Am J Cancer Res 2021;11(1):92-107
www.ajcr.us /ISSN:2156-6976/ajcr0122485

Original Article

Ese-3 contributes to colon cancer progression by downregulating EHD2 and transactivating INPP4B

Junqiang Li[1*], Jing Yang[1*], Lei Hua[1*], Ronglin Wang[1], Hong Li[1], Chao Zhang[1], Haihua Zhang[2], Shanshan Li[1], Liaoliao Zhu[1], Haichuan Su[1]

[1]*Department of Oncology, Tangdu Hospital, Air Force Medical University, Xi'an 710038, Shaanxi, China;* [2]*Department of Pulmonary and Critical Care Medicine, Tangdu Hospital, Air Force Medical University, Xi'an 710038, Shaanxi, China.* [*]*Equal contributors.*

Received September 16, 2020; Accepted November 21, 2020; Epub January 1, 2021; Published January 15, 2021

Abstract: Epithelium-specific Ets protein 3 (Ese-3), a member of the Ets family of transcription factors, plays an important role in the development of cancers. However, little is known concerning its role in colon cancer (CC). In this study, we demonstrate that the expression of Ese-3 is upregulated in CC tissues and elevated Ese-3 expression is relationship with advanced T stage (P=0.037) and poor disease-free survival (DFS, P=0.044). Univariate and multivariate cox regression analyses show that Ese-3 expression may be an independent prognostic value for CC patients. Moreover, Ese-3 knockdown suppresses CC cell proliferation in vitro and in vivo, while Ese-3 overexpression has the opposite result. Further, we first demonstrate that EHD2 and INPP4B are the downstream genes of Ese-3. Subsequent investigation find that EHD2 is downregulated in CC tissues and knockdown of EHD2 significantly increase CC cell proliferation in vitro and vivo. Our findings reveal that Ese-3 promotes CC cell proliferation by downregulating EHD2 and transactivating INPP4B, and targeting the pathway may be a promising therapeutic target for CC patients.

Keywords: Ese-3, proliferation, EHD2, INPP4B, colon cancer

Introduction

Colon cancer (CC), which has one of the highest incidence rates and one of the mortality rates among gastrointestinal tumors worldwide [1, 2]. Despite with the development of various treatment methods including chemotherapy, radiation, and biotherapy, the 5 years survival rate for the patients with CC is no more than 40% due to the proliferation, metastasis and recurrence of CC [3]. Therefore, the molecular mechanisms of CC need to be further explored and novel therapeutic targets need to be identified.

Ese-3/EHF, a member of the Ets family of transcription factors, is widely expressed in various epithelial cells [4]. All members of the Ets family contain a highly conserved DNA-binding domain and recognize a specific binding site (GGAA/T) of within their target genes regions [5]. Increasing evidence indicates that Ese-3 could be a tumor oncogene or tumor suppressor gene in the developmental programs of various tumors. Domenico and Giuseppina reported that the loss of Ese-3 could promote prostate tumor progression by activating the Lin28/let-7 axis and deregulating the miR-424/COP1/STAT3 axis pathways [6, 7]. Ese-3, as a negative tumor regulator, is downregulated and inhibits cell invasion and metastasis by upregulating E-Cadherin in pancreatic cancer [8, 9]. However, Ese-3 as an oncogene in some other tumor types, can induce tumor proliferation and metastasis in gastric cancer [10], oral squamous cell carcinoma [11] and ovarian Cancer [12]. Although, Ese-3 plays an important role in the development of tumors by certain specific behaviors and signaling pathways, its roles and molecular mechanism remain unclear in CC.

Eps15 homology domain-containing 2 (EHD2), a member of the EHD family, was initially re-

ported as a plasma membrane-associated protein, but its precise function has been mysterious [13]. A series of reports have shown that EHD2 is a negative regulatory gene and EHD2 expression is significantly correlated with clinicopathologic parameters in tumors [14]. Decreased expression of EHD2 facilitated tumor invasion and metastasis in breast cancer [15], papillary thyroid carcinoma [16], esophageal squamous cell carcinoma [17] and induced the proliferation of clear cell renal cell carcinoma (ccRCC) [18] and hepatocellular carcinoma [19]. However, the role and function of EHD2 and its link to Ese-3 in CC have not been reported.

In this study, we demonstrated that Ese-3, a member of the Ets family of transcription factors, was upregulated in CC and that elevated expression of Ese-3 was dramatically correlated with poor disease free survival and advanced clinical T stages. Next, in vitro and in vivo assays results demonstrated that Ese-3 could accelerate the proliferation of CC. Further results showed Ese-3 could promote the activation of AKT by restraining the expression of EHD2 and targeting the INPP4B promoter region. Thus, this novel pathway may be valuable as a therapeutic approach for patients with CC.

Materials and methods

Patient specimens

Five paired CC and peritumoral tissue samples were obtained from CC patients who underwents urgical excision in Tangdu hospital affiliated with Air Force Military University (Xi'an, China). Every tissue sample were divided into two parts, one part of them were frozened in liquid nitrogen until use, the other were made into paraffin sections. Characterization of CC patients were described (Supplementary Table 1). Our study was supported by the Ethics Committee of Tangdu hospital. All subjects patients with CC gave the informed consent to participate in this study.

Cell lines and cell culture

In our study, we purchased CC cells (HCT116, HT29, LoVo, WiDr, and SW620) from Procell Life Science & Technology Company (Wuhan, China) and BeNa Culture Collection (BNCC, Beijing, China). All these cells have the STR authentication report. HCT116 and HT29 cell lines were cultured in McCoy's 5A media (Gibco, USA), WiDr and SW620 cell lines were grown in Dulbecco's Modified Eagle Medium media (Gibco, USA), and LoVo cell lines were maintained in Dulbecco's Modified Eagle Medium/F12 media. All CC cells were cultured in media with 10% fetal bovine serum (FBS), penicillin-streptomycin (Gibco, USA) and were incubated at 37°C in 5% CO_2.

Immunohistochemistry (IHC)

We purchased the human CC tissue microarray from Outdo Biotech Company (HColA180su16, Shanghai, China). Tissue microarray and tumor tissue sections were deparaffinized using the xylene, then rehydrated using the different concentration of ethanol. 3% hydrogen peroxide was used to inhibit endogenous peroxide activities and immediately to repair antigens through heating in citrate buffer (pH 6.0) using a microwave. Then, the 5% normal goat serum was used to block the tissue microarray and sections for 30 minutes. After being washed in PBS, the microarray and sections were incubated overnight at 4°C with the primary antibodys against Ese-3 (1:100, NBP2-14942, Novus), EHD2 (1:100, bs-14526R, Bioss), and Ki67 (1:1000, 27309-1-AP, Proteintech). The next day, after washing in PBS solution for 10 minutes, the peroxidase-conjugated secondary antibody was used to incubate the microarray and sections for 30 minutes, and then these tissue microarray and tumor tissue sections were stained with diaminobenzidine for 3 minutes. Finally, the slides were counterstained using hematoxylin.

The IHC staining results were assessed based on the stained cells percentage of (0, 0-5%; 1, 6-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%) and staining intensity (0, no staining; 1, faint yellow; 2, reddish; 3, brown). Then the two scores were multiplied to obtain the final result. A final staining score <6 was suggested as low Ese-3 expression, while a score of staining >6 was considered as high Ese-3 expression.

Lentivirus infection and stable cell line construction

The pLent-U6 lentiviral plasmid (Vigene Biosciences, Jinan, China) was used to generate lentivirus with shEse-3#1, shEse-3#2, shEHD2

and shControl sequences. The shControl sequence was used as the negative control (Supplementary Table 2). Lentiviral vectors encoding the human Ese-3 and EHD2 genes were constructed in the pLent-EF1a plasmid (Vigene Biosciences, Jinan, China) and named Ese-3 and EHD2. All the plasmids with packaging plasmids (pMD2G and psPAX2) were cotransfected into HEK293T cells, respectively. After transfecting for 48-72 hours, we collected the viral supernatants. The CC cells were infected with lentivirus. After 12 hours, 4 μg/ml puromycin was used to select these cells (OriGene, USA) for one week to establish stable CC cell lines.

Western blotting

All CC cells, CC and matched control tissues were lysed using lysis buffer (Applygen, Beijing, China) containing a protease and phosphatase inhibitor cocktail (Roche, Branchburg, USA). SDS-PAGE was used to fractionate the protein samples, then the protein bands were transferred onto PVDF membranes. 3% nonfat milk was used to block the PVDF membranes for 3 hours, then the PVDF membranes were incubated using appropriate antibodies overnight at 4°C (Supplementary Table 3). The next day, the PVDF membranes were incubated with HRP-conjugated secondary antibodies. Finally, the protein bands were detected with a Tanon-5200 chemiluminescent system (Shanghai, China).

Cell proliferation assay

The cell counting chamber was used to assess the CC cell proliferation ability. Briefly, CC cells were plated into 6-well plates in triplicate at the density of 3×10^4/well. After digestion, each well cell number was counted using the cell counting chamber at the indicated time point.

Colony-formation assay

The cell colony formation assay was performed using 6-well plates. A total of 800 cells/well were seeded on the plates in triplicates. After 10 days, the cells of 6-well plate were fixed using the anhydrous methanol and stained using the 1% crystal violet buffer for 20 minutes, then the plates were washed with running water to visualize the colony-formation ability.

Flow cytometry analysis

For flow cytometry, the CC cells (4×10^5 cells/plate) were plated in 6-well plates in triplicate and cultured for 24 hours. Then, the CC cells were collected and fixed with 70% ethanol for 30 minutes. Finally, the cells were stained using the PI/RNase solution for 20 minutes (Thermo, USA) and subjected to flow cytometry to perform cell cycle detection (BD FACSCalibur, USA).

Label-free quantitative LC/MS proteomics analysis

Knockdown of Ese-3 in HCT116 cells and control group cells were seeded on 100 mm cell culture dishes in triplicate, respectively. When the cells reached 80-90% of the cell culture dish, the cells were digested with trypsinization and washed with cold PBS for two times. Label-free quantitative LC/MS proteomics was performed by Mhelix Biotech Company (Shanghai, China).

Luciferase reporter assay

Luciferase reporter assay was used to detect the luciferase activity (Promega, E1910, USA) according to the experimental protocol of the manufacturer. The CC cells were transfected with plasmids. After two days, the cells were lysed with lysis solution, and then the relative luciferase activity was detected by a GloMax-TM 20/20 Microplate Luminometer (Promega, E1910, USA). All experiments were performed three times independently.

Ch-IP and real-time PCR assays

Ch-IP assays were performed using the Pierce™ Agarose ChIP Kit (Thermo, USA). Briefly, CC cells were cross-linked for 20 minutes with 1% formaldehyde, and then cross-linked chromatin was sonicated into small DNA fragments. Sonicated DNA fragments were immunoprecipitated with Ese-3 antibody (Proteintech, 27195-1-AP) and subjected to real-time PCR to amplify the corresponding fragment. The primers are displayed in Supplementary Table 4.

Animal studies

We purchased male BALB/c nude mice (4-6 weeks) from Beijing Vital River Laboratory

Animal Technologies company (Beijing, China), and all nude mice were kept in an SPF environment. A total of 2-3×10^6 CC cells were injected into subcutaneous of the nude mice (n=4 or 5). Every three days, the tumor volume of the nude mice was monitored (volume = length × width^2 ×0.5). At the end of the assay, the nude mice were sacrificed, and their subcutaneous tumors were weighed and imaged. Lastly, the nude mice tumors were fixed with paraformaldehyde or frozen in liquid nitrogen until use. All animal experiments were approved by the Institutional Animal Care and Use Committee of Tangdu Hospital.

Statistical analysis

All quantitative experimental data are showed as the mean ± SD of three independent replicates and were compared between two groups using Student's t-test, while ANOVA was used to assess differences among more than two groups. The correlation between Ese-3 expression levels and clinicopathological parameters was assessed using the chi-square test. Pearson correlation coefficient analysis was used to assess the association of Ese-3 mRNA levels and EHD2, INPP4B mRNA levels in prospective_CPTAC_COAD samples. Kaplan-Meier curves and log-rank tests were used to assess the correlation between Ese-3 expression and disease-free survival. Univariate or multivariate cox regression analyses were performed. The diagnostic value of Ese-3 was assessed by ROC curve analysis. SPSS 19.0 or GraphPad Prism 5.0 were used to analyze these experimental data (SPSS, Chicago, Illinois, USA) (La Jolla, CA, USA). *P* value <0.05 was considered as statistically significant.

Results

Enhanced Ese-3 expression is associated with poor prognosis in CC

To illuminate the potential function of Ese-3 in CC, we first analyzed the mRNA expression levels of Ese-3 using TCGA and GTEx databases in CC samples (n=275) and the normal control group (n=349). Ese-3 mRNA expression levels in CC cancer tissues were significantly upregulated compared with normal control tissues (*P*<0.05, **Figure 1A**). Western blotting results showed that Ese-3 expression levels also were elevated in CC tissues compared with paired peritumor tissues (**Figure 1B**). This result was further confirmed by IHC staining. In CC cancer tissues Ese-3 staining was strongly positive, but Ese-3 staining was weakly positive in matched peritumor tissues (**Figure 1C**). Then IHC staining was used to analyze the association between Ese-3 expression and clinical parameters using a tissue microarray. Statistical analysis revealed that Ese-3 expression significantly correlated with advanced T stage (*P*= 0.037), but not age, gender, clinical grade or N stage, TNM stage (**Table 1**, Supplementary Table 5). Furthermore, Kaplan-Meier curves and the long-rank test analysis showed that higher Ese-3 mRNA expression levels were correlated with poorer DFS (*P*=0.044, **Figure 1D**). Next, TCGA samples were used to assess Ese-3 prognostic value by univariate and multivariate Cox regression analyses. Univariate analysis showed that Ese-3 expression, T stage, N stage, M stage and TNM stage could be used to predict the disease free survival time (**Figure 1E**). Importantly, multivariate analysis showed that the expression of Ese-3 was an independent predictor value of DFS in CC (HR=1.013, 95% CI (1.005-1.022, *P*=0.002) (**Figure 1F**). Taken together, these results reveals that the Ese-3 expression level is up-regulated in CC and might as an independent prognostic value in patients with CC.

Ese-3 promotes CC cell proliferation in vitro and in vivo

To further assess the functional role of Ese-3 in CC in vitro, firstly, the protein expression levels were detected in different tumor cells (Supplementary Figures 1A, 10H1, 10H2). Then, we established stable Ese-3 knockdown CC cells (HCT116 and HT29) using two different shRNA sequences, and stable Ese-3-overexpressing WiDr and HCT116 cells. Western blotting was performed to confirm the successful knockdown and overexpression of Ese-3 in CC cells (**Figure 2A-C**, Supplementary Figures 1B, 10H3-H5). The knockdown of Ese-3 obviously decreased CC cell proliferation (**Figure 2D**, **2E**) and clonogenic ability (**Figure 2G**, **2H**). Flow cytometry analysis of the cell cycle revealed that knockdown of Ese-3 significantly increased the number of G0/1, and G2/M phase cells and concomitantly decreased the number of S phase cells in HCT116 and HT29 cells (**Figure 2J**, **2K**). In contrast, enriched Ese-3 expression remarkably promoted CC cell growth (**Figure 2F**, Supplementary Figure 1C) and colony forma-

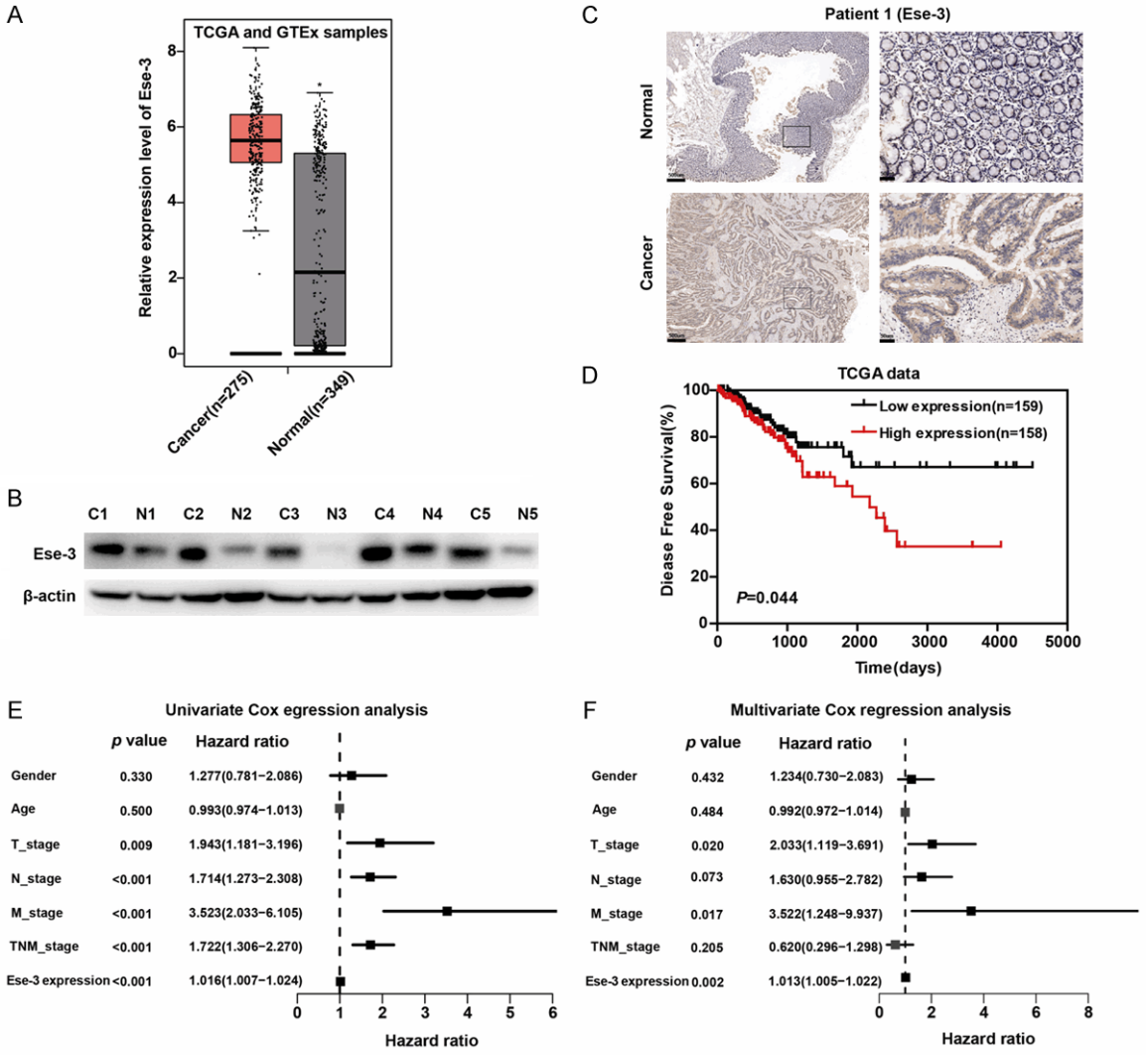


Figure 1. Elevated Ese-3 expression is associates with poor prognosis in CC. A. Ese-3 mRNA expression levels in CC and normal tissue samples were analyzed using TCGA and GTEx samples ($P<0.05$). B. Western blotting was used to analyze Ese-3 protein expression levels in 5 matched pairs of CC and peritumoral tissues (Original blots were showed in Supplementary Figure 3). C. Representative IHC staining of Ese-3 in matched CC and peritumoral tissues. Scale bar =500 μm (left) or Scale bar =50 μm (right). D. Kaplan-Meier disease-free survival analysis of Ese-3 expression in patients with CC using TCGA samples (n=317, $P=0.044$, log-rank test). E, F. Univariate (E) and multivariate (F) analyses of the relationship of disease free survival with clinicopathological characteristics and Ese-3 expression in TCGA samples.

tion (**Figure 2I**, Supplementary Figure 1D). Overexpression of Ese-3 resulted in an increase in G0/1 phase cells and a decrease in S phase cells in WiDr cells (**Figure 2L**). Then we further explored the impact of Ese-3 on CC cells in vivo. Ese-3-silenced HCT116 cells with luciferase and control cells, Ese-3-overexpressing HCT-116 cells and the corresponding control group were subcutaneously into the subcutaneous nude mice, the results showed that decreased Ese-3 cells reduced the bioluminescence intensity, tumor volume and tumor weight, while increasing Ese-3 expression increased tumor volume and tumor weight (**Figure 3A**, **3B**, Supplementary Figure 1E-G). Moreover, ki67 expression was decreased in Ese-3-silenced HCT116 cells compared with control group cells (**Figure 3C**).

Ese-3 regulates two tumor-related genes, EHD2 and INPP4B

To explore the underlying mechanisms of Ese-3 promoting CC proliferation, label-free quantitative proteomics technology was used to detect the changes of protein expression levels medi-

Table 1. Correlation of Ese-3 expression with clinicopathological features of CC

Parameters	Total (n)	Ese-3 expression (IHC)				
		Low	High	χ^2	*P* value	r
Gender						
Male	49	10	39	0.654	0.419	0.077
Female	51	15	36			
Age (years)						
<60	17	3	14	0.213	0.645	0.103
≥60	83	22	61			
T						
I	1	0	1	8.478	0.037*	0.3078
II	5	2	3			
III	75	14	61			
IV	18	9	9			
N						
N0	60	13	47	0.500	0.480	0.094
N1-2	40	12	28			
TNM stage						
I	6	2	4	3.957	0.266	0.1872
II	54	11	43			
III	49	11	38			
IV	1	1	0			
Pathological grade						
I	22	2	20	4.064	0.131	0.1976
II	73	22	51			
III	5	1	4			

Note: *Statistically significant (*P*<0.05).

ated by Ese-3. The results showed that 231 protein expression levels were upregulated and 234 protein expression level were downregulated in Ese-3 silenced HCT116 cells compared with control group cells (**Figure 3D**; Supplementary Table 6). Among these proteins, EHD2 protein levels were upregulated and INPP4B protein levels were reduced in Ese-3-silenced HCT116 cells compared with control group cells (**Figure 3E**). We further confirmed that Ese-3 expression levels were significantly negatively associated with EHD2 expression levels (R^2=0.05, r=-0.224, *P*=0.021) and obviously positively correlated with INPP4B expression levels (R^2=0.039, r=0.198, *P*=0.042) in 106 prospective_CPTAC_COAD samples (**Figure 3F**; Supplementary Table 7). In addition, Western blotting assays further revealed that EHD2 was significantly downregulated and INPP4B was remarkably upregulated in Ese-3 knockdown HCT116 and HT29 cells. Meanwhile, we also found that the phosphorylation levels of AKT were decreased in Ese-3-silenced cells, while Ese-3 overexpression in WiDr cells increased INPP4B expression and AKT phosphorylation levels and reduced EHD2 expression (**Figure 3G**).

EHD2 is downregulated in CC

In our current study, EHD2 protein expression levels were assessed in 5 matched pairs of CC tissues and normal tissues and EHD2 expression levels were significantly downregulated in CC tissues (**Figure 4A**). Further IHC staining analysis confirmed that EHD2 protein expression levels were also reduced in CC tissues compared with peritumoral tissues (**Figure 4B**). In agreement with these results, EHD2 mRNA levels were obviously lower in CC samples than that in normal control group by analyzing CPTCA and TCGA samples (**Figure 4C**, **4D**, *P*<0.001). Importantly, the mRNA expression levels of EHD2 in the normal control sample were significantly higher than that in the other T stages, including T1 (*P*<0.05), T2 (*P*<0.001), T3 (*P*<0.001) and T4 (*P*< 0.01) (**Figure 4E**). In addition, EHD2 mRNA levels were remarkably downregulated in TNM stage I (*P*<0.001), TNM stage II (*P*<0.001), TNM stage III (*P*< 0.001), and TNM stage IV (*P*<0.001) compared with the normal control in CPTCA and TCGA samples (**Figure 4F**, **4G**). ROC curve analysis showed that EHD2 as a good diagnostic value in patients with CC (AUC=0.798; *P*<0.0001) (**Figure 4H**). These findings suggest that EHD2 could be used as a promising biomarker in patients with CC.

EHD2 inhibits CC cell proliferation in vitro and in vivo

Firstly, the protein expression levels of EHD2 were assessed in different CC cells. The results indicated that EHD2 expression was lower in SW620 cells than in other CC cancer cells (**Figure 4I**). We then established stable EHD2-overexpressing SW620 cells, and EHD2-silenced HCT116 and HT29 cells with lentivirus infection. The downregulation of EHD2 significantly increased tumor cell proliferation and

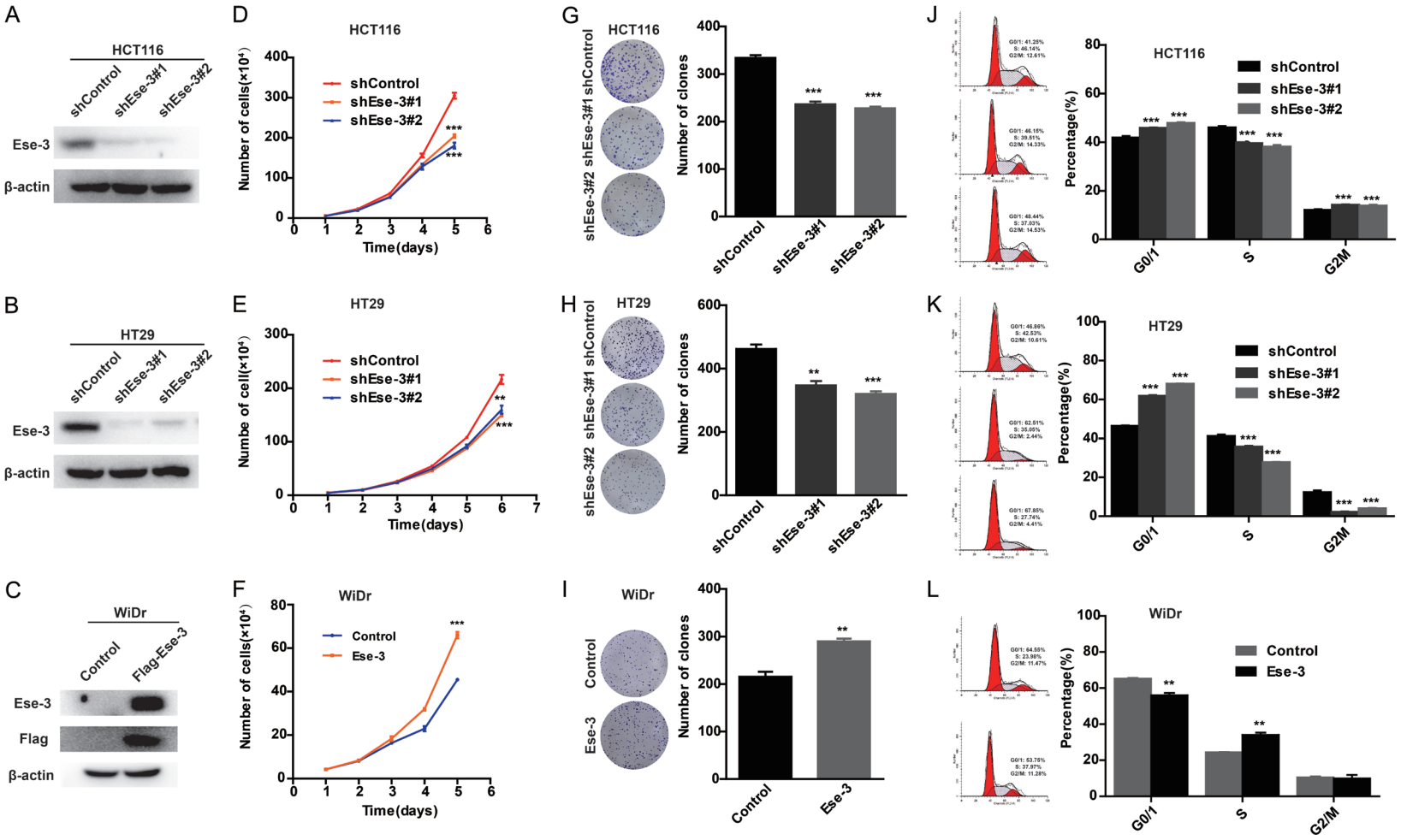


Figure 2. Ese-3 promotes CC cell proliferation in vitro. A-C. Western blotting analyzed the Ese-3 protein expression levels in Ese-3-silenced HCT116 cells, HT29 cells and Ese-3-overexpressing WiDr cells (Original blots were showed in Supplementary Figure 4). D-F. Cell proliferation assay analysis of the impact of Ese-3 on growth in Ese-3-silenced HCT116 and HT29 cells and Ese-3-overexpressing WiDr cells. G-I. Ese-3-silenced HCT116 and HT29 cells and Ese-3-overexpressing WiDr cells were subjected to colony formation assay. J-L. Ese-3-silenced HCT116 and HT29cells and Ese-3-overexpressing WiDr cells were used to evaluate the cell cycle by flow cytometry assay. Data are displayed as the mean ± SD of three in dependent replicates (*P<0.05; **P<0.01; ***P<0.001).

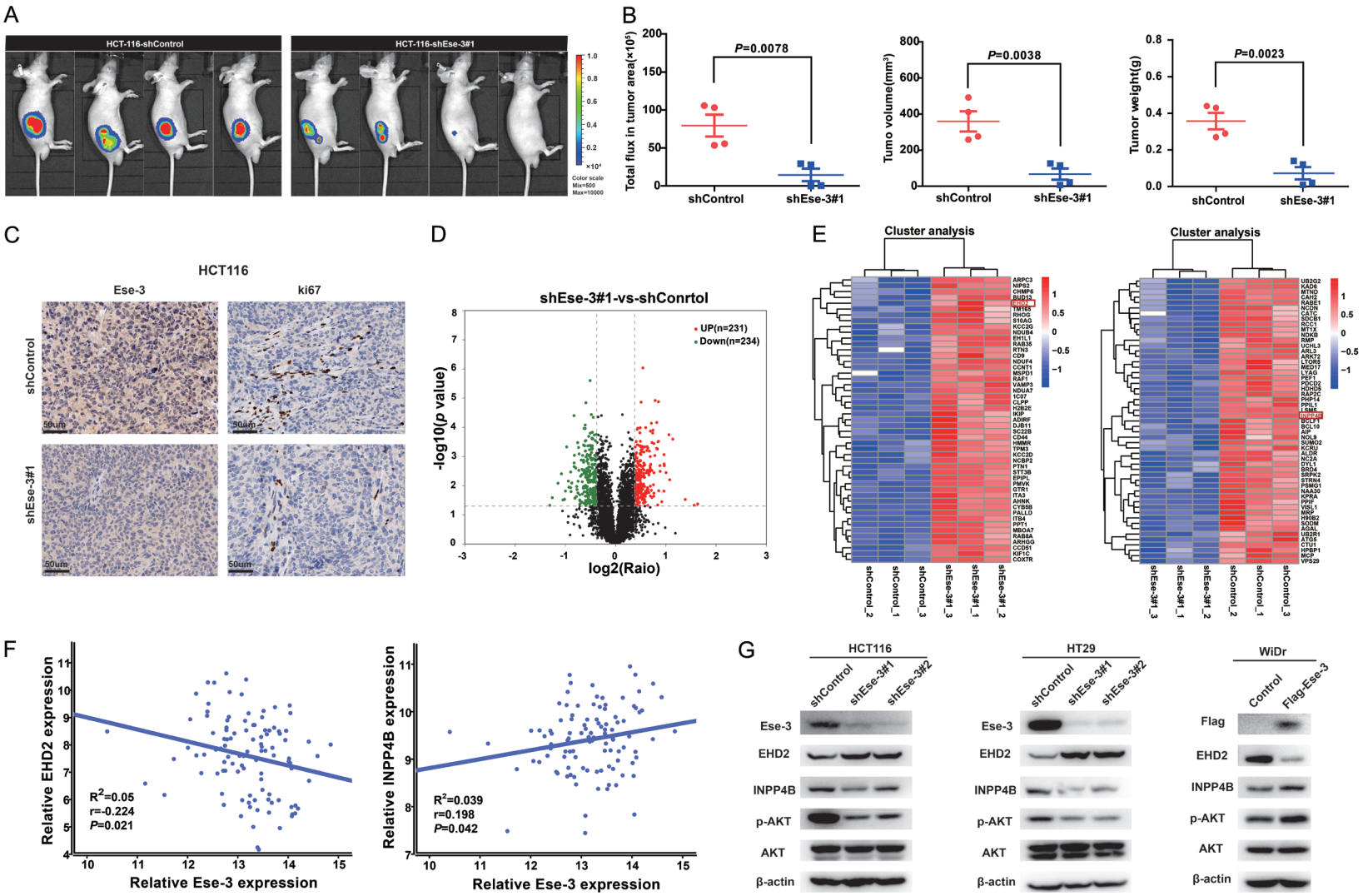


Figure 3. Ese-3 promotes CC cell growth in vivo and regulates the expression of EHD2, INPP4B and p-AKT. A. Representative bioluminescence imaging showed that knockdown of Ese-3 suppressed the growth of subcutaneous tumors in nude mice (n=4). B. Total flux, volume and weight of tumors were quantified and measured. C. IHC staining was used to measure the Ese-3 and Ki67 expression in nude mice tumor tissues (scale bars =50 μm). D, E. Volcano plot and heat map showing some differentially expressed proteins between Ese-3-silenced HCT116 and control group cells was identified by label-free quantitative proteomics technology. F. The expression level of Ese-3 was negatively correlated with that of EHD2 (P=0.021, r=-0.224) and positively correlated with that of INPP4B (P=0.42, r=0.198) (linear regression). G. Western blot analysis of the indicated proteins expression levels in Ese-3-silenced and Ese-3-overexpressing cells (Original blots were showed in Supplementary Figure 5).

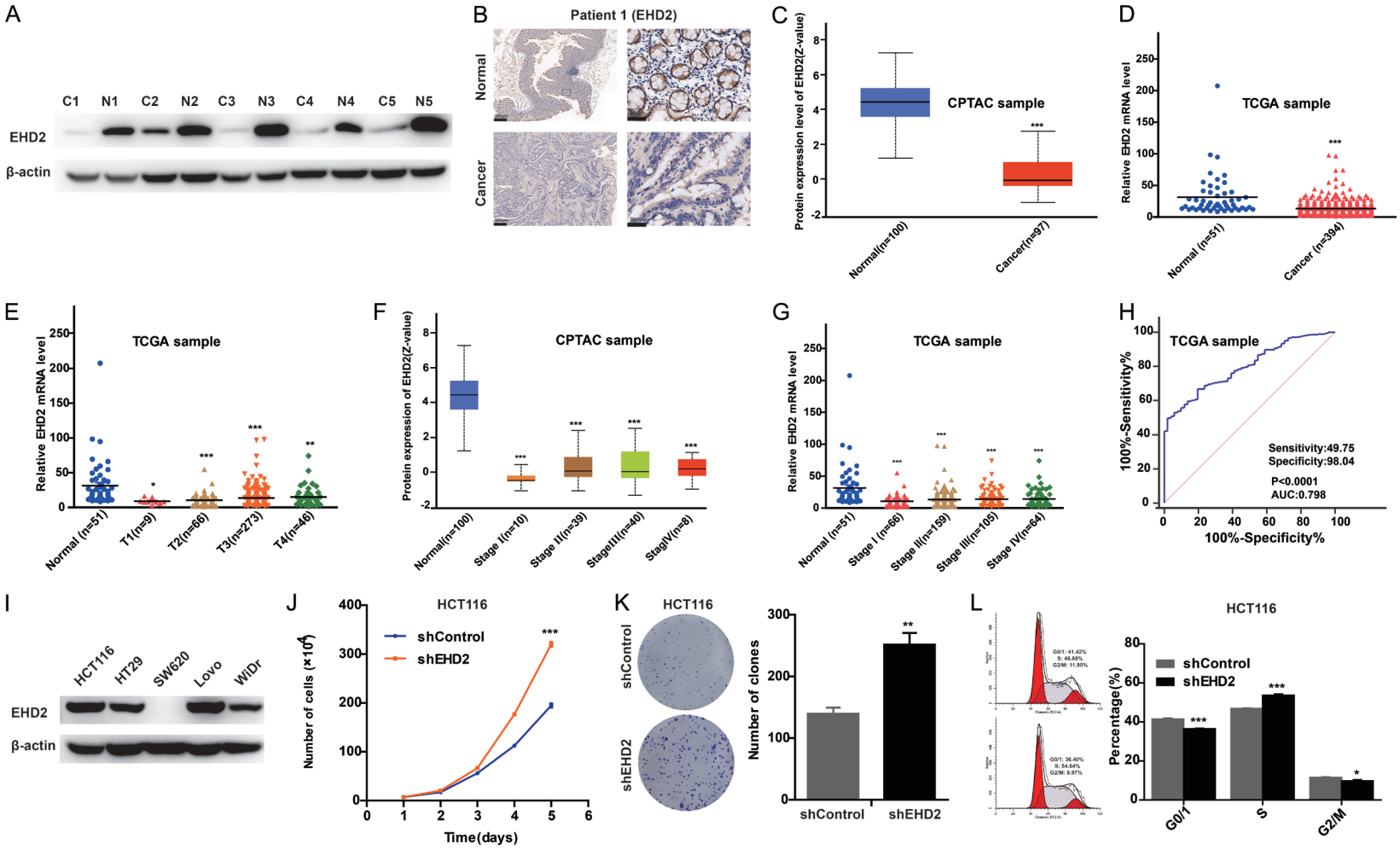


Figure 4. EHD2 is downregulated in CC. A. EHD2 protein expression levels were measured in 5 matched pairs of CC and peritumoral tissues (Original blots were showed in Supplementary Figure 6D1, 6D2). B. Representative IHC staining analysis of EHD2 expression in CC and peritumoral tissues. Scale bar =500 μm (left) or Scale bar =50 μm (right). C, D. EHD2 expression levels were analyzed in the CC and normal control groups using TCGA and CPTAC samples. E. The expression levels of EHD2 were analyzed in T1, T2, T3, T4 and normal tissues using TCGA samples. F, G. CPTAC and TCGA sample analysis of EHD2 expression levels in TNM stage I, TNM stage II, TNM stage III, TNM stage IV and normal control samples. H. The diagnostic value of EHD2 for CC was evaluated using the ROC curve. I. EHD2 protein expression levels were measured in different CC cells (Original blots were showed in Supplementary Figure 6D3, 6D4). J. Knockdown of EHD2 in HCT116 cells were performed cells proliferation assay. K. Colony formation assay was used to analyze the impact of EHD2 on growth in EHD2-silenced HCT116 cells. L. The cell cycle was examined in EHD2-silenced HCT116 cells using the flow cytometry assay. Data are shown as the mean ± SD of three independent replicates (**P*<0.05; ***P*<0.01; ****P*<0.001).

colony formation ability in HCT116 and HT29 cells, while EHD2 overexpression inhibited tumor cell growth and capacity of colony formation in SW620 cells (**Figures 4J**, **4K**, **5A-D**). Furthermore, flow cytometry analysis showed that EHD2-silenced in HCT116 and HT29 cells significantly decreased G0/1 phase cells and increased S phase cells (**Figures 4L**, **5E**). Consistent with these results, increased EHD2 expression remarkably increased the number of G0/1 phase cells and reduced the number of S-phase cells (**Figure 5F**). Next, we performed a nude mice tumorigenicity assay. Knockdown of EHD2 significantly increased the volume and weight of nude mice tumors (**Figure 5G-I**). In addition, IHC staining analysis indicated that ki67 expression levels were upregulated in EHD2-knockdown tumors compared with the control group (**Figure 5J**). Taken together, these experimental results suggest that EHD2 is a negative regulatory gene in CC.

EHD2 and INPP4B contribute to Ese-3-mediated CC cell proliferation

We further focused on examining the molecular mechanism of Ese-3-mediated CC cell proliferation. We first found that EHD2 knockdown can activate the phosphorylation of AKT in HCT116, HT29 and LoVo cells, while upregulated EHD2 decreased AKT phosphorylation levels in SW620 cell lines (**Figure 6A**, Supplementary Figures 2A, 11I1-I4), further implying that EHD2 is a tumor suppressor gene in CC by regulating AKT phosphorylation. To confirm the importance of EHD2 and INPP4B in Ese-3-mediated oncogenic function in CC, we knocked down the EHD2 gene in Ese-3-silenced HCT116, HT29, and LoVo cells, and the results showed that knockdown of EHD2 rescued the phosphorylation levels in Ese-3-knockdown HCT116, HT29, and LoVo cells (**Figure 6B**, Supplementary Figures 2B, 11I5-I9). Then, we transplanted Ese-3 plus EHD2-knockdown HCT116 cells with control Ese-3-silenced HCT116 cells into the back of nude mice, and the results showed that knockdown of EHD2 restored the growth capacity of HCT116 cells in the Ese-3-silenced xenograft group, which led to increase tumor volume and tumor weight (**Figure 6C-E**).

In our previous study, chromatin immunoprecipitation (ChIP) sequence assay analysis found that INPP4B might be a target gene of Ese-3. Further, we explored the molecular mechanism of Ese-3-mediated INPP4B. First, luciferase reporter assay results indicated that upregulated Ese-3 significantly transactivated the promoter activity of INPP4B (**Figure 6F**). Then, the INPP4B promoter sequence was analyzed, finding seven potential Ese-3 binding sites at the INPP4B promoter region. To further confirm which site of Ese-3 binds to the INPP4B promoter region, we established a serially truncated INPP4B promoter construct. We found that when Ese-3 binds to the region between -1043 to -640 bp, the luciferase activity was much higher in Ese-3- overexpressing HCT116 cells than in the control group (**Figure 6G**). In agreement with the results, the mutation between the -648 to -641 INPP4B promoter site significantly decreased the activity of the luciferase reporter compared with the wild-type group (**Figure 6H**). Ch-IP and qRT-PCR assays further confirmed that Ese-3 binds to the -648 to -641 bp site (**Figure 6I**, **6J**). The results suggest that the -648 to -641 bp region is vital for Ese-3-mediated INPP4B promoter transactivation.

Discussion

Great progress has been achieved in the diagnosis and treatment of CC, one of the most common malignant tumors of the digestive tract, but the duration of survival remains unfavorable due to a lack of good biomarkers as well as high rates of tumor metastasis and recurrence [20]. Thus, in order to identify new therapeutic targets, there is an urgent need to explore the molecular mechanisms underlying the development of CC. In our study, we found that Ese-3 knockdown inhibited the growth and colony formation of CC cells and induced cell cycle arrest in vitro. We also verified that knockdown of Ese-3 inhibited the tumorigenicity of nude mice in vivo. Furthermore, decreased Ese-3 expression in CC cells significantly inhibited the AKT phosphorylation. These results suggest that Ese-3 might be a potential therapeutic target in CC patients.

Ese-3/EHF, which belongs to the ESE subfamily, alongside Ese-1 and Ese-2, has a highly conserved 85-amino acid ETS DNA binding region that binds to the promoters of target genes [21]. A series of studies have shown that Ese-3 is expressed in differentiated epithelial cells of various organs and participates

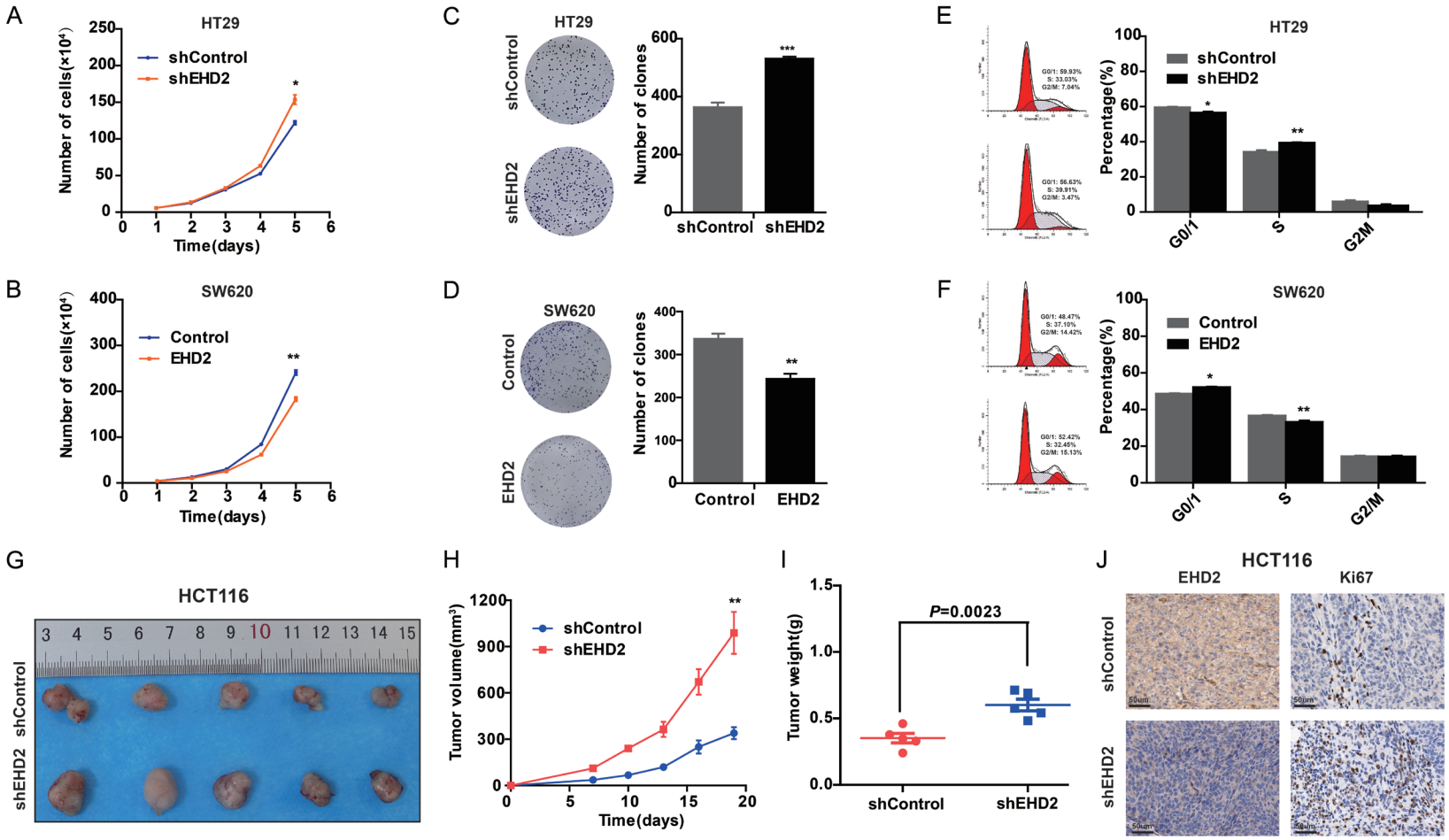


Figure 5. Effects of EHD2 on CC cell growth in vitro and vivo. A, B. Cell proliferation assay was performed to assess the impact of EHD2 knockdown (A) and overexpression (B) on CC cell growth. C, D. Colony formation assay was used to detect EHD2-silenced HT29 cells and EHD2-overexpressing SW620 cells. E, F. Flow cytometry assay was performed to examine the cell cycle in EHD2-silenced HT29 cells and EHD2-overexpressing SW620 cells. G. The subcutaneous tumors of nude mice were separated and measured. H. The volume of xenograft tumors was monitored every 3 days in EHD2-silenced HCT116 and control cells. I. The tumors of the two groups were weighed. J. EHD2 and ki67 expression levels in the two groups were measured by IHC staining. Data are presented as the mean ± SD of three independent replicates (**P*<0.05; ***P*<0.01; ****P*<0.001).

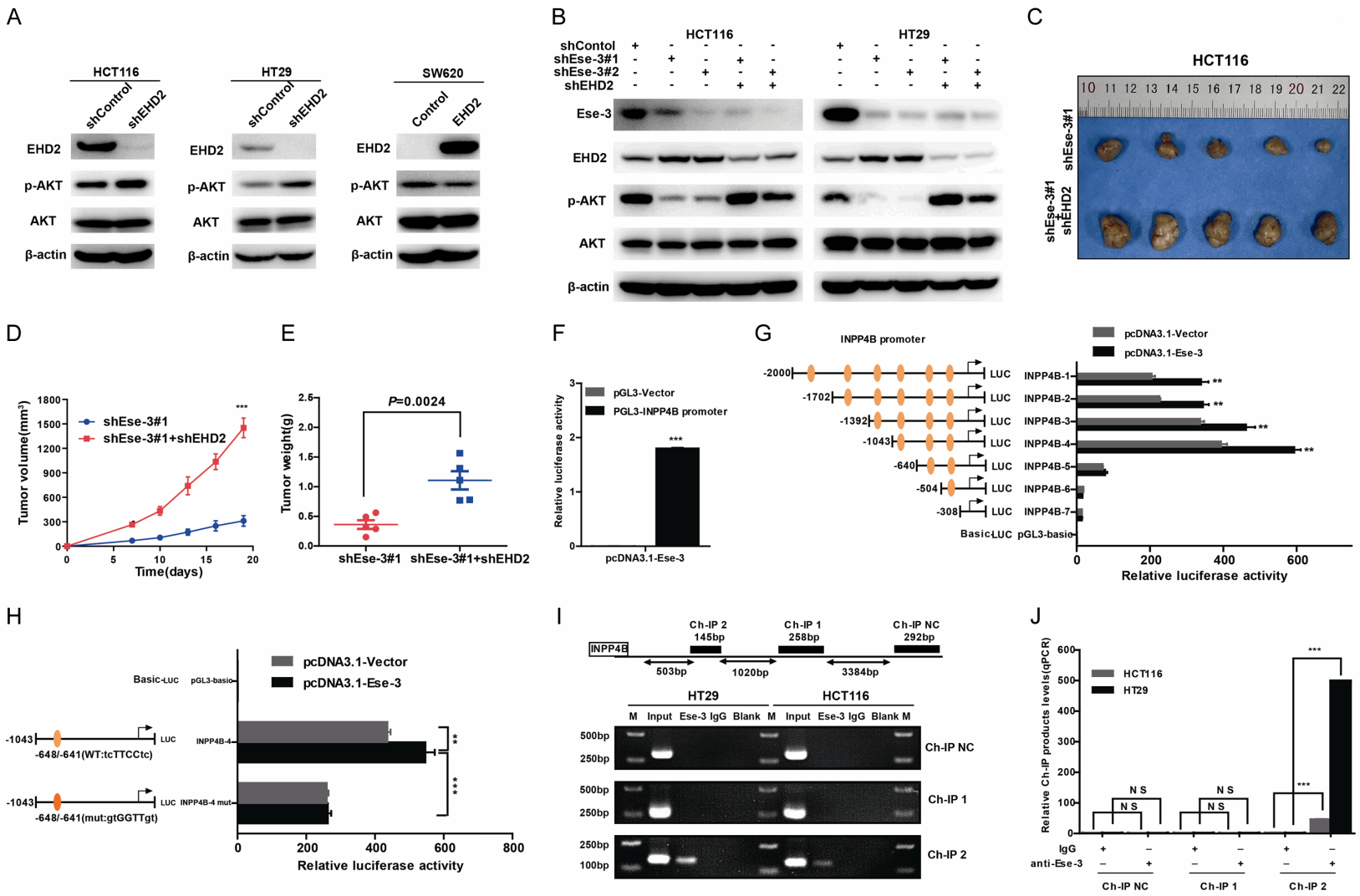


Figure 6. Ese-3 promotes CC cell proliferation by downregulating EHD2 and transactivating INPP4B. A. The indicated protein expression levels were detected in EHD2-knockdown and EHD2-overexpressing cells (Original blots were showed in Supplementary Figure 7). B. Western blotting analysis of the indicated protein expression levels in HCT116 and HT29 cells transduced with shControl, shEse-3 or shEse-3 plus shEHD2 (Original blots were showed in Supplementary Figure 8). C. ShEse-3#1-silenced HCT116 cells with or without co-transduction with shEHD2 were injected into the subcutaneous of nude mice (n=5). The tumors that developed in nude mice were isolated and measured at the end of this cell injection experiment. D. The growth curves of the two groups were compared. E. Comparison of the weight between the two groups. F. Relative luciferase activity was measured to analyze the effect of Ese-3 on INPP4B promoter activity in HCT116 cells. G. Luciferase reporter assays were performed to analyze the impact of Ese-3 on the serially truncated INPP4B promoter in HCT116 cells. H. The effect of Ese-3 on truncated

INPP4B-4 wild-type or mutant promoters were detected by luciferase reporter assays. I. Ch-IP assays were used to detect the binding site of Ese-3 to INPP4B in HCT116 and HT-29 cells (Original gels were showed in Supplementary Figure 9). J. The ability of Ese-3 bind to the INPP4B promoter was determined using qRT-PCR in HCT116 and HT29 cells. Data are presented as the mean ± SD of three independent replicate experiments (*P<0.05; **P<0.01; ***P<0.001).

in cell development, differentiation and homeostasis [22-24]. Studies on ovarian cancer have shown that decreased Ese-3 expression can inhibit tumor proliferation and invasion by activating the ERK and AKT signaling pathways [12]. Knockdown of Ese-3 expression can increase the conversion and expansion of regulatory T cells by decreasing the secretion of TGFβ1 and GM-CSF by the tumor, thereby increasing the effect of checkpoint immunotherapy [25]. In the present study, we found that Ese-3 was upregulated in CC tissues compared with normal control tissues using the TCGA and GTEx samples, and this result was corroborated by Western blotting and immunohistochemistry (IHC) assays. Our data also indicated that higher expression of Ese-3 predicted poorer disease-free survival for CC patients. Furthermore, univariate and multivariate analyses showed that Ese-3 could improve the predictive capacity of the clinical hazard ratio. However, the underlying molecular mechanism of Ese-3 in CC remains unclear.

To identify the underlying molecular mechanisms by which Ese-3 promote CC proliferation, we performed label-free quantitative proteomics analysis of differentially expressed proteins in control cells compared with Ese-3 silenced CC cells. Mass spectrometry (MS) has been developing rapidly, and modern proteomics can not only identify the types of proteins but also quantify their expression levels. Label-free quantitative proteomics technology has some advantages compared with tandem mass tags (TMT) and isobaric tags for relative and absolute quantitation (iTRAQ) in terms of throughput and the ability to bypass a great deal of manual operation and expensive isotope and metabolic labeling [26, 27]. Label-free quantitative proteomics analysis found that a total of 465 proteins were differentially regulated (231 up-regulated and 234 down-regulated) in Ese-3 silenced HCT116 cells compared with control cells, of which two novel genes involved in the Ese-3-mediated CC proliferation, namely EHD2 and INPP4B. Knockdown of Ese-3 in CC cells decreased the expression levels of EHD2 and increased the expression levels of INPP4B compared with control group cells. These results were further verified by Western blotting analysis and CC samples.

EHD2 is a member of Eps15 homology domain-containing 2, so far, only a little papers have reported that EHD2 is involved in the regulation of tumor development, such as cell proliferation, and metastasis. A series of researchs have shown that EHD2 as an oncogene in ccRCC, and high expression levels of EHD2 in ccRCC contributed to tumor proliferation, invasion, and migration and inhibited tumor cell apoptosis [18]. However, EHD2 has been reported as a negative regulatory gene, and elevated EHD2 expression can inhibit tumor metastasis, invasion and indicate poor prognosis in papillary thyroid carcinoma, hepatocellular carcinoma and esophageal squamous cell carcinoma [15-17]. In Colorectal Cancer, a reporter has shown that the expression of EHD2 is downregulated, but the function and underlying molecular mechanisms remain unclear [28]. In this study, we showed that EHD2 expression was downregulated in CC tissues, and the result was verified using the TCGA and CPTAC samples. Then we demonstrated for the first time that the downregulation of EHD2 in CC cells significantly improved tumor cell proliferation and colony formation ability in vitro assay. Conversely, overexpression of EHD2 markedly inhibited CC cell proliferation and colony formation. We also established for the first time that the mechanism by which EHD2 inhibit CC cell proliferation is mediated by the regulation of AKT phosphorylation. In accordance with the in vitro assay, decreased expression of EHD2 promoted tumor growth in nude mice xenograft models. EHD2 depletion resulted in AKT activation in Ese-3-knockdown CC cells, and restored their abolished proliferation capacity in Ese-3-knockdown xenograft nude mice. These results suggest that Ese-3 can promote CC cell proliferation by suppressing its downstream target gene, EHD2.

Inositol polyphosphate 4-phosphatase type II (INPP4B), a phosphoinositide phosphatase, plays an important role in the development of various cancers by the PI3K/Akt signaling

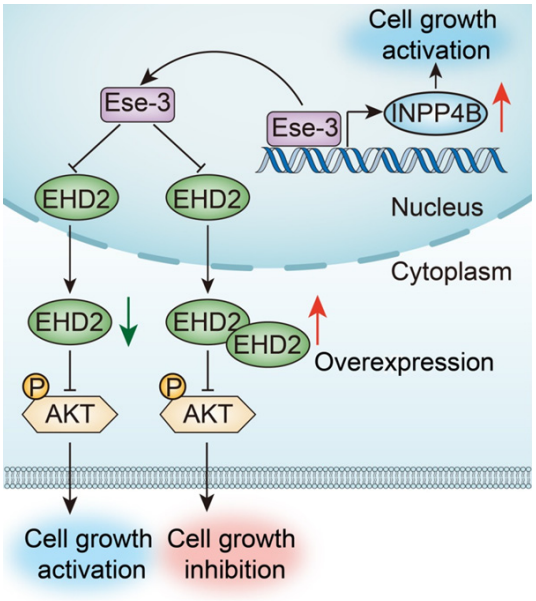


Figure 7. Ese-3 promotes CC cell proliferation. EHD2 as a tumor suppressor gene that suppresses CC cell proliferation by inactivating AKT signaling pathway. Ese-3 facilitates CC progression through downregulating the expression of EHD2 and promoting the transcription of INPP4B to activate AKT signaling pathway.

pathway [29, 30]. Previous works showed that INPP4B expression levels were upregulated and that elevated INPP4B expression could accelerate cell proliferation, metastasis, and invasion by regulating the AKT and SGK signaling pathways in cervical cancer [31], prostate cancer [32, 33], and hepatocellular carcinoma [34], suggesting that INPP4B is a negative tumor regulatory gene in these cancers. Interestingly, some reports have shown that INPP4B-mediated autophagy or SGK3, and AKT activation can promote cell survival and suppresses apoptosis in some other types of cancers, including leukemia [35, 36] and pancreatic cancer [37]. In CC, a series of studies have verified that INPP4B can promote tumor cell proliferation by AKT signaling of INPP4B-mediated PTEN [38] and contribute to colorectal cancer proliferation through the mTORC1 signaling pathway [39]. These results showed that INPP4B plays a vital role in promoting CC cell proliferation. In our study, elevated Ese-3 expression promoted the expression of INPP4B. Meanwhile, our Ch-IP and qRT-PCR results showed that INPP4B was the target gene of Ese-3. Furthermore, our results showed that Ese-3 directly binds to the INPP4B promoter at -648 to -641 bp to promote its transcription. These results suggest that Ese-3 promotes CC cell proliferation by promoting INPP4B transcription, and then activating AKT phosphorylation.

In conclusion, our study demonstrates that Ese-3 is upregulated in CC tissues, and is correlated with poor prognosis in patients with CC. Moreover, Ese-3 is an oncogene that promotes CC cell proliferation by downregulating the expression of EHD2 and promoting the transcription of INPP4B to activate the AKT signaling pathway (**Figure 7**). This new pathway may provide novel insights into CC, and targeting this new pathway could be a promising therapeutic strategy for patients with CC.

Acknowledgements

This study was supported by a grant from the National Natural Science Foundation of China to Haichuan Su (No. 81372255).

Disclosure of conflict of interest

None.

Abbreviations

Ese-3, Epithelium-specific Ets protein 3; EHD2, Eps15 homology domain-containing 2; CC, Colon Cancer; INPP4B, Inositol polyphosphate 4-phosphatase type II; TCGA, The Cancer Genome Atlas; GTEx, Genoytpe-Tissue Expression Program; CPTAC, Cancer Proteomic Tumor Analysis Consortium; iTRAQ, Isobaric Tags for Relative and Absolute Quantitation; TMT, Tandem Mass Tags; ROC, Receiver operating characteristic; SDS PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, Polyvinylidene fluoride; IHC, Immunohistochemistry; Ch-IP, Chromatin Immunoprecipitation; qRT-PCR, Reverse transcription quantitative real-time PCR; DFS, Disease Free Survival.

Address correspondence to: Haichuan Su, Department of Oncology, Tangdu Hospital, Air Force Medical University, 569 Xinsi Road, Xi'an 710038, Shaanxi, China. Tel: +86-29-84717681; E-mail: suhc@fmmu.edu.cn

References

[1] Hernández-Luna MA, López-Briones S and Luria-Pérez R. The four horsemen in colon cancer. J Oncol 2019; 2019: 5636272.

[2] Yang L, Cui J, Wang Y and Tan J. FAM83H-AS1 is upregulated and predicts poor prognosis in colon cancer. Biomed Pharmacother 2019; 118: 109342.
[3] Xiong W, Qin J, Cai X, Xiong W, Liu Q, Li C, Ju Y, Wang Q, Li Y and Yang Y. Overexpression LINC01082 suppresses the proliferation, migration and invasion of colon cancer. Mol Cell Biochem 2019; 462: 33-40.
[4] Gong YC, Ren GL, Liu B, Li F, Zhao HP, Chen JB, Li YP and Yu HH. miR-206 inhibits cancer initiating cells by targeting EHF in gastric cancer. Oncol Rep 2017; 38: 1688-1694.
[5] Seth A and Watson DK. ETS transcription factors and their emerging roles in human cancer. Eur J Cancer 2005; 41: 2462-2478.
[6] Albino D, Civenni G, Dallavalle C, Roos M, Jahns H, Curti L, Rossi S, Pinton S, D'Ambrosio G, Sessa F, Hall J, Catapano CV and Carbone GM. Activation of the Lin28/let-7 axis by loss of ESE3/EHF promotes a tumorigenic and stem-like phenotype in prostate cancer. Cancer Res 2016; 76: 3629-3643.
[7] Dallavalle C, Albino D, Civenni G, Merulla J, Ostano P, Mello-Grand M, Rossi S, Losa M, D'Ambrosio G, Sessa F, Thalmann GN, Garcia-Escudero R, Zitella A, Chiorino G, Catapano CV and Carbone GM. MicroRNA-424 impairs ubiquitination to activate STAT3 and promote prostate tumor progression. J Clin Invest 2016; 126: 4585-4602.
[8] Wolin E, Mita A, Mahipal A, Meyer T, Bendell J, Nemunaitis J, Munster PN, Paz-Ares L, Filvaroff EH, Li S, Hege K, de Haan H and Mita M. A phase 2 study of an oral mTORC1/mTORC2 kinase inhibitor (CC-223) for non-pancreatic neuroendocrine tumors with or without carcinoid symptoms. PLoS One 2019; 14: e0221994.
[9] Zhao T, Jiang W, Wang X, Wang H, Zheng C, Li Y, Sun Y, Huang C, Han ZB, Yang S, Jia Z, Xie K, Ren H and Hao J. ESE3 inhibits pancreatic cancer metastasis by upregulating E-cadherin. Cancer Res 2017; 77: 874-885.
[10] Shi J, Qu Y, Li X, Sui F, Yao D, Yang Q, Shi B, Ji M and Hou P. Increased expression of EHF via gene amplification contributes to the activation of HER family signaling and associates with poor survival in gastric cancer. Cell Death Dis 2016; 7: e2442.
[11] Huang WC, Jang TH, Tung SL, Yen TC, Chan SH and Wang LH. A novel miR-365-3p/EHF/keratin 16 axis promotes oral squamous cell carcinoma metastasis, cancer stemness and drug resistance via enhancing β5-integrin/c-met signaling pathway. J Exp Clin Cancer Res 2019; 38: 89.
[12] heng Z, Guo J, Chen L, Luo N, Yang W and Qu X. Knockdown of EHF inhibited the proliferation, invasion and tumorigenesis of ovarian cancer cells. Mol Carcinog 2016; 55: 1048-1059.
[13] Morén B, Hansson B, Negoita F, Fryklund C, Lundmark R, Göransson O and Stenkula KG. EHD2 regulates adipocyte function and is enriched at cell surface-associated lipid droplets in primary human adipocytes. Mol Biol Cell 2019; 30: 1147-1159.
[14] Pohl U, Smith JS, Tachibana I, Ueki K, Lee HK, Ramaswamy S, Wu Q, Mohrenweiser HW, Jenkins RB and Louis DN. EHD2, EHD3, and EHD4 encode novel members of a highly conserved family of EH domain-containing proteins. Genomics 2000; 63: 255-262.
[15] Yang X, Ren H, Yao L, Chen X and He A. Role of EHD2 in migration and invasion of human breast cancer cells. Tumour Biol 2015; 36: 3717-3726.
[16] Kim Y, Kim MH, Jeon S, Kim J, Kim C, Bae JS and Jung CK. Prognostic implication of histological features associated with EHD2 expression in papillary thyroid carcinoma. PLoS One 2017; 12: e0174737.
[17] Li M, Yang X, Zhang J, Shi H, Hang Q, Huang X, Liu G, Zhu J, He S and Wang H. Effects of EHD2 interference on migration of esophageal squamous cell carcinoma. Med Oncol 2013; 30: 396.
[18] Liu C, Liu S, Wang L, Wang Y, Li Y and Cui Y. Effect of EH domain containing protein 2 on the biological behavior of clear cell renal cell carcinoma. Hum Exp Toxicol 2019; 38: 927-937.
[19] Liu J, Ni W, Qu L, Cui X, Lin Z, Liu Q, Zhou H and Ni R. Decreased expression of EHD2 promotes tumor metastasis and indicates poor prognosis in hepatocellular carcinoma. Dig Dis Sci 2016; 61: 2554-2567.
[20] Ma R, Wang L, Yuan F, Wang S, Liu Y, Fan T and Wang F. FABP7 promotes cell proliferation and survival in colon cancer through MEK/ERK signaling pathway. Biomed Pharmacother 2018; 108: 119-129.
[21] Sharrocks AD. The ETS-domain transcription factor family. Nat Rev Mol Cell Biol 2001; 2: 827-837.
[22] Luk IY, Reehorst CM and Mariadason JM. ELF3, ELF5, EHF and SPDEF transcription factors in tissue homeostasis and cancer. Molecules 2018; 23: 2191.
[23] Wu J, Duan R, Cao H, Field D, Newnham CM, Koehler DR, Zamel N, Pritchard MA, Hertzog P, Post M, Tanswell AK and Hu J. Regulation of epithelium-specific Ets-like factors ESE-1 and ESE-3 in airway epithelial cells: potential roles in airway inflammation. Cell Res 2008; 18: 649-663.
[24] Kas K, Finger E, Grall F, Gu X, Akbarali Y, Boltax J, Weiss A, Oettgen P, Kapeller R and Liber-

mann TA. ESE-3, a novel member of an epithelium-specific ets transcription factor subfamily, demonstrates different target gene specificity from ESE-1. J Biol Chem 2000; 275: 2986-2998.
[25] Liu J, Jiang W, Zhao K, Wang H, Zhou T, Bai W, Wang X, Zhao T, Huang C, Gao S, Qin T, Yu W, Yang B, Li X, Fu D, Tan W, Yang S, Ren H and Hao J. Tumoral EHF predicts the efficacy of anti-PD1 therapy in pancreatic ductal adenocarcinoma. J Exp Med 2019; 216: 656-673.
[26] Goeminne LJE, Gevaert K and Clement L. Experimental design and data-analysis in label-free quantitative LC/MS proteomics: a tutorial with MSqRob. J Proteomics 2018; 171: 23-36.
[27] Tang J, Zhang Y, Fu J, Wang Y, Li Y, Yang Q, Yao L, Xue W and Zhu F. Computational advances in the label-free quantification of cancer proteomics data. Curr Pharm Des 2018; 24: 3842-3858.
[28] Lv J, Fan N, Wang Y, Wang X and Gao C. Identification of differentially expressed proteins of normal and cancerous human colorectal tissues by liquid chromatograph-mass spectrometer based on iTRAQ approach. Cancer Invest 2015; 33: 420-428.
[29] Woolley JF, Dzneladze I and Salmena L. Phosphoinositide signaling in cancer: INPP4B Akt(s) out. Trends Mol Med 2015; 21: 530-532.
[30] Rodgers SJ, Ferguson DT, Mitchell CA and Ooms LM. Regulation of PI3K effector signalling in cancer by the phosphoinositide phosphatases. Biosci Rep 2017; 37: BSR20160432.
[31] Chen Y, Sun Z, Qi M, Wang X, Zhang W, Chen C, Liu J and Zhao W. INPP4B restrains cell proliferation and metastasis via regulation of the PI3K/AKT/SGK pathway. J Cell Mol Med 2018; 22: 2935-2943.
[32] Chen H, Li H and Chen Q. INPP4B overexpression suppresses migration, invasion and angiogenesis of human prostate cancer cells. Clin Exp Pharmacol Physiol 2017; 44: 700-708.
[33] Chen H, Li H and Chen Q. INPP4B overexpression suppresses migration, invasion and angiogenesis of human prostate cancer cells. Clin Exp Pharmacol Physiol 2017; 44: 700-708.
[34] Tang W, Yang L, Yang T, Liu M, Zhou Y, Lin J, Wang K and Ding C. INPP4B inhibits cell proliferation, invasion and chemoresistance in human hepatocellular carcinoma. Onco Targets Ther 2019; 12: 3491-3507.
[35] Zhang F, Li J, Zhu J, Liu L, Zhu K, Cheng S, Lv R and Zhang P. IRF2-INPP4B-mediated autophagy suppresses apoptosis in acute myeloid leukemia cells. Biol Res 2019; 52: 11.
[36] Jin H, Yang L, Wang L, Yang Z, Zhan Q, Tao Y, Zou Q, Tang Y, Xian J, Zhang S, Jing Y and Zhang L. INPP4B promotes cell survival via SGK3 activation in NPM1-mutated leukemia. J Exp Clin Cancer Res 2018; 37: 8.
[37] Zhai S, Liu Y, Lu X, Qian H, Tang X, Cheng X, Wang Y, Shi Y and Deng X. INPP4B as a prognostic and diagnostic marker regulates cell growth of pancreatic cancer via activating AKT. Onco Targets Ther 2019; 12: 8287-8299.
[38] Guo ST, Chi MN, Yang RH, Guo XY, Zan LK, Wang CY, Xi YF, Jin L, Croft A, Tseng HY, Yan XG, Farrelly M, Wang FH, Lai F, Wang JF, Li YP, Ackland S, Scott R, Agoulnik IU, Hondermarck H, Thorne RF, Liu T, Zhang XD and Jiang CC. INPP4B is an oncogenic regulator in human colon cancer. Oncogene 2016; 35: 3049-3061.
[39] Ruan XH, Liu XM, Yang ZX, Zhang SP, Li QZ and Lin CS. INPP4B promotes colorectal cancer cell proliferation by activating mTORC1 signaling and cap-dependent translation. Onco Targets Ther 2019; 12: 3109-3117.

Supplementary Table 1. Clinical characteristics and pathology of patients

No.	Age	Gender	Pathological Type	TNM	AJCC Stage
1	58	M	Adenocarcinoma	pT3N0M0	IIA
2	62	F	Adenocarcinoma	pT2N0M0	IA
3	53	F	Adenocarcinoma	pT4N0M0	IIB
4	56	M	Adenocarcinoma	pT2N0M0	IB
5	60	M	Adenocarcinoma	pT3N0M0	IIA

Supplementary Table 2. Knockdown shRNA sequences used in this study

TRC Number	Squence
Ese-3	
shRNA#1	AGTCCACACACAATGTCATTGCTCGAGCAATGACATTGTGTGTGGACTTTTTTT
shRNA#2	GAAACACTACAGTCGATTAAACTCGAGTTTAATCGACTGTAGTGTTTCTTTTTT
EHD2	
shRNA	GGAAGGAGAACAAGAAGAAGCTTCAAGAGAGCTTCTTCTTGTTCTCCTTCCTTTTTT

Supplementary Table 3. The primary antibodies used of this study

Antibodies	Sources
anti-Ese-3	Proteintech, 67125-1-Ig
anti-EHD2	Proteintech, 11440-1-AP
anti-INPP4B	Abcam, ab81269
anti-Akt (pan)	Cell Signaling Technology, 4685
anti-p-Akt (Ser-473)	Cell Signaling Technology, 4060
anti-β-actin	Proteintech, 66009-1-Ig

Supplementary Table 4. Different primer sequences of this study

Primer Name	Primer sequences
INPP4B promoter primers	
INPP4B sense	5'-CGACGCGTATGTCCTGATATTATTTCAATCTCTC-3'
INPP4B antisense	5'-CCGCTGAGAGCGTGTGCGCGCCCAGGAGGAGCTG-3'
Truncated INPP4B promoter primers	
(-2000-0) INPP4B sense	5'-CGACGCGTATGTCCTGATATTATTTCAATCTCTC-3'
(-1702-0) INPP4B sense	5'-CGACGCGTGCTTCGGGGGCGCCAAGCAGTTTCTGGC-3'
(-1392-0) INPP4B sense	5'-CGACGCGTGTTTGTGAAACTGTTAAGCCCCGAC-3'
(-1043-0) INPP4B sense	5'-CGACGCGTGGGGGAGTTTTAGTCTCCGTCCAGACT-3'
(-640-0) INPP4B sense	5'-CGACGCGTCCGCGGGTTCTTTCTCTGTGGTTTC-3'
(-504-0) INPP4B sense	5'-CGACGCGTGGTCGGGGTACGGGGTGTGAGCACAC-3'
(-308-0) INPP4B sense	5'-CGACGCGTGGAGCGGGGAGGGTGGGGGTTGGGG-3'
Antisense	5'-CCGCTCGAGAGCGTGTGCGCGCCCAGGAGGAGCTG-3'
INPP4B promoter mutation primer	
(-1043-0) INPP4B mutation sense	5'-CCGCTCGAGAGCGTGTGCGCGCCCAGGAGGAGCTG-3'
(-1043-0) INPP4B mutation antisense	5'GAACCCGCGAACAACCACAAGCGCCCCAGACCCCGGCCTATGGCAGCGCAGTCCCTAG-3'
Primers used for ChIP Promoter	
Ch-IP NC sense	5'-GGAATCATAGATGCTATTCAG-3'
Ch-IP NC antisense	5'-CTGGAAGTAAAGGCGAAGGA-3'
Ch-IP 1 sense	5'-CTTTCCAGCAGATCTCTATG-3'
Ch-IP 1 antisense	5'-CTATAGGCCAGAAACTGCTTG-3'
Ch-IP 2 sense	5'-TCTTCCTCCCGCGGGTTCTTTC-3'
Ch-IP 2 antisense	5'-CCCTTTCTGCGGCCCCTCTGC-3'

Supplementary Table 5. The Ese-3, EHD2 mRNA levels and patients information of TCGA samples

Sample ID	Ese-3 mRNA levels	EHD2 mRNA levels	RFS.time	RFS.unit	disease
TCGA-AA-3875-01	19.63545654	7.705764135			colon cancer
TCGA-AA-3877-01	26.72893006	16.32360729	943	0	colon cancer
TCGA-AA-A00W-01	39.13884987	3.496088096	456	0	colon cancer
TCGA-AZ-4313-01	12.90510395	7.625891572	2310	0	colon cancer
TCGA-AZ-5407-01	33.89128956	8.189056672	2683	0	colon cancer
TCGA-CK-5916-01	125.881007	14.96193755	292	1	colon cancer
TCGA-CM-6163-01	26.61897645	8.922865322	427	0	colon cancer
TCGA-F4-6808-01	24.0687148	6.094302476	1024	0	colon cancer
TCGA-3L-AA1B-01	22.58503833	9.743523164	475	0	colon cancer
TCGA-A6-2684-01	108.1299225	7.891998	976	1	colon cancer
TCGA-A6-5656-01	10.91755168	2.827262	1001	0	colon cancer
TCGA-A6-5659-01	71.75485854	4.495021	926	0	colon cancer
TCGA-A6-6138-01	17.64146581	20.04248214	685	0	colon cancer
TCGA-A6-6653-01	30.33182239	18.80136077			colon cancer
TCGA-AA-3495-01	18.54793068	5.31029108	1127	0	colon cancer
TCGA-AA-3502-01	29.95841342	1.992555897			colon cancer
TCGA-AA-3506-01	11.47196644	13.49375849			colon cancer
TCGA-AA-3514-01	7.764499683	24.53165287			colon cancer
TCGA-AA-3526-01	55.37938036	6.435457594	580	0	colon cancer
TCGA-AA-3530-01	38.04057071	3.169530189	580	0	colon cancer
TCGA-AA-3538-01	16.89193627	10.16932085	791	0	colon cancer
TCGA-AA-3543-01	20.66516412	7.281923386			colon cancer
TCGA-AA-3544-01	7.413122972	15.81929745	426	0	colon cancer
TCGA-AA-3549-01	28.7954254	13.61806824	639	0	colon cancer
TCGA-AA-3553-01	13.87421043	8.81553115	730	0	colon cancer
TCGA-AA-3556-01	83.03153418	6.483085789			colon cancer
TCGA-AA-3667-01	13.82537442	13.5589696	426	0	colon cancer
TCGA-AA-3821-01	40.20940927	8.875585381			colon cancer
TCGA-AA-3850-01	32.83372894	11.88266344			colon cancer
TCGA-AA-3854-01	38.75296606	8.358301448	1096	0	colon cancer
TCGA-AA-3855-01	14.9515208	7.890001157			colon cancer
TCGA-AA-3858-01	31.6512682	14.75543384	945	0	colon cancer
TCGA-AA-3866-01	64.7767892	15.2854367	518	0	colon cancer
TCGA-AA-3968-01	20.86402667	15.39917304	669	0	colon cancer
TCGA-AA-3975-01	14.14785136	13.58443463	1036	0	colon cancer
TCGA-AA-3980-01	31.61187082	7.35738614	822	0	colon cancer
TCGA-AA-3986-01	24.11434466	9.985446332	580	0	colon cancer
TCGA-AA-A00D-01	20.55925917	9.200923601	578	0	colon cancer
TCGA-AA-A00R-01	25.39644493	19.91654367			colon cancer
TCGA-AA-A01I-01	58.70978686	3.062089719	943	0	colon cancer
TCGA-AA-A01V-01	28.04720278	5.592908493			colon cancer
TCGA-AA-A02W-01	22.37058499	4.314664328	1005	1	colon cancer
TCGA-AA-A02Y-01	26.98492092	1.29629564	1216	0	colon cancer
TCGA-AA-A03J-01	7.90807646	12.38201164	1246	0	colon cancer
TCGA-AD-6548-01	15.68186116	13.01810839	650	0	colon cancer
TCGA-AU-6004-01	38.26496143	17.82084347			colon cancer
TCGA-AY-A54L-01	22.60552661	1.700552268	396	1	colon cancer

TCGA-AY-A71X-01	33.40859889	1.995932286	588	0	colon cancer
TCGA-CM-4744-01	10.50567269	2.896691137	609	0	colon cancer
TCGA-CM-4746-01	20.98811934	4.320000167	1126	0	colon cancer
TCGA-CM-5864-01	19.2983778	4.115304546	457	0	colon cancer
TCGA-CM-6161-01	26.50974023	6.726177191	457	0	colon cancer
TCGA-CM-6166-01	21.85061916	8.522746084	669	0	colon cancer
TCGA-CM-6170-01	58.70978686	10.21446423	457	0	colon cancer
TCGA-CM-6171-01	20.58579144	6.080994846	427	0	colon cancer
TCGA-CM-6676-01	15.18106714	11.88617217	337	0	colon cancer
TCGA-D5-6530-01	68.65181799	5.400298256	621	0	colon cancer
TCGA-D5-6540-01	30.80311173	20.06700595	491	0	colon cancer
TCGA-D5-6898-01	29.2506044	14.38981721	229	0	colon cancer
TCGA-D5-6923-01	20.47341774	34.56361166	378	0	colon cancer
TCGA-D5-7000-01	44.37645664	13.24280505	312	0	colon cancer
TCGA-F4-6569-01	14.72598676	54.89223261	1087	0	colon cancer
TCGA-F4-6806-01	56.56768595	9.669629795	1132	1	colon cancer
TCGA-F4-6856-01	64.86674916	6.454542176	1074	0	colon cancer
TCGA-G4-6628-01	55.69334415	13.49375849	2424	0	colon cancer
TCGA-QG-A5Z2-01	68.83747592	1.85242168	952	0	colon cancer
TCGA-A6-2680-01	16.84406895	13.90600778	1068	0	colon cancer
TCGA-AA-3489-01	70.55901563	35.67117157			colon cancer
TCGA-AA-3492-01	15.64306424	6.37382117	92	0	colon cancer
TCGA-AA-3496-01	94.43468865	15.93630584			colon cancer
TCGA-AA-3509-01	21.97791568	6.513442793	1915	0	colon cancer
TCGA-AA-3510-01	21.39391262	7.269393328	1946	0	colon cancer
TCGA-AA-3520-01	25.88245814	13.39049508			colon cancer
TCGA-AA-3521-01	46.75809591	10.54419313			colon cancer
TCGA-AA-3524-01	20.41339504	4.811659985	1096	0	colon cancer
TCGA-AA-3655-01	57.41372447	10.39062445	1856	0	colon cancer
TCGA-AA-3660-01	32.91868856	8.225519541	2375	0	colon cancer
TCGA-AA-3663-01	25.69123162	4.866037303	212	0	colon cancer
TCGA-AA-3664-01	33.26134125	4.733764939			colon cancer
TCGA-AA-3673-01	35.52258984	9.203416322	1522	0	colon cancer
TCGA-AA-3675-01	39.01610306	6.676091988	1431	0	colon cancer
TCGA-AA-3685-01	15.78646466	5.184538007	1127	0	colon cancer
TCGA-AA-3697-01	47.82100553	8.619189964			colon cancer
TCGA-AA-3715-01	14.79557811	44.04367486	153	1	colon cancer
TCGA-AA-3864-01	36.12055025	7.349928601	1612	0	colon cancer
TCGA-AA-A01Q-01	44.99913972	4.001268654			colon cancer
TCGA-AA-A01Z-01	22.86232041	9.4626961	1126	1	colon cancer
TCGA-AA-A024-01	23.51839961	3.795605288	1188	0	colon cancer
TCGA-AA-A029-01	16.60103704	5.296248604	1581	0	colon cancer
TCGA-AA-A02O-01	12.28166983	7.745016285			colon cancer
TCGA-AZ-6601-01	30.13524186	16.03818205	2270	1	colon cancer
TCGA-G4-6295-01	36.9483284	5.817045086	254	0	colon cancer
TCGA-5M-AATE-01	36.37776921	4.915945444	810	1	colon cancer
TCGA-A6-2681-01	21.47748547	23.26724659	1150	1	colon cancer
TCGA-A6-2685-01	16.31786407	31.68954892	948	1	colon cancer
TCGA-A6-2686-01	6.244424294	9.529273509	1126	0	colon cancer

TCGA-A6-3808-01	32.96091	21.55404092	1014	0	colon cancer
TCGA-A6-3810-01	61.19477683	4.194791	1111	0	colon cancer
TCGA-A6-4105-01	40.87585141	15.39917304	364	1	colon cancer
TCGA-A6-5661-01	48.31796719	97.73777	1020	0	colon cancer
TCGA-A6-5665-01	112.8213669	19.7767	529	1	colon cancer
TCGA-A6-6140-01	49.93680786	6.390823531	734	0	colon cancer
TCGA-A6-6141-01	42.03852397	8.327779022			colon cancer
TCGA-A6-6650-01	52.31666063	3.713995	627	0	colon cancer
TCGA-AA-3517-01	27.05753619	9.952545629	821	1	colon cancer
TCGA-AA-3518-01	38.40823614	6.746716585			colon cancer
TCGA-AA-3522-01	37.48812377	2.853507974	1127	0	colon cancer
TCGA-AA-3527-01	9.645973432	20.49875849			colon cancer
TCGA-AA-3531-01	19.7389522	7.709753297	1035	0	colon cancer
TCGA-AA-3532-01	53.77599985	12.81896563			colon cancer
TCGA-AA-3534-01	11.13059922	8.206371691	882	0	colon cancer
TCGA-AA-3554-01	24.12583314	27.072553	546	0	colon cancer
TCGA-AA-3555-01	76.36234329	9.569189082	91	1	colon cancer
TCGA-AA-3561-01	18.36242374	3.767194183	424	0	colon cancer
TCGA-AA-3710-01	27.09991188	10.92068486	821	0	colon cancer
TCGA-AA-3812-01	12.23336229	24.99305218	762	1	colon cancer
TCGA-AA-3814-01	20.0086868	13.34383079			colon cancer
TCGA-AA-3815-01	26.30430965	7.428240298	1005	0	colon cancer
TCGA-AA-3818-01	29.10220659	5.298809218			colon cancer
TCGA-AA-3819-01	27.30329095	8.675659667	761	0	colon cancer
TCGA-AA-3831-01	30.20775445	9.393398758	547	0	colon cancer
TCGA-AA-3833-01	28.32244642	32.20434172	485	0	colon cancer
TCGA-AA-3837-01	42.14357317	13.61365557	1186	0	colon cancer
TCGA-AA-3841-01	13.63947495	17.282747	1124	0	colon cancer
TCGA-AA-3845-01	29.77896833	9.08123816			colon cancer
TCGA-AA-3846-01	44.04367486	5.329860323			colon cancer
TCGA-AA-3851-01	28.90646	7.245661469			colon cancer
TCGA-AA-3852-01	46.45134419	14.59431476			colon cancer
TCGA-AA-3856-01	19.15169434	5.872322096			colon cancer
TCGA-AA-3861-01	35.03590489	2.991579095	914	0	colon cancer
TCGA-AA-3862-01	29.50387718	4.974914859	914	0	colon cancer
TCGA-AA-3939-01	16.92399799	5.150662994			colon cancer
TCGA-AA-3950-01	8.055453137	16.66094085	730	0	colon cancer
TCGA-AA-3956-01	43.85519557	12.34387946			colon cancer
TCGA-AA-3966-01	24.18345092	19.1977861			colon cancer
TCGA-AA-3970-01	30.89380725	6.10022487	1096	0	colon cancer
TCGA-AA-3979-01	23.93036515	3.537624323	730	0	colon cancer
TCGA-AA-3984-01	31.96622873	14.49795581			colon cancer
TCGA-AA-A004-01	3.987190643	20.44726249	424	0	colon cancer
TCGA-AA-A00A-01	33.82228106	12.92129744			colon cancer
TCGA-AA-A00E-01	32.58923328	6.605333918	913	0	colon cancer
TCGA-AA-A00K-01	15.80315598	10.77882887			colon cancer
TCGA-AA-A00L-01	27.21519544	6.140338616	1157	0	colon cancer
TCGA-AA-A00Z-01	42.45683444	8.202133469	669	0	colon cancer
TCGA-AA-A017-01	19.71540838	11.33224085	457	0	colon cancer

TCGA-AA-A01G-01	43.25714341	5.508764675	365	0	colon cancer
TCGA-AA-A02R-01	30.17138603	17.11809465			colon cancer
TCGA-AD-6889-01	19.62774229	4.553795044	2532	0	colon cancer
TCGA-AM-5821-01	20.40516371	8.080633723			colon cancer
TCGA-AU-3779-01	45.11948827	9.262758482			colon cancer
TCGA-AY-A69D-01	96.61423047	4.905247872	543	0	colon cancer
TCGA-AZ-4315-01	26.78627751	6.459238626	1776	0	colon cancer
TCGA-CA-5254-01	44.41707357	10.68936817	386	0	colon cancer
TCGA-CA-5255-01	18.11437191	5.960895997	376	0	colon cancer
TCGA-CA-5256-01	23.60477991	2.511271066	379	0	colon cancer
TCGA-CA-5796-01	52.15522974	6.716160227	377	0	colon cancer
TCGA-CA-5797-01	23.48659552	12.25150935	383	0	colon cancer
TCGA-CA-6716-01	64.7767892	10.82490647	371	0	colon cancer
TCGA-CA-6717-01	34.96975199	28.36760547	388	0	colon cancer
TCGA-CA-6718-01	77.39523821	13.51530227	207	1	colon cancer
TCGA-CA-6719-01	56.37523384	21.58285102	354	1	colon cancer
TCGA-CK-4951-01	34.82556689	25.15465443	480	1	colon cancer
TCGA-CM-4743-01	11.12390525	13.31392203	701	0	colon cancer
TCGA-CM-4752-01	17.10539644	7.993180873	396	0	colon cancer
TCGA-CM-5349-01	12.14107971	25.18019045	915	0	colon cancer
TCGA-CM-5860-01	17.72094588	16.25969869	974	0	colon cancer
TCGA-CM-5861-01	21.89836933	15.15077584	457	0	colon cancer
TCGA-CM-6164-01	52.05183675	11.47886105	883	0	colon cancer
TCGA-CM-6165-01	49.39993448	13.0017844	488	0	colon cancer
TCGA-CM-6168-01	52.42502587	27.98662424	395	0	colon cancer
TCGA-CM-6169-01	22.84160215	25.23042664	396	0	colon cancer
TCGA-CM-6674-01	38.52260975	19.59484095	394	0	colon cancer
TCGA-CM-6677-01	24.20639115	14.14785136	337	0	colon cancer
TCGA-CM-6679-01	34.68432086	21.29273231	306	0	colon cancer
TCGA-D5-5540-01	19.76242834	2.183499585	1706	0	colon cancer
TCGA-D5-6529-01	41.94092237	23.95234643	386	1	colon cancer
TCGA-D5-6531-01	27.73104351	11.59984486	540	0	colon cancer
TCGA-D5-6532-01	46.67036801	4.91709075	555	0	colon cancer
TCGA-D5-6534-01	29.60602753	40.68376367	1316	0	colon cancer
TCGA-D5-6536-01	71.31730996	18.88958878	493	1	colon cancer
TCGA-D5-6541-01	59.20419049	17.04919066	474	0	colon cancer
TCGA-D5-6920-01	48.31796719	10.57690285	377	0	colon cancer
TCGA-D5-6924-01	12.79546083	20.05074948	435	0	colon cancer
TCGA-D5-6927-01	43.40552792	11.87904178			colon cancer
TCGA-D5-6928-01	17.47212476	18.71392815	354	0	colon cancer
TCGA-D5-6930-01	40.49071949	12.9978344	406	0	colon cancer
TCGA-D5-6932-01	28.89034047	17.47212476	346	0	colon cancer
TCGA-DM-A0X9-01	30.83919626	5.576676399	3641	0	colon cancer
TCGA-DM-A0XD-01	11.08826346	9.595145344	617	1	colon cancer
TCGA-DM-A1D0-01	18.97074147	2.332317667	3974	0	colon cancer
TCGA-DM-A1D4-01	8.820192565	2.541852942	1920	1	colon cancer
TCGA-DM-A1D6-01	41.83866132	3.684123854	1518	0	colon cancer
TCGA-DM-A1D7-01	19.7947362	6.209286713	154	1	colon cancer
TCGA-DM-A1D9-01	26.31755876	4.362711263	4270	0	colon cancer

TCGA-DM-A1DB-01	12.21072895	3.148234985	1348	0	colon cancer
TCGA-DM-A280-01	16.44024805	9.547785841	236	0	colon cancer
TCGA-DM-A282-01	13.28864158	7.173776119	4233	0	colon cancer
TCGA-DM-A28C-01	45.83650125	3.093764132	1929	1	colon cancer
TCGA-DM-A28E-01	68.74420003	2.209646047	3648	0	colon cancer
TCGA-DM-A28G-01	29.43635247	3.437811425	1849	0	colon cancer
TCGA-DM-A28K-01	21.40286691	6.494281728	2988	0	colon cancer
TCGA-DM-A28M-01	24.32380001	3.504018317	2895	0	colon cancer
TCGA-F4-6463-01	20.17499828	22.01687009	1087	0	colon cancer
TCGA-F4-6570-01	27.74621606	30.4573159	188	0	colon cancer
TCGA-F4-6703-01	28.77924517	60.51568277	1456	0	colon cancer
TCGA-F4-6805-01	35.30236689	32.62989718	1047	0	colon cancer
TCGA-F4-6854-01	29.81328786	15.54586521	16	0	colon cancer
TCGA-F4-6855-01	35.54717451	24.21845732	1442	0	colon cancer
TCGA-G4-6302-01	7.318579539	96.81957128	2047	0	colon cancer
TCGA-G4-6586-01	17.04273034	3.644707469	1089	0	colon cancer
TCGA-G4-6588-01	26.89912777	16.94270035	796	0	colon cancer
TCGA-G4-6625-01	43.85519557	17.01792414	2564	1	colon cancer
TCGA-G4-6626-01	40.74744052	6.116578895	1422	0	colon cancer
TCGA-G4-6627-01	28.09340026	22.87321614	2275	0	colon cancer
TCGA-T9-A92H-01	62.52241533	3.559689445	81	1	colon cancer
TCGA-AA-3511-01	27.74621606	18.728404	59	1	colon cancer
TCGA-AA-A022-01	9.607256533	11.98549064			colon cancer
TCGA-A6-2676-01	24.01065531	15.34713336	1305	0	colon cancer
TCGA-A6-3809-01	20.68242106	13.13628	996	0	colon cancer
TCGA-AA-3947-01	41.43778918	2.320882287	1004	0	colon cancer
TCGA-AA-A00N-01	32.91868856	27.44971817			colon cancer
TCGA-AA-A010-01	28.77924517	7.537320357	1064	0	colon cancer
TCGA-G4-6304-01	22.22281726	1.560429433	859	1	colon cancer
TCGA-SS-A7HO-01	12.9739096	7.987207984	1801	1	colon cancer
TCGA-A6-5666-01	45.59515785	3.577321943	970	1	colon cancer
TCGA-AA-3678-01	14.61839797	7.48243401	1430	0	colon cancer
TCGA-AA-A01X-01	13.12374789	11.93204063	791	1	colon cancer
TCGA-AA-3842-01	19.51671798	13.35184066	396	1	colon cancer
TCGA-AA-3976-01	26.34533533	7.467438006	791	0	colon cancer
TCGA-AA-A01C-01	24.88367144	18.27255801	457	0	colon cancer
TCGA-AZ-6608-01	24.29970122	3.205829622	59	0	colon cancer
TCGA-AA-3519-01	18.02508721	8.265718999	276	0	colon cancer
TCGA-AA-3666-01	16.86814591	6.83457337			colon cancer
TCGA-AA-3672-01	16.13469351	17.83423725			colon cancer
TCGA-AA-3681-01	35.47351475	7.518650785	182	0	colon cancer
TCGA-AA-3971-01	35.76967747	9.735591086	489	0	colon cancer
TCGA-AA-A01P-01	27.80489607	28.7954254	762	1	colon cancer
TCGA-AA-A01S-01	25.48609265	3.704330508			colon cancer
TCGA-AA-A01T-01	7.103540843	7.211651329	1005	0	colon cancer
TCGA-CK-4948-01	22.42115045	20.96153408	4502	0	colon cancer
TCGA-G4-6293-01	60.06243964	9.068705419	4051	0	colon cancer
TCGA-D5-5539-01	35.05998154	14.76562654	579	1	colon cancer
TCGA-D5-6922-01	33.84437555	19.82678507	308	0	colon cancer

TCGA-A6-2672-01	39.55693852	9.470836	1419	0	colon cancer
TCGA-A6-2678-01	98.27560783	5.716898857	1286	0	colon cancer
TCGA-A6-5657-01	18.12788256	32.04543579	962	0	colon cancer
TCGA-A6-6654-01	49.8392355	44.33527447	726	0	colon cancer
TCGA-A6-A56B-01	112.2468032	15.01853798	1678	1	colon cancer
TCGA-A6-A5ZU-01	11.60340402	12.78352623	293	0	colon cancer
TCGA-AA-3525-01	25.67854518	13.81625094	245	0	colon cancer
TCGA-AA-3860-01	26.13402878	13.12374789	945	0	colon cancer
TCGA-AA-3949-01	32.75213114	12.31044283	791	0	colon cancer
TCGA-AA-3982-01	26.1474824	9.661530103			colon cancer
TCGA-AA-3994-01	23.58385502	9.845891818	822	0	colon cancer
TCGA-AA-A00U-01	48.18335148	7.179002243	518	0	colon cancer
TCGA-AA-A01F-01	16.38227912	3.00310218			colon cancer
TCGA-AZ-4308-01	6.981949354	19.39166448	3324	0	colon cancer
TCGA-AZ-4615-01	30.85734568	9.132490486	1002	0	colon cancer
TCGA-CA-6715-01	44.57304959	6.298186464	383	0	colon cancer
TCGA-CK-4950-01	30.15328077	11.17587082	2599	0	colon cancer
TCGA-DM-A1HB-01	25.60004405	7.187960231	4126	0	colon cancer
TCGA-DM-A28F-01	38.18059351	3.756742953	1094	0	colon cancer
TCGA-F4-6460-01	13.21022725	27.50795517	972	0	colon cancer
TCGA-G4-6307-01	13.24280505	3.860112415	1674	0	colon cancer
TCGA-G4-6309-01	29.15041866	9.884474349	2176	1	colon cancer
TCGA-G4-6310-01	18.40624539	38.43827648	1935	0	colon cancer
TCGA-A6-A566-01	15.15077584	52.75864536	257	1	colon cancer
TCGA-AA-A00J-01	42.28343668	9.978544336	549	0	colon cancer
TCGA-AA-A00Q-01	26.87133632	7.760670559	1278	0	colon cancer
TCGA-AZ-6605-01	28.94012307	34.2680287	159	0	colon cancer
TCGA-CK-4947-01	38.95720949	10.23477092			colon cancer
TCGA-D5-6926-01	35.25516262	22.05682987	275	0	colon cancer
TCGA-CM-5341-01	13.35628826	4.076295336	884	0	colon cancer
TCGA-AD-6895-01	28.6051853	17.51059025	763	0	colon cancer
TCGA-AY-6386-01	37.90272143	5.475626103	542	0	colon cancer
TCGA-CM-5348-01	22.44087017	31.02437918	699	0	colon cancer
TCGA-CM-6162-01	70.77236076	34.35962277	365	0	colon cancer
TCGA-CM-6172-01	178.8089756	9.635223064	335	0	colon cancer
TCGA-D5-5541-01	24.25357044	8.076275729	1701	0	colon cancer
TCGA-CM-4748-01	27.02858175	15.39917304	792	0	colon cancer
TCGA-CM-4750-01	16.72752699	7.731211204	244	0	colon cancer
TCGA-A6-4107-01	16.57692647	10.35549579	987	0	colon cancer
TCGA-A6-6649-01	47.6403783	27.14305215	735	0	colon cancer
TCGA-AD-6888-01	23.2783584	1.88331622	343	1	colon cancer
TCGA-CM-4751-01	21.10412501	17.32870029	822	0	colon cancer
TCGA-CM-5344-01	23.00113582	24.04611243	670	0	colon cancer
TCGA-CM-5863-01	34.54034047	34.80242111	457	0	colon cancer
TCGA-D5-5538-01	34.94650207	11.28942975	1007	1	colon cancer
TCGA-4N-A93T-01	23.34448923	3.511078555	146	0	colon cancer
TCGA-A6-6137-01	77.13111569	8.471150511	824	0	colon cancer
TCGA-AA-3516-01	29.99303528	20.1829269			colon cancer
TCGA-AA-3712-01	44.45496818	13.90600778			colon cancer

TCGA-AA-3811-01	14.71179903	11.26962206			colon cancer
TCGA-AA-A01R-01	6.662734501	9.97172339	608	1	colon cancer
TCGA-AA-A03F-01	12.15163017	7.771450932			colon cancer
TCGA-D5-6538-01	26.6881007	7.635570994	521	0	colon cancer
TCGA-A6-2677-01	46.75809591	2.288043	740	0	colon cancer
TCGA-A6-3807-01	29.72643095	11.76476656	1054	0	colon cancer
TCGA-AA-3529-01	16.23602943	6.497542937			colon cancer
TCGA-AA-3542-01	5.998768252	6.063444205	395	0	colon cancer
TCGA-AA-3548-01	16.29473896	11.9571128	1034	0	colon cancer
TCGA-AA-3552-01	32.91868856	10.65297311	122	1	colon cancer
TCGA-AA-3560-01	25.79365303	6.274898769	608	0	colon cancer
TCGA-AA-3562-01	44.80474204	9.159122814			colon cancer
TCGA-AA-3844-01	32.44711096	5.212465294	365	1	colon cancer
TCGA-AA-3848-01	17.12470155	11.26336607			colon cancer
TCGA-AA-3952-01	15.88016059	14.17116243			colon cancer
TCGA-AA-A00F-01	21.85061916	20.33583173	1035	0	colon cancer
TCGA-AA-A00O-01	51.67976408	21.51597306	822	0	colon cancer
TCGA-AA-A01D-01	20.12550722	24.55575988			colon cancer
TCGA-AA-A01K-01	14.64691264	17.53616587	943	0	colon cancer
TCGA-AY-4070-01	9.900834029	12.17379948	186	1	colon cancer
TCGA-DM-A0XF-01	19.77074365	7.116293278	1162	0	colon cancer
TCGA-DM-A1DA-01	10.73551695	4.448250234	228	0	colon cancer
TCGA-DM-A1HA-01	12.78352623	2.573785968	4000	0	colon cancer
TCGA-DM-A288-01	19.26700648	4.053199476	320	1	colon cancer
TCGA-DM-A28A-01	42.14357317	11.6620907	805	0	colon cancer
TCGA-DM-A28H-01	23.2783584	4.268616025	484	1	colon cancer
TCGA-G4-6299-01	9.947024591	12.58901545	2268	0	colon cancer
TCGA-CK-4952-01	62.60397071	6.964698393			colon cancer
TCGA-D5-6931-01	43.40552792	12.69040214	365	0	colon cancer
TCGA-F4-6461-01	146.2353837	17.96422474	288	1	colon cancer
TCGA-AA-3955-01	32.96091	4.779974818	638	0	colon cancer
TCGA-CM-6680-01	86.13989718	11.45224714	366	0	colon cancer
TCGA-F4-6459-01	17.00593328	31.98697838	262	0	colon cancer
TCGA-NH-A50V-01	76.49627441	10.66505183	588	0	colon cancer
TCGA-A6-5660-01	38.18059351	7.587420916	888	0	colon cancer
TCGA-CM-6167-01	14.18999389	73.64931432	456	0	colon cancer
TCGA-F4-6807-01	46.58022045	28.6051853	1309	0	colon cancer
TCGA-AD-6965-01	22.16285868	6.249063071	648	1	colon cancer
TCGA-AA-3494-01	37.45963814	5.990159779			colon cancer
TCGA-AA-3713-01	60.51568277	8.09690876	579	0	colon cancer
TCGA-AA-A02J-01	19.60245006	3.280659514			colon cancer
TCGA-A6-6652-01	11.13383965	6.045936485	751	0	colon cancer
TCGA-AZ-4682-01	16.49990297	5.159420339	680	0	colon cancer
TCGA-A6-2683-01	18.51192606	9.159122814			colon cancer
TCGA-A6-A567-01	28.97288967	8.433628863	1069	1	colon cancer
TCGA-AA-3688-01	27.91104949	7.235123659	578	0	colon cancer
TCGA-AA-3696-01	31.35679323	8.347355984			colon cancer
TCGA-AA-3972-01	34.2680287	9.033054084	1216	1	colon cancer
TCGA-AA-A02E-01	11.45862123	5.126830349			colon cancer

TCGA-AA-A02F-01	28.54064974	8.906415729	1216	0	colon cancer
TCGA-CK-6748-01	49.88728114	29.60602753			colon cancer
TCGA-D5-6929-01	21.71499137	12.82302592	408	0	colon cancer
TCGA-G4-6294-01	24.73682728	2.851947648	517	1	colon cancer
TCGA-G4-6303-01	58.64536185	12.30680838	872	1	colon cancer
TCGA-G4-6315-01	21.89836933	6.836279755	1883	0	colon cancer
TCGA-F4-6809-01	14.37113899	32.50716035	403	0	colon cancer
TCGA-AA-3693-01	16.32360729	3.995220182			colon cancer
TCGA-AA-3973-01	41.77125645	7.564175735	397	0	colon cancer
TCGA-AZ-6600-01	19.12118581	21.65719485			colon cancer
TCGA-AZ-4614-01	7.114507044	9.011550636	172	0	colon cancer
TCGA-A6-2682-01	10.93973387	13.33067829	381	1	colon cancer
TCGA-AY-5543-01	43.36818073	2.956197013	1004	0	colon cancer
TCGA-A6-2671-01	54.77492735	21.36566579	1215	1	colon cancer
TCGA-A6-2674-01	115.5463	7.512978186	1219	1	colon cancer
TCGA-AA-3488-01	15.48104461	6.082450955			colon cancer
TCGA-AA-3679-01	12.07223413	9.45494344	457	0	colon cancer
TCGA-AA-3692-01	45.83650125	6.227597444	396	1	colon cancer
TCGA-AA-3867-01	31.45418253	22.25284256	394	1	colon cancer
TCGA-AA-3870-01	9.049753082	48.27293873	912	0	colon cancer
TCGA-AA-3930-01	13.4075515	8.308171049	61	0	colon cancer
TCGA-AA-3989-01	34.02269154	14.05104002			colon cancer
TCGA-AA-A02H-01	41.50052122	6.832869799			colon cancer
TCGA-AZ-4616-01	20.34461907	14.00583924	156	0	colon cancer
TCGA-DM-A285-01	77.39523821	18.17636427	179	0	colon cancer
TCGA-G4-6297-01	36.89693846	29.55368007	2394	1	colon cancer
TCGA-G4-6314-01	39.8961298	38.63745977	38	1	colon cancer
TCGA-A6-5662-01	38.26496143	9.132490486	661	1	colon cancer
TCGA-AZ-4684-01	19.00794722	14.07411474	411	1	colon cancer
TCGA-AA-3662-01	11.93568412	9.085979086	184	0	colon cancer
TCGA-AA-3680-01	21.37510365	6.168959881	335	0	colon cancer
TCGA-AA-3684-01	7.552511795	35.40094881			colon cancer
TCGA-AA-3869-01	27.40787297	9.529273509	822	0	colon cancer
TCGA-AA-3872-01	14.25048961	30.58242047			colon cancer
TCGA-AA-A02K-01	13.38179401	3.410196776	306	1	colon cancer
TCGA-AZ-4323-01	4.268616025	31.74760946	43	0	colon cancer
TCGA-AZ-6606-01	37.5146177	4.643771272	357	0	colon cancer
TCGA-AZ-6607-01	45.47683511	74.22690694	97	0	colon cancer
TCGA-AM-5820-01	30.33182239	14.2456324			colon cancer
TCGA-AY-A8YK-01	28.97288967	5.194666681	573	0	colon cancer
TCGA-NH-A8F8-01	32.0257883	13.32229371	511	0	colon cancer
TCGA-A6-6648-01	93.87970677	5.101868803	686	1	colon cancer
TCGA-NH-A50U-01	24.09085336	8.958698201			colon cancer
TCGA-A6-6142-01	28.63554338	28.1386087	654	1	colon cancer
TCGA-CM-5862-01	10.13524306	6.076490501	153	0	colon cancer
TCGA-CM-5868-01	21.68579559	12.69427479	518	0	colon cancer
TCGA-AA-3941-01	14.31074422	4.77416978			colon cancer
TCGA-CM-4747-01	7.68617645	11.68585244	761	0	colon cancer
TCGA-CM-6678-01	15.60462465	9.429264052			colon cancer

TCGA-5M-AAT6-01	38.72366392	21.26545997	219	1	colon cancer
TCGA-5M-AAT4-01	35.71918576	5.951951924	49	0	colon cancer
TCGA-NH-A6GC-01	41.97275559	14.7452347	389	0	colon cancer
TCGA-CM-6675-01	38.29160336	22.92602639	337	1	colon cancer

sampleID	gender	stage_M	stage_N	stage_T	TNM_stage	age
TCGA-AA-3875-01	FEMALE	M0	N0	T1	Stage I	78
TCGA-AA-3877-01	FEMALE	M0	N0	T1	Stage I	83
TCGA-AA-A00W-01	MALE	M0	N0	T1	Stage I	80
TCGA-AZ-4313-01	FEMALE	M0	N0	T1	Stage I	51
TCGA-AZ-5407-01	FEMALE	M0	N0	T1	Stage I	51
TCGA-CK-5916-01	FEMALE	M0	N0	T1	Stage I	71
TCGA-CM-6163-01	MALE	M0	N0	T1	Stage I	74
TCGA-F4-6808-01	FEMALE	M0	N0	T1	Stage I	54
TCGA-3L-AA1B-01	FEMALE	M0	N0	T2	Stage I	61
TCGA-A6-2684-01	FEMALE	M0	N0	T2	Stage I	75
TCGA-A6-5656-01	MALE	M0	N0	T2	Stage I	74
TCGA-A6-5659-01	MALE	M0	N0	T2	Stage I	82
TCGA-A6-6138-01	MALE	M0	N0	T2	Stage I	61
TCGA-A6-6653-01	MALE	M0	N0	T2	Stage I	82
TCGA-AA-3495-01	MALE	M0	N0	T2	Stage I	79
TCGA-AA-3502-01	MALE	M0	N0	T2	Stage I	73
TCGA-AA-3506-01	MALE	M0	N0	T2	Stage I	77
TCGA-AA-3514-01	FEMALE	M0	N0	T2	Stage I	81
TCGA-AA-3526-01	MALE	M0	N0	T2	Stage I	57
TCGA-AA-3530-01	MALE	M0	N0	T2	Stage I	80
TCGA-AA-3538-01	FEMALE	M0	N0	T2	Stage I	54
TCGA-AA-3543-01	MALE	M0	N0	T2	Stage I	84
TCGA-AA-3544-01	MALE	M0	N0	T2	Stage I	68
TCGA-AA-3549-01	MALE	M0	N0	T2	Stage I	69
TCGA-AA-3553-01	FEMALE	M0	N0	T2	Stage I	61
TCGA-AA-3556-01	MALE	M0	N0	T2	Stage I	78
TCGA-AA-3667-01	FEMALE	M0	N0	T2	Stage I	36
TCGA-AA-3821-01	FEMALE	M0	N0	T2	Stage I	81
TCGA-AA-3850-01	MALE	M0	N0	T2	Stage I	74
TCGA-AA-3854-01	FEMALE	M0	N0	T2	Stage I	71
TCGA-AA-3855-01	MALE	M0	N0	T2	Stage I	72
TCGA-AA-3858-01	MALE	M0	N0	T2	Stage I	67
TCGA-AA-3866-01	FEMALE	M0	N0	T2	Stage I	78
TCGA-AA-3968-01	FEMALE	M0	N0	T2	Stage I	55
TCGA-AA-3975-01	MALE	M0	N0	T2	Stage I	80
TCGA-AA-3980-01	FEMALE	M0	N0	T2	Stage I	89
TCGA-AA-3986-01	MALE	M0	N0	T2	Stage I	73
TCGA-AA-A00D-01	MALE	M0	N0	T2	Stage I	70
TCGA-AA-A00R-01	FEMALE	M0	N0	T2	Stage I	64
TCGA-AA-A01I-01	MALE	M0	N0	T2	Stage I	76
TCGA-AA-A01V-01	MALE	M0	N0	T2	Stage I	59
TCGA-AA-A02W-01	FEMALE	M0	N0	T2	Stage I	73
TCGA-AA-A02Y-01	MALE	M0	N0	T2	Stage I	73
TCGA-AA-A03J-01	FEMALE	M0	N0	T2	Stage I	65
TCGA-AD-6548-01	FEMALE	M0	N0	T2	Stage I	81

TCGA-AU-6004-01	FEMALE	M0	N0	T2	Stage I	69
TCGA-AY-A54L-01	FEMALE	M0	N0	T2	Stage I	74
TCGA-AY-A71X-01	FEMALE	M0	N0	T2	Stage I	54
TCGA-CM-4744-01	MALE	M0	N0	T2	Stage I	69
TCGA-CM-4746-01	MALE	M0	N0	T2	Stage I	61
TCGA-CM-5864-01	MALE	M0	N0	T2	Stage I	60
TCGA-CM-6161-01	FEMALE	M0	N0	T2	Stage I	36
TCGA-CM-6166-01	FEMALE	M0	N0	T2	Stage I	48
TCGA-CM-6170-01	FEMALE	M0	N0	T2	Stage I	73
TCGA-CM-6171-01	FEMALE	M0	N0	T2	Stage I	77
TCGA-CM-6676-01	MALE	M0	N0	T2	Stage I	82
TCGA-D5-6530-01	MALE	M0	N0	T2	Stage I	53
TCGA-D5-6540-01	MALE	M0	N0	T2	Stage I	66
TCGA-D5-6898-01	FEMALE	M0	N0	T2	Stage I	51
TCGA-D5-6923-01	MALE	M0	N0	T2	Stage I	57
TCGA-D5-7000-01	FEMALE	M0	N0	T2	Stage I	79
TCGA-F4-6569-01	MALE	M0	N0	T2	Stage I	60
TCGA-F4-6806-01	FEMALE	M0	N0	T2	Stage I	59
TCGA-F4-6856-01	MALE	M0	N0	T2	Stage I	45
TCGA-G4-6628-01	MALE	M0	N0	T2	Stage I	78
TCGA-QG-A5Z2-01	MALE	M0	N0	T2	Stage I	61
TCGA-A6-2680-01	FEMALE	M0	N0	T3	Stage II	72
TCGA-AA-3489-01	MALE	M0	N0	T3	Stage II	75
TCGA-AA-3492-01	FEMALE	M0	N0	T3	Stage II	90
TCGA-AA-3496-01	FEMALE	M0	N0	T3	Stage II	83
TCGA-AA-3509-01	FEMALE	M0	N0	T3	Stage II	54
TCGA-AA-3510-01	MALE	M0	N0	T3	Stage II	70
TCGA-AA-3520-01	FEMALE	M0	N0	T3	Stage II	86
TCGA-AA-3521-01	MALE	M0	N0	T3	Stage II	87
TCGA-AA-3524-01	MALE	M0	N0	T3	Stage II	85
TCGA-AA-3655-01	MALE	M0	N0	T3	Stage II	68
TCGA-AA-3660-01	FEMALE	M0	N0	T3	Stage II	51
TCGA-AA-3663-01	MALE	M0	N0	T3	Stage II	42
TCGA-AA-3664-01	FEMALE	M0	N0	T3	Stage II	74
TCGA-AA-3673-01	FEMALE	M0	N0	T3	Stage II	53
TCGA-AA-3675-01	MALE	M0	N0	T3	Stage II	84
TCGA-AA-3685-01	MALE	M0	N0	T3	Stage II	69
TCGA-AA-3697-01	MALE	M0	N0	T3	Stage II	77
TCGA-AA-3715-01	MALE	M0	N0	T3	Stage II	77
TCGA-AA-3864-01	MALE	M0	N0	T3	Stage II	71
TCGA-AA-A01Q-01	FEMALE	M0	N0	T3	Stage II	48
TCGA-AA-A01Z-01	MALE	M0	N0	T3	Stage II	68
TCGA-AA-A024-01	MALE	M0	N0	T3	Stage II	81
TCGA-AA-A029-01	MALE	M0	N0	T3	Stage II	67
TCGA-AA-A02O-01	MALE	M0	N0	T3	Stage II	82
TCGA-AZ-6601-01	MALE	M0	N0	T3	Stage II	68
TCGA-G4-6295-01	FEMALE	M0	N0	T3	Stage II	70
TCGA-5M-AATE-01	MALE	M0	N0	T3	Stage IIA	76
TCGA-A6-2681-01	FEMALE	M0	N0	T3	Stage IIA	73
TCGA-A6-2685-01	FEMALE	M0	N0	T3	Stage IIA	48

TCGA-A6-2686-01	FEMALE	M0	N0	T3	Stage IIA	81
TCGA-A6-3808-01	MALE	M0	N0	T3	Stage IIA	73
TCGA-A6-3810-01	MALE	M0	N0	T3	Stage IIA	62
TCGA-A6-4105-01	MALE	M0	N0	T3	Stage IIA	79
TCGA-A6-5661-01	FEMALE	M0	N0	T3	Stage IIA	80
TCGA-A6-5665-01	FEMALE	M0	N0	T3	Stage IIA	84
TCGA-A6-6140-01	MALE	M0	N0	T3	Stage IIA	62
TCGA-A6-6141-01	MALE	M0	N0	T3	Stage IIA	31
TCGA-A6-6650-01	FEMALE	M0	N0	T3	Stage IIA	69
TCGA-AA-3517-01	MALE	M0	N0	T3	Stage IIA	60
TCGA-AA-3518-01	FEMALE	M0	N0	T3	Stage IIA	81
TCGA-AA-3522-01	MALE	M0	N0	T3	Stage IIA	67
TCGA-AA-3527-01	FEMALE	M0	N0	T3	Stage IIA	90
TCGA-AA-3531-01	FEMALE	M0	N0	T3	Stage IIA	75
TCGA-AA-3532-01	MALE	M0	N0	T3	Stage IIA	63
TCGA-AA-3534-01	FEMALE	M0	N0	T3	Stage IIA	78
TCGA-AA-3554-01	FEMALE	M0	N0	T3	Stage IIA	62
TCGA-AA-3555-01	FEMALE	M0	N0	T3	Stage IIA	81
TCGA-AA-3561-01	MALE	M0	N0	T3	Stage IIA	72
TCGA-AA-3710-01	FEMALE	M0	N0	T3	Stage IIA	80
TCGA-AA-3812-01	FEMALE	M0	N0	T3	Stage IIA	82
TCGA-AA-3814-01	FEMALE	M0	N0	T3	Stage IIA	85
TCGA-AA-3815-01	FEMALE	M0	N0	T3	Stage IIA	65
TCGA-AA-3818-01	FEMALE	M0	N0	T3	Stage IIA	78
TCGA-AA-3819-01	FEMALE	M0	N0	T3	Stage IIA	41
TCGA-AA-3831-01	MALE	M0	N0	T3	Stage IIA	66
TCGA-AA-3833-01	FEMALE	M0	N0	T3	Stage IIA	63
TCGA-AA-3837-01	MALE	M0	N0	T3	Stage IIA	67
TCGA-AA-3841-01	MALE	M0	N0	T3	Stage IIA	66
TCGA-AA-3845-01	FEMALE	M0	N0	T3	Stage IIA	86
TCGA-AA-3846-01	FEMALE	M0	N0	T3	Stage IIA	74
TCGA-AA-3851-01	MALE	M0	N0	T3	Stage IIA	74
TCGA-AA-3852-01	MALE	M0	N0	T3	Stage IIA	88
TCGA-AA-3856-01	MALE	M0	N0	T3	Stage IIA	59
TCGA-AA-3861-01	MALE	M0	N0	T3	Stage IIA	72
TCGA-AA-3862-01	MALE	M0	N0	T3	Stage IIA	82
TCGA-AA-3939-01	MALE	M0	N0	T3	Stage IIA	83
TCGA-AA-3950-01	FEMALE	M0	N0	T3	Stage IIA	79
TCGA-AA-3956-01	MALE	M0	N0	T3	Stage IIA	65
TCGA-AA-3966-01	FEMALE	M0	N0	T3	Stage IIA	89
TCGA-AA-3970-01	MALE	M0	N0	T3	Stage IIA	65
TCGA-AA-3979-01	MALE	M0	N0	T3	Stage IIA	84
TCGA-AA-3984-01	FEMALE	M0	N0	T3	Stage IIA	61
TCGA-AA-A004-01	MALE	M0	N0	T3	Stage IIA	76
TCGA-AA-A00A-01	MALE	M0	N0	T3	Stage IIA	80
TCGA-AA-A00E-01	MALE	M0	N0	T3	Stage IIA	65
TCGA-AA-A00K-01	MALE	M0	N0	T3	Stage IIA	79
TCGA-AA-A00L-01	MALE	M0	N0	T3	Stage IIA	66
TCGA-AA-A00Z-01	MALE	M0	N0	T3	Stage IIA	70
TCGA-AA-A017-01	FEMALE	M0	N0	T3	Stage IIA	57

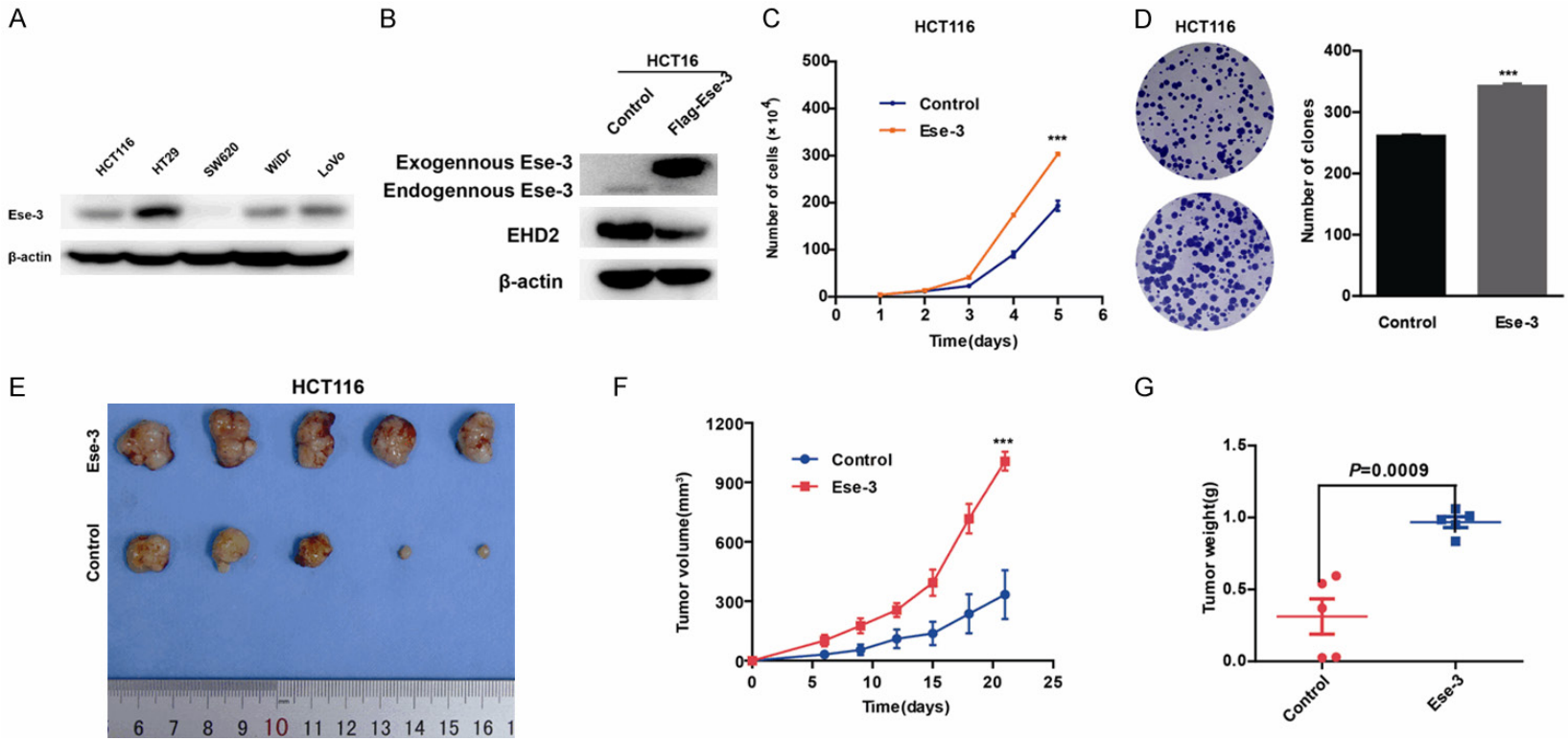
TCGA-AA-A01G-01	MALE	M0	N0	T3	Stage IIA	63
TCGA-AA-A02R-01	FEMALE	M0	N0	T3	Stage IIA	84
TCGA-AD-6889-01	MALE	M0	N0	T3	Stage IIA	76
TCGA-AM-5821-01	FEMALE	M0	N0	T3	Stage IIA	68
TCGA-AU-3779-01	FEMALE	M0	N0	T3	Stage IIA	80
TCGA-AY-A69D-01	FEMALE	M0	N0	T3	Stage IIA	55
TCGA-AZ-4315-01	MALE	M0	N0	T3	Stage IIA	61
TCGA-CA-5254-01	FEMALE	M0	N0	T3	Stage IIA	42
TCGA-CA-5255-01	MALE	M0	N0	T3	Stage IIA	45
TCGA-CA-5256-01	FEMALE	M0	N0	T3	Stage IIA	54
TCGA-CA-5796-01	FEMALE	M0	N0	T3	Stage IIA	52
TCGA-CA-5797-01	MALE	M0	N0	T3	Stage IIA	56
TCGA-CA-6716-01	MALE	M0	N0	T3	Stage IIA	65
TCGA-CA-6717-01	MALE	M0	N0	T3	Stage IIA	57
TCGA-CA-6718-01	MALE	M0	N0	T3	Stage IIA	46
TCGA-CA-6719-01	MALE	M0	N0	T3	Stage IIA	77
TCGA-CK-4951-01	FEMALE	M0	N0	T3	Stage IIA	79
TCGA-CM-4743-01	MALE	M0	N0	T3	Stage IIA	69
TCGA-CM-4752-01	MALE	M0	N0	T3	Stage IIA	58
TCGA-CM-5349-01	FEMALE	M0	N0	T3	Stage IIA	68
TCGA-CM-5860-01	MALE	M0	N0	T3	Stage IIA	44
TCGA-CM-5861-01	FEMALE	M0	N0	T3	Stage IIA	63
TCGA-CM-6164-01	FEMALE	M0	N0	T3	Stage IIA	46
TCGA-CM-6165-01	MALE	M0	N0	T3	Stage IIA	74
TCGA-CM-6168-01	FEMALE	M0	N0	T3	Stage IIA	84
TCGA-CM-6169-01	MALE	M0	N0	T3	Stage IIA	67
TCGA-CM-6674-01	MALE	M0	N0	T3	Stage IIA	39
TCGA-CM-6677-01	FEMALE	M0	N0	T3	Stage IIA	75
TCGA-CM-6679-01	MALE	M0	N0	T3	Stage IIA	58
TCGA-D5-5540-01	MALE	M0	N0	T3	Stage IIA	73
TCGA-D5-6529-01	MALE	M0	N0	T3	Stage IIA	69
TCGA-D5-6531-01	MALE	M0	N0	T3	Stage IIA	75
TCGA-D5-6532-01	MALE	M0	N0	T3	Stage IIA	61
TCGA-D5-6534-01	FEMALE	M0	N0	T3	Stage IIA	62
TCGA-D5-6536-01	MALE	M0	N0	T3	Stage IIA	73
TCGA-D5-6541-01	MALE	M0	N0	T3	Stage IIA	49
TCGA-D5-6920-01	FEMALE	M0	N0	T3	Stage IIA	77
TCGA-D5-6924-01	MALE	M0	N0	T3	Stage IIA	68
TCGA-D5-6927-01	MALE	M0	N0	T3	Stage IIA	34
TCGA-D5-6928-01	MALE	M0	N0	T3	Stage IIA	80
TCGA-D5-6930-01	MALE	M0	N0	T3	Stage IIA	67
TCGA-D5-6932-01	MALE	M0	N0	T3	Stage IIA	69
TCGA-DM-A0X9-01	FEMALE	M0	N0	T3	Stage IIA	71
TCGA-DM-A0XD-01	MALE	M0	N0	T3	Stage IIA	65
TCGA-DM-A1D0-01	FEMALE	M0	N0	T3	Stage IIA	79
TCGA-DM-A1D4-01	MALE	M0	N0	T3	Stage IIA	80
TCGA-DM-A1D6-01	MALE	M0	N0	T3	Stage IIA	88
TCGA-DM-A1D7-01	MALE	M0	N0	T3	Stage IIA	82
TCGA-DM-A1D9-01	FEMALE	M0	N0	T3	Stage IIA	67
TCGA-DM-A1DB-01	MALE	M0	N0	T3	Stage IIA	68

TCGA-DM-A280-01	FEMALE	M0	N0	T3	Stage IIA	70
TCGA-DM-A282-01	FEMALE	M0	N0	T3	Stage IIA	60
TCGA-DM-A28C-01	MALE	M0	N0	T3	Stage IIA	74
TCGA-DM-A28E-01	FEMALE	M0	N0	T3	Stage IIA	72
TCGA-DM-A28G-01	MALE	M0	N0	T3	Stage IIA	75
TCGA-DM-A28K-01	MALE	M0	N0	T3	Stage IIA	75
TCGA-DM-A28M-01	MALE	M0	N0	T3	Stage IIA	63
TCGA-F4-6463-01	MALE	M0	N0	T3	Stage IIA	51
TCGA-F4-6570-01	FEMALE	M0	N0	T3	Stage IIA	78
TCGA-F4-6703-01	MALE	M0	N0	T3	Stage IIA	64
TCGA-F4-6805-01	FEMALE	M0	N0	T3	Stage IIA	58
TCGA-F4-6854-01	FEMALE	M0	N0	T3	Stage IIA	77
TCGA-F4-6855-01	FEMALE	M0	N0	T3	Stage IIA	70
TCGA-G4-6302-01	FEMALE	M0	N0	T3	Stage IIA	90
TCGA-G4-6586-01	FEMALE	M0	N0	T3	Stage IIA	73
TCGA-G4-6588-01	FEMALE	M0	N0	T3	Stage IIA	58
TCGA-G4-6625-01	FEMALE	M0	N0	T3	Stage IIA	77
TCGA-G4-6626-01	MALE	M0	N0	T3	Stage IIA	90
TCGA-G4-6627-01	MALE	M0	N0	T3	Stage IIA	84
TCGA-T9-A92H-01	MALE	M0	N0	T3	Stage IIA	82
TCGA-AA-3511-01	MALE	M0	N0	T4	Stage II	64
TCGA-AA-A022-01	FEMALE	M0	N0	T4	Stage II	88
TCGA-A6-2676-01	FEMALE	M0	N0	T4	Stage IIB	75
TCGA-A6-3809-01	FEMALE	M0	N0	T4	Stage IIB	71
TCGA-AA-3947-01	FEMALE	M0	N0	T4	Stage IIB	60
TCGA-AA-A00N-01	MALE	M0	N0	T4	Stage IIB	75
TCGA-AA-A010-01	FEMALE	M0	N0	T4	Stage IIB	46
TCGA-G4-6304-01	FEMALE	M0	N0	T4	Stage IIB	66
TCGA-SS-A7HO-01	FEMALE	M0	N0	T4a	Stage IIB	44
TCGA-A6-5666-01	MALE	M0	N0	T4b	Stage IIC	78
TCGA-AA-3678-01	FEMALE	M0	N1	T2	Stage III	60
TCGA-AA-A01X-01	FEMALE	M0	N1	T2	Stage III	80
TCGA-AA-3842-01	MALE	M0	N1	T2	Stage IIIA	51
TCGA-AA-3976-01	MALE	M0	N1	T2	Stage IIIA	70
TCGA-AA-A01C-01	MALE	M0	N1	T2	Stage IIIA	75
TCGA-AZ-6608-01	FEMALE	M0	N1	T2	Stage IIIA	55
TCGA-AA-3519-01	MALE	M0	N1	T3	Stage III	63
TCGA-AA-3666-01	MALE	M0	N1	T3	Stage III	68
TCGA-AA-3672-01	FEMALE	M0	N1	T3	Stage III	90
TCGA-AA-3681-01	FEMALE	M0	N1	T3	Stage III	77
TCGA-AA-3971-01	MALE	M0	N1	T3	Stage III	58
TCGA-AA-A01P-01	FEMALE	M0	N1	T3	Stage III	80
TCGA-AA-A01S-01	FEMALE	M0	N1	T3	Stage III	47
TCGA-AA-A01T-01	FEMALE	M0	N1	T3	Stage III	63
TCGA-CK-4948-01	FEMALE	M0	N1	T3	Stage III	45
TCGA-G4-6293-01	FEMALE	M0	N1	T3	Stage III	49
TCGA-D5-5539-01	MALE	M0	N1	T3	Stage IIIA	60
TCGA-D5-6922-01	MALE	M0	N1	T3	Stage IIIA	76
TCGA-A6-2672-01	FEMALE	M0	N1	T3	Stage IIIB	82
TCGA-A6-2678-01	FEMALE	M0	N1	T3	Stage IIIB	43

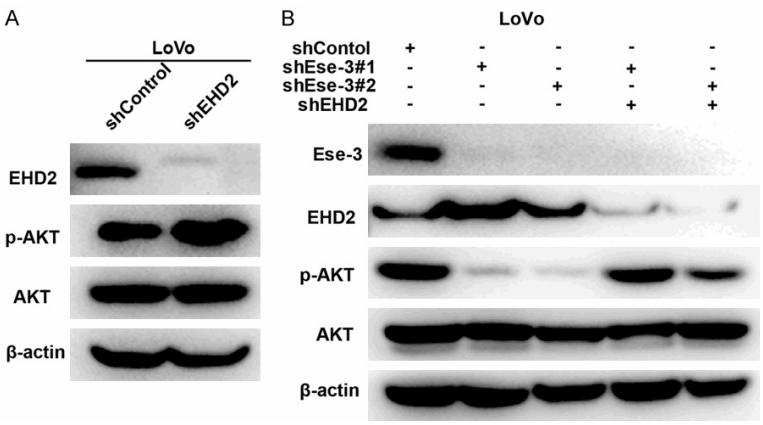
TCGA-A6-5657-01	MALE	M0	N1	T3	Stage IIIB	65
TCGA-A6-6654-01	FEMALE	M0	N1	T3	Stage IIIB	65
TCGA-A6-A56B-01	MALE	M0	N1	T3	Stage IIIB	57
TCGA-A6-A5ZU-01	MALE	M0	N1	T3	Stage IIIB	59
TCGA-AA-3525-01	MALE	M0	N1	T3	Stage IIIB	90
TCGA-AA-3860-01	FEMALE	M0	N1	T3	Stage IIIB	53
TCGA-AA-3949-01	FEMALE	M0	N1	T3	Stage IIIB	87
TCGA-AA-3982-01	MALE	M0	N1	T3	Stage IIIB	75
TCGA-AA-3994-01	MALE	M0	N1	T3	Stage IIIB	69
TCGA-AA-A00U-01	MALE	M0	N1	T3	Stage IIIB	50
TCGA-AA-A01F-01	MALE	M0	N1	T3	Stage IIIB	72
TCGA-AZ-4308-01	FEMALE	M0	N1	T3	Stage IIIB	47
TCGA-AZ-4615-01	MALE	M0	N1	T3	Stage IIIB	84
TCGA-CA-6715-01	MALE	M0	N1	T3	Stage IIIB	63
TCGA-CK-4950-01	FEMALE	M0	N1	T3	Stage IIIB	68
TCGA-DM-A1HB-01	MALE	M0	N1	T3	Stage IIIB	75
TCGA-DM-A28F-01	MALE	M0	N1	T3	Stage IIIB	73
TCGA-F4-6460-01	FEMALE	M0	N1	T3	Stage IIIB	51
TCGA-G4-6307-01	FEMALE	M0	N1	T3	Stage IIIB	37
TCGA-G4-6309-01	FEMALE	M0	N1	T3	Stage IIIB	40
TCGA-G4-6310-01	MALE	M0	N1	T3	Stage IIIB	69
TCGA-A6-A566-01	FEMALE	M0	N1	T4	Stage IIIB	55
TCGA-AA-A00J-01	MALE	M0	N1	T4	Stage IIIB	80
TCGA-AA-A00Q-01	FEMALE	M0	N1	T4	Stage IIIB	66
TCGA-AZ-6605-01	MALE	M0	N1	T4	Stage IIIB	77
TCGA-CK-4947-01	FEMALE	M0	N1	T4	Stage IIIB	46
TCGA-D5-6926-01	MALE	M0	N1	T4a	Stage IIIB	65
TCGA-CM-5341-01	FEMALE	M0	N1a	T2	Stage IIIA	82
TCGA-AD-6895-01	MALE	M0	N1a	T3	Stage IIIB	84
TCGA-AY-6386-01	FEMALE	M0	N1a	T3	Stage IIIB	66
TCGA-CM-5348-01	MALE	M0	N1a	T3	Stage IIIB	72
TCGA-CM-6162-01	FEMALE	M0	N1a	T3	Stage IIIB	48
TCGA-CM-6172-01	FEMALE	M0	N1a	T3	Stage IIIB	70
TCGA-D5-5541-01	MALE	M0	N1a	T3	Stage IIIB	63
TCGA-CM-4748-01	MALE	M0	N1a	T4a	Stage IIIB	53
TCGA-CM-4750-01	FEMALE	M0	N1b	T1	Stage IIIA	34
TCGA-A6-4107-01	FEMALE	M0	N1b	T3	Stage IIIB	57
TCGA-A6-6649-01	MALE	M0	N1b	T3	Stage IIIB	66
TCGA-AD-6888-01	MALE	M0	N1b	T3	Stage IIIB	73
TCGA-CM-4751-01	MALE	M0	N1b	T3	Stage IIIB	62
TCGA-CM-5344-01	FEMALE	M0	N1b	T3	Stage IIIB	39
TCGA-CM-5863-01	FEMALE	M0	N1b	T3	Stage IIIB	60
TCGA-D5-5538-01	FEMALE	M0	N1b	T3	Stage IIIB	60
TCGA-4N-A93T-01	MALE	M0	N1b	T4a	Stage IIIB	67
TCGA-A6-6137-01	MALE	M0	N1c	T3	Stage IIIB	55
TCGA-AA-3516-01	FEMALE	M0	N2	T3	Stage III	74
TCGA-AA-3712-01	MALE	M0	N2	T3	Stage III	65
TCGA-AA-3811-01	FEMALE	M0	N2	T3	Stage III	84
TCGA-AA-A01R-01	MALE	M0	N2	T3	Stage III	47
TCGA-AA-A03F-01	FEMALE	M0	N2	T3	Stage III	90

TCGA-D5-6538-01	FEMALE	M0	N2	T3	Stage IIIB	79
TCGA-A6-2677-01	FEMALE	M0	N2	T3	Stage IIIC	68
TCGA-A6-3807-01	FEMALE	M0	N2	T3	Stage IIIC	53
TCGA-AA-3529-01	FEMALE	M0	N2	T3	Stage IIIC	78
TCGA-AA-3542-01	MALE	M0	N2	T3	Stage IIIC	69
TCGA-AA-3548-01	FEMALE	M0	N2	T3	Stage IIIC	71
TCGA-AA-3552-01	MALE	M0	N2	T3	Stage IIIC	85
TCGA-AA-3560-01	FEMALE	M0	N2	T3	Stage IIIC	72
TCGA-AA-3562-01	MALE	M0	N2	T3	Stage IIIC	82
TCGA-AA-3844-01	FEMALE	M0	N2	T3	Stage IIIC	78
TCGA-AA-3848-01	FEMALE	M0	N2	T3	Stage IIIC	82
TCGA-AA-3952-01	MALE	M0	N2	T3	Stage IIIC	68
TCGA-AA-A00F-01	MALE	M0	N2	T3	Stage IIIC	66
TCGA-AA-A000-01	FEMALE	M0	N2	T3	Stage IIIC	83
TCGA-AA-A01D-01	FEMALE	M0	N2	T3	Stage IIIC	47
TCGA-AA-A01K-01	FEMALE	M0	N2	T3	Stage IIIC	74
TCGA-AY-4070-01	FEMALE	M0	N2	T3	Stage IIIC	50
TCGA-DM-A0XF-01	FEMALE	M0	N2	T3	Stage IIIC	68
TCGA-DM-A1DA-01	FEMALE	M0	N2	T3	Stage IIIC	71
TCGA-DM-A1HA-01	MALE	M0	N2	T3	Stage IIIC	82
TCGA-DM-A288-01	MALE	M0	N2	T3	Stage IIIC	68
TCGA-DM-A28A-01	MALE	M0	N2	T3	Stage IIIC	78
TCGA-DM-A28H-01	MALE	M0	N2	T3	Stage IIIC	50
TCGA-G4-6299-01	MALE	M0	N2	T3	Stage IIIC	69
TCGA-CK-4952-01	FEMALE	M0	N2	T4	Stage IIIC	48
TCGA-D5-6931-01	MALE	M0	N2	T4b	Stage IIIC	77
TCGA-F4-6461-01	FEMALE	M0	N2	T4b	Stage IIIC	41
TCGA-AA-3955-01	MALE	M0	N2a	T2	Stage IIIB	38
TCGA-CM-6680-01	FEMALE	M0	N2a	T3	Stage IIIB	78
TCGA-F4-6459-01	FEMALE	M0	N2a	T3	Stage IIIB	61
TCGA-NH-A50V-01	MALE	M0	N2a	T3	Stage IIIB	69
TCGA-A6-5660-01	MALE	M0	N2b	T3	Stage IIIC	73
TCGA-CM-6167-01	FEMALE	M0	N2b	T3	Stage IIIC	57
TCGA-F4-6807-01	FEMALE	M0	N2b	T3	Stage IIIC	51
TCGA-AD-6965-01	MALE	M0	N2b	T4a	Stage IIIC	62
TCGA-AA-3494-01	MALE	M1	N0	T3	Stage IV	55
TCGA-AA-3713-01	MALE	M1	N0	T3	Stage IV	68
TCGA-AA-A02J-01	FEMALE	M1	N0	T3	Stage IV	70
TCGA-A6-6652-01	MALE	M1	N0	T3	Stage IVA	59
TCGA-AZ-4682-01	MALE	M1	N0	T3	Stage IVA	61
TCGA-A6-2683-01	FEMALE	M1	N0	T4	Stage IV	57
TCGA-A6-A567-01	MALE	M1	N1	T3	Stage IV	56
TCGA-AA-3688-01	MALE	M1	N1	T3	Stage IV	80
TCGA-AA-3696-01	FEMALE	M1	N1	T3	Stage IV	75
TCGA-AA-3972-01	MALE	M1	N1	T3	Stage IV	72
TCGA-AA-A02E-01	FEMALE	M1	N1	T3	Stage IV	82
TCGA-AA-A02F-01	FEMALE	M1	N1	T3	Stage IV	68
TCGA-CK-6748-01	FEMALE	M1	N1	T3	Stage IV	45
TCGA-D5-6929-01	FEMALE	M1	N1	T3	Stage IV	49
TCGA-G4-6294-01	MALE	M1	N1	T3	Stage IV	75

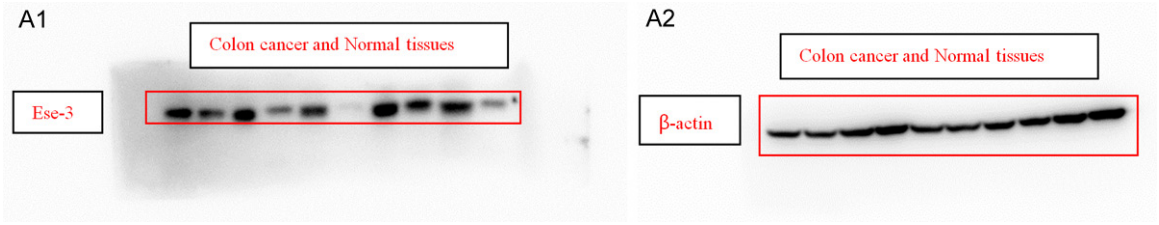
TCGA-G4-6303-01	FEMALE	M1	N1	T3	Stage IV	54
TCGA-G4-6315-01	MALE	M1	N1	T3	Stage IV	66
TCGA-F4-6809-01	FEMALE	M1	N1	T3	Stage IVA	52
TCGA-AA-3693-01	FEMALE	M1	N1	T4	Stage IV	77
TCGA-AA-3973-01	MALE	M1	N1	T4	Stage IV	69
TCGA-AZ-6600-01	MALE	M1	N1	T4	Stage IV	64
TCGA-AZ-4614-01	FEMALE	M1	N1	T4a	Stage IVA	71
TCGA-A6-2682-01	MALE	M1	N1	T4b	Stage IV	70
TCGA-AY-5543-01	FEMALE	M1	N1a	T3	Stage IVA	65
TCGA-A6-2671-01	MALE	M1	N2	T3	Stage IV	85
TCGA-A6-2674-01	MALE	M1	N2	T3	Stage IV	71
TCGA-AA-3488-01	MALE	M1	N2	T3	Stage IV	58
TCGA-AA-3679-01	MALE	M1	N2	T3	Stage IV	59
TCGA-AA-3692-01	FEMALE	M1	N2	T3	Stage IV	47
TCGA-AA-3867-01	MALE	M1	N2	T3	Stage IV	74
TCGA-AA-3870-01	FEMALE	M1	N2	T3	Stage IV	71
TCGA-AA-3930-01	MALE	M1	N2	T3	Stage IV	66
TCGA-AA-3989-01	MALE	M1	N2	T3	Stage IV	84
TCGA-AA-A02H-01	FEMALE	M1	N2	T3	Stage IV	74
TCGA-AZ-4616-01	FEMALE	M1	N2	T3	Stage IV	82
TCGA-DM-A285-01	FEMALE	M1	N2	T3	Stage IV	71
TCGA-G4-6297-01	FEMALE	M1	N2	T3	Stage IV	55
TCGA-G4-6314-01	FEMALE	M1	N2	T3	Stage IV	76
TCGA-A6-5662-01	MALE	M1	N2	T3	Stage IVA	46
TCGA-AZ-4684-01	MALE	M1	N2	T3	Stage IVA	49
TCGA-AA-3662-01	FEMALE	M1	N2	T4	Stage IV	80
TCGA-AA-3680-01	FEMALE	M1	N2	T4	Stage IV	67
TCGA-AA-3684-01	FEMALE	M1	N2	T4	Stage IV	65
TCGA-AA-3869-01	MALE	M1	N2	T4	Stage IV	76
TCGA-AA-3872-01	MALE	M1	N2	T4	Stage IV	45
TCGA-AA-A02K-01	MALE	M1	N2	T4	Stage IV	50
TCGA-AZ-4323-01	MALE	M1	N2	T4	Stage IV	37
TCGA-AZ-6606-01	MALE	M1	N2	T4	Stage IV	81
TCGA-AZ-6607-01	MALE	M1	N2	T4	Stage IV	69
TCGA-AM-5820-01	FEMALE	M1	N2	T4a	Stage IVA	59
TCGA-AY-A8YK-01	MALE	M1	N2a	T3	Stage IVA	44
TCGA-NH-A8F8-01	MALE	M1	N2b	T4a	Stage IV	79
TCGA-A6-6648-01	MALE	M1a	N0	T3	Stage IVA	56
TCGA-NH-A50U-01	MALE	M1a	N0	T4a	Stage IVA	42
TCGA-A6-6142-01	FEMALE	M1a	N1a	T3	Stage IVA	56
TCGA-CM-5862-01	MALE	M1a	N1a	T3	Stage IVA	80
TCGA-CM-5868-01	FEMALE	M1a	N1a	T4a	Stage IVA	59
TCGA-AA-3941-01	FEMALE	M1a	N1b	T4	Stage IVA	84
TCGA-CM-4747-01	MALE	M1a	N1b	T4a	Stage IVA	47
TCGA-CM-6678-01	FEMALE	M1a	N1c	T4a	Stage IVA	63
TCGA-5M-AAT6-01	FEMALE	M1a	N2b	T4a	Stage IV	40
TCGA-5M-AAT4-01	MALE	M1b	N0	T3	Stage IV	74
TCGA-NH-A6GC-01	FEMALE	M1b	N1b	T4b	Stage IVB	66
TCGA-CM-6675-01	MALE	M1b	N2b	T3	Stage IVB	35



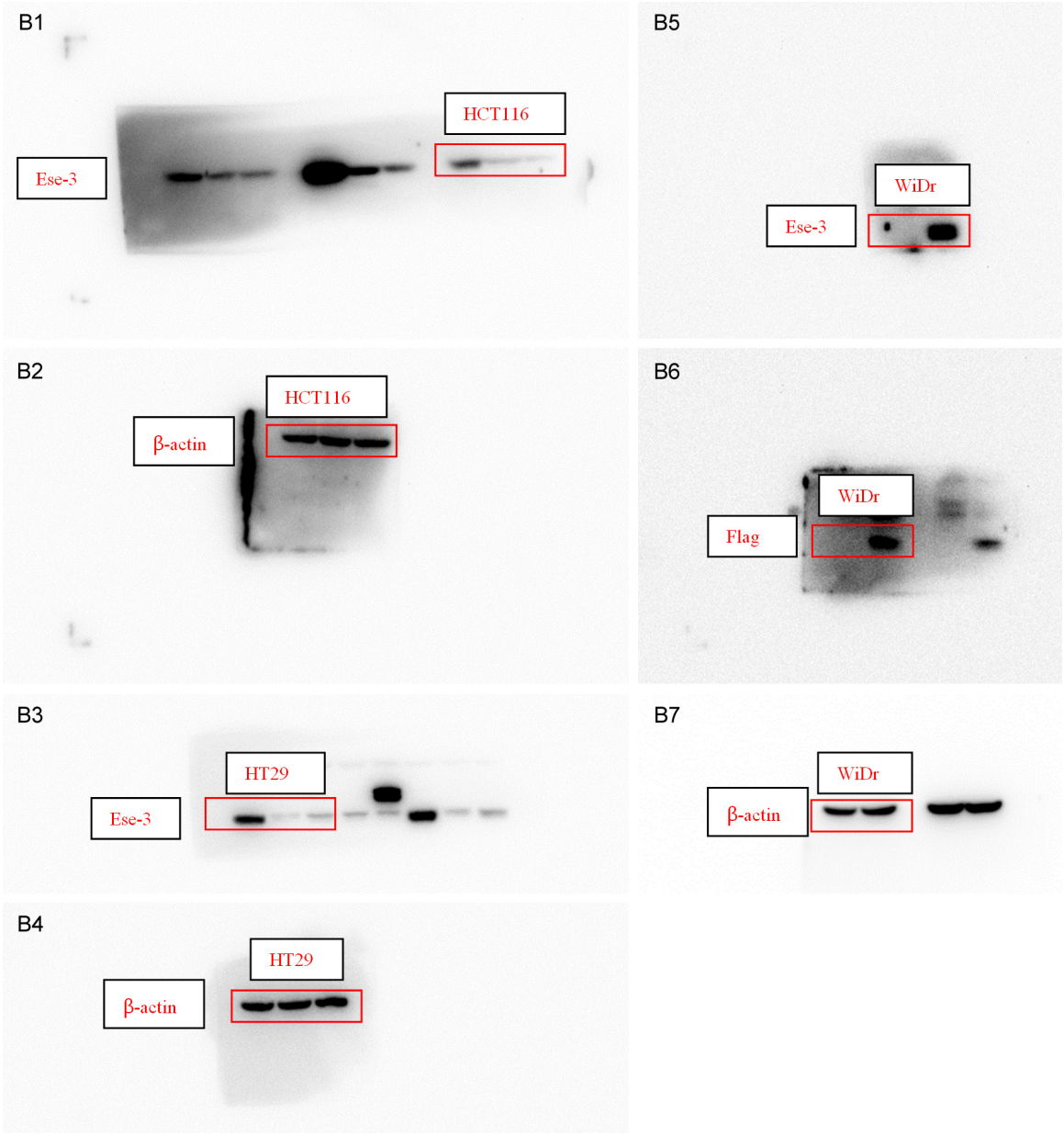
Supplementary Figure 1. Elevated expression of Ese-3 promotes CC cell proliferation in vitro and in vivo. A, B. Indicated protein expression levels were measured in different CC cells, Ese-3-overexpressing HCT116 cells and control cells (Original blots were showed in Supplementary Figure 10). C, D. Cell proliferation and colony formation assays were performed to analyze the effect of Ese-3 overexpression on HCT116 cells. E-G. Xenografts in nude mice showed the impact of upregulation Ese-3 on the tumorigenicity in the HCT116 cells.



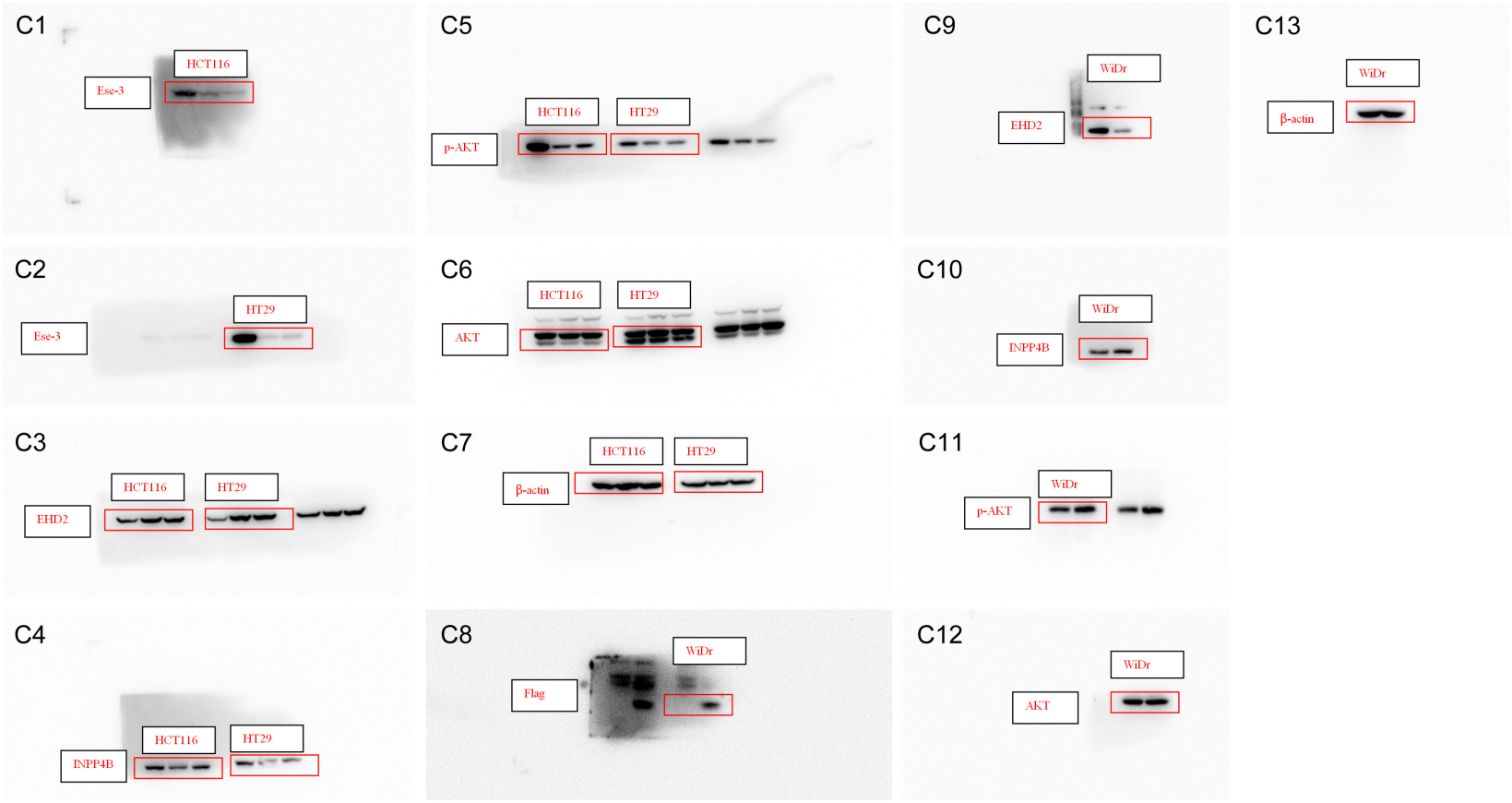
Supplementary Figure 2. Ese-3 upregulates the phosphorylation levels of AKT by suppressing EHD2 expression. A. The indicated protein expression levels were measured in EHD2-knockdown LoVo cells (Original blots were showed in Supplementary Figure 11I1-I4). B. Western blotting analysis of the indicated protein expression levels in LoVo cells transduced with shControl, shEse-3 or shEse-3 plus shEHD2 (Original blots were showed in Supplementary Figure 11I5-I9).



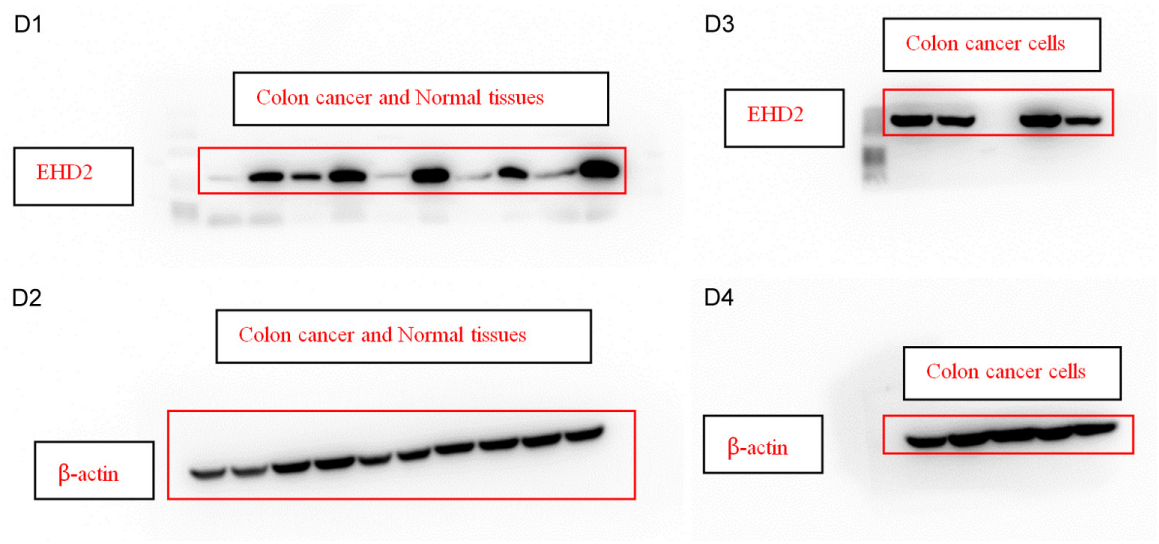
Supplementary Figure 3. The expression levels of Ese-3 in Colon cancer and normal tissues. A1, A2. Ese-3 and β-actin expression levels in Colon cancer and normal tissues.



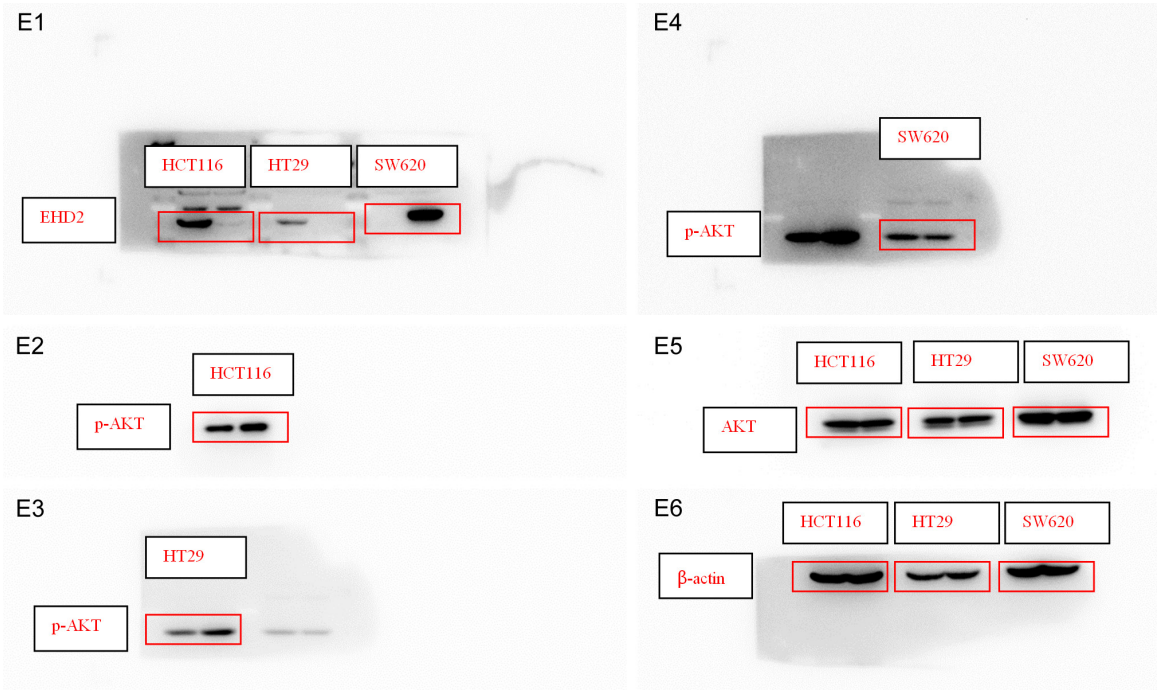
Supplementary Figure 4. The indicated protein expression levels in Colon cancer cells. B1, B2. Ese-3 expression levels in Ese-3-silenced HCT116 cells and control cells. B3, B4. Ese-3 expression levels in Ese-3-silenced HT29 cells and control cells. B5-B7. Ese-3 expression levels in Ese-3-overexpressing WiDr cells and control cells.



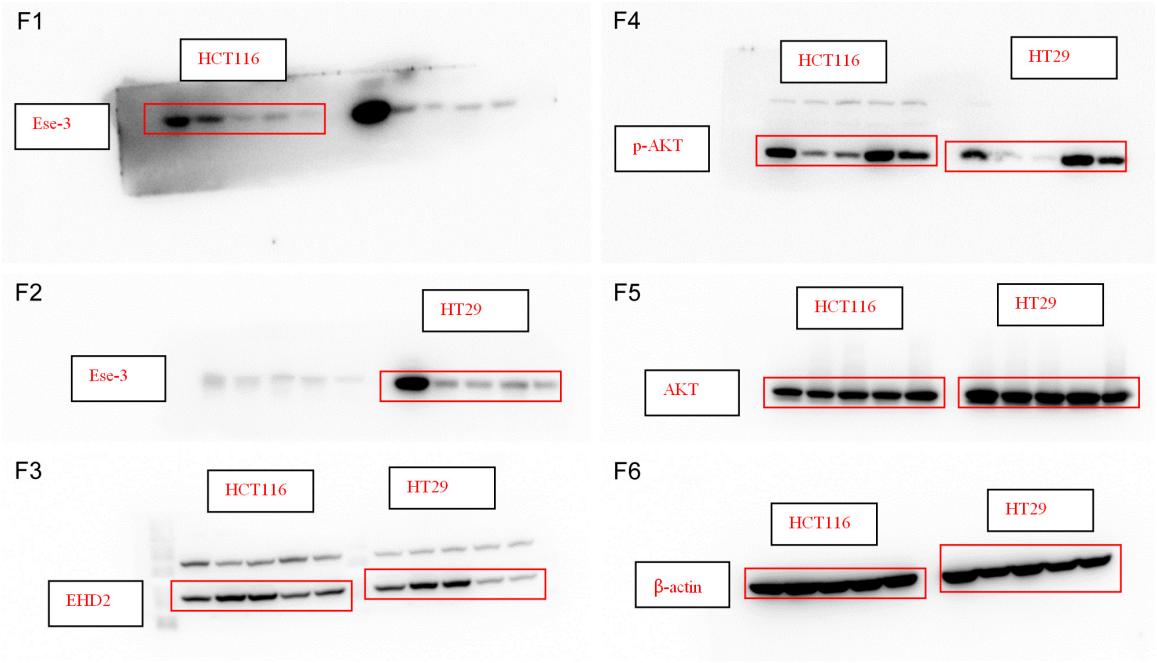
Supplementary Figure 5. The indicated protein expression levels in Colon cancer cells. C1, C2. Ese-3 expression levels in Ese-3 silenced HCT116, HT29 cells and control cells. C3-C7. EHD2, INPP4B, p-AKT, AKT, β-actin expression levels in Ese-3 silenced HCT116, HT29 cells and control cells. C8-C13. Flag, EHD2, INPP4B, p-AKT, AKT, β-actin expression levels in Ese-3 overexpressing WiDr cells and control cells.



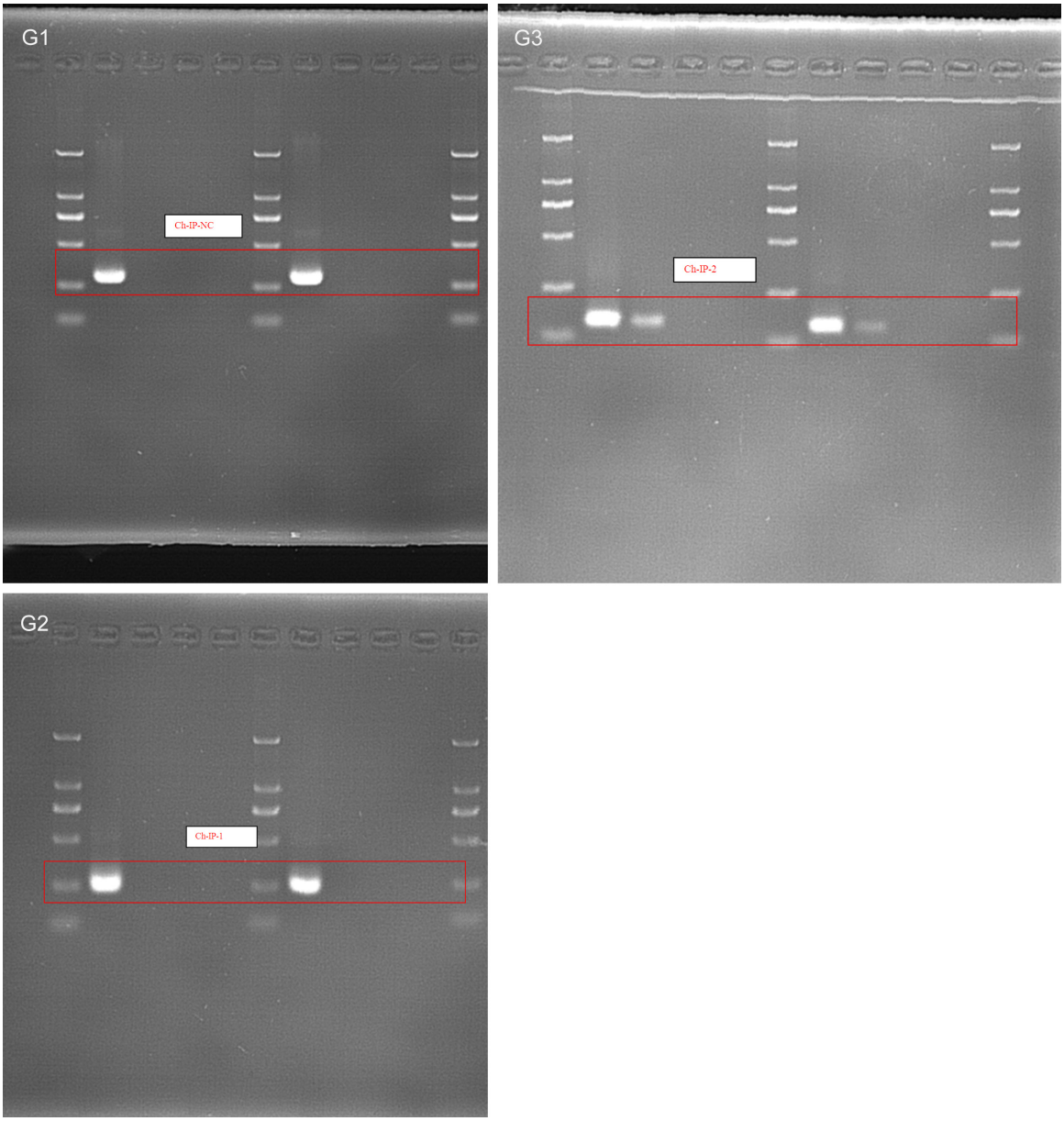
Supplementary Figure 6. The indicated protein expression levels in Colon cancer and normal tissues, Colon cancer cells. D1, D2. EHD2 expression levels in Colon cancer and Normal tissues. D3, D4. EHD2 expression levels in different Colon cancer cells.



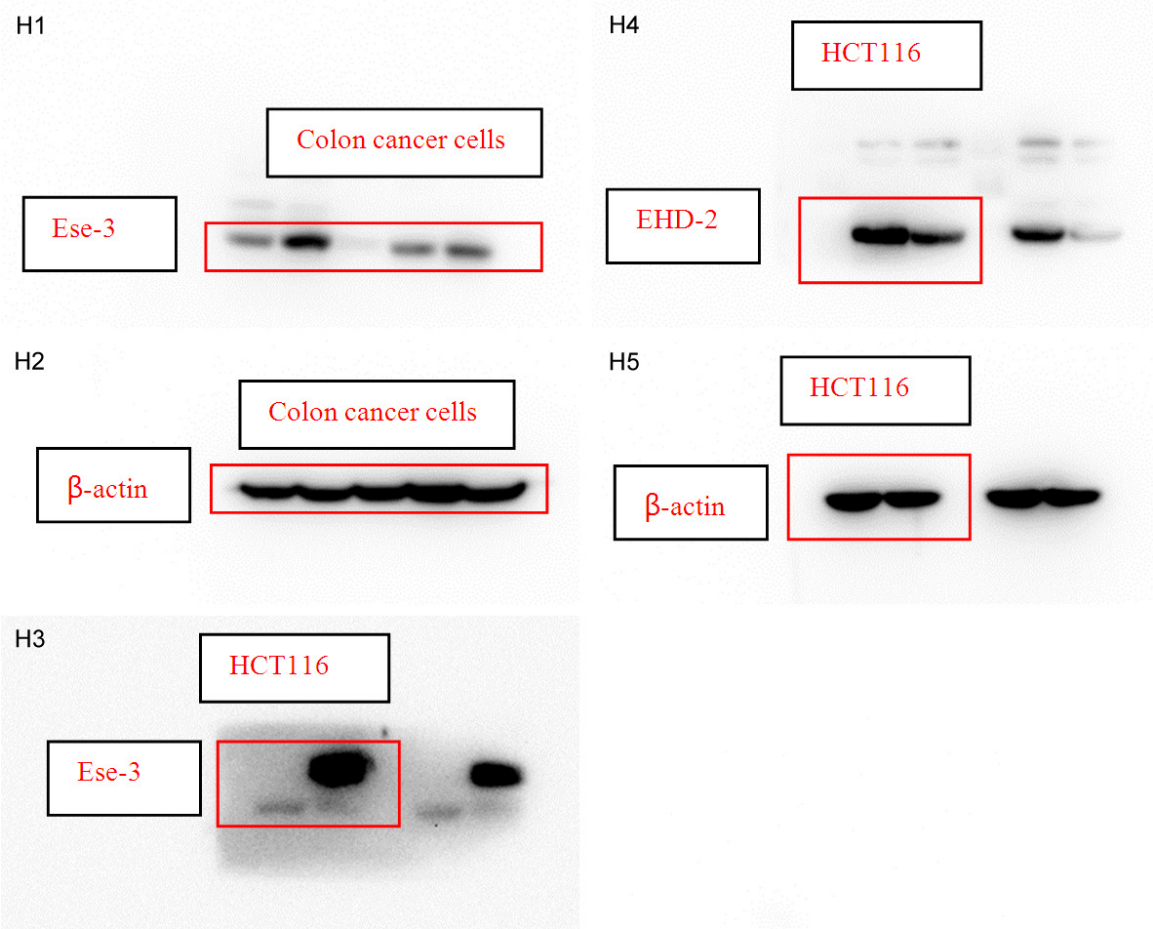
Supplementary Figure 7. The indicated protein expression levels in Colon cancer cells. E1. EHD2 expression level in EHD2 silenced HCT116, HT29 cells, EHD2 overexpressing SW620 cells and control cells. E2-E6. p-AKT, AKT, β-actin expression levels in EHD2 silenced HCT116, HT29 cells, EHD2 overexpressing SW620 cells and control cells.



Supplementary Figure 8. The indicated protein expression levels in Colon cancer cells. F1, F2. Ese-3 expression levels in Ese-3 silenced HCT116, HT29 cells and Ese-3 plus EHD2 silenced HCT116, HT29 cells and control cells. F3-F6. EHD2, p-AKT, AKT and β-actin expression levels in Ese-3 silenced HCT116, HT29 cells and Ese-3 plus EHD2 silenced HCT116, HT29 cells and control cells.



Supplementary Figure 9. Agarosegel electrophoresis assay showed the binding site of Ese-3 to INPP4B in HCT116 and HT29 cells. G1-G3. The site of Ese-3 bind to the INPP4B.



Supplementary Figure 10. The indicated protein expression levels in Colon cancer cells. H1, H2. Ese-3 expression levels in different Colon cancer cells. H3-H5. Ese-3, EHD2, β-actin expression levels in Ese-3 overexpressing HCT116 cells and control cells.

Supplementary Table 6. Label-free quantitative proteomics analysis of differentially expressed proteins in Ese-3-silenced HCT116 cells and control group cells

Protein	symbol	shEse-3#1-vs-shControl FC	*P* value
Q8TBF2	PXL2B	1.34	0.005614992
Q02127	PYRD	1.44	0.029301111
O15066	KIF3B	1.36	0.009238177
Q9Y291	RT33	1.33	0.031081333
Q00765	REEP5	1.42	0.008184668
O75396	SC22B	1.56	0.003020421
P46013	KI67	1.33	0.003618701
P50897	PPT1	2.09	0.000178559
Q9Y606	TRUA	1.35	0.003987691
P62820	RAB1A	1.40	0.000797717
Q9NS69	TOM22	1.35	0.000117185
P29144	TPP2	1.41	0.001364568
Q9NV06	DCA13	1.35	0.00513444
Q9BTU6	P4K2A	1.33	0.021019819
Q16762	THTR	1.52	0.018435459
P04156	PRIO	1.55	0.013289726
Q68CQ4	DIEXF	1.35	0.005534737
O14974	MYPT1	1.35	0.044545843
Q9H9A6	LRC40	1.30	0.008536083
P05783	K1C18	1.40	0.000153807
Q8IY81	SPB1	1.41	0.000432338
Q9HAV7	GRPE1	1.40	0.000422086
O43169	CYB5B	2.20	0.000253416
P48730	KC1D	1.32	0.029375558
Q02218	ODO1	1.42	0.009080367
P21926	CD9	1.84	0.002060557
P21291	CSRP1	1.42	0.000614788
Q9GZT3	SLIRP	1.35	0.00466429
Q9HCY8	S10AE	1.46	0.024905525
Q13442	HAP28	1.31	0.007082222
Q7Z434	MAVS	1.31	0.002915262
P62753	RS6	1.31	0.009610642
P18031	PTN1	1.67	0.000157124
P58107	EPIPL	1.54	0.000455475
P06400	RB	1.31	0.017574028
Q96C86	DCPS	1.34	0.005705124
P10321	1C07	1.48	0.001676553
P61916	NPC2	1.43	0.013089558
Q9NY33	DPP3	1.41	0.001942603
Q9H0G5	NSRP1	1.39	0.014486739
P52298	NCBP2	1.95	0.000863062
Q9P2M7	CING	1.39	0.017481857
Q9NTJ3	SMC4	1.34	0.034773096
Q6NW29	RWDD4	1.34	0.006334553
Q9Y5M8	SRPRB	1.39	0.002019973
Q15418	KS6A1	1.42	0.000858545

Q9UPT8	ZC3H4	1.49	0.020084172
Q9H4A5	GLP3L	2.61	0.029839408
Q8N6M0	OTU6B	1.37	0.016416054
Q15363	TMED2	1.39	0.011279068
Q9Y4E5	ZN451	1.42	0.021944905
Q96FZ7	CHMP6	1.48	0.00516945
A0MZ66	SHOT1	1.41	0.005542648
P09497	CLCB	2.95	0.046950778
Q9Y4P3	TBL2	1.40	0.006175778
Q9BSJ8	ESYT1	1.33	0.001076765
Q9Y4Z0	LSM4	1.31	0.00047347
Q9UJG1	MSPD1	1.95	0.001796161
O95182	NDUA7	1.81	1.33E-05
Q15836	VAMP3	1.65	0.000333611
Q14126	DSG2	1.43	0.000683092
Q9BVK6	TMED9	1.61	0.022655314
Q9NVA1	UQCC1	1.36	0.020848772
Q14669	TRIPC	1.40	0.000103729
O95168	NDUB4	1.65	0.003007834
O43805	SSNA1	1.40	0.006711123
Q7KZN9	COX15	1.42	0.026073289
Q9ULV3	CIZ1	1.49	0.033679856
Q8WXH0	SYNE2	1.70	0.018023549
O94842	TOX4	1.33	0.003818804
P16070	CD44	1.60	0.002849886
Q96NC0	ZMAT2	1.55	0.013548104
P08727	K1C19	1.31	0.008472763
P21589	5NTD	1.81	0.014917813
Q16740	CLPP	1.61	0.001007086
P04049	RAF1	1.56	0.000512906
P50238	CRIP1	1.37	0.006535097
Q9UBS4	DJB11	1.47	0.002113817
O00151	PDLI1	1.32	0.00101292
P05141	ADT2	1.34	0.002249548
Q6ZXV5	TMTC3	1.55	0.011658876
P50552	VASP	1.39	0.005386508
Q9P0V9	SEP10	1.42	0.002172997
Q9BYD6	RM01	1.38	0.004903072
Q9Y547	IFT25	1.42	0.009994027
O15228	GNPAT	1.44	0.000531593
O75116	ROCK2	1.36	0.013830249
Q9BQL6	FERM1	1.30	0.047473352
Q15286	RAB35	1.62	0.001850044
Q9P032	NDUF4	1.78	0.00055536
O14981	BTAF1	1.51	0.036386505
O14672	ADA10	1.30	0.014121824
Q9UHA3	RLP24	1.35	0.027243309
P61006	RAB8A	1.50	0.001955813
Q9NZN4	EHD2	1.47	0.008841268

P09601	HMOX1	1.42	1.37E-05
Q9Y3E2	BOLA1	1.45	0.021774544
O43318	M3K7	1.37	0.005888954
O15269	SPTC1	1.41	0.002060545
A1L0T0	ILVBL	1.37	0.002815108
P49207	RL34	1.73	1.20E-05
P20337	RAB3B	1.95	0.015337265
Q96RT1	ERBIN	1.37	0.012084749
Q9NYV4	CDK12	1.31	0.019925773
Q96N66	MBOA7	1.76	0.000525736
Q12830	BPTF	1.38	0.010902241
Q9Y2U8	MAN1	1.64	0.014434785
P17931	LEG3	1.32	0.005008802
P52655	TF2AA	1.30	0.007467619
Q5MNZ6	WIPI3	1.35	0.000107707
O94905	ERLN2	1.47	0.019740793
Q8IUI8	CRLF3	1.44	0.000546403
P35659	DEK	1.36	0.016688607
P23368	MAOM	1.44	0.000541033
Q5TBB1	RNH2B	1.44	0.029927895
Q9HD20	AT131	1.38	0.014489198
P10586	PTPRF	1.33	0.033808578
Q6NXR4	TTI2	1.61	0.010364332
Q8TCD5	NT5C	1.30	0.001455836
Q9NQ48	LZTL1	1.36	0.030814678
Q56VL3	OCAD2	1.36	0.0403844
P09234	RU1C	1.56	0.013695947
Q6P1L8	RM14	1.40	0.049792281
Q9HAU5	RENT2	1.55	0.01731922
Q15758	AAAT	1.42	0.016212451
Q99959	PKP2	1.31	0.005824929
Q13573	SNW1	1.34	0.001120132
O00233	PSMD9	1.32	0.026941123
Q9BYJ9	YTHD1	1.37	0.013233933
Q9BV40	VAMP8	3.10	0.042465504
Q09666	AHNK	1.81	0.000302325
P30050	RL12	1.32	0.002071677
O15145	ARPC3	1.57	0.003662675
Q8IWZ3	ANKH1	1.35	0.005630389
P26006	ITA3	1.48	0.000704365
A6NIH7	U119B	1.45	0.013304999
Q6PIU2	NCEH1	1.33	0.010654605
Q6DD88	ATLA3	1.41	0.000452117
Q13557	KCC2D	1.55	0.002987661
Q13555	KCC2G	1.47	0.004398084
P62877	RBX1	1.34	0.029466079
Q5VV41	ARHGG	1.72	0.000930027
Q86X29	LSR	1.34	0.004047917
P82664	RT10	1.85	0.003232807

P62318	SMD3	1.41	0.000302453
P84095	RHOG	1.58	0.004272833
P13987	CD59	1.49	0.029217418
P13984	T2FB	1.35	0.001422211
Q9ULC5	ACSL5	1.40	0.000431683
Q86WR7	PRSR2	1.42	0.017940545
Q8TAE8	G45IP	1.56	0.047534708
Q6ZMG9	CERS6	1.42	0.013873601
Q9Y3T9	NOC2L	1.33	0.000746731
P13473	LAMP2	1.34	0.005134415
Q9BRD0	BUD13	1.48	0.002921211
P06753	TPM3	2.00	0.003572797
Q05D32	CTSL2	1.58	0.017214699
P51648	AL3A2	1.45	0.024778903
Q01650	LAT1	1.51	0.023176293
Q8WX93	PALLD	1.61	0.000135158
P36551	HEM6	1.36	0.001664482
Q8IWS0	PHF6	1.49	0.010705247
O43663	PRC1	1.34	0.005314285
Q9UBX3	DIC	1.70	0.022836673
O43278	SPIT1	1.46	0.010467388
Q8N983	RM43	1.44	0.004730334
Q14116	IL18	1.37	0.002725915
P16144	ITB4	1.79	0.000312062
Q96DG6	CMBL	1.33	0.000308395
Q9Y2R4	DDX52	1.38	0.003377792
P61619	S61A1	1.44	0.000449539
P12429	ANXA3	1.36	0.00170974
Q9UL63	MKLN1	1.38	0.010740265
P58876	H2B1D	1.32	0.000817537
Q9Y5P4	C43BP	1.48	0.01853251
Q8TCJ2	STT3B	1.49	0.000466596
O75569	PRKRA	1.41	0.005604665
P14927	QCR7	1.39	0.026225718
Q07666	KHDR1	1.34	0.012428617
P14923	PLAK	1.40	0.001400359
O95197	RTN3	1.61	0.002517934
Q13769	THOC5	1.51	0.015674564
Q02543	RL18A	1.35	0.000397229
O60925	PFD1	1.46	0.002644117
Q9BRQ8	AIFM2	1.33	0.045019491
O15371	EIF3D	1.31	0.011013623
Q8N3D4	EH1L1	1.96	0.002076724
P11166	GTR1	1.76	6.87E-05
Q9Y3B3	TMED7	1.33	0.005579972
O75330	HMMR	1.47	0.003792086
Q02952	AKA12	1.43	0.003495108
Q9BVV7	TIM21	1.79	0.046114116
Q9NVD7	PARVA	1.38	0.004595092

Q8TAQ2	SMRC2	1.36	0.022058517
Q14696	MESD	1.33	0.011874813
Q9UPT5	EXOC7	1.39	0.037391502
Q27J81	INF2	1.33	0.016515272
Q9NP72	RAB18	1.35	0.018038082
Q14197	ICT1	1.41	0.002946111
Q16778	H2B2E	1.59	0.00065216
O14548	COX7R	1.67	0.000125005
P52943	CRIP2	1.41	0.004039113
P24539	AT5F1	1.39	0.004201704
Q15102	PA1B3	1.34	0.018490803
Q8TF05	PP4R1	1.36	0.029050056
Q70UQ0	IKIP	1.70	0.003603641
P25440	BRD2	1.31	0.011034502
Q5C9Z4	NOM1	1.42	0.012296964
Q9BRU9	UTP23	1.89	0.035627608
Q96ER9	CCD51	1.70	0.00036037
Q15847	ADIRF	2.11	0.006258677
Q5UIP0	RIF1	1.41	0.010720342
Q99590	SCAFB	1.32	0.001043282
Q15126	PMVK	1.51	0.000102493
Q96QD8	S38A2	1.47	0.03929663
Q9Y3D3	RT16	1.53	0.003614125
Q9Y3D9	RT23	1.49	0.005140536
P23497	SP100	1.40	0.004012385
Q96FQ6	S10AG	1.61	0.003223654
Q9Y6C9	MTCH2	1.37	0.001559194
Q15149	PLEC	1.46	9.23E-07
Q9H0U3	MAGT1	1.54	0.01540983
P46939	UTRO	1.40	0.008884219
P17301	ITA2	1.65	0.025584067
Q5T8P6	RBM26	1.41	0.001404231
Q9HC07	TM165	1.68	0.009328561
Q9HBL8	NMRL1	1.38	0.003764274
O60563	CCNT1	1.64	7.26E-05
O43896	KIF1C	1.54	0.000624562
Q9NZ01	TECR	1.40	0.009587379
O75323	NIPS2	1.61	0.002110139
Q9Y605	MOFA1	0.70	0.006301913
Q9NYK5	RM39	0.65	0.016152061
Q16890	TPD53	0.63	0.037347473
Q9H2P0	ADNP	0.75	0.000908901
Q9Y3C6	PPIL1	0.59	0.000335958
P04179	SODM	0.65	0.003679264
Q14061	COX17	0.57	0.02701643
Q96CW5	GCP3	0.74	0.001929048
P62841	RS15	0.65	0.002918869
P57088	TMM33	0.77	0.027312413
Q8TED1	GPX8	0.63	0.03695303

O43598	DNPH1	0.73	0.013617198
Q96BP3	PPWD1	0.75	0.005475589
Q9NQP4	PFD4	0.63	0.019153601
P62070	RRAS2	0.74	0.03973388
Q96II8	LRCH3	0.69	0.020692667
Q96TA1	NIBL1	0.76	0.000286304
Q9Y3L5	RAP2C	0.66	8.37E-05
P05204	HMGN2	0.61	0.033279079
P19404	NDUV2	0.74	0.001249109
Q9GZP4	PITH1	0.69	0.000939829
O15327	INPP	0.66	1.44E-05
Q9BYN0	SRXN1	0.75	0.001824328
Q9BV57	MTND	0.66	0.003298059
P30049	ATPD	0.74	0.033653191
P30040	ERP29	0.77	0.007914543
P30046	DOPD	0.75	0.005955307
Q15004	PAF15	0.71	0.01273358
O95905	ECD	0.74	0.021559273
O43447	PPIH	0.76	0.003623754
Q9NX74	DUS2L	0.74	0.041830893
Q16342	PDCD2	0.64	0.001334901
P62308	RUXG	0.76	0.001386408
P49711	CTCF	0.75	0.016649765
Q99436	PSB7	0.76	0.045215526
P22392	NDKB	0.67	0.001446751
Q8WW33	GTSF1	0.75	0.030016932
P15121	ALDR	0.67	0.003942917
Q9NR31	SAR1A	0.68	0.037446333
Q15043	S39AE	0.74	0.012039552
P16949	STMN1	0.73	0.002539625
P15374	UCHL3	0.64	0.001575737
Q96D46	NMD3	0.74	0.001949929
P20674	COX5A	0.69	0.000203884
P49366	DHYS	0.71	0.031578749
P63167	DYL1	0.45	0.008255225
Q14978	NOLC1	0.71	0.000158483
P06280	AGAL	0.68	0.000855433
O43795	MYO1B	0.72	0.000147429
P53634	CATC	0.60	0.007061892
P36954	RPB9	0.64	0.003009552
Q9NVC6	MED17	0.69	0.004619966
Q13137	CACO2	0.77	0.028282135
P61956	SUMO2	0.69	0.00771256
P60604	UB2G2	0.68	0.002933266
Q14919	NC2A	0.63	0.000924011
Q06124	PTN11	0.75	0.02565301
Q9NRX4	PHP14	0.60	0.000815753
P78362	SRPK2	0.69	0.001744057
Q9UET6	TRM7	0.76	0.03577335

O75340	PDCD6	0.70	0.007540546
Q8NFH3	NUP43	0.76	0.018444942
P48059	LIMS1	0.74	0.003922592
Q9H8Y8	GORS2	0.72	0.001331199
O75575	RPC9	0.52	0.006982862
P61970	NTF2	0.75	0.016978177
P61088	UBE2N	0.70	0.004775121
O00193	SMAP	0.63	0.037826836
Q9BPZ3	PAIP2	0.49	0.029283423
P27797	CALR	0.71	3.65E-05
P62273	RS29	0.40	0.047258079
Q9BVI4	NOC4L	0.72	0.016152609
Q99496	RING2	0.75	0.035037245
P07947	YES	0.71	0.019860505
P36405	ARL3	0.60	0.000490039
Q9UBQ0	VPS29	0.62	0.001324058
Q7L7V1	DHX32	0.53	0.011280145
Q13630	FCL	0.73	0.007003392
Q6YHU6	THADA	0.63	0.036658849
P19388	RPAB1	0.74	0.00493459
O43488	ARK72	0.66	0.000109929
Q9Y4Y9	LSM5	0.56	3.96E-05
O75794	CD123	0.72	0.000566696
Q02750	MP2K1	0.73	0.026023631
P61225	RAP2B	0.69	0.027071059
Q9BRT3	MIEN1	0.66	0.011576686
Q9Y530	OARD1	0.73	0.016986345
Q9Y3A4	RRP7A	0.70	2.51E-06
Q9Y3A2	UTP11	0.68	0.019786505
Q3KQU3	MA7D1	0.72	0.001855263
P49427	UB2R1	0.69	0.004982145
P47895	AL1A3	0.75	0.003867407
Q9HB90	RRAGC	0.74	0.005749163
P49247	RPIA	0.75	0.003308694
O43715	TRIA1	0.68	0.018071991
Q9UBU9	NXF1	0.70	0.004578679
O00170	AIP	0.68	0.002720675
Q12872	SFSWA	0.66	0.01171719
P46779	RL28	0.51	0.000468863
Q9Y5Y2	NUBP2	0.71	0.017291267
Q99733	NP1L4	0.77	0.001008882
Q7Z7K6	CENPV	0.74	0.033936655
Q9NQ29	LUC7L	0.73	0.047621712
Q13868	EXOS2	0.77	0.020218147
Q13867	BLMH	0.74	0.001200459
Q14558	KPRA	0.65	0.000109144
O43504	LTOR5	0.66	0.005116026
O95067	CCNB2	0.70	0.040582138
Q9NVR2	INT10	0.67	0.041773348

Q9NPD3	EXOS4	0.67	0.011123125
O15260	SURF4	0.66	0.032482849
P09012	SNRPA	0.70	0.003338956
Q92530	PSMF1	0.74	0.013567897
Q13242	SRSF9	0.72	0.002361033
P18754	RCC1	0.68	0.000429509
Q9NZL4	HPBP1	0.66	0.00989372
P52657	T2AG	0.72	0.019365706
Q9NPF4	OSGEP	0.68	0.031524251
P49419	AL7A1	0.72	0.001164874
Q96MU7	YTDC1	0.71	0.006934609
P20618	PSB1	0.64	0.012211392
Q9UEE9	CFDP1	0.75	0.021345153
Q5SY16	NOL9	0.55	0.008125267
Q16881	TRXR1	0.72	0.000528569
Q96SZ6	CK5P1	0.73	0.033381773
P80297	MT1X	0.56	0.00166115
Q13595	TRA2A	0.75	0.003304538
P13807	GYS1	0.70	0.004268675
P61599	NAA20	0.58	0.023581196
Q9NYJ1	COA4	0.54	0.015950786
Q9NX24	NHP2	0.76	0.035042735
Q3B726	RPA43	0.61	0.04258264
O60216	RAD21	0.73	0.00042652
Q13416	ORC2	0.67	0.022507373
P55327	TPD52	0.72	0.000250756
Q9BZX2	UCK2	0.75	0.003168627
P62081	RS7	0.66	0.000285222
Q6IQ49	SDE2	0.73	0.020715386
Q9NVZ3	NECP2	0.74	0.022125262
O43172	PRP4	0.72	0.00396482
Q7Z7A3	CTU1	0.66	0.000639179
Q96GD0	PLPP	0.75	0.03394636
Q9UBB6	NCDN	0.68	0.006416756
Q9UBB4	ATX10	0.73	4.54E-05
P62875	RPAB5	0.57	0.000911312
P32321	DCTD	0.73	0.007589337
P62312	LSM6	0.61	0.020599613
O14737	PDCD5	0.70	0.000416028
O15127	SCAM2	0.74	0.013298442
Q9Y4P1	ATG4B	0.72	0.000732173
O60256	KPRB	0.76	0.012071803
Q86W92	LIPB1	0.76	0.004125048
Q147X3	NAA30	0.65	0.000429197
Q9UDY8	MALT1	0.50	0.015290633
P49721	PSB2	0.71	0.001121128
O60885	BRD4	0.60	0.002984293
P50583	AP4A	0.73	0.006375771
O95219	SNX4	0.74	0.026581557

Q9BTZ2	DHRS4	0.68	0.013768189
Q8IYS1	P20D2	0.71	0.000471908
O95456	PSMG1	0.58	0.002491499
Q16637	SMN	0.71	0.03789242
Q8WW22	DNJA4	0.70	0.015973897
Q16822	PCKGM	0.75	0.027021702
Q96DE0	NUD16	0.58	0.010680218
P10253	LYAG	0.66	0.000655051
Q9GZY8	MFF	0.66	0.037375655
O43665	RGS10	0.74	0.00499359
Q96IU4	ABHEB	0.70	0.032362798
Q99871	HAUS7	0.59	0.017720778
Q8N183	NDUF2	0.74	0.046881002
P62760	VISL1	0.54	0.003390251
Q1ED39	KNOP1	0.64	0.010689042
P63172	DYLT1	0.76	0.010658177
Q04323	UBXN1	0.72	0.000236521
Q9BXW7	HDHD5	0.66	0.000295301
Q9NRF9	DPOE3	0.76	0.014901983
O00560	SDCB1	0.66	0.004170277
P00441	SODC	0.69	0.00095148
Q9NYF8	BCLF1	0.69	0.002377705
O43432	IF4G3	0.74	0.047909677
Q9HCG8	CWC22	0.66	0.022875907
Q9Y6I3	EPN1	0.59	0.013565094
P61960	UFM1	0.73	0.036598395
Q9NY61	AATF	0.70	0.003932473
O95999	BCL10	0.63	0.002774085
P15529	MCP	0.62	0.004731555
P82930	RT34	0.76	0.002826097
Q13371	PHLP	0.48	0.030759024
Q9BV86	NTM1A	0.69	0.023477107
O15514	RPB4	0.61	0.03094366
Q96EK9	KTI12	0.73	0.012813345
Q9H1Y0	ATG5	0.47	0.003918764
O94763	RMP	0.68	0.005908669
P30483	1B45	0.69	0.002257514
P78318	IGBP1	0.73	0.000250002
Q9Y3B8	ORN	0.67	0.016530363
P20226	TBP	0.75	0.003847079
Q92995	UBP13	0.73	0.023514662
Q9Y512	SAM50	0.74	0.001884487
Q13144	EI2BE	0.73	0.018043865
Q58FF8	H90B2	0.63	0.00737218
Q15276	RABE1	0.67	0.001031046
Q9H6Z4	RANB3	0.77	0.000173631
Q9NP77	SSU72	0.73	0.006690338
Q86Y56	DAAF5	0.70	0.00058506
Q9H788	SH24A	0.77	0.007314715

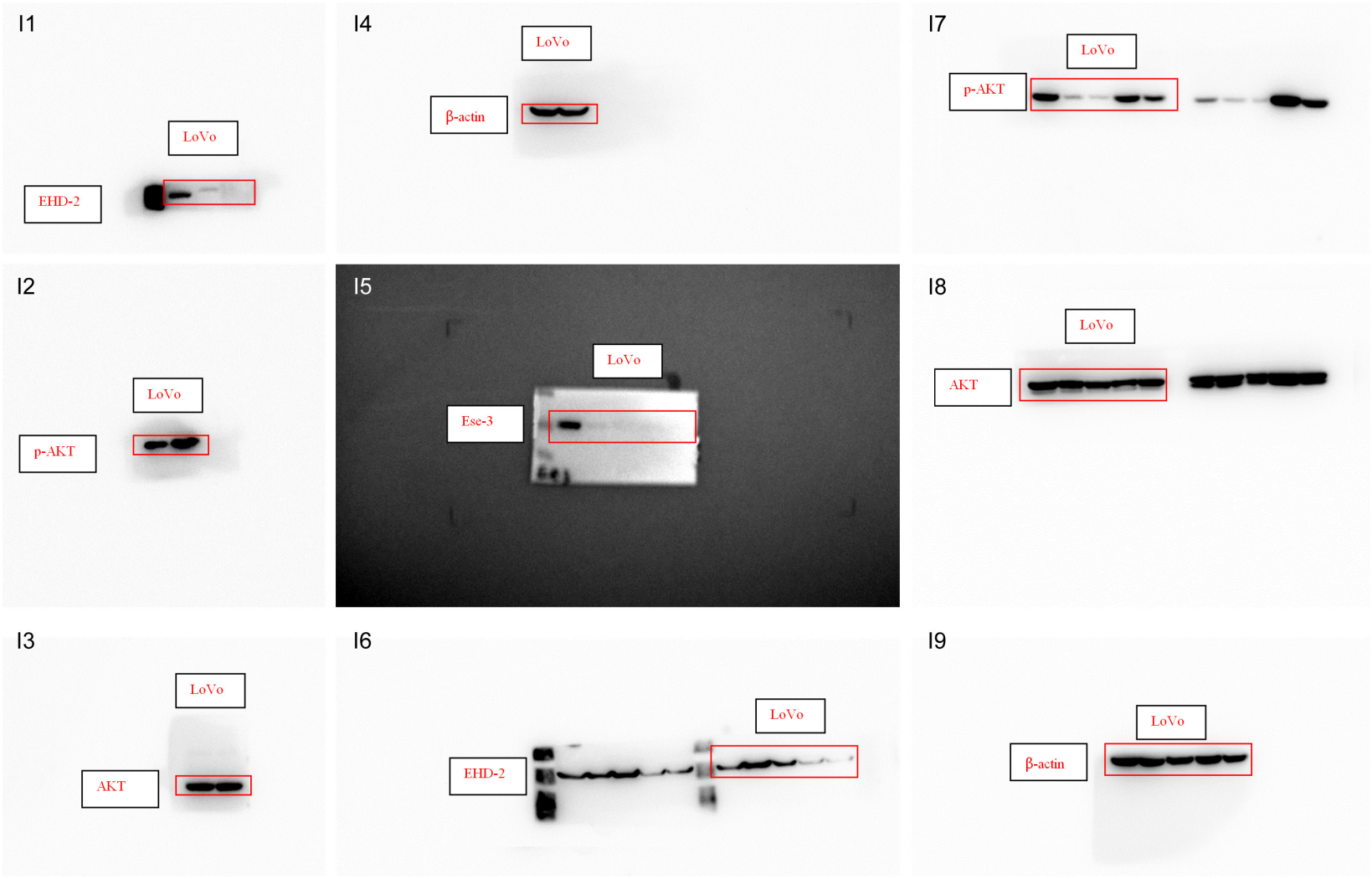
Q8TDN6	BRX1	0.75	0.002943187
P37802	TAGL2	0.76	0.004790142
Q6PK04	CC137	0.70	0.003073137
Q04760	LGUL	0.74	0.000183242
P46976	GLYG	0.74	0.00195066
P55210	CASP7	0.54	0.011729914
P00918	CAH2	0.67	0.002615756
P0DPI2	GAL3A	0.77	0.033773659
Q3KQV9	UAP1L	0.77	0.035821021
Q8IYB3	SRRM1	0.74	0.007405979
P23511	NFYA	0.74	0.035158694
Q9NPA8	ENY2	0.73	0.003651889
Q16718	NDUA5	0.67	0.034269339
Q9NRL3	STRN4	0.67	0.00334836
Q3YEC7	RABL6	0.66	0.048303505
Q96L92	SNX27	0.76	0.007327339
Q6NZY4	ZCHC8	0.68	0.010755862
P49005	DPOD2	0.71	0.014517056
P49006	MRP	0.56	0.002212027
Q9Y3D8	KAD6	0.68	0.001251625
Q9BVP2	GNL3	0.70	0.000877982
P11279	LAMP1	0.42	0.025872343
Q9UBV8	PEF1	0.64	0.000404678
Q9BQI0	AIF1L	0.65	0.040512654
P30405	PPIF	0.63	0.00416498
P12532	KCRU	0.67	0.005152131
Q86Y37	CACL1	0.70	0.000977434

Supplementary Table 7. The mRNA expression levels of Ese-3, EHD2, INPP4B in prospective_CPTAC_COAD samples

Patients ID	Ese-3 expression levels	EHD2 expression levels	INPP4B expression levels
X01C0001	14.5834354	7.592457037	10.30378075
X01C0005	13.94416299	8.174925683	9.479780264
X01C0006	13.81778312	8.495855027	10.29347165
X01C0008	14.4166656	6.686500527	10.59058705
X01C0013	14.4103851	8.118941073	9.419960178
X01C0014	13.6263935	6.189824559	9.411510988
X01C0015	12.68474862	6.209453366	9.162391329
X01C0019	14.01113988	7.400879436	8.918863237
X01C0022	13.813681	5.977279923	8.658211483
X05C0002	12.76942464	10.61562964	10.77396337
X05C0003	13.48381578	9.011227255	9.328674927
X05C0005	13.21916852	5.882643049	10.55842071
X05C0006	14.06743436	7.139551352	9.154818109
X05C0007	13.16537811	10.39016896	9.368506462
X05C0011	10.40194612	8.491853096	9.573647187
X05C0014	13.72195361	8.62935662	8.971543554
X05C0015	12.82057788	9.022367813	9.924812504
X05C0020	13.51199967	9.243173983	8.370687407

X05C0026	13.54351522	8.535275377	8.45532722
X05C0028	13.41587378	4.169925001	10.29001885
X05C0029	14.07531201	5.392317423	10.0768156
X05C0032	12.72131284	5.807354922	8.636624621
X05C0033	12.80735492	7.562242424	9.840777924
X05C0034	12.00807841	7.894817763	9.599912842
X05C0035	13.14402087	8.87958325	9.465566405
X05C0037	13.6263935	8.527477006	8.794415866
X05C0039	13.24362029	5.727920455	9.45532722
X05C0041	13.11585648	7.592457037	9.83447105
X05C0044	13.07564627	9.847057346	7.442943496
X05C0045	13.24198315	6.14974712	10.15481811
X05C0047	12.70908381	7.055282436	10.1382718
X05C0048	12.50605959	8.434628228	8.731319031
X05C0049	13.46416282	8.290018847	10.05799172
X05C0050	11.53964351	6.169925001	7.483815777
X05C0053	14.19644844	5.672425342	10.02652344
X05C0054	13.60733031	6	9.618385502
X05C0055	12.74965996	7.055282436	9.434628228
X06C0001	11.72621816	7.483815777	8.787902559
X06C0002	13.96018298	8.515699838	10.95274125
X09C0005	13.36454508	7.451211112	10.10066234
X09C0006	14.06600508	5.727920455	10.77971936
X09C0008	13.54737656	7.787902559	8.845490051
X09C0011	14.03943317	7.339850003	10.1176431
X09C0013	12.66378045	7.864186145	9.049848549
X09C0014	12.78606538	8.199672345	8.668884984
X09C0015	13.03411115	7.599912842	8.810571635
X09C0018	14.13995098	5.491853096	10.42836017
X09C0019	13.08613623	5.977279923	9.638435914
X09C0022	14.08480839	5.459431619	9.036173613
X11C0005	12.41494944	9.50779464	9.539158811
X11C0007	13.39030333	5.614709844	8.758223215
X11C0008	12.27262978	6.965784285	9.483815777
X11C0010	12.38936246	9.06608919	9.5980525
X11C0018	12.52991853	10.27612441	8.700439718
X11C0019	12.39446269	9.216745858	9.224001674
X11C0020	14.84534303	8.011227255	9.586839788
X11C0021	12.17023805	9.873444113	8.939579214
X11C0022	13.71392345	7.614709844	9.773139207
X11C0027	13.61907365	5.523561956	9.434628228
X11C0030	12.56319627	7.826548487	10.17617315
X11C0031	13.39124359	4.247927513	9.682994584
X11C0032	13.18843431	7.754887502	9.550746785
X11C0033	12.8591465	8.233619677	8.625708843
X11C0036	13.08015131	7.247927513	7.807354922
X11C0037	13.46786024	7.781359714	9.477758266
X11C0039	13.06204614	5.392317423	9.717676423
X11C0042	12.93073734	8.422064766	10.12670447

X11C0043	12.74462364	7.971543554	8.816983623
X11C0044	13.33161677	8.727920455	8.375039431
X11C0045	12.83664215	9.930737338	9.169925001
X11C0047	14.03118426	9.442943496	8.647458426
X11C0048	12.40407714	10.28655776	9.315149562
X11C0051	13.57672053	8.388017285	9.184875343
X11C0052	13.46518374	6.741466986	9.539158811
X11C0053	13.11634396	7.139551352	8.761551232
X11C0054	12.89500718	7.312882955	8.46760555
X11C0057	13.30349538	8.194756854	9.836050355
X11C0058	12.77148947	8.539158811	9.548821908
X11C0059	12.88436134	7.721099189	9.041659152
X11C0060	13.48896984	6.357552005	9.73470962
X11C0061	13.30691611	5	8.73470962
X11C0062	13.73830341	9.379378367	9.164906927
X11C0070	13.46199095	8.027905997	9.177419538
X11C0072	13.68693817	7.894817763	8.451211112
X11C0077	12.05256805	8.936637939	8.960001932
X11C0079	12.72940832	8.214319121	9.394462695
X14C0002	13.83575437	4.95419631	9.247927513
X14C0003	13.21234439	9.154818109	9.390168956
X14C0005	12.59339112	6.95419631	9.818582177
X15C0001	14.0454184	9.162391329	7.832890014
X15C0002	13.16600612	8.531381461	9.381542951
X16C0002	12.17492568	7.614709844	9.605479518
X16C0003	13.47560678	7.936637939	10.14465824
X16C0006	12.33091688	7.64385619	9.071462363
X16C0011	12.77396337	8.632995197	9.159871337
X16C0012	13.33803993	5.977279923	9.754887502
X20C0001	12.79014483	8.894817763	10.60455323
X20C0003	12.73089465	7.375039431	10.41679753
X20C0004	13.46607646	6.95419631	9.622051819
X20C0007	14.14664822	5.781359714	9.988684687
X21C0006	14.01828713	7.499845887	9.569855608
X21C0007	13.00123225	7.022367813	9.548821908
X22C0004	13.67364034	5.882643049	9.887220615
X22C0006	13.30848124	8.724513853	10.31854281
X24C0005	11.15671514	6.584962501	9.328674927
X27C0004	13.83526092	8.409390936	9.804131021



Supplementary Figure 11. The indicated protein expression levels in Colon cancer cells. I1-I4. EHD2, p-AKT, AKT, β-actin expression levels in Ese-3 silenced LoVo cells and control cells. I5-I9. Ese-3, EHD2, p-AKT, AKT, β-actin expression levels in Ese-3 silenced LoVo cells and Ese-3 plus EHD2 silenced LoVo cells and control cells.