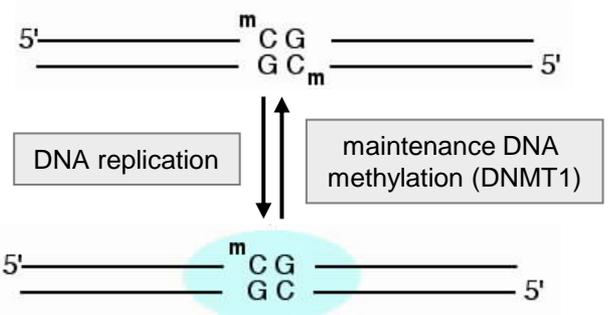
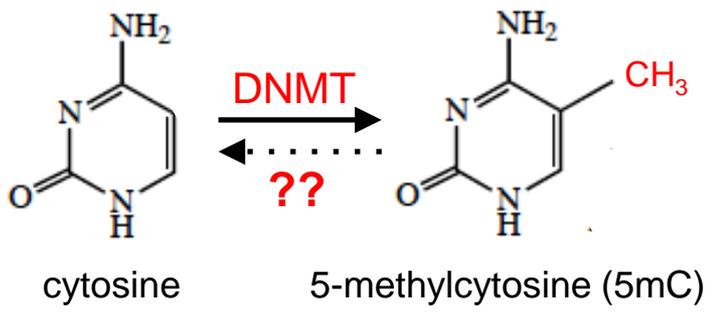
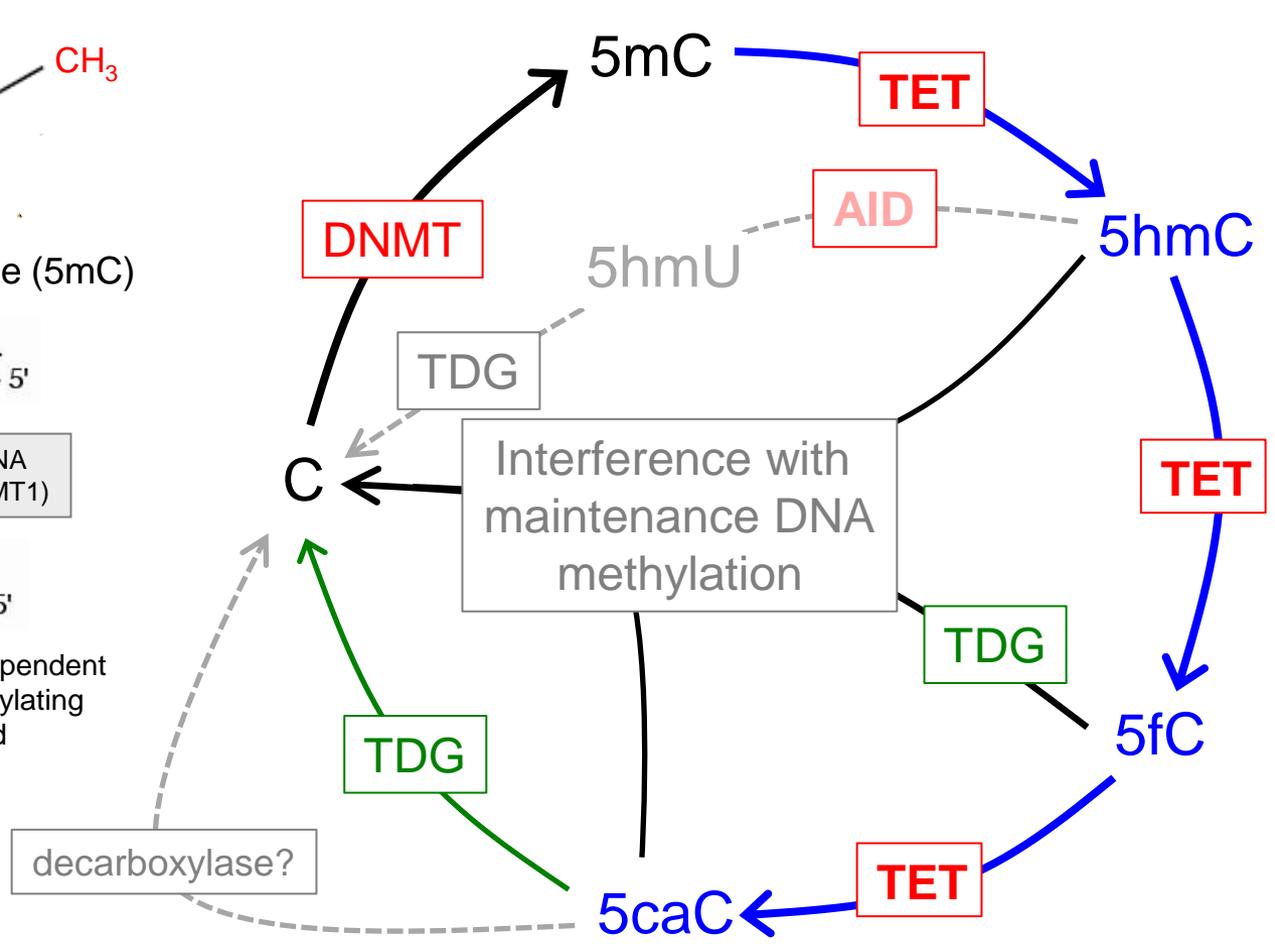
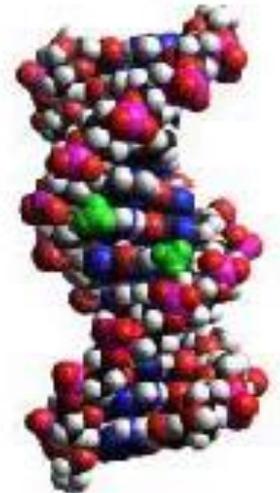


The DNA methylation-demethylation cycle

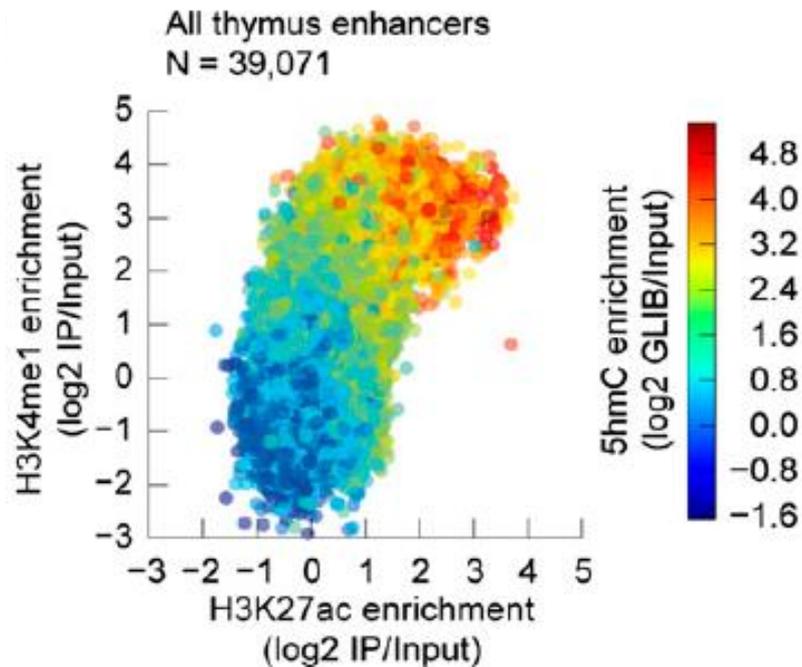
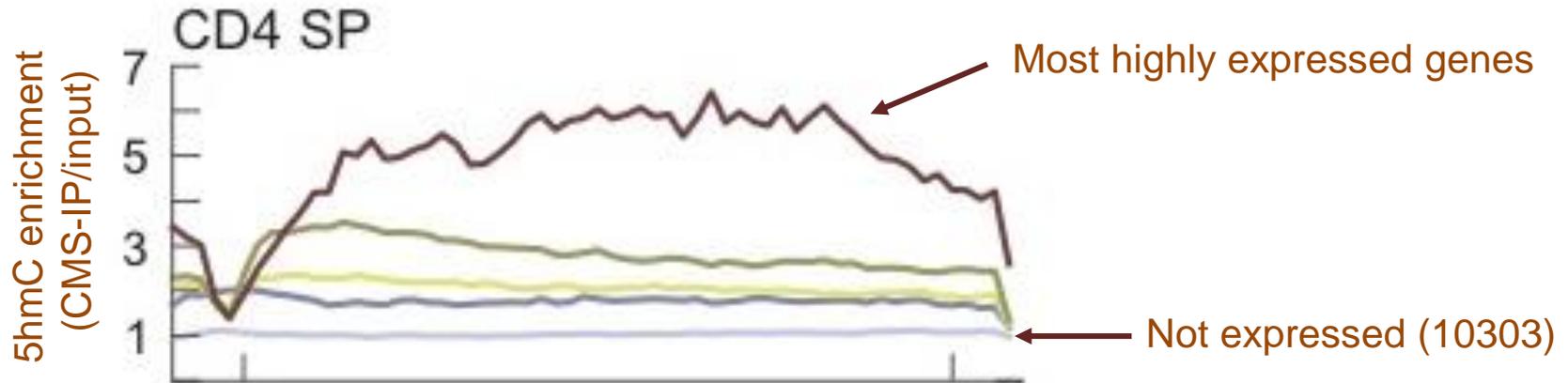


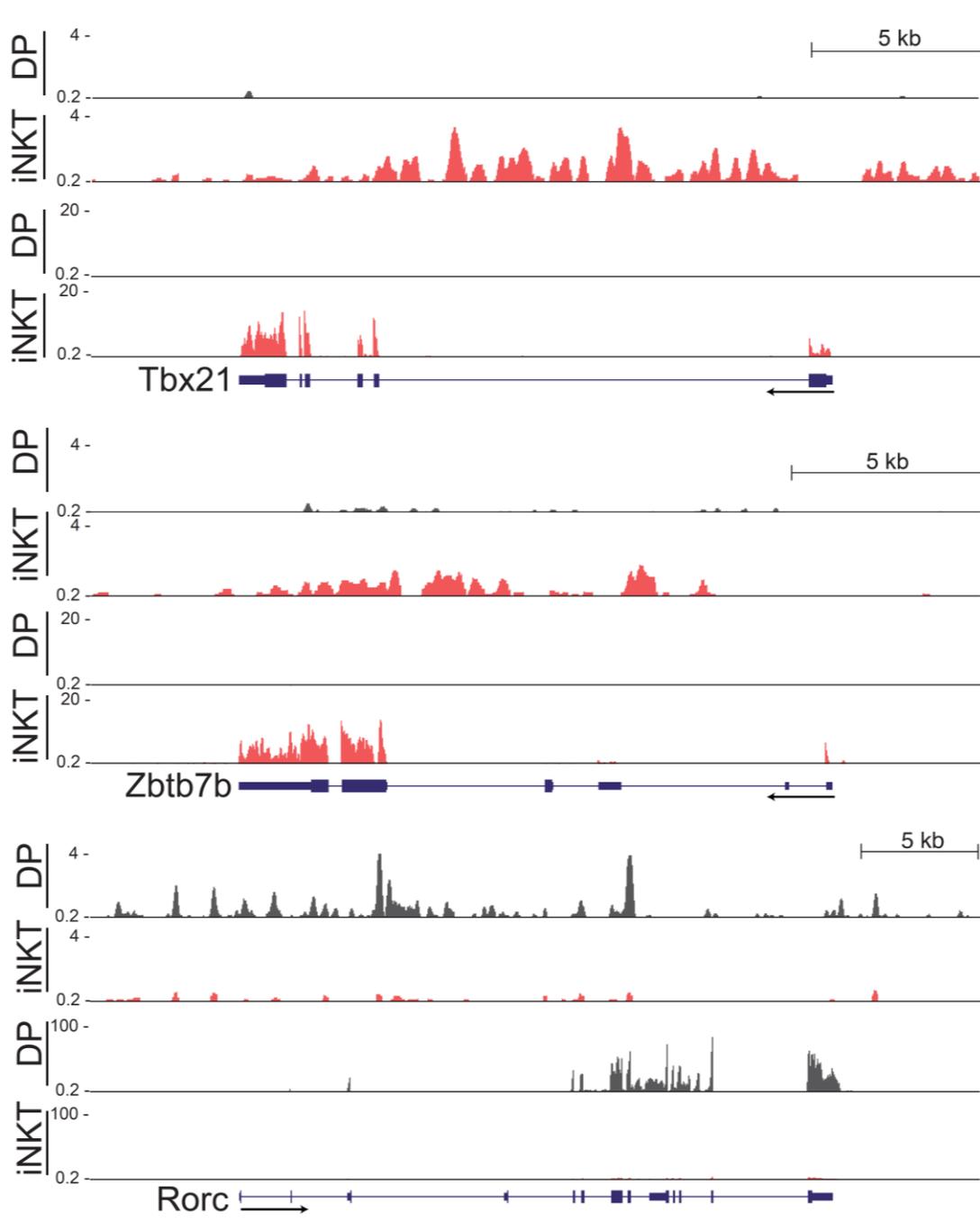
DNA demethylation occurs in a replication-dependent manner if DNMT1 is prevented from remethylating cytosine in the newly replicated strand



all modified cytosines can be epigenetic marks

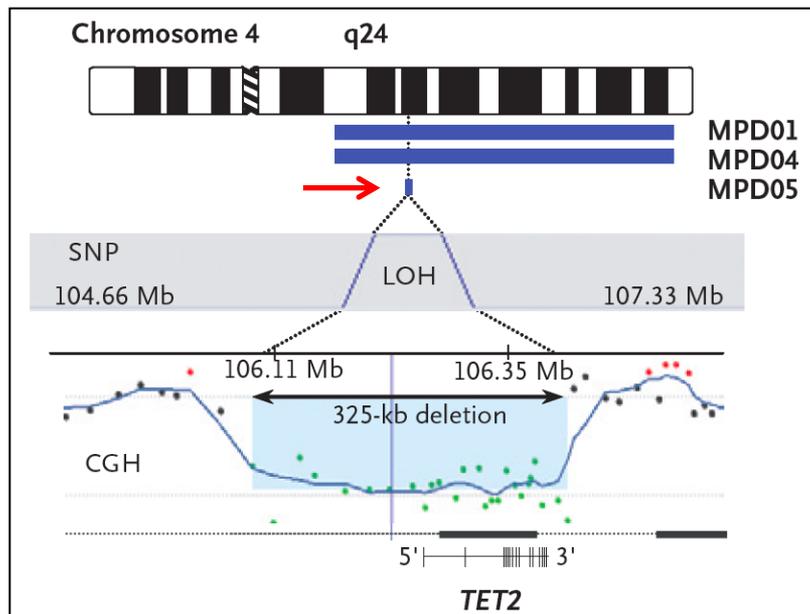
5hmC is highest in gene bodies of the most highly transcribed genes, and at active enhancers (H3K4me1+, H3K27Ac+)



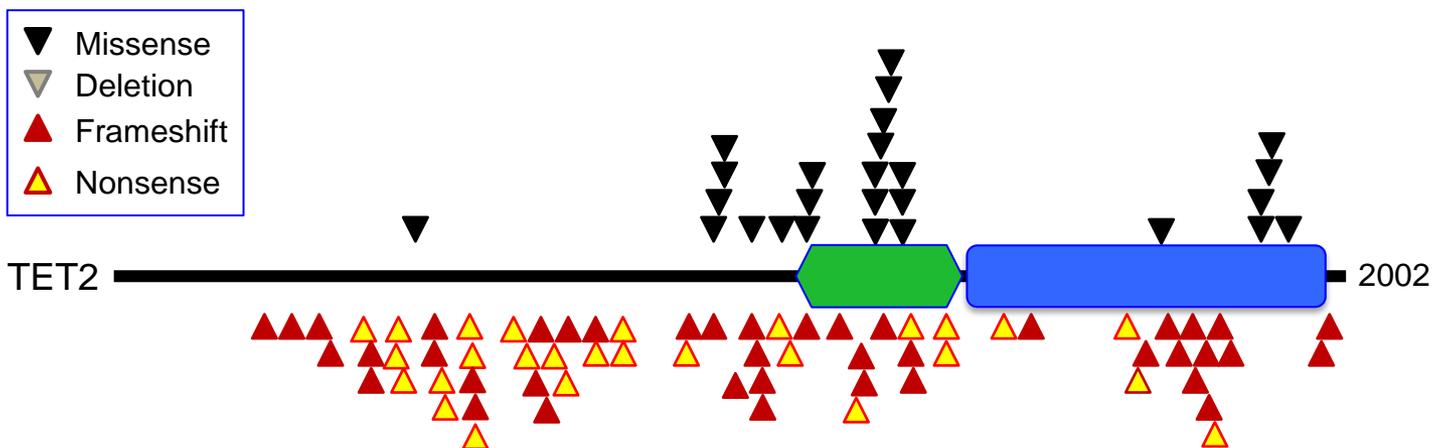


Cell type 1	}	5hmC
Cell type 2		
Cell type 1	}	RNA
Cell type 2		
Cell type 1	}	5hmC
Cell type 2		
Cell type 1	}	RNA
Cell type 2		
Cell type 1	}	5hmC
Cell type 2		
Cell type 1	}	RNA
Cell type 2		

TET2 deletions and mutations are frequently associated with haematologic malignancies



Delhommeau et al., *NEJM* 2009



TET2 mutations are loss-of-function

Loss of 5-hydroxymethylcytosine is accompanied with malignant cellular transformation

Yotaro Kudo,¹ Keisuke Tateishi,^{1,3} Keisuke Yamamoto,¹ Shinzo Yamamoto,¹ Yoshinari Asaoka,¹ Hideaki Ijichi,¹ Genta Nagae,² Haruhiko Yoshida,¹ Hiroyuki Aburatani² and Kazuhiko Koike¹

Cancer Science 2011

5-Hydroxymethylcytosine Is Strongly Depleted in Human Cancers but Its Levels Do Not Correlate with *IDH1* Mutations

Seung-Gi Jin, Yong Jiang, Runxiang Qiu, et al.

Cancer Res 2011;71:7360-7365. Published OnlineFirst November 3, 2011.

Cancer Research 2011

Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation

H Yang^{1,2,8}, Y Liu^{3,4,8}, F Bai⁵, J-Y Zhang¹, S-H Ma^{1,2}, J Liu^{3,4}, Z-D Xu^{3,4}, H-G Zhu^{3,4}, Z-Q Ling⁶, D Ye¹, K-L Guan^{1,4,7} and Y Xiong^{1,2,5}

Oncogene 2011

Loss of 5-Hydroxymethylcytosine Is an Epigenetic Hallmark of Melanoma

Christine Guo Lian,^{1,2,13} Yufei Xu,^{1,13} Craig Ceol,^{3,6} Feizhen Wu,⁹ Allison Larson,⁵ Karen Dresser,⁷ Wenqi Xu,⁹ Li Tan,⁹ Yeguang Hu,¹ Qian Zhan,² Chung-wei Lee,² Di Hu,¹ Bill Q. Lian,^{1,8} Sonja Kleffel,⁵ Yijun Yang,¹⁰ James Neiswander,⁶ Abraham J. Khorasani,¹ Rui Fang,¹ Cecilia Lezcano,² Lyn M. Duncan,⁴ Richard A. Scolyer,¹¹ John F. Thompson,¹¹ Hojabr Kakavand,¹¹ Yariv Houvras,^{3,12} Leonard I. Zon,³ Martin C. Mihm Jr.,⁵ Ursula B. Kaiser,¹ Tobias Schatton,⁵ Bruce A. Woda,⁷ George F. Murphy,^{2,*} and Yujiang G. Shi^{1,9,*}

Cell 2012

TET1 Suppresses Cancer Invasion by Activating the Tissue Inhibitors of Metalloproteinases

Chih-Hung Hsu,^{1,11,13} Kai-Lin Peng,^{1,2,11} Ming-Lun Kang,^{1,12} Yi-Ren Chen,^{3,12} Yu-Chih Yang,³ Chin-Hsien Tsai,^{3,4} Chi-Shuen Chu,^{1,5} Yung-Ming Jeng,⁶ Yen-Ting Chen,^{1,5} Feng-Mao Lin,⁷ Hsien-Da Huang,⁷ Yun-Yuh Lu,¹ Yu-Ching Teng,^{1,2} Shinn-Tsuen Lin,⁹ Ruo-Kai Lin,^{1,14} Fan-Mei Tang,⁹ Sung-Bau Lee,^{1,15} Huan Ming Hsu,¹⁰ Jyh-Cherng Yu,^{10,*} Pei-Wen Hsiao,^{3,*} and Li-Jung Juan^{1,2,5,*}

Cell Reports 2012

HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis

Miao Sun^{a,b}, Chun-Xiao Song^{c,1}, Hao Huang^{d,1}, Casey A. Frankenger^{a,1}, Devipriya Sankarasharma^{e,1}, Suzana Gomes^a, Ping Chen^d, Jianjun Chen^d, Kiran K. Chada^e, Chuan He^c, and Marsha R. Rosner^{a,b,2}

PNAS 2013

MicroRNA-Antagonism Regulates Breast Cancer Stemness and Metastasis via TET-Family-Dependent Chromatin Remodeling

Su Jung Song,¹ Laura Poliseo,^{1,6,8} Min Sup Song,^{1,7,8} Ugo Ala,¹ Kaitlyn Webster,¹ Christopher Ng,¹ Gary Beringer,^{2,4} Nicolai J. Brikbak,⁵ Xin Yuan,³ Lewis C. Cantley,^{2,4} Andrea L. Richardson,⁵ and Pier Paolo Pandolfi^{1,*}

Cell 2013

Is acute loss of TET function associated with cancer? **Yes**

Two model systems in mice:

- deletion of Tet2 and Tet3 with Mx1Cre and polyI:polyC injection,
or with Cre-ERT2 and tamoxifen injection
effects of deletion first seen in **haematopoietic stem/precursor cells**
→ aggressive myeloid leukemia
- deletion of Tet2 and Tet3 with CD4Cre **in T cells**
→ aggressive antigen-driven T cell leukemia

Both: cell-intrinsic, polyclonal, transmissible indefinitely to recipient mice

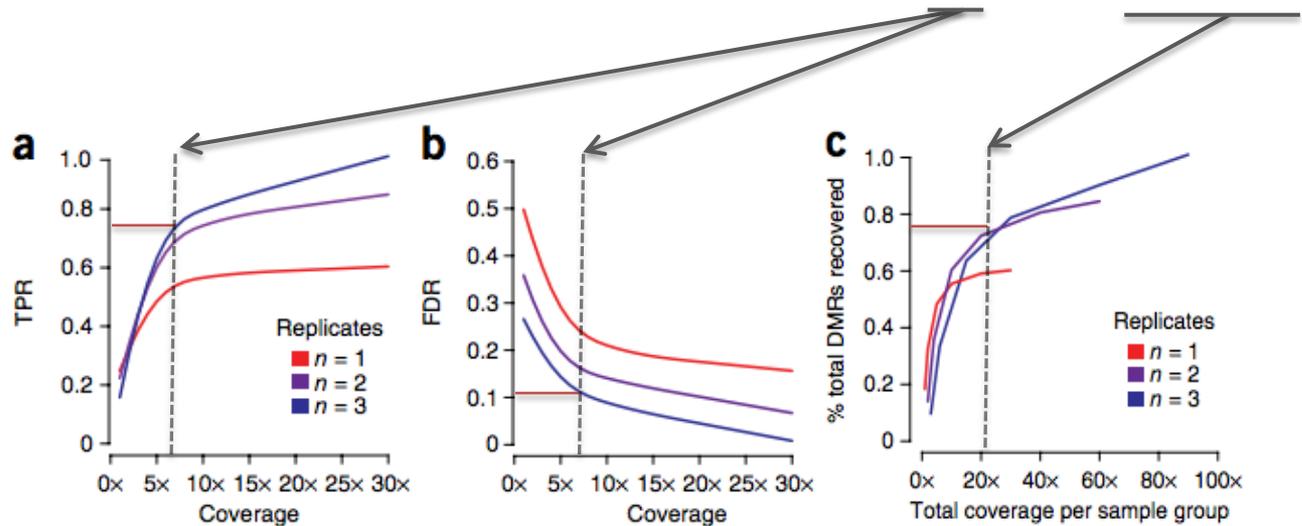
Cancer develops rapidly (< 5 weeks)

WGBS of WT and Tet2/3 DKO LSK haematopoietic stem/ precursor cells

Sequencing results

Covered basepairs = mapped reads x length of reads
mm9 Genome size = 2,725,765,481

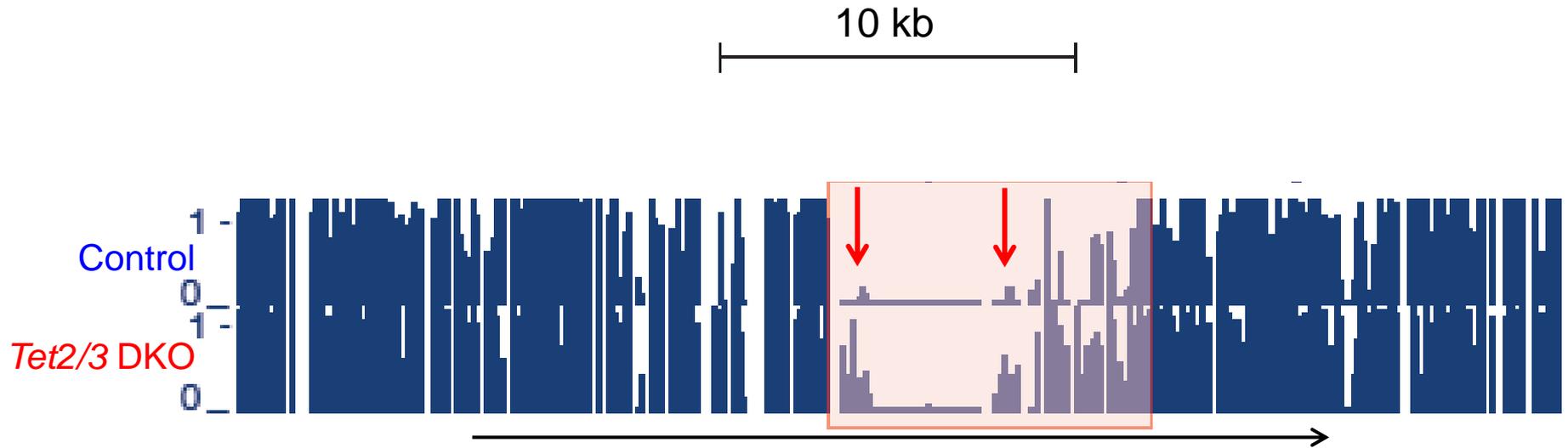
	Total Number of Reads	Mapped Reads	(%)	Covered Basepairs	Genome Coverage	Genome Coverage per Condition
CONTROLS	Ctr1	215,810,374	197,201,739	0.91	19,917,375,639	7.31
	Ctr2	192,188,864	177,428,469	0.92	17,920,275,369	6.57
	Ctr3	208,921,452	192,865,014	0.92	19,479,366,414	7.15
KNOCKOUTS	KO1	217,675,274	201,809,518	0.93	20,382,761,318	7.48
	KO2	230,150,082	211,497,322	0.92	21,361,229,522	7.84
	KO3	193,437,008	179,920,060	0.93	18,171,926,060	6.67



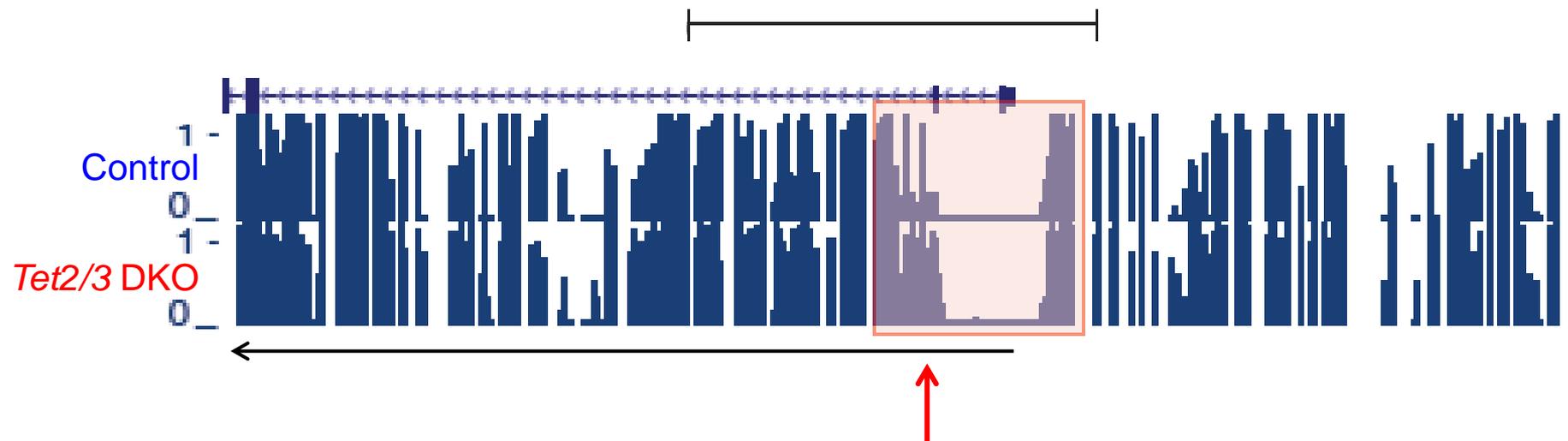
TPR = True positive regions (sensitivity) → refDMRs

FDR = False Discovery Rate (specificity) → refPositives

Narrowing of "canyons" of DNA methylation in *Tet2/3* DKO cells

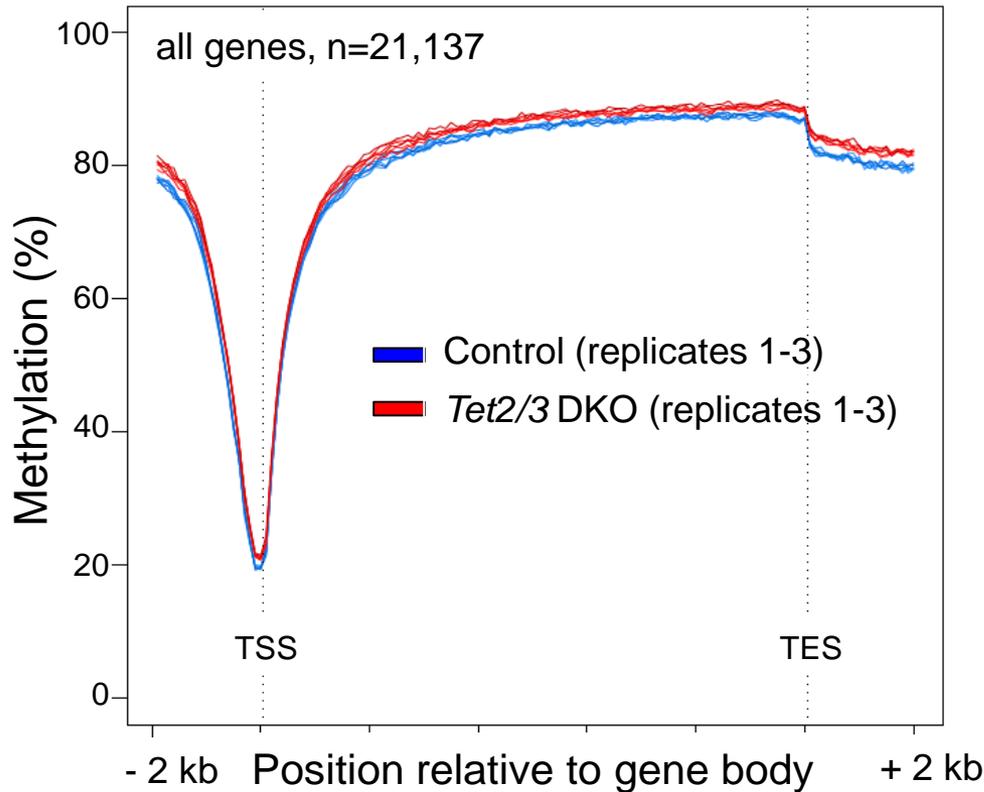


Both genes downregulated in *Tet2/3* DKO cells relative to WT

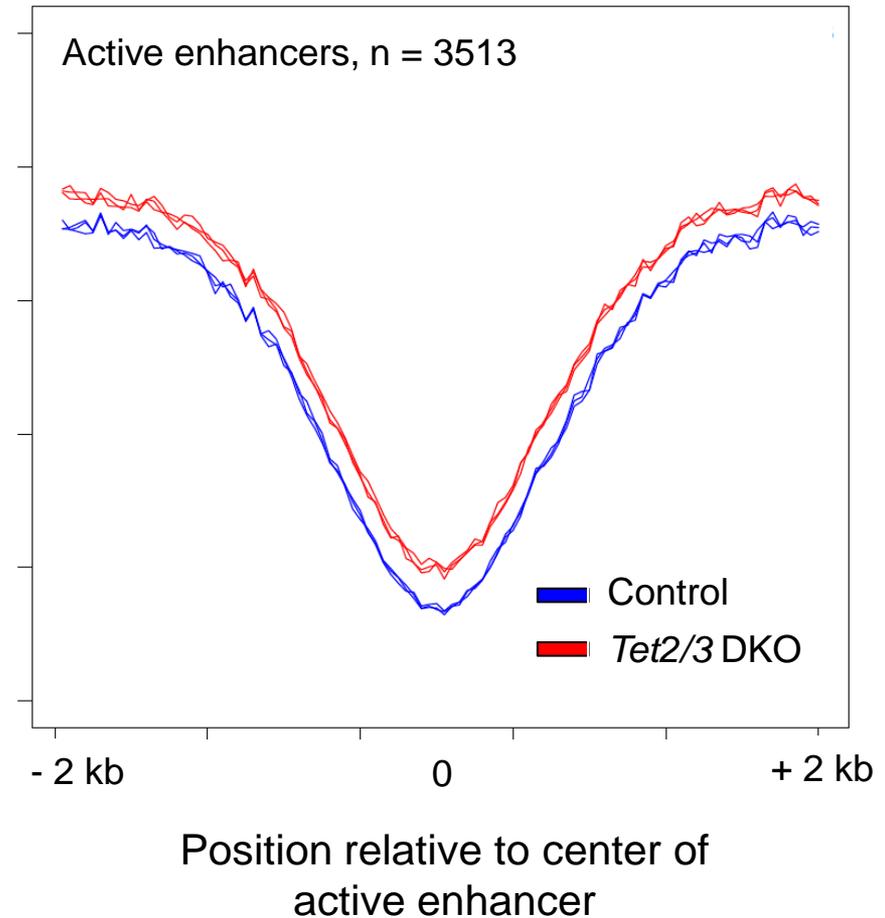


TET loss-of-function results in increased DNA methylation across the genome

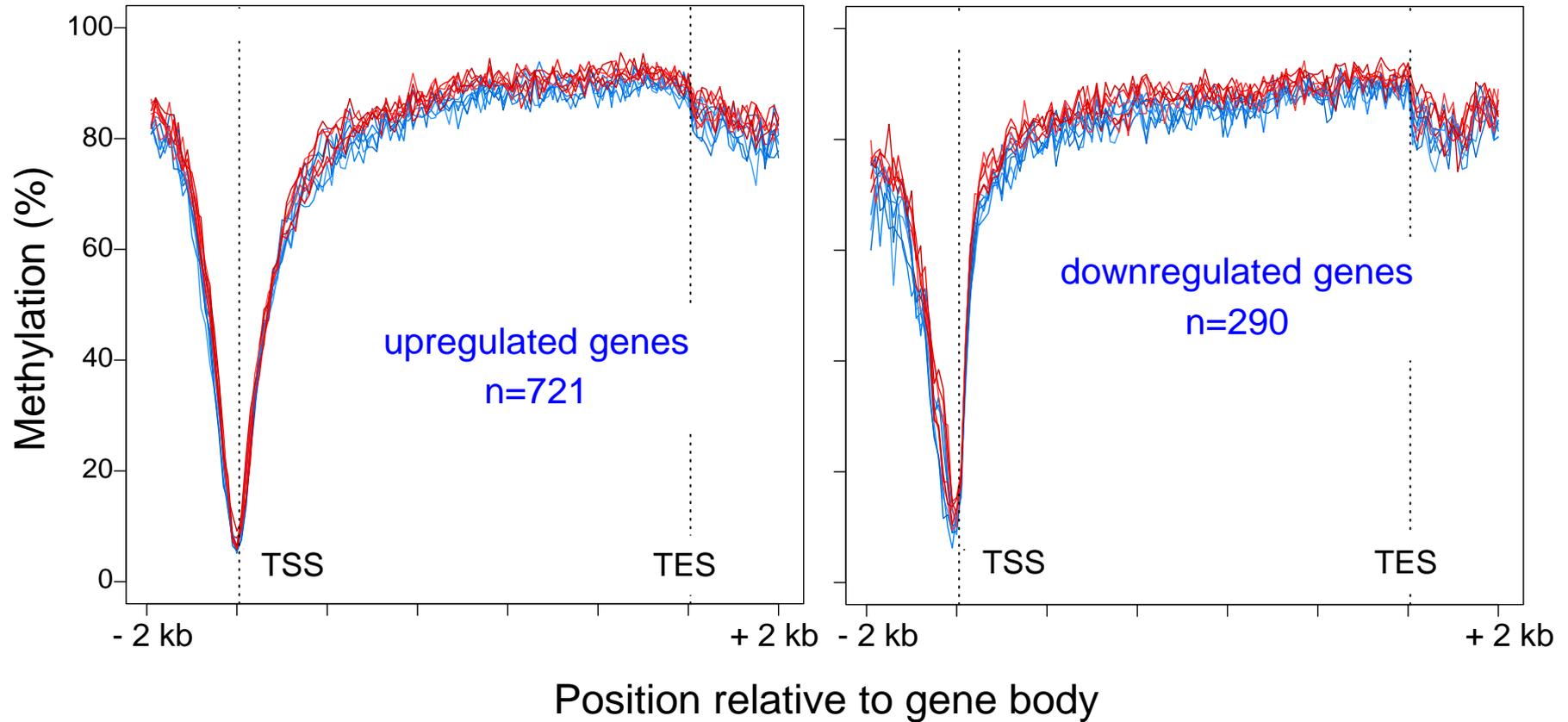
TSS and gene body methylation
(both strands, replicates separated)



Active enhancers (H3K4me1⁺, H3K27Ac⁺)

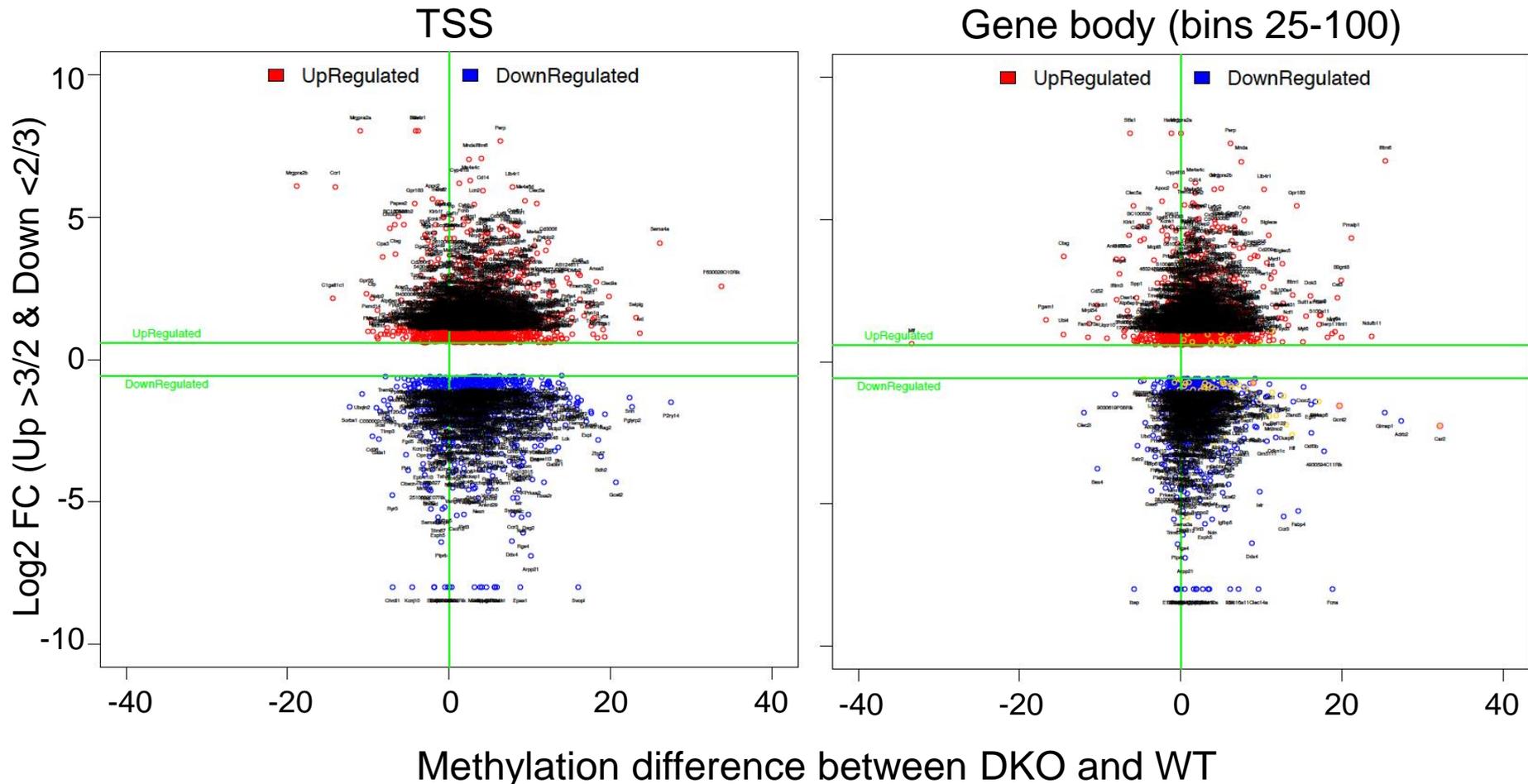


Increased DNA methylation in both classes of differentially-expressed genes
(plotted as averages across all genes)



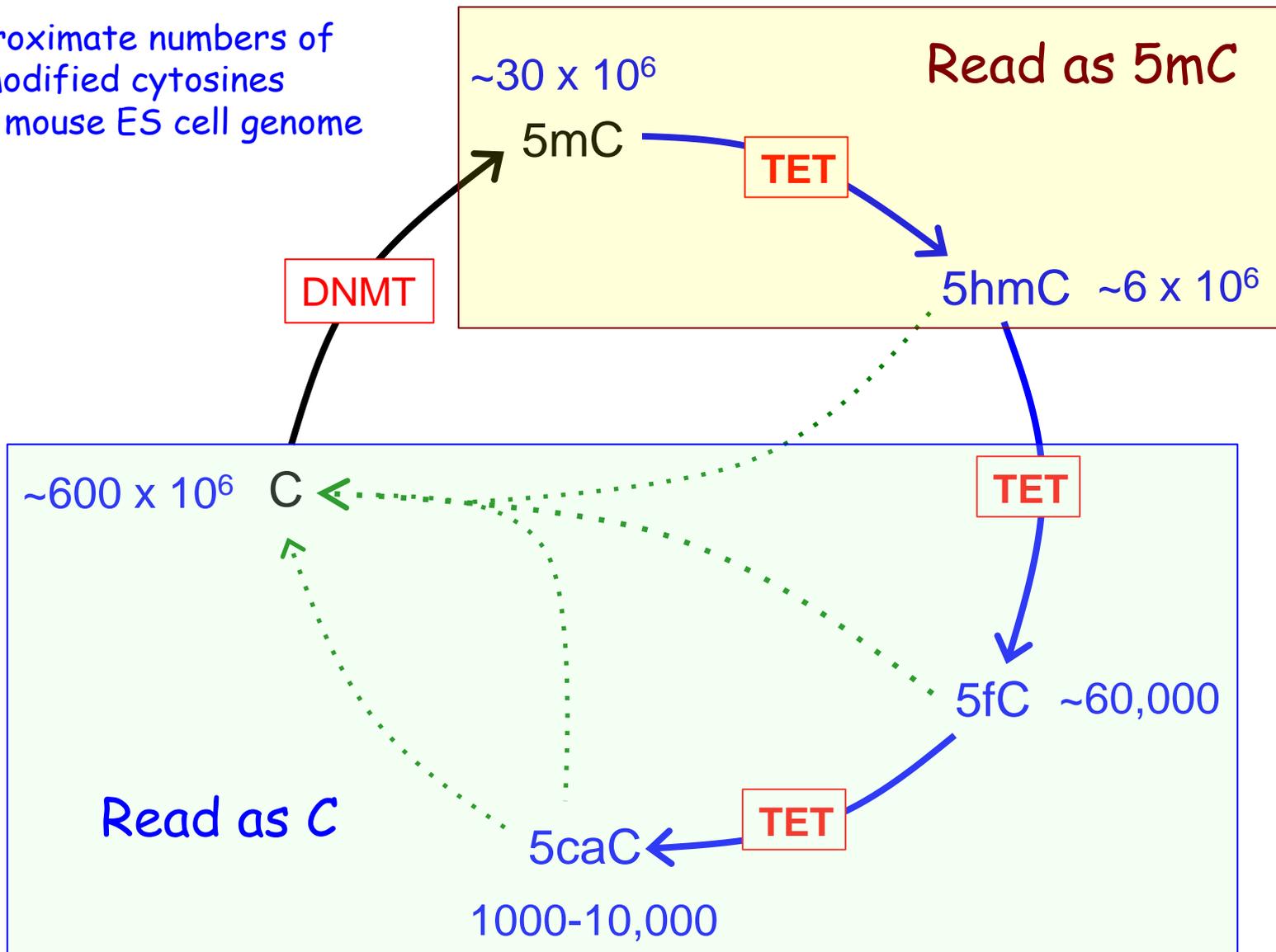
Increased DNA methylation in both up- and down-regulated genes (plotted at the single-gene level)

Methylation change in differentially expressed genes (721 up, 290 down)



Bisulfite sequencing conflates five bases into just two

Approximate numbers of modified cytosines in the mouse ES cell genome



Recommendations - 1

1. Include oxi-mC (or at least 5hmC) mapping in DNA methylation analysis

DNA methylation is not binary as previously thought:
5mC can be 5hmC; C can be 5fC or 5caC

Also, a 20% change in methylation level using bisulphite sequencing means that 20% of alleles have likely undergone a change in modification status

and some undefined proportion have changed state, from 5mC to 5hmC or vice versa, but have not been counted

2. Include perturbations and kinetic measurements

DNMTs and TETs are clearly sensitive to environment and metabolism

Changes may happen on very rapid timescales, as seen in these cancers

3. Encourage the development of new sequencing methods to map all modified cytosines in unamplified genomic DNA

8 bases altogether: A, G, T + 5 cytosine species: C, 5mC, 5hmC, 5fC, 5caC

Ideally, long reads (10 kb) to allow unambiguous mapping of repetitive DNA

Recommendations - 2

1. Shift some attention to purified primary cells, not just tissues or cell lines!

Cells examined *ex vivo* or *in situ* can be very different from cultured cells, established cell lines, or even primary cells cultured for just a few days *in vitro*

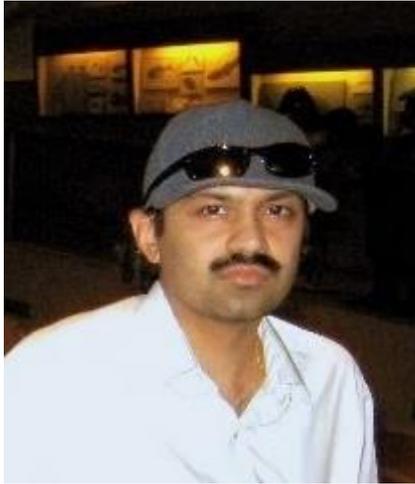
2. Corollary: enable technologies for looking at single cells or small numbers of cells preferably isolated *ex vivo*

e.g. exhausted T cells in mouse models or from humans are available as thousands, not millions

All the technologies: histone modifications, ChIP-seq, DNA modification mapping

3. Take-home message: model organisms are likely to be quite useful, even to a National "Human" Genome Research Institute

Collaborators



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