

## Commentary

# Commentary on: The actin bundling activity of ITPKA mainly accounts for its migration-promoting effect in lung cancer cells

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1,4,5-triphosphate 3-kinase A (ITPKA) was first described and characterized by Irvine *et al.* in 1986 and cloned by Takazawa *et al.* in 1990. It is one of the components of the Ca<sup>2+</sup> and calmodulin signaling pathway and a substrate for cAMP-dependent kinase (PKA) and protein kinase C (PKC), and is mainly involved in the regulation of intracellular inositol polyphosphate signaling molecules. Through a series of studies, Sabine's team has found that ITPKA expression was up-regulated in a variety of cancer cells, and silencing ITPKA inhibited while overexpressing ITPKA promoted cancer cell migration *in vitro* and metastasis *in vivo*. The latest research from Sabine's team has demonstrated that in H1299 lung cancer cells, the mechanism by which ITPKA promoted migration and invasion was predominantly depending on the ability of binding to F-actin, which will induce cancer cells to form a tight flexible actin networks. Small molecule compounds targeting the IP<sub>3</sub> kinase activity of ITPKA protein may only inhibit the migration and invasion of cancer cells caused by the enhanced ITPKA kinase activity under ATP stimulation, but not the cytoskeletal remodeling caused by the binding of ITPKA protein to F-actin and the driven migration and invasion of cancer cells. Therefore, targeted therapeutic strategy focusing on blocking the binding of ITPKA to F-actin is indispensable when designing the inhibitors targeting ITPKA protein.

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. The pathological classification of lung cancer primarily comprises two types: non-small cell lung cancer, which includes lung adenocarcinoma and lung squamous cell carcinoma and accounts for 75% of cases, and small cell lung cancer, which constitutes the mainly remaining cases [2]. In the recent years, with the development of high-throughput sequencing and targeted drugs, the prognosis of lung cancer patients has significantly improved [1]. However, pathological subtypes of lung cancer, such as squamous cell carcinoma and small cell lung cancer, remain lack of mutation sites and corresponding target, which significantly affects the outcome and survival of patients [3]. When tumor cells are not responsive to chemotherapeutic drugs, they are inclined to metastasize throughout the body, ultimately resulting in death due to multiple organ failure [4]. This finding prompts us to further investigate additional targets for drug action and enhance the scope of clinical therapeutic alternatives.

The prime targets of commonly used drugs in the clinical treatment of lung cancer function the anti-tumor role by blocking growth signals, inhibiting core signaling pathways, damaging DNA, inhibiting key metabolic enzymes, blocking the cell cycle, remodeling the microenvironment, destroying abnormal angiogenesis, and relieving immunosuppression [5–7]. However, the drugs targeting the movement of cancer cells seems to be scant. The underlying reasons include the absence of specific mechanisms explaining the movement of cancer cells, extensive crosstalk with the molecular network contributing to cell

Received: 12 April 2023  
Revised: 24 August 2023  
Accepted: 01 September 2023

Accepted Manuscript online:  
04 September 2023  
Version of Record published:  
11 September 2023

movement, and the lack of a clear pharmacodynamic hub for the skeleton proteins involved in cell movement. Therefore, in-depth understanding and investigation of the mechanism of cancer cell movement regulation is of great necessity for the identification of potential therapeutic targets.

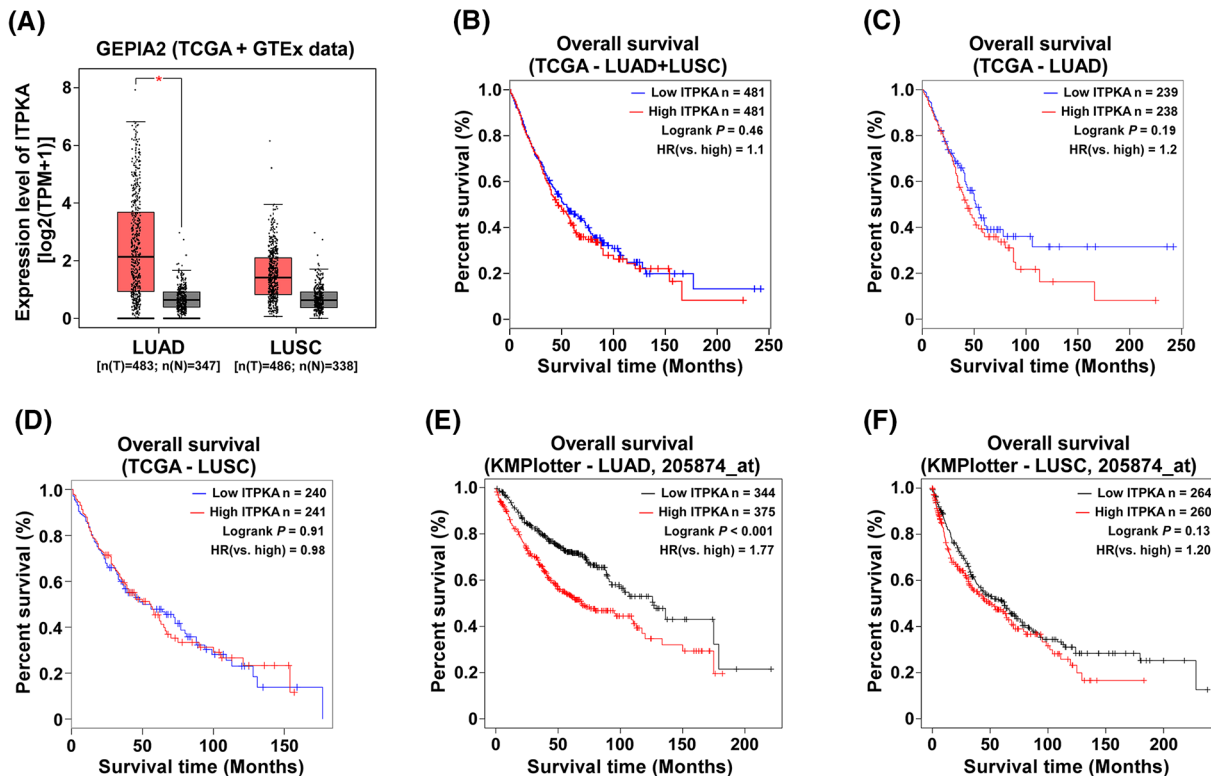
Cytoskeletal proteins are mainly divided into three categories: microtubules, microfilaments and intermediate fibers, and the basis of cell motility is the polymerization and depolymerization of cytoskeletal proteins [8]. Actin is the structural protein of microfilaments, and the monomer is called globular actin (G-actin) and the polymer is called filamentous actin (F-actin) [9]. G-actin can polymerize into F-actin under ATP supply and  $Mg^{2+}$  catalysis, while F-actin can spontaneously depolymerize under  $Ca^{2+}$  catalysis [9]. Therefore, G-actin and F-actin are in a dynamic process of polymerization and depolymerization in normal biological process, which is pivotal for the formation or degradation of microfilaments in the specific region [8,9]. The actin cytoskeleton is extensively involved in a multitude of cellular processes, including the positioning of intracellular organelles, cytoplasmic division, establishment of cell polarity, formation of pseudopods and adherent patches [10]. Moreover, it is closely associated with the division and motility of cancer cells and presents a potential therapeutic target.

The dynamic process of microfilament polymerization and depolymerization is influenced by calcium signals, so inositol 1,4,5-trisphosphate ( $IP_3$ ), a second messenger molecule capable of mobilizing  $Ca^{2+}$  release from the endoplasmic reticulum, is one of the key regulatory molecules [11]. The extracellular signaling molecule binds to the corresponding G protein-coupled receptor and transmits the activation signal to phospholipase C $\beta$  (PLC $\beta$ ) via the activated q type of G $\alpha$  subunit (G $\alpha_q$ ) [12]. PLC $\beta$  divides phosphatidyl-inositol-4,5-bisphosphate (PIP $_2$ ) into two intracellular second messengers: diacylglycerol (DAG) and  $IP_3$  are subsequently activating protein kinase C (PKC) and  $IP_3$  receptors, respectively [13]. The  $IP_3$  receptor is located on the endoplasmic reticulum (ER), and activates its coupled ligand-gated calcium channels by binding to  $IP_3$ , which further triggers the release of large amounts of  $Ca^{2+}$  into the cytoplasm and gives rise to the inward flow of extracellular  $Ca^{2+}$  [14,15].

The 1,4,5-triphosphate 3-kinase A (ITPKA) is one of the components of the  $Ca^{2+}$  and calmodulin signaling pathway and a substrate for cAMP-dependent kinase (PKA) and PKC, and is mainly involved in the regulation of intracellular inositol polyphosphate signaling molecules [16,17]. ITPKA can phosphorylate  $IP_3$  to inositol 1,3,4,5-trisphosphate ( $IP_4$ ), which can be further phosphorylated to inositol 1,3,4,5,6-pentakisphosphate ( $IP_5$ ) by inositol polyphosphate multikinase (IPMK) or dephosphorylated to inositol 1,4-bisphosphate ( $IP_2$ ) by inositol polyphosphate-4-phosphatase (INPP4) [16,18,19]. Since the affinity of INPP4 for  $IP_4$  is approximately 10-fold higher than that of  $IP_3$ , the increased concentration of  $IP_4$  will protect  $IP_3$  from dephosphorylation, where the duration and concentration of  $IP_3$ -mediated  $Ca^{2+}$  signaling depends on the ITPKA/INPP4 activity ratio [16,19,20].

Through a series of studies [16,21–23], Sabine's team found that ITPKA expression was up-regulated in a variety of cancer cells, and silencing ITPKA inhibited cancer cell migration *in vitro* and metastasis *in vivo*, while over-expressing ITPKA promoted these processes. By comparing cancer tissues from different sources, Sabine's team found that ITPKA expression was higher in lung adenocarcinoma, correlated with poor clinical and pathological staging in patients, and predicted poor survival outcomes in these patients [23], all of which could be verified in online open databases (Figure 1). Relevant mechanistic studies have shown that ITPKA promoted cancer cell migration through two different mechanisms: (1) without growth factor stimulation, highly expressed ITPKA binds to F-actin through the N-terminal actin-binding domain (ABD), increasing the polymerization level of cellular F-actin to stabilize microfilaments and subsequently promoting formation of large cellular protrusions [21]. (2) Under growth factor stimulation, ITPKA in cells can increase  $IP_4$  content through enzymatic activity, protect  $IP_3$  from dephosphorylation, enhance the duration and concentration of  $Ca^{2+}$  signaling mediated by  $IP_3$ , and thus enhance ITPKA-induced migration [21]. Also, Sabine's team has found that impaired repressor-element-1-silencing transcription factor (REST)/neuron-restrictive silencer factor (NRSF) repression of gene expression function in cancer cells relieved the state of ITPKA expression repression, leading to its aberrant high expression in cancer cells [22]. In addition, Sabine's team examined ITPKA expression in a variety of solid tumor tissues and cells and found that ITPKA expression was most significantly up-regulated in lung cancer and further highly expressed in metastatic lymph node, suggesting that ITPKA may be a potential target for anti-metastasis therapy against lung cancer [23].

The mutation rate of ITPKA is low, approximately 1.1%, in pan-cancer tissue (Figure 2A). Mutation types include missense and truncating variants, with the majority localized to the IPK region (Figure 2B). The ITPKA gene, comprising seven exons, is situated on the 15th chromosome, and it spans a length of 1825 nucleotides, with a coding sequence that initiates at position 55 and concludes at position 1440 (Figure 2B). There are 461 amino acid (aa) in ITPKA and aa 245–455 belongs to the IPA region, and Lys264 plays a critical role in the catalytic mechanism of  $IP_3$ -3K (Figure 2B,C). This residue interacts with the ligands in the substrate complex and the 3-phosphate in the product complex, potentially aiding the nucleophilic attack of the phosphate by neutralizing the transition state's negative charge and directing the phosphate group transfer (Figure 2D). This function is analogous to Lys168 in PKA

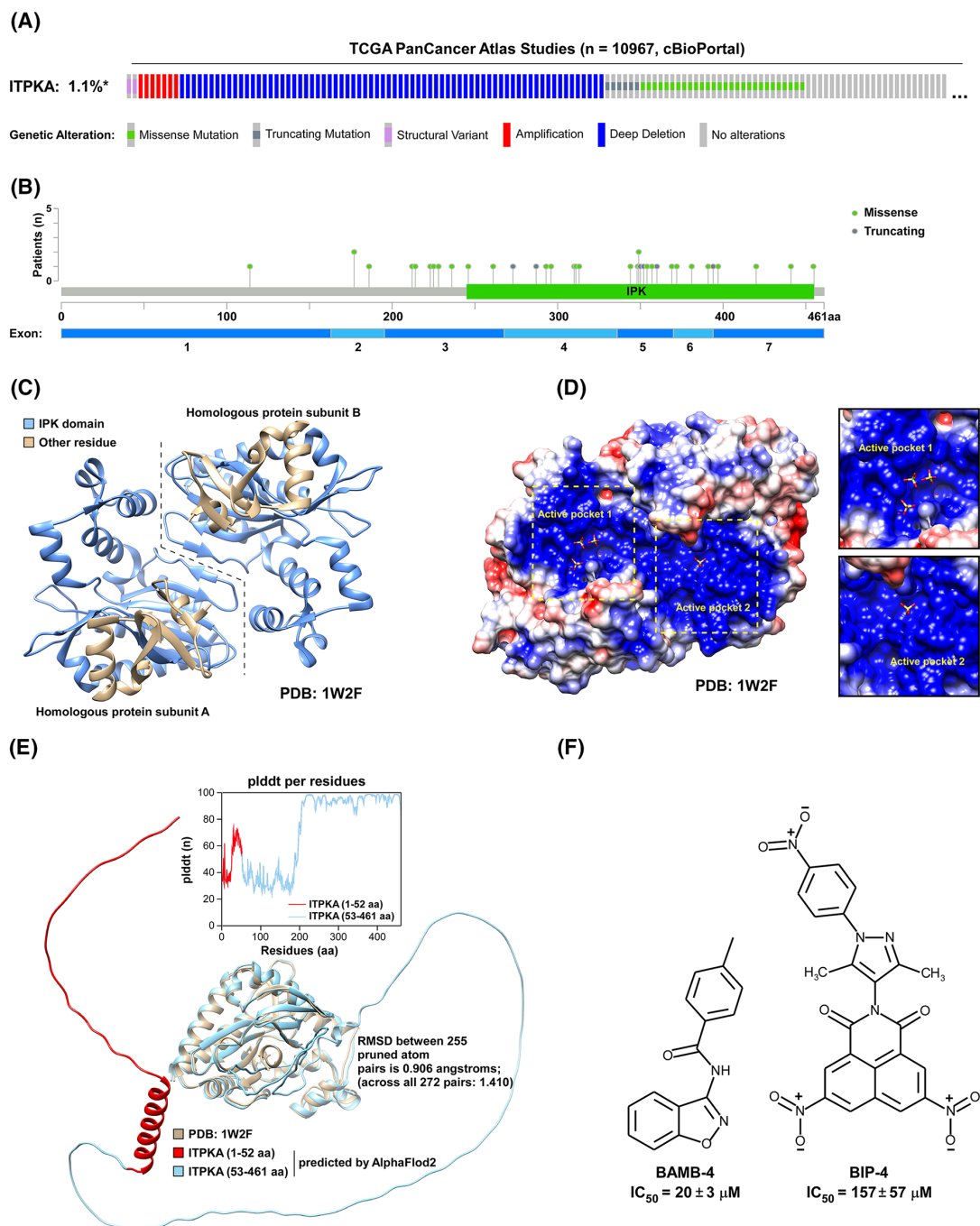


**Figure 1.** Online open databases have shown that ITPKA is highly expressed in lung adenocarcinoma tissues and predicts a worse prognosis for patients

(A) Compared with normal lung tissue (N), ITPKA expression was increased in lung adenocarcinoma (LUAD), but not significantly up-regulated in lung squamous cell carcinoma (LUSC). (B–D) In the TCGA database, high expression of ITPKA in LUAD predicted poor survival prognosis of patients (C), while there was no trend in overall lung cancer (B) or LUSC (D). (E,F) In the KMplotter database, high ITPKA expression in LUAD predicted poor prognosis in patients (E), while there was no correlation in LUSC (F). The data in (A–D) come from GEPIA2 (<http://gepia2.cancer-pku.cn/>) and the data in (E,F) come from Kaplan–Meier Plotter (<http://kmplot.com/>).

[24]. PKA also possesses another crucial catalytic residue, Asp166, which forms a tighter interaction with the serine hydroxyl. Asp166 primarily functions to choose the appropriate stereochemical isomer of the serine hydroxyl group in the substrate, thus enabling and enhancing the nucleophilic attack by the hydroxyl group [24]. Previous research teams claim that the N-terminal domain aa 1–52 of ITPKA binds actin filaments [25]. AlphaFold2 was used to predicted the 3D structure of the full length of ITPKA. Residues 1–197 constitute a flexible disordered domain with no fixed structure, characterized by lower pLDDT scores (Figure 2E). However, residues 31–50 are capable of forming an alpha helix. To confirm the existence of this structure and its involvement in actin binding, further experimental validation by the research team is required.

The Sabine research team conducted a high-throughput screen of 341,440 small molecule compounds using an ADP-Glo assay to identify potential inhibitors of ITPKA [26]. The initial screen yielded 237 hit compounds exhibiting inhibitory activity. The inhibitory effects of MEPTT-3 and BAMB-4 on ITPKA activity were validated using an orthogonal assay (coupled PK/LDH optical assay). The results showed that MEPTT-3 and BAMB-4 had IC<sub>50</sub> values of 35 ± 4 μM and 20 ± 3 μM, respectively. Cellular uptake experiments showed that BAMB-4 could be completely absorbed by lung cancer H1299 cells and remained stable after cellular uptake. The above evidence indicates that BAMB-4 is a druggable inhibitor of ITPKA [26]. To gain a deeper understanding of BAMB-4, its mechanism of inhibition and kinetic parameters were investigated. It was revealed that BAMB-4 acts as a mixed-type inhibitor, affecting both ATP and IP<sub>3</sub> binding. During their investigation, BAMB-4 demonstrated an IC<sub>50</sub> value of 20 μM for inhibiting InsP<sub>3</sub> kinase activity. In another research from the Sabine team [27], they found a novel InsP<sub>3</sub> kinase inhibitor, named BIP-4, with an IC<sub>50</sub> of 157 ± 57 nM, exhibiting stronger inhibitory activity compared with the previous inhibitor BAMB-4. In contrast, BIP-4 competitively obstructs InsP<sub>3</sub> binding to ITPKA but does not interfere with ATP binding. It occupies the binding site, overlapping with InsP<sub>3</sub>, resulting in selective inhibition. BIP-4 enters cells



**Figure 2. The gene and protein structures of ITPKA**

(A) Based on 10,967 Pancancer Atlas studies in the TCGA database within cBioPortal, the altered/profiled rate of ITPKA is 115/10,950, which corresponds to 1.1%. (B) The ITPKA gene consists of seven exons, with missense and truncating mutations being the most prevalent types. These mutations primarily occur in the IPK region. (C) ITPKA functions as a homodimer, where the IPK domain is represented in blue, and the remaining portions are shown in yellow. The PDB (1W2F) structure spans amino acids 185–461, and homologous subunits are indicated by dashed lines. (D) The colored surface represents the electrostatic potential, where red indicates a negative charge and blue indicates a positive charge. The two active pocket domains, which are positively charged, interact with ligands. (E) The structure of ITPKA predicted by AlphaFold2 (AF) (cyan) aligns well with the structure from the Protein Data Bank (yellow). The AF structure reveals that amino acids 1–185 form a flexible loop with a low plddt value. Additionally, the Schröder team found that amino acids 1–52 (red) bind to the actin filament, and there is a suggestion that amino acids 31–50 form an alpha helix. (F) The Sabine team discovered two compounds that act as inhibitors for ITPKA. Among these, BIP-4 demonstrated a better inhibitory effect with an IC<sub>50</sub> value of 157 ± 57 μM compared with BAMB-4.

through non-endocytic mechanisms and is capable of inhibiting ITPKA within the cellular environment. However, clinical trials of the aforementioned compounds have not been observed, and their pharmaceutical properties still need further evaluation.

When studying the specific molecular mechanism of ITPKA and F-actin interaction, Sabine's team further found that ITPKA not only binds to F-actin through the N-terminal ABD but also that the C-terminal IP<sub>3</sub> kinase domain is involved in the regulation of F-actin arrangement. This enables ITPKA to form tight and flexible actin networks and facilitates the formation of cellular pseudopods [28]. This finding supported the notion that the mechanism underlying the ITPKA regulation of cancer cell migration is a multi-pathway and multi-levels process, and the effect of related mechanisms on cancer cell metastasis needs to be literally investigated. To elucidate those mechanisms involved, Sabine's team performed the following experimental treatments in their latest research paper published in *Bioscience Reports* [19]: (1) inhibited the actin-binding regulatory activity of endogenous ITPKA using an ITPKA mutant overexpressing an interaction structural domain deletion (ITPKA<sup>L34P</sup>); (2) inhibited the actin-binding regulatory activity of endogenous ITPKA using an ITPKA mutant overexpressing an IP<sub>3</sub> catalytic activity loss ITPKA mutant (ITPKA<sup>D416N</sup>) or addition of IP<sub>3</sub> kinase inhibitor GNF362, to observe the alteration of IP<sub>3</sub> catalytic activity of ITPKA on the migration and invasion ability of lung cancer cells under normal or ATP-stimulated conditions.

In the present study [19], Sabine's team observed two important results: (1) Overexpression of ITPKA did not increase the migration and invasion of lung cancer H1299 cells under normal culture conditions; however, overexpression of ITPKA<sup>L34P</sup> significantly inhibited the migration of cancer cells and decreased the motility of filamentous pseudopods. (2) Overexpression of ITPKA slightly increased the migratory ability of cancer cells under ATP stimulation, but overexpression of ITPKA<sup>D416N</sup> had no significant effect, while GNF362 inhibited the migratory ability of cancer cells increased by ITPKA.

In conclusion, the data from Sabine's team suggested that in H1299 lung cancer cells, the mechanism by which ITPKA promoted migration and invasion was predominantly depend on the ability of binding to F-actin, which induces cancer cells to form a tight flexible actin networks [19]. Small molecule compounds targeting the IP<sub>3</sub> kinase activity of ITPKA protein may only inhibit the migration and invasion of cancer cells caused by the enhanced ITPKA kinase activity under ATP stimulation, but not the cytoskeletal remodeling caused by the binding of ITPKA protein to F-actin and the driven migration and invasion of cancer cells [19]. Therefore, the most important implication of the present study for subsequent research is that targeted therapeutic strategy focusing on blocking the binding of ITPKA to F-actin is indispensable when designing the inhibitors targeting ITPKA protein.

Overall, the findings in the present study from Sabine's team exhibit excellent sustainability. In the meantime, the present study offers novel scientific ideas for the development of ITPKA inhibitors.

## Data Availability

Online open databases were not generated nor owned by the authors and are available online. RNA expression levels of ITPKA in lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) and their correlation with survival and prognosis were obtained from database GEPIA2 (<http://gepia2.cancer-pku.cn/>) and Kaplan–Meier Plotter (<http://kmplot.com/>), respectively. Mutation information is sourced from the TCGA PanCancer Atlas Studies within cBioPortal (<https://www.cbioportal.org/>). The 3D crystal structure, identified by PDB ID 1w2f, originates from the Protein Data Bank. AlphaFold2 (Colab version) was utilized to predict ITPKA's complete 3D structure [29,30]. The electrostatic surface was calculated using DelPhi and visualized in UCSF Chimera [31,32]. All relevant data are provided within the manuscript, figures and supplementary data. Other data used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

## Funding

This work was supported by the grants from the National Natural Science Foundation of China [grant numbers 81802918 and 82173284]; China Postdoctoral Science Foundation Grant [grant number 2019M660206]; Science and Technology Project of Guangdong Province [grant numbers 2019A1515011565, 2018A030310007, and 2022A1515010055]; and Science and Technology Project of Jiangmen [grant numbers 2020030103140008978 and 2019030102430012905]. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

## CRediT Author Contribution

**Xin Zhang:** Conceptualization, Formal analysis, Supervision, Methodology, Writing—original draft, Project administration, Writing—review & editing. **Jiadi He:** Data curation, Formal analysis, Writing—review & editing. **Dong Ren:** Data curation, Investigation, Writing—original draft, Project administration, Writing—review & editing.

## Abbreviations

ABD, actin-binding domain; AF, AlphaFold2; DAG, diacylglycerol; ER, endoplasmic reticulum; F-actin, filamentous actin; G-actin, globular actin; G $\alpha$ q, q type of G protein  $\alpha$  subunit; INPP4, inositol polyphosphate-4-phosphatase; IP<sub>2</sub>, inositol 1,4-bisphosphate; IP<sub>3</sub>, inositol 1,4,5-trisphosphate; IP<sub>4</sub>, inositol 1,3,4,5-trisphosphate; IP<sub>5</sub>, 1,3,4,5,6-pentakisphosphate; IPMK, inositol polyphosphate multikinase; NRSF, neuron-restrictive silencer factor; PDB, Protein Data Bank; PIP<sub>2</sub>, phosphatidyl-inositol-4,5-bisphosphate; PKA, cAMP-dependent kinase; PKC, protein kinase C; PLC $\beta$ , phospholipase C $\beta$ ; REST, repressor-element-1-silencing transcription factor; TCGA, The Cancer Genome Atlas.

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