Supplementary Information

Evaluation of the p53 pathway in polycystic ovarian syndrome pathogenesis and apoptosis enhancement in human granulosa cells through transcriptome data analysis

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Supplementary Table 1: Transcriptome datasets from GCs employed in the current study.

GEO Reference Series[a]	PCOS Subset	Control Subset	Platform
GSE34526	7	3	Affymetrix Human Genome U133 Plus 2.0
GSE138518	3	3	Illumina HiSeq 2000
GSE155489[b]	4	4	HiSeq X Ten
GSE80432[c]	8	8	Affymetrix Human Gene 1.0 ST
GSE10946[d]	12	11	Affymetrix Human Genome U133 Plus 2.0

a: Data were taken from the https://www.ncbi.nlm.nih.gov/geo/

b: Data related to the cumulus GCs in this study was used but those associated with oocytes were excluded.

c & d: According to PCA analysis, these datasets were removed from this study.

Supplementary Table 2: List of differentially expressed p53 target genes in PCOS granulosa cells: PCOS women versus control with $p < 0.05$ and $|\log FC| \geq 1$.

GEO Reference	Ensemble Gene ID	Gene Symbol	Gene Name [a]
GSE34526	ENSG00000015475	*BID*	BH3 interacting domain death agonist
	ENSG00000087088	*BAX*	BCL2 associated X
	ENSG00000171862	*PTEN*	Phosphatase and tensin homolog
	ENSG00000064012	*CASP8*	Caspase 8
	ENSG00000026103	*FAS*	Fas cell surface death receptor
	ENSG00000134057	***CCNB1***	**Cyclin B1**
	ENSG00000157456	*CCNB2*	Cyclin B2
	ENSG00000112576	*CCND3*	Cyclin D3
	ENSG00000175305	*CCNE2*	Cyclin E2
	ENSG00000113328	*CCNG1*	Cyclin G1
	ENSG00000175793	*SFN*	Stratifin
	ENSG00000135679	***MDM2***	**Mouse double minute-2 homolog**
	ENSG00000137801	*THBS1*	Thrombospondin 1
	ENSG00000085117	*CD82*	Cluster of differentiation 82
	ENSG00000115107	*STEAP3*	Six-transmembrane epithelial antigen of prostate 3
GSE138518	ENSG00000170312	*CDK1*	Cyclin dependent kinase 1
	ENSG00000134057	*CCNB1*	Cyclin B1
	ENSG00000110092	***CCND1***	**Cyclin D1**
	ENSG00000181790	***ADGRB1***	**Adhesion G protein-coupled receptor B1**
	ENSG00000085117	***CD82***	**Cluster of differentiation 82**
	ENSG00000141682	*PMAIP1*	Phorbol-12-myristate-13-acetate-induced protein 1
	ENSG00000026103	*FAS*	Fas cell surface death receptor
GSE155489	ENSG00000170312	*CDK1*	Cyclin dependent kinase 1
	ENSG00000112576	*CCND3*	Cyclin D3
	ENSG00000137801	*THBS1*	Thrombospondin 1
	ENSG00000141682	*PMAIP1*	Phorbol-12-myristate-13-acetate-induced protein 1

	ENSG00000130222	*GADD45G*	Growth arrest and DNA damage inducible gamma
	ENSG00000106366	***SERPINE1***	**Serpin family E member 1**

a: Down-regulated genes were shown as bold.

Supplementary Table 3: The list of significantly enriched KEGG pathways of associated PCOS related p53 downstream target genes which were differentially expressed.

Term	Overlap	*p-value*	Adjusted *p-value*	Genes
p53 signaling pathway	21/73	2.59E-66	2.90E-64	*STEAP3; CD82; SERPINE1; PTEN; THBS1; CCNB2; CCND3 CCNB1; CASP8; CCND1; PMAIP1; SFN; GADD45G CCNE2; ADGRB1; CCNG1; CDK1; MDM2; FAS; BAX*
Cellular senescence	11/156	1.51E-23	8.47E-22	*SERPINE1; PTEN; GADD45G; CCNB2 CCND3; CCNB1; CND2; CCND1; CCNE2; DK1; MDM2*
Cell cycle	10/124	1.68E-20	6.26E-19	*CCNB2; CCND3; CCNB1; CCND2; CCND1; CCNE2 CDK1; MDM2; SFN; GADD45G*
FoxO signaling pathway	7/131	3.86E-12	4.32E-11	*CCNB2; CCNB1; CCND2; CCND1; PTEN; MDM2; GADD45G*
Apoptosis	6/142	4.60E-10	3.68E-09	*CASP8; PMAIP1; FAS; BAX; BID; GADD45G*
PI3K-Akt signaling pathway	7/354	1.05E-08	6.72E-08	*CCND3; CCND2; CCND1; CCNE2; PTEN; MDM2; THBS1*
Focal adhesion	5/201	5.40E-06	1.78E-05	*CCND3; CCND2; CCND1; PTEN; THBS1*
AGE-RAGE signaling pathway in diabetic complications	4/100	8.08E-06	2.51E-05	*CCND1; CDK4; SERPINE1; BAX*
Progesterone-mediated oocyte maturation	3/100	2.90E-04	7.22E-04	*CCNB2; CCNB1; CDK1*

Supplementary Table 4: The primers and their sequences were used for qRT-PCR experiments.
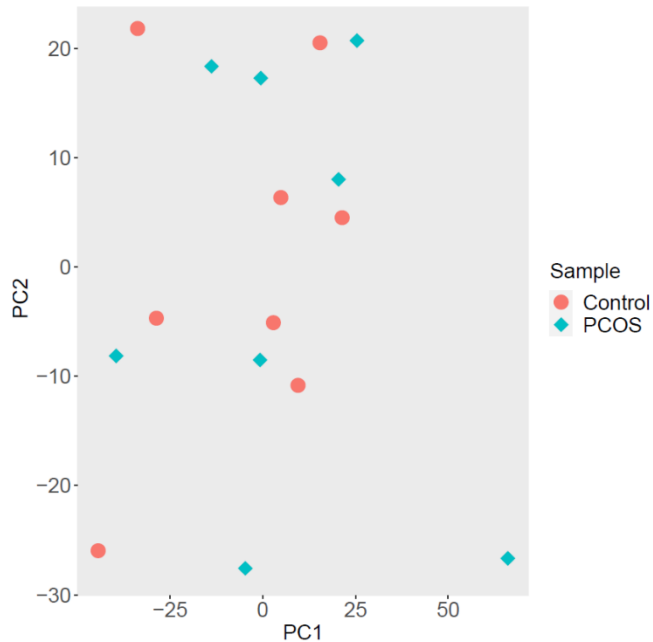F, Forward; R, Reverse.

|	
FAS	F- AGATTGTGTGATGAAGGACATGG
	R- TGTTGCTGGTGAGTGTGCATT
MDM2	F- AGTAGCAGTGAATCTACAGGGA
	R- CTGATCCAACCAATCACCTGAAT
PMAIP1	F -GGAGGTGCACGTTTCATCAATTT
	R - AGGGTCTTCCATTCTTGCTTTAA

Supplementary Figure 1

a: GSE10946
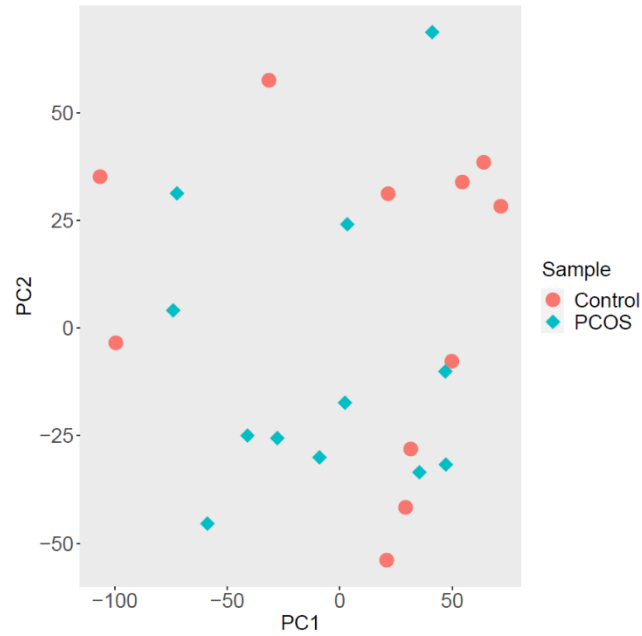
b: GSE80432

 Supplementary Figure 1: PCA (Principal component analysis) plots were constructed by using a normalized gene expression matrix. (A) GSE10946 dataset, (B) GSE80432 dataset. PCA of transcriptome in GCs obtained from PCOS and control cases. The samples are represented by different colours as indicated in the right.
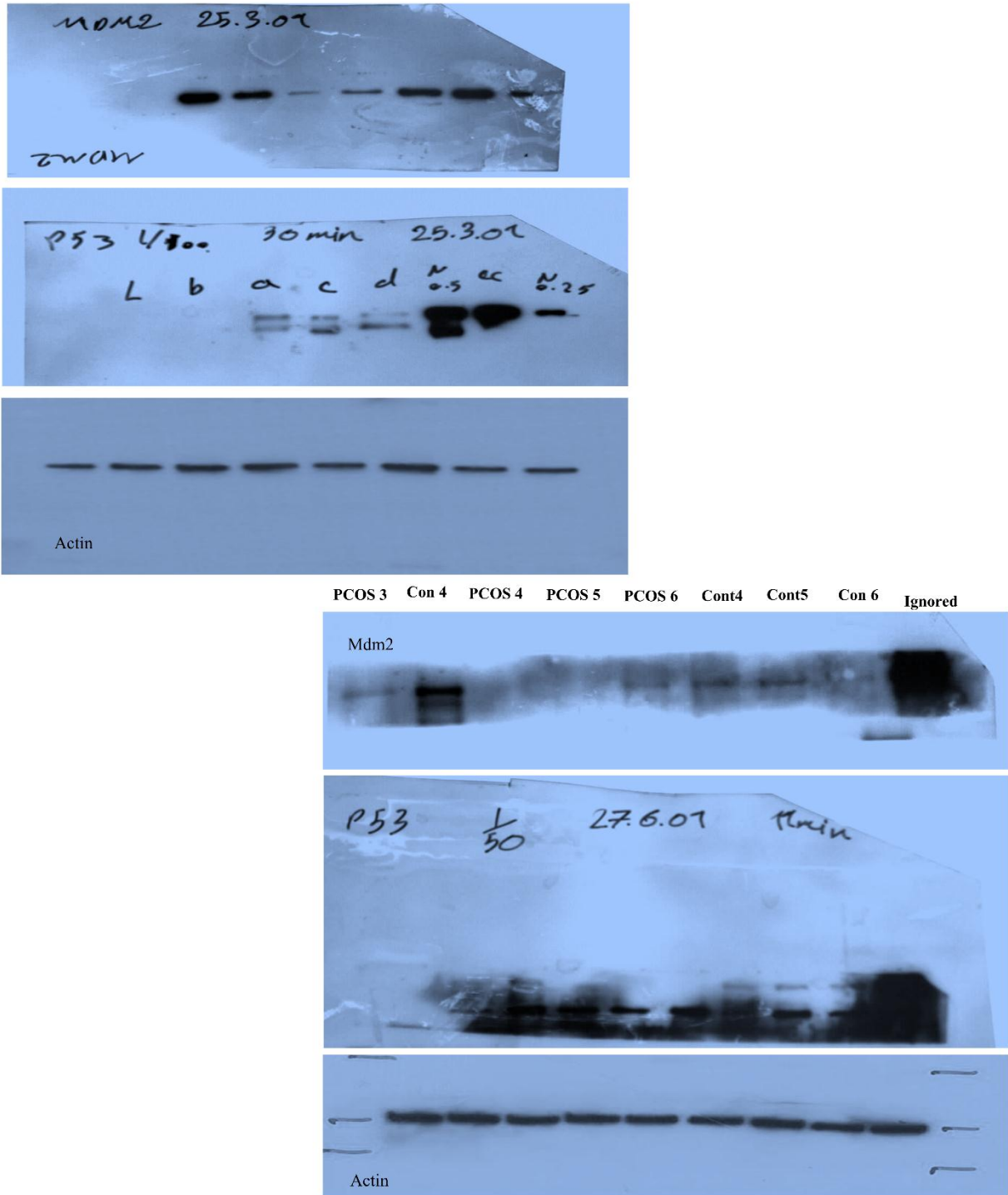
Supplementary Figure 2
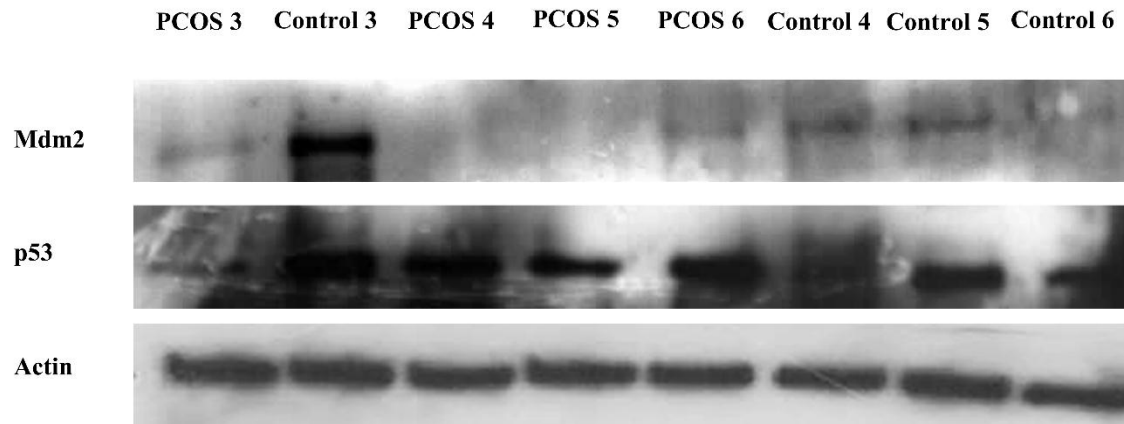
Supplementary Figure 2: Western blot analysis of p53 and Mdm2 proteins in PCOS women (PCOS 3-6) compared to the controls (Controls 3-6). The Thermo scientific protein ladder, (10 to 180 kDa) was used as size standards for monitoring protein migration, protein transfer to membranes, and sizing proteins. The blots were cut into 3 pieces at ~ 70 kDa, a little under ~ 55 kDa, and a little under ~ 40 kDa for Mdm2 (90 kDa), p53 (53 kDa), and actin (42 kDa), respectively, prior to hybridization with antibodies.

Supplementary Figure 3

L: Deleted p53 cell line, B & a: control women (non-PCOS) C & d: PCOS women
N (0.5 RG7388), CC, & N (0.25 RG7388): 3 positive control cell line for p53, Con: Control

Supplementary Figure 3: The original unedited blots presenting Mdm2 (90 kDa), p53 (53 kDa), and actin (42 kDa) in Figure 5B and Supplementary Figure 2.