Supplementary Tables and Figures

Supplementary Table S1 Sequence of primers used in PCR amplification

Gene name	GenBank accession No.	Primer Sequence (5'-3')	Tm/°C	Size/bp
Gapdh	XM_005680968.3	Fwd: CCACGCCATCACTGCCACCC Rev: CAGCCTTGGCAGCGCCAGTA	58	116
Dmrt1	XM_005683769.3	Fwd:CCCGTGCCTGATGATTGA Rev:GGCGATTCTCCGAGTTTT	58	380
TNF-α	NM_013693.3	Fwd: CCTGTAGCCCACGTCGTAG Rev: GGGAGTAGACAAGGTACAACCC	58	148
IL-6	NM_001314054.1	Fwd: GAAACCGCTATGAAGTTCCTCTCTG Rev: TGTTGGGAGTGGTATCCTCTGTGA	58	136
IFNα	NM_010503.2	Fwd: TACTCAGCAGACCTTGAACCT Rev: CAGTCTTGGCAGCAAGTTGAC	58	307
IFNβ	NM_010510.1	Fwd: GGATCCTCCACGCTGCGTTCC Rev: CCGCCCTGTAGGTGAGGTTGA	58	153
Plzf	JX047313.1	Fwd: CACCGCAACAGCCAGCACTAT Rev: CAGCGTACAGCAGGTCATCCAG	58	127
Csf1r	XM_006525586.3	Fwd: AACTATGTTGTCAAGGGCAATGC Rev: GGACCACACATCACTCTGAACTG	58	99
Nanog	FJ970651.1	Fwd: GGAACTGCTGGGGAAAATTA Rev:TACAAATCTTCAGGCTGTATGTTG	58	118
Pcna	NM_011045.2	Fwd: AGTGGAGAACTTGGAAATGGAA Rev: GAGACAGTGGAGTGGCTTTTGT	58	167
IL-8	NM_011339.2	Fwd: TGATAGCAGTCCCAAAAAATG Rev: CCAACAGTAGCCTTCACCCAT	58	73
IL-10	NM_010548.2	Fwd: CTGGACAACATACTGCTAACCGA Rev: CCTGGGGCATCACTTCTACC	58	112
TLR2	XM_018060889.1	Fwd: CGTGTTATCAGTTATGAGAAT Rev: GAGCGTCACAGCGGTAG	58	203
TLR4	NM_001285574.1	Fwd: TAAAGAACTTGGAGGAGGGCG Rev: GGGACACCACGACAATCACC	58	138
TLR5	NM_001285699.1	Fwd: GGGACTCAGCCTCAGAGACAA Rev: TGACCCAATGGATAAAAGCACTA	58	197
TRAF3	XM_013973268.2	Fwd: GAGTCCCTCCAGAACCG Rev: TCCGCCGCTTGTAGTCG	58	223

Supplementary Table S2 H1 vs. shDmrt1 GO enrichment histogram

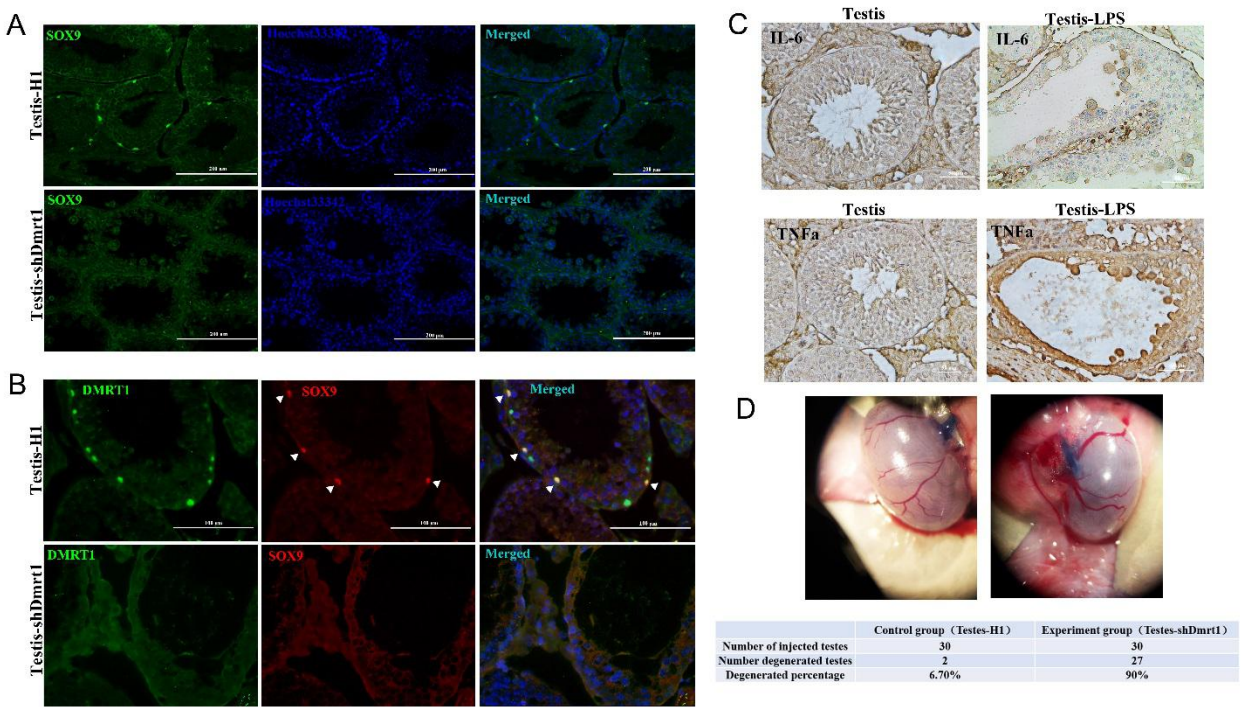
Description	Term_type	Corrected_pValue	DEG_item
oxidation-reduction process	biological_process	0	110
proteolysis	biological_process	0.000425	81
cell adhesion	biological_process	0.000562	17
transmembrane transport	biological_process	0.000738	78
signal transduction	biological_process	0.00145	52
metabolic process	biological_process	0.004103	58
immune response	biological_process	0.014321	27
oxidoreductase activity	molecular_function	0	60
protein binding	molecular_function	0.000439	384
catalytic activity	molecular_function	0.001278	46
cysteine-type endopeptidase inhibitor activity	molecular_function	0.002236	11
oxidoreductase activity, acting on the CH-CH group of donors	molecular_function	0.032465	11
viral envelope	cellular_component	0.001751	8
myosin complex	cellular_component	0.018035	13
extracellular matrix	cellular_component	0.032871	9

Supplementary Table S3 H1 vs. shDmrt1 KEGG enriched pathways

#Pathway	KO	Enrichment Factor	Q-value	Gene Number
Fatty acid metabolism	ko01212	3.57	5.20E-08	27
Valine, leucine and isoleucine degradation	ko00280	3.38	2.81E-07	27
Cytokine-cytokine receptor interaction	ko04060	1.89	3.49E-06	74
Propanoate metabolism	ko00640	4.25	3.73E-06	17
Fatty acid degradation	ko00071	3.31	4.06E-06	24
Malaria	ko05144	3.17	2.23E-05	23
Rheumatoid arthritis	ko05323	2.51	6.22E-05	32
Glycine, serine and threonine metabolism	ko00260	3.29	7.14E-05	20
Amoebiasis	ko05146	2.2	9.59E-05	41
PPAR signaling pathway	ko03320	2.47	0.000226	30
Phagosome	ko04145	1.92	0.000875	48
Osteoclast differentiation	ko04380	2.05	0.001633	38
ECM-receptor interaction	ko04512	2.25	0.002916	29
Protein digestion and absorption	ko04974	2.2	0.004783	29
Tuberculosis	ko05152	1.81	0.004833	48
Leishmaniasis	ko05140	2.38	0.005676	24
Biosynthesis of unsaturated fatty acids	ko01040	3.38	0.006636	13
TNF signaling pathway	ko04668	2.04	0.007265	33
Mineral absorption	ko04978	2.7	0.008961	18
beta-Alanine metabolism	ko00410	2.95	0.021098	14

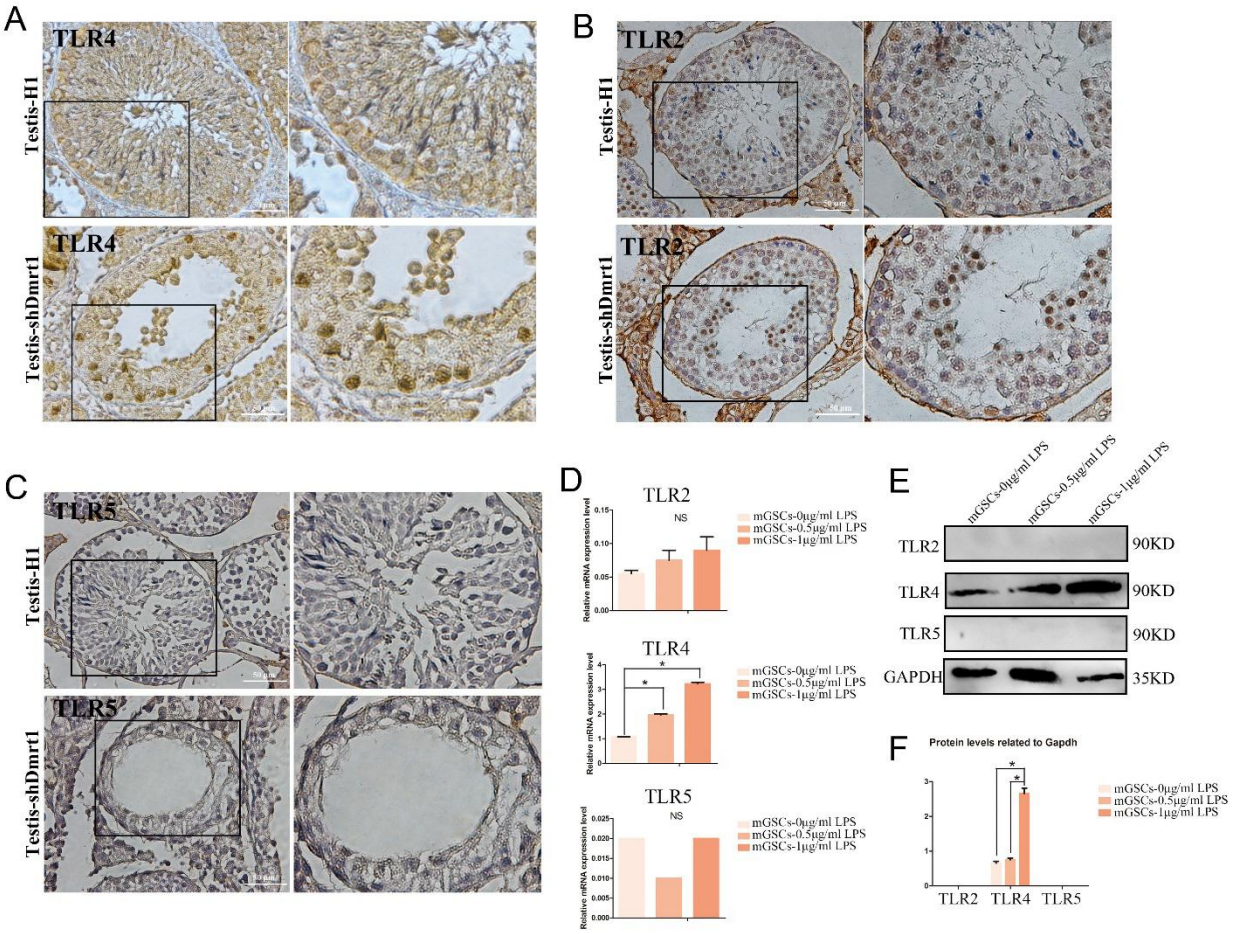
Supplementary Table S4 H1 vs. shDmrt1 differentially expressed genes

#ID	H1-9_Count	shDmrt1-9_Count	H1-9_FPKM	shDmrt1-9_FPKM	FDR	log2FC	regulated
Dmrt1(ENSMUSG00000024837)	360	39	8.348695	0.938961	6.88E-15	-3.18009	down
Zbtb16(ENSMUSG00000066687)	452	54	2.932735	0.350825	8.55E-15	-3.05989	down
Kit(ENSMUSG00000005672)	1965	239	22.20534	2.820006	0	-3.07551	down
Etv5(ENSMUSG00000013089)	1092	317	14.60727	4.274957	1.58E-06	-1.82524	down
Gata4(ENSMUSG00000021944)	2478	444	41.45055	7.917682	1.69E-12	-2.52272	down
Sox9(ENSMUSG00000000567)	956	299	12.80558	4.161969	9.56E-06	-1.71747	down
Il1b(ENSMUSG00000027398)	64	1234	2.58645	50.2955	0	4.173474	up
Tnf(ENSMUSG00000024401)	280	2511	9.265935	82.96671	0	3.106113	up
Tlr4(ENSMUSG00000039005)	772	2064	6.374413	14.34809	0.001531	1.367774	up
Tlr2(ENSMUSG00000027995)	1552	8647	32.42067	186.2071	1.66E-11	2.427948	up
Tlr6(ENSMUSG00000051498)	354	1139	5.478673	17.37242	3.80E-05	1.631319	up
Nfkb2(ENSMUSG00000025225)	1408	3705	23.66947	65.2301	0.002421	1.346008	up
Tlr13(ENSMUSG00000033777)	943	2415	12.0945	30.3329	0.003568	1.306223	up
Cxcl10(ENSMUSG00000034855)	515	1290	33.12505	82.51052	0.004899	1.272612	up

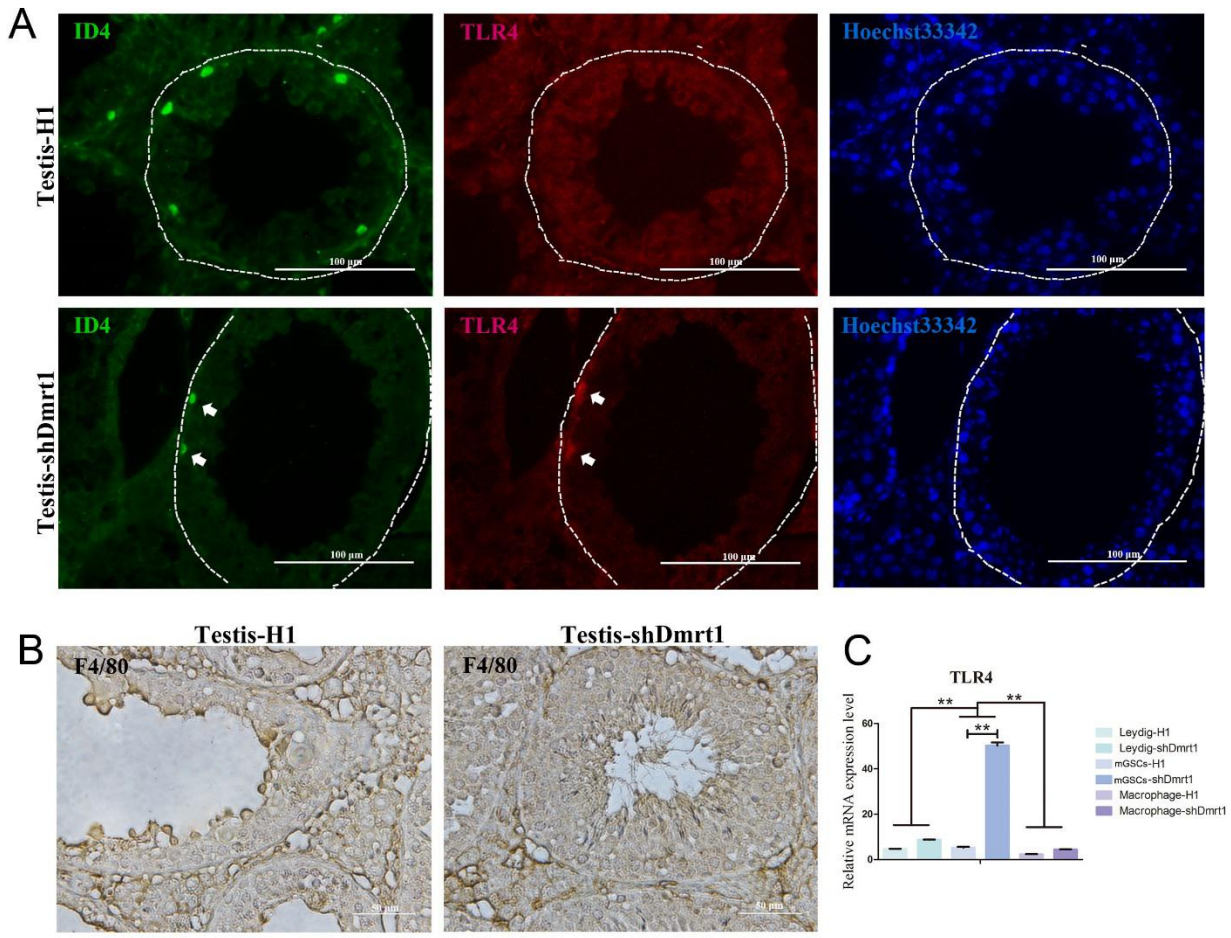


	Control group（Testes-H1）	Experiment group（Testes-shDmrt1）
Number of injected testes	30	30
Number degenerated testes	2	27
Degenerated percentage	6.70%	90%

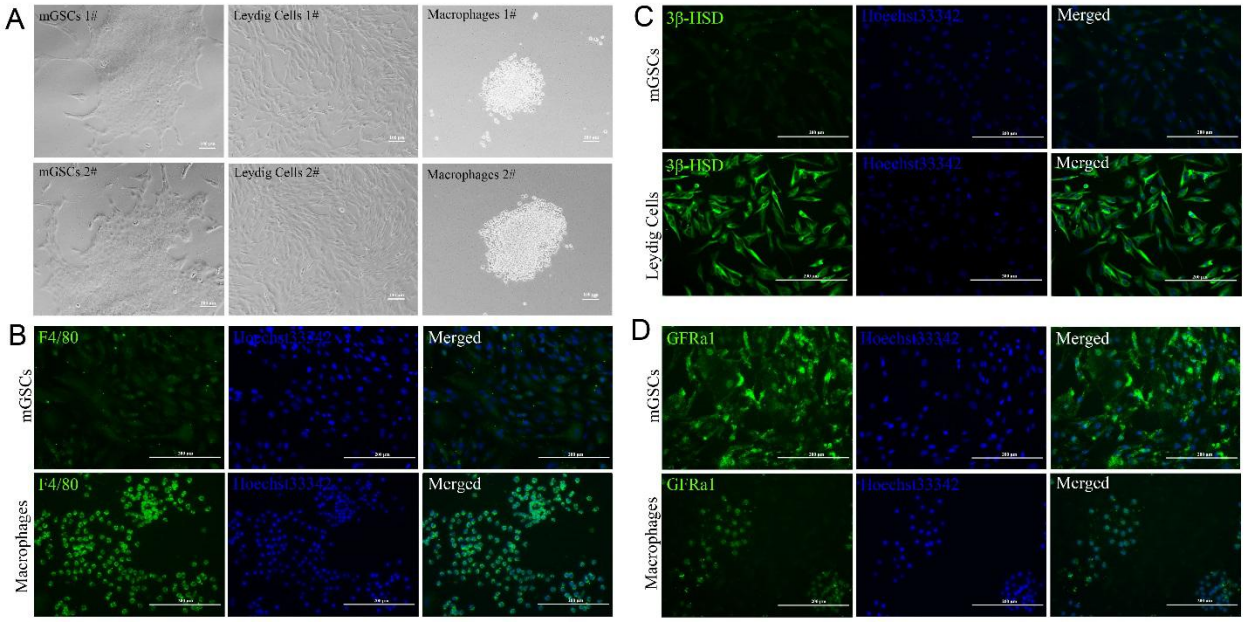
Supplementary Figure S1. Knockdown of Dmrt1 in seminiferous tubules resulted in widespread degeneration of Sertoli cells. A: Immunofluorescence staining of Sox9 in pSIH-H1-shDmrt1 or pSIH-H1 lentivirus-injected testes. Scale bar: 200 μm. B: Immunofluorescence staining of Dmrt1 and Sox9 in pSIH-H1-shDmrt1 or pSIH-H1 lentivirus-injected testes. Scale bar: 100 μm. C: Immunohistochemical detection of IL-6 and TNFα in normal testes and LPS-injected testes. Scale bar: 50 μm. D: shDmrt1 and H1 lentivirus were injected into seminiferous tubules of mouse testes harmlessly.



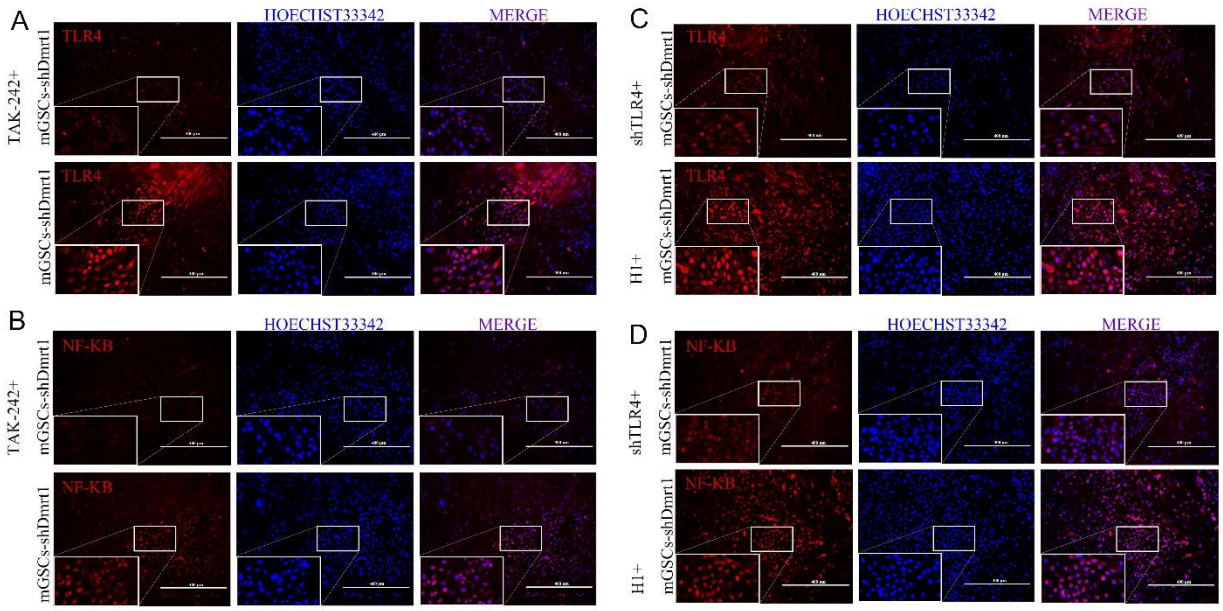
Supplementary Figure S2. TLR4 was mainly expressed in mGSCs and resulted in immune response. A: TLR4 was mainly located in spermatogonial stem cells. Knockdown of Dmrt1 expression in testicular cells led to up-regulation of TLR4. Scale bar: 50 μm. B TLR2 was mainly located in spermatocytes. Knockdown of Dmrt1 expression in testicular cells led to up-regulation of TLR2. Scale bar: 50 μm. C: Immunohistochemical staining of TLR5. Scale bar: 50 μm. D: mRNA expression levels of TLRs after mGSCs were treated with different concentrations of LPS. E: Protein expression levels of TLRs after mGSCs were treated with different concentrations of LPS. F: Protein levels were quantified using ImageJ software and normalized to GAPDH. For all statistical analyses, **P*<0.05, ***P*<0.01, ****P*<0.001.



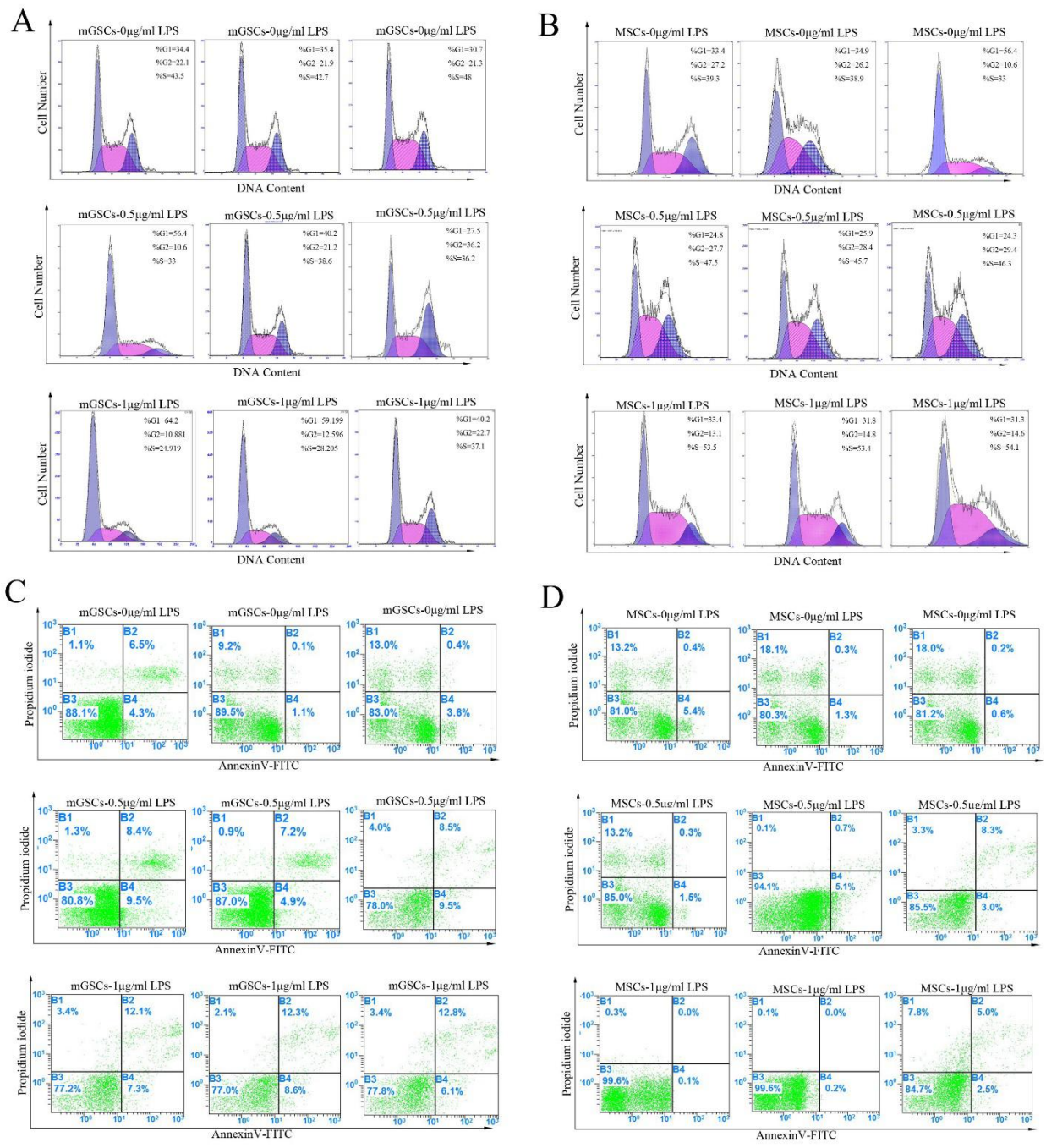
Supplementary Figure S3. TLR4 located in mGSCs was mainly responsible for immune response in seminiferous tubules. A: Co-immunofluorescence staining of ID4 and TLR4 in Testis-H1 and Testis-shDmrt1. Scale bar: 100 μm. B: Immunohistochemical staining of F4/80 in Testis-H1 and Testis-shDmrt1. Scale bar: 50 μm. C: mRNA expression level of TLR4 in Leydig cells, macrophages, and mGSCs in Dmrt1-knockdown testes, respectively. For all statistical analyses, **P*<0.05, ***P*<0.01, ****P*<0.001



Supplementary Figure S4. Isolation and identification of mGSCs, Leydig cells, and macrophages. A: Characteristics of mGSCs, Leydig cells, and macrophages. Scale bar: 100 μm. B: Immunofluorescence staining of F4/80 in mGSCs and macrophages. Scale bar: 200 μm. C: Immunofluorescence staining of 3β-HSD in mGSCs and Leydig cells. Scale bar: 200 μm. D: Immunofluorescence staining of GFRα1 in mGSCs and macrophages. Scale bar: 200 μm.

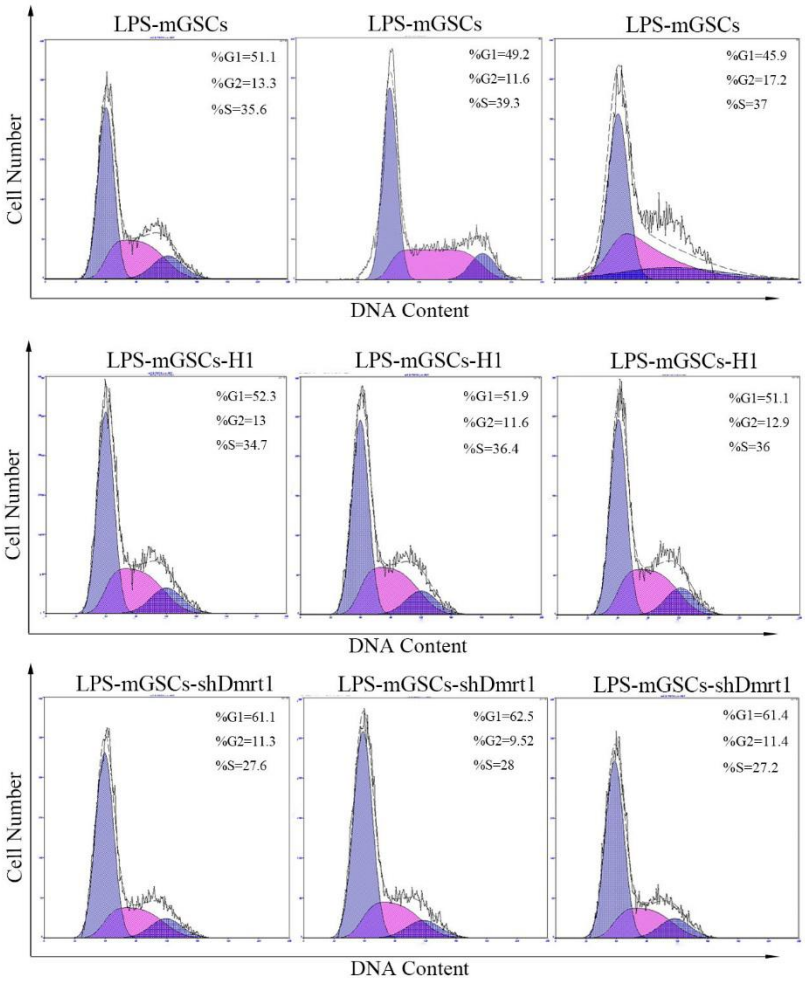


Supplementary Figure S5. Dmrt1 inhibited TLR4 signaling pathways in mGSCs. A: Immunofluorescence detection of TLR4 in TAK-242-treated mGSCs-shDmrt1 and mGSCs-shDmrt1. Scale bar: 400 μm. B: Immunofluorescence detection of NF-κB in TAK-242-treated mGSCs-shDmrt1 and mGSCs-shDmrt1. Scale bar: 400 μm. C: Immunofluorescence detection of TLR4 in shTLR4 lentivirus-treated mGSCs-shDmrt1 and H1 lentivirus-treated mGSCs-shDmrt1. Scale bar: 400 μm. D: Immunofluorescence detection of NF-κB in shTLR4 lentivirus-treated mGSCs-shDmrt1 and H1 lentivirus-treated mGSCs-shDmrt1. Scale bar: 400 μm.



Supplementary Figure S6. Flow cytometry data of cell cycle and apoptosis. A: Flow cytometry of cell cycle in mGSCs under LPS treatment (0, 0.5, and 1 μg/mL), respectively. B: Flow cytometry of cell cycle in MSCs under LPS treatment (0, 0.5, and 1 μg/mL), respectively. C: Flow cytometry of apoptosis in mGSCs under LPS treatment (0, 0.5, and 1 μg/mL), respectively. D:

Flow cytometry of apoptosis in MSCs under LPS treatment (0, 0.5, and 1 μg/mL), respectively. For each test, three biological replicates were conducted.



Supplementary Figure S7. Flow cytometry data of cell cycle in LPS-mGSCs. Flow cytometry data of cell cycle in LPS-mGSCs, LPS-mGSCs-H1, and LPS-mGSCs-shDmrt1, respectively. For each test, three biological replicates were conducted.