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CELL SCIENCE AT A GLANCE

YAP/TAZ functions and their regulation at a glance

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ABSTRACT

YAP and TAZ proteins are transcriptional coactivators encoded by paralogous genes, which shuttle between the cytoplasm and the nucleus in response to multiple inputs, including the Hippo pathway. In the nucleus, they pair with DNA-binding factors of the TEAD family to regulate gene expression. Nuclear YAP/TAZ promote cell proliferation, organ overgrowth, survival to stress and dedifferentiation of post-mitotic cells into their respective tissue progenitors. YAP/TAZ are required for growth of embryonic tissues, wound healing and organ regeneration, where they are activated by cell-intrinsic and extrinsic cues. Surprisingly, this activity is dispensable in many adult self-renewing tissues, where YAP/TAZ are constantly kept in check. YAP/TAZ lay at the center of a complex regulatory network including cell-autonomous factors but also cell- and tissue-level structural features such as the mechanical properties of the cell microenvironment, the establishment of cell–cell junctions and of basolateral tissue polarity. Enhanced levels and activity of YAP/TAZ are observed in many cancers, where they sustain tumor growth, drug resistance and malignancy. In this Cell Science at a Glance article and the accompanying poster, we review the biological functions of YAP/TAZ and their regulatory mechanisms, and highlight their position at the center of a complex signaling network.

KEY WORDS: Hippo pathway, TEAD transcription factors, YAP/TAZ, Mechanotransduction

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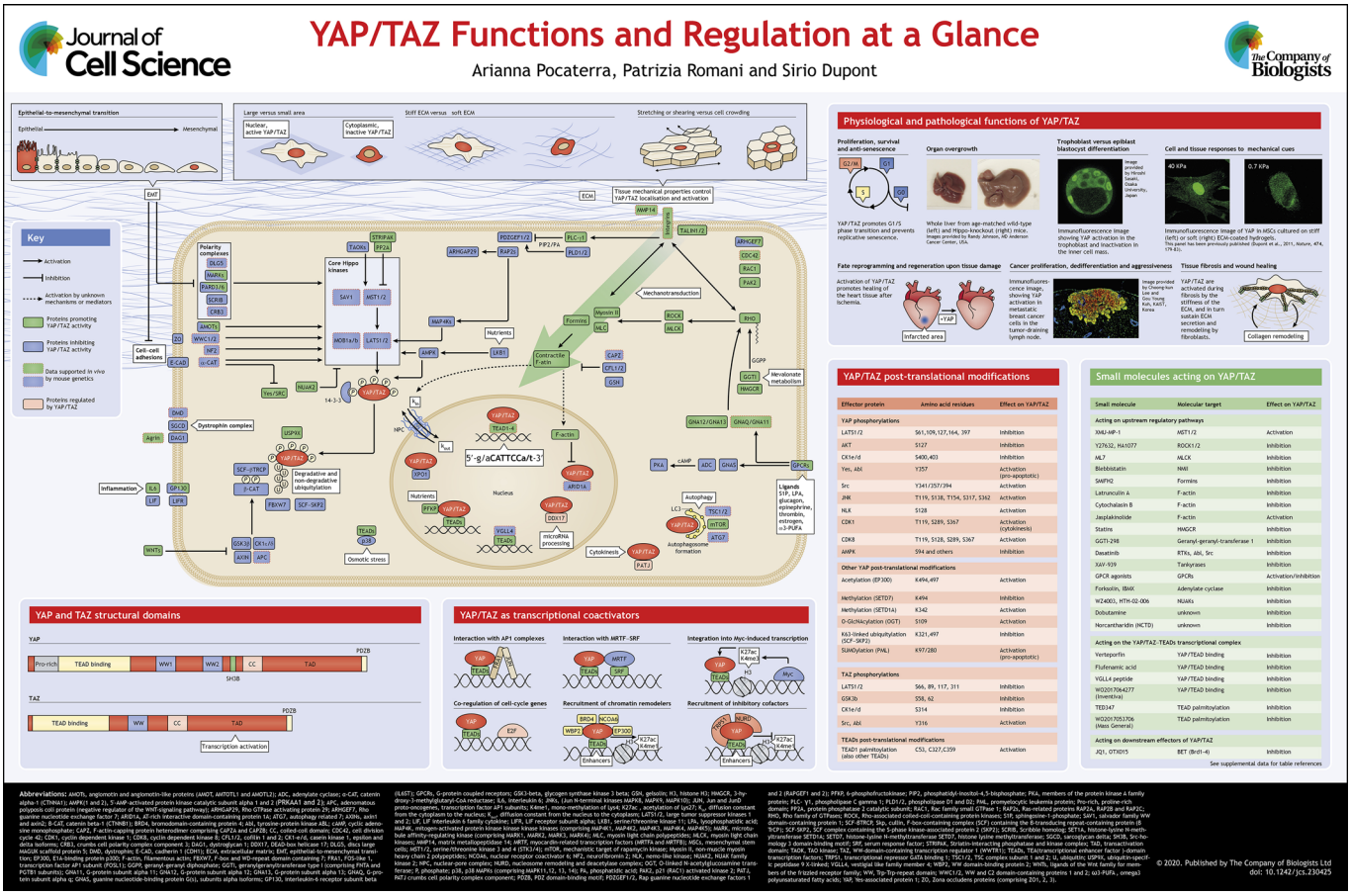
Introduction

Yes-associated protein 1 (YAP1; hereafter referred to as YAP) and WW-domain-containing transcription regulator 1 (WWTR1; also known as and hereafter referred to as TAZ) are transcriptional coactivators with 46% amino acid identity, which are encoded by paralogous genes. Both proteins interact with the members of the transcriptional enhancer factor (TEA)-domain (TEAD) family of



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DNA-binding factors (TEAD1, TEAD2, TEAD3, TEAD4) to regulate transcription. YAP and TAZ (hereafter referred to as YAP/TAZ) can be regulated in response to multiple cellular and tissue-level signals through many mechanisms, including regulation of nuclear complexes, and shuttling between the nucleus and the cytoplasm, where they are inactive and/or degraded. YAP has been independently isolated as an interactor of Yes kinase (Sudol, 1994), as a cofactor of the TEAD factors (Vassilev, 2001) and as an interactor of 14-3-3 proteins (Basu et al., 2003). TAZ has also been isolated as a 14-3-3 protein family-binding protein (Kanai, 2000). YAP/TAZ came, however, to prominence when their *Drosophila melanogaster* homolog Yki (Yorkie) was identified as the downstream mediator of the organ-size-controlling Hippo pathway (Huang et al., 2005). This was closely followed by the seminal findings that YAP is able to trigger hyperproliferation, organ overgrowth, anchorage-independent growth and cancer (Camargo et al., 2007; Dong et al., 2007; Ota and Sasaki, 2008; Overholtzer et al., 2006; Zhao et al., 2007). YAP/TAZ have since become of wide interest in many fields, ranging from developmental to cancer biology, from mechanobiology to stem cells and regeneration. In this Cell Science at a Glance article and the accompanying poster, we provide an introduction to the main functions of YAP/TAZ, the mechanisms by which they regulate gene transcription, their upstream regulatory inputs, and some hints to the experimental methods used to measure their activity (Box 1).

Box 1. Monitoring YAP/TAZ activity in cells and tissues

Several independent assays can be used to monitor YAP/TAZ activity in cells and tissues. Visualization of the nucleo-cytoplasmic shuttling of YAP/TAZ is a general assay that is also amenable to quantitative automated analysis (Sero and Bakal, 2017; Sorrentino et al., 2014) and sensitive enough to detect even slight variations in YAP/TAZ localization. This can be performed by immunofluorescence of fixed cells for endogenous YAP/TAZ (Dupont et al., 2011; Kingston et al., 2019; Zhao et al., 2007). The use of exogenous epitope-tagged YAP can be misleading, as it is prone to overexpression artifacts. Imaging of endogenous YAP and TAZ in both mouse and human tissue sections is also possible (Haderk et al., 2019; Kingston et al., 2019; Pocaterra et al., 2019) but requires careful controls (ideally, by using YAP/TAZ-knockout tissue to gauge specificity). Typically, immunofluorescence analysis of YAP/TAZ localization is accompanied by analysis of YAP phosphorylation at S127 and S381, the two main target residues of LATS1/2. For both, commercial antibodies are available or the PHOS-Tag technology can be used (Chen et al., 2019). However, phosphorylation is not ideal if used as the primary read-out for YAP activity because it is difficult to obtain quantitative data, there is multiple feedback between YAP and Hippo components (see main text), and YAP phosphorylated at S127 was found to be fully nuclear and active (Wada et al., 2011). Whatever the observed localization and phosphorylation status of YAP/TAZ, evidence with regards to transcriptional activity should always be provided. To this end, it is possible to monitor the expression levels of multiple experimentally validated YAP/TAZ target genes by using *in silico* GSEA correlative analyses (Calvo et al., 2013; Cordenonsi et al., 2011; Dupont et al., 2011; Santinon et al., 2018) or, directly, by RNA sequencing, microarray profiling and qPCR (Cao and Zhao, 2019). If YAP/TAZ is activated or inhibited, a significant number of genes should respond coherently. The use of a limited subset of genes, such as *CTGF*, *CYR61* and *ANKRD1,* has been a powerful approach in the past, but it can be misleading because these genes are also regulated by other transcription factors, including the actin-sensitive MRTF–SRF complex (Foster et al., 2017). As additional evidence, the specific transcriptional activity of YAP/TAZ can be monitored with luciferase reporters (Dupont, 2019).

Biological functions of YAP/TAZ

YAP/TAZ have two main activities. *In vitro*, under standard tissue-culture conditions, most adherent cells display active YAP/TAZ, which are required for cell proliferation (Ma et al., 2019). Accordingly, forced expression of YAP or TAZ in cells or tissues potently promotes proliferation, leading to organ overgrowth and, almost invariably, to the development of cancer (Ma et al., 2019; Zanconato et al., 2016). Moreover, in multiple instances, expression of YAP/TAZ is also sufficient to 'reprogram' mature differentiated cells into their corresponding less-differentiated progenitors, often resembling embryonic precursors, which can occur both *in vivo* and *in vitro* (Moya and Halder, 2019; Panciera et al., 2018; Schlegelmilch et al., 2011; Yimlamai et al., 2014; Yui et al., 2018). This led to the initial idea of YAP/TAZ as general factors required for stem-cell self-renewal; however, loss-of-function experiments in mouse showed that YAP and TAZ activity is dispensable for the proliferative homeostasis of many adult tissues, such as of skin and intestine (Azzolin et al., 2014; Cai et al., 2010; Gregorieff et al., 2015; Zanconato et al., 2015). By contrast, YAP and TAZ are required during embryonic development, as well as under non-homeostatic conditions, where tissues undergo high rates of proliferation, such as during wound healing, regeneration and cancer development (Ma et al., 2019; Moya and Halder, 2019; Zanconato et al., 2016). Thus, in normal tissues YAP/TAZ are constantly kept in check by multiple negative regulators, and activated under conditions of regenerative or malignant growth.

YAP/TAZ also perform other important functions, including those indicated in the poster, induction of epithelial-to-mesenchymal transition (EMT) (Lei et al., 2008; Overholtzer et al., 2006; Shao et al., 2014), regulation of angiogenesis and heart development (Astone et al., 2018; Chen et al., 1994; Kim et al., 2017; Murakami et al., 2005; Wang et al., 2018; Wang et al., 2017), promotion of peripheral axons myelination (Deng et al., 2017; Grove et al., 2017; Poitelon et al., 2016) as well as regulation of endocrine pancreas differentiation (George et al., 2012; Mamidi et al., 2018; Rosado-Olivieri et al., 2019). We refer the reader to comprehensive recent reviews for more details (Ma et al., 2019; Piccolo et al., 2014; Zheng and Pan, 2019).

Most data indicate that YAP and TAZ, generally, have interchangeable functions. Very often, expression of one protein recapitulates the effects seen by expressing the other, and combined knockdown or knockout experiments have additive quantitative effects (Piccolo et al., 2014). For example, YAP and TAZ are both required for embryonic development but their combined inactivation causes much stronger defects, being incompatible with blastocyst formation (Sasaki, 2017). However, at least in the kidney, YAP and TAZ can perform different functions. In this organ, YAP is required for normal nephron morphogenesis, whereas TAZ opposes cystic dilation of tubules (Hossain et al., 2007; Makita et al., 2008; Reginensi et al., 2013). In principle, a way to experimentally address the question whether and where YAP and TAZ perform different functions would be to engineer mice, in which the endogenous TAZ alleles are substituted for YAP and vice-versa, as done for other factors in the past (Dunn et al., 2005).

Their biological activities make YAP and TAZ interesting therapeutic targets in oncology, because they are activated downstream several oncogenes or in response to inactivation of tumor-suppressor genes, and they are specifically required for proliferation and survival of tumor cells (Ma et al., 2019; Zanconato et al., 2016). YAP/TAZ activity is also interesting in regenerative applications, as modulation of YAP/TAZ has been shown to enhance endogenous repair mechanisms in damaged tissues, such

as wounded skin and infarcted heart (Lee et al., 2014; Lin et al., 2014; Wang et al., 2018; Xin et al., 2013), as well as owing to their ability to reprogram cell differentiation in a cell lineage-specific manner (Panciera et al., 2018; Yimlamai et al., 2014). Not surprisingly, the quest for small-molecule drugs that are able to modulate YAP/TAZ activity is an active field of research (Crawford et al., 2018; Johnson and Halder, 2014) (see poster and Table S1).

YAP/TAZ regulate transcription

The identification of TEAD factors as a DNA-binding platform for YAP/TAZ in mammals and flies (Vassilev, 2001; Wu et al., 2008; Zhang et al., 2008), provided the first hints to understand the role of YAP/TAZ in transcription regulation (Ota and Sasaki, 2008; Zhang et al., 2009; Zhao et al., 2008). All TEAD family members share a highly conserved TEA domain that facilitates binding to the 5′-g/aCATTCCa/t-3′ DNA consensus sequence (also known as MCAT element; lowercase letters indicate less frequently observed nucleotides), which is often found as closely spaced repeats (Davidson et al., 1988; Farrance et al., 1992; Galli et al., 2015; Stein et al., 2015; Zanconato et al., 2015). Multiple studies, including high-resolution chromatin-immunoprecipitation (ChIP) assays, later confirmed that YAP/TAZ interact with TEAD factors to regulate gene transcription (Croci et al., 2017; Galli et al., 2015; Stein et al., 2015; Zanconato et al., 2015). This interaction has been finely mapped, including crystal structures comprising the C-terminal part of TEADs together with the N-terminal part of YAP (Kaan et al., 2017; Li et al., 2010; Tian et al., 2010). YAP and/or TAZ can also bind to other transcription factors, including TP73, RUNX2, NKX2-1, TBX5 and CTNNB1 (hereafter referred to as β-CAT) (Piccolo et al., 2014), but most of the transcriptional studies performed so far focused on interaction with TEADs. This is also indicated by the lack of biological activity of YAP and TAZ mutants that are unable to physically interact with TEADs in several assays. Surprisingly, the YAP- and TEAD-binding sites in genes are not found in close vicinity to transcriptional start sites but, preferentially, on distant enhancers, where YAP/TAZ recruit multiple chromatin modifiers to regulate gene activity (Galli et al., 2015; Stein et al., 2015; Zanconato et al., 2015; 2018) (see poster). Moreover, complexes between YAP and TEAD or TAZ and TEAD (YAP–TEAD or TAZ–TEAD, respectively) interact and/or cooperate with other transcription factor complexes, which are bound to their own DNA motifs, such as the AP-1 heterodimer (comprising Fos and Jun; Zanconato et al., 2015), E2F transcription factors (Kapoor et al., 2014; Santinon et al., 2018), the serum response factor (SRF) (Foster et al., 2017) and Myc (Croci et al., 2017) (see poster). Most experimental data typically focus on the genes that are positively regulated by YAP/TAZ; however, ∼50% of YAP/TAZ target genes are negatively regulated, probably owing to recruitment of inhibitory cofactors by YAP/TAZ (Elster et al., 2018; Kim et al., 2015). Also, direct YAP/TAZ targets remain difficult to define because assignment of YAP/TEAD-bound enhancers to actual genes is challenging owing to the distances that can span megabases. Finally, YAP and TAZ do not interact with each other, and it remains unexplored whether YAP–TEAD and TAZ–TEAD complexes have distinct activities. Finally, TEADs can also bind members of the vestigial-like family of transcriptional coactivators (VGLL1, VGLL2, VGLL3, VGLL4), which can represent an alternative input for TEAD-driven transcription (Chen et al., 2004; Figeac et al., 2019; Günther et al., 2004; Halder et al., 1998; Maeda et al., 2002), but also a mechanism to outcompete YAP/TAZ from TEADs (Jiao et al., 2014; Zhang et al., 2014).

It must also be considered that, apart from gene expression, YAP/TAZ can also regulate non-transcriptional processes. In the nucleus, YAP can bind to the component of the microRNA-processing machinery DDX17, thereby regulating general microRNA biogenesis (Mori et al., 2014). In the cytoplasm, YAP/TAZ promotes the degradation of β-CAT by using at least two concurrent mechanisms (Azzolin et al., 2014; Varelas et al., 2010). Moreover, during cytokinesis, YAP localizes with PATJ to the midbody, where it helps to ensure the timely separation of daughter cells (Bui et al., 2016).

YAP/TAZ target genes

A general analysis of YAP/TAZ targets in multiple cell systems indicates that YAP/TAZ regulate a coherent gene program involved in proliferation, including genes driving G1/S phase transition, DNA replication and repair, nucleotide metabolism, and mitosis (Dong et al., 2007; Santinon et al., 2018; Zanconato et al., 2015; Zhao et al., 2008). This reflects the potent ability of YAP/TAZ to promote cell growth and to prevent cell senescence, and probably also warrants their potent pro-tumorigenic activity. YAP/TAZ can also regulate the expression of upstream regulators of the Hippo pathway (Gill et al., 2018; Moroishi et al., 2015; Yuan et al., 2018) and of the integrin and cytoskeletal machinery (Calvo et al., 2013; Nardone et al., 2017; Porazinski et al., 2015; Qiao et al., 2017), thus, respectively, limiting and reinforcing their own activity (see below). As many YAP/TAZ targets are regulated the same way across different cell types and tissues, it was possible to describe YAP/TAZ transcriptional signatures to infer YAP/TAZ activity in gene-expression datasets (Calvo et al., 2013; Cordenonsi et al., 2011; Dupont et al., 2011; see also Box 1).

However, which target genes account for the role of YAP/TAZ in cell dedifferentiation has been less explored, and so far it remains unclear whether the ‘reprogramming’ activity of YAP and TAZ represents a specific gene program or, rather, their ability to promote proliferation (see above) and chromatin remodeling (Monroe et al., 2019) – both being integral elements of cell fate reprogramming and self-renewing ability. A link has recently been established between YAP/TAZ and regulation of the Notch pathway in some systems (Totaro et al., 2018), and this might represent a general mechanism to regulate cell fate.

YAP/TAZ as transducers of the Hippo pathway

The Hippo pathway was discovered in genetic screens of *Drosophila*, and it is conserved across animals. Mutation of the pathway components causes overgrowth of fly embryonic tissues, hence the name ‘hippo’. In mammals, the hippo serine/threonine kinases 3 and 4 (STK3 and STK4, hereafter referred to as MST2 and MST1, respectively), together with the scaffolding protein salvador family WW-domain-containing protein 1 (SAV1) directly phosphorylate and activate the large tumor suppressor kinases 1 and 2 (LATS1/2). LATS1/2 in turn, in conjunction with the scaffolding proteins MOB kinase activator1A and 1B (MOB1A and MOB1B, respectively), directly phosphorylate and, therefore, inhibit YAP/TAZ (Ma et al., 2019; Zheng and Pan, 2019; see poster and Table S2). Multiple upstream inputs can modulate the activity of MST1/2 and LATS1/2 kinases, including the tumor suppressor neurofibromin 2 (NF2) (Ma et al., 2019; Zheng and Pan, 2019; see poster). So far, knockout studies in mice indicate that the Hippo pathway acts as a tonic inhibitor of YAP/TAZ in embryonic and adult tissues; but it is less clear whether activity of the Hippo kinase cascade can be patterned in space and/or time within tissues, thereby giving rise to patterned YAP/TAZ activity.

YAP/TAZ integrate multiple inputs at cell and tissue level

In contrast to classic signal transduction pathways that are controlled by a dedicated ligand (e.g. Notch, Wnt, TGFβ), activity of YAP/TAZ is regulated by an ever-expanding network of factors and mechanisms. These include the Hippo pathway, cell–cell adhesions, cell polarity, extracellular forces exerted by the cell microenvironment (including the elasticity of the extracellular matrix, tissue stretching and shear forces), metabolic pathways and extracellular growth factors (see poster). Several of these inputs reflect the structure and organization of cells themselves, leading to the idea that YAP/TAZ integrate the 'architectural' features of cells and tissues (Gaspar and Tapon, 2014; Halder et al., 2012; Irvine, 2012).

One important architectural feature of a tissue that regulates YAP/TAZ is the formation of cell–cell contacts (both adherens and tight junctions) and the resulting establishment of apicobasal cell polarity, leading to the notion that a normal epithelial architecture restricts YAP/TAZ activity. Indeed, CTNNA1 (hereafter referred to as α-CAT) and the apical and basolateral polarity complexes (i.e. Dlg–Scrib, Crumbs) exert tumor-suppressive functions by keeping YAP/TAZ inactive (Cordenonsi et al., 2011; Kwan et al., 2016; Li et al., 2016; Liu et al., 2017; Schlegelmilch et al., 2011; Silvis et al., 2011; Szymaniak et al., 2015). Moreover, several other regulators and interaction partners of YAP/TAZ localize to cell–cell junctions (see poster). Thus, even a partial EMT, which disengages cell–cell adhesions or disables polarity, is sufficient to unleash YAP/TAZ activity (Cordenonsi et al., 2011). The general tenet that epithelial polarity inhibits YAP/TAZ does not, however, always hold true because YAP/TAZ are active in the trophoblast of the blastocyst, even if it is fully polarized (Sasaki, 2017). Furthermore, other polarity proteins, such as microtubule affinity-regulating kinases 1–4 (MARK1, 2, 3 or 4), and par-3 and par-6 family cell-polarity regulators (PARD3 and PARD6, respectively) can both activate and inhibit YAP/TAZ, depending on the system (Heidary Arash et al., 2017; Kwan et al., 2016; Liu et al., 2018; Lv et al., 2015; Mohseni et al., 2014). This indicates that the relationship between cell polarity and YAP/TAZ is far from being fully understood.

Another important regulator of YAP/TAZ is the mechanical property of the cell microenvironment (Dupont, 2016). Maturation of integrin-mediated adhesion to the ECM, which is induced by extracellular resisting forces, and the corresponding establishment of cytoskeletal contractility represent a fundamental basal input to keep YAP/TAZ nuclear and active (Dupont, 2016; Iskratsch et al., 2014). Thus, inhibition of the cellular machinery that is required to build-up cytoskeletal contractility – including RHO GTPases, ROCK and MLCK kinases, diaphanous F-actin elongation factors, non-muscle myosin II (NMII) and F-actin itself – result in YAP/TAZ inhibition on stiff substrata. By contrast, inactivation of F-actin inhibitory factors, such as capping actin protein of muscle Z-line (CAPZB), cofilin-1 or -2 and gelsolin result in YAP/TAZ activation on soft substrata (Aragona et al., 2013; Dupont et al., 2011; Wada et al., 2011; Zhao et al., 2012). Since cells need to spread to develop cytoskeletal contractility (Fu et al., 2010), YAP/TAZ are also inhibited when cells are forced to take on a small cell geometry, which can be imposed by reducing the area of substrate adhesion, can occur because of overcrowding during contact inhibition or by caging of cells within a dense ECM that cells cannot remodel (Aragona et al., 2013; Dupont et al., 2011; Gjorevski et al., 2016; Tang et al., 2013; Wada et al., 2011). Surprisingly, cell–substrate adhesion through the dystrophin glycoprotein complex (DGC) has the opposite effect in mature cardiomyocytes, limiting YAP/TAZ activity (Morikawa et al., 2017). Finally, regulation of YAP/TAZ through tissue forces is not limited to the ECM but also includes stretching and shear stresses exerted by blood flow (Nakajima et al., 2017; Wang et al., 2016a; 2016b).

The link between YAP/TAZ and tissue forces is the reason for several biological processes. It explains the switch from proliferation to quiescence observed in many cell types cultured on stiff (YAP/TAZ on) versus soft (YAP/TAZ off) ECM substrata (Dupont, 2016). It also represents an important and often overlooked difference between cultured cells (on plastics, where YAP/TAZ are active) and normal tissues (where tissue softness keeps YAP/TAZ in check). This is instrumental to maintain organ homeostasis – at least in the liver – because increasing cell contractility through inactivation of the CAPZ F-actin inhibitors is sufficient to activate YAP/TAZ in hepatocytes and induce hyperproliferation, organ overgrowth and dedifferentiation (Pocaterra et al., 2019). The same mechanism accounts for the observation that a soft adipose tissue, by inhibiting YAP/TAZ, skews mesenchymal stem cell (MSC) differentiation towards adipocytes, whereas a stiff bone niche, by activating YAP/TAZ, skews their differentiation towards osteoblasts (Dupont et al., 2011; Engler et al., 2006; Tang et al., 2013). During tissue fibrosis, several inputs initiate the deposition of collagen, whose stiffness activates YAP/TAZ. In turn, YAP/TAZ regulate expression of cytoskeletal and ECM genes to maintain the activated and contractile state of fibroblasts, and further promote ECM remodeling in a positive feedback loop (Calvo et al., 2013; Liu et al., 2015; Mannaerts et al., 2015). Fibrosis also represents a risk factor for many cancers. Indeed, cancer cells cooperate with cancer-associated fibroblasts to stiffen the tumor ECM, which promotes tumor growth and metastasis (Butcher et al., 2009). Given the powerful oncogenic properties of YAP/TAZ, they represent the ideal candidates to mediate those effects. More recently, it was found that hemodynamic shearing forces induce YAP/TAZ activity in endothelia with atheroprotective effects (Nakajima et al., 2017; Wang et al., 2016a; 2016b). Finally, cells can actively tune their own mechanics to override tissue forces; for example, in the fish granulosa 'winner' cells enlarge and compress their neighboring 'loser' cells in a process akin to mechanical competition, resulting in differential TAZ activity (Brás-Pereira and Moreno, 2018; Dupont and Morsut, 2019; Xia et al., 2019). A similar mechanism driven by oncogene activation might promote the survival of single transformed cells at the very beginning of tumor development.

Besides the above-mentioned inputs, YAP/TAZ activity can be regulated by more classic extracellular signaling pathways, including WNTs and ligands activating GPCRs and the interleukin-6 receptor subunit beta (IL6ST; hereafter referred to as GP130) (see poster). The crosstalk with WNT is bidirectional because the β-CAT destruction complex can degrade YAP/TAZ and regulate their activity; however, YAP/TAZ themselves can also regulate β-CAT degradation – as noted above – thus, modulating Wnt signaling (Azzolin et al., 2012; 2014; Cai et al., 2015; Heallen et al., 2011; Park et al., 2015; Varelas et al., 2010). GPCRs can have different effects, depending on the prevalent downstream pathway: activation of heterotrimeric G protein complexes that contain the G-protein α subunits GNAQ, GNA11, GNA12 or GNA13 induces YAP/TAZ through the activation of RHO, whereas activation of G-protein subunit alpha S (GNAS) inhibits YAP/TAZ through activation of members of the protein kinase A (PKA) family (Feng et al., 2014; Iglesias-Bartolome et al., 2015; Kim et al., 2013; Yu et al., 2012; 2013; 2014). Similarly, activation of the cytokine receptor GP130 can promote YAP/TAZ in the context of inflammation, and inhibit

them when forming a dimer with the leukemia inhibitory factor receptor (LIFR) in metastatic breast cancer cells (Chen et al., 2012; Taniguchi et al., 2015).

Finally, YAP/TAZ can be regulated by cell metabolism. For example, cholesterol-lowering drugs that target the mevalonate pathway interfere with the prenylation of members of the Rho family of GTPases leading to RHO inhibition and, thus, to YAP/TAZ inhibition (Sorrentino et al., 2014; Wang et al., 2014). Glucose and energy metabolism were identified as additional metabolic pathways – including that of PRKAA1 and 2 (also known as AMPK1 and 2, respectively) – to regulate YAP/TAZ activity, and these studies provided evidence for a reinforcing loop between the acquisition of aerobic glycolysis and proliferation in cancer cells (DeRan et al., 2014; Enzo et al., 2015; Mo et al., 2015; Wang et al., 2015). Also, the mTOR pathway can regulate YAP by mediating its autophagic degradation (Liang et al., 2014), further linking the nutrient status of cells to YAP activity.

Taken together, it is tempting to speculate that YAP/TAZ represent a cellular system through which multiple inputs from the cell microenvironment can license 'emergency' proliferation and cell fate plasticity when needed. Although many molecular pathways have been described to activate YAP/TAZ in cancer cells, the mechanisms that activate YAP/TAZ in tissues during physiological regeneration processes – and safely de-activate them once tissue integrity is reconstituted – remain to be elucidated.

Multiple molecular mechanisms regulate YAP/TAZ activity

Regulation of YAP/TAZ can occur at multiple levels, such as at *YAP1* and *WWTR1* gene expression (Wu et al., 2013; Xia et al., 2019), the protein level through both the ubiquitin-proteasome system and autophagy (Liang et al., 2014; Ma et al., 2019), and through post-translational modifications (see poster and Table S2) that affect the subcellular localization, protein-protein interaction partners and transcriptional activity of YAP/TAZ (He et al., 2016). Owing to space constraints, we illustrate these mechanisms on the poster and only briefly discuss two regulatory mechanisms of broad interest in detail below.

YAP/TAZ constantly shuttle between the nucleus and the cytoplasm, such that regulation of their relative nuclear-cytoplasmic ratio can influence YAP/TAZ activity in both compartments (see Box 1 for technical considerations). YAP/TAZ are kept in the nucleus by interaction with TEADs and by at least another mechanism involving the PDZ-binding domain of YAP/TAZ (Chan et al., 2009; Ege et al., 2018). Activation of the Hippo pathway and, thus, of LATS1/2-mediated phosphorylation, induces cytoplasmic accumulation of YAP/TAZ, where they interact with the 14-3-3 proteins and where they can undergo proteasomal degradation (Ma et al., 2019). Importantly, when cytoplasmic accumulation of YAP/TAZ is observed, the proteins are not stably 'anchored' in the cytoplasm but, because of increased nuclear export rates, dynamically accumulate there; i.e. they continue to pass through the nucleus but spend more time in the cytoplasm (Chan et al., 2009; Dupont et al., 2011; Ege et al., 2018; Ren et al., 2010). It remains unclear whether LATS1/2 phosphorylate YAP/TAZ in the nucleus (Li et al., 2014) or while they transiently pass through the cytoplasm – as might be suggested by the physical and functional interactions LATS1/2 and YAP/TAZ undergo with cell–cell junction proteins (Ma et al., 2019; Zheng and Pan, 2019; see poster and above discussion). It is also possible that phosphorylation by LATS1/2 occurs as a secondary effect of other mechanisms that favor YAP/TAZ accumulation in the cytoplasm and, thus, their interaction with LATS1/2.

With regard to inhibition of YAP/TAZ in cells cultured on soft ECM or under conditions of reduced actomyosin contractility, at least three mechanisms have been proposed. (i) Activation of LATS1/2, followed by phosphorylation of YAP/TAZ (Meng et al., 2018; Zhao et al., 2012). For this, there are supporting biochemical data but contrasting functional evidence was observed (Aragona et al., 2013; Feng et al., 2014; Pocaterra et al., 2019). (ii) Closure of the nuclear pores due to rounding up of the nucleus, which is predicted to affect many other proteins in addition to YAP/TAZ (Aureille et al., 2019; Elosegui-Artola et al., 2017). (iii) Inhibition of nuclear F-actin, which enables the nuclear actin-binding factor ARID1A to sequester YAP/TAZ away from TEADs (Chang et al., 2018). It will be interesting to understand which factors regulate this nuclear F-actin pool and how contractility of cytoplasmic F-actin is connected to nuclear F-actin synthesis; perhaps, deformation of the nucleus might bridge these two F-actin pools. ARID1A, as a subunit of the SWI–SNF chromatin-remodeling complex, additionally serves as mediator of YAP/TAZ activity during mammary lineage commitment and, so, facilitate access of YAP/TAZ to chromatin during liver regeneration (Li et al., 2019; Skibinski et al., 2014). This is likely to reflect the dual function of ARID1A as a tumor suppressor and a tumor promoter (Fang et al., 2015; Sun et al., 2016; 2018). Notice that the second and third mechanism act independently of phosphorylation, indicating that the mechanical control of YAP/TAZ entails multiple redundant mechanisms.

Conclusions and future directions

In the past decade, YAP/TAZ have become the object of intense research efforts. Although the main regulatory mechanisms have been elucidated, several questions at the subcellular, cellular and tissue levels remain open and provide the grounds for future investigations. These include: which mechanisms activate YAP/TAZ in tissues during normal regeneration processes and safely de-activate them once tissue integrity is reconstituted? Are the same mechanisms active in cancer cells? What are the molecular mechanisms that regulate nucleo-cytoplasmic shuttling dynamics of YAP/TAZ? What is the location of LATS-induced phosphorylation of YAP/TAZ? Another important issue is the identification of the mechanosensor that translates extracellular forces into biochemical regulation of YAP/TAZ. Finally, which target genes account for the ability of YAP/TAZ to dedifferentiate cells in multiple tissues? Clearly, the extensive biological effects of YAP/TAZ signaling make them attractive drug targets in cancer therapy and regenerative medicine, which is likely to represent an important research avenue in the coming years, including the development of specific small-molecule inhibitors.

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Competing interests

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Supplementary information

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Summary: A hitchhiker's guide to the rapidly expanding YAP/TAZ regulatory network, centered on the Hippo pathway and on mechanosensing of the physical properties of the cell microenvironment.

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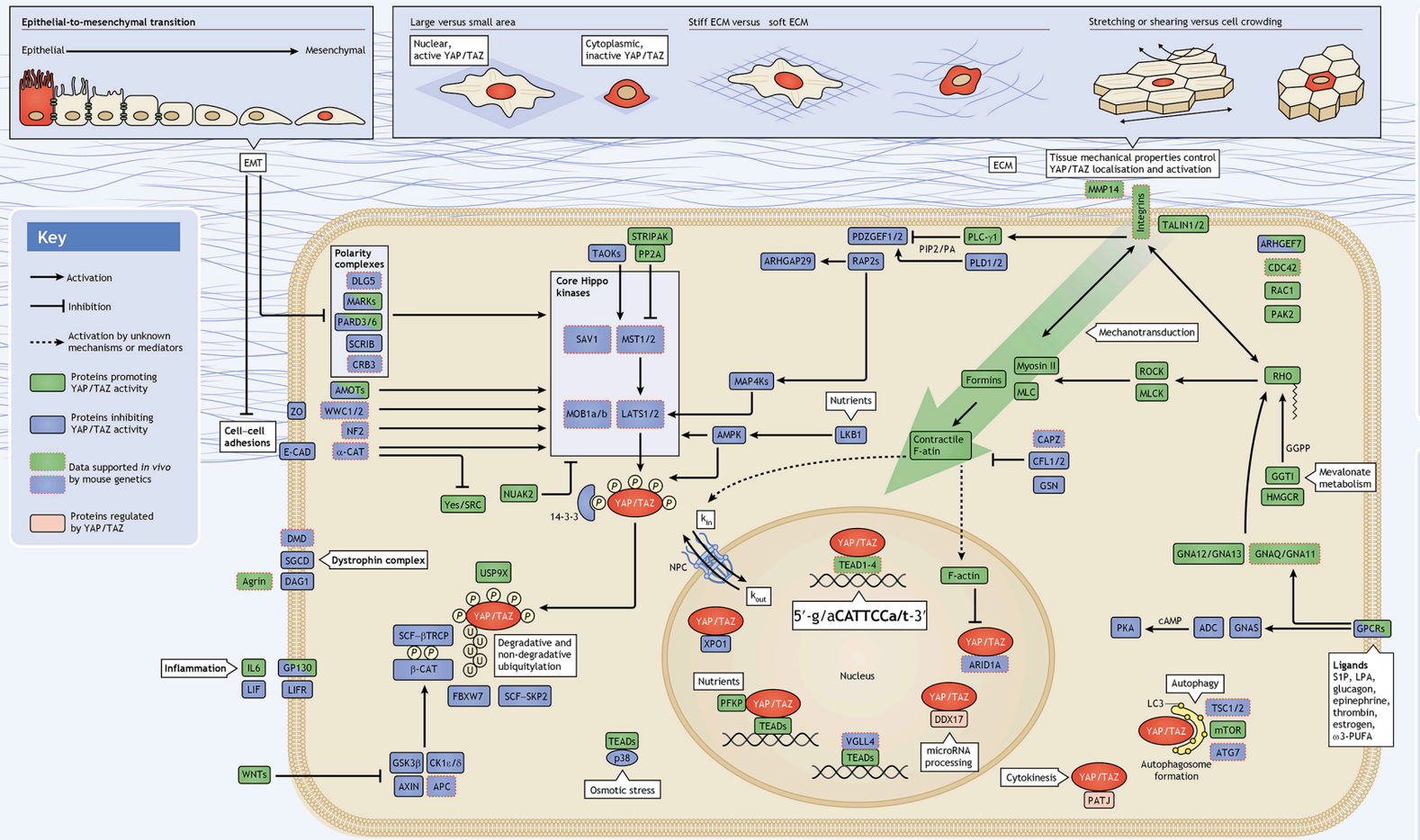
Journal of Cell Science

YAP/TAZ Functions and Regulation at a Glance

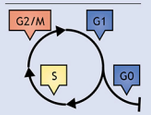
Arianna Pocaterra, Patrizia Romani and Sirio Dupont

The Company of Biologists



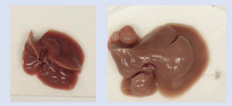
Physiological and pathological functions of YAP/TAZ

Proliferation, survival and anti-senescence



YAP/TAZ promotes G1/S phase transition and prevents replicative senescence.

Organ overgrowth



Whole liver from age-matched wild-type (left) and Hippo-knockout (right) mice. Images provided by Randy Johnson, MD Anderson Cancer Center, USA.

Trophoblast versus epiblast blastocyst differentiation

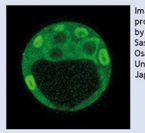
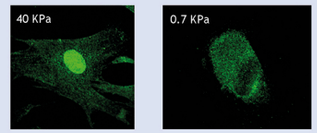


Image provided by Hiroshi Sasaki, Osaka University, Japan

Immunofluorescence image showing YAP activation in the trophoblast and inactivation in the inner cell mass.

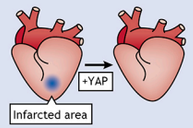
Cell and tissue responses to mechanical cues



Immunofluorescence image of YAP in MSCs cultured on stiff (left) or soft (right) ECM-coated hydrogels. This panel has been previously published (Dupont et al., 2011, *Nature*, **474**, 179-83).

Fate reprogramming and regeneration upon tissue damage

Activation of YAP/TAZ promotes healing of the heart tissue after ischemia.



Cancer proliferation, dedifferentiation and aggressiveness

Immunofluorescence image, showing YAP activation in metastatic breast cancer cells in the tumor-draining lymph node.

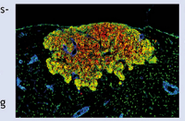
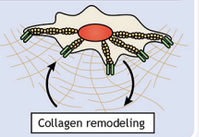


Image provided by Choong-kun Lee and Gou Young Koh, KAIST, Korea

Tissue fibrosis and wound healing

YAP/TAZ are activated during fibrosis by the stiffness of the ECM, and in turn sustain ECM secretion and remodeling by fibroblasts.



YAP/TAZ post-translational modifications

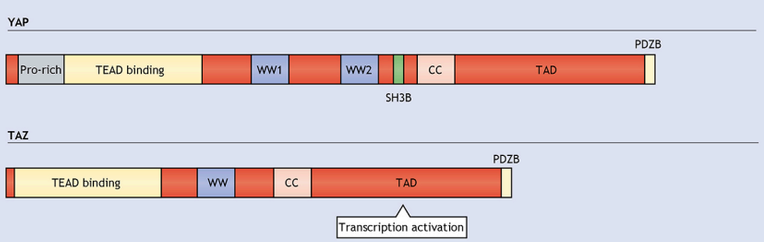
Effector protein	Amino acid residues	Effect on YAP/TAZ
YAP phosphorylations		
LATS1/2	S61,109,127,164, 397	Inhibition
AKT	S127	Inhibition
CK1e/d	S400,403	Inhibition
Yes, Abl	Y357	Activation (pro-apoptotic)
Src	Y341/357/394	Activation
JNK	T119, S138, T154, S317, S362	Activation
NLK	S128	Activation
CDK1	T119, S289, S367	Activation (cytokinesis)
CDK8	T119, S128, S289, S367	Activation
AMPK	S94 and others	Inhibition
Other YAP post-translational modifications		
Acetylation (EP300)	K494,497	Activation
Methylation (SETD7)	K494	Inhibition
Methylation (SETD1A)	K342	Activation
O-GlcNAcylation (OGT)	S109	Activation
K63-linked ubiquitylation (SCF–SKP2)	K321,497	Inhibition
SUMOylation (PML)	K97/280	Activation (pro-apoptotic)
TAZ phosphorylations		
LATS1/2	S66, 89, 117, 311	Inhibition
GSK3b	S58, 62	Inhibition
CK1e/d	S314	Inhibition
Src, Abl	Y316	Activation
TEADs post-translational modifications		
TEAD1 palmitoylation (also other TEADs)	C53, C327,C359	Activation

Small molecules acting on YAP/TAZ

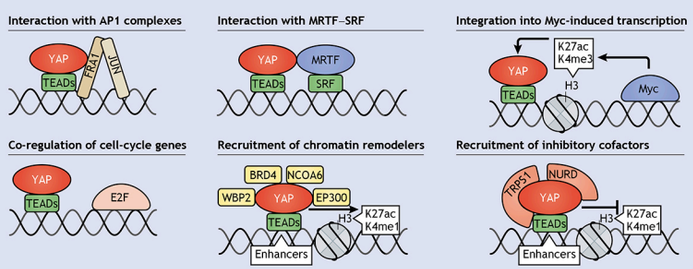
Small molecule	Molecular target	Effect on YAP/TAZ
Acting on upstream regulatory pathways		
XMU-MP-1	MST1/2	Activation
Y27632, HA1077	ROCK1/2	Inhibition
ML7	MLCK	Inhibition
Blebbistatin	NMII	Inhibition
SMIFH2	Formins	Inhibition
Latrunculin A	F-actin	Inhibition
Cytochalasin B	F-actin	Inhibition
Jasplakinolide	F-actin	Activation
Statins	HMGCR	Inhibition
GGTI-298	Geranyl-geranyl-transferase 1	Inhibition
Dasatinib	RTKs, Abl, Src	Inhibition
XAV-939	Tankyrases	Inhibition
GPCR agonists	GPCRs	Activation/Inhibition
Forksolin, IBMX	Adenylate cyclase	Inhibition
WZ4003, HTH-02-006	NUAKs	Inhibition
Dobutamine	unknown	Inhibition
Norcantharidin (NCTD)	unknown	Inhibition
Acting on the YAP/TAZ–TEADs transcriptional complex		
Verteporfin	YAP/TEAD binding	Inhibition
Flufenamic acid	YAP/TEAD binding	Inhibition
VGLL4 peptide	YAP/TEAD binding	Inhibition
WO2017064277 (Inventiva)	YAP/TEAD binding	Inhibition
TED347	TEAD palmitoylation	Inhibition
WO2017053706 (Mass General)	TEAD palmitoylation	Inhibition
Acting on downstream effectors of YAP/TAZ		
JQ1, OTX015	BET (Brd1-4)	Inhibition

See supplemental data for table references

YAP and TAZ structural domains



YAP/TAZ as transcriptional coactivators



Abbreviations: AMOTs, angiomotin and angiomotin-like proteins (AMOT, AMTOTL1 and AMOTL2); ADC, adenylate cyclase; α-CAT, catenin alpha-1 (CTNNA1); AMPK(1 and 2), 5′-AMP-activated protein kinase catalytic subunit alpha 1 and 2 (PRKAA1 and 2); APC, adenomatous polyposis coli protein (negative regulator of the WNT-signaling pathway); ARHGAP29, Rho GTPase activating protein 29; ARHGEF7, Rho guanine nucleotide exchange factor 7; ARID1A, AT-rich interactive domain-containing protein 1A; ATG7, autophagy related 7; AXINs, axin1 and axin2; B-CAT, catenin beta-1 (CTNNB1); BRD4, bromodomain-containing protein 4; Abl, tyrosine-protein kinase ABL; cAMP, cyclic adenosine monophosphate; CAPZ, F-actin-capping protein heterodimer comprising CAPZA and CAPZB; CC, coiled-coil domain; CDC42, cell division cycle 42; CDK1, cyclin dependent kinase 1; CDK8, cyclin dependent kinase 8; CFL1/2, cofilin 1 and 2; CK1-e/d, casein kinase 1, epsilon and delta isoforms; CRB3, crumbs cell polarity complex component 3; DAG1, dystroglycan 1; DDX17, DEAD-box helicase 17; DLG5, discs large MAGUK scaffold protein 5; DMD, dystrophin; E-CAD, cadherin 1 (CDH1); ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; EP300, E1A-binding protein p300; F-actin, filamentous actin; FBXW7, F-box and WD-repeat domain containing 7; FRA1, FOS-like 1, transcription factor AP1 subunit (FOSL1); GGPP, geranyl-geranyl diphosphate; GGTI, geranylgeranyltransferase type I (comprising FNTA and PGTB1 subunits); GNA11, G-protein subunit alpha 11; GNA12, G-protein subunit alpha 12; GNA13, G-protein subunit alpha 13; GNAQ, G-protein subunit alpha q; GNAS, guanine nucleotide-binding protein G(s), subunits alpha isoforms; GP130, interleukin-6 receptor subunit beta (IL6ST); GPCRs, G-protein coupled receptors; GSK3-beta, glycogen synthase kinase 3 beta; GSN, gelsolin; H3, histone H3; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; IL6, interleukin 6; JNKs, (Jun N-terminal kinases MAPK8, MAPK9, MAPK10); JUN, Jun and JunD proto-oncogenes, transcription factor AP1 subunits; K4me1, mono-methylation of Lys4; K27ac , acetylation of Lys27; K_{in}, diffusion constant from the cytoplasm to the nucleus; K_{out}, diffusion constant from the nucleus to the cytoplasm; LATS1/2, large tumor suppressor kinases 1 and 2; LIF, LIF interleukin 6 family cytokine; LIFR, LIF receptor subunit alpha; LKB1, serine/threonine kinase 11; LPA, lysophosphatidic acid; MAP4K, mitogen-activated protein kinase kinase kinase kinases (comprising MAP4K1, MAP4K2, MAP4K3, MAP4K4, MAP4K5); MARK, microtubule affinity-regulating kinase (comprising MARK1, MARK2, MARK3, MARK4); MLC, myosin light chain polypeptides; MLCK, myosin light chain kinases; MMP14, matrix metallopeptidase 14; MRTF, myocardin-related transcription factors (MRTFA and MRTFB); MSCs, mesenchymal stem cells; MST1/2, serine/threonine kinase 3 and 4 (STK3/4); mTOR, mechanistic target of rapamycin kinase; Myosin II, non-muscle myosin heavy chain 2 polypeptides; NCOA6, nuclear receptor coactivator 6; NF2, neurofibromin 2; NLK, nemo-like kinase; NUAK2, NUAK family kinase 2; NPC, nuclear-pore complex; NURD, nucleosome remodeling and deacetylase complex; OGT, O-linked N-acetylglucosamine transferase; P, phosphate; p38, p38 MAPKs (comprising MAPK11,12, 13, 14); PA, phosphatidic acid; PAK2, p21 (RAC1) activated kinase 2; PATJ, PATJ crumbs cell polarity complex component; PDZB, PDZ domain-binding motif; PDZGEF1/2, Rap guanine nucleotide exchange factors 1 and 2 (RAPGEF1 and 2); PFKP, 6-phosphofructokinase; PIP2, phosphatidyl-inositol-4,5-bisphosphate; PKA, members of the protein kinase A family protein; PLC- γ1, phospholipase C gamma 1; PLD1/2, phospholipase D1 and D2; PML, promyelocytic leukemia protein; Pro-rich, proline-rich domain; PP2A, protein phosphatase 2 catalytic subunit; RAC1, Rac family small GTPase 1; RAP2s, Ras-related proteins RAP2A, RAP2B and RAP2C; RHO, Rho family of GTPases; ROCK, Rho-associated coiled-coil-containing protein kinases; S1P, sphingosine-1-phosphate; SAV1, salvador family WW domain-containing protein 1; SCF-BTRCP, Skp, cullin, F-box-containing complex (SCF) containing the β-transducing repeat-containing protein (β TrCP); SCF-SKP2, SCF complex containing the S-phase kinase-associated protein 2 (SKP2); SCRIB, Scribble homolog; SET1A, histone-lysine N-methyltransferase SETD1A; SETD7, histone-lysine N-methyltransferase SETD7, histone lysine methyltransferase; SGCD, sarcoglycan delta; SH3B, Src-homology 3 domain-binding motif; SRF, serum response factor; STRIPAK, Striatin-interacting phosphatase and kinase complex; TAD, transactivation domain; TAOK, TAO kinase; TAZ, WW-domain-containing transcription regulator 1 (WWTR1); TEADs, TEA(transcriptional enhancer factor)-domain transcription factors; TRPS1, transcriptional repressor GATA binding 1; TSC1/2, TSC complex subunit 1 and 2; U, ubiquitin; USP9X, ubiquitin-specific peptidase 9 X-linked; VGLL4, vestigial like family member 4; WBP2, WW domain-binding protein 2; WNTs, ligands of the Wnt family for members of the frizzled receptor family; WW, Trp-Trp-repeat domain; WWC1/2, WW and C2 domain-containing proteins 1 and 2; ω3-PUFA , omega3 polyunsaturated fatty acids; YAP, Yes-associated protein 1; ZO, Zona occludens proteins (comprising ZO1, 2, 3).

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