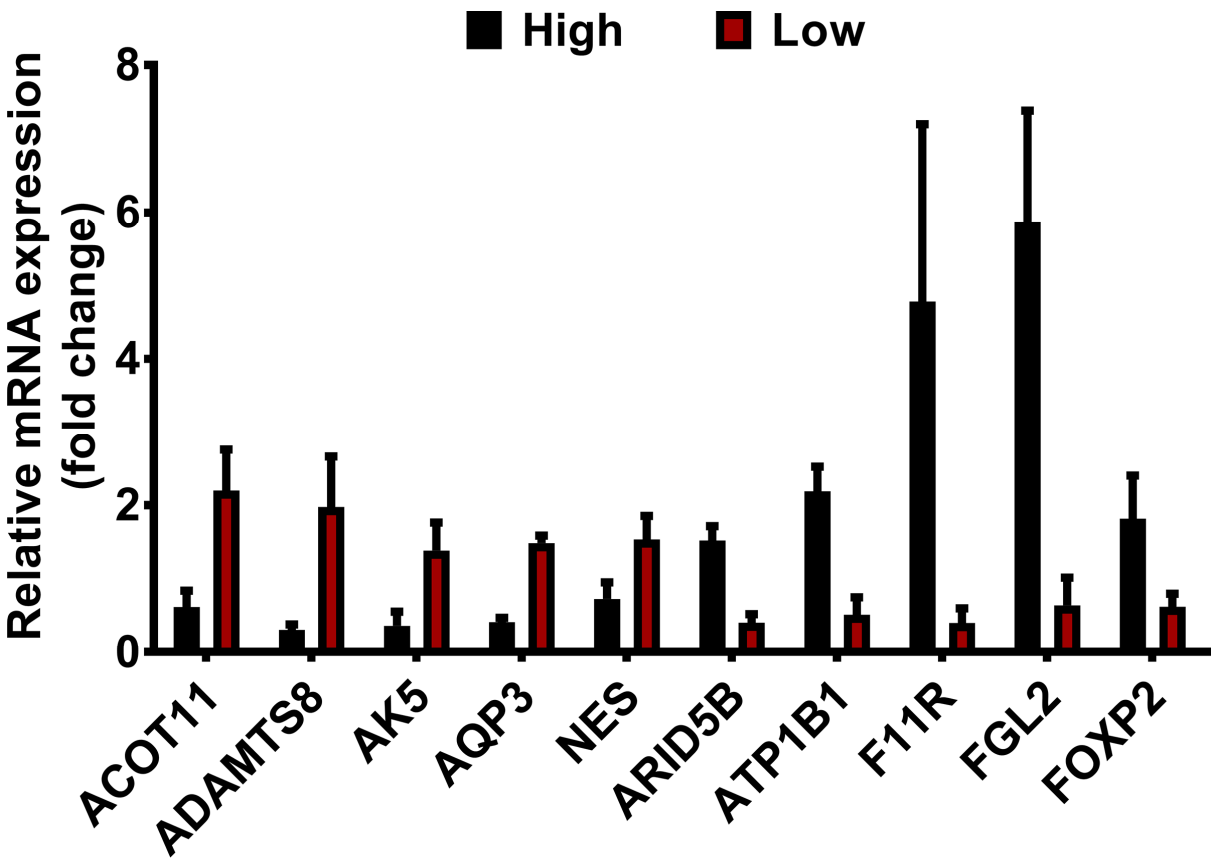

Fig. S1. Validation of differentially expressed genes (DEGs) by qRT-PCR assay. Expression data were normalized to that of *Tuba1a*. Experiments were repeated in triplicate.

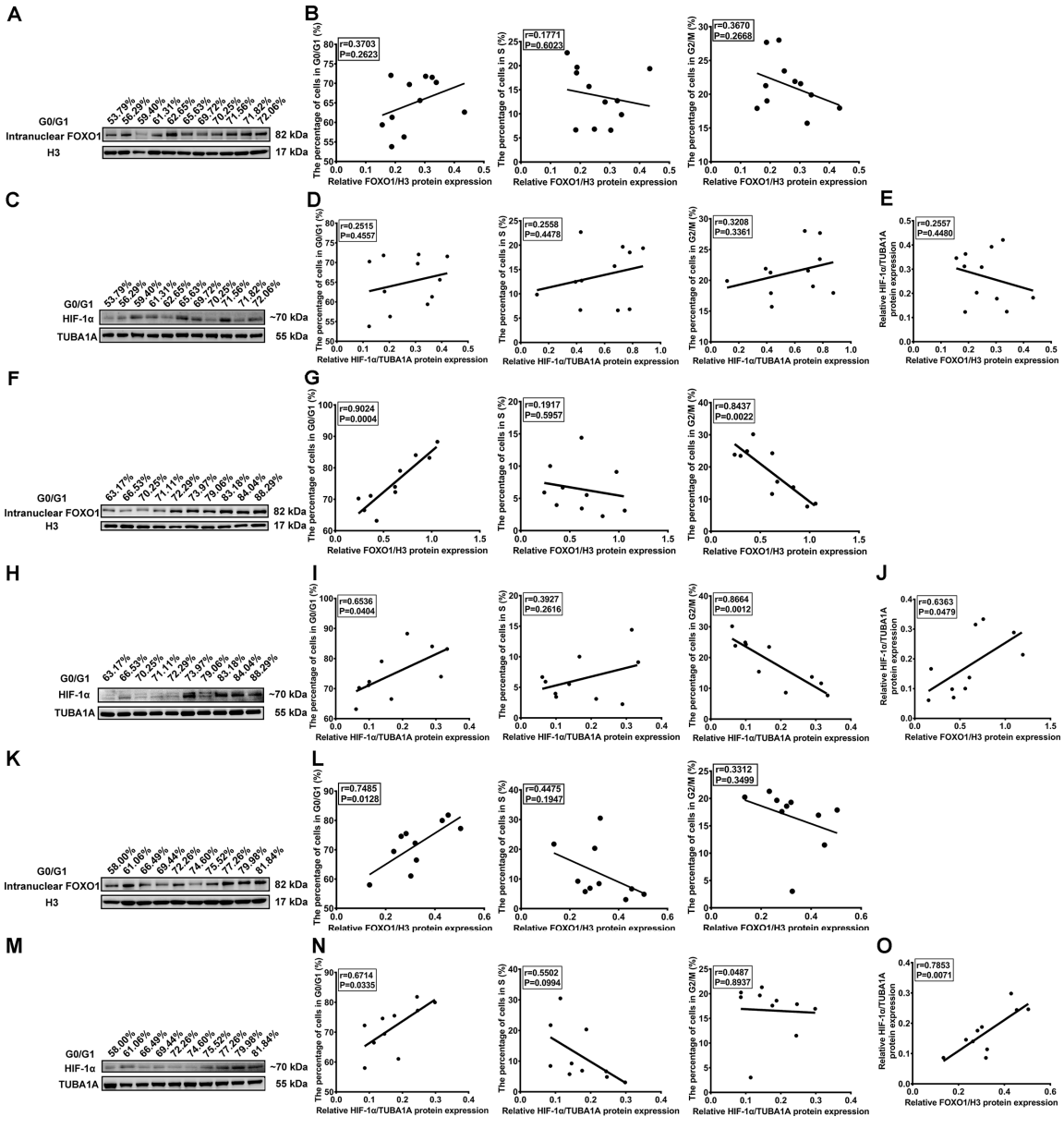

Fig. S2. The relationship among hypoxia, FOXO1 signaling, and GC cell cycle. **A-D**, **F-I**, **K-N** The relationship between protein levels of intranuclear FOXO1/HIF-1α and GCs cell cycle was assessed by using linear regression in porcine antral follicles with different sizes, including small follicles (1-3 mm; A-D), medium follicles (3-5 mm; F-I), and large follicles (≥5 mm; K-N). **E, J, O** The relationship between intranuclear level of FOXO1 and HIF-1α accumulation was analyzed by linear regression in small follicles (1-3 mm; E), medium follicles (3-5 mm; J), and large follicles (≥5 mm; O).

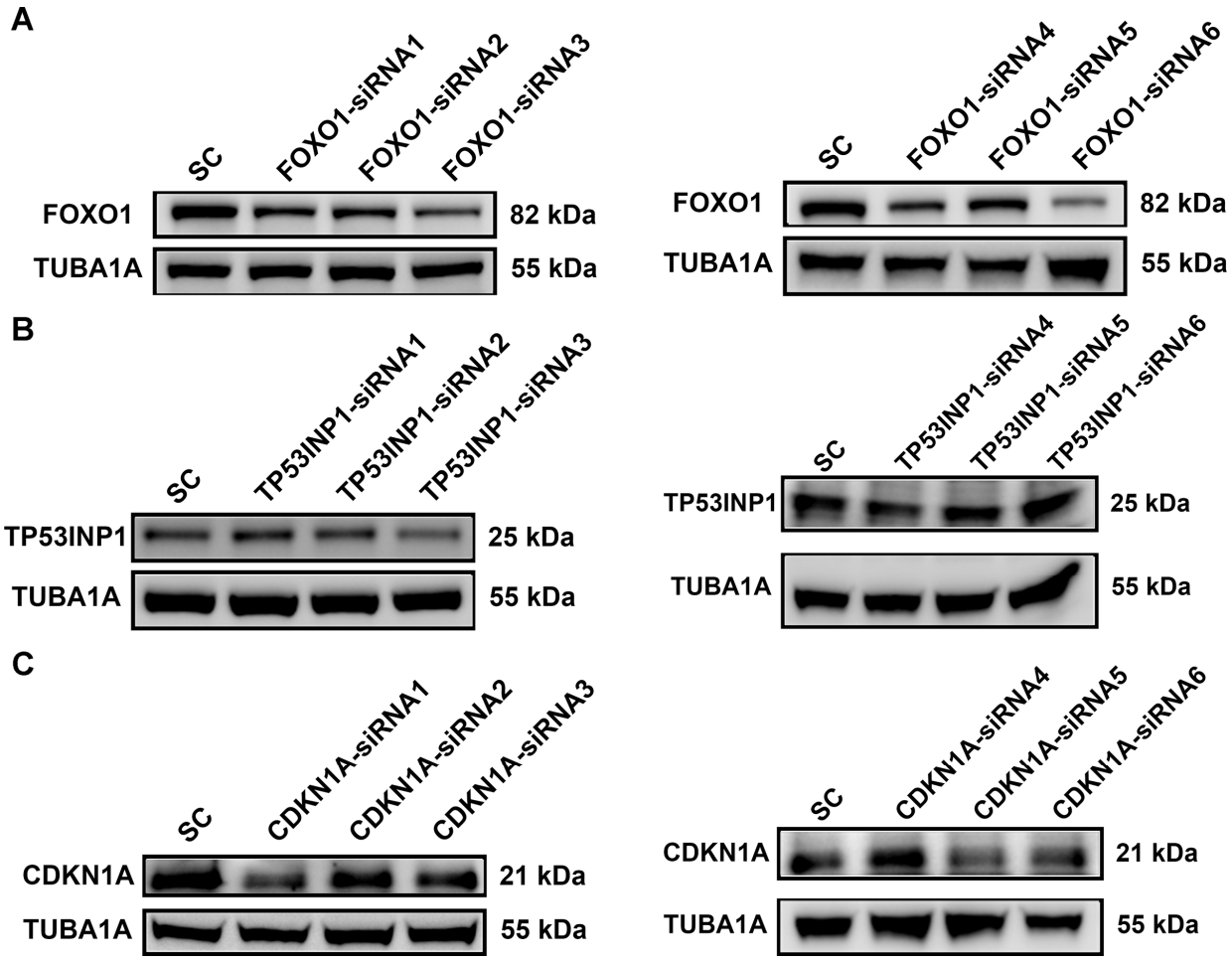

Fig. S3. The gene silencing efficiency of siRNAs against FOXO1, TP53INP1, and CDKN1A. Primary cultured GCs were transfected with FOXO1 siRNAs (siRNA1-3, siRNA4-6), TP53INP1 siRNAs (siRNA1-3, siRNA4-6), CDKN1A siRNAs (siRNA1-3, siRNA4-6), or scrambled control siRNA for 12 h, and then collected for western blotting detection of the protein levels as indicated. Two out of the candidate siRNAs in each group were identified as the most potent and specific siRNAs, and were used for the subsequent experiments. TUBA1A served as the control for loading.

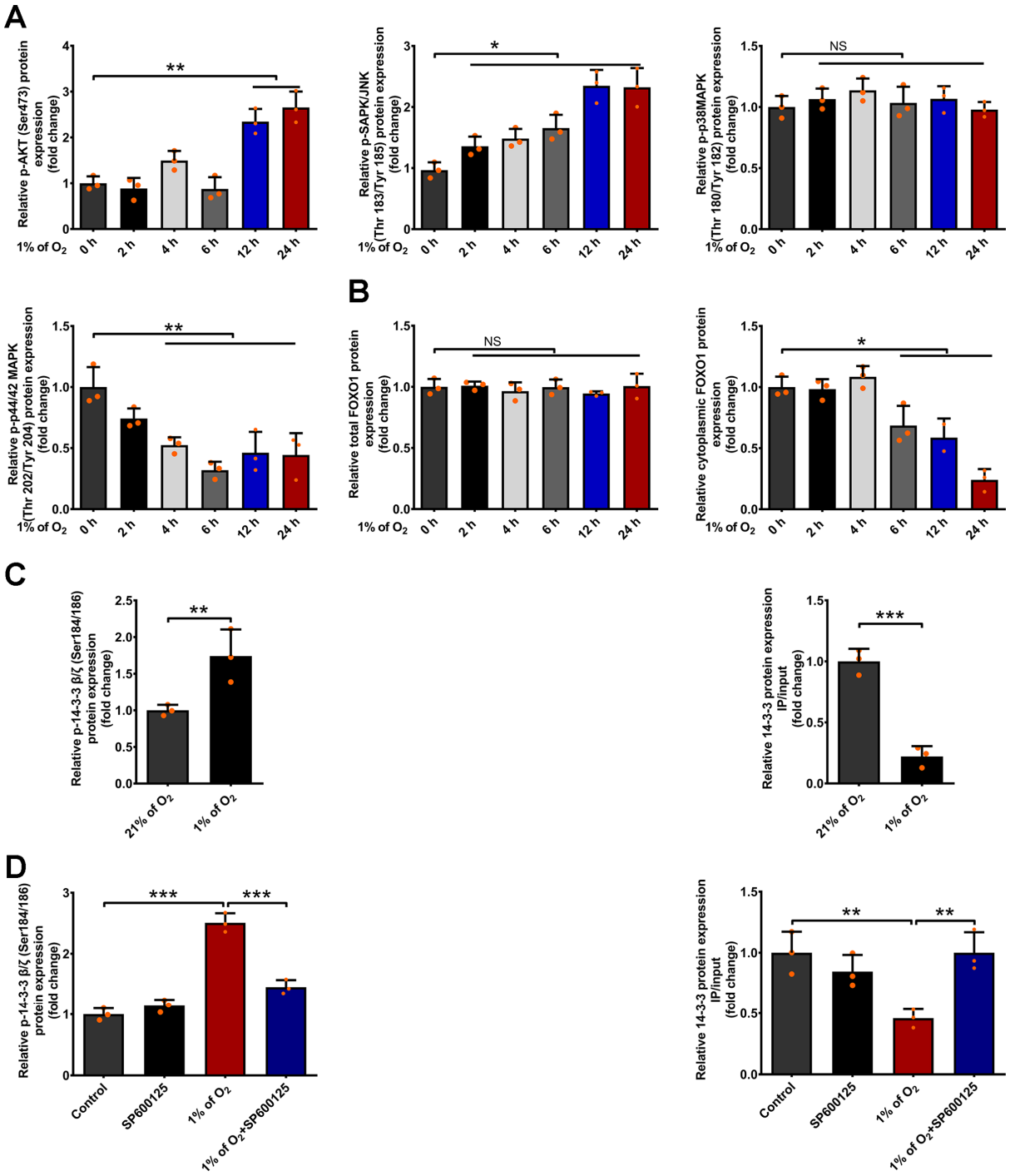

Fig. S4. Hypoxia induces nuclear translocation of FOXO1 through the JNK-14-3-3 pathway. **A-C** Quantification of immunoblot signal for Fig. 3A-C. **D, E** Quantification of immunoblot signal for Fig. 3H and I. The corresponding data above are represented as mean ± SEM; n = 3. *P < 0.05, **P < 0.01, ***P < 0.001; NS, not significant, P > 0.05.

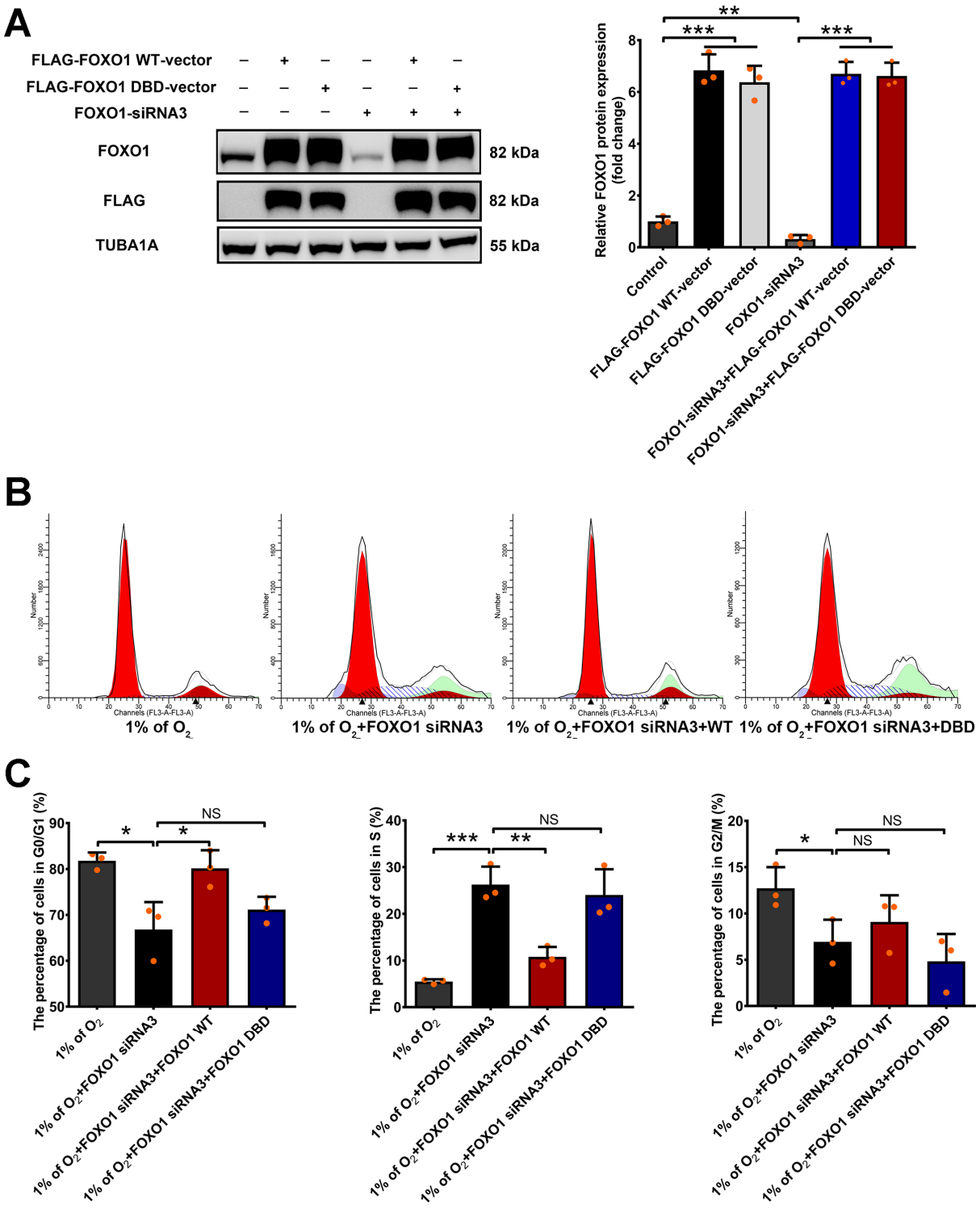

Fig. S5. The DNA-binding activity of FOXO1 is indispensable for hypoxia-induced G0/G1 arrest in GCs. **A-C** After treatment with FOXO1 siRNA for 12 h, FlAG-tagged FOXO1-expressing vectors, including FOXO1-WT (WT) and FOXO1N208A,H212R (DBD), were transfected into GCs, which were then cultured under normoxia (20% of O2) condition. 12 h later, cell lysates were collected to determine the protein levels of exogenous FOXO1 (FLAG) and total FOXO1 using western blotting **(A)**. TUBA1A served as the control for loading. **B**, **C** GCs consecutively transfected with FOXO1 siRNA and FOXO1-expressing vectors as mentioned above were grown under hypoxia (1% of O2) for 24 h, and then collected for the flow cytometric detection of cell cycle **(B)**. The cell cycle distribution was quantified using the ModFit LT 3.2 software **(C)**. The corresponding data above are represented as mean ± SEM; $n = 3$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; NS, not significant, $P > 0.05$.

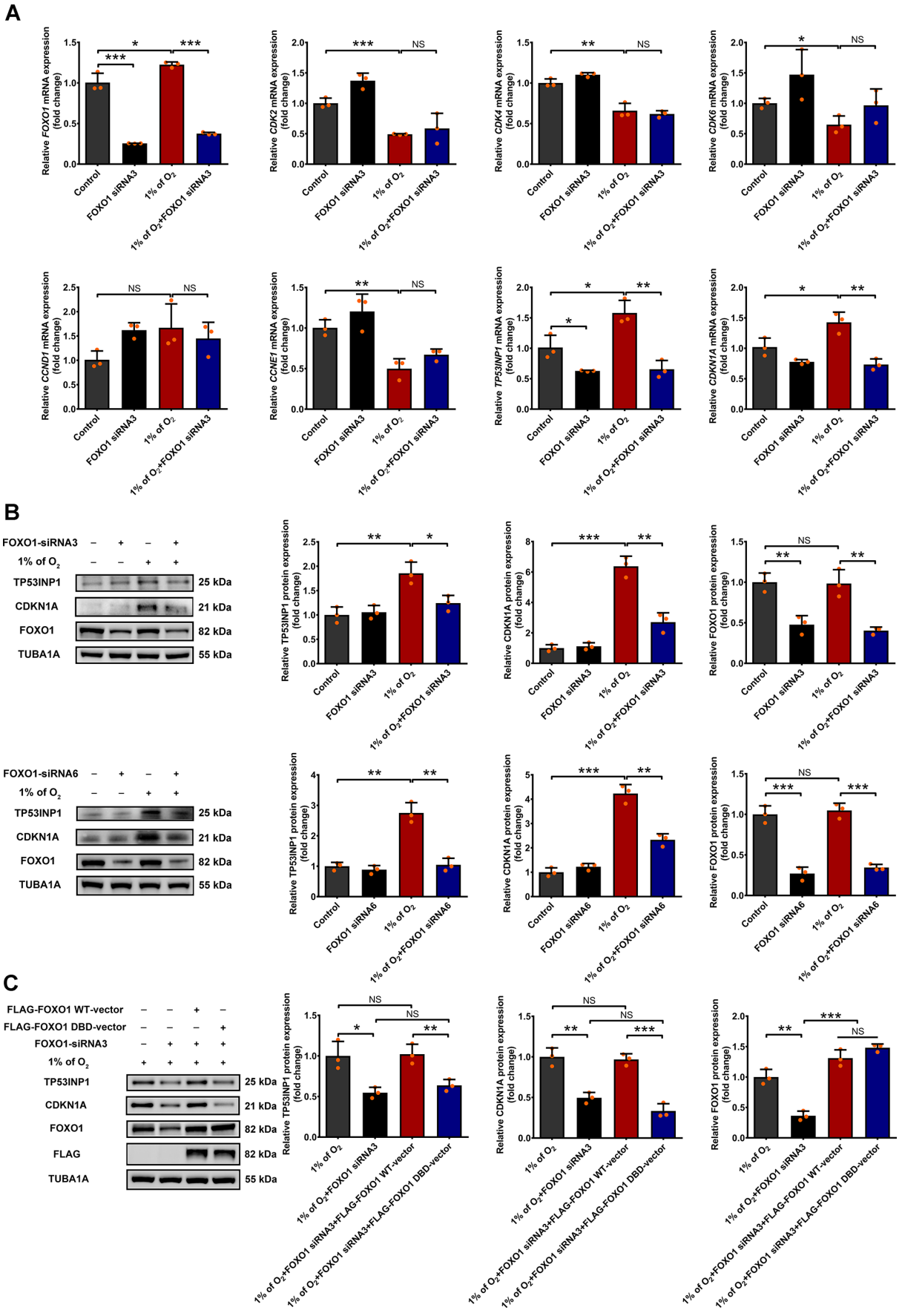

Fig. S6. Effects of FOXO1 on the expression of genes related to G1-S transition under hypoxic conditions. A, B GCs transfected with FOXO1 siRNAs or scramble control siRNA for 12 h were cultured for an additional 12 h under normoxia (20% of O2) or hypoxia (1% of O2) conditions. qRT-PCR was performed to measure the mRNA levels of genes related to G1-S transition in GCs (**A**). The protein levels of TP53INP1, CDKN1A, and FOXO1 were determined by western blotting (**B**). C After treatment with FOXO1 siRNA for 12 h, GCs were transfected with FOXO1-WT (WT) or FOXO1N208A,H212R (DBD), grown under hypoxia (1% of O2) for 12 h, and then collected for western blot detection of TP53INP1, CDKN1A, and FOXO1 levels in GCs. The corresponding data above are represented as mean ± SEM; $n = 3$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; NS, not significant, $P > 0.05$.

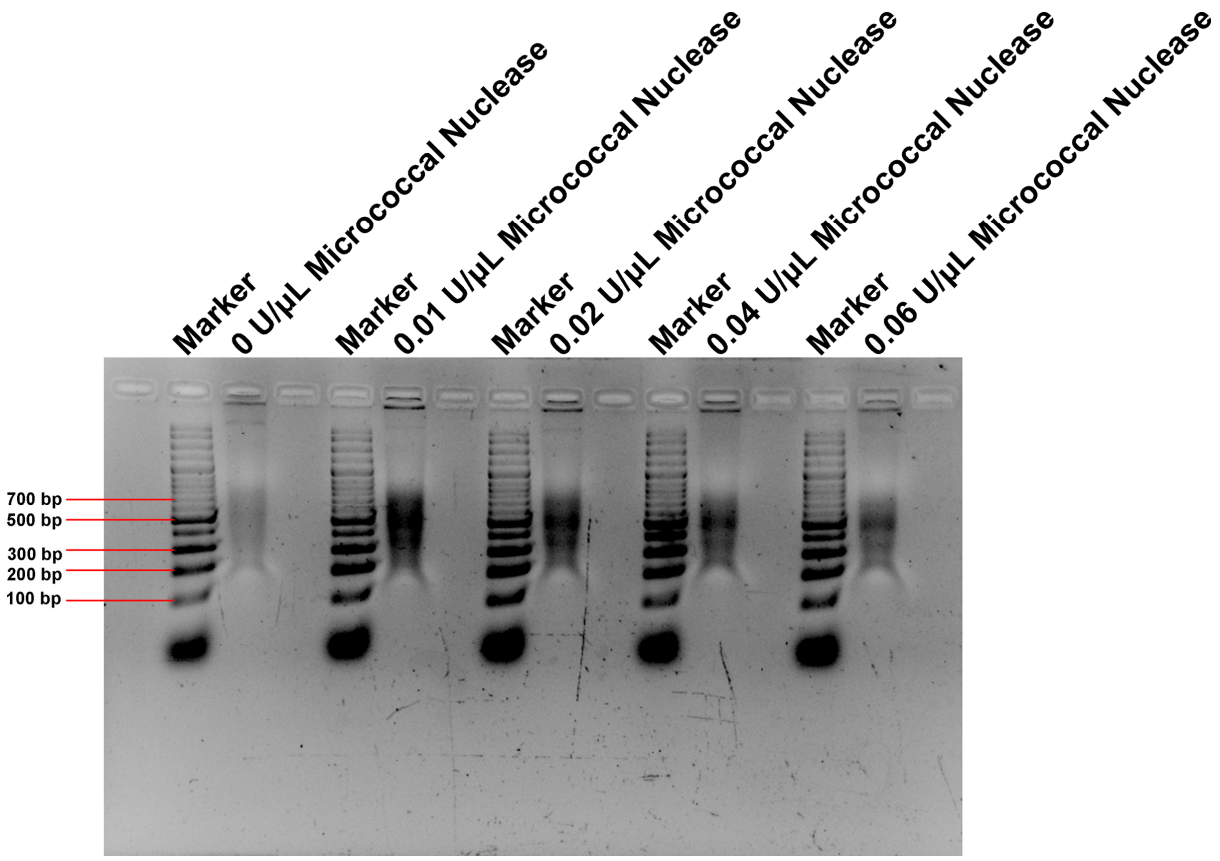

Fig. S7. Chromatin in GCs was digested with micrococcal nuclease to generate 250-700 bp fragments. Columns represent electrophoretic results of the DNA sample digested with micrococcal nuclease at different working concentrations.

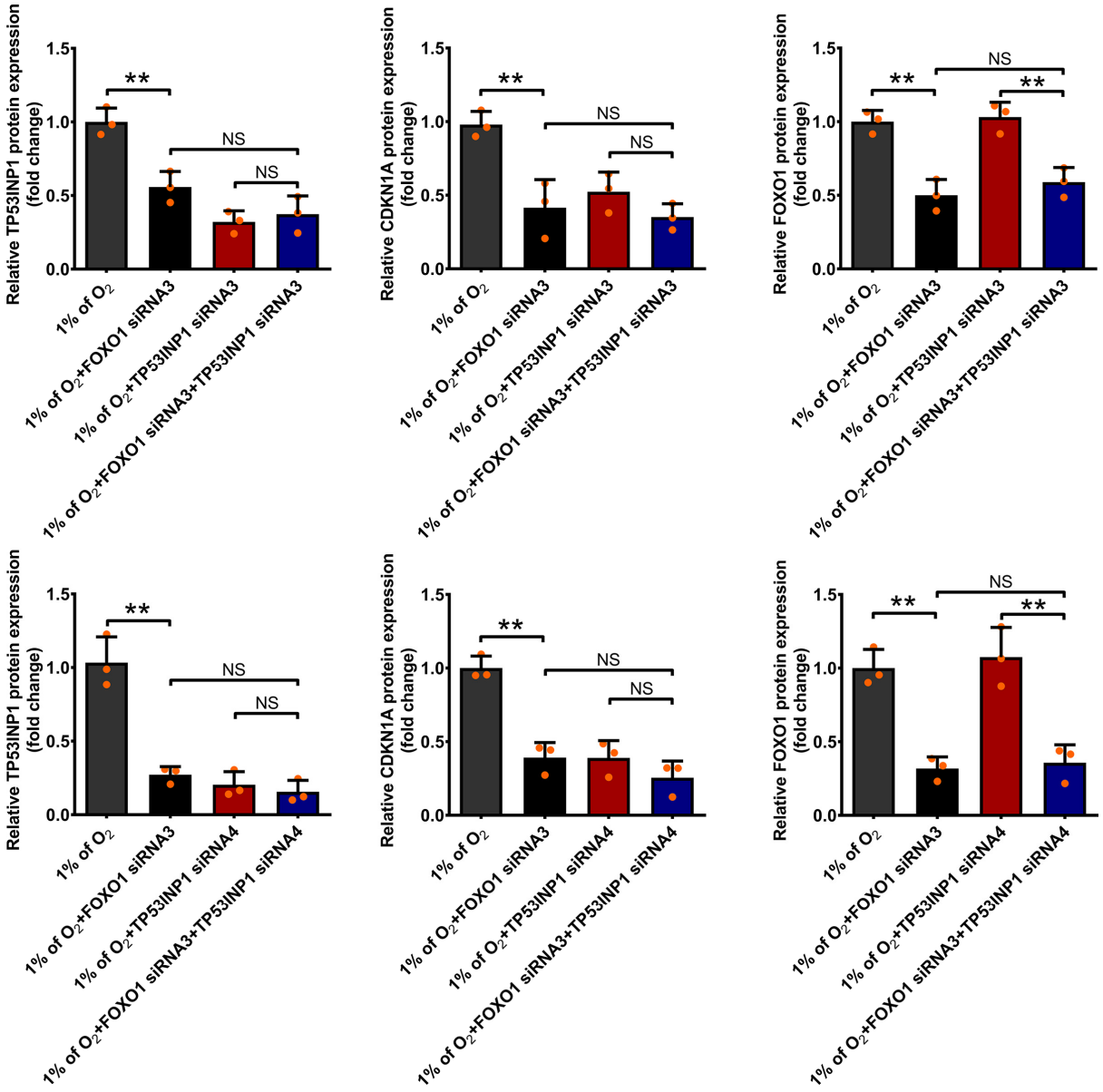

Fig. S8. Quantitative analysis of the protein levels for the western blot data as shown in Fig. 4H. The corresponding data above are represented as mean ± SEM; $n = 3$. $^{**}P < 0.01$; NS, not significant, $P > 0.05$.

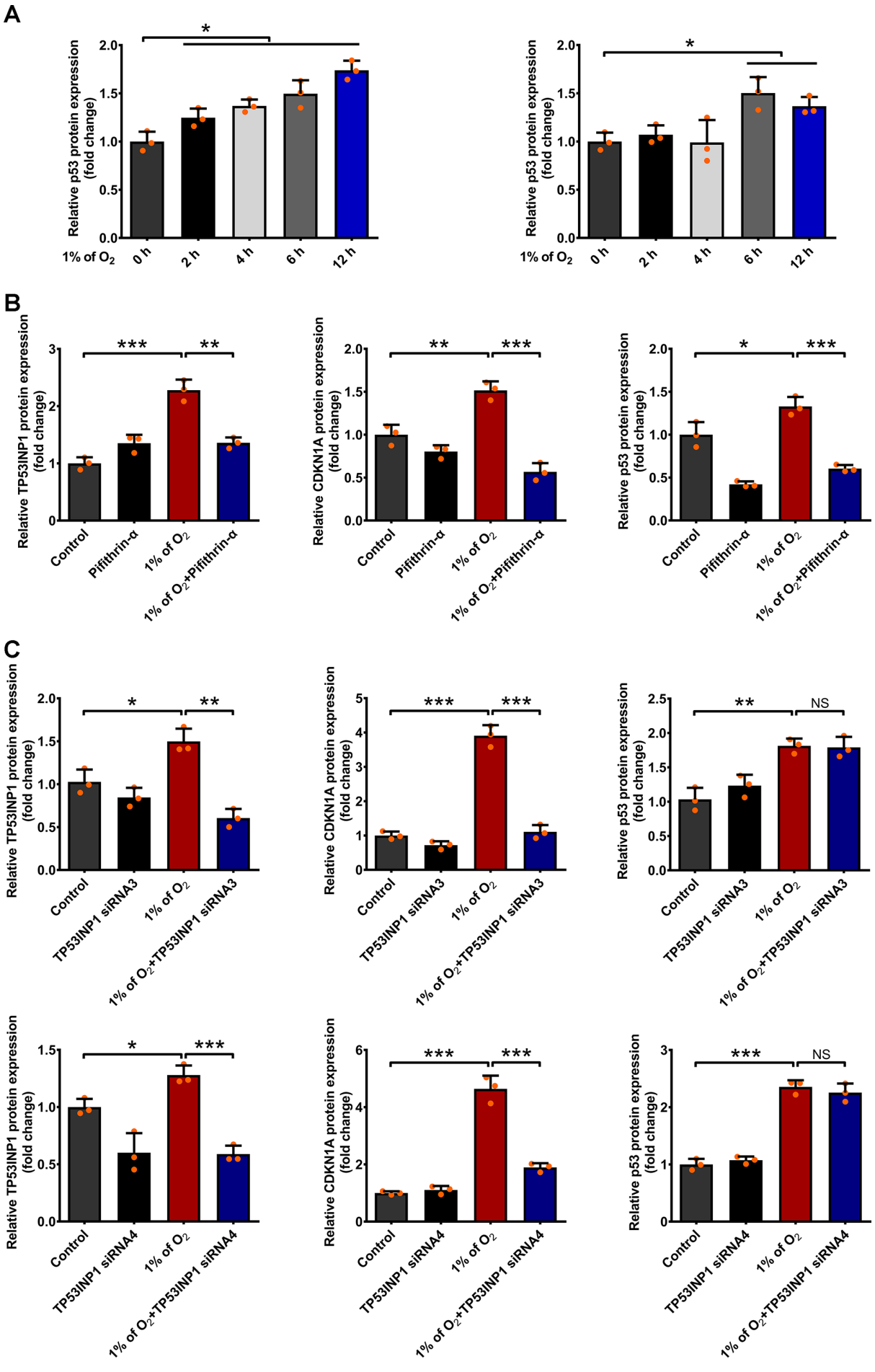

Fig. S9. Quantitative analysis of the protein levels for the western blot data as shown in Fig. 5A-C. The corresponding data above are represented as mean ± SEM; $n = 3$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; NS, not significant, $P > 0.05$.

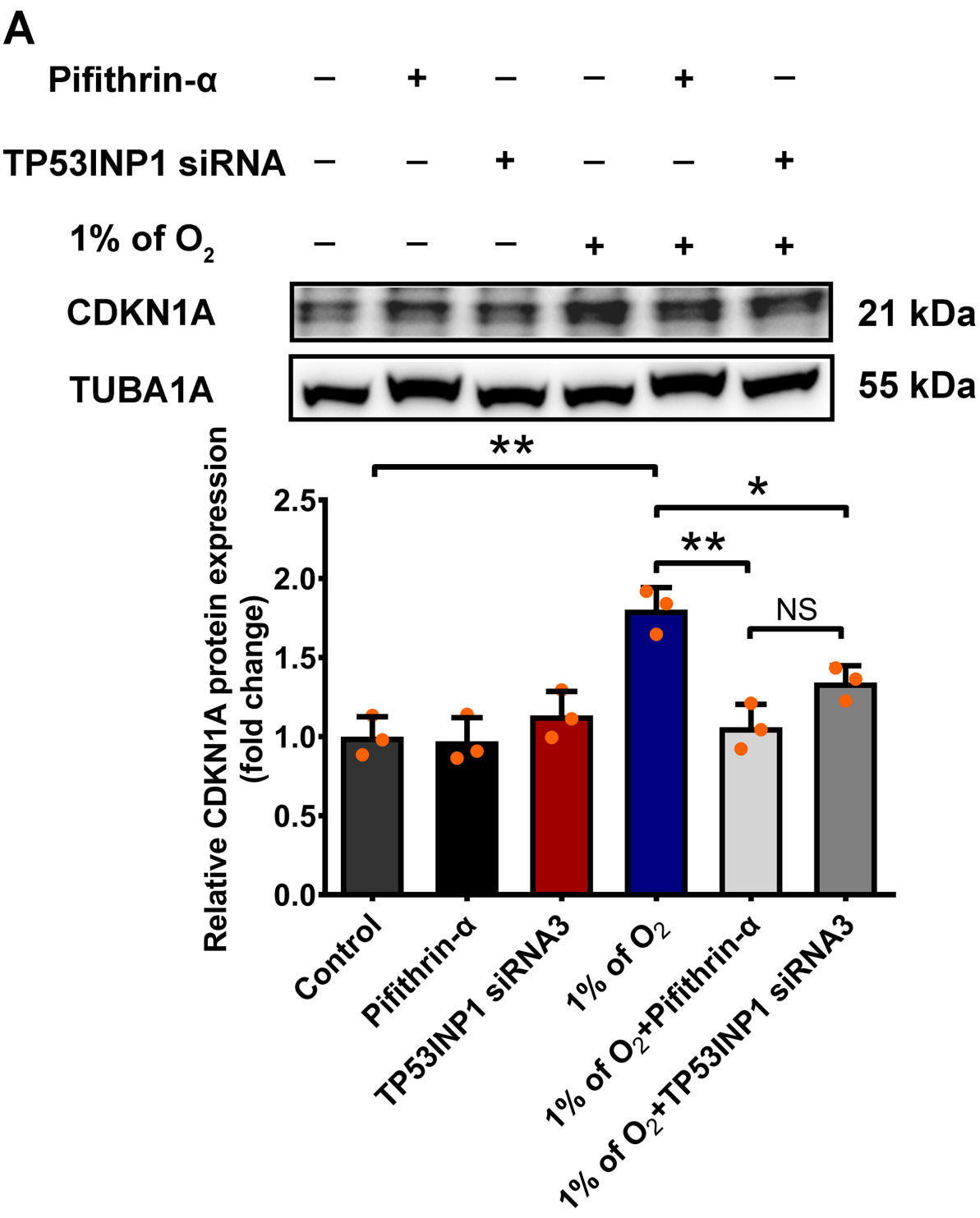

Fig. S10. p53 and TP53INP1 confer similar effects on CDKN1A expressions. wGeCre pretreated with the p53 inhibitor Pifithrin-α (30 μM) for 2 h or transfected with TP53INP1 siRNA for 12 h, and then cultured for an additional 12 h under normoxia (20% of O2) or hypoxia (1% of O2) conditions. The protein level of CDKN1A was determined by western blotting. The protein bands were quantified densitometrically using ImageJ 1.42q software. The corresponding data above are represented as mean ± SEM; $n = 3$. *$P < 0.05$, **$P < 0.01$; NS, not significant, $P > 0.05$.

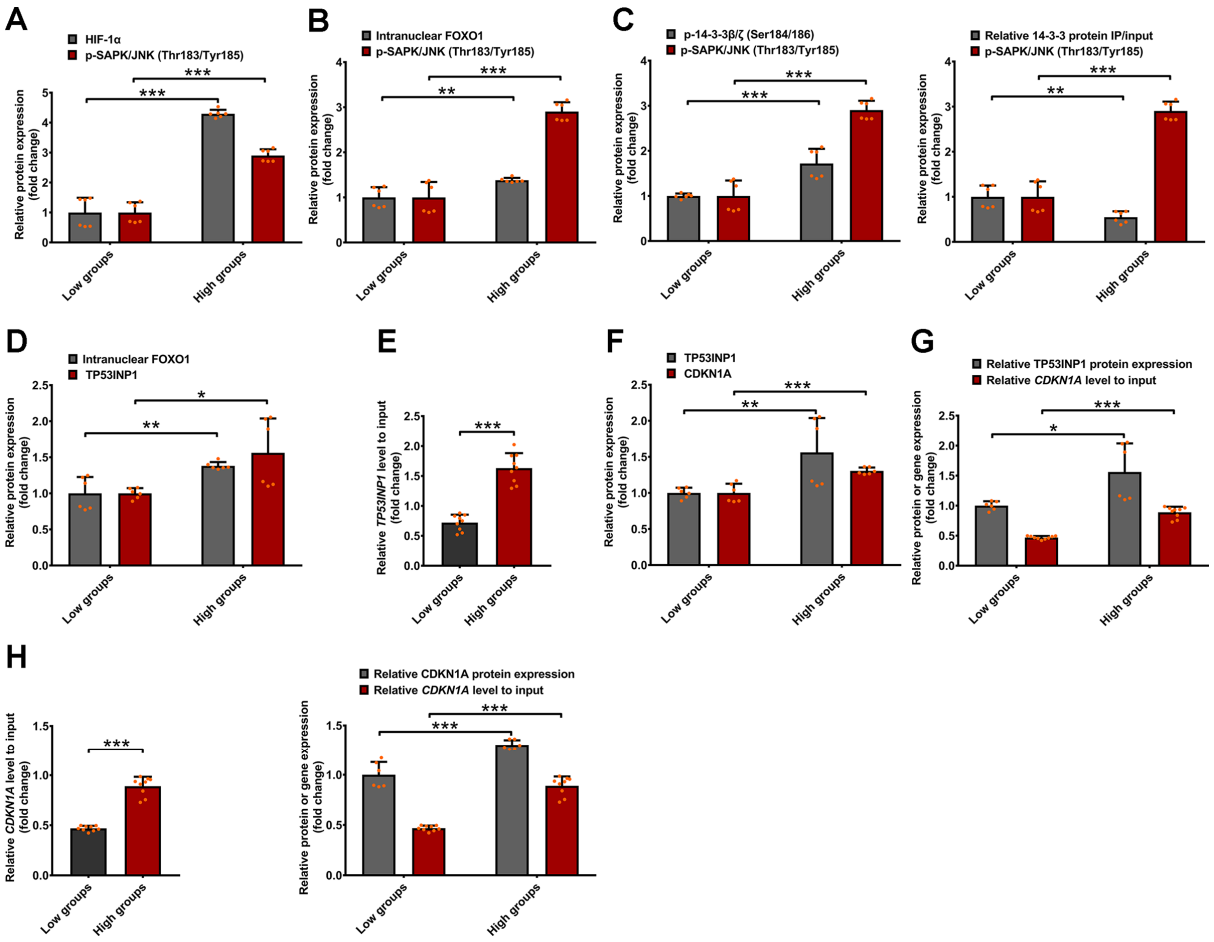

Fig. S11. Quantitative measurements and statistical analysis of the data as shown in Fig. 6. GCs collected from each of 90 follicles around 4 mm in diameter were labeled with PI for cell cycle analysis. Based on the ratio of cells in G0/G1 phase, GCs were classified into high G0/G1 distribution groups (high groups) and low G0/G1 distribution groups (low groups). A, B levels of HIF-1α, Phospho-SAPK/JNK (Thr183/Tyr185), and intranuclear FOXO1 were quantified by densitometry in low and high groups. C Quantitative analysis showed a close relationship among JNK activity, 14-3-3 phosphorylation, and the binding status between 14-3-3 and FOXO1. D Quantitative data compared TP53INP1 expression and the level of intranuclear FOXO1 in low and high groups. E The binding of FOXO1 to the TP53INP1 promoter in GCs was detected with ChI Passa y . D N A was isolated from the precipitated complexes as a template for qRT-PCR. The qRT-PCR products were then analyzed on a 2% agarose gel and quantified with densitometry using Image J 1.42q software. F Quantitative data compared the expression of TP53INP1 and CDKN1A in low and high groups. G ChIP assay was conducted to detect the binding of p53 to the CDKN1A promoter. Bar graph compared CDKN1A expression and the p53 binding level at CDKN1A promoter in low and high groups. H Quantitative data compared TP53INP1 expression and the p53 binding level at CDKN1A promoter in low and high groups. The corresponding data above are represented as mean ± SEM; n = 3. *P < 0.05, **P < 0.01, ***P < 0.001; NS, not significant, P > 0.05.

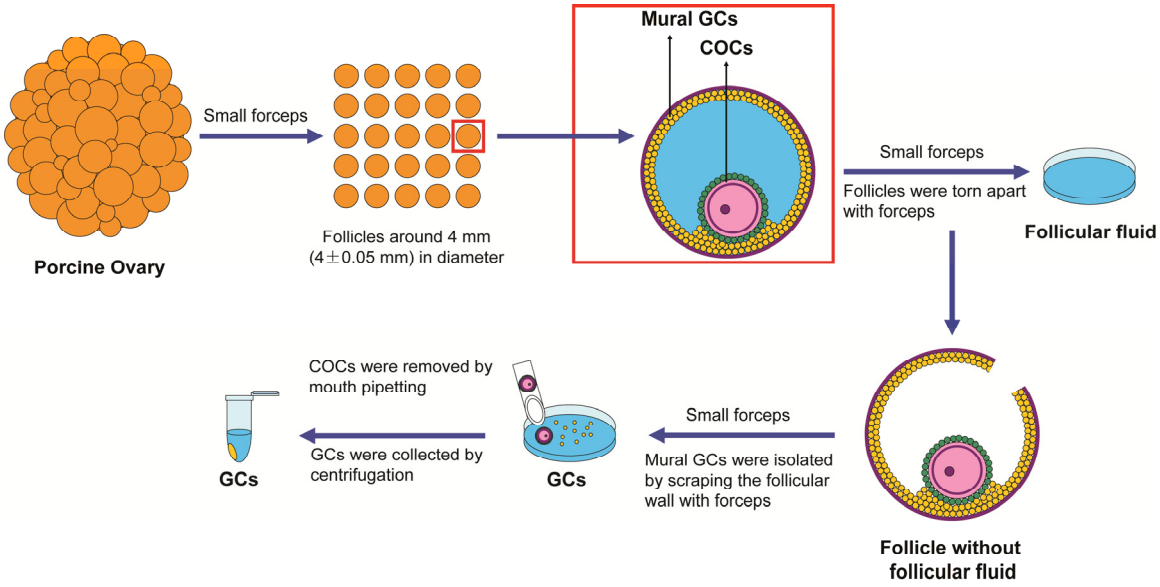

Fig. S12. A schematic diagram depicting the collection procedure of ovarian GCs. Porcine ovaries obtained from a local slaughterhouse were immediately transferred to the laboratory. Individual follicles at various stages, including small antral follicles (1-3 mm), medium antral follicles (3-5 mm), large antral follicles (≥5 mm) were dissected from the ovaries, and torn apart to discharge the follicular fluid with small forceps. A mixture of mural GCs and cumulus-oocyte complexes (COCs) was collected by scraping the follicular wall. After removing the COCs via mouth pipetting, GCs were harvested by centrifugation (1,000 g, 5 min).

Table S1. Primer sequences for qRT-PCR

Gene Name	GenBank Accession NO.	Primer Sequence(5'→3')
Tuba1a	[GenBank: NM_001315710.1]	F: AAGAGTCGCGCTGTAAGAAG R: AATGACTGTGGGTTCCAGGTC
CDKN1A	[GenBank: XM_013977858.2]	F: CCCCTGGAGGGTGACTT R: CGGCGTTTGGAGTGGTA
FOXO1	[GenBank: NM_214014.3]	F: AAGACCGCTTTACAAGTGCC R: TCAATGAACATGCCATCCAA
TP53INP1	[GenBank: XM_001925224.6]	F: CTCAGCAGCAGAAGACGA R: ATGGGACAGGACTCAAAC
CDK2	[GenBank: XM_005655594.2]	F: CACCTGAAATCCTTCTGGGCT R: GGTAGACACTGCGGTCATCC
CDK4	[GenBank: NM_001123097.1]	F: TGAACCAGTGGCGGAGATTG R: GCAGTAAGGCCACTTCACGA
CDK6	[GenBank: XR_002347034.1]	F: CAAGCAACCAACCATCCACG R: GGCACCATGAGGAGGGTAAC
CCND1	[GenBank: XM_021082686.1]	F: TTGAAGGCGAGGTTCCAGTC R: GCTGGTTCTCTAGGTCAGCC
CCNE1	[GenBank: XM_005653265.2]	F: GAGTGCTCCAGATGCTGCTAA R: ACAACGCTCACAACCACCTG
ACOT11	[GenBank: XM_021096589.1]	F: GGCGGCACATCAACAGC R: CGAGCATCTTCAGGGAGG
ADAMTS8	[GenBank: XM_003130083.4]	F: GAACGACGGCAACTACCT R: GTGGCGATGGAACCACT
AK5	[GenBank: XM_003127921.6]	F: AGCAGCAATAGGAAATGG R: GGAAACCCGTCAATAACA
AQP3	[GenBank: NM_001110172.1]	F: GTGACCTTCGCTATGTGCT R: CTGTGCCGATGAACTGG
ARID5B	[GenBank: XM_021072755.1]	F: TAGGCAGTGACGACATCCA R: ATCCCTCGCAATCAGTTTC
ATP1B1	[GenBank: NM_001001542.1]	F: AAAAGCCAAGGAGGAGGG R: CAGCCAGGCAGCCATAAA
F11R	[GenBank: NM_001128444.1]	F: ACAATCAGTGTTCCCTCC R: CAAGTGTAATCTCCAGCATC
FGL2	[GenBank: NM_001005152.2]	F: GGTGGTTTGATGCTTGTC R: CAGGTTGTGCCTCACTTAT
FOXP2	[GenBank: XM_021078421.1]	F: CAAGCAATGATGACCCAC R: TGAATGTCGCCTTCGTAT
NES	[GenBank: XM_005663265.3]	F: AGTGAGGACAAGGCAGAC R: CAACACGGGCTCTATCA

Table S2. siRNA sequences

siRNA Name	Sense（5'-3'）	Antisense（5'-3'）
Scrambled siRNA (SC)	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
FOXO1-1115 (FOXO1-siRNA1)	CCUACACAGCAAGUUCAUUTT	AAUGAACUUGCUGUGUAGGTT
FOXO1-1515 (FOXO1-siRNA2)	GCGGCAAAGAUGGCUUCUATT	UAGAAGCCAUCUUUGCCGCTT
FOXO1-2272 (FOXO1-siRNA3)	GCAUGUUCAUUGAGCGCUUTT	AAGCGCUCAAUGAACAUGCTT
FOXO1-925 (FOXO1-siRNA4)	CAGCAACGAUGACUUUGAUTT	AUCAAAGUCAUCHUUGCUGTT
FOXO1-1097 (FOXO1-siRNA5)	CCCAGUCUGUCUGAGAUAATT	UUAUCUCAGACAGACUGGGTT
FOXO1-638 (FOXO1-siRNA6)	GCAGGCUGGAAGAAUUCAATT	UUGAAUUCUUCCAGCCUGCTT
TP53INP1-450 (TP53INP1-siRNA1)	GGUGAAGUCAAUACUUCUUTT	AAGAAGUAUUGACUUCACCTT
TP53INP1-732 (TP53INP1-siRNA2)	GCAGGUGGCUUAACCACUATT	UAGUGGUUAAGCCACCUGCTT
TP53INP1-1117 (TP53INP1-siRNA3)	GCCCACGUCAGUACAAUUATT	UAAUUGUACUGACGUGGGCTT
TP53INP1-1 (TP53INP1-siRNA4)	GUUCCAGAGACUGAAUAAATT	UUUAUUCAGUCUCUGGAACTT
TP53INP1-314 (TP53INP1-siRNA5)	GGUGGCUUAACCACUAUCATT	UGAUAGUGGUUAAGCCACCTT
TP53INP1-599 (TP53INP1-siRNA6)	CAGUCUCUGAACAGAAAUATT	UAUUUCUGUUCAGAGACUGTT
CDKN1A-155 (CDKN1A-siRNA1)	CCAGGGAUGCACAUCAGAUTT	AUCUGAUGUGCAUCCCUGGTT
CDKN1A-282 (CDKN1A-siRNA2)	GCGAUGGAACUUCGACUUCTT	GAAGUCGAAGUUCCAUCGCTT
CDKN1A-464 (CDKN1A-siRNA3)	AGGACCAUGUGGACCUGUUTT	AACAGGUCCACAUGGUCCUTT
CDKN1A-76 (CDKN1A-siRNA4)	CGCCUCUUUGGCCCAGUGGTT	CCACUGGGCCAAAGAGGCGTT
CDKN1A-141 (CDKN1A-siRNA5)	GAUGGAACUUCGACUUCAUTT	AUGAAGUCGAAGUUCCAUCTT
CDKN1A-435 (CDKN1A-siRNA6)	GCAUGACAGAUUUCUACCATT	UGGUAGAAAUCUGUCAUGCTT

Table S3. Primer sequences for ChIP-qRT-PCR

Gene Name	GenBank Accession NO.	Primer Sequence(5'→3')
TP53INP1	[GenBank:NC_010446.5]	F: CGGAATTTTCCTAGTAGTACGGAAC R: GTAATGATGTAATCCTCGGGGTATC
CDKN1A	[GenBank:NC_010449.5]	F: AGCTGAGTCTCACTCTGTCCCTAAG R: TCTTCTATGCCAAGGCTCAACAT

Table S4. Primer sequences for amplifying *TP53INP1* promoter

Gene Name	ID	Primer Sequence(5'→3')
pGL3-TP53INP1	[G0149257-1-A_1]	TGCAGGTGCCAGAACATTTCTCTATC GATAGGTACCGCAGAAGCTGTCAGGC TTGCTTAAGG
	[G0149257-1-C_12]	TGCCAAGCTTACTTAGATCGCAGATCT CGAGAGCCGCTGCGCCCGCCCGGCC GCCCCGCCC

Table S5. Differentially expressed genes between high groups and low groups

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