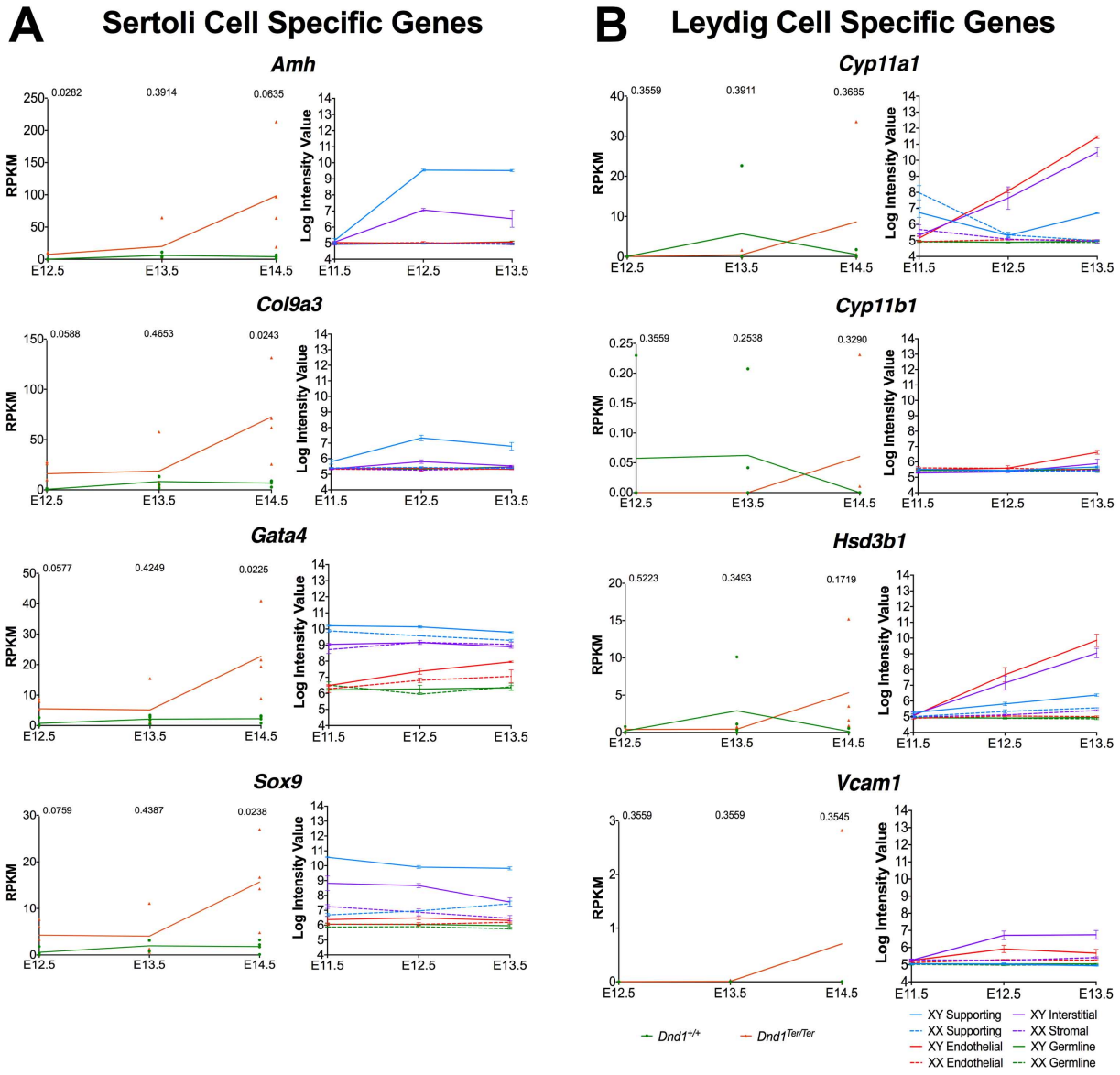


Fig. S1. Examples of genes detected in the *Dnd1*$^{Ter/Ter}$ transcriptome data that are specific to Sertoli or Leydig cell lineages in the gonad based on the Jameson data (2). Typically, expression of these genes spikes in the E14.5 *Dnd1*$^{Ter/Ter}$ data (orange), a stage when *Dnd1*$^{Ter/Ter}$ germ cell numbers are reduced and expression of Oct4-GFP declines, which may have led to errors in threshold settings during FACS. Graphs of transcriptome data from each cell type in the gonad performed at E11.5, E12.5 and E13.5 are shown for comparison. The germ cell lineage is green; supporting cells are blue; interstitial/stromal cells are purple; endothelial cells are red.

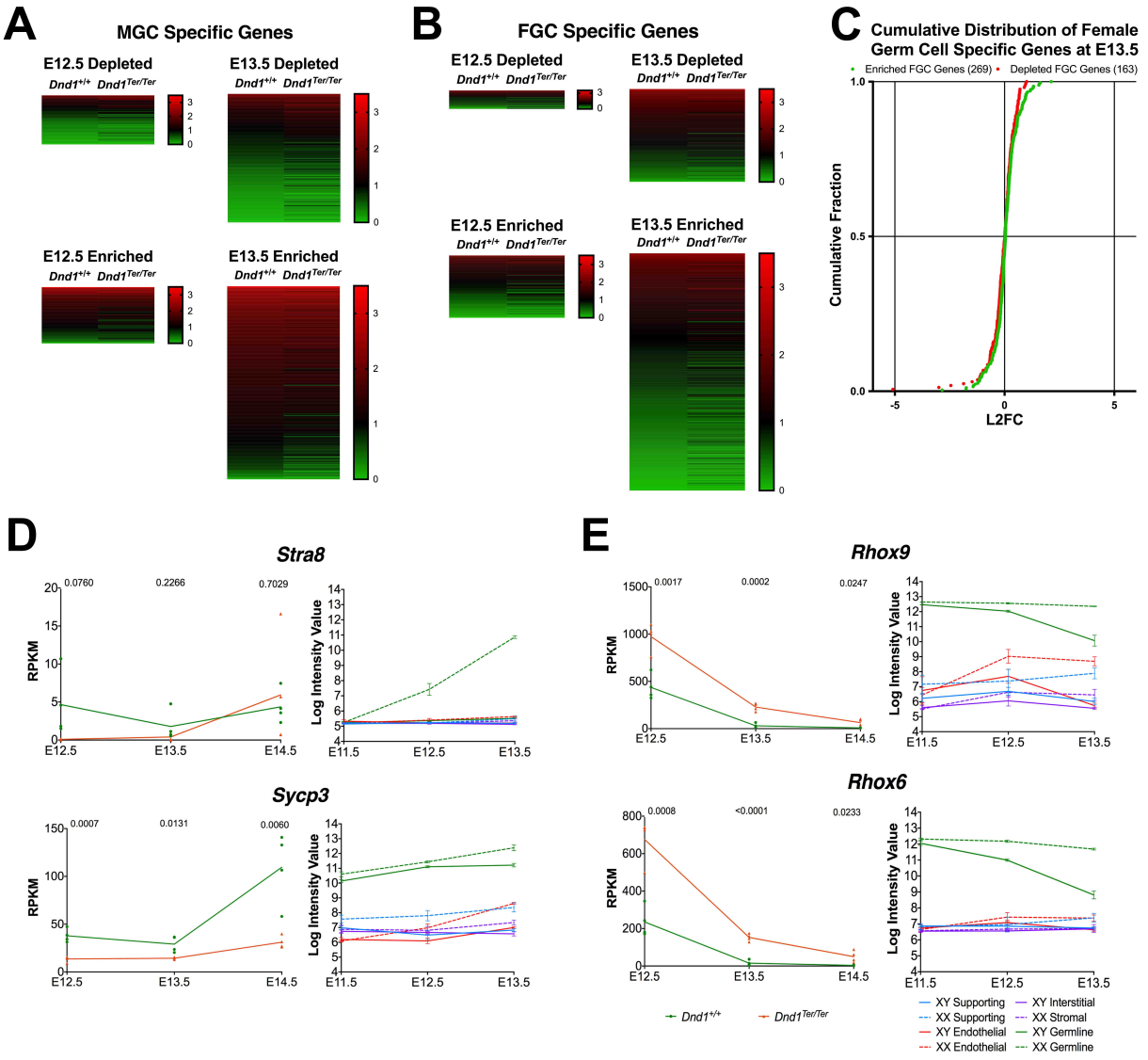


Fig. S2. Male germ cells do not acquire a female (or meiotic) identity, but retain expression of pluripotency genes shared with female germ cells. (A) Heat map analysis showing that *Dnd1*$^{Ter/Ter}$ male germ cells retain a profile very similar to wild type at E12.5 and E13.5 with respect to genes that are specifically depleted in male germ cells, and genes that are specifically enriched. **(B)** Similarly, heat maps comparing expression of female-specific genes in XY *Dnd1*$^{+/+}$ to XY *Dnd1*$^{Ter/Ter}$ samples show few changes for depleted or enriched genes at E12.5 or E13.5. **(C)** A cumulative distribution plot of the log2 fold change between mutant male germ cells and wild type male germ cells at E13.5 for female germ cell enriched (green) or depleted (red) genes. Genes typically enriched (green) or depleted (red) in female germ cells show no clear bias for the direction of change in mutant germ cells. **(D)** Genes specifically upregulated as female germ cells enter meiosis (*Stra8* and *Sycp3*) were not activated by E14.5 in *Dnd1*$^{Ter/Ter}$ mutants. Graphs to the right show expression of the gene in all gonadal lineages (green broken line = XX germ cells; green solid line = XY germ cells; data from (2)). **(E)** *Rhox9* and *Rhox6*, which normally become specific to XX germ cells by abrupt down-regulation in male germ cells, show a delay in down-regulation similar to other pluripotency genes.

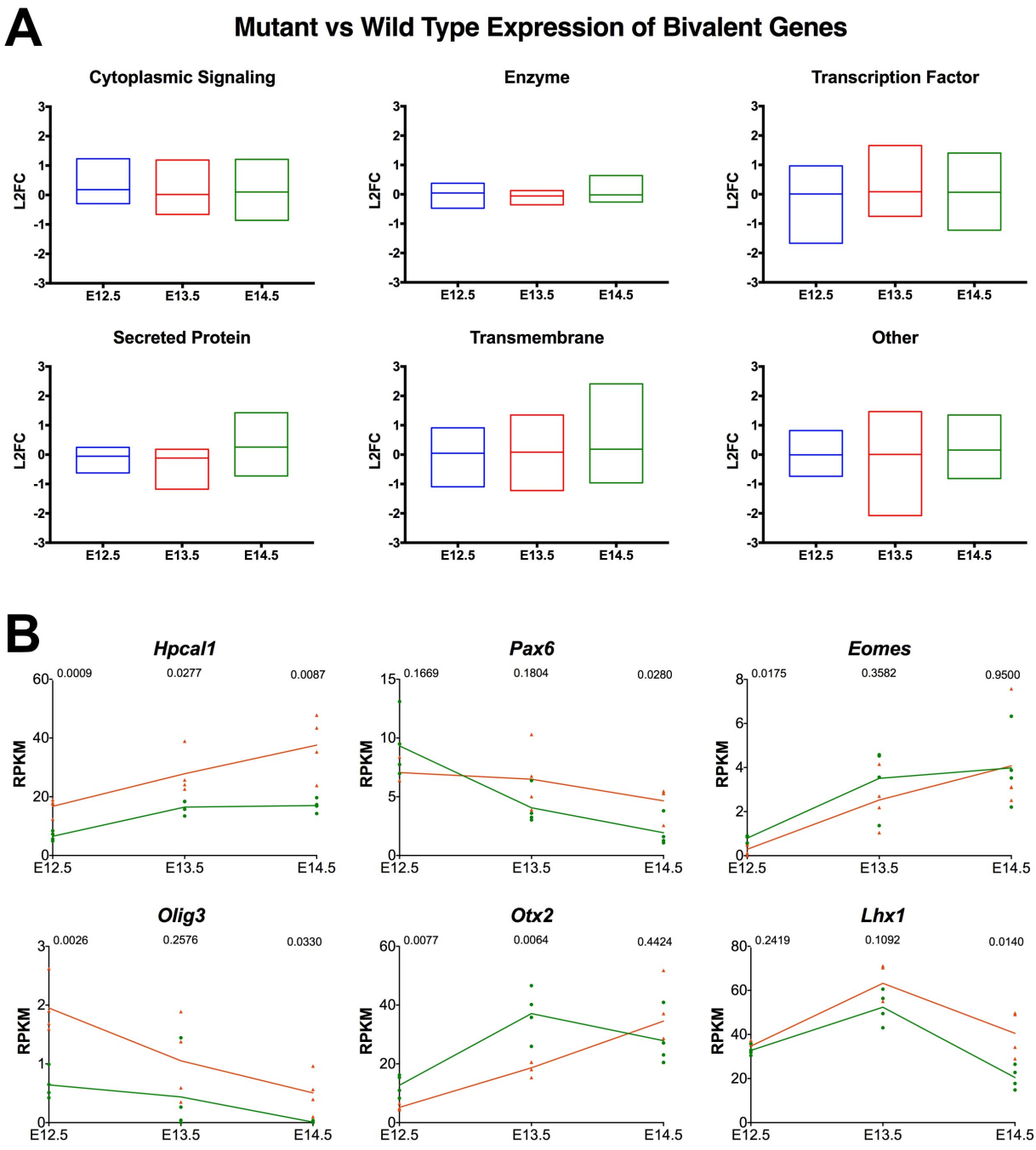


Fig. S3. *Dnd1*$^{Ter/Ter}$ germ cells show no evidence of lineage infidelity between E12.5-E14.5. Despite the failure to activate many chromatin regulators in *Dnd1*$^{Ter/Ter}$ mutants, **(A)** expression of bivalent genes grouped by category (cytoplasmic signaling proteins, enzymes, transcription factors, secreted proteins, transmembrane proteins, and other) was not elevated, and **(B)** with the exception of *Hpcal1,* neither neuronal genes, nor other transcription factors associated with somatic cell differentiation (*Pax6*, *Eomes*, *Olig3*, *Otx2*, and *Lhx1*) were significantly elevated in mutants.

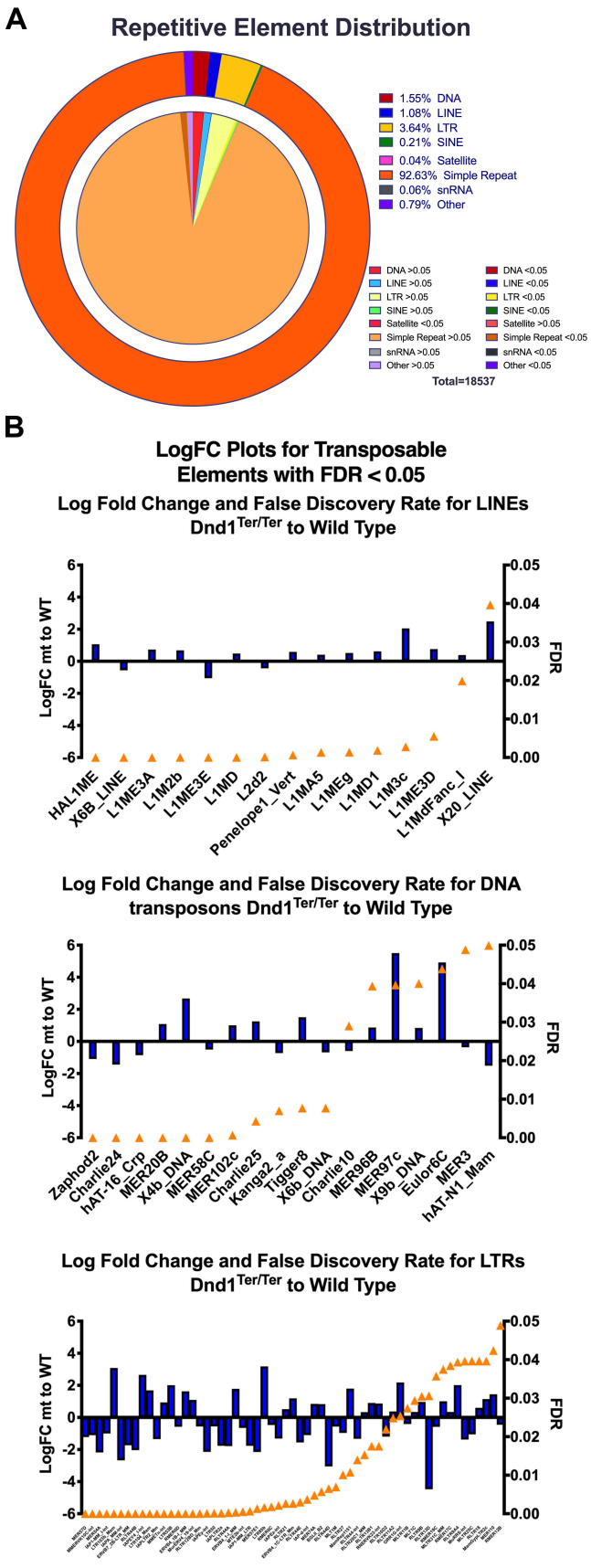


Fig. S4. Repetitive elements in general do not exhibit aberrant activity. (A) A survey of repetitive element distribution organized by class. Outer ring: class distribution of elements represented in reads at E14.5 (Red=DNA transposons; Blue=LINEs; gold=LTRs; green=SINEs; Magenta=satellite; Orange=simple repeats; Dark Green=snRNAs; Purple=other). Inner circle divides specific classes into reads with significant differences between mutant and wildtype and reads without significant differences between mutant and wildtype (bold colors: FDR<0.05, significant; pale colors: FDR>0.05). **(B)** Waterfall plots of Log Fold Change (LogFC) between mutant and wildtype in each transposable element class (LINEs, LTRs, and DNA transposons) with FDR<0.05 (significant). LogFC shown to left of plots; FDR shown by orange triangle placement relative to right scale on each graph.

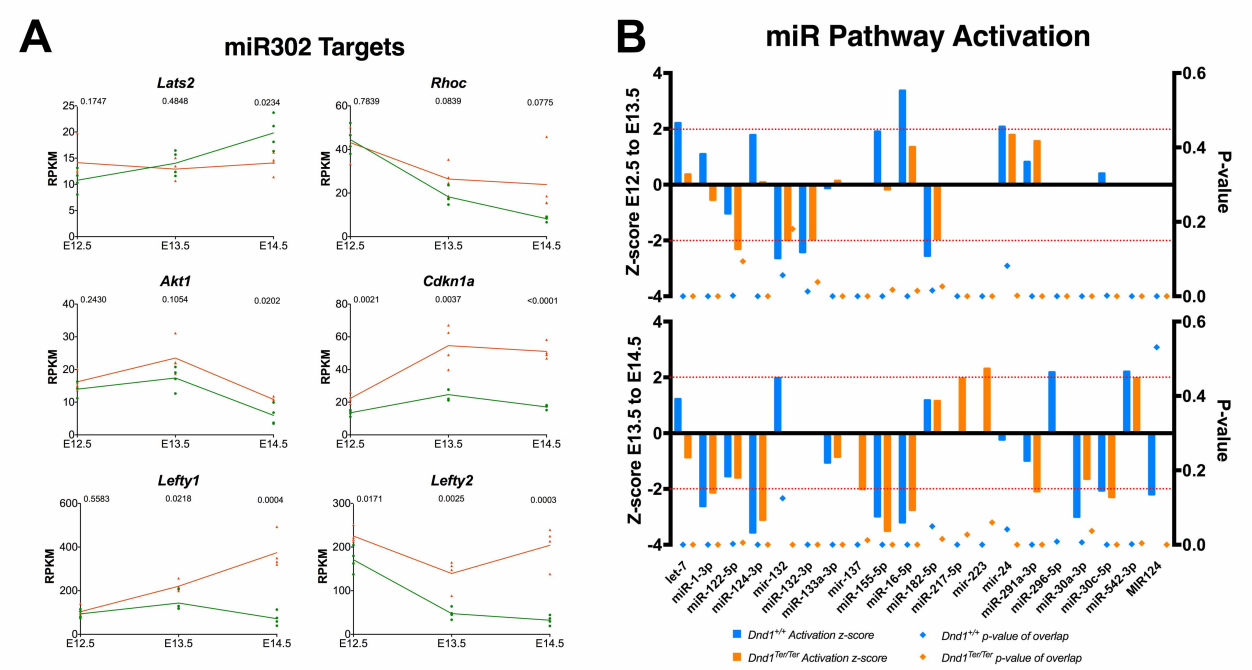


Fig. S5. In general, miRNAs did not show activation based on downstream target analysis. **(A)** Although DND1 is believed to block degradation of zebrafish miR430 targets, with the exception of *Lats2*, targets of the orthologous miRNA in mice (miR320) are not down-regulated in mutants as predicted. **(B)** Waterfall graphs of Ingenuity Pathway Analysis (Qiagen) of miRNA activation based on expression changes in predicted targets at E12.5-E13.5 (top) and E13.5-E14.5 (bottom) for wild type (blue) and mutant (orange) samples. Only miRNA pathways with an activation Z-score of >2 or <-2 (left y-axis) in at least one transition period for mutant or wild type data are shown. P-values are plotted on the right Y-axis and represented as diamonds.

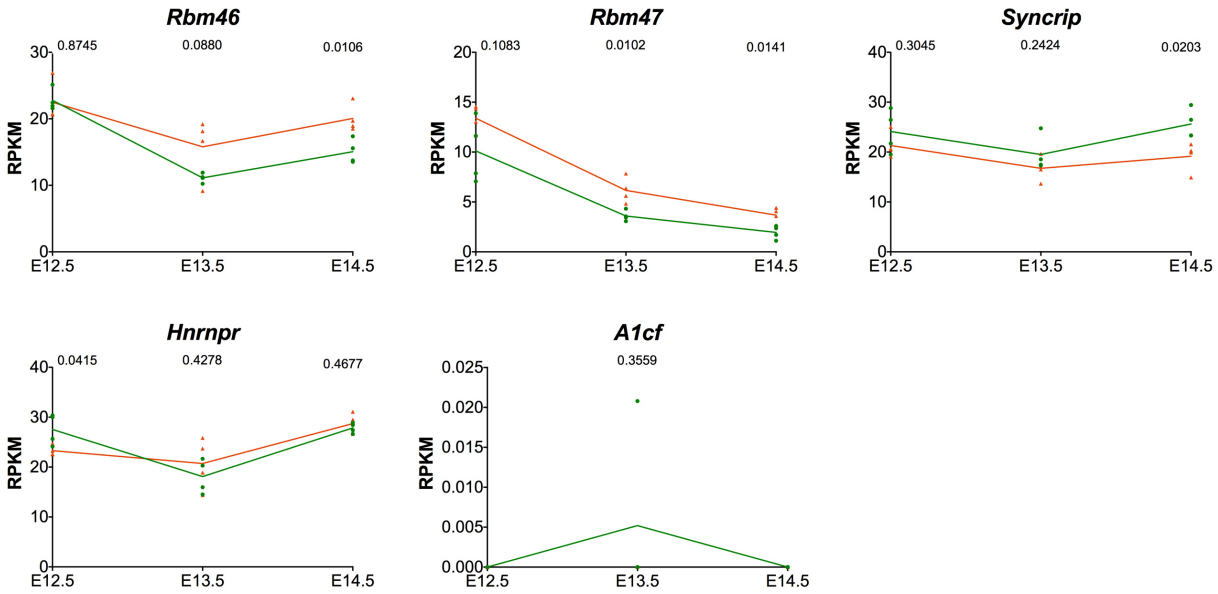


Fig. S6. Four RBPS closely related to DND1 are expressed in germ cells. Four of the five RBPs (*Rbm47*, *Rbm46*, *Syncrip*, *Hnrnpr*, but not *A1cf*) related to DND1 (Fig. 3) are expressed in male germ cells during the transcriptome timecourse.

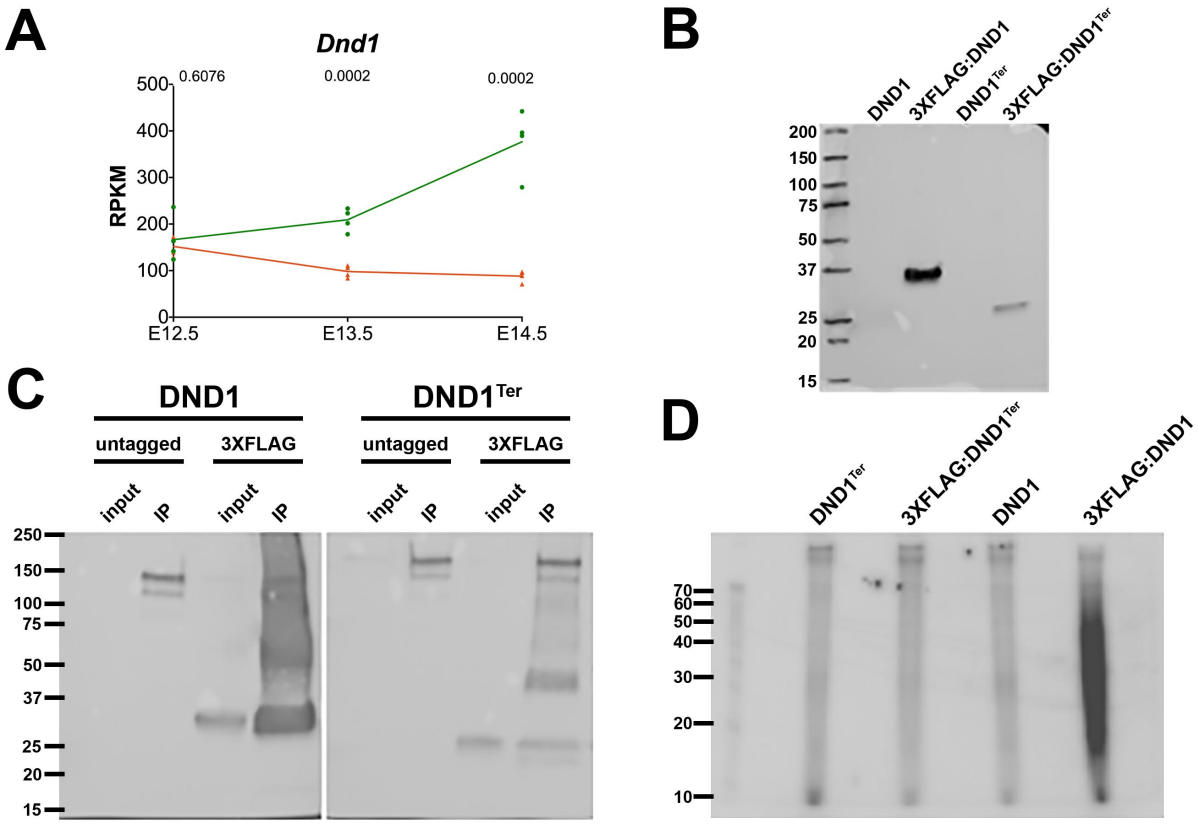


Figure S7. Levels of the *Dnd1-Ter* transcript decline relative to wild type in mutants, and the flag-tagged allele is poorly represented relative to the wild type allele when over-expressed in HEK293 cells. (A) *Dnd1*^{Ter} transcript level in mutants is similar at E12.5, but >4-fold lower that wild type by E14.5. **(B)** While the flag-tagged mutant protein is expressed in HEK293 cells based on Western analysis, it is detected at much lower levels. **(C)** Immunoprecipitation of the tagged mutant protein relative to input yields much lower levels of DND1-Ter than wild type DND1. **(D)** Autoradiography of the RNA associated with DND1-Ter vs the wild type allele shows no difference above background.

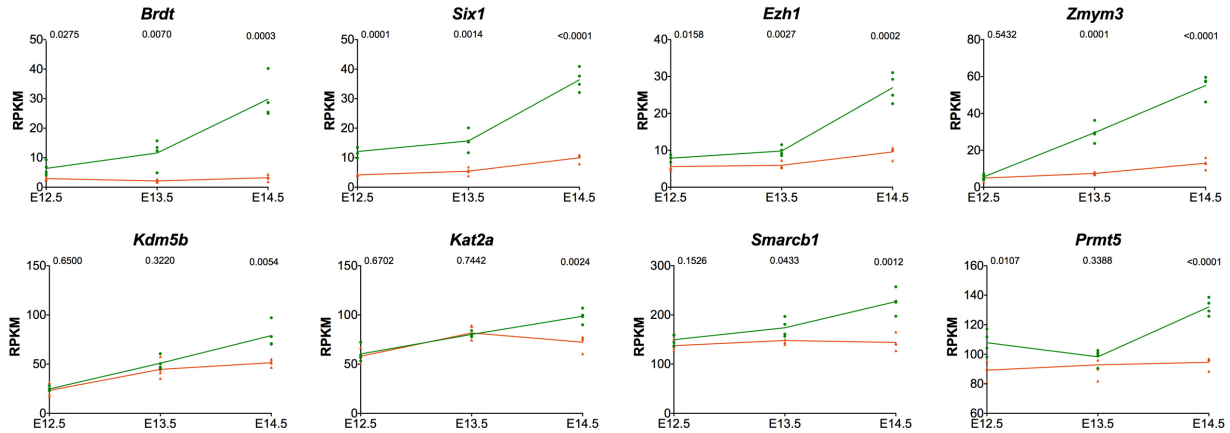
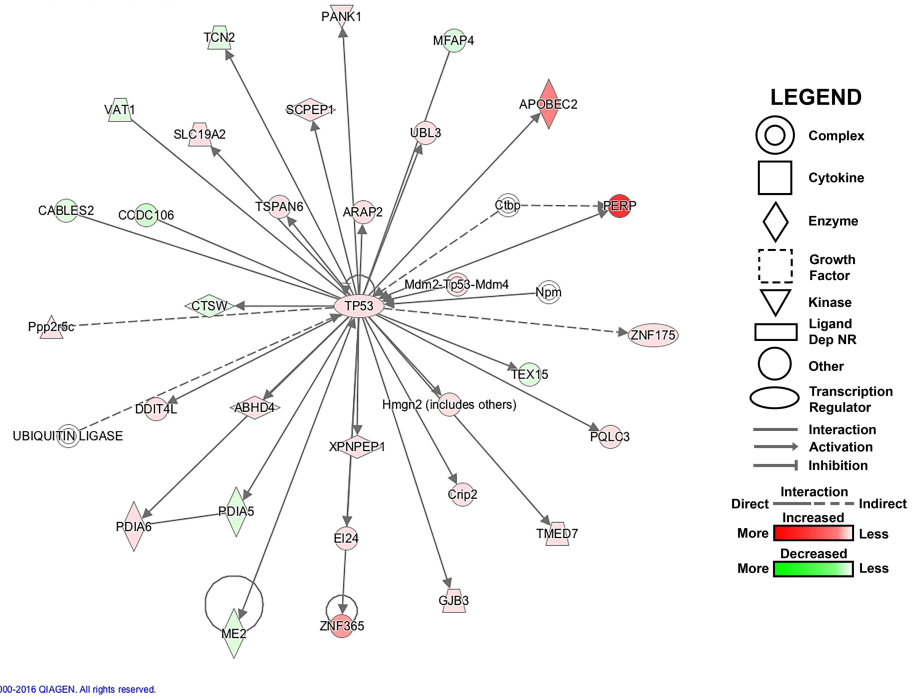


Fig. S8. Other chromatin regulators that are not expressed in HEK293 cells, thus did not map as targets of DND1 in RIP assays, fail to be activated in mutants. Nine additional chromatin regulators (*Brdt*, *Six1*, *Ezh1*, *Zmym3*, *Kdm5b*, *Kat2a*, *Smarcb1*, and *Prmt5*) are all expressed at lower levels in *Dnd1*$^{Ter/Ter}$ mutants likely reflecting a failure to activate the male pathway.

A

Pathway	P-value	# Affected Genes
Cell Death and Survival	$1.05E^{-04} – 3.21E^{-22}$	433
Cellular Growth and Proliferation	$1.06E^{-04} – 5.18E^{-20}$	482

B



C

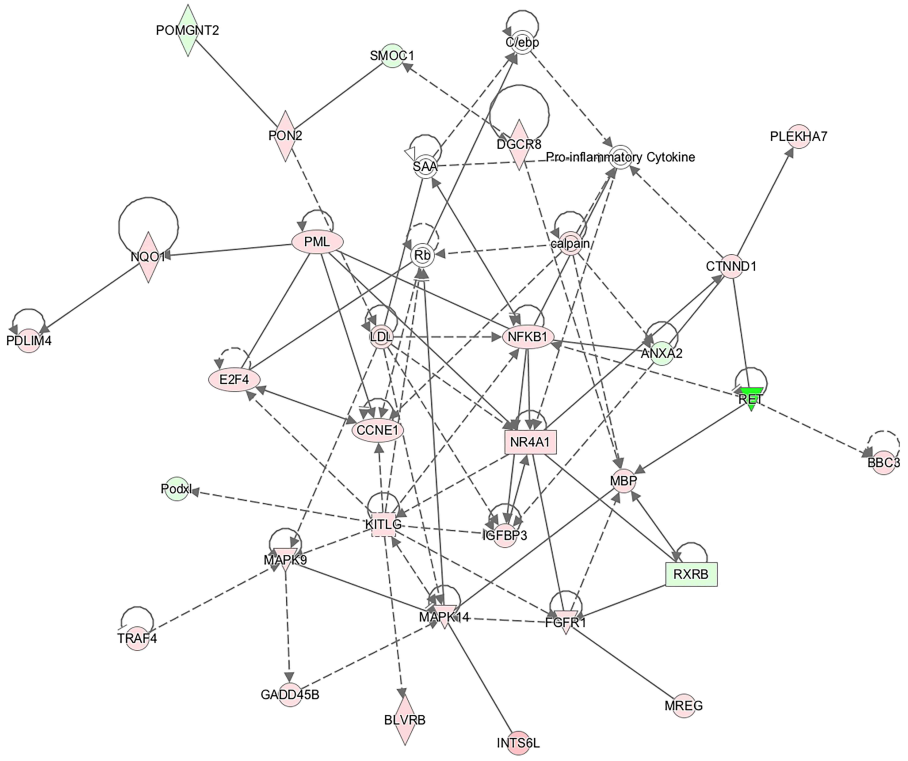


Fig. S9. Cell death and cell cycle pathways are strongly affected in *Dnd1*$^{Ter/Ter}$ mutants. (A) Ingenuity pathway analysis identified cell death (433 genes) and cell cycle (482 genes) pathways as the most strongly affected in *Dnd1*$^{Ter/Ter}$ mutants. **(B)** Network diagram centered on *Tp53*, depicting the relationship of many cell death and survival genes. **(C)** Network diagram centered on *Ccne1*, depicting the relationship of many cell cycle genes, most of which are up-regulated.

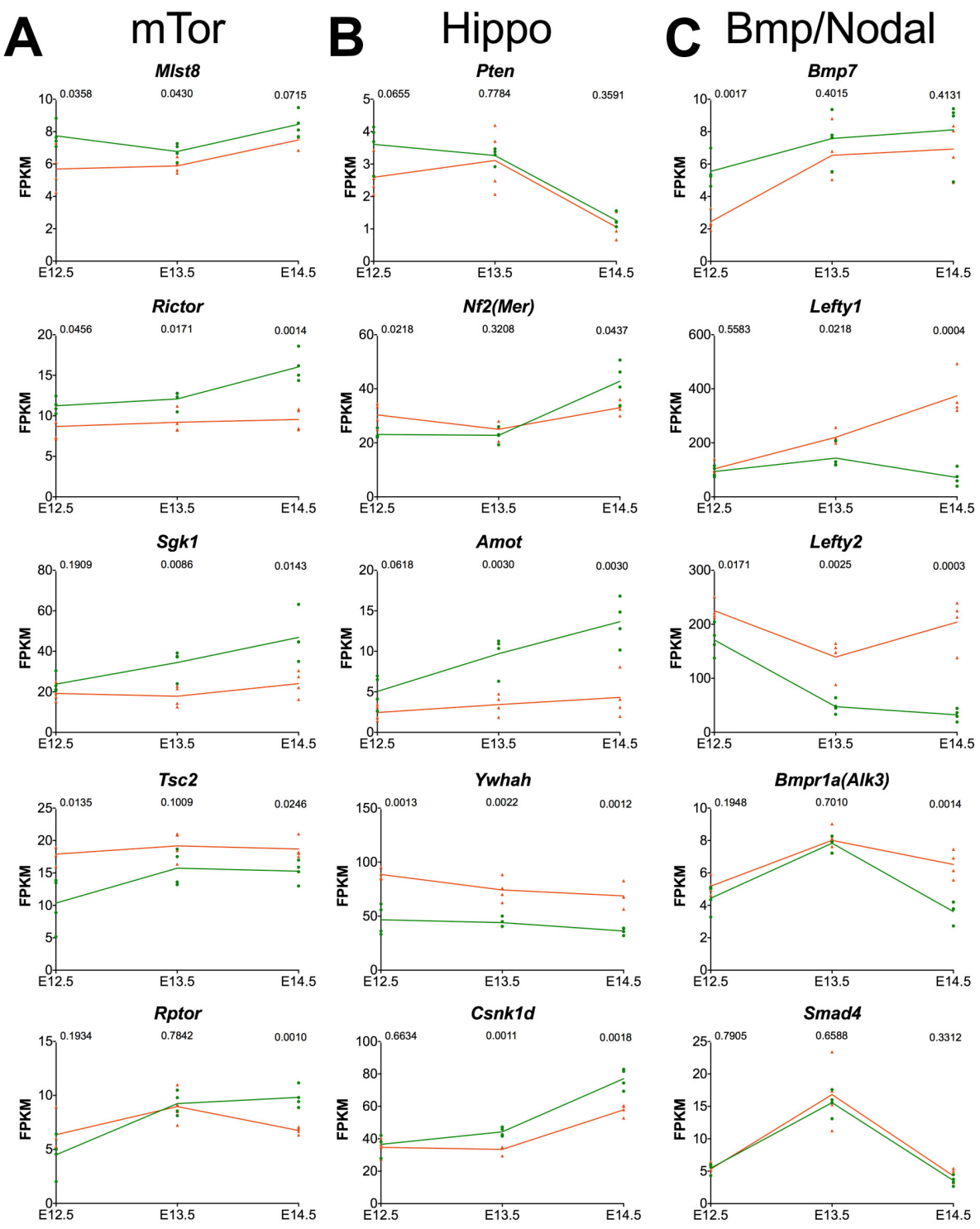


Fig. S10. Expression of genes in the mTor, Hippo and Bmp/Nodal pathways are altered in *Dnd1*$^{Ter/Ter}$ mutants. Expression of other members of the **(A)** mTor, **(B)** Hippo and **(C)** Bmp/Nodal pathways from Fig. 5 are misregulated in mutants with some mapping as transcript targets of DND1 (Fig. 5A).

Table S1. RNAseq gene expression graphing. We provide an excel file with **(A)** the RPKM values used in our analysis and **(B)** the graphic format used to display gene expression in wild type and *Dnd1*$^{Ter/Ter}$ mutants over time. The user can copy any row from **(A)** with all values into row 2 of **(B)** to generate the graph for that gene.

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Table S2. Lists of genes significantly up-regulated or down-regulated in *Dnd1*$^{Ter/Ter}$ mutants at E12.5, E13.5 and E14.5, ordered from the highest to the lowest log2-fold change.

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Table S3. The list of changed genes defined by GSEA as part of the "DPPA4 Targets".

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Table S4. List of genes that are **(A)** up-regulated or **(B)** down-regulated in *Nanos2* mutants, annotated for activity in *Dnd1*$^{Ter/Ter}$ mutants.

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Table S5. (A) List of all genes (expressed or not expressed in HEK293 cells), indicating genes identified in DO-RIP and/or PAR-CLIP (1). **(B)** List of all binding sites enriched in DO-RIP-seq. **(C)** List of genes with reproducible 3'UTR binding sites enriched in both DO-RIP-seq experiments. This table was used to label targets in Figure 5A. **(D)** List of genes unique to Ruthig study, unique to Yamaji study, or identified in both studies.

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Supporting Materials - References

1. Yamaji, M., Jishage, M., Meyer, C., Suryawanshi, H., Der, E., Yamaji, M., Garzia, A., Morozov, P., Manickavel, S., McFarland, H.L. *et al.* (2017) DND1 maintains germline stem cells via recruitment of the CCR4-NOT complex to target mRNAs. *Nature*, **543**, 568-572.
2. Jameson, S.A., Natarajan, A., Cool, J., DeFalco, T., Maatouk, D.M., Mork, L., Munger, S.C. and Capel, B. (2012) Temporal transcriptional profiling of somatic and germ cells reveals biased lineage priming of sexual fate in the fetal mouse gonad. *PLoS Genet*, **8**, e1002575.