LINC00665 enhances tumorigenicity of endometrial carcinoma by interacting with high mobility group AT-hook 1

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Primary research

Keywords: endometrial carcinoma, LINC00665, HMGA1

Posted Date: June 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-35106/v1

Version of Record: A version of this preprint was published on January 6th, 2021. See the published version at https://doi.org/10.1186/s12935-020-01657-2.

Abstract

Background: Endometrial carcinoma is a frequently diagnosed cancer among females. LncRNAs are reported to be associated with various cancers. Their biological roles in endometrial carcinoma progression is an emerging scientific area. LINC00665 can exert a significant role in many cancers. Up to now, its potential function in endometrial carcinoma is still poorly known.

Method: qRT-PCR was carried out to test expression of LINC00665 and HMGA1. Western blot analysis was carried out to detect protein expression of HMGA1. Cell proliferation was evaluated using Cell Counting Kit-8 (CCK-8) and EdU assay. Flow cytometry assay was used to determine cell apoptosis and cell cycle. Would healing and transwell invasion assay was carried out to test cell migration and invasion. Immunohistochemical staining and HE staining were conducted to assess Ki-67 and tumor growth respectively.

Results: Expression of LINC00665 in clinical endometrial carcinoma tissues and cells was obviously up-regulated. Loss of LINC00665 could repress endometrial carcinoma cell viability, induce cell apoptosis and blocked cell cycle in G1 phase. KLE and HHUA cell migration and invasion ability were depressed by LINC00665 shRNA. Decrease of LINC00665 suppressed endometrial carcinoma tumorigenicity in vivo. RIP assay evidenced LINC00665 directly bound with HMGA1 protein. shRNA of HMGA1 obviously restrained endometrial carcinoma cell growth and cell invasion.

Conclusions: LINC00665 might promote endometrial carcinoma progression by positively modulating HMGA1.

Background

Endometrial carcinoma is a malignant tumor among women with a leading cause of mortality worldwide [1]. Endometrioid endometrial carcinoma is a frequent subtype of endometrial cancer, which can account for about 90% endometrial carcinoma cases [2]. The primary surgery in endometrial cancer is still generally not satisfactory [3]. Nevertheless, these treatments are not effective for advanced TNM stage or distant metastases [4]. The molecular mechanism underlying endometrial cancer development remains poorly known. Therefore, it is of great significance to investigate the mechanism and identify effective therapeutic strategy for endometrial cancer.

LncRNAs are ncRNA transcripts with more than 200 nts and they are crucial modulators in various cellular processes, including cell proliferation, cell apoptosis and cell cycle progression [5, 6]. Many lncRNAs are reported to be dys-regulated in human cancers [7-9]. Besides these, the function of lncRNAs are widely described in endometrial carcinoma [10-12]. For example, lncRNA TDRG1 can enhance endometrial carcinoma tumorigenicity via binding to VEGF-A protein [13]. LncRNA H19 can modulate the expression of HOXA10 in endometrial carcinoma by sponging miR-612 [14]. LncRNA GAS5 can increase PTEN expression via repressing miR-103 in endometrial cancer [15].

In our current work, LINC00665 (ENST00000590622, NR_038278) was investigated. It was markedly elevated in endometrial cancer. The functions of LINC00665 in endometrial cancer have not been reported previously. Hence, we studied the impact of LINC00665 on the progression of endometrial cancer. Furthermore, mechanistic analysis indicated LINC00665 regulated the expression of HMGA1, thereby inducing endometrial cancer progression.

Materials And Methods

Clinical samples

10 endometrial carcinoma and 10 normal endometrial specimens were obtained from patients with surgical resection. No patients received any chemotherapy or radiotherapy before the surgery. Tissue samples were processed based on the ethical standards. We had obtained the informed consent form all the patients before enrollment. This research was approved by the Ethics Committee Beijing Friendship Hospital Affiliated to Capital Medical University.

Cell culture

RL-95-2, Ishikawa, HEC-1B, KLE and HHUA cells and hESCs and were purchased from Cell Bank of the Chinese Academy of Science (Shanghai, China). DMEM added with 100 U/mL penicillin/streptomycin and 10% FBS. A 5% CO_2 incubator at 37 °C was used to incubate the cells.

Cell transfection

shRNAs for LINC00665 were constructed by Invitrogen (Carlsbad, CA, USA). 1×10^6 cells per well were grown into six-well plates overnight and infected with the vector. The efficacy of transfection was tested using PCR. HMGA1 siRNA (RiboBio, Guangzhou, China). Lipofectamine 3000 (Invitrogen, CA, USA) was utilized to do cell transfection.

CCK-8 assay

Cell viability was assessed by CCK-8 assay. 2000 cells were seeded into 96-well plates. Absorption was tested using a CCK-8 kit (Dojindo, Japan) at different time points. OD values were evaluated at 490 nm by a spectrophotometer (BioTek, Winooski, VT, USA).

EdU assays

A Cell-Light EdU DNA Cell Proliferation Kit (KeyGEN BioTECH, China) was carried out to evaluate cell proliferation. Briefly, cells were grown into 96-well plates. Then, cells were treated with 50 μmol/L EdU for 2 hours. Then, cells were fixed using 4 % paraformaldehyde and then 1×Apollo reaction cocktail was used. Cell nuclei was stained using DAPI for 15 min. We captured the images using a fluorescence microscope (Nikon, Tokyo, Japan).

Apoptosis assays

PI and FITC-labeled annexin V (BD Biosciences, New Jersey, USA) staining was used to assess cell apoptosis. Cells were washed twice using PBS and resuspended using 100 μL 1× binding buffer, and then incubated with 5μL FITC-annexin V and PI with no light. Then, 400 μL 1× binding buffer was used. Within 1 hour, cells were exposed to flow cytometry analysis.

Cell cycle assays

Cells cultured in six-well plates, were trypsinized for cell cycle analysis. Then, after cells were washed using PBS, 70% ice-cold ethanol was added to cells at -20°C for 2 hours for a whole night. Cell cycle detection kit and flow cytometry were used to determine cell cycle.

Wound healing assay

Cells were seeded into 6-well plates. To form wounded gaps, cell layers were scratched using a 200 μL tip. Cells were cultured in FBS-free medium with 20μg/mL mitomycin C. We photographed and analyzed the wounded gaps were at 0 and 24.

Cell invasion assays

To carry out cell invasion experiment, we used matrigel Transwell Cell Culture chambers (BD Biosciences, San Jose, CA, USA). Matrix was put into the upper chamber of the chamber. 5×10^4 cells were incubated in 200 μL FBS-free culture medium and then, added to the upper chambers. Then, 600 μL culture medium containing 10% FBS was placed to the lower chambers. Then, cells were fixed using 4% paraformaldehyde. Cells in the lower chamber were stained using crystal violet and imaged under an Olympus fluorescence microscope.

qRT-PCR

TRIzol reagent (Takara, Tokyo, Japan) was used to extract total RNA. PrimeScript RT Reagent Kit (Takara, Tokyo, Japan) was employed to do reverse transcription. Then, SYBR green (Takara, Tokyo, Japan) was conducted to do qRT-PCR on Bio-Rad CFX96 system to test the expression of LINC00665 and HMGA1 mRNA. $2^{-\Delta\Delta Ct}$ method was carried out to analyze gene expression with primers listed in **Table 1**.

Western blot

Protein was separated on 10% SDS polyacrylamide gels. Afterwards, proteins were transferred onto PVDF membranes. The membranes were blocked using 5% skimmed milk. Then, primary antibodies against HMGA1 and GAPDH (1:1000, CST, Boston, MA, USA) were used for a whole night. Next, the membranes were washed using TBST and incubated with the secondary antibodies (1:2000, CST, Boston, MA, USA) for 2 hours. Finally, we visualized the protein bands using the enhanced chemiluminescence reagent.

RIP assay

RIP assay was performed using Magna RIP Immunoprecipitation Kit (Millipore, Bedford, MA, USA). Briefly, KLE cells were lysed using RIP lysis buffer. 100μL cell extract was incubated with magnetic beads conjugated to human anti-HMGA1 or normal mouse IgG.

Animal assays

Twelve 8-week-old female mice were bought from Shanghai Animal Laboratory Center. The mice were maintained in specific conditions with no pathogen. Then, mice were divided into 2 groups and injected with 5×10^6 KLE cells infected with LV-shLINC00665 or LV-NC, respectively. We measured tumor volume every week. 7 weeks later, the animals were sacrificed for future histopathological determination. Approval of this animal study was obtained from the Animal Research Ethics Committee of Beijing Friendship Hospital Affiliated to Capital Medical University.

Histology

Tumor tissues were fixed using 4 % paraformaldehyde and embedded in paraffin. H&E staining (Beijing Solarbio, Beijing, China) was carried out based on the manufacturer's protocols. To carry out immunohistochemistry analysis, Ki-67 were detected in xenograft tumor tissues. The sections were deparaffinized, hydrated, and then antigen retrieved. Primary antibody (Ki-67, 1:500, Abcam, UK) was utilized and then a solution of anti-rabbit IgG was used for 15 min. Then, a 3,3-diaminobenzidine color kit was used. We captured the pictures using a light microscope.

Statistical analysis

Data were analyzed by SPSS 20.0 (Chicago, USA) and GraphPad Prism 7 (GraphPad, CA, USA). Statistical significance between various groups was analyzing using Student's t tests or one-way ANOVA analysis. $P < 0.05$ indicated the statistical significance.

Results

Expression of LINC00665 in endometrial tissues and cells.

Real-time PCR was used and we found that LINC00665 was obviously elevated in endometrial carcinoma in comparison to normal endometrial tissues as shown in **Figure 1A**. In addition, LINC00665 was also increased in endometrial cancer cells (RL-95-2, Ishikawa, HEC-1B, KLE and HHUA cells) than in hESCs H (**Figure 1B**). These indicated LINC00665 was significantly increased in endometrial carcinoma.

LINC00665 repressed endometrial carcinoma cell proliferation.

Moreover, **Figure 2A and 2B** revealed that LINC00665 was successfully down-regulated in KLE and HHUA cells. In **Figure 2C and 2D,** we found that decrease of LINC00665 significantly depressed KLE and HHUA cell survival as proved using CCK-8 assay. Additionally, it was proved that KLE and HHUA cell proliferation was restrained by the down-regulation of LINC0066 as indicated in **Figure 2E and 2F**.

Knockdown of LINC00665 triggered endometrial carcinoma cell apoptosis and depressed cell cycle progression.

Then, in **Figure 3A and 3B,** KLE and HHUA cell apoptosis was induced by loss of LINC00665. Meanwhile, we confirmed that LINC00665 greatly blocked KLE and HHUA cell cycle progression at G1 phase as exhibited in **Figure 3C and 3D**.

Down-regulation of LINC00665 restrained endometrial carcinoma cell migration and invasion capacity.

Moreover, we evaluated the migration and invasion capability of KLE and HHUA cells by wound-healing assays and transwell invasion assay. In **Figure 4A and 4B,** shRNA of LINC00665 significantly reduced the wound closure in A KLE and HHUA cells. Then, transwell invasion assay indicated that decrease of LINC00665 greatly retarded AN3CA and HEC1-A cell invasion ability in **Figure 4C and 4D**.

Silence of LINC00665 inhibited endometrial carcinoma cell growth in vivo.

To explore the effect of LINC00665 on the endometrial carcinoma cells tumorigenesis in vivo, KLE cells silencing LINC00665 were transplanted into nude mice subcutaneously. As displayed in **Figure 5A**, LINC00665 expression in grafted tumor tissues was evidenced. shRNA of LINC00665 was able to reduce tumor growth and tumor volume in **Figure 5B and 5C**. Furthermore, IHC analysis of xenografted tumors displayed LINC00665 shRNA significantly reduced cell growth and cell proliferation rate (Ki-67) as shown in **Figure 5D and 5E.** Therefore, the data manifested that lack of LINC00665 suppressed endometrial carcinoma cell tumorigenesis in vivo.

LINC00665 co-immunoprecipitated with HMGA1 protein.

Subsequently, in this study, by using bioinformatical prediction, many miRNAs contained binding sites between LINC00665 and HMGA1 were found. RIP assays were used to test whether HMGA1 protein interacted with LINC00665. RNA obtained from RIP assay using a HMGA1 antibody was exposed to qPCR analysis and an enrichment of LINC00665 was demonstrated (**Figure 6A**). Loss of LINC00665 significantly repressed HMGA1 mRNA and protein expression in **Figure 6B and 6C**. KLE cell viability and invasion was markedly reduced by HMGA1 siRNA in **Figure 6D and 6E**.

Discussion

In recent years, lncRNAs are identified to be closely associated with many cancers [16, 17]. In addition, increasing studies have displayed ncRNAs are differentially expressed in endometrial cancer [18]. Numerous lncRNAs are altered in cancers and they have been linked to the progression of tumor cells. LncRNAs may represent candidate biomarkers for the diagnosis or treatment of cancers. LINC00665 can induce resistance to gefitinib via recruiting EZH2 and the activation of PI3K/AKT signaling in lung cancer [19]. LINC00665 can promote tumorigenesis of gastric cancer via regulating miR-149-3p and RNF2 [20]. In addition, LINC00665 contributes to breast cancer development via modulating miR-379-5p and LIN28B

[21]. In hepatocellular carcinoma, LINC00665 modulates viability, apoptosis, and autophagy through sponging miR-186-5p and regulating MAP4K3 [22].

In our current research, we found LINC00665 was greatly up-regulated in endometrial cancer tissues and cells compared to the corresponding controls. Therefore, endometrial cancer can serve as an important oncogene in the progression of endometrial cancer. Loss of LINC00665 repressed proliferation, G1-S progression, migration and invasion capacity and induced apoptosis in KLE and HHUA cells. Moreover, a tumorigenesis assay proved that silence of LINC00665 restrained endometrial tumor growth. This was consistent with the biological roles of LINC00665 in other cancers. Then, we explored the mechanism by which LINC00665 enhanced endometrial carcinoma tumorigenicity.

LncRNAs can be involved in various pathological processes, including various cancers. LncRNAs can interact with both nucleic acids and proteins directly [23]. LncRNAs exert significant roles in regulating protein expression [24–26]. In this study, we found that many miRNAs contained binding sites with both LINC00665 and HMGA1, which indicated that there might be an interaction between them. Through carry outing RIP assay, we observed that LINC00665 interacted with the HMGA1 protein. Additionally, silencing HMGA1 in KLE cells reduced the proliferation and invasion capacity. These results suggested LINC00665 might regulate endometrial carcinoma by interacting with HMGA1.

Studies have shown that the silence of HMGA1 represses cancer developmenyt [27, 28]. For example, HMGA1 can promote breast cancer angiogenesis via supporting the transcriptional activity of FOXM1 [29]. HMGA1 exacerbates cervical cancer growth via regulating cell cycle and migration/invasion capacity through targeting miR-221/222 [30]. HMGA1 is correlated with breast cancer malignant status [31]. In addition, HMGA1 can facilitate endometrial cancer progression by regulating Wnt/β-catenin signaling [32]. Thus, we suggested that LINC00665 may induce tumorigenesis by binding with HMGA1 in endometrial carcinoma.

Conclusion

This study demonstrated LINC00665 promoted endometrial carcinoma tumorigenesis and progression through interacting with HMGA1. These data could pave a new way to develop novel diagnostic and treatment strategy for endometrial carcinoma.

Table

Table 1. Primers used for real-time PCR

Genes	Forward (5’-3’)	Reverse (5’-3’)
GAPDH	CAAGGTCATCCATGACAACTTTG	GTCCACCACCCTGTTGCTGTAG
HMGA1	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
LINC00665	GGTGCAAAGTGGGAAGTGTG	CGGTGGACGGATGAGAAACG

Declarations

Ethics approval and consent to participate

We had obtained the informed consent form all the patients before enrollment. This research was approved by the Ethics Committee Beijing Friendship Hospital Affiliated to Capital Medical University, and the approval of this animal study was obtained from the Animal Research Ethics Committee of Beijing Friendship Hospital Affiliated to Capital Medical University.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Beijing Natural Science Foundation [Grant No. 7184211]; National Natural Science Foundation of China [Grant No. 81801402]; Beijing Natural Science Foundation Program and Scientific Research Key Program of Beijing Municipal Commission of Education [Grant No. KM201610025023], and the Beijing key clinical specialty project.

Authors’ contributions

Yun Liu designed and supervised the study; **Yun Liu** and **Yue Chang** performed the experiments; **Yixuan Cai** collected and did the analysis; **Yue Chang** prepared the manuscript; **Yun Liu** revised the manuscript; All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Felix AS, Scott McMeekin D, Mutch D, Walker JL, Creasman WT, Cohn DE, Ali S, Moore RG, Downs LS, Ioffe OB, Park KJ, Sherman ME and Brinton LA. Associations between etiologic factors and mortality after endometrial cancer diagnosis: the NRG Oncology/Gynecologic Oncology Group 210 trial. Gynecologic oncology. 2015; 139(1):70-76.
2. Morice P, Leary A, Creutzberg C, Abu-Rustum N and Darai E. Endometrial cancer. Lancet. 2016; 387(10023):1094-1108.
3. Kupets R, Le T, Sogc-Goc-Scc P, Practice Guidelines C and Special C. The role of adjuvant therapy in endometrial cancer. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC. 2013; 35(4):375-376.
4. Humber C, Tierney J, Symonds P, Collingwood M, Kirwan J, Williams C and Green J. Chemotherapy for advanced, recurrent or metastatic endometrial carcinoma. The Cochrane database of systematic reviews. 2005; (3):CD003915.
5. Bhan A, Soleimani M and Mandal SS. Long Noncoding RNA and Cancer: A New Paradigm. Cancer research. 2017; 77(15):3965-3981.
6. St Laurent G, Wahlestedt C and Kapranov P. The Landscape of long noncoding RNA classification. Trends in genetics : TIG. 2015; 31(5):239-251.
7. Sanchez Calle A, Kawamura Y, Yamamoto Y, Takeshita F and Ochiya T. Emerging roles of long non-coding RNA in cancer. Cancer science. 2018; 109(7):2093-2100.
8. Schmitt AM and Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer cell. 2016; 29(4):452-463.
9. Kondo Y, Shinjo K and Katsushima K. Long non-coding RNAs as an epigenetic regulator in human cancers. Cancer science. 2017; 108(10):1927-1933.
10. Vallone C, Rigon G, Gulia C, Baffa A, Votino R, Morosetti G, Zaami S, Briganti V, Catania F, Gaffi M, Nucciotti R, Costantini FM, Piergentili R, et al. Non-Coding RNAs and Endometrial Cancer. Genes. 2018; 9(4).
11. Liu H, Wan J and Chu J. Long non-coding RNAs and endometrial cancer. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2019; 119:109396.
12. Ferlita A, Battaglia R, Andronico F, Caruso S, Cianci A, Purrello M and Pietro CD. Non-Coding RNAs in Endometrial Physiopathology. International journal of molecular sciences. 2018; 19(7).
13. Chen S, Wang LL, Sun KX, Liu Y, Guan X, Zong ZH and Zhao Y. LncRNA TDRG1 enhances tumorigenicity in endometrial carcinoma by binding and targeting VEGF-A protein. Biochimica et biophysica acta Molecular basis of disease. 2018; 1864(9 Pt B):3013-3021.
14. Zhang L, Wang DL and Yu P. LncRNA H19 regulates the expression of its target gene HOXA10 in endometrial carcinoma through competing with miR-612. European review for medical and pharmacological sciences. 2018; 22(15):4820-4827.

15. Guo C, Song WQ, Sun P, Jin L and Dai HY. LncRNA-GAS5 induces PTEN expression through inhibiting miR-103 in endometrial cancer cells. Journal of biomedical science. 2015; 22:100.
16. Fang Y and Fullwood MJ. Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. Genomics, proteomics & bioinformatics. 2016; 14(1):42-54.
17. Bolha L, Ravnik-Glavac M and Glavac D. Long Noncoding RNAs as Biomarkers in Cancer. Disease markers. 2017; 2017:7243968.
18. Smolle MA, Bullock MD, Ling H, Pichler M and Haybaeck J. Long Non-Coding RNAs in Endometrial Carcinoma. International journal of molecular sciences. 2015; 16(11):26463-26472.
19. Liu X, Lu X, Zhen F, Jin S, Yu T, Zhu Q, Wang W, Xu K, Yao J and Guo R. LINC00665 Induces Acquired Resistance to Gefitinib through Recruiting EZH2 and Activating PI3K/AKT Pathway in NSCLC. Molecular therapy Nucleic acids. 2019; 16:155-161.
20. Qi H, Xiao Z and Wang Y. Long non-coding RNA LINC00665 gastric cancer tumorigenesis by regulation miR-149-3p/RNF2 axis. OncoTargets and therapy. 2019; 12:6981-6990.
21. Ji W, Diao YL, Qiu YR, Ge J, Cao XC and Yu Y. LINC00665 promotes breast cancer progression through regulation of the miR-379-5p/LIN28B axis. Cell death & disease. 2020; 11(1):16.
22. Shan Y and Li P. Long Intergenic Non-Protein Coding RNA 665 Regulates Viability, Apoptosis, and Autophagy via the MiR-186-5p/MAP4K3 Axis in Hepatocellular Carcinoma. Yonsei medical journal. 2019; 60(9):842-853.
23. Zhao XY and Lin JD. Long Noncoding RNAs: A New Regulatory Code in Metabolic Control. Trends in biochemical sciences. 2015; 40(10):586-596.
24. Wang D, Wang D, Wang N, Long Z and Ren X. Long Non-Coding RNA BANCR Promotes Endometrial Cancer Cell Proliferation and Invasion by Regulating MMP2 and MMP1 via ERK/MAPK Signaling Pathway. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2016; 40(3-4):644-656.
25. Guo Q, Qian Z, Yan D, Li L and Huang L. LncRNA-MEG3 inhibits cell proliferation of endometrial carcinoma by repressing Notch signaling. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2016; 82:589-594.
26. Zhao X, Wei X, Zhao L, Shi L, Cheng J, Kang S, Zhang H, Zhang J, Li L, Zhang H and Zhao W. The rs6983267 SNP and long non-coding RNA CARLo-5 are associated with endometrial carcinoma. Environmental and molecular mutagenesis. 2016; 57(7):508-515.
27. Sumter TF, Xian L, Huso T, Koo M, Chang YT, Almasri TN, Chia L, Inglis C, Reid D and Resar LM. The High Mobility Group A1 (HMGA1) Transcriptome in Cancer and Development. Current molecular medicine. 2016; 16(4):353-393.
28. De Martino M, Forzati F, Arra C, Fusco A and Esposito F. HMGA1-pseudogenes and cancer. Oncotarget. 2016; 7(19):28724-28735.
29. Zanin R, Pegoraro S, Ros G, Ciani Y, Piazza S, Bossi F, Bulla R, Zennaro C, Tonon F, Lazarevic D, Stupka E, Sgarra R and Manfioletti G. HMGA1 promotes breast cancer angiogenesis supporting the

stability, nuclear localization and transcriptional activity of FOXM1. Journal of experimental & clinical cancer research : CR. 2019; 38(1):313.

30. Fu F, Wang T, Wu Z, Feng Y, Wang W, Zhou S, Ma X and Wang S. HMGA1 exacerbates tumor growth through regulating the cell cycle and accelerates migration/invasion via targeting miR-221/222 in cervical cancer. Cell death & disease. 2018; 9(6):594.
31. Huang R, Huang D, Dai W and Yang F. Overexpression of HMGA1 correlates with the malignant status and prognosis of breast cancer. Molecular and cellular biochemistry. 2015; 404(1-2):251-257.
32. Han X, Cao Y, Wang K and Zhu G. HMGA1 facilitates tumor progression through regulating Wnt/beta-catenin pathway in endometrial cancer. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2016; 82:312-318.

Figures

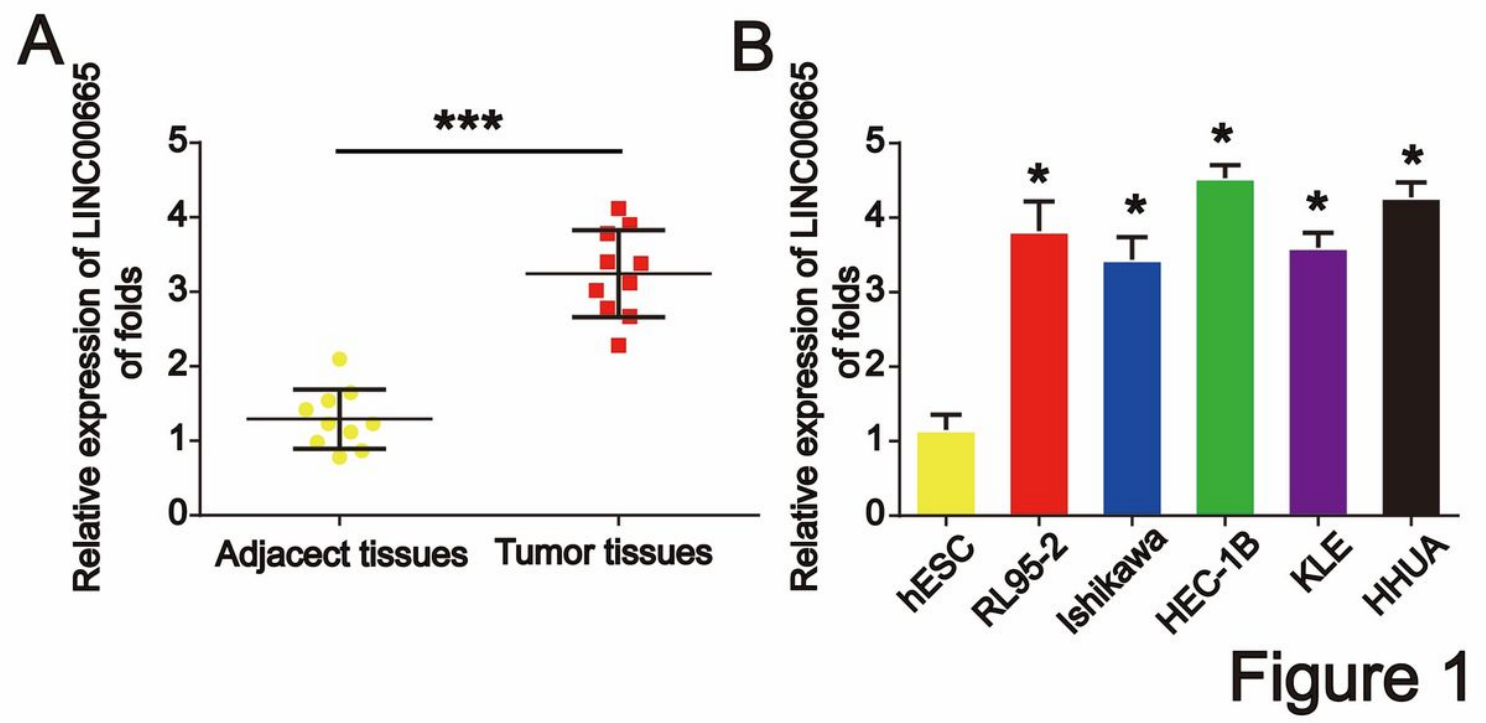

Figure 1

LINC00665 expression was up-regulated in endometrial cancer tissues and cell lines. (A) LINC00665 was highly expressed in endometrial cancer tissue than in normal ovarian tissues (ten pairs). (B) LINC00665 was highly expressed in endometrial cancer cellines (RL-95-2, Ishikawa, HEC-1B, KLE and HHUA cells) than in hESCs. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05, ***P<0.001.

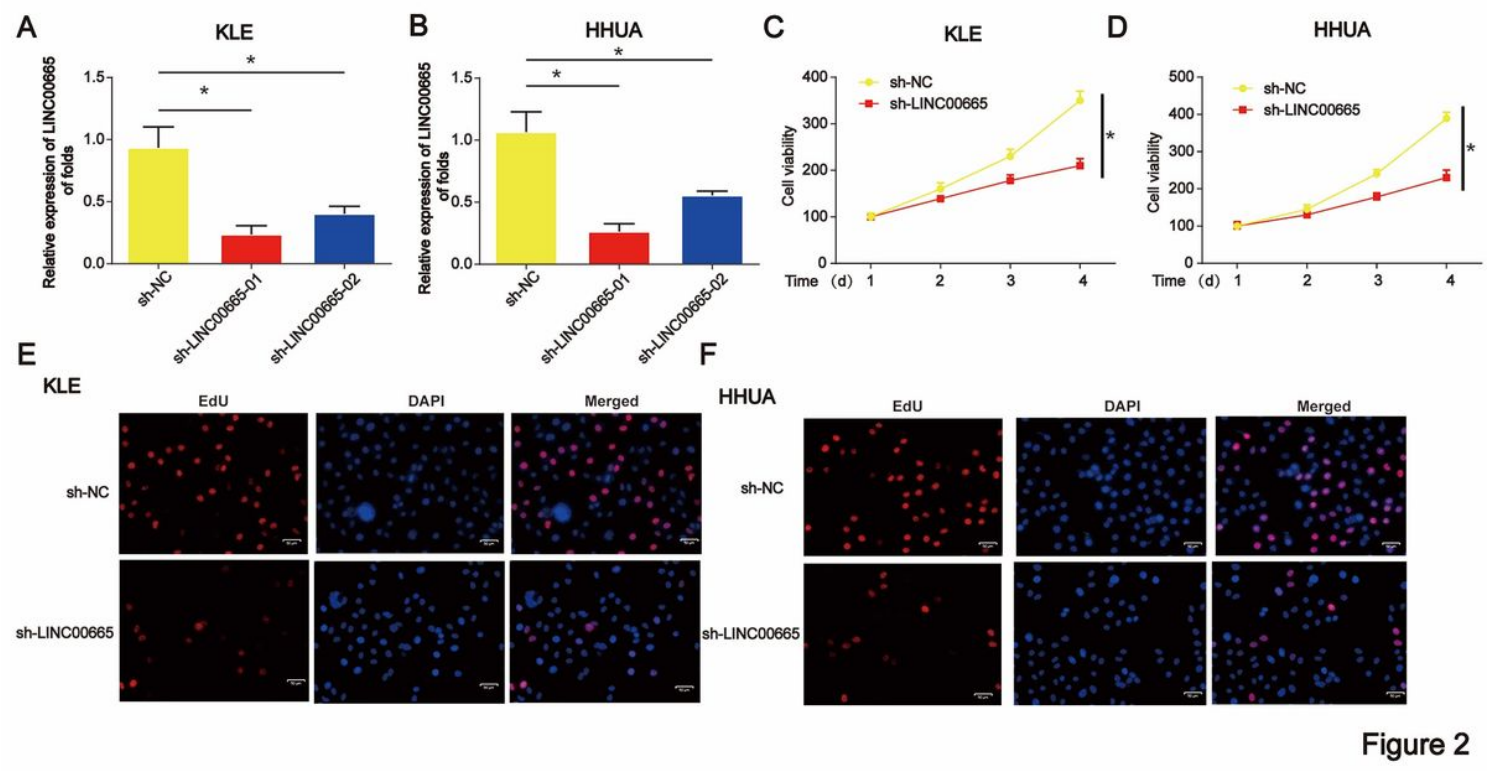

Figure 2

Figure 2

Loss of LINC00665 repressed proliferation and induced apoptosis in endometrial carcinoma cells. (A and B) LINC00665 expression in KLE and HHUA cells. Cells were infected with LV-shLINC00665 or LV-NC for 48 hours. (C and D) Effects of LINC00665 on KLE and HHUA cells survival. CCK-8 assay was conducted to detect cell viability. (E and F) Effects of LINC00665 on KLE and HHUA cell proliferation. EdU assay was performed to test cell proliferation. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

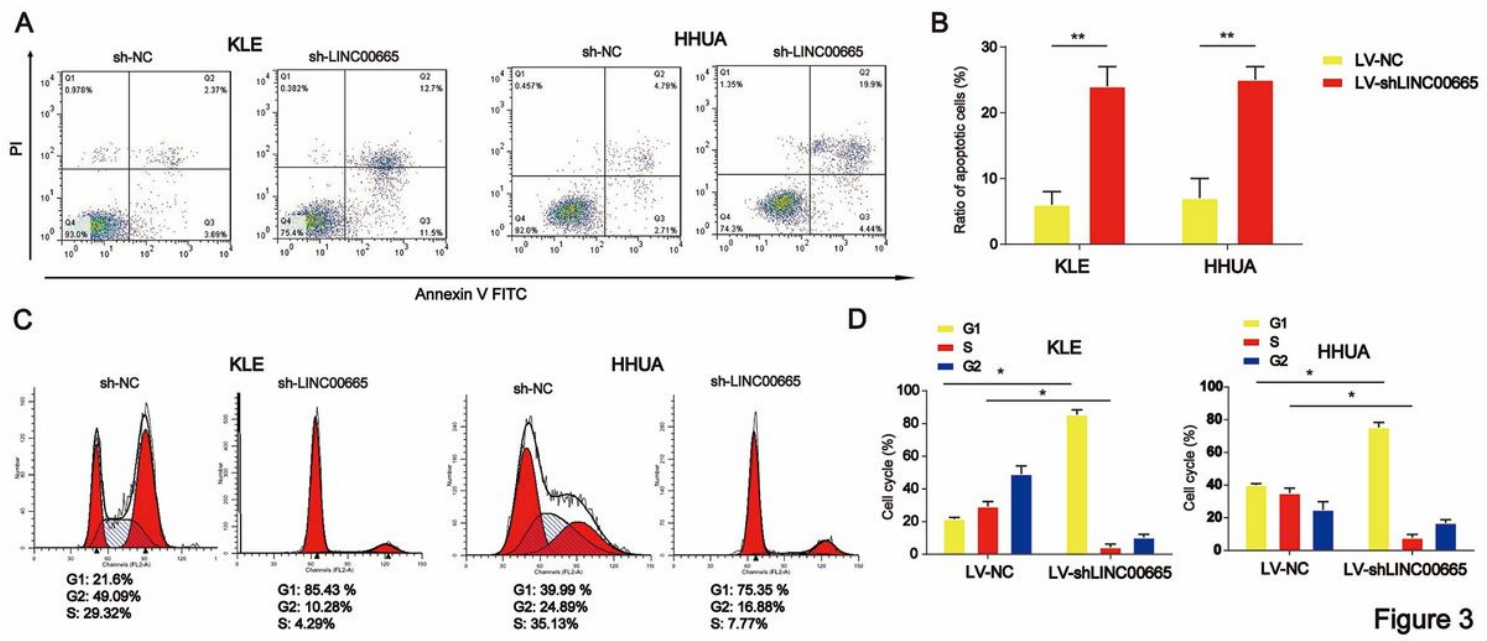

Figure 3

Figure 3

Effects of LINC00665 on endometrial carcinoma cell apoptosis and cell cycle. (A and B) Effects of LINC00665 on KLE and HHUA cell apoptosis. Flow cytometry was used to detect cell apoptosis. (C and D) Effects of LINC00665 on KLE and HHUA cell cycle. Flow cytometry was conducted to detect cell cycle. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05, ** P<0.01.

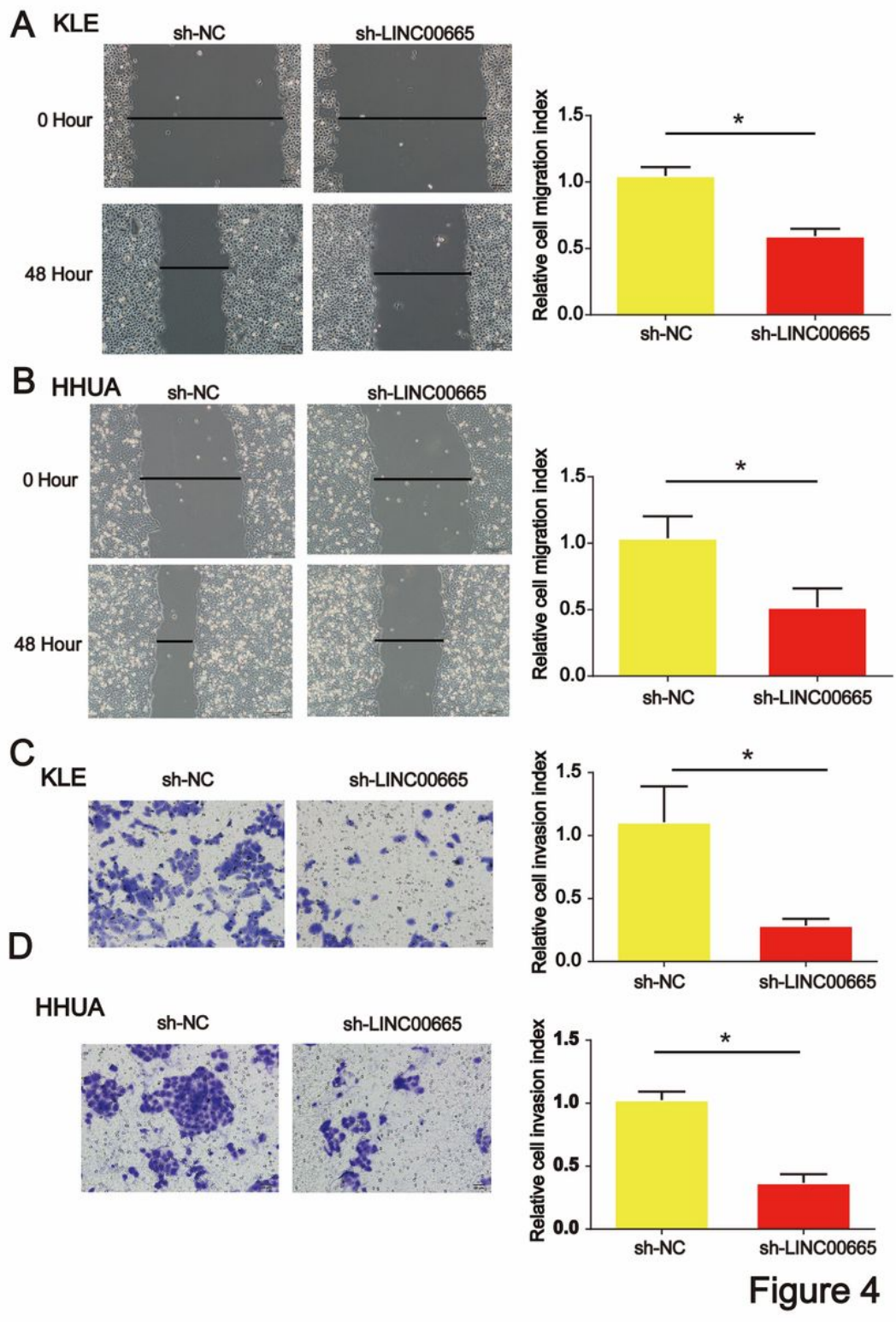

Figure 4

Effects of LINC00665 on endometrial carcinoma cell migration and invasion. (A and B) Effects of LINC00665 on KLE and HHUA cell migration. Would healing assay was performed to detect cell migration capacity. (C and D) Effects of LINC00665 on KLE and HHUA cell invasion. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

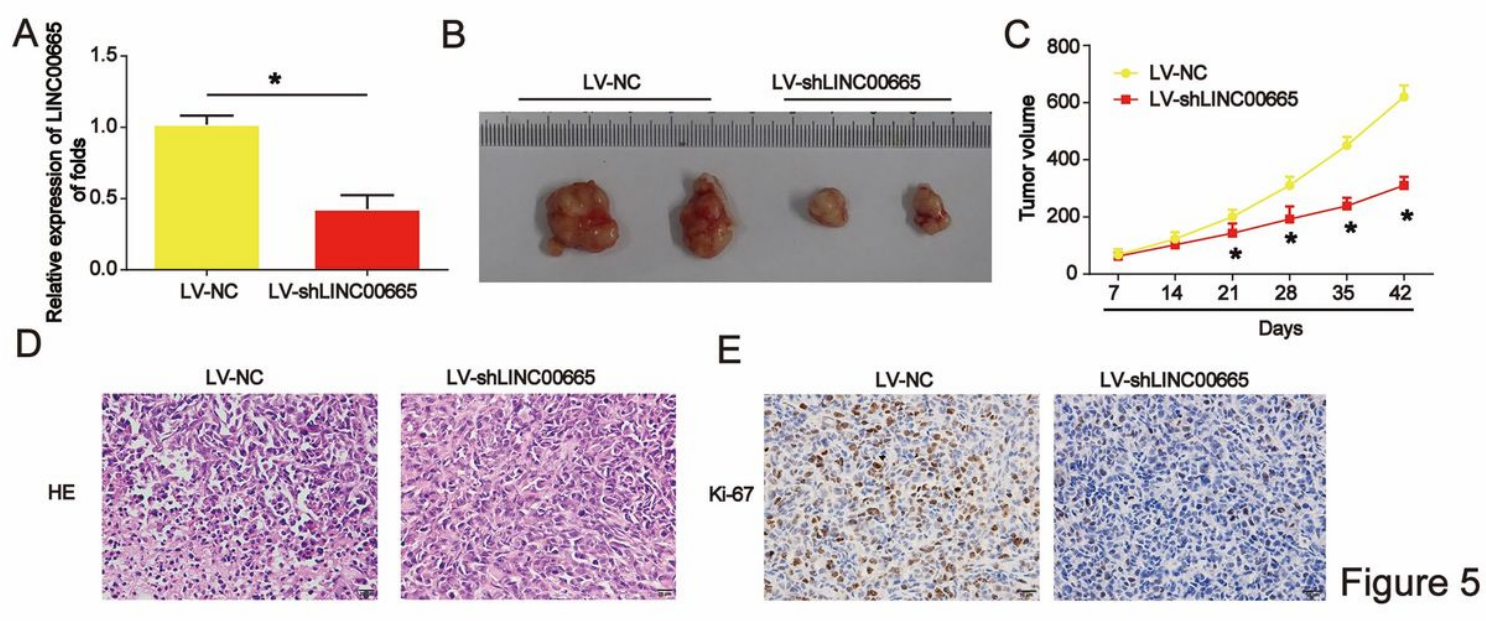

Figure 5

Loss of LINC00665 repressed endometrial carcinoma in vivo. Twelve 8-week old female BALB/c nude mice were injected with KLE cells infected with LV-NC (six mice) or LV-shLINC00665(six mice). (A) Expression of LINC00665 in the tumor tissues. (B) Tumors isolated from the two groups. (C) Tumor volume. (D and E) IHC staining of Ki-67 in tumor tissues. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

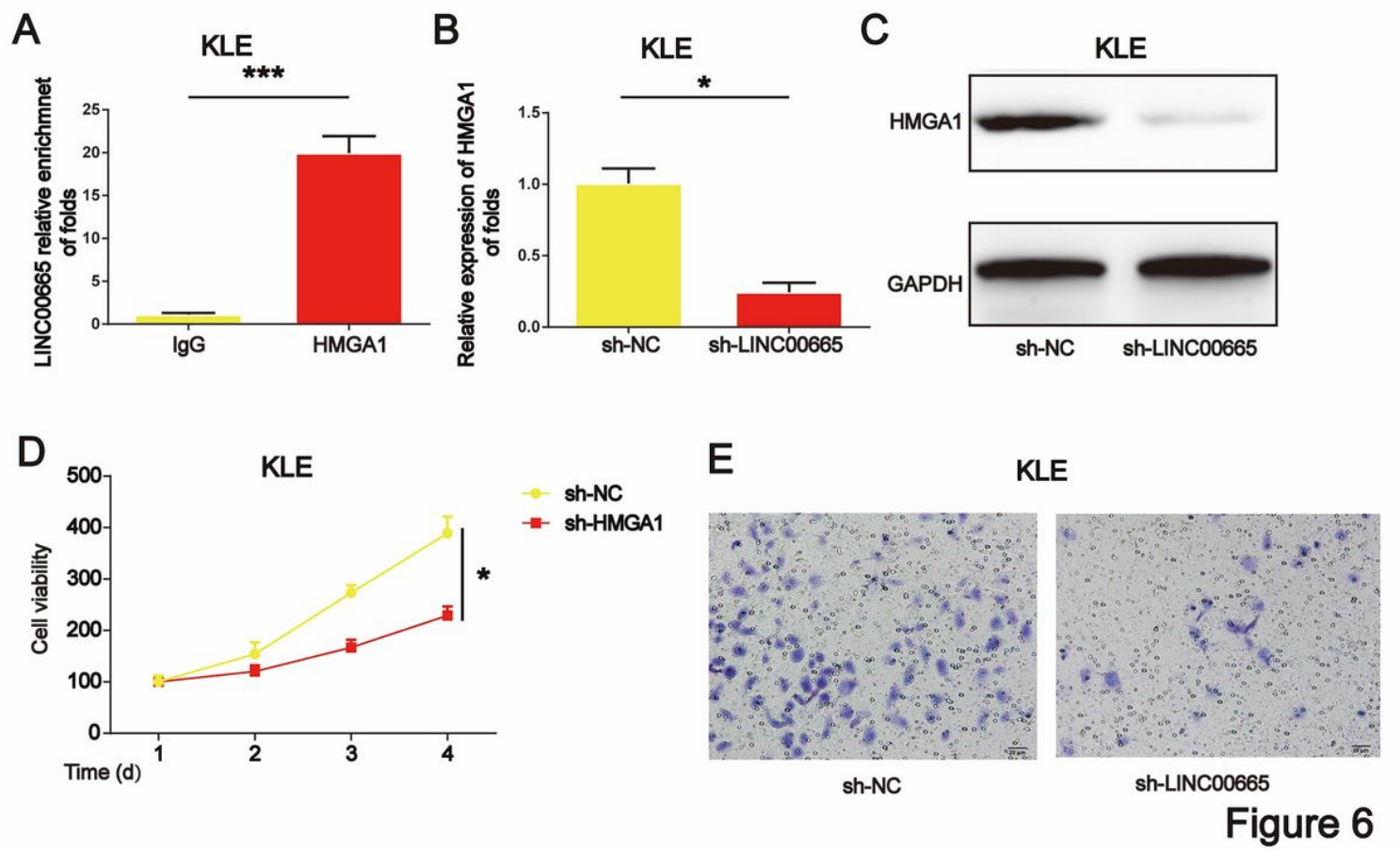

Figure 6

HGMA1 with LINC00665. (A) HMGA1 protein interacted with LINC00665. (B and C) HMGA1 mRNA and protein expression in KLE cells. (D) KLE cell proliferation. Cells were infected with HMGA1 siRNA. (E) KLE cell invasion. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05, ***P<0.001.