**C-MYC inducible onco-lncRNA LINC00036 acting as EGFR mRNA stabilizer via RNA-protein and RNA-RNA interactions decreases the** **sensitivity of** **gefitinb in human cancer**

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**Supplementary information**

**Additional file 1:**

**Table S1**. Sequence of siRNAs and shRNAs used in this study.

**Table S2**. Antibody uesd in this study.

**Table S3**. Sequence of qRT-PR primers to detect RNA expression.

**Table S4**. Sequence of qRT-PR primers to detect microRNAs expression.

**Table S5**. Putative TF-binding site in the transcriptional start of LINC00036 locus.

**Table S6**. Predict microRNAs binding to the 3’-UTR of EGFR using public databases TargetScan and RNA22.

**Additional file 2:**

**Figure S1.** C-MYC mRNA expression is positively correlated with LINC00036 expression in human cancer. **A.** Knockdown of c-MYC decreases the expression of LINC00036 in GSE5823. **B**-**D.**c-MYC mRNA expression is positively correlated with LINC00036 expression in human cancer in LUSC (**B**), CESC (**C**) and ESCA (**D**) in TCGA data.

**Figure S2.** Knockdown LINC00036 inhibits cell growth and migration/invasion and accelerates cell apoptosis *in vitro*. **A.** Knockdown efficiency of LINC00036 in UACC-812, MDA-MB-453 cells via qRT-PCR analysis. **B.** CCK-8 assays for LINC00036 knockdown and Sh-NC group in UACC-812 cells and MDA-MB-453 cells. **C** and **D.** Representative images for flow cytometry(**C**) and quantification analysis (**D**) of analysis of UACC-812 cells and MDA-MB-453 cells after transfection**. E.** The protein level of Bax and Bcl-2 in LINC00036 knockdown group and Sh-NC group via western blot analysis. **F**-**I**. Cell migration and invasion and quantification analysis in MDA-MB-453and UACC-812 cells with LINC00036 knockdown. **J.** Knockdown efficiency of LINC00036 in NCI-H1975cells, SGC-7901 cells, SKOV-3 cells, 786-O cells, U251 cells, Hep-3B cells via qRT-PCR analysis.The data are presented as the mean± SD, \**P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001.

**Figure S3.** Characteristics of TTN and its toxicity effects on vital organs. **A.**The TUNEL assay for apoptotic cells in the TTN treatment group and in the other groups. **B.** H&E image for liver, kidney, heart, lung, spleen and skin treated with different treatments. (Original magnification. 200 ×. Scale bar: 200 µm). **C** and **D.**TUNEL assays for liver, kidney, heart, lung, spleen and skin treated with different treatments. (Original magnification. 200 ×. Scale bar: 100 µm). **E.** Serum biochemical indexes for detection of GPT, GGT, ALP, TBIL, CRE and BUN with different treatments. The data are presented as the mean± SD, \*\*\* *P* < 0.001.

**Figure S4.** LINC00036 expression is positively correlated with EGFR mRNA expression in human cancer. **A**-**I.** LINC00036 expression is positively correlated with EGFR mRNA expression in COAD (**A**), CESC (**B**), ESCA (**C**), HNSC (**D**), LUSD (**E**), PRAD (**F**), STAD (**G**), THCA (**H**) and THYM (**I**) in TCGA data. The data are presented as the mean± SD,\**P* < 0.05; \*\*\* *P* < 0.001.

**Figure S5.** LINC00036 promotes EGFR expression via RNA-protein interaction. **A.** LINC00036 sub-cellular localization assays with the nuclear and cytoplasmic fractions of UACC-812, MCF-7, 47D, NCI-H1950, NCI-H1650, SGC-7901, MKN-45, and MDA-MB-453 cells. The U1 small nuclear RNA was the nuclear positive control and GAPDH was the cytoplasmic positive control. **B.** RNA-FISH assays for LINC00036 sub-cellular localization in UACC-812, MDA-MB-452, SGC-7901 and NCI-H1975 cells. LINC00036 probes are shown in red. The U6 small nuclear RNA acted as the positive control (Original magnification: 1,000 ×; scale bar: 50 µm). **C.** Knockdown efficiency of si-PPP1R150 in T47D UACC-812 and SKBR3 cells via qRT-PCR analysis. **D.** Predicting the potential microRNAs that can bind to the 3′ UTR of *EGFR* using TargetScan and RNA22 databases. Seventy-four overlapping microRNAs were filtered between upregulated microRNAs (FC > 1.5, *P* < 0.05) in the BGISEQ-500 RNA-seq of the HMUCC cohort and the above predicted microRNAs in databases. Venn diagrams were drawn using the Venny online software analysis. **E** and **F.** Expression of LINC00036 in T47D and UACC-812 cells treated with microRNA inhibitors of miR-424-5p, miR-196b-5p, miR-301a-5p, miR-708-5p, miR-125b-2-3p, miR-143-3p, miR-503-5p, miR-324-3p, miR-1260a, miR-1260b, miR-4510, and miR-6720-5p. **G** and **H.** Expression of EGFR mRNA in T47D and UACC-812 cells treated with microRNA inhibitors or mimics of miR-424-5p, miR-196b-5p, miR-301a-5p, miR-708-5p, miR-125b-2-3p, miR-143-3p, miR-503-5p, miR-324-3p, miR-1260a, miR-1260b, miR-4510, and miR-6720-5p. The data are presented as the mean ± SD, \**P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001.

**Figure S6.** LINC00036-miR-125b-2-3p/miR-424-5p-EGFR axis promotes cell proliferation. **A** and **B.** Cell proliferation was analyzed by CCK-8 assay in NCI-H1975 and SGC-7901cell. The data are presented as the mean± SD, \*\* *P* < 0.01; \*\*\* *P* < 0.001; #*P* < 0.05. \*\* *P* < 0.01; \*\*\* *P* < 0.001 vs Sh-NC; #*P* < 0.05 vs Sh-LINC00036-1.

**Figure S7.** Downregulation of LINC00036 increases the sensitivity of cancer cells to gefitinib. **A**-**H.** CCK8 assays were measure the IC50 ability of LINC00036 knockdown in MDA-MB-231, UACC-812, NCI-H1975, A549, SGC-7901, 786-O, Hep3B and SK-OV-3 cells after various concentration of gefitinib treatment for 48 h. **I.** Cell apoptosis in combination of LINC00036 knockdown and gefitinib reatment group, LINC00036 knockdown group and gefitinib treatment alone group in UACC-812 tumor mice models by TUNEL assays.The data are presented as the mean± SD, \**P* < 0.05; \*\*\* *P* < 0.001