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KIF15 promotes pancreatic cancer proliferation via the MEK–ERK signalling pathway

Jie Wang[1,2,6], Xingjun Guo[3,6], Chencheng Xie[4] and Jianxin Jiang*[,1,5]

[1]*Department of Hepatic-Biliary-Pancreatic Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, China;* [2]*Department of Hepatic-Biliary-Pancreatic Surgery, The Affiliated Hospital of Guizhou Medical University, Guiyang 550000, China;* [3]*Department of Biliary-Pancreatic Surgery, Affiliated Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China;* [4]*University of South Dakota, Sanford School of Medicine, Department of Internal Medicine, Vermillion, SD 57105, USA and* [5]*Hubei Key Laboratory of Digestive System Disease, Wuhan 430060, China*

Background: Pancreatic cancer is highly malignant and characterised by rapid and uncontrolled growth. While some of the important regulatory networks involved in pancreatic cancer have been determined, the cancer relevant genes have not been fully identified.

Methods: We screened genes that may control proliferation in pancreatic cancer in seven pairs of matched pancreatic cancer and normal pancreatic tissue samples. We examined KIF15 expression in pancreatic cancer tissues and the effect of KIF15 on cell proliferation *in vitro* and *in vivo*. The mechanisms underlying KIF15 promotion of cell proliferation were investigated.

Results: mRNA microarray and functional analysis identified 22 genes that potentially play an important role in the proliferation of pancreatic cancer. High-content siRNA screening evaluated whether silencing these 22 genes affected proliferation of pancreatic cancer. Notably, silencing KIF15 exhibited the most potent inhibition of proliferation compared with the rest of the 22 genes. KIF15 was upregulated in human pancreatic cancer tissues, and higher KIF15 expression levels correlated with shorter patient survival times. Upregulation KIF15 promoted pancreatic cancer growth. KIF15 upregulated cyclin D1, CDK2, and phospho-RB and also promoted G1/S transition in pancreatic cancer cells. KIF15 upregulation activated MEK–ERK signalling by increasing p-MEK and p-ERK levels. MEK–ERK inhibitors successfully inhibited cell cycle progression, and PD98059 blocked KIF15-mediated pancreatic cancer proliferation *in vivo* and *in vitro*.

Conclusions: This study identified KIF15 as a critical regulator that promotes pancreatic cancer proliferation, broadening our understanding of KIF15 function in tumorigenesis.

Pancreatic cancer (PC) is generally acknowledged as being difficult to diagnose and treat. Despite improvements in treatment, the low patient survival rate is partly attributed to early, extensive local tumour invasion and distant metastasis, as well as multiple chemoresistance (Rombouts *et al*, 2015). The development of PC is a multistep process based on the accumulation of genetic and epigenetic alterations. Therefore, obtaining a better understanding of the molecular mechanisms responsible for the rapid growth, distant metastasis and tumorigenic properties of PC is likely to lead to novel therapeutic strategies for this disease.

We screened for genes with abnormal overexpression in PC using seven matched pairs of PC and adjacent, normal pancreatic

*Correspondence: Professor J Jiang; E-mail: jjx731003@163.com
[6]These authors contributed equally to this work.

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tissues with a gene expression profile microarray. We verified the function of the identified genes by high-content siRNA screening. Using this approach, we found that *KIF15*, a gene encoding a member of the kinesin family of proteins, may play a vital role in PC proliferation.

The kinesin superfamily comprises a group of proteins that share a highly conserved motor domain (Minakawa *et al*, 2013; Goulet *et al*, 2014). Most KIF proteins have ATP-dependent activity and can drive microtubule-dependent plus-end motion (Florian and Mayer, 2011). Kinesins participate in several essential cellular processes including mitosis, meiosis, and the transport of macromolecules (Liu *et al*, 2010). Increasing evidence indicates that kinesin proteins play critical roles in the genesis and development of human cancers (Yokota *et al*, 2012; Minakawa *et al*, 2013). Some kinesin proteins are associated with malignancy as well as with drug resistance in solid tumours (Buster *et al*, 2003). Thus, targeting KIF may be a promising anticancer strategy. KIF inhibitors such as kinesin spindle protein have been investigated as a monotherapy or in combination with other drugs in clinical trials. Other kinesins with potential anticancer characteristics are still being discovered (Song, 2015).

In this study, we demonstrate that KIF15 promotes PC proliferation via the MEK–ERK signalling pathway. This result provides insight into the molecular mechanisms of pancreatic carcinogenesis and suggests a novel therapeutic strategy for PC.

MATERIALS AND METHODS

Ethical statement. This study was approved by the Human Research Ethics Committees at the Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST), and was carried out in accordance with the principles embodied in the Declaration of Helsinki (more details can be found in the Supplementary Materials and Methods).

All *in vivo* animal experiments were approved by the Committee on the Ethics of Animal Experimentation of HUST (Permit no. 2015-S207). All treatments were carried out according to the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals. All surgical procedures were conducted under sodium pentobarbital anaesthesia and every effort was made to minimise animal suffering.

Cell culture. MIA-PaCa-2 and PANC-1 cell lines were purchased from ATCC (Manassas, USA). The two cell lines were previously authenticated by ATCC via STR typing. MIA-PaCa-2 and PANC-1 cells were grown in DMEM medium (Gibco, Waltham, USA) supplemented with 10% fetal bovine serum (Gibco), 100 U ml^{-1} penicillin G, and 100 μg ml^{-1} streptomycin (Sigma, Shanghai, China) at 37 °C in a humidified atmosphere containing 5% CO_2.

Human tissue samples. A PC tissue microarray (HPan-Ade180-Sur-01) was obtained from Shanghai Outdo Biotech (Shanghai, China).

Surgical specimens of PCs and adjacent normal pancreatic tissue were obtained from 27 PC patients who underwent surgical resection from January 2013 to December 2015. The 27 PC patients comprised 12 men and 15 women with a mean age of 60.4 years (range, 45–79 years). All 27 cancer specimens were histologically classified as adenocarcinomas. This study was approved by the Human Research Ethics Committees at the Tongji Hospital, Tongji Medical College, HUST.

RNA microarrays. Total RNA extracted from seven PC tissue samples and matching normal pancreatic tissues were screened for differentially expressed genes using an Agilent RNA 6000 Nano Kit (Agilent, Santa Clara, CA, USA). In another experiment, total RNA was extracted from three KIF15 knocked-down (KIF15D) samples and three negative control (NC) samples using an Agilent RNA 6000 Nano Kit and a PrimeView Human GeneChip (Agilent) was used for microarray analysis. RNA labelling and hybridisation to Agilent miRNA microarray chips were performed with a GeneChip Hybridization Wash and Stain Kit (Agilent). Microarray data were deposited in the NCBI Gene Expression Omnibus public database (http://www.ncbi.nlm.nih.gov/geo/).

High-content screening and cell growth curve analysis. PANC-1 cells were transfected with a KIF15-KD or NC lentivirus (Supplementary Material) and seeded into 48-well plates. GFP expression was observed using a fluorescence microscope. When cells reached 80% confluency, they were collected for further experiments. A total of 2000 cells per well were analysed once a day using a Cellomics ArrayScan System. By adjusting the input parameters, cells could be quantified by measuring the green fluorescence signal in each well. Data were collected for statistical analysis using 5-day cell proliferation curves. Cells on the scanned image were counted using image analysis software. The number of cells at each time point was compared with the cell count on day 1 to obtain a cell proliferation ratio for each time point for each experimental group, and the fold change in proliferation was used to produce a cell growth curve. The cell proliferation ratio was calculated as follows: fold change (NC *vs* experimental group) = proliferation ratio on day 5 for the NC group/proliferation ratio on day 5 for the experimental group. A fold change in the proliferation ratio of two or more indicated that cell proliferation had slowed down sufficiently to allow the effect of RNAi lentivirus infection on cell proliferation to be measured.

Animals. Female 6-week-old Balb/c nude mice were purchased from HFK Bioscience (Beijing, China). All mice were maintained under specific pathogen-free conditions in the Central Animal Laboratory, HUST.

Orthotopic transplantation of mouse PC cells in Balb/c nude mice. Female 8-week-old wild-type Balb/c nude mice were used in all experiments. For orthotopic implantation, hair was removed from the abdomen under pentobarbital sodium anesthesia. An abdominal longitudinal incision was then made to expose the pancreas, and a 20 μl cell suspension (approximate 2×10^6 cells) was injected directly into the pancreas. The incision was then closed with sutures. PD98059 was diluted in dimethyl sulfoxide (DMSO) solution to a concentration of 1.0 mg ml^{-1}, and from day 1 onward, mice were subcutaneously injected with 40 μg kg^{-1} · per day (Chen *et al*, 2015). All mice were routinely weighed and checked for signs of distress. Abdominal palpation was used to monitor tumour size. Mice were killed using CO_2 narcosis followed by cervical dislocation at the end of the study period, or earlier if they appeared moribund or exhibited $>$15% weight loss. Tumours were evaluated both macroscopically and microscopically.

Subcutaneous transplantation of human PC cells in Balb/c nude mice. Female 6-week-old Balb/c nude mice were used in all experiments. A total of 2×10^6 transfected cells were subcutaneously injected into the right armpit of Balb/c nude mice. The weight of each mouse and the tumour diameter were measured every week. All mice were killed 9 weeks after initiation of treatment. Tumours were evaluated macroscopically and microscopically.

Statistical analyses. Results for continuous variables are presented as means ± s.d. unless otherwise stated. Treatment groups were compared using independent sample *t*-tests. Pairwise multiple comparisons used one-way ANOVA (two-sided). A *P* value of $<$0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics software version 17.0 (Chicago, IL, USA).

RESULTS

Identification of KIF15 as a critical gene that promotes PC proliferation. To identify genes with an essential role in PC tumorigenesis, we used mRNA microarray analysis to compare the mRNA expression profiles of seven pairs of matched PC and normal pancreatic tissue samples. The results identified 892 upregulated mRNAs and 568 downregulated mRNAs in the PC group compared with the control group (Figure 1A). On the basis of a functional analysis (Supplementary Table 1), 22 genes were found to potentially play an important role in the proliferation of PC. A literature review found that VEGFA and BIRC5, proteins encoded by two of the identified genes, were previously shown to promote PC cell proliferation (Lang *et al*, 2008; Glienke *et al*, 2010). All 22 candidate genes were then silenced in PANC-1 cells to examine the potential effect on the proliferation of PC cells *in vitro* (Figure 1B). Knockdown of four candidate genes, *FAM54A*, *GMFB*, *KIF15*, and *ZWINT*, in PANC-1 cells reduced the cell proliferation rate to a greater extent than *VEGFA* or *BIRC5*

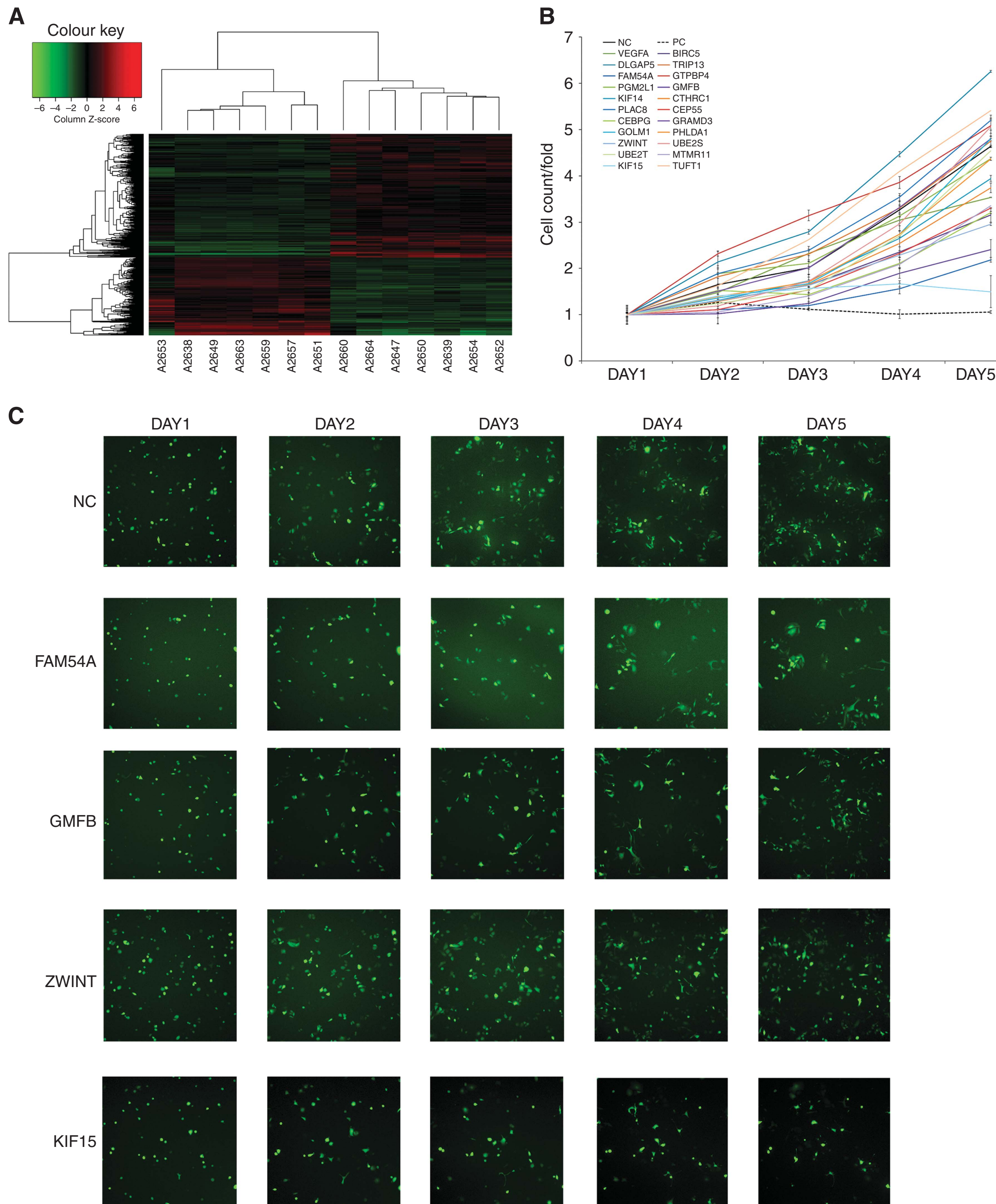

Figure 1. Agilent mRNA microarray and high-content siRNA screening identified KIF15 as a critical gene in promoting PC proliferation. (**A**) Heat map showing gene expression profiles. Each row represents a gene and each column represents a sample. Red indicates high expression, whereas green indicates low expression. (**B**) A total of 22 genes were selected for validation by high-content screening. NC: negative control siRNA, PC: positive control siRNA targeting β-actin. (**C**) Representative fluorescence images of high-content siRNA screening for *FAM54A*, *GMFB*, *KIF15*, and *ZWINT*.

knockdown (Figure 1B and C). *KIF15* knockdown showed the greatest effect on reducing the proliferation rate and thus we focused on *KIF15* in subsequent studies.

KIF15 upregulation in PC tissues is associated with poor patient prognosis. Immunohistochemical (IHC) staining of tissue microarrays shows that KIF15 was mainly localised to the cytoplasm of human PC cells (Figure 2A), whereas no KIF15 staining was present on adjacent, normal pancreatic tissue. Quantitative analysis indicated KIF15 was upregulated in human PC tissues compared with adjacent, normal pancreas tissues (Figure 2B). However, KIF15 expression levels did not vary significantly among different grades of human PC (Supplementary Table 2). Higher KIF15 expression (Supplementary Figure S1) was associated with poorer tumour differentiation (according to NCCN staging (Tempero *et al*, 2012), Supplementary Table 2) and shorter overall patient survival time (Figure 2C). Real-time PCR analysis of 27 pairs of matched PC and adjacent normal pancreatic tissue samples verified that the *KIF15* mRNA expression was upregulated in PC tissues (Figure 2D). We have sequenced 20 cases of pancreatic cancer tissues and found no activating mutations in KIF15. According to the exon sequencing result, the observations in this study are not due to epiphenomenon. KIF15 in pancreatic cancer is not an epiphenomenon and is directly involved in pathogenesis of pancreatic cancer (Supplementary Figure S2).

KIF15 promotes PC cell proliferation *in vitro* and *in vivo*. Stable KIF15 knockdown in the PANC-1 cell line was established using a lentiviral delivery system, and we confirmed downregulation of both KIF15 protein and mRNA in this cell line (Supplementary Figure S3). KIF15 knockdown suppressed PC cell proliferation, as measured by the CCK-8 assay and cell growth curve analysis using a fluorescence imaging system (Figure 3A–C). To further explore whether KIF plays a role in PC proliferation *in vivo*, a subcutaneous xenograft model of human PC cells in Balb/c nude mice was established. The KIF15 knockdown group showed a slower increase in tumour diameter and volume and less weight loss compared with controls (Figure 3D–F). In addition, Ki-67 staining was reduced in the KIF15 knockdown group compared with the control group (Supplementary Figure S4).

KIF15 promotes G1/S phase transition. Flow cytometry was used to explore whether KIF15 promotes PC proliferation through regulating the cell cycle and/or apoptosis. KIF15 overexpression induced a dramatic alteration in cell cycle distribution in both PANC-1 and MIA-PaCa-2 cells: the fraction of cells in G1 phase decreased, while the fraction of cells in both S phase and G2/M phase increased compared with controls (Figure 4A and B). In both cell lines with KIF15 knockdown, the number of cells arrested in G1 phase increased compared with controls. However, changes in KIF15 levels had no effect on the apoptotic ratio (Figure 4C). Western blot analysis showed that KIF15 overexpression upregulated cyclin D1, CDK2 and p-RB, which promote cell cycle progression, while KIF15 knockdown had the opposite effect (Figure 4D and E).

KIF15 activates the MEK–ERK pathway. To further elucidate the mechanisms underlying KIF15 promotion of PC proliferation, KIF15 knockdown cells and negative control cells were analysed by mRNA microarray. Surprisingly, many key genes in the MEK–ERK

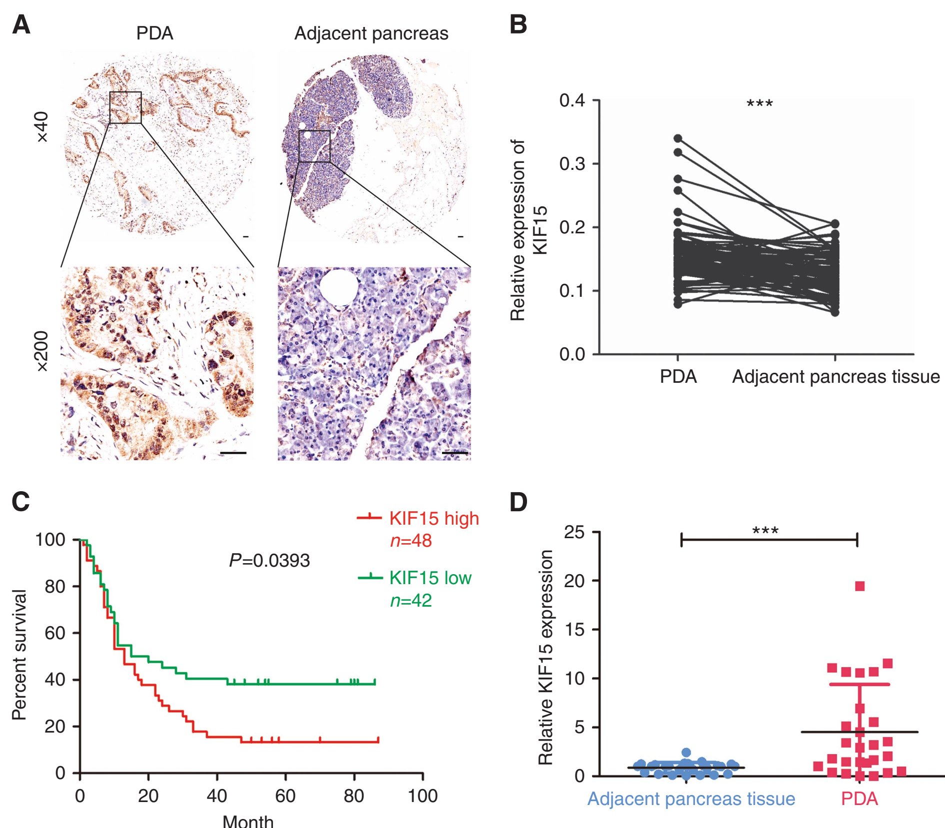

Figure 2. KIF15 is upregulated in PC tissues and KIF15 overexpression is associated with poor prognosis. (**A**) Immunohistochemical staining of KIF15 and representative images of one sample pair comprising PC tissue and adjacent, normal pancreas tissue. Magnification: ×40 (upper panel), ×200 (lower panel) (bar: 50 μm). (**B**) Quantitative analysis of KIF15 expression in PC tissues and adjacent, normal pancreas tissues. ***$P<0.001$. (**C**) By setting a relative KIF15 expression value of 0.5 as the demarcation point, PC patients were classified into high and low KIF15 expression groups. Kaplan–Meier analysis shows the correlation between KIF15 expression and overall survival of PC patients ($P=0.0167$). (**D**) PCR was used to quantify *KIF15* mRNA expression in 27 pairs of PC tissue and adjacent, normal pancreas tissues. ***$P<0.001$.

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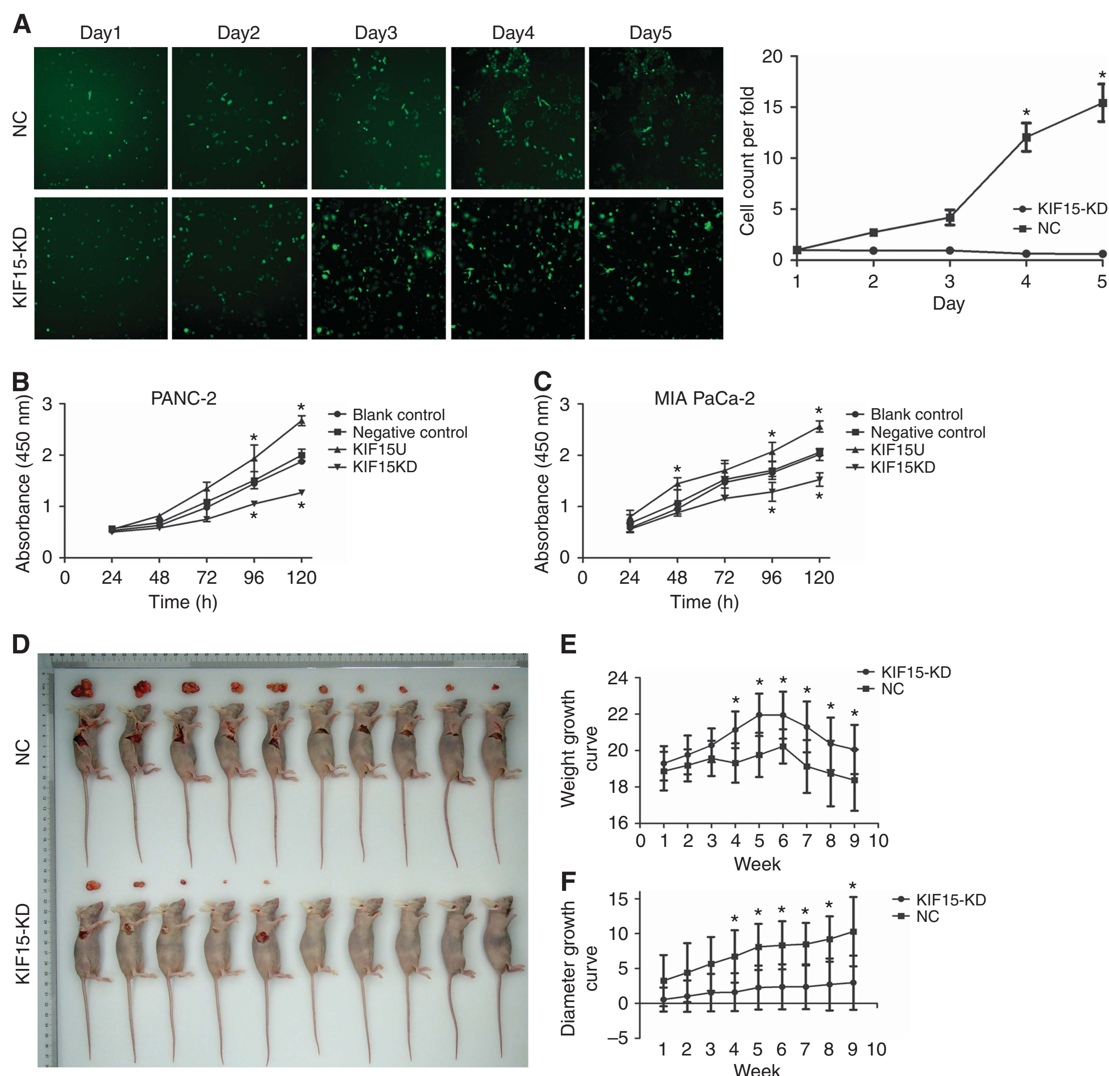

Figure 3. KIF15 promotes PC cell proliferation *in vivo* and *in vitro*. (**A**) Cell growth curve analysis comparing KIF15 knockdown (KIF15KD) with negative control (NC) PC cells. $n=3$, *$P<0.05$. (**B** and **C**) CCK-8 assays comparing proliferation of KIF15KD, KIF15-overexpressing (KIF15U), NC and blank control (BC) PANC-1 (**B**) and MIA-PaCa-2 (**C**) cells. $n=3$, *$P<0.05$. (**D**) Images of the subcutaneous xenografts from the KIF15KD and NC groups. $n=10$. (**E** and **F**) Mice weight change curves (**E**) and tumour volume growth curves for subcutaneous xenografts. *$P<0.05$.

signalling pathway were downregulated in the KIF15 knockdown group compared with control cells. Pathway analysis confirmed that the MEK–ERK pathway was most closely connected with KIF15 (Figure 5A; Supplementary Figure S5A). We thus next investigated whether changes in KIF15 expression could affect activation of the MEK–ERK signalling pathway. Western blot and IHC analysis showed that KIF15 overexpression increased p-ERK expression, whereas KIF15 knockdown inhibited p-ERK expression in PC cells (Figure 5B; Supplementary Figure S6). Furthermore, KIF15 co-localised with p-c-Raf and p-MEK in PANC-1 (Figure 5C and D) and MIA-PaCa-2 cells (Supplementary Figure S5C and D). Moreover, KIF15 coimmunoprecipitated with both MEK and Raf (Figure 5E). KIF15 knockdown in PANC-1 and MIA-PaCa-2 cells suppressed p-ERK translocation from the plasma membrane to the nucleus (Figure 5F, Supplementary Figure S5B). MEK–ERK signalling pathway inhibitors U0126, AZD6244 and PD98059 inhibited KIF15 activation of the MEK–ERK signalling pathway (Figure 5G).

MEK–ERK pathway inhibitors block KIF15-dependent G1/S phase transition. We have shown that KIF15 promotes G1/S phase transition in PC cells. To investigate whether this effect is mediated by the MEK–ERK pathway, KIF15-overexpressing cell lines were treated with the MEK–ERK pathway inhibitors U0126, AZD6244, and PD98059 and effects on the cell cycle were examined. All the inhibitors blocked KIF15-dependent cell cycle transition (Figure 6A and B), indicating that KIF15-mediated inhibition of the G1/S phase transition occurs via the MEK–ERK pathway. Furthermore, KIF15-dependent increases in cyclin-D1, CDK2, and p-RB expression were blocked by treatment with U0126, AZD6244 and PD98059 (Figure 6C).

MEK–ERK inhibitors block KIF15-dependent PC proliferation *in vivo* and *in vitro*. We next examined whether the effect of KIF15 on promoting PC cell proliferation was mediated by the MEK–ERK pathway. We found that the enhancement of cell proliferation was counteracted to different extents by treatment with U0126, AZD6244 or PD98059 in CCK-8 assays (Figure 7A and B). Among the three inhibitors, PD98059 completely blocked KIF15-mediated PC cell proliferation.

To examine the relationship between KIF15 function and the MEK–ERK pathway *in vivo*, we established a Balb/c nude mice orthotopic transplantation model and examined three groups of mice: (1) a negative control (NC) group; (2) a group injected with KIF15-overexpressing cells (KIF15U); and (3) a group injected with KIF15-overexpressing cells plus a MEK–ERK pathway

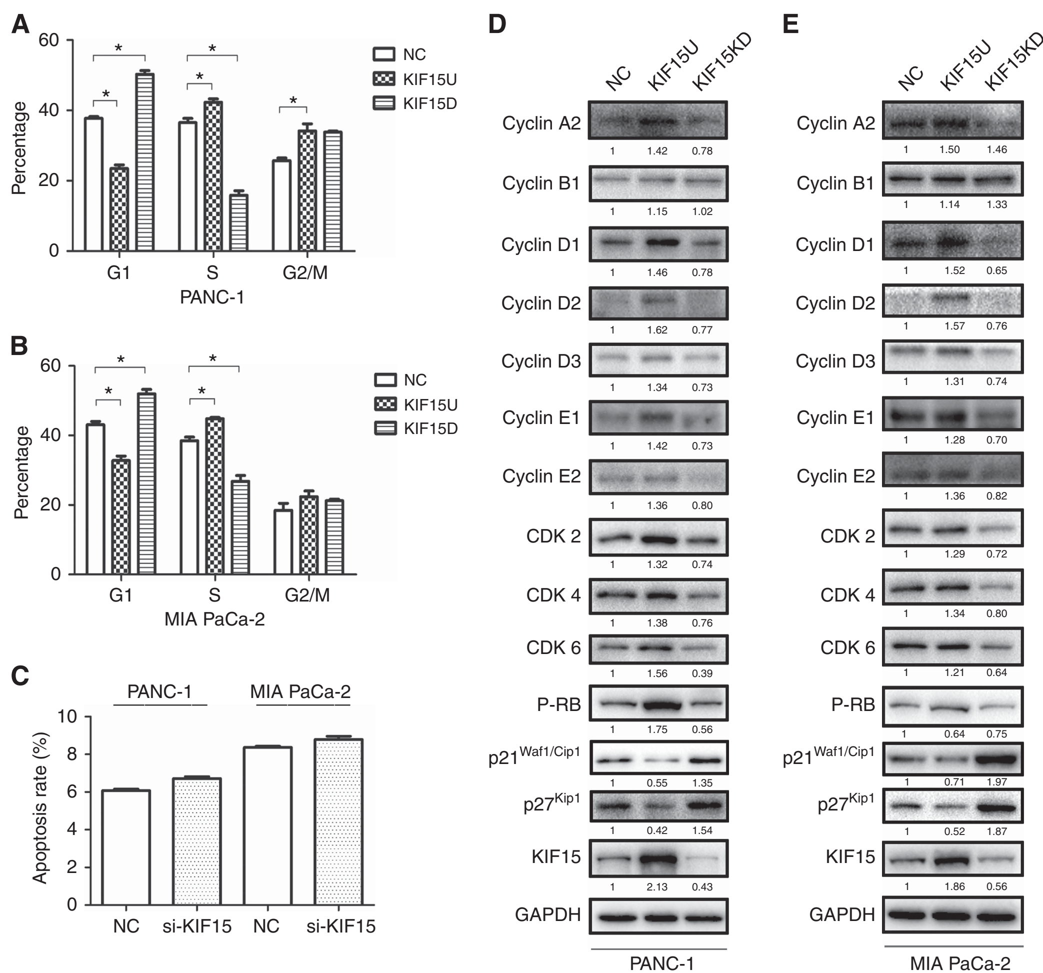

Figure 4. KIF15 regulates PC cell proliferation via promoting G1/S phase transition. (**A** and **B**) Flow cytometry analysis of the cell cycle in KIF15 knockdown (KIF15KD), KIF15-overexpressing (KIF15U), and negative control (NC) PANC-1 (**A**) and MIA-PaCa-2 (**B**) cells. (**C**) Flow cytometry analysis of apoptosis in KIF15KD, KIF15U and NC PANC-1 and MIA-PaCa-2 cells. (**D** and **E**) Western blotting shows cell cycle protein expression in KIF15KD, KIF15U, and NC PANC-1 (**D**) and MIA-PaCa-2 (**E**) cells. $n=3$. $^*P<0.05$.

inhibitor (KIF15U + PD98059). Survival analyses revealed the mice in the KIF15U + PD98059 group had the longest survival time, whereas the KIF15U group had the shortest survival time (Figure 7C). The KIF15 overexpressing group showed a more rapid weight loss compared with the KIF15U + PD98059 group and NC group (Figure 7D). Together, these results suggest that MEK–ERK pathway inhibitors block KIF15-mediated PC proliferation both *in vivo* and *in vitro*.

DISCUSSION

Although the structure and function of KIF15 have been studied for 10 years, the role of KIF15 in regulating the behavior of cancer cells has not yet been elucidated.

Kinesins are a superfamily of proteins with important roles in eukaryotic intracellular trafficking and cell division (Klejnot *et al*, 2014). The genomes of higher vertebrates contain as many as 45 genes encoding different kinesins (Florian and Mayer, 2011). Most of these molecular machines are implicated in intracellular transport, and one third of kinesin superfamily members play key roles at different stages of mitosis and cytokinesis (Messin and Millar, 2014). For example, the mitotic kinesin KIF11 drives glioblastoma invasion, proliferation and self-renewal (Venere *et al*, 2015). KIF1B promotes glioma migration and invasion via inducing the cell surface localisation of MT1-MMP (Chen *et al*, 2016). Silencing of KIF2A inhibits the proliferation and migration of breast cancer cells and correlates with an unfavourable prognosis for breast cancer patients (Wang *et al*, 2014). High levels of KIF18A expression are related to metastasis and significantly affect cancer progression (Shichijo *et al*, 2005; Nagahara *et al*, 2011; Rucksaken *et al*, 2012; Kasahara *et al*, 2016). KIF23 expression levels provide additional prognostic information for patients undergoing lung cancer surgery. KIF23 may also be a novel therapeutic target in this cancer type (Sun *et al*, 2015; Iltzsche *et al*, 2017; Kato *et al*, 2016; Sun *et al*, 2016). Therefore, several kinesin superfamily members have important effects on tumour behaviour.

Drechsler *et al* reported that KIF15 is a second tetrameric spindle motor (in addition to kinesin-5, Eg5) and reported the mechanisms by which hKIF15 and its inhibitor hTpx2 modulate spindle microtubule architecture (Tanenbaum *et al*, 2009; Drechsler *et al*, 2014; Eskova *et al*, 2014). The motor domain structure of KIF15 was captured in the 'ATP-like' configuration, with the neck linker docked onto the catalytic core. The interaction of KIF15 with microtubules was also investigated and structural differences between these two motors indicate profound differences in their modes of action, consistent with current models of microtubule crosslinking and sliding (Klejnot *et al*, 2014). KIF15 mediates plasma membrane localisation of the alternative clathrin adaptor Dab2, thus impinging on pathways that regulate α2-integrin internalisation. KIF15 can also drive centrosome

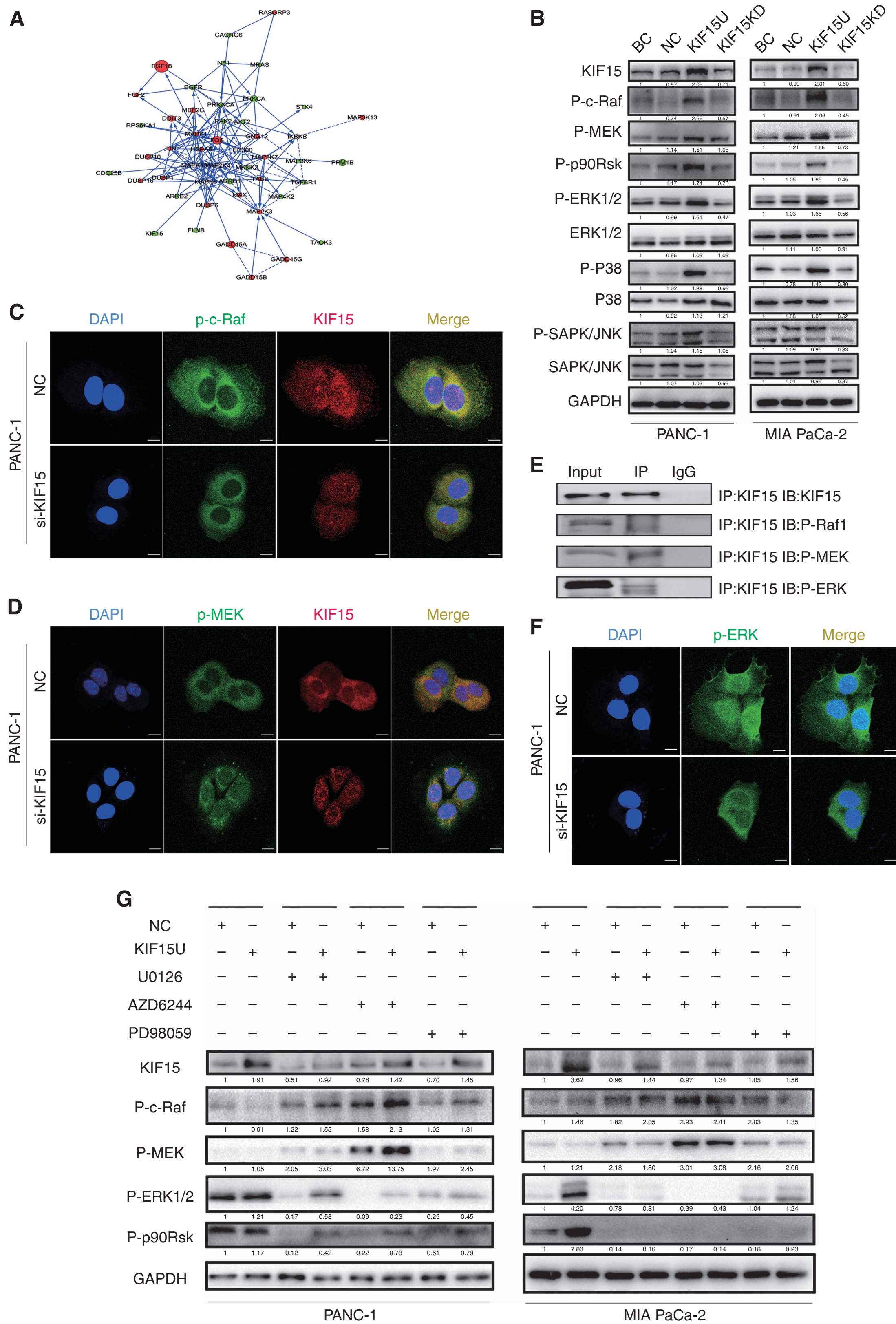

Figure 5. KIF15 activated the MEK–ERK signalling pathway. (**A**) Network analysis of KIF15-associated signalling pathways in PC tissues using data obtained from mRNA microarrays. (**B**) Protein expression levels of key modulators of the MEK–ERK signalling pathway were analysed by western blotting in KIF15 knockdown (KIF15KD), KIF15-overexpressing (KIF15U), negative control (NC) and blank control (BC) PANC-1 and MIA-PaCa-2 cells. $n = 3$. (**C** and **D**) Immunofluorescence staining shows p-c-Raf and p-MEK co-localisation with KIF15 in PANC-1 cells (bar: 10 μm). (**E**) KIF15 protein was immunoprecipitated with KIF15 antibody and levels of bound p-Raf, p-MEK, and p-ERK were detected by western blotting. (**F**) Immunofluorescence staining shows p-ERK expression in the nuclei of KIF15 knockdown (KIF15KD) and NC PANC-1 cells (bar: 10 μm). (**G**) Western blotting of protein lysates from NC, KIF15U, and KIF15U PANC-1 and MIA-PaCa-2 cells treated with three different MEK–ERK signalling pathway inhibitors: U0126(25 μM), AZD6244(25 μM), and PD98059(25 μM).

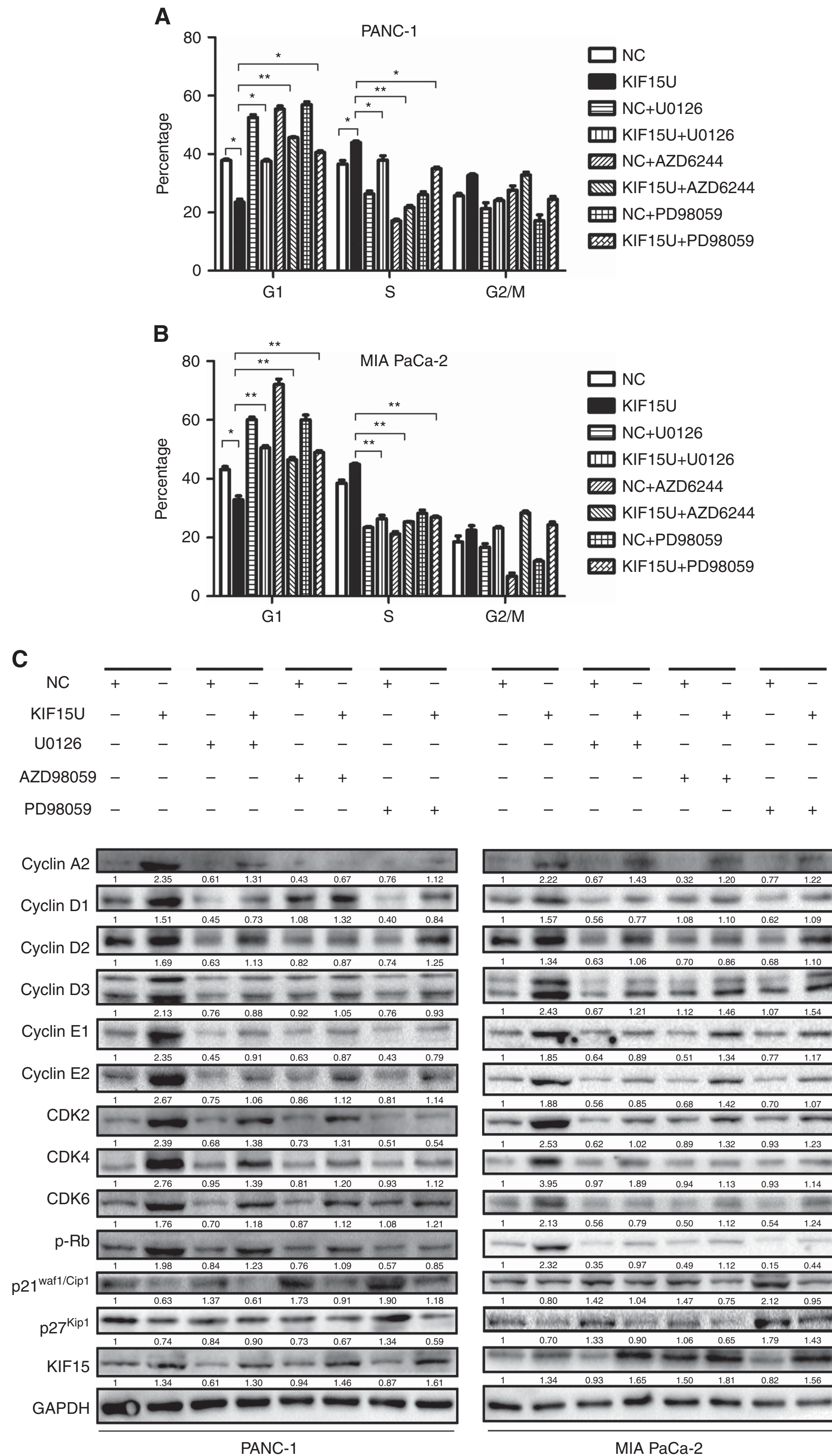

Figure 6. KIF15 promotion of G1/S phase transition via the MEK–ERK signalling pathway is ablated by MEK–ERK pathway inhibitors. (**A** and **B**) Flow cytometry analysis of the cell cycle in negative control (NC), KIF15-overexpressing (KIF15U), and NC or KIF15U PANC-1 (**A**) and MIA-PaCa-2 (**B**) cells treated with three different MEK–ERK signalling pathway inhibitors: U0126(25 μM), AZD6244(25 μM), and PD98059(25 μM). (**C**) Western blotting shows cell cycle protein expression in NC, KIF15U and NC or KIF15U PANC-1 (left panel) and MIA-PaCa-2 (right panel) cells treated with three different MEK–ERK signalling pathway inhibitors: U0126; AZD6244; and PD98059. $n=3$. $^*P<0.05$, $^{**}P<0.01$.

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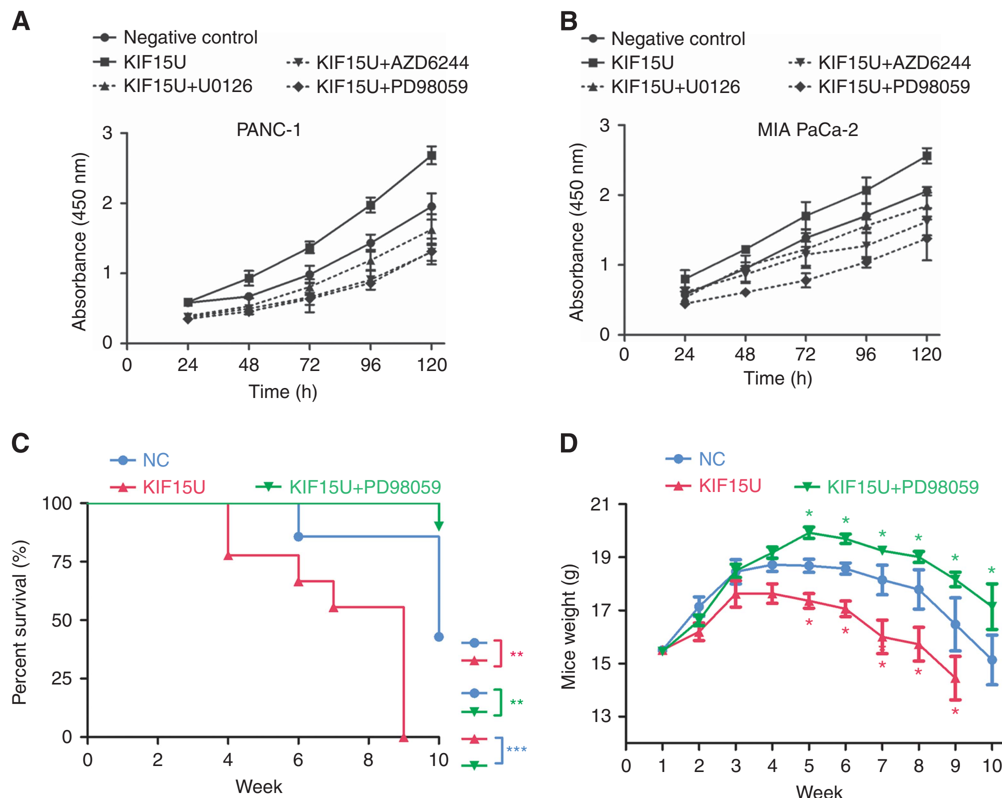

Figure 7. MEK–ERK signalling pathway inhibitors block KIF15-induced PC proliferation *in vivo* and *in vitro*. (**A** and **B**) Negative control (NC), KIF15-overexpressing (KIF15U) and KIF15U cells were treated with three different MEK–ERK signalling pathway inhibitors, U0126(25 μM), AZD6244(25 μM), and PD98059(25 μM), and proliferation rates were measured using the CCK-8 assay in PANC-1 (**A**) and MIA-PaCa-2 (**B**) cells. (**C**) Kaplan–Meier survival curves for different experimental mouse groups: NC, KIF15U, or KIF15U + PD98059 (10 mg kg^{-1}), $n=8$, $^{**}P<0.01$, $^{***}P<0.001$. (**D**) Mice weight changes in PANC-1 KIF15U and PANC-1 KIF15U + PD98059(10 mg kg^{-1}) groups *vs* the NC group. $^{*}P<0.05$.

separation during bipolar spindle assembly (Eskova *et al*, 2014). This activity requires both the KIF15 motor domain and interaction with TPX2 (Sturgill *et al*, 2014). KIF15 and TPX2 can crosslink and slide through two antiparallel microtubules, thereby driving centrosome separation. Studies on rodent brain development showed a pronounced enrichment of KIF15 in migratory neurons compared with other neurons. KIF15 opposes the capacity of other motors to generate independent microtubule movements within key regions of developing neurons (Buster *et al*, 2003; Drechsler *et al*, 2014). KIF15 also plays an important role in several tumours. KIF15 is required for maintenance of spindle bipolarity and is the breast cancer tumour antigen (Scanlan *et al*, 2001). KIF15 is essential for K5I resistance in HeLa cells, even in cases that necessitate additional factors such as the Eg5 rigor mutant (Sturgill *et al*, 2016). KIF15 is overexpressed in breast cancer cells and can have important values as both a prognostic factor and new therapeutic target for endocrine therapy-resistant breast cancer (Zou *et al*, 2014). KIF15 is also overexpressed in lung adenocarcinoma and may play a vital role in regulating the cell cycle (Bidkhori *et al*, 2013). In this study, we report for the first time that KIF15 promotes PC cell proliferation via the MEK–ERK pathway.

The MEK–ERK pathway plays pivotal role in cell proliferation (Park, 2014) and its deregulation is a signature of many epithelial cancers. The MEK–ERK signalling pathway is involved in controlling diverse cellular processes such as proliferation, survival, differentiation and motility (Giordano *et al*, 2015; Vajravelu *et al*, 2015). This pathway is often upregulated in human tumours and is thus an attractive target for the development of anticancer drugs (Hayashido *et al*, 2014). The mitogen-activated protein kinase 1/2 (MAP2K1/2) inhibitor PD98059 effectively inhibits ERK1/2 phosphorylation and enhances the radiosensitivity of rhabdomyosarcoma cells (Asati *et al*, 2016; Ding *et al*, 2016). Currently, dozens of MEK inhibitors targeting the MEK–ERK pathway have been included in clinical trials for cancer therapy (Johnson *et al*, 2014). The key step for quiescent cells to enter the cell cycle is the formation of an active cyclin D–CDK4/6 complex. ERK regulates cyclin D1 transcriptional induction via Fos family members and myc (Daksis *et al*, 1994). Furthermore, formation of the cyclin E/CDK2 complex seems to be indirectly regulated by ERK at two levels. First, ERK activity is required for nuclear translocation of CDK2. CDK2 is translocated to a nuclear compartment in which ERK is activated by threonine-160 phosphorylation via the CDK-activating kinase (CAK) and threonine-14 and tyrosine-16 dephosphorylation via the CDC25 phosphatase. However, blocking ERK activation does not modify the levels of cyclin E–CDK2 complexes: ERK activation is solely necessary for the nuclear localisation of CDK2. Second, ERK activity has been shown to regulate phosphorylation of CDK2 at threonine-160, an activating site. The pathways linking ERK activation to ERK-dependent CDK2 nuclear translocation and activating threonine-160 phosphorylation are not yet known. Further studies are necessary to resolve these questions. ERK activation plays a fundamental role in G1/S phase transition because it is required for the induction of cyclin D1 protein via several mechanisms; sustained activity of ERK is also required for the downregulation of many antiproliferative genes throughout the G1 phase of the cell cycle (Chambard *et al*, 2007).

This study thus reports a novel role for KIF15 in promoting cancer cell proliferation. Previous research efforts have focused on KIF15 functions related to microtubules and mitosis. This study reveals that KIF15 also promotes cancer proliferation and identifies

a potential link between its cancer-promoting effects and the MEK–ERK signalling pathway (Supplementary Figure S7).

In conclusion, our study provides evidence that supports KIF15 as a key regulator that promotes the proliferation of PC. This is accomplished by promoting G1/S phase transition via regulating the MEK–ERK signalling pathway. Thus, MEK–ERK signalling pathway inhibitors may form the basis of novel therapies to block cancer promotion by KIF15.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception and design: XG, JJ. Development of methodology: XG, JW. Acquisition of the data: JW, XG, JJ. Analysis and interpretation of data: JW, XG, JJ. Writing, review, and/or revision of the manuscript: XG, CX. Study supervision: JJ.

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