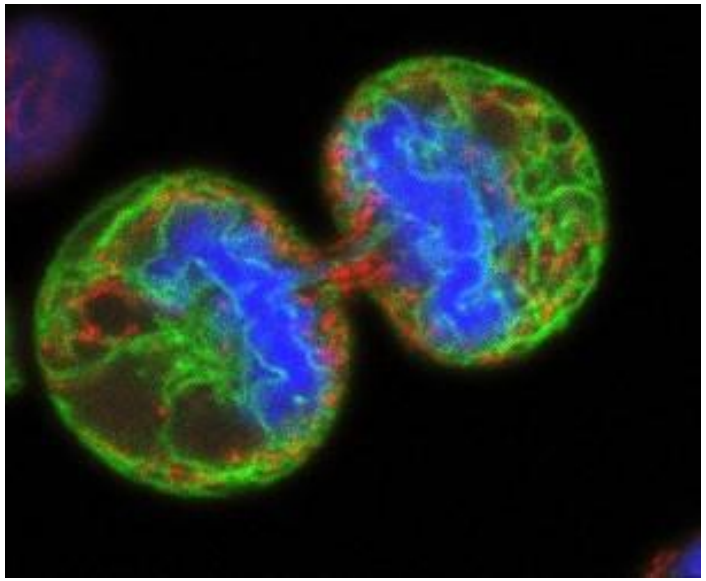
Cancer Genomics

2022.04.18

Cem Meydan, Ph.D

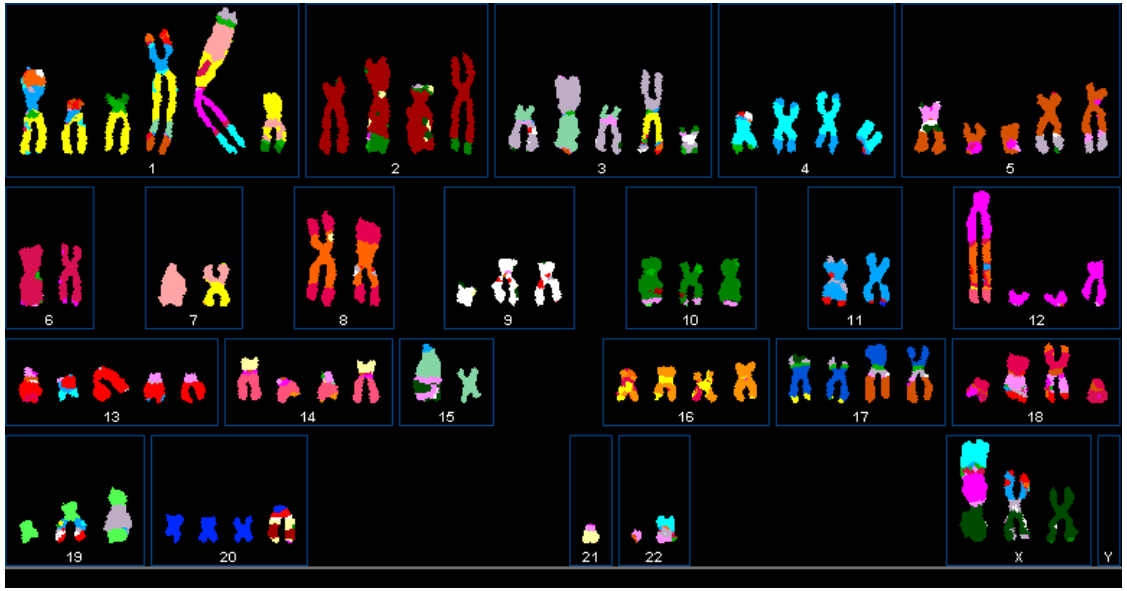
Cancer

- All cancers derive from single cells that have acquired the characteristics of continually dividing in an unrestrained manner and invading surrounding tissues.
- Cancer cells behave in this abnormal manner because of changes in the DNA sequence of key genes, which are known as cancer genes. Therefore all cancers are genetic diseases.



Human melanoma cell undergoing cell division

Paul Smith & Rachel Errington, Wellcome Images

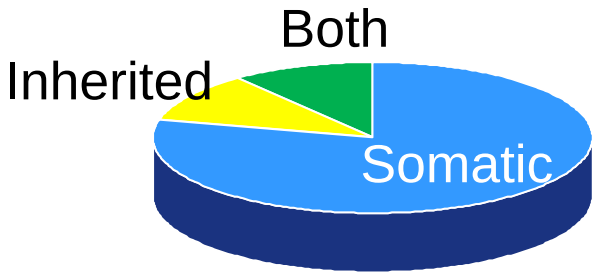


Mira Grigorova and Paul Edwards,
www.path.cam.ac.uk/~pawefish/BreastCellLineDescriptions/HCC38.html

yourgenome.org, Wellcome Sanger

Genetics of cancer

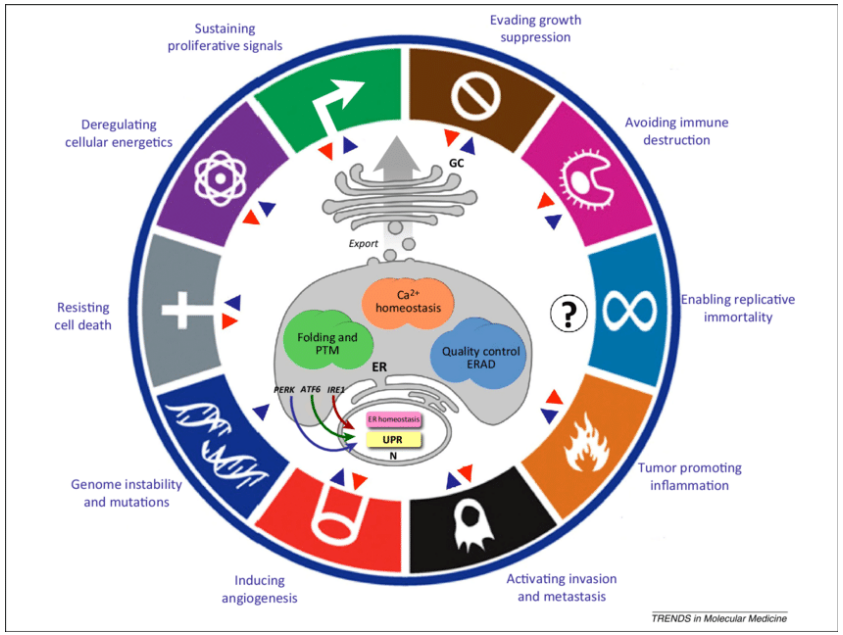
- Inherited germline mutations → 5-10% of all cancers
  - TP53, BRCA1, BRCA2, PTEN…
- Somatic mutations



- Mutations:
  - SNVs, Insertion-deletions, Structural variants and rearrangements
  - Missense, nonsense, frameshift, splicing site

- Region:
  - Coding regions/exome
  - non-coding regions

- Genes
  - Oncogenes / tumor suppressors
  - Other (regulatory elements, epigenetic modifiers, …)



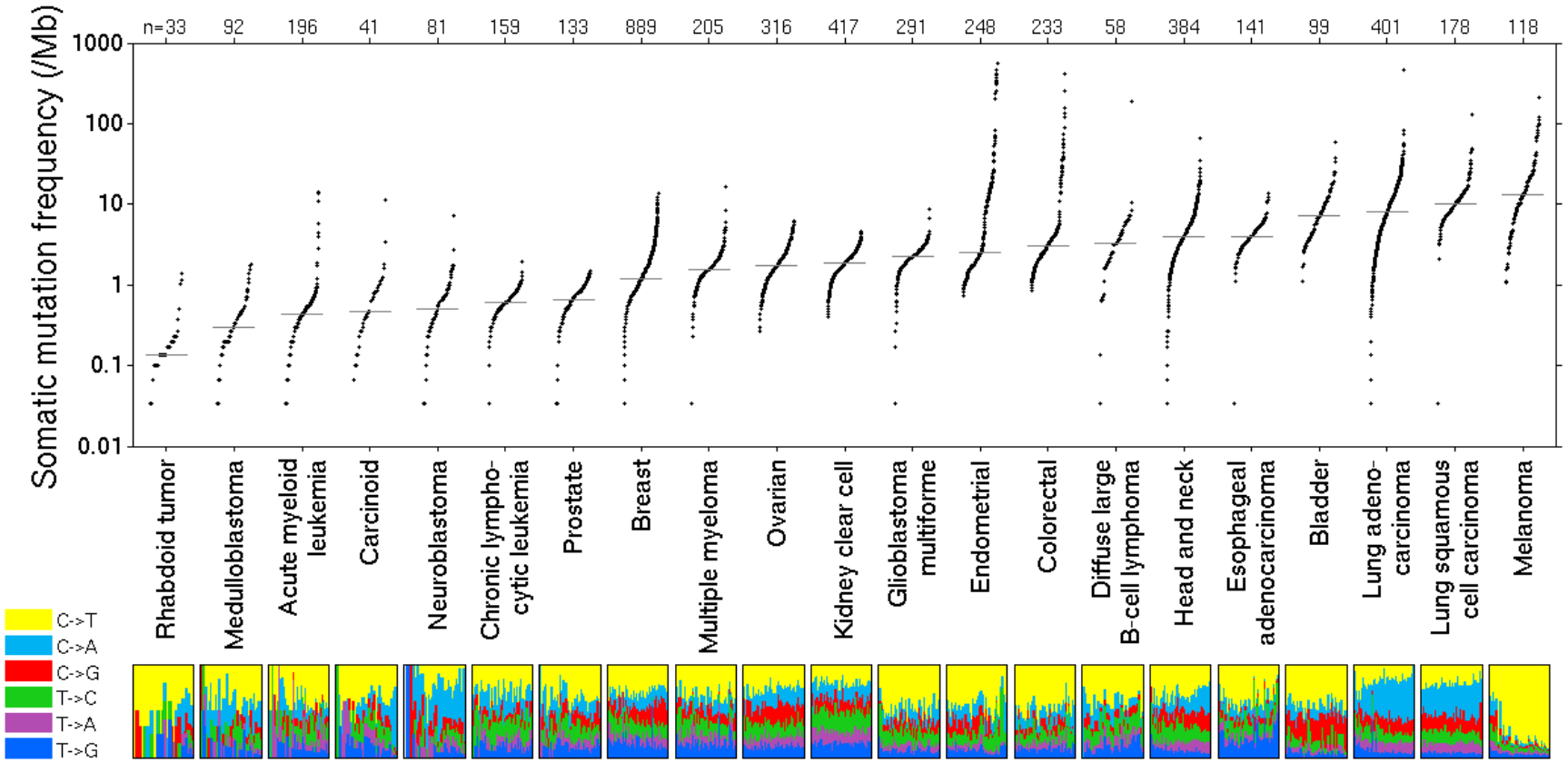
Trends in Molecular Medicine

Genomics in cancer

- Clinical & translational:
  - Early diagnosis → liquid biopsy, cfDNA/ctDNA, exosomes
  - Detection & characterization for optimal therapy → WGS, WES, …
  - Personalized treatments, precision medicine
  - …

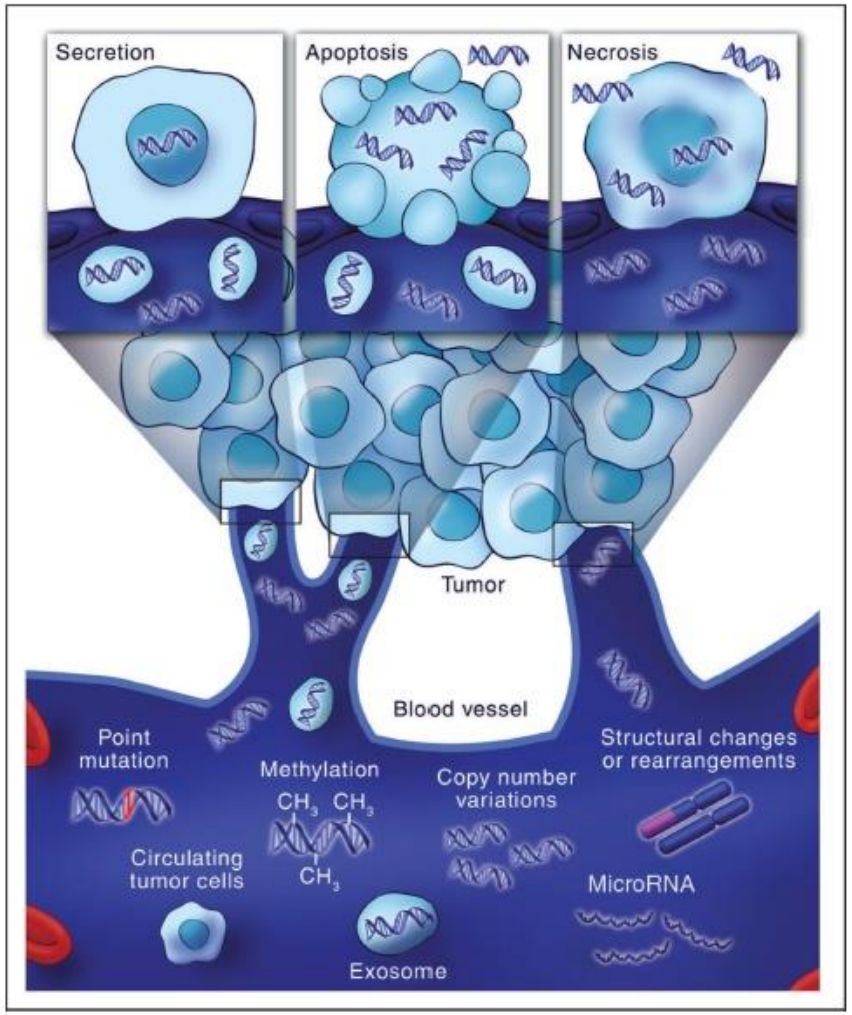
- Research
  - Mechanism
  - Evolution
  - Emergence of therapy resistance
  - → Novel therapies

Mutation Frequencies in Common Cancers



Cancer Genome Group, Broad Institute

Liquid Biopsy



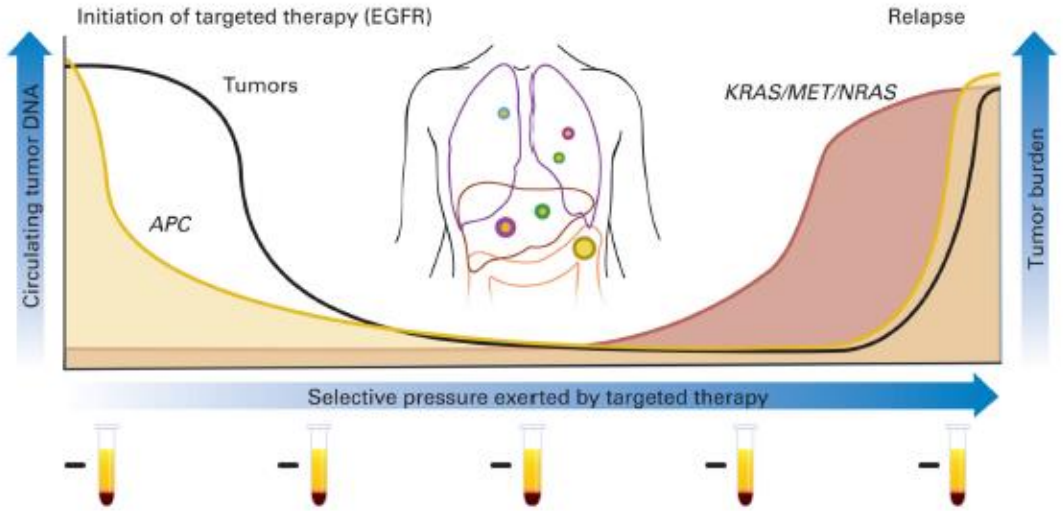
Minimal residual disease

Resistance mutations

Initial genotyping when no tissue available

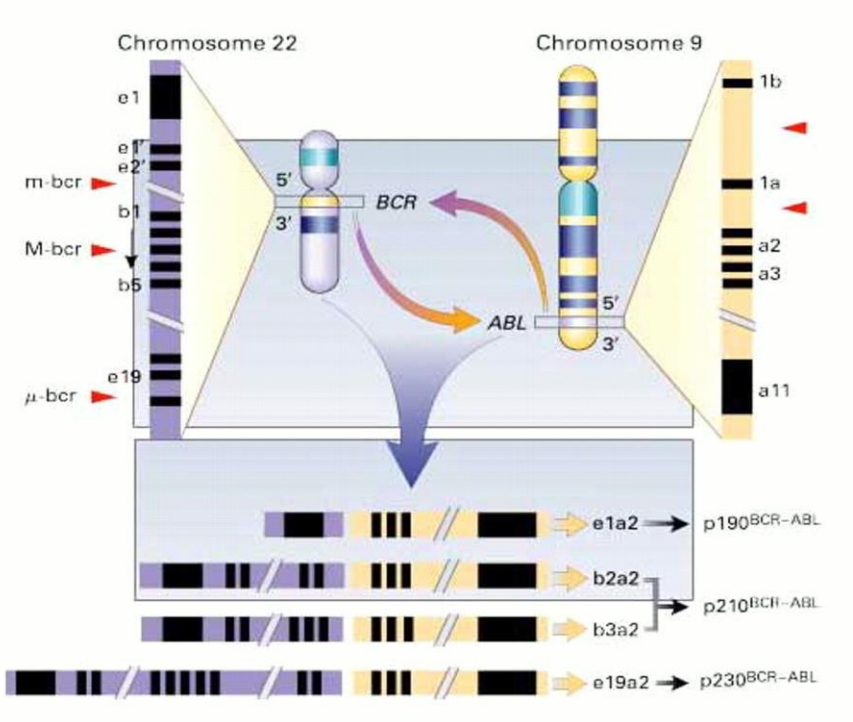
Early detection/screening

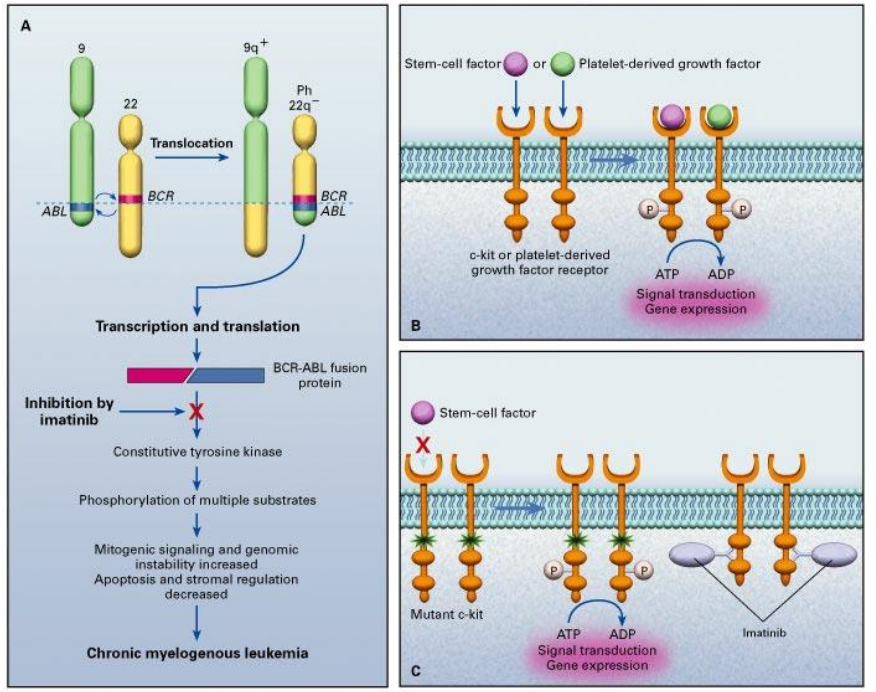
Research of heterogeneity

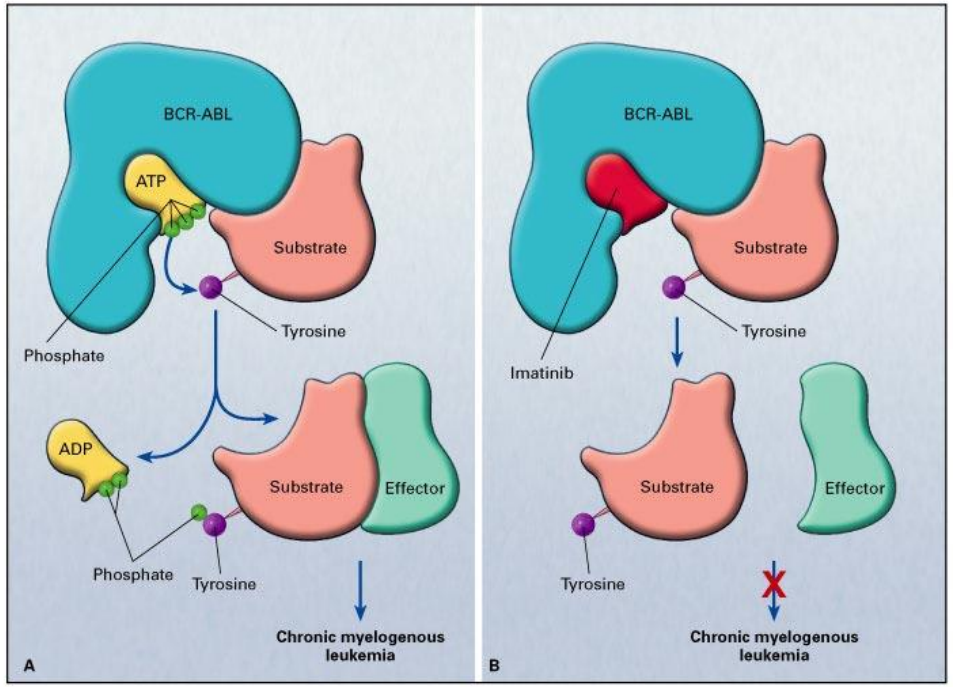


Liquid biopsies: genotyping circulating tumor DNA.
Diaz LA Jr, Bardelli A. J Clin Oncol. 2014 Feb 20;32(6):579-86.

Chronic Myeloid Leukemia

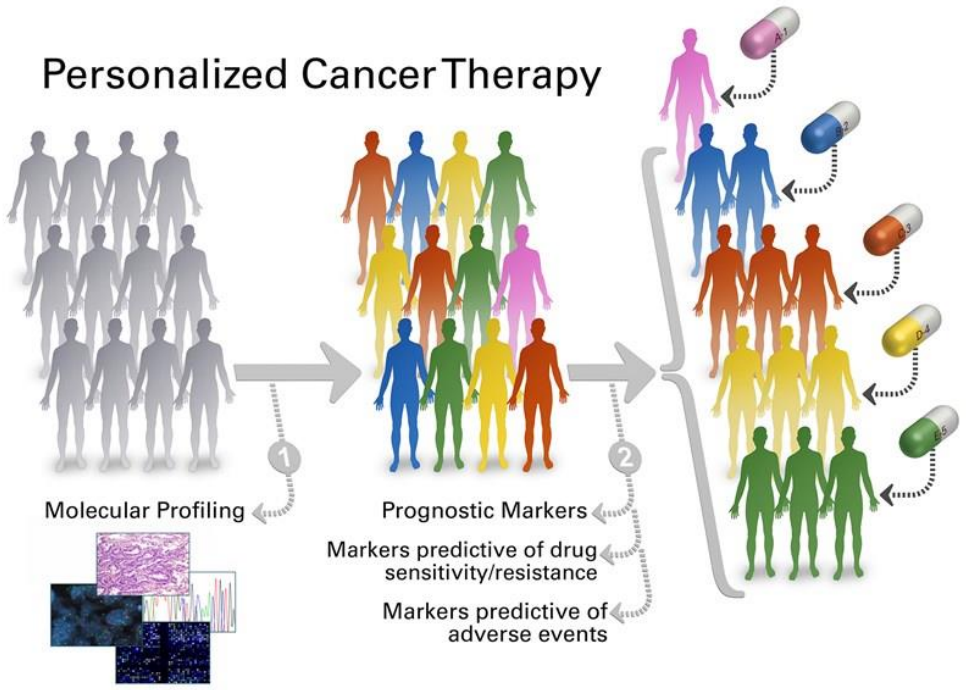




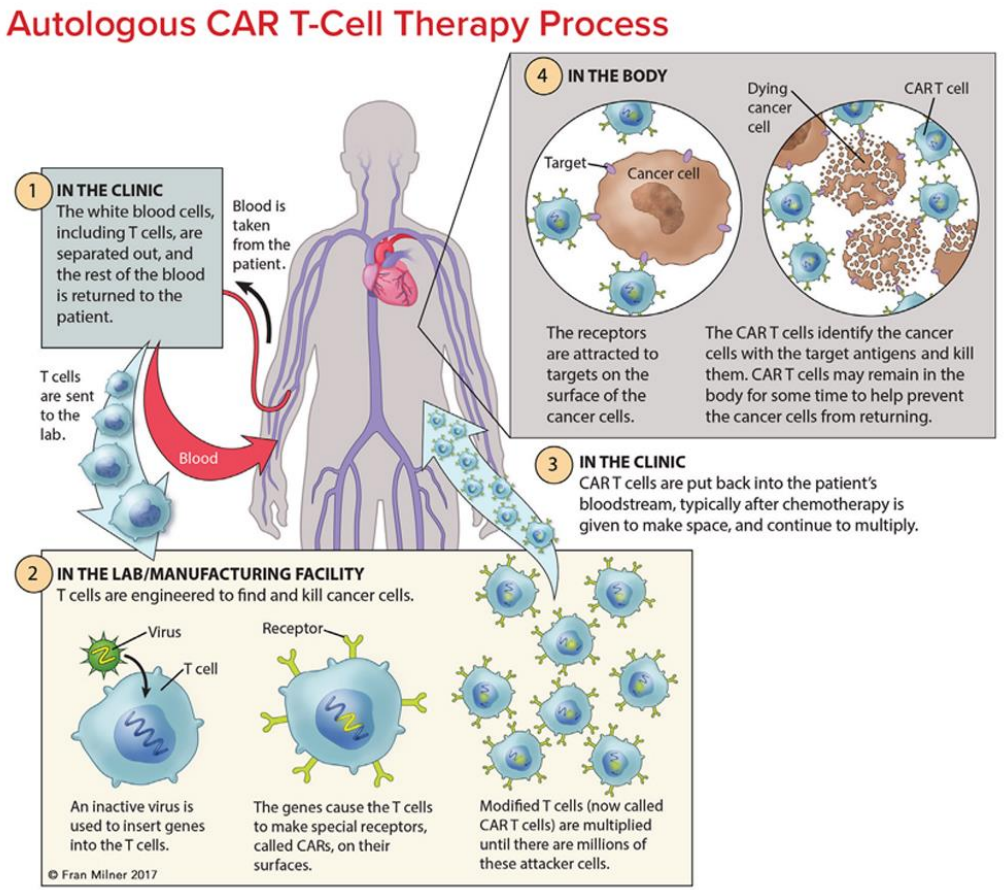


- Target for inhibition: Tyrosine kinase
- Aim: to design a small chemical compound that would compete with ATP for its binding site in the kinase domain.
- By blocking the ATP site, no phosphate groups would be transferred to tyrosine residues on the BCR-ABL substrate → unphosphorylated substrate protein would not be able to undergo a conformational change to allow it to associate with downstream effectors → the downstream reactions would then be impeded → interrupting transmission of the oncogenic signal to the nucleus.

http://cinjweb.umdnj.edu/sites/molmdweb/documents



pct.mdanderson.org



Fran Milner

Not just coding genes

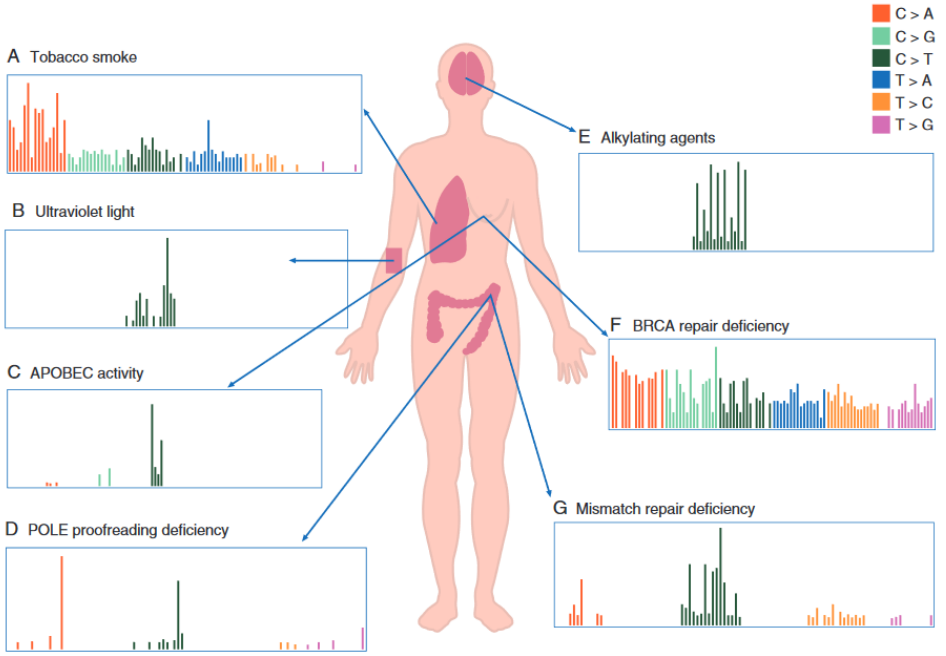


Figure 2. Several mutational signatures identified so far have important clinical or epidemiological implications. Some signatures, such as those associated with tobacco smoke (A), ultraviolet light (B) and alkylating agents (E), can serve as markers of previous mutagenic exposure. Signatures associated with altered DNA damage response including deficiency in *POLE* (D), *BRCA* (F) and mismatch repair pathways (G) may serve as markers for prognosis and efficacy of certain types of therapy.

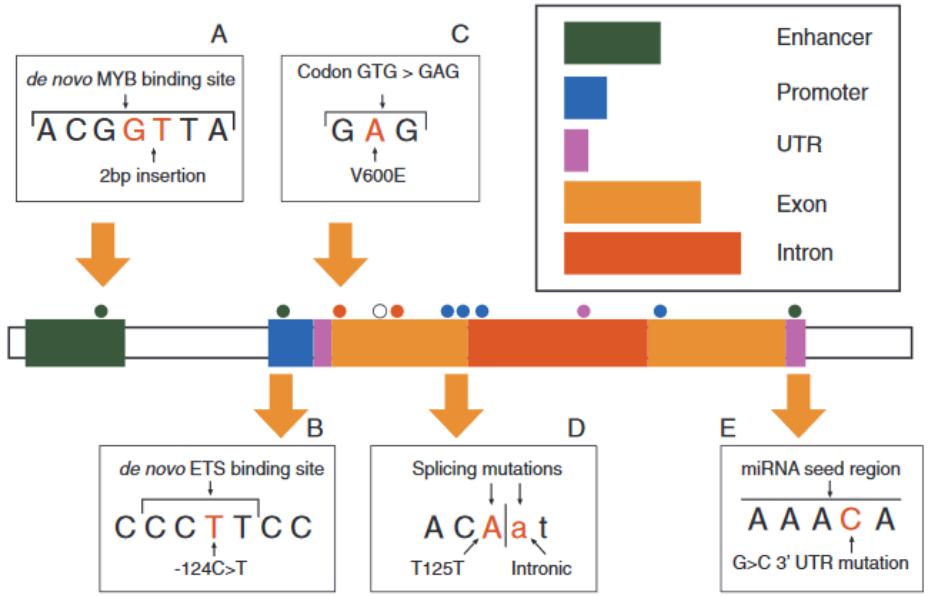
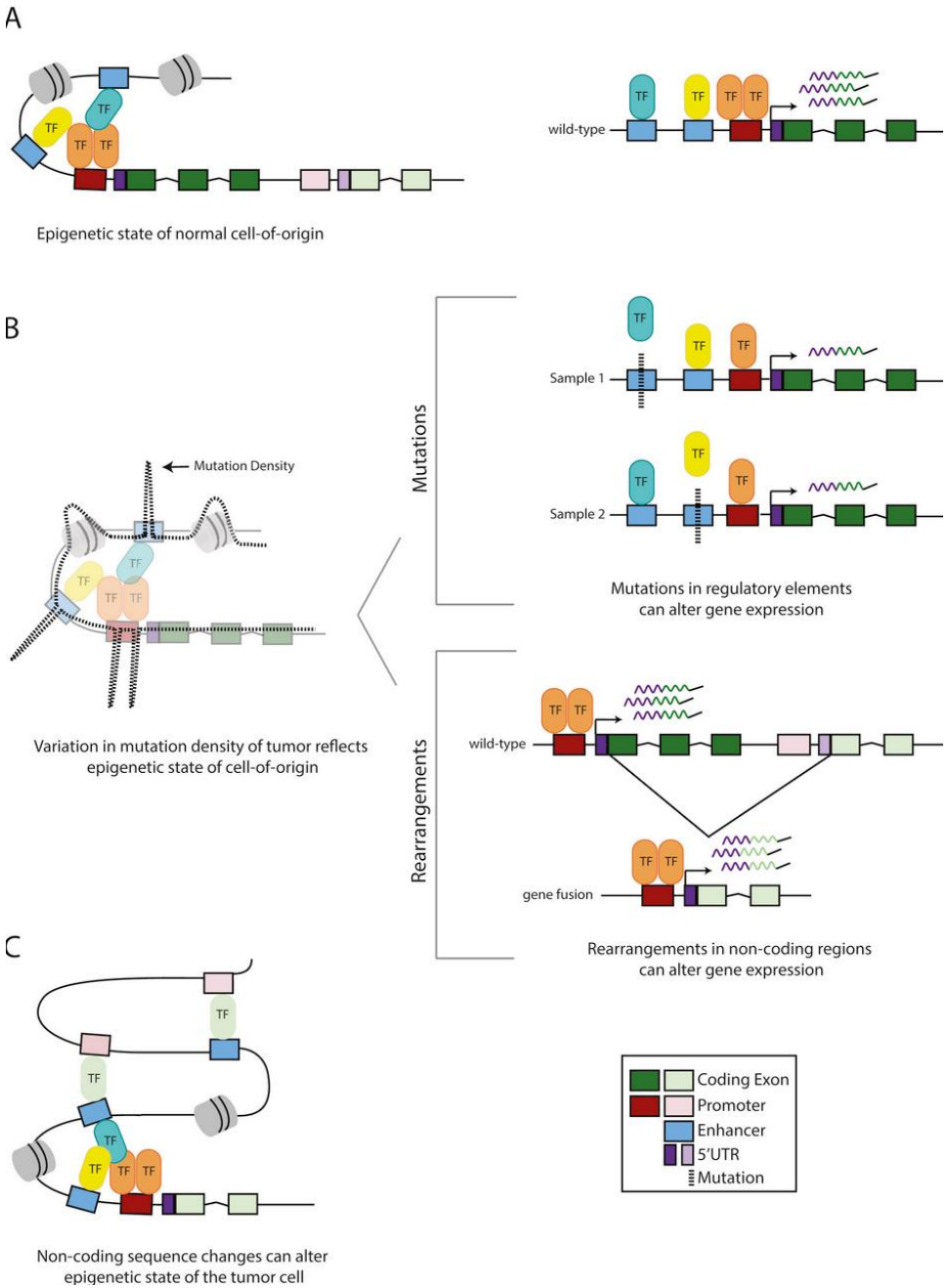
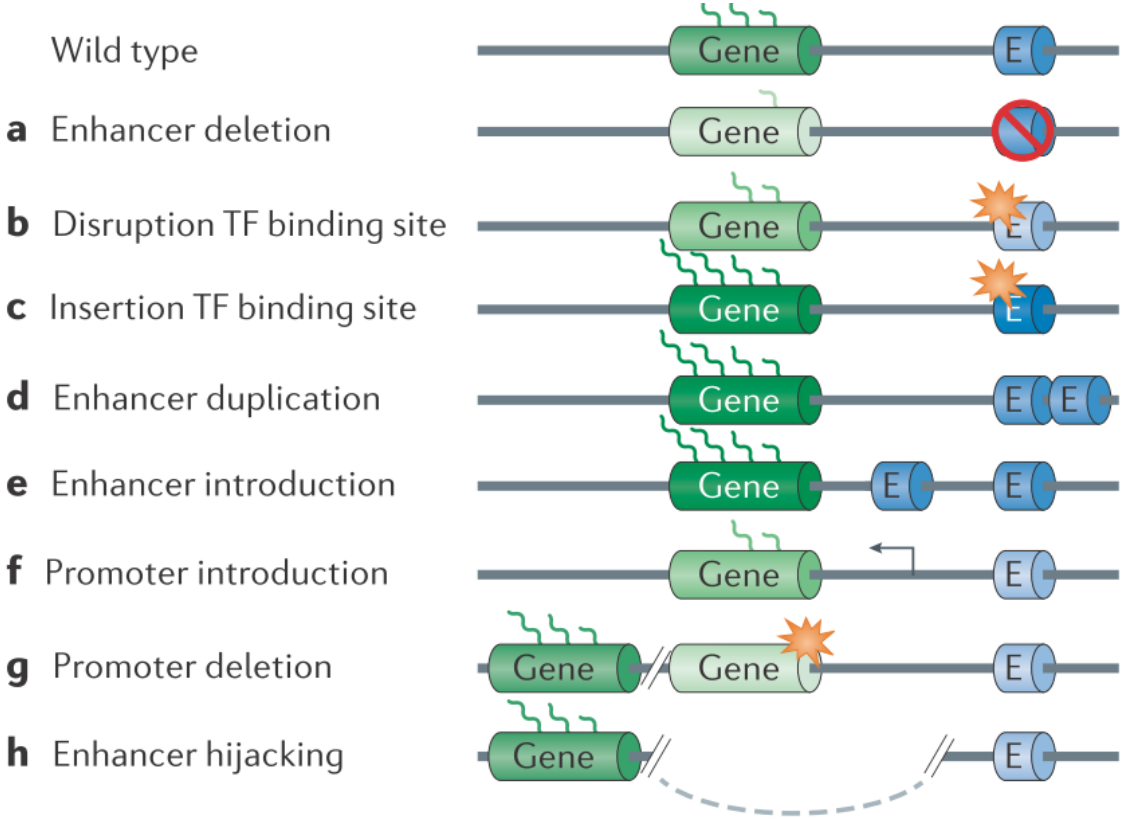


Figure 3. Several mechanisms have been discovered which can result in driver mutations, within both coding and non-coding regions of the genome. Enhancer mutations (A) may induce the binding of regulatory factors that either promote or inhibit gene expression. Promoter mutations (B) can similarly create or destroy binding sites that affect transcription. Coding mutations (C) can have many effects, such as altering critical amino acids, causing constitutive protein activation or disrupting protein folding. Splice site mutations (D) alter the splicing of genes. UTR mutations (E) can have various effects, including altering miRNA targeting.

Piraino et al. Beyond the exome: the role of non-coding somatic mutations in cancer



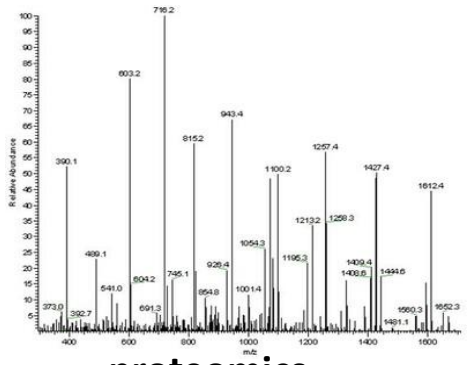
Cuykendall et al. Non-coding genetic variation in cancer



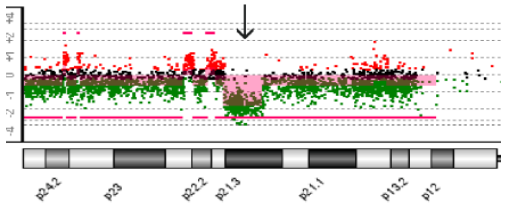
Disease	Affected gene	Enhancer	Refs
β-Thalassaemia	β-globin genes	LCR	3,4
HPE	*SHH*	SBE2	36
PDD2	*SHH*	ZRS	35,122
Lung adenocarcinoma	*MYC*	3′~450 kb SE	124
T-ALL	*TAL1*	NA	121
α-Thalassaemia	α-Globin genes	α-Globin enhancers	46
α-Thalassaemia*	α-Globin genes/*NME4*	α-Globin enhancers	44
Burkitt lymphoma	*MYC*	IgH enhancer	116,117

Figure 3 | **Erroneous regulatory wiring between enhancers and target genes causing disease.** Erroneous regulatory

Data analysis



proteomics



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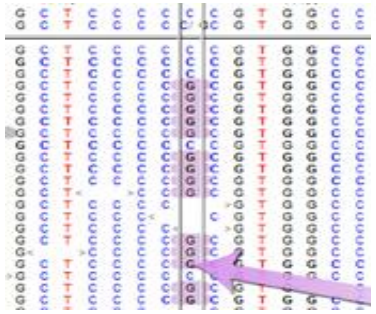
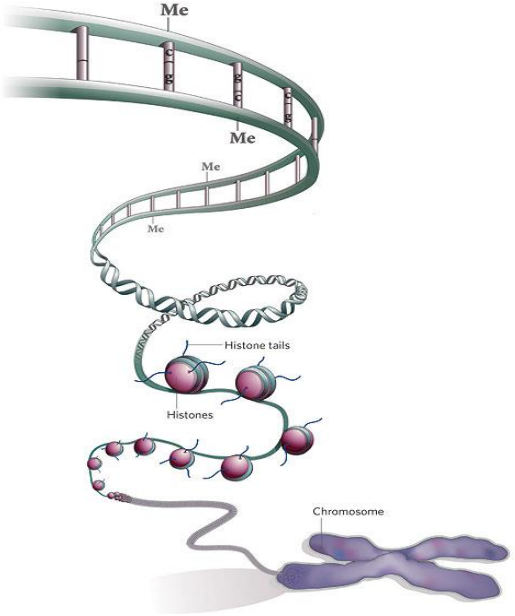
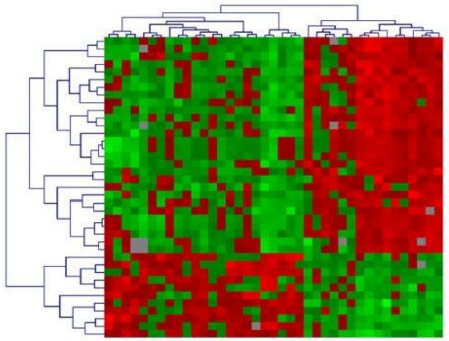


point mutations and in/dels

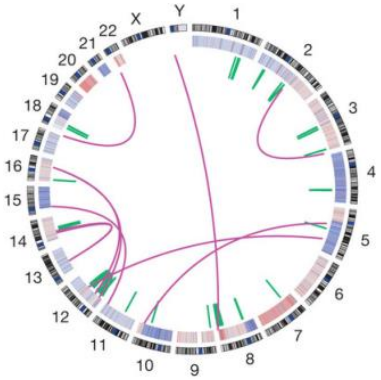


epigenetics

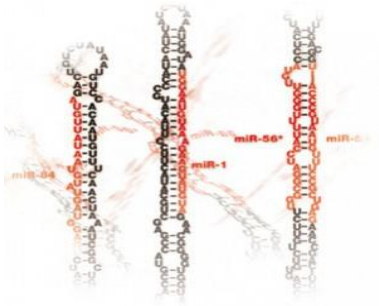


gene expression

rearrangements



non-coding RNAs

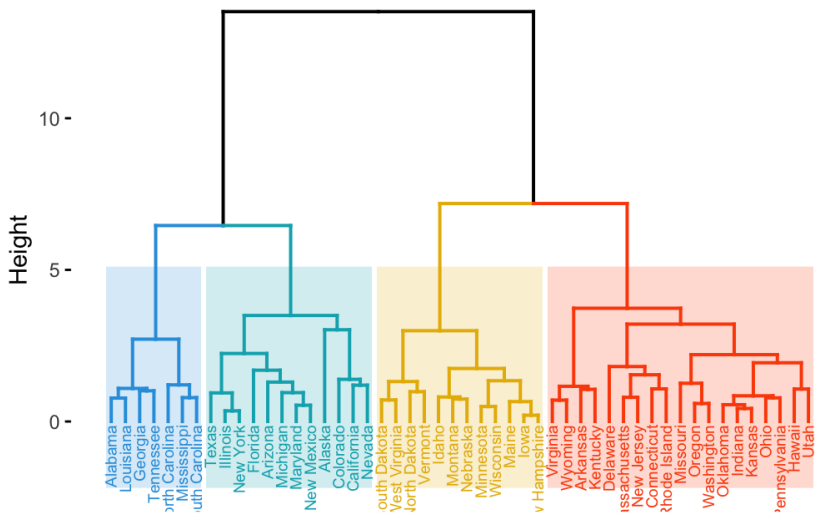


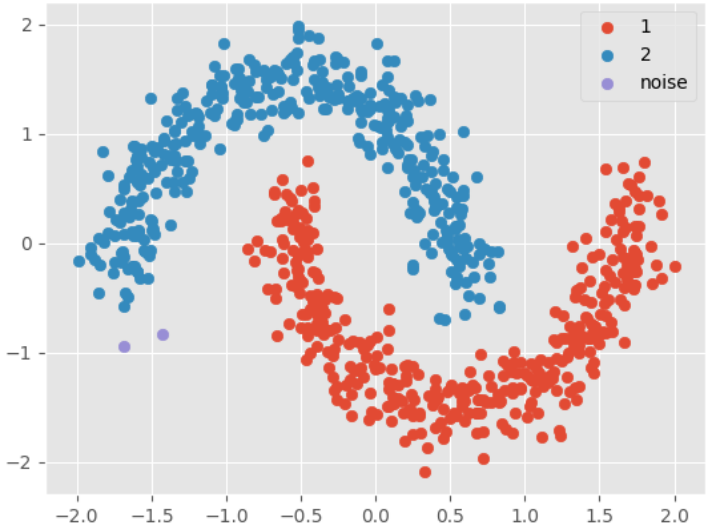
Bulk
Single-cell
Spatial
Spatiotemporal

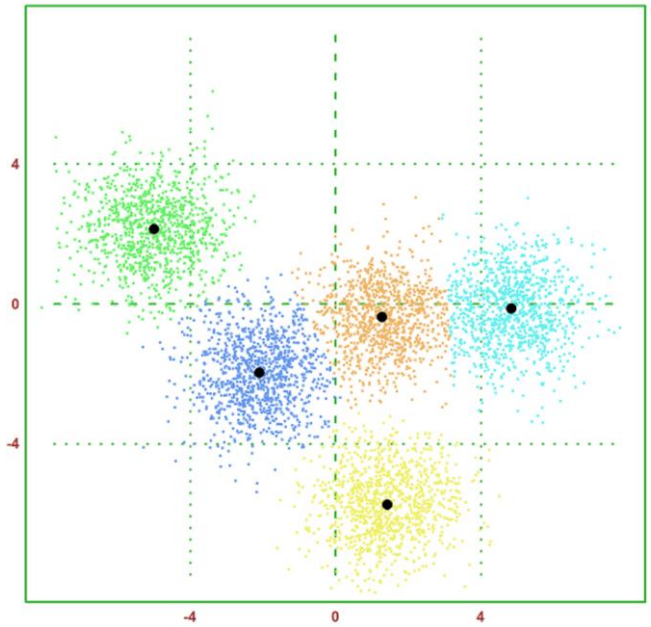
DNA modifications
Chromatin modifications
Chromatin structure and accessibility

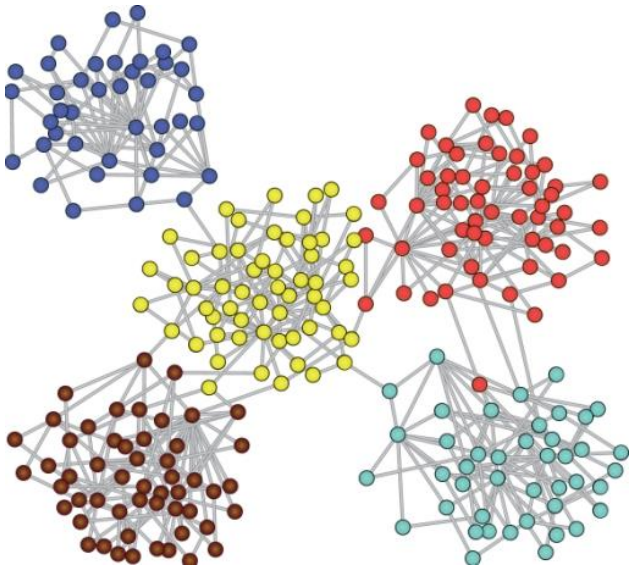
Clustering

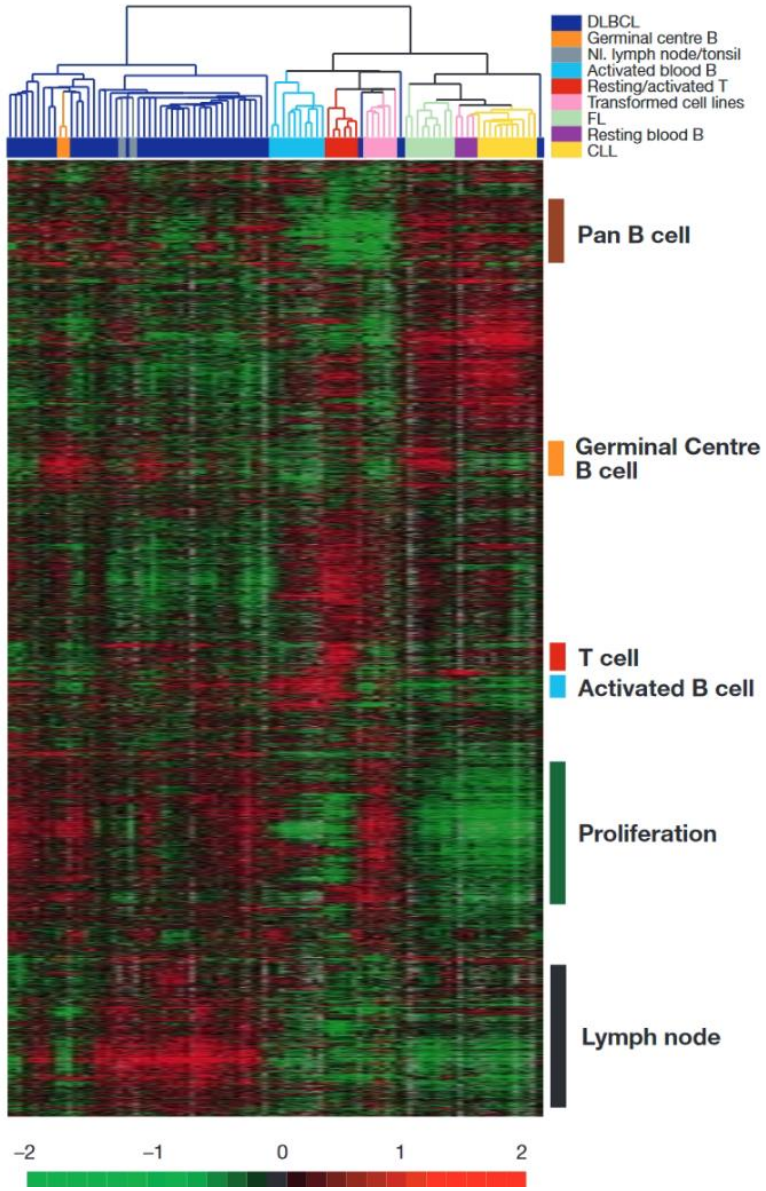
- Hierarchical
  - Agglomerative
  - Divisive
  - → Neighbor joining, UPGMA…
- Partitioning
  - Centroid → K-means, PAM…
  - Distribution
- Partitioning with outliers
  - Density-based → DBSCAN, OPTICS
- Overlapping clustering
- Graph clustering
- Spectral clustering
- …



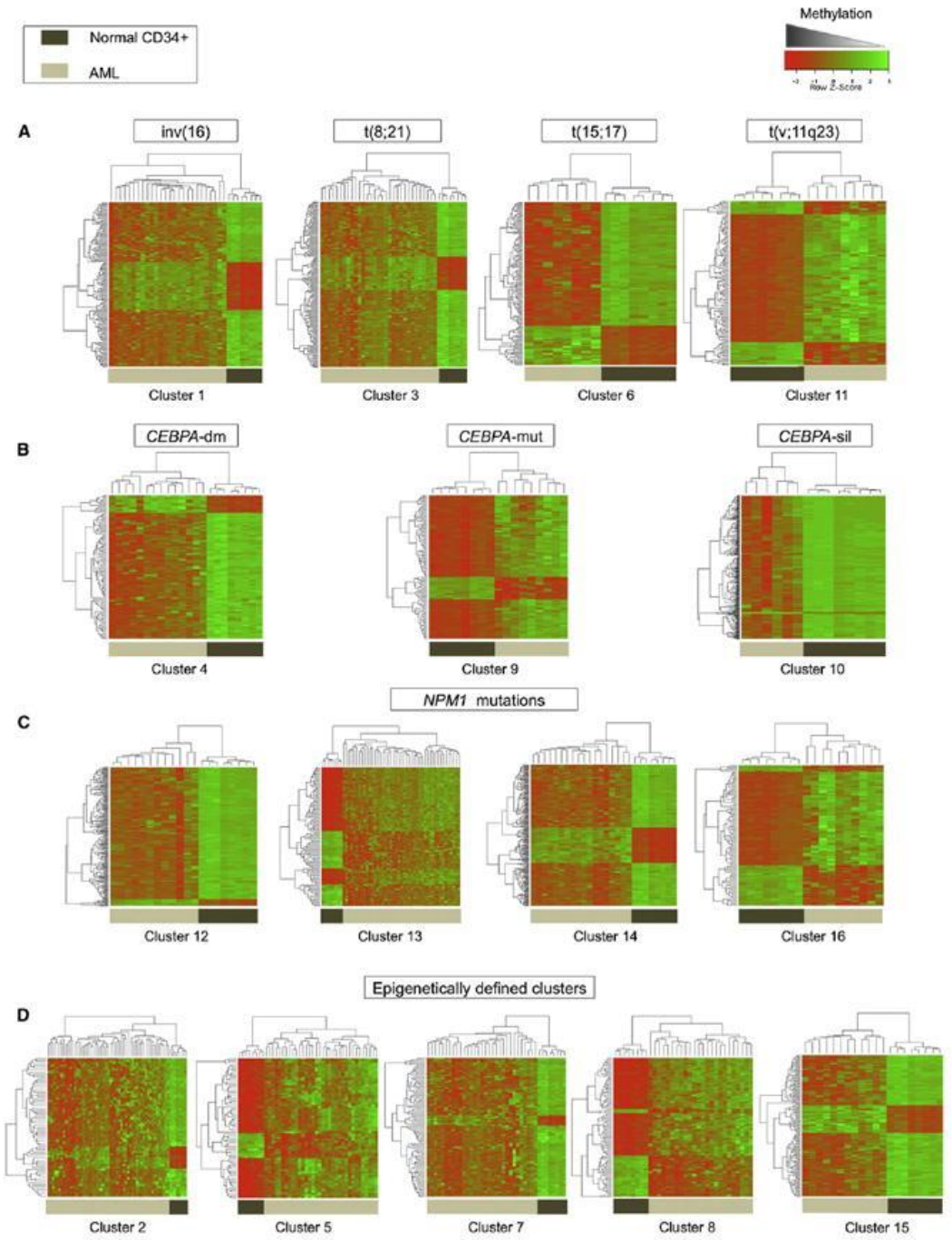




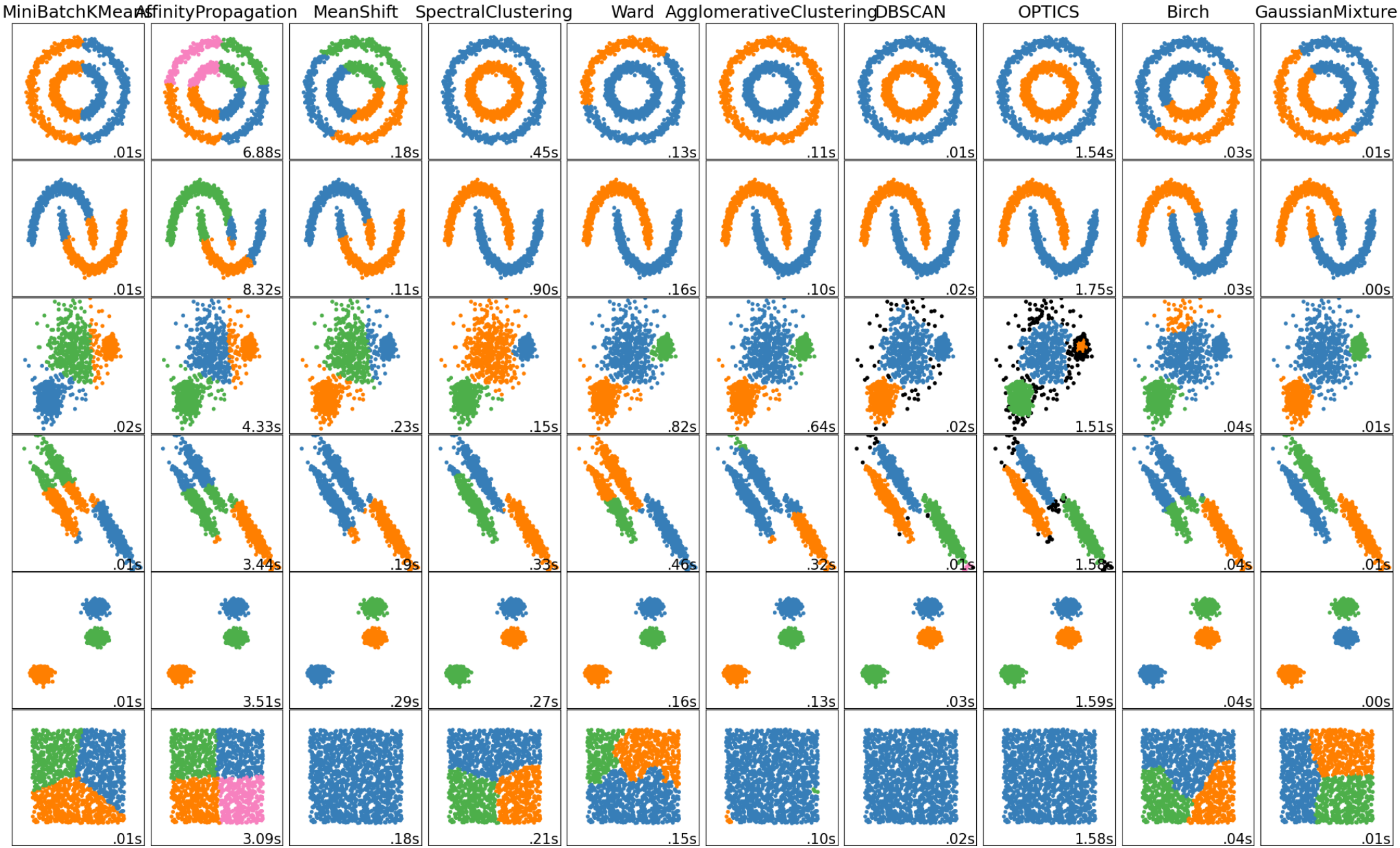




Alizadeh et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling, Nature 2000

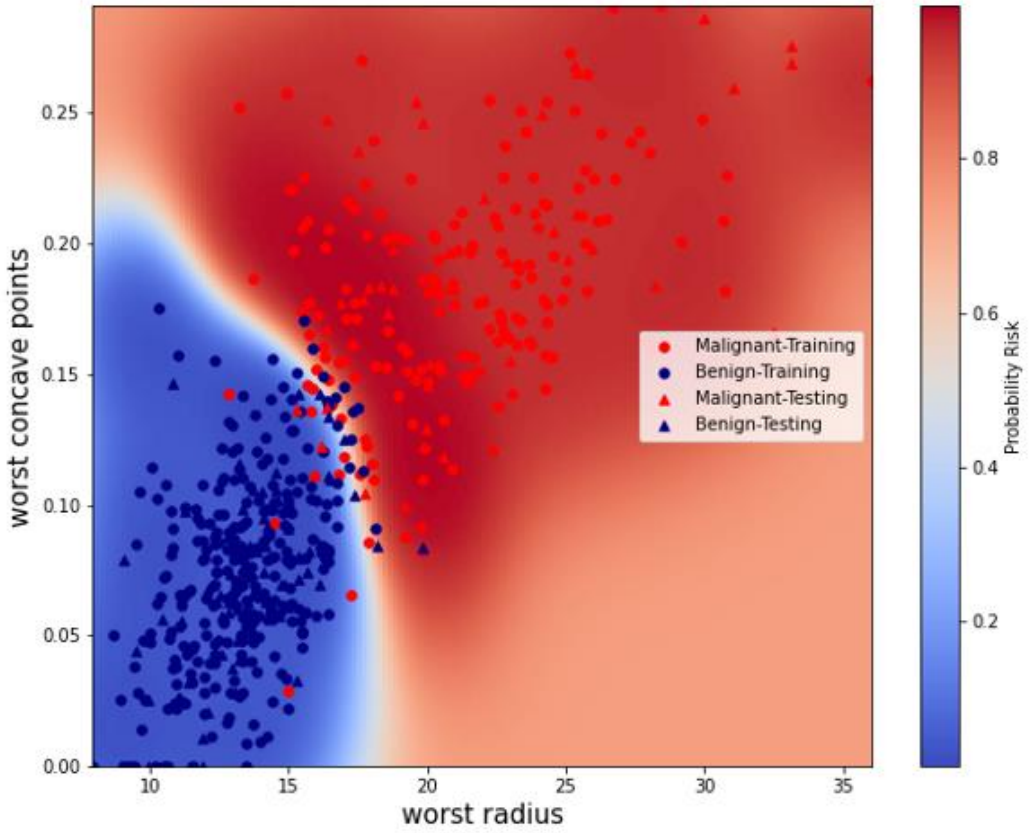


Figueroa et al. DNA Methylation Signatures Identify Biologically Distinct Subtypes in Acute Myeloid Leukemia



scikit-learn.org

Supervised methods



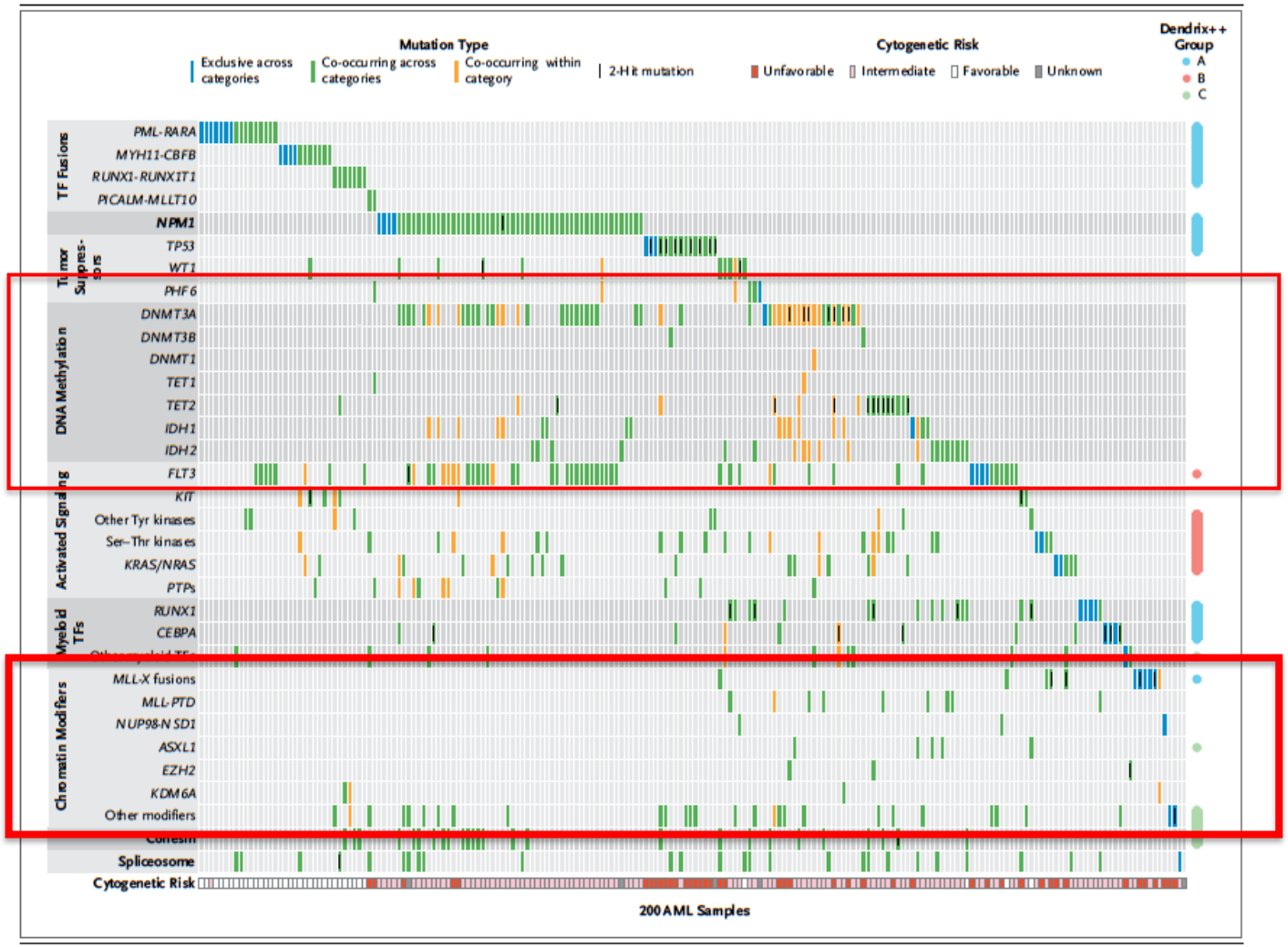
towardsdatascience.com

Case study:
TET2+FLT3-ITD or IDH2+FLT3-ITD double mutant AML

Genomic Analysis of AML Identifies Mutations in Genes Which Regulate DNA Methylation and Chromatin State

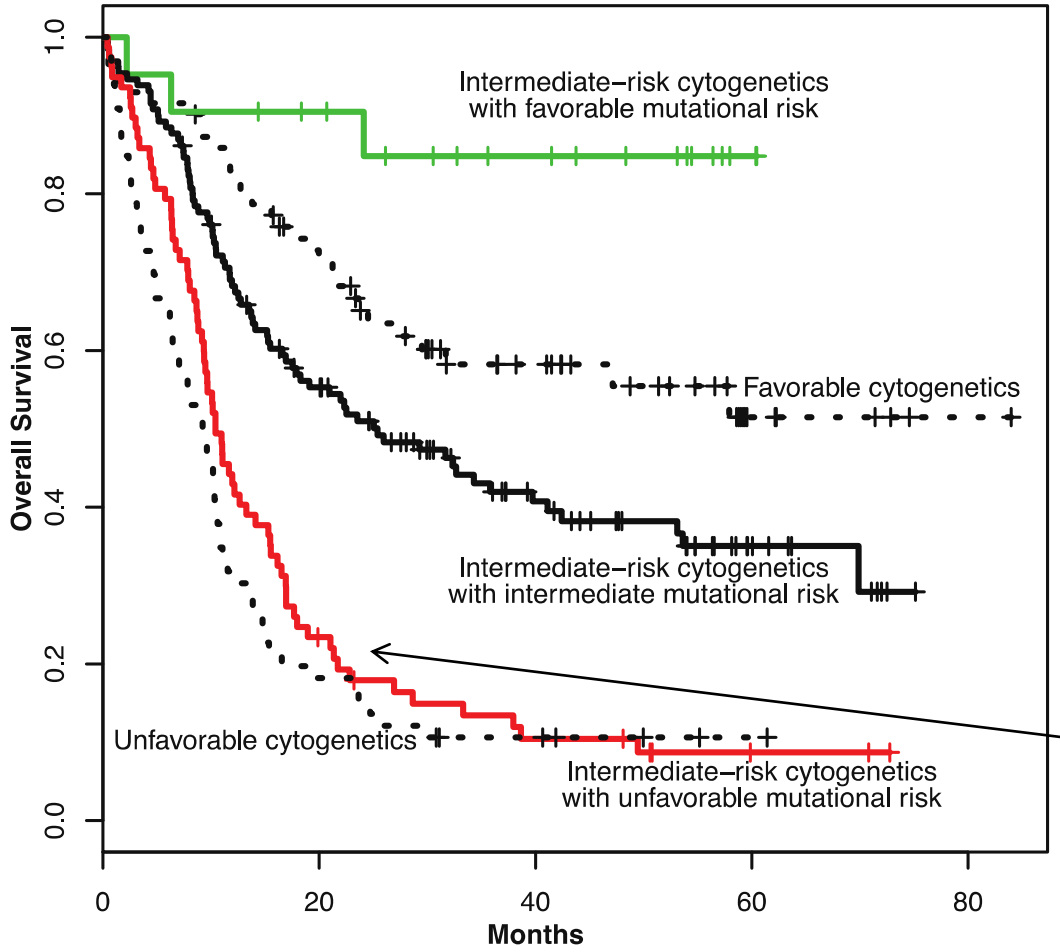
DNA Methylation

Chromatin State



TCGA AML NEJM 2013

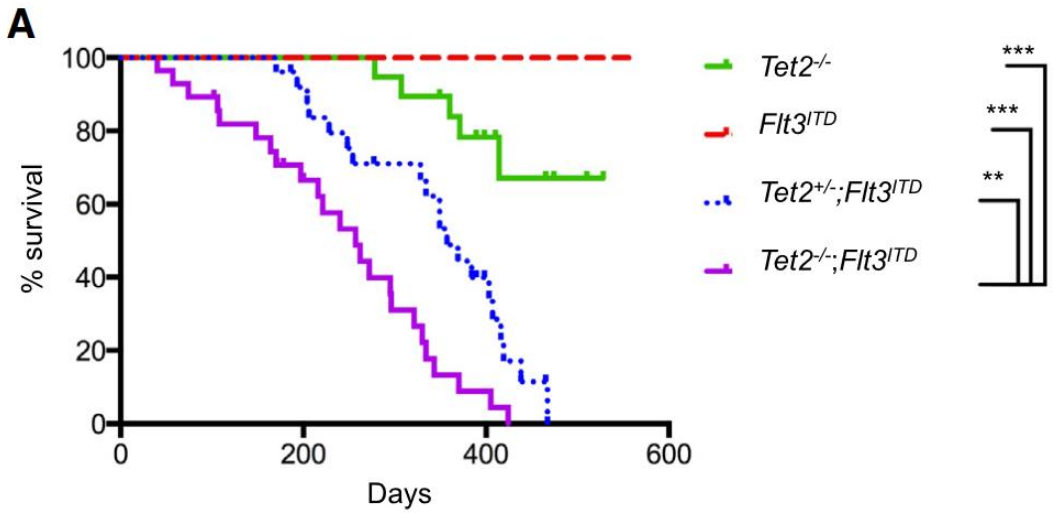
Adverse Outcome in AML Patients With Mutations in Epigenetic Modifiers

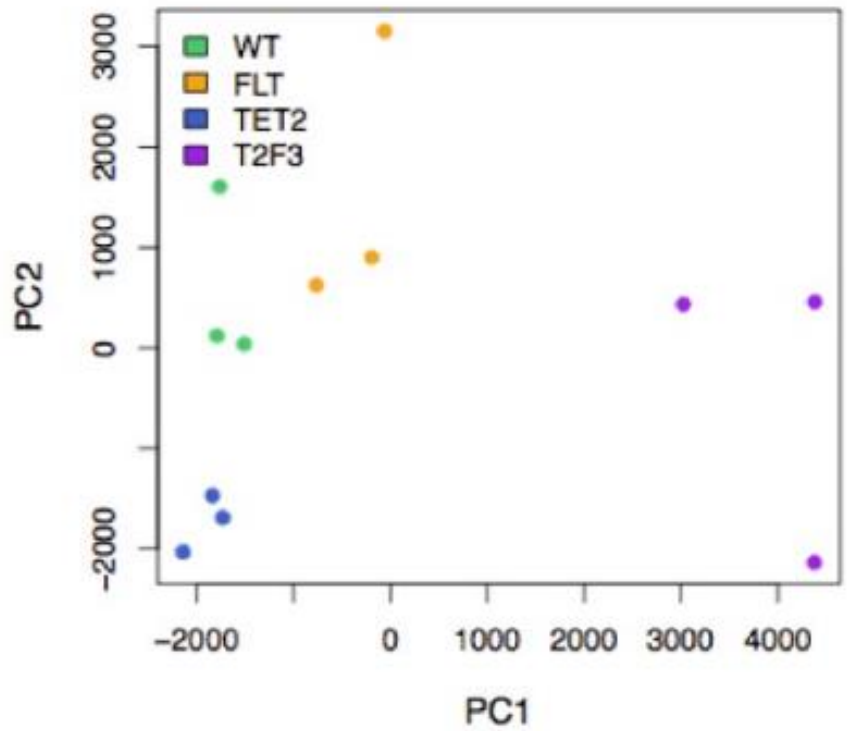


(p<0.001)

TET2, ASXL1, DNMT3A, MLL mutations

Patel et al. NEJM 2012

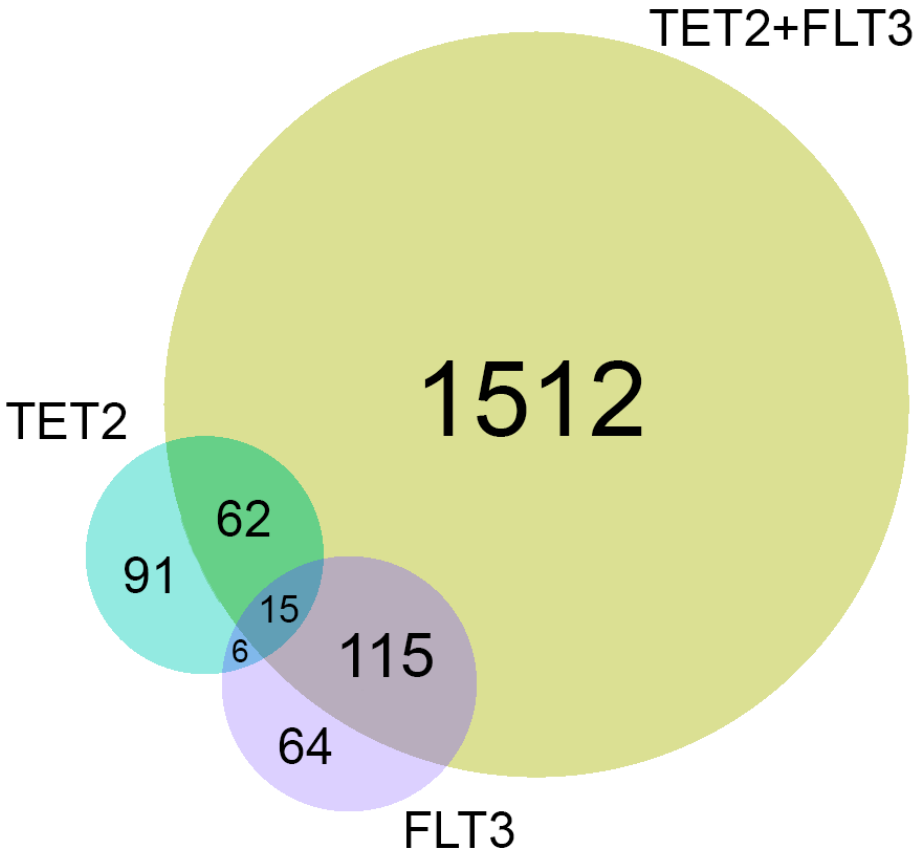

A
100
80
60
40
20
0
% survival
0
200
400
600
Days
Tet2-/-
Flt3ITD
Tet2+/-;Flt3ITD
Tet2-/-;Flt3ITD
***
***
**

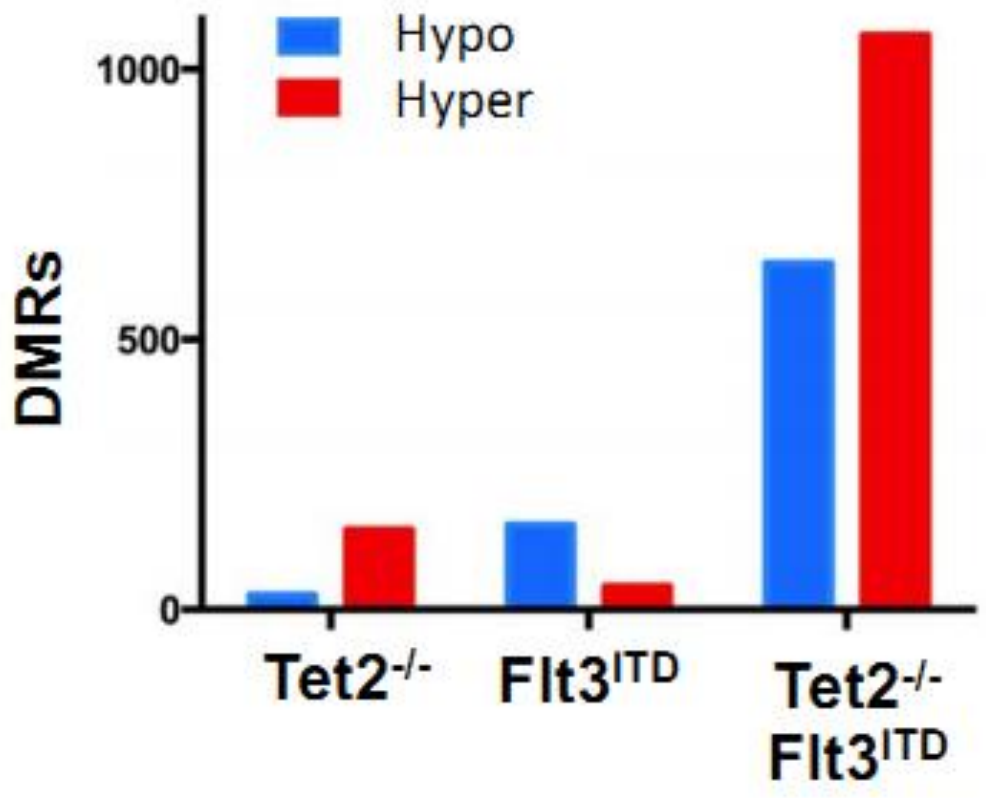

WT
FLT
TET2
T2F3
PC2
3000
2000
1000
0
−2000
−2000
0
1000
2000
3000
4000
PC1

TET2/FLT3 interaction causes disproportional epigenetic changes compared to the single mutants

of DMRs

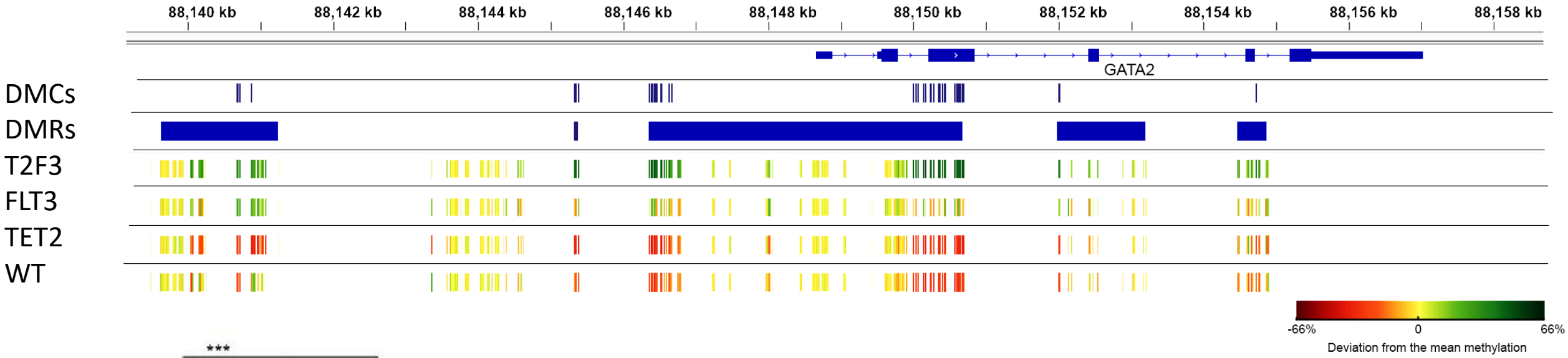
Differentially Methylated Regions

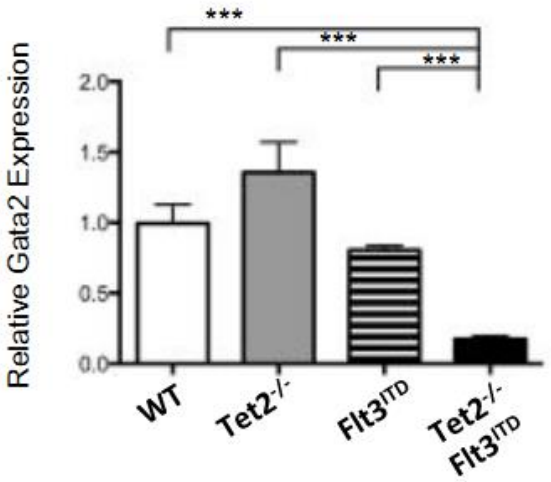




GATA2

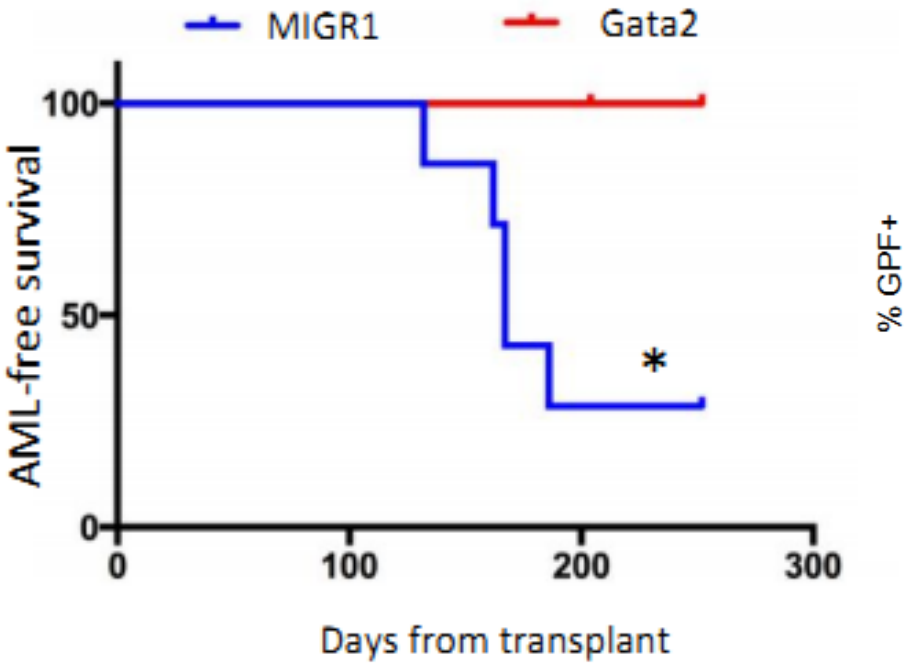
Gata2: transcription factor, regulator of gene expression in hematopoietic cells, associated with AML

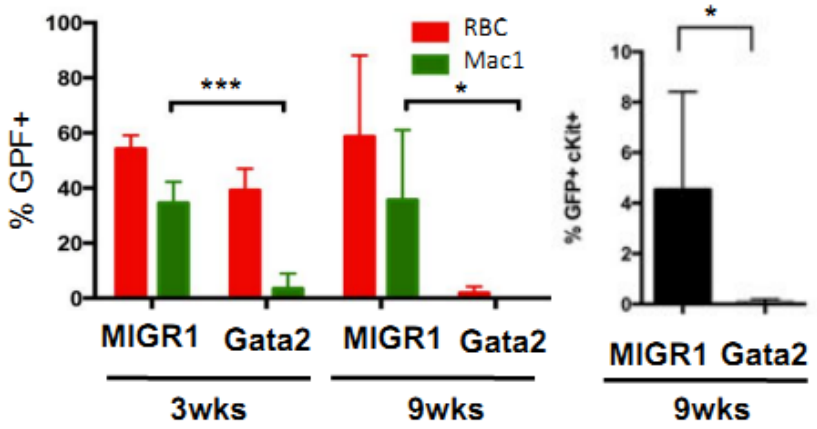


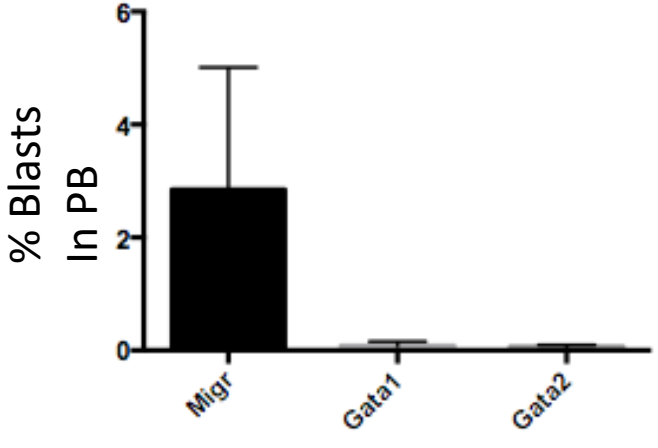


TET2 and FLT3-ITD mutations in LSKs led to significant decreased levels of Gata2 RNA compared to wild type or single mutants

GATA2 re-expression restores differentiation and attenuates leukemogenesis

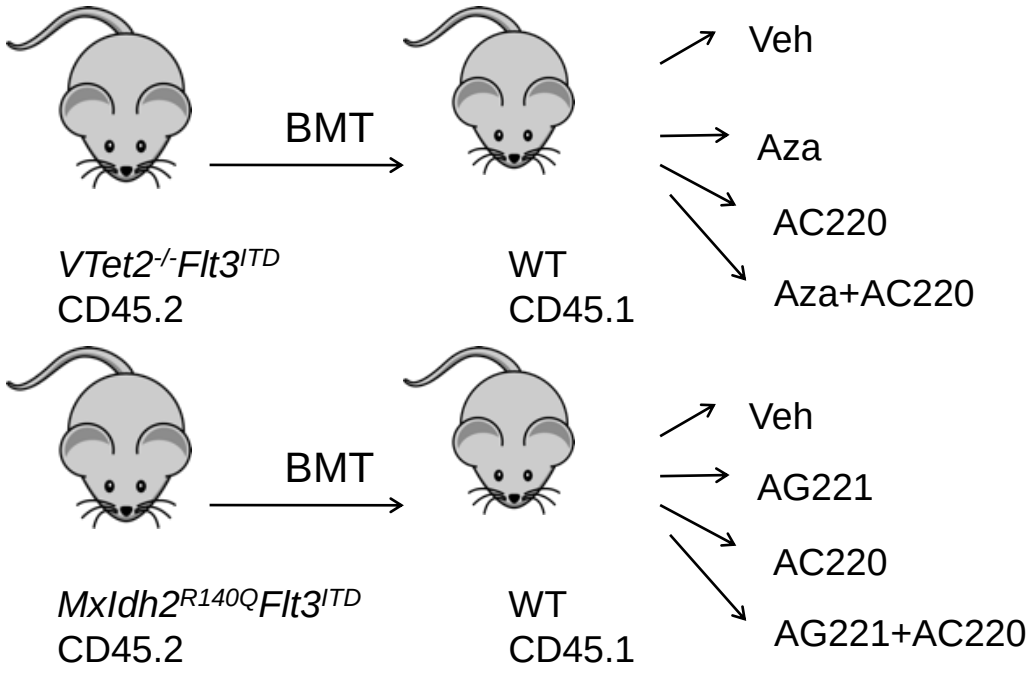


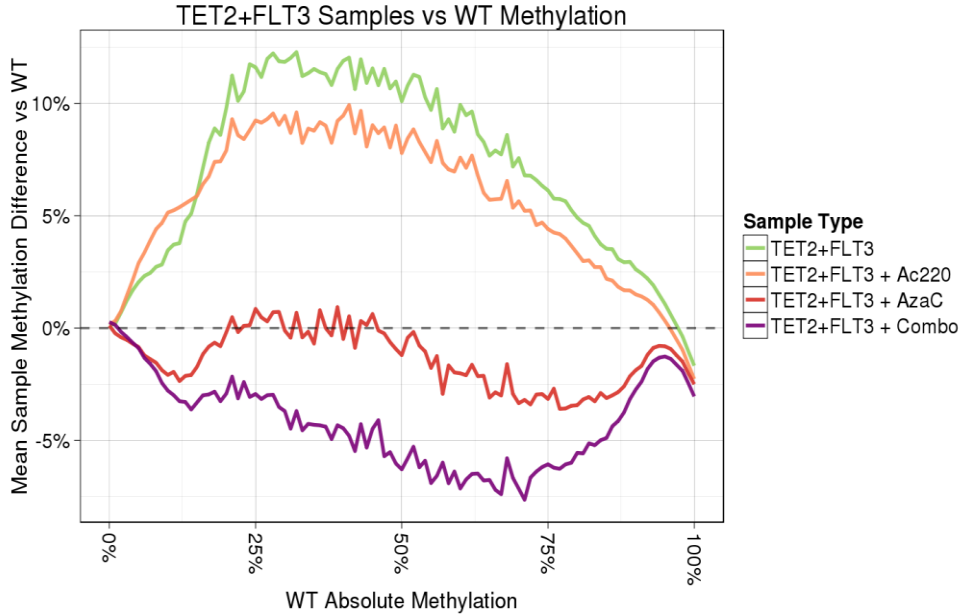


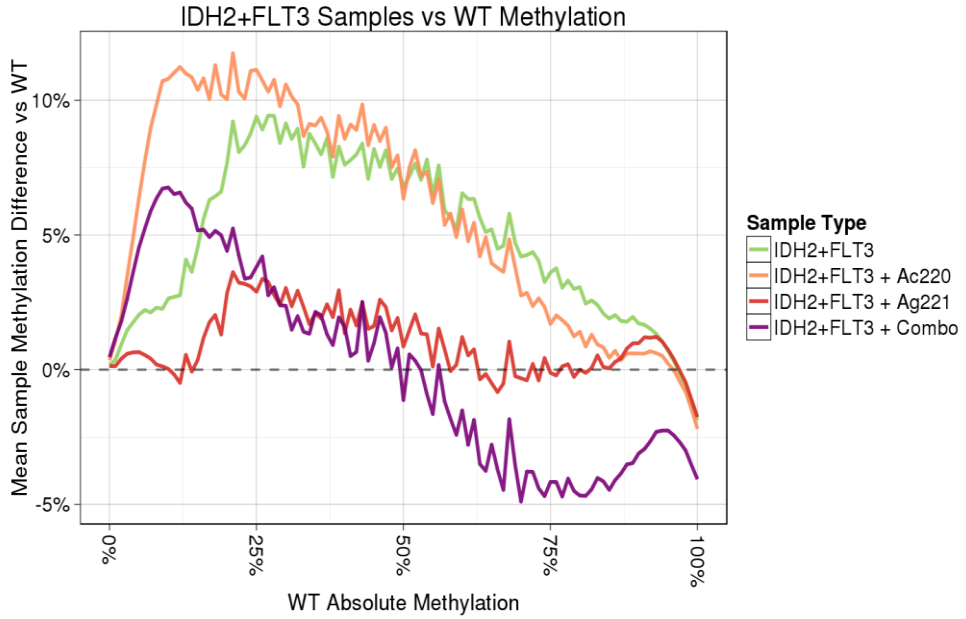


mice expressing vector alone succumbed to leukemia, whereas no mice expressing GATA2 developed lethal AML

Gata2 expression, but not expression of vector control, resulted in a progressive reduction in the proportion of c-kit+ AML cells, consistent with disappearance of the AML clone in vivo


BMT
VTet2-/-Flt3ITD
CD45.2
WT
CD45.1
Veh
Aza
AC220
Aza+AC220
BMT
MxIdh2R140QFlt3ITD
CD45.2
WT
CD45.1
Veh
AG221
AC220
AG221+AC220


TET2+FLT3 Samples vs WT Methylation
Mean Sample Methylation Difference vs WT
10%
5%
0%
-5%
0%
25%
50%
75%
100%
WT Absolute Methylation
Sample Type
TET2+FLT3
TET2+FLT3 + Ac220
TET2+FLT3 + AzaC
TET2+FLT3 + Combo

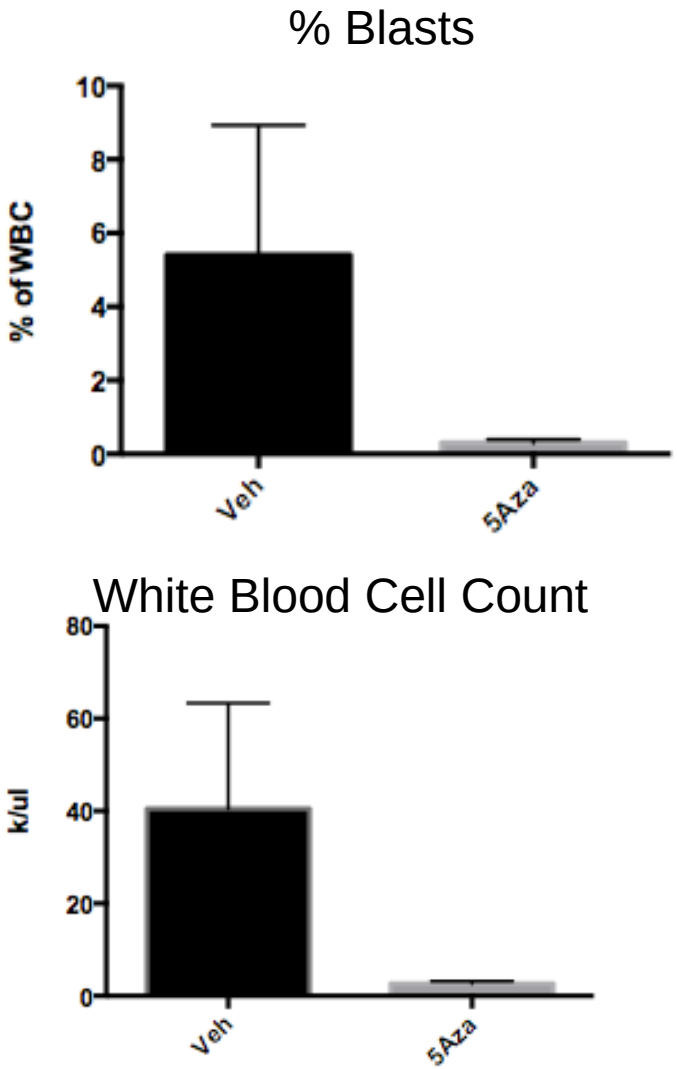

IDH2+FLT3 Samples vs WT Methylation
Mean Sample Methylation Difference vs WT
10%
5%
0%
-5%
0%
25%
50%
75%
100%
WT Absolute Methylation
Sample Type
IDH2+FLT3
IDH2+FLT3 + Ac220
IDH2+FLT3 + Ag221
IDH2+FLT3 + Combo

AzaC reverts methylation of GATA2 to WT levels

- Complete reversal of aberrant hypermethylation at FLT3/TET2 synergistic target loci, including GATA2, MN1, HOXA3 which are aberrantly methylated in FLT3/TET2-mutant AMLs

- See synergistic activity with FLT3 targeted therapies (dose, sequence matter)
- What about inhibition of mutant IDH1/2?

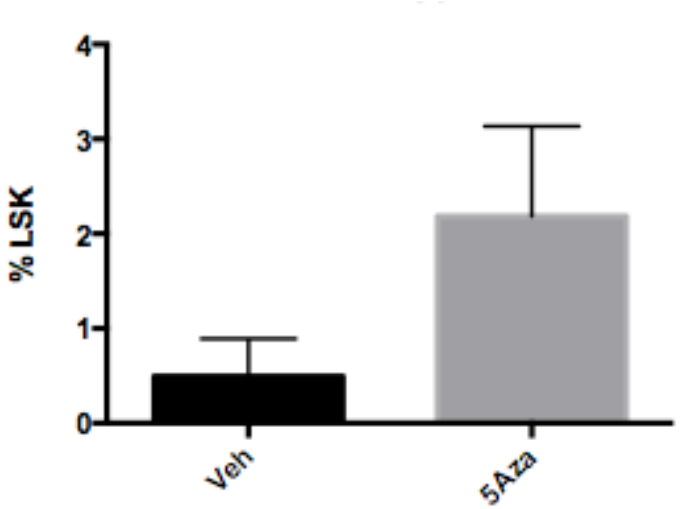
- Azacytidine therapy results in clearance of AML cells from peripheral blood
  - Marked reduction in white blood cell count, blast percentage
  - Reduction in spleen size
  - Reversal of anemia/thrombocytopenia with normalization of hematocrit/platelet counts



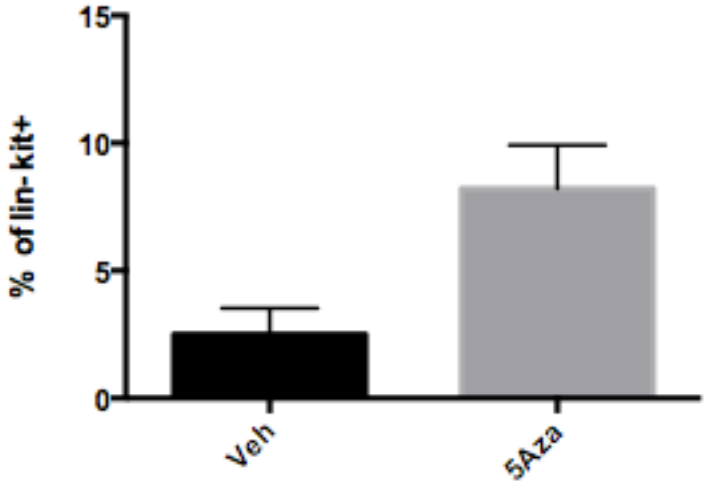
- **AzaC therapy normalizes differentiation in TET2-mutant AML**

Restores Long-term HSC Compartment

LT-HSCs

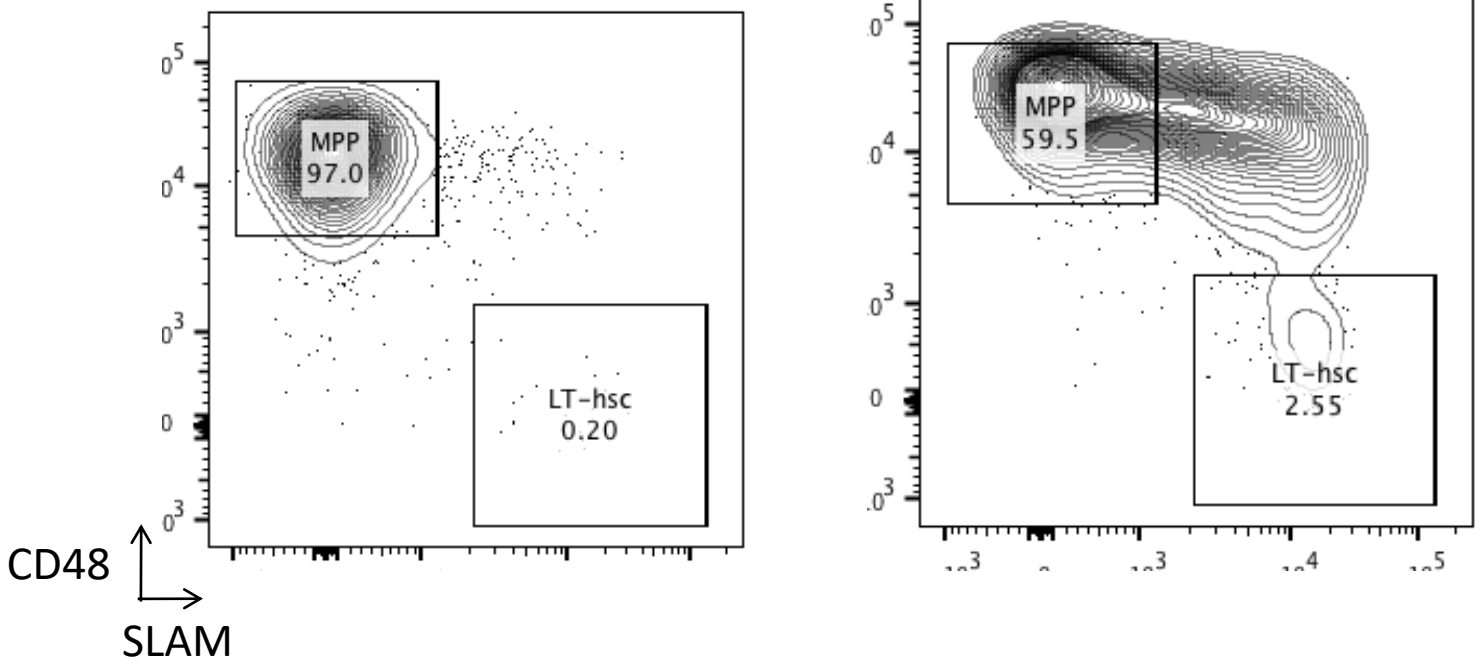


Meg-Erythroid Progenitors

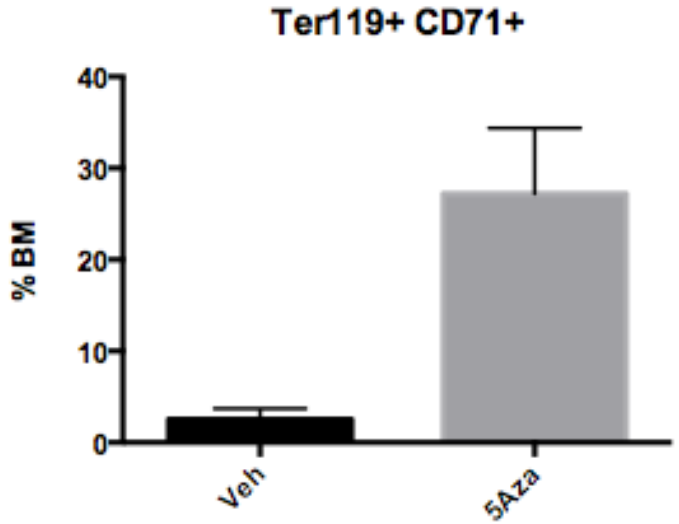


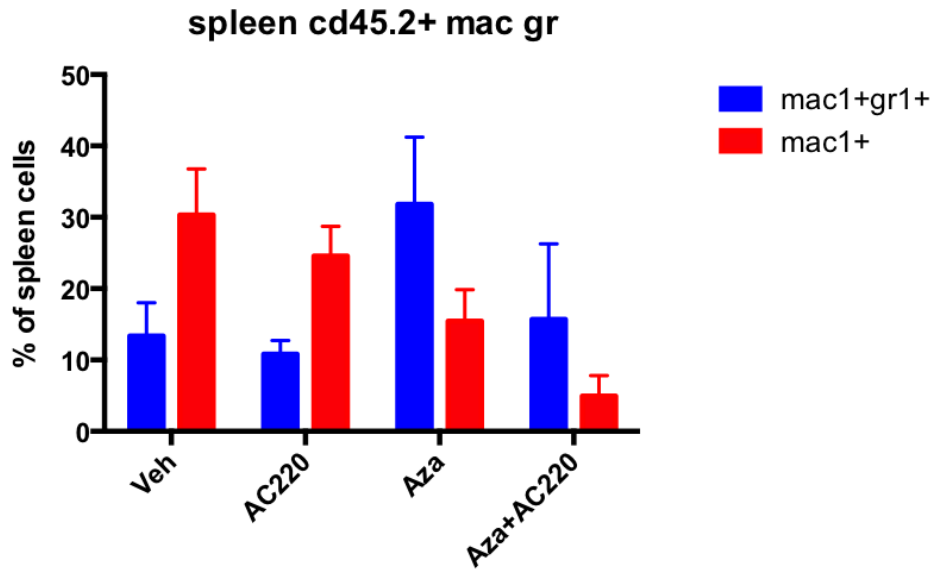
Vehicle

Azacytidine



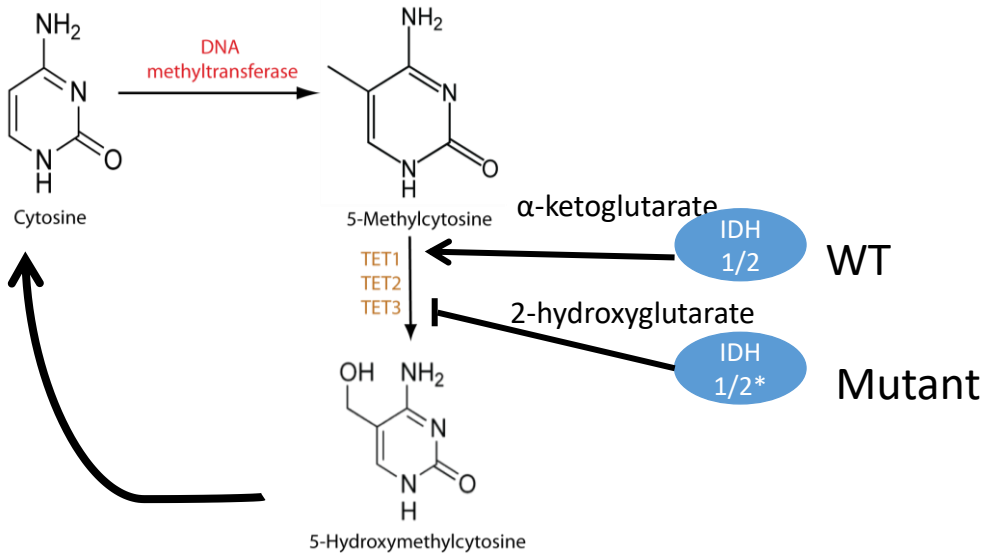
AzaC treatment in TET2+FLT3 causes differentiation response

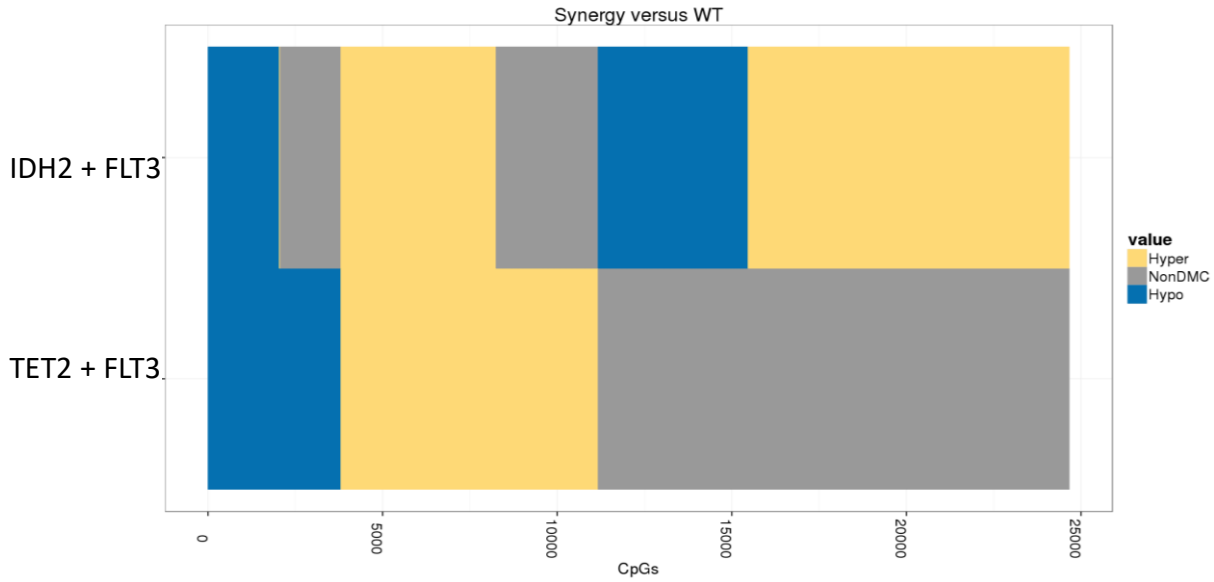




Monocytes, Mac+Gr- are reduced

Overlap in Epigenetic Signature in TET2 & IDH2 + FLT3





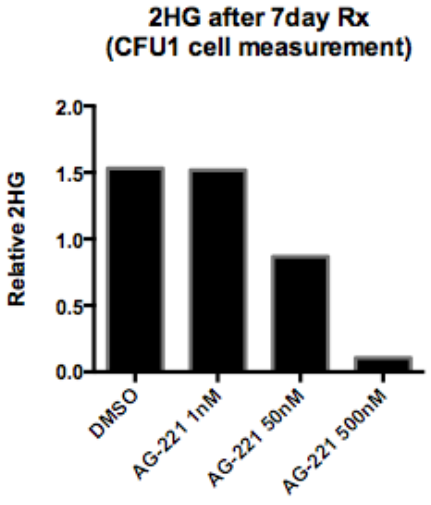
Figueroa, Abdel-Wahab, Lu *et al,* ***Cancer Cell*** 2010

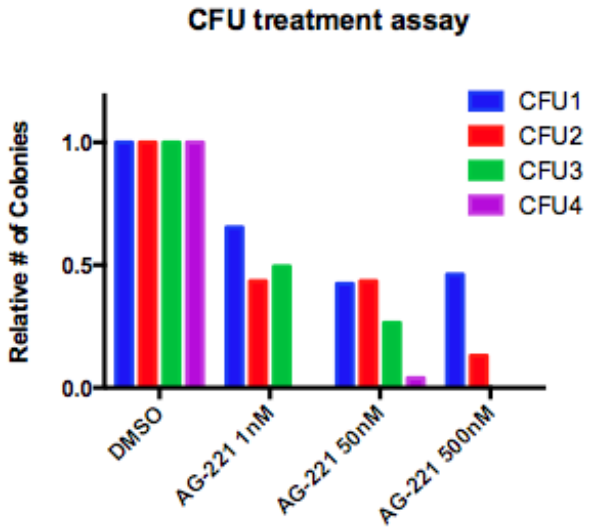
GATA2 Hypermethylation in IDH2/FLT3

- IDH2/FLT3 shows hypermethylation/silencing of GATA2 not present in IDH2-only mutant mice
- Similar signature to TET2/FLT3

IDH2 Inhibitor

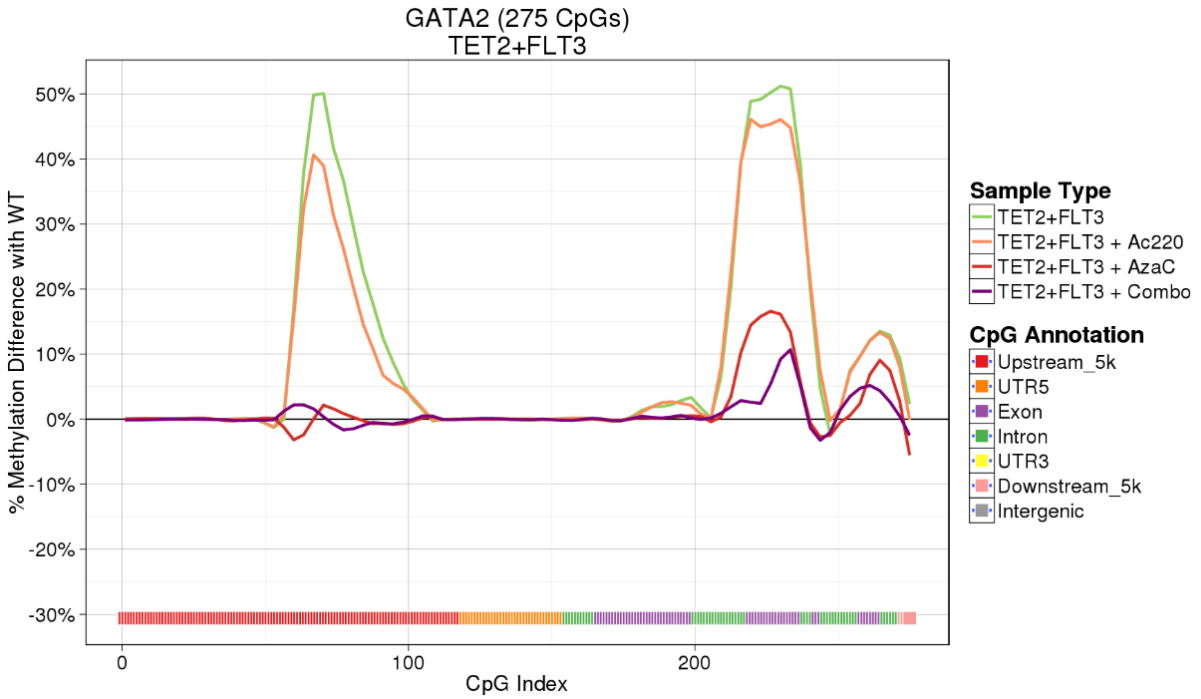
- AG221 - orally available, selective, potent inhibitor of the mutated IDH2 protein

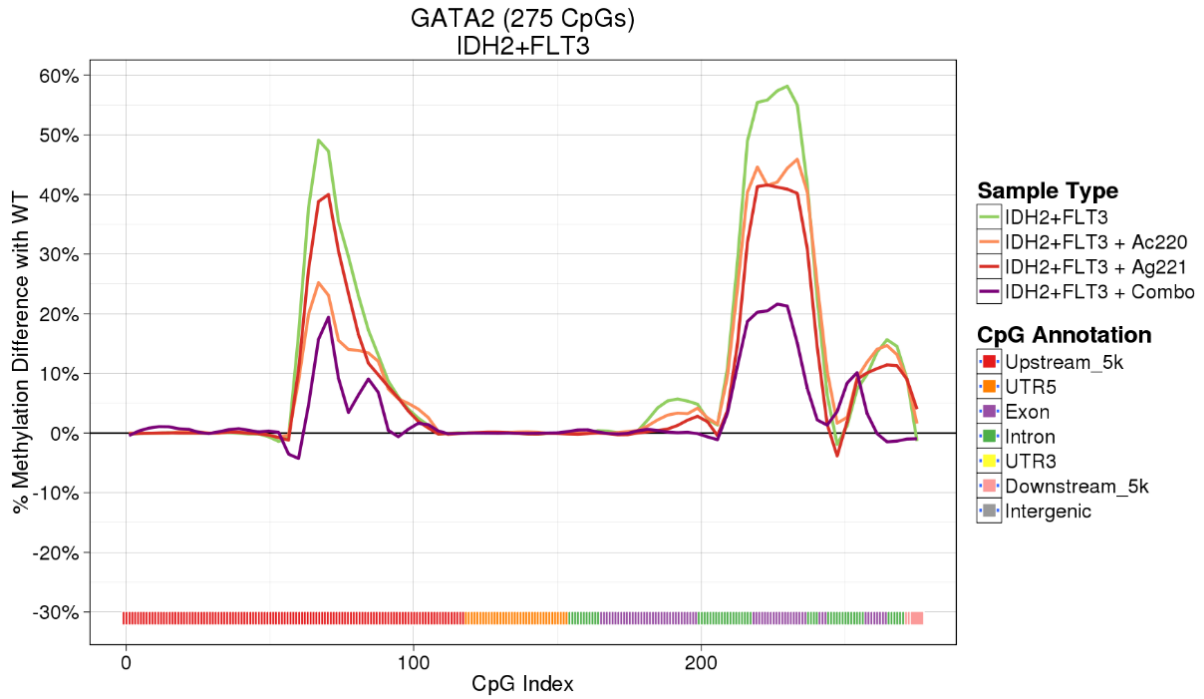


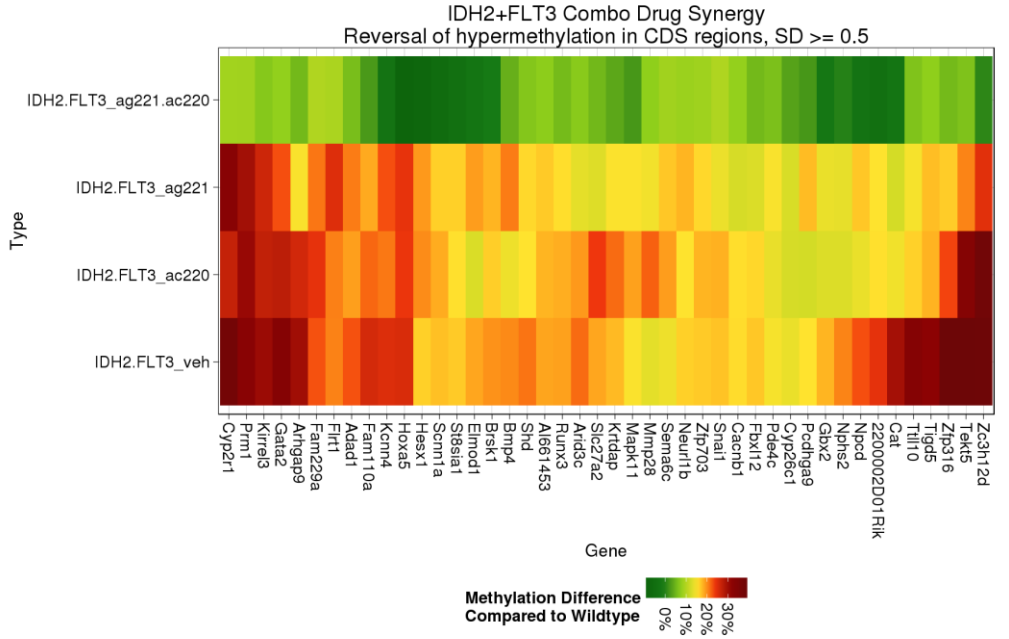


IDH2-inhibition Inhibits FLT3/IDH2-mutant Replating

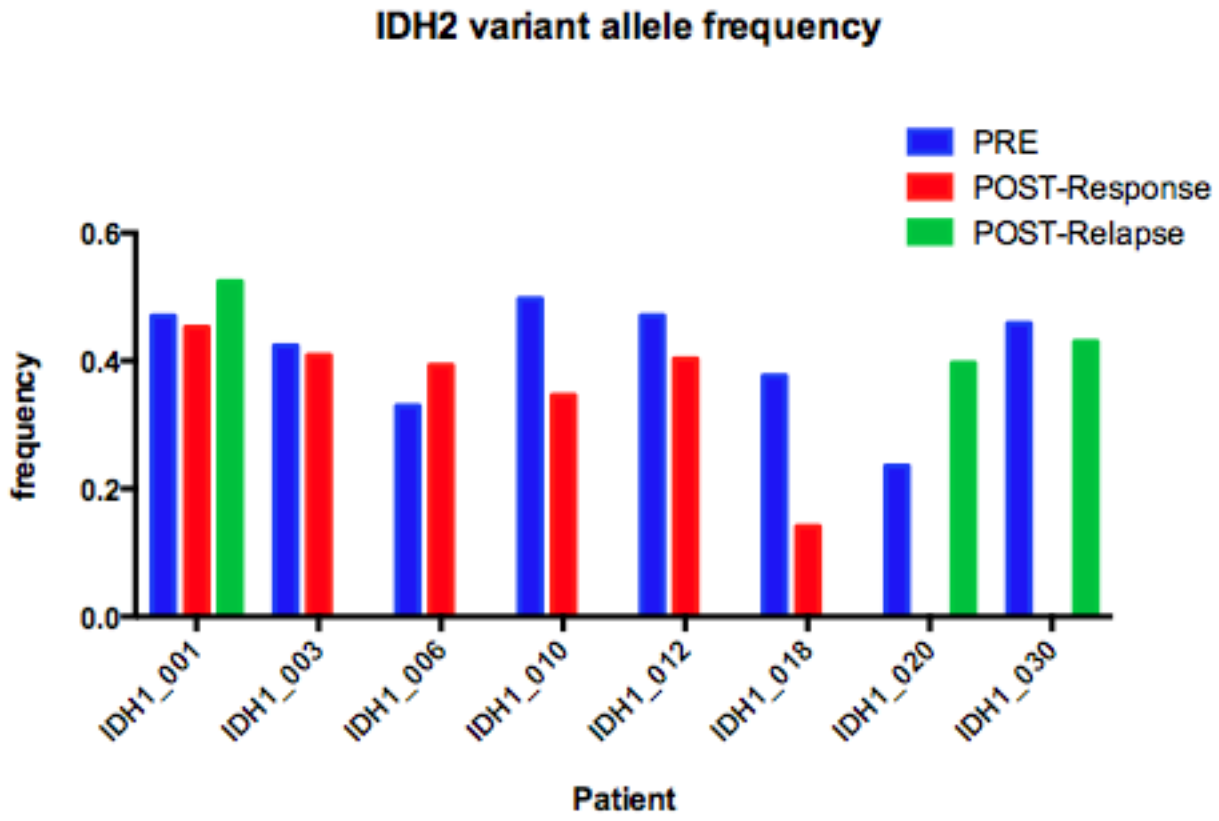
No effect on TET2-mutant cells consistent with mutant-specific effects on 2-HG and self-renewal in vitro


GATA2 (275 CpGs)
TET2+FLT3
% Methylation Difference with WT
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
0
100
200
CpG Index
Sample Type
TET2+FLT3
TET2+FLT3 + Ac220
TET2+FLT3 + AzaC
TET2+FLT3 + Combo
CpG Annotation
Upstream_5k
UTR5
Exon
Intron
UTR3
Downstream_5k
Intergenic

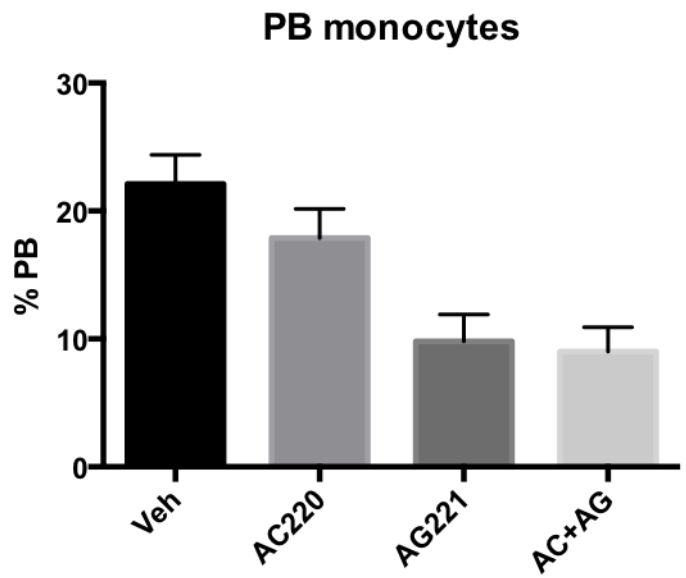

GATA2 (275 CpGs)
IDH2+FLT3
% Methylation Difference with WT
60%
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
0
100
200
CpG Index
Sample Type
IDH2+FLT3
IDH2+FLT3 + Ac220
IDH2+FLT3 + Ag221
IDH2+FLT3 + Combo
CpG Annotation
Upstream_5k
UTR5
Exon
Intron
UTR3
Downstream_5k
Intergenic

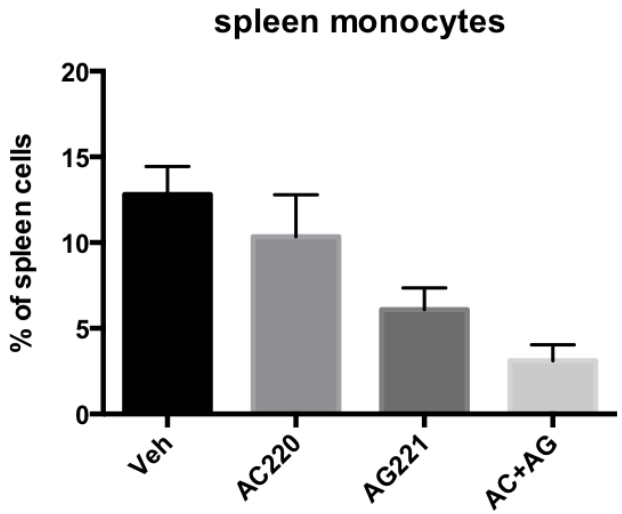

IDH2+FLT3 Combo Drug Synergy
Reversal of hypermethylation in CDS regions, SD >= 0.5
Type
IDH2.FLT3_ag221.ac220
IDH2.FLT3_ag221
IDH2.FLT3_ac220
IDH2.FLT3_veh
Cyp2r1
Prm1
Kirrel3
Gata2
Arhgap9
Fam229a
Flrt1
Adad1
Fam110a
Kcnn4
Hoxa5
Hesx1
Scnn1a
St8sia1
Eimod1
Brsk1
Bmp4
Shd
Al661453
Arid3c
Runx3
Slc27a2
Krtdap
Mapk11
Mmp28
Sema6c
Neurl1b
Zfp703
Snai1
Cacnb1
Fbxl12
Pde4c
Cyp26c1
Pcdhga9
Gbx2
Nphs2
Npcd
2200002D01Rik
Cat
Ttll10
Tigd5
Zfp316
Tekt5
Zc3h12d
Gene
Methylation Difference
Compared to Wildtype
0%
10%
20%
30%

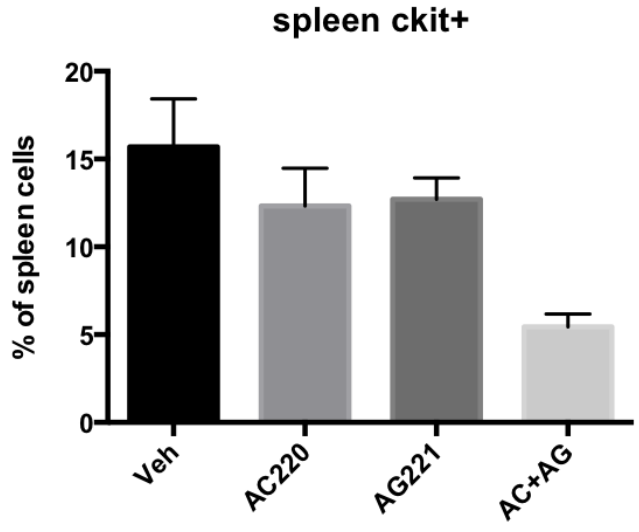
AG221 reatment does not significantly alter IDH2-mut allele frequency



AG221 treatment in IDH+FLT3 model causes differentiation changes







agios

Press Release

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FDA Grants Approval of IDHIFA®, the First Oral Targeted Therapy for Adult Patients with Relapsed/Refractory Acute Myeloid Leukemia and an IDH2 Mutation

IDHIFA is the first and only oral, targeted inhibitor of IDH2[1]

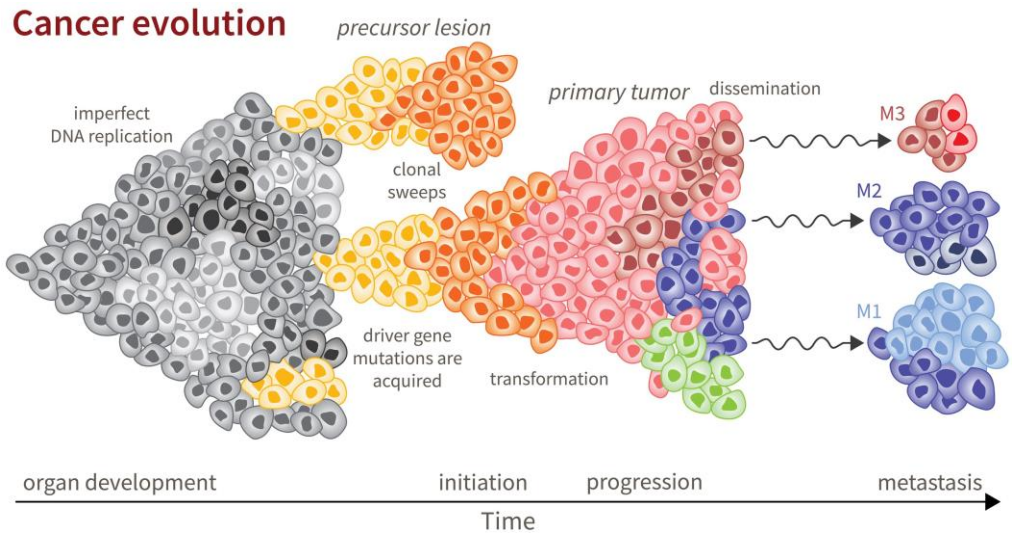
FDA approval of IDHIFA was based on results from the phase I/II AG-221 AML-001 study including safety, rate and duration of complete response (CR) or CR with partial hematologic recovery (CRh) and rate of conversion to transfusion independence[1]

Relapsed and refractory AML is a debilitating disease with a significant unmet medical need[2]

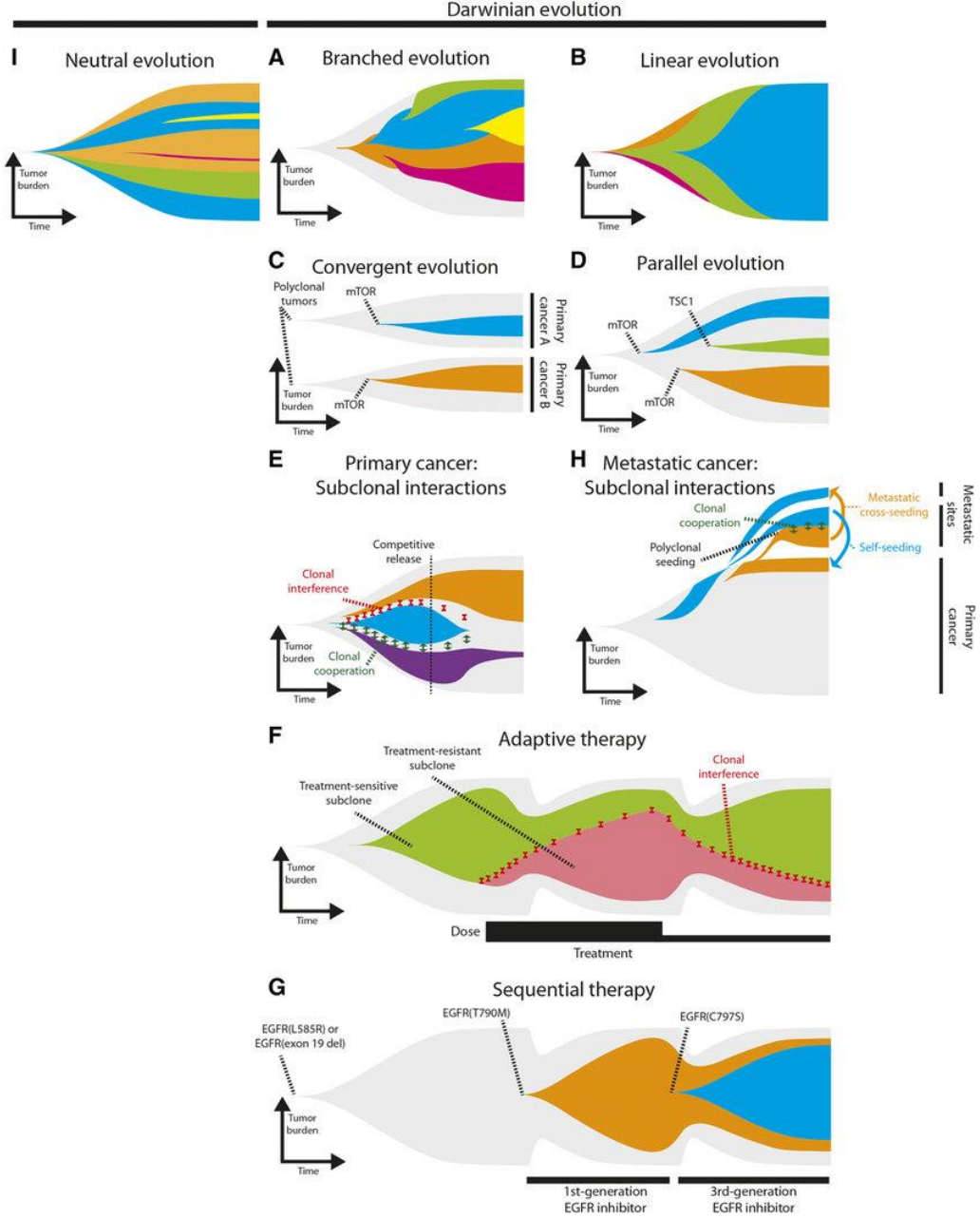
SUMMIT, N.J., & CAMBRIDGE, Mass.--(BUSINESS WIRE)-- Celgene Corporation (NASDAQ:CELG) and Agios Pharmaceuticals, Inc. (NASDAQ:AGIO) today announced that IDHIFA® (enasidenib) was granted approval from the U.S. Food and Drug Administration (FDA) for the treatment of adult patients with relapsed or refractory AML (R/R AML) with an isocitrate dehydrogenase-2 (IDH2) mutation as detected by an FDA approved test.1 IDHIFA, an oral targeted inhibitor of the IDH2 enzyme, is the first and only FDA-approved therapy for patients with R/R AML and an IDH2 mutation, which represents between 8 and 19 percent of AML patients.3

This Smart News Release features multimedia. View the full release here: http://www.businesswire.com/news/home/20170801006281/en/

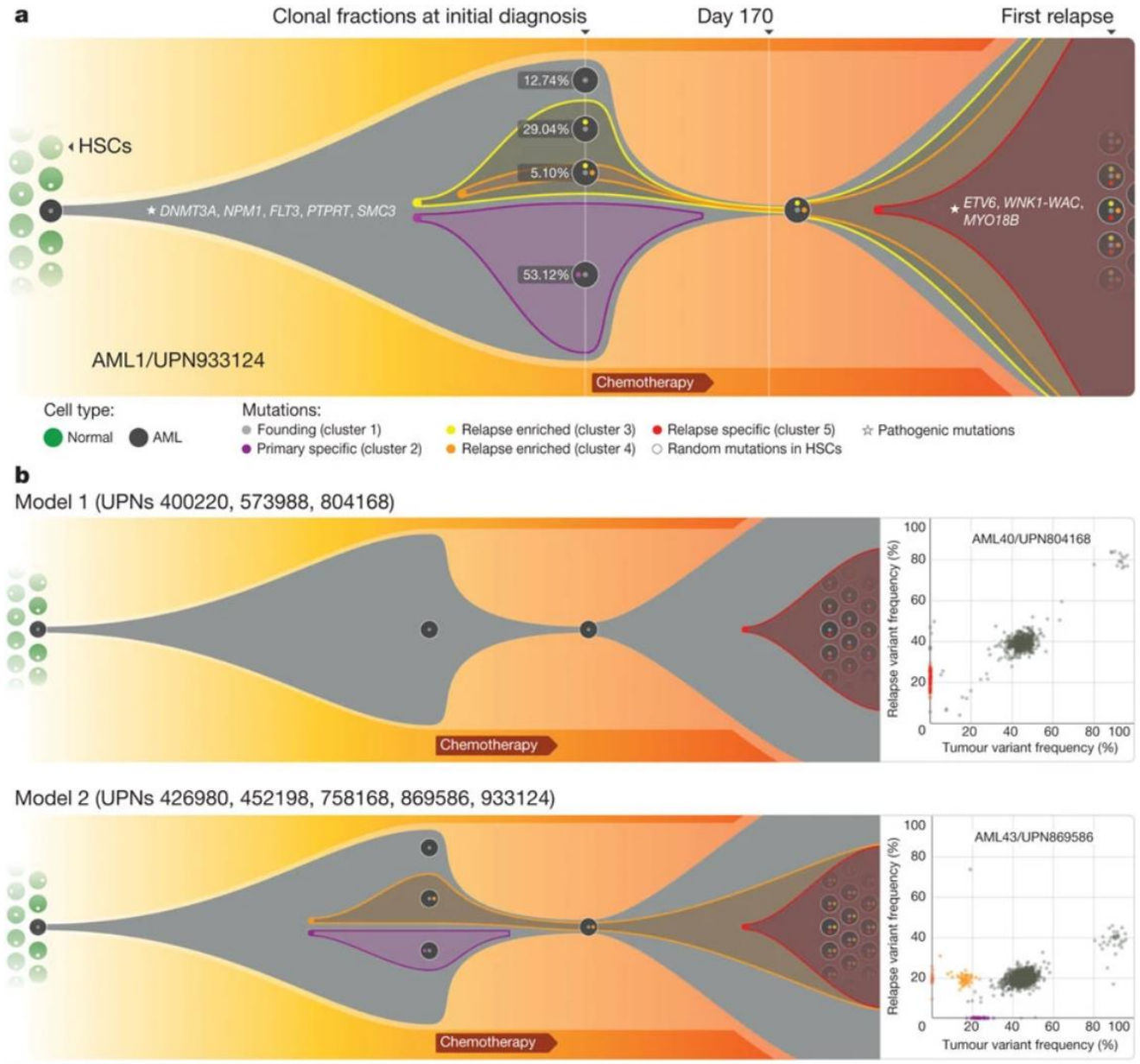
Cancer evolution



Sarah Haurin, Duke university



Venkatesan et al. Tumor Evolutionary Principles: How Intratumor Heterogeneity Influences Cancer Treatment and Outcome

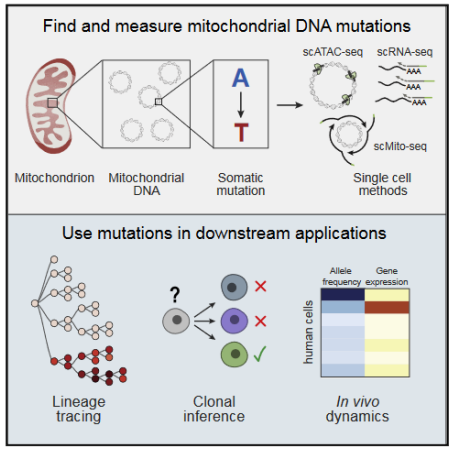


Ding et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing

Cell

Lineage Tracing in Humans Enabled by Mitochondrial Mutations and Single-Cell Genomics

Graphical Abstract



Authors

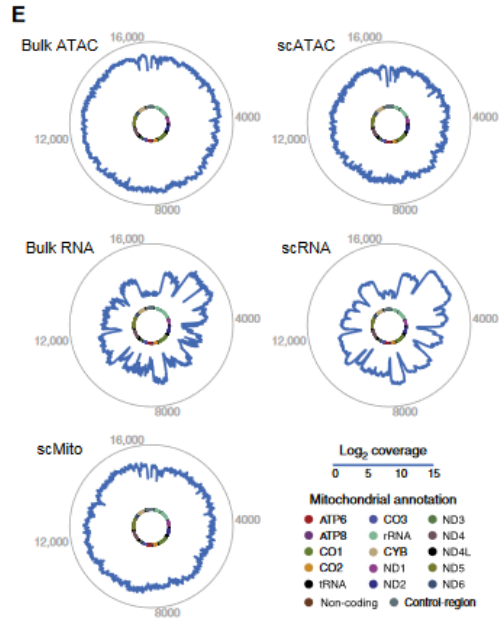
Leif S. Ludwig, Caleb A. Lareau, Jacob C. Ulirsch, ..., Jason D. Buenrostro, Aviv Regev, Vijay G. Sankaran

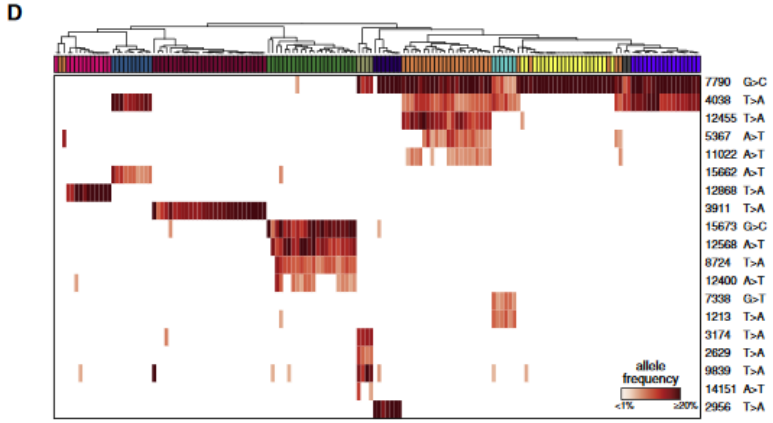
Correspondence

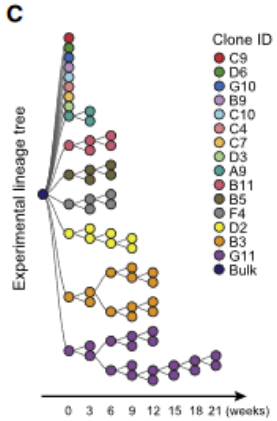
ludwig@broadinstitute.org (L.S.L.), aregev@broadinstitute.org (A.R.), sankaran@broadinstitute.org (V.G.S.)

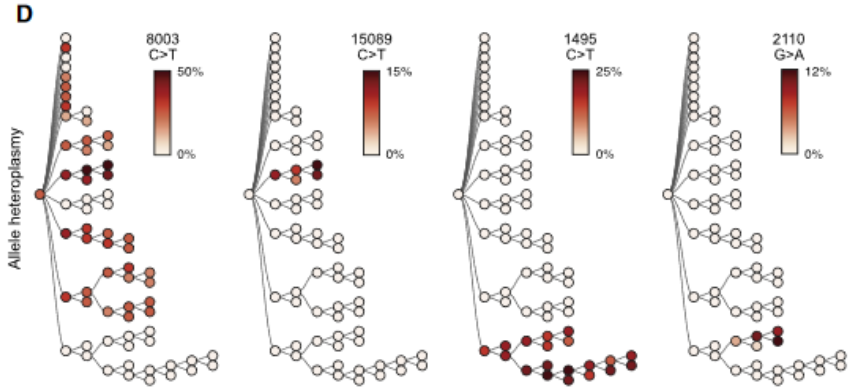
In Brief

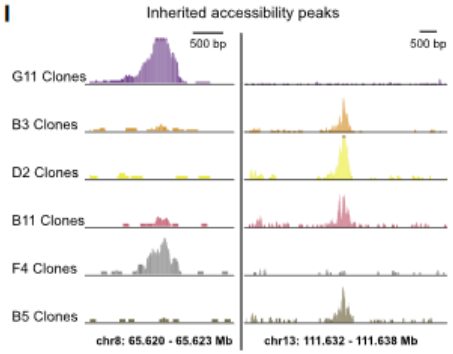
Using single-cell sequencing technologies, somatic mutations in mtDNA can be used as natural genetic barcodes to study cellular states and clonal dynamics.











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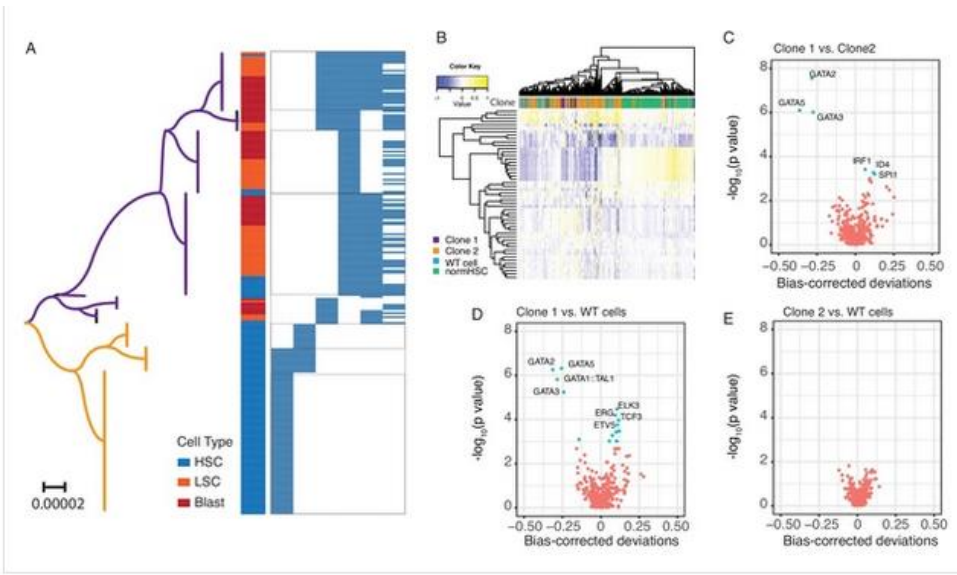
Single-cell lineage tracing by endogenous mutations enriched in transposase accessible mitochondrial DNA

Jin Xu[1,2,3], Kevin Nuno[4,5], Ulrike M Litzenburger[1,2,3], Yanyan Qi[1,2,3], M Ryan Corces[1,2,3], Ravindra Majeti[4,5]*, Howard Y Chang[1,2,3,6]*

[1]Center for Personal Dynamic Regulomes, Stanford, United States; [2]Department of Dermatology, Stanford University School of Medicine, Stanford, United States; [3]Department of Genetics, Stanford University School of Medicine, Stanford, United States; [4]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, United States; [5]Division of Hematology, Department of Medicine, Stanford University School of Medicine, Stanford, United States; [6]Howard Hughes Medical Institute, Stanford University, Stanford, United States

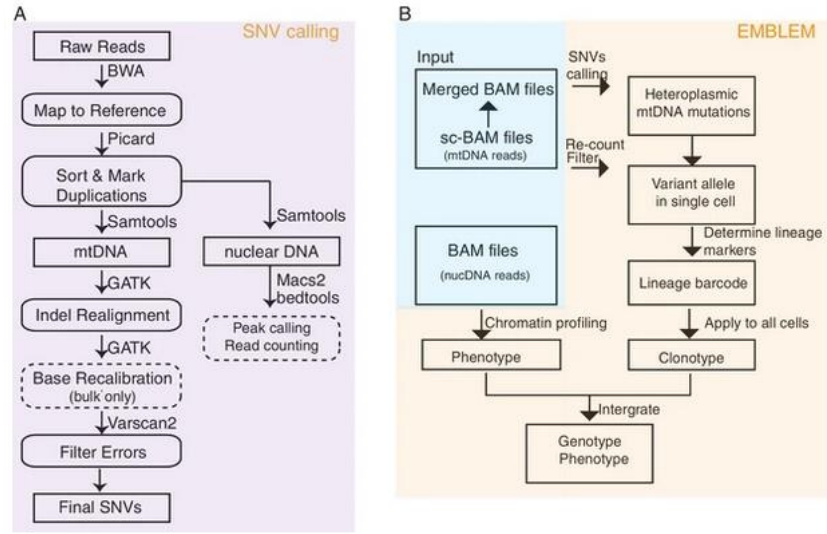
Abstract Simultaneous measurement of cell lineage and cell fates is a longstanding goal in biomedicine. Here we describe EMBLEM, a strategy to track cell lineage using endogenous mitochondrial DNA variants in ATAC-seq data. We show that somatic mutations in mitochondrial DNA can reconstruct cell lineage relationships at single cell resolution with high sensitivity and specificity. Using EMBLEM, we define the genetic and epigenomic clonal evolution of hematopoietic stem cells and their progenies in patients with acute myeloid leukemia. EMBLEM extends lineage tracing to any eukaryotic organism without genetic engineering.

DOI: https://doi.org/10.7554/eLife.45105.001



Single cell chromatin accessibility.

(A) Phylogenetic relationship of cells from SU353 was inferred using the Neighbor-Joining method. The phylogenetic tree is drawn to scale, with branch lengths in the units of the number of base difference



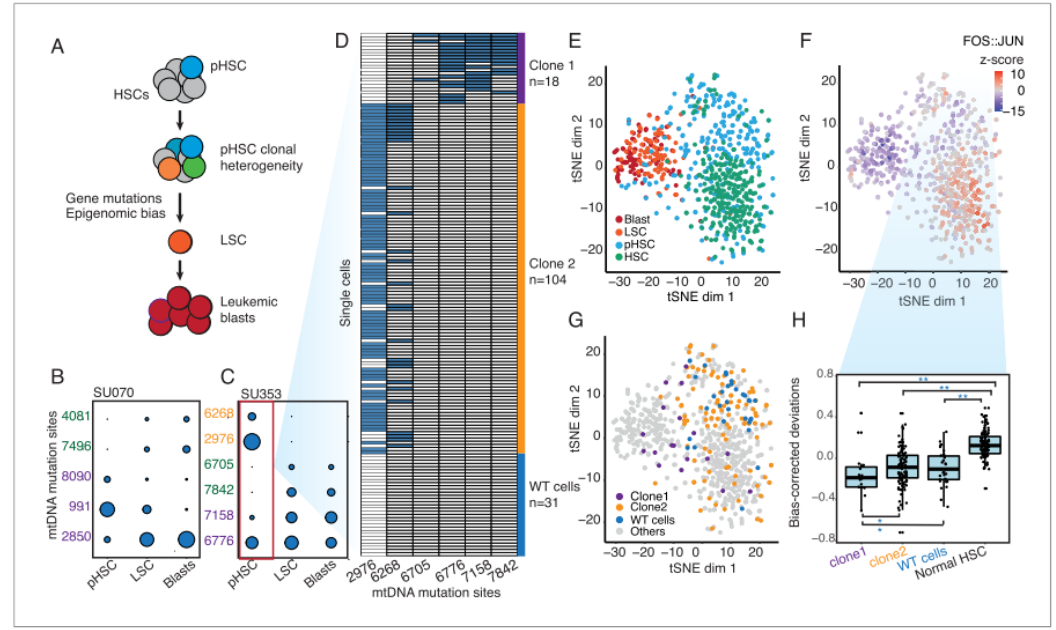


Figure 2. Clonal evolution of pre-leukemic HSCs inferred from joint lineage tracing and single cell chromatin accessibility. (**A**) Lineage hierarchy in acute myeloid leukemia based on EMBLEM and prior genetic information. mtDNA mutations reveals pHSC clonal heterogeneity. The clonal precursor of the leukemic stem cell is not the clone with most representation in the pHSC pool, but rather the clone with epigenomic bias towards the leukemic regulatory program, as depicted by related color schemes. (**B**) EMBLEM deconvolutes AML clonal heterogeneity. Heteroplasmic mtDNA mutations in

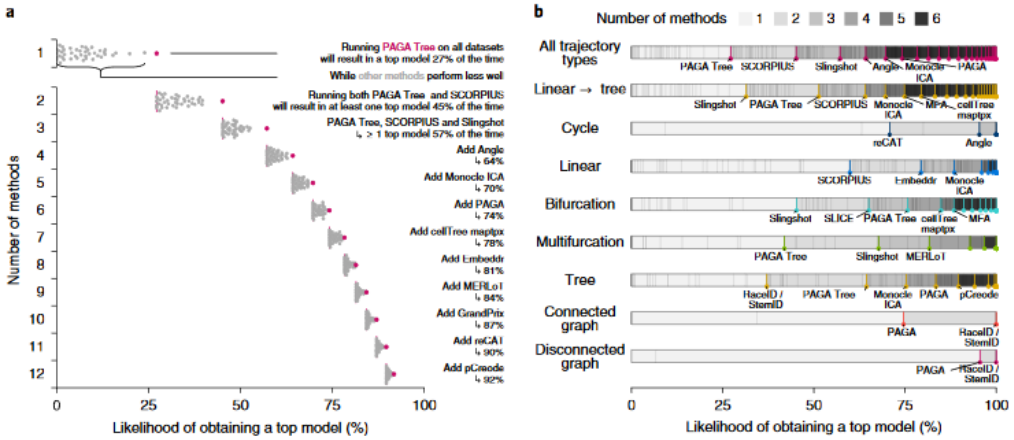
nature biotechnology

ARTICLES

https://doi.org/10.1038/s41587-019-0071-9

A comparison of single-cell trajectory inference methods

Wouter Saelens[1,2,6], Robrecht Cannoodt[1,3,4,6], Helena Todorov[1,2,5] and Yvan Saeys[1,2*]

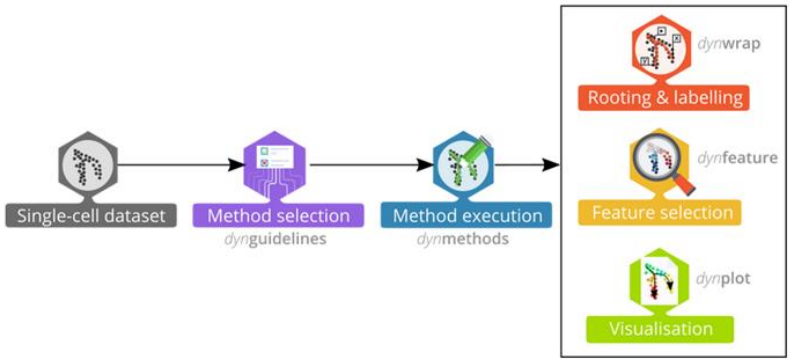


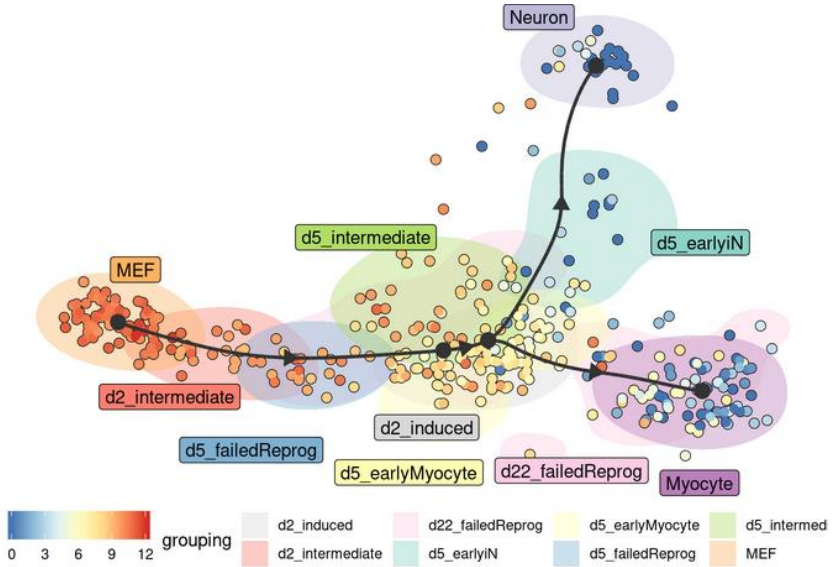
Inferring trajectories using dyno

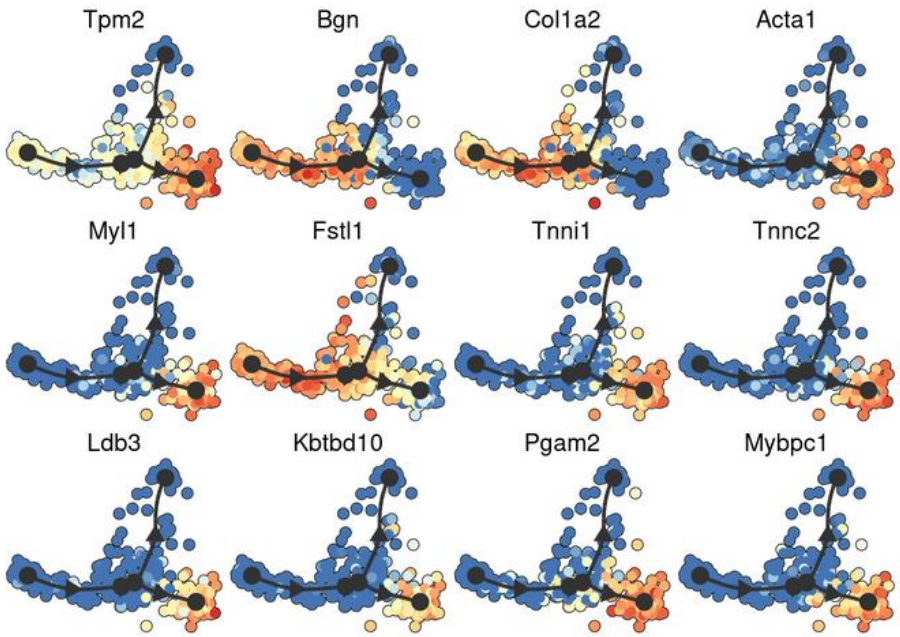
The **dyno** package offers **end-users** a complete TI pipeline. It features:

- a uniform interface to 59 TI methods,
- an interactive guideline tool to help the user select the most appropriate method,
- streamlined interpretation and visualisation of trajectories, including colouring by gene expression or clusters, and
- downstream analyses such as the identification of potential marker genes.

For information on how to use dyno, check out the installation instructions, tutorials and documentation at dynverse.org





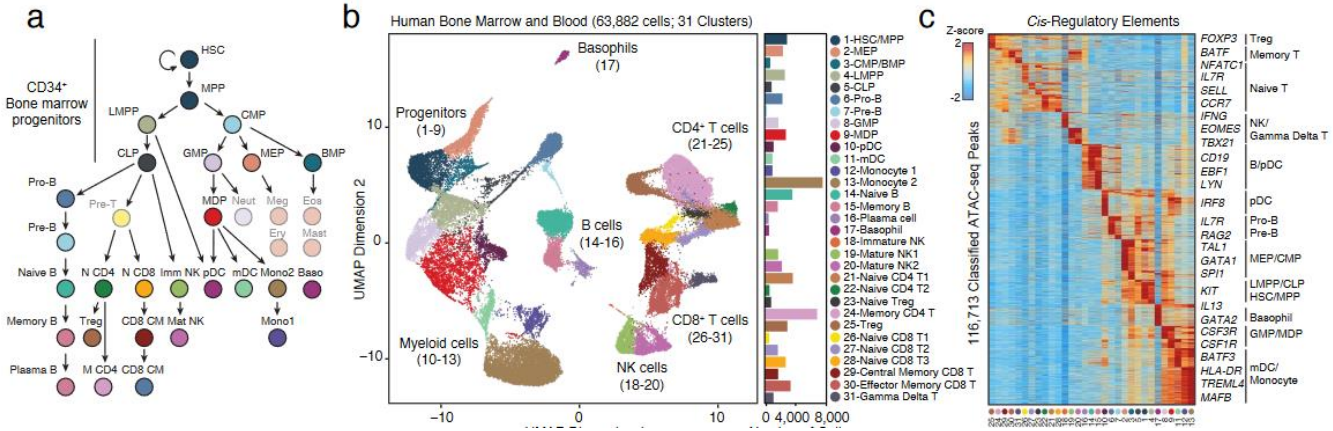


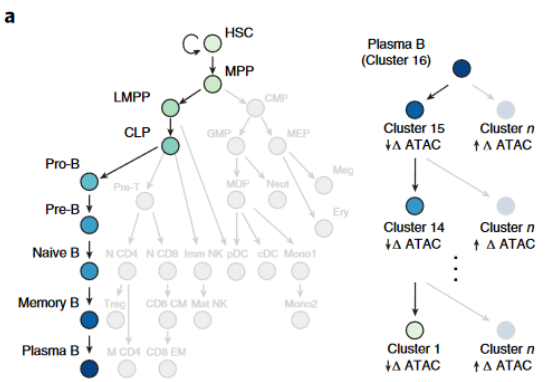
Article | Published: 02 August 2019

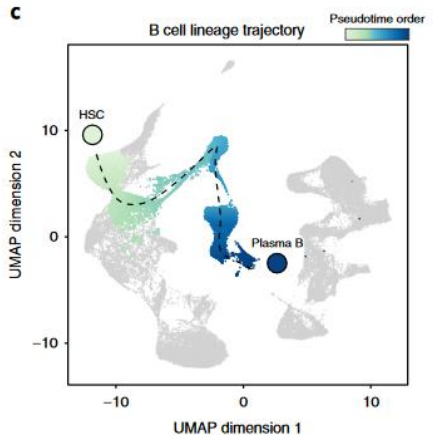
Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion

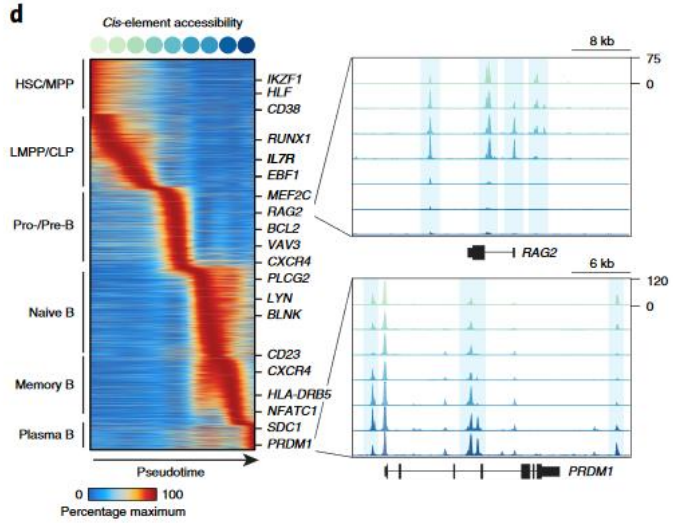
Ansuman T. Satpathy, Jeffrey M. Granja, Kathryn E. Yost, Yanyan Qi, Francesca Meschi, Geoffrey P. McDermott, Brett N. Olsen, Maxwell R. Mumbach, Sarah E. Pierce, M. Ryan Corces, Preyas Shah, Jason C. Bell, Darisha Jhutty, Corey M. Nemec, Jean Wang, Li Wang, Yifeng Yin, Paul G. Giresi, Anne Lynn S. Chang, Grace X. Y. Zheng ✉, William J. Greenleaf ✉ & Howard Y. Chang ✉

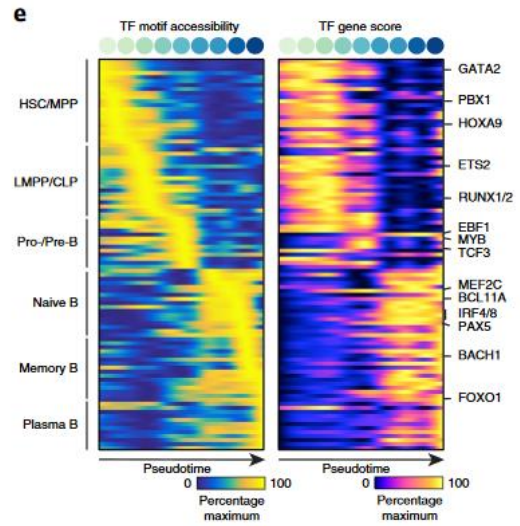
Nature Biotechnology **37**, 925–936(2019) | Cite this article



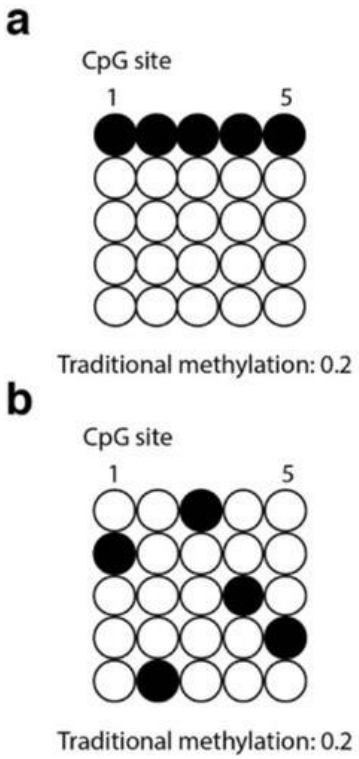




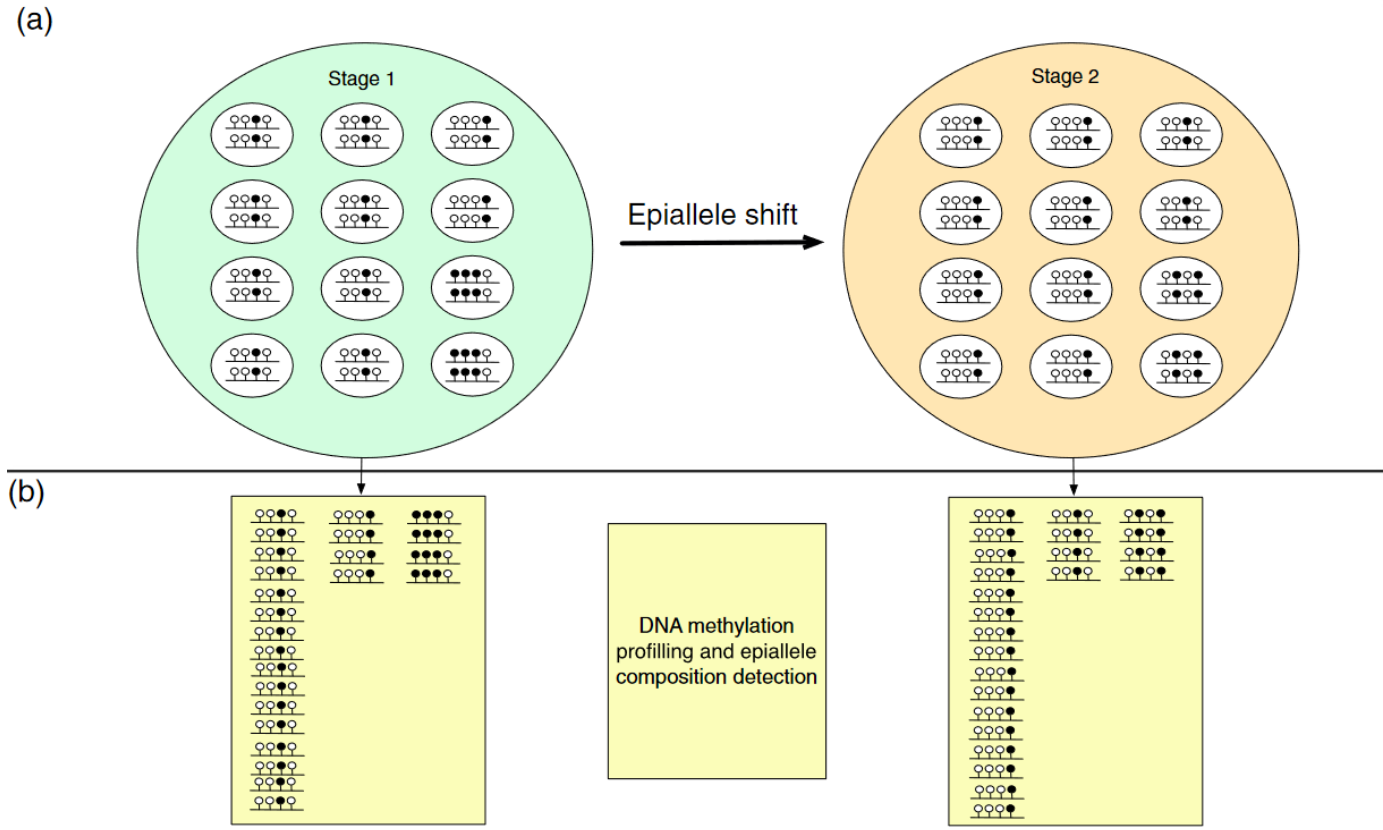




Epigenetic clonality



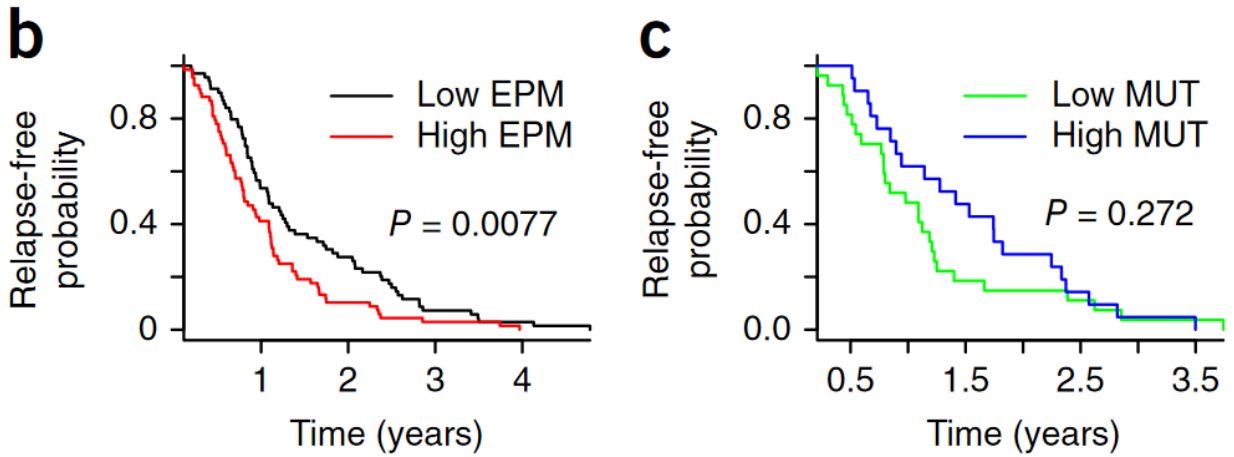
Xu et al. Cellular heterogeneity–adjusted clonal methylation (CHALM) provides better prediction of gene expression

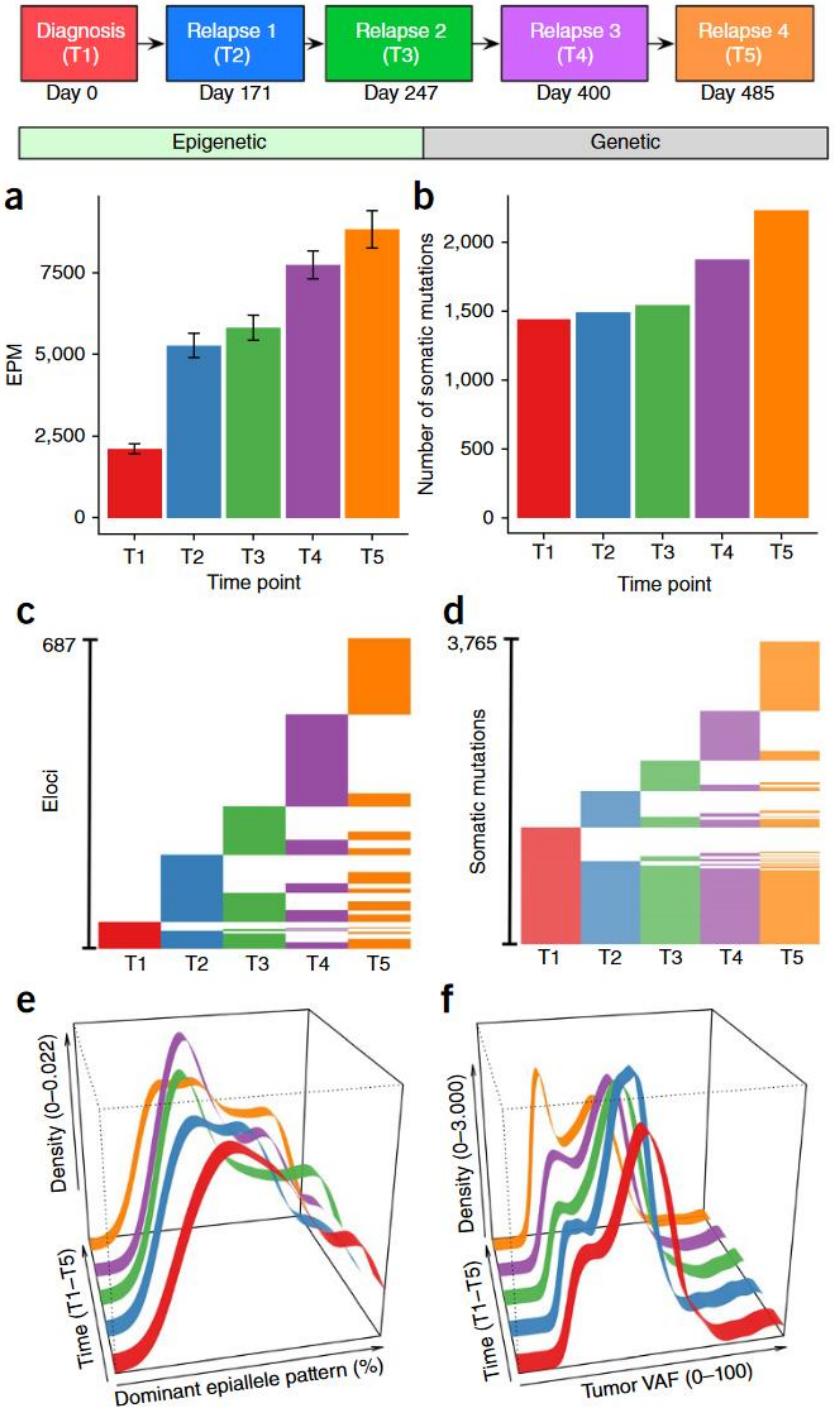


Li et al. Dynamic evolution of clonal epialleles revealed by methclone

Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia

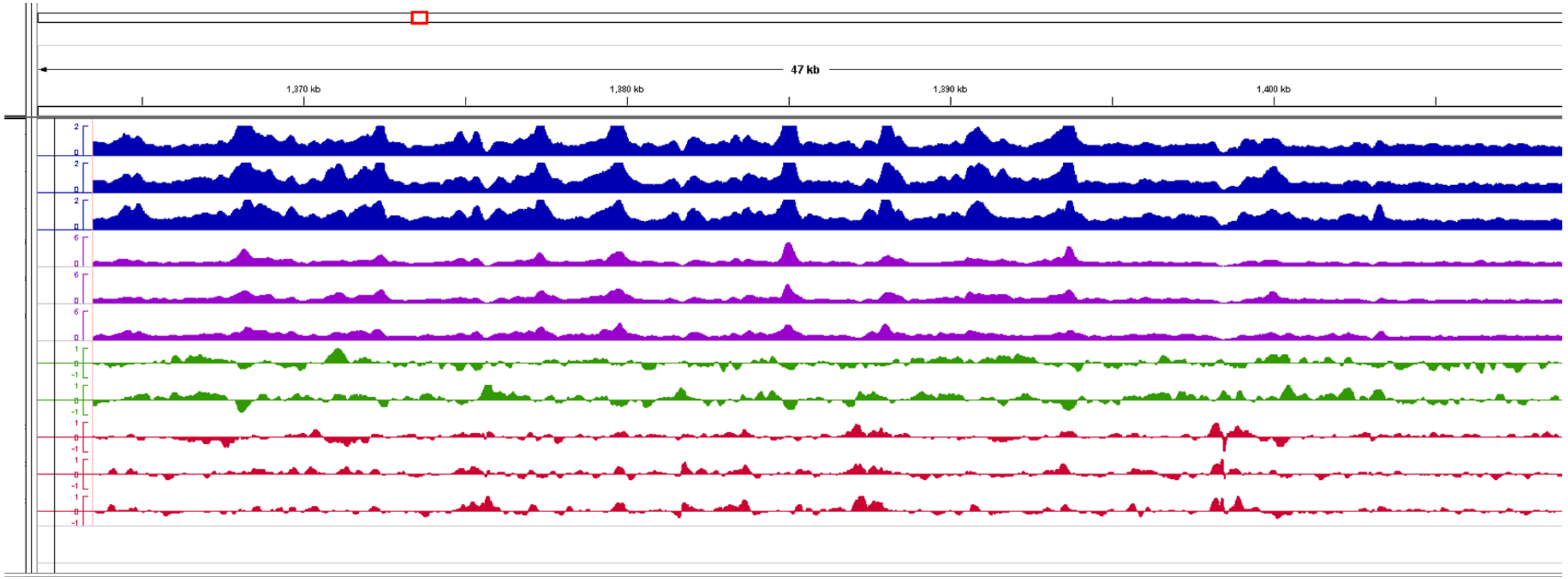
Sheng Li[1,18,19], Francine E Garrett-Bakelman[2,19], Stephen S Chung[3], Mathijs A Sanders[4], Todd Hricik[3],



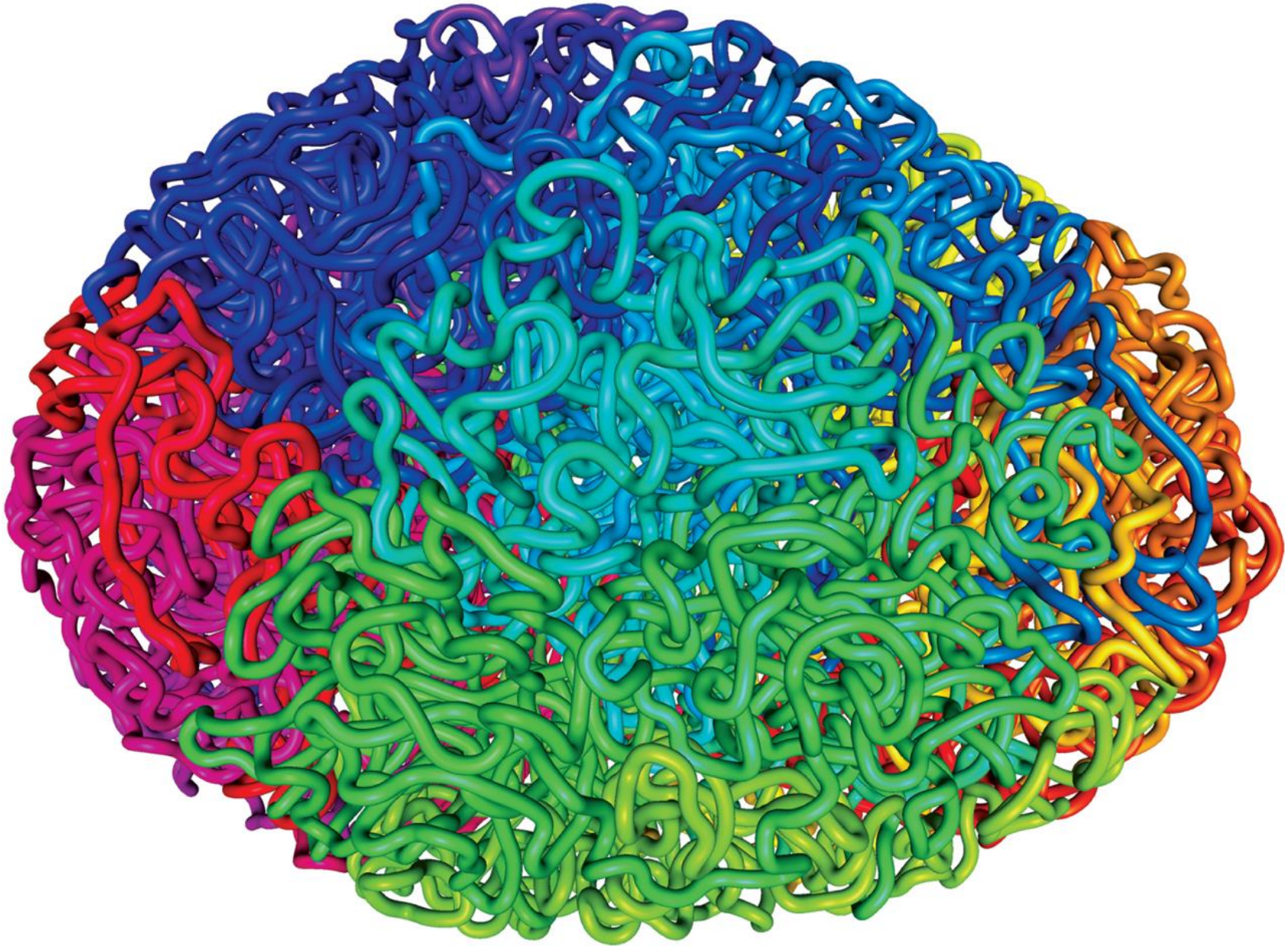


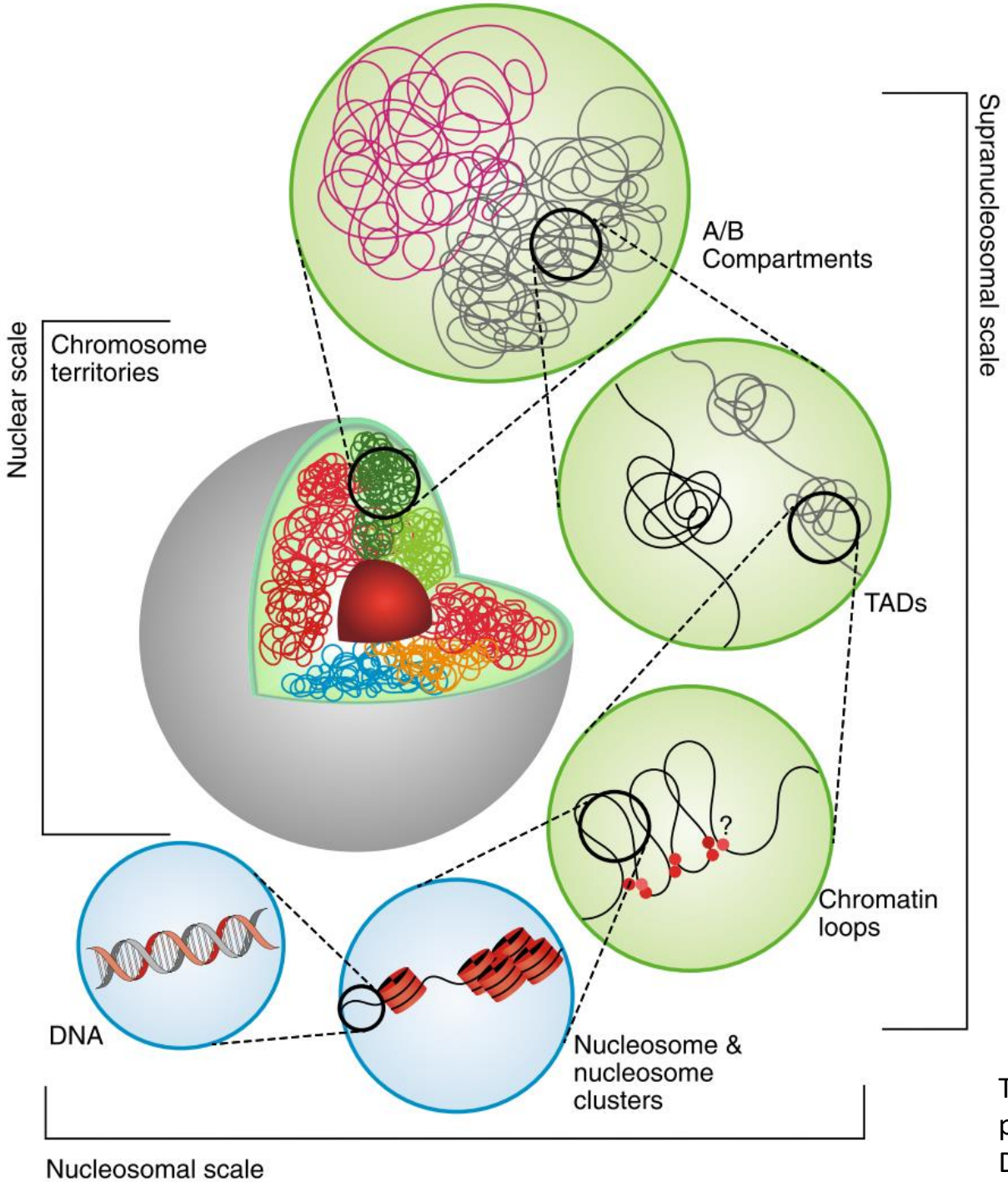
Chromatin Structure

Genome



Genome





Three-dimensional chromatin packing and positioning of plant genomes
Dogan et al.

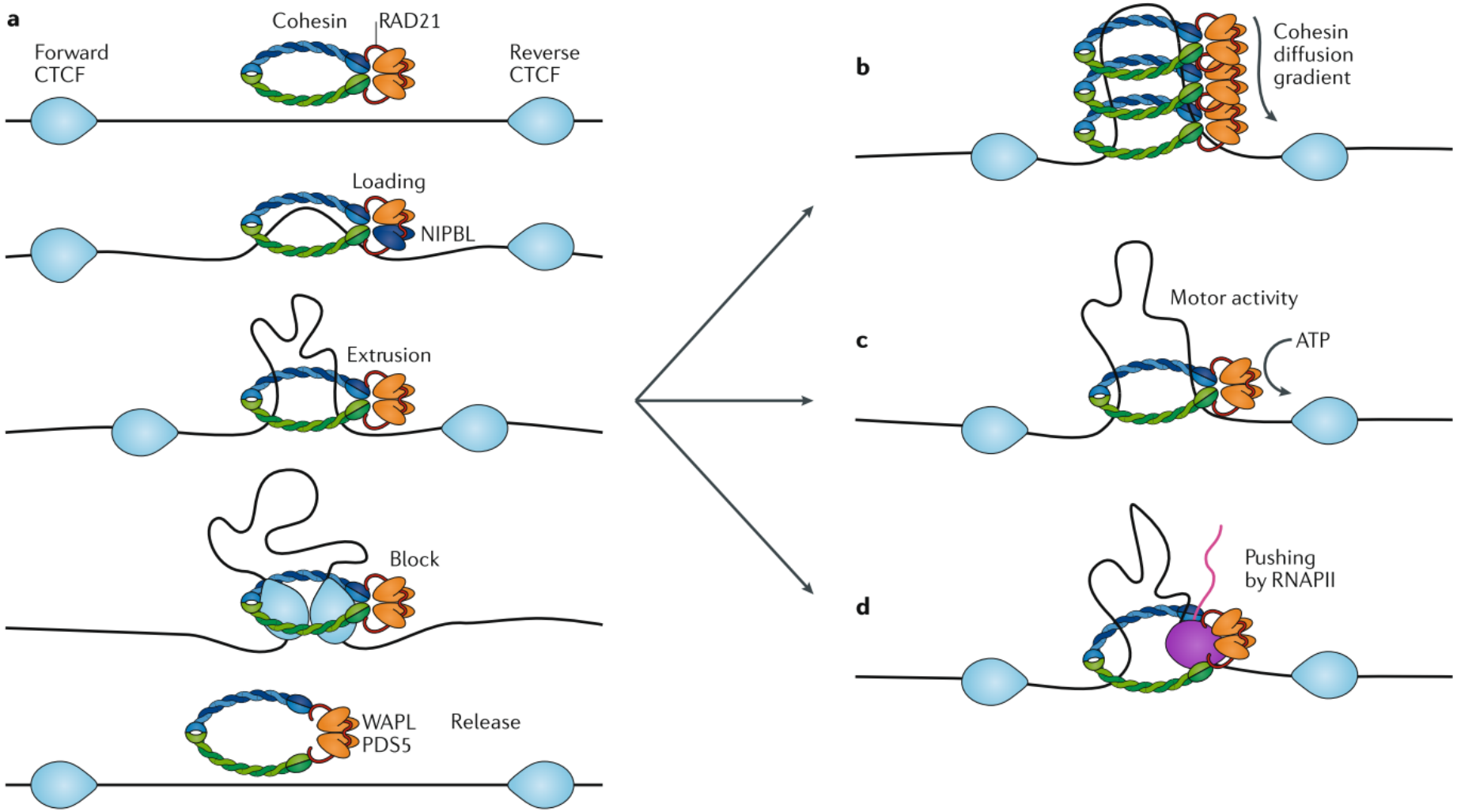
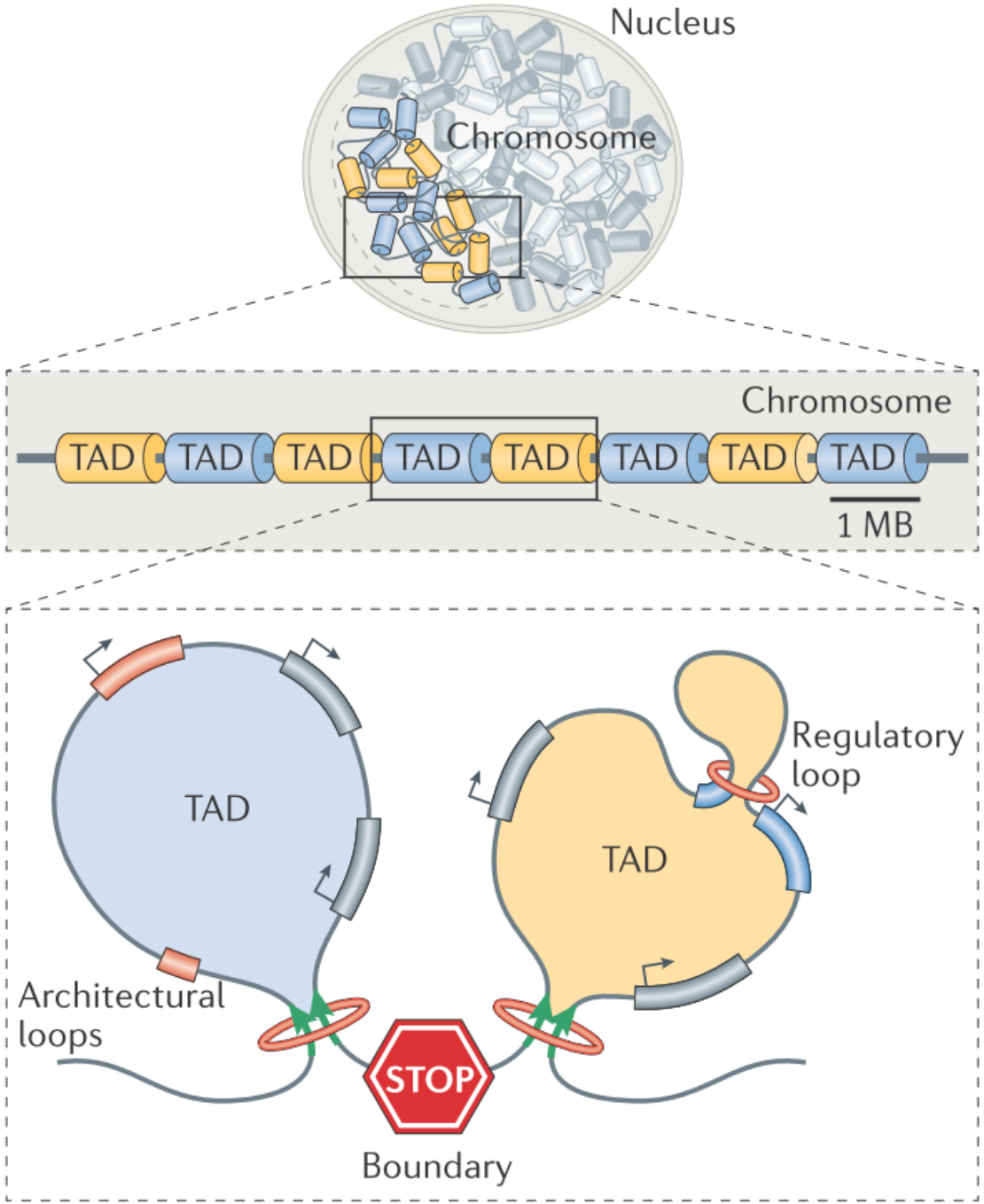
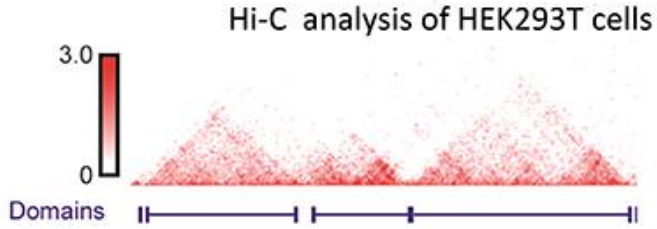


Fig. 3 | **Mechanisms of loop extrusion. a** | General model of loop extrusion. The extrusion process involves cohesin composed of structural maintenance of chromosomes (SMC) proteins SMC1 and SMC3 and RAD21; cohesin is loaded onto chromatin via NIPBL[94]. Extrusion is blocked at CTCF sites arranged in a convergent head-to-head orientation[45–49]. Some proportion of cohesin is released throughout this process by the activity of WAPL and PDS5 (REF.[94]). **b** | Extrusion via cohesin diffusion. Extrusion may occur by constant loading of cohesin resulting in a diffusion gradient[70]. **c** | Extrusion via cohesin motor activity. An alternative explanation for extrusion is that the process is driven by the motor activity of cohesin via ATP hydrolysis[54,72]. **d** | Extrusion via pushing of cohesin by RNA polymerase II (RNAPII). Other factors able to move along chromatin, such as RNAPII (purple), may help cohesin to extrude DNA[50,51,73–76].

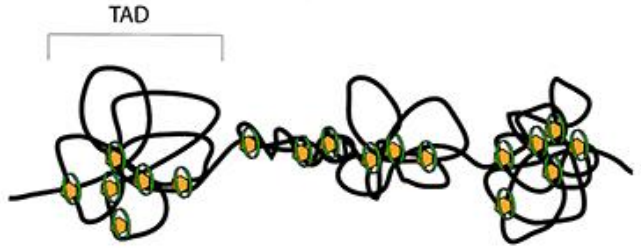
Organizational principles of 3D genome architecture
Rowley et al



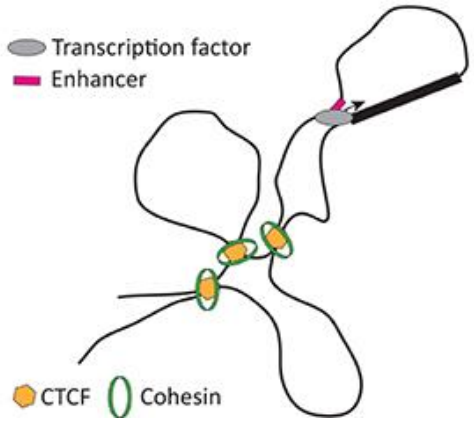
Chromatin fibers are orgnaized in TADs



TADs are chromatin loop clusters



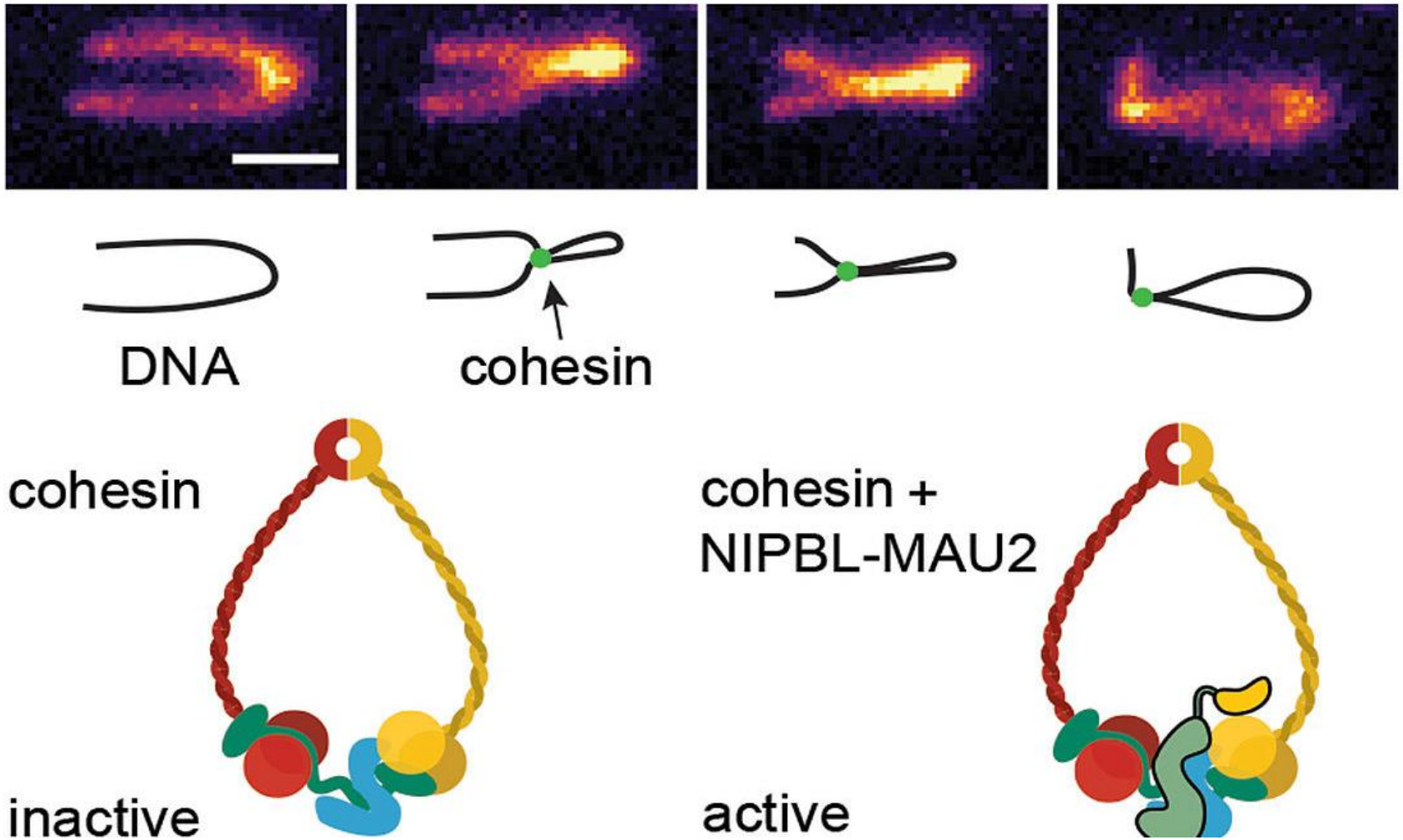
Cohesin and CTCF shape chromatin loops and promote promoter-enhancer interactions



Regulation of disease-associated gene expression in the 3D genome, Krijger et al

Cohesin in chromatin structure and gene regulation, Erasmus MC

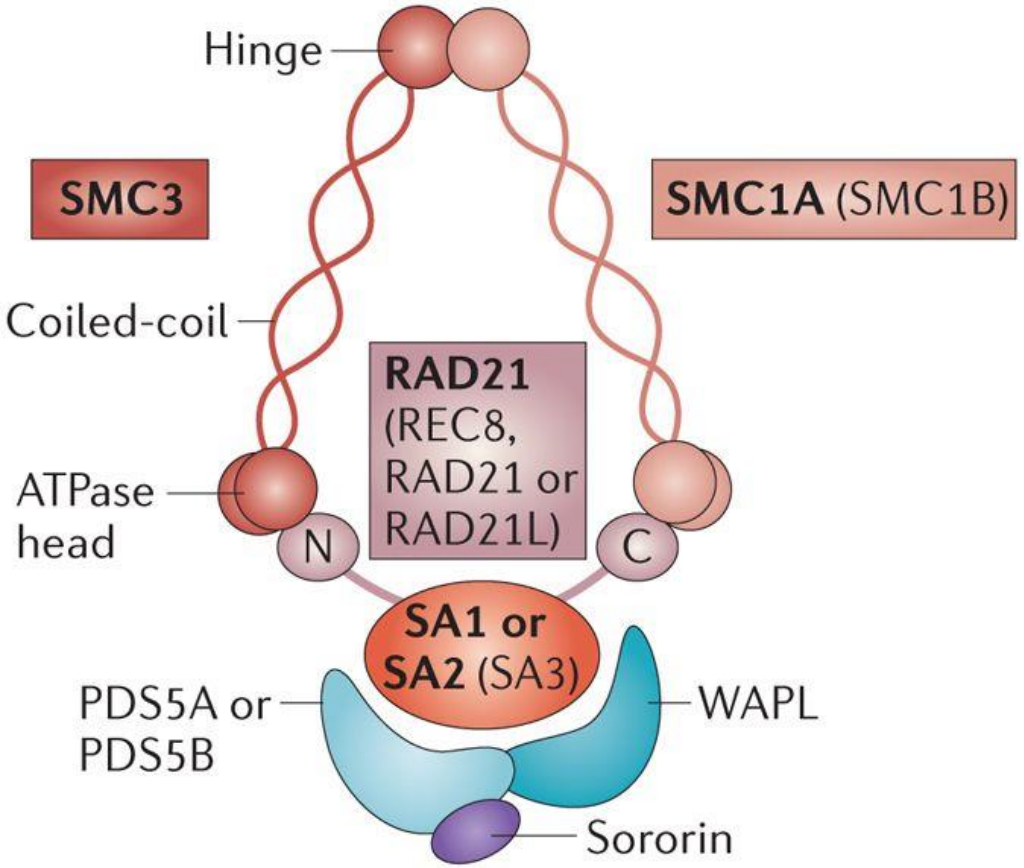
DNA loop extrusion by cohesin



DNA loop extrusion by human cohesion
Davidson et al. Science 2019

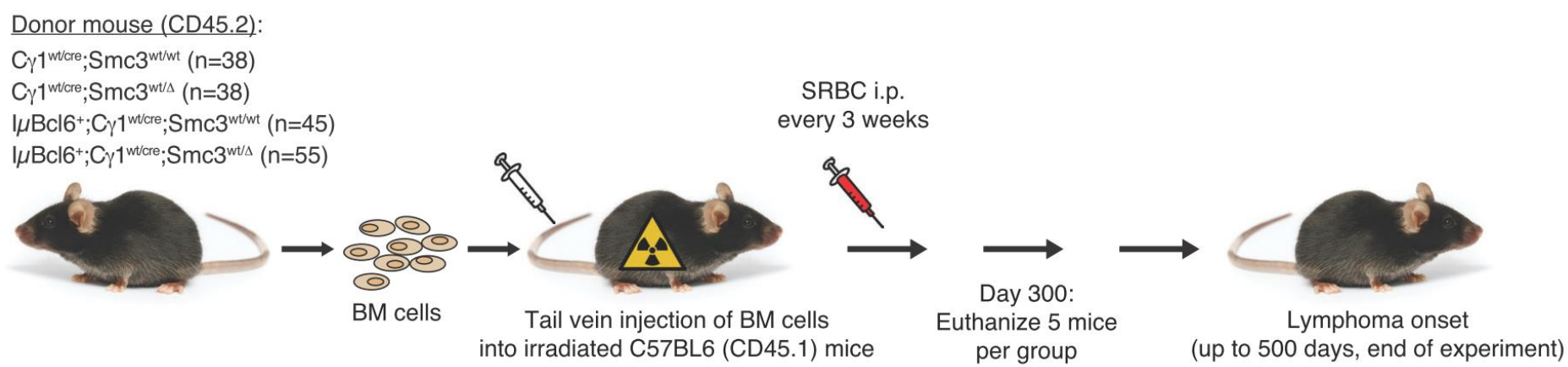
SMC3

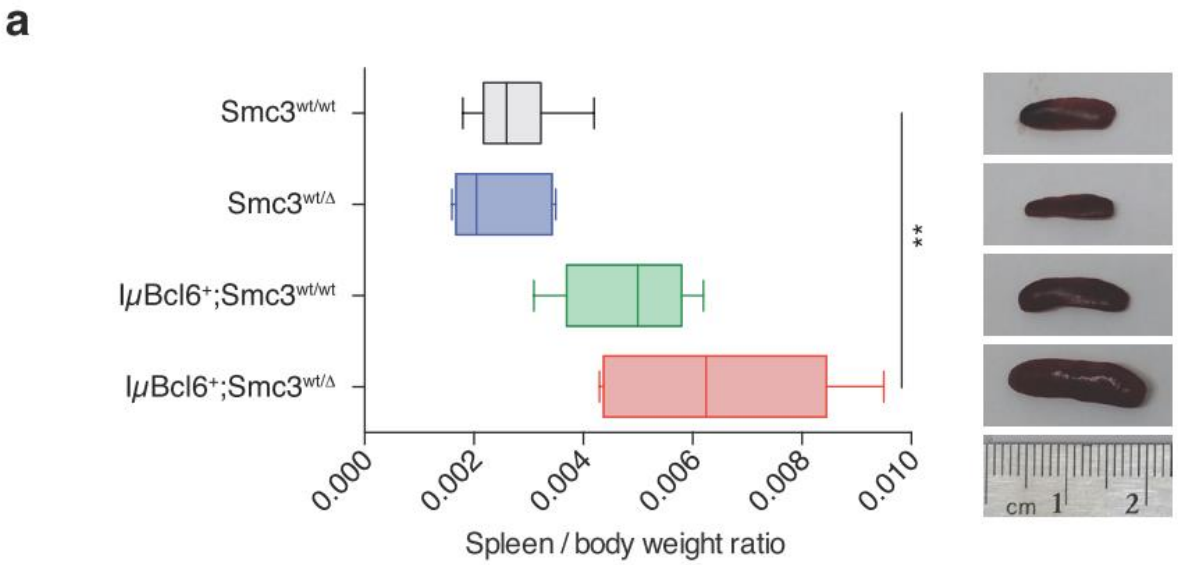
- Structural Maintenance Of Chromosomes 3
- Part of cohesin complex
- Important for
  - regulation of gene expression (enhancer/promoter interactions, insulators)
  - cell cycle (separation of sister chromatids
  - etc
- Abnormal copies (heterozygous) were found in myeloid and lymphoid cancers and more

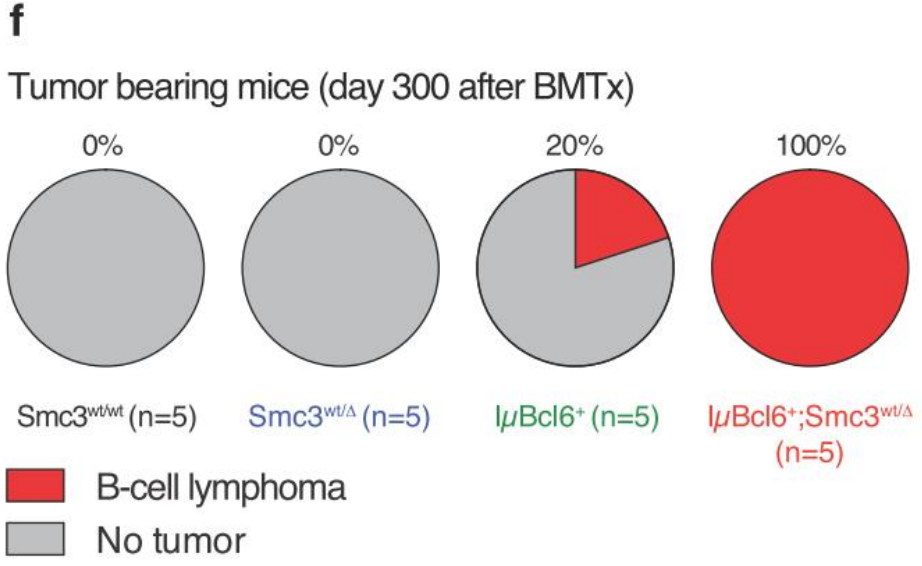


Cohesin in cancer: chromosome segregation and beyond, Nat Rev Cancer 2014

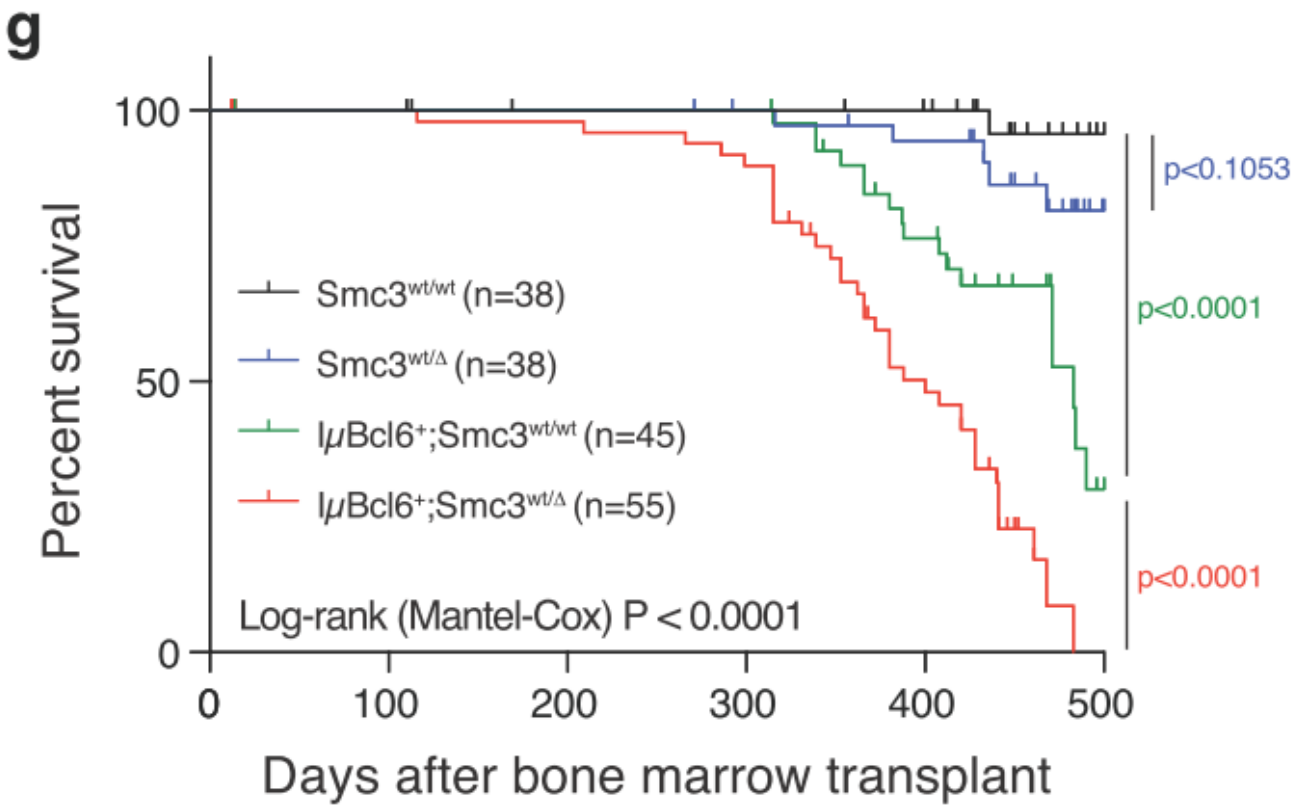
Smc3 deficiency accelerates malignant transformation of GC B-cells and is linked to inferior outcome of DLBCL patients

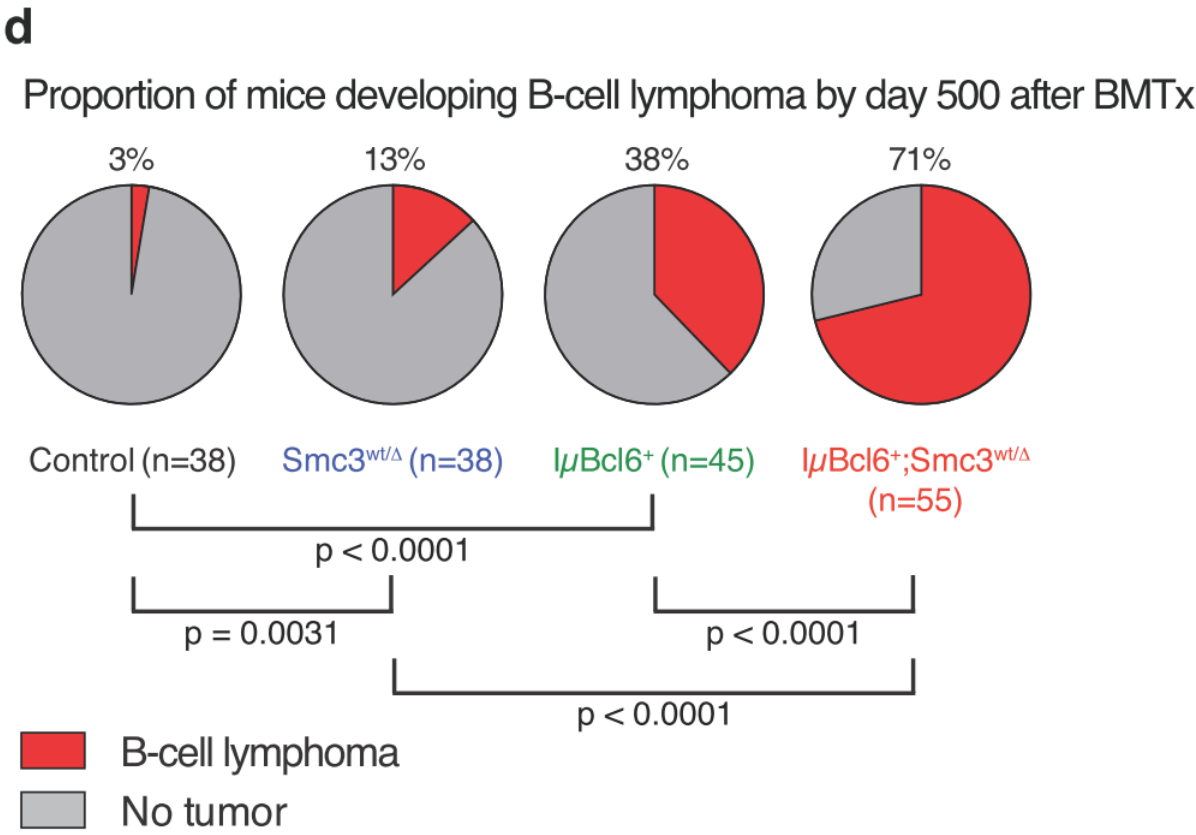


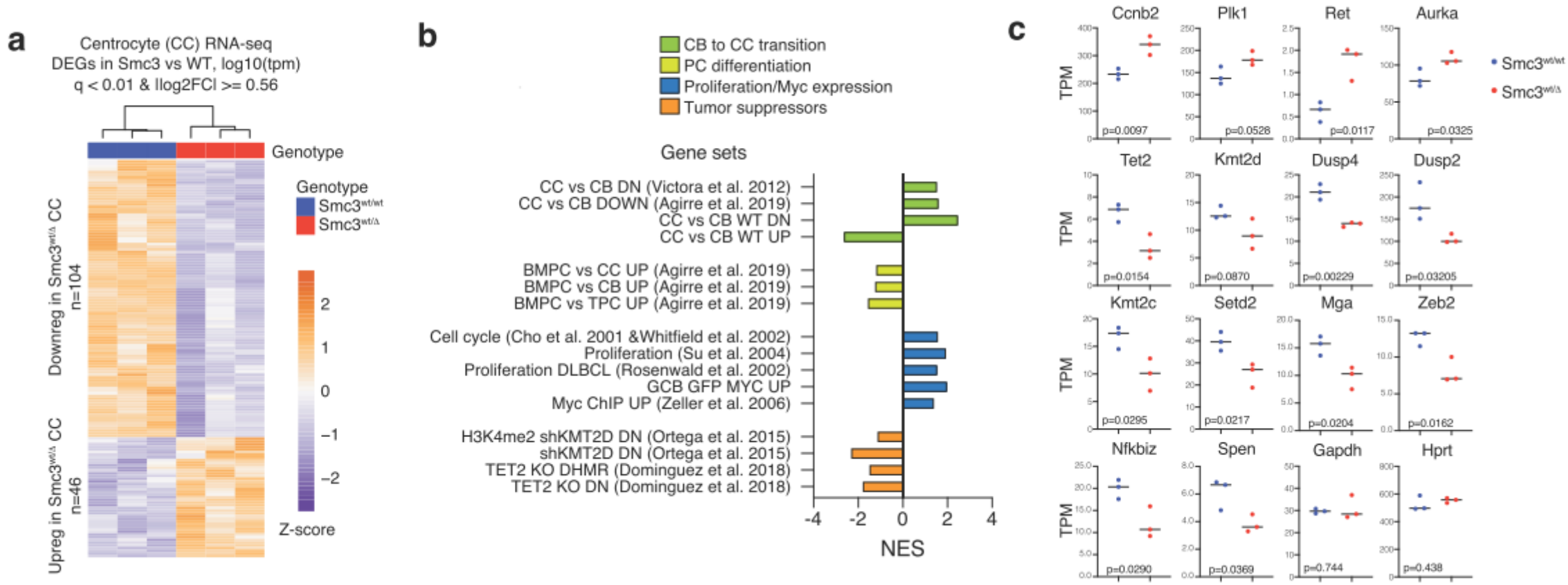


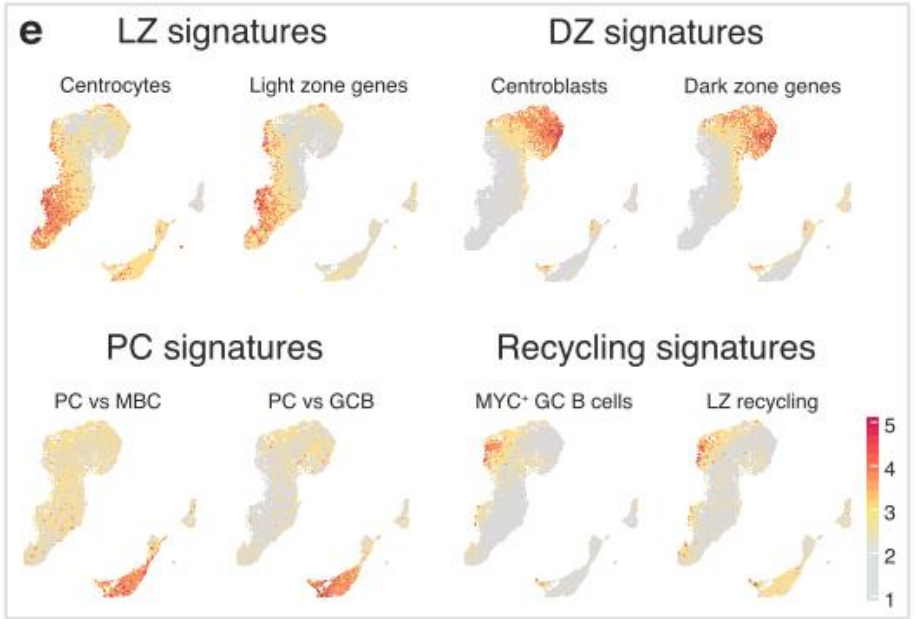


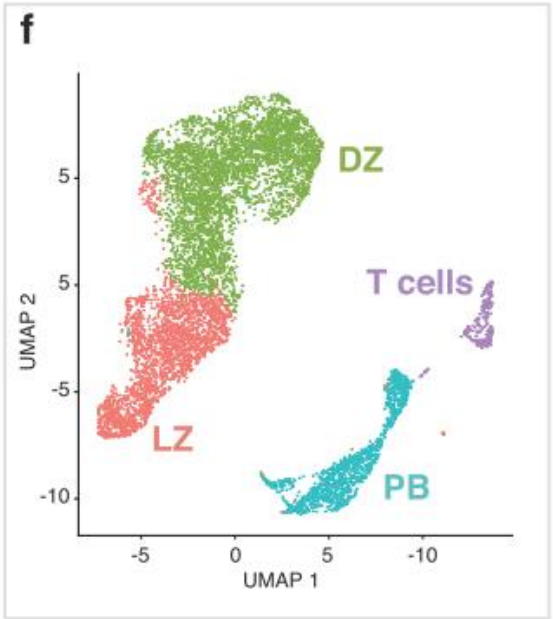
Smc3 deficiency accelerates malignant transformation of GC B-cells and is linked to inferior outcome of DLBCL patients

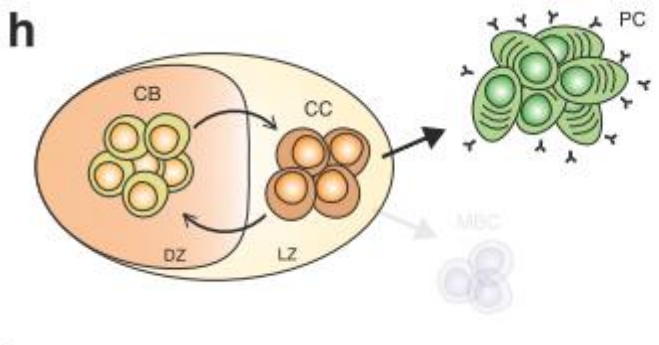


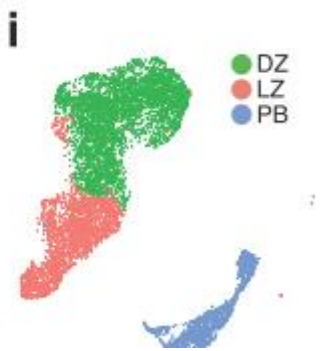


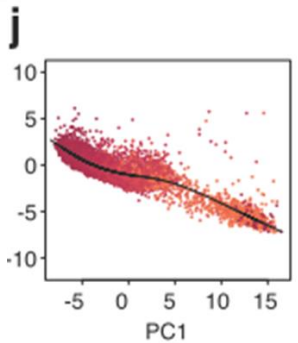

a
Centrocyte (CC) RNA-seq
DEGs in Smc3 vs WT, log10(tpm)
q < 0.01 & |log2FC| >= 0.56
Genotype
Genotype
Smc3wt/wt
Smc3wt/Δ
Downreg in Smc3wt/Δ CC
n=104
Upreg in Smc3wt/Δ CC
n=46
2
1
0
−1
−2
Z-score
b
CB to CC transition
PC differentiation
Proliferation/Myc expression
Tumor suppressors
Gene sets
CC vs CB DN (Victora et al. 2012)
CC vs CB DOWN (Agirre et al. 2019)
CC vs CB WT DN
CC vs CB WT UP
BMPC vs CC UP (Agirre et al. 2019)
BMPC vs CB UP (Agirre et al. 2019)
BMPC vs TPC UP (Agirre et al. 2019)
Cell cycle (Cho et al. 2001 &Whitfield et al. 2002)
Proliferation (Su et al. 2004)
Proliferation DLBCL (Rosenwald et al. 2002)
GCB GFP MYC UP
Myc ChIP UP (Zeller et al. 2006)
H3K4me2 shKMT2D DN (Ortega et al. 2015)
shKMT2D DN (Ortega et al. 2015)
TET2 KO DHMR (Dominguez et al. 2018)
TET2 KO DN (Dominguez et al. 2018)
-4
-2
0
2
4
NES
c
Ccnb2
Plk1
Ret
Aurka
Tet2
Kmt2d
Dusp4
Dusp2
Kmt2c
Setd2
Mga
Zeb2
Nfkbiz
Spen
Gapdh
Hprt
TPM
p=0.0097
p=0.0528
p=0.0117
p=0.0325
p=0.0154
p=0.0870
p=0.00229
p=0.03205
p=0.0295
p=0.0217
p=0.0204
p=0.0162
p=0.0290
p=0.0369
p=0.744
p=0.438
Smc3wt/wt
Smc3wt/Δ

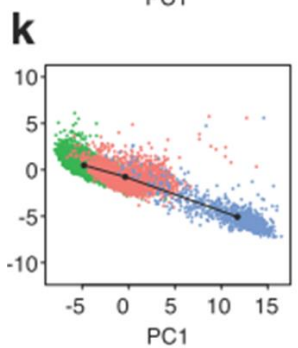

e
LZ signatures
DZ signatures
Centrocytes
Light zone genes
Centroblasts
Dark zone genes
PC signatures
Recycling signatures
PC vs MBC
PC vs GCB
MYC+ GC B cells
LZ recycling
5
4
3
2
1


f
DZ
T cells
LZ
PB
UMAP 2
UMAP 1
5
0
-5
-10


h
CB
CC
DZ
LZ
PC
MBC


i
DZ
LZ
PB


j
PC1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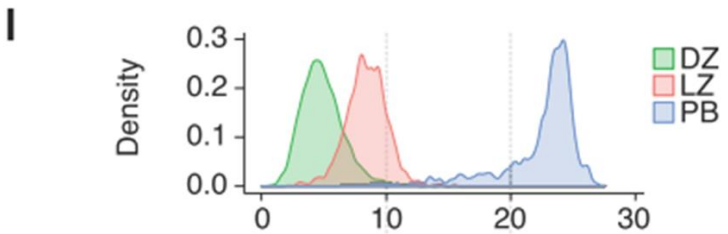
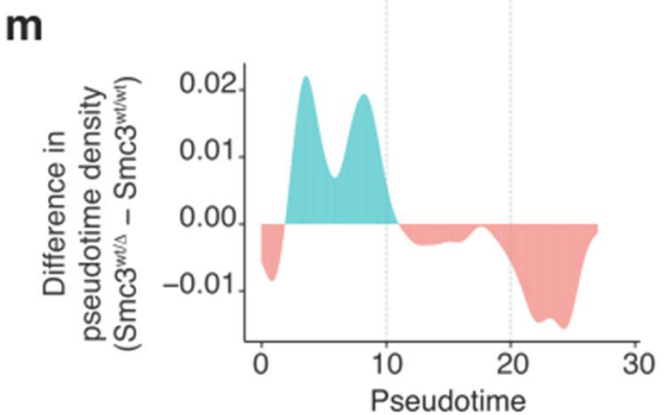
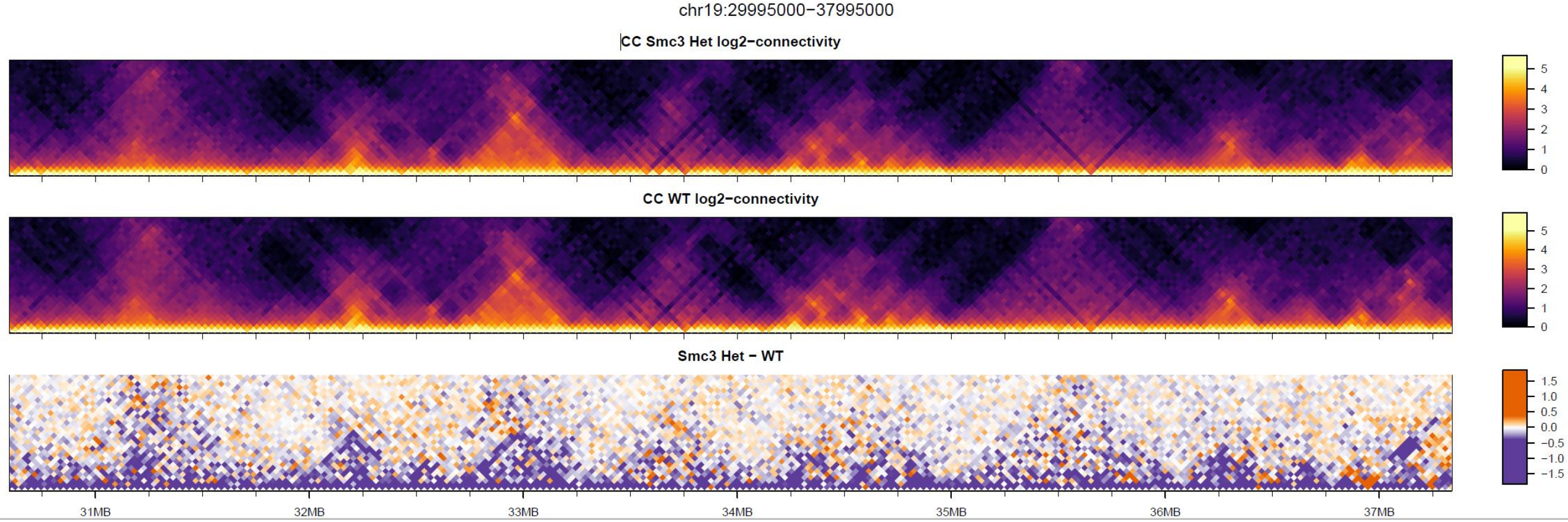
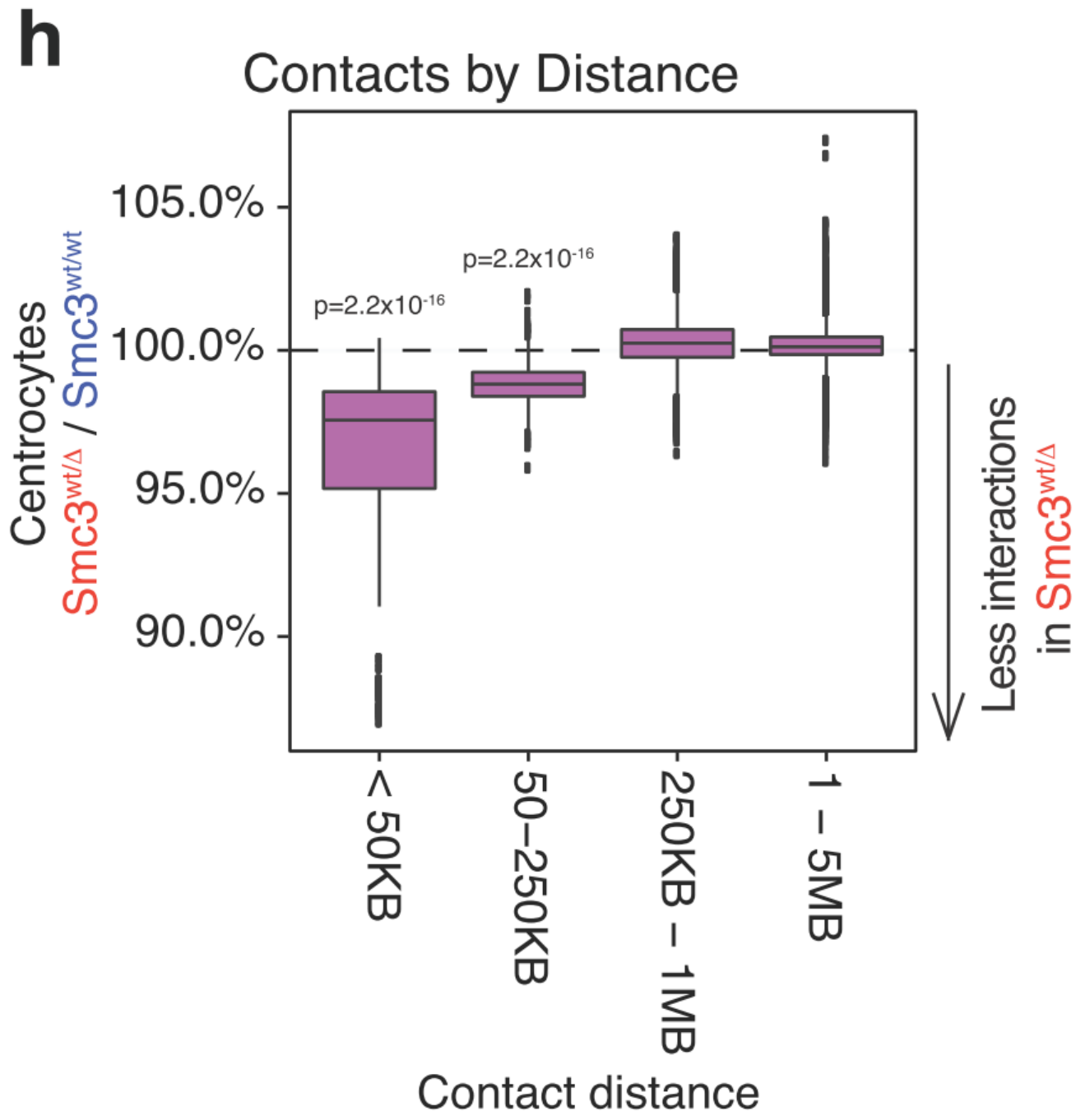
PC1


l
Density
DZ
LZ
PB


m
Difference in pseudotime density (Smc3w/Δ – Smc3w/wt)
Pseudotime

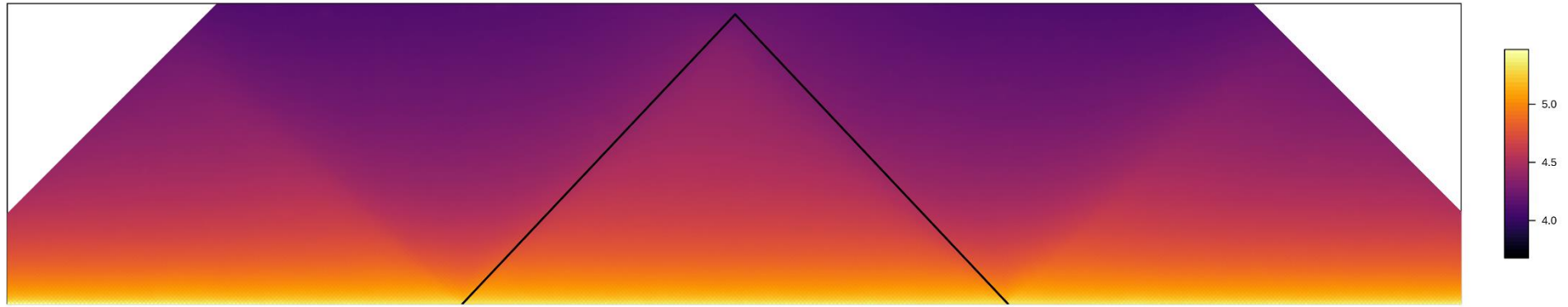
Chris Chin


chr19:29995000−37995000
CC Smc3 Het log2−connectivity
5
4
3
2
1
0
CC WT log2−connectivity
5
4
3
2
1
0
Smc3 Het − WT
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
31MB
32MB
33MB
34MB
35MB
36MB
37MB

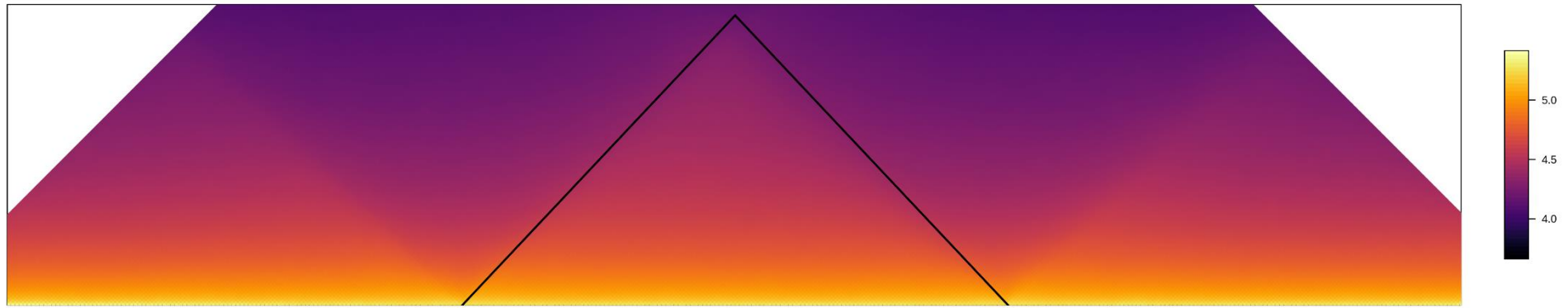

h
Contacts by Distance
105.0%
100.0%
95.0%
90.0%
p=2.2x10-16
p=2.2x10-16
Centrocytes
Smc3wt/Δ / Smc3wt/wt
Less interactions
in Smc3wt/Δ
< 50KB
50–250KB
250KB – 1MB
1 – 5MB
Contact distance

CC, All TADs +- 1*TAD length, stretched to same size

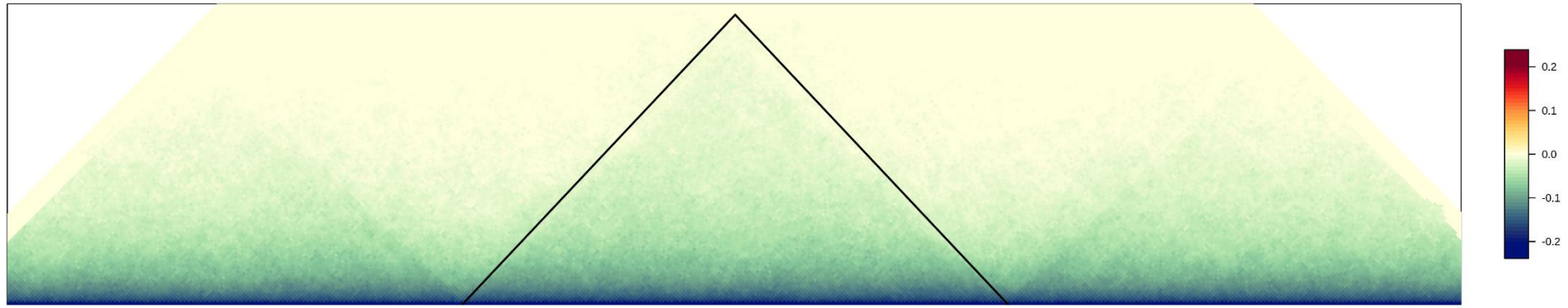
CC CTRL



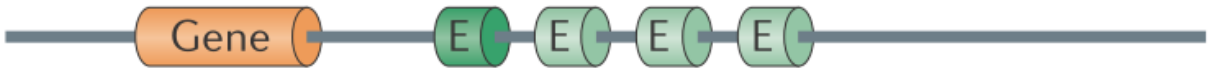
CC SMC3



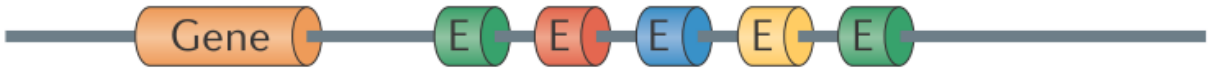
CC SMC3vsWT Log2Ratio



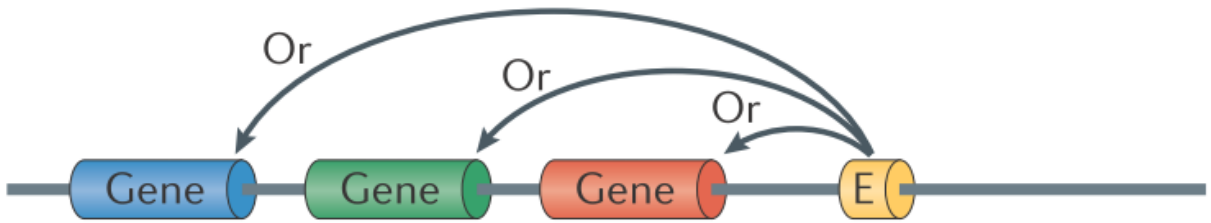
a One gene, multiple enhancers, one tissue



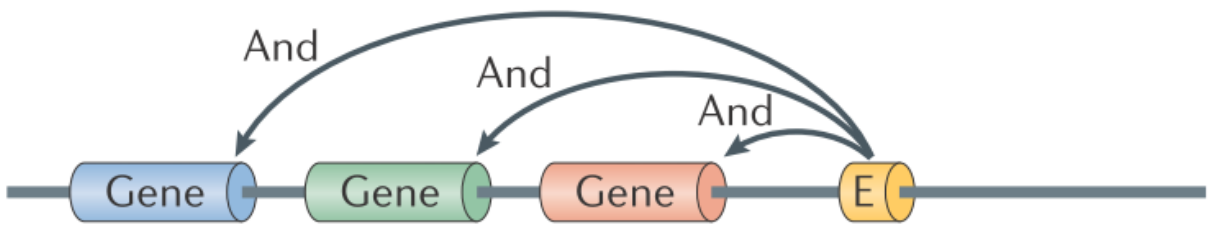
b One gene, multiple enhancers, more tissues



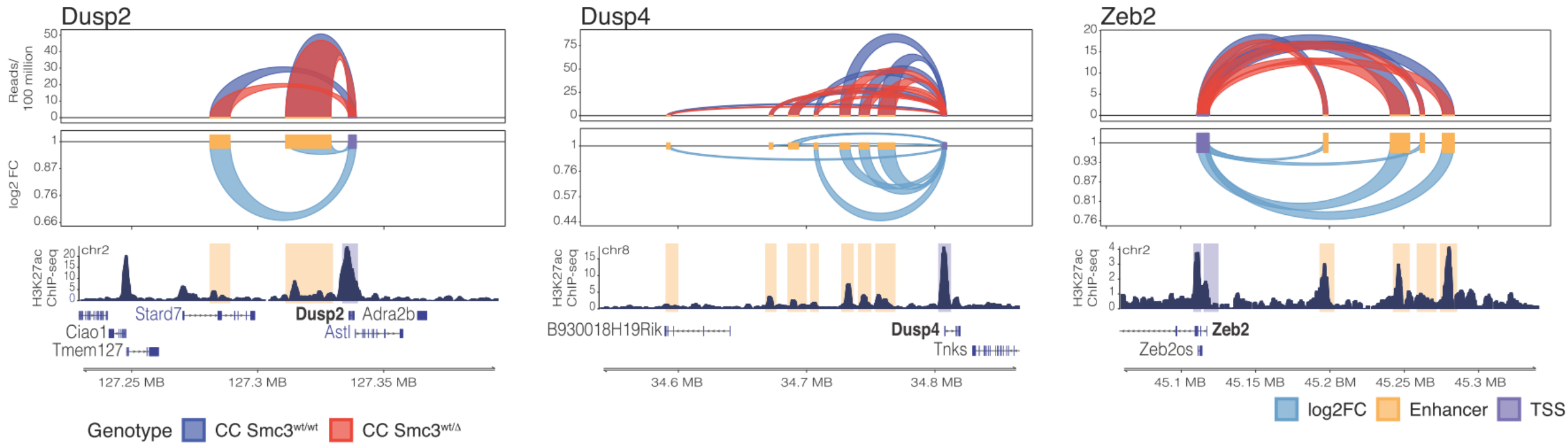
c Gene competition for a shared enhancer: winner takes all



d Gene competition for a shared enhancer: we are all winners



Regulation of disease-associated gene expression in the 3D genome, Krijger et al


Dusp2
Reads/ 100 million
log2 FC
H3K27ac ChIP-seq
chr2
Stard7
Dusp2
Adra2b
Ciao1
Astl
Tmem127
127.25 MB
127.3 MB
127.35 MB
Dusp4
chr8
B930018H19Rik
Dusp4
Tnks
34.6 MB
34.7 MB
34.8 MB
Zeb2
chr2
Zeb2
Zeb2os
45.1 MB
45.15 MB
45.2 BM
45.25 MB
45.3 MB
Genotype
CC Smc3wt/wt
CC Smc3wt/Δ
log2FC
Enhancer
TSS