosis
- Higher abundance of lymphocytes in fibrotic samples

Josep Carreras
LEUKAEMIA
Research Institute

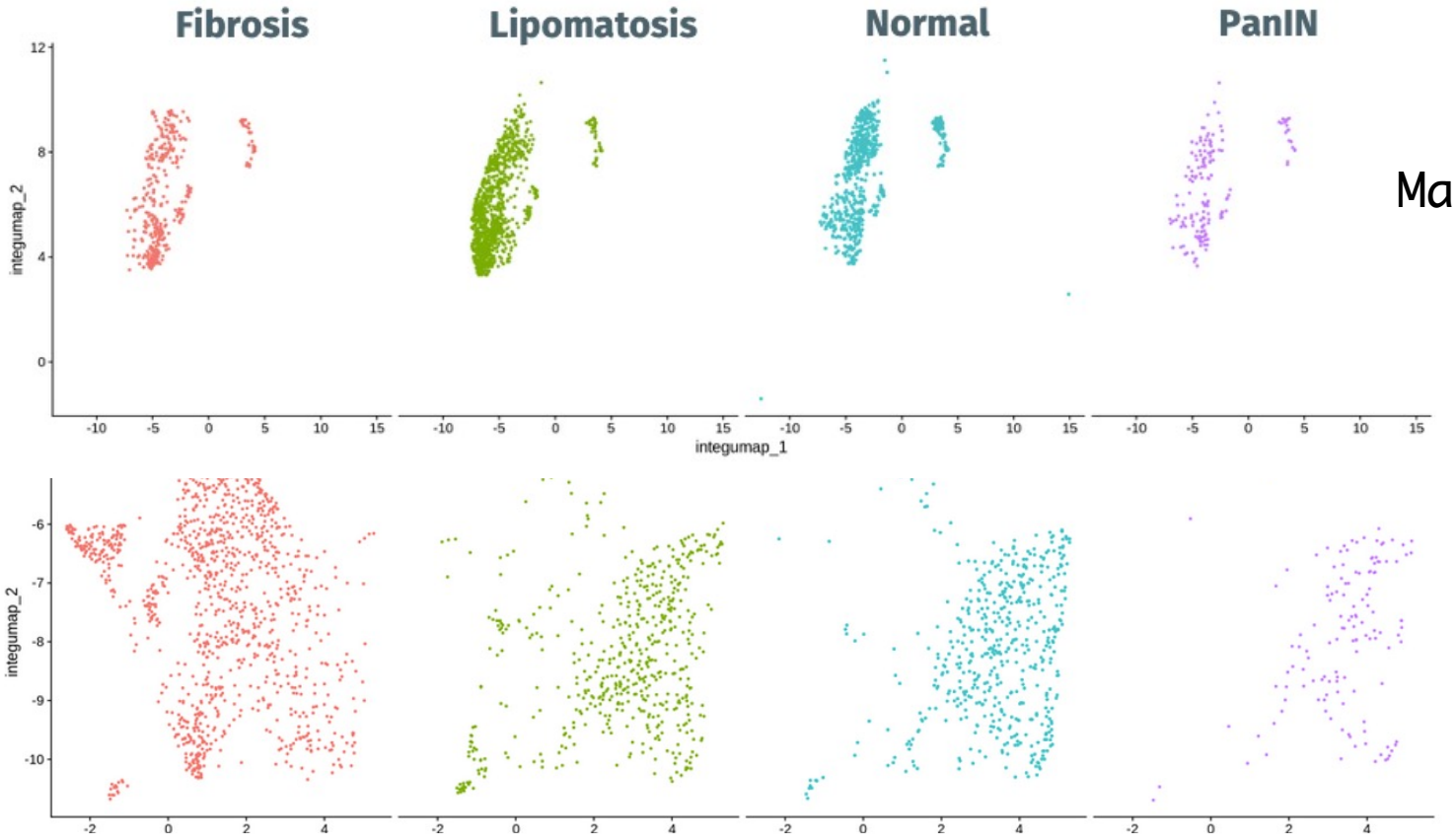
Cell-type composition changes in micropathologies



- Higher abundance of macrophages in lipomatosis
- Higher abundance of lymphocytes in fibrotic samples
- No changes in composition for PanIN (integration with PDAC will serve to reconstruct the early stages of PDAC initiation)

Josep Carreras LEUKAEMIA Research Institute

Transcriptional and epigenetic changes in micropathologies

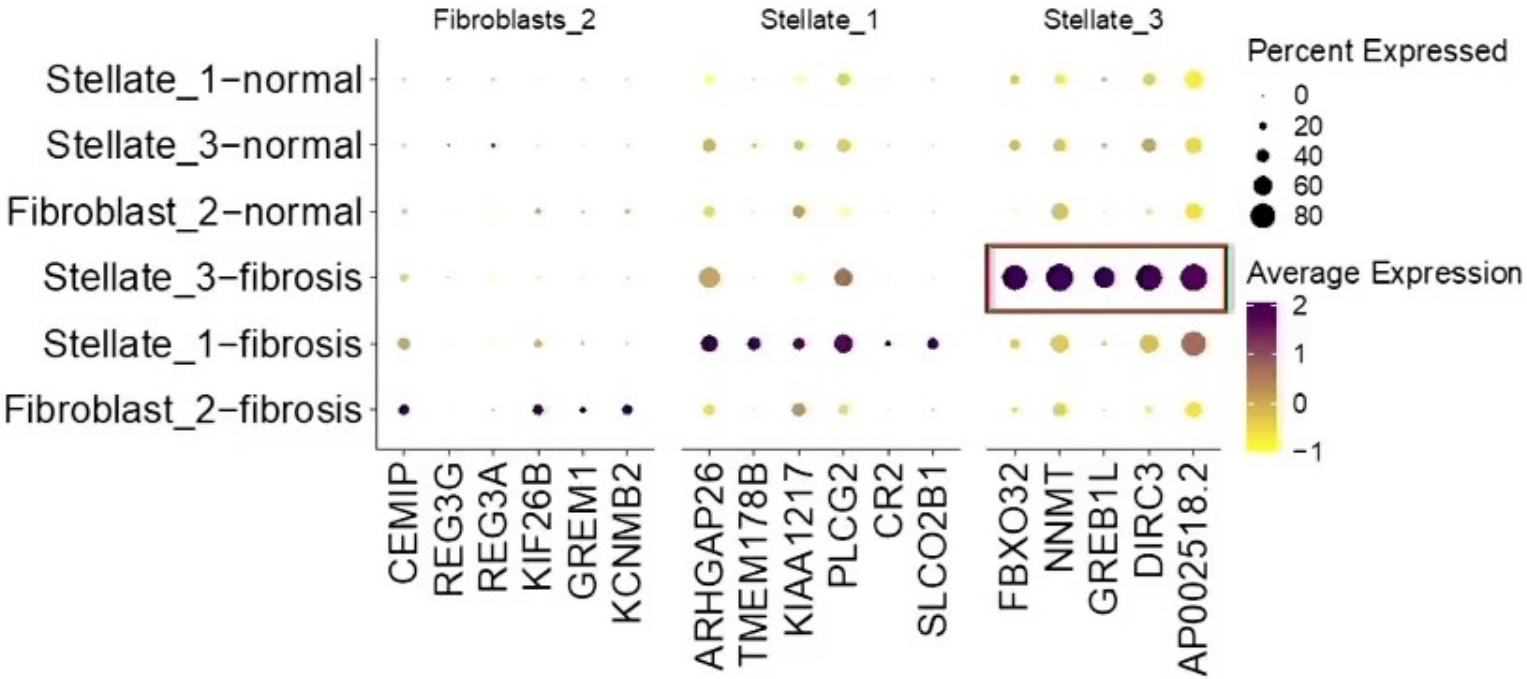


Macrophages

T cells

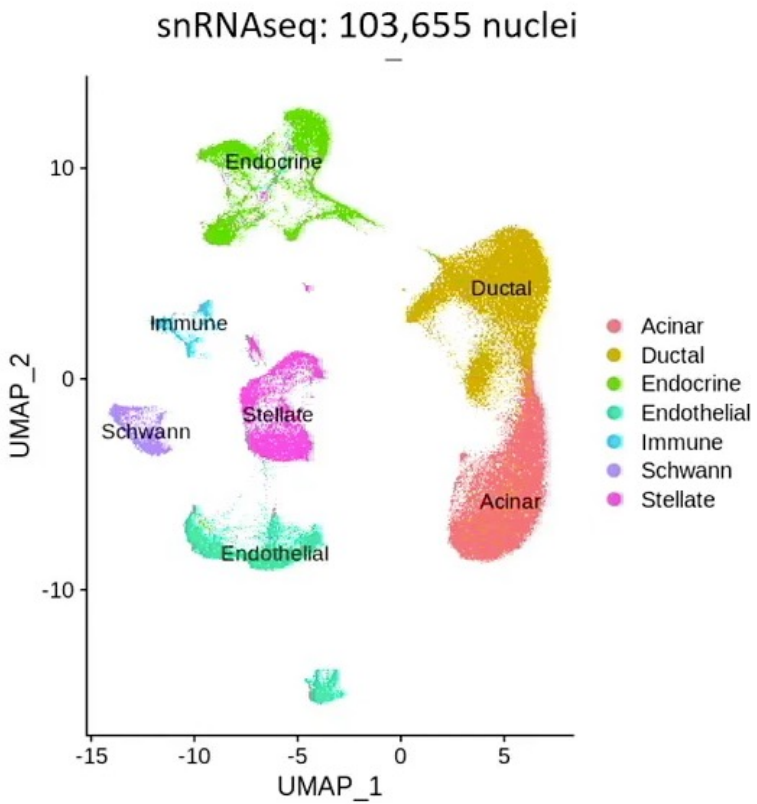
Josep Carreras
LEUKAEMIA
Research Institute

Transcriptional and epigenetic changes in micropathologies



Josep Carreras
LEUKAEMIA
Research Institute

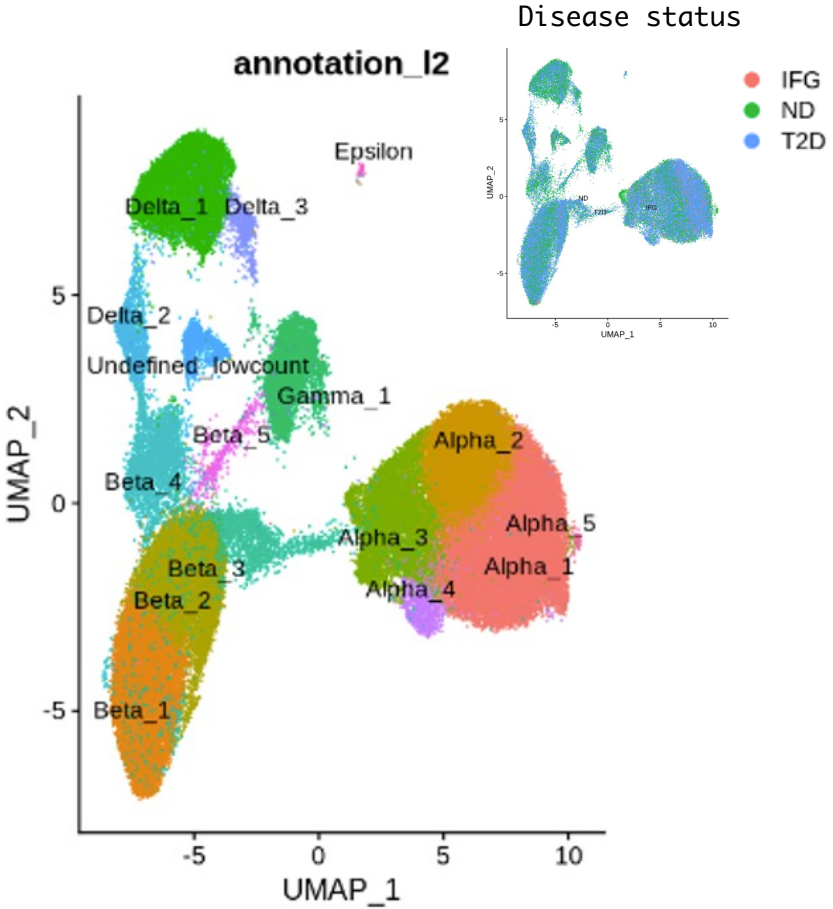
4. Temporal single-cell analysis of fetal samples shed light on pancreas development



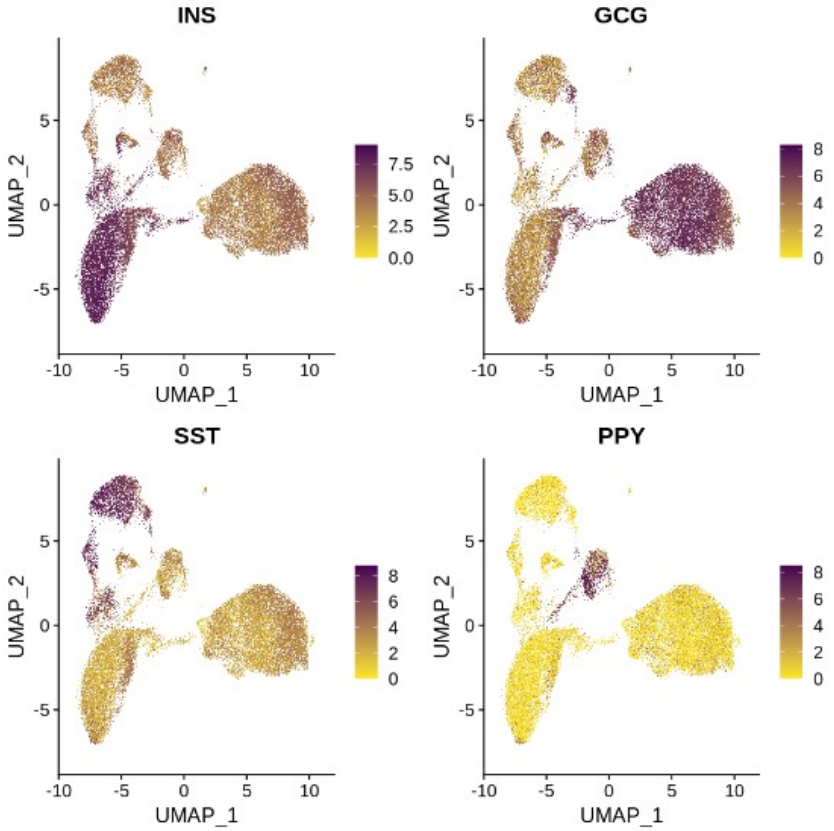
- Characterization of pancreatic cell-type progenitors
- Understanding *signalling pathways* that regulate pancreas development, direct cell fate decisions and cellular plasticity in adult pancreas.
- Particularly relevant in understanting human pancreatic disorders and regenerative medicine

Josep Carreras
LEUKAEMIA
Research Institute

5. Characterization of the endocrine cells in health and T2 diabetes

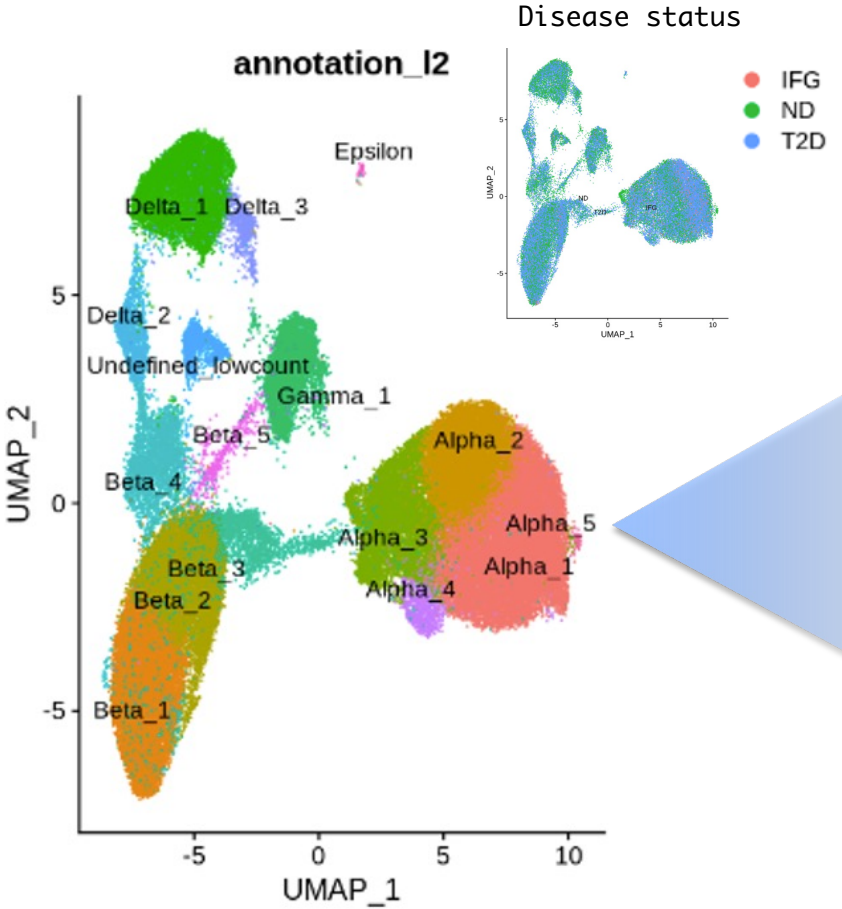


Disease status

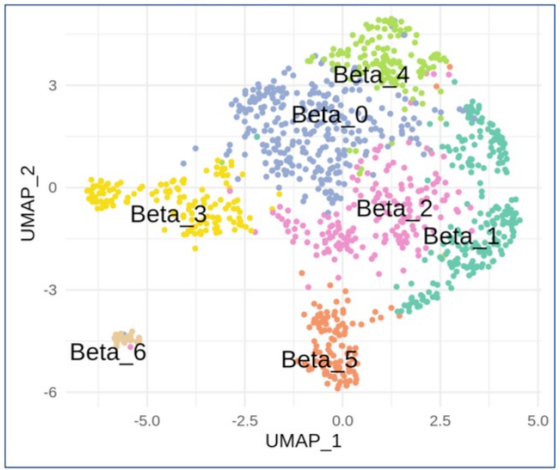


Josep Carreras LEUKAEMIA Research Institute

Characterization of the endocrine cells in health and T2 diabetes

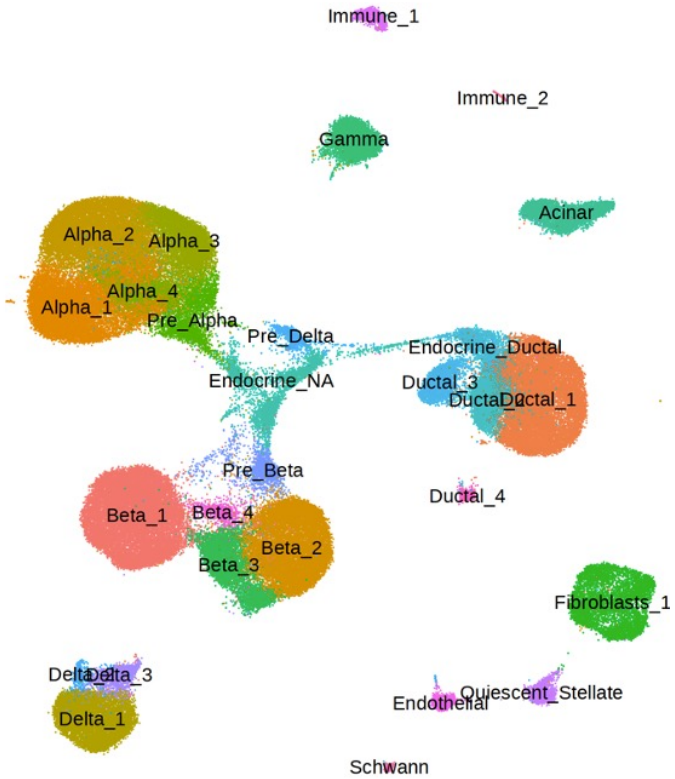


VASA-seq enables the full-length transcriptomics profiling of Beta cells

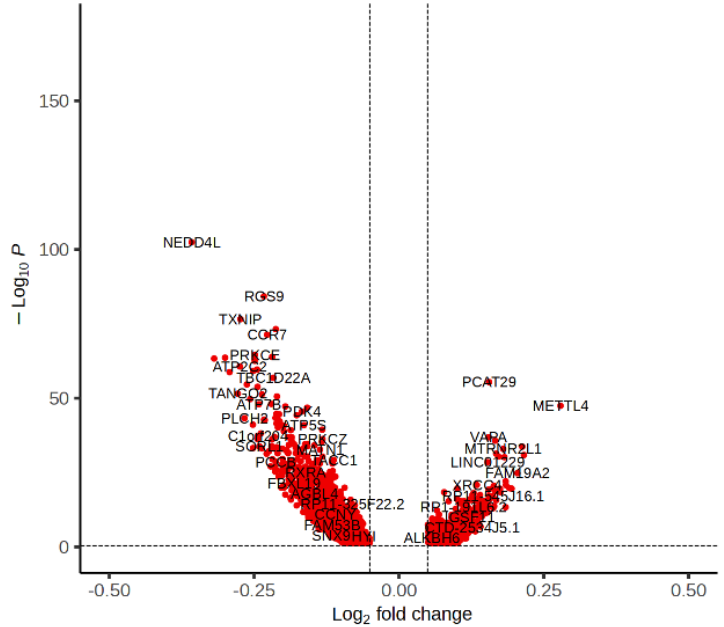


Josep Carreras LEUKAEMIA Research Institute

Changes in chromatin accessibility at different levels of glucose



Changes in chromatin accessibility in Beta cells related to glucose levels



Josep Carreras LEUKAEMIA Research Institute

Summary

- We have generated a comprehensive single-cell reference atlas of ~1M of pancreatic cells across:

  - *45 «healthy» adults* mapped by snRNA/snATAC-seq
  - *10 Fetal pancreas* mapped by snRNA/snATAC-seq
  - *20 T2D/IFG/Controls adults*. Islet cells mapped by scRNA-seq, scATAC-seq, scVASA-seq and Spatial information.

- Our resource permit the exhaustive identification and characterization of >50 pancreatic cell types and states, enabling the identification of:

  - A previously unappreciated *Acinar heterogeneity*;
  - *Cell-type pancreatic progenitors* involved in embrionic development;
  - Critical cell-types/states associated with *common histo-pathological features* and *PDAC initiation*;
  - Differential cell-type changes in composition and cellular processes underlying T2 diabetes and pre-diabetic samples.

Cellular Systems Genomics Group & Friends

Computational Postdoc WANTED!!

Berlin 2022– Final ESPACE meeting

Acknowledgements

GOBIERNO DE ESPAÑA

espace
THE HCA PANCREAS INITIATIVE

Josep Carreras
LEUKAEMIA
Research Institute