

CENPU Promotes Growth and Metastasis in Nasopharyngeal Carcinoma by Inhibiting DUSP6

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Abstract

Background: Centromere protein U (CENPU), a centromere component, is key for mitosis and involved in the carcinogenesis of cancers. The role and mechanisms of CENPU in nasopharyngeal carcinoma (NPC) has not been described.

Methods: CENPU expression in NPC cells and tissues was evaluated by RT-PCR and western blotting. Clinical significance of CENPU was evaluated by Immunohistochemistry. Biological functions of CENPU were evaluated by cell growth assay, colony formation assay, apoptosis assay, migration assay and invasion assays. Xenograft growth and lung metastasis model were conducted to investigate the effect of CENPU in vivo. Gene chip analysis, Ingenuity Pathway Analysis (IPA), and co-immunoprecipitation (Co-IP) experiments were used to explore the mechanisms of CENPU in NPC.

Results: CENPU was highly expressed in NPC cells and samples. Patients with CENPU positive expression were closely associated with poor overall survival. Knockdown of CENPU inhibited proliferation and migration in vitro and in vivo in NPC. Gene chip analysis and IPA suggested that differentially expressed genes (DEGs) were significantly enriched in cancer and functions, including cellular movement, cellular development, cell growth and death, and proliferation when CENPU was downregulated. Dual specificity phosphatase 6 (DUSP6) was one of the DEGs and significantly decreased in NPC samples, and inversely correlated with expression with CENPU. Mechanism studies confirmed that CENPU increased the activation of ERK1/2 and p38 signal pathways by suppressing the expression of DUSP6. Therefore, our results suggested that CENPU might act as an oncogene in NPC and promote the development of NPC via inhibition of DUSP6, resulting in the inactivation of Erk1/2 and p38 pathways.

Conclusions: CENPU facilitated cell proliferation and invasion by interacting with DUSP6. CENPU might be a promising prognostic biomarker and a potential target for NPC therapy.

Introduction

Nasopharyngeal carcinoma is characterized by unique geographic distribution and is particularly prevalent in southeast China [1]. Over the past decade, with the advance of Tumor staging, intensity-modulated radiation therapy (IMRT), and therapeutic strategy, the overall survival of NPC has remarkably improved. However, the exact underlying mechanisms of tumor local recurrence and metastasis remain elusive and warrant further research[2].

Centromere protein U (CENPU) is a centromere component crucial for mitosis and has a vital role in orchestrating kinetochore-microtubule attachment[3–5]. Studies reported that CENPU has a positive impact on tumorigenesis and is implicated in growth and metastasis in multiple cancers[6–10]. Overexpression of CENPU increased proliferation and migration in triple-negative breast cancer (TNBC), and a high level of CENPU was correlated with poorer outcomes in patients with TNBC[6]. Downregulation of CENPU significantly inhibited growth and metastasis in lung adenocarcinoma (LAC)[10]. Additionally, Crawford et al. found that CENPU was a germline modifier of aggressive prostate cancer by comparative

genetics[11], which was confirmed by Zhang et al. that CNEPU promoted prostate cancer cell proliferation[12]. However, the roles of CENPU have not been investigated in NPC.

Dual Specificity Phosphatase 6 (DUSP6) belongs to dual specificity protein phosphatase subfamily, which negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily (MAPK/ERK, SAPK/JNK, p38) and are associated with cell growth, metastasis and apoptosis in various cancers[13–18]. Wong, V. C reported that DUSP6 suppress the tumorigenesis and metastasis of NPC cells through negatively regulating the activity of ERK pathway[19]. Yet, the relationship between DUSP6 and CENPU remains unknown.

This study was to explore clinical significance and functions of CENPU, and to study the underlying molecular mechanisms of CENPU in the pathogenesis of NPC. Here, we found that CNEPU act as a cancer-promoting gene that significantly promoted growth and metastasis through inhibiting DUSP6 in NPC cells. Our study highlights the value of CENPU as a promising biomarker and potential target for NPC.

Materials And Methods

Cell lines

NPC cells, including CNE-1, CNE-2, SUNE-1 and 5-8F, were cultured in RPMI medium 1640 (NCS, Hyclone, Invitrogen) supplemented with 10%FBS (Gibco BRL, Grand Island, NY, USA). The immortalized normal nasopharyngeal epithelial cell line (NP69) was cultured in Keratinocyte-SFM medium supplemented with epidermal growth factor (Invitrogen, Carlsbad, NM, USA). All cells were maintained at 37°C under 5% CO₂.

shRNA and lentiviruses

Short hairpin RNA targeting CENPU (shCENPU-1 and shCENPU-2) and a scrambled control shRNA (shCtrl) were synthesized by Shang-hai Genechem. NPC cells were infected with shRNA-encoding lentiviruses, and CNE-2 cells were chosen for further experiments in *vitro* and in *vivo*.

Quantitative real-time PCR

Total RNA was extracted using TRIzol (Invitrogen, USA). To measure CENPU mRNA expression, complementary DNA (cDNA) was synthesized Using Transcriptor cDNA Synth Kit (Roche, USA). Real-time PCR was performed using the FastStart Universal SYBR Green Mast (Roche, USA). The primer sequences were: CENPU forward 5' -ATGAACTGCTTCGGTTAGAGC-3' and reverse 5' -TATTTTCGCAGATGGCTTTCGG-3'. The primer sequences were: DUSP6 forward 5' - GAACTGTGGTGTCTTGGTACATT -3' and reverse 5' - GAACTGTGGTGTCTTGGTACATT-3'. GAPDH was used to normalize the expression of mRNA. The fold

changes were calculated using relative quantification method ($2^{-\Delta\Delta Ct}$). All reactions were performed in triplicate times.

Cell growth and Clone formation assay

Celigo (Nexcelom), a cell count instrument, was used to detect cell growth. 2000 cell/well were seeded and each group has 3 multiple holes. From the second day after laying, the Celigo reading board is tested once a day for 5 days continuously. By adjusting the input parameters of analysis settings, the number of cells with green fluorescence in each scanning orifice plate was accurately calculated and then cell proliferation curve for 5 days was drawn.

Migration and invasion assays

For migration and invasion assays, CNE-2 cells in serum-free medium were added to the upper chamber of 8.0 μ m pore size (Corning, USA) without or with Matrigel (BD Biosciences, USA), and 20% FBS was added to the lower chamber. After 24h (migration assays) or 48h (invasion assays), cells in the lower chamber were fixed, stained, and photographed.

Western blotting analyses and Immunohistochemistry (IHC)

Western blotting was performed using standard protocols. Primary antibodies, including rabbit anti-CENPU polyclonal antibody (1:2000; ImmunoWay Biotechnology Company, USA), rabbit anti-p-NF- κ B monoclonal antibody (1:1000; Cell Signaling Technology, USA), rabbit anti-Erk1/2 monoclonal antibody (1:1000; Cell Signaling Technology, USA), rabbit anti-p-Erk1/2 monoclonal antibody (1:1000; Cell Signaling Technology, USA). Rabbit anti-GAPDH polyclonal antibody (1:10000; Abcam, USA) was used as a loading control. IHC staining was performed according to standard protocols. Paraffin slices were incubated with primary antibodies against CENPU (1:400; ImmunoWay Biotechnology Company, USA). The scores of CENPU were determined by multiplying the intensity score and percentage score to figure out a semiquantitative H-score. The mean H-score from all patients was used as the cutoff value to define CENPU positivity, which was described in our previous article[20].

Xenograft growth and lung metastasis model

For the xenograft growth model, CNE-2 cells were subcutaneously injected into nude mouse with a concentration of 2×10^7 cells. Tumor volume and average of total fluorescence expression were measured and compared. For the lung metastasis model, CNE-2 cells were intravenously injected through the tail vein with a concentration of 1.0×10^6 cells. Fluorescent imaging and total radiant efficiency were taken and compared. Number of metastatic nodes in lung were sampled and quantified after the mice were

sacrificed. The xenograft model in our study is in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

GeneChip

GeneChip primeview human was performed to identify differentially expressed genes (DEG) between knock down groups and normal control groups (NC). KD refers to the downregulation of CENPU in CNE-2 cells, then stably transfected cells were selected, and NC refers to the transfection of control into CNE-2 cells. Then, linear model analysis based on empirical Bayesian distribution to calculate the significant difference level (p -value), and Benjamini-Hochberg method was used to correct the false discovery rate (FDR). Genes with p value ≤ 0.05 and fold change (FC) > 2.0 were considered significant. Hierarchical Clustering and Volcano Plot of the significant genes were plotted using R programming language. Then, Ingenuity Pathway Analysis was used to perform classical pathway analysis, biological downstream effect analysis, disease and functional analysis, regulatory effect analysis and interaction network analysis according to the DEGs.

Ingenuity Pathway Analysis

Ingenuity pathways analysis (Ingenuity Systems, Mountain View, CA) is a robust and expertly curated database containing up-to-date information on over 20,000 mammalian genes and proteins, 1.4 million biological interactions, and 100 canonical pathways incorporating over 6,000 discrete gene concepts. This information is integrated with other relevant databases such as Entrez- Gene and Gene Ontology. The experimental data sets were used to query the IPA and to compose a set of interactive networks taking into consideration canonical pathways, the relevant biological interactions, and the cellular and disease processes.

Co-immunoprecipitation experiments

Co-immunoprecipitation (Co-IP) experiments were conducted using a Pierce™ Co-IP kit (Thermo Scientific) and performed according to the protocol. Briefly, cell lysates were incubated with antibodies against CENPU (Abcam, USA) or DUSP6 (Abcam, USA) in 4 °C overnight. After incubation, the antigen/antibody complex was combined with magnetic beads for 1 hour at room temperature. Then, magnetic beads were washed twice using immunoprecipitation lysis and washed once using pure water. Then, protein was eluted using elution buffer. IgG (Santa Cruz Biotechnology) was used as a negative control. Lastly, samples were resolved by SDS-PAGE and analyzed by western blot.

Statistical analysis

All statistical analyses were performed using the software SPSS version 24.0 (SPSS, Chicago, USA) and Graph Pad Prism 8 (GraphPad Prism, USA). P value less than 0.05 was considered statistically significant.

Results

CENPU is overexpressed in Nasopharyngeal carcinoma and associated with poor survival

To explore the involvement of CENPU in NPC, we first examined CENPU expression in 4 NPC cell lines and immortalized normal nasopharyngeal epithelial cell line (NP69) by Western Blotting and Real-Time PCR. The results showed that protein and mRNA levels of CENPU were both upregulated in NPC cells, especially in CNE-2 cells (Fig. 1a). Then, we evaluate CENPU expression in 10 normal nasopharyngeal epithelial tissues and 20 NPC tissues (Fig. 1b), and the result was further confirmed using the Gene Expression Omnibus database (GSE12452), which also revealed that CENPU mRNA was significantly upregulated in NPC tissues (Fig. 1c).

To examine the relationship between CENPU expression and patient prognosis, we performed IHC staining of CENPU in a tissue microarray (TMA) of 98 NPC specimens and 33 normal nasopharyngeal epithelial samples (Fig. 1d). The results showed that CENPU was overexpressed in 32.65% (32/98) NPC patients. While CENPU was almost lost in normal nasopharynx epithelia, with 12.12% (4/33) positive rates. Kaplan–Meier analysis indicated that patients with CENPU positive expression had worse overall survival (OS) compared to those with lower CENPU negative ($p < 0.001$, Fig. 1e). Taken together, these data reveal that CENPU is overexpressed in NPC patients and is associated with poorer survival.

Knockdown of CENPU in NPC cells suppress growth in vitro and in vivo

As CENPU mRNA and protein were highly upregulated in CNE-2 cells, CNE-2 cells were selected for further biological study. CNE2 cells were transfected with control vector (shCtrl) and lentiviral vector that downregulated CENPU expression (shCENPU-1 and shCENPU-2). Western blotting and qRT-PCR were then performed to confirm transduction efficiency (Fig.2a). By performing Celigo and colony-forming assays, we found that downregulation of CENPU remarkably attenuated CEN2 growth and colony formation (Fig.2b and 2c). Meanwhile, apoptosis assay indicated that knockdown of CENPU expression dramatically promote death of CNE-2 cells (Fig.2d). Moreover, subcutaneous xenograft tumor growth *in vivo* further confirmed that CENPU showed a negative effect on tumor proliferation (Fig. 2e).

Knockdown of CENPU in NPC cells suppress metastasis in vitro and in vivo

Transwell assay, Matrigel invasion assay (Fig. 3a) and Wound-healing assay (Fig. 3b) were done to evaluate the effect of CENPU on NPC metastasis. Notably, CENPU significantly attenuated the mobility of CNE-2 cells. To determine the effect of CENPU function *in vivo*, we injected cancer cells into the tail vein of nude mice to observe the rate of nodule formation in the lungs. Compared with controls, lower radiant efficiency and smaller lung metastatic nodules were found in the lungs of mouse xenograft injected with shCENPU-2 (Fig. 3c).

Gene chip analysis and Ingenuity Pathway Analysis

To explore the molecular mechanisms by which CENPU contributes to the proliferation and invasion abilities of NPC cells, we conducted a microarray analysis comparing the differentially expressed genes in CENPU knockdown group (KD) and the normal control group (NC). Each group had 3 samples to reduce systemic error and increase statistic accuracy. Genes with p value ≤ 0.05 and fold change (FC) > 2.0 were considered significant and were chosen for further analysis. According to the Gene chip, 172 genes were upregulated, while 397 genes were downregulated (Supplement Table 1), as was shown in Volcano Plot upper right quadrant and upper left quadrant (Fig. 4a). Hierarchical Clustering showed that the similarity of the data pattern within the group is high, and the similarity of the data pattern between the groups is low (Fig. 4b). Then, classical signal pathways based on IPA were performed. Data suggested that p38/MAPK and ERK1/2 were strongly activated when CENPU was knocked down (Fig. 4c).

Moreover, diseases and function analysis based on IPA revealed that DEGs were significantly enriched in cancer and functions including cellular movement, cell death and survival, cellular development, cell growth, and proliferation (Fig.5a). Network of DEGs involved in cell movement of tumor cell lines suggested that DUSP6 had a negative effect in the movement of NPC cell lines (Fig.5b).Heat map of diseases and function that affected by DEGs showed that knock down of CENPU suppress cellular movement (Fig. 5c), which was closely consistent with our study.

The possible regulation effects of upstream and downstream of DEGs were also analyzed via IPA. Upstream regulatory network suggested that lipopolysaccharide is predicted to be strongly inhibited when CENPU was downregulated (Fig. 6a). Down regulatory network of interactions between DEGs, regulators and functions indicated that CENPU not only regulated proliferation and migration of cell lines, but also participated in the activity of lymphocytes, monocytes and phagocytes (Fig. 6b).

Knockdown of CENPU inhibited p38 and ERK1/2 pathway by interacting with DUSP6

In order to confirm the signals indicated in Gene chip analysis, p38 and ERK1/2 pathway-related proteins were tested. Western bolt showed that knockdown of CENPU reduced phosphorylation of p38, ERK1/2 and MARK (Fig. 7a).

To study the downstream genes that lead to the carcinogenesis of NPC, 30 candidate downstream genes enriched in Cyclins and Cell Cycle Regulation, Integrin Signaling, ERK/MAPK Signaling and p38 MAPK Signaling were chosen and tested by Real-Time PCR (Fig. 7b). Then, 6 candidate genes were further tested by western blotting. Our data suggested that knockdown of CENPU downregulated expression of CCND3 and CKD6, while upregulated tumor suppressor dual-specificity phosphatase 6 (DUSP6) expression (Fig. 7c), which was in line with mRNA expression. Using our own samples and database (GSE12452), we found that DUSP6 was downregulated in NPC tissues (Fig. 7d and 7e), and the expression of CENPU was inversely correlated with DUSP6 in NPC samples (Fig. 7f). To further confirm the interaction between CENPU and DUSP6 in NPC cells, we performed co-immunoprecipitation and reciprocal western blotting analysis. Our data revealed that CENPU was co-immunoprecipitated with DUSP6 and, conversely, DUSP6 was co-immunoprecipitated with CENPU in CNE-2 cells (Fig. 7g). Taken together, these findings suggest that CENPU promoted development of NPC by negatively regulating the expression of DUSP6.

Discussion

CENPU is a key member of the Centromere proteins (CENPs) family, which was associated with centromeres mitosis and chromosome movement. In our study, we found that expression of CENPU was significantly increased in NPC samples and was associated with a worse prognosis. Moreover, downregulation of CENPU significantly inhibited tumor growth and metastasis in NPC cells *in vitro*, and that was further confirmed by the mouse xenograft model. Gene chip analysis and Ingenuity Pathway Analysis indicated that knocking down CENPU expression repressed NPC tumorigenesis by suppressing Erk1/2 and p38 pathways, which was also demonstrated by Western blot. Preliminary mechanism analysis suggested that CENPU interacted with DUSP6 and decreased expression of DUSP6, a tumor suppressor that negatively regulates the activity of ERK, ultimately leading to inhibition of progression in NPC. Taken together, we provide a novel insight into the involvement of CENPU in NPC.

Recently studies have shown that CENPU act as an oncogene in breast cancer, non-small-cell lung cancer, ovarian cancer, and bladder cancer [6–10, 21]. In TNBC, CENPU promoted angiogenesis via inhibiting the ubiquitination of COX-2, and through activation of COX-2-p-ERK-HIF-1 α -VEGFA pathway[6]. In ovarian cancer, CENPU facilitates metastasis via targeting HMGB2[8]. While, in non-small-cell lung cancer, CENPU facilitates growth and metastasis involving Wnt/beta-catenin pathway[9], and may also be mediated by PI3K/AKT signaling[10]. In line with the finding in TNBC, we found activation of the ERK signaling pathway plays an important role in CENPU knockdown NPC cells. ERK1/2 and p38 all belong to Mitogen-activated protein kinase (MAPK). ERK1/2 signaling pathway is frequently activated and promotes proliferation and metastasis in cancers [22]. In NPC, activation of ERK1/2 pathway facilitates cancer cell growth and metastasis in NPC cell [23, 24] and overexpression of p-ERK was associated with advanced clinical stage and worse outcome in patients with NPC [25].

To understand the deep mechanisms that CENPU involved in NPC, Gene chip and IPA were performed, and candidate downstream pathway genes were examined according to published literature. Interestingly,

we found DUSP6 was considered to have a tumor suppressive effect in NPC and acted as a negative feedback regulator for ERK/MAPK in NPC[26], which is consistent with the results of Gene chip and IPA. In addition, our study demonstrated that knockdown of CENPU increased DUSP6 expression by Western blot and PCR, and Spearman's correlation analysis revealed a negative correlation between CENPU and DUSP6 expression. Thus, we put on a hypothesis that CENPU may contribute to the tumor progressive effects through downregulation of DUSP6, leading to the inactivation of the MAPK signaling pathway in NPC cells. Fortunately, co-immunoprecipitation confirmed our hypothesis that CENPU interacted with DUSP6. Of note, further investigation is warranted to reveal the concrete mechanism of the gap between CENPU and DUSP6, and the upstream regulatory network of CENPU also remain exploration.

Conclusions

our study shows that CENPU acts as an oncogene in NPC and has a vital role in the pathogenesis of NPC. More importantly, our study raises the potential that CENPU could be a promising prognostic biomarker and a novel molecular target for NPC.

Abbreviations

CENPU: Centromere protein U; NPC: nasopharyngeal carcinoma; DUSP6: dual specificity phosphatase 6; DEG: differentially expressed genes; Co-IP: Co-immunoprecipitation; IPA: Ingenuity Pathway Analysis; IHC: Immunohistochemistry; OS: overall survival; NC: normal control; KD: knockdown; TMA: tissue microarray.

Declarations

Acknowledgements

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Authors' contributions

All authors helped to perform the research; LC participated in manuscript writing and data analysis; LMF, LC and PJJ participated in study concept and study design. ZJF and XY participated in data collection. All authors approved the final manuscript.

Availability of data and material

The datasets generated and/or analyzed in our study are available from the corresponding author.

Ethical statement and consent to participate

The study protocol was designed in accordance with the guidelines outlined in the Declaration of Helsinki. All information was retrospectively extracted in the context of compliance with the relevant regulations and protection of patients' privacy. All the participants signed an informed consent form. This study was approved by the Ethical Review Committee of Fujian Cancer Hospital. All animal studies were performed in accordance with the ARRIVE and IACUC guidelines. All methods were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

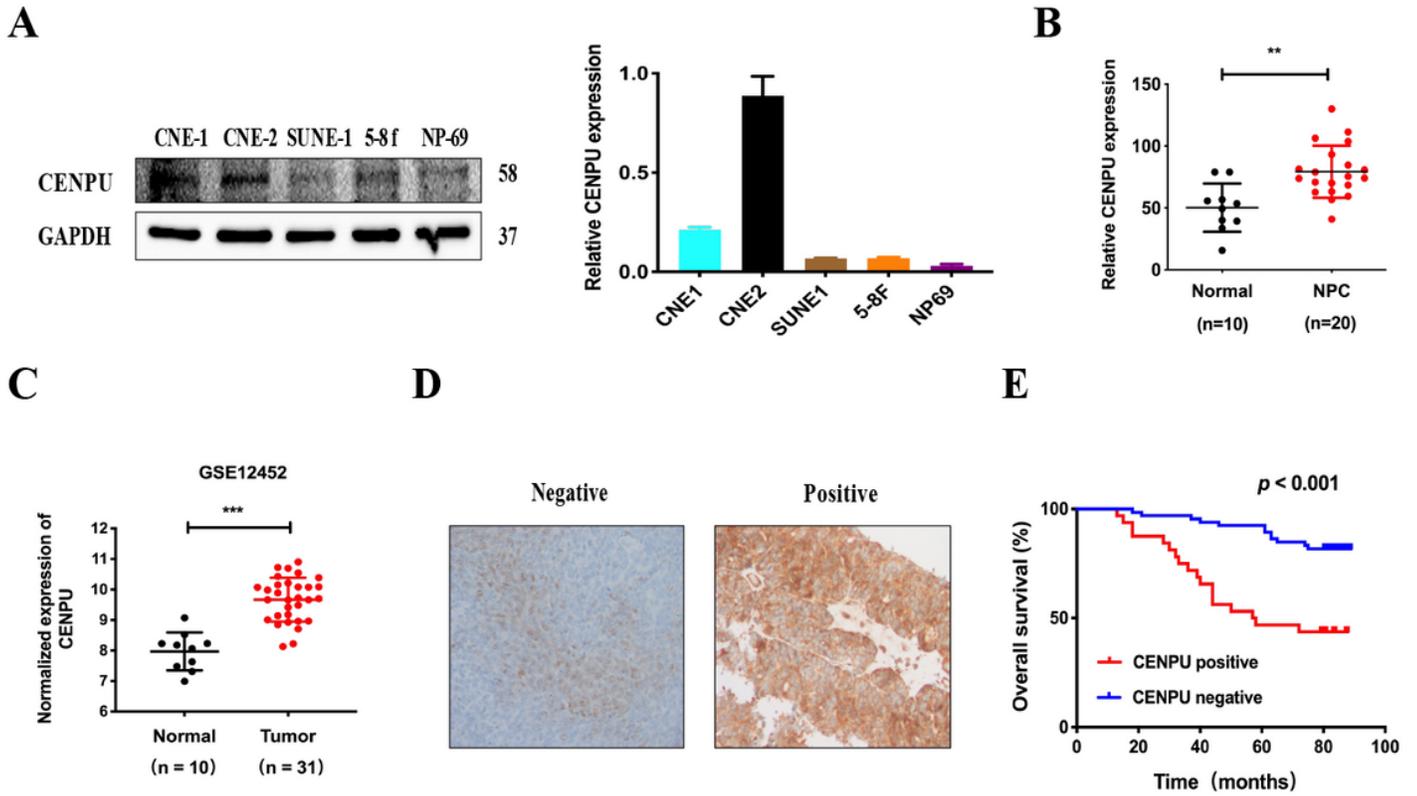


Figure 1

CENPU expression in nasopharyngeal carcinoma cells, tissue and its clinical significance. **A** The protein and mRNA expression of CENPU in CNE-1, CNE-2, SUNE-1, 5-8F and NP 69 cells by western blot (Left column) and PCR (Right column). **B** CENPU expression in 10 normal nasopharyngeal tissues and 20 NPC tissues by PCR. **C** CENPU expression in NPC tissues using Gene Expression Omnibus database (GSE12452). **D** Representative images of negative (Left column) and positive (Right column) CENPU expression by immunohistochemistry in nasopharyngeal carcinoma. **E** Kaplan–Meier analysis of overall survival in NPC patients according to different CENPU expression levels. Statistical significance was presented as $*p \leq 0.05$, $** p \leq 0.01$ or $*** p \leq 0.001$.

Figure 2

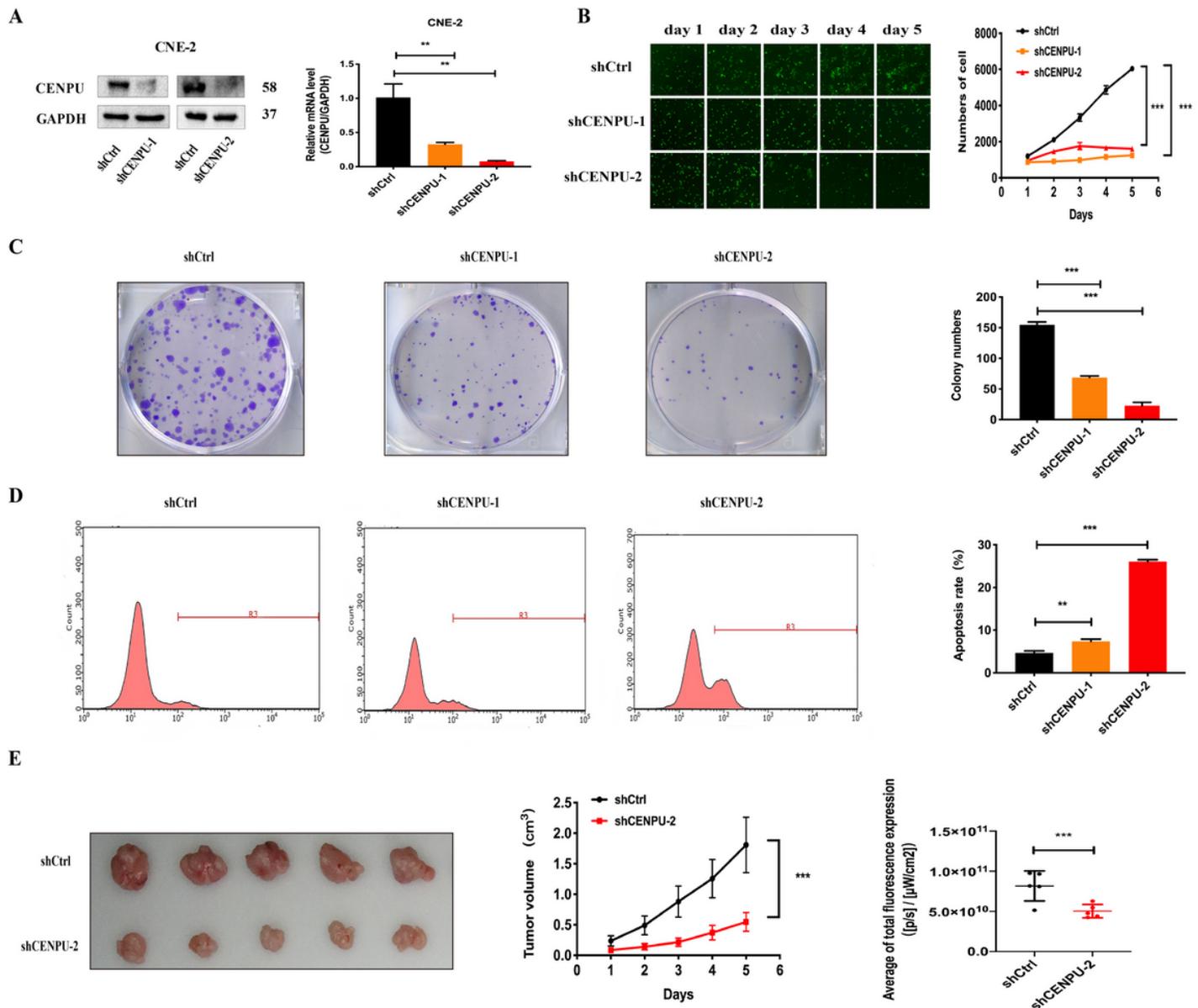
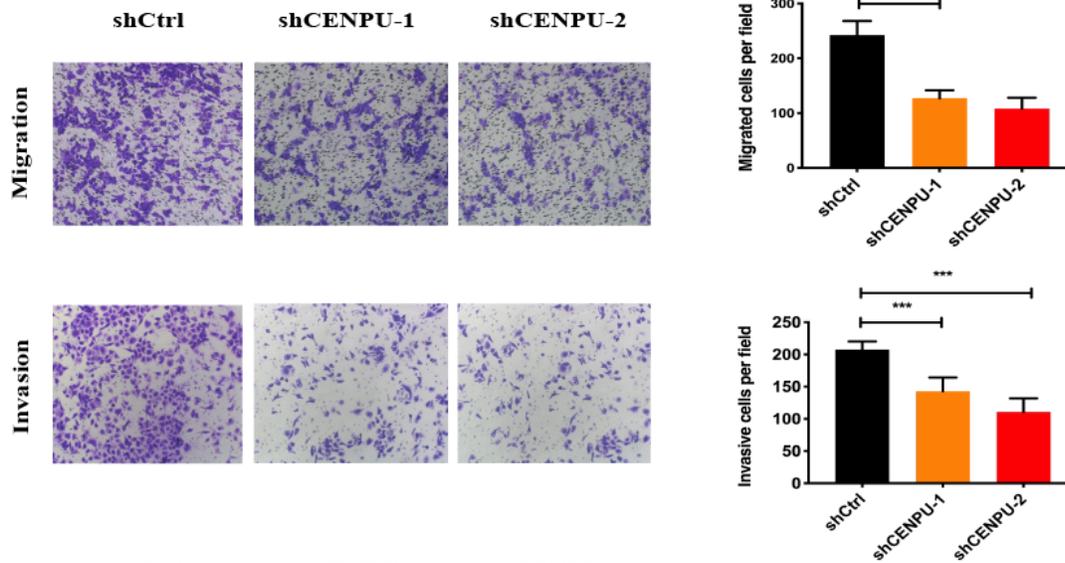


Figure 2

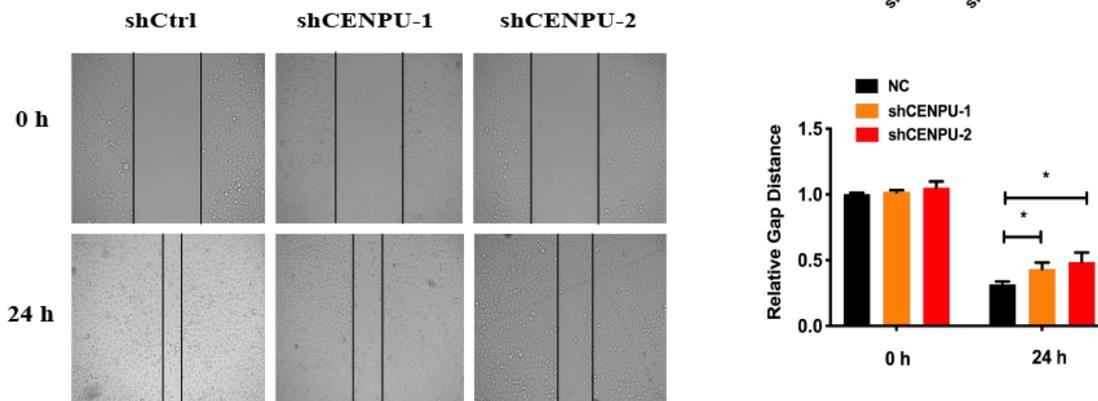
Effect of CENPU on NPC cells proliferation and apoptosis in. **A** CNE-2 cells transfected with si-CNEPU-1 and si-CENPU-2 lentiviral vector (shCENPU-1 and shCENPU-2) or control vector (shCtrl), and transfection efficiency were verified by western (Left column) and PCR (Right column). **B** The proliferation ability of CNE-2 cells transfected with shCNEPU-1 and shCENPU-2 were determined by cell growth and clone formation assay (**C**). **D** Apoptosis rate was analyzed by flow cytometry after knockdown of CENPU. **E** Representative images of tumors from nude mice that injected with subcutaneous xenografts of CNE-2 cells transfected with shCENPU-2 (Left column). Quantitative analysis of xenografted tumor volumes (Middle column) and fluorescence expression (Right column). Statistical significance was presented as * $p \leq 0.05$, ** $p \leq 0.01$ or *** $p \leq 0.001$.

Figure 3

A



B



C

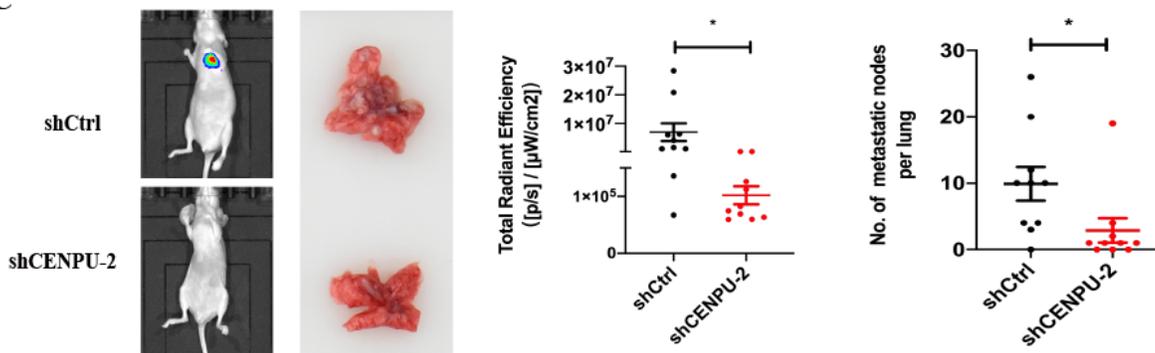


Figure 3

Effect of CNEPU on CNE-2 cells migration and invasion in vitro and in vivo. **A** The metastatic ability of CNE-2 cells transfected with shCNEPU-1 and shCNEPU-2 were determined by migration and invasion assay. **B** The migration ability of transfected CNE-2 cells by cell wound healing assays. **C** Representative

images of nude mice and metastatic nodes of lung in nude mice that were intravenously injected with CNE-2 cells transfected with shCENPU-2 via the tail vein (n = 5) (Left column). Quantitative analysis of radiant efficiency (Middle column) and metastatic nodes of lung in xenografted tumor (Right column). Statistical significance was presented as * $p \leq 0.05$, ** $p \leq 0.01$ or *** $p \leq 0.001$.

Figure 4

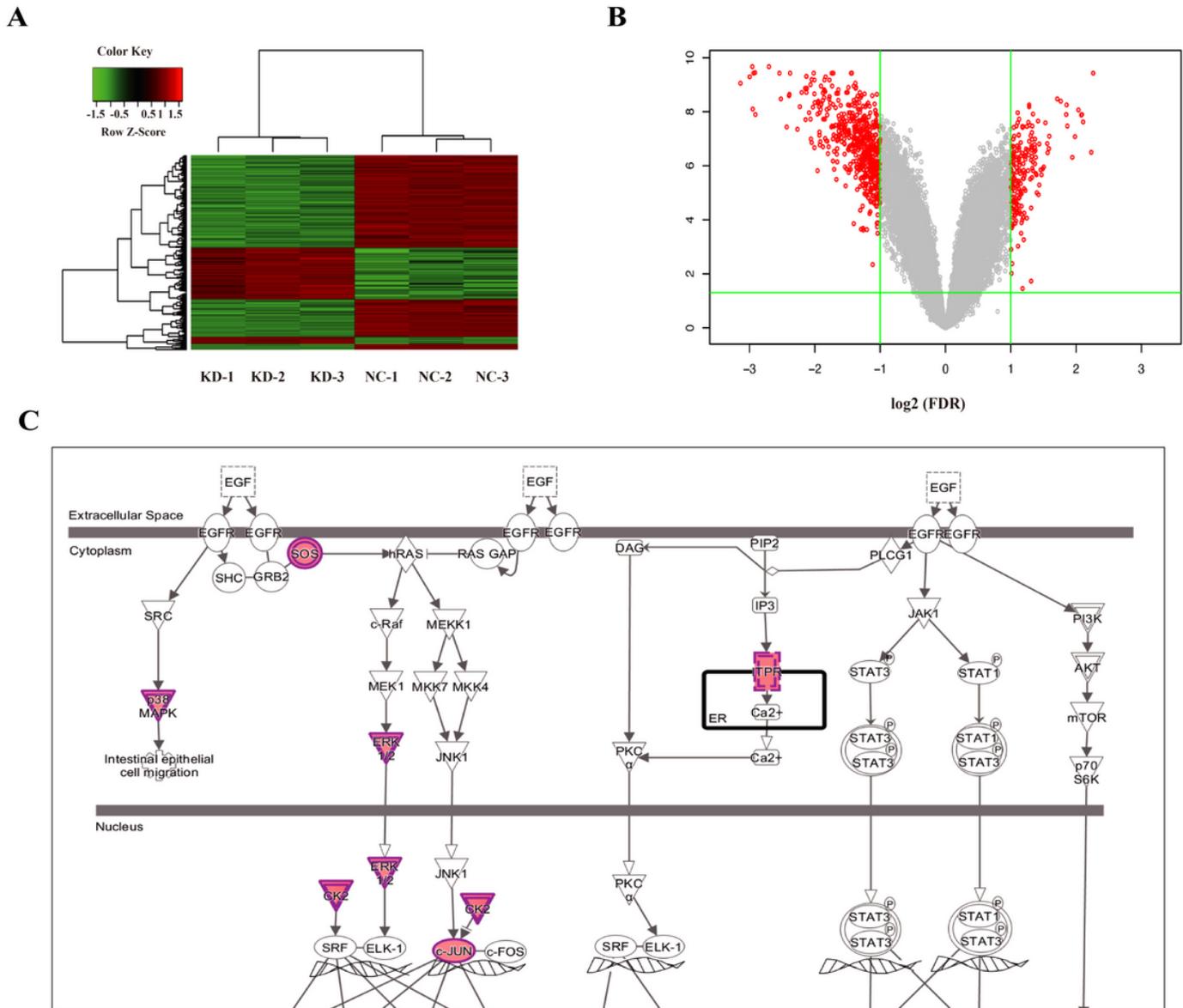


Figure 4

Gene chip analysis and Ingenuity Pathway Analysis (IPA). **A** The Hierarchical Clustering of the differentially expressed genes (DEGs) in CENPU knockdown group (KD) and normal control group (NC). **B** The volcano plot of the DEGs. The red dots are the significant differentially genes screened by the standard of Fold Change > 2 and FDR < 0.05. The gray dot are the other genes with no significant

difference. **C** Classical signal pathways based on DEGs according to IPA. The red shapes mean significantly up-regulated genes in KD group, compared to NC group.

Figure 5

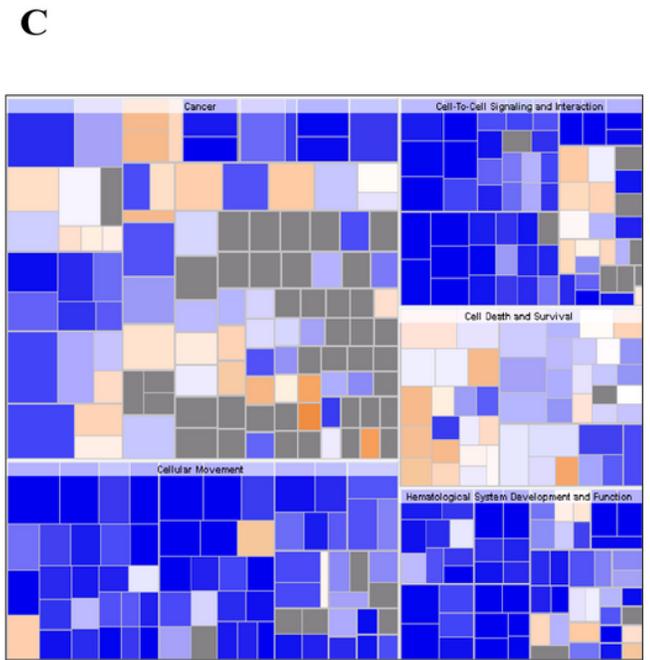
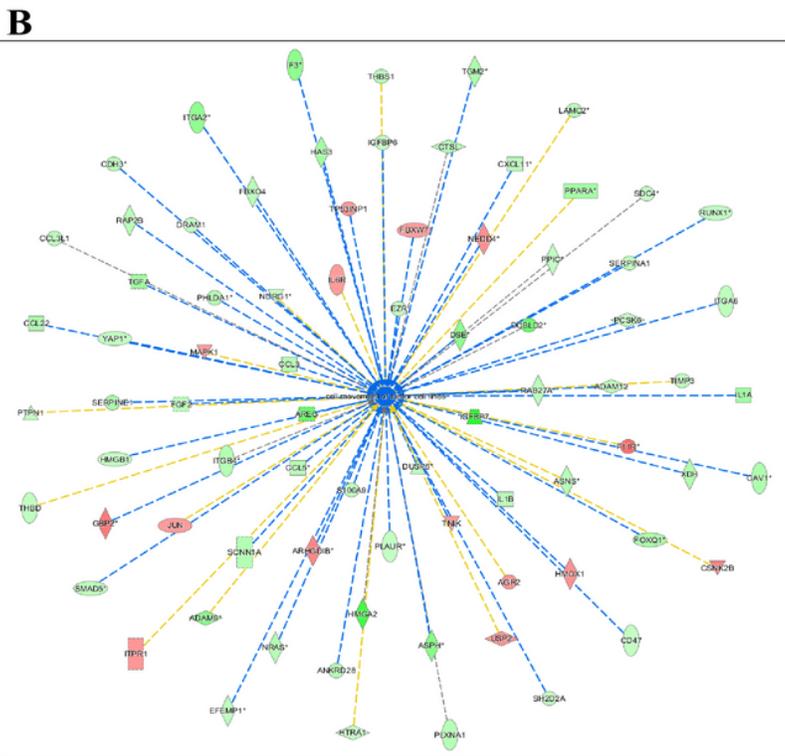
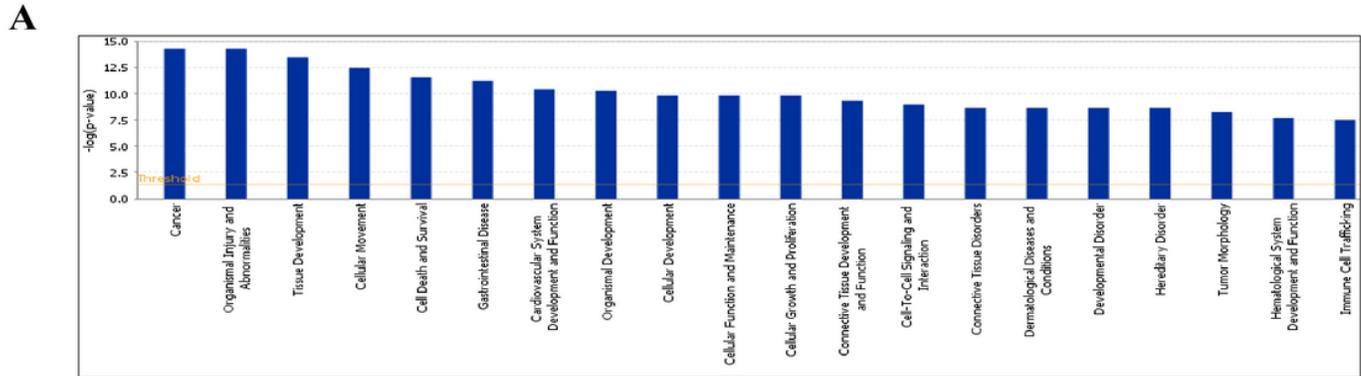
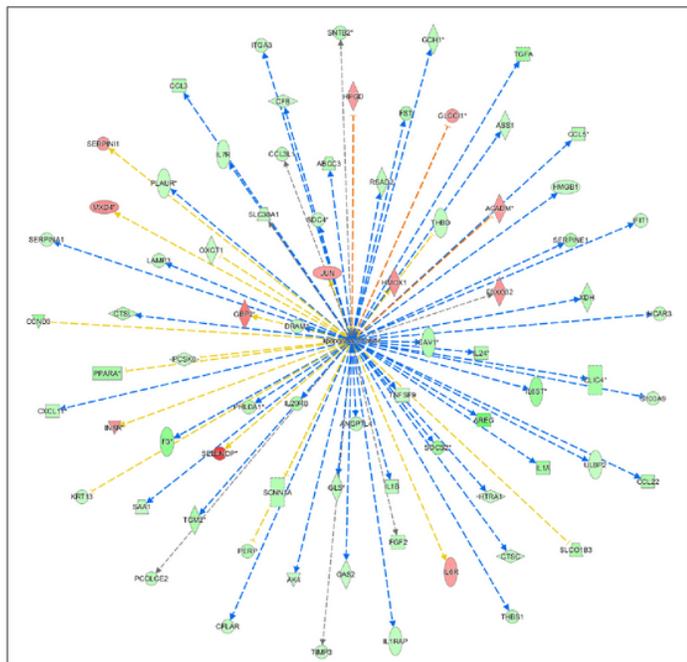


Figure 5

Ingenuity pathway analysis (IPA) of the differentially expressed genes (DEGs). **A** Diseases and function enrichment analysis of DEGs. **B** Network diagram of DEGs involved in cell movement of tumor cell lines. **C** Heat map of diseases and function that affected by DEGs. The orange line and blue line indicates that genes activate or inhibit cell movement of tumor cell lines, respectively. The gray line indicates that the regulatory relationship is unknown.

Figure 6

A



B

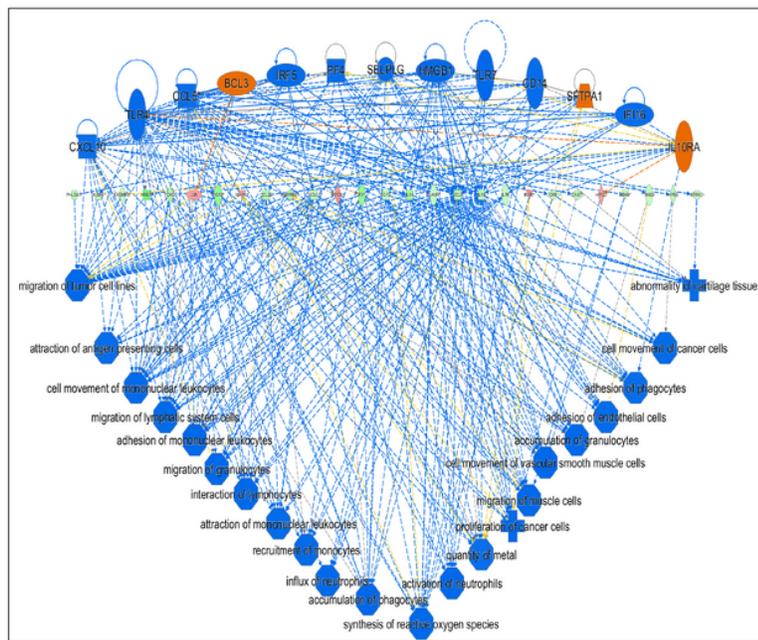


Figure 6

Upstream and downstream analysis of the differentially expressed genes (DEGs) based on Ingenuity pathway analysis (IPA). **A** Upstream regulatory network that can affect the expression of CENPU. **B** Down regulatory network of interactions between DEGs, regulators and functions. The orange line and blue line indicates that genes activate or inhibit cell movement of tumor cell lines, respectively. The gray line indicates that the regulatory relationship is unknown.

Figure 7

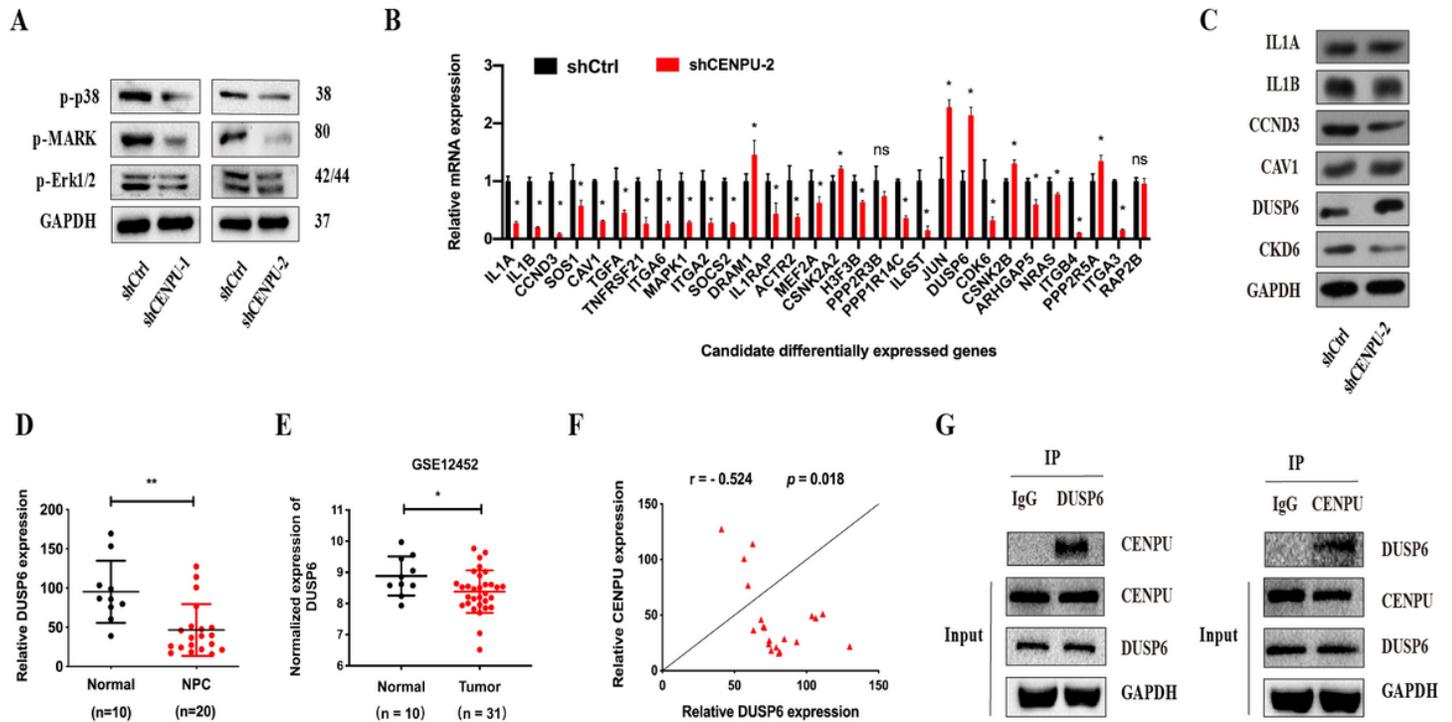


Figure 7

Effects of CENPU on signal pathways. **A** Expression of p-ERK1/2, p-p38 and p-MAPK by Western blot in CNE-2 cells transfected with shCENPU-1 and shCENPU-2. **B** Levels of downstream genes in CENPU knockdown CNE-2 cells by PCR. **C** 6 candidate downstream genes that were further tested by Western bolt. **D** Expression of DUSP6 in normal nasopharyngeal mucosa and NPC samples. **E** Expression of DUSP6 using database (GSE12452). **F** Correlations between CENPU and DUSP6 in NPC samples. **G** The lysate of CNE-2 cells was immunoprecipitated with CENPU, DUSP6 or IgG antibodies, followed by immunoblot analysis. * Statistically significant ($p < 0.05$), ns No significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.xlsx](#)