ERIC International Meeting
New frontiers in CLL Research
25-27 October 2018
Barcelona

ERIC
european research initiative on CLL

Aberrant cell processes in CLL
Beyond genomic aberrations

Iñaki Martin-Subero, PhD

Biomedical Epigenomics Group

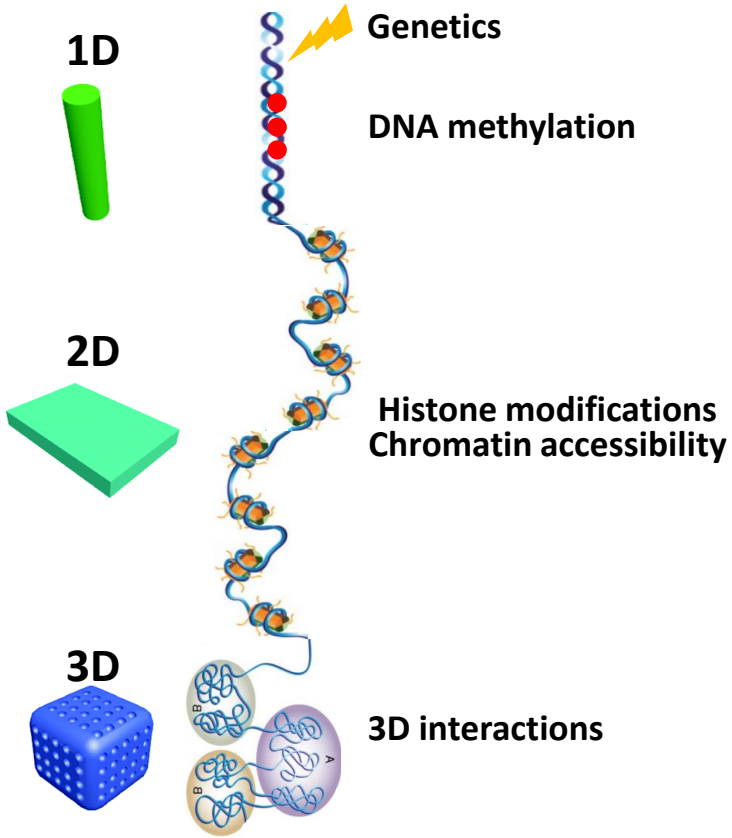
IDIBAPS

ciberonc

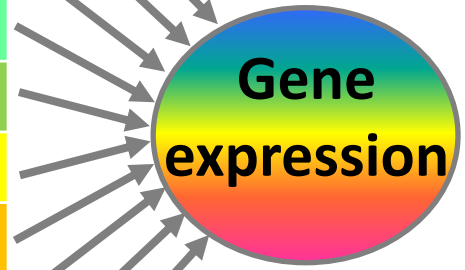
IDIBAPS

UB

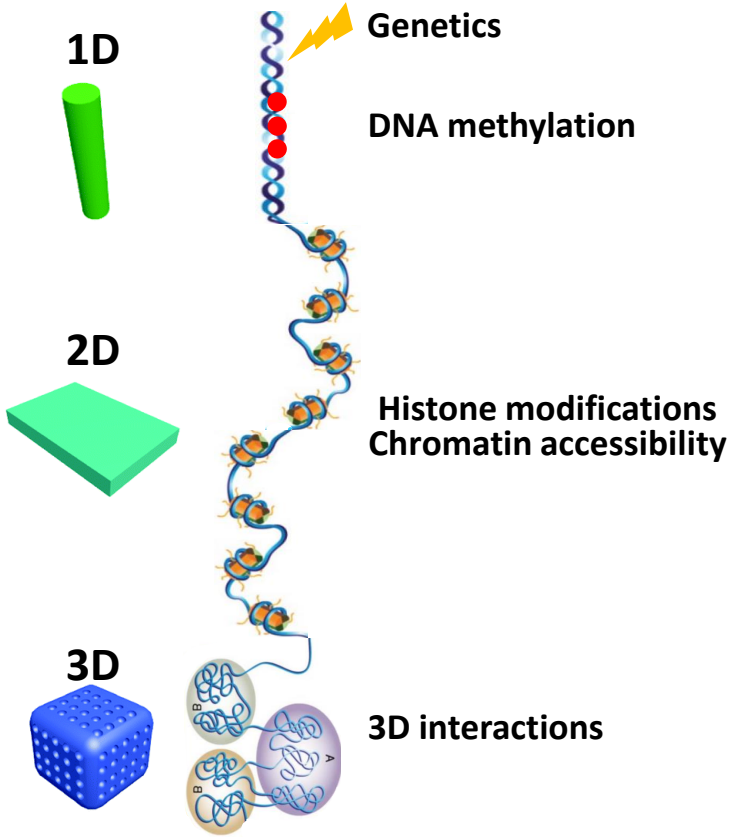
Epigenetic information controls gene expression



LAYER	FUNCTION
Genome	Information storage
DNA methylation	Context dependent
H3K4me3	Promoters
H3K4me1	Enhancers
H3K27ac	Active reg. element
H3K36me3	Txn. Elongation
H3K27me3	Polycomb repression
H3K9me3	Heterochromatin
Chr. accessibility	TF binding
3D interactions	Distant regulation



Three epigenetic stories in CLL



Story 1: DNA methylation
Insights into cellular origin and clinical behavior

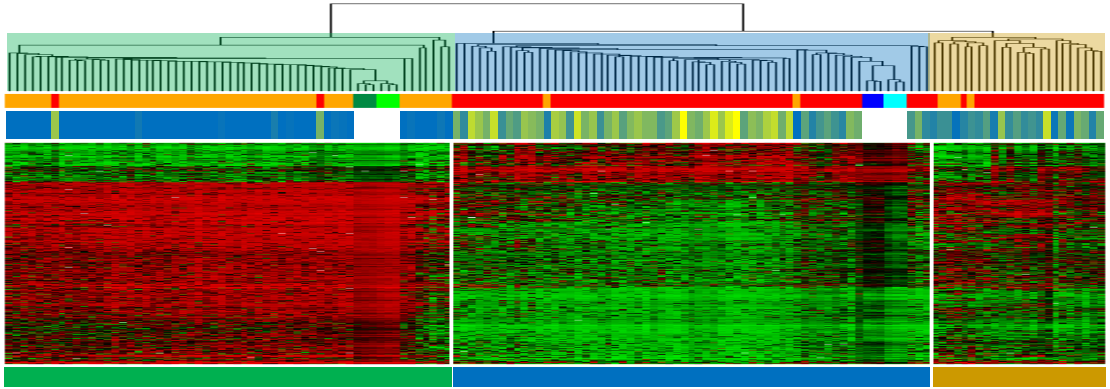
Story 2: Chromatin marks
Alterations in the chromatin landscape

Story 3: 3D stucture
Broad changes in 3D folding

DNA methylation profiling: 3 epigenetic subgroups

Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia

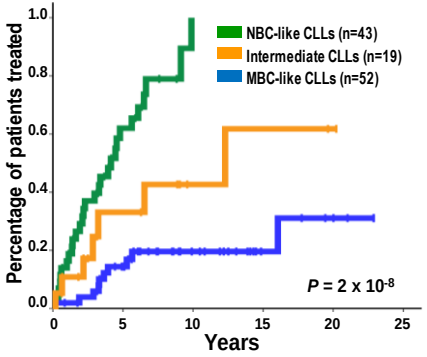
Marta Kulis[1,11], Simon Heath[2,11], Marina Bibikova[3,11], Ana C Queirós[4,11], Alba Navarro[1], Guillem Clot[1], Alejandra Martínez-Trillos[5], Giancarlo Castellano[1], Isabelle Brun-Heath[2], Magda Pinyol[6], Sergio Barberán-Soler[7], Panagiotis Papasaikas[7], Pedro Jares[1], Sílvia Beà[1], Daniel Rico[8], Simone Ecker[8], Miriam Rubio[8], Romina Royo[9], Vincent Ho[3], Brandy Klotzle[3], Lluis Hernández[1], Laura Conde[1], Mónica López-Guerra[1], Dolors Colomer[1], Neus Villamor[1], Marta Aymerich[1], María Rozman[1], Mónica Bayes[2], Marta Gut[2], Josep L Gelpí[9], Modesto Orozco[9], Jian-Bing Fan[3], Víctor Quesada[10], Xose S Puente[10], David G Pisano[8], Alfonso Valencia[8], Armando López-Guillermo[5], Ivo Gut[2], Carlos López-Otín[10], Elías Campo[1] & José I Martín-Subero[4]



Naïve-like CLLs
Low-programmed CLLs
GC-inexperienced CLLs

Memory-like CLLs
High-programmed CLLs
GC-experienced CLLs

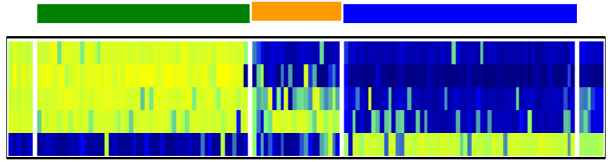
Intermediate CLLs

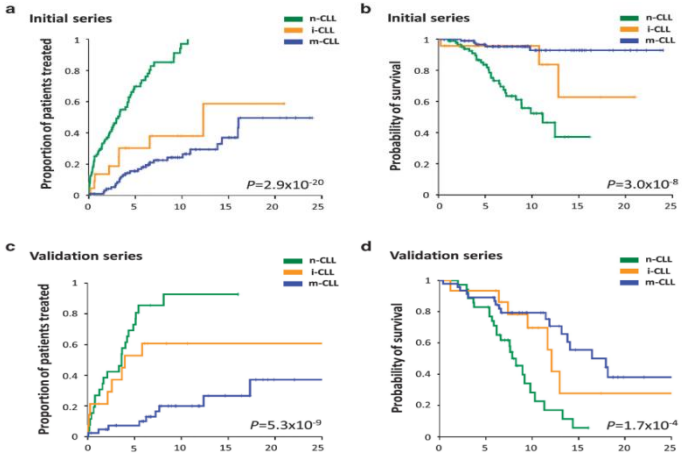


Kulis et al., Nat Genet 2012

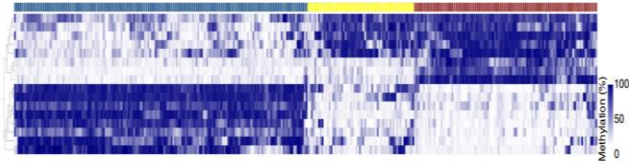
Validation of the 3 clinico-biological subgroups of CLL

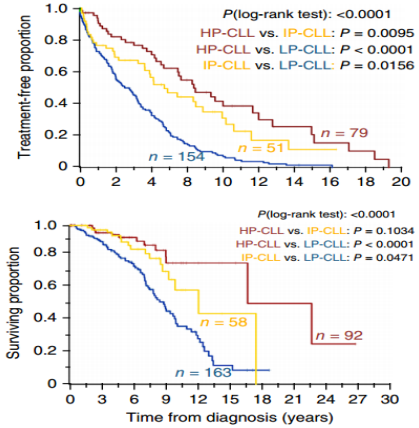
Queiros et al., Leukemia 2015



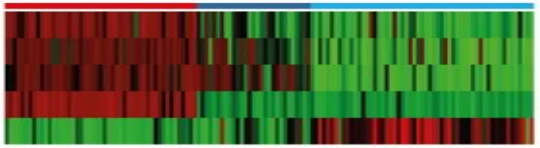


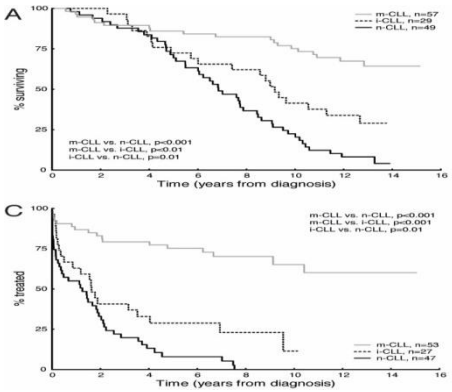
Oakes et al., Nat Genet 2016





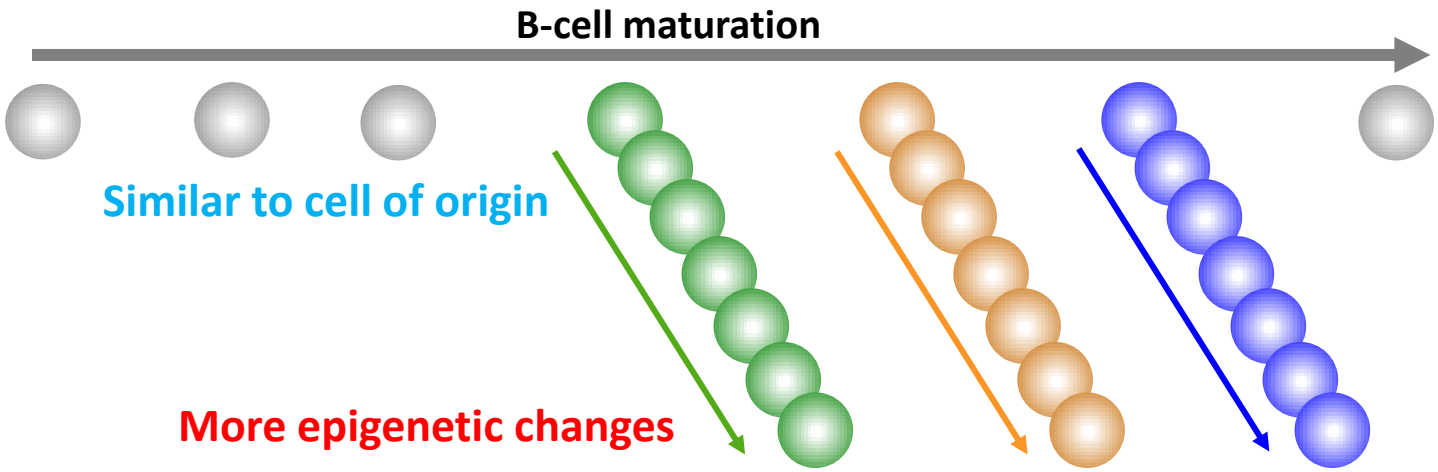
Bhoi et al., Epigenetics 2016





Cell of origin imprints and epigenetic evolution

Less mature CLLs	Intermediate CLLs	More mature CLLs
Naive-like	Intermediate	Memory-like
Low-programmed	Int-programmed	High-programmed
Worse prognosis	*Int-prognosis*	*Better prognosis*



DNA methylation changes in repressed regions represent the proliferative history of a cell

Yang et al. Genome Biology (2016) 17:205
DOI 10.1186/s13059-016-1064-3

Genome Biology

RESEARCH Open Access

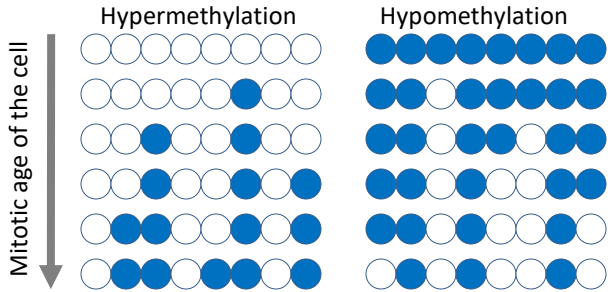
Correlation of an epigenetic mitotic clock with cancer risk

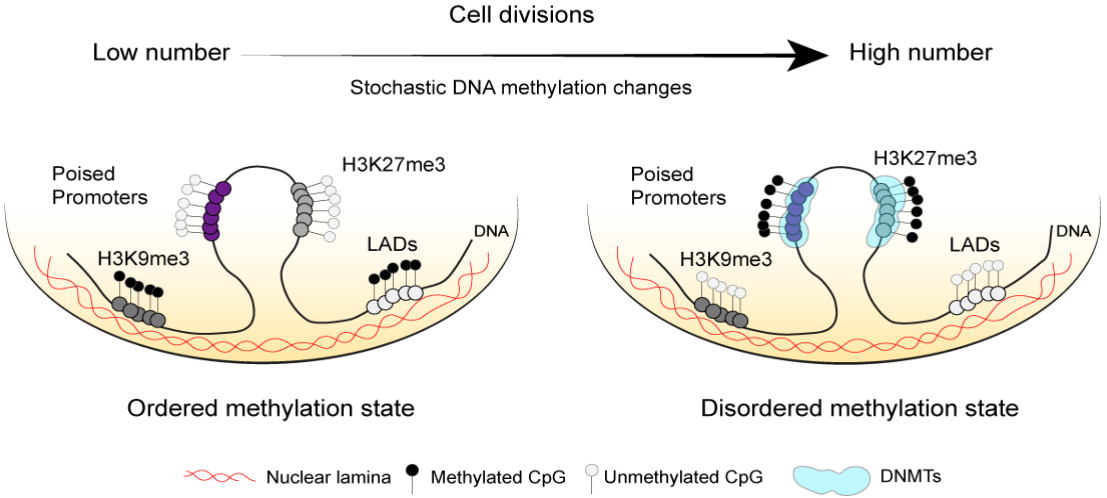
Zhen Yang[1†], Andrew Wong[2], Diana Kuh[2], Dirk S. Paul[3], Vardhman K. Rakyan[4], R. David Leslie[4], Shijie C. Zheng[1], Martin Widschwendter[5], Stephan Beck[3] and Andrew E. Teschendorff[1,5,6*†]

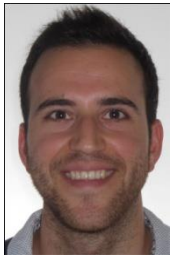
nature genetics ARTICLES
https://doi.org/10.1038/s41588-018-0073-4

DNA methylation loss in late-replicating domains is linked to mitotic cell division

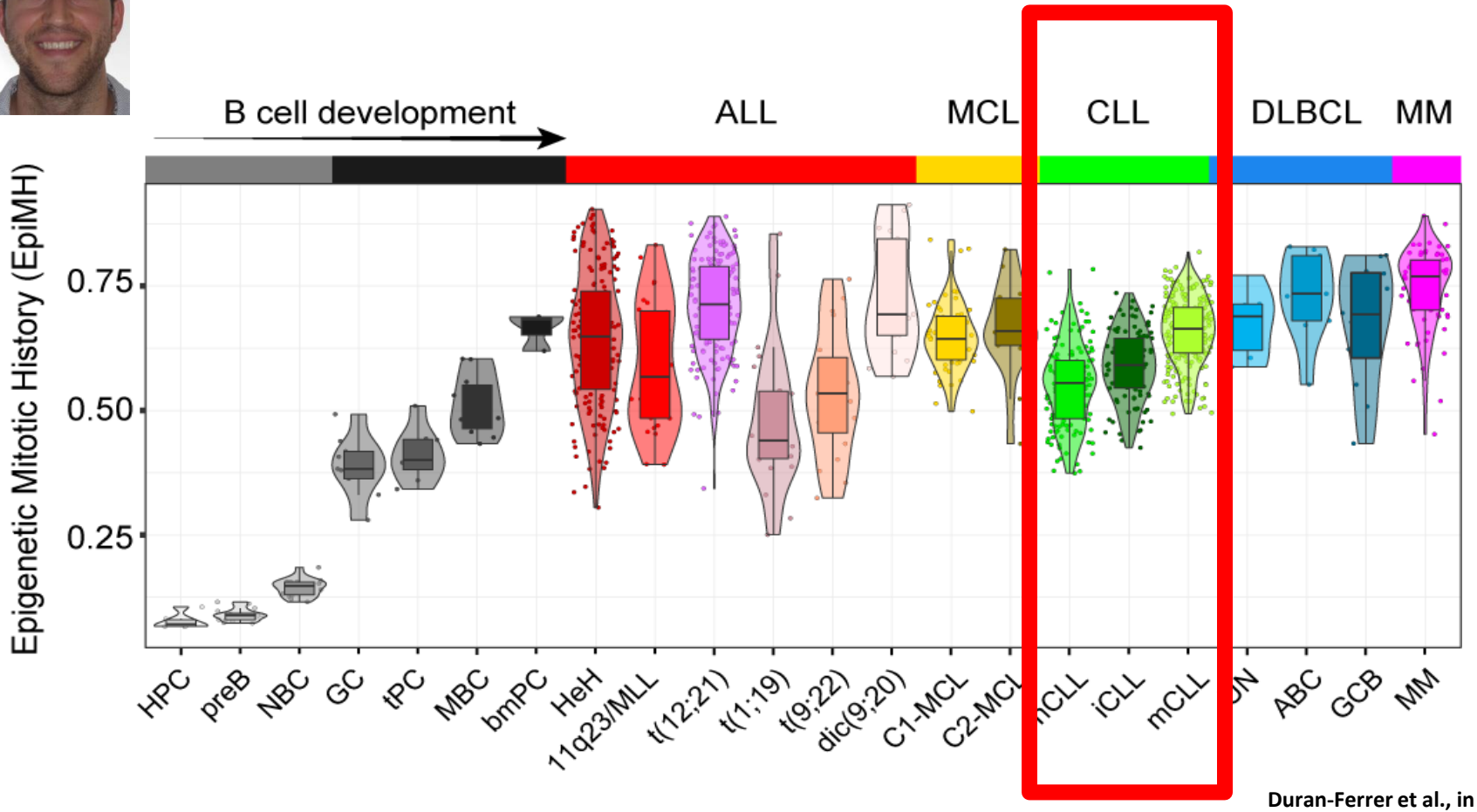
Wanding Zhou[1,5], Huy Q. Dinh[2,5], Zachary Ramjan[3], Daniel J. Weisenberger[4], Charles M. Nicolet[4], Hui Shen[1,6*], Peter W. Laird[1,6*] and Benjamin P. Berman[2,6*]





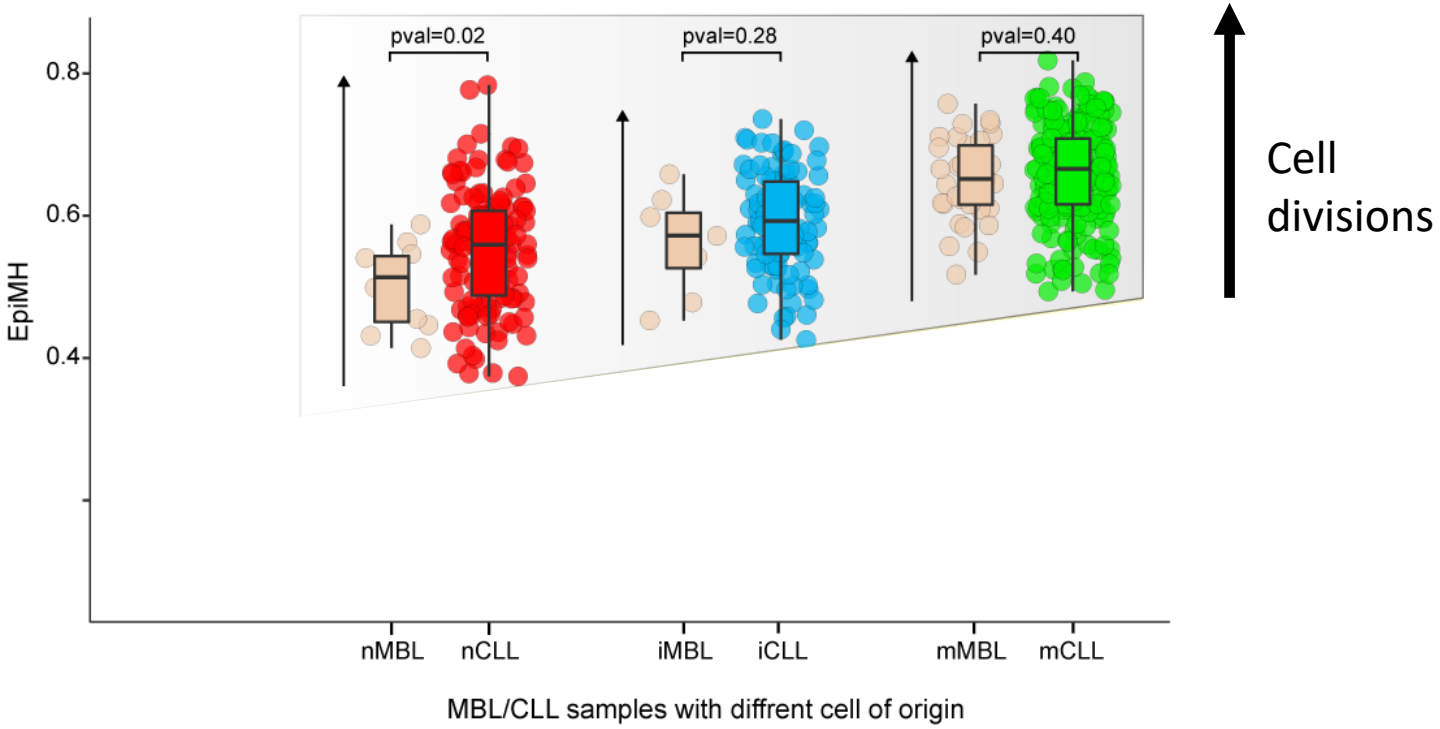


Mitotic history score in normal and neoplastic B cells



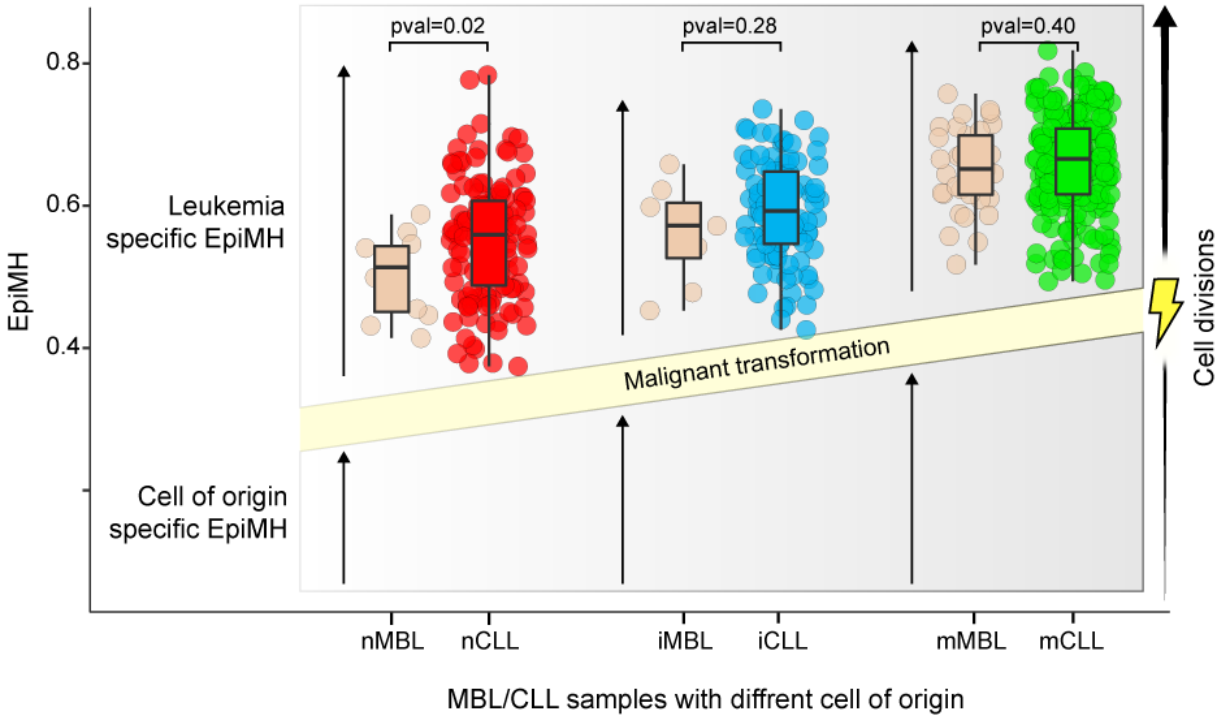
Duran-Ferrer et al., in preparation

Mitotic history in the 3 MBL/CLL subgroups



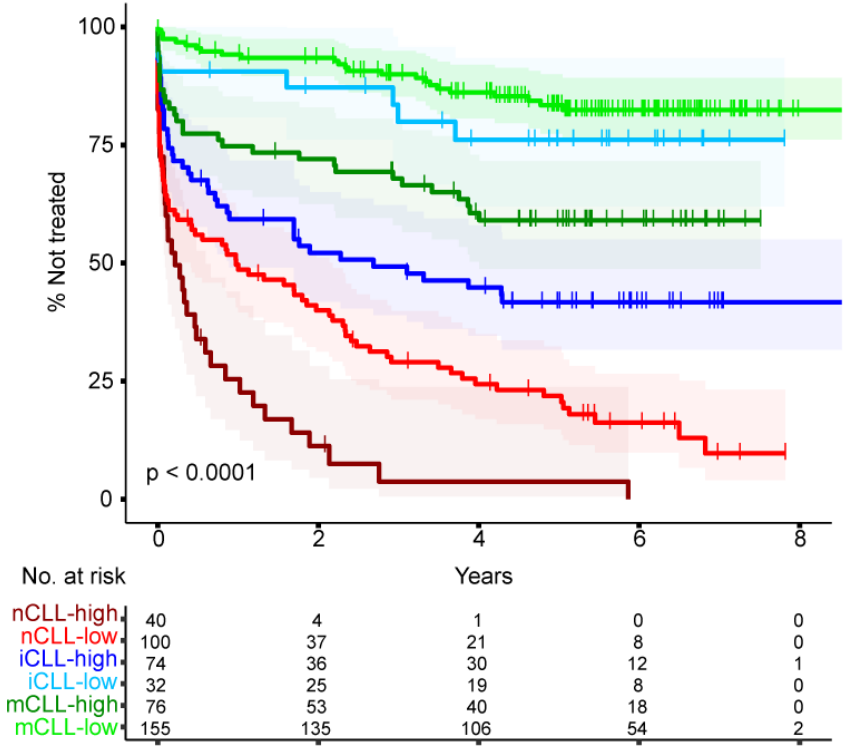
Duran-Ferrer et al., in preparation

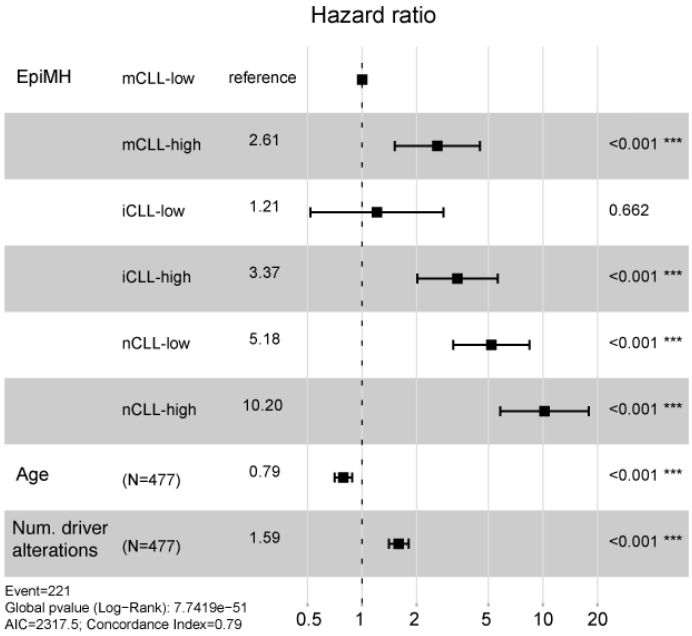
Mitotic history in the 3 MBL/CLL subgroups



Duran-Ferrer et al., in preparation

Cell of origin, mitotic history and clinical behavior





Duran-Ferrer et al., in preparation

Reference epigenomes of CLL: experimental design

	Naive B cells		Germinal center B cells	Memory B cells	Plasma cells	CLL with unmutated IGVH	CLL with mutated IGVH
Surface markers	CD19^{+} IgD^{+} CD27^{-}	CD19^{+} CD20^{+} CD38$^{-/low}$ CD23^{+}	CD19^{+} CD20^{++} CD38^{+}	CD19^{+} IgA^{+}/IGD^{+} CD27^{+}	CD19^{+} CD20^{+} CD38^{++}	CD19^{+}	CD19^{+}
Source	Peripheral blood	Tonsil	Tonsil	Peripheral blood	Tonsil	Peripheral blood	Peripheral blood
Samples (chIP/RNA-seq)	3	3	3	3	3	2	5
Samples (WGBS)	2	0	2	2	2	2	5

BLUEPRINT epigenome

Whole genome bisulfite sequencing (WGBS)

ChIP-seq of H3K4me1, H3K4me3, H3K36me3, H3K27me3, H3K27ac, H3K9me3

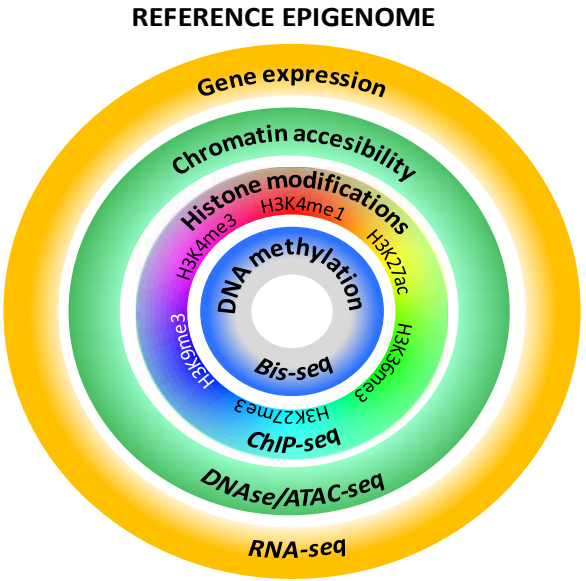
Stranded RNA-seq

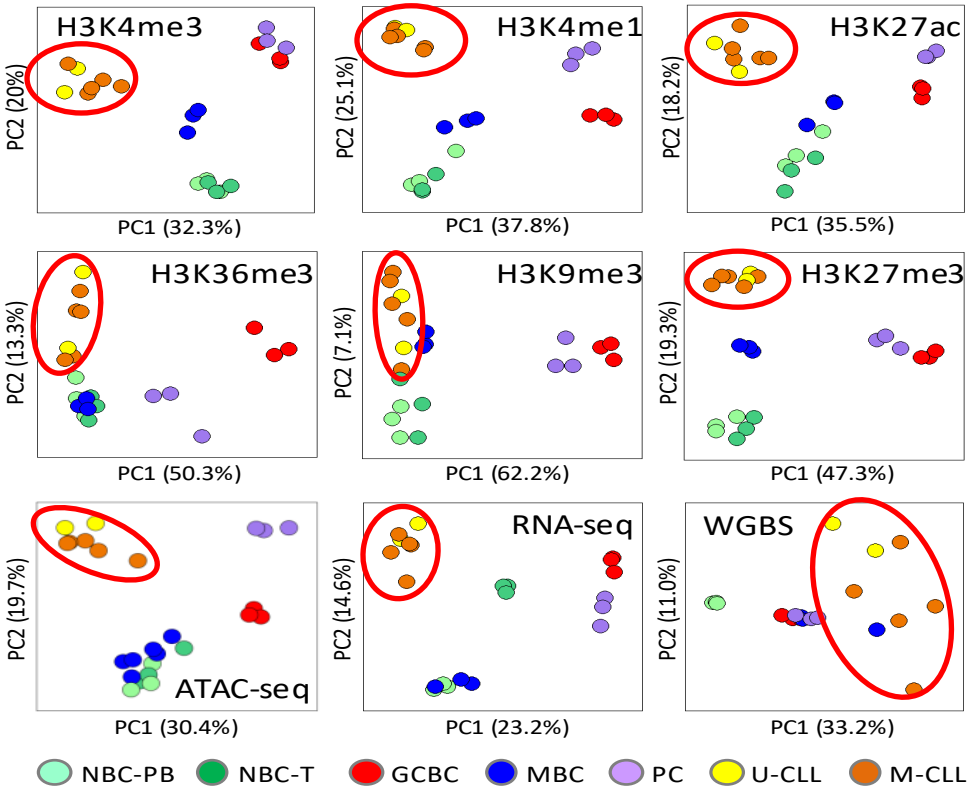
Whole genome sequencing (n=5)
Whole exome sequencing (n=2)
Copy number arrays (n=7)

International Cancer Genome Consortium

Beekman et al., Nat Med 2018

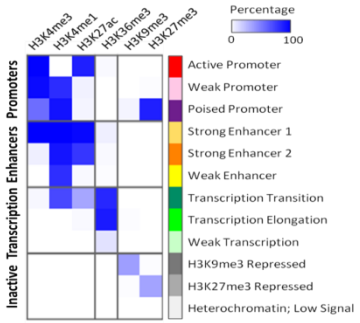
The different layers of a reference epigenome separate CLL from normal B cells

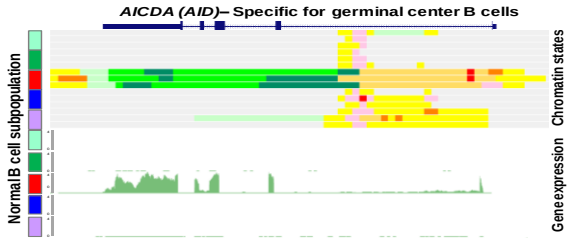


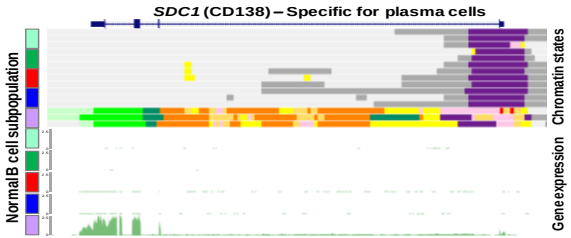


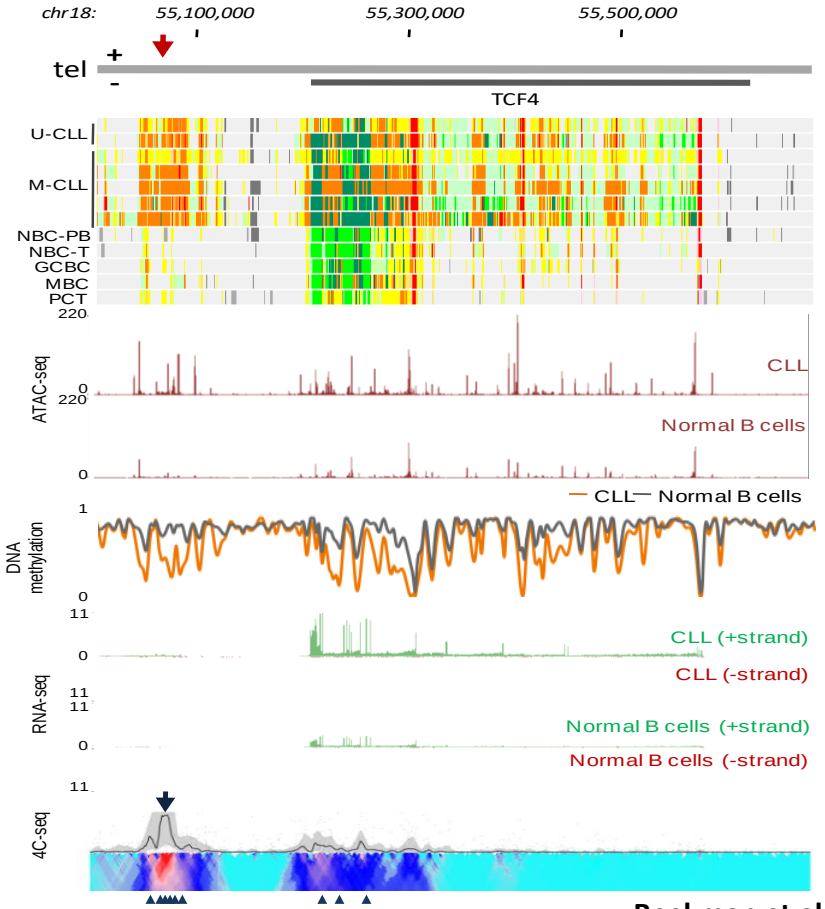
Beekman et al., Nat Med 2018

Integrative analysis of histone modifications



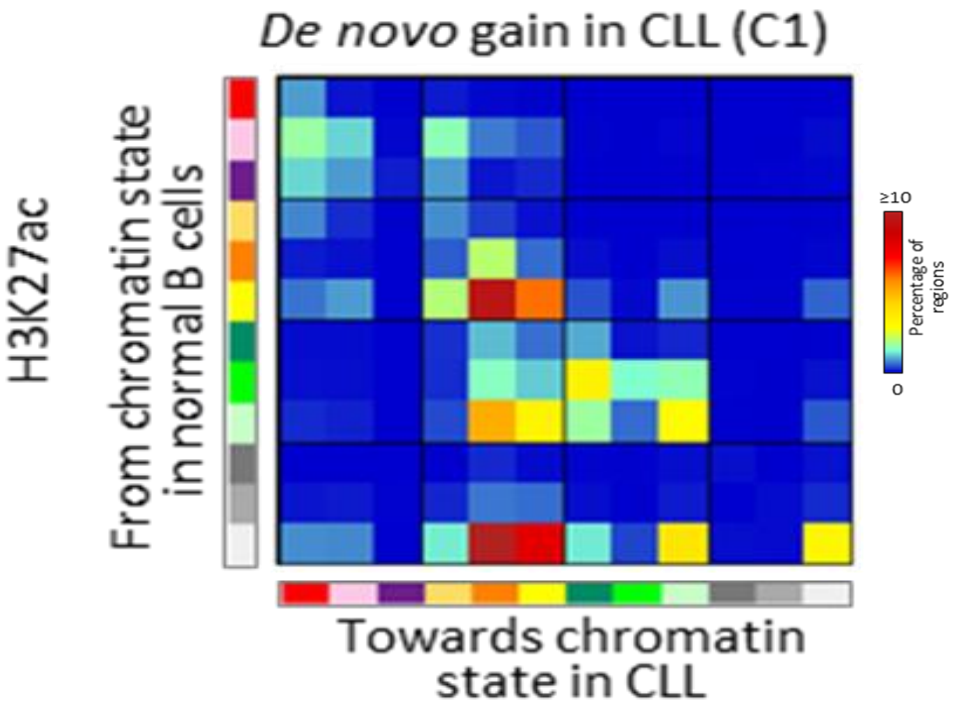


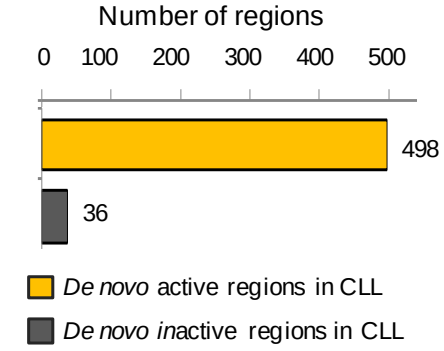




Beekman et al., Nat Med 2018

Integrative analysis of histone marks





NFAT family (P val=6e-16)

FOX family (P val=1e-11)

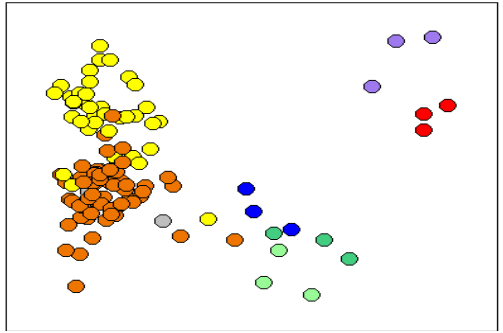
TCF/LEF family (P val=5e-9)

Beekman et al., Nat Med 2018

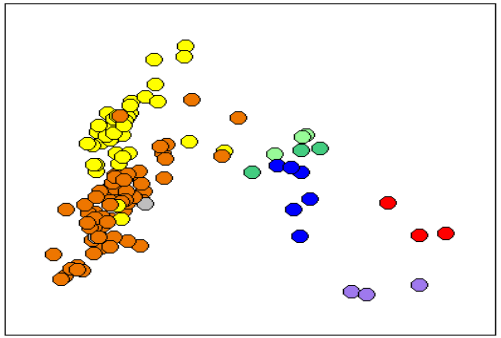
Active chromatin landscape in 107 CLLs

	CLL with unmutated IGVH	CLL with mutated IGVH	CLL with unknown IGVH
Surface markers	CD19+	CD19+	CD19+
Source	Peripheral blood	Peripheral blood	Peripheral blood
Samples	38	66	3

H3K27ac



ATAC



NBC-PB, NBCB-T, GCBC, MBC, PC, NA-CLL, U-CLL, M-CLL

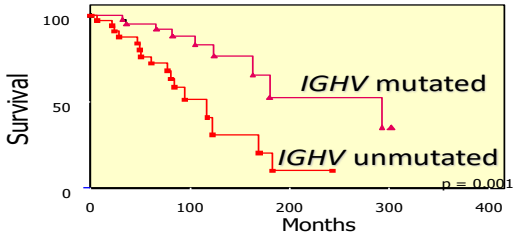
BLUEPRINT epigenome

DNA methylation arrays (450K)

ChIP-seq of H3K27ac and ATAC-seq

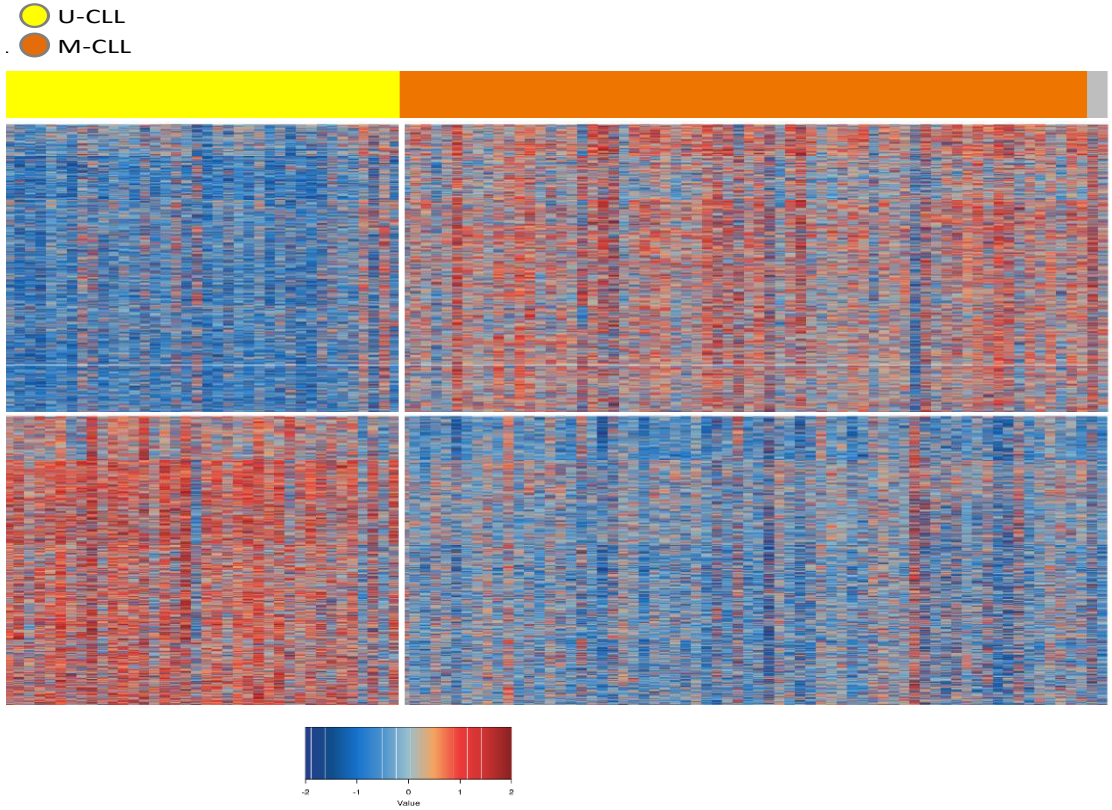
International Cancer Genome Consortium

Whole genome sequencing (n=42)
Whole exome sequencing (n=63)
Copy number arrays (n=107)
RNA-seq (n=74)



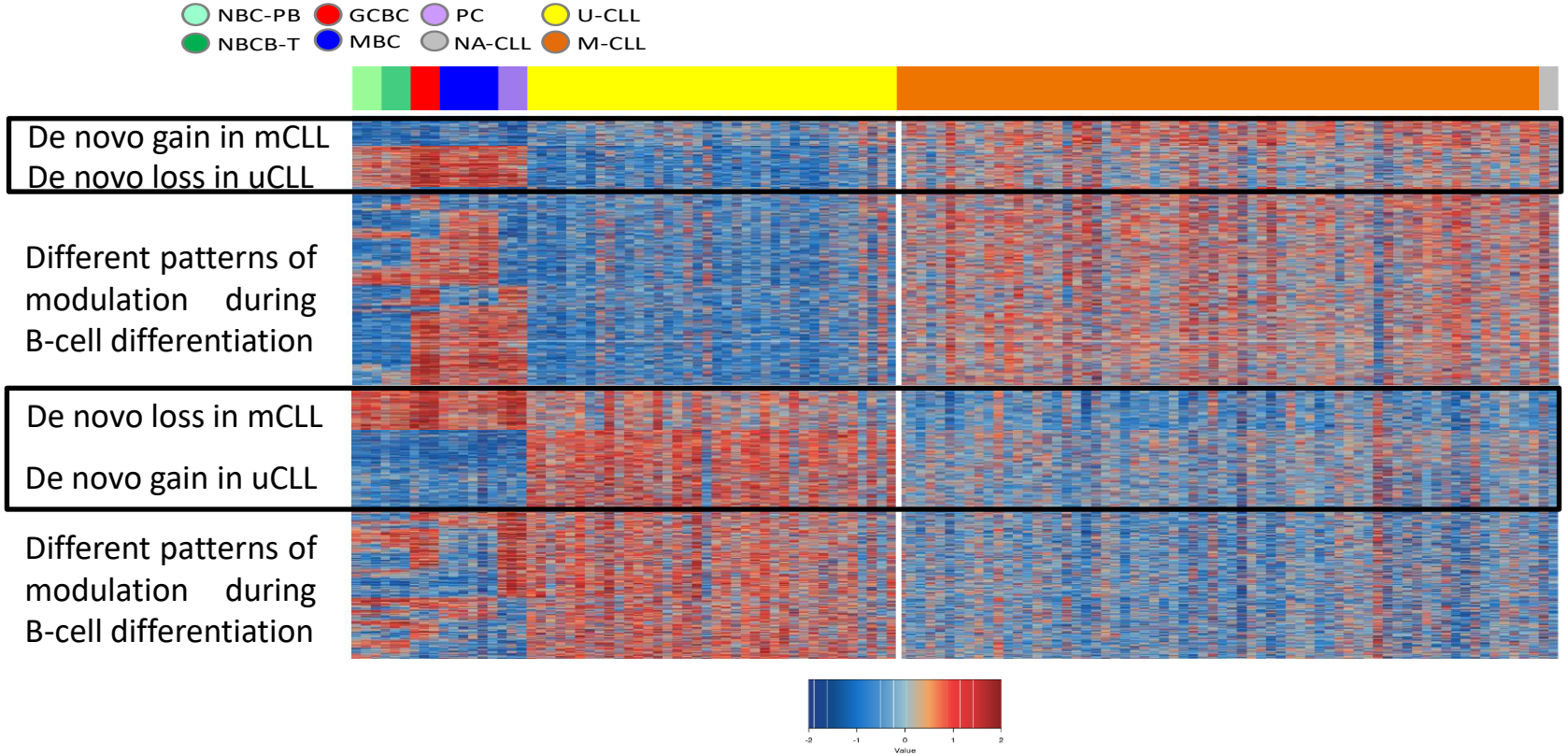
Beekman et al., Nat Med 2018

Differential chromatin accessibility in mCLL and uCLL



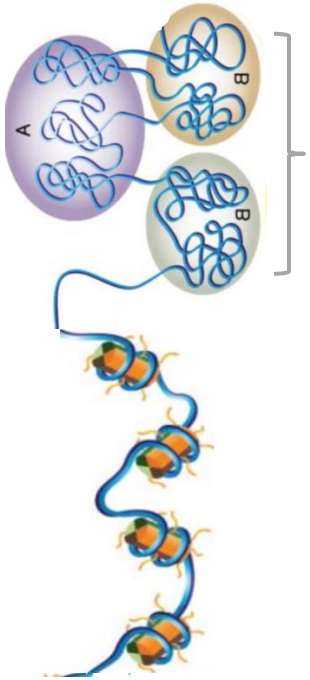
Beekman et al., Nat Med 2018

Differential chromatin accessibility in mCLL and uCLL



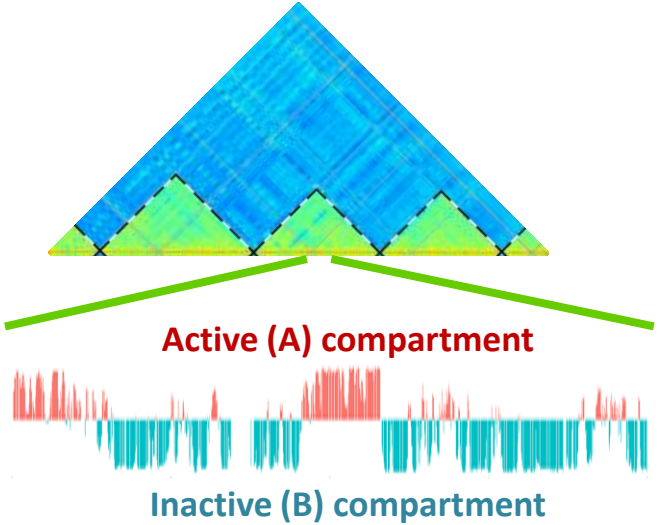
Beekman et al., Nat Med 2018

The 3D genome structure in CLL

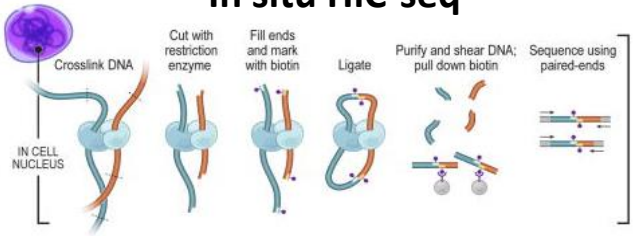


Active (A) compartment

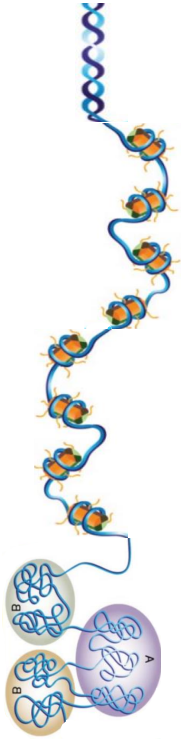
Inactive (B) compartment



In situ HiC-seq

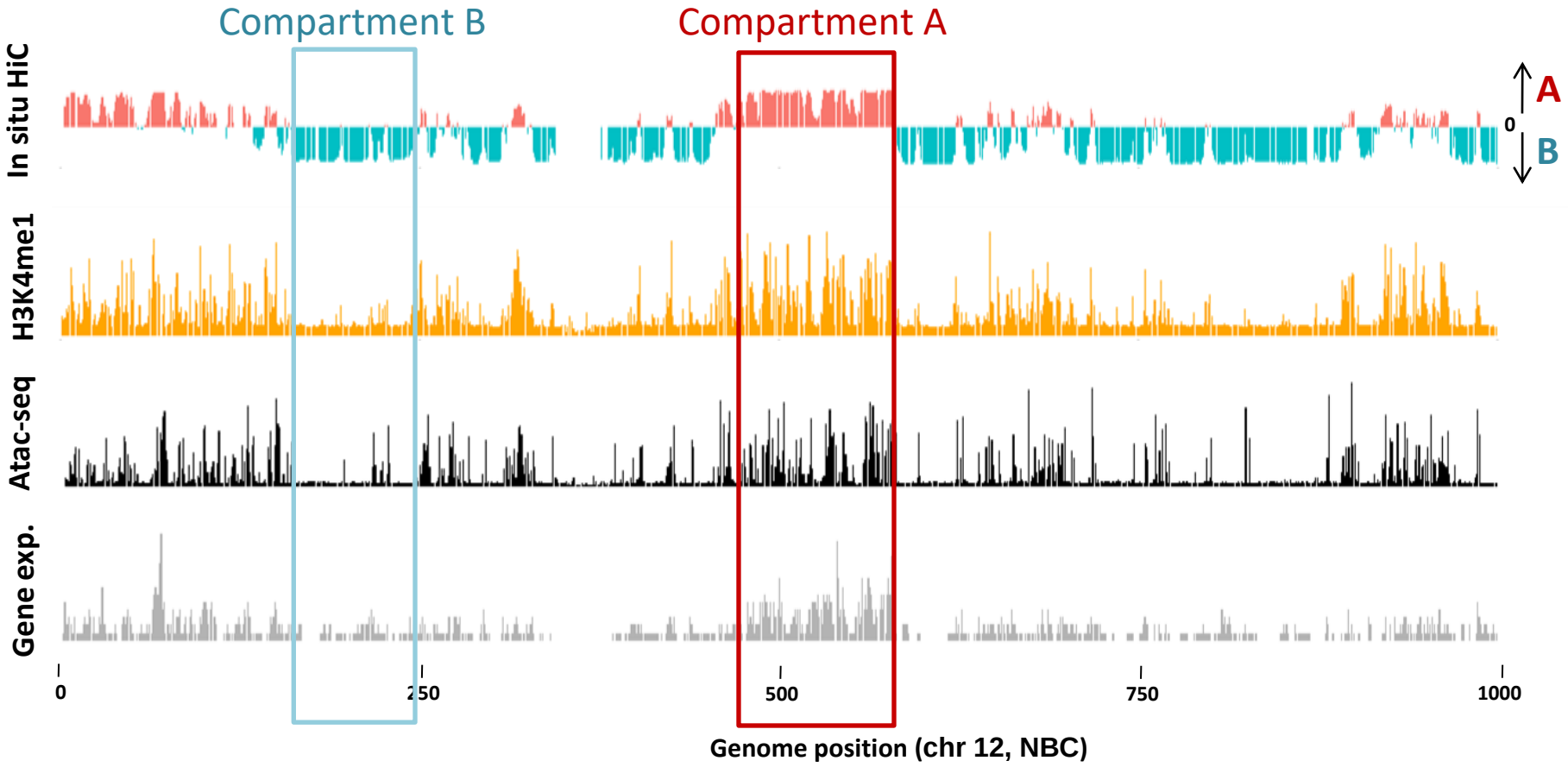


Experimental design: 1D, 2D and 3D CLL genomes



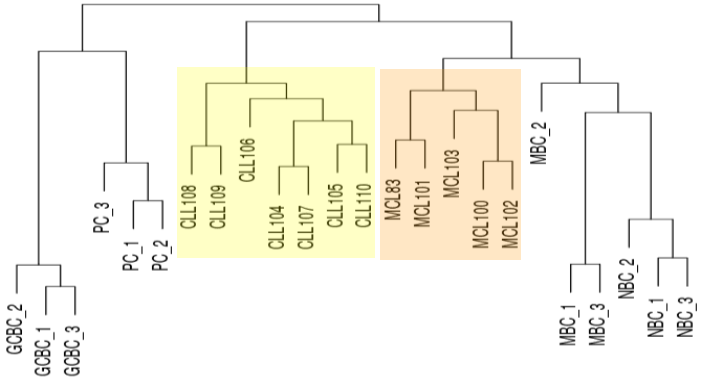
	Normal B cell differentiation				Neoplastic B cells		
	Naive B cells	CG B cells	Memory B cells	Plasma cells	CLL	MCL	Function
RNA-seq	3	3	3	3	7	5	Gene expression
WGBS	3	3	3	3	7	5	DNA methylation Context-dependent
ChIP-seq (H3K4me3)	3	3	3	3	7	5	Promoter
ChIP-seq (H3K4me1)	3	3	3	3	7	5	Enhancer
ChIP-seq (H3K27ac)	3	3	3	3	7	5	Active promoter/enhancer
ChIP-seq (H3K36me3)	3	3	3	3	7	5	Txn. Elongation
ChIP-seq (H3K27me3)	3	3	3	3	7	5	Polycomb-repression
ChIP-seq (H3K9me3)	3	3	3	3	7	5	Long-term repression
ATAC-seq	3	3	3	3	7	5	Accessibility, TF binding
In situ HiC-seq	3	3	3	3	7	5	3D interactions

Classifying the genome in 3D compartments

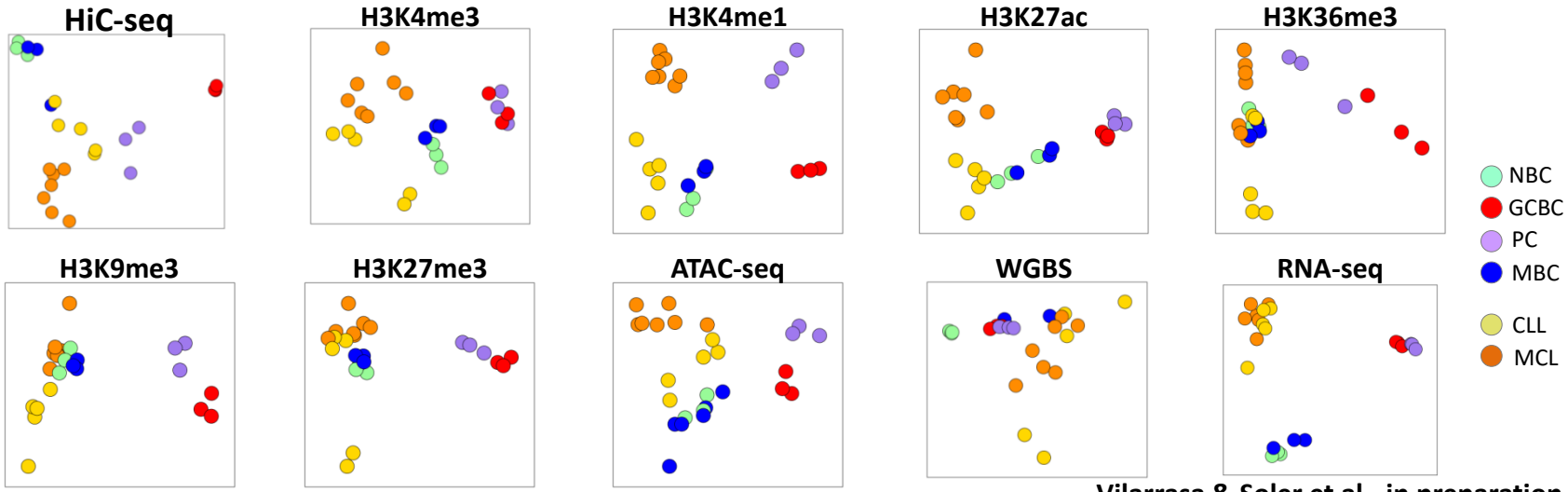


Vilarrasa & Soler et al., in preparation

Unsupervised view of 3D genomes



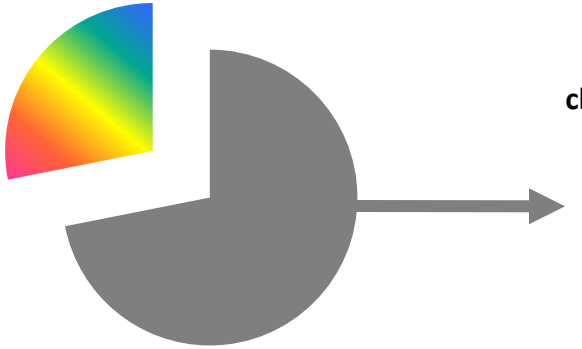
- The 3D genome structure changes during cell differentiation and is maturation stage-specific
- CLLs and MCLs cluster are in the same cluster as NBCs and MBCs
- CLLs and MCL show differential 3D structures



Vilarrasa & Soler et al., in preparation

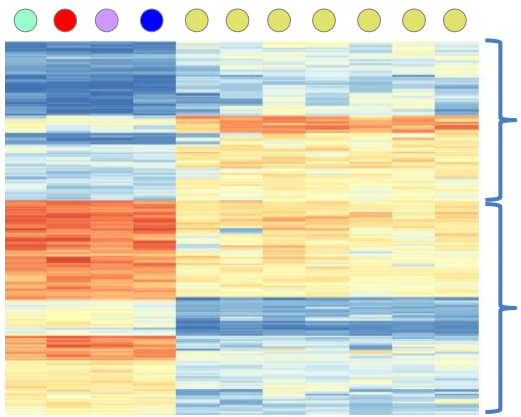
3D genome dynamics in normal B cells and CLL

28% of the 3D genome changes during B-cell differentiation



72% is stable in normal B cells

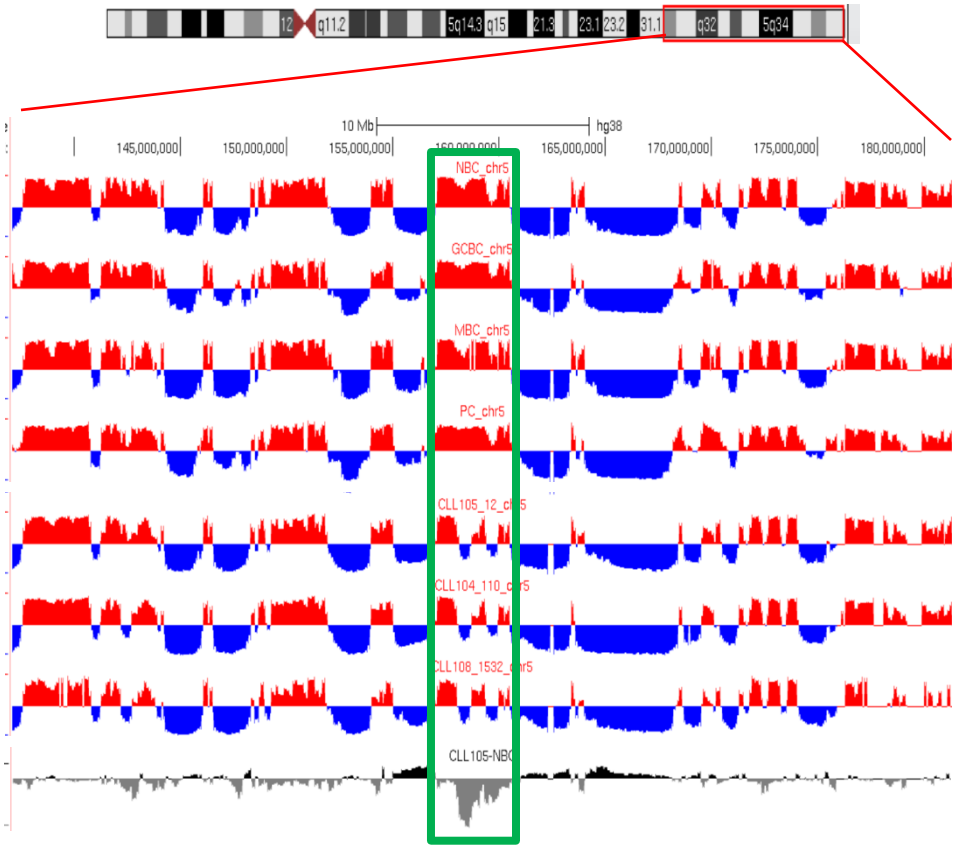
CLL
(348 regions de novo change 3D interactions)

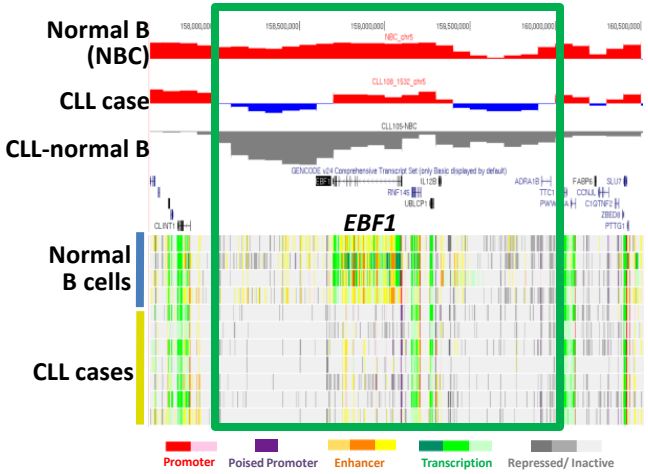


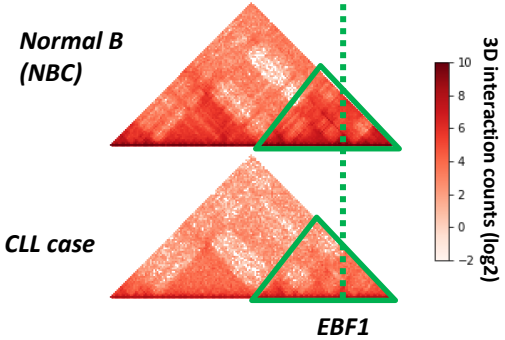
Can we detect tumor-specific changes in the 3D CLL genome structure?

Vilarrasa & Soler et al., in preparation

A block of 2 Mb in chr5 becomes inactive in CLL







Vilarrasa & Soler et al., in preparation

Conclusions

- **DNA methylation imprints of normal B-cell differentiation are useful to determine the cellular origin and clinical behavior CLL.**
- **The proliferative history of CLLs at diagnosis is a strong independent prognostic factor within each CLL subgroup.**
- **The regulatory chromatin landscape is extensively altered in CLL and seems to be mediated by few TF families.**
- **CLL shows an altered 3D genome structure and DNA blocks changing their level of 3D interactions contain genes related to disease pathogenesis.**

Acknowledgements

IDIBAPS, Hospital Cinic and Universitat de Barcelona, Spain

Marta Kulis
Martí Duran-Ferrer
Renee Beekman
Roser Vilarrasa-Blasi
Vicente Chapaprieta
Núria Russiñol
Núria Verdaguer-Dot
Giancarlo Castellano
Ana C. Queirós
Guillem Clot
Sílvia Beà
Marta Aymerich
Armando Lopez-Guillermo
Neus Villamor
Elías Campo

Unversidad de,Oviedo, Spain

Xose Puente
Carlos López-Otín

Radboud University Nijmegen, The Netherlands

Joost H.A. Martens
Hendrik G. Stunnenberg

Euopean Bioinformatics Institute, Hinxton, UK

Avik Datta
Ernesto Lowy
Paul Flicek

University of Navarra, Spain

Xabier Agirre
Felipe Prosper

Max Planck Institue for Molecular Genetics, Berlin, Germany

Alexander Kovacsovics
Marie-Laure Yaspo

Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain

Emanuele Raineri
Angelika Merkel
Simon Heath
Julie Blanc
Marta Gut
Ivo Gut
Paula Soler
François le Dilly
Davide Baù
Marc Martí Renom

Babraham Institute, Cambridge, UK

Biola Javierre
Peter Fraser

Center for Genomic Regulation (CRG), Barcelona, Spain

Sebastian Ullrich
Anna Vlasova
Emilio Palumbo
Roderic Guigo Serra