# Additional file 1

**Oct4 transcriptionally regulates the expression of long non-coding RNAs *NEAT1* and *MALAT1* to promote lung cancer progression**

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# Supplementary methods

### Anchorage-independent growth assay

Anchorage-independent growth assay was performed by seeding A549 and CL1-0 at $3 \times 10^3$ cells/well in 0.4% bactoagar on a bottom layer of solidified 0.6% bactoagar in 6-well plates. After 12~17 days for A549 cells and 7~10 days for CL1-0 cells, colonies consisted of > 30 cells were counted.

### Tumor-sphere formation assay

Cells were expanded as spheres in a 10-cm ultra-low adhesion culture dish (Corning Inc., Corning, NY, USA) containing DMEM/F-12 with N2 supplement (Invitrogen), 20 ng/ml epithelial growth factor (EGF), and 20 ng/ml basic fibroblast growth factor (bFGF; PeproTech Inc., Rocky Hill, NJ, USA), referred to as stem cell medium. Tumor spheres consisting of > 30 cells were counted and expressed as the means ± SEM of triplicate within the same experiment.

### Tumor formation assay

5-6-week-old BALB/c nude female mice were subcutaneously implanted with varying number ($1 \times 10^2$, $1 \times 10^3$, or $5 \times 10^3$) of vector control (vector) or Oct4-stably expressing A549 cells (Oct4#1). Vector and Oct4#1 cells in 50 μl Hanks' balanced salt solution (HBSS) were mixed with 50 μl matrigel (2.5 mg/ml, Sigma-Aldrich, St. Louis, MO, USA), and then subcutaneously injected into the flanks of mice. The incidence of tumor formation was monitored within 8 weeks after implantation.

**Western blot analysis**

Samples containing equal amounts of protein (50 μg) were separated on an 8% sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto Immobilon-P membranes (Millipore Co., Billerica, MA, USA). Immunoblotting was performed by incubating membranes with a 1:1000 dilution of anti-Oct4 antibody (Abcam, Cambridge, UK; Cat. ab-19857) overnight. GAPDH expression was used as internal control. Proteins expression was detected with indicated secondary antibody for 1 h at room temperature.

## Supplementary tables

**Table S1.** The plasmids and their characteristics used in the current study.

| Plasmid | Target | Insert (bp) | Function | Source |
|---|---|---|---|---|
| pPyCAGIP-vector | --[c] | 0 | Vector control | From Dr. Ying Jin[d] |
| pPyCAGIP-Oct4 | Wild-type Oct4 | 1,083 | Overexpression | From Dr. Ying Jin[d] |
| pMIR-REPORT-vector | --[c] | 0 | Vector control | From Dr. Tetsuro Hirose[e] |
| pMIR-REOPRT-NEAT1 | *NEAT1* | 3,756 | Overexpression | From Dr. Tetsuro Hirose[e] |
| pCMV-vector | --[c] | 0 | Vector control | From Dr. Kannanganattu V. Prasanth[f] |
| pCMV-MALAT1 | *MALAT1* | 7,161 | Overexpression | From Dr. Kannanganattu V. Prasanth[f] |
| pGL3-Basic vector | --[c] | 0 | Vector control | Promega |
| pGL3-NEAT1 WT[a] | *NEAT1* promoter (WT) | 4,422 | Promoter activity assay | Homemade |
| pGL3-NEAT1 Mut[b] | *NEAT1* promoter (Mut) | 4,422 | Promoter activity assay | Homemade |
| pGL3-MALAT1-pro | *MALAT1* promoter-luc | 468 | Enhancer activity assay | Homemade |
| pGL3-MALAT1-enh WT[a] | *MALAT* promoter-luc-enhancer (WT) | 435 | Enhancer activity assay | Homemade |
| pGL3-MALAT1-enh Mut[b] | *MALAT* promoter-luc-enhancer (Mut) | 435 | Enhancer activity assay | Homemade |
| pGL3-UCA1-pro | *UCA1* promoter-luc | 519 | Enhancer activity assay | Homemade |
| pGL3-UCA1-enh WT[a] | *UCA1* promoter-luc-enhancer (WT) | 512 | Enhancer activity assay | Homemade |
| pGL3-UCA1-enh Mut[b] | *UCA1* promoter-luc-enhancer (Mut) | 512 | Enhancer activity assay | Homemade |

[a] WT: wild-type Oct4 binding sites within *NEAT1* promoter, *MALAT1* enhancer and *UCA1* enhancer

[b] Mut: mutated Oct4 binding sites within *NEAT1* promoter, *MALAT1* enhancer and *UCA1* enhancer

[c] Not applicable.

[d] Plasmid was kindly provided by Dr. Ying Jin at Shanghai Stem Cell Institute, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

[e] Plasmid was kindly provided by Dr. Tetsuro Hirose at Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan.

[f] Plasmid was kindly provided by Dr. Kannanganattu V. Prasanth at Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, USA.

**Table S2.** The ChIP-PCR primers used in the current study.

| Gene | Primer | Sequences (5' → 3') | Application[a] | PCR size (bp) | Tm (°C) |
|---|---|---|---|---|---|
| *BACE1AS* | Forward | TGG TGG GTG TTG ACT GTG ACA | ChIP-PCR | 150 | 54 |
| | Reverse | AAA CTC ACC TGC CGA CAA GCT | | | |
| *IGF2AS* | Forward | CTC AAT TGT TTT GTG AAG GGA AAA | ChIP-PCR | 157 | 50 |
| | Reverse | AAT GTA CAT ATG GAC CCC CAA GTC | | | |
| *MALAT1* | Forward | CCT GGC CAC AGT TGC GTA T | ChIP-PCR | 150 | 61 |
| | Reverse | GTG AAC CGA GAT CGC AAC ACT | | | |
| *NEAT1* | Forward | AGG ACA GTT GGG AAG GAG GAA | ChIP-PCR | 188 | 65 |
| | Reverse | GTG CAC TCT CAG CCA CAC GTT | | | |
| *SRA* | Forward | GGG CAC AGT CCT GGA ATC AG | ChIP-PCR | 150 | 61 |
| | Reverse | AGT AGG GCA AGC GTT TTT ATC CT | | | |
| *TUG1* | Forward | ACC ACT TTG GCA GAG CCT TTT | ChIP-PCR | 200 | 65 |
| | Reverse | TGA ATG GGA ACA AGC ACA CAA | | | |
| *UCA1* | Forward | TGC TTC TGC TTC ATC CCA GTA G | ChIP-PCR | 202 | 61 |
| | Reverse | CCT GTA GCG GAG ACA GAG ACA GT | | | |
| *GAS5* | Forward | AAT GAT ATG TAA GAA AAG GAG GCT ATA GG | ChIP-PCR | 123 | 54 |
| | Reverse | GAG GCT TGG GAA ATA GTG GTA GTA GA | | | |

[a] ChIP-PCR: Chromatin immunoprecipitation coupled with polymerase chain reaction.

**Table S3.** The cDNA primers used in the current study.

| Gene | Primer | Sequences (5'→ 3') | Application[a] | PCR size (bp) | Tm (°C) |
|---|---|---|---|---|---|
| *BACE1AS* | Forward | GAA GGG TCT AAG TGC AGA CAT CTT | qRT-PCR | 61 | 60 |
| | Reverse | AGG GAG GCG GTG AGA GT | | | |
| *GAPDH* | Forward | GAG TCA ACG GAT TTG GTC GT | qRT-PCR | 238 | 60 |
| | Reverse | TTG ATT TTG GAG GGA TCT CG | | | |
| *IGF2AS* | Forward | AGC TCT GCT TGA CGA GGC CA | qRT-PCR | 119 | 60 |
| | Reverse | TCT GTT GCA CCC TGG ACC CA | | | |
| *MALAT1* | Forward | TCTCCCCACAAGCAACTTCT | qRT-PCR | 150 | 60 |
| | Reverse | ACCTCGACACCATCGTTACC | | | |
| *NEAT1* | Forward | TCG GGT ATG CTG TTG TGA AA | qRT-PCR | 95 | 60 |
| | Reverse | TGA CGT AAC AGA ATT AGT TCT TAC CA | | | |
| *Oct4* | Forward | CGA AAG AGA AAG CGA ACC AG | qRT-PCR | 157 | 60 |
| | Reverse | GCC GGT TAC AGA ACC ACA CT | | | |
| *SRA* | Forward | AGG ATG GAT CCC CCA GAG T | qRT-PCR | 87 | 60 |
| | Reverse | TGG GAG CCT TAC TTG AAG GAG | | | |
| *TUG1* | Forward | TAG CAG TTC CCC AAT CCT TG | qRT-PCR | 116 | 60 |
| | Reverse | CAC AAA TTC CCA TCA TTC CC | | | |
| *UCA1* | Forward | CTT CTG CAT AGG ATC TGC AAT CAG | qRT-PCR | 136 | 60 |
| | Reverse | TTT TGT CCC CAT TTT CCA TCA TAC G | | | |
| *GAS5* | Forward | AGC TGG AAG TTG AAA TGG | qRT-PCR | 149 | 60 |
| | Reverse | CAA GCC GAC TCT CCA TAC C | | | |

[a] qRT-PCR: Quantitative reverse-transcriptase polymerase chain reaction

**Table S4.** The construction primers of promoters and enhancers used in the current study.

| Gene[a] | Primer | Sequences (5' → 3') | Application[b] | PCR size (bp) | Tm (°C) |
|---|---|---|---|---|---|
| *NEAT1* promoter WT | Forward | AAG GTA CCA GGA CAG TTG GGA AGG AGG AAG | Promoter construct PCR | 4,422 | 61 |
| | Reverse | AGC TAG CCT TCC TCC CCC ACA ACT ACA CC | | | |
| *NEAT1* promoter-1[c] Mut | Forward | AAG GTA CCA GGA CAG TTG GGA AGG AGG AAG GGC CCG T | Site-directed mutagenesis PCR | 118 | 54 |
| | Reverse | GCC CTG GTT TAC GGA TCC GAG GTG AGG TGA | | | |
| *NEAT1* promoter-2 Mut | Forward | TCA CCT CAC CTC GGA TCC GTA AAC CAG GGC | Site-directed mutagenesis PCR | 4,334 | 54 |
| | Reverse | AGC TAG CCT TCC TCC CCC ACA ACT ACA CCC AGG CGC | | | |
| *MALAT1* promoter | Forward | AAG GTA CCA GAG CCG GTT AGA ACC AGT G | Promoter construct PCR | 468 | 61 |
| | Reverse | CTA AGC TTT CCT CCA AAC CCC | | | |
| *MALAT*1 enhancer WT | Forward | TGG ATC CGC CTT CCA AAG TGC TGA GAT | Enhancer construct PCR | 435 | 54 |
| | Reverse | CCC GTC GAC CCA ACA ACA ATG GCA AGA AA | | | |
| *MALAT*1 enhancer Mut | Forward | TGA AGT GTC TGT TCA TCC CGG TTG TGT TTT TCT CTT | Site-directed mutagenesis PCR | 5,721 | 54 |
| | Reverse | AAG AGA AAA ACA CAA CCG GGA TGA ACA GAC ACT TCA | | | |
| *UCA1* promoter | Forward | AAG GTA CCA GAA ATG ACC CAG GAG CTG A | Promoter construct PCR | 519 | 65 |
| | Reverse | CTA AGC TTT CAG CGA AGG GAG ATA GGA G | | | |
| *UCA1* enhancer WT | Forward | TGG ATC CTC ATC CCA GTA GGA GGC TCT | Enhancer construct PCR | 512 | 65 |
| | Reverse | CCC GTC GAC ACG TGT GTG TGT GTT GGT TTT | | | |
| *UCA1* enhancer Mut | Forward | ACT GAG CCC AAA TCG CCT TAT TTA TCC CTC | Site-directed mutagenesis PCR | 5,849 | 50 |
| | Reverse | GAG GGA TAA ATA AGG CGA TTT GGG CTC AGT | | | |

[a] WT: Wild-type Oct4 binding site; Mut: Mutant Oct4 binding site

[b] PCR: polymerase chain reaction

[c] The PCR fragments promoter-1 and promoter-2 were ligated and then used to construct *NEAT1* promoter mutant vector.

## Supplementary figure and figure legend

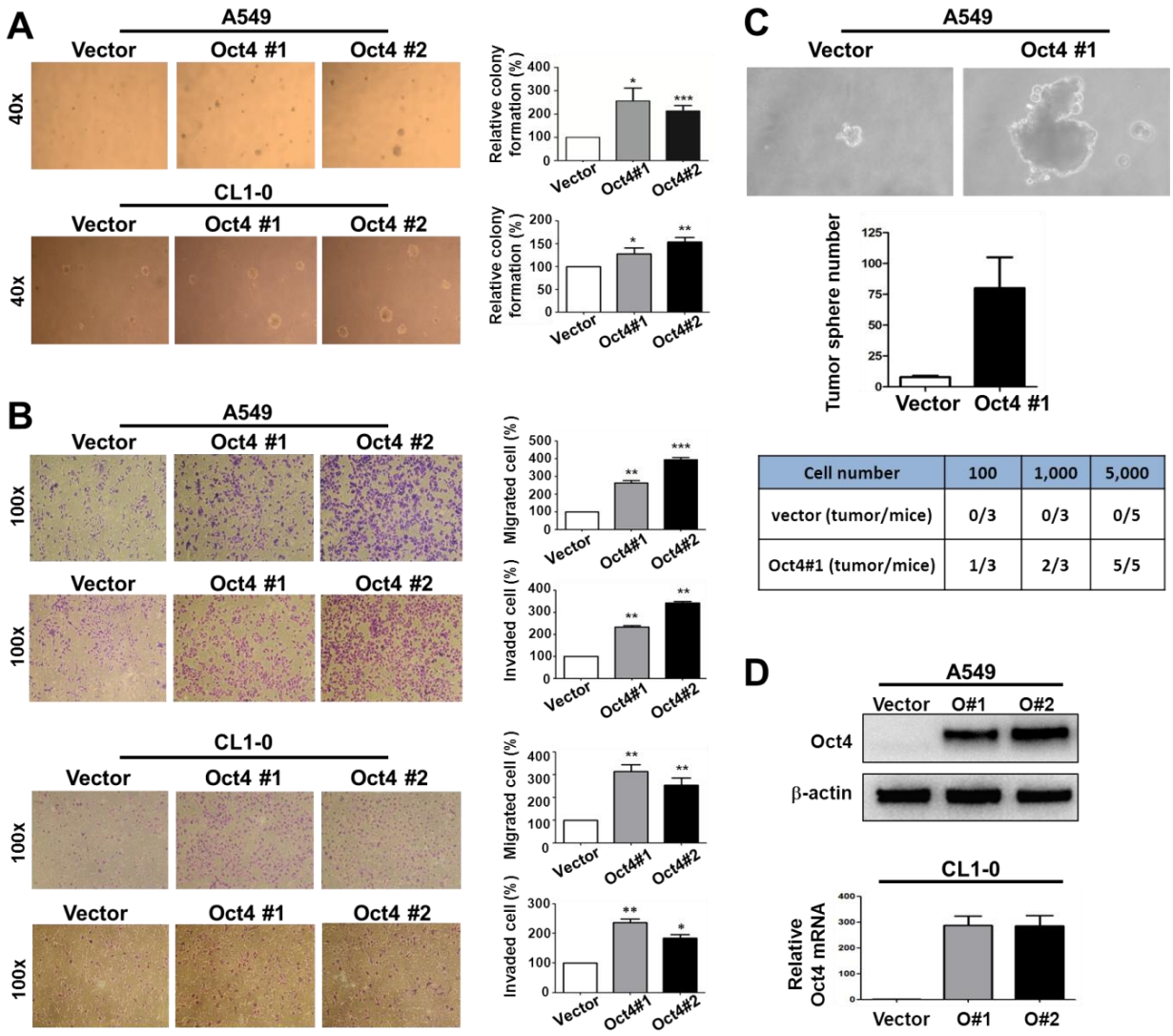


**Figure S1** Oct4 promoted lung cancer tumorigenesis *in vitro* and *in vivo*. **A** Anchorage-independent assays in empty vector stably-transfected cell line (vector) and two biological replicates of Oct4 stably-overexpressed A549 and CL1-0 cell lines (Oct4#1, Oct4#2). Results were photographed (left) and quantified (right). **B** Transwell migration and invasion assay analysis of stably-transfected cell lines in A549 and CL1-0 cells. Results were photographed (left) and quantified (right). *P*-values were determined by two-tailed Student's *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. **C** *In vitro* tumor sphere formation assay of A549 lung cancer cells stably expressing Oct4#1 or vector photographed (top) and quantified (middle). *In vivo* tumor formation assay using limited cell number (100, 1000, and 5000 cells) of vector and Oct4#1 cells. Tumor incidence of mice was analyzed at 8 weeks after implantation. **D** The immunoblots (upper) and qRT-PCR (lower) confirmed Oct4 expression in A549 and CL1-0 stable clones.

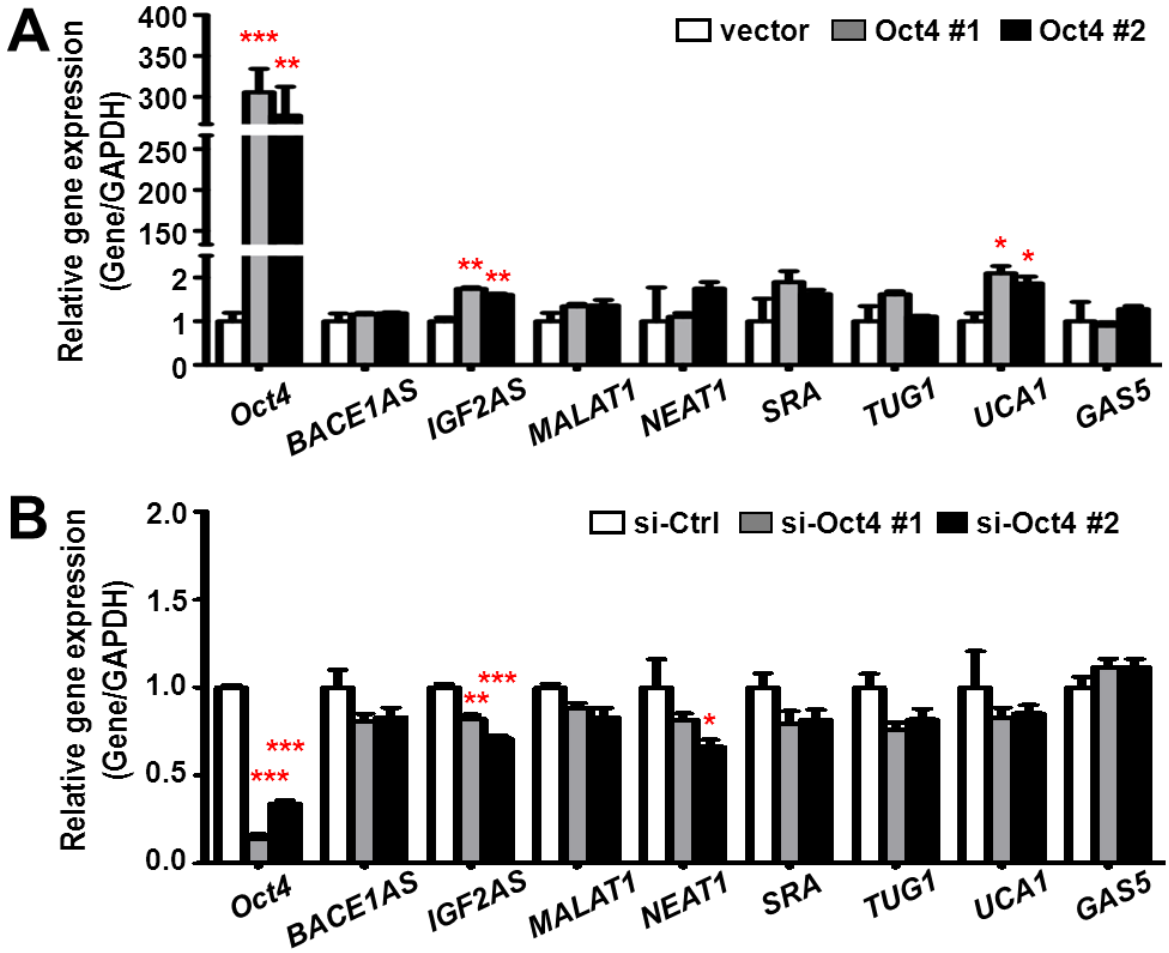


**Figure S2** Expression of lncRNAs in CL1-0 lung cancer cells manipulated for Oct4. **A**, **B** qRT-PCR analysis of eight lncRNAs expression in CL1-0 cells stably overexpressing Oct4 (Oct4#1, Oct4#2) (**A**) or Oct4-silenced CL1-0 cells (si-Oct4#1, si-Oct4#2) (**B**). Target lncRNA expression levels were normalized to *GAPDH* expression levels. Data represent mean ± SEM. *P*-values were determined by two-tailed Student's *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

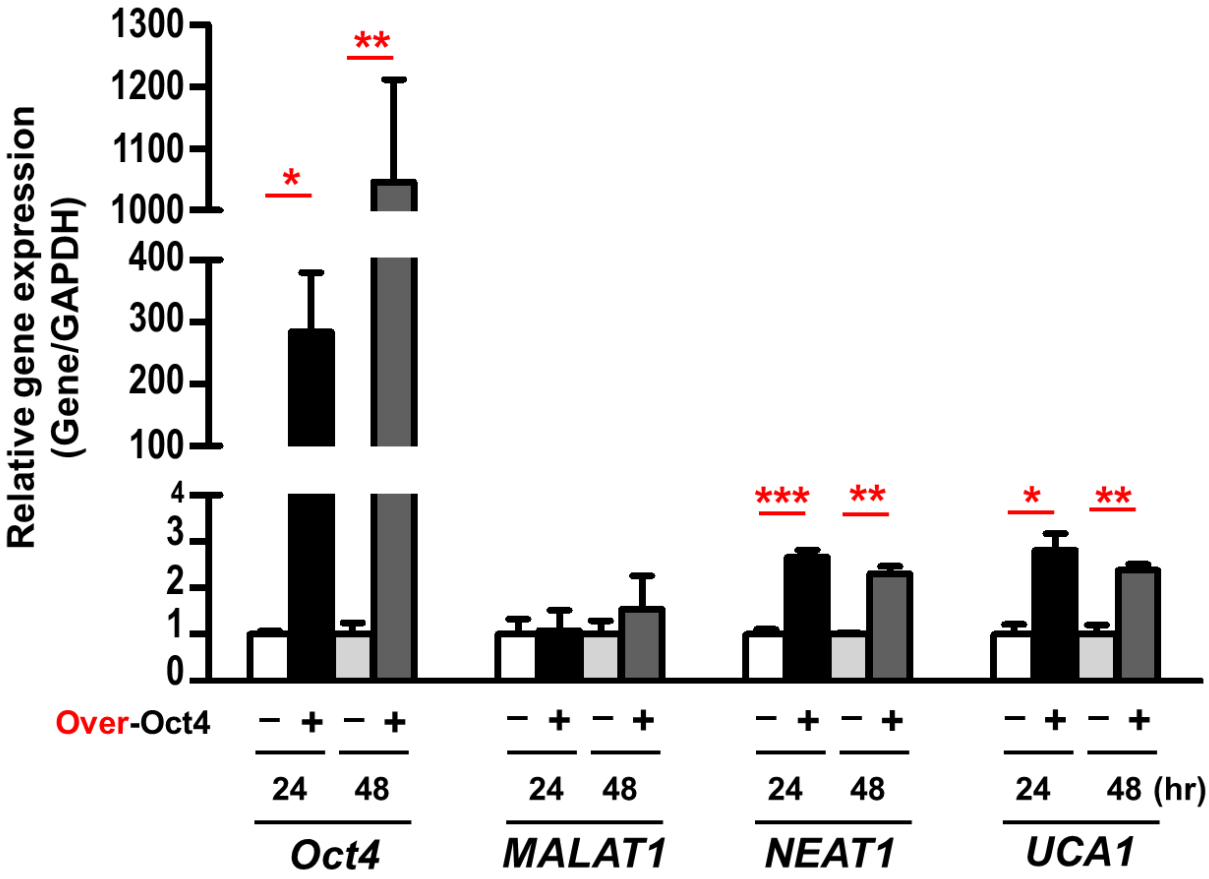


**Figure S3** Oct4 positively regulated *NEAT1* and *UCA1* lncRNAs transcription in normal bronchial epithelial BEAS-2B cells. qRT-PCR analysis of selected lncRNAs expressions in BEAS-2B cells overexpressing Oct4. Target lncRNA expression levels were normalized to *GAPDH* expression levels. Data represent mean ± SEM. *P*-values were determined by two-tailed Student's *t*-test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

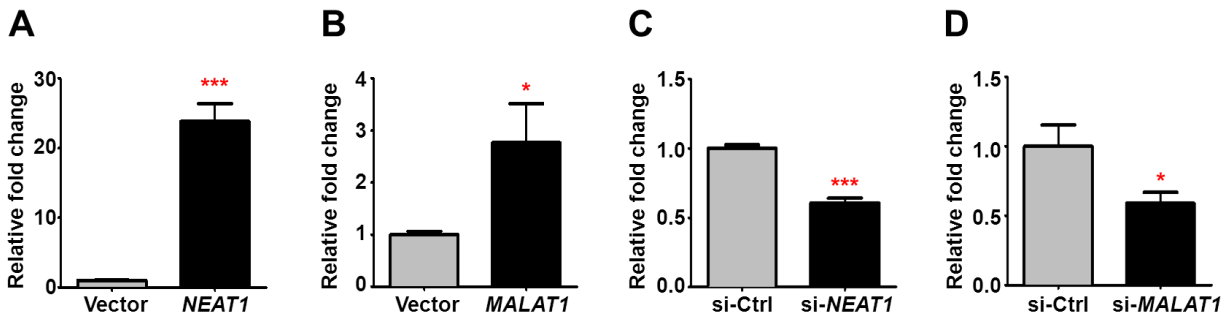


**Figure S4** RNA expression level of the manipulated *NEAT1* and *MALAT1* in A549 lung cancer cells. A549 cells transfected with *NEAT1* expression vector (**A**) or *MALAT1* expression vector (**B**), si-*NEAT1* oligo (si-*NEAT1*) (**C**) or si-*MALAT1* oligo (si-*MALAT1*) (**D**) were harvested and subjected to qRT-PCR assays for *NEAT1* and *MALAT1* RNA expression. Data are mean ± SEM. *P*-values were determined by two-tailed Student's *t*-test. **P* < 0.05; ****P* < 0.001.

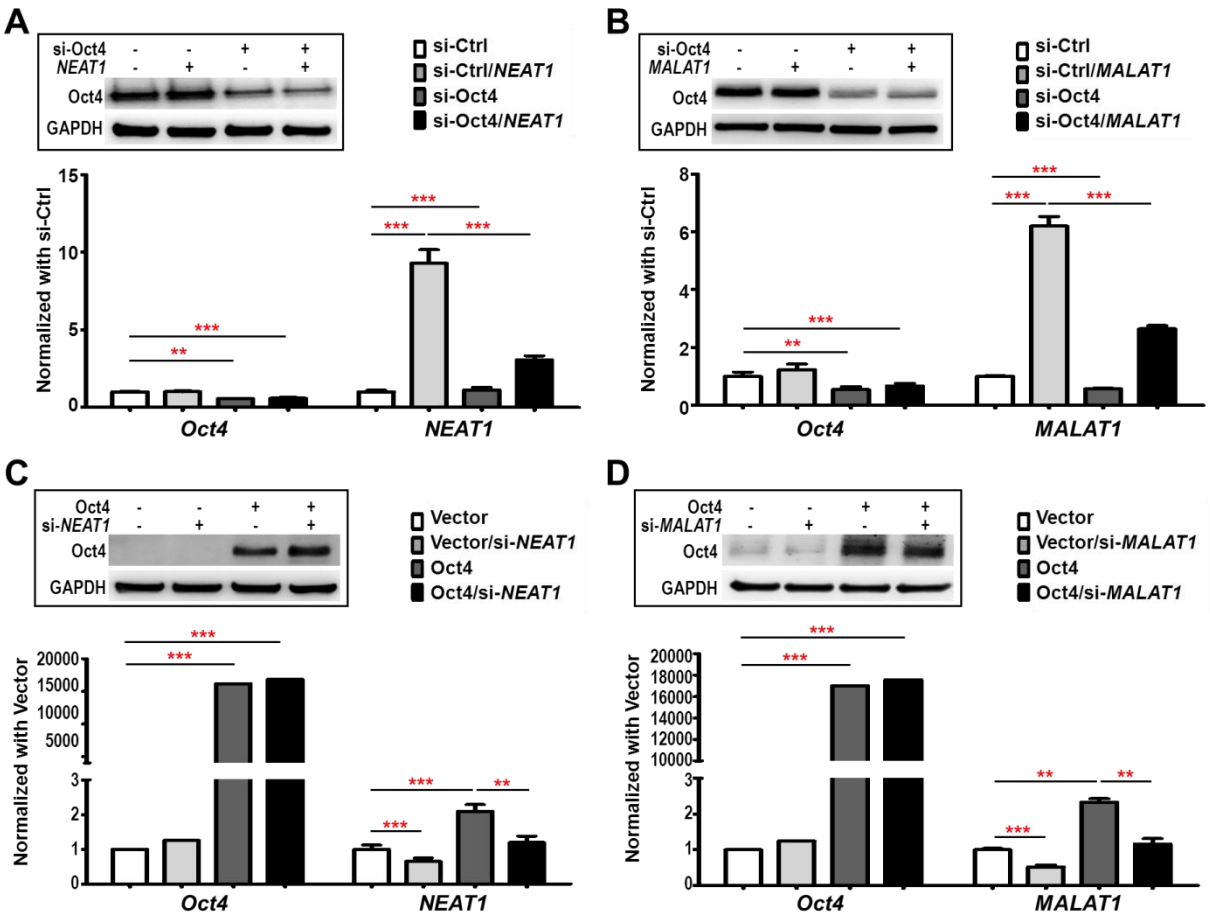


**Figure S5** RNA and protein expression level of the manipulated Oct4, *NEAT1* and *MALAT1* in A549 lung cancer cells. A549 cells were transfected with expression vectors of *NEAT1* (**A**) or *MALAT1* (**B**) alone or together with si-Oct4 oligo (si-Oct4). A549 cells were transfected with si-*NEAT1* oligo (si-*NEAT1*) (**C**) or si-*MALAT1* oligo (si-*MALAT1*) (**D**) alone or together with Oct4 expression vector. Cell lysates were subjected to qRT-PCR assays for *Oct4*, *NEAT1* and *MALAT1* RNA expression or Western blot analysis for Oct4 protein expression (inset). GAPDH serves as an internal control. Data are mean ± SEM. *P*-values were determined by two-way ANOVA. $^{**}P < 0.01$; $^{***}P < 0.001$.