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Supplemental information

PPT1 regulation of HSP90 α

depalmitoylation participates

in the pathogenesis of hyperandrogenism

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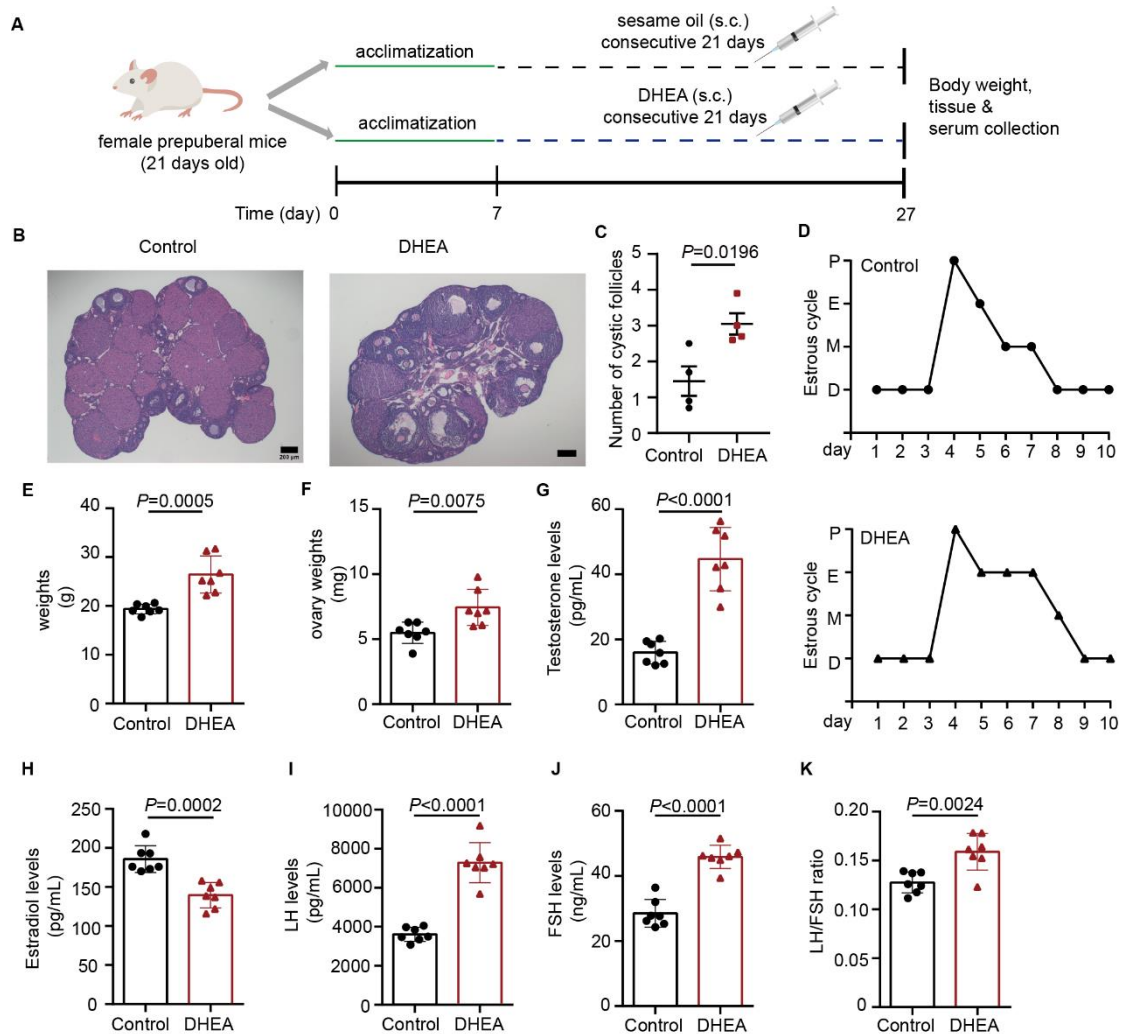


Figure S1 The DHEA-induced mouse model displayed hyperandrogenism phenotypes, related to Figure 1. (A) Timeline of DHEA treatment of mice. (B) Representative hematoxylin-eosin (H&E)-stained sections from the ovaries of the control and DHEA groups. Scale bar: 200 μm . The asterisk (*) represents the corpus luteum, and the pound sign (#) represents the ovary vacuoles. (C) Number of cystic follicles in the control and DHEA groups. (D) Representative estrous cycles. (E) Weight (n=7 mice per group). (F) Ovary weight (n=7 mice per group). (G) Testosterone levels (n=7 mice per group). (H) Estradiol levels (n=7 mice per group). (I) LH levels (n=7 mice per group). (J) FSH levels (n=7 mice per group). (K) LH/FSH (n=7 mice per group). For (C, E-K), P values were determined by two-tailed Student's t test, and data are presented as the mean values \pm SD.

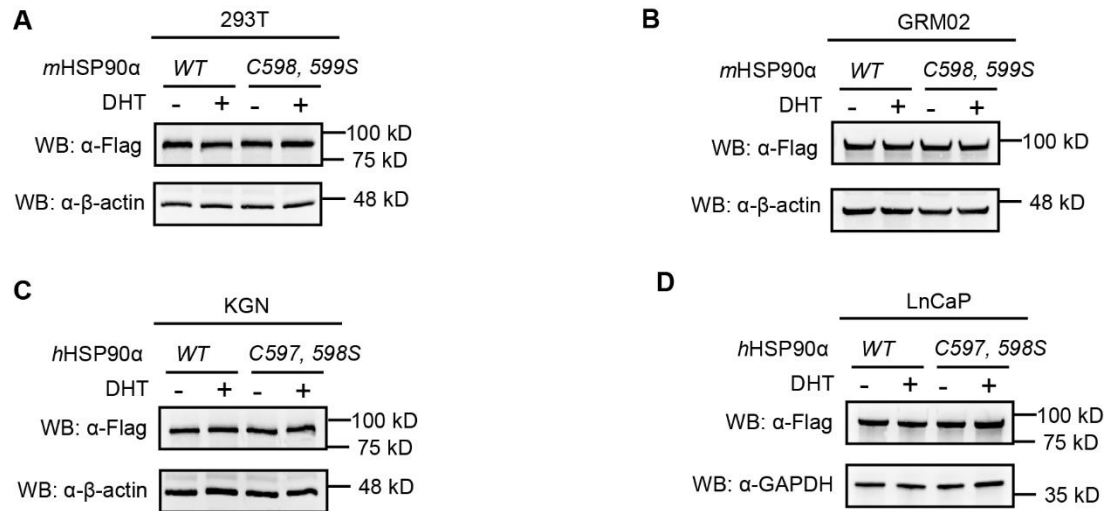


Figure S2 HSP90α equal protein expression between WT and mutants after plasmids transfection and DHT treatment, related to Figure 4. (A) Immunoblot analysis showing *mHSP90α* protein level in HEK293T cells transfected with Flag-tagged *mHSP90α*-WT or *mHSP90α*-C598, 599S treated with methanol or DHT (100 nM) for 24 hours. (B) Immunoblot analysis showing *mHSP90α* protein level in GRM02 cells transfected with Flag-tagged *mHSP90α*-WT or *mHSP90α*-C598, 599S treated with methanol or DHT (100 nM) for 24 hours. (C) Immunoblot analysis showing *mHSP90α* protein level in KGN cells transfected with Flag-tagged *hHSP90α*-WT or *hHSP90α*-C597, 598S treated with methanol or DHT (10 nM) for 24 hours. (D) Immunoblot analysis showing *hHSP90α* protein level in LnCaP cells transfected with Flag-tagged *hHSP90α*-WT or *hHSP90α*-C597, 598S treated with methanol or DHT (10 nM) for 24 hours.

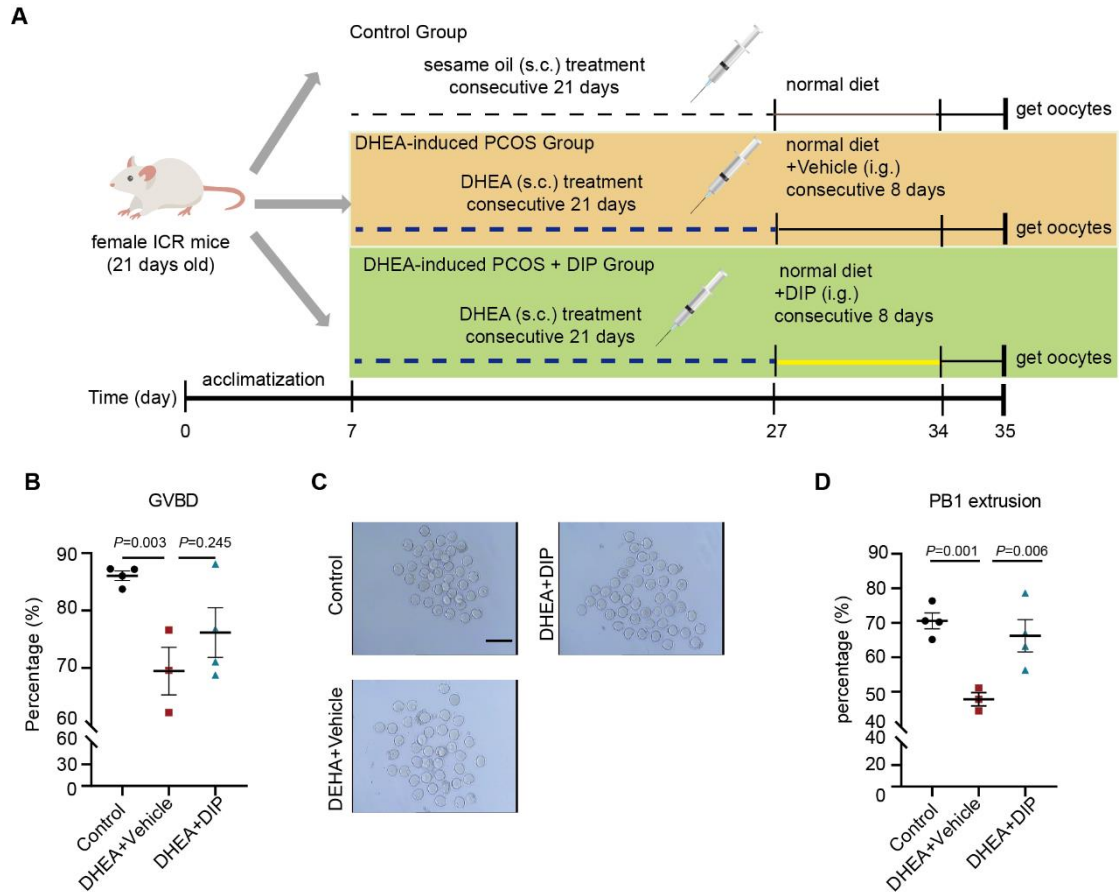


Figure S3 Dipyridamole treatment protects against a DHEA-induced poor-quality oocyte phenotype, related to Figure 7. (A) Timeline of dipyridamole treatment in hyperandrogenism phenotype or control mice. (B) Germinal vesicle breakdown (GVBD) percentage ($n=4$ mice per group). (C and D) The first polar body extrusion (PBE) rate ($n=4$ mice per group). For (B and D), P values were determined by Student's t test, and the data are presented as the mean values \pm SEMs.

Table S1 Increased level of S-palmitoylation proteins, related to Figure 3.

Gene	Full name
DYH5	Dynein heavy chain 5
CAC1E	Voltage-dependent R-type calcium channel subunit alpha-1E
SEPT7	Septin-7
H12	Histone H1.2
KPYM	Pyruvate kinase PKM
STOM	Erythrocyte band 7 integral membrane protein
DAD1	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit
RANT	GTP-binding nuclear protein Ran,
1433Z	14-3-3 protein zeta/delta
CO6A2	Collagen alpha-2(VI) chain
TNR6A	Trinucleotide repeat-containing gene 6A protein
LTBP1	Latent-transforming growth factor beta-binding protein 1
FIBB	Fibrinogen beta chain
ZDHC1	Palmitoyltransferase ZDHHC1
NB5R3	NADH-cytochrome b5 reductase 3

Table S2 Decreased level of S-palmitoylation proteins, related to Figure 3.

Gene	Full name
TITIN	Titin
DPYL3	Dihydropyrimidinase-related protein 3
PRDX6	Peroxiredoxin-6
CD151	CD151 antigen
PDIA4	Protein disulfide-isomerase A4
CO3A1	Collagen alpha-1(III) chain
MDHM	Malate dehydrogenase
GNAI2	Guanine nucleotide-binding protein G(i) subunit alpha-2
GPX1	Glutathione peroxidase 1
AT1B2	Sodium/potassium-transporting ATPase subunit beta-2
HSP72	Heat shock-related 70 kDa protein 2
ENOG	Gamma-enolase
3BHS1	3 beta-hydroxysteroid dehydrogenase/Delta 5--4-isomerase type 1
GRP75	Stress-70 protein
CHD1	Chromodomain-helicase-DNA-binding protein 1
H15	Histone H1.5
HSP90A	Heat shock protein 90, alpha (Cytosolic), class A member 1
CDN1B	Cyclin-dependent kinase inhibitor 1B
ASPX	Acrosomal protein SP-10
RHG39	Rho GTPase-activating protein 39
RS8	40S ribosomal protein S8
RAP1B	Ras-related protein Rap-1b
UB2L3	Ubiquitin-conjugating enzyme E2 L3
1433T	14-3-3 protein theta
TERA	Transitional endoplasmic reticulum ATPase
PGBM	Basement membrane-specific heparan sulfate proteoglycan core protein
SPB6	Serpin B6
ADRO	NADPH:adrenodoxin oxidoreductase
ZYX	Zyxin
HUMMR	Protein MGARP
THIKA	3-ketoacyl-CoA thiolase A
MCCA	Methylcrotonoyl-CoA carboxylase subunit alpha
RRBP1	Ribosome-binding protein 1
ACBG1	Long-chain-fatty-acid--CoA ligase ACSBG1
UQCC2	Ubiquinol-cytochrome-c reductase complex assembly factor 2
CRIP2	Cysteine-rich protein 2
AL9A1	4-trimethylaminobutyraldehyde dehydrogenase
K1C17	Keratin, type I cytoskeletal 17
PEPL	Periplakin
INSRR	Insulin receptor-related protein
ASAH1	Acid ceramidase
SAE2	SUMO-activating enzyme subunit 2

Table S3. Clinical characteristics in women with or without ovarian hyperandrogenism in PCOS, related to Figure 5.

	Control (n=5)	ovarian hyperandrogenism in PCOS (n=5)	<i>P</i> value
Age (year)	26.5±2.26	24.0±1.79	0.059
Body Mass Index	21.3±1.71	24.0±4.33	0.189
FSH (IU/L)	8.05±1.94	4.79±1.22	0.006
LH (IU/L)	9.3±3.20	16.8±6.25	0.033
LH/FSH	1.2±0.33	3.4±0.48	<0.0001
Estradiol (nmol/L)	0.22±0.088	0.185±0.056	0.449
Testosterone (nmol/L)	4.35±0.78	4.94±1.39	0.387
Dehydroepiandrosterone (nmol/L)	4.12±0.82	7.83±1.78	0.001

FSH, follicle-stimulating hormone; LH, luteinizing hormone; *P* value were analyzed by two-tailed Student's *t*-test (for two groups).