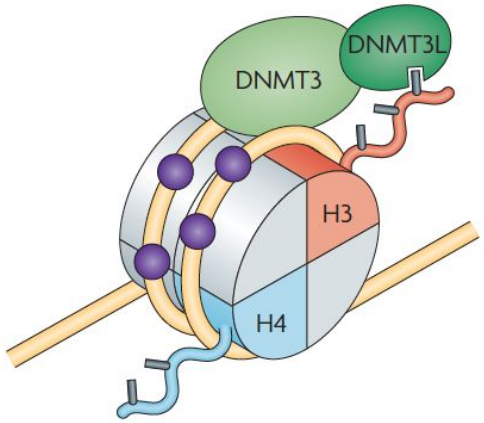
Background: de novo DNA methylation by Dnmt3b



DNA Methyltransferases Dnmt3a and Dnmt3b Are Essential for De Novo Methylation and Mammalian Development

Masaki Okano,* Daphne W. Bell,† Daniel A. Haber,† and En Li*‡

Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation

Tuncay Baubec[1], Daniele F. Colombo[1], Christiane Wirbelauer[1], Juliane Schmidt[1], Lukas Burger[1,2], Arnaud R. Krebs[1], Altuna Akalin[1]† & Dirk Schübeler[1,3]

Background: Gene body methylation in cancer

- Global DNA hypomethylation in tumours
- Abnormal transcripts in cancer

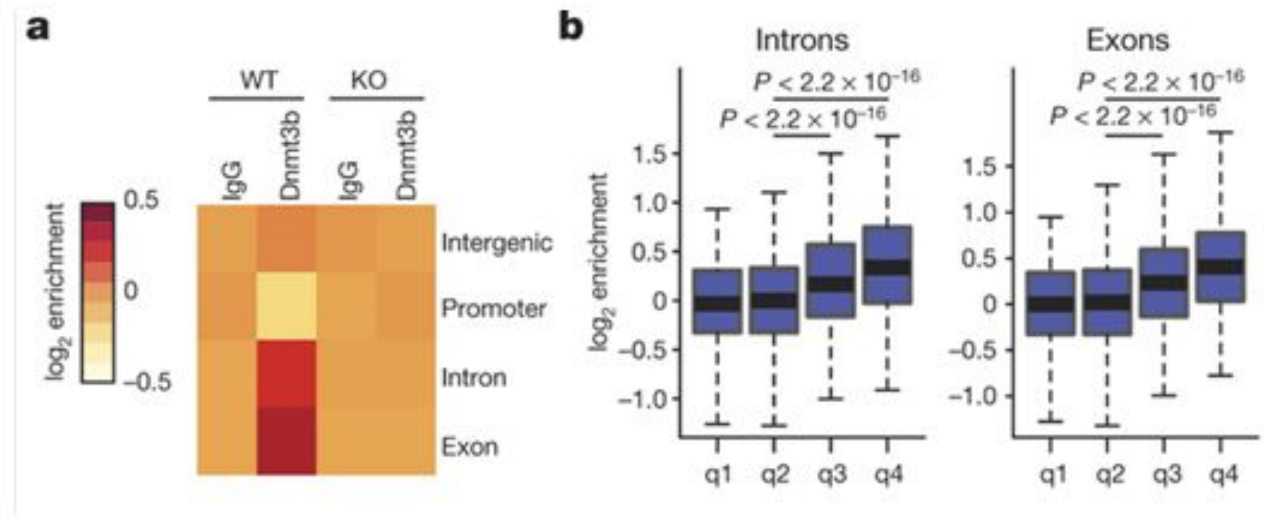
Induction of Tumors in Mice by Genomic Hypomethylation

François Gaudet[1,2,3], J. Graeme Hodgson[4], Amir Eden[1], Laurie Jackson-Grusby[1], Jessica Dausman[1], Joe W. Gray[4], Heinrich Leonhardt[2,3], Rudolf Jaenisch[1,*]

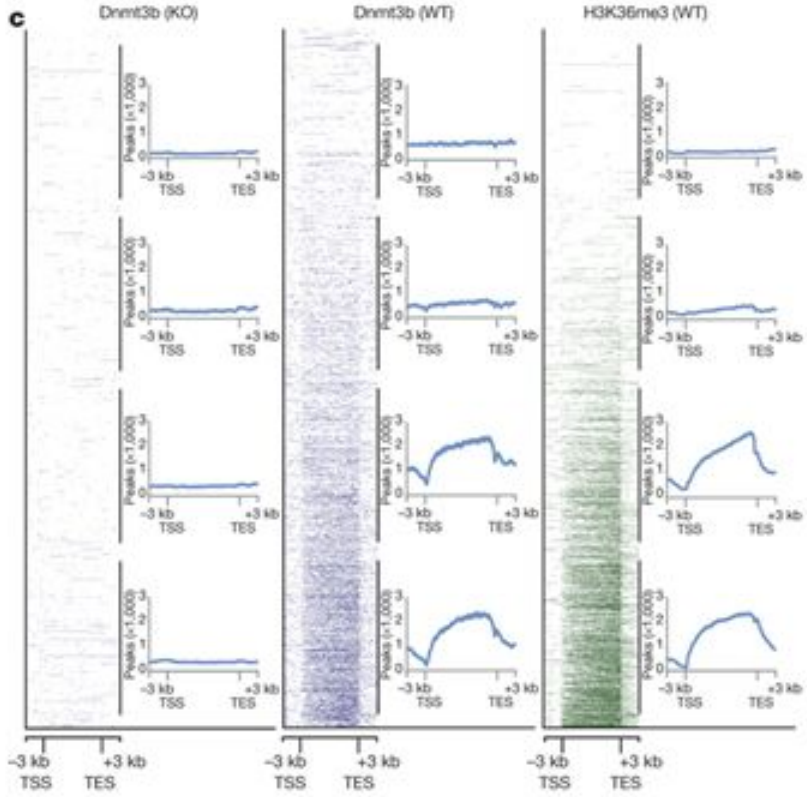
Hypomethylation distinguishes genes of some human cancers from their normal counterparts

Andrew P. Feinberg & Bert Vogelstein

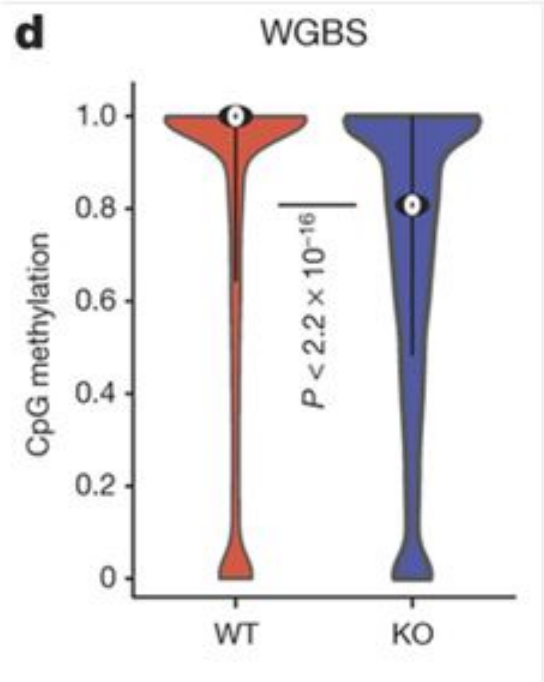
Dnmt3b binds preferentially in genes in third and fourth quartiles

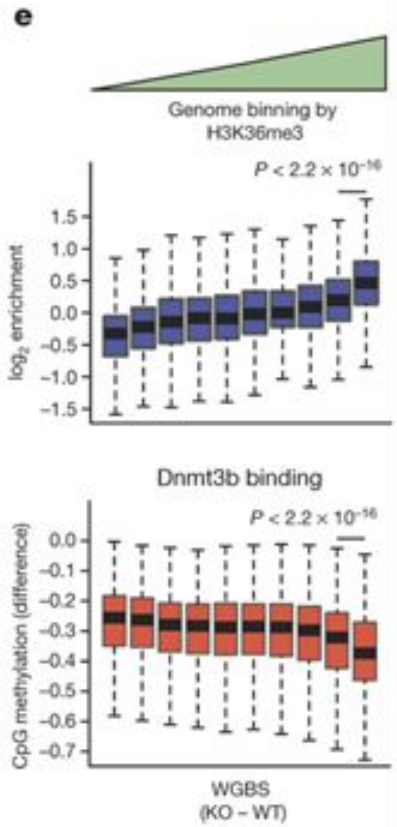


Dnmt3b binding correlates with H3K36me3 histone modification



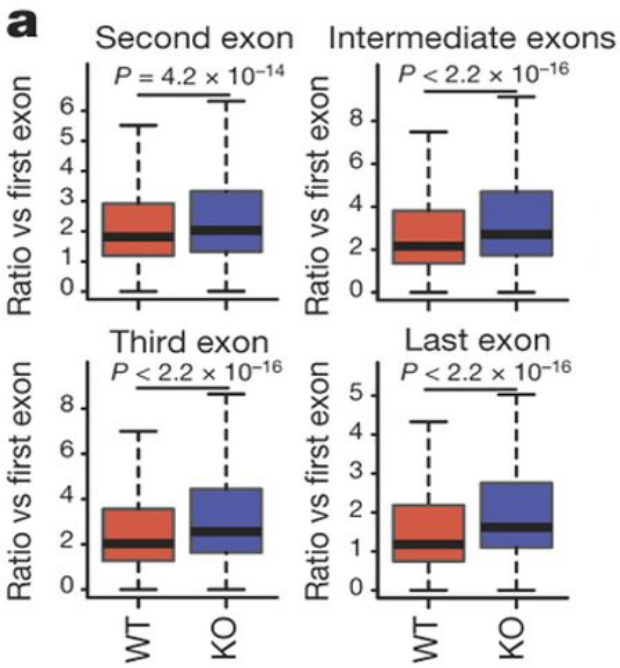
Dnmt3b-/- leads to reduced DNA methylation



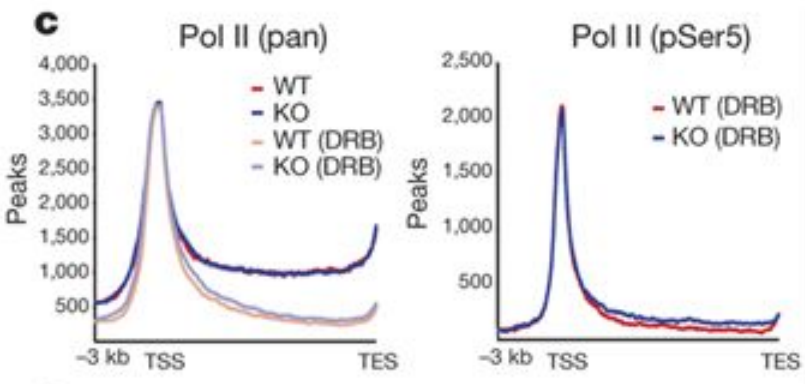


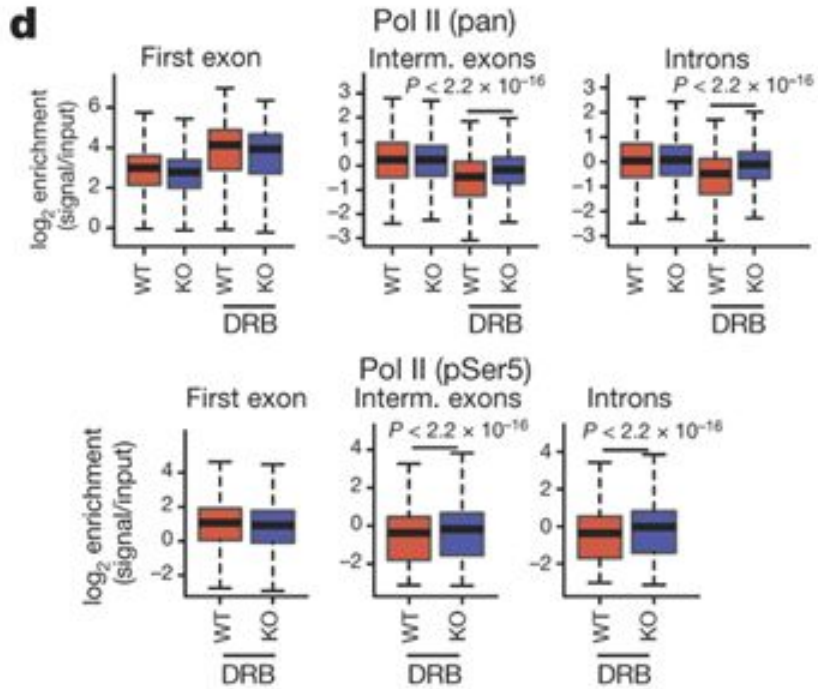
Increased amount of RNAs transcribed within gene body of Dnmt3b-/-

$$Ratio = \frac{RPKM_{later}}{RPKM_{first}}$$



Increased Pol II binding on intragenic regions in Dnmt3b-/-

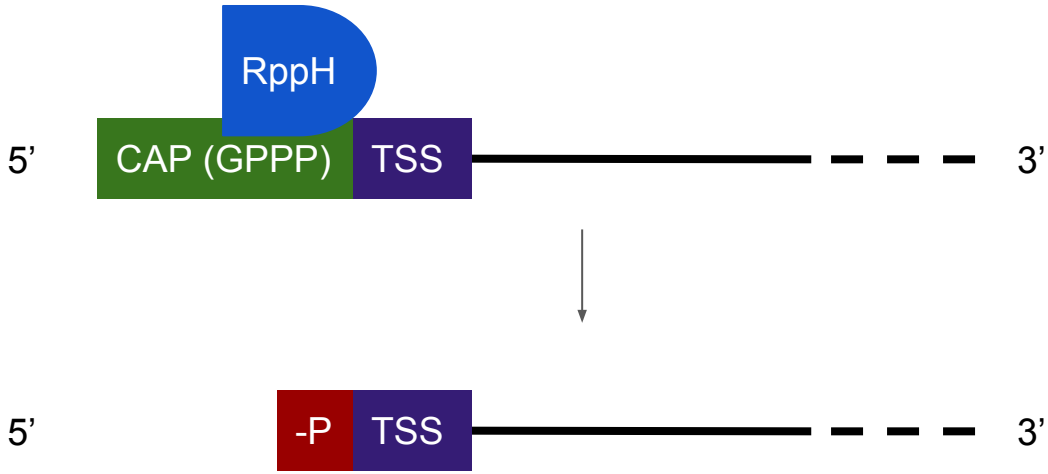




DECAP-Seq: How it works

Recall: 5′ cap (and 3′ poly-A tail) added to transcripts as RNA processing steps

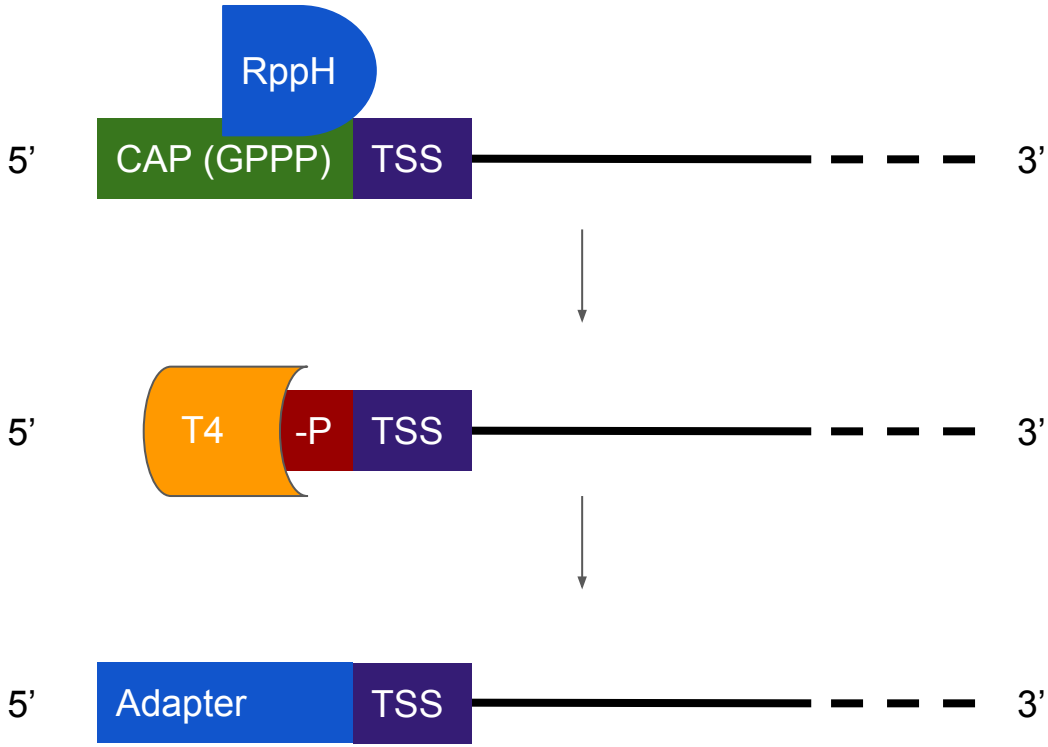
DECAP-Seq: How it works



Recall: 5′ cap (and 3′ poly-A tail) added to transcripts as RNA processing steps.

RNA 5' pyrophosphohydrolase (RppH) removes the cap, leaving a single phosphate

DECAP-Seq: How it works

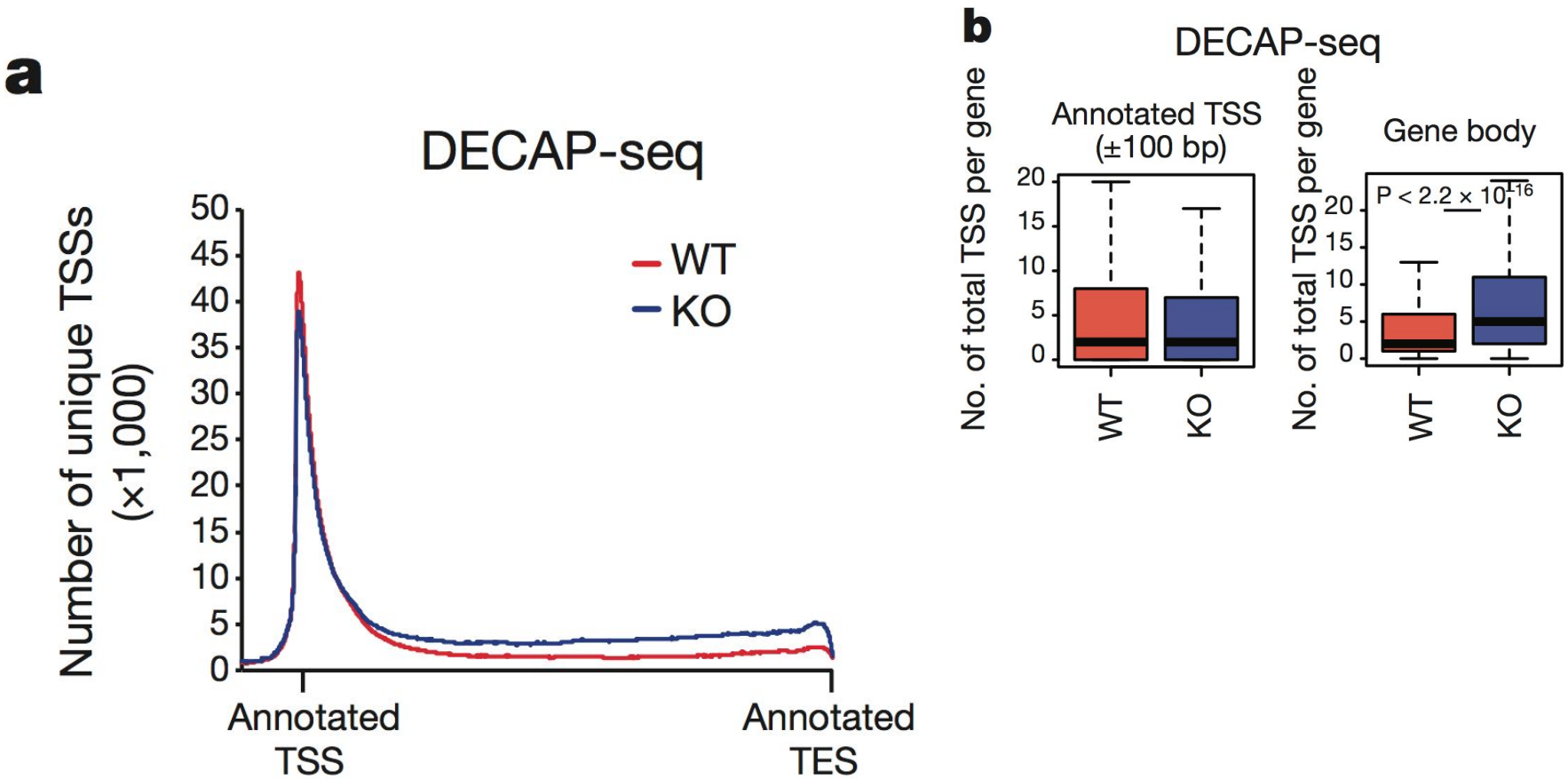


Recall: 5′ cap (and 3′ poly-A tail) added to transcripts as RNA processing steps.

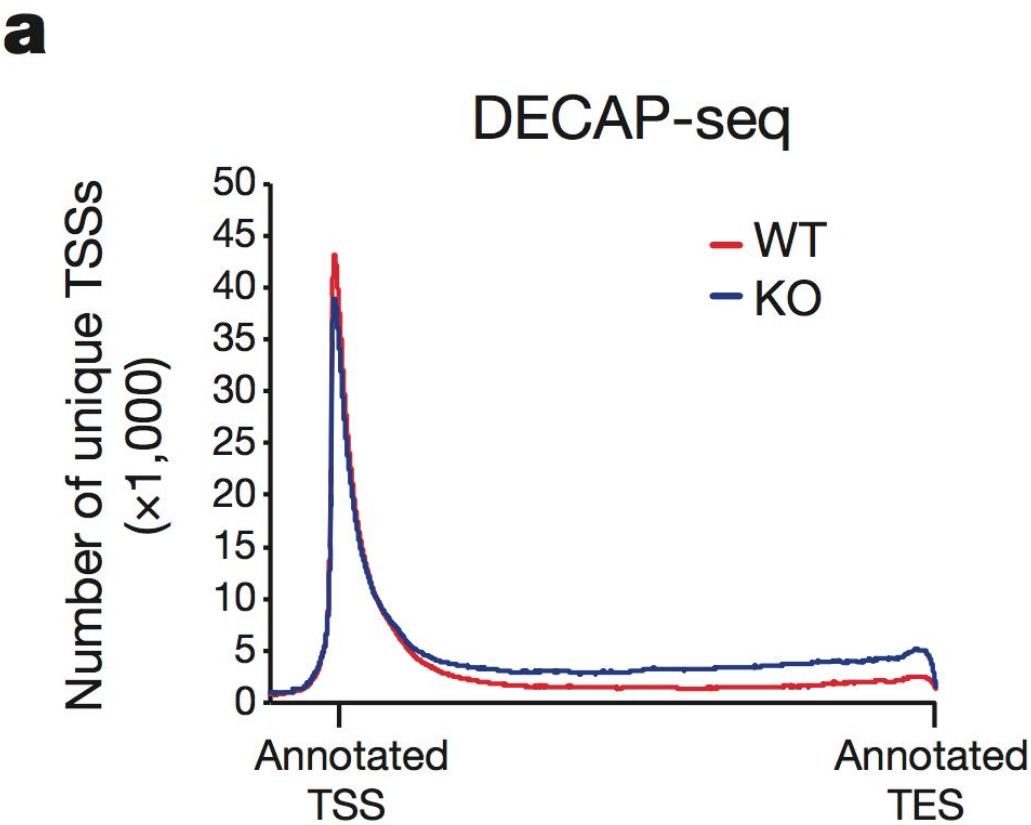
RNA 5' pyrophosphohydrolase (RppH) removes the cap, leaving a single phosphate

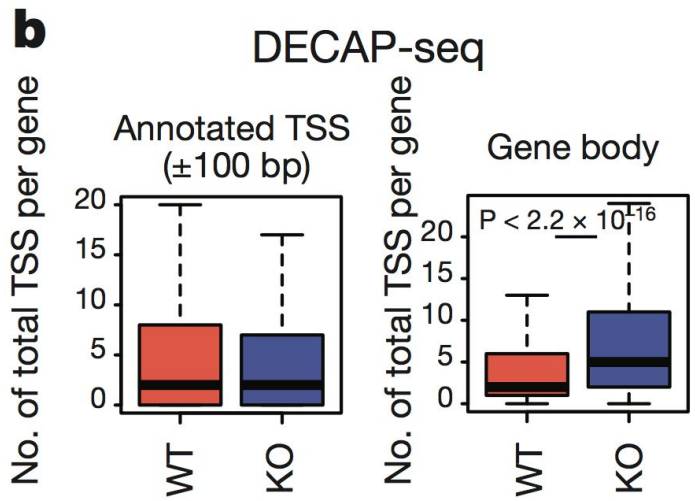
T4 RNA ligase attaches a known adapter sequence

DECAP-Seq : Distribution of TSSs



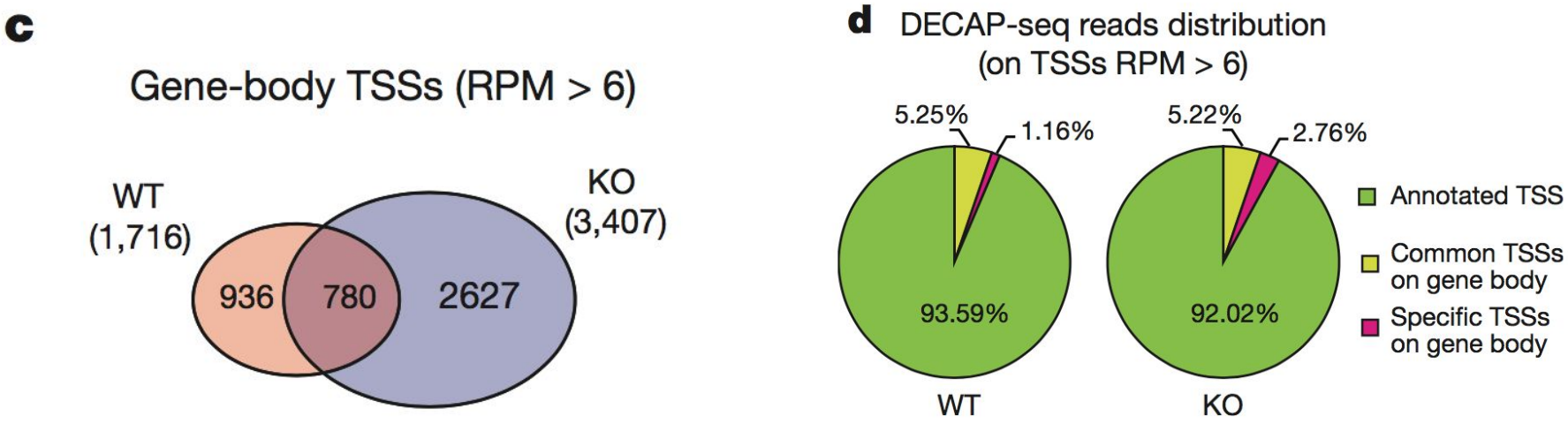
DECAP-Seq : Distribution of TSSs



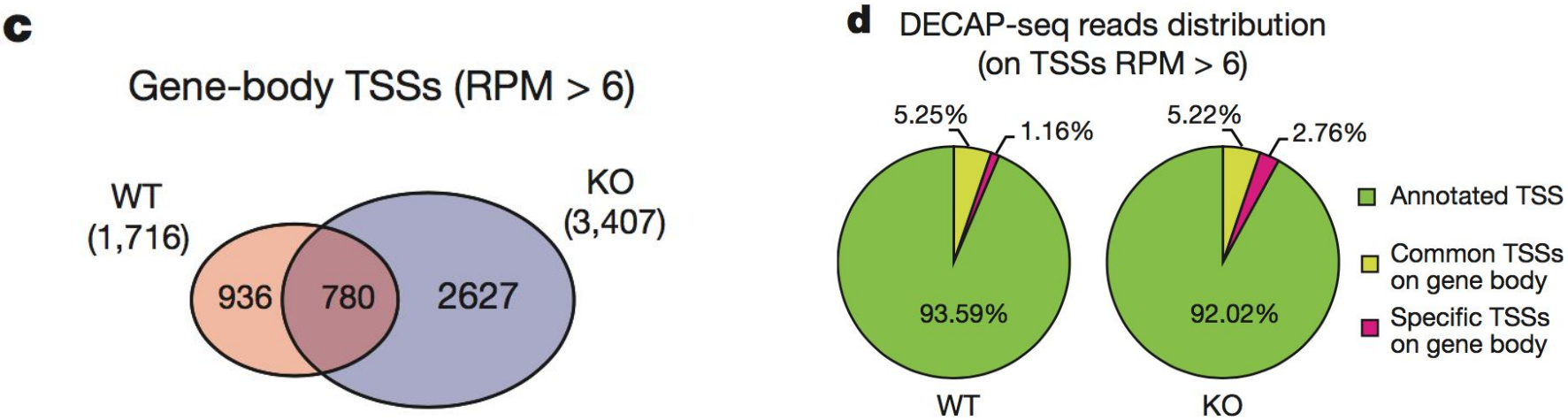


More TSSs appear on gene bodies in Dnmt3b knockout cells

DECAP-Seq : ‘De novo’ TSSs

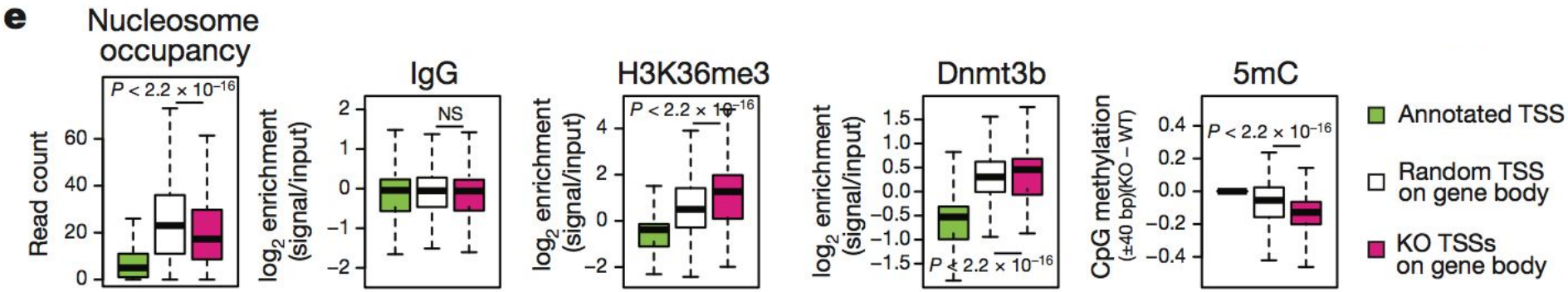


DECAP-Seq : ‘De novo’ TSSs

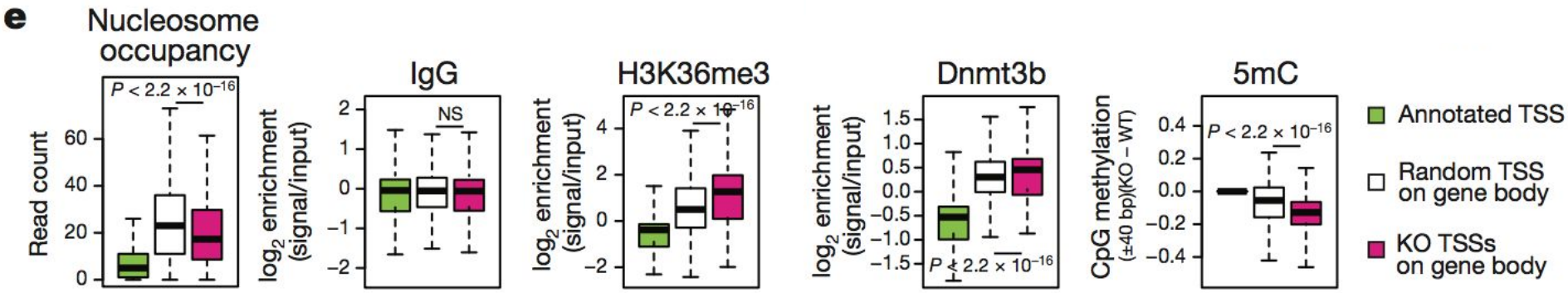


The increased number of TSSs in Dmnt3b knockout cells are largely *de novo* (do not appear in the wild type).

DECAP-Seq : Binding profiles

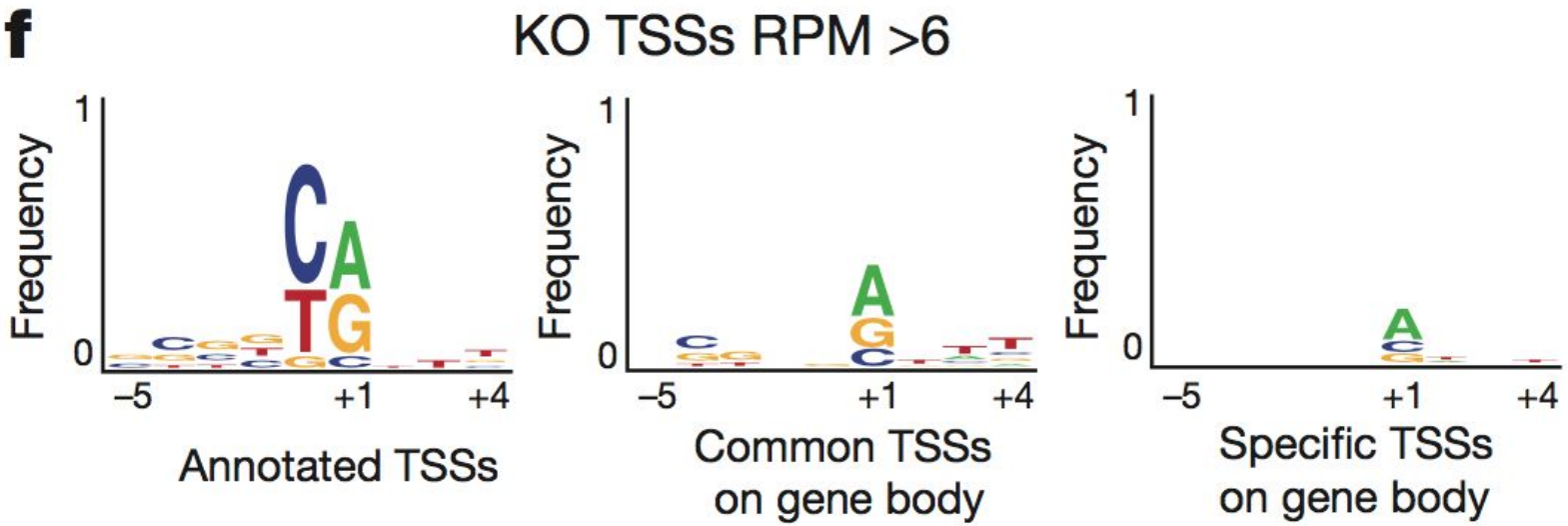


DECAP-Seq : Binding profiles

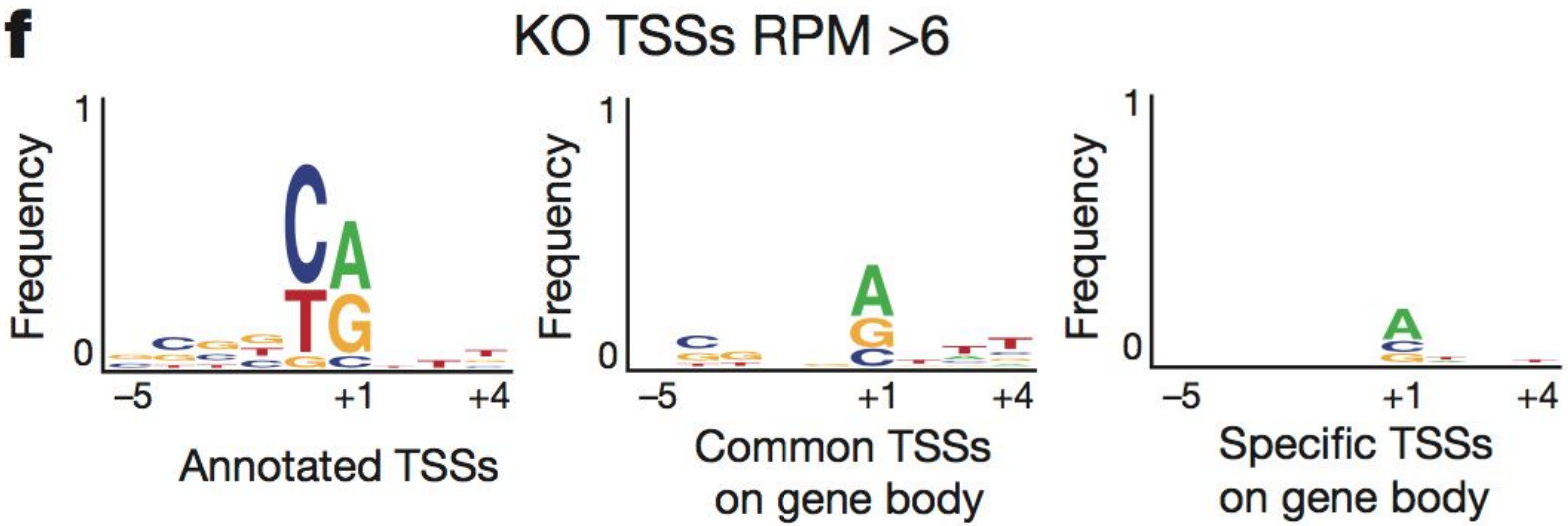


TSSs found only in Dmnt3b knockout cells appear in areas of the genome where there would normally be high H3K35me3 and dnmt3b binding.

DECAP-Seq : Sequence Context

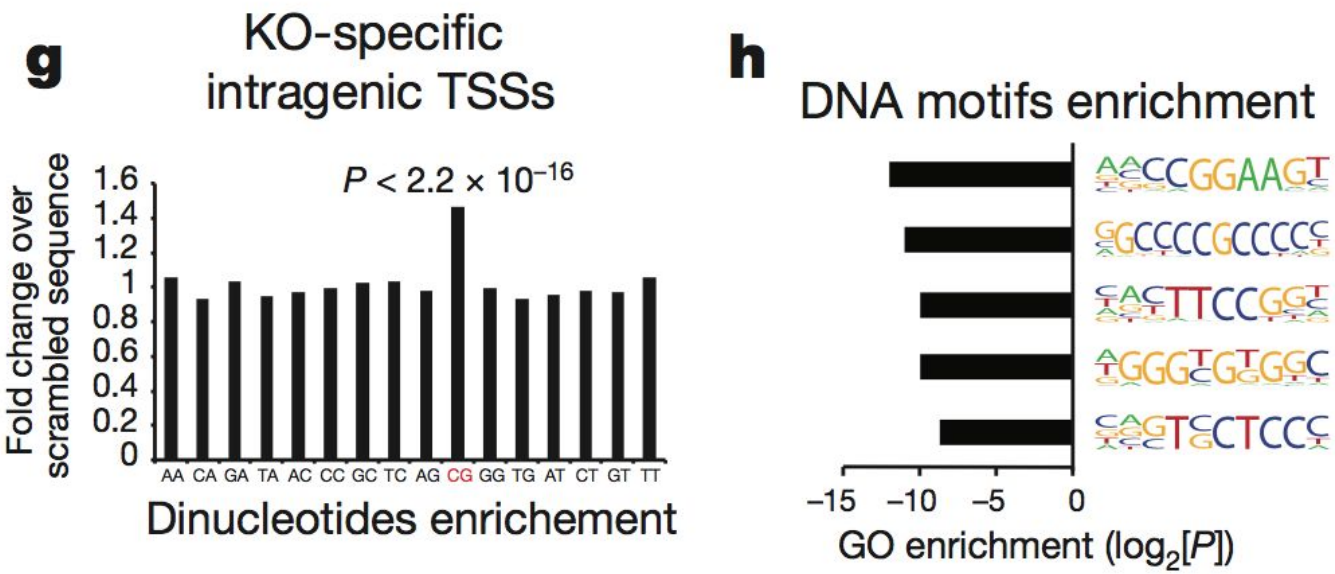


DECAP-Seq : Sequence Context

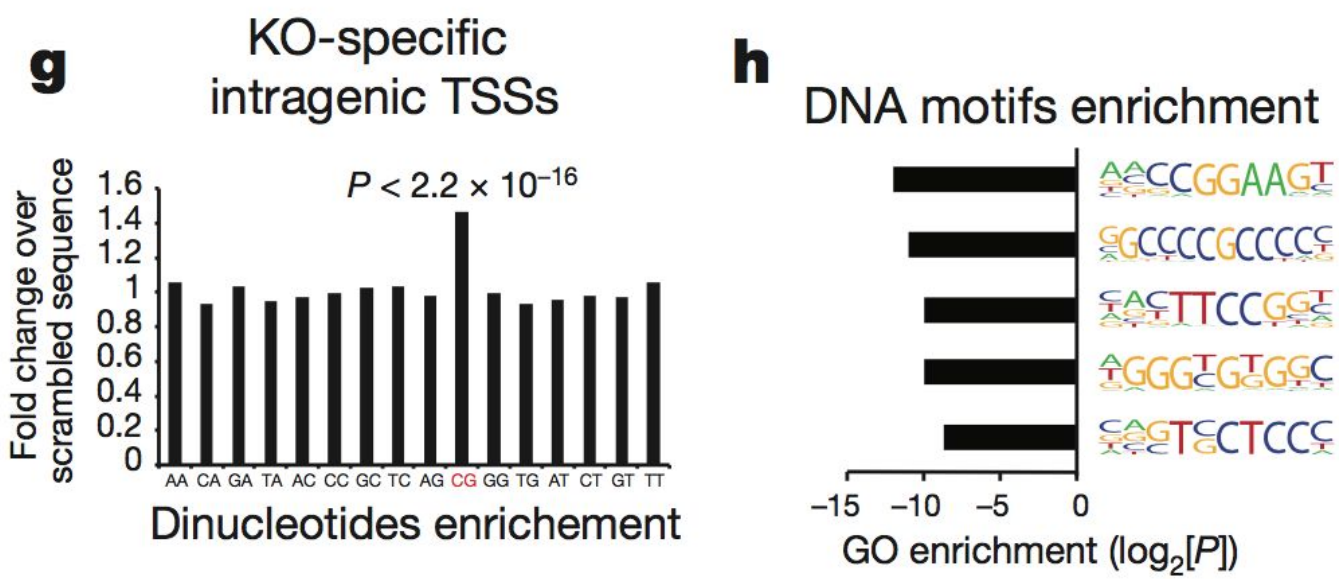


There is a loss of pyrimidine enrichment, and a reduction of purine enrichment in gene body TSSs as compared with the canonical ones.

DECAP-Seq : Sequence Context

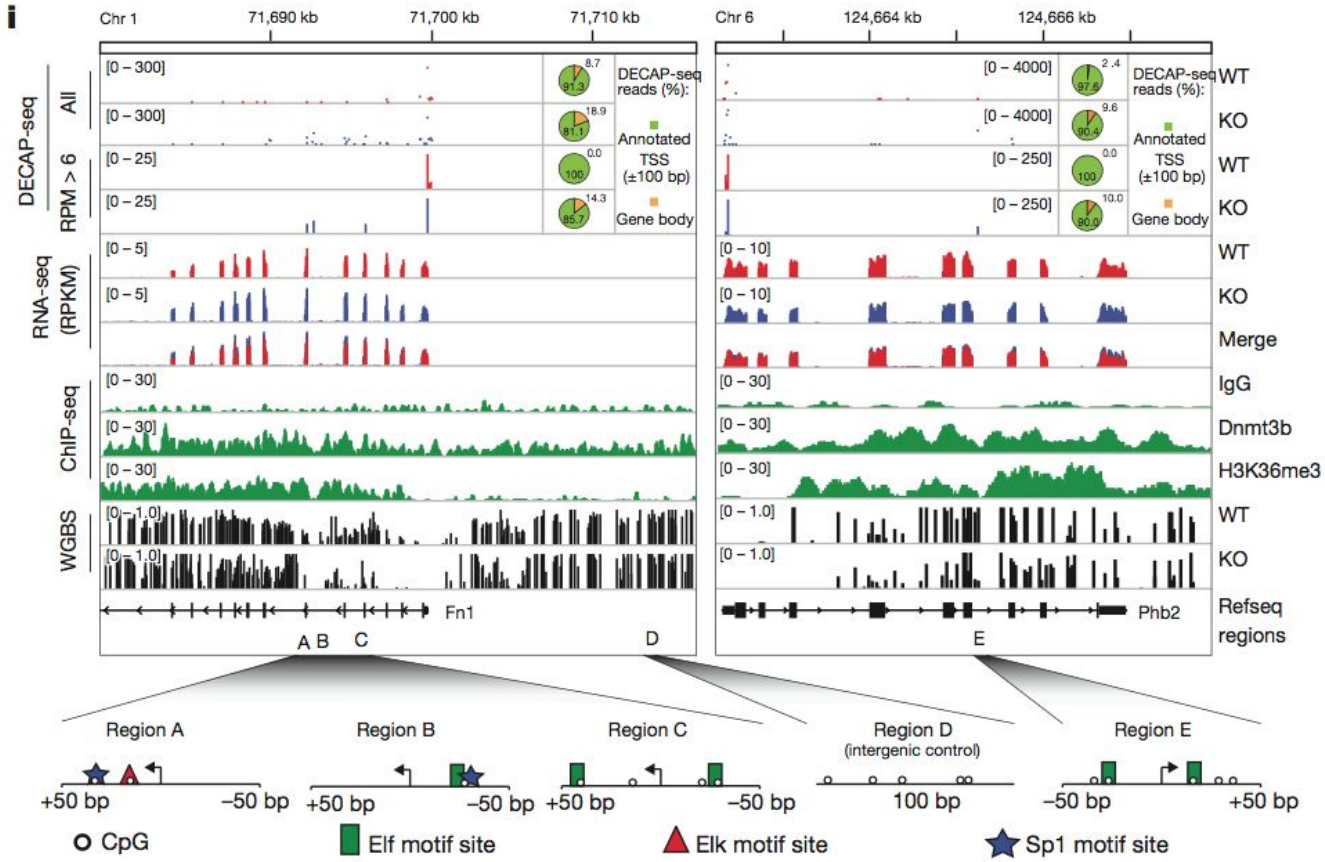


DECAP-Seq : Sequence Context



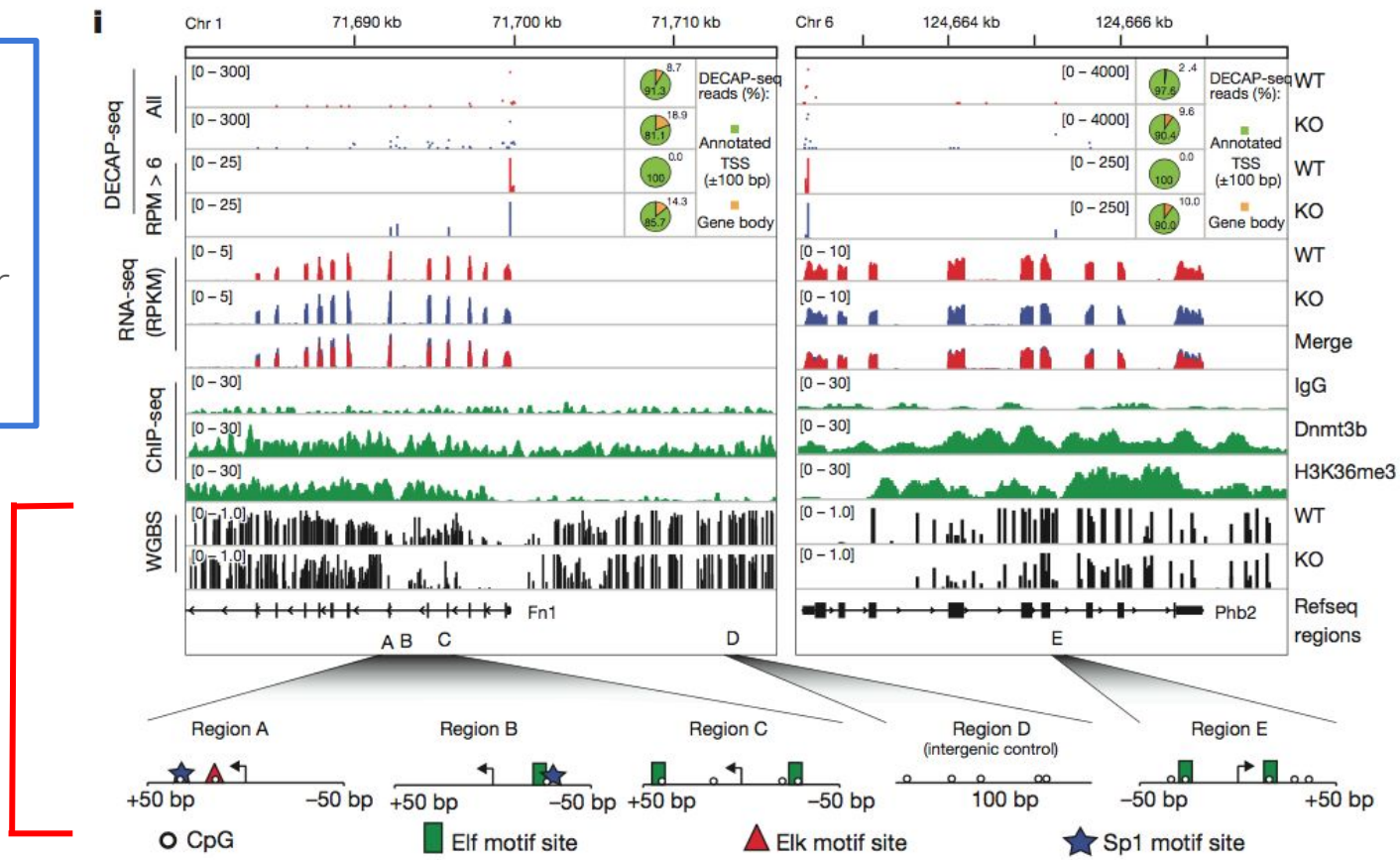
Within 50 bp of dnmt3b dependent TSSs, there is enrichment for CGs and CG-dependent transcription factor binding motifs.

DECAP-Seq : Global View

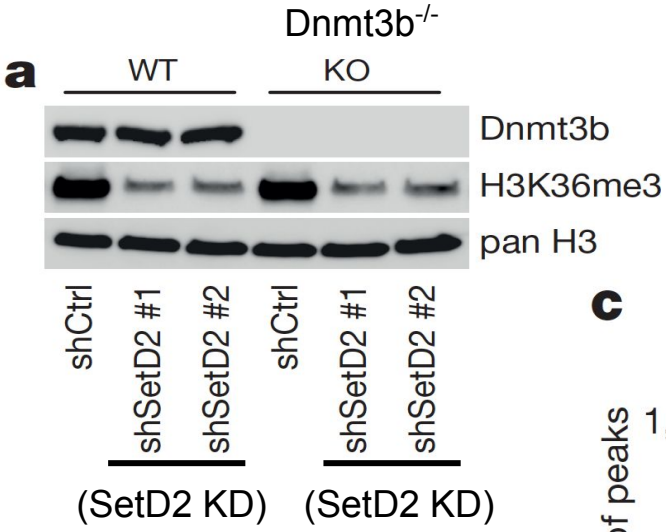


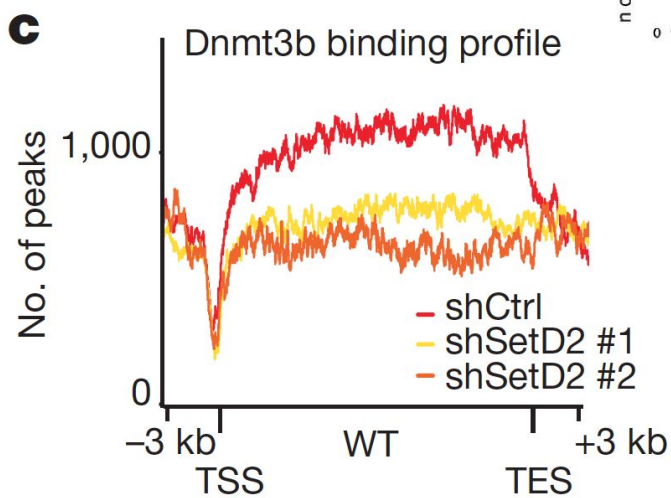
DECAP-Seq : Global View

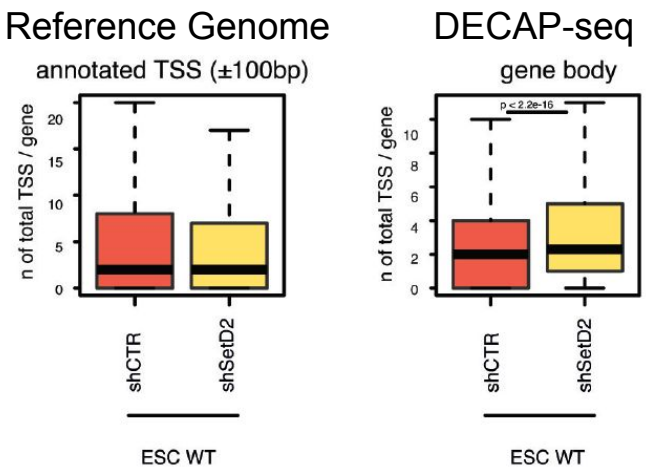
Hypomethylated CpGs are co-localized with transcription factor motifs.



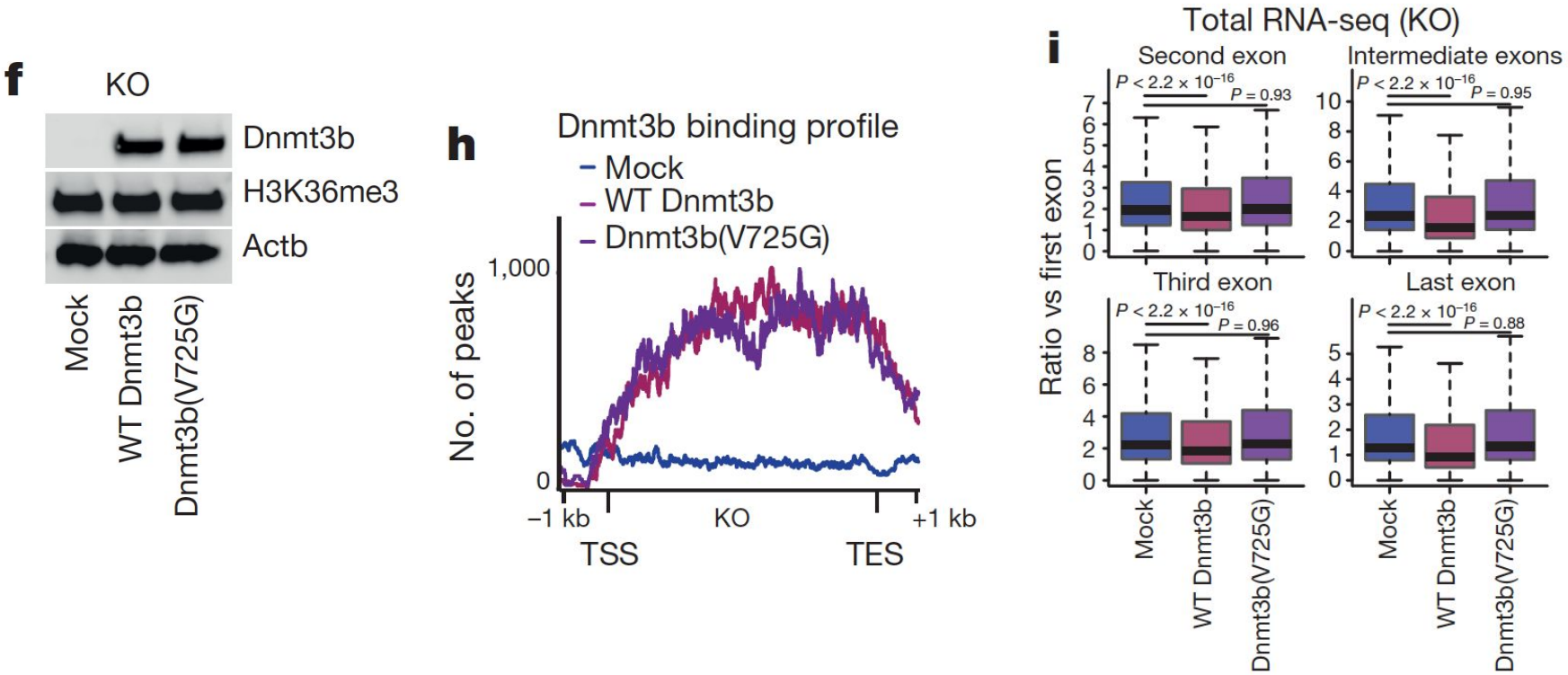
Knockdown of SetD2 results in reduced H3K36me3 and Dnmt3b binding and increased spurious transcription



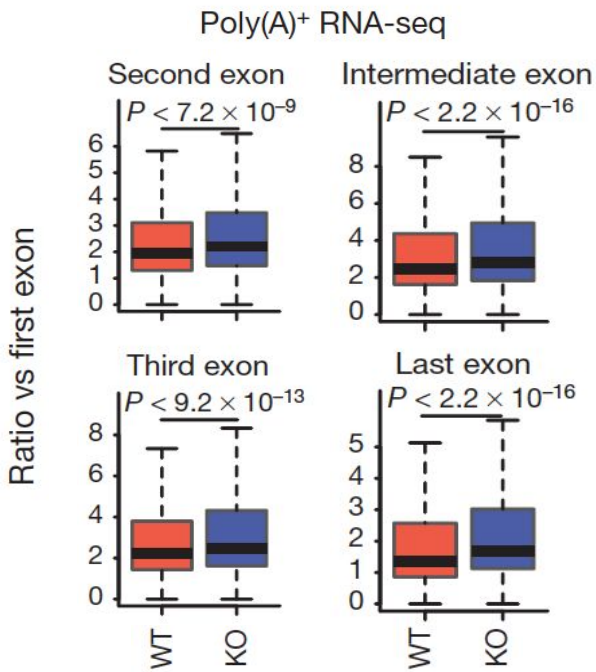


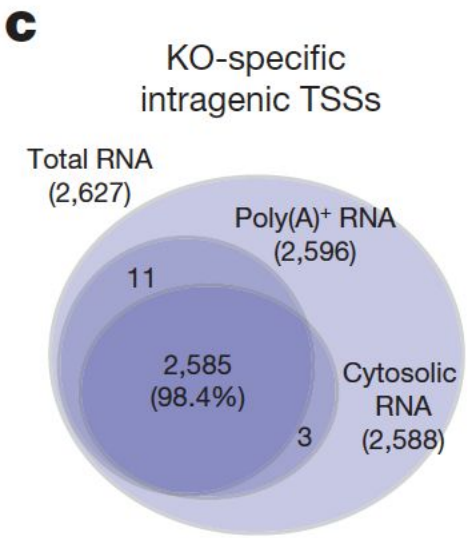


Dnmt3b must be catalytically active for H3K36me3-dependent transcription inhibition

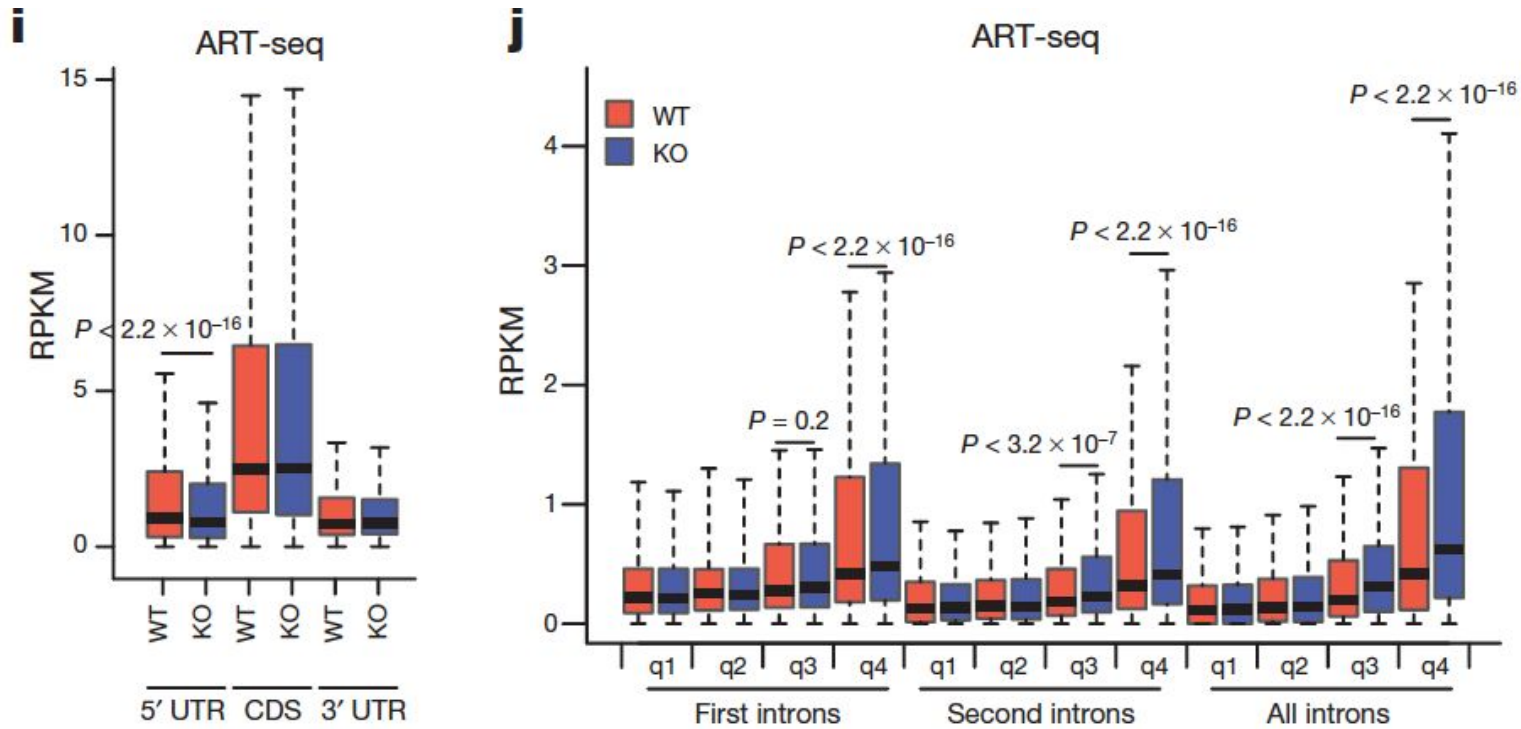


Spurious transcription generates stable, polyadenylated RNAs





Spurious transcripts associate with the ribosome



Summary

-Dnmt3b recruitment dependent on H3K36me3

-Insufficient Dnmt3b results in spurious transcription events on the gene body

-Interaction between Dnmt3b and H3K36me3 is essential for proper transcriptional regulation

-Spurious transcription due to Dnmt3b defects can result in aberrant proteins