Supporting Information

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SI Text

Bisulfite Sequencing. GFP positive E13.5 germ cells from *Dmrt1*$^{-/-}$ *Oct4ΔPE:GFP*$^{+}$ males were sorted into RNAprotect (Qiagen) by using a FACSDiva cell sorter. DNA was prepared by using AllPrep Micro kit (Qiagen) and bisulfate-treated by using EpiTect Bisulfite kit (Qiagen). *H19*, *Lit1*, and *Xist* loci were amplified and sequenced as described in ref. 1 and analyzed by using CpG Viewer (2).

Array Comparative Genome Hybridization (CGH). DNA was phenol: chloroform:isoamyl alcohol-extracted from adult 129Sv wild-type and 129Sv *Dmrt1*$^{-/-}$ testes and spleen tissue and from adult B6 wild-type and B6 *Dmrt1*$^{-/-}$ testes. CGH was carried out on an Agilent mouse genome 4 × 44 K oligonucleotide microarray.

Expression Array Data Analysis. Cel files were normalized by using the GC-RMA algorithm (3) as implemented in the Genedata Expressionist Software Package. Affymetrix probe set ID's were converted to Entrez Gene Symbols by using Affymetrix mappings for the array. Two group *t* tests were carried out to determine genes that were differentially expressed between genotypes. Genes found to have a P value <0.05 and an average fold change of greater than absolute value of 2 are included in the figure. Gene profiles in *Dmrt1*$^{-/-}$ are shown relative to the expression pattern observed in wild-type.

1. Hajkova P, et al. (2002) Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev* 117:15–23.
2. Carr IM, Valleley EM, Cordery SF, Markham AF, Bonthron DT (2007) Sequence analysis and editing for bisulfite genomic sequencing projects. *Nucleic Acids Res* 35:e79.
3. Wu Z, Irizarry R, Gentleman R, Martinez-Murillo F, Spencer F (2004) A model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 99:909–917.
4. Ancelin K, et al. (2006) Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nat Cell Biol* 8:623–630.
5. Looijenga LH, et al. (2006) Genomic and expression profiling of human spermatocytic seminomas: Primary spermatocyte as tumorigenic precursor and DMRT1 as candidate chromosome 9 gene. *Cancer Res* 66:290–302.

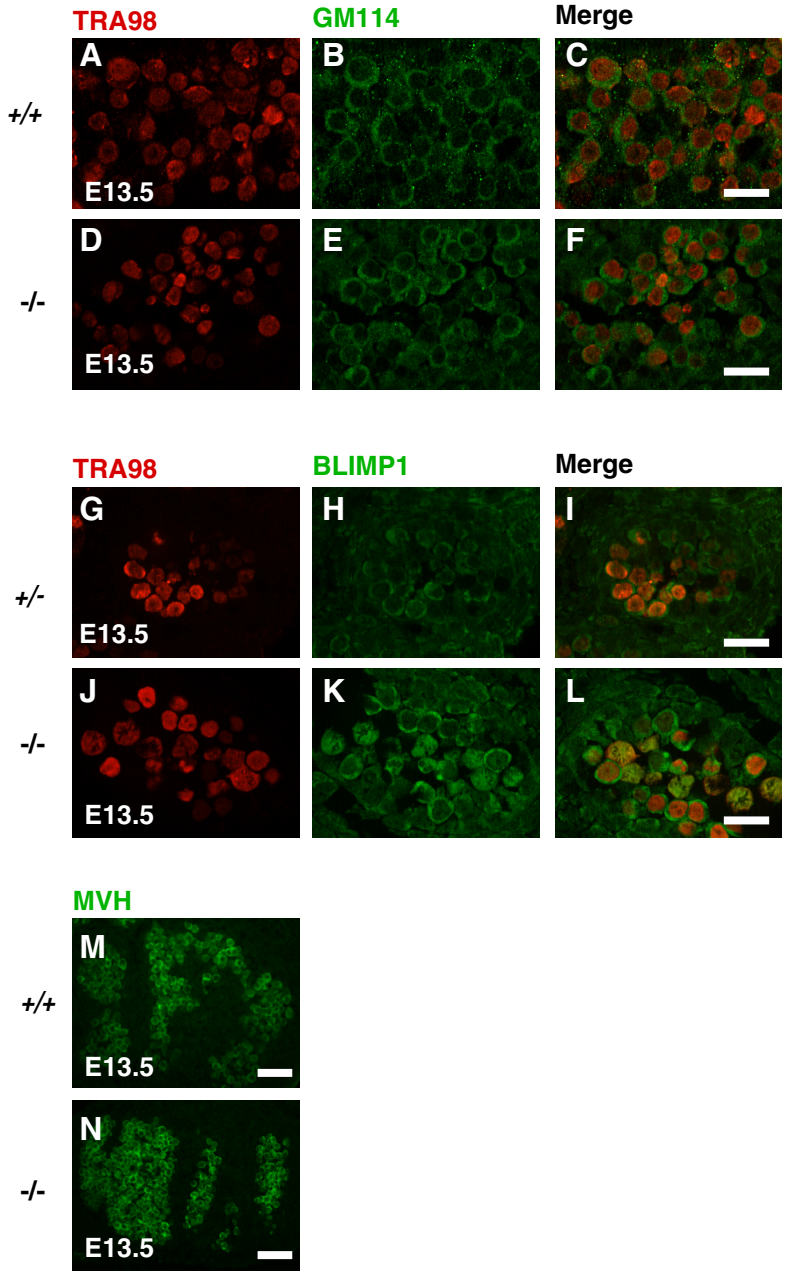

Fig. S1. Early gonocyte developmental markers are normal in *Dmrt1* mutant germ cells. (*A–F*) GM114 is expressed at similar levels in germ cells of *Dmrt1* mutants and wild-type controls. (*G–L*) BLIMP1 translocation to the cytoplasm (4) is complete by E13.5. (*M* and *N*) Similar numbers of MVH positive germ cells are present in *Dmrt1* mutant testes compared to wild-type controls. In addition, the number of germ cells (MVH+) was counted for 50 tubules for $Dmrt1^{-/-}$ (avg = 5.0, SD = 0.5) and wild-type (avg = 4.8, SD = 0.3) at P0; $P = 0.69$ ($n = 2$). (Scale bars, 50 μm.)

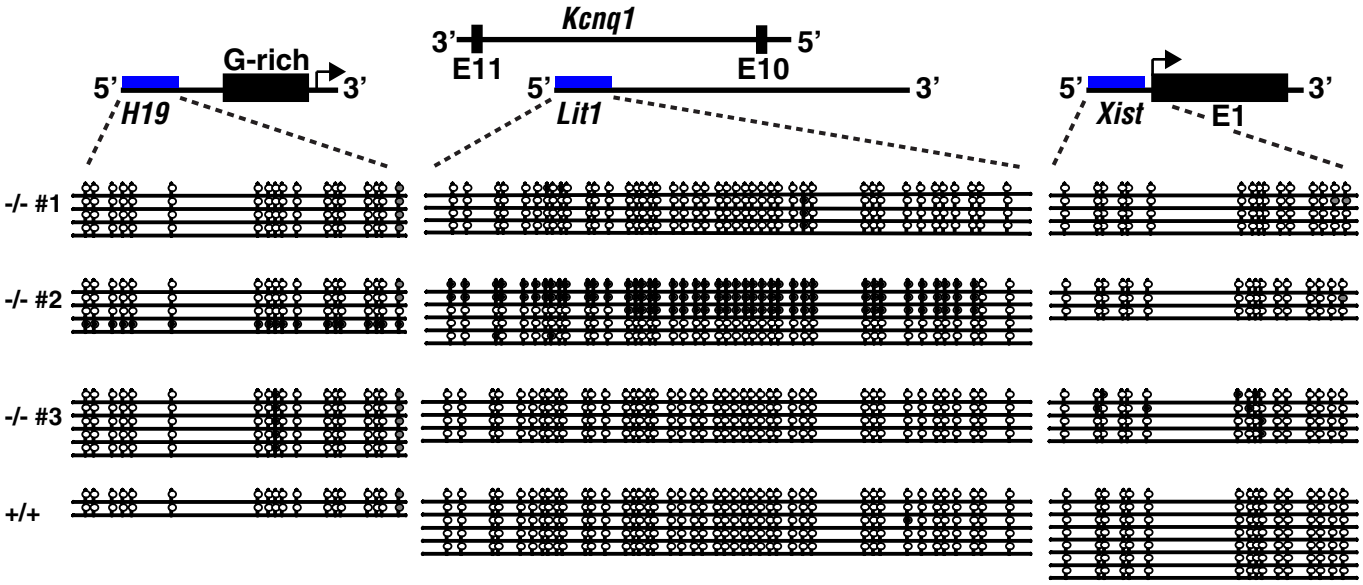

Fig. S2. Parental imprinting is mostly erased by E13.5 in *Dmrt1* mutant germ cells. Methylation of the paternally imprinted locus *H19* (*Left*) and the maternally imprinted locus *Lit1* within the *Kcnq1* gene (*Center*) is erased in *Dmrt1* mutant germ cells. The *Xist* locus (*Right*) underwent normal demethylation by E13.5 in *Dmrt1* mutant germ cells. *Dmrt1*$^{-/-}$ mutant #2 had not completed parental imprinting erasure by E13.5. Open circles, unmethylated; black filled circles, methylated; gray circles, sequence did not align to reference.

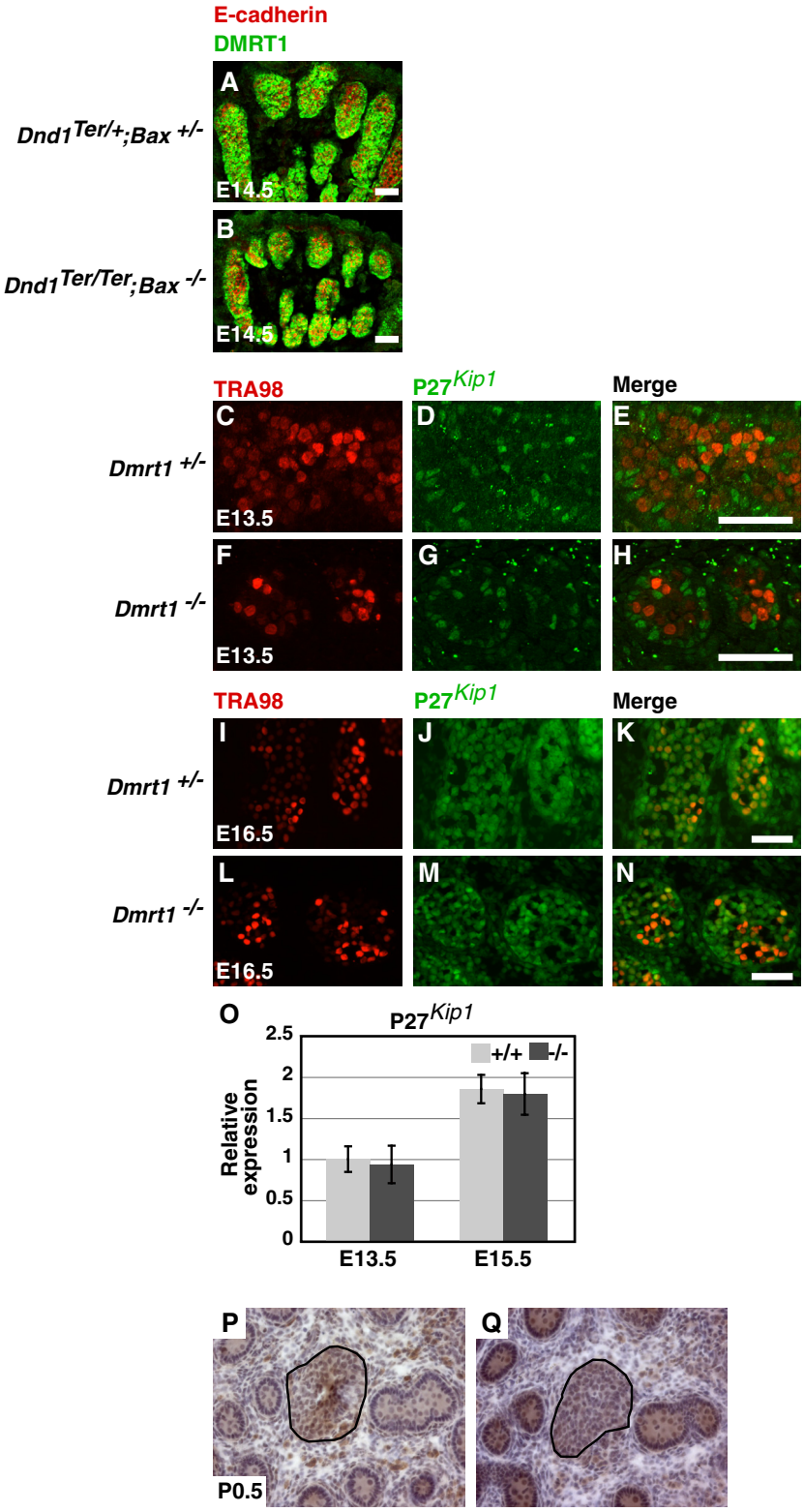

Fig. S3. *Dmrt1* likely acts independently of *Dnd1*. (*A* and *B*) DMRT1 is expressed at similar levels in $Dnd^{Ter/Ter};Bax^{-/-}$ and $Dnd^{Ter/+};Bax^{+/-}$ germ cells at E14.5. Germ cells are marked with E-cadherin, which is expressed in all germ cells at this stage. (*C–N*) Double staining for the germ cell marker TRA98 and the cell cycle inhibitor $P27^{Kip1}$ at E13.5 (*C–H*) and E16.5 (*I–N*). $P27^{Kip1}$ is expressed in *Dmrt1* mutant germ cells at a similar level compared to heterozygous control germ cells at E13.5 and E16.5. (Scale bars, 50 μm.) (*O*) $P27^{Kip1}$ is expressed in E13.5 and E15.5 $Dmrt1^{-/-}$ gonads at similar levels to wild-type controls. Expression was normalized to *Hprt*. Error bars: SD from three animals of each genotype. (*P* and *Q*) Serial sections of $Dmrt1^{-/-}$ gonad at P0.5. (*P*) PTEN and (*Q*) P-AKT Thr-308 are expressed at similar levels in EC cluster, outlined in black, compared to adjacent tubules.

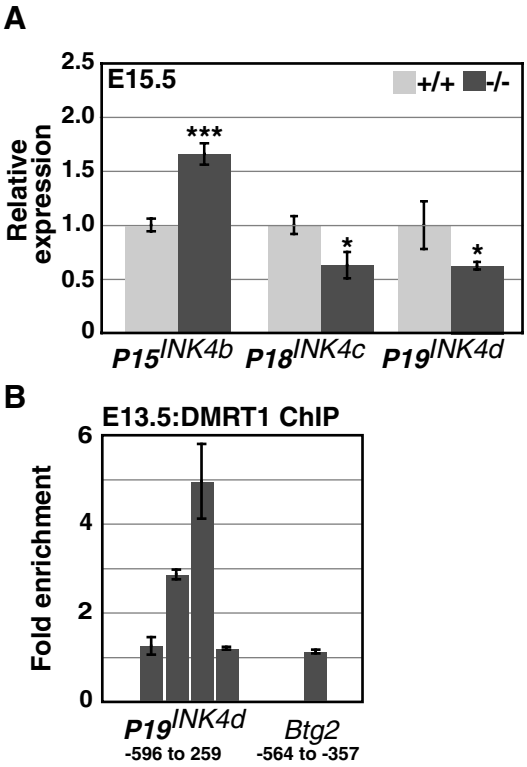

Fig. S4. DMRT1 regulates $P15^{INK4b}$, $P18^{INK4c}$, and $P19^{INK4d}$. (*A*) $Dmrt1^{-/-}$ testes have elevated expression of $p15^{INK4b}$ and have reduced expression of $P18^{INK4c}$ and $P19^{INK4d}$ relative to wild-type controls at E15.5 based on qRT-PCR. Expression is normalized to *Hprt* expression. Error bars: SD from three animals of each genotype. *, $P < 0.05$; ***, $P < 0.0005$. (*B*) DMRT1 binds to $P19^{INK4d}$ promoter in E13.5 testes. ChIP-qPCR of $P19^{INK4d}$ and *Btg2* regulatory regions, comparing enrichment in chromatin immunoprecipitated with DMRT1 relative to input chromatin. Numbering below gene name indicates region covered by amplicons tested, relative to start of transcription. Error bars: SD of duplicate qPCR of sample. The negative control promoter *Btg2* was chosen because it is expressed at similar levels in germ cells and Sertoli cells and does not change expression in *Dmrt1* mutant testes.

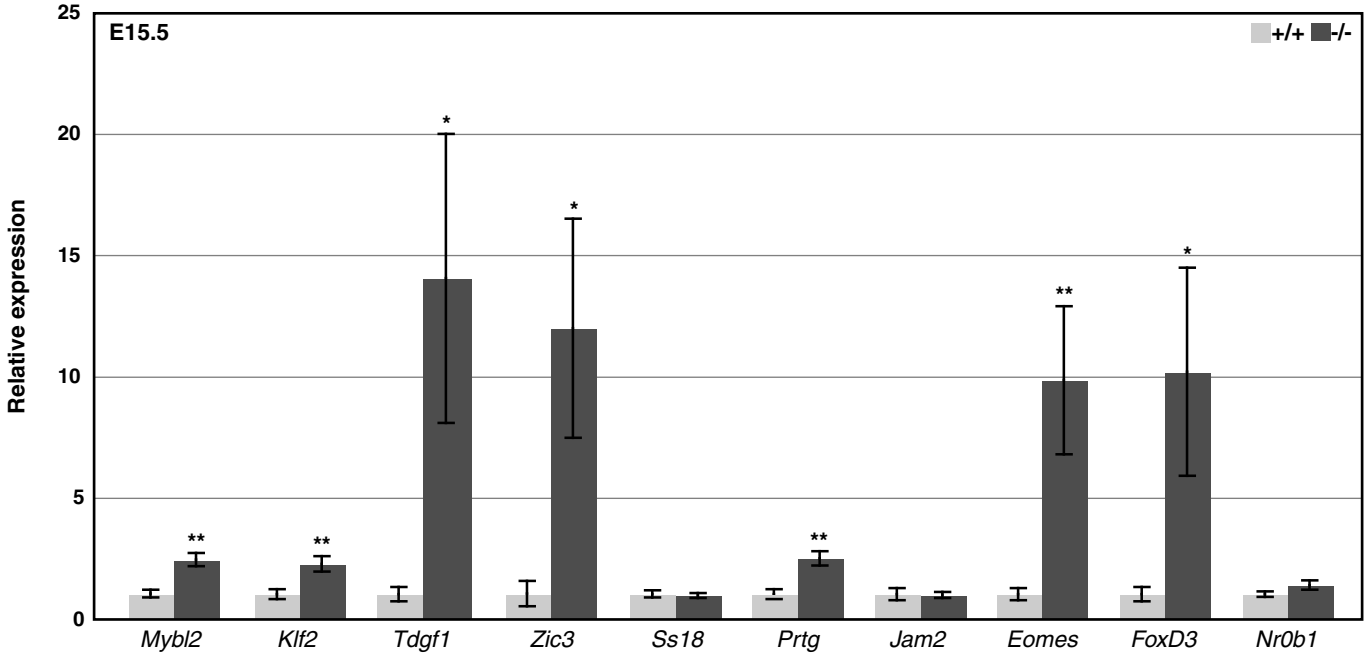

Fig. S5. Early direct response genes of OCT3/4 are overexpressed in *Dmrt1*$^{-/-}$ gonads. Seven out of 10 genes examined by qRT-PCR were significantly up-regulated in *Dmrt1*$^{-/-}$ gonads compared to wild-type at E15.5. Expression was normalized to *Hprt*. Error bars: SD from three animals of each genotype. *, $P < 0.05$; **, $P < 0.005$.

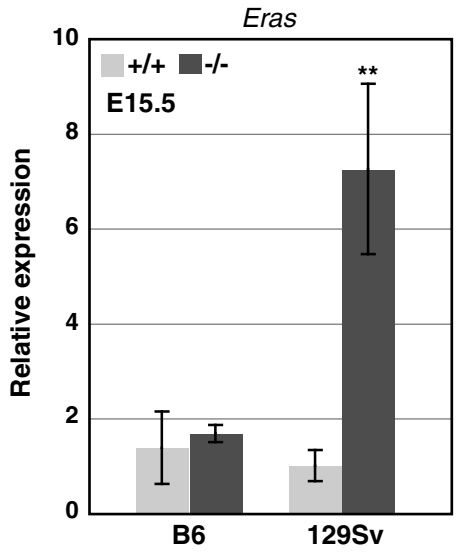

Fig. S6. *Eras* is significantly up-regulated in 129Sv *Dmrt1*$^{-/-}$ gonads relative to 129Sv wild-type and B6 gonads of either genotype. Expression was normalized to *Hprt*. Error bars: SD from three animals of each genotype. **, $P < 0.005$.

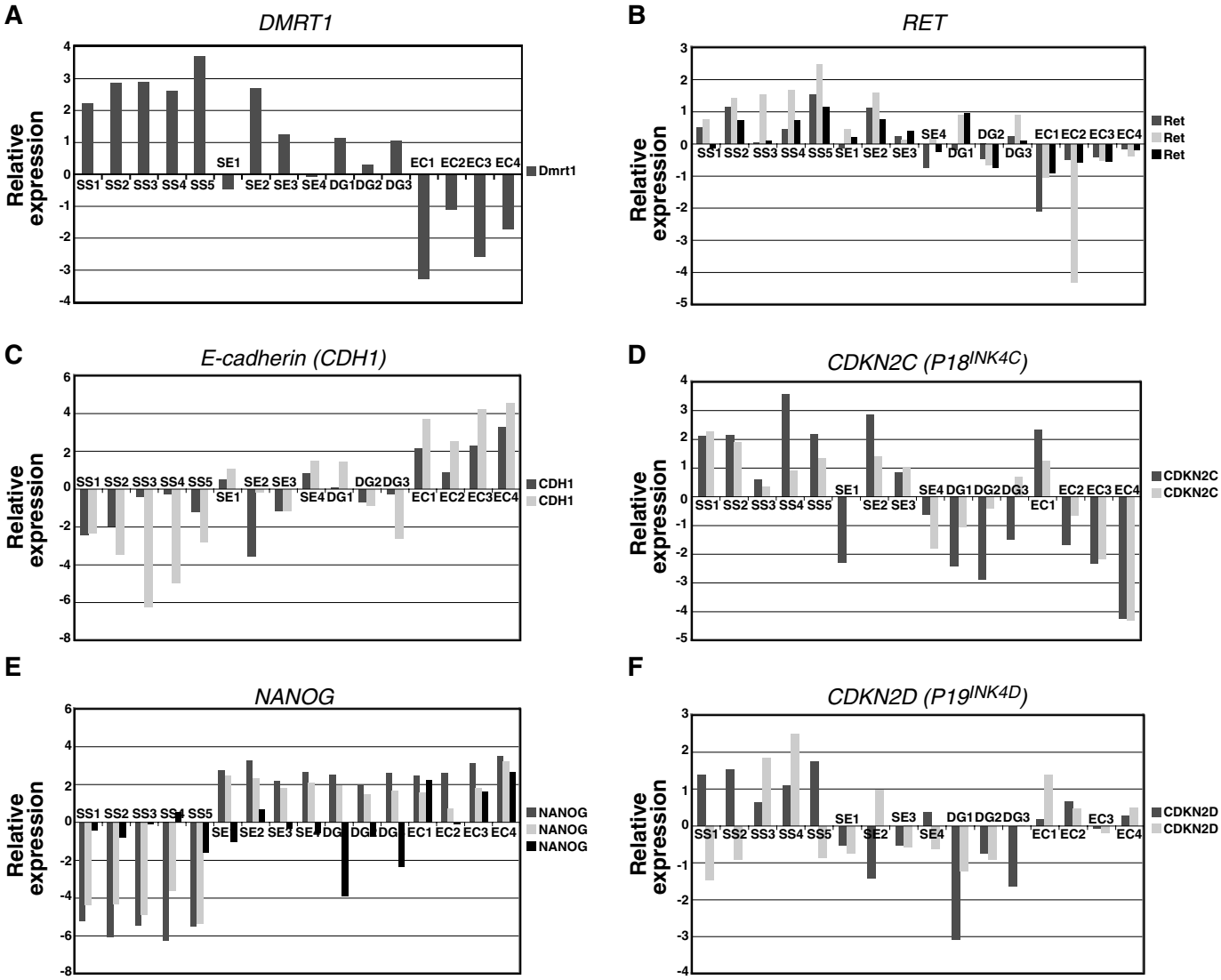

Fig. S7. Gene expression in human germ cell tumors correlates with DMRT1 expression level. Expression profiling of spermatocytic seminomas (SS), seminomas (SE), dysgerminomas (DG), and embryonal carninomas (EC). (*A*) *DMRT1*, (*B*) *RET*, (*C*) *E-CADHERIN*/*CDH1*, (*D*) *CDKN2C* (*P18*^{INK4C}), (*E*) *NANOG*, and (*F*) *CDKN2D* (*P19*^{INK4D}). Tumor samples with high *DMRT1* expression (SS1–5, SE2) also have high *RET*, *CDKN2C*, and *CDKN2D* expression and reduced *E-CADHERIN* expression. EC cells have low *DMRT1* expression and have low *RET* and *CDKN2C* expression and elevated *E-CADHERIN* expression. *NANOG* expression was reduced in SS and elevated in SE, DG, and EC. Relative expression was determined in relation to the geometric mean of hybridization intensities for each patient sample. Differences in relative expression represent deviations from the geometric mean (5).

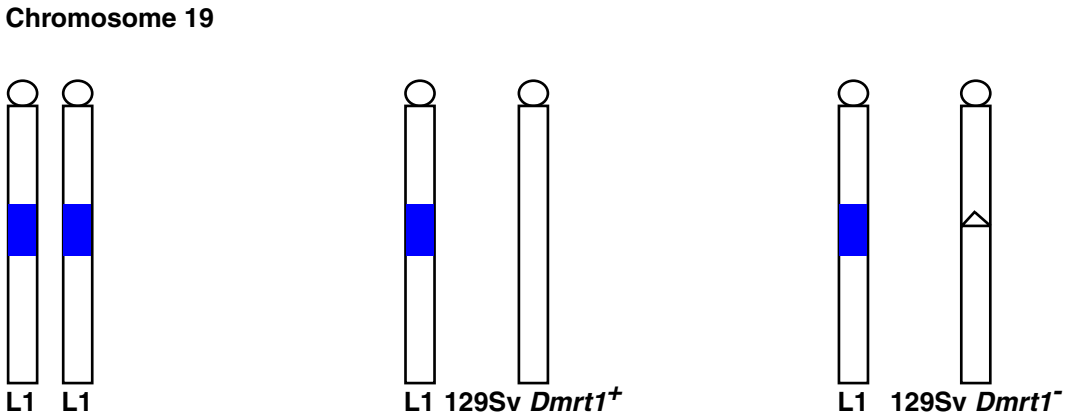

Fig. S8. Diagram of complementation test with L1 and 129Sv *Dmrt1*. Blue bar represents region of MOLF-derived DNA substituted for 129Sv DNA in congenic L1 chromosome 19. Triangle indicates position of *Dmrt1* null mutation.

Table S1. Antibodies

Antibody	Supplier	Catalog no.	Diluton
RET	Neuromics	GT15002	1:100
TRA98	Bio Academia	73–003	1:500
BLIMP1/PRDM1	Lifespan Biosciences	LC-C2538	1:50
Ki67	Thermo Scientific	RM-9106-S1	1:250
E-CADHERIN	Zymed Laboratories	12–1900	1:500
DMRT1 140A	David Zarkower		1:500
STRA8	Pierre Chambon		1:200
SOX2	Chemicon	AB5603	1:300
OCT3/4	Santa Cruz	SC-9081	1:150
NANOG	Cosmo Bio	REC-RCAAB0002PF	1:250
MVH	Abcam	AB13840	1:300
$P27^{Kip1}$	Santa Cruz	Sc-528	1:100
P-AKT Thr 308	Cell Signaling	244F9H2	1:50
PTEN	Cell Signaling	138G6	1:100
GM114	Blanche Capel		1:500
Goat α rat Alexa 594	Molecular Probes	A11007	1:500
Goat α rabbit Alexa 488	Molecular Probes	A11034	1:500
Goat α mouse Alexa	Moleular Probes	A11029	1:500
Biotinylated α rabbit IgG	Vector Labs	BA-1000	1:200

Table S2. qRT-PCR primer sequences

qRT-PCR	Forward	Reverse
Ret	TTCCGAGGAAATTCCCACTT	CAGGGCTTCCCAATCAGTTA
E-cadherin	TGAGCTGCCTCACAAAAACA	AGCCTGAACCACCAGAGTGT
Sox2	GAGTGGAAACTTTTGTCCGAGA	GAAGCGTGTACTTATCCTTCTTCAT
Oct3/4	GAAGCAGAAGAGGATCACCTTG	TTCTTAAGGCTGAGCTGCAAG
Nanog	CCTCAGCCTCCAGCAGATGC	CCGCTTGCACTTCATCCTTTG
p15^{INK4b}	AATAACTTCCTACGCATTTTCTGC	CCCTTGGCTTCAAGGTGAG
P27^{Kip1}	TGGAGGGCAGATACGAATG	CGGGGGCCTGTAGTAGAACT
P18^{INK4c}	CAGATTAACCATCCCAGTCCTT	CCCCTTTCCTTTGCTCCTAA
P19^{INK4d}	AATGTGACCCAAGGCCACT	TTTCCTCTTTTGTTGAGAAGTAACC
Mybl2	AGGACAAGGAACAGCACCAG	GCAGCTATGGCAATCTCCTC
Klf2	ACCAAGAGCTCGCACCTAAA	CTGTGACCTGTGTGCTTTCG
Tdgf1	GGAAGGCACAAACTGGAAAG	GTTTGAATTTGGACCCGTTG
Zic3	TCCACAAGAGGACCCATACAG	TATAGGGCTTGTCCGAGGTG
ss18	ACTGCTGACCTTGACCCTGA	ACCTCCACAGCAAGGATACC
Eomes	CAATGTTTTCGTGGAAGTGG	GTGGGAGCCAGTGTTAGGAG
Prtg	GGGGAAGAACCTGGAGAGAG	GACCGTAGCTATTGATGATCAGG
Jam2	CCTCCTGATGCTGCTGCT	TGTGACTTCTTGACGGTGGT
FoxD3	CAGCAACCGTTTTCCGTACT	GGGTCCAGGGTCCAGTAGTT
Nr0b1	TATCTGAAAGGGACCGTGCT	CAGCGGATCTGATCTGGTACT
Eras	CCTCTGGAGATCTGGTGCAT	GCCCCTCATCAGACTGCTAC
HPRT	TCCTCCTCAGACCGCTTTT	CCTGGTTCATCATCGCTAATC

Table S3. ChIP primer sequences

ChIP primers	Forward	Reverse	Size	Distance from transcription start	
				F primer	R primer
Sox2 4	CAGCAGCCACATCTCAGAAA	TTTTCTCCTTAATCGGGGTGT	125	−2,104	−1,980
Sox2 5	ATAAGTCCTTTCCGGGTTCC	ATGCCCACTTTAGACCCAGA	148	−1,937	−1,790
Sox2 2	AGCCAAGCCTGGGAGAAT	GCTCCGCTCATTGTCCTTAC	108	−1,766	−1,659
Sox2 6	GGGCAGAGAGGGAAGGATAA	TTTTTCCCTGCAATACTCTCG	135	−1,710	−1,568
Sox2 3	TGGCATCAGGACTTTCTTCC	GCCCCGTCTAAGTTTCCTTC	100	−1,499	−1,400
p19^{INK4d} 2	GGGCCCATCTTGGATCTTTA	GCGCTTTACCACTCAGAAGC	189	−408	−596
p19^{INK4d} 4	TTGTCTCTCCCGTAGCAGTG	CGCTGGGGTCAGTTAAACC	141	−243	−383
p19^{INK4d} 1	GACTCACCCTCCCTCCTTTC	GGCTCTCGCTACTCTGTTGC	160	−6	−165
p19^{INK4d} 3	CCTCCATTGTCACAAAAGCA	GATCCAACTCCCCAAAATGA	119	259	157
Btg2	GACACTGACAGAGCCGTTCA	ACACTCCTCCCACCAAACAG	208	−357	−564

All ChIP qPCR reactions performed in the presence of 0.5 M Betain.