The Epigenetic Features of CLL: DNA Methylation

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Disclosures

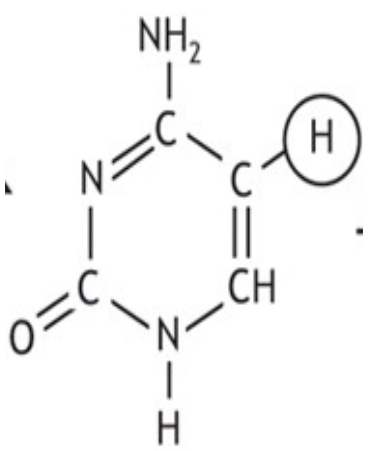
- None

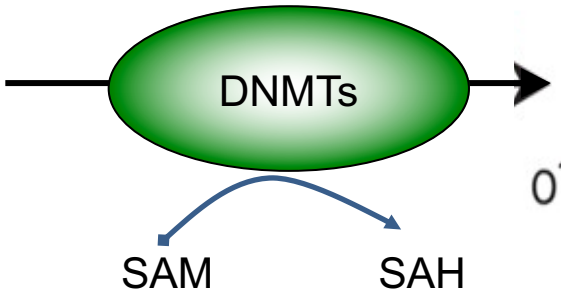
Epigenetic modifications

DNA Methylation

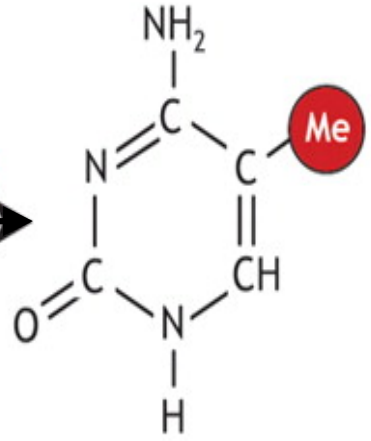
5‘-CpG-3‘
3‘-GpC-5‘

Cytosine





5 Methyl-Cytosine



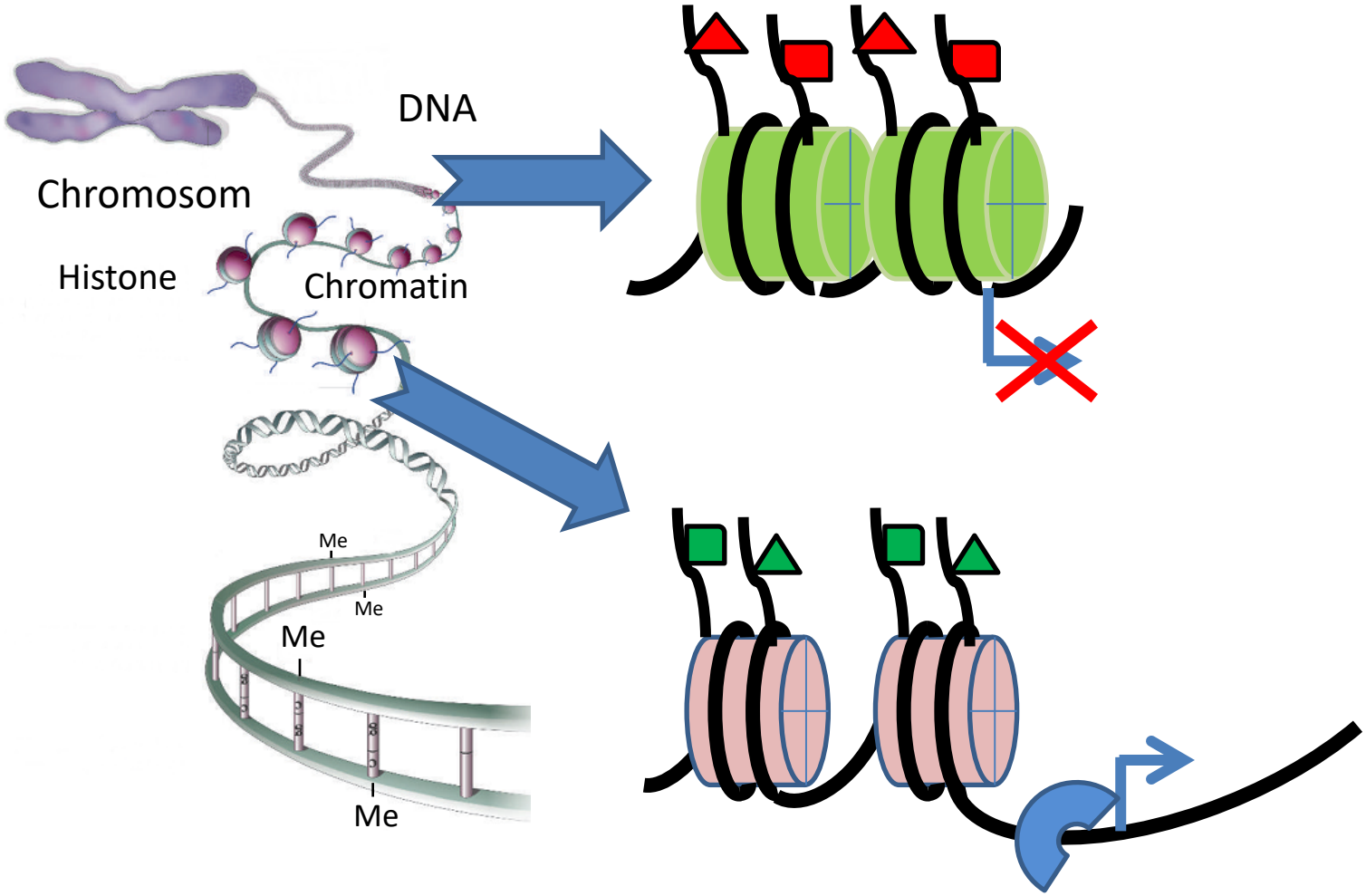
DNMT1
DNMT3a
DNMT3b

DNMT: DNA Methyltransferase

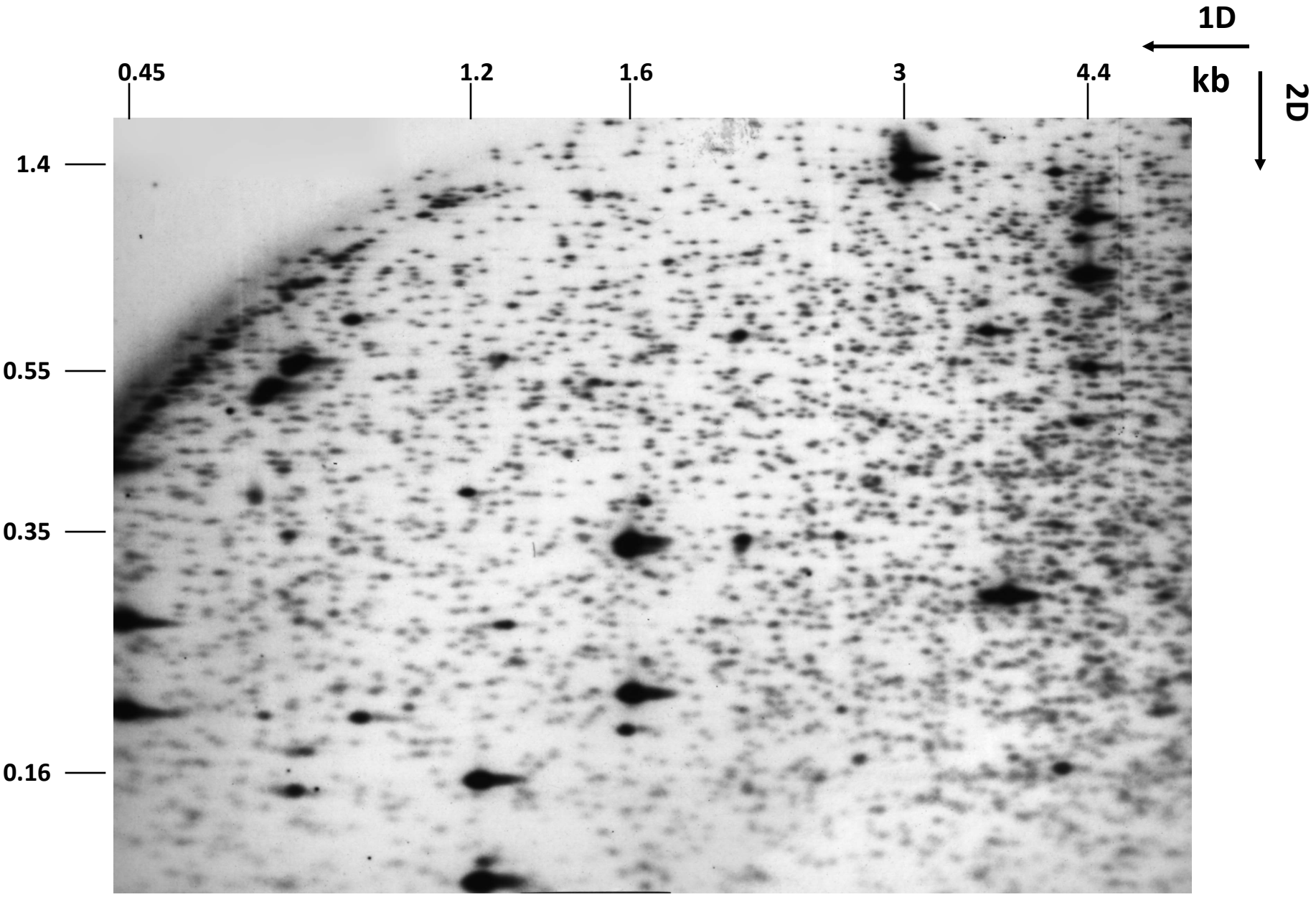
SAM: S-Adenosyl methionine, SAH: S-Adenosyl homocysteine

Epigenetic Modifications

Histone Modifications

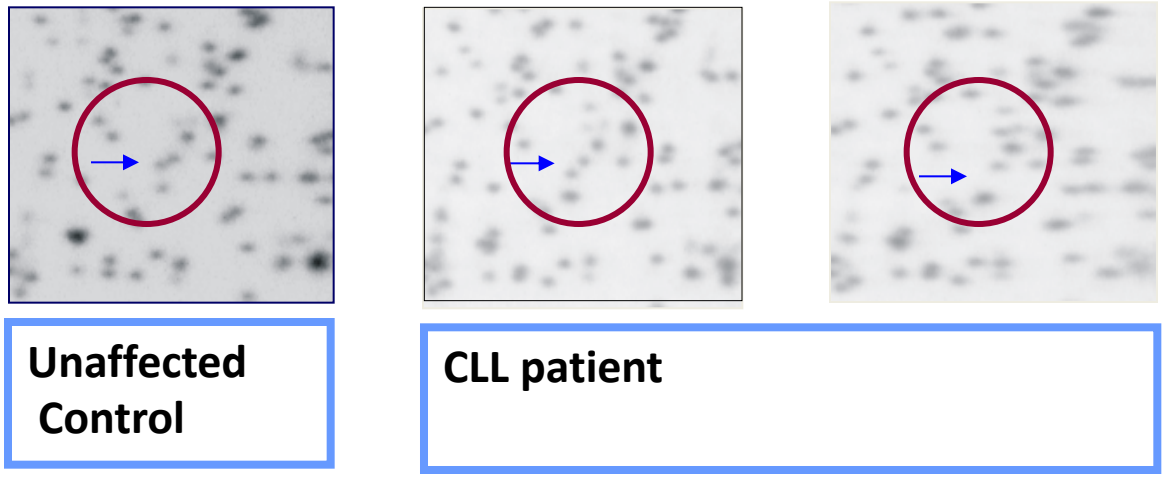


Restriction Landmark Genomic Scanning (RLGS)



Results of a genome scan for DNA methylation in CLL

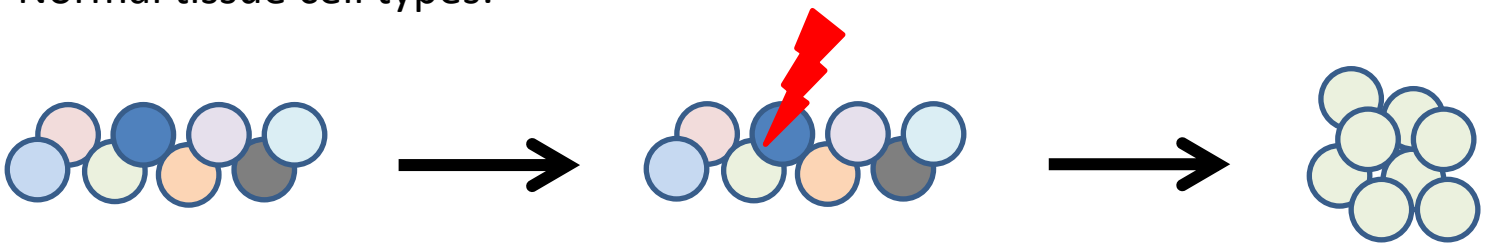
- over 3000 CpG islands were assayed in each sample.
- **2.5% to 8.1%** (mean 4.8%) of the CpG islands in CLL samples were aberrantly methylated
- Patterns of methylation were **non-random**



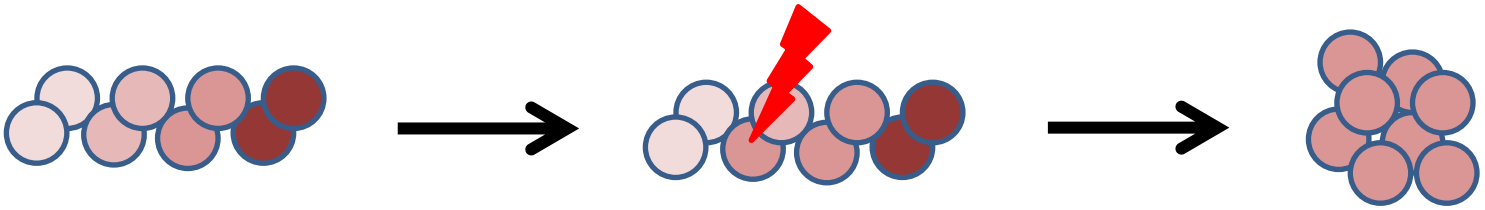
Rush et al. **Cancer Res**. 64:2424-2433, 2004.

Cancer: Cell-of-origin and Epigenetics

Normal tissue cell types:

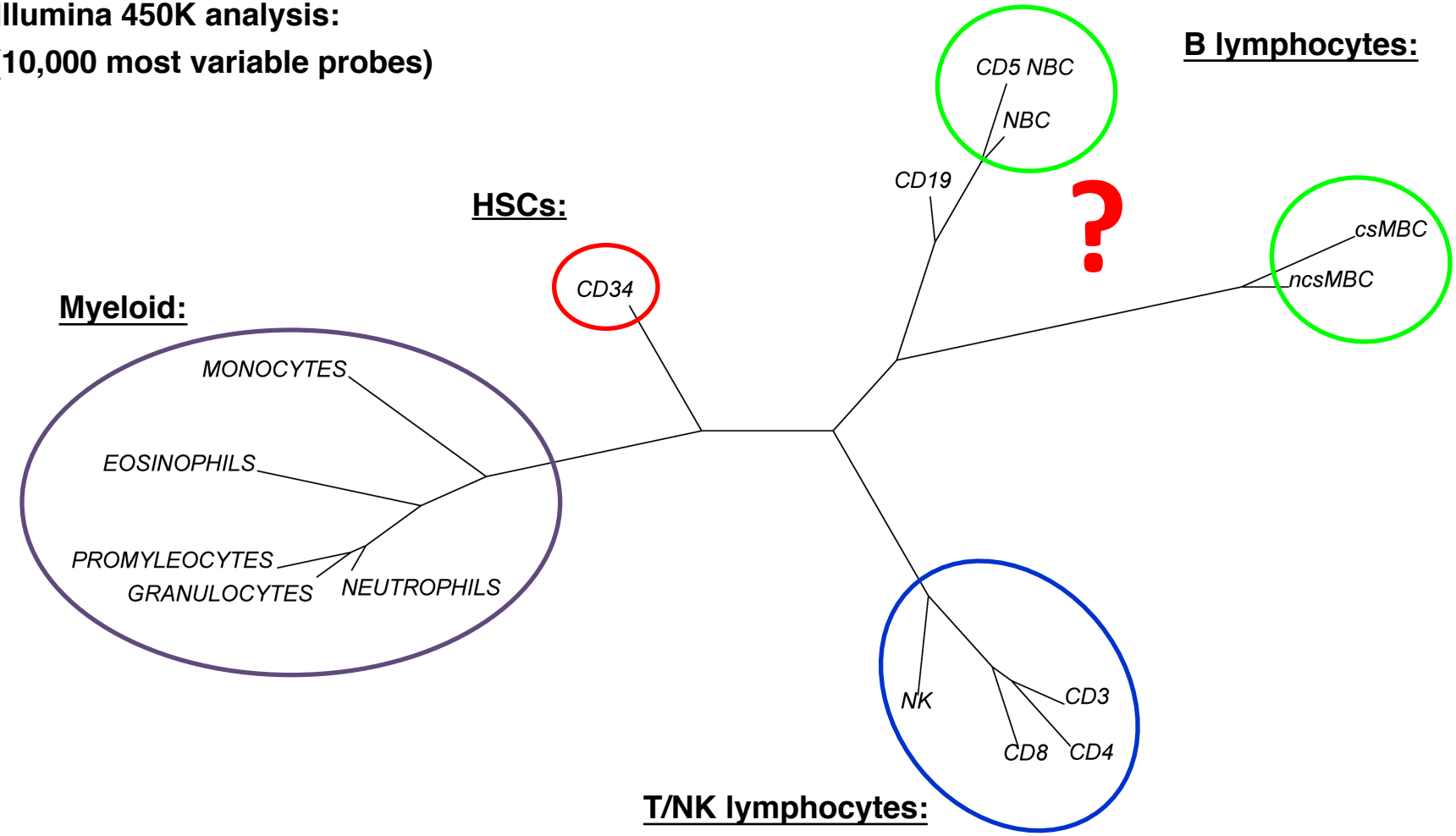


Different developmental stages, aging and microenvironment:



Phylo(epi)genetic analysis of the development of blood cell types:

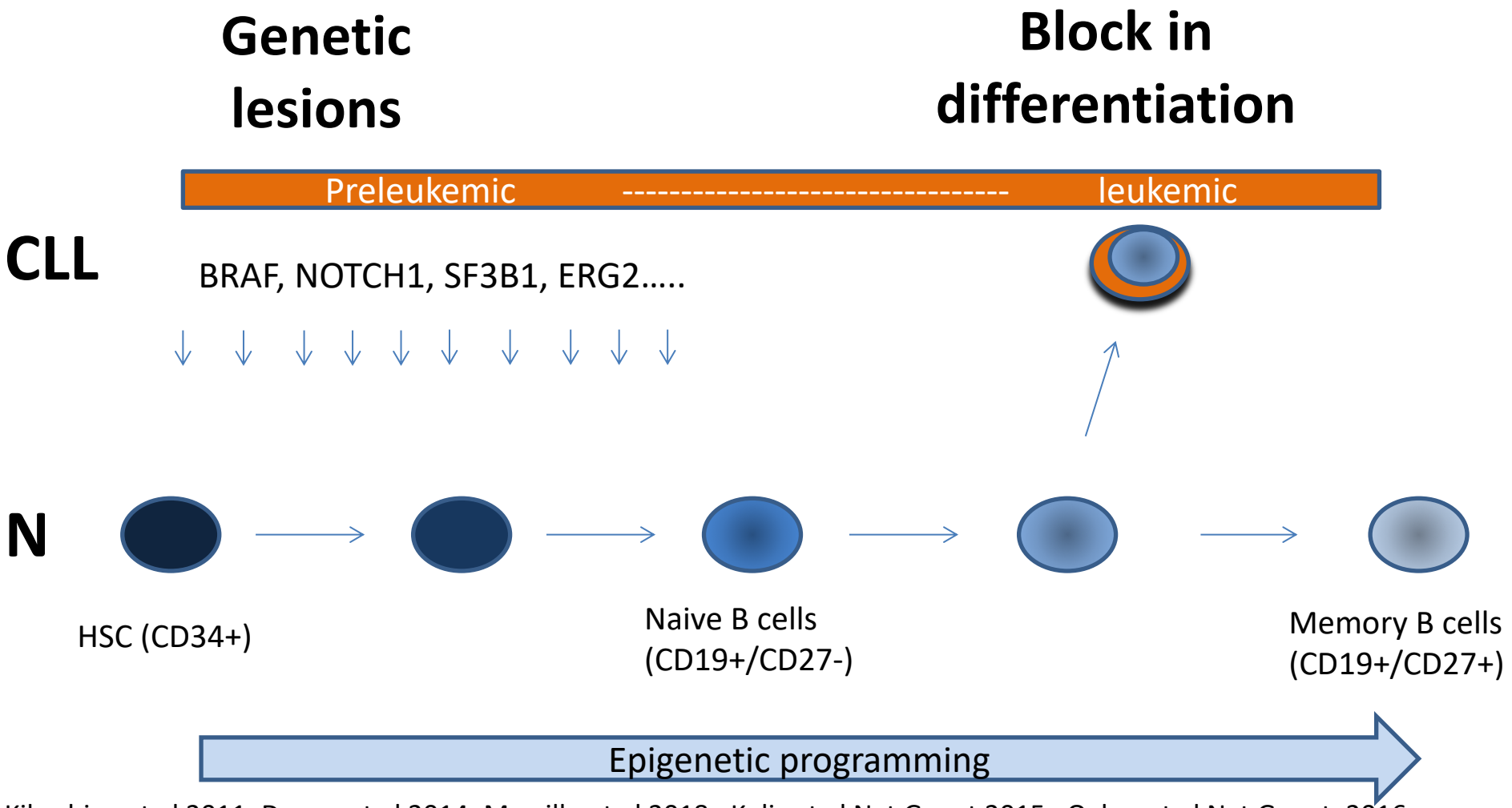
Illumina 450K analysis:
(10,000 most variable probes)



APE package in Bioconductor

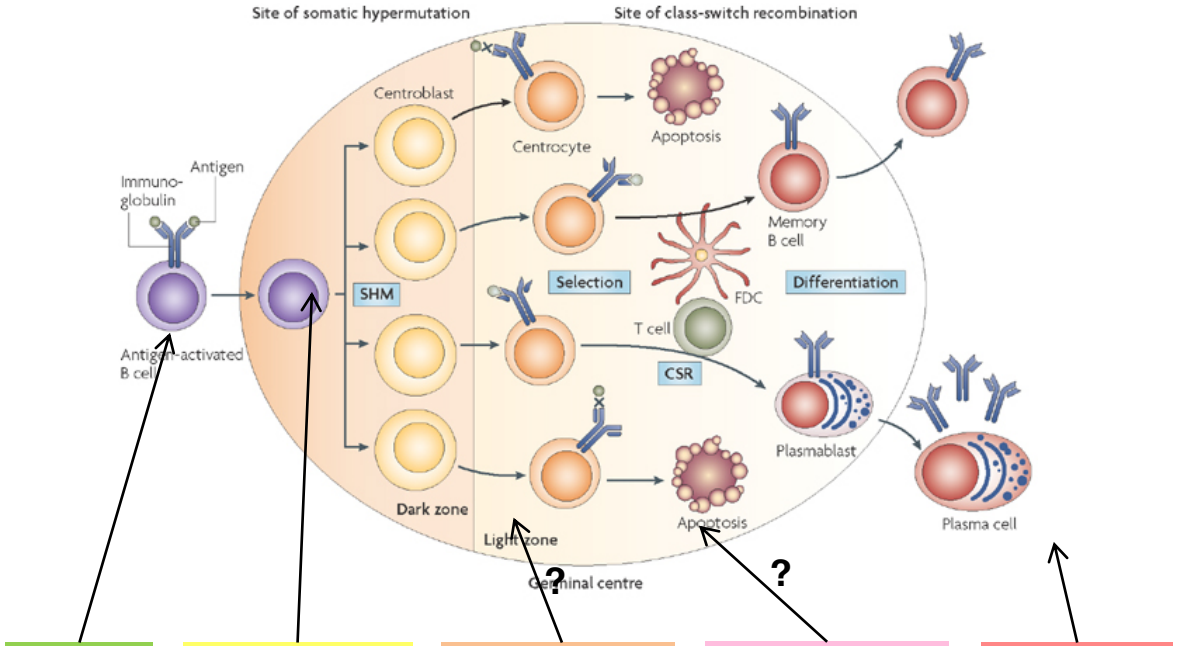
Profiles downloaded from: ICGC Data Portal (http://dcc.icgc.org/web)
GEO Database (http://www.ncbi.nlm.nih.gov/geo)

The cellular origin of the CLL methylome



Kikushige et al 2011; Damm et al 2014; Marsillo et al 2018; Kulis et al Nat Genet 2015; Oakes et al Nat Genet. 2016

Isolation of B cells at various stages of maturation

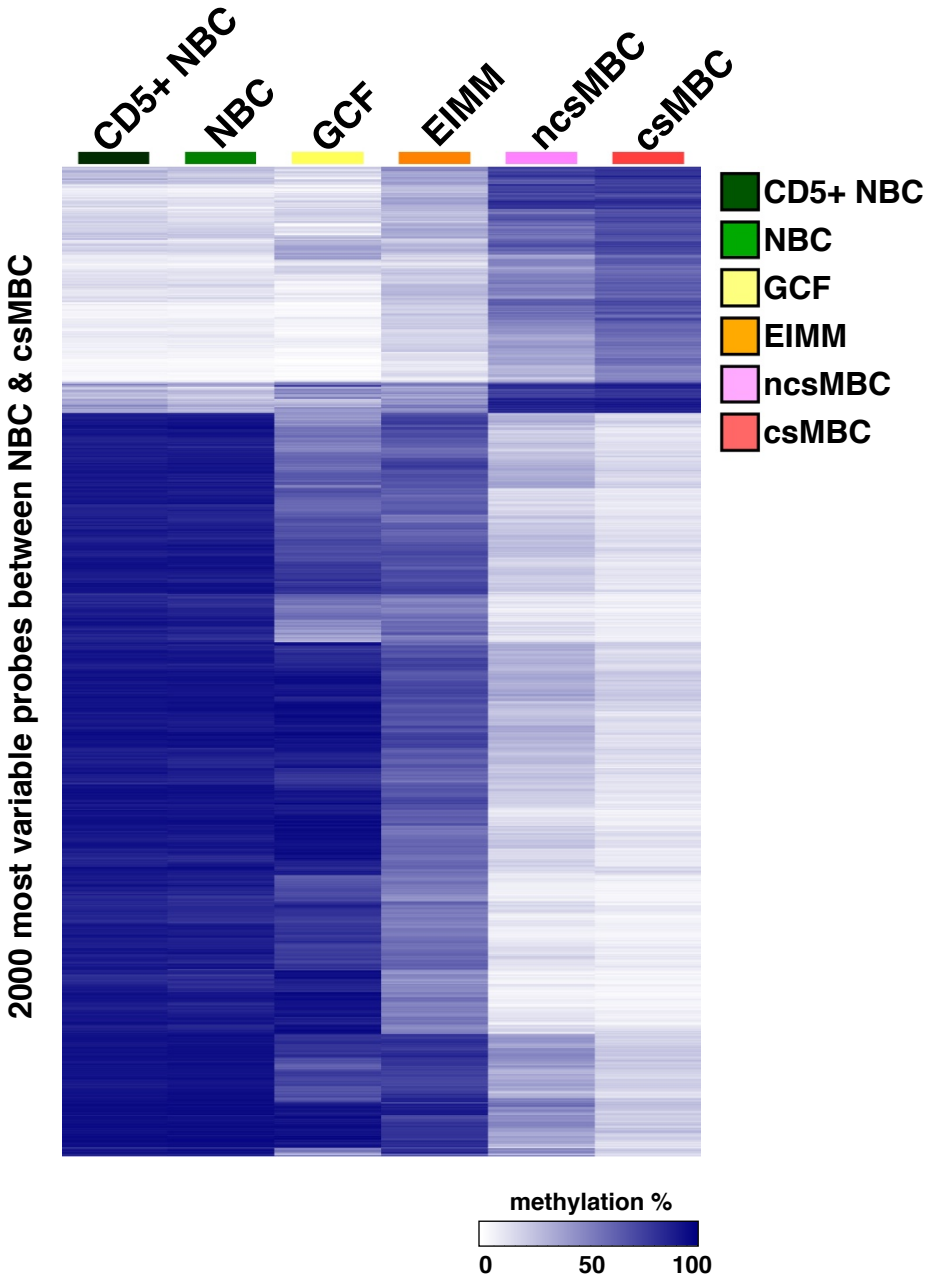


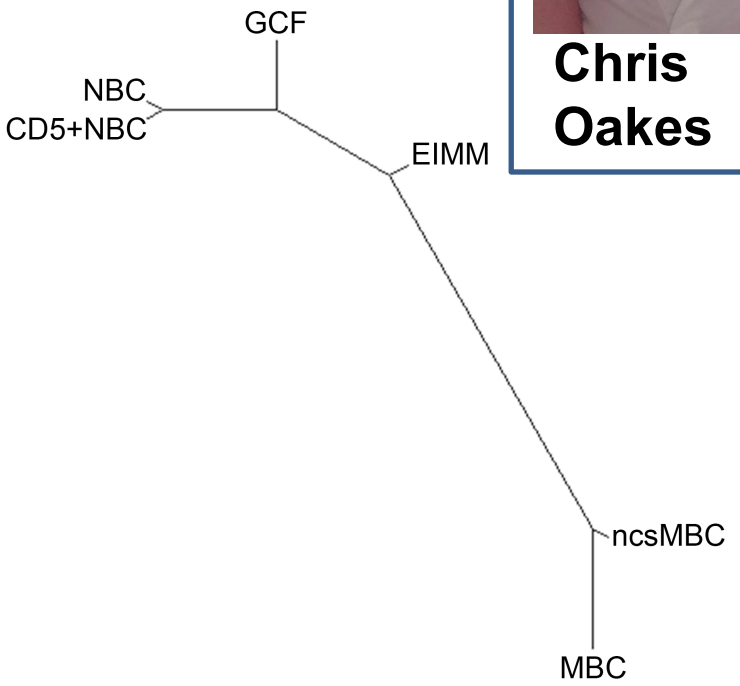
Naïve B cells	Germinal center founder B cells	Early IgM+ Memory cells	Conventional IgM+ Memory cells	Memory B cells
(NBC)	(GCF)	(EIMM)	(ncsMBC)	(csMBC)
CD19+ CD27- IgD+	CD19+ IgD (low) IgM (high) CD80 (high) CD38 (int) CD27+ CD20+	CD19+ IgM+ IgD (low) CD27- CD23- Rhodamine+	CD19+ IgM+ IgD+ CD27+	CD19+ IgA+/IgG+ CD27+

Collaboration with Ralf Küppers and Marc Seifert

Figure from *Nature Reviews Immunology*. 2008; 8, 22-33

DNA methylation programming during B cell maturation

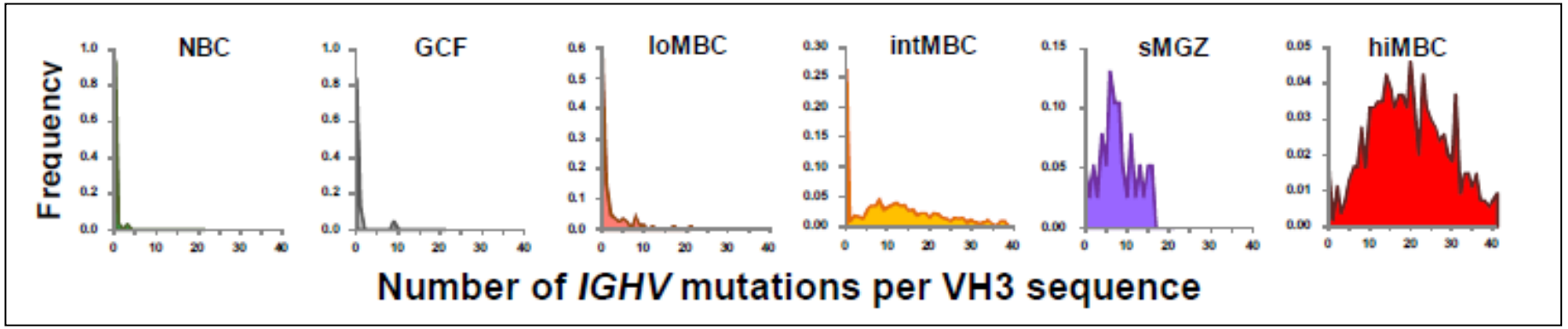


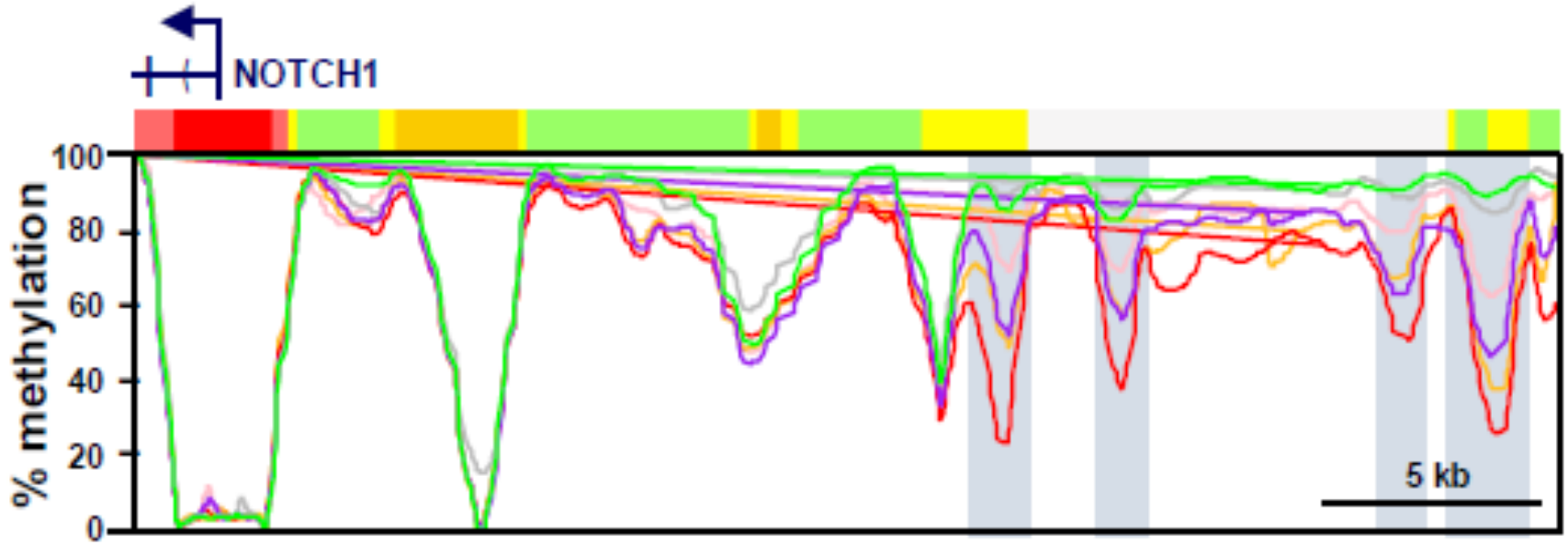


Chris Oakes

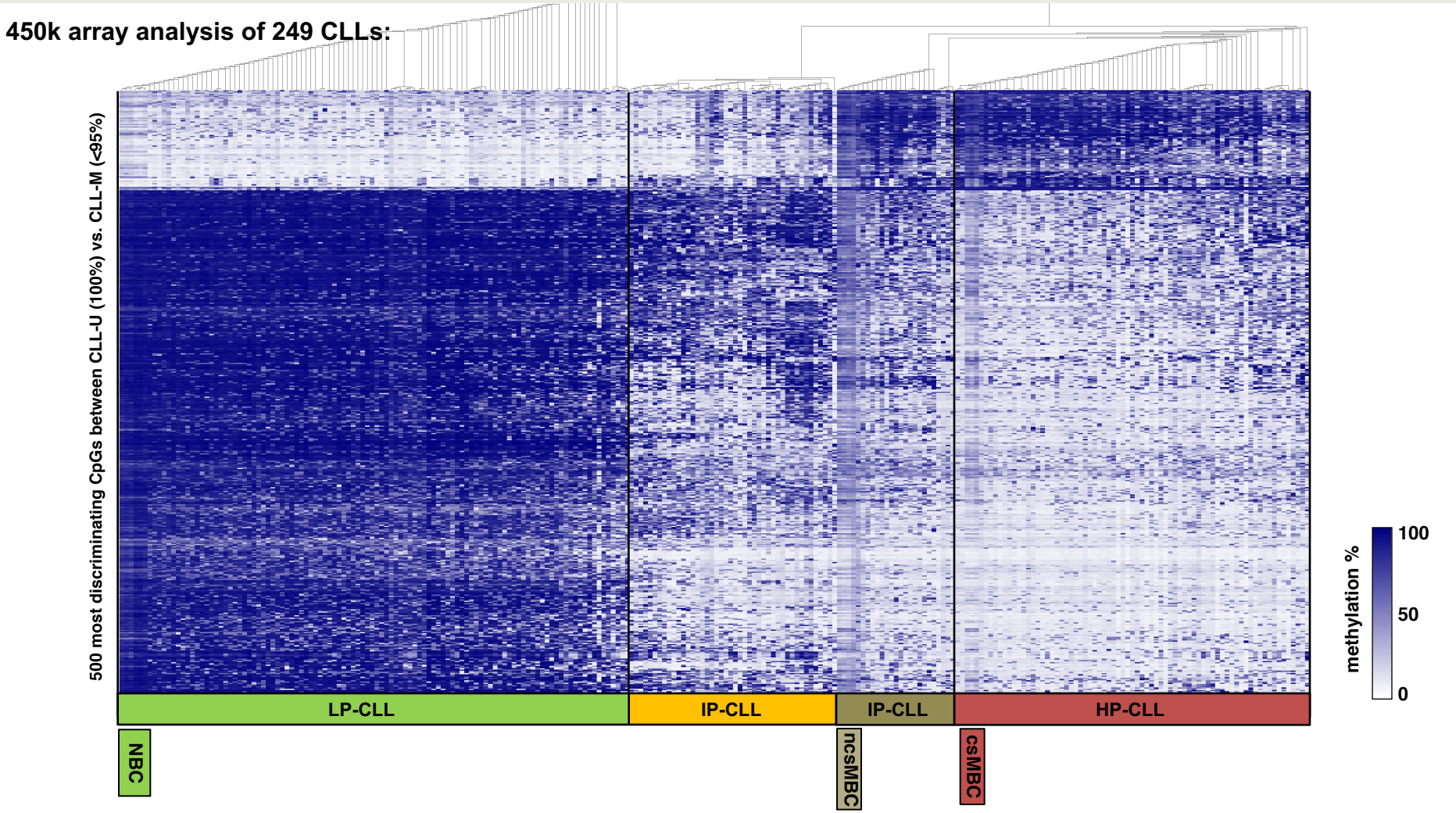
Oakes et al. Nat Gent 2016

Progressive DNA methylation changes during B cell maturation





CLL patients form distinct clusters analogous to healthy B cell subtypes:

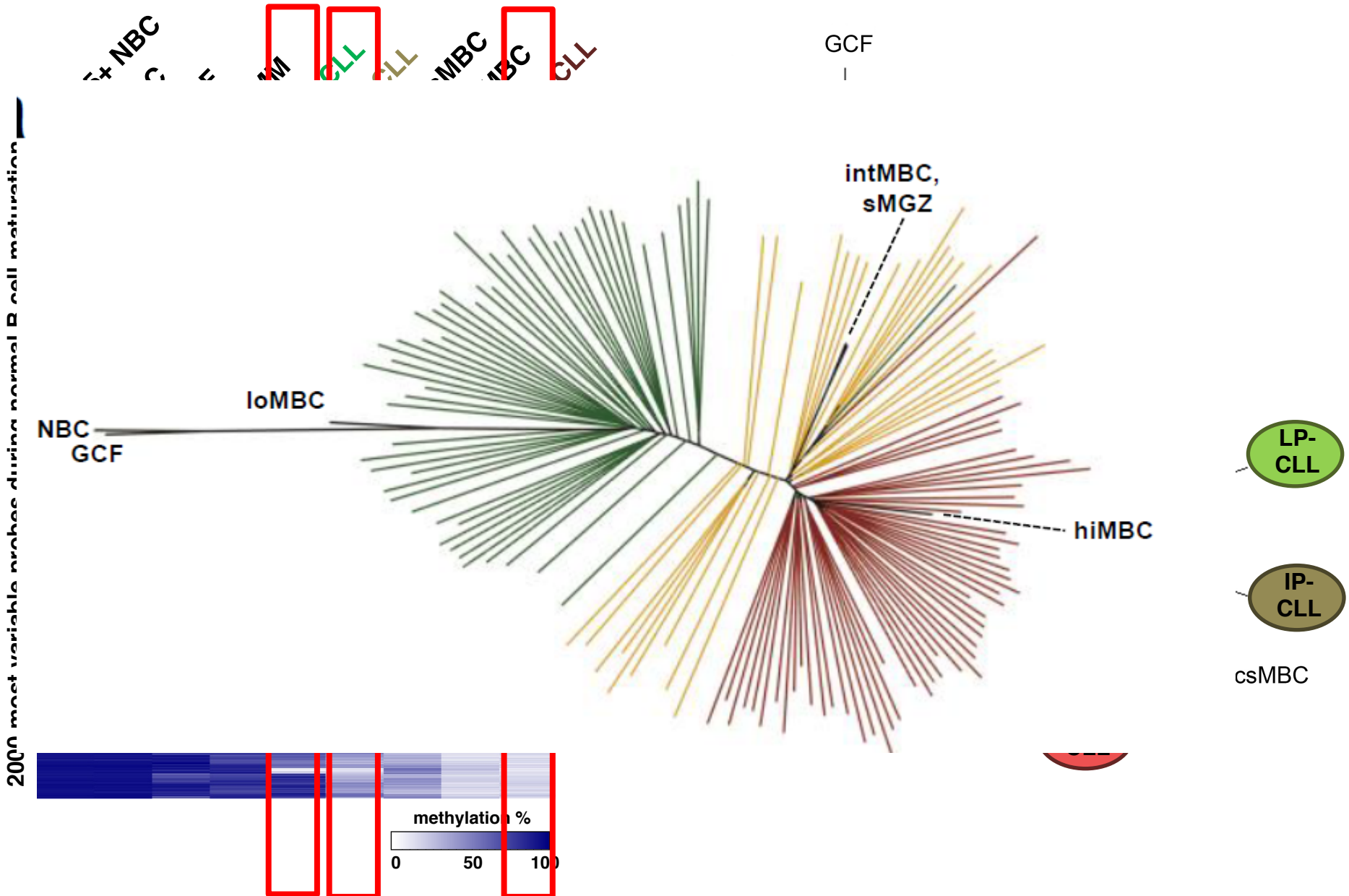


The degree of epigenetic programming normally achieved during affinity maturation defines CLL subgroups:

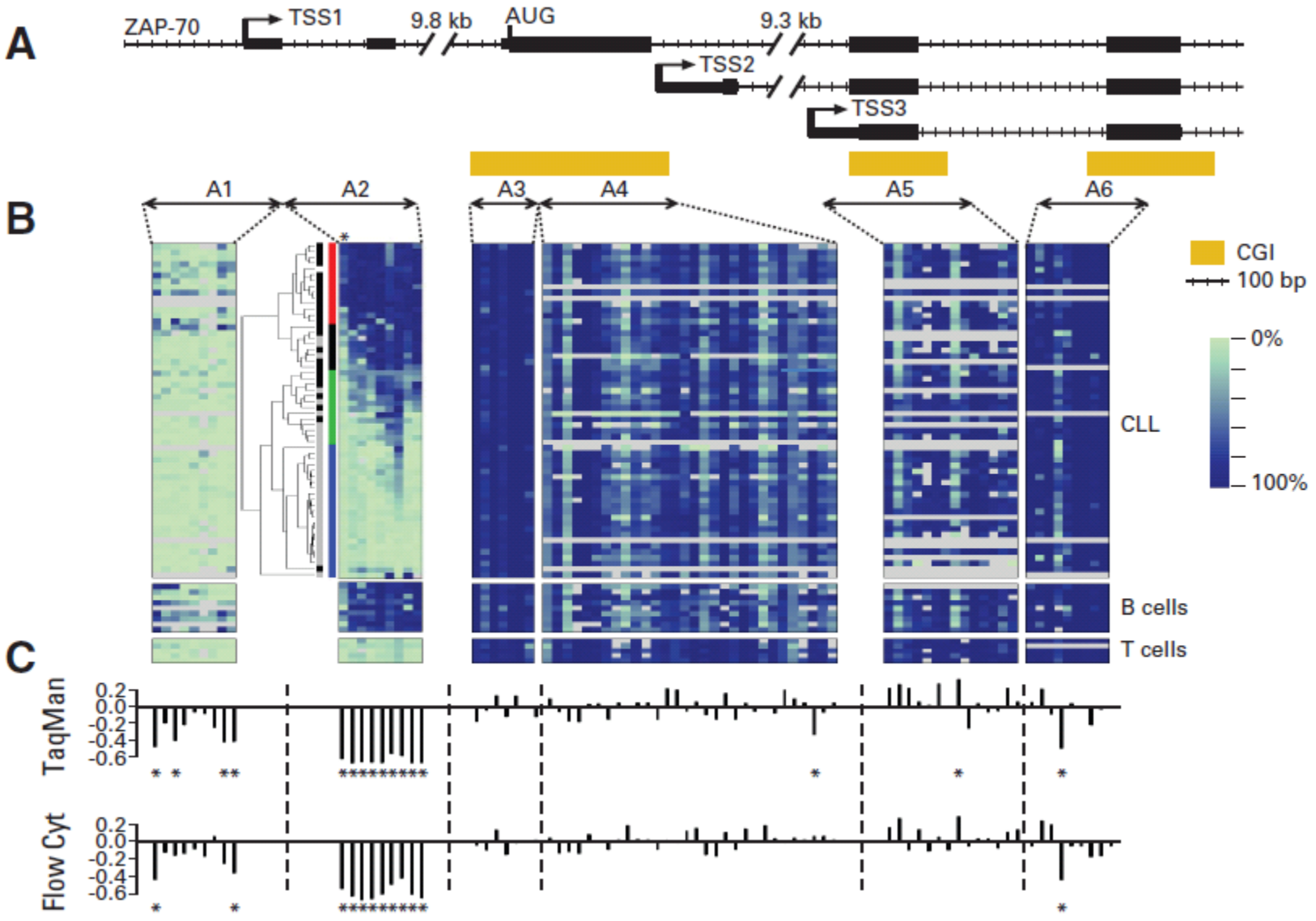
LP-CLL	**(Less Programmed)**	**≈ *IGHV* Unmutated**
IP-CLL	**(Intermediate Programmed)**	
HP-CLL	**(Highly Programmed)**	**≈ *IGHV* Mutated**

139 CLL profiles downloaded from the ICGC Data Portal (http://dcc.icgc.org/web)

Using DNA methylation to estimate the cellular origin of the B cell in which the block of differentiation occurred:



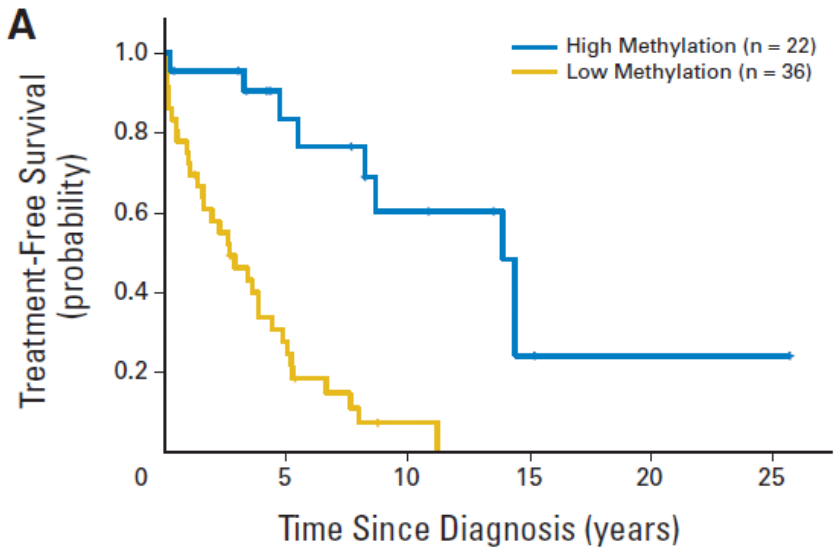
ZAP70 promoter methylation in CLL

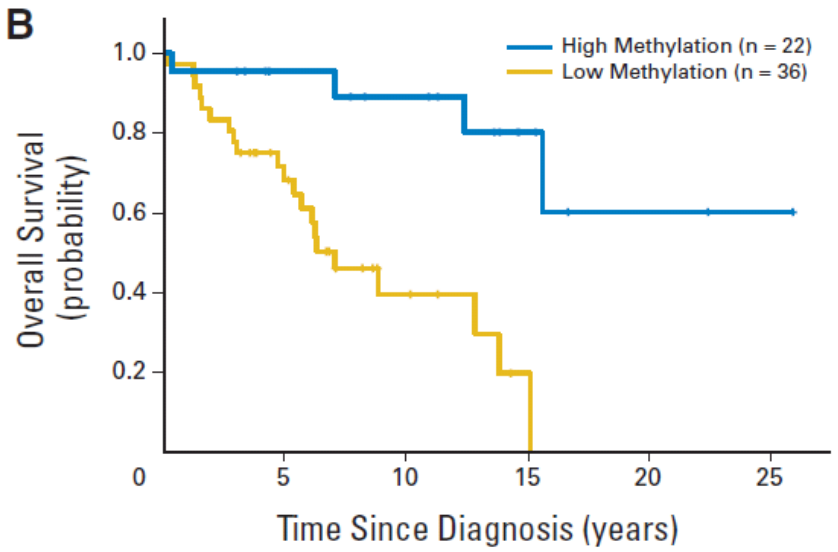


Claus et al. J Clin Oncol. 2012

ZAP70 promoter methylation in CLL

- DNA methylation at a single CpG dinucleotide in the *ZAP-70* promoter region impacts on ZAP-70 transcriptional regulation and is prognostic in CLL



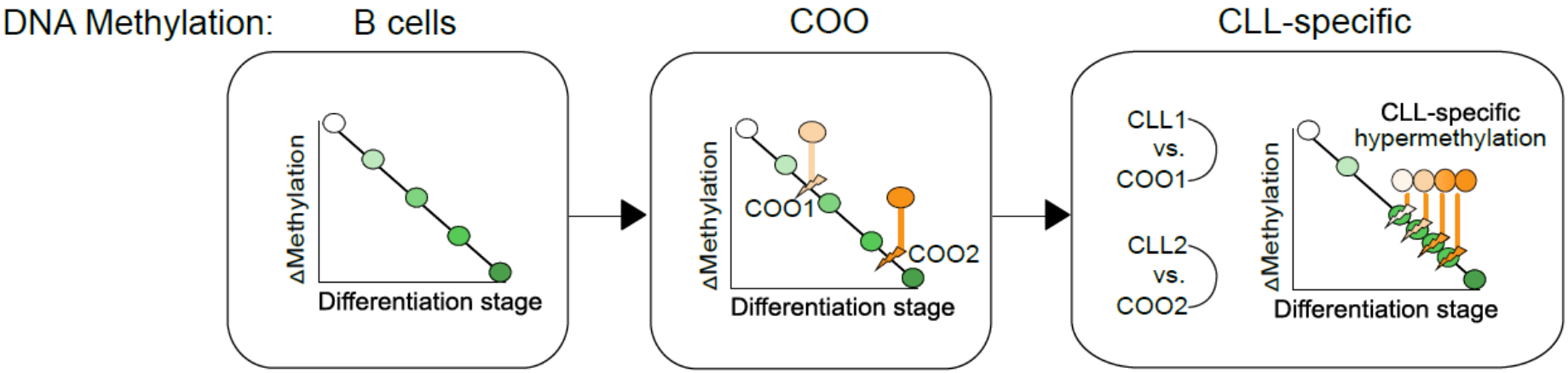


Claus et al. J Clin Oncol. 2012

Discriminating CLL-specific epigenetic alterations from developmental epigentic alterations

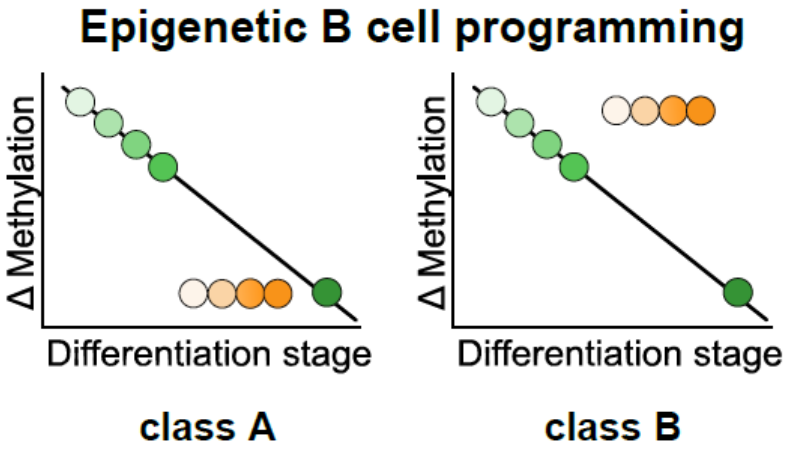
Problem: discrimination between sites that are epigenetically remodeled during B cell differentiation and sites that are modified during leukemogenesis.

Solution: Modelling the epigenome of the cellular origin for CLLs



Wierzbinska JA *et al.* unpublished

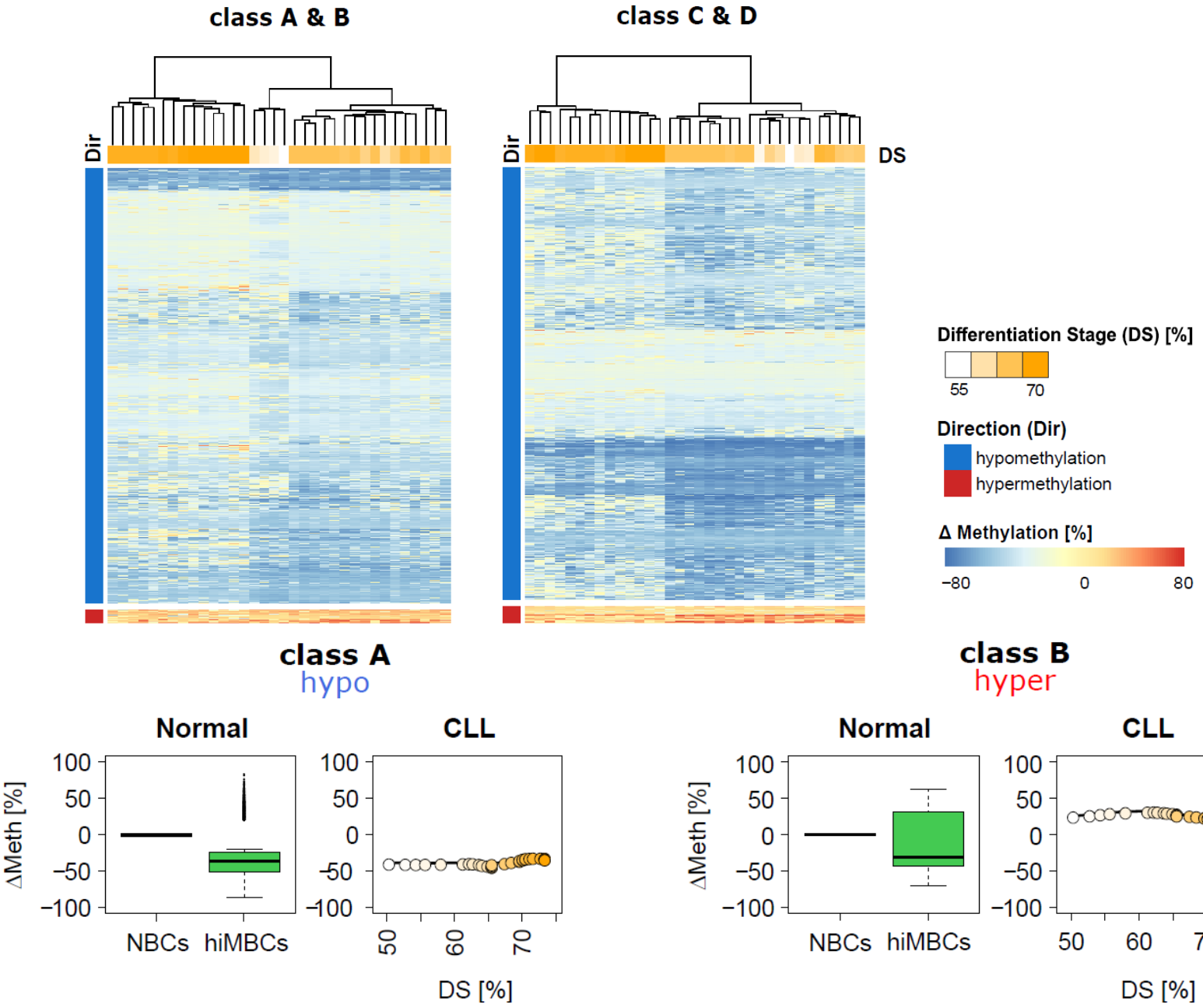
Dissecting CLL-specific epigenetic alterations



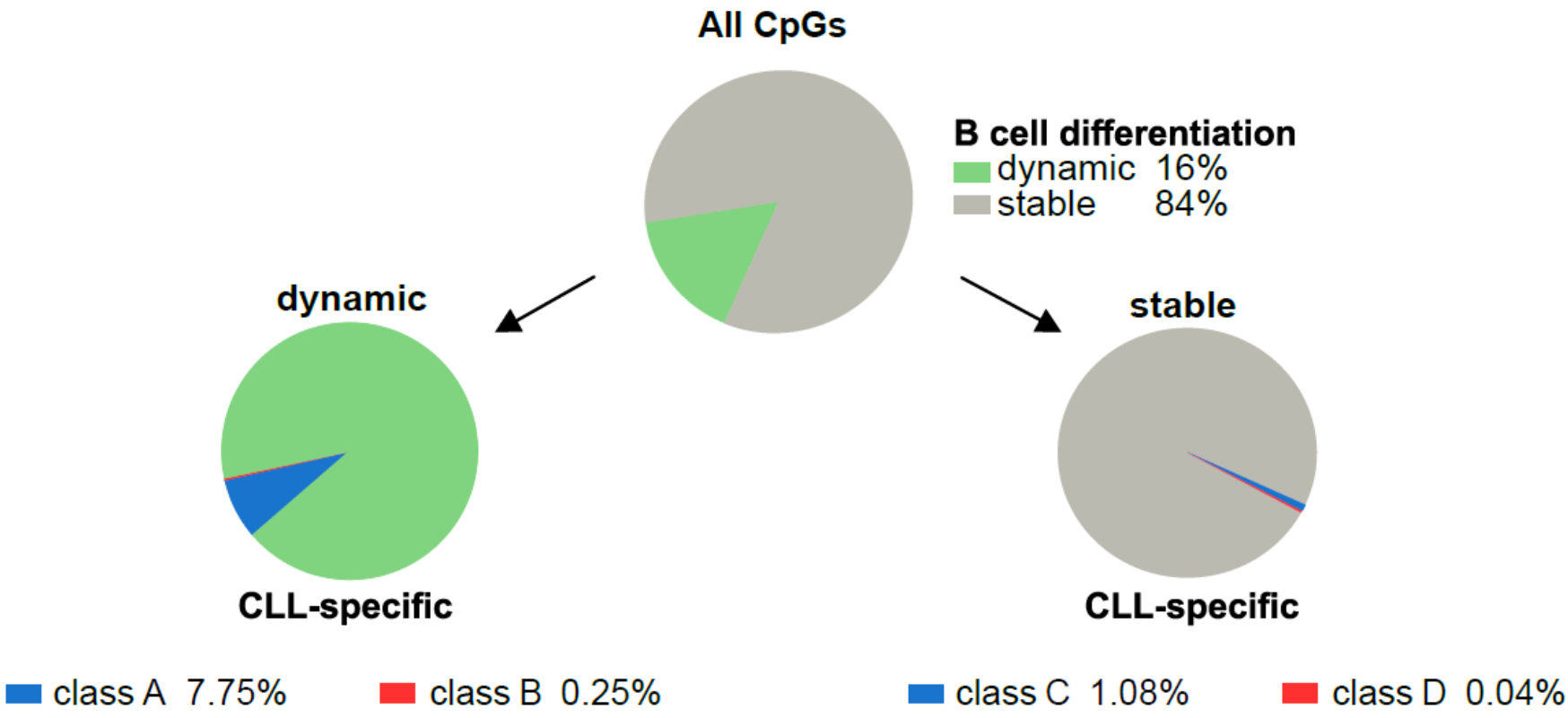
Sites with epigenetic B-cell programming

CLL-specific reprogramming

Sites with epigenetic B-cell programming

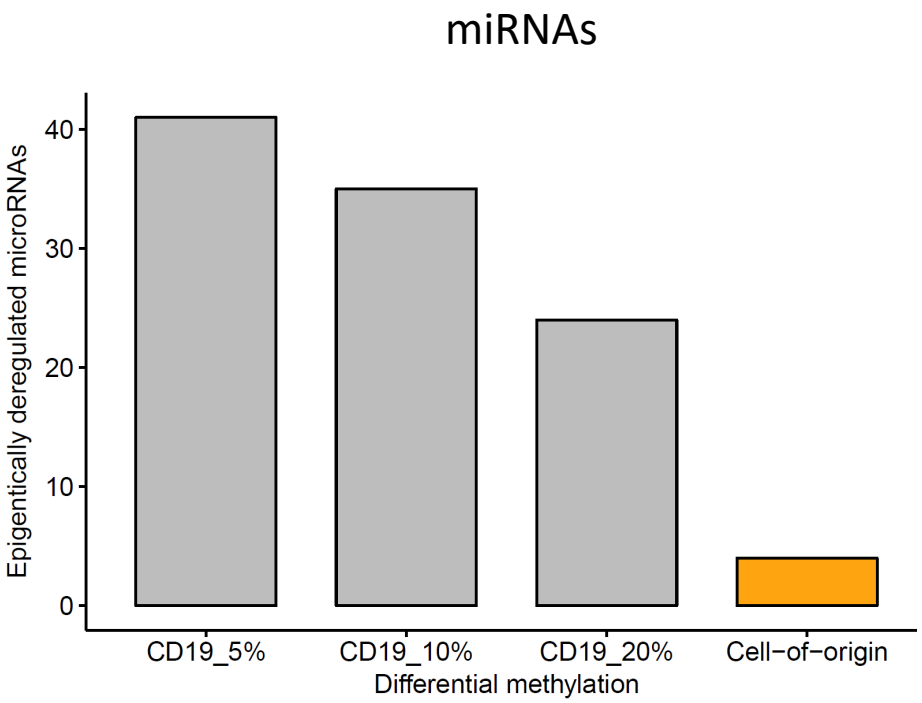


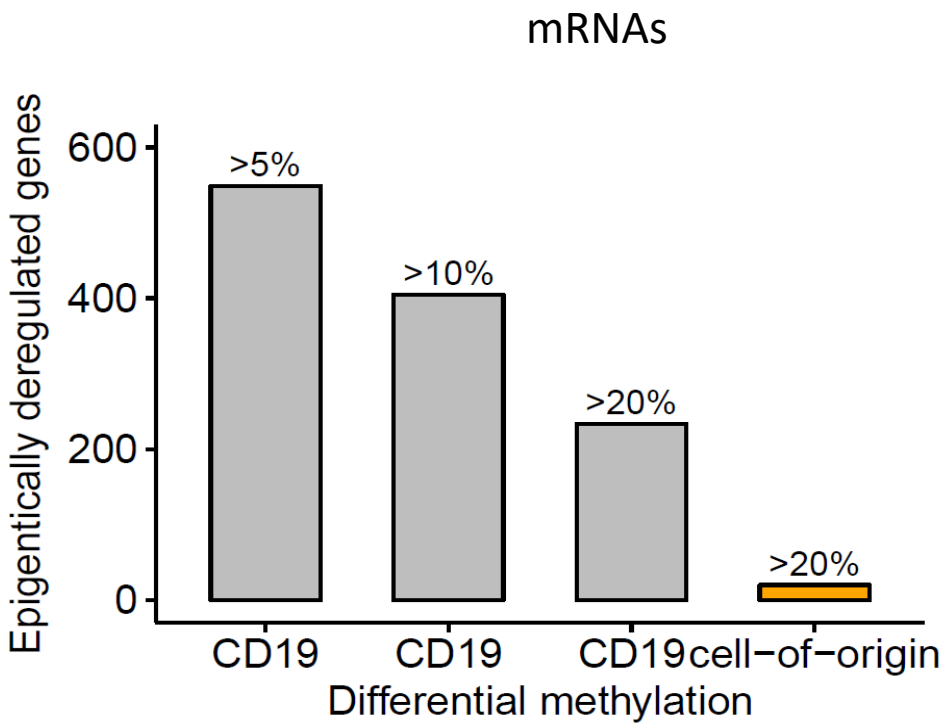
Proportion of disease-specific events



- **Hypomethylation** accounts for majority of disease-specific methylation events
- The **vast proportion** of CLL-specific methylation occurs **on CpG sites not affected** during **B-cell programming**
- CLL-specific methylation events are rare

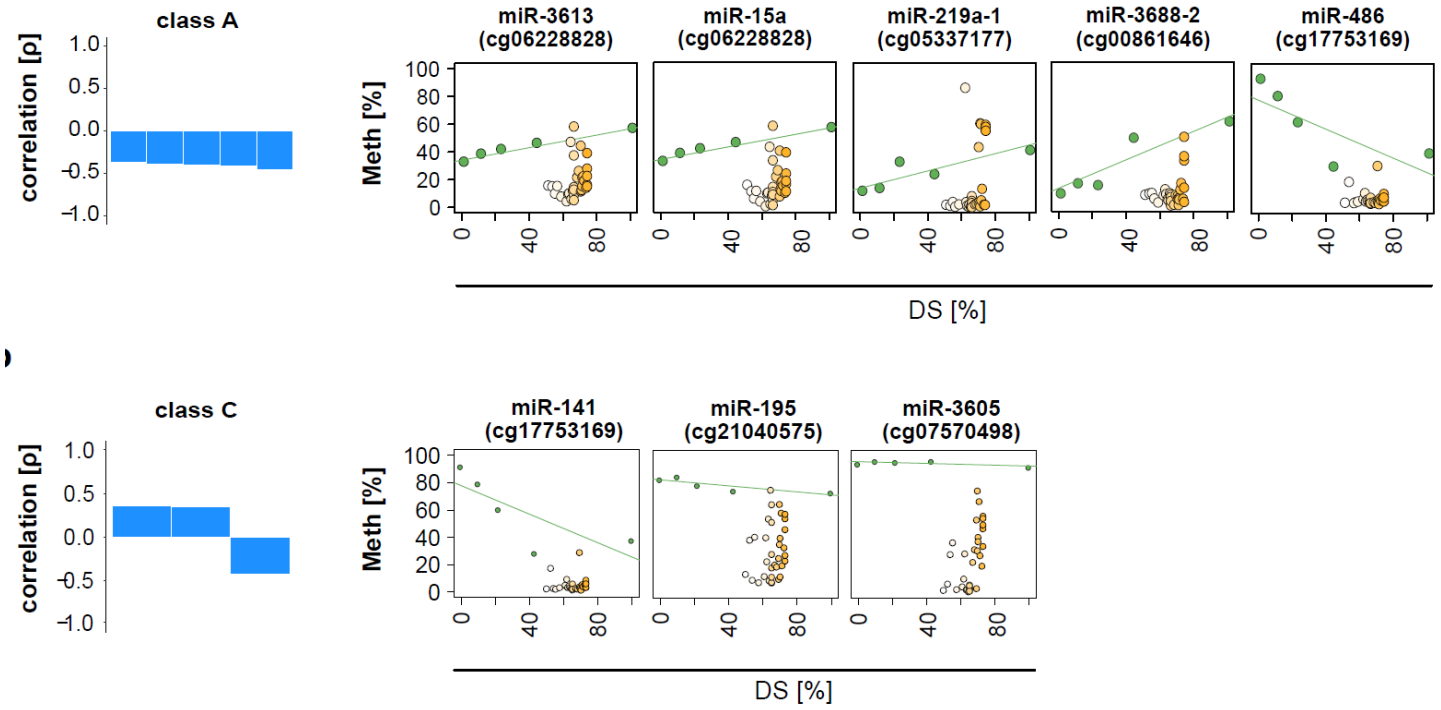
Differential methylation: CD19+ B vers. cell of origin



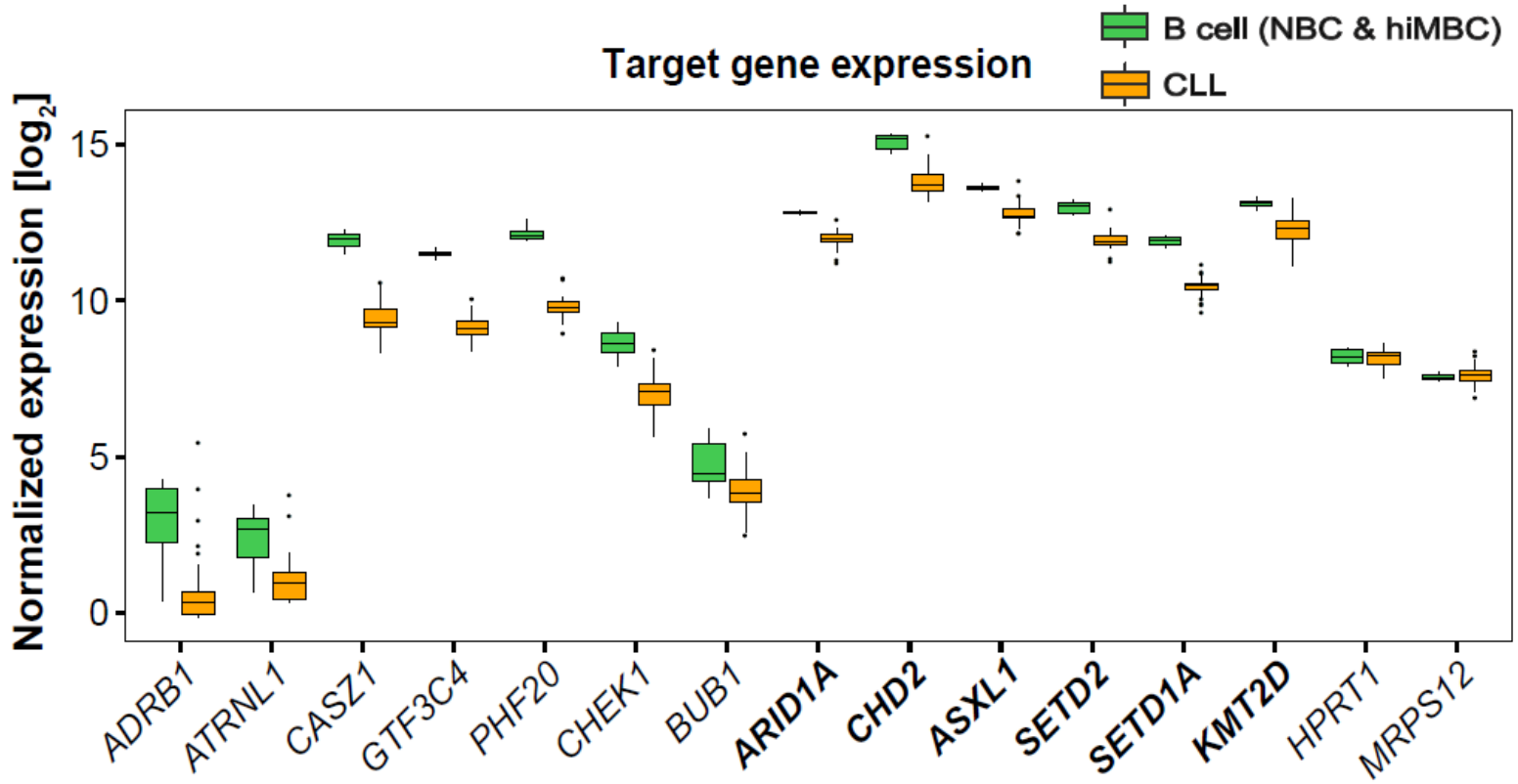


More than 10-fold overestimation of epigenetic contribution to gene silencing using CD19+ controls

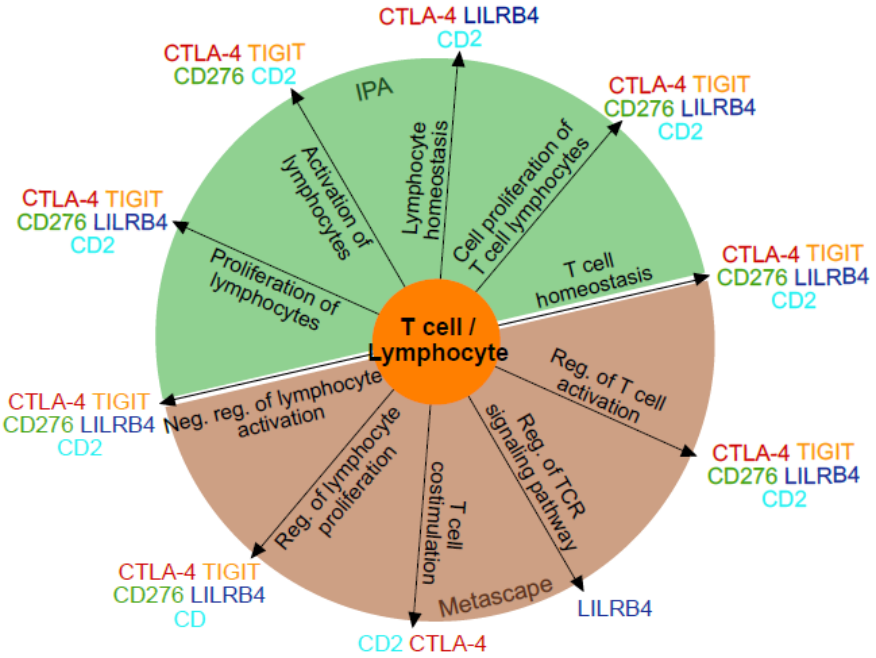
Disease-specific epigenetic alterations in regulatory sequences for miRNAs



Target genes enriched for epigenetic enzymes

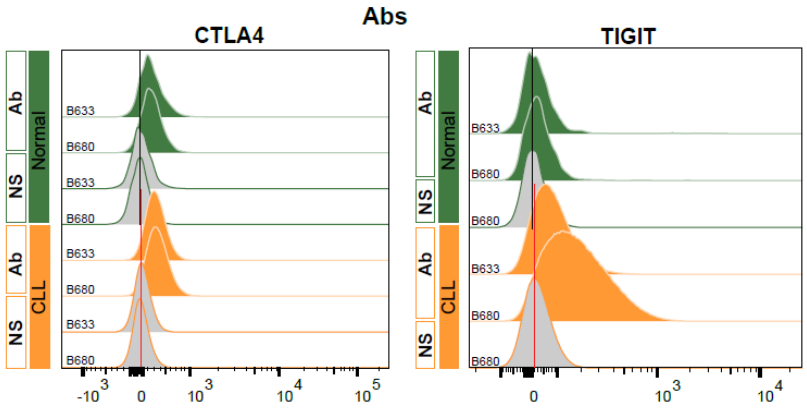


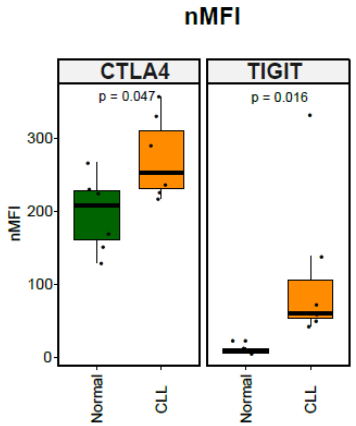
Epigenetically deregulated transcripts show aberrant protein expression in CLL



Activated genes:

lymphocyte/T-lymphocyte related processes





Conclusions

- Normal B cells undergo massive epigenetic reprogramming during the germinal center reaction
- Linear regression modeling allows to compute the methylome of the cell-of-origin for each CLL
- Previous DNA methylation analysis overestimated the number of epigenetically silenced genes in CLL

Acknowledgments:

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Martina Seifert / Philipp Rößner

NCT Heidelberg

Molecular Therapy in Hematology and Oncology

Thorsten Zenz

Bioquant

Genome Organization and Function

Karsten Rippe

Daniel Mertens

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Essen: Marc Seifert

Augsburg: Rainer Claus

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