

# CCCTC-Binding Factor (CTCF)

- Binding sites universally present in all mammalian differentially methylated regions
- Highly conserved
- Sensitive to methylation,
- Contributes to formation and structure of chromatin

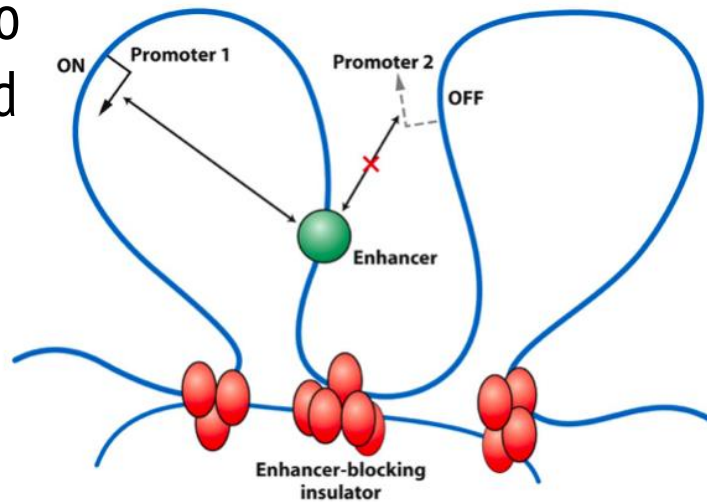
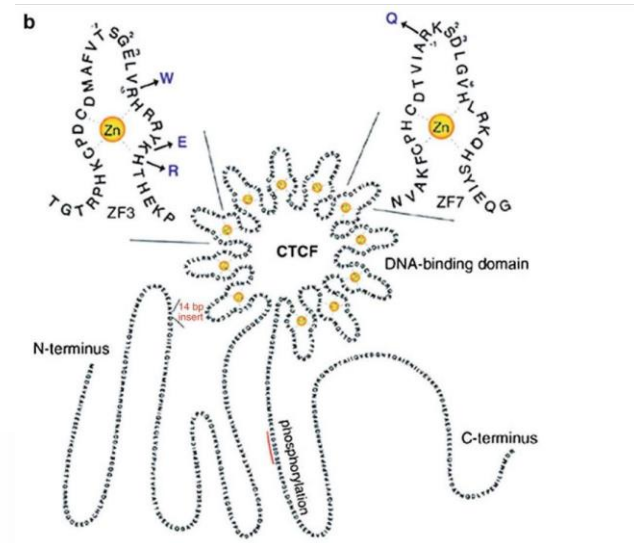
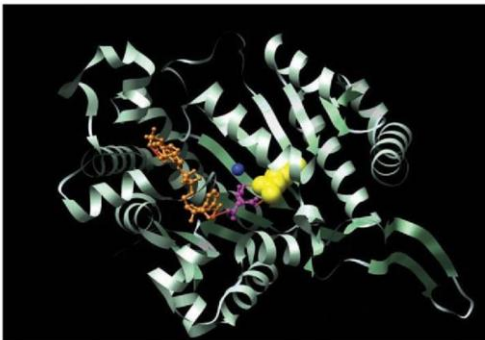


Figure 12-21

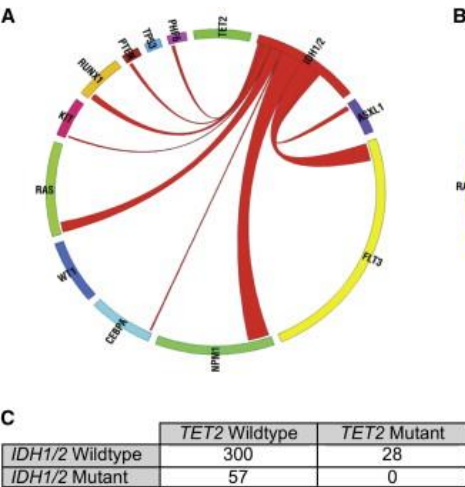


# Overview of IDH and its mutations

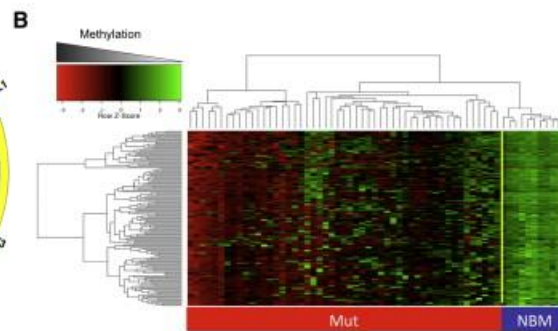
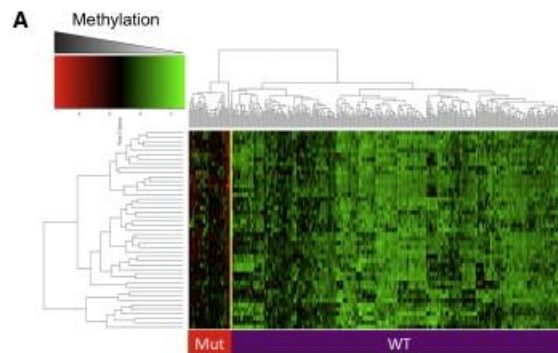
- IDH proteins involved in Krebs's cycle, where it catalyzes the production of alpha-ketoglutarate
- Some mutations of IDH lead to the production of abnormal 2-hydroxyglutarate
  - 2-HG is an inhibitor of alpha-ketoglutarate dependent enzymes, including demethylases



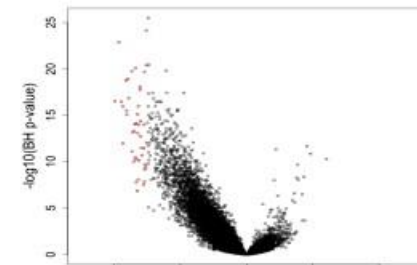
Previous work established the prevalence of DNA hypermethylation due to IDH mutations in patients with leukemia



p value: 0.009 (left-tailed Fisher test)



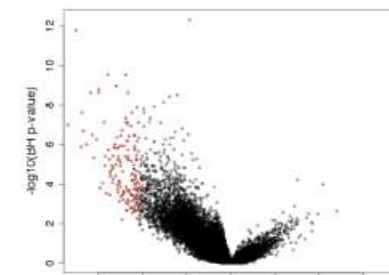
**C**



Log2 Methylation difference: IDH-mut - IDH-wt

More methylated in mutant IDH1/2

More methylated in wild type IDH1/2



Log2 Methylation Difference: IDH mutant - NBM

More methylated in mutant IDH1/2

More methylated in NBM

**D**

**Table 2.** Most frequently altered GBM *CAN*-genes. All *CAN*-genes are listed in table S7.

Gene	Point mutations*		Amplifications†		Homozygous deletions‡		Fraction of tumors with any alteration (%)	Passenger probability‡
	No. of tumors	Fraction of tumors (%)	No. of tumors	Fraction of tumors (%)	No. of tumors	Fraction of tumors (%)		
CDKN2A	0/22	0	0/22	0	11/22	50	50	<0.01
TP53	37/105	35	0/22	0	1/22	5	40	<0.01
EGFR	15/105	14	5/22	23	0/22	0	37	<0.01
PTEN	27/105	26	0/22	0	1/22	5	30	<0.01
NF1	16/105	15	0/22	0	0/22	0	15	0.04
CDK4	0/22	0	3/22	14	0/22	0	14	<0.01
RB1	8/105	8	0/22	0	1/22	5	12	0.02
IDH1	12/105	11	0/22	0	0/22	0	11	<0.01
PIK3CA	10/105	10	0/22	0	0/22	0	10	0.10
PIK3R1	8/105	8	0/22	0	0/22	0	8	0.10

Table of gene alterations in glioblastoma

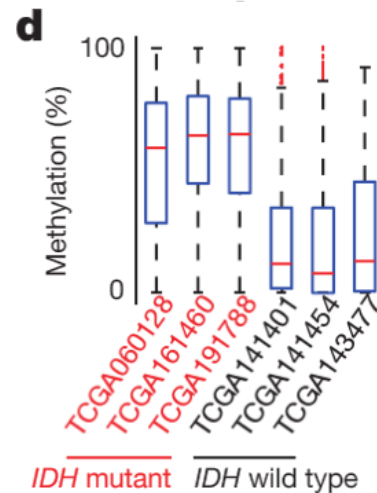
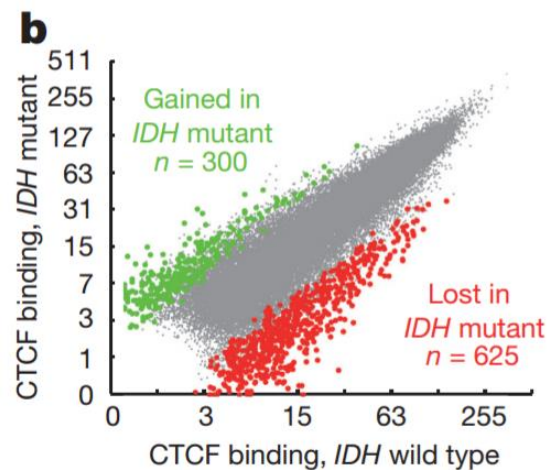
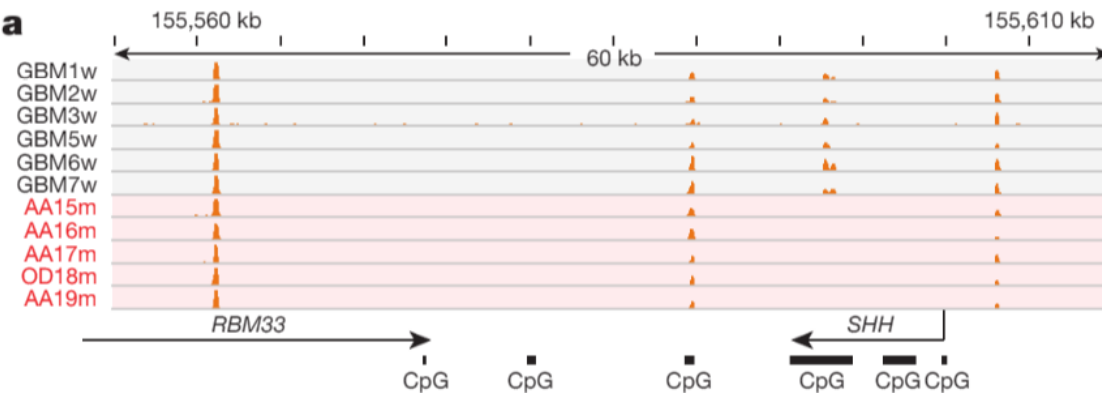
Survival Rates of patients  
With GBM IDH1 mutations

Significant because a significant  
portion of young patients have  
IDH1 mutations, and were  
associated with an increase in  
overall survival

Patient ID	Patient age (years)*	Sex	Recurrent GBM†	Secondary GBM‡	Overall survival (years)§	IDH1 mutation		Mutation of TP53	Mutation of PTEN, RB1, EGFR, or NF1
						Nucleotide	Amino acid		
Br10P	30	F	No	No	2.2	G395A	R132H	Yes	No
Br11P	32	M	No	No	4.1	G395A	R132H	Yes	No
Br12P	31	M	No	No	1.6	G395A	R132H	Yes	No
Br104X	29	F	No	No	4.0	C394A	R132S	Yes	No
Br106X	36	M	No	No	3.8	G395A	R132H	Yes	No
Br122X	53	M	No	No	7.8	G395A	R132H	No	No
Br123X	34	M	No	Yes	4.9	G395A	R132H	Yes	No
Br237T	26	M	No	Yes	2.6	G395A	R132H	Yes	No
Br211T	28	F	No	Yes	0.3	G395A	R132H	Yes	No
Br27P	32	M	Yes	Yes	1.2	G395A	R132H	Yes	No
Br129X	25	M	Yes	Yes	3.2	C394A	R132S	No	No
Br29P	42	F	Yes	Unknown	Unknown	G395A	R132H	Yes	No
IDH1 mutant patients (n=12)	33.2	67% M	25%	42%	3.8	100%	100%	83%	0%
IDH1 wild-type patients (n=93)	53.3	65% M	16%	1%	1.1	0%	0%	27%	60%

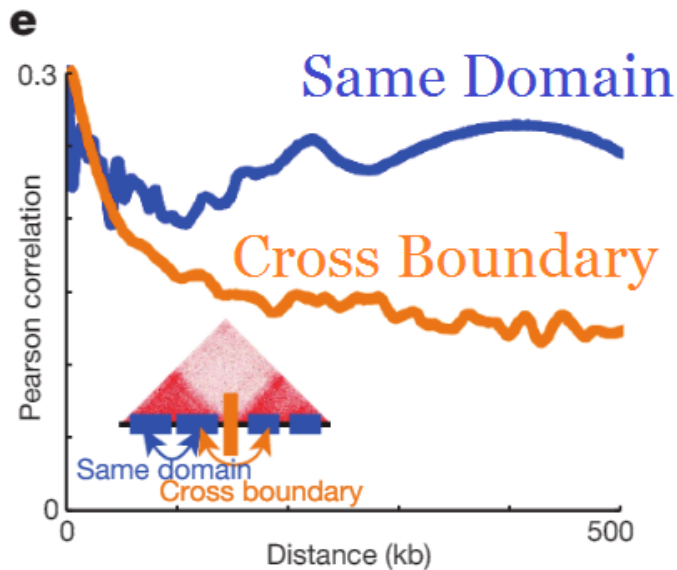
# CTCF Binding and Gene Insulation

- Used ChIP-Seq to map CTCF binding for loci in *IDH* mutant (red) vs wild type (black) tumors

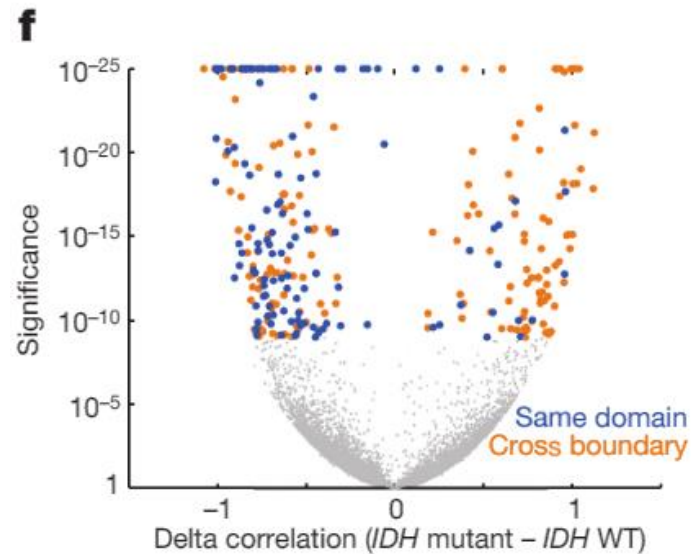


- Used WGBS data to determine methylation of loci with reduced CTCF binding

# Effect of Hypermethylation on Boundaries



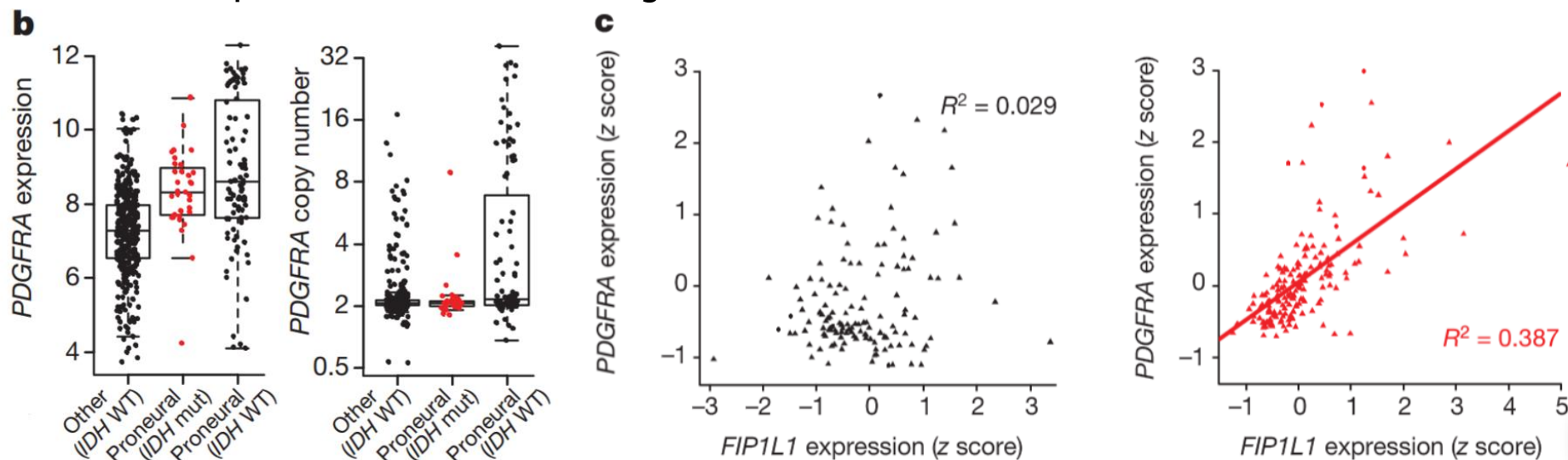
- Used Hi-C to determine TAD boundaries
- Gene correlation for normal brain tissue samples



- *IDH* mutant gliomas exhibit inverse effect (stronger correlation across boundaries)

# Topological Domain Boundaries

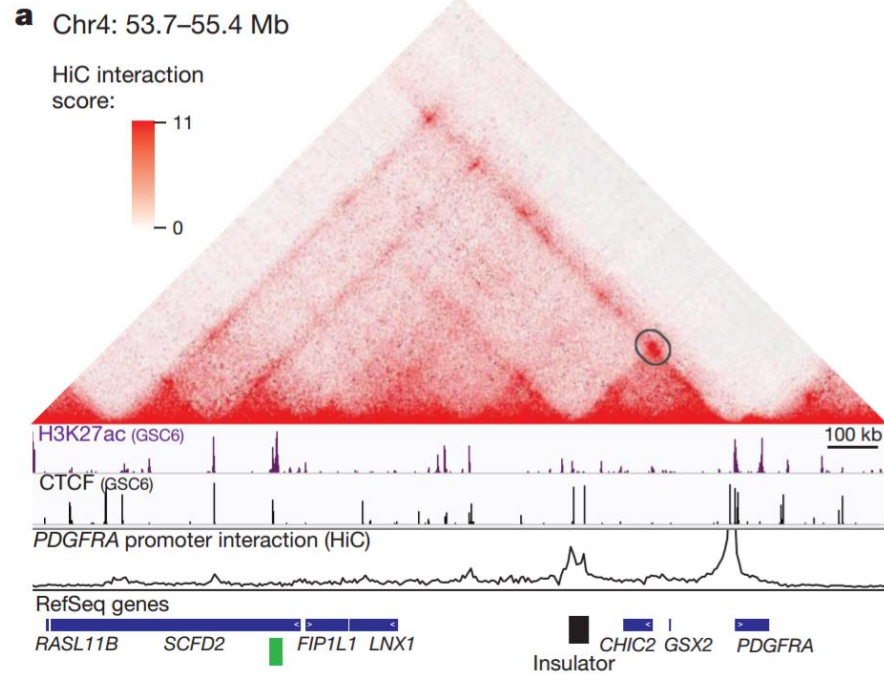
- Pinpointed boundaries disrupted by *IDH* mutations- 203
- *IDH* mutants have boundaries with higher DNA methylation and lower CTCF binding compared to wild-type tumours
- Gliomagenesis: *PDGFRA* one of the genes in the top scoring domains of overexpression in *IDH* mutant gliomas





# PDGFRA

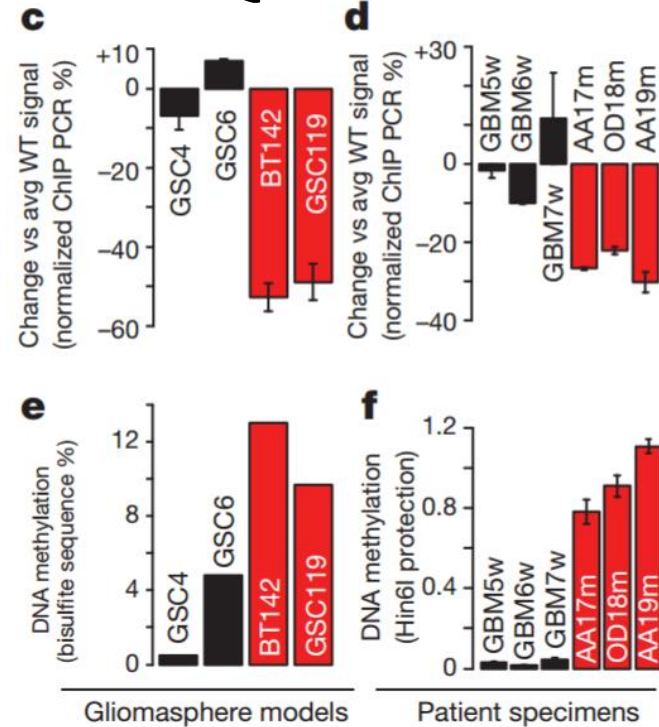
- HiC data (kb) to investigate topology



- H3K27ac = enhancer-associated (*FIP1L1*)

- Used ChIP-Seq data to confirm boundary contains a CTCF motif with a CpG dinucleotide

- Quantitative ChIP-PCR

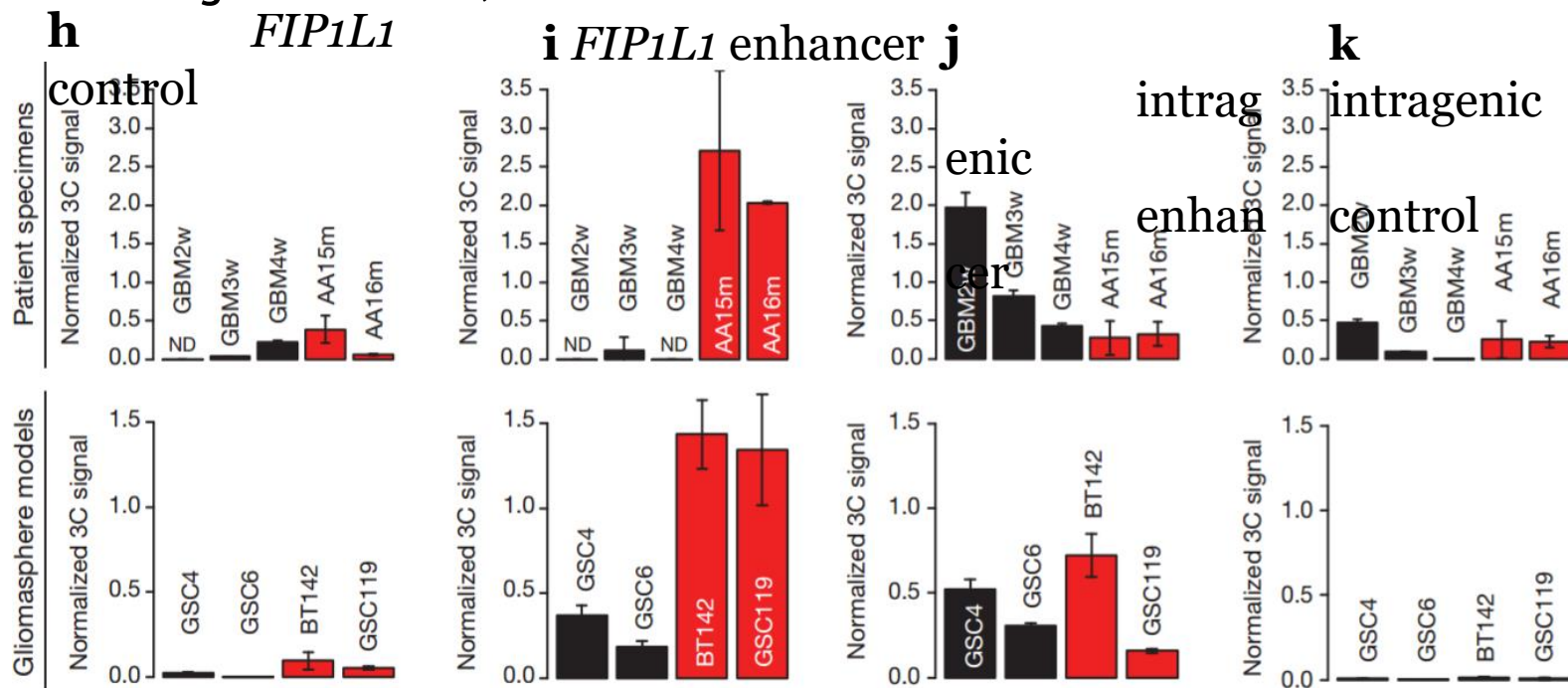


- Change in CTCF binding over
- DNA methylation at CTCF-motif

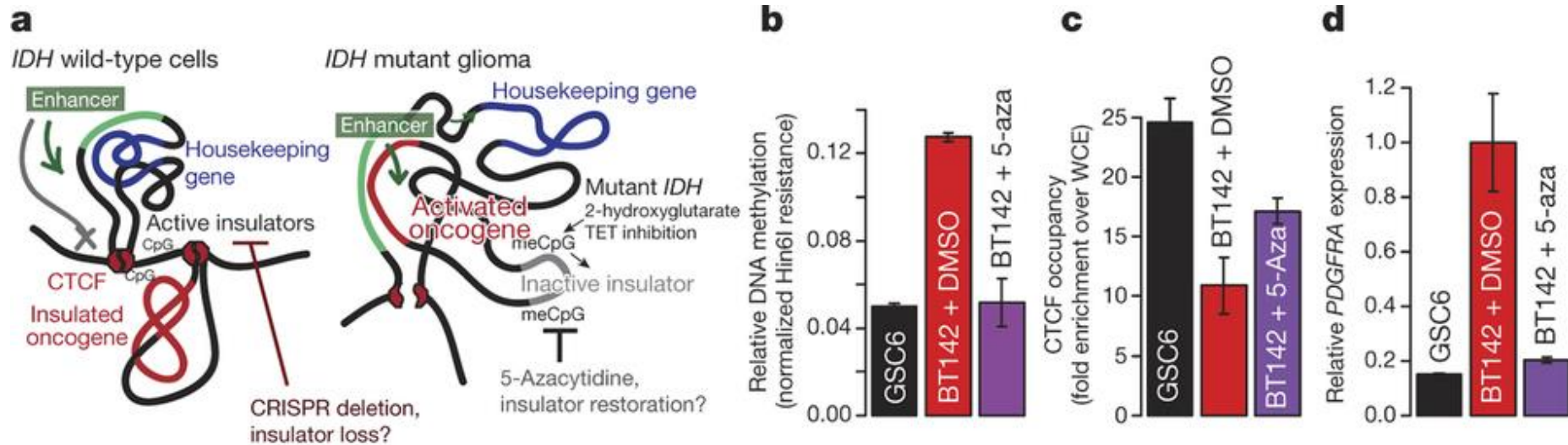


# *PDGFRA*: Identifying Regulatory Elements

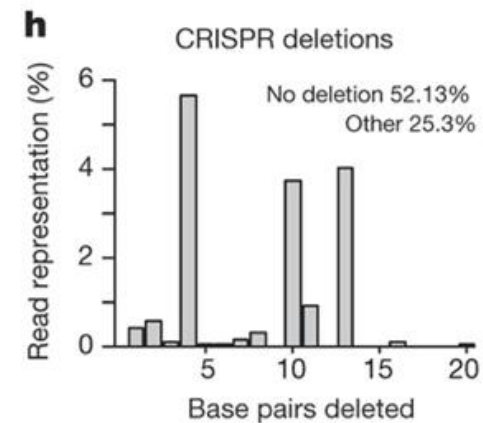
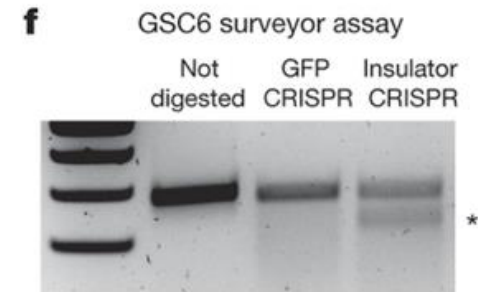
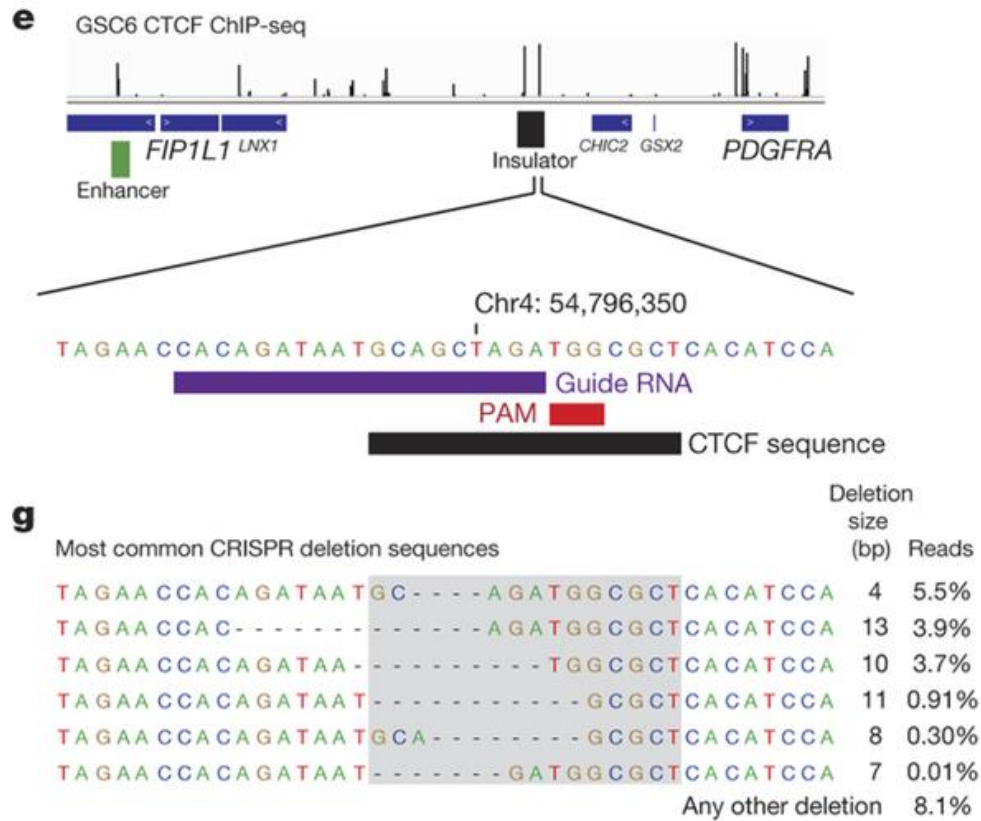
- Enhancer upstream of *FIP1L1* - strong acetylation
- Used Chromosome Conformation Capture (3C)
- Intragenic: ~50kb; *FIP1L1*: ~900kb



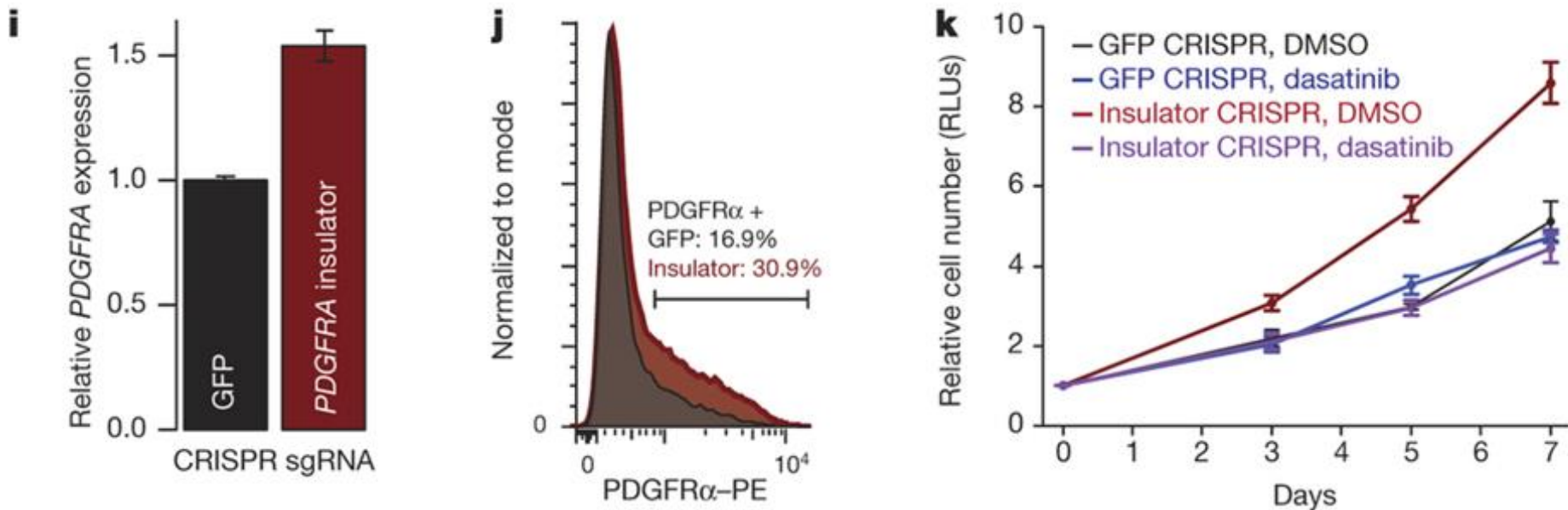
# Removing Methylation Causes Effect Reversal



# Insulator CRISPR Deletion



# Insulator Deletion Results in PDGFR $\alpha$ Expression



# Research with Potential Implications



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## CTCF/cohesin-binding sites are frequently mutated in cancer

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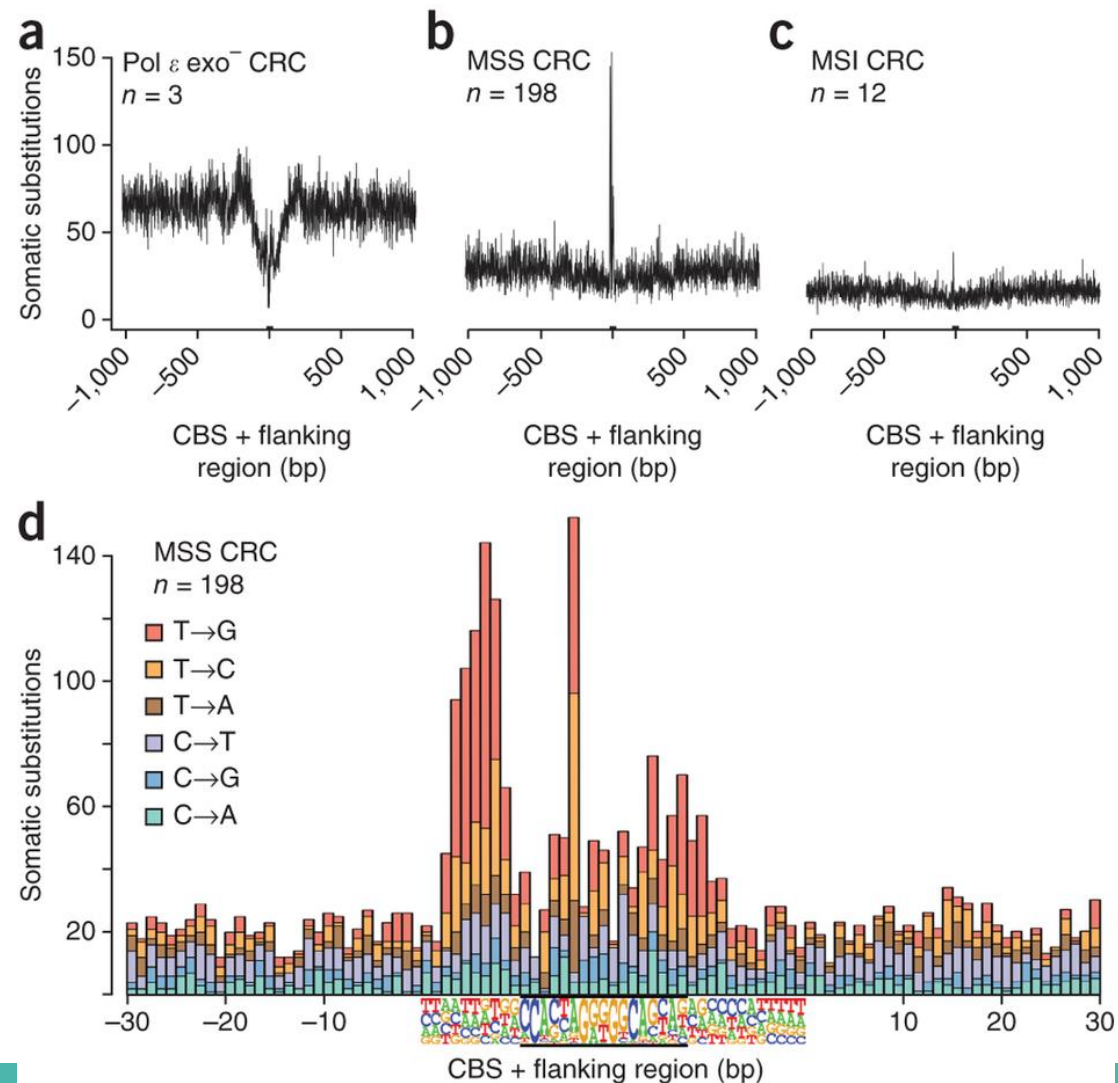
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# Mutations at CTCF/cohesin sites

Mainly mutations to C and G

Occurs most at sites bound by CTCF and cohesin

Not due to  $\text{POL}\epsilon$  proofreader



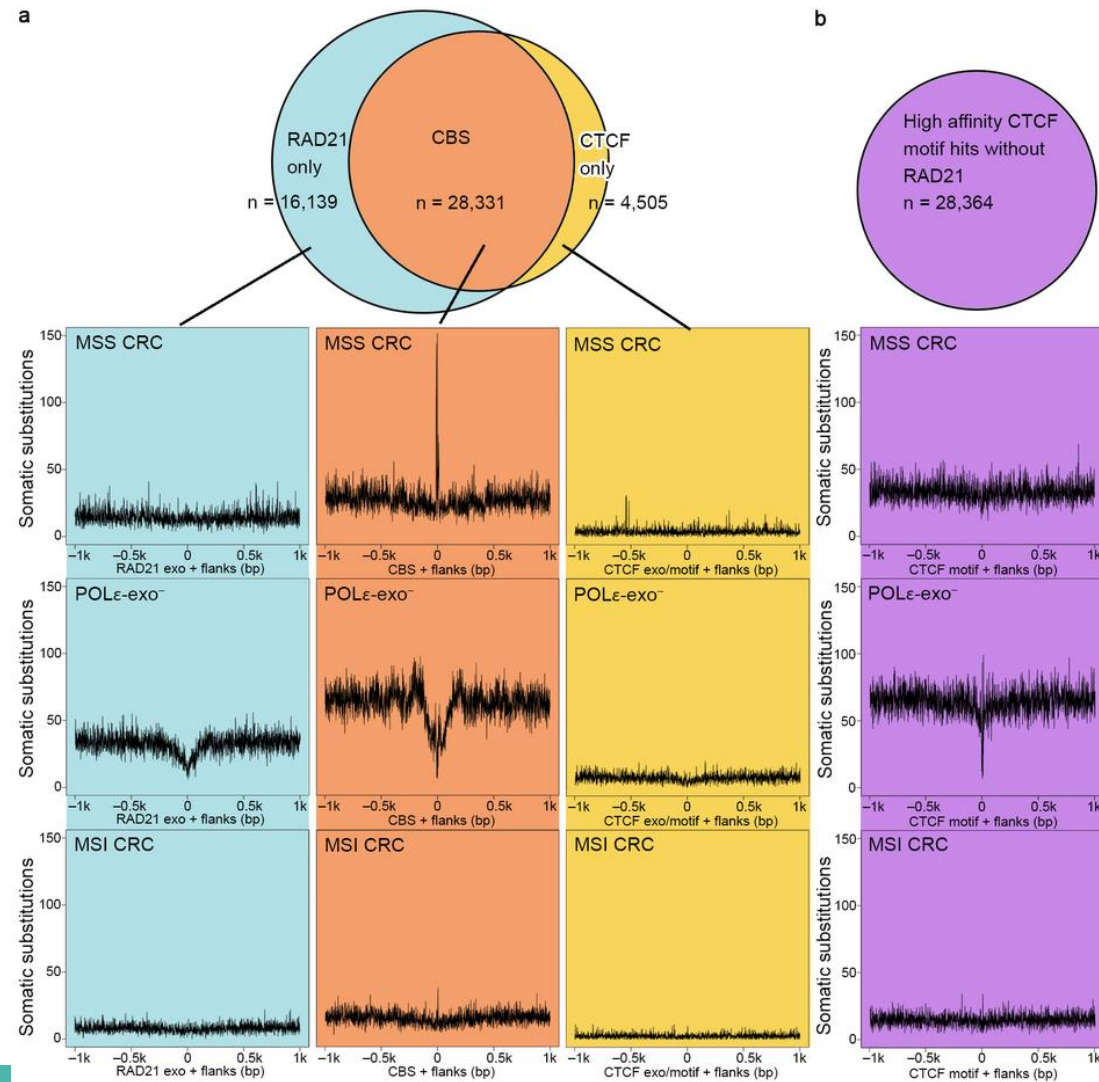


# Mutations at CTCF/cohesin sites

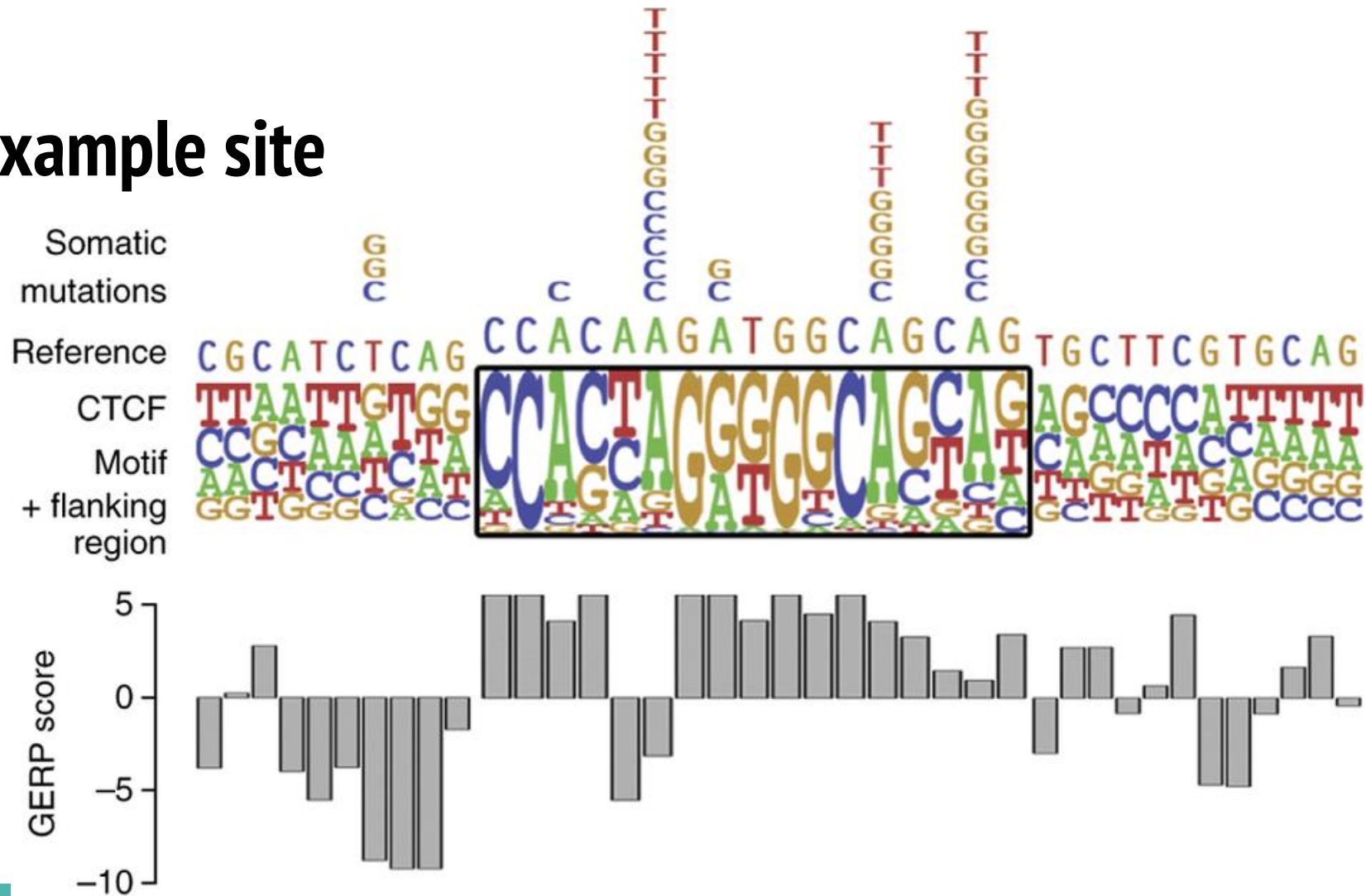
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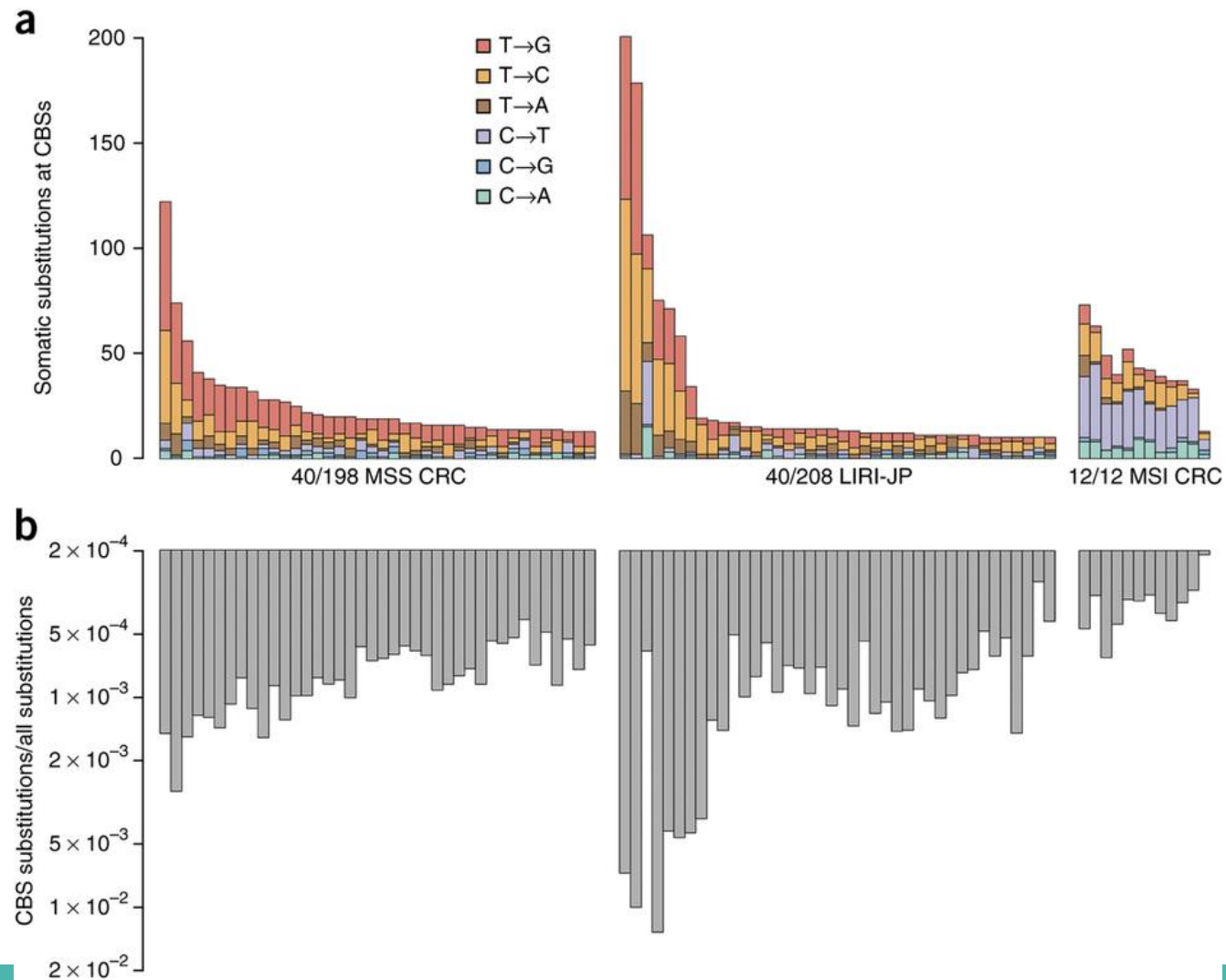
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# Example site



# Observed in other cancer types



# Questions?

