





ERK1/2

5mC 2i

## RESULTS

### Epigenome of Ground State ESCs

To address these questions we carried out genome-wide bisulphite sequencing (BS-seq) and transcriptomics (RNA-seq), comparing ESCs either grown in serum or switched from serum to 2i conditions for 24 days. 2i induced a striking loss of DNA

pattern of demethylation is also characteristic of PGCs during their migration phase (Seisenberger et al., 2012), while ICRs and germline-specific genes are demethylated upon their arrival in the gonads (Seisenberger et al., 2012; Guibert et al., 2012; Hajkova et al., 2002). As in PGCs, global demethylation in 2i did not result in promiscuous transcription of demethylated genes (Figure 2A). Indeed, a substantial number of genes in serum cultured ESCs have promoters that are highly methylated with the majority of them being transcriptionally silenced (blue group of promoters in Figure 2A); while these promoters are demethylated in 2i, this does not result in upregulation of the associated genes.

Rapid Dnmt3b Transcriptional Changes Are Effected  
through a 2i-Responsive *cis*-Element  
Examining



binding sites (Figure S1



the transition to epiblast. This suggests that early ICM cells resemble 2i ESCs in their ground state epigenome, while later stage ICM cells resemble serum ESCs before transiting to an





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