Title

Hippo Pathway in Organ Size Control, Tissue Homeostasis, and Cancer

Journal

Cell, 163(4)

ISSN

0092-8674

Authors

Yu, Fa-Xing
Zhao, Bin
Guan, Kun-Liang

Publication Date

2015-11-01

DOI

10.1016/j.cell.2015.10.044

Peer reviewed

HHS Public Access

Author manuscript

Cell. Author manuscript; available in PMC 2016 November 05.

Published in final edited form as:

Cell. 2015 November 5; 163(4): 811–828. doi:10.1016/j.cell.2015.10.044.

Hippo Pathway in Organ Size Control, Tissue Homeostasis, and Cancer

Fa-Xing Yu[1], **Bin Zhao**[2], and **Kun-Liang Guan**[3]

[1]Children's Hospital and Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China

[2]Life Sciences Institute and Innovation Center for Cell Signaling Network, Zhejiang University, Hangzhou, Zhejiang 310058, China

[3]Department of Pharmacology and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Abstract

Two decades of studies in multiple model organisms have established the Hippo pathway as a key regulator of organ size and tissue homeostasis. By inhibiting YAP and TAZ transcription co-activators, the Hippo pathway regulates cell proliferation, apoptosis, and stemness in response to a wide range of extracellular and intracellular signals, including cell-cell contact, cell polarity, mechanical cues, ligands of G-protein coupled receptors, and cellular energy status. Dysregulation of the Hippo pathway exerts a significant impact on cancer development. Further investigation of the functions and regulatory mechanisms of this pathway will help uncovering the mystery of organ size control and identify new targets for cancer treatment.

The emergence of multicellular organisms is an evolutionary milestone. Among the most fundamental mechanisms supporting multicellularity, are those that ensure the proper size and shape of tissues and organs to meet the need of functionality. However, despite intensive investigations into the underlying principles behind a “preset” size of organs, we are far from having a clear picture to this basic question in developmental biology. Nevertheless, investigations of the Hippo pathway on organ size control have shed light into this mystery (Halder and Johnson, 2011; Pan, 2010; Yu and Guan, 2013).

In 1995, two studies in *Drosophila* discovered that deletion of *Warts* (*Wts*) gene resulted in dramatic overgrowth of multiple tissues (Justice et al., 1995; Xu et al., 1995). Several years later, a flurry of studies showed that *Salvador* (*Sav*) (Kango-Singh et al., 2002; Tapon et al., 2002), *Hippo* (*hpo*) (Harvey et al., 2003; Jia et al., 2003; Pantalacci et al., 2003; Udan et al., 2003; Wu et al., 2003), and *Mob as tumor suppressor* (*Mats*) (Lai et al., 2005) mutant *Drosophila* phenocopy *Wts* mutants with regards to tissue overgrowth. Hpo, Sav, Wts, and

Correspondence: kuguan@ucsd.edu.

Mats interact genetically and physically, and the remarkable organ size phenotype elicited by their mutation is unprecedented in other established developmental pathways, thus they were grouped into a new signaling module — the Hippo pathway — named after the enormous size of *Hpo* mutant organs which resemble that of a hippopotamus. *Yorkie* (*yki*), the key functional effector of the Hippo pathway in organ size regulation, was soon discovered in a screen for Wts-interacting proteins (Huang et al., 2005).

The Hippo pathway is highly conserved in mammals. The mammalian orthologs of *Hpo*, *Sav*, *Wts*, *Mats*, and *Yki* are *Mammalian sterile 20-like 1 /2* (*MST1/2*, also called *STK4/3*), *Salvador* (*SAV1*), *Large tumor suppressor homolog 1/2* (*LATS1/2*), *MOB kinase activator 1A/B* (*MOB1a/b*), and Yes-associated protein (YAP) / Transcriptional co-activator with PDZ binding motif (*TAZ*, also called *WWTR1*), respectively (Halder and Johnson, 2011; Pan, 2010; Yu and Guan, 2013) (Table 1). The Hippo pathway quickly attracted broad attention due to its remarkable potency in regulating organ size, as well as its apparent relevance to tissue regeneration and cancer. Here, with an emphasis on recent developments, we review the current understanding of the organization, regulation, and function of the Hippo pathway and discuss some key open questions.

YAP/TAZ and Yki as Major Effectors of Hippo Signaling

In *Drosophila*, deleting *Yki* suppresses the overgrowth phenotypes of *Hpo*, *Sav*, or *Wts* mutants (Huang et al., 2005). In mice, deleting *YAP* also diminishes the overgrowth phenotypes caused by deficiency of *MST1/2* or other upstream regulators (Zhang et al., 2010; Zhou et al., 2011). Thus, Yki and YAP/TAZ are the evolutionarily-conserved key effectors of the Hippo pathway.

Yki and YAP/TAZ are believed to mediate the biological functions of the Hippo pathway by regulating gene transcription. As transcriptional co-activators, Yki and YAP/TAZ cannot bind DNA directly, and they must interact with DNA-binding transcription factors to regulate target gene expression. In *Drosophila*, Scalloped (Sd) is a transcription factor and key partner of Yki which mediates the function of Yki in tissue growth (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008; Zhao et al., 2008). Similarly, in mammalian cells, the TEAD family transcription factors (TEAD1-4, orthologs of Sd) are key partners for YAP (Zhao et al., 2008). Several lines of evidence indicate that TEADs are the major partners of YAP in transcriptional regulation. For instance, a TEAD-binding deficient YAP mutant lost its ability to induce transcription of known YAP target genes (Zhao et al., 2008), and knock-in of the TEAD-binding deficient YAP (Y94A mutant) phenotypically mimics YAP knockout in the skin and heart (Schlegelmilch et al., 2011; von Gise et al., 2012). In addition, the majority of YAP and TEAD occupied sites in the genome are shared (Stein et al., 2015; Zanconato et al., 2015; Zhao et al., 2008), and when TEAD is fused with a VP16 transactivation domain, it produces a gene expression profile similar to that induced by active YAP (Ota and Sasaki, 2008). TEAD1-4 or Sd can bind to a consensus motif similar to the GTIIC sequence (TGGAATGT or ACATTCCA), and transcription reporters under control of GTIIC concatemers are now widely used to measure Hippo pathway activity (Dupont et al., 2011; Mohseni et al., 2014; Ota and Sasaki, 2008). Besides TEAD1-4, YAP/TAZ have also been shown to interact with other transcription factors including Smad,

RUNX1/2, p63/p73, and ErbB4 (reviewed in (Varelas, 2014)), although the functional significance of these transcription factors in Hippo pathway is less clear.

A strong transcriptional activation domain is present in YAP/TAZ, but absent in Yki. Nevertheless, human YAP can rescue the lethality resulting from Hippo pathway hyperactivation in *Drosophila*, indicating a functional conservation (Huang et al., 2005). Genome-wide assessment of chromatin binding status reveals that, in addition to occupancy at proximal promoters of target genes, YAP and TEAD largely exert their transcriptional activity by interacting with distal enhancers, suggesting that YAP, and probably TAZ and Yki, may regulate transcription via multiple mechanisms including recruitment of general transcription factors, modification of epigenetic markers, and modulation of chromatin structure (Lian et al., 2010; Stein et al., 2015; Zanconato et al., 2015) (Figure 1). Indeed, recent evidence shows that Yki can interact with the Brahma complex, GAGA factors, nuclear receptor coactivator 6 (NCoA6, a subunit of the Trithorax-related histone methyltransferase), and the Mediator complex (Jin et al., 2013; Oh et al., 2013; Qing et al., 2014), and TAZ can interact with the chromatin-remodeling complex SWI/SNF (Skibinski et al., 2014).

The transcriptional activity of Yki and YAP/TAZ is regulated in the nucleus by Tondu-domain-containing growth inhibitor (Tgi) and Vestigial-like family member 4 (VGLL4, an ortholog of Tgi). Tgi can directly compete with Yki for Sd binding, resulting in inhibition of Yki-regulated transcription (Guo et al., 2013; Koontz et al., 2013). When Hippo signaling is on, Tgi and Sd form a complex, leading to transcriptional repression; on the contrary, when Hippo signaling is off, Yki enters the nucleus and displaces Tgi from Sd, leading to expression of Yki target genes and tissue growth (Koontz et al., 2013). In mammals, VGLL4 similarly competes with YAP/TAZ for TEAD binding (Jiao et al., 2014; Zhang et al., 2014b) (Figure 1). However, whether YAP/TAZ functions simply by relieving a default repression of TEAD by VGLL4 has not been demonstrated. Interestingly, the expression of VGLL4 appears to be repressed by miR-130a—a microRNA (miRNA) that is directly induced by YAP, leading to amplification of YAP activity (Shen et al., 2015). A similar mechanism is also present in *Drosophila*, in which *Bantam*—a well-known Yki-induced miRNA (Nolo et al., 2006; Thompson and Cohen, 2006), can repress expression of Tgi (Shen et al., 2015).

Although their role as transcriptional co-activators is widely appreciated, YAP/TAZ and Yki may also repress expression of certain genes when bound to specific factors. For instance, YAP/TAZ can interact with the nucleosome remodeling and histone deacetylase (NuRD) complex, resulting in transcriptional repression (Kim et al., 2015). Moreover, YAP has been identified as a regulator for global miRNA biogenesis via modulation of miRNA processing enzymes Microprocessor or Dicer complex, suggesting a transcription-independent role for YAP (Chaulk et al., 2014; Mori et al., 2014). Hence YAP/TAZ and Yki may regulate gene expression via multiple mechanisms.

Gene expression signatures under YAP/TAZ and Yki hyperactivation (or ectopic expression) in *Drosophila* and different mammalian cell types have been profiled by independent studies. However, the overlap between these different gene profiling studies is

not high, suggesting that YAP/TAZ and Yki may regulate target gene expression in a tissue or cell type-specific manner. In *Drosophila*, some common Yki target genes are *Bantam*, *Diap1* and *cyclin E*, which may mediate the effect of Yki in inhibiting cell death and promoting cell proliferation (Huang et al., 2005; Nolo et al., 2006; Tapon et al., 2002; Thompson and Cohen, 2006). In mammals, connective tissue growth factor (CTGF) is a commonly used marker of YAP activation (Zhao et al., 2008), and many genes involved in cell proliferation, cell adhesion, and cell migration have also been identified as YAP targets (Stein et al., 2015). It is likely that a panel of genes work together to exert biological functions of YAP/TAZ.

MST1/2 and LATS1/2 Kinases Restrict YAP Activity

In the Hippo pathway, LATS1/2 directly phosphorylate and inhibit YAP/TAZ. Interestingly, YAP has five LATS1/2 target consensus motifs (HXRXXS), four of which are conserved in TAZ (Zhao et al., 2010). Phosphorylation of YAP on serine 127 (S127) generates a 14-3-3 binding site, and binding with 14-3-3 sequesters YAP in the cytoplasm (Dong et al., 2007; Zhao et al., 2007). In addition, phosphorylation of YAP on serine 381 (S381) triggers a subsequent phosphorylation by casein kinase 1 (CK1δ/ε) and activation of a phosphodegron, resulting in recruitment of $SCF^{beta\text{-}TRCP}$ E3 ligase, ubiquitination, and proteasomal degradation of YAP (Zhao et al., 2010). Thus, through regulating both YAP subcellular localization and protein stability, LATS1/2 ensures a spatial and temporal control of YAP activity (Figure 1). TAZ is regulated by LATS1/2 in a similar fashion, although degradation plays a more prominent role in TAZ regulation possibly due to an additional phosphodegron at its N-terminus (Lei et al., 2008; Liu et al., 2010a). The subcellular localization of Yki is regulated similarly by Wts phosphorylation and 14-3-3 binding. However, the phosphodegron and the phosphorylation-mediated degradation mechanisms are not conserved in Yki (Huang et al., 2005; Oh and Irvine, 2008). In addition, YAP and Yki have also been shown to be degraded via the autolysosomal pathway (Kwon et al., 2013; Liang et al., 2014), suggesting a potential role of YAP and Yki in vesicular membrane dynamics and related cellular processes such as autophagy.

LATS1/2 are activated by MST1/2 through several mechanisms. MST1/2 phosphorylate LATS1/2 at the C-terminal hydrophobic motif, which promotes LATS1/2 autophosphorylation at its activation loop. Furthermore, phosphorylation of MOB1 by MST1/2 enhances MOB1 interaction with the autoinhibitory domain of LATS1/2, leading to full activation of LATS1/2 (Callus et al., 2006; Chan et al., 2005; Praskova et al., 2004; Wu et al., 2003). In addition, SAV1 is also phosphorylated by MST1/2, and SAV1 functions as a partner of MST1/2 in promoting LATS1/2 phosphorylation (Callus et al., 2006; Tapon et al., 2002) (Figure 1). In *Drosophila*, Wts is regulated by Hpo, Sav, and Mats by a similar mechanism (Wehr et al., 2013).

Merlin (Mer), Expanded (Ex), and Kibra, three cell cortex-localized and cytoskeleton-interacting proteins, may function as a scaffold for core Hippo components at the apical domain for activation, as Sav, Hpo, and Wts have been shown to physically interact with Ex/Mer/Kibra (Baumgartner et al., 2010; Genevet et al., 2010; Hamaratoglu et al., 2006; Yu et al., 2010) (Table 1). In addition, the effect of Ex/Mer/Kibra on the Hippo pathway may be

mediated by Tao kinase 1 (Tao-1), which can directly phosphorylate and activate Hpo (Boggiano et al., 2011; Poon et al., 2011). In mammalian cells, Neurofibromin 2 (NF2, Mer ortholog) appears to play a more direct role in regulating LATS1/2 activity: it can directly interact with LATS1/2 and recruit LATS1/2 to the plasma membrane for activation by MST1/2 (Yin et al., 2013; Zhang et al., 2010) (Figures 2 and 3).

The *Drosophila* Hpo or MST1/2 are not absolutely required for regulation of Wts or LATS1/2. It has been observed that in mouse embryonic fibroblast (MEF) cells, MST1/2 double knockout did not abolish YAP phosphorylation, suggesting the existence of additional Hippo-like activity (Zhou et al., 2009). Indeed, a recent study in *Drosophila* has identified Misshapen (Msn) as another kinase responsible for Wts activation. This mechanism is also conserved in mammals, as MAP4K4 (Msn ortholog) overexpression promotes phosphorylation of LATS1/2 (Li et al., 2014), and MAP4K4 knockdown induces activity of a YAP reporter (Mohseni et al., 2014). It is possible that additional kinases, especially some STE20 family members, may activate LATS1/2 in response to different upstream signals or in different tissue contexts (Figures 2 and 3).

YAP/TAZ have also been shown to be phosphorylated by many other kinases such as cyclin-dependent kinase 1 (CDK1), Jun N-terminal kinases (JNK), homeodomain interacting protein kinases (HIPK), ABL, and Src family tyrosine kinases (reviewed in (Varelas, 2014)), suggesting that YAP/TAZ can be regulated by mechanisms independent of Hippo pathway kinases.

Cell Polarity and Cell Adhesion Regulate Hippo Signaling

In searching for upstream regulators of the Hippo pathway, many proteins involved in cell polarity and cell adhesion have been identified. Echinoid (Ed), a cell adhesion molecule in *Drosophila*, can interact with and stabilize Sav, and leads to activation of Hpo (Yue et al., 2012). In mammalian cells, several proteins at adherens and tight junctions, such as Angiomotin (AMOT), protein tyrosine phosphatase non-receptor type 14 (PTPN14), and α-Catenin can sequester YAP/TAZ at cell junctions (reviewed in (Yu and Guan, 2013)). Therefore cell adhesion and formation of intercellular junctions serve as a mechanism to repress YAP/TAZ transcriptional activity (Figures 2 and 3).

Crumbs (Crb), a component of apical-basal polarity, interacts with Ex which is critical for the apical localization of Ex/Mer/Kibra complex (Chen et al., 2010; Ling et al., 2010; Robinson et al., 2010). In addition, Scribble (SCRIB) interacts with both MST1/2 and LATS1/2, thus promoting LATS1/2 activation (Cordenonsi et al., 2011; Mohseni et al., 2014). Fat, a protocadherin which plays a key role in planar cell polarity, also activates the Hippo pathway, possibly through regulating Ex protein levels and localization (Bennett and Harvey, 2006; Silva et al., 2006; Tyler and Baker, 2007; Willecke et al., 2006) (Figure 2). However, mammalian Fat orthologs do not seem to be major regulators of the Hippo pathway (Sharma and McNeill, 2013). It is noteworthy that the link between cell polarity and the Hippo pathway may be indirect, and some proteins, such as Fat, may regulate cell polarity and Hippo signaling via different mechanisms (Matakatsu and Blair, 2012).

Cell Contact and Mechanical Cues Regulate Hippo Signaling

Cells in solid tissues communicate with neighboring cells and their extracellular matrix, and perceive constant physical signals from their local environment. Cell-cell contact was discovered as the first signal regulating the Hippo pathway (Zhao et al., 2007). In a sparse culture, YAP and TAZ are primarily localized in the nucleus to promote target gene transcription and cell proliferation. On the contrary, at high cell density, YAP and TAZ are primarily cytoplasmic, corresponding to growth inhibition. It is known that a cell ceases to proliferate following physical contact with surrounding cells, and loss of cell contact inhibition is an indicator of oncogenic transformation. Thus, regulation of YAP/TAZ by cell density suggests a critical role for the Hippo pathway in contact inhibition, tissue growth, and tumorigenesis.

Mechanical cues, such as ECM stiffness and cell geometry, are also potent regulators of YAP/TAZ (Dupont et al., 2011) (Figure 3). When cells are grown on a stiff matrix or are spread across a large surface, YAP and TAZ are activated. In contrast, when cells are seeded on a soft matrix or are compressed into a small area, YAP and TAZ are inactivated. ECM stiffness and cell geometry are important for cell proliferation and differentiation, and YAP/TAZ activity plays a role in these cellular processes. Cell geometry has also been proposed as a mechanism underlying Hippo pathway regulation by cell density: at low density, cells are flat and spread, leading to YAP activation; whereas at high density, cells adopt a round and compact geometry, resulting in YAP inactivation (Aragona et al., 2013; Wada et al., 2011). Further support for a role of YAP/TAZ in mechanosensing, mechanical strain and shear stress have been shown to stimulate YAP/TAZ, and YAP activation is required for mechanical strain-induced cell cycle reentry (Benham-Pyle et al., 2015; Codelia et al., 2014; Kim et al., 2014), and YAP also mediates changes in three dimensional body shape in response to tissue tension (Porazinski et al., 2015).

The attachment of cells to the ECM is an important mechanism for maintaining cell survival, and cells normally undergo anoikis when detached from the ECM. The attachment status of a cell to its ECM can also regulate Hippo pathway activity. YAP is activated during the process of cell attachment but inactivated when cells are detached (Zhao et al., 2012). Expression of constitutively active YAP promotes survival of detached cells, suggesting that cancer cells with high YAP activity may escape anoikis and undergo metastasis. In support of this notion, LATS1/2 expression was found to be selectively reduced in metastatic but not primary prostate tumors (Zhao et al., 2012).

Soluble Factors Regulate Hippo Signaling

The primary function of YAP/TAZ is to promote growth, and many mitogenic hormones and growth factors act through G-protein coupled receptors (GPCR) to induce cell proliferation (Figure 3). GPCR ligands signal through $G\alpha_{12/13}$ or $G\alpha_{q/11}$, such as lysophosphatidic acid, thrombin, angiotensin II, and estrogen, can activate YAP/TAZ; in contrast, ligands signal through $G\alpha_s$ and protein kinase A (PKA), such as epinephrine and glucagon, can repress YAP/TAZ activity (Kim et al., 2013; Miller et al., 2012; Mo et al., 2012; Wennmann et al., 2014; Yu et al., 2013a; Yu et al., 2012; Zhou et al., 2015).

Interestingly, activation of protein kinase C (PKC) by $G\alpha_{q/11}$ can either activate or inhibit YAP, with conventional PKC activating YAP and novel PKC inhibiting YAP (Gong et al., 2015). The remarkable differential functions of PKC in YAP regulation provide a mechanism to explain some of the cell type-specific responses to PKC activation. GPCR is the largest family of membrane receptors mediating diverse physiological or pathological responses. The demonstration of Hippo regulation by GPCRs links the Hippo pathway to a wide range of upstream signals and biological functions.

The Wnt/β-catenin pathway is a key signaling cascade in development and carcinogenesis. A destruction complex including Axin, adenomatous polyposis coli (APC) and glycogen synthase kinase-3 (GSK3) causes constant degradation of β-catenin. Wnt stimulation disrupts the destruction complex and leads to accumulation of β-catenin. Interestingly, recent studies suggest that YAP/TAZ are also activated by diverse Wnt family ligands. YAP/TAZ have been shown to be components of the destruction complex, and are regulated by Wnt in a fashion similar to that of β-catenin (Azzolin et al., 2014; Azzolin et al., 2012). However, a recent study suggests that Wnt activates YAP/TAZ via Frizzled (a GPCR-like Wnt receptor), $G\alpha_{12/13}$, Rho GTPases, and LATS1/2 (Park et al., 2015). In addition, APC has been shown to act as a scaffold protein for SAV1 and LATS1, and *Apc* deletion lead to YAP activation and tumorigenesis (Cai et al., 2015). More studies may be needed to verify the mechanism of YAP/TAZ activation by Wnt.

Epidermal growth factor (EGF) and insulin have also been shown to regulate YAP/Yki activity in cultured mammalian cells and *Drosophila*, and these signals are mediated by the Ras-Raf-MAPK signaling cascade or phosphoinositide-dependent kinase (PDK1) (Fan et al., 2013; Reddy and Irvine, 2013; Strassburger et al., 2012). TAZ is also stabilized upon PI3K activation, which is mediated by direct phosphorylation by GSK3 (Huang et al., 2012). However, no significant effect of EGF and IGF on YAP was observed in several other studies, and YAP activity appeared to be normal in the presence of inhibitors of PI3K or AKT, or in PDK1 null embryonic cells (Yu et al., 2012; Zhao et al., 2007). These discrepancies could be due to differences in experimental settings or cell types, and should be clarified by future studies.

Effect of Cellular Metabolic Status on Hippo Signaling

Recently, a link between cellular metabolic status and the Hippo pathway has been reported (Figure 3). Under energy deprivation, such as glucose starvation, the AMPK activated protein kinase (AMPK) can directly phosphorylate YAP at serine 61 (S61) and serine 94 (S94) (Mo et al., 2015; Wang et al., 2015). Phosphorylation of YAP S94 abolishes the interaction between YAP and TEADs, leading to inhibition of YAP activity. In addition, energy stress also inhibits YAP by increasing kinase activity of LATS1/2, either in an AMPK-dependent or -independent manner (Mo et al., 2015; Wang et al., 2015). AMPK can phosphorylate AMOTL1, which in turn facilitates YAP phosphorylation by LATS1/2 (DeRan et al., 2014). A similar mechanism is also present in *Drosophila*, in which Ampk inactivates Yki and affects cell proliferation in the larval central brain and central nerve cord (Gailite et al., 2015). It appears that during energy stress, both AMPK and LATS1/2 are unleashed to restrict YAP activity. These findings also suggest that the Hippo pathway may

mediate the anticancer effect of metformin, which is known to activate AMPK (DeRan et al., 2014; Mo et al., 2015). Other than AMPK, glucose may also promote YAP/TAZ activity through phosphofructokinase, which stimulates the interaction between YAP/TAZ and TEADs (Enzo et al., 2015).

YAP/TAZ activity has also been linked to oxygen availability. Under hypoxic conditions, the hypoxia-inducible factor 1 (HIF1) stimulates expression of SIAH1/2, two E3 ubiquitin ligases. SIAH1/2 then promote ubiquitination and degradation of LATS2, leading to YAP/TAZ activation (Ma et al., 2015; Xiang et al., 2014) (Figure 3). In addition, HIF1 directly induces transcription of TAZ (Xiang et al., 2014), and YAP interacts with and stabilizes HIF1 to enhance transcription of HIF1 target genes (Ma et al., 2015).

YAP/TAZ are potent stimulators of cell growth and proliferation, which are energy-consuming processes. The regulation of Hippo signaling by AMPK suggests that metabolic status can function as a checkpoint for growth–promoting activity of YAP/TAZ. Under conditions of nutrient deprivation or energy crisis, YAP/TAZ activity needs to be restricted to prevent energy exhaustion caused by anabolic processes. Oxygen also plays a critical role in cellular metabolism, and hypoxia is involved in different pathological processes such as cancer. The link between hypoxia and YAP/TAZ activity indicates a role of YAP/TAZ in mediating oncogenic effect of hypoxia.

Actin Cytoskeleton Integrates Upstream Signals

The actin cytoskeleton and Rho GTPases are not only important in maintaining cell morphology, but also play important roles in regulating cell proliferation and differentiation (Jaffe and Hall, 2005). Manipulation of the actin cytoskeleton, such as overexpression of Rho GTPases or inhibition of Rho by C3 toxin, dramatically modulates YAP/TAZ activity (Dupont et al., 2011; Yu et al., 2012; Zhao et al., 2012). Rho GTPases and changes in actin cytoskeleton dynamics have been demonstrated to be key mediators of mechanical cues, GPCR ligands, and cell attachment in regulating the Hippo pathway (Figure 3). Consistently, deletion of different regulators of the actin cytoskeleton impacts YAP and Yki activity. For instance, loss of actin-capping proteins or the *Capulet* gene (inhibits actin polymerization) in *Drosophila* results in Yki activation and tissue overgrowth (Fernandez et al., 2011; Sansores-Garcia et al., 2011). Similarly, knockdown of actin-capping proteins or filamentous actin (F-actin) severing proteins (cofilin or gelsolin) in mammalian cells also leads to YAP activation (Aragona et al., 2013). In general, Rho GTPase activity and F-actin appear to activate YAP/TAZ, whereas destabilization of F-actin inhibits YAP/TAZ (Dupont et al., 2011; Miller et al., 2012; Wada et al., 2011; Yu et al., 2012; Zhao et al., 2012).

Spectrin proteins, in association with short actin filaments, are organized into an elastic polygonal meshwork which lines the intracellular side of the plasma membrane. In epithelial cells, localization of the spectrin network is usually polarized and present in both apical and basolateral domains. In addition to a supporting role for cell structure, the spectrin network may transmit diverse signals from cell microenvironment to regulate cellular functions (Bennett and Gilligan, 1993). Recently, three independent studies revealed a regulatory role of the spectrin network on the Hippo pathway, and disruption of the spectrin network in

multiple *Drosophila* tissues leads to activation of Yki and tissue outgrowth, and a similar phenomenon is also observed in mammalian cells (Deng et al., 2015; Fletcher et al., 2015; Wong et al., 2015) (Figures 2 and 3). Therefore, spectrins or associated actin filaments may function as a major node for integrating upstream signals, such as mechanical cues.

Despite its apparent importance, it remains unclear how the actin cytoskeleton regulates activity of Hippo pathway core kinases. One possibility is that multiple Hippo pathway components are enriched at the apical domain via an actin-mediated mechanism which facilitates signal transduction, and actin remodeling may reinforce or disrupt the clustering of Hippo pathway components. In another scenario, the Hippo pathway could be regulated by contractile actomyosin and cellular tension (Figure 3). Inhibition of tension-related enzymes, such as non-muscle myosin (by Blebbistatin), Rho kinases (ROCK, by Y27632), and myosin light chain kinase (by ML-7) results in YAP/TAZ inhibition (Dupont et al., 2011; Wada et al., 2011). However, how tension is sensed by the Hippo pathway kinases is not fully understood. Treating cells with small molecules to inhibit cellular tension may also affect actin dynamics, so it is difficult to separate the effects of actin remodeling and tension generated by actomyosin. However, it has been proposed that in *Drosophila*, Ajuba (Jub) plays a critical role in tension-induced Yki regulation. In response to high tension, Jub recruits Wts to intercellular junctions via interactions with α-catenin, thereby inhibiting Wts activity (Rauskolb et al., 2014). In addition, JNK activation upon mechanical strain has also been shown to repress LATS1 (Codelia et al., 2014).

Although it has been initially reported that YAP regulation by mechanical signals is independent of LATS1/2, recent studies suggest that LATS1/2 are involved in YAP/TAZ regulation by the actin cytoskeleton (Miller et al., 2012; Wada et al., 2011; Yu et al., 2012; Zhao et al., 2012; Zhao et al., 2007). This discrepancy may be due to incomplete LATS1/2 depletion in knockdown experiments reported in earlier studies. Supporting a role for LATS1/2, the phosphorylation status and *in vitro* kinase activity of LATS1/2 are clearly regulated upon actin cytoskeleton rearrangement, and the kinetics are similar to that of YAP/TAZ phosphorylation (Yu et al., 2012; Zhao et al., 2012). The regulation of Yki/YAP/TAZ by spectrins or cell attachment/detachment has been shown dependent on Wts and LATS1/2 (Deng et al., 2015; Fletcher et al., 2015; Zhao et al., 2012). Moreover, during the preimplantation stage of mouse embryo development, LATS1/2 are essential for YAP regulation by small molecules targeting the actin cytoskeleton (Kono et al., 2014). Collectively, mechanical signals most likely act through Wts and LATS1/2 to influence the activity and function of Yki and YAP/TAZ.

Negative Feedback Regulation and Crosstalk

High Yki or YAP/TAZ activity, especially over long-term, results in tissue overgrowth or cancer (see below). Thus the physiological fluctuation of Hippo signaling must be tightly controlled to avoid detrimental effects. In *Drosophila*, regulation of the Hippo pathway is fine-tuned by a built-in negative feedback loop in which activation of Yki turns on the expression of upstream reglators including Four-jointed, Ex, Mer, Kibra, and Wts (Cho et al., 2006; Genevet et al., 2010; Hamaratoglu et al., 2006; Jukam et al., 2013). Consistently, a similar negative feedback mechanism also exists in mammalian cells. YAP/TAZ directly

induce the transcription of *NF2*, *LATS2*, and probably *MST1*, leading to LATS1/2 activation and YAP/TAZ inhibition (Chen et al., 2015b; Dai et al., 2015; Moroishi et al., 2015b). In addition, YAP/TAZ induce expression of angiomotin-like protein 2 (AMOTL2), a negative regulator of YAP (Mohseni et al., 2014; Zhao et al., 2008). This negative feedback loop is critical for maintaining the proper transient activation of YAP/TAZ upon stimulation. By these mechanisms, YAP and TAZ antagonize each other and may provide a buffer for fluctuations in Hippo pathway activity to ensure tissue homeostasis. When this negative feedback is disrupted, dysregulation of the Hippo pathway may lead to tumorigenesis.

Elucidating the Hippo pathway regulation and function is complicated by crosstalk between the Hippo pathway and many other developmental signaling pathways (reviewed in (Varelas, 2014)). Besides Wnt, YAP/TAZ may also be regulated by sonic hedgehog (SHH) signaling (Fernandez et al., 2009). On the other hand, YAP/TAZ has been shown to regulate the expression of ligands for Wnt, Shh, transforming growth factor beta (TGF-β), JAK-STAT, EGFR, and Notch pathways (reviewed in (Yu et al., 2015)). The extensive signaling crosstalk may create a microenvironment rich in different factors, which in turn regulates cell fate through autocrine or paracrine mechanisms in both cell autonomous and non-autonomous manners.

Hippo Pathway in Early Embryonic Development

YAP/TAZ are critical during early embryonic development. Although TAZ knockout mice are viable, YAP knockout mice die at E8.5, and blastomeres stop dividing before the morula (16-32 cells) stage when YAP and TAZ are both deleted (Hossain et al., 2007; Makita et al., 2008; Morin-Kensicki et al., 2006; Nishioka et al., 2009; Tian et al., 2007). Therefore, the role of YAP and TAZ in early development is partially overlapping.

The first cell-fate specification during embryogenesis occurs during preimplantation stage, in which the trophectoderm (TE) and inner cell mass (ICM) are formed. The TE consists of the outer cells of the blastocyst and forms extraembryonic tissues, while the ICM contains the inner cells of the blastocyst and give rise to the embryo proper and other tissues. The formation of the TE and ICM is mainly due to the position or polarity of cells in the morula, in which the inner and apolar cells form the ICM, while the outer and polar cells give rise to the TE (Sasaki, 2015). As early as the 16-cell stage, YAP/TAZ already show differential subcellular localization between inner and outer cells, and this difference lasts to the blastocyst stage. The different distribution of YAP/TAZ in the TE and ICM results in different gene expression signatures, especially the induction of TE-specific genes, such as *Cdx2* in outer cells, thus directing cell fate specification (Nishioka et al., 2009). Indeed, mouse embryos with TEAD4 knockout failed to develop TE cells, with all cells transforming into ICM. On the other hand, depleting LATS1/2, NF2, or AMOT/AMOTL2 turns all cells into TE linage, and these embryos failed to develop ICM-derived tissues (Cockburn et al., 2013; Hirate et al., 2013; Lorthongpanich et al., 2013). These results suggest that the Hippo pathway plays a key role in early embryonic cell specification.

Hippo Signaling in Organ Size Control and Tissue Homeostasis

The effect on organ size is the best-known physiological function of the Hippo pathway. In *Drosophila*, mutation of Hippo pathway kinases (*Hpo* and *Wts*) or upstream regulators (*Ex*, *Mer*, *Kibra*, *Ft*, etc.) leads to overgrowth of organs such as eyes, wings, or other appendages, and transgenic expression of *Yki* results in a similar phenotype (reviewed in (Halder and Johnson, 2011; Pan, 2010)). The increased tissue/organ size is mainly due to Yki-induced cell proliferation and survival.

The effect of the Hippo pathway on organ size is highly conserved in mammals as revealed by many studies performed in mice (summarized in Table 2). For instance, liver specific transgenic *Yap* expression in mice can produce a dramatically enlarged liver. Remarkably, the liver returns to its normal size via apoptosis once *Yap* overexpression is turned off (Camargo et al., 2007; Dong et al., 2007). Similarly, liver specific knockout of *Mst1/2*, *Sav1*, or *Nf2* also results in liver enlargement (Yin et al., 2013; Zhang et al., 2010; Zhou et al., 2009). The mouse embryonic heart is enlarged when *Sav1*, *Mst1/2*, or *Lats1/2* is deleted, and proliferation or apoptosis of cardiomyocytes is sensitive to genetic manipulation of *Yap* (Del Re et al., 2013; Heallen et al., 2011; Lin et al., 2014; von Gise et al., 2012; Xin et al., 2013; Xin et al., 2011).

However, not all organs are equally sensitive to Hippo pathway mutations. For instance, *Mst1/2* knockout results in dramatic overgrowth of liver, heart, stomach, and spleen, but not of kidney, lung, or limbs (Song et al., 2010). A possible explanation is that there are MST1/2-independent regulators of YAP/TAZ in these tissues. In the breast and intestine, tissue-specific deletion of *Yap* does not result in any defects in tissue structure or size (Cai et al., 2010; Chen et al., 2014). *Yap* knockout in the mouse liver leads to bile duct defects but not a general reduction of liver size. This lack of effect could be due to the presence of TAZ, which should be activated due to the loss of feedback inhibition upon *Yap* deletion. Alternatively, the Hippo pathway, or YAP/TAZ activity, may be negligible for the size control of some organs. Nevertheless, the organ specific effects of the Hippo pathway in size control may suggest that different principles are utilized for size regulation in different organs. For example, organ size could be determined by proliferation of differentiated cells or the number of progenitor cells in a pre-allocated pool.

Physiological signals upstream of the Hippo pathway important in organ size determination have yet to be identified. Mechanical force or tension may change as a result of organ growth and inhibit YAP/TAZ when the organ reaches its final size. Alternatively, organ size may be restricted/induced by a soluble factor via autocrine/paracrine mechanisms, and the concentration of this factor is controlled by organ size. These two models are not mutually exclusive, and further investigations are needed to address how the Hippo pathway senses physiological cues to modulate organ size.

High YAP/TAZ activity has been observed in the stem or progenitor cells of multiple tissues, suggesting a role for YAP/TAZ in stem cell maintenance. For example, YAP is highly nuclear in basal progenitor cells and in intestinal stem cells localized at the crypt base (Barry et al., 2013; Camargo et al., 2007; Schlegelmilch et al., 2011; Zhang et al., 2011).

Activation of YAP, either by transgenic expression of YAP or deletion of upstream regulators, usually results in expansion of progenitor cells, impaired cell differentiation, and hyperplasia of target tissues such as intestine, liver, skin, and nervous system (Cai et al., 2010; Camargo et al., 2007; Cao et al., 2008; Lee et al., 2008; Lee et al., 2010; Lu et al., 2010; Zhou et al., 2011).

The role of YAP/TAZ on cell proliferation and stem cell expansion suggests a critical function of YAP in normal tissue development and homeostasis. Indeed, tissue-specific deletion of *Yap* results in abnormalities of the heart, skin, and kidney (Reginensi et al., 2013; Schlegelmilch et al., 2011; von Gise et al., 2012; Xin et al., 2011). However, mammary glands and the intestine remain relatively normal upon *Yap* deletion (Cai et al., 2010; Chen et al., 2014; Zhou et al., 2011). These findings suggest that YAP is required for development and homeostasis of some, but not all, tissues in mice. In human, *TEAD1* mutations are found in Sveinsson chorioretinal atrophy, a disease characterized by chorioretinal degeneration, and Aicardi syndrome, a congenital neurodevelopmental disorder (Fossdal et al., 2004; Schrauwen et al., 2015). In addition, loss-of-function mutations of *YAP* have been identified in both isolated and syndromic optic fissure closure defects (Williamson et al., 2014). Hence, the loss of TEAD-mediated YAP transcriptional activity plays a role in some degeneration related disorders in humans.

Even though it is not required for development and normal homeostasis of some tissues, YAP activity is critical for tissue regeneration upon certain types of damage. For example, *Yap* deletion severely compromises pregnancy-induced mammary tissue growth, although virgin mammary development was normal (Chen et al., 2014). Likewise, in wild type mice, the intestines can effectively regenerate following colitis induced by dextran sulfate sodium (DSS) treatment; however, the regenerative capability is severely impeded in conditional *Yap* knockout mice (Cai et al., 2010). Similar results have also been observed in *Drosophila* midgut regeneration upon DSS-induced injury (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010). Normally the liver regenerates efficiently following liver damage. For instance, after partial hepatectomy, hepatocytes start to proliferate to restore liver mass in a few days, and in this process, YAP activity is induced and is most likely required for complete liver regeneration (Grijalva et al., 2014; Su et al., 2015; Wu et al., 2013; Yimlamai et al., 2014). In contrast to the intestine and liver, tissue regeneration of the adult heart is very limited. However, inactivation of the Hippo pathway or transgenic expression of *Yap* restores some myocardial regenerative capability, although the efficiency is low. In contrast, cardiac-specific deletion of *Yap* impedes regeneration of the neonatal heart. (Heallen et al., 2013; Lin et al., 2014; Xin et al., 2013). Taken together, these results indicate that YAP plays a significant role in regeneration of multiple tissues.

Hippo Signaling in Cancer

Long-term YAP activation, such as transgenic expression of *Yap* in the mouse liver, results in cell transformation and tumor development (Dong et al., 2007), indicating the power of the Hippo pathway in cancer initiation and progression. Evidence of the Hippo pathway in tumorigenesis based on mouse models is summarized in Table 2, which generally supports

an oncogenic role for YAP and TAZ, as well as a tumor suppressive function for Hippo pathway upstream components.

The tumor-promoting activity of YAP is largely dependent on a TEAD-mediated transcription program, as YAP-induced liver cancer is fully blocked by expression of a dominant negative TEAD which is able to sequester both YAP and TAZ (Liu-Chittenden et al., 2012). At the cellular level, YAP activation is important for cell proliferation, survival, migration, and invasion. High YAP or TAZ activity enables the cell to escape contact inhibition and anoikis, and support anchorage-independent growth (Chan et al., 2008; Zhao et al., 2012; Zhao et al., 2007). YAP induces expression of ZEB1/2 to stimulate the epithelial-to-mesenchymal transition (EMT), which is a key step for tumor metastasis (Gao et al., 2014; Lei et al., 2008; Liu et al., 2010b; Overholtzer et al., 2006). In addition, YAP is able to promote genomic instability (Fernandez et al., 2012), and TAZ is required to sustain self-renewal and the tumor-initiation capacity of breast cancer stem cells (Cordenonsi et al., 2011).

Accumulating evidence suggests that the Hippo pathway is dysregulated in many human cancers. Elevated YAP/TAZ expression or nuclear enrichment of YAP/TAZ has been observed in many types of cancers, including liver, breast, lung, colon, ovary, and others (Chan et al., 2008; Moroishi et al., 2015a; Steinhardt et al., 2008). However, the majority of cancers with high YAP/TAZ activity have not been linked to genetic mutations of the Hippo pathway, and the overall genetic alteration rate of Hippo pathway components in human cancer is relatively low (Table 3).

One well-characterized example of a Hippo pathway mutation associated with cancer is in *NF2*, which causes neurofibromatosis 2 lesions including schwannomas and meningiomas (Xiao et al., 2003). Moreover, inactivating *NF2* mutations are also observed in 40-50% of malignant mesothelioma (Sekido, 2011). Importantly, even heterozygous deletion of *Yap* completely blocks liver tumorigenesis induced by *Nf2* knockout in mice, indicating that YAP activation is the major mechanism mediating the tumorigenic potential of *Nf2* mutations (Zhang et al., 2010).

YAP gene amplification may contribute to a portion of hepatocellular carcinomas, medulloblastomas, and esophageal squamous cell carcinomas (Fernandez et al., 2009; Overholtzer et al., 2006; Song et al., 2014; Zender et al., 2006). Gene fusions involving *YAP* and *TAZ* have also been discovered in human cancers. Remarkably, virtually all epithelioid hemangioendotheliomas contain gene fusions of *TAZ-CAMTA1*, *TAZ-FOSB*, or *YAP-TFE3* (Antonescu et al., 2013; Errani et al., 2011; Flucke et al., 2014; Tanas et al., 2011). In addition, *YAP* gene fusions with MAMLD1 or *C11orf95* have been discovered in a subset of ependymal tumors (Pajtler et al., 2015; Parker et al., 2014). It is worth noting that in both epithelioid hemangioendotheliomas and ependymal tumors, all YAP/TAZ fusions proteins retain their N-terminal TEAD binding domain, but lose the C-terminal transactivation domain. These observations suggest that these YAP fusions may still bind to and activate the TEAD dependent transcriptional program to promote tumorigenesis. Indeed, neural stem cells carrying the *YAP-C11orf95* fusion gene can effectively form brain tumors when grafted into mice (Parker et al., 2014). In addition, a familial *YAP* point mutation (R331W) has also

been reported to correlate with a high incidence of lung adenocarcinomas (Chen et al., 2015a).

Aberrant GPCR signaling often results in tumorigenesis, so it is possible that GPCR dysregulation can cause cancer by activating YAP/TAZ. *GNAQ* or *GNA11* (encoding $G\alpha_q$ or $G\alpha_{11}$, respectively) activating mutations have been identified in approximately 80% of uveal melanomas and function as driver mutations (Van Raamsdonk et al., 2009; Van Raamsdonk et al., 2010). Recent studies showed that YAP is constitutively activated in mutant *GNAQ* or *GNA11* mutant uveal melanomas and the high YAP activity contributes to tumor growth (Feng et al., 2014; Yu et al., 2014). In mice, deletion of *GNAS* (encoding $G\alpha_S$) in skin stem cells initiates basal-cell carcinogenesis, which is partially dependent on YAP (Iglesias-Bartolome et al., 2015). Moreover, expression of a viral GPCR induces tumorigenesis in Kaposi's sarcoma, where YAP/TAZ also play a critical role (Liu et al., 2015).

LATS1/2 mutations or gene fusion have been sporadically identified in different cancers which may lead to YAP/TAZ activation (Table 3). In addition, Crosstalk with other cancer-related signaling pathways also likely contributes to high YAP/TAZ activity in cancers that have no mutations of Hippo pathway components. For example, *KRAS*, *APC*, and *LKB1* mutations have all been reported to activate YAP/TAZ (Azzolin et al., 2014; Gao et al., 2014; Mohseni et al., 2014; Zhang et al., 2014a).

YAP/TAZ activity is also linked to drug resistance and cancer relapse. Cultured breast cancer cells with high YAP/TAZ activity show resistance to drugs such as taxol, 5-fluorouracil, and doxorubicin (Cordenonsi et al., 2011; Lai et al., 2011; Touil et al., 2014). Furthermore, lung and colon cancer cells with high YAP activity are resistant to RAF- and MEK-targeted therapies (Lin et al., 2015b). Tamoxifen is commonly used to treat estrogen receptor (ER, a nuclear receptor) positive breast cancer; however, some ER positive breast cancers are insensitive to tamoxifen. Recently, tamoxifen has been shown to activate YAP/TAZ by stimulating the membrane estrogen receptor GPER, a GPCR. Therefore, activation of GPER by tamoxifen or estrogen may contribute to tumor growth and drug resistance (Zhou et al., 2015). Amplification of the *YAP* gene has been associated with cancer relapse in *KRAS*-driven colon and pancreatic cancers (Kapoor et al., 2014; Shao et al., 2014). Thus, inhibition of YAP/TAZ will not only target tumor initiation and progression, but also potentially sensitize cancer cells to chemotherapies and prevent cancer relapse.

Notably, in contrast to its oncogenic function in most solid tumors, YAP seems to play a tumor-suppressor role in hematological cancers. The *YAP* gene locus is frequently deleted in hematological cancer and expression of YAP or inhibition of MST1 leads to growth inhibition and increased apoptosis (Cottini et al., 2014). Currently, the underlying mechanism responsible for this tumor suppressor function of YAP in hematological cancers is not well understood.

Therapeutic Targeting of Hippo Signaling

The core Hippo pathway is a kinase cascade, and protein kinases are usually druggable. Thus, inhibitors for MAP4K4, MST1/2, or LATS1/2 may be developed to induce YAP/TAZ

activity and facilitate the process of wound healing, tissue repair, or regeneration, and possibly for treating degenerative diseases (Figure 4). For example, temporal inhibition of MST1/2 or LATS1/2 may promote myocardial regeneration or survival that would be beneficial for heart attack patients. It is also possible that inhibitors of MST1/2 or LATS1/2 could be used for treating hematological cancers.

Generally, MST1/2 and LATS1/2 are tumor suppressors, and inhibition of MST1/2 or LATS1/2 may promote tumor growth in most instances. On the other hand, inhibiting YAP/TAZ activity would offer a new and attractive anti-cancer strategy (Park and Guan, 2013). The function of YAP/TAZ is primarily mediated by TEADs, so small molecules disrupting the YAP/TAZ-TEAD interaction will function as YAP/TAZ inhibitors. Indeed, porphyrin family molecules, especially verteporfin, are able to disrupt the interaction between YAP/TAZ and TEADs, and verteporfin can block transcription of YAP/TAZ target genes and suppress liver overgrowth induced by YAP over-expression or NF2 inactivation in mice (Liu-Chittenden et al., 2012). However, verteporfin has general cellular toxicity and low aqueous solubility. Based on structural information from the YAP-TEAD and VGLL4-TEAD complex, a polypeptide termed “super-TDU” has been designed to block YAP-TEAD interaction, and has been shown to suppress tumor growth in mouse models (Jiao et al., 2014).

It is challenging to design direct activators for protein kinases. However, LATS1/2 may be activated indirectly by molecules targeting their upstream regulators. The very first small molecule (dobutamine) identified with an inhibitory effect on YAP is an antagonist of a GPCR receptor (Bao et al., 2011). Since then, many indirect inhibitors for YAP/TAZ have been identified, including phosphodiesterase inhibitors rolipram and ibudilast (Yu et al., 2013a). The Rho family GTPases have a strong inhibitory effect on LATS1/2, and membrane localization is important for Rho cellular function. Indeed, mevalonate metabolic pathway inhibitor statins can block membrane translocation of Rho GTPases and indirectly inhibit YAP/TAZ activity (Mi et al., 2015; Sorrentino et al., 2014; Wang et al., 2014). It will be interesting to test whether these drugs are effective in suppressing tumor growth in mouse models and perform epidemiologic studies to determine whether patients using statins or rolipram have a lower incidence of cancer. Given the important function of the Hippo pathway in regulating cell proliferation and tissue homeostasis, it represents an exciting and previously unexplored field for cancer therapy.

Acknowledgements

KLG is supported by grants from NIH (CA196878, CA132809, DE015964, EY022611, GM51586). FXY is supported by grants from "Thousand Youth Talents", Shanghai "Oriental Scholar", and NSFC (31571479). BZ is supported by grants from NSFC (31422036, 31271508, 31471316), State Key Development Program for Basic Research of China (2013CB945303), and the NSF of Zhejiang (LR12C07001). We thank Jean Guan, Hyun Woo Park, and Steven Plouffe for critical reading of the manuscript.

References

Antonescu CR, Le Loarer F, Mosquera JM, Sboner A, Zhang L, Chen CL, Chen HW, Pathan N, Krausz T, Dickson BC, et al. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. Gene Chromosome Canc. 2013; 52:775–784.

Aragona M, Panciera T, Manfrin A, Giulitti S, Michielin F, Elvassore N, Dupont S, Piccolo S. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. Cell. 2013; 154:1047–1059. [PubMed: 23954413]

Azzolin L, Panciera T, Soligo S, Enzo E, Bicciato S, Dupont S, Bresolin S, Frasson C, Basso G, Guzzardo V, et al. YAP/TAZ Incorporation in the beta-Catenin Destruction Complex Orchestrates the Wnt Response. Cell. 2014; 158:157–170. [PubMed: 24976009]

Azzolin L, Zanconato F, Bresolin S, Forcato M, Basso G, Bicciato S, Cordenonsi M, Piccolo S. Role of TAZ as mediator of Wnt signaling. Cell. 2012; 151:1443–1456. [PubMed: 23245942]

Bai H, Zhang N, Xu Y, Chen Q, Khan M, Potter JJ, Nayar SK, Cornish T, Alpini G, Bronk S, et al. Yes-associated protein regulates the hepatic response after bile duct ligation. Hepatology. 2012; 56:1097–1107. [PubMed: 22886419]

Bao YJ, Nakagawa K, Yang Z, Ikeda M, Withanage K, Ishigami-Yuasa M, Okuno Y, Hata S, Nishina H, Hata Y. A cell-based assay to screen stimulators of the Hippo pathway reveals the inhibitory effect of dobutamine on the YAP-dependent gene transcription. J Biochem. 2011; 150:199–208. [PubMed: 21586534]

Barry ER, Morikawa T, Butler BL, Shrestha K, de la Rosa R, Yan KS, Fuchs CS, Magness ST, Smits R, Ogino S, et al. Restriction of intestinal stem cell expansion and the regenerative response by YAP. Nature. 2013; 493:106–110. [PubMed: 23178811]

Baumgartner R, Poernbacher I, Buser N, Hafen E, Stocker H. The WW domain protein Kibra acts upstream of Hippo in Drosophila. Dev Cell. 2010; 18:309–316. [PubMed: 20159600]

Benham-Pyle BW, Pruitt BL, Nelson WJ. Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and beta-catenin activation to drive cell cycle entry. Science. 2015; 348:1024–1027. [PubMed: 26023140]

Benhamouche S, Curto M, Saotome I, Gladden AB, Liu CH, Giovannini M, McClatchey AI. Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. Genes Dev. 2010; 24:1718–1730. [PubMed: 20675406]

Bennett FC, Harvey KF. Fat cadherin modulates organ size in Drosophila via the Salvador/Warts/Hippo signaling pathway. Curr Biol. 2006; 16:2101–2110. [PubMed: 17045801]

Bennett V, Gilligan DM. The spectrin-based membrane skeleton and micron-scale organization of the plasma membrane. Annu Rev Cell Biol. 1993; 9:27–66. [PubMed: 8280463]

Boggiano JC, Vanderzalm PJ, Fehon RG. Tao-1 phosphorylates Hippo/MST kinases to regulate the Hippo-Salvador-Warts tumor suppressor pathway. Dev Cell. 2011; 21:888–895. [PubMed: 22075147]

Cai J, Maitra A, Anders RA, Taketo MM, Pan D. beta-Catenin destruction complex-independent regulation of Hippo-YAP signaling by APC in intestinal tumorigenesis. Genes Dev. 2015; 29:1493–1506. [PubMed: 26193883]

Cai J, Zhang N, Zheng Y, de Wilde RF, Maitra A, Pan D. The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program. Genes Dev. 2010; 24:2383–2388. [PubMed: 21041407]

Callus BA, Verhagen AM, Vaux DL. Association of mammalian sterile twenty kinases, Mst1 and Mst2, with hSalvador via C-terminal coiled-coil domains, leads to its stabilization and phosphorylation. FEBS J. 2006; 273:4264–4276. [PubMed: 16930133]

Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, Brummelkamp TR. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol. 2007; 17:2054–2060. [PubMed: 17980593]

Cao X, Pfaff SL, Gage FH. YAP regulates neural progenitor cell number via the TEA domain transcription factor. Genes Dev. 2008; 22:3320–3334. [PubMed: 19015275]

Chan EH, Nousiainen M, Chalamalasetty RB, Schafer A, Nigg EA, Sillje HH. The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1. Oncogene. 2005; 24:2076–2086. [PubMed: 15688006]

Chan SW, Lim CJ, Guo K, Ng CP, Lee I, Hunziker W, Zeng Q, Hong W. A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. Cancer Res. 2008; 68:2592–2598. [PubMed: 18413727]

Chaulk SG, Lattanzi VJ, Hiemer SE, Fahlman RP, Varelas X. The Hippo Pathway Effectors TAZ/YAP Regulate Dicer Expression and MicroRNA Biogenesis through Let-7. J Biol Chem. 2014; 289:1886–1891. [PubMed: 24324261]

Chen CL, Gajewski KM, Hamaratoglu F, Bossuyt W, Sansores-Garcia L, Tao C, Halder G. The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. Proc Natl Acad Sci U S A. 2010; 107:15810–15815. [PubMed: 20798049]

Chen HY, Yu SL, Ho BC, Su KY, Hsu YC, Chang CS, Li YC, Yang SY, Hsu PY, Ho H, et al. R331W Missense Mutation of Oncogene YAP1 Is a Germline Risk Allele for Lung Adenocarcinoma With Medical Actionability. J Clin Oncol. 2015a; 33:2303–2310. [PubMed: 26056182]

Chen Q, Zhang N, Gray RS, Li H, Ewald AJ, Zahnow CA, Pan D. A temporal requirement for Hippo signaling in mammary gland differentiation, growth, and tumorigenesis. Genes Dev. 2014; 28:432–437. [PubMed: 24589775]

Chen Q, Zhang N, Xie R, Wang W, Cai J, Choi KS, David KK, Huang B, Yabuta N, Nojima H, et al. Homeostatic control of Hippo signaling activity revealed by an endogenous activating mutation in YAP. Genes Dev. 2015b; 29:1285–1297. [PubMed: 26109051]

Cho E, Feng Y, Rauskolb C, Maitra S, Fehon R, Irvine KD. Delineation of a Fat tumor suppressor pathway. Nat Genet. 2006; 38:1142–1150. [PubMed: 16980976]

Chung C, Kim T, Kim M, Kim M, Song H, Kim TS, Seo E, Lee SH, Kim H, Kim SK, et al. Hippo-Foxa2 signaling pathway plays a role in peripheral lung maturation and surfactant homeostasis. Proc Natl Acad Sci U S A. 2013; 110:7732–7737. [PubMed: 23620511]

Cockburn K, Biechele S, Garner J, Rossant J. The Hippo pathway member Nf2 is required for inner cell mass specification. Curr Biol. 2013; 23:1195–1201. [PubMed: 23791728]

Codelia VA, Sun GP, Irvine KD. Regulation of YAP by Mechanical Strain through Jnk and Hippo Signaling. Curr Biol. 2014; 24:2012–2017. [PubMed: 25127217]

Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A, et al. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell. 2011; 147:759–772. [PubMed: 22078877]

Cottini F, Hideshima T, Xu C, Sattler M, Dori M, Agnelli L, ten Hacken E, Bertilaccio MT, Antonini E, Neri A, et al. Rescue of Hippo coactivator YAP1 triggers DNA damage-induced apoptosis in hematological cancers. Nat Med. 2014; 20:599–606. [PubMed: 24813251]

Dai X, Liu H, Shen S, Guo X, Yan H, Ji X, Li L, Huang J, Feng XH, Zhao B. YAP activates the Hippo pathway in a negative feedback loop. Cell Res. 2015 doi: 10.1038/cr.2015.1101.

Del Re DP, Yang Y, Nakano N, Cho J, Zhai P, Yamamoto T, Zhang N, Yabuta N, Nojima H, Pan D, et al. Yes-associated protein isoform 1 (Yap1) promotes cardiomyocyte survival and growth to protect against myocardial ischemic injury. J Biol Chem. 2013; 288:3977–3988. [PubMed: 23275380]

Deng H, Wang W, Yu J, Zheng Y, Qing Y, Pan D. Spectrin regulates Hippo signaling by modulating cortical actomyosin activity. Elife. 2015; 4:e06567. [PubMed: 25826608]

DeRan M, Yang J, Shen CH, Peters EC, Fitamant J, Chan P, Hsieh M, Zhu S, Asara JM, Zheng B, et al. Energy stress regulates hippo-YAP signaling involving AMPK-mediated regulation of angiomotin-like 1 protein. Cell Rep. 2014; 9:495–503. [PubMed: 25373897]

Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell. 2007; 130:1120–1133. [PubMed: 17889654]

Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011; 474:179–183. [PubMed: 21654799]

Enzo E, Santinon G, Pocaterra A, Aragona M, Bresolin S, Forcato M, Grifoni D, Pession A, Zanconato F, Guzzo G, et al. Aerobic glycolysis tunes YAP/TAZ transcriptional activity. EMBO J. 2015; 34:1349–1370. [PubMed: 25796446]

Errani C, Zhang L, Sung YS, Hajdu M, Singer S, Maki RG, Healey JH, Antonescu CR. A Novel WWTR1-CAMTA1 Gene Fusion Is a Consistent Abnormality in Epithelioid Hemangioendothelioma of Different Anatomic Sites. Gene Chromosome Canc. 2011; 50:644–653.

Fan R, Kim NG, Gumbiner BM. Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1. Proc Natl Acad Sci U S A. 2013; 110:2569–2574. [PubMed: 23359693]

Feng XD, Degese MS, Iglesias-Bartolome R, Vaque JP, Molinolo AA, Rodrigues M, Zaidi MR, Ksander BR, Merlino G, Sodhi A, et al. Hippo-Independent Activation of YAP by the GNAQ Uveal Melanoma Oncogene through a Trio-Regulated Rho GTPase Signaling Circuitry. Cancer Cell. 2014; 25:831–845. [PubMed: 24882515]

Fernandez BG, Gaspar P, Bras-Pereira C, Jezowska B, Rebelo SR, Janody F. Actin-Capping Protein and the Hippo pathway regulate F-actin and tissue growth in Drosophila. Development. 2011; 138:2337–2346. [PubMed: 21525075]

Fernandez LA, Northcott PA, Dalton J, Fraga C, Ellison D, Angers S, Taylor MD, Kenney AM. YAP1 is amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic hedgehog-driven neural precursor proliferation. Genes Dev. 2009; 23:2729–2741. [PubMed: 19952108]

Fernandez LA, Squatrito M, Northcott P, Awan A, Holland EC, Taylor MD, Nahle Z, Kenney AM. Oncogenic YAP promotes radioresistance and genomic instability in medulloblastoma through IGF2-mediated Akt activation. Oncogene. 2012; 31:1923–1937. [PubMed: 21874045]

Fletcher GC, Elbediwy A, Khanal I, Ribeiro PS, Tapon N, Thompson BJ. The Spectrin cytoskeleton regulates the Hippo signalling pathway. EMBO J. 2015; 34:940–954. [PubMed: 25712476]

Flucke U, Vogels RJ, de Saint Aubain Somerhausen N, Creytens DH, Riedl RG, van Gorp JM, Milne AN, Huysentruyt CJ, Verdijk MA, van Asseldonk MM, et al. Epithelioid Hemangioendothelioma: clinicopathologic, immunhistochemical, and molecular genetic analysis of 39 cases. Diagn Pathol. 2014; 9:131. [PubMed: 24986479]

Fossdal R, Jonasson F, Kristjansdottir GT, Kong A, Stefansson H, Gosh S, Gulcher JR, Stefansson K. A novel TEAD1 mutation is the causative allele in Sveinsson's chorioretinal atrophy (helicoid peripapillary chorioretinal degeneration). Hum Mol Genet. 2004; 13:975–981. [PubMed: 15016762]

Gailite I, Aerne BL, Tapon N. Differential control of Yorkie activity by LKB1/AMPK and the Hippo/Warts cascade in the central nervous system. Proc Natl Acad Sci U S A. 2015 doi: 10.1073/pnas.1505512112.

Gao T, Zhou D, Yang C, Singh T, Penzo-Mendez A, Maddipati R, Tzatsos A, Bardeesy N, Avruch J, Stanger BZ. Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. Gastroenterology. 2013; 144:1543–1553. 1553 e1541. [PubMed: 23454691]

Gao Y, Zhang W, Han X, Li F, Wang X, Wang R, Fang Z, Tong X, Yao S, Li F, et al. YAP inhibits squamous transdifferentiation of Lkb1-deficient lung adenocarcinoma through ZEB2-dependent DNp63 repression. Nat Commun. 2014; 5:4629. [PubMed: 25115923]

Genevet A, Wehr MC, Brain R, Thompson BJ, Tapon N. Kibra is a regulator of the Salvador/Warts/Hippo signaling network. Dev Cell. 2010; 18:300–308. [PubMed: 20159599]

George NM, Day CE, Boerner BP, Johnson RL, Sarvetnick NE. Hippo signaling regulates pancreas development through inactivation of Yap. Mol Cell Biol. 2012; 32:5116–5128. [PubMed: 23071096]

Gong R, Hong AW, Plouffe SW, Zhao B, Liu G, Yu FX, Xu Y, Guan KL. Opposing roles of conventional and nove PKC in the Hippo-YAP pathway regulation. Cell Res. 2015; 25:985–988. [PubMed: 26206313]

Goulev Y, Fauny JD, Gonzalez-Marti B, Flagiello D, Silber J, Zider A. SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in Drosophila. Curr Biol. 2008; 18:435–441. [PubMed: 18313299]

Grijalva JL, Huizenga M, Mueller K, Rodriguez S, Brazzo J, Camargo F, Sadri-Vakili G, Vakili K. Dynamic alterations in Hippo signaling pathway and YAP activation during liver regeneration. Am J Physiol Gastrointest Liver Physiol. 2014; 307:G196–204. [PubMed: 24875096]

Guo T, Lu Y, Li PX, Yin MX, Lv DK, Zhang WJ, Wang HZ, Zhou ZC, Ji HB, Zhao Y, et al. A novel partner of Scalloped regulates Hippo signaling via antagonizing Scalloped-Yorkie activity. Cell Res. 2013; 23:1201–1214. [PubMed: 23999857]

Halder G, Johnson RL. Hippo signaling: growth control and beyond. Development. 2011; 138:9–22. [PubMed: 21138973]

Hamaratoglu F, Willecke M, Kango-Singh M, Nolo R, Hyun E, Tao C, Jafar-Nejad H, Halder G. The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. Nat Cell Biol. 2006; 8:27–36. [PubMed: 16341207]

Harvey KF, Pfleger CM, Hariharan IK. The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis. Cell. 2003; 114:457–467. [PubMed: 12941274]

Heallen T, Morikawa Y, Leach J, Tao G, Willerson JT, Johnson RL, Martin JF. Hippo signaling impedes adult heart regeneration. Development. 2013; 140:4683–4690. [PubMed: 24255096]

Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, Martin JF. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. Science. 2011; 332:458–461. [PubMed: 21512031]

Hirate Y, Hirahara S, Inoue K, Suzuki A, Alarcon VB, Akimoto K, Hirai T, Hara T, Adachi M, Chida K, et al. Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. Curr Biol. 2013; 23:1181–1194. [PubMed: 23791731]

Hossain Z, Ali SM, Ko HL, Xu J, Ng CP, Guo K, Qi Z, Ponniah S, Hong W, Hunziker W. Glomerulocystic kidney disease in mice with a targeted inactivation of Wwtr1. Proc Natl Acad Sci U S A. 2007; 104:1631–1636. [PubMed: 17251353]

Huang J, Wu S, Barrera J, Matthews K, Pan D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell. 2005; 122:421–434. [PubMed: 16096061]

Huang W, Lv X, Liu C, Zha Z, Zhang H, Jiang Y, Xiong Y, Lei QY, Guan KL. The N-terminal phosphodegron targets TAZ/WWTR1 protein for SCFbeta-TrCP-dependent degradation in response to phosphatidylinositol 3-kinase inhibition. J Biol Chem. 2012; 287:26245–26253. [PubMed: 22692215]

Iglesias-Bartolome R, Torres D, Marone R, Feng X, Martin D, Simaan M, Chen M, Weinstein LS, Taylor SS, Molinolo AA, et al. Inactivation of a Galphas-PKA tumour suppressor pathway in skin stem cells initiates basal-cell carcinogenesis. Nat Cell Biol. 2015; 17:793–803. [PubMed: 25961504]

Jaffe AB, Hall A. Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol. 2005; 21:247–269. [PubMed: 16212495]

Jia JH, Zhang WS, Wang B, Trinko R, Jiang J. The Drosophila Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Gene Dev. 2003; 17:2514–2519. [PubMed: 14561774]

Jiao S, Wang HZ, Shi ZB, Dong AM, Zhang WJ, Song XM, He F, Wang YC, Zhang ZZ, Wang WJ, et al. A Peptide Mimicking VGLL4 Function Acts as a YAP Antagonist Therapy against Gastric Cancer. Cancer Cell. 2014; 25:166–180. [PubMed: 24525233]

Jin Y, Xu J, Yin MX, Lu Y, Hu L, Li P, Zhang P, Yuan Z, Ho MS, Ji H, et al. Brahma is essential for Drosophila intestinal stem cell proliferation and regulated by Hippo signaling. Elife. 2013; 2:e00999. [PubMed: 24137538]

Judson RN, Tremblay AM, Knopp P, White RB, Urcia R, De Bari C, Zammit PS, Camargo FD, Wackerhage H. The Hippo pathway member Yap plays a key role in influencing fate decisions in muscle satellite cells. J Cell Sci. 2012; 125:6009–6019. [PubMed: 23038772]

Jukam D, Xie B, Rister J, Terrell D, Charlton-Perkins M, Pistillo D, Gebelein B, Desplan C, Cook T. Opposite feedbacks in the Hippo pathway for growth control and neural fate. Science. 2013; 342:1238016. [PubMed: 23989952]

Justice RW, Zilian O, Woods DF, Noll M, Bryant PJ. The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes Dev. 1995; 9:534–546. [PubMed: 7698644]

Kango-Singh M, Nolo R, Tao C, Verstreken P, Hiesinger PR, Bellen HJ, Halder G. Shar-pei mediates cell proliferation arrest during imaginal disc growth in Drosophila. Development. 2002; 129:5719–5730. [PubMed: 12421711]

Kapoor A, Yao W, Ying H, Hua S, Liewen A, Wang Q, Zhong Y, Wu CJ, Sadanandam A, Hu B, et al. Yap1 activation enables bypass of oncogenic Kras addiction in pancreatic cancer. Cell. 2014; 158:185–197. [PubMed: 24954535]

Karpowicz P, Perez J, Perrimon N. The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. Development. 2010; 137:4135–4145. [PubMed: 21098564]

Kim KM, Choi YJ, Hwang JH, Kim AR, Cho HJ, Hwang ES, Park JY, Lee SH, Hong JH. Shear Stress Induced by an Interstitial Level of Slow Flow Increases the Osteogenic Differentiation of Mesenchymal Stem Cells through TAZ Activation. Plos One. 2014; 9:e92427. [PubMed: 24658423]

Kim M, Kim T, Johnson RL, Lim DS. Transcriptional Co-repressor Function of the Hippo Pathway Transducers YAP and TAZ. Cell Rep. 2015; 11:270–282. [PubMed: 25843714]

Kim M, Lee S, Kuninaka S, Saya H, Lee H, Lim DS. cAMP/PKA signalling reinforces the LATS-YAP pathway to fully suppress YAP in response to actin cytoskeletal changes. EMBO J. 2013; 32:1543–1555. [PubMed: 23644383]

Kono K, Tamashiro DA, Alarcon VB. Inhibition of RHO-ROCK signaling enhances ICM and suppresses TE characteristics through activation of Hippo signaling in the mouse blastocyst. Dev Biol. 2014; 394:142–155. [PubMed: 24997360]

Koontz LM, Liu-Chittenden Y, Yin F, Zheng YG, Yu JZ, Huang B, Chen Q, Wu S, Pan DJ. The Hippo Effector Yorkie Controls Normal Tissue Growth by Antagonizing Scalloped-Mediated Default Repression. Dev Cell. 2013; 25:388–401. [PubMed: 23725764]

Kwon Y, Vinayagam A, Sun XY, Dephoure N, Gygi SP, Hong PY, Perrimon N. The Hippo Signaling Pathway Interactome. Science. 2013; 342:737–740. [PubMed: 24114784]

Lai D, Ho KC, Hao YW, Yang XL. Taxol Resistance in Breast Cancer Cells Is Mediated by the Hippo Pathway Component TAZ and Its Downstream Transcriptional Targets Cyr61 and CTGF. Cancer Res. 2011; 71:2728–2738. [PubMed: 21349946]

Lai ZC, Wei X, Shimizu T, Ramos E, Rohrbaugh M, Nikolaidis N, Ho LL, Li Y. Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. Cell. 2005; 120:675–685. [PubMed: 15766530]

Lange AW, Sridharan A, Xu Y, Stripp BR, Perl AK, Whitsett JA. Hippo/Yap signaling controls epithelial progenitor cell proliferation and differentiation in the embryonic and adult lung. J Mol Cell Biol. 2015; 7:35–47. [PubMed: 25480985]

Lavado A, He Y, Pare J, Neale G, Olson EN, Giovannini M, Cao X. Tumor suppressor Nf2 limits expansion of the neural progenitor pool by inhibiting Yap/Taz transcriptional coactivators. Development. 2013; 140:3323–3334. [PubMed: 23863479]

Lavado A, Ware M, Pare J, Cao X. The tumor suppressor Nf2 regulates corpus callosum development by inhibiting the transcriptional coactivator Yap. Development. 2014; 141:4182–4193. [PubMed: 25336744]

Lee JH, Kim TS, Yang TH, Koo BK, Oh SP, Lee KP, Oh HJ, Lee SH, Kong YY, Kim JM, et al. A crucial role of WW45 in developing epithelial tissues in the mouse. EMBO J. 2008; 27:1231–1242. [PubMed: 18369314]

Lee KP, Lee JH, Kim TS, Kim TH, Park HD, Byun JS, Kim MC, Jeong WI, Calvisi DF, Kim JM, et al. The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci U S A. 2010; 107:8248–8253. [PubMed: 20404163]

Lei QY, Zhang H, Zhao B, Zha ZY, Bai F, Pei XH, Zhao S, Xiong Y, Guan KL. TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. Mol Cell Biol. 2008; 28:2426–2436. [PubMed: 18227151]

Li Q, Li S, Mana-Capelli S, Roth Flach RJ, Danai LV, Amcheslavsky A, Nie Y, Kaneko S, Yao X, Chen X, et al. The conserved misshapen-warts-Yorkie pathway acts in enteroblasts to regulate intestinal stem cells in Drosophila. Dev Cell. 2014; 31:291–304. [PubMed: 25453828]

Li SQ, Shen D, Shao JY, Crowder R, Liu WB, Prat A, He XP, Liu SY, Hoog J, Lu C, et al. Endocrine-Therapy-Resistant ESR1 Variants Revealed by Genomic Characterization of Breast-Cancer-Derived Xenografts. Cell Rep. 2013; 4:1116–1130. [PubMed: 24055055]

Lian I, Kim J, Okazawa H, Zhao J, Zhao B, Yu J, Chinnaiyan A, Israel MA, Goldstein LS, Abujarour R, et al. The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. Genes Dev. 2010; 24:1106–1118. [PubMed: 20516196]

Liang N, Zhang C, Dill P, Panasyuk G, Pion D, Koka V, Gallazzini M, Olson EN, Lam H, Henske EP, et al. Regulation of YAP by mTOR and autophagy reveals a therapeutic target of tuberous sclerosis complex. J Exp Med. 2014; 211:2249–2263. [PubMed: 25288394]

Lin C, Yao E, Chuang PT. A conserved MST1/2-YAP axis mediates Hippo signaling during lung growth. Dev Biol. 2015a; 403:101–113. [PubMed: 25912685]

Lin L, Sabnis AJ, Chan E, Olivas V, Cade L, Pazarentzos E, Asthana S, Neel D, Yan JJ, Lu X, et al. The Hippo effector YAP promotes resistance to RAF- and MEK-targeted cancer therapies. Nat Genet. 2015b; 47:250–256. [PubMed: 25665005]

Lin Z, von Gise A, Zhou P, Gu F, Ma Q, Jiang J, Yau AL, Buck JN, Gouin KA, van Gorp PR, et al. Cardiac-specific YAP activation improves cardiac function and survival in an experimental murine MI model. Circ Res. 2014; 115:354–363. [PubMed: 24833660]

Ling C, Zheng Y, Yin F, Yu J, Huang J, Hong Y, Wu S, Pan D. The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. Proc Natl Acad Sci U S A. 2010; 107:10532–10537. [PubMed: 20498073]

Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, Liu JO, Pan D. Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev. 2012; 26:1300–1305. [PubMed: 22677547]

Liu CY, Zha ZY, Zhou X, Zhang H, Huang W, Zhao D, Li T, Chan SW, Lim CJ, Hong W, et al. The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCF{beta}-TrCP E3 ligase. J Biol Chem. 2010a; 285:37159–37169. [PubMed: 20858893]

Liu G, Yu FX, Kim YC, Meng Z, Naipauer J, Looney DJ, Liu X, Gutkind JS, Mesri EA, Guan KL. Kaposi sarcoma-associated herpesvirus promotes tumorigenesis by modulating the Hippo pathway. Oncogene. 2015; 34:3536–3546. [PubMed: 25195862]

Liu Y, Xin Y, Ye F, Wang W, Lu Q, Kaplan HJ, Dean DC. Taz-tead1 links cell-cell contact to zeb1 expression, proliferation, and dedifferentiation in retinal pigment epithelial cells. Invest Ophthalmol Vis Sci. 2010b; 51:3372–3378. [PubMed: 20207963]

Lorthongpanich C, Messerschmidt DM, Chan SW, Hong W, Knowles BB, Solter D. Temporal reduction of LATS kinases in the early preimplantation embryo prevents ICM lineage differentiation. Genes Dev. 2013; 27:1441–1446. [PubMed: 23824537]

Lu L, Li Y, Kim SM, Bossuyt W, Liu P, Qiu Q, Wang Y, Halder G, Finegold MJ, Lee JS, et al. Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver. Proc Natl Acad Sci U S A. 2010; 107:1437–1442. [PubMed: 20080689]

Ma B, Chen Y, Chen L, Cheng H, Mu C, Li J, Gao R, Zhou C, Cao L, Liu J, et al. Hypoxia regulates Hippo signalling through the SIAH2 ubiquitin E3 ligase. Nat Cell Biol. 2015; 17:95–103. [PubMed: 25438054]

Mahoney JE, Mori M, Szymaniak AD, Varelas X, Cardoso WV. The hippo pathway effector Yap controls patterning and differentiation of airway epithelial progenitors. Dev Cell. 2014; 30:137–150. [PubMed: 25043473]

Makita R, Uchijima Y, Nishiyama K, Amano T, Chen Q, Takeuchi T, Mitani A, Nagase T, Yatomi Y, Aburatani H, et al. Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. Am J Physiol Renal Physiol. 2008; 294:F542–553. [PubMed: 18172001]

Matakatsu H, Blair SS. Separating planar cell polarity and Hippo pathway activities of the protocadherins Fat and Dachsous. Development. 2012; 139:1498–1508. [PubMed: 22399682]

McClatchey AI, Saotome I, Mercer K, Crowley D, Gusella JF, Bronson RT, Jacks T. Mice heterozygous for a mutation at the Nf2 tumor suppressor locus develop a range of highly metastatic tumors. Gene Dev. 1998; 12:1121–1133. [PubMed: 9553042]

Mi W, Lin Q, Childress C, Sudol M, Robishaw J, Berlot CH, Shabahang M, Yang W. Geranylgeranylation signals to the Hippo pathway for breast cancer cell proliferation and migration. Oncogene. 2015; 34:3095–3106. [PubMed: 25109332]

Miller E, Yang J, Deran M, Wu C, Su AI, Bonamy GM, Liu J, Peters EC, Wu X. Identification of Serum-Derived Sphingosine-1-Phosphate as a Small Molecule Regulator of YAP. Chem Biol. 2012; 19:955–962. [PubMed: 22884261]

Mitani A, Nagase T, Fukuchi K, Aburatani H, Makita R, Kurihara H. Transcriptional coactivator with PDZ-binding motif is essential for normal alveolarization in mice. Am J Respir Crit Care Med. 2009; 180:326–338. [PubMed: 19498055]

Miyanaga A, Masuda M, Tsuta K, Kawasaki K, Nakamura Y, Sakuma T, Asamura H, Gemma A, Yamada T. Hippo pathway gene mutations in malignant mesothelioma: revealed by RNA and targeted exon sequencing. J Thorac Oncol. 2015; 10:844–851. [PubMed: 25902174]

Mo JS, Meng Z, Kim YC, Park HW, Hansen CG, Kim S, Lim DS, Guan KL. Cellular energy stress induces AMPK-mediated regulation of YAP and the Hippo pathway. Nat Cell Biol. 2015; 17:500–510. [PubMed: 25751140]

Mo JS, Yu FX, Gong R, Brown JH, Guan KL. Regulation of the Hippo-YAP pathway by protease activated receptor PAR. Genes Dev. 2012; 29:2138–2143. [PubMed: 22972936]

Mohseni M, Sun J, Lau A, Curtis S, Goldsmith J, Fox VL, Wei C, Frazier M, Samson O, Wong KK, et al. A genetic screen identifies an LKB1-MARK signalling axis controlling the Hippo-YAP pathway. Nat Cell Biol. 2014; 16:108–117. [PubMed: 24362629]

Mori M, Triboulet R, Mohseni M, Schlegelmilch K, Shrestha K, Camargo FD, Gregory RI. Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. Cell. 2014; 156:893–906. [PubMed: 24581491]

Morin-Kensicki EM, Boone BN, Howell M, Stonebraker JR, Teed J, Alb JG, Magnuson TR, O'Neal W, Milgram SL. Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. Mol Cell Biol. 2006; 26:77–87. [PubMed: 16354681]

Moroishi T, Hansen CG, Guan KL. The emerging roles of YAP and TAZ in cancer. Nat Rev Cancer. 2015a; 15:73–79. [PubMed: 25592648]

Moroishi T, Park HW, Qin B, Chen Q, Meng Z, Plouffe SW, Taniguchi K, Yu FX, Karin M, Pan D, et al. A YAP/TAZ-induced feedback mechanism regulates Hippo pathway homeostasis. Genes Dev. 2015b; 29:1271–1284. [PubMed: 26109050]

Murakami H, Mizuno T, Taniguchi T, Fujii M, Ishiguro F, Fukui T, Akatsuka S, Horio Y, Hida T, Kondo Y, et al. LATS2 is a tumor suppressor gene of malignant mesothelioma. Cancer Res. 2011; 71:873–883. [PubMed: 21245096]

Nishio M, Hamada K, Kawahara K, Sasaki M, Noguchi F, Chiba S, Mizuno K, Suzuki SO, Dong Y, Tokuda M, et al. Cancer susceptibility and embryonic lethality in Mob1a/1b double-mutant mice. J Clin Invest. 2012; 122:4505–4518. [PubMed: 23143302]

Nishioka N, Inoue K, Adachi K, Kiyonari H, Ota M, Ralston A, Yabuta N, Hirahara S, Stephenson RO, Ogonuki N, et al. The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. Dev Cell. 2009; 16:398–410. [PubMed: 19289085]

Nolo R, Morrison CM, Tao C, Zhang X, Halder G. The bantam microRNA is a target of the hippo tumor-suppressor pathway. Curr Biol. 2006; 16:1895–1904. [PubMed: 16949821]

Oh H, Irvine KD. In vivo regulation of Yorkie phosphorylation and localization. Development. 2008; 135:1081–1088. [PubMed: 18256197]

Oh H, Slattery M, Ma L, Crofts A, White KP, Mann RS, Irvine KD. Genome-wide association of Yorkie with chromatin and chromatin-remodeling complexes. Cell Rep. 2013; 3:309–318. [PubMed: 23395637]

Ota M, Sasaki H. Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling. Development. 2008; 135:4059–4069. [PubMed: 19004856]

Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, Deng CX, Brugge JS, Haber DA. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A. 2006; 103:12405–12410. [PubMed: 16894141]

Pajtler KW, Witt H, Sill M, Jones DTW, Hovestadt V, Kratochwil F, Wani K, Tatevossian R, Punchihewa C, Johann P, et al. Molecular Classification of Ependymal Tumors across All CNS

Compartments, Histopathological Grades, and Age Groups. Cancer Cell. 2015; 27:728–743. [PubMed: 25965575]

Pan D. The hippo signaling pathway in development and cancer. Dev Cell. 2010; 19:491–505. [PubMed: 20951342]

Pantalacci S, Tapon N, Leopold P. The Salvador partner Hippo promotes apoptosis and cell-cycle exit in Drosophila. Nat Cell Biol. 2003; 5:921–927. [PubMed: 14502295]

Park HW, Guan KL. Regulation of the Hippo pathway and implications for anticancer drug development. Trends Pharmacol Sci. 2013; 34:581–589. [PubMed: 24051213]

Park HW, Kim YC, Yu B, Moroishi T, Mo JS, Plouffe SW, Meng Z, Lin KC, Yu FX, Alexander CM, et al. Alternative Wnt Signaling Activates YAP/TAZ. Cell. 2015; 162:780–794. [PubMed: 26276632]

Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, Lee R, Tatevossian RG, Phoenix TN, Thiruvenkatam R, et al. C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. Nature. 2014; 506:451–455. [PubMed: 24553141]

Poon CL, Lin JI, Zhang X, Harvey KF. The sterile 20-like kinase Tao-1 controls tissue growth by regulating the Salvador-Warts-Hippo pathway. Dev Cell. 2011; 21:896–906. [PubMed: 22075148]

Porazinski S, Wang H, Asaoka Y, Behrndt M, Miyamoto T, Morita H, Hata S, Sasaki T, Krens SF, Osada Y, et al. YAP is essential for tissue tension to ensure vertebrate 3D body shape. Nature. 2015; 521:217–221. [PubMed: 25778702]

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. [PubMed: 15109305]

Qing Y, Yin F, Wang W, Zheng Y, Guo P, Schozer F, Deng H, Pan D. The Hippo effector Yorkie activates transcription by interacting with a histone methyltransferase complex through Ncoa6. Elife. 2014; 3:e02564.

Rauskolb C, Sun S, Sun G, Pan Y, Irvine KD. Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex. Cell. 2014; 158:143–156. [PubMed: 24995985]

Reddy BV, Irvine KD. Regulation of Hippo signaling by EGFR-MAPK signaling through Ajuba family proteins. Dev Cell. 2013; 24:459–471. [PubMed: 23484853]

Reginensi A, Scott RP, Gregorieff A, Bagherie-Lachidan M, Chung C, Lim DS, Pawson T, Wrana J, McNeill H. Yap- and Cdc42-dependent nephrogenesis and morphogenesis during mouse kidney development. PLoS Genet. 2013; 9:e1003380. [PubMed: 23555292]

Ren F, Wang B, Yue T, Yun EY, Ip YT, Jiang J. Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. Proc Natl Acad Sci U S A. 2010; 107:21064–21069. [PubMed: 21078993]

Robinson BS, Huang J, Hong Y, Moberg KH. Crumbs regulates Salvador/Warts/Hippo signaling in Drosophila via the FERM-domain protein Expanded. Curr Biol. 2010; 20:582–590. [PubMed: 20362445]

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoangxuan K, Demczuk S, Desmaze C, Plougastel B, et al. Alteration in a New Gene Encoding a Putative Membrane-Organizing Protein Causes Neurofibromatosis Type-2. Nature. 1993; 363:515–521. [PubMed: 8379998]

Sansores-Garcia L, Bossuyt W, Wada K, Yonemura S, Tao C, Sasaki H, Halder G. Modulating F-actin organization induces organ growth by affecting the Hippo pathway. EMBO J. 2011; 30:2325–2335. [PubMed: 21556047]

Sasaki H. Position- and polarity-dependent Hippo signaling regulates cell fates in preimplantation mouse embryos. Semin Cell Dev Biol. 2015 doi:10.1016/j.semcdb.2015.1005.1003.

Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR, Zhou D, Kreger BT, Vasioukhin V, Avruch J, Brummelkamp TR, et al. Yap1 acts downstream of alpha-catenin to control epidermal proliferation. Cell. 2011; 144:782–795. [PubMed: 21376238]

Schrauwen I, Szelinger S, Siniard AL, Corneveaux JJ, Kurdoglu A, Richholt R, De Both M, Malenica I, Swaminathan S, Rangasamy S, et al. A De Novo Mutation in TEAD1 Causes Non-X-Linked Aicardi Syndrome. Invest Ophthalmol Vis Sci. 2015; 56:3896–3904. [PubMed: 26091538]

Sekido Y. Inactivation of Merlin in malignant mesothelioma cells and the Hippo signaling cascade dysregulation. Pathol Int. 2011; 61:331–344. [PubMed: 21615608]

Shao DD, Xue W, Krall EB, Bhutkar A, Piccioni F, Wang X, Schinzel AC, Sood S, Rosenbluh J, Kim JW, et al. KRAS and YAP1 converge to regulate EMT and tumor survival. Cell. 2014; 158:171–184. [PubMed: 24954536]

Sharma P, McNeill H. Fat and Dachsous cadherins. Prog Mol Biol Transl Sci. 2013; 116:215–235. [PubMed: 23481197]

Shaw RL, Kohlmaier A, Polesello C, Veelken C, Edgar BA, Tapon N. The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. Development. 2010; 137:4147–4158. [PubMed: 21068063]

Shen S, Guo X, Yan H, Lu Y, Ji X, Li L, Liang T, Zhou D, Feng XH, Zhao JC, et al. A miR-130a-YAP positive feedback loop promotes organ size and tumorigenesis. Cell Res. 2015; 25:997–1012. [PubMed: 26272168]

Silva E, Tsatskis Y, Gardano L, Tapon N, McNeill H. The tumor-suppressor gene fat controls tissue growth upstream of expanded in the hippo signaling pathway. Curr Biol. 2006; 16:2081–2089. [PubMed: 16996266]

Skibinski A, Breindel JL, Prat A, Galvan P, Smith E, Rolfs A, Gupta PB, Labaer J, Kuperwasser C. The Hippo transducer TAZ interacts with the SWI/SNF complex to regulate breast epithelial lineage commitment. Cell Rep. 2014; 6:1059–1072. [PubMed: 24613358]

Song H, Mak KK, Topol L, Yun K, Hu J, Garrett L, Chen Y, Park O, Chang J, Simpson RM, et al. Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression. Proc Natl Acad Sci U S A. 2010; 107:1431–1436. [PubMed: 20080598]

Song Y, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, et al. Identification of genomic alterations in oesophageal squamous cell cancer. Nature. 2014; 509:91–95. [PubMed: 24670651]

Sorrentino G, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S, Manfrin A, Ingallina E, Sommaggio R, Piazza S, et al. Metabolic control of YAP and TAZ by the mevalonate pathway. Nat Cell Biol. 2014; 16:357–366. [PubMed: 24658687]

Stein C, Bardet AF, Roma G, Bergling S, Clay I, Ruchti A, Agarinis C, Schmelzle T, Bouwmeester T, Schubeler D, et al. YAP1 Exerts Its Transcriptional Control via TEAD-Mediated Activation of Enhancers. PLoS Genet 11, e1005465. 2015

Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA, Anders RA. Expression of Yes-associated protein in common solid tumors. Hum Pathol. 2008; 39:1582–1589. [PubMed: 18703216]

Strassburger K, Tiebe M, Pinna F, Breuhahn K, Teleman AA. Insulin/IGF signaling drives cell proliferation in part via Yorkie/YAP. Dev Biol. 2012; 367:187–196. [PubMed: 22609549]

Su T, Bondar T, Zhou X, Zhang C, He H, Medzhitov R. Two-signal requirement for growth-promoting function of Yap in hepatocytes. Elife. 2015; 4:e02948.

Tanas MR, Sboner A, Oliveira AM, Erickson-Johnson MR, Hespelt J, Hanwright PJ, Flanagan J, Luo Y, Fenwick K, Natrajan R, et al. Identification of a disease-defining gene fusion in epithelioid hemangioendothelioma. Sci Transl Med. 2011; 3:98ra82.

Tapon N, Harvey KF, Bell DW, Wahrer DC, Schiripo TA, Haber DA, Hariharan IK. salvador Promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines. Cell. 2002; 110:467–478. [PubMed: 12202036]

Thompson BJ, Cohen SM. The Hippo pathway regulates the bantam microRNA to control cell proliferation and apoptosis in Drosophila. Cell. 2006; 126:767–774. [PubMed: 16923395]

Tian Y, Kolb R, Hong JH, Carroll J, Li D, You J, Bronson R, Yaffe MB, Zhou J, Benjamin T. TAZ promotes PC2 degradation through a SCFbeta-Trcp E3 ligase complex. Mol Cell Biol. 2007; 27:6383–6395. [PubMed: 17636028]

Touil Y, Igoudjil W, Corvaisier M, Dessein AF, Vandomme J, Monte D, Stechly L, Skrypek N, Langlois C, Grard G, et al. Colon Cancer Cells Escape 5FU Chemotherapy-Induced Cell Death by Entering Stemness and Quiescence Associated with the c-Yes/YAP Axis. Clin Cancer Res. 2014; 20:837–846. [PubMed: 24323901]

Tyler DM, Baker NE. Expanded and fat regulate growth and differentiation in the Drosophila eye through multiple signaling pathways. Dev Biol. 2007; 305:187–201. [PubMed: 17359963]

Udan RS, Kango-Singh M, Nolo R, Tao C, Halder G. Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. Nat Cell Biol. 2003; 5:914–920. [PubMed: 14502294]

Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature. 2009; 457:599–602. [PubMed: 19078957]

Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, Obenauf AC, Wackernagel W, Green G, Bouvier N, et al. Mutations in GNA11 in uveal melanoma. N Engl J Med. 2010; 363:2191–2199. [PubMed: 21083380]

Varelas X. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. Development. 2014; 141:1614–1626. [PubMed: 24715453]

von Gise A, Lin Z, Schlegelmilch K, Honor LB, Pan GM, Buck JN, Ma Q, Ishiwata T, Zhou B, Camargo FD, et al. YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. Proc Natl Acad Sci U S A. 2012; 109:2394–2399. [PubMed: 22308401]

Wada K, Itoga K, Okano T, Yonemura S, Sasaki H. Hippo pathway regulation by cell morphology and stress fibers. Development. 2011; 138:3907–3914. [PubMed: 21831922]

Wang W, Xiao ZD, Li X, Aziz KE, Gan B, Johnson RL, Chen J. AMPK modulates Hippo pathway activity to regulate energy homeostasis. Nat Cell Biol. 2015; 17:490–499. [PubMed: 25751139]

Wang Z, Wu Y, Wang H, Zhang Y, Mei L, Fang X, Zhang X, Zhang F, Chen H, Liu Y, et al. Interplay of mevalonate and Hippo pathways regulates RHAMM transcription via YAP to modulate breast cancer cell motility. Proc Natl Acad Sci U S A. 2014; 111:E89–98. [PubMed: 24367099]

Watt KI, Turner BJ, Hagg A, Zhang X, Davey JR, Qian H, Beyer C, Winbanks CE, Harvey KF, Gregorevic P. The Hippo pathway effector YAP is a critical regulator of skeletal muscle fibre size. Nat Commun. 2015; 6:6048. [PubMed: 25581281]

Wehr MC, Holder MV, Gailite I, Saunders RE, Maile TM, Ciirdaeva E, Instrell R, Jiang M, Howell M, Rossner MJ, et al. Salt-inducible kinases regulate growth through the Hippo signalling pathway in Drosophila. Nat Cell Biol. 2013; 15:61–71. [PubMed: 23263283]

Wennmann DO, Vollenbroker B, Eckart AK, Bonse J, Erdmann F, Wolters DA, Schenk LK, Schulze U, Kremerskothen J, Weide T, et al. The Hippo pathway is controlled by Angiotensin II signaling and its reactivation induces apoptosis in podocytes. Cell Death Dis. 2014; 5:e1519. [PubMed: 25393475]

Willecke M, Hamaratoglu F, Kango-Singh M, Udan R, Chen CL, Tao C, Zhang X, Halder G. The fat cadherin acts through the hippo tumor-suppressor pathway to regulate tissue size. Curr Biol. 2006; 16:2090–2100. [PubMed: 16996265]

Williamson KA, Rainger J, Floyd JA, Ansari M, Meynert A, Aldridge KV, Rainger JK, Anderson CA, Moore AT, Hurles ME, et al. Heterozygous loss-of-function mutations in YAP1 cause both isolated and syndromic optic fissure closure defects. Am J Hum Genet. 2014; 94:295–302. [PubMed: 24462371]

Wong KK, Li W, An Y, Duan Y, Li Z, Kang Y, Yan Y. beta-Spectrin regulates the hippo signaling pathway and modulates the basal actin network. J Biol Chem. 2015; 290:6397–6407. [PubMed: 25589787]

Wu H, Xiao Y, Zhang S, Ji S, Wei L, Fan F, Geng J, Tian J, Sun X, Qin F, et al. The Ets transcription factor GABP is a component of the hippo pathway essential for growth and antioxidant defense. Cell Rep. 2013; 3:1663–1677. [PubMed: 23684612]

Wu S, Huang J, Dong J, Pan D. hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. Cell. 2003; 114:445–456. [PubMed: 12941273]

Wu S, Liu Y, Zheng Y, Dong J, Pan D. The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway. Dev Cell. 2008; 14:388–398. [PubMed: 18258486]

Xiang L, Gilkes DM, Hu H, Takano N, Luo W, Lu H, Bullen JW, Samanta D, Liang H, Semenza GL. Hypoxia-inducible factor 1 mediates TAZ expression and nuclear localization to induce the breast cancer stem cell phenotype. Oncotarget. 2014; 5:12509–12527. [PubMed: 25587023]

Xiao GH, Chernoff J, Testa JR. NF2: The wizardry of Merlin. Gene Chromosome Canc. 2003; 38:389–399.

Xin M, Kim Y, Sutherland LB, Murakami M, Qi X, McAnally J, Porrello ER, Mahmoud AI, Tan W, Shelton JM, et al. Hippo pathway effector Yap promotes cardiac regeneration. Proc Natl Acad Sci U S A. 2013; 110:13839–13844. [PubMed: 23918388]

Xin M, Kim Y, Sutherland LB, Qi X, McAnally J, Schwartz RJ, Richardson JA, Bassel-Duby R, Olson EN. Regulation of insulin-like growth factor signaling by Yap governs cardiomyocyte proliferation and embryonic heart size. Sci Signal. 2011; 4:ra70. [PubMed: 22028467]

Xu T, Wang W, Zhang S, Stewart RA, Yu W. Identifying tumor suppressors in genetic mosaics: the Drosophila lats gene encodes a putative protein kinase. Development. 1995; 121:1053–1063. [PubMed: 7743921]

Yimlamai D, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B, Shrestha K, Cahan P, Stanger BZ, Camargo FD. Hippo pathway activity influences liver cell fate. Cell. 2014; 157:1324–1338. [PubMed: 24906150]

Yin F, Yu J, Zheng Y, Chen Q, Zhang N, Pan D. Spatial organization of Hippo signaling at the plasma membrane mediated by the tumor suppressor Merlin/NF2. Cell. 2013; 154:1342–1355. [PubMed: 24012335]

Yu FX, Guan KL. The Hippo pathway: regulators and regulations. Gene Dev. 2013; 27:355–371. [PubMed: 23431053]

Yu FX, Luo J, Mo JS, Liu GB, Kim YC, Meng ZP, Zhao L, Peyman G, Ouyang H, Jiang W, et al. Mutant Gq/11 Promote Uveal Melanoma Tumorigenesis by Activating YAP. Cancer Cell. 2014; 25:822–830. [PubMed: 24882516]

Yu FX, Meng Z, Plouffe SW, Guan KL. Hippo pathway regulation of gastrointestinal tissues. Annu Rev Physiol. 2015; 77:201–227. [PubMed: 25293527]

Yu FX, Zhang Y, Park HW, Jewell JL, Chen Q, Deng Y, Pan D, Taylor SS, Lai ZC, Guan KL. Protein kinase A activates the Hippo pathway to modulate cell proliferation and differentiation. Gene Dev. 2013a; 27:1223–1232. [PubMed: 23752589]

Yu FX, Zhao B, Panupinthu N, Jewell JL, Lian I, Wang LH, Zhao J, Yuan H, Tumaneng K, Li H, et al. Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. Cell. 2012; 150:780–791. [PubMed: 22863277]

Yu J, Zheng Y, Dong J, Klusza S, Deng WM, Pan D. Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. Dev Cell. 2010; 18:288–299. [PubMed: 20159598]

Yu T, Bachman J, Lai ZC. Evidence for a tumor suppressor role for the large tumor suppressor genes LATS1 and LATS2 in human cancer. Genetics. 2013b; 195:1193–1196. [PubMed: 24026096]

Yue T, Tian A, Jiang J. The cell adhesion molecule echinoid functions as a tumor suppressor and upstream regulator of the Hippo signaling pathway. Dev Cell. 2012; 22:255–267. [PubMed: 22280890]

Zanconato F, Forcato M, Battilana G, Azzolin L, Quaranta E, Bodega B, Rosato A, Bicciato S, Cordenonsi M, Piccolo S. Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nat Cell Biol. 2015; 17:1218–1227. [PubMed: 26258633]

Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, Fan ST, Luk JM, Wigler M, Hannon GJ, et al. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell. 2006; 125:1253–1267. [PubMed: 16814713]

Zhang H, Pasolli HA, Fuchs E. Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. Proc Natl Acad Sci U S A. 2011; 108:2270–2275. [PubMed: 21262812]

Zhang L, Ren F, Zhang Q, Chen Y, Wang B, Jiang J. The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control. Dev Cell. 2008; 14:377–387. [PubMed: 18258485]

Zhang N, Bai H, David KK, Dong J, Zheng Y, Cai J, Giovannini M, Liu P, Anders RA, Pan D. The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. Dev Cell. 2010; 19:27–38. [PubMed: 20643348]

Zhang W, Nandakumar N, Shi Y, Manzano M, Smith A, Graham G, Gupta S, Vietsch EE, Laughlin SZ, Wadhwa M, et al. Downstream of mutant KRAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma. Sci Signal. 2014a; 7:ra42. [PubMed: 24803537]

Zhang WJ, Gao YJ, Li PX, Shi ZB, Guo T, Li F, Han XK, Feng Y, Zheng C, Wang ZY, et al. VGLL4 functions as a new tumor suppressor in lung cancer by negatively regulating the YAP-TEAD transcriptional complex. Cell Res. 2014b; 24:331–343. [PubMed: 24458094]

Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). Genes Dev. 2010; 24:72–85. [PubMed: 20048001]

Zhao B, Li L, Wang L, Wang CY, Yu J, Guan KL. Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis. Genes Dev. 2012; 26:54–68. [PubMed: 22215811]

Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, Xie J, Ikenoue T, Yu J, Li L, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev. 2007; 21:2747–2761. [PubMed: 17974916]

Zhao B, Ye X, Yu J, Li L, Li W, Li S, Yu J, Lin JD, Wang CY, Chinnaiyan AM, et al. TEAD mediates YAP-dependent gene induction and growth control. Genes Dev. 2008; 22:1962–1971. [PubMed: 18579750]

Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, Lauwers GY, Thasler W, Lee JT, Avruch J, et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell. 2009; 16:425–438. [PubMed: 19878874]

Zhou D, Zhang Y, Wu H, Barry E, Yin Y, Lawrence E, Dawson D, Willis JE, Markowitz SD, Camargo FD, et al. Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. Proc Natl Acad Sci U S A. 2011; 108:E1312–1320. [PubMed: 22042863]

Zhou X, Wang S, Wang Z, Feng X, Liu P, Lv XB, Li F, Yu FX, Sun Y, Yuan H, et al. Estrogen regulates Hippo signaling via GPER in breast cancer. J Clin Invest. 2015; 125:2123–2135. [PubMed: 25893606]

Outstanding Questions

Despite the rapid research progress in the Hippo pathway, some key questions remain unanswered and new questions are emerging. Listed below are some of the key questions in the Hippo field.

1. What are the molecular mechanisms regulating MST1/2 activity? Many upstream signals have been convincingly shown to regulate LATS1/2 phosphorylation and kinase activity. However, neither MST1/2 phosphorylation nor its kinase activity is strongly modulated by upstream signals. *Drosophila* misshapen (a member of MAP4K in mammals) acts upstream of Wts. An interesting question is whether MST1/2 and MAP4Ks mediate different or similar upstream signals to activate LATS1/2.
2. Where are Hippo pathway components localized in mammalian cells? This may be key to understanding how the Hippo pathway is regulated in response to upstream signals. It is obvious that phosphorylated YAP/TAZ are enriched in the cytoplasm and dephosphorylated YAP/TAZ in the nucleus. However, it is less clear where YAP/TAZ phosphorylation and dephosphorylation occur. A related question is what are the mechanisms underlying YAP/TAZ translocation between the nucleus and cytoplasm.
3. How are LATS1/2 regulated by actin remodeling and/or cellular tension? This is one of the key questions in understanding the biochemical mechanism of the Hippo kinase cascade regulation. Accumulating evidence suggests that the actin cytoskeleton and cellular tension play a key role in LATS1/2 regulation and appear to act downstream of many, if not most, upstream signals. The actin cytoskeleton and cellular tension are intertwined, thus the question is: which one plays a more direct role in regulating Hippo core components?
4. What is the mechanism of organ size sensing? Although many signals are reported to regulate the Hippo pathway *in vitro*, so far none have been demonstrated to play a key role in organ size control *in vivo*. Uncovering this magic signal will solve a key question in developmental biology.
5. How does YAP become deregulated in cancer? It is clear that YAP/TAZ activation is observed in a broad spectrum of human cancers, although mutations in Hippo pathway genes are rare. This interesting conundrum indicates that the Hippo pathway maybe regulated broadly by many other cancer driving pathways.

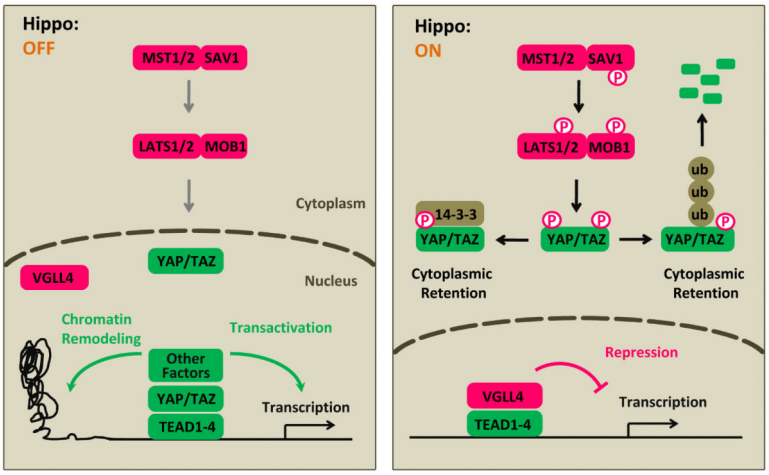

Figure 1.

Inhibition of YAP/TAZ transcriptional coactivators by LATS1/2. (A) When Hippo signaling is off, YAP/TAZ enters the nucleus, competes with VGLL4 for TEADs, and recruits other factors to induce gene transcription. YAP/TAZ may bind proximal promoters or distal enhancers of target genes to induce transcription. (B) When Hippo signaling is on, YAP/TAZ is phosphorylated by LATS1/2 on multiple sites, resulting interaction with 14-3-3 and cytoplasmic retention; phosphorylation also leads to YAP/TAZ poly-ubiquitination and degradation. VGLL4 interacts with TEADs and represses target gene transcription.

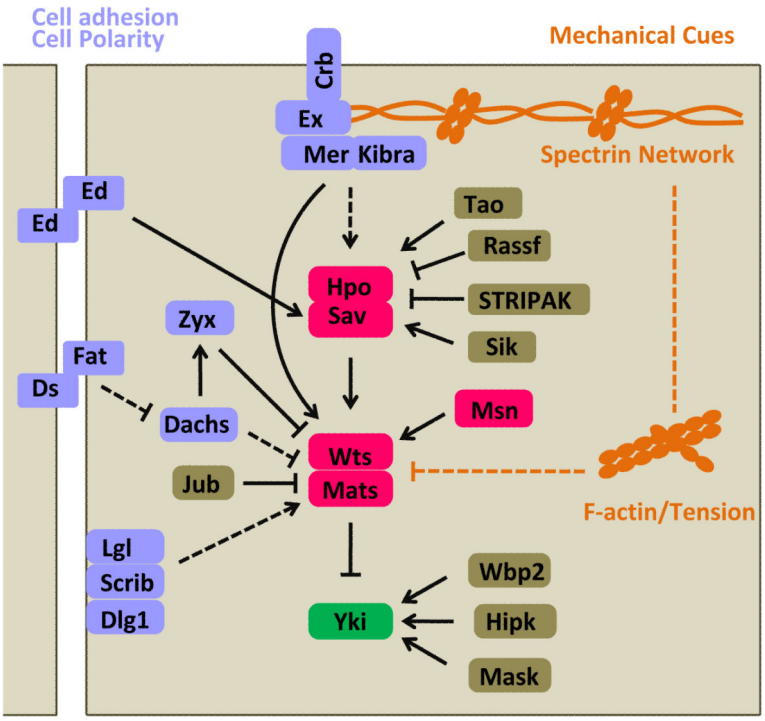

Figure 2.

Regulation of the Hippo pathway in *Drosophila*. The Hippo pathway is regulated by cell adhesion molecules (Ed), determinants of cell polarity (Crb, Fat/Ds, Scrib complex), and mechanical cues (spectrin, F-actin, or cellular tension). In addition, Hpo is regulated by Tao, salt induced kinase (Sik), Ras-associated factor (Rassf), striatin-interacting phosphatase and kinase (STRIPAK) complex; Wts is regulated by Zyxin (Zyx) and Jub; Yki is regulated by WW Domain Binding Protein 2 (Wbp2), Hipk, and Multiple ankyrin repeats single KH domain (Mask) (refer to (Varelas, 2014)). Arrows, blunt ends, and dashed lines indicate activation, inhibition, and indirect regulation, respectively.

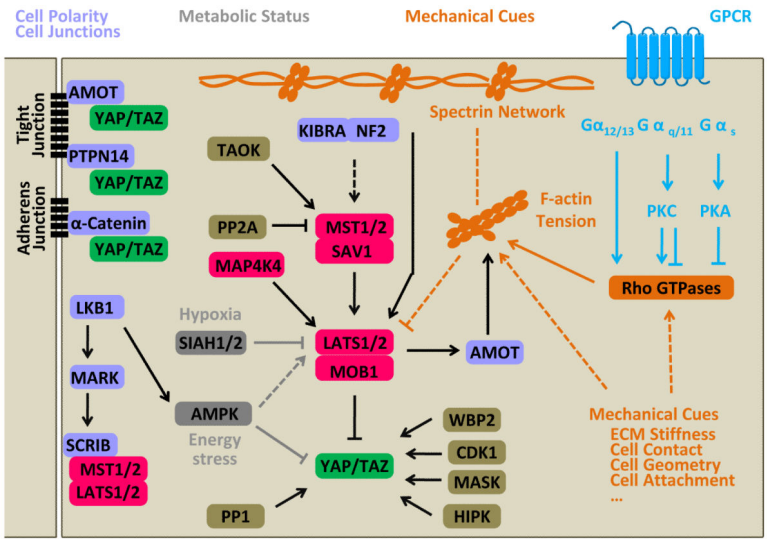

Figure 3.

Regulation of the Hippo pathway in mammals. The Hippo pathway is regulated by diverse signals: 1) Determinants of cell polarity and cell-cell junctions, such as SCRIB that interacts with MST1/2 and LATS1/2, AMOT, PTPN14, and α-Catenin, which can sequester YAP/TAZ to cell junctions; 2) Mechanical cues, such as stiffness, cell contact, cell geometry, and cell attachment status that regulate the Hippo pathway by modulating activity of Rho GTPases, remodeling the actin cytoskeleton, or altering cellular tension. Both apical and basolateral spectrin networks may function as sensors for mechanical cues in Hippo pathway regulation; 3) Soluble factors, especially ligands for GPCRs, regulate LATS1/2 likely through Rho GTPases and actin dynamics; 4) Metabolic status, such as cellular energy and oxygen stress, also regulate Hippo signaling. Many other proteins such as protein phosphatase 2A (PP2A), protein phosphatase 1 (PP1), WBP2, CDK1, MASK, and HIPK can also regulate activities of different Hippo pathway components (refer to (Varelas, 2014)). Arrows, blunt ends, and dashed lines indicate activation, inhibition, and indirect regulation, respectively.

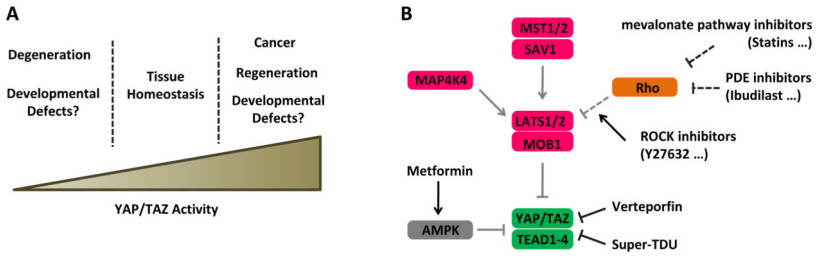

Figure 4.

Therapeutic targeting of the Hippo pathway. (A) Potential roles of YAP/TAZ activity in tissue development and diseases. A confined window of YAP/TAZ activity is required for normal tissue development and homeostasis. (B) Strategies for targeting YAP/TAZ activity. Inhibitors for MST1/2, MAP4K4, and LATS1/2 can activate YAP/TAZ. YAP/TAZ-TEAD interaction may be disrupted by small molecules directly (Verteporfin) or AMPK activators (Metformin). Small molecules inhibiting Rho family GTPases or ROCK can indirectly activate LATS1/2, leading to YAP/TAZ inhibition.

Table 1

Hippo pathway components and major functions.

Drosophila	Mammals	Major functions in Hippo pathway
Hippo (Hpo)	MST1/2	Phosphorylate LATS1/2, MOB1, and SAV1, leading to LATS1/2 activation
Salvador (Sav)	SAV1	Interacts with MST1/2, promotes phosphorylation of LATS1/2 by MST1/2
Warts (Wts)	LATS1/2	Phosphorylate and inactivate YAP/TAZ
Mats	MOB1A/B	Scaffold protein of LATS1/2
Yorkie (Yki)	YAP/TAZ	Transcription co-activator, major effectors of the Hippo pathway
Scalloped (Sd)	TEAD1-4	Transcription factors mediate the effect of YAP/TAZ
Tgi	VGLL4	Competes with YAP/TAZ for TEADs, inhibits YAP/TAZ functions
misshapen (Msn)	MAP4K4	Activates LATS1/2
Merlin (Mer)	Merlin/NF2	May form a complex and mediates upstream signals (from plasma membrane) to MST1/2. NF2 may bring LATS1/2 to plasma membrane and facilitate its activation by MST1/2
Kibra	KIBRA	
Expanded (Ex)	FRMD6?	
	AMOT	Sequesters YAP/TAZ to cell junctions, binding and indirectly activates LATS1/2; a substrate of LATS1/2

Table 2

Physiological and pathological functions of Hippo pathway genes in mice.

Organ	Phenotypes	References
Liver	*Yap* inducible expression results in hepatomegaly in a reversible manner, and in long term, leads to development of liver tumors.	(Camargo et al., 2007; Dong et al., 2007)
	Nf2, *Sav1*, or *Mst1/2* deletion causes hepatomegaly and results in hepatocellular carcinoma, cholangiocarcinoma, or bile duct hamartoma. *Mob1a/b* deletion also causes liver cancer.	(Benhamouche et al., 2010; Lee et al., 2010; Lu et al., 2010; McClatchey et al., 1998; Nishio et al., 2012; Song et al., 2010; Yin et al., 2013; Zhang et al., 2010; Zhou et al., 2009)
	Yap deletion causes bile duct defect, and YAP activity is involved in liver regereation upon tissue damage.	(Bai et al., 2012; Su et al., 2015; Yimlamai et al., 2014; Zhang et al., 2010)
	Lkb1-deficiency-induced liver over growth is dependent on YAP activation.	(Mohseni et al., 2014)
Intestine	*Yap* transgenic expression causes intestinal dysplasia.	(Camargo et al., 2007)
	Deletion of *Sav1* or *Mst1/2* in mouse intestine results in expansion of progenitor cells, colonic polyps or adenoma.	(Cai et al., 2010; Lee et al., 2008; Zhou et al., 2011)
	Deletion of *Yap* in mouse intestine shows no obvious phenotype, but affects regeneration upon tissue damage.	(Cai et al., 2010)
	Yap transgenic expression (intestine specific) results in rapid loss of intestinal crypts by repressing Wnt signaling.	(Barry et al., 2013)
	Apc deletion induced expansion of intestinal crypts in a Yap/Taz dependent manner.	(Azzolin et al., 2014; Cai et al., 2015)
Skin	Deletion of *Sav1* or transgenic expression of *Yap* leads to expansion of basal progenitor cells and skin thickening, long term activation of YAP results in squamous cell carcinoma. *Mob1a/b* deletion also causes skin cancer.	(Camargo et al., 2007; Lee et al., 2008; Nishio et al., 2012)
	Deletion of *Mst1/2* shows no clear phenotype. Deletion of *Ctnna1* (α-Catenin) leads to keratinocyte hyperproliferation and squamous cell carcinoma likely mediated by YAP activation.	(Schlegelmilch et al., 2011)
	Gnas (Gs) deletion causes Basal-cell carcinoma partially dependent on YAP activation.	(Iglesias-Bartolome et al., 2015)
Heart	Deletion of *Sav1*, *Mst1/2*, or *Lats2* at embryonic stage, or transgenic expression of active *Yap* mutant, results in hyperproliferation of cardiomyocytes and enlargement of heart.	(Del Re et al., 2013; Heallen et al., 2011; Lin et al., 2014; von Gise et al., 2012; Xin et al., 2013; Xin et al., 2011)
	Deletion of *Yap* leads to heart hypoplasia, and more severe phenotype is observed when both *Yap* and *Taz* are deleted.	(von Gise et al., 2012; Xin et al., 2011)
	In adult heart, high YAP/TAZ activity enhances heart regeneration following cardiac damages such as myocardial infarction	(Heallen et al., 2013; Lin et al., 2014; Xin et al., 2013)
Kidney	*Taz* deletion causes polycystic kidney disease, whereas *Yap* deletion leads to hypoplastic kidneys with severe defect in nephron morphogenesis.	(Hossain et al., 2007; Makita et al., 2008; Reginensi et al., 2013; Tian et al., 2007)
	Kidney with *Mst1/2* or *Sav1* deletion appears normal.	(Reginensi et al., 2013; Song et al., 2010)
Lung	*Taz* deleted mice display abnormal alveolar structures.	(Makita et al., 2008; Mitani et al., 2009; Tian et al., 2007)
	Mst1/2 deletion leads to disrupted lung structures, and neonatal lethality, which is dependent on high YAP/TAZ activity. *Mst1/2* deletion in adult lung bronchiolar epithelial cells results in airway hyperplasia and altered differentiation. YAP appears critical in regulating proximal-distal patterning of	(Lange et al., 2015; Lin et al., 2015a; Mahoney et al., 2014)

Organ	Phenotypes	References
	the lung, and a decrease in YAP activity ensures epithelial cells differentiation.	
	Mob1a/b deletion causes lung tumor.	(Nishio et al., 2012)
	Forkhead box A2 (FOXA2) has also been shown critical in mediating the effect of *Mst1/2* in lung development.	(Chung et al., 2013)
Pancreas	The effect of Hippo pathway is mainly in exocrine compartment of the pancreas. During postnatal stage, deletion of *Mst1/2* increases ratio of ductal and acinar cells, leads to pancreatitis-like autodigestion and a reduced size of pancreas.	(Gao et al., 2013; George et al., 2012)
	Kras (K12D) mutant induced Pancreatic ductal adenocarcinoma requires YAP activity.	(Zhang et al., 2014a)
Nervous System	*Nf2* deletion results in a reduction in hippocampus size, whereas the pool of Neural and neocortical progenitor cells. *Nf2* deletion also affects development of corpus callosum, in which Yap mediated overexpression of SLIT2 disrupts callosal axon pathfinding.	(Lavado et al., 2013; Lavado et al., 2014)
Mammary glands	*Yap* and *Sav1* are dispensable in mammary glands development. During pregnancy, *Yap* deletion results in hypoplasia and reduced alveolar structures, on the other hand *Sav1* deletion or transgenic expression of *Yap* prevents terminal differentiation of mammary cells.	(Chen et al., 2014)
	Mob1a/b deletion causes breast tumor.	(Nishio et al., 2012)
	Yap deletion delays mammary tumor growth induced by polyoma middle T antigen (PyMT).	(Chen et al., 2014)
Muscle	*Yap* overexpression promotes proliferation of satellite cells and represses their differentiation. YAP down-regulation reduces basal skeletal muscle fibre size, and YAP activity is required to relief neurogenic muscle atrophy following injuries.	(Judson et al., 2012; Watt et al., 2015)

Table 3

Genetic alterations of Hippo pathway genes in human cancers.

Gene	Alteration	Cancer type	References
NF2	Mutation or deletion	Mesothelioma neurofibromatosis type 2 (schwannoma, meningioma)	(Sekido, 2011) (Rouleau et al., 1993)
LATS1/2	Gene Fusion (*LATS1-PSEN1*)	Mesothelioma	(Miyanaga et al., 2015)
	LATS2 deletion	Mesothelioma	(Murakami et al., 2011)
	LATS/2 mutations	Sporadic in different cancers	(Yu et al., 2013b)
YAP	Amplification	hepatocellular carcinoma medulloblastoma Esophageal squamous cell carcinoma	(Fernandez et al., 2009) (Overholtzer et al., 2006) (Song et al., 2014) (Zender et al., 2006)
	Mutation (R331W)	lung adenocarcinoma	(Chen et al., 2015a)
	Gene Fusion (*YAP-TFE3*, *YAP-ESR1*, *YAP- C11orf95*, and *YAP-MAMLD1*)	epithelioid hemangioendothelioma luminal breast cancer Ependymal tumors	(Antonescu et al., 2013) (Flucke et al., 2014) (Li et al., 2013) (Pajtler et al., 2015) (Parker et al., 2014)
	Deletion	hematological cancer	(Cottini et al., 2014)
TAZ	Gene Fusion (*TAZ-CAMTA1*, and *TAZ-FOSB*)	epithelioid hemangioendothelioma	(Errani et al., 2011) (Flucke et al., 2014) (Tanas et al., 2011)
GNAQ/GNA11	Activating mutation	Uveal Melanoma	(Van Raamsdonk et al., 2009) (Van Raamsdonk et al., 2010)
vGPCR	Viral oncogene	Kaposi's sarcoma	(Liu et al., 2015)