

# ***Zfp57* inactivation illustrates the role of ICR methylation in imprinted gene expression during neural differentiation of mouse ESCs**

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## **SUPPLEMENTARY INFORMATION**

## Legends to supplementary Figures

**Supplementary Figure 1. Immunofluorescence (IF) staining of Nestin and Pou5f1(Oct3-4) in wild-type and *Zfp57*<sup>-/-</sup> at d12 JB1 cells of cyclopamine-mediated differentiation.** Representative images of undifferentiated and mESC-derived neural precursor wild-type and *Zfp57*<sup>-/-</sup> JB1 cells examined by immuno-staining using anti-Nestin and anti-Pou5f1 antibodies. Nuclei were stained with DAPI. Merge: Nestin/ Oct3-4/DAPI signals. Scale bars, 100  $\mu$ m

**Supplementary Figure 2. Differentiation and imprinted genes expression analysis of wild-type and *Zfp57*<sup>-/-</sup> inbred cells.** (a) Representative images of undifferentiated and mESC-derived neural precursor wild-type and *Zfp57*<sup>-/-</sup> cells examined by immuno-staining using anti-Nestin and anti-Pou5f1 antibodies. Nuclei were stained with DAPI. Merge: Nestin/ Oct3-4/DAPI signals. Scale bars, 100  $\mu$ m. (b,c) Expression analysis of imprinted genes (b,c) in wild-type and *Zfp57*<sup>-/-</sup> E14 cells at day 0 and day 12 assayed by quantitative RT-qPCR. The histograms show the average gene expression levels of three independent experiments, after normalization against the level of  $\beta$ -actin. Error bars represent the SD.

**Supplementary Figure 3. Analysis of DMR methylation at ICRs in wild-type and *Zfp57*<sup>-/-</sup> NPCs.** (a). Allele-specific DNA methylation results of three DMRs (*Plagl1*, *Peg13* and *Igf2r*) obtained by bisulfite treatment followed by cloning and sequencing. Each row corresponds to a single template DNA molecule cloned; each circle corresponds to a CpG dinucleotide. Filled circles designate methylated cytosines; open circles, unmethylated cytosines. The SNP used to distinguish the parental alleles is reported. (b, c) DNA methylation analysis of the *Snrpn*:TSS-DMR, *Peg3*:TSS-DMR, *Kcnq1ot1*:TSS-DMR and *Meg3/Dlk1*:IG-DMR by bisulfite treatment and pyrosequencing in JB1(b) and E14 (c) cell lines (wild-type and *Zfp57*<sup>-/-</sup>) at day 12. Violin-plots represent the average methylation level of several CpG of the DMR expressed in %mCpG. 'n' represents the number of CpGs analysed. The box-plot inside the violin-plot indicates interquartile range (25%-75%) and median value. (d) DNA methylation profiling of the *Mest*:TSS-DMR, *Rasgrf1*:TSS-DMR and *H19/Igf2*:IG-DMR, by bisulfite treatment and Sanger sequencing. The arrows above the electropherograms indicate the position of the CpGs. After bisulfite conversion, methylated cytosines appear as C and non-methylated cytosines as T.

**Supplementary Figure 4. Allele-specific expression analysis of imprinted genes in wild-type and *Zfp57*<sup>-/-</sup> NPCs.** Transcribed regions encompassing SNPs were analysed by Sanger sequencing after RT-PCR. Red arrows indicate the position of SNPs used to distinguish paternal (P) and maternal (M) alleles are indicated by red arrows.

**Supplementary Figure 5. Deregulated expression at the polycistronic *Meg3* RNA in *Zfp57*<sup>-/-</sup> NPCs.** (A) Screen shot from the UCSC Genome Browser depicting location of transcripts, and the results of bulk (black) and allele-specific (pink for maternal, blue for paternal) RNA-seq analysis of wild-type and *Zfp57*<sup>-/-</sup> JB1 NPCs. (B-D) Deregulation of bulk RNA level of the miRNA 379/410 cluster (B), their target imprinted genes *Mkrn3*, *Plagl1* and *Peg3* (C) and their target synaptic transmission and activity markers (D), expressed as log-ratio between *Zfp57*<sup>-/-</sup> and wild-type NPCs.

## Supplementary Tables

Supplementary Table 1. RNA-seq data retrieved from GEO

Supplementary Table 2. Differentially expressed genes in *Zfp57*<sup>-/-</sup> day 12-JB1 cells (n=4912)

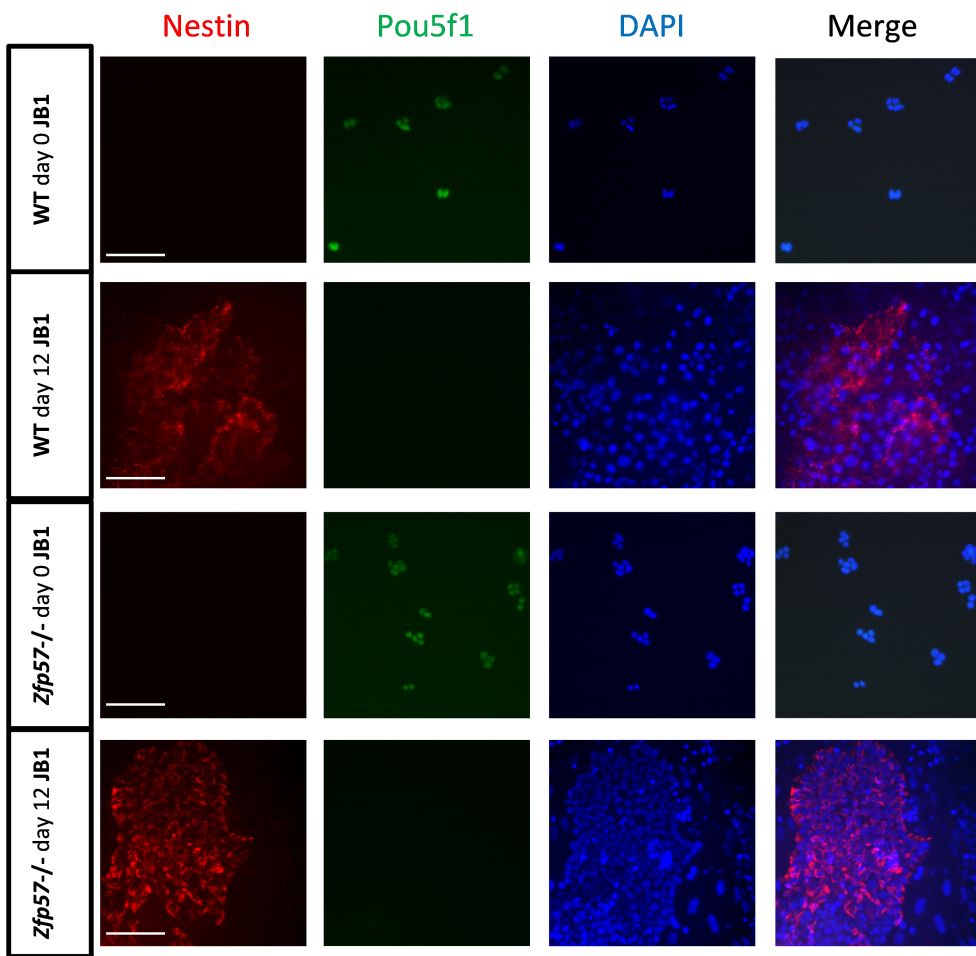
Supplementary Table 3. Downregulated genes within 100 kb from the ZFP57 binding sites in *Zfp57*<sup>-/-</sup> JB1 day 12-cells.

Supplementary Table 4. Allele-specific gene expression analysis in wild-type and *Zfp57*<sup>-/-</sup> JB1 day 12-cells.

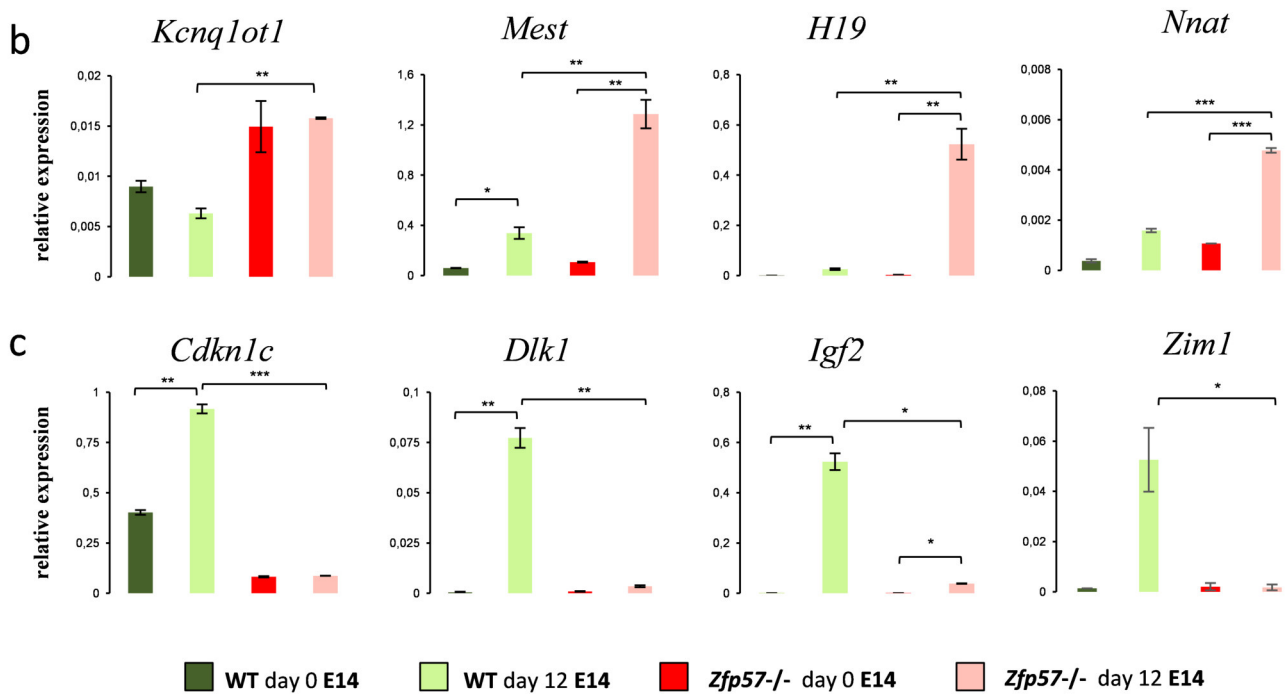
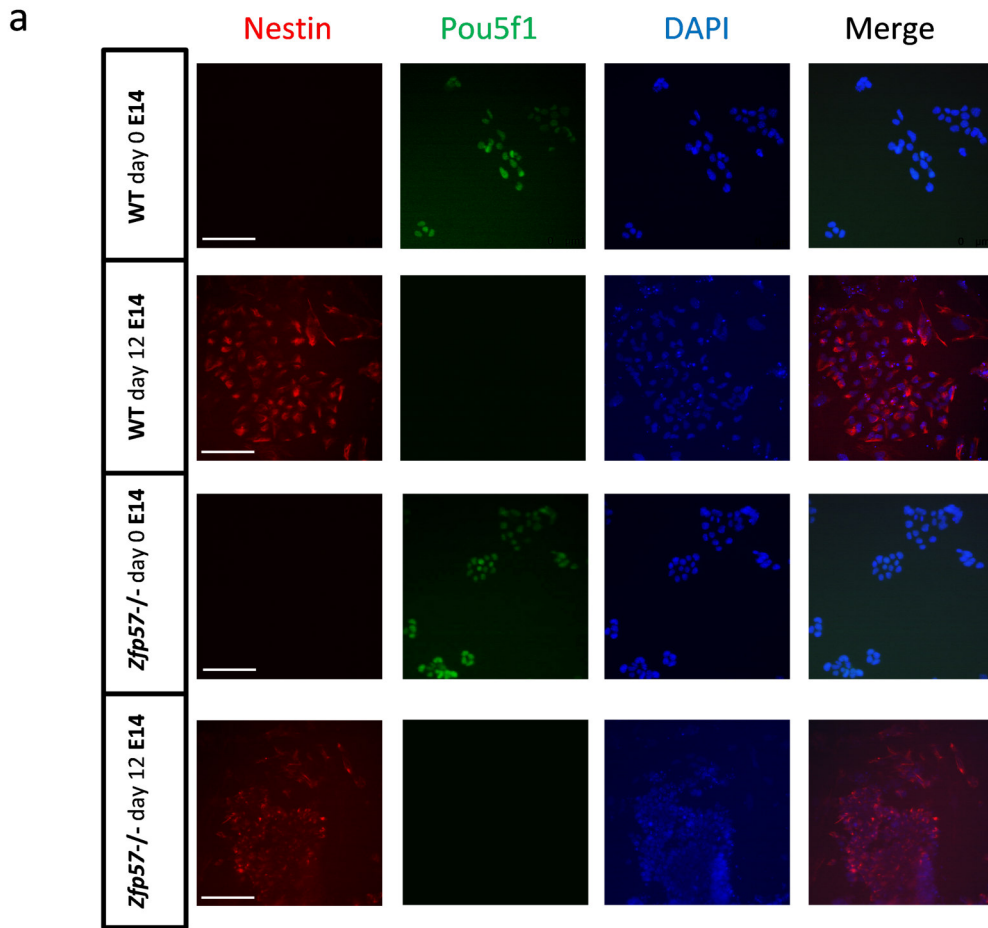
Supplementary Table 5. Allele-specific expression of imprinted genes in wild-type and *Zfp57*<sup>-/-</sup> JB1 day 0 and day 12-JB1 cells.

Supplementary Table 6. Sequence of PCR primers used in this study.

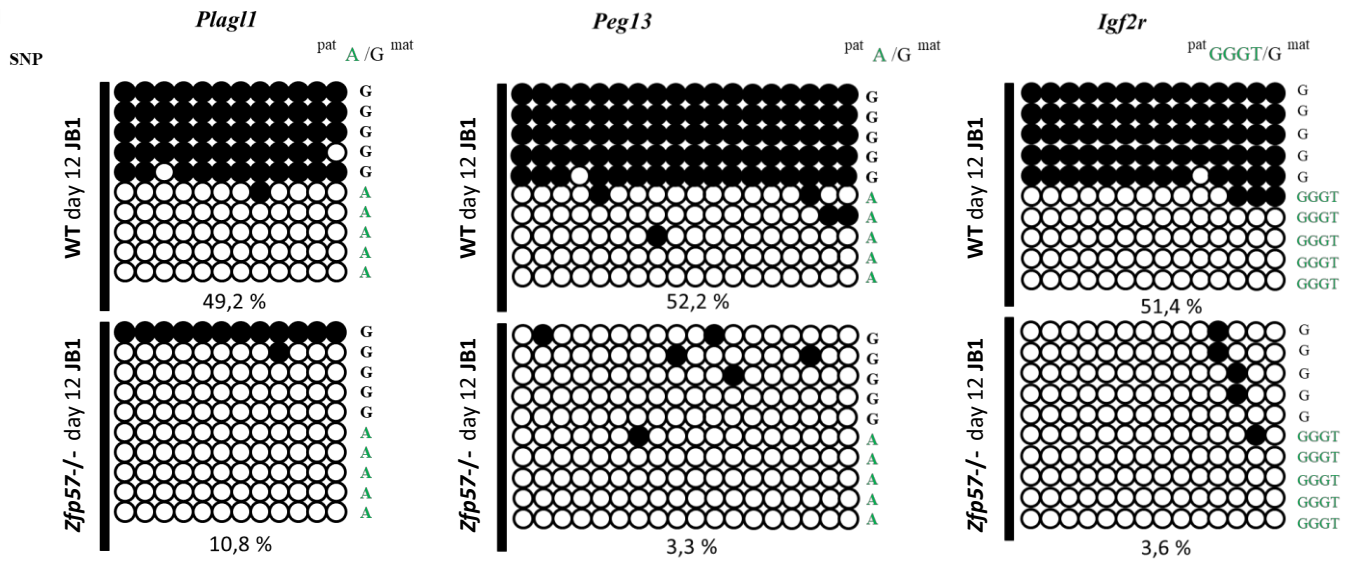
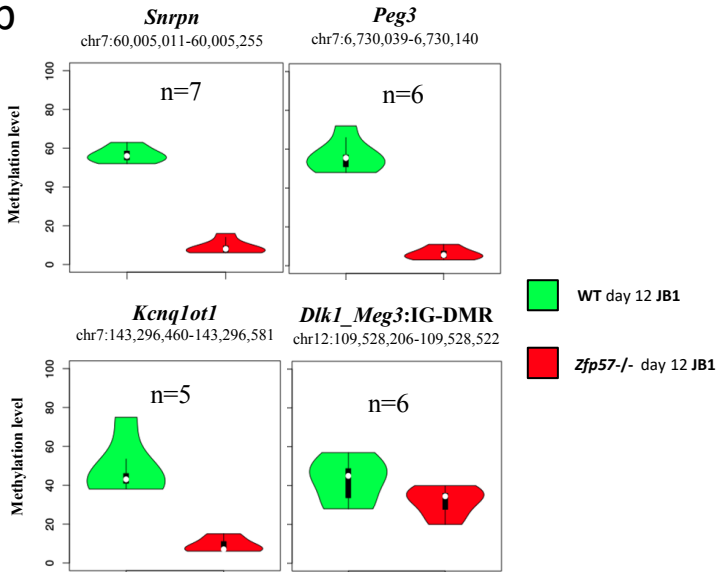
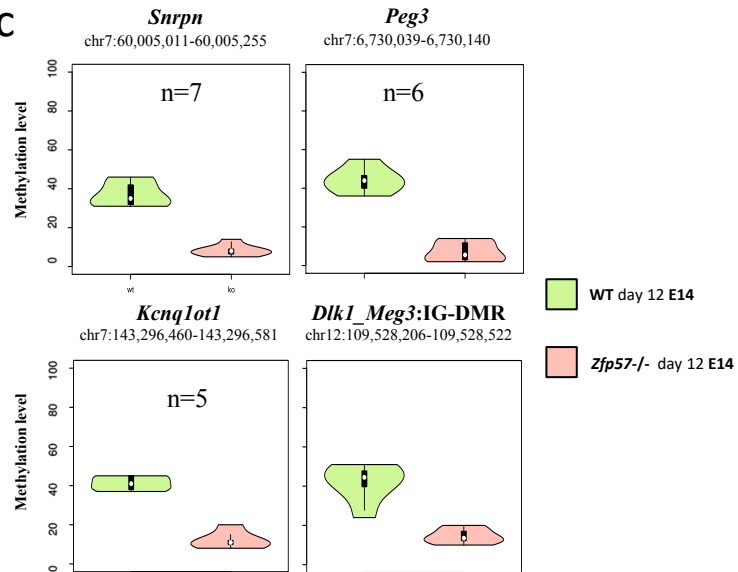
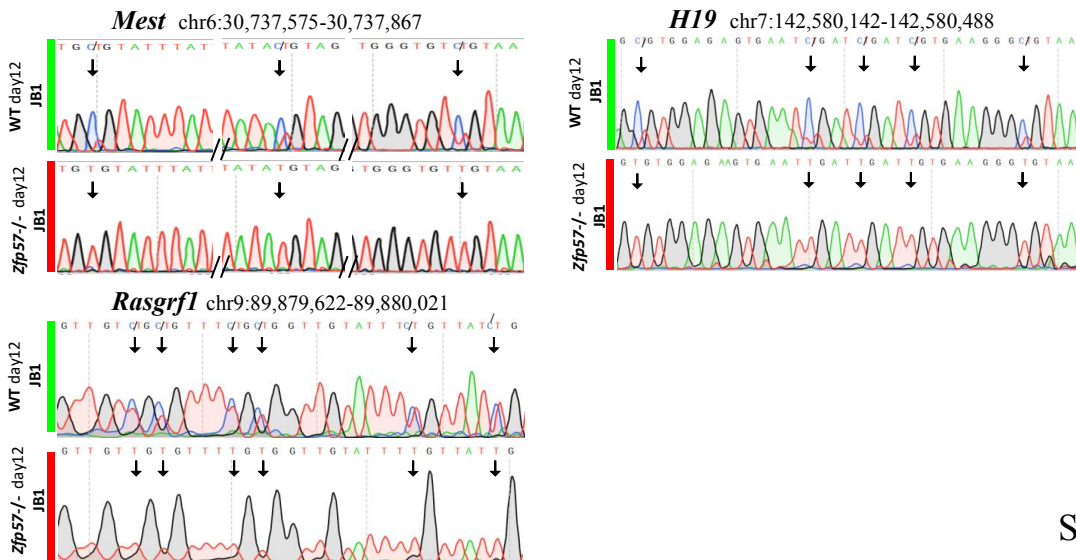
Supplementary Table 7. ZFP57 and KAP1 overlapping peaks in wild-type day 0-JB1 cells.

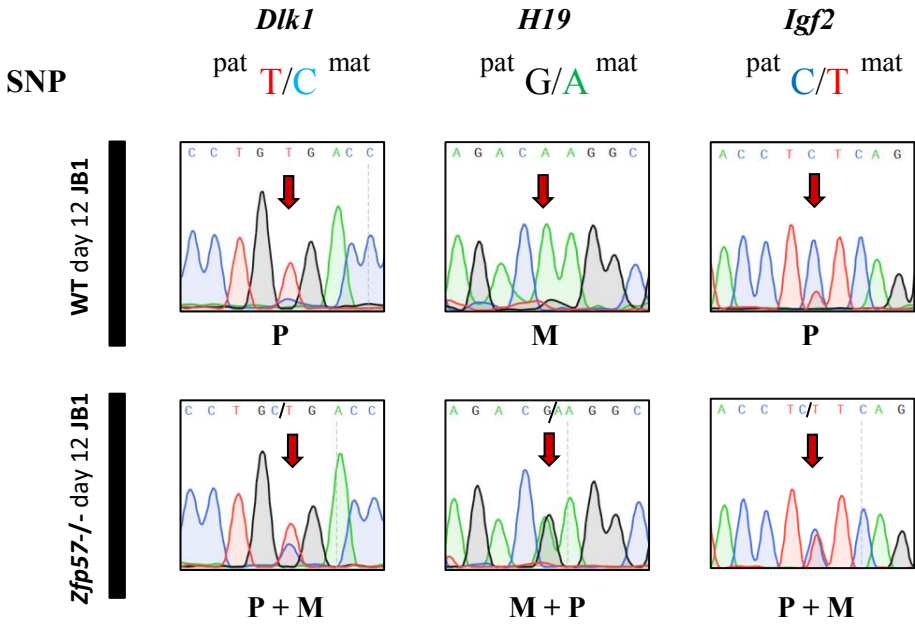
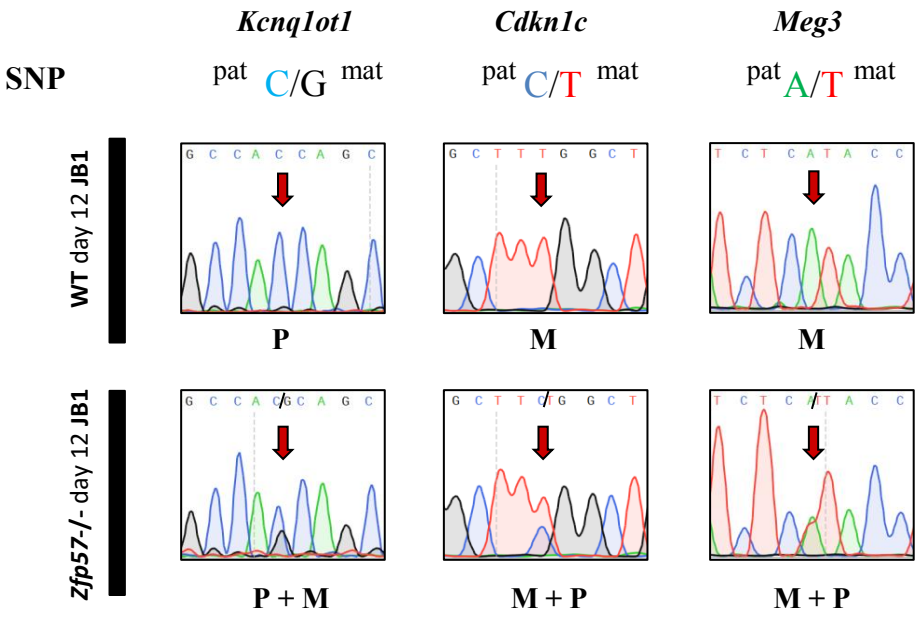


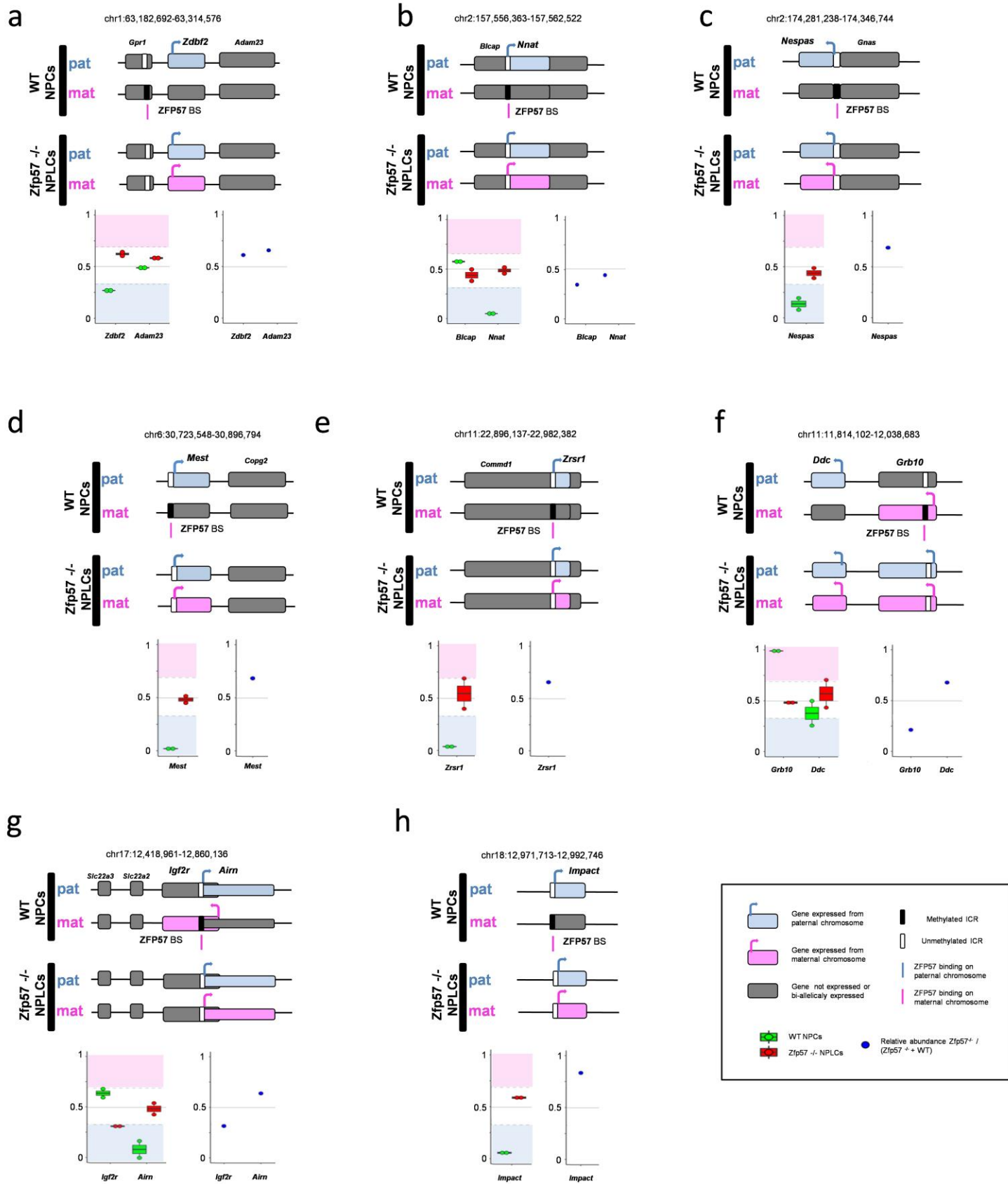
Supplementary Figure 1



Supplementary Figure 2

**a****b****c****d**





Supplementary Figure 5



chr12:109,539,638-109,750,301

