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Supplemental Information

Long non-coding RNA lnc-CCNL1-3:1 promotes granulosa cell apoptosis and suppresses glucose uptake in women with polycystic ovary syndrome

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Table S1. Small interfering RNA sequences used for transfection

Target genes	**Sequences (5’ → 3’)**
FOXO1	F:5’- GGACAACAACAGUAAAUUUdTdT -3’

Notes: F, forward; R, reverse; FOXO1, forkhead box protein O1.

Table S2. Primer sequences and amplification conditions used for real-time PCR

Genes	**Primer sequences (5’ → 3’)**	**Amplification condition**
CCNL	F:5’-TTTGCAGCATTTCCTCCTCT-3’	Stage 1: 95°C, 10 sec Stage 2: 95°C, 5 sec 60°C, 34 sec Number of cycles:40
	R:5’-CTCCCAAAGTGCTGGGTTTA-3’	
FOXO1	F:5’-TCGTACGCCGACCTCATCA-3’	
	R:5’-TCCTTGAAGTAGGGCACGCTC-3’	
ACTB	F:5’-CTCCATCCTGGCCTCGCTGT-3’	
	R:5’-GCTGTCACCTTCACCGTTCC-3’	
U6	F:5’-CTCGCTTCGGCAGCACA-3’	
	R:5’-AACGCTTCACGAATTTGCGT-3’	
GADD45α	F:5’-TGCTGGTGACGAATCCAC-3’	
	R:5’-TAGCGACTTTCCCGGCAA-3’	
TRAIL	F:5’-ACATTGTCTTCTCCAAACTCCA-3’	
	R:5’-GCTCAGGAATGAATGCCCA-3’	
Bim	F:5’-GCTTACTATGCAAGGAGGGTAT -3’	
	R:5’- ACAAGAGAACCGCTGGCT -3’	

Notes: F, forward; R, reverse; CCNL: Lnc-CCNL1-3:1; FOXO1: forkhead box protein O1; ACTB: β-Actin. GADD45α: Growth arrest and DNA damage inducible protein 45α; TRAIL: TNF related apoptosis inducing ligand; Bim: Bcl-2 interacting mediator of cell death.

Table S3. Primer sequences and amplification conditions used for real-time PCR in RLM-RACE

Fragmented primers		Sequences (5’ → 3’)	Amplification condition
	Lnc-CCNL1-F	GGGTCTCTAGATGTCCATGA	Stage 1: 94°C, 2 min Stage 2: 94°C, 30 sec 62°C, 30 sec 68°C, 30 sec Number of cycles: 38
	Lnc-CCNL1-R	GCTGGGTTTACAGGTGTAAG	
5’RACE	rLnc-CCNL1-R1	GATAGCCTATGCCCAGGTTTCTGAGGAGTT	
	rLnc-CCNL1-R2	CCTGGTGTTGTAACTGGGATGCAGTCTTG	
3’RACE	rLnc-CCNL1-F1	GGTCAAAGGCATGAGTTTCCAGGAGAT	
	rLnc-CCNL1-F2	GACTAGAGGATGACTGTTGACTAGATT	

Notes: RLM-RACE, RNA ligase-mediated rapid amplification of 5’and 3’cDNA ends; F, forward; R, reverse; CCNL: Lnc-CCNL1-3:1;

Table S4. Primary antibodies used for western blot and Immunofluorescence assay

Protein target	Host	Dilution used	Manufacturer
β-Actin	Mouse Monoclonal	1:3000	Proteintech Group Inc
GAPDH	Mouse Monoclonal	1:3000	Proteintech Group Inc
Lamin A/C	Mouse Monoclonal	1:3000	Cell Signaling Technology
Tubulin	Mouse Monoclonal	1:3000	Proteintech Group Inc
FOXO1	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Total PARP	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Cleaved PARP	Rabbit Monoclonal	1:500	Invitrogen
Total Caspase-3	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Cleaved Caspase-3	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Bcl-2	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Bax	Mouse Monoclonal	1:1000	Cell Signaling Technology
GLUT4	Rabbit Polyclonal	1:1000	Abcam
IRS1	Rabbit Polyclonal	1:1000	Cell Signaling Technology
Phospho-Rb	Rabbit Polyclonal	1:1000	Cell Signaling Technology
Rb	Mouse Monoclonal	1:2000	Cell Signaling Technology
Phospho-CDK2	Rabbit Polyclonal	1:1000	Cell Signaling Technology
CDK2	Mouse Monoclonal	1:1000	Cell Signaling Technology
P21 (CDKN1A)	Rabbit Polyclonal	1:1000	Cell Signaling Technology
Alexa Fluor 488 (green)-conjugated	Goat Polyclonal	1:100	Invitrogen

goat anti-rabbit IgG

Notes: FOXO1: forkhead box protein O1; PARP: poly-ADP-ribose polymerase; Bcl-2: B cell lymphoma-2; Bax: BCL2-associated X; GLUT4: glucose transporter 4; IRS1: insulin receptor substrate 1; CDK2: cyclin-dependent kinase 2; P21 (CDKN1A): cyclin-dependent kinase inhibitor 1A.

Table S5. The relative endocrine parameters in patients with PCOS

	PCOS (n = 43)
BMI (kg/m^2)	22.73 ± 3.34
FBG (mmol/L)	5.46 ± 0.55
2HPG (IU/L)	6.97 ± 1.99
Flns (mIU/L)	17.50 ± 16.53
HOMA-IR	4.46 ± 0.73

Notes:All data are mean ± SD value. FPG: Fasting blood glucose; 2HPG: 2 hour postprandial blood glucose; Flns: fasting insulin; HOMA-IR: Homeostasis model assessment of insulin resistance.

Table S6. Range of HOMA-IR in women with PCOS

HOMA-IR		
Minimum		0.71
Maximum		20.73
Tertile	33.33	2.04
	66.66	3.89

Notes: HOMA-IR: Homeostasis model assessment of insulin resistance

Table S7. Prediction of Lnc-CCNL1-3:1 and FOXO1 affinity using the RPISeq

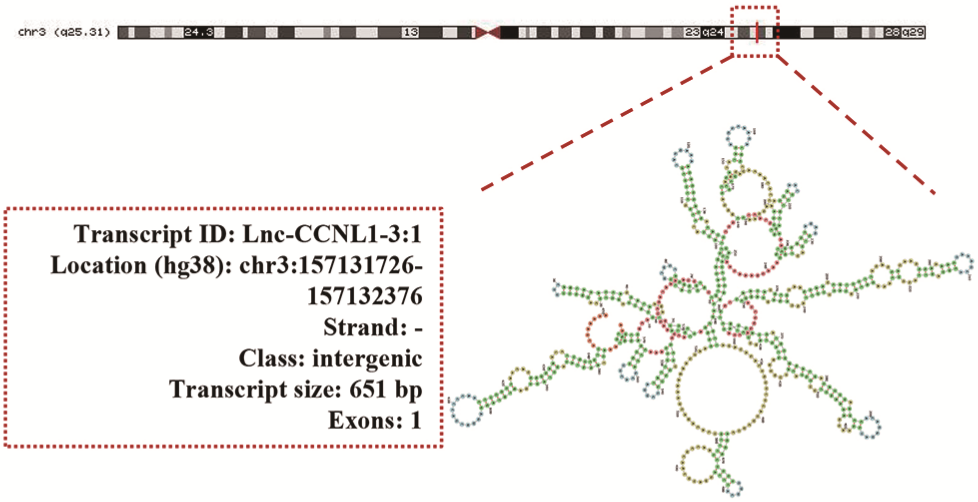
RNA-Protein Interaction Prediction (RPISeq)	
Protein Sequence (FOXO1)	
MRSKHTANCLAFRCILAWPGGLAQNCWCQNSIRHNLSLHSKFIRVQNEGTGKSSWWMLNPEGGKSGKSPRRRAASMDNNSKFAKSRSRAAKKKASLQSGQEGAGDSPGSQFSKWPASPGSHSNDDFDNWSTFRPRTSSNASTISGRLSPIMTEQDDLGEGDVHSMVYPPSAAKMASTLPSLSEISNPENMENLLDNLNLLSSPTSLTVSTQSSPGTMMQQTPCYSFAPPNTSLNSPSPNYQKYTYGQSSMSPLPQMPIQTLQDNKSSYGGMSQYNCAPGLLKELLTSDSPPHNDIMTPVDPGVAQPNSRVLGQNVMMGPNSVMSTYGSQASHNKMMNPSSHTHPGHAQQTSAVNGRPLPHTVSTMPHTSGMNRLTQVKTPVQVPLPHPMQMSALGGYSSVSSCNGYGRMGLLHQEKLPSDLDGMFIERLDCDMESIIRNDLMDGDTLDFNFDNVLPNQSFPHSVKTTTHSWVSG	
RNA Sequence (lnc-CCNL1-3:1)	
AGCTCTTCAGTGTTGTGCTCATTGGTTTCATCAAAGCAGGACAATACTTGGCACTAAGTCGACAAAATTCTCACTTCTGCAGTGGGTCTCTAGATGTCCATGACCCTGCCTTCTCAGGAAAAGAGGGAGGACATGCGGCTGGAGGGACACAGAGGGCAAGACTGCATCCCAGTTACAACACCAGGAAGCCACGTCTGAAAGGAAACTCCTCAGAAACCTGGGCATAGGCTATCGGGAAAGTAAAGAAGACATCTGGAGAGGCGAATATTCTGAAGGAATCTCTGCCAGAGGAGAAGGGAAAAGGAAAGCACTTATCCAGGTCAAAGGCATGAGTTTCCAGGAGATTATTCATCGTGACTAGAGGATGACTGTTGACTAGATTTATTCCACAGGTATGGAAGGAAAAGTCTTGGTACATTTTTTGCAGCATTTCCTCCTCTCCATATAGACTGTTCTCATTCTGCAGGGCTGGGGTTCACCCAGTATTCAACTCCAAGCCCTTCACATAGTAAAAGAAGTAAGGGGAGCCAGGCATGGTGGCTTACACCTGTAAACCCAGCACTTTGGGAGGGCAAAGCAGGCAGATTCATTGAGCTCAGGAGTTCGACCCCAACCTGGGCAACATGGTGAAACCCTGTCTCTACTAAAAATACAAAAATTAGCTGGGTGTGGTGGTGCATGCCTGTGGTCCCAGCTACTCTGGAGGCTGAGGTGGTAGGATTGCTTGAGCCCACATGTGTGAGGCTGCAGTGAGCTGTGATCACACCACTGCTCTCCAGCCTGGGTGACAGAGTGAGAGACCCTGTCCCCCCAACCACCCCCCCCAACAAAAAAAAAAAAAA	
Interaction probabilities	
Prediction using RF classifier	0.75
Prediction using SVM classifier	0.65

Notes: The sequence of lnc-CCNL1-3:1 (CCNL) was obtained from the RACE assay. Interaction probabilities generated by RPISeq range from 0 to 1. In performance evaluation experiments, predictions with probabilities > 0.5 were considered “positive,” indicating that the corresponding RNA and protein are likely to interact.

Table S8. Prediction of Lnc-CCNL1-3:1 and EZH2 affinity using the RPISeq

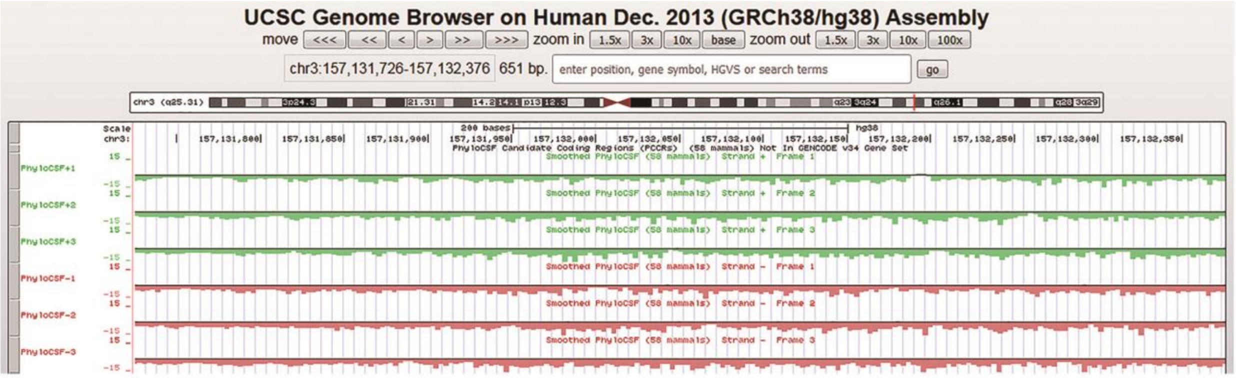
RNA-Protein Interaction Prediction (RPISeq)	
Protein Sequence (EZH2)	
MGQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQKILERTEILNQEWKQRRIQPVHILTSCSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSPLQQNFMVEDETVLHNIPYMGDEVLDQDGTFIEELIKNYDGKVHGDRECGFINDEIFVELVNALGQYNDDDDDDDGDDPEEREEKQKDLEDHRDDKESRPPRKFPSDKIFEAISSMFPDKGTAEELKEKYKELTEQQLPGALPPECTPNIDGPNAKSVQREQSLHSFHTLFCRRCFKYDCFLHPFHATPNTYKRKNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVLESKDTDSDREAGTETGGENNDKEEEEKKDETSSSSEANSRCQTPIKMKPNIEPPENVEWSGAEASMFRVLIGTYYDNFCAIARLIGTKTCRQVYEFRVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHCRKIQLKKGQNRFPGCRCKAQCNTKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCSIQRGSKKHLLLAPSDVAGWGIFIKDPVQKNEFISEYCGEIISQDEADRRGKVYDKYMCSFLFNLNNDFVVDATRKGNKIRFANHSVNPNCYAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIP	
RNA Sequence (lnc-CCNL1-3:1)	
AGCTCTTCAGTGTTGTGCTCATTGGTTTCATCAAAGCAGGACAATACTTGGCACTAAGTCGACAAAATTCTCACTTCTGCAGTGGGTCTCTAGATGTCCATGACCCTGCCTTCTCAGGAAAAGAGGGAGGACATGCGGCTGGAGGGACACAGAGGGCAAGACTGCATCCCAGTTACAACACCAGGAAGCCACGTCTGAAAGGAAACTCCTCAGAAACCTGGGCATAGGCTATCGGGAAAGTAAAGAAGACATCTGGAGAGGCGAATATTCTGAAGGAATCTCTGCCAGAGGAGAAGGGAAAAGGAAAGCACTTATCCAGGTCAAAGGCATGAGTTTCCAGGAGATTATTCATCGTGACTAGAGGATGACTGTTGACTAGATTTATTCCACAGGTATGGAAGGAAAAGTCTTGGTACATTTTTTGCAGCATTTCCTCCTCTCCATATAGACTGTTCTCATTCTGCAGGGCTGGGGTTCACCCAGTATTCAACTCCAAGCCCTTCACATAGTAAAAGAAGTAAGGGGAGCCAGGCATGGTGGCTTACACCTGTAAACCCAGCACTTTGGGAGGGCAAAGCAGGCAGATTCATTGAGCTCAGGAGTTCGACCCCAACCTGGGCAACATGGTGAAACCCTGTCTCTACTAAAAATACAAAAATTAGCTGGGTGTGGTGGTGCATGCCTGTGGTCCCAGCTACTCTGGAGGCTGAGGTGGTAGGATTGCTTGAGCCCACATGTGTGAGGCTGCAGTGAGCTGTGATCACACCACTGCTCTCCAGCCTGGGTGACAGAGTGAGAGACCCTGTCCCCCCAACCACCCCCCCCAACAAAAAAAAAAAAAA	
Interaction probabilities	
Prediction using RF classifier	0.65
Prediction using SVM classifier	0.89

Notes: The sequence of lnc-CCNL1-3:1 (CCNL) was obtained from the RACE assay. Interaction probabilities generated by RPISeq range from 0 to 1. In performance evaluation experiments, predictions with probabilities > 0.5 were considered “positive,” indicating that the corresponding RNA and protein are likely to interact.



Transcript ID: Lnc-CCNL1-3:1
Location (hg38): chr3:157131726-157132376
Strand: -
Class: intergenic
Transcript size: 651 bp
Exons: 1

Figure S1. Basic information for Lnc-CCNL1-3:1 from the NONCODE database (Transcript ID: NONHSAT092887.2).



UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly

chr3:157,131,726-157,132,376 651 bp.

Figure S2. The PhyloCSF score indicating that lnc-CCNL1-3:1 is more likely to be a non-coding RNA

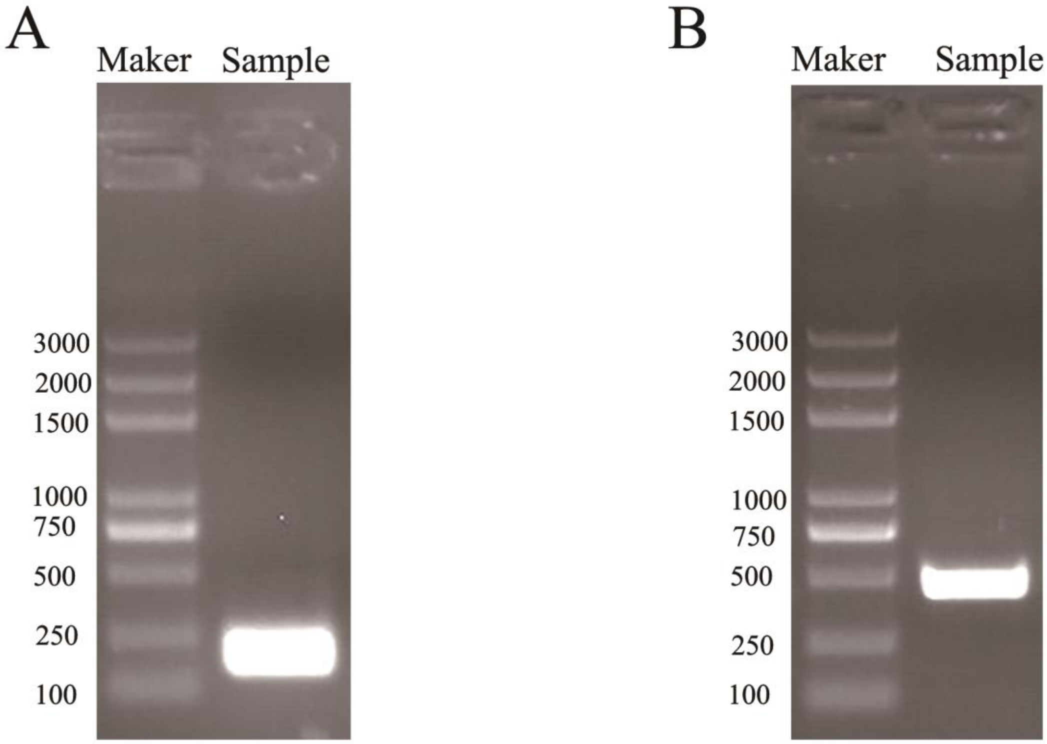


Figure S3. 5’and 3’rapid amplification of cDNA ends (RACE) assays in KGN cells to detect the whole sequence of CCNL. A: a gelelectrophoresis image of PCR products from the 5’RACE assays. B: a gelelectrophoresis image of PCR products from the 3’RACE assays.

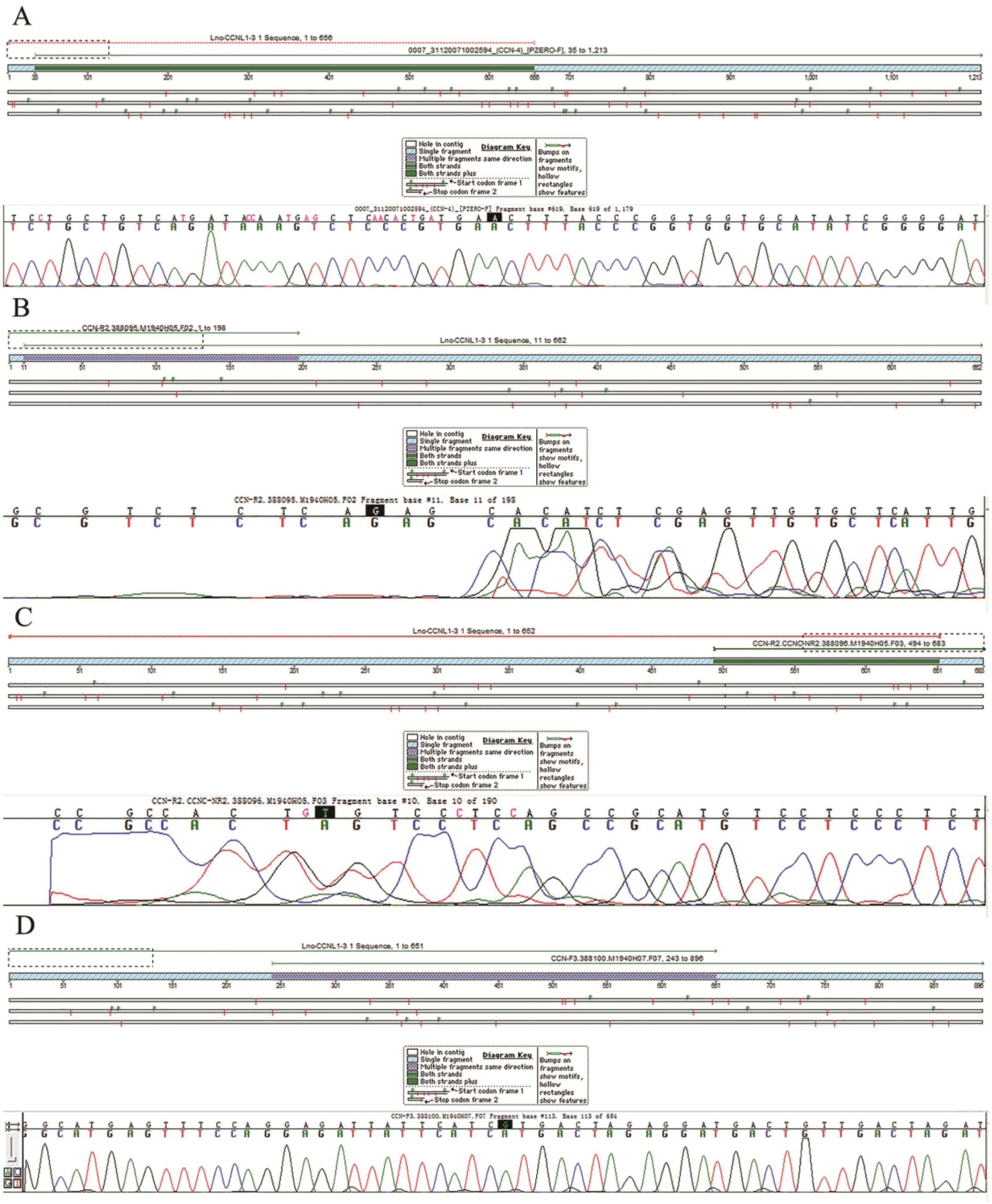


Figure S4. The validation of CCNL sequence using Sequencher (Gene Codes Corporation, Michigan, USA). Lnc-CCNL 1-3:1 sequence was obtained from the NONCODE database (Transcript ID: NONHSAT092887.2) and consistent with our results from RACE experiment. A: The part of CCNL sequence from PCR products compared with Lnc-CCNL 1-3:1 sequence. B, C: The CCNL sequence from 5’RACE PCR products compared with Lnc-CCNL 1-3:1 sequence. D: The CCNL sequence from 3’RACE PCR products compared with Lnc-CCNL 1-3:1 sequence.

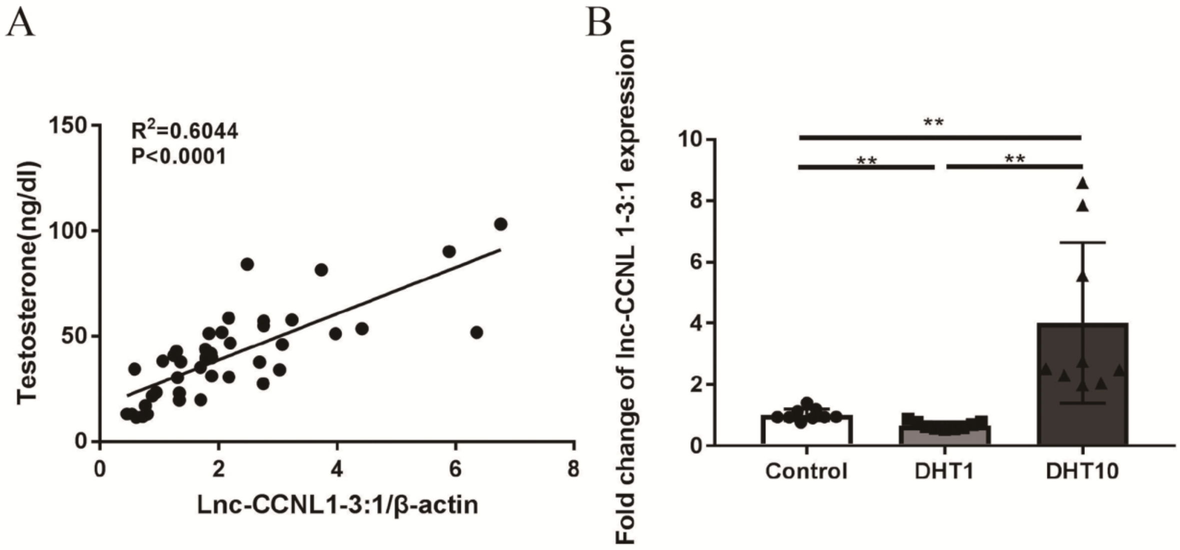


Figure S5. Androgen excess induces Lnc-CCNL1-3:1 expression. A: The correlation between the expression level of *CCNL* in the serum concentration of testosterone in patients with PCOS (n = 43). The expression level was detected via qRT-PCR and normalized against *β-actin*. B: Androgen excess induces *CCNL* expression in KGN cells. KGN cell lines were preincubated with 1 or 10mM double hydrogen testosterone (DHT) medium for 24 hours then subjected to qRT-PCR analysis and normalized against *β-actin* to measure *CCNL* expression. Error bars represent SDs of at least 3 independent experiments. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ correspond to two–tailed Student's tests.

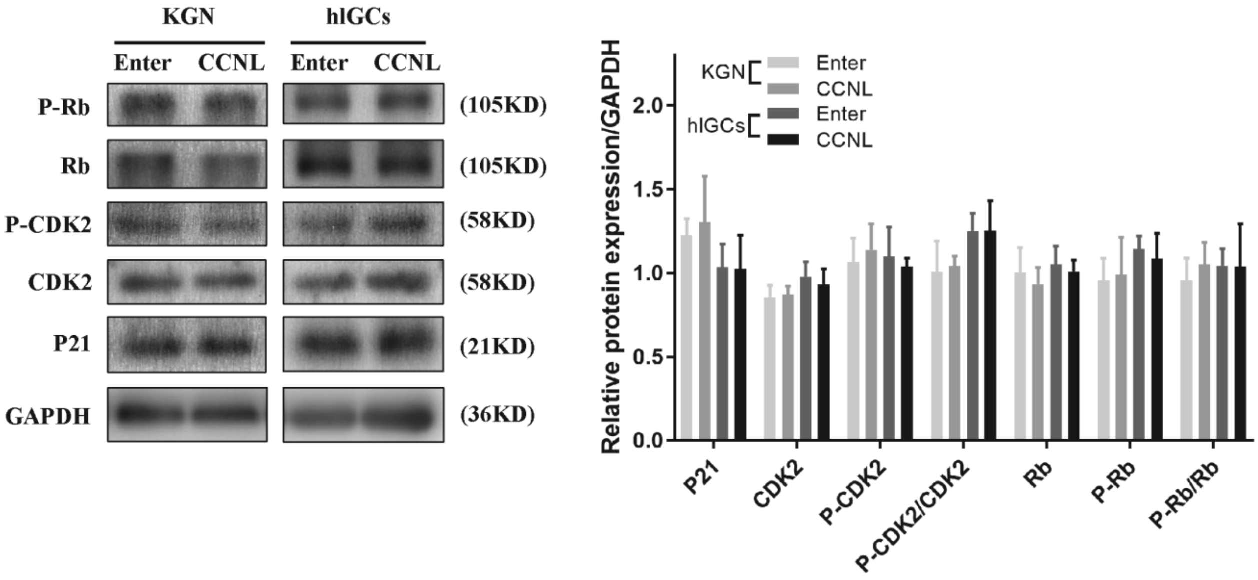


Figure S6. Western blotting analysis to measure relative protein expression (P-Rb: Phospho-Rb, Rb, P-CDK2: Phospho-CDK2, CDK2, P21: CDKN1A) from KGN and hlGCs cells (n=3) after transfected with vectors for 72 h.***: Error bars represent SDs of at least 3 independent experiments. $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ correspond to two–tailed Student's tests.

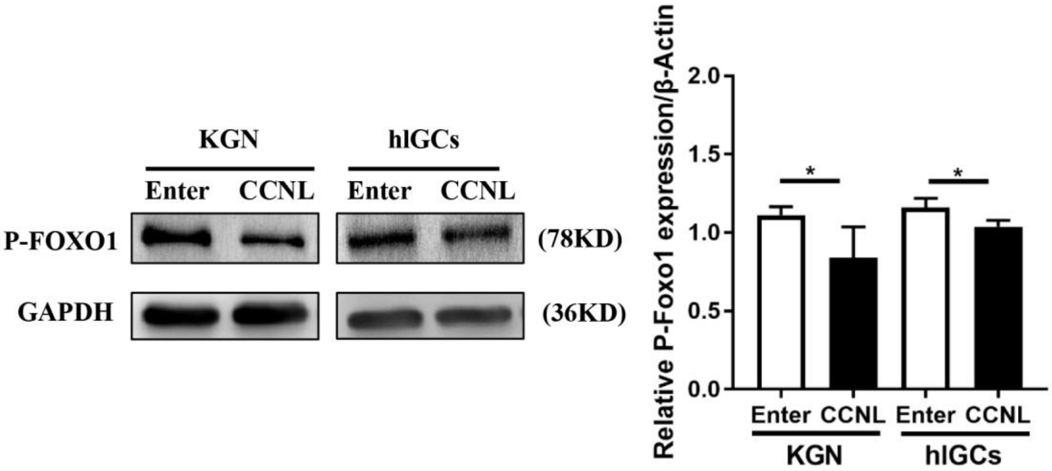


Figure S7. Western blotting analysis to measure relative p-FOXO1 expression (p-FOXO1: Phospho- FOXO1) from KGN and hlGCs cells after transfected with vectors for 72 h.***: Error bars represent SDs of at least 3 independent experiments. $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ correspond to two–tailed Student's tests. FOXO1: forkhead box protein O1.

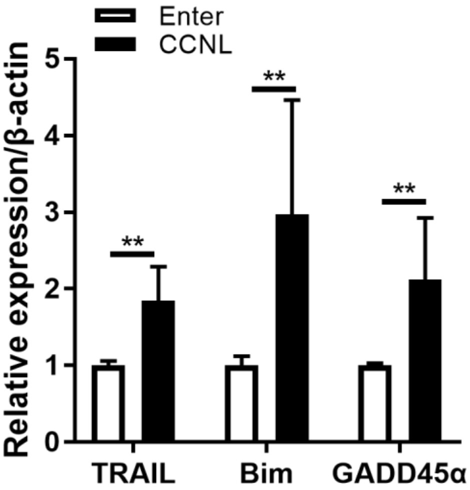


Figure S8. QPCR analysis to measure relative expression (TRAIL, Bim and GADD45α) from KGN cells after transfected with vectors for 72 h.***: Error bars represent SDs of at least 3 independent experiments. $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$ correspond to two–tailed Student's tests. GADD45α: Growth arrest and DNA damage inducible protein 45α; TRAIL: TNF related apoptosis inducing ligand; Bim: Bcl-2 interacting mediator of cell death.

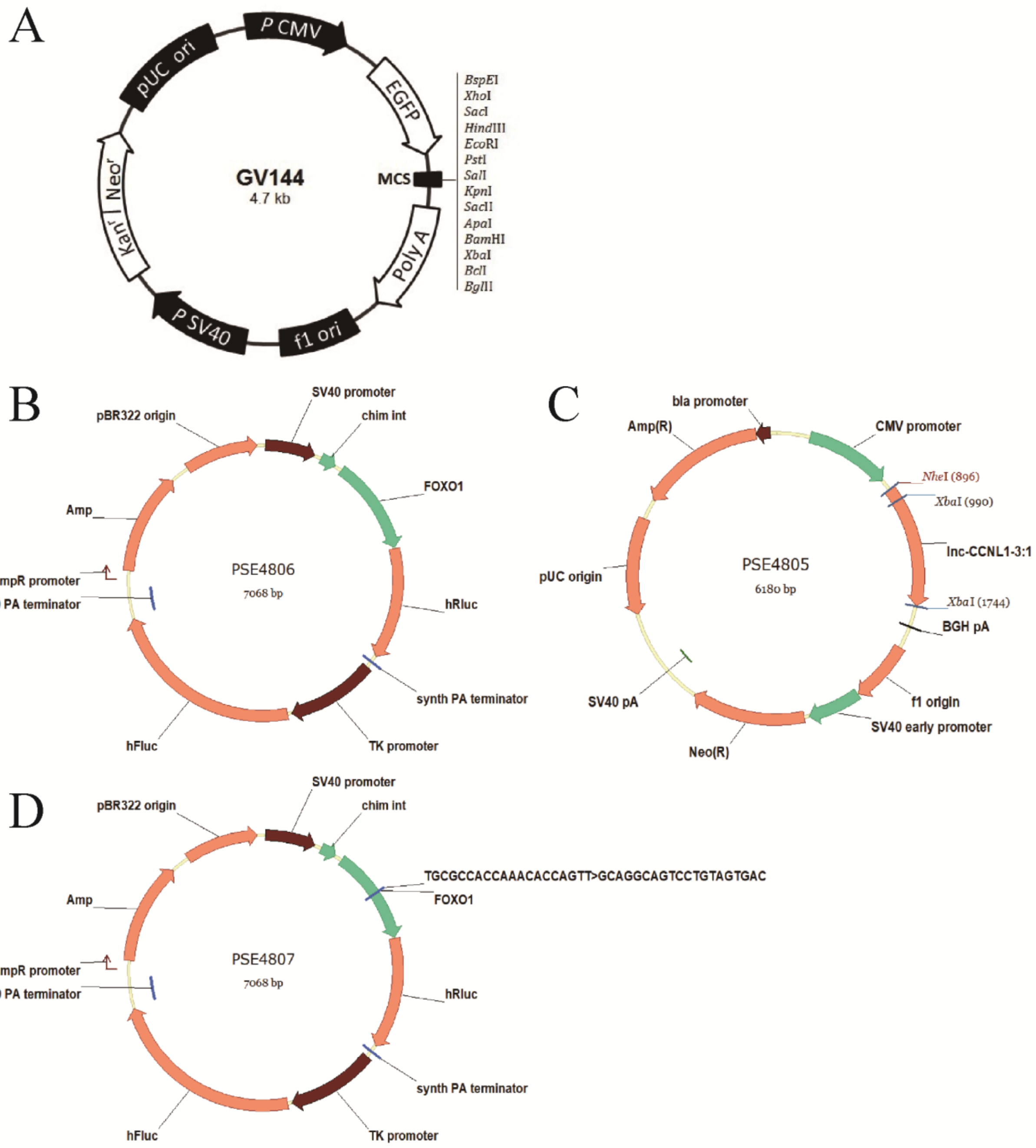


Figure S9. Vector backbone for transfection and luciferase reporter assays. A: Vector backbone for CCNL transfection. B-D: Vector backbone for luciferase reporter assays. B: pcDNA3.1-CCNL. C: pcDNA3.1-FOXO1. D: pcDNA3.1-FOXO1-mut. FOXO1: forkhead box protein O1.

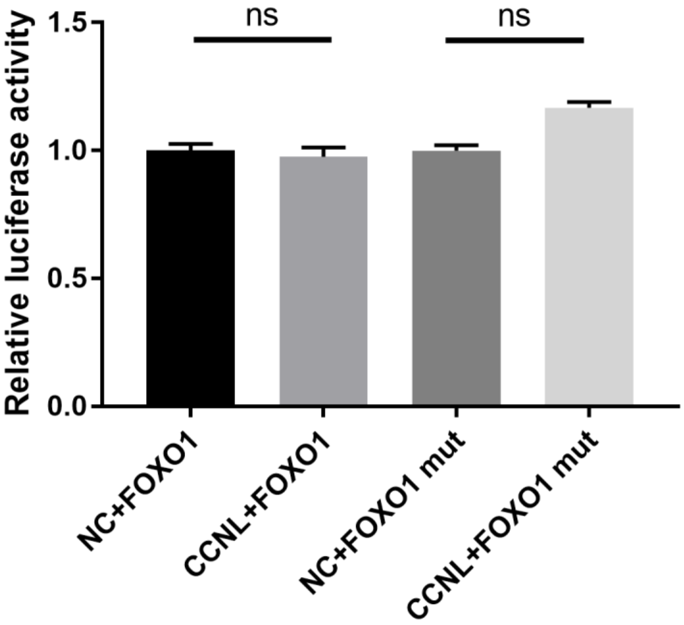


Figure S10. After co-transfection of pcDNA3.1-FOXO1, or pcDNA3.1-FOXO1-mut with pcDNA3.1, pcDNA3.1-CCNL into 293T cells. Results are shown as the relative ratio of firefly luciferase activity to Renilla luciferase activity. FOXO1: forkhead box protein O1.