

Expanded View Figures

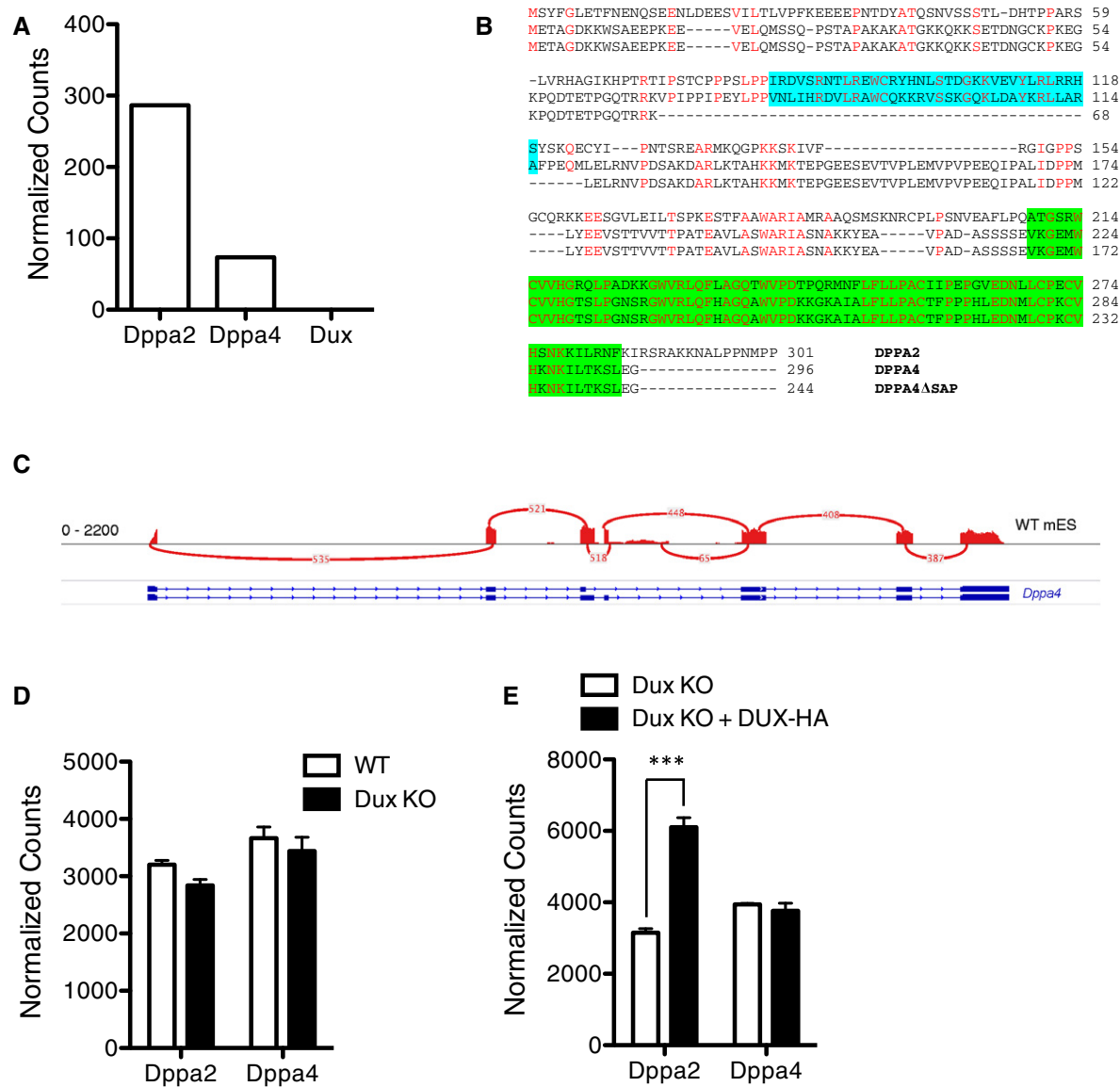


Figure EV1. DUX is not necessary for DPPA2 and DPPA4 expression.

A Comparative expression of *Dppa2*, *Dppa4*, and *Dux* in murine oocytes from [14].
B Protein alignment of murine DPPA2, DPPA4, and DPPA4 lacking the SAP domain. SAP domain is represented in cyan and C-terminal domain in green.
C Sashimi plot representing the coverage on the *Dppa4* gene of an RNA sequencing analysis of mESCs.
D, E Comparative expression of *Dppa2* and *Dppa4* in WT and *Dux* KO mESCs (D), and in *Dux* KO mESCs with or without ectopic expression of DUX (E). Bars represent the average and error bars the SD; $n = 3$. *** $P \leq 0.001$, unpaired t-test.

Figure EV2. DPPA2 and DPPA4 regulate expression of DUX-dependent and -independent genes.

- A qPCR of *Dux*, *Dppa2*, and *Dppa4* in mESCs sorted for their expression of GFP under control of a MERVL promoter. Bars represent the average and error bars the SD; $n = 3$
- B Comparative expression of DUX-regulated genes ($n = 300$) or the rest of the expressed genes in *Dppa2* KO, *Dppa4* KO, and WT mESCs. Box limits, twenty-fifth and seventy-fifth percentiles; lines in the boxes, median. The upper whisker extends from the hinge to the largest value, no further than 1.5× the interquartile range (IQR) from the hinge. The lower whisker extends from the hinge to the smallest value, at most 1.5× the IQR of the hinge (paired t-test).
- C, D Plots of gene set enrichment analysis (GSEA) of downregulated genes in *Dppa2* KO with genes deregulated in *Dppa4* KO (C) and vice versa (D) (fold change [FC], > 5 ; $P < 0.05$). NES, normalized enrichment score; fdr, false discovery rate.
- E Venn diagram showing overlap between DUX-regulated, DPPA2-/DPPA4-regulated, 2C-like, and ZGA-specific genes.
- F Dynamics of expression of DPPA2/4-dependent and DUX-dependent genes during pre-implantation embryo development. Gray lines represent the Z-score of all genes belonging to a cluster, and black lines represent their median values. Percentage values depict the proportion of the two subsets of genes that belong to a specific cluster.
- G Distance of promoters belonging to specific subgroups of genes from the MERVL regulatory region, MT2_mm.

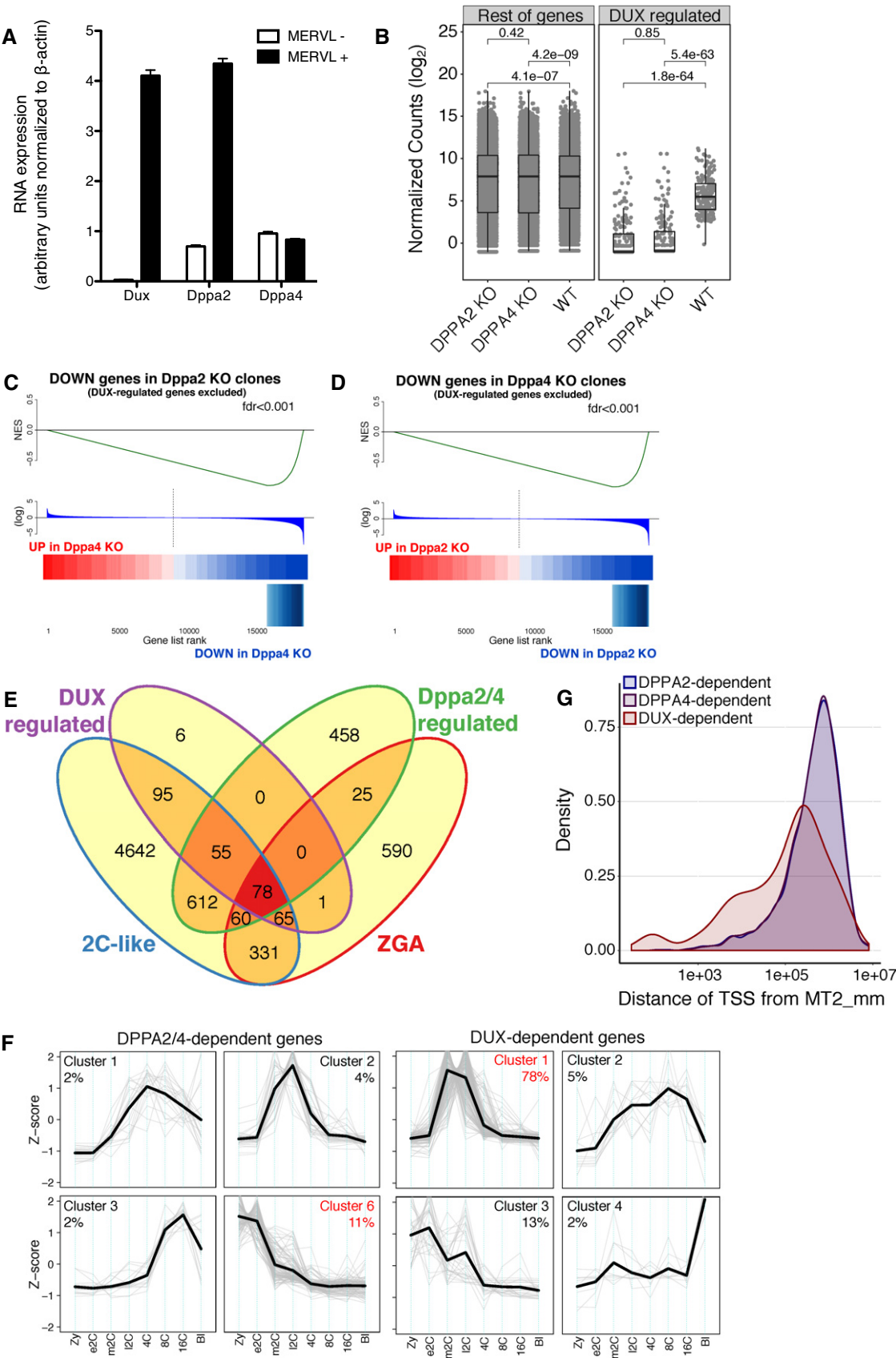


Figure EV2.

Figure EV3. DPPA2 and DPPA4 directly regulate expression of target genes.

- A, B Fraction of GFP⁺ cells (A) or *Dux*, *Zscan4*, *Tdpz4*, *Mael*, and *Tdrd1* gene expression (B) in mESCs carrying a MERVL-GFP reporter and overexpressing DUX alone, DPPA2 and DPPA4, or the three transgenes together. Expression was normalized to *Actb*. Bars represent the average and error bars the SD; *n* = 3.
- C, D Fraction of GFP⁺ cells (C) or *Zscan4*, *Tdpz4*, *Mael*, and *Tdrd1* gene expression (D) in *Dux* KO mESCs carrying a MERVL-GFP reporter and overexpressing DUX or DPPA2 and DPPA4. Expression was normalized to *Actb*. Bars represent the average and error bars the SD; *n* = 3.
- E Heatmap representing the enrichment of DUX, DPPA2, DPPA4, and DPPA4ΔSAP binding at TSS of DPPA2/4-dependent or DUX-dependent genes or a random set of 1,000 genes (analysis in Fig 3C).
- F Comparative expression of genes bound by DPPA2 and DPPA4 at the promoter (1,155) or a random set of genes in *Dppa2* KO, *Dppa4* KO, and WT mESCs. Box limits, twenty-fifth and seventy-fifth percentiles; lines in the boxes, median. The upper whisker extends from the hinge to the largest value, no further than 1.5× the interquartile range (IQR) from the hinge. The lower whisker extends from the hinge to the smallest value, at most 1.5× the IQR of the hinge (paired *t*-test).
- G Comparative expression of DUX-dependent (*n* = 300) and DPPA2-/DPPA4-dependent (*n* = 1,288) genes and the rest of the genes in WT or *Dppa4* KO mESCs expressing ectopically DPPA4 or DPPA4ΔSAP. Box limits, twenty-fifth and seventy-fifth percentiles; lines in the boxes, median. The upper whisker extends from the hinge to the largest value, no further than 1.5× the interquartile range (IQR) from the hinge. The lower whisker extends from the hinge to the smallest value, at most 1.5× the IQR of the hinge (paired *t*-test).
- H Fold enrichment of DPPA2 and DPPA4 ChIP-qPCR in *Dppa2* or *Dppa4* KO mESCs at the promoter of *Zscan4c* (negative control), *Dux*, and two direct targets of DPPA4 (*Mael* and *Tdrd1*), compared to *Tubb* promoter. Bars represent the average and error bars the SD; *n* = 3. **P* ≤ 0.05, ****P* ≤ 0.001, unpaired *t*-test.

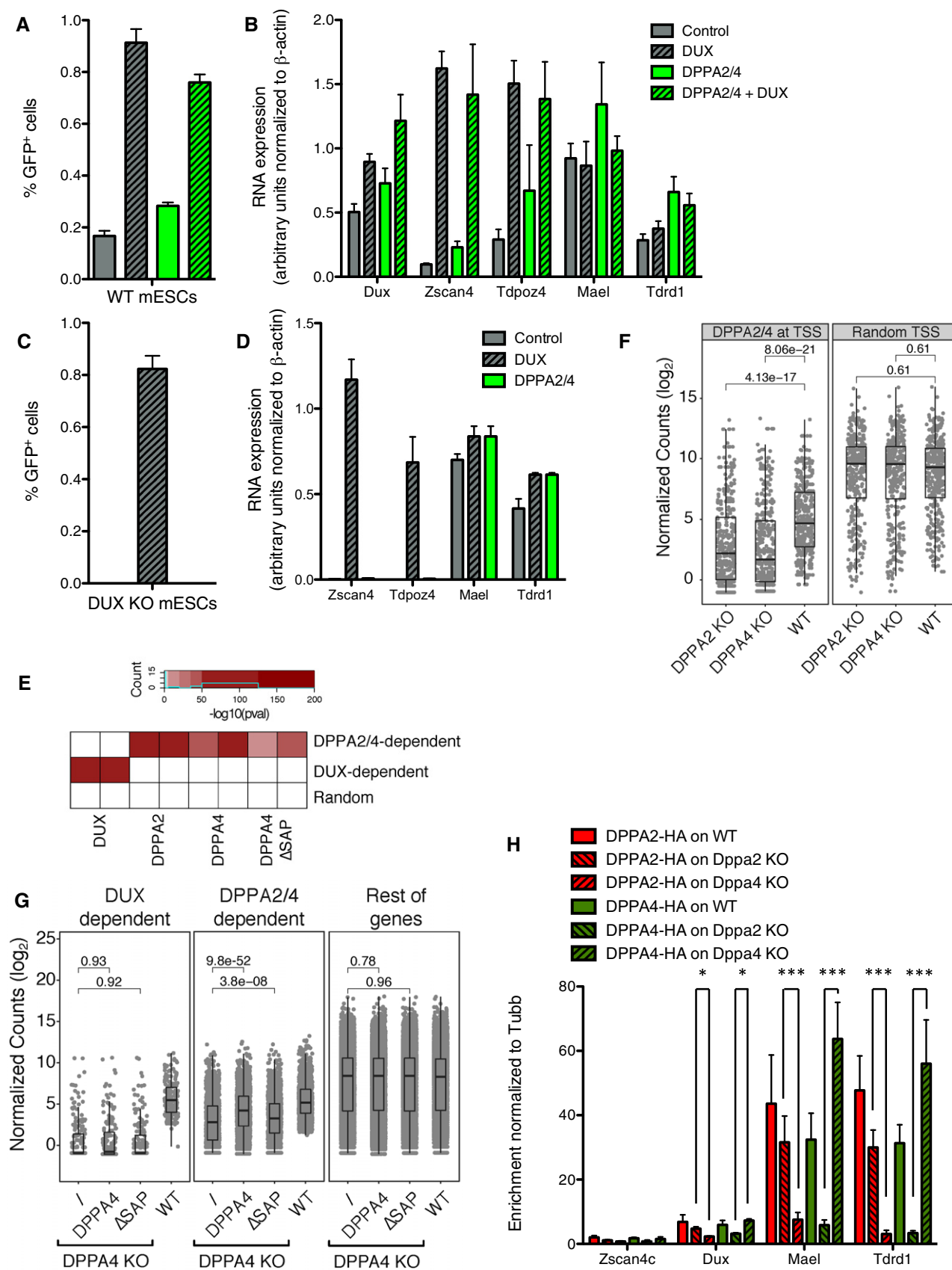


Figure EV3.

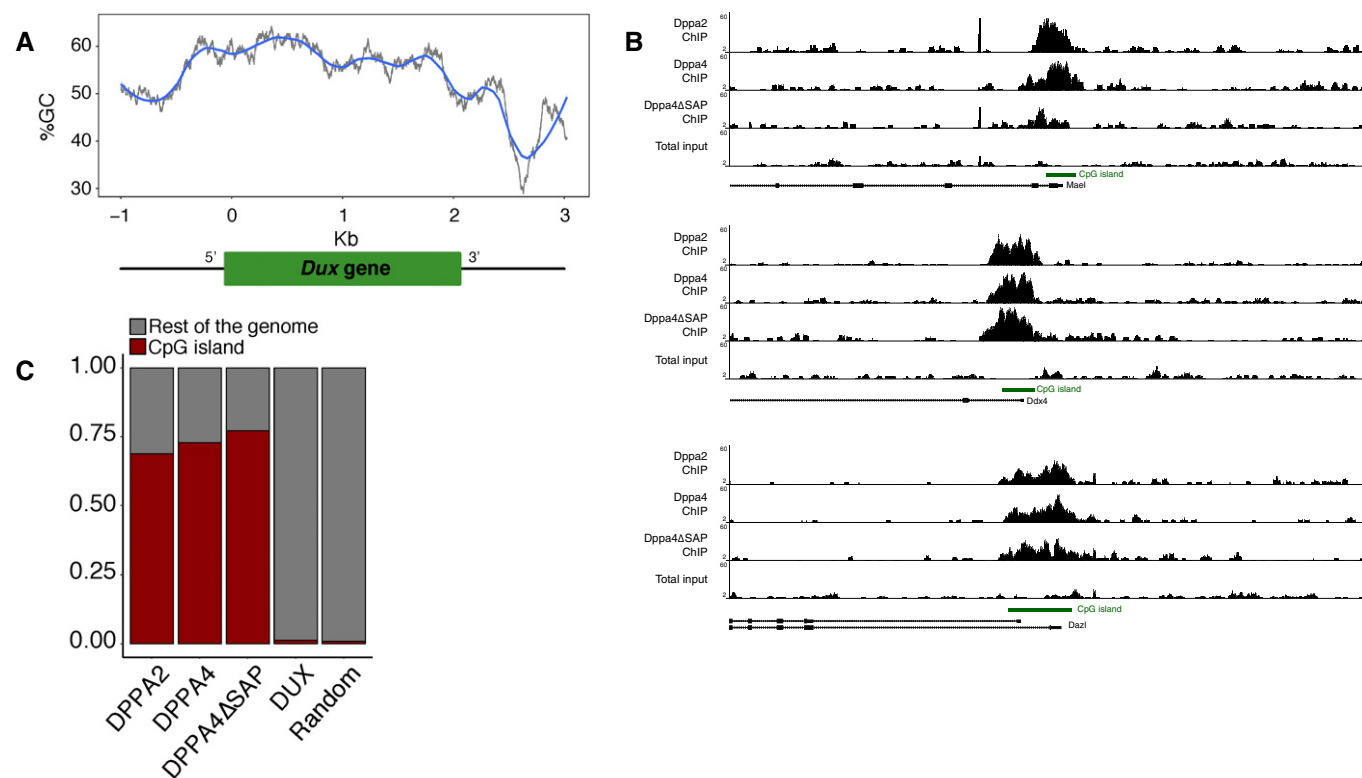


Figure EV4. DPPA2 and DPPA4 bind GC-rich regions.

A Percentage of GC in a window of $\pm 1,000$ bp around the *Dux* gene, using a 200-bp sliding window.

B UCSC screenshots representing the genomic location of the promoters of three genes: *Mael*, *Ddx4*, and *Dazl*. Each screenshot represents from top to bottom: DPPA2, DPPA4, and DPPA4ΔSAP ChIP-seq, total input, CpG island track, and RefSeq gene annotation.

C Fraction of DPPA2, DPPA4, DPPA4ΔSAP, and DUX ChIP-seq peaks and a random set of sequences of similar size enriched at CpG island.

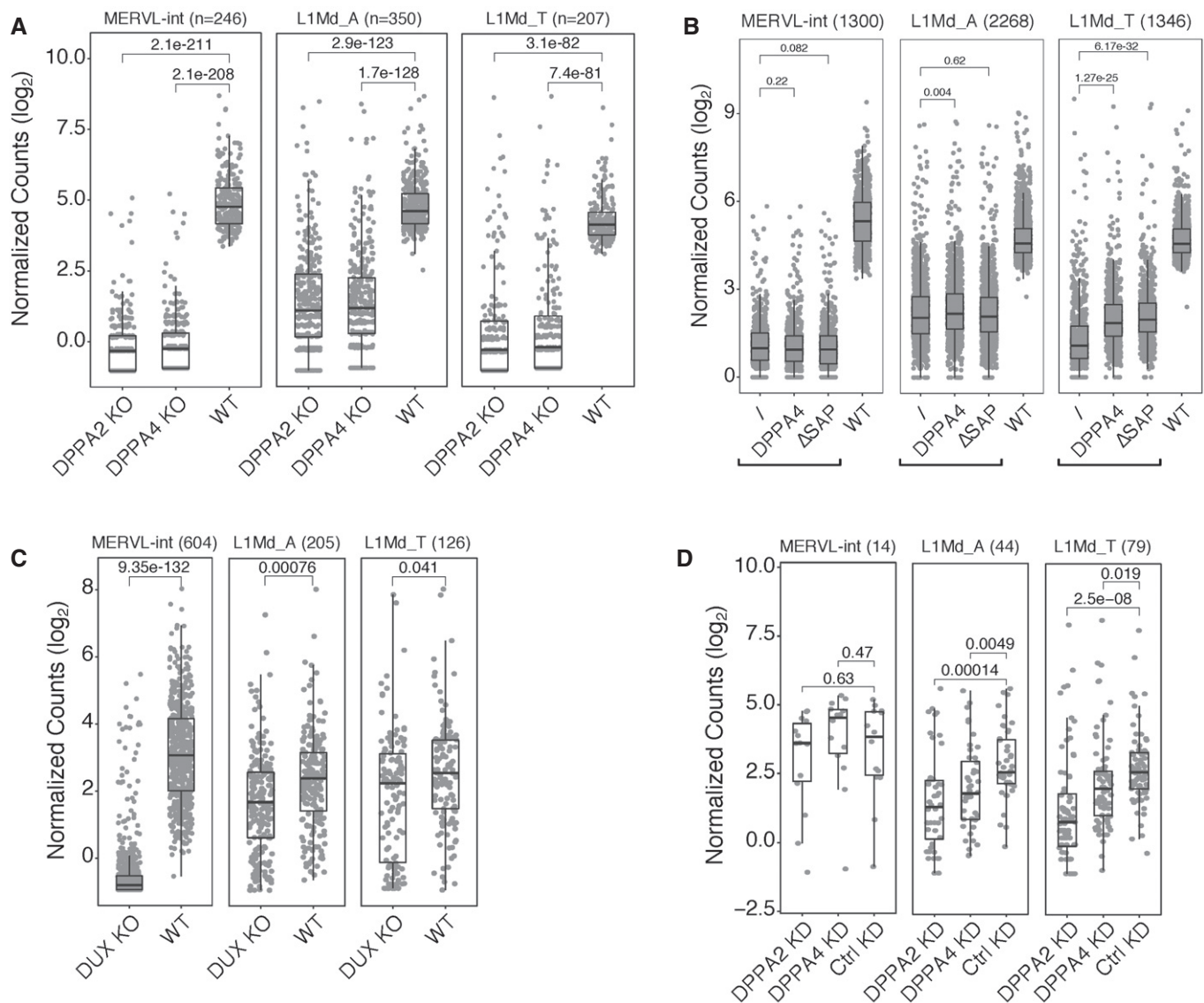


Figure EV5. Young LINE-1 families are regulated by DPPA2 and DPPA4.

- A Comparative expression of single TE integrants belonging to MERVL-int ($n = 246$), L1Md_A ($n = 350$), and L1Md_T ($n = 207$) families in *Dppa2* KO, *Dppa4* KO, or WT mESC clones.
- B Comparative expression of single TE integrants belonging to MERVL-int ($n = 1,300$), L1Md_A ($n = 2,268$), and L1Md_T ($n = 1,346$) families in WT or *Dppa4* KO mESCs expressing ectopically DPPA4 or DPPA4 Δ SAP.
- C Comparative expression of MERVL-int ($n = 604$), L1Md_A ($n = 205$), and L1Md_T ($n = 126$) in WT and *Dux* KO mESCs.
- D Comparative expression of MERVL-int ($n = 14$), L1Md_A ($n = 44$), and L1Md_T ($n = 79$) in *Dux* KO mESCs depleted of DPPA2 or DPPA4 or a control. Box limits, twenty-fifth and seventy-fifth percentiles; lines in the boxes, median. The upper whisker extends from the hinge to the largest value, no further than 1.5 $\times$ the interquartile range (IQR) from the hinge. The lower whisker extends from the hinge to the smallest value, at most 1.5 $\times$ the IQR of the hinge (paired *t*-test).