Allelic reprogramming of the histone modification H3K4me3 in early mammalian development

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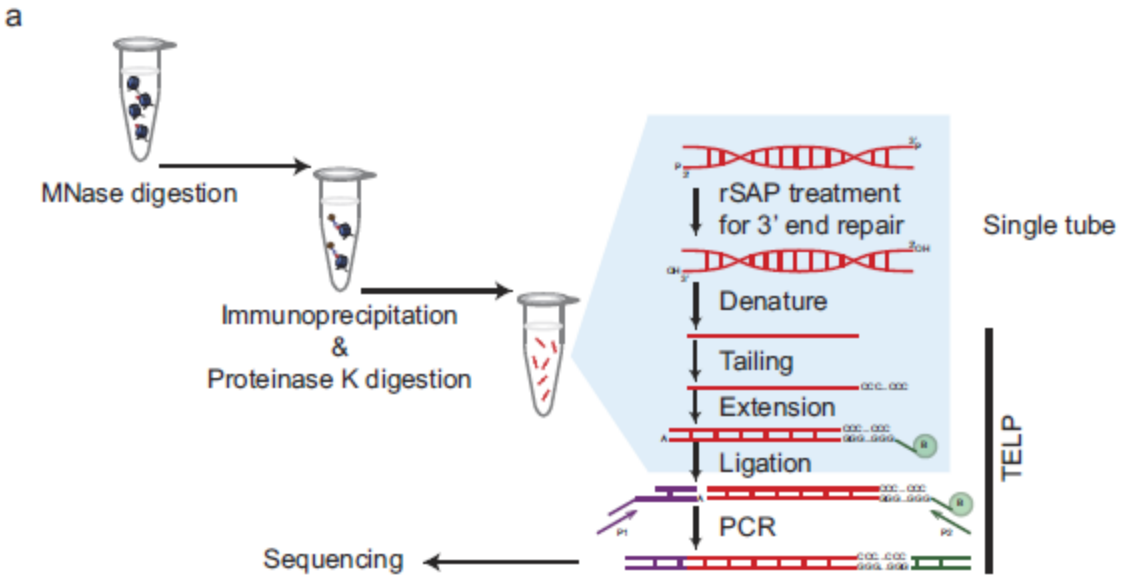
Method and material

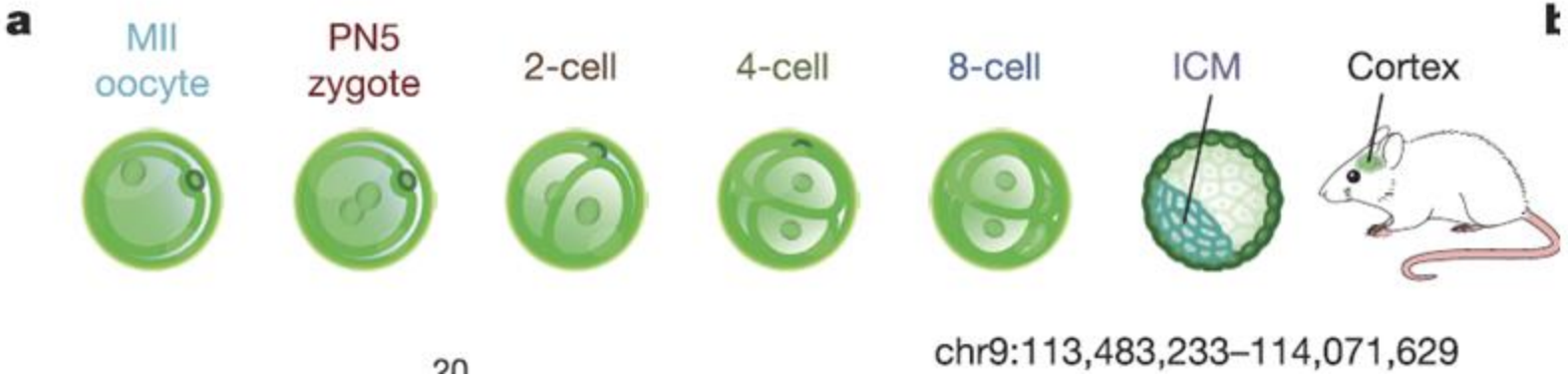
- STAR ChIP–seq (small-scale TELP-assisted rapid ChIP–seq)
- 200 mouse embryonic stem cells
- PWK/PhJ (male) and C57BL/6N(female)



A brief schematic of STAR ChIP–seq.

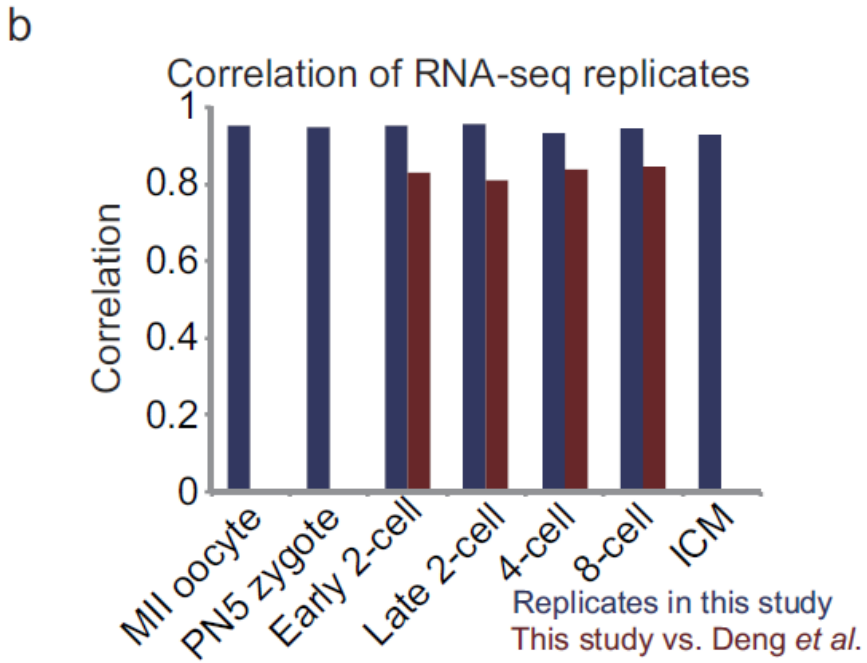
Genome-wide H3K4me3

- metaphase II (MII) oocytes；
- pronuclear stage 5 (PN5) zygotes；
- ZGA/two-cell（early two-cell and late two-cell）；
- four-cell；
- eight-cell embryos；
- inner cell masses (ICMs)；
- cortex

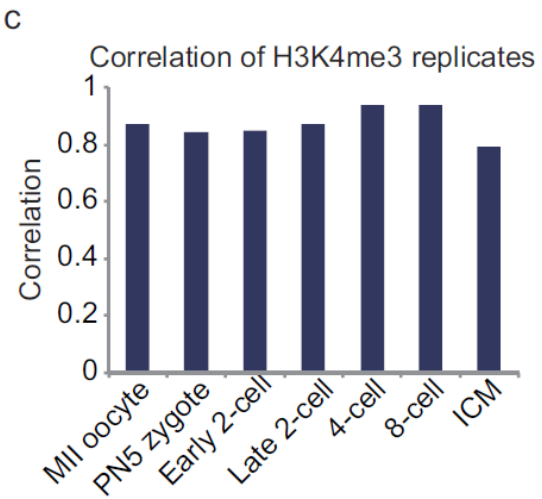


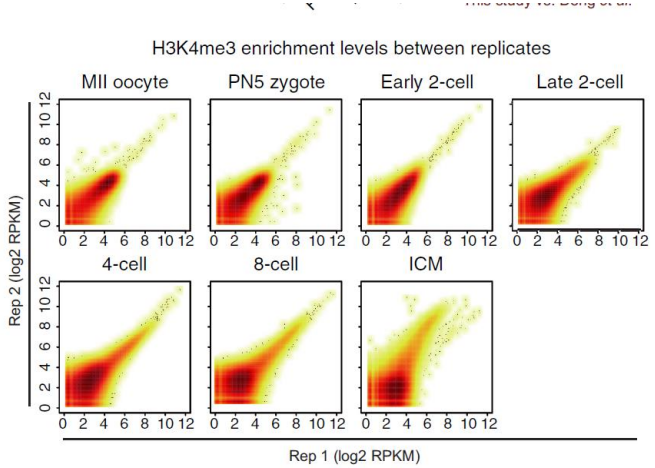
Data Examine

RNA-seq was performed using Smart-seq2 to examine the transcription activities in these samples

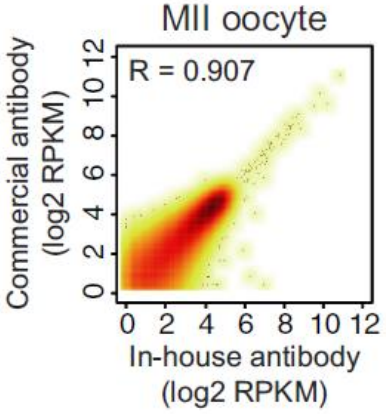


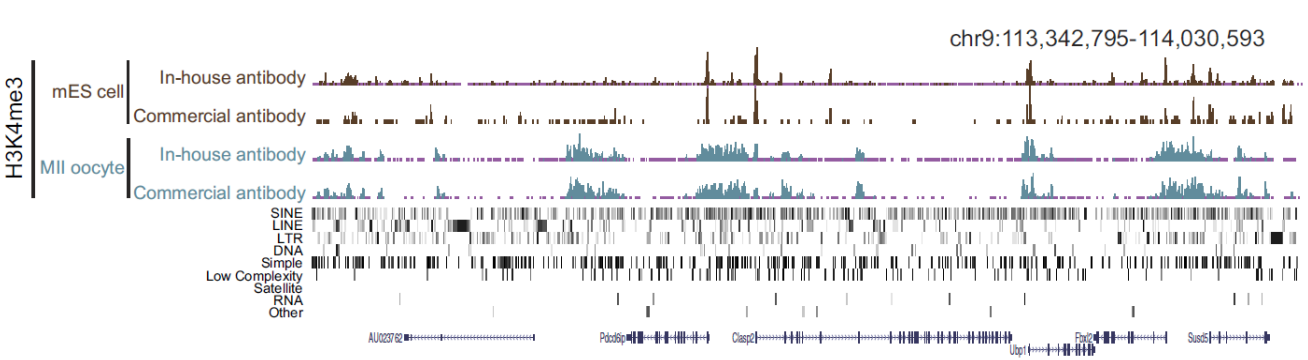
reproducible between replicates

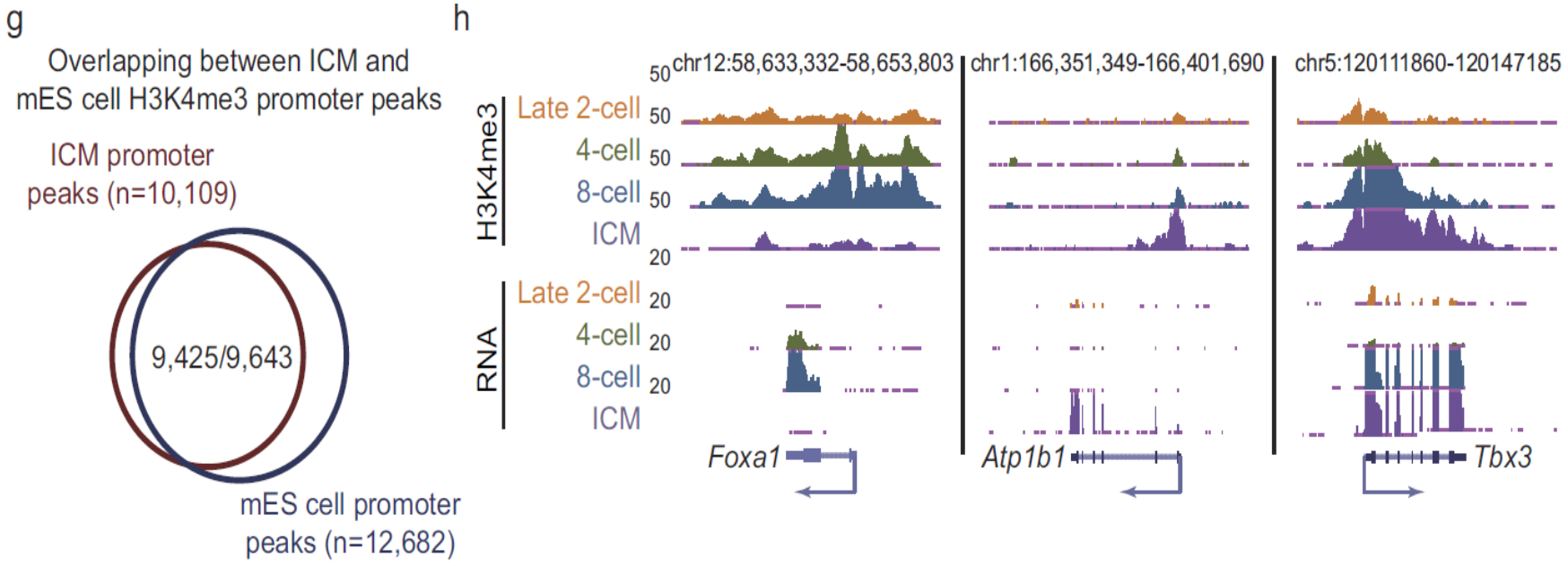




recapitulated using a different antibody





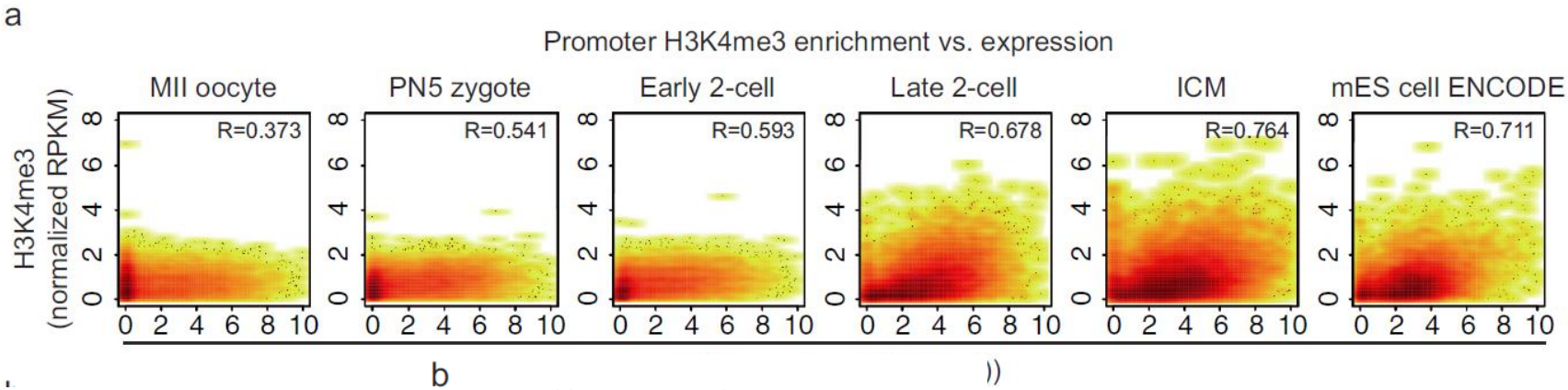


The H3K4me3 peaks in mES cells showed strong overlap with those in ICMs from which mES cells were derived

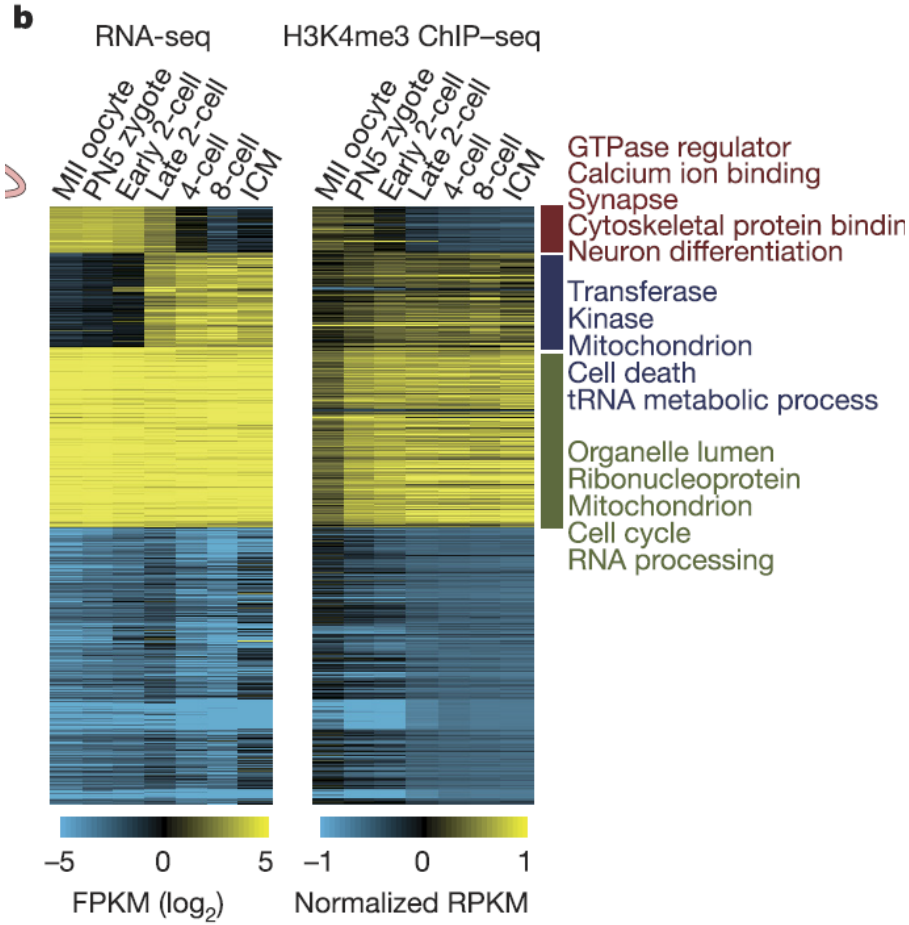
H3K4me3 is correlated with transcription at genes known to be developmentally regulated

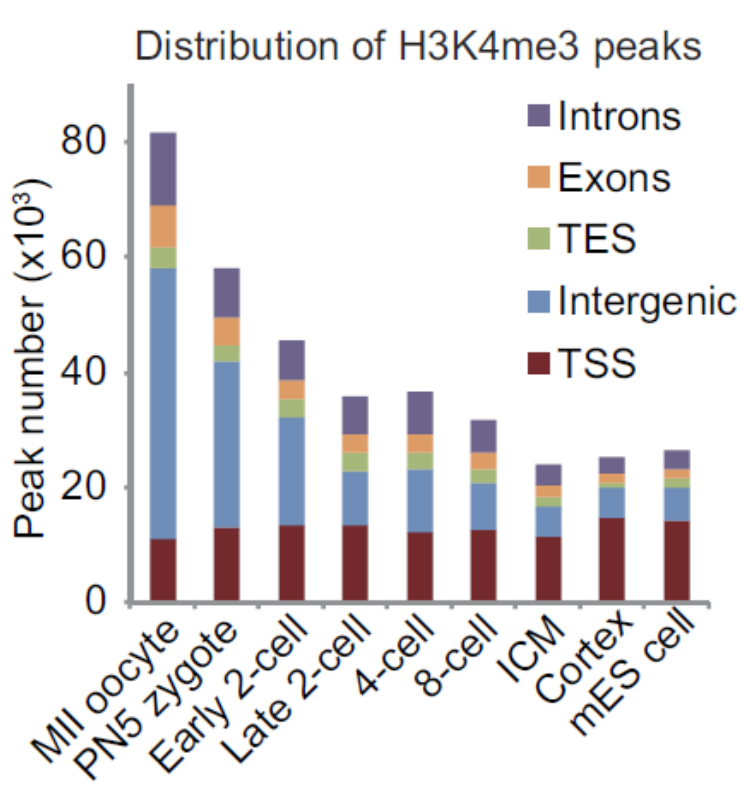
result

- 1、the association between H3K4me3 and transcription genome wide in early embryos

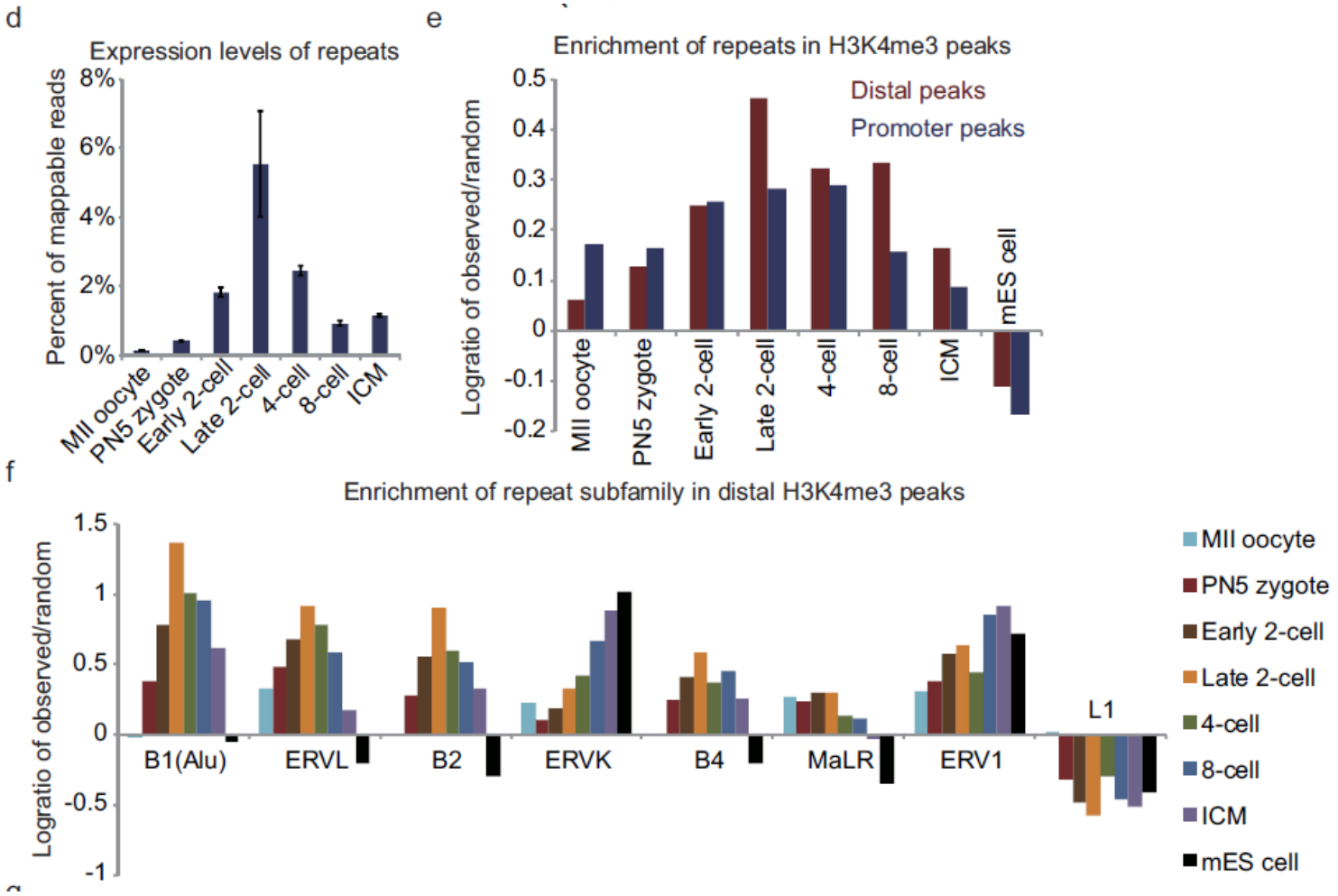


the relationship of H3K4me3 and gene expression by grouping all genes into four clusters



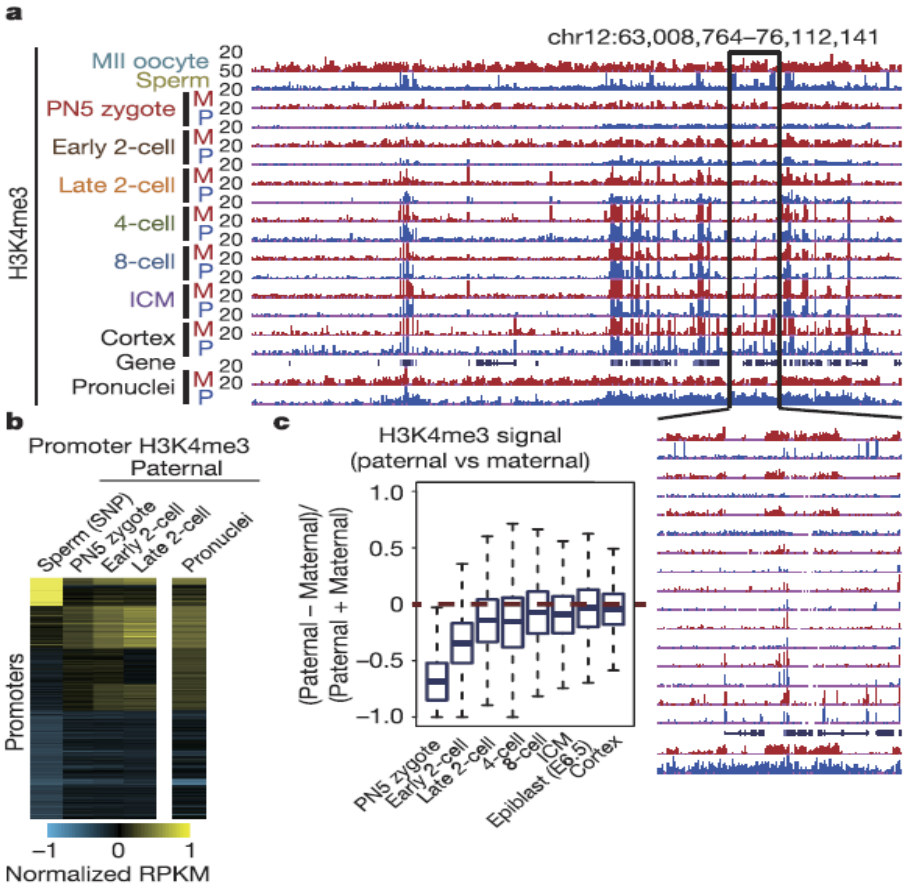


2、distal H3K4me3 peaks



3、Dynamic reprogramming of H3K4me3

1) on the paternal genome

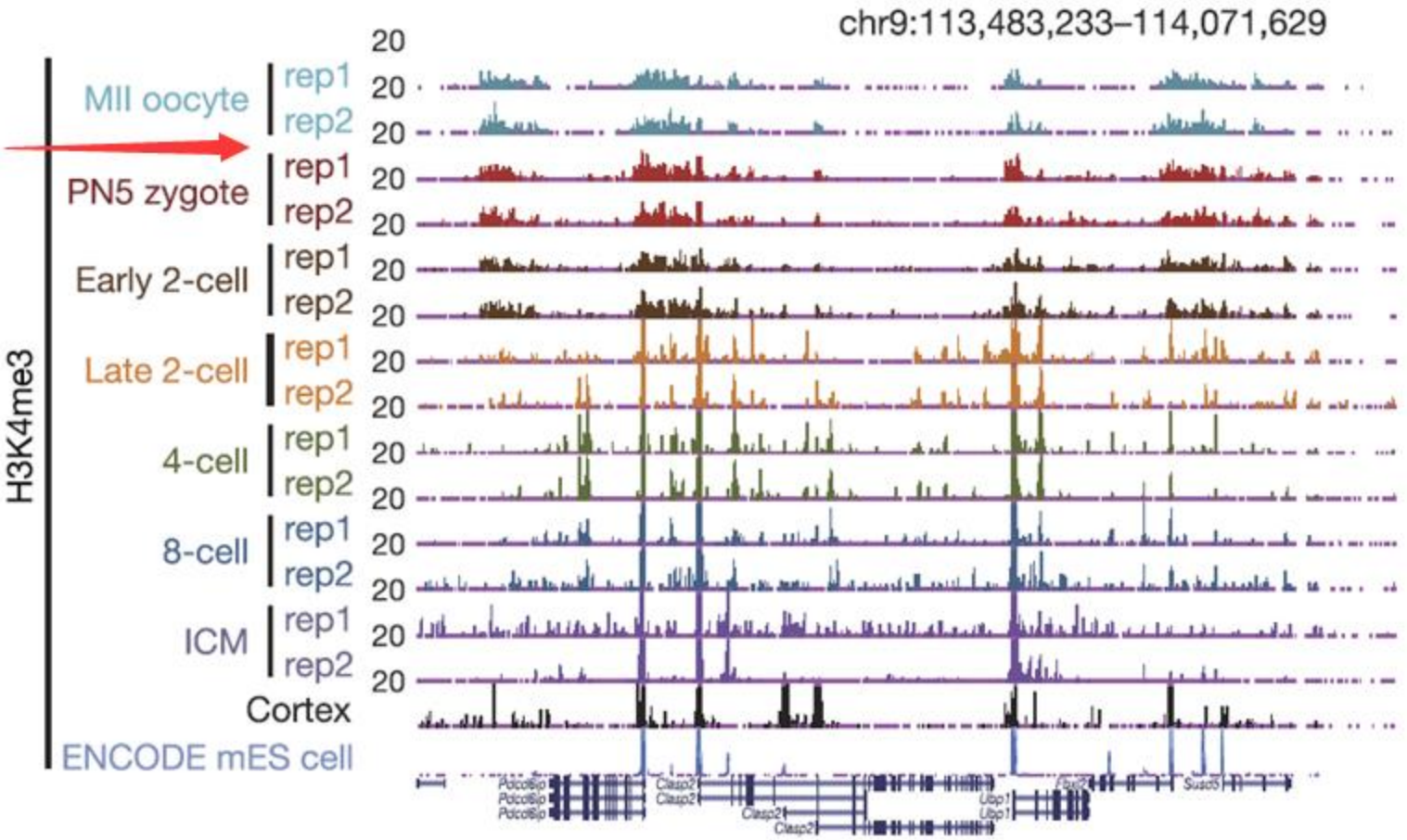


(a) Strong paternal H3K4me3 peaks then reappear particularly the late two-cell stage onward

(b) extensive reprogramming occurs for paternal H3K4me3 after fertilization

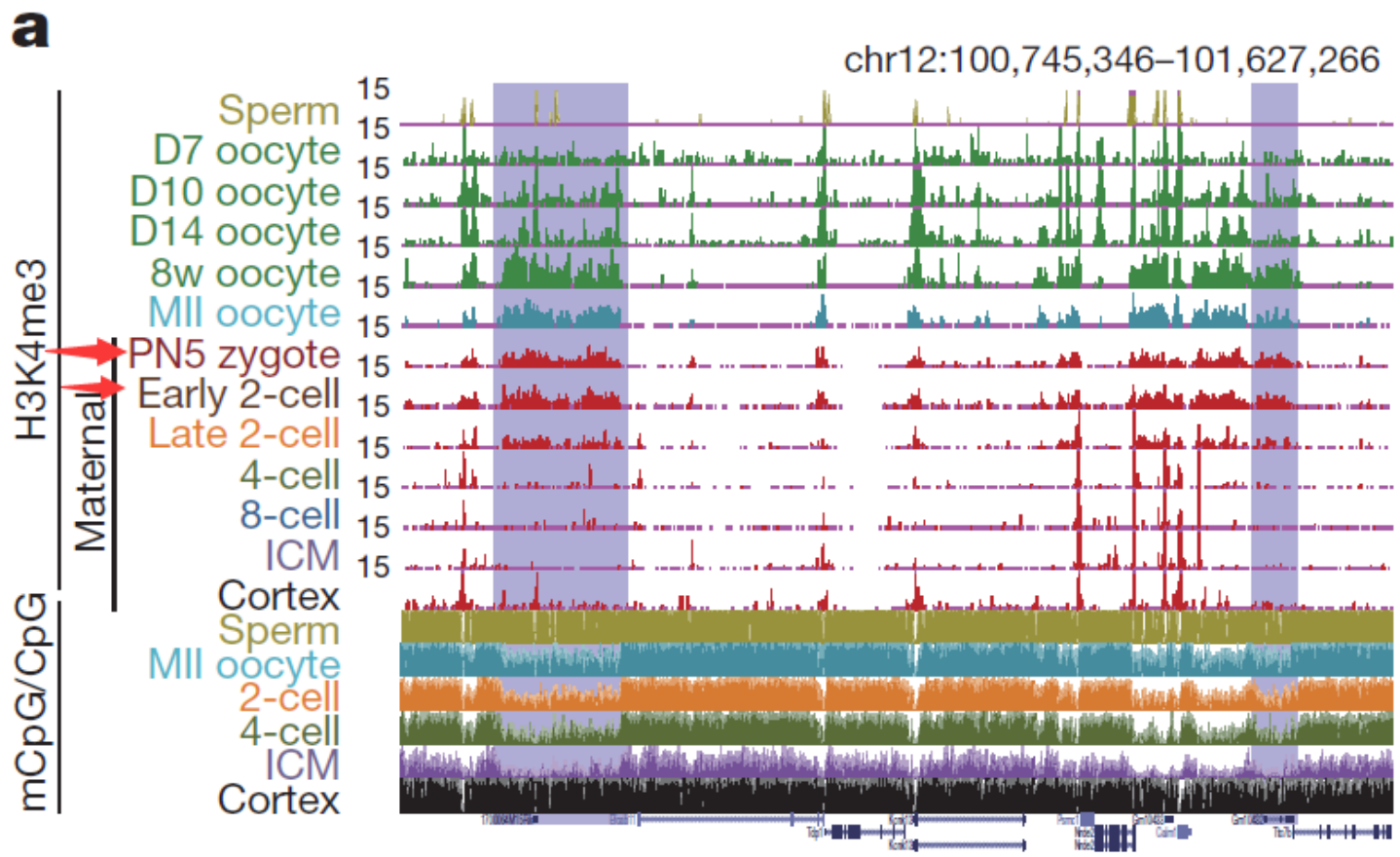
(c)the allelic H3K4me3 enrichment becomes comparable in post-implantation embryos

2） on the maternal genome



Two patterns： ‘non-canonical H3K4me3’ (ncH3K4me3)

‘canonical H3K4me3’ patterns



(a) The similarity indicate possible inheritance of ncH3K4me3 from oocytes

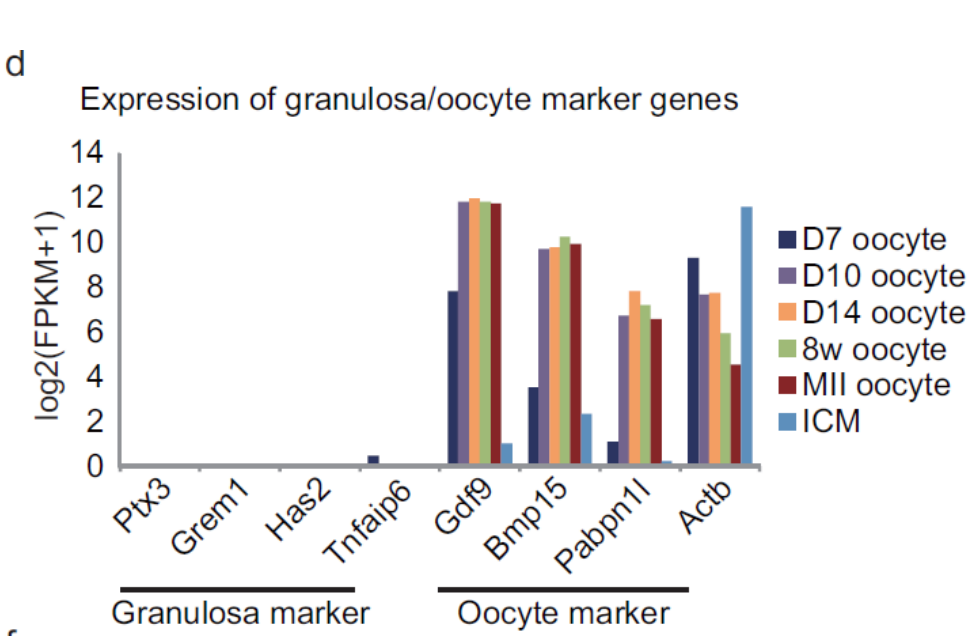
(b) ncH3K4me3 in distal regions is significantly reduced in the late two-cell embryos and is nearly erased by the four-cell stage

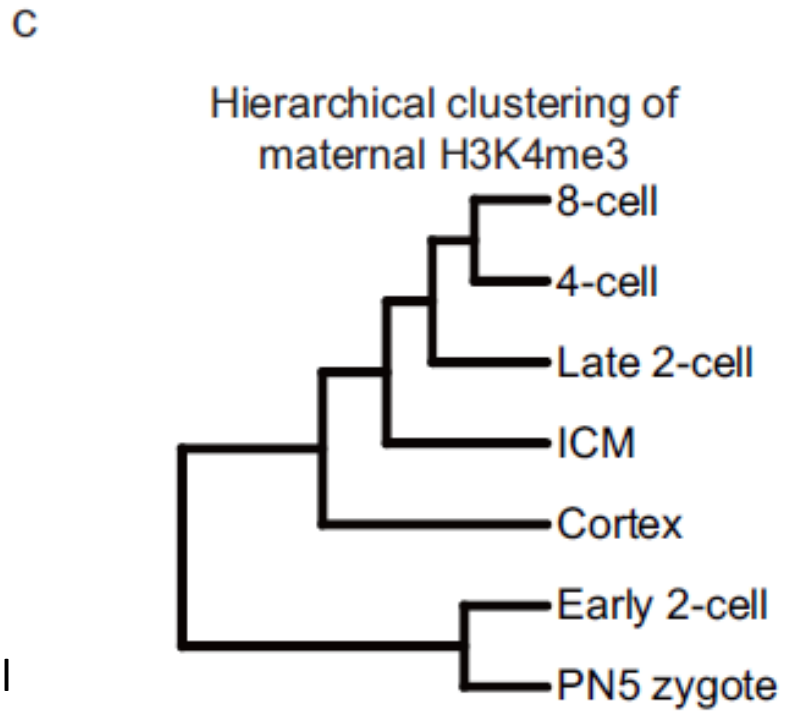
(c) promoter H3K4me3 starts to adopt a canonical form from the late two-cell stage

(d) indicating possible involvement of both global demethylation and methylation

4、ncH3K4me3 established during oogenesis

1）Established stage

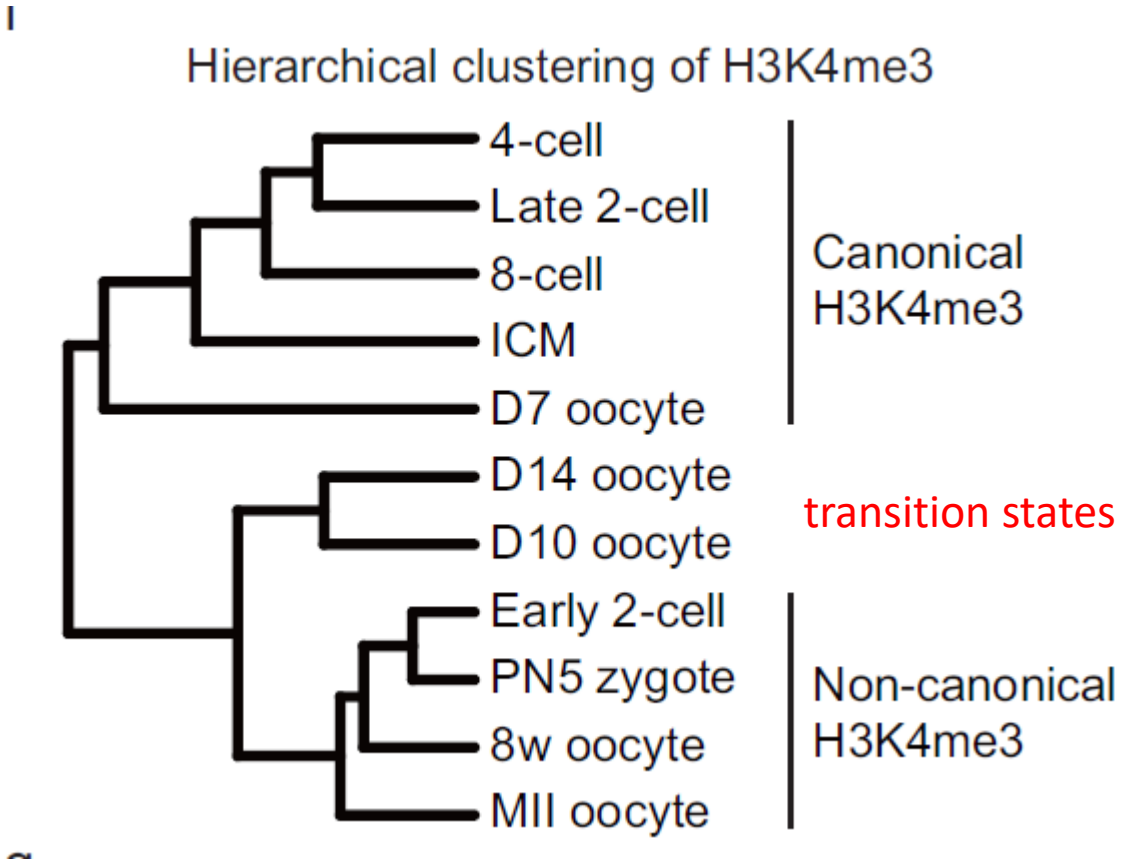




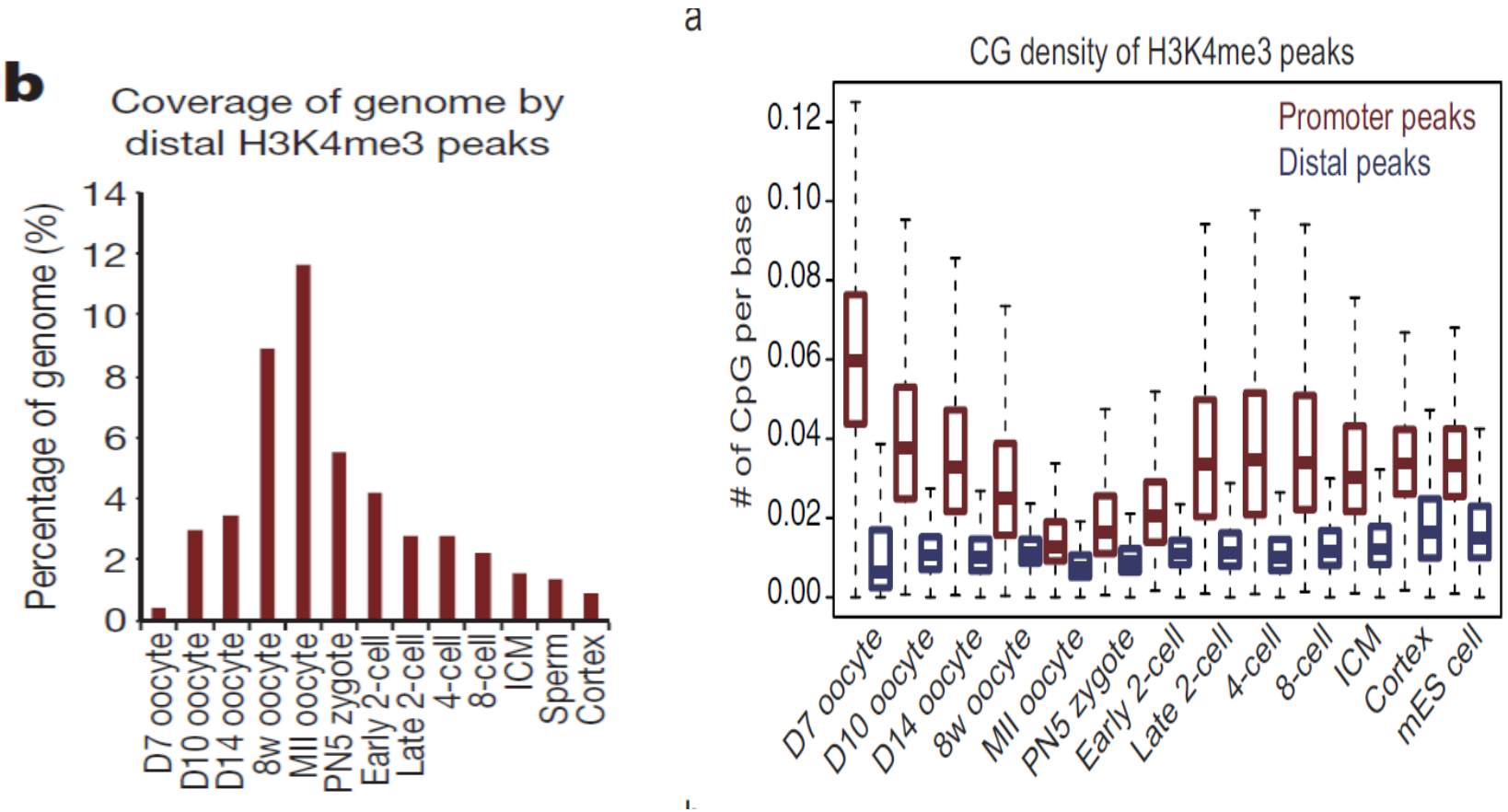
day 7–14 full-grown oocytes-2-cell

The switch of maternal ncH3K4me3 to canonical patterns at the two-cell stage

the global ncH3K4me3 pattern is readily established in full-grown oocytes

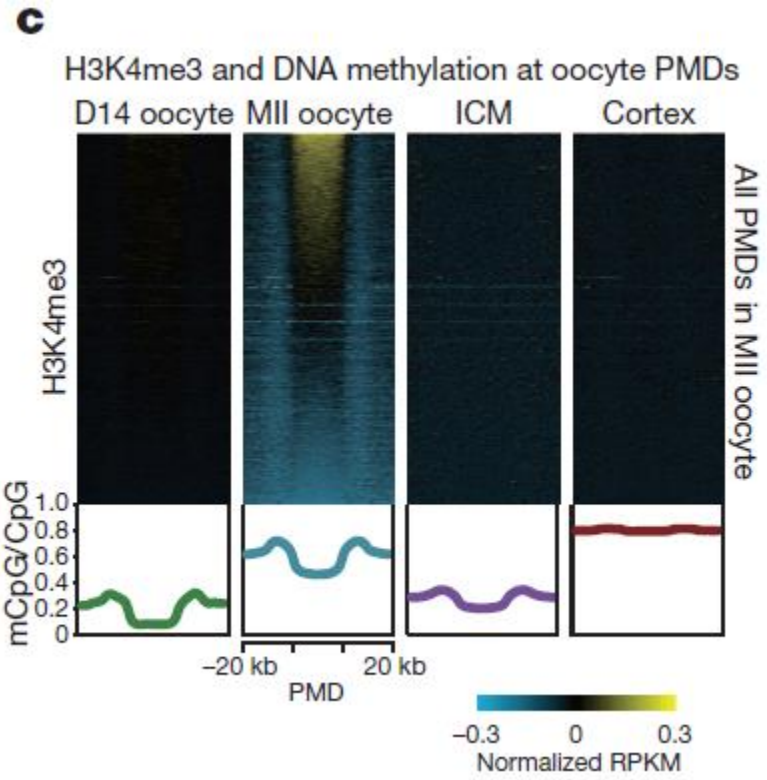

Hierarchical clustering of H3K4me3
4-cell
Late 2-cell
8-cell
ICM
D7 oocyte
D14 oocyte
D10 oocyte
Early 2-cell
PN5 zygote
8w oocyte
MII oocyte
Canonical H3K4me3
transition states
Non-canonical H3K4me3

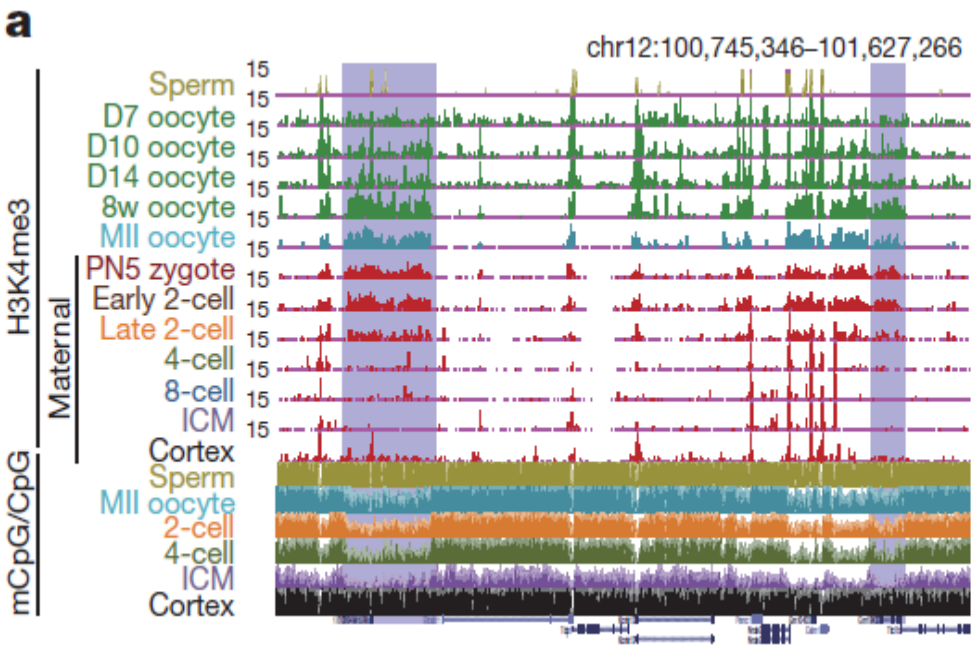
2）distal ncH3K4me3 peaks during oocyte development



These peaks cover large fractions of the genome in grown and mature oocytes preferentially in low CG regions

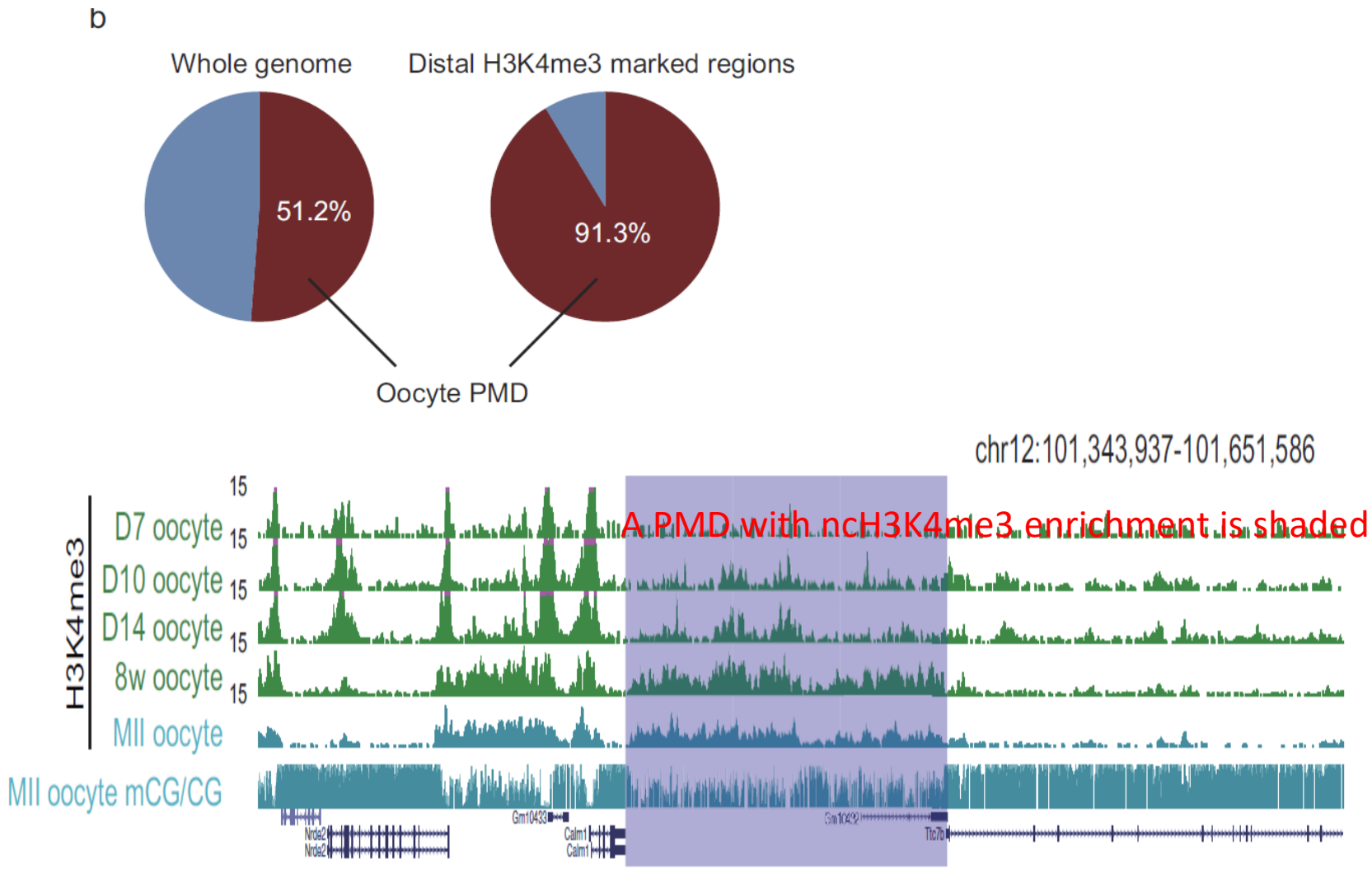
3）association with PMDS





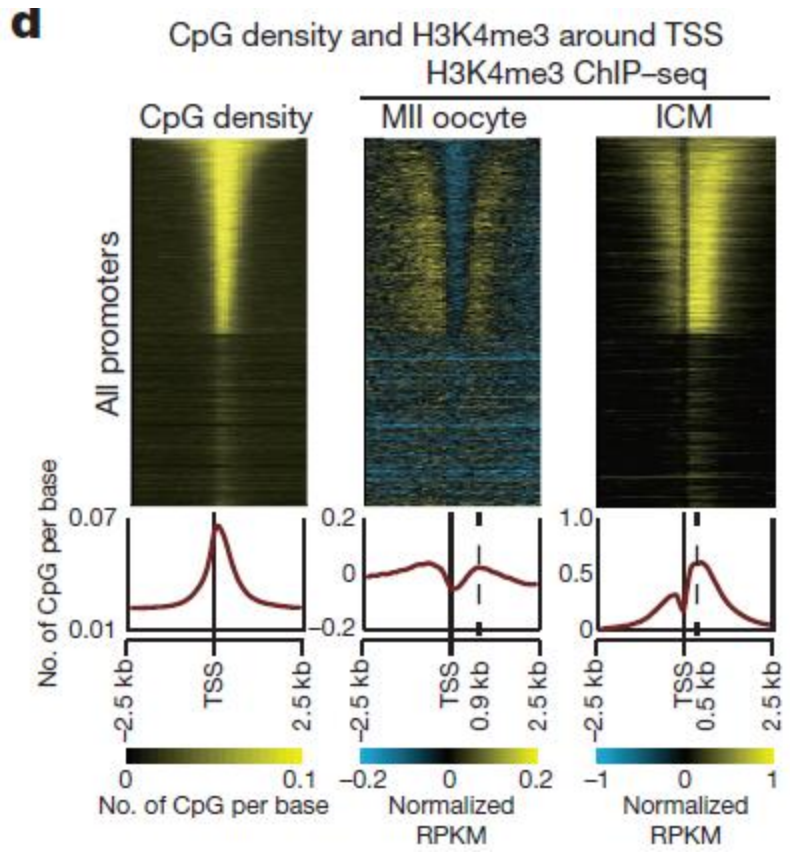
distal H3K4me3 almost exclusively overlaps with DNA hypomethylated regions in MII oocytes

'partially methylated domains' (PMDs)：
intergenic regions and non-transcribing gene bodies are poorly methylated



distal ncH3K4me3 is highly correlated with PMDs in oocytes

4）ncH3K4me3 in promoter



The promoter ncH3K4me3 shows highest enrichment in CpG-poor loci flanking the TSSs

In all， both promoter and distal H3K4me3 demonstrate non-canonical patterns in MII oocytes

5、validation

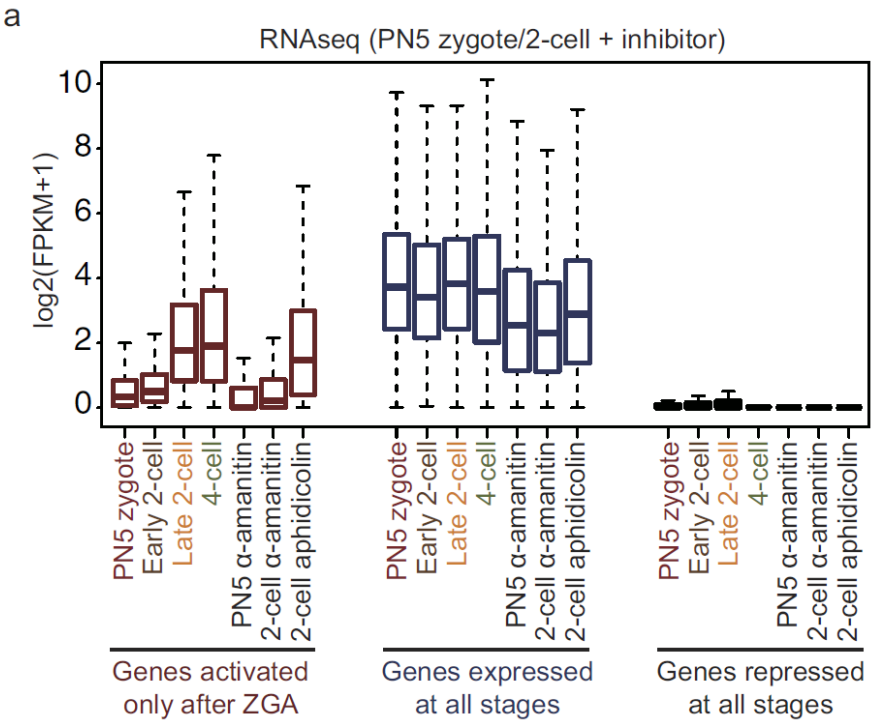
As ncH3K4me3 appears to be replaced by canonical H3K4me3 in the late two-cell embryos which coincides with major ZGA,

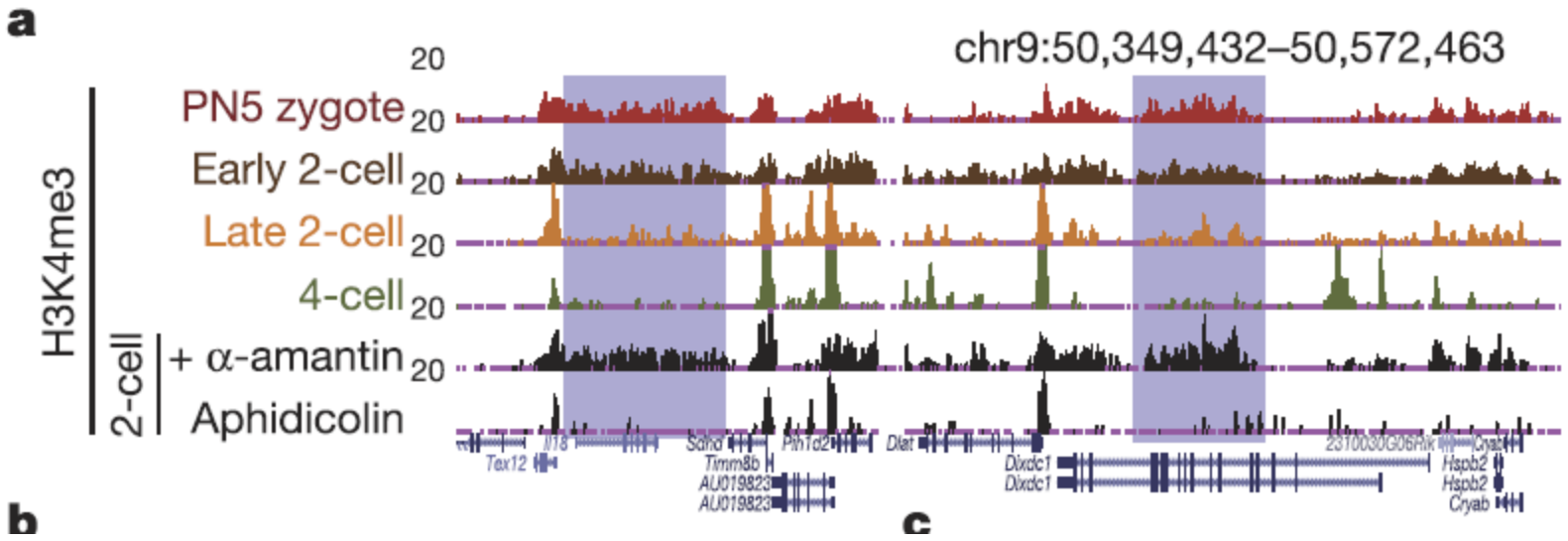
1）we asked if such reprogramming depends on zygotic transcription

inhibited transcription by treating embryos with α –amanitin（票菌素） before major ZGA (starting from the stages of late zygote or early two-cell

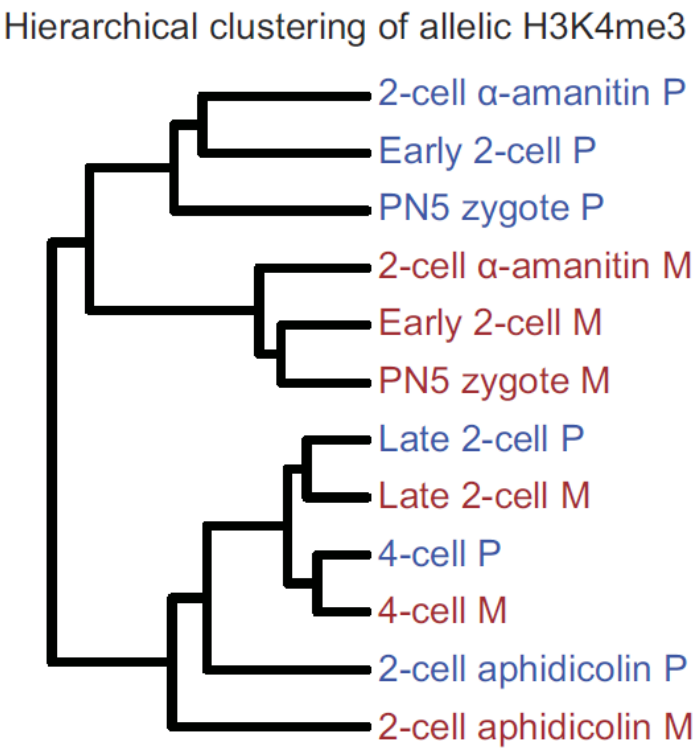
2）We then asked if the loss of ncH3K4me3 is caused by active histone demethylation or passive dilution in cell division

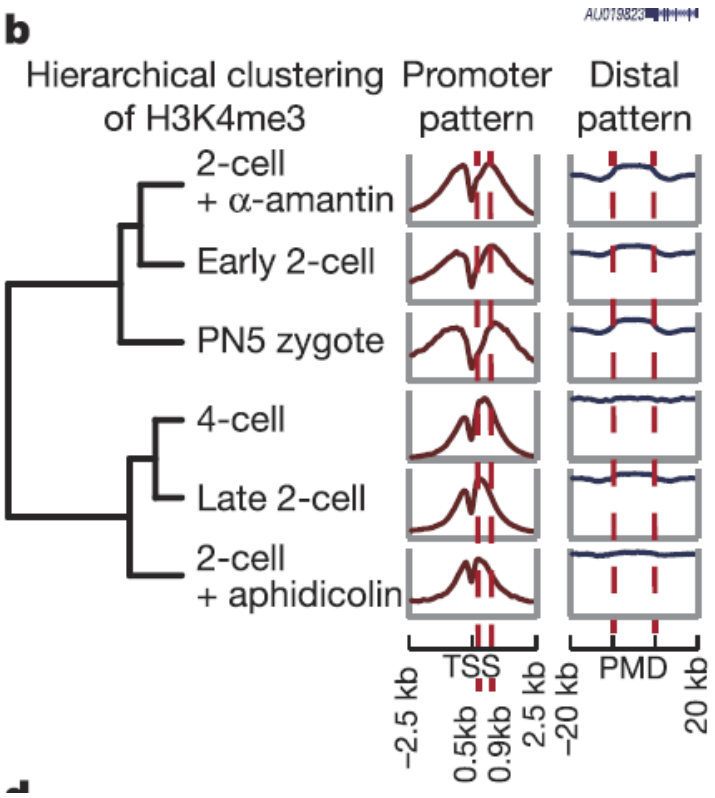
treated late zygotes/early two-cell embryos with aphidicolin（阿非地霉素） which arrested embryos in the S phase at the two-cell stage





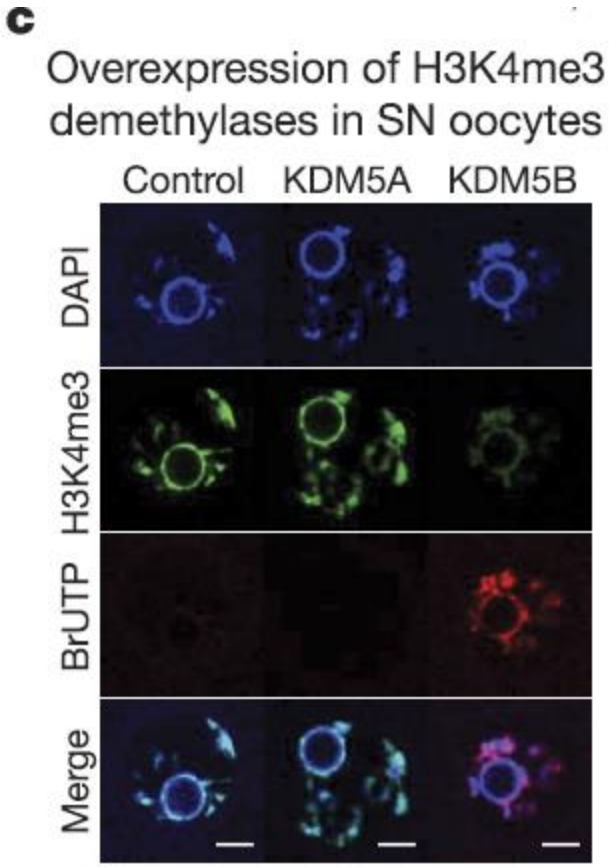
the reprogramming of H3K4me3 at the two-cell stage requires zygotic transcription





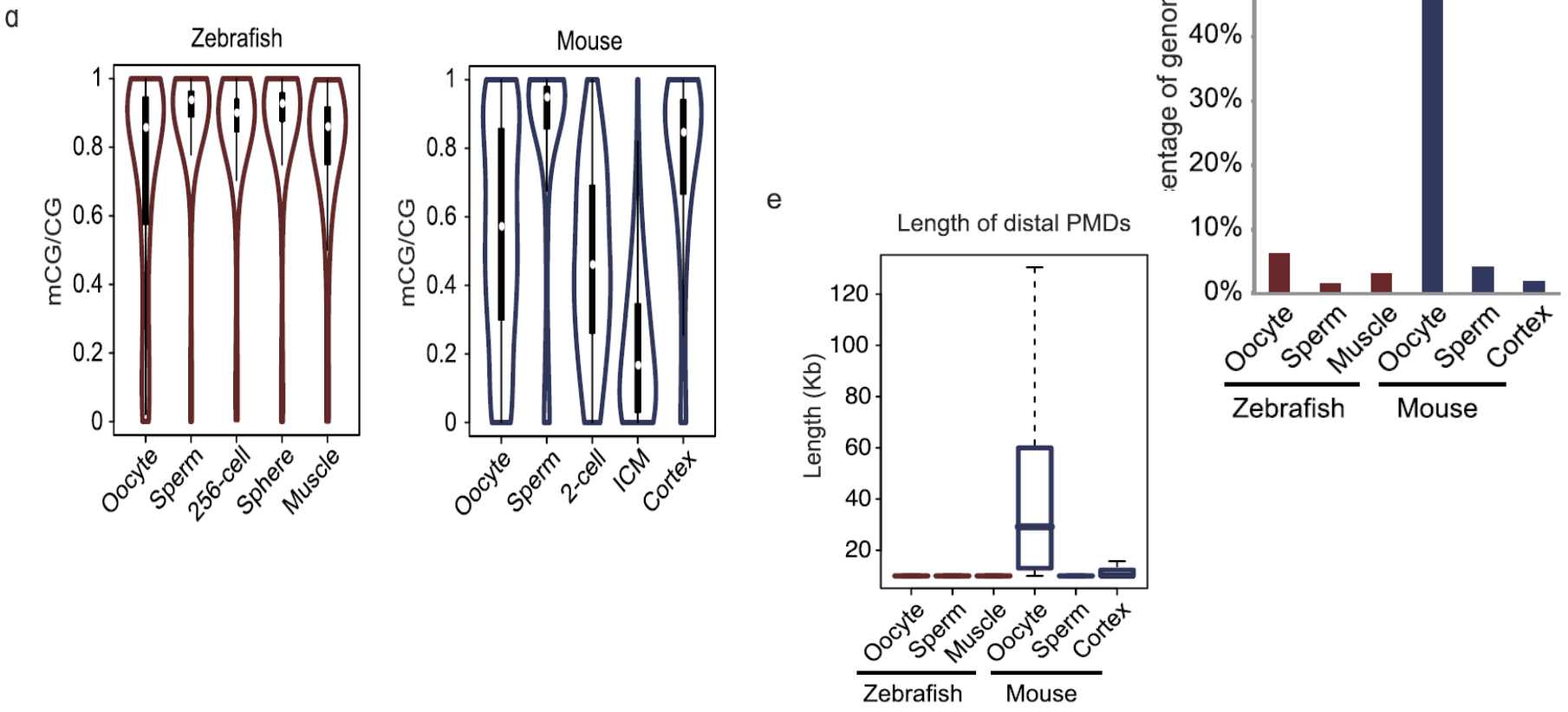
the erasure of ncH3K4me3 in the two-cell embryos probably requires active demethylation but not passive dilution

3）the absence of KMT2B



These data indicate that ncH3K4me3 may play a role in genome silencing during oogenesis.

4）ncH3K4me3 in zebrafish



The absence of ncH3K4me3 in zebrafish oocytes may be related to the characteristics of their DNA methylomes which generally lack PMDs

The strong correlation between the presence of PMDs and ncH3K4me3 indicates that the reprogramming of histone modifications and DNA methylation is inherently linked

conclusion

- The ncH3K4me3 pattern appears to be inherited from oocytes to early embryos before ZGA
- Extensive reprogramming also occurs on the paternal genome in zygotes where H3K4me3 peaks are largely depleted
- very broad H3K4me3 domains at weak levels were observed which could be considered as a type of paternal ncH3K4me3

- distal ncH3K4me3 can work as ‘molecular sponges’ by absorbing and sequestering transcriptional resources to prevent spontaneous and inadvertent transcription
- Eliminating ncH3K4me3 may be important for restoring the quiescent genome back to the active state during ZGA

- **Innovation：1）**not only discover a highly dynamic landscape of modified histones in pre-implantation development, but also shed light on the fundamental mechanisms for inheritance and reprogramming of epigenetic modifications；2）Star chip-seq
- **Shortcomings：** only the example of zebrafish oocytes is thin