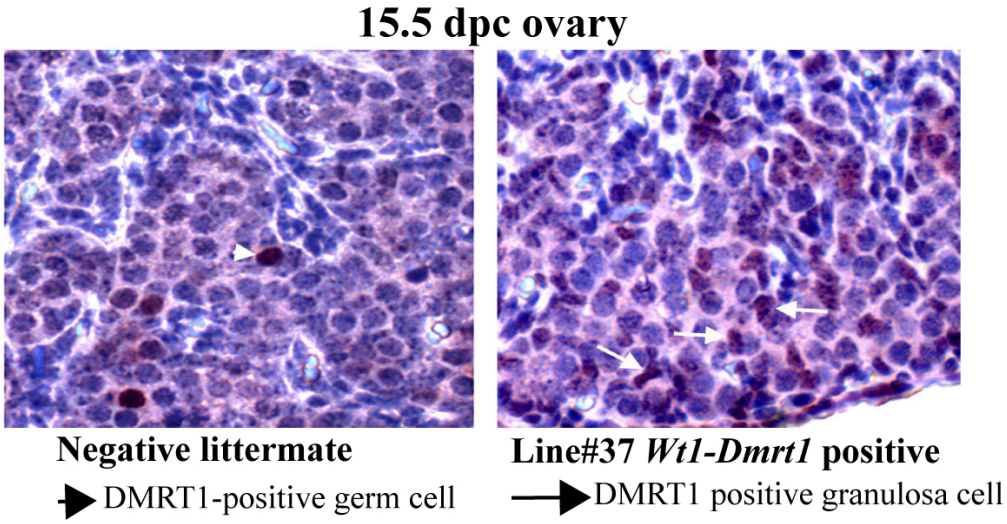
BOR-Papers in Press. Published as DOI: 10.1095/biolreprod.112.103135

Agbor et al., Supplemental Figures

Supplementary Figure 1:



Supplemental Figure S1. Expression of transgenic DMRT1 in somatic cells of 15.5dpc ovary. Immunocytochemistry was used to examine DMRT1 expression in wild type (negative littermate, left) and transgenic ovaries isolated 15.5dpc embryos. In the wild type ovary (left) a few DMRT1-positive germ cells (arrowheads) were observed. As previously shown, DMRT1 was absent from ovarian somatic cells of 15.5dpc [1]. In the transgenic ovary (right), numerous DMRT1-positive somatic cells (some noted by arrows) were observed and few DMRT1-postive germ cells were present. Magnification x400.

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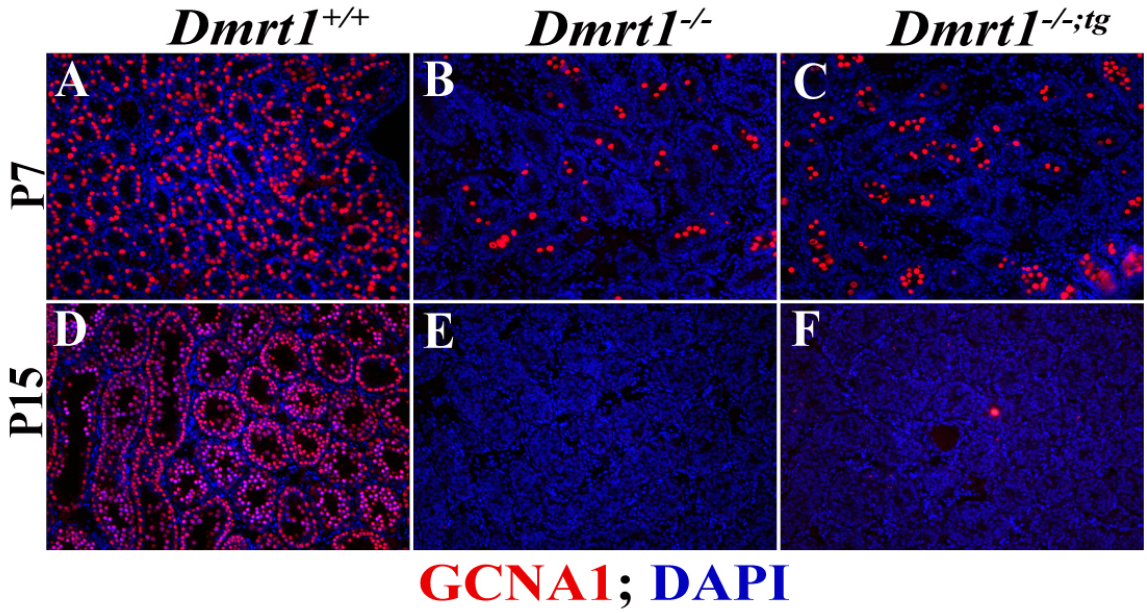
Supplementary Figure 2:



Supplemental Figure S2. Germ cell loss in *Dmrt1*$^{-/-}$ and *Dmrt1*$^{-/-;tg}$ mice testes at P7 and P15. GCNA1, a germ cell-specific protein, was examined to evaluate germ cell numbers in testes from different genotypes. GCNA1 immunofluorescence (red) was examined in testis sections from P7 (**A**-**C**) and P15 (**D**-**F**) wild type (**A** and **D**), *Dmrt1*$^{-/-}$ (**B** and **E**) and *Dmrt1*$^{-/-;tg}$ (**C** and **F**) mice. DAPI (blue) was used to stain nuclei. Final magnification for all micrographs is x400.

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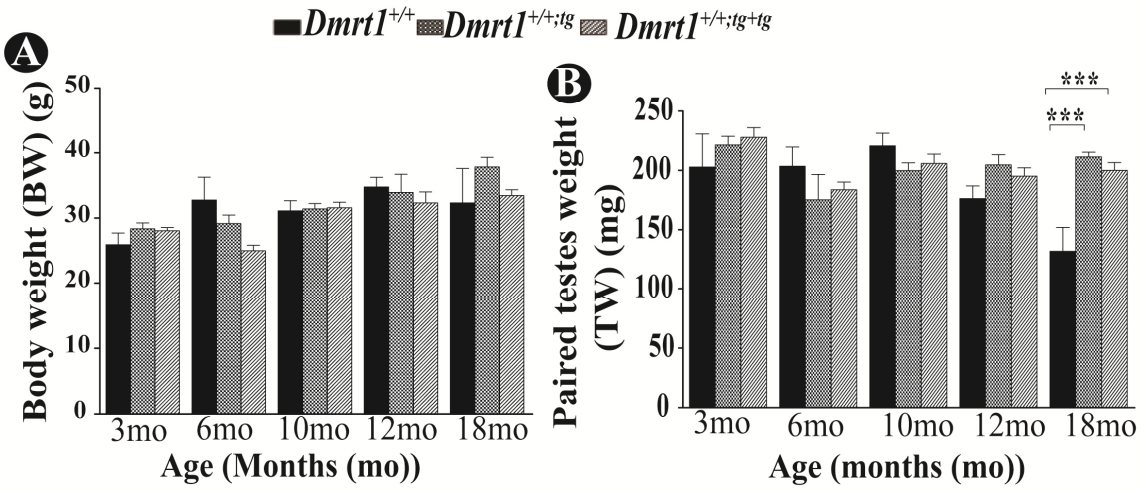
Supplementary Figure 3:



Supplemental Figure S3. Effect of exogenous *Dmrt1* on testes weights and sperm motility. Average body (A) and paired testes (B) weights were determined for wild type mice (*Dmrt1*$^{+/+}$; black bar) and mice with either one (*Dmrt1*$^{+/+;tg}$; stipple bar) or two (*Dmrt1*$^{+/+;\ tg+tg}$; stripe bar) copies of the transgene was determined at 3, 6, 10, 12, and 18 months of age. For each genotype, a minimum of three animals were used per time point. Bars represent mean ± SEM of three to 20 animals. ***$p<0.0001$ (Two tail Student T-Test).

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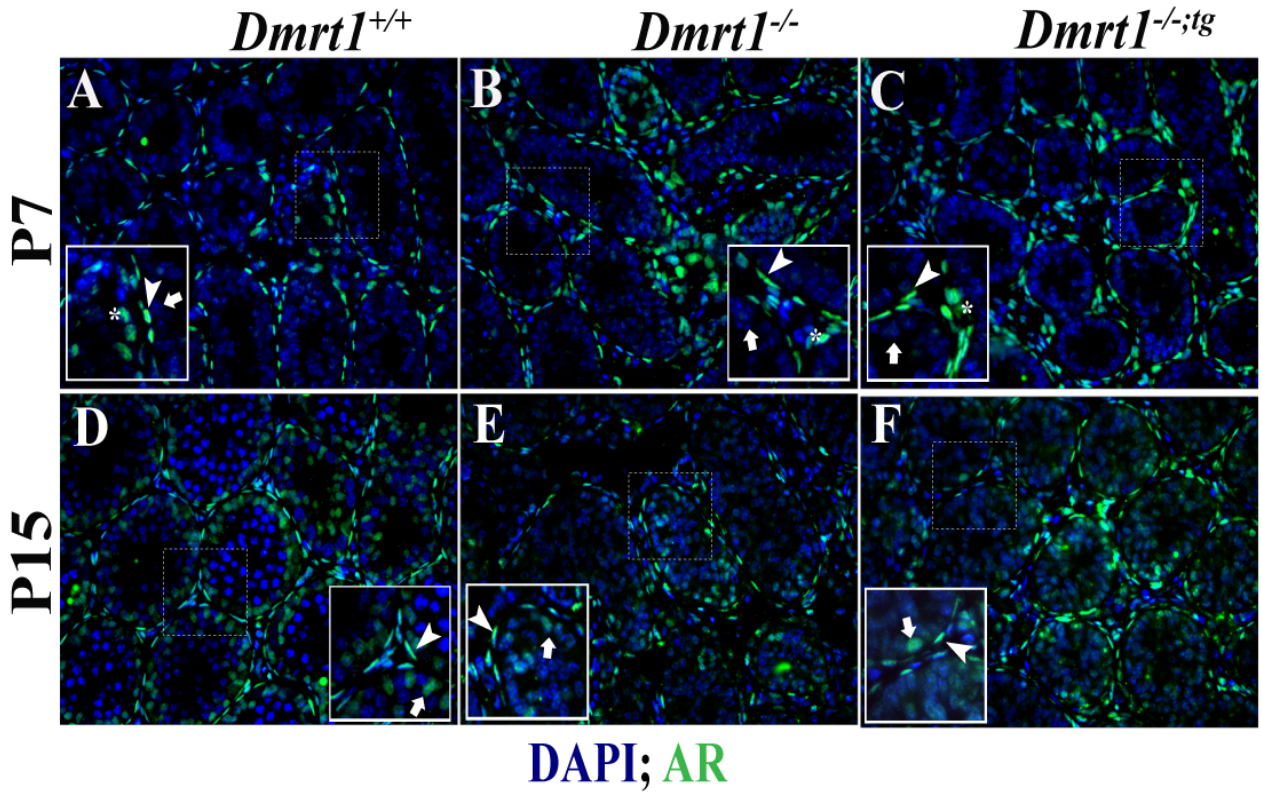
Supplementary Figure 4:



Supplemental Figure S4. AR expression in P7 and P15 testes. AR immunofluorescence of P7 (A-C) and P15 (D-F) testes from *Dmrt1*$^{+/+}$ (A & D), *Dmrt1*$^{-/-}$ (B & E), and *Dmrt1*$^{-/-;tg}$ (C & F) mice. Arrows denote AR-positive Sertoli cells. Asterisks denote AR-positive Leydig cells. Arrowheads denote AR-positive peritubular myoid cells. DAPI (blue) was used to stain for nuclei. Final Magnification x400.

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Supplemental Tables

Supplemental Table S1. Primers used to PCR-amplify components of targeting vector.

Clone	Sequence (5'→3')	Direction	Product size (bp)	Polylinkers
Left arm *(Wt1* 5′)	GCGCAAGCTTGAGCATCCTGGCTCCTCCTC	F	825	HindIII
	GCGCACGCGTCGTCGTTAGGCATGAGGTGCGGCTCGG	R		MluI
Right arm *(Wt1* 3′)	GCGCCCGCGGTTCCGACGTGCGGGACCTG	F	776	SacII
	GCGCCCGCGGATCGATCCCTAAACCACAGCACCACTC	R		SacII
HPRT1	GCGCTCTAGAGTCGAGGACTTCAGGGATTTG	F	1312	XbaI
	GCGCTCTAGAATTCAAAAAGTGGCGAATT	R		XbaI
LYS2	AGAGAGCGGCCGCCACTTGCAATTACATAAAAAATTCCGG	F	4804	NotI
	AGAGAGCGGCCGCCGCAAGTATTCATTTTAGACCCATG	R		NotI
Dmrt1 m*MluI*	GCGCACGCGTTCGGCAAGCCCTCTGCACCG	F	237	MluI
	GCGCACGCGTAGCCGTGGTTCCTGCAGCGA	R		MluI

Supplemental Table S2. Primers used for genotyping.

	Primer Name	Sequence (5'→3')	Direction	Product size (bp)
Genotyping of Transgenic founders	*Wt1*-355	GCCTCAGAACCCAGGAGAG	F	873
	Dmrt1.16A	GTGAGGAACCTCCGTCGG	R	
Sex Genotyping	SRY Forward	AAGCGCCCCATGAATGCATT	F	250
	SRY Reverse	CGATGAGGCTGATATTTATA	R	
Genotyping of Transgenic pups	TGIF0088	GTCCTCTGAACCTAGCAGCTACG	F	689
	TGIF0090	CCATGGAGACTTTCTAACTGCTCCTG	R	
Transgene expression in Transgenic mice	*Dmrt1 3'*	CACAGGGTATTAGGAGGCTTG	F	434
	HPRT1	GGCCTATAGGCTCATAGTGCAA	R	
Genotying of $Dmrt1^{+/+}$ allele	TGIF0105 (KOs 1N)	GATCTATCTGGAGCCAGGTGGTAG	F	277
	TGIF0107 (KOs 2N)	TGCACACGTGCACCCTCGCCATCG	R	
Genotying of $Dmrt1^{-/-}$ allele	TGIF0105 (KOs 1N)	GATCTATCTGGAGCCAGGTGGTAG	F	420
	TGIF0106 (KOs 3N)	TCATGGCAGCTCTCCCAGTGGAGC	R	
Probe to identify positive YAC*Wt1*-*Dmrt1* clones	*Dmrt1* ΔMluI Up	GCGCACGCGTTCGGCAAGCCCTCTGCACCG	F	237
	Dmrt1 ΔMluI Down	GCGCACGCGTAGCCGTGGTTCCTGCAGCGA	R	
Probe for Tg*Dmrt1* copy number determination	*Dmrt1* exon 5 forward	GCCCAGCAGTCAAGATTCTG	F	165
	Dmrt1 exon 5 reverse	CGACTCAGTTCACAGGGTATT	R	

Supplemental Table S3. Primers used for quantitative PCR.

Transcript	Sequence	Tm	Direction	Product Length (bp)	Function	Reference
Ar	TGGCGGTCCTTCACTAATGTC	59	F	72	Marker of Somatic cells (Sertoli, Leydig & myoid cells) in the testis	[2]
	TGCGGTACTCATTGAAAACCAA	59	R			
Gata1	GTCAGAACCGGCCTCTCATC	58	F	59	Sertoli cell maturation marker (nuclear and cytoplasmic)	[3]
	TGCCTGCCCGTTTGCT	58	R			
Krt18	CTT GCT GGA GGA TGG AGA AG	58	F	72	Immaturity marker	[4]
	CTG CAC AGT TTG CAT GGA GT	58	R			
Gata4	GGGCCAACCCTGGAAGAC	59	F	67	Sertoli cell nuclei marker	[5, 6]
	GACACACTCTCTGCCTTCTGAGAA	59	R			
Espin	TTACATGCAGACCAAGAACAAGCT	59	F	64	Adherens junction Protein	[7, 8]
	CCACCTTGGGCTCCTTGAG	59	R			
TJP	GCAATGGAGGAAACAGCTATATGG	60	F	61	Tight and adherens junction protein	[8, 9]
	AACCCAGGAGCCCTGTGAA	59	R			
Ocln	CTGGACATTTTGCTCATCATAAAGA	58	F	102	Tight junction protein	[8, 9]
	GTTTGAATTCATCAGGTCTGTAAGGA	59	R			
Cldn11	GGACGAACTGGGCTCCAA	58	F	104	Tight junction protein	[8, 9]
	TGCACGTAGCCTGGAAGGA	59	R			
Rpl7	CAACAAGGCTTCAATTAACATGCT	59	F	59	Structural constituent of ribosome	[10]
	GGGTACCCCCATGCAATG	59	R			

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