The application of epigenomic profiling strategies to study cancer and other common diseases

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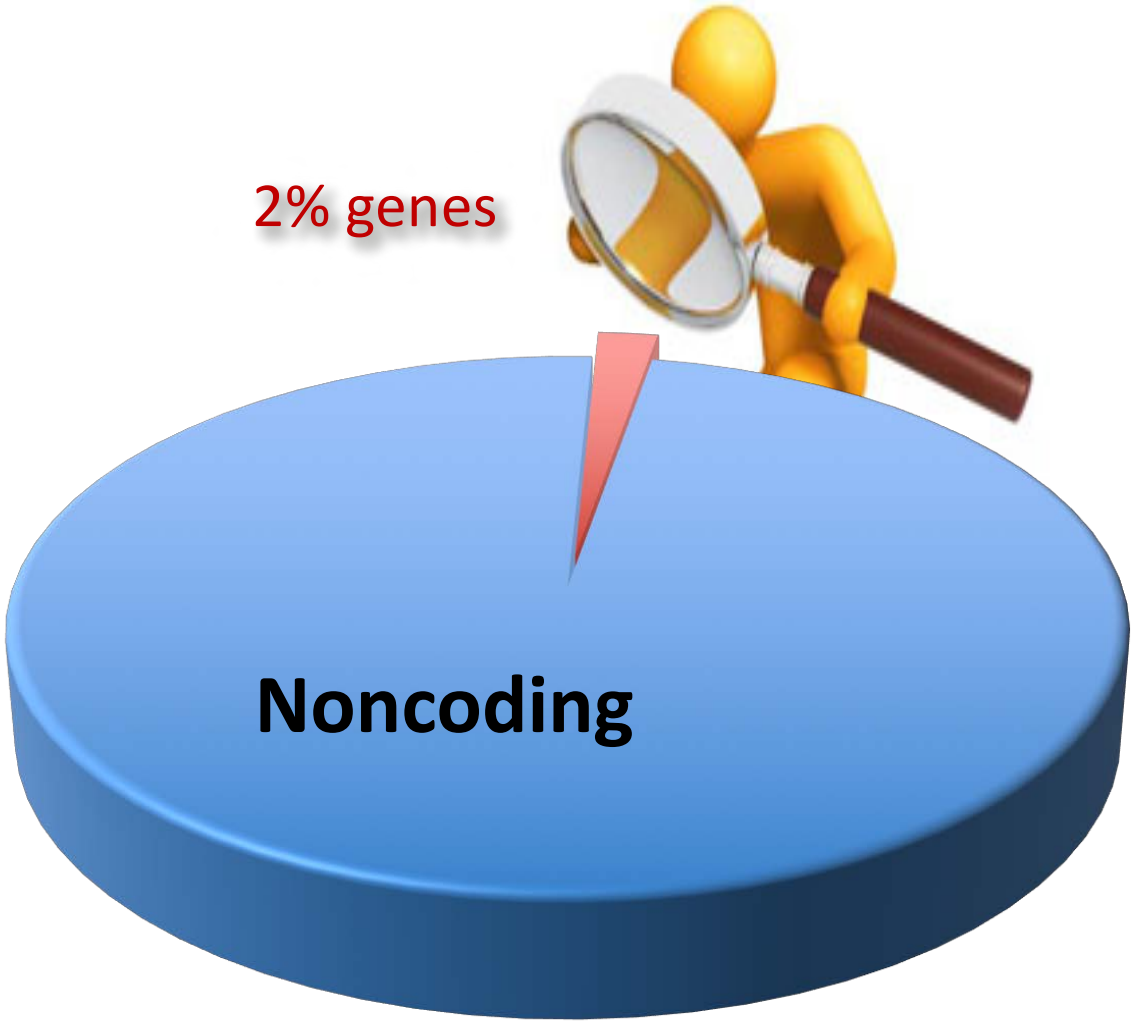
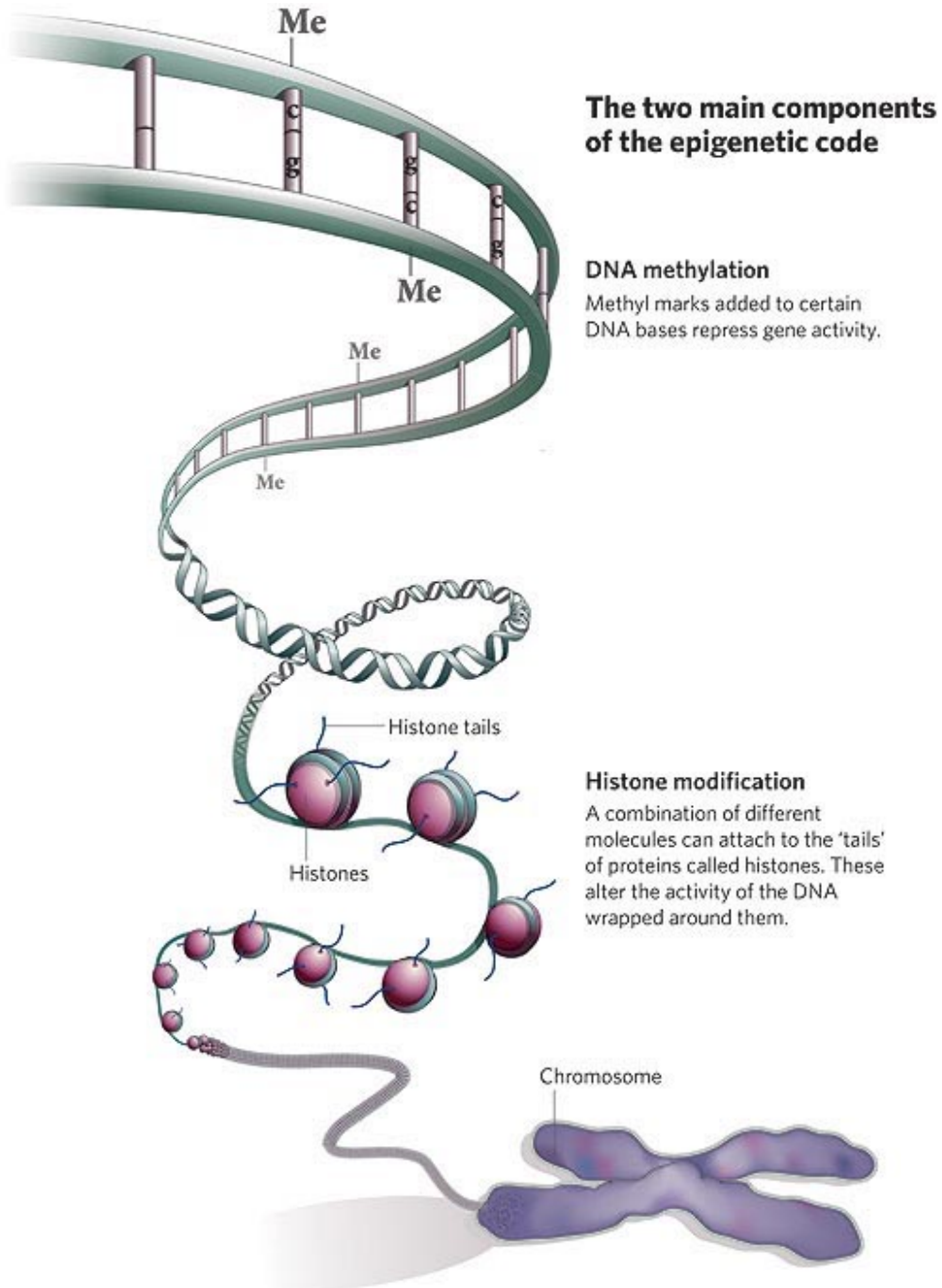
Outline

- Introduction
  - Non-coding regions and the epigenome
  - Techniques for epigenomic profiling
  - Repositories and browsers for epigenomics datasets
  - Data analysis
- Applications of epigenomic profiling (Focus on enhancer elements and cancer)
  - Tumorigenesis
  - Clinical subtyping
  - Metastasis
  - Drug resistance
  - Identification of non-coding driver mutations
  - GWAS
  - Biomarkers
- Opportunities for Precision Oncology

Most of the human genome is non-coding DNA



The Epigenome



Profiling Techniques for DNA methylation

Technique	Method	Advantages	Limitations
Whole-Genome Bisulfite Sequencing (WGBS)	Bisulfite converted DNA is amplified and sequenced	Genome-wide, single nucleotide resolution	Costly and computationally intensive
Reduced-Representation Bisulfite Sequencing (RRBS)	Methylation-insensitive restriction enzymes digest DNA, enriching for CpG regions	Cheaper than WGBS with relatively high coverage	Enzymatic digestion covers most but not all CpG sites
Pyrosequencing	DNA is bisulfite converted, amplified, with the ratio of C/T nucleotides measured	Genome-wide or targeted, single nucleotide resolution. Allele-specific primers	Relatively expensive
Methylated DNA Immunoprecipitation (MeDip)	Methylated DNA is enriched by immunoprecipitation followed by sequencing or microarray analysis	Random fragmentation by sonication avoids restriction enzyme bias	Varying CpG density can confound methylation estimates
Methylation Sensitive Restriction Enzyme Sequencing (MSRE/MRE-Seq or Methyl-seq)	Unmethylated DNA is restriction enzyme digested while methylated DNA is amplified	No bisulfite conversion bias	DNA may be partially digested, limited coverage
Combined Bisulfite Restriction Analysis (COBRA)	Bisulfite converted DNA is amplified and restriction enzyme digested	Simple, fast, inexpensive, works on FFPE-treated DNA	DNA may be partially digested, limited coverage
Methylation Specific PCR	Bisulfite converted DNA is amplified with methylation specific primers	Simple and inexpensive	Purely qualitative
High Resolution Melt Analysis (HRM)	Bisulfite converted DNA is amplified by q-PCR	Most sensitive method for determining methylation at a specific region	Single base resolution not possible
Illumina MethylationEPIC BeadChip Microarray (previously 450k, 27k)	Bisulfite (or oxidized + bisulfite) converted DNA is interrogated on a microarray chip	Relatively simple and inexpensive. Extremely popular	Data has limited coverage and requires pre-processing
Global DNA Methylation	Methods include LINE1, Alu, LUMA, HPLC-UV	Relatively inexpensive	Does not identify differentially methylated regions
Tet-assisted Bisulfite Sequencing (TAB-seq)	5hmC is protected then oxidized to 5caC then uracil by TET	Differentiation between 5mC and 5hmC at single base resolution	Sensitivity and specificity depends on sequencing depth
Oxidative Bisulfite Sequencing (OxBis)	DNA is oxidized then bisulfite converted to 5fC and subsequently uracil	Quantitative genome-wide coverage	Bias to regions of low 5mC. Must be performed in parallel with bisulfite techniques
APOBEC-coupled epigenetic sequencing (ACE-seq)	Non-destructive DNA deaminase enzymes discriminate between 5hmC and 5mC	Genome-wide, single nucleotide resolution. Very low DNA input required	Not yet extensively tested
Hydroxymethylated DNA Immunoprecipitation (hMeDIP)	Immunoprecipitation and sequencing of hydroxymethylated DNA	Simple and inexpensive	Only semi quantitative and bias to regions of low 5hmC

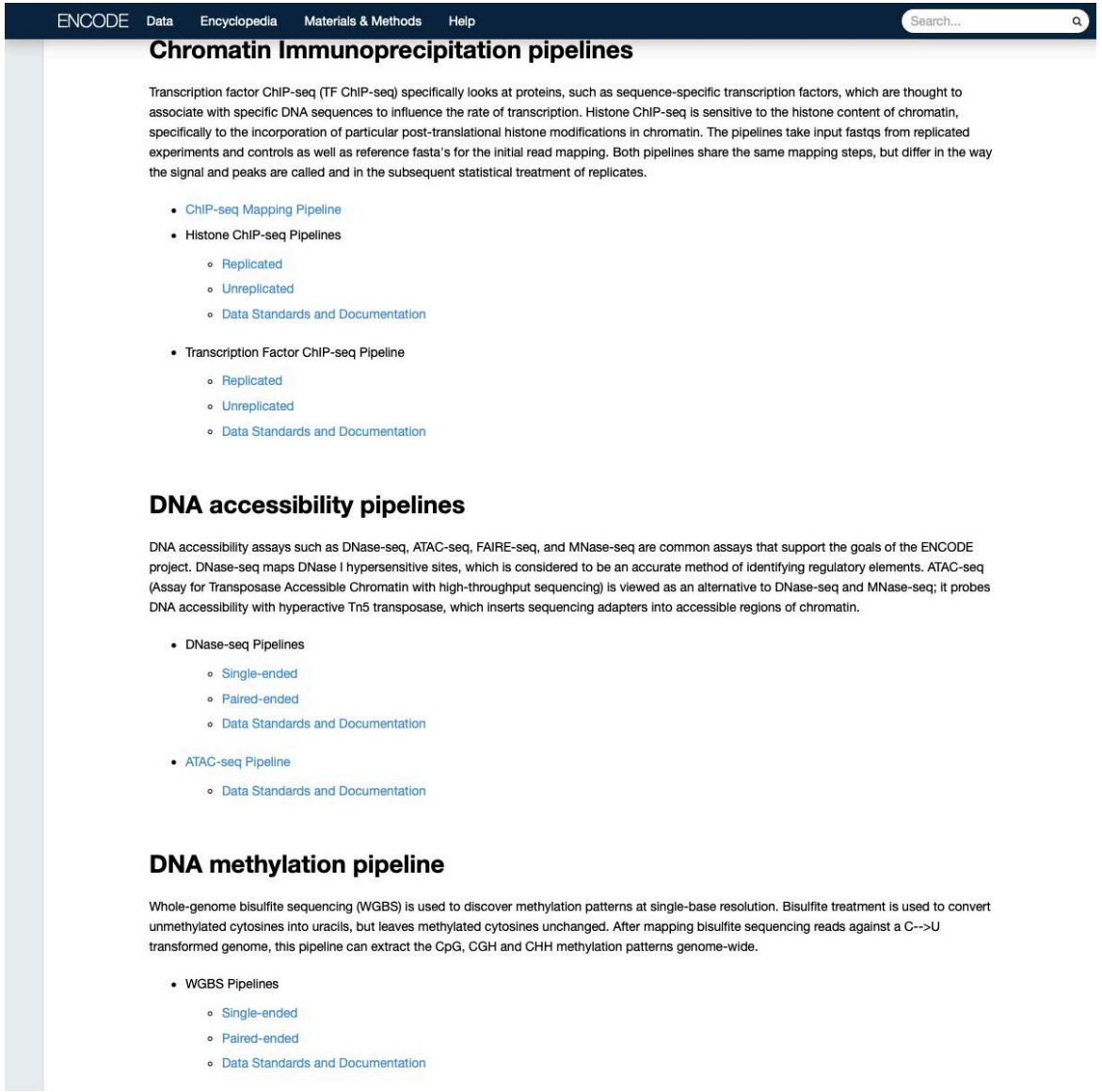
Cazaly et al., *Front Pharm* 2019

Profiling techniques for histones and chromatin accessibility

Technique	Method	Advantages	Limitations
Chromatin Immunoprecipitation (ChIP)	Couples highly specific antibodies for DNA-binding proteins with sequencing, microarrays or PCR	Detect DNA associated proteins and histone modifications	Requires intact cells and chromatin
Digital DNase	Enzymes digest nuclease-accessible regions, indicating open chromatin	Maps both nucleosomes and non-histone proteins	High sequencing depth required. Potential actin contamination.
NOMe-seq	Single-molecule, high-resolution nucleosome positioning assay	Maps both DNA methylation and nucleosomes at high resolution	Relies on presence of CpG residues
Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq)	Measures chromatin accessibility based on Tn5 transposase activity. Maps nucleosomes and non-histone proteins	Simple, fast, low input of cells with single nucleotide resolution	Distance between binding sites may bias results
Chromosome Conformation Capture (3C, 4C, 5C, Hi-C) & Hi-ChIP	Assess spatial organization of chromatin in a cell	Various modified versions	Often lack genome-wide, single nucleotide resolution
CUT&RUN (Cleavage Under Targets and Release Using Nuclease)	antibody-targeted controlled cleavage by micrococcal nuclease releases specific protein-DNA complexes into the supernatant for paired-end DNA sequencing.	In situ, simple, fast, low input, Less sequencing depth required	Need good antibodies

Cazaly et al., *Front Pharm* 2019

Lots of methods for processing datasets

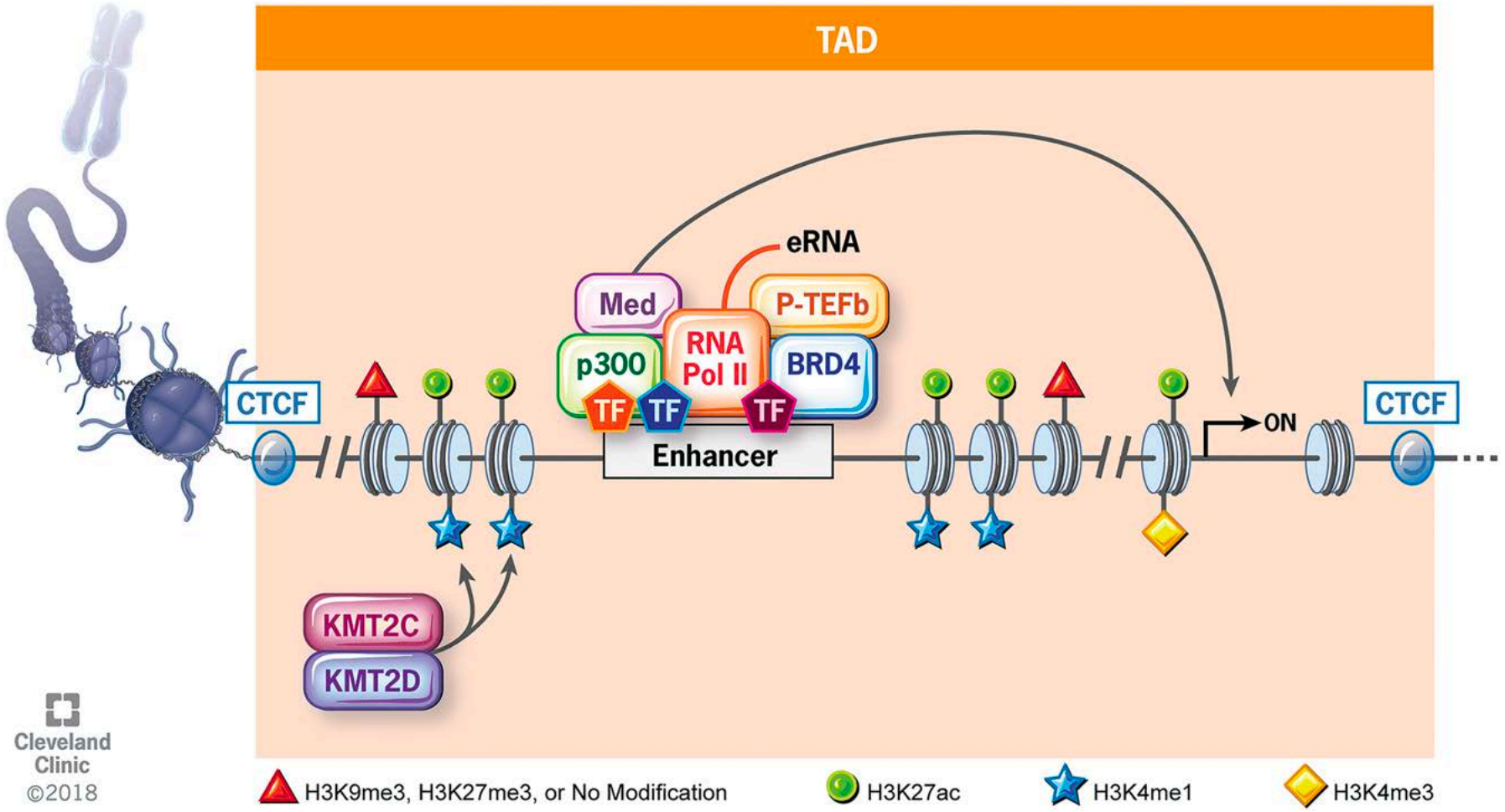


Epigenetic data repositories and browsers

Consortia and resources	Data availability	URLs
The International Human Epigenome Consortium (IHEC)	Reference epigenomes generated by NIH Roadmap, ENCODE, CEEHRC, BLUEPRINT, DEEP, AMED/CREST, and KEP	IHEC Data Portal http://epigenomesportal.ca/ihec
NIH Roadmap Epigenomics	Maps of histone modifications, chromatin accessibility, DNA methylation and mRNA Expression in stem cells and primary *ex vivo* human tissues	VizHub http://vizhub.wustl.edu
Canadian Epigenetics, Environment and Health Research Consortium (CEEHRC) Network	Reference epigenomes including histone modifications, DNA methylation, mRNA and miRNA of human cancer and normal cells	CEEHRC Data http://www.epigenomes.ca/site-data Software Tools http://www.epigenomes.ca/tools-and-software
BLUEPRINT Epigenome	Reference epigenomes of human normal and malignant hematopoietic cells	BLUEPRINT Portal http://blueprint-data.bsc.es
The German epigenome programme (DEEP)	Reference epigenomes of human cells and tissues in normal and complex disease states	DEEP Data Portal http://deep.dkfz.de
IHEC Team Japan (AMED-CREST)	Reference epigenomes of human gastrointestinal epithelial cells, vascular endothelial cells and cells of reproductive organs	IHEC Data Portal http://epigenomesportal.ca/ihec
Korea Epigenome Project (KEP)	Reference epigenome map for common complex diseases	IHEC Data Portal http://epigenomesportal.ca/ihec
DeepBlue	Epigenomic data server for storing and working with genomic and epigenomic data. Collection of over 30,000 experiment files from the main epigenome mapping projects available. Uploading own data allowed	DeepBlue server http://deepblue.mpi-inf.mpg.de
Allelic Epigenome Project	Allelic DNA methylome, histone modifications, and transcriptome in human cells and tissues	Genboree http://genboree.org/genboreeKB/projects/allelic-epigenome
GTEx	Genotype and expression profiles in different tissues enabling eQTL studies	GTEx Portal http://www.gtexportal.org
BRAINEAC	Brain eQTL Almanac provides genotype and expression profile across 10 brain regions	BRAINEAC http://braineac.org
MQTLdb	Methylation and genotype data on mother-child pairs providing access to meQTL mapping across five different stages of life	mQTL Database http://www.mqtldb.org
Fetal brain meQTLs	Epigenome-wide significant meQTLs observed in fetal brain	Fetal Brain meQTL http://epigenetics.essex.ac.uk/mQTL
Pancan-meQTL	Database of *cis*- and *trans*- meQTLs across 23 cancer types from The Cancer Genome Atlas	Pancan-meQTL http://bioinfo.life.hust.edu.cn/Pancan-meQTL
Epigenome Browser	UCSC genome browser with tracks from ENCODE project	UCSC Epigenome Browser http://www.epigenomebrowser.org
WashU Epigenome Browser	Web browser with tracks from ENCODE and Roadmap Epigenomics projects	WashU Epigenome Browser http://epigenomegateway.wustl.edu
Ensembl	ENCODE data used in the regulatory build	Ensembl ENCODE https://www.ensembl.org
RMBase	Database listing over 100 RNA modifications	http://rna.sysu.edu.cn/rmbase/

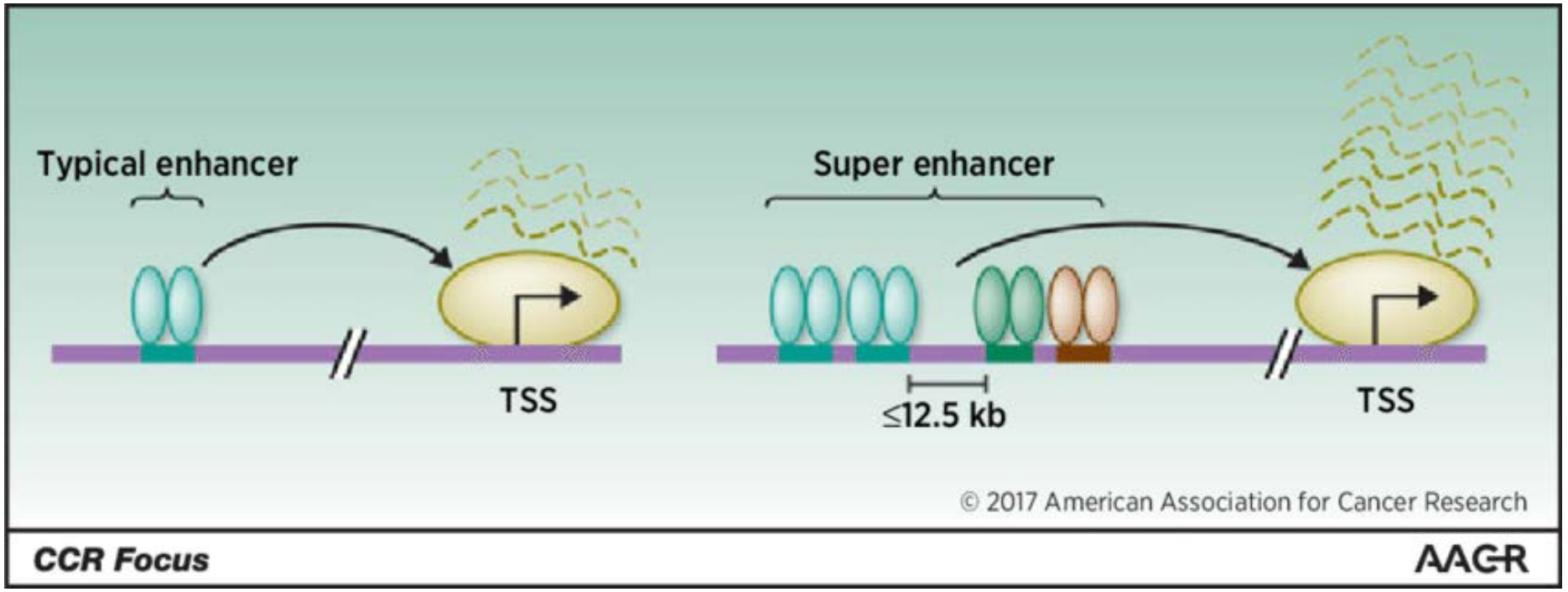
Cazaly et al., *Front Pharm* 2019

The Epigenome and Gene Enhancer Elements



Karnuta, Jaret M., and Peter C. Scacheri. "Enhancers: Bridging the Gap between Gene Control and Human Disease." *Human Molecular Genetics,* 2018.

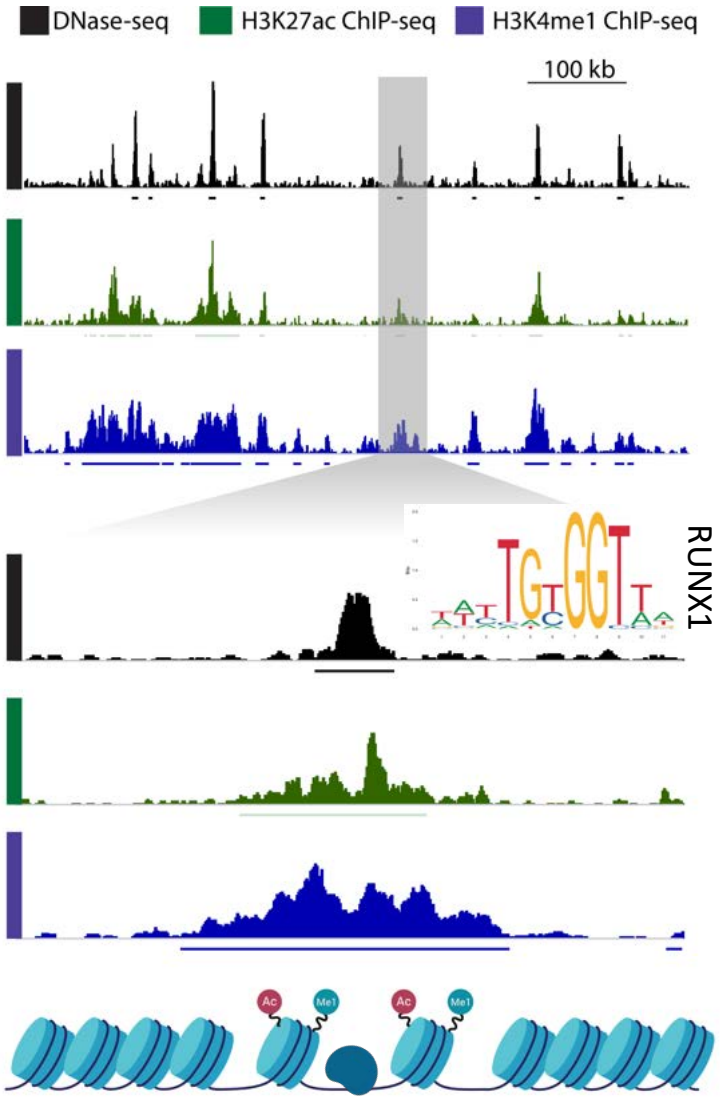
Superenhancers

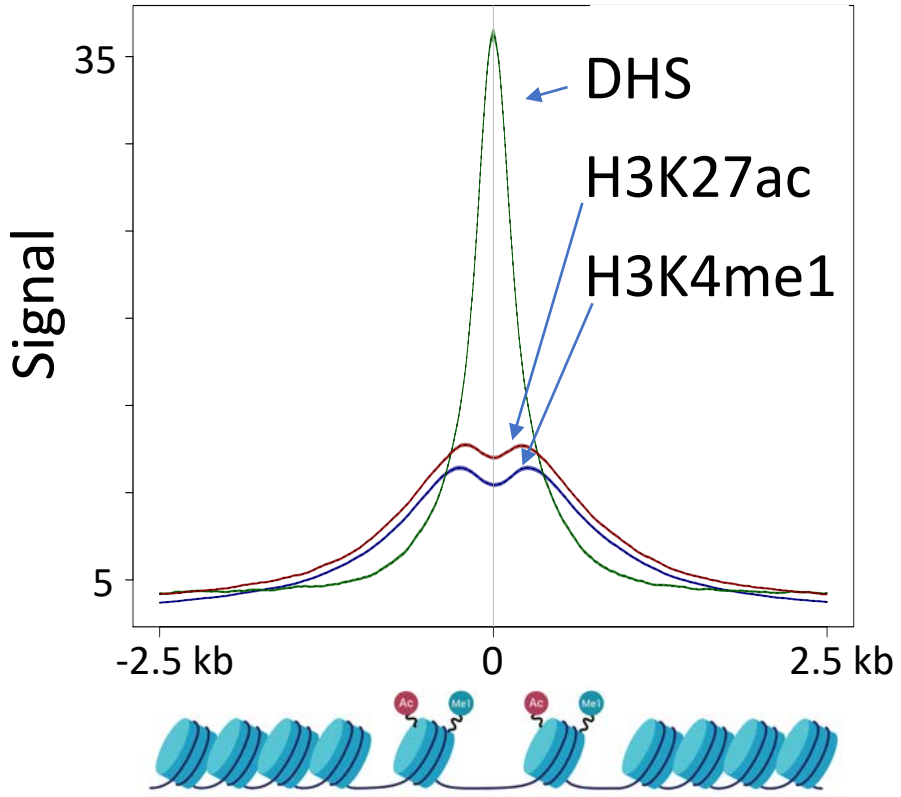


"Super enhancers are typically an order of magnitude larger than typical enhancers in size, have higher transcription factor density, and greater ability for transcriptional activation."

Evan et al, CCR 2017

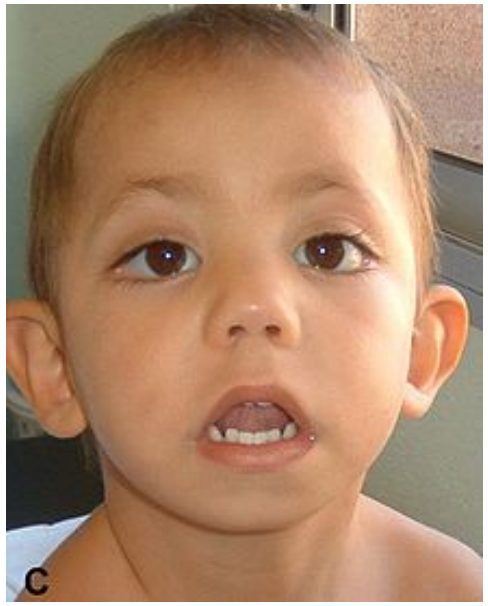
Genome-wide identification of active enhancer elements based on signature chromatin features



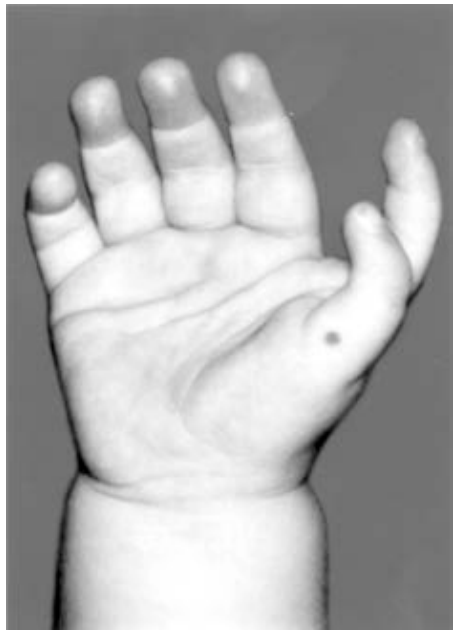


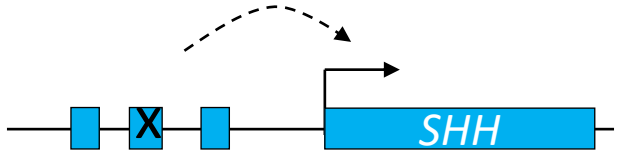
”Enhanceropathies”

Kabuki syndrome

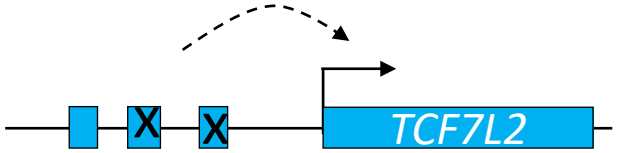


Preaxial Polydactyly





Type II diabetes



Karnuta and Scacheri, *HMG* 2018
Scacheri and Scacheri, *Curr Opin Pediatr* 2015
Corradin and Scacheri, *Genome Med* 2014

Studying the epigenome can provide insights into various aspects of cancer

- Tumorigenesis
- Clinical subtyping
- Metastasis
- Drug resistance
- Identification of non-coding driver mutations
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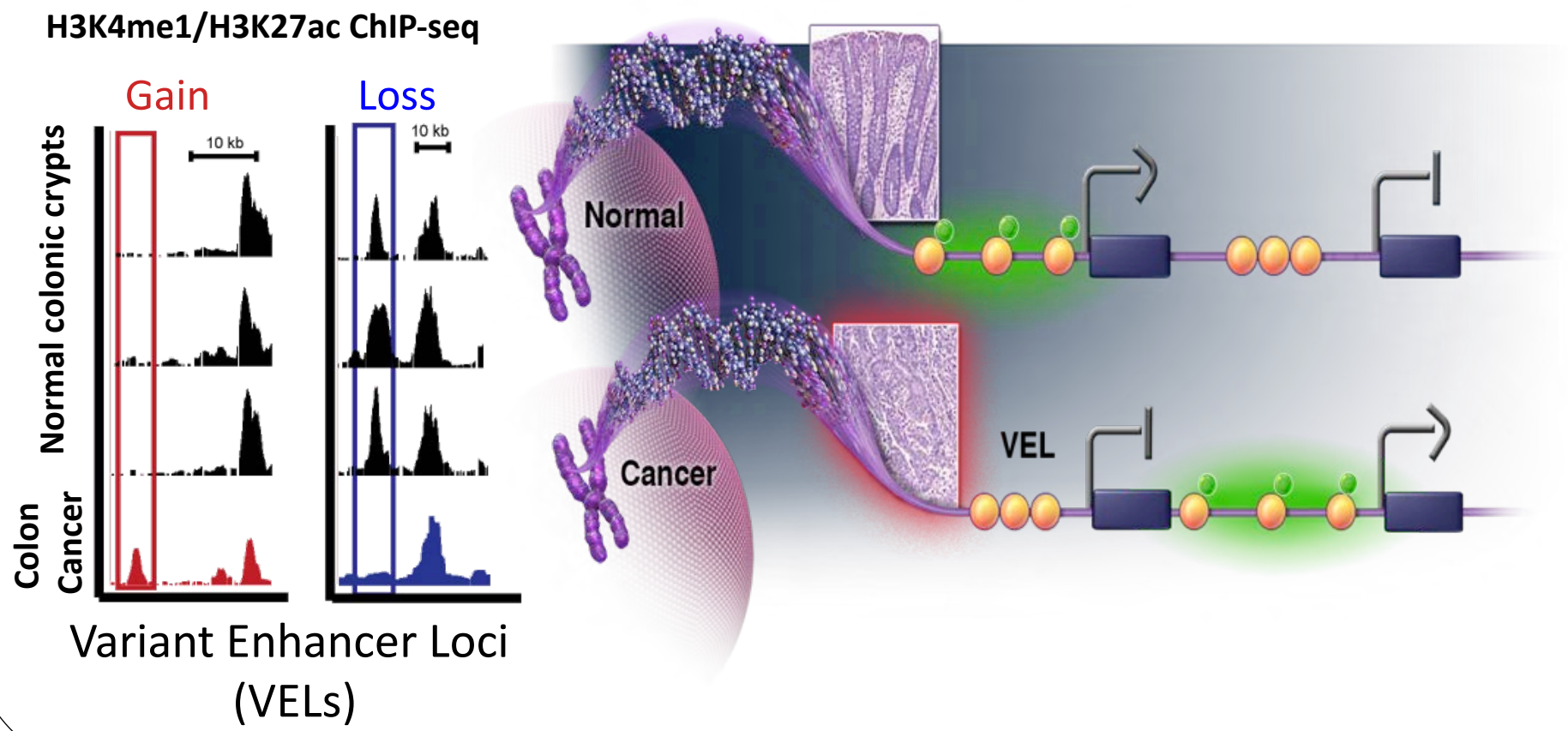
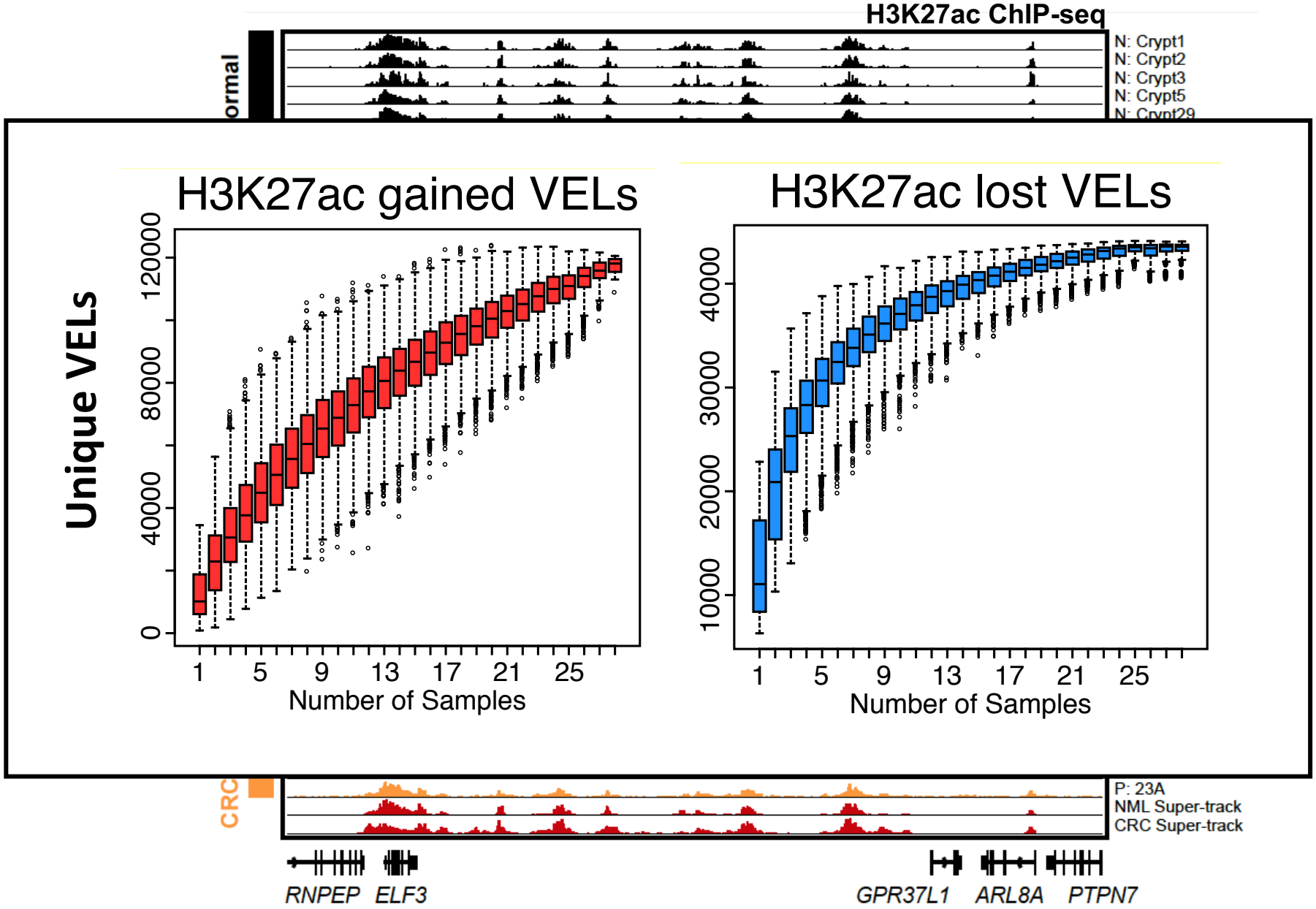
- Biomarkers

Enhancers alterations in cancer

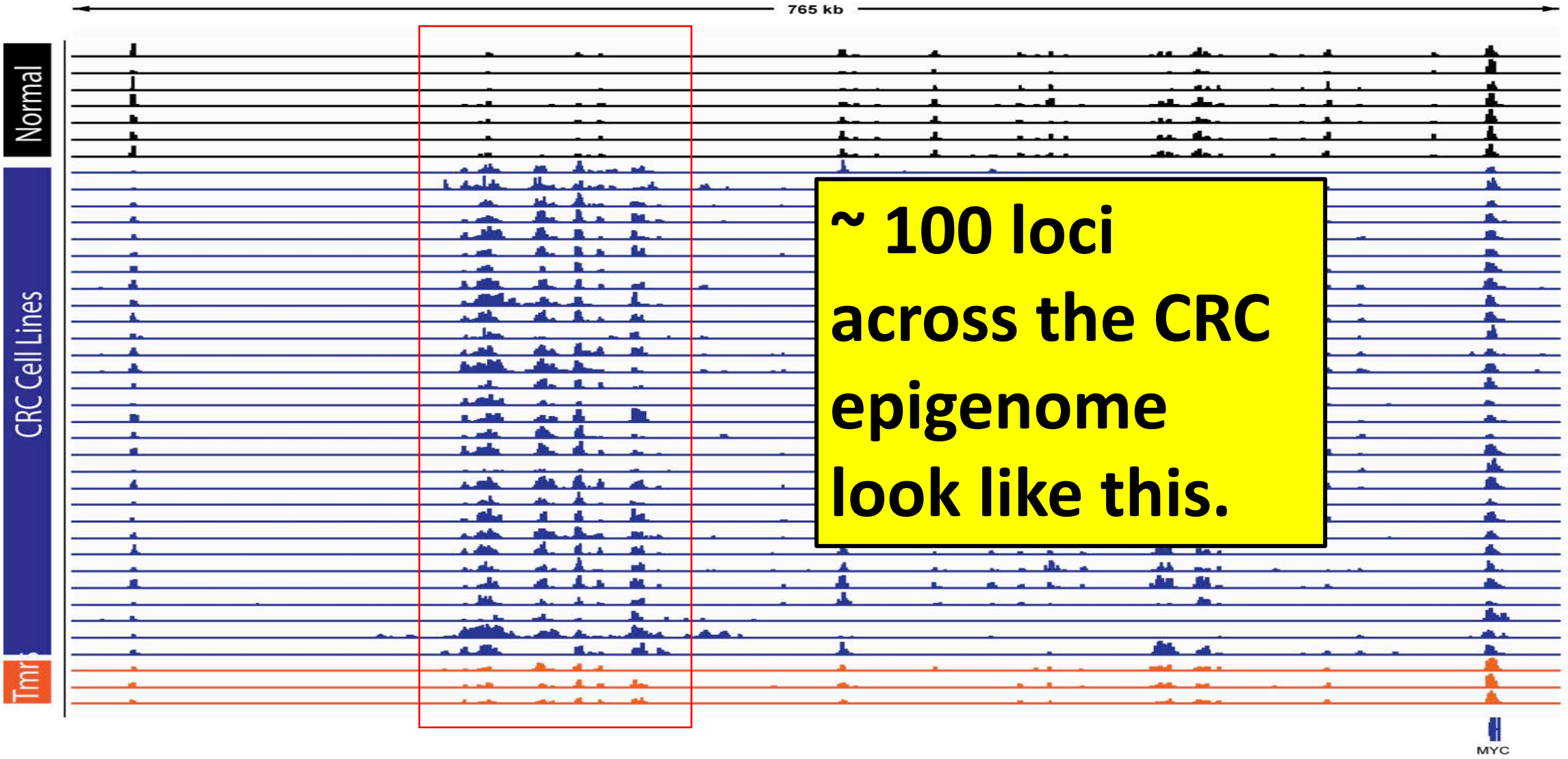


Akhtar-Zaidi et al, *Science* 2012
Cohen et al, *Nat Comm* 2017

Expansion of enhancer profiling studies



Recurrent enhancer alterations in CRC



Studying the epigenome can provide insights into various aspects of cancer

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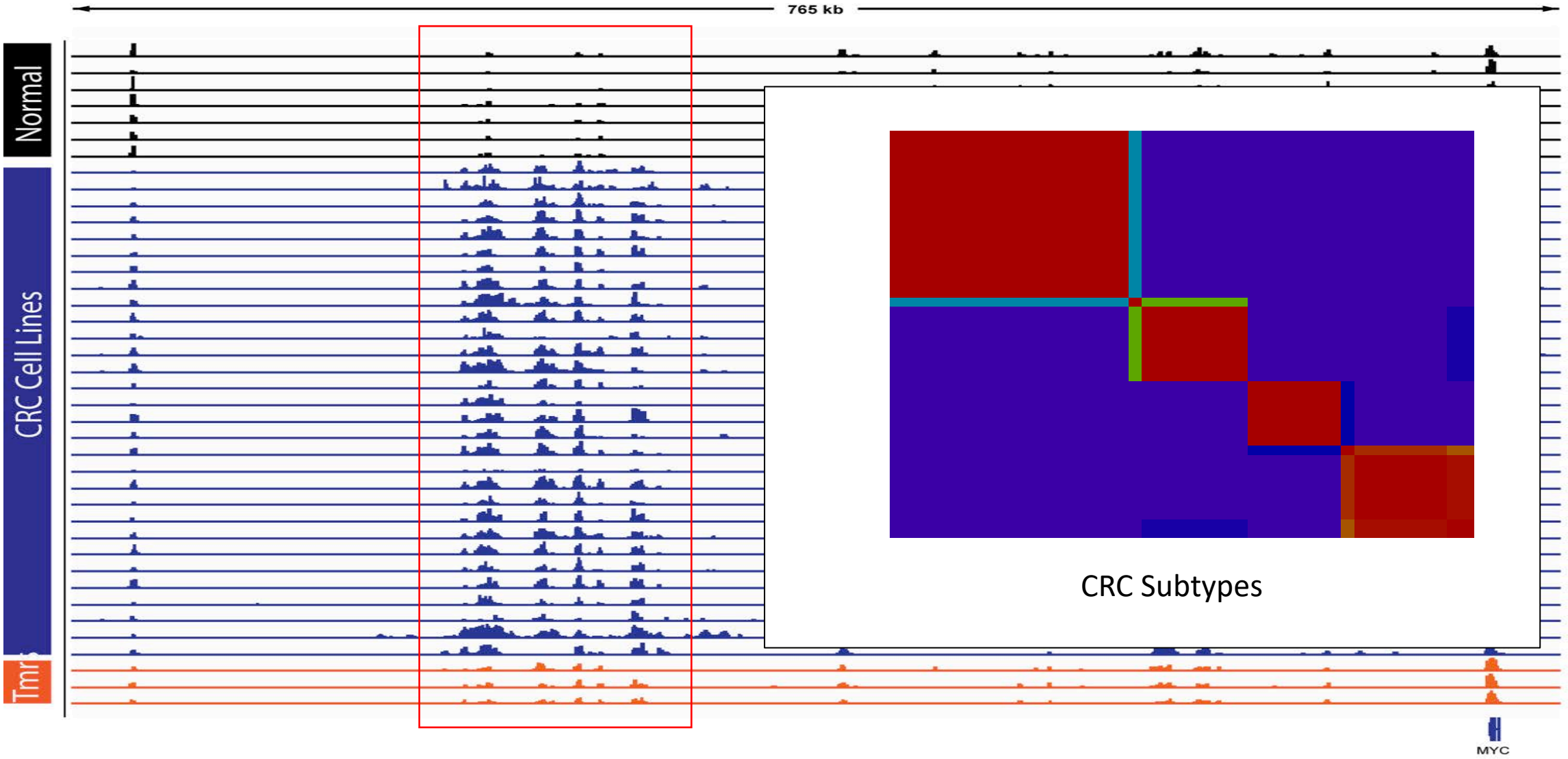
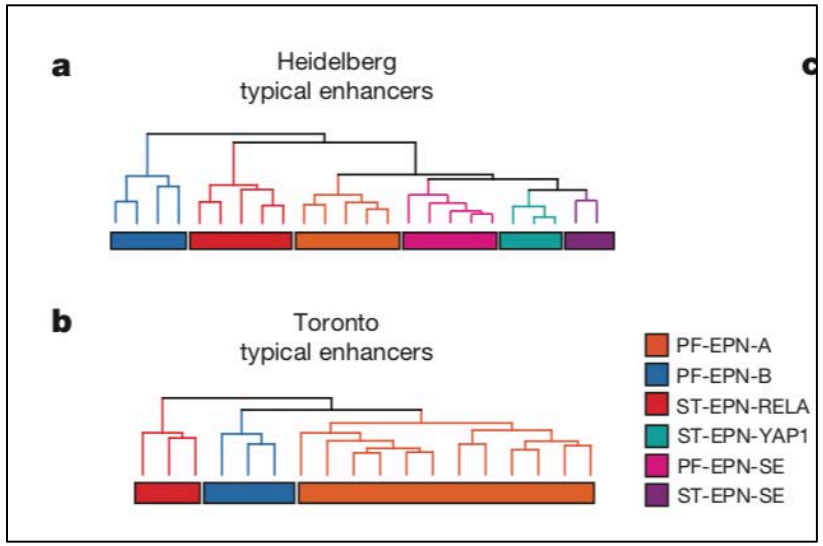
Recurrent enhancer alterations in CRC



Therapeutic targeting of ependymoma as informed by oncogenic enhancer profiling

Nature 2018

Stephen C. Mack[1,2,3,4]*, Kristian W. Pajtler[5,6,7]*, Lukas Chavez[5,6,8]*, Konstantin Okonechnikov[5,6], Kelsey C. Bertrand[1,2,9], Xiuxing Wang[3,4,10], Serap Erkek[5,6,11], Alexander Federation[12], Anne Song[3,4], Christine Lee[3,4], Xin Wang[13], Laura McDonald[13], James J. Morrow[14], Alina Saiakhova[14], Patrick Sin-Chan[13], Qiulian Wu[3,4,10], Kulandaimanuvel Antony Michaelraj[13], Tyler E. Miller[3,4,15], Christopher G. Hubert[3,4], Marina Ryzhova[16], Livia Garzia[13], Laura Donovan[13], Stephen Dombrowski[3,4,17],



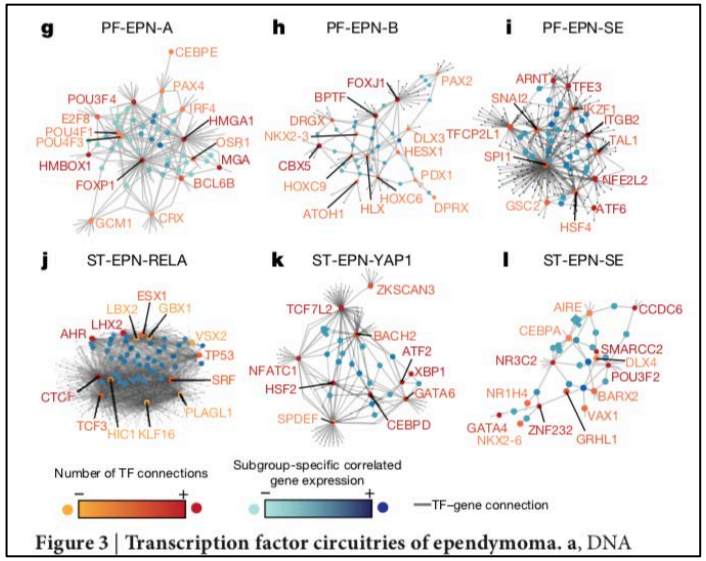
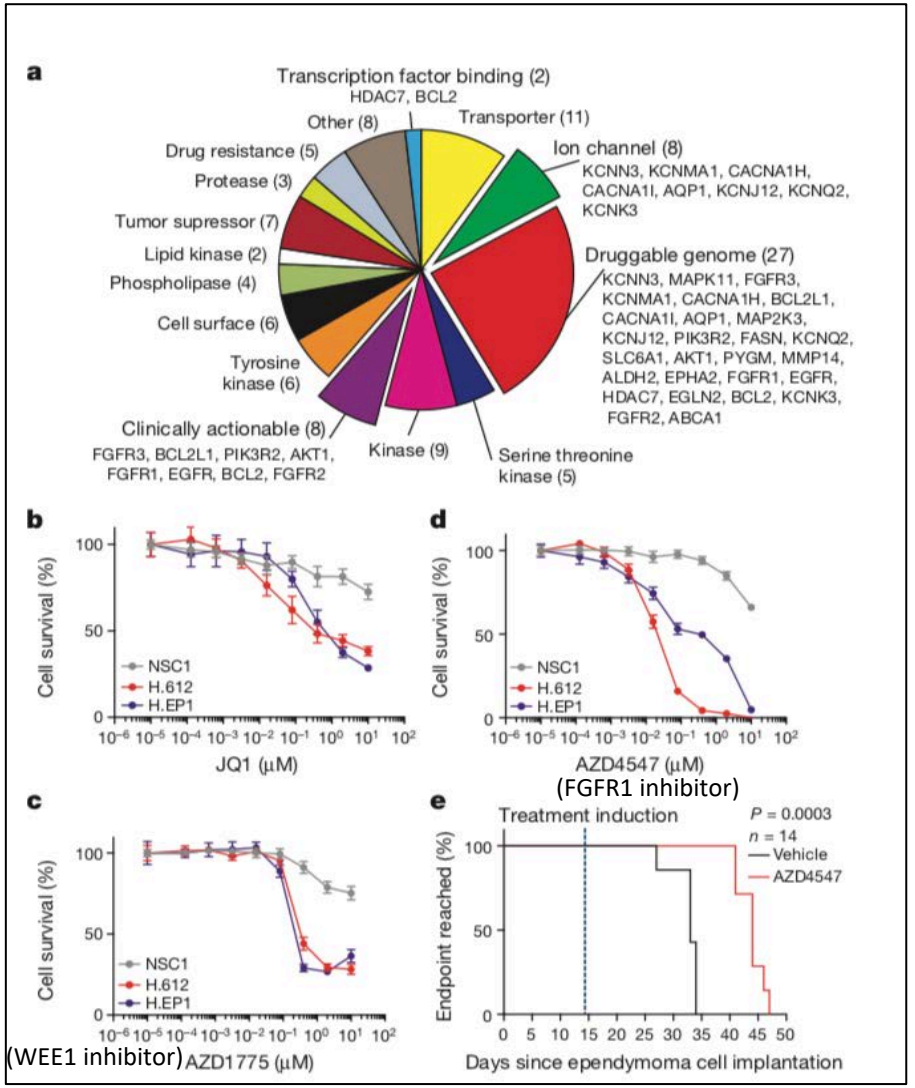
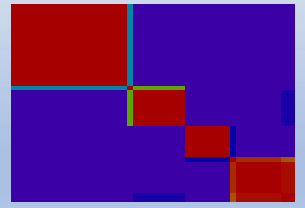


Figure 3 | Transcription factor circuitries of ependymoma. a, DNA

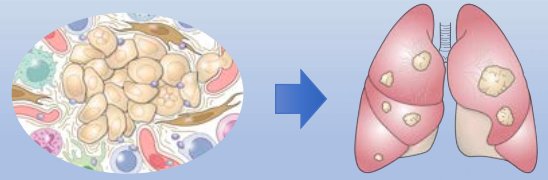


Enhancer changes mediating acquisition of traits

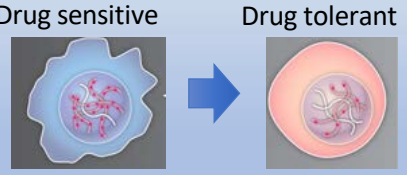
Subtypes



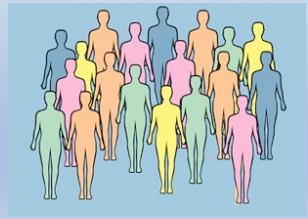
Metastasis



Drug resistance



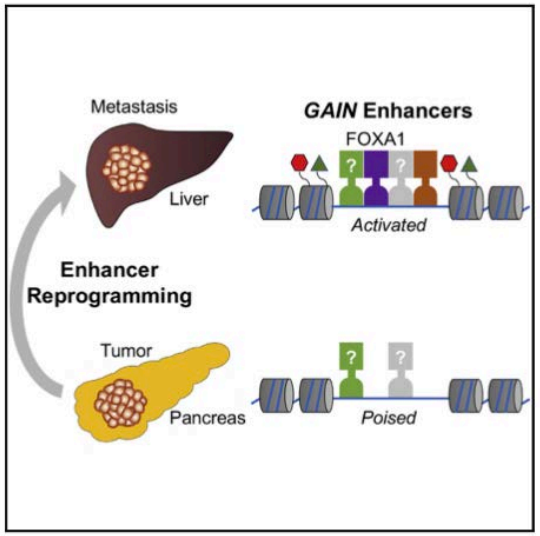
Predisposition



Enhancer Reprogramming Promotes Pancreatic Cancer Metastasis

Cell 2017

Jae-Seok Roe,[1,9] Chang-Il Hwang,[1,2,9] Tim D.D. Somerville,[1] Joseph P. Milazzo,[1] Eun Jung Lee,[1,2] Brandon Da Silva,[1,2] Laura Maiorino,[1] Hervé Tiriac,[1,2] C. Megan Young,[1,2] Koji Miyabayashi,[1,2] Dea Filippini,[1,2] Brianna Creighton,[1,2] Richard A. Burkhart,[3] Jonathan M. Buscaglia,[4] Edward J. Kim,[5] Jean L. Grem,[6] Audrey J. Lazenby,[7] James A. Grunkemeyer,[8] Michael A. Hollingsworth,[8] Paul M. Grandgenett,[8] Mikala Egeblad,[1] Youngkyu Park,[1,2] David A. Tuveson,[1,2,*] and Christopher R. Vakoc[1,10,*]

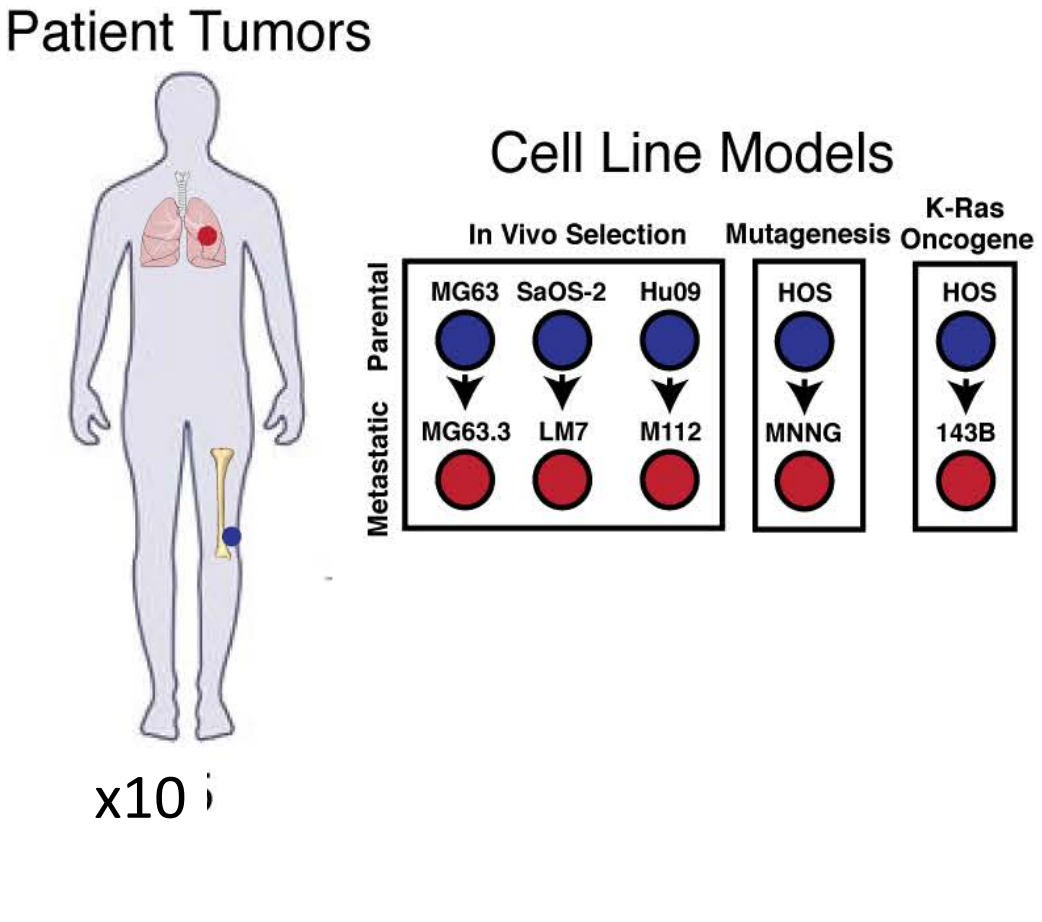


Nature Medicine 2018

Positively selected enhancer elements endow osteosarcoma cells with metastatic competence

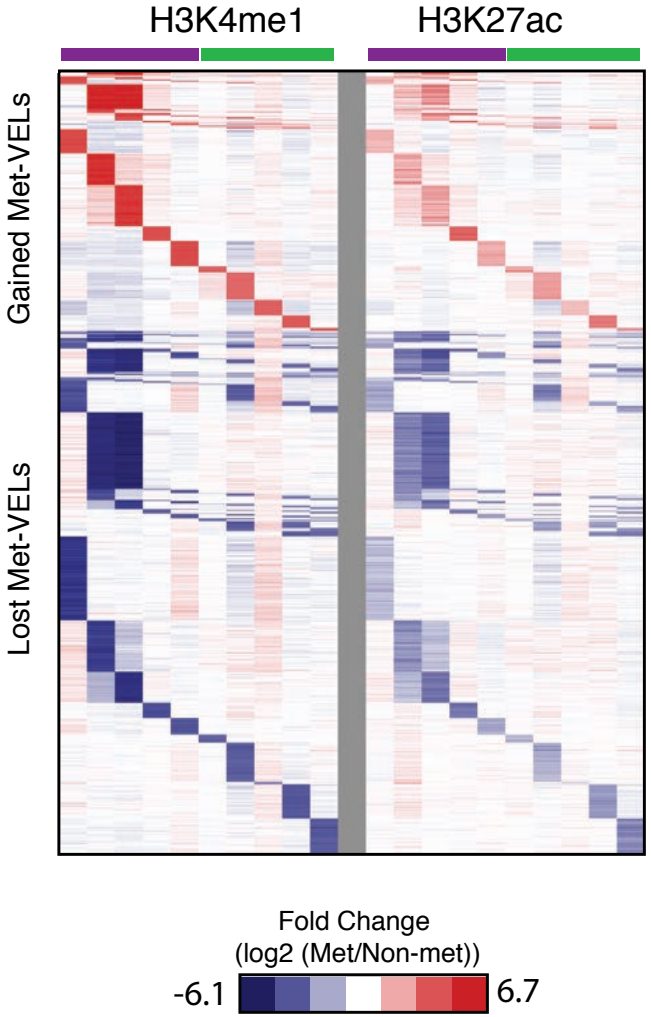
James J Morrow[1,2], Ian Bayles[2], Alister P W Funnell[3], Tyler E Miller[1], Alina Saiakhova[2], Michael M Lizardo[4], Cynthia F Bartels[2], Maaike Y Kapteijn[5], Stevephen Hung[2], Arnulfo Mendoza[4], Gursimran Dhillon[2], Daniel R Chee[6], Jay T Myers[7], Frederick Allen[1], Marco Gambarotti[8], Alberto Righi[8], Analisa DiFeo[9], Brian P Rubin[10], Alex Y Huang[1,7], Paul S Meltzer[11], Lee J Helman[4], Piero Picci[8], Henri H Versteeg[5], John A Stamatoyannopoulos[3], Chand Khanna[4,12] & Peter C Scacheri[2,8]

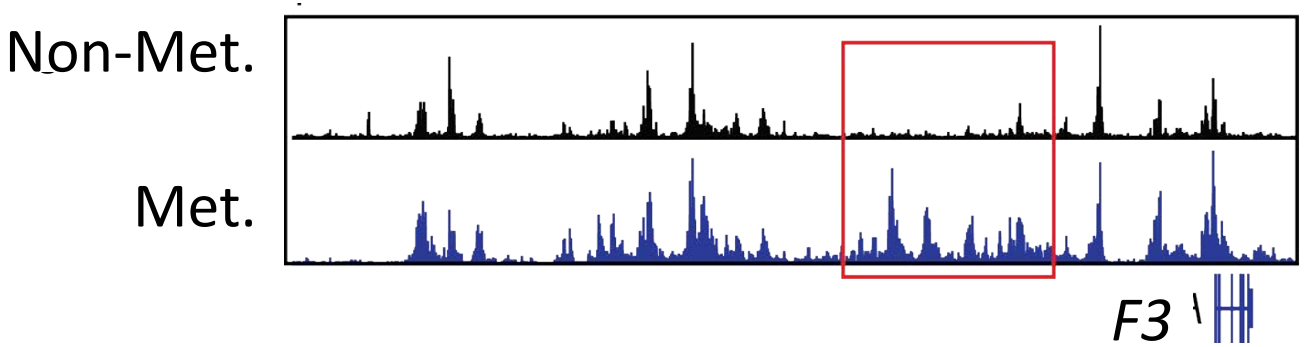
Metastatic tumors show extensive enhancer alterations (Met-VELs)

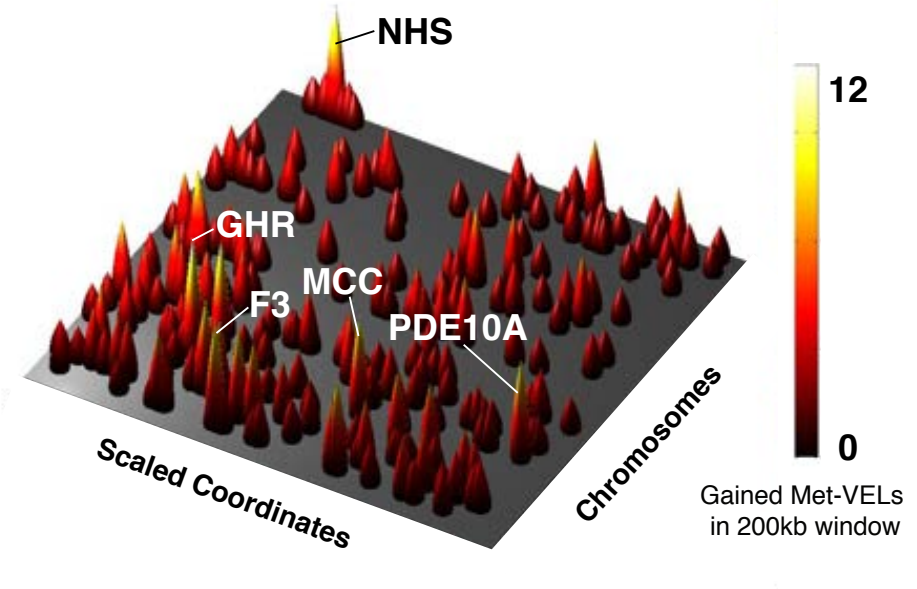


Morrow et al, *Nature Med* 2018

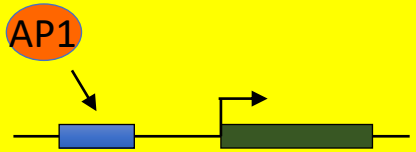
Enhancer alterations (Met-VELs) in metastatic osteosarcoma



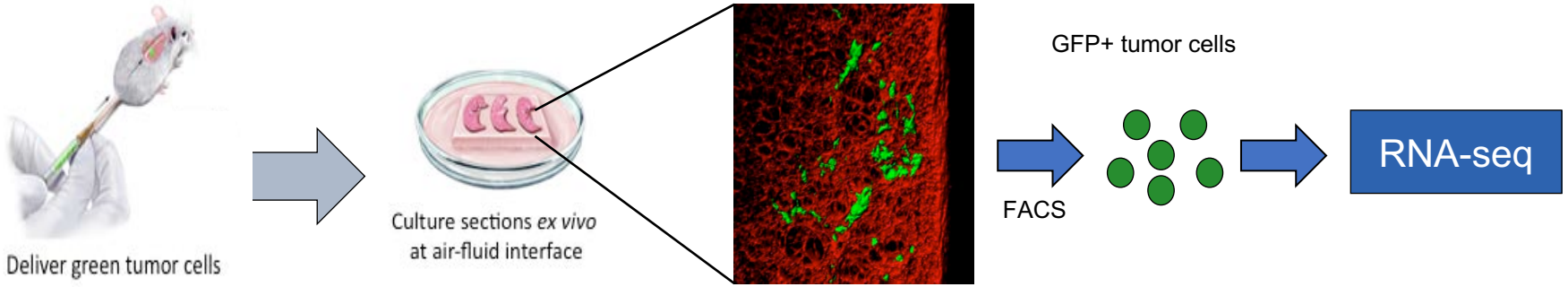


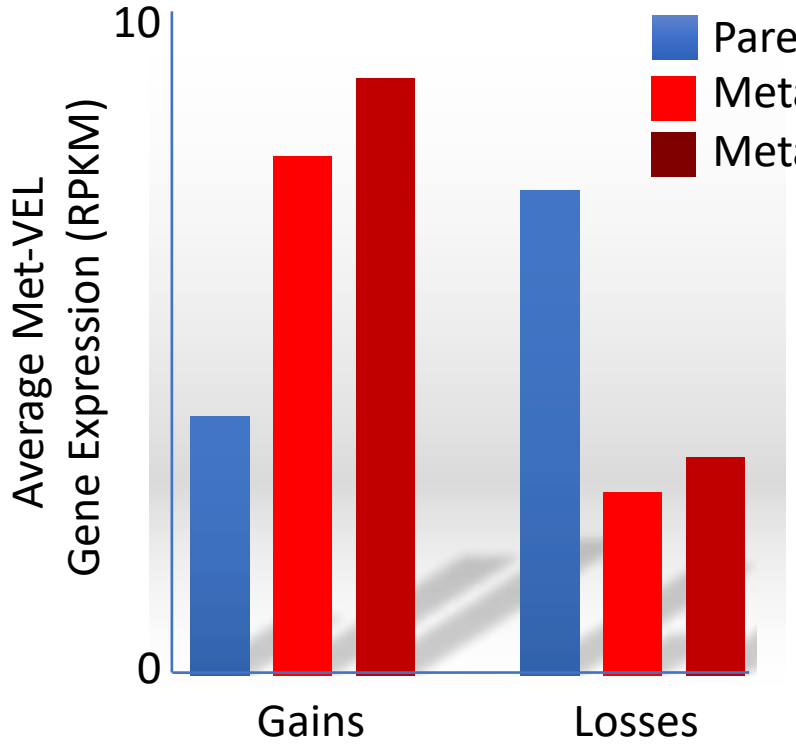


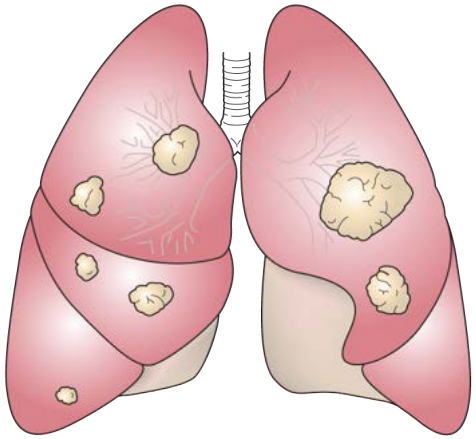
*AP-1 transcription factors bind to Met-VELs
(FOS, FOSL1, JUN, JUNB, JUND)



Met-VEL genes switch on/off in the lung microenvironment

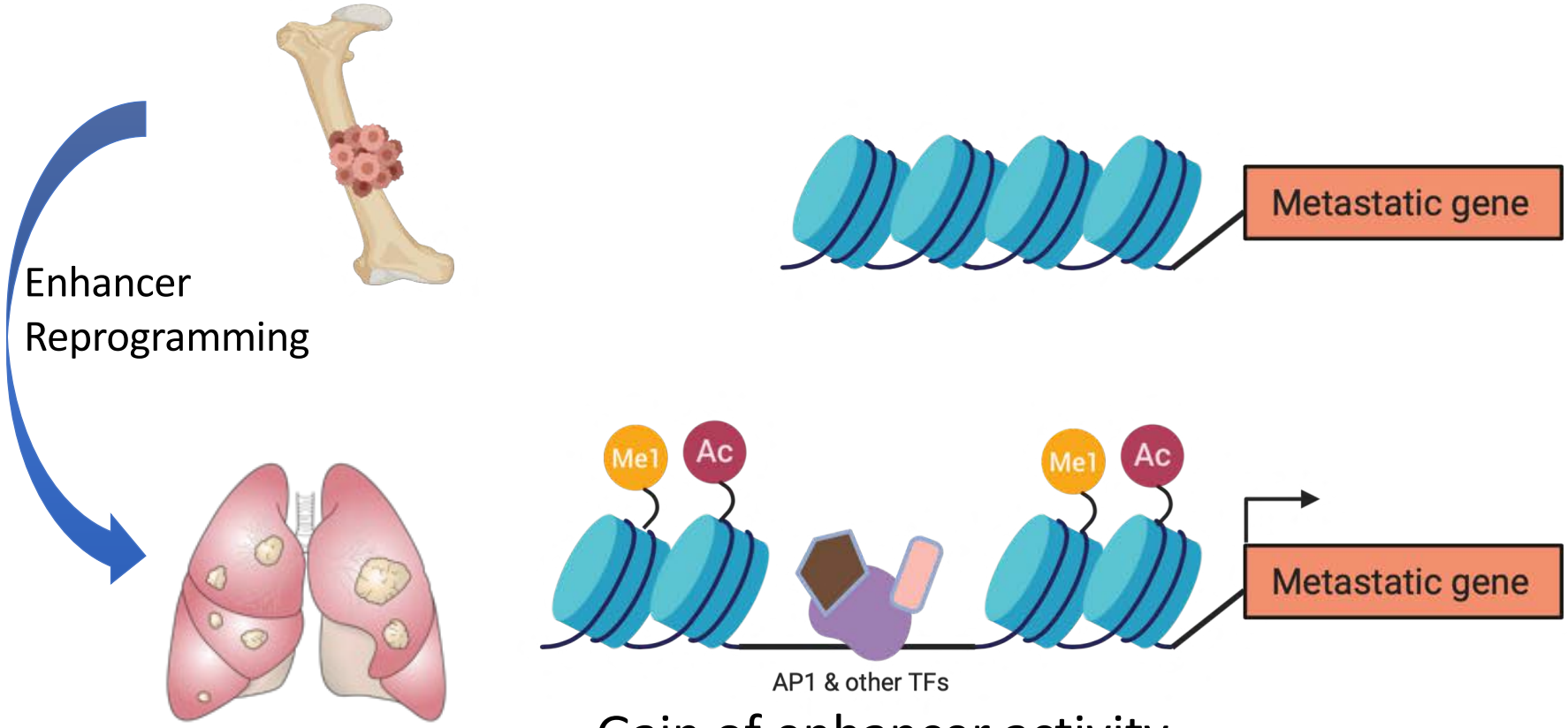






Environment of the lung is changing the way the Met-VELs regulate genes

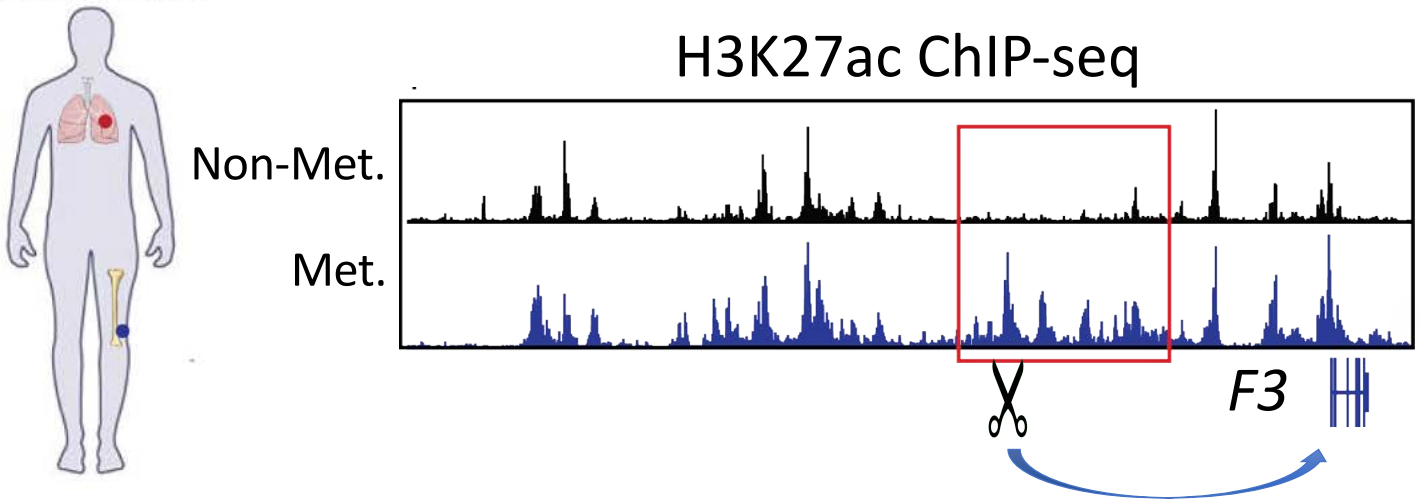
Model of enhancer function in metastasis

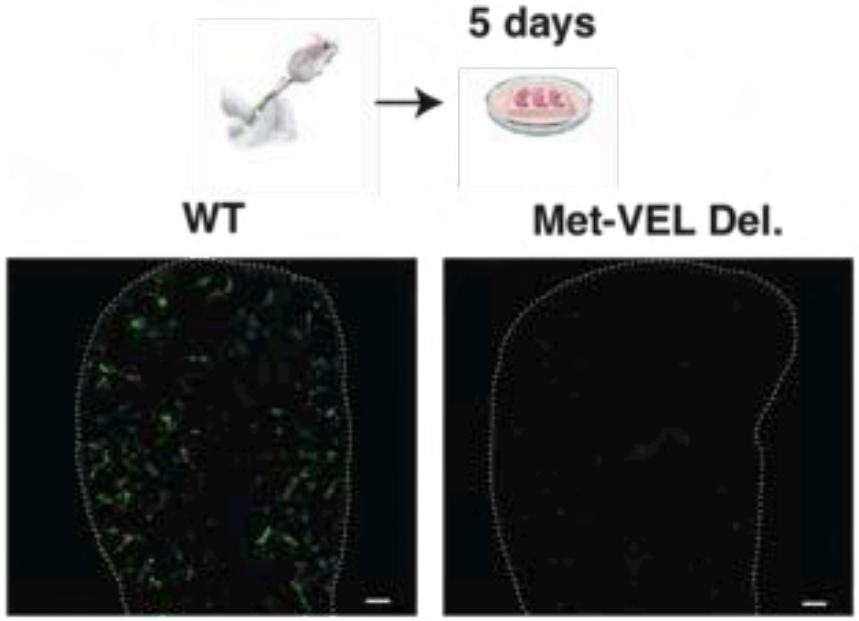


Gain of enhancer activity

Morrow et al, *Nature Med* 2018

Metastasis is dependent on the *F3* enhancer





Morrow et al, *Nature Med* 2018

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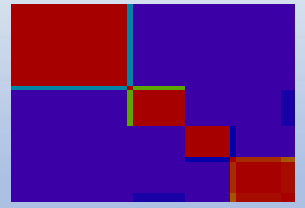
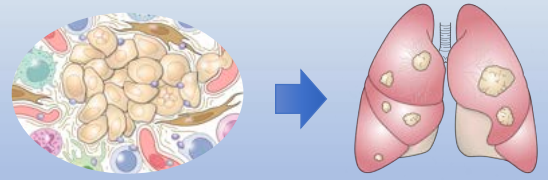


Metastasis

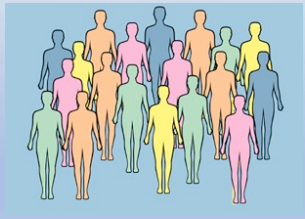


Drug resistance

Drug sensitive

Drug tolerant

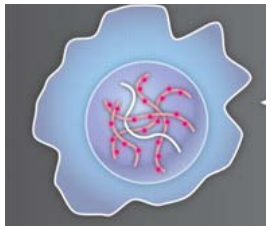
Predisposition



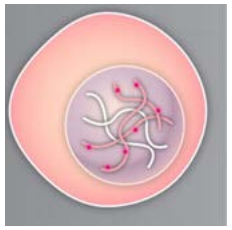
Genome-wide reprogramming of the chromatin landscape underlies endocrine therapy resistance in breast cancer

Luca Magnani[a,1], Alexander Stoeck[b], Xiaoyang Zhang[a], András Lánczky[c], Anne C. Mirabella[a,2], Tian-Li Wang[b], Balázs Gyorffy[c,3], and Mathieu Lupien[a,d,e,f,3]

Drug sensitive



Drug tolerant



Genome and Epigenome

Cancer Research

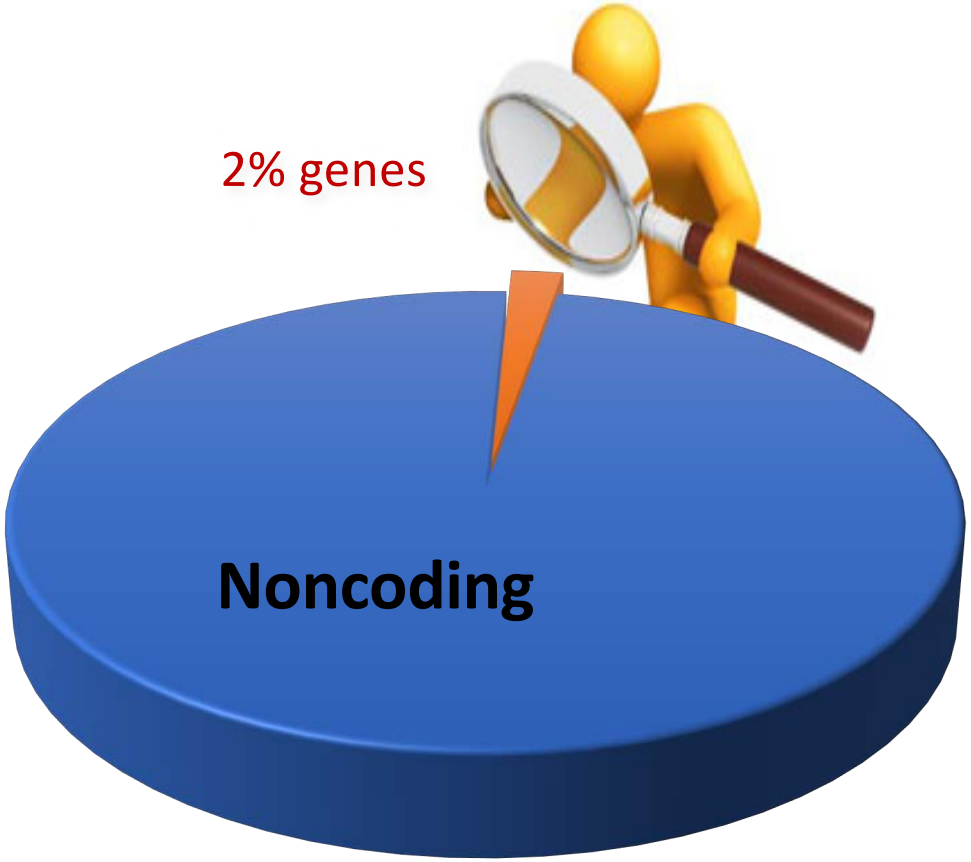
Chemotherapy-Induced Distal Enhancers Drive Transcriptional Programs to Maintain the Chemoresistant State in Ovarian Cancer

Stephen Shang[1], Jiekun Yang[1], Amir A. Jazaeri[2], Alexander James Duval[1], Turan Tufan[1], Natasha Lopes Fischer[1], Mouadh Benamar[1,3], Fadila Guessous[3], Inyoung Lee[1], Robert M. Campbell[4], Philip J. Ebert[4], Tarek Abbas[1,3], Charles N. Landen[5], Analisa Difeo[6], Peter C. Scacheri[6], and Mazhar Adli[1]

Studying the epigenome can provide insights into various aspects of cancer

- Tumorigenesis
- Clinical subtyping
- Metastasis
- Drug resistance
- **Identification of non-coding driver mutations**
- GWAS
- Biomarkers

Search for cancer driver mutations in the human genome



		BLCA	BRCA	COAD/READ	GBM	HNSC	KIRC	AML	LUAD	LUSC	OV	UCEC	Pan-Cancer	
MAPK signalling		0.0	0.8	**45.1**	0.7	0.3	0.2	4.0	26.3	1.2	0.6	20.0	6.7	*KRAS*
		7.1	2.5	1.0	11.0	2.7	1.7	1.0	**11.8**	10.3	3.8	3.5	4.4	*NF1*
		3.1	**7.2**	0.0	2.1	1.0	1.2	0.0	1.8	1.7	0.3	3.5	2.7	*MAP3K1*
		2.0	0.4	3.6	2.1	1.0	0.2	0.0	**6.6**	4.6	0.6	0.9	1.5	*BRAF*
		2.0	0.1	**8.8**	0.3	0.0	0.0	7.5	1.8	0.6	0.6	2.6	1.5	*NRAS*
		0.0	**4.1**	2.6	0.0	0.3	0.0	0.0	1.3	0.6	0.3	1.3	1.4	*MAP2K4*
		2.0	0.3	**2.1**	0.7	0.7	0.5	0.0	1.8	1.2	0.3	0.4	0.7	*MAPK8IP1*
PI(3)K signalling		17.4	33.6	17.6	11.0	20.6	2.9	0.0	4.4	14.9	0.6	**52.2**	17.8	*PIK3CA*
		3.1	3.8	1.0	30.7	1.3	4.3	0.0	2.2	8.1	0.6	**63.5**	9.7	*PTEN*
		1.0	2.5	2.1	11.4	1.7	0.5	0.0	1.3	0.6	0.3	30.9	4.4	*PIK3R1*
		2.0	1.2	0.0	0.3	2.0	0.5	0.5	**11.4**	5.8	1.0	0.4	1.9	*TLR4*
		2.0	0.4	0.5	2.4	2.7	0.7	0.0	5.3	**7.5**	1.0	1.3	1.7	*PIK3CG*
		0.0	**2.5**	0.0	0.3	0.7	0.5	0.0	0.0	0.6	0.0	1.3	0.9	*AKT1*
TGF-β signalling		2.0	0.4	**9.8**	0.3	2.0	0.5	0.0	3.1	2.9	0.0	0.0	1.4	*SMAD4*
		3.1	0.4	2.6	0.7	3.0	0.2	0.0	0.9	1.7	1.0	1.3	1.1	*TGFBR2*
		0.0	0.7	**3.6**	0.0	1.3	1.0	0.0	2.2	1.2	0.3	1.7	1.0	*ACVR1B*
		1.0	0.5	**5.7**	0.0	1.0	0.5	0.0	0.9	1.2	0.0	1.3	0.9	*SMAD2*
		1.0	0.5	**2.6**	0.0	0.7	0.2	0.0	0.9	1.2	0.0	0.4	0.6	*ACVR2A*
Wnt/β-catenin signalling		4.1	0.5	**81.9**	0.3	4.0	1.4	0.0	9.2	4.0	2.2	5.7	7.3	*APC*
		2.0	0.1	4.7	0.3	0.7	0.2	0.0	3.5	1.7	0.6	28.3	2.9	*CTNNB1*
		3.1	0.1	**3.6**	0.3	1.7	0.2	0.0	0.9	0.6	0.3	2.6	0.9	*AXIN2*
		2.0	1.1	0.0	0.0	1.0	0.7	0.0	**2.2**	1.2	0.3	1.3	0.8	*TBL1XR1*

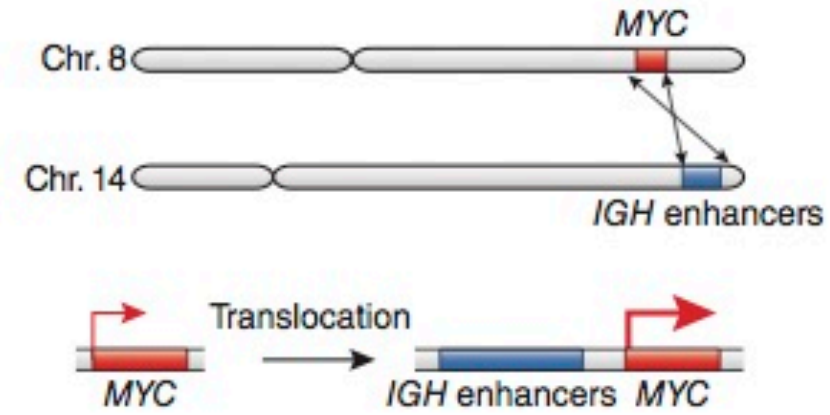
Kandoth et al, *Nature* (2013)

Challenges with finding mutations in non-coding regions

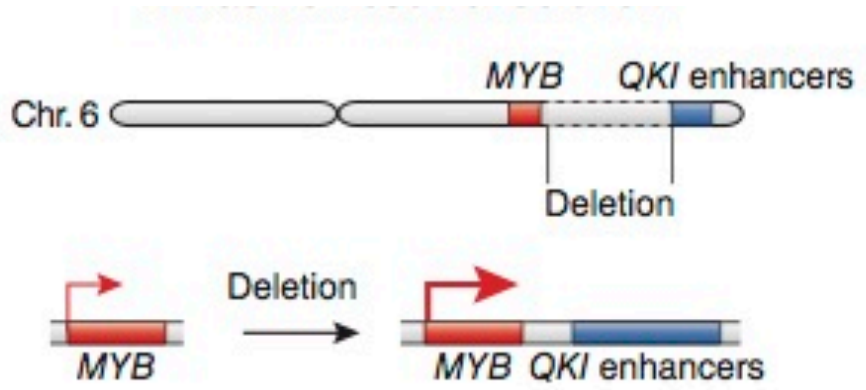
- Mutation rates vary between cancers
- Mutation rates are influenced by chromatin states
  - Active chromatin – low mutation rate
  - Inactive chromatin – high mutation rate
- Epigenome varies between tumors
- Cell type of origin is unknown or unavailable

Cancer driver mutations in noncoding regions

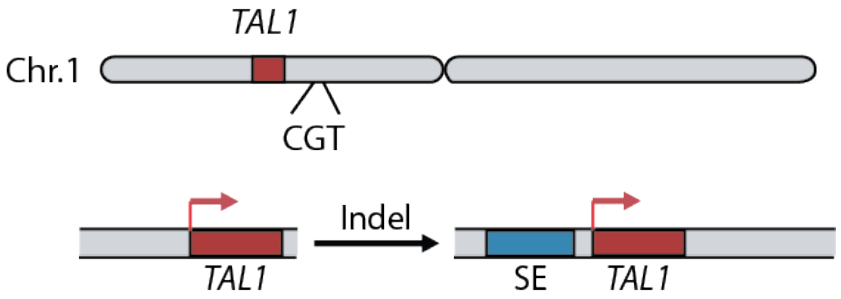
Structural Rearrangements



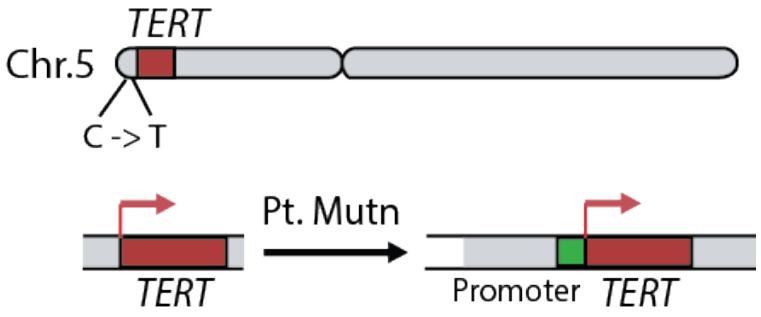
Copy Number Alterations



Indels

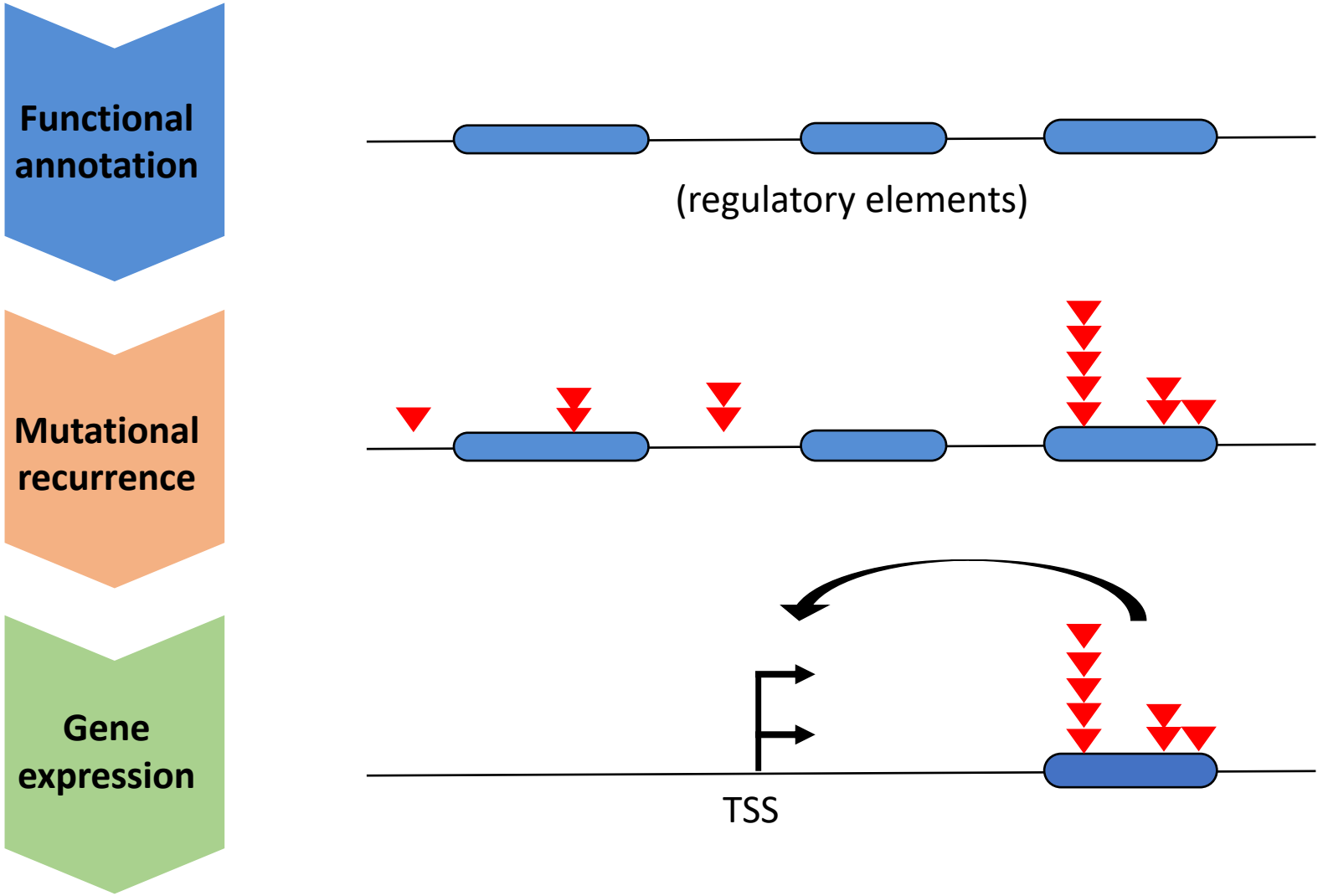


Point Mutations



Beroukhim, R. et al. 2017
Mansour et al, *Science* 2014

Common workflow for detecting candidate drivers



Adapted from Khurana et al, *Science* (2013)

Recurrent driver mutations in noncoding regions are rare

PCAWG
PanCancer Analysis
OF WHOLE GENOMES

New Results

bioRχiv
THE PREPRINT SERVER FOR BIOLOGY

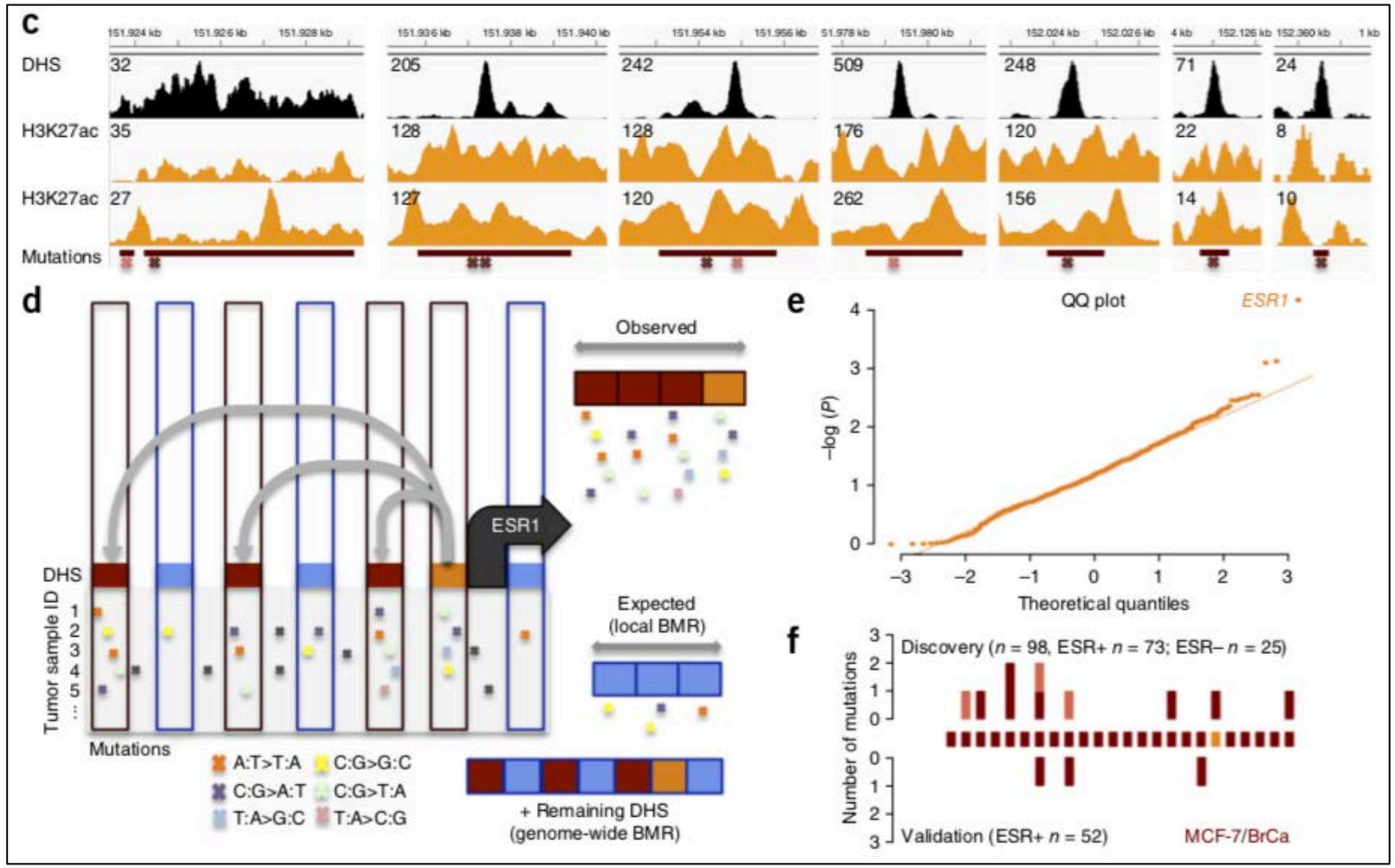
Discovery and characterization of coding and non-coding driver mutations in more than 2,500 whole cancer genomes

”Perhaps the most striking finding is the relative paucity of point mutations driving cancer in non-coding genes and regulatory elements.”

Mark P Hamilton, Chen Hong, André Kahles, Youngwook Kim, Kjong-Van Lehmann,
Todd Andrew A Johnson, Abdullah Kahraman, Keunchil Park, Gordon Saksena, Lina Sieverling,
Nicholas A Sinnott-Armstrong, Peter J Campbell, Asger Hobolth, Manolis Kellis, Michael S Lawrence,
Ben Raphael, Mark A Rubin, Chris Sander, Lincoln Stein, Josh Stuart, Tatsuhiko Tsunoda,
David A Wheeler, Rory Johnson, Jüri Reimand, Mark B Gerstein, Ekta Khurana,
Nuria Lopez-Bigas, Inigo Martincorena, Jakob Skou Skou Pedersen, Gad Getz,

Noncoding somatic and inherited single-nucleotide variants converge to promote *ESR1* expression in breast cancer

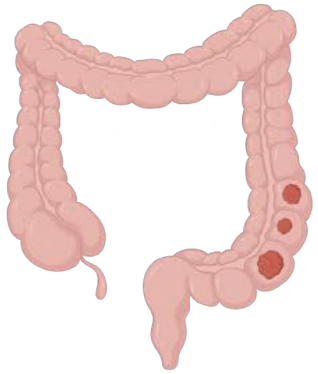
Swneke D Bailey[1,2,11], Kinjal Desai[3,11], Ken J Kron[1,2], Parisa Mazrooei[1,2], Nicholas A Sinnott-Armstrong[4], Aislinn E Treloar[1,2], Mark Dowar[1], Kelsie L Thu[5], David W Cescon[1,5], Jennifer Silvester[5], S Y Cindy Yang[1,2], Xue Wu[1,10], Rossanna C Pezo[1], Benjamin Haibe-Kains[1,2,6], Tak W Mak[2,5], Philippe L Bedard[1,7], Trevor J Pugh[1,2], Richard C Sallari[8] & Mathieu Lupien[1,2,9]



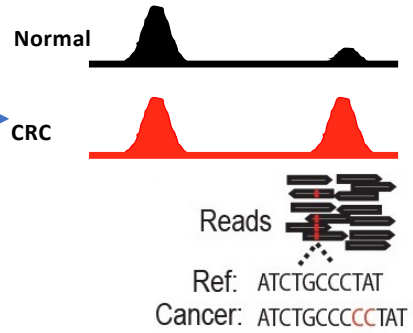
Mismatch repair-signature mutations activate gene enhancers across human colorectal cancer epigenomes

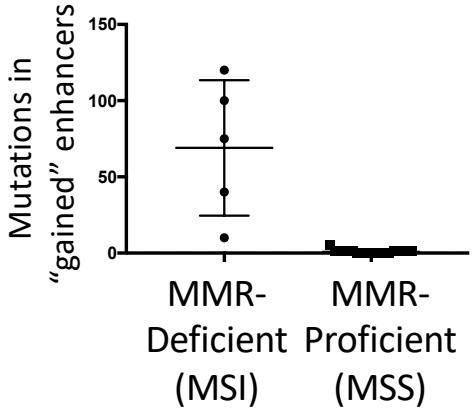
eLife 2019

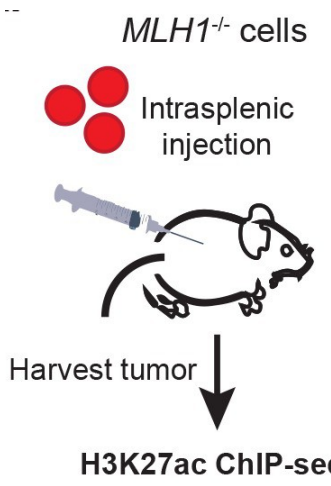
Stevephen Hung[1], Alina Saiakhova[1], Zachary J Faber[1], Cynthia F Bartels[1], Devin Neu[1], Ian Bayles[1], Evelyn Ojo[2], Ellen S Hong[1], W Dean Pontius[3], Andrew R Morton[1], Ruifu Liu[2], Matthew F Kalady[3,4,5], David N Wald[2,6], Sanford Markowitz[1,6,7], Peter C Scacheri[1,6]*

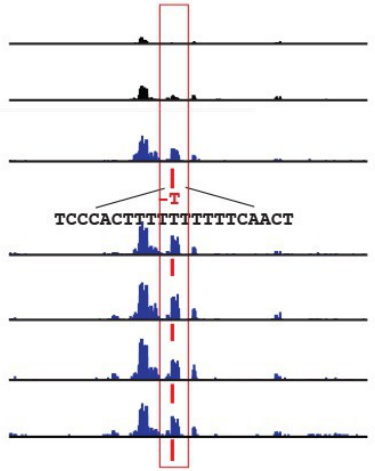


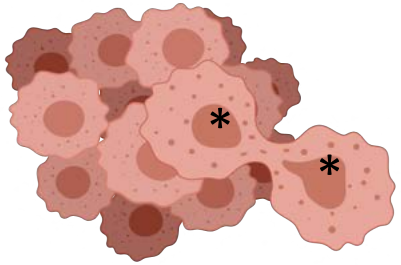
H3K27ac ChIP-seq (n = 40)









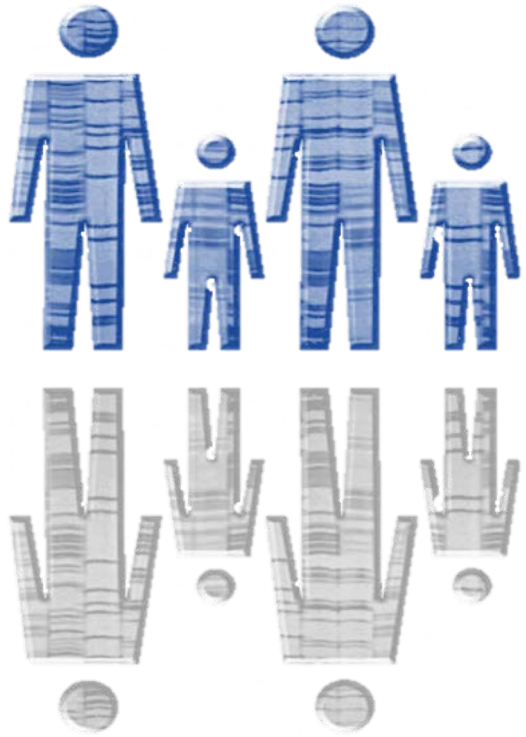


Enhancer mutation: Selective growth advantage

Studying the epigenome can provide insights into various aspects of cancer

- Tumorigenesis
- Clinical subtyping
- Metastasis
- Drug resistance
- Identification of non-coding driver mutations
- **GWAS**
- Biomarkers

GWAS: Genome Wide Association Studies



Cases

Controls

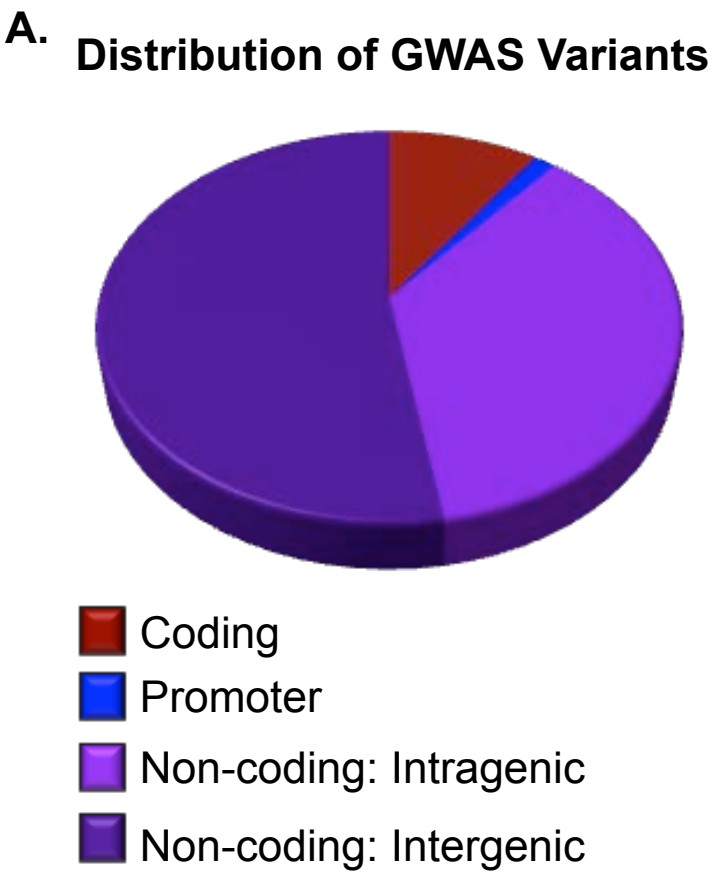
Variants associated with increased risk for disease

GWAS: Genome Wide Association Studies

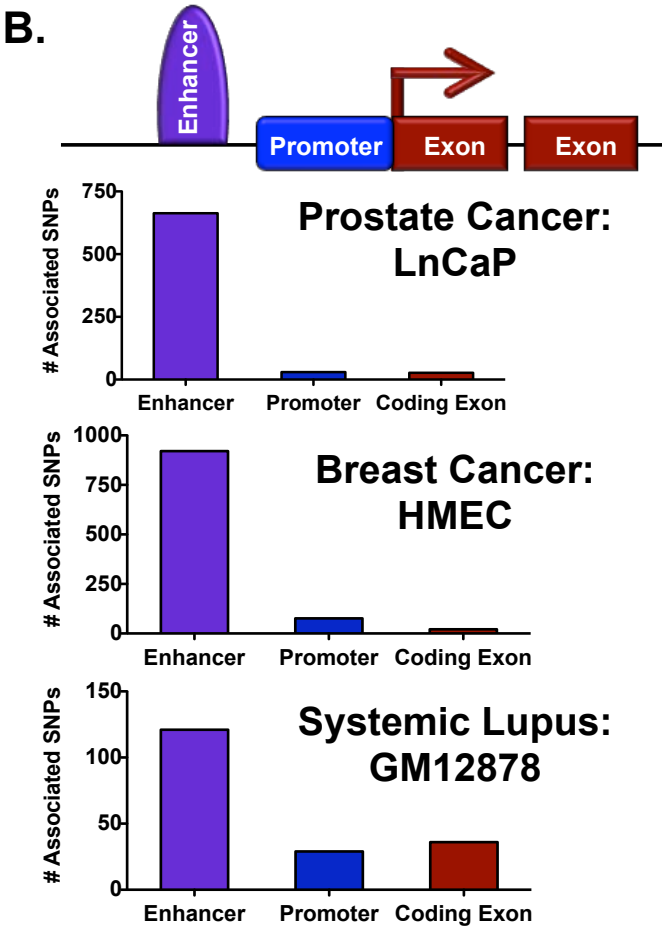
- Thousands of associations
- Most SNPs lie in non-coding regions

Transcriptional enhancer elements are hotspots for SNPs that predispose to disease

A.



B.

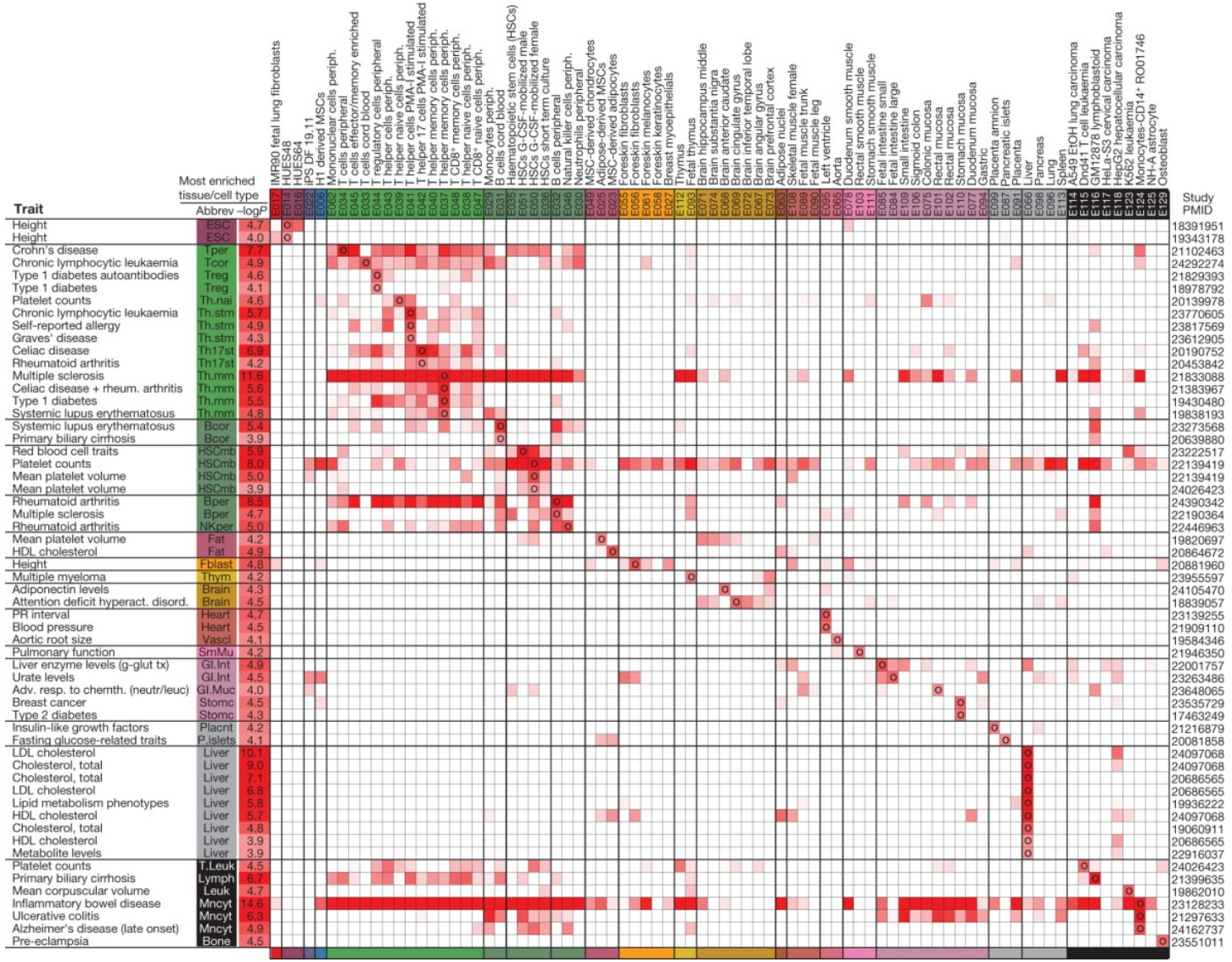


Corradin and Scacheri Genome Medicine 2014

Of the known heritability estimates from GWAS, variants in regulatory elements are estimated to account for 79%.

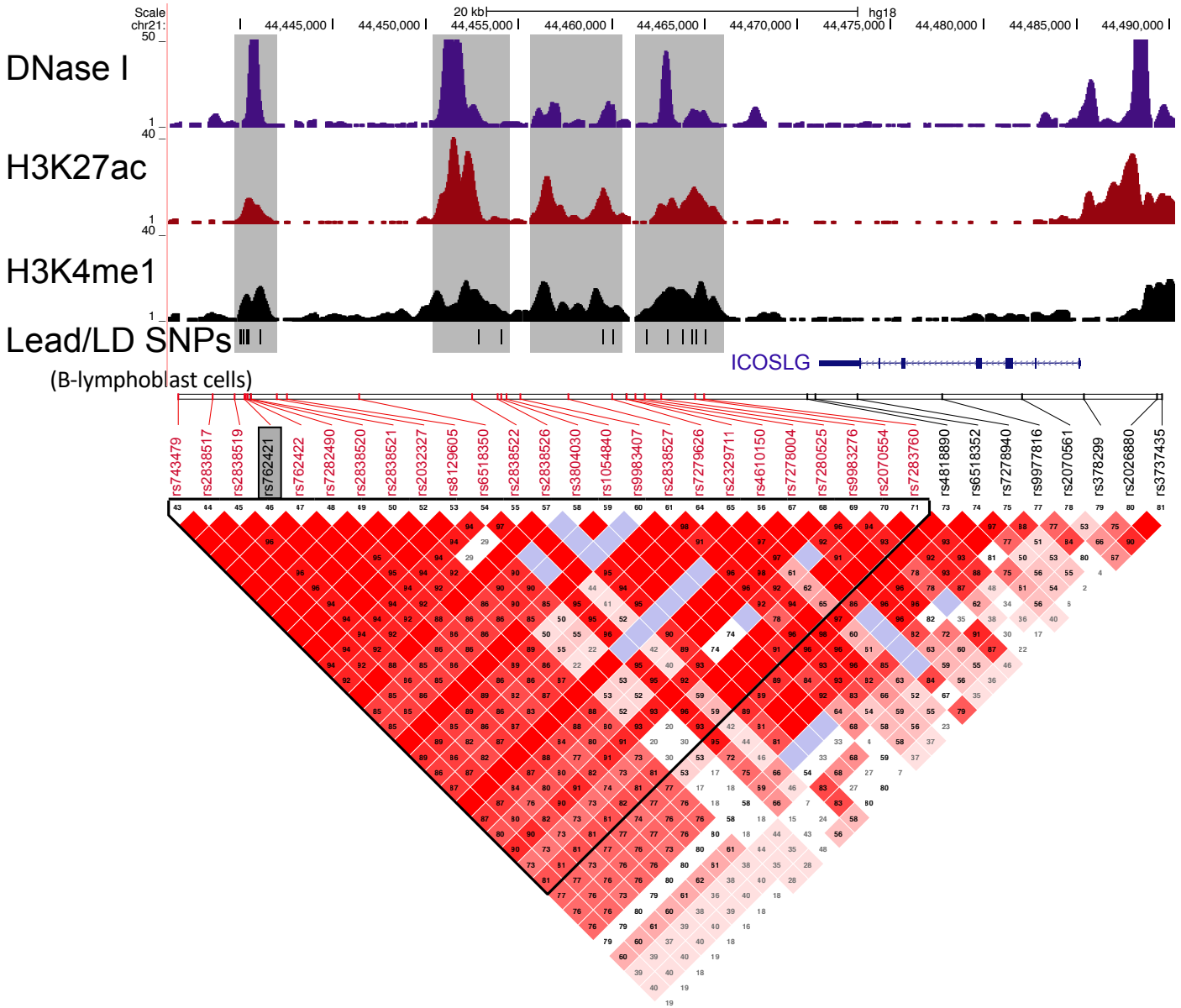
Gusev et al. AJHG 2014

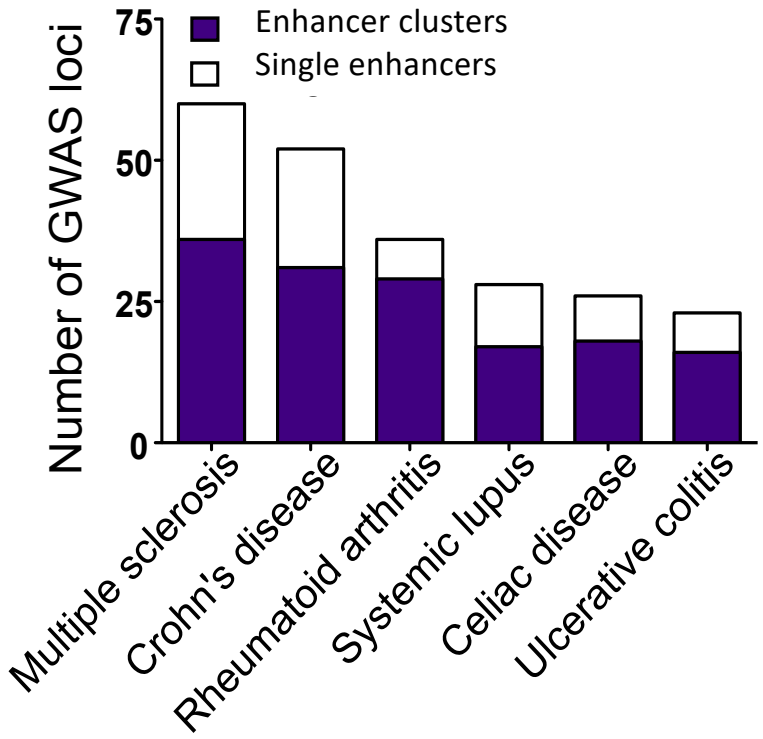
Epigenomic enrichments of genetic variants associated with diverse traits.



Roadmap Epigenomics Consortium *et al. Nature* **518**, 317-330 (2015) doi:10.1038/nature14248

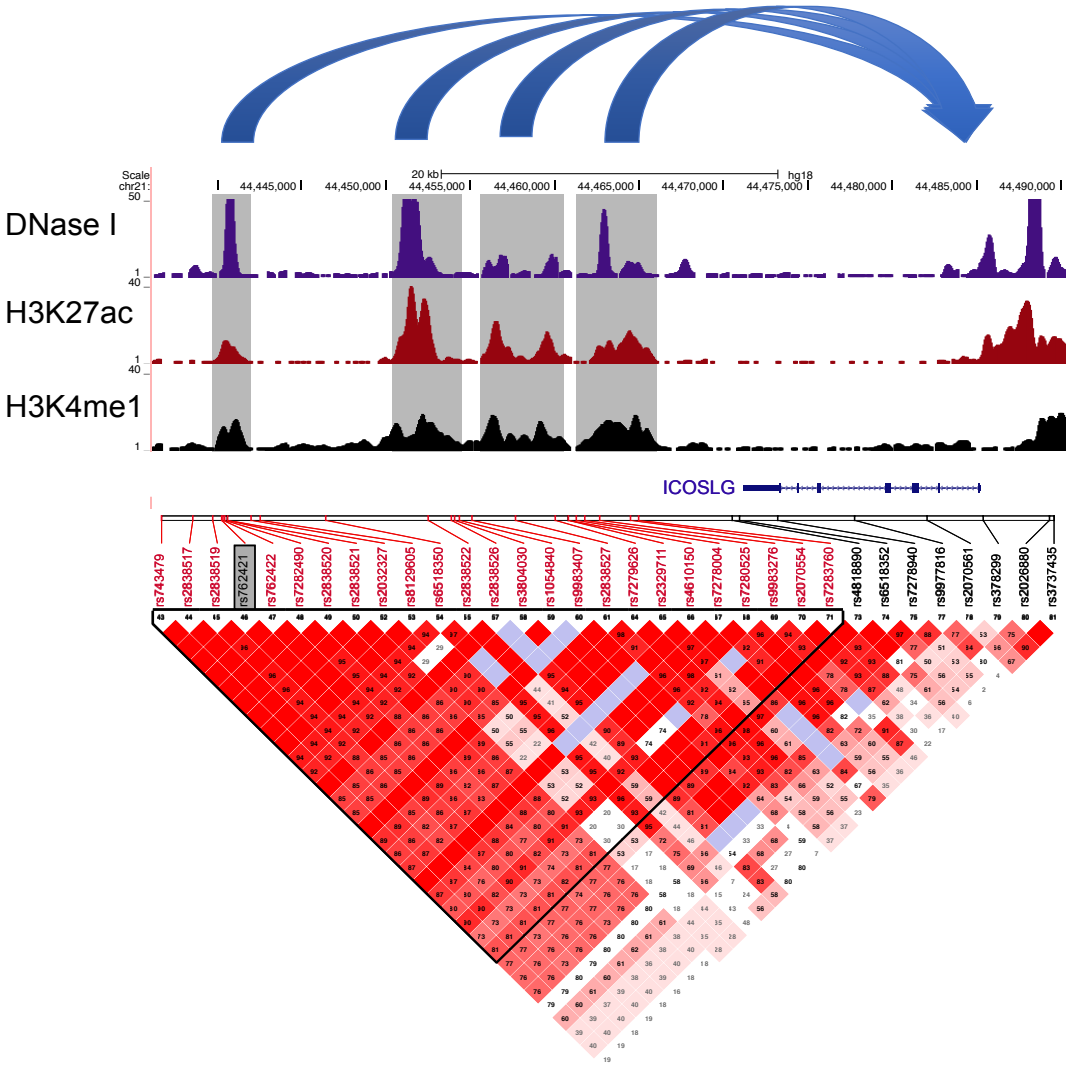
GWAS risk SNPs often lie in enhancer clusters





Hnisz et al, Cell 2013
Parker, Stitzel et al, PNAS 2013
Corradin et al. Genome Res 2014
Pasquali et al, Nat. Gen 2014

Constituents of enhancer clusters collude to regulate genes



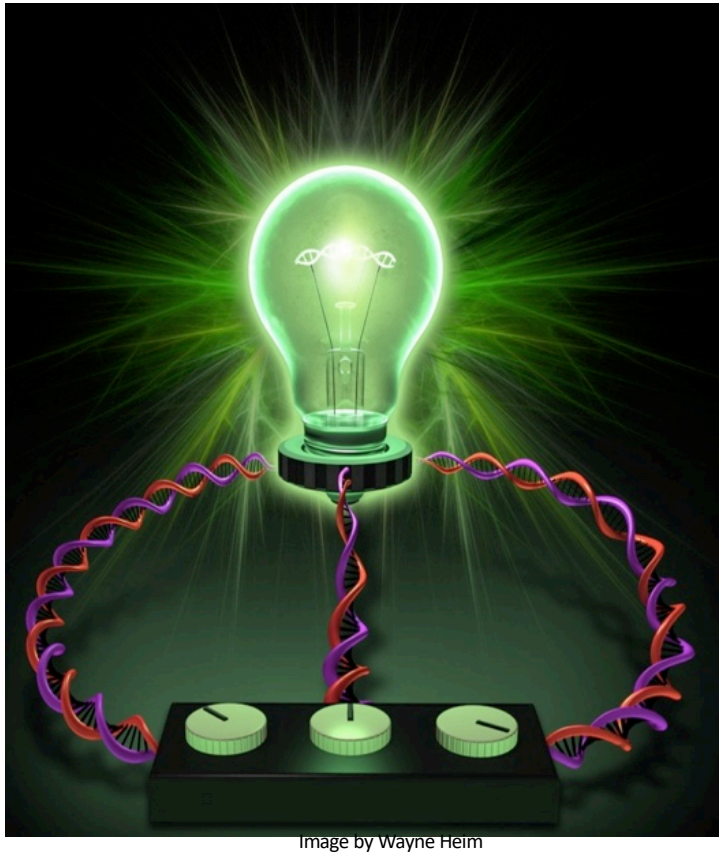
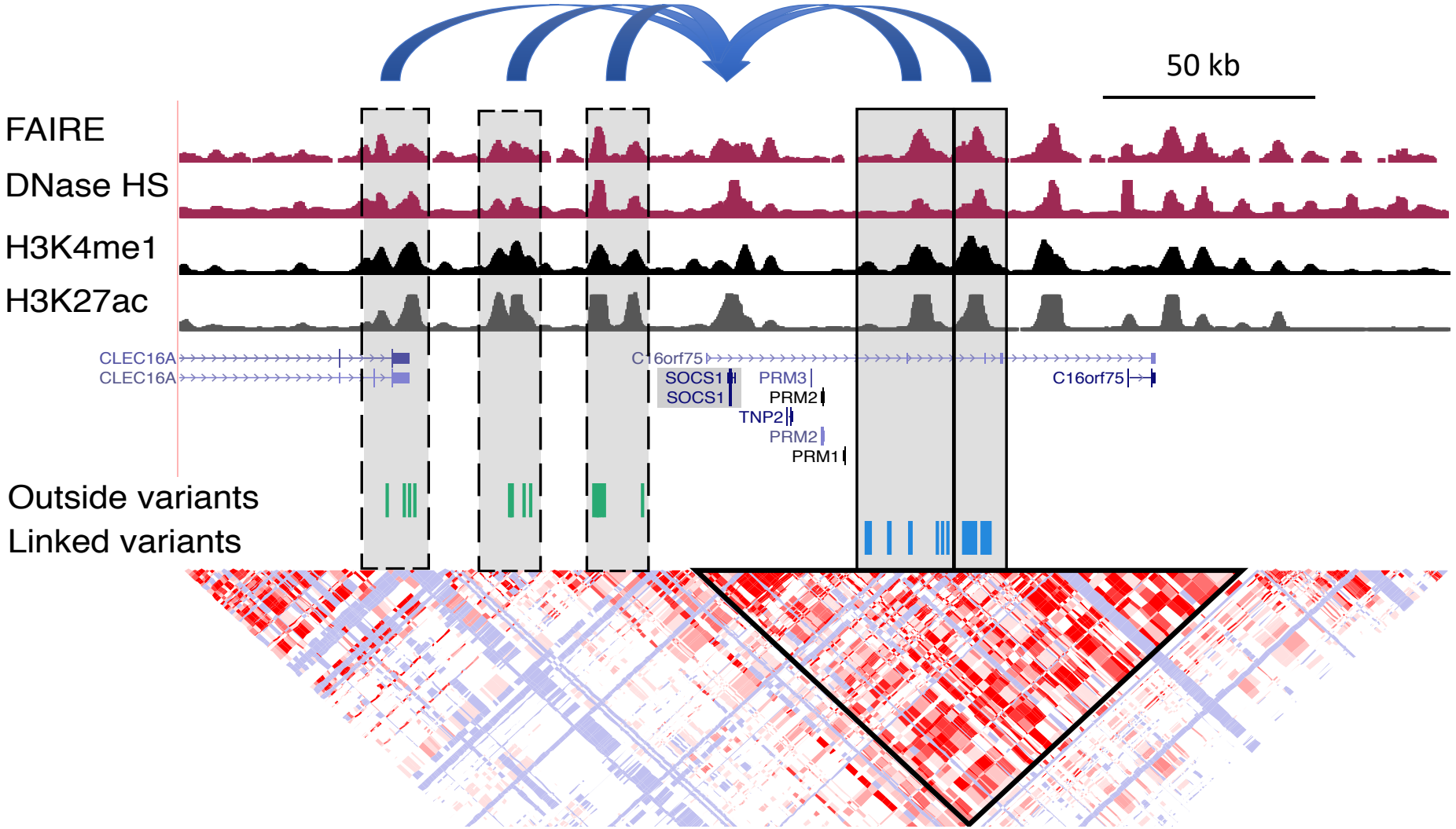
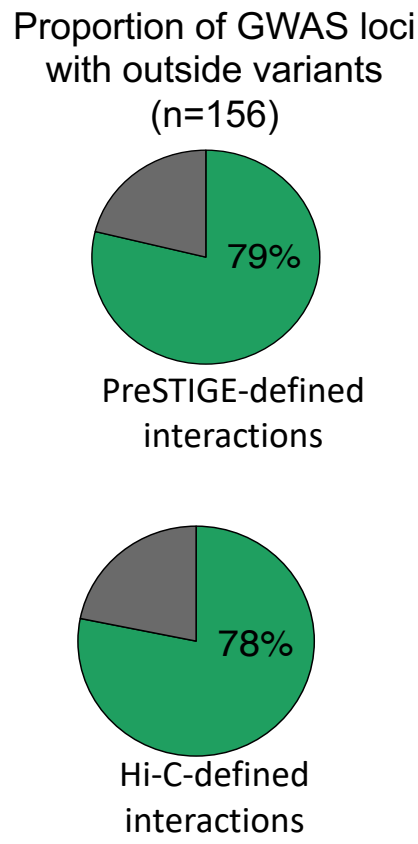


Image by Wayne Heim

May be more than 1 causal SNP at a locus!

Regulatory circuitry at GWAS loci extends beyond LD blocks

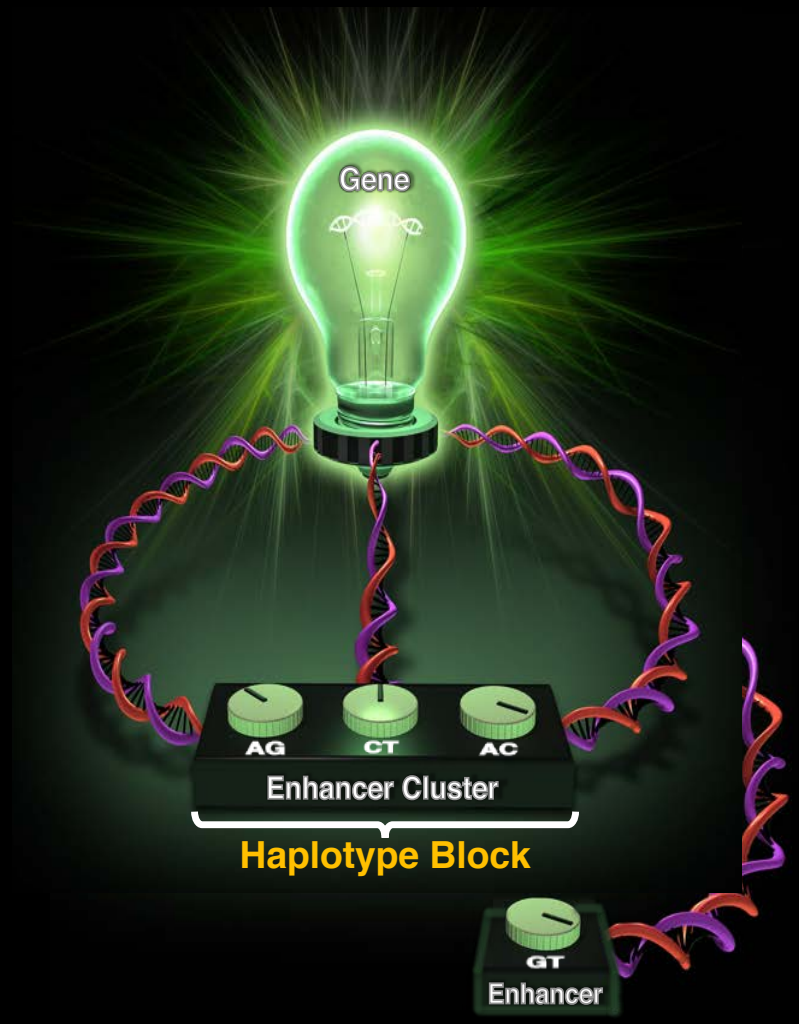




"Outside variants" – SNPs inherited independently of GWAS linked variants that are within the regulatory circuit of the same gene target

Corradin et al, *Nat Genet* 2016
Factor et al, *Cell* 2020

The Regulatory Circuitry of Gene Expression



SNPs that lie outside the GWAS-associated region, but are part of the same regulatory circuit can influence disease risk

Studying the epigenome can provide insights into various aspects of cancer

- Tumorigenesis
- Clinical subtyping
- Metastasis
- Drug resistance
- Identification of non-coding driver mutations
- GWAS
- **Biomarkers**

Sensitive tumour detection and classification using plasma cell-free DNA methylomes

Shu Yi Shen[1,12], Rajat Singhania[1,12], Gordon Fehringer[2,12], Ankur Chakravarthy[1,12], Michael H. A. Roehrl[1,3,4], Dianne Chadwick[1], Philip C. Zuzarte[5], Ayelet Borgida[2], Ting Ting Wang[1,4], Tiantian Li[1], Olena Kis[1], Zhen Zhao[1], Anna Spreafico[1], Tiago da Silva Medina[1], Yadon Wang[1], David Roulois[1,6], Ilias Ettayebi[1,4], Zhuo Chen[1], Signy Chow[1], Tracy Murphy[1], Andrea Arruda[1], Grainne M. O'Kane[1], Jessica Liu[4], Mark Mansour[4], John D. McPherson[7], Catherine O'Brien[1], Natasha Leighl[1], Philippe L. Bedard[1], Neil Fleshner[1], Geoffrey Liu[1,4,8], Mark D. Minden[1], Steven Gallinger[9,10], Anna Goldenberg[11], Trevor J. Pugh[1,4], Michael M. Hoffman[1,4,11], Scott V. Bratman[1,4], Rayjean J. Hung[2,8]* & Daniel D. De Carvalho[1,4]*

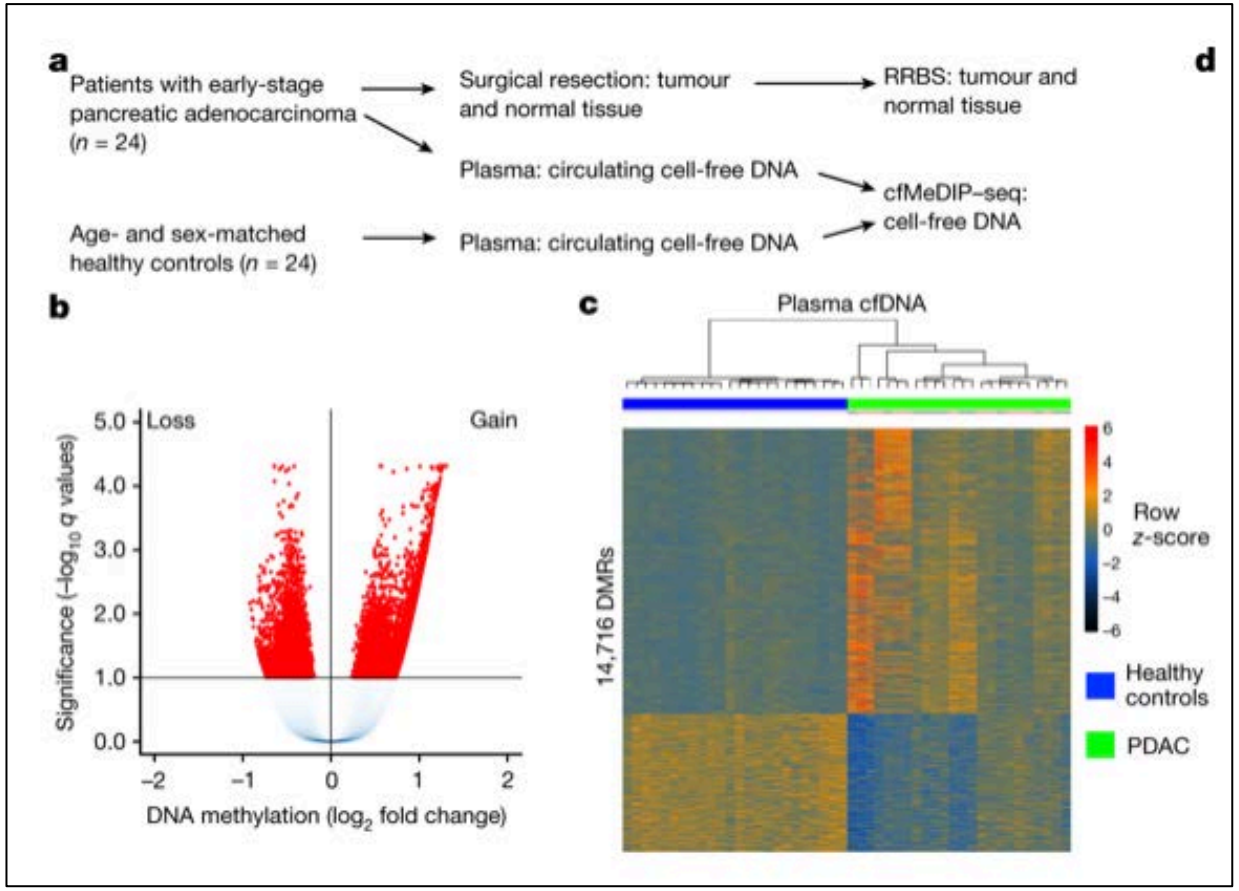
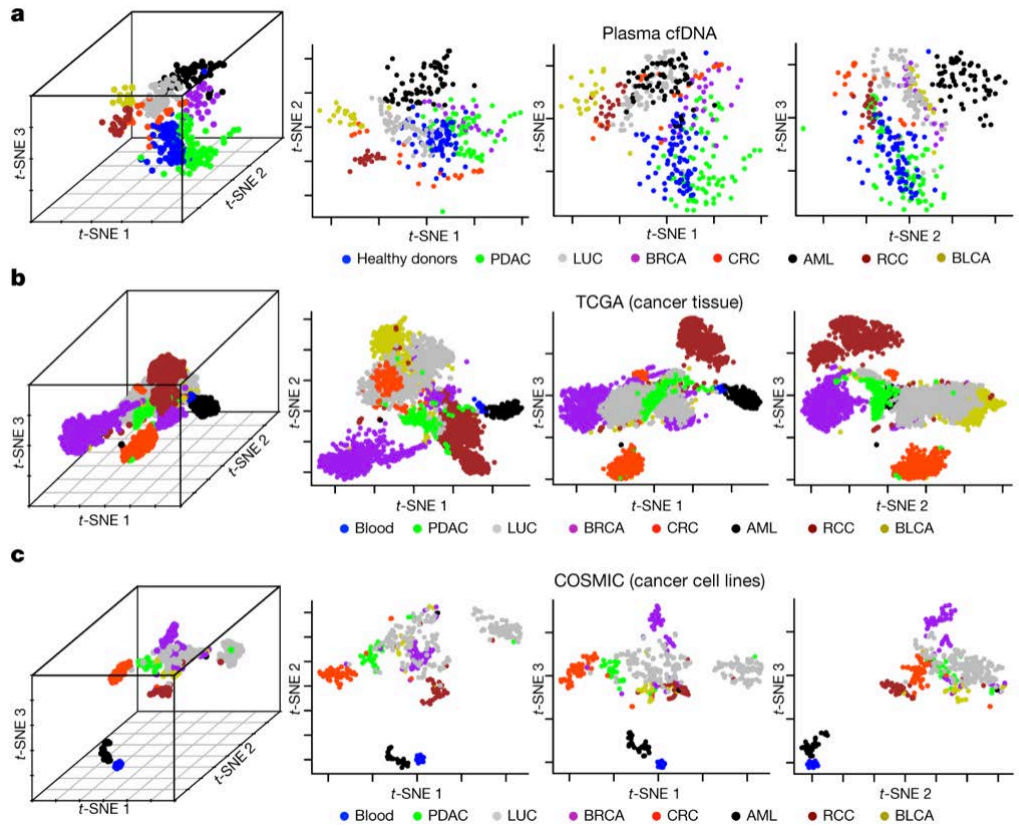


Fig. 4: Plasma-derived DMRs are informative of cancer type.

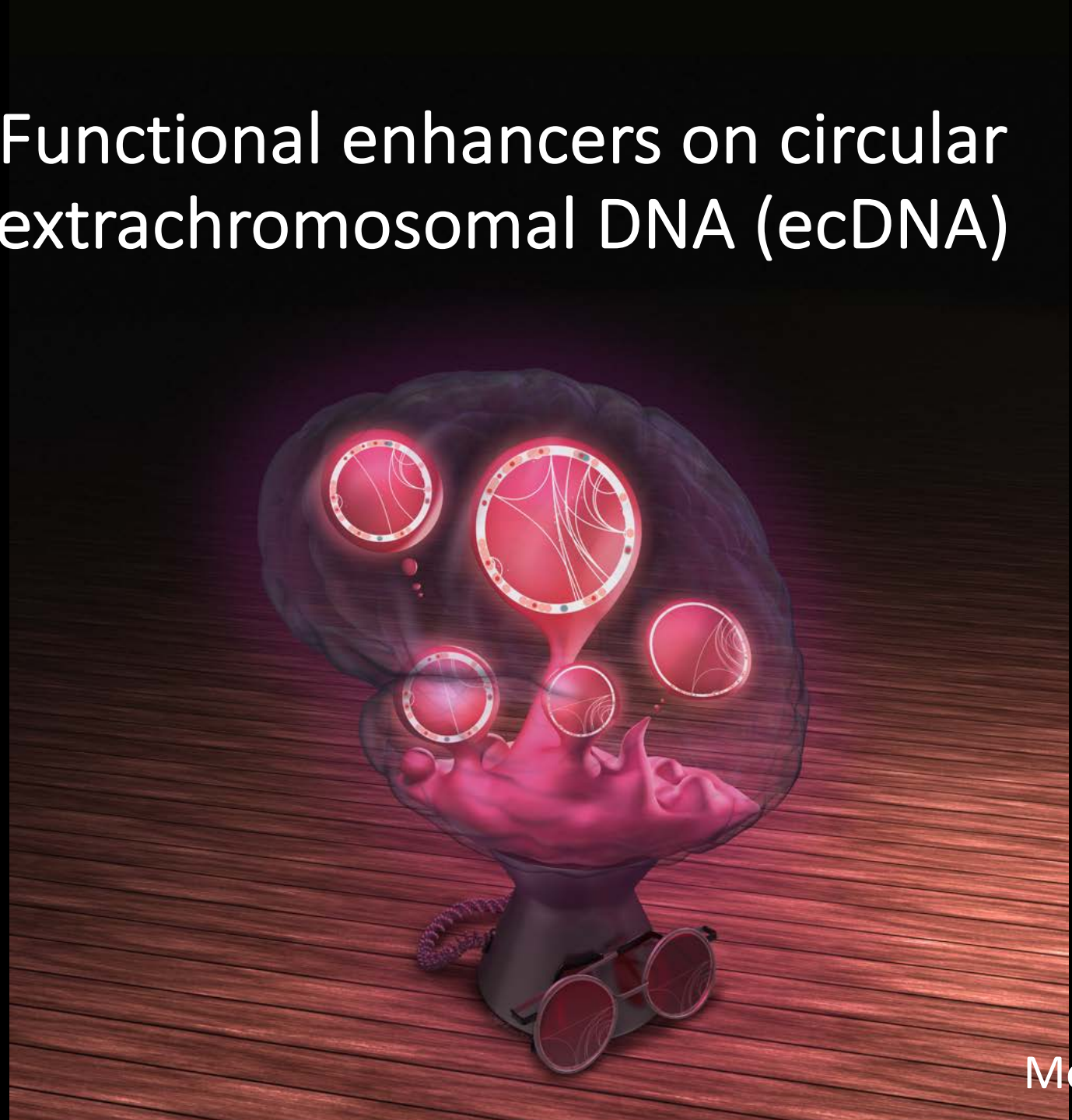
From: Sensitive tumour detection and classification using plasma cell-free DNA methylomes



Studying the epigenome can provide insights into various aspects of cancer

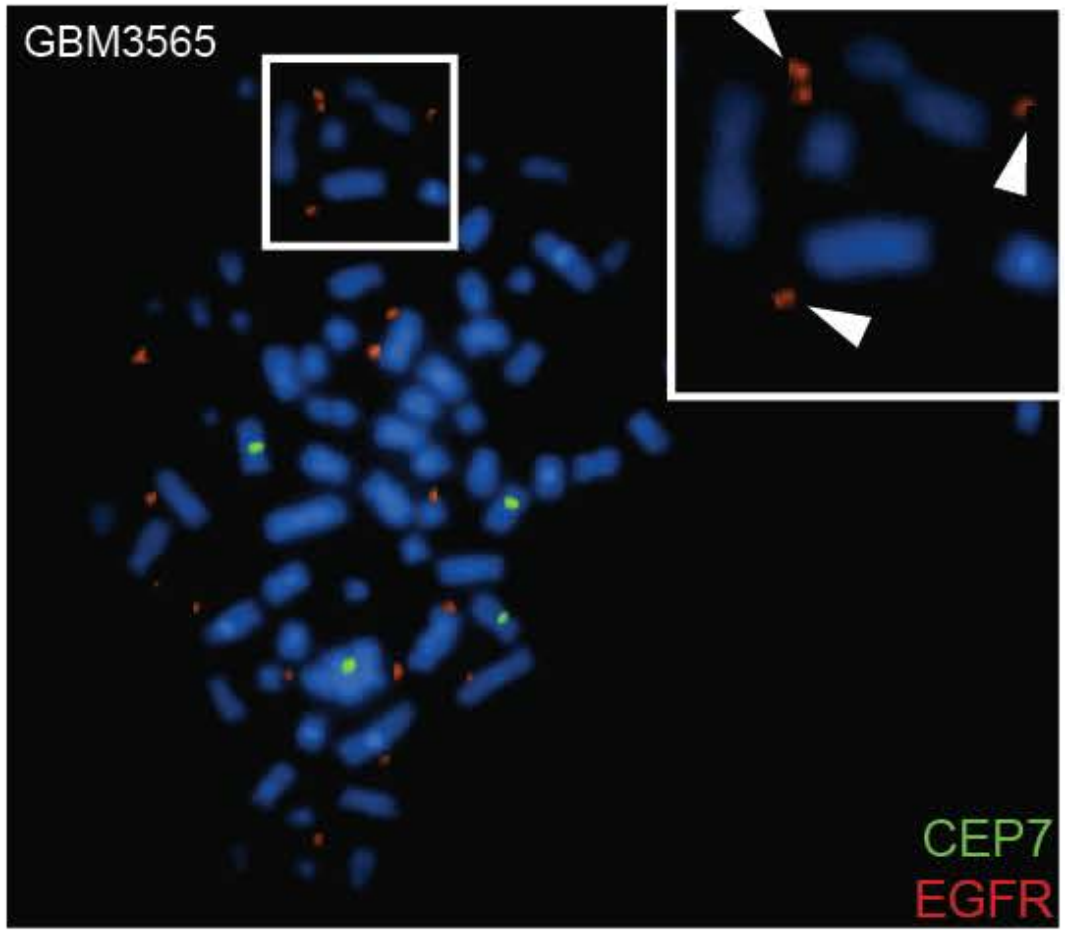
- Tumorigenesis
- Clinical subtyping
- Metastasis
- Drug resistance
- Identification of non-coding driver mutations
- GWAS
- Biomarkers
- **One final story**

Functional enhancers on circular extrachromosomal DNA (ecDNA)



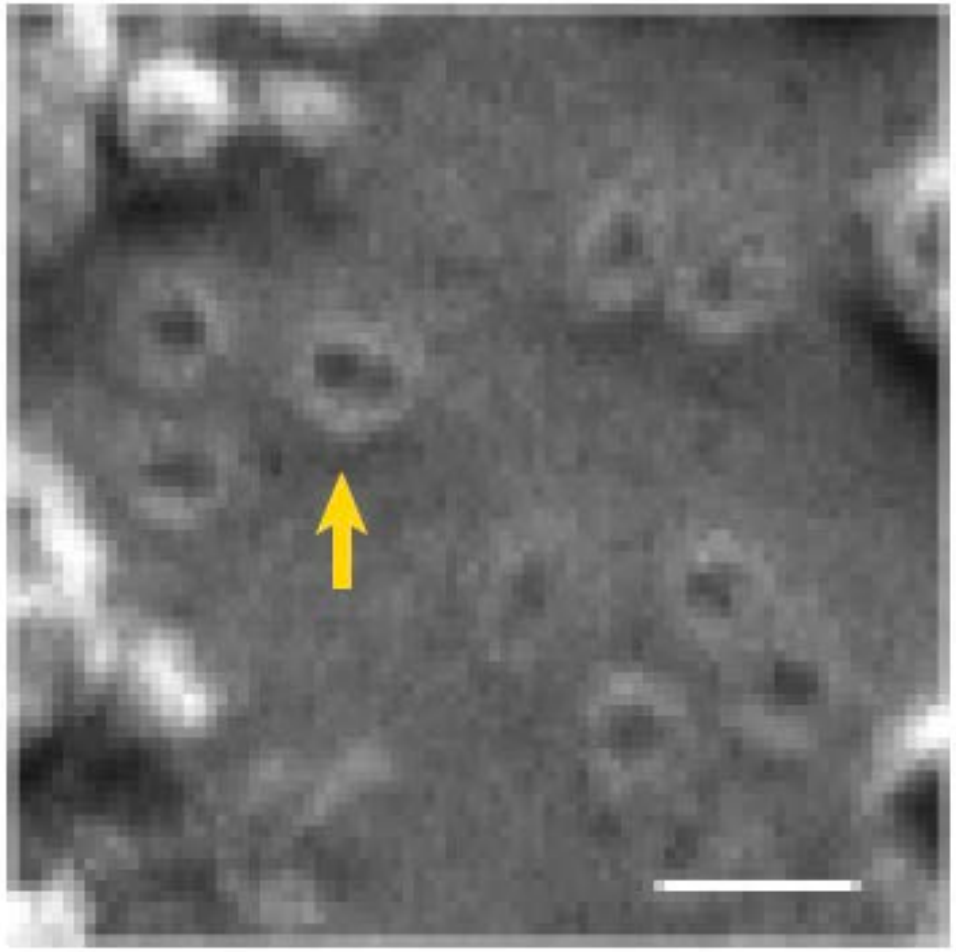
Morton et al, *Cell* 2019

DNA FISH



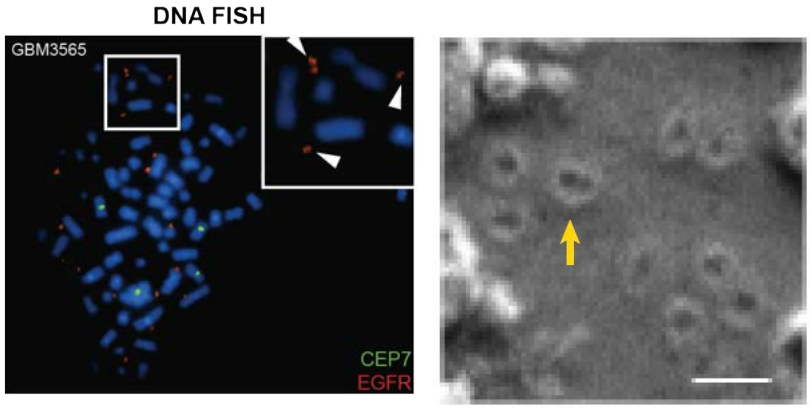
Morton et al, *Cell* 2019

Electron Micrograph



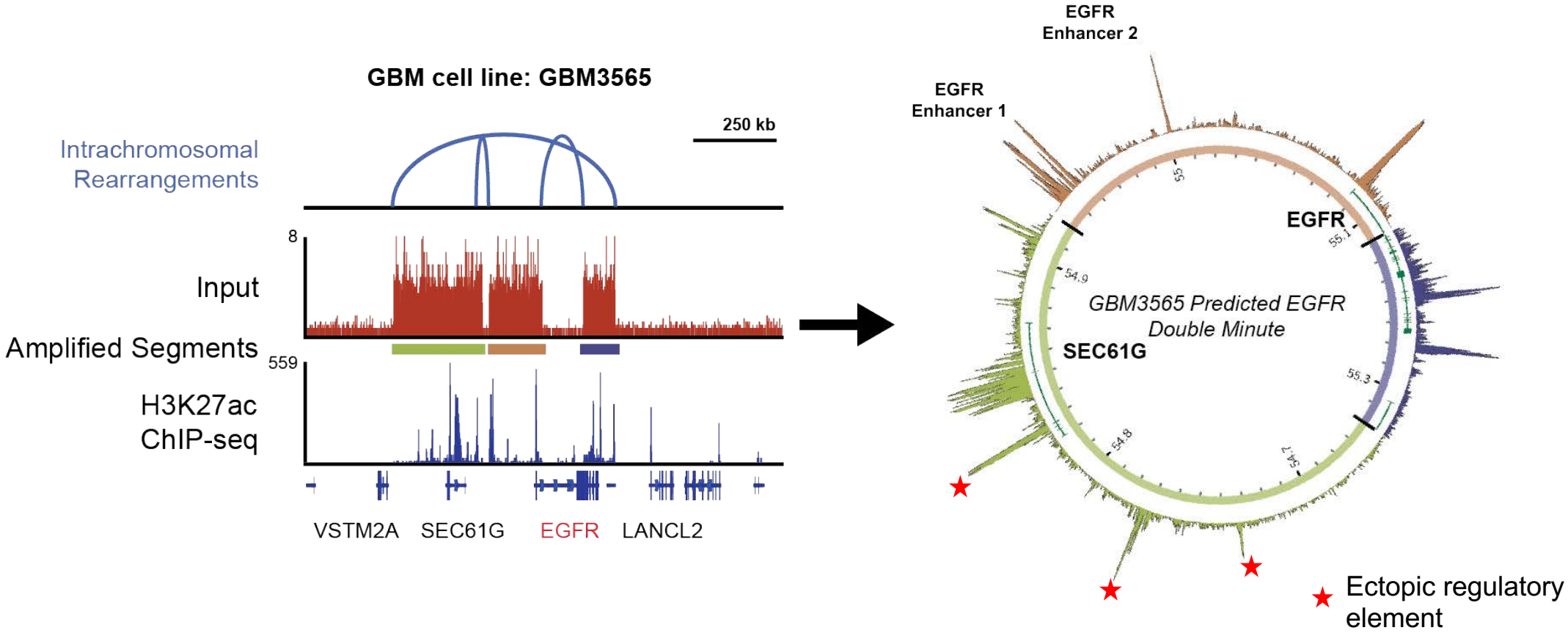
Wu et al, *Nature* 2019

Extrachromosomal DNA (ecDNA)
AKA: “double minutes”

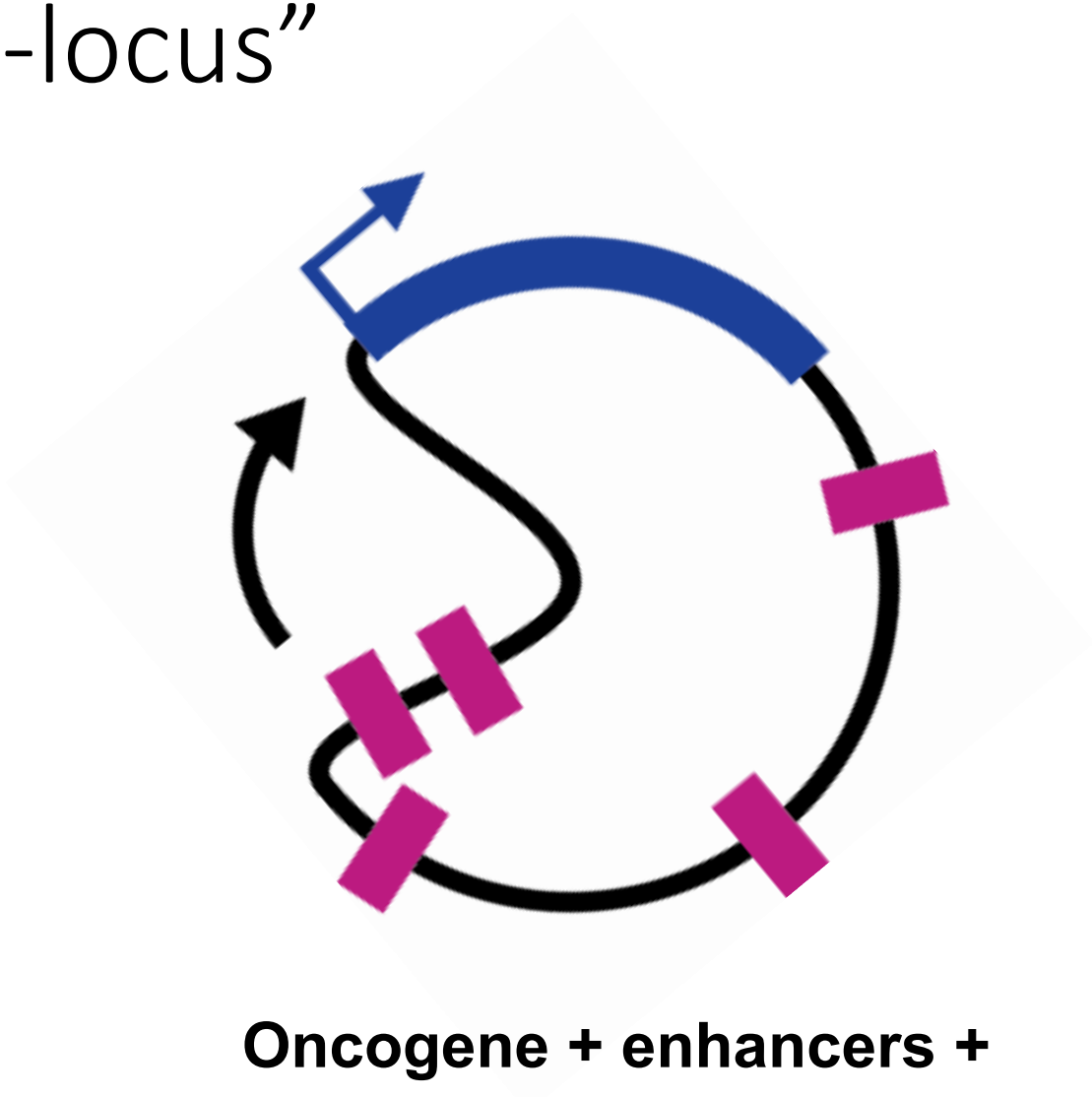


- Found in many forms of cancer.
  - Particularly prevalent in aggressive cancers notoriously difficult to treat
- Massive focal DNA amplifications
  - 10-100s of copies per cell
  - 0.5 – 2.5 Million base pairs in size
- Complex structures → can incorporate multiple oncogenes from different chromosomes
  - Extensive sub-clonality
- Can hop back into the genome and remodel it
- Provides a means for rapid tumor evolution and emergence of drug resistance phenotypes

Selection of enhancers on ecDNA



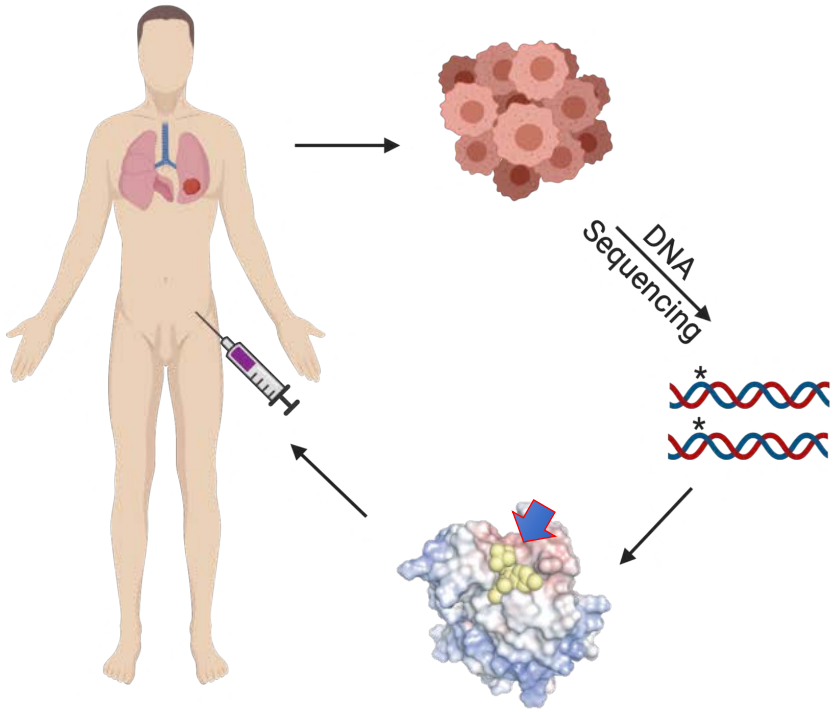
The “Onco-locus”



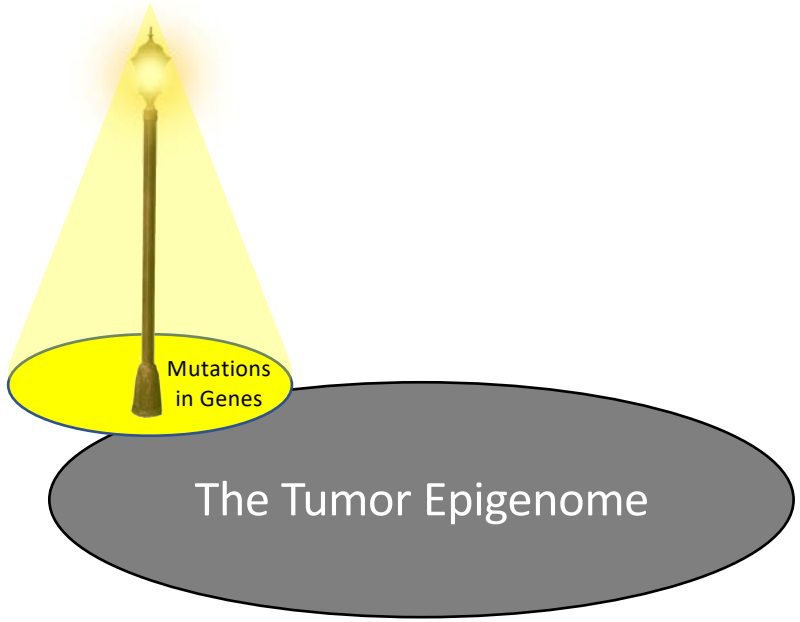
Oncogene + enhancers +
other selected elements contributing to fitness

Precision cancer medicine – A vision for an epigenomics based approach

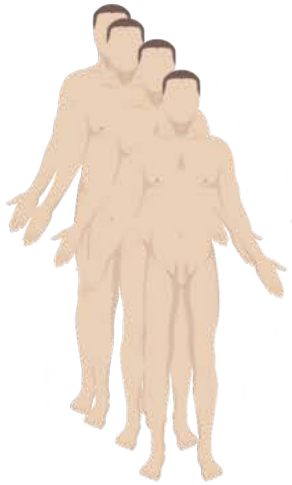
- Leverage knowledge about the genetic makeup of cancer for precisely targeted therapy.



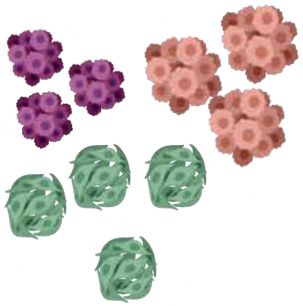
- Some success
- Most patients don't meet the clinical criteria



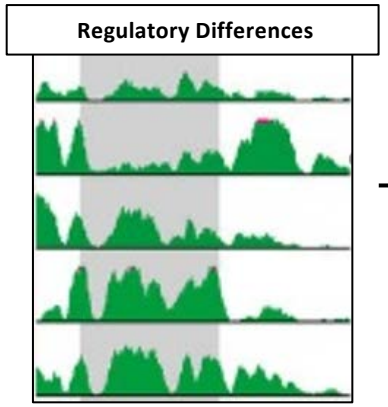
Precision cancer medicine – An epigenomics based approach



Consented Patients



Tumor Collection



Epigenomic Profiling

Clinical Integration

Deliverables

Identification of markers for prognosis, tumor behavior, and treatment response