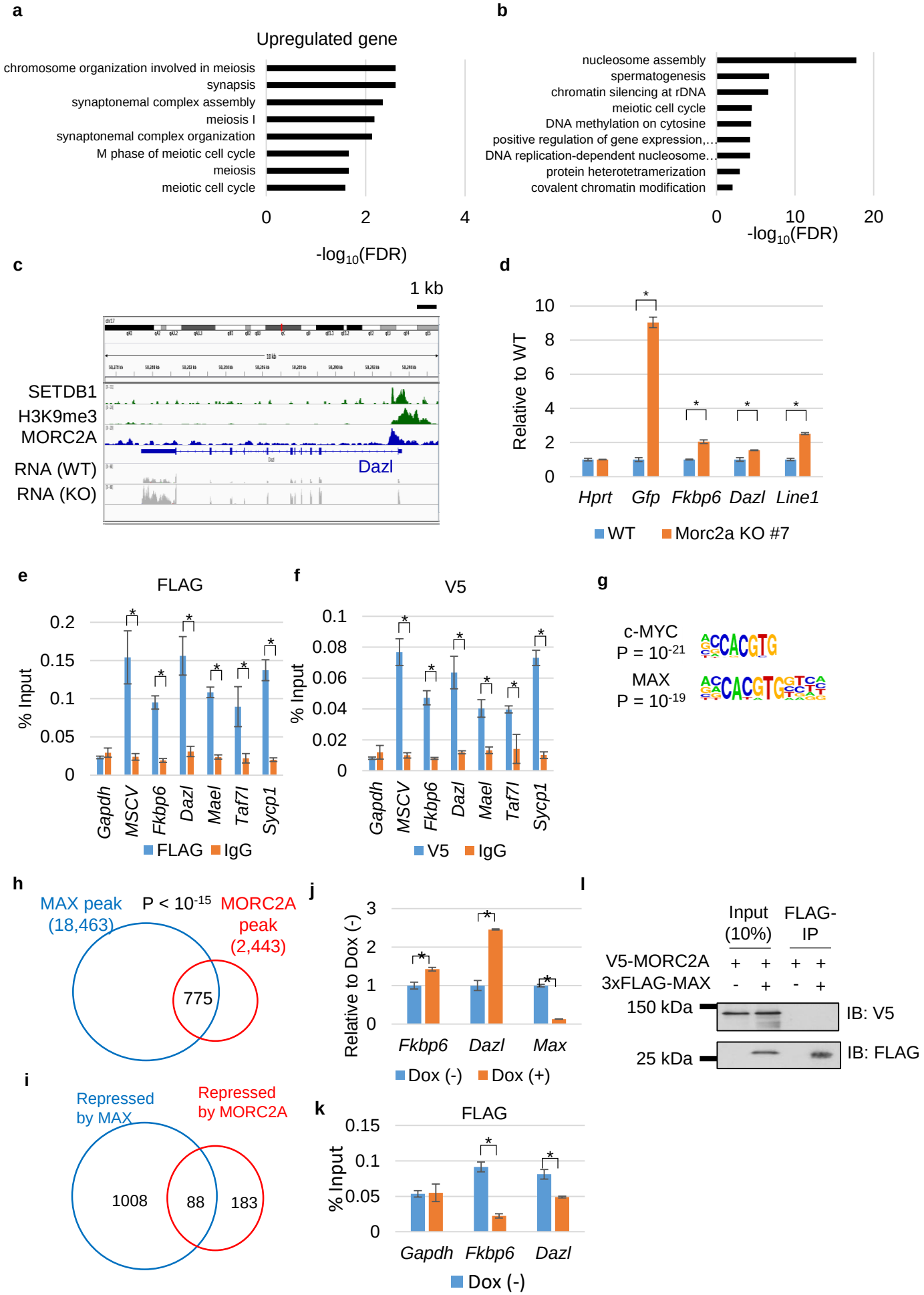
Fukuda_Fig_S4



Supplemental Figure 4. L1 retrotransposons were repressed by MORC2A. (a) GO term enrichment analysis of upregulated genes in *Morc2a* KO cells. X-axis represents $-\log_{10}$(FDR). (b) GO term enrichment analysis of genes with MORC2A binding sites in their promoters. (c) Colocalization of SETDB1, H3K9me3 and MORC2A at a representative panel of germ-cell related gene promoter. (d) RT-qPCR analysis of germ-cell related genes and LINE1 retrotransposon expression in *Morc2a* KO cell line. (e-f) ChIP-qPCR confirmation of MORC2A binding to promoter of germ-cell related genes. MORC2A ChIPs were performed with *Morc2a* KO mESCs stably expressing FLAG- (e) or V5- (f) tagged WT MORC2A. Anti-FLAG, V5 or MORC2A Ab were used. (g) Motif analysis of MORC2A-binding sites. MYC and MAX binding motifs were significantly enriched in MORC2A binding sites. (h) Enrichment of MAX in MORC2A binding sites. Publicly available MAX ChIP-seq data in mESCs (GSE48175) were analyzed to identify MAX binding sites. Venn diagram shows that overlap between MORC2A and MAX peaks: 775 of 2,443 MORC2A peaks were overlapped with MAX peaks. We calculated the significance of overlap between MORC2A and MAX peaks by the following procedure. A set of genomic regions was randomly selected so that its total number and length equaled those of the MORC2A peaks, and regions overlapping MAX peaks were counted. We generated 1,000 such data sets and calculated the average number of regions containing that retrotransposon, which became the expected number. Then, significance of overlap between MAX and MORC2A peaks were calculated by Chi-squared test. (i) Venn diagram of target genes of MAX and MORC2A. These were significantly overlapped (P-value = $3.3\text{x}10^{-46}$, hypergeometric test). Publicly available microarray data in *Max* KO mESCs (GSE45181) was used for this analysis. These microarray data were analyzed by GEO2R and genes repressed by MAX were identified the following criteria: adjusted P-value < 0.01, fold change > 2. (j,k) Effect of MAX depletion on MORC2A enrichment in germ-cell related genes. *Max*-null mESCs, which stably expresses FLAG-MORC2A, with MAX cDNA under the control of the tetracycline-off were cultured in ESC culture medium with 1 mg ml^{-1} doxycycline (Dox) for 3 days, followed by RNA extraction and ChIP using anti-FLAG antibody. Enhanced expression of germ-cell related genes and depletion of MAX in *Max*-null mESCs were validated by RT-qPCR (j). Enrichment of MORC2A in germ-cell related genes was examined by ChIP-qPCR (k). (l) Coimmunoprecipitation of V5-MORC2A with 3XFLAG-MAX from transfected cells. 293FT cells were transfected with plasmids expressing 3XFLAG-MAX and V5-MORC2A. 48 hours after the transfection, the cell lysates were immunoprecipitated with anti-FLAG antibody, and then the immunoprecipitated complexes were subjected to Western blot analysis with an anti-V5 antibody or anti-FLAG antibody.

Data represent mean ± SE (n = 3). *P-value < 0.01 (*t*-test).