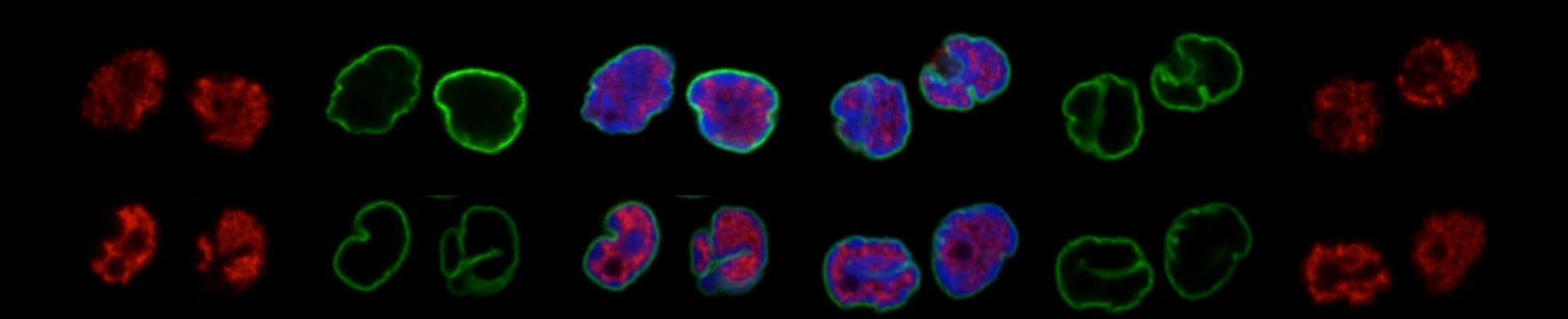
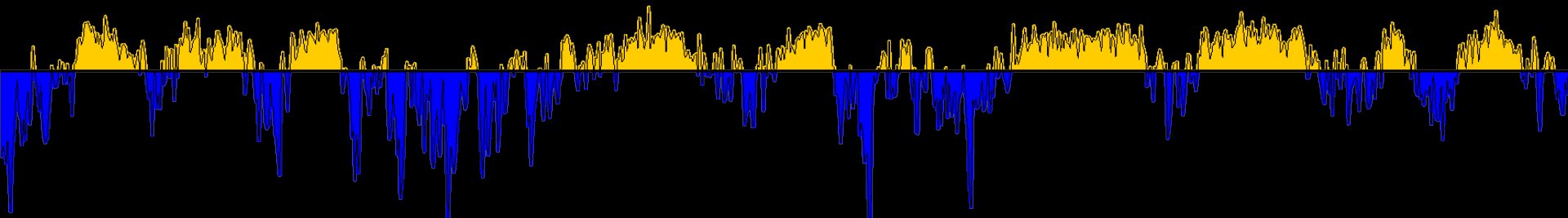


SAMMY-seq: a new technology to capture dysfunctional chromatin landscapes

Chiara Lanzuolo

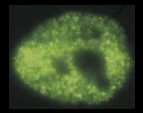
Chromatin and Nuclear Architecture Laboratory, CNR and INGM



The crosstalk between the nuclear and genome structure

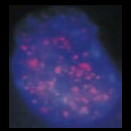
ACTIVE COMPARTMENT

Nucleoli

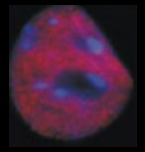


Transcriptional factories

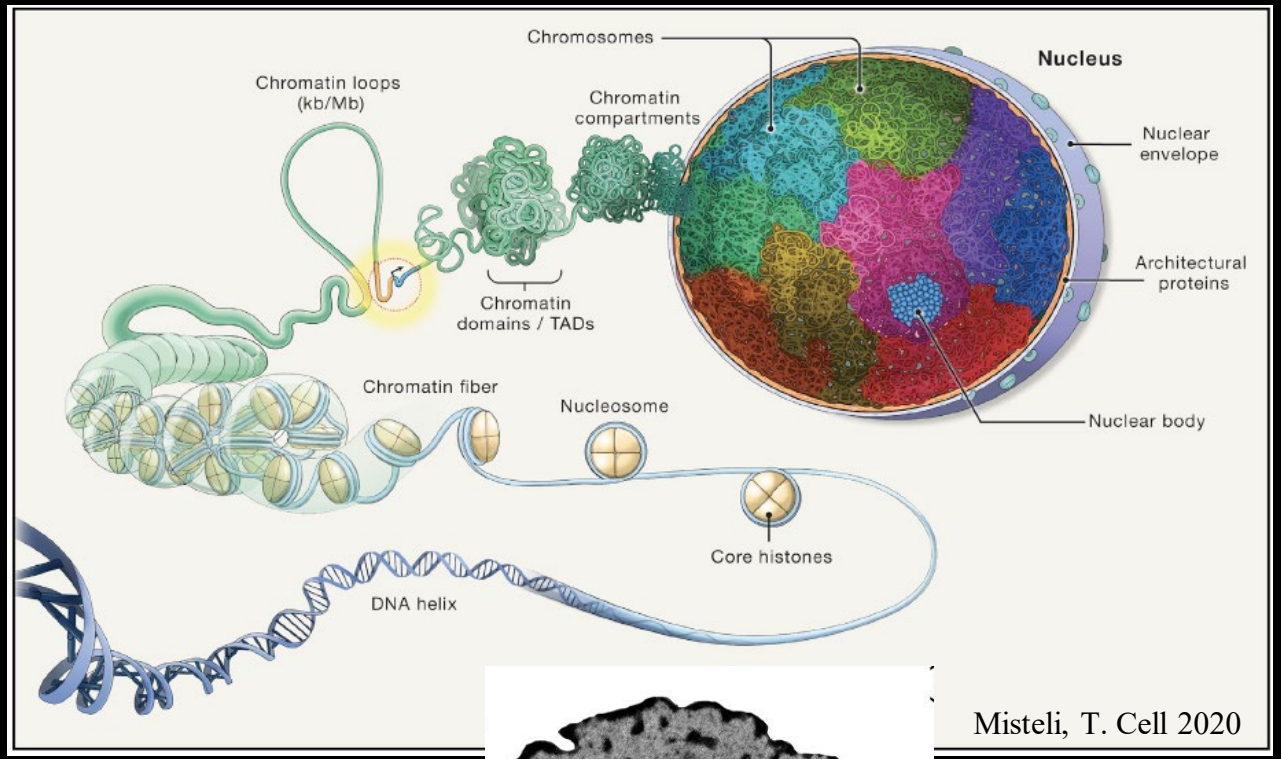
Cajal Bodies

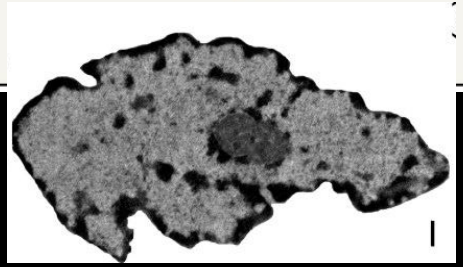


Nuclear speckles

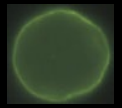


Euchromatin

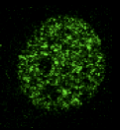




REPRESSIVE COMPARTMENT



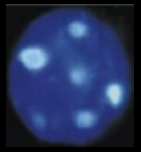
Nuclear Lamin



Polycomb bodies

Centromeres

Telomeres



Heterochromatin

INGM
ISTITUTO NAZIONALE GENETICA MOLECOLARE
"Romeo ed Enrica Invernizzi"

Chromatin and Nuclear Architecture Laboratory

Francesca Gorini, postdoc

Valentina Rosti, postdoc

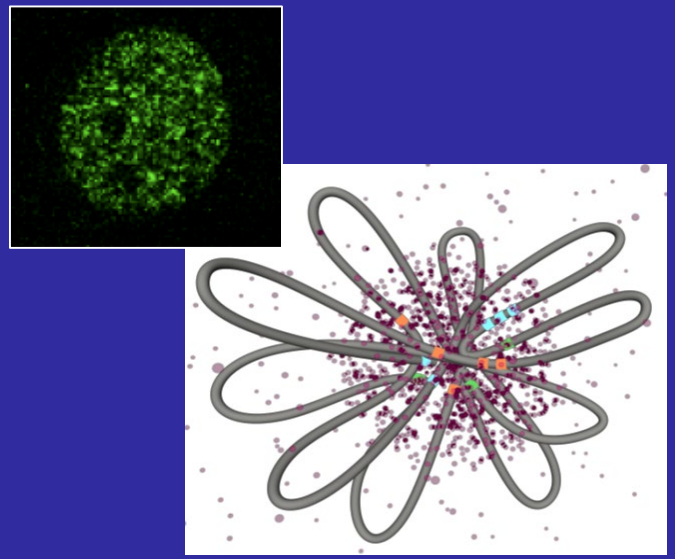
Philina Santarelli, PhD student

Emanuele Soldateschi, PhD student

Consiglio Nazionale delle Ricerche

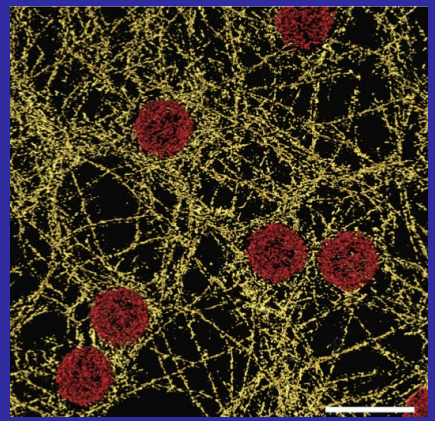
Our research interest: Nuclear and genome architecture in gene regulation

PcG bodies



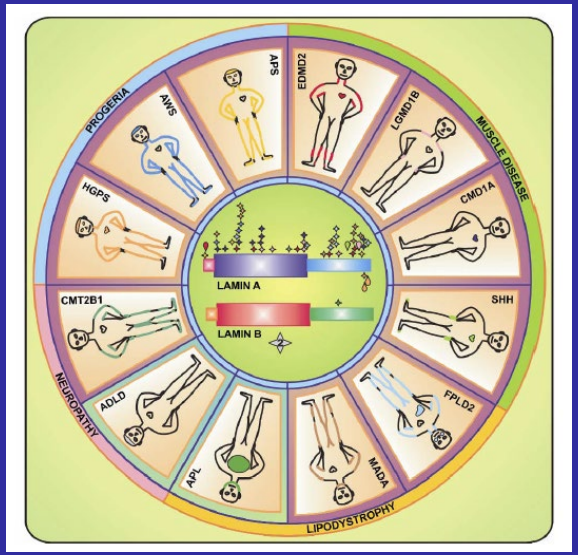
Lanzuolo Nat Cell Biol. 2007

The nuclear Lamin



Turgay et al., *Nature* 2017

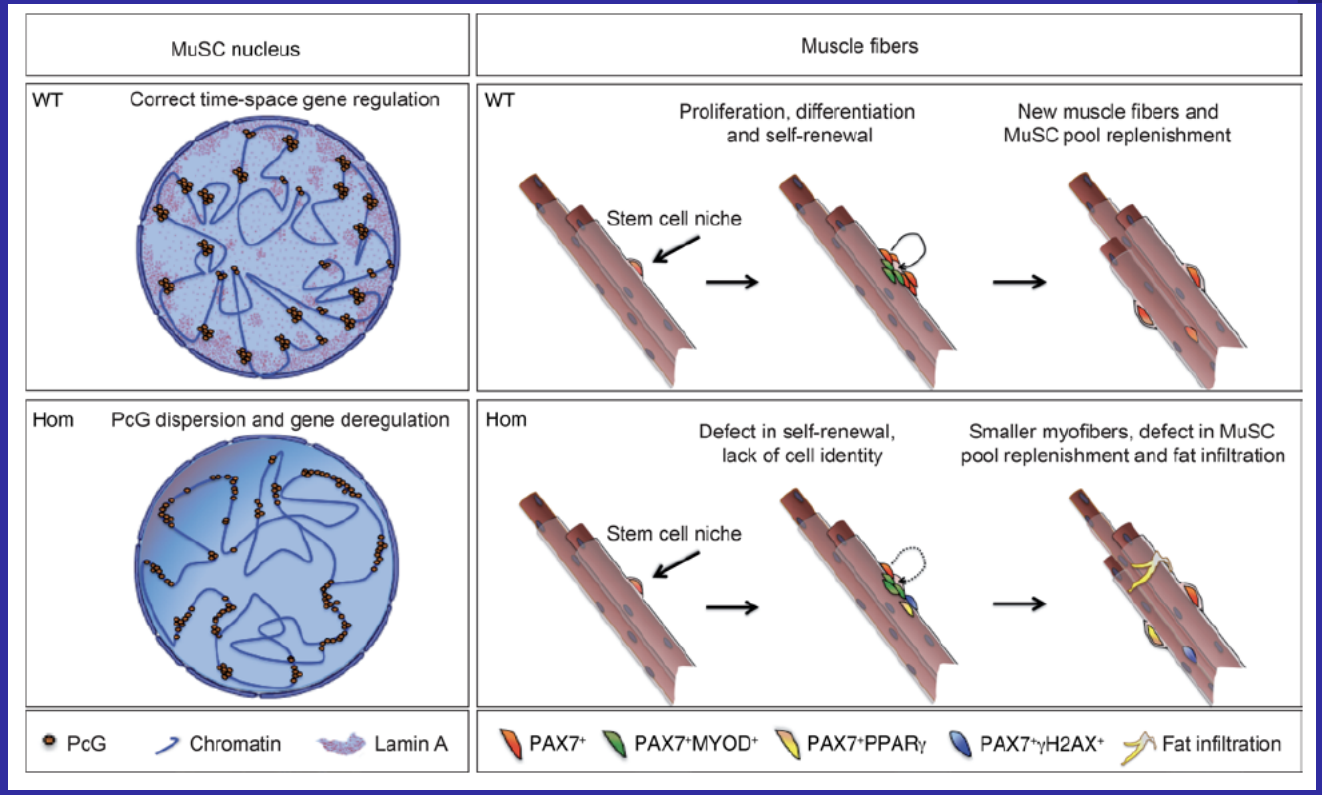
The laminopathies



- Cesarini, E. et al., JCB 2015; Marullo, F. et al., Nucleus 2016; Sebestyen, E. et al., Nat Comm 2020

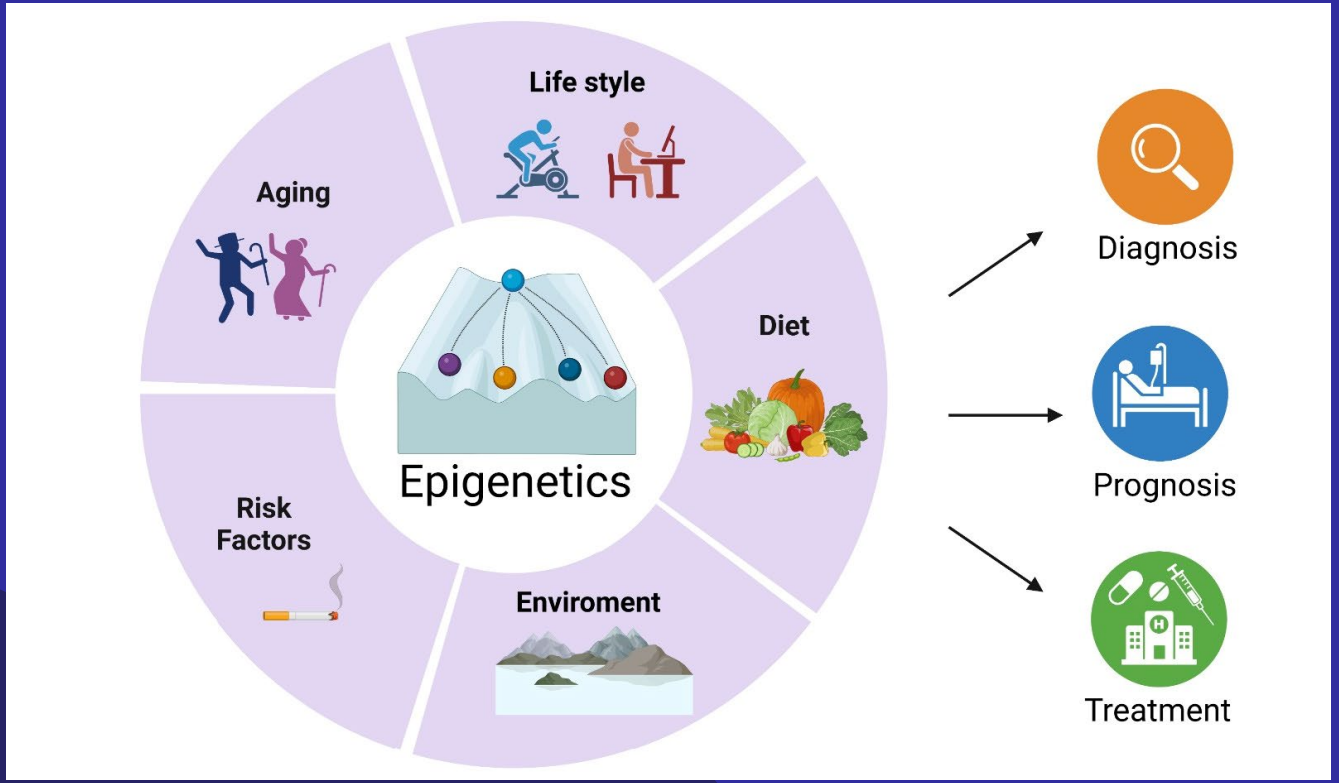
Our research interest: Nuclear and genome architecture in gene regulation

Lamin A -/-

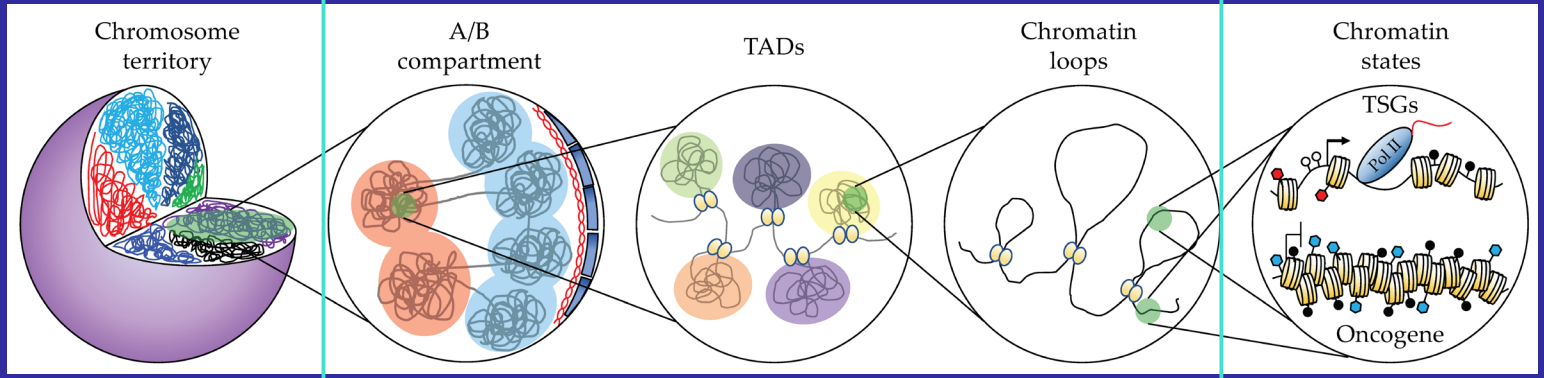


Bianchi A. et al., JCI 2020, Pegoli et al., JoVE 2020; Pegoli et al., Biomolecules 2021

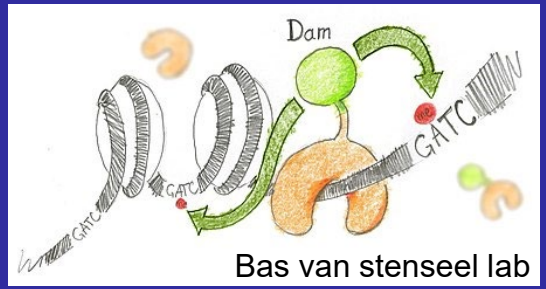
Chromatin architecture and human disease



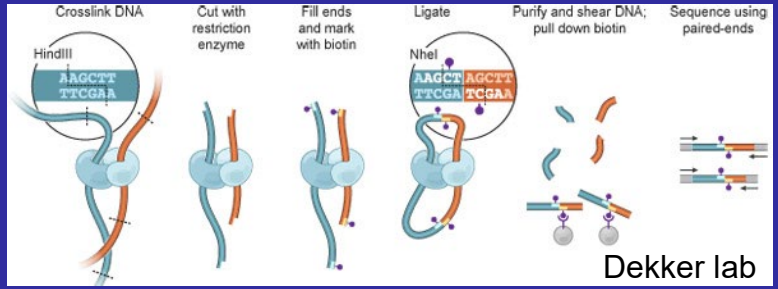
The Methods:



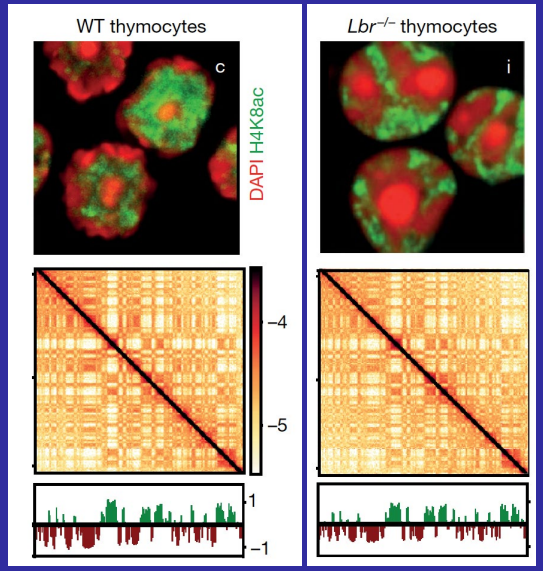
DamID



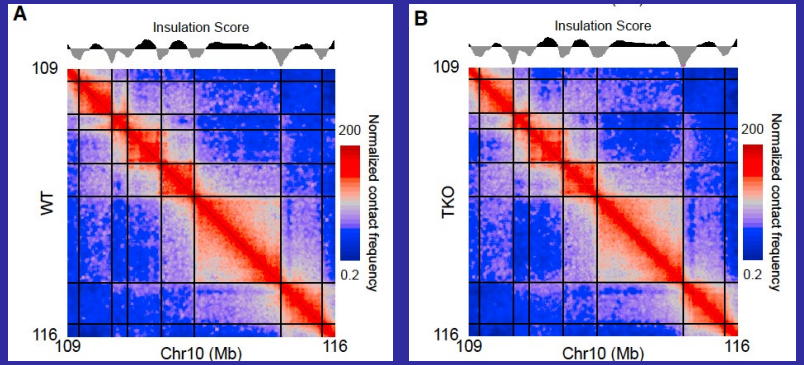
HiC



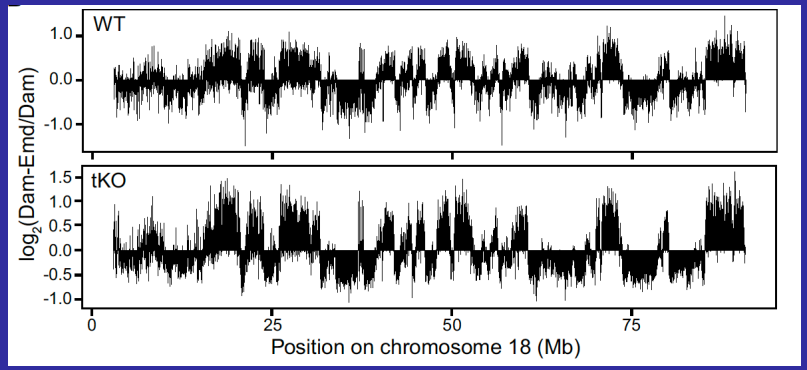
Genome conformation technologies do not capture lamin dependent alterations:



Falk, Nature 2019



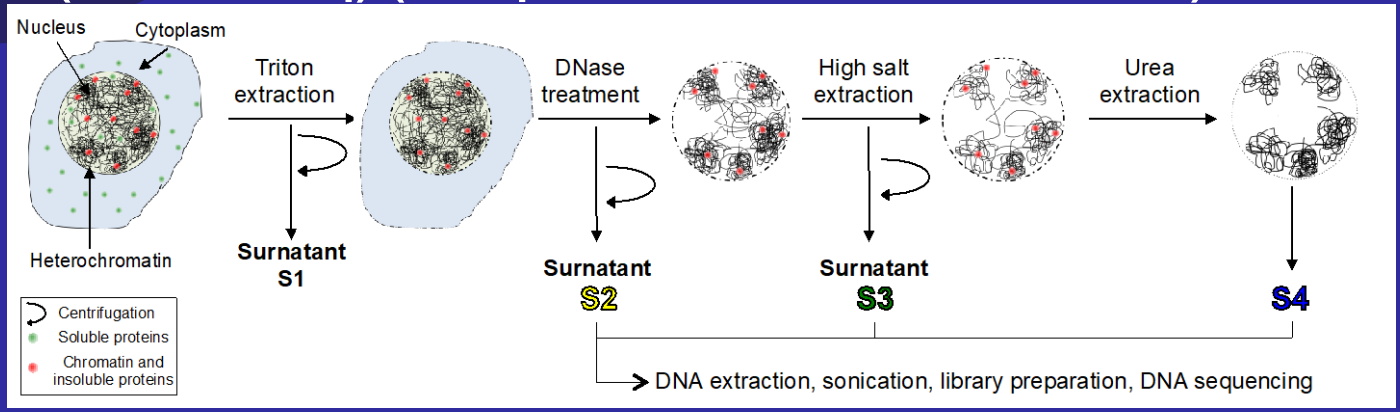
Zheng, Mol Cell 2018



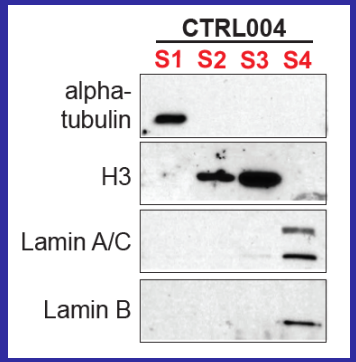
Amendola, Embo Rep. 2015

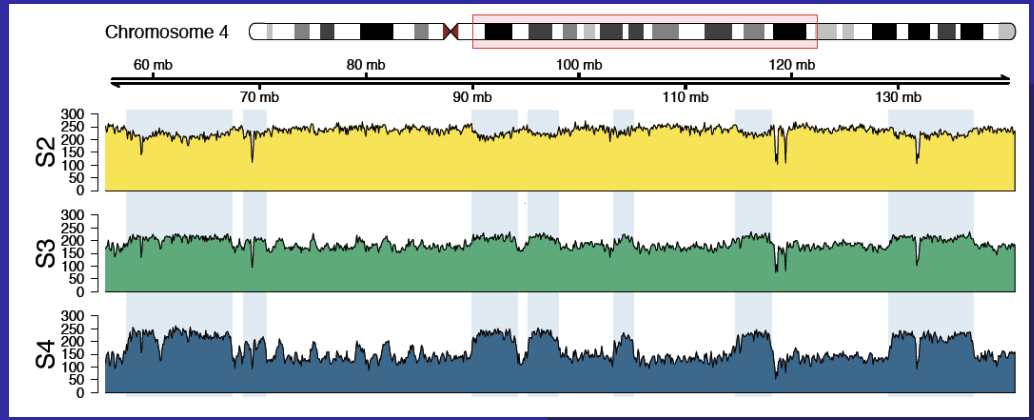
Ourtechnology :

The Sequential Analysis of MacroMolecules accessibilitY (SAMMYseq) (European Patent No. 18200482.0)



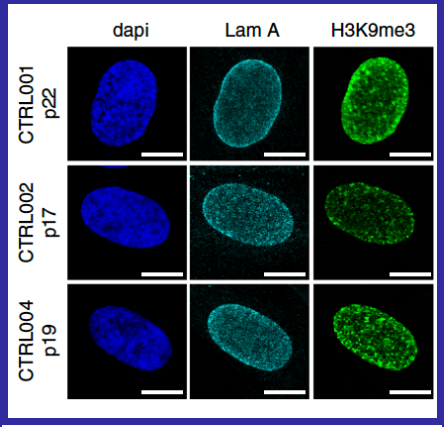
HUMAN FIBROBLASTS

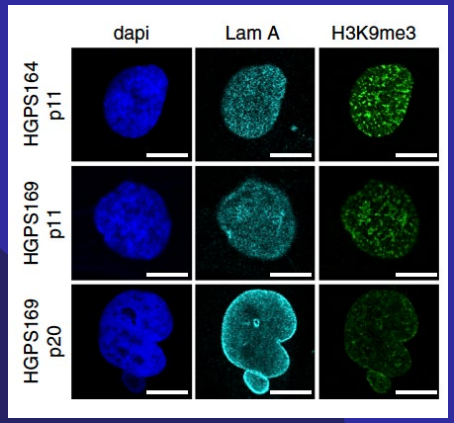


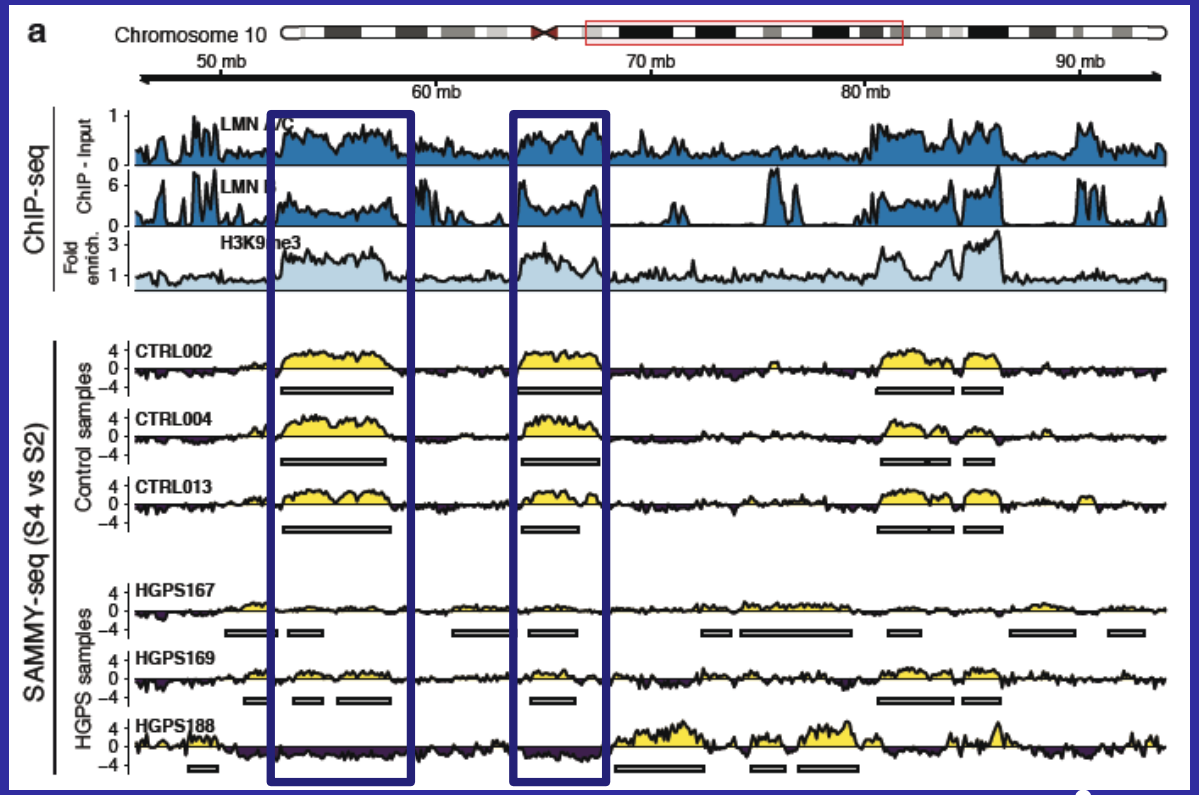


Sebestyén, Marullo Nat Comm 2020

SAMMY-seq highlights a patient-specific increase of LAD solubility in progeria

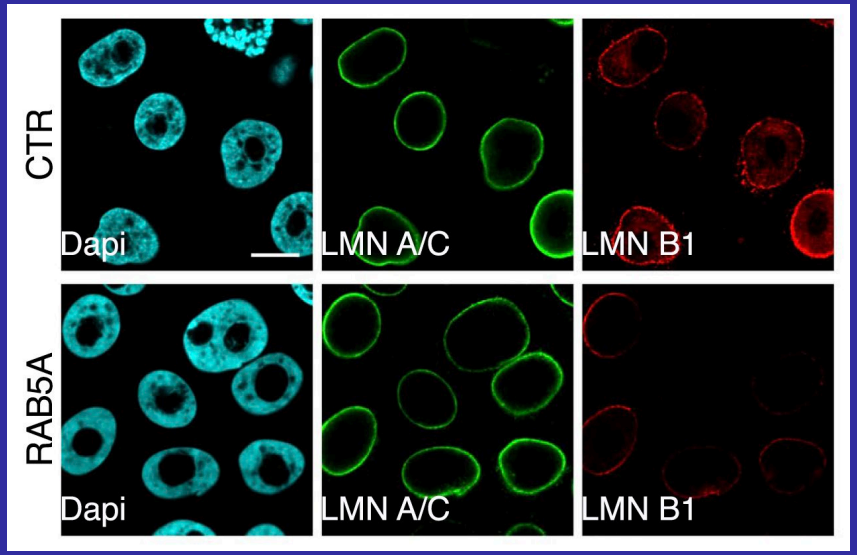


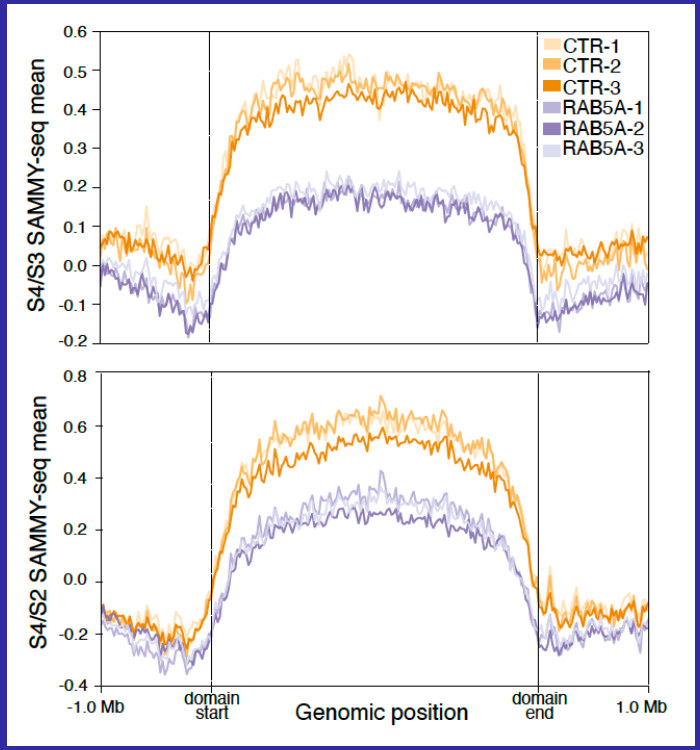




Sebestyén, Marullo Nat Comm 2020

SAMMY-seq detects loss of lamin interactions induced by mechanical stress

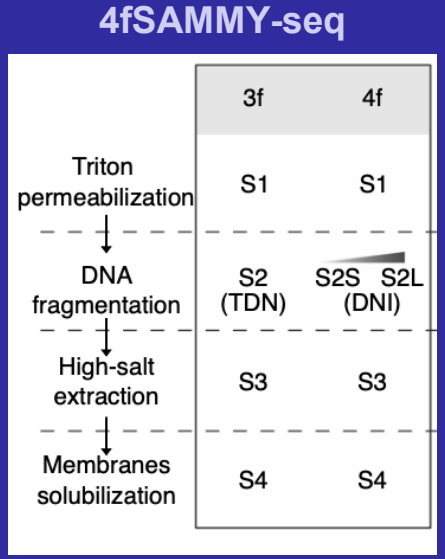


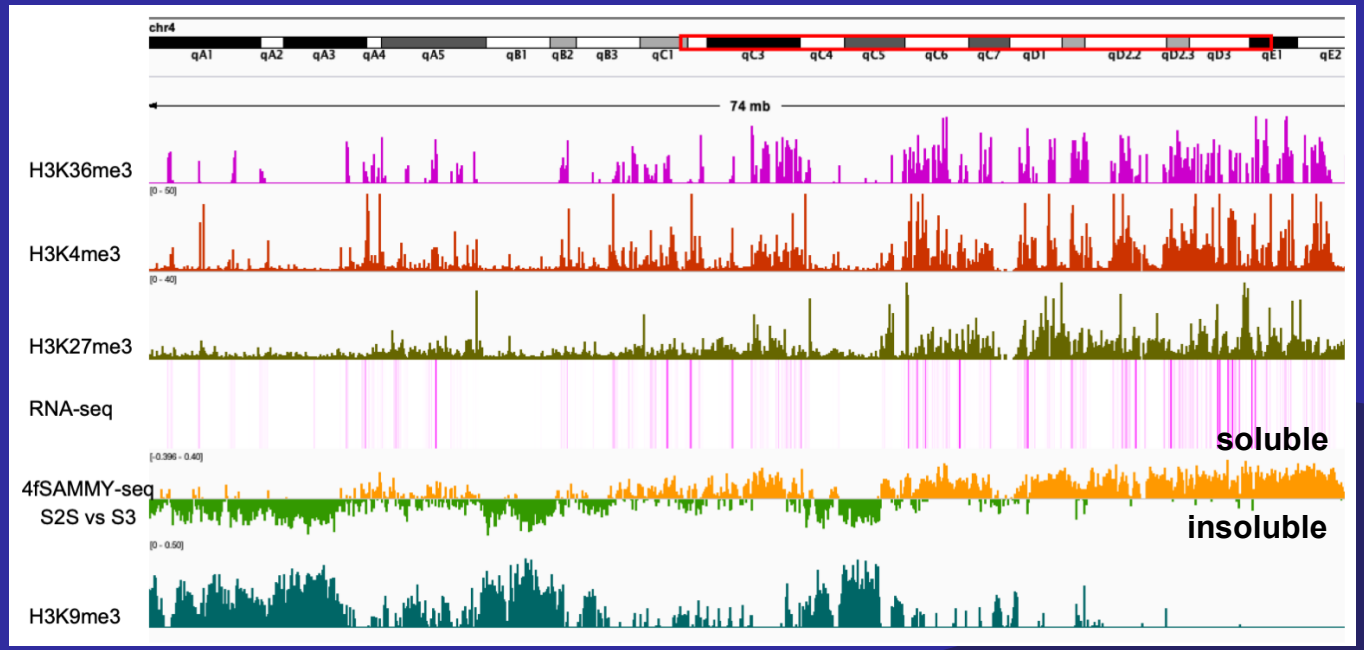


In collaboration with Giorgio Scita, IFOM

Frittoli, Palmidessi Nat Mater 2022

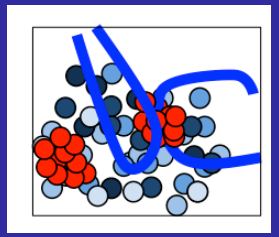
Upgrade of SAMMYseq adding a subfractioning step



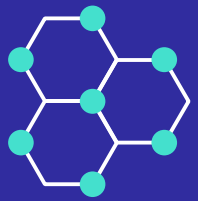


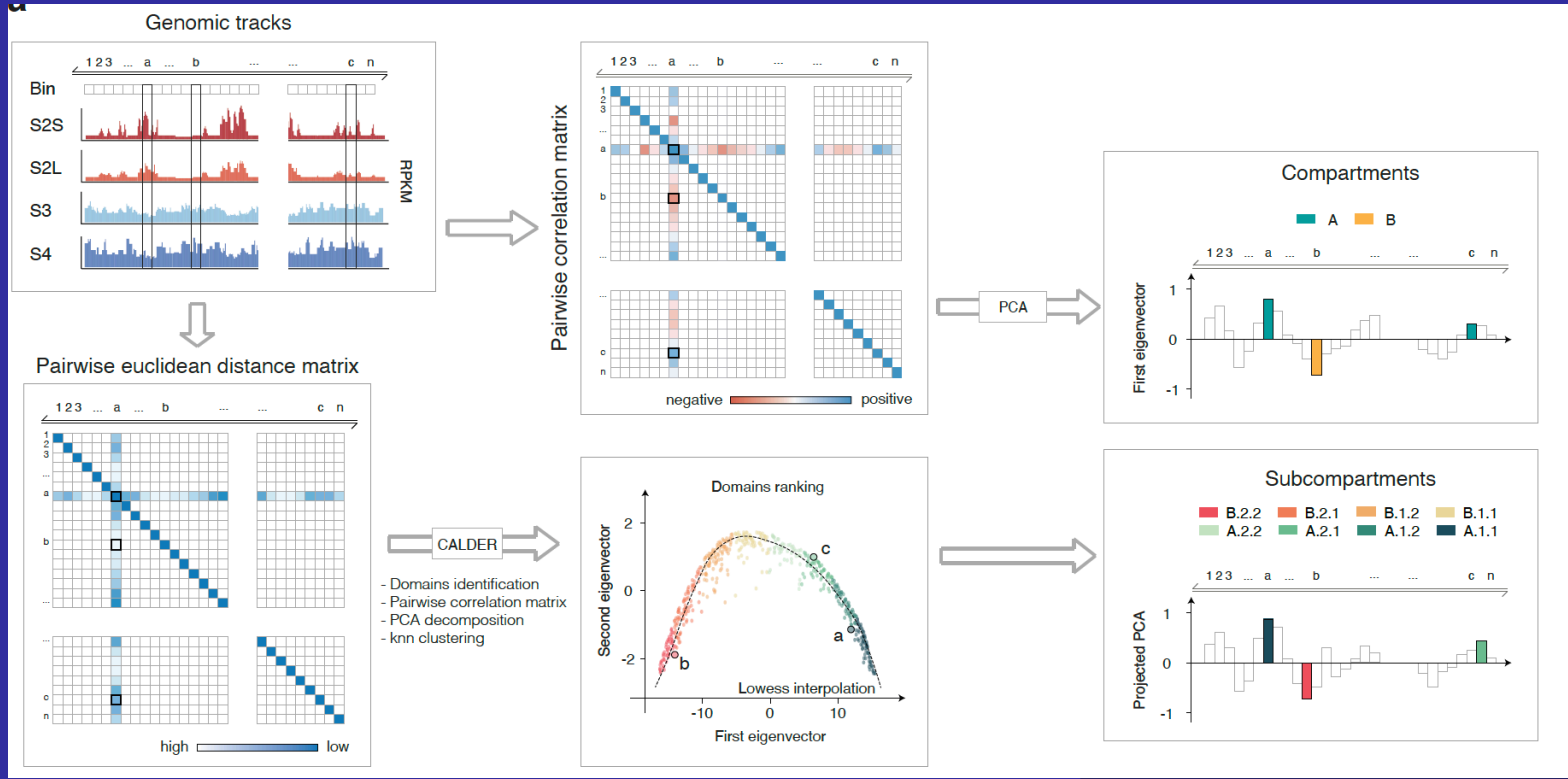
Lucini F., Petrini C., Salviato E., Pal K. et al., unpublished

4fSAMMY-seq compartment analysis



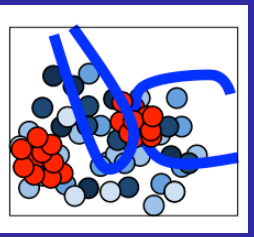
Same nuclear positions ≡ same solubility?



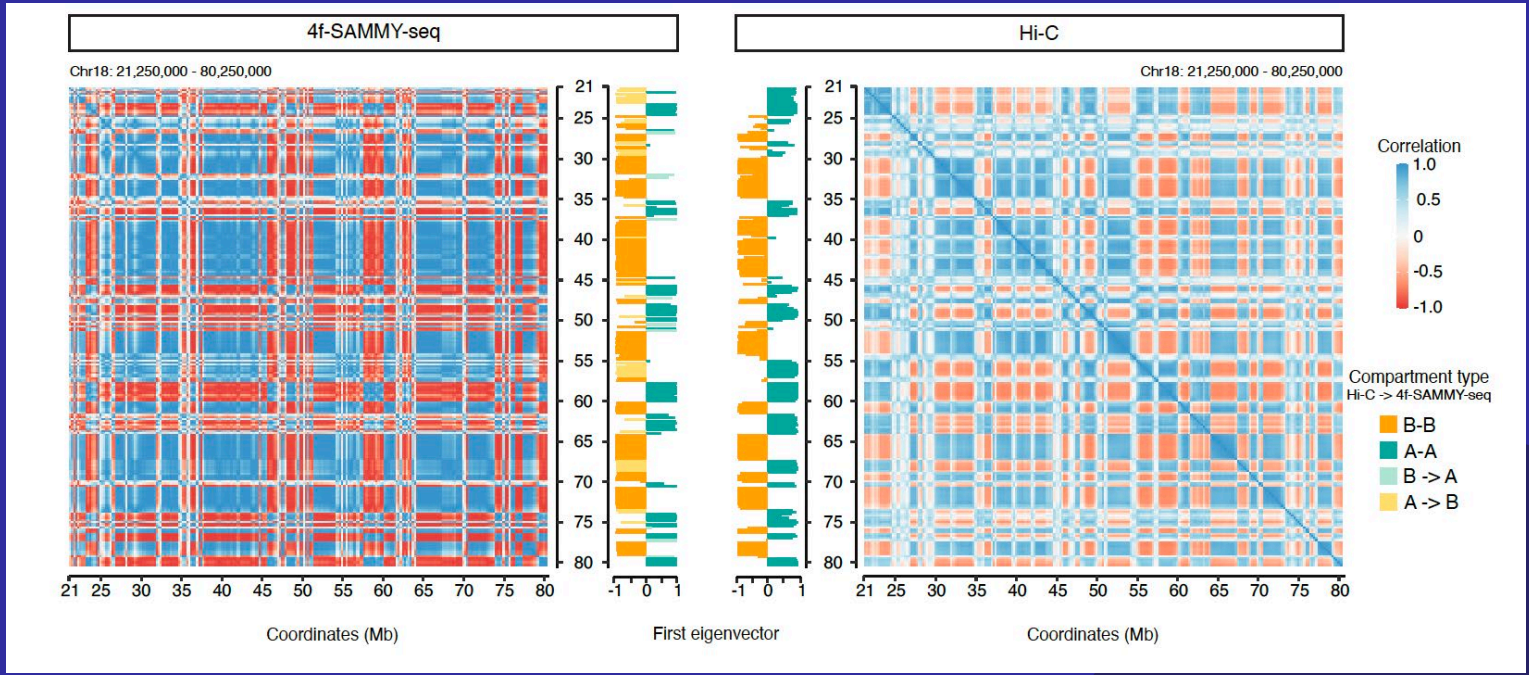


Lucini F., Petrini C., Salviato E., Pal K. et al., unpublished

4fSAMMY-seq ricapitulates compartment analysis as seen by HiC

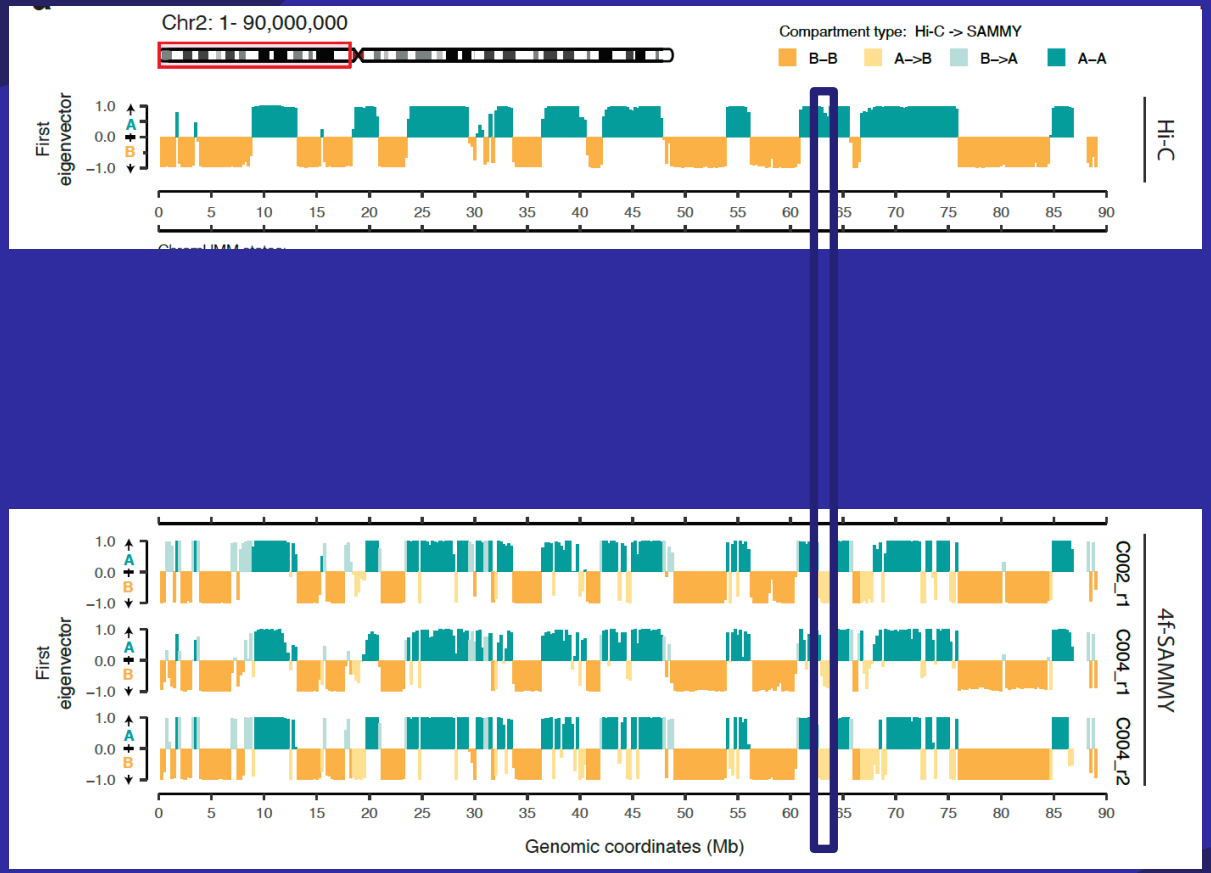


Same nuclear positions ≡ same solubility?



Lucini F., Petrini C., Salviato E., Pal K. et al., unpublished

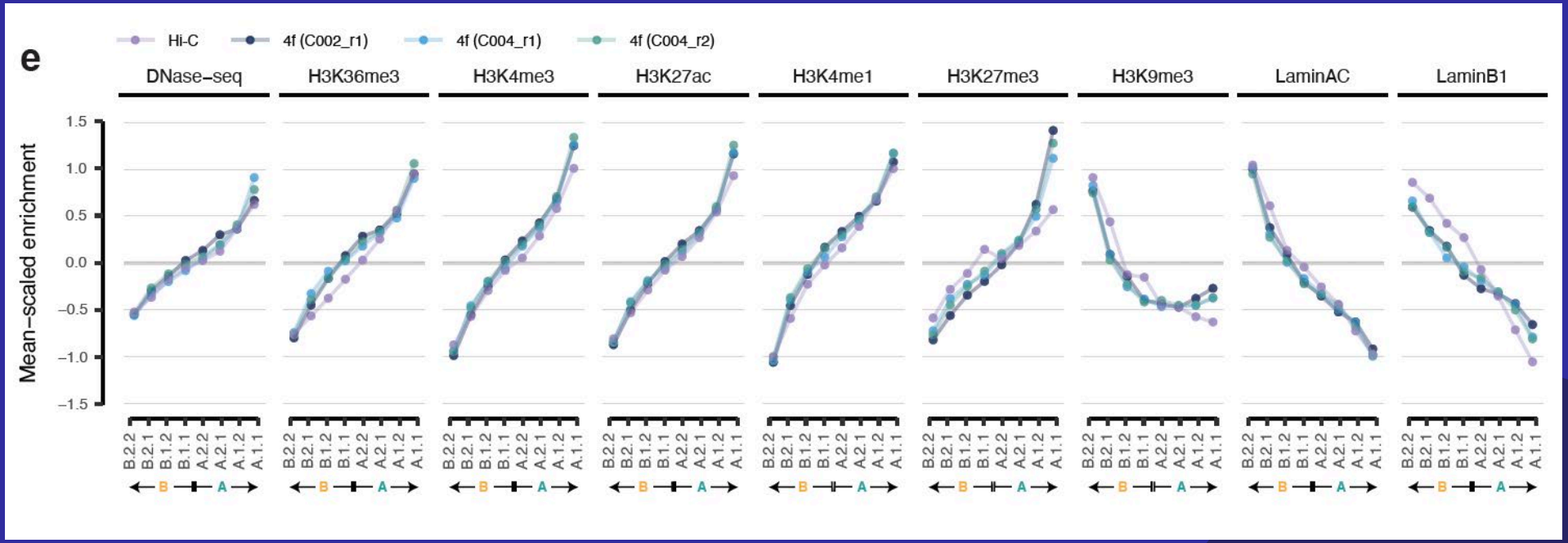
4fSAMMY-seq captures Polycomb targets:



Lucini F., Petrini C., Salviato E., Pal K. et al., unpublished

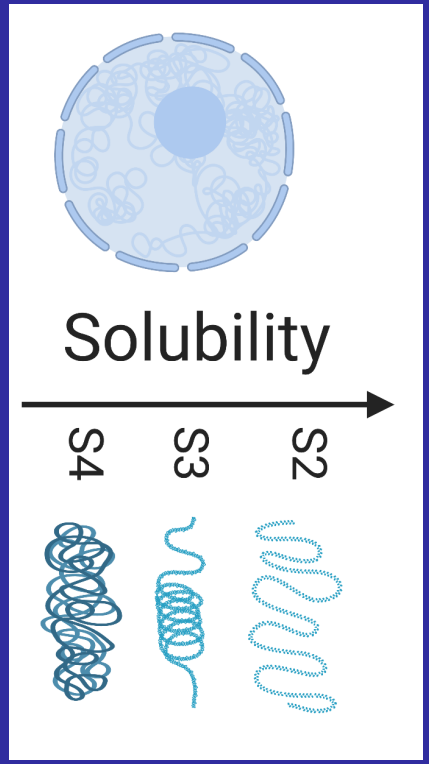
Subcompartment analysis highlights SAMMY-seq strenghts

Euchromatin markers | Heterochromatin markers | Lamins



Lucini F., Petrini C., Salviato E., Pal K. et al., unpublished

4fSAMMY-seq advantages



Advantages:

- Cells required (10.000)
- Few protocol steps
- Absence of crosslinking agents
- Independency on antibody or protein levels

4fSAMMY-seq detects lamin specific changes of solubility in MEF

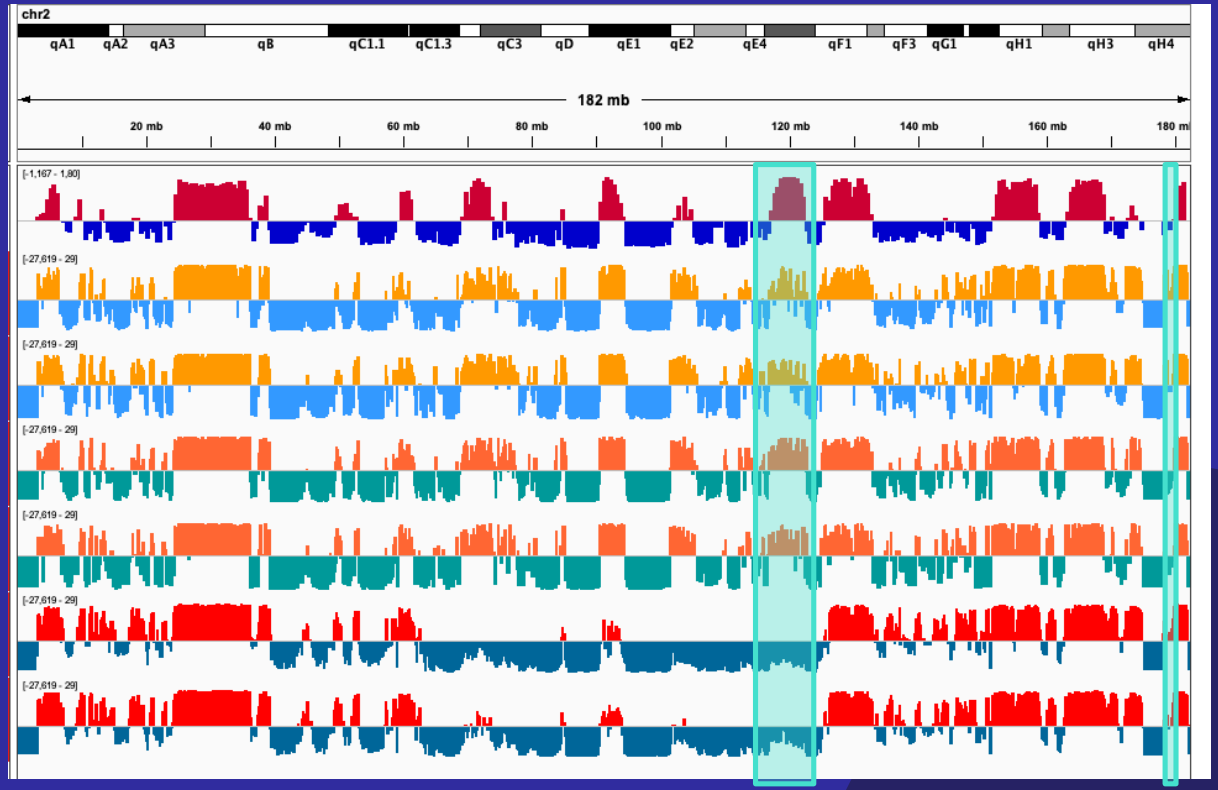
SAMMY-seq

HiC

wt

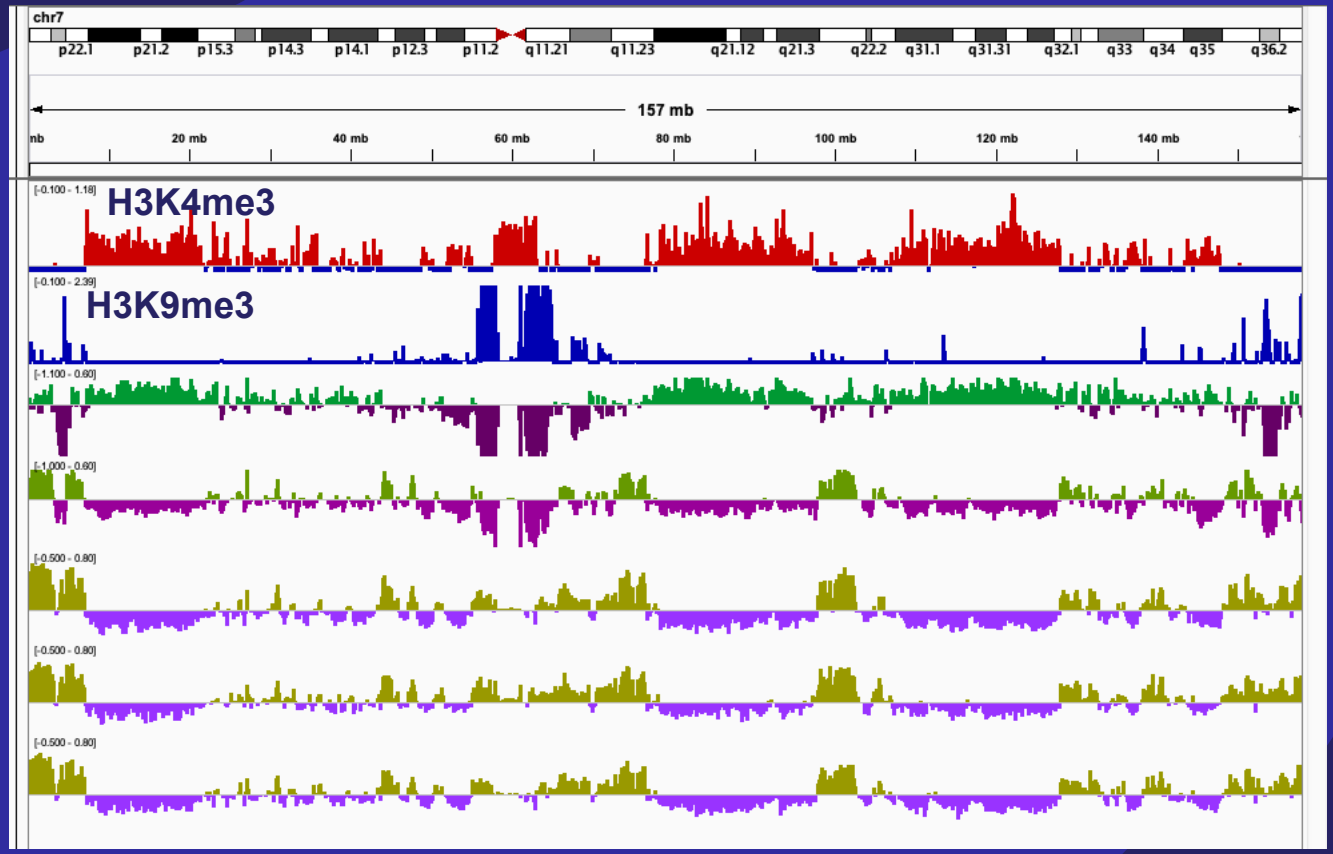
Lamin A/C K/O

Lamin B K/O



Valentina Rosti in collaboration with Ohad Medalia, University of Zurich, unpublished

SAMMY-seq in iPSC, skeletal muscle differentiation , and whole tissue



ChIPseq iPSC

SAMMY-seq

iPSC

Myogenic precursors

Frozen muscle

Frozen cells

soluble

insoluble

In collaboration with Claudia Bearzi, ITB, CNR, and Yvan Torrente University of Milan and Policlinico Hospital unpublished

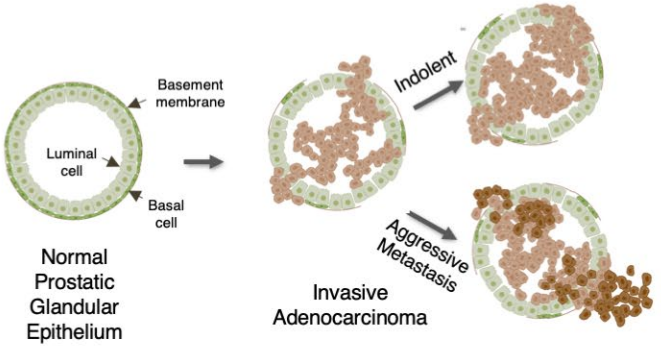
The biological question: Prostate Cancer (Pca)

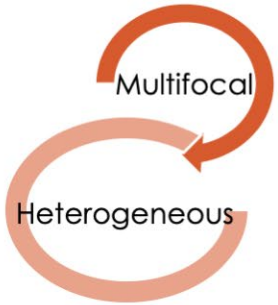
Initial Screening

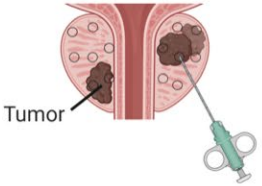
Serum Prostate-Specific Antigen (PSA) > 4 ng/ml

Diagnosis

Transrectal ultrasound guided prostate biopsy (TRUS)







Indolent disease
Monitoring strategies:
Active surveillance

Aggressive disease
Treatment: Radical prostatectomy

Siegel et al., *A Cancer Journal for Clinicians* 2021

PCamolecular profiling : the state of the art

Genomic profiling

Cell

> Cell. 2015 Nov 5;163(4):1011-25. doi: 10.1016/j.cell.2015.10.025.

The Molecular Taxonomy of Primary Prostate Cancer

Cancer Genome Atlas Research Network

nature genetics

Published: 20 May 2012

Exome sequencing identifies recurrent *SPOP*, *FOXA1* and *MED12* mutations in prostate cancer

Christopher E Barbieri, Sylvan C Baca, ... Levi A Garraway + Show authors

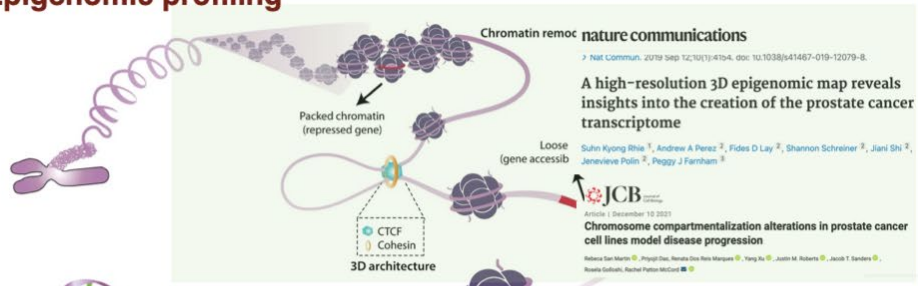
nature

Published: 01 February 2009

ETS rearrangements and prostate cancer initiation

Brett S. Carver, Jennifer Tran, Zhenbang Chen, Arkaitz Carracedo-Perez, Andrea Alimonti, Caterina Nardella, Anuradha Gopalan, Peter T. Scardino, Carlos Cordon-Cardo, William Gerald & Pier Paolo Pandolfi

Epigenomic profiling



nature communications

> Nat Commun. 2019 Sep 12;10(1):4170. doi: 10.1038/s41467-019-12079-8.

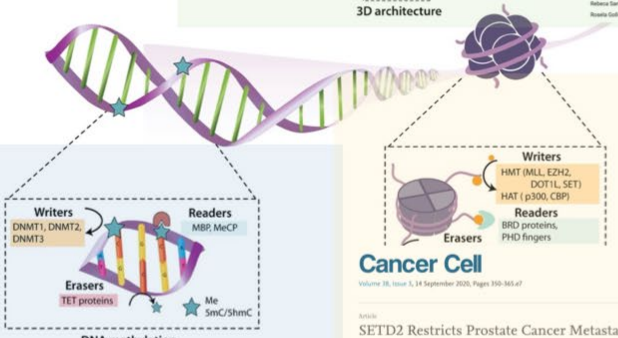
A high-resolution 3D epigenomic map reveals insights into the creation of the prostate cancer transcriptome

Suhn Kyong Rhie, Andrew A Perez, Fides D Lay, Shannon Schreiner, Jiani Shi, Jenevieve Polin, Peggy J Farnham

JCB

Article | December 10 2021

Chromosome compartmentalization alterations in prostate cancer cell lines model disease progression



GENOME RESEARCH

> Genome Res. 2011 Jul;21(7):1028-41. doi: 10.1101/gr.119347.110.

Deep sequencing reveals distinct patterns of DNA methylation in prostate cancer

Jung H Kim, Saravana M Dhanasekaran, John R Prensner, Xuhong Cao, Daniel Robinson, Shanker Kalyana-Sundaram, Christina Huang, Sunita Shankar, Xiaojun Jing, Matthew Iyer, Ming Hu, Lee Sam, Catherine Grasso, Christopher A Maher, Nallasivam Palanisamy, Rohit Mehra, Hal D Kominsky, Javed Siddiqui, Jindan Yu, Zhaohui S Qin, Arul M Chinnaiyan

Cancer Cell

Volume 38, Issue 3, 14 September 2020, Pages 350-365.e7

SETD2 Restricts Prostate Cancer Metastasis by Integrating EZH2 and AMPK Signaling Pathways

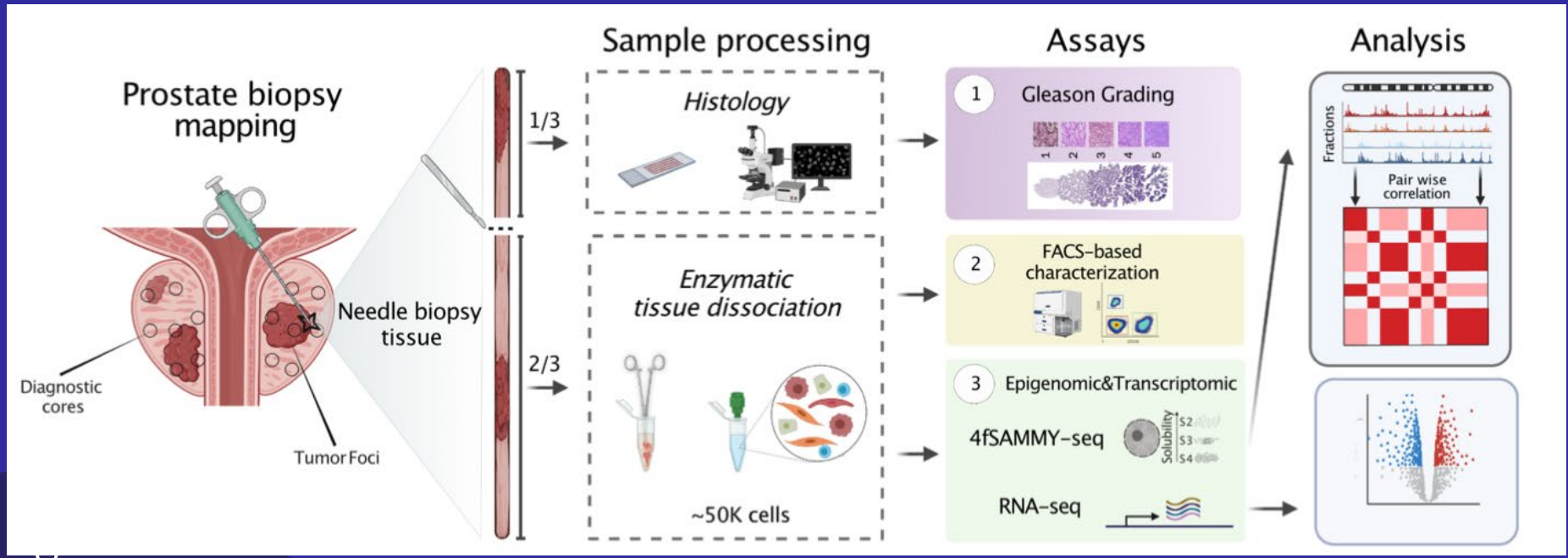
nature

Published: 30 June 2005

Global histone modification patterns predict risk of prostate cancer recurrence

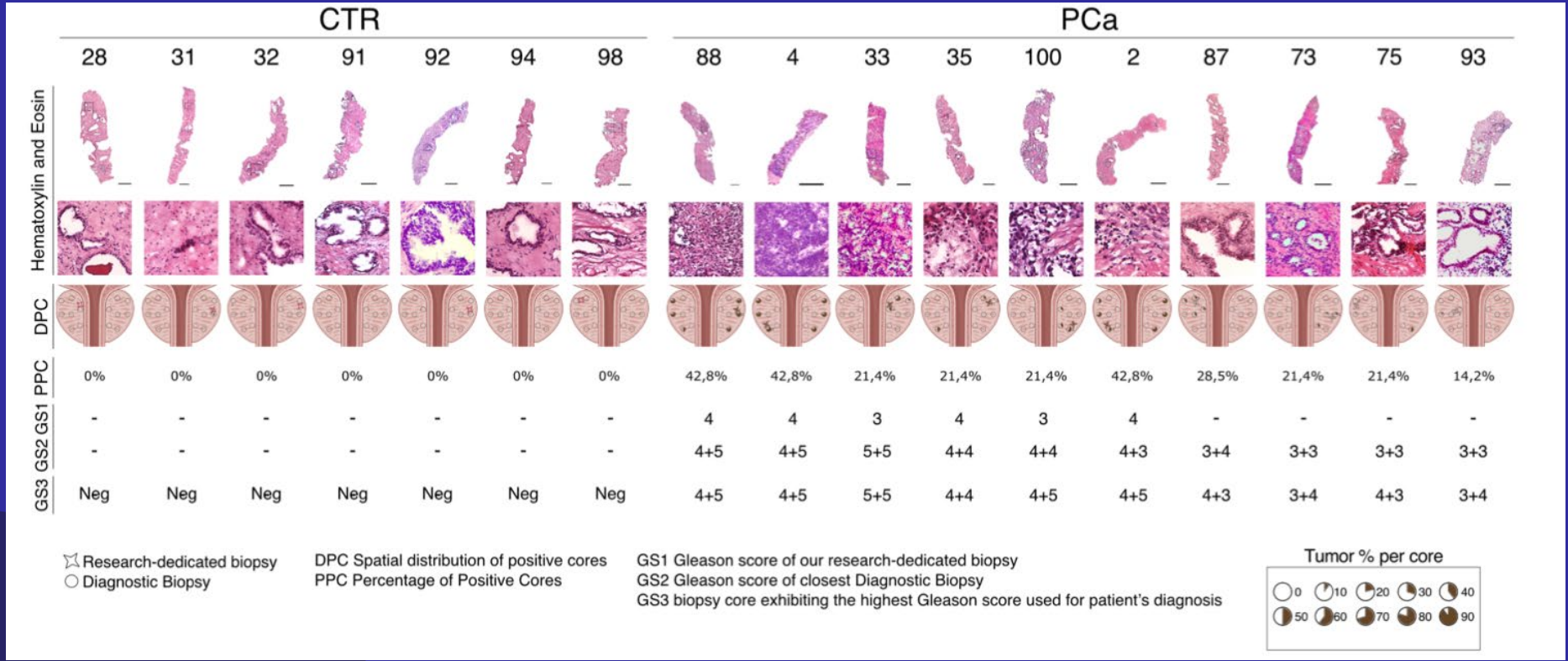
David B. Seligson, Steve Horvath, Tao Shi, Hong Yu, Sheila Tze, Michael Grunstein & Siavash K. Kurdistani

The experimental model:



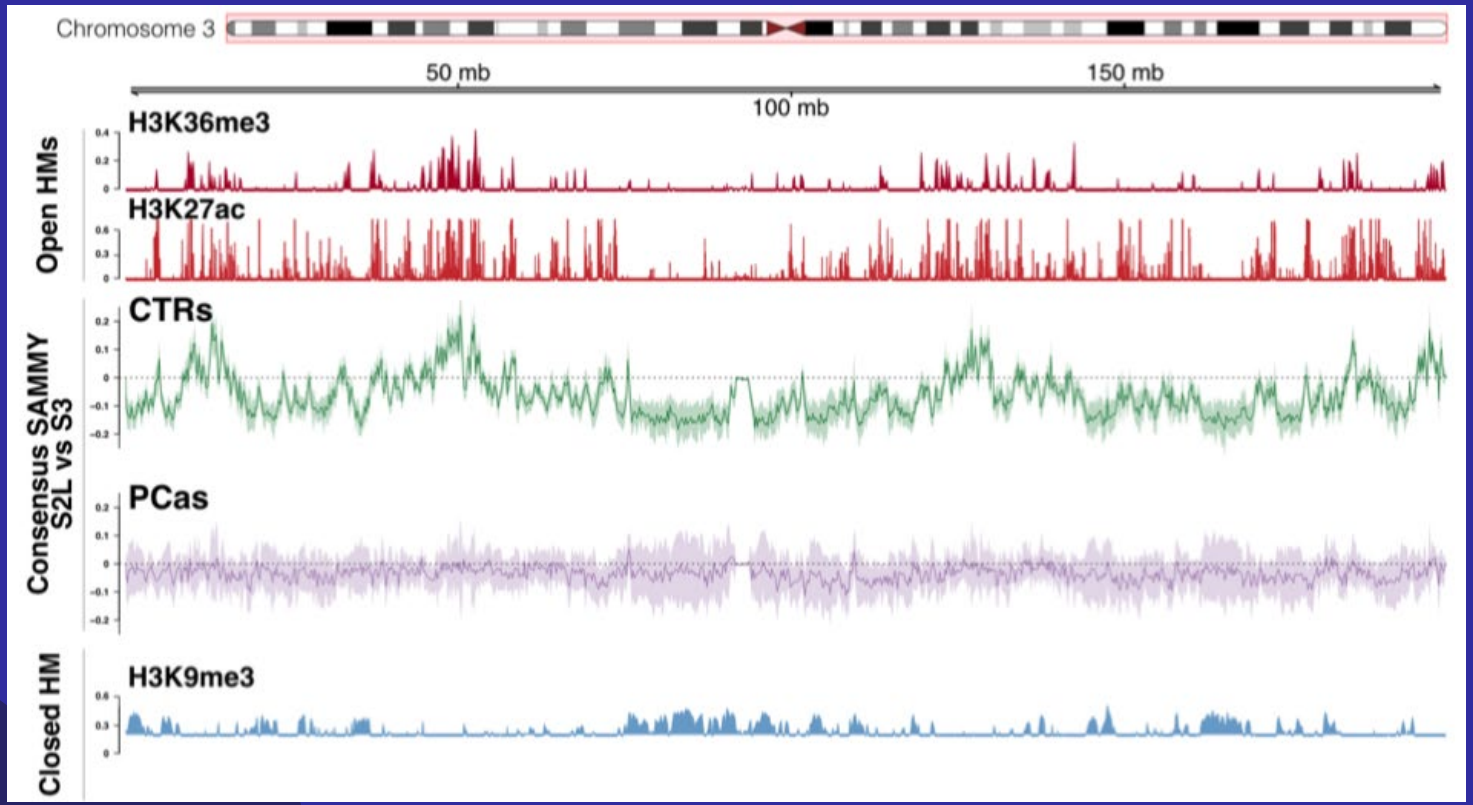
Rosti, Petrini, unpublished data

Solubility and compartment analysis on fresh , 15mg biopsy



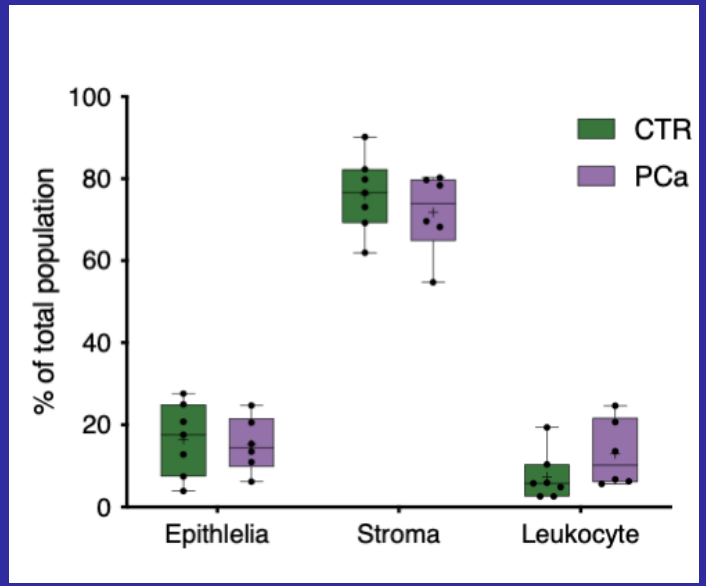
Rosti, Petrini, unpublished data

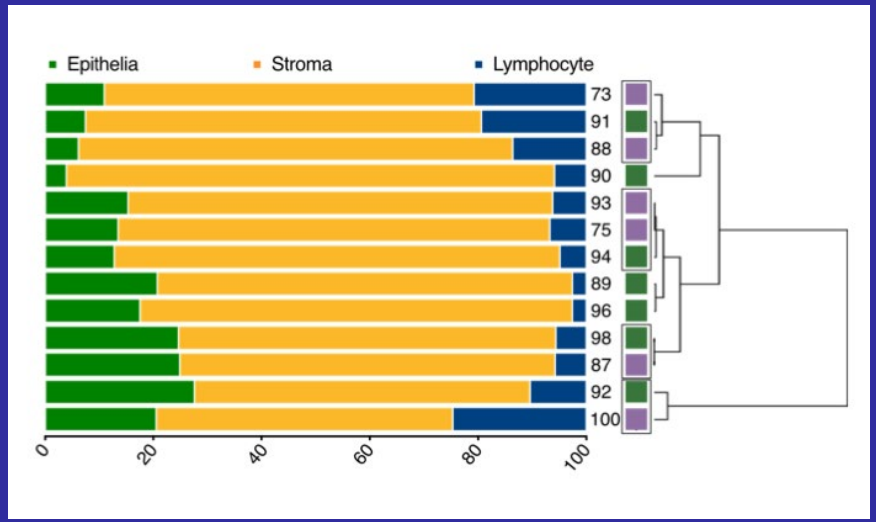
Tumor samples exhibit individual-specific chromatin remodeling



Rosti, Petrini, unpublished data

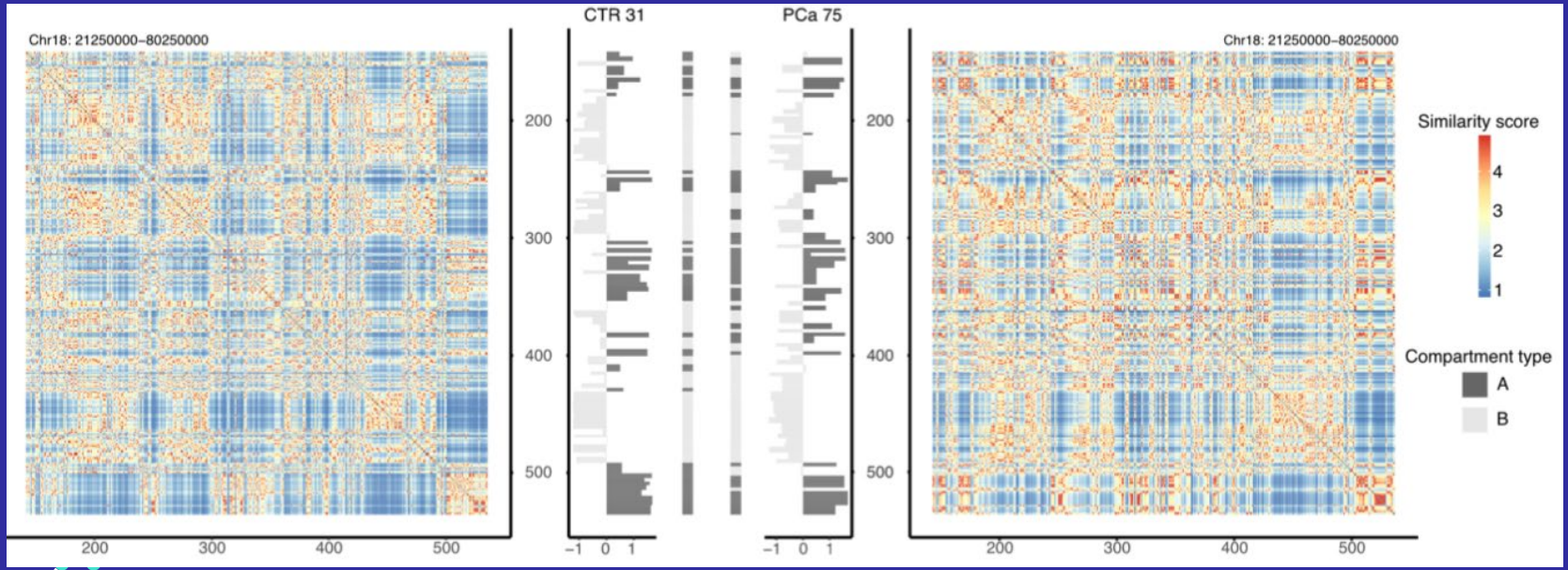
Chromatin profile differences are NOT due to biopsy-specific cell composition





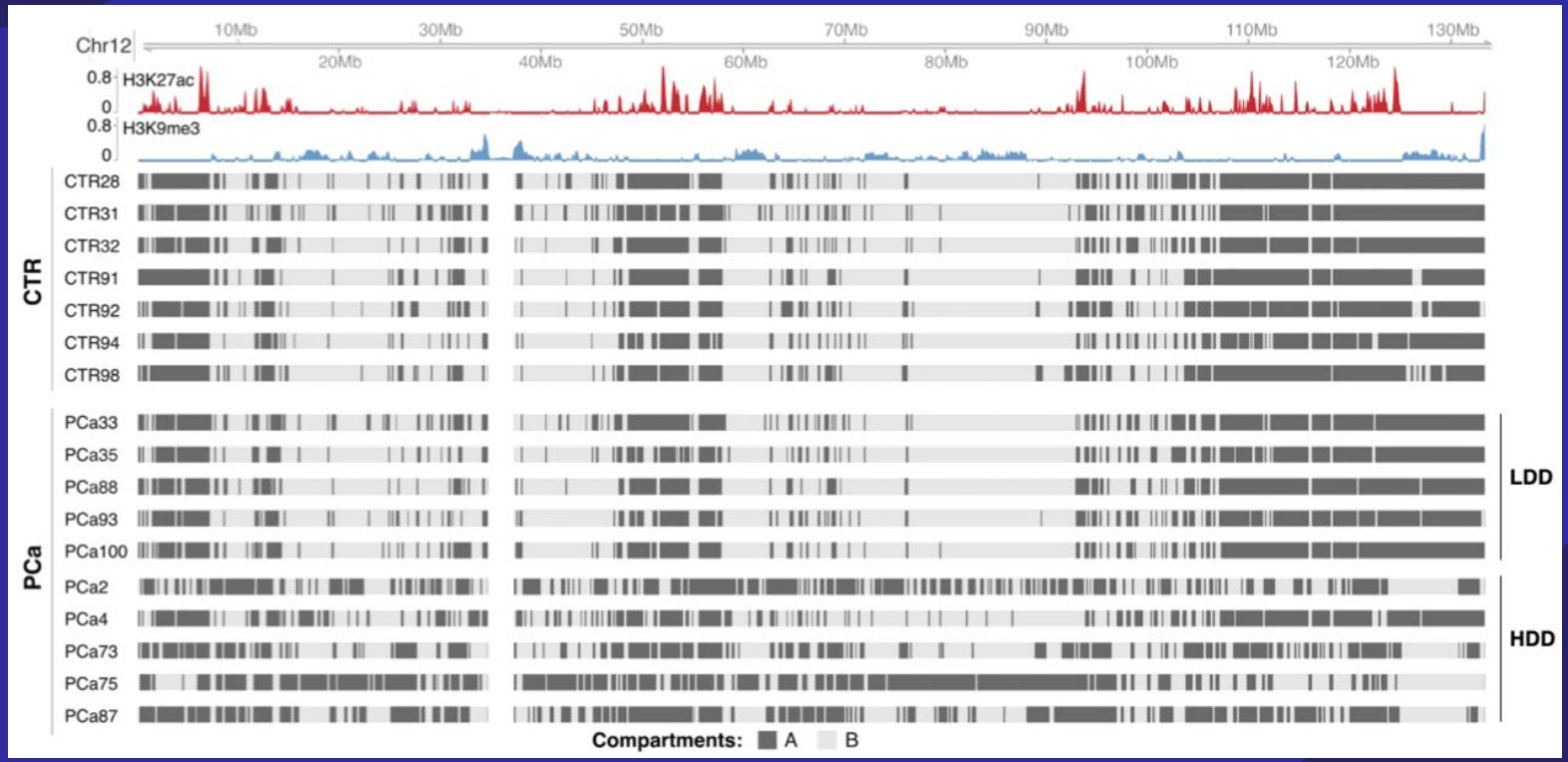
Rosti, Petrini, unpublished data

Definition of two distinct tumor subtypes on the basis of compartments



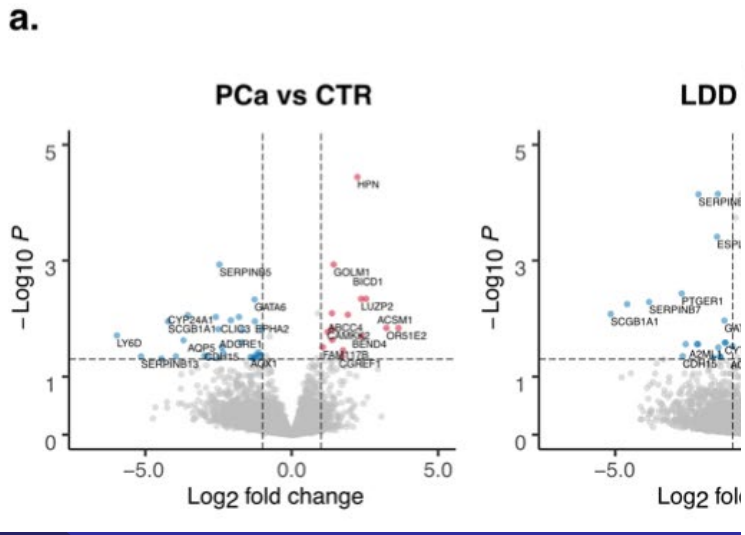
Rosti, Petrini, unpublished data

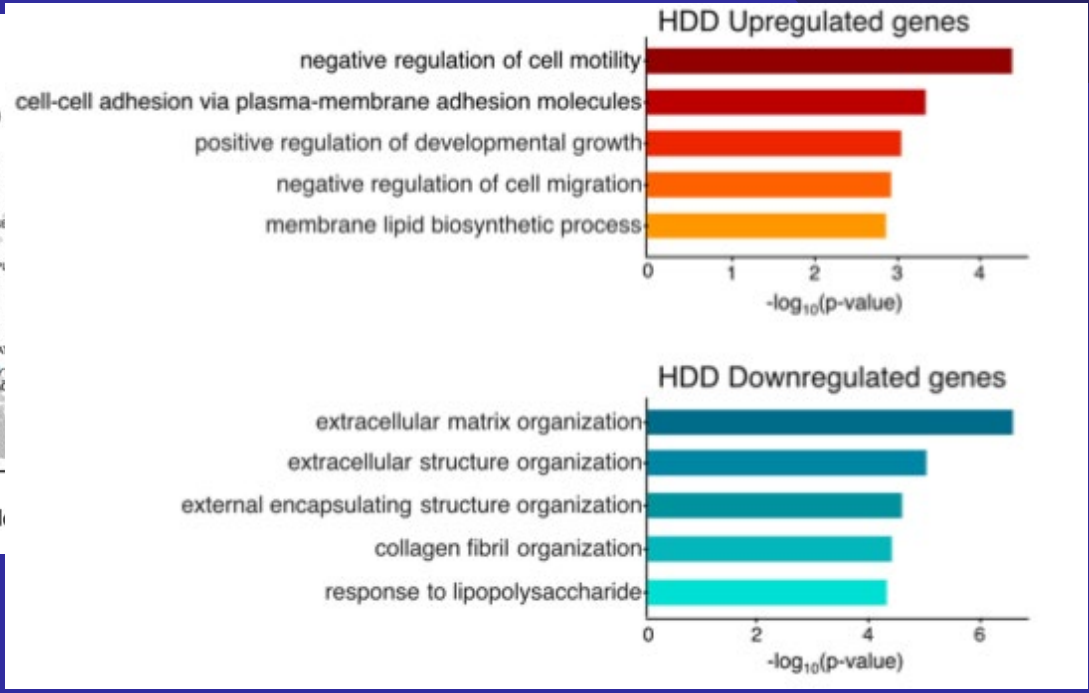
High (HDD) and low (LDD) degree of decompartmentalization



Rosti, Petrini, unpublished data

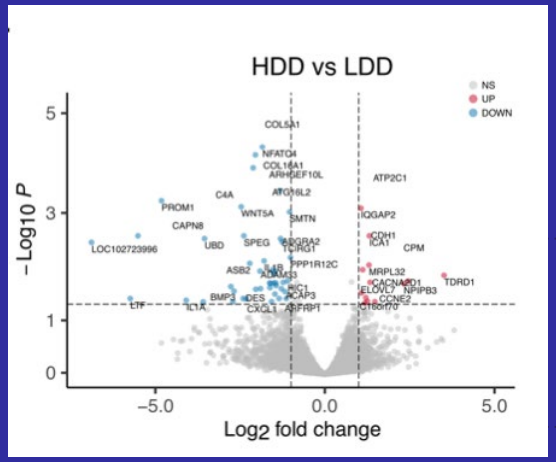
Changesin solubility are associated with dysfunctional transcription of antitumoral pathways



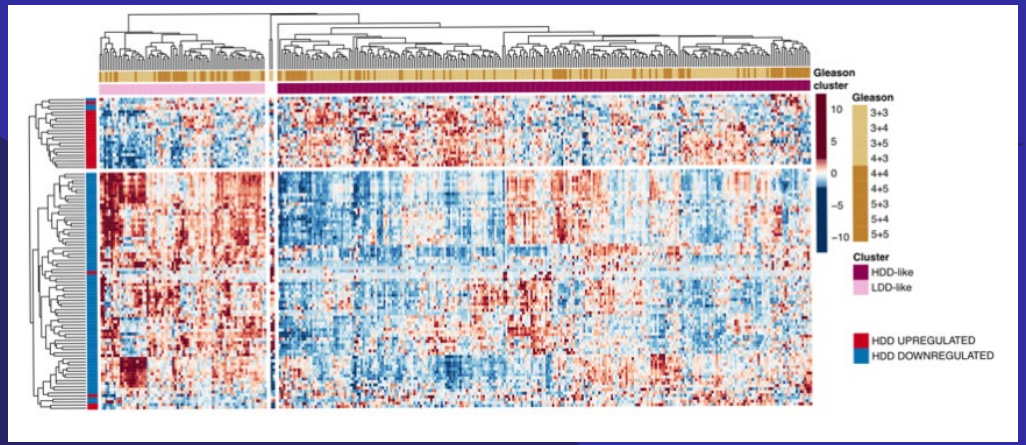


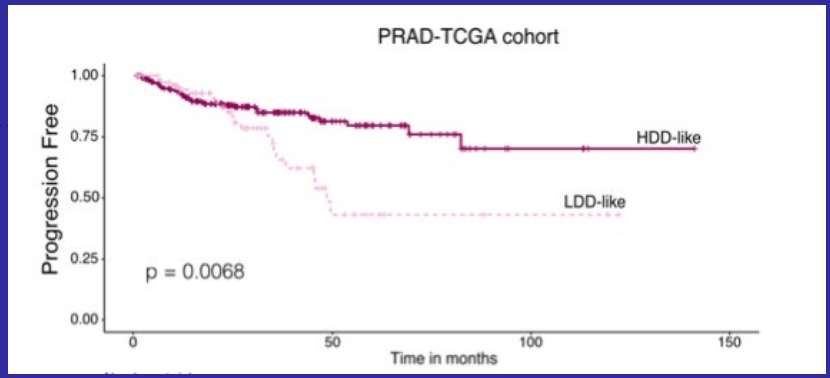
Rosti, Petrini, unpublished data

HDD signature (101 DEGs) correlates with good prognosis



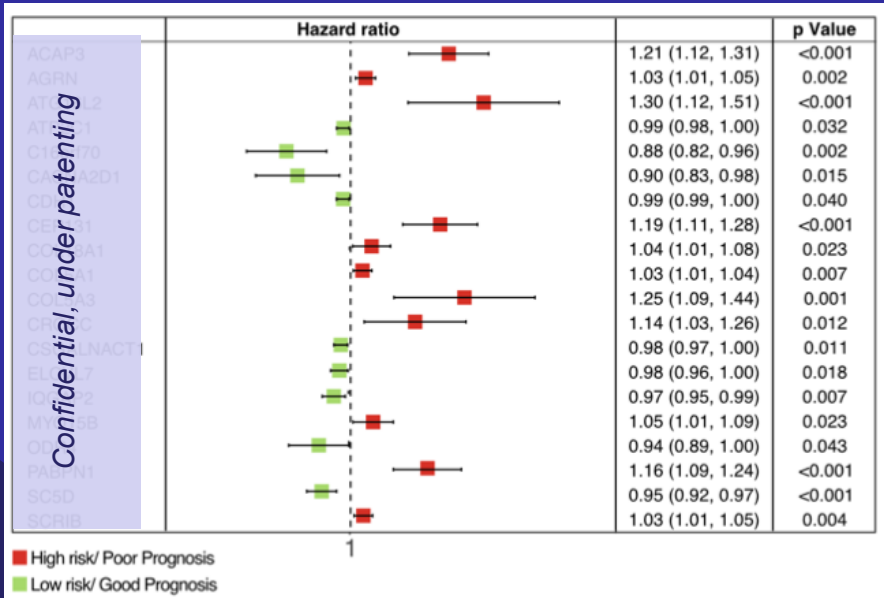
Our signature:
Include up and downregulated genes
Donot overlap with previously published signatures

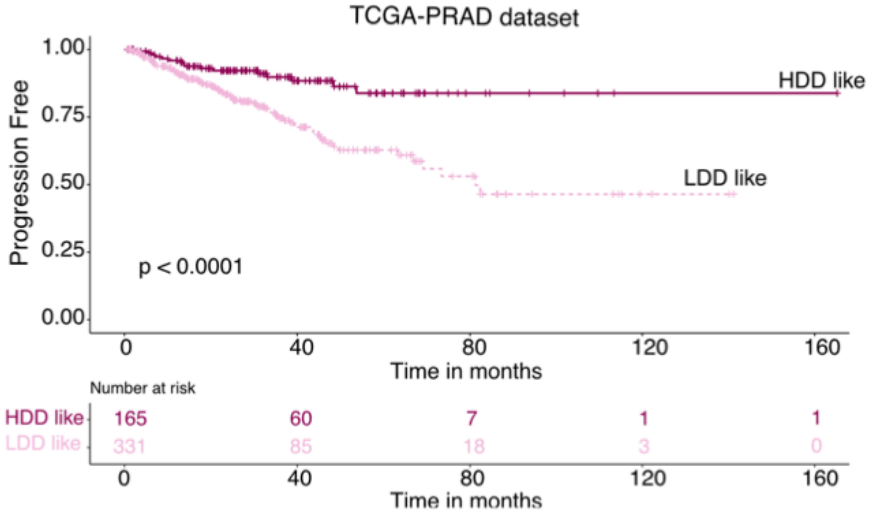




Rosti, Petrini, unpublished data

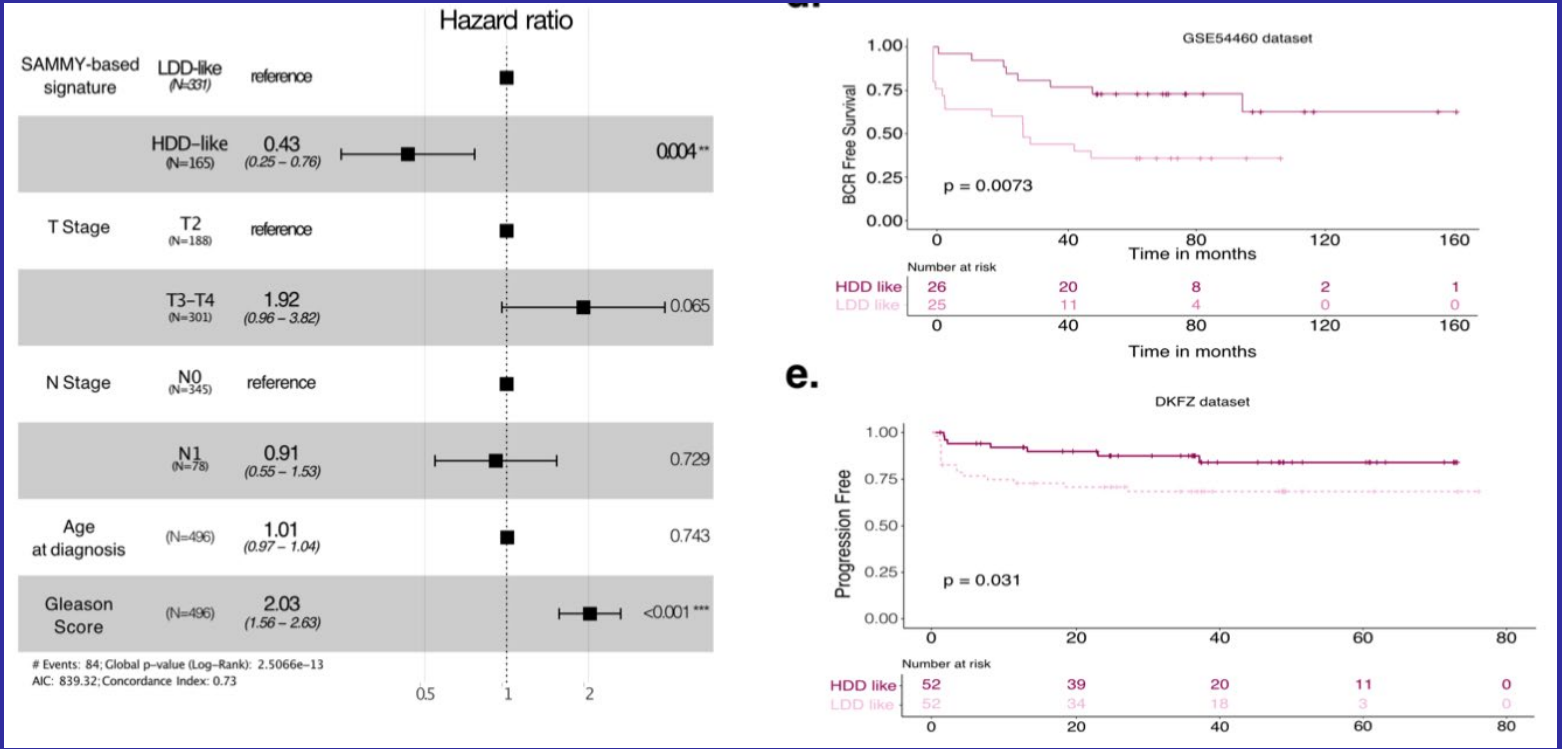
Restriction of signature to 20 genes improves prognostic power





Rosti, Petrini, unpublished data

Chromatin dependent signature allows a prognosis at the time of diagnosis



Rosti, Petrini, unpublished data

Conclusions

- *4fSAMMY-seq captures euchromatin, heterochromatin and compartments with a unique protocol*
- *4fSAMMY-seq identifies functional chromatin solubility states in normal prostate biopsies*
- *Cancer-containing biopsies exhibit alterations in the chromatin compartimentalization*
- *PCa tissues with a severe solubility remodelling show an antitumoral transcriptional activity*
- *HDD signature is associated with good prognosis*

OPEN JUNIOR AND SENIOR POSITIONS IN MY LAB:

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