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The Hippo pathway in organ development, homeostasis, and regeneration

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Abstract

The Hippo pathway is a universal governor of organ size, tissue homeostasis, and regeneration. A growing body of work has advanced our understanding of Hippo pathway regulation of cell proliferation, differentiation, and spatial patterning not only in organ development but also upon injury-induced regeneration. The pathway's central role in stem cell biology thus implicates its potential for therapeutic manipulation in mammalian organ regeneration. In this review, we survey recent literature linking the Hippo pathway to the development, homeostasis, and regeneration of various organs, including Hippo-independent roles for YAP, defined here as YAP functions that are not regulated by the Hippo pathway kinases LATS1/2.

Introduction to the *Drosophila* and mammalian Hippo pathway

Discovery of the Hippo pathway in *Drosophila* was a pivotal point of entry to understanding the molecular mechanisms that govern organ growth during development and regeneration. The *Drosophila* tumor suppressors *Hippo* (*hpo*), *Salvador* (*sav*), *Warts* (*wts*), and *Mob as tumor suppressor* (*mats*) comprise the pathway's core kinase cascade. The *hpo-sav* complex phosphorylates and activates the *wts-mats* complex, which phosphorylates *Yorkie* (*yki*), a transcriptional co-activator and the downstream effector of the Hippo pathway.

The pathway's mammalian orthologs consist of Mammalian Sterile 20-like kinases 1 and 2 (MST1/2), Salvador homolog 1 (SAV1), large tumor suppressor 1 and 2 (LATS1/2), and MOB kinase activator 1A and 1B (MOB1A/B), which regulate the downstream effectors Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) (Figure 1). Extensive studies have established the Hippo pathway's prominent role in human cancer, and this topic has been covered by recent reviews [1,2]. However, given the limited capacity of certain mammalian organs for regeneration upon injury, the Hippo pathway's dual roles in organ growth and stem cell maintenance is an area of great interest

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Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

with the potential to be therapeutically harnessed for regenerative purposes. Thus, in this review, we survey recent studies of the Hippo pathway in the development, homeostasis, and regeneration of various organs, including Hippo-independent roles for YAP, defined here as YAP functions that are not regulated by the Hippo pathway kinases LATS1/2. Furthermore, we briefly discuss the planarian as an emerging model organism for studying the Hippo pathway in tissue regeneration.

The Hippo pathway in the development and regeneration of different organs

Brain/central nervous system

Brain development requires a rigorous balance between the expansion of neural progenitors and the production of post-mitotic neurons and glial cells, and disrupting this equilibrium leads to structural abnormalities and dysfunction in the nervous system [3]. Several lines of evidence have implicated YAP in the regulation of neural stem cell (NSC) behavior. For instance, YAP is reported to interact with Smad to mediate bone morphogenetic protein (BMP) suppression of NSC differentiation and mediate Sonic hedgehog-induced neural progenitor proliferation [4]. During murine brain development, neurofibromatosis 2 (NF2) suppresses YAP activity to promote differentiation of guidepost cells in the corpus callosum [3]. In the developing central nervous system (CNS), downregulation of YAP is correlated with neural cell cycle exit, which is required for their terminal differentiation [5]. Furthermore, in *Drosophila*, *yki* is critical for maintaining neuroepithelial cells in an undifferentiated state, while *YAP* knockdown reduces neuron production in zebrafish embryos [6,7]. Together, these observations support an indispensable role for YAP in neural progenitor proliferation and differentiation.

Moreover, there is sufficient evidence that YAP plays an important role during brain development, and dysregulation of YAP can have severe consequences on brain size. One study identified a YAP-positive subpopulation of early, pre-migratory neural crest mesenchymal cells, and demonstrated that *YAP* expression was inversely correlated with human neuronal differentiation [8]. Furthermore, YAP acted synergistically with retinoic acid signaling to regulate neural crest fate and migration. In *Drosophila*, *yki* was recently linked to neuroblast biology downstream of the energy-sensing *Lkb1* and *AMPK* kinases, where *lkb1* restricted *yki* activity in larval neuronal cells in the central brain and ventral nerve cord (VNC) independently of *wts* [9]. The Hippo pathway was most recently shown to restrict *Drosophila* brain size by modulating larval neuroblasts, where depletion of upstream kinases *tao-1*, *hpo*, or *wts* by RNAi led to a significant increase in brain volume; deletion of *hpo* or *tao-1*, or mutation of *wts*, in VNC neuroblasts also significantly increased clonal volume [10].

Given the Hippo pathway's critical role in brain development, its recent association with several neurodevelopmental diseases is perhaps unsurprising. During brain development, NSCs coordinate cell cycle entry and exit with developmental timing to ensure that the correct neuron type and number are specified in a strict temporal sequence. NSC deregulation can thus give rise to either an under-sized or oversized brain, and microcephaly

is one such disorder resulting in an undersized brain along with other developmental complications. Microcephaly is associated with mutations in Cyclin-dependent kinase 5 regulatory subunit 2 (CDK5RAP2), and MST1 was recently identified as a CDK5RAP2 interaction partner, while CDK5RAP2 was also found to regulate YAP/TAZ, suggesting a potential role for the Hippo pathway in CDK5RAP2-associated microcephaly [11]. Another neurodevelopmental disease linked to the Hippo pathway is hydrocephalus, a disease characterized by enlarged cerebrospinal fluid (CSF)-filled ventricles, severe mental retardation, and motor dysfunction. Hydrocephalus is caused by abnormal generation and maturation of the ependymal cells that line the ventricular surface, and a recent study demonstrated that YAP was required for generating ependymal cells and maintaining structural integrity of the ventricular system. Nervous system-specific deletion of *YAP* led to severe hydrocephalus phenotypes in mice, and Yap overexpression rescued a lysophosphatidic acid-induced model of hydrocephalus [12[●]]. These results highlight a novel function of YAP in maintaining tissue junctions during normal development and after fetal brain injury, thereby suggesting its potential as a therapeutic target for certain neurodevelopmental diseases.

Heart

Organ size is especially critical during heart development: the heart must be sufficiently large to generate a physiological cardiac output, yet not so large as to inhibit cardiac outflow, as is the case with cardiomyopathies [13]. The Hippo pathway plays a well-established role in the developmental control of mammalian cardiac size. Deletion of MST1/2 or LATS1/2 increases cardiomyocyte proliferation during mouse embryogenesis, and overexpression of a constitutively active YAP in either the embryonic or post-natal mouse heart increases cardiomyocyte proliferation and cardiac size [13–15]. YAP and TAZ play redundant roles in cardiac growth, and dual deletion of both proteins leads to dose-dependent cardiomyopathy [16]. While cardiomyocytes retain some regenerative capacity at birth, this capacity is largely lost during postnatal development, during which the Hippo pathway inhibits cardiomyocyte proliferation to limit cardiac regeneration in the adult heart [13,17].

Though its role in cardiac size control has been well-established, the mechanism by which the Hippo pathway restricts cardiac growth has yet to be fully elucidated. Recent work has shown that the murine FAT tumor suppressor homolog 4 (FAT4) restricts cardiomyocyte proliferation through a non-canonical mechanism involving the scaffold protein Angiomotin-like 1 (AMOTL1), independent of LATS1/2-mediated YAP phosphorylation [18]. AMOTL1 physically interacts with both YAP and FAT4, and this FAT4 complex sequesters YAP in the cytoplasm, thereby preventing excessive YAP-mediated cardiac growth.

The Hippo pathway's regulation of cardiac development has also garnered interest in the potential roles that the pathway might play in cardiac regeneration following injury. For instance, SAV knockout adult cardiomyocytes have been shown to increase proliferative capacity and expression levels of YAP target genes, many of which are normally expressed in the fetal mammalian heart, including components of the dystrophin–glycoprotein complex (DGC), a complex critical for cardiac regeneration [19]. Indeed, it was more recently shown

that the DGC inhibits cardiomyocyte proliferation through direct interaction of one of its components, dystroglycan 1 (DAG1) with YAP. Specifically, Hippo-mediated YAP phosphorylation promotes the interaction between DAG1 and YAP, and the DGC and Hippo pathway cooperatively inhibits YAP nuclear localization to prevent its transcriptional activity [20●●]. Finally, a recent study by Tian *et al.* explored the use of microRNAs (miRNAs) in Hippo-mediated cardiac regeneration. The authors identified the microRNA cluster miR302–367 as a Hippo pathway inhibitor that mediated cardiomyocyte proliferation during development via repression of *MST1*, *LATS2*, and *MOB1B*. Importantly, administration of miR302–367 following induced myocardial infarction reactivated the cardiomyocyte cell cycle and reduced scar formation, thus providing a proof-of-concept for microRNA-based approaches to transiently induce cardiac regeneration following injury [21●]. These collective studies hold exciting implications for the therapeutic harnessing of the Hippo pathway in promoting transient regeneration during cardiac development or upon injury.

Liver

Adult liver cells are normally quiescent, yet the liver possesses a striking capacity for regeneration following surgical resection or injury, during which fully differentiated adult hepatocytes emerge from quiescence to divide and proliferate [22]. The Hippo pathway is already a well-established regulator of liver size: *MST1/2* are required for maintaining quiescence in the adult liver and their dual deletion leads to YAP activation, liver overgrowth, and hepatocellular carcinoma (HCC) [23]. Early studies already established that *YAP* overexpression in the liver similarly leads to liver overgrowth and HCC in mice [24–26]. Furthermore, YAP is partly responsible for regulating the transcriptional program associated with the fetal-to-adult switch during liver maturation, through modulation of transcriptional regulators of hepatocyte specification and function including hepatocyte nuclear factor 4 (HNF4a) and forkhead box A2 (FOXA2) [27,28].

In addition to its role in restricting YAP/TAZ to prevent overgrowth, the Hippo pathway may also play a role in liver fibrosis. Hepatic stellate cells (HSCs) are activated in response to liver damage and are the major cell type involved in liver fibrosis. Recent work has shown that YAP/TAZ are overexpressed in fibrotic liver and activated HSCs, suggesting that elevated levels of YAP/TAZ play a role in HSC activation. Interestingly, omega-3 polyunsaturated fatty acids (ω -3 PUFAs) inhibited HSC activation and CCl₄-induced liver fibrosis by promoting YAP/TAZ degradation [29]. Another recent study demonstrated that YAP activation selectively eliminates damaged hepatocytes by switching cell fate from proliferation to migration/apoptosis in response to cell stresses such as ethanol damage in both liver sinusoidal epithelial cells and hepatocytes [30].

During liver development, hepatoblasts arising from the endoderm develop into mature hepatocytes or biliary epithelial cells. Recent work has shown that *LATS1/2* are redundantly required for differentiation and maturation during mouse liver development in both the biliary epithelial and hepatocyte lineages [31]. Deletion of *LATS1/2* resulted in perinatal lethality, excess biliary epithelial proliferation, and a failure of hepatoblast maturation, while *YAP* overexpression repressed the transcriptional network associated with normal

hepatocyte maturation [31]. Thus, this study demonstrates the appreciable fine-tuning of YAP activity required in regulating the balance between organ growth and differentiation in the liver.

Intestine

The gut epithelium must maintain its integrity and function amidst constant microbial and environmental threats. Indeed, intestinal epithelial cells (IECs) have evolved the capacity for rapid regeneration upon insult and harbor an appropriately short half-life to do so: the small intestinal epithelia, for instance, turn over completely every 4–5 days [32]. While YAP/TAZ are not required for normal intestinal turnover [33], their upstream regulators MST1/2 and SAV1 are indispensable for intestinal homeostasis. For example, mice harboring conditional knockouts of *MST1/2* in IECs exhibit drastic changes in intestinal architecture, including disorganized villi, epithelial dysplasia, and adenomas [34]. Furthermore, mice with *SAV1*-deficient small and large intestines display higher levels of polyp formation and enlarged crypt structures [35]. In light of the considerable body of work on Hippo in intestinal homeostasis, a comprehensive survey can be found in a more extensive recent review [32].

Crosstalk between the Hippo pathway and Wnt/ β -catenin signaling is central to the regulation of intestinal regeneration and homeostasis [32]. Several studies have demonstrated that the Hippo and Wnt pathways are intimately tied in regulation and function, yet the exact mechanistic role of YAP in Wnt signaling is still a subject of debate. Recent work identified the lysine methyltransferase suppressor of variegation 3–9-Enhancer of zeste-Trithorax domain 7 (SETD7) as an important cross-regulator between the two pathways in intestinal regeneration [36]. SETD7 was shown to be required for Wnt-mediated intestinal regeneration, and its control over Wnt signaling in turn required YAP [36].

In the colon, inflammatory cytokine prostaglandin E2 (PGE₂) plays an important role in regeneration and tumorigenesis [37]. Recent work has shown that PGE₂ positively regulates *YAP* expression in the mouse colon and human colon cancer cells, creating a positive feedback loop that then promoted colon regeneration following colitis *in vivo*.

Lung

During lung development, temporal coordination of proliferation and differentiation is critical for proper organization of the complex branch structures comprising the airway epithelia. Loss of *YAP* in the lung epithelium is sufficient to cause defective lung development [38], while deleting *MST1/2* in the developing lung results in alveolar defects independent of YAP localization [39], indicating that the Hippo pathway plays a critical role in regulating lung development. Consistent with these findings, recent work has shown that loss of *MST1/2* in the lung epithelium results in neonatal lethality from lung defects in mice; analysis of the mutant lungs revealed denser lung architecture, reduced airway space, and increased proliferation, all reflective of disrupted lung epithelial differentiation [40]. Genetic deletion of one allele of *YAP*, or one allele of both *YAP* and *TAZ*, is sufficient to rescue the lethality seen from loss of *MST1/2*, thereby supporting a conserved MST1/2-YAP/TAZ axis regulating lung growth during development [40].

Furthermore, the precise regulation of YAP localization is critical for distal–proximal patterning in the terminal differentiation of both human and mouse lung epithelium. Nuclear YAP is required for progenitor specification, while cytoplasmic YAP is a marker for proximal airway maturation; thus, YAP localization is intricately coordinated with airway epithelial cell fate [38]. Building upon this notion, Szymaniak *et al.* recently outlined a polarity-directed mechanism of YAP regulation by Crumbs protein homolog 3 (CRB3) in airway epithelial cell fate [41[●]]. In mouse and human airway epithelia, CRB3 controls apical-basal polarity and promotes interaction between LATS1/2 and YAP at apical junctions to increase YAP phosphorylation and cytoplasmic retention, thereby initiating airway progenitor differentiation.

Breast

The mammary epithelium undergoes various developmental phases: ductal elongation in the virgin mammary gland, proliferation and differentiation during pregnancy, and post-lactation-induced epithelial cell death [42]. Though the Hippo pathway has had longstanding associations with breast cancer, its role in mammary gland development has remained poorly understood until recent years. The Hippo pathway is dispensable in the virgin gland and is specifically required during pregnancy, when mammary epithelia undergo rapid proliferation and differentiation [42]. Surprisingly, Yap hyper-activation alone is insufficient to drive hyperplasia, only leading to failed terminal differentiation of the mammary epithelia. These observations parallel the role of the Hippo pathway in the intestine, where YAP is dispensable for intestinal development but required for injury-induced regeneration. Thus, the temporally restricted roles of the Hippo pathway suggest it is particularly important during times of robust tissue growth and morphogenesis [42].

Recent work has continued to advance our understanding of the Hippo pathway's role in mammary development. Britschgi *et al.* identified LATS1 and LATS2 as regulators of human breast epithelial cell fate using a high-content, image-based short hairpin RNA (shRNA) screen [43[●]]. The authors found that *LATS1/2* deletion promoted a luminal phenotype via expansion of bipotent and luminal progenitors. Interestingly, *LATS1/2* deletion stabilized both YAP/TAZ and the estrogen receptor alpha (ER α), suggesting non-canonical crosstalk between the Hippo pathway and ER α signaling that may have far-reaching implications in cell fate and development in other tissue types. Another recent study delineated a mechanism of Hippo-mediated regulation of breast epithelial cell fate, where poly(rC)-binding proteins (PCBPs) form a complex with the Hippo pathway components SAV1, MST1/2, and LATS1 to suppress YAP/TAZ activity [44].

Kidney

In the developing mammalian kidney, YAP promotes nephron formation within the nephron progenitor cell (NPC) population, while TAZ prevents renal cyst formation [45,46]. Meanwhile, two recent studies from the McNeill group have implicated additional Hippo pathway components in mammalian nephrogenesis. One study demonstrated a critical role for LATS1/2 in restricting YAP activity to promote NPC differentiation where genetic deletion of *LATS1/2* impaired epithelialization of NPCs by converting NPCs into myofibroblasts [47]. A second study identified an unexpected role for the Hippo pathway in

branching morphogenesis in the mouse kidney; deletion of either *NF2* or *LATS1/2* in the ureteric bud led to severe branching defects, an effect that was rescued by lowering expression of *YAP* or *TAZ* levels and was phenocopied by *YAP* overexpression [48].

Muscle

Several observations support a potential role for the Hippo pathway in the growth and remodeling of mammalian skeletal musculature. *YAP* was previously identified as a novel regulator of C2C12 myogenesis, while *Mst1* overexpression enhances skeletal muscular atrophy [49,50]. Recently, *YAP/TAZ* were reported to promote myogenic differentiation through activating the MEK5/ ERK5 pathway. Overexpression of either *YAP* or *TAZ* activated the ERK5 pathway to stimulate C2C12 myoblast differentiation [51]. Mechanistically, *YAP* and *TAZ* interact with different components of the ERK5 pathway, with *YAP* interacting with MEKK3 and ERK5, and *TAZ* with c-Abl; all of these interactions induce *YAP/TAZ* nuclear translocation and myogenic differentiation. Therefore, beyond their canonical role as transcriptional co-activators, *YAP/TAZ* also have a novel function in ERK5 regulation. In addition, *YAP* was recently found to be an important regulator of skeletal muscle fiber size, regulating muscle mass preservation following injury or disruption of the neuromuscular junction [52]. *YAP* levels declined during postnatal skeletal fiber maturation, while *YAP* overexpression induced hypertrophy. Finally, the authors found that *YAP* expression was induced in a *SOD^{G93A}* mouse model for neuromuscular disease upon denervation as a mechanism of minimizing neurogenic atrophy.

Craniofacial

Recent work has examined potential roles the Hippo pathway may play in craniofacial and dental development. *YAP* overexpression results in defective tooth morphogenesis and enamel knot mislocation [53,54]. In addition, Zhang *et al.* monitored the spatio-temporal expression of *Yap* in the developing rat molar and found that *YAP* and *TAZ* expression levels fluctuate during tooth development, suggesting that *YAP* and *TAZ* expression promotes enamel and crown formation, promotes stem cell maintenance, and decreased *YAP* and *TAZ* expression is correlated with ameloblast and odontoblast differentiation [55]. Together, these studies suggest that the Hippo pathway may play an important role in orofacial and tooth development.

In addition, a recent study by Wang *et al.* examined the function of *YAP* and *TAZ* in the mouse cranial neural crest (CNC) by generating compound *YAP* and *TAZ* conditional CNC mutants, and collecting embryos at different developmental stages to analyze the morphogenesis of several CNC-derived structures. *YAP* and *TAZ* mutant embryos exhibited defective craniofacial vascular development, reduced proliferation, and increased apoptosis in the mandibular mesenchyme, while later-stage embryos also developed hydrocephalus [56]. Moreover, human genetic studies have demonstrated that heterozygous nonsense mutations in *YAP* are associated with various phenotypes in affected families, including orofacial clefts and intellectual disability [57]. Together, these studies suggest a potential, critical role for the Hippo pathway in craniofacial and tooth development.

Bone formation

The role of the Hippo pathway in osteogenesis has only recently been described. Using mouse and human mesenchymal stem/stromal cells (MSCs), Tang *et al.* reported that the transcription factors Snail and Slug regulate skeletal stem/stromal cell proliferation and differentiation by forming complexes with YAP/TAZ [58[●]]. YAP/TAZ protein levels were markedly reduced in the absence of Snail/Slug, as were the expression levels of Yap/Taz target genes, including *CTGF*, *DKK1*, and *CYR61*. It will be interesting to further elucidate how the Snail/Slug-YAP/TAZ axis is regulated, and whether Snail/Slug regulation of YAP/TAZ is confined to MSC development or is also relevant in the adult setting following maturation (*i.e.* self-renewal, regeneration following bone injury, *etc.*). [59].

The Hippo pathway in planarian size and regeneration

The freshwater planarian flatworm *Schmidtea mediterranea* has emerged as a unique and powerful model organism for studying adult stem cell (ASC) biology *in vivo*. The planarian's large ASC population (10%) accounts for the organism's remarkable ability to regenerate following nearly any injury, regardless of severity, while rescaling the entire organism in proportion to the amount of remaining tissue [60,61]. The planarian can thus be used to understand the active processes of growth — proliferation, spatial patterning, and scaling — within the context of adult regeneration.

S. mediterranea contains a single ortholog of Yap, *Smed-yki*, which plays a conserved role in maintaining the structural homeostasis and patterning of the organism's excretory system. Interestingly, while *Smed-yki* is required for restricting the size and proliferation of the stem cell compartment, *Smed-yki(RNAi)* planarians also cannot regenerate [60]. These observations indicate pleiotropic roles for *Smed-yki* in planarians, and it will be interesting to further dissect the multi-faceted roles of *Smed-yki* signaling in tissue-specific contexts.

Recent work has also begun to characterize the role of *Smed-yki* in response to injury, specifically in stem cell proliferation and early transcriptional response. *Smed-yki* is critical for regulating the wound response. *Smed-yki (RNAi)* animals exhibited temporally dysregulated expression of injury-induced genes, and increased muscle mass and epidermal populations [61]. Furthermore, *Smed-yki*-deficient animals were unable to maintain proper size and scaling of the brain, midline, and gut during regeneration. These data suggest that *Smed-yki* is a key restrictive regulator that ensures the correct scaling during the regenerative response to injury.

Concluding remarks and areas of future inquiry

The Hippo pathway has emerged as an exciting and potent regulator of organ development, homeostasis, and regeneration. A growing body of work has advanced our understanding of the Hippo pathway's involvement in mechanisms of progenitor differentiation, proliferative control, and spatial patterning during organ development, thus supporting the potential for therapeutic manipulation of this pathway for transient regeneration following injury. Here we highlight some remaining questions for future inquiry.

In the majority of studies, YAP/TAZ promote stemness and inhibit differentiation, although YAP/TEAD have also been shown to promote differentiation of trophoblasts [62]. More recently, YAP/TAZ were shown to play dual roles in both stem cell proliferation and differentiation of intestinal epithelia into goblet cells [63[●]]. Is there a unifying set of signals applicable to all cell types/organisms that dictates the renewal/differentiation roles of YAP/TAZ?

Many signals are capable of regulating the Hippo pathway at the cellular level. What are the physiological signals responsible for size regulation by the Hippo pathway at the organ and tissue levels? Do different organs use different signals to control organ/tissue mass by modulating the Hippo pathway? Given the Hippo pathway's cell-type-dependent roles in the regulation of cell size, one might speculate that it would similarly behave in an organ-specific manner in its control of organ size.

How does the size and mass of a particular organ coordinate with that of other organs? Is the Hippo pathway involved in this process?

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increases bipotent and luminal progenitors, the proposed cells-of-origin of most human breast cancers. Mechanistically, the authors reveal a direct interaction between Hippo components and oestrogen receptor- α (ER α) signalling. Their findings reveal a novel, noncanonical role of LATS1/2 in the regulation of human breast cell fate.

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Figure 1.

Schematic of the mammalian Hippo pathway. The core Hippo pathway comprises the MST1/2 and LATS1/2 kinases, their cofactors SAV1 and MOB1A/B, transcriptional co-activators YAP and TAZ, and the TEAD1–4 family of transcription factors. When the pathway is active, the LATS kinases phosphorylate YAP/TAZ, which are sequestered and degraded in the cytoplasm. When the kinases are inactive, YAP/TAZ accumulate in the nucleus where they cooperate with TEAD factors to promote the expression of target genes. Core Hippo pathway components are shown in green, with the exception of YAP/TAZ, which are shown in pink. Recently identified interactors and regulators of the Hippo pathway described in this review are shown in purple. Dashed circles represent all possible complexes and interactions formed by Hippo components. The yellow arrow represents upregulation of the LATS-YAP interaction by CRB3. Abbreviations: PCBP = poly(rC)-binding protein; ER α = estrogen receptor alpha; DCAF = Ddb1-cullin4-associated-factor;

DAG1 = dystroglycan; FAT4 = FAT tumor suppressor homolog 4; AMOTL1 = angiomin-like 1; CRB3 = crumbs protein homolog 3.

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