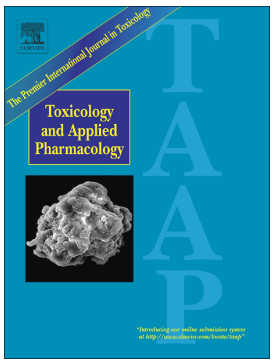

Sesamin suppresses NSCLC cell proliferation and induces apoptosis via Akt/p53 pathway

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Title page

Sesamin suppresses NSCLC cell proliferation and induces apoptosis via Akt/p53 pathway

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Running title: Sesamin inhibits NSCLC via Akt/p53

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Abstract

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer with a disappointing prognosis. The aim of this study was to investigate the anticancer effect of sesamin and the underlying mechanism. The MTT assay was used to detect the proliferation of NSCLC cells. The cell cycle and apoptosis were analyzed by flow cytometry. The protein levels of Akt, p-Akt (Ser473), p53, cyclin D1, CDK2, MDM2, p-MDM2 (Ser166) were detected by western blotting. The expression of p-Akt (Ser473), p53 and Ki67 *in vivo* was analyzed by IHC. Histopathologic analyses of major organs (heart, liver, spleen, lung and kidney) were performed by H&E staining. The results show that sesamin suppressed cell proliferation and induced apoptosis of NSCLC cells (A549 and H1792) in a dose-dependent manner. Treatment with sesamin caused cell cycle arrest at G1 phase and inhibited cyclin D1 and CDK2 expression. In addition, sesamin inhibited Akt activity and upregulated p53 expression both *in vivo* and *in vitro*. When Akt and p53 were suppressed by LY294002 and PFTα, respectively, sesamin exerted no additional effects. The *in vivo* results mostly matched the *in vitro* findings. Specifically, sesamin exerted little damage to major organs. Taken together, this study demonstrates that sesamin suppresses NSCLC cell proliferation by induction of G1 phase cell cycle arrest and apoptosis via Akt/p53 pathway. Therefore, sesamin may be a promising adjuvant treatment for NSCLC therapy.

Keywords

sesamin; NSCLC; cyclin D1; cell cycle arrest; p53; apoptosis

Abbreviations

NSCLC, non-small cell lung cancer; LY, LY294002; PFT*α,* Pifithrin-α

1. Introduction

Non-small cell lung cancer (NSCLC) takes up almost 85% of lung cancer cases, and is one of the leading causes of cancer-related mortalities in both men and women all over the world (Zheng et al., 2018). Although many new effective ways against lung cancer have been found in recent years, their severe toxicities *in vivo* greatly limit their application. Hence, it is vital to develop a safe and effective adjuvant therapy against NSCLC.

Cancer is characterized by uncontrollable cell proliferation due to the deregulated activity of various cell cycle-related proteins (Otto et al., 2017). CCND1 (a key gene determining G1/S transition) is amplified and its coded protein cyclin D1 is frequently overexpressed in NSCLC. The overexpression of cyclin D1 in NSCLC is a key factor involved in its malignant progression (Gautschi et al., 2007). Therefore, inhibition of redundant cyclin D1 in NSCLC may be an effective strategy to suppress its growth.

To find out the effective, nontoxic and economical phytochemicals is in urgent need to develop intervention therapy against cancer (Deep et al., 2008; Shu et al., 2010). Lignans are a large group of fiber-associated phenolic compounds widely distributed in edible plants (Saarinen et al., 2007). Increasing evidences have proved that lignans exert anti-cancer effect in various cancer types including breast cancer and lung cancer (Wu et al., 2013; Kim et al., 2010; Choi et al., 2015).

Sesamin, the most abundant lignan in sesame seed oil, has been well documented to have anti-hypertension, anti-thrombogenesis and anti-hypercholesterolemia properties (Tomoya Yokota et al., 2007; Noguchi et al., 2004). Besides, sesamin has also been shown to induce growth inhibition in MCF-7 cells by induction of cell cycle arrest via inhibition of cyclin D1 (Tomoya Yokota et al., 2007). A recent study revealed that sesamin could also induce apoptosis in lung cancer cells possibly through inhibition of COX-2/PI3K/Akt axis (Fang et al., 2019). However, the comprehensive mechanisms underlying sesamin-induced cyclin D1 downregulation and apoptosis in cancer cells remain unclear. Importantly, a previous report showed that no accumulation of sesamin was observed in the plasma and sesamin was confirmed to be safe and tolerable in healthy subjects (Tomimori et al., 2015). Nevertheless, whether sesamin suppresses NSCLC viability with a safe profile has not been identified.

This study was designed to explore the effect of sesamin on proliferation and apoptosis of NSCLC cells both *in vitro* and *in vivo*, as well as the underlying mechanisms. Our data demonstrate that sesamin induces G1 phase arrest of cell cycle and apoptosis in NSCLC cells via Akt/p53 pathway and causes little damage to major organs.

2. Materials and methods

2.1 Chemicals and antibodies

Sesamin (99.75% purity, S2392), Pifithrin-α (PFTα, S2929*)* and LY294002 (LY, S1105) were purchased from Selleck chemicals. 3-(4,5-Dimethylthiazol-2-yl)-2,5-

diphenyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and RIPA lysis buffer were purchased from Sigma (Beverly, MA, USA). Primary antibodies such as anti-p53 antibody (Cat#131442), anti-Ki67 antibody (Cat#15580) and anti-β-Tubulin antibody (Cat#6046) were purchased from Abcam. The anti-p-Akt (Ser473) antibody (Cat#4060), anti-Akt antibody (Cat#9272), anti-cyclin D1 antibody (Cat#2978), anti-p-MDM2 (Ser166) antibody (Cat#3521), anti-MDM2 antibody (Cat#86934), anti-CDK2 antibody (Cat#2546) and (HRP)–labeled anti-rabbit secondary antibody (Cat#7074) were purchased from Cell Signaling Technology.

2.2 Cell lines and culture

Human NSCLC cell lines (A549 and H1792) and the normal lung epithelial cell line (BEAS-2B) were originally obtained from ATCC. All these cell lines were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640, Gibco-BRL, USA). The culture medium was supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, USA) and penicillin/streptomycin (pen-strep, Gibco-BRL, USA). Cells were cultured in a humidified incubator at 37°C in a 5% CO_2 atmosphere.

2.3 Bioinformatic tool

STITCH (Version 5.0, http://stitch.embl.de/) is a web-based resource to explore known and predicted interactions of proteins and chemicals. Sesamin was submitted to the STITCH database under Homo sapiens organism (Hu et al., 2019). The biological process (BP) and cellular component (CC) of sesamin by GO analysis were shown.

2.4 Cell viability/proliferation assay

The protocol used for MTT assay of detection of cell viability was strictly according

to our previous study (Zhang et al., 2014). For detection of cell viability, 5×10^3 cells in 200 μL of serum-free culture RPMI-1640 medium were seeded in 96-well plates and incubated for 24 hours and 48 hours. And then, MTT was added to each well (with the final concentration of 0.5 mg/ml). After incubation at 37 °C for 4 hours, the plates were centrifuged at 450 × g for 5 minutes. Untransformed MTT was removed by aspiration, and formazan crystals were dissolved in DMSO (150 μl/well) and quantified spectrophotometrically at 563 nm.

2.5 Flow cytometry (FCM) analysis of cell cycle

The protocol of FCM analysis of cell cycle was according to a previous report (Du et al., 2016). Briefly, cells were washed and resuspended in cold PBS and incubated in ice-cold 70% ethanol for 4 hours. The cells were then centrifuged at 1000 rpms for 10 mins and resuspended in propidium iodide (PI) master mix (40 μg/ml PI and 100 μg/ml RNase A in PBS) at a density of 3×10^5 cells/ml and incubated at 37 °C for 30 mins before analysis with flow cytometry (BD FACS Calibur, USA) as described. The excitation and emission wavelengths of PI were 535/615 nm. The cell cycle phase analysis was performed by FlowJo V10 software.

2.6 Flow cytometry (FCM) analysis of apoptosis

Apoptotic cell death was determined by flow cytometry analysis using Annexin V-FITC and propidium iodide (PI) assay kit (BestBio, BB-4101). After 24-hour drug treatments, A549 and H1792 cells were collected. The cells were washed twice by cold PBS and resuspended by 400 μL 1x binding buffer. 5 μL of Annexin V-FITC was added to the cell suspension, gently mixed, and incubated at 4°C for 15 mins in the dark.

Finally, 10 μL of PI was added to the cell suspension, gently mixed, and incubated at 4°C for 5 mins in the dark. The samples were analyzed by flow cytometry (BD FACS Calibur, USA). The excitation and emission wavelengths of FITC were 490/525 nm. The excitation and emission wavelengths of PI were 535/615 nm. The apoptosis analysis was performed by FlowJo V10 software.

2.7 Western blotting analysis

After treating NSCLC cells (2×10^5 cells/well) with sesamin, the cells were collected, washed with PBS and lysed with RIPA buffer (0.5% protease inhibitor cocktail (APExBIO, #K1007) and 1% phosphatase inhibitor cocktail I). Protein quantification was performed using BCA Protein Assay Kit (Thermo Fisher Sientific, #23227). Equal amounts of protein were loaded onto the 15% SDS-PAGE gels for electrophoresis and transferred to polypropylene difluoride (PVDF) membranes. After blocking with 5% non-fatty milk in TBST for 1.5 hours, membranes were probed with their specific primary antibodies (diluted with 5% BSA to 1: 1000). And then, membranes were probed with horseradish peroxidase (HRP)–labeled anti-rabbit secondary antibody (diluted with 5% BSA to 1: 5000). Antibody binding was detected by enhanced chemiluminescence detection kit (ECL) (Thermo Fisher Scientific, #32016). The integrated density of each band was analyzed with Image J.

2.8 Xenograft models assay in vivo

The animal experiment was approved by the animal center of Guangdong pharmaceutical university (No. GDPU20170298). A549 cells (approximately 1.5×10^6 cells) were subcutaneously inoculated into the left flank of 5-6-week-old female nude

mice. When the tumors developed to about 80-100 mm^3, the mice were divided into three groups (n=5 for each group): 1) control group: treatment with solvent orally (thrice per week); 2) low dosage: treatment with sesamin orally (thrice per week, 100 mg/kg); 3) high dosage: treatment with 0sesamin orally (thrice per week, 150 mg/kg). The treatment lasted for 21 days and the tumor size was measured thrice per week (Wang et al., 2018). Tumor volumes were calculated with the formula: (mm^3) = ($L \times W^2$) $\times 0.5$.

The tumor tissues were analyzed by immunohistochemistry (IHC) using indicated antibodies as anti-p53, anti-p-Akt and anti-Ki67. The images were captured with the AxioVision Rel.4.6 computerized image analysis system (Carl Zeiss). The positive area was determined based on both the proportion of positively stained tumor cells and the intensity of staining with an analysis tool (Image J, version 1.80.0). The positive area of each image was analyzed by GraphPad 7 software.

The organ tissues (heart, liver, spleen, lung and kidney) were fixed in 4% paraformaldehyde, embedded in paraffin and cut into 2 μm sections, and subsequently stained with H&E for pathological analysis. The images were captured with the AxioVision Rel.4.6 computerized image analysis system (Carl Zeiss).

2.9 Statistical analysis

Experimental data were presented as means ± S.D. from three or more independent experiments and were analyzed with the unpaired Student's t test by using GraphPad 7 software. For the *in vivo* study, a log-linear mixed model with random intercept was used to compare the significance of the mean tumor volumes among each group. *P*

value of <0.05 was considered statistically significant.

3. Results

3.1 Sesamin suppresses cell cycle and cyclin D1 expression in NSCLC cells

Sesamin is a lignan with the molecular weight of 354.35 g/mol and its structure was shown in Fig. 1A. We then measured the effect of sesamin on NSCLC cells by MTT assay. Fig. 1B and Fig. 1C show that sesamin suppressed cell proliferation in A549 and H1792 cells in a dose-dependent manner. However, sesamin (10-30 μM) had little cytotoxicity in normal human bronchial epithelial BEAS-2B cells (supplementary Fig. 1). As shown in Fig. 1D and Fig. 1E, sesamin could induce apoptosis in A549 and H1792 cells in a dose-dependent manner after 24-hour incubation.

To further detect the effect of sesamin on NSCLC cells, we searched its interaction by STITCH (a search tool for interactions of chemicals). As shown in supplementary Fig. 2, sesamin might affect G1/S transition of mitotic cell cycle and cyclin-dependent protein kinase holoenzyme complex in cellular component (GO database). Therefore, we analyzed the effect of sesamin treatment on cell cycle of A549 and H1792 cells by flow cytometry assay. Fig. 2A and Fig. 2B show that sesamin significantly inhibited cell cycle by arresting G1 phase in A549 and H1792 cells in a dose-dependent manner. Besides, we found that sesamin down-regulated the protein level expression of cyclin D1 and CDK2 in a dose-dependent manner (Fig. 2C).

3.2 Sesamin exerts anti-proliferation and cell cycle arrest effects via the up-regulation of p53 expression

Next, we sought to explore the mechanism of sesamin-induced cell cycle arrest and apoptosis in NSCLC cells. P53 is a vital regulator of cell cycle in cancer (Ho et al., 2005). As shown in Fig. 3A and Fig. 3B, treatment with p53 inhibitor PFTα (20 μM) alone produced no obvious effect on NSCLC proliferation. However, PFTα was able to efficiently negate the sesamin's inhibitory effect on cell proliferation in both the NSCLC cell lines.

As shown in Fig. 3C and Fig. 3D, sesamin plus PFTα reduced the cell cycle distribution at G1 phase compared with sesamin group in A549 and H1792 cells. Similarly, PFTα significantly counteracted sesamin-induced apoptosis (Fig. 3E and Fig. 3F). Besides, Fig. 3G shows that the expression of p53 was upregulated by sesamin, which was significantly reduced by PFTα.

These data strongly support that p53 majorly mediates sesamin-induced proliferation inhibition and apoptosis in NSCLC cells.

3.3 Sesamin inhibits proliferation and cyclin D1 expression in NSCLC cells via Akt/p53 signalling

Akt inhibition results in cell cycle arrest (Rassidakis et al., 2005). Therefore, to determine the mechanism of sesamin in NSCLC cells, we detected the effect of sesamin on p-Akt (Ser473) expression. Fig. 4A and Fig. 4B show that LY294002 (a selective PI3K/Akt inhibitor) treatment produced a similar effect of cell proliferation inhibition with sesamin. When Akt activity was suppressed by LY294002, sesamin exerted no additional anti-proliferative effect. Similarly, the G1 phase and apoptosis of combination group (sesamin plus LY294002) had no significant changes compared with

LY294002 group (Fig. 4C-Fig. 4F). In addition, we also found that sesamin inhibited the expression of p-Akt (Ser473) and p-MDM2 (Ser 166) in A549 and H1792 cells (Fig. 4G).

To further determine the role of Akt in sesamin-induced proliferation inhibition, we detected the p53 and cyclin D1 expression in A549 and H1792 cells. The alterations of p53 and cyclin D1 caused by sesamin were similar to those by LY294002 treatment (Fig. 5A). In addition, the expression of p53 and cyclin D1 in combination group (sesamin plus LY294002) showed no significant alterations compared with LY294002 group (Fig. 5A). In addition, we observed that PFT-α significantly reversed down-regulation of cyclin D1 caused by sesamin (Fig. 5B).

Taken together, the above results indicate that sesamin induces NSCLC cell cycle arrest and apoptosis via inhibition of Akt/p53 pathway.

3.4 Inhibition of tumor growth by sesamin in xenograft models

To extend our observation *in vitro*, we evaluated the antitumor efficacy of treatment with low dosage and high dosage of sesamin for 21 days in the nude mice xenograft models. The tumor volume of sesamin group showed the significant alteration since 7th day compared with control group (Fig. 6A and Fig. 6B). After 21-day treatment, we observed that the dosage of 100 mg/kg (low dosage) and 150 mg/kg (high dosage) of sesamin significantly reduced tumor weight compared with control group (Fig. 6C). Importantly, the data of histological analysis of heart, liver, spleen, lung and kidney tissues showed no significant alterations between control group and sesamin treatment groups (Fig. 6D).

We performed IHC analysis of tumor tissues to examine the expression of Ki67 *in vivo*, which is a common proliferation marker. IHC analysis showed a markedly reduced Ki67 expression in tumors treated with sesamin (Fig. 7). Besides, the levels of p-Akt were decreased whereas the levels of p53 were increased in sesamin treatment groups compared with those in control group. These *in vivo* data strongly support that sesamin suppresses NSCLC growth via Akt/p53 pathway.

4. Discussion

Many anticancer agents can exert strong tumor growth inhibition *in vitro* but sometimes fail to inhibit tumor growth *in vivo* or cause severe side effects. As mentioned above, cyclin D1 is overexpressed in NSCLC both *in vivo* and *in vitro*. Overexpression of cyclin D1 results in imbalance of CDK activity and rapid cell growth out of control (Qie et al., 2016). In the present study, we found that sesamin suppressed cell proliferation in A549 and H1792 cells by induction of cell cycle arrest via inhibiting cyclin D1 expression

The p53 tumor suppressor belongs to a small family of related proteins that includes two other members-p63 and p73 (Levine et al., 2009). P53 is subjected to several oncogenic signals, which results in inhibition of cell growth. In addition, p53 also regulates many cellular fates including cell cycle arrest, apoptosis and senescence (Vousden et al., 2002). In our study, we found that sesamin up-regulated p53 expression both *in vitro* and *in vivo*. Inhibition of p53 reversed sesamin-induced proliferation inhibition and apoptosis in NSCLC cells. These data strongly support that sesamin

inhibits cell proliferation and induces apoptosis via up-regulation of p53 expression.

Akt alteration plays an important role in human malignancy including aberrant growth, apoptosis resistance and invasiveness of cancer cells. As a member of AGC (PKA/PKG/PKC) protein kinase family, activated Akt (p-Akt) has been shown to be present in 43–90% of NSCLC cases (Balsara et al., 2004). Besides, many clinical studies have shown that activation of Akt in NSCLC results in more aggressive diseases which correlates with poor prognosis for patients (Heavey et al., 2014). Inhibition of Akt has the potential to restore sensitivity to other modalities of treatments when administered as part of combination regimens (Curigliano at al., 2019). Therefore, inhibition of Akt may be an effective therapeutic approach in NSCLC (Ji et al., 2002). In this study, we found that sesamin and LY294002 induced G1 phase arrest and apoptosis in NSCLC cells. However, the combination group (sesamin plus LY294002) could not exert an additional effect compared with LY294002 group. Besides, the results clearly showed that sesamin could suppress Akt activity in NSCLC cells. Therefore, these results indicate that sesamin exerts its anti-cancer effect through inhibiting Akt activity

In this study, we found that either sesamin or LY294002 treatment enhanced p53 expression in NSCLC cells. When Akt activity was blocked by LY294002, sesamin could not induce further increase in p53 expression. Previous studies have confirmed that inhibition of Akt reduces MDM2-mediated degradation of p53 (Yoko et al., 2002; Wade et al., 2013). Our study observed that sesamin inhibited p-MDM2 expression in NSCLC cells. Therefore, it is suggested from our results that sesamin upregulates p53

expression via reducing MDM2-mediated degradation of p53 through Akt inhibition in NSCLC cells.

The present study also showed that sesamin significantly inhibited tumor growth *in vivo*. After 21-day administration, there were no obvious changes on major organs (heart, liver, spleen, lung and kidney) between control group and sesamin groups. Besides, the IHC data revealed that the levels of p-Akt were decreased whereas the levels of p53 were increased in sesamin treatment groups compared with those in control group. Taken together, these data suggest that sesamin exerts anti-proliferation effect via inhibition of Akt and enhancement of p53 without severe side effects *in vivo*.

In conclusion, our study demonstrates that sesamin suppresses proliferation and induces apoptosis in NSCLC cells via Akt/p53 pathway. Specifically, sesamin inhibits tumor growth *in vivo* without obvious damages to major organs. Therefore, sesamin may be a promising adjuvant agent for NSCLC therapy.

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Conflict of interests

The authors declare that they have no competing interests.

Authors' contributions

LZ and BL designed this study drafted the manuscript. YC and HL contributed equally to this work. YC, HL, WZ, WQ, CL, HH and ZY performed the experiments. YC and HL conducted the statistical analysis. All authors read and approved the final manuscript.

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Figure legends

Fig. 1. Sesamin suppresses cell proliferation and induces apoptosis in NSCLC cells. (A) The structure of sesamin. (B-C) The effects of sesamin on the cell viability in A549 and H1792 cells determined by MTT assay at the indicated concentrations after 24-hour and 48-hour incubation. *Significantly different from 24-hour control group, n=3, *$p<0.05$, **$p<0.01$, ***$p<0.001$; #Significantly different from 48-hour control group, n=3, #$p<0.05$, ##$p<0.01$, ###$p<0.001$. (D-E) Sesamin induced apoptosis in NSCLC cells at indicated concentrations after 24-hour incubation. *Significantly different from control group, n=3, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Fig. 2. Sesamin suppresses cell cycle and cyclin D1 expression in NSCLC cells. (A-B) Sesamin induced cell cycle arrest at G1 phase in A549 and H1792 cells at indicated concentrations after 24-hour incubation. *Significantly different from control group, n=3, *$p<0.05$, **$p<0.01$, ***$p<0.001$. (C) Sesamin down-regulated the expression of cyclin D1 and CDK2 at the indicated concentrations analyzed by western blotting after 12-hour incubation in A549 and H1792 cells. *Significantly different from A549 cell control group, n=3, *$p<0.05$, **$p<0.01$, ***$p<0.001$. #Significantly different from H1792 cell control group, n=3, #$p<0.05$, ##$p<0.01$, ###$p<0.001$.

Fig. 3. Sesamin exerts anti-proliferation, cell cycle arrest and apoptosis via up-regulation of p53 expression. (A-B) After administration of sesamin (30 μM), sesamin (30 μM) plus PFTα (20 μM) or PFTα (20 μM), the cell viability of A549 and H1792 cells was determined by MTT assay. NS, not significantly different from control groups. *Significantly different from 24-hour control group, n=3, ***$p<0.001$. #Significantly

different from 48-hour control group, n=3, ###$p<0.001$. +Significantly different from 24-hour sesamin treatment group, n=3, +++$p<0.001$. &Significantly different from 48-hour sesamin treatment group, n=3, &&&$p<0.001$. (C-D) PFTα (20 μM) reversed sesamin (30 μM)-induced cell cycle arrest in A549 and H1792 cells after 24-hour incubation. NS, not significantly different from control group, n=3. *Significantly different from control group, n=3, ***$p<0.001$, #Significantly different from sesamin group, n=3, ##$p<0.01$. (E-F) PFTα (20 μM) reversed sesamin (30 μM)-induced apoptosis in A549 and H1792 cells after 24-hour incubation. *Significantly different from control group, n=3, ***$p<0.001$, #Significantly different from sesamin group, n=3, ##$p<0.01$. (G) The expression of p53 in control, sesamin (30 μM), PFTα (20 μM) or sesamin (30 μM) plus PFTα (20 μM) groups in A549 and H1792 cells after 12-hour incubation. NS, not significantly different from A549 or H1792 control group. *Significantly different from A549 cell control group, n=3, **$p<0.01$. #Significantly different from H1792 cell control group, n=3, ##$p<0.01$. +Significantly different from A549 cell sesamin group, n=3, ++$p<0.01$. &Significantly different from H1792 cell sesamin group, n=3, ++$p<0.01$.

Fig. 4. Sesamin induces cell cycle arrest and apoptosis in NSCLC cells via Akt inhibition. (A-B) After administration of sesamin (30 μM), LY294002 (20 μM) or sesamin (30 μM) plus LY294002 (20 μM), the cell viability of A549 and H1792 cells was determined by MTT assay. NS, not significantly different from LY294002 group. *Significantly different from 24-hour control group, n=3, ***$p<0.001$. #Significantly different from 48-hour control group, n=3, ###$p<0.001$. (C-D) LY294002 (20 μM)

plus sesamin (30 μM) group showed no additional inhibition compared with LY294002 (20 μM) group on cell cycle in A549 and H1792 cells after 24-hour incubation. NS, not significantly different from LY294002 group. *Significantly different from control group, n=3, ***$p<0.001$. (E-F) LY294002 (20 μM) plus sesamin (30 μM) group showed no additional induction compared with LY294002 (20 μM) group on apoptosis in A549 and H1792 cells after 24-hour incubation. NS, not significantly different from LY294002 group. *Significantly different from control group, n=3, ***$p<0.001$. (G) Sesamin (30 μM) inhibited the expression of p-Akt (Ser473) and p-MDM2 (Ser 166) in A549 and H1792 cells after 12-hour incubation. *Significantly different from A549 cell control group, n=3, **$p<0.01$. #Significantly different from H1792 cell control group, n=3, ##$p<0.01$.

Fig. 5. Sesamin inhibits proliferation via Akt/p53 pathway in NSCLC cells. (A) The expression of p53 and cyclin D1 in sesamin (30 μM), LY294002 (20 μM) or sesamin (30 μM) plus LY294002 (20 μM) groups detected by western blot in A549 and H1792 cells after 12 hours incubation. NS, not significantly different from A549 or H1792 LY294002 group. *Significantly different from A549 cell control group, n=3, **$p<0.01$. #Significantly different from H1792 cell control group, n=3, ##$p<0.01$. (B) The expression of cyclin D1 in sesamin (30 μM) and sesamin (30 μM) plus PFTα (20 μM) groups detected by western blot in A549 and H1792 cells after 12 hours incubation. NS, not significantly different from control group. *Significantly different from A549 cell control group, n=3, **$p<0.01$. #Significantly different from H1792 cell control group, n=3, ##$p<0.01$.

Fig. 6. Inhibition of tumor growth by sesamin in xenograft models. (A) The tumor growth was inhibited in sesamin treatment groups compared with the control group. (B) Tumor volumes were significantly decreased in sesamin treatment groups compared with control group. *Significantly different from control group, n=5, *$p<0.05$,**$p<0.01$, ***$p<0.001$. (C) Tumor weight was significantly reduced in sesamin groups compared with control group. *Significantly different from control group, n=5, **$p<0.01$, ***$p<0.001$. (D) Histopathologic analyses of major organs from sesamin treatment groups and control group.

Fig. 7. Sesamin inhibits Akt activity and enhances p53 expression *in vivo*. The tumor tissues were stained with antibodies of p-Akt (Ser473), p53 and Ki67. Magnification, 20×. The positive staining of p-Akt (Ser473), p53 and Ki67 expression per field from paraffin-embedded sections of control groups or sesamin treatment groups was determined by immunohistochemistry and morphometric quantification. *Significantly different from control group. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

1. Sesamin suppresses NSCLC cell proliferation and viability.
2. Sesamin induces G1 phase cell cycle arrest and apoptosis via Akt/p53 pathway.
3. Sesamin inhibits NSCLC growth in vivo without damage to major organs.

Authors' contributions

Luyong Zhang and Bing Liu designed this study drafted the manuscript. Yueming Chen and Huachao Li contributed equally to this work. Yueming Chen, Huachao Li, Weinan Zhang, Wanchen Qi, Changpeng Lu, Huiliang Huang and Zhicheng Yang performed the experiments. Yueming Chen and Huachao Li conducted the statistical analysis. All authors read and approved the final manuscript.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplementary Fig.1. The effects of sesamin on the cell viability in BEAS-2B cell determined by MTT assay at the indicated concentrations after 24-hour and 48-hour incubation. NS, not significantly different from 24-hour or 48-hour control group. *Significantly different from 24-hour control group, n=3, *$p < 0.05$; #Significantly different from 48-hour control group, n=3, #$p < 0.05$.

Supplementary Fig.2. The Chemical-protein interaction networks of sesamin were analysed by STITCH.

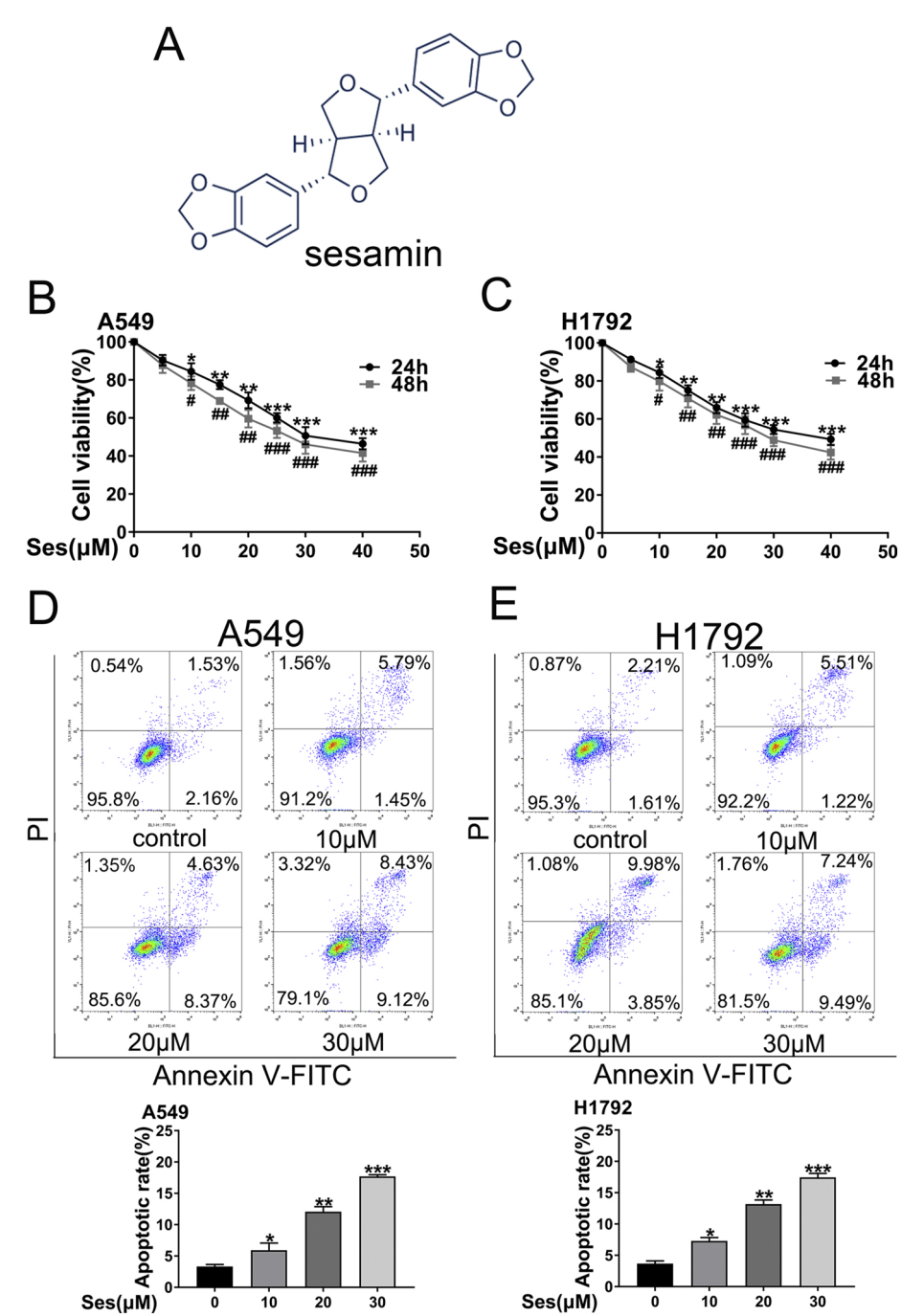

A
sesamin
B
A549
Cell viability(%)
24h
48h
Ses(μM)
C
H1792
Cell viability(%)
24h
48h
Ses(μM)
D
A549
0.54%
1.53%
95.8%
2.16%
control
1.56%
5.79%
91.2%
1.45%
10μM
1.35%
4.63%
85.6%
8.37%
20μM
3.32%
8.43%
79.1%
9.12%
30μM
PI
Annexin V-FITC
E
H1792
0.87%
2.21%
95.3%
1.61%
control
1.09%
5.51%
92.2%
1.22%
10μM
1.08%
9.98%
85.1%
3.85%
20μM
1.76%
7.24%
81.5%
9.49%
30μM
PI
Annexin V-FITC
A549
Apoptotic rate(%)
Ses(μM)
H1792
Apoptotic rate(%)
Ses(μM)

Figure 1

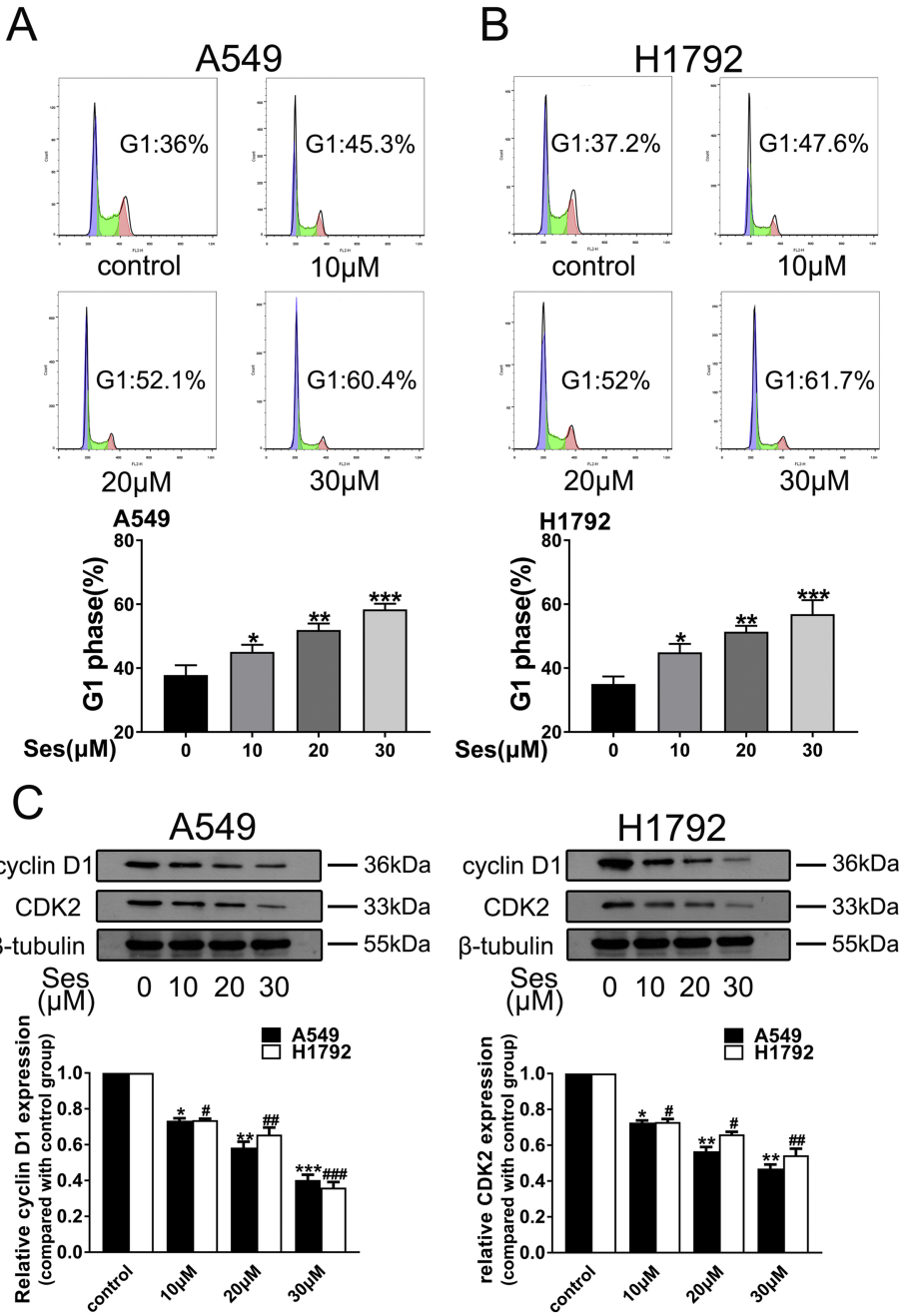

A
A549
G1:36%
G1:45.3%
control
10μM
G1:52.1%
G1:60.4%
20μM
30μM
B
H1792
G1:37.2%
G1:47.6%
control
10μM
G1:52%
G1:61.7%
20μM
30μM
A549
G1 phase(%)
Ses(μM)
0
10
20
30
H1792
G1 phase(%)
Ses(μM)
0
10
20
30
C
A549
cyclin D1
CDK2
β-tubulin
36kDa
33kDa
55kDa
Ses (μM)
0 10 20 30
H1792
cyclin D1
CDK2
β-tubulin
36kDa
33kDa
55kDa
Ses (μM)
0 10 20 30
A549
H1792
Relative cyclin D1 expression (compared with control group)
control
10μM
20μM
30μM
A549
H1792
relative CDK2 expression (compared with control group)
control
10μM
20μM
30μM

Figure 2

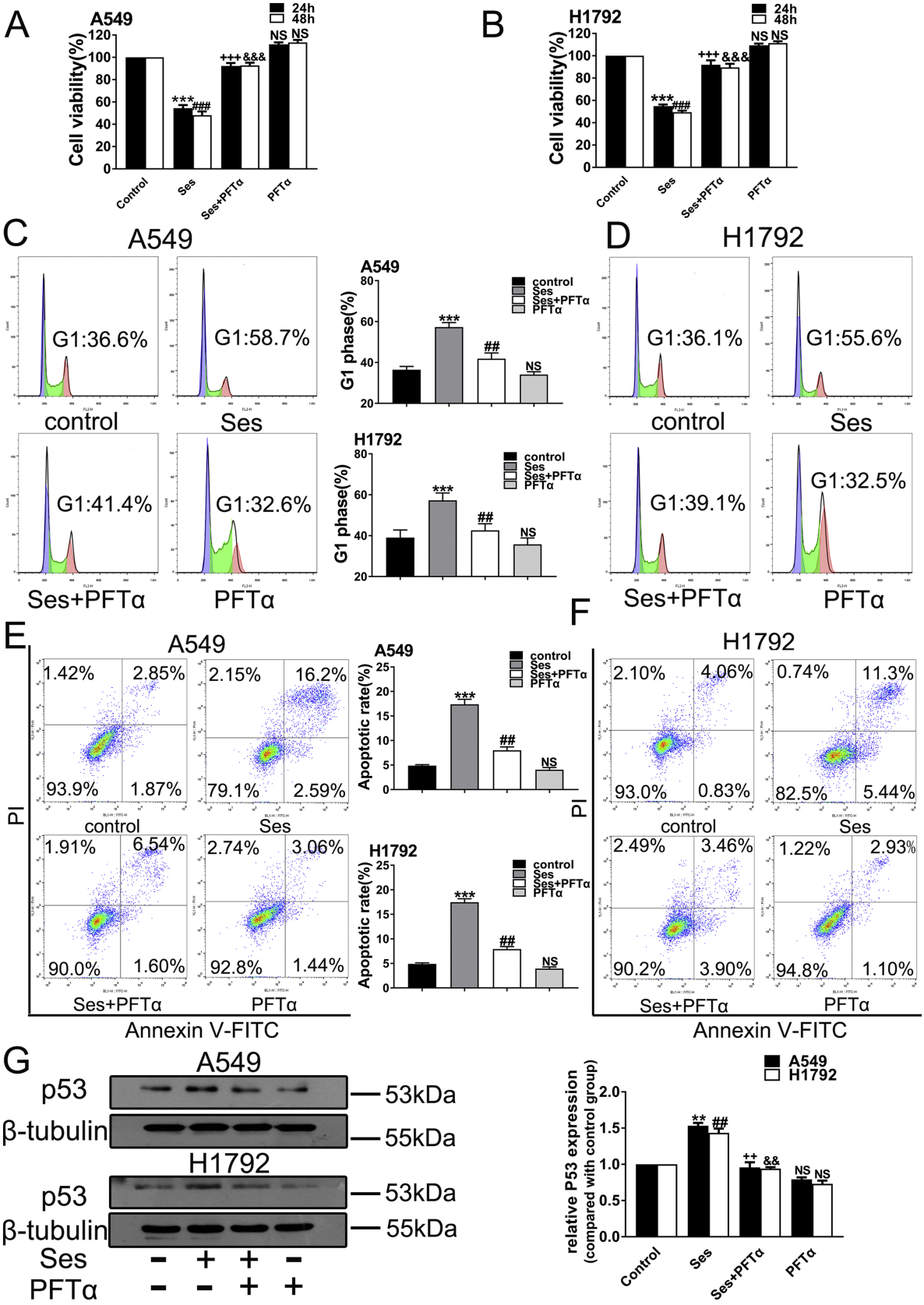

A
A549
24h
48h
Cell viability(%)
Control
Ses
Ses+PFTα
PFTα
B
H1792
C
A549
G1:36.6%
G1:58.7%
G1:41.4%
G1:32.6%
control
Ses
Ses+PFTα
PFTα
G1 phase(%)
D
H1792
G1:36.1%
G1:55.6%
G1:39.1%
G1:32.5%
E
A549
1.42%
2.85%
93.9%
1.87%
2.15%
16.2%
79.1%
2.59%
1.91%
6.54%
90.0%
1.60%
2.74%
3.06%
92.8%
1.44%
PI
Annexin V-FITC
Apoptotic rate(%)
F
H1792
2.10%
4.06%
93.0%
0.83%
0.74%
11.3%
82.5%
5.44%
2.49%
3.46%
90.2%
3.90%
1.22%
2.93%
94.8%
1.10%
G
p53
β-tubulin
53kDa
55kDa
Ses
PFTα
relative P53 expression
(compared with control group)

Figure 3

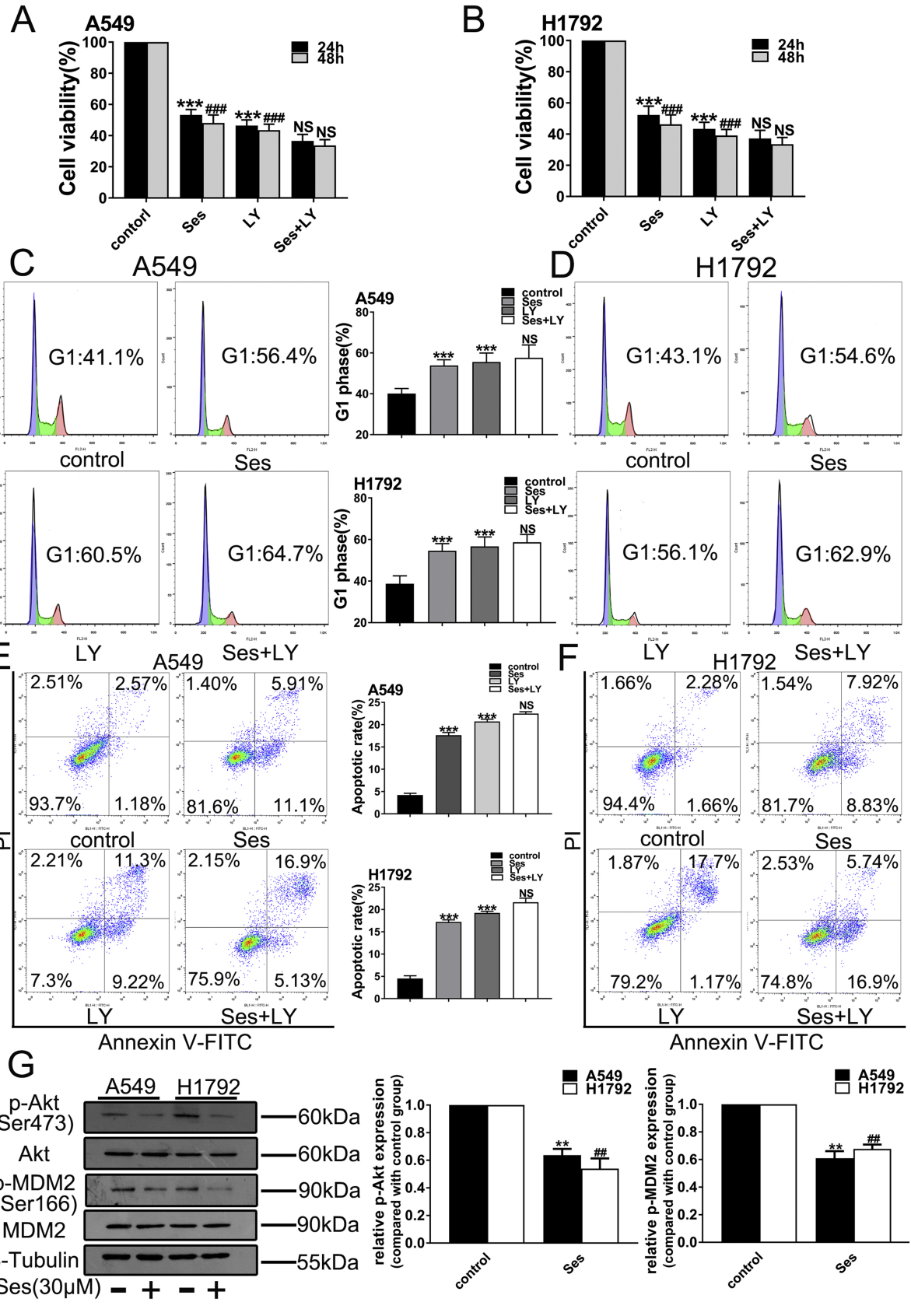

A
A549
Cell viability(%)
24h
48h
***
###
NS
contorl
Ses
LY
Ses+LY
B
H1792
control
C
A549
G1:41.1%
G1:56.4%
G1 phase(%)
control
Ses
LY
Ses+LY
G1:60.5%
G1:64.7%
D
H1792
G1:43.1%
G1:54.6%
G1:56.1%
G1:62.9%
E
A549
2.51%
2.57%
93.7%
1.18%
1.40%
5.91%
81.6%
11.1%
2.21%
11.3%
7.3%
9.22%
2.15%
16.9%
75.9%
5.13%
Apoptotic rate(%)
PI
Annexin V-FITC
F
H1792
1.66%
2.28%
94.4%
1.66%
1.54%
7.92%
81.7%
8.83%
1.87%
17.7%
79.2%
1.17%
2.53%
5.74%
74.8%
16.9%
G
A549
H1792
p-Akt (Ser473)
Akt
p-MDM2 (Ser166)
MDM2
β-Tubulin
60kDa
90kDa
55kDa
Ses(30μM)
relative p-Akt expression (compared with control group)
relative p-MDM2 expression (compared with control group)
**
##

Figure 4

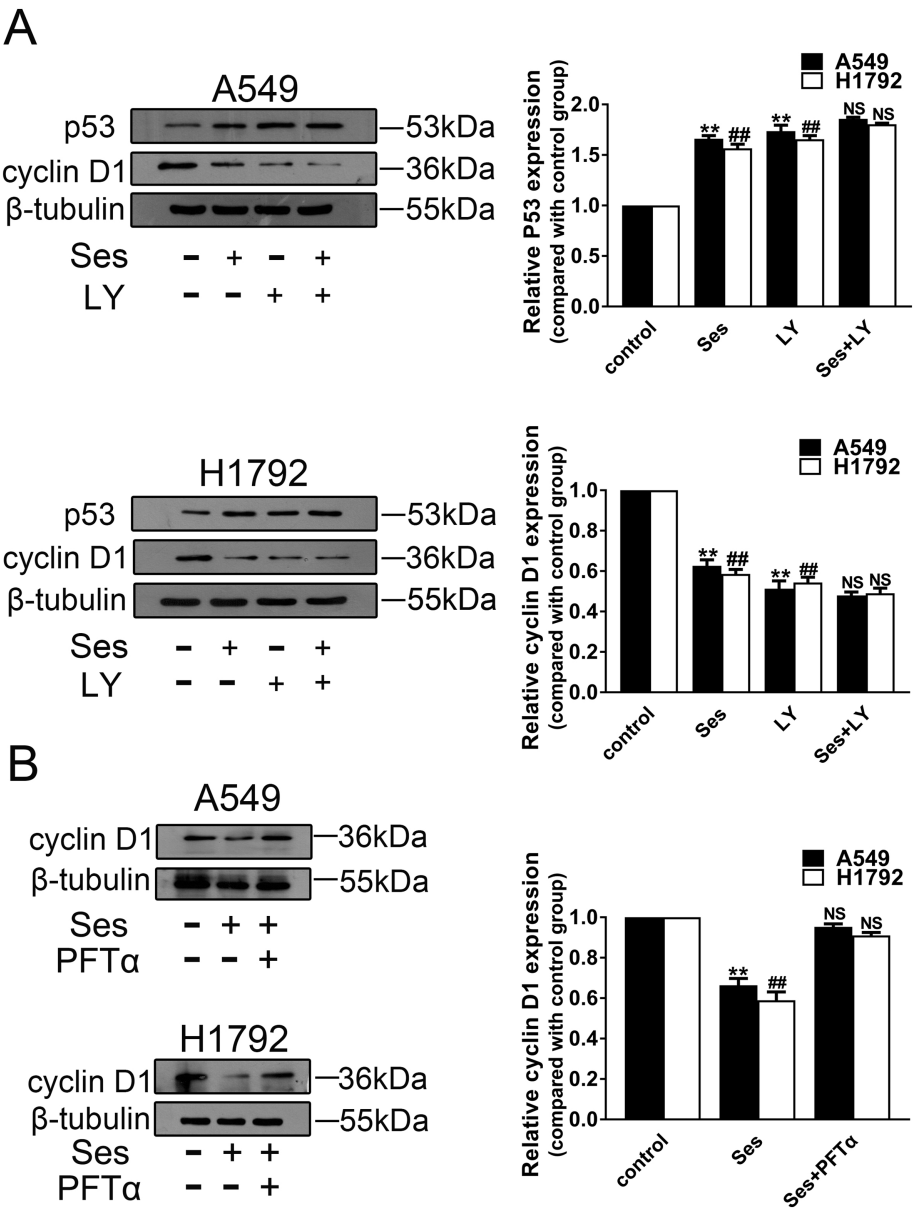

A
A549
p53
cyclin D1
β-tubulin
—53kDa
—36kDa
—55kDa
Ses
LY
Relative P53 expression
(compared with control group)
A549
H1792
control
Ses
LY
Ses+LY
H1792
Relative cyclin D1 expression
(compared with control group)
B
PFTα
Ses+PFTα

Figure 5

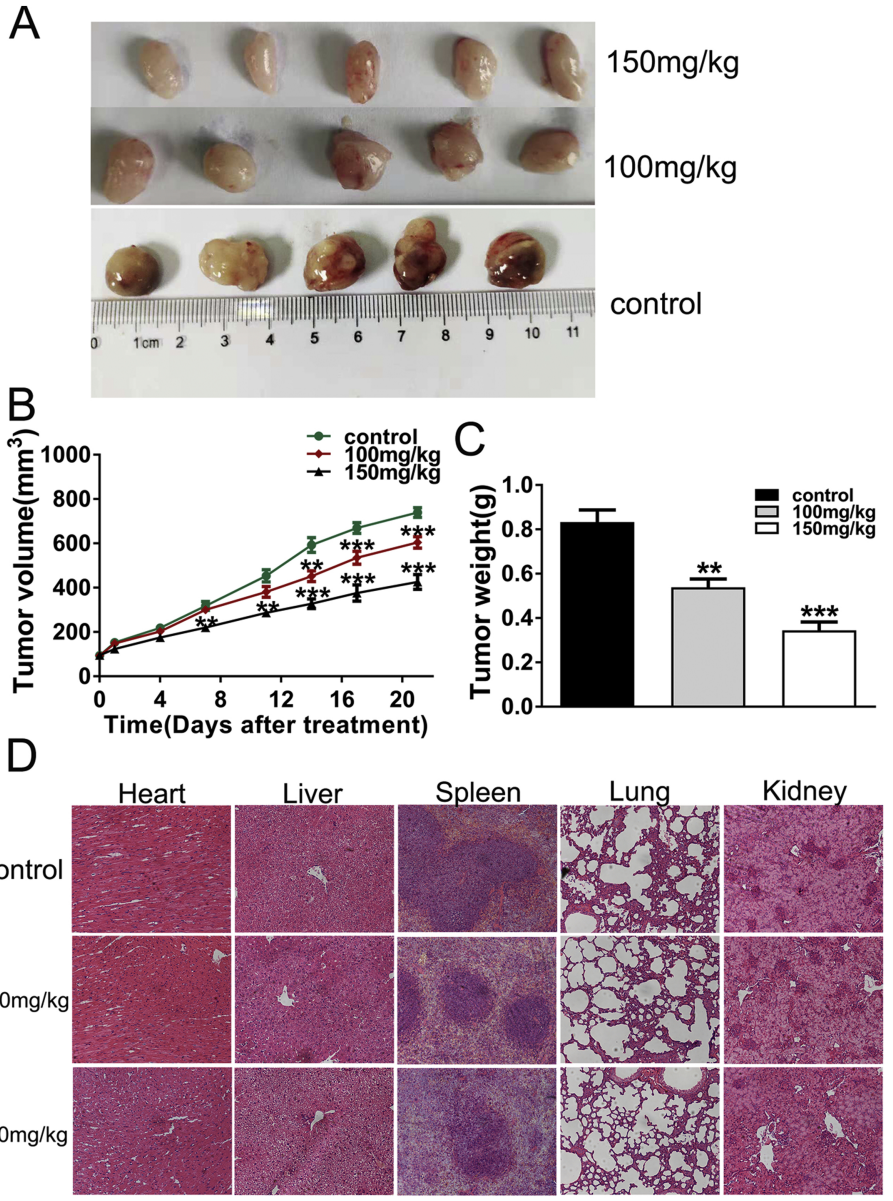
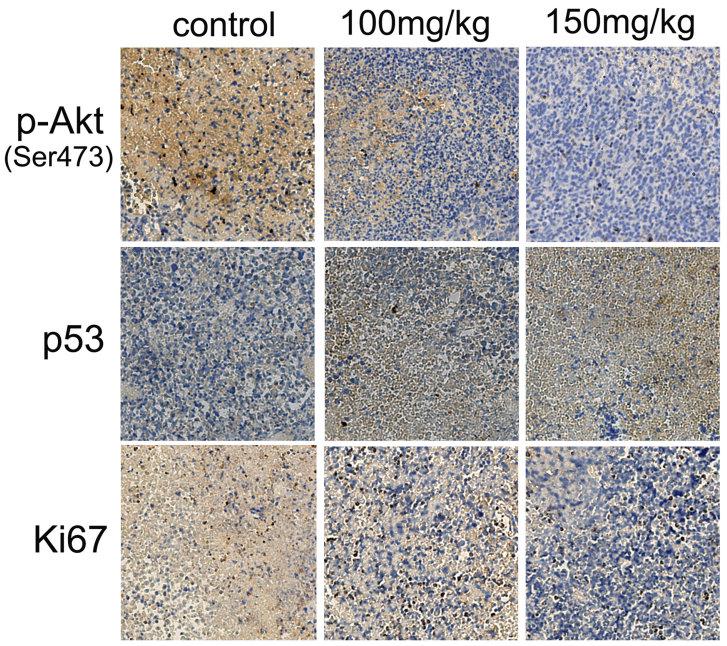
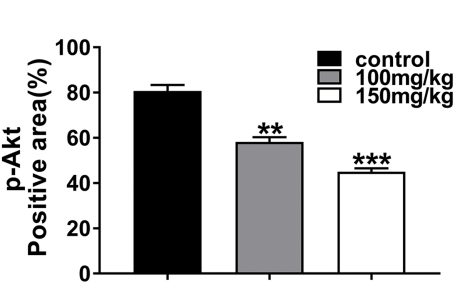
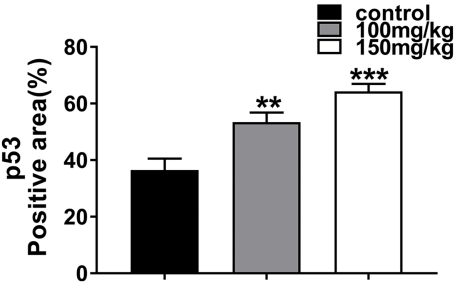

A
150mg/kg
100mg/kg
control
0
1cm
2
3
4
5
6
7
8
9
10
11
B
Tumor volume(mm3)
1000
800
600
400
200
0
control
100mg/kg
150mg/kg
**
***
0
4
8
12
16
20
Time(Days after treatment)
C
Tumor weight(g)
1.0
0.8
0.6
0.4
0.2
0.0
control
100mg/kg
150mg/kg
**
***
D
Heart
Liver
Spleen
Lung
Kidney
control
100mg/kg
150mg/kg

Figure 6


control
100mg/kg
150mg/kg
p-Akt
(Ser473)
p53
Ki67


p-Akt
Positive area(%)
100
80
60
40
20
0
control
100mg/kg
150mg/kg
**
***


p53
Positive area(%)
80
60
40
20
0
control
100mg/kg
150mg/kg
**
***

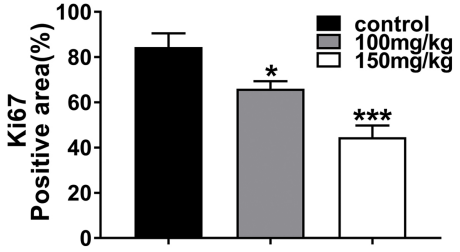

Ki67
Positive area(%)
100
80
60
40
20
0
control
100mg/kg
150mg/kg
*
***

Figure 7

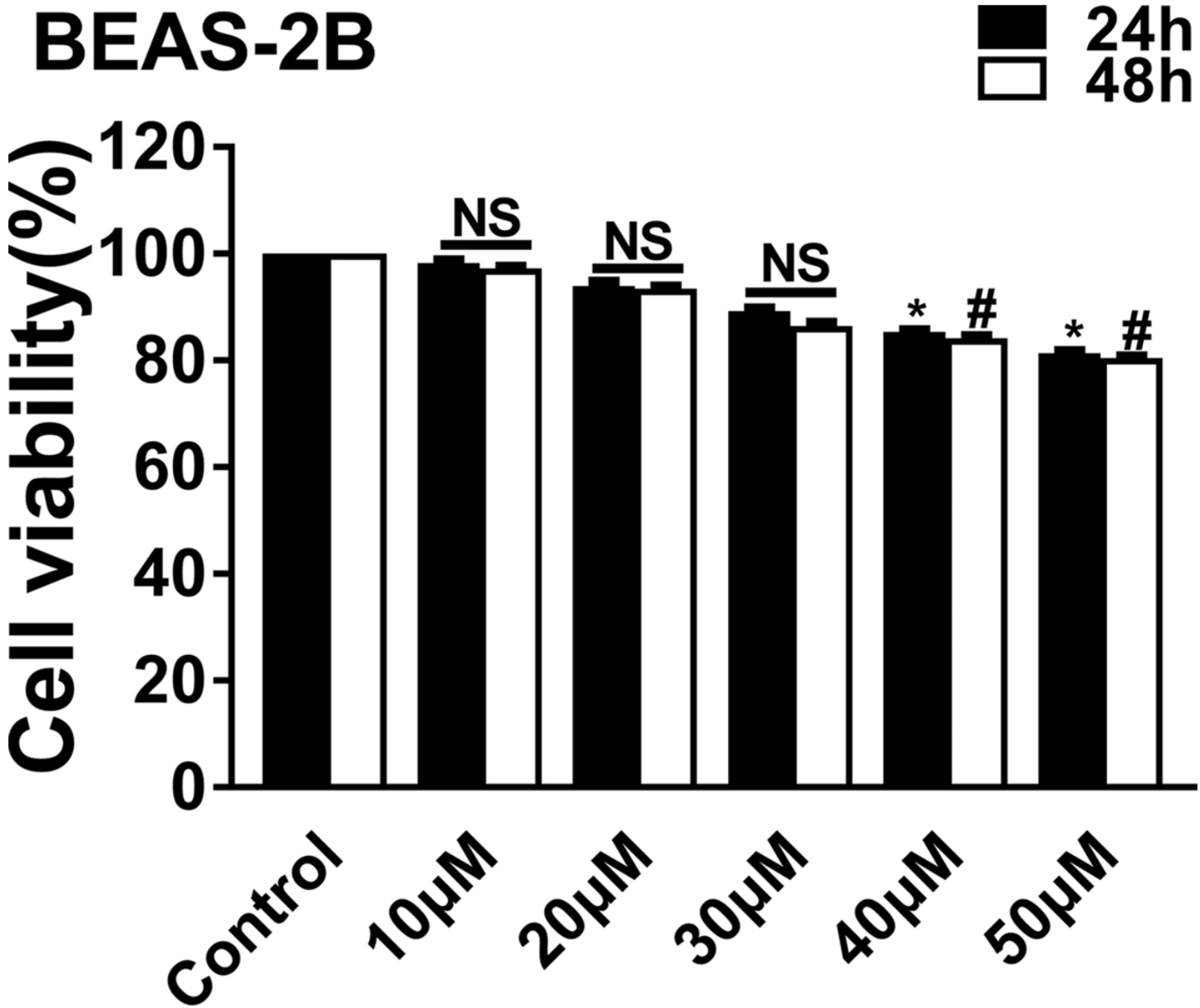
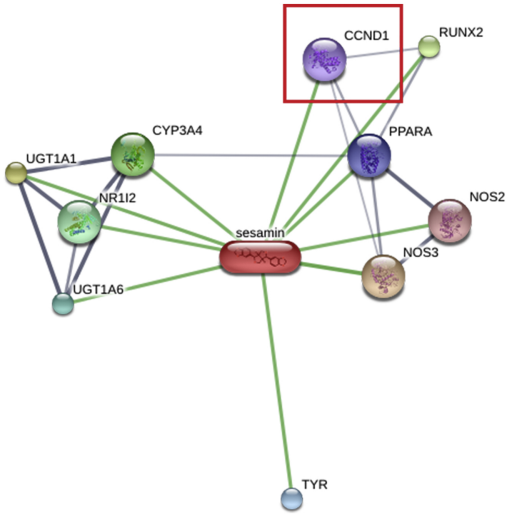

BEAS-2B
24h
48h
Cell viability(%)
120
100
80
60
40
20
0
NS
NS
NS
*
#
*
#
Control
10μM
20μM
30μM
40μM
50μM

Figure 8



Biological Process (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0000082	G1/S transition of mitotic cell cycle	10	2.54e-12
GO:0044772	mitotic cell cycle phase transition	11	7.46e-12
GO:0033993	response to lipid	13	5.8e-11
GO:0009628	response to abiotic stimulus	13	3.79e-09
GO:0000278	mitotic cell cycle	11	8.51e-08
			(more ...)

Cellular Component (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0000307	cyclin-dependent protein kinase holoenzyme complex	5	3.33e-09
GO:0005654	nucleoplasm	14	1.06e-05
GO:0031981	nuclear lumen	14	6.87e-05
GO:0070557	PCNA-p21 complex	2	0.000274
GO:0005634	nucleus	17	0.00041
			(more ...)

Figure 9