Title: Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species

Authors: Dan Xie, Chieh-Chun Chen, Leon M. Ptaszek, Shu Xiao, Xiaoyi Cao, Harris A. Lewin, Chad Cowan, Sheng Zhong

Supplementary Figures

Figure S1. Clustering homologous genes in three species. Blue clusters exhibit conserved expression patterns in all three species. Green clusters exhibit conserved expression in two of the three species. Red clusters exhibit different expression in every species.

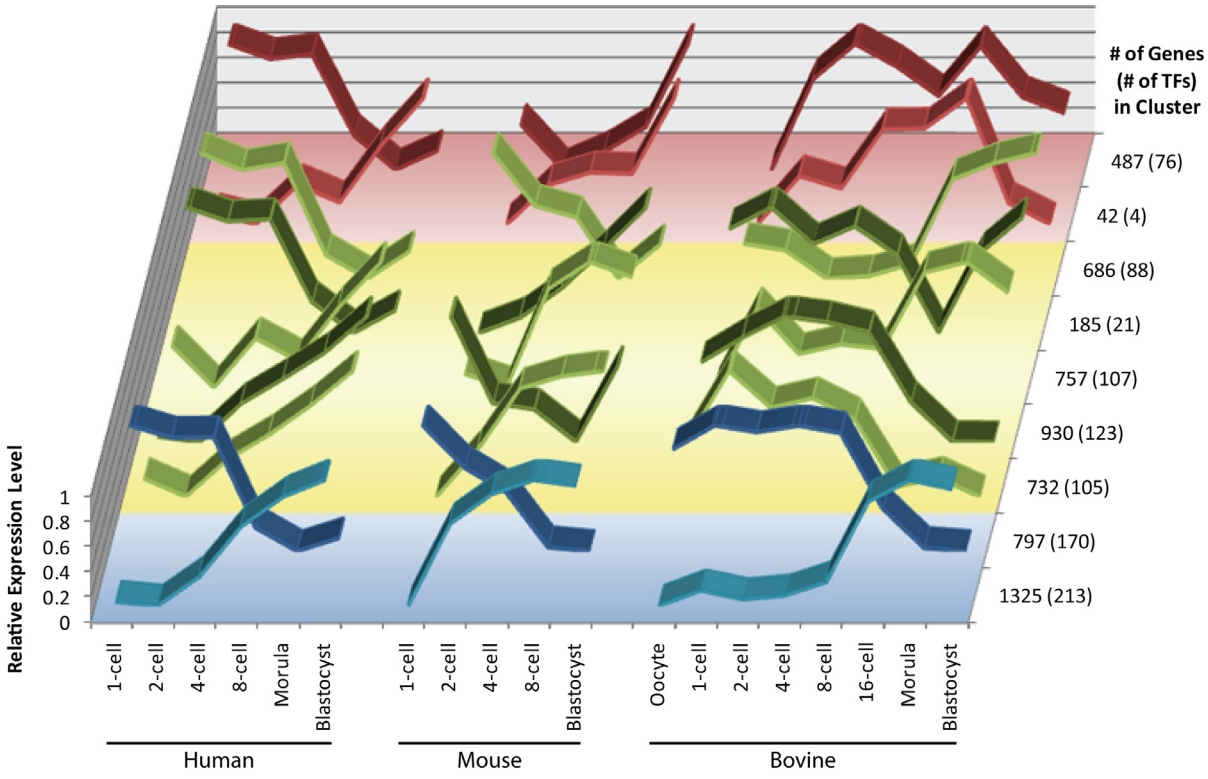
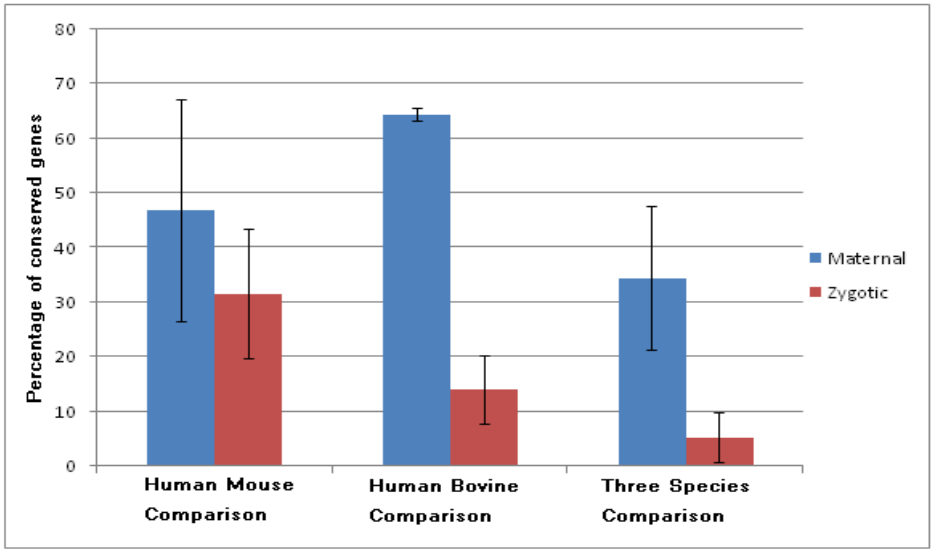


Figure S2. Re-wiring of ZGATs by TFBS usage. (A) Conservation levels of MT and ZGA genes. The percentages were calculated based on the total numbers of human MT and ZGA genes. The error bars reflect standard errors computed by varying the thresholds for detecting MT and ZGAT. (B) Intersection of human and mouse genes upregulated during ZGA with the genes bound by ZGA-related TFs in human and mouse ES cells.

A



B

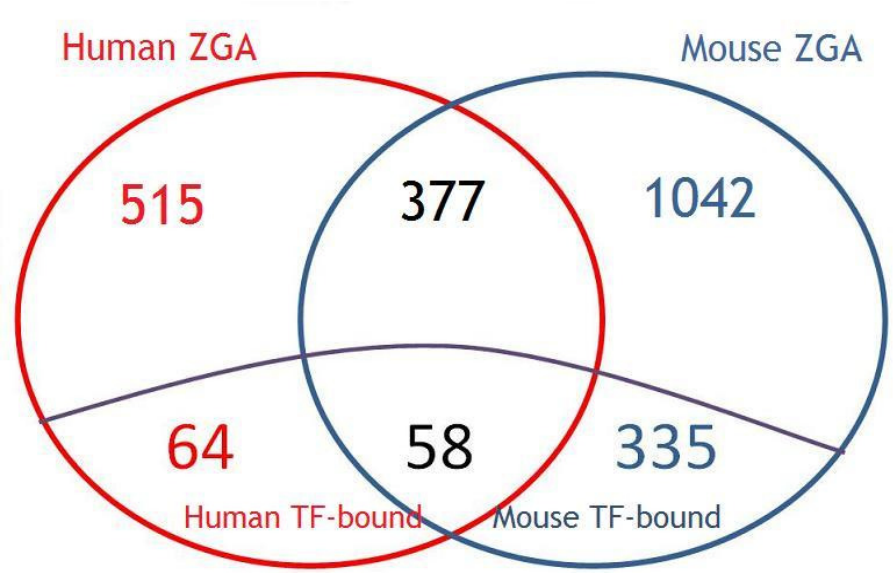


Figure S3. Estimated insertion times of two transposons into the mouse genome. Lx8 and Orr1b1-int are both murine-specific transposons, which were inserted to the murine genomes about 60 and 40 million years ago. Lx8 carries a Pou5f1 binding site, and Orr1b1-int carries Nanog and Pou5f1 binding sites.

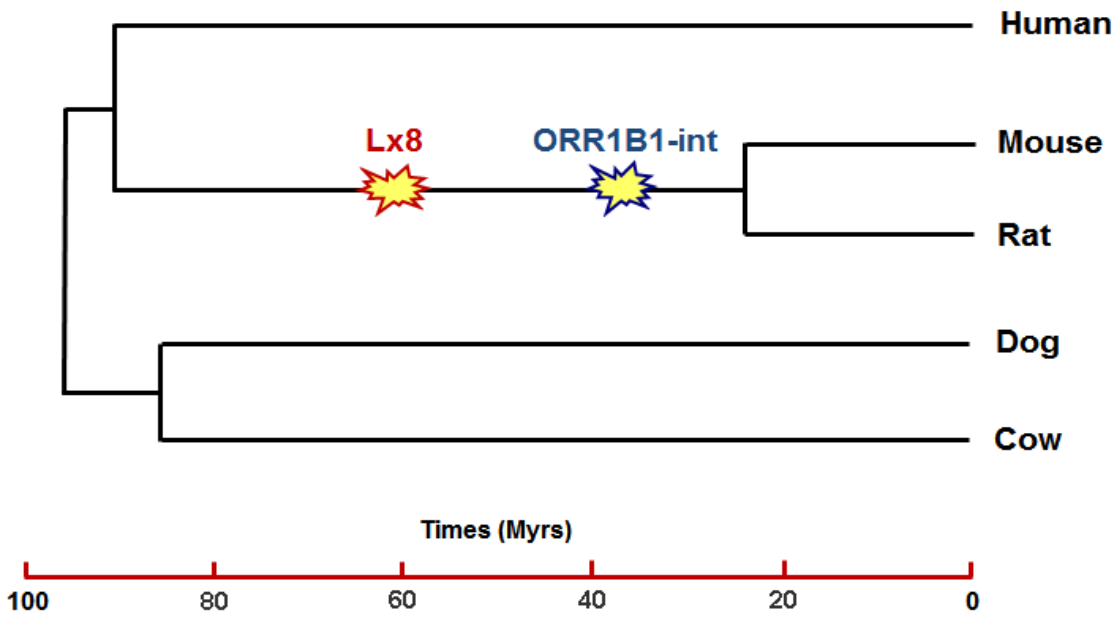


Figure S4. Lack of expression of the mouse *Frat2* gene is due to a mutation on its Pou5f1 binding site. (A) Both human and bovine *FRAT2* genes respond to the upregulation of *POU5F1* in the late stages of preimplantation. But the mouse *Frat2* gene is not transcribed at any time during PED. (B) POU5F1 and SOX2 binding in the *FRAT2* promoter is detected in human ES cells, but neither ChIP-PET nor ChIP-seq detected any binding of Pou5f1 or Sox2 near the *Frat2* gene in mouse ES cells. (C) The human POU5F1 and SOX2 binding regions contain POU5F1 and SOX2 binding sites that match the POU5F1 and SOX2 binding motifs. (D) The loss of Pou5f1 binding in the mouse is consistent with the mutation (A to C) at the central position in the Pou5f1 binding site. Primates (humans and rhesus monkeys) carry another mutation (T to A) at the second position of the binding site, which makes the primate binding sites more similar to the POU5F1 consensus sequence. This is consistent with the larger induction of *FRAT2* expression in the human than in the bovine.

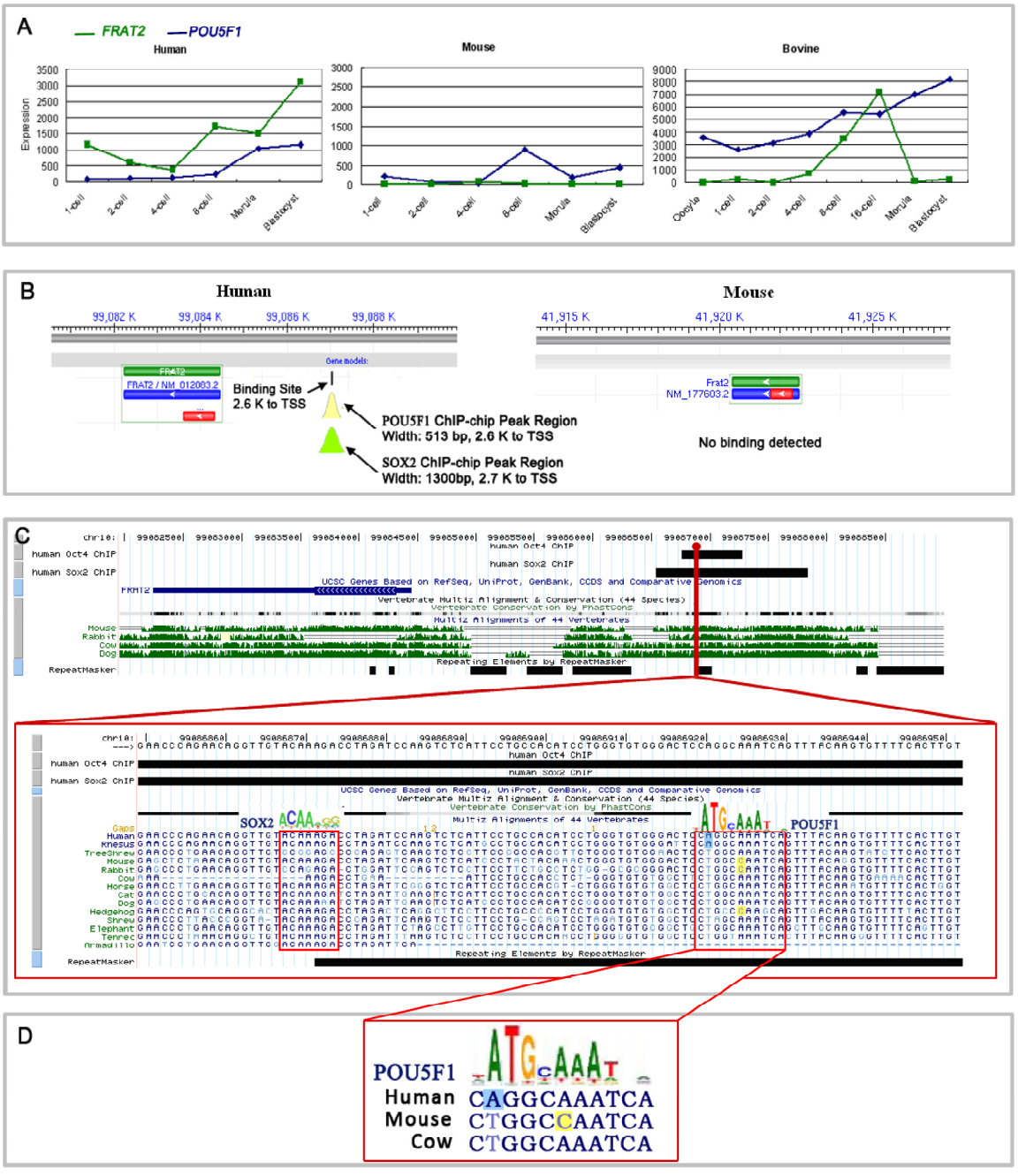


Figure S5. Protein domains and expression levels of SOX2 and HMGB1. (A) One and two HMG-boxes were detected on the SOX2 and HMGB1 proteins by InterPro. No other protein domain was found on these proteins. (B) Gene expression levels measured by real-time PCR. Two replicates of mouse E14 ES cells, human H1 ES cells and bovine morula embryos were analyzed. Gene expression levels were first normalized by ACTB expression levels and then by the first replicate of SOX2 in E14 cells.