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REVIEW

STRIPAK complexes in cell signaling and cancer

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Striatin-interacting phosphatase and kinase (STRIPAK) complexes are striatin-centered multicomponent supramolecular structures containing both kinases and phosphatases. STRIPAK complexes are evolutionarily conserved and have critical roles in protein (de) phosphorylation. Recent studies indicate that STRIPAK complexes are emerging mediators and regulators of multiple vital signaling pathways including Hippo, MAPK (mitogen-activated protein kinase), nuclear receptor and cytoskeleton remodeling. Different types of STRIPAK complexes are extensively involved in a variety of fundamental biological processes ranging from cell growth, differentiation, proliferation and apoptosis to metabolism, immune regulation and tumorigenesis. Growing evidence correlates dysregulation of STRIPAK complexes with human diseases including cancer. In this review, we summarize the current understanding of the assembly and functions of STRIPAK complexes, with a special focus on cell signaling and cancer.

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INTRODUCTION

Recent proteomic studies identified a group of novel multi-component complexes named striatin (STRN)-interacting phosphatase and kinase (STRIPAK).[1–3] STRIPAK complexes are evolutionarily conserved from fungi to human, and are involved in a variety of critical cellular process.[4] Loss of function or dysregulation of STRIPAK components has been increasingly linked to many human diseases including cancer. In mammalian STRIPAK complexes, the STRN family proteins as B‴ regulatory subunits of serine/threonine-protein phosphatase 2A (PP2A) recruit PP2A catalytic subunit (C subunit, PP2Ac) via PP2A scaffold subunit (A subunit, PP2Aa) on the one hand, and members of germinal center kinase (GCK) family via adaptor molecules including cerebral cavernous malformations 3 (CCM3, also named PDCD10 and TFAR15) on the other hand (Figure 1a). Other major components of STRIPAK complexes include STRN-interacting protein 1/2 (STRIP1/2, also named FAM40A/B), sarcolemmal membrane-associated protein (SLMAP), tumor necrosis factor receptor-associated factor 3 (TRAF3)-interacting protein 3 (TRAF3IP3, also known as T3JAM), suppressor of IKBKE 1 (SIKE1), fibroblast growth factor receptor 1 (FGFR1) oncogene partner 2 (FGFR1OP2), cortactin-binding protein 2 (CTTNBP2), CTTNBP2 N-terminal-like protein (CTTNBP2NL) and MOB4 (also known as phocein, MOB3 and MOBKL3). SLMAP/TRAF3IP3 – SIKE1/FGFR1OP2 and CTTNBP2/CTTNBP2NL may form mutually exclusive complexes with STRNs via STRIP1/2.[2] In this review, we summarize major components of STRIPAK complexes, as well as the topology and assembly of these complexes, and then focus on frontier advances regarding their function and mechanism mainly in a context of cell signaling and cancer.

MAJOR COMPONENTS AND ARCHITECTURE OF STRIPAK COMPLEXES

STRNs and PP2A

The human STRN family of proteins includes STRN, STRN3 (also named SG2NA) and STRN4 (also named zinedin). STRN is enriched in the central nervous system and STRN4 is mostly abundant in the brain and lung, whereas STRN3 is ubiquitously expressed in almost all tissues.[5–9] STRNs share a conserved caveolin (CAV)-binding region, a coiled-coil domain, a calmodulin-binding region, a CCM3-binding region and a C-terminal WD40-repeat domain (Figure 1b).[5,9–12] The CAV-binding domain of STRNs can directly interact with the structural components of caveolae CAVs, which regulate signal transduction via binding to signaling molecules such as endothelial nitric oxide synthase (eNOS).[13,14] STRNs might be recruited to membrane compartments by CAVs and engaged in the modulation of nuclear receptors (NRs).[15] The calmodulin-binding region of STRNs can directly interact with calmodulin in a calcium-dependent manner.[5,16] Downregulation of STRN impairs the growth of dendrites in cultured motoneurons.[17] As dendritic spines are abundant in calcium, it is likely that STRNs are involved in the regulation of dendritic calcium signaling and the development of neuron. The CCM3-binding region of STRNs can directly interact with CCM3, a binding partner of GCKIII kinases. The WD40-repeat domain of STRNs contain seven WD40 repeats, which mainly mediate protein–protein interactions.[18] Potential binding partners for the WD40-repeat domain of STRNs include MOB4 and adenomatous polyposis coli protein.[19,20] STRN and adenomatous polyposis coli, a multifunctional protein governing cellular behavior and function,[21] colocalized at cell–cell tight junctions in epithelial cells and their colocalization are dependent on each other.[20]

PP2A holoenzyme is a heterotrimeric complex composed of a catalytic subunit (PP2Ac), a scaffold subunit (PP2Aa) and a regulatory subunit.[22] The PP2Aa subunit mediates the interaction between PP2Ac and various regulatory subunits that determine substrate specificity. The regulatory subunits can be divided into four families: B (also known as B55 or PR55), B′ (also known as B56 or PR61), B″ (including PR48/PR72/PR130/G5PR) and B‴ (also known as PR93/PR110). STRNs were identified as B‴ subunits of PP2A, which could activate PP2Aa/c to dephosphorylate histone H1.[23] The coiled-coil domain of STRNs forms an asymmetric homodimer that directly binds to two PP2Aa subunits

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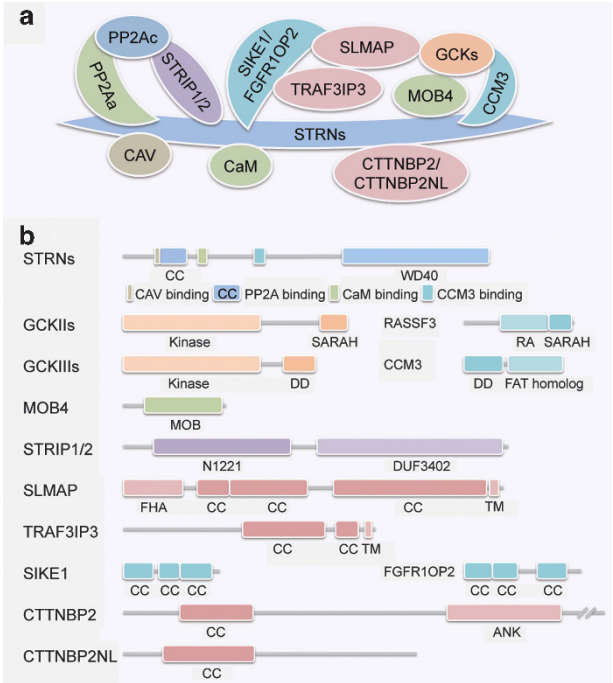

Figure 1. (**a**) A proposed model for the architecture of STRIPAK complexes. STRN proteins as a center mediate the association of phosphatase PP2A and GCK kinases via a series of adaptor proteins. (**b**) Domain organization of STRIPAK components. Abbreviations: ANK, ankyrin repeats; CaM, calmodulin; CC, coiled coil; DD, dimerization domain; FAT, focal adhesion targeting.

in a side-by-side manner.[12,19,24] Thus, STRNs can form a PP2A holoenzyme with PP2Aa and PP2Ac, with a stoichiometry of 2:2:2 (PP2Aa:PP2Ac:STRNs).

GCKII kinases and RASSFs

The GCKII kinases are key components of the Hippo pathway and have important roles in development and tissue homeostasis.[25] Mammalian GCKII subfamily contains two kinases: MST1 (also named STK4) and MST2 (also named STK3). Both MST1 and MST2 contain a highly conserved N-terminal kinase domain and a C-terminal SARAH (Sav/Rassf/Hpo) domain that is also shared by salvador homolog 1 (SAV1, also known as WW45) and Ras association domain-containing protein (RASSF) 1–6 (Figure 1b). The SARAH domain mediates homo- or heterodimerization of MST1/2, SAV1 and RASSFs.[26] SAV1 can bind to MST1/2 to facilitate the activation of Hippo signaling.

The RASSF family contains 10 members, namely RASSF1–10, all of which have a conserved Ras association domain.[27] Unlike RASSF1–6, RASSF7–10 are named N-terminal RASSF proteins because of the lack of a SARAH domain.[28] Most RASSFs function as tumor suppressors that inhibit cell growth and promote apoptosis. In multiple tumors, methylation of the promoter regions were observed for RASSFs with their expression decreased. RASSF1, RASSF5 and RASSF6 suppress MST1 auto-phosphorylation and RASSF5 may recruit MST1 to the plasma membrane during Ras-induced apoptosis.[29–31] Meanwhile, RASSF1A may stabilize MST1/2 and enhance their activities to promote Fas ligation-induced apoptosis or regulate mitosis.[32–34] RASSF2 is also reported to stabilize and activate MST2.[35,36] Therefore, the influence of RASSFs on MST1/2 function may be spatiotemporally regulated depending on specific cellular context.

GCKIII kinases and CCM3

GCKIII kinases include MST3 (also named STK24), MST4 (also named STK26 and MASK) and sterile 20/oxidant stress-response kinase 1 (YSK1, also named STK25 and SOK1). GCKIII kinases share conserved N-terminal kinase domain and C-terminal dimerization domain with a linker region in between. GCKIII kinases have been implicated in multiple biological processes including cell growth, proliferation, migration, polarity, apoptosis and cell cycle progression.[37] For example, an epidermal growth factor receptor agonist EGF can activate MST4 in prostate tumor cell lines.[38] Knockdown of *Drosophila* GckIII or Cka (homolog of STRNs in *Drosophila*) partially suppressed ectopic wing veins caused by the epidermal growth factor receptor gain-of-function, suggesting that the GCKIII kinases are involved in epidermal growth factor receptor signaling.[39,40] The liver kinase B1 (LKB1) activator MO25 (also called CAB39) can markedly enhance the activity of GCKIII kinases, whereas phosphatases in the STRIPAK complex such as PP2A may negatively regulate their activities.[19,41,42]

CCM3 was firstly identified as a cell death-related gene.[43] Further studies found that similar to CCM1 and CCM2, loss-of-function mutations in CCM3 are also associated with CCMs, which are vascular lesions histologically characterized by abnormally enlarged capillary cavities without intervening brain parenchyma.[44] CCM3 is composed of an N-terminal dimerization domain similar to that of GCKIII and a C-terminal focal adhesion targeting homology domain.[45] Thus CCM3 can form a homodimer or heterodimer with GCKIII kinases.[46,47] STRNs, CCM2 or paxillin can directly interact with the focal adhesion targeting homology domain of CCM3 via a conserved binding mode, suggesting that CCM1–CCM2–CCM3- and CCM3-containing STRIPAK complexes are mutually exclusive.[12] A primary function of CCM3 in STRIPAK complexes is to act as a molecular bridge recruiting GCKIII kinases to STRNs. Meanwhile, the GCKIII family member MST4 has been shown to localize preferentially to Golgi apparatus in CCM3-depleted HeLa cells, but mainly retain in the cytosol when STRNs are depleted, suggesting that CCM3 and STRNs have different effects on the subcellular localization of GCKIII kinases.[12] We and others have found that MST4 acts together with MO25 to promote apoptosis of HEK293T cells,[42] but cooperates with CCM3 to enhance proliferation of HeLa cells,[48] suggesting the differential functions of GCKIII kinases dependent on a specific partner and cellular context.

MOB4

MOB4 belongs to MOB family of proteins but shows lower sequence similarity with other members of the family. MOB4 was identified as a STRN/PP2Ac-binding protein and was highly expressed in the adrenal gland and central nervous system.[49,50] Similar to STRN, MOB4 is also enriched in dendritic spines where it associates with nucleoside diphosphate kinase, epidermal growth factor receptor substrate 15 and dynamin-1, all of which are involved in endocytosis and vesicular trafficking.[51–54]

MOB4 undergoes phosphorylation under physiological condition, which can be enhanced by treatment of cells using PP2A inhibitor okadaic acid. Owing to the presence of GCK kinases in STRIPAK complexes, MOB4, as well as STRNs, could be phosphorylated by GCK kinases. Yet, at this point, it remains to be further investigated whether MOB4 is a physiological substrate of GCKIII kinases, and if so what is the functional consequence. Other MOB proteins such as MOB1 can be phosphorylated by upstream kinases and then activate downstream nuclear DBF2-related kinase/large tumor suppressor (LATS) family kinases in signaling transduction.[55] Nuclear DBF2-related kinase 1/2 kinases have been identified as substrates of MST3, which may regulate cell cycle progression.[56,57] Moreover, *Drosophila* Mob4 is involved in spindle focusing.[58] Therefore, MOB4 perhaps also uses similar manner as

does MOB1 to recruit and activate nuclear DBF2-related kinase or nuclear DBF2-related kinase-like kinases.

STRIP1/2

STRIP1 and STRIP2 were identified as regulators of cell morphology and cytoskeletal organization.[59] STRIP1/2 proteins contain two conserved domains, N1221 and DUF3402. The biological function of both domains is yet to be defined. Depletion of STRIP1 in PC3 prostate carcinoma cells led to flatter phenotype with an increase in F-actin-rich lamellae around the cell periphery and broad lamellipodium, whereas loss of STRIP2 caused an elongated phenotype with very long thin protrusions containing microtubules. In contrast to STRIP2-depleted PC3 cells, HeLa cells depleted of STRIP2 did not display elongated phenotype, but had reduced cell–cell adhesions. Therefore, STRIP1/2 may function in a cellular context-dependent manner. Either knockdown or overexpression of STRIP1 reduced PC3 cell area, indicating that STRIP1 levels are critical for cell spreading. However, knockdown of STRIP1 or STRIP2 did not significantly influence migration of PC3 cells into scratch wounds. Knockdown of STRIP2 in differentiating mouse embryoid bodies caused increased transcription of pluripotency factors and epigenetic factors.[60] STRIP2-depleted mouse embryonic stem cells lost the ability of differentiation into functional cardiomyocytes. The indispensable role of STRIP2 for lineage commitment of mouse embryonic stem cells is mechanistically related to pluripotency and epigenetic networks as well as to cytoskeleton dynamics.

In *Drosophila*, Strip regulates neural development together with dynein regulator Glued (Gl) and Sprint (Spri), a guanine nucleotide exchange factor of small GTPase Rab5.[61] In this process, Strip and Gl are involved in clustering of early endosome organization, whereas Strip and Spri regulate fusion via activating Rab5. Strip-depleted neuroblast was defective in neuronal guidance and targeting, whereas Strip-deficient projection neurons were defective in axon elongation and dendrite branching. Knockdown of Strip1 or Rab5 in mouse cerebral cortex at E14 also caused defect in neuron migration.

SLMAP

SLMAP is a tail-anchored membrane protein possessing one of two alternative-splicing-generated mutually exclusive transmembrane domains (TM1/2 or TA1/2), which target it to different membrane compartments including endoplasmic reticulum, mitochondrion and nuclear membrane.[62–65] Besides the TM domain, SLMAP contains an N-terminal forkhead-associated (FHA) domain, which is classically known to recognize phosphopeptide present in many regulatory proteins. Between the TM and FHA domains, there are multiple regions of coiled coil, which could mediate homodimerization of SLMAP. The FHA domain of SLMAP is required for its centrosomal localization.[66] Overexpression of SLMAP with a truncation of the FHA domain suppressed cell proliferation, leading to increased number of cells at the G2/M phase of cell cycle.

There are multiple isoforms of SLMAP because of alternative splicing.[62] The expression and distribution of SLMAP isoforms are highly regulated in distinct biological processes of development. For example, the ~90 kDa isoform of SLMAP was detected in both proliferating myoblasts and differentiated myotubes with a relatively constant level, whereas the ~80 kDa isoform was expressed only during myoblast differentiation.[67] Alteration of the expression level or mutations of SLMAP has been associated with diseases that occurred in the heart and muscle. For instance, the expression of SLMAP was increased in group I leiomyosarcoma, a malignant smooth muscle tumor, and identified as an immunohistochemistry marker.[68,69] Two mutations of SLMAP V269I and E710A were found in patients with Brugada syndrome, a cardiac channelopathy.[70]

TRAF3IP3

TRAF3IP3 was initially identified as a TRAF3-interacting protein.[71] TRAF3IP3 is a paralog of SLMAP, which also contains several coiled-coil regions and a C-terminal TM domain. Instead of an FHA domain observed for SLMAP, the N terminus of TRAF3IP3 is a region with uncharacterized structure and function. TRAF3IP3 can associate with autophagy-related protein 16-1 via its potential autophagy-related protein 16-1-binding motif (318-WRSQYEALKEDWRTL-332) and the WD domain of autophagy-related protein 16-1.[72] TRAF3IP3 is abundant in the bone marrow, spleen and thymus.[71] Recently, TRAF3IP3 has been reported to have a critical role in the development of T and B cells through the regulation of autophagy and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK) signaling.[73,74]

SIKE1

SIKE1 contains three-coiled-coil domains, and is widely expressed in many human tissues, mostly abundant in the brain, heart, kidney and placenta.[75] Upon poly(I:C) and vesicular stomatitis virus stimulation, SIKE1 associates with kinases inhibitor of nuclear factor-κB kinase subunit ε and TANK-binding kinase 1 via their coiled coils. SIKE1 also functions as a substrate of nuclear factor-κB kinase subunit epsilon and TANK-binding kinase 1 to suppress the phosphorylation of transcription factor interferon regulatory factor 3, thus negatively regulating Toll-like receptor 3 and retinoic acid-inducible gene I signaling.[75,76] Expression of SIKE1 was elevated in chronic hepatitis C cells, indicating that hepatitis C virus may use SIKE1 to inhibit anti-viral response and escape host defense.[77] SIKE1, together with CXCL12 and miR-146a-5p, has been implicated in the regulation of mesenchymal stem cell proliferation and migration.[78]

FGFR1OP2

FGFR1OP2 is a paralog of SIKE1, which also includes three-coiled-coil domains. It was initially found that the expression of FGFR1OP2 is robustly enhanced in oral mucosa undergoing tooth extraction wound healing.[79] FGFR1OP2 is indispensible for collagen gel contraction of oral wound fibroblasts and oral wound closure.[80,81] Several SNPs of FGFR1OP2 were detected in patients with long-term atrophy of edentulous mandible.[82,83] Lately, FGFR1OP2 has been identified as a potential oncogene in non-small-cell lung cancer.[84] A fusion protein FGFR1OP2-FGFR1 was found in patients with 8p11 myeloproliferative syndrome, an aggressive hematological malignancy.[85] It is most likely that FGFR1OP2 can form homodimer via its coiled-coil domains. Thus, similar to other FGFR1 fusion partners such as zinc-finger protein 198 (also known as ZMYM2), the fusion of FGFR1OP2 to FGFR, may lead to forced dimerization and ligand-independent spontaneous activation of FGFR1, which is a major trigger of 8p11 myeloproliferative syndrome.[86–88]

CTTNBP2 and CTTNBP2NL

The N-terminal region of CTTNBP2 contains a coiled-coil region and a cortactin-binding proline-rich region, both of which are conserved in CTTNBP2NL. There are six ankyrin repeats in the middle region of CTTNBP2, while the C-terminal region is largely uncharacterized. CTTNBP2NL, as the name suggests, lacks the middle and C-terminal parts observed for CTTNBP2. As a cortactin-binding protein, CTTNBP2 can interact with cortactin via its proline-rich region and the SH3 domain of cortactin.[89] CTTNBP2 is expressed in the brain, kidney and pancreas, whereas CTTNBP2NL is expressed in the skin, lung and spleen.[89–91] CTTNBP2 is methylated in prostate cancer cell line LNCaP, but not in primary prostate cells PrEC, suggesting that the expression of CTTNBP2 is altered in prostate cancer.[92] Deletion of CTTNBP2NL gene was

also detected in oral squamous cell carcinoma by array-based comparative genomic hybridization, but its association with oral squamous cell carcinoma should be further investigated.[93]

STRIPAK COMPLEXES AND HIPPO PATHWAY

The highly conserved Hippo pathway has a key role in organ size control and tissue homeostasis.[25,26,94,95] Dysregulation of the Hippo pathway may result in developmental defect and cancer. The core of Hippo pathway is a kinase cascade. GCKII kinases MST1/2 (Hippo or Hpo in *Drosopila*) phosphorylate MOB1 (Mats or Mob1 in *Drosophila*) – LATS1/2 (Warts or Wts in *Drosopila*) complex together with SAV1 (salvador or Sav in *Drosopila*) (Figure 2). Activated LATS1/2 or Wts further phosphorylates transcriptional coactivator Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) or Yorkie (Yki) to promote their cytoplasmic retention and degradation. When Hippo signaling is absent, YAP/TAZ or Yki translocate into the nucleus, where it binds TEA domain family member 1–4 or scalloped to form a hybrid transcriptional factor and regulate target gene transcription. Therefore, the Hippo kinase cascade functions as a tumor suppressor to control the oncogenic activity of YAP/TAZ. Elevated expression and nuclear localization of YAP are closely associated with tumorigenesis. Meanwhile, YAP/TAZ is also important for self-renewal and expansion of stem cell and progenitor cell, which have pivotal roles in tissue repair and regeneration. Recently, we and others have revealed nuclear regulation of Hippo pathway by identification of VGLL4 as a natural YAP antagonist.[96–99]

MST1/2 kinases are not only the central kinase of Hippo pathway but also are the critical components of certain types of STRIPAK complexes.[3,100,101] Thus, it is conceivable that STRIPAK complex would directly regulate Hippo signaling. On the one hand, the phosphatase component of the STRIPAK complex may negatively regulate the kinase activity of MST1/2 in the Hippo pathway.[102] On the other hand, components other than phosphatase in the STRIPAK complex may also have a role in tuning the activity of Hippo signaling. Indeed, in a small interfering RNA screen study in HEK293T cells, knockdown of TRAF3IP3, a component of the STRIPAK complex, significantly enhanced the levels of YAP protein and phosphorylated YAP, as well as YAP reporter activity.[103]

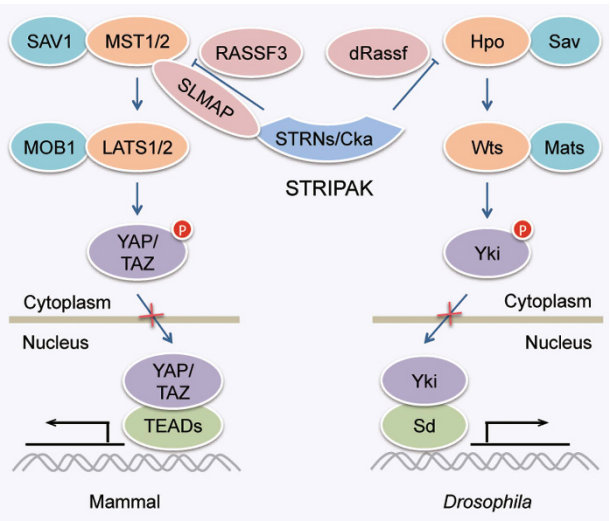

Figure 2. STRIPAK complexes and Hippo signaling. STRIPAK complexes negatively regulate Hippo signaling via decreasing the phosphorylation of MST1/2 or Hpo, causing the activation of YAP/TAZ–TEA domain family members (TEADs) or Yki–scalloped (Sd) and promoting cell proliferation.

In *Drosophila*, Hpo and dRassf associate with *Drosphila* STRIPAK components Cka, Mts (homolog of PP2Ac in *Drosophila*), Fgfr1op2 and Mob4.[3] dRassf may mediate the interaction between Hpo and STRIPAK complex. dRassf, Cka and Mts can inhibit Hpo activation and therefore promote Yki phosphorylation. Mechanistically, dRassf antagonizes Hpo function possibly by competing with Sav for binding Hpo.[104] Loss of several STRIPAK components including Cka, Mob4, Fgfr1op2, Ccm3, Strip and Slmap suppressed the Hpo-depletion-induced overgrowth phenotype in *Drosophila* wing. In human STRIPAK complex, SLMAP and RASSF3 might link MST1/2 kinases to the scaffold STRNs.[100,101] It is reported that SLMAP associates with MST1/2 in a phosphorylation-dependent manner. Further biochemical and structural studies are required to define the precise mechanism of MST1/2 recruitment and the assembly of the MST1/2-SLMAP subcomplex.

Recently, the tumor suppressor LKB1 has been linked with Hippo-YAP signaling cascade.[103] As LKB1 is also known able to act together with MST4, a GCKIII kinase and a component of the STRIPAK complex, in the control of intestinal cell polarity and brush border formation,[105,106] thus it would be intriguing to speculate that LKB1 may cooperate with different kinase components of STRIPAK complexes to form a signaling network during its cross-talk with Hippo pathway. Given that the LKB1 substrate 5'-AMP-activated protein kinase can directly phosphorylate YAP and regulate its activity in parallel with MST1/2–LATS1/2 (Hpo–Wts) kinase cascade,[107,108] it is also possible that STRIPAK complexes could have a role in the differential control of such signaling network.

STRIPAK COMPLEXES AND MAPK PATHWAY

MAPK signaling regulates a variety of cellular processes in response to distinct stimuli including hormones, growth factors, cytokines, other G-protein-coupled receptors ligands, pathogen-associated molecular patterns, danger-associated molecular patterns and environmental stresses.[109,110] The three-tiered 'core signaling module' of MAPK pathway is composed of MAPK kinase kinases (MAPKKKs), MAPK kinases and MAPKs.[109] Activated MAPKKKs by stimulus phosphorylates MAPK kinase, which in turn phosphorylates and activates MAPKs. MAPK can be divided into three major groups, ERK, c-Jun N-terminal kinase (JNK) and p38. ERK is activated by Ras-dependent manner in response to insulin and mitogen, and also by Ras-independent manner in response to proinflammatory stimuli, pathogen-associated molecular patterns and danger-associated molecular patterns. JNK and p38 can be activated by environmental stresses, proinflammatory cytokines, pathogen-associated molecular patterns and danger-associated molecular patterns. JNK is also activated by growth factors.

Multiple evidences support the notion that STRIPAK complexes participate in the regulation of MAPK signaling (Figure 3). GCK family members are the kinases of MAPKKKs and are so-called MAP4Ks.[111] Therefore, GCKs might directly activate MAPKKKs. For example, the STRIPAK kinase MST4 together with its adaptor CCM3 can enhance MEK1-dependent ERK activation to promote cell growth and transformation.[48,112] In this regard, MST4 that is highly expressed in multiple cancer cell lines and tumors can promote prostate cancer cell growth *in vitro* and *in vivo*.[38,113,114] MST4 also accelerates hepatocellular carcinoma proliferation, invasion and epithelial–mesenchymal transition *in vitro*.[113] Recently, a novel microRNA, miR-4728-3p found within an intron of the *ERBB2* gene, directly targets MST4 and thus modulates ERK activation to inhibit tumor growth *in vitro* and in a xenograft model.[115] Under hypoxia, MST4 protects pituitary cell from death and enhances proliferation and colony formation of pituitary gonadotrope cells through activating p38 and RAC-alpha serine/threonine-protein kinase (AKT).[114] Under oxidative stress, MST3 inhibits JNK activation to promote cell death.[116] In *Drosophila,* GckIII is required for wing development via activating ERK.[39]

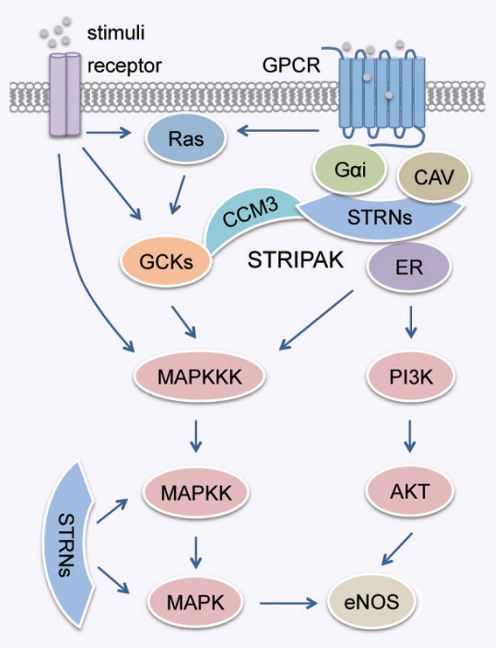

Figure 3. STRIPAK complexes and MAPK and nongenomic NR signaling. GCK kinases as MAP4K activate MAPK pathway. STRNs and CAV recruit ER, Gαi and eNOS to forming a signaling complex, mediating nongenomic effects. Abbreviation: PI3K, phosphoinositide-3-kinase.

In addition to GCKs, other STRIPAK components have also been implicated in MAPK signaling. Cka activates *Drosophila* JNK pathway to function in embryonic dorsal closure and apoptosis in wing imaginal disk.[117] Knockdown of Cka lead to decreased MAPK activity and expression of Ras–MAPK target genes, resulting in defective eye development.[40,118] Consistently, depletion of STRN or STRN3 reduced ERK phosphorylation in human HEK293T cells. Depletion of other STRIPAK components such as Strip, Slmap, Fgfr1op2 and Mob4 also led to compromised MAPK activation.[118] Besides its effects on the development of T and B cells via regulating MEK signaling, TRAF3IP3 also promotes growth of HEK293T cells by enhancing JNK-dependent Elk-1 activation possibly through binding TRAF3.[71,73,74,119]

STRIPAK COMPLEXES AND NONGENOMIC NR PATHWAY

NRs translocate from the cytosol into the nucleus to regulate gene expression when sensing steroid and thyroid hormones, which is called genomic NR signaling. This genomic response occurs several hours and even days after hormones' entry into cells. Meanwhile, NRs also function in a rapid-and-nongenomic manner even seconds after sensing hormones, a process not involving the transcriptional activity of NRs.[120]

In vascular endothelial cells, estradiol activates ERK/MAPK and phosphoinositide-3-kinase–AKT pathways and ultimately leads to the phosphorylation of eNOS. Estrogen receptors (ERs) and Gαi also participate in this process, during which they form a signaling complex with eNOS in the caveolae (Figure 3).[15] STRN acts as a scaffolding to interact with ER, Gαi and eNOS, which is important for the assembly of this signaling complex. Disrupting the association between STRN and ER impairs estradiol-induced activation of MAPK and AKT, as well as phosphorylation of eNOS *in vitro* and *in vivo*. Studies on a transgenic mouse model overexpressing the peptide that blocks the ER–STRN association suggested that STRN-related nongenomic ER signaling is required for the protective effects of estrogen against vascular injury.[121]

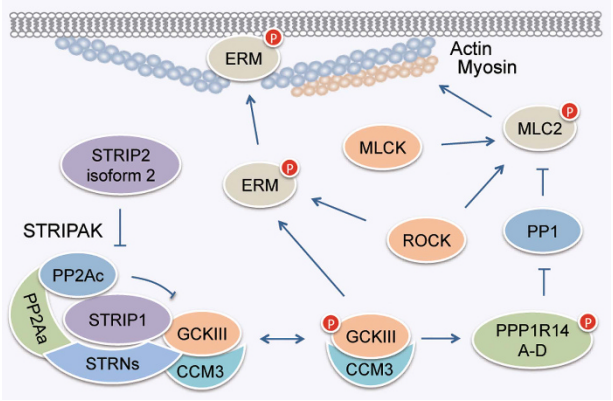

Figure 4. STRIPAK complexes and cytoskeleton remodeling. GCKIII kinases are recruited by CCM3 to sites of actomyosin contraction and promote the colocalization of contractile actomyosin machinery (phosphorylated myosin regulatory light chain 2 (MLC2)) and actin-plasma linkage (phosphorylated ERM) by phosphorylating PPP1R14A–D and inhibiting protein phosphatase 1 (PP1) activity, promoting tumor cell migration and metastasis. STRNs and STRIP1 suppress activity of MST3 and MST4 to regulate negatively this process, which is antagonized by STRIP2 isoform 2. Abbreviations: MLCK, myosin light chain kinase; ROCK, Rho-associated protein kinase.

Moreover, the estradiol-induced eNOS phosphorylation is further augmented by aldosterone, which promotes STRN expression.[122,123] Aldosterone also stimulates rapid phosphorylation of ERK through mineralocorticoid receptor in endothelial cells in an STRN-dependent manner. Caveolae component CAV1 mediates mineralocorticoid receptor binding to STRN, indicating that STRN and CAV have important roles in assembling the nongenomic NR signaling complex. Given that both STRNs and GCKIII kinases are involved in the regulation of MAPK and AKT, it is also possible that the STRIPAK complex as a whole may participate in nongenomic NR signaling.

STRIPAK COMPLEXES AND CYTOSKELETON REMODELING

Cytoskeleton exists as a delicate and dynamic network, which not only controls cell shape, architecture and motility but also mediates communication of the cell with external environment.[124] Actin filaments, microtubules and intermediate filaments are three major types of cytoskeletal building blocks. Actin filaments support filopodial protrusions and the leading edge of motile cells to generate force required for cell shape change. Microtubules are essential for cell migration, mitotic spindle formation and generation of cilia and flagella. STRIPAK components STRN and CTTNBP2 are associated with microtubule dynamics. Knockdown of STRN leads to depolymerization of microtubules and inhibition of cell proliferation.[125] CTTNBP2 associates with distinct cytoskeleton molecules and colocalizes with microtubule and F-actin, respectively, in premature and mature hippocampal neurons.[126,127] CTTNBP2 associates with microtubule to increase microtubule stability. Loss of CTTNBP2 reduces the density and width of dendritic spines and attenuates the electrophysiological response of neurons in hippocampal neurons. Disruption of the interaction between CTTNBP2 and cortactin, a protein involved in tumor invasion and metastasis,[128] also decreases the density of dendritic spines.

In addition to STRN and CTTNBP2, multiple kinases of STRIPAK complexes have been implicated in cytoskeleton remodeling often through phosphorylation of ERM (Ezrin, Radixin and Moesin) proteins (Figure 4). As key players in development, ERM proteins can regulate many signaling pathways such as RhoA and Hedgehog.[129] Ezrin is an actin-binding protein that links

actin cytoskeleton to the plasma membrane. Intestinal brush border is the actin-supported microvilli-covered apical surface of polarized epithelial cells.[130] During brush border formation, LKB1 is activated by the pseudokinase STRAD and the scaffold protein MO25, resulting in translocation of MST4 to apical domain, where it phosphorylates and activates Ezrin.[106] CCM3 mediates the phosphorylation of ERM proteins by MST4 to protect cells from oxidative stress-induced apoptosis.[131] In addition, STRIPAK kinases MST3 and YSK1 can phosphorylate Moesin, and together with CCM3, these kinases can inhibit RhoA activity and actin stress fiber formation to regulate endothelial cell junctions.[132] Considering their membrane localization,[133] other STRIPAK components might also participate in these processes by modulating the recruitment of GCKIIIs to membrane compartment.

Cell migration is a central process for the development and physiology of multicellular organisms and is tightly associated with the dynamics of actin cytoskeleton.[134,135] Membrane blebs are one of several ways for cell migration. The actomyosin contractility enhances hydrostatic pressure, resulting in weakened association of actin cortex and plasma membrane, which generates membrane blebs and pushes membrane forward.[136] Phosphorylation of myosin regulatory light chain 2 (also named MYL9) by many kinases including myosin light chain kinase and Rho-associated protein kinase induces actomyosin contractility (Figure 4).[137] Protein phosphatase 1 dephosphorylates myosin regulatory light chain 2 and negatively regulates actomyosin contractility. STRIPAK kinases MST3 and MST4 can be recruited by CCM3 to sites of actomyosin contraction and promote the colocalization of contractile actomyosin machinery and actin-plasma linkage by phosphorylating protein phosphatase 1 regulatory subunit 14A–D (PPP1R14A–D), the inhibitors of protein phosphatase 1-β catalytic subunit.[138] This colocalization benefits cancer cell migration in confined environment, promoting cancer metastasis. In contrast, other STRIPAK components including STRNs and STRIP1 appear to suppress the activity of MST3/4 kinases to regulate negatively this process. Moreover, STRIP2 has two isoforms that have different roles in cell migration. The isoform 1 of STRIP2 can bind PP2Ac like STRIP1, whereas the isoform 2 lacks a C-terminal region present in isoform 1 and does not bind PP2Ac. It was suggested that the isoform 2 of STRIP2 promotes three-dimensional cell migration and cancer metastasis likely through antagonizing the function of STRIP1 and the isoform 1 of STRIP2. Consistent with its role in cancer cell migration, STRIP2 undergoes more amplification and mutation in multiple tumor samples when compared with STRIP1. Meanwhile, the expression of MST3, MST4 and CCM3 is elevated in more aggressive breast cancer subtypes and their overexpression correlates with poor prognosis.[138]

PERSPECTIVE

STRIPAK complexes have critical roles in tumorigenesis and metastasis, whereas new functions of these complexes are increasingly uncovered during past several years. Growing evidence indicate that in addition to GCKII and GCKIII kinases, the GCKIV members misshapen-like kinase 1, TRAF2 and NCK-interacting protein kinase and MAP4K4 may also associate with STRN4 of STRIPAK complexes.[139] Meanwhile, other STRIPAK components such as STRIP1/2 and SLMAP are also identified as misshapen-like kinase 1-associating proteins. Misshapen-like kinase 1 and STRN4 together regulate cytokinesis after mitosis, in which STRN4 may negatively regulate misshapen-like kinase 1 activity. Similar to yeast STRIPAK complex, the mammalian STRIPAK complexes are implicated in cell cycle regulation.[133] The dysregulation of mitosis is tightly associated with tumorigenesis. STRNs and STRIP1/2, as well as GCKIIIs,[140] localize at the Golgi. SLMAP exists in the outer nuclear envelope during interphase but concentrates on centrosome during mitosis. Knockdown of STRIPAK component such as STRN3, STRIP1, SLMAP or MOB4 results in mitosis failure. Thus, STRIPAK complexes may link nuclear envelope, centrosome and Golgi during mitosis, which is required for proper cell cycle progression.

It appears that the biological roles of STRIPAK complexes are even more diversified than initially expected. For example, a recent study revealed that STRIPAK complexes may control the phosphorylation and transcriptional activity of circadian locomotor output cycles protein kaput in the *Drosophila* circadian clock.[141] In *Caenorhabditis elegans*, STRIPAK may regulate tube extension involving cell division control protein 42 signaling.[142] Meanwhile, dysregulation or mutation of STRIPAK components has been frequently linked to various diseases. Therefore, the activity of STRIPAK components and the related protein–protein interaction could be promising targets for drug development. For instance, targeting MST3 and MST4 kinase activity or their association with the activator MO25 would suppress cancer cell migration and metastasis. Several groups have screened chemical compounds directly inhibiting MST3 or MST4 activity, but their specificity and physiological activity need to be further examined.[143,144] Owing to the inhibitory role of STRIPAK in Hippo signaling, therapeutic targeting of STRIPAK complexes might also benefit cancer patients.

Despite considerable recent progresses on the understanding of STRIPAK complexes, there are still a great deal of questions and puzzles to be addressed. Currently, the overall architecture and assembly of STRIPAK complexes remain largely unknown. It is not clear which types of STRIPAK complexes or subcomplexes are involved in a specific biological process and how these complexes are regulated. Particularly, the molecular mechanism through which STRIPAK complexes regulate distinct cellular signaling pathways is poorly defined, which impedes rational targeting of STRIPAK for disease treatment. Apparently, further studies are required to define in-depth their regulatory machineries in certain physiological and/or pathological settings. Finally, the biological functions of the STRIPAK complexes studied to date are mostly based on the functions of their individual components. Future efforts should be directed towards understanding the role of STRIPAK complexes as a whole in various signaling.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Glatter T, Wepf A, Aebersold R, Gstaiger M. An integrated workflow for charting the human interaction proteome: insights into the PP2A system. *Mol Syst Biol* 2009; **5**: 237.
2 Goudreault M, D'Ambrosio LM, Kean MJ, Mullin MJ, Larsen BG, Sanchez A *et al.* A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein. *Mol Cell Proteomics* 2009; **8**: 157–171.
3 Ribeiro PS, Josue F, Wepf A, Wehr MC, Rinner O, Kelly G *et al.* Combined functional genomic and proteomic approaches identify a PP2A complex as a negative regulator of Hippo signaling. *Mol Cell* 2010; **39**: 521–534.
4 Hwang J, Pallas DC. STRIPAK complexes: structure, biological function, and involvement in human diseases. *Int J Biochem Cell Biol* 2014; **47C**: 118–148.
5 Castets F, Bartoli M, Barnier JV, Baillat G, Salin P, Moqrich A *et al.* A novel calmodulin-binding protein, belonging to the WD-repeat family, is localized in dendrites of a subset of CNS neurons. *J Cell Biol* 1996; **134**: 1051–1062.

6 Kachidian P, Vuillet J, Bartoli M, Castets F, Nieoullon A, Kerkerian-Le Goff L. Relationships between striatin-containing neurons and cortical or thalamic afferent fibres in the rat striatum. An ultrastructural study by dual labelling. *Neuroscience* 1998; **85**: 111–122.
7 Moqrich A, Mattei MG, Bartoli M, Rakitina T, Baillat G, Monneron A *et al.* Cloning of human striatin cDNA (STRN), gene mapping to 2p22-p21, and preferential expression in brain. *Genomics* 1998; **51**: 136–139.
8 Salin P, Kachidian P, Bartoli M, Castets F. Distribution of striatin, a newly identified calmodulin-binding protein in the rat brain: an *in situ* hybridization and immunocytochemical study. *J Comp Neurol* 1998; **397**: 41–59.
9 Castets F, Rakitina T, Gaillard S, Moqrich A, Mattei MG, Monneron A. Zinedin, SG2NA, and striatin are calmodulin-binding, WD repeat proteins principally expressed in the brain. *J Biol Chem* 2000; **275**: 19970–19977.
10 Muro Y, Chan EK, Landberg G, Tan EM. A cell-cycle nuclear autoantigen containing WD-40 motifs expressed mainly in S and G2 phase cells. *Biochem Biophys Res Commun* 1995; **207**: 1029–1037.
11 Gaillard S, Bartoli M, Castets F, Monneron A. Striatin, a calmodulin-dependent scaffolding protein, directly binds caveolin-1. *FEBS Lett* 2001; **508**: 49–52.
12 Kean MJ, Ceccarelli DF, Goudreault M, Sanches M, Tate S, Larsen B *et al.* Structure-function analysis of core STRIPAK proteins: a signaling complex implicated in Golgi polarization. *J Biol Chem* 2011; **286**: 25065–25075.
13 Hansen CG, Nichols BJ. Exploring the caves: cavins, caveolins and caveolae. *Trends Cell Biol* 2010; **20**: 177–186.
14 Sotgia F, Martinez-Outschoorn UE, Howell A, Pestell RG, Pavlides S, Lisanti MP. Caveolin-1 and cancer metabolism in the tumor microenvironment: markers, models, and mechanisms. *Annu Rev Pathol* 2012; **7**: 423–467.
15 Lu Q, Pallas DC, Surks HK, Baur WE, Mendelsohn ME, Karas RH. Striatin assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NO synthase by estrogen receptor alpha. *Proc Natl Acad Sci USA* 2004; **101**: 17126–17131.
16 Bartoli M, Monneron A, Ladant D. Interaction of calmodulin with striatin, a WD-repeat protein present in neuronal dendritic spines. *J Biol Chem* 1998; **273**: 22248–22253.
17 Bartoli M, Ternaux JP, Forni C, Portalier P, Salin P, Amalric M *et al.* Down-regulation of striatin, a neuronal calmodulin-binding protein, impairs rat locomotor activity. *J Neurobiol* 1999; **40**: 234–243.
18 Stirnimann CU, Petsalaki E, Russell RB, Muller CW. WD40 proteins propel cellular networks. *Trends Biochem Sci* 2010; **35**: 565–574.
19 Gordon J, Hwang J, Carrier KJ, Jones CA, Kern QL, Moreno CS *et al.* Protein phosphatase 2a (PP2A) binds within the oligomerization domain of striatin and regulates the phosphorylation and activation of the mammalian Ste20-Like kinase Mst3. *BMC Biochem* 2011; **12**: 54.
20 Breitman M, Zilberberg A, Caspi M, Rosin-Arbesfeld R. The armadillo repeat domain of the APC tumor suppressor protein interacts with Striatin family members. *Biochim Biophys Acta* 2008; **1783**: 1792–1802.
21 Nelson S, Nathke IS. Interactions and functions of the adenomatous polyposis coli (APC) protein at a glance. *J Cell Sci* 2013; **126**: 873–877.
22 Shi Y. Serine/threonine phosphatases: mechanism through structure. *Cell* 2009; **139**: 468–484.
23 Moreno CS, Park S, Nelson K, Ashby D, Hubalek F, Lane WS *et al.* WD40 repeat proteins striatin and S/G(2) nuclear autoantigen are members of a novel family of calmodulin-binding proteins that associate with protein phosphatase 2A. *J Biol Chem* 2000; **275**: 5257–5263.
24 Chen C, Shi Z, Zhang W, Chen M, He F, Zhang Z *et al.* Striatins contain a noncanonical coiled coil that binds protein phosphatase 2 A A subunit to form a 2:2 heterotetrameric core of striatin-interacting phosphatase and kinase (STRIPAK) complex. *J Biol Chem* 2014; **289**: 9651–9661.
25 Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. *Cell* 2015; **163**: 811–828.
26 Shi Z, Jiao S, Zhou Z. Structural dissection of Hippo signaling. *Acta Biochim Biophys Sin (Shanghai)* 2015; **47**: 29–38.
27 Volodko N, Gordon M, Salla M, Ghazaleh HA, Baksh S. RASSF tumor suppressor gene family: Biological functions and regulation. *FEBS Lett* 2014; **588**: 2671–2684.
28 Sherwood V, Recino A, Jeffries A, Ward A, Chalmers AD. The N-terminal RASSF family: a new group of Ras-association-domain-containing proteins, with emerging links to cancer formation. *Biochem J* 2010; **425**: 303–311.
29 Khokhlatchev A, Rabizadeh S, Xavier R, Nedwidek M, Chen T, Zhang XF *et al.* Identification of a novel Ras-regulated proapoptotic pathway. *Curr Biol* 2002; **12**: 253–265.
30 Praskova M, Khoklatchev A, Ortiz-Vega S, Avruch J. Regulation of the MST1 kinase by autophosphorylation, by the growth inhibitory proteins, RASSF1 and NORE1, and by Ras. *Biochem J* 2004; **381**: 453–462.
31 Ikeda M, Kawata A, Nishikawa M, Tateishi Y, Yamaguchi M, Nakagawa K *et al.* Hippo pathway-dependent and -independent roles of RASSF6. *Sci Signal* 2009; **2**: ra59.
32 Oh HJ, Lee KK, Song SJ, Jin MS, Song MS, Lee JH *et al.* Role of the tumor suppressor RASSF1A in Mst1-mediated apoptosis. *Cancer Res* 2006; **66**: 2562–2569.
33 Guo C, Tommasi S, Liu L, Yee JK, Dammann R, Pfeifer GP. RASSF1A is part of a complex similar to the *Drosophila* Hippo/Salvador/Lats tumor-suppressor network. *Curr Biol* 2007; **17**: 700–705.
34 Guo C, Zhang X, Pfeifer GP. The tumor suppressor RASSF1A prevents dephosphorylation of the mammalian STE20-like kinases MST1 and MST2. *J Biol Chem* 2011; **286**: 6253–6261.
35 Cooper WN, Hesson LB, Matallanas D, Dallol A, von Kriegsheim A, Ward R *et al.* RASSF2 associates with and stabilizes the proapoptotic kinase MST2. *Oncogene* 2009; **28**: 2988–2998.
36 Song H, Oh S, Oh HJ, Lim DS. Role of the tumor suppressor RASSF2 in regulation of MST1 kinase activity. *Biochem Biophys Res Commun* 2010; **391**: 969–973.
37 Sugden PH, McGuffin LJ, Clerk A. SOcK, MiSTs, MASK and STicKs: the GCKIII (germinal centre kinase III) kinases and their heterologous protein-protein interactions. *Biochem J* 2013; **454**: 13–30.
38 Sung V, Luo W, Qian D, Lee I, Jallal B, Gishizky M. The Ste20 kinase MST4 plays a role in prostate cancer progression. *Cancer Res* 2003; **63**: 3356–3363.
39 Friedman A, Perrimon N. A functional RNAi screen for regulators of receptor tyrosine kinase and ERK signalling. *Nature* 2006; **444**: 230–234.
40 Horn T, Sandmann T, Fischer B, Axelsson E, Huber W, Boutros M. Mapping of signaling networks through synthetic genetic interaction analysis by RNAi. *Nat Methods* 2011; **8**: 341–346.
41 Hao Q, Feng M, Shi Z, Li C, Chen M, Wang W *et al.* Structural insights into regulatory mechanisms of MO25-mediated kinase activation. *J Struct Biol* 2014; **186**: 224–233.
42 Shi Z, Jiao S, Zhang Z, Ma M, Zhang Z, Chen C *et al.* Structure of the MST4 in complex with MO25 provides insights into its activation mechanism. *Structure* 2013; **21**: 449–461.
43 Wang Y, Liu H, Zhang Y, Ma D. cDNA cloning and expression of an apoptosis-related gene, humanTFAR15 gene. Sci China C. *Sci China C* 1999; **42**: 323–329.
44 Bergametti F, Denier C, Labauge P, Arnoult M, Boetto S, Clanet M *et al.* Mutations within the programmed cell death 10 gene cause cerebral cavernous malformations. *Am J Hum Genet* 2005; **76**: 42–51.
45 Li X, Ji W, Zhang R, Folta-Stogniew E, Min W, Boggon TJ. Molecular recognition of leucine-aspartate repeat (LD) motifs by the focal adhesion targeting homology domain of cerebral cavernous malformation 3 (CCM3). *J Biol Chem* 2011; **286**: 26138–26147.
46 Zhang M, Dong L, Shi Z, Jiao S, Zhang Z, Zhang W *et al.* Structural mechanism of CCM3 heterodimerization with GCKIII kinases. *Structure* 2013; **21**: 680–688.
47 Ceccarelli DF, Laister RC, Mulligan VK, Kean MJ, Goudreault M, Scott IC *et al.* CCM3/PDCD10 heterodimerizes with germinal center kinase III (GCKIII) proteins using a mechanism analogous to CCM3 homodimerization. *J Biol Chem* 2011; **286**: 25056–25064.
48 Ma X, Zhao H, Shan J, Long F, Chen Y, Chen Y *et al.* PDCD10 interacts with Ste20-related kinase MST4 to promote cell growth and transformation via modulation of the ERK pathway. *Mol Biol Cell* 2007; **18**: 1965–1978.
49 Baillat G, Moqrich A, Castets F, Baude A, Bailly Y, Benmerah A *et al.* Molecular cloning and characterization of phocein, a protein found from the Golgi complex to dendritic spines. *Mol Biol Cell* 2001; **12**: 663–673.
50 Moreno CS, Lane WS, Pallas DC. A mammalian homolog of yeast MOB1 is both a member and a putative substrate of striatin family-protein phosphatase 2 A complexes. *J Biol Chem* 2001; **276**: 24253–24260.
51 Haeberle AM, Castets F, Bombarde G, Baillat G, Bailly Y. Immunogold localization of phocein in dendritic spines. *J Comp Neurol* 2006; **495**: 336–350.
52 Baillat G, Gaillard S, Castets F, Monneron A. Interactions of phocein with nucleoside-diphosphate kinase, Eps15, and dynamin I. *J Biol Chem* 2002; **277**: 18961–18966.
53 Bailly YJ, Castets F. Phocein: a potential actor in vesicular trafficking at Purkinje cell dendritic spines. *Cerebellum* 2007; **6**: 344–352.
54 Schulte J, Sepp KJ, Jorquera RA, Wu C, Song Y, Hong P *et al.* DMob4/Phocein regulates synapse formation, axonal transport, and microtubule organization. *J Neurosci* 2010; **30**: 5189–5203.
55 Hergovich A. MOB control: reviewing a conserved family of kinase regulators. *Cell Signal* 2011; **23**: 1433–1440.
56 Stegert MR, Hergovich A, Tamaskovic R, Bichsel SJ, Hemmings BA. Regulation of NDR protein kinase by hydrophobic motif phosphorylation mediated by the mammalian Ste20-like kinase MST3. *Mol Cell Biol* 2005; **25**: 11019–11029.
57 Cornils H, Kohler RS, Hergovich A, Hemmings BA. Human NDR kinases control G (1)/S cell cycle transition by directly regulating p21 stability. *Mol Cell Biol* 2011; **31**: 1382–1395.
58 Trammell MA, Mahoney NM, Agard DA, Vale RD. Mob4 plays a role in spindle focusing in *Drosophila* S2 cells. *J Cell Sci* 2008; **121**: 1284–1292.

59 Bai SW, Herrera-Abreu MT, Rohn JL, Racine V, Tajadura V, Suryavanshi N *et al.* Identification and characterization of a set of conserved and new regulators of cytoskeletal organization, cell morphology and migration. *BMC Biol* 2011; **9**: 54.
60 Wagh V, Doss MX, Sabour D, Niemann R, Meganathan K, Jagtap S *et al.* Fam40b is required for lineage commitment of murine embryonic stem cells. *Cell Death Dis* 2014; **5**: e1320.
61 Sakuma C, Kawauchi T, Haraguchi S, Shikanai M, Yamaguchi Y, Gelfand VI *et al.* *Drosophila* Strip serves as a platform for early endosome organization during axon elongation. *Nat Commun* 2014; **5**: 5180.
62 Wigle JT, Demchyshyn L, Pratt MA, Staines WA, Salih M, Tuana BS. Molecular cloning, expression, and chromosomal assignment of sarcolemmal-associated proteins. A family of acidic amphipathic alpha-helical proteins associated with the membrane. *J Biol Chem* 1997; **272**: 32384–32394.
63 Wielowieyski PA, Sevinc S, Guzzo R, Salih M, Wigle JT, Tuana BS. Alternative splicing, expression, and genomic structure of the 3′ region of the gene encoding the sarcolemmal-associated proteins (SLAPs) defines a novel class of coiled-coil tail-anchored membrane proteins. *J Biol Chem* 2000; **275**: 38474–38481.
64 Byers JT, Guzzo RM, Salih M, Tuana BS. Hydrophobic profiles of the tail anchors in SLMAP dictate subcellular targeting. *BMC Cell Biol* 2009; **10**: 48.
65 Guzzo RM, Salih M, Moore ED, Tuana BS. Molecular properties of cardiac tail-anchored membrane protein SLMAP are consistent with structural role in arrangement of excitation-contraction coupling apparatus. *Am J Physiol Heart Circ Physiol* 2005; **288**: H1810–H1819.
66 Guzzo RM, Sevinc S, Salih M, Tuana BS. A novel isoform of sarcolemmal membrane-associated protein (SLMAP) is a component of the microtubule organizing centre. *J Cell Sci* 2004; **117**: 2271–2281.
67 Guzzo RM, Wigle J, Salih M, Moore ED, Tuana BS. Regulated expression and temporal induction of the tail-anchored sarcolemmal-membrane-associated protein is critical for myoblast fusion. *Biochem J* 2004; **381**: 599–608.
68 Beck AH, Lee CH, Witten DM, Gleason BC, Edris B, Espinosa I *et al.* Discovery of molecular subtypes in leiomyosarcoma through integrative molecular profiling. *Oncogene* 2010; **29**: 845–854.
69 Mills AM, Beck AH, Montgomery KD, Zhu SX, Espinosa I, Lee CH *et al.* Expression of subtype-specific group 1 leiomyosarcoma markers in a wide variety of sarcomas by gene expression analysis and immunohistochemistry. *Am J Surg Pathol* 2011; **35**: 583–589.
70 Ishikawa T, Sato A, Marcou CA, Tester DJ, Ackerman MJ, Crotti L *et al.* A novel disease gene for Brugada syndrome: sarcolemmal membrane-associated protein gene mutations impair intracellular trafficking of hNav1.5. *Circ Arrhythm Electrophysiol* 2012; **5**: 1098–1107.
71 Dadgostar H, Doyle SE, Shahangian A, Garcia DE, Cheng G. T3JAM, a novel protein that specifically interacts with TRAF3 and promotes the activation of JNK (1). *FEBS Lett* 2003; **553**: 403–407.
72 Boada-Romero E, Letek M, Fleischer A, Pallauf K, Ramon-Barros C, Pimentel-Muinos FX. TMEM59 defines a novel ATG16L1-binding motif that promotes local activation of LC3. *EMBO J* 2013; **32**: 566–582.
73 Peng S, Wang K, Gu Y, Chen Y, Nan X, Xing J *et al.* TRAF3IP3, a novel autophagy up-regulated gene, is involved in marginal zone B lymphocyte development and survival. *Clin Exp Immunol* 2015; **182**: 57–68.
74 Zou Q, Jin J, Xiao Y, Hu H, Zhou X, Jie Z *et al.* T cell development involves TRAF3IP3-mediated ERK signaling in the Golgi. *J Exp Med* 2015; **212**: 1323–1336.
75 Huang J, Liu T, Xu LG, Chen D, Zhai Z, Shu HB. SIKE is an IKK epsilon/TBK1-associated suppressor of TLR3- and virus-triggered IRF-3 activation pathways. *EMBO J* 2005; **24**: 4018–4028.
76 Marion JD, Roberts CF, Call RJ, Forbes JL, Nelson KT, Bell JE *et al.* Mechanism of endogenous regulation of the type I interferon response by suppressor of IkappaB kinase epsilon (SIKE), a novel substrate of TANK-binding kinase 1 (TBK1). *J Biol Chem* 2013; **288**: 18612–18623.
77 Ito M, Masumi A, Mochida K, Kukihara H, Moriishi K, Matsuura Y *et al.* Peripheral B cells may serve as a reservoir for persistent hepatitis C virus infection. *J Innate Immun* 2010; **2**: 607–617.
78 Hsieh JY, Huang TS, Cheng SM, Lin WS, Tsai TN, Lee OK *et al.* miR-146a-5p circuitry uncouples cell proliferation and migration, but not differentiation, in human mesenchymal stem cells. *Nucleic Acids Res* 2013; **41**: 9753–9763.
79 Sukotjo C, Abanmy AA, Ogawa T, Nishimura I. Molecular cloning of wound inducible transcript (wit 3.0) differentially expressed in edentulous oral mucosa undergoing tooth extraction wound-healing. *J Dent Res* 2002; **81**: 229–235.
80 Sukotjo C, Lin A, Song K, Ogawa T, Wu B, Nishimura I. Oral fibroblast expression of wound-inducible transcript 3.0 (wit3.0) accelerates the collagen gel contraction *in vitro*. *J Biol Chem* 2003; **278**: 51527–51534.
81 Lin A, Hokugo A, Choi J, Nishimura I. Small cytoskeleton-associated molecule, fibroblast growth factor receptor 1 oncogene partner 2/wound inducible transcript-3.0 (FGFR1OP2/wit3.0), facilitates fibroblast-driven wound closure. *Am J Pathol* 2010; **176**: 108–121.
82 Suwanwela J, Lee J, Lin A, Ucer TC, Devlin H, Sinsheimer J *et al.* A genetic association study of single nucleotide polymorphisms in FGFR1OP2/wit3.0 and long-term atrophy of edentulous mandible. *PLoS One* 2011; **6**: e16204.
83 Kim JH, Oh MY, Paek J, Lee J. Association between FGFR1OP2/wit3.0 polymorphisms and residual ridge resorption of mandible in Korean population. *PLoS One* 2012; **7**: e42734.
84 Al Zeyadi M, Dimova I, Ranchich V, Rukova B, Nesheva D, Hamude Z *et al.* Whole genome microarray analysis in non-small cell lung cancer. *Biotechnol Biotechnol Equip* 2015; **29**: 111–118.
85 Grand EK, Grand FH, Chase AJ, Ross FM, Corcoran MM, Oscier DG *et al.* Identification of a novel gene, FGFR1OP2, fused to FGFR1 in 8p11 myeloproliferative syndrome. *Genes Chromosomes Cancer* 2004; **40**: 78–83.
86 Xiao S, McCarthy JG, Aster JC, Fletcher JA. ZNF198-FGFR1 transforming activity depends on a novel proline-rich ZNF198 oligomerization domain. *Blood* 2000; **96**: 699–704.
87 Jackson CC, Medeiros LJ, Miranda RN. 8p11 Myeloproliferative syndrome: a review. *Hum Pathol* 2010; **41**: 461–476.
88 Macdonald D, Reiter A, Cross NC. The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. *Acta Haematol* 2002; **107**: 101–107.
89 Ohoka Y, Takai Y. Isolation and characterization of cortactin isoforms and a novel cortactin-binding protein, CBP90. *Genes Cells* 1998; **3**: 603–612.
90 Chen YK, Chen CY, Hu HT, Hsueh YP. CTTNBP2, but not CTTNBP2NL, regulates dendritic spinogenesis and synaptic distribution of the striatin-PP2A complex. *Mol Biol Cell* 2012; **23**: 4383–4392.
91 Cheung J, Petek E, Nakabayashi K, Tsui LC, Vincent JB, Scherer SW. Identification of the human cortactin-binding protein-2 gene from the autism candidate region at 7q31. *Genomics* 2001; **78**: 7–11.
92 Coolen MW, Stirzaker C, Song JZ, Statham AL, Kassir Z, Moreno CS *et al.* Consolidation of the cancer genome into domains of repressive chromatin by long-range epigenetic silencing (LRES) reduces transcriptional plasticity. *Nat Cell Biol* 2010; **12**: 235–246.
93 Cha JD, Kim HJ, Cha IH. Genetic alterations in oral squamous cell carcinoma progression detected by combining array-based comparative genomic hybridization and multiplex ligation-dependent probe amplification. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; **111**: 594–607.
94 Pan D. Hippo signaling in organ size control. *Genes Dev* 2007; **21**: 886–897.
95 Zhao B, Li L, Lei Q, Guan KL. The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. *Genes Dev* 2010; **24**: 862–874.
96 Jiao S, Wang H, Shi Z, Dong A, Zhang W, Song X *et al.* A peptide mimicking VGLL4 function acts as a YAP antagonist therapy against gastric cancer. *Cancer Cell* 2014; **25**: 166–180.
97 Koontz LM, Liu-Chittenden Y, Yin F, Zheng Y, Yu J, Huang B *et al.* The Hippo effector Yorkie controls normal tissue growth by antagonizing scalloped-mediated default repression. *Dev Cell* 2013; **25**: 388–401.
98 Guo T, Lu Y, Li P, Yin MX, Lv D, Zhang W *et al.* A novel partner of Scalloped regulates Hippo signaling via antagonizing Scalloped-Yorkie activity. *Cell Res* 2013; **23**: 1201–1214.
99 Zhang W, Gao Y, Li P, Shi Z, Guo T, Li F *et al.* VGLL4 functions as a new tumor suppressor in lung cancer by negatively regulating the YAP-TEAD transcriptional complex. *Cell Res* 2014; **24**: 331–343.
100 Couzens AL, Knight JD, Kean MJ, Teo G, Weiss A, Dunham WH *et al.* Protein interaction network of the mammalian Hippo pathway reveals mechanisms of kinase-phosphatase interactions. *Sci Signal* 2013; **6**: rs15.
101 Hauri S, Wepf A, van Drogen A, Varjosalo M, Tapon N, Aebersold R *et al.* Interaction proteome of human Hippo signaling: modular control of the co-activator YAP1. *Mol Syst Biol* 2013; **9**: 713.
102 Hata Y, Timalsina S, Maimaiti S. Okadaic acid: a tool to study the hippo pathway. *Mar Drugs* 2013; **11**: 896–902.
103 Mohseni M, Sun J, Lau A, Curtis S, Goldsmith J, Fox VL *et al.* A genetic screen identifies an LKB1-MARK signalling axis controlling the Hippo-YAP pathway. *Nat Cell Biol* 2014; **16**: 108–117.
104 Polesello C, Huelsmann S, Brown NH, Tapon N. The *Drosophila* RASSF homolog antagonizes the hippo pathway. *Curr Biol* 2006; **16**: 2459–2465.
105 Gloerich M, ten Klooster JP, Vliem MJ, Koorman T, Zwartkruis FJ, Clevers H *et al.* Rap2A links intestinal cell polarity to brush border formation. *Nat Cell Biol* 2012; **14**: 793–801.
106 ten Klooster JP, Jansen M, Yuan J, Oorschot V, Begthel H, Di Giacomo V *et al.* Mst4 and Ezrin induce brush borders downstream of the Lkb1/Strad/Mo25 polarization complex. *Dev Cell* 2009; **16**: 551–562.
107 Wang W, Xiao ZD, Li X, Aziz KE, Gan B, Johnson RL *et al.* AMPK modulates Hippo pathway activity to regulate energy homeostasis. *Nat Cell Biol* 2015; **17**: 490–499.
108 Mo JS, Meng Z, Kim YC, Park HW, Hansen CG, Kim S *et al.* Cellular energy stress induces AMPK-mediated regulation of YAP and the Hippo pathway. *Nat Cell Biol* 2015; **17**: 500–510.

109 Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. *Physiol Rev* 2012; **92**: 689–737.
110 Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 2001; **81**: 807–869.
111 Dan I, Watanabe NM, Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. *Trends Cell Biol* 2001; **11**: 220–230.
112 Lin JL, Chen HC, Fang HI, Robinson D, Kung HJ, Shih HM. MST4, a new Ste20-related kinase that mediates cell growth and transformation via modulating ERK pathway. *Oncogene* 2001; **20**: 6559–6569.
113 Lin ZH, Wang L, Zhang JB, Liu Y, Li XQ, Guo L *et al.* MST4 promotes hepatocellular carcinoma epithelial-mesenchymal transition and metastasis via activation of the p-ERK pathway. *Int J Oncol* 2014; **45**: 629–640.
114 Xiong W, Knox AJ, Xu M, Kiseljak-Vassiliades K, Colgan SP, Brodsky KS *et al.* Mammalian Ste20-like kinase 4 promotes pituitary cell proliferation and survival under hypoxia. *Mol Endocrinol* 2015; **29**: 460–472.
115 Schmitt DC, Madeira da Silva L, Zhang W, Liu Z, Arora R, Lim S *et al.* ErbB2-intronic MicroRNA-4728: a novel tumor suppressor and antagonist of oncogenic MAPK signaling. *Cell Death Dis* 2015; **6**: e1742.
116 Chen CB, Ng JK, Choo PH, Wu W, Porter AG. Mammalian sterile 20-like kinase 3 (MST3) mediates oxidative-stress-induced cell death by modulating JNK activation. *Biosci Rep* 2009; **29**: 405–415.
117 Chen HW, Marinissen MJ, Oh SW, Chen X, Melnick M, Perrimon N *et al.* CKA, a novel multidomain protein, regulates the JUN N-terminal kinase signal transduction pathway in *Drosophila*. *Mol Cell Biol* 2002; **22**: 1792–1803.
118 Ashton-Beaucage D, Udell CM, Gendron P, Sahmi M, Lefrancois M, Baril C *et al.* A functional screen reveals an extensive layer of transcriptional and splicing control underlying RAS/MAPK signaling in *Drosophila*. *PLoS Biol* 2014; **12**: e1001809.
119 Ma X, Wang X, Gao X, Wang L, Lu Y, Gao P *et al.* Identification of five human novel genes associated with cell proliferation by cell-based screening from an expressed cDNA ORF library. *Life Sci* 2007; **81**: 1141–1151.
120 Losel R, Wehling M. Nongenomic actions of steroid hormones. *Nat Rev Mol Cell Biol* 2003; **4**: 46–56.
121 Bernelot Moens SJ, Schnitzler GR, Nickerson M, Guo H, Ueda K, Lu Q *et al.* Rapid estrogen receptor signaling is essential for the protective effects of estrogen against vascular injury. *Circulation* 2012; **126**: 1993–2004.
122 Coutinho P, Vega C, Pojoga LH, Rivera A, Prado GN, Yao TM *et al.* Aldosterone's rapid, nongenomic effects are mediated by striatin: a modulator of aldosterone's effect on estrogen action. *Endocrinology* 2014; **155**: 2233–2243.
123 Pojoga LH, Coutinho P, Rivera A, Yao TM, Maldonado ER, Youte R *et al.* Activation of the mineralocorticoid receptor increases striatin levels. *Am J Hypertens* 2012; **25**: 243–249.
124 Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. *Nature* 2010; **463**: 485–492.
125 Kazmierczak-Baranska J, Peczek L, Przygodzka P, Cieslak MJ. Downregulation of striatin leads to hyperphosphorylation of MAP2, induces depolymerization of microtubules and inhibits proliferation of HEK293T cells. *FEBS Lett* 2015; **589**: 222–230.
126 Shih PY, Lee SP, Chen YK, Hsueh YP. Cortactin-binding protein 2 increases microtubule stability and regulates dendritic arborization. *J Cell Sci* 2014; **127**: 3521–3534.
127 Chen YK, Hsueh YP. Cortactin-binding protein 2 modulates the mobility of cortactin and regulates dendritic spine formation and maintenance. *J Neurosci* 2012; **32**: 1043–1055.
128 Hashimoto S, Hirose M, Hashimoto A, Morishige M, Yamada A, Hosaka H *et al.* Targeting AMAP1 and cortactin binding bearing an atypical src homology 3/proline interface for prevention of breast cancer invasion and metastasis. *Proc Natl Acad Sci USA* 2006; **103**: 7036–7041.
129 Fehon RG, McClatchey AI, Bretscher A. Organizing the cell cortex: the role of ERM proteins. *Nat Rev Mol Cell Biol* 2010; **11**: 276–287.
130 Crawley SW, Mooseker MS, Tyska MJ. Shaping the intestinal brush border. *J Cell Biol* 2014; **207**: 441–451.
131 Fidalgo M, Guerrero A, Fraile M, Iglesias C, Pombo CM, Zalvide J. Adaptor protein cerebral cavernous malformation 3 (CCM3) mediates phosphorylation of the cytoskeletal proteins ezrin/radixin/moesin by mammalian Ste20-4 to protect cells from oxidative stress. *J Biol Chem* 2012; **287**: 11556–11565.
132 Zheng X, Xu C, Di Lorenzo A, Kleaveland B, Zou Z, Seiler C *et al.* CCM3 signaling through sterile 20-like kinases plays an essential role during zebrafish cardiovascular development and cerebral cavernous malformations. *J Clin Invest* 2010; **120**: 2795–2804.
133 Frost A, Elgort MG, Brandman O, Ives C, Collins SR, Miller-Vedam L *et al.* Functional repurposing revealed by comparing *S. pombe* and *S. cerevisiae* genetic interactions. *Cell* 2012; **149**: 1339–1352.
134 Rorth P. Collective cell migration. *Annu Rev Cell Dev Biol* 2009; **25**: 407–429.
135 Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J. Actin dynamics, architecture, and mechanics in cell motility. *Physiol Rev* 2014; **94**: 235–263.
136 Ridley AJ. Life at the leading edge. *Cell* 2011; **145**: 1012–1022.
137 Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol* 2009; **10**: 778–790.
138 Madsen CD, Hooper S, Tozluoglu M, Bruckbauer A, Fletcher G, Erler JT *et al.* STRIPAK components determine mode of cancer cell migration and metastasis. *Nat Cell Biol* 2015; **17**: 68–80.
139 Hyodo T, Ito S, Hasegawa H, Asano E, Maeda M, Urano T *et al.* Misshapen-like kinase 1 (MINK1) is a novel component of striatin-interacting phosphatase and kinase (STRIPAK) and is required for the completion of cytokinesis. *J Biol Chem* 2012; **287**: 25019–25029.
140 Preisinger C, Short B, De Corte V, Bruyneel E, Haas A, Kopajtich R *et al.* YSK1 is activated by the Golgi matrix protein GM130 and plays a role in cell migration through its substrate 14-3-3zeta. *J Cell Biol* 2004; **164**: 1009–1020.
141 Andreazza S, Bouleau S, Martin B, Lamouroux A, Ponien P, Papin C *et al.* Daytime CLOCK dephosphorylation is controlled by STRIPAK complexes in *Drosophila*. *Cell Rep* 2015; **11**: 1266–1279.
142 Lant B, Yu B, Goudreault M, Holmyard D, Knight JD, Xu P *et al.* CCM-3/STRIPAK promotes seamless tube extension through endocytic recycling. *Nat Commun* 2015; **6**: 6449.
143 Record CJ, Chaikuad A, Rellos P, Das S, Pike AC, Fedorov O *et al.* Structural comparison of human mammalian ste20-like kinases. *PLoS One* 2010; **5**: e11905.
144 Henderson JL, Kormos BL, Hayward MM, Coffman KJ, Jasti J, Kurumbail RG *et al.* Discovery and preclinical profiling of 3-[4-(morpholin-4-yl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl]benzonitrile (PF-06447475), a highly potent, selective, brain penetrant, and *in vivo* active LRRK2 kinase inhibitor. *J Med Chem* 2015; **58**: 419–432.