

**Supplementary Data for**

***Per1/Per2 disruption reduces testosterone synthesis and impairs fertility  
in elderly male mice***

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**Table S1** Feed formulation of experimental diets

Ingredient	Proportion
Corn starch	40%
Soybean meal	20%
Fish Meal	7%
Wheat flour	23%
Dry yeast	4.5%
Calcium hydrophosphate	2%
Mountain flour	1.5%
Vit-min premix	0.3%
Mineral premix	1.7%

**Table S2** qPCR primer of this study

Gene	Forward Primer(5'- 3')	Reverse Primer(5'- 3')
$\beta$ -actin	CTACCTCATGAAGATCCTGACC	CACAGCTTCTCTTGATGTCAC
<i>Star</i>	TCAACTGGAAGCAACACTCTAT	ATCTTACTTAGCACTCGTCCC
<i>Hsd3b1</i>	AAAGGTACCCAGAACCTATTGG	CTGTATGGGTATGGATCAGACC
<i>Hsd3b6</i>	AAGTTCTTCAGACCAGAAACCA	TACTGGGTGTCAAGAATGTCTC
<i>Cyp11a1</i>	AGTATTATCAGAGGCCATTGG	AACATCTGGTAGACAGCATTGA
<i>Cyp17a1</i>	GAGGTGAAGAGGAAGATCCAAA	ATACGAAGCACCTCTCGGATAG
<i>Cyp19a1</i>	TCATGAAGCACAGTCACTACAT	AAACTTCCACCATTGAAACAAG
<i>Hsd17b3</i>	CGCCGATGAGTTGTTAAAGAA	GGATCCGGTTCAGAATTATTGC
<i>Cyp21a1</i>	CAAGATGTGGTGGTGTCAAATT	GCCTTCCACATGAGAGAGTAAT
<i>Cyp11b1</i>	GAATGTGTATCGAGAGCTGGCAGAG	TTGATGTCGTGTCAGTGCTTCCAG
<i>Ugt1a6a</i>	ATCCAAAGACTCGGGCATTCATCAC	ATCATCACCATCGGAACTCCATTGC
<i>Ugt1a7c</i>	ATCCAAAGACTCGGGCATTCATCAC	ATCATCACCATCGGAACTCCATTGC

**Table S3** Changes in the ratio of DKO/ WT-related differential genes in KEGG pathways

KEGG Pathway	Gene	Description	log2FC	P value
	Symbol			
Steroid hormone biosynthesis	<i>Cyp17a1</i>	cytochrome P450, family 17, subfamily a, polypeptide 1	0.538 ↓	5.87*10 <sup>-17</sup>
	<i>Cyp11a1</i>	cytochrome P450, family 11, subfamily a, polypeptide 1	0.487 ↓	1.67*10 <sup>-13</sup>
	<i>Cyp21a1</i>	cytochrome P450, family 21, subfamily a, polypeptide 1	0.788 ↓	0.028
	<i>Ugt1a6a</i>	UDP glucuronosyltransferase 1 family, polypeptide A6A	1.220 ↓	3.00*10 <sup>-14</sup>
	<i>Ugt1a10</i>	UDP glycosyltransferase 1 family, polypeptide A10	5.246 ↑	0.00087
	<i>Ugt1a7c</i>	UDP glucuronosyltransferase 1 family, polypeptide A7C	0.608 ↓	0.00095
	<i>Cyp21a1</i>	cytochrome P450, family 21, subfamily a, polypeptide 1	0.788 ↓	0.00119
	<i>Hsd17b3</i>	hydroxysteroid (17-beta) dehydrogenase 3	0.383 ↓	0.0028
	<i>Sult1e1</i>	sulfotransferase family 1E, member 1	0.511 ↓	0.0054
	<i>Cyp2e1</i>	cytochrome P450, family 2, subfamily e, polypeptide 1	2.086 ↑	0.0075
	<i>Hsd3b1</i>	hydroxy-delta-5-steroid dehydrogenase, beta- and steroid delta-isomerase 1	0.182 ↓	0.0223
				0.0456
	<i>Cyp3a41a</i>	cytochrome P450, family 3, subfamily a, polypeptide 41A	3.002 ↓	0.0499
Cortisol synthesis and secretion	<i>Cyp17a1</i>	cytochrome P450, family 17, subfamily a, polypeptide 1	0.538 ↓	5.87*10 <sup>-17</sup>
	<i>Cyp11a1</i>	cytochrome P450, family 11, subfamily a, polypeptide 1	0.485 ↓	1.67*10 <sup>-13</sup>
	<i>Star</i>	steroidogenic acute regulatory protein	0.541 ↓	7.88*10 <sup>-9</sup>
	<i>Plcb4</i>	phospholipase C, beta 4	0.427 ↓	2.11*10 <sup>-17</sup>
	<i>Cyp21a1</i>	cytochrome P450, family 21, subfamily a, polypeptide 1	0.788 ↓	0.000280

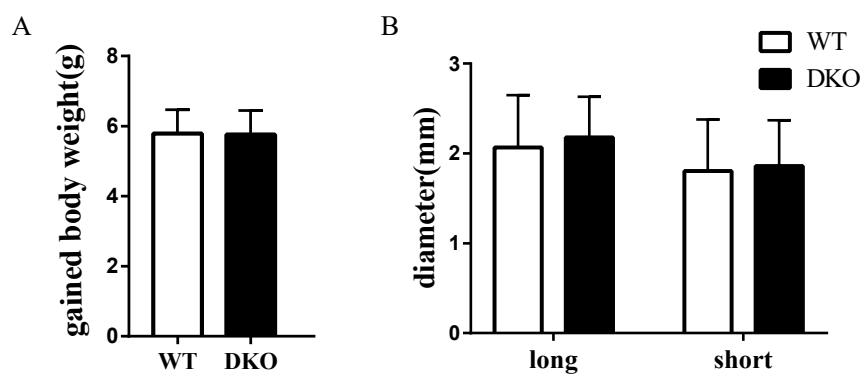
	<i>Agt</i>	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	0.208 ↓	0.0103
	<i>Creb3l1</i>	cAMP responsive element binding protein 3-like 1	0.360 ↓	0.0157
	<i>Cacna1h</i>	calcium channel, voltage-dependent, T type, alpha 1H subunit	0.158 ↑	0.0177
	<i>Scarb1</i>	scavenger receptor class B, member 1 hydroxy-delta-5-steroid dehydrogenase, 3	0.124 ↓	0.0279
	<i>Hsd3b1</i>	beta- and steroid delta-isomerase 1	0.182 ↓	0.0456
	<i>Ldlr</i>	low density lipoprotein receptor	0.439 ↓	0.0477
Aldosterone synthesis and secretion	<i>Cyp11a1</i>	cytochrome P450, family 11, subfamily a, polypeptide 1	-0.485 ↓	$1.67 \times 10^{-13}$
	<i>Star</i>	steroidogenic acute regulatory protein	-0.541 ↓	$7.88 \times 10^{-9}$
	<i>Plcb4</i>	phospholipase C, beta 4	-0.427 ↓	$2.11 \times 10^{-9}$
	<i>Cyp21a1</i>	cytochrome P450, family 21, subfamily a, polypeptide 1	-0.788 ↓	$4 \times 10^{-9}$
	<i>Agt</i>	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	-0.208 ↓	0.0103
	<i>Creb3l1</i>	cAMP-responsive element binding protein 3-like 1	-0.360 ↓	0.0157
	<i>Cacna1h</i>	calcium channel, voltage-dependent, T type, alpha 1H subunit	0.158 ↑	0.0177
	<i>Scarb1</i>	scavenger receptor class B, member 1	-0.124 ↓	
	<i>Prkcg</i>	protein kinase C, gamma	0.701 ↑	0.0279
	<i>Atp1a2</i>	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, alpha 2 polypeptide	0.157 ↑	0.0327
	<i>Dagla</i>	diacylglycerol lipase, alpha	0.522 ↑	
	<i>Hsd3b1</i>	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	-0.182 ↓	0.0431
	<i>Ldlr</i>	low-density lipoprotein receptor	-0.439 ↓	
				0.0477

**Table S4** plasma of T, ALD, CORT, DOC, A4, P, LH levels in WT and DKO mice

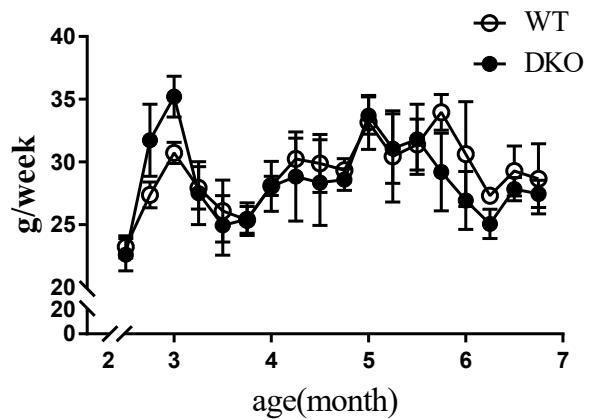
	WT (n=8)	DKO (n=8)	P value
Testosterone (ng/mL)	0.3371 ± 0.05291	0.116 ± 0.006902	0.0060 **
Aldosterone (ng/mL)	0.1937 ± 0.03848	0.1759 ± 0.03639	0.7419
Corticosterone (ng/mL)	93.11 ± 4.814	105.1 ± 8.333	0.2322
11-Deoxycorticosterone (ng/mL)	1.803 ± 0.2599	3.098 ± 0.9717	0.2189
Androstendione (ng/mL)	0.09029 ± 0.07627	0.1531 ± 0.08578	0.6131
Progesterone (ng/mL)	0.6761 ± 0.08387	0.7625 ± 0.2151	0.6851
Luteinizing hormone (mIU/mL)	1.98 ± 0.6555	1.02 ± 0.4048	0.2444

Values are mean ± SD; \* P<0.05, \*\* P<0.01

## SUPPLEMENTARY FIGURES



**Figure S1 Testicular morphology data of two types.** DKO and WT male mice (A) weight gain (15-month-age - 9-month-age weight). (B) The testicular minor and major axis



**Figure S2** Feed intake of mice per week of WT and DKO mice from 2-month-age to 7-month-age.

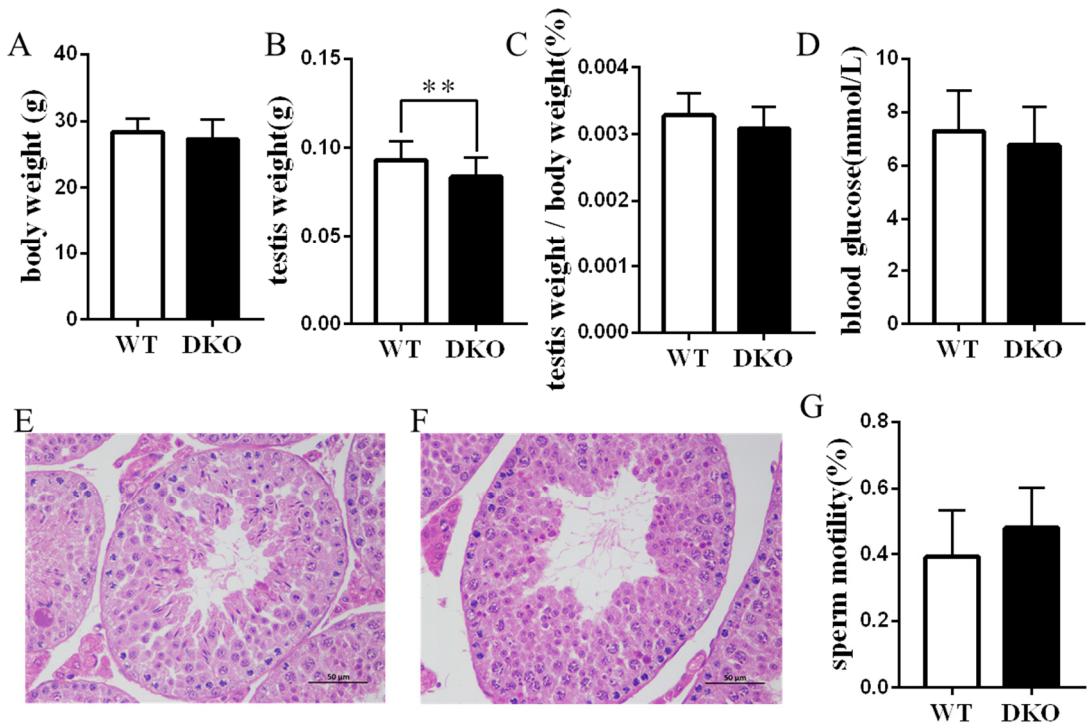
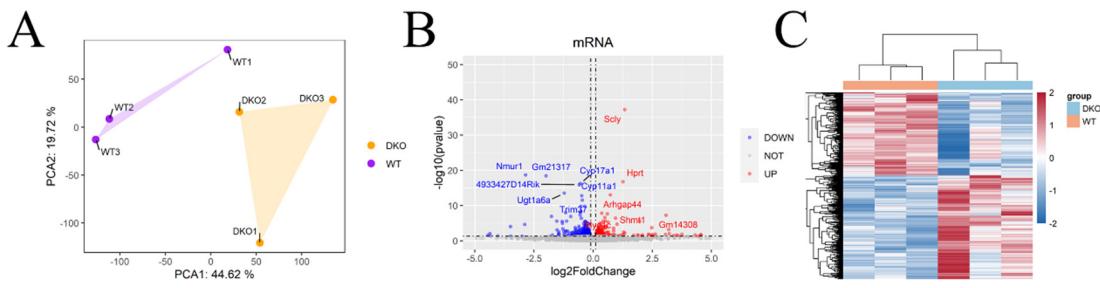
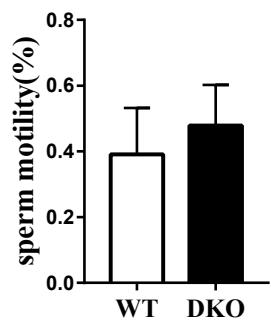


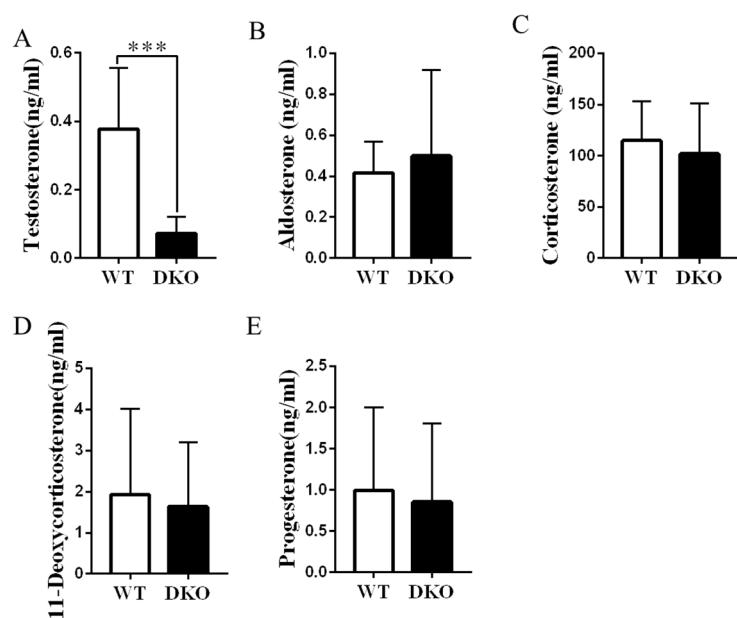
Figure S3. Physiological effect indices and HE staining results of 9-month DKO and WT mice. (A) Bodyweight growth curve; (B) Testicular weight; (C) Ratio of testis weight/body weight; (D) Fasting blood glucose levels; (E) HE staining of WT testis; (F) HE staining of DKO testis; (G) Sperm motility; ( $n=10$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ )



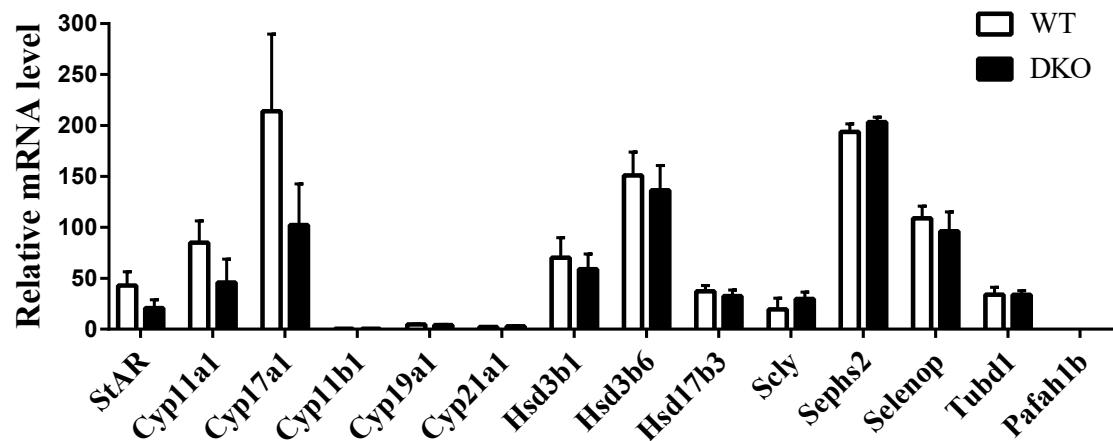
**Figure S4 Differentially expressed testis RNA between DKO and WT mice ( $|\log_2 \text{fold change}| > 0.1$ ,  $P\text{value} < 0.05$ ).** Compared with normal samples, 532 mRNA were upregulated and 425 mRNA were downregulated in DKO mice. (A) Principal component analysis (PCA) of DKO samples and WT samples; (B) is a volcano map for differentially expressed mRNAs. Red stands for up-regulation, blue stands for down-regulations, and gray stands for intermediate in volcanoes. Each point represents a gene. (C) is the heatmap for differentially expressed mRNAs. Red stands for DKO male mice, and blue stands for WT male mice.



**Figure S5 Sperm motility of WT and DKO mice in 9-month-age.**



**Figure S6. Plasma steroid hormone levels in 9-month-age WT and DKO male mice. (A)-(G):**  
T, ALD, CORT, DOC, P ( $n=6$ ). \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .



**Figure S7. Expression of genes associated with testicular steroid biosynthesis, tubulin dynamics and Seleno-compound metabolic loci.**