

# Histone demethylase JMJD3 disrupts spectrin-dependent cytoskeleton in Pancreatic Ductal Adenocarcinoma cells by regulating H exokinase domain containing 1 expression

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## Research

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# Abstract

**Background:** JMJD3 is a jmj domain containing histone demethylase which can remove methyl groups from lysine 27 of histone 3 (H3K27) to activate histone methylated genes. Previous studies have demonstrated that JMJD3 played a crucial role in inflammation.

**Methods:** Our study showed that JMJD3 was significantly down-regulated in pancreatic ductal adenocarcinoma (PDAC) cell lines and tissues. Restored expression of JMJD3 inhibited oncogenic phenotypes of PDAC cells, including cell proliferation, cell migration, and *in vivo* tumorigenicity, indicating a tumor suppressive role. Gene-expression microarray revealed that Hexokinase domain containing 1 (HKDC1) was one of the JMJD3 downstream targets.

**Results:** The expression of JMJD3 and HKDC1 in PDAC tissues was positively correlated. High H3K27 trimethylation (H3K27me<sub>3</sub>) status in HKDC1 gene was attenuated by ectopic expression of JMJD3 in PDAC cells, suggested that JMJD3 regulated HKDC1 expression by histone demethylation activity. The tumor suppressive role of HKDC1 in PDAC was also proved. Moreover, HKDC1 was demonstrated to competitively bind to spectrin beta  $\alpha$  to induce cytoskeleton disruption, which may contribute to tumor suppression.

**Conclusion:** Taken together, our study indicates that JMJD3 may disrupt spectrin-dependent cytoskeleton via activation of HKDC1 to suppress PDAC.

## Background

Pancreatic cancer ranked the fourth in cancer deaths and more than 53000 new cases were estimated in 2016[1]. Pancreatic ductal adenocarcinoma (PDAC) is making up more than 80% of all pancreatic cancer with high incidence and mortality rate[2, 3]. Recent studies showed that tumor suppressor genes were mutated or down-regulated in human PDAC tumors, which accelerated tumor progression and resulted in invasive and metastatic malignancies[4]. The repressive modification of histone tail histone H3 lysine 27 (H3K27) trimethylation is catalysed by the polycomb group protein EZH2[5]. JMJD3 and UTX are the only two proteins[6]that demethylate H3K27me<sub>3</sub> to H3K27me<sub>2</sub> or H3K27me<sub>1</sub>, and dissociate polycomb group complexes[7, 8]. Evidences showed that alteration of the enzymes activity controlling H3K27 methylation contributed to carcinogenesis. Polycomb group proteins exhibit oncogenic phenotypes by repressing tumor suppressor genes in a variety of cancers, such as lymphoma[9, 10], bladder cancer[11], PDAC[12], breast cancer and prostate cancer[13]. JMJD3 and UTX reverse polycomb group-mediated transcriptional repression by demethylation of H3K27me<sub>3</sub>.

Inactivating somatic mutations of UTX frequently occur in cancers[14]. However, the roles of JMJD3 in cancers were highly controversial. It was reported that JMJD3 expression was upregulated in prostate cancer and promoted melanoma progression and metastasis[15, 16]. However, accumulating studies indicated the tumor suppressive role of JMJD3 in cancers. Agger et al reported that JMJD3 was recruited to the INK4A-ARF locus which encoded the tumor suppressor proteins p16INK4A and p14ARF, and

activated p16INK4A expression in human fibroblasts[8, 17]. Another study reported that JMJD3 acted as a tumor suppressor by regulating p53 protein nuclear stabilization in glioblastoma stem cells[18]. Till now, the functional role of JMJD3 in PDAC is still unknown, providing a comprehensive picture depicting JMJD3 dysregulation during pancreatic tumorigenesis may promote the development of JMJD3-directed diagnostics and therapeutics in PDAC.

## **Materials And Methods**

### **Clinical specimens and cell lines**

The tissue microarray containing tissues from 132 PDAC patients and 12 normal individuals was purchased from Biomax (US). 25 pairs of PDAC tumor and surrounding normal tissues were retrieved from the tissue bank of Department of Anatomic Pathology, the Prince of Wales Hospital (Hong Kong). Overall survival in relation to JMJD3 and HKDC1 staining were from 25 pairs of PDAC tumor and surrounding normal tissues. Use of these tissues had been approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. PDAC cell lines, HEK-293 and 293T cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Human pancreatic ductal epithelial (HPDE) cell line was a gift from DR. Ming-Sound Tsao (Ontario Cancer Institute and Princess Margaret Hospital Site, Toronto), All cells were maintained according to the publications[12, 19].

### **In Vivo Tumor Growth**

Male BALB/c nude mice aged 4 to 6 weeks were acquired from Laboratory Animal Services Centre of the Chinese University of Hong Kong. Animal handling and experimental procedures were approved by the Animal Experimental Ethics Committee of the institute. Treated cells were injected subcutaneously into the right flank of the nude mice (five mice per group). Tumor growth was monitored twice a week, the tumor volume was measured by a caliper and calculated by the equation:  $\text{volume} = (\text{Length} \times \text{width}^2) / 2$ .

### **Whole-genome Mrna Expression Array**

Panc-1 cells were transfected with wt JMJD3 or mutant JMJD3 plasmids. Total RNA was extracted using TRIzol reagent (Life Technologies, CA). mRNA expression was profiled using HG-U133 plus 2.0 according to standard Affymetrix protocols (Affymetrix, CA). The dataset is available from NCBI database (GSE85933).

### **Chromatin Immunoprecipitation (chip) Assay**

ChIP was performed according to previous study[20]. Cross-linked chromatin was incubated overnight at 4 °C with anti-H3 (Positive ctrl), anti-IgG (Negative ctrl), anti-H3K27me3 (Millipore), anti-RNA polymerase II (Abcam) and anti-JMJD3 (Abcam). The precipitated DNA were quantitated absolutely by real-time PCR and normalized by their 2% ChIP input. All the primers mention in this study were listed in **supplementary table 1**.

## Immunohistochemistry

Immunohistochemistry analysis of JMJD3 and HKDC1 was conducted on two cohorts of pancreatic cancer tissues. Cohort 1 contained 132 PDAC tissues and 12 normal cases (Rockville, MD). Cohort 2 contained 25 paired PDAC clinical tissues with survival information. Immunohistochemical staining were performed using anti-JMJD3 or anti-HKDC1 antibodies (BD Biosciences) with standard avidin-biotin method. Measurement of IHC staining was based on a semiquantitative scoring method. For the intensity of staining, 0 = negative, 1 = very weak, 2 = weak, 3 = moderate, 4 = strong, 5 = very strong. During scoring for JMJD3 and HKDC1 in PDAC clinic tissues, the scores were further subdivided into high-expression (3, 4, 5) and low-expression groups (0, 1, 2).

## Statistical analysis

GraphPad Prism 5 was used for statistical analysis. All results were expressed as mean  $\pm$  SD from at least three independent experiments. For multiple comparisons, each value was compared by one-way ANOVA following Dunnett test, Tukey test and Student t-test. Overall survival in relation to JMJD3 and HKDC1 staining were evaluated by the Kaplan–Meier survival curve and the log-rank test. P values of less than 0.05 were considered statistically significant.

## Results

### JMJD3 acts as a tumor suppressor in PDAC

JMJD3 expressions in tissues from 132 PDAC patients and 12 normal individuals were analyzed by immunohistochemical staining. JMJD3 expression levels were much lower in tumor tissues compared to the normal pancreatic tissues (Fig. 1A). The primary tumors showed higher overall JMJD3 expression than the metastatic tumors, and a statistically significant correlation between JMJD3 expression levels and tumor grades were found (Fig. 1B). Importantly, low expression of JMJD3 was correlated with shorter overall survival in PDAC patients (Fig. 1C), which was further validated by using GEO data (Fig. S1A). We examined the *in vivo* anti-cancer activities of JMJD3 by over-expressing wild type (wt) JMJD3, or mutant JMJD3 which lacked H3K27me3 demethylase activity, in PDAC cells. The tumors in control group showed a faster and more stable growth. The mutant JMJD3 treated group showed a significant tumor inhibition, and there was no tumor formed after transfected with wt JMJD3 (Fig. 1D, E, F). In *in vitro* studies also revealed a tumor suppressive role of JMJD3 in PDAC (Fig. S1B, C, D, E, F, G).

# Jmjd3 Regulates Hkdc1 Expression By Histone Demethylation

We found that JMJD3 caused no changes on the p16<sup>INK4A</sup>, p19<sup>Arf</sup> and p53 expression levels in PDAC cell (Fig. S2A). To further elucidate the role of JMJD3 in PDAC, we performed the genome-wide gene expression microarray to screen differentially expressed genes in wt JMJD3/mutant JMJD3 over-expressed Panc-1 cells. AGR2 and HKDC1 were the most up-regulated genes after ectopic expression of wt JMJD3. However, AGR2 was also up-regulated upon mutant JMJD3 over-expression, indicated that AGR2 was not involved in JMJD3 demethylase activity. Then, HKDC1 was further studied in PDAC (Fig. S2B, C, D and supplementary Table 2).

Both mRNA and protein level of HKDC1 were upregulated by wt JMJD3 in PDAC cells (Fig. 2A, Fig. S3A). The expression of HKDC1 was decreased by the knockdown of JMJD3 in HPDE cells (Fig. 2B). To determine whether HKDC1 was regulated under histone modification, we firstly treated PDAC cells with DZnep which was an H3K27me3 inhibitor[21]. DZnep increased HKDC1 expression in PDAC cells (Fig. 2C), indicated that histone H3K27me3 may serve to inhibit HKDC1 transcription. By ChIP assay, a higher H3K27me3 mark at HKDC1 promoter in PDAC cells (Panc-1 and SW1990) was identified when compared to that in human pancreatic duct epithelial (HPDE) cells (Fig. 2D). JMJD3 was reported to pave the way for the RNA pol  $\alpha$  progression, then activated the transcription of JMJD3 downstream targets[22]. By ChIP assay with Pol  $\alpha$  antibody, we found a significant loss of pol  $\alpha$  and JMJD3 enrichment at HKDC1 promoters in PDAC cells when compared to that in HPDE cells (Fig. 2D). As a high H3K27me3 level was observed at HKDC1 promoter in PDAC cells, we speculated whether ectopic expression of JMJD3 alone would be sufficient to induce HKDC1 transcription by reducing H3K27me3 at HKDC1 loci. By ChIP assay using antibodies specific for JMJD3, we found that ectopic expression of JMJD3 increased the binding of JMJD3 to HKDC1 promoter and correlated with a decrease of H3K27me3 levels at HKDC1 locus (Fig. 2E). These results suggested that JMJD3 contributed to the transcriptional induction of HKDC1 by demethylating H3K27me3. We also performed ChIP assays using JMJD3-knockdown HPDE cells. The result showed that depletion of JMJD3 impaired the H3K27me3 reduction and repressed HKDC1 transcription (Fig. 2F). These results suggested that JMJD3 activated HKDC1 expression by demethylating H3K27me3 associated with the locus.

## The Tumor Suppressive Role Of Hkdc1 In Pdac

We performed immunohistochemistry to examine HKDC1 expression in a PDAC tissue microarray. We found that HKDC1 expression was much lower in tumor tissues compared to tumor-adjacent normal tissues (Fig. 3A), and negatively correlated with tumor grades (Fig. 3B). A significant positive correlation between expression of JMJD3 and HKDC1 was proved in PDAC tissues (Fig. 3C). Low expression of HKDC1 was correlated with shorter overall survival in 25 PDAC patients (Fig. 3D). Meanwhile, In *in vitro* studies also proved a tumor suppressive role of HKDC1 in PDAC (Fig. S3B, C, D, E). Low expression of

HKDC1 was correlated with shorter overall survival which was also validated by using GEO data (Fig. S3F).

We next studied the effect of HKDC1 knockdown to the growth inhibition induced by JMJD3. It showed that the expression of HKDC1 decreased significantly in JMJD3 over-expressed PDAC cells upon knockdown of HKDC1 (Fig. 3E). By MTT assay, we found that JMJD3-induced growth inhibition could be reversed by knockdown of HKDC1 (Fig. 3F). The colony formation assay also showed that knockdown of HKDC1 increased the number of colony formation by PDAC cells when compared to cells over-expressing JMJD3 only (Fig. 3G).

## HKDC1 bond to Spectrin beta $\alpha$ to suppress PDAC cell growth

Bioinformatics prediction suggested that HKDC1 contain a spectrin binding domain (SBD) (Fig. S4A). We found that HKDC1 co-localized with spectrin in HPDE cells (Fig. 4A). We then constructed a series of plasmids which prokaryotically expressed truncated or full length HKDC1, Spectrin alpha  $\alpha$  and Spectrin beta  $\alpha$  recombinant proteins (Fig. S5A, B, C, D). By binding assay, the interaction between full length HKDC1 and C-terminal domain of Spectrin beta  $\alpha$  were observed (Fig. 4B). To further prove whether HKDC1 bound to Spectrin beta  $\alpha$  through SBD, two other HKDC1 recombinant proteins contain SBD only or the SBD-deleted (HKDC1 $\Delta$ <sup>SBD</sup>) were used in binding assay. The SBD only protein bond to spectrin beta  $\alpha$  fragments (Fig. 4C). These results indicated that HKDC1 bond to C-terminal domain of spectrin beta  $\alpha$  through SBD.

We next examined the interaction between HKDC1 and Spectrin beta  $\alpha$  *in vitro*. Firstly, we found that HKDC1 bound to Spectrin beta  $\alpha$  (Fig. 4D). When HKDC1 was inhibited, the interaction between HKDC1 and Spectrin beta  $\alpha$  was diminished (Fig. 4D). By reverse-immunoprecipitation, knockdown of Spectrin beta  $\alpha$  also diminished the interaction between these two proteins (Fig. 4E). Moreover, after ectopic expression of HKDC1 and Spectrin beta  $\alpha$  with specific tags in Panc-1 cells, the binding between HKDC1 and Spectrin beta  $\alpha$  was further confirmed (Fig. 4F). We then identified that SBD domain was needed while HKDC1 exhibited its anti-cancer activities in PDAC cells (Fig. S4B, C).

## HKDC1 disrupted the Spectrin beta $\alpha$ -Spectrin alpha $\alpha$ tetramerization by competing with Spectrin alpha $\alpha$ for binding to Spectrin beta $\alpha$ in PDAC cells

Previous study reported that Spectrin alpha  $\alpha$  and Spectrin beta  $\alpha$  formed a stable tetramer[23]. Our study showed that HKDC1 bound to Spectrin beta  $\alpha$  and ectopic expression of JMJD3 caused no expression changes of Spectrin beta  $\alpha$  in PDAC cells (Fig. S6A). We found that Spectrin beta  $\alpha$  bound to Spectrin alpha  $\alpha$  in PDAC cells (Fig. 5A). In turn, we showed that there was a decreased binding between these two

proteins in PDAC cells with JMJD3 overexpression (Fig. 5A). From the extracellular binding assay, we found the binding between spectrin beta  $\beta$  and spectrin alpha  $\alpha$  was diminished by presenting HKDC1 recombinant protein (Fig. 5B). These results showed that HKDC1 competed with Spectrin alpha  $\alpha$  for binding with Spectrin beta  $\beta$  in PDAC cells.

Spectrin beta  $\beta$ -Spectrin alpha  $\alpha$  tetramerization was one of the important components of cytoskeleton. We further asked whether ectopic expression of HKDC1 disrupted the cytoskeleton in PDAC cells. We found that co-localization of HKDC1 and spectrin beta  $\beta$  (orange color) in JMJD3 over-expressed cells but not in vehicle control (Fig. 5C). Spectrin beta  $\beta$  and Spectrin alpha  $\alpha$  co-localization was diminished in JMJD3 over-expressed cells (Fig. 5D). Finally, the binding between HKDC1 and Spectrin beta  $\beta$  was found to disrupt the cytoskeleton in PDAC cells which may be involved in PDAC progression ((Fig. S6A, B, C, D).

## Discussion

The role of JMJD3 in cancer is still controversial, the discrepancies between these studies might be due to differential roles played by JMJD3 in different cancer types and/or different signaling mechanisms. In this study, we demonstrated that JMJD3 and its target HKDC1 played tumor suppressive roles in PDAC. The function of HKDC1 was poorly studied. Previous studies of hexokinase activity in diverse vertebrates failed to observe hexokinase activity in HKDC1[24, 25]. Recent study identified that HKDC1 was a novel potential therapeutic target for lung cancer[26], indicated that HKDC1 has critical function in cancers. To our knowledge, we are the first to show the impact of histone modification on the tumor suppressive role of HKDC1 in PDAC.

Most PDAC patients are diagnosed with metastatic disease, and majority of patients will develop metastatic disease even after the localized lesions are resected[27, 28]. Therefore, the development of more effective strategies to combat PDAC metastasis is of paramount importance. The process of metastasis is regulated in a highly complex manner, however, cytoskeletal reorganization is a cellular phenomenon observed to be involved during the process[29]. We found that ectopic expression of JMJD3 inhibited PDAC cell migration and anchorage-independent survival which were required for metastatic process. We also found that JMJD3-HKDC1-spectrin pathway played a crucial role in suppressing PDAC by inducing cytoskeleton disruption, suggesting that the PDAC cells may form more stable cytoskeleton to exhibit oncogenic phenotypes. This is quite consistent with the previous studies that spectrin alpha  $\alpha$ -spectrin beta  $\beta$  tetramers contributed to anticancer drug resistance and the cytoskeleton protein were upregulated in several types of human cancers[30–32]. Though JMJD3-HKDC1-spectrin pathway may be involved in PDAC metastasis, the mechanism is still unclear which requires investigation.

Demethylation-independent mechanisms also contributed to tumor suppressive role of JMJD3 in PDAC. We showed that mutant JMJD3 without demethylation activity also partially suppressed PDAC. Previous study demonstrated that JMJD3 played a role in general chromatin remodeling that is independent to their H3K27 demethylase potential[33, 34]. Przanowski et al. also demonstrated that JMJD3 stimulated

the expression of LPS-induced inflammatory genes which was independent to its demethylase activity[35].

## Conclusion

In summary, we have elucidated that the JMJD3-HKDC1-spectrin pathway involved in suppressing PDAC via demethylation dependent mechanism. We suggested that JMJD3 may also suppress PDAC through chromatin remodeling or regulating inflammatory genes independent of its demethylase activity, which warranted investigation in future study.

## Abbreviations

PDAC, pancreatic ductal adenocarcinoma; H3K27, lysine 27 of histone; MTT,3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromid; HKDC1, Hexokinase domain containing 1; H3K27me3,H3K27 trimethylation; HPDE, Human pancreatic ductal epithelial; KSFM, Keratinocyte Serum Free Medium; CHIP, Chromatin immunoprecipitation; SBD, spectrin binding domain; EZH2, Enhancer of Zeste homolog 2; Pol II, RNA polymerase II; siRNA, small interfering RNA; qRT-PCR, quantitative reverse transcriptional polymerase chain reaction.

## Declarations

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### Consent for publication

All authors have reviewed the manuscript and consented for publication.

### Author Contributions

JS, QZ contributed to acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis. CHL and YX contributed to acquisition of data and drafting of the manuscript. JHMT contributed to obtain research material. KFT and CHC commented on the manuscript. ZX and YC contributed to study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, material support and study supervision.

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### **Competing interests**

The authors declare no conflict of interest.

### **Ethics approval and consent to participate**

All studies involving animal have get the Ethical Approval.

### **Availability of data and materials**

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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## Supplementary Figure Legends

**Figure S1. JMJD3 acts as a tumor suppressor in PDAC.** **A**, Low JMJD3 expression was correlated with shortened overall survival in PDAC patients. **B**, RT-QPCR and western blotting showed the expression of wt JMJD3 and mutant JMJD3 in Panc-1 cells. **C**, MTT assay showed the cell growth inhibition of wt JMJD3 and mutant JMJD3 on PDAC cells. **D**, Wound healing assay showed the cell migration inhibition of wtJMJD3 and mutant JMJD3 on PDAC cells. **E**, Colony formation assay showed the cell proliferation inhibition of wtJMJD3 and mutant JMJD3 on PDAC cells. **F**, Soft agar assay showed the anchorage-independent survival inhibition of wtJMJD3 and mutant JMJD3 on PDAC cells. **G**, Knock down of JMJD3 by shJMJD3 lenti-virus in HPDE cells. **H**, MTT assay showed the cell growth activation after knock down of JMJD3 in HPDE cells.

**Figure S2. Human mRNA expression in JMJD3 ectopic expressed Panc-1 cells was profiled by gene expression array.** **A**, Overexpression of JMJD3 decreased H3K27m3 levels caused no changes on the expression of p16<sup>INK4A</sup>, p19<sup>Arf</sup> and p53 in PDAC cells. **B**, Top ten changed genes profiled in wtJMJD3 and mutant JMJD3 ectopic expressed Panc-1 cells. **C**, Distribution of genes changed ontology groups from the microarray profiling.

**Figure S3. HKDC1 exhibited tumor suppressive role in PDAC cells.** **A**, The expression of HKDC1 was increased in wt JMJD3 but not mutant JMJD3 ectopic expressed Panc-1 cell. **B**, RT-QPCR and western blotting showed HKDC1 expression profiling in HPDE and PDAC cell lines. **C**, MTT assay showed the cell growth inhibition of HKDC1 on PDAC cells. **D**) Colony formation assay showed the cell proliferation inhibition of HKDC1 on PDAC cells. **E**, Wound healing assay showed the cell migration inhibition of HKDC1 on PDAC cells. **F**, Low HKDC1 expression was correlated with shortened overall survival in PDAC patients.

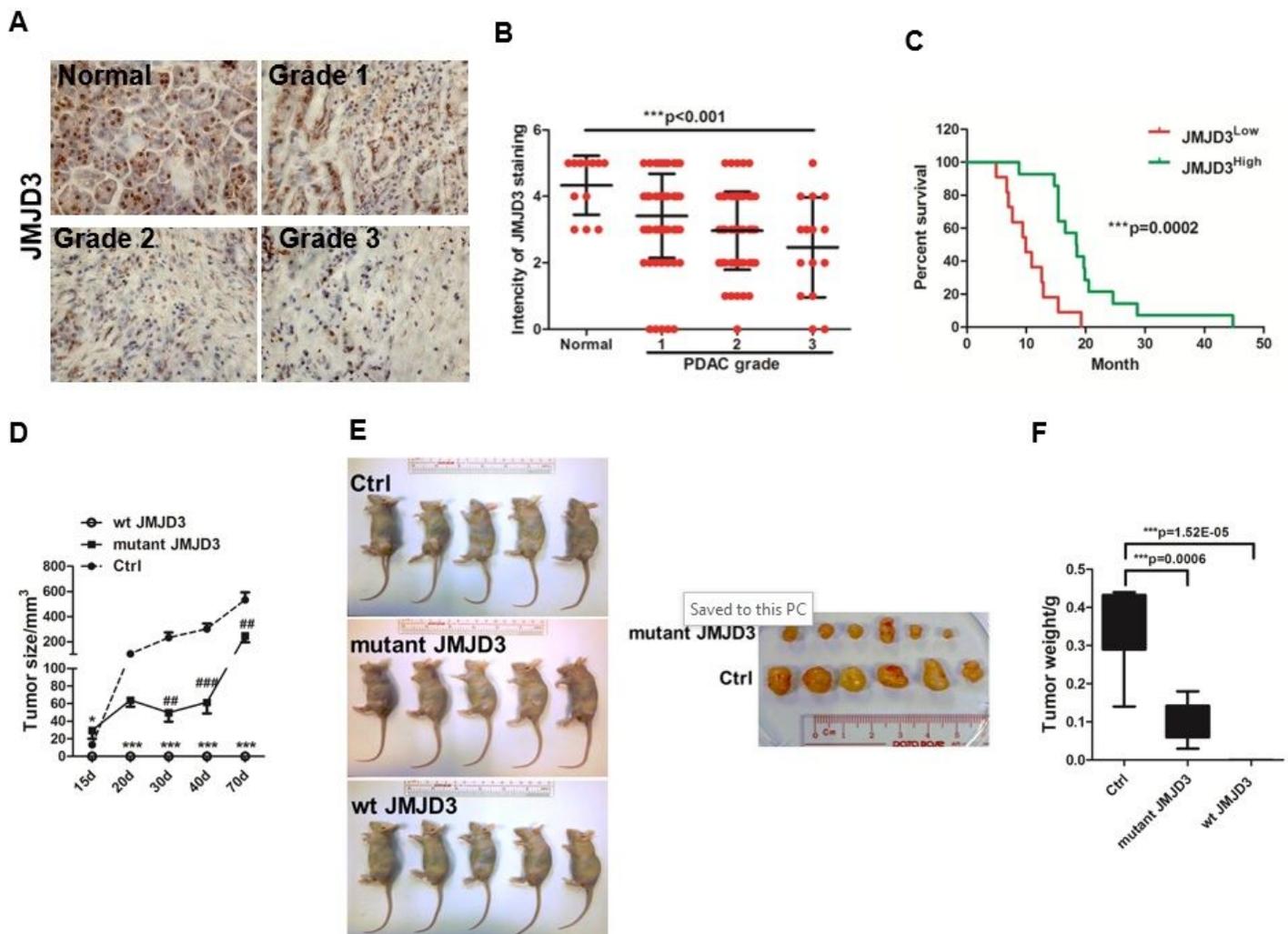
**Figure S4. HKDC1 suppressed PDAC cell growth through SBD domain.** **A**, A photograph showed the bioinformatics predicted domains of HKDC1 including HK and spectrin binding domain (SBD). **B**, HKDC1 constructs including including blank control, the full coding region of HKDC1 and the SBD deleted constructs (HKDC1<sup>ΔSBD</sup>). **C**, MTT assay showed the cell growth inhibition of HKDC1<sup>ΔSBD</sup> on Panc-1 cells. **D**, Colony formation assay showed the cell proliferation inhibition of HKDC1<sup>ΔSBD</sup> on Panc-1 cells.

**Figure S5. The scheme of constructs for binding assay.** **A**, The strategy of cloning for the generation of wtHKDC1, the SBD deleted or only domain contained PET-28a prokaryotic expression plasmids with the His tags. **B**, Spectrin alpha II was divided into four fragments. Each part was cloned into pGEX-4T-2 vector with GST tags, named α1, α2, α3, α4 respectively. **C**, Spectrin beta II was divided into five fragments. Each fragment was cloned into prokaryotic expression vector with GST tags, named β1, β2, β3, β4, β5

respectively. **D**, Expression, purification and identification of recombinant proteins including HKDC1, HKDC1 (SBD), HKDC $\Delta^{SBD}$ ,  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\beta 1$ ,  $\beta 2$ ,  $\beta 3$ ,  $\beta 4$  and  $\beta 5$ .

**Figure S6. Cytoskeleton disruption in JMJD3 ectopic expressed Panc-1 cells.** **A**, Spectrin beta II expression in JMJD3 ectopic expressed Panc-1 cells. **B**, Knock down of spectrin beta II by siSpectrin beta II in PDAC cells. **C**, MTT assay showed that cell growth inhibition of siSpectrin beta II on PDAC cells. **D**, Confocal laser-scanning microscope images showed the disruption of actin cytoskeleton and microtubule cytoskeleton in JMJD3 ectopic expressed PDAC cells.

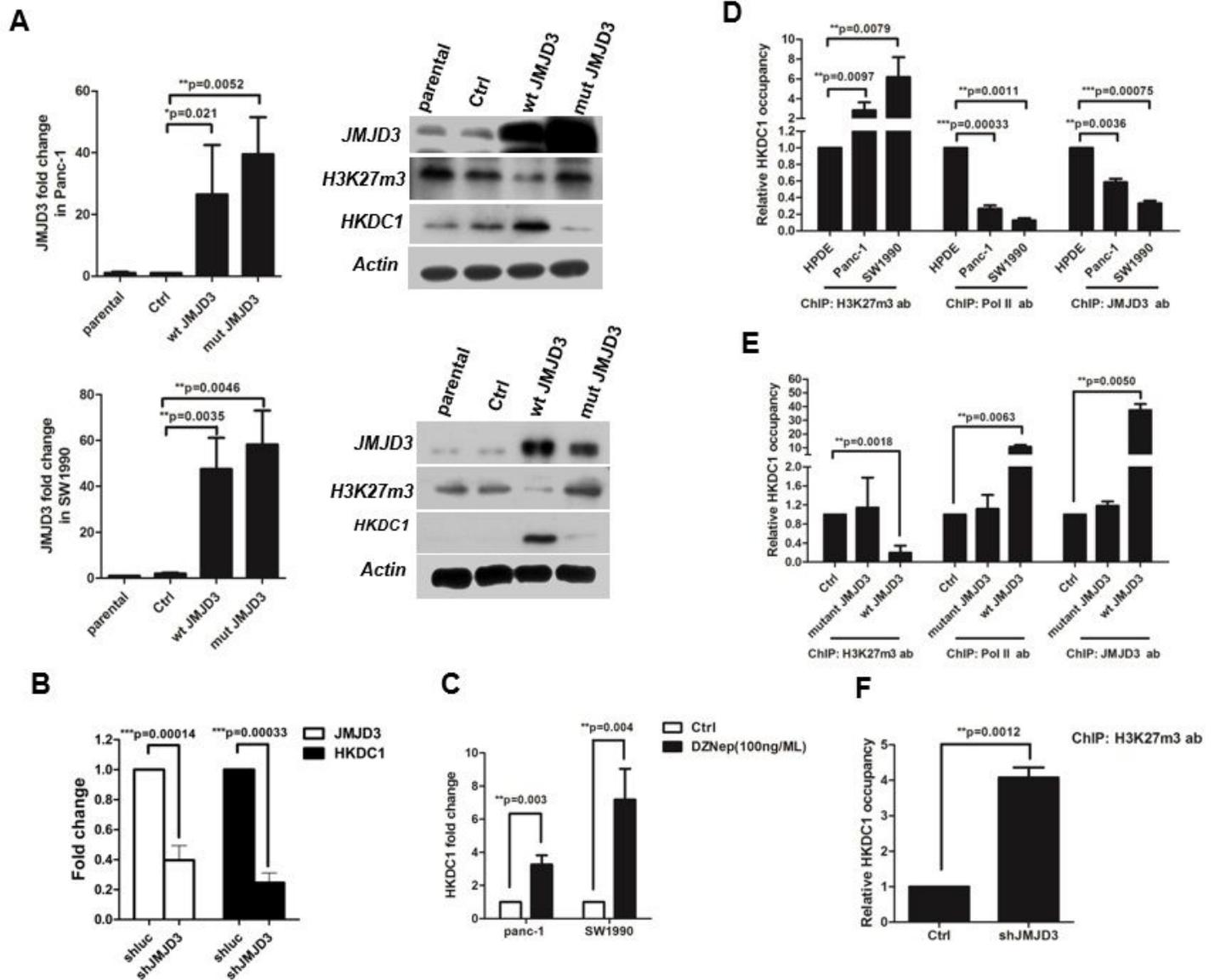
## Figures



**Figure 1**

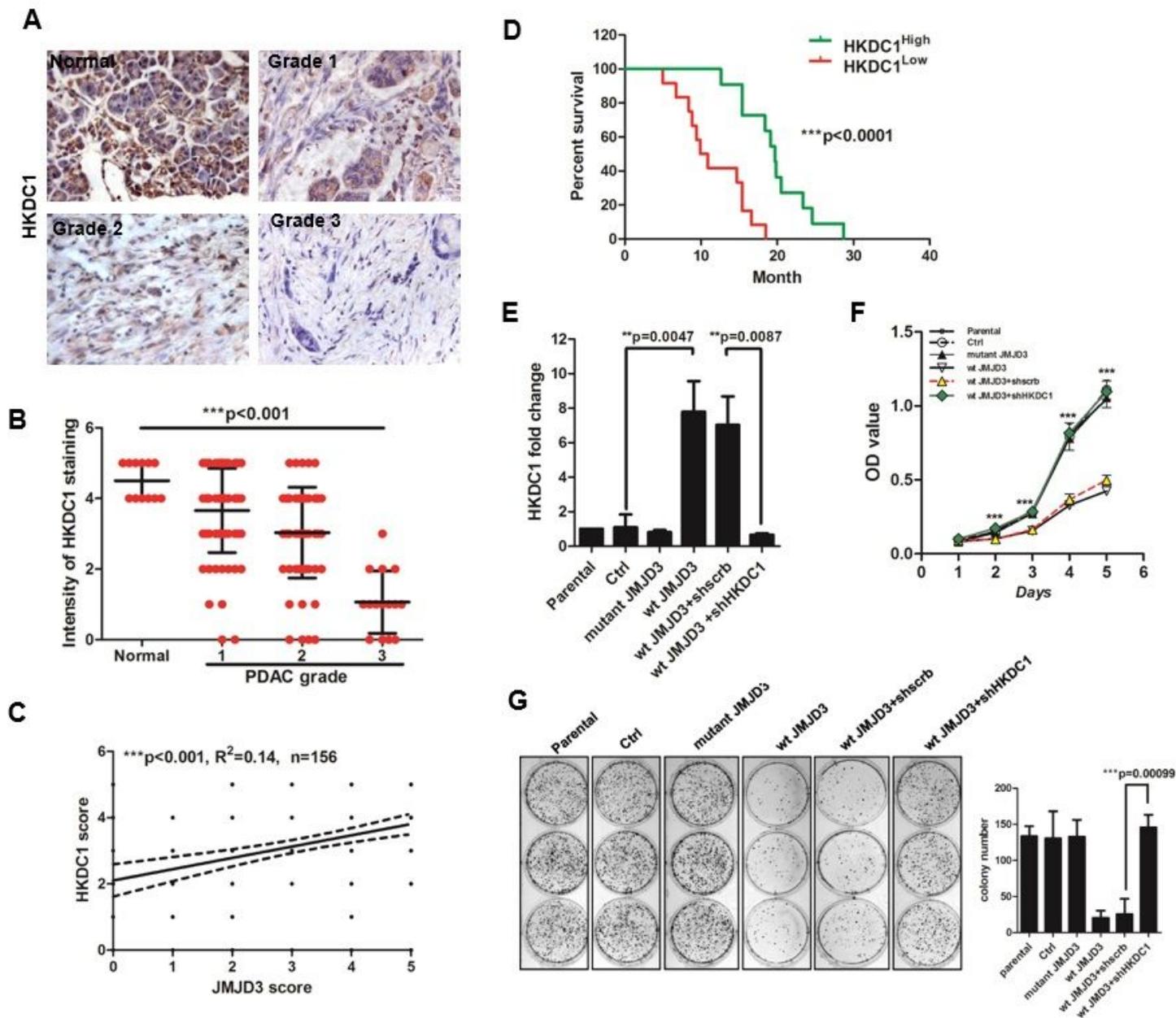
Loss of JMJD3 associated with PDAC development. **A**, JMJD3 immunohistochemistry of human tissue microarray of normal pancreas (n = 41) and PDAC grade 1 (n = 59), 2 (n = 96), 3 (n = 51). **B**, Loss of JMJD3 expression in high-grade PDAC. JMJD3 expression levels exhibited a statistically significant negative correlation with and tumor grades. P value is calculated by one-way ANOVA: \*\*\*, P < 0.001. **C**,

Representative immunohistochemical staining of JMJD3 in 25 PDAC tissues (low and high JMJD3 staining) are shown. D, Low JMJD3 expression was correlated with shortened overall survival in PDAC patients. (E, F, G) JMJD3 suppressed PDAC growth in vivo.



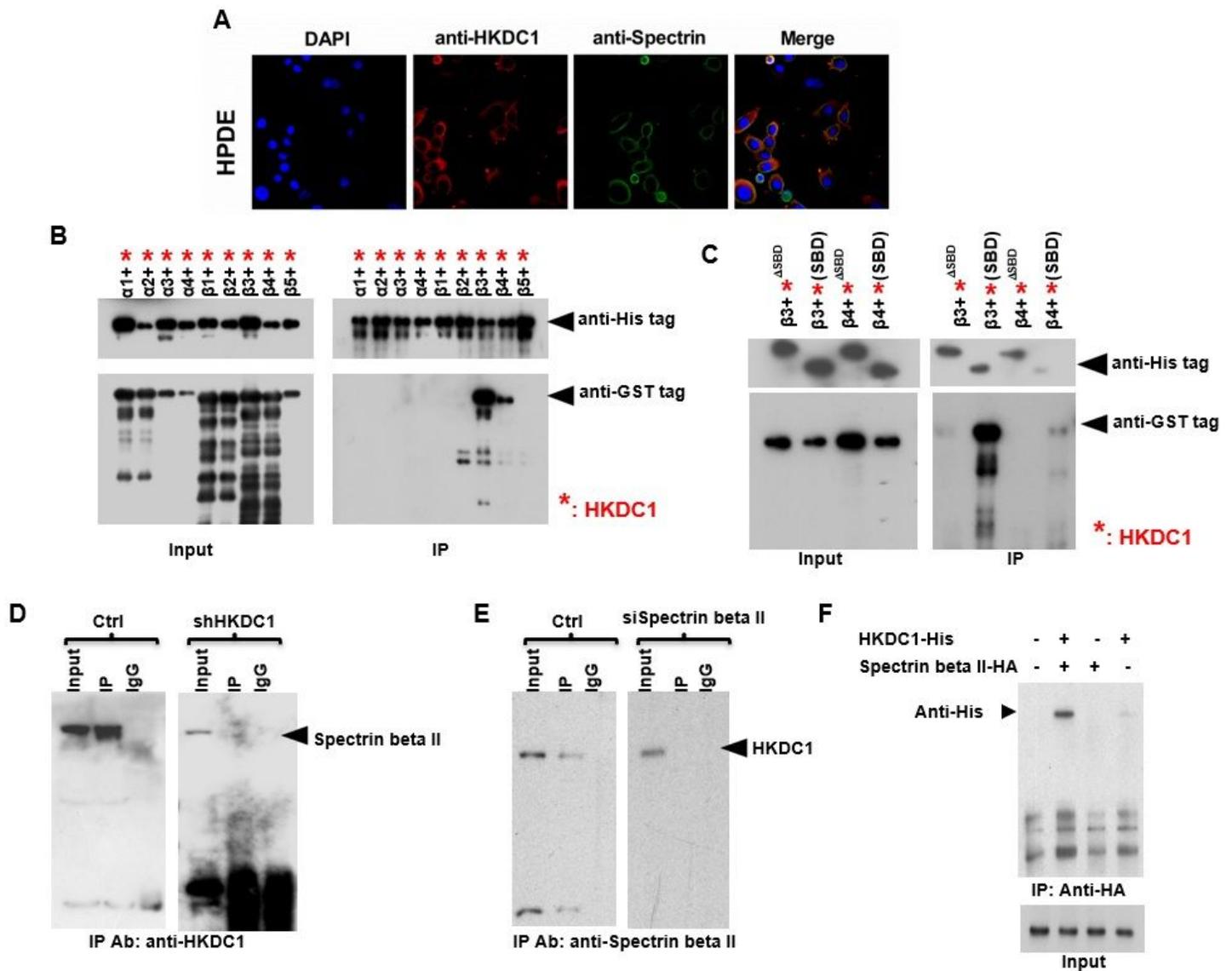
**Figure 2**

JMJD3 regulated HKDC1 expression by histone demethylation. A, The expression of HKDC1 mRNA and protein level was accelerated by wt JMJD3 which attenuated H3K27me3 level in Panc-1 and SW1990 cells. B, The expression of HKDC1 was decreased by knockdown of JMJD3 in Panc-1 and SW1990 cells. C, The expression of HKDC1 was increased by DZNep treatment in Panc-1 and SW1990 cells. D, ChIP assay showed the higher H3K27me3 mark but significant loss enrichment of pol  $\alpha$  and JMJD3 at HKDC1 promoter in PDAC cells (Panc-1 and SW1990) compared to the HPDE cells. E, ChIP assay showed that ectopic expression of JMJD3 led to a decrease of H3K27me3 level and increased binding of pol  $\alpha$ , JMJD3 to HKDC1 promoter region. F, ChIP assay showed that depletion of JMJD3 impaired the H3K27me3 reduction and repressed HKDC1 transcription.



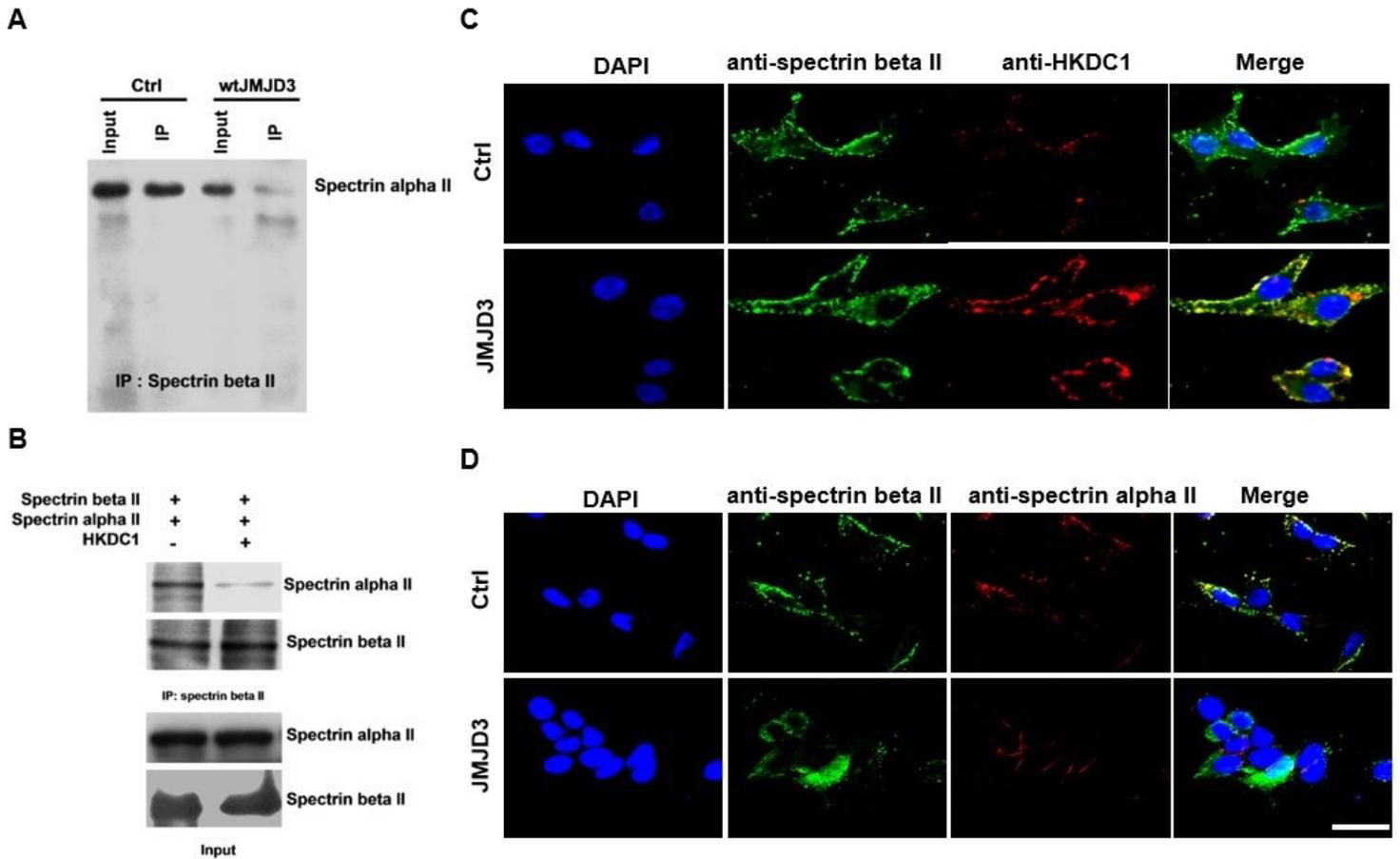
**Figure 3**

HKDC1 acted as a tumor suppressor in PDAC. A, HKDC1 immunohistochemistry of human tissue microarray of normal pancreas (n = 41) and PDAC grade 1 (n = 59), 2 (n = 96), 3 (n = 51). B, Loss of HKDC1 expression in high-grade PDAC. HKDC1 expression levels exhibited a statistically significant negative correlation with and tumor grades. P value is calculated by one-way ANOVA: \*\*\*, P < 0.001. C, Spearman rank correlation test showed a significant positive correlation between expression of JMJD3 and HKDC1. D, Low HKDC1 expression was correlated with shorter overall survival in PDAC patients. E, qRT-PCR showed that the expression of HKDC1 decreased significantly in shHKDC1 lentivirus infected Panc-1 cells with JMJD3 overexpression. F, MTT assay showed that JMJD3-induced growth inhibition could be reversed by knockdown of HKDC1 expression. G, The colony formation assay revealed that knockdown of HKDC1 expression increased the number of colony formation in Panc-1 cells when compared to cells ectopically expressing JMJD3 only.



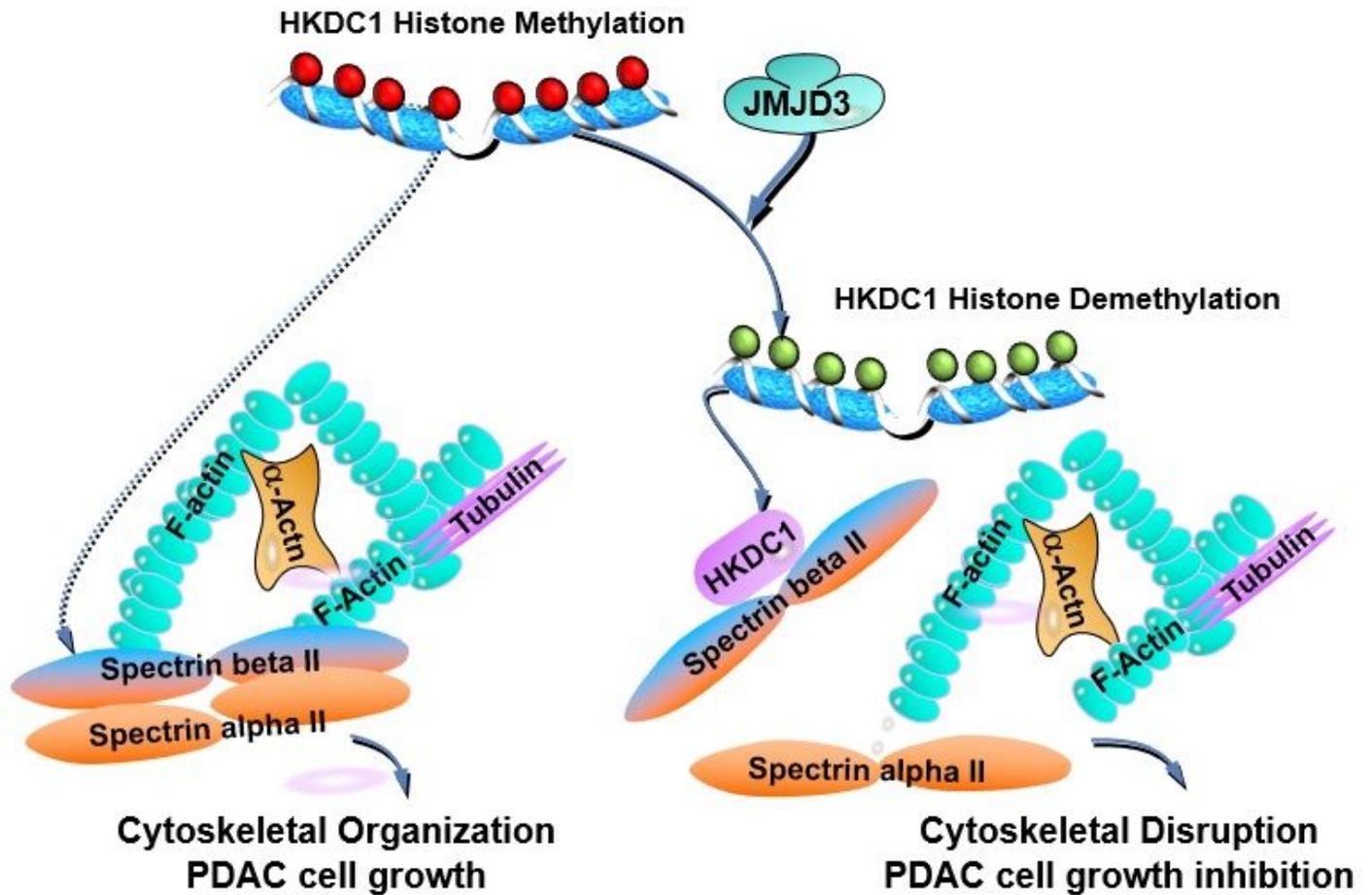
**Figure 4**

HKDC1 bound to spectrin beta  $\boxtimes$ . A, HKDC1 co-localized with spectrin in HPDE cells. B, Binding assay showed that the full length HKDC1 was bound to C-terminal domain of spectrin beta  $\boxtimes$ . C, Binding assay showed the SBD-domain only HKDC1 protein bound to spectrin beta  $\boxtimes$  fragments but not the SBD-deleted recombinant protein (HKDC1 $\Delta$ SBD). D, HKDC1 bound to spectrin beta  $\boxtimes$  in PDAC cells. The binding between HKDC1 and Spectrin beta  $\boxtimes$  was diminished by knockdown of HKDC1. E, By reverse-immunoprecipitation, knockdown of spectrin beta  $\boxtimes$  diminished the interaction of HKDC1 and spectrin beta  $\boxtimes$ . F, The binding between HKDC1 and spectrin beta  $\boxtimes$  was further confirmed by ectopic expression of HKDC1 and spectrin beta  $\boxtimes$  with specific tags in Panc-1 cells.



**Figure 5**

HKDC1 competed with spectrin alpha  $\beta$  for binding with spectrin beta  $\beta$ . A, Spectrin beta  $\beta$  bound to spectrin alpha  $\beta$  in Panc-1 cells. The binding between spectrin alpha  $\beta$  and spectrin beta  $\beta$  was attenuated in JMJD3 ectopically expressed Panc-1 cells. B, The extracellular binding assay showed HKDC1 competed with spectrin alpha  $\beta$  to bind with spectrin beta  $\beta$  by presenting HKDC1 recombinant proteins. C, The co-localization of HKDC1 and spectrin beta  $\beta$  (orange color) was found in JMJD3 over-expressed Panc-1 cells but not in vehicle control. D, Spectrin beta  $\beta$  and spectrin alpha  $\beta$  co-localization was diminished in JMJD3 over-expressed cells.



**Figure 6**

Schematic model of HKDC1-mediated cytoskeleton organization that was regulated by JMJD3. JMJD3 activated HKDC1 pathway in PDAC cells via demethylation dependent mechanism to suppress PDAC. In PDAC cells, the expression of HKDC1 was silenced by histone methylation. Ectopic expression of JMJD3 up-regulated HKDC1 expression by histone demethylation, and HKDC1 suppressed PDAC cell growth by disrupting the Spectrin beta-Spectrin alpha tetramerization.

## Supplementary Files

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